Phosphoproteomic Investigation of Differential Signalling Downstream of Class IA PI3K Isoforms

PhD Thesis of Michael Hartley Walsh

Principal Supervisor: Professor Bart Vanhaesebroeck

Second Supervisor: Dr Pedro Cutillas

Submitted in fulfilment of the requirements of the Degree of Doctor of Philosophy

Statement of Originality

I, Michael Hartley Walsh, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

I attest that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge break any UK law, infringe any third party's copyright or other Intellectual Property Right, or contain any confidential material.

I accept that the College has the right to use plagiarism detection software to check the electronic version of the thesis.

I confirm that this thesis has not been previously submitted for the award of a degree by this or any other university.

Signature: M. H. Walsh

Date: 22/11/2014

Acknowledgements

I must first thank Bart Vanhaesebroeck for giving me the opportunity to work in an environment which engenders personal growth and independence, and on projects which hold such interest and importance. The knowledge and guidance of my second supervisor Pedro Cutillas have been supremely useful, and I wish to thank him for always being there when called upon.

The Centre for Cell Signalling has been a wonderful place to work, and everyone in it past and present has played a part in getting me to where I am. Ardent thanks go to Larissa for doing much more than she ever needed to, and to Claire for her support in some of the hardest times. I thank Salma for her work from which much of this project sprang, and for her help throughout. Ed, Michalina, Aine and Alex P have provided much needed material and moral support which has been indispensable. Thanks also to Alex M, Alex S, Benoit, Daniele, Ellie, Ezra, Inma, J.C., Khaled, Luisa, Maria, Pedro Casado, Regine, Roberto, Samira, Sandra, Veronica, Vinni, Wayne and York for their help along the way.

I would like to thank the Biotechnology and Biological Sciences Research Council and AstraZeneca for the funding which allowed the project and my PhD to happen. I am grateful to Kevin Hudson for his discussions, and to the graduate tutors who have helped my progression.

I am especially grateful to the BBSRC and the Society of Biology for providing the means and opportunity for me to explore a non-academic path, and to Bart for recognising my need for this, and for his support in granting me the freedom to pursue it.

Final, great, and sincere thanks go to my parents and family, whose limited knowledge of my project has never precluded concern, enquiry, or encouragement.

Abstract

The PI3K family is central to numerous cellular processes in both health and disease. The class IA isoforms of PI3K control such outputs as proliferation, metabolism and survival through their well-characterised function as lipid kinases, with their signalling thought to predominantly mediated by the Akt/PKB protein kinase. However there exist other signalling routes, including from the lipid kinase activity through other effectors, but also through a protein kinase function of the class IA isoforms themselves.

Mass spectrometry is a powerful tool which has been central to the recent advances in phosphoproteomic techniques. It is now possible to use mass-spectrometry to probe the phosphoproteome of any number of systems in an unbiased and global manner.

In this project, we aimed to advance our understanding of two aspects of class IA PI3K signalling which are relatively poorly understood. We used phosphoproteomic techniques which allowed us to provide answers to some old questions which have up to now proved elusive.

First, we investigated the protein kinase activity of p110α. We used an *in vitro* protein kinase assay and coupled this to mass spectrometry techniques to identify direct substrates of p110α. We proposed two novel protein substrates and attempted to characterise them further, although we were hampered by lack of available biochemical tools.

Second, we investigated the differential phosphoproteomes of the ubiquitously expressed class IA PI3K isoforms p110α and p110β in a panel of breast cancer cell lines. We used mass spectrometry-based phosphoproteomics and found significant differences in signalling between p110α and p110β in 4T1 cells, including differential regulation of previously described PI3K effectors, amongst them the Akt substrate PRAS40, and potential novel targets. Additionally, we found that some of these effects were conserved between cell lines.

Table of Contents

Abbreviations

4EBP1	Eukaryotic translation initiation factor 4E-binding protein 1
ACN	Acetonitrile
ATP	Adenosine triphosphate
BH	Bcr homology
BTK	Bruton's tyrosine kinase
CID	Collision induced dissociation
DDA	Data dependent analysis
DTT	Dithiothreitol
DMSO	Dimethyl sulfoxide
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EGTA	Ethylene glycol tetraacetic acid
ESI	Electrospray ionisation
ETD	Electron transfer dissociation
GAP	GTPase activating protein
GEF	Guanine nucleotide exchange factor
GA	Glycolic acid
GPCR	G protein-coupled receptor
GSK3	Glycogen synthase kinase 3
GTP	Guanosine-5'-triphosphate
HILIC	Hydrophilic interaction liquid chromatography
IC50	Concentration achieving 50% maximal inhibition
ICAT	Isotope-coded affinity tags
IMAC	Immobilised metal affinity chromatography
IRS-1	Insulin receptor substrate-1
iSH2	inter-SH2
iTRAQ	Isobaric tags for relative and absolute quantitation
KD	Kinase-dead
MALDI	Matrix-assisted laser desorption/ionisation
MAPK	Mitogen-activated protein kinase
MDM2	Mouse double minute 2 homolog

MEF	Mouse embryonic fibroblast
$MnCl_2$	Manganese (II) chloride
MS	Mass spectrometry
mTORC	Mechanistic target of rapamycin complex
m/z	Mass/charge
NaF	Sodium fluoride
$NaVO_4$	Sodium orthovanadate
NH_4OH	Ammonium hydroxide
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
PDK1	Phosphoinositide-dependent kinase-1
PH	Plekstrin homology
PHLPP	PH domain and leucine rich repeat protein phosphatase
PI	Phosphatidylinositol
PI(3)P	Phosphatidylinositol-3-phosphate
$PI(3,4)P_2$	Phosphatidylinositol (3,4) bisphosphate
$PI(4,5)P_2$	Phosphatidylinositol (4,5) bisphosphate
$PI(3,4,5)P_3$	Phosphatidylinositol (3,4,5) trisphosphate
PI3K	Phosphatidylinositol 3-kinase
PKC	Protein kinase C
PMSF	Phenylmethylsulfonyl fluoride
PP2A	Protein phosphatase 2A
PRAS40	Proline-rich Akt substrate of 40 kDa
PTEN	Phosphatase and tensin homologue
PTM	Post-translational modification
pTyr	Phosphorylated tyrosine
PX	Phox homology
RAPTOR	Regulatory-associated protein of mTOR
RBD	Ras-binding domain
RICTOR	Rapamycin-insensitive companion of mTOR
RSK	p90 ribosomal S6 kinase
RTK	Receptor tyrosine kinase
S6	Ribosomal protein S6
S6K	p70 ribosomal S6 kinase

SCX	Strong cation exchange
SDS	Sodium dodecyl sulfate
SGK	Serum-and glucocorticoid-induced protein kinase
SH2	Src homology 2
SILAC	Stable isotope labelling by amino acids in cell culture
SHIP	SH2 domain-containing inositol-5'- phosphatase
TSC	Tuberous Sclerosis Complex
TFA	Trifluoroacetic acid
TiO_2	Titanium dioxide
TMT	Tandem mass tags
TOF	Time of flight
Vps15	Vacuolar protein sorting 15
Vps34	Vacuolar protein sorting 34
WT	Wild-type
XIC	Extracted ion chromatograms

1. Introduction

1.1. Phosphoinositide 3-kinases (PI3Ks)

The phosphoinositide 3-kinase (PI3K) family is made up of eight enzymes which are conserved across species and have key roles in intracellular signalling. PI3Ks primarily act as lipid kinases and phosphorylate the inositol ring of phosphatidylinositol (PI) and its derivatives. This phosphate addition occurs at the 3' position, a reaction which leads to the production of a number of new lipid products – PI(3)P from PI, $PI(3,4)P_2$ from PI(4)P, and $PI(3,4,5)P_3$ (PIP_3) from $PI(4,5)P_2$. These second messengers then recruit effector proteins to cellular membranes and further influence multiple downstream pathways. PI3K activity contributes to a diverse range of cellular processes, including cell division, metabolism and intracellular vesicular trafficking, and while its signalling is crucial for healthy cells, it is also implicated in a number of disease processes, most notably in cancer where the PI3K pathway is one of the most frequently dysregulated pathways.

1.1.1. PI3K classification

The eight PI3K isoforms are subdivided into three classes, based on structural homology and substrate preference [1]. There are four class I PI3Ks which are heterodimers made up of a p110 catalytic subunit bound to a regulatory protein, three monomeric class II PI3Ks, and a sole class III member called vacuolar protein sorting (Vps) 34, which is found in complex with Vps15 (Fig. 1.1.). PI3Ks share a common core made up of a helical, catalytic, and C2 lipid-binding domain. The class I isoforms will be discussed in greatest detail in this and later chapters, as their signalling pathways are the primary focus of this project.

The first signs of a PI3K activity were observed in the 1980s when an association between viral oncoproteins and a PI kinase was noticed, which was discovered by the Cantley group to phosphorylate PI at the 3' position. Further work saw the generation of PIP3 from PIP2 following RTK or GPCR activation, and purification of a PI3K activity was found to correlate with proteins of 110 kDa and 85 kDa. Cloning of the 85 kDa protein showed that it had SH2 domains

but no intrinsic kinase activity, and subsequent cloning of the p110 kDa protein formally established it as holding the kinase activity in a p110-p85 heterodimer. This cloning revealed homology with the yeast Vps34 which was later shown also to have a PI3K activity, and further genetic and biochemical work saw genes for multiple PI3K family members identified and split into three classes.

1.1.1.1. Class I PI3Ks

The four members of class I PI3Ks are subdivided further depending on which regulatory subunit they bind: the class IA isoforms (p110α, β, and δ) bind to a p85 subunit, while p110γ binds to either p87 or p101 [2].

1.1.1.2. Class II PI3Ks

There are three mammalian class II isoforms, PI3K-C2α, PI3K-C2β and PI3K–C2γ. Class II PI3Ks are monomers and consist of the conserved PI3K core, as well as extensions at both termini; proline-rich regions at the N-terminus, and a lipid-binding PX domain plus an extra C2 domain at the C-terminus [1]. While the tissue distribution of PI3K-C2α and PI3K-C2β is broad, PI3K–C2γ is found only in a limited number of tissues such as the liver [3].

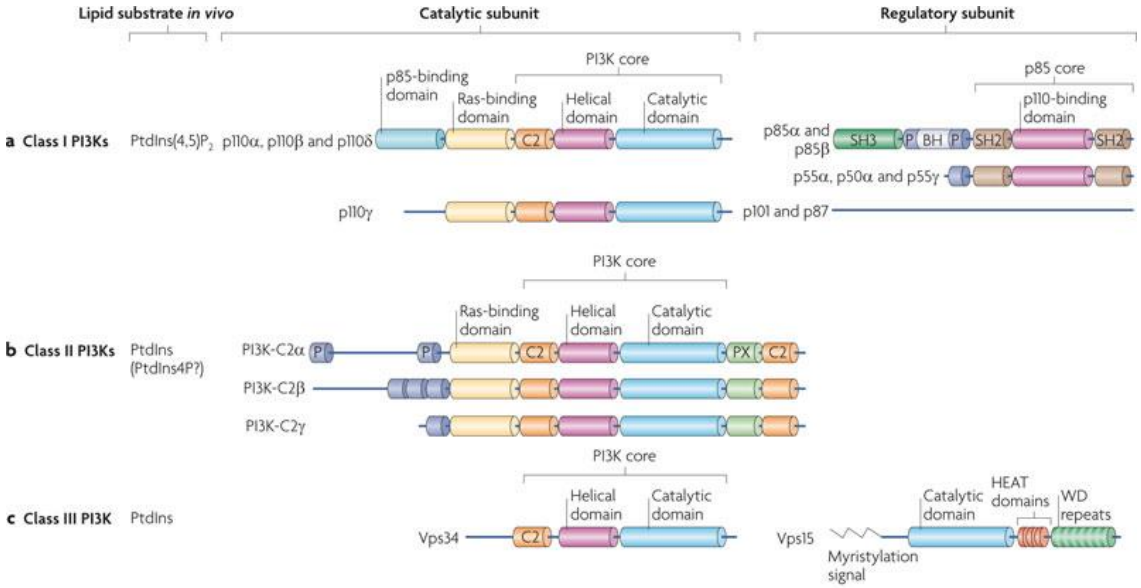


Figure 1.1. Classification of PI3Ks. Adapted from [1]. The seven PI3K isoforms display a large degree of structural homology. A) The four class I PI3K family members are heterodimers composed of a catalytic and regulatory subunit. B) The three class II members are monomers with N- and C-terminal extensions, while C) the sole class III PI3K is a heterodimer which is membrane-bound.

Class II PI3Ks are the least studied isoforms, and our current understanding of their signalling and physiological roles is limited. Studies suggest that activation is achieved by GPCRs, RTKs and GTPases, and at least *in vitro*, the class II PI3Ks can utilise PI, PI(4)P and PI(4,5)P_2 as substrates [4], with PI thought to be the preferred substrate *in vitro* and *in vivo* (Fig. 1.2.) [5, 6]. The class II PI3Ks have been implicated in a number of processes including vesicle trafficking, cell migration and metabolism [1], but exactly how they bind to membrane receptors and exert control over such biological outputs is unclear.

1.1.1.3. Class III PI3Ks

The sole class III member is Vps34, which is evolutionarily conserved and ubiquitously expressed in mammals [1]. Similar to the class I isoforms, it exists as a constitutive heterodimer with its partner Vps15. The Vps15 subunit is myristoylated, which targets class III PI3K to intracellular membranes where its primary substrate PI is found [7]. In addition to Vps15, Vps34 forms higher order complexes with other proteins which are required for its cellular functions [7, 8].

Vps34 is localised to the endosomal compartment, and it has been implicated in various processes involving vesicle trafficking such as autophagy and endocytosis [8, 9].

1.1.2. Class I PI3K structure

In resting cells, the class I regulatory subunit maintains the catalytic p110 subunit in a closed, inactive conformation [11]. Upon stimulation, the regulatory subunit is required to recruit the heterodimer to activated receptors [12, 13]. The regulatory subunit further acts to stabilise the p110 proteins which are unstable as monomers [14].

All four p110 proteins share the conserved PI3K core plus a Ras-binding domain, with the class IA members having an extra p85-binding domain which dictates the preference for p85 as a regulatory partner [15]. The p85 proteins are transcribed from multiple different genes: p85α, p55α and p50α are produced from *PIK3R1*, while p85β is transcribed from *PIK3R2* and p55γ from *PIK3R3*. The p85 proteins have multiple domains including a proline-rich region,

and a p110-binding domain which is flanked by two SH2 domains which allow binding to phosphorylated tyrosine (pTyr) residues, such as those at activated receptors [1]. p85α and β also possess an extra proline-rich region, as well as Bcr homology (BH) and SH3 domains, which may allow extra signalling outputs [1]. The structure and contribution of p87 and p101 to p110γ signalling are unclear, but they are able to help to recruit p110γ to Ras and GPCRs.

Class I PI3Ks catalyse the production of PIP_3 from $PI(4,5)P_2$ [16], which allows recruitment of proteins which contain lipid-binding domains, such as pleckstrin homology (PH) domains, to the plasma membrane [17]. Class I PI3Ks are present in all cell types, although the distribution of the different isoforms varies: p110α and β are ubiquitously expressed, while p110γ and δ are enriched in leukocytes [3]. Perhaps because of the ubiquitous tissue distribution of p110α and p110β, mice lacking these isoforms display embryonic lethality [18, 19], while p110γ and p110δ deficient mice are viable but have immune defects [20, 21]. It is important to note that both the upstream and downstream signalling of PI3Ks can vary in different cell- and signal-specific contexts.

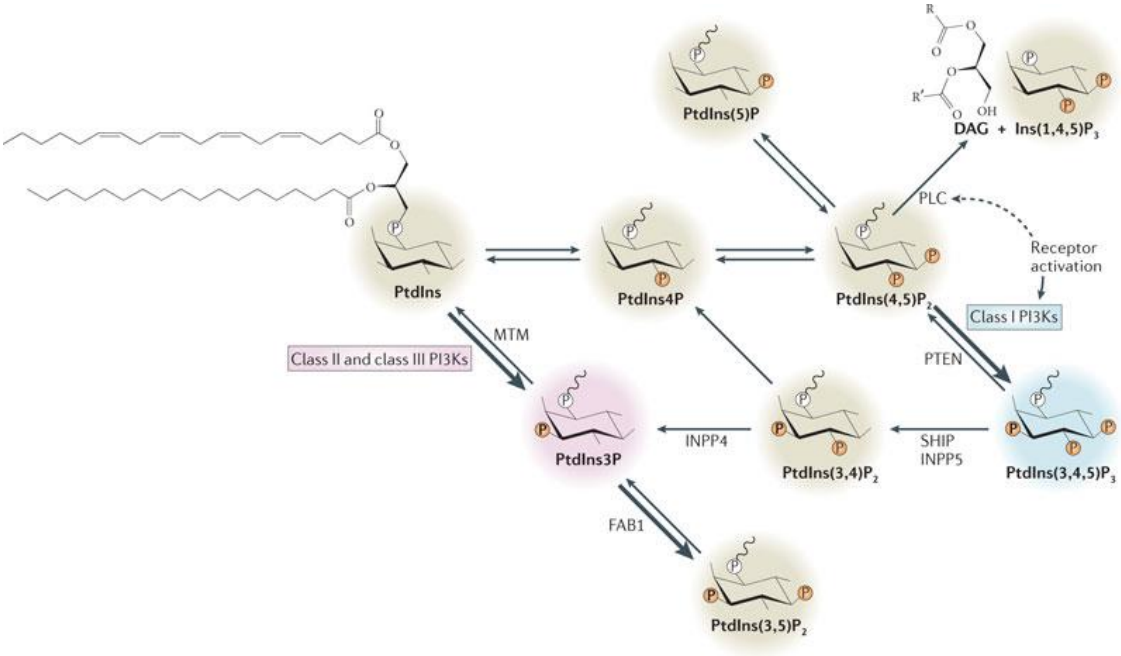


Figure 1.2. PI3K lipid substrates and products. Adapted from [10]. Phosphoinositde species are produced by the interplay between PI3K isoforms and the phosphatases which oppose their action. Class I PI3Ks produce PIP_3 from $PI(4,5)P_2$, while the class II and class III isoforms are thought to prefer PI as a substrate. The dynamic production of these species influence signalling cascades via binding of effector proteins.

As well as this lipid kinase activity, PI3Ks also have protein kinase activity [22]. The best characterised substrates of this activity are the PI3Ks themselves; p110α phosphorylates p85α at the Ser608 residue [23], while the other isoforms autophosphorylate the p110 subunit [22]. For the class IA isoforms (auto)phosphorylation causes a reduction in their lipid kinase activity, and so has been proposed to be a potential mode of negative regulation. Exogenous substrates have also been identified, although little work has been done to uncover the potential roles of those phosphorylations. The protein kinase function of PI3Ks will be discussed in more detail below.

While the majority of studied PI3K outputs are predominantly kinase-dependent, the class I PI3K members also have roles as scaffolding proteins, with both p110 and p85 proteins reported to have non-PI3K binding partners such as the small GTPase Rab5 and Cdc42 [24, 25, 26, 27]. This non-catalytic function is less well studied but supported by different phenotypes of knock-out and knock-in strategies (discussed later), and few interactions of this nature have been reported. It is certainly true that although the lipid kinase activity of PI3K is the best characterised effector of its own functions, other processes (i.e. protein kinase activity, scaffolding) can contribute to the diverse cellular outputs found in the PI3K pathway.

1.1.3. Signalling upstream of class I PI3K

Class I PI3Ks were originally subdivided based on differences in the perceived view of activation by RTKs and GPCRs (which has since changed), and in regulatory subunits, (which still stands) (Fig. 1.3.). However, the real picture is much more complex than a simple separation into class IA and IB. While p110α and p110δ are mainly activated by recruitment of the p85/p110 complex to pTyr residues in receptor tyrosine kinases (RTK) or adaptor proteins, they can also be activated by Ras [28, 29]. The class IB p110γ is activated downstream of Ras and G protein coupled receptor (GPCR) Gβγ subunits [30], whereas there is evidence for activation of p110β by all three modes, and possibly also by families of other small GTPases such as Rab5 [24, 31].

The canonical method of class I activation is downstream of activated growth factor receptors [2]. For example, PDGF binds its receptor causing activation

and autophosphorylation of intracellular portions of the receptor. These pTyr residues are recognised and bound by the SH2 domains of a variety of proteins, including p85 isoforms, leading to recruitment of class IA PI3Ks to the plasma membrane where they catalyse the production of PIP_3 from $PI(4,5)P_2$. Indirect recruitment of class I heterodimers to RTKs can also occur via adaptor proteins such as Grb2 or the insulin receptor substrate (IRS) -1. While p110β and p110δ seem to require both p85 SH2 domains to be bound to pTyr, p110α may be fully activated by binding of only the N-terminal SH2 domain [32]. This less stringent

requirement for activation, along with its ubiquitous tissue expression, may help explain the fact that p110α has been the only PI3K found mutated in cancer [33].

The class IB PI3K p110γ is activated downstream of GPCRs [30]. Upon activation, the Gβγ subunit dissociates from Gα and recruits p110γ, although the exact mechanism of this switch is still unclear. Expression of the p110γ adaptors p87 and p101 varies between cell types, and the two have different properties with regards to affinity and activation with p110γ-p101 heterodimers being more sensitive to activation by Gβγ, and less reliant on Ras [34] (see below). GPCRs have also been shown to be upstream of p110β [35], which is also activated through direct interaction with the Gβγ subunit.

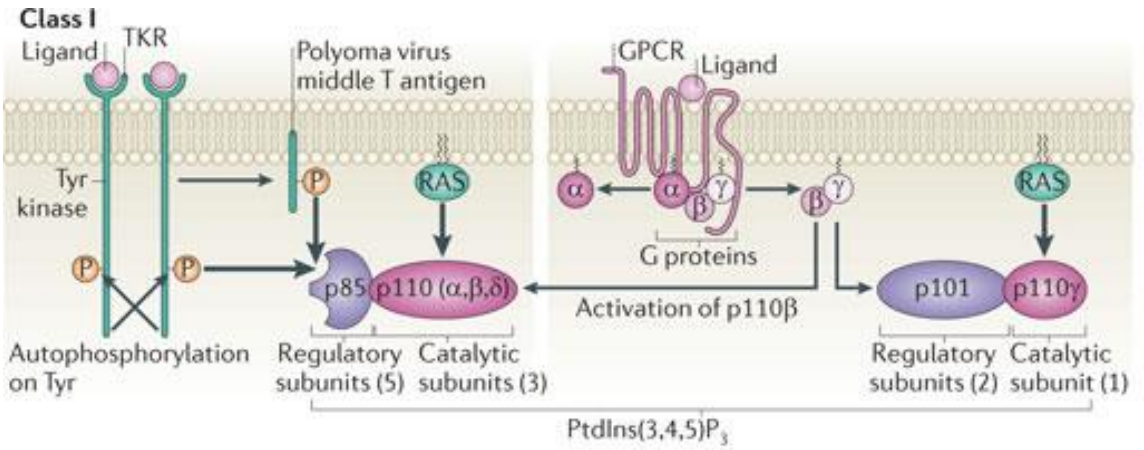


Figure 1.3. Class I PI3K structure and signalling input. Adapted from [10]. The canonical view of PI3K signalling is of the class IA isoforms being regulated by RTKs and p110γ being downstream of GPCRs, with Ras able to influence all isoforms. The emerging picture has p110β also regulated by GPCRs, with evidence for a role of other small GTPases.

All class I isoforms possess a Ras binding domain (RBD) and Ras family members have long been thought to be activators of all four isoforms. However, recent evidence suggests that p110β is an exception and is instead activated downstream of other small GTPases [31]. There are multiple different Ras family members, and Ras/PI3K interactions probably vary in cell- and agonist-specific contexts. It has been shown that the class IA isoforms are activated downstream of Ras but in an RTK dependent manner [36], as p85 can block stimulation by Ras. For p110γ, p101 and p87 display the opposite dependency on upstream agonists: p110γ-p87 is more sensitive to Ras, while p110γ-p101 is more sensitive to Gβγ [37].

While there is a high level of sequence homology between p110α and p110β, differences exist in the RBD, suggesting varying sensitivity to small GTPases [31]. As well as inputs from both RTKs and GPCRs, p110β has the potential to be activated by Rab and the Rho family of GTPases [31]. Rab5 is found in endosomes and binding to p110β occurs in clathrin-coated vesicles [24, 38], suggesting a role for p110β in endosome formation, although this link has not been fully explored. Ras was also thought to activate p110β but recent evidence has emerged that this interaction does not take place, and it is in fact the Rho superfamily which is upstream of p110β [31]. The association of PI3K with various families of small GTPases may need to be re-examined, as the complexity and contexts may be more intricate than currently stated.

The complexity of upstream PI3K activation has yet to be fully understood – questions still remain as to the role of the different regulatory isoforms, and the extent of overlap between the different inputs. In different contexts with spatial and temporal considerations the input to and output from PI3Ks will vary, and it has been shown that there is a certain amount of functional redundancy between isoforms. Cells and tissues are not presented with a single input and so signalling studies which typically only monitor the effect of one agonist over-simplify the real physiological range of stimuli.

1.1.4. Signalling downstream of class I PI3K

As dual specificity kinases, PI3Ks are able to phosphoryate both lipid species and proteins [1, 22].

1.1.4.1. Lipid kinase activity

The lipid kinase activity of PI3Ks is the best characterized function of these kinases and is largely responsible for its physiological effects.

Phosphoinositide species are recognised with varying specificity by effector proteins which contain lipid-binding domains (such as PH or PX domains) leading to membrane recruitment [16]. This change in localisation may in turn affect protein function, activity or binding partners. Despite the variety of upstream signalling to the class I isoforms, they all catalyse the acute production of PIP_3 from $PI(4,5)P_2$, a lipid species which is ubiquitous in cells [16]. PIP_3 is short-lived and recognised by proteins with PH domains, including the best characterised PI3K effector, the serine/threonine kinase Akt/protein kinase B (PKB), whose activity is discussed in detail below. It has been estimated that phosphoinositides make up around 10% of total cellular lipids, with $PI(4,5)P_2$ present at low levels (~2-30 μM) and PIP_3 more than 25 times lower still [39]. As well as providing platforms for signalling cascades, the presence or absence of PI species can help to define cellular membranes; PIP_3 is enriched at the plasma membrane, while PI3P is present mainly on intracellular membranes [16].

Recruitment of effector proteins to the plasma membrane allows them to continue the signalling cascade. For example, Akt is phosphorylated and activated by the kinase PDK1 [40], both of which are responsive to PIP_3 levels. Akt then goes on to phosphorylate a number of proteins leading to changes in diverse processes such as metabolism, survival and proliferation [41]. There are over 200 proteins which contain putative PH domains, although not all will be responsive to PIP_3. These include other kinases, such as Bruton's tyrosine kinase (BTK), as well as some GTPase activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs), which are effectors of PI3K function [42].

1.1.4.2. Akt

Akt is one of the best characterised effectors of PI3K activity. Akt was originally identified through its homology with previously known protein kinases, and is a member of the AGC family of kinases which includes PKA, PDK and serum-and

glucocorticoid-induced protein kinase (SGK) [41]. There are three isoforms of the kinase, Akt1, 2 and 3, which have differential tissue distribution and are encoded by separate genes. The isoforms all have a similar structure including three conserved domains: an N-terminal lipid binding PH domain which allows membrane binding, a catalytic kinase domain, and a C-terminal hydrophobic motif which is found in other members of the AGC family. Isoform-specific knock-out mice are viable with different phenotypes, evidence for non-redundant functions of the isoforms. Akt1 is highly expressed in all tissue types, and genetic knock-out leads to a reduction in body weight [43]. Akt2 and Akt3 have more restricted tissue distribution: Akt2 is highly expressed in insulin-responsive tissues such as liver and adipose tissues and is implicated in the control of metabolism [44] while Akt3 has high expression in the brain [45]. Genetic knock-outs of Akt2 and Akt3 cause metabolic and brain defects respectively [44, 45].

Through its PH domain Akt binds to both $PI(3,4)P_2$ and the PIP_3 lipid product of class I PI3Ks, leading to relocalisation from the cytoplasm to the plasma membrane [41]. This translocation event anchors Akt to the membrane, brings it into contact with its upstream activating kinases, and causes a small increase in kinase activity by way of a conformational change, but its activity is dramatically increased, by around 1000 fold, by two phosphorylation events. First, Thr308 is phosphorylated by phosphoinositide-dependent protein kinase 1 (PDK1) [40], which is also recruited to the plasma membrane through its own PH domain. This increases the kinase activity of Akt by around 100 fold, with a further 10 fold increase being achieved by phosphorylation of the Ser473 residue [46], an event catalysed by mTORC2 [47].

Full activation of the kinase requires phosphorylation of these two residues which are located in separate domains. The Thr308 residue of Akt1 is located in a region termed the activation or T-loop, a domain which is conserved among kinases; phosphorylation of residues in this area is required for substrate recognition, and also efficient transfer of the phosphate group from the ATP donor molecule. The Ser473 residue is found in a C-terminal hydrophobic motif, and phosphorylation of residues in this region are commonly seen in the activation of other AGC family members [48]. The phosphorylations are

removed by phosphatases: protein phosphatase 2A (PP2A) targets Thr308 and Ser473 [49, 50], while PH domain and Leucine rich repeat Protein Phosphatase (PHLPP) targets Ser473 [51]. However, these phosphorylations are not always a reliable readout of activity of upstream or downstream pathways, or of the activity of Akt. It has been shown that the activating phosphorylations may be present when the activity of Akt is reduced [52], and inhibition of Akt by ATP-competitive compounds can bring about conformational changes which shield the phosphorylations from phosphatase attack [53], which is also seen upon ATP binding.

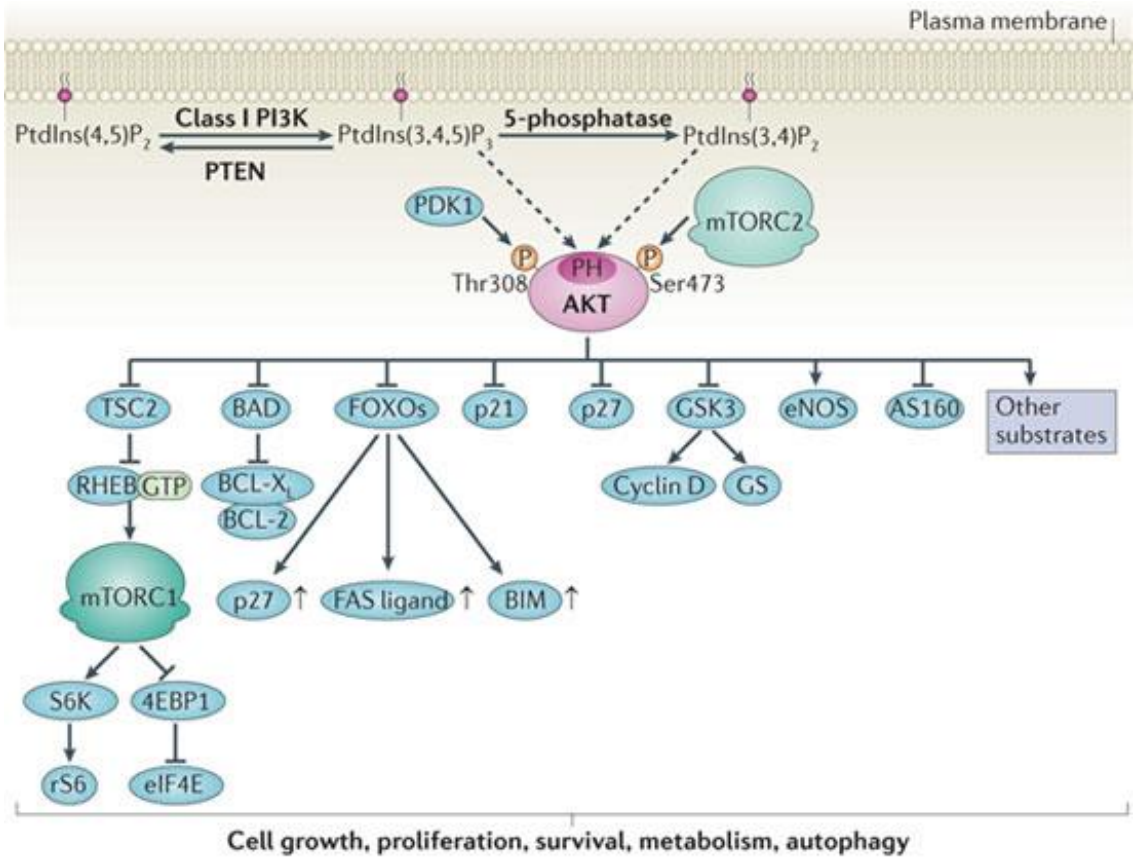


Figure 1.4. Akt phosphorylates and controls diverse substrates and processes. Adapted from [10]. Akt is thought of as the primary effector of class I PI3K function. It binds the PIP_3 lipid product at the plasma membrane and is targeted by two activating phosphorylations from PDK1 and mTORC2. In turn it phosphorylates a range of substrates, affecting many central cellular processes.

Akt is a central node in multiple signalling pathways, and it connects growth factor signalling and PI3K activity to numerous cell processes, many of which have been implicated in oncogenesis. These pathways are not under the control of one single Akt substrate, and some substrates may influence more than one output; the multiple Akt targets may change in a signal and cell-specific context, allowing fine tuning of the response. To date, more than 100 Akt substrates have been identified, allowing Akt to control multiple and diverse cellular signalling pathways such as survival, metabolism and proliferation (Fig. 1.4.) [41]. Like many other kinases Akt has a peptide consensus motif, in this case RxRxxpS/pT, which it recognises and phosphorylates [54], although it is absent from some reported substrates. Phosphorylation by Akt often provides a docking site for 14-3-3 scaffolding proteins; binding of 14-3-3 isoforms to Akt substrates can cause sequestration and inactivation. An example of this is the phosphorylation of Foxo3a by Akt at two sites which leads to 14-3-3 binding and relocalisation from the nucleus to the cytoplasm, hence the Foxo3a transcription factor is unable to influence expression of genes important in processes such as apoptosis [55].

One of the best described effects of Akt activation is subsequent activation of the mechanistic target of rapamycin complex 1 (mTORC1). mTORC1 is itself a key controller of cell growth, proliferation and metabolism, and Akt contributes to its regulation by phosphorylating and inhibiting two mTORC1 negative regulators; TSC2 [56, 57, 58] and proline-rich Akt substrate of 40 kDa (PRAS40) [59, 60]. TSC2 acts in a complex with TSC1 as a GAP for Rheb, which is an upstream activator of mTORC1 when GTP bound. Phosphorylation of the mTORC1 inhibitor PRAS40 causes dissociation from the complex and 14-3-3 binding [60]. mTORC1 is then active and exerts its control over autophagy, protein synthesis and cell growth [61] (discussed in more depth later).

Cell survival mechanisms are also triggered by Akt activation, mainly through direct phosphorylation and inactivation of proapoptotic proteins, or indirectly through down regulation of their expression. Akt phosphorylates Bim and Bad [62] to inhibit their function through 14-3-3 binding and sequestration, and phosphorylation of Bax inhibits mitochondrial translocation and membrane

disruption [63]. Bcl-2 is inhibited through the phosphorylation of mouse double minute 2 homolog (MDM2) by Akt [64], while, through its direct negative regulation of the Foxo family of transcription factors, Akt can reduce the expression of proapoptotic factors [65]. Akt aids cell cycle progression through phosphorylation and inhibition of glycogen synthase kinase 3 (GSK3) [66]. GSK3 has a negative impact on the cell cycle through phosphorylation of transcription factors and other regulators such as Myc [67] and cyclin D [68], and the inactivation of GSK3 by Akt releases its block on progression through checkpoints.

As the primary Akt isoform in insulin-responsive tissues, Akt2 plays a key role in cellular metabolism. This includes direct phosphorylation of the glycolytic 6-phosphofructo-2-kinase isoforms to activate them [69], or of GSK3 [66] or PGC-1α [70] to inhibit them, which pushes the cell towards a more glycolytic profile [71], a phenotype which is frequently seen in tumours [72]. At the receptor level too, Akt promotes translocation of the glucose transporter Glut4 to the plasma membrane through its phosphorylation and inhibition of AS160 which prevents translocation through its Rab-GAP activity [73]. Akt also inhibits the phosphatase PTB1B which dephosphorylates the insulin receptor and targets it for degradation, thereby allowing an extension of signalling [74]. Through regulation of mTOR and HIF-1α amongst other proteins, Akt regulates transcription of metabolic enzymes such as the glucose transporter Glut1 [75].

Akt is primarily thought of in terms of the PI3K signalling pathway, but there is also a degree of involvement in other signalling axes. This crosstalk allows further fine-tuning of signalling, and means that Akt has a wider reach than a first look would suggest, as it communicates with (among others) mitogen-activated protein kinase (MAPK) pathways [76, 77, 78].

1.1.4.3. mTOR

mTOR is an atypical serine/threonine protein kinase, and through its enzymatic activity it regulates crucial signalling pathways. It is able to respond to environmental stimuli to integrate such inputs as growth factors and stress with metabolism and cell fate, a function which is vital for maintaining a balance between nutrient availability in the environment, and cellular growth. mTOR is

often thought of as a sensor, which allows the cell to switch between energy conservation and recycling during periods of starvation, and metabolic growth when nutrients and cellular building blocks are freely available. Its central position as such a regulator means that it has been implicated in metabolic diseases such as diabetes [79], and also as the cell's energy factory in cancer [80].

mTOR is part of the PI3K-related protein kinase family, and it exists in two discrete complexes (mTORC1 and mTORC2) which are formed of multiple regulatory proteins (Fig. 1.5.), thus allowing the two complexes to exhibit non-redundant functions [81]. Both complexes have mTOR, deptor [82], mLST8 [83] and the homodimer Tti1/Tel2 [84] in common, while regulatory-associated protein of mTOR (RAPTOR) [85, 86] and PRAS40 [60, 87] are only found in mTORC1, and rapamycin-insensitive companion of mTOR (RICTOR) [88], mSin1 [89] and protor1/2 [90] are only found in mTORC2. A further difference is in the response of the two complexes to rapamycin, the bacterium-derived macrolide from which mTOR takes its name. Rapamycin interacts with the small protein FKBP12, and when bound the two are able to inhibit mTORC1 [91]. The complex does not seem to directly inhibit mTORC2, however there is evidence that long-term rapamycin treatment can reduce mTORC2 signalling [92]. Activation of the mTOR complexes impacts PI3K signalling through feedback loops [93]. mTORC1 activates p70 ribosomal S6 kinase (S6K) which in turn phosphorylates IRS1 to inhibit it (Manning 2004, [94]. Conversely, mTORC2 is part of a positive feedback loop, in which Akt is further activated [47].

The two mTOR complexes have been shown to be key regulators of growth and metabolism in cells, and control these and other processes by acting on a number of different targets [79]. Of the two complexes, mTORC1 is the better characterised, and positively regulates anabolic pathways including protein and lipid synthesis while negatively regulating catabolic activities such as autophagy. Since the processes it controls require large amounts of energy, regulation of mTORC1 is closely linked to the energy state of the cell. Being under the control of four different regulatory inputs (growth factors, nutrients, energy and stress) means that the signalling output of mTORC1 is tightly

controlled, and biosynthesis occurs only when environmental conditions are favourable.

mTORC1 has been shown to localise to the surface of lysosomes where it is activated, which is a two stage process. First, stimulation by amino acids promote activation of Rag GTPases, which recruit mTORC1 to the lysosome where it localises with Rheb [95, 96]. Second, growth factors promote the phosphorylation and inhibition of the TSC1/2 complex, which relieves the inhibition of Rheb. The now-active Rheb GTPase is able to activate mTORC1 to initiate down-stream signalling cascades. Phosphorylation by mTORC1 can be activating or inhibitory to the target: in the case of protein synthesis, S6K1 is activated and 4E-BP1 releases an inhibitory interaction in order to positively regulate mRNA transcription and ribosome biogenesis, while phosphorylation inhibits ATG1 to negatively impact autophagy [79, 97].

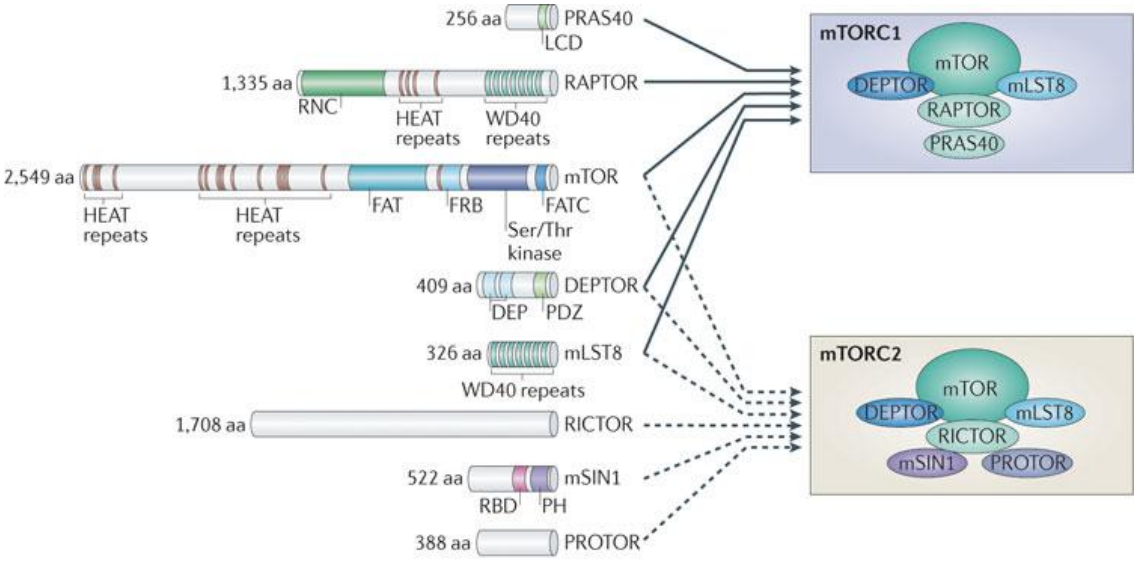


Figure 1.5. mTOR exists in two complexes. Adapted from [81]. The protein mTOR forms two functionally distinct complexes due to the binding of different partners. Both complexes contain DEPTOR and mLST8, while mTORC1 also comprises RAPTOR and PRAS40, and mTORC2 is also formed from RICTOR, mSIN1 and PROTOR.

The signalling networks around mTORC2 are less well defined, but one important downstream target is Akt, where phosphorylation of Ser473 is required for full activation [47]. It is thought that out of the four regulatory pathways governing mTORC1, only growth factors (such as insulin) control mTORC2. It has been suggested that by varying the members of the complex through different splice variants, specificity in terms of sensitivity to upstream activators and downstream targets may be achieved. A requirement of ribosomes for mTORC2 signalling has also been proposed [98], and because mTORC1 regulates ribosome biogenesis, activity of the two complexes may be linked in this way. Few targets have been identified downstream of mTORC2, however it has been shown to phosphorylate and activate three members of the AGC kinase subfamily: Akt, SGK [99] and protein kinase C (PKC) [100, 101]. Through the activity of these substrates, mTORC2 is able to control processes such as metabolism, survival, growth and cytoskeletal polarity.

1.1.4.4. Akt-independent pathways

Akt is the best understood PI3K effector, and its prominence in the literature can give the impression that is it the sole transducer of PI3K function. However Akt is only one of many proteins downstream of class I PI3Ks, and a variety of other catalytically active or scaffolding proteins are increasingly being shown to play important roles. This is especially true in cancer, where the importance of Akt-independent PI3K pathways have been identified [102].

PDK1 is the kinase responsible for phosphorylation of Akt at Thr308 [40]. PDK1 possesses a PH domain sensitive to both $PI(4,5)P_2$ and PIP_3 [48, 103], which may contribute to its wide cellular distribution profile; it is found in the cytosol and nucleus, as well as at the plasma membrane. It has constitutive activity due to its ability to transphosphorylate itself on Ser241 [104, 105]. A region in its catalytic domain allows it to interact with, phosphorylate, and activate a number of other AGC kinases, including serum and glucocorticoid regulated kinases (SGK) [106], S6K [106], p90 ribosomal S6 kinase (RSK) [107] and PKC [108] isoforms once these substrates display a facilitating change in conformation. This constitutive activity provides a means of signal transduction which is different from other kinases such as Akt, which require an activating event such

as phosphorylation. Such “substrate-directed” regulation allows PDK1 to control branches of different signalling pathways which have diverse signalling inputs and are largely independent of each other, such as PI3K and MAPK [109].

SGK isoforms are serine/threonine kinases which are closely related to Akt. There are three isoforms, of which SGK1 is the best studied [110]. SGK and Akt appear to have similar requirements in terms of activation and substrate consensus motifs, but differences do exist. In contrast to Akt, SGK isoforms lack a PH domain and are primarily cytosolic, although SGK3 has a PX domain by which it can be recruited to PI3P-containing membranes [111]. As is the case with other AGC kinases, phosphorylation at two sites is required for full activation [48]. PDK1 phosphorylates SGK1 in the T-loop at Thr256 [112] which is equivalent to Thr308 in AKT1. While mTORC2 is responsible for the second activating phosphorylation of Akt on Ser473, both mTORC1 and mTORC2 are able to phosphorylate the equivalent site in SGK1 (Ser422) [99, 113].

Both SGK and Akt can recognise and phosphorylate residues within the consensus motif RxRxxS/T and share a number of substrates [48]. For example, in a similar manner to Akt, SGK1 phosphorylation of p27 leads to its accumulation in the cytoplasm where it is unable to inhibit of the cyclin-E/Cdk2 complex [113], hence promoting cell cycle progression. In addition, phosphorylation of Foxo3a provides a 14-3-3 binding site which allows sequestration in the cytoplasm, hence inhibiting Foxo3a transcriptional activity, and subsequent cell cycle arrest and apoptosis. Both Akt and SGK1 can phosphorylate Foxo3a at Thr32, while SGK1 has a preference of Ser315 over Akt’s preferred Ser253 site [114]. This shows overlapping but also complementary functions of the two kinases.

A number of studies have shown that SGK has an important role to play downstream of PI3K in cancer. A panel of *PIK3CA* mutated cell lines displayed minimal Akt activation, and were dependent on SGK3 for viability [115]. A further study showed that Akt inhibitor resistance in breast cancer cell lines was due to increased expression of SGK1, and that either its knock-down or mTOR inhibition could reduce proliferation [116]. While Akt may be the primary PI3K

effector in healthy tissues, SGK has an important function in at least a subset of cancer cells.

S6K also lacks a PH domain, and is activated through phosphorylation in its hydrophobic motif at Thr389 by mTORC2, which promotes a second activating phosphorylation by PDK1 of Thr229 [48]. Activation allows initiation of protein synthesis through direct phosphorylation and activation of S6. Multiple PKC isoforms can also be activated by PDK1, which control a variety of downstream cellular processes such as cell growth and transcription. The control of multiple (more than 20) other kinases allows PDK1 to exert control over a wide range of cell functions, and to integrate parallel signalling pathways from diverse inputs [48, 109].

Kinase-independent PI3K pathways exist, where small GTPases and their GEFs and GAPs have important functions. One such example is the link between the PI3K-Akt and MAPK pathways, which is still somewhat unclear. It has been shown that some trials of PI3K inhibitors have been unsuccessful in part because of feedback loops which allow MAPK activation. In a panel of breast cancer cell lines with *PIK3CA* mutations, PI3K inhibition brought about Akt inhibition, as well as inhibition of c-Raf/MEK/ERK through Rac-1 and its GEF P-Rex1. This led to induction of apoptosis through Bim, displaying a role for both Akt and MAPK pathways in transducing the PI3K survival signal [117].

1.1.4.5. Phosphatases

While the proteins discussed above are responsible for transducing the PI3K signal, others are responsible for ending it. The primary antagonist of class I PI3Ks is phosphatase and tensin homologue (PTEN), a lipid phosphatase which removes the 3' phosphate group of PIP_3 to generate $PI(4,5)P_2$, thereby terminating the PI3K-transduced signal [118, 119]. As a key regulator of a pathway implicated in many aspects of cancer development, it is not surprising that PTEN is second only to p53 in terms of its frequency of loss in cancer, where it is absent at the chromosome level, or expressed in truncated forms [120].

In a manner analogous to that of PI3Ks being both lipid and protein kinases, PTEN is able to act as both a lipid and a protein phosphatase [105, 120]. This dual action was explained when the crystal structure of PTEN was solved [121], which showed that the active site of the protein is sufficiently large to also accommodate phosphorylated serine, threonine and tyrosine.

The protein SH2 domain-containing inositol-5'-phosphatase (SHIP) is also able to dephosphorylate PIP_3, removing the 5' phosphate to produce $PI(3,4)P_2$ [119, 122]. In this way, metabolism of PIP_3 terminates the PI3K signal, but this step is also important in providing opportunities for further signalling by phosphatases and effector proteins which recognise the new lipid species [16].

As well as direct dephosphorylation of the PI3K lipid substrate, phosphatases are also important to terminate signalling by dephosphorylating PI3K protein effectors. For example, the activating phosphorylations on Akt (Thr308 and Ser473) have both been shown to be removed by PP2A [49, 123], while PHLPP1 and 2 dephosphorylate Ser473 [51, 124], leading to a down-regulation in the kinase output. Further along this signalling pathway, other phosphatases will antagonise the activity of Akt, and by removing the phosphate group will reverse changes in activity or localisation.

1.1.4.6. Protein kinase activity

As well as their canonical lipid kinase activity, PI3Ks are able to phosphorylate proteins [22]. This activity has been reported for all three PI3K classes, but many questions still remain. While the lipid kinase activity of class I PI3Ks has been relatively well characterised, even though their protein kinase function has been known about for decades, it remains largely unexplored. Autophosphorylation is a potential means of self-regulation, and a number of other substrates have been identified for various isoforms (Fig. 1.6.), but whether or not the protein kinase activity has *in vivo* significance has not yet been established.

1.1.4.6.1. Class I PI3K autophosphorylation

Indications that PI3Ks have dual specificity came soon after discovery. Sequence similarity of a newly described p110 protein to Vps34 indicated that the two were related, and presence of motifs found in PKA suggested a protein kinase activity [125]. Soon after, p85 was shown to be phosphorylated on Ser608 by p110 with a good stoichiometry *in vitro*, and this phosphorylation negatively regulated the PI3K lipid kinase activity [126, 127], a finding which has since been shown repeatedly. Barring an examination of the *in vivo* relevance of the activity of p110α and basic biochemical characteristics of other isoforms and novel substrates, very few significant advances have been made to our knowledge of the protein kinase activity of PI3Ks. Discovery of novel protein kinase substrates is challenging, and PI3K lipid kinase activity remains by far the better characterised output.

Of the protein kinase activity of the class I PI3Ks, most of the work has focused on p110α. Due to its ubiquitous tissue expression, early reports which analysed "p110" were likely examining p110α. Creation of a mutant p110 to remove its phosphotransferase ability (R916P) allowed interrogation of the p110-p85 relationship. The two proteins were still able to form a heterodimer but the mutation removed the phosphorylation of p85, thereby demonstrating that p110 is the kinase responsible [127]. The same study however also stated that the protein kinase activity of p110 was reliant on the presence of Mn^{2+}; the absence of this cofactor in cells has led others to query the *in vivo* relevance of the activity. More recently however, this dependency has been called into question. While making a comparison of the protein kinase activity of the class I proteins, it was observed that p110α and two of its mutants displayed robust phosphorylation of p85α in the presence of Mg^{2+} alone [128]. An *in vivo* role for protein kinase activity was shown with the p110α agonists insulin and PDGF both being able to increase the level of pSer608 in CHO-IR and 3T3 cells, an effect which is reversed by treatment with wortmannin [23]. Furthermore, injection of mice with insulin or okadaic acid also increased pSer608 levels in liver and muscle extracts respectively [23]. This clear phenotype in addition to *in*

vitro activity in the presence of Mg^{2+} alone strongly support the case for the protein kinase activity of p110α having a true signalling relevance.

Because of its frequent mutation in cancer [129], the contribution of the protein kinase activity to the signalling output of p110α is clearly of interest. In a functional comparison of two common p110α mutants (E545K in the helical domain, and H1047R in the kinase domain), it was shown that compared to wild-type p110α, the oncogenic mutants are able to phosphorylate p85α to a much greater extent [130]. This was confirmed more recently, both in terms of autophosphorylation and targeting of an exogenous substrate [128], and in AML cell lines. However, creation of lipid kinase deficient mutants of p110α and p110γ showed that the protein kinase activity is not sufficient for *in vitro* transformation, although the possibility remains that the protein kinase activity may contribute in some way once foci or tumours form [131].

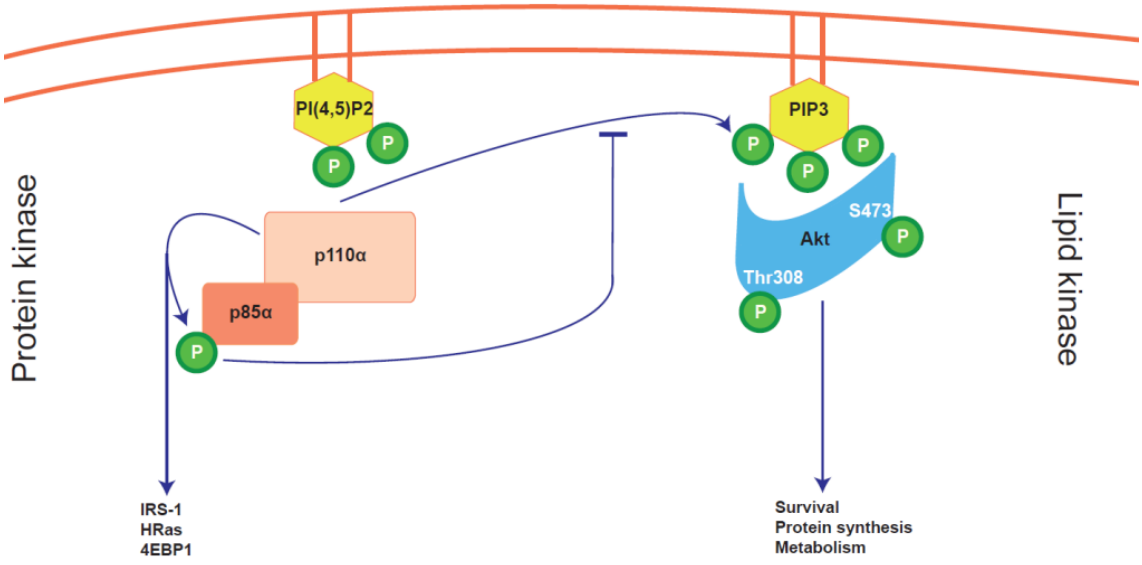


Figure 1.6. p110α is has both protein and lipid kinase activity. p110α directs its lipid kinase activity towards $PI(4,5)P_2$ to produce PIP_3, which leads to activation of Akt and downstream signalling. The protein kinase activity of p110α can be directed towards p85α, an event which has an inhibitory effect on the lipid kinase activity of p110α. Other p110α protein kinase substrates have been identified, such as IRS-1, HRas, and 4EBP1.

Although p110α phosphorylates p85α [23], the other three class I PI3Ks have been shown to autophosphorylate the C-terminus of the p110 subunit [132, 133]. Using a catalytically inactive mutant of p110δ, it was shown that the p110 subunit was the main target of p110δ protein kinase activity [132], in contrast to p110α. Sequencing and mutation studies showed that the target residue was Ser1039 on p110δ, and that its phosphorylation reduced lipid kinase activity [132]. Ser1039 phosphorylation was stimulated by T cell agonists and displayed a reasonable stoichiometry of phosphorylation of 0.5 mol phosphate/mol p110δ. Further, both lipid and protein kinase activities display equal sensitivity to the PI3K inhibitor LY294002. Taken together this evidence suggests that autophosphorylation may have a functional significance *in vivo*.

The existence and relevance of p110β protein kinase activity is much less clear. One study observed no *in vitro* autophosphorylation of the p110β subunit while p85α was phosphorylated. However, *in vivo* phosphorylation of p85α was not significantly decreased in cells expressing a kinase-dead p110β compared to wild-type kinase. Further to this, using a phospho-specific antibody, Foukas *et al* found that Ser608 phosphorylation was not increased upon p110β and p85α co-expression [23, 130]. In addition, an independent study observed limited p85α phosphorylation *in vitro* but found much more phosphate was to be incorporated into the p110β subunit, and sequencing and mutation studies revealed Ser1070 to be the target [133]. This autophosphorylation however was not found to be stimulated by either pTyr or Gβγ, although phosphomimetic mutants did bring about a down-regulation in lipid kinase activity, thereby suggesting similarities but also differences among the class IA PI3Ks, but still leaving unanswered questions about a physiological role.

In contrast to the protein kinase activity of the class IA isoforms, p110γ autophosphorylates in the presence of Mg^{2+} alone, and this autophosphorylation does not negatively regulate its lipid kinase activity [133]. Again, using sequencing and mutation studies the autophosphorylation site was mapped to Ser1101 in the C-terminus [43]. There are differing reports on the ability of Gβγ to stimulate autophosphorylation of p110γ, but it has been shown that lipid vesicles enhance the effect of Gβγ suggesting a requirement of orientation with

a cellular membrane [18]. This autophosphorylation has no effect on observed lipid kinase activity of p110γ, and so physiological relevance remains unclear.

A recent study undertook a comparison of the class I proteins protein kinase activities bringing some clarity to this issue. Using *in vitro* kinase assays, they confirmed that p110α mainly phosphorylates p85α, while p110β p110γ and p110δ autophosphorylate the p110 subunit [128]. In addition and to a lesser extent, p110α demonstrated autophosphorylation activity while p110β could also phosphorylate p85α.

1.1.4.6.2. Biochemical characteristics

While multiple studies have demonstrated that p110 has protein kinase activity, questions remain: how it is regulated, what is the full complement of exogenous substrates, and most importantly, what is the physiological consequence of this activity?

One elegant study showed that the lipid and protein kinase activities of class I PI3K can have separate physiological signalling output. The study exchanged the lipid binding domain of p110γ for one of several proteins to create p110γ mutants which retained protein kinase activity but had different lipid-binding specificities. In this way, it was shown that while the lipid kinase activity is required for Akt activation, the protein kinase activity activates the MAPK pathway [134]. Additionally, the lipid and protein kinase activities were found to occur in different subcellular localisation. The authors showed that membrane targeting (which mimicked recruitment to activated receptors) of p110γ resulted in Akt activation, while protein kinase mediated activation of MAPK occurred when p110γ was free in the cytosol. Building on this work, membrane binding was further shown to inhibit p110γ autophosphorylation, suggesting a more general inhibition of p110γ protein kinase activity.

A subsequent study demonstrated that the two kinase activities of p110γ may be differentially controlled by varying concentrations of Gβγ subunits of heterodimeric G proteins. p110γ lipid kinase activity was found to increase with increasing Gβγ concentrations, while the opposite was true for phosphorylation of an uncharacterised site on MEK1, or for p110γ itself [135]. While a clear

reduction in MEK1 phosphorylation was observed with increasing Gβγ, a less drastic change was seen in autophosphorylation, with still around 70% of maximum activity seen even with the highest Gβγ concentrations. Nevertheless, the data do point to physiological scenarios where PI3K protein and lipid kinase activities can function separately to produce different outputs. As we learn more about the regulation of PI3Ks, it would be worthwhile to revisit these hypotheses to comprehensively assess the effect of different stimuli (e.g. RTKs, GPCRs, Ras and small GTPases) on the class I isoforms.

1.1.4.6.3. Non-PI3K substrates

While analysis of a known phosphorylation can be a difficult enough task, and identification and characterisation of novel targets is even more challenging, non-PI3K substrates have also been identified. Early on in the characterisation of PI3K protein kinase activity, IRS1 was identified as a substrate [136, 137, 138]. Insulin-stimulation of adipocytes increased phosphorylation of p85α and IRS-1, which was sensitive to treatment with the pan-PI3K inhibitor wortmannin [136, 138]. Such tools helped to first display a role for PI3K as a protein kinase and suggested a functional purpose in the insulin response in which PI3K was already implicated, but were not enough to assign a specific responsible isoform. More recently, both p110α and p110δ were shown to phosphorylate the GM-CSF/IL-3 receptor, which led to increased survival of two leukaemia cell lines [139]. The authors showed a detachment of lipid and kinase activities depending on the concentration of receptor agonists, with lower cytokine concentrations positively regulating protein kinase function.

When searching for new substrates of a kinase, a targeted approach such as looking to known pathways has clear benefits. However, identification of less obvious proteins is unlikely. Foukas *et al* used a targeted method to identify novel PI3K substrates, by looking for sites that had similar characteristics to p85 pSer608: phosphorylation sites that were surrounded by acidic residues and were wortmannin-sensitive. This led to the identification of Ser111 of 4EBP1 as an *in vitro* substrate of p110α and p110γ [140]. However, comparison of this activity to that of ERK1 (an established 4EBP1 kinase) showed that the stoichiometry of phosphorylation was very low: 0.16 compared to 0.6 mol of

phosphate/mol 4EBP1 for p110γ and ERK1 respectively [140]. The same study also identified H-Ras as an *in vitro* target of p110α, and p110γ but not p110β. However, functional effects were not elucidated for either phosphorylation, and *in vivo* relevance remains unclear.

In the context of class I protein kinase activity, physiological effects of non-PI3K targets have been described. The use of lipid kinase- or protein kinase-only p110γ allowed the identification of non-muscle tropomyosin as a substrate [141], both in a whole cell context and in an *in vitro* kinase assay, although use of a more specific inhibitor than wortmannin would have been beneficial. Use of cells derived from p110γ knock-out mice led to the finding that p110α can compensate to phosphorylate non-muscle tropomyosin, but when both isoforms are lost phosphorylation is vastly reduced. Knock-down of non-muscle tropomyosin, as well as serine-to-alanine mutation of the PI3K target site, both resulted in a clear phenotype of a block in the internalisation of β-adrenergic receptor.

1.1.5. Tools for studying PI3K function

Although the class I PI3Ks are structurally conserved which implies redundancy, other characteristics suggest independent functions [1]. Tissue distribution, upstream signalling input, and regulation by heterodimer partners all point to the isoforms having non-redundant roles. While there is redundancy in terms of signalling between isoforms in different cell types, pharmacological and animal models have elucidated isoform-specific roles in healthy and disease processes. In our and other laboratories, model systems have been developed which target the genes of the various PI3K isoforms allowing activation (such as are found in cancer) or inactivation of the kinase activity [142, 143]. These lines have been key in delineating signalling pathways, assigning isoform-specific functions, and providing models to mimic disease states and assess efficacy of pharmacological compounds.

The first approach, termed “knock-out”, involves the deletion of a gene so that it is not expressed. This can be cell- or tissue-specific, or it may be that the protein is absent from the whole organism, and is achievable in a wide range of systems, from yeast to mice.

A second approach, termed “knock-in”, involves the introduction of a point mutation to the gene of interest, so that it is still fully expressed, but with altered function. This may also be in a specific or general context. Our laboratory has developed mice which have activating and inactivating mutations for a range of PI3K isoforms. For example, the introduction of the oncogenic H1047R mutation into the *Pik3ca* gene produces an activated p110α and can be used to model cancer initiation and progression. The reverse can be achieved with mutation of the DFG motif in the kinase domain which coordinates ATP binding; substitution of DFG with AFG produces a catalytically inactive kinase [144, 145]. The inactivating knock-in model has a number of advantages over the knock-out system. First and foremost, knock-out models can suffer from compensation from other isoforms. Because the targeted gene is still present (albeit in a modified form) in the knock-in system, problems of compensation by other isoforms are less likely to occur, and so the knock-in approach more accurately represents small molecule inhibition. Another important factor is that a point mutation is less disruptive than gene deletion in terms of off-target effects; it has been shown that even when one gene is targeted for specific deletion, genes at distant loci can be affected, thereby clouding any conclusions drawn from its use. Complementary pharmacological and animal studies have been central to developing our knowledge of the PI3K pathway.

1.1.6. Isoform-specific functions of class IA PI3K

Both p110α and p110β have overlapping functions in downstream processes such as survival, proliferation and metabolism. However, it has long been known that the two isoforms have different contributions from upstream activators (see chapter 1.1.3) and mouse models and pharmacological tools have provided insights into functional differences.

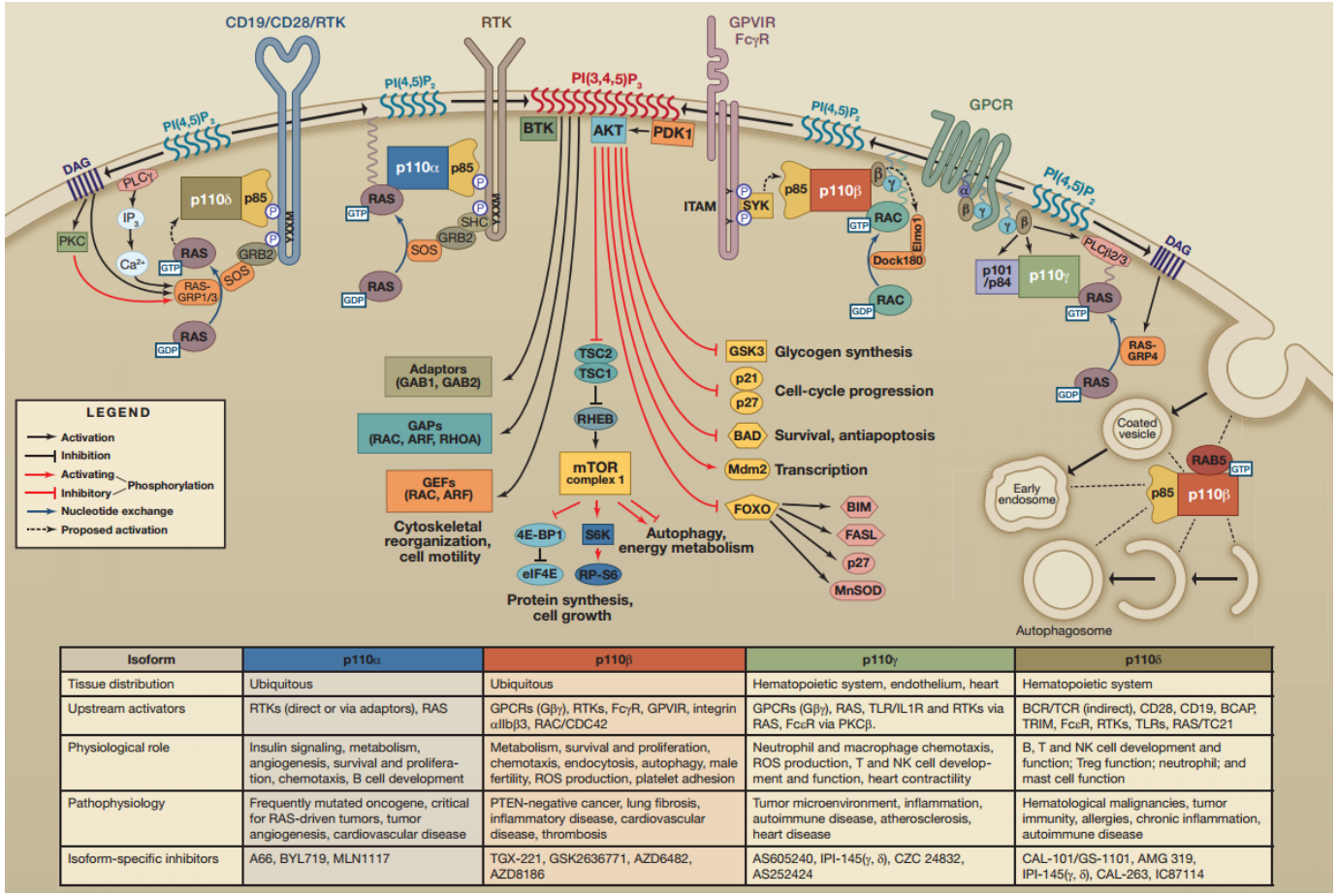
Homozygous loss of p110α causes embryonic lethality in mice [18]. This is a kinase- dependent phenotype as knock-in of a kinase-dead p110α is also embryonic lethal [144]. Mice heterozygous for the knock-in allele are viable and fertile but have reduced insulin sensitivity [144]. That p110α is critically positioned downstream of the insulin receptor, while p110β plays only a minor (or no) part, was confirmed by use of pharmacological compounds [146]. A key

role for p110α in the control of angiogenesis has also been demonstrated: in a comparative study of the class IA isoforms, it was only p110α that was critical for endothelial cell migration and vascular remodelling, acting through the small GTPase RhoA [145].

Mice homozygous for loss of p110β are also embryonic lethal [19], with heterozygous litters viable and fertile. Interestingly, mice which express low levels of kinase-dead p110β are also embryonic lethal, while those which express higher levels of the same gene survive to adulthood [147]. This suggests that the kinase activity of p110β is dispensable and may have a scaffolding function. An early isoform-specific role for p110β in platelet function and thrombosis was demonstrated through inhibitor use. Binding and stability of integrin bonds were reduced upon p110β inhibition [148]. Conditional knock-out of p110β in platelets also showed that isoform to be responsible for normal signalling downstream of both RTKs and GPCRs, and aggregation of platelets was defective [149]. These studies demonstrate the potential of p110β as a therapeutic target in thrombosis. A role for p110β in endocytosis has also been suggested, as it is able to bind the endocytotic regulator Rab5 [24, 38]. This may be a kinase-independent function, as ablation of p110β causes defects in receptor internalisation, which can be rescued by expression of kinase-dead p110β [147, 150].

p110α and p110β also appear to have differing contributions to cancer, which are discussed below. Briefly, p110α is the only PI3K found mutated in cancer [129], while p110β has been shown to be required for oncogenesis in certain PTEN-null prostate cancers [152].

In the same way that p110α and p110β share a tissue distribution but can control different functions, the two isoforms enriched in haematopoietic cells are able to produce distinct biological outputs. In terms of disease, p110γ has been shown to be important for cardiac function and p110δ for immunity and the tumour microenvironment [153]. As discussed previously there are also differences in terms of substrates for the protein kinase activities of the isoforms [128].



Isoform	p110α	p110β	p110γ	p110δ
Tissue distribution	Ubiquitous	Ubiquitous	Hematopoietic system, endothelium, heart	Hematopoietic system
Upstream activators	RTKs (direct or via adaptors), RAS	GPCRs (Gβγ), RTKs, FcγR, GPVIR, integrin αIIbβ3, RAC/CDC42	GPCRs (Gβγ), RAS, TLR/IL1R and RTKs via RAS, FcεR via PKCβ.	BCR/TCR (indirect), CD28, CD19, BCAP, TRIM, FcεR, RTKs, TLRs, RAS/TC21
Physiological role	Insulin signaling, metabolism, angiogenesis, survival and prolifera-tion, chemotaxis, B cell development	Metabolism, survival and proliferation, chemotaxis, endocytosis, autophagy, male fertility, ROS production, platelet adhesion	Neutrophil and macrophage chemotaxis, ROS production, T and NK cell develop-ment and function, heart contractility	B, T and NK cell development and function; Treg function; neutrophil; and mast cell function
Pathophysiology	Frequently mutated oncogene, critical for RAS-driven tumors, tumor angiogenesis, cardiovascular disease	PTEN-negative cancer, lung fibrosis, inflammatory disease, cardiovascular disease, thrombosis	Tumor microenvironment, inflammation, autoimmune disease, atherosclerosis, heart disease	Hematological malignancies, tumor immunity, allergies, chronic inflammation, autoimmune disease
Isoform-specific inhibitors	A66, BYL719, MLN1117	TGX-221, GSK2636771, AZD6482, AZD8186	AS605240, IPI-145(γ, δ), CZC 24832, AS252424	CAL-101/GS-1101, AMG 319, IPI-145(γ, δ), CAL-263, IC87114

Figure 1.7. Class I PI3K isoforms display differential signalling in health and disease. Adapted from [151]. The emerging picture of upstream and downstream class I PI3K signalling is more complex than originally thought. Class IA isoforms are activated by RTKs and Ras, and p110γ by GPCRs and Ras, while p110β may also receive additional input from GPCRs and small GTPases. Both p110α and p110β have ubiquitous tissue expression, while p110γ and p110δ are expressed mainly in haematopoietic cell lines. They also have different roles in normal cellular and disease signalling.

1.1.7. Disease

Since the PI3K-Akt-mTOR axis is at the heart of pathways critical for cell growth, survival and metabolism, it follows that their signalling is often dysregulated in a number of diseases. PI3Ks are activated downstream of growth factors such as insulin, and are highly expressed in immune cells, and so it stands to reason that they play a role in pathologies such as diabetes and inflammatory diseases. Many of the aberrations that enable a cell to grow uncontrollably and transform are controlled by the PI3K/Akt/mTOR pathway, and so mutations here are often observed in cancer [154]. That these pathways and proteins are so active in disease has resulted in a large amount of interest from academia and industry as well as a push to develop modulators of nodes of these pathways, particularly in cancer [155]. Mutations which increase the activity of oncogenes (gain of function) or decrease the activity of tumour suppressor genes (loss of function) contribute to the formation and survival of tumours. Both types of mutation affect the PI3K pathway in different cancers and mutations can occur at multiple nodes [154]. These include activating mutations upstream of PI3K in RTKs and Ras, and downstream in Akt, as well as in the regulatory subunit p85α [156, 157], which cluster in and around the SH2 domains thereby allowing decreased inhibition of the class I PI3K catalytic isoforms [158]. The two most frequently observed PI3K pathway mutations are

in the genes coding for p110α [129] and PTEN, and in fact these are two of the most commonly mutated genes in cancer.

The only PI3K isoform to have been found mutated in cancer is p110α, with mutations clustering around two “hotspots” in the helical and kinase domains [129]. Both mutations increase kinase activity though through different mechanisms: mutations at E542K or E545K in the helical domain relieve p85’s inhibition of p110α by mimicking the binding of pTyr residues, while the H1047R kinase domain mutation mimics activation by Ras, and this mutant can still be further activated by pTyr [130, 159].

Loss of the phosphatase PTEN provides another means of activating the PI3K pathway, and it has been associated with a reliance on p110β in the context of prostate cancer [152]. Loss of p110β but not p110α prevented tumour formation

in PTEN-null mice [150]. In other models of prostate cancer, inhibition or siRNA-mediated knock-down of p110β negatively impacted growth and colony formation of PTEN-null cell lines [160] and tumour progression [152]. While PTEN loss and *PIK3CA* mutations allow essentially the same biochemical output, it has been shown that they can coexist in tumours, and a recent study demonstrated that inhibition of p110α protected PTEN-null mice from thyroid cancer, as well as a number of other diseases [152].

PI3Ks other than p110α are part of signalling pathways which are important for tumour initiation and maintenance, and it is perhaps surprising that p110α is the only PI3K to be mutated in cancer. A number of features of p110α structure and signalling may help to explain this, as well as its ability to contribute to many of the hallmarks of cancer. Though not due to mutation, a role in angiogenesis not shared by other isoforms means that p110α can provide a lifeline to tumours [145], which cannot grow past ~1mm^3 without a blood supply. Second, p110α is ubiquitously expressed [15] meaning that it can contribute to a wide range of tumour types, whereas in theory p110δ could only contribute to haematological malignancies. This, however, does not explain the lack of common oncogenic mutations in p110β which also has a widespread tissue distribution. Structural examination of the class I PI3K isoforms has suggested differences in the inhibitory pressures which the regulatory subunit places on the p110 protein. While p110α binds the nSH2 and iSH2 domains of p85α, other class I isoforms have an extra inhibitory contact at the cSH2, hence making p110α more amenable to oncogenic mutation than the other class I PI3Ks [32]. This is also the case with upstream signalling: p110α is activated primarily by RTKs, in contrast to p110β and p110γ which have additional input from GPCRs [30, 35].

The importance of the class I PI3Ks to a range of cellular outputs means that their role in disease is not limited to cancer. For example, both p110α and p110β have been implicated in cardiovascular disease, and p110γ and p110δ are known to contribute to autoimmunity (Figure 1.7 lists more examples). One interesting and recently characterised phenotype is an overgrowth syndrome caused by somatic mutations in p110α; sequencing studies found an amino acid substitution at H1047 (to R or L), which caused dramatic overgrowth of bone as well as fibrous and adipose tissues [161]. Interestingly, while H1047 is the same

residue mutated in many cancers, in these syndromes the mutation was found to induce benign mosaic hyperplasia instead of tumour formation. This provides an interesting aspect to the thoughts around whether or not p110α is important in tumour initiation and maintenance.

An analogous mutation in the p110δ kinase domain has recently been characterised. A single amino acid substitution (E1021K) resulted in increased activation and membrane association of the isoform [162], which led to a range of clinical phenotypes including respiratory disease and splenomegaly. The mutant was able to be inhibited by the p110δ-specfic inhibitor IC87114.

1.1.8. Pharmacological Intervention

Because of the relevance of the PI3K-Akt-mTOR signalling pathway in a variety of disease states and clinical settings, an effort has been made to target various nodes with pharmacological compounds. Typically, it has been the case that the down-regulation of an enzyme is more easily achieved than up-regulation, and so more progress has been made in trying to inhibit kinase activity rather than increasing phosphatase activity.

With 518 kinases present in the human genome [163], they play roles in most if not all signal transduction cascades. With this in mind, kinases have long been attractive targets for therapeutic intervention, and both specific and general inhibitors have been developed for use in the laboratory and through clinical trials. These inhibitors are generally small molecules which competitively target the ATP-binding site of the kinase [164]. Inhibitors targeting the active site can be type I or II ATP competitive (binding to the active or inactive kinase conformation respectively) or covalent, or may bind a region away from the active site (allosteric inhibitors).

Because of the degree of conservation of the catalytic region within members of the kinase family, achieving specificity for different kinases and isoforms is challenging, and so next generation inhibitors may seek to exploit differences in the rest of the molecule to realise this desired specificity. It is also true that in a cancer setting, the selective pressure on the kinase to mutate which is caused by the drug, alongside the rapidly growing tumour promote the rapid outgrowth

of resistant cells. This resistance can occur due to mutations in the ATP-binding site, and often to a specific ‘gate-keeper’ residue which controls access to this site, but may also be due to increased activity of other parts of the signalling cascade, for example by mutation of receptors, or differential expression of other enzymes, such as by loss of phosphatases like PTEN.

In spite of the large amount of effort devoted to seeking to block the PI3K pathway at various points and in various cancers, there has been a disappointing amount of success [155]. This is the case when therapy has been given to groups both with and without PI3K pathway mutations. Despite its central role in many pathways which tumour cells are dependent on, it would seem that inhibition of PI3K or its allies is not enough to produce large and long term beneficial effects in the clinic.

In both the laboratory setting and the clinic, it is becoming increasingly clear that targeting more than one enzyme or signalling pathway is necessary for optimal therapeutic benefit. This is also true in terms of resistance to inhibitors, as it is harder for a cancer to become resistant to multiple therapies compared to one. However, the use of multiple inhibitors can complicate the readout of their effect, especially when feedback loops are considered. Through the build-up of knowledge of individual and integrated signalling pathways, as well as structural insights gained from crystallography, a more targeted approach is now being taken in terms of design of novel inhibitors. In the same way that drug design is informed by our knowledge of the target and related kinases, the decision on which patients receive these drugs is beginning to be directed by genetic factors and biomarker readout.

The pan PI3K-isoform inhibitors wortmannin and LY294002 were first described in the early 1990s, and are still in use today to make general observations about PI3K activity. Both compounds were originally thought to be more specific than they in fact are, with off target effects including inhibition of mTOR meaning that conclusions drawn from their early use may have to be re-evaluated. Nevertheless, they were very useful for the initial characterisations of PI3K function.

More recently, specific and pan-isoform inhibitors have become available for class I PI3Ks, as well as for pan-Akt and mTORC1/2; these are listed in Table 1.1. The most promising translational results have been with p110δ inhibitors: the first isoform-specific compound described was IC87114 which targets p110δ, and CAL101 is now in late stage clinical trials for use in haematological malignancies. Even though it is not actually mutated in cancer, it has been found that targeting p110δ can lead to clear positive outcomes in a clinical setting. Trials using the p110δ-selective inhibitor CAL-101 in patients with chronic lymphocytic leukaemia saw a large reduction in tumour burden, and the drug is well into phase III. The cause of the modulation has been suggested to be directly on the tumour cells by causing cell death, and also indirectly by inhibiting their retention in the microenvironment [165, 166].

As the only PI3K isoform to be found mutated in cancer p110α is an attractive target for inhibition and specific inhibitors have recently become available. Some of these compounds, including BYL-719, are now progressing through clinical trials. Another such compound, A66, has around 40 times more selectivity for p110α over the other class I isoforms, and is also potent against the E545K and H1047R mutants [167].

Alongside these encouraging results however, a number of findings suggest that PI3Ks may have a different role in cancer than previously thought. While the PI3K pathway is frequently found to be mutated in cancer, studies using specific or pan-isoform PI3K inhibitors have been less effective than anticipated at inducing cancer cell death, and it is also true that there is a lack of correlation between *PIK3CA* and PTEN mutation, and the ability of p110α-specific/pan-class I PI3K inhibitors to reverse tumour growth. The failure of many anti-class I PI3K compounds in the clinical setting is due, at least in part, to the unwanted suppression of negative feedback loops, ultimately leading to increased not decreased signalling downstream of PI3K [93].

Table 1.1. Compounds available for inhibition of PI3K pathway components

Compound	Target	Clinical Setting
Wortmannin	Pan-PI3K, mTOR	Anti-inflammatory, displayed *in vivo* anti-tumour effects, but also liver toxicity
LY294002	Pan-PI3K, mTOR	Displayed *in vivo* anti-tumour effects, but also dermal toxicity.
GDC-0941	Pan-class I PI3K	Trials for solid tumours (including breast, ovarian, melanoma, pancreatic)
BYL-719	p110α	Trials for solid tumours (including breast, cervical, endometrial, ovarian, head and neck)
TGX221	p110β	Thrombosis
CAL-101	p110δ	Trials for haematological cancers (including mantle cell lymphoma, chronic lymphocytic leukemia)
IPI-145	p110δ/γ	Trials for haematological cancers (chronic lymphocytic leukemia, small lymphocytic lymphoma)
MK-2206	Akt	Trials for solid tumours (including neuroendocrine)
Rapamycin	mTORC1 (mTORC2 with long-term treatment)	Approved for renal cancer, in trials for various other solid tumours, as well as ageing studies.
AZD8055	mTORC1/2	Trials for solid tumours (gliomas, hepatocellular carcinoma)

Inhibitors have also been generated for Akt [168], and the pan-isoform allosteric inhibitor MK-2206 is in phase II clinical trials for use in a number of carcinomas, both as a single agent and in combination with other drugs. While it is known that the different Akt isoforms display non-redundant roles, isoform-specific compounds are currently unavailable.

mTOR therapies began with the discovery of the macrolide rapamycin, and progressed when it was found that rapamycin acutely targets mTORC1 through FKBP12, although long term exposure can affect mTORC2. Rapamycin has immune-suppressive effects, and since its discovery various rapalogs have been developed and moved into clinical trials with varying success. Next-generation compounds (some of which also target PI3K) are also in early phase trials for use in solid tumours, and inhibitors are available which are selective for one or both mTOR complexes.

1.2. Proteomics

Over the past few decades, the advances in sample processing, mass spectrometers and analytical techniques have allowed the large-scale identification and quantification of thousands of proteins and post-translational modifications in a single experiment. There are two general mass spectrometry methods for protein analysis: first is the ‘top down’ approach, in which whole proteins are examined intact, and the second is the ‘bottom up’ approach which looks at the peptides after digestion of a protein [169]. These techniques benefit from the unbiased manner in which proteins can be analysed, with no prior knowledge of the content of the sample required.

While intact proteins can be analysed by mass spectrometry, it is more common for at least one digestion step to be used to produce peptides which are more amenable to LC-MS. Trypsin is typically the enzyme of choice, and works by cleavage of bonds C-terminal to lysine or arginine residues [170]; because of its high specificity, bioinformatic analysis is made simpler than if peptides with random termini were produced. This specificity is not always beneficial however, as some proteins may not be digested efficiently and some may be digested too well, causing fragments with m/z ratios which fall outside of the typical MS window of detection to be produced. The use of enzymes with different specificities can be used to combat this; for example, the combination of databases derived from samples treated with one or both of trypsin and Lys-N typically allows greater coverage of the proteome compared to a database from the use of one enzyme alone [171].

1.2.1. Post-translational modifications

Signalling pathways are an essential means of coordinating and synchronising cellular processes with each other, and with the extracellular environment [172]. It has been found that the expression of a protein is not necessarily the best indicator of its signalling capacity, and a number of other factors affect activity. PTMs play a key role in regulating and integrating signalling pathways, as they can affect localisation and interactions of effector proteins, as well as protein stability. In addition they are a much more rapid method than alterations at the gene expression level. PTMs typically involve the addition of a chemical tag to the amino acid side chain of proteins, a change which can influence a variety of characteristics. Some PTMs are well characterised and understood: ubiquitination on specific lysine residues targets the protein for different processes (including degradation) [173], while phosphorylation of kinases in specific motifs typically confers higher activity [48]. Hundreds of PTMs have been identified including the above mentioned phosphorylation and ubiquitination, as well as acetylation, methylation and palmitoylation. The variety of modifications, the potential for dynamic addition and removal and the multiple functional effects that can occur, provides great scope for rapid and specific fine-tuning of signalling pathways, both inter- and intra-cellularly [172]. Phosphorylation is historically one of the most extensively studied PTMs and this is still the case today, in part due to the ease with which it can be monitored with the currently available tools; tools which continue to evolve and improve because of the extent of their use.

As their correct function is required for correct signalling in healthy cells, it follows that aberrations in PTMs which control critical networks can be at the heart of disease processes. This is especially true for phosphorylation, where kinases and phosphatases are often mutated. The ability to delineate signalling networks, to assess functionality of PTMs, and to assign substrates to effector enzymes is increasingly important in our aim to understand and tackle diseases such as cancer.

1.2.1.1. Phosphoproteomics

Phosphorylation is a reversible post-translational modification in which a phosphate group is covalently added to a serine, threonine or tyrosine residue [174]; some estimates suggest that more than half of proteins can be phosphorylated [175]. Phosphorylation of other amino acids can also occur, although these are less well studied. The process of phosphorylation is carried out by a group of proteins called kinases, of which there are over 500 in the human genome [163], and is antagonised by phosphatases, of which there are around 150. The addition of the negatively charged bulky phosphate group can cause a change in protein structure which may affect a protein's activity or binding partners, and in this way phosphorylation is able to control the amount, activity and cellular location of the protein target [176]. It is therefore an important regulator of most, if not all, cellular pathways which can be regulated at each level, allowing the capacity for both positive and negative feedback, and input from different and diverse signalling networks [93].

While the study of genes (genomics) and their transcription (transcriptomics) can provide valuable information about the state of a cellular or biological system, a major limitation is that they do not necessarily correlate with presence or activity of the cellular protein product. Therefore, it is important to look to other fields in order to study signalling outputs and cellular processes, and proteomics is increasingly being used to delineate and functionally analyse pathways. While some sites in the phosphoproteome will not impact function or be dynamically regulated, a significant number affect protein activity or localisation. This means that monitoring phosphorylation of a protein can give useful information on the signalling output of a particular system and so can be more relevant than genomic or transcriptomic data. However, when comparisons are being made of samples over long time points (>6 h), especially regarding PTMs, thought must be given to potential discrepancies in protein expression. Where appropriate, experiments should take information on the abundance of both proteins and PTMs, which would allow stoichiometry to be established.

1.2.1.2. Enrichment Techniques

It is possible to identify and quantify many thousands of phosphorylation events from a single experiment using similar techniques employed for typical proteomic analysis. However, in order to gain significant insight into the phosphosites present in a sample, great care needs to be taken to conserve the modification, which is typically present at low abundance. This requires inhibition of phosphatases which would otherwise attack and dephosphorylate it, throughout sample processing until those enzymes are inactivated through denaturing or digestion. Phosphatases are thought to have faster reaction kinetics than kinases [177], adding to the importance of their inhibition. Identification and quantification of modified peptides is more challenging than when analysing their unmodified counterparts. This is because while unmodified proteins will have a number of peptide fragments produced upon digestion which can be used for analysis, only the peptide which is post-translationally tagged is of interest and used for characterisation in the case of modified proteins.

Other problems also need to be addressed. Ionisation of the peptide and dissociation along its backbone are key steps required to allow identification, however there is evidence that phosphopeptides ionise less efficiently than their non-phosphorylated counterparts [178]. A further challenge arises when one considers that the phosphate group attached to the amino acid side chain is often the first bond to break under the pressure of the internalised energy from whichever collision method is employed [179]. This means that instead of allowing dissociation of the peptide backbone and better identification of the sample, the desired information on PTM and location is difficult to obtain. Different strategies for fragmentation avoid this problem, for example electron capture dissociation (ECD) and electron transfer dissociation (ETD) which achieve fragmentation through electron bombardment rather than through vibrational energy transfer [180, 181].

As well as these problems due to the chemistry of phosphopeptides, issues with the technology used to identify them exist. Because of their relatively low abundance compared to their non-phosphorylated counterpart, phosphoproteins

and phosphopeptides are unlikely to be detected using standard non-targeted discovery MS techniques. This is due to the limitations of the mass spectrometer, which permit only a fraction of peptides from a complex sample mixture to be selected for fragmentation and analysis [182]. Because of this under-sampling, phosphopeptides should be enriched to facilitate detection and analysis; there are a number of techniques in use for this purpose, many based on affinity purification.

Antibodies have been raised to a number of different phospho-motifs, both in specific and more general contexts. For example, antibodies exist to pSer473 of Akt, and also to pTyr motifs which will recognise a wide variety of proteins. These can be used in the same way as immobilised metal affinity chromatography (IMAC) to enrich for phosphoproteins, in much the same way as immunoprecipitation for whole proteins. While this may work well for IP and WB, commercially available antibodies specific for pSer, pThr and pTyr most probably lack the phosphoproteome coverage required for large-scale global analysis [183]. Different IMAC approaches may favour proteins with different numbers of phosphorylations but do not discriminate based on the amino acid residue to which the modification is attached. There are large differences in the proportion of pSer, pThr and pTyr found in nature, and differences in affinity of antibodies will dramatically impact enrichment.

Exploitation of the chemical properties of the phosphate group can produce high enrichment yields. Because of the negative charge which the phosphate species incorporates into the modified protein, positively charged “baits” can be used to pull down phosphoproteins in a relatively specific manner. For example in IMAC, positively charged metal ions such as Fe^{3+} are bound to a solid support which will then bind any proteins with a large negative charge [184, [185]. One limitation of this method is that the positive ions also bind with high affinity to acidic proteins, and measures need to be taken to remove these proteins before mass spectrometry, where they may interfere with detection of the less abundant phosphoproteins of interest. Solutions include modification of acidic amino acid side chains to prevent them from binding the metal ion, and varying solvents and pH, although these do not always fully resolve the issue [186, 187, 188]. A further problem with IMAC-based enrichment is the

preference for binding peptides with more than one phosphate which can introduce a bias which should be borne in mind during analysis [189].

Titanium dioxide (TiO_2) –based affinity chromatography is essentially a modified version of IMAC [190, 191]. Introduction of steps during sample processing have greatly reduced the presence of non-phosphorylated proteins after the purification round, for example use of acidic buffers and various combinations of washes after binding of proteins to the TiO_2 [192, 193]. Due to its higher enrichment efficiency than IMAC, and by being more resilient to various buffers [177], TiO_2 is now widely used. In contrast to IMAC methods which use transition metal ions as "bait", TiO_2-based enrichment favours identification of peptides with a single phosphorylation [193]; higher numbers of phosphates increase affinity of binding which makes elution of multiply-phosphorylated challenging. Peptides which are rich in acidic residues can bind non-specifically to TiO_2 in a similar manner to conventional IMAC techniques, thereby reducing specificity of the methodology for phosphopeptides. Measures can however be taken to counter this, such as by the use of compounds which bind TiO_2 in a manner intermediate to that of phosphopeptides and acidic peptides; thereby allowing binding of the former but competing with the binding of the latter.

Different approaches have different benefits, and so there may be advantages to combining one or more methods. Separation steps such as hydrophilic interaction liquid chromatography (HILIC) or strong cation exchange (SCX) can also be used in concert along with IMAC methods to reduce sample complexity and hence improve enrichment, as a number of studies have shown [177].

As well as these affinity based methods, it is possible to chemically modify the phosphate bond in order to subsequently capture the peptide to which it is attached, which may be done with and without the loss of the phosphate ion itself. The peptide can be linked to biotin via reactions which expel the phosphate, and then this complex can be captured through biotin-avadin affinity [194]. Alternatively, by keeping the phosphate attached to the peptide and coupling it to iodoacetyl beads, peptides can be immobilised and subsequently eluted. Both of these strategies can suffer from chemical modification of non-phosphate bonds, while the former method does not work for pTyr residues.

Vast improvements in proteome coverage have been made in recent years which are attributable to developments in handling techniques and analysers; these improvements will continue. Stimulation as well as spatial and temporal contexts must also be kept in mind, which may mean that a specific PTM is not present in a sample. Also, despite the best efforts to maximise proteome coverage and PTM identification, not all proteins or modifications actually present in a sample will be detected. The duty cycle of a mass spectrometer is a limiting factor, meaning that less abundant peptides are not selected for analysis, but it must be remembered that just because a peptide is not detected it may still be present in the sample.

1.2.2. Instrumentation

While small numbers of proteins can be detected and analysed by techniques such as western blotting, the analysis of many thousands of proteins needs a more sophisticated approach provided by mass spectrometry and its associated systems. Today's tools allow detection of thousands of proteins in a single sample, and the increase over what was achievable even just a few years ago is due in no small part to improvements in instrumentation and analysers. Mass spectrometers all employ the same basic components and consist of a source which provides the ionised sample, an analyser which separates ions based on their mass-to-charge (m/z) ratio, and a detector which measures relative abundance.

In order to be identified by the mass spectrometer, the sample must be introduced to it in gaseous form. Two different methods are used to achieve this: electrospray ionisation (ESI) which uses the sample in liquid phase [195], and matrix-assisted laser desorption/ionisation (MALDI) which uses the sample in the solid phase [196]. ESI is more commonly used, partly because it can be coupled more easily to the LC system and ionisation can take place on-line, although MALDI has added sensitivity and is more tolerant to the presence of contaminants such as salts or small amount of detergent [197]. ESI is based on coulombic repulsion, whereby the liquid sample is reduced to a spray of charged droplets after evaporation of the solvent by application of a high voltage, and the charge density reaches such a point that the charged ions are

produced in the gas phase. The same result is achieved using MALDI, but in this case a laser beam is used to both sublimate and ionise the sample.

Once inside the mass spectrometer ions can be fragmented by their excitement and contact with inert gases [198], a process termed collision-induced dissociation (CID). Collision allows internalisation of the kinetic energy which then leads to peptide fragmentation. Ideally this occurs along the backbone of the peptide, but may instead bring about loss of the phosphate ion as discussed earlier [179, 199]. A further problem of CID is that low mass ions will not be fragmented, but this may be resolved by using higher energies. Instead of collision with inert gases, the peptide ions can be bombarded with electrons (ECD or ETC) to achieve the same goal [180, 181]. While these approaches can have high sensitivity, they are much less effective when analysing ions of low charge.

A number of different methods may be used in analysers for sample detection. Time of flight (TOF) instruments rely on measurement of the time taken for an ion to reach the detector from the source, while other instruments manipulate electrical or magnetic fields to control how ions of different mass/charge ratio (m/z) move and behave. Instruments which use the latter technique may be referred to as ion traps, and include quadrupole and orbitrap analysers. How an ion oscillates in two or three dimensions when a specific field is applied relates to its m/z, and equations which relate the two aid detection and quantification. Orbitrap analysers have higher mass accuracy and resolution than other mass spectrometers while maintaining a wide dynamic range, which makes them the analyser of choice for many groups.

While improvements have been made with instrumentation and methodology, there are still limitations of MS analysis. One significant challenge is the reproducibility in terms of identification of a specific peptide. Some estimates suggest that replicate analyses will only yield a 60-70% overlap of phosphopeptides [200].

1.2.3. Quantification

While protein/peptide identification alone was the object of early proteomic studies utilising mass spectrometry, quantification is now often a requirement especially when comparing across samples and conditions. There are two main approaches currently being employed to achieve either absolute or relative quantification; one involves the addition of differential labels to either the whole protein or digested peptide, while the other avoids this and so is referred to as 'label-free' (Fig. 1.8.).

1.2.3.1. Labelling methods

There are various labelling techniques currently being employed in the field, usually involving the modification of the separate samples with tags which may be of the same or different mass (isobaric and isotopic respectively) [201]. These small differences can be resolved by the mass spectrometer, meaning that different samples can be pooled for concurrent identification and analysis. Labelling approaches mean that different conditions can be mixed early in the methodology and so samples are handled in the same way, thereby reducing the effect of any errors; the earlier that the samples are mixed, the greater the reduction in artificial differences between them. Strategies which allow high throughput identification and quantitation of proteins from multiple samples, such as the isobaric tags for relative and absolute quantitation (iTRAQ) method, are now widely used.

Metabolic labelling approaches provide the earliest point at which a label can be introduced. One such method involves using amino acids made from heavy and light isotopes of carbon or nitrogen which are added to cell culture medium taken up by the growing cells, and hence incorporated at the translational level [202]. Different combinations of heavy and light isotopes can be used, so that a number of conditions may be analysed simultaneously using this stable isotope labelling by amino acids in cell culture (SILAC) method. After sample pooling, standard digestion and mass spectrometry processing procedures can then be applied.

Tags can also be introduced at later stages of sample processing by chemical derivation methods. The iTRAQ method introduces tags which are chemically identical, and uses their fragmentation into specific reporter ions to obtain quantification by measuring their relative intensities [203]. The iTRAQ method allows comparison of up to eight samples and the identification and quantitation of proteins and post-translational modifications, while the tandem mass tags (TMT) technique can compare up to six samples.

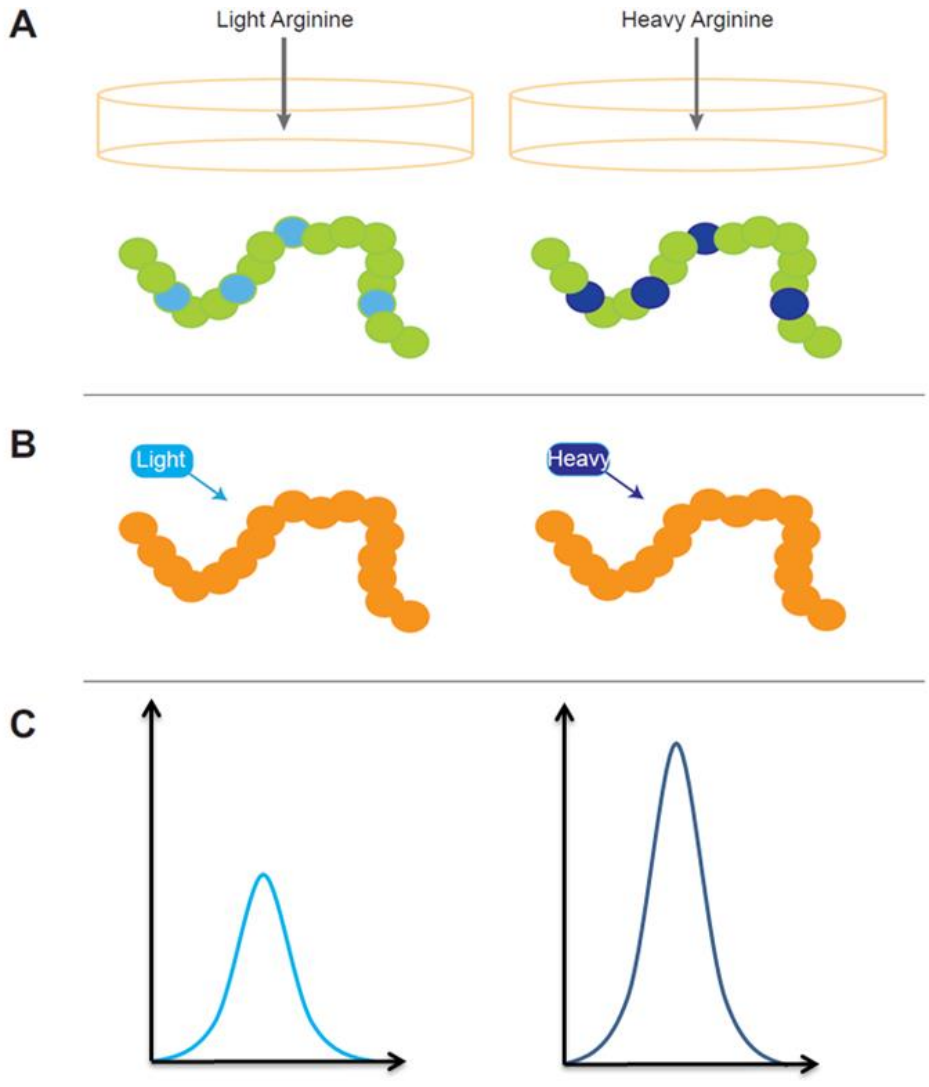


Figure 1.8. Approaches to quantification. A variety of techniques are used for the purpose of quantifying proteins and post-translational modifications. (A) The SILAC metabolic labelling method introduces amino acids with light or heavy isotopes, (B) methods like iTRAQ and ICAT add tags of different mass to the protein, whereas other methods are termed (C) label-free, and rely on comparisons of peptide intensity between samples.

Among the isotopic labelling techniques is the isotope-coded affinity tags (ICAT) method [204]. This involves the addition of a biotin affinity tag which aids purification of the sample, and either a heavy or light region which links this to a reactive group to allow incorporation into the protein sample. Isotopic labelling can also be achieved through modification of the carboxyl or amino groups, or the side chains within the peptide by addition of heavy or light tags based on incorporation of deuterium. This is the case with the ICAT method [204] which modifies cysteine residues in order to add the tag. Variations of the ICAT method include addition of fluorescent labels to aid quantification, and affinity tags to aid purification. A second approach incorporates light or heavy oxygen atoms from water during trypsinisation to create the mass difference [205], but this ^{16}O or ^{18}O incorporation means that only two samples can be analysed at the same time.

Despite its inherent quantitative value, there exist a number of drawbacks of the labelling techniques. Due to the fact that there is a limit to the number of combinations of light/heavy isotopes which can be introduced to the samples, there is a limit to the number of samples which may be mixed and analysed together. A further problem is that the costly reagents required add extra expense to any experiments and so may prohibit some laboratories from applying it to their projects. Recently, a “SILAC-mouse” was reported [206], but this is expensive and technically challenging for widespread use.

1.2.3.2. Label-free methods

Label-free approaches permit the direct comparison of an unlimited number of samples and avoid additional sample processing steps required to add the metabolic or chemical tag, which can allow the introduction of error. Limitations of label-free methods, such as difficulty in confidently assigning spectra for peak identification and comparison, have been greatly reduced by advances in analysers and bioinformatics, and many approaches display high accuracy and precision. Two broad categories of label-free analysis exist; one based on the abundance of detected peptides (spectral counting), and the other based on the measurement of peak height or area.

The technique of spectral counting is based on the theory that in a given sample, more abundant proteins will produce more peptides when digested [207]. This means that the ionic intensities of these peptides will be greater, and therefore more likely to be selected for analysis in the mass spectrometer than the peptides produced from a less abundant protein. It has been shown that there is a correlation between this spectral count and overall protein concentration, but a number of other factors can complicate interpretation of results. In the same way that more abundant proteins provide more peptides upon digestion, larger proteins will be more highly represented than smaller proteins; optimisation and normalisation are therefore required to improve accuracy, which may need to be carried out for each sample or experiment type. This method has a requirement for reproducibility with regard to the selection and fragmentation of ions, and this is not always achievable with highly complex samples. The use of internal standards may aid accuracy and precision.

Alternatively non-labelling methods may use a peptide's peak height or area to quantify it, in this instance using peptide ion intensities for the inference of protein abundance and concentration rather than a specific label. An approach based on this technique has been developed and previously published by Pedro Cutillas' group [191] and was employed for the work contained in this thesis. In this method, peptides are identified using the freely available MASCOT database, and quantified with an in-house program named Pescal. The extracted ion chromatograms (XIC) for peptides are used for correlation with peptide abundance. Internal standards (enolase) spiked into each sample are then used to align chromatograms from different samples so that peptide peaks can be matched and directly compared between MS runs. This overcomes problems caused by shifts in retention times that can occur across different sample runs and which can be large as well as non-linear.

Non-labelling approaches suffer from the uneven distribution of errors introduced due to sample handling; labelled samples can be mixed at an early stage and so any such errors affect all samples equally, whereas this is not the case with non-labelled samples. Additionally the mass spectrometry and data

analyses can be more involved and more complex, and are more reliant on stability of elution and reproducibility of spectra due to the importance of alignment for quantification. Despite the weaknesses of non-labelling approaches, they may (at least in theory) be used to analyse an unlimited number of samples while avoiding costly and time-consuming addition of labels. These methods are especially useful when comparing clinical samples, of which there are often large numbers and so the use of a finite number of labels is limiting [208].

While each enrichment or quantification method will have inherent advantages and disadvantages, choices must be made during experimental design. Many of these techniques can in fact be complementary, and in the same way that running the same sample multiple times can improve proteome or phosphoproteome coverage, this is also true of employing more than one approach for different stages of sample processing and analysis. Ideally, there would be scope for many different combinations of the above to be used to best maximise coverage, but in reality we are limited by time and financial constraints.

1.2.4. Validation and characterisation of phosphosites

While PTMs can now be identified and quantified with high degrees of confidence, their functional relevance is often not known. Analyses of some abundant and important (with regard to specific signalling pathways) phosphosites have been well characterised, such as phosphorylation of Ser473 on Akt, but this is lacking for the vast majority of sites in the phosphoproteome. What phosphoproteomics must now seek to do is assign functional significance to the sites which it detects, which is a huge challenge. A phosphorylation is the result of interplay between (potentially multiple) kinases and phosphatases, but less dynamic phosphorylation of sites suggests that they are not as important as those which are acutely regulated upon different stimuli. It is not a trivial task to separate phosphorylations which play key roles in dynamic signal transduction from those which are “silent” but frequently identified in screens. It should however not be discounted that these phosphosites which appear to be silent do actually have a purpose, much in the same way that some stretches of

DNA and RNA once thought to be redundant are now being shown to have important functions. More realistic short-term goals of the field which can be met include the reduction of the time from obtaining a sample to quantifying its contents, and more widely applying these techniques in a clinical setting, both of which are achievable considering recent advances and ever-improving technology.

Bearing in mind the points discussed previously, characterisation of phosphosites is an important aim. Of the many thousands of phosphopeptides identified and quantified in a typical MS screen, there will inevitably be some false positives and so proper validation is an important component of an experimental plan. This can be done in a number of different ways depending on the protein of interest, MS experimental design, as well as time and financial constraints.

If no kinase is suspected as being responsible, consideration of the amino acid sequence around the phosphosite may reveal a motif particular to a kinase or family, from which further studies can be based. Once a specific kinase is hypothesized to be responsible for a phosphorylation event, *in vitro* kinase assays with purified kinase and radio-labelled phosphate may be performed to test this theory. Incorporation of radioactivity can be monitored to assess both the presence and strength of catalysis, and some quantitation is possible. The ability of a kinase to phosphorylate a substrate *in vitro* is often the first step in validation/characterization. However as kinases are often promiscuous *in vitro*, and this setting does not fully mimic characteristics such as localisation and binding partners, further work is necessary to confidently claim a relationship.

Studies in which the phosphorylation is examined *in vivo* are the next step, and simple tools such as monitoring a possible shift on a western blot caused by the phosphorylation can be very helpful, in the context of different stimuli or inhibitors. If possible, antibodies can be raised against the sequence around the phosphorylated residue, although this may be costly and time-consuming. Use of inhibitors or siRNA specific to the kinase of interest can also be of use *in vitro* and *in vivo*, assuming that these exist. Mutation of the site to a non-phosphorylatable residue (alanine) or to a phospho-mimetic one

(aspartic/glutamic acid) may offer information about the function of the site. However it should be noted that phosphomimetic mutations do not always reproduce the effect of phosphorylation on the tertiary structure of the protein, and hence its function. If a protein of interest is not well characterised, relevant knowledge and tools may not be available, meaning that functional studies can be challenging. While further studies of MS data are always useful, it must be kept in mind that these screens are in themselves informative and powerful tools.

1.3. Aims

The three class IA PI3K catalytic isoforms display structural homology and preference for regulatory binding partners, though there exist differences in both upstream activation and downstream effects of these signalling nodes. The mechanisms around such differences have yet to be fully elucidated and while it is the case that the lipid kinase activity of class IA PI3K isoforms is relatively well-characterised, certain aspects of the signalling of these proteins is less clear.

The aim of this research project was to explore these differences in signalling and had two main branches, focusing on the ubiquitously expressed class IA PI3K isoforms p110α and p110β.

First, we aimed to discover novel targets of the protein kinase activity of p110α in a global and unbiased manner. Only a small number of protein substrates of p110α have been identified, and full characterisation of most of these has not been completed. Large scale analyses of the protein complement of p110α substrates are also lacking. Therefore, we designed a p110α protein kinase assay coupled to phosphoproteomic and mass spectrometry techniques with the goal of identifying a wider range of substrates than has previously been achieved, along with subsequent characterisation.

Second, we compared and contrasted the phosphoproteomes associated with p110α and p110β. We used conditions of serum starvation and stimulation, with which differential phenotypes have been observed in our laboratory following selective isoform-inhibition. Using similar analytical techniques as when

addressing our first aim, we sought to identify individual and groups of phosphopeptides which might account for these differences in signalling, as well as the effectors which may be responsible for these differences.

2. Materials and Methods

2.1. Antibodies

Table 2.1 Antibody reagents

Target	Specification	Manufacturer	Product number	WB conc.
Anti-mouse secondary	HRP conjugated	GE Healthcare	NA931	1:2500
Anti-rabbit secondary	HRP conjugated	GE Healthcare	NA934	1:2500
p-NDRG1 p-Ser330	Rabbit pAb	Cell Signalling	3506	1:1000
p-PRAS40 p-Thr246	Rabbit pAb	Cell Signalling	2640	1:1000
p-S6 p-Ser235/236	Rabbit pAb	Cell Signalling	2211	1:1000
Vinculin	Mouse mAb	Sigma-Aldrich	V9131	1:10000

2.2. Other materials and reagents

Table 2.2. Other materials and reagents

Reagent	Manufacturer	Product number
A66	Symansis	SY-A66
ATP	Sigma-Aldrich	01930-5G
[γ-^{32}P] ATP	Hartmann Analytic	SRP-401

Protein assay dye reagent	BioRad	500-0006
Dulbecco's Modified Eagle's Medium (DMEM)	Sigma-Aldrich	D8062
Foetal bovine serum (FBS)	Invitrogen	16000-044
Immobilon-P	Milipore	IPVH00010
L-glutamine	Invitrogen	25030-081
Lambda protein phosphatase	BioLabs	P0753S
OASIS HLB 1 cc cartridges	Waters	WAT094225
p110α (recombinant)	AstraZeneca	Gift
P81 phosphocellulose paper		Gift
PD MiniTrap G-25 column	GE Healthcare	28-9180-07
Penicillin/Streptomycin	PAA	P11-010
PepClean C18 spin columns	Thermo Scientific	89870
Phosphatase inhibitor cocktail	Roche	88667
Protease inhibitor cocktail	Sigma-Aldrich	P8340
RPMI-1640	Sigma-Aldrich	R5886
TGX221	AstraZeneca	Gift
Titanosphere TiO_2 beads (10 micron)	GL Sciences	5020-75010
TPCK-Trypsin	Thermo Scientific	20230
Trypsin-EDTA	Sigma-Aldrich	T4049

2.3. Cell culture

All cell culture work was carried out in a laminar flow hood using standard sterile techniques. Cell lines were grown at 37°C with 5% CO_2 using the corresponding media outlined in Table 2.3.

2.3.1. Cell lines

Table 2.3. Cell lines

Cell line	Cell type	Species	Growth Medium
4T1	Breast	Mouse	RPMI supplemented with 10% FBS, 1% Pen/Strep, 1% L-glutamine
MCF-7	Breast	Human	DMEM supplemented with 10% FBS, 1% Pen/Strep
MDA-MB-231	Breast	Human	DMEM supplemented with 10% FBS, 1% Pen/Strep
MDA-MB-453	Breast	Human	DMEM supplemented with 10% FBS, 1% Pen/Strep
MEFs	Immortalised embryonic fibroblasts	Mouse	DMEM supplemented with 10% FBS, 1% Pen/Strep

2.3.2. Growth maintenance

Cells were grown in a humidified incubator with 5% CO2, and kept at 37ºC. Growth media for each cell line is as described in Table 2.3.

Cells were passaged or seeded for experiments when they reached 80-90% confluency. Using standard sterile conditions, cells were washed with PBS and detached using 0.05% trypsin-EDTA. Trypsin was inactivated by addition of an excess of growth media. Cells were then further diluted in growth media in a fresh tissue culture flask, or seeded at the required density in 6-well plates or 15 cm dishes, depending on the use.

2.3.3. Inhibitor treatments

Table 2.4. Inhibitors and concentrations used

Compound	Target	Concentration	Source	Chapter
A66 [167]	p110α	3 µM	Symansis	3, 4
TGX221 [148]	p110β	100 nM	AstraZeneca	4

Table 2.5. IC50 of inhibitors towards class IA PI3K isoforms

	IC50 of compounds towards class IA PI3K isoforms (nM)			
Compound	**p110α**	**p110β**	**p110δ**	**p110γ**
A66 [167]	32	>12500	>1250	3480
TGX221 [148]	~5000	5	100	>10000

2.4. Cell lysate preparation

2.4.1. Cell lysis

Table 2.6. Lysis buffer composition

Lysis Buffer Composition	Use
40 mM Hepes (pH 7.5), 0.3% CHAPS, 120 mM NaCl, 1mM EDTA, supplemented with protease inhibitor and phosphatase inhibitor cocktails	Western blotting
20 mM Tris-HCl pH 7.0 1% Triton X-100, supplemented with 15 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml aprotinin, 10 µg/ml leupeptin,100 mM Na_3VO, 1 mM NaF, 1 mM β-Glycerol phosphate, 2.5 mM $Na_2H_2P_2O_7$	Phosphoproteomic assays

Cells were lysed from tissue culture plates when 80-90% confluent. Plates were placed on ice, washed 2x with ice cold PBS supplemented with phosphatase inhibitors, and the corresponding lysis buffer was added. Cells were scraped into Eppendorf tubes and vortexed then incubated at 4°C for 30 min with rotation to allow full lysis of cells. Lysates were then centrifuged at 13,000 rpm for 10 min at 4°C, and the supernatant collected for determination of protein concentration and further processing.

2.4.2. Determination of protein concentration

Protein concentration was determined using protocols and reagents supplied by Bio-Rad, and listed in Table 2.2. 200 µl of Bio-Rad reagent was dispensed into the wells of a 96-well plate and either BSA protein standard to produce a calibration curve, or sample was added. All wells of the plate were then measured at absorbance of 595 nm on a TECAN plate reader. Absorbance of the known standards was used to create a calibration curve, from which protein concentration of the samples could be calculated.

2.5. Sodium dodecyl sulphate polyacrylamide gel electrophophoresis (SDS-PAGE)

Lysed samples were diluted with 4x sample buffer (200 mM Tris-HCl pH 6.8, 400 mM DTT, 8% SDS, Bromophenol Blue (0.0025%), 40% glycerol) and incubated for 5 min at 100ºC to denature proteins, then separated by SDS-PAGE. 1.5 mm 8 – 12.5% acrylamide resolving gels (acrylamide as indicated, 375 mM Tris pH 8.8, 0.1% SDS, 0.1 ammonium persulphate) were cast using BioRad apparatus. A layer of stacking gel (5% acrylamide, 375 mM Tris HCl pH 8.8, 0.1% SDS, 0.1% ammonium persulphate) was poured above, containing wells for sample loading. Gels were placed into a tank containing running buffer (25 mM Tris HCl, 200 mM glycine, 0.1% SDS) protein ladder or samples were loaded into wells, and electrophoresis performed at 75 V for 20 min then at 125 V until the dye front reached the bottom of the gel.

2.5.1. Western Blotting

Proteins separated by SDS-PAGE were transferred from the gel to a PVDF membrane activated with ethanol for 1 min. “Semi-dry” transfer was achieved by assembly into a Bio-Rad Trans-Blot Turbo Blot System cassette of the following which had been previously incubated in transfer buffer (25 mM Tris HCl, 200 mM glycine, 0.1% SDS, 20% ethanol): 3 pieces Whatman paper, gel, PVDF membrane, 3 pieces Whatman paper. Transfer of proteins was performed at 25 V for 40 min.

After transfer, membranes were blocked by 30 min incubation with 5% BSA in TBS-T (150 mM NaCl, 50 mM Tris HCl pH 7.6, 0.1% Tween-20). Membranes were then incubated for 18 h with primary antibody prepared in 5% BSA (concentrations of antibodies are shown in Table 2.1). After incubation with primary antibody, membranes were washed for 6 x 5 min with 2.5% milk prepared in TBS-T. Membranes were incubated with secondary antibody diluted (as described in Table 2.1) in 2.5% milk prepared in TBS-T for 1 h at room temperature. Membranes were then washed 6 x 3 min in 2.5% milk prepared in TBS-T, followed by 2 x 3 min washes in TBS-T, followed by incubation for 2 min

in enhanced chemiluminescent (ECL) solution, after which time antibody binding was visualised by exposure to X-ray film.

As required, membranes were stripped by 3x 5 min washes in distilled water then 20 s incubation in 8 M guanidine hydrochloride solution, followed by 3x 10 min washes in distilled water and 3x 5 min washes in TBS-T.

2.5.2. Staining

Where necessary, gels were rehydrated in coomassie staining solution (50% methanol, 10% acetic acid, 0.025% Coomassie Brilliant Blue) for 1h and destained (30% methanol, 10% acetic acid) to visualise proteins.

2.6. *In vitro* protein kinase assay

2.6.1. With recombinant proteins

PI3K protein kinase assays were performed in a final volume of 30 µL in a buffer containing 50 mM Tris-HCl pH 7.4, 1 mM EGTA, 1 mM DTT, 10 mM $MgCl_2$, 10 mM $MnCl_2$ and 100 uM ATP, plus 5 µCi [γ-^{32}P]-ATP per reaction condition. 1µg of protein kinase was incubated with the buffer for 20 minutes at 25°C, and reactions were terminated by addition of 5x SDS-PAGE sample buffer and boiling for 5 minutes. Samples were separated by SDS-PAGE, and gels subsequently fixed with 10% acetic acid, 20% methanol for 30 minutes and dried down. Visualisation of phosphorylation was achieved with PhosphoImager and a Typhoon scanner.

When recombinant peptides were included as substrates, 1 µg of each was added per reaction condition. When the inhibitor A66 was used, this was incubated with the kinase at the concentrations indicated for 30 minutes on ice before initiation of the kinase reaction.

2.6.2. With peptides

As described in Hastie *et al*. [209]. PI3K protein kinase assays were performed in a final volume of 50 µL containing 50 mM Tris-HCl pH 7.4, 1 mM EGTA, 1 mM DTT, 10 mM $MgCl_2$, 10 mM $MnCl_2$ and 100 uM ATP, plus 5 µCi [γ-^{32}P]-ATP per reaction condition. 1 µg each of recombinant p110α and peptide substrate

were incubated together in this buffer for 10 min at 30ºC. 40 µL of solution was spotted onto P81 phosphocellulose paper which was immediately immersed in 75 mM phosphoric acid for 4x 5 min washes. The papers were briefly rinsed with acetone, left to air-dry, then bound radioactivity measured by Cerenkov counting using a ^{32}P program.

2.7. Preparation of samples for MS

After cell lysis as described above (without inclusion of phosphatase inhibitors), size exclusion chromatography was performed on samples using PD MiniTrap G-25 to remove small molecules such as ATP. Proteins were eluted into 1 mL of equilibration buffer (40 mM Tris-HCl pH 7.0, 1 mM DTT, 0.1 mM EGTA, 0.1% Triton X-100). Samples were pooled and protein quantified. Total cell lysate was dephosphorylated through incubation with Lambda protein phosphatase for 1 h at 37ºC. Phosphatase activity was inhibited by addition of sodium fluoride (to a final concentration of 50 nM), sodium orthovanadate (to a final concentration of 10 mM) and EDTA (to a final concentration of 50 mM), and proteases inhibitor cocktail was added at a 1:100 dilution. Aliquots equivalent to 500 µg were taken and made up to 800 µl in equilibration buffer.

2.7.1. In vitro protein kinase assay for MS

1ml of reaction buffer (60 mM Tris-HCl pH 7.5, 2 mM EGTA, 1 mM DTT, 20 mM $MnCl_2$, 20 mM $MnCl_2$) was added to the sample along with 200 µL of ATP (final concentrations of 0 µM, 50 µM, 100 µM). 2 µg of recombinant p110α was then added, and the reaction mixture was incubated for 5 min at 30°C. The reaction was terminated through addition of urea to a final concentration of 8M, buffered in 20 mM HEPES pH 8.0.

2.7.2. In-solution digestion

Samples were incubated with 4.1 mM DTT and 8.3 mM iodoacetamide for 15 min each to achieve reduction and alkylation. Samples were then diluted 1 in 4 with 20 mM HEPES, and incubated with immobilized TLCK-trypsin for 18 h at 37°C with agitation. Tryptic digestion was inhibited by addition of trifluroacetic acid (TFA) at a final concentration of 1%. Samples were centrifuged at 2000 g

for 5 min at 5ºC and the supernatant removed to a fresh tube and beads discarded.

2.7.3. Desalting

Samples were then desalted and concentrated using OASIS HLB 1cc extraction cartridges. Cartridges were assembled into a vacuum manifold with a pressure <15 inHg and washed sequentially with 1 ml 100% acetonitrile (ACN), 1.5 ml 1% ACN, 0.1 TFA. Peptide samples were then loaded and washed with 1 ml 1% ACN, 0.1 TFA, and eluted in 0.5 ml 1 M glycolic acid (GA), 1% ACN, 0.1% TFA.

2.7.4. Phosphopeptide enrichment

Sample volumes were normalised to 1000 µL with 1M GA, 1% ACN, 0.1% TFA then incubated with 50 µL of re-suspended TiO_2 beads for 5 min with rotation, centrifuged for 30 s at 2,000 rpm and TiO_2 beads applied to spin columns previously equilibrated with 100% ACN. Beads were washed 2 x 1 M GA, 80% ACN, 5% TFA, 1 x 100 mM ammonium acetate, 25% ACN, 4 x 1% ACN, with 3 min centrifugation at 1,500 rpm for each wash. Peptides were eluted with 4 x 50 µL 5% NH_4OH, 1% ACN, also with 3 min centrifugation at 1,500 rpm. Samples were snap-frozen and dried under a vacuum, and stored at -20ºC. Immediately before LC-MS/MS, samples were reconstituted in reconstitution buffer.

2.8. Liquid chromatography mass spectrometry

Analysis was carried out as detailed in [215]. Briefly, phosphopeptide pellets were dissolved in 13 µl of 0.1% TFA and run in a ThermoFisher LTQ-Orbitrap XL mass spectrometer (full scan survey spectra, m/z 375–1800, 30,000 resolution at m/z 400) which was coupled to a NanoAcquity UPLC system from Waters. Separation of peptides was achieved by C18 reverse-phase column (inner diameter = 100 µm, length = 100 mm, particle size = 1.7 µm, pore size = 300 Å). 4 µl of sample were loaded at a flow rate of 2 µL/min for 8 min followed by a gradient elution at 400 nL/min, during which the instrument operated with a back pressure of around 3,000 psi. Gradient elution was from 99% solution A (0.1% formic acid in LC-MS grade H2O), 1% solution B (0.1% formic acid in LC-

MS grade acetonitrile) to 65% solution A, 35% B in 100 min, followed by a 10 min wash using 15% solution A, 85% solution B, and an equilibration with 99% solution A, 1% solution B for 10 min. Data dependent analysis (DDA) was used in the LTQ-Orbitrap XL, whereby the five most abundant multiply charged ions present in the survey spectrum were selected, and CID used for fragmentation.

MS analysis was carried out as in [215]. Briefly, Mascot Daemon was used to analyse the MS data generated. This software functions to automate the use of Mascot Distiller in order to smooth and centroid the MS/MS data, and also the Mascot search engine [210], which searches the processed files against the peptide sequence library in the Swiss Prot database (restricted to either human or mouse entries, depending on the cell line origin). The search parameters included: human/mouse taxonomy; digestion enzyme: trypsin, up to two missed cleavages permitted; fixed modification: carbamidomethyl (C); variable modifications: Pyro-glu (N-term), Oxidation (M), Phospho (STY). Datasets were searched with a mass tolerance of ±7 ppm and a fragment mass tolerance of 0.6 Da. Hits were considered significant when returned with an expectation value < 0.05 as returned by Mascot. False discovery rates were ~2% as determined by decoy database searches. Identifications of peptide of interest and parent protein were returned with confidence scores representing probability. Mascot was used to assign site of phosphorylation, with Delta score returned to represent confidence through probability (Figure 2.1.).

Mascot results were extracted to Microsoft Excel files using an in-house script. Identification of the correct position of phosphorylation for peptides with multiple potential was achieved using the delta score between the first and second hits as reported by Mascot. The in-house program Pescal [211] was used to automate the generation of extracted ion chromatograms (XIC) and to calculate the peak heights and areas.

In experiments using DDA, the nature of undersampling and stochasticity of peak selection for fragmentation of ions means that MS/MS data were not obtained for all phosphopeptides across all runs. To overcome this issue, phosphopeptides identified by Mascot above a statistically significant threshold were placed in a database of peptides quantifiable by LC-MS. Pescal was used

to quantify the intensities of the peptides present in the database across all the samples, using the m/z and retention time of the selected peptides to construct extracted ion chromatograms (XICs) for the first three isotopes of each ion. This applies restrictions on the molecular mass, retention time, charge, and isotope distribution, thereby permitting high confidence (false positive discovery rate ~ 5%) identification of the LC-MS elution profiles corresponding to the studied phosphopeptides (windows for XIC construction: m/z = 7 ppm, retention time = 5), following which the peak height and areas of each individual XIC could then be determined. Internal peptide standards (enolase) were spiked into each sample to allow chromatographic alignment. The resulting quantitative data were parsed into Microsoft Excel files for further normalization and statistical analysis. Peptide intensities were normalised to the total chromatogram intensity, and expressed relative to the greatest intensity value across all samples. Data were statistically analysed in Microsoft Excel files.

2.9. Bio-informatic analysis

2.9.1. Peptide production

Water solubility (vital for reconstitution of solid state manufactured peptides) and pH of peptides was predicted using Innovagen calculator (http://pepcalc.com/peptide-solubility-calculator.php). The amino acid sequence of residues surrounding candidate phosphorylation site of interest was used as input, and additional lysine residues were included to ensure binding of peptides to P81 phosphocellulose paper; a net positive charge at low pH indicates likelihood of peptide binding.

2.9.2. Motif analysis

The freely available online program motif-x [212] was used to interrogate the phosphopeptide sequences identified in MS screens for conserved motifs. When necessary, sequences were manually extended to five residues on either side of the phosphorylation site of interest. Selections were: MS/MS foreground format, extend from=IPI mouse proteome, central character=S/T/Y, width=11, occurrences=5, significance=0.0499, background=IPI mouse proteome.

2.9.3. Sequence analysis

The ClustalW2 tool (http://www.ebi.ac.uk/Tools/msa/clustalw2/) was used for the alignment of protein sequences and identification of conservation across species. 30 amino acids surrounding the phosphorylation site of interest were compared to the equivalent human, rat, and bull sequences, and ClustalW2 returned qualitative data on conservation of residues.

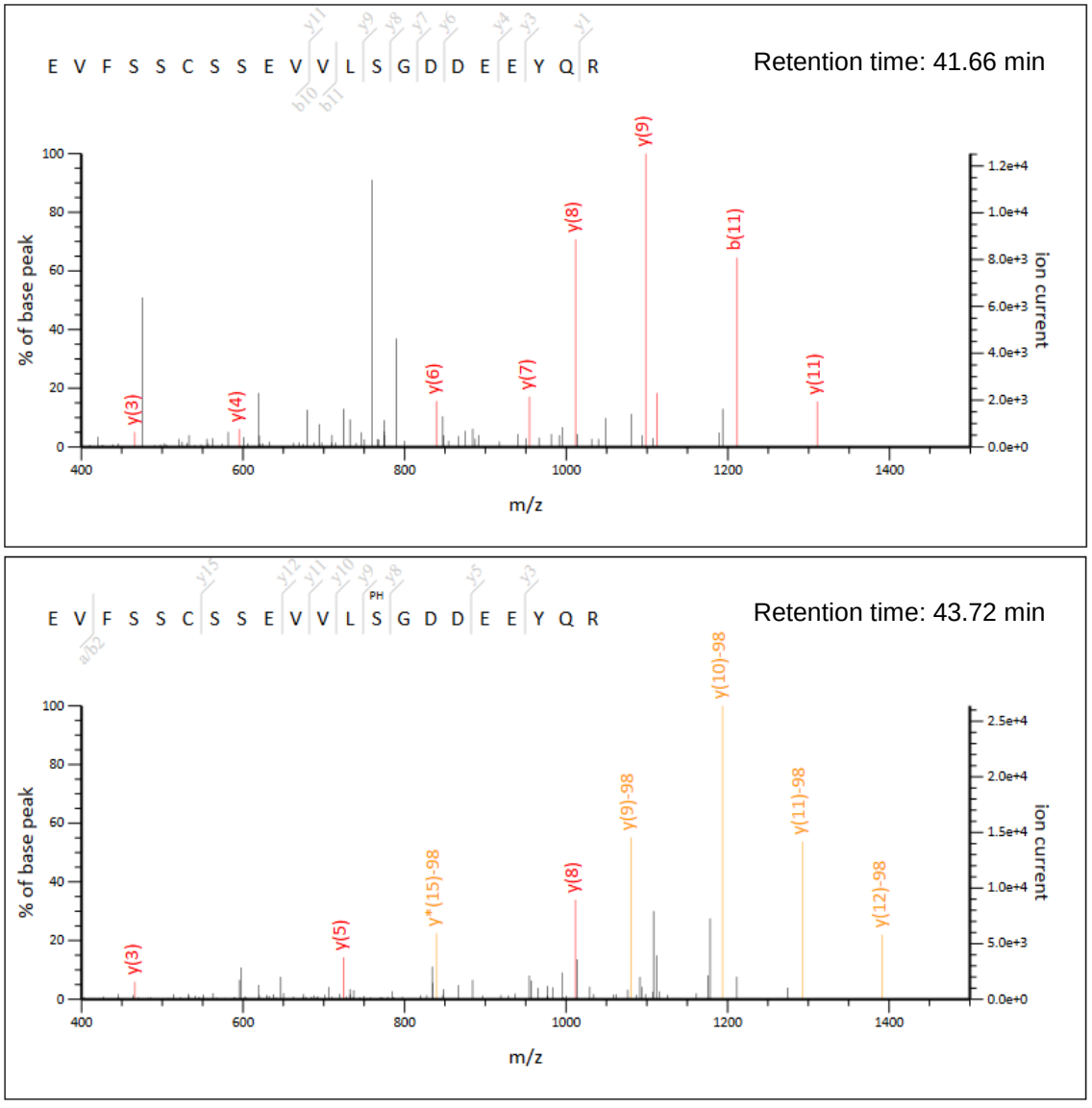


Figure 2.1. Illustration of peptide and phosphopeptide spectra. MS/MS fragmentation of the peptide EVFSSCSSEVVLSGDDEEYQR is shown with **A)** no modifications and **B)** phosphorylation of residue 13 (serine). Elimination/neutral loss of H_3PO_4 (97.9769 Da) is represented by y(*residue*)-98 for y ions which include the modified residue.

3. Investigation into the protein kinase activity of p110α

3.1. Introduction and aim of study

While the lipid kinase activity of the PI3K family has been studied extensively, relatively little is known about the protein kinase function which the PI3K isoforms also exhibit. As lipid kinases, PI3Ks have been shown to have important roles in a number of normal cellular and pathological processes including cancer. The protein kinase activity of class IA PI3Ks has been shown to be capable of down-regulating their own lipid kinase activity, suggesting a means of self-regulation and supporting the case for further study.

All three classes of PI3K have been shown to display a protein kinase activity with the class I isoforms capable of autophosphorylation: in the case of p110α the regulatory subunit p85α is phosphorylated [126, 127], while the remaining class I isoforms phosphorylate the p110 subunit [132, 133]. For the class IA PI3K isoforms, this phosphorylation causes a down-regulation of their lipid kinase activity, and so is a potential method of autoregulation. In addition, a number of non-PI3K targets have been identified, and the possibility of the existence of undiscovered protein substrates remains.

Mass spectrometry and proteomic techniques have advanced significantly over the past decade, and they are powerful tools capable of characterising intracellular signalling pathways. Many of these pathways rely heavily on protein phosphorylation as a means of transmitting and regulating the intensity and duration of the signal, and so phosphoproteomics is an area of increasing relevance. Techniques developed by Dr Cutillas and others allow for unbiased identification and quantitation of thousands of phosphosites from the starting point of a whole cell lysate [191, 211], making phosphoproteomics a powerful discovery tool, and suitable for delineating signalling pathways and uncovering novel kinase-substrate pairings.

The aim of the work presented in this chapter was to uncover novel substrates of the protein kinase activity of p110α, using an *in vitro* kinase assay coupled to innovative mass spectrometry techniques available in our laboratory.

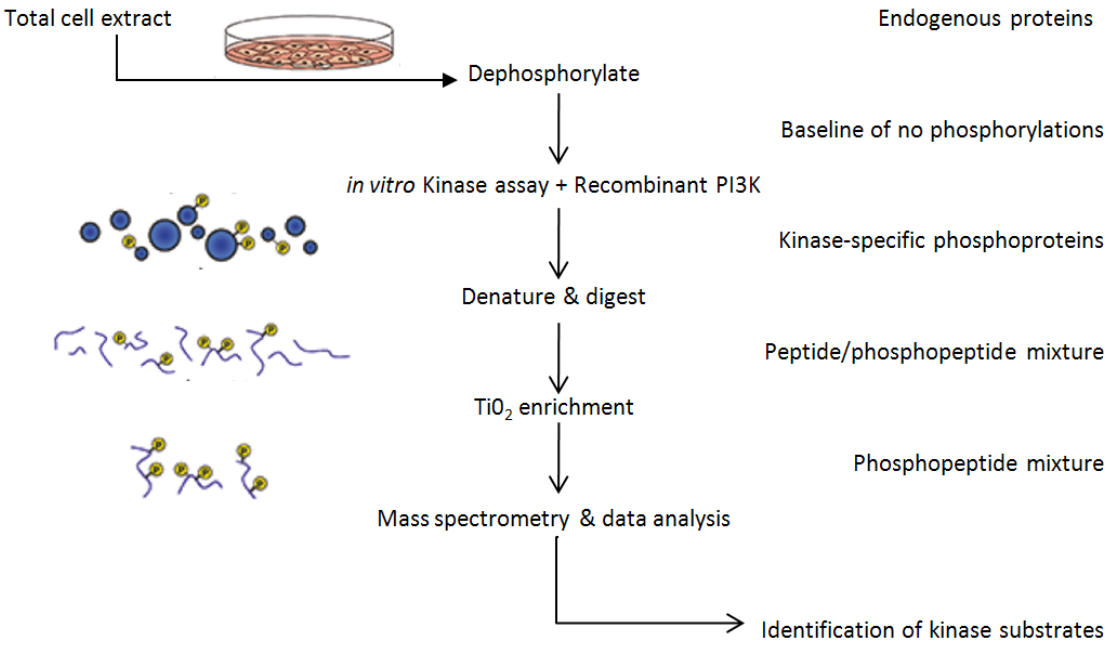


Figure 3.1. Summary of phosphoproteomic approach. A total cell lysate of MEF cells was dephosphorylated using exogenous lambda phosphatase and used as a substrate for an *in vitro* kinase assay with p110α. Samples were digested using trypsin, and phosphopeptides enriched by titanium dioxide affinity purification, and analysed by LC-MS/MS. Data analysis was carried out using a combination of in-house and freely available software programs.

3.2. Optimisation of experimental conditions

Since a number of studies have shown conflicting reports on the conditions necessary for robust p110α protein kinase activity, we first sought to optimise the conditions for an *in vitro* kinase assay. We performed a number of experiments with the aim of identifying the necessary cofactors, as well as inhibitor concentration and time point for a phosphoproteomic screen using recombinant p110α in an *in vitro* kinase assay. To assay p110α protein kinase activity, we carried out *in vitro* kinase assays with recombinant p110α/ p85α heterodimers using [γ-^{33}P]-ATP as a phosphate donor, and monitored phosphorylation of p85α as a read-out of the protein kinase activity of p110α.

Conflicting reports have been published as to the divalent cation preference of p110α in terms of its protein kinase activity [127, 128]. Therefore, we first sought to address which of Mg^{2+} or Mn^{2+} supports the strongest *in vitro* protein kinase activity of p110α. *In vitro* kinase assays were carried out as detailed in

Figure 3.2A, which shows a representative autoradiogram. As is clear, robust phosphorylation of the p85α subunit is observed with either cofactor. Use of 10 mM Mg^{2+} produces slightly lower p85α phosphorylation than 10 mM Mn^{2+} alone, which is in turn similar to the use of both cofactors together. Therefore, we decided to use a concentration of 10 mM of each cofactor in future assays, including in the MS screen.

Next, we sought to evaluate the kinetics of phosphorylation of p85α by p110α and to determine an optimal time point for the MS screen. We proceeded to carry out *in vitro* kinase assay time courses as described in Figure 3.2B, which shows a representative autoradiogram and quantification of three experiments. Phosphorylation of p85α is clearly seen at the shortest time point of 1 min and increases across the time points used. Based on these data, we decided to use for the MS screen a time of 5 min for the kinase assay, in order to allow p110α sufficient time to phosphorylate its substrates, while also minimising the inevitable background phosphorylation events catalysed by other kinases present in the cell lysate.

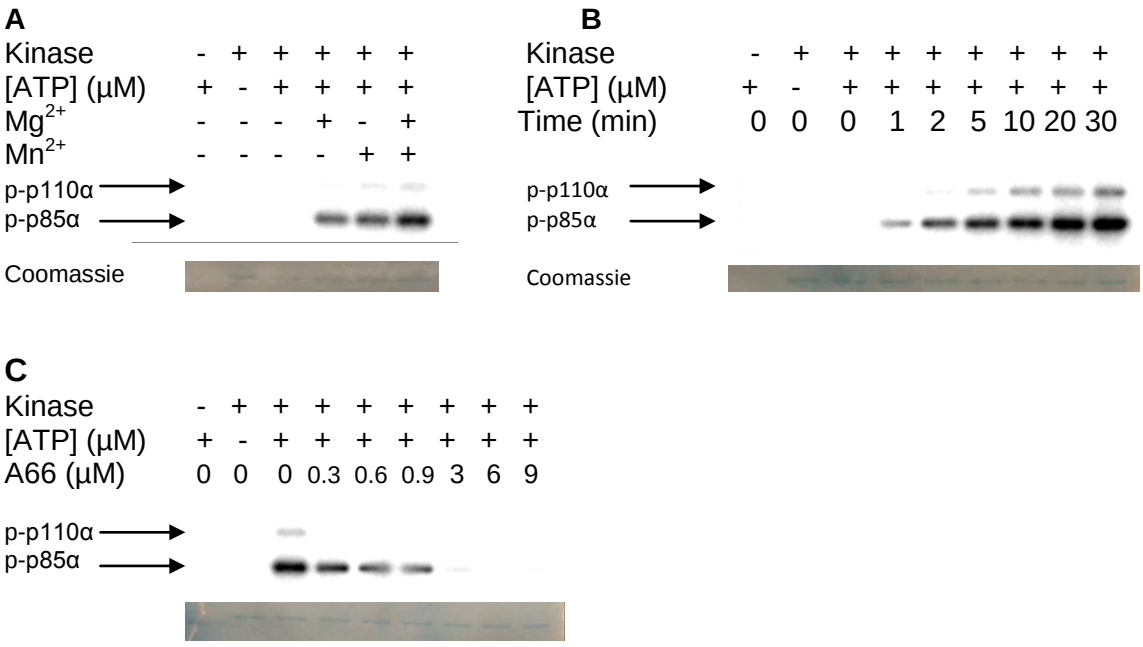


Figure 3.2. p110α phosphorylates p85α *in vitro*. *In vitro* kinase assays were performed using recombinant p110α/p85α heterdimers and [γ^{32}P], with the stated conditions: kinase assays were carried out **(A)** for 20 min at 30°C in the presence or absence of 10 mM Mg^{2+};or 10 mM Mn^{2+} ; **(B)** at 30°C for the stated time with 10 mM Mg^{2+} and 10 mM Mg^{2+}; **(C)** at 30°C for 5 min in the presence or absence A66 at 30°C with 10 mM Mg^{2+} and 10 mM Mg^{2+} with the indicated concentrations of A66. Proteins were separated by SDS-PAGE, and gels were fixed and dried then incorporation of [γ^{32}P] visualised using a Typhoon scanner and PhosphoImager. ATP was included at a final concentration of 100 μM where indicated.

Two controls are present in the MS screen. In one, the cell lysate is left untreated as a measure of the level of background phosphorylation present in the samples and in the other, inactive p110α is added. Therefore, we next sought to determine how best to achieve full deactivation of p110α. A66 is a highly selective inhibitor of wild-type and oncogenic p110α isoforms, with an IC50 around two orders of magnitude lower for p110α than other class IA PI3K isoforms, and extremely limited inhibition of other kinases. In order to determine the optimal concentration of A66 for full inhibition of the protein kinase activity of p110α, we performed *in vitro* kinase assays as described in Figure 3.2C, which shows a representative autoradiogram. A dose-dependent effect is observed, with the lowest concentration of A66 used eliciting a negative effect in terms of the phosphorylation of p85α. Maximum inhibition is achieved using A66 at a concentration of 3 μM, and so we decided to use this concentration to achieve inactivation of p110α in the MS screen, in line with the reported IC_{50} values (Chapter 2.3.3.).

It should be noted that for all kinase assays described above, phosphorylation of p110α is also observed, though to a much smaller degree than the phosphorylation of p85α, and that observed profiles of the phosphorylation of p85α and p110α are similar.

3.3. Use of a phosphoproteomic screen to analyse p110α protein kinase activity

Having established optimum conditions for its protein kinase activity, we next moved on to identifying the phosphorylation events catalysed by p110α. The approach we used, in which an *in vitro* kinase assay was coupled to mass spectrometric analysis, is based on the work of the Cohen group (MRC Protein Phosphorylation and Ubiquitylation Unit, University of Dundee) [213], and further developed by the Cutillas laboratory (Integrative Cell Signalling and Proteomics, Barts Cancer Institute, Queen Mary University of London) [214].

Briefly, 500 μg of a whole cell lysate was taken from MEFs, the proteins from which were dephosphorylated by addition of recombinant lambda phosphatase

and used as the substrate for an *in vitro* kinase assay using p110α. This assay was designed so as to provide qualitative and quantitative information about the

phosphorylation events. First, three conditions were compared: cell lysate only (termed “cell lysate”) to which no recombinant p110α was added, cell lysate which was treated with recombinant wild-type p110α and 3 μM of the p110α-specific inhibitor A66 (termed “p110α/A66”), and cell lysate which was treated with recombinant wild-type p110α (termed “p110α”). This enabled us to compare the data from p110α and both cell lysate and p110α/A66 conditions to ensure that we could discount background phosphorylation events not specific to the protein kinase activity of p110α. Second, each condition was subjected to three separate kinase assays with three different ATP concentrations (0 μM, 50 μM and 100 μM), and it is expected that intensity of a valid kinase-dependent phosphorylation would increase with an increase in ATP concentration. Also relevant and a reason for the experimental design is inclusion of multiple ATP concentrations allows approximations of reaction kinetics to be made.

After an *in vitro* kinase assay, proteins were denatured by reduction and alkylation, followed by digestion using trypsin, followed by enrichment of phosphopeptides based on TiO_2 affinity. The samples were then subjected to LC-MS/MS using an Orbitrap-XL, and phosphopeptides were identified and quantified using in-house techniques [191, 211]. The experiment was performed so as to have three biological replicates; therefore there were 27 samples in total (3 ATP concentrations x 3 kinase conditions x 3 biological replicates). Targets were chosen for validation based on comparison of phosphopeptide levels in p110α samples with cell lysate and A66 samples, and also the kinetic profile of phosphorylation which was determined by analysis of intensity of phosphorylation at multiple ATP concentrations.

The data from the MS analysis of the samples were used to interrogate the MASCOT protein database, and peptides which were returned with an expectation value of <0.05 were chosen for further analysis. This resulted in a group of 641 peptides which were quantified using the in-house program Pescal [215] (Appendix 1).

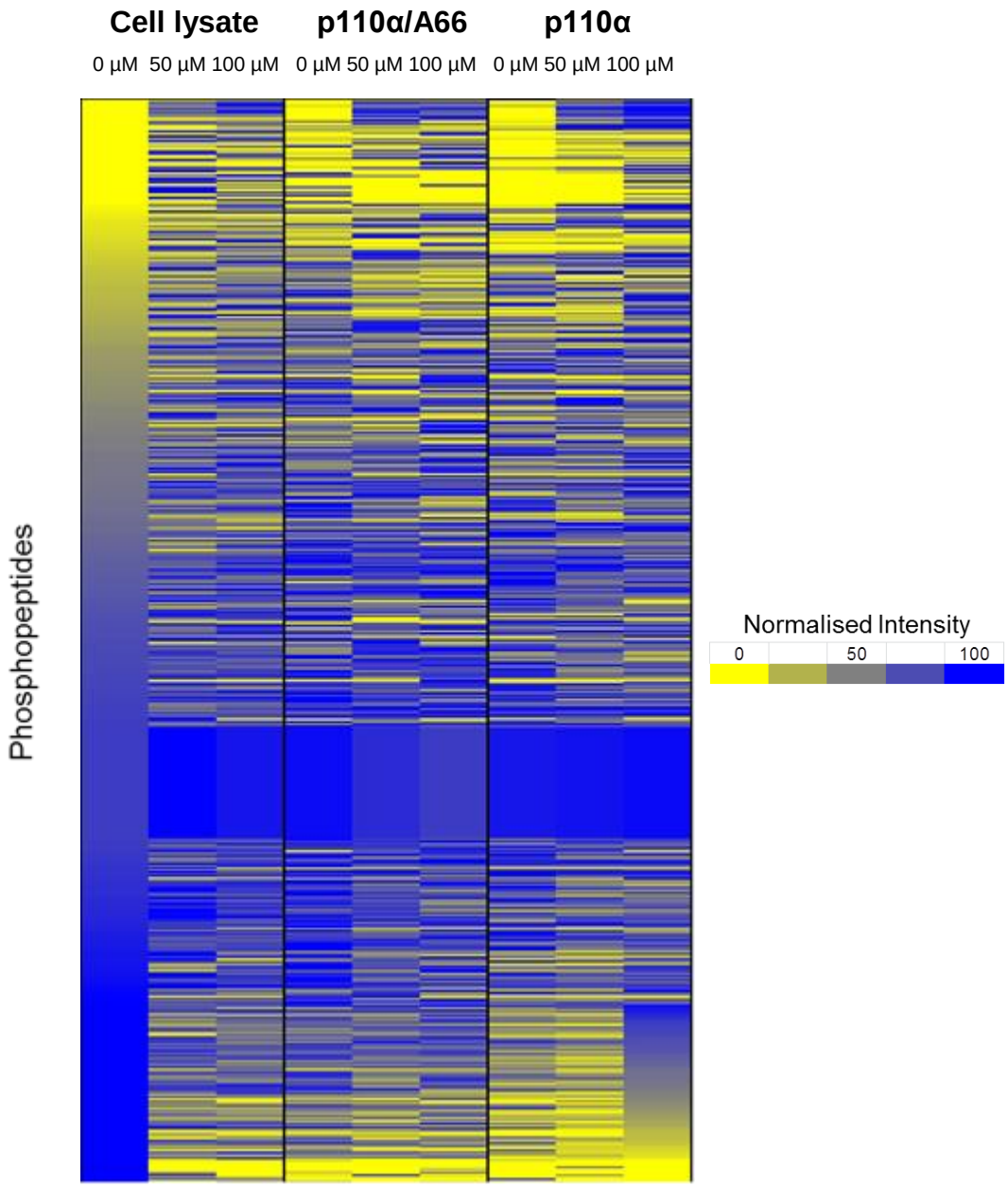


Figure 3.3. Quantitative analysis of response of phosphorylation events to *in vitro* p110α kinase assay. MEF cell lysate was dephosphorylated using exogenous lambda phosphatase and incubated with 0, 50, 100 µM ATP, in the presence or absence of recombinant p110α and its specific inhibitor A66 for 5 min at 30°C. Phosphorylated proteins were subjected to phosphoproteomic analysis (n=3). Quantification of 641 phosphopeptides identified was visualised as a heat map, where intensity of phosphorylation for a given phosphopeptide was expressed relative to the maximum intensity for that phosphopeptide.

3.3.1. Comparison of *in vitro* kinase assay conditions

After averaging the values from the three replicates, we used a heat map to graphically represent patterns of phosphorylation of peptides across all samples (Fig. 3.3). Quantification of all 641 phosphopeptides identified in this experiment shows that the conditions broadly behave in a similar manner, with high intensity of some phosphorylation events present in the control samples. This includes in the cell lysate, p110α/A66, and 0 μM ATP p110α conditions. While some individual events do display a profile expected of true p110α targets, there is a greater number of phosphorylation events which are positively regulated in the p110α+ATP samples and also in one or both control samples. This suggests that the effect on the phosphoproteome of adding recombinant p110α is minor, and that the effects caused by the addition of recombinant p110α in this context are subtle, further implying that its protein kinase activity is not significant, at least when tested under the indicated conditions.

Principle component analysis (PCA) can be used to assess the similarities between sets of conditions. We subjected the whole dataset to PCA, the results of which are shown in Figure 3.4A, which reveals an effect which reinforces the conclusion drawn above. Instead of a clear separation being observed between the cell lysate and A66, and p110α conditions, the data points showed a more indiscriminate grouping with none of the conditions clustering. This is also true of the 0 μM ATP samples for cell lysate, A66 and p110α conditions which would be expected to behave similarly, and also the replicates of a given condition. Both the cell lysate and A66 controls appear to be similar to the p110α variable in terms of PCA and the majority of peptides in the heat map. Had it been the case that in this context cell lysate displayed different characteristics to A66 and p110α, it could have been argued that full inhibition in the A66-treated samples had not been achieved. However, this is unlikely to be true, and it is more reasonable to consider p110α as being inhibited to a similar level to Figure 3.2.

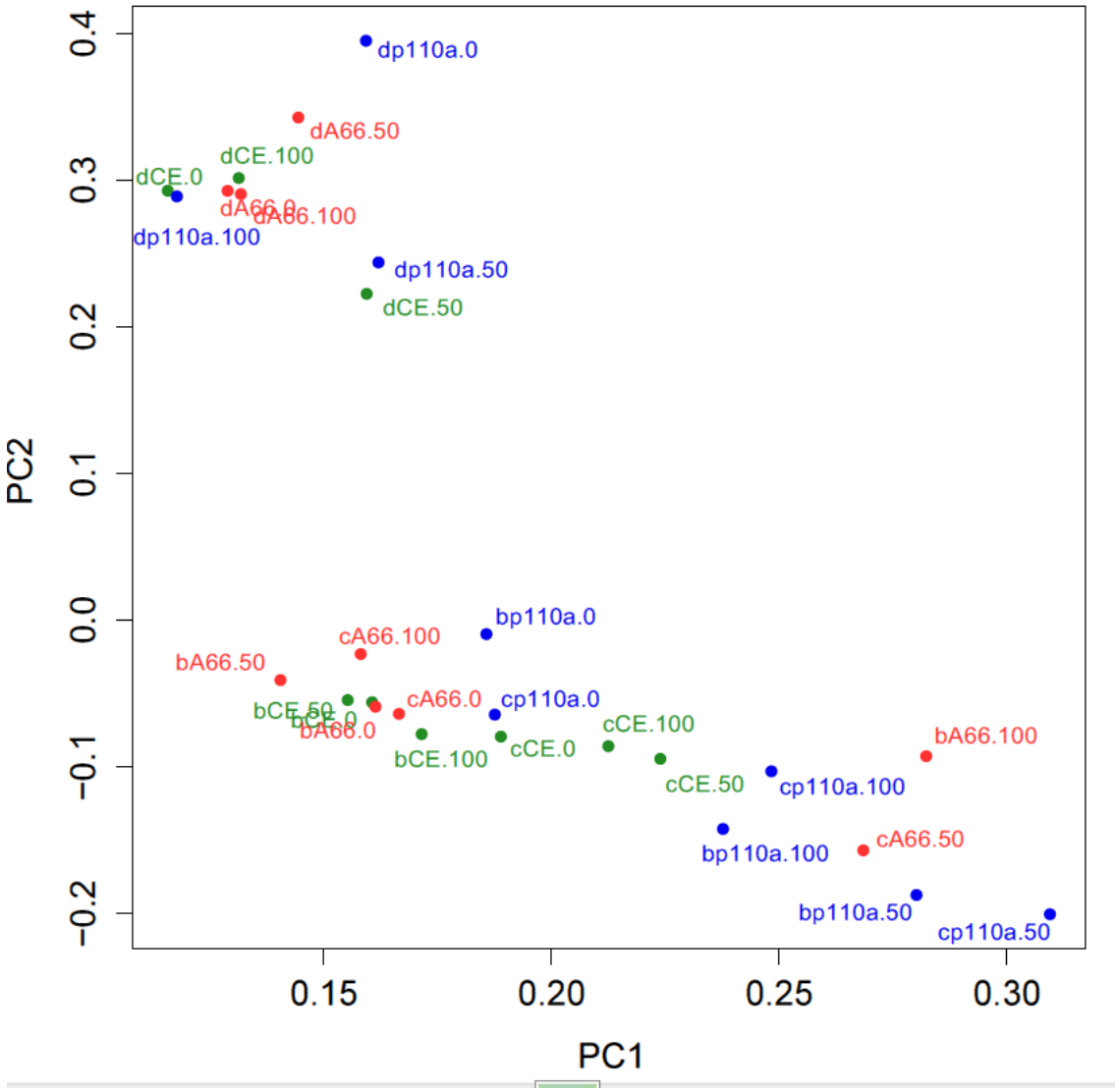


Figure 3.4. PCA indicates little effect of addition of recombinant p110α. MEF cell lysate was dephosphorylated using exogenous lambda phosphatase and incubated with 0, 50, 100 μM ATP, in the presence or absence of p110α and its specific inhibitor A66 for 5 min at 30°C. Phosphorylated proteins were subjected to phosphoproteomic analysis, and three independent experiments were carried out (n=3). Green = cell lysate; red = p110α/A66; blue = p110α. Number = ATP concentration (μM).

3.3.2. Identification of potential substrates of p110α protein kinase activity

We then took the average of the individual replicates so that a single value was obtained which was then used for comparison and subsequent analysis, and then proceeded to define arbitrary criteria to define p110α substrates. To ensure that phosphorylation increased with an increase in ATP concentration, we defined a cut-off of a Pearson correlation value of >0.6 for the p110α-treated samples. This resulted in a list of 254 phosphopeptides, whose quantitative data are graphically represented by way of a heat map in Figure 3.5. As is clearly seen, intensity of phosphorylation increases with ATP concentration for the p110α condition, however within this list are phosphopeptides whose phosphorylation is present at high levels in either or both of the control samples. Examples of such profiles are shown in Figure 3.6. It is important to note the variation in the levels of intensity of phosphorylation in the controls, with some phosphopeptides displaying higher intensity than in the p110α samples, even at the 0 µM ATP concentration

Because of this level of background phosphorylation present in the controls, we included a second criterion for defining potential candidates. Phosphorylation events were considered to be due to addition of recombinant p110α if there was at least a two-fold increase from the 50 µM and 100 µM ATP concentrations in both the cell lysate and A66 samples, to the p110α condition.

Two phosphopeptides were found to meet these criteria, and their profiles of phosphorylation are shown in Figure 3.7. Both phosphopeptides had two phosphorylation sites, all of which were previously undescribed. FBX7 (Ser283, Ser294) is the substrate recognition component of SCF E3 ubiquitin protein ligase complex, and GRIA1 (Ser873, Ser881) is a membrane-bound glutamate receptor, whose putative phosphorylation sites are in its cytoplasmic portion.

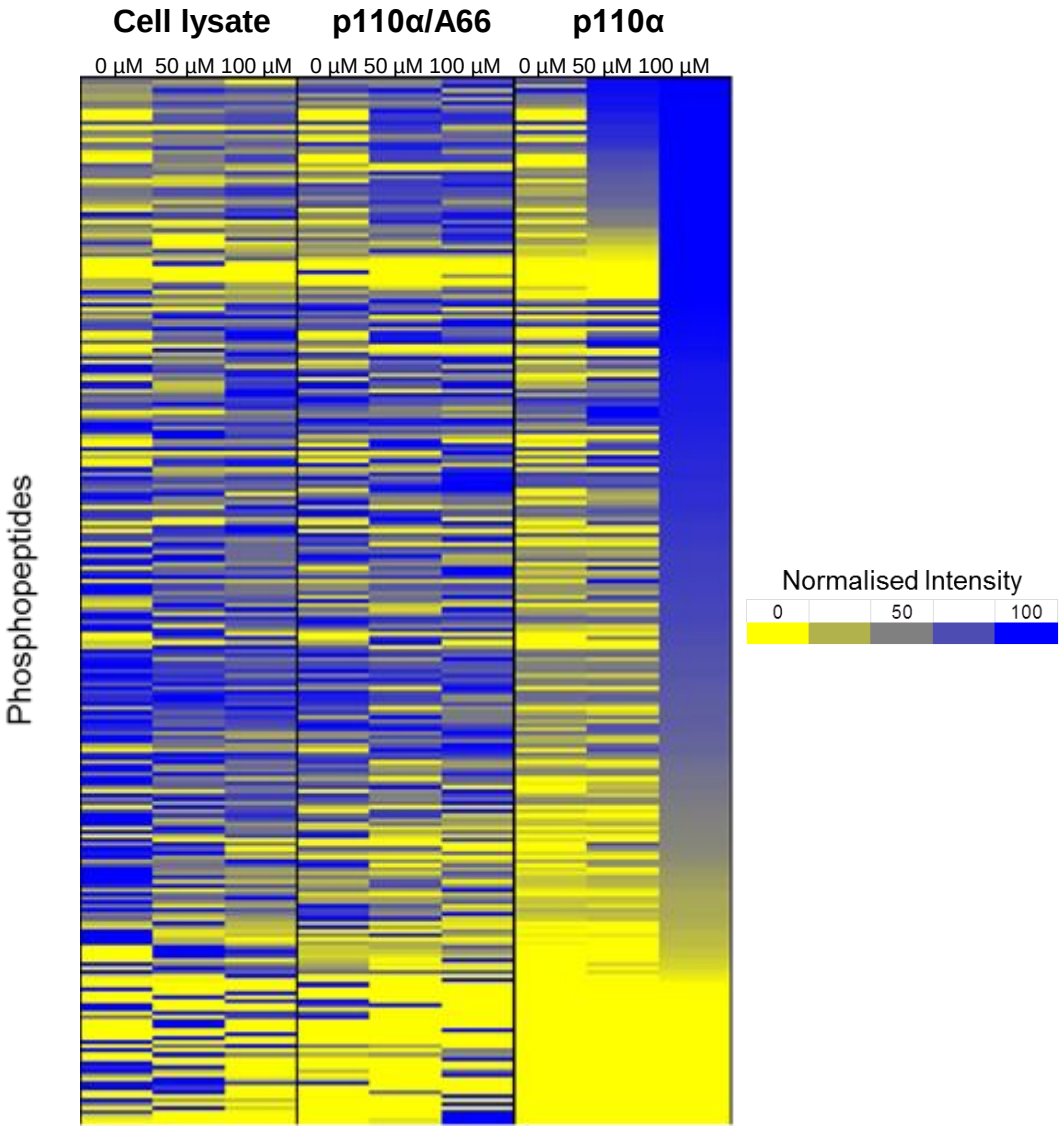


Figure 3.5. Quantitative analysis reveals phosphoproteomic response to an *in vitro* kinase assay using total cell lysate and recombinant p110α. MEF cell lysate was dephosphorylated using exogenous lambda phosphatase and incubated with 0, 50, 100 µM ATP, in the presence or absence of p110α and its specific inhibitor A66 for 5 min at 30°C. Phosphorylated proteins were subjected to phosphoproteomic analysis, and three independent experiments were carried out (n=3). Quantification of 254 phosphopeptides with a Pearson correlation >0.6 for the p110α-treated samples was visualised as a heat map, where intensity of phosphorylation for a given phosphopeptide was expressed relative to the maximum intensity for that phosphopeptide.

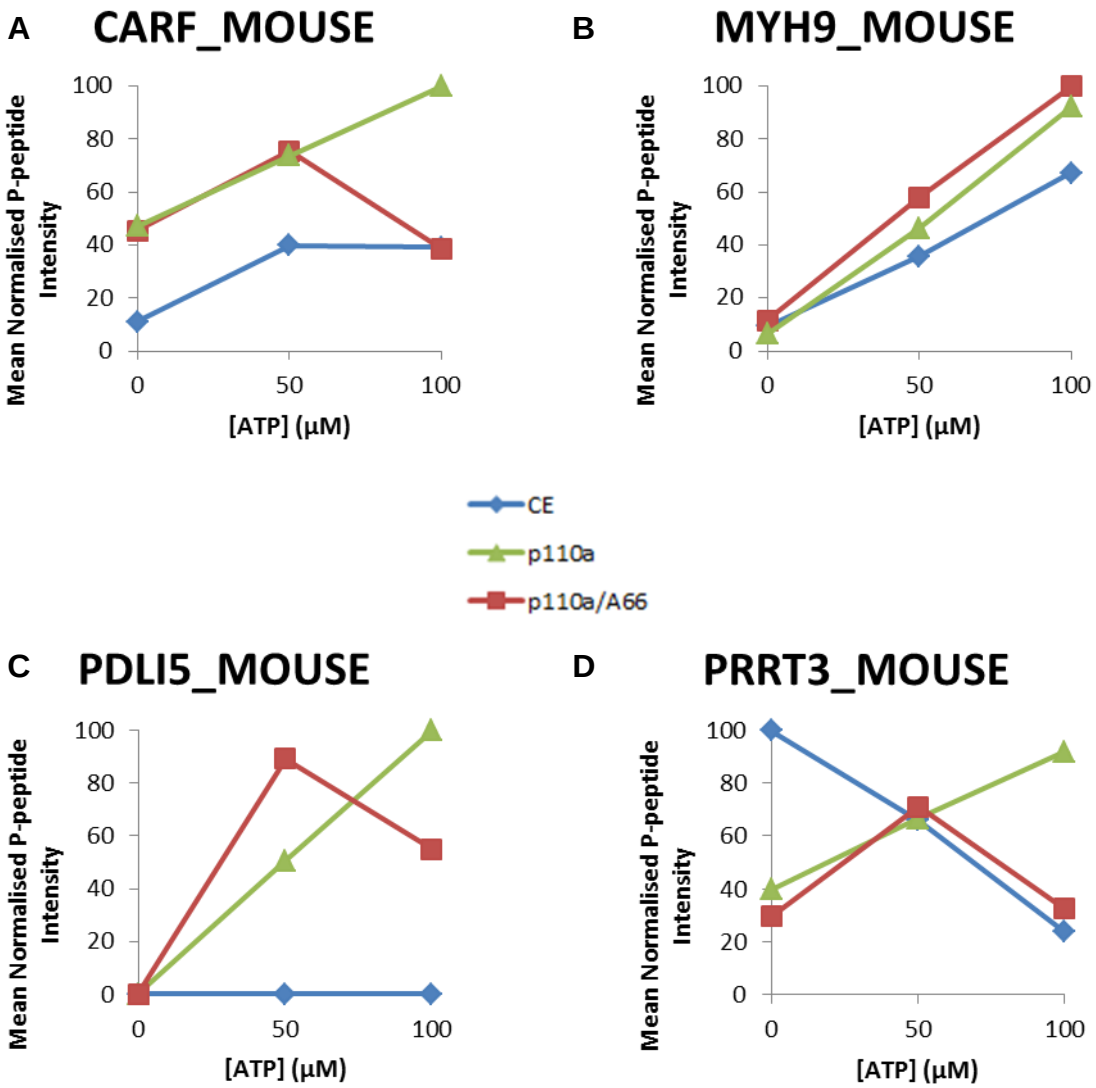


Figure 3.6. Examples of phosphorylation events identified in p110α *in vitro* kinase assay which display high background intensity in control samples. MEF cell lysate was dephosphorylated using exogenous lambda phosphatase and incubated with 0, 50, 100 µM ATP, in the presence or absence of p110α and its specific inhibitor A66 for 5 min at 30°C. Phosphorylated proteins were subjected to phosphoproteomic analysis. Mean (n=3) normalised phosphopeptide intensities for four candidate p110α substrates are shown. Many phosphorylation events which display an increase in intensity with an increase in ATP concentration for the p110α condition, such as **A-D**, are also observed to have high background phosphorylation in the control cell lysate and A66 samples.

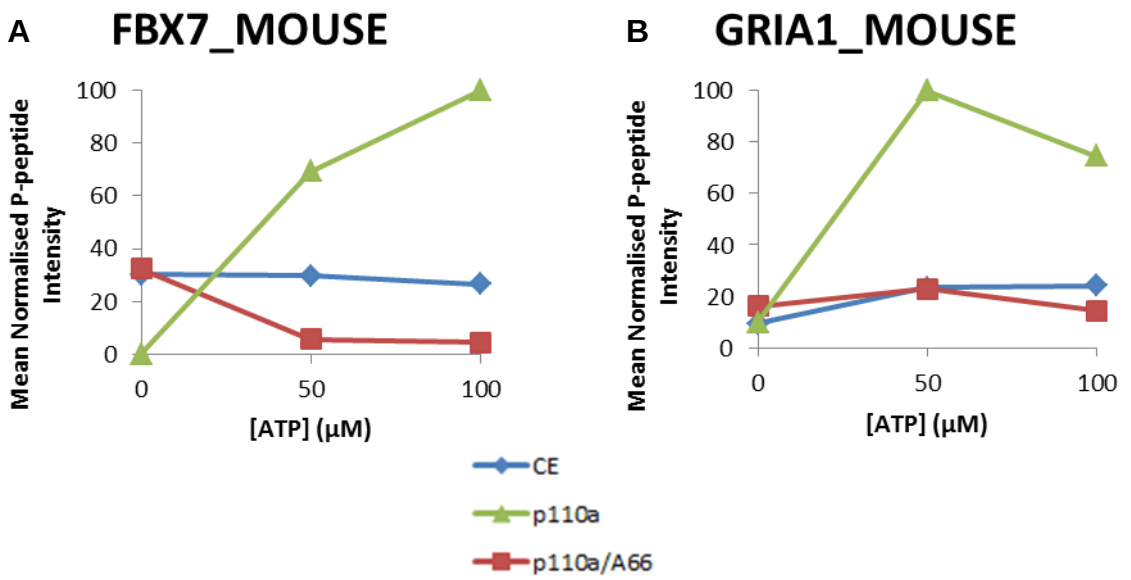


Figure 3.7. Candidate p110α substrates identified using an *in vitro* p110α protein kinase assay. MEF cell lysate was dephosphorylated using exogenous lambda phosphatase and incubated with 0, 50, 100 µM ATP, in the presence or absence of p110α and its specific inhibitor A66 for 5 min at 30°C. Phosphorylated proteins were subjected to phosphoproteomic analysis. Mean (n=3) normalised phosphopeptide intensities for two candidate p110α substrates, as defined by arbitrary criteria, are shown, **(A)** FBX7_MOUSE and **(B)** GRIA1_MOUSE.

3.3.3. Sequence analysis

It has been noted that the site of phosphorylation on previously described p110α protein substrates, including Ser608 of p85α, is often within an acidic primary protein structure. While the small number of characterised p110α substrates means that describing a definitive consensus motif is challenging, no striking similarities have been elucidated. Software which allows comparison of the primary protein structure around a phosphosite is available, however these are typically most useful when large numbers of sites are being compared, and so in this context we chose to do this comparison manually.

Sequence comparison between the two candidate proteins and previously described p110α substrates revealed no obvious similarities in terms of preference for a specific amino acid at a particular position relative to the phosphorylation site. However, three of the four phosphorylation sites (Ser294 of FBX7 and both sites for GRIA1) have an acidic amino acid within two residues, though none are in a region as acidic as Ser608 of p85α (Fig. 3.8.). These acidic residues are also conserved between mouse and human sequences for glutamate receptor 1.

The degree of overall sequence conservation around these residues should also be noted. Ser608 of p85α lies in a region of extremely high conservation, and all residues from 529 to the C-terminus are conserved between the mouse, human, rat and bovine sequences. For FBX7, Ser283 is conserved between the mouse, human and bovine sequences, and Ser294 between the mouse and rat sequences. Identical residues make up 33% of the amino acid sequence 271-300, with a further 33% displaying some conservation (Fig. 3.8.). There is a higher level of sequence homology in the C-terminal of GRIA1 between the mouse, human, rat and bovine proteins. Between residues 861-890, 90% of the amino acids are identical, including both candidate phosphorylation sites. Well-conserved sequences or phosphorylation sites are indicative of an important or conserved function, and it is more likely that functional phosphorylation events are conserved between species.

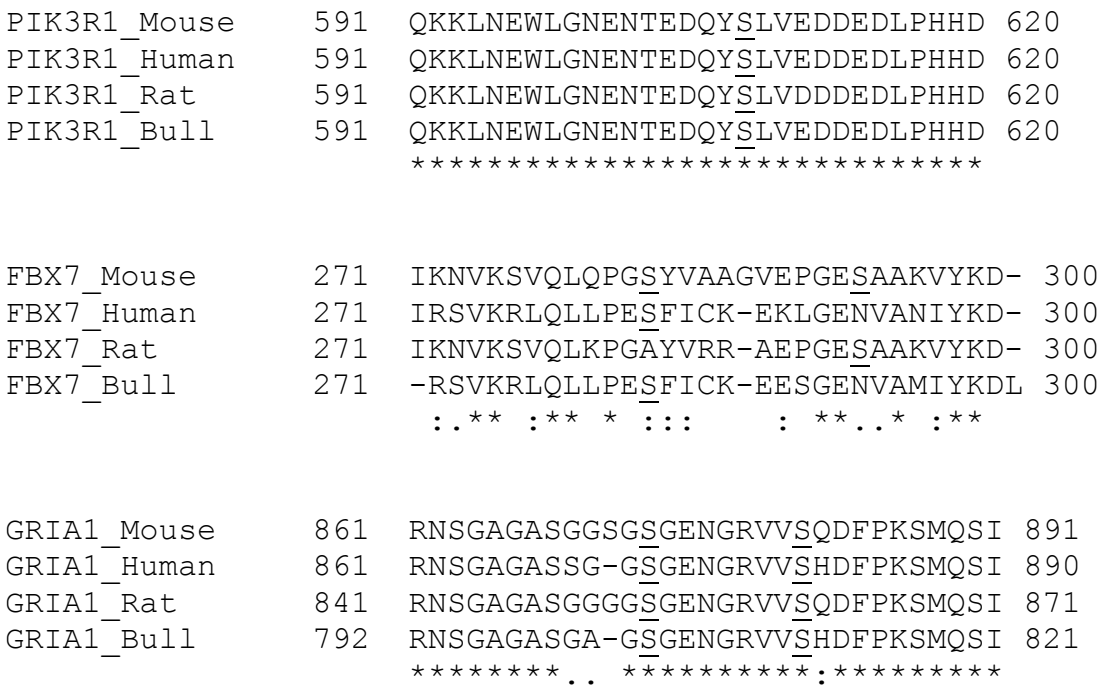


Figure 3.8. Sequence alignment of known and candidate p110α substrates. The amino acid sequences of previously described and candidate p110α substrates show varying degrees of conservation between species. An area of 30 amino acids around the phosphosites of interest from human and mouse sequences were analysed and annotated using the freely available ClustalW2 program. Key: Single letter amino acids code; "*"=identical; ":"=conserved substitution; "."=semi-conserved substitution. S indicates phosphosite.

3.3.4. Quantification of reaction kinetics

The *in vitro* p110α kinase assays for the MS screen were performed with three ATP concentrations, and therefore correlation of the ATP concentration and abundance of a given phosphorylation event can be used to quantify the affinity of the kinase for that particular phosphorylation site. Using the principles of Michaelis-Menten kinetics, it is possible to deduce dynamics of the kinase-substrate interaction in the same way as one would for other enzyme-substrate reactions. However, these experimental conditions may not follow those set out for a typical Michaelis-Menten reaction, for instance the substrates in the *in vitro* kinase assay may not be in excess over kinase. The experimental setup was one which allowed some approximation of reaction kinetics, but which was mindful of practical considerations such as time and cost. Therefore the number of ATP concentrations included is a compromise and does not allow for a perfect characterisation of kinetics, but gives a quantitative estimate.

In order to better characterise the phosphorylation events chosen for validation, we used the data for intensity of phosphorylation to generate quantitative data for reaction kinetics for each phosphosite to grade the potential substrates. In Michaelis-Menten kinetics, K_m is the substrate concentration at which the reaction rate is half of the maximum (Fig. 3.9.). We defined K_{ATP} (the p110α-ATP affinity for a given phosphorylation event) as the ATP concentration required to reach half the maximum rate of reaction (Fig. 3.9.), and used this to calculate K_{ATP} for both candidates identified as potential p110α substrates, based on our criteria (Fig. 3.7). These are shown in Table 3.2. The data suggest that the p110α-GRIA1 interaction has a higher affinity for ATP than the p110α-FBX7 interaction, however it is assumed that for each protein candidate, the two phosphorylation events have equal kinetics.

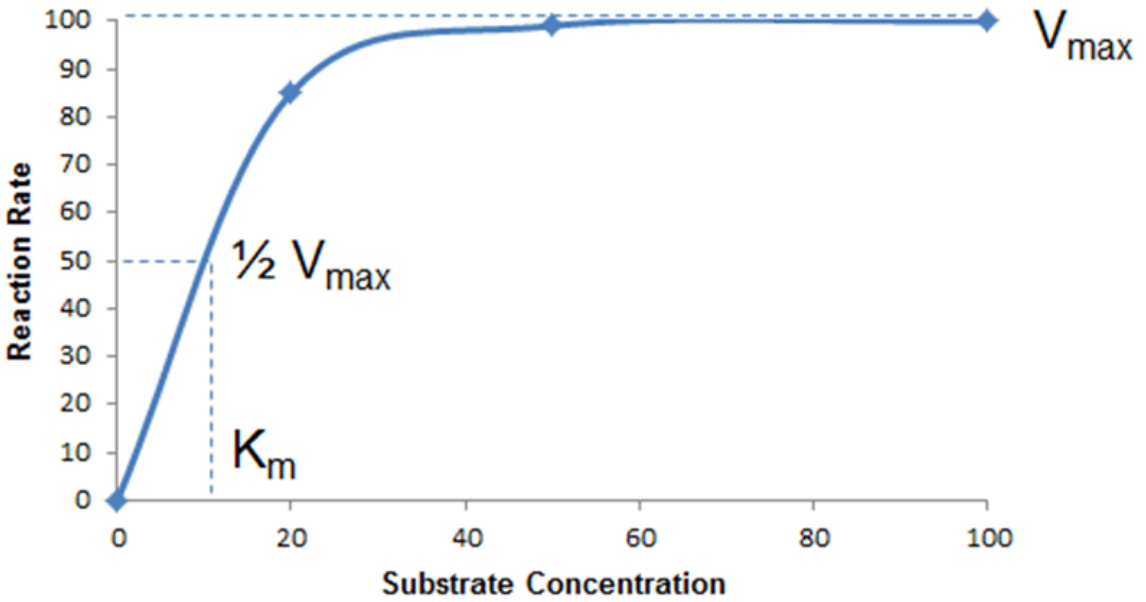


Figure 3.9. Principles of Km and estimation of K_{ATP}. The principles of Michaelis-Menten kinetics allow quantification of affinity of reaction (K_m) from knowledge of substrate concentration and maximum rate of reaction (V_{max}). Substitution of phosphopeptide intensity for reaction rate allows us to estimate affinity of a kinase-ATP interaction for a given substrate, hence K_{ATP} is an estimation of K_m.
Reaction rate = phosphopeptide intensity; $K_m = K_{ATP}$

Table 3.2. Affinity of phosphorylation for candidate p110α substrates

Protein	Phosphosite	K_{ATP} (μM)
FBX7_MOUSE	pSer283 + pSer294	36.07
GRIA1_MOUSE	pSer873 + pSer881	27.63

3.4. Validation of targets identified in phosphoproteomic screen

After selection of candidates which, based on our criteria, had the potential to be substrates for the protein kinase activity of p110α, we next moved on to attempt to validate these links. The easiest way to provide further evidence for a kinase-substrate relationship is to use *in vitro* kinase assays in which the association can be directly probed without possible contaminating factors (such as other kinases) which may be present in the original MS screen. Incubation of recombinant kinase and substrate with [γ-^{32}P]-ATP allows incorporation of the radiolabelled phosphate, which can then be visualised.

Neither candidate was commercially available as recombinant protein for use as a substrate in an *in vitro* kinase assay. Therefore, we decided to use as a substrate peptide fragments of each phosphosite and the amino acid sequence directly adjacent to it. Engineering of peptide fragments also allows the substitution of a non-phosphorylatable amino acid for the phosphorylated serine residue for use as a negative control. The peptides were designed so as to be able to bind P81 phosphocellulose paper, a requirement for one of our chosen methods of validation (see below). Therefore, basic lysine residues were incorporated into the N-termini of peptides to facilitate binding. The sequences of the peptide fragments are shown in Table 3.3.

We employed two different approaches for validation via *in vitro* kinase assays using the peptide fragments, both involving presentation of the substrate to the kinase in an appropriate buffer with [γ-^{32}P]-ATP. While the first method involves use of SDS-PAGE and subsequent detection of incorporated ^{32}P by autoradiography, phosphorylated peptide fragments must be immobilised onto P81 phosphocellulose paper prior to measurement of incorporated radioactivity for the latter [209]. This approach has not previously been shown to work for a peptide based on the sequence around Ser608 of p85α, but given the biochemical tools it was one of the few options open to us for our attempted validation.

First, we performed *in vitro* kinase assays using the peptides in Table 3.3 as substrates, and separated components of the assays by SDS-PAGE. Gels were

dried and incorporated ^{32}P was used as a measure of phosphorylation, and visualised by exposure to a PhosphoImager screen scanned on a Typhoon scanner. A challenge with this method is that the small molecular weight of the peptides used means that they migrate at a similar rate and to a similar position as the

Table 3.3. Sequences of peptides synthesised for validation of p110α *in vitro* kinase assay

Protein	Phosphosite	Sequence
p85α	pSer608	KKKTEDQY**S**LVEDD
p85α	pSer608	KKKTEDQY**A**LVEDD
FBX7_MOUSE	pSer283 + pSer294	KKQLQPG**S**YVAAGVEPGE**S**AAK
FBX7_MOUSE	pSer283 + pSer294	KKQLQPG**A**YVAAGVEPGE**S**AAK
FBX7_MOUSE	pSer283 + pSer294	KKQLQPG**S**YVAAGVEPGE**A**AAK
FBX7_MOUSE	pSer283 + pSer294	KKQLQPG**A**YVAAGVEPGE**A**AAK
GRIA1_MOUSE	pSer873 + pSer881	KKGSG**S**GENGRVV**S**QDFPK
GRIA1_MOUSE	pSer873 + pSer881	KKGSG**A**GENGRVV**S**QDFPK
GRIA1_MOUSE	pSer873 + pSer881	KKGSG**S**GENGRVV**A**QDFPK
GRIA1_MOUSE	pSer873 + pSer881	KKGSG**A**GENGRVV**A**QDFPK

unincorporated ^{32}P-ATP. This means that any signal from ^{32}P incorporated into peptides during the kinase assay may be masked by the free ^{32}P-ATP. Therefore, the unincorporated ^{32}P-ATP must be run off the edge of the SDS-PAGE gel, or else the very edge of the gel cut off, but in a way so as to leave the peptides from which to detect a signal.

Despite performing the assay three times and attempting to optimise the removal of the free ^{32}P-ATP, we were unable to do so in a way as to detect a signal suggesting phosphorylation of the peptide substrates over the background of free ^{32}P-ATP (data not shown). Therefore, we proceeded to attempt to validate the interaction by a different method. We performed the same *in vitro* kinase assays, but used phosphocellulose paper to immobilise the peptides after the reaction. Unbound ^{32}P-ATP was washed away, and incorporated ^{32}P again used as a measure of phosphorylation and quantified. The experiment was performed with four replicates, and the values were averaged and shown in Figure 3.10.

We included a peptide based on the sequence around Ser608 of p85α as a control, and observed an increase in incorporated ^{32}P-ATP (shown as cpm) relative to the ATP and kinase only control, which reduced to background levels when the p85α Ser608Ala mutant was used, indicating specificity of phosphorylation of that residue. However the absolute level of ^{32}P-ATP incorporation was low, relative to control. We again observed increased ^{32}P-ATP incorporation for FBX7 relative to control, with decreases when using either of the Ser283Ala or Ser294Ala mutants and a further decrease with the double mutant, though again the absolute changes relative to control were small. For GRIA1, the levels of ^{32}P-ATP incorporation for all four peptides was below the ATP and kinase only control. The values observed are lower than would be expected for a strong kinase with a valid substrate. Because of the low cpm seen even for the previously characterised Ser608 residue, it may be argued that either this assay or the peptides designed are not suitable for our goal of validation.

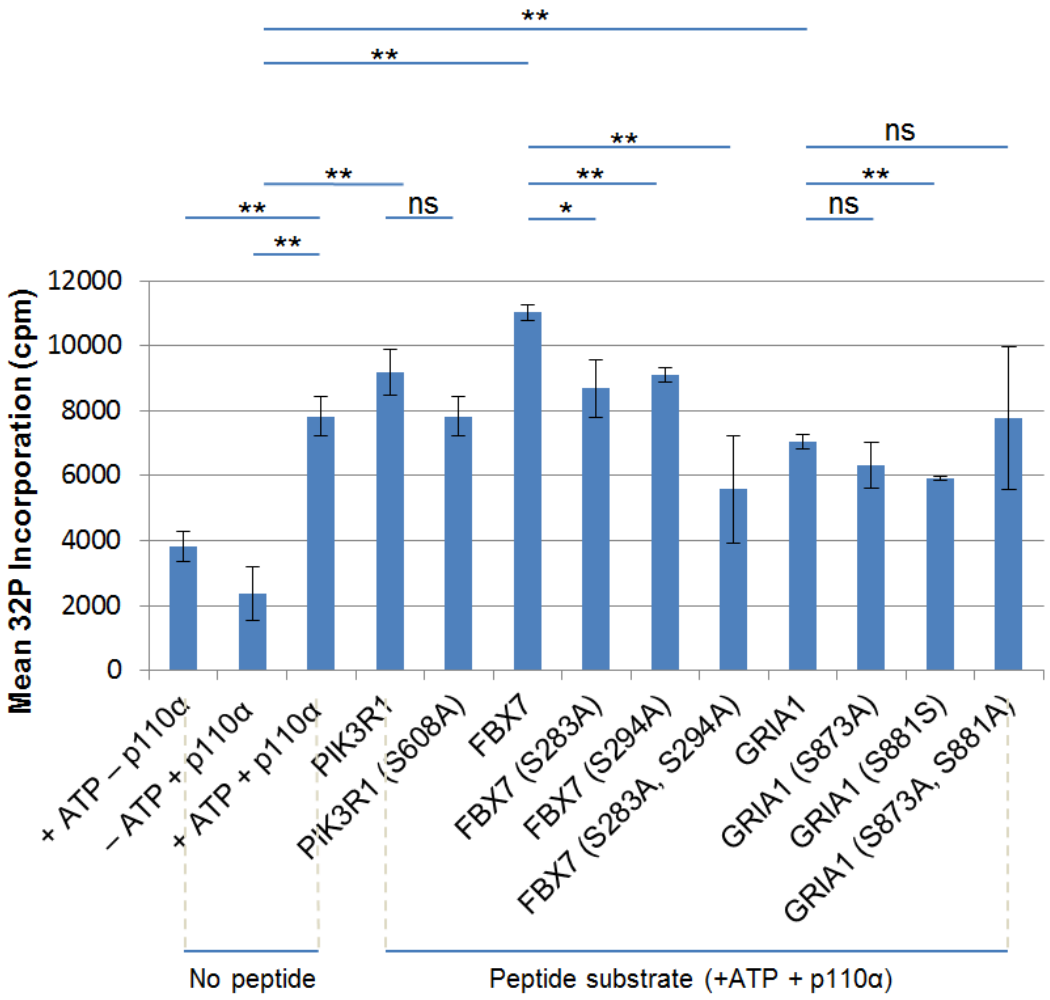


Figure 3.10. p110α is a weak protein kinase towards peptide substrates. An *in vitro* protein kinase assay was performed with p110α and peptide substrates as shown. Recombinant p110α was incubated with or without ATP and peptide substrates for 10 min at 30°C, peptides were bound to P81 phosphocellulose paper and washed, then incorporated radiolabeled phosphate was measured by scintillation counting. Four replicates were performed, and the mean count per minute are displayed.
*p<0.05 **p<0.005

3.5. Discussion

PI3K isoforms have been well-characterised as lipid kinases and exert control over many important cellular processes through this catalytic activity [1, 41]. PI3Ks also exhibit a protein kinase function which has been less comprehensively studied [23]. All class I PI3K isoforms have been described as being able to autophosphorylate and some non-PI3K substrates have been identified, although comprehensive analyses are lacking [23, 132, 133, 140].

p110α is the best characterised PI3K in terms of protein kinase activity, and it can phosphorylate the Ser608 residue of its regulatory partner p85α [23]. It has been reported that this phosphorylation event brings about a reduction in the lipid kinase activity, and so is a means of regulation [23]. The ubiquitously expressed p110α is central to a number of vital cellular processes and is the only PI3K isoform found mutated in cancer [1, 129].

We therefore decided to investigate PI3K protein kinase activity further, focusing on the p110α isoform. Using a quantitative phosphoproteomic approach, we employed a strategy to search in an unbiased and global manner for novel substrates of p110α protein kinase activity, which we then sought to validate candidate proteins by independent means.

3.5.1. Optimisation of the *in vitro* p110α protein kinase model

We used a method based on work from the Cohen laboratory (MRC Protein Phosphorylation and Ubiquitylation Unit, University of Dundee) [213] and further developed by the Cutillas laboratory (Integrative Cell Signalling and Proteomics, Barts Cancer Institute, Queen Mary University of London) [214], which has previously been used to examine the signalling pathways downstream of Akt. A total cell lysate is hereby used as the substrate for an *in vitro* kinase assay using a purified/recombinant protein kinase, and the resulting phosphopeptides are analysed using mass spectrometry. While there are relatively few data in the literature regarding the activation of PI3K protein kinase activity, some long-held beliefs have recently been challenged. For instance in terms of p110α, it was thought that phosphorylation of Ser608 of p85α brought about a reduction

in p110α lipid kinase activity [23, 126, 127], but a recent study found this not to be the case [217].

We optimised the conditions for our *in vitro* kinase assay by using an *in vitro* protein kinase assay in which radiolabelling of p85α was used as a readout of p110α protein kinase activity. Protein kinases require a metal ion cofactor to allow catalytic activity; often this is in the form of Mg^{2+}, but p110α has been reported to be inactive in the presence of Mg^{2+} and to instead require Mn^{2+} [127]. In terms of p110α activity as measured by phosphorylation of p85α we found there to be little difference between using either Mg^{2+} or Mn^{2+}, with robust phosphorylation observed for either cofactor. However use of both cofactors together brought about the highest level of p110α activity.

An important consideration is the length of time for the assay itself. Due to a whole cell lysate being used as a substrate for the kinase assay, there will inevitably be active endogenous protein kinases present, as well as protein phosphatases. These will both increase the background level of phosphorylation through reactions not specific to addition of exogenous p110α kinase and potentially reduce the signal. Therefore, a time point is required which allows robust p110α protein kinase activity while minimising this background contamination. We observed robust p85α phosphorylation at the shortest time point examined (1 min) which continued to increase with time, and so chose 5 minutes as the length of time for all future assays.

3.5.2. A phosphoproteomic screen to analyse p110α protein kinase activity

Following optimisation of conditions for p110α protein kinase activity, we used these specifications for an MS-coupled *in vitro* kinase assay. We used these conditions and presented recombinant p110α with a total cell lysate of mouse embryonic fibroblasts (MEFs) in the presence or absence of the p110α-specific inhibitor A66. We coupled the kinase assay to an enrichment process for phosphopeptides based on TiO_2 affinity purification and analysis using label-free quantification which allows the comparison of an unlimited number of samples [191, [211].

3.5.2.1. General characterisation of phosphoproteomic screen

We identified and quantified 641 phosphopeptides, and used heat maps and PCA to visualise the data. This approach suggested that there were only minor differences between the cell lysate only, p110α/A66, and p110α samples. While there were phosphopeptides which appeared to display an increase in intensity with increasing ATP concentration in the p110α condition, these were in the minority and large groups of such phosphopeptides were not obvious. It is also the case that large differences were not observed between the conditions when visualised by a heat map, and this may explain the lack of a clear separation when all 641 phosphopeptides were used as the input in a PCA.

Similar experiments in our laboratory using more well-characterised protein kinases such as Akt have yielded larger datasets comprising thousands of proteins [218]. The output from our screen was significantly smaller, with 641 phosphopeptides identified and quantified. This observation has three implications.

The first is that the small number of phosphopeptides identified and quantified in this screen is evidence that the dephosphorylation step of the methodology worked well. If this was not the case, a greater number of phosphopeptides would have been found across the samples. Due to the method employed, all material from the *in vitro* kinase assay was processed and analysed by LC-MS/MS, and so none was available to be used for analysis of relative levels of total phosphorylation by, for example, immunoblotting. However, there are arguments against this conclusion (discussed below).

Secondly, this suggests that the protein kinase activity of p110α is relatively limited in the context of this experiment. This may be considered to be in keeping with the literature, in that very few p110α protein substrates have been identified. A potential way around this could be to increase the amount of exogenous p110α in the assay, or to increase the length of time for which the assay was performed. While the earlier radiometric *in vitro* kinase assay controls showed good p110α activity in a simple reaction mixture, this is likely to be diluted due to the cell lysate in the MS-based assay. However making either or both of these changes would probably lead to more non-specific

phosphorylation events being produced, thus increasing the background through which to detect real substrates.

Finally, it is likely that the majority of phosphopeptides generated during the *in vitro* kinase assays and present in the samples were identified. The phenomenon of under-sampling is frequently a problem in MS studies which yield large (many thousands) of hits, due to the limited duty cycle of mass spectrometers. However due to the relatively small number of peptides identified in this study and the duty cycle of the Orbitrap, it is unlikely that identification of a large number of phosphopeptides was missed.

Another important point to note is the high level of background phosphorylation observed in the 0 µM ATP condition of the cell lysate. The substrate for the *in vitro* kinase assays is a dephosphorylated cell lysate, from which small molecules such as ATP have been removed. It is possible that either the steps involving dephosphorylation or exclusion of small molecules were incompletely performed, leading to background phosphorylation events observed. However, there is also variation across the conditions at the 0 µM ATP concentrations. This was also observed during previous experiments in our laboratory employing a similar method, with variability and background phosphorylation seen [218]. It may be the case that removal of ATP from the cell lysate has not been completely achieved, and so it would be of interest to include extra steps in future experiments to make sure that this aim is met.

We included an extra control in our experimental design, and used kinase with its specific inhibitor. This was so that candidates taken for validation would be as robust as possible. However it may be the case that this extra control means that comparisons of intensity of phosphorylation across samples in the search for true novel substrates are confounded due to the variability present in the system. It may be of benefit to perform additional similar experiments of a different, perhaps more simple, design and compare these datasets to identify robust candidates suitable for validation. For instance, changing the amount of recombinant kinase instead of ATP concentration as done alongside the Akt *in vitro* kinase assay discussed above [218], or using as a substrate cell lysate, the proteins of which have been enzymatically digested, as described by Xue *et*

al. [219]. The latter approach should have the added benefit of also reducing background as kinases present in the sample would be digested and hence inactive. These strategies would however increase time, cost and complexity, and in the latter case peptides may not be as good a substrate as full length proteins.

We note that previously identified p110α substrates, including Ser608 of p85α, were not identified in our MS screen. Because of the incomplete characterisation of these substrates in the literature, it is not known which conditions produce robust phosphorylation, for example cell stimulation, other priming phosphorylation events, or binding to an adaptor. Therefore we do not know whether or not the conditions required to achieve these specific (or indeed wider) phosphorylation events were replicated in this assay. Pilot experiments were carried out using a similar experimental design, and phosphorylation of Ser608 was observed, though not across all experiments. This suggests that the assay is capable of bringing about phosphorylation and identification of Ser608 and in theory other valid substrates, though not with a 100% success rate.

It is not a trivial task to design an experiment which assays the endogenous protein kinase activity of p110α while excluding the effects of its lipid kinase activity. Were we to analyse the protein kinase activity in intact cells with stimulation using, for instance, insulin, this stimulation would also activate signalling cascades downstream of the lipid kinase arm, and separating phosphorylation events due to the two activities would be impossible. However the use of conditions which optimally induce p110α protein kinase *in vitro* and *in vivo*, would of course be the ideal scenario.

Work in other laboratories has reported the creation of p110γ mutants in which the lipid and protein kinase activities have been separated. Through the swapping of binding domains, Wymann *et al*. [134] produced proteins which were deficient for lipid kinase activity, but with protein kinase activity intact. It would in theory be possible to introduce these mutants (or similar versions for p110α) into cells in which the endogenous protein has been knocked out, and attempt to identify novel substrates in this way. However issues exist around the

ability of these mutants to retain some capacity for lipid phosphorylation, though it may be reduced or directed towards a different substrate. These mutants have the potential to be a useful tool in the context of our experiments, but it would be challenging to assign phosphorylation events directly to p110α, or even to conclusively separate them from the lipid kinase activity.

3.5.2.2. Selection of candidate phosphorylation events for validation as p110α substrates

It was necessary to define a set of arbitrary criteria in order to narrow the hundreds of phosphorylation events identified and quantified in our screen down to likely candidates for true p110α substrates. The inclusion of multiple ATP concentrations for each condition allows a basic understanding of the reaction kinetics to be inferred. Therefore we used this to set one criterion as a correlation of intensity of phosphorylation with ATP concentration in the p110α condition, and set a Pearson correlation of >0.6. 254 phosphopeptides exceeded this threshold, however many had background phosphorylation in one or both of the control (cell lysate or p110α/A66) conditions. For that reason, we used a second criterion which was a requirement of at least a two-fold increase in intensity of phosphorylation from both control 50 µM and 100 µM ATP conditions to the same concentrations in the p110α condition. When both criteria were applied, two phosphopeptides remained, each with two phosphorylation events identified, none of which had been previously identified: FBX7 (Ser283, Ser294) and GRIA1 (Ser873, Ser881). It is interesting to note that peptides with two phosphorylation events have been identified for both proteins. In this context, it is not possible to thus far separate the characterisation of the two phosphorylation events, especially in the absence of previously described features.

FBX7 codes for the substrate recognition element of the ubiquitin ligase machinery, which targets proteins for proteasomal degredation by tagging them with ubiquitin. GRIA1 codes for the protein glutamate receptor 1, a serpentine receptor located at the plasma membrane. The identified phosphorylation sites in GRIA1 lie within the cytoplasmic portion of the protein, meaning that p110α could access them.

Because none of these phosphorylation events have previously been characterised, it is not possible to speculate in great depth over the effect of the potential p110α action. Both proteins function as part of signalling cascades: Phosphorylation of receptor-associated proteins by p110α has been described with IRS1, and it may be the case that p110α acts in a similar manner towards GRIA1.

It is important to note the variety of phosphorylation profiles observed for the events which we do not assign as being p110α-dependent. Phosphorylation events in the dataset with high or low intensity exist for all combinations of conditions (e.g. low in cell lysate, high in p110α/A66, low in p110α; high in cell lysate, low in p110α/A66, low in p110α etc). Given that each ATP concentration for each condition had as its starting material the same cell lysate, this variability is interesting especially when considering control and 0 µM ATP samples, though perhaps not entirely unexpected. As discussed previously, experiments within our laboratory using a similar design with Akt also saw variability between replicates and conditions [218]. While the protein substrates and kinases present in each reaction condition should be largely the same, it is not true that the same reactions will occur or products be formed; this would be expected in closed system, but variability is anticipated in a biological context. It is clear that this complex mixture of substrates and products makes analysis more challenging.

While optimising conditions, radiometric *in vitro* kinase assays using phosphorylation of p85α as a readout of p110α protein kinase activity showed activity to be high in a simple reaction mixture (i.e. recombinant p110α/p85α only; in the absence of cell lysate). However due to the unavoidable dilution effect of generating a cell lysate this activity is likely to be diluted somewhat in the *in vitro* kinase assays used prior to the MS screens, and it could be argued that there is a suggestion implicit in the literature that having few characterised substrates of p110α protein kinase activity means that it is a weak kinase.

Additionally, it is certainly true that the same residue of a particular protein may be targeted for phosphorylation by more than one kinase, and it is realistic that any p110α substrates have additional kinases that phosphorylate them. If it is

accepted that in such a complex reaction mixture the products (phosphoproteins) will not be identical across the conditions, then this could therefore lead to any true substrates of p110α being masked by their phosphorylation by more active kinases still present in the total cell lysate substrate, even with the amount of exogenous p110α used.

3.5.2.3. Biochemical characteristics of candidate phosphorylation events

For many well-characterised protein kinases, there exist consensus motifs which are present in their substrates at the primary sequence level, and recognised by the kinase which then phosphorylates the target [220]. For example, Akt phosphorylates residues within the RxRxxpS/pT motif, though not all substrates conform to this sequence [54]. Had a larger number of phosphorylation events been found to be potential p110α substrates, it would have been informative to use bio-informatic analysis tools such as motif-x to interrogate these sequences in p110α substrates in search of sequence similarities around the phosphosite pointing to a conserved motif. This approach works best with a larger dataset, and as we only took two substrates for validation, we decided to compare the sequences around the candidate phosphorylation events to the sequences of previously described p110α substrates manually, as well as examining conservation across species. Even this approach can be informative; a measure of how little work has been done regarding the protein kinase activity of p110α is the limited characterisation of its substrates, even those discovered decades ago. This includes IRS1, where the phosphosite targeted by p110α has not yet been mapped.

We first looked at the degree of conservation around the Ser608 residue of p85α between mouse and human sequences, and found 100% conservation between amino acids 591-620. Of the two phosphorylation sites for each protein, FBX7 has Ser283 conserved between mouse and human proteins, while GRIA1 has both candidate phosphosites conserved. We then analysed the wider sequences of our candidates and found 33% conservation between mouse, human, rat and bovine sequences in the residues around the phosphosites of FBX7, and 90% conservation for GRIA1. This degree of

sequence conservation for GRIA1 is evidence for a conserved function for that area of the protein, one which may include one or both phosphorylation events.

The MS assay was designed to include different concentrations of ATP for each condition (cell lysate, p110α/A66, p110α) so as to be able provide quantitative data on reaction kinetics. These data can then be used to infer qualitative information for the kinase-substrate pairing, including its validity and strength of interaction compared to other previously characterised pairings or others found in the MS screen. A shortcoming of this approach applied to our experiments is the limited number of ATP concentrations that we have used: while it is preferable to have as many as possible over as large a range as possible, practical considerations such as cost and time need to be taken into account. We therefore decided to use three separate ATP concentrations for each condition, which allowed us to reasonably consider a phosphorylation event in terms of the relationship between the control and p110α conditions, but could only give an approximation of reaction kinetics.

Data on reaction kinetics can provide valuable information on the strength of an enzyme-substrate pairing, which can be used to infer physiological relevance. The principles of Michaelis-Menten kinetics can be applied to a kinase-substrate interaction to produce the quantitative data mentioned above. It is important to note that these principles were set out for an idealised interaction, and not all of the clauses will be met. For instance, the substrate concentrations may not be saturating, and the curves of phosphorylation intensity may not show a plateau were more ATP concentrations to be included. Additionally, an assumption was made that the two phosphorylation events on each protein had equal kinetics which is unlikely to be the case in reality. We redefined the components of the Michaelis-Menten model so that K_m becomes an estimation of the affinity of a substrate for ATP (K_{ATP}). Using these approximations, we found that the affinity for ATP was higher for the p110α-GRIA1 interaction (K_{ATP} = 27.63 μM) than for the p110α-FBX7 interaction (K_{ATP} = 36.07 μM). Affinity in these terms has not been defined for previously characterised p110α substrates, and so it is not possible to make a direct comparison here. However, this approach has been used in our laboratory previously in an attempt to identify novel Akt substrates. This work resulted in identification of phosphorylation events which could be

grouped; events with relatively high affinity for ATP included those with K_{ATP} of lower than 10 µM, those with relatively low affinity for ATP had K_{ATP} calculated to be above 10 µM. This comparison gave further credence to the suggestion that the protein kinase activity of p110α is more limited than better characterised kinases. In combination with the sequence analysis of the two candidate proteins, it could be argued that GRIA1 is the stronger candidate, as its sequence homology and affinity of interaction are higher than for FBX7.

That we observed the effect of the protein kinase activity of p110α as being relatively small in this experimental context is perhaps unsurprising for a number of reasons. Firstly, given the small number of described substrates previously published in the literature, it could be argued that a large physiological role for the p110α protein kinase activity is unlikely to have been hitherto overlooked. It is disappointing to note the absence of previously described substrates of p110α in the MS screen, though the phosphorylation of Ser608 on p85α was observed in pilot experiments. This may be due to the experimental setup, specifically that a dephosphorylation step removed any previously present phosphorylations from the lysate to produce as low a background as possible, which was followed by an *in vitro* model with an absence of any cell stimulation/p110α activation. Therefore, it may be that the protein kinase activity of p110α is dependent on the correct but unknown context of stimuli, scaffold, or membrane, which was not reproduced faithfully enough in these experiments.

3.5.3. Validation of targets identified in p110α *in vitro* kinase phosphoproteomic screen

The most straightforward method for validating a kinase-substrate interaction is by carrying out *in vitro* kinase assays, presenting the recombinant protein substrate to the kinase in a simple reaction mixture including ATP and a suitable buffer. Inclusion of ATP labelled with a radioisotope of phosphorus allows incorporation of ATP to be visualised, following separation of reaction components by SDS-PAGE and exposure of the resultant gel to X-ray film.

We wished to validate both candidate interactions by this method, but due to the relatively poor level of characterisation of both proteins and all phosphorylation events, the desired biochemical tools were not available, and neither was commercially available as a full length recombinant protein. We therefore decided to produce peptides which included the phosphosites of interest, as well as a number of amino acids on either side. Protein kinases generally use only a small number of amino acids directly adjacent to a phosphorylation site to aid recognition, so we reasoned that these short peptide fragments would be suitable for *in vitro* kinase assays. A variation on the method outlined above involves the immobilisation of the peptide onto phosphocellulose paper following the kinase reaction, and quantification of the radiolabelled ATP incorporated into the peptide [209]. Binding of the peptide to the phosphocellulose paper is aided by the addition of basic amino acids to a peptide terminus, and so we chose to add lysine residues to each peptide we produced. All peptides which we had synthesised were predicted by freely available online bio-informatic tools to be water soluble.

As well as using peptides which replicate the protein sequence around the phosphosites of interest, we decided to also test peptides with serine-to-alanine substitutions for each phosphosite, as well as dual substitutions for each candidate protein. In this way it is possible to glean more qualitative and quantitative data, and have more confidence in a true kinase-substrate interaction, as well as separating the characterisation of the individual phosphorylation events.

We first attempted to use an *in vitro* kinase assay followed by 1D SDS-PAGE to validate the candidate substrates. Due to the small size of the peptides (~2kDa), we anticipated that they would migrate to a similar position on a gel as the unincorporated radiolabeled ATP. This would mean that upon exposure to X-ray film, any free radiolabelled ATP would be indistinguishable from bands due to radiolabelled ATP incorporated into the peptides during the kinase assay. Therefore we endeavoured to overcome this by using Tricine gels to enable maximum separation of low molecular weight substances. Unfortunately, we were unable to achieve sufficient resolution of peptides and free radiolabelled ATP to counter this problem, and our multiple attempts resulted in

visualisation of a large area of exposure, with free radiolabeled ATP masking any possible signal from incorporated ATP.

We then proceeded to validate *in vitro* phosphorylation of peptides using an alternative method. We again performed *in vitro* kinase assays, followed by peptide immobilisation onto phosphocellulose paper, a process aided by the incorporation of basic lysine residues in the peptides, and scintillation counting [209]. These experiments revealed only low incorporation of radiolabelled ATP into any of the peptides, including the p85α sequence which included Ser608, one of our controls. The overall increase from the kinase+ATP control to the non-mutated peptides was small, however comparing each peptide (p85α, FBX7, and GRIA1) with their single and double serine-to-alanine mutants, there was a trend towards a decrease in incorporation of radiolabelled ATP.

We included peptides with the sequence around the Ser608 residue of p85α as controls, hoping to see radiolabelled ATP incorporate in the non-mutated which was not observed in the serine-alanine mutant. This trend was observed, but with very small differences, and with counts which were only slightly higher than the kinase+ATP control. The phosphorylation of Ser608 by p110α has been observed by us while using similar conditions (Fig. 3.2) and so we would expect that phosphorylation could be achieved in this assay. That we did not detect robust incorporation of radiolabelled ATP suggests either that the partial p85α peptide is not a good *in vitro* substrate for p110α and so incorporation does not occur, or that the p85α peptide has not bound well to the phosphocellulose paper and so incorporation occurs but is not observed. While designing the peptides for this assay, we included basic lysine residues so that the required immobilisation of the peptide onto phosphocellulose paper would occur, and used freely available prediction tools to assess pH and thereby inform sequence design. In using partial peptides rather than full length recombinant proteins there is a risk that the substrate will not optimally interact with the kinase because of some missing secondary or tertiary structure. These factors would suggest that it is likely that the peptides did indeed bind the phosphocellulose paper, but that they were not a suitable substrate for the kinase. Another possibility is that in this assay, the phosphorylation of full length recombinant p85α by p110α which we have previously shown to be strong (Fig. 3.2) and

residual binding to the phosphocellulose paper is sufficient to mask the weak phosphorylation of the candidate peptides.

The lack of high levels of incorporation of radiolabelled ATP in the p85α Ser608 peptide that served as a positive control of p110α protein kinase activity, suggests the need for an alternative positive control. It is possible that the protein kinase of p110α only occurs when the full-length p85α substrate is in close proximity with p110α by virtue of forming a heterodimeric protein complex with high affinity between p110α and p85α. While the protein kinase activity of p110α has not been well-characterised, it would be possible to use a better-studied kinase such as Akt or S6 kinase II which have been shown to phosphorylate synthetic peptides (Aktide and kemptide respectively) [221, 222]. Inclusion of such a control would at least allow verification of the assay as a whole, but the activity of such a robust protein kinase may not be directly comparable to the activity of the weaker p110α protein kinase.

4. Investigation into differential p110α and p110β signalling in the mouse 4T1 breast cancer cell line model

4.1. Introduction and aim of study

Of the four class I PI3K isoforms, only p110α and p110β are ubiquitously expressed. PI3Ks control many important cellular functions, and p110α and p110β share a number of outputs including in the regulation of cell survival, proliferation and migration, but also have differential functions as detailed below. We therefore explored potential difference in global protein kinase pathways downstream of these PI3K isoforms.

Activation of class I PI3Ks is through recruitment to activated receptors and small GTPases. Traditionally, class IA PI3Ks were thought to be downstream of RTKs, and the class IB p110γ downstream of GPCRs, with all four class I PI3Ks responsive to input from Ras or other small GTPases. While p110α has RTKs and Ras as upstream activators, the relative contribution of agonists appears to be more complex for p110β. RTKs, GPCRs and small GTPases other than Ras (i.e. Rac and cdc42) are now known to all activate p110β.

Binding of the class I PI3K homodimer to membrane-bound receptors brings the complex to the plasma membrane, where the p110 subunit catalyses the production of PIP_3 from $PI(4,5)P_2$. This 3-phosphoinositide lipid is recognised by a subset of proteins through their lipid-binding domains, including Akt. These proteins may also be kinases, act as molecular scaffolds, or transduce the signal in some other way. This binding event causes translocation to the plasma membrane where signalling cascades can be initiated or affected. Akt is the best characterised PI3K effector, but there are other proteins which have been shown to have a growing importance such as the related AGC kinase, SGK.

Differences in both upstream activation of and downstream signalling from p110α and p110β are becoming increasingly apparent. The two PI3K isoforms control different biological outputs: p110α but not p110β regulates developmental and tumour angiogenesis and insulin signalling/glucose metabolism [144, 145]. Although p110α is the only PI3K found mutated in

cancer, there is a growing body of evidence that non-mutated p110β plays a role in PTEN-null cancers such as prostate cancer.

Unpublished work from our laboratory by Dr Salma Taboubi has shown that inhibition of p110α or p110β in the murine BALB/c 4T1 breast tumour cell line results in differential effects on a number of phenotypes. The 4T1 cell line expresses wild-type *PIK3CA* and *PTEN*, and is deficient for *TP53*. Dr Taboubi found that *in vitro* proliferation and invasion, as well as levels of *in vivo* metastasis were *enhanced* upon inhibition of p110β but unaltered following inhibition of p110α. The initial attempts of Dr Taboubi to characterise the signalling of these cells by western blotting in terms of levels of phosphorylation of Akt and its downstream targets found no obvious causes for the phenotypic outputs. The specific inhibitors A66 and TGX211 were used to inhibit the p110α and p110β isoforms respectively [167, 148]. While both isoforms are ubiquitously expressed and signal through similar effectors such as Akt, they control different processes (see Chapter 1.1.6.). Although Akt is likely the main effector of the two isoforms and certainly the best characterised, the specific signalling pathways for the differential phenotypes have not been fully elucidated and were what we aimed to characterise. While the efficacy and specificity of our inhibitors for the PI3K isoforms under investigation has been determined previously in *in vitro* assays [167, 148], the possibility exists that off-target effects on other kinases may occur, and so work to validate findings is key. The 4T1 cell line is frequently used in our laboratory in other experiments [153] and was therefore an interesting model system to further characterise, in terms of the specific phenotypes observed but also more generally.

We decided to characterise biological differences by probing the phosphoproteome upon selective inactivation of p110α or p110β. The aim of the work presented in this chapter was to compare phosphorylation events downstream of p110α and p110β in 4T1 cells in a global and unbiased manner, using label-free quantitative mass spectrometry. The experimental design (Fig. 4.1) replicates the conditions under which Dr Taboubi observed differential phenotypes following inactivation of p110α and p110β.

4.2. Phosphoproteomic investigation of signalling downstream of p110α and p110β

Exponentially growing 4T1 cells were starved for 18 h in media which lacked serum, followed by pre-treatment with DMSO or drug as indicated for 2 h, also in media without serum. Cells were then left unstimulated (subsequently referred to as “Starved”), or stimulated with 0.5% serum which contained either DMSO (“DMSO”), the p110α-specific inhibitor A66 (“A66”) or the p110β-specific inhibitor TGX221 (“TGX221”), at 3 μM and 100 nM respectively (Fig. 4.1). Three biological replicates were performed with two technical replicates analysed for each condition.

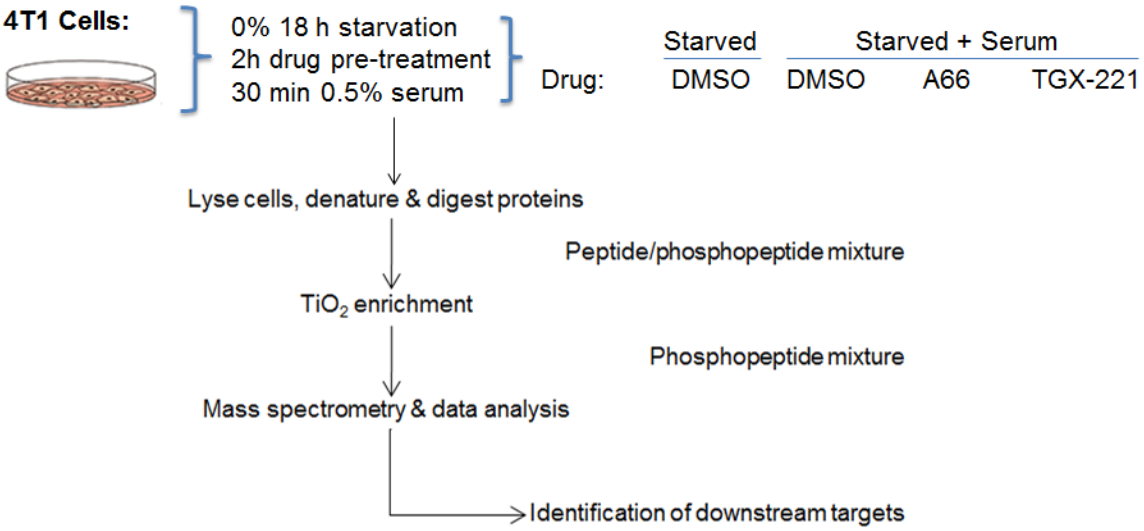


Figure 4.1. Summary of phosphoproteomic approach. 4T1 cells were starved for 18 h in 0% serum, followed by a 2 h incubation with or without inhibitor. Cells were then left untreated or stimulated with 0.5% serum with or without inhibitor for 30 min, and subjected to phosphoproteomic analysis. Samples were lysed, digested using trypsin, and phosphopeptides enriched by titanium dioxide affinity then analysed by LC-MS/MS. Data analysis was carried out using in-house and freely available programs. Three biological replicates were performed for each condition, each of which was analysed in duplicate (n=6).

Cell lysates were harvested and analysed using methodology published previously by the Cutillas laboratory [191]. Briefly, 500 μg of total protein was isolated and denatured, reduced and alkylated. Proteins were then subjected to overnight trypsin digestion, phosphopeptides were enriched by TiO_2 affinity

chromatography, after which they were analysed by LC-MS/MS using an Orbitrap-XL and identified and quantified by in-house techniques.

The data from the MS analysis of the samples were used to interrogate the MASCOT protein database, and peptides which were returned with an expectation value of <0.05 were chosen for further analysis. The in-house program Pescal (developed by Dr Cutillas) was then used to quantify this database of 2121 peptides across all 24 samples (Appendix 2).

Quantification of intensity of phosphorylation across the samples was visualised in the form of a heat map (Fig. 4.2). The data show groups of phosphopeptides which are positively or negatively regulated upon inhibition of one or both PI3K isoforms.

Interestingly, whereas almost half of the phosphopeptides increased in intensity upon serum-stimulation relative to the starved control, a slightly larger number of phosphopeptides showed the reverse pattern, with phosphorylation being higher in starved cells.

Of interest, a number of phosphorylation events which have previously been shown to be downstream of class I PI3Ks are found in the dataset, giving us confidence in the output as a whole. These include phosphorylations on AKTS1 (PRAS40; pThr257) and RS6 (40S ribosomal S6; pSer236). Interestingly, these phosphopeptides display different profiles in terms of relative change of intensity upon inhibition of p110α or p110β. Indeed, intensity of AKTS1-Thr257 is reduced to 13% of control following inhibition of p110α and 79% of control following p110β inhibition, and intensity of RS6-Ser236 reduced to 43% of control after inhibition of p110α, and 118% of control after inhibition of p110β (discussed in more detail later).

This analysis showed that although there are clear differences in the way that individual phosphopeptides change, the inclusion of all peptides from the dataset masks these variables and so these differences are not sufficiently large to enable the conditions to separate out.

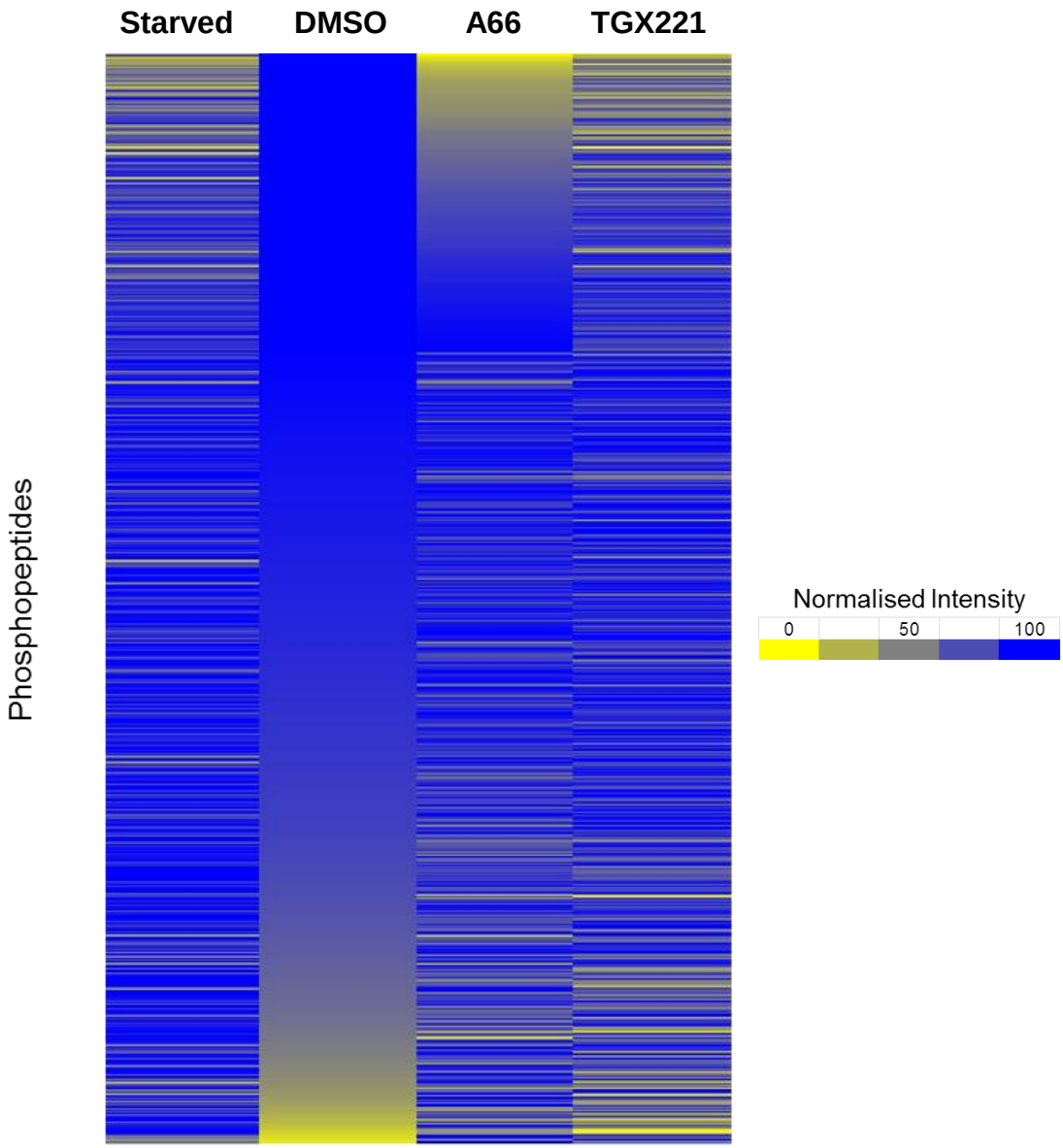


Figure 4.2. Quantitative analysis reveals phosphoproteomic response to an *in vitro* kinase assay. 4T1 cells were starved for 18 h in 0% serum, followed by a 2 h incubation with or without inhibitor. Cells were then left untreated or stimulated with 0.5% serum with or without inhibitor for 30 min, and subjected to phosphoproteomic analysis (n=6). Quantification of 2121 phosphopeptides identified was visualised as a heat map, where intensity of phosphorylation for a given phosphopeptide was expressed relative to the maximum intensity for that phosphopeptide.

To assess the differences between the conditions on a large scale, we performed principle component analysis (PCA) on the entire dataset (Fig. 4.3A).

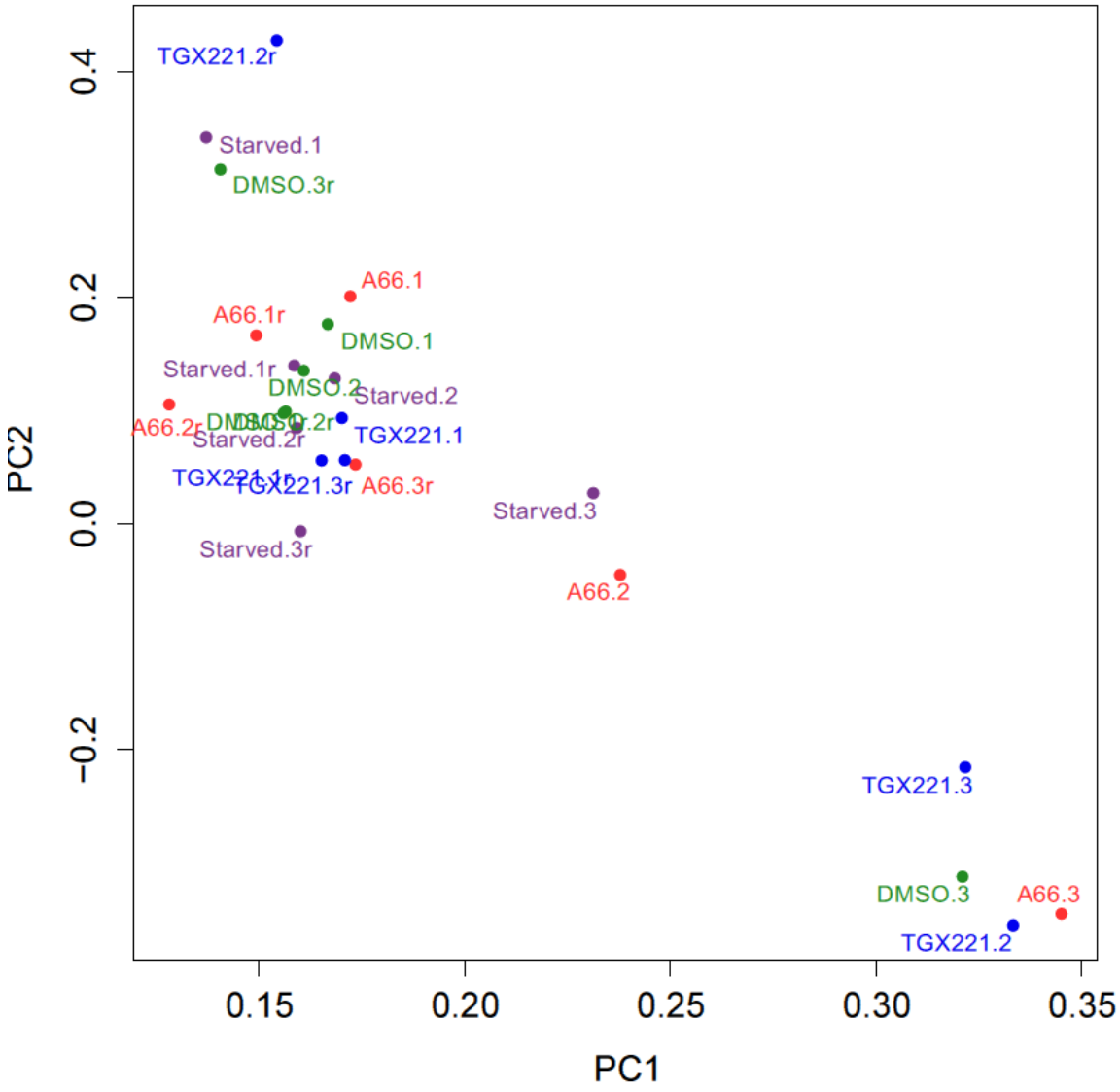


Figure 4.3. PCA shows similar profiles following PI3K isoform inhibition. 4T1 cells were starved for 18 h in 0% serum, followed by a 2 h incubation with or without inhibitor. Cells were then left untreated or stimulated with 0.5% serum with or without inhibitor for 30 min, and subjected to phosphoproteomic analysis (n=6). PCA was performed using all identified and quantified phosphopeptides. Purple = starved; green = DMSO; red = A66, blue = TGX221.

The aim of the experiment was to compare the phosphorylation events downstream of p110α and p110β, hence sites of phosphorylation which are either mutually or differentially regulated were of specific interest to us. These include phosphorylation events which either increased or decreased upon PI3K isoform-inhibition. Those which decrease upon PI3K isoform-inhibition are likely to be under direct control of effector protein kinases downstream of either p110α or p110β, and those which increase upon PI3K isoform-inhibition may be part of a feedback loop. We decided to focus our analysis mainly on phosphorylation events which are negatively regulated upon PI3K isoform-inhibition, as these are more likely to be amenable to future investigation.

A summary of the data is shown in Table 4.1. Inhibition of either p110α or p110β elicits a response of a similar magnitude, with around 10% of phosphorylation events in the dataset reduced by more than twofold, and around one third of these being significantly altered as determined by a Student's t-test. Within these categories will be phosphorylation events which are regulated by one or both PI3K isoforms. We next proceeded to define some (arbitrary) criteria which would allow us to categorise phosphorylation events to aid further analysis.

Table 4.1. Phosphorylation events identified as regulated by p110α or p110β.

DMSO *vs*	>2x Decrease	P<0.05	>2x Increase	P<0.05
A66	180	54	148	28
TGX221	195	59	108	9

A phosphorylation event was considered to be regulated by both PI3K isoforms when, upon inhibition, there was at least a twofold change in its intensity when comparing the impact of inhibition of each PI3K isoform to the DMSO control (Fig. 4.4).

A phosphorylation event was considered to be regulated by only one PI3K isoform when (1) there was at least a twofold change in its intensity when comparing inhibition of that PI3K isoform to the DMSO control, and (2) the fold

change of intensity upon inhibition of the other PI3K isoform compared to the DMSO control was less than 1.5-fold (Fig. 4.4).

Unless otherwise stated, comparisons are made between the intensity of a phosphopeptide in an inhibitor-treated sample (A66 or TGX221) relative to the DMSO control condition.

These criteria were applied to the whole dataset (2121 peptides), and the summary of the categories is shown in Table 4.2. As may be expected, a large proportion of phosphopeptides lie outside of the boundaries for fold change of intensity which we defined, and so are not stated to be under the control of one or both PI3K isoforms. By our criteria, 150 phosphorylation events are under the control of both p110α and p110β, 106 phosphorylation events are under selective control of p110α, and 93 phosphorylation events are under selective control of p110β. These values include those phosphorylation events which decrease and also those which increase upon PI3K isoform-inhibition and are summarised in Table 4.2.

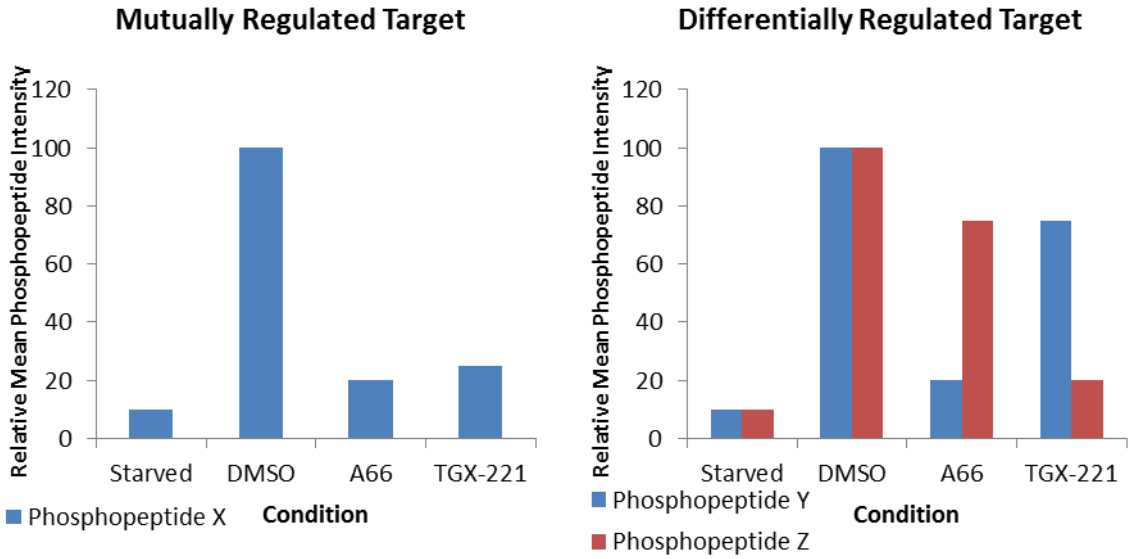


Figure 4.4. Criteria for categorising targets. 4T1 cells were starved for 18 h in 0% serum, followed by a 2 h incubation with or without inhibitor. Cells were then left untreated or stimulated with 0.5% serum with or without inhibitor for 30 min, and subjected to phosphoproteomic analysis (n=6). **(A)** Phosphopeptides were defined as being regulated by both p110α and p110β when there was ≥ 2x reduction in phosphopeptide intensity after inhibition of each PI3K isoform compared to DMSO control ($p<0.05$ in each case). **(B)** Phosphopeptides were defined as being regulated by either p110α or p110β only when there was ≥ 2x reduction in phosphopeptide intensity after inhibition of that PI3K isoform compared to DMSO control ($p<0.05$), and a < 1.5x reduction in phosphopeptide intensity after inhibition of the other PI3K isoform compared to DMSO control.

4.2.1. Results of phosphoproteomic screen

Both p110α and p110β are ubiquitously expressed, can control similar processes, and share a number of downstream effectors such as Akt. Surprisingly, when we applied our criteria we found that there were fewer mutually regulated phosphorylation events than events regulated by either p110α or p110β. It is likely that the emerging complexity of signalling upstream of class I PI3K contributes to this PI3K isoform-selective downstream signalling. For example the interplay and contribution from different RTKs, GPCRs, Ras and other small GTPases feeding into class I PI3Ks has yet to be fully described, and they are likely to promote different signalling networks.

4.2.1.1. Categorisation of phosphorylation events

Of the 150 phosphorylation events found to be regulated by both p110α and p110β, 67 were positively regulated, and intensity of phosphorylation of 12 of these reduced significantly as determined by a Student's t-test using a threshold of $p<0.05$. A heat map was used to visually represent these quantitative data, and shows negative regulation of phosphorylation upon inhibition of either p110α or p110β (Fig. 4.5A).

Table 4.2. Phosphorylation events identified as mutually or differentially regulated.

Regulated by	>2x Decrease	P<0.05	Group
p110α and p110β	67	12	A
p110α	66	17	B
p110β	76	9	C

Again based on the criteria outlined above, 106 phosphorylation events were found to be regulated by p110α alone. Intensity of 66 of these phosphorylation events reduced, and 17 of these were significantly altered as determined by a

Student's t-test using a threshold of $p<0.05$. A heat map was used to visually represent the quantitative data, and shows negative regulation of phosphorylation upon inhibition of p110α but not p110β (Fig. 4.5B).

In a similar manner, 93 phosphorylation events were found to be regulated by p110β alone, 76 of which reduced upon p110β inhibition. Of these, intensity of 9 phosphorylation events reduced significantly as determined by a Student's t-test using a threshold of $p<0.05$. A heat map was used to visually represent the quantitative data, and shows negative regulation of phosphorylation upon inhibition of p110β but not p110α (Fig. 4.5C).

Interestingly, while intensity of phosphorylation for mutually or p110α-regulated substrates appears to increase following serum starvation (from the Starved to DMSO controls), the reverse pattern is seen in the p110β-regulated category. Intensity of phosphorylation is often relatively high in the serum-starved control, and reduces after stimulation. Examples of phosphopeptides from each category are shown in Figure 4.6.

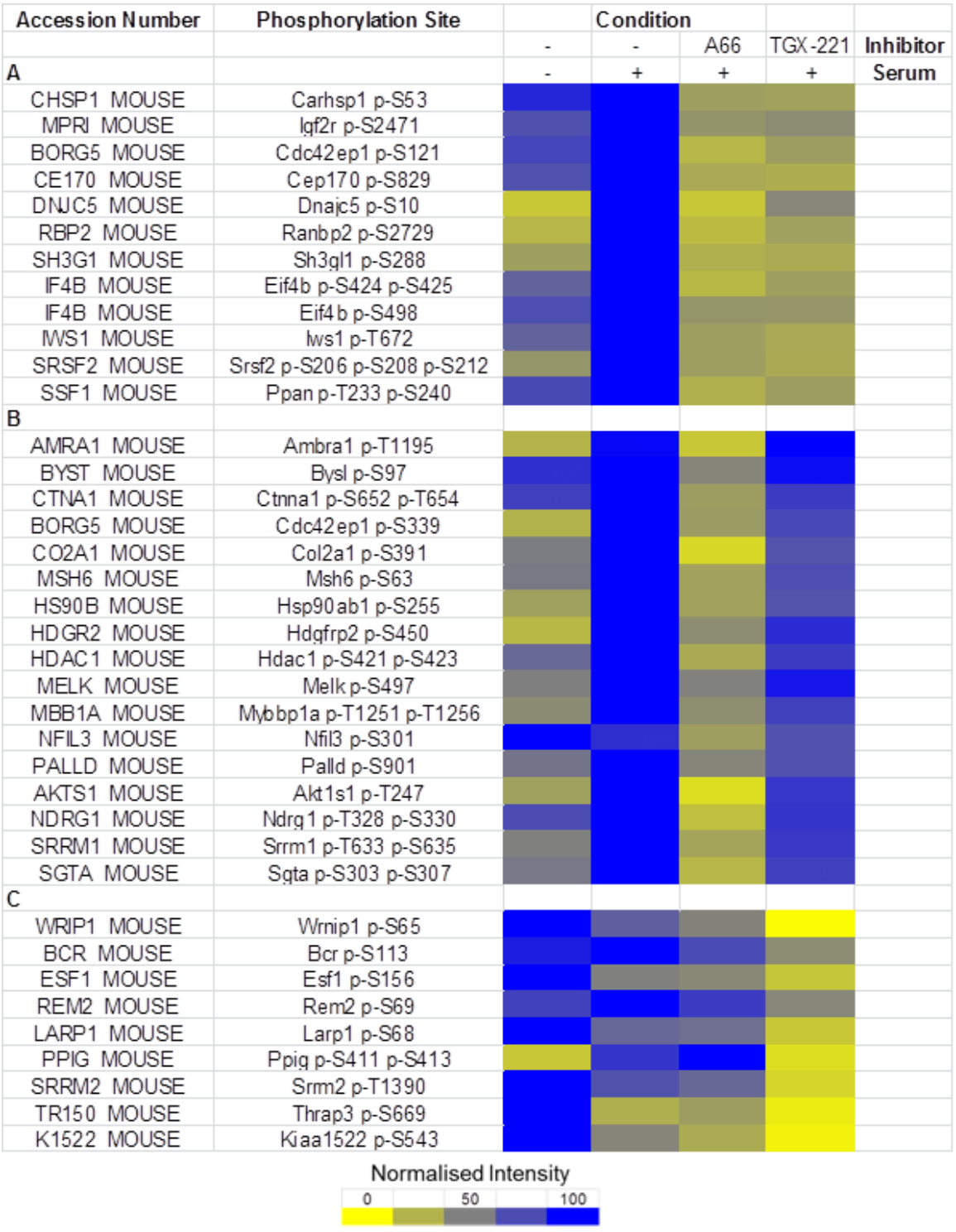


Figure 4.5. Examples of phosphorylation events identified following inhibition of p110α or p110β PI3K. 4T1 cells were starved for 18 h in 0% serum, followed by a 2 h incubation with or without inhibitor. Cells were then left untreated or stimulated with 0.5% serum with or without inhibitor for 30 min, and subjected to phosphoproteomic analysis (n=6). Mean normalised phosphopeptide intensity are represented as a heat map for events described as regulated by **(A)** p110α and p110β, **(B)** p110α only, **(C)** p110β are shown, where intensity of phosphorylation for a given phosphopeptide was expressed relative to the maximum intensity for that phosphopeptide

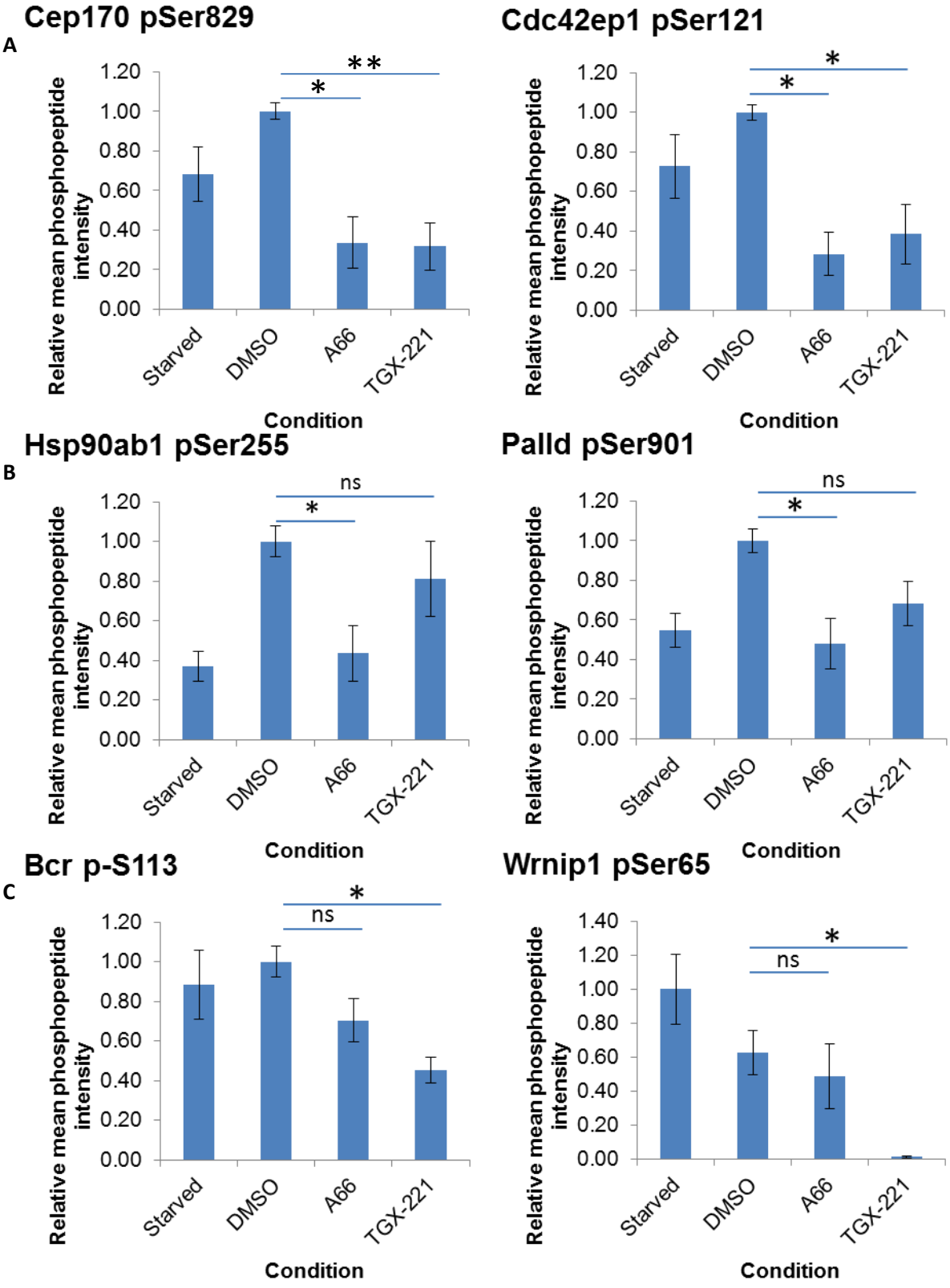


Figure 4.6. Examples of phosphorylation events identified following inhibition of p110α or p110β PI3K. 4T1 cells were starved for 18 h in 0% serum, followed by a 2 h incubation with or without inhibitor. Cells were then left untreated or stimulated with 0.5% serum with or without inhibitor for 30 min, and subjected to phosphoproteomic analysis (n=6). Mean normalised phosphopeptide intensity for examples of events described as regulated by **(A)** p110α and p110β, **(B)** p110α only, **(C)** p110β are shown, where intensity of phosphorylation for a given phosphopeptide was expressed relative to the maximum intensity for that phosphopeptide. Error bars represent one standard deviation from the mean. *p<0.05 **p<0.005

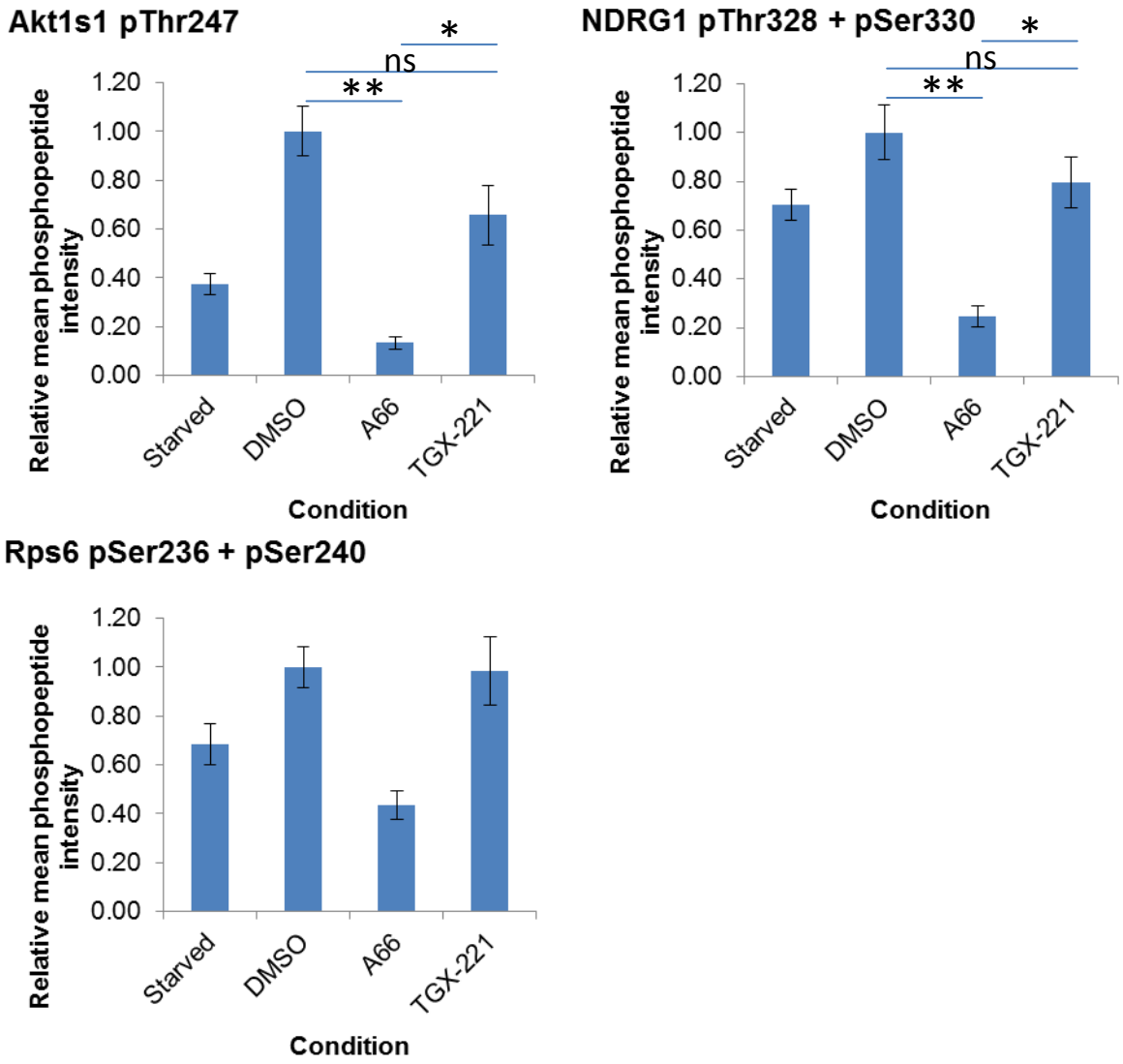


Figure 4.7. Phosphorylation events downstream of PI3K/Akt are under the control of p110α but not p110β. 4T1 cells were starved for 18 h in 0% serum, followed by a 2 h incubation with or without inhibitor. Cells were then left untreated or stimulated with 0.5% serum with or without inhibitor for 30 min, and subjected to phosphoproteomic analysis (n=6). Mean normalised phosphopeptide intensity for examples of events previously described as being regulated by PI3K/Akt are shown where intensity of phosphorylation for a given phosphopeptide was expressed relative to the maximum intensity for that phosphopeptide. Error bars represent one standard deviation from the mean. *p<0.05 **p<0.005

As previously mentioned, the Akt substrates AKTS1 (pThr247) and 40S ribosomal protein S6 (pSer236) were both identified in this MS screen as being negatively regulated upon PI3K inhibition (Fig. 4.7). Because Akt is a downstream effector of PI3K, this observation serves to increase confidence in the dataset as a whole. Interestingly, these two phosphoproteins display similar profiles in terms of the relative intensity of phosphorylation, which suggests

different roles of p110α and p110β. Serum-stimulation brings about an increase in phosphorylation relative to the starved control. Inhibition of p110α causes a reduction in phosphorylation to a level lower than the starved control. However, this is not repeated upon inhibition of p110β. Instead, only a minor reduction in phosphorylation of AKTS1 is seen, and a slight increase is observed for phosphorylation of ribosomal protein S6. This observation suggests that in this context, p110α and p110β have different absolute contributions to cellular signalling through Akt.

This p110α-specific dataset also includes pSer330 of NDRG1. This phosphorylation event has previously been described as being a direct target of SGK [216], a kinase which has been shown to signal downstream of PI3K [112]. This phosphorylation event will be discussed in more detail later.

4.2.2. Motif analysis

Phosphorylation of a protein is the result of the interplay between a kinase and a phosphatase. Many kinases have been described to have specific motifs which they preferentially phosphorylate, for example the motif RxRxxS/T is present in a number of Akt substrates. Therefore we reasoned that Akt, which is known to be a prominent PI3K effector, or one or more other kinases may be responsible for phosphorylating a large number of the proteins identified in our MS screen.

Identified phosphorylation events which conform to the previously described Akt motif [54] are listed in Table 4.3. These proteins represent around one third (13/38) of the phosphorylation events identified as being significantly down-regulated by PI3K isoform inhibition in the screen, suggesting a rather substantial possible involvement of non-Akt-directed pathways in this experimental context. Only one phosphorylation event from the p110β-regulated category is seen to have an Akt motif. It is important to bear in mind that many of the identified phosphorylation events will be one or several layers below the first PI3K effector, be it Akt or other kinases such as SGK.

One of these phosphorylation events is pSer330 of NDRG1. Interestingly, the kinase SGK1 which has previously been described as being responsible for this phosphorylation has similar substrate specificity to Akt.

Table 4.3. Phosphorylation events identified which conform to the Akt consensus motif.

Accession Number	Phosphosite	Sequence (* indicates phosphosite)	Full/Partial Akt motif	Group
AKTS1_MOUSE	Akt1s1 p-T247	RPRLNT*SDFQ	Full	B
CE170_MOUSE	Cep170 p-S829	LVRQGS*FTID	Partial	A
CHSP1_MOUSE	Carhsp1 p-S53	RTRTFS*ATVR	Full	A
DNJC5_MOUSE	Dnajc5 p-S10	RQRSLS*TSGES	Full	A
HDGR2_MOUSE	Hdgfrp2 p-S450	RTRKRS*EGLS	Full	B
MELK_MOUSE	Melk p-S497	ERRCRS*MDVD	Partial	B
NDRG1_MOUSE	Ndrg1 p-T328 p-S330	LMRSRT*AS*GS	Partial/Full	B
PALLD_MOUSE	Palld p-S901	TARIAS*DEEI	Partial	B
PPIG_MOUSE	Ppig p-S411 p-S413	DHRHMS*ES*PNRK	Partial/No	C
RS6_MOUSE	Rps6 p-S236 p-S240	RRRLSS*LRAS*	Full/No	B
SGTA_MOUSE	Sgta p-S303 p-S307	IRSQVVRS*RTPS*ASH	No/Full	B
SRRM1_MOUSE	Srrm1 p-T633 p-S635	KRRT*AS*PPPP	No/Partial	B
SRSF2_MOUSE	Srsf2 p-S206 p-S208 p-S212	KSRSRS*KS*PPKS*PEEE	Partial/No/No	A

We used the freely available web-based program motif-x [212] in order to identify common motifs within the dataset. Identifying motifs from small datasets is challenging unless the motif is extremely well-represented. Therefore, we decided to increase the number of sequences from which we aimed to detect

motifs, and included phosphopeptides which fell within our categories although did not meet the required significance of $p<0.05$ (Table 4.2). Consensus motifs are typically made up of a small number of amino acids adjacent to the phosphosite, and so using the UniProt database, we extended the sequences of our identified phosphopeptides to account for tryptic cleavages and avoid missing motifs. The top ranking motifs are shown in Tables 4.4-6 and Fig. 4.8.

While no one motif is obviously enriched in the dataset, a full or partial Akt motif (RxRxxS/T) is prominent within the phosphorylation events of each category (Table 4.7). Two other features of the motifs also stand out: a proline residue in the +1 position, and acidic residues prominent at various positions both N- and C-terminal to the phosphorylation site. Proline-directed kinases are in the minority among the protein kinase family but include cyclin-dependent protein kinases (CDKs), mitogen-activated protein kinases (MAPKs) and the Akt effector GSK-3. Acidic residues have been shown to be important for recognition of substrates by casein kinase 2 and polo-like kinase 1. These motifs appear to show different profiles within the categories. All three are well represented in the mutually regulated phosphorylation events, while acidic and SP motifs are most prominent in p110α- and p110β-regulated phosphorylation events, respectively (Table 4.7).

That an Akt consensus motif is found in a minority of the interrogated sequences, and that no one other motif is dominant suggests that multiple other kinases or families are involved in transducing the PI3K signal in this context. It should be noted that because of the nature of tryptic digestion, some sequence information will be lost, therefore some motifs will be under-represented by this method of analysis.

Table 4.4. Motifs enriched in phosphorylation events categorised as being mutually regulated by p110α and p110β

#	Motif	Matches (/67)	Fold Increase
1.	xxxDxS*PEPxx	3	1476.25
2.	xxRxxS*PxxSx	3	74.08
3.	xxxxxS*PTxxx	4	14.45
4.	xxxxxS*xDExD	3	178.80
5.	RxRxxS*xxxxx	6	17.86
6.	ExxxxS*DxDxx	4	262.73
7.	xxxExS*Pxxxx	3	15.47
8.	xxxxxS*DxExx	5	23.97
9.	xxxxxS*Pxxxx	11	3.88
10.	xxRRxS*xxxxx	4	27.64
11.	xxDxxS*xxSxx	3	21.21
12.	xxxxxS*xEExx	4	21.76
13.	xxxxxS*xxxPx	6	4.18
14.	xxxxxS*xESxx	3	38.58
15.	xxxxxS*Sxxxx	6	3.85
16.	xxxxxS*Fxxxx	3	9.08

Fold increase = increase in observations of motif while searching this group of sequences versus a group of randomly generated sequences.

Table 4.5. Motifs enriched in phosphorylation events categorised as being regulated by p110α only

#	Motif	Matches (/66)	Fold Increase
1.	xxxxxS*DEExx	6	164.81
2.	xNxxxS*Pxxxx	3	22.78
3.	xxRxxS*Pxxxx	4	15.18
4.	xxxQxS*xxExx	4	23.33
5.	xxSxxS*Pxxxx	5	12.57
6.	xxxxxS*xxEDx	4	20.59
7.	RxRxxS*xxxxx	5	23.75
8.	xxxxxS*PxRxx	3	25.45
9.	xxxxxS*xxEEE	3	85.52
10.	xxxQxS*xxDxx	3	45.43
11.	xxxxxS*xDxxx	5	3.85
12.	xxxxxS*xxxxS	6	2.59
13.	xxxxxS*xxxEx	4	3.72
14.	xxxxAS*xxxxx	4	4.40

Fold increase = increase in observations of motif while searching this group of sequences versus a group of randomly generated sequences.

Table 4.6. Motifs enriched in phosphorylation events categorised as being regulated by p110β only

#	Motif	Matches (/76)	Fold Increase
1.	AxAxxS*PxKxx	3	2522.27
2.	xxKAxS*PSxxx	3	1618.34
3.	xxxxxS*PxxxR	6	22.16
4.	xxSxxS*PxKxx	3	179.37
5.	xxxxxS*PxxxK	4	22.58
6.	xxxxxS*PxxxT	4	19.59
7.	xxxxxS*PxRxx	3	20.76
8.	xxRRxS*xxxxx	4	20.19
9.	xxxxxS*PTxxx	3	25.37
10.	RxxxxS*xPxxx	4	31.10
11.	xxxxxS*PxxxL	3	17.26
12.	xxxxDS*DxDxx	3	278.48
13.	xxxxxS*ExxxD	3	40.60
14.	xxxRxS*xxxxx	5	4.81
15.	xxxxxS*xxxDx	4	5.49
16.	xxRxxS*xxxxx	3	4.38
17.	xxxxxS*xExxx	3	5.34

Table 4.7. Motifs enriched in phosphorylation events across categories

Motif	Mutual (/67)	p110α (/66)	p110β (/76)
RxxS*	13	9	7
S*P	24	15	32
Acidic	28	29	13

Fold increase = increase in observations of motif while searching this group of sequences versus a group of randomly generated sequences.

Figure 4.8. Specific motifs are enriched upon PI3K isoform inhibition. 4T1 cells were starved for 18 h in 0% serum, followed by a 2 h incubation with or without inhibitor. Cells were then left untreated or stimulated with 0.5% serum with or without inhibitor for 30 min, and subjected to phosphoproteomic analysis (n=6). Sequences of phosphopeptides found to exhibit a >2-fold reduction upon PI3K isoform-inhibition were interrogated using the motif-x website. Top ranking consensus sites as returned by motif-x are displayed for **(A)** mutually regulated phosphorylation events and events regulated by **(B)** p110α or **(C)** p110β only.

4.2.3. Pathway analysis

The PI3K pathway is known to exert control over a number of cellular functions such as survival and proliferation, and does so through well-defined networks. We therefore decided to look for known connections between the identified proteins, including between the phosphorylation events and PI3Ks. While it is sensible to ordinarily take only robust candidates forward for validation, when attempting to elucidate connections within a network it may be preferable to use less stringent parameters. This is endorsed by our finding of 40S ribosomal protein S6 (pSer236) being regulated by p110α alone, but at p=0.07 falling short of reaching significance although the change was a greater than 2-fold reduction. Therefore we decided to seek connections in the groups of phosphopeptides which we classified as described above (Fig. 4.4), but this time not taking significance into account.

We used the freely available online tool STRING to produce maps which plot previously described connections between the proteins identified in our MS screen, with proteins lacking connections omitted. Figures 4.9.A-C show the proteins categorised as being mutually regulated, or regulated by p110α or p110β alone, respectively. For each group of proteins, distinct nodes exist where connections are made between effectors of transcription (1) and translation (2), as well as DNA binding proteins (3). Other smaller nodes also exist for each group, relating to cell adhesion (4). It is interesting to note that even though the three groups which we defined (phosphorylation events regulated in common and exclusively) contain different phosphorylation events, the proteins within them represent similar pathways (e.g. transcription and translation). This is noteworthy that PI3Ks may act through different targets for the same ends, and possibly points towards redundant signalling.

The experiment was primarily designed to explore the signalling downstream of p110α and p110β, which could potentially be used to help to explain the differences in observed phenotypes such as proliferation, invasion and metastasis. However, we could not discriminate obvious differences in terms of pathways represented which could explain the observed differences in phenotypes following PI3K isoform-selective inhibition observed by Dr Taboubi.

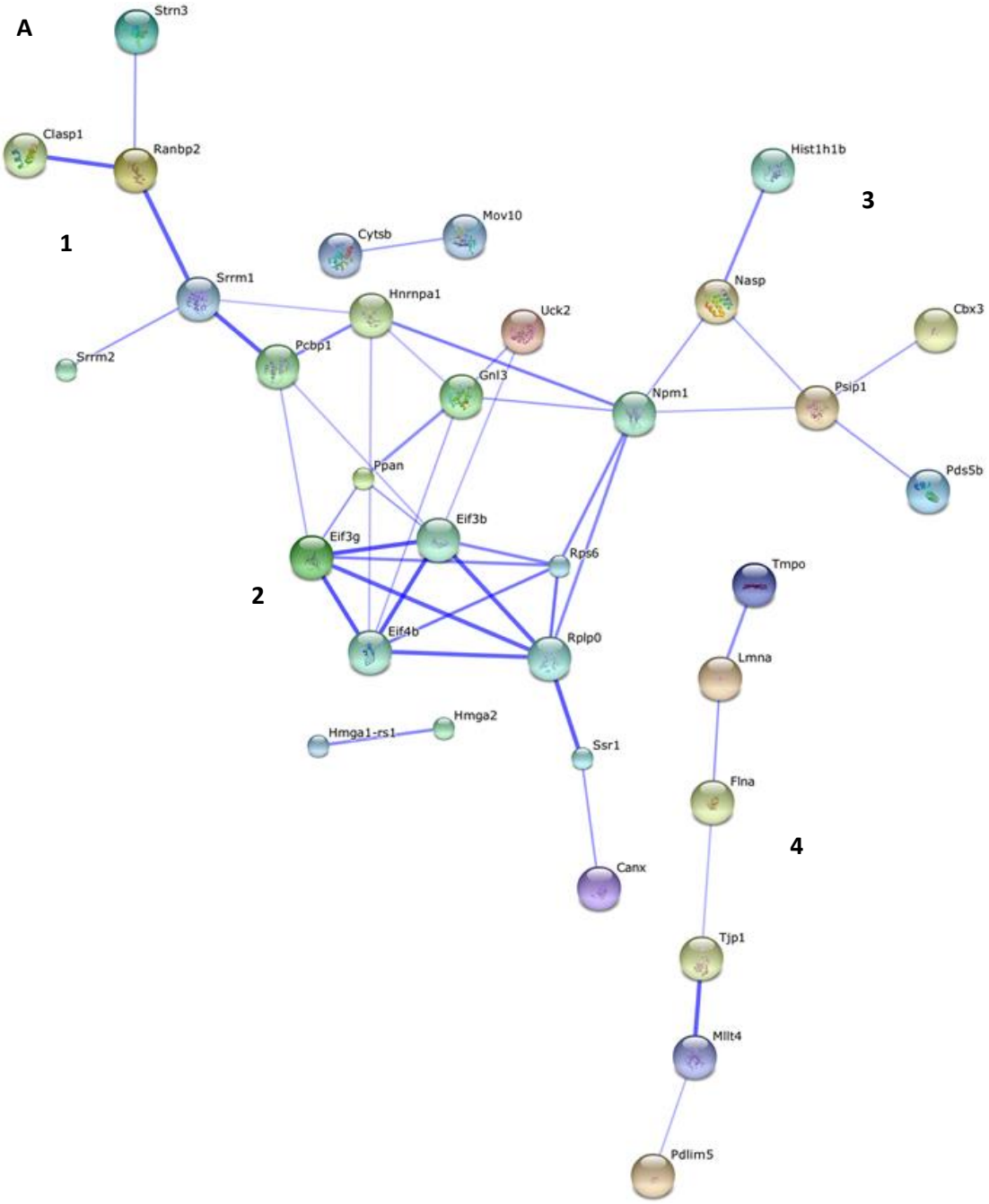


Figure 4.9. Analysis of pathways regulated by PI3K isoforms. 4T1 cells were starved for 18 h in 0% serum, followed by a 2 h incubation with or without inhibitor. Cells were then left untreated or stimulated with 0.5% serum with or without inhibitor for 30 min, and subjected to phosphoproteomic analysis (n=6). The online tool STRING was used to map networks mapped of phosphorylation events described as being regulated by **(A)** p110α and p110β, **(B)** p110α only, **(C)** p110β only. Previously described interactions (lines) between proteins (circles) from each category are depicted, and proteins without connections omitted.

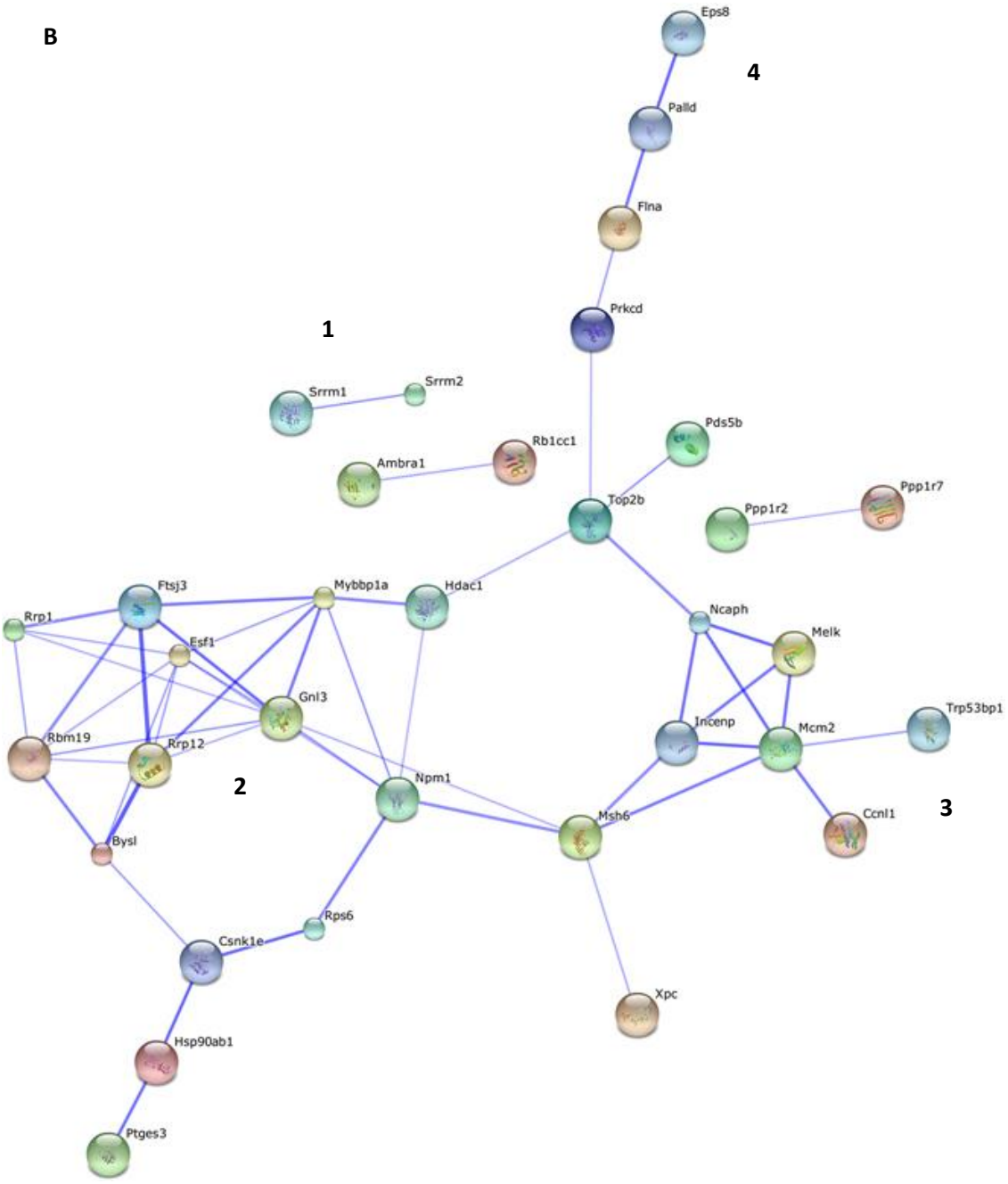


Figure 4.9. Analysis of pathways regulated by PI3K isoforms. 4T1 cells were starved for 18 h in 0% serum, followed by a 2 h incubation with or without inhibitor. Cells were then left untreated or stimulated with 0.5% serum with or without inhibitor for 30 min, and subjected to phosphoproteomic analysis (n=6). The online tool STRING was used to map networks mapped of phosphorylation events described as being regulated by **(A)** p110α and p110β, **(B)** p110α only, **(C)** p110β only. Previously described interactions (lines) between proteins (circles) from each category are depicted, and proteins without connections omitted.

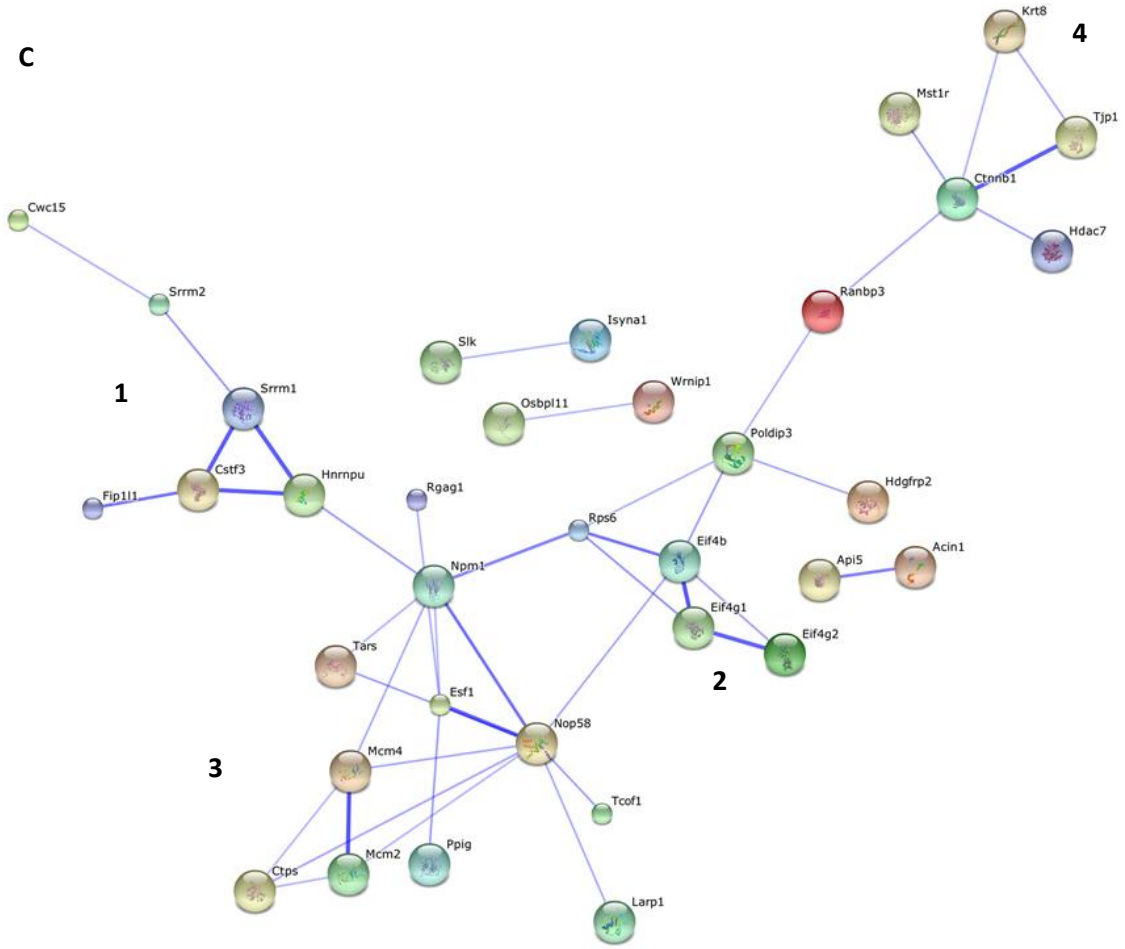


Figure 4.9. Analysis of pathways regulated by PI3K isoforms. 4T1 cells were starved for 18 h in 0% serum, followed by a 2 h incubation with or without inhibitor. Cells were then left untreated or stimulated with 0.5% serum with or without inhibitor for 30 min, and subjected to phosphoproteomic analysis (n=6). The online tool STRING was used to map networks mapped of phosphorylation events described as being regulated by **(A)** p110α and p110β, **(B)** p110α only, **(C)** p110β only. Previously described interactions (lines) between proteins (circles) from each category are depicted, and proteins without connections omitted.

4.3. Validation

While large-scale MS screens are of themselves informative, it is prudent to follow up on some aspects so as to have greater confidence in the data generated. Therefore, we chose a number of targets identified in the MS screen for validation by independent methods. These targets were chosen based on a number of criteria, including an established link to the PI3K pathway, involvement in other well-characterised pathways, and, importantly, availability of biochemical tools for analysis. We focused our attention on phosphorylation events which were negatively regulated upon PI3K isoform-inhibition, as these are likely to be directly downstream of PI3K, rather than phosphorylation events which were positively regulated and possibly part of a feedback loop.

Using these criteria, we chose the targets listed in Table 4.4. This list includes pSer236 of 40S ribosomal protein S6 which is a direct target of Akt and found to be negatively regulated in the MS screen upon inhibition of p110α, although it did not meet our set cut-off threshold for significance. Also chosen is pSer330 of NDRG1 which is a direct target of SGK1, a kinase which has shown to be able to transduce the PI3K signal, and pThr247 of AKTS1 which is a direct target of Akt. Figure 4.7 shows the relative mean intensity of phosphorylation within the MS screen of the proteins taken for validation.

Table 4.4. Phosphoproteins identified in MS screen and chosen for validation

Accession Number	Protein	Phosphosite	Significance
Negatively regulated upon inhibition			
RS6_MOUSE	40S ribosomal protein S6	p-Ser236	Akt substrate
NDRG1_MOUSE	NDRG1	p-Ser330	SGK1 substrate
AKTS1_MOUSE	Proline-rich AKT1 substrate 1	p-Thr247	Akt substrate

The proteins listed in Table 4.4 all have commercially available antibodies targeted to the phosphorylation event identified in the MS screen, which was a limiting factor in choosing candidates for validation. It would have been preferable for more phosphorylation events to be studied, and for these to cover all categories, but unfortunately this was not possible due to a lack of available biochemical tools such as protein- and phosphosite-specific antibodies.

Our next step was to probe by western blot the relative levels of phosphorylation of the candidates. 4T1 cells were treated in the same way as in the MS screen, and then subjected to western blot analysis, representative blots are shown in Figure 4.10.

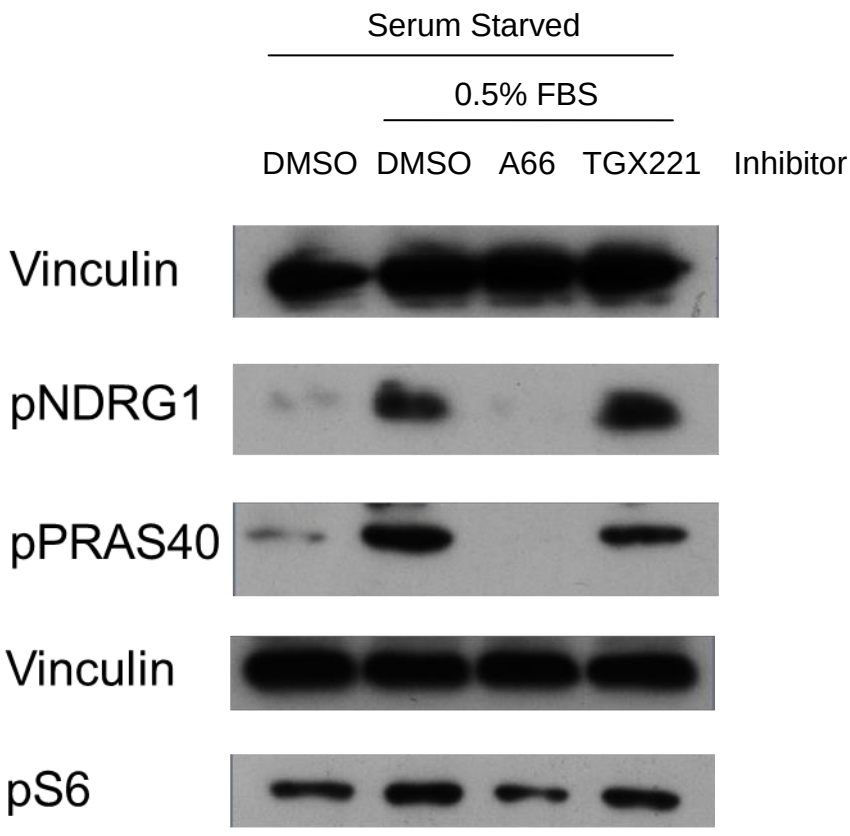


Figure 4.10. Validation of phosphorylation events categorised as being regulated by p110α but not p110β. 4T1 cells were starved for 18 h in 0% serum, followed by a 2 h incubation without or with inhibitor. Cells were then left untreated (S) or stimulated with 0.5% serum without (D) or with inhibitor (A=A66, T=TGX221) for 30 min, and subjected to WB analysis with antibodies to the indicated proteins. Representative blots are shown. Vinculin was used as a loading control.

The results show that for the phosphorylation events under investigation which decrease upon PI3K isoform-inhibition, the same pattern is observed by western blot as in the MS screen. Each of the phosphorylation sites of 40S ribosomal protein S6, NDRG1, and AKTS1 are observed to strongly reduce upon inhibition of p110α, but not p110β. This confirms a PI3K isoform-specific role for p110α in the regulation of these proteins, which likely extends to others.

4.4. Comparison with other cell lines

After observing differences in the signalling of p110α and p110β in 4T1 cells, we decided to test other breast cancer cell lines to test whether or not these findings would be specific to 4T1 cells. We chose a panel of breast cancer cell lines which had previously shown similar phenotypes to 4T1 cells upon class I PI3K inhibition (Dr Taboubi – unpublished). These cell lines are listed in Table 4.5, along with genotypes related to the PI3K pathway and some other genes important in breast cancer cell biology.

Table 4.5. Breast cancer cell lines chosen for comparison with 4T1

Cell line	*PIK3CA*	PTEN	p53	ERBB2	Oestrogen Receptor
4T1	WT	Positive	Negative		
MCF-7	E545K	Positive	Positive	Negative	Positive
MDA-MB-231	WT	Positive	Negative	Negative	Negative
MDA-MB-453	H1047R	Positive	Negative	Positive	Negative

Each cell line was treated in the same way as the 4T1 cells prior to the MS screen (Fig. 4.1) and validation studies (Fig. 4.10), and subjected to analysis by western blot. We probed for the same proteins and phosphorylation events as in Fig. 4.10, and representative blots are shown in Fig. 4.11 (and summarised in Table 4.6.).

It is clear that the overall 4T1 phosphorylation signature of the three proteins tested is not replicated in any of the three other cell lines examined. Indeed while as in 4T1 cells phosphorylation of NDRG1 is greatly reduced upon p110α inhibition in both MCF7 and MDA-MB-231 cell lines, this is not the case with phosphorylation of PRAS40 any other cell line studied. Additionally, the reduction of phosphorylation of RS6 following inhibition of p110α seen in 4T1 cells is not replicated any of the three cell lines. In fact the signalling profile of these three cell lines is more similar to each other than 4T1 cells. Of the cell lines we assessed, regulation of phosphorylation of AKTS1-Thr247 and RS6-Ser236 is specific to p110α only in 4T1 cells, while regulation of NDRG1-Ser330 is dependent on p110α in 4T1, MCF7 and MDA-MB-231 cells.

Table 4.6. PI3K isoform-inhibition on phosphorylation events compared to 4T1 cell line

Cell line	**4T1**		**MCF-7**		**MDA-MB-231**		**MDA-MB-453**	
Compound Phosphosite	A66	TGX221	A66	TGX221	A66	TGX221	A66	TGX221
pNDRG1	↓	–	↓	↓	↓	–	–	–
pPRAS40	↓	–	–	–	–	–	–	–
pS6	↓	–	–	–	–	–	–	–

(↓) = decrease, (–) = no change in phosphorylation relative to DMSO control

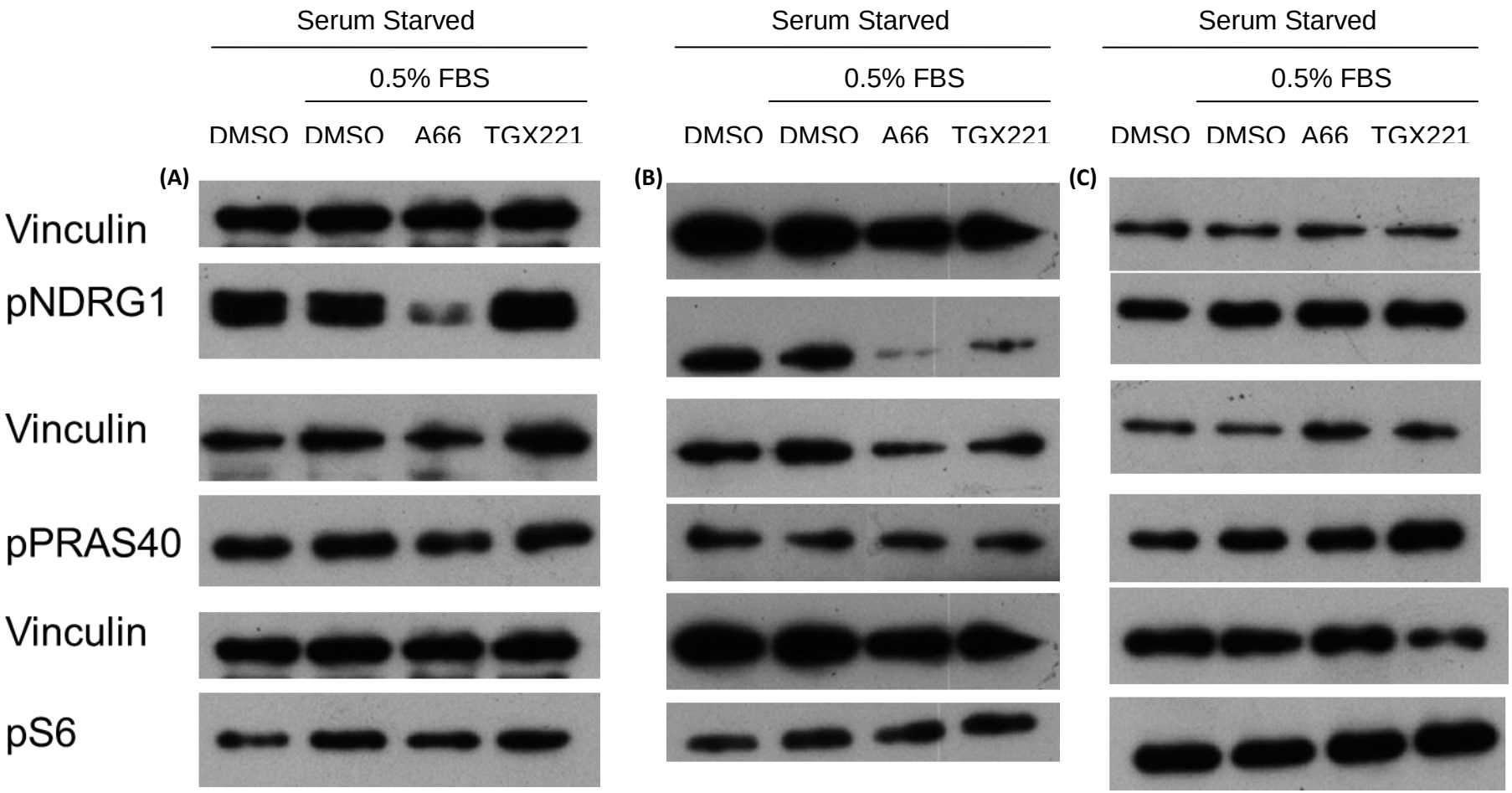


Figure 4.11. Differences in signalling downstream of class IA PI3K isoforms in a panel of breast cancer cell lines. Cells were starved for 18 h in 0% serum, followed by a 2 h incubation with or without inhibitor. Cells were then left untreated or stimulated with 0.5% serum with or without inhibitor for 30 min, and subjected to WB analysis with the antibodies described. Representative blots are shown for the **(A)** MCF7 cell line, **(B)** MDA-MB-231 cell line and **(C)** MDA-MB-453 cell line. Vinculin was used as a loading control.

The genetic background of the panel of cell lines varies and is shown in Table 4.5. In terms of PI3K-related signalling, all cells lines are positive for PTEN, 4T1 and MDA-MB-231 have wild-type p110α, while the remaining two cell lines have oncogenic p110α mutants. The signature of each cell line is different and does not appear to correlate with PI3K pathway mutational status, and it is likely that other aberrations in these cell lines contribute to the observed signalling output.

4.5. Discussion

Alongside our examination of less well-studied aspects of p110α signalling, we wanted to compare its wider downstream signalling profile (of lipid and protein kinase activity) to that of the other ubiquitously expressed class I PI3K, p110β. While both PI3K isoforms are expressed across cell types, there is a growing body of evidence showing differential upstream regulation, and downstream signalling [24, 28, 31, 38]. p110α appears to be mainly activated by RTKs and Ras, while p110β has input from GPCRs and Rho GTPases. These differences in upstream signalling may go some way to account for the differences in cellular processes controlled by the two PI3K isoforms. This extends to differences in phenotypes observed within our laboratory after PI3K isoform inhibition in a 4T1 murine breast cancer cell model (unpublished data from our laboratory).

Following activation of class IA PI3Ks, phosphorylation signalling cascades are initiated which involve transduction through effector kinases such as Akt. Other AGC kinases are also involved in relay of the PI3K signal, but these networks are less well-characterised than the Akt signalling node.

Therefore, we decided to use a quantitative phosphoproteomic approach to interrogate the signalling pathways downstream of p110α and p110β in an unbiased and global manner using mass spectrometry and more traditional biochemical tools for validation of identified phosphorylation events.

4.5.1. Phosphoproteomic investigation of signalling downstream of p110α and p110β

Building on previous experiments performed in our laboratory which observed different cell biological phenotypes upon p110α or p110β inhibition in 4T1 cells, we designed a strategy which would allow us to characterise the signalling pathways downstream of those class IA PI3K. We used the same conditions with which differential phenotypes were observed in 4T1 cells, and so compared serum-starved cells with cells which were stimulated with 0.5% serum, with or without inhibition of either p110α or p110β. Isoform inhibition was achieved with the specific inhibitors A66 (for p110α) and TGX221 (for p110β), reducing signalling from these isoforms and through the well-characterised PI3K effector Akt and other proteins. In this way, we hoped to match signalling profiles in terms of phosphorylation events with previously observed differential phenotypic outputs. Off-target effects of inhibitors on other kinases may occur and should be kept in mind when interpreting data, with validation by additional methods preferred.

While there are imperfections in this model, we decided to use it because of its relation to the cancer context, and its interesting and differential biology upon p110α and p110β inhibition. Flaws will always exist in any cell line model, especially around questions as to the physiological relevance of the system being assessed. In this case, we were seeking to answer questions raised by specific observations from previous work, and so it was necessary to use the same system. The 4T1 cell line is of murine mammary gland in origin, and closely resembles human breast cancer progression in terms of growth and spontaneous metastatic spread to a number of sites, and so for these reasons is a model relevant to oncology, and also basic biology.

The specificity of interaction for both A66 and TGX221 has been determined previously [167, 148], and both compounds have been shown to have highest efficacy against p110α and p110β respectively, out of the panels of kinases assayed. It is the case that efficacy and specificity of inhibition of these isoforms is analysed in the context of *in vitro* methodology and criteria defined in the pharmacological (often cell-free) assays, and other considerations must be

taken into account when analysing data from more complex systems such as ours. The use of a more intricate system such as our 4T1 cell model allows for the possibility of a wider range of interactions between the pharmacological compounds and the proteins, lipids, metabolites etc. that are present in the cell mixture. This in turn suggests that the inhibitors used may have characteristics (such as IC50) which are different to those analysed or derived while using simpler models, and so the possibility exists that concentrations which were used were not the optimal ones for the isoforms under study.

It is also true that with a much greater number of kinases present in the 4T1 cell system compared to some pharmacological assays which have only a single kinase, off-target effects of the inhibitor may occur. There are more than 500 kinases coded in the human genome, and it is possible that A66 and TGX221 display some efficacy regarding the inhibition of some of these enzymes, and so it may be that regulation of some phosphorylation sites was in fact due to kinases other than p110α and p110β. It is also true that one specific phosphosite may have more than one parent kinase. These points should be kept in mind when interpreting data, and there is always a need for follow up work and subsequent validation. We therefore used complementary techniques to confirm some of our findings, and western blotting was able to strengthen the conclusions we reached; western blotting has among its advantages speed, ease and cost. Another option for validation is the knock-down of a protein of interest using siRNA targeted to the transcript product of a specific gene. This technique would be useful in confirming the specificity of the inhibitors and so similar results obtained while using inhibitors or siRNA would add weight to a specific claim. However no system or technique is without its disadvantages, and the use of siRNA might also elicit off-target effects in terms of knocking-down other proteins which play some part in a specific pathway. Therefore caution must always be exercised when analysing data and drawing conclusions.

Whereas in Chapter 3 we used a number of different concentrations of ATP so as to have an idea of reaction kinetics and identify dose-response relationships, in this investigation we decided to use a single concentration of each inhibitor. We reasoned that having a large number of replicates informs the validity of the

inhibition of a given phosphorylation event, but equally additional inhibitor concentrations would also be of benefit. The use of only one concentration means that we must be aware of false positives, but generally these should be able to be accounted for by analysing the replicates, and of course validation is always preferred. Were we to carry out this investigation again, the inclusion of an additional inhibitor concentration and use of three replicates would mean that the same resources may be distributed more effectively, although the success which we achieved with our validation studies does in part vindicate our approach.

Using this model, we treated cells and subjected samples to processing in such a way as to maximise identification and quantification of phosphorylation events. We performed the experiment triplicate, and analysed each replicate twice by LC-MS, thereby having six replicates for each of the four conditions. In a similar manner to the experiments in Chapter 3, we used TiO_2-based enrichment of phosphopeptides, and analysed them using label-free methods [191, 211]. Following LC-MS, we again used the freely available online Mascot tool to identify peptides present in our samples. The program PESCAL, developed in-house, was then used to quantify these phosphopeptides across all samples.

4.5.1.1. General characterisation of phosphoproteomic data

A total of 2121 phosphopeptides were identified and quantified, and we used heat maps and PCA to visualise the quantitative data generated. Heatmaps showed that different profiles of intensity of phosphorylation were observed, with some phosphorylation events showing similarities, and some differences, when comparing p110α- and p110β-inhibited samples.

Following quantification, we defined arbitrary criteria to categorise phosphorylation events as mutually or differentially regulated. We defined events at being mutually regulated when there was a two-fold change in intensity ($p<0.05$) upon inhibition of each isoform *versus* DMSO control. As even small changes in the relative phosphorylation of a protein can induce an effect on, for example, localisation or activity of that protein, it may be argued that by placing a lower limit of a two-fold change is too stringent, but we chose

this criterion along with requiring a significance of $p<0.05$ to increase our chances of finding phosphorylation events which truly fitted into each category. We defined differentially regulated phosphorylation events as those with a two-fold change in intensity ($p<0.05$) upon inhibition of one isoform compared to DMSO control, with an upper limit of a 1.5 fold-change in intensity upon inhibition of the other isoform compared to DMSO control. The upper limit of a fold change of 1.5 for an event following inhibition of "the second isoform" was set so as to categorise events which had a clear difference in intensity for inhibition of the two isoforms. A consequence of these criteria is that phosphorylation events which would, under less stringent criteria and perhaps physiologically, be considered to be in a different category instead fall into the "no difference" category. It may be the case that this approach limits false positives, but increases false negatives. However we wanted to be able to choose strong candidates to take forward for validation, and the success of the validation vindicates this approach.

While phosphorylation events which decrease upon isoform-inhibition are likely under the direct positive control of a kinase, phosphorylation events which increase upon PI3K isoform-inhibition may be part of a feedback loop. Phosphopeptides in either category were of interest to us, although we thought it likely that the former may be easier to characterise.

It is interesting to note the different profiles of intensity of phosphorylation which are observed between the categories. While phosphorylation events which are negatively regulated by both p110α and p110β, and those which are negatively regulated by only p110α show an intensity which increases from the starved to DMSO condition, the majority of those which are defined as being regulated by p110β only have an intensity which is already high in the starved condition and decreases to the DMSO control. This suggests a separation of the types of signalling which the two PI3K isoforms are involved in.

A scenario can be considered in which p110α is the PI3K isoform primarily responsible for the acute response to stimuli such as those found in the fetal bovine serum used in this experiment, and p110β is the isoform responsible for low level continual tonic signalling necessary to sustain a cell. The experimental

design used serum starvation to remove stimulating factors and reduce the majority of signalling pathways down to a baseline level. Pathways responsive to stimuli like growth factors would therefore be at a low level and elevated upon addition of serum, and these contain the phosphorylation events which tend to be regulated by p110α alone or with p110β. Pathways necessary for tonic signalling would be high in the starved condition relative to the DMSO condition, and these tend to be regulated by p110β only.

4.5.1.2. Biochemical consideration of motifs and kinases

In contrast to the work carried out in Chapter 3, here we were not exclusively interested in substrates of PI3K protein kinase activity. These experiments focused on the inhibition of p110α and p110β and the effect on downstream signalling pathways, the majority of which are likely to be initiated by the lipid kinase function, and which will include multiple effector kinases. As outlined previously, kinases often phosphorylate residues in the context of a specific amino acid motif, such as Akt which targets the RxRxxpS/pT motif, or SGK which phosphorylates amino acids in a similar context [48]. We therefore decided to interrogate our database of phosphopeptides for the presence of common motifs. We first did this in a targeted manner, and looked for the Akt consensus motif amongst the sequences. The Akt motif was found in 13/38 phosphorylation events we defined as being positively regulated by PI3K (phosphorylation decreased upon PI3K inhibition). It is important to remember that these phosphorylation events will be part of a signalling cascade, and so may not be phosphorylated by the primary effector of PI3K signalling, i.e. some will be targeted by kinases further downstream. It is interesting to note that only one of these phosphorylation events was from the p110β-regulated category.

We then searched for common motifs within the dataset, the size of which we increased by including peptides which met our criteria for fold change, but had a significance of $p>0.05$. It could be argued that our stringent criteria mean that phosphorylation events will be missed from the relevant groups, but that those present in these groups are strong candidates and more likely to be successfully validated. For the question of motifs, it is reasonable to apply less stringent criteria because the success of the approach relies on larger numbers

of sequences being analysed and picking up patterns, and so in expanding the dataset by removing the p value requirement we increase our chances of finding relevant commonalities.

We were able to pick out a number of common features from our increased sets of peptide sequences. At least a partial Akt motif (RxxS*) was again observed for a relatively higher proportion of phosphopeptides in the mutual or p110α-only categories compared to the p110β-only category, however this was still only for a minority of phosphorylation events (mutual=13/67; p110α=9/66; p110β=7/76). Two other motifs were represented more widely in all categories: the S*P motif (common=24/67; p110α=15/66; p110β=32/76), and a motif characterised by prominence of acidic residues around the phosphosite (mutual=28/67; p110α=29/66; p110β=13/76).

It has been reported the majority of serine/threonine kinases disfavour a protein substrate which contains a proline residue immediately to the C terminus of the phosphorylated residue, but that over a quarter of identified phosphorylation sites are S*P or T*P [174]. The values which we observe are therefore roughly in keeping with other global studies. This motif has a higher relative representation for p110β-regulated than p110α-regulated phosphorylation events, while the opposite is true of acidic motifs. Proline-directed kinases include cyclin-dependent kinase-2 and casein kinase II; the latter displays a preference for acidic motifs [223]. It may be that these kinases or others are more heavily involved in transduction of p110α or p110β than previously appreciated.

These findings may reflect a difference in the effector kinases downstream of p110α and p110β: it could be that p110α is more reliant on Akt (or SGK), while p110β has a different range of effectors. It is known that the upstream input to these kinases is different in terms of RTKs, GPCRs and small GTPases, and it is feasible that this somehow translates to differences downstream.

4.5.1.3. Interpretation of data in a biological pathways context

Another approach in looking for common features in datasets is to analyse connections between identified proteins. Therefore we mapped the interactions

for the proteins (rather than phosphorylation events themselves) within the same datasets used for our motif analysis by using the bio-informatic program STRING, which visually represents previously described interactions between proteins. We observed nodes which were present in all three categories (mutually regulated events, and those regulated only by p110α or p110β). These nodes, though made up of different phosphoproteins, represented transcription- and translation-related processes. These two hubs were the best represented in each dataset, with proteins involved in cell adhesion also forming nodes. The remainder of the components were diverse and unconnected.

Transcription and translation are central to protein synthesis, and are vital cellular processes. It is well established that the PI3K-Akt-mTOR pathway is a significant driver in protein synthesis [79], and so it is not surprising that proteins regulated by each category have these nodes. It may be reasoned from our earlier conclusions that p110α could be producing this effect through Akt, while p110β may well be using an alternative pathway.

It is not entirely unexpected that other nodes did not appear through the observation of more direct interactions. The assay we designed is one which characterises phosphorylation events, but phosphorylation events do not need to occur at each step of a signalling pathway. For instance a phosphorylation event X may occur, and Protein Y which binds this new phosphosite can be regulated spatially and temporally without itself being phosphorylated. Therefore while Protein Y is in fact regulated by our assay, it would not show in our screen. This diverse set of phosphorylation events, and pathways which they regulate, are therefore likely representative of the transduction of the PI3K signal from p110α or p110β.

Unfortunately, we have not been able to relate the observed differences between p110α and p110β in terms of signalling pathways to the differential phenotypes seen by Dr Taboubi. We chose to explore PI3K signalling upon acute activation at early time points and for short periods, and it may be that longer time points or different stimuli are necessary to exactly replicate the conditions used by Dr Taboubi.

We note that we identified a similar proportion of phosphorylation events whose intensity was decreased, as those whose intensity increased. It may seem counterintuitive that inhibition of a kinase or signalling pathway results in an increase in a phosphorylation event, but the complexity of signalling networks which contain feedback loops and other regulatory elements helps to explain this [93]. The PI3K pathway is a prime example of such feedback events: mTORC1 exerts a negative influence over the pathway through a feedback loop, and studies which targeted it for inhibition in cancer models found hyperphosphorylation of Akt [224, 225]. It is therefore likely that many of the phosphorylation events which increase in intensity upon PI3K isoform-inhibition result from such feedback loops which impact on phosphorylation status through direct modulation of other kinases, mechanisms which prevent protein degradation, the activity of phosphatases or access to their phosphorylated substrate.

4.5.2. Validation of phosphorylation events regulated in an isoform-specific manner

We identified a number of phosphorylation events which defined as mutually or differentially regulated in 4T1 cells by p110α and p110β, and decided to validate these by independent means. Western blotting has long been used to analyse abundance of proteins and post-translational modifications, and is a relatively fast, accurate, and inexpensive method using antibodies raised against a specific target. We therefore chose to use it to validate our phosphorylation sites of interest. Unfortunately not all candidates had suitable reagents for analysis by western blotting, which limited our potential choices in terms of targets picked for subsequent analysis. It would have been preferable to validate more targets across more categories (specifically to include mutually regulated and p110β-regulated phosphorylation events) than we were able to, especially in light of the success achieved with the phosphorylation events analysed.

Based on the reagents available, we took three candidate phosphosites for validation: two of which are known components of the PI3K pathway, and a third which could also be a part of that network. These were RS6 and AKTS1, both

previously described direct targets of Akt, and NDRG1 which has been shown to be phosphorylated by SGK1. We used the same conditions and inhibitor treatments as described previously, however in this case we coupled the method to SDS-PAGE and western blot rather than LC-MS/MS. After probing for phosphorylation of each of these proteins, we observed the same patterns of phosphorylation for each candidate as in the MS screen, and the relative ratios of intensity of phosphorylation were also similar between the conditions. That is to say that phosphorylation of both all proteins is reduced much more strongly upon p110α inhibition compared to p110β, although there is some variability between the MS and western blot data. This is to be expected because of the differing sensitivities of the two techniques, but it is encouraging that the same general outcomes are observed.

Direct phosphorylation of both RS6 and AKTS1 by Akt positively regulates protein synthesis. RS6 is a component of ribosomes, organelles which are responsible for translation. Its phosphorylation at Ser236 has been shown to be directed by Akt [87, 226], an event which activates the protein and hence promotes protein synthesis. AKTS1, also known as PRAS40, is a negative regulator of mTOR [60, 87]. The mTOR pathway positively regulates protein synthesis amongst other pathways, and phosphorylation of Thr247 on AKTS1 by Akt promotes its binding to 14-3-3 and hence sequestration away from mTOR, thereby allowing activation of the mTOR pathway and protein synthesis [59, 60].

NDRG1 is a tumour suppressor involved in cell growth and differentiation. Ser330 of NDRG1 has previously been shown to be a direct target of SGK1, and phosphorylation primes NDRG1 for subsequent phosphorylation by GSK3 [216].

That inhibition of p110α has a greater effect on the phosphorylation of previously described Akt and SGK1 substrates than inhibition of p110β, and that it also appears to regulate more phosphorylation events with Akt/SGK1 motifs, are interesting findings. While it is known that p110α and p110β have differential signalling inputs, they both produce PIP_3 and explanations for non-redundant processes are not complete. The evidence which we put forward for

p110α relying more on Akt to transduce its signal could go some way to explaining the differences in the phenotypic outputs of the two isoforms.

4.5.3. Cross species comparison of phosphorylation events regulated in an isoform-specific manner

After validating in a 4T1 cell model a number of phosphorylation events from our MS screen by western blot, we proceeded to investigate the phosphorylation status of these events in a panel of other breast cancer cell models. These cell lines were used originally in experiments by Dr Taboubi which revealed differential phenotypes upon inhibition of p110α or p110β, and differ in the mutational status of several common oncogenes, including *PIK3CA*. We treated the panel of cell lines in the same way as during the validation of candidate phosphorylation events in the 4T1 cell line, and probed for the same phosphosites.

Compared to 4T1 cells, we observed different signatures in terms of phosphorylation events analysed in the three human cell lines studied. While some significant similarities were observed with 4T1 cells, there were also clear differences, for instance phosphorylation of NDRG1 was significantly decreased upon inhibition of p110α but not p110β in MCF7 and MDA-MB-231 cells, while little difference was seen following inhibition of either isoform in MDA-MB-453 cells. Indeed all other changes upon isoform-inhibition are much less striking than this effect on NDRG1, and it is clear that the phosphorylation of PRAS40 and S6 is less dependent on p110α in these three cell lines than in 4T1 cells. It would be interesting to compare the effects of Akt inhibition on these phosphorylation events in light of these cell line-specificities, to determine whether or not they are Akt-dependent in 4T1 cells and if this dependence remains in other cell lines but is directed by something other than p110α. The variation observed between the cell lines may be in part accounted by the differences in species of origin, in that 4T1 cells are murine and the remaining three are derived from human breast tissue. However it is also interesting to note the differences in phosphorylation signatures observed between the human cell lines themselves.

It is challenging to account for these observations in terms of the genetics of the PI3K pathway; all four cell lines are positive for PTEN, and MCF7, MDA-MB-231 and MDA-MB-453 have p110α that is oncogenic (E545K), wild-type, and oncogenic (H1047R) respectively, and the differences in phosphorylation signature to not display a clear pattern. It is likely that the variations are due to the interplay between these and other key signalling proteins, such as those listed in Table 4.5, though it would be necessary to characterise the pathways further to explore this hypothesis.

5. Discussion

The PI3K pathway is central to both normal cellular and disease signalling. Its control over crucial cellular functions such as metabolism, growth and survival mean that a better understanding of this signalling pathway can contribute to our understanding of the cell in both health and disease. This project sought to investigate aspects of the PI3K pathway which are incompletely understood.

Modern proteomic techniques provide opportunities to address questions which have until now proven difficult to answer by more traditional methods. They allow interrogation of the phosphoproteome of a given pathway, process or condition on a global scale. In an unbiased manner, these novel techniques allow the characterisation of both new and previously described relationships, as well as elucidation of quantitative data for thousands of phosphopeptides. We therefore used mass spectrometry-based phosphoproteomics to explore signalling pathways downstream of class I PI3K isoforms.

The protein kinase activity of p110α was first reported over a decade ago, yet a comprehensive and unbiased assessment of its potential substrates has been lacking. We have used novel and unbiased quantitative phosphoproteomic techniques to globally examine targets of p110α protein kinase activity, and identified two potential novel substrates (Chapter 3).

Another poorly understood aspect of PI3K signalling is the difference between downstream signalling of the p110α and p110β isoforms of PI3K. We applied a similar unbiased and quantitative approach to examine the phosphoproteomes of mouse 4T1 breast cancer cells after inhibition of either p110α or p110β. We found evidence of phosphopeptides which were mutually or exclusively controlled by p110α or p110β and were able to validate these findings by independent means. Furthermore, we showed that the control of these phosphorylation events varies between cell types (Chapter 4).

5.1. Investigation into the protein kinase activity of p110α

The work described in Chapter 3 aimed to identify novel substrates of the protein kinase activity of p110α using an unbiased quantitative MS-based

phosphoproteomic approach, after which we sought to validate candidate proteins by independent means.

We performed *in vitro* kinase assays using radiolabelled ATP to monitor phosphorylation of p85α as a readout of p110α protein kinase activity. This allowed us to define the optimum conditions for robust p110α protein kinase activity for use in our MS-based screen. We observed robust p85α phosphorylation from our shortest time-point (1 min) in the presence of one or both of the cofactors Mg^{2+} and Mn^{2+}.

Employing a MEF cell lysate as a substrate for *in vitro* protein kinase assays with recombinant p110α/p85α protein, we used a time-point of 5 min to ensure good p110α protein kinase activity but also to also minimise non-specific phosphorylation events due to other protein kinases present In the sample. We included both Mg^{2+} and Mn^{2+} as cofactors, and were able to identify and quantify the resulting 641 phosphopeptides. This is a relatively low number when compared to previous experiments in our laboratory using Akt kinase, and suggests that the protein kinase activity of p110α is low, which could be inferred from the sparse number of published substrates in the literature. We were also unable to identify previously described p110α protein kinase substrates, though p85α was observed in pilot studies. Despite observing robust activation of p110α protein kinase activity in our radiometric *in vitro* kinase assays, the different conditions which were used when using a MEF cell lysate as a substrate may mean that the optimal conditions were not met. It would be of use to explore this future (discussed later).

PCA and heatmaps based on all 641 phosphopeptides also suggested that the effect of the protein kinase activity of p110α in this context was minor, and that only a few phosphorylation events were modulated in the samples using recombinant p110α. Phosphorylation of a large number of peptides was also seen in the control samples (cell lysate without p110α kinase, or p110α and its specific inhibitor A66). This is interesting and unexpected. It could be argued that the high levels of phosphorylation present in the control samples is due to incomplete dephosphorylation before the *in vitro* kinase assay. However there is variability between the two sets of control samples and the recombinant p110α-

treated samples, and since the substrate for all assays was the same MEF cell lysates, the same patterns of phosphorylation would be expected to be seen. It would be of benefit to have some of this starting material to reanalyse, but all was used for the initial MS assay. Since this is not the case the variability is not accounted for, though it should be acknowledged that incomplete dephosphorylation may play a part. Equally, kinases which are present in the cell lysate and which are more active or more potent than p110α may contribute to the high background phosphorylation. Though the same MEF cell lysate is used as a substrate, a different mixture of kinase-substrate interactions will occur in each sample, leading to the different observed phosphorylation events. It is likely that this, along with incomplete dephosphorylation, plays a role in the high and variable background phosphorylation.

Following identification and quantification of these phosphopeptides, we next proceeded to define criteria to categorise proteins as potential p110α substrates. We reasoned that a true kinase-substrate interaction would see intensity of phosphorylation increase with ATP concentration in our assays. Therefore we defined criteria concerning this correlation to exclude phosphorylation events which did not see this positive trend of ATP concentration with phosphopeptide intensity in the p110α-treated samples. This relatively low stringency measure alongside manual verification of the profiles of candidates to ensure that the control samples did not see significant peptide phosphorylation, resulted in only two phosphopeptides being chosen as suitable for validation as p110α substrates, in agreement with the above proposition that our system observed weak p110α protein kinase activity. The candidate proteins were GRIA1, a membrane receptor; and FBX7, a protein involved in ubiquitylation.

Following the classification of phosphosites, we moved on to use the available data of intensity of phosphorylation at a given ATP concentration to profile the two candidates based on reaction kinetics and efficiency of phosphorylation. We estimated affinity of the kinase-substrate interaction by using the three ATP concentration points present for each sample set (cell lysate only; recombinant p110α+A66; recombinant p110α), and we found p110α-GRIA1 to be the stronger partnership. In addition to this, we analysed the sequence homology of

the amino acid residues around the phosphosites of interest. A functional phosphosite will often be conserved across species, and we reasoned that sequence analysis might provide information as to the relevance of the site, with areas of primary protein structure which are superfluous to function being less likely to be evolutionarily conserved. By way of reference, the Ser608 residue of p85α which is phosphorylated by p110α has 100% conservation for the 30 residue fragment surrounding it for the species which we looked at (mouse, human, rat, bull). We found the phosphorylations under analysis for GRIA1 to have strong (90%) homology across four analysed species, while 33% of residues were conserved for FBX7.

We then proceeded to attempt to validate our two candidate proteins as p110α substrates by independent means. Ideally validation would take the form of *in vitro* kinase assays using recombinant p110α and [γ-32P]-ATP, and the protein of interest. Unfortunately neither candidate was available as a recombinant protein, and so we instead produced peptide fragments which included the phosphosites of interest for use as substrates in these kinase assays. Following incubation of p110α, peptide substrate and [γ-32P]-ATP, we then utilised SDS-PAGE to separate, visualise and quantify peptides and phosphorylation. However due to the small molecular weight of the peptides and their migration close to the [γ-32P]-ATP used in the assay, this approach proved unsuccessful, as any phosphorylation present was masked by the large signal from the free [γ-32P]-ATP. Therefore we attempted validation using an alternative means, and employed scintillation counting which uses *in vitro* kinase assays performed in a similar manner, though is able to capture [γ-32P]-ATP bound to the peptides and discard unbound [γ-32P]-ATP, thereby theoretically allowing a “cleaner” quantification. Here, we observed incorporation of radiolabelled ATP into our peptides of interest as well as control p85α peptides, and the levels of incorporation decreased when using serine-to-alanine mutants, suggesting that our candidates may in fact be substrates of p110α. The relative levels of incorporation were however low, in keeping with the theory that p110α is a relatively weak protein kinase.

In conclusion, we provided evidence for novel substrates of the protein kinase activity of p110α. However, we also suggest that this activity may be limited a)

in the context of this experiment due to investigation of the basal state and lack of stimulation, or b) more generally in a physiological setting.

5.2. Investigation into differential p110α and p110β signalling in the mouse 4T1 breast cancer cell line model

The work described in Chapter 4 aimed to compare the phosphoproteomes regulated by the p110α and p110β class IA isoforms of PI3K. With the goal of gaining insight in the remarkable differences in cell biological phenotypes observed upon inhibition of these PI3K isoforms in the 4T1 murine breast cancer cell line, we compared downstream signalling of p110α and p110β in an unbiased manner using MS-based phosphoproteomics. We used the same experimental design which produced differential phenotypes following isoform inhibition, and so limited ourselves in this respect.

Comparing serum-starved cells with those which had been starved and stimulated, and those which had additionally been treated with inhibitors of p110α or p110β allowed us to identify mutually and differentially regulated phosphopeptides. We used the specific inhibitors A66 and TGX221 to inhibit p110α and p110β respectively, and analysed the signalling profiles in terms of phosphorylation events downstream of these isoforms. These inhibitors have been shown to be specific for the relevant isoforms through *in vitro* testing of panels of kinases, though the possibility exists that they exhibit off-target inhibition of other kinases in the more complex cell model which we employed. Therefore we moved to validate a number of our results and were successful, though these caveats must be kept in mind for the wider dataset. As mentioned previously, we followed an experimental design which had previously shown interesting results and so chose only one concentration for each inhibitor though it is possible that the same resources could have been distributed differently, for example by having two concentrations per inhibitor and three rather than six replicates.

We defined arbitrary criteria to help us categories phosphopeptides as being downstream of either p110α, p110β, or both. It is desirable to have criteria which minimise false negatives and positives, and this is difficult to completely

achieve without modelling or attempts at validation. Using the criteria which we defined, we made the following main observations:

(1) more phosphorylation events are differentially than mutually controlled. This is surprising given the similarities in upstream signalling and cellular processes controlled by the two PI3K isoforms. It is also interesting to note that phosphorylation of the previously characterised Akt substrates AKTS1 and ribosomal protein S6 appear to be under the control of p110α but not p110β, and that the phosphorylation events share a functional output and serve to promote protein synthesis.

(2) many phosphorylation sites were found to be increased upon PI3K inhibition. It is possible that this is a consequence of the induction of feedback loops that turn off the initial pathways, a phenomenon frequently observed in the PI3K pathway.

It was also interesting to note that phosphorylation sites which we defined as being positively regulated by both p110α and p110β, or by p110α alone, usually had low intensity of phosphorylation in the serum-starved state, high intensity in the serum+DMSO control, and low intensity after isoform inhibition. However those phosphopeptides which we defined as being positively regulated by p110β only usually displayed high intensity of phosphorylation in the serum-starved basal state. This could be related to the suggestion that p110α is responsible for acute signalling and the response to stimuli, while p110β is involved in tonic signalling.

Following categorisation, moved on to analyse the groups of phosphorylation sites. Due to the importance of sequence motifs for substrate recognition by kinases, we interrogated the primary sequences around our phosphorylation sites of interest for any signs of consensus. In a targeted analysis, we found the Akt motif in a third of all peptides which we defined as being positively regulated by our PI3K isoforms, though the majority of these came from phosphorylation events regulated by both p110α and p110β, or by p110α alone. This suggests a reliance of p110α on Akt for mediation of its signalling, whereas p110β perhaps relies on other signal transducers.

We then looked in an unbiased way for any motifs in the peptides within our categories, and found motifs associated with proline residues adjacent to the phosphosite, and phosphosites rich in acidic residues nearby. These types of motifs have been shown to be recognised by cyclin-dependent kinase 2 and casein kinase II respectively, and it may be that these kinases are important in PI3K signalling in this context.

We next attempted to uncover links between the proteins in our categories in terms of function and mutual processes. Though the categories contain different phosphosites and proteins, similar processes were found each time, namely transcription, translation, and cell adhesion. It is unsurprising that these nodes were found as the importance of the PI3K-Akt-mTOR pathway here is well-established.

We then proceeded to take some candidate phosphorylation events for validation by independent methods. Western blotting has long been used as a relatively simple way of analysing protein expression and post-translational modifications, and it is frequently the first choice in terms of follow up for such studies. Unfortunately we were limited in terms of the number of candidates which we could validate due to a lack of antibodies to either the proteins or phosphosites of interest, and it would have been preferable to validate more candidates and across more categories. It could be the case that subsequent studies could use mass spectrometry as a tool to look for the phosphorylation of a specific protein in a targeted way, and hence a wider range of candidates could be assayed.

Of the proteins which displayed a dependence on p110α in the MS screen, we used western blotting to successfully confirm the p110α-specific role in regulation of phosphorylation of the 40S ribosomal protein S6 (pSer256), AKTS1 (pThr257), and NDRG1 (pSer330) in 4T1 cells. The first two phosphorylation events are known to be under the control of PI3K, and promote protein synthesis. The validation of these substrates and the suggestion of p110α rather than p110β displaying a preference for Akt is in line with our motif analyses which imply a similar result. It may be that differential use of Akt is a means of non-redundant p110α/p110β signalling.

Having made novel observations in 4T1 cells, we next analysed the extent and profile of phosphorylation of the same panel of proteins in other breast cancer cell lines with different genetic backgrounds. We found that each cell line differed in terms of the dependence on p110α or p110β of phosphorylation of the proteins assessed, for reasons that are as yet unclear. This is remarkable, and points towards cell line-specific regulation of p110α and p110β signal transduction pathways.

Unfortunately, we were unable to ascribe the original observations of Dr Taboubi of differential phenotypes following inhibition of p110α or p110β to specific phosphorylation events or defined pathways. While this is somewhat disappointing we have still explored the differences in downstream signalling of the two PI3K isoforms, and uncovered specific and general aspects of differential signalling, indicating a greater complexity of the PI3K pathway than previously appreciated.

In summary, we have provided evidence of differential regulation of signalling pathways downstream of p110α and p110β.

5.3. Implications of study and future directions

Our objectives focused on two main parts: to characterise the protein kinase activity of the PI3K isoform p110α, and to compare and contrast the signalling pathways downstream of p110α and p110β.

Addressing the first point, our strategy of coupling an *in vitro* kinase assay to MS-based phosphoproteomic methods identified and quantified hundreds of phosphorylation events but it turned out to be challenging to ascribe these to selective activity of the kinase under study, namely p110α PI3K. Due to the relatively poor characterisation of p110α as a protein kinase, little is known about the upstream input that may be required to stimulate the activity of this kinase. This means that planning an experiment such as the one carried in Chapter 3 is not a trivial task, and there will always be an unknown element relating to whether or not the p110α protein kinase has been sufficiently activated. While it is likely that the conditions chosen for our *in vitro* kinase assay are the optimum for our scenario and this is supported by the data, it may

well be the case that there are additional requirements in order to observe phosphorylation of physiologically relevant substrates. For instance, stimuli such as growth factors may be necessary, or an association with membranes or scaffolds which induce or enhance the protein kinase activity of p110α, and further examination of optimal conditions could be carried out.

It may however be possible to redesign the assay with different controls or comparisons, with the aim of making true p110α substrates more obvious within a dataset. The levels of background within the control samples should be as low as possible to help valid substrates stand out. This could be better achieved by adding steps with the aim of completely removing ATP, such as extra fractionation or purification of the cell extract, such as further size exclusion. Additionally, dephosphorylation of the cell lysate may benefit from the use of additional phosphatases or longer time points.

Two controls are present in our MS-based assay: cell lysate only, and cell lysate plus recombinant p110α and its specific inhibitor A66. We used recombinant kinase-dead p110α in pilot experiments, but this was unavailable at the time the MS-based assay was performed. We instead used A66, but this is an added variable in our system. Were kinase-dead p110α available, it would be interesting to use it in future experiments. It can be challenging to achieve an experimental setup which optimally assays kinase activity, especially so with a protein kinase as poorly characterised as p110α. Improvements could be made learning from the experiments described here: an experimental redesign could also compare the full or scaled down version of the current assay, with a related variation such as one which altered the amount of recombinant kinase added rather than ATP concentration, or one which used enzymatically digested proteins as a substrate, such as described by Xue *et al.* [219]. In this setting, a redesign would mean that the two experiments complement and act as a validation for each other, but would also increase time taken and cost.

We have provided evidence for novel substrates of the protein kinase activity of p110α – GRIA1 and FBX7. Estimation of reaction kinetics and analysis of the degree sequence conservation suggested GRIA1 to be a stronger candidate, or a stronger p110α substrate. While our options have been limited in terms of

validation of these substrates and attempts at validation hampered, it would still be of use to continue these efforts and examine the kinase-substrate relationship further. Attempts at validation with SDS-PAGE proved inconclusive, and the findings of *in vitro* assays with peptides do not rule out a valid interaction.

Having the substrates available as recombinant proteins would be extremely valuable. Initial validation is more relevant when using full length recombinant proteins because protein secondary and tertiary structures can impact on a kinase-substrate interaction, and further investigation of the relationship is also aided. Though time and cost will be factors, it would be hugely beneficial to future experiments to produce these either commercially or in-house through e.g. bacterial or yeast protein expression systems. *In vitro* kinase assays using recombinant full length protein substrate in place of peptides would provide more evidence for a relationship, and these tools could be used alongside A66 to analyse biochemical characteristics such as reaction stoichiometry and kinetics. Additionally, comparison of the extent of phosphorylation of these substrates using wild-type p110α or the E545K/H1047R oncogenic mutants as the kinase would also be interesting and informative. Looking much further ahead, raising specific antibodies to the proteins and phosphorylation sites of interest would be a useful exercise so that *in vivo* studies could be carried out which would enable questions around physiological relevance and upstream stimuli to be probed.

As suggested previously, our results are in keeping with what may be inferred from the literature, in that the protein kinase activity of p110α is likely to be limited in a physiological setting. While the existence of p110α protein kinase activity has been previously established, it may be that this is dependent on a narrow set of as yet determined criteria and stimuli.

To address our second aim, we used a 4T1 mouse breast cancer cell line model to compare the phosphoproteomes of p110α- or p110β-inhibited conditions, and found evidence of mutual and differential signalling.

Based on motif analyses, as expected we found that Akt appears to play a role in a number of phosphorylation events downstream of both p110α and p110β,

but in neither case is it responsible for the majority of phosphorylations. We suggest that this is due to 1) some of these events being carried out at levels below the primary effector kinase of PI3K function and 2) other kinases such as SGK1 playing a role alongside Akt in transducing the PI3K signal. It would therefore be interesting to characterise these pathways more completely. Initial work has been done to validate some identified phosphorylation events, but lack of biochemical tools has meant that this was limited – in future it would be attractive to revisit these results. Were specific antibodies to the proteins and phosphorylation sites identified within our screen available, these would be valuable tools in validating our results more widely, and across more of our defined categories. The success of the validation which we carried out is a strong justification for continuing these efforts if the necessary tools become available. We focused our analysis on phosphorylation events which were positively regulated by PI3Ks, and it would also be worthwhile to investigate further phosphorylation events which are negatively regulated by PI3Ks, though extensive characterisation of these would likely be harder.

The validation and analyses which we conducted stemmed from our initial approach of interrogating signalling pathways downstream of p110α and p110β in an untargeted manner. These analyses suggested that p110α signals through Akt more than p110β does, and so it would be interesting to revisit this area in a more objective manner. Akt-specific inhibitors exist, such as the compound MK-2206, and could be used in conjunction with PI3K isoform-specific inhibitors to further dissect these pathways.

Additionally, while we have identified and validated previously described Akt substrates as being differentially regulated by p110α and p110β, there remain many other Akt substrates which could be specifically assayed in a similar manner to those described in this chapter. For example phosphorylation of GSK3, Bad and TSC2 among others could be analysed, to examine whether or not specific pathways downstream of Akt are differentially regulated. Phosphorylation of proteins such as TSC2 and AKTS1 could be interrogated in parallel to look at the extent of functional redundancy in the system, as phosphorylation of either negatively impacts on the ability of the protein to inhibit mTOR.

Exploration of these networks in terms of effector kinases active downstream of p110α and p110β would also be a valuable prospect in order to better understand the extent to which Akt, SGK1 and other kinases transduce the signalling of individual PI3K isoforms, a task which would have additional benefits in a clinical setting with PI3K and Akt inhibitors in trials for various diseases.

We were unable to assign specific phosphorylation events to the differential observed phenotypes which led to this study, but it would still be of benefit to continue efforts in this area. We chose a relatively short time point for inhibition of p110α or p110β which included serum starvation and a short stimulation, but this could be altered to mimic the longer time points at which the different phenotypes were seen. Such further experiments could obviously complement the current study, and it would be interesting to look at the phosphorylation of events identified in these experiments in such a context.

An attractive future direction would be to analyse the differences in signalling of both p110α and p110β through Akt and other effector kinases, alongside p110δ and/or p110γ in an immunological background. Additionally, while we focused our attention on the 4T1 cell line specifically and on breast cancer cells in general, there are obvious questions as to whether or not these patterns of phosphorylation and phenotypes would be seen in other cell types. Our results have demonstrated the general principle that signalling downstream of p110α and p110β is likely to be more different than perhaps originally appreciated, and given specific examples of this divergence.

5.4. Concluding remarks

While the importance of the PI3K pathway in both normal and pathological cellular processes is well established, there remain a number of features of signalling which remain underexplored and unclear. We chose to specifically target two of these lesser-known aspects of PI3K pathway and characterise them further: namely the protein kinase activity of p110α, and the differences between the downstream signalling of the two ubiquitous class IA PI3K isoforms p110α and p110β.

To achieve our aims, we used mass spectrometry-based phosphoproteomics and employed these powerful tools to address fundamental questions in a way which has not previously been possible. We have proposed novel protein substrates of p110α, and uncovered differences between signalling of p110α and p110β in terms of specific substrates and on a larger scale than perhaps previously appreciated. These experiments build on previous knowledge, and provide opportunities for further exploration.

Bibliography

1. Vanhaesebroeck B, et al. *The emerging mechanisms of isoform-specific PI3K signalling*. Nature Reviews Molecular Cell Biology. 2010; **11**(5): p.329-41.
2. Cantley LC. *The phosphoinositide 3-kinase pathway*. Science. 2002; **296**(5573): p.1655-7.
3. Kok K, et al. *Regulation of phosphoinositide 3-kinase expression in health and disease*. Trends in Biochemical Sciences. 2009; **34**(3): p.115-27.
4. Domin J, et al. *Cloning of a human phosphoinositide 3-kinase with a C2 domain that displays reduced sensitivity to the inhibitor wortmannin*. Biochemical Journal. 1997; **326**: p.139-47.
5. Domin J, et al. *The class II phosphoinositide 3-kinase PI3K-C2 beta regulates cell migration by a PtdIns(3)P dependent mechanism*. J Cell Physiol. 2005; **205**(3): p.452-62.
6. Falasca M, et al. *The role of phosphoinositide 3-kinase C2 alpha in insulin signaling*. Journal of Biological Chemistry. 2007; **282**(38): p.28226-36.
7. Backer JM. *The regulation and function of Class III PI3Ks: novel roles for Vps34*. Biochemical Journal. 2008; **410**: p.1-17.
8. Kihara A, et al. *Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in Saccharomyces cerevisiae*. Journal of Cell Biology. 2001; **152**(3): p.519-30.
9. Vieira OV, et al. *Distinct roles of class I and class III phosphatidylinositol 3-kinases in phagosome formation and maturation*. Journal of Cell Biology. 2001; **155**(1): p.19-25.
10. Vanhaesebroeck B, et al. *PI3K signalling: the path to discovery and understanding*. Nature reviews Molecular cell biology. 2012; **13**(3): p.195-203. Epub 2012/02/24.
11. Kodaki T, et al. *The activation of phosphatidylinositol 3-kinase by Ras*. Curr Biol. 1994; **4**(9): p.798-806.
12. Rordorfnikolic T, et al. *Regulation of phosphatidylinositol 3'-knase by tyrosyl phosphoproteins - full activation requires occupancy of both SH2 domains in the 85-kDa regulatory subunit*. Journal of Biological Chemistry. 1995; **270**(8): p.3662-6.
13. Brock C, et al. *Roles of G beta gamma in membrane recruitment and activation of p110 gamma/p101 phosphoinositide 3-kinase gamma*. Journal of Cell Biology. 2003; **160**(1): p.89-99.
14. Yu JH, et al. *Regulation of the p85/p110 phosphatidylinositol 3 '-kinase: Stabilization and inhibition of the p110 alpha catalytic subunit by the p85 regulatory subunit*. Molecular and Cellular Biology. 1998; **18**(3): p.1379-87.
15. Vanhaesebroeck B, et al. *Signalling by PI3K isoforms: insights from gene-targeted mice*. Trends in Biochemical Sciences. 2005; **30**(4): p.194-204.
16. Balla T. *Phosphoinositides: Tiny Lipids With Giant Impact on Cell Regulation*. Physiological Reviews. 2013; **93**(3): p.1019-137.
17. Harlan JE, et al. *PLECKSTRIN HOMOLOGY DOMAINS BIND TO PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE*. Nature. 1994; **371**(6493): p.168-70.
18. Bi L, et al. *Proliferative defect and embryonic lethality in mice homozygous for a deletion in the p110 alpha subunit of phosphoinositide 3-kinase*. Journal of Biological Chemistry. 1999; **274**(16): p.10963-8.
19. Bi L, et al. *Early embryonic lethality in mice deficient in the p110 beta catalytic subunit of PI 3-kinase*. Mammalian Genome. 2002; **13**(3): p.169-72.
20. Clayton E, et al. *A crucial role for the p110 delta subunit of phosphatidylinositol 3-kinase in B cell development and activation*. J Exp Med. 2002; **196**(6): p.753-63.
21. Hirsch E, et al. *Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation*. Science. 2000; **287**(5455): p.1049-53.
22. Foukas LC, et al. *Phosphoinositide 3-kinase: the protein kinase that time forgot*. Biochemical Society Transactions. 2004; **32**: p.330-1.

23. Foukas LC, et al. *Regulation of phosphoinositide 3-kinase by its intrinsic serine kinase activity in vivo*. Molecular and Cellular Biology. 2004; **24**(3): p.966-75.
24. Christoforidis S, et al. *Phosphatidylinositol-3-OH kinases are Rab5 effectors*. Nature Cell Biology. 1999; **1**(4): p.249-52.
25. Patrucco E, et al. *PI3K gamma modulates the cardiac response to chronic pressure overload by distinct kinase-dependent and -independent effects*. Cell. 2004; **118**(3): p.375-87.
26. Garcia Z, et al. *A PI3K activity-independent function of p85 regulatory subunit in control of mammalian cytokinesis*. Embo Journal. 2006; **25**(20): p.4740-51.
27. Chamberlain MD, et al. *Disrupted RabGAP function of the p85 subunit of phosphatidylinositol 3-kinase results in cell transformation*. Journal of Biological Chemistry. 2008; **283**(23): p.15861-8.
28. Rodriguezviciana P, et al. *Phosphatidylinositol-3-OH kinase as a direct target of Ras*. Nature. 1994; **370**(6490): p.527-32.
29. RodriguezViciana P, et al. *Activation of phosphoinositide 3-kinase by interaction with Ras and by point mutation*. Embo Journal. 1996; **15**(10): p.2442-51.
30. Stoyanov B, et al. *Cloning and Characterisation of a G-Protein-activated Human Phosphoinositide-3 Kinase*. Science. 1995; **269**(5224): p.690-3.
31. Fritsch R, et al. *RAS and RHO Families of GTPases Directly Regulate Distinct Phosphoinositide 3-Kinase Isoforms*. Cell. 2013; **153**(5): p.1050-63.
32. Burke JE, et al. *Dynamic steps in receptor tyrosine kinase mediated activation of class IA phosphoinositide 3-kinases (PI3K) captured by H/D exchange (HDX-MS)*. Advances in Biological Regulation. 2013; **53**(1): p.97-110.
33. Okkenhaug K. *Signaling by the Phosphoinositide 3-Kinase Family in Immune Cells*. Annual Review of Immunology, Vol 31. 2013; **31**: p.675-704.
34. Walser R, et al. *PKC beta Phosphorylates PI3K gamma to Activate It and Release It from GPCR Control*. Plos Biology. 2013; **11**(6).
35. Kurosu H, et al. *Heterodimeric phosphoinositide 3-kinase consisting of p85 and p110 beta is synergistically activated by the beta gamma subunits of G proteins and phosphotyrosyl peptide*. Journal of Biological Chemistry. 1997; **272**(39): p.24252-6.
36. Chan TO, et al. *Small GTPases and tyrosine kinases coregulate a molecular switch in the phosphoinositide 3-kinase regulatory subunit*. Cancer Cell. 2002; **1**(2): p.181-91.
37. Kurig B, et al. *Ras is an indispensable coregulator of the class I-B phosphoinositide 3-kinase p87/p110 gamma*. Proceedings of the National Academy of Sciences of the United States of America. 2009; **106**(48): p.20312-7.
38. Shin HW, et al. *An enzymatic cascade of Rab5 effectors regulates phosphoinositide turnover in the endocytic pathway*. Journal of Cell Biology. 2005; **170**(4): p.607-18.
39. Saarikangas J, et al. *Regulation of the Actin Cytoskeleton-Plasma Membrane Interplay by Phosphoinositides*. Physiological Reviews. 2010; **90**(1): p.259-89.
40. Alessi DR, et al. *Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase B alpha*. Curr Biol. 1997; **7**(4): p.261-9.
41. Manning BD, et al. *AKT/PKB signaling: Navigating downstream*. Cell. 2007; **129**(7): p.1261-74.
42. Scheffzek K, et al. *Pleckstrin homology (PH) like domains - versatile modules in protein-protein interaction platforms*. FEBS Lett. 2012; **586**(17): p.2662-73.
43. Cho H, et al. *Akt1/PKB alpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice*. Journal of Biological Chemistry. 2001; **276**(42): p.38349-52.
44. Cho H, et al. *Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta)*. Science. 2001; **292**(5522): p.1728-31.
45. Easton RM, et al. *Role for Akt3/Protein kinase B gamma in attainment of normal brain size*. Molecular and Cellular Biology. 2005; **25**(5): p.1869-78.

46. Alessi DR, et al. *Mechanism of activation of protein kinase B by insulin and IGF-1*. Embo Journal. 1996; **15**(23): p.6541-51.
47. Sarbassov DD, et al. *Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex*. Science. 2005; **307**(5712): p.1098-101.
48. Pearce LR, et al. *The nuts and bolts of AGC protein kinases*. Nature Reviews Molecular Cell Biology. 2010; **11**(1): p.9-22.
49. Andjelkovic M, et al. *Activation and phosphorylation of a pleckstrin homology domain containing protein kinase (RAC-PK/PKB) promoted by serum and protein phosphatase inhibitors*. Proceedings of the National Academy of Sciences of the United States of America. 1996; **93**(12): p.5699-704.
50. Meier R, et al. *Inactivation and dephosphorylation of protein kinase B alpha (PKB alpha) promoted by hyperosmotic stress*. Embo Journal. 1998; **17**(24): p.7294-303.
51. Brognard J, et al. *PHLPP and a second isoform, PHLPP2, differentially attenuate the amplitude of Akt signaling by regulating distinct Akt isoforms*. Molecular Cell. 2007; **25**(6): p.917-31.
52. Han EKH, et al. *Akt inhibitor A-443654 induces rapid Akt Ser-473 phosphorylation independent of mTORC1 inhibition*. Oncogene. 2007; **26**(38): p.5655-61.
53. Lin K, et al. *An ATP-Site On-Off Switch That Restricts Phosphatase Accessibility of Akt*. Science Signaling. 2012; **5**(223).
54. Alessi DR, et al. *Molecular basis for the substrate specificity of protein kinase B; Comparison with MAPKAP kinase-1 and p70 S6 kinase*. FEBS Lett. 1996; **399**(3): p.333-8.
55. Brunet A, et al. *Akt promotes cell survival by phosphorylating and inhibiting a forkhead transcription factor*. Cell. 1999; **96**(6): p.857-68.
56. Inoki K, et al. *TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling*. Nature Cell Biology. 2002; **4**(9): p.648-57.
57. Manning BD, et al. *Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-Kinase/Akt pathway*. Molecular Cell. 2002; **10**(1): p.151-62.
58. Potter CJ, et al. *Akt regulates growth by directly phosphorylating Tsc2*. Nature Cell Biology. 2002; **4**(9): p.658-65.
59. Kovacina KS, et al. *Identification of a proline-rich Akt substrate as a 14-3-3 binding partner*. Journal of Biological Chemistry. 2003; **278**(12): p.10189-94.
60. Vander Haar E, et al. *Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40*. Nature Cell Biology. 2007; **9**(3): p.316-U126.
61. Laplante M, et al. *mTOR Signaling in Growth Control and Disease*. Cell. 2012; **149**(2): p.274-93.
62. delPeso L, et al. *Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt*. Science. 1997; **278**(5338): p.687-9.
63. Gardai SJ, et al. *Phosphorylation of Bax Ser(184) by Akt regulates its activity and apoptosis in neutrophils*. Journal of Biological Chemistry. 2004; **279**(20): p.21085-95.
64. Mayo LD, et al. *A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus*. Proceedings of the National Academy of Sciences of the United States of America. 2001; **98**(20): p.11598-603.
65. Tzivion G, et al. *FoxO transcription factors; Regulation by AKT and 14-3-3 proteins*. Biochimica Et Biophysica Acta-Molecular Cell Research. 2011; **1813**(11): p.1938-45.
66. Cross DAE, et al. *Inhibition of glycogen-synthase kinase-3 by insulin-mediated by protein-kinase-B*. Nature. 1995; **378**(6559): p.785-9.
67. Yeh E, et al. *A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells*. Nature Cell Biology. 2004; **6**(4): p.308-18.
68. Diehl JA, et al. *Glycogen synthase kinase 3 beta regulates cyclin D1 proteolysis and subcellular localization*. Genes Dev. 1998; **12**(22): p.3499-511.

69. Deprez J, et al. *Phosphorylation and activation of heart 6-phosphofructo-2-kinase by protein kinase B and other protein kinases of the insulin signaling cascades*. Journal of Biological Chemistry. 1997; **272**(28): p.17269-75.
70. Li X, et al. *Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1 alpha transcription coactivator*. Nature. 2007; **447**(7147): p.1012-U8.
71. Elstrom RL, et al. *Akt stimulates aerobic glycolysis in cancer cells*. Cancer Research. 2004; **64**(11): p.3892-9.
72. Warburg O. *Origin of cancer cells*. Science. 1956; **123**(3191): p.309-14.
73. Sano H, et al. *Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation*. Journal of Biological Chemistry. 2003; **278**(17): p.14599-602.
74. Ravichandran LV, et al. *Phosphorylation of PTP1B at Ser(50) by Akt impairs its ability to dephosphorylate the insulin receptor*. Molecular Endocrinology. 2001; **15**(10): p.1768-80.
75. Majumder PK, et al. *mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways*. Nature Medicine. 2004; **10**(6): p.594-601.
76. Kim AH, et al. *Akt phosphorylates and negatively regulates apoptosis signal-regulating kinase 1*. Molecular and Cellular Biology. 2001; **21**(3): p.893-901.
77. Rommel C, et al. *Differentiation stage-specific inhibition of the Raf-MEK-ERK pathway by Akt*. Science. 1999; **286**(5445): p.1738-41.
78. Zimmermann S, et al. *Phosphorylation and regulation of Raf by Akt (protein kinase B)*. Science. 1999; **286**(5445): p.1741-4.
79. Wullschleger S, et al. *TOR signaling in growth and metabolism*. Cell. 2006; **124**(3): p.471-84.
80. Guertin DA, et al. *Defining the role of mTOR in cancer*. Cancer Cell. 2007; **12**(1): p.9-22.
81. Zoncu R, et al. *mTOR: from growth signal integration to cancer, diabetes and ageing*. Nature Reviews Molecular Cell Biology. 2011; **12**(1): p.21-35.
82. Peterson TR, et al. *DEPTOR Is an mTOR Inhibitor Frequently Overexpressed in Multiple Myeloma Cells and Required for Their Survival*. Cell. 2009; **137**(5): p.873-86.
83. Kim DH, et al. *G beta L, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR*. Molecular Cell. 2003; **11**(4): p.895-904.
84. Kaizuka T, et al. *Tti1 and Tel2 Are Critical Factors in Mammalian Target of Rapamycin Complex Assembly*. Journal of Biological Chemistry. 2010; **285**(26): p.20109-16.
85. Hara K, et al. *Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action*. Cell. 2002; **110**(2): p.177-89.
86. Kim DH, et al. *MTOR interacts with Raptor to form a nutrient-sensitive complex that signals to the cell growth machinery*. Cell. 2002; **110**(2): p.163-75.
87. Sancak Y, et al. *PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase*. Molecular Cell. 2007; **25**(6): p.903-15.
88. Sarbassov DD, et al. *Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton*. Curr Biol. 2004; **14**(14): p.1296-302.
89. Jacinto E, et al. *SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity*. Cell. 2006; **127**(1): p.125-37.
90. Pearce LR, et al. *Identification of Protor as a novel Rictor-binding component of mTOR complex-2*. Biochemical Journal. 2007; **405**: p.513-22.
91. Sabatini DM, et al. *RAFT1 - a mammalian protein that binds to FKB12 in a rapamycin-dependent fashion and is homologous to yeast TORs*. Cell. 1994; **78**(1): p.35-43.
92. Sarbassov DD, et al. *Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB*. Molecular Cell. 2006; **22**(2): p.159-68.

93. Rozengurt E, et al. *Suppression of Feedback Loops Mediated by PI3K/mTOR Induces Multiple Overactivation of Compensatory Pathways: An Unintended Consequence Leading to Drug Resistance*. Molecular Cancer Therapeutics. 2014; **13**(11): p.2477-88.
94. Harrington LS, et al. *Restraining PI3K: mTOR signalling goes back to the membrane*. Trends in Biochemical Sciences. 2005; **30**(1): p.35-42.
95. Kim E, et al. *Regulation of TORC1 by Rag GTPases in nutrient response*. Nature Cell Biology. 2008; **10**(8): p.935-45.
96. Sancak Y, et al. *The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1*. Science. 2008; **320**(5882): p.1496-501.
97. Ma XM, et al. *Molecular mechanisms of mTOR-mediated translational control*. Nature Reviews Molecular Cell Biology. 2009; **10**(5): p.307-18.
98. Zinzalla V, et al. *Activation of mTORC2 by Association with the Ribosome*. Cell. 2011; **144**(5): p.757-68.
99. Garcia-Martinez JM, et al. *mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1)*. Biochemical Journal. 2008; **416**: p.375-85.
100. Facchinetti V, et al. *The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C*. Embo Journal. 2008; **27**(14): p.1932-43.
101. Ikenoue T, et al. *Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling*. Embo Journal. 2008; **27**(14): p.1919-31.
102. Moniz LS, et al. *AKT-ing out: SGK kinases come to the fore*. Biochemical Journal. 2013; **452**(3): p.e11-3.
103. Currie RA, et al. *Role of phosphatidylinositol 3,4,5-trisphosphate in regulating the activity and localization of 3-phosphoinositide-dependent protein kinase-1*. Biochemical Journal. 1999; **337**: p.575-83.
104. Wick MJ, et al. *Mouse 3-phosphoinositide-dependent protein kinase-1 undergoes dimerization and trans-phosphorylation in the activation loop*. Journal of Biological Chemistry. 2003; **278**(44): p.42913-9.
105. Casamayor A, et al. *Phosphorylation of Ser-241 is essential for the activity of 3-phosphoinositide-dependent protein kinase-1: identification of five sites of phosphorylation in vivo*. Biochemical Journal. 1999; **342**: p.287-92.
106. Biondi RM, et al. *The PIF-binding pocket in PDK1 is essential for activation of S6K and SGK, but not PKB*. Embo Journal. 2001; **20**(16): p.4380-90.
107. Frodin M, et al. *A phosphoserine-regulated docking site in the protein kinase RSK2 that recruits and activates PDK1*. Embo Journal. 2000; **19**(12): p.2924-34.
108. Le Good JA, et al. *Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1*. Science. 1998; **281**(5385): p.2042-5.
109. Mora A, et al. *PDK1, the master regulator of AGC kinase signal transduction*. Seminars in Cell & Developmental Biology. 2004; **15**(2): p.161-70.
110. Tessier M, et al. *Serum and glucocorticoid-regulated protein kinases: Variations on a theme*. Journal of Cellular Biochemistry. 2006; **98**(6): p.1391-407.
111. Tessier M, et al. *Role of the Phox homology domain and phosphorylation in activation of serum and glucocorticoid-regulated kinase-3*. Journal of Biological Chemistry. 2006; **281**(33): p.23978-89.
112. Kobayashi T, et al. *Activation of serum- and glucocorticoid-regulated protein kinase by agonists that activate phosphatidylinositide 3-kinase is mediated by 3-phosphoinositide-dependent protein kinase-1 (PDK1) and PDK2*. Biochemical Journal. 1999; **339**: p.319-28.
113. Hong F, et al. *mTOR-raptor binds and activates SGK1 to regulate p27 phosphorylation*. Molecular Cell. 2008; **30**(6): p.701-11.

114. Brunet A, et al. *Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 (FOXO3a)*. Molecular and Cellular Biology. 2001; **21**(3): p.952-65.
115. Vasudevan KM, et al. *AKT-Independent Signaling Downstream of Oncogenic PIK3CA Mutations in Human Cancer*. Cancer Cell. 2009; **16**(1): p.21-32.
116. Sommer EM, et al. *Elevated SGK1 predicts resistance of breast cancer cells to Akt inhibitors*. Biochemical Journal. 2013; **452**: p.499-508.
117. Ebi H, et al. *PI3K regulates MEK/ERK signaling in breast cancer via the Rac-GEF, P-Rex1*. Proceedings of the National Academy of Sciences of the United States of America. 2013; **110**(52): p.21124-9.
118. Maehama T, et al. *The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate*. Journal of Biological Chemistry. 1998; **273**(22): p.13375-8.
119. Vanhaesebroeck B, et al. *Synthesis and function of 3-phosphorylated inositol lipids*. Annual Review of Biochemistry. 2001; **70**: p.535-602.
120. Li J, et al. *PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer*. Science. 1997; **275**(5308): p.1943-7.
121. Lee JO, et al. *Crystal structure of the PTEN tumor suppressor: Implications for its phosphoinositide phosphatase activity and membrane association*. Cell. 1999; **99**(3): p.323-34.
122. Damen JE, et al. *The 145-kDa protein induced to associate with Shc by multiple cytokines is an inositol tetraphosphate and phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase*. Proceedings of the National Academy of Sciences of the United States of America. 1996; **93**(4): p.1689-93.
123. Ugi S, et al. *Protein phosphatase 2A negatively Akt (protein kinase B) activity in 3T3-L1 adipocytes*. Molecular and Cellular Biology. 2004; **24**(19): p.8778-89.
124. Gao TY, et al. *PHLPP: A phosphatase that directly dephosphorylates akt, promotes apoptosis, and suppresses tumor growth*. Molecular Cell. 2005; **18**(1): p.13-24.
125. Hiles ID, et al. *Phosphatidylinositol 3-Kinase - Structure and expression of the 110kd Catalytic Subunit*. Cell. 1992; **70**(3): p.419-29.
126. Carpenter CL, et al. *A tightly associated serine threonine protein-kinase regulates phosphoinositide 3-kinase activity*. Molecular and Cellular Biology. 1993; **13**(3): p.1657-65.
127. Dhand R, et al. *PI-3-Kinase is a dual-specificity enzyme - autoregulation by an intrinisic protein-serine kinase-activity*. Embo Journal. 1994; **13**(3): p.522-33.
128. Buchanan CM, et al. *Oncogenic Mutations of p110 alpha Isoform of PI 3-Kinase Upregulate Its Protein Kinase Activity*. Plos One. 2013; **8**(8).
129. Samuels Y, et al. *High frequency of mutations of the PIK3CA gene in human cancers*. Science. 2004; **304**(5670): p.554-.
130. Chaussade C, et al. *Functional differences between two classes of oncogenic mutation in the PIK3CA gene*. Biochemical and Biophysical Research Communications. 2009; **381**(4): p.577-81.
131. Kang S, et al. *Oncogenic transformation induced by the p110 beta, -gamma, and -delta isoforms of class I phosphoinositide 3-kinase*. Proceedings of the National Academy of Sciences of the United States of America. 2006; **103**(5): p.1289-94.
132. Vanhaesebroeck B, et al. *Autophosphorylation of p110 delta phosphoinositide 3-kinase: a new paradigm for the regulation of lipid kinases in vitro and in vivo*. Embo Journal. 1999; **18**(5): p.1292-302.
133. Czupalla C, et al. *Identification and characterization of the autophosphorylation sites of phosphoinositide 3-kinase isoforms beta and gamma*. Journal of Biological Chemistry. 2003; **278**(13): p.11536-45.
134. Bondeva T, et al. *Bifurcation of lipid and protein kinase signals of PI3K gamma to the protein kinases PKB and MAPK*. Science. 1998; **282**(5387): p.293-6.

135. Bondev A, et al. *Differential regulation of lipid and proteins kinase activities of phosphoinositide 3-kinase gamma in vitro*. Biol Chem. 1999; **380**(11): p.1337-40.
136. Lam K, et al. *The phosphatidylinositol 3-kinase serine kinase phosphorylates IRS-1 - stimulation by insulin and inhibition by wortmannin*. Journal of Biological Chemistry. 1994; **269**(32): p.20648-52.
137. Tanti JF, et al. *Insulin-receptor substrate-1 is phosphorylated by the serine kinase-activity of phosphatidylinositol 3-kinase*. Biochemical Journal. 1994; **304**: p.17-21.
138. Freund GG, et al. *The PI3-kinase serine kinase phosphorylates its p85 subunit and IRS-1 in PI3-kinase IRS-1 complexes*. Biochemical and Biophysical Research Communications. 1995; **206**(1): p.272-8.
139. Thomas D, et al. *Protein Kinase Activity of Phosphoinositide 3-Kinase Regulates Cytokine-Dependent Cell Survival*. Plos Biology. 2013; **11**(3).
140. Foukas LC, et al. *eIF4E binding protein 1 and H-Ras are novel substrates for the protein kinase activity of class-I phosphoinositide 3-kinase*. Biochemical and Biophysical Research Communications. 2004; **319**(2): p.541-9.
141. Naga Prasad SV, et al. *Protein kinase activity of phosphoinositide 3-kinase regulates beta-adrenergic receptor endocytosis*. Nat Cell Biol. 2005; **7**(8): p.785-96.
142. Foukas LC, et al. *Activity of any class IA PI3K isoform can sustain cell proliferation and survival*. Proceedings of the National Academy of Sciences of the United States of America. 2010; **107**(25): p.11381-6.
143. Aksoy E, et al. *The p110delta isoform of the kinase PI(3)K controls the subcellular compartmentalization of TLR4 signaling and protects from endotoxic shock*. Nature immunology. 2013; **13**(11): p.1045-54.
144. Foukas LC, et al. *Critical role for the p110 alpha phosphoinositide-3-OH kinase in growth and metabolic regulation*. Nature. 2006; **441**(7091): p.366-70.
145. Graupera M, et al. *Angiogenesis selectively requires the p110 alpha isoform of PI3K to control endothelial cell migration*. Nature. 2008; **453**(7195): p.662-6.
146. Knight ZA, et al. *A pharmacological map of the PI3-K family defines a role for p110 alpha in insulin signaling*. Cell. 2006; **125**(4): p.733-47.
147. Ciraolo E, et al. *Phosphoinositide 3-Kinase p110 beta Activity: Key Role in Metabolism and Mammary Gland Cancer but Not Development*. Science Signaling. 2008; **1**(36).
148. Jackson SP, et al. *PI 3-kinase p110 beta: a new target for antithrombotic therapy*. Nature Medicine. 2005; **11**(5): p.507-14.
149. Martin V, et al. *Deletion of the p110 beta isoform of phosphoinositide 3-kinase in platelets reveals its central role in Akt activation and thrombus formation in vitro and in vivo*. Blood. 2010; **115**(10): p.2008-13.
150. Jia S, et al. *Essential roles of PI(3)K-p110 beta in cell growth, metabolism and tumorigenesis*. Nature. 2008; **454**(7205): p.776-U102.
151. Fritsch R, et al. *SnapShot: Class I PI3K Isoform Signaling*. Cell. 2013; **154**(4): p.940-+.
152. Berenjeno IM, et al. *Both p110 alpha and p110 beta isoforms of PI3K can modulate the impact of loss-of-function of the PTEN tumour suppressor*. Biochemical Journal. 2012; **442**: p.151-9.
153. Ali K, et al. *Inactivation of PI(3)K p110delta breaks regulatory T-cell-mediated immune tolerance to cancer*. Nature. 2014; **509**(7505): p.407-11.
154. Vivanco I, et al. *The phosphatidylinositol 3-kinase-AKT pathway in human cancer*. Nature Reviews Cancer. 2002; **2**(7): p.489-501.
155. Engelman JA. *Targeting PI3K signalling in cancer: opportunities, challenges and limitations*. Nature Reviews Cancer. 2009; **9**(8): p.550-62.
156. Urick ME, et al. *PIK3R1 (p85 alpha) Is Somatically Mutated at High Frequency in Primary Endometrial Cancer*. Cancer Research. 2011; **71**(12): p.4061-7.

157. Jaiswal BS, et al. *Somatic Mutations in p85 alpha Promote Tumorigenesis through Class IA PI3K Activation*. Cancer Cell. 2009; **16**(6): p.463-74.
158. Wu H, et al. *Regulation of Class IA PI 3-kinases: C2 domain-iSH2 domain contacts inhibit p85/p110 alpha and are disrupted in oncogenic p85 mutants*. Proceedings of the National Academy of Sciences of the United States of America. 2009; **106**(48): p.20258-63.
159. Zhao L, et al. *Helical domain and kinase domain mutations in p110 alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms*. Proceedings of the American Association for Cancer Research Annual Meeting. 2008; **49**: p.845-.
160. Wee S, et al. *PTEN-deficient cancers depend on PIK3CB*. Proceedings of the National Academy of Sciences of the United States of America. 2008; **105**(35): p.13057-62.
161. Lindhurst MJ, et al. *Mosaic overgrowth with fibroadipose hyperplasia is caused by somatic activating mutations in PIK3CA*. Nature Genetics. 2012; **44**(8): p.928-+.
162. Angulo I, et al. *Phosphoinositide 3-Kinase delta Gene Mutation Predisposes to Respiratory Infection and Airway Damage*. Science. 2013; **342**(6160): p.866-71.
163. Manning G, et al. *The protein kinase complement of the human genome*. Science. 2002; **298**(5600): p.1912-+.
164. Zhang J, et al. *Targeting cancer with small molecule kinase inhibitors*. Nature Reviews Cancer. 2009; **9**(1): p.28-39.
165. Fruman DA, et al. *PI3K and cancer: lessons, challenges and opportunities*. Nature Reviews Drug Discovery. 2014; **13**(2): p.140-56.
166. Macias-Perez IM, et al. *GS-1101: A Delta-Specific PI3K Inhibitor in Chronic Lymphocytic Leukemia*. Current Hematologic Malignancy Reports. 2013; **8**(1): p.22-7.
167. Jamieson S, et al. *A drug targeting only p110 alpha can block phosphoinositide 3-kinase signalling and tumour growth in certain cell types*. Biochemical Journal. 2011; **438**: p.53-62.
168. Hirai H, et al. *MK-2206, an Allosteric Akt Inhibitor, Enhances Antitumor Efficacy by Standard Chemotherapeutic Agents or Molecular Targeted Drugs In vitro and In vivo*. Molecular Cancer Therapeutics. 2010; **9**(7): p.1956-67.
169. Walther TC, et al. *Mass spectrometry-based proteomics in cell biology*. Journal of Cell Biology. 2010; **190**(4): p.491-500.
170. Olsen JV, et al. *Trypsin cleaves exclusively C-terminal to arginine and lysine residues*. Molecular & Cellular Proteomics. 2004; **3**(6): p.608-14.
171. Gauci S, et al. *Lys-N and Trypsin Cover Complementary Parts of the Phosphoproteome in a Refined SCX-Based Approach*. Analytical Chemistry. 2009; **81**(11): p.4493-501.
172. Choudhary C, et al. *Decoding signalling networks by mass spectrometry-based proteomics*. Nature Reviews Molecular Cell Biology. 2010; **11**(6): p.427-39.
173. Pickart CM. *Mechanisms underlying ubiquitination*. Annual Review of Biochemistry. 2001; **70**: p.503-33.
174. Ubersax JA, et al. *Mechanisms of specificity in protein phosphorylation*. Nature Reviews Molecular Cell Biology. 2007; **8**(7): p.530-41.
175. Olsen JV, et al. *Status of Large-scale Analysis of Post-translational Modifications by Mass Spectrometry*. Molecular & Cellular Proteomics. 2013; **12**(12): p.3444-52.
176. Olsen JV, et al. *Global, in vivo, and site-specific phosphorylation dynamics in signaling networks*. Cell. 2006; **127**(3): p.635-48.
177. Engholm-Keller K, et al. *Technologies and challenges in large-scale phosphoproteomics*. Proteomics. 2013; **13**(6): p.910-31.
178. Miliotis T, et al. *Analysis of regulatory phosphorylation sites in ZAP-70 by capillary high-performance liquid chromatography coupled to electrospray ionization or matrix-assisted laser desorption ionization time-of-flight mass spectrometry*. Journal of Chromatography B. 2001; **752**(2): p.323-34.
179. Boersema PJ, et al. *Phosphopeptide fragmentation and analysis by mass spectrometry*. Journal of Mass Spectrometry. 2009; **44**(6): p.861-78.

180. Zubarev RA. *Electron-capture dissociation tandem mass spectrometry*. Current Opinion in Biotechnology. 2004; **15**(1): p.12-6.
181. Chi A, et al. *Analysis of phosphorylation sites on proteins from Saccharomyces cerevisiae by electron transfer dissociation (ETD) mass spectrometry*. Proceedings of the National Academy of Sciences of the United States of America. 2007; **104**(7): p.2193-8.
182. Michalski A, et al. *More than 100,000 Detectable Peptide Species Elute in Single Shotgun Proteomics Runs but the Majority is Inaccessible to Data-Dependent LC-MS/MS*. Journal of Proteome Research. 2011; **10**(4): p.1785-93.
183. Gronborg M, et al. *A mass spectrometry-based proteomic approach for identification of serine/threonine-phosphorylated proteins by enrichment with phospho-specific antibodies - Identification of a novel protein, Frigg, as a protein kinase A substrate*. Molecular & Cellular Proteomics. 2002; **1**(7): p.517-27.
184. Andersson L, et al. *Isolation of phosphoproteins by imobilized metal (Fe3+) affinity-chromatography*. Analytical Biochemistry. 1986; **154**(1): p.250-4.
185. Gaberc-Porekar V, et al. *Perspectives of immobilized-metal affinity chromatography*. Journal of Biochemical and Biophysical Methods. 2001; **49**(1-3): p.335-60.
186. Ficarro SB, et al. *Phosphoproteome analysis by mass spectrometry and its application to Saccharomyces cerevisiae*. Nat Biotechnol. 2002; **20**(3): p.301-5.
187. Ye J, et al. *Optimized IMAC-IMAC Protocol for Phosphopeptide Recovery from Complex Biological Samples*. Journal of Proteome Research. 2010; **9**(7): p.3561-73.
188. Tsai C-F, et al. *Immobilized metal affinity chromatography revisited: pH/Acid control toward high selectivity in phosphoproteomics*. Journal of Proteome Research. 2008; **7**(9): p.4058-69.
189. Jensen SS, et al. *Evaluation of the impact of some experimental procedures on different phosphopeptide enrichment techniques*. Rapid Communications in Mass Spectrometry. 2007; **21**(22): p.3635-45.
190. Pinkse MWH, et al. *Selective isolation at the femtomole level of phosphopeptides from proteolytic digests using 2D-nanoLC-ESI-MS/MS and titanium oxide precolumns*. Analytical Chemistry. 2004; **76**(14): p.3935-43.
191. Montoya A, et al. *Characterization of a TiO(2) enrichment method for label-free quantitative phosphoproteomics*. Methods. 2011; **54**(4): p.370-8.
192. Aryal UK, et al. *Enrichment and analysis of phosphopeptides under different experimental conditions using titanium dioxide affinity chromatography and mass spectrometry*. Rapid Communications in Mass Spectrometry. 2010; **24**(2): p.219-31.
193. Larsen MR, et al. *Highly selective enrichment of phosphorylated peptides from peptide mixtures using titanium dioxide microcolumns*. Molecular & Cellular Proteomics. 2005; **4**(7): p.873-86.
194. Adamczyk M, et al. *Selective analysis of phosphopeptides within a protein mixture by chemical modification, reversible biotinylation and mass spectrometry*. Rapid Communications in Mass Spectrometry. 2001; **15**(16): p.1481-8.
195. Fenn JB, et al. *Electrospray ionization for mass-spectrometry of large biomolecules*. Science. 1989; **246**(4926): p.64-71.
196. Karas M, et al. *Laser desorption ionization of proteins with molecular masses exceeding 10000 Daltons*. Analytical Chemistry. 1988; **60**(20): p.2299-301.
197. Domon B, et al. *Review - Mass spectrometry and protein analysis*. Science. 2006; **312**(5771): p.212-7.
198. Hunt DF, et al. *Protein sequencing by tandem mass-spectrometry*. Proceedings of the National Academy of Sciences of the United States of America. 1986; **83**(17): p.6233-7.
199. Paizs B, et al. *Fragmentation pathways of protonated peptides*. Mass Spectrometry Reviews. 2005; **24**(4): p.508-48.

200. Elias JE, et al. *Comparative evaluation of mass spectrometry platforms used in large-scale proteomics investigations*. Nature Methods. 2005; **2**(9): p.667-75.
201. Yates JR, et al. *Proteomics by Mass Spectrometry: Approaches, Advances, and Applications*. Annual Review of Biomedical Engineering. 2009; **11**: p.49-79.
202. Ong SE, et al. *Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics*. Molecular & Cellular Proteomics. 2002; **1**(5): p.376-86.
203. Ross PL, et al. *Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents*. Molecular & Cellular Proteomics. 2004; **3**(12): p.1154-69.
204. Gygi SP, et al. *Quantitative analysis of complex protein mixtures using isotope-coded affinity tags*. Nat Biotechnol. 1999; **17**(10): p.994-9.
205. Stewart, II, et al. *O-18 Labeling: a tool for proteomics*. Rapid Communications in Mass Spectrometry. 2001; **15**(24): p.2456-65.
206. Krueger M, et al. *SILAC mouse for quantitative proteomics uncovers kindlin-3 as an essential factor for red blood cell function*. Cell. 2008; **134**(2): p.353-64.
207. Lu P, et al. *Absolute protein expression profiling estimates the relative contributions of transcriptional and translational regulation*. Nat Biotechnol. 2007; **25**(1): p.117-24.
208. Zhu W, et al. *Mass Spectrometry-Based Label-Free Quantitative Proteomics*. Journal of Biomedicine and Biotechnology. 2010.
209. Hastie CJ, et al. *Assay of protein kinases using radiolabeled ATP: a protocol*. Nature Protocols. 2006; **1**(2): p.968-71.
210. Perkins DN, et al. *Probability-based protein identification by searching sequence databases using mass spectrometry data*. Electrophoresis. 1999; **20**(18): p.3551-67.
211. Cutillas PR, et al. *Quantitative profile of five murine core proteomes using label-free functional proteomics*. Molecular & Cellular Proteomics. 2007; **6**(9): p.1560-73.
212. Schwartz D, et al. *An iterative statistical approach to the identification of protein phosphorylation motifs from large-scale data sets*. Nat Biotechnol. 2005; **23**(11): p.1391-8.
213. Knebel A, et al. *A novel method to identify protein kinase substrates: eEF2 kinase is phosphorylated and inhibited by SAPK4/p38 delta*. Embo Journal. 2001; **20**(16): p.4360-9.
214. Beltran L, et al. *Global profiling of protein kinase activities in cancer cells by mass spectrometry*. Journal of Proteomics. 2012; **77**: p.492-503.
215. Casado P, et al. *A Self-validating Quantitative Mass Spectrometry Method for Assessing the Accuracy of High-content Phosphoproteomic Experiments*. Molecular & Cellular Proteomics. 2011; **10**(1).
216. Murray JT, et al. *Exploitation of KESTREL to identify NDRG family members as physiological substrates for SGK1 and GSK3*. Biochemical Journal. 2004; **384**: p.477-88.
217. Layton MJ, et al. *Autophosphorylation of serine 608 in the p85 regulatory subunit of wild type or cancer-associated mutants of phosphoinositide 3-kinase does not affect its lipid kinase activity*. Bmc Biochemistry. 2012; **13**.
218. Beltran L. *Proteomic Investigation of the Class IA Phosphoinositide 3-Kinase Signalling Pathway*. PhD Thesis. 2012.
219. Xue L, et al. *Sensitive kinase assay linked with phosphoproteomics for identifying direct kinase substrates*. Proceedings of the National Academy of Sciences of the United States of America. 2012; **109**(15): p.5615-20.
220. Amanchy R, et al. *A curated compendium of phosphorylation motifs*. Nat Biotechnol. 2007; **25**(3): p.285-6.
221. Madhusudan, et al. *CAMP-DEPENDENT PROTEIN-KINASE - CRYSTALLOGRAPHIC INSIGHTS INTO SUBSTRATE RECOGNITION AND PHOSPHOTRANSFER*. Protein Science. 1994; **3**(2): p.176-87.

222. Obata T, et al. *Peptide and protein library screening defines optimal substrate motifs for AKT/PKB*. Journal of Biological Chemistry. 2000; **275**(46): p.36108-15.
223. Marin O, et al. *THE CONSENSUS SEQUENCES FOR CDC2 KINASE AND FOR CASEIN KINASE-2 ARE MUTUALLY INCOMPATIBLE - A STUDY WITH PEPTIDES DERIVED FROM THE BETA-SUBUNIT OF CASEIN KINASE-2*. FEBS Lett. 1992; **301**(1): p.111-4.
224. Shi YJ, et al. *Mammalian target of rapamycin inhibitors activate the AKT kinase in multiple myeloma cells by up-regulating the insulin-like growth factor receptor/insulin receptor substrate-1/phosphatidylinositol 3-kinase cascade*. Molecular Cancer Therapeutics. 2005; **4**(10): p.1533-40.
225. O'Reilly KE, et al. *mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt*. Cancer Research. 2006; **66**(3): p.1500-8.
226. Zhang H, et al. *Phosphoprotein analysis using antibodies broadly reactive against phosphorylated motifs*. Journal of Biological Chemistry. 2002; **277**(42): p.39379-87.

Appendix 1

Summary of phosphorylation events identified following *in vitro* p110α protein kinase assay (Chapter 3).

		Relative mean phosphopeptide intensity								
		Cell lysate, ATP]			p110α /A66, ATP]			p110α, ATP]		
Accession number	Phosphopeptide identification	0 µM	50 µM	100 µM	0 µM	50 µM	100 µM	0 µM	50 µM	100 µM
A2MP_MOUSE	A2mp p-Y1018 (z= 3)	88.7	87.2	65.1	82.9	73.8	74.3	100.0	81.3	82.8
AAA1_MOUSE	AAA1 p-S5 p-S9 (z= 3) + Oxi	98.3	72.5	74.0	46.1	76.8	94.4	54.5	52.4	100.0
ABCA2_MOUSE	Abca2 p-T1173 p-S1177 (z= 3)	100.0	33.4	52.5	95.9	55.0	87.5	64.1	57.6	68.7
ABCBA_MOUSE	ABCBA p-S494 (z= 4)	100.0	7.3	64.2	76.3	48.7	47.6	21.2	13.2	71.8
ABCD3_MOUSE	Abcd3 p-T222 p-T245 (z= 3) + Oxi	69.8	72.6	93.5	92.0	63.5	100.0	83.6	64.2	62.8
ABHGA_MOUSE	Abhd16a p-S91 p-Y99 p-T102 (z= 5)	100.0	32.1	45.4	57.5	46.2	37.8	6.7	0.0	75.5
ABI1_MOUSE	Abi1 p-S183 (z= 2)	89.5	66.2	71.8	90.3	100.0	82.0	88.4	89.3	82.9
ABI1_MOUSE	Abi1 p-S183 (z= 3)	74.2	75.9	78.5	100.0	67.9	62.6	71.2	73.9	78.0
ACADV_MOUSE	Acadvl p-T348 (z= 3) + Oxi	87.0	73.2	54.9	100.0	62.5	37.5	65.3	21.9	65.7
ACSF4_MOUSE	Aasdh p-S1053 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
ACTT3_MOUSE	Actrt3 p-S2 p-Y4 (z= 3) + Oxi	14.8	31.7	66.6	23.3	19.5	43.3	55.4	100.0	36.8
ADA17_MOUSE	Adam17 p-S322 p-S330 (z= 4) + Oxi	59.3	71.3	82.2	100.0	54.9	66.4	67.8	41.9	65.2
ADCY4_MOUSE	Adcy4 p-T833 p-T835 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
ADCY6_MOUSE	Adcy6 p-T1029 p-T1054 (z= 5) +	100.0	28.2	19.1	26.1	21.8	84.2	17.8	5.8	83.8
ADCY7_MOUSE	Adcy7 p-T176 p-T195 p-S207 (z= 5)	100.0	71.7	76.3	84.7	50.7	77.3	26.9	17.9	52.4
ADCY9_MOUSE	ADCY9 p-S394 p-T404 (z= 3)	100.0	73.8	54.2	45.4	66.4	72.1	42.5	48.8	74.1
ADCYA_MOUSE	Adcy10 p-T161 (z= 4)	17.7	41.5	49.5	49.5	100.0	44.9	20.1	31.6	6.6
AFAD_MOUSE	Mllt4 p-S1182 (z= 3)	100.0	18.1	15.2	20.8	25.3	64.3	20.9	23.7	17.3
AFF3_MOUSE	Aff3 p-T585 p-T598 (z= 4)	100.0	85.3	78.6	77.9	74.1	64.6	35.2	44.5	57.4
AKT3_MOUSE	Akt3 p-S238 (z= 5) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
AL3A1_MOUSE	Aldh3a1 p-S383 p-T387 p-T396 (z=	9.9	0.0	3.0	83.7	69.9	54.3	100.0	43.6	78.2
ALDH2_MOUSE	Aldh2 p-Y499 (z= 3) + Oxi	78.7	54.5	70.2	67.0	66.0	100.0	98.1	74.4	62.2
ALEX_MOUSE	Gnas p-T590 p-S591 (z= 4)	79.8	52.2	84.0	100.0	63.1	67.8	55.5	60.8	57.7
AMER3_MOUSE	Fam123c p-S141 (z= 3)	57.2	48.9	31.3	56.8	45.1	100.0	26.4	23.3	30.1
AMGO1_MOUSE	Amigo1 p-S480 p-T488 (z= 3) + Oxi	81.7	74.9	77.5	73.8	25.1	100.0	31.0	46.9	0.0
ANK1_MOUSE	Ank1 p-S1473 p-S1482 (z= 3) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
AP5B1_MOUSE	Ap5b1 p-S19 p-S21 (z= 3)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
APMAP_MOUSE	Apmap p-T189 p-T205 (z= 4) + Oxi	96.3	100.0	81.3	88.5	82.2	81.9	64.2	62.3	72.0
ARG33_MOUSE	Arhgef33 p-T145 p-S148 p-S152 (z=	31.2	32.2	100.0	81.0	29.0	23.6	12.6	0.0	94.5
ARGL1_MOUSE	Arglu1 p-S75 (z= 2)	76.1	50.5	59.8	41.0	94.1	91.1	97.7	100.0	74.8
ARHG6_MOUSE	Arhgef6 p-S487 (z= 3)	62.9	68.4	94.1	75.8	79.1	65.4	100.0	75.2	75.8
ARHL1_MOUSE	Adprhl1 p-Y19 (z= 3) + Oxi	27.2	54.9	66.9	100.0	67.4	31.3	75.4	15.8	56.0
ARX_MOUSE	Arx p-S142 (z= 3)	87.6	47.4	83.3	100.0	48.8	99.3	72.6	62.6	39.8
ASH2L_MOUSE	Ash2l p-S270 p-S287 (z= 3)	63.4	65.7	76.4	93.6	75.6	96.3	100.0	61.2	62.7
ASPD_MOUSE	Aspdh p-S173 (z= 5) + Oxi	60.5	53.0	94.6	100.0	55.7	34.4	59.7	23.6	61.5
ASXL2_MOUSE	Asxl2 p-S590 (z= 3)	85.6	100.0	67.7	79.5	69.1	64.1	75.3	75.3	87.1
AT2A1_MOUSE	Atp2a1 p-T729 p-S731 p-S741 (z=	32.2	54.5	39.3	32.3	68.6	100.0	47.2	61.5	60.3
ATG4D_MOUSE	Atg4d p-T312 (z= 3)	4.9	77.0	92.2	100.0	43.7	21.1	69.4	89.5	83.8
ATPG_MOUSE	Atp5c1 p-T122 (z= 4)	70.3	100.0	69.8	86.0	72.9	37.1	26.5	76.1	10.0
BCOR_MOUSE	Bcor p-S453 (z= 3)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
BCORL_MOUSE	Bcorl1 p-S657 (z= 4)	100.0	42.4	59.6	57.4	37.5	36.8	29.3	11.5	56.8
BCR_MOUSE	Bcr p-S113 (z= 4)	88.8	60.3	70.0	66.3	50.4	100.0	72.1	76.6	59.6
BCR_MOUSE	Bcr p-S122 (z= 3)	42.5	35.6	52.2	45.5	85.9	100.0	76.7	82.2	83.8
BCR_MOUSE	Bcr p-S124 (z= 4)	92.7	22.8	77.1	100.0	64.6	96.4	28.9	52.2	90.3
BEGIN_MOUSE	Begain p-S307 (z= 3)	80.6	0.0	11.5	35.5	20.2	67.7	37.8	24.6	100.0
BICD2_MOUSE	Bicd2 p-T393 (z= 3)	100.0	46.5	53.3	68.5	50.3	56.0	42.4	29.7	73.9
BICR1_MOUSE	Ccdc64 p-S390 p-S417 (z= 6)	66.7	100.0	68.9	88.8	0.0	0.0	64.2	65.3	0.0
BIG1_MOUSE	Arfgef1 p-T1130 (z= 3)	100.0	78.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
BPHL_MOUSE	BPHL p-S139 p-Y155 (z= 4)	100.0	54.1	30.7	38.6	7.0	62.3	0.9	0.0	54.7
BSSP4_MOUSE	Prss22 p-S4 p-T34 p-S38 (z= 7) +	23.4	100.0	26.4	25.5	4.9	9.7	0.0	0.0	30.6
C1QL3_MOUSE	C1ql3 p-Y35 p-T38 p-S42 p-T43 (z=	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
C209B_MOUSE	Cd209b p-Y27 p-S28 p-S31 p-S32	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
CAC1H_MOUSE	Cacna1h p-S2326 (z= 6) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
CAH3_MOUSE	CAH3 p-S85 (z= 2) + Oxi	89.7	68.5	47.2	61.0	59.4	60.9	100.0	50.5	77.9
CAHD1_MOUSE	Cachd1 p-T351 (z= 3) + Oxi	44.4	81.3	59.8	100.0	52.0	3.7	27.1	15.5	37.2
CAN10_MOUSE	CAN10 p-T494 p-S498 (z= 4)	98.9	100.0	86.9	75.2	78.0	78.0	41.9	44.5	74.9
CAP1_MOUSE	Cap1 p-T77 (z= 4) + Oxi	93.8	96.1	95.7	100.0	74.9	67.7	82.8	78.7	79.5
CAPG_MOUSE	Capg p-S131 (z= 3)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
CARF_MOUSE	Cdkn2aip p-S355 p-T356 p-S362	11.0	39.8	39.2	45.4	75.5	38.6	47.2	73.8	100.0
CASC3_MOUSE	Casc3 p-S27 (z= 2)	73.4	59.8	46.2	51.2	89.7	89.1	78.3	100.0	87.0
CBL_MOUSE	Cbl p-S9 (z= 3) + Oxi	100.0	43.3	65.2	57.8	54.6	50.6	35.2	28.6	87.9
CBPA3_MOUSE	Cpa3 p-T302 p-S305 p-Y306 (z= 4)	82.9	100.0	88.6	62.0	71.0	82.2	51.7	51.5	59.8
CBS_MOUSE	Cbs p-T138 p-Y160 (z= 4) + Oxi	0.1	100.0	80.8	69.5	0.1	0.1	0.1	0.1	0.1
CBX6_MOUSE	CBX6 p-T194 (z= 4)	91.7	100.0	57.2	89.8	63.0	31.7	61.2	71.4	75.5
CC103_MOUSE	Ccdc103 p-T203 (z= 5) + Oxi	17.3	100.0	54.1	16.7	11.9	15.2	0.0	0.0	48.6
CC105_MOUSE	Ccdc105 p-T374 (z= 2) + Oxi	72.0	80.5	69.2	25.9	99.0	78.0	100.0	58.4	68.3
CC105_MOUSE	Ccdc105 p-Y372 (z= 3) + Oxi	82.0	88.3	84.1	80.9	69.4	69.9	100.0	48.7	52.6
CCD18_MOUSE	CCD18 p-Y8 p-S19 p-S26 (z= 3) +	53.3	100.0	78.8	60.9	53.3	86.9	46.3	53.5	54.9
CCNC_MOUSE	Ccnc p-Y76 p-S77 p-S95 (z= 4) +	91.3	100.0	39.7	33.9	25.8	59.4	14.9	16.5	45.4
CD021_MOUSE	Cntnap1 p-S649 p-S663 (z= 3)	51.0	100.0	97.3	91.4	71.7	58.6	81.0	72.4	72.8
CD158_MOUSE	Ccdc158 p-S1054 p-T1057 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
CD158_MOUSE	Ccdc158 p-S219 p-T230 (z= 4)	69.0	54.0	79.6	100.0	0.0	46.0	52.4	33.8	0.0
CD19_MOUSE	Cd19 p-S477 p-S482 p-Y493 p-	100.0	16.4	47.5	0.0	16.4	23.4	58.3	18.1	56.9
CD2AP_MOUSE	Cd2ap p-S434 p-T439 (z= 3)	63.3	85.8	100.0	93.8	88.9	92.1	60.9	99.4	41.5
CD80_MOUSE	Cd80 p-S146 (z= 4)	71.6	87.1	92.8	100.0	64.2	69.8	51.4	67.3	55.2
CDK1_MOUSE	Cdk1 p-Y15 (z= 2)	57.6	57.8	55.1	71.4	78.1	86.8	68.7	100.0	77.4
CDK13_MOUSE	Cdk13 p-S343 (z= 2)	0.0	27.9	41.1	45.6	100.0	64.1	58.1	67.3	55.3
CDK18_MOUSE	CDK18 p-T134 (z= 2)	57.6	57.8	55.1	71.4	78.1	86.8	68.7	100.0	77.4
CDT1_MOUSE	Cdt1 p-S328 (z= 4) + Oxi	57.2	48.0	21.0	0.0	0.0	10.9	44.5	68.2	100.0
CDV3_MOUSE	Cdv3 p-S214 (z= 3)	0.0	52.9	74.1	0.0	74.5	72.7	0.0	71.9	100.0
CDV3_MOUSE	Cdv3 p-Y213 (z= 3)	0.0	52.9	74.1	0.0	74.5	72.7	0.0	71.9	100.0
CE034_MOUSE	Tmem130 p-S154 p-S160 (z= 3)	100.0	9.6	25.5	47.2	84.9	34.7	6.7	9.4	27.3

CEA16_MOUSE	Ceacam16 p-T327 p-T330 (z= 4) +	98.2	8.3	23.5	31.5	51.4	70.7	3.5	0.0	100.0
CEA18_MOUSE	Ceacam18 p-S156 (z= 3)	100.0	51.3	0.0	0.0	52.7	71.9	63.1	56.1	60.1
CELR3_MOUSE	Celsr3 p-T1784 p-T1787 (z= 3) +	68.6	100.0	77.5	66.8	34.0	61.1	7.4	5.7	97.0
CENPB_MOUSE	Cenpb p-T117 (z= 3)	20.4	32.6	100.0	41.3	7.4	29.2	72.0	65.0	90.0
CEP85_MOUSE	CEP85 p-S84 p-T87 p-T90 (z= 4)	74.0	63.8	57.7	52.7	51.9	23.5	100.0	45.1	72.7
CF132_MOUSE	Fgf17 p-S1098 (z= 4) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
CF132_MOUSE	M21D2 p-T799 (z= 3)	100.0	74.5	53.9	79.8	56.8	72.4	54.1	35.8	54.6
CHIP_MOUSE	Stub1 p-S20 (z= 2)	63.1	57.0	67.5	55.5	100.0	86.4	79.2	97.4	95.6
CHIP_MOUSE	Stub1 p-S20 (z= 3)	82.1	72.5	75.8	100.0	83.3	66.6	60.9	61.2	79.9
CHIP_MOUSE	Stub1 p-S20 (z= 3)	69.5	80.7	96.2	100.0	64.7	82.1	79.2	96.1	76.6
CHSP1_MOUSE	Carhsp1 p-S33 (z= 2)	0.0	0.0	24.5	40.5	14.6	13.6	42.4	100.0	61.4
CI172_MOUSE	CI172 p-S571 p-S572 (z= 3)	71.7	59.8	70.3	71.5	77.6	78.0	100.0	48.0	69.9
CJ088_MOUSE	Myzap p-S174 (z= 4)	44.5	51.9	100.0	65.2	49.3	71.2	25.9	98.9	26.9
CKP2L_MOUSE	Ckap2l p-S225 p-T232 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
CLC1B_MOUSE	Clec1b p-S39 p-S40 p-Y60 (z= 4) +	100.0	76.8	83.8	57.9	78.8	24.3	0.0	0.0	49.8
CLCKA_MOUSE	Clcnka p-T322 p-S323 p-Y327 p-	80.1	70.3	66.1	61.9	68.1	33.8	100.0	54.3	81.2
CLCN6_MOUSE	Clcn6 p-T538 p-T568 (z= 4) + Oxi	100.0	27.0	0.0	25.6	0.0	60.6	0.0	0.0	0.0
CLH_MOUSE	Cltc p-S77 (z= 4) + Oxi	0.1	0.1	0.1	0.1	0.1	100.0	0.1	0.1	0.1
CLUA1_MOUSE	Cluap1 p-S308 p-S326 p-S334 (z=	99.0	0.1	0.1	77.8	100.0	0.1	0.1	0.1	0.1
CM033_MOUSE	CM033 p-S243 (z= 4)	100.0	46.5	46.7	33.1	6.9	13.7	0.0	17.0	16.8
CMAH_MOUSE	Cmah p-T398 p-T415 p-S417 (z= 3)	100.0	70.7	32.4	43.8	70.3	79.4	32.7	21.5	60.8
CMLO5_MOUSE	Cml5 p-Y198 p-T209 (z= 4)	100.0	65.2	73.9	53.1	61.3	77.1	26.6	19.8	14.2
CN079_MOUSE	Usp37 p-S284 (z= 2)	53.2	37.6	51.6	77.2	43.4	100.0	24.1	23.7	33.3
CNDP2_MOUSE	Cndp2 p-Y311 p-S324 (z= 4)	92.0	75.7	82.3	93.4	69.5	100.0	74.5	23.8	36.1
CNN1_MOUSE	Cnn1 p-Y261 (z= 2)	0.0	85.4	92.8	0.0	100.0	65.6	0.0	67.5	84.4
CNN3_MOUSE	Cnn3 p-Y261 (z= 2)	0.0	67.9	100.0	0.0	81.1	63.1	0.0	48.1	69.4
CNO6L_MOUSE	Cnot6l p-T129 p-S138 (z= 4) + Oxi	100.0	87.5	44.3	42.1	77.8	96.8	20.8	17.6	70.7
CNTLN_MOUSE	Cntln p-T624 p-T642 (z= 4) + Oxi	71.5	73.1	52.8	29.1	100.0	85.3	30.4	48.1	18.4
CNTN2_MOUSE	Cntn2 p-T80 p-S88 (z= 3) + Oxi	73.9	86.8	100.0	93.0	67.9	82.4	57.1	52.6	64.1
CO4A2_MOUSE	Col4a2 p-S1480 p-S1482 (z= 5) +	6.0	0.0	0.0	0.0	12.0	100.0	0.0	0.0	0.0
CO4A3_MOUSE	Col4a3 p-T1348 (z= 5) + Oxi	100.0	0.0	0.0	0.0	0.0	51.1	0.0	0.0	0.0
CO6A5_MOUSE	Col6a5 p-T1235 p-S1251 p-Y1252	100.0	86.1	81.4	82.7	91.3	89.7	46.7	85.0	88.3
COBL_MOUSE	Cobl p-S314 (z= 3)	62.1	80.6	100.0	90.8	87.6	91.3	55.1	95.0	38.4
COFA1_MOUSE	Col15a1 p-S1333 p-T1337 (z= 3)	78.2	84.2	90.1	100.0	67.7	74.1	98.5	62.0	60.3
COG4_MOUSE	Cog4 p-S468 p-S469 (z= 2) + Oxi	71.0	72.4	82.6	31.4	100.0	52.6	51.2	55.1	62.7
COG4_MOUSE	Cog4 p-S468 p-S469 (z= 3) + Oxi	83.7	100.0	80.6	89.1	75.5	43.9	57.6	63.0	63.8
COKA1_MOUSE	Col20a1 p-S809 p-S821 (z= 3) + Oxi	72.6	84.8	76.8	100.0	44.8	71.5	71.5	54.0	41.2
COMT_MOUSE	Comt p-S261 (z= 2)	54.0	84.7	81.3	91.5	74.5	69.7	84.4	100.0	87.9
COOA1_MOUSE	Col24a1 p-S1513 p-T1528 p-S1531	0.0	100.0	58.0	19.5	31.9	65.6	0.0	0.0	25.2
COPG1_MOUSE	COPG1 p-T135 (z= 4) + Oxi	60.9	65.1	66.6	100.0	76.6	64.1	55.5	61.7	71.8
CP110_MOUSE	Ccp110 p-S306 p-S309 (z= 5) + Oxi	0.0	57.4	100.0	53.1	34.5	0.0	0.0	0.0	0.0
CRK_MOUSE	Crk p-Y221 (z= 4)	93.0	32.3	54.8	50.0	35.2	100.0	36.0	22.6	67.2
CRK_MOUSE	Crk p-Y221 (z= 5)	50.8	22.4	49.2	23.7	63.2	100.0	43.6	49.4	33.6
CRKL_MOUSE	Crkl p-S197 (z= 3)	69.7	52.0	84.9	65.5	47.0	27.5	62.5	100.0	89.2
CRKL_MOUSE	Crkl p-T212 (z= 5)	20.4	61.2	69.4	100.0	57.5	37.5	58.7	57.9	73.1
CRKL_MOUSE	Crkl p-Y198 (z= 4)	60.6	61.6	83.1	97.6	77.5	48.9	83.1	67.7	100.0
CS069_MOUSE	Gm7092 p-S15 (z= 3)	50.1	50.3	100.0	50.0	33.9	16.6	93.7	19.9	51.2
CTF8A_MOUSE	Chtf8 p-T305 p-T309 (z= 4)	31.0	100.0	0.0	35.3	47.7	5.5	75.2	34.4	37.6
CTNA1_MOUSE	Ctnna1 p-S641 (z= 2)	55.1	54.8	53.4	30.1	62.6	68.0	55.9	100.0	68.9
CTND2_MOUSE	Ctnnd2 p-S1094 (z= 4) + Oxi	27.2	26.5	53.3	34.2	26.8	19.9	100.0	48.4	57.4
CTR4_MOUSE	Slc7a4 p-T548 (z= 3) + Oxi	80.0	100.0	21.5	18.8	21.7	40.1	12.2	8.2	15.5
CTRO_MOUSE	Cit p-S826 (z= 5)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
CTRO_MOUSE	Cit p-T1582 p-S1586 (z= 3)	75.4	83.8	67.3	100.0	77.1	82.0	71.7	65.0	43.2
CYHR1_MOUSE	Cyhr1 p-S168 p-S171 (z= 3)	100.0	31.1	20.9	33.2	31.6	45.3	34.6	32.3	12.3
CYTSA_MOUSE	Specc1l p-S33 p-S50 (z= 4)	68.8	57.1	71.0	100.0	0.0	60.3	0.0	42.0	42.6
DBPA_MOUSE	Csda p-S328 (z= 2)	0.0	12.2	19.5	0.0	76.7	78.6	0.0	64.9	100.0
DBPA_MOUSE	Csda p-S328 (z= 3)	0.0	40.0	58.5	0.0	97.4	62.2	0.0	91.8	100.0
DCLK1_MOUSE	Dclk1 p-S352 (z= 2)	0.0	27.2	38.1	0.0	100.0	92.4	0.0	72.8	59.9
DDR1_MOUSE	Ddr1 p-T3 p-T17 (z= 3) + Oxi	79.3	85.4	80.7	92.3	80.4	100.0	97.9	95.6	87.8
DDX3Y_MOUSE	Ddx3y p-S227 (z= 5) + Oxi	55.8	43.0	88.5	76.8	55.6	55.4	100.0	37.5	79.0
DDX42_MOUSE	Ddx42 p-T724 p-S725 p-S726 (z= 5)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
DEND_MOUSE	DEND p-S309 p-S318 p-S327 (z= 4)	90.2	71.4	74.4	100.0	65.6	79.6	94.6	52.2	63.1
DEPD5_MOUSE	DEPD5 p-S779 p-S780 p-S781 (z=	100.0	9.5	18.1	10.3	6.3	48.4	0.0	0.0	28.0
DESP_MOUSE	Dsp p-Y28 p-T31 p-Y32 p-S33 (z=	100.0	41.9	36.5	28.0	16.3	67.8	8.8	9.1	40.6
DHI1_MOUSE	Hsd11b1 p-T40 p-S55 (z= 3)	60.5	17.6	38.8	100.0	82.8	17.4	19.7	25.4	32.1
DI3L1_MOUSE	Dis3l p-T568 p-S569 p-Y570 (z= 4)	70.8	0.0	28.7	19.8	100.0	0.0	25.6	41.8	79.3
DIEXF_MOUSE	Diexf p-S350 (z= 3)	100.0	76.5	60.4	62.4	69.6	72.0	67.7	57.4	69.5
DISP1_MOUSE	Disp1 p-S18 p-T19 p-S20 (z= 7) +	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	85.0
DJC15_MOUSE	DJC15 p-T8 (z= 3) + Oxi	82.0	100.0	77.3	64.0	59.9	72.6	89.9	84.6	71.8
DKKL1_MOUSE	Dkkl1 p-T167 (z= 3)	54.9	84.8	61.2	95.4	100.0	43.2	67.9	47.1	84.6
DLGP2_MOUSE	Dlgap2 p-S574 p-S584 p-T594 (z=	16.0	42.2	26.3	100.0	33.8	6.6	4.1	0.0	30.5
DMRT1_MOUSE	Dmrt1 p-Y31 (z= 3) + Oxi	88.1	92.0	61.1	83.0	59.6	59.5	100.0	88.6	44.5
DMWD_MOUSE	Dmwd p-S645 p-S647 p-S653 (z= 3)	43.5	11.0	0.0	18.5	0.0	100.0	24.8	0.0	36.0
DMXL2_MOUSE	Dmxl2 p-S483 p-T488 (z= 3) + Oxi	100.0	48.1	42.9	17.2	55.6	35.7	25.3	34.2	49.0
DNLI1_MOUSE	Lig1 p-S49 (z= 2)	79.0	53.7	74.0	68.7	100.0	94.2	75.9	90.1	93.8
DOCK1_MOUSE	Dock1 p-T721 (z= 3)	45.5	56.7	56.2	45.0	60.9	63.3	50.6	100.0	70.3
DOCK9_MOUSE	Dock9 p-T1587 (z= 3) + Oxi	42.9	46.9	57.7	55.3	42.0	40.1	100.0	36.6	51.4
DPOLZ_MOUSE	Rev3l p-S1777 p-S1778 (z= 2)	63.2	84.4	88.1	100.0	67.7	81.7	82.5	78.7	80.4
DPYL3_MOUSE	Dpysl3 p-T524 (z= 3)	86.0	81.9	69.2	100.0	54.0	85.0	67.4	87.1	70.8
DRD3_MOUSE	Drd3 p-S331 (z= 3) + Oxi	95.7	74.9	69.4	82.8	100.0	72.3	87.9	99.2	68.7
DSG1B_MOUSE	DSG1B p-S979 p-S984 p-T1022 (z=	76.0	52.8	71.0	100.0	55.0	62.6	52.9	48.4	38.6
DTNB_MOUSE	Dtnb p-S131 (z= 3) + Oxi	100.0	51.9	66.8	66.9	46.3	56.3	38.6	29.7	82.0
DUS7_MOUSE	Dusp7 p-T49 p-T50 (z= 5) + Oxi	100.0	27.3	28.0	28.3	66.6	10.3	23.1	29.4	38.2
E41L1_MOUSE	Epb41l1 p-S407 (z= 3)	26.6	21.9	38.2	33.4	18.2	13.7	100.0	28.3	52.1
EAA1_MOUSE	Slc1a3 p-Y80 p-S82 p-S116 (z= 4) +	100.0	4.5	31.2	27.5	0.0	19.2	11.0	9.6	0.0
EAA3_MOUSE	Slc1a1 p-S73 p-S74 (z= 5) + Oxi	56.1	49.8	42.5	61.7	49.8	35.0	2.4	0.0	100.0
ECM29_MOUSE	ECM29 p-T900 (z= 3)	76.5	70.3	65.2	80.5	82.1	100.0	76.1	83.2	70.4
EDRF1_MOUSE	Edrf1 p-S26 (z= 4)	100.0	26.8	0.0	0.0	0.0	0.0	0.0	0.0	26.6
EF1D_MOUSE	Eef1d p-S162 (z= 3)	0.0	52.4	100.0	0.0	91.6	59.9	0.0	58.0	96.9
EIF3B_MOUSE	Eif3b p-S120 (z= 3)	100.0	37.4	61.8	80.4	51.6	91.1	68.5	52.7	77.6
EMAL6_MOUSE	Eml6 p-T1462 p-T1473 p-S1479 (z=	80.7	77.5	100.0	63.5	67.0	72.3	87.2	37.9	57.5
EME1_MOUSE	Eme1 p-S397 (z= 3) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
EMIL1_MOUSE	EMIL1 p-S723 (z= 4)	92.5	31.5	28.4	100.0	69.9	30.6	14.9	0.0	25.7
EPHX4_MOUSE	Ephx4 p-S284 p-T294 (z= 6) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
ERAP1_MOUSE	Erap1 p-T512 p-T514 (z= 4)	100.0	61.9	87.0	68.6	67.6	44.1	41.0	9.7	12.6
ERG28_MOUSE	ORF11 p-S16 p-S26 (z= 3) + Oxi	100.0	74.9	58.0	48.2	69.1	74.9	47.3	48.0	76.6
ERLEC_MOUSE	Erlec1 p-Y189 p-T197 p-S200 p-	92.8	85.9	76.7	100.0	81.7	58.3	93.0	71.7	64.9
ERR1_MOUSE	Esrra p-S72 p-S73 p-Y91 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3

EVI2A_MOUSE	Evi2a p-S84 p-T85 p-S86 (z= 3)	81.4	64.7	66.8	100.0	87.9	69.9	57.0	50.8	38.1
F1142_MOUSE	F1142 p-S2 (z= 4)	72.4	70.8	79.4	100.0	82.6	63.3	52.7	57.6	67.1
F178A_MOUSE	Fam178a p-S21 (z= 3)	71.7	59.8	70.3	71.5	77.6	78.0	100.0	48.0	69.9
F178A_MOUSE	Fam178a p-S591 (z= 4)	42.4	67.8	63.1	100.0	13.4	82.8	26.6	12.6	32.7
F187A_MOUSE	Fam187a p-S150 (z= 4)	30.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
F190A_MOUSE	Fam190a p-S6 (z= 2) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
FA40A_MOUSE	Fam40a p-S335 (z= 2)	38.9	47.7	42.6	45.0	75.2	75.8	71.1	100.0	56.0
FA63B_MOUSE	Fam63b p-Y280 (z= 5) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
FA83D_MOUSE	Fam83d p-S411 p-T417 p-T419 (z=	83.5	75.5	63.4	85.0	64.0	42.9	100.0	71.0	52.1
FA86A_MOUSE	Fam86a p-S320 p-T330 p-S331 (z=	53.4	45.6	68.6	75.3	52.2	100.0	67.4	51.0	66.2
FAS_MOUSE	Fasn p-S1879 p-Y1880 (z= 3)	76.6	79.6	100.0	89.1	73.0	41.5	34.0	71.5	55.6
FAT2_MOUSE	Fat2 p-Y529 (z= 3)	50.6	27.8	24.7	0.0	53.1	0.0	0.0	100.0	0.0
FBLI1_MOUSE	Fblim1 p-S193 (z= 3)	32.1	31.6	32.1	60.0	100.0	60.0	21.3	24.4	12.0
FBLL1_MOUSE	FBLL1 p-S268 p-S275 (z= 3)	83.7	100.0	80.6	87.9	75.5	43.9	57.6	63.0	63.8
FBSP1_MOUSE	Fbxo45 p-S33 p-S41 (z= 4)	100.0	50.8	59.6	51.7	61.6	59.6	32.6	9.3	21.2
FBX16_MOUSE	Fbxo16 p-T166 (z= 4) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
FBX7_MOUSE	Fbxo7 p-S283 p-S294 (z= 4)	30.2	29.8	32.2	32.4	5.6	4.5	0.0	69.3	100.0
FBXL5_MOUSE	Fbxl5 p-S331 p-T334 p-S340 (z= 4)	100.0	62.9	73.3	79.4	51.0	51.6	32.1	15.7	62.6
FCSD2_MOUSE	FCSD2 p-Y524 p-S545 (z= 4)	100.0	75.9	53.0	49.1	51.8	52.8	11.7	35.7	24.5
FEM1B_MOUSE	FEM1B p-S40 (z= 2)	64.3	62.0	61.3	71.1	60.6	39.5	100.0	57.9	60.7
FEM1B_MOUSE	Fem1b p-Y34 (z= 2)	64.3	62.0	61.3	71.1	60.6	39.5	100.0	57.9	61.0
FKB1A_MOUSE	Fkbp1a p-S9 (z= 2)	74.7	63.6	76.0	98.8	96.9	55.7	100.0	98.6	91.0
FLIP1_MOUSE	Filip1 p-T1180 (z= 4) + Oxi	100.0	65.1	63.5	58.2	60.6	68.0	0.0	14.3	10.6
FLNA_MOUSE	Flna p-S1084 (z= 2)	65.4	51.7	55.6	98.0	90.0	100.0	75.1	84.1	88.4
FLNA_MOUSE	Flna p-S2128 (z= 2)	49.3	32.2	32.7	28.9	75.0	100.0	62.3	76.4	56.7
FLNA_MOUSE	Flna p-S2128 (z= 3)	66.1	36.5	56.0	61.7	51.7	100.0	70.3	60.3	42.3
FLNA_MOUSE	Flna p-S2180 (z= 2)	57.8	35.9	0.0	41.9	45.2	66.9	77.8	96.0	100.0
FLNA_MOUSE	Flna p-S2327 (z= 3)	67.1	37.1	37.4	45.1	49.5	100.0	58.8	62.2	61.9
FLNB_MOUSE	Flnb p-S2130 (z= 3)	100.0	43.2	50.7	64.2	70.4	74.0	98.0	54.6	66.5
FLNB_MOUSE	Flnb p-S2137 (z= 4)	100.0	0.0	35.5	74.6	0.0	49.5	57.1	36.1	25.4
FLNC_MOUSE	Flnc p-S2234 (z= 2)	0.0	0.0	16.6	0.0	77.9	24.8	0.0	84.0	100.0
FLT3_MOUSE	Flt3 p-S980 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
FLVC2_MOUSE	Flvcr2 p-T315 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
FMN1_MOUSE	Fmn1 p-T91 (z= 2)	39.5	27.4	40.3	45.8	20.1	27.4	100.0	22.1	26.6
FMO5_MOUSE	Fmo5 p-S508 p-T515 (z= 3) + Oxi	0.1	100.0	60.3	82.6	0.1	0.1	0.1	0.1	77.5
FND3A_MOUSE	Fndc3a p-T911 p-Y913 (z= 4)	38.7	100.0	77.6	50.8	23.8	29.1	40.1	0.0	41.1
FOXP2_MOUSE	Foxp2 p-S25 (z= 4) + Oxi	90.1	71.4	74.4	100.0	65.6	79.6	94.6	52.2	63.1
FOXP2_MOUSE	FOXP2 p-T7 (z= 3) + Oxi	51.1	50.3	58.7	42.8	63.3	100.0	82.3	53.3	81.4
FRAS1_MOUSE	Fras1 p-Y3442 p-Y3443 p-S3465 p-	63.9	67.7	58.8	61.9	51.5	27.7	100.0	55.7	74.6
FREM2_MOUSE	Frem2 p-S197 p-S218 (z= 4)	100.0	83.8	49.2	51.1	43.3	53.2	21.6	32.2	38.4
FSIP2_MOUSE	Fsip2 p-S4863 p-S4866 p-Y4878	92.2	100.0	0.0	47.7	62.3	74.5	0.0	34.0	82.3
FSIP2_MOUSE	Fsip2 p-Y3219 p-S3220 p-T3222 p-	77.8	59.4	77.1	85.9	100.0	74.0	96.2	73.3	91.6
FUMH_MOUSE	FUMH p-T282 (z= 3) + Oxi	78.4	39.6	44.5	100.0	34.3	42.5	47.6	10.6	31.3
GBB4_MOUSE	Gnb4 p-T34 p-T47 (z= 3)	100.0	56.5	79.7	62.6	52.2	30.1	64.8	35.9	50.2
GBRB3_MOUSE	Gabrb3 p-Y168 p-Y182 p-T201 (z=	83.5	75.0	63.4	85.0	64.0	42.9	100.0	71.0	52.3
GBX1_MOUSE	Gbx1 p-S59 p-S88 (z= 3) + Oxi	18.1	0.0	29.1	22.9	63.4	90.7	0.0	32.5	100.0
GCNT3_MOUSE	Gcnt3 p-Y297 p-S301 (z= 4) + Oxi	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	100.0
GDF5_MOUSE	GDF5 p-S128 p-S129 p-S136 (z= 3)	100.0	73.8	54.2	45.4	66.4	72.1	42.5	48.8	74.1
GFRA3_MOUSE	Gfra3 p-Y179 (z= 3)	41.6	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
GIT1_MOUSE	Git1 p-S419 p-S422 p-Y435 (z= 7) +	20.3	0.0	30.0	30.7	100.0	0.0	0.0	0.0	69.4
GLGB_MOUSE	Gbe1 p-T220 (z= 2)	48.5	58.3	49.8	37.7	56.4	45.3	100.0	82.7	57.2
GMPR1_MOUSE	Gmpr p-T44 p-S46 p-T57 (z= 4)	67.2	32.7	91.8	100.0	63.4	66.5	83.9	39.1	46.2
GNTK_MOUSE	Idnk p-S13 p-S18 p-T19 (z= 3)	30.0	100.0	52.7	83.3	0.0	40.9	0.0	0.0	56.9
GON4L_MOUSE	Gon4l p-S1412 p-S1414 (z= 4) +	100.0	36.3	24.9	0.0	0.0	0.0	0.0	0.0	0.0
GP128_MOUSE	Gpr128 p-S243 (z= 6)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
GPAT1_MOUSE	Gpam p-Y494 (z= 6) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
GPC5B_MOUSE	Gprc5b p-T11 p-S25 p-S28 (z= 5)	94.6	96.1	70.5	100.0	58.9	35.9	37.6	34.8	35.8
GPR82_MOUSE	Gpr82 p-S300 (z= 3)	100.0	43.9	45.3	36.9	36.2	64.9	24.4	22.0	33.8
GPR88_MOUSE	Gpr88 p-S39 p-T46 p-S57 (z= 5) +	52.5	12.2	37.4	34.9	54.9	94.0	45.8	48.4	100.0
GRB10_MOUSE	Grb10 p-S503 p-S508 (z= 4) + Oxi	76.2	71.5	72.9	100.0	78.2	66.1	53.2	69.1	80.4
GREB1_MOUSE	GREB1 p-S293 p-S295 p-S309 (z=	77.4	100.0	83.9	0.0	62.7	79.7	91.6	78.9	9.9
GRIA1_MOUSE	Gria1 p-S873 p-S881 (z= 3)	9.5	23.4	24.2	16.0	23.0	14.4	9.9	100.0	74.4
GRIN1_MOUSE	Gprin1 p-T493 p-S519 (z= 3)	21.7	60.3	60.9	71.1	64.5	59.9	37.6	58.5	100.0
GRIN1_MOUSE	Gprin1 p-T569 (z= 4)	91.9	100.0	73.4	88.3	0.0	0.0	0.0	0.0	0.0
GSDA3_MOUSE	Gsdma3 p-T324 p-Y344 (z= 6) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
GSDC2_MOUSE	Gsdmc2 p-S195 (z= 3)	33.4	77.8	32.6	34.4	88.1	90.2	21.5	11.6	100.0
GSK3A_MOUSE	Gsk3a p-Y279 (z= 2)	75.2	45.4	66.6	74.1	100.0	98.0	84.8	83.0	82.2
GTR7_MOUSE	Slc2a7 p-S15 (z= 3) + Oxi	61.5	67.1	69.1	100.0	55.2	75.7	82.1	68.6	33.0
GTR8_MOUSE	Slc2a8 p-S99 p-T109 (z= 5) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
H12_MOUSE	Hist1h1c p-S36 (z= 2)	36.1	44.1	49.8	64.1	77.8	38.5	82.7	65.1	100.0
H12_MOUSE	Hist1h1c p-S36 (z= 3)	39.4	19.1	37.1	63.5	82.3	37.1	43.3	59.4	100.0
HELQ_MOUSE	Helq p-T1007 p-S1012 (z= 3) + Oxi	57.0	12.1	36.2	100.0	66.5	10.9	18.3	9.2	33.7
HES1_MOUSE	Hes1 p-S12 p-T17 p-S20 (z= 3) +	81.6	85.8	92.8	97.9	74.6	100.0	91.0	47.0	45.8
HIRA_MOUSE	Hira p-S79 (z= 4) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
HMCS2_MOUSE	Hmgcs2 p-Y387 p-S392 p-S395 p-	100.0	85.6	46.1	42.2	98.7	60.8	87.3	70.1	68.9
HNRPK_MOUSE	Hnrnpk p-S214 (z= 3)	47.1	73.2	53.1	83.8	94.7	89.6	100.0	96.1	94.4
HUWE1_MOUSE	Huwe1 p-Y4234 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
HXA10_MOUSE	HXA10 p-S328 (z= 4)	100.0	68.8	58.8	85.0	57.6	57.1	59.4	47.1	55.2
I2BP2_MOUSE	Irf2bp2 p-S443 (z= 2)	74.4	75.4	91.0	100.0	65.4	69.1	82.1	62.4	57.4
I2BP2_MOUSE	Irf2bp2 p-S443 (z= 2) + Oxi	44.2	61.8	57.2	71.8	64.7	78.3	35.5	96.4	100.0
IDHC_MOUSE	Idh1 p-Y272 p-S278 p-S280 p-S287	0.0	0.0	0.0	99.1	0.0	0.0	0.0	0.0	100.0
IF2B1_MOUSE	Igf2bp1 p-S181 (z= 2)	63.0	60.0	68.0	77.2	67.7	88.9	69.8	100.0	74.9
IF2B1_MOUSE	Igf2bp1 p-S181 (z= 2)	49.0	50.3	72.2	68.7	52.1	97.5	63.0	100.0	64.7
IF2B1_MOUSE	Igf2bp1 p-S181 (z= 3)	65.2	100.0	93.6	66.6	76.7	66.9	84.0	61.4	87.9
IF2M_MOUSE	Mtif2 p-S180 (z= 3)	94.5	100.0	91.8	87.4	74.2	74.6	77.0	71.7	85.7
IF4B_MOUSE	Eif4b p-S504 (z= 2)	28.2	54.6	33.3	46.6	34.8	65.4	28.4	100.0	42.8
IF4B_MOUSE	Eif4b p-S504 (z= 4)	71.7	66.5	76.9	100.0	72.9	93.3	68.0	64.8	28.6
IF4B_MOUSE	Eif4b p-T506 (z= 3)	89.9	68.0	74.3	100.0	94.3	78.3	81.7	57.0	97.2
IFI4_MOUSE	Ifi204 p-S94 p-T106 p-T108 (z= 3)	53.2	100.0	54.6	55.1	88.5	82.2	59.4	50.6	52.7
INCE_MOUSE	Incenp p-T121 p-T124 p-S135 (z= 4)	49.1	68.1	97.4	57.3	15.4	100.0	35.6	37.7	72.5
INF2_MOUSE	Inf2 p-S327 (z= 3)	56.6	59.9	48.1	100.0	60.8	0.0	59.5	35.8	57.6
INMT_MOUSE	Inmt p-S213 p-Y216 (z= 2)	23.7	16.8	49.4	45.4	49.0	7.0	41.9	100.0	6.6
INO1_MOUSE	Isyna1 p-S331 (z= 4) + Oxi	100.0	50.2	39.5	55.9	37.5	34.9	15.7	0.0	40.8
INP4B_MOUSE	Inpp4b p-S425 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
IPO4_MOUSE	Ipo4 p-S973 (z= 3)	40.8	9.8	31.7	27.2	15.9	31.6	100.0	50.5	55.0
IQCD_MOUSE	Iqcd p-T24 p-T34 p-S40 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
IRF2_MOUSE	Irf2 p-Y272 p-T282 (z= 4)	0.1	100.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
JARD2_MOUSE	Jarid2 p-S368 p-S376 (z= 4)	100.0	77.6	65.5	72.4	65.9	68.0	49.5	50.8	67.2

K0494_MOUSE	K0494 p-T220 p-T221 p-S228 (z= 3)	64.7	83.3	93.3	78.7	71.3	55.5	100.0	56.7	51.1
K1468_MOUSE	K1468 p-S243 (z= 3)	12.7	32.0	30.0	26.2	100.0	38.4	16.7	24.3	4.6
K1958_MOUSE	Dmkn p-T502 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
K22E_MOUSE	Krt2 p-S596 p-Y600 p-S620 p-S622	0.0	100.0	91.8	20.9	20.7	40.7	0.0	0.0	22.1
K22E_MOUSE	Krt2 p-S596 p-Y600 p-Y614 p-S622	0.0	100.0	73.5	16.0	9.5	22.8	0.0	0.0	21.7
K22O_MOUSE	Krt76 p-S559 p-S562 p-T564 (z= 6)	0.1	88.6	0.1	0.1	0.1	0.1	0.1	0.1	100.0
KAP2_MOUSE	Prkar2a p-T101 (z= 3)	47.8	12.0	13.3	22.1	12.7	100.0	39.5	43.8	28.8
KAPCA_MOUSE	Prkaca p-S66 p-Y70 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
KCD16_MOUSE	KCD16 p-T208 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
KCNQ5_MOUSE	Kcnq5 p-S20 (z= 3) + Oxi	0.0	36.8	28.5	0.0	0.0	100.0	0.0	16.3	0.0
KDM3A_MOUSE	Kdm3a p-S994 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
KDM4B_MOUSE	Kdm4b p-S1027 p-S1029 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
KDM5B_MOUSE	Kdm5b p-T1138 (z= 4) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
KDM5B_MOUSE	Kdm5b p-T203 (z= 2)	11.2	2.1	29.0	14.1	0.0	8.8	100.0	8.6	12.2
KI26B_MOUSE	Kif26b p-S1495 (z= 3)	35.9	48.0	47.6	66.1	68.0	34.0	54.1	93.5	100.0
KIF7_MOUSE	Kif7 p-S398 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
KLC4_MOUSE	Klc4 p-T465 p-T466 (z= 3)	58.2	81.2	73.1	92.7	78.2	47.9	100.0	81.5	79.3
KNTC1_MOUSE	Kntc1 p-T1304 p-S1308 (z= 3)	100.0	30.2	94.6	0.0	9.3	17.1	12.6	21.8	6.5
KPCG_MOUSE	Prkcg p-S192 (z= 4) + Oxi	13.3	100.0	72.4	41.4	0.0	4.6	0.0	0.0	15.1
KRT83_MOUSE	Krt83 p-T485 p-S489 (z= 3)	73.9	61.9	64.0	100.0	88.0	96.3	72.4	91.7	77.5
LAMC3_MOUSE	Lamc3 p-S4 p-S8 p-T12 (z= 3) + Oxi	0.0	100.0	0.0	0.0	0.0	0.0	0.0	60.6	0.0
LC7L2_MOUSE	Luc7l2 p-S2 p-T17 (z= 5) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
LIN41_MOUSE	Trim71 p-S21 p-S42 (z= 3)	8.7	57.9	40.4	22.9	100.0	88.5	34.4	90.6	59.7
LRBA_MOUSE	Lrba p-T1955 p-S1974 (z= 4)	100.0	70.2	77.6	79.9	47.0	43.9	37.1	21.0	55.8
LRC30_MOUSE	LRC30 p-S113 p-S117 p-S136 (z=	93.0	22.9	78.1	100.0	65.6	99.1	31.8	52.4	90.6
LRP6_MOUSE	Lrp6 p-S1450 p-S1455 p-S1456 (z=	0.0	100.0	45.8	29.1	37.7	46.3	0.0	0.0	0.0
LRRC7_MOUSE	Lrrc7 p-S1428 (z= 3)	100.0	76.5	60.4	62.4	69.6	72.0	67.7	57.4	69.5
LRRK1_MOUSE	Lrrk1 p-T102 (z= 4)	47.1	100.0	74.9	49.4	38.2	29.8	42.7	31.0	46.8
M4K1_MOUSE	M4K1 p-S270 (z= 4) + Oxi	0.1	100.0	0.1	0.1	0.1	0.1	0.1	0.1	70.2
MA7D1_MOUSE	Map7d1 p-S401 (z= 2)	80.0	9.7	62.0	68.6	84.0	96.6	92.7	100.0	66.0
MA7D1_MOUSE	Map7d1 p-S401 (z= 2)	47.0	9.6	19.1	53.0	100.0	62.7	44.3	65.6	40.3
MA7D2_MOUSE	Map7d2 p-S235 (z= 4)	97.2	97.6	100.0	94.6	83.4	68.6	87.7	79.2	86.1
MA7D2_MOUSE	Map7d2 p-S4 (z= 3) + Oxi	50.5	100.0	72.9	76.2	55.6	30.0	66.4	68.1	51.3
MACD1_MOUSE	Macrod1 p-S248 (z= 5)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
MAGI3_MOUSE	Magi3 p-S1300 p-S1307 p-S1311	0.1	0.1	100.0	0.1	0.1	0.1	0.1	0.1	0.1
MAP2_MOUSE	Map2 p-S333 (z= 3)	54.1	58.5	75.7	100.0	70.2	64.5	81.7	83.2	89.8
MAP4_MOUSE	Map4 p-S667 (z= 3)	76.5	70.3	65.2	80.5	82.1	100.0	76.1	83.2	70.4
MAP4_MOUSE	Map4 p-S667 (z= 3)	76.7	71.0	70.6	78.0	76.1	100.0	69.3	82.9	67.9
MAP4_MOUSE	Map4 p-S667 (z= 4)	55.9	72.7	76.2	93.5	69.8	100.0	72.5	74.9	58.6
MAP6_MOUSE	MAP6 p-S261 (z= 3)	85.5	98.2	100.0	89.8	70.9	90.4	62.2	80.0	68.4
MARK3_MOUSE	Mark3 p-T78 (z= 3)	24.1	26.2	56.5	40.6	7.3	16.5	100.0	10.6	27.3
MBL2_MOUSE	Mbl2 p-S137 (z= 2)	70.0	100.0	94.3	94.0	68.2	50.1	43.2	37.4	29.1
MBOA5_MOUSE	Lpcat3 p-S226 p-T227 (z= 4) + Oxi	92.5	66.3	82.4	100.0	56.7	59.7	64.2	47.4	36.8
MBP_MOUSE	Mbp p-S245 (z= 2) + Oxi	71.9	50.3	45.0	100.0	48.8	57.7	9.5	41.1	82.0
MCAF2_MOUSE	MCAF2 p-S32 p-T33 p-T41 (z= 3)	100.0	54.9	69.4	90.9	62.1	87.1	78.9	44.1	55.2
MCH_MOUSE	Pmch p-T5 p-S7 p-Y9 (z= 6) + Oxi	0.0	100.0	16.3	17.5	0.0	0.0	0.0	0.0	0.0
MDR3_MOUSE	Abcb4 p-T495 (z= 2)	68.3	64.3	100.0	50.8	93.7	29.0	62.8	22.9	10.7
MED1_MOUSE	Med1 p-S1149 p-T1151 (z= 2) + Oxi	69.8	68.5	69.6	59.7	76.0	79.1	100.0	53.9	62.7
MED25_MOUSE	Med25 p-S516 p-S517 (z= 3) + Oxi	75.7	79.9	79.7	74.0	39.9	43.2	100.0	52.8	70.0
MED4_MOUSE	Med4 p-S25 (z= 3) + Oxi	62.4	64.4	60.7	100.0	67.7	54.1	88.1	66.5	55.4
MESD1_MOUSE	Mesdc1 p-T10 (z= 3) + Oxi	21.0	37.7	43.4	43.5	23.6	48.0	44.5	100.0	35.6
METH_MOUSE	Mtr p-T1192 p-S1194 (z= 3) + Oxi	7.6	18.1	15.5	4.7	21.6	100.0	8.6	26.2	14.2
MEX3B_MOUSE	Mex3b p-S320 p-S321 p-Y332 (z=	57.7	40.3	43.6	45.5	38.3	38.2	17.8	8.0	100.0
MFGM_MOUSE	Mfge8 p-S149 (z= 5) + Oxi	100.0	66.0	0.0	63.7	64.2	0.0	0.0	46.1	46.8
MGAT3_MOUSE	Mgat3 p-S522 p-S527 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
MICLK_MOUSE	MICLK p-S276 p-S281 (z= 3)	12.7	32.0	29.8	25.9	100.0	38.4	16.5	24.3	4.6
MIER1_MOUSE	Mier1 p-T234 (z= 3)	90.8	67.6	91.5	85.5	100.0	34.2	85.4	24.5	29.8
MINK1_MOUSE	Mink1 p-S903 (z= 3)	100.0	54.9	65.3	76.4	64.9	60.7	57.1	46.7	44.2
MIPEP_MOUSE	Mipep p-T177 (z= 4)	100.0	75.6	40.0	45.5	43.3	22.3	59.3	37.3	61.3
MLF2_MOUSE	Mlf2 p-S237 (z= 2)	86.8	81.9	86.2	97.5	78.0	87.1	98.5	100.0	89.0
MLL3_MOUSE	Mll3 p-S3108 (z= 4) + Oxi	82.5	61.7	59.7	100.0	64.1	73.3	60.7	23.5	38.9
MLL3_MOUSE	Mll3 p-Y1165 p-S1178 p-S1182 (z=	83.1	100.0	85.2	71.9	80.7	88.1	78.1	81.3	58.6
MOGT2_MOUSE	Mogat2 p-S157 (z= 2)	100.0	20.9	26.4	61.2	60.4	70.6	85.1	18.1	43.1
MOGT2_MOUSE	Mogat2 p-Y154 p-S157 (z= 2)	100.0	36.6	54.4	86.8	85.6	96.0	77.3	78.3	75.0
MOGT2_MOUSE	Mogat2 p-Y154 p-S157 (z= 3)	100.0	38.4	52.7	76.5	40.7	61.9	48.2	50.3	55.2
MON2_MOUSE	MON2 p-T329 (z= 3)	100.0	69.4	58.1	87.6	88.3	85.1	80.4	69.0	93.8
MRC1_MOUSE	Mrc1 p-S1385 p-S1386 p-T1393 (z=	25.9	29.1	100.0	66.9	15.5	0.0	61.2	16.8	45.5
MRF_MOUSE	MRF p-T964 (z= 4)	97.9	100.0	65.4	89.6	64.2	32.1	65.7	58.4	67.3
MTCH1_MOUSE	Mtch1 p-S4 (z= 3)	18.9	22.0	75.9	55.9	84.8	52.7	92.2	44.3	100.0
MTOR_MOUSE	Mtor p-T484 p-S487 (z= 4)	100.0	79.9	64.1	76.5	67.7	67.4	50.5	51.4	68.1
MTUS2_MOUSE	Mtus2 p-T370 p-S373 p-S379 (z= 6)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
MUM1_MOUSE	Mum1 p-S581 p-T585 p-Y603 (z= 7)	29.4	46.8	94.3	100.0	25.3	43.1	0.0	0.0	91.1
MYCB2_MOUSE	Mycbp2 p-S3694 p-S3699 p-S3702	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
MYCT_MOUSE	Slc2a13 p-T311 (z= 3)	47.3	100.0	60.9	76.1	58.2	8.4	51.5	64.3	44.8
MYH9_MOUSE	Myh9 p-S1943 (z= 2)	0.0	0.0	53.0	0.0	80.3	100.0	0.0	80.6	79.0
MYH9_MOUSE	Myh9 p-S1943 (z= 3)	9.3	35.8	67.3	11.6	57.9	100.0	6.3	46.4	92.2
MYL1_MOUSE	Myl1 p-T140 (z= 3) + Oxi	100.0	61.9	79.6	63.1	53.9	56.1	6.1	26.5	54.2
MYLK_MOUSE	Mylk p-S263 (z= 2)	27.4	94.0	75.9	89.5	63.1	41.2	80.8	34.1	100.0
MYO10_MOUSE	Myo10 p-S1761 (z= 3)	27.1	42.8	59.3	100.0	37.5	61.5	44.0	65.3	12.8
MYO1H_MOUSE	Myo1h p-T356 p-S364 p-S365 (z=	41.1	38.7	100.0	53.7	16.0	53.4	86.6	68.6	83.2
MYOF_MOUSE	Myof p-T231 (z= 3)	72.8	51.3	45.4	70.4	52.8	100.0	14.4	18.9	77.8
NAA16_MOUSE	Naa16 p-T759 p-S760 (z= 3)	63.4	65.7	76.4	93.6	75.6	96.3	100.0	61.2	62.7
NACAM_MOUSE	Naca p-T1535 p-S1559 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
NAGAB_MOUSE	Naga p-T134 (z= 3) + Oxi	100.0	66.6	79.1	76.0	57.8	92.5	55.3	17.6	34.3
NAR2A_MOUSE	Art2a p-S259 (z= 3)	56.1	79.2	75.0	100.0	86.5	55.2	94.7	58.5	58.7
NCOR1_MOUSE	Ncor1 p-S1993 p-Y2008 (z= 3) +	100.0	97.7	85.8	89.7	87.8	88.2	78.1	19.0	28.7
NDKA_MOUSE	Nme1 p-S120 (z= 2)	0.0	0.0	73.5	0.0	28.5	100.0	0.0	25.1	0.0
NDKB_MOUSE	Nme2 p-S120 (z= 2)	0.0	10.6	53.3	0.0	44.4	100.0	0.0	34.9	53.1
NDKB_MOUSE	Nme2 p-T94 (z= 3)	41.3	52.0	94.4	100.0	64.4	39.0	84.0	50.5	41.7
NDUA4_MOUSE	Ndufa4 p-T27 (z= 3) + Oxi	52.3	70.0	83.5	59.1	74.4	56.1	84.0	83.7	100.0
NDUAB_MOUSE	Ndufa11 p-T74 p-S77 (z= 4) + Oxi	0.0	42.9	41.4	52.1	100.0	0.0	13.7	48.4	86.6
NEK3_MOUSE	Nek3 p-T477 (z= 3)	100.0	5.7	0.0	54.6	12.8	0.0	75.7	13.8	16.7
NEUA_MOUSE	Cmas p-S11 p-S20 (z= 4) + Oxi	100.0	77.6	65.5	72.4	65.9	68.0	49.5	50.8	67.2
NF1_MOUSE	Nf1 p-S1142 p-T1147 p-Y1171 (z=	71.6	50.0	56.8	100.0	43.7	44.9	33.7	31.4	26.8
NF2IP_MOUSE	Nfatc2ip p-S81 (z= 3)	39.2	68.4	64.8	19.1	57.4	100.0	15.6	75.0	73.5
NIPBL_MOUSE	Nipbl p-S1301 p-T1307 p-T1308 (z=	72.3	64.2	73.1	85.9	66.1	82.5	100.0	53.4	78.5
NMS_MOUSE	Nms p-T81 (z= 4) + Oxi	100.0	82.9	93.1	85.0	37.3	80.9	48.8	41.9	52.6
NPA1P_MOUSE	Urb1 p-T25 (z= 3)	100.0	73.7	0.0	11.0	87.9	16.5	0.0	42.9	0.0

NPAS2_MOUSE	Npas2 p-S607 p-S611 (z= 3)	67.4	58.0	66.9	100.0	64.0	33.5	77.9	47.0	57.4
NPM_MOUSE	Npm1 p-S70 (z= 4)	46.3	0.0	78.3	64.5	51.4	86.3	38.4	27.2	100.0
NPM_MOUSE	Npm1 p-S70 (z= 4) + Oxi	34.0	30.9	28.9	27.3	82.6	75.8	67.2	100.0	84.9
NR2E3_MOUSE	Nr2e3 p-S3 p-T4 (z= 3)	6.2	29.5	19.8	21.2	31.5	19.3	7.9	100.0	20.7
NSD1_MOUSE	Nsd1 p-T2255 p-S2262 (z= 3)	100.0	84.1	76.9	93.3	78.5	57.7	89.6	76.3	82.4
NSE2_MOUSE	Nsmce2 p-S6 (z= 5)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
NSF1C_MOUSE	Nsfl1c p-S165 (z= 2)	0.0	65.3	70.9	0.0	77.7	84.2	0.0	87.9	100.0
NSF1C_MOUSE	Nsfl1c p-Y167 (z= 2)	0.0	65.3	70.9	0.0	77.7	84.2	0.0	87.9	100.0
NTAN1_MOUSE	Ntan1 p-S252 p-S256 (z= 3)	50.4	77.8	47.4	76.0	53.2	31.1	100.0	30.1	69.8
NTKL_MOUSE	Scyl1 p-T275 (z= 3)	35.7	50.8	100.0	78.4	45.7	21.9	32.7	42.9	44.8
NU2M_MOUSE	Mtnd2 p-S245 p-T258 (z= 4) + Oxi	99.6	53.1	37.0	41.9	6.1	100.0	0.5	0.0	64.9
OPHN1_MOUSE	Ophn1 p-T699 (z= 3)	100.0	43.9	43.1	65.5	50.1	40.2	36.5	13.2	44.8
OPHN1_MOUSE	Ophn1 p-T780 (z= 2)	32.0	57.4	53.4	50.3	64.6	100.0	91.5	32.2	49.5
OSTF1_MOUSE	Ostf1 p-T47 (z= 4) + Oxi	87.1	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
OTOF_MOUSE	Otof p-Y1214 p-S1224 (z= 4)	76.2	73.5	81.2	100.0	80.5	61.0	59.6	62.3	67.8
PACR_MOUSE	Adcyap1r1 p-S465 p-S475 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
PAK6_MOUSE	Pak6 p-T213 (z= 3)	100.0	54.3	57.5	14.0	0.0	24.4	0.0	0.0	34.4
PAR3L_MOUSE	Pard3b p-S350 p-S352 (z= 3)	100.0	11.1	17.9	21.5	0.6	1.3	17.3	0.0	1.1
PAX4_MOUSE	Pax4 p-S120 p-S122 p-S123 (z= 4)	71.7	77.4	52.8	34.3	100.0	81.3	30.4	48.1	18.4
PCBP3_MOUSE	PCBP3 p-T346 p-S351 p-Y355 (z=	100.0	84.9	78.9	89.0	93.3	42.8	70.9	70.2	52.0
PCX1_MOUSE	Pcnx p-S1836 p-S1837 (z= 3)	19.2	59.5	43.2	13.1	80.8	87.5	67.8	100.0	44.7
PCX2_MOUSE	Pcnxl2 p-S1831 (z= 3)	76.7	44.0	53.2	81.8	85.4	86.4	100.0	50.6	69.8
PCX2_MOUSE	Pcnxl2 p-T809 (z= 3)	100.0	48.0	42.4	46.7	54.5	26.5	34.0	23.6	41.8
PCY1A_MOUSE	Pcyt1a p-S203 (z= 4) + Oxi	0.1	100.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
PDE4C_MOUSE	Pde4c p-T500 (z= 3) + Oxi	57.3	53.0	25.6	63.4	30.2	28.3	100.0	21.5	34.1
PDGFC_MOUSE	Pdgfc p-T51 (z= 3)	0.0	0.0	0.0	0.0	0.0	42.7	0.0	0.0	100.0
PDLI5_MOUSE	Pdlim5 p-S228 (z= 2)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
PDLI5_MOUSE	Pdlim5 p-S228 (z= 3)	0.0	0.0	0.0	0.0	89.2	55.0	0.0	50.5	100.0
PEG3_MOUSE	Peg3 p-S303 p-S309 p-S311 (z= 7)	0.0	100.0	67.9	22.7	0.0	17.4	0.0	0.0	51.2
PF21A_MOUSE	Phf21a p-T424 p-T426 p-S427 (z=	43.4	7.7	24.1	42.6	41.5	25.8	15.0	14.6	100.0
PHC2_MOUSE	Phc2 p-S828 (z= 4) + Oxi	55.7	80.6	77.3	100.0	41.4	34.5	67.5	62.3	57.0
PIAS1_MOUSE	Pias1 p-S428 p-S437 p-S438 (z= 6)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
PIGV_MOUSE	Pigv p-S192 (z= 4)	100.0	29.1	69.8	79.7	38.1	42.0	22.1	17.4	49.5
PITM2_MOUSE	Pitpnm2 p-S820 p-S823 (z= 3)	64.5	47.8	58.3	0.0	35.4	29.2	100.0	47.9	96.7
PKHG5_MOUSE	PKHG5 p-S255 p-S259 (z= 4)	99.1	66.3	73.8	100.0	61.1	52.0	61.5	50.2	41.9
PLAP_MOUSE	Plaa p-T481 p-S482 (z= 3)	40.8	73.3	81.5	98.7	76.6	100.0	41.8	59.3	37.3
PLCG1_MOUSE	Plcg1 p-T33 p-T36 (z= 5) + Oxi	100.0	17.5	39.9	37.8	39.7	62.6	41.8	31.2	72.0
PLD2_MOUSE	Pld2 p-Y75 p-S79 p-T83 p-T85 (z=	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
PLIN1_MOUSE	Plin1 p-T370 (z= 4)	100.0	80.7	54.1	56.0	71.6	81.7	27.1	22.1	66.5
PLPL1_MOUSE	PLPL1 p-S433 p-T435 p-S446 (z=	52.1	75.3	56.9	57.7	89.0	68.8	77.4	100.0	98.8
POC5_MOUSE	Poc5 p-S501 (z= 3) + Oxi	50.7	66.4	69.4	100.0	57.9	33.4	80.4	45.4	71.8
POL2_MOUSE	Pol p-T3 p-S27 (z= 4) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
POLK_MOUSE	Polk p-S309 p-S323 (z= 3) + Oxi	23.5	35.9	34.2	60.3	56.4	36.3	36.3	100.0	32.8
PON3_MOUSE	Pon3 p-S220 (z= 4)	69.6	39.0	71.6	0.0	75.8	100.0	26.0	11.9	50.8
PP14C_MOUSE	Ppp1r14c p-S2 p-T5 p-S25 (z= 4)	35.1	8.5	6.5	100.0	43.5	7.9	19.2	12.0	15.2
PPAC2_MOUSE	Ppapdc2 p-S31 (z= 3)	43.4	86.4	74.0	78.8	93.7	33.2	92.0	95.4	100.0
PPIC_MOUSE	Ppic p-T116 (z= 3)	35.7	57.1	51.8	36.5	59.7	87.0	58.1	94.3	100.0
PPR3F_MOUSE	Ppp1r3f p-S201 p-S229 p-S232 (z=	94.1	100.0	53.6	84.2	73.8	28.8	97.3	65.3	56.6
PPRC1_MOUSE	Pprc1 p-S440 p-S442 (z= 3)	48.8	0.0	0.0	53.0	0.0	100.0	32.6	20.7	0.0
PR8A8_MOUSE	Prl8a8 p-S103 p-T106 (z= 3)	54.2	81.9	52.2	41.9	90.0	68.0	55.0	78.3	100.0
PREP_MOUSE	Pitrm1 p-T912 p-S921 (z= 4)	45.0	87.5	87.5	76.5	58.8	80.6	76.5	100.0	55.8
PRGC1_MOUSE	PRGC1 p-S312 p-S318 (z= 3)	82.0	100.0	74.7	85.4	65.8	38.4	81.5	49.4	42.3
PROM2_MOUSE	Prom2 p-T803 (z= 2)	66.5	81.4	77.7	53.3	64.9	63.7	100.0	66.2	39.8
PRRT3_MOUSE	Prrt3 p-S760 (z= 4)	100.0	65.9	23.8	29.5	71.0	32.5	39.7	66.6	92.0
PSA_MOUSE	Npepps p-Y301 (z= 4)	42.7	26.3	77.6	65.6	28.0	100.0	59.6	11.4	27.7
PTBP2_MOUSE	Ptbp2 p-S26 (z= 4) + Oxi	50.5	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
PTBP3_MOUSE	PTBP3 p-S445 p-T447 p-S451 (z=	54.2	100.0	74.6	71.2	47.3	59.5	45.9	47.2	52.1
PTGR1_MOUSE	Ptgr1 p-S77 p-S97 p-T103 (z= 3)	98.3	66.8	36.0	58.2	85.7	29.9	100.0	77.0	57.5
PTN3_MOUSE	Ptpn3 p-S394 p-Y405 p-S421 (z= 3)	71.0	59.9	60.0	46.7	76.1	100.0	86.9	70.9	65.5
PTPRK_MOUSE	Ptprk p-T365 p-T381 (z= 3)	76.7	100.0	81.3	78.7	68.1	60.5	70.4	73.1	89.7
PTPRS_MOUSE	Ptprs p-Y205 p-S218 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
R51A1_MOUSE	Rad51ap1 p-S309 p-S311 (z= 3)	70.9	71.1	57.2	86.5	75.9	66.5	100.0	40.9	50.4
RADIL_MOUSE	Radil p-T728 (z= 3)	100.0	80.5	57.3	80.4	33.3	58.1	21.9	24.3	48.3
RAI1_MOUSE	Rai1 p-S1093 p-S1102 p-S1104 (z=	11.6	100.0	35.2	20.9	22.7	14.1	3.5	0.0	72.5
RANB3_MOUSE	Ranbp3 p-S40 (z= 3)	52.6	58.8	76.3	54.0	92.1	86.6	41.9	65.7	100.0
RANB3_MOUSE	Ranbp3 p-S40 (z= 3)	0.0	57.8	74.7	0.0	69.2	100.0	40.6	93.2	75.4
RANB3_MOUSE	Ranbp3 p-S40 (z= 4)	54.8	69.0	82.2	65.8	73.7	70.5	31.4	62.2	100.0
RASF4_MOUSE	RASF4 p-T200 p-T205 (z= 5) + Oxi	52.9	39.1	94.2	69.4	57.0	48.0	100.0	29.4	74.1
RAVR1_MOUSE	Raver1 p-S676 (z= 3) + Oxi	22.3	100.0	23.5	72.8	37.0	23.0	15.0	32.3	16.9
RBBP5_MOUSE	Rbbp5 p-S130 p-T136 (z= 3) + Oxi	74.1	74.0	85.5	100.0	87.0	47.2	89.1	35.7	43.3
RBM40_MOUSE	Rnpc3 p-S306 p-S314 (z= 4) + Oxi	84.1	100.0	68.2	41.2	69.4	48.5	19.3	25.8	38.0
RC3H1_MOUSE	Rc3h1 p-S315 (z= 5) + Oxi	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RDH13_MOUSE	Rdh13 p-S221 p-T224 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
RED1_MOUSE	Adarb1 p-S291 p-S306 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
RERE_MOUSE	Rere p-S1105 (z= 3)	65.4	47.7	74.5	91.0	73.4	100.0	69.7	50.8	49.6
RGP1_MOUSE	Rgp1 p-T139 (z= 3)	72.7	100.0	63.1	71.0	39.7	54.8	32.6	39.2	33.6
RGRF2_MOUSE	Rasgrf2 p-T1150 p-Y1152 (z= 4) +	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
RGS19_MOUSE	Rgs19 p-S159 (z= 4)	29.4	100.0	70.4	67.5	58.0	60.9	62.5	45.2	56.7
RGS3_MOUSE	Rgs3 p-S699 (z= 3)	69.1	0.0	13.0	42.7	0.0	0.0	83.9	100.0	58.7
RHBL1_MOUSE	Rhbdl1 p-S220 p-T237 (z= 3) + Oxi	52.2	43.5	70.9	86.7	65.8	22.1	100.0	55.3	72.4
RHBL2_MOUSE	Rhbdl2 p-S242 p-S245 p-Y261 (z=	58.4	66.5	47.9	48.1	49.1	23.1	100.0	70.1	45.1
RHG35_MOUSE	Arhgap35 p-S1085 (z= 4) + Oxi	30.4	49.7	55.7	36.0	41.4	58.0	50.9	100.0	84.8
RHGBA_MOUSE	Arhgap11a p-T908 (z= 3)	47.6	50.7	60.7	100.0	64.1	27.9	56.0	42.7	66.9
RIMB3_MOUSE	Rimbp3 p-T1402 p-S1404 p-Y1408	27.4	100.0	96.8	72.2	0.0	31.5	0.0	0.0	98.4
RIPK1_MOUSE	Ripk1 p-S181 p-T183 (z= 2)	34.4	6.8	34.5	54.6	37.4	100.0	6.9	8.1	35.1
RLA1_MOUSE	Rplp1 p-S104 (z= 2)	11.0	62.3	100.0	6.9	91.6	76.7	16.0	77.2	96.9
RLA1_MOUSE	Rplp1 p-S104 (z= 2) + Oxi	2.4	55.3	69.5	0.0	83.5	50.4	12.1	100.0	91.0
RLA2_MOUSE	Rplp2 p-S105 (z= 2)	1.0	36.2	81.0	0.0	57.6	68.1	0.0	53.7	100.0
RLA2_MOUSE	Rplp2 p-S105 (z= 2) + Oxi	0.0	48.4	52.0	0.0	57.4	55.6	0.0	82.4	100.0
RM36_MOUSE	Mrpl36 p-S8 p-S12 p-S21 (z= 4)	39.8	89.7	95.8	90.6	76.3	88.3	66.4	100.0	25.9
RMD3_MOUSE	Fam82a2 p-S57 (z= 3)	55.1	25.5	24.6	34.3	29.0	26.3	100.0	20.7	32.8
RN139_MOUSE	Rnf139 p-Y449 p-Y450 p-Y462 (z=	0.0	50.7	100.0	24.7	18.5	0.0	10.6	10.1	0.0
RN169_MOUSE	Rnf169 p-T8 (z= 3)	38.3	83.9	59.6	55.9	80.2	12.9	100.0	82.9	65.7
RNF12_MOUSE	Rlim p-T404 p-Y414 p-S417 p-S419	23.7	74.4	39.5	52.4	15.7	49.6	18.9	0.0	100.0
ROA2_MOUSE	Hnrnpa2b1 p-S324 p-S344 (z= 4) +	0.1	0.2	0.1	100.0	0.1	0.1	0.1	0.1	0.1
ROA3_MOUSE	Hnrnpa3 p-S356 p-S367 p-Y374 (z=	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
ROS1_MOUSE	Ros1 p-S1099 p-S1101 p-S1108 (z=	0.0	0.1	80.2	100.0	56.7	0.0	0.1	0.1	99.7
RPA1_MOUSE	Polr1a p-S450 (z= 3)	39.3	72.3	65.2	46.0	71.2	86.8	82.5	100.0	57.9

RPN2_MOUSE	Rpn2 p-T489 (z= 3)	30.8	79.6	100.0	65.8	0.0	43.1	0.0	0.0	63.0
RPP29_MOUSE	Pop4 p-T122 (z= 7)	12.6	74.3	35.5	0.0	100.0	87.5	0.0	33.7	0.0
RT22_MOUSE	Mrps22 p-T106 (z= 5) + Oxi	0.0	100.0	60.2	0.0	0.0	0.0	0.0	0.0	95.3
RTN4_MOUSE	Rtn4 p-T171 (z= 3)	0.0	49.1	54.1	0.0	100.0	89.5	0.0	76.4	86.0
RUSC1_MOUSE	Rusc1 p-S829 p-S832 (z= 3)	83.7	62.4	59.3	44.2	54.9	100.0	22.0	26.6	75.1
RXFP2_MOUSE	Rxfp2 p-S265 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
RXFP2_MOUSE	Rxfp2 p-S706 p-T712 (z= 4)	0.1	0.1	100.0	0.1	0.1	0.1	0.1	0.1	0.1
RYR1_MOUSE	Ryr1 p-S3056 (z= 4)	49.4	51.6	57.4	43.2	69.3	59.3	50.5	60.3	100.0
S12A7_MOUSE	Slc12a7 p-S775 p-T790 (z= 5) + Oxi	75.5	100.0	0.0	0.0	65.0	0.0	0.0	57.0	0.0
S1PR5_MOUSE	S1pr5 p-T91 p-S96 p-T100 (z= 4) +	24.6	21.2	36.3	100.0	37.3	6.6	67.0	22.3	0.0
S35D3_MOUSE	Slc35d3 p-Y36 p-T48 p-S49 p-S50	100.0	0.0	0.0	8.1	0.0	72.6	13.5	0.0	47.9
S39A5_MOUSE	S39A5 p-T273 p-S276 p-T302 (z= 4)	62.6	61.9	88.2	97.5	79.4	52.3	87.2	71.6	100.0
S39AC_MOUSE	Slc39a12 p-S346 (z= 3) + Oxi	80.1	100.0	58.3	94.7	100.0	47.5	92.6	85.6	89.9
SAHH3_MOUSE	Ahcyl2 p-T155 (z= 3)	27.7	52.2	55.3	47.2	50.5	33.4	42.9	100.0	88.9
SAMD8_MOUSE	Samd8 p-T143 p-S153 p-S155 (z=	40.3	38.2	70.7	69.2	38.1	22.5	100.0	36.2	39.5
SARM1_MOUSE	Sarm1 p-S574 (z= 3)	55.5	36.7	46.1	100.0	45.3	36.7	96.5	49.1	25.4
SBK1_MOUSE	Sbk1 p-T101 p-S103 p-S105 (z= 2)	59.2	69.8	86.8	65.9	77.6	56.6	100.0	63.2	57.5
SC61B_MOUSE	Sec61b p-S7 (z= 3)	100.0	71.1	64.7	100.0	47.3	63.3	49.1	67.4	51.4
SCRT1_MOUSE	Scrt1 p-S138 p-S166 (z= 3)	59.5	73.1	57.7	40.7	100.0	99.4	76.2	77.6	66.1
SF01_MOUSE	Sf1 p-S82 (z= 2)	57.9	49.1	65.7	88.0	86.9	85.6	68.8	80.6	100.0
SF01_MOUSE	Sf1 p-S82 (z= 2)	43.3	51.3	59.0	87.1	86.3	100.0	63.7	98.6	79.3
SF01_MOUSE	Sf1 p-S82 (z= 3)	61.9	69.8	73.8	90.2	75.7	100.0	74.3	95.5	78.5
SFI1_MOUSE	Sfi1 p-T414 p-S416 (z= 3)	100.0	0.0	0.0	0.0	0.0	0.0	0.0	56.1	0.0
SFPQ_MOUSE	Sfpq p-T468 (z= 3)	25.6	51.0	36.5	32.2	47.5	57.9	52.8	100.0	54.7
SFR1_MOUSE	Sfr1 p-S115 (z= 3)	99.9	62.5	74.9	100.0	96.6	94.8	73.9	68.3	71.6
SFR1_MOUSE	Sfr1 p-S67 (z= 2)	80.1	82.8	78.9	89.6	95.5	95.3	99.2	100.0	92.8
SFR1_MOUSE	Sfr1 p-S67 (z= 3)	68.7	84.6	81.2	100.0	65.6	76.4	70.5	74.2	64.1
SGCE_MOUSE	Sgce p-T315 p-S325 (z= 5)	100.0	83.1	68.5	82.1	64.0	71.5	43.3	39.9	63.5
SHPRH_MOUSE	Shprh p-T504 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
SHSA7_MOUSE	Shisa7 p-S248 p-S249 p-S250 (z=	42.8	75.3	89.3	100.0	52.0	78.2	38.1	50.0	31.0
SIA8E_MOUSE	St8sia5 p-Y236 p-T238 (z= 4)	100.0	62.9	73.3	79.4	51.0	51.6	32.1	15.7	62.8
SIN3B_MOUSE	Sin3b p-S261 p-S270 (z= 4)	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
SKIT6_MOUSE	Skint6 p-S524 p-S525 (z= 4) + Oxi	72.2	56.8	77.1	100.0	50.9	60.7	78.3	32.9	39.7
SLIT3_MOUSE	Slit3 p-S1273 p-T1286 p-S1289 (z=	0.0	11.0	100.0	29.3	1.5	27.0	61.1	57.7	81.3
SMK2A_MOUSE	Smok2a p-T354 p-S358 p-T362 (z=	100.0	25.3	51.4	46.5	43.3	51.4	58.4	8.8	26.0
SN_MOUSE	Siglec1 p-T1515 p-T1519 (z= 3)	72.9	100.0	92.8	85.6	78.4	57.6	93.6	84.0	65.0
SNX13_MOUSE	SNX13 p-Y115 p-Y120 p-S128 (z=	65.7	100.0	84.5	61.3	66.0	83.6	49.0	56.2	65.1
SO1A5_MOUSE	Slco1a5 p-S525 (z= 3) + Oxi	100.0	32.4	15.9	37.4	30.0	18.9	4.6	0.0	26.3
SODM_MOUSE	Sod2 p-T136 p-S145 (z= 3)	39.3	72.3	65.2	46.0	71.2	86.8	82.5	100.0	57.9
SPB12_MOUSE	Serpinb12 p-S360 (z= 3)	36.5	44.2	100.0	68.0	15.6	17.7	23.4	6.5	5.4
SPCS2_MOUSE	Spcs2 p-S4 p-S6 p-S11 (z= 5)	52.8	63.6	100.0	30.5	35.3	47.6	21.9	25.1	34.0
SPG20_MOUSE	Spg20 p-S537 p-S548 p-T555 (z= 3)	71.1	71.2	71.2	40.8	90.7	100.0	95.5	77.1	61.7
SPRE1_MOUSE	Spred1 p-T7 p-S8 p-S13 (z= 4) +	54.8	69.0	82.2	63.0	73.7	70.7	30.2	62.2	100.0
SPY2_MOUSE	Spry2 p-S111 p-T112 (z= 3)	100.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
SRB4D_MOUSE	Srcrb4d p-S184 (z= 3) + Oxi	84.1	95.6	93.4	95.7	89.0	46.9	100.0	83.2	74.2
SRF_MOUSE	SRF p-S99 p-T136 (z= 4) + Oxi	100.0	57.8	74.1	79.3	50.6	52.5	31.4	15.2	60.3
SRRM1_MOUSE	Srrm1 p-S391 (z= 2)	64.8	49.0	50.4	65.3	80.5	100.0	84.3	81.5	46.8
SRRM1_MOUSE	Srrm1 p-S400 (z= 3)	32.8	13.3	39.2	78.7	100.0	72.5	88.4	93.5	34.8
SRRM1_MOUSE	Srrm1 p-S574 (z= 2)	48.2	46.0	49.6	58.3	89.9	100.0	70.3	76.1	67.4
SRRM1_MOUSE	Srrm1 p-S574 (z= 3)	73.0	86.8	79.7	87.9	75.1	100.0	87.9	98.0	68.5
SRRM1_MOUSE	Srrm1 p-S574 (z= 3)	52.5	59.0	59.7	69.2	71.2	100.0	73.7	76.1	54.7
SRRM1_MOUSE	Srrm1 p-S602 (z= 2)	61.4	48.4	46.8	50.2	50.9	100.0	50.0	70.5	98.1
SRRM1_MOUSE	Srrm1 p-S602 (z= 2)	3.5	0.0	44.8	56.4	50.3	51.0	64.2	100.0	89.2
SRRM1_MOUSE	Srrm1 p-S781 (z= 2)	86.4	66.1	60.8	66.1	71.1	100.0	84.4	93.7	79.4
SRRM1_MOUSE	Srrm1 p-S810 (z= 3)	51.1	50.8	47.4	56.6	78.2	100.0	91.7	42.8	0.0
SRRM1_MOUSE	Srrm1 p-T736 (z= 2)	74.5	48.4	57.3	70.5	97.1	100.0	80.6	94.6	97.7
SRRM2_MOUSE	Srrm2 p-S1068 (z= 2)	52.9	75.2	73.3	33.6	45.8	94.1	52.2	93.3	100.0
SRRM2_MOUSE	Srrm2 p-S1572 (z= 3)	100.0	51.7	87.4	85.5	64.3	99.7	75.7	82.3	100.0
SRRM2_MOUSE	Srrm2 p-S1608 (z= 3)	74.3	36.4	40.7	62.1	56.5	100.0	47.7	48.2	51.6
SRRM2_MOUSE	Srrm2 p-S1650 (z= 2)	100.0	57.1	53.8	66.9	44.7	98.8	69.1	75.6	54.9
SRRM2_MOUSE	Srrm2 p-S1669 (z= 2)	0.0	0.0	40.5	41.2	0.0	6.5	21.7	100.0	95.6
SRRM2_MOUSE	Srrm2 p-S1683 (z= 2)	60.6	51.1	67.0	70.9	79.8	100.0	76.5	96.9	63.4
SRRM2_MOUSE	Srrm2 p-S1683 (z= 3)	77.6	42.0	56.8	75.1	56.3	100.0	57.3	41.1	50.1
SRRM2_MOUSE	Srrm2 p-S1998 (z= 2)	74.8	37.2	60.1	80.8	82.7	100.0	88.8	78.0	73.3
SRRM2_MOUSE	Srrm2 p-S2535 (z= 2)	54.8	58.0	73.5	77.8	87.5	100.0	93.0	85.3	39.8
SRRM2_MOUSE	Srrm2 p-S778 (z= 2)	100.0	72.0	54.3	0.0	0.0	0.0	88.4	0.0	0.0
SRRM2_MOUSE	Srrm2 p-S945 (z= 3) + Oxi	39.5	53.3	47.2	66.3	50.8	96.5	29.2	95.9	100.0
SRRM2_MOUSE	Srrm2 p-S946 (z= 2)	100.0	58.6	71.1	79.5	45.6	53.4	66.3	32.9	67.4
SRRM2_MOUSE	Srrm2 p-S946 (z= 2) + Oxi	41.4	46.4	37.2	12.2	45.0	63.1	23.8	83.7	100.0
SRRM2_MOUSE	Srrm2 p-T1574 (z= 2)	18.4	19.7	30.8	38.4	100.0	80.7	44.3	76.7	100.0
SRRM2_MOUSE	Srrm2 p-T1654 (z= 2)	53.6	19.9	48.1	39.5	43.4	78.5	52.6	65.0	100.0
SRRM2_MOUSE	Srrm2 p-T2096 p-S2099 (z= 4) +	0.1	80.5	100.0	0.1	0.1	0.1	0.1	0.1	78.2
SRRM2_MOUSE	Srrm2 p-T955 (z= 3)	95.5	75.7	77.0	100.0	54.5	52.4	70.3	44.2	61.6
SRSF2_MOUSE	Srsf2 p-S208 (z= 2)	64.8	45.7	58.4	69.2	93.5	100.0	70.3	75.6	82.4
SRSF2_MOUSE	Srsf2 p-S208 (z= 3)	95.1	65.6	80.1	100.0	53.1	96.0	68.3	66.5	83.6
SRSF2_MOUSE	Srsf2 p-S26 (z= 3)	100.0	39.5	31.3	76.8	26.4	96.6	58.1	57.1	36.5
SRSF2_MOUSE	Srsf2 p-T22 (z= 2)	59.2	20.6	18.9	35.1	47.9	100.0	48.8	60.3	52.1
SSFA2_MOUSE	Ssfa2 p-T742 (z= 4)	65.2	58.5	100.0	71.0	93.8	96.5	32.4	51.3	89.8
ST32C_MOUSE	Stk32c p-S353 (z= 4) + Oxi	0.0	100.0	0.0	0.0	57.7	0.0	0.0	0.0	0.0
STAR9_MOUSE	Stard9 p-T360 p-S362 p-T366 (z= 4)	72.6	81.5	84.0	80.6	71.8	87.8	100.0	58.1	67.7
STON2_MOUSE	Ston2 p-S342 (z= 3)	88.4	65.8	100.0	54.6	51.8	77.8	89.7	28.1	55.8
STON2_MOUSE	Ston2 p-S759 p-S762 p-S766 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
STRN3_MOUSE	STRN3 p-S389 p-S395 p-S397 (z=	92.4	80.4	54.9	54.8	77.0	47.9	100.0	74.8	55.9
STS_MOUSE	Sts p-S99 p-T113 (z= 3) + Oxi	64.2	100.0	50.9	46.7	94.5	78.4	74.0	74.0	68.0
STT3B_MOUSE	Stt3b p-T519 (z= 4)	47.0	63.7	57.4	77.1	100.0	0.0	0.0	24.6	54.1
SYAP1_MOUSE	Syap1 p-S226 p-S239 (z= 3)	0.0	100.0	17.7	0.0	0.0	7.2	0.0	0.0	0.0
SYFM_MOUSE	Fars2 p-S33 p-S34 p-S49 (z= 3)	82.0	94.2	80.7	100.0	62.6	57.0	65.1	39.9	56.1
SYLC_MOUSE	Lars p-Y266 p-T267 (z= 3)	100.0	44.9	49.9	57.6	37.8	68.0	31.8	22.2	38.3
SYNE2_MOUSE	Syne2 p-S2300 p-T2310 (z= 4)	74.1	87.6	70.6	100.0	87.0	44.6	85.6	40.8	73.5
SYNP2_MOUSE	Synpo2 p-T525 (z= 4) + Oxi	28.0	61.3	32.4	0.0	0.0	0.0	0.0	0.0	100.0
SYVC_MOUSE	Vars p-S7 p-S15 (z= 4)	84.8	100.0	75.4	84.4	75.8	58.2	54.9	46.3	55.6
T2FA_MOUSE	T2FA p-S431 p-S433 (z= 3)	54.9	71.6	65.6	11.5	99.3	100.0	6.5	45.3	82.4
T30A1_MOUSE	Ttc30a1 p-T230 (z= 3) + Oxi	33.8	100.0	57.7	97.8	99.0	28.4	45.6	39.1	75.6
TAF1C_MOUSE	TAF1C p-T20 (z= 3) + Oxi	40.6	69.4	48.5	38.4	99.0	88.4	67.9	100.0	86.2
TAF3_MOUSE	Taf3 p-T384 p-T387 p-S391 (z= 3)	67.4	100.0	79.4	78.7	65.8	63.7	73.0	67.2	56.5
TBC8B_MOUSE	Tbc1d8b p-Y34 p-T43 p-S52 p-S56	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
TBD_MOUSE	Tubd1 p-S397 (z= 3)	53.7	100.0	51.4	72.1	89.8	70.8	36.3	20.7	49.6
TBD2B_MOUSE	Tbc1d2b p-T171 (z= 2)	55.3	46.1	63.8	100.0	85.4	17.2	75.4	53.3	77.1

TC1D3_MOUSE	Tcte3 p-T15 p-S18 (z= 3)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
TCEA1_MOUSE	Tcea1 p-T241 (z= 2)	80.2	90.9	82.1	100.0	75.0	62.0	89.3	78.7	73.1
TCPG_MOUSE	Cct3 p-S45 (z= 3) + Oxi	26.7	51.9	45.5	34.1	51.4	57.5	53.3	100.0	56.5
TCPG_MOUSE	Cct3 p-S45 (z= 3) + Oxi	69.7	76.2	75.6	74.8	69.8	83.7	100.0	67.7	62.8
TDT_MOUSE	Dntt p-S200 p-S207 (z= 4)	45.0	87.5	87.5	76.5	58.8	80.6	76.5	100.0	55.8
TET2_MOUSE	Tet2 p-Y1095 p-T1096 (z= 2)	15.4	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
TEX14_MOUSE	Tex14 p-S310 (z= 4) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
TEX14_MOUSE	Tex14 p-T1248 (z= 4)	85.9	86.2	65.6	89.8	100.0	80.5	19.8	69.1	62.3
TGT_MOUSE	Qtrt1 p-S235 (z= 3)	74.9	70.2	73.9	80.8	88.9	78.4	92.4	87.6	100.0
THOP1_MOUSE	Thop1 p-S655 (z= 3) + Oxi	85.0	100.0	89.0	98.8	77.0	43.7	66.6	47.3	69.2
THY1_MOUSE	Thy1 p-S6 p-S12 p-S17 (z= 4) + Oxi	53.4	0.1	75.8	100.0	0.1	0.1	0.1	0.1	0.1
TISD_MOUSE	Zfp36l2 p-T9 p-S18 (z= 3) + Oxi	100.0	60.5	67.4	76.3	61.7	29.8	64.5	16.3	23.0
TITIN_MOUSE	Ttn p-S566 (z= 3)	100.0	37.2	58.7	64.8	61.7	60.1	38.6	30.8	76.2
TITIN_MOUSE	Ttn p-T16223 p-S16238 (z= 4)	73.7	62.8	73.2	81.4	56.7	19.8	100.0	68.7	28.9
TITIN_MOUSE	Ttn p-T32932 (z= 2)	74.0	94.8	92.9	99.5	75.5	93.8	79.8	100.0	81.3
TITIN_MOUSE	Ttn p-Y25882 (z= 3)	71.2	100.0	80.2	65.0	71.3	74.4	47.3	98.2	30.9
TLN1_MOUSE	Tln1 p-S1328 (z= 2)	71.9	75.2	66.1	74.6	78.7	97.9	80.0	100.0	63.6
TLR3_MOUSE	Tlr3 p-S315 p-S318 p-S323 (z= 4)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
TLR9_MOUSE	Tlr9 p-S710 p-S711 p-S713 (z= 3)	3.8	100.0	24.8	9.3	14.2	19.9	26.0	27.6	3.5
TM135_MOUSE	Tmem135 p-S232 (z= 4) + Oxi	100.0	75.2	88.8	76.0	71.9	89.5	41.3	32.5	91.3
TM158_MOUSE	Tmem158 p-T246 (z= 4)	0.1	100.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TM158_MOUSE	Tmem158 p-T246 (z= 5)	100.0	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1
TM245_MOUSE	Tmem245 p-S12 (z= 2)	66.8	0.0	67.1	0.0	67.0	100.0	71.8	76.9	0.0
TM63C_MOUSE	Tmem63c p-S679 p-S682 (z= 4)	29.2	21.8	91.9	65.3	66.4	34.5	100.0	25.5	63.3
TMM74_MOUSE	Tmem74 p-S180 p-S195 (z= 5)	24.9	100.0	79.1	28.3	40.7	28.7	0.0	0.0	87.0
TMM9B_MOUSE	Tmem9b p-S14 (z= 4) + Oxi	0.0	34.9	75.5	0.0	100.0	67.5	0.0	44.4	0.0
TP4A1_MOUSE	Ptp4a1 p-T13 (z= 3)	44.1	61.2	100.0	51.7	0.0	19.1	91.1	0.0	0.0
TPC2L_MOUSE	Trappc2l p-T74 p-S76 (z= 4) + Oxi	89.8	62.4	71.0	100.0	81.7	70.3	47.7	65.6	62.4
TRA2A_MOUSE	Tra2a p-S261 (z= 2)	33.8	20.2	58.0	79.8	77.4	92.7	44.8	62.5	100.0
TRA2A_MOUSE	Tra2a p-T200 p-T202 p-T211 (z= 3)	79.5	100.0	93.5	90.0	69.6	62.6	74.3	60.9	78.7
TRA2A_MOUSE	Tra2a p-T211 p-S213 (z= 3) + Oxi	14.6	0.0	100.0	0.0	0.0	6.8	0.0	0.0	8.4
TRI32_MOUSE	Trim32 p-T301 p-S307 (z= 4) + Oxi	100.0	38.9	21.4	0.0	0.0	0.0	0.0	0.0	45.0
TRUA_MOUSE	Pus1 p-T141 p-S153 (z= 4)	0.0	26.6	0.0	24.7	0.0	100.0	0.0	0.0	18.5
TS1R2_MOUSE	Tas1r2 p-T480 p-Y481 p-S483 p-	0.0	25.7	72.5	100.0	14.8	19.0	0.0	0.0	48.1
TSC2_MOUSE	Tsc2 p-S687 (z= 2)	53.5	93.9	88.3	77.3	100.0	16.5	80.8	79.7	56.4
TSSC4_MOUSE	Tssc4 p-S64 (z= 3)	11.7	8.1	100.0	40.4	7.5	61.8	50.0	15.6	77.3
TTC38_MOUSE	Ttc38 p-T59 p-T77 (z= 4) + Oxi	52.4	83.6	100.0	73.7	35.4	61.7	22.8	11.5	78.6
TTL10_MOUSE	Ttll10 p-S637 p-S640 (z= 3) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
TTLL8_MOUSE	Ttll8 p-S664 (z= 4) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
TYRP1_MOUSE	Tyrp1 p-T199 p-T203 (z= 4)	47.7	100.0	51.1	0.0	0.0	0.0	0.0	67.9	47.7
U119A_MOUSE	Unc119 p-S12 p-S24 p-T28 (z= 3)	49.1	63.0	51.9	54.3	62.5	46.2	39.7	92.7	100.0
UBE3C_MOUSE	Ube3c p-S135 p-T139 (z= 3)	100.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
UBP14_MOUSE	Usp14 p-S222 p-T226 (z= 3)	73.4	56.7	39.6	71.3	65.2	64.0	100.0	39.2	48.2
UBP2_MOUSE	Usp2 p-S92 p-S94 p-Y115 (z= 7) +	2.9	19.4	66.3	13.6	4.0	6.5	2.2	5.7	100.0
UBP3_MOUSE	Usp3 p-T491 p-T493 (z= 3)	100.0	66.0	75.9	86.1	48.3	54.5	62.3	16.3	23.5
UBP37_MOUSE	Usp37 p-S298 (z= 2)	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
UH1BL_MOUSE	Uhrf1bp1l p-S443 (z= 4)	69.5	70.7	97.5	47.1	78.5	92.9	34.5	50.6	100.0
UHRF2_MOUSE	Uhrf2 p-T509 (z= 2)	54.9	77.0	65.6	100.0	62.0	22.8	68.5	69.6	55.7
USH2A_MOUSE	Ush2A p-S3872 p-S3873 p-T3883	70.6	63.5	77.7	79.7	100.0	70.9	90.3	70.5	85.8
VCIP1_MOUSE	Vcpip1 p-T1072 p-S1075 p-S1076	0.0	100.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1
VINC_MOUSE	Vcl p-S290 (z= 3)	28.3	16.8	21.5	61.8	41.1	70.0	100.0	38.1	36.5
VINC_MOUSE	Vcl p-S346 (z= 2)	45.4	55.0	59.2	100.0	62.9	88.9	89.7	92.1	70.4
VINC_MOUSE	Vcl p-S346 (z= 2)	60.8	0.0	28.8	42.8	89.2	100.0	39.9	68.5	45.3
VINC_MOUSE	Vcl p-S787 p-S795 (z= 3)	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
VITRN_MOUSE	VITRN p-S267 (z= 3)	23.7	24.6	37.5	25.6	8.4	13.4	100.0	18.3	24.9
WBP11_MOUSE	Wbp11 p-S600 (z= 3)	40.2	63.3	45.8	54.4	47.4	69.7	35.0	35.8	100.0
WDR11_MOUSE	WDR11 p-S606 p-S620 p-S625 (z=	100.0	90.0	66.2	75.6	51.7	28.6	31.3	32.7	33.4
WDR24_MOUSE	Wdr24 p-T450 p-S458 p-S463 (z= 3)	74.8	72.3	63.2	55.2	68.4	100.0	68.2	64.6	82.5
WDR33_MOUSE	Wdr33 p-S1212 (z= 3)	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
WDR78_MOUSE	Wdr78 p-T58 (z= 5) + Oxi	15.9	100.0	94.9	65.4	3.3	38.3	0.0	0.0	68.2
WNK2_MOUSE	Wnk2 p-T279 p-T282 (z= 3) + Oxi	76.2	100.0	92.2	95.8	83.3	76.6	91.4	93.4	96.3
WNT2_MOUSE	Wnt2 p-S49 (z= 4)	84.8	94.8	76.8	50.1	85.9	96.5	100.0	63.2	60.4
YBOX1_MOUSE	Ybx1 p-S2 p-S3 p-T41 (z= 4) + Oxi	67.1	67.0	48.7	55.3	52.4	27.7	100.0	68.3	46.9
YBOX1_MOUSE	Ybx1 p-S2 p-S3 p-T7 (z= 3) + Oxi	69.2	75.3	39.1	20.1	61.7	27.7	100.0	77.3	45.7
YIF1A_MOUSE	Yif1a p-T251 (z= 3)	89.8	74.6	77.7	100.0	89.9	66.5	52.9	52.3	65.4
YIPF6_MOUSE	Yipf6 p-S196 p-T201 (z= 4) + Oxi	0.0	5.7	5.1	4.1	0.0	100.0	0.0	0.0	4.6
ZFHX4_MOUSE	Zfhx4 p-T417 p-T420 (z= 4)	59.2	73.3	86.8	72.5	90.0	60.4	100.0	65.2	63.0
ZN503_MOUSE	Znf503 p-S140 p-S141 (z= 4)	72.8	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
ZNFX1_MOUSE	Znfx1 p-S1877 p-T1895 (z= 6) + Oxi	0.1	0.1	79.9	100.0	0.1	0.1	0.1	0.1	0.1

Appendix 2

Summary of phosphorylation events identified in phosphoproteomic screen for proteins differentially regulated by p110α and p110β (Chapter 4).

		Relative mean phosphopeptide intensity of condition:			
Accession Number	Phosphopeptide identification	Starved	DMSO	A66	TGX221
1433E_MOUSE	Ywhae p-S213 (z= 2)	61.2	100.0	80.8	84.8
3BP1_MOUSE	Sh3bp1 p-S553 (z= 3)	55.0	100.0	91.8	94.6
3BP5L_MOUSE	Sh3bp5l p-S377 (z= 2)	72.3	84.8	100.0	47.9
4ET_MOUSE	Eif4enif1 p-S563 (z= 2)	91.7	90.7	100.0	85.0
4ET_MOUSE	Eif4enif1 p-S949 (z= 2)	100.0	56.7	58.1	97.8
A7L3B_MOUSE	Atxn7l3b p-S92 (z= 3)	81.8	85.0	100.0	78.1
AAK1_MOUSE	Aak1 p-S635 (z= 2)	69.7	100.0	45.3	59.6
AAK1_MOUSE	Aak1 p-S674 (z= 2)	100.0	58.9	98.5	58.0
AAK1_MOUSE	Aak1 p-T618 p-S622 (z= 2)	100.0	80.2	62.6	81.4
AATF_MOUSE	Aatf p-S283 p-S287 p-S288 (z= 3)	85.7	81.4	100.0	99.0
AB1IP_MOUSE	Apbb1ip p-S507 (z= 2)	85.0	100.0	52.8	43.1
AB1IP_MOUSE	Apbb1ip p-S507 (z= 3)	100.0	97.8	69.0	87.2
ABCAD_MOUSE	Abca13 p-S4703 p-T4710 (z= 4)	100.0	77.0	87.5	85.3
ABCF1_MOUSE	Abcf1 p-S107 (z= 2)	93.7	90.7	99.8	100.0
ABCF1_MOUSE	Abcf1 p-S138 (z= 2)	95.9	87.1	100.0	84.1
ABCF1_MOUSE	Abcf1 p-S138 (z= 3)	82.0	67.4	88.1	100.0
ABI1_MOUSE	Abi1 p-S183 (z= 2)	72.4	100.0	55.7	14.6
ABI1_MOUSE	Abi1 p-S183 (z= 3)	100.0	92.4	65.8	51.2
ABI1_MOUSE	Abi1 p-S225 (z= 2)	100.0	57.1	77.9	55.6
ABL1_MOUSE	Abl1 p-S838 p-S840 p-T844 (z= 3) + Oxi	92.2	50.6	47.5	100.0
ABL2_MOUSE	Abl2 p-S632 (z= 2)	100.0	93.3	87.9	79.1
ABLM1_MOUSE	Ablim1 p-S475 (z= 2)	87.0	100.0	77.1	89.4
ABLM1_MOUSE	Ablim1 p-S789 (z= 2)	100.0	80.3	85.5	83.7
ACACA_MOUSE	Acaca p-S79 (z= 2)	45.0	56.3	56.7	100.0
ACINU_MOUSE	Acin1 p-S1003 (z= 2)	89.1	57.7	82.0	100.0
ACINU_MOUSE	Acin1 p-S216 (z= 3)	91.7	100.0	98.6	65.4
ACINU_MOUSE	Acin1 p-S477 p-S491 (z= 2)	100.0	37.6	55.2	61.2
ACINU_MOUSE	Acin1 p-S477 p-S491 (z= 3)	100.0	36.7	62.7	57.8
ACINU_MOUSE	Acin1 p-S479 (z= 2)	74.5	100.0	54.4	66.3
ACINU_MOUSE	Acin1 p-S710 (z= 4)	51.7	100.0	71.8	33.4
ACLY_MOUSE	Acly p-S455 (z= 2)	54.3	78.7	75.6	100.0
ACSM1_MOUSE	Acsm1 p-T226 p-Y228 (z= 3) + Oxi	100.0	68.2	82.1	87.0
ACY3_MOUSE	Acy3 p-S7 (z= 2)	81.7	32.5	100.0	57.4
ADCY8_MOUSE	Adcy8 p-Y1147 (z= 3)	63.2	4.3	100.0	32.3
ADDA_MOUSE	Add1 p-S465 (z= 2)	23.9	100.0	50.0	45.4
ADNP_MOUSE	Adnp p-S679 p-S681 (z= 3)	75.0	96.5	94.6	100.0
AF1L2_MOUSE	Afap1l2 p-T674 (z= 2)	39.1	0.0	9.2	100.0
AFAD_MOUSE	Mllt4 p-S1107 (z= 3)	67.3	100.0	45.7	35.5
AFAD_MOUSE	Mllt4 p-S1173 p-S1182 (z= 3)	100.0	83.8	73.4	81.6
AFAD_MOUSE	Mllt4 p-S1795 (z= 2)	64.7	87.0	65.0	100.0
AFAP1_MOUSE	Afap1 p-S669 (z= 2)	88.5	100.0	74.3	76.9
AFAP1_MOUSE	Afap1 p-T342 p-S343 (z= 3)	98.1	100.0	76.6	61.8
AFTIN_MOUSE	Aftph p-S151 (z= 2)	100.0	85.4	85.9	91.7
AGAP2_MOUSE	Agap2 p-S224 p-S226 p-T227 (z= 3)	47.8	63.2	97.8	100.0
AGFG1_MOUSE	AGFG1 p-S179 p-S181 (z= 4)	100.0	58.5	61.4	63.5
AGFG1_MOUSE	Agfg1 p-S181 (z= 2)	73.6	0.0	100.0	0.0
AGFG1_MOUSE	Agfg1 p-T177 p-S181 (z= 3)	100.0	56.0	61.0	62.6
AJUBA_MOUSE	Ajuba p-S132 p-S133 p-S146 (z= 4)	92.4	79.4	79.2	100.0
AKAP2_MOUSE	Akap2 p-S163 (z= 4)	100.0	70.6	87.5	75.4
AKAP2_MOUSE	Akap2 p-S740 (z= 2)	76.6	93.0	100.0	84.7
AKAP2_MOUSE	Akap2 p-S799 (z= 4)	99.9	100.0	93.8	93.8
AKAP8_MOUSE	Akap8 p-S320 p-S325 (z= 2)	50.7	59.1	100.0	77.2
AKC1H_MOUSE	Akr1c18 p-Y259 (z= 3)	100.0	94.7	81.5	88.8
AKIR2_MOUSE	Akirin2 p-S21 (z= 2)	89.0	90.6	100.0	83.8
AKT1_MOUSE	Akt1 p-S122 (z= 3)	67.0	68.4	82.1	100.0
AKTS1_MOUSE	Akt1s1 p-S184 (z= 2)	41.8	38.3	55.1	100.0
AKTS1_MOUSE	Akt1s1 p-S204 p-S213 (z= 2)	56.9	94.9	70.2	100.0
AKTS1_MOUSE	Akt1s1 p-S213 (z= 2)	99.3	46.3	100.0	98.3
AKTS1_MOUSE	Akt1s1 p-T247 (z= 2)	37.2	100.0	13.4	65.6
AMGO1_MOUSE	AMGO1 p-S407 p-S412 (z= 4) + Oxi	56.7	72.8	86.6	100.0
AMOT_MOUSE	Amot p-T785 (z= 3) + Oxi	89.9	58.3	92.2	100.0
AMPD2_MOUSE	Ampd2 p-S87 (z= 2)	100.0	76.8	60.4	94.3
AMRA1_MOUSE	Ambra1 p-T1195 (z= 3)	29.3	97.1	21.9	100.0
AN32B_MOUSE	Anp32b p-T265 (z= 2)	100.0	56.6	60.5	35.3
ANLN_MOUSE	Anln p-S180 (z= 2)	63.1	100.0	54.1	39.5
ANLN_MOUSE	Anln p-S293 (z= 2)	100.0	89.3	95.5	97.0
ANLN_MOUSE	Anln p-S318 (z= 3)	100.0	91.4	89.5	67.1
ANLN_MOUSE	Anln p-S557 (z= 2)	55.7	67.6	54.6	100.0
ANLN_MOUSE	Anln p-S637 (z= 2)	82.5	100.0	52.8	81.8
ANLN_MOUSE	Anln p-T316 (z= 2)	100.0	93.7	99.7	67.1
ANLN_MOUSE	Anln p-T359 (z= 2)	82.5	100.0	32.5	6.0
ANLN_MOUSE	Anln p-T505 (z= 3)	77.7	100.0	74.1	91.3
ANM3_MOUSE	Prmt3 p-S22 p-S24 (z= 3)	93.3	94.4	100.0	80.6
ANR17_MOUSE	Ankrd17 p-S2401 (z= 3)	77.8	100.0	73.9	64.3
ANR52_MOUSE	Ankrd52 p-Y349 p-T357 p-T360 p-T365 (z= 4) + Oxi	82.9	100.0	67.1	86.8
ANR55_MOUSE	Ankrd55 p-T5 p-S9 p-S11 (z= 3) + Oxi	76.2	97.0	100.0	83.9
AP3D1_MOUSE	Ap3d1 p-S755 p-S760 (z= 2)	58.4	100.0	88.0	66.2
AP3D1_MOUSE	Ap3d1 p-S760 (z= 2)	100.0	92.1	87.5	70.1
AP3D1_MOUSE	Ap3d1 p-S784 (z= 3)	81.8	95.6	100.0	72.9
API5_MOUSE	Api5 p-S462 (z= 2)	98.7	100.0	39.4	49.3
API5_MOUSE	Api5 p-S464 (z= 2)	54.6	76.1	100.0	24.6
ARAF_MOUSE	Araf p-S580 (z= 2)	100.0	74.9	79.4	68.4
ARBK1_MOUSE	Adrbk1 p-S670 (z= 2)	56.4	100.0	70.9	75.1

ARGL1_MOUSE	Arglu1 p-S75 (z= 2)	42.9	10.1	100.0	11.6
ARGL1_MOUSE	Arglu1 p-S75 (z= 2)	100.0	73.9	76.0	71.1
ARGL1_MOUSE	Arglu1 p-S75 (z= 3)	100.0	81.9	88.9	86.4
ARH_MOUSE	Ldlrap1 p-S198 (z= 2)	87.9	100.0	83.0	89.2
ARHG1_MOUSE	Arhgef1 p-S905 (z= 3)	57.8	100.0	63.3	70.6
ARHG2_MOUSE	Arhgef2 p-S151 (z= 3)	100.0	88.3	89.9	71.3
ARHG2_MOUSE	Arhgef2 p-S174 (z= 2)	92.5	81.5	82.2	100.0
ARHG2_MOUSE	Arhgef2 p-S885 (z= 2)	49.3	26.9	100.0	63.6
ARHG7_MOUSE	Arhgef7 p-S497 (z= 2) + Oxi	100.0	80.7	69.8	72.2
ARHG7_MOUSE	Arhgef7 p-S673 (z= 2)	69.1	97.2	100.0	84.5
ARHGH_MOUSE	Arhgef17 p-S906 (z= 2)	100.0	92.5	54.5	39.2
ARI1A_MOUSE	Arid1a p-S697 (z= 2)	68.1	100.0	74.5	74.5
ARIP4_MOUSE	Rad54l2 p-S1141 (z= 3) + Oxi	36.0	72.1	19.4	100.0
ASAP2_MOUSE	Asap2 p-S704 (z= 2)	80.0	100.0	94.3	65.5
ASHWN_MOUSE	Lyg1 p-S189 p-T197 (z= 3)	87.8	97.0	81.7	100.0
ASPC1_MOUSE	Aspscr1 p-S199 (z= 3)	79.2	77.2	100.0	70.0
ASPC1_MOUSE	Aspscr1 p-S285 (z= 3)	83.3	100.0	89.4	98.9
ATRX_MOUSE	Atrx p-S1223 p-S1224 (z= 3)	71.7	83.5	100.0	82.9
ATRX_MOUSE	Atrx p-S1290 (z= 2)	78.3	100.0	68.6	86.7
ATRX_MOUSE	Atrx p-S312 (z= 2)	100.0	88.5	71.8	77.8
ATRX_MOUSE	Atrx p-S315 (z= 3)	100.0	95.7	81.2	93.5
ATRX_MOUSE	Atrx p-S590 (z= 2)	57.7	92.9	77.3	100.0
ATRX_MOUSE	Atrx p-S590 (z= 3)	100.0	99.0	89.1	93.7
ATRX_MOUSE	Atrx p-S717 p-S719 (z= 3)	80.2	100.0	65.7	94.8
ATRX_MOUSE	Atrx p-S92 (z= 2)	64.3	100.0	60.5	78.1
ATRX_MOUSE	Atrx p-S92 (z= 3)	100.0	66.6	83.3	67.9
ATX2_MOUSE	Atxn2 p-T710 (z= 2)	100.0	76.7	49.3	50.9
ATX2L_MOUSE	Atxn2l p-S109 (z= 2)	75.9	91.9	95.8	100.0
ATX2L_MOUSE	Atxn2l p-S339 (z= 2)	80.0	100.0	72.7	75.7
ATX2L_MOUSE	Atxn2l p-S562 (z= 2)	100.0	76.3	87.6	89.6
ATX2L_MOUSE	Atxn2l p-S597 (z= 2)	74.6	91.5	95.4	100.0
ATX2L_MOUSE	Atxn2l p-S597 (z= 2) + Oxi	92.2	100.0	70.2	86.6
ATX2L_MOUSE	Atxn2l p-S681 p-S687 (z= 2)	100.0	2.9	46.4	57.1
ATX2L_MOUSE	Atxn2l p-S687 (z= 2)	93.9	100.0	94.7	18.7
ATX2L_MOUSE	Atxn2l p-T505 (z= 2)	81.0	100.0	66.1	88.5
ATX2L_MOUSE	Atxn2l p-T635 (z= 4)	17.2	90.1	56.8	100.0
BAG3_MOUSE	Bag3 p-S270 p-S274 (z= 3)	84.5	58.9	100.0	78.4
BAG3_MOUSE	Bag3 p-S289 p-S297 (z= 3)	100.0	88.5	72.5	67.9
BAG6_MOUSE	Bag6 p-S995 (z= 2)	45.9	100.0	53.7	93.2
BAG6_MOUSE	Bag6 p-S995 (z= 3)	94.5	100.0	81.6	76.7
BAIP2_MOUSE	Baiap2 p-S367 (z= 2)	80.0	100.0	68.0	70.9
BAIP2_MOUSE	Baiap2 p-S453 (z= 3)	100.0	37.1	33.5	20.9
BAP18_MOUSE	Bap18 p-S96 (z= 2)	95.0	97.3	94.3	100.0
BAZ1A_MOUSE	Baz1a p-T732 (z= 3)	100.0	90.4	59.4	74.3
BAZ1B_MOUSE	Baz1b p-S1464 (z= 3)	100.0	67.9	42.5	49.3
BCAS1_MOUSE	Bcas1 p-S544 (z= 2)	100.0	67.8	88.5	99.4
BCLF1_MOUSE	Bclaf1 p-S177 (z= 2)	66.4	100.0	95.4	70.2
BCLF1_MOUSE	Bclaf1 p-S177 (z= 2)	97.6	89.8	100.0	58.8
BCLF1_MOUSE	Bclaf1 p-S177 (z= 3)	69.7	100.0	90.4	78.9
BCLF1_MOUSE	Bclaf1 p-S177 (z= 3)	98.0	100.0	99.7	70.2
BCLF1_MOUSE	Bclaf1 p-S221 (z= 2)	55.8	78.1	100.0	66.1
BCLF1_MOUSE	Bclaf1 p-S284 p-S289 (z= 3)	67.3	100.0	90.7	70.7
BCLF1_MOUSE	Bclaf1 p-S284 p-S296 (z= 2)	66.0	100.0	94.3	79.6
BCLF1_MOUSE	Bclaf1 p-S383 (z= 3)	70.9	95.6	82.5	100.0
BCLF1_MOUSE	Bclaf1 p-S494 (z= 2)	99.4	100.0	95.7	66.3
BCLF1_MOUSE	Bclaf1 p-S510 (z= 2)	100.0	82.2	44.3	36.8
BCLF1_MOUSE	Bclaf1 p-S510 (z= 2)	87.0	83.9	100.0	92.5
BCLF1_MOUSE	Bclaf1 p-S510 (z= 3)	100.0	89.7	78.4	79.7
BCLF1_MOUSE	Bclaf1 p-S529 (z= 2)	100.0	58.3	63.9	50.9
BCLF1_MOUSE	Bclaf1 p-S646 (z= 2)	80.6	100.0	73.8	64.1
BCLF1_MOUSE	Bclaf1 p-S656 (z= 2)	91.5	99.0	94.9	100.0
BCLF1_MOUSE	Bclaf1 p-S658 (z= 2)	100.0	81.0	83.4	79.9
BCR_MOUSE	Bcr p-S113 (z= 4)	88.4	100.0	70.5	45.2
BICD2_MOUSE	Bicd2 p-S578 (z= 2)	100.0	92.8	99.4	83.4
BICD2_MOUSE	Bicd2 p-S817 (z= 2)	100.0	84.5	62.9	65.0
BIN1_MOUSE	Bin1 p-S296 (z= 2)	70.9	100.0	58.6	90.8
BIN1_MOUSE	Bin1 p-S298 (z= 3)	92.0	100.0	83.2	92.8
BIN1_MOUSE	Bin1 p-S324 (z= 2)	100.0	72.7	74.9	60.5
BIN1_MOUSE	Bin1 p-T328 (z= 3)	87.5	93.8	100.0	69.1
BLM_MOUSE	Blm p-T1311 (z= 3)	100.0	85.8	80.4	82.9
BNI3L_MOUSE	Bnip3l p-S165 (z= 2) + Oxi	67.2	100.0	8.8	53.4
BNIP2_MOUSE	Bnip2 p-S114 (z= 2)	77.6	94.4	100.0	85.7
BORG5_MOUSE	Cdc42ep1 p-S113 (z= 2)	54.5	100.0	99.9	58.4
BORG5_MOUSE	Cdc42ep1 p-S121 (z= 2)	72.9	100.0	28.4	38.4
BORG5_MOUSE	Cdc42ep1 p-S19 (z= 4)	65.3	100.0	80.9	59.6
BORG5_MOUSE	Cdc42ep1 p-S207 (z= 2)	36.1	85.7	100.0	86.4
BORG5_MOUSE	Cdc42ep1 p-S207 (z= 2)	93.0	97.9	100.0	94.4
BORG5_MOUSE	Cdc42ep1 p-S207 p-S210 (z= 2)	31.7	78.6	54.0	100.0
BORG5_MOUSE	Cdc42ep1 p-S339 (z= 2)	30.0	100.0	39.2	71.8
BORG5_MOUSE	Cdc42ep1 p-S368 p-S371 (z= 3)	0.0	6.1	0.0	100.0
BRD2_MOUSE	Brd2 p-S297 p-S300 (z= 3)	91.5	79.0	94.4	100.0
BRD3_MOUSE	Brd3 p-S262 (z= 2)	75.4	100.0	65.0	90.7
BRD8_MOUSE	Brd8 p-S617 (z= 2)	92.5	52.8	3.6	100.0
BRE1A_MOUSE	Rnf20 p-S138 (z= 2)	100.0	80.9	79.2	82.0
BRWD3_MOUSE	Brwd3 p-T1014 (z= 2) + Oxi	89.8	100.0	48.4	81.4
BTBD3_MOUSE	Btbd3 p-S44 p-S47 p-S49 (z= 3)	62.2	38.6	100.0	68.9
BUD13_MOUSE	Bud13 p-S420 p-S423 (z= 3)	56.8	3.9	100.0	6.4
BUD13_MOUSE	Bud13 p-T131 p-S135 (z= 2)	100.0	35.9	95.3	80.8
BUD13_MOUSE	Bud13 p-T144 p-S148 (z= 2)	93.5	16.0	100.0	81.9
BUD13_MOUSE	Bud13 p-T196 p-S200 (z= 3)	100.0	77.0	80.8	84.2
BUD13_MOUSE	Bud13 p-T196 p-S200 (z= 4)	100.0	68.8	72.4	62.1
BUD13_MOUSE	Bud13 p-T222 p-S226 (z= 3)	100.0	40.9	56.8	84.7
BYST_MOUSE	Bysl p-S97 (z= 2)	81.5	100.0	47.8	95.4
BYST_MOUSE	Bysl p-S97 (z= 3)	100.0	91.8	99.5	88.7
BZW1_MOUSE	Bzw1 p-S411 p-S413 (z= 2)	61.6	100.0	56.4	0.0
C1TC_MOUSE	Mthfd1 p-S549 (z= 2)	100.0	74.9	63.5	67.9
CA052_MOUSE	Eps8l2 p-S156 (z= 2)	82.0	100.0	42.0	46.7
CA112_MOUSE	CA112 p-S725 p-T735 (z= 4)	100.0	79.8	61.2	74.8

CA2D4_MOUSE	Cacna2d4 p-S804 p-S814 p-T815 (z= 4) + Oxi	100.0	35.6	64.5	37.7
CALX_MOUSE	Canx p-S553 p-S563 (z= 3)	27.6	100.0	30.6	47.3
CALX_MOUSE	Canx p-S553 p-S563 (z= 3)	56.9	100.0	93.6	95.6
CALX_MOUSE	Canx p-S582 (z= 2)	54.3	100.0	83.4	57.1
CALX_MOUSE	Canx p-S582 (z= 3)	58.8	100.0	68.4	62.6
CAMP1_MOUSE	Camsap1 p-S1069 (z= 3)	80.8	100.0	69.6	76.2
CAMP1_MOUSE	Camsap1 p-S1133 (z= 2)	94.3	100.0	60.8	94.5
CAMP1_MOUSE	Camsap1 p-T559 (z= 2)	96.6	100.0	88.8	97.7
CAMP2_MOUSE	Camsap2 p-S573 (z= 2)	100.0	72.8	51.5	80.9
CAPG_MOUSE	Capg p-S341 (z= 3)	82.9	65.6	100.0	55.1
CASC3_MOUSE	Casc3 p-S263 (z= 3)	100.0	93.7	87.4	90.5
CASP8_MOUSE	Casp8 p-S188 (z= 2)	58.3	100.0	94.8	62.1
CASP8_MOUSE	Casp8 p-S188 (z= 2) + Oxi	100.0	65.0	78.7	51.7
CB071_MOUSE	Rpap3 p-S653 (z= 2)	82.9	96.2	89.3	100.0
CBX3_MOUSE	Cbx3 p-S93 p-S95 (z= 3)	100.0	69.4	65.0	60.0
CBX3_MOUSE	Cbx3 p-S93 p-S95 p-S99 (z= 2)	100.0	92.7	33.4	37.5
CBX3_MOUSE	Cbx3 p-S93 p-S97 (z= 2)	100.0	98.3	84.7	78.6
CBX3_MOUSE	Cbx3 p-S95 (z= 2)	39.4	32.6	100.0	71.4
CBX5_MOUSE	Cbx5 p-S11 p-S12 p-S13 (z= 2)	52.8	48.6	100.0	92.1
CBX5_MOUSE	Cbx5 p-S93 (z= 2)	52.7	100.0	74.5	75.8
CC020_MOUSE	Srrm2 p-T103 p-T109 p-Y114 (z= 4)	87.3	68.9	100.0	67.9
CCD12_MOUSE	Ccdc12 p-S165 (z= 2)	77.0	78.5	100.0	82.1
CCD25_MOUSE	Ccdc25 p-S204 (z= 2)	79.4	69.0	90.3	100.0
CCD43_MOUSE	Ccdc43 p-T137 (z= 4)	96.1	87.2	75.9	100.0
CCD86_MOUSE	Ccdc86 p-S18 (z= 3)	81.8	100.0	85.6	61.6
CCD86_MOUSE	Ccdc86 p-S18 (z= 3)	100.0	96.7	56.7	71.3
CCD94_MOUSE	CCD94 p-S211 p-S213 (z= 2)	70.9	34.1	100.0	70.7
CCG8_MOUSE	Cacng8 p-S290 p-S301 p-Y307 p-Y337 (z= 4)	80.3	100.0	85.2	27.0
CCKAR_MOUSE	Cckar p-S265 p-S266 (z= 3)	90.3	22.3	100.0	90.5
CCNK_MOUSE	Ccnk p-S325 p-S329 (z= 2)	85.1	50.2	71.4	100.0
CCNK_MOUSE	Ccnk p-S341 (z= 2)	98.8	87.4	100.0	67.7
CCNL1_MOUSE	Ccnl1 p-S341 p-S344 p-S347 (z= 3)	82.2	83.1	100.0	76.9
CCNL1_MOUSE	Ccnl1 p-S344 p-S347 (z= 3)	4.8	77.8	2.6	100.0
CCNL1_MOUSE	Ccnl1 p-S358 (z= 2)	77.0	100.0	95.2	75.6
CD11B_MOUSE	Cdk11b p-S270 (z= 2)	59.7	75.4	100.0	76.6
CD11B_MOUSE	Cdk11b p-S47 (z= 2)	73.6	100.0	95.3	70.3
CD11B_MOUSE	Cdk11b p-T740 p-S741 (z= 3)	61.5	85.1	97.0	100.0
CD226_MOUSE	Cd226 p-Y188 p-S190 p-T196 (z= 3)	84.9	100.0	78.4	71.4
CD2AP_MOUSE	Cd2ap p-S404 (z= 2)	100.0	58.6	82.4	55.3
CD2AP_MOUSE	Cd2ap p-S458 (z= 2)	100.0	52.6	52.5	67.5
CDCA3_MOUSE	Cdca3 p-S67 (z= 2)	69.2	86.5	100.0	82.3
CDK12_MOUSE	Cdk12 p-S382 p-S384 (z= 3)	69.8	100.0	86.7	67.1
CDK13_MOUSE	Cdk13 p-S384 (z= 2)	70.8	91.0	100.0	99.3
CDK13_MOUSE	Cdk13 p-T1245 (z= 3)	100.0	86.5	68.5	66.7
CDK16_MOUSE	CDK16 p-S119 (z= 2)	83.4	100.0	95.2	98.2
CDK17_MOUSE	Cdk17 p-S180 (z= 2)	72.3	92.9	81.4	100.0
CDK18_MOUSE	Cdk18 p-S111 (z= 2)	49.9	96.5	90.3	100.0
CDK3_MOUSE	Cdk3 p-T14 p-Y15 (z= 2)	67.0	94.8	61.0	100.0
CDK3_MOUSE	Cdk3 p-Y15 (z= 2)	97.2	100.0	96.7	92.3
CDK4_MOUSE	Cdk4 p-S300 (z= 2)	100.0	75.2	90.0	46.7
CDN1A_MOUSE	Cdkn1a p-S81 (z= 2)	80.8	100.0	66.9	82.3
CE104_MOUSE	CE104 p-S327 (z= 2)	100.0	72.7	57.2	67.1
CE170_MOUSE	Cep170 p-S1102 (z= 2)	89.0	100.0	56.8	79.6
CE170_MOUSE	Cep170 p-S1184 (z= 2)	100.0	71.1	59.3	58.1
CE170_MOUSE	Cep170 p-S829 (z= 3)	68.0	100.0	33.6	31.7
CE170_MOUSE	Cep170 p-S829 (z= 3)	72.2	100.0	69.5	64.9
CENPC_MOUSE	Cenpc1 p-S158 (z= 3)	100.0	77.6	52.0	65.8
CEP55_MOUSE	Cep55 p-S423 p-S426 (z= 3)	100.0	55.3	66.1	72.9
CEP55_MOUSE	Cep55 p-S428 (z= 2)	65.2	90.1	93.6	100.0
CEP55_MOUSE	Cep55 p-T422 p-S426 (z= 3)	70.4	85.2	79.1	100.0
CF132_MOUSE	Dync1li1 p-S1026 (z= 2)	0.0	0.0	100.0	0.0
CF132_MOUSE	Frmd4a p-S1131 (z= 2)	61.6	64.4	100.0	36.4
CF132_MOUSE	SMCE1 p-S368 (z= 3)	100.0	85.8	78.3	78.6
CGNL1_MOUSE	Cgnl1 p-S199 p-S203 (z= 2)	100.0	80.6	68.1	92.1
CGNL1_MOUSE	Cgnl1 p-S252 (z= 2)	75.3	100.0	73.5	69.8
CGNL1_MOUSE	Cgnl1 p-S284 (z= 2)	58.9	59.7	100.0	60.1
CGNL1_MOUSE	Cgnl1 p-S284 (z= 3)	77.9	100.0	92.0	88.0
CGNL1_MOUSE	Cgnl1 p-S416 (z= 3)	82.9	100.0	73.7	52.7
CGNL1_MOUSE	Cgnl1 p-S602 (z= 3)	70.2	100.0	39.6	55.0
CGNL1_MOUSE	Cgnl1 p-Y197 p-S203 (z= 2)	63.4	22.2	100.0	54.1
CH10_MOUSE	Hspe1 p-T27 (z= 3)	100.0	73.9	79.3	64.1
CHAP1_MOUSE	Champ1 p-S416 (z= 3)	84.0	100.0	90.3	90.4
CHAP1_MOUSE	Champ1 p-S416 p-S421 p-S425 (z= 3)	78.1	82.3	95.0	100.0
CHAP1_MOUSE	Champ1 p-S603 (z= 2)	72.1	89.9	100.0	57.4
CHERP_MOUSE	Cherp p-S822 p-S824 p-S826 (z= 3)	80.9	82.4	100.0	100.0
CHERP_MOUSE	Cherp p-S824 p-S826 p-T828 (z= 3)	77.6	85.0	100.0	98.8
CHIP_MOUSE	Stub1 p-T15 (z= 2)	100.0	89.6	85.5	71.4
CHIP_MOUSE	Stub1 p-T15 (z= 3)	100.0	93.4	72.6	58.4
CHM2B_MOUSE	Chmp2b p-S199 (z= 2)	100.0	71.0	53.9	54.1
CHRC1_MOUSE	Chrac1 p-S122 (z= 2)	100.0	80.3	85.4	84.9
CHRC1_MOUSE	Chrac1 p-S122 (z= 3)	100.0	97.6	100.0	91.1
CHSP1_MOUSE	Carhsp1 p-S31 (z= 2)	76.4	85.2	100.0	74.4
CHSP1_MOUSE	Carhsp1 p-S31 p-S33 (z= 2)	53.5	45.6	100.0	48.5
CHSP1_MOUSE	Carhsp1 p-S31 p-S33 (z= 3)	91.2	81.8	100.0	94.6
CHSP1_MOUSE	Carhsp1 p-S31 p-S33 p-S42 (z= 3)	98.1	85.9	92.0	100.0
CHSP1_MOUSE	Carhsp1 p-S42 (z= 2)	66.7	94.7	92.8	100.0
CHSP1_MOUSE	Carhsp1 p-S42 (z= 3)	100.0	95.8	95.3	88.5
CHSP1_MOUSE	Carhsp1 p-S53 (z= 2)	85.0	100.0	37.7	36.4
CI078_MOUSE	PPDPF p-S261 (z= 3)	92.8	81.1	100.0	83.9
CIC_MOUSE	Cic p-S1080 (z= 2)	74.8	100.0	76.0	64.2
CIC_MOUSE	Cic p-S1403 (z= 2)	63.6	92.3	100.0	49.4
CIC_MOUSE	Cic p-S2280 p-S2284 (z= 3)	89.8	83.8	67.2	100.0
CING_MOUSE	Cgn p-T283 (z= 2)	100.0	44.9	44.0	39.8
CJ088_MOUSE	Gtf2b p-S423 (z= 2)	93.4	58.4	100.0	84.4
CLAP1_MOUSE	Clasp1 p-S1088 (z= 2)	96.5	100.0	48.5	38.7
CLAP1_MOUSE	Clasp1 p-S1193 (z= 2)	100.0	29.5	59.4	82.1
CLAP1_MOUSE	Clasp1 p-S1220 (z= 2)	100.0	62.1	28.1	36.6
CLASR_MOUSE	Clasrp p-S541 (z= 3)	100.0	97.6	77.9	88.7

CLPX_MOUSE	Clpx p-S97 (z= 3)	100.0	91.6	75.1	54.2
CLSPN_MOUSE	Clspn p-S787 (z= 2)	97.1	100.0	97.1	75.9
CND1_MOUSE	Ncapd2 p-S1320 p-S1323 (z= 3)	86.1	81.2	73.3	100.0
CND1_MOUSE	Ncapd2 p-T1319 (z= 3)	100.0	71.3	76.7	79.4
CND2_MOUSE	Ncaph p-S25 (z= 2)	100.0	41.0	64.7	98.4
CND2_MOUSE	Ncaph p-S83 (z= 3)	43.4	89.1	26.5	100.0
CNOT2_MOUSE	Cnot2 p-S165 (z= 2)	72.1	100.0	97.8	78.8
CO2A1_MOUSE	Col2a1 p-S391 (z= 3)	51.4	100.0	15.3	67.4
COBL1_MOUSE	Cobll1 p-S1214 (z= 2)	75.1	93.3	87.1	100.0
COBL1_MOUSE	Cobll1 p-S546 p-T550 (z= 3)	74.5	87.5	89.3	100.0
COBL1_MOUSE	Cobll1 p-T1091 (z= 3)	100.0	77.3	80.3	73.2
COKA1_MOUSE	Col20a1 p-Y731 p-S734 p-S737 (z= 2)	80.5	36.7	100.0	72.2
COMT_MOUSE	Comt p-S261 (z= 2)	100.0	67.4	93.4	86.6
COPD_MOUSE	Arcn1 p-Y58 (z= 3) + Oxi	72.7	100.0	71.7	89.2
CP088_MOUSE	Tsg118 p-S57 (z= 2)	60.5	86.3	100.0	89.0
CP088_MOUSE	Tsg118 p-S57 (z= 3)	76.7	82.5	100.0	76.4
CP135_MOUSE	Cep135 p-S441 (z= 2)	100.0	0.1	25.0	0.1
CPSF2_MOUSE	Cpsf2 p-S419 p-S420 p-S423 (z= 3)	76.0	72.7	86.8	100.0
CQ085_MOUSE	Iqch p-S30 (z= 3)	73.1	88.2	100.0	69.7
CR025_MOUSE	Sorbs1 p-S66 p-S67 (z= 3)	98.0	100.0	94.3	97.1
CRKL_MOUSE	Crkl p-Y207 (z= 4)	6.5	66.1	3.7	100.0
CRTC2_MOUSE	Crtc2 p-S70 (z= 4)	43.3	100.0	63.6	57.7
CS021_MOUSE	Gjd3 p-S346 (z= 2)	100.0	27.6	39.6	30.2
CS021_MOUSE	Sdad1 p-S364 (z= 2)	100.0	50.3	59.5	58.9
CS044_MOUSE	Atn1 p-S228 (z= 3)	100.0	68.3	95.1	73.4
CSTF3_MOUSE	Cstf3 p-S691 (z= 3)	100.0	41.9	30.1	12.0
CSTF3_MOUSE	CSTF3 p-S691 (z= 4)	100.0	39.9	29.1	11.7
CT2NL_MOUSE	Cttnbp2nl p-S522 (z= 3)	100.0	98.0	90.2	90.1
CT2NL_MOUSE	Cttnbp2nl p-S548 (z= 2)	24.4	40.0	100.0	9.0
CT2NL_MOUSE	Cttnbp2nl p-S559 p-S567 (z= 3)	82.8	83.0	100.0	71.7
CT2NL_MOUSE	Cttnbp2nl p-S567 (z= 3)	100.0	73.9	62.9	40.1
CTBP2_MOUSE	Ctbp2 p-S428 (z= 4)	49.8	100.0	64.4	70.2
CTF18_MOUSE	CTF18 p-S70 (z= 4)	87.4	75.3	78.5	100.0
CTGE5_MOUSE	Ctage5 p-S514 p-T517 (z= 3)	56.9	100.0	25.4	47.1
CTNA1_MOUSE	Ctnna1 p-S641 (z= 2)	94.7	90.7	100.0	89.1
CTNA1_MOUSE	Ctnna1 p-S641 (z= 3)	87.4	79.2	95.8	100.0
CTNA1_MOUSE	Ctnna1 p-S652 p-T654 (z= 2)	75.1	100.0	38.3	76.6
CTNA1_MOUSE	Ctnna1 p-S652 p-T654 p-S655 (z= 3)	100.0	75.1	57.5	95.5
CTNB1_MOUSE	Ctnnb1 p-S191 (z= 3)	100.0	56.3	50.6	25.8
CTND1_MOUSE	Ctnnd1 p-S268 (z= 2)	100.0	73.7	49.2	37.1
CTND1_MOUSE	Ctnnd1 p-S268 p-S269 (z= 2)	97.8	100.0	66.4	95.4
CTND1_MOUSE	Ctnnd1 p-S346 p-S349 p-S352 (z= 2)	99.2	76.2	80.8	100.0
CTND1_MOUSE	Ctnnd1 p-S349 (z= 2)	100.0	75.7	72.2	73.9
CTND1_MOUSE	Ctnnd1 p-S349 (z= 3)	100.0	85.0	78.2	85.6
CTND1_MOUSE	Ctnnd1 p-S349 p-S352 (z= 2)	79.9	77.2	70.8	100.0
CTND1_MOUSE	Ctnnd1 p-S349 p-S352 (z= 3)	52.8	62.5	62.5	100.0
CTND1_MOUSE	Ctnnd1 p-S53 (z= 3)	80.1	75.6	79.8	100.0
CTND1_MOUSE	Ctnnd1 p-S920 (z= 2)	68.1	75.8	62.9	100.0
CTR9_MOUSE	Ctr9 p-T925 (z= 2)	71.6	76.6	100.0	63.9
CTR9_MOUSE	Ctr9 p-T925 (z= 3)	82.2	91.2	79.8	100.0
CUL4B_MOUSE	Cul4b p-S149 (z= 3)	99.0	94.7	100.0	67.0
CWC15_MOUSE	Cwc15 p-T110 p-S121 (z= 3)	85.6	100.0	85.9	1.3
CWC15_MOUSE	Cwc15 p-T110 p-S121 (z= 4)	73.2	59.0	45.2	100.0
CWC22_MOUSE	Cwc22 p-S831 (z= 2)	60.6	89.3	100.0	56.9
CX023_MOUSE	Lama3 p-S78 p-S80 (z= 3)	91.3	95.7	100.0	89.4
CX023_MOUSE	Srrm1 p-S410 (z= 2)	100.0	53.8	84.6	93.0
CXAR_MOUSE	Cxadr p-S332 (z= 2)	55.4	100.0	53.6	84.3
CXAR_MOUSE	Cxadr p-S332 (z= 3)	100.0	80.3	76.9	78.5
CYTSB_MOUSE	Specc1 p-S133 (z= 2)	43.3	100.0	41.5	28.7
CYTSB_MOUSE	Specc1 p-S847 (z= 2)	100.0	91.6	92.9	99.3
CYTSB_MOUSE	Specc1 p-T357 p-S359 (z= 3)	85.4	81.0	76.8	100.0
DAB2_MOUSE	Dab2 p-S401 (z= 2)	28.0	100.0	78.9	90.8
DAP1_MOUSE	Dap p-S51 (z= 3)	81.2	67.6	68.9	100.0
DBNL_MOUSE	Dbnl p-S291 (z= 2)	78.2	100.0	88.5	75.7
DBPA_MOUSE	Csda p-S328 (z= 2)	78.1	95.3	100.0	99.5
DBPA_MOUSE	Csda p-S52 (z= 3)	100.0	57.4	75.1	52.4
DBPA_MOUSE	Csda p-S52 p-S66 (z= 4)	64.0	76.9	86.1	100.0
DC1I2_MOUSE	Dync1i2 p-S84 (z= 3)	45.3	89.8	78.9	100.0
DC1L1_MOUSE	Dync1li1 p-S207 (z= 3)	98.5	98.8	100.0	98.8
DC1L1_MOUSE	Dync1li1 p-S405 (z= 2)	62.5	0.0	100.0	9.5
DC1L1_MOUSE	Dync1li1 p-S421 (z= 2)	100.0	93.3	81.4	69.1
DC1L1_MOUSE	Dync1li1 p-S516 (z= 2)	72.4	75.6	100.0	94.5
DC1L1_MOUSE	Dync1li1 p-T513 p-T515 (z= 2)	69.9	100.0	70.1	78.6
DCLK3_MOUSE	Dclk3 p-S221 p-S229 p-S236 (z= 3)	94.1	90.8	89.9	100.0
DCP1A_MOUSE	Dcp1a p-S372 (z= 3)	100.0	87.7	83.8	67.1
DDX10_MOUSE	Ddx10 p-T587 (z= 3)	100.0	80.9	84.3	82.9
DDX20_MOUSE	Ddx20 p-S569 (z= 2)	62.0	14.7	100.0	88.2
DDX21_MOUSE	Ddx21 p-S118 (z= 2)	100.0	96.0	94.3	89.5
DDX21_MOUSE	Ddx21 p-S118 (z= 2) + Oxi	100.0	70.1	50.3	53.1
DDX21_MOUSE	Ddx21 p-S118 (z= 3)	100.0	20.7	22.2	22.9
DDX21_MOUSE	Ddx21 p-S144 (z= 2)	51.6	61.5	100.0	44.7
DDX21_MOUSE	Ddx21 p-S211 (z= 3)	100.0	49.2	60.0	38.2
DDX21_MOUSE	Ddx21 p-S218 (z= 2)	100.0	47.0	92.8	53.9
DDX21_MOUSE	Ddx21 p-S243 (z= 3)	91.6	100.0	98.1	70.9
DDX21_MOUSE	Ddx21 p-S244 p-S245 (z= 3)	87.1	100.0	76.2	77.1
DDX21_MOUSE	Ddx21 p-S245 p-T247 (z= 2)	100.0	81.2	54.9	75.6
DDX24_MOUSE	Ddx24 p-S170 (z= 2)	100.0	84.5	81.5	78.2
DDX24_MOUSE	Ddx24 p-S288 (z= 3)	97.9	100.0	98.7	81.0
DDX3L_MOUSE	D1Pas1 p-S592 (z= 2)	95.6	97.8	74.9	100.0
DDX42_MOUSE	Ddx42 p-S104 p-S111 (z= 3)	12.2	100.0	70.8	7.1
DDX42_MOUSE	Ddx42 p-S109 p-S111 (z= 3)	62.5	86.6	61.0	100.0
DDX42_MOUSE	Ddx42 p-S185 (z= 3)	87.7	77.7	85.0	100.0
DDX46_MOUSE	Ddx46 p-S804 (z= 3)	11.9	0.0	100.0	0.0
DDX54_MOUSE	Ddx54 p-S74 (z= 2)	95.0	89.6	96.8	100.0
DDX54_MOUSE	Ddx54 p-S74 (z= 3)	76.6	74.6	82.4	100.0
DEK_MOUSE	Dek p-S306 p-S308 p-S311 (z= 2)	34.1	1.2	100.0	31.8
DEK_MOUSE	Dek p-S306 p-S308 p-S311 (z= 2)	100.0	55.0	93.8	90.3
DEK_MOUSE	Dek p-S306 p-S308 p-S311 (z= 3)	100.0	62.5	96.0	86.3

DEK_MOUSE	Dek p-S33 (z= 3)	65.0	99.6	74.8	100.0
DEN5A_MOUSE	Dennd5a p-S13 (z= 3)	73.9	100.0	38.8	72.6
DENR_MOUSE	Denr p-S73 (z= 3)	95.0	100.0	99.0	95.2
DESP_MOUSE	Dsp p-S2620 (z= 3)	79.0	100.0	61.2	9.8
DESP_MOUSE	Dsp p-S2622 (z= 2)	91.5	53.6	52.8	100.0
DFFB_MOUSE	Dffb p-T270 p-T274 (z= 3)	54.6	95.8	86.3	100.0
DGCR8_MOUSE	Dgcr8 p-S271 p-S275 (z= 3)	88.7	92.2	77.4	100.0
DGCR8_MOUSE	Dgcr8 p-S377 (z= 3)	81.2	100.0	87.3	76.9
DGCR8_MOUSE	Dgcr8 p-Y267 p-S275 (z= 3)	93.8	87.8	100.0	85.5
DHSB_MOUSE	DHSB p-S124 (z= 2)	100.0	48.9	53.6	60.0
DKC1_MOUSE	Dkc1 p-S451 p-S453 p-S455 (z= 3)	100.0	81.0	93.9	98.3
DKC1_MOUSE	Dkc1 p-S451 p-S455 (z= 3)	100.0	79.6	83.0	89.9
DKC1_MOUSE	Dkc1 p-S481 (z= 2)	100.0	77.3	57.5	52.3
DLGP5_MOUSE	Dlgap5 p-S328 (z= 2)	100.0	95.1	81.4	83.7
DLGP5_MOUSE	Dlgap5 p-T453 (z= 2)	67.8	91.3	76.1	100.0
DMBX1_MOUSE	Dmbx1 p-S227 p-S232 p-Y254 (z= 4)	100.0	80.1	71.2	83.4
DNJC5_MOUSE	Dnajc5 p-S10 (z= 2)	12.6	100.0	11.0	5.8
DNJC5_MOUSE	Dnajc5 p-S10 (z= 3)	21.5	100.0	22.0	46.9
DNLI1_MOUSE	Lig1 p-S51 (z= 2)	78.2	100.0	74.0	73.0
DNLI1_MOUSE	Lig1 p-S51 (z= 3)	100.0	87.4	47.7	71.8
DNLI1_MOUSE	Lig1 p-S907 p-S911 (z= 2)	100.0	80.1	82.9	97.1
DNLI1_MOUSE	Lig1 p-T195 (z= 3)	68.6	99.5	90.0	100.0
DNLI1_MOUSE	Lig1 p-T77 (z= 2)	100.0	74.2	71.0	57.8
DNLI1_MOUSE	Lig1 p-T77 (z= 2)	95.3	100.0	60.4	58.2
DNLI1_MOUSE	Lig1 p-T77 (z= 3)	100.0	87.1	67.4	75.6
DNLI3_MOUSE	Lig3 p-S211 (z= 2)	55.2	100.0	52.5	93.0
DNM1L_MOUSE	Dnm1l p-S622 (z= 2)	77.9	100.0	93.5	95.9
DNMT1_MOUSE	Dnmt1 p-S717 (z= 2)	64.5	100.0	67.7	76.6
DOCK5_MOUSE	Dock5 p-S1765 (z= 2)	100.0	83.6	80.7	85.4
DOCK7_MOUSE	Dock7 p-S1428 (z= 2)	100.0	28.5	96.9	69.3
DOCK7_MOUSE	Dock7 p-S452 (z= 3)	18.8	100.0	56.3	4.3
DOCK7_MOUSE	Dock7 p-S888 (z= 3)	97.0	100.0	41.8	68.3
DOCK7_MOUSE	Dock7 p-S898 (z= 2)	100.0	53.5	42.6	52.8
DPM1_MOUSE	Dpm1 p-S9 p-S13 (z= 4)	84.0	88.8	77.5	100.0
DPOA2_MOUSE	Pola2 p-S126 (z= 2)	52.4	88.5	96.6	100.0
DPOD3_MOUSE	Pold3 p-S306 (z= 2)	53.2	78.3	100.0	65.9
DPOLA_MOUSE	Pola1 p-S215 (z= 3)	82.4	98.9	100.0	68.5
DPTOR_MOUSE	Deptor p-S238 (z= 3)	100.0	99.6	73.6	74.5
DPYL2_MOUSE	Dpysl2 p-S522 (z= 2)	100.0	0.0	2.5	0.0
DPYL2_MOUSE	Dpysl2 p-T509 (z= 2)	82.2	74.0	100.0	90.0
DREB_MOUSE	Dbn1 p-S142 (z= 2)	100.0	69.7	52.4	48.7
DREB_MOUSE	Dbn1 p-S387 (z= 2)	79.5	90.3	100.0	98.9
DTD1_MOUSE	Dtd1 p-S197 (z= 2)	100.0	91.2	97.8	94.9
DUS6_MOUSE	Dusp6 p-S331 (z= 2)	65.0	0.6	100.0	74.4
DUS6_MOUSE	Dusp6 p-S331 (z= 3)	76.1	100.0	79.2	3.1
DYH2_MOUSE	Dnah2 p-S2571 (z= 2)	83.8	100.0	67.2	50.4
DYHC1_MOUSE	Dync1h1 p-S4366 (z= 2)	82.3	100.0	84.8	90.4
DYHC1_MOUSE	Dync1h1 p-S4366 (z= 3)	82.3	95.9	91.1	100.0
DYR1A_MOUSE	Dyrk1a p-Y321 (z= 2)	96.8	66.2	100.0	89.2
E2F8_MOUSE	E2f8 p-S71 (z= 2)	76.5	100.0	80.3	97.6
E41L1_MOUSE	Epb41l1 p-S782 (z= 2)	82.5	100.0	86.6	84.5
E41L2_MOUSE	Epb41l2 p-S38 (z= 3)	53.7	100.0	39.1	63.3
E41L2_MOUSE	Epb41l2 p-S38 (z= 4)	100.0	85.8	93.8	70.1
E41L2_MOUSE	Epb41l2 p-S86 (z= 2)	100.0	75.6	75.3	93.4
E41L2_MOUSE	Epb41l2 p-T35 (z= 3)	100.0	83.0	74.4	51.7
E41L2_MOUSE	Epb41l2 p-T669 (z= 3)	100.0	67.0	59.4	75.7
EAF1_MOUSE	Eaf1 p-S158 p-S165 (z= 3)	80.9	85.3	94.0	100.0
EDC3_MOUSE	Edc3 p-S131 (z= 2)	81.7	100.0	44.7	54.7
EDC4_MOUSE	Edc4 p-S727 (z= 3)	81.7	43.3	100.0	71.5
EDC4_MOUSE	Edc4 p-S727 (z= 4)	94.3	94.5	77.8	100.0
EDC4_MOUSE	Edc4 p-S733 (z= 3)	75.3	0.8	100.0	40.4
EDC4_MOUSE	Edc4 p-S745 (z= 3)	85.0	2.2	100.0	41.3
EDC4_MOUSE	Edc4 p-S895 (z= 3)	81.0	89.9	90.1	100.0
EF1B_MOUSE	Eef1b p-S106 (z= 3)	73.3	75.3	84.5	100.0
EF1B_MOUSE	Eef1b p-S106 (z= 3)	51.6	76.3	71.2	100.0
EF1B_MOUSE	Eef1b p-S106 (z= 3)	100.0	66.4	79.6	74.0
EF1B_MOUSE	Eef1b p-S106 (z= 4)	98.0	81.8	100.0	98.3
EF1D_MOUSE	Eef1d p-S119 (z= 2)	100.0	22.9	24.9	24.7
EF1D_MOUSE	Eef1d p-S133 (z= 2)	83.5	93.4	100.0	93.5
EF1D_MOUSE	Eef1d p-S133 (z= 2) + Oxi	100.0	83.4	68.8	45.9
EF1D_MOUSE	Eef1d p-S133 (z= 3)	79.9	10.4	100.0	82.7
EF1D_MOUSE	Eef1d p-S133 (z= 3) + Oxi	100.0	92.3	77.7	77.7
EF1D_MOUSE	Eef1d p-S162 (z= 3)	74.7	84.5	88.5	100.0
EF1D_MOUSE	Eef1d p-S162 (z= 3)	99.4	85.6	92.2	100.0
EF1D_MOUSE	Eef1d p-S162 (z= 4)	84.0	84.5	88.6	100.0
EF1D_MOUSE	Eef1d p-T147 p-S162 (z= 3)	100.0	76.6	86.4	86.7
EF1D_MOUSE	Eef1d p-T147 p-S162 (z= 4)	96.3	85.7	93.4	100.0
EF1G_MOUSE	Eef1g p-T46 (z= 2)	65.6	90.5	79.9	100.0
EFHD2_MOUSE	Efhd2 p-S74 (z= 2)	100.0	51.0	97.6	79.3
EFHD2_MOUSE	Efhd2 p-S74 (z= 2)	100.0	45.3	65.1	62.9
EFHD2_MOUSE	Efhd2 p-S74 (z= 3)	100.0	51.8	50.3	36.0
EFHD2_MOUSE	Efhd2 p-S76 (z= 3)	99.1	88.8	100.0	90.3
EHD1_MOUSE	Ehd1 p-T454 (z= 3)	76.0	100.0	53.8	63.8
EI2BE_MOUSE	Eif2b5 p-S540 (z= 2)	98.2	89.7	100.0	86.4
EIF3B_MOUSE	Eif3b p-S120 p-S123 (z= 2)	84.9	100.0	53.7	19.4
EIF3B_MOUSE	Eif3b p-S120 p-S123 (z= 3)	100.0	91.0	83.2	87.3
EIF3B_MOUSE	Eif3b p-S143 (z= 3)	100.0	0.2	98.3	0.4
EIF3B_MOUSE	Eif3b p-S143 p-S153 (z= 3)	40.2	100.0	16.5	48.6
EIF3B_MOUSE	Eif3b p-S75 (z= 3)	100.0	87.1	92.2	89.6
EIF3B_MOUSE	Eif3b p-S75 p-S79 (z= 2)	94.1	100.0	76.6	73.5
EIF3B_MOUSE	Eif3b p-S75 p-S79 (z= 3)	59.1	99.8	56.3	100.0
EIF3B_MOUSE	Eif3b p-S75 p-S79 (z= 4)	100.0	58.8	33.0	59.9
EIF3B_MOUSE	Eif3b p-T74 p-S75 p-S79 (z= 4)	100.0	60.6	62.4	46.9
EIF3C_MOUSE	Eif3c p-S39 (z= 2)	97.6	93.2	96.5	100.0
EIF3C_MOUSE	Eif3c p-S39 (z= 2)	100.0	87.0	87.4	88.4
EIF3C_MOUSE	Eif3c p-S39 (z= 3)	95.9	88.2	92.0	100.0
EIF3G_MOUSE	Eif3g p-S42 (z= 2)	37.0	100.0	31.0	41.4
EIF3G_MOUSE	Eif3g p-S42 (z= 3)	100.0	49.7	65.8	67.1

EIF3G_MOUSE	Eif3g p-S42 (z= 3)	88.5	96.1	100.0	92.7
EIF3G_MOUSE	Eif3g p-T41 p-S42 (z= 3)	76.9	67.5	100.0	77.3
EMAL4_MOUSE	Eml4 p-S146 (z= 2)	100.0	56.8	81.7	63.5
EMIL1_MOUSE	Emilin1 p-S508 (z= 2)	100.0	28.5	54.9	46.4
ENPP1_MOUSE	ENPP1 p-S685 p-S690 p-Y694 (z= 4)	89.1	57.2	76.7	100.0
ENSA_MOUSE	Ensa p-S67 (z= 2)	75.6	95.5	93.7	100.0
ENSA_MOUSE	Ensa p-S67 (z= 2) + Oxi	100.0	97.6	66.1	66.3
EPCR_MOUSE	Procr p-S206 p-S214 p-T237 (z= 4) + Oxi	100.0	76.5	67.2	81.2
EPIPL_MOUSE	Eppk1 p-S1537 (z= 2)	100.0	75.8	66.8	69.3
EPN2_MOUSE	Epn2 p-S173 (z= 2)	71.3	77.6	90.7	100.0
EPN2_MOUSE	Epn2 p-S192 p-S195 (z= 2)	58.4	100.0	53.3	37.4
EPN2_MOUSE	Epn2 p-T441 (z= 3)	92.8	99.9	100.0	96.0
EPS8_MOUSE	Eps8 p-S624 (z= 3)	77.8	94.4	100.0	86.5
EPS8_MOUSE	Eps8 p-S658 (z= 2)	71.1	77.9	36.1	100.0
ERRFI_MOUSE	Errfi1 p-S251 (z= 3)	100.0	84.4	65.1	67.0
ERRFI_MOUSE	Errfi1 p-T126 p-T130 (z= 2)	56.4	78.7	78.8	100.0
ES8L2_MOUSE	Eps8l2 p-S483 (z= 3)	100.0	94.5	88.0	86.5
ESF1_MOUSE	Esf1 p-S156 (z= 2)	82.5	100.0	45.1	94.5
ESF1_MOUSE	Esf1 p-S156 (z= 3)	100.0	49.6	46.8	22.9
ESF1_MOUSE	Esf1 p-S651 (z= 3)	95.2	94.7	100.0	68.1
ESF1_MOUSE	Esf1 p-S651 p-S657 (z= 3)	85.9	70.3	100.0	79.7
ESF1_MOUSE	Esf1 p-S686 p-S688 p-S689 (z= 2)	79.9	55.3	57.1	100.0
ESF1_MOUSE	Esf1 p-T305 p-S306 p-S307 (z= 3)	100.0	57.2	36.2	20.2
ESPL1_MOUSE	Espl1 p-S1504 (z= 3)	85.4	91.4	95.6	100.0
ETV6_MOUSE	Etv6 p-S205 (z= 2)	90.2	78.1	100.0	97.9
ETV6_MOUSE	Etv6 p-S215 (z= 2)	100.0	81.7	47.5	37.3
ETV6_MOUSE	Etv6 p-Y17 p-S22 (z= 2)	91.6	62.2	100.0	95.1
ETV6_MOUSE	Etv6 p-Y17 p-S22 (z= 3)	99.5	100.0	96.6	86.0
EZH2_MOUSE	Ezh2 p-S406 p-S407 p-S408 (z= 3)	100.0	0.0	0.0	0.0
F10A1_MOUSE	St13 p-S74 p-S75 p-S78 (z= 4)	100.0	49.1	52.5	73.8
F110A_MOUSE	Fam110a p-S190 (z= 2)	100.0	77.0	65.1	59.7
F110C_MOUSE	Fam110c p-S350 (z= 2)	77.3	87.6	90.6	100.0
F122A_MOUSE	Fam122a p-S34 (z= 3)	94.3	100.0	96.3	68.4
F122A_MOUSE	Fam122a p-S73 (z= 2)	100.0	87.9	84.4	79.5
F195B_MOUSE	Fam195b p-S21 (z= 2)	96.9	100.0	96.9	96.3
F195B_MOUSE	Fam195b p-S25 p-S26 (z= 3)	100.0	61.2	51.8	72.0
F195B_MOUSE	Fam195b p-T23 (z= 3)	86.7	92.1	92.6	100.0
F207A_MOUSE	Fam207a p-S38 (z= 2)	76.8	100.0	71.6	82.8
FA40A_MOUSE	Fam40a p-S335 (z= 2)	87.0	98.1	100.0	96.3
FA54B_MOUSE	Fam54b p-S100 (z= 2)	100.0	84.8	69.3	78.3
FA54B_MOUSE	Fam54b p-S235 (z= 2)	60.4	100.0	60.7	50.9
FA65A_MOUSE	FA65A p-Y78 (z= 2)	84.2	100.0	96.9	85.7
FA76B_MOUSE	Fam76b p-S193 (z= 2)	96.9	95.2	96.9	100.0
FA83H_MOUSE	Fam83h p-S522 (z= 2)	100.0	60.8	65.9	52.4
FA83H_MOUSE	Fam83h p-S871 (z= 2)	82.8	94.0	100.0	69.6
FA83H_MOUSE	Fam83h p-S871 (z= 3)	83.1	96.2	100.0	86.5
FA83H_MOUSE	Fam83h p-S904 (z= 2)	99.0	100.0	97.3	49.0
FA83H_MOUSE	Fam83h p-S915 (z= 2)	83.5	90.8	100.0	42.2
FA83H_MOUSE	Fam83h p-S926 (z= 2)	81.3	96.7	100.0	74.8
FA83H_MOUSE	Fam83h p-S948 (z= 2)	100.0	87.4	87.3	66.5
FA83H_MOUSE	Fam83h p-S970 (z= 2)	100.0	87.2	94.0	66.8
FAK1_MOUSE	Ptk2 p-S948 (z= 2)	60.9	90.9	100.0	90.2
FAM21_MOUSE	Fam21 p-S1169 p-S1172 p-S1173 (z= 3)	96.8	48.3	100.0	63.5
FAM21_MOUSE	Fam21 p-S157 p-S159 (z= 3)	67.7	100.0	98.2	91.3
FAM21_MOUSE	Fam21 p-S533 (z= 3)	74.2	80.5	100.0	78.1
FAM21_MOUSE	Fam21 p-S613 p-S614 (z= 3)	75.8	88.4	84.3	100.0
FAM21_MOUSE	Fam21 p-S723 (z= 3)	65.1	86.6	100.0	73.7
FAT3_MOUSE	Fat3 p-S1659 (z= 4) + Oxi	73.5	88.2	78.4	100.0
FBX15_MOUSE	Fbxo15 p-S185 (z= 2)	100.0	98.2	56.2	44.9
FCHO2_MOUSE	Fcho2 p-S387 (z= 2)	77.5	100.0	99.6	73.1
FGD6_MOUSE	Fgd6 p-S557 (z= 3)	100.0	98.9	50.5	36.1
FHOD1_MOUSE	Fhod1 p-S502 (z= 2)	73.5	10.1	100.0	73.3
FHOD1_MOUSE	Fhod1 p-S524 (z= 2)	100.0	73.6	51.2	40.7
FHOD1_MOUSE	Fhod1 p-S524 p-S527 (z= 2)	88.0	72.2	100.0	97.9
FIL1L_MOUSE	Filip1l p-S789 (z= 2)	94.7	94.0	100.0	94.4
FIP1_MOUSE	Fip1l1 p-S235 (z= 2)	96.8	98.5	86.2	100.0
FIP1_MOUSE	Fip1l1 p-S280 (z= 2)	40.9	84.9	100.0	19.7
FIP1_MOUSE	Fip1l1 p-S479 (z= 2)	99.8	97.8	100.0	82.8
FIP1_MOUSE	Fip1l1 p-S479 (z= 3)	77.2	94.2	100.0	69.0
FIP1_MOUSE	Fip1l1 p-S479 p-S487 (z= 3)	100.0	74.9	80.7	71.2
FIP1_MOUSE	Fip1l1 p-S483 p-S487 (z= 2)	71.2	89.7	53.0	100.0
FIP1_MOUSE	Fip1l1 p-T481 (z= 3)	100.0	90.6	72.8	44.1
FLNA_MOUSE	Flna p-S1084 (z= 2)	100.0	82.5	26.4	39.0
FLNA_MOUSE	Flna p-S1459 (z= 2)	91.3	100.0	63.0	71.4
FLNA_MOUSE	Flna p-S2152 (z= 3)	8.7	100.0	66.2	80.5
FLNA_MOUSE	Flna p-S2327 (z= 3)	99.1	99.2	100.0	85.6
FLNA_MOUSE	Flna p-S968 (z= 2)	73.6	85.3	0.1	100.0
FLNC_MOUSE	Flnc p-S2234 (z= 2)	64.1	100.0	60.8	99.9
FMNL_MOUSE	Fmnl1 p-S184 (z= 2)	100.0	94.8	88.2	91.0
FNBP4_MOUSE	Fnbp4 p-S19 (z= 2)	100.0	86.9	82.7	90.5
FNBP4_MOUSE	Fnbp4 p-S19 (z= 3)	79.3	69.7	69.9	100.0
FOSL2_MOUSE	Fosl2 p-S200 (z= 2)	100.0	77.4	75.4	79.0
FOXA1_MOUSE	Foxa1 p-S283 (z= 2)	93.7	100.0	57.6	44.0
FOXK1_MOUSE	Foxk1 p-S402 p-S406 (z= 2)	91.1	100.0	91.5	80.8
FOXK2_MOUSE	Foxk2 p-S389 (z= 2)	71.8	100.0	73.4	77.5
FOXP2_MOUSE	Foxp2 p-S5 (z= 4) + Oxi	94.0	100.0	94.0	80.3
FRK_MOUSE	Frk p-T407 (z= 2)	64.3	52.4	100.0	61.9
FRM4A_MOUSE	Frmd4a p-S782 (z= 2)	80.9	89.2	79.1	100.0
FRM4A_MOUSE	Frmd4a p-T511 (z= 2)	95.7	100.0	83.4	66.3
FUBP2_MOUSE	Khsrp p-S182 (z= 2)	55.4	56.2	100.0	66.5
FXR1_MOUSE	Fxr1 p-S641 (z= 3)	100.0	88.9	84.4	79.2
FXR2_MOUSE	Fxr2 p-S602 (z= 3)	82.2	82.7	84.6	100.0
FXR2_MOUSE	Fxr2 p-S602 p-S604 (z= 3)	88.8	89.6	100.0	95.4
G3BP1_MOUSE	G3bp1 p-S149 (z= 2)	97.6	65.6	95.2	100.0
G3BP1_MOUSE	G3bp1 p-S149 (z= 3)	89.3	85.1	100.0	86.5
G3BP1_MOUSE	G3bp1 p-S231 (z= 2)	97.3	97.5	80.1	100.0
G3BP1_MOUSE	G3bp1 p-S231 (z= 3)	79.8	80.2	79.5	100.0
G3BP2_MOUSE	G3bp2 p-T227 (z= 2)	100.0	91.4	81.5	71.7

GAPD1_MOUSE	Gapvd1 p-S452 p-S453 p-S454 (z= 3) + Oxi	100.0	59.9	49.0	39.5
GAPD1_MOUSE	Gapvd1 p-S761 p-T762 (z= 4)	78.0	80.1	76.6	100.0
GFPT1_MOUSE	Gfpt1 p-T260 (z= 2)	82.3	89.7	94.9	100.0
GIT1_MOUSE	Git1 p-S370 (z= 2)	100.0	87.3	88.1	93.4
GIT1_MOUSE	Git1 p-S394 p-S397 (z= 3)	100.0	85.2	92.4	87.9
GIT1_MOUSE	Git1 p-S601 p-Y607 (z= 3)	80.9	98.4	100.0	82.5
GMIP_MOUSE	Gmip p-S436 p-S440 (z= 2)	52.7	100.0	37.6	35.9
GNL3_MOUSE	Gnl3 p-S505 (z= 2)	75.0	100.0	46.3	43.0
GNL3_MOUSE	Gnl3 p-S505 (z= 3)	61.1	100.0	42.2	68.9
GNL3_MOUSE	Gnl3 p-S95 (z= 2)	76.7	85.1	100.0	63.7
GNL3_MOUSE	Gnl3 p-S95 (z= 3)	56.0	100.0	41.0	57.1
GOG7B_MOUSE	GOLGA7B p-S142 p-S157 p-S158 (z= 3)	36.5	59.0	100.0	76.6
GOGA1_MOUSE	Golga1 p-S41 p-S47 p-S50 (z= 4) + Oxi	22.4	100.0	30.7	12.5
GOGA4_MOUSE	Golga4 p-S93 (z= 2)	100.0	79.6	83.0	59.5
GOLP3_MOUSE	Golph3 p-S35 (z= 2)	100.0	88.2	33.7	86.0
GPN1_MOUSE	Gpn1 p-S314 (z= 2)	56.0	100.0	91.2	81.9
GPN1_MOUSE	Gpn1 p-S338 (z= 2)	100.0	89.5	71.4	76.4
GPN1_MOUSE	Gpn1 p-S338 (z= 3)	80.1	44.6	100.0	81.7
GPR98_MOUSE	Gpr98 p-S133 p-T135 p-S155 (z= 3)	19.0	100.0	58.4	79.1
GPTC4_MOUSE	Gpatch4 p-S130 (z= 4)	100.0	75.7	76.7	50.5
GPTC4_MOUSE	Gpatch4 p-S258 (z= 2)	57.0	100.0	60.2	50.0
GPTC4_MOUSE	Gpatch4 p-S258 (z= 2)	100.0	90.5	59.6	15.0
GPTC4_MOUSE	Gpatch4 p-S258 (z= 3)	100.0	55.3	88.8	89.1
GRAA_MOUSE	Gzma p-S5 (z= 2)	100.0	75.6	50.2	86.0
GSK3A_MOUSE	Gsk3a p-Y279 (z= 2)	98.1	85.5	81.7	100.0
GTPB1_MOUSE	Gtpbp1 p-S6 p-S25 (z= 3) + Oxi	100.0	61.0	56.5	62.7
GTPB1_MOUSE	Gtpbp1 p-S8 p-S25 (z= 3)	82.2	82.6	83.2	100.0
GUAA_MOUSE	Gmps p-T331 (z= 2)	100.0	71.1	95.6	83.4
H15_MOUSE	Hist1h1b p-S18 (z= 2)	69.0	81.0	45.3	100.0
H15_MOUSE	Hist1h1b p-T135 (z= 2)	29.3	100.0	24.2	23.9
HAP28_MOUSE	Pdap1 p-S60 p-S63 (z= 2)	75.8	100.0	39.4	45.0
HAP28_MOUSE	Pdap1 p-S60 p-S63 (z= 2)	79.2	24.9	100.0	86.4
HAP28_MOUSE	Pdap1 p-S60 p-S63 (z= 3)	100.0	42.6	89.3	84.5
HAVR1_MOUSE	HAVR1 p-S270 p-S279 (z= 3)	100.0	81.9	88.9	86.4
HCDH_MOUSE	Hadh p-S13 (z= 3) + Oxi	100.0	87.1	43.5	35.8
HCN3_MOUSE	HCN3 p-S648 (z= 3)	70.9	85.3	89.7	100.0
HDAC1_MOUSE	Hdac1 p-S393 (z= 3)	100.0	99.2	79.8	78.8
HDAC1_MOUSE	Hdac1 p-S393 (z= 3) + Oxi	100.0	67.0	66.8	82.9
HDAC1_MOUSE	Hdac1 p-S393 (z= 4)	95.7	100.0	95.5	98.2
HDAC1_MOUSE	Hdac1 p-S393 (z= 4) + Oxi	100.0	71.8	61.4	97.5
HDAC1_MOUSE	Hdac1 p-S393 (z= 4) + Oxi	100.0	55.5	51.1	70.8
HDAC1_MOUSE	Hdac1 p-S421 p-S423 (z= 2)	58.9	100.0	33.5	76.4
HDAC1_MOUSE	Hdac1 p-S421 p-S423 (z= 2)	100.0	90.8	55.0	68.0
HDAC1_MOUSE	Hdac1 p-S421 p-S423 (z= 3)	88.6	59.8	100.0	87.0
HDAC2_MOUSE	Hdac2 p-S394 (z= 4)	100.0	85.3	92.2	65.4
HDAC2_MOUSE	Hdac2 p-S394 (z= 4) + Oxi	84.7	77.4	78.7	100.0
HDAC2_MOUSE	Hdac2 p-S394 (z= 4) + Oxi	100.0	56.9	51.8	62.4
HDAC2_MOUSE	Hdac2 p-S422 p-S424 (z= 3)	81.4	100.0	99.3	82.9
HDAC7_MOUSE	Hdac7 p-S204 (z= 2)	100.0	78.2	64.5	24.5
HDGF_MOUSE	Hdgf p-S132 p-S133 (z= 2)	50.6	81.8	89.2	100.0
HDGF_MOUSE	Hdgf p-S132 p-S133 (z= 2)	81.3	94.7	91.2	100.0
HDGF_MOUSE	Hdgf p-S132 p-S133 (z= 3)	74.9	100.0	96.6	68.1
HDGF_MOUSE	Hdgf p-S133 (z= 3)	74.4	100.0	83.6	97.6
HDGF_MOUSE	Hdgf p-S165 (z= 2)	65.3	100.0	73.9	76.0
HDGF_MOUSE	Hdgf p-S165 (z= 2)	100.0	63.5	84.4	47.6
HDGF_MOUSE	Hdgf p-S165 (z= 2)	86.8	100.0	99.5	72.8
HDGF_MOUSE	Hdgf p-S165 (z= 3)	93.6	100.0	98.6	69.4
HDGF_MOUSE	Hdgf p-S165 (z= 3)	100.0	82.4	80.9	56.9
HDGF_MOUSE	Hdgf p-T200 (z= 3)	77.0	83.8	99.6	100.0
HDGR2_MOUSE	Hdgfrp2 p-S366 p-S367 (z= 2)	92.2	52.6	85.1	100.0
HDGR2_MOUSE	Hdgfrp2 p-S366 p-S367 (z= 2)	78.4	54.1	100.0	87.4
HDGR2_MOUSE	Hdgfrp2 p-S366 p-S367 (z= 2)	100.0	84.9	91.6	96.9
HDGR2_MOUSE	Hdgfrp2 p-S366 p-S367 (z= 3)	100.0	72.1	45.7	61.0
HDGR2_MOUSE	Hdgfrp2 p-S450 (z= 2)	27.7	100.0	44.9	83.0
HDGR2_MOUSE	Hdgfrp2 p-S450 (z= 3)	65.4	100.0	29.5	48.5
HDGR2_MOUSE	Hdgfrp2 p-S659 (z= 2)	100.0	61.3	42.7	20.8
HEBP1_MOUSE	Hebp1 p-T158 p-Y162 (z= 4) + Oxi	65.3	76.5	100.0	95.7
HECD1_MOUSE	Hectd1 p-S358 (z= 2)	82.8	100.0	57.7	28.2
HELB_MOUSE	Helb p-S1015 (z= 3)	100.0	92.9	82.2	55.6
HELB_MOUSE	HELB p-S1068 (z= 3)	98.0	83.9	100.0	98.8
HEXI1_MOUSE	Hexim1 p-S114 (z= 4)	75.7	46.0	100.0	95.6
HIRP3_MOUSE	Hirip3 p-S134 p-T135 (z= 2)	80.0	100.0	66.1	67.5
HIRP3_MOUSE	Hirip3 p-S152 p-S153 (z= 2)	39.9	70.2	45.9	100.0
HIRP3_MOUSE	Hirip3 p-S163 p-T167 (z= 2)	84.7	100.0	98.4	79.3
HIRP3_MOUSE	Hirip3 p-S163 p-T167 (z= 3)	73.3	100.0	76.5	61.2
HIRP3_MOUSE	Hirip3 p-S205 p-S207 p-S208 (z= 2)	50.4	83.3	66.3	100.0
HIRP3_MOUSE	Hirip3 p-S575 (z= 2)	80.9	90.0	83.0	100.0
HJURP_MOUSE	Hjurp p-S388 (z= 2)	68.0	80.3	68.3	100.0
HM20A_MOUSE	Hmg20a p-S104 (z= 2)	68.2	45.6	100.0	28.1
HMGA1_MOUSE	Hmga1 p-S102 p-S103 (z= 2)	0.0	0.0	100.0	0.3
HMGA1_MOUSE	Hmga1 p-S102 p-S103 (z= 2)	100.0	68.2	71.2	63.2
HMGA1_MOUSE	Hmga1 p-S102 p-S103 (z= 3)	100.0	73.5	81.4	75.3
HMGA1_MOUSE	Hmga1 p-S103 (z= 3)	100.0	64.8	67.6	69.0
HMGA1_MOUSE	Hmga1 p-S36 p-T53 (z= 3)	100.0	72.2	97.1	95.1
HMGA1_MOUSE	Hmga1 p-S99 p-S102 p-S103 (z= 2)	83.5	100.0	39.7	42.9
HMGA1_MOUSE	Hmga1 p-S99 p-S102 p-S103 (z= 2)	73.1	73.1	64.1	100.0
HMGA1_MOUSE	Hmga1 p-S99 p-S102 p-S103 (z= 3)	100.0	98.2	87.8	89.0
HMGA1_MOUSE	Hmga1 p-T53 (z= 3)	100.0	70.0	80.5	67.3
HMGA2_MOUSE	Hmga2 p-S44 (z= 2)	67.3	100.0	80.0	60.8
HMGA2_MOUSE	Hmga2 p-T99 p-S104 (z= 2)	37.9	100.0	39.7	33.1
HMGN1_MOUSE	Hmgn1 p-S84 p-S87 (z= 3)	79.8	96.9	100.0	92.9
HMGN1_MOUSE	Hmgn1 p-S84 p-S87 (z= 4)	76.6	78.4	54.7	100.0
HMGN1_MOUSE	Hmgn1 p-S87 (z= 3)	91.6	100.0	88.9	96.6
HMGN1_MOUSE	Hmgn1 p-S87 (z= 3)	100.0	92.2	71.5	55.1
HMGN1_MOUSE	Hmgn1 p-S87 (z= 4)	89.3	93.9	100.0	73.7
HMGN1_MOUSE	Hmgn1 p-T70 p-T71 (z= 3)	68.2	100.0	36.8	65.8
HN1_MOUSE	Hn1 p-S88 (z= 2)	46.3	89.6	81.5	100.0
HN1_MOUSE	Hn1 p-T54 (z= 2)	100.0	83.3	89.2	69.0

HN1L_MOUSE	Hn1l p-S97 (z= 3)	100.0	92.8	90.9	78.8
HNRH1_MOUSE	Hnrnph1 p-S104 (z= 2)	100.0	73.7	82.4	71.0
HNRH1_MOUSE	Hnrnph1 p-T107 (z= 3)	99.3	100.0	95.4	87.7
HNRL2_MOUSE	Hnrnpul2 p-S159 (z= 2)	84.3	77.8	74.4	100.0
HNRL2_MOUSE	Hnrnpul2 p-S226 (z= 4)	100.0	28.4	30.4	4.2
HNRPC_MOUSE	Hnrnpc p-S232 (z= 2)	73.9	93.1	84.5	100.0
HNRPC_MOUSE	Hnrnpc p-S232 (z= 2)	66.3	88.1	100.0	71.6
HNRPC_MOUSE	Hnrnpc p-S268 (z= 3)	53.5	71.8	100.0	93.4
HNRPC_MOUSE	Hnrnpc p-S268 (z= 3) + Oxi	47.8	61.0	46.0	100.0
HNRPC_MOUSE	Hnrnpc p-S268 (z= 3) + Oxi	69.0	71.4	87.6	100.0
HNRPC_MOUSE	Hnrnpc p-S268 (z= 4)	55.5	100.0	97.8	99.6
HNRPC_MOUSE	Hnrnpc p-S268 (z= 4) + Oxi	83.4	96.2	79.2	100.0
HNRPC_MOUSE	Hnrnpc p-S306 (z= 2)	63.7	100.0	67.7	97.4
HNRPC_MOUSE	Hnrnpc p-S306 (z= 3)	42.5	100.0	55.6	85.8
HNRPD_MOUSE	Hnrnpd p-S83 (z= 3)	72.6	90.5	100.0	83.5
HNRPK_MOUSE	Hnrnpk p-S116 (z= 3)	61.7	56.5	78.1	100.0
HNRPK_MOUSE	Hnrnpk p-S116 (z= 4)	39.6	100.0	52.6	38.5
HNRPK_MOUSE	Hnrnpk p-S116 (z= 4)	0.1	0.1	0.1	100.0
HNRPK_MOUSE	Hnrnpk p-S216 (z= 2)	85.1	97.6	94.5	100.0
HNRPK_MOUSE	Hnrnpk p-S216 (z= 3)	100.0	87.0	82.1	95.3
HNRPK_MOUSE	Hnrnpk p-S284 (z= 2)	74.8	68.0	100.0	84.2
HNRPK_MOUSE	Hnrnpk p-S284 (z= 2)	94.7	100.0	93.5	77.0
HNRPK_MOUSE	Hnrnpk p-S284 (z= 2) + Oxi	100.0	60.6	71.3	40.6
HNRPK_MOUSE	Hnrnpk p-S379 (z= 2)	57.9	88.3	100.0	87.3
HNRPU_MOUSE	Hnrnpu p-S247 (z= 4)	100.0	40.0	37.2	14.5
HPBP1_MOUSE	Hspbp1 p-T351 (z= 2)	75.7	88.7	84.6	100.0
HS105_MOUSE	Hsph1 p-S810 (z= 2)	100.0	54.3	55.9	26.7
HS90A_MOUSE	Hsp90aa1 p-S252 (z= 2)	49.0	100.0	86.6	81.6
HS90A_MOUSE	Hsp90aa1 p-S263 (z= 2)	50.4	92.1	100.0	97.5
HS90A_MOUSE	Hsp90aa1 p-S263 (z= 3)	54.4	97.9	90.2	100.0
HS90A_MOUSE	Hsp90aa1 p-S263 (z= 3)	58.6	94.4	100.0	85.9
HS90A_MOUSE	Hsp90aa1 p-S263 (z= 3)	49.9	100.0	86.2	71.2
HS90A_MOUSE	Hsp90aa1 p-S263 (z= 4)	54.6	100.0	97.8	97.6
HS90B_MOUSE	Hsp90ab1 p-S255 (z= 2)	77.7	100.0	62.7	33.7
HS90B_MOUSE	Hsp90ab1 p-S255 (z= 2)	67.3	100.0	97.7	88.8
HS90B_MOUSE	Hsp90ab1 p-S255 (z= 2)	37.1	100.0	43.6	81.0
HS90B_MOUSE	Hsp90ab1 p-S255 (z= 3)	58.5	88.3	87.9	100.0
HS90B_MOUSE	Hsp90ab1 p-S255 (z= 3)	75.5	100.0	95.4	82.6
HS90B_MOUSE	Hsp90ab1 p-S255 (z= 4)	72.9	99.6	100.0	88.7
HSF1_MOUSE	Hsf1 p-S314 (z= 3)	71.5	8.7	100.0	80.4
HSF1_MOUSE	Hsf1 p-S314 p-S319 (z= 3)	45.7	100.0	18.9	35.5
HSPB8_MOUSE	Hspb8 p-S87 (z= 2)	62.9	85.1	92.1	100.0
HSPB8_MOUSE	Hspb8 p-S87 (z= 3)	100.0	86.9	90.3	82.9
HTSF1_MOUSE	Htatsf1 p-S705 (z= 3)	72.3	95.1	82.9	100.0
HTSF1_MOUSE	Htatsf1 p-S724 (z= 2)	93.2	74.4	100.0	82.1
HUWE1_MOUSE	Huwe1 p-S1907 (z= 2)	100.0	75.4	93.9	88.5
HYDIN_MOUSE	Hydin p-T2739 p-Y2740 (z= 2)	22.7	92.1	43.7	100.0
I2BP1_MOUSE	Irf2bp1 p-S453 (z= 3)	60.8	100.0	50.5	58.8
I2BP2_MOUSE	Irf2bp2 p-S169 (z= 2)	100.0	66.9	52.6	63.5
I2BP2_MOUSE	Irf2bp2 p-S169 (z= 3)	100.0	77.4	79.5	75.2
I2BP2_MOUSE	Irf2bp2 p-S71 (z= 2)	81.6	100.0	71.2	80.7
I2BPL_MOUSE	Irf2bpl p-S195 (z= 4)	98.8	78.5	91.1	100.0
I2BPL_MOUSE	Irf2bpl p-S526 (z= 2)	86.3	99.8	100.0	63.1
I2BPL_MOUSE	Irf2bpl p-S636 p-S638 (z= 2)	50.3	100.0	65.8	80.6
I2BPL_MOUSE	Irf2bpl p-S636 p-S638 p-S641 (z= 2)	65.8	100.0	30.2	67.7
I2BPL_MOUSE	Irf2bpl p-S638 (z= 2)	88.9	77.4	100.0	95.4
IASPP_MOUSE	Ppp1r13l p-S485 (z= 3)	100.0	2.4	95.0	1.9
ICAL_MOUSE	Cast p-S219 (z= 3)	75.4	100.0	95.5	83.5
ICAL_MOUSE	Cast p-S444 (z= 2)	100.0	50.7	92.3	71.7
ICAL_MOUSE	Cast p-T321 (z= 3)	60.1	100.0	72.6	81.6
ICAL_MOUSE	Cast p-T479 (z= 2)	82.1	82.4	100.0	87.1
IF2B1_MOUSE	Igf2bp1 p-S181 (z= 2)	89.4	100.0	57.8	40.3
IF2B1_MOUSE	Igf2bp1 p-S181 (z= 2)	100.0	72.4	97.8	49.5
IF2P_MOUSE	Eif5b p-S114 (z= 2)	100.0	80.7	76.0	82.2
IF2P_MOUSE	Eif5b p-S137 (z= 3)	100.0	85.9	70.1	68.9
IF2P_MOUSE	Eif5b p-S137 (z= 3)	68.3	100.0	59.0	81.0
IF2P_MOUSE	Eif5b p-S137 p-S139 (z= 3)	100.0	90.8	81.1	77.2
IF2P_MOUSE	Eif5b p-S139 (z= 4)	100.0	60.2	50.8	38.0
IF2P_MOUSE	Eif5b p-S165 (z= 2)	100.0	72.1	56.5	63.0
IF2P_MOUSE	Eif5b p-S183 p-S184 p-S187 (z= 3)	90.2	79.7	73.6	100.0
IF2P_MOUSE	Eif5b p-S215 (z= 2)	100.0	14.7	18.7	22.9
IF2P_MOUSE	Eif5b p-S215 (z= 2)	77.6	79.5	100.0	95.2
IF4B_MOUSE	Eif4b p-S424 p-S425 (z= 2)	61.2	100.0	27.7	37.6
IF4B_MOUSE	Eif4b p-S424 p-S425 (z= 2)	37.1	61.0	46.6	100.0
IF4B_MOUSE	Eif4b p-S424 p-S425 (z= 3)	58.7	57.5	100.0	99.7
IF4B_MOUSE	Eif4b p-S498 (z= 2)	69.6	100.0	41.6	41.2
IF4B_MOUSE	Eif4b p-S93 (z= 3)	5.7	100.0	56.4	5.2
IF4B_MOUSE	Eif4b p-T427 (z= 2)	74.2	100.0	68.7	47.6
IF4B_MOUSE	Eif4b p-T461 (z= 4)	96.9	100.0	87.0	99.3
IF4B_MOUSE	Eif4b p-T500 p-S504 (z= 2)	88.9	0.0	100.0	79.3
IF4G1_MOUSE	Eif4g1 p-S1096 (z= 3)	100.0	51.4	58.3	50.2
IF4G1_MOUSE	Eif4g1 p-S1187 p-S1189 (z= 2)	81.0	79.3	100.0	71.0
IF4G1_MOUSE	Eif4g1 p-S1189 (z= 2)	100.0	57.5	73.5	48.2
IF4G1_MOUSE	Eif4g1 p-S1201 (z= 3)	100.0	49.5	45.8	15.8
IF4G1_MOUSE	Eif4g1 p-S1211 (z= 2)	100.0	55.8	51.6	60.7
IF4G1_MOUSE	Eif4g1 p-S1597 (z= 2)	100.0	56.9	94.6	76.6
IF4G1_MOUSE	Eif4g1 p-T1213 (z= 2)	89.4	54.4	100.0	54.8
IF4G2_MOUSE	Eif4g2 p-S394 (z= 2)	71.3	34.3	100.0	98.2
IF4G2_MOUSE	Eif4g2 p-S394 (z= 4)	100.0	80.5	89.4	8.4
IFIH1_MOUSE	Ifih1 p-S289 (z= 2)	100.0	98.3	76.1	98.2
IFIH1_MOUSE	Ifih1 p-S645 p-S648 (z= 3)	59.9	79.6	89.7	100.0
IGEB_MOUSE	Iap p-S178 (z= 2)	67.9	90.6	59.4	100.0
ILF3_MOUSE	Ilf3 p-S382 (z= 3)	78.1	83.3	100.0	58.3
ILF3_MOUSE	Ilf3 p-S477 p-S482 (z= 4)	58.3	1.8	100.0	24.8
ILF3_MOUSE	Ilf3 p-S482 (z= 4)	58.5	100.0	93.2	77.7
ILFT1_MOUSE	Ifltd1 p-T112 p-S113 (z= 3)	100.0	48.3	66.3	48.9
IMA2_MOUSE	Kpna2 p-S62 (z= 2)	71.6	74.5	76.4	100.0
IMA3_MOUSE	Kpna3 p-S60 (z= 2)	68.1	75.7	86.9	100.0

IMA3_MOUSE	Kpna3 p-T72 (z= 4)	100.0	18.4	39.7	1.5
IMDH2_MOUSE	Impdh2 p-S444 (z= 3)	65.8	100.0	69.3	64.3
INCE_MOUSE	Incenp p-S259 p-S273 (z= 3)	83.2	100.0	47.9	84.1
INO1_MOUSE	Isyna1 p-S524 (z= 4)	99.2	100.0	66.9	39.9
IPP2_MOUSE	Ppp1r2 p-S122 p-S123 (z= 2)	100.0	67.4	82.7	79.7
IPP2_MOUSE	Ppp1r2 p-S122 p-S123 (z= 2)	46.3	100.0	47.9	56.5
IPP2_MOUSE	Ppp1r2 p-S122 p-S123 (z= 3)	100.0	11.4	9.6	16.6
IPP2_MOUSE	Ppp1r2 p-S122 p-S123 (z= 3)	100.0	89.0	44.2	84.8
IPP2_MOUSE	Ppp1r2 p-S88 (z= 3)	73.7	100.0	86.3	79.3
IPP2_MOUSE	Ppp1r2 p-S88 (z= 3) + Oxi	100.0	78.1	78.2	63.2
IPP2_MOUSE	Ppp1r2 p-S90 (z= 4)	82.6	93.6	100.0	83.2
IRS2_MOUSE	Irs2 p-S1089 (z= 2)	60.3	100.0	70.4	58.4
IRS2_MOUSE	Irs2 p-S678 (z= 2)	62.2	83.4	93.9	100.0
IRX2_MOUSE	Irx2 p-S2 p-Y9 p-S14 p-Y18 (z= 4) + Oxi	100.0	51.0	48.3	25.6
ITSN1_MOUSE	Itsn1 p-S335 (z= 2)	53.2	100.0	71.8	59.7
IWS1_MOUSE	Iws1 p-S183 (z= 2)	100.0	95.1	70.4	83.0
IWS1_MOUSE	Iws1 p-S183 p-S185 (z= 2)	71.9	66.3	100.0	68.9
IWS1_MOUSE	Iws1 p-S183 p-S185 (z= 3)	77.6	77.6	100.0	93.1
IWS1_MOUSE	Iws1 p-S198 p-S200 p-S209 (z= 4)	41.7	13.7	100.0	57.0
IWS1_MOUSE	Iws1 p-S343 p-S345 (z= 2)	100.0	44.3	91.7	81.0
IWS1_MOUSE	Iws1 p-S343 p-S345 (z= 2)	100.0	73.7	63.8	69.2
IWS1_MOUSE	Iws1 p-S343 p-S345 (z= 2)	94.7	100.0	83.4	73.9
IWS1_MOUSE	Iws1 p-S366 (z= 2)	100.0	78.6	89.2	86.3
IWS1_MOUSE	Iws1 p-S366 p-S368 (z= 2)	100.0	84.6	73.3	100.0
IWS1_MOUSE	Iws1 p-T672 (z= 2)	61.0	100.0	37.5	33.7
JIP3_MOUSE	JIP3 p-S330 p-T358 (z= 3) + Oxi	46.6	100.0	62.8	87.7
JIP4_MOUSE	Spag9 p-S730 p-S733 (z= 3)	100.0	60.9	73.7	65.7
JIP4_MOUSE	Spag9 p-S732 p-S733 (z= 2)	66.9	84.8	83.4	100.0
JUNB_MOUSE	Junb p-S248 p-T252 p-S256 (z= 3)	86.5	74.4	100.0	73.0
JUNB_MOUSE	Junb p-T252 p-S256 (z= 2)	59.3	87.1	73.0	100.0
JUNB_MOUSE	Junb p-T252 p-S256 (z= 3)	64.9	86.3	62.1	100.0
JUND_MOUSE	Jund p-S100 (z= 2)	68.2	100.0	85.2	78.6
JUND_MOUSE	Jund p-S249 p-S253 (z= 3)	82.5	66.3	91.1	100.0
JUND_MOUSE	Jund p-S249 p-S253 (z= 4)	99.6	100.0	91.2	91.3
K0284_MOUSE	Kiaa0284 p-S1122 (z= 2)	100.0	68.7	67.3	70.2
K0284_MOUSE	Kiaa0284 p-S843 (z= 3)	100.0	46.9	52.7	36.4
K1143_MOUSE	Map4 p-S50 (z= 4)	100.0	65.1	68.6	41.2
K1430_MOUSE	Kiaa1430 p-S121 (z= 2)	100.0	71.9	87.7	40.4
K1522_MOUSE	Kiaa1522 p-S543 (z= 3)	100.0	47.7	32.9	5.2
K1522_MOUSE	Kiaa1522 p-S909 (z= 3)	70.0	96.3	100.0	97.7
K1704_MOUSE	Kiaa1704 p-S146 (z= 3)	100.0	97.9	72.8	75.4
K1C18_MOUSE	Krt18 p-S35 (z= 2)	96.8	97.4	69.9	100.0
K1C18_MOUSE	Krt18 p-S7 (z= 2)	75.4	100.0	76.1	89.8
K22E_MOUSE	Krt2 p-S563 p-T564 p-S571 (z= 3)	66.5	82.9	83.5	100.0
K2C7_MOUSE	Krt7 p-S32 (z= 3)	43.3	72.6	71.3	100.0
K2C8_MOUSE	Krt8 p-S24 (z= 2)	100.0	93.0	43.7	53.5
K2C8_MOUSE	Krt8 p-S37 (z= 2)	99.7	100.0	55.0	51.5
K2C8_MOUSE	Krt8 p-S47 (z= 2)	16.3	1.2	100.0	23.6
K2C8_MOUSE	Krt8 p-T54 (z= 4)	25.0	52.2	100.0	0.0
K6PL_MOUSE	Pfkl p-S775 (z= 2)	47.5	100.0	72.5	99.4
KANK3_MOUSE	Kank3 p-S163 p-S167 p-S176 (z= 3)	88.1	79.6	100.0	96.9
KANK3_MOUSE	Kank3 p-S166 p-S167 (z= 3)	100.0	73.1	74.0	79.2
KANK3_MOUSE	Kank3 p-S167 (z= 3)	81.5	95.6	98.7	100.0
KAP0_MOUSE	Prkar1a p-S83 (z= 2)	100.0	80.2	80.7	81.8
KAP2_MOUSE	Prkar2a p-S96 (z= 3)	100.0	80.7	76.8	78.8
KAP3_MOUSE	Prkar2b p-S112 (z= 3)	100.0	66.5	56.1	31.5
KAP3_MOUSE	Prkar2b p-S83 p-S85 (z= 3)	99.9	85.7	100.0	83.0
KAPCA_MOUSE	Prkaca p-T198 (z= 2)	45.9	1.4	100.0	47.6
KC1D_MOUSE	Csnk1d p-S382 (z= 2)	91.5	56.6	100.0	67.9
KC1E_MOUSE	Csnk1e p-S363 (z= 2)	100.0	58.7	28.3	74.0
KCNQ4_MOUSE	Kcnq4 p-S411 (z= 3)	100.0	97.8	80.5	75.2
KIF15_MOUSE	Kif15 p-S568 (z= 2)	76.8	100.0	69.1	91.5
KIF1A_MOUSE	Kif1a p-S1490 p-T1492 p-S1498 (z= 4) + Oxi	54.8	100.0	44.8	61.8
KIF1C_MOUSE	KIF1C p-S1020 (z= 2)	94.1	25.3	100.0	34.7
KIFC2_MOUSE	Kifc2 p-S342 (z= 2) + Oxi	100.0	87.5	72.1	65.3
KITH_MOUSE	Tk1 p-Y61 p-S62 p-S66 p-T67 (z= 3)	86.3	47.4	81.5	100.0
KKCC1_MOUSE	Camkk1 p-S74 p-T93 p-S97 (z= 4)	31.9	41.4	58.0	100.0
KLC1_MOUSE	Klc1 p-S520 (z= 2)	90.8	100.0	94.8	87.4
KLC3_MOUSE	Klc3 p-S467 (z= 2)	96.2	87.5	100.0	75.8
KLC3_MOUSE	Klc3 p-S501 (z= 2)	100.0	84.2	76.7	75.5
KLF1_MOUSE	Klf1 p-S347 (z= 3) + Oxi	100.0	51.9	70.3	49.2
KPCD_MOUSE	Prkcd p-S643 (z= 3)	5.2	60.8	4.5	100.0
KPRB_MOUSE	Prpsap2 p-S227 (z= 4)	100.0	65.3	44.1	38.3
KRI1_MOUSE	Kri1 p-S148 (z= 2)	86.3	100.0	77.5	82.6
KRI1_MOUSE	Kri1 p-S148 (z= 3)	88.7	100.0	97.6	96.0
KS6A1_MOUSE	Rps6ka1 p-S721 (z= 2)	94.2	85.6	94.7	100.0
KS6A3_MOUSE	Rps6ka3 p-S715 (z= 2)	100.0	82.3	71.0	81.5
KS6B1_MOUSE	KS6B1 p-S441 (z= 2)	100.0	83.5	74.9	56.4
LAD1_MOUSE	Lad1 p-S336 (z= 2)	100.0	84.1	84.5	77.6
LAD1_MOUSE	Lad1 p-S367 (z= 3)	71.7	84.2	91.8	100.0
LAD1_MOUSE	Lad1 p-S367 (z= 3)	100.0	81.0	86.4	90.5
LAD1_MOUSE	Lad1 p-S62 (z= 4)	10.3	100.0	85.6	68.7
LAD1_MOUSE	Lad1 p-S62 (z= 4)	63.3	100.0	84.2	62.2
LAD1_MOUSE	Lad1 p-S62 p-S72 (z= 4)	100.0	86.5	75.8	71.9
LAD1_MOUSE	Lad1 p-T522 (z= 2)	73.8	76.5	82.2	100.0
LAP2A_MOUSE	Tmpo p-S308 (z= 2)	100.0	72.1	87.5	76.5
LAP2A_MOUSE	Tmpo p-S422 (z= 2)	90.4	82.8	88.9	100.0
LAP2A_MOUSE	Tmpo p-T74 (z= 3)	87.5	94.1	75.3	100.0
LAP2B_MOUSE	Tmpo p-S158 (z= 2)	78.5	100.0	50.0	56.5
LAP2B_MOUSE	Tmpo p-S179 (z= 3)	100.0	90.3	58.8	66.0
LAP2B_MOUSE	Tmpo p-S179 p-S183 (z= 3)	100.0	57.2	67.5	89.9
LAP2B_MOUSE	Tmpo p-S179 p-S183 (z= 3)	78.3	100.0	43.1	61.6
LAP2B_MOUSE	Tmpo p-S179 p-Y182 (z= 3)	87.5	100.0	73.9	78.6
LAP2B_MOUSE	Tmpo p-S183 (z= 3)	96.2	100.0	60.5	84.3
LAP2B_MOUSE	Tmpo p-S66 p-S67 (z= 3)	89.0	86.5	100.0	99.8
LAP2B_MOUSE	Tmpo p-T207 (z= 2)	95.5	100.0	41.5	16.2
LAP2B_MOUSE	Tmpo p-Y182 p-S183 (z= 3)	100.0	88.4	45.0	71.9
LAR4B_MOUSE	LAR4B p-S603 (z= 3)	90.9	94.4	100.0	90.2

LAR4B_MOUSE	Larp4b p-S721 (z= 2)	100.0	70.0	73.2	52.4
LAR4B_MOUSE	Larp4b p-S739 (z= 2)	33.0	43.9	100.0	2.3
LARP1_MOUSE	Larp1 p-S299 p-S302 (z= 3)	100.0	68.2	63.3	64.3
LARP1_MOUSE	Larp1 p-S494 p-S498 (z= 2)	88.0	84.6	58.2	100.0
LARP1_MOUSE	Larp1 p-S498 (z= 2)	81.3	100.0	51.3	34.9
LARP1_MOUSE	Larp1 p-S525 (z= 2)	56.2	100.0	25.0	53.8
LARP1_MOUSE	Larp1 p-S68 (z= 3)	79.5	100.0	92.3	78.3
LARP1_MOUSE	Larp1 p-S68 (z= 4)	100.0	59.5	56.5	21.9
LARP1_MOUSE	Larp1 p-S743 p-S751 (z= 2)	50.5	77.5	39.9	100.0
LARP1_MOUSE	Larp1 p-S751 (z= 2)	100.0	84.1	85.1	87.1
LARP1_MOUSE	Larp1 p-S81 (z= 4)	100.0	16.1	14.2	19.6
LARP1_MOUSE	Larp1 p-T297 (z= 3)	100.0	58.5	64.5	39.6
LARP1_MOUSE	Larp1 p-T503 (z= 2)	100.0	92.6	78.8	61.8
LARP1_MOUSE	Larp1 p-T503 (z= 3)	100.0	69.6	70.0	63.2
LARP4_MOUSE	Larp4 p-S578 (z= 4)	62.4	100.0	90.0	94.8
LARP4_MOUSE	Larp4 p-T583 (z= 3)	70.7	98.6	100.0	95.7
LARP7_MOUSE	Larp7 p-S253 p-S256 (z= 2)	89.4	55.9	49.6	100.0
LARP7_MOUSE	Larp7 p-S253 p-S256 (z= 2)	69.6	0.0	100.0	0.0
LARP7_MOUSE	Larp7 p-S253 p-S256 (z= 3)	100.0	75.5	93.3	96.8
LARP7_MOUSE	Larp7 p-S253 p-S256 (z= 3)	70.2	100.0	61.8	74.8
LASP1_MOUSE	Lasp1 p-T104 (z= 2)	100.0	71.3	70.2	69.1
LATS1_MOUSE	Lats1 p-S463 (z= 2)	75.0	97.7	100.0	91.0
LC7L2_MOUSE	Luc7l2 p-S281 p-S283 p-S285 (z= 2)	100.0	60.4	32.5	87.7
LEG1_MOUSE	Lgals1 p-S30 (z= 2)	48.9	100.0	60.5	61.7
LEGLA_MOUSE	Lgalsla p-S25 (z= 3)	79.5	90.9	100.0	68.4
LEO1_MOUSE	Leo1 p-S631 (z= 3)	100.0	63.0	93.3	72.1
LEO1_MOUSE	Leo1 p-S659 (z= 2)	79.4	100.0	95.7	72.6
LGMN_MOUSE	Lgmn p-S218 p-Y222 p-Y222 p-Y230 (z= 3)	0.1	0.1	0.1	100.0
LIMA1_MOUSE	Lima1 p-S488 (z= 3)	100.0	77.7	84.5	48.3
LIMA1_MOUSE	Lima1 p-S488 (z= 4)	100.0	67.7	95.8	70.2
LIMA1_MOUSE	Lima1 p-S607 (z= 2)	78.8	100.0	53.7	27.5
LIPB1_MOUSE	Ppfibp1 p-S37 (z= 3)	80.9	63.5	94.0	100.0
LIPB1_MOUSE	Ppfibp1 p-S435 (z= 2)	100.0	84.8	83.3	80.7
LIPB1_MOUSE	Ppfibp1 p-S500 (z= 3)	100.0	84.5	51.0	68.7
LMNA_MOUSE	Lmna p-S390 (z= 2)	100.0	73.8	79.0	61.6
LMNA_MOUSE	Lmna p-S390 p-S395 (z= 2)	100.0	99.6	55.8	57.1
LMNA_MOUSE	Lmna p-S392 (z= 2)	46.1	100.0	56.0	45.8
LMNA_MOUSE	Lmna p-S404 (z= 2)	74.8	100.0	77.8	74.8
LMNA_MOUSE	Lmna p-T19 p-S22 (z= 2)	42.2	100.0	40.3	33.8
LMNA_MOUSE	Lmna p-T24 (z= 2)	82.9	100.0	78.3	77.3
LMNB1_MOUSE	Lmnb1 p-T21 (z= 2)	90.6	100.0	97.2	61.9
LMNB2_MOUSE	Lmnb2 p-S385 (z= 2)	100.0	79.0	56.1	58.2
LRBA_MOUSE	Lrba p-S979 (z= 3)	98.6	100.0	75.1	55.9
LRRF1_MOUSE	Lrrfip1 p-S302 (z= 3)	97.1	99.0	91.1	100.0
LRRF1_MOUSE	Lrrfip1 p-S547 (z= 2)	100.0	51.8	60.4	22.6
LRRF1_MOUSE	Lrrfip1 p-S547 (z= 3)	100.0	74.9	44.0	66.6
LRRF1_MOUSE	Lrrfip1 p-S83 (z= 2)	96.9	100.0	90.1	80.0
LRRF2_MOUSE	Lrrfip2 p-S111 (z= 2)	88.3	100.0	97.1	66.0
LS14A_MOUSE	Lsm14a p-S182 p-S183 (z= 4)	93.7	73.0	100.0	98.5
LS14A_MOUSE	Lsm14a p-S183 (z= 3)	78.0	97.2	100.0	88.0
LS14A_MOUSE	Lsm14a p-S192 (z= 3)	38.8	57.2	53.0	100.0
LUZP1_MOUSE	Luzp1 p-S660 (z= 2)	99.8	80.2	100.0	97.0
M3K1_MOUSE	Map3k1 p-S411 p-S413 p-T415 (z= 4) + Oxi	100.0	69.2	0.0	0.0
M3K7_MOUSE	Map3k7 p-S431 p-S432 p-S434 (z= 4)	30.1	80.0	50.8	100.0
M4K5_MOUSE	Map4k5 p-T333 (z= 3)	92.0	100.0	63.5	70.6
MA7D1_MOUSE	Map7d1 p-S315 (z= 2)	36.5	2.5	35.7	100.0
MA7D1_MOUSE	Map7d1 p-S401 (z= 2)	55.5	63.9	100.0	65.3
MA7D1_MOUSE	Map7d1 p-S401 (z= 3)	100.0	69.8	59.3	35.8
MA7D3_MOUSE	Map7d3 p-S49 (z= 3)	100.0	57.7	62.0	45.1
MAP1B_MOUSE	Map1b p-S1260 (z= 2)	69.2	100.0	71.2	92.4
MAP1B_MOUSE	Map1b p-S1293 p-S1307 (z= 3)	56.3	92.3	57.3	100.0
MAP1B_MOUSE	Map1b p-S1307 (z= 3)	95.7	100.0	69.7	99.6
MAP1B_MOUSE	Map1b p-S1373 (z= 2)	63.3	88.9	92.6	100.0
MAP1B_MOUSE	Map1b p-S1391 p-S1395 (z= 3)	35.3	100.0	35.1	65.0
MAP1B_MOUSE	Map1b p-S1395 (z= 3)	83.6	100.0	83.7	85.6
MAP1B_MOUSE	Map1b p-S1438 (z= 3)	100.0	93.6	78.6	55.3
MAP1B_MOUSE	Map1b p-S1438 (z= 4)	100.0	87.6	95.8	82.9
MAP1B_MOUSE	Map1b p-S1775 (z= 2)	63.7	100.0	55.4	58.4
MAP1B_MOUSE	Map1b p-S1775 p-S1778 p-S1781 (z= 3)	63.5	80.9	84.7	100.0
MAP1B_MOUSE	Map1b p-S1789 p-S1793 (z= 2)	79.0	76.6	63.3	100.0
MAP1B_MOUSE	Map1b p-S1793 (z= 2)	100.0	87.4	98.1	84.9
MAP1B_MOUSE	Map1b p-S1813 (z= 3)	100.0	96.0	98.4	98.8
MAP1B_MOUSE	Map1b p-S2030 (z= 2)	51.0	88.9	64.2	100.0
MAP1B_MOUSE	Map1b p-S2094 (z= 3)	82.5	99.2	58.6	100.0
MAP1B_MOUSE	Map1b p-S609 (z= 3)	96.3	100.0	88.4	79.8
MAP1B_MOUSE	Map1b p-S828 p-S829 (z= 3)	41.8	100.0	30.9	57.9
MAP1B_MOUSE	Map1b p-T1499 (z= 3)	49.9	93.5	61.4	100.0
MAP1S_MOUSE	Map1s p-S462 (z= 2)	90.2	97.8	72.7	100.0
MAP1S_MOUSE	Map1s p-S658 p-S660 (z= 3)	100.0	83.9	67.9	47.2
MAP1S_MOUSE	Map1s p-S724 (z= 4)	100.0	66.0	77.6	68.1
MAP1S_MOUSE	Map1s p-T659 (z= 3)	100.0	96.3	87.9	75.8
MAP4_MOUSE	Map4 p-S1046 (z= 2)	100.0	54.4	67.1	57.2
MAP4_MOUSE	Map4 p-S1046 (z= 3)	100.0	57.7	73.0	67.9
MAP4_MOUSE	Map4 p-S254 (z= 3)	81.4	99.9	100.0	96.7
MAP4_MOUSE	Map4 p-S345 (z= 3)	68.4	100.0	98.9	69.0
MAP4_MOUSE	Map4 p-S475 (z= 2)	65.4	97.0	96.8	100.0
MAP4_MOUSE	Map4 p-S475 (z= 2) + Oxi	87.8	86.4	81.4	100.0
MAP4_MOUSE	Map4 p-S475 (z= 3)	100.0	72.1	75.3	80.5
MAP4_MOUSE	Map4 p-S517 (z= 2)	40.5	100.0	41.2	62.9
MAP4_MOUSE	Map4 p-S517 (z= 2) + Oxi	100.0	76.3	95.8	81.0
MAP4_MOUSE	Map4 p-S517 (z= 3)	100.0	71.0	72.3	57.2
MAP4_MOUSE	Map4 p-S667 (z= 3)	81.2	100.0	77.7	80.5
MAP4_MOUSE	Map4 p-S760 (z= 3)	85.8	100.0	98.0	77.8
MAP4_MOUSE	Map4 p-S785 (z= 2)	100.0	86.3	79.4	90.8
MAP4_MOUSE	Map4 p-S798 (z= 2)	79.1	100.0	59.8	75.2
MAP4_MOUSE	Map4 p-S914 (z= 2)	100.0	54.3	57.8	42.5
MAP4_MOUSE	Map4 p-T847 (z= 2)	96.5	100.0	90.5	79.8
MAP6_MOUSE	Map6 p-T774 p-T779 (z= 3)	81.9	100.0	61.7	32.1

MARCS_MOUSE	Marcks p-S141 (z= 2)	85.8	100.0	69.8	55.7
MARCS_MOUSE	Marcks p-S163 (z= 2)	16.4	87.6	85.7	100.0
MARCS_MOUSE	Marcks p-S163 (z= 2)	45.8	100.0	89.6	94.1
MARK2_MOUSE	Mark2 p-S453 (z= 2)	100.0	99.7	79.2	92.9
MARK2_MOUSE	Mark2 p-S480 (z= 2)	100.0	73.7	73.6	48.4
MARK2_MOUSE	MARK2 p-S483 (z= 3)	100.0	93.4	75.9	60.1
MATR3_MOUSE	Matr3 p-S188 (z= 2)	26.7	62.7	100.0	78.2
MATR3_MOUSE	Matr3 p-S188 (z= 4)	44.9	34.2	59.9	100.0
MBB1A_MOUSE	Mybbp1a p-S1164 (z= 2)	89.2	86.6	100.0	96.5
MBB1A_MOUSE	Mybbp1a p-S1164 (z= 2)	100.0	97.3	74.7	82.4
MBB1A_MOUSE	Mybbp1a p-S1187 (z= 3)	65.9	100.0	49.2	78.0
MBB1A_MOUSE	Mybbp1a p-S1248 (z= 3)	100.0	29.6	26.3	28.3
MBB1A_MOUSE	Mybbp1a p-S1253 (z= 2)	73.7	100.0	42.4	54.4
MBB1A_MOUSE	Mybbp1a p-S1253 p-S1255 (z= 3)	100.0	75.1	32.0	45.1
MBB1A_MOUSE	Mybbp1a p-S1280 (z= 2)	79.2	100.0	96.8	67.1
MBB1A_MOUSE	Mybbp1a p-S6 (z= 3)	66.0	100.0	78.0	81.6
MBB1A_MOUSE	Mybbp1a p-T1251 p-T1256 (z= 2)	45.6	100.0	44.2	74.5
MCAF1_MOUSE	Atf7ip p-S593 (z= 2)	100.0	86.9	56.3	28.1
MCES_MOUSE	Rnmt p-S100 (z= 2)	72.0	51.2	82.8	100.0
MCES_MOUSE	Rnmt p-S15 (z= 2)	100.0	46.9	88.8	77.9
MCM2_MOUSE	Mcm2 p-S139 (z= 2)	100.0	93.6	92.7	93.9
MCM2_MOUSE	Mcm2 p-S139 (z= 3)	100.0	77.1	76.5	57.1
MCM2_MOUSE	Mcm2 p-S139 p-S140 (z= 2)	100.0	82.7	94.1	99.7
MCM2_MOUSE	Mcm2 p-S139 p-S140 (z= 3)	100.0	67.3	77.6	80.0
MCM2_MOUSE	Mcm2 p-S139 p-S140 (z= 3)	81.0	80.0	100.0	89.2
MCM2_MOUSE	Mcm2 p-S140 (z= 3)	91.5	100.0	89.1	77.8
MCM2_MOUSE	Mcm2 p-S21 p-S26 (z= 2)	83.7	99.4	100.0	90.0
MCM2_MOUSE	Mcm2 p-S26 (z= 2)	100.0	82.1	92.6	75.7
MCM2_MOUSE	Mcm2 p-S26 p-S27 (z= 2)	78.0	100.0	45.7	75.0
MCM2_MOUSE	Mcm2 p-S27 (z= 2)	84.6	100.0	84.8	75.4
MCM2_MOUSE	Mcm2 p-S40 p-S41 (z= 2)	57.4	2.1	100.0	48.2
MCM2_MOUSE	Mcm2 p-S41 (z= 2)	82.5	96.3	100.0	74.9
MCM2_MOUSE	Mcm2 p-S41 (z= 4)	100.0	58.7	57.2	9.7
MCM3_MOUSE	Mcm3 p-S668 p-S672 (z= 3)	98.3	100.0	74.9	89.3
MCM3_MOUSE	Mcm3 p-S672 (z= 3)	91.1	91.2	64.2	100.0
MCM3_MOUSE	Mcm3 p-S672 (z= 3)	100.0	91.2	87.9	99.2
MCM3_MOUSE	Mcm3 p-S672 (z= 3)	100.0	81.8	72.5	73.9
MCM3_MOUSE	Mcm3 p-T719 (z= 3)	86.5	93.6	100.0	84.0
MCM4_MOUSE	Mcm4 p-S119 (z= 2)	84.7	100.0	94.0	72.2
MCM4_MOUSE	Mcm4 p-S31 (z= 2)	100.0	0.0	0.0	37.1
MCM4_MOUSE	Mcm4 p-S53 (z= 4)	90.3	4.5	100.0	0.0
MCM6_MOUSE	Mcm6 p-S704 (z= 3)	71.5	85.2	100.0	68.7
MCPT2_MOUSE	Mcpt2 p-S217 p-Y219 p-T228 (z= 4)	74.4	100.0	82.2	80.8
MD1L1_MOUSE	Mad1l1 p-T9 p-S13 (z= 4)	38.9	10.0	100.0	32.1
MDC1_MOUSE	Mdc1 p-S168 (z= 2)	100.0	69.2	73.0	67.5
MED1_MOUSE	Med1 p-S1078 p-S1079 p-Y1083 (z= 4) + Oxi	88.3	39.3	100.0	90.5
MED24_MOUSE	Med24 p-S871 (z= 2)	100.0	79.7	82.6	93.2
MELK_MOUSE	Melk p-S497 (z= 2)	50.3	100.0	49.4	91.9
MELK_MOUSE	Melk p-S521 (z= 2)	100.0	78.9	81.1	96.3
MELT_MOUSE	Veph1 p-S783 (z= 2)	81.5	63.9	100.0	87.3
MEPCE_MOUSE	Mepce p-T188 p-S191 (z= 2)	88.7	53.6	100.0	70.5
METL6_MOUSE	Mettl6 p-T276 p-S281 (z= 3)	58.2	0.0	100.0	0.0
MFAP1_MOUSE	Mfap1 p-S116 p-S118 (z= 2)	2.3	71.7	100.0	95.8
MFAP1_MOUSE	Mfap1 p-S116 p-S118 (z= 3)	96.6	76.0	94.7	100.0
MFAP1_MOUSE	Mfap1 p-S132 p-S133 (z= 2)	74.1	86.4	95.0	100.0
MFAP1_MOUSE	Mfap1 p-S132 p-S133 (z= 3)	82.8	76.6	86.5	100.0
MFAP1_MOUSE	Mfap1 p-S132 p-S133 (z= 3)	100.0	78.0	79.7	83.8
MFAP1_MOUSE	Mfap1 p-S52 p-S53 (z= 3)	88.3	70.9	100.0	78.2
MFAP1_MOUSE	Mfap1 p-S52 p-S53 (z= 3)	100.0	78.0	76.1	58.0
MFAP1_MOUSE	Mfap1 p-T267 (z= 2)	86.5	89.6	100.0	72.2
MFAP1_MOUSE	Mfap1 p-T267 (z= 3)	87.2	52.1	100.0	99.9
MIA3_MOUSE	Mia3 p-S1766 (z= 2)	77.9	100.0	53.8	30.6
MILK1_MOUSE	Micall1 p-S496 (z= 3)	100.0	64.1	76.1	87.7
MK01_MOUSE	Mapk1 p-T183 p-Y185 (z= 3)	70.3	92.3	88.7	100.0
MK03_MOUSE	Mapk3 p-T203 p-Y205 (z= 3)	70.2	91.3	76.7	100.0
MKL1_MOUSE	Mkl1 p-T488 p-S492 (z= 2)	100.0	45.1	61.2	56.1
MLF2_MOUSE	Mlf2 p-S237 (z= 2)	100.0	5.0	93.3	75.2
MLL2_MOUSE	Mll2 p-S4789 (z= 2)	100.0	81.2	67.5	95.8
MOR2A_MOUSE	MOR2A p-S977 (z= 2)	100.0	58.6	85.4	44.5
MOR2A_MOUSE	Morc2a p-S614 (z= 2)	100.0	98.4	88.0	88.6
MOV10_MOUSE	Mov10 p-S967 (z= 3)	26.1	100.0	32.2	0.4
MPP10_MOUSE	Mphosph10 p-S164 p-S168 p-S172 (z= 3)	89.0	93.6	100.0	74.2
MPRI_MOUSE	Igf2r p-S2471 (z= 2)	68.7	100.0	42.0	45.1
MPRIP_MOUSE	Mprip p-S992 (z= 3)	100.0	73.4	81.4	44.2
MRE11_MOUSE	Mre11a p-S686 (z= 2)	90.5	88.7	100.0	94.2
MRE11_MOUSE	Mre11a p-S686 (z= 3)	95.7	79.9	100.0	100.0
MRP_MOUSE	Marcksl1 p-S104 (z= 2)	79.4	100.0	77.4	80.8
MRP_MOUSE	Marcksl1 p-S22 (z= 2)	70.3	74.9	85.3	100.0
MRP_MOUSE	Marcksl1 p-T148 (z= 2)	64.6	100.0	42.3	61.3
MRP_MOUSE	Marcksl1 p-T85 (z= 2)	95.9	100.0	77.5	89.4
MRP_MOUSE	Marcksl1 p-T85 (z= 3)	75.6	100.0	61.7	34.9
MSH6_MOUSE	Msh6 p-S252 p-S254 p-S261 (z= 3)	100.0	78.2	87.2	97.4
MSH6_MOUSE	Msh6 p-S63 (z= 2)	52.7	100.0	36.6	69.9
MTA1_MOUSE	Mta1 p-S449 (z= 2)	100.0	84.6	51.3	54.3
MTA1_MOUSE	Mta1 p-S449 (z= 3)	100.0	98.4	69.9	87.1
MTA1_MOUSE	Mta1 p-S576 (z= 2)	86.1	82.0	88.2	100.0
MTA3_MOUSE	Mta3 p-S516 (z= 2)	100.0	70.9	89.7	73.7
MTMR2_MOUSE	Mtmr2 p-S6 (z= 2)	96.7	100.0	84.9	98.0
MTR1_MOUSE	Ftsjd2 p-S28 p-S30 (z= 3)	81.3	93.8	100.0	98.5
MTR1B_MOUSE	MTR1B p-T342 (z= 3)	83.2	100.0	48.4	97.0
MY18A_MOUSE	Myo18a p-S140 (z= 2)	100.0	94.9	87.2	59.0
MY18A_MOUSE	Myo18a p-S1966 p-S1970 (z= 3)	100.0	79.1	84.9	88.8
MY18A_MOUSE	Myo18a p-S2037 p-T2041 (z= 2)	67.5	100.0	85.7	73.2
MYBB_MOUSE	Mybl2 p-S505 p-T522 p-T524 (z= 3) + Oxi	80.1	53.6	100.0	74.1
MYC_MOUSE	Myc p-T58 p-S62 (z= 3)	93.1	83.5	100.0	69.9
MYH10_MOUSE	Myh10 p-S1956 (z= 2)	63.2	63.7	100.0	83.4
MYH9_MOUSE	Myh9 p-S1943 (z= 2)	43.7	30.0	100.0	48.1
MYH9_MOUSE	Myh9 p-S1943 (z= 2)	81.6	93.7	87.1	100.0

MYH9_MOUSE	Myh9 p-S1943 (z= 2)	93.0	93.4	100.0	65.9
MYH9_MOUSE	Myh9 p-S1943 (z= 2)	61.3	100.0	86.6	67.7
MYH9_MOUSE	Myh9 p-S1943 (z= 3)	76.0	100.0	78.1	84.5
MYH9_MOUSE	Myh9 p-T1939 (z= 3)	0.2	2.1	100.0	11.6
MYH9_MOUSE	Myh9 p-T1939 p-S1943 (z= 3)	57.7	92.4	65.4	100.0
MYL9_MOUSE	Myl9 p-S20 (z= 2)	28.9	91.3	64.3	100.0
MYL9_MOUSE	Myl9 p-S20 (z= 3) + Oxi	16.3	33.6	70.2	100.0
MYL9_MOUSE	Myl9 p-T19 p-S20 (z= 2)	11.8	43.8	100.0	89.2
MYL9_MOUSE	Myl9 p-T19 p-S20 (z= 3)	28.7	45.7	100.0	88.6
MYO9B_MOUSE	Myo9b p-S1220 (z= 3)	100.0	87.6	80.0	94.7
MYO9B_MOUSE	Myo9b p-S1264 (z= 3)	88.1	91.9	82.3	100.0
MYPT1_MOUSE	Ppp1r12a p-S417 (z= 3)	100.0	71.3	81.9	60.6
MYPT1_MOUSE	Ppp1r12a p-T419 (z= 3)	80.6	100.0	82.1	90.7
NACAM_MOUSE	Naca p-S2138 (z= 3)	100.0	86.3	91.4	90.8
NADK_MOUSE	Nadk p-S48 (z= 2)	100.0	44.9	66.4	28.7
NASP_MOUSE	Nasp p-S712 (z= 2)	36.4	100.0	0.0	0.0
NBEL2_MOUSE	Nbeal2 p-T929 p-S940 (z= 4)	97.8	88.8	90.1	100.0
NBN_MOUSE	Nbn p-S398 (z= 2)	100.0	77.2	54.1	73.0
NCBP1_MOUSE	Ncbp1 p-S22 (z= 3)	74.6	100.0	90.2	84.9
NCOA5_MOUSE	Ncoa5 p-S29 p-S34 (z= 2)	89.5	100.0	99.1	73.0
NCOA5_MOUSE	Ncoa5 p-S29 p-S34 (z= 3)	100.0	65.2	72.3	56.2
NCOAT_MOUSE	Mgea5 p-S364 (z= 3)	63.3	67.4	100.0	80.8
NCOR1_MOUSE	Ncor1 p-S2165 (z= 3)	83.0	84.4	100.0	66.7
NCOR1_MOUSE	Ncor1 p-S2198 (z= 2)	100.0	77.2	59.4	98.3
NDRG1_MOUSE	Ndrg1 p-S332 p-S333 (z= 2)	76.4	56.3	71.1	100.0
NDRG1_MOUSE	Ndrg1 p-S333 p-T335 p-S336 (z= 2)	60.8	76.9	48.6	100.0
NDRG1_MOUSE	Ndrg1 p-T328 (z= 2)	78.3	100.0	43.7	55.2
NDRG1_MOUSE	Ndrg1 p-T328 p-S330 (z= 2)	70.3	100.0	24.7	79.7
NDRG1_MOUSE	Ndrg1 p-T366 (z= 2)	47.2	82.7	9.2	100.0
NED4L_MOUSE	Nedd4l p-S475 (z= 2)	80.2	95.5	70.7	100.0
NEDD4_MOUSE	Nedd4 p-S281 (z= 3) + Oxi	100.0	63.1	54.7	70.9
NEDD4_MOUSE	Nedd4 p-T287 (z= 3)	83.7	86.6	84.7	100.0
NEK3_MOUSE	Nek3 p-T477 (z= 3)	100.0	88.5	94.0	85.0
NELFB_MOUSE	Cobra1 p-S557 (z= 3)	44.9	1.0	100.0	21.1
NEST_MOUSE	Nes p-S1127 (z= 4)	100.0	66.9	56.9	14.5
NEST_MOUSE	Nes p-S1481 (z= 4)	87.1	95.1	76.4	100.0
NEST_MOUSE	Nes p-S1541 (z= 3)	35.1	100.0	70.3	68.9
NEST_MOUSE	Nes p-S169 (z= 3)	100.0	59.0	46.9	12.8
NEST_MOUSE	Nes p-S169 (z= 4)	60.0	100.0	62.9	26.0
NEST_MOUSE	Nes p-S1837 (z= 2)	83.9	100.0	86.1	83.1
NEST_MOUSE	Nes p-S1837 (z= 3)	84.3	100.0	94.0	94.7
NEST_MOUSE	Nes p-S353 (z= 4)	5.0	100.0	85.5	2.3
NEST_MOUSE	Nes p-S623 (z= 3)	54.7	100.0	78.3	78.2
NEST_MOUSE	Nes p-S623 (z= 3) + Oxi	20.6	100.0	80.5	8.0
NEST_MOUSE	Nes p-S688 (z= 2)	70.2	100.0	79.5	93.7
NEST_MOUSE	Nes p-S688 (z= 3)	67.1	100.0	90.5	88.7
NEST_MOUSE	Nes p-S731 (z= 2)	80.2	59.5	100.0	81.4
NEST_MOUSE	Nes p-S894 (z= 2)	100.0	69.5	58.5	55.5
NEST_MOUSE	Nes p-S894 (z= 3)	100.0	80.3	73.3	91.2
NEST_MOUSE	Nes p-S963 (z= 3)	87.4	98.3	100.0	95.7
NF2IP_MOUSE	Nfatc2ip p-S79 p-S81 p-S83 (z= 3)	70.8	57.1	100.0	71.7
NFAC2_MOUSE	Nfatc2 p-S136 (z= 3)	94.2	100.0	82.4	78.8
NFH_MOUSE	Nefh p-S70 p-S71 p-S79 (z= 4)	80.2	83.6	73.8	100.0
NFIB_MOUSE	Nfib p-S328 (z= 3)	100.0	90.6	92.5	82.6
NFIL3_MOUSE	Nfil3 p-S301 (z= 3)	100.0	81.4	37.9	68.2
NFIX_MOUSE	Nfix p-S284 (z= 2)	77.2	79.2	97.7	100.0
NFIX_MOUSE	Nfix p-S301 (z= 3)	88.4	94.0	91.5	100.0
NFKB2_MOUSE	Nfkb2 p-T425 (z= 3)	100.0	80.3	77.2	76.0
NHRF1_MOUSE	Slc9a3r1 p-S285 (z= 2)	100.0	96.1	92.1	62.8
NHRF1_MOUSE	Slc9a3r1 p-S285 (z= 2)	64.9	100.0	50.4	67.5
NIBL1_MOUSE	Fam129b p-S574 (z= 4)	68.4	100.0	58.3	21.1
NIBL1_MOUSE	Fam129b p-S669 p-S670 p-S695 (z= 4)	71.4	72.5	100.0	66.1
NIPA_MOUSE	Zc3hc1 p-S343 (z= 3)	58.1	100.0	77.4	66.4
NIPA_MOUSE	Zc3hc1 p-S394 (z= 3) + Oxi	100.0	90.1	79.0	71.7
NKAP_MOUSE	Nkap p-S147 (z= 2)	62.6	79.4	100.0	80.7
NKPD1_MOUSE	Nkpd1 p-T408 p-T412 (z= 3)	93.4	97.6	100.0	91.3
NKTR_MOUSE	Nktr p-S1148 (z= 3)	52.4	92.5	100.0	94.0
NMT1_MOUSE	Nmt1 p-S47 (z= 2)	57.2	100.0	51.4	66.8
NOC2L_MOUSE	Noc2l p-S673 p-S674 (z= 2)	4.6	1.8	2.3	100.0
NOC2L_MOUSE	Noc2l p-S673 p-S674 (z= 3)	100.0	59.1	41.8	7.0
NOC2L_MOUSE	Noc2l p-S673 p-S674 p-T680 (z= 2)	58.1	40.6	100.0	62.7
NODAL_MOUSE	Nodal p-S175 (z= 2)	77.9	48.8	1.6	100.0
NOG1_MOUSE	Gtpbp4 p-S468 p-S470 p-S472 (z= 2)	66.1	100.0	92.7	69.4
NOL7_MOUSE	Nol7 p-S129 (z= 3)	92.6	100.0	62.1	79.1
NOL8_MOUSE	Nol8 p-S1064 p-S1065 p-S1077 (z= 4)	94.5	81.9	100.0	91.1
NOL8_MOUSE	Nol8 p-S300 p-S306 (z= 2)	46.3	100.0	42.8	60.6
NOP14_MOUSE	Nop14 p-S96 (z= 2)	82.1	100.0	44.1	54.9
NOP56_MOUSE	Nop56 p-S513 (z= 2)	73.5	100.0	43.4	55.3
NOP56_MOUSE	Nop56 p-S513 (z= 2)	21.4	100.0	55.2	69.8
NOP56_MOUSE	Nop56 p-S513 (z= 3)	31.4	78.1	100.0	77.2
NOP56_MOUSE	Nop56 p-S513 (z= 3)	100.0	94.8	77.2	73.0
NOP56_MOUSE	Nop56 p-S513 (z= 3)	100.0	75.9	58.5	57.3
NOP56_MOUSE	Nop56 p-S513 (z= 3)	100.0	26.4	17.2	14.8
NOP56_MOUSE	Nop56 p-S513 (z= 3) + Oxi	78.8	83.1	100.0	87.0
NOP56_MOUSE	Nop56 p-S513 (z= 3) + Oxi	100.0	78.4	69.9	78.5
NOP56_MOUSE	Nop56 p-S528 p-T545 p-T546 (z= 3)	100.0	91.9	91.3	84.1
NOP56_MOUSE	Nop56 p-S529 p-S543 (z= 3)	100.0	49.3	65.4	66.7
NOP56_MOUSE	Nop56 p-S529 p-S543 (z= 3)	87.1	100.0	74.1	84.2
NOP56_MOUSE	Nop56 p-S536 (z= 2)	100.0	67.2	65.9	73.9
NOP56_MOUSE	Nop56 p-S536 (z= 2)	51.9	100.0	69.5	77.9
NOP56_MOUSE	Nop56 p-S536 (z= 3)	100.0	48.8	64.8	41.9
NOP56_MOUSE	Nop56 p-S536 (z= 3)	91.5	100.0	95.6	65.3
NOP56_MOUSE	Nop56 p-S536 p-S543 (z= 2)	100.0	50.0	89.6	86.6
NOP56_MOUSE	Nop56 p-S536 p-S543 (z= 2)	0.0	0.0	100.0	0.0
NOP56_MOUSE	Nop56 p-S536 p-S543 p-T545 (z= 3)	75.7	64.8	95.2	100.0
NOP56_MOUSE	Nop56 p-S536 p-S543 p-T546 (z= 2)	59.6	0.0	100.0	0.0
NOP56_MOUSE	Nop56 p-S536 p-T545 (z= 3)	100.0	56.8	72.2	60.5
NOP56_MOUSE	Nop56 p-S554 (z= 2)	66.4	100.0	75.9	78.7

NOP58_MOUSE	Nop58 p-S509 (z= 3)	100.0	22.1	44.5	0.0
NOP58_MOUSE	Nop58 p-S509 (z= 3)	100.0	91.2	91.0	73.9
NOP58_MOUSE	Nop58 p-S509 p-S521 (z= 3)	60.3	75.1	79.5	100.0
NOP58_MOUSE	Nop58 p-S509 p-S521 (z= 3)	100.0	77.9	72.0	69.9
NOP58_MOUSE	Nop58 p-S509 p-T517 (z= 3)	86.5	86.6	96.6	100.0
NOP58_MOUSE	Nop58 p-T515 p-S521 (z= 4)	100.0	86.9	81.3	86.6
NP1L1_MOUSE	Nap1l1 p-T62 (z= 2)	87.7	91.6	100.0	74.7
NP1L4_MOUSE	Nap1l4 p-S125 (z= 3)	100.0	89.7	69.6	81.2
NP1L4_MOUSE	Nap1l4 p-S125 (z= 4)	100.0	87.7	79.1	90.1
NPM_MOUSE	Npm1 p-S10 (z= 3) + Oxi	43.9	100.0	39.4	59.7
NPM_MOUSE	Npm1 p-S125 (z= 3)	71.5	93.3	83.5	100.0
NPM_MOUSE	Npm1 p-S125 (z= 4)	34.3	51.2	42.8	100.0
NPM_MOUSE	Npm1 p-S125 (z= 4)	92.1	100.0	69.6	97.1
NPM_MOUSE	Npm1 p-S125 (z= 4) + Oxi	100.0	61.5	37.5	67.5
NPM_MOUSE	Npm1 p-S139 (z= 2)	80.1	93.6	100.0	72.7
NPM_MOUSE	Npm1 p-S252 (z= 2)	74.8	70.6	100.0	69.3
NPM_MOUSE	Npm1 p-S4 (z= 4) + Oxi	79.9	38.8	100.0	79.7
NPM_MOUSE	Npm1 p-S4 p-S10 (z= 4) + Oxi	100.0	72.2	65.9	83.1
NPM_MOUSE	Npm1 p-S4 p-Y17 (z= 4)	43.5	100.0	42.9	43.1
NPM_MOUSE	Npm1 p-S70 (z= 4)	100.0	99.7	75.5	29.8
NPM_MOUSE	Npm1 p-S70 (z= 4)	23.4	100.0	0.0	11.8
NPM_MOUSE	Npm1 p-S70 (z= 4) + Oxi	46.0	100.0	32.7	91.7
NPM_MOUSE	Npm1 p-T198 (z= 2)	62.4	70.0	100.0	58.8
NPM_MOUSE	Npm1 p-T217 (z= 2)	83.6	100.0	89.2	65.0
NRBP_MOUSE	Nrbp1 p-T431 (z= 2)	96.8	100.0	58.7	81.5
NRDC_MOUSE	Nrd1 p-S85 (z= 2)	59.0	100.0	50.7	25.9
NS1BP_MOUSE	Ivns1abp p-S338 (z= 3)	66.7	100.0	95.3	6.7
NSF1C_MOUSE	Nsfl1c p-S114 (z= 2)	30.3	0.0	100.0	44.1
NSF1C_MOUSE	Nsfl1c p-S114 (z= 3)	100.0	0.0	0.0	0.0
NSF1C_MOUSE	Nsfl1c p-S140 (z= 3)	100.0	73.4	56.6	57.1
NSRP1_MOUSE	Nsrp1 p-S31 p-S33 (z= 3)	81.1	75.5	85.6	100.0
NSUN2_MOUSE	Nsun2 p-S23 (z= 2)	64.3	100.0	60.7	68.5
NSUN2_MOUSE	Nsun2 p-S723 (z= 2)	66.3	80.7	100.0	88.6
NSUN2_MOUSE	Nsun2 p-S723 (z= 3)	64.1	76.2	63.6	100.0
NSUN2_MOUSE	Nsun2 p-S723 (z= 3)	99.6	100.0	90.1	84.2
NU160_MOUSE	Nup160 p-S1123 (z= 3)	70.5	100.0	81.4	88.0
NUCKS_MOUSE	Nucks1 p-S181 (z= 2)	100.0	1.4	5.9	12.7
NUCKS_MOUSE	Nucks1 p-S181 (z= 2)	100.0	53.5	40.9	40.3
NUCKS_MOUSE	Nucks1 p-S181 (z= 2)	70.0	100.0	62.0	84.2
NUCKS_MOUSE	Nucks1 p-S181 (z= 2)	66.5	100.0	55.7	51.3
NUCKS_MOUSE	Nucks1 p-S181 (z= 3)	100.0	96.2	63.8	66.1
NUCKS_MOUSE	Nucks1 p-S19 (z= 2)	37.4	27.8	94.1	100.0
NUCKS_MOUSE	Nucks1 p-S19 (z= 2)	68.0	82.5	81.1	100.0
NUCKS_MOUSE	Nucks1 p-S19 (z= 3)	33.9	64.0	59.2	100.0
NUCKS_MOUSE	Nucks1 p-S19 (z= 3)	65.8	79.2	81.6	100.0
NUCKS_MOUSE	Nucks1 p-S19 (z= 3)	100.0	63.8	73.7	80.3
NUCKS_MOUSE	Nucks1 p-S214 (z= 2)	68.4	89.5	100.0	66.1
NUCKS_MOUSE	Nucks1 p-S214 (z= 3)	98.2	98.4	100.0	98.2
NUCKS_MOUSE	Nucks1 p-S214 (z= 4)	75.1	90.2	100.0	77.5
NUCKS_MOUSE	Nucks1 p-S58 (z= 2)	64.8	93.5	92.2	100.0
NUCKS_MOUSE	Nucks1 p-S58 p-S61 (z= 2)	85.2	72.6	91.8	100.0
NUCKS_MOUSE	Nucks1 p-S58 p-S61 (z= 3)	100.0	89.7	79.0	74.8
NUCKS_MOUSE	Nucks1 p-T179 p-S181 (z= 2)	70.2	41.7	65.3	100.0
NUCKS_MOUSE	Nucks1 p-T179 p-S181 (z= 2)	100.0	79.2	65.0	74.3
NUCL_MOUSE	Ncl p-S145 p-S157 (z= 3)	83.2	72.5	100.0	87.3
NUCL_MOUSE	Ncl p-S145 p-S157 (z= 4)	49.3	73.1	81.7	100.0
NUCL_MOUSE	Ncl p-S189 p-S212 (z= 3)	57.0	43.1	100.0	61.5
NUCL_MOUSE	Ncl p-S189 p-S212 (z= 3) + Oxi	93.6	74.8	68.9	100.0
NUCL_MOUSE	Ncl p-S189 p-S212 (z= 4)	61.1	51.7	67.6	100.0
NUCL_MOUSE	Ncl p-S28 p-S34 p-S40 (z= 3)	40.6	47.2	87.4	100.0
NUCL_MOUSE	Ncl p-S28 p-S34 p-S40 (z= 3)	82.6	64.5	92.2	100.0
NUCL_MOUSE	Ncl p-S28 p-S34 p-S40 (z= 3)	79.4	81.9	93.5	100.0
NUCL_MOUSE	Ncl p-S28 p-S34 p-S40 (z= 3) + Oxi	100.0	61.9	58.6	59.4
NUCL_MOUSE	Ncl p-S28 p-S34 p-S40 (z= 3) + Oxi	56.6	81.7	53.3	100.0
NUCL_MOUSE	Ncl p-S28 p-S34 p-S40 (z= 4)	44.8	47.6	100.0	64.1
NUCL_MOUSE	Ncl p-S28 p-S34 p-S40 (z= 4)	75.1	71.5	100.0	91.0
NUCL_MOUSE	Ncl p-S28 p-S34 p-S40 (z= 4) + Oxi	100.0	41.2	43.0	65.0
NUCL_MOUSE	Ncl p-S28 p-S34 p-S40 (z= 4) + Oxi	77.8	81.0	96.6	100.0
NUCL_MOUSE	Ncl p-S403 (z= 2)	73.5	77.0	100.0	82.3
NUCL_MOUSE	Ncl p-T76 (z= 2)	100.0	75.9	83.9	68.3
NUCL_MOUSE	Ncl p-T84 (z= 2)	89.2	44.2	100.0	42.1
NUDC3_MOUSE	Nudcd3 p-S218 p-S223 (z= 4) + Oxi	100.0	83.2	63.6	97.1
NUDT5_MOUSE	Nudt5 p-T7 (z= 3)	100.0	71.0	67.8	69.3
NUFP1_MOUSE	Nufip1 p-S329 p-S331 p-S333 (z= 3)	86.5	80.8	100.0	73.7
NUFP2_MOUSE	Nufip2 p-S626 (z= 2)	81.4	100.0	85.6	79.1
NUFP2_MOUSE	Nufip2 p-S626 (z= 3)	0.9	62.1	1.2	100.0
NUFP2_MOUSE	Nufip2 p-S649 (z= 2)	85.2	89.8	100.0	75.1
NVL_MOUSE	Nvl p-S190 (z= 2)	85.6	90.3	93.2	100.0
ODP2_MOUSE	Dlat p-T66 (z= 3)	68.0	100.0	83.5	79.9
ODPA_MOUSE	Pdha1 p-S232 (z= 2)	80.6	91.9	100.0	98.9
ODPA_MOUSE	Pdha1 p-S293 p-S300 (z= 3)	100.0	92.8	95.0	62.7
ODPA_MOUSE	Pdha1 p-S293 p-Y301 (z= 3)	100.0	92.8	94.9	62.5
OGFR_MOUSE	Ogfr p-S403 (z= 2)	67.1	100.0	98.6	98.3
OSB11_MOUSE	Osbpl11 p-S186 (z= 2)	100.0	80.2	78.2	82.3
OSB11_MOUSE	Osbpl11 p-S194 (z= 3)	84.1	63.0	100.0	25.5
OSBL3_MOUSE	Osbpl3 p-S271 (z= 3)	90.7	94.2	100.0	91.3
OSTF1_MOUSE	Ostf1 p-S214 (z= 2)	100.0	81.8	96.1	98.4
OTU7A_MOUSE	Otud7a p-S593 (z= 2)	100.0	66.0	70.6	49.1
OTUD4_MOUSE	Otud4 p-S1000 (z= 2)	59.8	62.4	82.8	100.0
OTUD4_MOUSE	Otud4 p-S1016 p-S1017 (z= 3)	68.3	79.2	100.0	88.6
P3C2A_MOUSE	Pik3c2a p-S261 (z= 2)	53.8	100.0	66.3	75.5
P3C2A_MOUSE	Pik3c2a p-S339 (z= 2)	82.5	99.1	76.8	100.0
P4R3B_MOUSE	Smek2 p-T358 (z= 3) + Oxi	54.0	1.2	100.0	52.3
P66A_MOUSE	Gatad2a p-S96 p-S103 p-S110 (z= 3)	76.1	79.1	68.3	100.0
P66B_MOUSE	Gatad2b p-T121 p-S123 p-S130 (z= 3)	67.4	80.1	100.0	65.2
P66B_MOUSE	Gatad2b p-T121 p-S123 p-S136 (z= 3)	95.1	81.8	96.1	100.0
PA1_MOUSE	Pa1 p-S236 (z= 2)	71.7	81.6	100.0	84.7
PA24A_MOUSE	Pla2g4a p-S437 (z= 2)	78.8	84.9	94.0	100.0

PACN2_MOUSE	Pacsin2 p-T424 (z= 3)	71.1	65.6	100.0	70.4
PAIRB_MOUSE	Serbp1 p-S25 (z= 2)	73.7	100.0	87.0	81.2
PAIRB_MOUSE	Serbp1 p-T232 (z= 3)	100.0	77.6	72.8	74.0
PAK1_MOUSE	Pak1 p-T146 (z= 2)	70.1	100.0	72.3	90.9
PAK1_MOUSE	Pak1 p-T230 (z= 2)	100.0	68.0	80.9	74.9
PAK2_MOUSE	Pak2 p-S141 (z= 3)	100.0	91.3	83.9	73.8
PAK4_MOUSE	Pak4 p-S476 (z= 2)	50.1	100.0	45.3	14.9
PALLD_MOUSE	Palld p-S1141 p-S1143 p-S1146 (z= 3)	100.0	77.6	76.4	83.8
PALLD_MOUSE	Palld p-S901 (z= 2)	54.7	100.0	48.0	68.3
PALLD_MOUSE	Palld p-S901 (z= 3)	100.0	53.0	31.9	45.8
PARD3_MOUSE	Pard3 p-S144 (z= 3)	100.0	85.4	81.6	90.8
PATL1_MOUSE	Patl1 p-S179 (z= 2)	100.0	80.8	99.6	70.1
PAXI_MOUSE	Pxn p-S322 (z= 3)	100.0	99.9	80.7	97.3
PAXI_MOUSE	Pxn p-S83 (z= 2)	100.0	88.9	86.5	80.7
PCBP1_MOUSE	Pcbp1 p-S173 (z= 2)	75.2	100.0	39.5	10.5
PCBP1_MOUSE	Pcbp1 p-S173 (z= 2)	40.8	100.0	15.1	62.4
PCBP1_MOUSE	Pcbp1 p-S173 (z= 3)	48.1	7.8	100.0	47.0
PCBP1_MOUSE	Pcbp1 p-S189 (z= 2)	85.2	87.4	100.0	67.7
PCBP1_MOUSE	Pcbp1 p-S189 (z= 3)	90.3	82.4	100.0	74.0
PCBP1_MOUSE	Pcbp1 p-S190 (z= 3) + Oxi	100.0	80.5	81.2	91.8
PCBP2_MOUSE	Pcbp2 p-S183 (z= 3)	100.0	80.3	78.6	79.7
PCBP2_MOUSE	Pcbp2 p-T175 (z= 4)	100.0	92.2	81.8	92.7
PCM1_MOUSE	Pcm1 p-S1766 p-S1769 (z= 3)	100.0	71.8	81.0	80.4
PCM1_MOUSE	Pcm1 p-S1769 (z= 3)	81.9	71.0	100.0	92.7
PCM1_MOUSE	Pcm1 p-S65 (z= 2)	79.7	100.0	73.2	81.5
PCM1_MOUSE	Pcm1 p-S65 p-S69 (z= 2)	100.0	0.0	73.9	41.0
PCM1_MOUSE	Pcm1 p-S957 (z= 3)	100.0	74.1	81.2	66.3
PCY1A_MOUSE	Pcyt1a p-S315 (z= 2)	72.1	100.0	81.7	64.0
PDCD4_MOUSE	Pdcd4 p-S94 (z= 2)	100.0	83.2	85.7	82.3
PDCD5_MOUSE	Pdcd5 p-S119 (z= 2)	76.3	89.6	100.0	83.3
PDCD5_MOUSE	Pdcd5 p-S119 (z= 2)	88.3	100.0	96.3	82.1
PDCD5_MOUSE	Pdcd5 p-S119 (z= 2) + Oxi	100.0	68.4	55.3	59.6
PDCL3_MOUSE	Pdcl3 p-S235 p-S237 (z= 2)	100.0	23.6	30.2	13.1
PDIA6_MOUSE	Pdia6 p-S428 (z= 2)	41.5	72.9	82.9	100.0
PDIA6_MOUSE	Pdia6 p-S428 (z= 3)	100.0	39.2	59.5	81.3
PDIP3_MOUSE	Poldip3 p-S127 (z= 3)	100.0	46.0	57.3	16.6
PDIP3_MOUSE	Poldip3 p-T132 (z= 2)	89.1	85.4	79.6	100.0
PDLI2_MOUSE	Pdlim2 p-S199 p-S204 (z= 2)	100.0	69.5	97.4	88.0
PDLI2_MOUSE	Pdlim2 p-S199 p-S204 (z= 3)	100.0	90.5	89.9	81.4
PDLI5_MOUSE	Pdlim5 p-S137 (z= 3)	100.0	60.5	73.6	65.4
PDLI5_MOUSE	Pdlim5 p-S228 (z= 2)	82.7	100.0	10.4	4.3
PDS5B_MOUSE	Pds5b p-S1166 (z= 2)	63.2	100.0	44.8	84.1
PDS5B_MOUSE	Pds5b p-S1255 (z= 2)	75.7	100.0	89.6	77.0
PDS5B_MOUSE	Pds5b p-S1356 (z= 2)	94.4	100.0	66.0	44.1
PDS5B_MOUSE	Pds5b p-S1356 p-T1368 (z= 2)	30.7	100.0	43.5	34.6
PDS5B_MOUSE	Pds5b p-S1381 (z= 2)	100.0	79.2	79.5	32.7
PDS5B_MOUSE	Pds5b p-S1381 (z= 2)	100.0	63.3	42.3	51.3
PDXD1_MOUSE	Pdxdc1 p-T687 (z= 2)	100.0	95.7	99.5	91.3
PDXD1_MOUSE	Pdxdc1 p-T687 p-T691 (z= 2)	57.4	98.1	71.1	100.0
PEA15_MOUSE	Pea15 p-S116 (z= 2)	100.0	80.4	89.4	93.5
PEAK1_MOUSE	Peak1 p-S282 (z= 2)	67.6	77.2	70.6	100.0
PEBP1_MOUSE	Pebp1 p-S52 (z= 3)	77.9	100.0	97.7	68.4
PELP1_MOUSE	Pelp1 p-S7 p-S13 (z= 3)	95.1	87.0	100.0	73.2
PERE_MOUSE	Epx p-S671 p-S673 (z= 2)	37.5	98.8	54.6	100.0
PERQ1_MOUSE	Gigyf1 p-S408 (z= 2)	96.0	94.5	100.0	87.2
PERQ2_MOUSE	Gigyf2 p-S20 p-S30 (z= 2)	97.0	80.6	87.9	100.0
PERQ2_MOUSE	Gigyf2 p-S26 (z= 2)	99.5	90.5	100.0	91.2
PERQ2_MOUSE	Gigyf2 p-T383 (z= 4)	99.7	91.1	100.0	93.2
PGAM1_MOUSE	Pgam1 p-Y119 (z= 3) + Oxi	100.0	73.4	66.4	98.0
PGK1_MOUSE	Pgk1 p-S203 (z= 2)	78.2	100.0	73.3	84.8
PGM1_MOUSE	Pgm1 p-S117 (z= 3)	26.5	94.1	31.9	100.0
PGRC1_MOUSE	Pgrmc1 p-S181 (z= 2)	57.9	100.0	53.8	47.7
PGRC1_MOUSE	Pgrmc1 p-S181 (z= 3)	100.0	32.2	26.7	25.1
PGRC1_MOUSE	Pgrmc1 p-S181 (z= 3)	82.1	100.0	60.0	80.9
PGRC2_MOUSE	Pgrmc2 p-T205 (z= 4)	81.4	100.0	63.4	51.6
PHAR4_MOUSE	Phactr4 p-S118 (z= 2)	70.1	100.0	74.4	65.5
PHIP_MOUSE	Phip p-S1281 p-S1283 (z= 3)	98.2	100.0	74.2	83.1
PHIP_MOUSE	Phip p-S1783 (z= 2)	84.4	69.8	85.8	100.0
PHLB1_MOUSE	Phldb1 p-S463 (z= 2)	100.0	88.1	99.1	81.9
PHLB2_MOUSE	Phldb2 p-S465 (z= 2)	100.0	87.4	77.3	68.5
PHLB2_MOUSE	Phldb2 p-S510 (z= 3)	100.0	91.4	70.0	54.0
PHLB2_MOUSE	Phldb2 p-S976 (z= 3)	88.4	93.5	84.2	100.0
PHLP_MOUSE	Pdcl p-S293 p-S296 (z= 2)	75.6	98.7	73.9	100.0
PHLP_MOUSE	Pdcl p-S296 (z= 2)	90.7	87.6	100.0	88.0
PHRF1_MOUSE	Phrf1 p-S1201 (z= 2)	92.3	100.0	68.2	48.9
PI4KB_MOUSE	Pi4kb p-S428 (z= 2)	100.0	88.9	91.0	71.7
PI4KB_MOUSE	Pi4kb p-S428 (z= 3)	84.0	100.0	71.3	96.5
PIGH_MOUSE	Pigh p-S8 (z= 2)	87.1	100.0	65.4	85.0
PININ_MOUSE	Pnn p-S100 (z= 3)	100.0	75.3	81.8	83.6
PININ_MOUSE	Pnn p-S100 (z= 3)	100.0	78.7	69.0	46.2
PININ_MOUSE	Pnn p-S380 (z= 3)	14.8	11.2	14.3	100.0
PININ_MOUSE	Pnn p-S380 (z= 3)	100.0	19.6	46.7	19.2
PITM1_MOUSE	Pitpnm1 p-S621 (z= 2)	54.8	0.0	100.0	88.0
PJA1_MOUSE	Pja1 p-T231 (z= 3)	94.7	90.5	85.8	100.0
PKHG3_MOUSE	Plekhg3 p-S1136 p-S1141 (z= 2)	88.0	92.5	81.3	100.0
PKHG3_MOUSE	Plekhg3 p-S76 (z= 2)	83.0	99.6	95.2	100.0
PKHO2_MOUSE	Plekho2 p-S395 (z= 2)	100.0	47.3	53.6	57.8
PKN2_MOUSE	Pkn2 p-S582 (z= 2)	85.6	74.3	100.0	93.4
PKN3_MOUSE	Pkn3 p-S292 p-S302 p-T308 (z= 4) + Oxi	75.9	15.9	100.0	79.4
PLEC_MOUSE	Plec p-S1443 (z= 2)	55.5	100.0	55.8	80.3
PLEC_MOUSE	Plec p-S1729 (z= 2)	60.0	100.0	82.0	68.9
PLEC_MOUSE	Plec p-S4393 (z= 2)	81.1	78.1	82.0	100.0
PLEC_MOUSE	Plec p-S4393 p-S4396 (z= 2)	69.3	100.0	86.7	89.8
PLEC_MOUSE	Plec p-S4413 (z= 3)	74.8	57.9	81.8	100.0
PLEC_MOUSE	Plec p-S4633 (z= 2)	100.0	47.4	79.2	51.5
PLEC_MOUSE	Plec p-T4037 (z= 2)	100.0	56.9	55.3	79.3
PLEC_MOUSE	Plec p-T731 (z= 2)	50.4	100.0	58.6	71.2
PLTP_MOUSE	Pltp p-T330 (z= 3)	100.0	93.1	76.9	50.1

PMF1_MOUSE	Pmf1 p-S18 (z= 3)	100.0	91.1	82.3	96.5
PML_MOUSE	Pml p-S528 (z= 3)	100.0	71.3	76.3	64.7
PNISR_MOUSE	Pnisr p-S211 (z= 2)	52.1	100.0	81.7	97.7
PNISR_MOUSE	Pnisr p-S211 (z= 3)	100.0	69.3	67.2	67.4
PP1R7_MOUSE	Ppp1r7 p-S24 p-S27 (z= 2)	100.0	68.7	25.7	57.1
PP1RA_MOUSE	Ppp1r10 p-S313 (z= 2)	69.0	100.0	99.6	78.0
PP1RA_MOUSE	Ppp1r10 p-T315 (z= 3)	82.1	99.6	100.0	66.3
PP6R3_MOUSE	Ppp6r3 p-S588 (z= 2)	87.1	80.4	88.6	100.0
PPHLN_MOUSE	Pphln1 p-S219 (z= 2)	78.3	100.0	44.9	26.8
PPIA_MOUSE	Ppia p-T5 (z= 3)	32.5	100.0	14.0	61.0
PPIG_MOUSE	Ppig p-S354 p-T356 (z= 2)	91.7	100.0	80.6	97.3
PPIG_MOUSE	Ppig p-S411 p-S413 (z= 2)	21.9	79.7	100.0	13.1
PPIL4_MOUSE	PPIL4 p-S178 (z= 4)	100.0	94.0	60.9	8.4
PPM1G_MOUSE	Ppm1g p-T212 p-S215 p-S216 (z= 4)	90.4	85.6	81.2	100.0
PPR18_MOUSE	Ppp1r18 p-S146 (z= 2)	80.1	78.1	100.0	44.2
PPR18_MOUSE	Ppp1r18 p-S212 (z= 2)	54.3	44.1	100.0	55.8
PR38A_MOUSE	Prpf38a p-S193 p-S194 (z= 3)	58.5	73.2	89.1	100.0
PR38A_MOUSE	Prpf38a p-S193 p-S194 p-S209 (z= 4)	100.0	46.3	45.9	47.3
PR38A_MOUSE	Prpf38a p-S226 (z= 2)	100.0	79.5	89.2	56.5
PR40A_MOUSE	Prpf40a p-T928 p-S929 p-S931 (z= 3)	68.2	82.4	93.4	100.0
PRC2A_MOUSE	Prrc2a p-S1083 p-S1087 p-S1090 (z= 3)	79.3	0.5	100.0	74.7
PRC2A_MOUSE	Prrc2a p-S1217 (z= 2)	90.7	100.0	93.8	94.5
PRC2A_MOUSE	Prrc2a p-S808 (z= 3)	58.9	100.0	54.0	88.8
PRC2C_MOUSE	Prrc2c p-S1514 (z= 2)	77.2	100.0	38.4	33.9
PRC2C_MOUSE	Prrc2c p-S851 (z= 2)	100.0	74.7	29.3	52.9
PRDBP_MOUSE	Prkcdbp p-S165 p-S166 (z= 3)	85.9	75.7	100.0	93.6
PRP4B_MOUSE	Prpf4b p-S278 (z= 3)	100.0	87.0	83.6	51.1
PRP4B_MOUSE	Prpf4b p-S292 p-S294 (z= 2)	76.4	100.0	74.2	81.7
PRP4B_MOUSE	Prpf4b p-S366 p-S368 (z= 2)	86.6	100.0	87.3	97.5
PRP4B_MOUSE	Prpf4b p-S410 p-S411 (z= 2)	70.7	61.9	100.0	43.9
PRP4B_MOUSE	Prpf4b p-S411 (z= 2)	62.9	37.0	100.0	37.2
PRP4B_MOUSE	Prpf4b p-Y849 (z= 3)	96.2	88.3	94.6	100.0
PSA3_MOUSE	Psma3 p-S250 (z= 2)	79.8	100.0	77.0	97.3
PSA3_MOUSE	Psma3 p-S250 (z= 2) + Oxi	100.0	37.6	82.9	77.8
PSA5_MOUSE	Psma5 p-S56 (z= 2)	73.8	100.0	87.6	91.4
PSIP1_MOUSE	Psip1 p-S106 (z= 2)	46.7	100.0	43.5	56.3
PSIP1_MOUSE	Psip1 p-S270 p-S274 (z= 3)	100.0	65.9	54.5	44.6
PSIP1_MOUSE	Psip1 p-S272 p-S274 (z= 4)	100.0	68.4	61.2	55.1
PSIP1_MOUSE	Psip1 p-T141 (z= 2)	100.0	84.7	43.8	69.4
PSIP1_MOUSE	Psip1 p-T269 p-S272 p-S274 (z= 3)	66.5	43.7	100.0	77.0
PSIP1_MOUSE	Psip1 p-T269 p-S274 (z= 3)	51.0	100.0	32.6	47.2
PSIP1_MOUSE	Psip1 p-T269 p-S274 (z= 3)	88.2	100.0	75.3	78.4
PSIP1_MOUSE	Psip1 p-T271 p-S272 p-S274 (z= 3)	69.2	75.4	100.0	87.0
PSIP1_MOUSE	Psip1 p-T271 p-S272 p-S274 (z= 3)	100.0	74.4	61.7	69.1
PTN12_MOUSE	Ptpn12 p-S323 (z= 4)	100.0	85.4	98.7	87.6
PTN12_MOUSE	Ptpn12 p-S434 (z= 2)	69.7	74.6	78.6	100.0
PTN12_MOUSE	Ptpn12 p-S603 p-S606 p-S608 (z= 3)	65.3	70.7	100.0	67.6
PTN21_MOUSE	Ptpn21 p-S637 (z= 3)	100.0	43.6	30.1	39.9
PTN21_MOUSE	Ptpn21 p-S658 (z= 2)	83.3	100.0	60.3	95.1
PTN22_MOUSE	PTN22 p-Y440 p-S443 (z= 2)	68.0	9.1	100.0	56.6
PTRF_MOUSE	Ptrf p-S171 (z= 2)	34.5	100.0	54.0	44.7
PTRF_MOUSE	Ptrf p-S204 p-S205 (z= 4)	100.0	96.6	51.6	61.8
PUM2_MOUSE	Pum2 p-S136 (z= 2)	100.0	90.1	87.9	95.1
PUM2_MOUSE	Pum2 p-S181 (z= 2)	100.0	74.9	95.0	97.7
PUR9_MOUSE	Atic p-S554 p-T566 (z= 3)	100.0	97.1	87.3	95.9
PURB_MOUSE	Purb p-S310 (z= 2)	71.3	100.0	67.6	28.0
PURB_MOUSE	Purb p-S316 (z= 2)	100.0	48.3	29.4	51.2
PWP1_MOUSE	Pwp1 p-S49 (z= 3)	100.0	71.7	99.3	93.2
PWP1_MOUSE	Pwp1 p-S493 (z= 2)	100.0	99.9	95.2	71.0
PWP2_MOUSE	Pwp2 p-T891 p-S898 (z= 3)	81.2	80.7	100.0	68.0
PWP2_MOUSE	Pwp2 p-T891 p-S898 p-S902 (z= 3)	78.6	68.1	100.0	66.6
PYRG1_MOUSE	PYRG1 p-S562 (z= 2)	100.0	55.8	45.9	16.4
RAB7A_MOUSE	Rab7a p-S72 (z= 2)	13.6	100.0	89.7	57.0
RAGP1_MOUSE	Rangap1 p-S444 (z= 3)	70.6	1.2	100.0	63.6
RAI14_MOUSE	Rai14 p-S414 (z= 3)	56.9	57.6	100.0	65.8
RAI3_MOUSE	Gprc5a p-S344 (z= 2)	63.5	100.0	67.8	62.8
RALY_MOUSE	Raly p-S135 (z= 2)	81.2	100.0	66.7	62.1
RALY_MOUSE	Raly p-S270 p-T274 (z= 3)	100.0	47.0	97.6	96.1
RALY_MOUSE	Raly p-S294 p-S301 p-T304 (z= 3)	91.3	83.6	99.9	100.0
RALY_MOUSE	Raly p-T268 p-S270 (z= 2)	86.1	100.0	59.2	62.8
RALY_MOUSE	Raly p-T268 p-S270 p-T274 (z= 3)	100.0	68.6	74.8	96.8
RALY_MOUSE	Raly p-T274 (z= 2)	100.0	62.2	91.8	84.2
RALY_MOUSE	Raly p-T274 (z= 3)	100.0	69.4	83.9	88.3
RALY_MOUSE	Raly p-T304 (z= 3)	75.6	83.0	100.0	91.4
RAM_MOUSE	Fam103a1 p-S36 (z= 3)	100.0	90.7	93.8	99.6
RANB3_MOUSE	Ranbp3 p-S31 p-S40 (z= 3)	100.0	62.9	81.8	76.8
RANB3_MOUSE	Ranbp3 p-S32 p-S33 p-S40 (z= 3)	100.0	68.0	82.7	87.5
RANB3_MOUSE	Ranbp3 p-S40 (z= 3)	100.0	82.1	83.7	40.1
RANB3_MOUSE	Ranbp3 p-S58 (z= 2)	64.0	100.0	61.3	70.7
RAVR1_MOUSE	Raver1 p-S576 (z= 2)	73.7	92.4	89.2	100.0
RB15B_MOUSE	Rbm15b p-S107 (z= 2)	100.0	63.0	26.1	36.8
RB40C_MOUSE	Rab40c p-S224 p-S226 p-Y241 (z= 4) + Oxi	0.0	100.0	64.5	77.9
RB40C_MOUSE	Rab40c p-Y241 p-S245 p-S252 (z= 4)	58.2	83.5	74.0	100.0
RBBP6_MOUSE	Rbbp6 p-S1179 (z= 2)	84.8	100.0	82.4	73.5
RBBP6_MOUSE	Rbbp6 p-S1179 (z= 3)	100.0	60.0	64.1	48.8
RBBP6_MOUSE	Rbbp6 p-S1179 (z= 3)	68.2	100.0	75.3	66.7
RBCC1_MOUSE	Rb1cc1 p-S237 (z= 2)	100.0	4.7	1.9	5.5
RBM10_MOUSE	Rbm10 p-S723 (z= 2)	100.0	84.5	86.1	80.6
RBM10_MOUSE	Rbm10 p-S723 (z= 3)	100.0	82.7	89.4	86.0
RBM14_MOUSE	Rbm14 p-S618 (z= 2)	59.0	100.0	97.5	61.2
RBM14_MOUSE	Rbm14 p-T206 (z= 2)	94.7	84.2	100.0	83.6
RBM14_MOUSE	Rbm14 p-T206 (z= 2)	94.0	87.3	100.0	81.0
RBM19_MOUSE	Rbm19 p-S944 (z= 3)	37.6	41.8	18.4	100.0
RBM25_MOUSE	Rbm25 p-S581 (z= 3)	64.6	100.0	79.1	83.9
RBM25_MOUSE	Rbm25 p-S673 (z= 2)	63.6	100.0	56.8	45.7
RBM25_MOUSE	Rbm25 p-S675 (z= 2)	100.0	34.1	30.3	19.5
RBM25_MOUSE	Rbm25 p-S675 (z= 3)	100.0	61.3	64.8	54.3
RBM39_MOUSE	Rbm39 p-S136 (z= 2)	97.0	100.0	65.7	59.3

RBM39_MOUSE	Rbm39 p-S136 (z= 3)	100.0	91.2	87.3	86.2
RBM39_MOUSE	Rbm39 p-S97 (z= 2)	100.0	80.3	98.6	72.8
RBM5_MOUSE	Rbm5 p-S624 (z= 2)	48.7	69.4	71.9	100.0
RBP1_MOUSE	Ralbp1 p-S29 (z= 3)	100.0	77.8	80.5	76.0
RBP2_MOUSE	Ranbp2 p-S2641 (z= 3)	70.1	100.0	86.0	83.6
RBP2_MOUSE	Ranbp2 p-S2729 (z= 4)	28.6	100.0	26.4	37.2
REEP4_MOUSE	Reep4 p-S152 (z= 2)	99.0	100.0	87.7	93.3
REM2_MOUSE	Rem2 p-S27 (z= 2)	99.4	54.1	96.7	100.0
REM2_MOUSE	Rem2 p-S27 (z= 2)	98.3	86.1	100.0	73.7
REM2_MOUSE	Rem2 p-S334 (z= 2)	63.9	100.0	89.5	97.5
REM2_MOUSE	Rem2 p-S69 (z= 2)	82.6	100.0	94.2	76.8
REM2_MOUSE	Rem2 p-S69 (z= 3)	74.4	100.0	76.7	47.0
REPS1_MOUSE	Reps1 p-S561 (z= 2)	75.8	100.0	47.1	81.7
REQU_MOUSE	Dpf2 p-S142 (z= 2)	100.0	76.6	81.4	67.4
RERE_MOUSE	Rere p-S656 (z= 2)	99.1	100.0	99.6	65.3
RFC1_MOUSE	Rfc1 p-S107 p-T109 (z= 3)	100.0	77.0	54.5	73.9
RFIP1_MOUSE	Rab11fip1 p-S358 (z= 2)	100.0	95.9	87.4	92.5
RFIP5_MOUSE	Rab11fip5 p-S307 (z= 2)	93.5	50.2	85.4	100.0
RGAG1_MOUSE	Rgag1 p-S1023 p-T1024 p-T1030 (z= 3) + Oxi	44.1	97.3	100.0	5.6
RGPS1_MOUSE	Ralgps1 p-T390 p-T393 p-S397 (z= 4) + Oxi	75.1	100.0	61.9	98.6
RHG01_MOUSE	Arhgap1 p-S51 (z= 2)	81.7	100.0	74.4	73.1
RHG05_MOUSE	Arhgap5 p-T1216 (z= 2)	88.5	65.9	77.0	100.0
RHG12_MOUSE	Arhgap12 p-T229 p-S238 (z= 3)	91.2	64.1	100.0	83.1
RHG17_MOUSE	Arhgap17 p-T562 (z= 3)	100.0	85.2	91.3	65.9
RHG17_MOUSE	Arhgap17 p-T730 p-T734 p-T736 (z= 3)	68.5	71.7	83.3	100.0
RHG22_MOUSE	Arhgap22 p-S387 (z= 2)	56.1	100.0	50.3	29.2
RHG35_MOUSE	Arhgap35 p-S1179 (z= 2)	100.0	78.8	80.2	95.2
RIC8A_MOUSE	Ric8a p-S435 p-T440 (z= 3)	45.9	73.5	70.9	100.0
RIN2_MOUSE	Rin2 p-S495 (z= 2)	0.0	88.3	83.4	100.0
RIR2_MOUSE	Rrm2 p-S20 (z= 3)	100.0	87.8	78.3	69.0
RL12_MOUSE	Rpl12 p-S38 (z= 2)	100.0	94.0	88.9	93.9
RL14_MOUSE	Rpl14 p-S139 (z= 2)	100.0	74.5	69.2	68.4
RL18_MOUSE	Rpl18 p-S130 (z= 2)	84.9	95.0	100.0	93.0
RL4_MOUSE	Rpl4 p-S295 (z= 2)	100.0	86.2	61.0	62.0
RL4_MOUSE	Rpl4 p-S295 (z= 3)	99.6	100.0	70.1	76.9
RL6_MOUSE	Rpl6 p-S21 (z= 3)	100.0	87.6	77.7	53.4
RLA0_MOUSE	Rplp0 p-S304 p-S307 (z= 2)	61.5	71.8	100.0	89.6
RLA0_MOUSE	Rplp0 p-S304 p-S307 (z= 2) + Oxi	48.8	100.0	24.7	62.0
RLA0_MOUSE	Rplp0 p-S304 p-S307 (z= 3)	100.0	56.8	55.1	95.3
RLA0_MOUSE	Rplp0 p-S304 p-S307 (z= 3) + Oxi	100.0	53.9	23.2	2.2
RLA0_MOUSE	Rplp0 p-S304 p-S307 (z= 4)	0.5	0.6	0.5	100.0
RLA0_MOUSE	Rplp0 p-T286 p-S304 (z= 4) + Oxi	91.3	77.1	75.5	100.0
RLA1_MOUSE	Rplp1 p-S101 p-S104 (z= 2)	42.9	69.3	66.9	100.0
RLA1_MOUSE	Rplp1 p-S101 p-S104 (z= 2) + Oxi	100.0	67.2	91.4	88.7
RLA2_MOUSE	Rplp2 p-S102 p-S105 (z= 2)	57.9	58.0	100.0	77.3
RLA2_MOUSE	Rplp2 p-S102 p-S105 (z= 2)	99.9	92.7	84.9	100.0
RLA2_MOUSE	Rplp2 p-S102 p-S105 (z= 2) + Oxi	69.2	78.5	94.5	100.0
RLA2_MOUSE	Rplp2 p-S102 p-S105 (z= 3)	50.5	40.8	100.0	71.9
RLA2_MOUSE	Rplp2 p-S105 (z= 2)	35.6	100.0	28.4	65.9
RLA2_MOUSE	Rplp2 p-S16 (z= 3)	100.0	73.6	70.9	86.7
RLA2_MOUSE	Rplp2 p-S17 (z= 2)	89.1	100.0	57.7	7.1
RM32_MOUSE	RM32 p-T134 (z= 2)	94.8	100.0	90.7	68.9
RMXL1_MOUSE	Rbmxl1 p-S113 p-S121 (z= 3)	72.7	64.0	100.0	80.6
RMXL1_MOUSE	Rbmxl1 p-S165 (z= 2)	100.0	71.5	66.7	42.5
RMXL1_MOUSE	Rbmxl1 p-S205 (z= 1)	100.0	75.9	92.9	78.2
RMXL1_MOUSE	Rbmxl1 p-S205 (z= 2)	93.2	100.0	78.2	91.9
RMXL1_MOUSE	Rbmxl1 p-S349 (z= 2)	88.9	100.0	99.2	85.9
RNPS1_MOUSE	Rnps1 p-S27 (z= 2)	91.0	68.0	100.0	48.2
ROA1_MOUSE	Hnrnpa1 p-S6 (z= 2)	92.6	100.0	86.5	72.3
ROA1_MOUSE	Hnrnpa1 p-S6 (z= 3)	70.8	100.0	41.5	33.0
ROA1_MOUSE	Hnrnpa1 p-S6 (z= 3)	100.0	82.8	81.7	56.1
ROA2_MOUSE	Hnrnpa2b1 p-S259 (z= 3)	86.6	79.6	83.9	100.0
ROA3_MOUSE	Hnrnpa3 p-S359 (z= 2)	100.0	60.5	89.9	85.3
RON_MOUSE	Mst1r p-S216 p-S218 (z= 2)	93.3	71.5	100.0	3.1
RPA34_MOUSE	Cd3eap p-S173 (z= 3)	100.0	90.4	93.4	90.0
RPAP3_MOUSE	Rpap3 p-S429 (z= 2)	100.0	72.2	41.7	23.2
RPB1_MOUSE	Polr2a p-S1913 (z= 2)	90.0	100.0	71.3	84.8
RPR1B_MOUSE	Rprd1b p-S166 (z= 3)	97.0	56.5	93.9	100.0
RPRD2_MOUSE	Rprd2 p-S392 (z= 2)	72.4	91.5	96.5	100.0
RPRD2_MOUSE	Rprd2 p-S749 (z= 3)	99.2	99.8	100.0	93.9
RPTOR_MOUSE	Rptor p-S863 (z= 2)	69.2	87.2	100.0	92.0
RRMJ3_MOUSE	Ftsj3 p-S335 p-S336 (z= 2)	0.0	65.1	1.3	100.0
RRP1_MOUSE	Rrp1 p-S434 (z= 2)	100.0	80.6	39.5	67.2
RRP12_MOUSE	Rrp12 p-S1081 (z= 2)	15.8	100.0	37.7	91.1
RRP5_MOUSE	Pdcd11 p-S1468 (z= 2)	26.9	100.0	66.6	75.4
RS17_MOUSE	Rps17 p-S113 (z= 2)	93.4	100.0	39.5	57.2
RS3_MOUSE	Rps3 p-T221 (z= 2)	83.1	94.8	98.2	100.0
RS6_MOUSE	Rps6 p-S235 p-S236 (z= 2)	37.4	98.7	54.5	100.0
RS6_MOUSE	Rps6 p-S235 p-S247 (z= 2)	88.1	100.0	8.6	30.5
RS6_MOUSE	Rps6 p-S236 (z= 2)	53.4	100.0	71.1	69.9
RS6_MOUSE	Rps6 p-S236 p-S240 (z= 3)	68.3	100.0	43.5	98.5
RS6_MOUSE	Rps6 p-S240 (z= 2)	100.0	48.2	35.9	19.4
RSRC2_MOUSE	Rsrc2 p-S45 (z= 2)	59.4	13.5	100.0	31.6
RTKN_MOUSE	Rtkn p-S220 (z= 2)	65.9	49.1	70.5	100.0
RTN4_MOUSE	Rtn4 p-S165 (z= 3)	55.0	100.0	37.7	36.5
RTN4_MOUSE	Rtn4 p-S768 (z= 3)	100.0	86.1	61.4	47.8
RTN4_MOUSE	Rtn4 p-S832 p-T834 (z= 3)	11.6	100.0	84.7	6.2
RTN4_MOUSE	Rtn4 p-T171 (z= 3)	100.0	58.2	56.0	14.8
RTN4_MOUSE	Rtn4 p-T98 (z= 3)	74.9	94.0	76.0	100.0
RTP4_MOUSE	Rtp4 p-S179 p-S181 (z= 3)	79.1	68.4	100.0	88.2
RU17_MOUSE	Snrnp70 p-S226 (z= 3)	100.0	93.5	96.0	71.4
RU17_MOUSE	Snrnp70 p-S408 (z= 2)	80.2	44.6	100.0	70.9
SAFB1_MOUSE	Safb p-S366 (z= 2)	62.2	63.7	100.0	81.5
SAFB1_MOUSE	Safb p-S366 (z= 2)	100.0	74.7	72.2	80.2
SAFB1_MOUSE	Safb p-S366 (z= 3)	88.0	19.2	100.0	68.2
SAFB1_MOUSE	Safb p-S366 (z= 4)	100.0	76.4	79.3	61.8
SAFB1_MOUSE	Safb p-S372 (z= 3)	100.0	70.5	70.2	51.5
SAFB1_MOUSE	Safb p-S626 (z= 2)	100.0	95.6	71.8	72.3

SAFB1_MOUSE	Safb p-T362 (z= 2)	100.0	58.8	48.2	40.7
SALL3_MOUSE	Sall3 p-S889 p-S892 p-S893 (z= 4)	53.1	61.7	61.7	100.0
SAMH1_MOUSE	Samhd1 p-T603 (z= 3)	74.5	100.0	45.3	50.8
SASH1_MOUSE	Sash1 p-S805 (z= 2)	81.0	100.0	85.0	71.7
SASH1_MOUSE	Sash1 p-S83 (z= 3)	82.7	100.0	62.5	70.0
SC61B_MOUSE	Sec61b p-S17 (z= 2)	61.3	100.0	69.9	52.8
SC61B_MOUSE	Sec61b p-S17 (z= 3)	77.3	100.0	60.5	69.6
SDE2_MOUSE	Sde2 p-S269 (z= 2)	100.0	88.0	73.5	86.1
SDE2_MOUSE	Sde2 p-S269 (z= 3)	100.0	94.7	84.5	98.6
SDPR_MOUSE	Sdpr p-S203 p-S204 p-S218 (z= 4)	69.3	100.0	36.6	25.2
SDPR_MOUSE	Sdpr p-S284 p-S293 (z= 3)	100.0	27.9	35.9	32.8
SDS3_MOUSE	Suds3 p-S236 p-S237 (z= 3)	100.0	73.0	56.9	70.3
SEM6A_MOUSE	Sema6a p-S539 p-S549 (z= 4)	95.7	100.0	95.4	98.1
SENP7_MOUSE	Senp7 p-S12 (z= 2)	2.0	100.0	75.7	73.5
SEPT2_MOUSE	Sept2 p-S218 (z= 2)	26.0	53.1	69.3	100.0
SEPT2_MOUSE	Sept2 p-S218 (z= 2)	100.0	80.3	65.6	48.6
SEPT2_MOUSE	Sept2 p-S218 (z= 3)	47.9	80.8	72.2	100.0
SEPT2_MOUSE	Sept2 p-S218 (z= 3)	63.2	97.1	100.0	97.1
SEPT2_MOUSE	Sept2 p-S218 (z= 4)	94.8	93.2	91.3	100.0
SEPT7_MOUSE	Sept7 p-T425 (z= 2)	100.0	85.7	78.6	72.3
SEPT9_MOUSE	Sept9 p-S85 (z= 2)	100.0	74.4	61.5	36.5
SEPT9_MOUSE	Sept9 p-S89 (z= 3)	100.0	15.6	18.6	16.0
SET_MOUSE	Set p-S30 (z= 2)	81.9	66.7	77.5	100.0
SETBP_MOUSE	Setbp1 p-T541 p-S551 p-T556 (z= 4) + Oxi	48.0	0.0	100.0	0.0
SETX_MOUSE	SETX p-T364 (z= 2)	100.0	96.2	57.5	77.9
SF01_MOUSE	Sf1 p-S80 p-S82 (z= 3)	100.0	60.8	63.7	61.3
SF01_MOUSE	Sf1 p-S80 p-S89 (z= 3)	83.5	79.3	100.0	97.1
SF01_MOUSE	Sf1 p-S80 p-Y87 (z= 4)	100.0	65.8	81.2	87.6
SF3B1_MOUSE	Sf3b1 p-T125 p-T142 (z= 3)	50.9	1.5	100.0	64.6
SF3B1_MOUSE	Sf3b1 p-T223 p-T227 (z= 3)	100.0	85.5	87.5	94.9
SF3B1_MOUSE	Sf3b1 p-T326 p-T328 p-S332 (z= 3)	62.4	93.5	54.2	100.0
SFR1_MOUSE	Sfr1 p-S115 (z= 2)	100.0	88.9	78.1	60.9
SFR1_MOUSE	Sfr1 p-S67 (z= 2)	100.0	98.8	63.6	82.0
SFR1_MOUSE	Sfr1 p-S67 p-S71 (z= 2)	54.2	0.1	100.0	49.4
SFR1_MOUSE	Sfr1 p-S67 p-T70 (z= 3)	71.9	100.0	72.5	89.7
SFR19_MOUSE	Scaf1 p-S240 (z= 3)	70.2	100.0	47.9	64.1
SFR19_MOUSE	Scaf1 p-S491 p-S493 (z= 2)	72.6	74.2	100.0	87.5
SFR19_MOUSE	Scaf1 p-S676 p-S682 (z= 2)	86.2	82.5	92.9	100.0
SGTA_MOUSE	Sgta p-S303 p-S307 (z= 2)	53.0	100.0	28.2	74.7
SGTA_MOUSE	Sgta p-S309 (z= 2)	71.1	100.0	87.5	72.5
SH2B1_MOUSE	Sh2b1 p-S127 (z= 3)	94.4	96.2	93.2	100.0
SH2B3_MOUSE	Sh2b3 p-S129 (z= 3)	100.0	87.3	87.6	88.9
SH3G1_MOUSE	Sh3gl1 p-S288 (z= 2)	37.8	100.0	30.9	33.1
SH3K1_MOUSE	Sh3kbp1 p-S274 (z= 2)	81.0	66.6	56.3	100.0
SH3K1_MOUSE	Sh3kbp1 p-S631 (z= 2)	100.0	68.0	74.7	49.6
SH3K1_MOUSE	Sh3kbp1 p-S631 (z= 3)	100.0	73.8	72.6	67.7
SI1L1_MOUSE	Sipa1l1 p-S1507 (z= 2)	41.4	5.9	73.4	100.0
SI1L1_MOUSE	Sipa1l1 p-S1528 (z= 2)	100.0	84.1	94.0	91.6
SI1L1_MOUSE	Sipa1l1 p-S162 (z= 2)	94.9	87.7	94.0	100.0
SKA3_MOUSE	Ska3 p-S154 (z= 3)	77.6	76.2	69.1	100.0
SKT_MOUSE	Skt p-S361 (z= 2)	100.0	48.7	69.0	57.7
SL9A1_MOUSE	Slc9a1 p-S707 (z= 2)	94.1	100.0	60.2	95.9
SLIRP_MOUSE	Slirp p-S105 (z= 2)	100.0	78.6	55.9	47.0
SLK_MOUSE	Slk p-S189 (z= 3)	100.0	88.5	85.9	18.6
SLTM_MOUSE	Sltm p-S289 (z= 2)	100.0	93.8	70.7	68.2
SLTM_MOUSE	Sltm p-S289 (z= 3)	100.0	85.3	94.7	76.6
SLTM_MOUSE	Sltm p-S552 (z= 2)	98.7	100.0	96.9	86.0
SLU7_MOUSE	Slu7 p-S215 (z= 2)	68.8	50.3	100.0	66.2
SMAP_MOUSE	Smap p-S17 (z= 2)	99.3	86.1	100.0	98.0
SMAP_MOUSE	Smap p-S17 (z= 3)	82.5	76.3	89.8	100.0
SMAP_MOUSE	Smap p-S17 (z= 3)	100.0	76.7	81.3	82.4
SMAP_MOUSE	Smap p-S24 (z= 3)	100.0	89.9	91.3	94.0
SMCA4_MOUSE	Smarca4 p-S1419 (z= 3)	100.0	94.4	93.3	83.3
SMCA5_MOUSE	Smarca5 p-S65 (z= 3)	41.8	78.4	54.5	100.0
SMCA5_MOUSE	Smarca5 p-S65 (z= 4)	70.5	100.0	89.4	95.4
SMGC_MOUSE	Muc19 p-S583 p-S600 (z= 4) + Oxi	82.2	80.7	75.9	100.0
SMN_MOUSE	Smn1 p-S25 (z= 2)	94.7	74.5	100.0	89.0
SMN_MOUSE	Smn1 p-S25 p-S28 (z= 2)	100.0	75.7	74.7	60.4
SMRC1_MOUSE	Smarcc1 p-S327 p-S329 (z= 2)	100.0	72.5	54.9	50.3
SMRC1_MOUSE	Smarcc1 p-S327 p-S329 (z= 3)	94.5	18.2	100.0	93.1
SMRC1_MOUSE	Smarcc1 p-S327 p-T336 (z= 2)	100.0	52.1	61.7	37.0
SMRC2_MOUSE	Smarcc2 p-S302 p-S306 (z= 2)	100.0	66.8	65.3	51.4
SMRCD_MOUSE	Smarcad1 p-S124 p-S127 (z= 3)	73.1	75.6	78.5	100.0
SMRCD_MOUSE	Smarcad1 p-S144 p-S145 (z= 3)	100.0	91.2	86.8	65.6
SMTN_MOUSE	Smtn p-S304 (z= 2)	100.0	93.4	94.1	83.2
SMTN_MOUSE	Smtn p-T440 (z= 3)	76.1	100.0	89.8	85.5
SN_MOUSE	Siglec1 p-S1331 p-S1335 p-Y1340 (z= 4)	2.1	0.0	1.6	100.0
SNAG_MOUSE	SNAG p-Y19 (z= 2)	49.0	100.0	86.7	97.0
SNIP1_MOUSE	Snip1 p-S18 (z= 3)	100.0	99.5	50.8	42.7
SNP23_MOUSE	Snap23 p-S110 (z= 2)	52.6	100.0	38.3	42.7
SNR27_MOUSE	Snrnp27 p-S59 p-S61 p-S63 (z= 3)	87.1	6.7	100.0	21.6
SNTB2_MOUSE	Sntb2 p-S75 (z= 2)	65.2	87.1	100.0	78.7
SNTB2_MOUSE	Sntb2 p-S88 (z= 3)	70.7	87.1	100.0	79.9
SNTB2_MOUSE	Sntb2 p-S90 (z= 2)	79.5	100.0	73.3	92.8
SNW1_MOUSE	Snw1 p-S224 p-S232 (z= 2)	89.0	66.1	89.5	100.0
SNW1_MOUSE	Snw1 p-S224 p-S232 (z= 2)	71.1	56.0	100.0	30.3
SNW1_MOUSE	Snw1 p-S224 p-S234 (z= 3)	82.2	57.0	100.0	92.0
SNW1_MOUSE	Snw1 p-S224 p-S234 (z= 3)	83.9	100.0	94.2	80.9
SON_MOUSE	Son p-S2027 p-S2029 p-S2031 (z= 3)	100.0	78.8	84.7	76.8
SOX6_MOUSE	Sox6 p-S527 p-S534 p-T538 (z= 4) + Oxi	4.7	100.0	58.0	74.7
SPA3A_MOUSE	Serpina3a p-Y400 (z= 4) + Oxi	100.0	96.2	87.7	84.6
SPAG5_MOUSE	Spag5 p-S229 (z= 3)	73.7	100.0	84.8	58.8
SPD2B_MOUSE	Sh3pxd2b p-S291 (z= 3)	83.6	95.1	92.9	100.0
SPDLY_MOUSE	Ccdc99 p-S558 (z= 3)	91.0	93.9	100.0	94.2
SPF45_MOUSE	Rbm17 p-S155 (z= 2)	85.4	100.0	92.0	70.6
SPF45_MOUSE	Rbm17 p-S155 (z= 3)	77.7	100.0	93.8	88.4
SPF45_MOUSE	Rbm17 p-S222 (z= 3)	65.0	83.0	93.3	100.0
SPT5H_MOUSE	Supt5h p-S664 (z= 2)	96.3	96.7	100.0	85.4

SPT5H_MOUSE	Supt5h p-T1028 (z= 3)	100.0	59.9	60.5	47.2
SPTB2_MOUSE	Sptbn1 p-S2102 (z= 2)	72.1	100.0	78.1	92.4
SPTB2_MOUSE	Sptbn1 p-S2102 (z= 3)	85.0	99.3	100.0	95.0
SPTB2_MOUSE	Sptbn1 p-S2163 p-S2164 p-S2168 (z= 2)	100.0	59.9	57.3	81.1
SPTB2_MOUSE	Sptbn1 p-S2163 p-S2164 p-S2168 (z= 3)	97.1	100.0	76.2	82.6
SQSTM_MOUSE	Sqstm1 p-S345 (z= 3)	72.7	92.1	100.0	69.3
SRBS2_MOUSE	Sorbs2 p-S27 (z= 2)	100.0	70.9	72.5	70.8
SRBS2_MOUSE	Sorbs2 p-S339 (z= 2)	62.8	87.8	100.0	76.8
SRBS2_MOUSE	Sorbs2 p-S339 (z= 3)	90.5	90.7	100.0	71.7
SRBS2_MOUSE	Sorbs2 p-S382 (z= 2)	100.0	81.5	52.8	89.5
SRC8_MOUSE	Cttn p-T401 p-S405 p-S417 (z= 4)	54.4	75.6	87.2	100.0
SRC8_MOUSE	Cttn p-T401 p-S405 p-S417 (z= 4)	100.0	87.1	99.2	93.3
SRC8_MOUSE	Cttn p-T401 p-S405 p-Y421 (z= 3)	59.3	82.9	94.4	100.0
SRC8_MOUSE	Cttn p-T401 p-S405 p-Y421 (z= 4)	100.0	86.3	96.4	98.3
SRC8_MOUSE	Cttn p-T401 p-S407 p-S417 (z= 3)	59.3	82.9	94.4	100.0
SRC8_MOUSE	Cttn p-T401 p-S417 (z= 4)	68.1	80.9	95.2	100.0
SRC8_MOUSE	Cttn p-T401 p-S418 (z= 3)	71.1	84.8	100.0	94.6
SRC8_MOUSE	Cttn p-T401 p-S418 (z= 4)	100.0	76.0	79.7	73.2
SRC8_MOUSE	Cttn p-T401 p-Y421 (z= 4)	68.3	81.3	94.8	100.0
SREK1_MOUSE	Srek1 p-S377 (z= 2)	100.0	45.5	98.3	92.5
SREK1_MOUSE	Srek1 p-S465 (z= 2)	80.4	36.2	100.0	35.1
SRF_MOUSE	Srf p-S220 (z= 3)	100.0	71.0	85.2	63.6
SRPK1_MOUSE	Srpk1 p-S51 (z= 3)	85.4	82.1	100.0	91.8
SRPR_MOUSE	Srpr p-S295 p-S296 p-S297 (z= 3)	92.3	99.5	90.1	100.0
SRRM1_MOUSE	Srrm1 p-S220 (z= 3)	100.0	99.0	76.5	82.2
SRRM1_MOUSE	Srrm1 p-S260 (z= 3)	100.0	59.9	70.3	54.0
SRRM1_MOUSE	Srrm1 p-S260 (z= 3)	67.0	100.0	80.0	63.6
SRRM1_MOUSE	Srrm1 p-S265 (z= 4)	100.0	78.6	76.3	50.0
SRRM1_MOUSE	Srrm1 p-S387 p-S389 p-S391 (z= 2)	57.5	100.0	47.3	53.0
SRRM1_MOUSE	Srrm1 p-S387 p-S391 (z= 2)	100.0	95.3	79.2	89.5
SRRM1_MOUSE	Srrm1 p-S389 p-S391 (z= 3)	100.0	87.1	67.7	95.8
SRRM1_MOUSE	Srrm1 p-S400 p-T404 (z= 2)	87.4	72.9	100.0	76.3
SRRM1_MOUSE	Srrm1 p-S401 p-T404 (z= 3)	89.2	78.2	100.0	79.5
SRRM1_MOUSE	Srrm1 p-S427 p-S429 (z= 2)	100.0	87.2	75.2	98.9
SRRM1_MOUSE	Srrm1 p-S429 (z= 2)	87.3	100.0	60.3	72.3
SRRM1_MOUSE	Srrm1 p-S448 (z= 2)	100.0	62.2	68.5	41.8
SRRM1_MOUSE	Srrm1 p-S448 (z= 3)	100.0	61.6	70.7	45.5
SRRM1_MOUSE	Srrm1 p-S461 p-S463 (z= 2)	99.8	92.5	100.0	97.9
SRRM1_MOUSE	Srrm1 p-S461 p-S463 (z= 3)	97.1	85.7	89.1	100.0
SRRM1_MOUSE	Srrm1 p-S469 (z= 3)	100.0	88.7	79.0	57.6
SRRM1_MOUSE	Srrm1 p-S522 p-S524 p-S526 (z= 3)	80.8	0.0	100.0	16.0
SRRM1_MOUSE	Srrm1 p-S561 p-S563 (z= 2)	100.0	98.2	56.2	44.9
SRRM1_MOUSE	Srrm1 p-S561 p-S563 (z= 3)	61.3	89.8	71.5	100.0
SRRM1_MOUSE	Srrm1 p-S572 p-S574 (z= 2)	67.3	3.7	100.0	51.5
SRRM1_MOUSE	Srrm1 p-S572 p-S574 (z= 3)	100.0	68.0	90.2	89.0
SRRM1_MOUSE	Srrm1 p-S572 p-S574 (z= 3)	100.0	94.6	99.1	92.5
SRRM1_MOUSE	Srrm1 p-S572 p-S574 (z= 4)	98.9	69.4	100.0	88.4
SRRM1_MOUSE	Srrm1 p-S616 (z= 2)	100.0	89.0	81.1	78.0
SRRM1_MOUSE	Srrm1 p-S624 p-S626 (z= 2)	59.9	100.0	45.3	37.8
SRRM1_MOUSE	Srrm1 p-S635 (z= 3)	100.0	97.4	90.7	81.3
SRRM1_MOUSE	Srrm1 p-S645 p-S647 (z= 2)	77.0	60.2	97.4	100.0
SRRM1_MOUSE	Srrm1 p-S645 p-S647 (z= 2)	100.0	75.0	64.7	68.6
SRRM1_MOUSE	Srrm1 p-S645 p-S647 (z= 3)	70.9	55.9	100.0	82.0
SRRM1_MOUSE	Srrm1 p-S655 p-S657 (z= 3)	52.4	64.3	100.0	49.1
SRRM1_MOUSE	Srrm1 p-S664 (z= 2)	76.6	100.0	45.3	43.1
SRRM1_MOUSE	Srrm1 p-S713 (z= 2)	76.1	37.0	100.0	67.8
SRRM1_MOUSE	Srrm1 p-S713 (z= 3)	100.0	44.1	48.3	18.8
SRRM1_MOUSE	Srrm1 p-S723 p-S725 (z= 2)	100.0	7.4	96.2	38.0
SRRM1_MOUSE	Srrm1 p-S723 p-S725 p-S731 (z= 3)	90.8	66.0	49.8	100.0
SRRM1_MOUSE	Srrm1 p-S723 p-S725 p-S731 (z= 3)	100.0	63.6	91.1	86.4
SRRM1_MOUSE	Srrm1 p-S723 p-S725 p-S731 (z= 3)	100.0	55.6	65.4	65.4
SRRM1_MOUSE	Srrm1 p-S743 p-T745 (z= 3)	100.0	67.3	65.4	65.3
SRRM1_MOUSE	Srrm1 p-S779 (z= 2)	83.6	59.3	100.0	33.1
SRRM1_MOUSE	Srrm1 p-S779 p-S781 (z= 2)	63.2	68.9	100.0	0.0
SRRM1_MOUSE	Srrm1 p-S810 p-S814 p-S822 (z= 3)	67.2	82.9	100.0	84.9
SRRM1_MOUSE	Srrm1 p-S810 p-S816 (z= 3)	75.4	65.3	83.7	100.0
SRRM1_MOUSE	Srrm1 p-S810 p-S816 (z= 3)	98.1	81.1	100.0	80.3
SRRM1_MOUSE	Srrm1 p-S810 p-T819 p-S822 (z= 3)	85.4	75.4	99.5	100.0
SRRM1_MOUSE	Srrm1 p-S832 p-T834 p-S838 (z= 3)	90.6	61.6	44.9	100.0
SRRM1_MOUSE	Srrm1 p-S915 (z= 2)	100.0	79.7	80.1	80.0
SRRM1_MOUSE	Srrm1 p-T600 p-S602 (z= 2)	100.0	69.5	79.5	93.4
SRRM1_MOUSE	Srrm1 p-T600 p-S602 (z= 3)	100.0	78.5	73.7	53.4
SRRM1_MOUSE	Srrm1 p-T633 (z= 2)	100.0	28.0	39.2	1.8
SRRM1_MOUSE	Srrm1 p-T633 p-S635 (z= 2)	49.8	100.0	35.8	77.8
SRRM1_MOUSE	Srrm1 p-T913 (z= 3)	100.0	90.7	94.2	98.3
SRRM1_MOUSE	Srrm1 p-T913 p-S915 (z= 2)	100.0	79.2	84.1	84.6
SRRM1_MOUSE	Srrm1 p-T913 p-S915 (z= 3)	95.1	86.8	100.0	85.7
SRRM1_MOUSE	Srrm1 p-Y623 p-S624 (z= 3)	61.5	100.0	43.1	65.8
SRRM2_MOUSE	Srrm2 p-S1067 (z= 2)	69.1	98.6	100.0	87.7
SRRM2_MOUSE	Srrm2 p-S1068 p-T1074 (z= 2)	100.0	64.6	68.3	59.8
SRRM2_MOUSE	Srrm2 p-S1097 (z= 2)	100.0	27.1	41.4	12.6
SRRM2_MOUSE	Srrm2 p-S1097 (z= 2)	100.0	98.7	57.8	83.3
SRRM2_MOUSE	Srrm2 p-S1097 (z= 3)	100.0	56.7	69.5	48.4
SRRM2_MOUSE	Srrm2 p-S1151 (z= 3)	100.0	84.6	77.8	53.0
SRRM2_MOUSE	Srrm2 p-S1179 (z= 2)	82.4	64.7	100.0	50.7
SRRM2_MOUSE	Srrm2 p-S1214 (z= 3)	43.6	83.8	97.9	100.0
SRRM2_MOUSE	Srrm2 p-S1214 (z= 4)	76.9	100.0	78.7	80.0
SRRM2_MOUSE	Srrm2 p-S1216 (z= 3)	100.0	97.5	86.2	85.2
SRRM2_MOUSE	Srrm2 p-S1229 p-S1230 (z= 3)	67.6	85.9	100.0	67.2
SRRM2_MOUSE	Srrm2 p-S1229 p-S1230 (z= 3) + Oxi	79.7	74.9	84.3	100.0
SRRM2_MOUSE	Srrm2 p-S1229 p-S1230 (z= 4)	64.2	77.9	100.0	61.8
SRRM2_MOUSE	Srrm2 p-S1269 (z= 2)	95.7	83.3	100.0	78.2
SRRM2_MOUSE	Srrm2 p-S1269 (z= 3)	88.2	79.5	100.0	89.5
SRRM2_MOUSE	Srrm2 p-S1334 p-S1335 (z= 3)	100.0	27.8	46.7	36.8
SRRM2_MOUSE	Srrm2 p-S1338 (z= 3)	76.4	100.0	99.6	81.9
SRRM2_MOUSE	Srrm2 p-S1338 p-S1339 (z= 2)	89.4	100.0	71.3	86.1
SRRM2_MOUSE	Srrm2 p-S1338 p-S1339 p-S1343 (z= 2)	81.2	100.0	83.9	81.8
SRRM2_MOUSE	Srrm2 p-S1340 (z= 2)	100.0	58.8	92.9	69.2

SRRM2_MOUSE	Srrm2 p-S1360 (z= 2)	62.1	100.0	38.0	57.0
SRRM2_MOUSE	Srrm2 p-S1360 p-S1372 (z= 3)	100.0	52.5	51.5	47.5
SRRM2_MOUSE	Srrm2 p-S1400 (z= 2)	100.0	66.0	92.1	57.7
SRRM2_MOUSE	Srrm2 p-S1434 p-S1438 p-S1439 (z= 2)	22.6	100.0	38.8	42.1
SRRM2_MOUSE	Srrm2 p-S1438 p-S1439 (z= 2)	100.0	62.3	10.4	0.0
SRRM2_MOUSE	Srrm2 p-S1453 p-S1455 p-S1458 (z= 2)	35.7	88.2	100.0	75.5
SRRM2_MOUSE	Srrm2 p-S1453 p-S1457 p-S1458 (z= 3)	80.3	50.7	100.0	20.5
SRRM2_MOUSE	Srrm2 p-S1535 p-S1537 p-S1538 (z= 2)	82.0	0.0	100.0	84.2
SRRM2_MOUSE	Srrm2 p-S1572 p-T1574 p-S1576 (z= 2)	74.1	58.7	100.0	99.0
SRRM2_MOUSE	Srrm2 p-S1572 p-T1574 p-S1576 (z= 3)	79.2	43.2	92.0	100.0
SRRM2_MOUSE	Srrm2 p-S1646 p-S1650 (z= 3)	100.0	82.6	98.0	76.6
SRRM2_MOUSE	Srrm2 p-S1703 p-S1705 p-S1706 (z= 3)	100.0	64.2	20.6	81.1
SRRM2_MOUSE	Srrm2 p-S1798 p-T1800 (z= 2)	64.7	65.6	100.0	75.4
SRRM2_MOUSE	Srrm2 p-S1798 p-T1800 (z= 3)	100.0	66.5	66.9	48.1
SRRM2_MOUSE	Srrm2 p-S1820 p-S1822 p-S1825 (z= 3)	100.0	61.3	87.2	64.2
SRRM2_MOUSE	Srrm2 p-S1834 p-T1836 (z= 2)	44.7	1.1	100.0	81.0
SRRM2_MOUSE	Srrm2 p-S1846 p-S1849 (z= 2)	27.7	100.0	21.7	79.3
SRRM2_MOUSE	Srrm2 p-S1849 (z= 2)	99.9	45.8	100.0	61.2
SRRM2_MOUSE	Srrm2 p-S1864 p-S1866 p-S1869 (z= 3)	100.0	58.2	49.8	64.1
SRRM2_MOUSE	Srrm2 p-S1866 p-S1869 (z= 2)	73.4	7.6	100.0	9.7
SRRM2_MOUSE	Srrm2 p-S1876 p-S1878 p-T1880 (z= 2)	74.9	66.3	59.1	100.0
SRRM2_MOUSE	Srrm2 p-S1876 p-S1878 p-T1880 (z= 3)	64.0	100.0	41.1	51.2
SRRM2_MOUSE	Srrm2 p-S1878 p-T1880 (z= 2)	71.3	50.7	100.0	71.5
SRRM2_MOUSE	Srrm2 p-S1924 p-S1927 (z= 2)	91.9	63.5	37.2	100.0
SRRM2_MOUSE	Srrm2 p-S1972 p-T1974 (z= 2)	84.6	100.0	70.3	83.8
SRRM2_MOUSE	Srrm2 p-S1982 p-S1984 p-T1986 (z= 3)	100.0	70.7	91.4	87.6
SRRM2_MOUSE	Srrm2 p-S1984 p-T1986 (z= 2)	100.0	78.0	69.0	80.2
SRRM2_MOUSE	Srrm2 p-S1996 p-S1998 (z= 2)	86.3	100.0	47.1	48.8
SRRM2_MOUSE	Srrm2 p-S2019 p-T2021 p-S2023 (z= 2)	82.7	49.2	89.0	100.0
SRRM2_MOUSE	Srrm2 p-S2019 p-T2021 p-S2023 (z= 3)	66.1	47.7	100.0	88.4
SRRM2_MOUSE	Srrm2 p-S2052 p-S2054 p-T2056 (z= 2)	88.1	74.6	99.5	100.0
SRRM2_MOUSE	Srrm2 p-S2052 p-S2054 p-T2056 (z= 3)	90.6	100.0	73.9	99.8
SRRM2_MOUSE	Srrm2 p-S2052 p-T2056 (z= 3)	100.0	75.6	81.4	70.7
SRRM2_MOUSE	Srrm2 p-S2070 (z= 3) + Oxi	100.0	63.7	59.2	74.7
SRRM2_MOUSE	Srrm2 p-S2073 (z= 3)	94.3	76.9	100.0	71.5
SRRM2_MOUSE	Srrm2 p-S2073 p-S2075 (z= 2)	100.0	69.5	80.9	69.9
SRRM2_MOUSE	Srrm2 p-S2084 (z= 2)	82.9	94.6	98.3	100.0
SRRM2_MOUSE	Srrm2 p-S2084 (z= 2)	98.1	52.4	100.0	33.7
SRRM2_MOUSE	Srrm2 p-S2084 (z= 2) + Oxi	100.0	64.5	58.5	59.9
SRRM2_MOUSE	Srrm2 p-S2224 (z= 2)	19.1	0.0	100.0	43.0
SRRM2_MOUSE	Srrm2 p-S2224 (z= 3)	94.3	86.1	100.0	94.1
SRRM2_MOUSE	Srrm2 p-S2335 (z= 2)	88.4	77.8	100.0	80.6
SRRM2_MOUSE	Srrm2 p-S2351 (z= 2)	100.0	92.4	99.6	76.8
SRRM2_MOUSE	Srrm2 p-S2351 (z= 2) + Oxi	100.0	79.6	58.9	56.1
SRRM2_MOUSE	Srrm2 p-S2360 p-T2362 (z= 2)	53.9	100.0	33.7	53.0
SRRM2_MOUSE	Srrm2 p-S2404 (z= 2)	83.4	100.0	69.0	87.8
SRRM2_MOUSE	Srrm2 p-S2404 (z= 4)	100.0	97.6	69.4	61.3
SRRM2_MOUSE	Srrm2 p-S2404 (z= 4) + Oxi	100.0	83.9	55.9	79.3
SRRM2_MOUSE	Srrm2 p-S2535 (z= 2)	100.0	96.2	51.1	36.2
SRRM2_MOUSE	Srrm2 p-S2642 (z= 3)	86.2	100.0	95.4	60.4
SRRM2_MOUSE	Srrm2 p-S2644 p-S2646 p-S2648 (z= 2)	93.0	59.5	73.9	100.0
SRRM2_MOUSE	Srrm2 p-S2648 (z= 2)	100.0	58.6	84.8	68.0
SRRM2_MOUSE	Srrm2 p-S2656 (z= 2)	95.3	95.3	93.3	100.0
SRRM2_MOUSE	Srrm2 p-S2656 p-S2660 (z= 2)	86.1	57.8	0.0	100.0
SRRM2_MOUSE	Srrm2 p-S351 (z= 3)	100.0	97.1	68.1	57.3
SRRM2_MOUSE	Srrm2 p-S433 p-S434 p-S435 (z= 2)	91.4	100.0	62.7	28.6
SRRM2_MOUSE	Srrm2 p-S433 p-S434 p-S435 (z= 3)	50.8	0.0	100.0	36.9
SRRM2_MOUSE	Srrm2 p-S433 p-S434 p-S435 (z= 3)	100.0	64.0	84.6	79.2
SRRM2_MOUSE	Srrm2 p-S433 p-S434 p-S447 (z= 2)	100.0	66.7	76.5	75.1
SRRM2_MOUSE	Srrm2 p-S433 p-S435 (z= 3)	100.0	96.3	82.9	89.9
SRRM2_MOUSE	Srrm2 p-S453 p-S454 (z= 2)	72.6	41.6	100.0	56.3
SRRM2_MOUSE	Srrm2 p-S757 p-S759 p-S761 (z= 2)	95.4	0.0	73.6	100.0
SRRM2_MOUSE	Srrm2 p-S759 p-S761 p-S762 (z= 2)	100.0	40.4	47.1	92.6
SRRM2_MOUSE	Srrm2 p-S773 p-S775 p-S778 (z= 2)	93.1	63.9	100.0	91.7
SRRM2_MOUSE	Srrm2 p-S876 p-S882 (z= 2)	82.5	58.1	91.2	100.0
SRRM2_MOUSE	Srrm2 p-S982 (z= 3)	100.0	98.7	99.4	99.4
SRRM2_MOUSE	Srrm2 p-T1032 (z= 3)	87.1	75.7	100.0	58.2
SRRM2_MOUSE	Srrm2 p-T1153 (z= 2)	100.0	73.4	67.2	54.4
SRRM2_MOUSE	Srrm2 p-T1390 (z= 3)	100.0	67.7	59.3	16.5
SRRM2_MOUSE	Srrm2 p-T2689 p-S2691 (z= 2)	51.4	50.4	100.0	7.8
SRRM2_MOUSE	Srrm2 p-T2689 p-S2691 (z= 3)	100.0	61.8	78.1	70.5
SRRM2_MOUSE	Srrm2 p-T288 p-T328 (z= 4)	100.0	86.6	96.1	97.0
SRRM2_MOUSE	Srrm2 p-T823 (z= 3)	73.9	96.5	100.0	58.3
SRRM2_MOUSE	Srrm2 p-T823 p-T831 (z= 3)	70.5	88.0	66.8	100.0
SRRM2_MOUSE	Srrm2 p-T823 p-T841 (z= 3) + Oxi	95.0	100.0	39.5	64.0
SRRM2_MOUSE	Srrm2 p-T955 (z= 2)	55.2	100.0	94.7	86.2
SRRM2_MOUSE	Srrm2 p-T955 (z= 2) + Oxi	90.6	100.0	23.6	80.4
SRRM2_MOUSE	Srrm2 p-T973 (z= 2)	67.0	90.6	100.0	88.1
SRRM2_MOUSE	Srrm2 p-T973 p-S982 (z= 3) + Oxi	100.0	34.5	13.6	38.0
SRRM2_MOUSE	Srrm2 p-T973 p-S984 (z= 3)	97.8	91.5	100.0	85.7
SRRT_MOUSE	Srrt p-S492 (z= 2)	100.0	65.2	62.0	50.0
SRRT_MOUSE	Srrt p-S492 (z= 3)	83.5	69.9	100.0	73.5
SRRT_MOUSE	Srrt p-S74 (z= 2)	95.4	100.0	99.4	61.6
SRRT_MOUSE	Srrt p-S74 (z= 3)	85.0	100.0	95.2	66.3
SRRT_MOUSE	Srrt p-T543 (z= 3)	82.7	62.2	93.2	100.0
SRSF1_MOUSE	Srsf1 p-S199 p-S201 (z= 3)	90.1	100.0	51.0	81.4
SRSF1_MOUSE	Srsf1 p-S199 p-S201 p-S205 (z= 2)	53.2	44.4	68.6	100.0
SRSF1_MOUSE	Srsf1 p-S223 p-S225 p-S227 (z= 2)	84.9	70.1	79.5	100.0
SRSF2_MOUSE	Srsf2 p-S187 p-S189 p-S191 (z= 3)	66.3	38.5	100.0	75.8
SRSF2_MOUSE	Srsf2 p-S189 p-S191 (z= 2)	46.2	100.0	50.2	42.8
SRSF2_MOUSE	Srsf2 p-S206 p-S208 p-S212 (z= 2)	41.7	100.0	37.9	33.4
SRSF2_MOUSE	Srsf2 p-S206 p-S208 p-S212 (z= 2)	0.0	0.0	100.0	0.0
SRSF2_MOUSE	Srsf2 p-S26 (z= 2)	69.5	62.6	100.0	57.5
SRSF2_MOUSE	Srsf2 p-S26 (z= 3)	94.4	100.0	79.2	67.5
SRSF2_MOUSE	Srsf2 p-T25 (z= 2)	99.2	98.7	100.0	74.7
SRSF3_MOUSE	Srsf3 p-S108 (z= 2)	100.0	54.3	44.6	18.8
SRSF3_MOUSE	Srsf3 p-S108 (z= 2)	75.2	32.2	100.0	34.9
SRSF7_MOUSE	Srsf7 p-S202 p-S204 (z= 2)	73.9	53.6	100.0	65.6

SRSF7_MOUSE	Srsf7 p-S208 p-S210 (z= 2)	100.0	71.5	88.3	68.8
SRSF7_MOUSE	Srsf7 p-S208 p-S210 p-S212 (z= 2)	99.2	64.2	51.2	100.0
SRSF7_MOUSE	Srsf7 p-S210 (z= 2)	70.9	70.3	100.0	82.6
SRSF7_MOUSE	Srsf7 p-S210 p-S212 (z= 2)	86.1	26.4	100.0	86.5
SRSF7_MOUSE	Srsf7 p-S262 (z= 2)	66.7	100.0	78.8	51.9
SSF1_MOUSE	Ppan p-S362 (z= 3)	97.4	78.2	91.2	100.0
SSF1_MOUSE	Ppan p-T233 p-S240 (z= 3)	71.0	100.0	30.8	38.4
SSFA2_MOUSE	Ssfa2 p-S738 (z= 2)	100.0	86.3	97.5	70.5
SSFA2_MOUSE	SSFA2 p-T245 p-T246 p-Y255 (z= 4)	100.0	50.9	64.3	45.3
SSH3_MOUSE	Ssh3 p-S639 (z= 2)	100.0	82.3	65.3	89.0
SSRA_MOUSE	Ssr1 p-T260 (z= 3)	87.0	100.0	2.4	14.0
SSRP1_MOUSE	Ssrp1 p-S444 (z= 3)	87.1	78.7	81.6	100.0
STALP_MOUSE	Stambpl1 p-S242 (z= 2)	84.9	82.8	95.2	100.0
STIP1_MOUSE	Stip1 p-S481 (z= 2)	100.0	59.7	72.7	48.8
STK10_MOUSE	Stk10 p-S952 (z= 3)	85.9	100.0	67.9	76.3
STK3_MOUSE	Stk3 p-S316 (z= 3)	86.7	85.2	94.6	100.0
STK4_MOUSE	Stk4 p-S320 (z= 2)	84.6	86.3	100.0	90.5
STMN1_MOUSE	Stmn1 p-S16 (z= 3)	100.0	85.5	60.8	53.9
STMN1_MOUSE	Stmn1 p-S25 (z= 2)	100.0	38.2	63.9	44.0
STMN1_MOUSE	Stmn1 p-S25 (z= 3)	41.5	100.0	89.7	90.2
STMN1_MOUSE	Stmn1 p-S38 (z= 2)	77.6	100.0	94.8	92.1
STMN1_MOUSE	Stmn1 p-S38 (z= 2)	50.0	100.0	91.7	69.7
STRN3_MOUSE	Strn3 p-S257 (z= 3)	40.2	100.0	14.8	29.0
STRN3_MOUSE	Strn3 p-S257 (z= 3) + Oxi	22.1	100.0	52.8	9.4
STX4_MOUSE	Stx4 p-S15 (z= 2)	68.5	100.0	60.2	68.2
SURF6_MOUSE	Surf6 p-S97 (z= 2)	100.0	37.0	42.3	35.3
SVIL_MOUSE	Svil p-S227 (z= 2)	100.0	73.1	77.8	70.6
SVIL_MOUSE	Svil p-S227 (z= 3)	100.0	78.2	90.4	86.8
SVIL_MOUSE	Svil p-S50 (z= 3)	79.9	100.0	71.5	67.2
SVIL_MOUSE	Svil p-S960 (z= 3)	66.6	100.0	99.7	83.7
SYHM_MOUSE	Hars2 p-S66 (z= 2)	100.0	63.9	62.3	85.4
SYMC_MOUSE	Mars p-S827 (z= 3)	85.1	100.0	78.3	77.0
SYMC_MOUSE	Mars p-S827 (z= 4)	100.0	86.0	67.0	98.4
SYT7_MOUSE	Syt7 p-T98 p-T100 p-S102 (z= 4) + Oxi	93.8	81.2	86.7	100.0
SYTC_MOUSE	Tars p-S8 (z= 3)	100.0	24.2	28.0	6.5
SZRD1_MOUSE	Szrd1 p-S107 (z= 3)	100.0	99.1	95.4	81.6
T2FA_MOUSE	Gtf2f1 p-S217 p-S218 p-S221 (z= 3)	72.4	62.8	100.0	71.3
T2FA_MOUSE	Gtf2f1 p-S305 p-S307 p-S308 (z= 3)	89.8	75.4	60.9	100.0
T2FA_MOUSE	Gtf2f1 p-S385 p-T389 (z= 2)	59.6	100.0	55.3	72.0
TACC2_MOUSE	Tacc2 p-S510 p-S514 (z= 3)	55.2	100.0	49.0	84.7
TARA_MOUSE	Triobp p-S1604 (z= 3)	100.0	68.3	45.1	60.7
TB182_MOUSE	Tnks1bp1 p-S1063 (z= 2)	68.5	50.3	100.0	83.5
TB182_MOUSE	Tnks1bp1 p-S1063 (z= 3) + Oxi	100.0	76.5	50.8	81.9
TB182_MOUSE	Tnks1bp1 p-S1131 (z= 2)	93.2	58.1	100.0	89.8
TB182_MOUSE	Tnks1bp1 p-S1133 (z= 2)	80.7	80.6	100.0	67.8
TB182_MOUSE	Tnks1bp1 p-S1373 p-S1375 (z= 2)	88.1	84.7	94.1	100.0
TB182_MOUSE	Tnks1bp1 p-S1375 (z= 2)	82.5	98.6	97.8	100.0
TB182_MOUSE	Tnks1bp1 p-S1437 (z= 2)	70.8	100.0	90.1	89.3
TB182_MOUSE	Tnks1bp1 p-S1611 p-S1612 (z= 2)	33.2	0.0	100.0	44.8
TB182_MOUSE	Tnks1bp1 p-S1611 p-S1612 (z= 2)	100.0	86.4	70.5	78.7
TB182_MOUSE	Tnks1bp1 p-S1657 (z= 2)	88.3	100.0	83.2	96.9
TB182_MOUSE	Tnks1bp1 p-S298 (z= 2)	100.0	78.3	85.2	80.1
TB182_MOUSE	Tnks1bp1 p-S568 (z= 2)	59.4	100.0	84.3	71.5
TB182_MOUSE	Tnks1bp1 p-S568 (z= 3)	58.0	97.1	100.0	71.9
TB182_MOUSE	Tnks1bp1 p-S602 (z= 3)	100.0	92.2	96.0	85.9
TB182_MOUSE	Tnks1bp1 p-S692 (z= 2)	53.6	100.0	41.3	64.6
TB182_MOUSE	Tnks1bp1 p-S763 (z= 2)	100.0	65.0	50.0	56.8
TB182_MOUSE	Tnks1bp1 p-S763 (z= 2)	40.1	100.0	67.4	71.1
TB182_MOUSE	Tnks1bp1 p-T1065 (z= 3)	72.5	43.0	100.0	78.4
TB182_MOUSE	Tnks1bp1 p-T533 (z= 2)	97.7	100.0	75.0	60.3
TB182_MOUSE	Tnks1bp1 p-T533 p-S539 (z= 2)	42.2	100.0	53.7	79.5
TBC15_MOUSE	Tbc1d15 p-S623 (z= 4)	11.1	17.6	100.0	4.6
TBCB_MOUSE	Tbcb p-S110 (z= 2)	55.7	100.0	89.4	90.9
TBCD1_MOUSE	Tbc1d1 p-S231 (z= 2)	75.2	39.1	100.0	77.6
TCEA1_MOUSE	Tcea1 p-S100 (z= 2)	74.8	99.1	94.2	100.0
TCEA1_MOUSE	Tcea1 p-S100 (z= 3)	100.0	86.9	81.2	57.1
TCEA1_MOUSE	Tcea1 p-S97 (z= 2)	100.0	75.8	59.4	46.9
TCOF_MOUSE	Tcof1 p-S1128 (z= 2)	62.1	100.0	87.3	91.2
TCOF_MOUSE	Tcof1 p-S1191 (z= 2)	100.0	90.7	66.2	67.4
TCOF_MOUSE	Tcof1 p-S1216 (z= 2)	81.5	100.0	74.9	61.5
TCOF_MOUSE	Tcof1 p-S1216 (z= 2) + Oxi	100.0	18.8	32.7	1.5
TCOF_MOUSE	Tcof1 p-S1224 (z= 3)	96.5	100.0	93.9	80.1
TCOF_MOUSE	Tcof1 p-S1242 (z= 2)	95.9	85.6	100.0	69.9
TCOF_MOUSE	Tcof1 p-S151 (z= 3)	100.0	75.5	77.3	61.3
TCOF_MOUSE	Tcof1 p-S154 (z= 2)	100.0	55.4	60.4	44.5
TCOF_MOUSE	Tcof1 p-S169 (z= 3)	100.0	61.5	74.5	58.8
TCOF_MOUSE	Tcof1 p-S413 p-S414 p-S417 (z= 3)	91.0	100.0	62.2	66.0
TCOF_MOUSE	Tcof1 p-S413 p-S414 p-S417 (z= 4)	96.1	98.9	100.0	82.0
TCOF_MOUSE	Tcof1 p-S593 (z= 2)	100.0	85.1	72.8	83.8
TCOF_MOUSE	Tcof1 p-S794 (z= 2)	62.4	100.0	74.4	33.6
TCOF_MOUSE	Tcof1 p-S794 (z= 3)	87.4	43.8	100.0	45.7
TCOF_MOUSE	Tcof1 p-S83 p-S84 p-S85 (z= 3)	65.2	36.1	100.0	62.2
TCOF_MOUSE	Tcof1 p-T171 (z= 3)	97.6	92.2	96.0	100.0
TE2IP_MOUSE	Terf2ip p-S200 (z= 2)	100.0	86.3	83.4	92.9
TEBP_MOUSE	Ptges3 p-S113 (z= 2)	68.4	82.7	100.0	99.9
TEBP_MOUSE	Ptges3 p-S113 (z= 2) + Oxi	90.6	83.1	87.2	100.0
TEBP_MOUSE	Ptges3 p-S113 (z= 3)	63.5	96.3	100.0	99.1
TEBP_MOUSE	Ptges3 p-S148 (z= 3)	72.7	76.1	100.0	81.1
TEBP_MOUSE	Ptges3 p-S148 p-S151 (z= 3)	73.4	54.3	100.0	86.9
TEBP_MOUSE	Ptges3 p-S148 p-S151 (z= 3) + Oxi	57.3	100.0	42.9	92.3
TEBP_MOUSE	Ptges3 p-S148 p-S151 (z= 3) + Oxi	76.7	83.3	47.1	100.0
TENS3_MOUSE	Tns3 p-S1436 (z= 2) + Oxi	100.0	17.7	61.7	0.0
TENS3_MOUSE	Tns3 p-Y773 (z= 3)	75.5	90.3	89.8	100.0
TF3B_MOUSE	Brf1 p-S586 p-T587 (z= 3)	81.4	100.0	61.3	31.4
TFDP1_MOUSE	Tfdp1 p-S23 (z= 2)	54.1	83.2	82.9	100.0
TFE3_MOUSE	Tfe3 p-S553 (z= 2)	100.0	94.0	97.4	91.0
TFP11_MOUSE	Tfip11 p-S96 p-S99 (z= 3)	100.0	86.3	81.6	67.2
TFPT_MOUSE	Tfpt p-S180 (z= 2)	100.0	96.7	57.6	53.4

TGFR2_MOUSE	Tgfbr2 p-S377 (z= 2)	70.0	100.0	66.7	79.8
THUM1_MOUSE	Thumpd1 p-S86 p-S88 (z= 3)	80.7	100.0	42.3	71.8
THUM1_MOUSE	Thumpd1 p-S86 p-S88 (z= 4)	84.7	100.0	91.5	75.9
TIAM1_MOUSE	Tiam1 p-S231 (z= 2)	100.0	23.6	84.1	33.1
TIF1B_MOUSE	Trim28 p-S471 (z= 3)	76.8	98.8	100.0	98.3
TIF1B_MOUSE	Trim28 p-S473 (z= 2)	68.8	100.0	58.2	61.6
TIF1B_MOUSE	Trim28 p-S51 (z= 4)	89.6	100.0	62.6	80.8
TIF1B_MOUSE	Trim28 p-S601 (z= 3)	100.0	82.7	83.9	82.4
TIF1B_MOUSE	Trim28 p-T599 (z= 4)	86.3	100.0	90.5	86.4
TIM_MOUSE	Timeless p-S1165 (z= 2)	100.0	84.7	68.5	86.8
TISB_MOUSE	Zfp36l1 p-S334 (z= 2)	66.2	100.0	75.1	96.7
TISD_MOUSE	Zfp36l2 p-S98 (z= 2)	100.0	66.9	65.8	53.6
TJAP1_MOUSE	Tjap1 p-S527 (z= 2)	89.9	91.9	93.0	100.0
TLE3_MOUSE	Tle3 p-S286 (z= 2)	90.4	75.6	100.0	85.1
TLN1_MOUSE	Tln1 p-S1328 (z= 2)	89.6	93.8	100.0	74.0
TM10A_MOUSE	Trmt10a p-S22 (z= 2)	73.9	11.4	100.0	67.9
TM10A_MOUSE	Trmt10a p-S22 (z= 2)	35.8	100.0	84.3	70.8
TM10A_MOUSE	Trmt10a p-S22 (z= 3)	74.3	13.8	100.0	73.8
TM87A_MOUSE	Tmem87a p-S540 (z= 3)	52.5	96.1	95.3	100.0
TMM47_MOUSE	Tmem47 p-S3 (z= 3) + Oxi	90.8	96.6	67.4	100.0
TNAP3_MOUSE	Tnfaip3 p-S571 (z= 2)	100.0	0.0	0.0	0.0
TOM1_MOUSE	Tom1 p-S462 (z= 3)	73.0	74.0	100.0	53.9
TOM34_MOUSE	Tomm34 p-S186 (z= 2)	100.0	91.9	51.0	37.1
TOM70_MOUSE	Tomm70a p-S94 (z= 3)	96.5	100.0	76.9	86.7
TOP2A_MOUSE	Top2a p-S1328 p-S1333 p-T1350 (z= 3)	2.4	2.6	2.7	100.0
TOP2A_MOUSE	Top2a p-S28 (z= 2)	62.7	100.0	68.4	63.6
TOP2B_MOUSE	Top2b p-S1568 (z= 3)	29.4	98.0	14.4	100.0
TOX4_MOUSE	Tox4 p-S178 p-S182 (z= 3)	52.1	87.2	62.5	100.0
TP53B_MOUSE	Tp53bp1 p-S1090 (z= 3)	85.2	97.7	100.0	96.5
TP53B_MOUSE	Tp53bp1 p-S1103 (z= 3)	83.1	100.0	64.9	55.9
TP53B_MOUSE	Tp53bp1 p-S1347 (z= 2)	90.7	100.0	89.8	61.0
TP53B_MOUSE	Tp53bp1 p-S262 (z= 2)	100.0	62.5	58.7	45.5
TP53B_MOUSE	Tp53bp1 p-S262 (z= 3)	90.5	99.0	100.0	68.3
TP53B_MOUSE	Tp53bp1 p-S376 (z= 2)	80.8	93.0	94.9	100.0
TP53B_MOUSE	Tp53bp1 p-S527 (z= 2) + Oxi	100.0	10.6	0.0	11.2
TPD54_MOUSE	Tpd52l2 p-S180 (z= 2)	100.0	42.8	39.1	52.2
TPD54_MOUSE	Tpd52l2 p-S180 (z= 3)	100.0	48.5	55.4	73.9
TPX2_MOUSE	Tpx2 p-S486 (z= 2)	67.2	94.3	82.4	100.0
TPX2_MOUSE	Tpx2 p-S737 (z= 2)	93.3	92.3	78.1	100.0
TR150_MOUSE	Thrap3 p-S243 (z= 2)	100.0	91.8	84.8	80.1
TR150_MOUSE	Thrap3 p-S248 p-S253 (z= 2)	100.0	74.7	77.8	79.2
TR150_MOUSE	Thrap3 p-S379 (z= 2)	67.6	100.0	43.3	39.0
TR150_MOUSE	Thrap3 p-S572 (z= 3)	74.0	100.0	93.8	53.6
TR150_MOUSE	Thrap3 p-S669 (z= 3)	100.0	31.3	38.4	7.5
TR150_MOUSE	Thrap3 p-S679 (z= 2)	87.8	97.4	93.0	100.0
TR150_MOUSE	Thrap3 p-S679 (z= 2)	100.0	82.2	91.5	82.8
TR150_MOUSE	Thrap3 p-S679 (z= 3)	100.0	50.6	43.9	31.8
TRA2A_MOUSE	Tra2a p-S259 p-S261 (z= 2)	100.0	81.9	81.2	94.8
TRA2A_MOUSE	Tra2a p-S259 p-S261 (z= 3)	85.1	100.0	91.0	76.8
TRA2A_MOUSE	Tra2a p-S271 p-S273 p-Y276 (z= 3)	72.7	55.5	87.4	100.0
TRA2A_MOUSE	Tra2a p-S271 p-S275 p-Y276 (z= 2)	70.4	32.5	51.5	100.0
TRA2A_MOUSE	Tra2a p-S94 p-S96 p-S98 (z= 2)	93.1	100.0	39.7	31.1
TRA2A_MOUSE	Tra2a p-S94 p-S96 p-S98 (z= 3)	59.9	100.0	52.3	35.6
TRA2A_MOUSE	Tra2a p-S96 p-S98 (z= 2)	95.3	32.5	48.5	100.0
TRI47_MOUSE	Trim47 p-S591 (z= 2)	76.1	90.0	88.7	100.0
TRI56_MOUSE	Trim56 p-S7 (z= 2)	100.0	70.7	65.2	59.7
TRIO_MOUSE	Trio p-S14 (z= 4) + Oxi	67.2	76.9	100.0	71.5
TRIO_MOUSE	Trio p-S2319 p-S2320 p-S2323 (z= 4)	100.0	75.6	74.8	79.0
TRIPC_MOUSE	Trip12 p-S312 (z= 2)	100.0	99.2	89.3	99.3
TRPS1_MOUSE	Trps1 p-S1081 p-S1085 (z= 2)	79.9	89.1	100.0	82.9
TRPS1_MOUSE	Trps1 p-S178 (z= 3)	72.6	94.6	67.7	100.0
TRPS1_MOUSE	Trps1 p-Y1080 p-S1085 (z= 2)	96.6	52.8	97.6	100.0
TTI2_MOUSE	Tti2 p-S41 p-S42 (z= 3)	57.3	100.0	73.4	61.6
TTPAL_MOUSE	Ttpal p-S196 p-S199 (z= 4)	0.3	100.0	0.2	0.4
TXLNA_MOUSE	Txlna p-S523 (z= 2)	54.8	0.0	100.0	41.7
TYDP1_MOUSE	Tdp1 p-S61 (z= 3)	96.2	1.2	100.0	76.1
TYDP1_MOUSE	TYDP1 p-S136 (z= 3)	90.3	100.0	92.1	74.8
U3IP2_MOUSE	Rrp9 p-S470 (z= 2)	100.0	89.5	92.3	81.2
U3IP2_MOUSE	Rrp9 p-S50 p-S51 p-S53 (z= 2)	70.7	0.3	100.0	46.9
U5S1_MOUSE	Eftud2 p-Y6 p-S19 (z= 3)	52.1	0.7	100.0	50.6
UBA1_MOUSE	Uba1 p-S46 (z= 2)	85.3	89.5	93.4	100.0
UBAP2_MOUSE	Ubap2 p-S634 (z= 3)	100.0	81.6	64.8	65.8
UBAP2_MOUSE	Ubap2 p-T406 (z= 4)	72.6	70.4	100.0	85.8
UBL7_MOUSE	Ubl7 p-S230 (z= 3)	97.9	55.2	86.4	100.0
UBL7_MOUSE	Ubl7 p-S230 (z= 3) + Oxi	100.0	73.4	61.7	79.0
UBP10_MOUSE	Usp10 p-S208 (z= 2)	100.0	80.2	80.6	81.8
UBP10_MOUSE	Usp10 p-S208 (z= 3)	56.5	100.0	69.0	79.4
UBP10_MOUSE	Usp10 p-T566 (z= 3)	82.2	75.8	86.0	100.0
UBP15_MOUSE	Usp15 p-S229 (z= 2)	100.0	96.1	82.6	89.3
UBP2L_MOUSE	Ubap2l p-S491 (z= 3)	100.0	70.6	74.6	91.4
UBP2L_MOUSE	Ubap2l p-S624 p-S629 (z= 3)	100.0	90.0	81.4	75.5
UBP2L_MOUSE	Ubap2l p-S628 (z= 2)	68.4	100.0	89.6	73.3
UBP2L_MOUSE	Ubap2l p-S628 (z= 3)	100.0	61.3	68.6	63.5
UBP7_MOUSE	Usp7 p-S19 (z= 3)	78.1	94.1	100.0	97.2
UBP8_MOUSE	Usp8 p-S680 (z= 3)	100.0	96.1	88.4	99.9
UBQL1_MOUSE	Ubqln1 p-Y267 p-T268 p-S289 p-S292 (z= 4)	100.0	81.1	61.6	53.1
UBR4_MOUSE	Ubr4 p-S2716 (z= 2)	100.0	84.2	96.5	79.0
UBR5_MOUSE	Ubr5 p-S133 p-S148 (z= 4)	79.6	88.9	81.3	100.0
UBR5_MOUSE	Ubr5 p-S1543 (z= 2)	100.0	83.3	50.3	73.1
UBXN1_MOUSE	Ubxn1 p-S199 (z= 3)	89.2	88.7	100.0	75.2
UCK2_MOUSE	Uck2 p-S254 (z= 2)	47.8	100.0	23.1	10.4
UFD1_MOUSE	Ufd1l p-S247 (z= 2)	78.4	100.0	77.4	67.3
UIMC1_MOUSE	Uimc1 p-S665 (z= 2)	95.2	49.9	100.0	76.1
USO1_MOUSE	Uso1 p-S940 (z= 3)	86.2	79.2	92.4	100.0
USO1_MOUSE	Uso1 p-S940 (z= 4)	100.0	70.4	72.9	93.9
UTP15_MOUSE	Utp15 p-T515 (z= 4)	100.0	92.3	93.2	99.5
UTP15_MOUSE	Utp15 p-T519 (z= 3)	100.0	76.2	85.8	84.9
UTP18_MOUSE	Utp18 p-S114 p-S115 p-S118 (z= 2)	44.5	100.0	44.5	60.9

UTP18_MOUSE	Utp18 p-S201 p-S202 p-S206 (z= 3)	100.0	67.0	81.2	81.8
UVSSA_MOUSE	Uvssa p-S481 p-T488 (z= 4)	91.1	91.2	80.0	100.0
VGLL4_MOUSE	Vgll4 p-T52 p-S60 (z= 2)	90.1	97.3	100.0	84.1
VIGLN_MOUSE	Hdlbp p-S31 (z= 2)	100.0	77.3	95.3	97.0
VIME_MOUSE	Vim p-S419 (z= 2)	77.2	56.2	100.0	57.6
VIME_MOUSE	Vim p-S459 (z= 2)	100.0	88.9	81.1	84.0
VIME_MOUSE	Vim p-S56 (z= 2)	89.8	92.8	77.2	100.0
VINC_MOUSE	Vcl p-S290 (z= 2)	82.5	85.1	100.0	93.6
VINC_MOUSE	Vcl p-S290 (z= 3)	100.0	76.7	78.9	78.1
VINC_MOUSE	Vcl p-S346 (z= 2)	74.2	100.0	53.3	75.1
VINC_MOUSE	Vcl p-S721 (z= 2)	72.0	100.0	82.1	91.6
VINC_MOUSE	Vcl p-S820 (z= 2)	82.2	91.7	100.0	77.8
VINC_MOUSE	Vcl p-Y822 (z= 2)	62.2	91.7	100.0	75.5
VIR_MOUSE	Kiaa1429 p-S1578 (z= 2)	100.0	83.1	89.6	86.1
WAPL_MOUSE	Wapal p-S77 (z= 2)	100.0	90.1	94.6	97.7
WAPL_MOUSE	Wapal p-S77 (z= 3)	99.8	61.7	100.0	84.7
WDHD1_MOUSE	Wdhd1 p-S821 (z= 3)	100.0	78.8	75.9	74.5
WDR26_MOUSE	Wdr26 p-S101 (z= 2)	100.0	83.9	87.7	73.5
WDR70_MOUSE	Wdr70 p-S641 (z= 3)	89.2	85.8	75.0	100.0
WNK1_MOUSE	Wnk1 p-S2000 (z= 4)	100.0	2.9	2.8	0.0
WNK1_MOUSE	Wnk1 p-S378 (z= 2)	100.0	61.7	75.4	42.2
WRIP1_MOUSE	Wrnip1 p-S153 (z= 2)	88.5	100.0	90.8	69.1
WRIP1_MOUSE	Wrnip1 p-S65 (z= 2)	100.0	62.6	48.7	1.2
XPC_MOUSE	Xpc p-S875 p-S876 (z= 3)	90.6	72.5	20.6	100.0
XRCC1_MOUSE	Xrcc1 p-S445 p-T452 (z= 2)	57.3	100.0	53.0	78.4
XRN2_MOUSE	Xrn2 p-S499 p-S501 (z= 2)	47.5	0.0	100.0	40.7
XRN2_MOUSE	Xrn2 p-S499 p-S501 (z= 2)	80.3	66.3	90.7	100.0
XRN2_MOUSE	Xrn2 p-S499 p-S501 (z= 3)	100.0	90.0	92.0	99.6
XRN2_MOUSE	Xrn2 p-S499 p-S501 (z= 3)	100.0	81.1	70.2	60.7
YAP1_MOUSE	Yap1 p-S113 (z= 4)	100.0	80.4	66.5	55.4
YAP1_MOUSE	Yap1 p-S149 (z= 2)	87.5	45.9	100.0	83.6
YAP1_MOUSE	Yap1 p-S46 (z= 2)	43.4	100.0	20.9	65.9
YAP1_MOUSE	Yap1 p-T48 (z= 3)	0.0	0.0	100.0	0.0
YAP1_MOUSE	Yap1 p-T95 (z= 2)	100.0	54.5	33.8	20.1
YAP1_MOUSE	Yap1 p-T95 (z= 2)	100.0	75.4	85.2	71.4
YAP1_MOUSE	Yap1 p-T95 (z= 3)	100.0	74.7	75.2	60.8
YBOX1_MOUSE	Ybx1 p-S163 (z= 3)	70.5	100.0	44.0	58.0
YBOX1_MOUSE	Ybx1 p-S312 (z= 2)	98.0	70.4	59.8	100.0
YD021_MOUSE	Hjurp p-S553 (z= 2)	75.9	30.3	100.0	25.8
Z280D_MOUSE	Znf280d p-S901 p-S911 (z= 3)	96.1	87.6	83.4	100.0
ZC3H4_MOUSE	Zc3h4 p-S1270 p-S1276 (z= 3)	80.3	67.7	100.0	82.1
ZC3H4_MOUSE	Zc3h4 p-S1276 (z= 2)	34.0	100.0	44.5	50.7
ZC3H4_MOUSE	Zc3h4 p-T1101 p-T1119 (z= 3)	95.8	39.9	100.0	78.4
ZC3H6_MOUSE	Zc3h6 p-T100 p-S101 (z= 2)	35.8	100.0	13.5	56.0
ZC3HE_MOUSE	Zc3h14 p-S515 (z= 2)	85.2	100.0	77.5	74.8
ZCCHV_MOUSE	Zc3hav1 p-S324 (z= 2)	100.0	41.7	47.2	36.7
ZCCHV_MOUSE	Zc3hav1 p-S490 (z= 2)	48.2	87.2	100.0	95.0
ZCH18_MOUSE	Zc3h18 p-S33 (z= 3)	82.2	85.9	87.3	100.0
ZCH18_MOUSE	Zc3h18 p-S530 (z= 2)	88.2	100.0	57.6	78.2
ZCH18_MOUSE	Zc3h18 p-S863 (z= 2)	55.1	82.9	100.0	72.7
ZCH18_MOUSE	Zc3h18 p-S888 (z= 2)	99.8	92.1	100.0	78.4
ZCH18_MOUSE	Zc3h18 p-S92 (z= 3)	73.6	90.5	94.5	100.0
ZCH24_MOUSE	Zcchc24 p-S93 (z= 3)	100.0	99.1	40.7	55.3
ZFX_MOUSE	Zfx p-S269 (z= 3)	100.0	87.5	27.4	47.0
ZN276_MOUSE	Znf276 p-S21 (z= 3)	73.6	29.2	100.0	79.1
ZN382_MOUSE	Znf382 p-S30 (z= 3) + Oxi	96.2	95.2	93.7	100.0
ZN609_MOUSE	Znf609 p-S1057 (z= 2)	93.2	100.0	57.3	90.5
ZN638_MOUSE	Znf638 p-T407 p-S409 (z= 4) + Oxi	52.6	74.0	50.3	100.0
ZN740_MOUSE	Znf740 p-S19 (z= 2)	91.4	100.0	62.3	72.4
ZO1_MOUSE	Tjp1 p-S125 p-S131 (z= 3)	100.0	26.8	17.5	0.6
ZO1_MOUSE	Tjp1 p-S125 p-S131 (z= 4)	91.6	85.6	93.8	100.0
ZO1_MOUSE	Tjp1 p-S125 p-S131 (z= 4)	100.0	69.3	86.5	80.2
ZO1_MOUSE	Tjp1 p-S125 p-Y132 (z= 3)	100.0	61.3	79.4	39.3
ZO1_MOUSE	Tjp1 p-S1614 (z= 3)	96.6	95.9	88.4	100.0
ZO1_MOUSE	Tjp1 p-S175 p-S178 (z= 2)	100.0	95.7	41.8	37.7
ZO1_MOUSE	Tjp1 p-S175 p-S178 p-S179 (z= 2)	100.0	0.0	50.4	35.7
ZO1_MOUSE	Tjp1 p-S617 (z= 2)	100.0	56.3	46.9	27.6
ZO2_MOUSE	Tjp2 p-S107 (z= 3)	100.0	95.7	92.6	96.5
ZO2_MOUSE	Tjp2 p-S111 (z= 3)	62.6	85.2	83.7	100.0
ZO2_MOUSE	Tjp2 p-S111 (z= 4)	84.7	100.0	77.1	87.4
ZO2_MOUSE	Tjp2 p-S1136 (z= 2)	80.9	60.5	100.0	93.2
ZO2_MOUSE	Tjp2 p-S1136 (z= 3)	86.0	95.1	100.0	70.4
ZO2_MOUSE	Tjp2 p-S395 (z= 2)	100.0	93.7	91.7	88.2
ZO2_MOUSE	Tjp2 p-S947 (z= 2)	75.2	81.6	100.0	61.0
ZO2_MOUSE	Tjp2 p-S968 (z= 2)	84.6	100.0	91.2	85.0
ZO2_MOUSE	Tjp2 p-S968 (z= 3)	91.0	50.8	100.0	88.1
ZRAB2_MOUSE	Zranb2 p-S153 (z= 3)	19.6	37.6	100.0	34.3
ZYX_MOUSE	Zyx p-S336 (z= 2)	83.5	100.0	95.6	97.9