

New table numbers given, with old numbers in brackets.

Ch II S1 Table 1 (1): Example of sort performance metrics.

Purity %	Yield %	Orig %	-log(Y-)	log(Fe)
95	100	50	1.28	1.28
95	90	50	1.32	1.28
95	10	50	2.28	1.28
99	90	1	4.04	3.99
95.6	90	0.1	4.38	4.34

Ch II S2.2 Table 2 (1): Expected purities, yields and processing times for different starting cell concentrations

Total cells/mL	10^6	10^7	10^8
Purity in Yield Sort[%]	96	69	18
Yield in Purity Sort[%]	96	64	11
Time to process 10^9 cells	309	31	3:05

Ch III S1 Table 3 (1): The consequences of using positive and negative populations with differing autofluorescence.

	Fluorescence (MFI)		
	FITC	PE	SOV
Cells (+)	3,135	903	n/a
Cells (-)	95	78	27%
Beads (-)	107	228	22%

Ch III S2 Table 4 (1): Suggested maintenance intervals for different instrument components.

System	Component	Observation/ Read out	Reason/ Maintenance	Frequency of checking
Optics	Laser/ LED/ Light source	measured decline of power output	exchange light source	upon request
		high %CVs (performance test failed)	Realign Optic	upon request
	Filter/ Beam Splitter	Weak or shifting signals Performance fail	Laser Delay Ageing	routinely seldom
		Impaired Scatter or Fluorescence signals	Dust	frequently
	PMT/ Diode/ Detector	When loss of sensitivity is observed Define Min. Volt and Max. Volt to know the linear detection range for each detector	Sensitivity Linearity (3)	routinely after initial installation or changing components
	Saline-Filter	Weak or no signals unexpected scatter signals / high background	Venting Replacement	routinely every 6 month
Tubing		High carry over between samples Unwanted dead cell staining for the sample	Cleaning Bleaching	after usage after usage of DNA-Dyes
	Fluidics	Flow cell/ Cuvette	No signals/ Clog	Sonicate or exchange
unexpected scatter signals / high background			Cleaning inside	upon request
Pressure system		Unstable flow	Storage over night Cleaning or replace sealing, if leaky	after usage upon request
		Ball Seal	No signals / Sample tube is filling up	Replacement
Pump tubing		e.g. wrong volumetric counting	Exchange	every 6 month
Sheath Tank			Refill Cleaning	routinely 2-3 times/

	Waste tank			Empty	year
	HTS	Carry over between samples		Cleaning	routinely
		Stop during operation		Alignment	after usage
	Volumetric Pump	Wrong cell counting		Replace tubing	upon request
Computer/ Software	Database	System is slowing down		Backup / Size control	Upon request/ bi-monthly
	Hard drive	System is slowing down		Defragmentation	usage dependent
	Deflection plates	Spray in drop deflection		Cleaning	monthly
	Camera optics	Additional scattering signals			routinely
	ACDU	Poor plating efficiency		Cleaning Adjustment	in front of sort
	Drop delay	Poor Yield, lower purity		Adjustment	In front of sort
Cell Sorting		Unstable droplet break off		Cleaning or degas Sheath tank	upon request
	Nozzle	Leakage		Exchange	upon failure
		Unsterile sorting		Cleaning	
	Cuvette/ Flow cell	Additional background/ lower sensitivity		Remove dust and salt	Upon request
	Cooling System	Bacterial growth in water bath		Cleaning and replacing cooling water	1-2 times/ year

Ch III S2 Table 5 (2): Summary of critical parameters defining the optical performance of a flow cytometer.

Parameter	A measure for ...	Recommended Value
SD_{EN}	... electronical noise	as low as possible
%rCV	... Laser alignment	as low as possible
Q_r	... Detector efficiency	as high as possible
B_r	... the channel background	as low as possible
Signal to Noise ratio	... sensitivity of Detector	as high as possible

Ch V S3 Table 6 (1). The number of acquired events, for a cell population with final frequency 0.01%.

Acquired events (N)	100,000	1,000,000	4,010,000	10,000,000
Positive (R)	10	100	401	1,000
Proportion (P)	0.0001	0.0001	0.0001	0.0001
Variance (Var)	10.0	100.0	400.6	999.9
Standard deviation (SD)	3.16	10.0	20.1	31.62
Coefficient of Variation (CV)	31.62	10.00	4.99	3.16

Ch VI S3 Table 7 (1): Comparison of two data sets X and Y in a rank analysis^a.

Y-group		y_1				y_2		y_3		y_4
X-group	x_1	x_2	x_3	x_4	x_5					
values	3	7	9	15	23	31	36	44	51	
Rank	1	2	3	4	5	6	7	8	9	

Ch VI S3 Table 8 (2): Part of the Mann-Whitney probability table example for the X-group size of Table 7 ($N=5$).

U	$N_1 = 1$	$N_1 = 2$	$N_1 = 3$	$N_1 = 4$	$N_1 = 5$
0	.167	.047	.018	.008	.004
1	.323	.095	.036	.016	.008
2	.500	.190	.071	.032	.016
3	.667	.286	.125	.056	.023
4		.429	.196	.095	.048
5		.571	.286	.143	.075
6			.393	.206	.111
7			.500	.278	.155
8			.607	.365	.210
9				.452	.271

Ch VI S3 Table 9 (3): Kolmogorov-Smirnoff (K-S) statistic critical values, D_c , with their associated P -values (probabilities)

D_c	1.0727	1.2238	1.3581	1.5174	1.6276	1.7317	1.8585	1.9525
p	0.200	0.100	0.050	0.020	0.010	0.005	0.002	0.001

Ch VI S3 Table 10 (4): Hypothetical results of the same determinations from two different laboratories.

Sample	1	2	3	4	5	6	7	8	9	10
Lab A	.61	.23	.31	.11	.41	.19	.10	.03	.07	.17
Lab B	.54	.38	.42	.20	.36	.27	.21	.11	.14	.12

Ch VI S3 Table 11 (5): Ranking of the data from Table 10 with rank differences, d, and d²

Sample	1	2	3	4	5	6	7	8	9	10
Lab A	10	7	8	4	9	6	3	1	2	5
Lab B	10	8	9	4	7	6	5	1	3	2
Rank difference, d	0	-1	-1	0	2	0	-2	0	-1	3
d ²	0	1	1	0	4	0	4	0	1	9

Ch VI S3 Table 12 (6): Differences between values from Table 4 by subtracting Lab B results from those of Lab A.

Sample	1	2	3	4	5	6	7	8	9	10
Lab A	.61	.23	.31	.11	.41	.19	.10	.03	.07	.17
Lab B	.54	.38	.42	.20	.36	.27	.21	.11	.14	.12
Sample difference, d	.07	-.15	-.11	-.09	.05	-.08	-.11	-.08	-.07	.05
d ²	.0049	.0225	.0121	.0081	.0025	.0064	.0121	.0064	.00	.0025

Ch VI S3 Table 13 (7): Results of the immunofluorescence analysis example, taken from Watson (2001) [17]

Sample	1	2	3	4	5	6	7	8	9	10
Lab A	.61	.23	.31	.11	.41	.19	.10	.03	.07	.17
Lab B	.54	.38	.42	.20	.36	.27	.21	.11	.14	.12
Sample difference, d	.07	-.15	-.11	-.09	.05	-.08	-.11	-.08	-.07	.05
d ²	.0049	.0225	.0121	.0081	.0025	.0064	.0121	.0064	.0029	.0025

Ch VI S3 Table 14 (8): Illustration of potential interpretation problems when counting extremely rare cells^a.

Sample	1	2	3	4
	6	2	6	8
	3	7	1	6
	1	3	5	3
	1	4	5	6
	1	4	6	3
Mean	2.4	4	4.6	5.2
St.Dev	2.2	1.9	2.1	2.2

Overall mean: 4.1

Overall St.Dev: 2.2

Ch VI S4 Table 15 (1): Important provided data for cytometric publications. (*part of MiFlowCyte).

Data set	Details
Sample/Specimen	Type*, source*, source treatment*, taxonomy, age*, gender*, phenotype*, genotype*, location*
Sample Treatment	Analytes*, Ab clone*, names/numbers*, manufacturer*, catalogue numbers*
Reagents	Concentration, purity
Controls	Quality Control Measures*, FMOs*, Positive/negative control*
Instrument	Manufacturer*, model*, configuration*, settings*, detector voltages*, optical filters*
Data Analysis	List-mode data file*, compensation*, gating*

Ch VI S5 Table 16 (1): Overview of repositories for flow cytometry data.

Name	URLs and References	Technical Notes and Highlighted Features
Cytobank	http://www.cytobank.org/ PMID: 24590675 PMID: 20578106	Free community version, requires registration Web access Advanced online data analysis options in paid versions Extended CyTOF data support
FlowRepository	https://flowrepository.org/ PMID: 22887982 PMID: 22752950	Free and open source, no registration required to download data Web access, R library, FlowJo plugin Full MiFlowCyt support Basic online data analysis options Integrated FCS de-identification (optional) Included in Thomson Reuters Data Citation Index Recommended by Nature, Cytometry Part A and PLOS journals
ImmPort	https://immport.niaid.nih.gov/ PMID: 24791905	Free, requires registration and approval Web access Data from dozens of assay types including cytometry Online data analysis tools Templates for data deposition, management and dissemination Used mainly for NIAID/DAIT funded studies
ImmuneSpace	https://www.immunespace.org/ PMID: 24441472	Free, requires registration Web access, R library Database and analysis engine that leverages ImmPort infrastructure Exploring, integration and analyses of data across assays Ontology support through standards-aware data templates Used mainly for HIPC data

Ch VII S4 Table 17 (1). Cytokine assay reagents.

Target	Bead ID
IL-10	A4
IFN-g	A5
IL-5	A6
IL-2	A7
TNF-a	A8
CM-CSF	A10
IL-4	B2
IL-17F	B3
IL-9	B4
IL-17A	B5
IL-13	B6
IL-22	B7
IL-6	B9

Ch VII S6.2 Table 18 (1): Methods for the detection of antigen-specific T cells.

Detection Method	Duration	Commonly used markers	Cell Type	Disadvantages
Proliferation	3-5 days		CD4+ and CD8+	Bystander proliferation may occur
				Selective outgrowth of single clones
				No direct quantification of specific cells
				Phenotypical and functional changes during long-term in vitro culture
Cytokine secretion	5-12 hours (different cytokines may have different kinetics)	TNF- α	CD4+ and CD8+	Restricted to preselected cytokine producers; Non-cytokine producing Tcells (e.g. naive, Treg) are neglected
		IFN- γ	CD4+ and CD8+	
		IL-2	CD4+ and CD8+	
		IL-4, IL-5, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, GM-CSF, ...	mainly CD4+	
		GARP/LAP/TGF- β	Treg	
Activation marker	5 hours - several days (different activation markers have different kinetics)	CD69 (3 till 24 hours)	CD4+ and CD8+	Sensitive to bystander activation
		CD25 (24 till <72 hours)	CD4+ and CD8+	Sensitive to bystander activation; late up-regulation; constitutively expressed by Treg
		HLA-DR (24 till <72 hours)	CD4+ and CD8+	Late up-regulation
		CD134 (OX-40) (48 till 72h)	CD4+ and CD8+	Late up-regulation
		CD154 (CD40L) (6 till 16 hours)	mainly CD4+	Restricted to CD4+ T cells; not expressed on Treg
		CD137 (4-1BB) (6 till 24 hours)	Treg (6h), later also on CD4+Tcon and CD8+	Detection Treg requires co-staining with CD154; On CD4+ and CD8+ Tcon sensitive to bystander activation
Cytotoxicity	1-6 hours	Perforin	mainly CD8+	Restricted to preselected cytotoxic marker; non-cytotoxic T cells are neglected
		Granzyme A	mainly CD8+	
		Granzyme B	mainly CD8+	
		CD107a	mainly CD8+	

Ch VII S7 Table 19 (7.1 or 1): Approaches for determining cell proliferation.

Nucleotide incorporation/dye dilution	Determination of cell divisions
5-bromo-2'-deoxyuridine (BrdU)	1-2
BrdU / Hoechst / PI (quenching) technique	3-4
Dye dilution	> 4

Ch VII S8.6 Table 20 (1): Main fluorescent probes used to stain mitochondria in intact, living cells.

Full name	Short name	Abs (nm)	Em (nm)	Fixable
Mitochondrial membrane potential				
3,3'-dihexyloxacarbocyanine iodide	DiOC ₆	484	501	No
Rhodamine 123	Rh123	507	529	No
5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimidazolcarbocyanine	JC-1	514	529/590	No
3,3'-dimethyl- α -naphthox-acarbocyanine iodide	JC-9	522	535/635	No
Tetramethylrhodamine ethyl ester	TMRE	549	574	No
Tetramethylrhodamine methyl ester	TMRM	548	573	No
Mitotracker Red CMXRos		578	599	Yes
Mitochondrial mass				
Nonyl Acridine Orange	NAO	495	519	No
Mito ID Red		558	690	Yes
Mitotraker Green FM		489	517	No
Mitotraker Deep Red 633		644	665	Yes
Mitotracker Red 580		581	644	No
Mitochondrial reactive oxygen species				
MitoSOX Red mitochondrial superoxide indicator	MitoSOX	510	580	
Mitochondria Peroxy Yellow-1	MitoPY-1	510	528/540	

Ch VII S10 Table 21 (1): Autophagy inducers for primary immune cells.

Primary Cell Type	Autophagy Inducer
T cells	α CD3/CD28 (highest after 4 days)
B cells	IgM, megaCD40L (CD40L construct, Enzo life sciences)
Monocytes	LPS, IFN- γ
Macrophages	LPS, IFN- γ

Ch VII S15 Table 22 (1): Most common transcription factors measured by flow cytometry.

Transcription Factors	Cell type	Cellular location
AHR	Liver, Treg and Th17 cells	cytoplasm
Aiolos	B, T and NK cells	nucleus
AIRE	dendritic cells, lymph node, lymphoid stromal cells, and monocytes	perinuclear region (??)
BATF	B and T cells	nucleus
Bcl-6	B cells and CD4+ T follicular helper cells and memory T cells	nucleus
β Catenin	several non-immune tissues, B, T, and hematopoietic stem cells	cytoplasm / nucleus
Blimp1	B, T, dendritic and some NK cells	cytoplasm
c-Maf	neural, ocular and hematopoietic systems	nucleus
c-Rel	Treg, mature T cells	cytoplasm / nucleus
E4BP4	NK, NKT, and dendritic cells	nucleus
Egr1	B, T and myeloid cells.	cytoplasm / nucleus
Egr2	(B), T and NKT cells	cytoplasm / nucleus
Eomesodermin / TBR2	NK and T cells	nucleus
Eos	T cells and nervous system	transmembrane
FoxJ1	ciliated epithelial cells, naive B and T cells	nucleus
FoxP3	CD4+CD25+ regulatory T cells (Treg cells), and CD4+CD25- cells	cytoplasm / nucleus
Gata-3	central nervous system, kidney, mammary glands, skin, and T cells	nucleus
Helios / IKZF2	T and hematopoietic stem cells	nucleus
IκB-zeta	macrophages, monocytes, B and T cells	nucleus
IRF4	macrophages, B and T cells	cytoplasm / nucleus
Nanog	blastocyst, embryonic stem (ES) cells, and embryonic germ (EG) cells.	nucleus
NFκB	almost all cell types	cytoplasm / nucleus
NFTA	T cells	cytoplasm / nucleus / transmembrane
Notch1	thymocytes, bone marrow hematopoietic stem cells, T and NK cells	cytoplasm / Golgi/ nucleus / transmembrane
Notch2	activated peripheral T cells, bone marrow and thymocytes,	cytoplasm / Golgi/ nucleus / transmembrane
Notch3	CNS, some thymocyte subsets, vascular smooth muscle, and T cells	cytoplasm / Golgi/ nucleus / transmembrane
Notch4	CD8+ splenic dendritic cells, endothelial cells, and macrophages	cytoplasm / Golgi/ nucleus / transmembrane
Nurr77	thymocytes and T cell	cytoplasm / nucleus

OCT3/4	embryonic stem (ES) and induced pluripotent stem (iPS) cells	nucleus
Pax5	hematopoietic cells, B cells	nucleus
PLZF	CD4 and CD8+ T cells, gamma delta T cells and NK.	cytoplasm / nucleus
RORγ	heart, kidney, liver, lung, muscle, and CD4+CD8+ thymocyte cells	nucleus
Runx1 / AML1	hematopoietic, myeloid, B and T cells	cytoplasm / nucleus
Sox2	embryonic stem (ES) cells and neural cells	cytoplasm / nucleus
T-bet	B cells and CD4+ T cell lineage	nucleus
ThPOK	hematopoietic cells, skin, heart, smooth muscle, and liver, invariant natural killer T (iNKT) cells and gamma delta T cells	nucleus
TOX	thymocytes, T lymphocytes, NK cells, and lymphoid tissue-inducer (LTi) cells	nucleus

Ch VII S16 Table 23 (1): Worksheet for timed addition of reagents for 15 minute (max) LPS activation of whole blood.

Work Sheet for Kinetics of LPS Activation Experiment (Figure 4)

Tube No.	Tube Label	Time of Addition		Time of Addition
		LPS	Formaldehyde	Triton X-100
1	LPS 15'	0:00	15:00	25:00
2	LPS 10'	5:10	15:10	25:10
3	LPS 8'	7:20	15:20	25:20
4	LPS 6'	9:30	15:30	25:30
5	LPS 4'	11:40	15:40	25:40
6	LPS 2'	13:50	15:50	25:50
7	Unstimulated control		16:00	26:00
8	CD14-PC7/CD45-KrO only		16:10	26:10

Unstimulated control: Vortex and put into 37C water bath at 14:00

CD14-PC7/CD45-KrO only: Vortex and put into 37C water bath at 14:10

Blood samples: 100uL

Addition of LPS: 2uL of 50ug/mL PBS; final concentration 100ng per 100uL blood

Addition of Formaldehyde: 65uL of 10% solution: final concentration 4%

Addition of 0.1% Triton X-100: 1mL of 0.1% Triton X-100/PBS

Ch VII S17 Table 24 (1): List of cell permeable dyes described in this chapter, along with solvents, working concentrations and storage conditions.

Solution	Supplier ^{a)}	Solvent	(Stock) Working concentration	Storage
6-NBGD	(Life Technologies, #N23106)	dH ₂ O	(100 mM) 300 μM	-20°C
DCFDA	Thermo Fisher (#D399)	DMSO	(100 mM) 1 μM	-20°C
DIOC6	Sigma-Adrich (#318426)	EtOH	(40μM) 1nM- 40nM	-20°C
MitoTracker Green FM	Cell signalling (#9074S)	DMSO	(1mM) 5-10 nM	-20°C
MitoTracker Red FM	Thermo Fisher (#M22425)	DMSO	(1mM) 50-100 nM	-20°C
TMRE	Life Technologies (#T668)	MeOH or DMSO	(20 μM) 20nM	-20°C

Ch VIII S2 Table 25 (1): Phenotypic differentiation of B lineage cell subsets based on their characteristic expression of surface markers*.

B cell population (CD19+)	Phenotype/Subphenotype	
Transitional		
T1+T2	CD24++CD38++CD10+CD27-IgM++	
Naive		
Resting	CD24+/-CD38+/-CD27-IgM++/+IgD++CD21+CD95-	
Activated	CD24-CD38-CD27-IgM++IgD++CD21-CD95+MTG+	
Memory (Ki-67-)		
Pre-switched	IgM+IgD+/-CD27+CD1c+	
Switched	IgG/IgA+CD27+CD21+	
Atypical memory	a) Double negative	IgD-CD27-
	b) activated double negative	IgD-CD27-CD95+
	c) Syk++	IgD+/-CD27-CD95+/-CD21+/-CD38-MTO-Syk++
	d) tissue-resident	IgM/IgG/IgA+CD27-FcRL4+
Marginal Zone		
Spleen	IgD+IgM+CD27++CD21++CD1c+	
Circulating	IgD+IgM+CD27+CD1c+	
Antibody secreting cells		
Circulating	PB	CD38++CD27++CD138-Ki-67+
	PC	CD38++CD27++CD138-Ki-67+
Bone marrow	a) CD19+ PC	CD19+CD38++CD27++CD138+Ki-67-
	b) CD19- PC	CD19-CD38++CD27++CD138+Ki-67-

Ch VIII S4 Table 26 (1): Selection of important markers for flow cytometry analysis of mouse and human ILC.

Marker	Mouse					Human				
	NK cells	CD127 ⁺ ILC1	ILC2	NCR ⁻ ILC3	NCR ⁺ ILC3	NK cells	CD127 ⁺ ILC1	ILC2	NCR ⁻ ILC3	NCR ⁺ ILC3
CD127	-	+	+	+	+	lo/-	+	+	+	+
CD117	lo/-	-	+/-	-	lo	lo/-	-	+/-	+	+
CD25	-	lo	+	+	ND	+/-	lo	+	+/-	lo
IL-23R	-	lo/-	-	+	+	lo	+/-	lo	+	+
IL-17RB	-	-	+	-	-	-	lo/-	+	ND	-
ST2	-	-	+	-	-	-	ND	+	ND	-
IL-1R1	-	lo	ND	+	+	+/-	lo/-	lo	+	+
CCR6	-	-	-	+/-	-	-	+	+	+	+
RANKL	lo/-	ND	ND	+	+	-	ND	ND	+	+
CRTH2	ND	ND	ND	ND	ND	-	-	+	-	-
ICOS	-	ND	+	+	+	-	+	+	+	+
NK1.1/CD161	+	+	-	-	lo/-	+/lo	+	+	+	+
CD56	NA	NA	NA	NA	NA	+	-	-	+/-	+/-
CD94	+/-	ND	+/-	-	+/-	+/-	-	-	-	-
CD16	+/-	ND	-	-	-	+/-	-	-	-	-
NKp30	NA	NA	NA	NA	NA	+	ND	+/lo	+/-	+
NKp44	NA	NA	NA	NA	NA	^a	-	-	-	+
NKp46	+	+	-	-	+	+	-	-	-	+
Ly49/KIR	+/-	lo	-	-	-	+/-	-	-	-	-
CD57	NA	NA	NA	NA	NA	+/-	ND	ND	ND	ND
CD27	+/-	+	-	-	-	+/-	+	-	-	-
CD11b	+/-	-	-	ND	ND	+/-	ND	ND	ND	ND
Perforin	+	lo	-	-	-	+	-	-	-	-

Transcription factors										
T-bet	+	+	-	+/-	+	+	+	-	-	-
Eomes	+	-	-	-	+	+	-	-	-	-
ROR γ t	-	-	-	+	+	-	-	-/lo	+	+
GATA3	lo	lo	+	lo	lo	lo	lo	+	lo	lo
Cytokines										
IFN γ	+	+	-/lo	-/lo	-/lo	+	+	-	-	-
IL-22	-	-	lo	+	+	-	-	lo	lo/-	+
IL-17	-	-	-	+/-	-	-	-	-	+	-
IL-13	-	-	+	-	-	lo	-	+	-	lo
IL-5	-	-	+	-	-	-	-	+	-	-

Ch VIII S5 Table 27 (1): NK cell phenotypes.

(A)	Receptor	Ligand	CD56 ^{bright}	CD56 ^{dim}
Activation	NKG2C (CD159a)	HLA-E	-	subsets
	NKG2D (CD314)	MIC-A - MIC-B - ULPBs	-	Subsets
	KIR2DS1 (CD158h)	HLA-C2	-	subsets
	KIR2DS2/3 (CD158j)	???	-	subsets
	KIR2DL4 (CD158d)	HLA-G	-	subsets
	KIR2DS4 (CD158i)	HLA-A*11 and HLA-C	-	subsets
	KIR2DS5 (CD158f)	???	-	subsets
	KIR3DS1 (CD158e1)	HLA-Bw4	-	subsets
	NKp30 (CD337)	B7-H6 - BAG6/BAT3	++	+
	NKp44 (CD336)	21spe-MLL5	only on activated cells	
	NKp46 (CD335)	CFP (properdin), haemagglutinin, PfEMP1	++	+
	NKp80	AICL	+	+
	DNAM1 (CD226)	Nectin-2 (CD112), PVR (CD155)	+	+
	2B4 (CD244)	CD48	All mature NK cells	
	NTB-A (CD352)	NTB-A (CD352)	All mature NK cells	
	CRACC/CS1 (CD319)	CRACC/CS1 (CD319)	All mature NK cells	
	Tactile (CD96)	PVR (CD155)	All mature NK cells	
	FcγRIII (CD16)	IgG	-/+	+ / ++

Inhibition	NKG2A/KLRD1 (CD159a/CD94)	HLA-E	+	subsets
	KIR2DL1 (CD158a)	HLA-C2	-	subsets
	KIR2DL2/3 (CD158b)	HLA-C1	-	subsets
	KIR2DL4 (CD158d)	HLA-G	-	subsets
	KIR2DL5 (CD158f)	???	-	subsets
	KIR3DL1 (CD158e1)	HLA-Bw4	-	subsets
	KIR3DL2 (CD158k)	HLA-A*03 and *11	-	subsets
	ILT2/LIR-1 (CD85J)	Different MHC-I alleles	-	subsets
	PD-1 (CD279)	PDL1 (CD274) PDL2 (CD273)	-	subsets
	Siglec-7 (CD328)	Ganglioside DSGb5	+	+
	IRP60 (CD300a)	α -herpes virus Pseudorabid virus Phosphatidylserine Phosphatidylethanolamine	+	+
	TIGIT	PVR (CD155)	All mature NK cells	

(B)

Receptor	Ligand	CD56 ^{bright}	CD56 ^{dim}
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Adhesion	LFA-1 (CD11a/CD18)	ICAM-1, ICAM-2, ICAM-3	-/+	++
	LFA-2 (CD2)	CD15, CD58, CD59	Most of mature NK cells	
	LFA-3 (CD58)	CD2, CD48, CD58	Most of mature NK cells	
	MAC-1 (CD11b/CD18)	iC3b, C4b, ICAM-1, fibrinogen	most of circulating NK, up-regulated upon activation	
	ICAM-1 (CD54)	LFA-1, MAC-1	++	+/-
	N-CAM (CD56)	???, FGFR	++	+
	HNK-1 (CD57)	???	-	subsets
	L-Selectin (CD62L)	GlyCAM-1 MadCAM-1	++	subsets

Cytokine / Chemokine receptors	IL-2R α (CD25)	IL-2	+	-
	IL-2R β /IL-2R γ (CD122/CD132)	IL-2 AND IL.15	Almost all PB NK cells	
	c-Kit (CD117)	SCF (KL)	+	-
	IL7R α (CD127)	IL-7	+	-
	CXCR1 (CD181)	CXCL8 (IL-8)	-	++
	CXCR3 (CD183)	CXCL9, CXCL10, CXCL11	++	Subsets
	CXCR4 (CD184)	CXCL2	Subsets of PB NK cells	
	CCR5 (CD195)	RANTES, CCL3 (MIP1 α) and CCL4 (MIP1 β)	Subsets of PB NK cells	
	CCR7 (CD197)	CCL19, CCL21	+	-
	IL-18R (CD218a)	IL-18	++	+
	ChemR23	Chemerin	-	+
	CX3CR1	Fraktaline	-	+

Death Receptors	Fas/APO-1 (CD95)	Fas ligand (CD95L)	Activated NK cells	
	Fas ligand (CD95L)	Fas/APO-1 (CD95)	Activated NK cells They induce target apoptosis	
	CD40L (CD154)	CD40		
	TRAIL (CD253)	DR4 (TRAIL-R1), DR5 (TRAIL-R2)		

Other surface molecules	LAMP1 (CD107a)	---	Briefly expressed on NK cell surface after degranulation	
	LAMP2 (CD107b)	---		
	LAMP3 (CD63)	---		
	TNFRSF7 (CD27)	CD70	+	-

Ch VIII S6 Table 28 (1): Selected commonly used surface markers for murine mononuclear phagocytes.

	<i>phenotype</i>	<i>selected references</i>
Monocytes		
classical	CD115 ⁺ CX ₃ CR1 ^{int} CCR2 ⁺ CD62L ⁺ CD43 ^{lo} Ly6C ^{hi}	[4],[7],[20]
non-classical	CX ₃ CR1 ^{hi} CCR2 ⁺ CD62L ⁺ CD43 ^{hi}	[4],[7],[20]
Macrophages		
Kupffer cells (liver)	CD11b ⁺ F4/80 ⁺ CD68 ^{+/-} Clec4F ⁺	[5],[21],[22]
microglia (brain)	CX ₃ CR1 ^{hi} CD45 ^{int} CD11b ⁺ F4/80 ⁺ Siglec ⁺	[23],[24]
intestinal lamina propria macrophages	CD64 ⁺ CX3CR1 ⁺ CD11c ^{+/-} F4/80 ⁺ CD11b ⁺ (lamina propria)	[25]-[29]
Dendritic cells		
lymphoid organ	CD11c ⁺ MHCII ⁺ CD24 ^{+/-} XCR1 ^{+/-}	[2],[3]
non-lymphoid organ (intestine)	CD11c ⁺ CD103 ^{+/-} MHCII ⁺ CD24 ^{+/-} CD11b ^{+/-}	[3],[30],[31]

Ch VIII S7 Table 29 (1): Selection of most important markers for flow cytometry analysis of granulocytes.

Cell type	Mouse	Human
Basophil	CD45 ^{pos} , CD11b ^{pos} , Ly6C ^{low} , CD200R3 ^{pos} , CD49b ^{pos} , FcεR1α ^{pos}	CD45 ^{pos} , CD11b ^{pos} , CD15 ^{neg} , CD16 ^{neg} , CCR3 ^{pos} , FcεR1α ^{pos} , CD203 ^{pos} , CD117 ^{neg}
Eosinophil	CD45 ^{pos} , CD11b ^{pos} , Ly6C ^{low/int} , Siglec-F ^{pos} , CCR3 ^{pos} , FcεR1α ^{pos}	CD45 ^{pos} , CD11b ^{pos} , CD15 ^{pos} , CD16 ^{neg} , Siglec-8 ^{pos} , CCR3 ^{pos} , FcεR1α ^{pos}
Neutrophil	CD45 ^{pos} , CD11b ^{pos} , Ly6C ^{int} , Ly6G ^{pos}	CD45 ^{pos} , CD11b ^{pos} , CD15 ^{pos} , CD16 ^{pos} , CD66b ^{pos}

Ch VIII S8 Table 30 (1): Antibodies for bone marrow stromal cells.

Antibody	Clone	Company
CD45	30-F11	Biolegend
Ter119	Ter-119	Biolegend
CD31	390	Biolegend
CD51	RMV-7	eBioscience
PDGFRα	APA5	eBioscience

Ch VIII S9 Table 1. Selection of most important markers for flow cytometry analysis of mouse BM hematopoietic stem cells.

Cell type	Mouse	Human
Long-term self-renewing pluripotent hematopoietic stem cells (LT-pHSC)	<u>Negative:</u>	<u>Negative:</u>
	F4/80, Mac1, Gr1, CD11c, CD4, CD8, CD3, CD5, CD19, B220, NK1.1, Ter119	CD1c, CD14, CD15, CD16, CD20, CD41, CD11c, CD56, CD203c, CD235a, BDCA2, Ter119
	Thy1.1 ^{low} , Flk2, CD34, CD48	CD38, CD45RA
	Rho123 ^{low} / Hoechst ^{low}	Rho123 ^{low} / Hoechst ^{low}
	<u>Positive:</u>	<u>Positive:</u>
	c-Kit, Sca1, CD201 ^{high} , CD150	CD34, CD90

Ch VIII S10 Table 31 (10.2.1): Collection of surface molecules for flow cytometry cell sorting staining of human solid tumor cells

Antigen	molecules / synonyms	antibody / clone (selection)
MHC class I complex	HLA class I, all HLA-A, -B, -C alleles	W6/32, HC10
	beta ₂ -microglobuline, β_2m	HB28, B2M-01, 2M2
MHC class II	HLA-DR; HLA-DQ; HLA-DP	L243; TÜ169, SK10; B7/21
NKG2D ligands	MICA; MICB; ULBP1; ULBP2; ULBP3	MAB1300; MAb1599; MAB1380; MAB1289; MAB1517
ICAM-1	CD54	9H21L19; LB-2
VCAM	CD106	51-10C9
Ep-CAM	CD326	EBA-1, 9C4, 22HCLC
VE-cadherin	CD144	BV13, 55-7H1, BV9
E-cadherin	CD234	36/E-cadherin, 5HCLC, 67A4
EGFR	HER1	EGFR.1, H11, 199.12,
PDGFR	CD140a (alpha chain) CD140b (beta chain)	AlphaR1, 16A1, 28D4, 18A2, Y92,
c-Met	HGFR	3D6, ebioclone97
pan-cytokeratin	pan-cytokeratin	C-11, PAN-CK
cytokeratin 18	CK18	CK2, C-04, DC10, AE1, E431-1
cytokeratin 8	CK8	K8.8, 5D3, C-43, M20
CD99		TÜ12, 3B2/TA8, EPR3096,

Ch VIII S10 Table 32 (10.2.2): Collection of surface molecules for flow cytometry cell sorting staining of murine solid tumor cells

Antigen	molecules / synonyms	antibody / clone (selection)
MHC class I complex	MHC class I all H-2 molecules	M1/42
	H-2K; H-2D; H-2L	Kd+Dd (ab131404); Dd (ab25590); Kb (ab93364);
	beta ₂ -microglobuline, β ₂ m	S19.8
MHC class II	I-A, I-E	M5/114.15.2
NKG2D ligands	Rae-1, H60, MULT1	(Rae-1g (CX1); H60 (MAB1155); MULT1 (5D0)
ICAM-1	CD54	YN1/1.7.4
VCAM	CD106	429
Ep-CAM	CD326	G8.8
VE-cadherin	CD144	ab33168, MC13.3
E-cadherin	CD234	DECMA-1, M168
EGFR	HER1	EP38Y,
PDGFR	CD140a (alpha chain)	APA-5
	CD140b (beta chain)	APB-5
c-Met	HGFR	ebioclone7, EP1454Y
pan-cytokeratin	pan-cytokeratin	C-11, ab9377, AE1/AE3
cytokeratin 18	CK18	6-19
cytokeratin 8	CK8+CK18	EP1628Y
CD24		J11d, M1/69, 30-F1
CD34		RAM34, MEC14.7, MAB6518
CD44		IM7,
CD133		13A4, 315-2C11,

Ch VIII S10 Table 33 (10.4.2): Overview of the most frequent human carcinomas

carcinoma tissue	most frequent form of carcinoma	originating cell	Ref
lung cancer	non-small cell lung cancer (NSCLC)	type I / II alveolar epithelial cells	[12]
breast cancer	mammary carcinoma	epithelial cells of the milk duct	[15]
colon cancer	colorectal carcinoma (CRC)	epithelial cells of inner mucosal layer	[12]
prostate cancer	prostate carcinoma	epithelial basal cells of the prostate	[16]
liver cancer	hepatocellular carcinoma (HCC)	Hepatocytes	[12]
stomach cancer	stomach carcinoma	epithelial cells transformed by H. pylori	[12]
cervical cancer	cervical carcinoma	cervical epithelial cells after HPV infection	[17]
oesophagus cancer	oesophagus carcinoma	Epithelial cells lining the oesophagus	[18]
bladder cancer	bladder carcinoma	transitional epithelium of the bladder wall	[19]
pancreatic cancer	pancreatic carcinoma	endocrine ductal epithelial cells	[20]
kidney cancer	renal cell carcinoma (RCC)	proximal tubular epithelial cells	[12]
ovarian cancer	ovarian carcinoma	ovarian tubal-type epithelium	[12]
squamous cancer	squamous cell carcinoma	epithelial cells of skin or glands	[12]

Ch VIII S10 Table 34 (10.4.3): Overview of the most frequent human sarcomas

sarcoma tissue	mesenchymal tumor	originating cell	Ref.
Ewing sarcoma	Ewing's sarcomas (bone, bone marrow, lung, kidney)	soft tissue cell of the respective organ	[22, 24]
Kaposi's sarcoma	soft tissue sarcoma	Induced after infection with HHV-8	[23]

Ch VIII S10 Table 35 (10.4.4): Overview of the most frequent human neuroectoderma tumors

tumor tissue	neuroectodermal tumor	originating cell	Ref.
black skin cancer	malignant melanoma	melanocytes of the skin	[25-27]
brain cancer	gioblastoma, glioma	glial cells of the brain	[28-29]
brain cancer	Astrocytoma	Astrocytes of the brain	[30]