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Supplemental Figure Legends

Supplemental Figure 1. Significant myofibroblast activation is observed in kidneys subjected to UUO for 4days. (A) Staining with H&E (top panel) or Sirius red (bottom panel) of kidney sections from obstructed (UUO; 4days after surgery) or contralateral sham-operated kidneys (Control) at 5x magnification (top panel). N=3. (B) Western blot of whole-kidney lysates for αSMA, vimentin and pERK1/2 expression. Membranes were subsequently stripped and re-probed for tubulin and ERK1/2 as loading control. A photomicrograph from N=1 western blots with N=3 animals in each group is shown. Optical density of the αSMA (C) and vimentin (D) bands in (B) were normalised against tubulin. Optical density of the pERK1/2 (E) bands in (B) were normalised against ERK1/2. The normalised density of the sham-vehicle treated samples was arbitrarily set to 1. For all graphs error bars represent the means ± S.E. of data from N=3 animals per group. *, $p < 0.05$ versus the control.

Supplemental Figure 2. Delayed administration of trametinib attenuated ERK1/2, AKT and mTORC1 pathway activation in UUO kidneys. (A) Representative image of western blot analysis (N=2) of kidney lysates from our experimental animals for phospho-ERK1/2 and phospho-AKT. Protein loading was corrected against total-ERK1/2 or total-AKT, as appropriate. (B-C) Densitometric analysis for normalised phosphorylated ERK1/2 and phosphorylated AKT levels from the western blots shown in A. (D) Kidney sections were immunostained for phospho-ERK1/2 and positive staining was quantified as percentage of total area (E). (F) Whole-kidney lysates were subjected to western blot and probed with specific antibodies against the phosphorylated forms of P70S6K, 4E-BP1 and S6. Protein was normalised against tubulin or total-S6, as appropriate. Densitometric analysis of normalised phospho-P70S6K, phospho-4E-BP1 and phospho-S6 levels are shown (G-I). For all graphs error bars represent the means ± S.E. of data from N=4-6 animals per group. *, $p < 0.05$ versus the control.

Supplemental Figure 3. Delayed trametinib administration attenuated Nox4 upregulation and suppressed STAT3, NF-κB, Smad2/3 phosphorylation and macrophage infiltration in UUO kidneys. (A) Representative image from western blot analysis of kidney lysates probed with a specific anti-Nox4 antibody. (B) Densitometric analysis of Nox4 bands in A normalised for protein loading against tubulin. (C) Representative image from N=2 western blots probed with specific antibodies against the phosphorylated forms of STAT3, P65 and Smad2/3. (D-G) Densitometric analysis of the western blots shown in C. Protein loading was normalised against total-STAT3, total-P65 or tubulin as appropriate. (H) Immunostainig of kidney sections for macrophage infiltration with F4/F80. (I) Quantification of positively stained area

as percentage of total area from the section in H. For all graphs error bars represent the means ± S.E. of data from N=4-6 animals per group. *, p < 0.05 versus the control.

Supplemental Figure 4. Trametinib suppressed ERK1/2 activation and myofibroblast proliferation in the kidneys of adenine-fed animals. (A) Representative western blot (N=2) from kidney lysates of control and adenine-fed animals treated with trametinib as indicated. Membranes were probed for phospho-AKT and phospho-ERK1/2. Protein loading was determined by probing for total-ERK1/2 and tubulin. Densitometric analyses of blots from A for normalised phospho-ERK1/2 (B) and phospho-AKT (C) levels as described in Figure 1. (D) Immunstaining of kidney sections for phospho-ERK1/2 and quantification of positive pERK1/2 staining (E). (F) Immunostaining for positive Ki67 nuclei in kidney sections from our experimental groups. (G) Quantification of Ki67 positive nuclei per field of view from the experiment in (F). For all graphs error bars represent the means ± S.E. of data from N=4-6 animals per group for western blots and N=7-8 for immunostaining experiments. *, p < 0.05 versus the control.
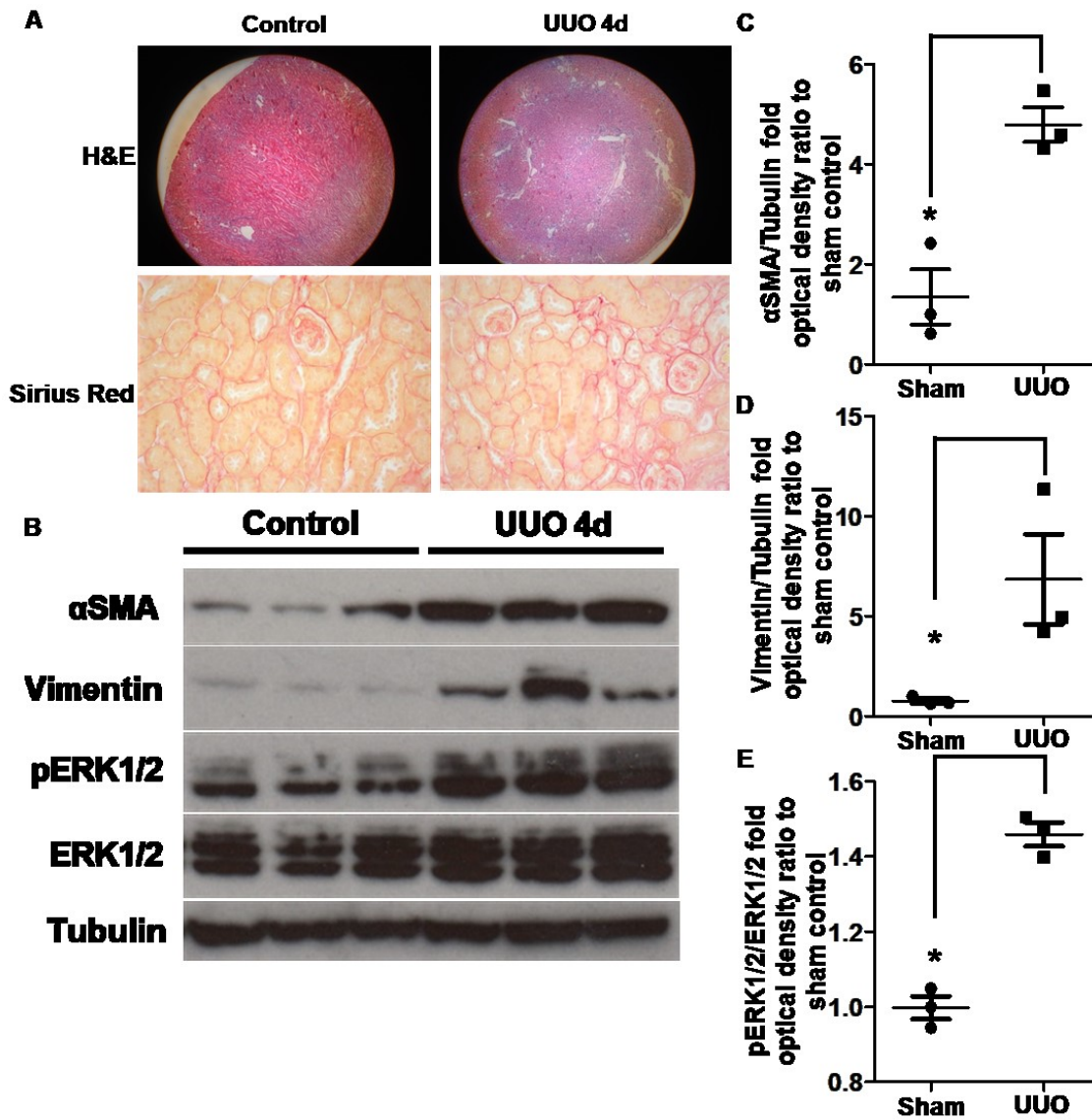
Supplemental Figure 5. Trametinib treatment ameliorated mTORC1 activation in the kidneys of adenine-fed mice. (A) Western blot analysis of kidney lysates for the activation of the downstream mTORC1 effectors pP70S6K, p4E-BP1and pS6. (B-D) Densitometric analysis of western blots in A. Protein loading was normalised with tubulin or total-S6 as appropriate. For all graphs error bars represent the means ± S.E. of data from N=4-6 animals per group. *, p < 0.05 versus the control.

Supplemental Figure 6. Trametinib ameliorated macrophage infiltration in the kidneys of adenine-fed mice. (A) Immunostainig of kidney sections for macrophage infiltration with F4/F80. (B) Quantification of positively stained area as percentage of total area from the section in (A). For all graphs error bars represent the means ± S.E. of data from N=7-8 animals per group. *, p < 0.05 versus the control.
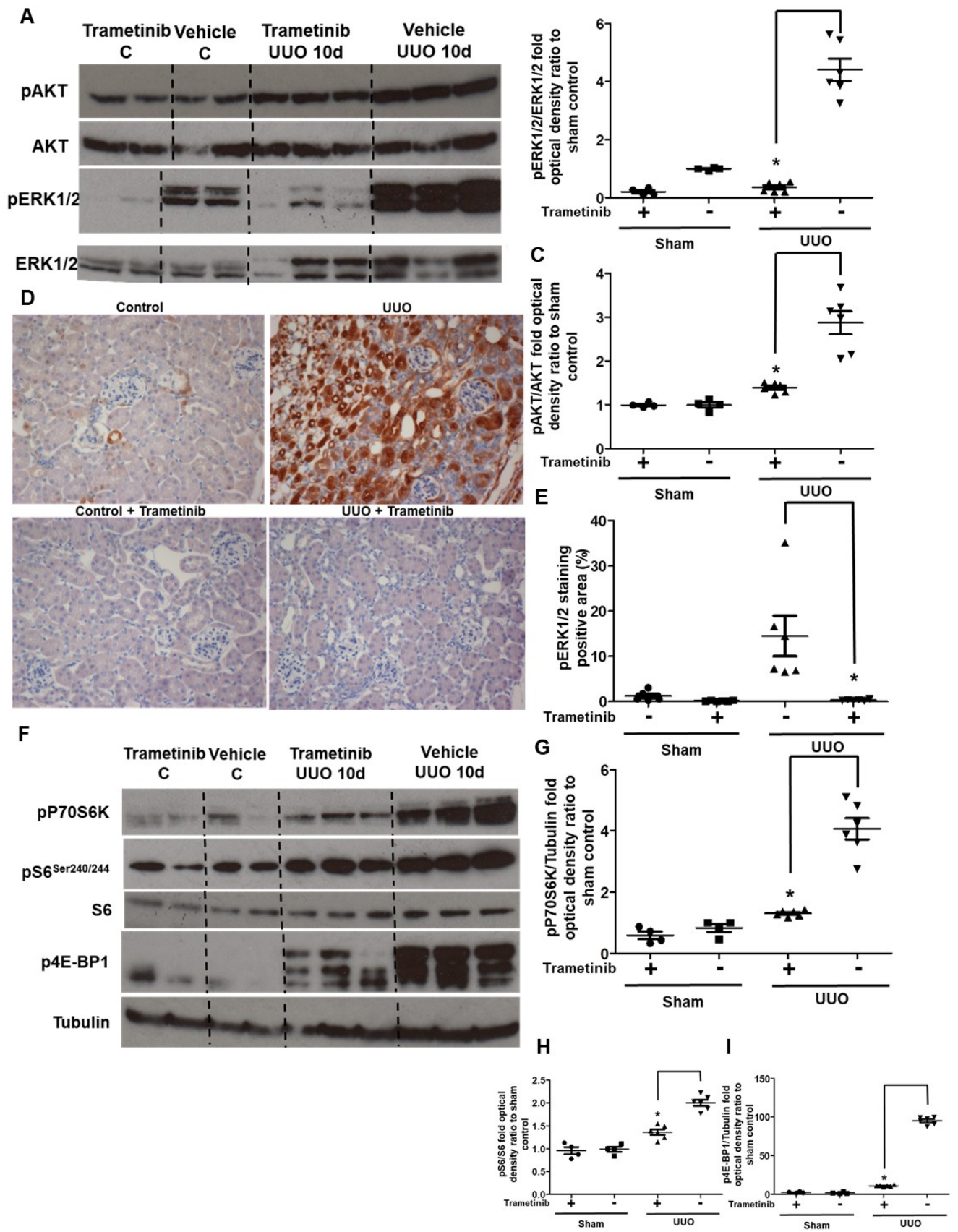
Supplemental Figure 7.Animal weights for the course of the adenine-diet experiment. The weights of the animals in the adenine diet experiment were measured approximately twice per week. Weights are expressed relative to the animal's initial weight set arbitrarily to 100%. Error bars represent the means ± S.E. of data from N=7-8 animals per group. *, p < 0.05 versus the control.
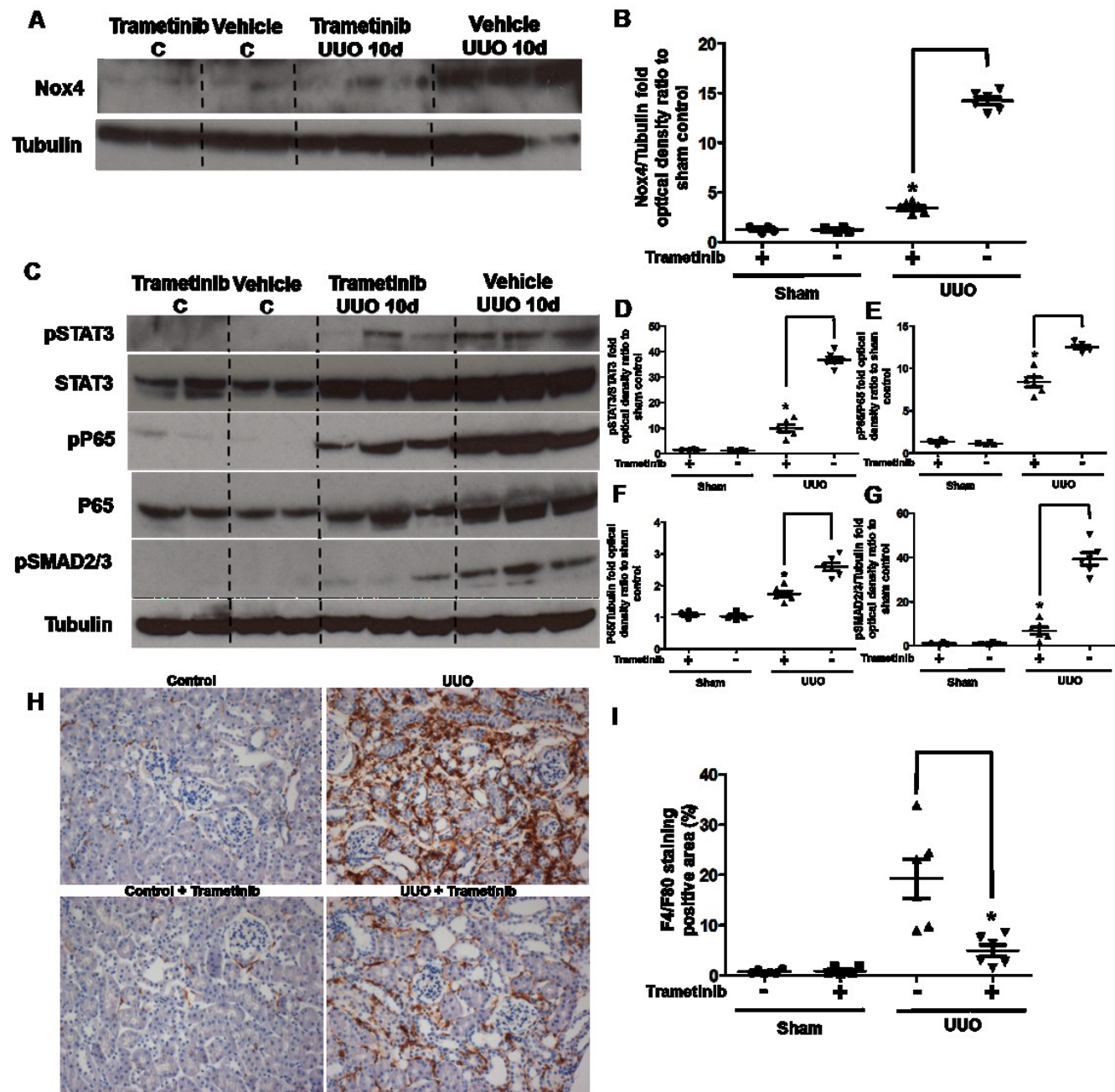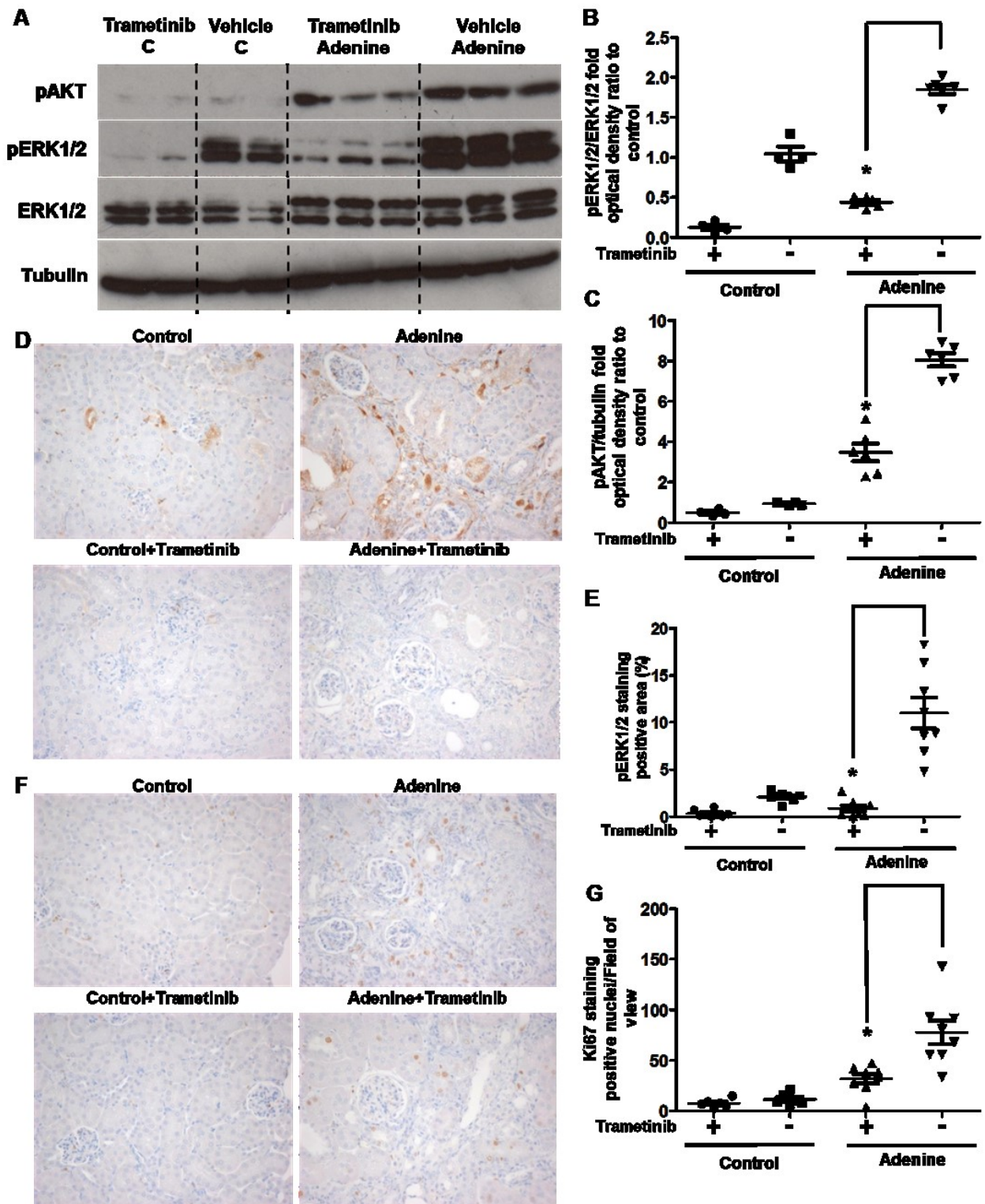
Supplemental Figures

Supplemental Figure 1

Supplemental Figure 2

A

B

pERK1/2/ERK1/2 fold optical density ratio to sham control

Trametinib   +   -   +   -
Sham   UUO

C

pAKT/AKT fold optical density ratio to sham control

Trametinib   +   -   +   -
Sham   UUO

D

Control   UUO

Control + Trametinib   UUO + Trametinib

E

pERK1/2 staining positive area (%)

Trametinib   -   +   -   +
Sham   UUO

F

G

pP70S6K/Tubulin fold optical density ratio to sham control

Trametinib   +   -   +   -
Sham   UUO

H

pS6/S6 fold optical density ratio to sham control

Trametinib   +   -   +   -
Sham   UUO

I

p4E-BP1/Tubulin fold optical density ratio to sham control

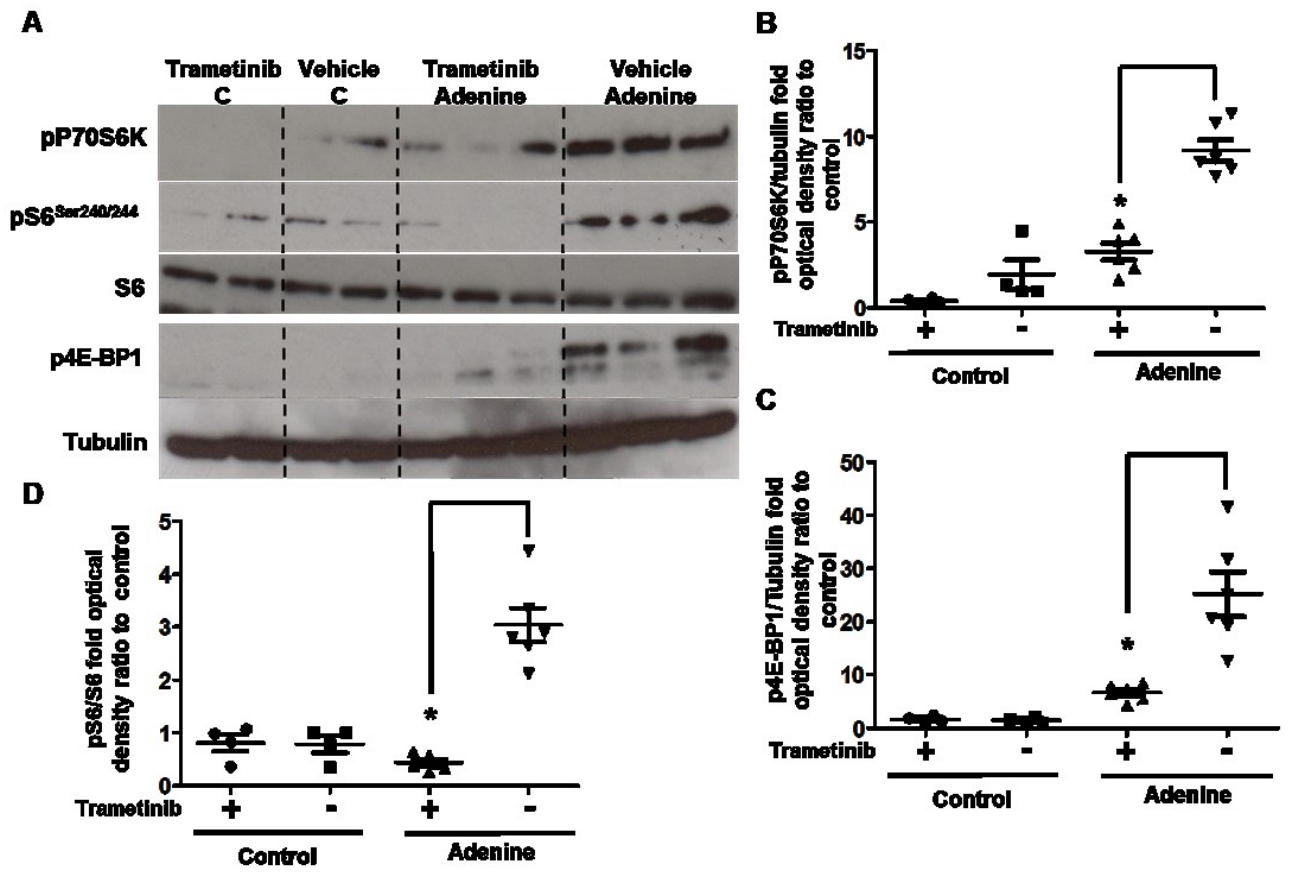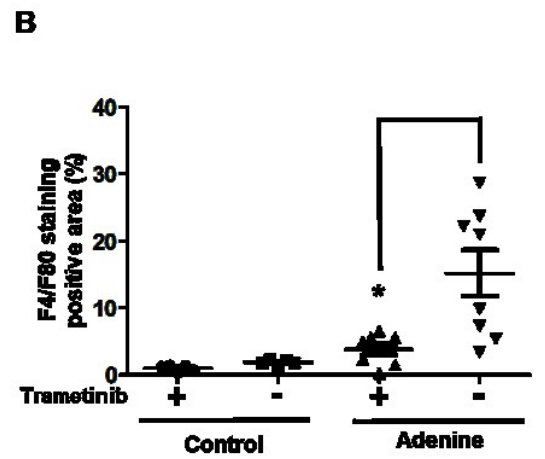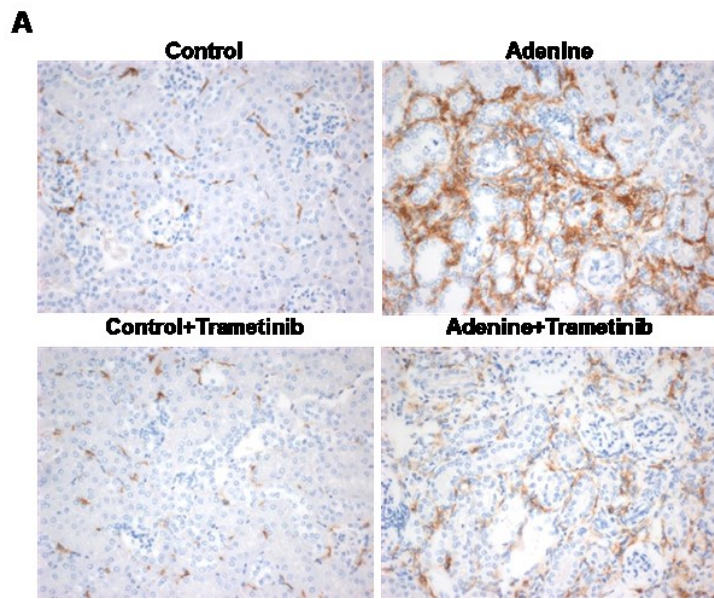Trametinib   +   -   +   -
Sham   UUO

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Supplemental Figure 3

Supplemental Figure 4

Supplemental Figure 5

Supplemental Figure 6

A

B

Supplemental Figure 7