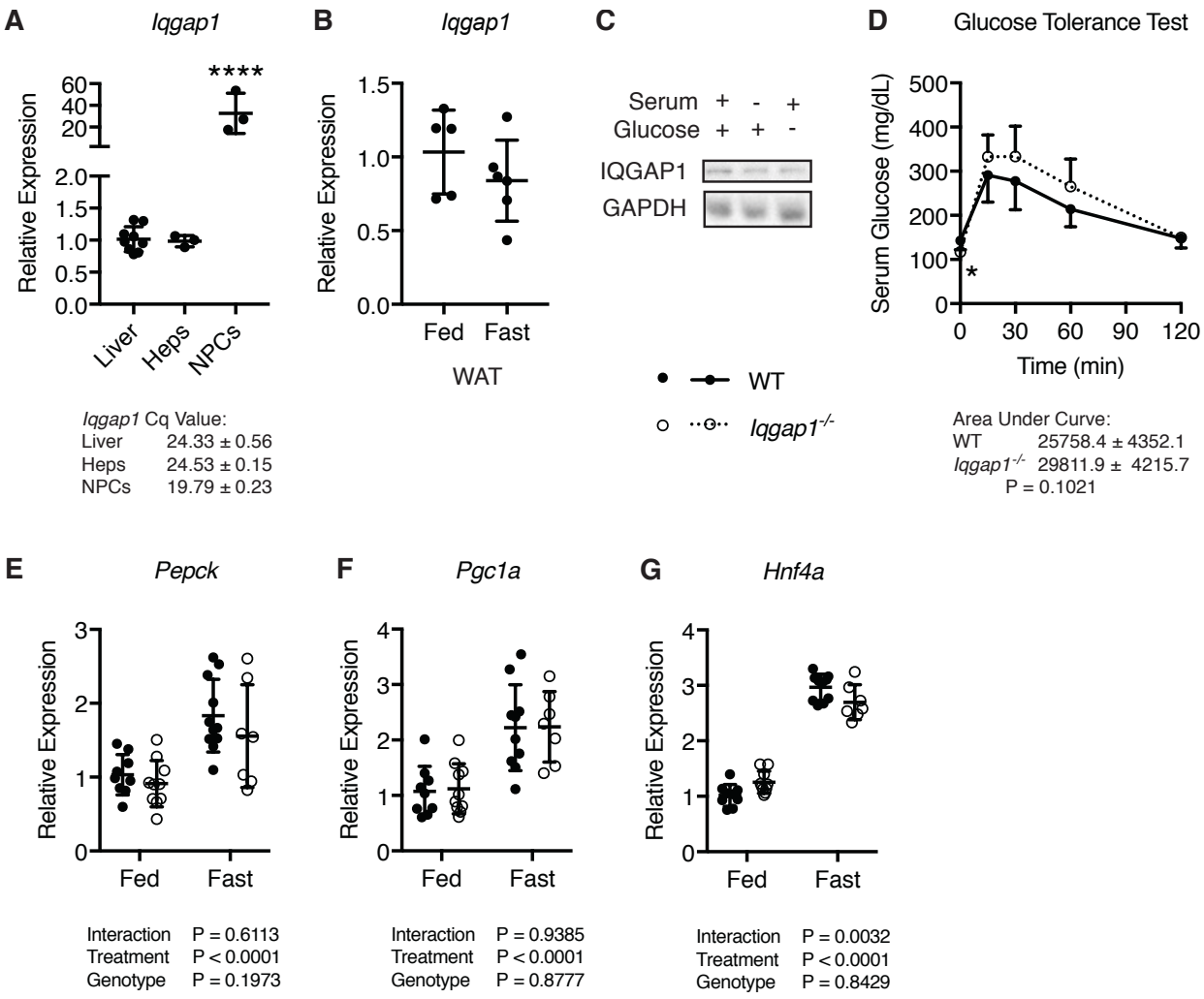
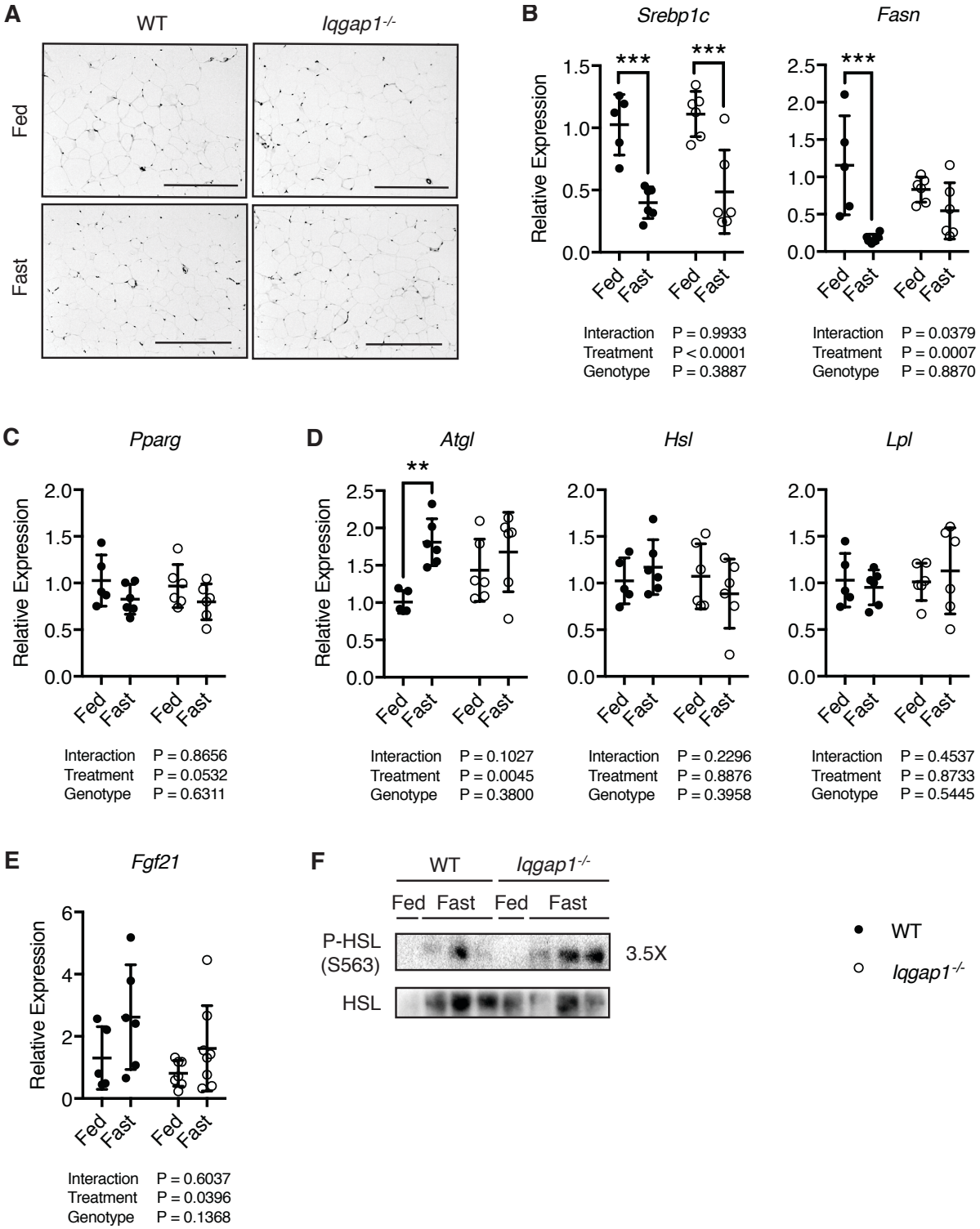


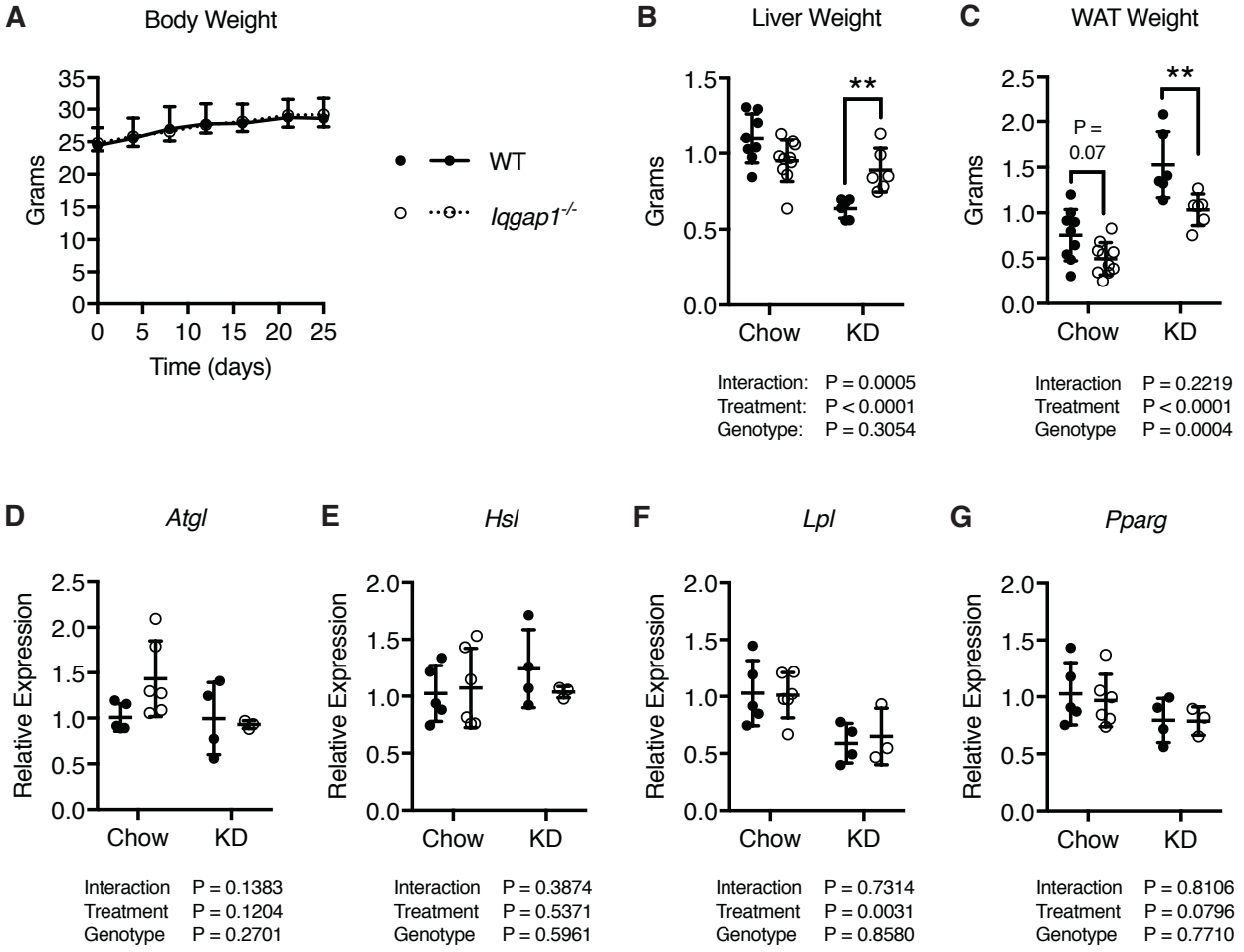
Supplemental Figure 1. Characterization of *Iqgap1* transcript expression and glucose metabolism during fasting. WT and *Iqgap1*$^{-/-}$ mice were fed *ad libitum* or fasted 24 hours (n = 5-9 mice per group). (A) *Iqgap1* gene expression normalized to *Gapdh* expression in liver, hepatocytes, and non-parenchymal cells (NPCs) of fed WT mice. (B) Gene expression of *Iqgap1* in gonadal white adipose tissue (WAT) of fed and fasted WT mice normalized to *Gapdh* expression. (C) Immunoblot of protein extracts from HepG2 cells subjected to serum or glucose deprivation. Each lane contains pooled protein from three separate wells. (D) Serum glucose in fed and 24 hour fasted states. (E) Intraperitoneal glucose tolerance test (n = 7 mice per group).

Mean area under curve (AUC) is listed. (F) Hepatic gene expression of *Pepck*, *Pgc1a,* and *Hnf4a* normalized to *Gapdh* expression in WT and *Iqgap1*$^{-/-}$ mice. Values are displayed as mean ± SD. One-way ANOVA with Bonferroni multiple comparisons test was used to determine significance between three groups under one condition. Two-way ANOVA with Bonferroni multiple comparisons test was used to determine significance between two groups under two conditions. Significance is indicated with **** $p < 0.0001$.

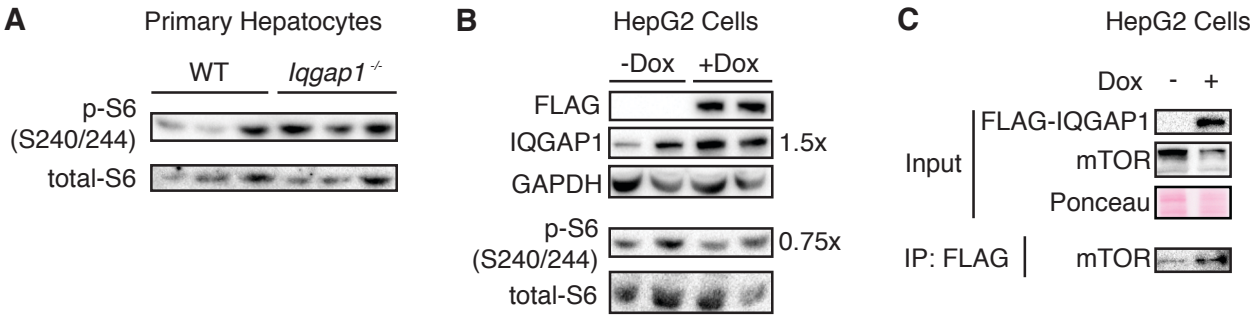


Supplemental Figure 2. White adipose tissue response to fasting is comparable between WT and *Iqgap1*$^{-/-}$ mice. Both groups of mice were fed *ad libitum* or fasted for 24 hours. (A)

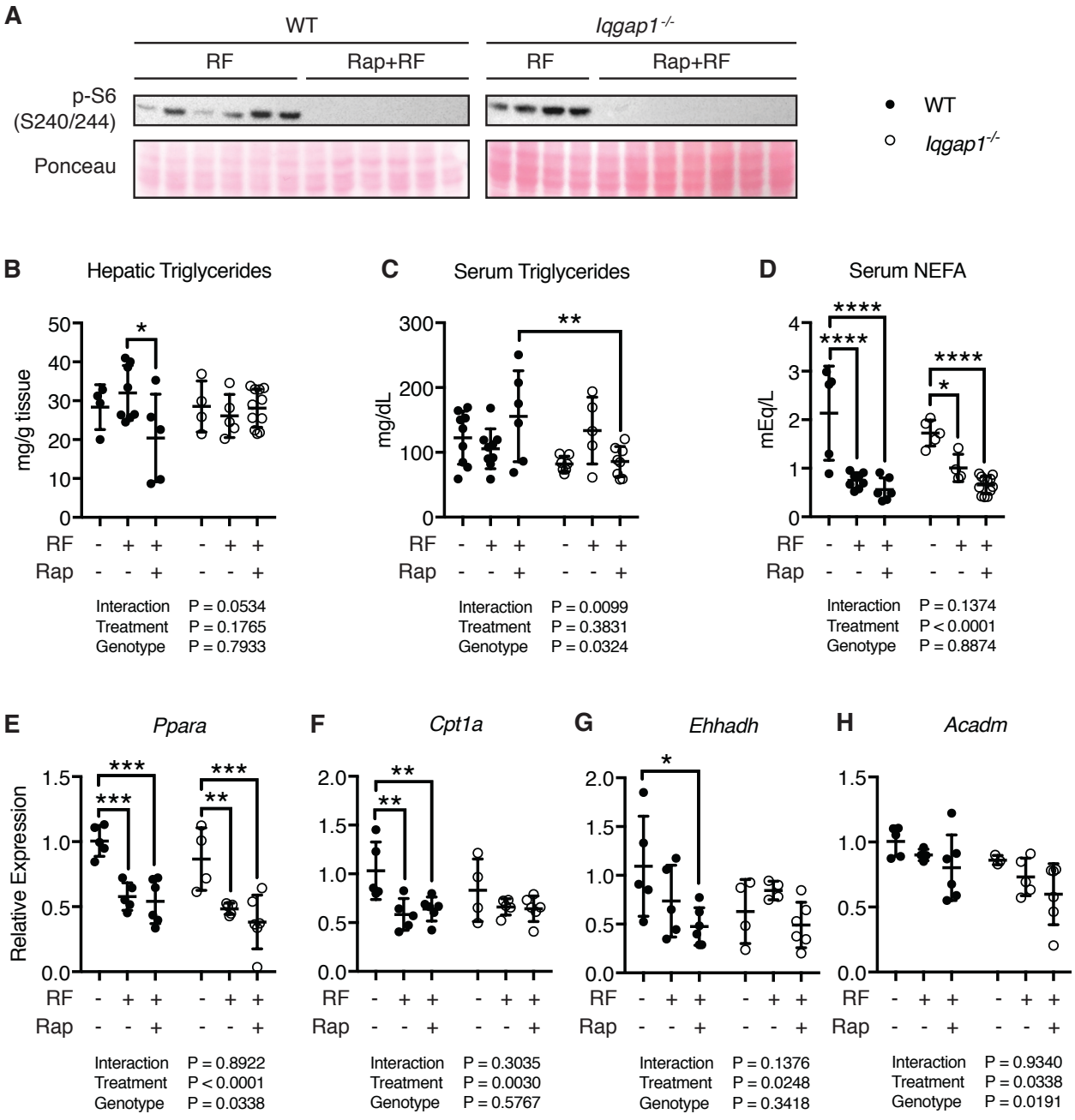
Representative images of gonadal WAT sections stained with hematoxylin/eosin (H&E). (B-E) WAT gene expression of (B) lipogenic genes *Srebp1c, and Fasn,* (C) *Pparg,* (D), lipases, *Atgl, Hsl, and Lpl* and (E) *Fgf21* normalized to *36b4* expression in WT and *Iqgap1*$^{-/-}$ mice. (F) Immunoblot of WT and *Iqgap1*$^{-/-}$ WAT protein extracts. Each lane contains WAT extracts from a single mouse. Values are displayed as mean ± SD. Two-way ANOVA with Bonferroni multiple comparisons test was used to determine significance between two groups under three conditions. Significance is indicated with ** $p < 0.01$ and *** $p < 0.001$.



Supplemental Figure 3. Gravimetric analysis and gonadal gene expression of WT and *Iqgap1*$^{-/-}$ mice on KD. WT and *Iqgap1*$^{-/-}$ mice were fed KD for 4 weeks. (A) Body weight measured over time during KD feeding (n = 6 mice per group). (B) Liver weight. (C) WAT weight. (D) WAT expression of (D) *Atgl,* (E) *Hsl*, (F) *Lpl,* and (G) *Pparg* is not affected by the absence of IQGAP1. Gene expression normalized to *36b4* expression. Values are displayed as mean ± SD. Two-way ANOVA with Bonferroni multiple comparisons test was used to determine significance between two groups under two conditions. Significance is indicated with ** $p < 0.01$.

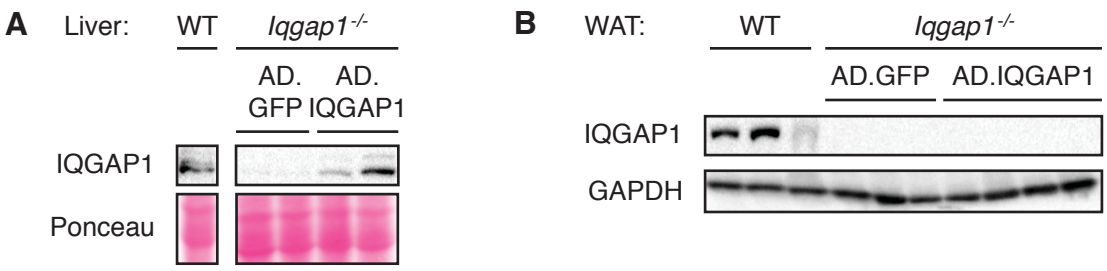


Supplemental Figure 4. IQGAP1 can interact with mTOR and regulates its activity. (A) Immunoblot of protein extracts from WT and *Iqgap1*$^{-/-}$ primary hepatocytes cultured for 24 hours on collagen-coated plates. Each lane represents hepatocytes from an individual mouse (n = 3 mice per group). (B) HepG2 cells were transfected with dox-inducible FLAG-IQGAP1 construct and treated with 2 ng/μL doxycycline (+Dox) or vehicle (-Dox) for 24 hours. Immunoblot of protein extracts. Each lane represents an individual well. IQGAP1 levels were normalized to GAPDH expression and p-S6 (S240/244) levels were normalized to total-S6 expression. Average relative level in presence of dox relative to vehicle is indicated. (C) FLAG-tagged IQGAP1 was immunoprecipitated using anti-FLAG antibodies from equal amounts of cell lysate from HepG2 cells expressing FLAG-IQGAP1 (+Dox) or controls (-Dox). Overexpression of FLAG-IQGAP1 was confirmed and mTOR levels were examined in whole cell lysates (input). Ponceau S was used as a loading control. Pulled down proteins were then separated by SDS-PAGE and blotted for mTOR (IP:FLAG). Values are displayed as mean ± SD. Two-way ANOVA with Bonferroni multiple comparisons test was used to determine significance between two groups. Significance is indicated with ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.



Supplemental Figure 5. Acute mTORC1 inhibition does not restore β-oxidation defect seen in *Iqgap1*$^{-/-}$ mice. (A) Immunoblot of protein extracts from WT and *Iqgap1*$^{-/-}$ mice fasted for 24 hours and refed 2 hours or mice treated with 10 mg/kg rapamycin 1 hour prior to refeeding. Ponceau S was used as a loading control. Each lane represents an individual mouse.

(B) Hepatic triglycerides, (C) serum triglycerides, and (D) serum NEFA were measured (n = 4-8 mice per group). (E-H) Hepatic gene expression of (E) *Ppara*, (F) *Cpt1a*, (G) *Ehhadh*, and (H) *Acadm* normalized to *Gapdh* expression (n = 4-6 mice per group). Values are displayed as mean ± SD. Two-way ANOVA with Bonferroni multiple comparisons test was used to determine significance between two groups under two conditions. Significance is indicated with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.



Supplemental Figure 6. Viral overexpression of IQGAP1 is specific to the liver. (A) Immunoblot of liver extracts from WT mice and *Iqgap1*$^{-/-}$ mice injected with adenoviruses-AD.GFP and AD.IQGAP1 by tail vein injection. (B) Immunoblot from gonadal WAT protein extracts from WT mice and *Iqgap1*$^{-/-}$ mice infected with AD.GFP and AD.IQGAP1 adenoviruses by tail vein injection. Each lane contains protein from a single mouse.

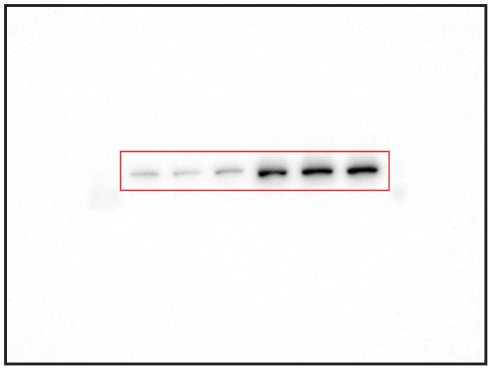
Supplemental Table 1. Primers used.

Gene	Forward (5' → 3')	Reverse (5' → 3')
36b4	AGATGCAGCAGATCCGCAT	GTTCTTGCCCATCAGCACC
Acadm	GATGAAGGTTGAACTCGCTAGG	CCTTGCAATCGAGGCATAGTA
Atgl	TCCGTGGCTGTCTACTAAAGA	TGGGATATGATGACGTTCTCTCC
Bdh1	GTTAACAACGCAGGCATCTC	CACTTCAGCCACCTCCTTAT
Cpt1a	TGATGACGGCTATGGTGTTTC	CAAACAAGGTGATAATGTCCATC
Cyp4a10	GCTGAGGTGGACACATTCAT	AGGCTCTGAACTTCCTCTCT
Ehhadh	GCCATCAAGGAAGAAGCAAAG	CTGAGGGAGTTGACCACTTATT
Fasn	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT
Fgf21	CAAATCCTGGGTGTCAAAGC	CATGGGCTTCAGACTGGTAC
Gapdh	AACTTTGGCATTGTGGAAGG	ACACATTGGGGGTAGGAACA
Hadha	CGAAGTGGGTGTGGATGTAG	CCTTGGAGACCATCTGTTTCA
Hmgcs2	CCTCTGTGAATCCTGGGTGT	CTGTGGGGAAAGATCTGCAT
Hnf4a	AGGTGCCAACCTCAATTCATC	TCGAGGCTCCGTAGTGTTT
Hsl	CTGGTGCAGAGAGACACTTC	CTTGCGTCCACTTAGTTCCA
Lpl	AACAAGGTCAGAGCCAAGAG	CCATCCTCAGTCCCAGAAAAG
Pepck	GTTCCCAGGGTGCATGAAAG	AGGGCGAGTCTGTCAGTTCAA
Pgc1a	CCCACAGAAAACAGGAACAG	CTGGGGTCAGAGGAAGAGAT
Ppara	ACAAGGCCTCAGGGTACCA	GCCGAAAGAAGCCCTTACAG
Pparg	CAAGAATACCAAAGTGCGATCAA	GAGCTGGGTCTTTTCAGAATAATAAG
Srebp1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT

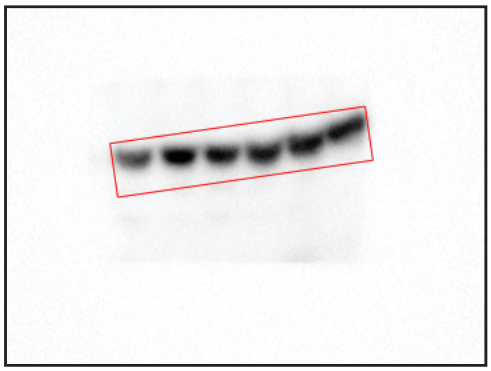
Supplemental Figure 7

Full unedited gels for Figure 1A.

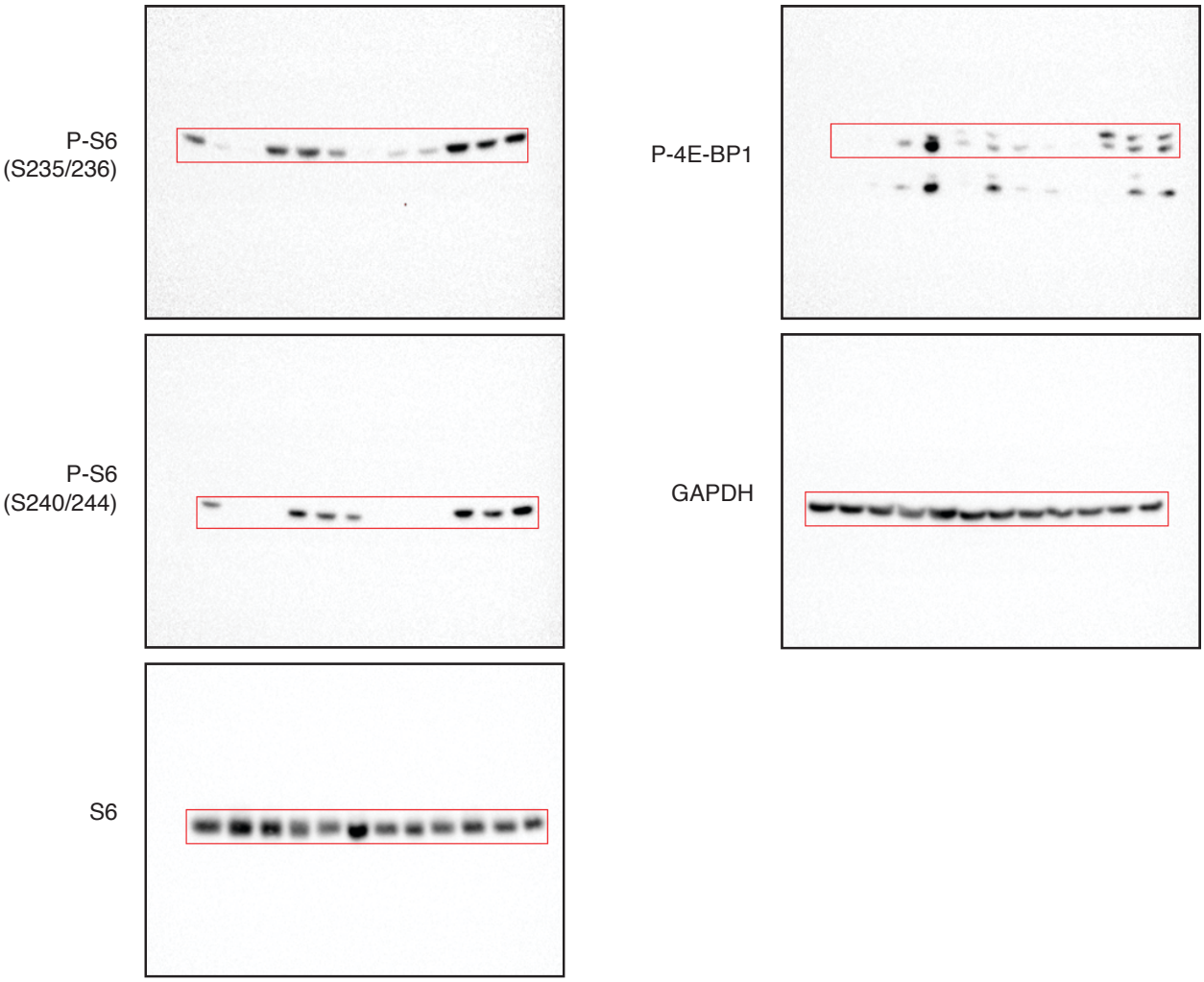
IQGAP1



GAPDH

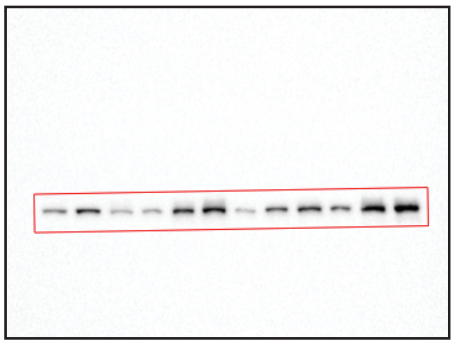


Full unedited gels for Figure 5A.

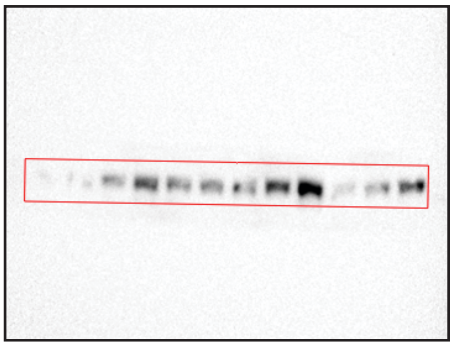


Full unedited gels for Figure 5B.

P-mTOR (S2448)

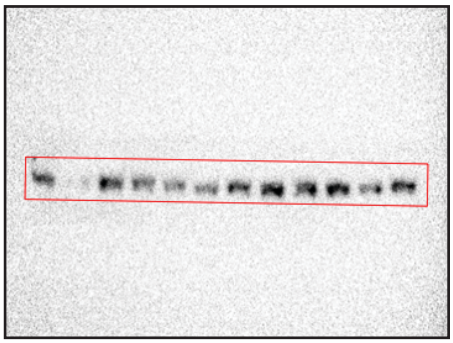


P-AMPKα (T172)

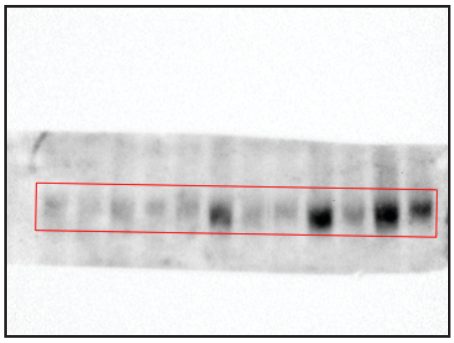


mTOR

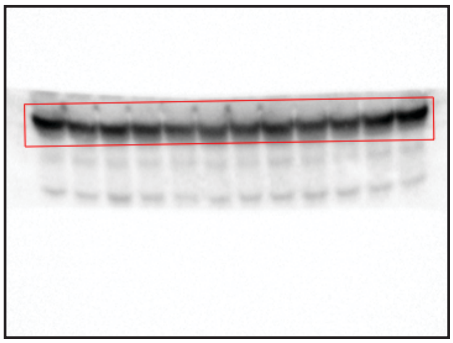
AMPKα



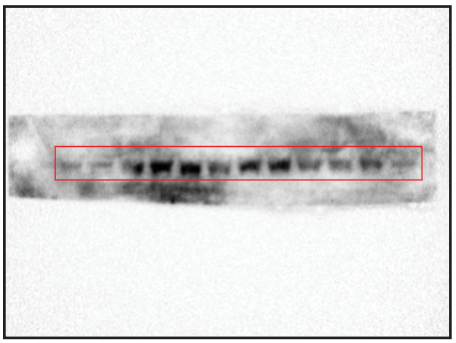
P-AKT (S473)



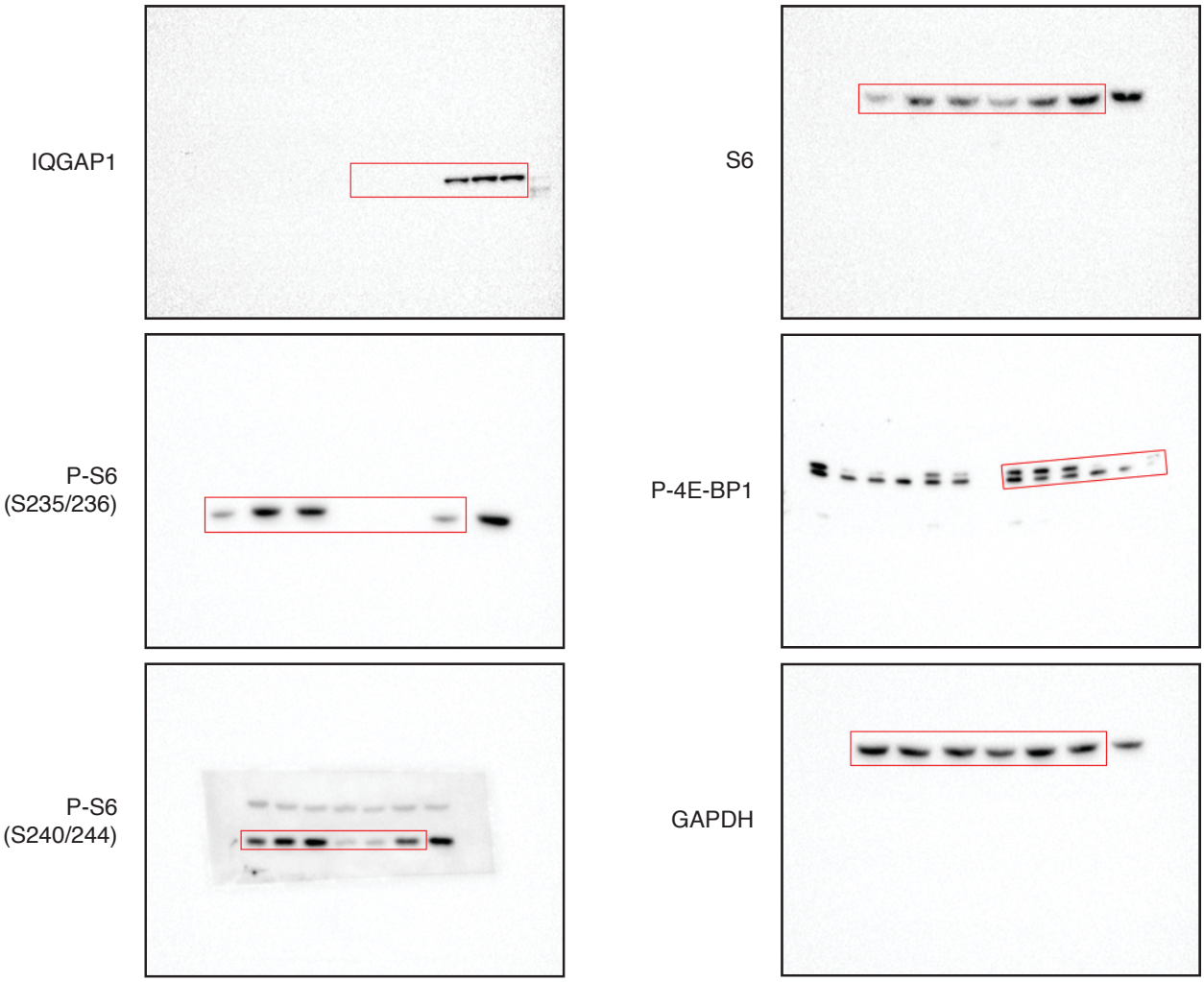
GAPDH



Pan AKT

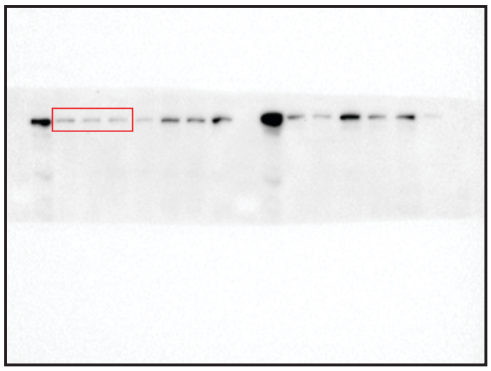


Full unedited gels for Figure 6A.

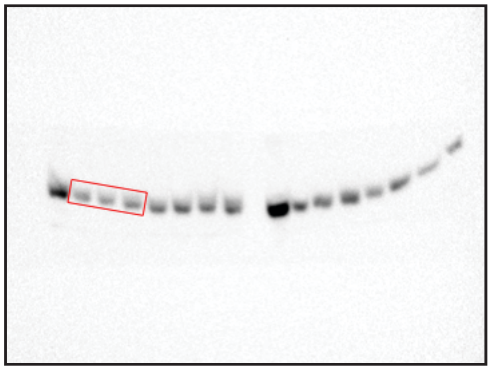


Full unedited gels for Supplemental Figure 1C.

IQGAP1

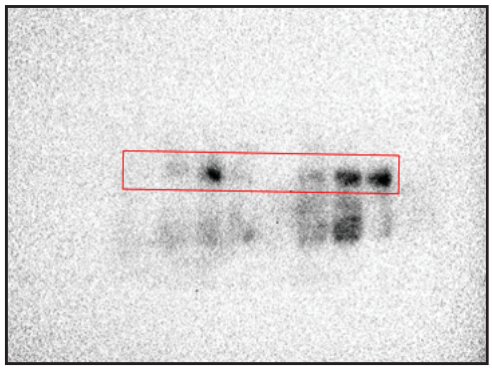


GAPDH

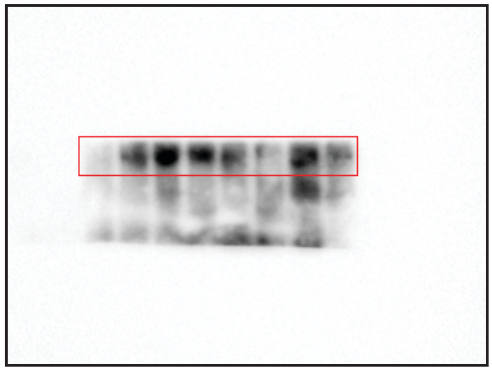


Full unedited gels for Supplemental Figure 2F.

P-HSL
(S563)

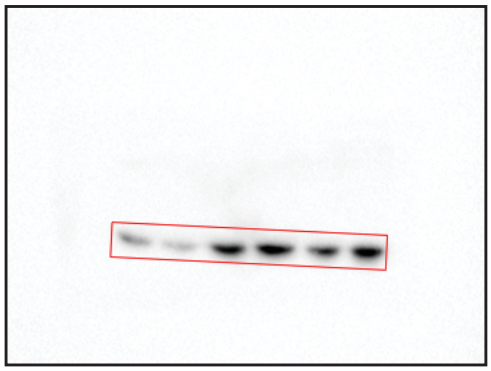


HSL

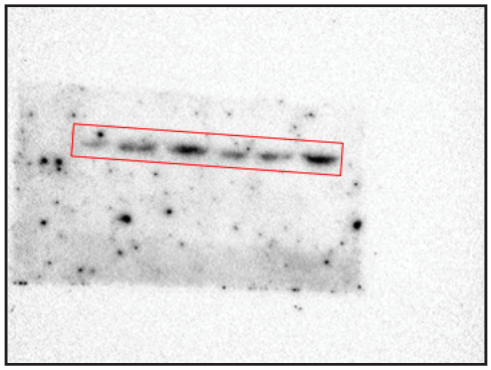


Full unedited gels for Supplemental Figure 4A.

P-S6
(S240/244)



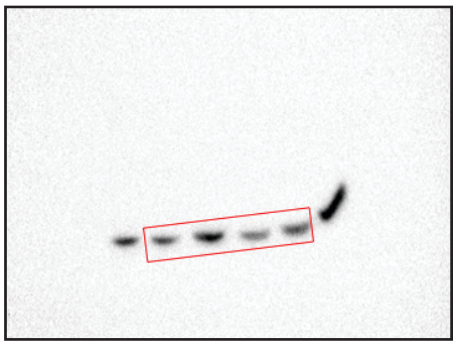
S6



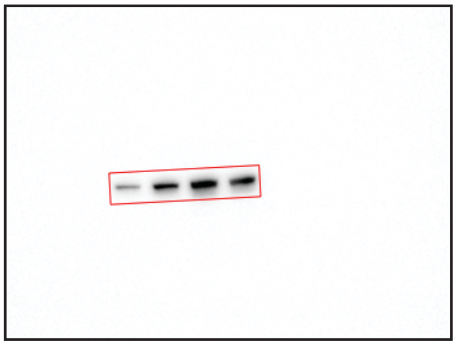
Full unedited gels for Supplemental Figure 4B.

FLAG

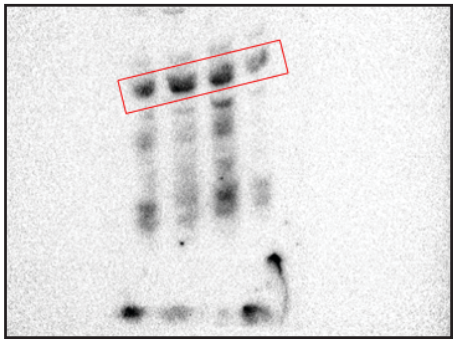
P-S6
(S240/244)



IQGAP1



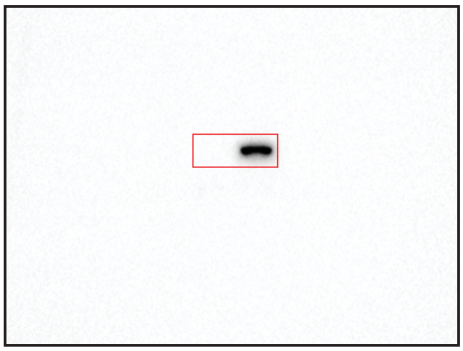
S6



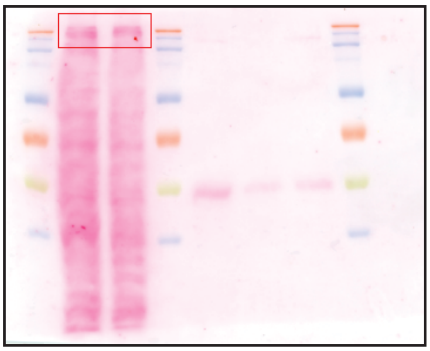
GAPDH

Full unedited gels for Supplemental Figure 4C.

FLAG

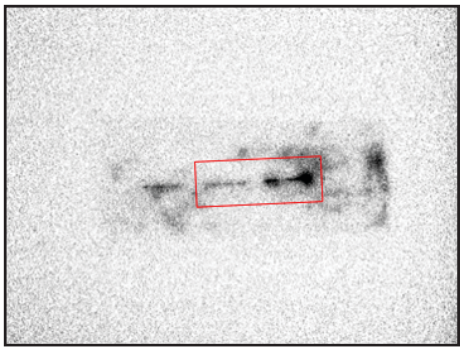


Ponceau S



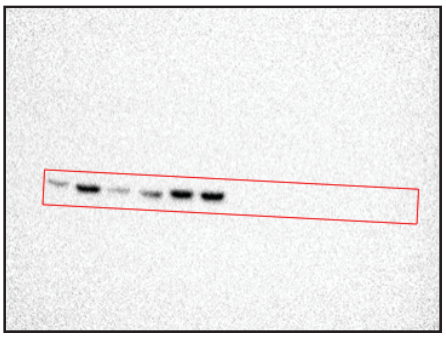
mTOR

IP: FLAG
IB: mTOR



Full unedited blots for Supplemental Figure 5A.

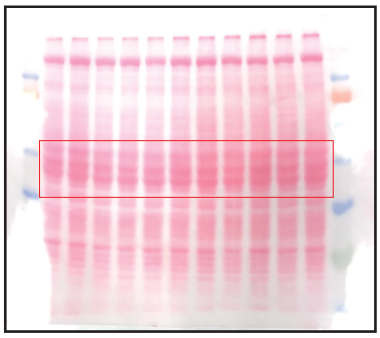
P-S6
(S240/244)



Ponceau S

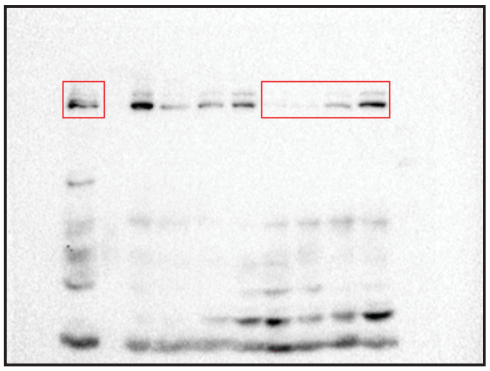
P-S6
(S240/244)

Ponceau S

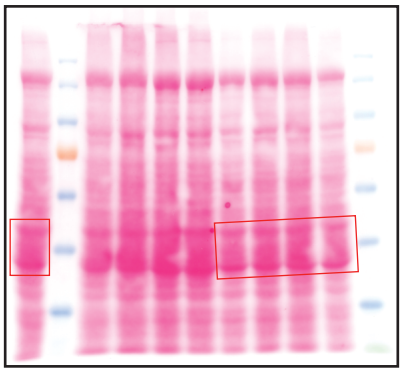


Full unedited gels for Supplemental Figure 6A.

IQGAP1

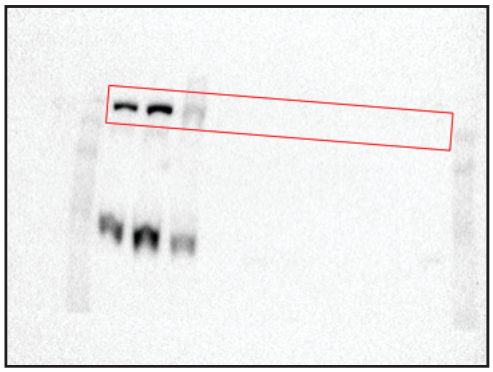


Ponceau S



Full unedited gels for Supplemental Figure 6B.

IQGAP1



GAPDH