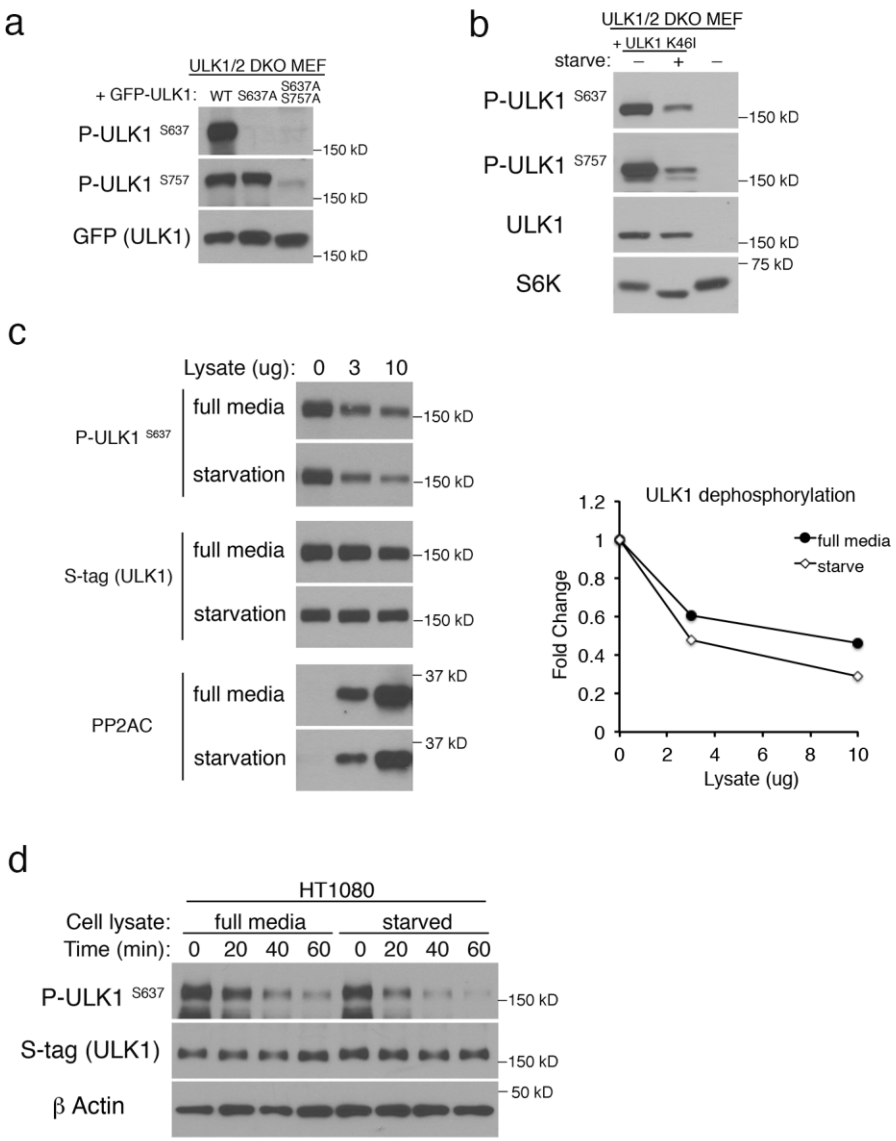
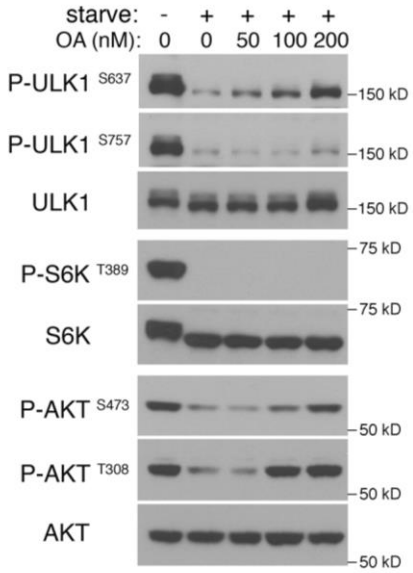
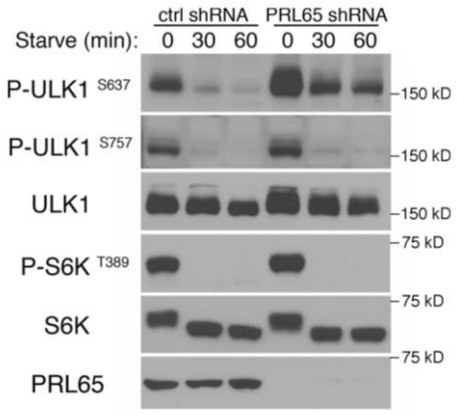


Supplementary Figure 1 Phosphatase activity is required for starvation induced autophagy. (**a, b**) ULK1 S637 and S757 phospho antibodies are specific. In (a), the antibodies cannot recognize ULK1 when the respective sites of ULK1 (S637, S757) are mutated to alanine. In (b), the antibodies recognized ectopic ULK1 (K46I mutant) expressed in ULK1/2DKO MEFs, and the signal was reduced upon starvation, correspondent with dephosphorylation of ULK1. (**c**) Starvation increases phosphatase activity in MEF cells. In vitro phosphatase assay was carried out using Flag-S-ULK1 as a substrate and increasing amounts of total cell lysate from MEFs that were kept in full media or starvation media. Reactions were incubated at 30^{o}C for 30 min. (**d**) Starvation increases phosphatase activity in HT1080 cells. In vitro phosphatase assay was carried out using 10 μg of total cell lysate from HT1080 cells that were kept in full media or starvation media. Reactions were terminated at the indicated time.

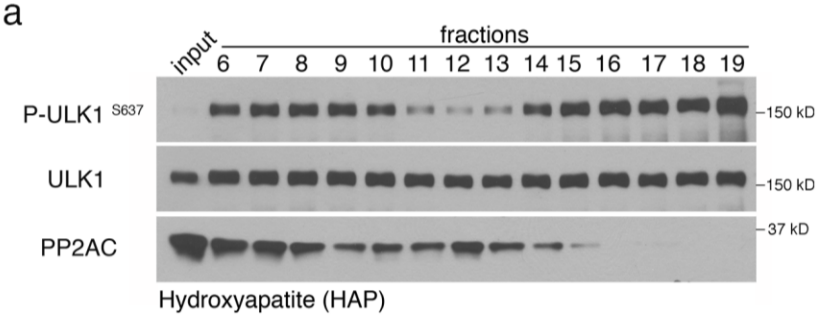


Supplementary Figure 2 Okadaic acid (OA) inhibits the dephosphorylation of ULK1 at S637 in a dose dependent manner. MEF cells were incubated in starvation media with increasing amounts of OA for 1 hr. Phosphorylation on ULK1 and S6K were monitored using site specific phospho-antibodies. AKT, previously reported as OA sensitive, was included as a positive control for OA treatment.



Supplementary Figure 3 PRL65 is required for ULK1 S637 dephosphorylation. MEF cells were transduced with lentivirus containing control shRNA or shRNA targeting PRL65, the scaffolding subunit of PP2A. 48-72 hrs post transduction, cells were incubated in starvation media for the indicted time and analysed by immunoblotting.

a



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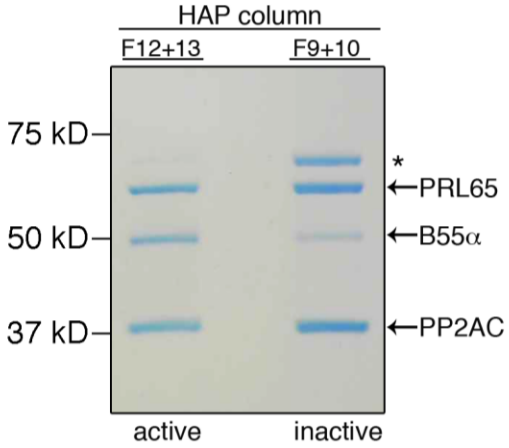


c

Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit gamma isoform (PPP2R5C)

```
  1 MLTCNKAGSG MVVDAASSNG PFQPVALLHI RDVPPADQEK LFIQKLRQCC
 51 VLFDFVSDPL SDLKWKEVKR AALSEMVEYI THNRNVITEP IYPEAVHMFA
101 VNMFRTLPPS SNPTGAEFDP EEDEPTLEAA WPHLQLVYEF FLRFLESPDF
151 QPNIAKKYID QKFVLQLLEL FDSEDPRERD FLKTTLHRIY GKFLGLRAYI
201 RKQINNIFYR FIYETEHHNG IAELLEILGS IINGFALPLK EEHKIFLLKV
251 LLPLHKVKSL SVYHPQLAYC VVQFLEKDST LTEPVVMALL KYWPKTHSPK
301 EVMFLNELEE ILDVIEPSEF VKIMEPLFRQ LAKCVSSPHF QVAERALYYW
351 NNEYIMSLIS DNAAKILPIM FPSLYRNSKT HWNKTIHGLI YNALKLFMEM
401 NQKLFDDCTQ QFKAEKLKEK LKMKEREEAW VKIENLAKAN PQAQKELKKD
451 RPLVRRKSEL PQDPHTEKAL EAHCRASELL SQDGR
```

sequence coverage: 24%

Supplementary Figure 4 Only a subset of PP2A complexes in the cell can dephosphorylate ULK1. (**a**) Not all fractions containing PP2AC have activity against ULK1. Fractions from Hydroxyapatite column were assessed for activity against ULK1 and presence of PP2AC. Active fractions (11-13) were combined as input for the next purification step. (**b, c**) Regulatory subunit purified from inactive fraction of HAP column. (b) shows a Coomassie blue gel of PP2A purified from active and inactive fractions of the HAP column. * indicates unknown band that was excised and analyzed by mass spectrometry, which was identified as the B'γ regulatory subunit. (c) shows results of mass spectrometry analysis. Peptide fragments matched to the B'γ regulatory subunit of PP2A with a sequence coverage of 24%. Identified proteins: At $p<0.01$, average false discovery rate, FDR: 0%.

a

Serine/threonine-protein phosphatase 2A catalytic subunit beta isoform (PPP2CB)

```
  1 MDDKAFTKEL DQWVEQLNEC KQLNENQVRT LCEKAKEILT KESNVQEVRC
 51 PVTVCGDVHG QFHDLMELFR IGGKSPDTNY LFMGDYVDRG YYSVETVTLL
101 VALKVRYPER ITILRGNHES RQITQVYGFY DECLRKYGNA NVWKYFTDLF
151 DYLPLTALVD GQIFCLHGGL SPSIDTLDHI RALDRLQEVP HEGPMCDLLW
201 SDPDDRGGWG ISPRGAGYTF GQDISETFNH ANGLTLVSRA HQLVMEGYNW
251 CHDRNVVTIF SAPNYCYRCG NQAAIMELDD TLKYSFLQFD PAPRRGEPHV
301 TRRTPDYFL
```

sequence coverage: 38.5%

b

Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform (PPP2R1A)

```
  1 MAAADGDDSL YPIAVLIDEL RNEDVQLRLN SIKKLSTIAL ALGVERTRSE
 51 LLPFLTDTIY DEDEVLLALA EQLGTFTTLV GGPEYVHCLL PPLESLATVE
101 ETVVRDKAVE SLRAISHEHS PSDLEAHFVP LVKRLAGGDW FTSRTSACGL
151 FSVCYPRVSS AVKAELRQYF RNLCSDDTPM VRRAAASKLG EFAKVLELDN
201 VKSEIIPMFS NLASDEQDSV RLLAVEACVN IAQLLPQEDL EALVMPTLRQ
251 AAEDKSWRVR YMVADKFTEL QKAVGPEITK TDLVPAFQNL MKDCEAEVRA
301 AASHKVKEFC ENLSADCREN VIMTQILPCI KELVSDANQH VKSALASVIM
351 GLSPILGKDN TIEHLLPLFL AQLKDECPEV RLNIISNLDC VNEVIGIRQL
401 SQSLLPAIVE LAEDAKWRVR LAIIEYMPLL AGQLGVEFFD EKLNSLCMAW
451 LVDHVYAIRE AATSNLKKLV EKFGKEWAHA TIIPKVLAMS GDPNYLHRMT
501 TLFCINVLSE VCGQDITTKH MLPTVLRMAG DPVANVRFNV AKSLQKIGPI
551 LDNSTLQSEV KPILEKLTQD QDVDVKYFAQ EALTVLSLA
```

sequence coverage: 37.2%

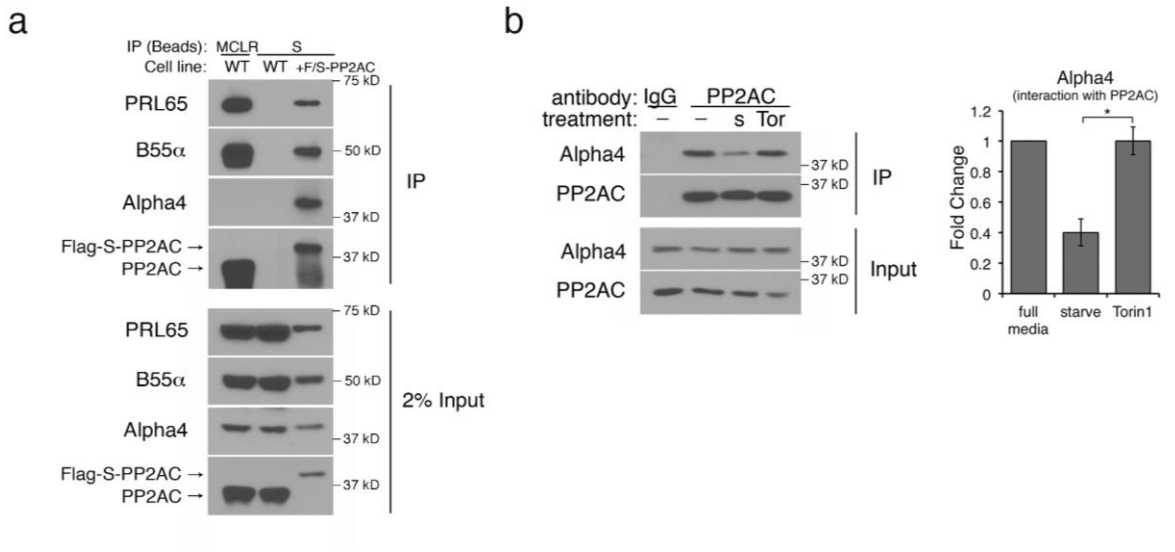
c

Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform (PPP2R2A)

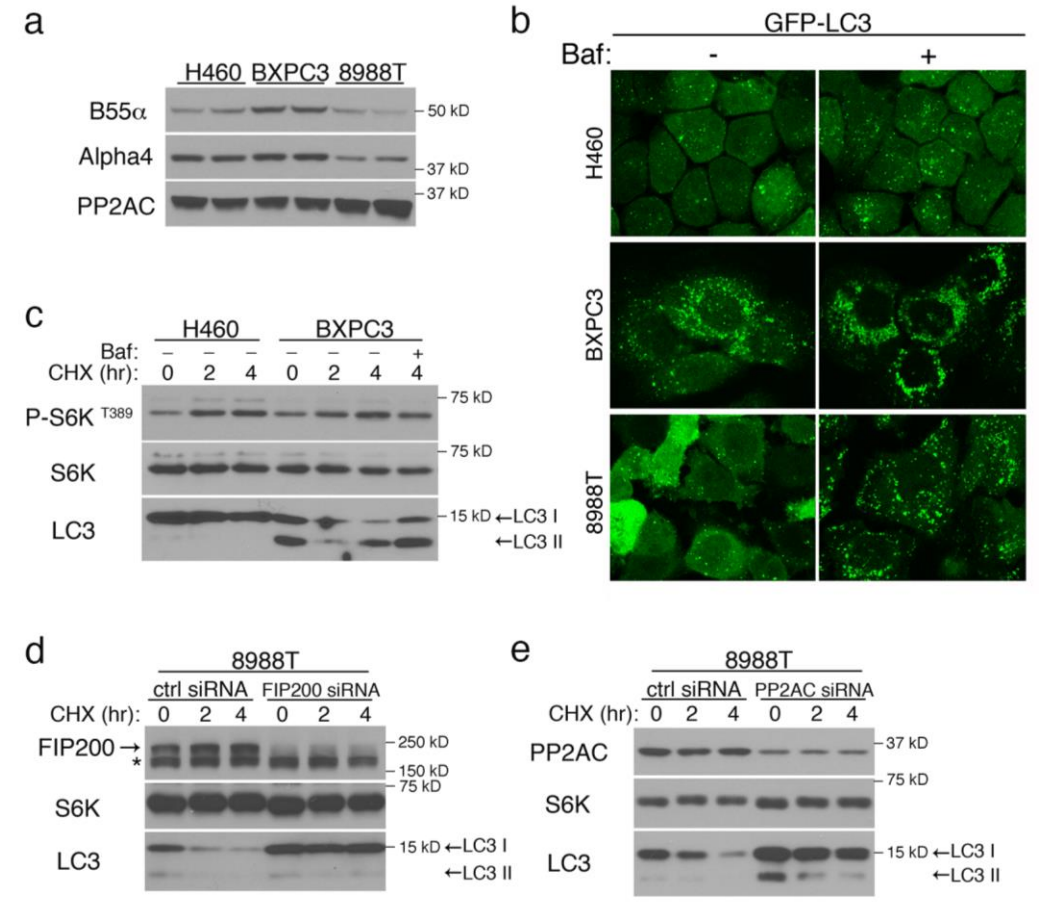
```
  1 MAGAGGGNDI QWCFSQVKGA VDDDVAEADI ISTVEFNHSG ELLATGDKGG
 51 RVVIFQQEQE NKIQSHSRGE YNVYSTFQSH EPEFDYLKSL EIEEKINKIR
101 WLPQKNAAQF LLSTNDKTIK LWKISERDKR PEGYNLKEED GRYRDPTTVT
151 TLRVPVFRPM DLMVEASPRR IFANAHTYHI NSISINSDYE TYLSADDLRI
201 NLWHLEITDR SFNIVDIKPA NMEELTEVIT AAEFHPNSCN TFVYSSSKGT
251 IRLCDMRASA LCDRHSKLFE EPEDPSNRSF FSEIISSISD VKFSHSGRYM
301 MTRDYLSVKI WDLNMENRPV ETYQVHEYLR SKLCSLYEND CIFDKFECCW
351 NGSDSVVMTG SYNNFFRMFD RNTKRDITLE ASRENNKPRT VLKPRKVCAS
401 GKRKKDEISV DSLDFNKKIL HTAWHPKENI IAVATTNNLY IFQDKVN
```

sequence coverage: 43.2%

Supplementary Figure 5 Mass spectrometry analysis of p36, p65 and p50 bands from ULK1 phosphatase purification. The peptides detected during mass spectrometry analysis are shown in red. (**a**) The p36 band produced peptide fragments that matched to the catalytic subunit of PP2A with a sequence coverage of 38.5%. (**b**) Peptide fragments from the p65 band matched to the scaffolding subunit of PP2A with a sequence coverage of 37.2%. (**c**) Peptide fragments from the p50 band matched to the B55α regulatory subunit of PP2A with a sequence coverage of 43.2%. Identified proteins: At $p<0.01$, average false discovery rate, FDR: 0%.



Supplementary Figure 6 Starvation induces Alpha4 dissociation from PP2AC. (**a**) Endogenous PP2AC-Alpha4 complex is catalytically inactive. WT MEFs (ctrl) were lysed and incubated with microcystin-LR (MCLR) beads which bind to the catalytic pocket of PP2AC. As a comparison, MEFs expressing Flag-S-PP2AC were lysed and incubated with S-beads to pull down total PP2A complexes in the cell. (**b**) WT MEFs were incubated in starvation media or media containing 1 μM Torin1 for 1 hour, lysed and incubated with control antibody or antibody against PP2AC. The amount of Alpha4 interacting with PP2AC under each condition was monitored by Immunoblotting and quantitated on the right (fold change relative to full media ± s.d., n=5. two-tail student's t-Test, * $p<0.05$).



Supplementary Figure 7 High basal autophagy in Pancreatic Ductal Adenocarcinoma cell lines is ULK1 complex dependent. (**a**) Immunoblot for endogenous levels of PP2A regulatory proteins B55α and Alpha4 in control cell line H460 and PDAC cell lines BXPC3 and 8988T. (**b**) Cell lines stably expressing GFP-LC3 were kept in complete media in the presence or absence of 20 nM bafilomycin (Baf) for 90 min. Representative images from two independent experiments shown. (**c**) BXPC3 has high basal autophagy. BXPC3 and H460 were kept in complete media with 20 μg ml^{-1} cycloheximide (CHX) for the indicated amount of time. Where indicated, 10 nM bafilomycin was added at the start of CHX treatment. Cells were lysed and immunoblotted for endogenous LC3. (**d**) FIP200 is required for basal autophagy in 8988T cells. 8988T was transduced with control siRNA or siRNA targeting FIP200, another member of the ULK1 complex. Cells were then treated as in (c). * indicates non-specific band. (**e**) PP2AC is required for basal autophagy in 8988T cells. 8988T was transduced with control siRNA or siRNA targeting the catalytic subunit of PP2A. Cells were then treated as in (c).

Figure 1c

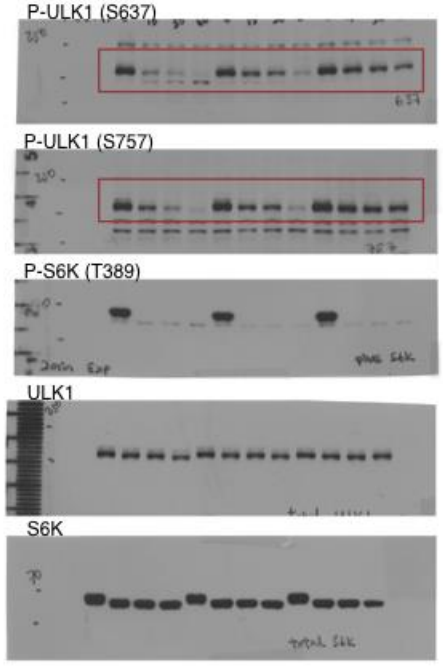


Figure 1e

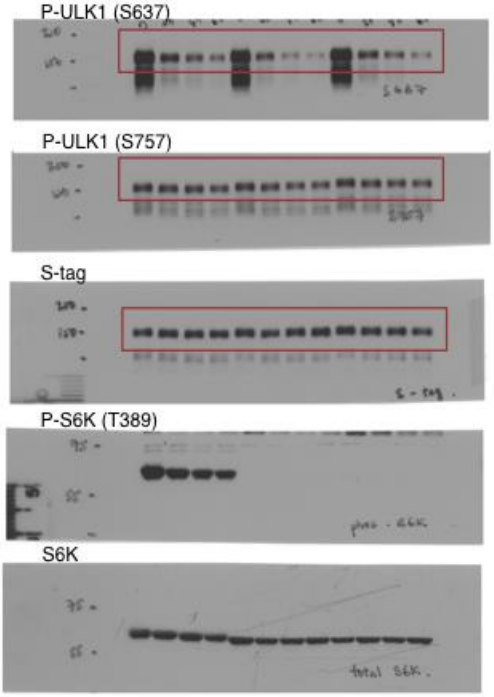


Figure 2a

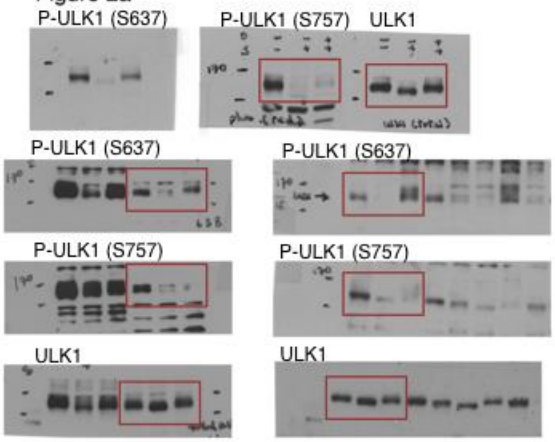


Figure 2b

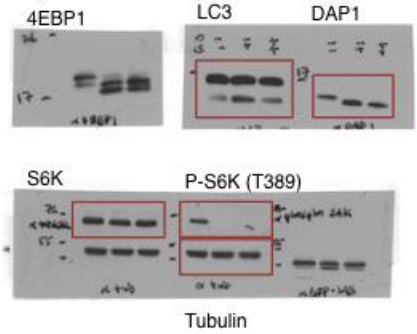
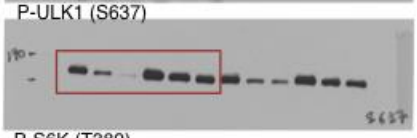


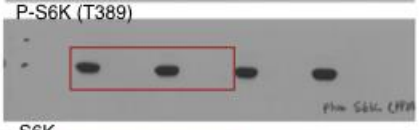
Figure 3a

P-ULK1 (S757)

P-ULK1 (S637)



P-S6K (T389)



S6K

PP2AC

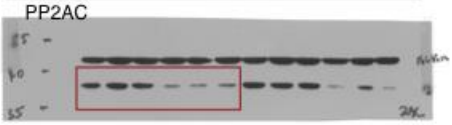
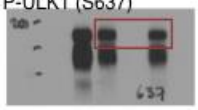


Figure 3c

P-ULK1 (S637)



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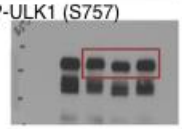
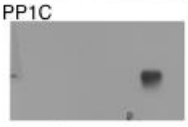


PP1C



PP2AC

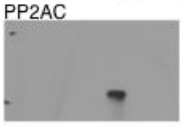
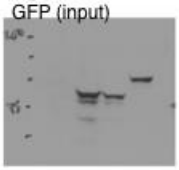


Figure 5d

GFP (input)



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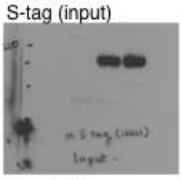

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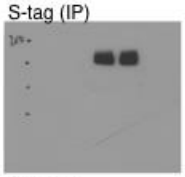

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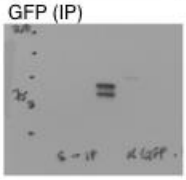
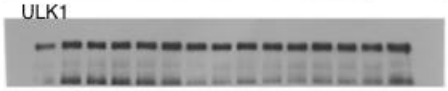


Figure 4e

P-ULK1 (S637)

ULK1



PP2AC

B55α

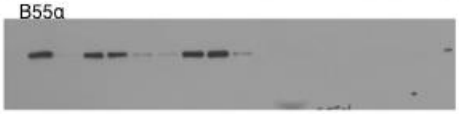
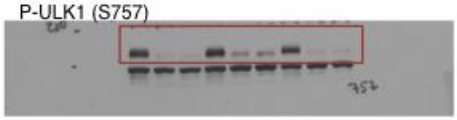
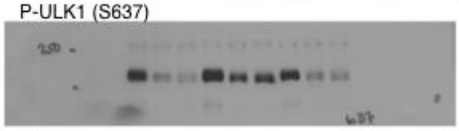


Figure 5b

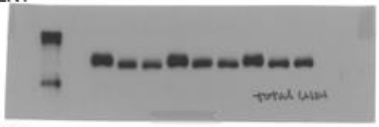
P-ULK1 (S757)



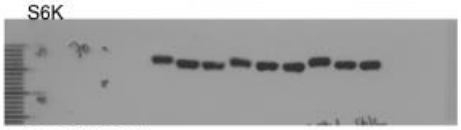
P-ULK1 (S637)



ULK1



S6K



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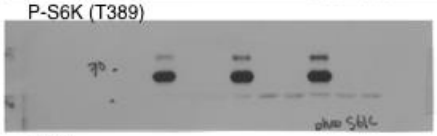


B55α

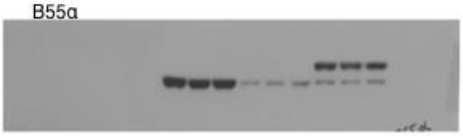


Figure 6B

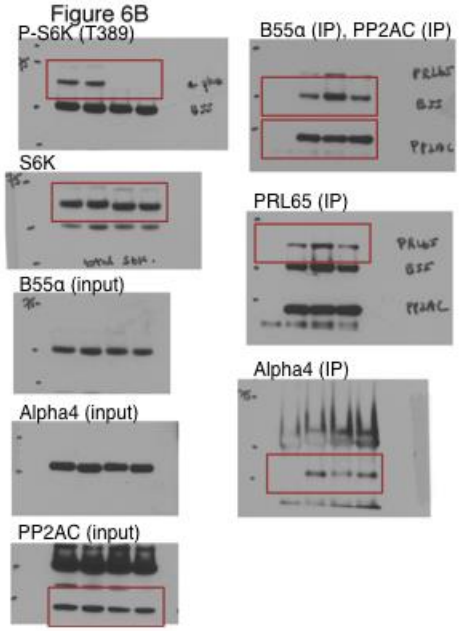


Figure 6C

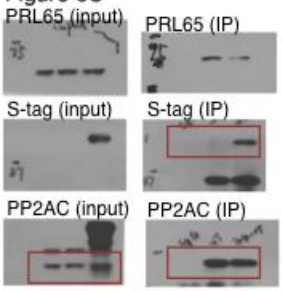


Figure 6D

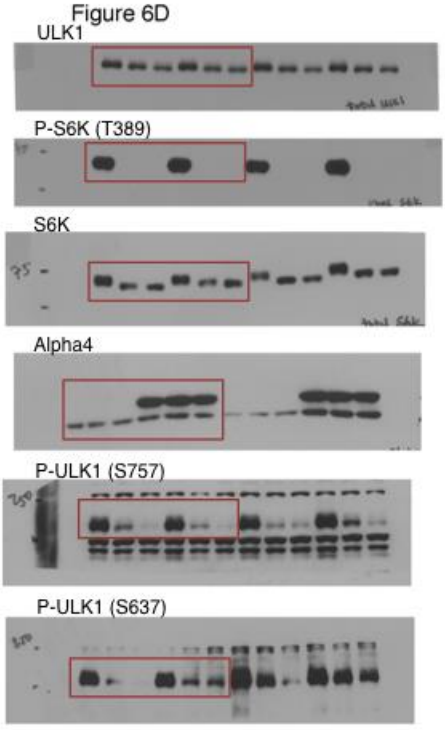
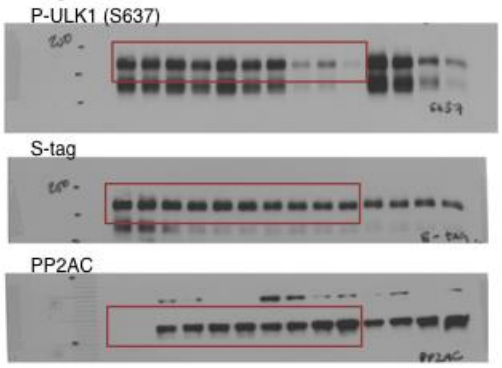
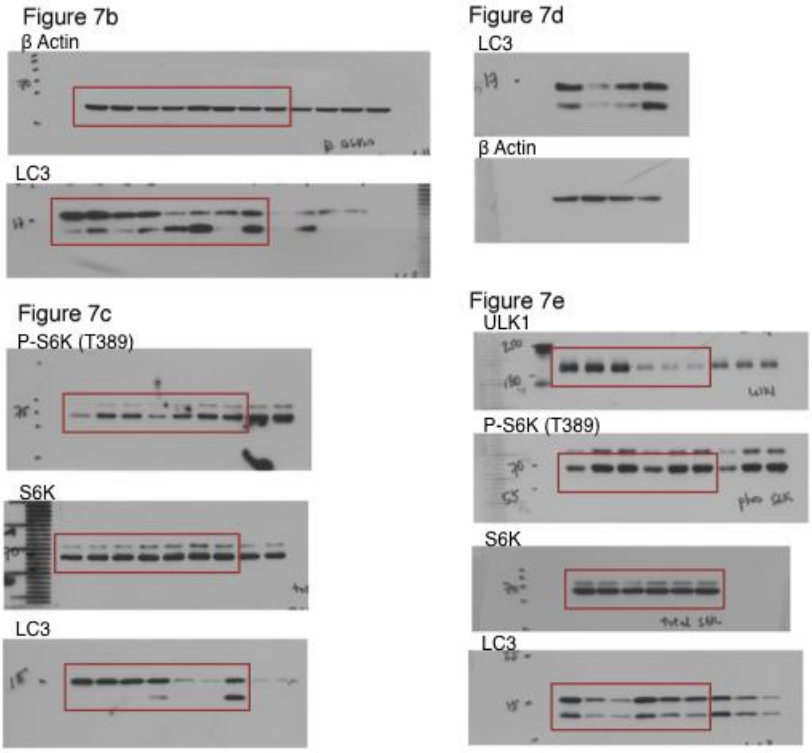


Figure 7a





Supplementary Figure 8 Full scans of western blots related to respective figures as indicated. The protein of interest being detected is labeled on the top left hand corner of each blot.