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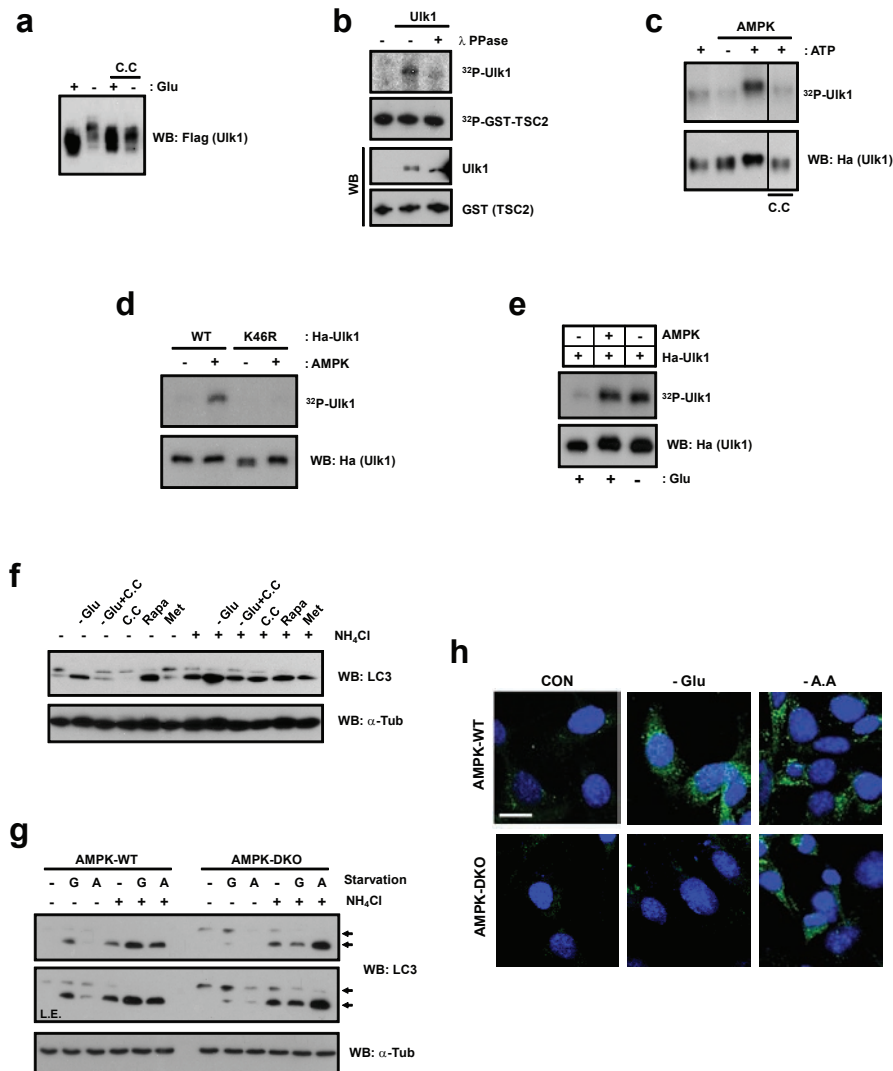


Figure S1 Ulk1 is activated by glucose starvation and by AMPK. **(a)** AMPK inhibitor blocks glucose starvation induced Ulk1 phosphorylation. Flag-Ulk1 was transfected into HEK293 cells and the cells were starved with glucose for 4hrs in the presence or absence of 20 μ M Compound C (C.C) before lysis. Total cell lysates were examined for Ulk1 mobility by a Phos-tag gel, which produced a bigger mobility shift of phosphorylated protein. **(b)** Endogenous Ulk1 kinase is activated by phosphorylation. ULK1-WT MEFs were starved with glucose for 4hrs and endogenous Ulk1 protein was immunoprecipitated. Purified Ulk1 immune-complex was treated with lambda phosphatase (λ PPase) before Ulk1 autophosphorylation reaction as described in *Methods*. 32 P-pre-labeled GST-TSC2 was also added to Ulk1 autophosphorylation reaction mixture to monitor the possible phosphatase contaminations after RIPA buffer washing. Ulk1 autophosphorylation level was determined by 32 P- autoradiogram. Total protein levels for Ulk1 and GST-TSC2 were determined by western blots. **(c)** AMPK directly stimulates Ulk1 autophosphorylation activity *in vitro*. Ha-Ulk1 was immuno-purified from the transfected HEK293 cells and pre-incubated with purified AMPK complex (Cell signaling) for 15 min under the KA buffer supplemented with 0.2 mM AMP in the presence or absence of 0.1 mM cold ATP as indicated. Also, AMPK inhibitor (Compound C, 10 μ M, denoted as C.C) was added to the reaction containing 0.1 mM ATP to confirm the reaction specificity toward AMPK. After *in vitro* AMPK reaction, Ulk1 immune-complex was extensively washed with RIPA buffer to remove AMPK and the Ulk1-bead was recovered by a centrifugation. The resulting Ulk1 immune-complex was used for Ulk1 autophosphorylation assay. **(d)** 32 P-incorporation

in Ulk1 autophosphorylation is mediated by Ulk1 kinase. Ha-Ulk1 wild-type (WT) or kinase inactive (K46R) mutant was immunoprecipitated from the transfected cells under glucose-rich medium. The Ulk1 immune complex was pre-incubated with AMPK *in vitro* for 15 min and then, washed to remove AMPK. Ulk1 autophosphorylation activity was measured as described in Fig. S1b. 32 P-incorporation in Ulk1 autophosphorylation was barely detected in Ulk1 K46R mutant even treated with AMPK. **(e)** AMPK co-transfection activates Ulk1 activity. Ha-Ulk1 and AMPK (α , β , and γ) were co-transfected into HEK293 cells. The cells were starved with glucose for 4hrs as indicated and then Ha-Ulk1 was immunoprecipitated to measure the Ulk1 autophosphorylation activity. **(f)** Glucose starvation and rapamycin stimulate autophagy. MEFs were starved with glucose (Glu) for 4hrs in the presence or absence of 20 μ M Compound C (C.C). In parallel, cells were also treated with 50 nM rapamycin (Rapa) or 2 mM Metformin (Met) for 4hrs. To examine the autophagic flux, 10 mM NH_4Cl was added as indicated. The cell lysates were probed for LC3 antibody and α -Tubulin, respectively. **(g-h)** Glucose starvation induces autophagic markers, LC3 lipidation and LC3GFP-LC3 punctuate formation, in an AMPK-dependent manner. **(g)** AMPK-WT and DKO MEFs were incubated in either glucose-free (G) or amino acid-free (A) medium for 4hrs with or without 10 mM NH_4Cl as indicated. LC3 lipidation was monitored by LC3 western. **(h)** AMPK-WT and DKO MEFs stably expressing GFP-LC3 were incubated in either glucose-free (-Glu) or amino acid free (-A.A) media for 4hrs and GFP-positive autophagosome was analyzed by confocal microscopy. Bar, 20 μ m.

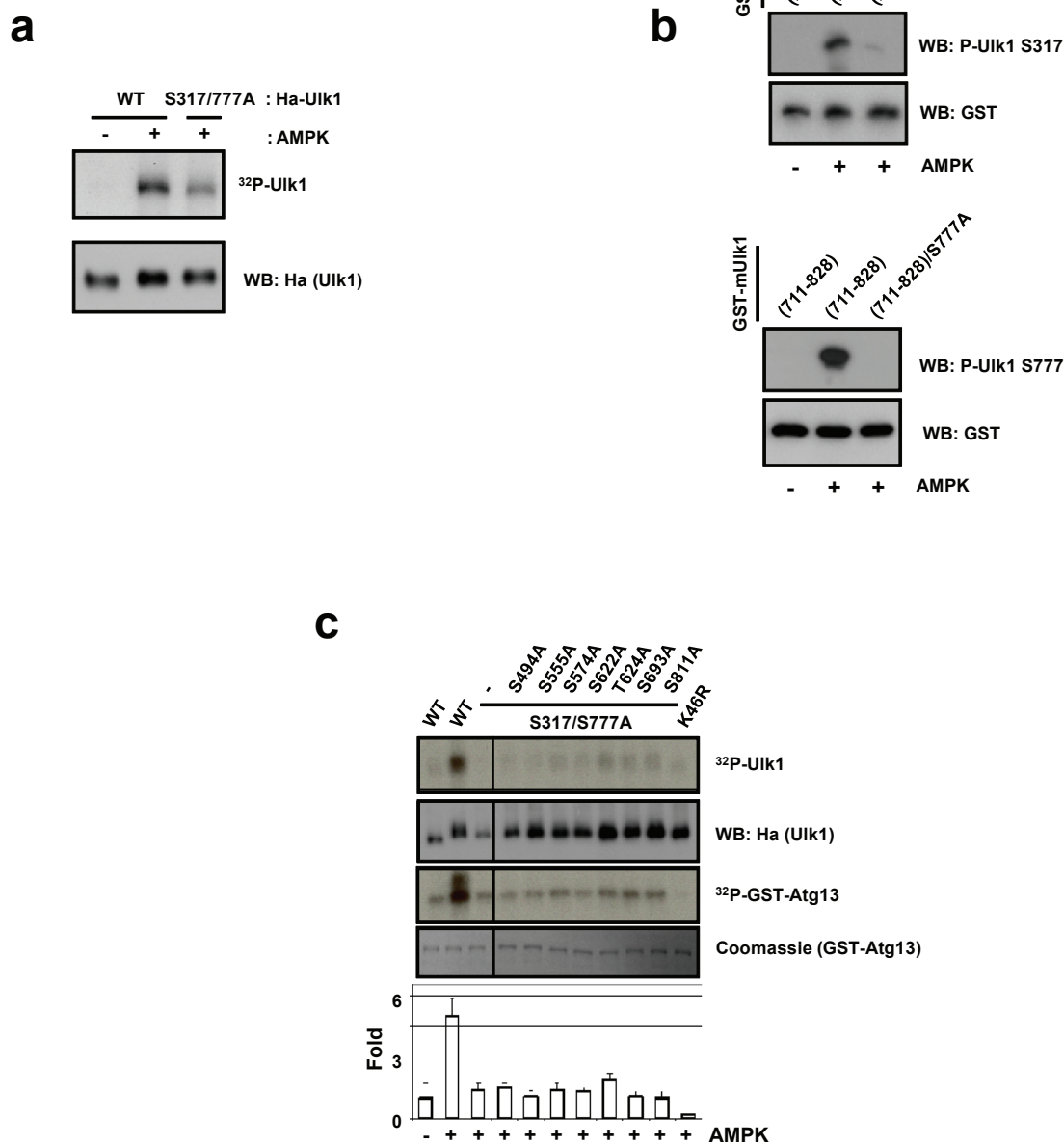


Figure S2 AMPK can activate Ulk1 by phosphorylating Ulk1 at S317 and S777 *in vitro*. **(a)** Mutation of S317 and S777 in Ulk1 decreases Ulk1 phosphorylation by AMPK *in vitro*. Ha-Ulk1 WT or S317/777A mutant were immunoprecipitated from the transfected HEK293 cells and used as substrates for *in vitro* AMPK phosphorylation. Phosphorylation level was determined by 32 P- autoradiogram. **(b)** Characterizations of Ulk1 S317 and S777 phospho-specific antibodies. Recombinant GST-mUlk1 fragments were purified from bacteria and 500 ng of the indicated recombinant fragments were used as a substrate for *in vitro* AMPK phosphorylation. After reaction, 5 ng of the phosphorylated GST-mUlk1 fragments were used to test specificity of S317 and S777 phospho-antibodies by western blot. Two phosphorylation defective mutant fragments, (279-425)/S317A and

(711-828)/S777A, were used as negative controls. **(c)** S317 and S777 are the major sites important for Ulk1 activation by AMPK. Seven putative AMPK consensus sites in Ulk1 were individually mutated in the S317/777A background and Ulk1 proteins were prepared by immuno-purification from the transfected HEK293 cells. The immunopurified Ulk1 was subjected to *in vitro* activation by AMPK and then used in Ulk1 kinase assays. Ulk1 activity was determined by autophosphorylation (32 P-Ulk1) and Atg13 phosphorylation (32 P-GST-Atg13) and normalized to Ulk1 protein levels. Western/Coomassie staining analyses were performed on a duplicate gel to that used for autoradiogram analysis. The quantification data were obtained from three-independent experiments and one representative result was shown (mean \pm S.D).

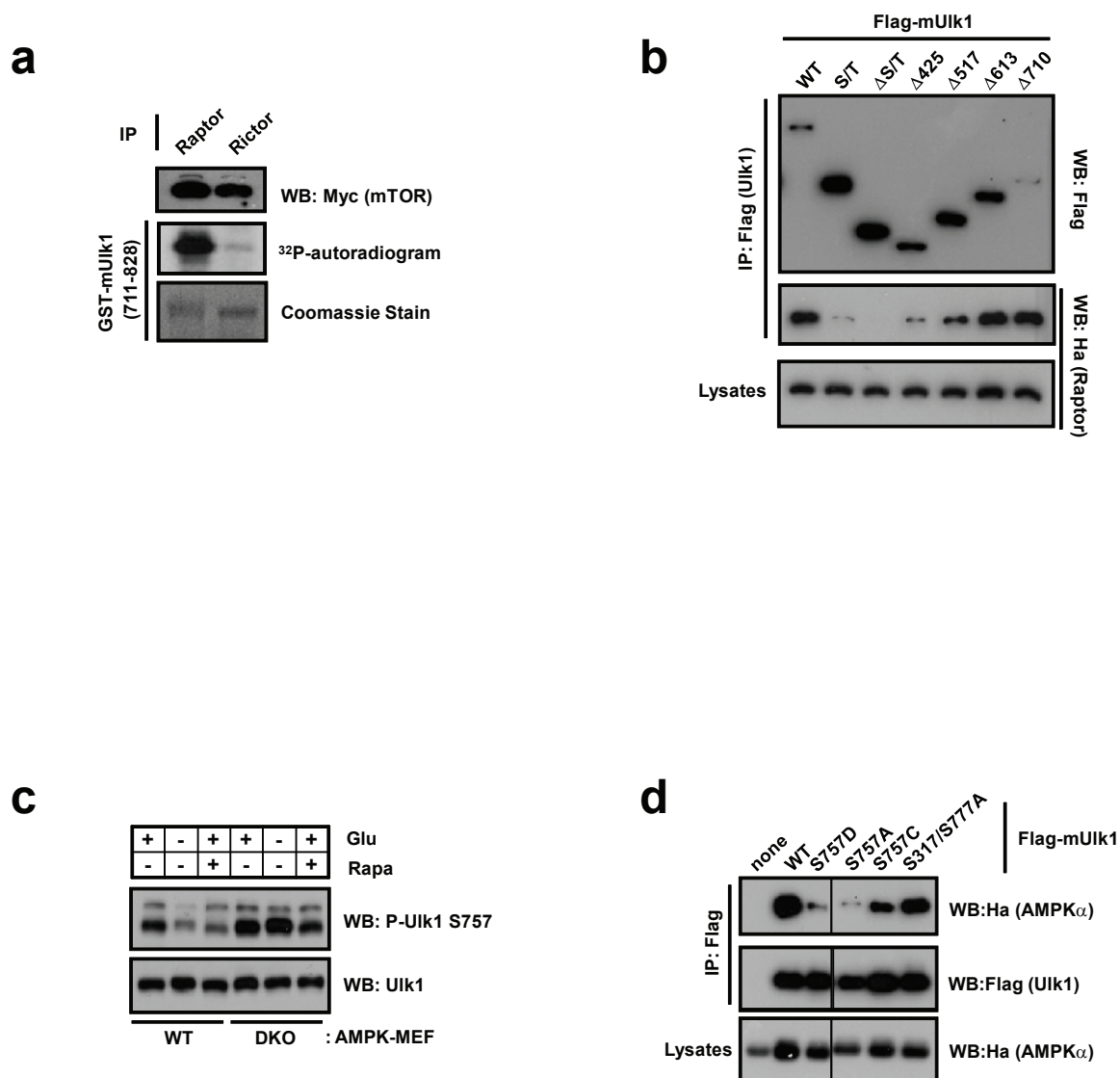


Figure S3 mTORC1 phosphorylates S757 in Ulk1. **(a)** mTORC1, but not mTORC2, phosphorylates Ulk1 *in vitro*. mTORC1 (by Raptor) and mTORC2 (by Rictor) were immuno-purified from the transfected HEK293 cells. The immune complexes were incubated with bacterially purified GST-mUlk1 (711-828) and phosphorylation of the GST-Ulk1 fragment was determined by ³²P-autoradiogram. Protein levels for mTOR and GST-mUlk1 (711-828) were shown by western blot and Coomassie staining, respectively. **(b)** Determination of Ulk1 domain responsible for Raptor interaction. Ha-Raptor was co-transfected with various Flag-Ulk1 deletion constructs. Ulk1 proteins

were immunoprecipitated and Co-IP of Raptor was examined by western blot. **(c)** Glucose starvation fails to inhibit Ulk1 S757 phosphorylation in AMPK-DKO MEFs. AMPK WT an DKO MEF cells were starved with glucose (4hrs) or treated with 50 nM rapamycin (Rapa, 1hr), as indicated. Endogenous Ulk1 proteins were immunoprecipitated and the phosphorylation of S757 was examined. **(d)** S757 is important for Ulk1-AMPK interaction. The indicated Flag-Ulk1 mutants and Ha-AMPKα were co-transfected into HEK293 cells. Flag-Ulk1 proteins were immunoprecipitated and Co-IP of Ha-AMPKα was examined by western blot.

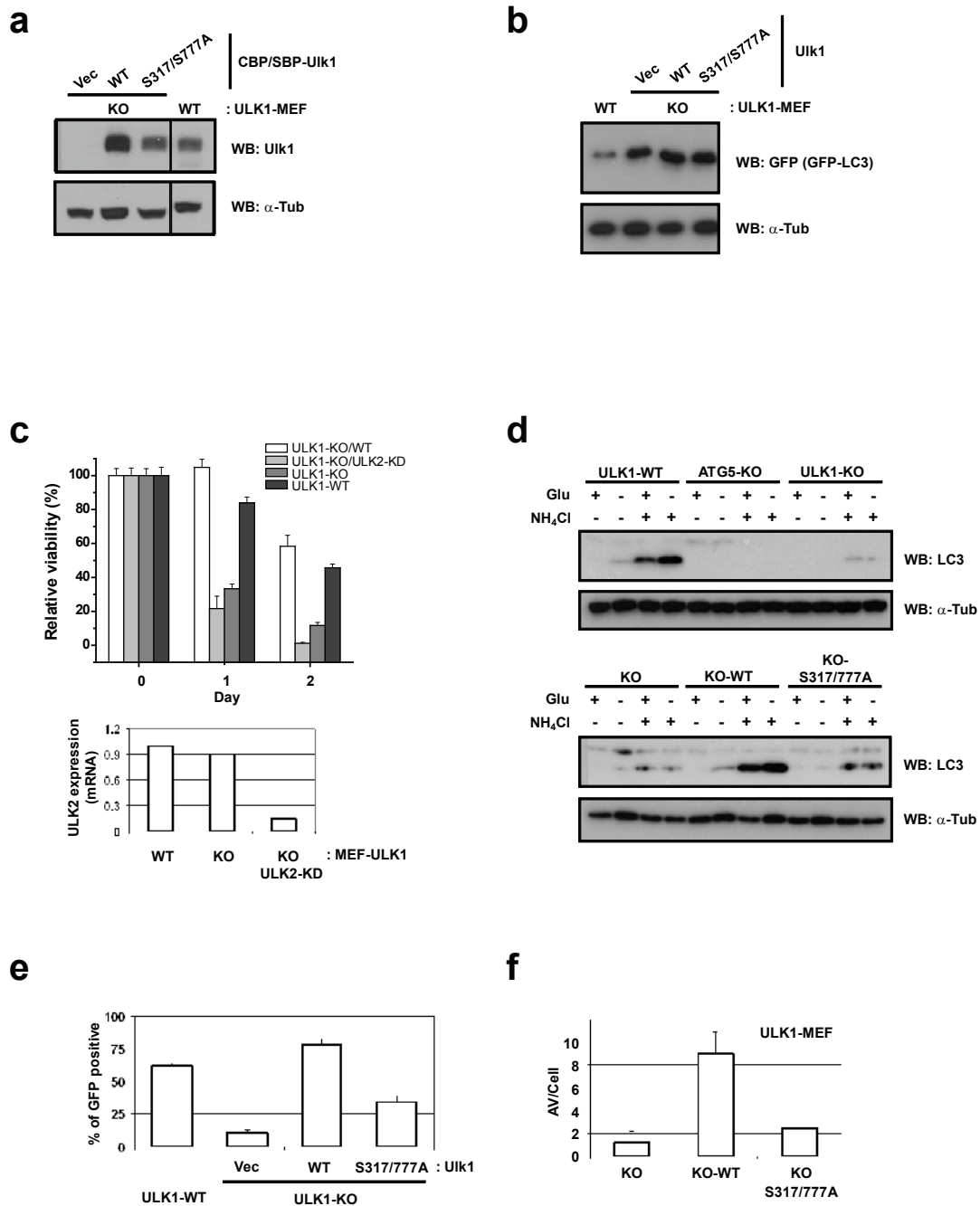


Figure S4 Analyses of Ulk1 reconstituted ULK1^{-/-} MEFs. **(A)** Expression levels of ectopic Ulk1 proteins in ULK1^{-/-} (KO) MEFs. The ULK1-KO MEFs stably expressing wild-type Ulk1 or S317/777A mutant were prepared as described in *Methods*. Expression levels of Ulk1 proteins were examined by western blot using an Ulk1 antibody. The levels of ectopic Ulk1 expression were comparable to that of the endogenous Ulk1 protein in the ULK1-WT MEFs. Protein levels were normalized against α -tubulin (α -Tub). **(b)** Expression levels of GFP-LC3 in the ectopic Ulk1 expression cell lines. GFP-LC3 was introduced to the cells by retroviral infection and the cells stably expressing GFP-LC3 were obtained by puromycin selection. The expression levels of GFP-LC3 were examined by western blot using anti-GFP antibody. **(c)** Ulk1 plays pivotal roles in cell survival under starvation. ULK1-WT, ULK1-KO, ULK1-KO/WT, and ULK1^{-/-} with ULK2 knockdown (ULK1-KO/ULK2-KD) MEFs were starved with glucose for the indicated time. Knock-down efficiency of ULK2 was determined by quantitative RT-PCR and shown in the lower panel (mean \pm S.D., n=2). Data was normalized

by GAPDH. Cell viability was determined by trypan blue staining (mean \pm S.D., n=3). Cell viability is represented as % of corresponding MEFs before starvation, which is set as 100%. **(d)** The Ulk1 S317/777A mutant is compromised in supporting glucose starvation-induced autophagy. ULK1-WT, KO, and KO expressing Ulk1-WT (KO-WT) or Ulk1 S317/777A mutant (KO-S317/777A) MEFs were starved with glucose (Glu) for 4hrs. Also, 10 mM NH₄Cl was added to determine the autophagic flux in these cells. Autophagy induction was monitored by LC3-II accumulation by LC3 western. **(e)** Quantification of GFP-LC3 punta formation for Fig.7C. The cells displaying strong GFP positive dots on confocal microscopy were counted and quantified (mean \pm S.D., n=30-40 cells) as described in *Supplementary Methods*. **(f)** Expression of wild type but not the S317/777A mutant restores autophagosome/autolysosome-like structures in ULK1^{-/-} cells. The numbers of autophagosome/autolysosome-like structures (AV) from 5-7 AV positive cells were counted and mean \pm S.D. are shown. This is the quantification for Fig.7d.

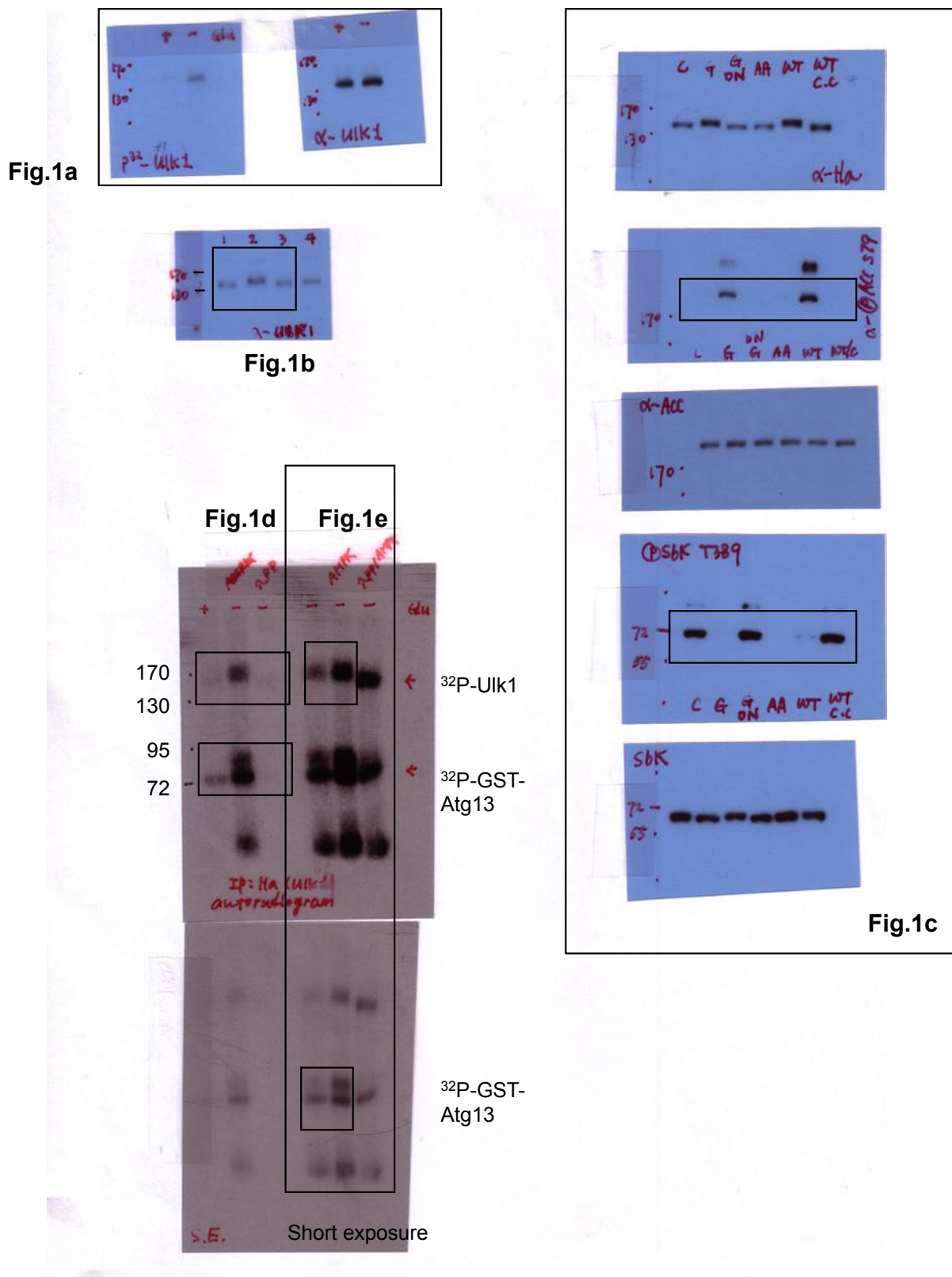


Figure S5 Full scans of original blots for data in Fig. 1, 2, 3, 4, 5, and 6. Panels corresponding to the figures in the paper are indicated.

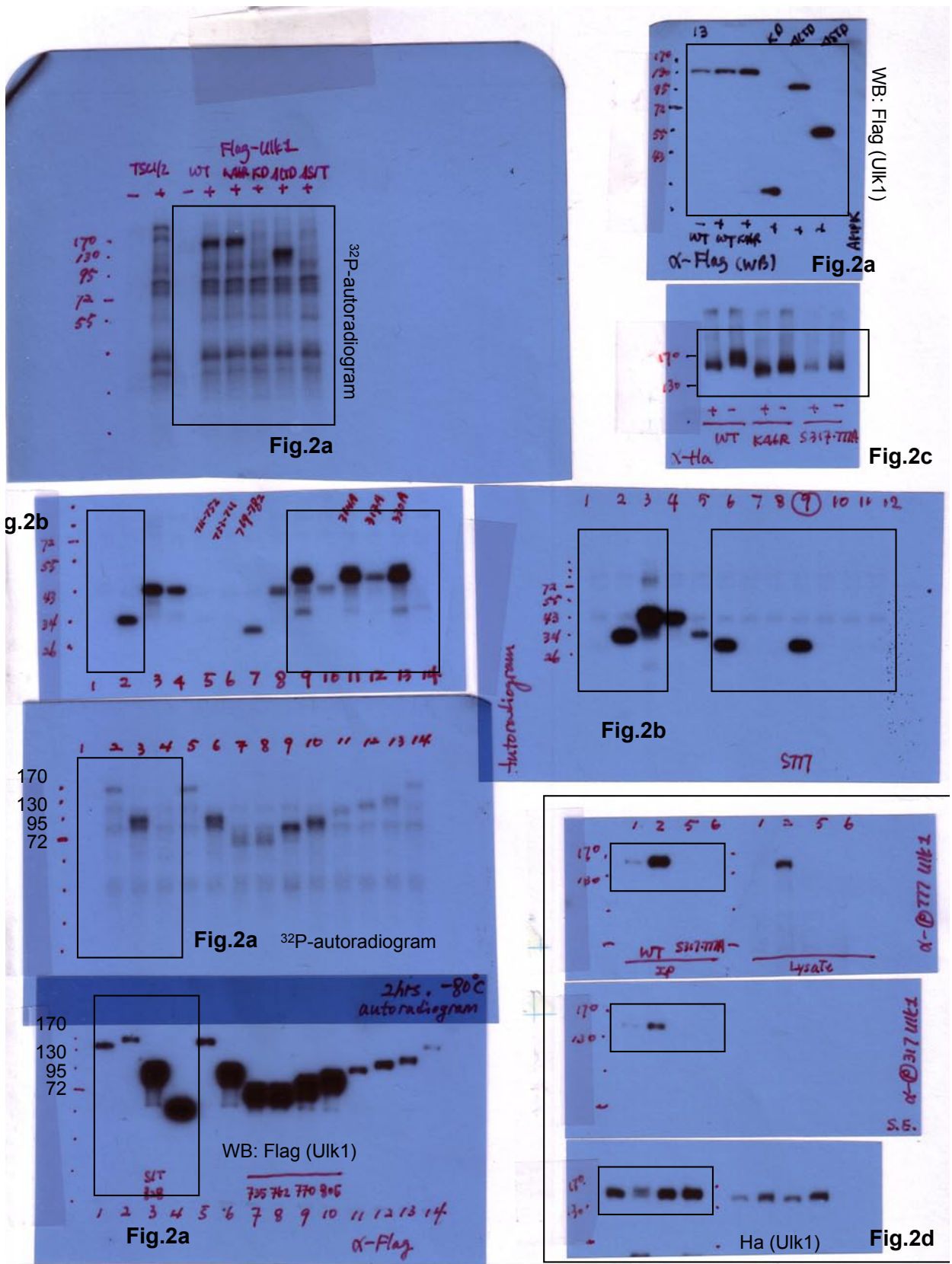


Figure S5 continued

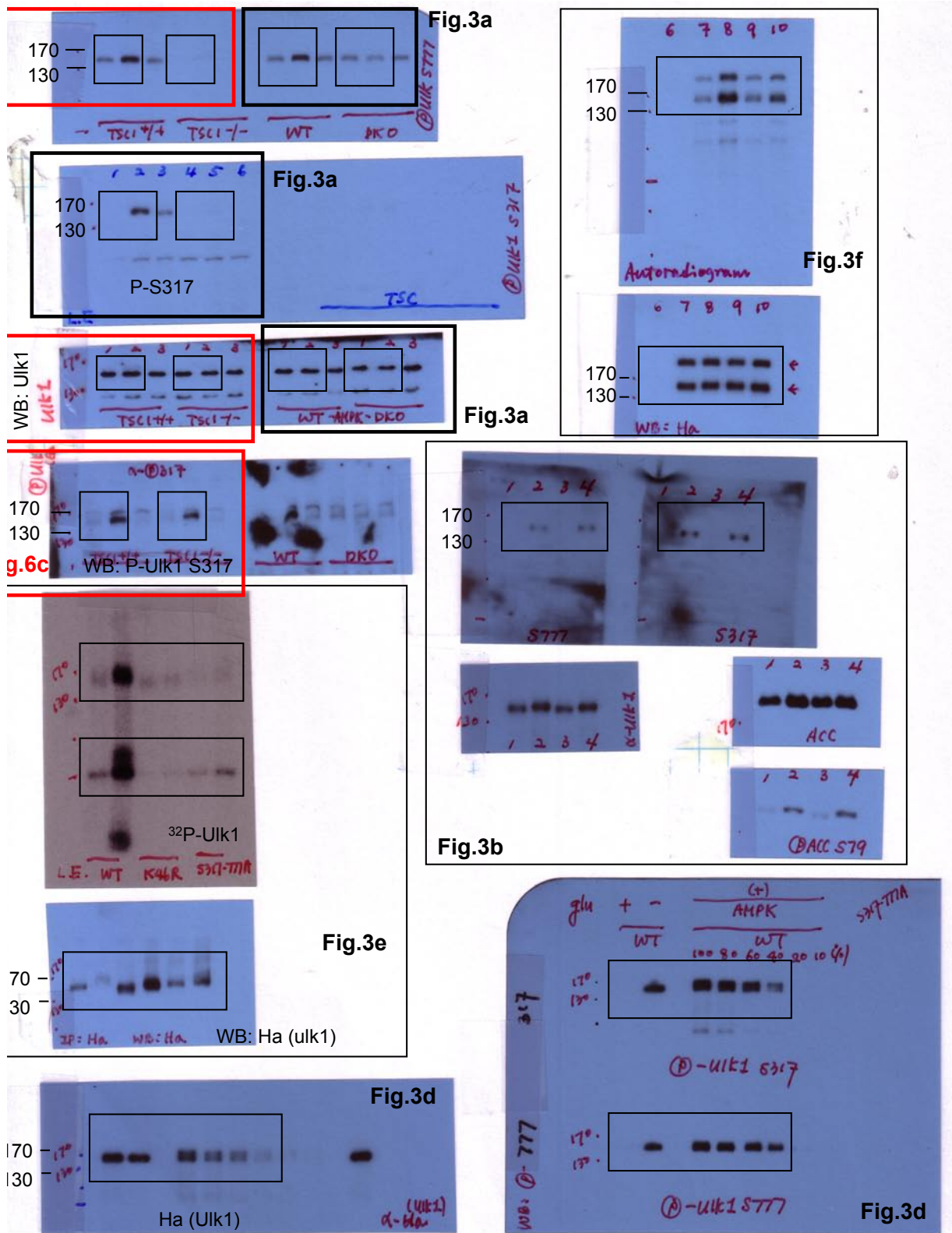


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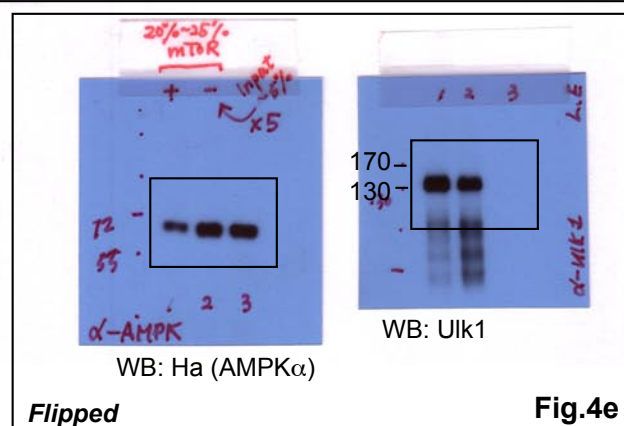
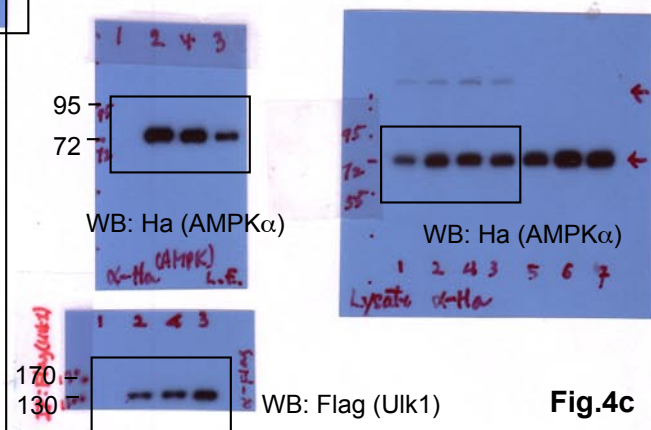
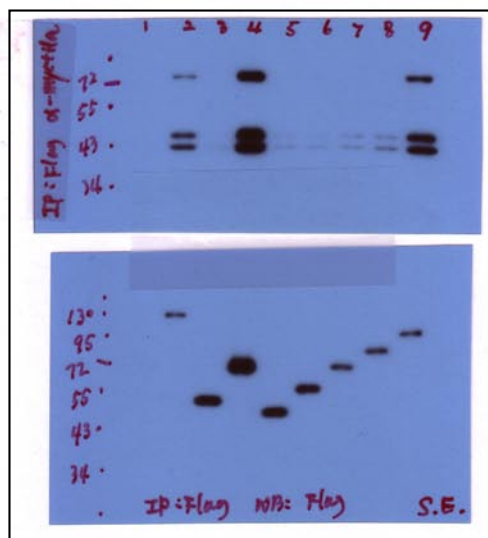
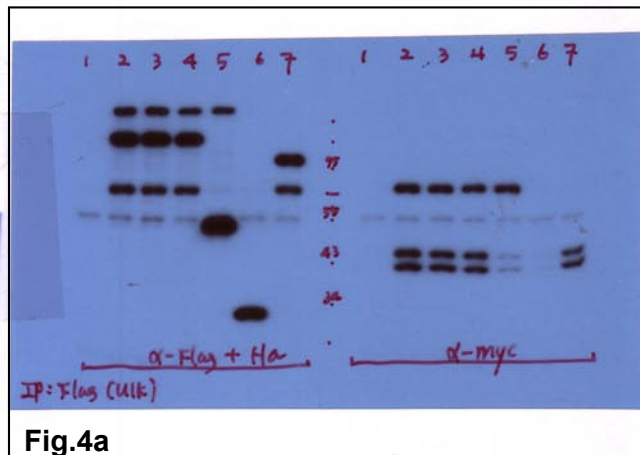
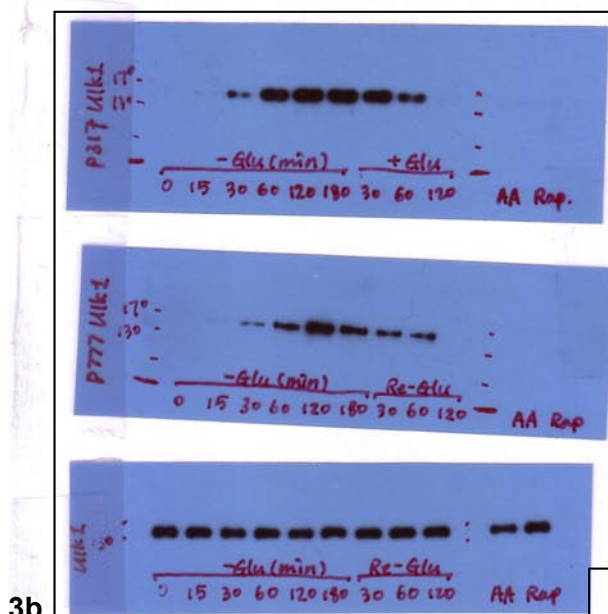


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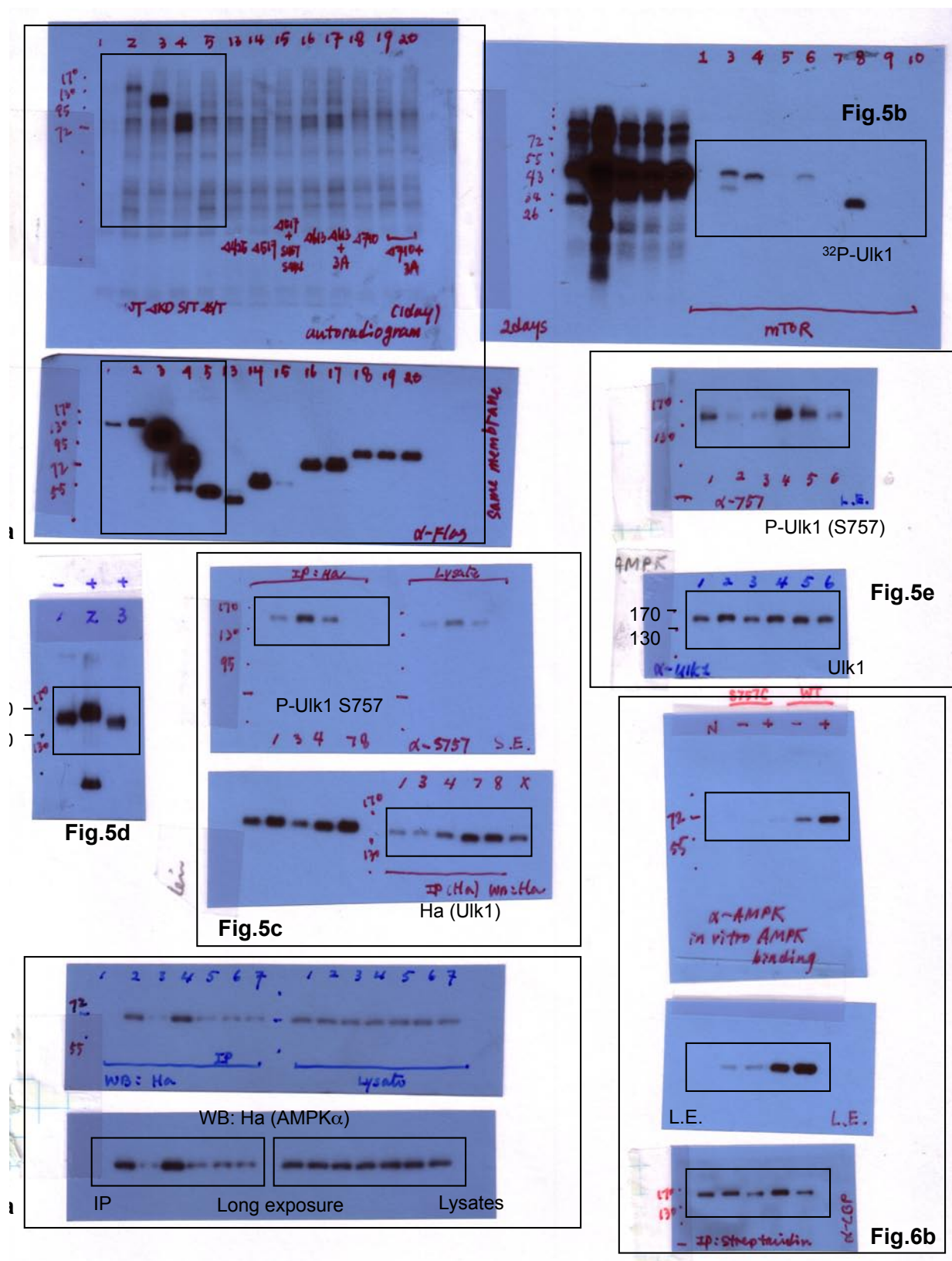


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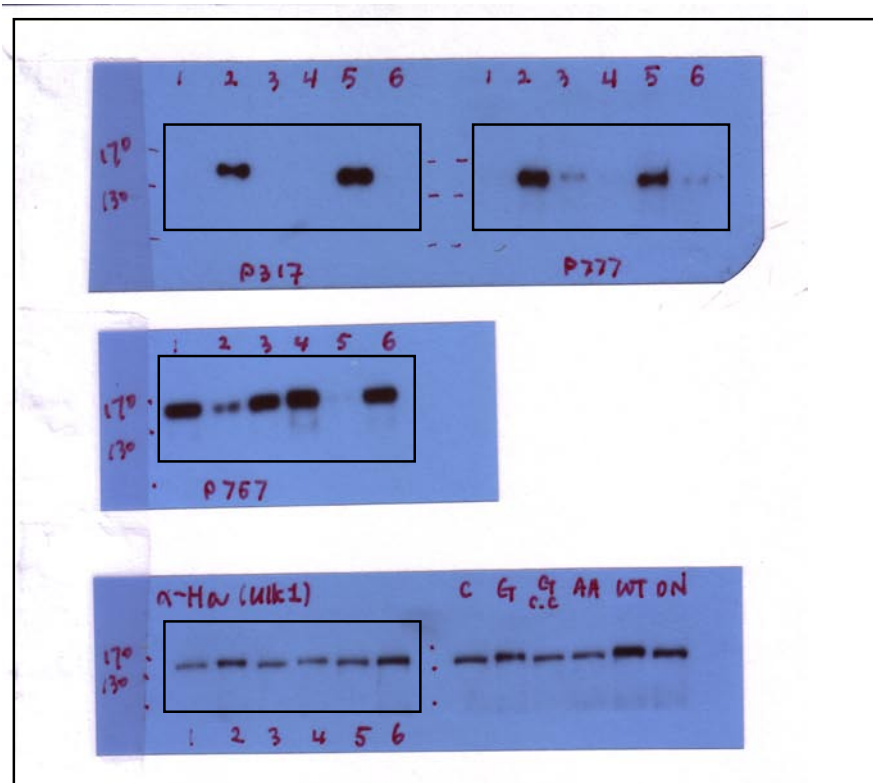


Fig.6d

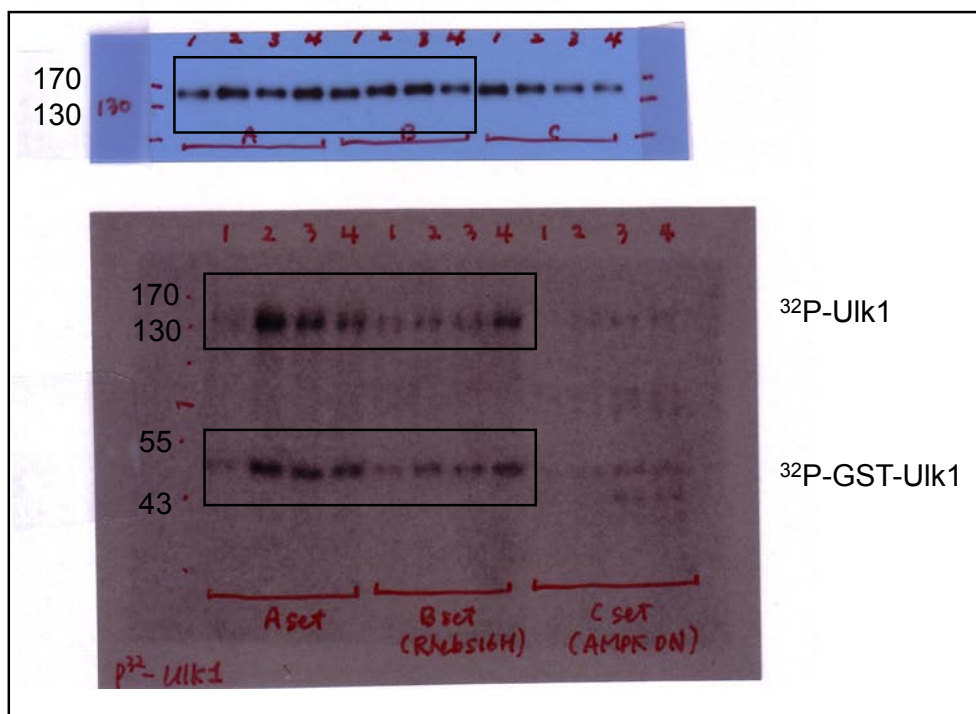


Fig.6e

Figure S5 continued