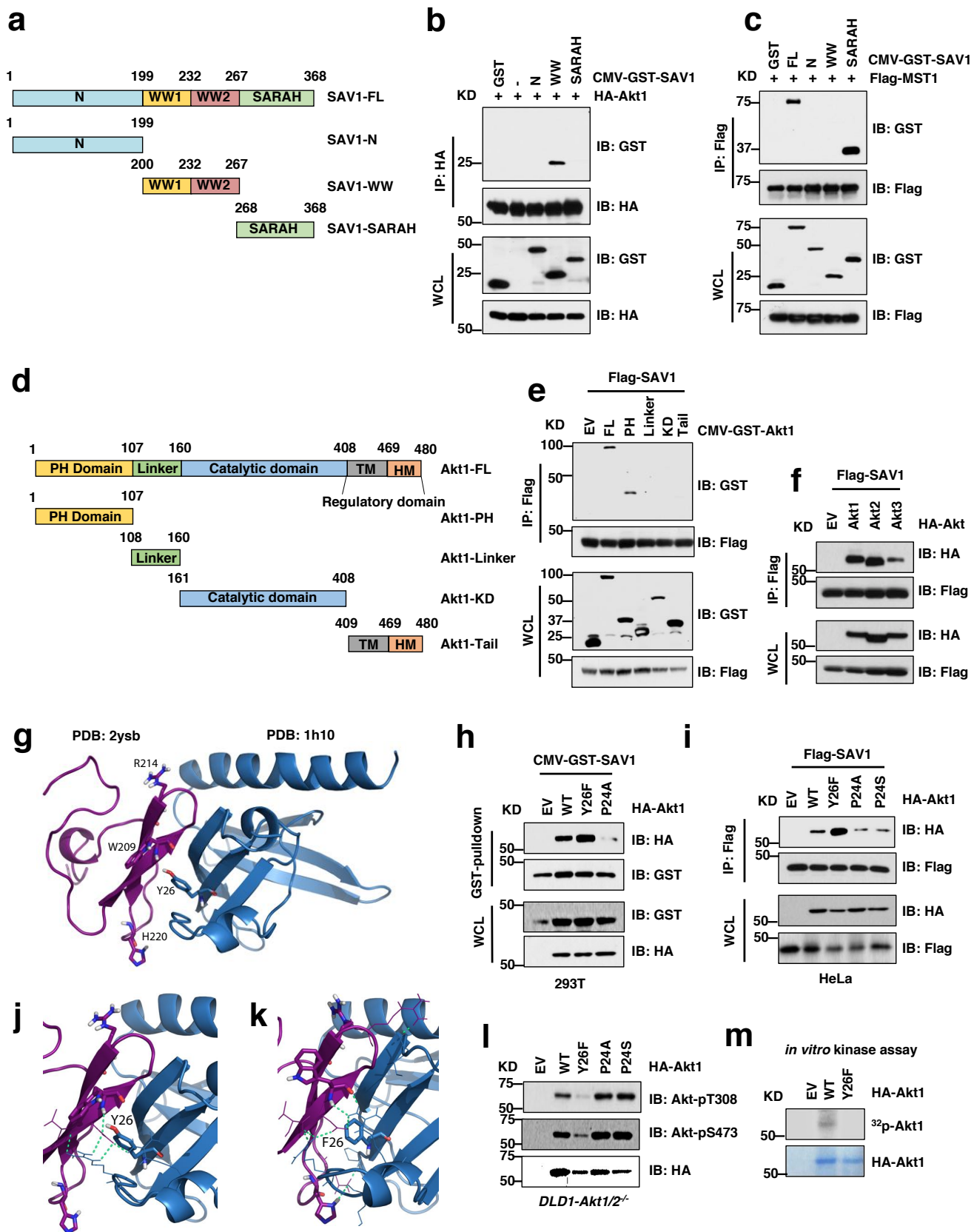
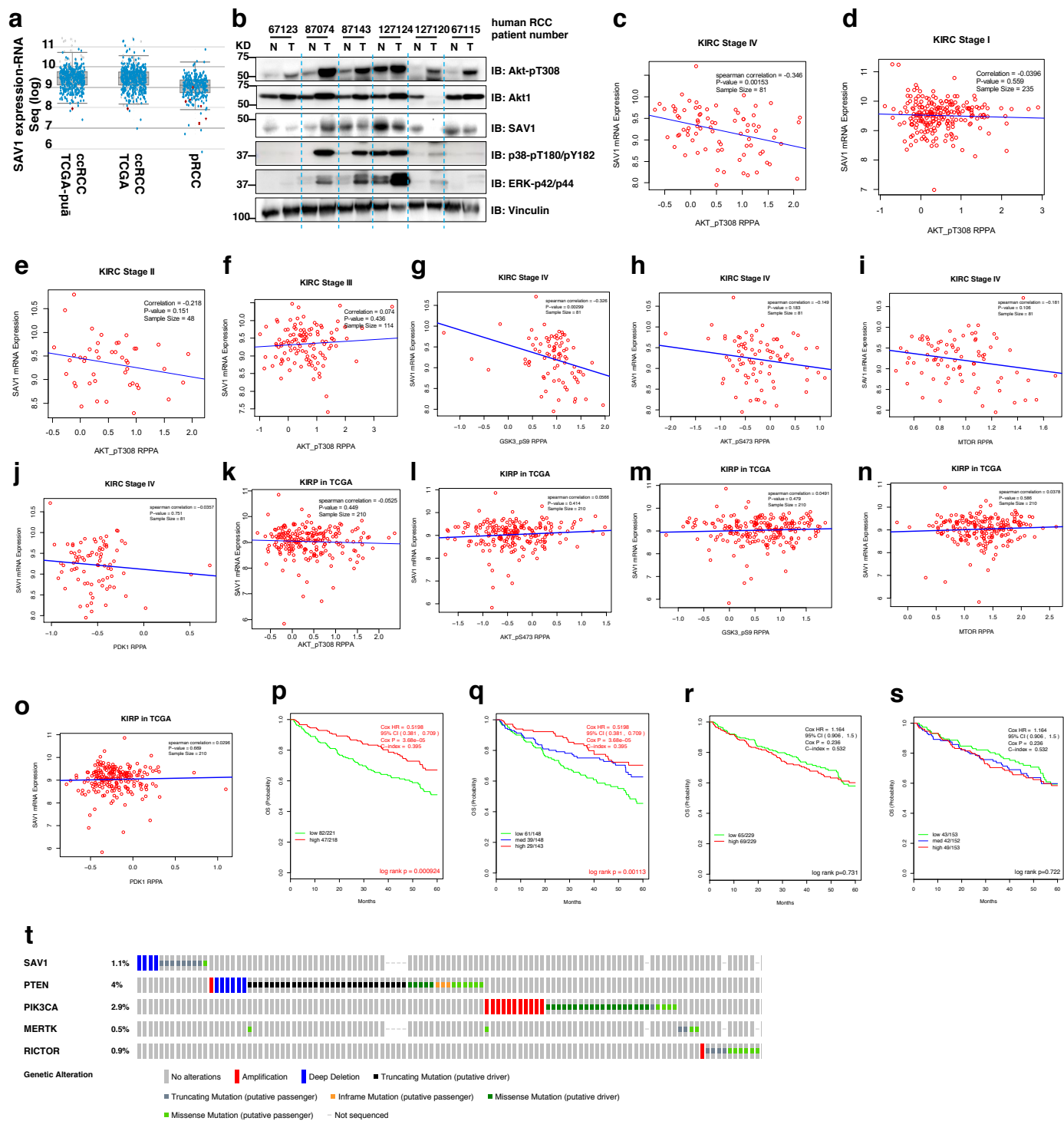
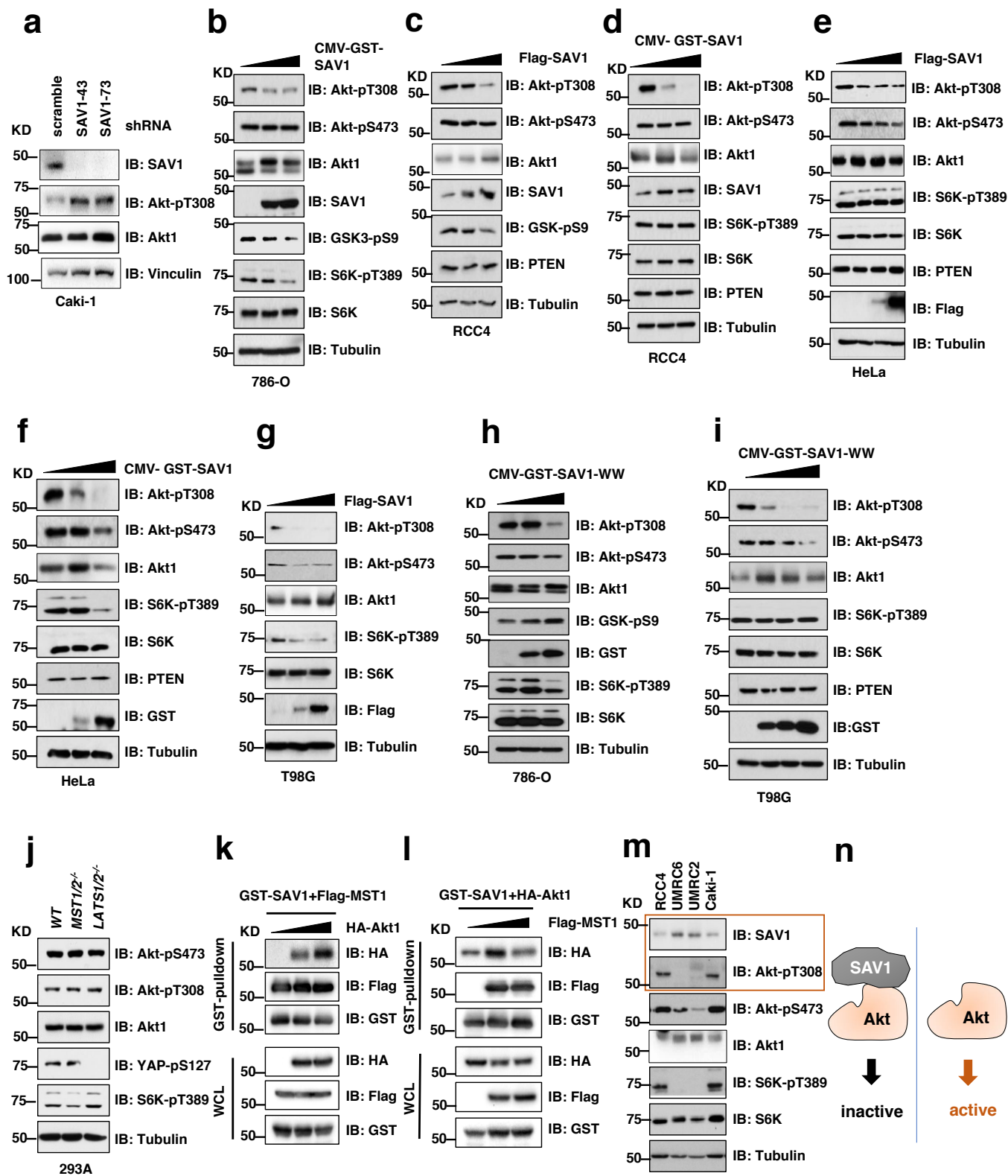




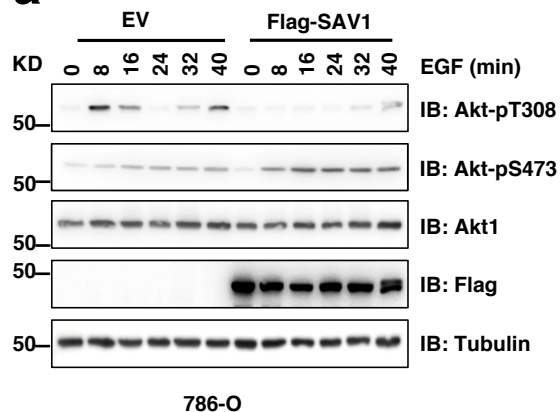
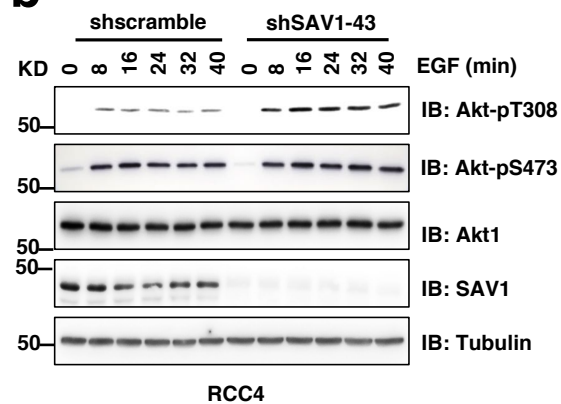
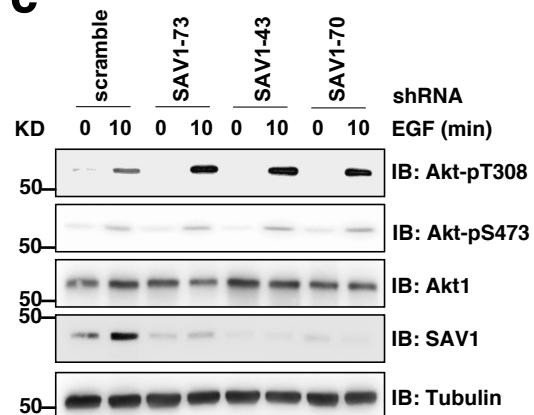
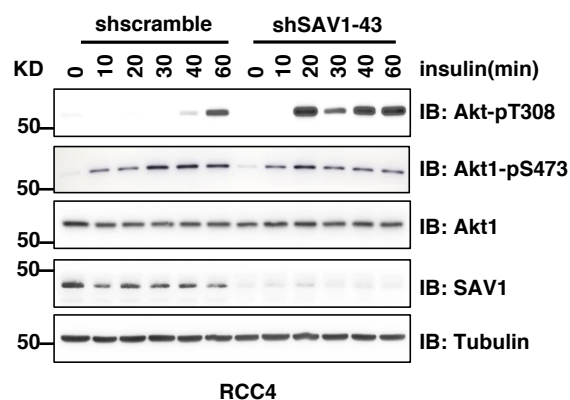
Supplementary Figure 1. Akt binds the WW domain of SAV1 through a PxY motif in its PH domain. (a) A cartoon illustration of domain structure for SAV1. (b-c) Immunoblot (IB) analyses of HA (b) or Flag (c) immunoprecipitates (IP) and whole cell lysates (WCL) derived from HEK293 cells transfected with indicated DNA constructs. (d) A cartoon illustration of domain structure for Akt1. (e-f) IB analyses of Flag-IP and WCL derived from HEK293 cells transfected with indicated DNA constructs. (g, j and k). A simulation model for the SAV1-WW domain/Akt1-PH domain structure. Please refer to the method section for details. (h) IB analysis of GST-pulldowns and WCL derived from HEK293T cells transfected with indicated DNA constructs. (i) IB analysis of Flag-IP and WCL derived from HeLa cells transfected with indicated DNA constructs. (l) IB analysis of *DLD1-Akt1/2^{-/-}* cells transfected with indicated DNA constructs. (m) *In vitro* kinase assays to examine Akt activity. Please refer to the method section for details.



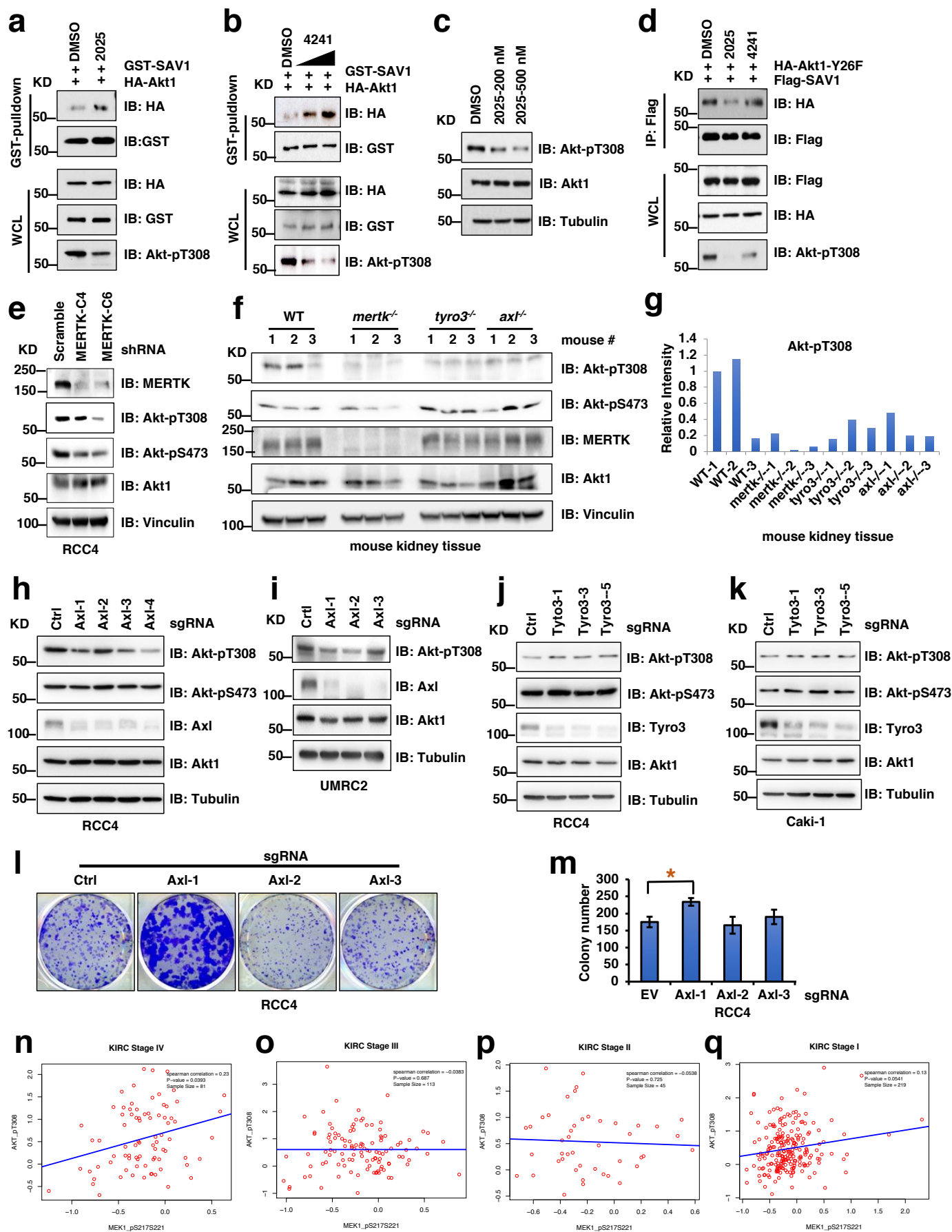
Supplementary Figure 2. An inverse correlation between SAV1 expression and Akt activation is observed in RCC patients. (a) mRNA expression data obtained from RNA-seq experiments in TCGA provided by cBioportal. (b) Immunoblot (IB) analysis of whole cell lysates (WCL) derived from RCC patient samples. N: non-tumor, T: tumor. (c-f) Examination of SAV1 expression (from TCGA KIRC RNA-seq data) and Akt-pT308 levels (from TCGA KIRC RPPA data) in kidney cancer patients with indicated disease stage. (g-j) Examination of the correlation of SAV1 expression (from TCGA KIRC RNA-seq data) with GSK3-pS9 (g), Akt-pS473 (h), mTOR (i) and PDK1 (j) protein levels (from TCGA KIRC RPPA data) in stage IV kidney cancer patients. (k) Examination of the correlation of SAV1 expression (from TCGA KIRP RNA-seq data) with Akt-pT308 (k), Akt-pS473 (l), GSK3-pS9 (m), mTOR (n) and PDK1 (o) protein levels (from TCGA KIRP RPPA data) in stage IV kidney cancer patients. (p-s) Kaplan-Meier plots indicating either low SAV1 expression or high Akt activity predicts poor prognosis. p and q: green line (low SAV1 expression); blue line (medium SAV1 expression) and red line (high SAV1 expression). r and s: red line (high Akt-pT308); blue line (medium Akt-pT308) and green line (low Akt-pT308). (t) Oncoprint from cBioportal indicating that SAV1 genetic changes are not concurrent with genetic alternations with canonical Akt upstream signaling components such as *PTEN* and *PIK3CA*.



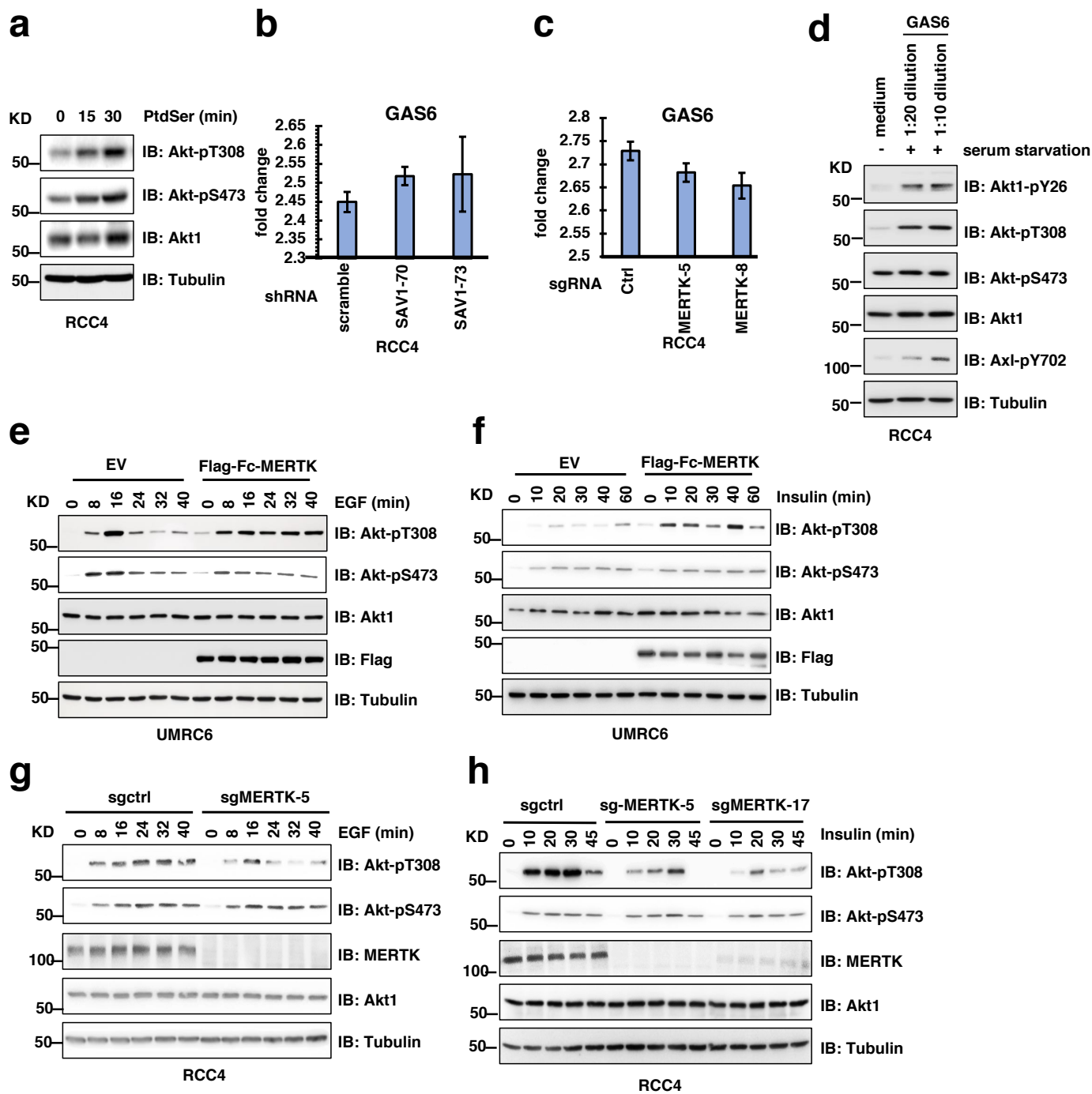
Supplementary Figure 3. The WW domain of SAV1 binds Akt and suppresses Akt activity. (a) Immunoblot (IB) analysis of whole cell lysates (WCL) derived from Caki-1 cells depleted of endogenous SAV1 by shRNAs. (b-g) IB analysis of WCL derived from 786-O (b), RCC4 (c-d), HeLa (e-f) and T98G (g) cells transfected with increasing doses of indicated SAV1 constructs. (h-i) IB analysis of WCL derived from 786-O (h) or T98G (i) cells that ectopically express GST-tagged SAV1-WW domain constructs. (j) IB analysis of WCL derived from HEK293A cells deleted of indicated endogenous genes. (k-l) IB analysis of GST-pulldown and WCL derived from HEK293 cells transfected with indicated DNA constructs. (m) IB analysis of WCL derived from indicated RCC cells. (n) A cartoon illustration of SAV1-mediated Akt activity inhibition.

a**b****c****d**

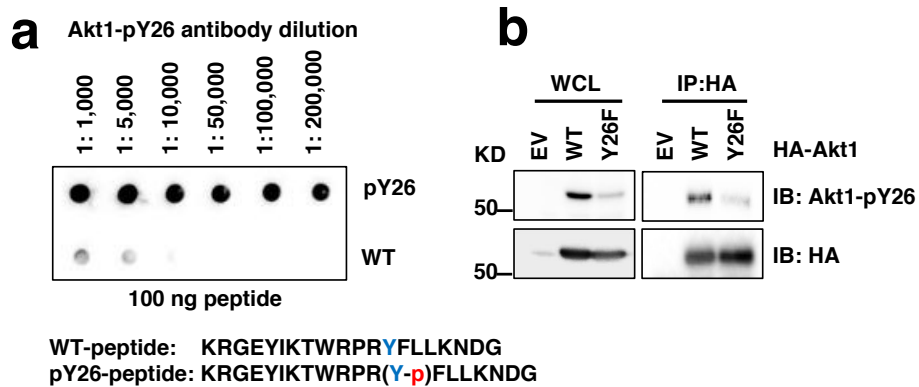
Supplementary Figure 4. SAV1 suppresses Akt-T308 phosphorylation upon EGF or insulin stimulation. (a) Immunoblot (IB) analysis of whole cell lysates (WCL) derived from 786-O cells transfected with indicated DNA constructs. Where indicated, cells were serum starved overnight and then treated with 100 nM EGF for indicated time periods before cell collection. (b-d) IB analyses of WCL derived from RCC4 (b, d) or Caki-1 (c) cells depleted of endogenous SAV1. Where indicated, cells were was serum starved overnight and then treated with 100 nM EGF (b, c) or insulin (d) for indicated time periods before cell collection.



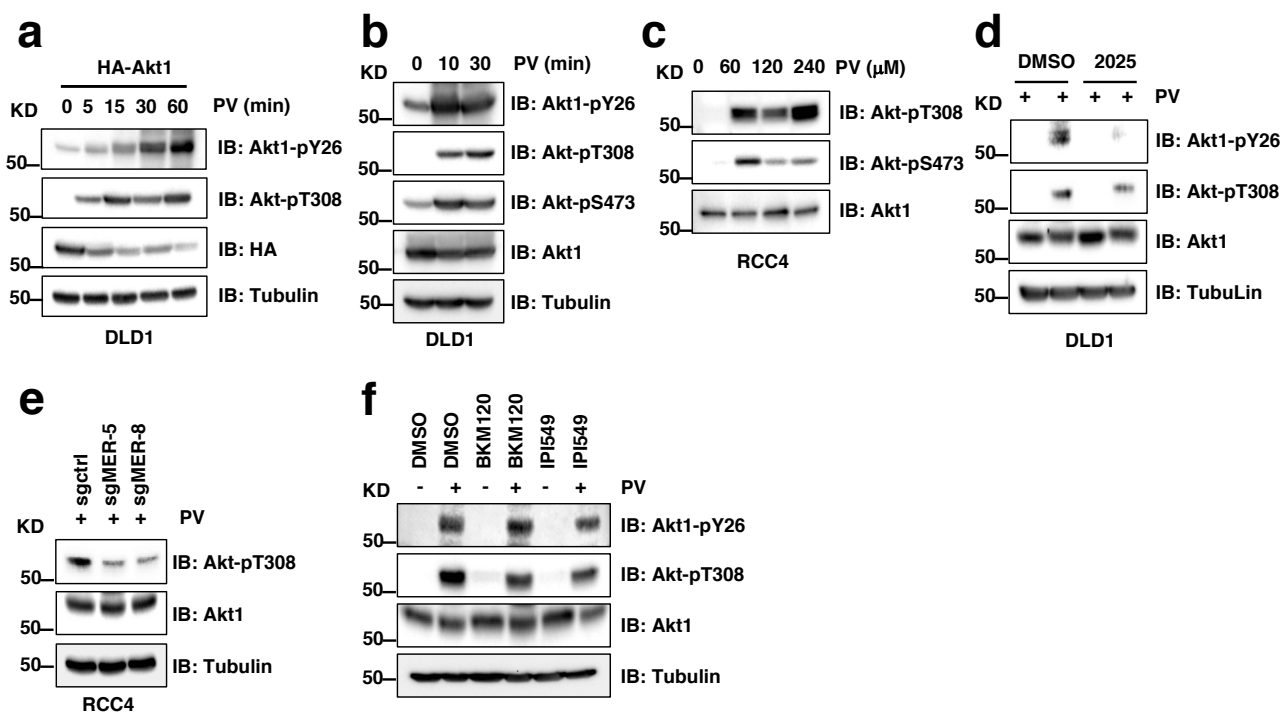
Supplementary Figure 5. MERTK inhibition leads to enhanced Akt1/SAV1 binding and attenuated Akt activity. (a-b) Immunoblot (IB) analysis of GST-pulldowns and whole cell lysates (WCL) derived from HeLa cells transfected with indicated DNA constructs. Where indicated, 300 nM UNC2025 (a) or 300 nM UNC4241 (b) was added for 2 hrs before cells were harvested. (c) IB analysis of WCL derived from 293T cells treated with indicated doses of UNC2025 for 2 hrs. (d) IB analysis of Flag-IP and WCL derived from HEK293 cells transfected with indicated DNA constructs. Where indicated, 300 nM UNC2025 or 300 nM UNC4241 was added for 2 hrs before cells were harvested. (e) IB analysis of WCL derived from RCC4 cells depleted of endogenous MERTK by shRNAs. (f-g) IB analysis of WCL derived from kidney tissues obtained from mice with indicated genotypes (f) and corresponding quantification for Akt-pT308 signals (g). (h-i) IB analysis of WCL derived from RCC4 (h) or UMRC2 (i) cells depleted of endogenous Axl by shRNAs. Infected cells were selected with 1 μ g/ml puromycin for 72 hours to eliminate non-infected cells before cell collection. (j-k) IB analysis of WCL derived from RCC4 (j) or Caki-1 (k) cells depleted of endogenous Tyro3 by shRNAs. Infected cells were selected with 1 μ g/ml puromycin for 72 hours to eliminate non-infected cells before cell collection. (l-m) 600 cells obtained in (h) were plated on 6-well plates and 15 days later formed colonies were fixed and visualized by crystal violet staining (l) and quantified (m). (n-q) Examination of Akt-pT308 levels (from TCGA KIRC RPPA data) and MEK1-pS217pS221 levels (an indicator of MERTK activity, from TCGA KIRC RPPA data) in kidney cancer patients with indicated disease stage.



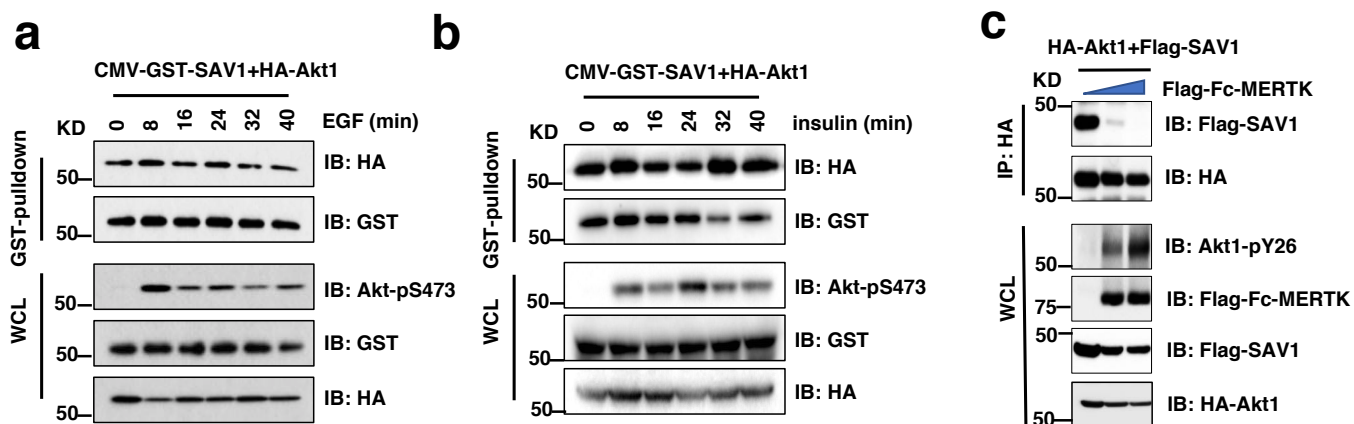
Supplementary Figure 6. METK activation promotes Akt-T308 phosphorylation. (a) IB analysis of WCL derived from RCC4 cells that were serum starved overnight and stimulated with 80 μ M PtdSer for the indicated time period. (b-c) RT-PCR indicating that GAS6 are expressed in RCC4 cells. (d) IB analysis of WCL derived from RCC4 cells that were serum starved overnight and stimulated with indicated dilutions of home-made GAS6 (from Earp lab) for 30min before cell collection. (e-f) IB analysis of WCL derived from UMRC6 cells transfected with indicated constructs. Where indicated, cells were serum starved overnight and stimulated with either EGF (e) or insulin (f) for indicated time periods before cell collection. (g-h) IB analysis of WCL derived from indicated RCC4 cells. Where indicated, cells were serum starved overnight and stimulated with either EGF (g) or insulin (h) for indicated time periods before cell collection.



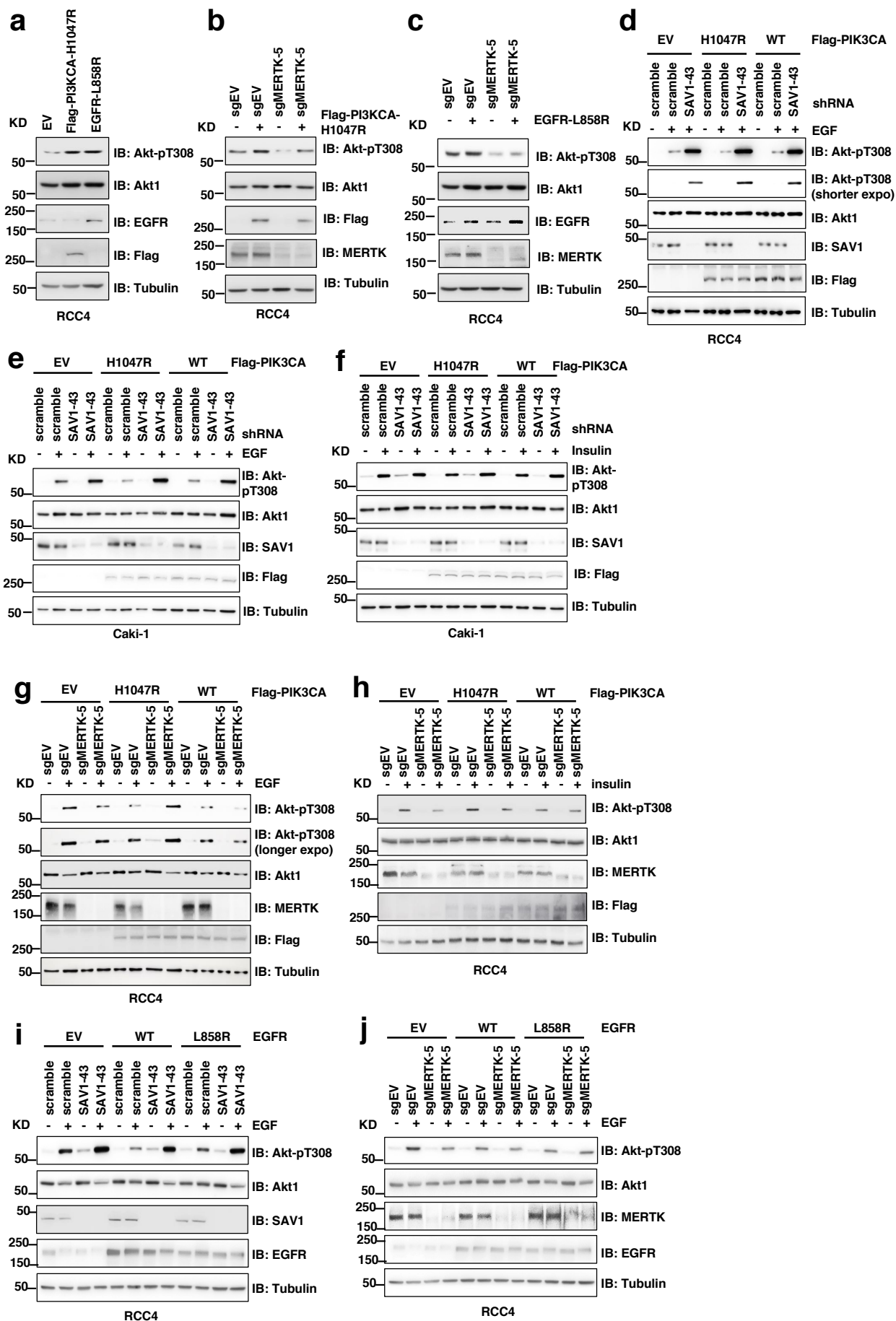
Supplementary Figure 7. Validation of the Akt1-pY26 antibody. (a) Dot blot assays to examine the specificity for the Akt1-pY26 antibody. 100 ng of the antigen peptides were spotted on nitrocellulose membrane and the Akt1-pY26 antibody was diluted with indicated factors and used for western blot analyses. (b) IB analyses of HA-IP and WCL derived from HEK293 cells transfected with indicated HA-Akt1 constructs.



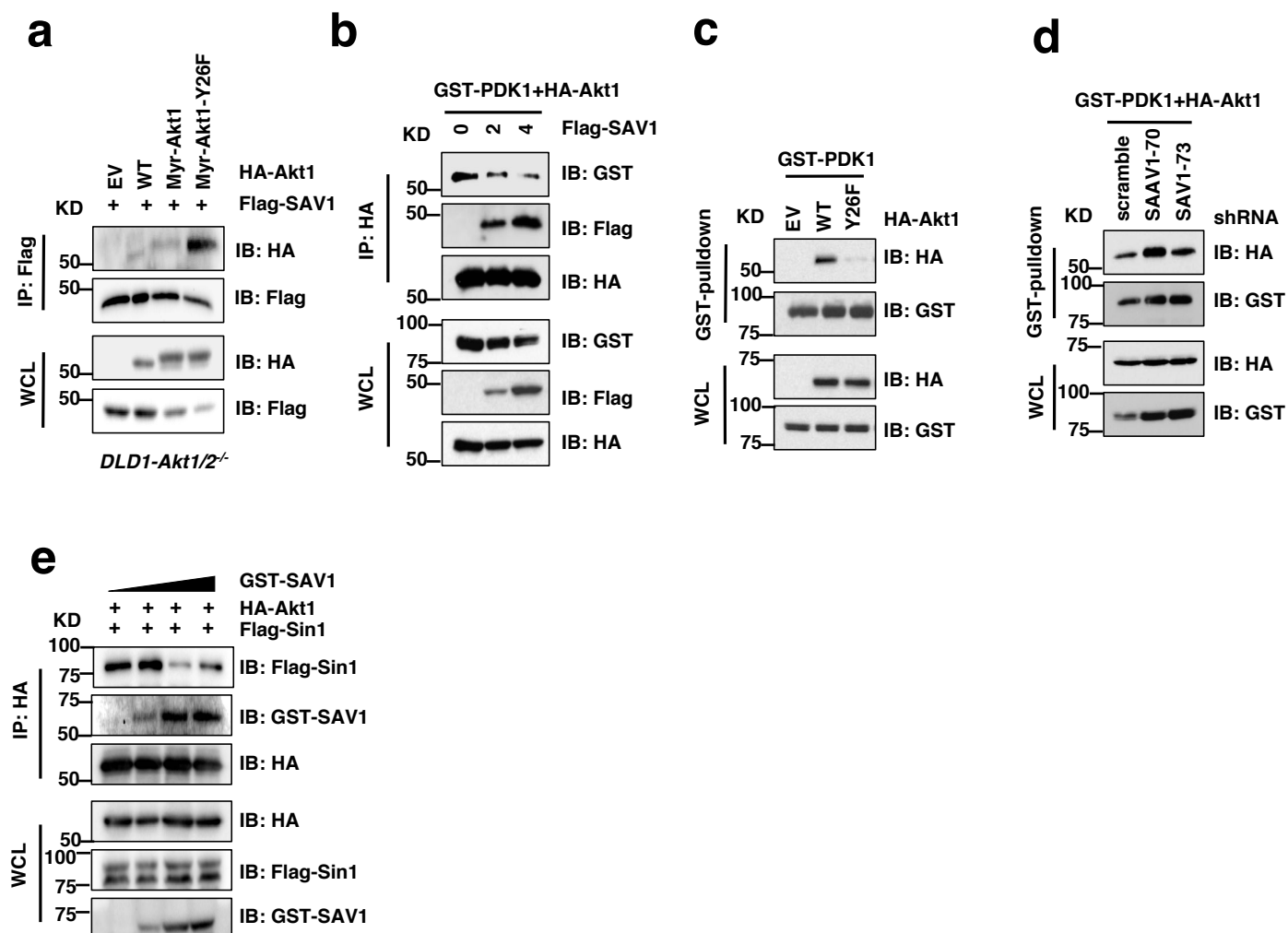
Supplementary Figure 8. MERTK activation facilitates Akt1-pY26 and Akt activation. (a) IB analysis of WCL derived from DLD1 cells transfected with HA-Akt1 and treated with 60 μ M PV for indicated time periods. (b-c) IB analysis of WCL derived from DLD1 (b) or RCC4 (c) cells treated with 60 μ M PV for indicated time periods. (d) IB analysis of WCL derived from DLD1 cells treated with 60 μ M PV for 30min. Where indicated, 300 nM UNC2025 was added for 2hrs before cell collection. (e) IB analysis of WCL derived from WT or MERTK-deleted RCC4 cells treated with 60 μ M PV for 30 min. (f) IB analysis of WCL derived from RCC4 cells treated with 60 μ M PV for 30 min. Where indicated, BKM120 (200 nM) or IPI549 (500 nM) were added for 10 hrs before cells were stimulated with PV.



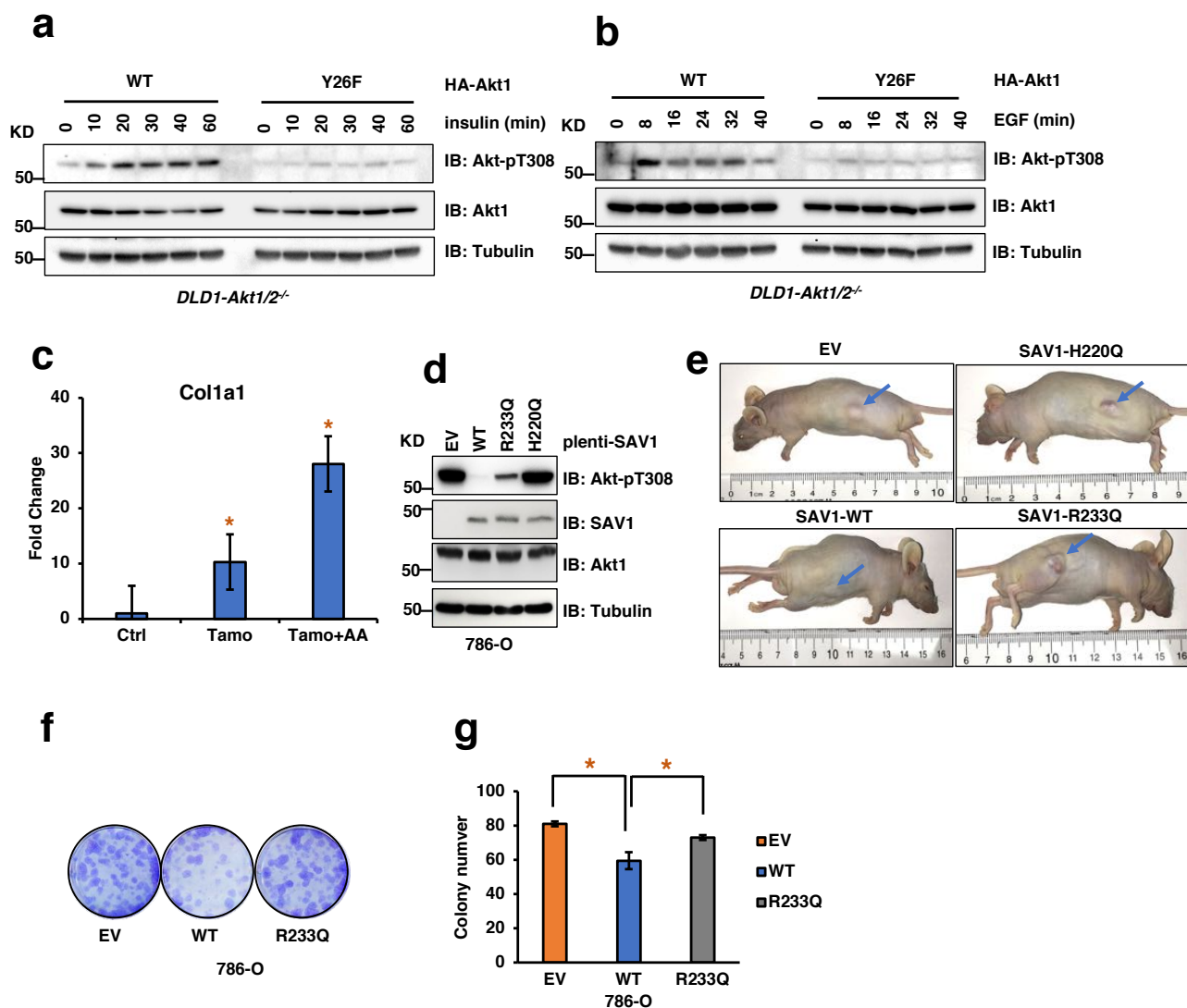
Supplementary Figure 9. Growth signaling does not regulate Akt binding with SAV1. (a-b) IB analysis of GST-pulldowns and WCL derived HeLa cells transfected with GST-SAV1 and HA-Akt1 constructs. Where indicated, cells were serum starved for 10 hrs followed by EGF (100 ng/ml) (a) or insulin (100 nM) (b) stimulation for indicated periods. (c) IB analysis of HA-IPs and WCL derived from HEK293 cells transfected with indicated constructs.



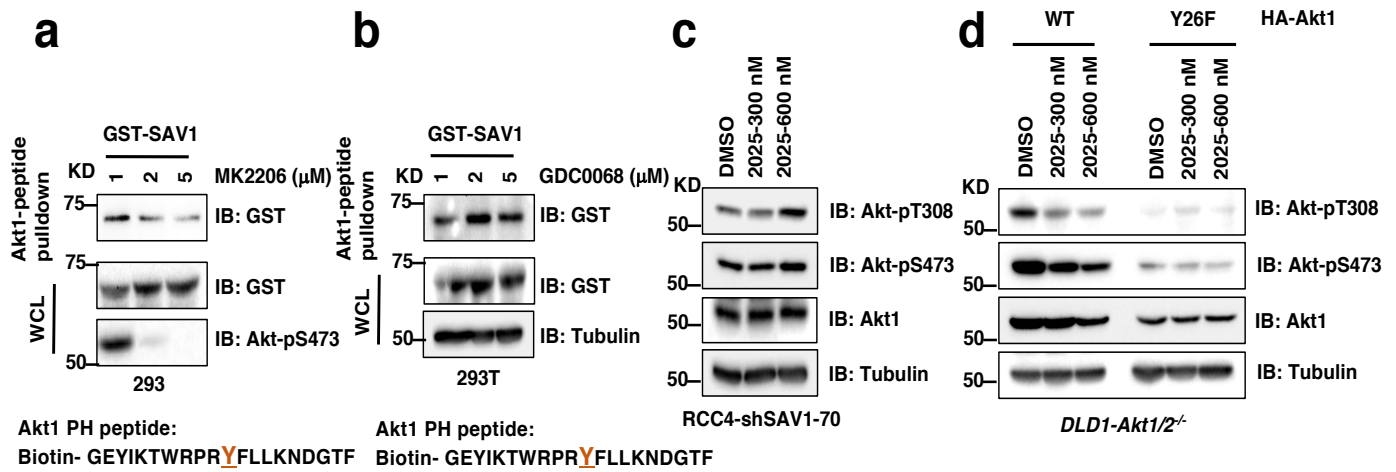
Supplementary Figure 10. Expression of constitutively active PI3K or EGFR mutants cannot compensate for MERTK loss in activating Akt. (a-c) IB analysis of WCL derived from indicated RCC4 cells transfected with indicated DNA constructs to demonstrate that PIK3CA and EGFR oncogenic mutations can activate Akt at basal levels. (d-e) IB analysis of WCL derived from indicated RCC4 (d) or Caki-1 (e) cells transfected with indicated DNA constructs. Where indicated, cells were serum starved overnight and stimulated with 100 nM EGF for 10 min before cell collection. (f) IB analysis of WCL derived from indicated Caki-1 cells transfected with indicated DNA constructs. Where indicated, cells were serum starved overnight and stimulated with 100 ng/ml insulin for 30 min before cell collection. (g-h) IB analysis of WCL derived from indicated RCC4 cells transfected with indicated DNA constructs. Where indicated, cells were serum starved overnight and stimulated with either 100 nM EGF for 10 min (g) or 100 ng/ml insulin for 30 min (h) before cell collection. (i-j) IB analysis of WCL derived from indicated RCC4 cells transfected with indicated DNA constructs. Where indicated, cells were serum starved overnight and stimulated with 100 nM EGF for 10 min before cell collection.



Supplementary Figure 11. SAV1 binding attenuates Akt1 interaction with PDK1 and mTORC2. (a) IB analysis of Flag-IP and WCL derived from *DLD1-Akt1/2^{-/-}* cells transfected with indicated DNA constructs. (b) IB analysis of HA-IP and WCL derived from HEK293 cells transfected with indicated DNA constructs. (c) IB analyses of GST-pulldowns and WCL derived from HEK293 cells transfected with indicated DNA constructs. (d) IB analyses of GST-pulldowns and WCL derived from WT or SAV1-depleted RCC4 cells transfected with indicated DNA constructs. (e) IB analysis of HA-IP and WCL derived from HEK293 cells transfected with indicated DNA constructs.



Supplementary Figure 12. SAV1 binding to Akt attenuates growth factor triggered Akt activation. (a-b) IB analyses of WCL derived from *DLD1-Akt1/2^{-/-}* cells stably expressing either WT-Akt1 or Y26F-Akt1 by lenti-viral infection. Where indicated, cells were serum starved for 10 hrs and stimulated by either 100 nM insulin (a) or 100 ng/ml EGF (b) for indicated time periods. (c) Analyses of mRNA changes in *Col1a1* fibrosis genes in *SAV1^{KS}* mouse kidneys receiving indicated treatments. (d) IB analysis of WCL derived from 786-O cells stably expressing indicated SAV1 constructs by lenti-viral infection and selected in 10 μ g/ml blasticidin for 72 hours to eliminate non-infected cells before cell collection. (e) Representation tumor images for mouse xenograft experiments using cells generated in (d). (f-g) 600 cells from (d) were plated into 6-well plates and 21 days later cells were fixed, stained with crystal violet (f) and quantified (g).



Supplementary Figure 13. MERTK inhibition governed Akt inactivation requires SAV1. (a-b) Akt1 peptide pull-down assays to indicate that an Akt allosteric inhibitor, MK2206 (a) but not an Akt-ATP analog inhibitor, GDC0068 (b) competes with SAV1 to bind the Akt PH domain. (c) IB analysis of WCL derived from RCC4 cells depleted of endogenous SAV1 and treated with indicated doses of UNC2025 for 2 hrs. (d) IB analyses of WCL derived from *DLD1-Akt1/2^{-/-}* cells stably expressing either WT-Akt1 or Y26F-Akt1 by lenti-viral infection that were treated with indicated doses of UNC2025 for 2 hrs before cell collection.

Fig. 1a

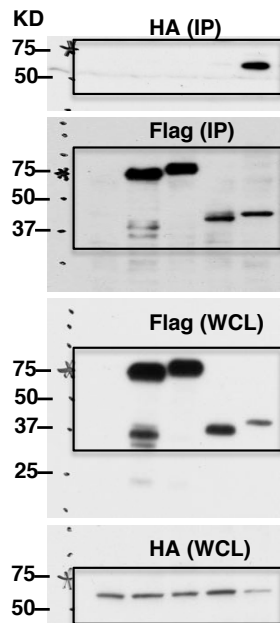


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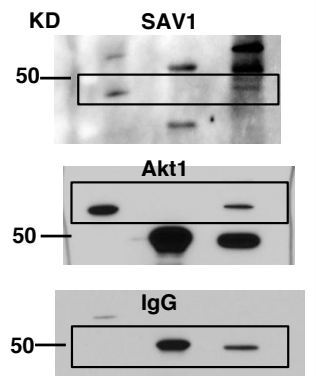


Fig. 1e

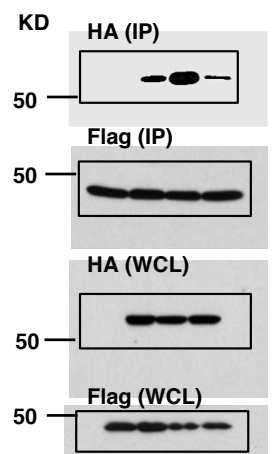


Fig. 1f

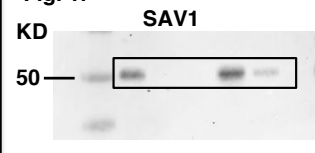


Fig. 1g

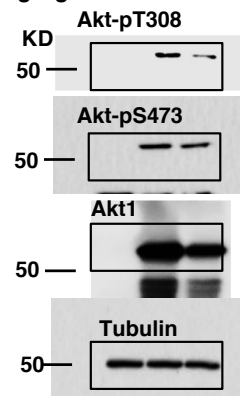


Fig1h

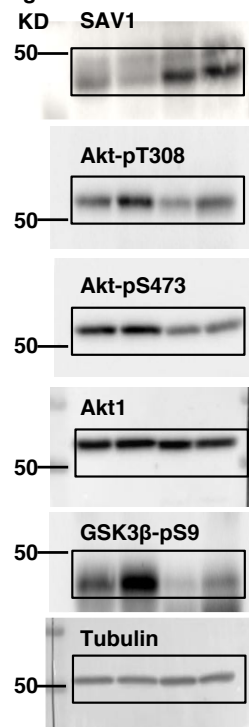


Fig1i

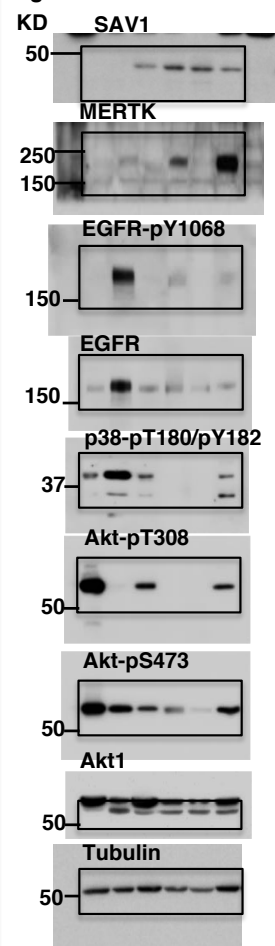


Fig1j

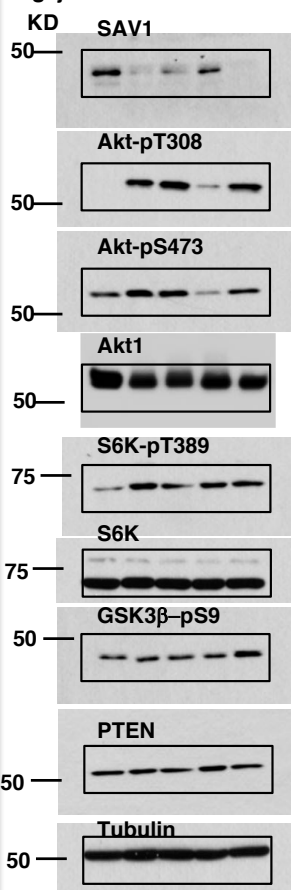
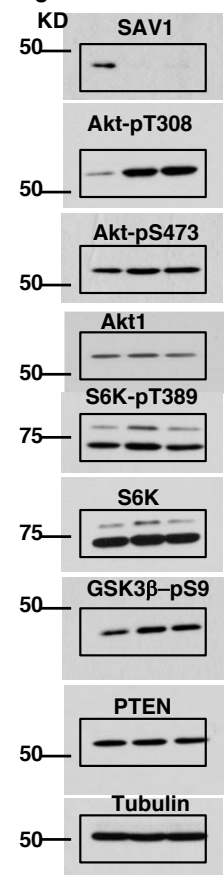
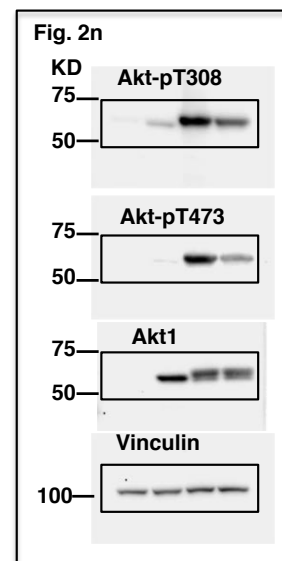
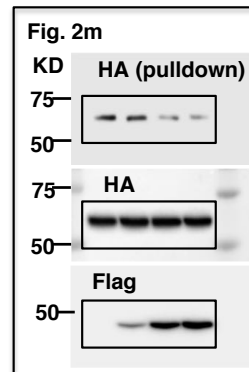
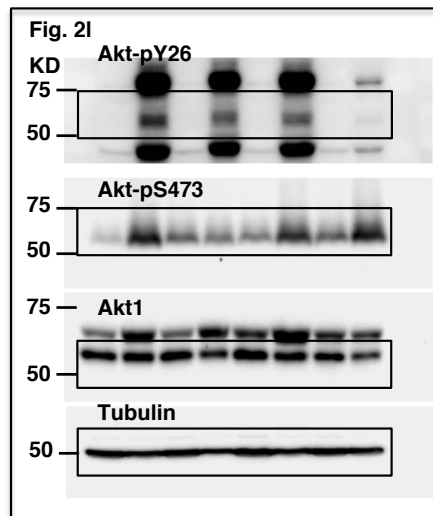
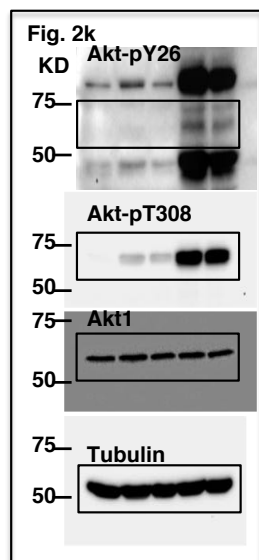
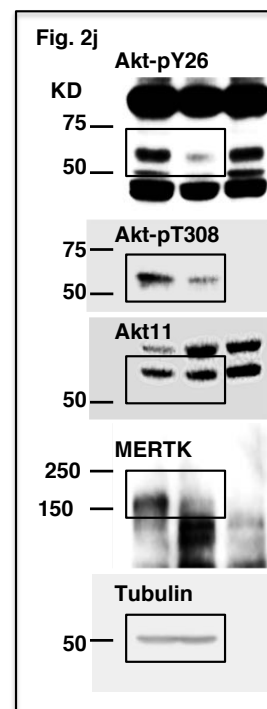
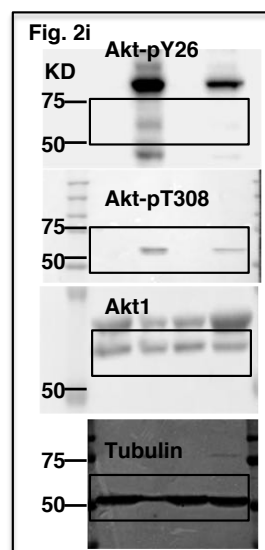
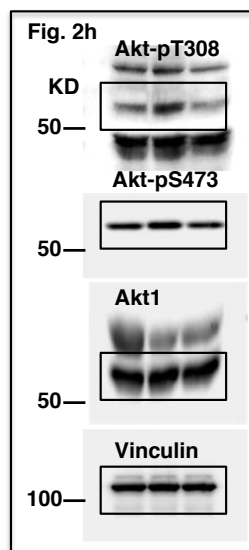
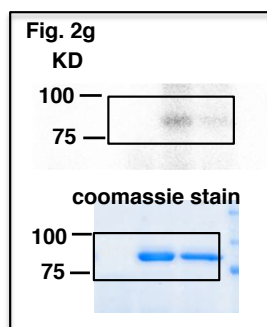
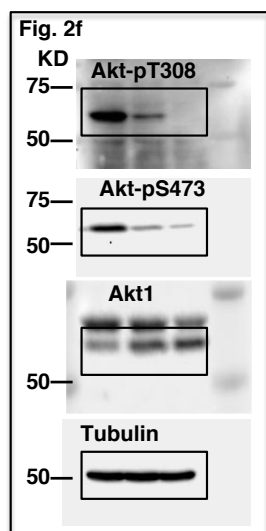
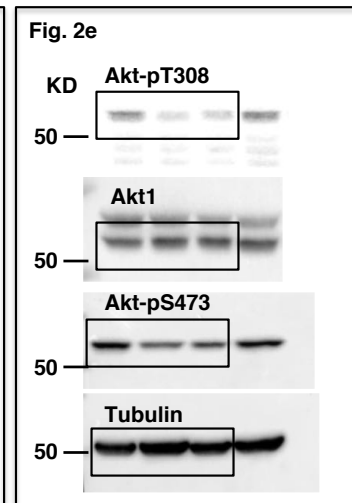
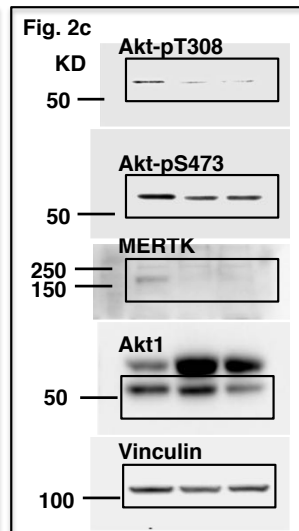
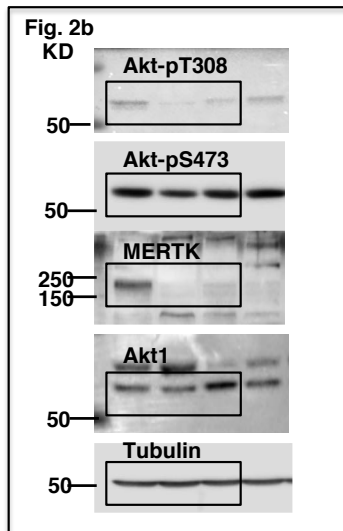
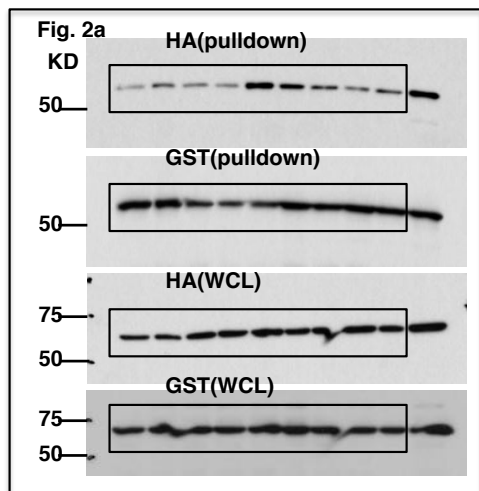


Fig1k





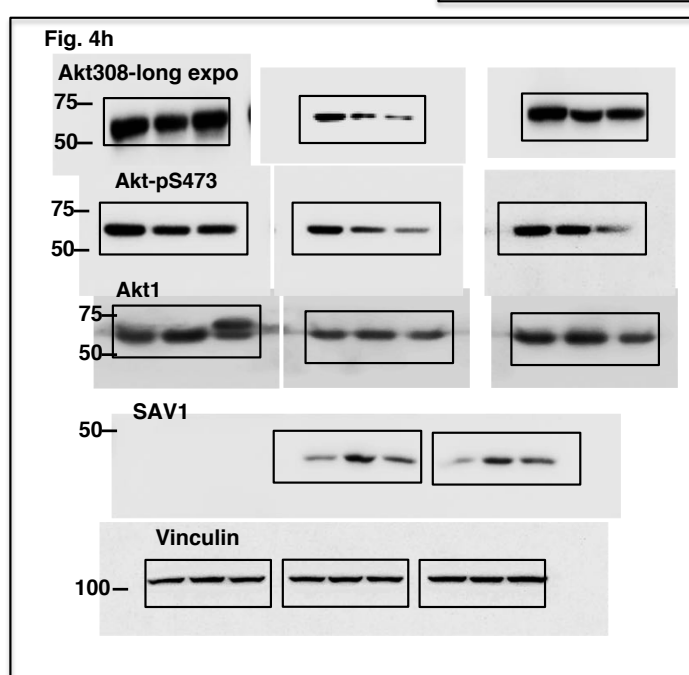
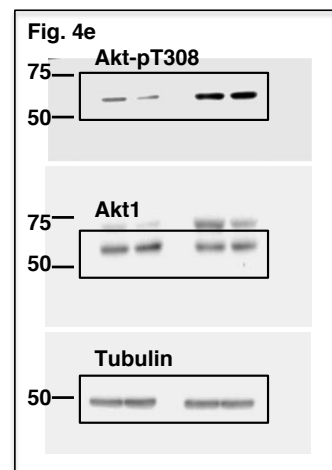
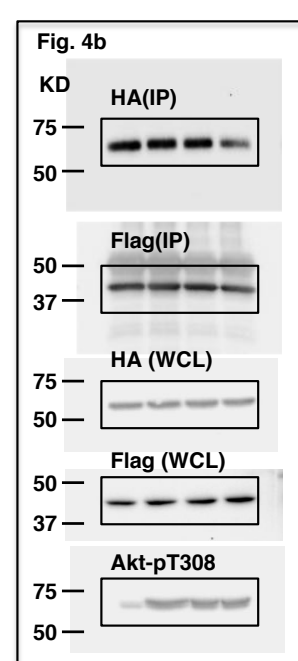
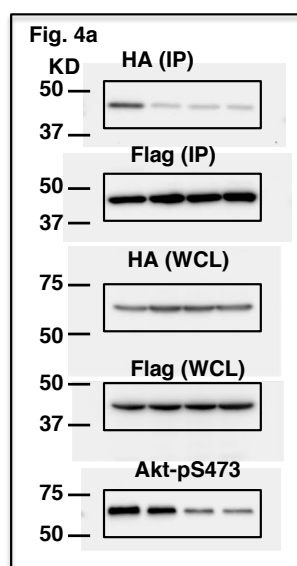
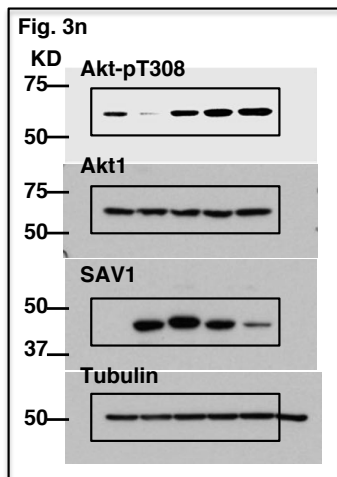
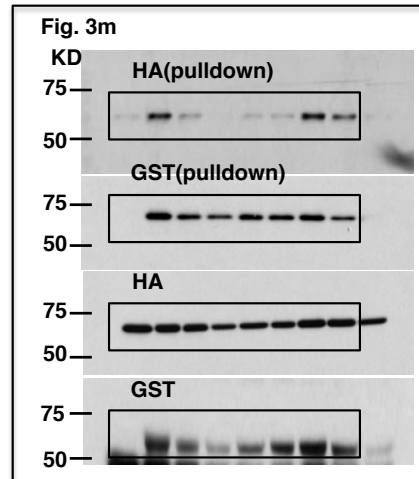
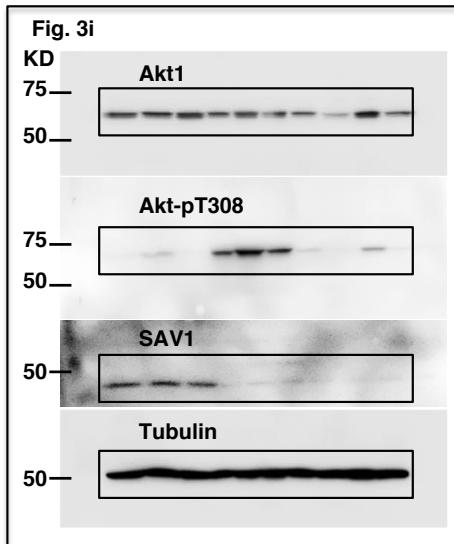
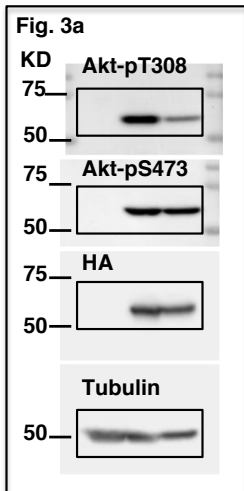


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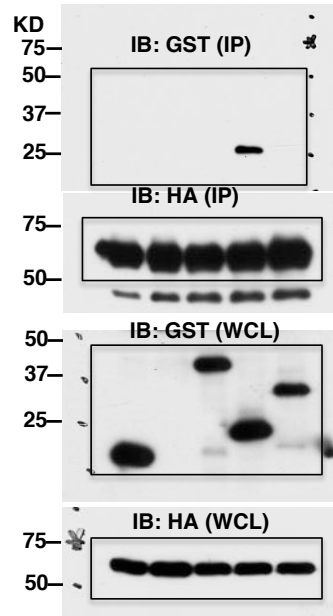


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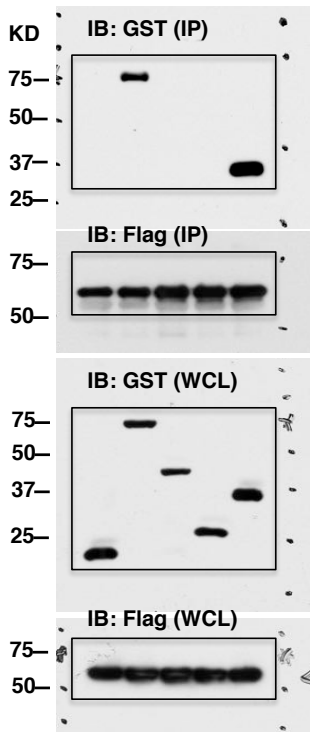


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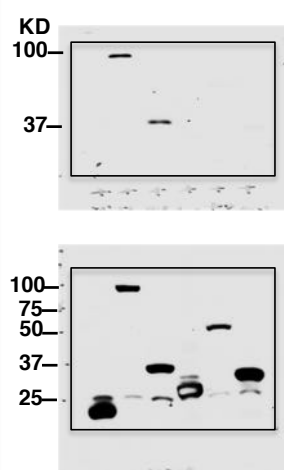


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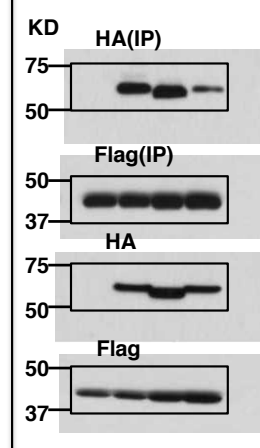


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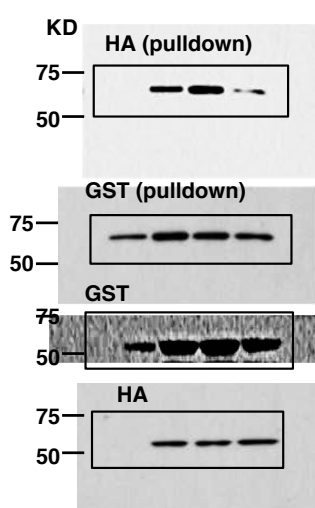


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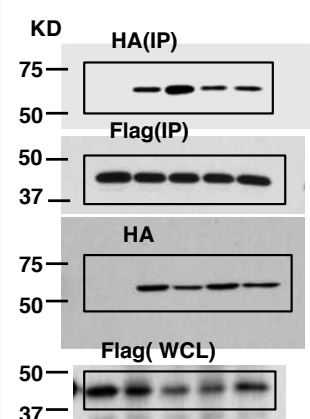


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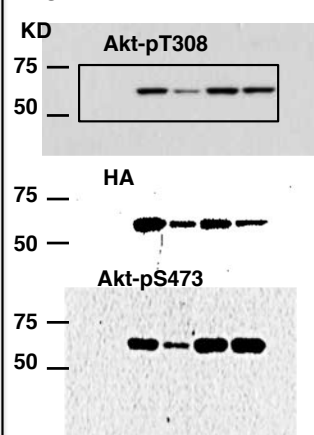


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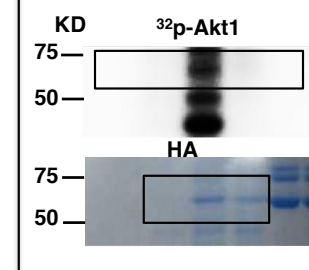


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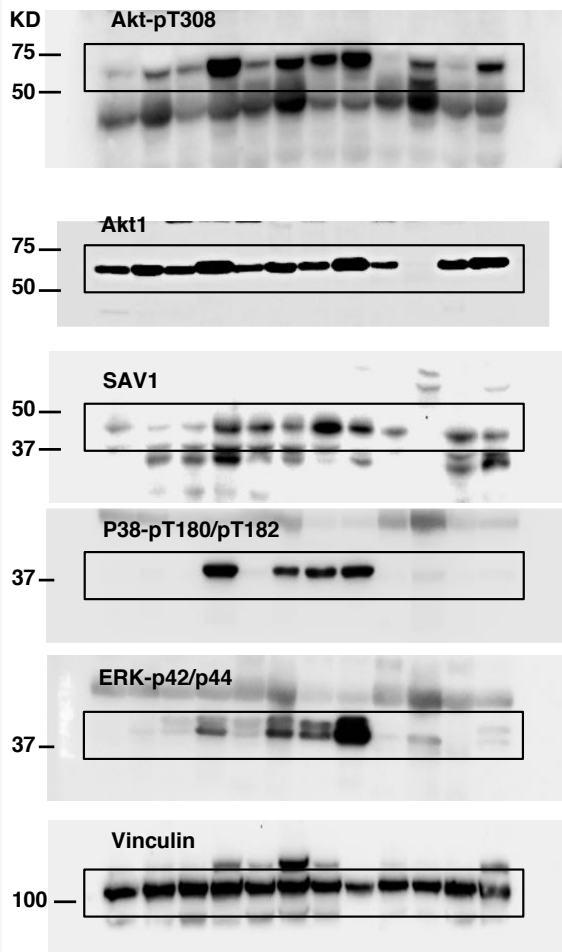


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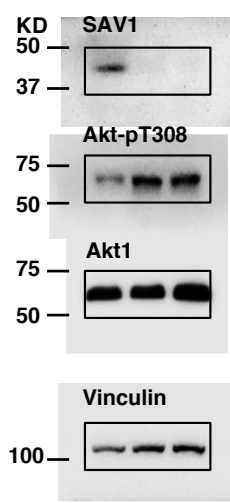


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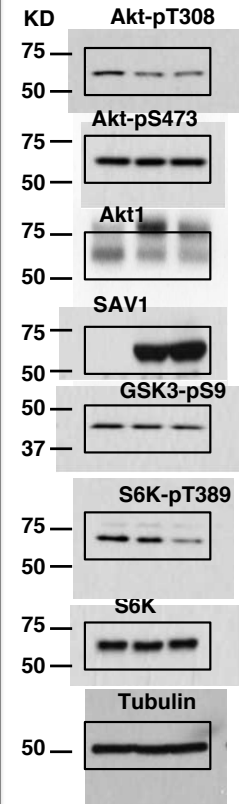


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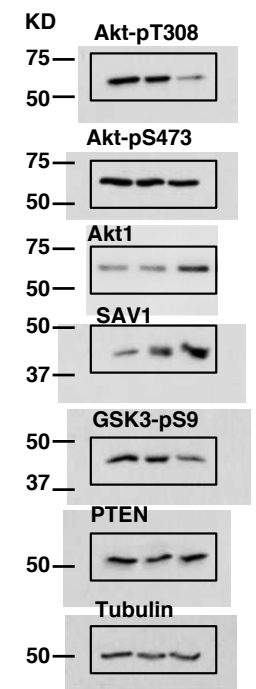


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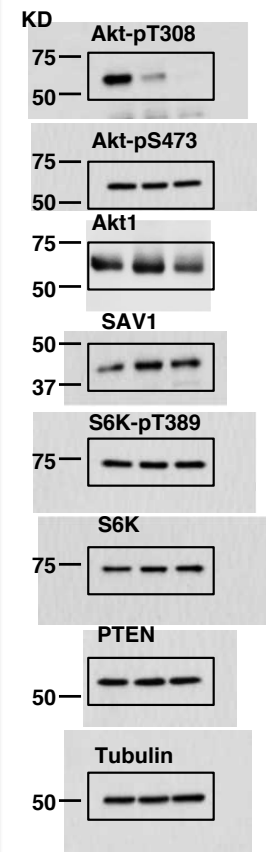


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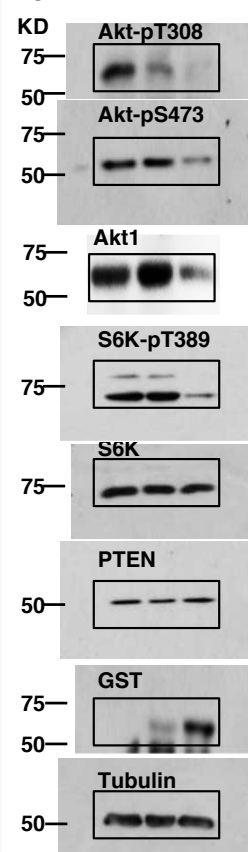


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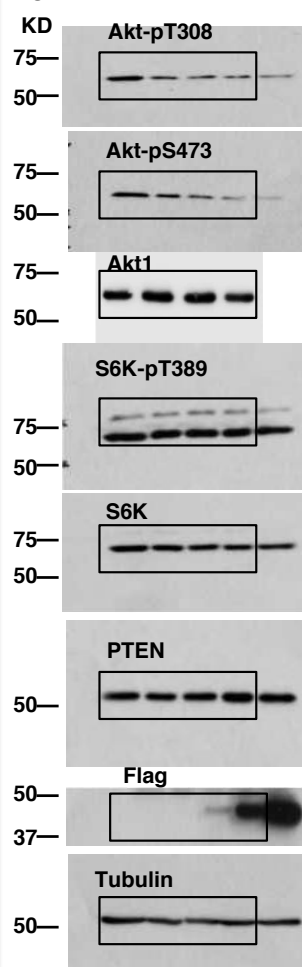


Fig. S3g

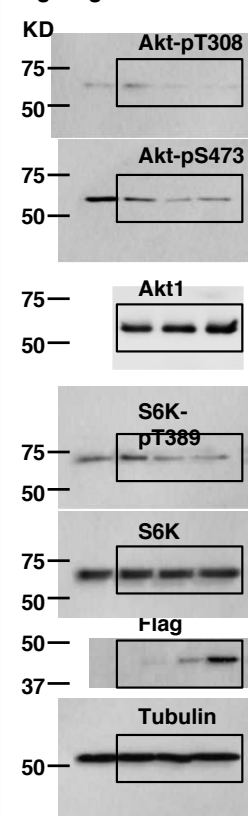
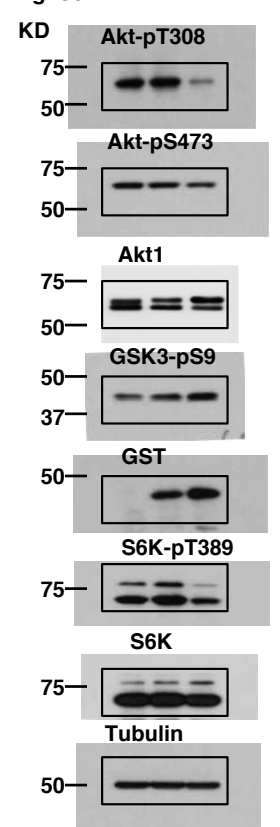


Fig. S3h



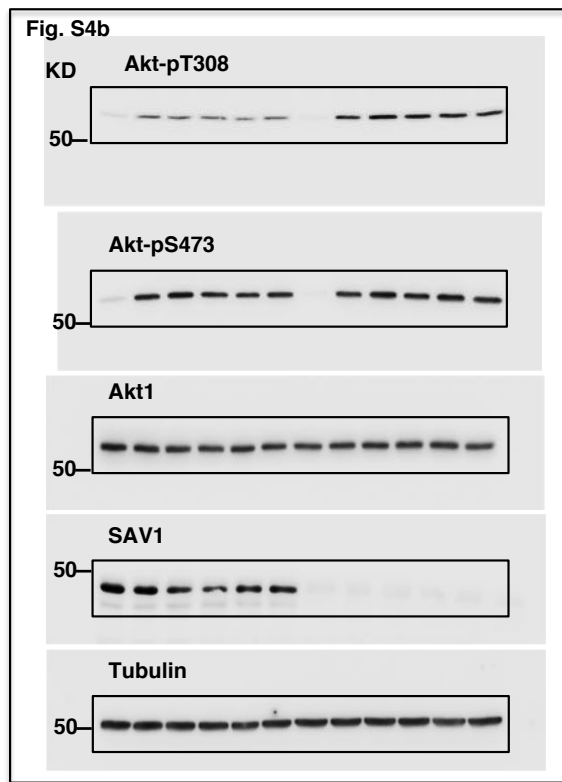
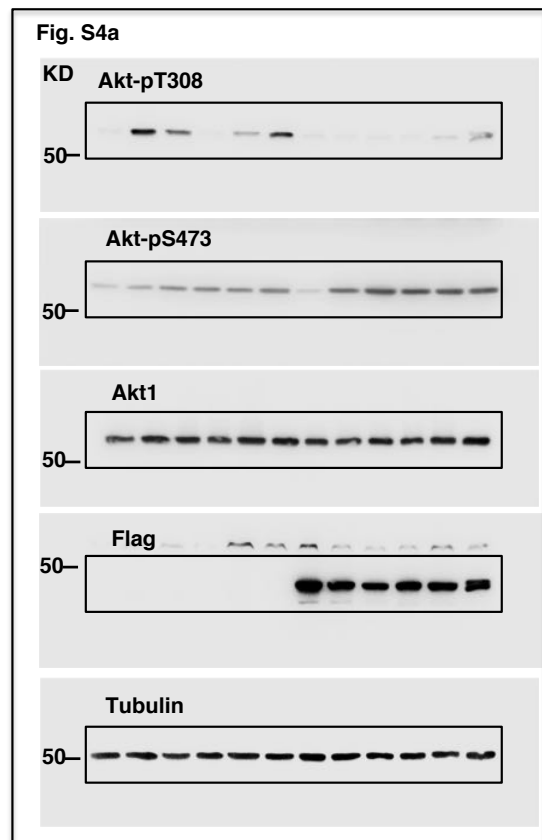
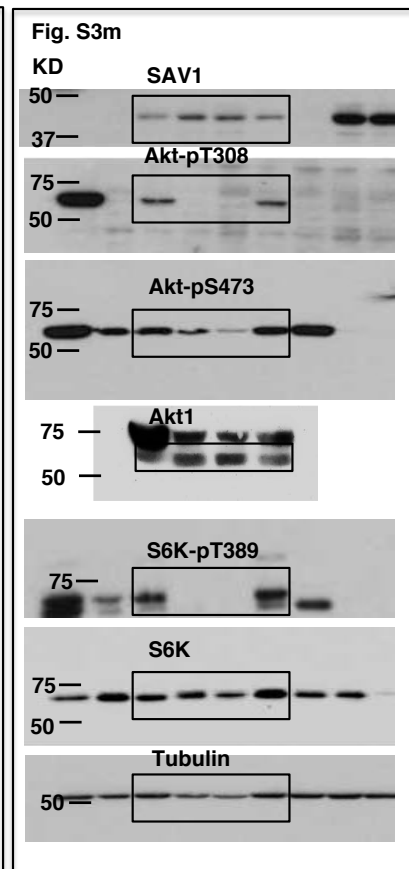
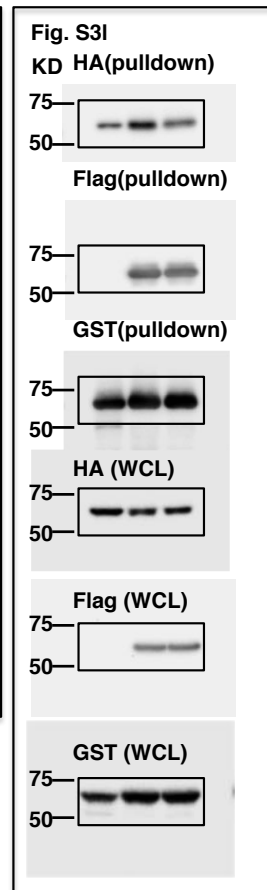
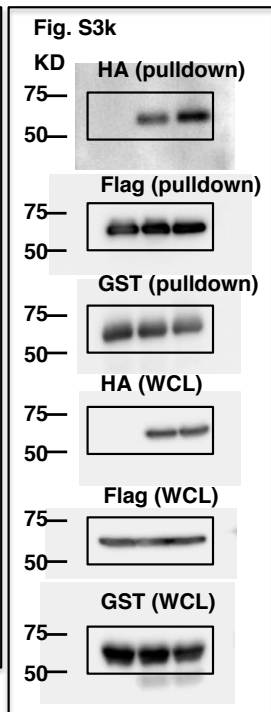
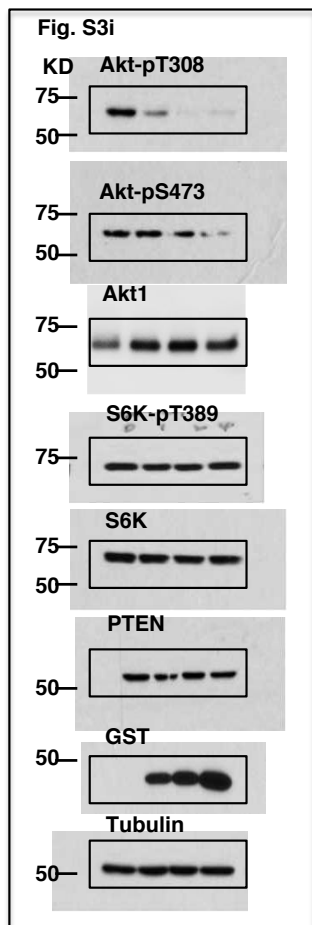


Fig. S4c

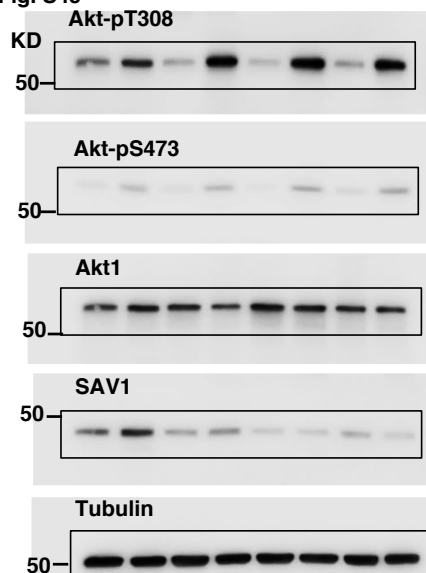


Fig. S4d

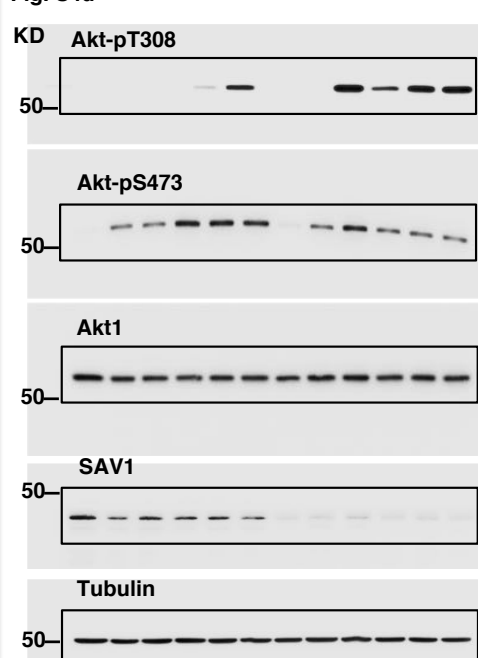


Fig. S5a

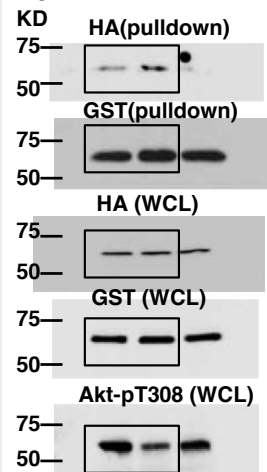


Fig. S5b

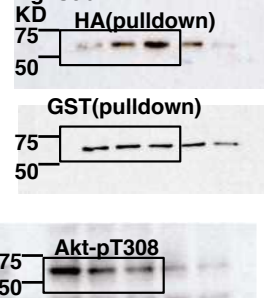


Fig. S5c

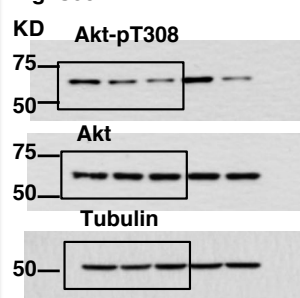


Fig. S5d

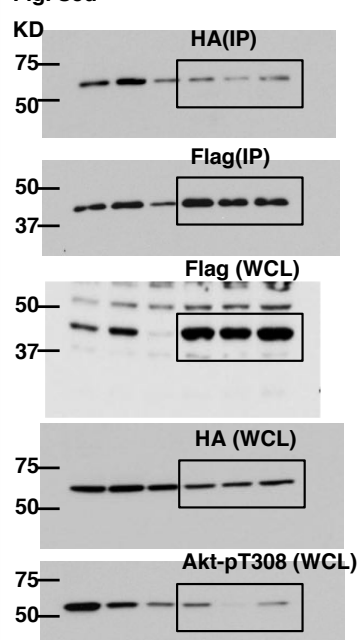


Fig. S5e

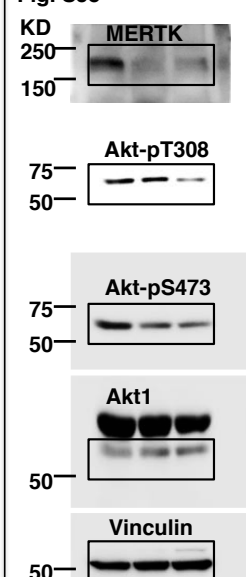


Fig. S5f

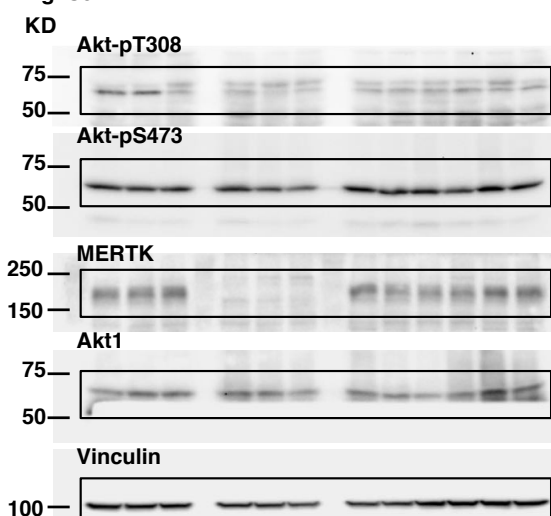


Fig. S5h

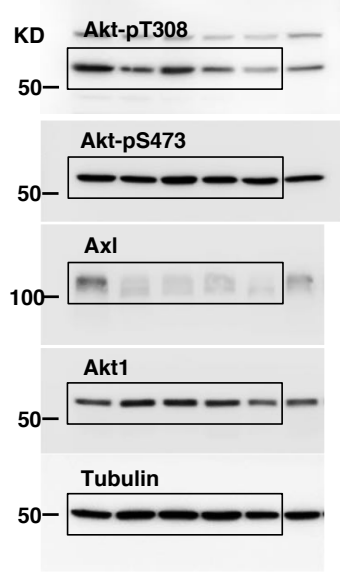


Fig. S5i

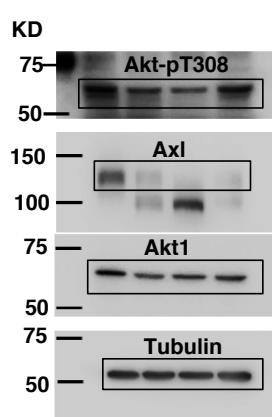


Fig. S5j

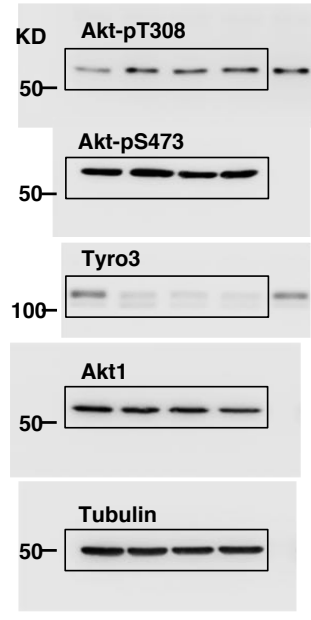


Fig. S5k

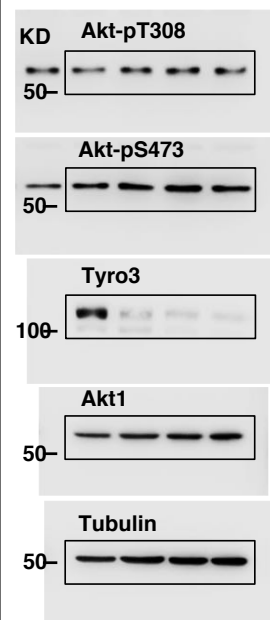


Fig. S6a

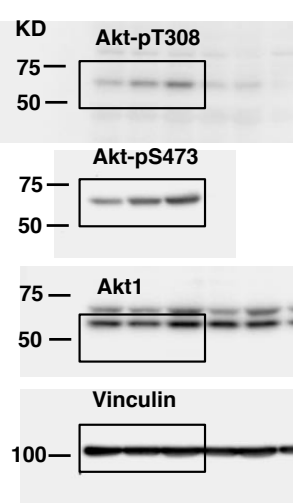


Fig. S6d

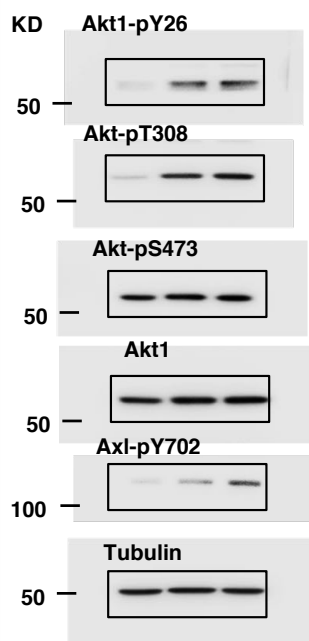


Fig. S6e

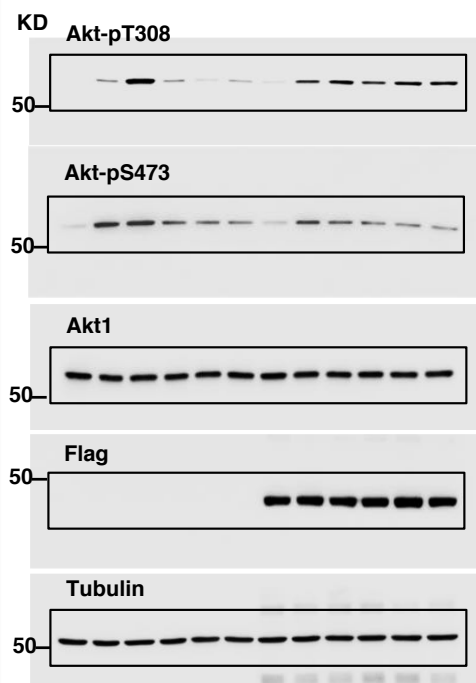


Fig. S6f

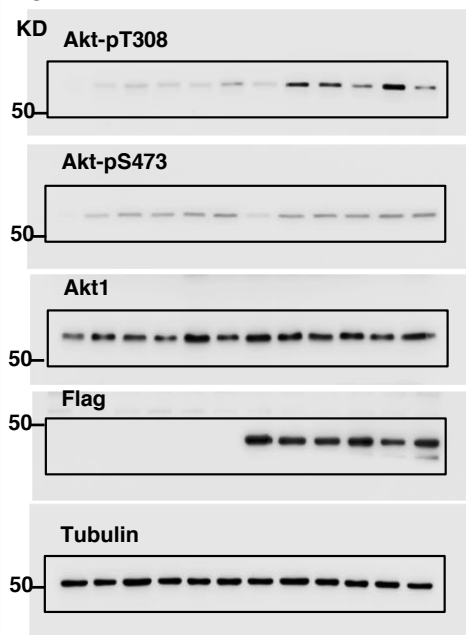


Fig. S6g

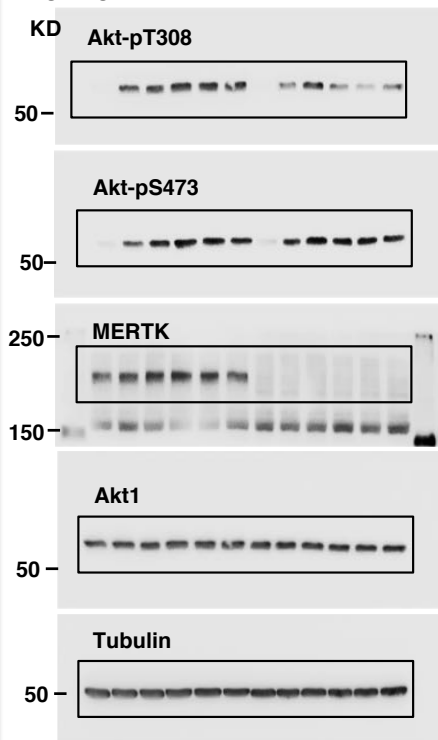


Fig. S6h

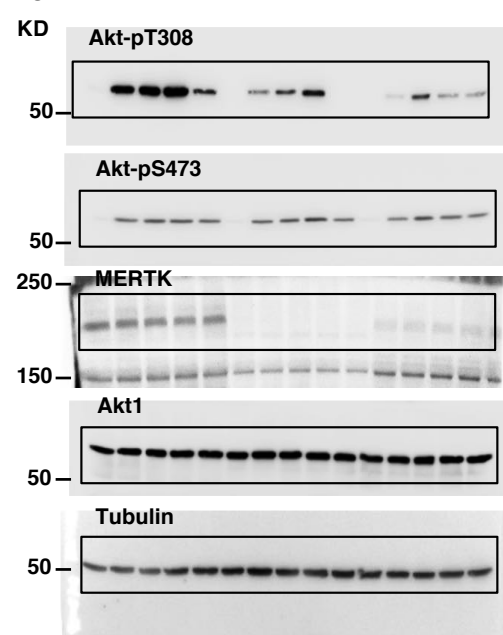


Fig. S7b

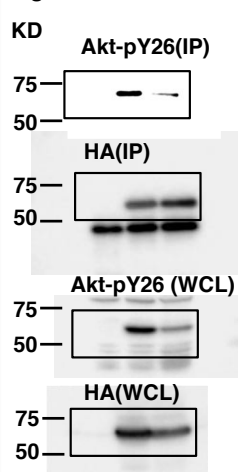


Fig. S8b

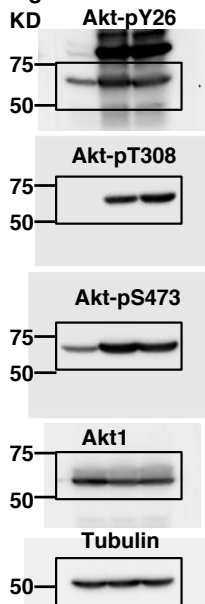


Fig. S8a

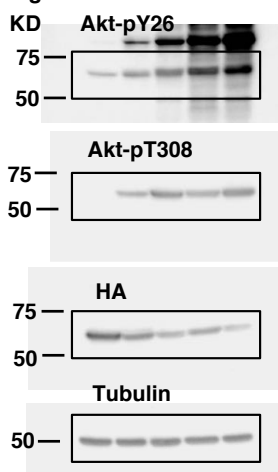


Fig. S8c

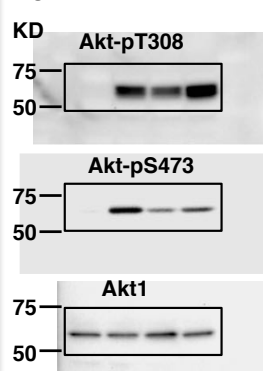
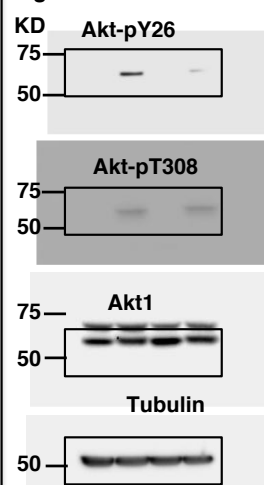


Fig. S8d



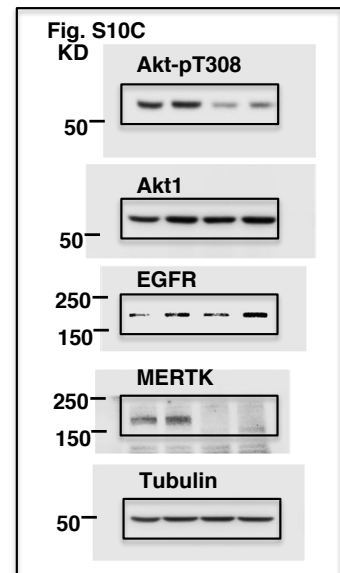
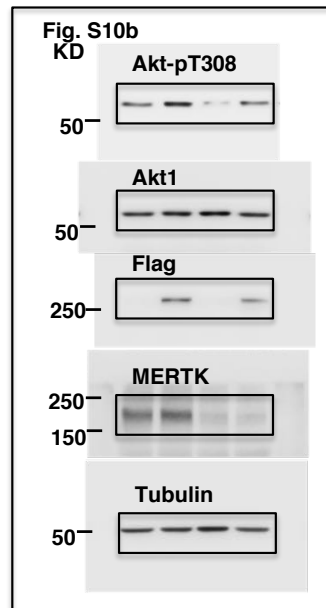
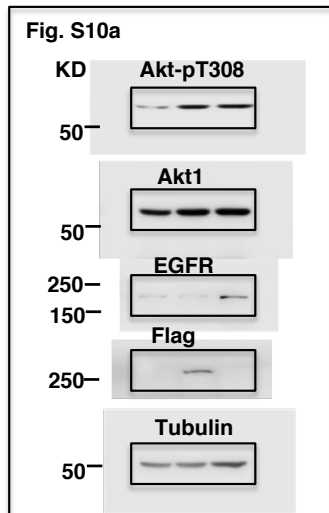
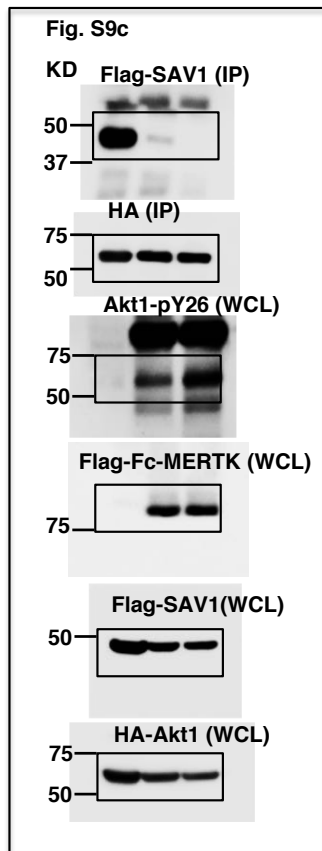
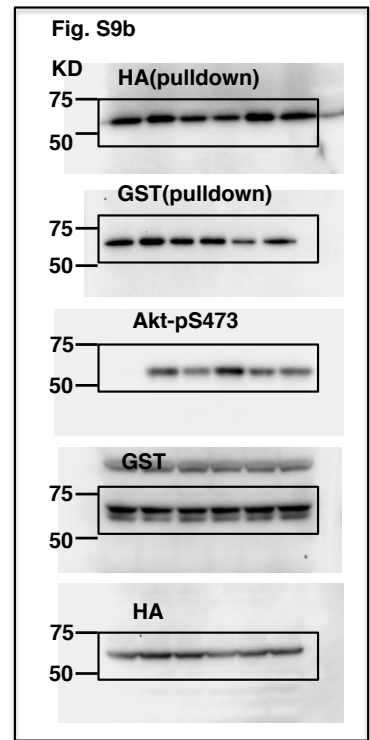
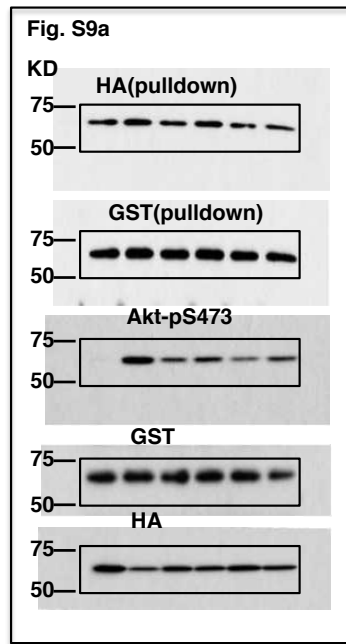
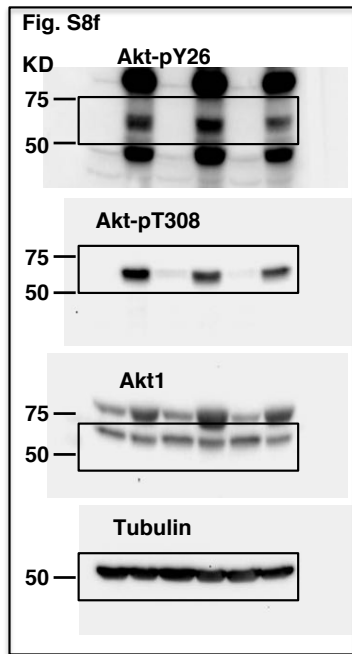
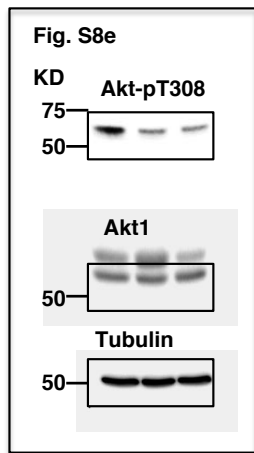


Fig. S10d

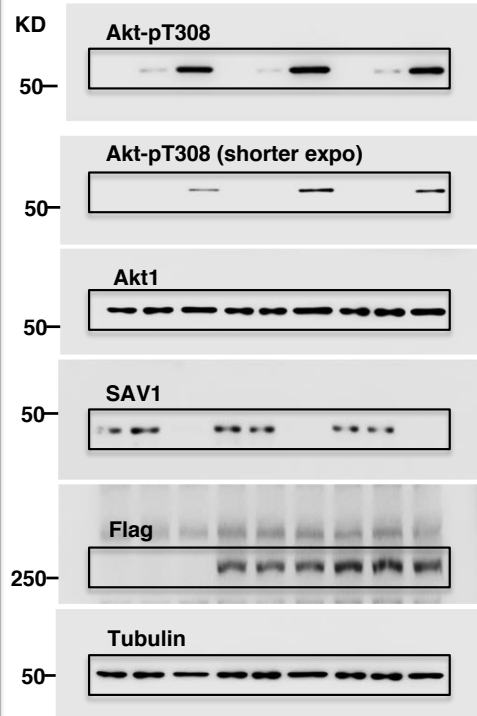


Fig. S10e

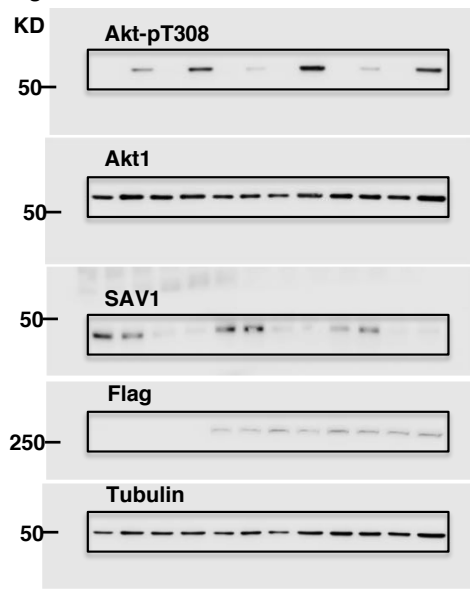


Fig. S10f

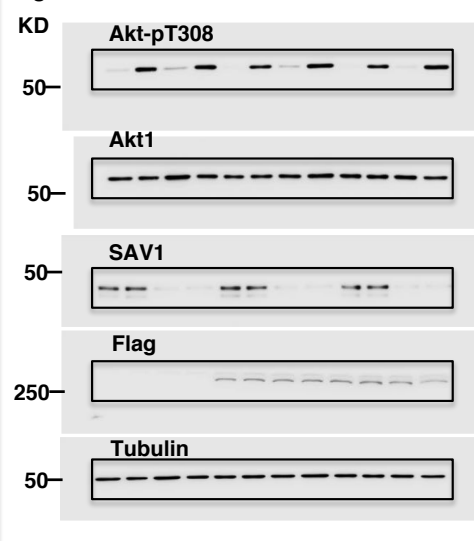


Fig. S10g

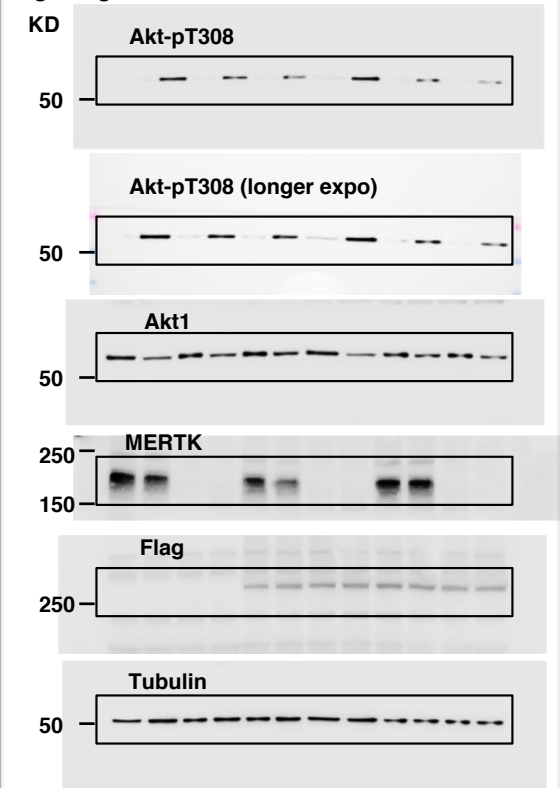


Fig. S10h

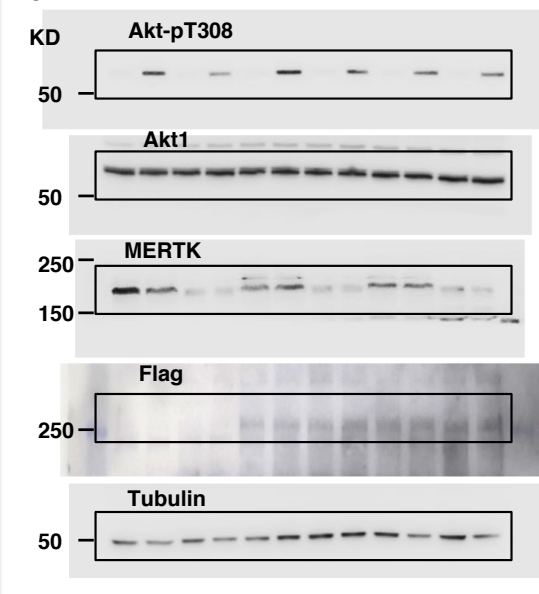


Fig. S10i

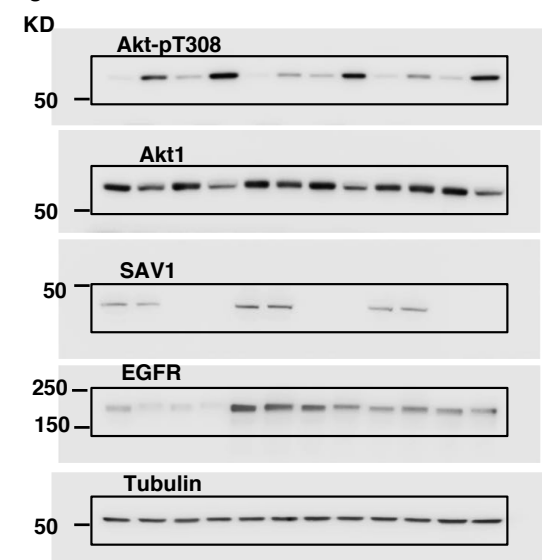


Fig. S10j

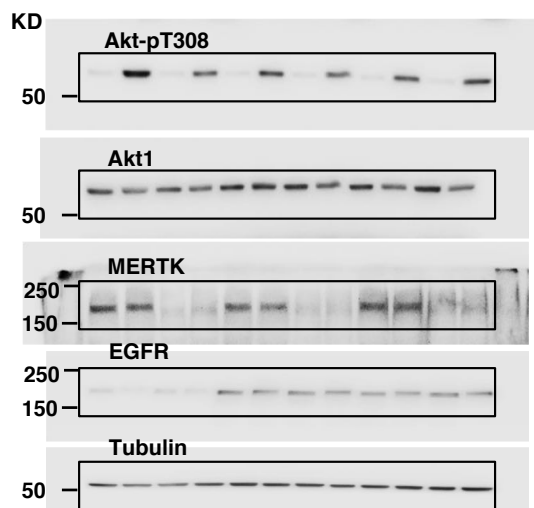


Fig. S11a

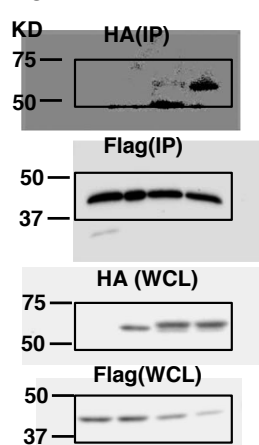


Fig. S11b

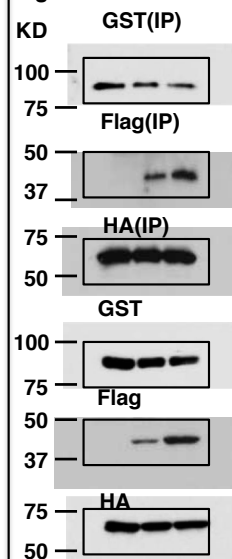


Fig. S11c

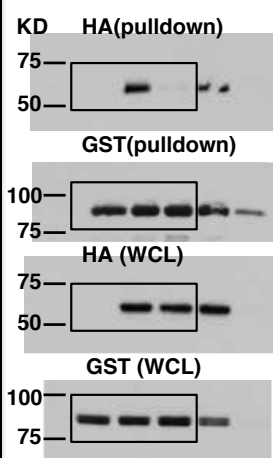


Fig. S11d

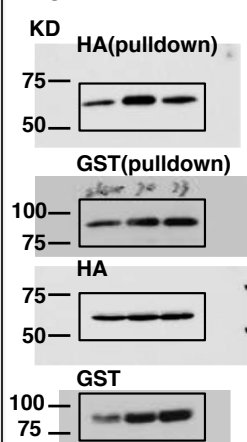


Fig. S12a

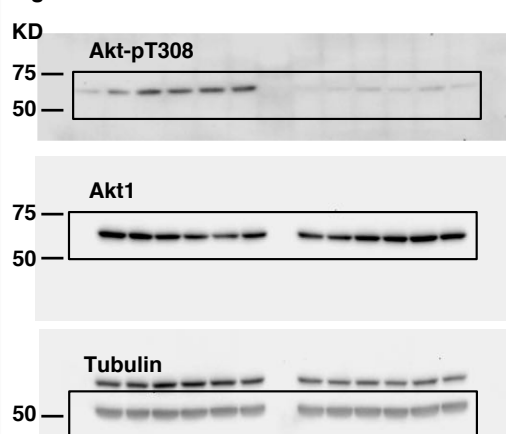


Fig. S12b

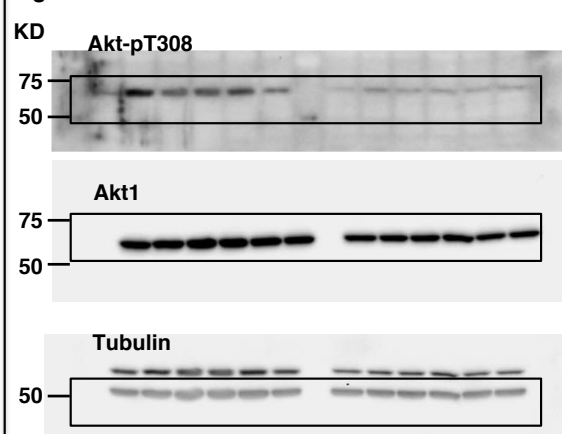


Fig. S11e

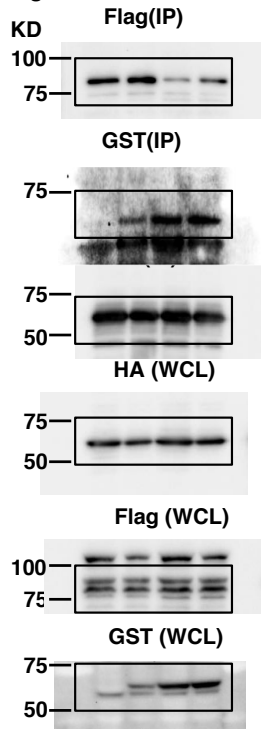


Fig. S12d

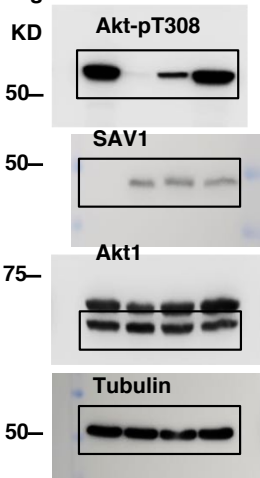


Fig. S13a

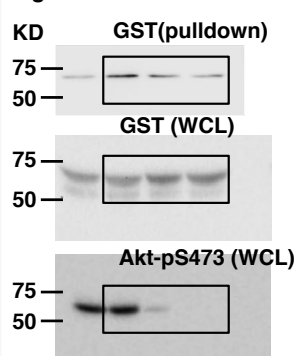


Fig. S13b

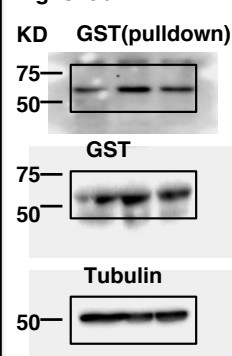


Fig. S13c

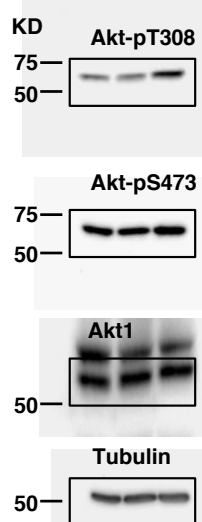
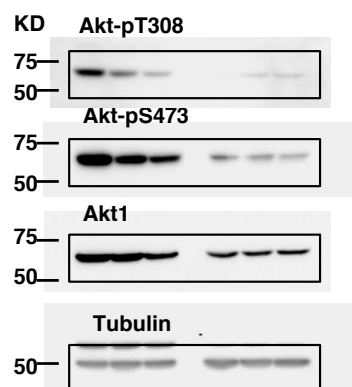


Fig. S13d



Supplementary Figure 14. Original uncropped scans for western blots.