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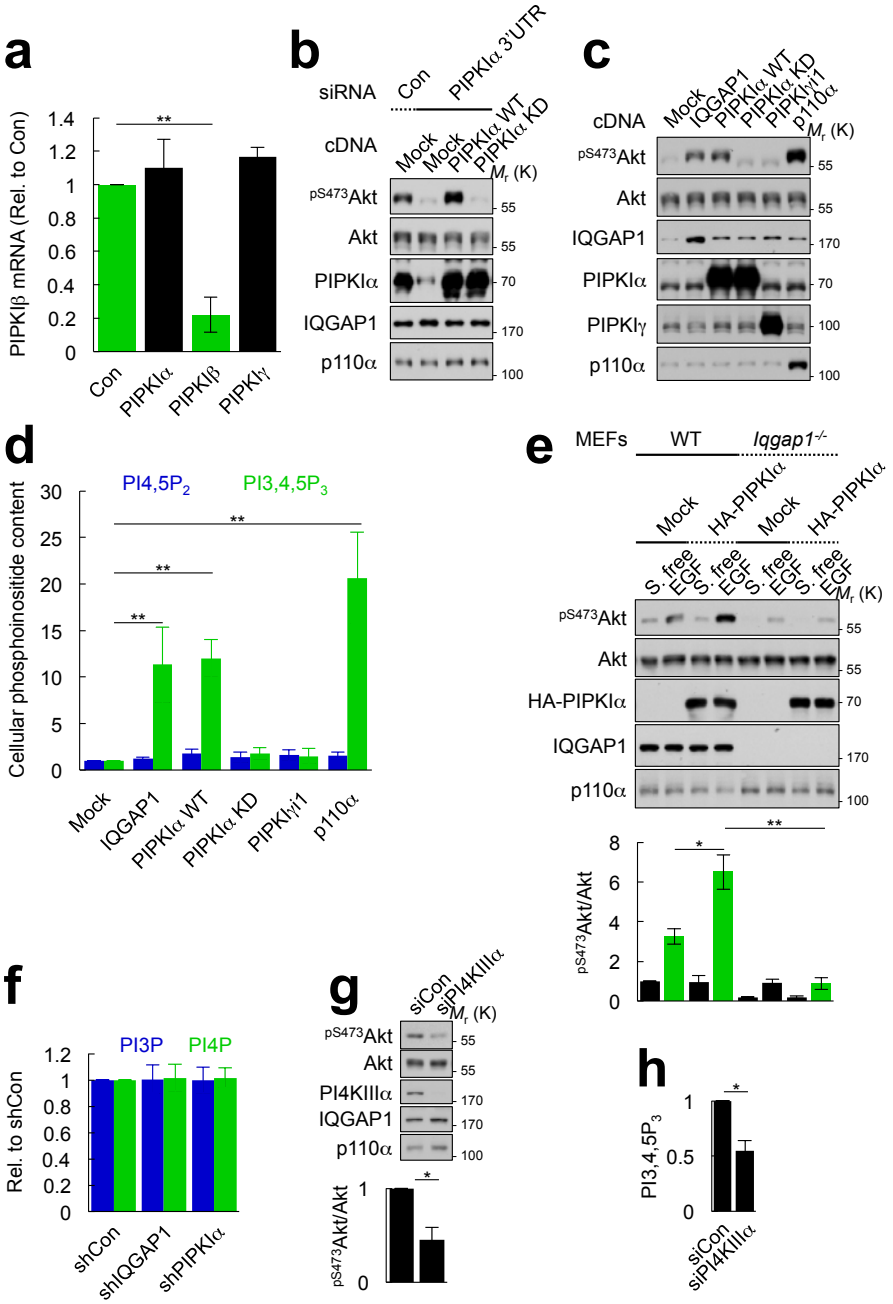
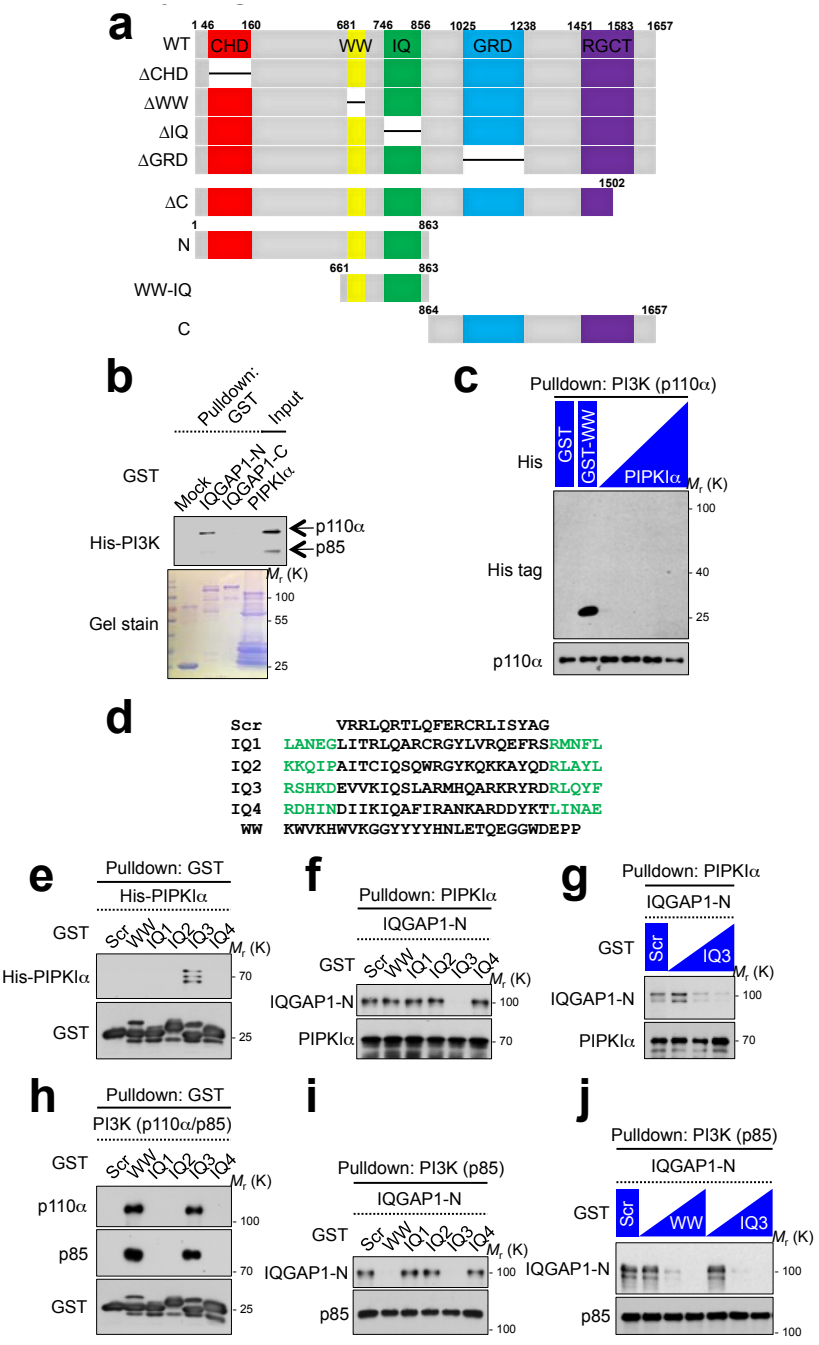
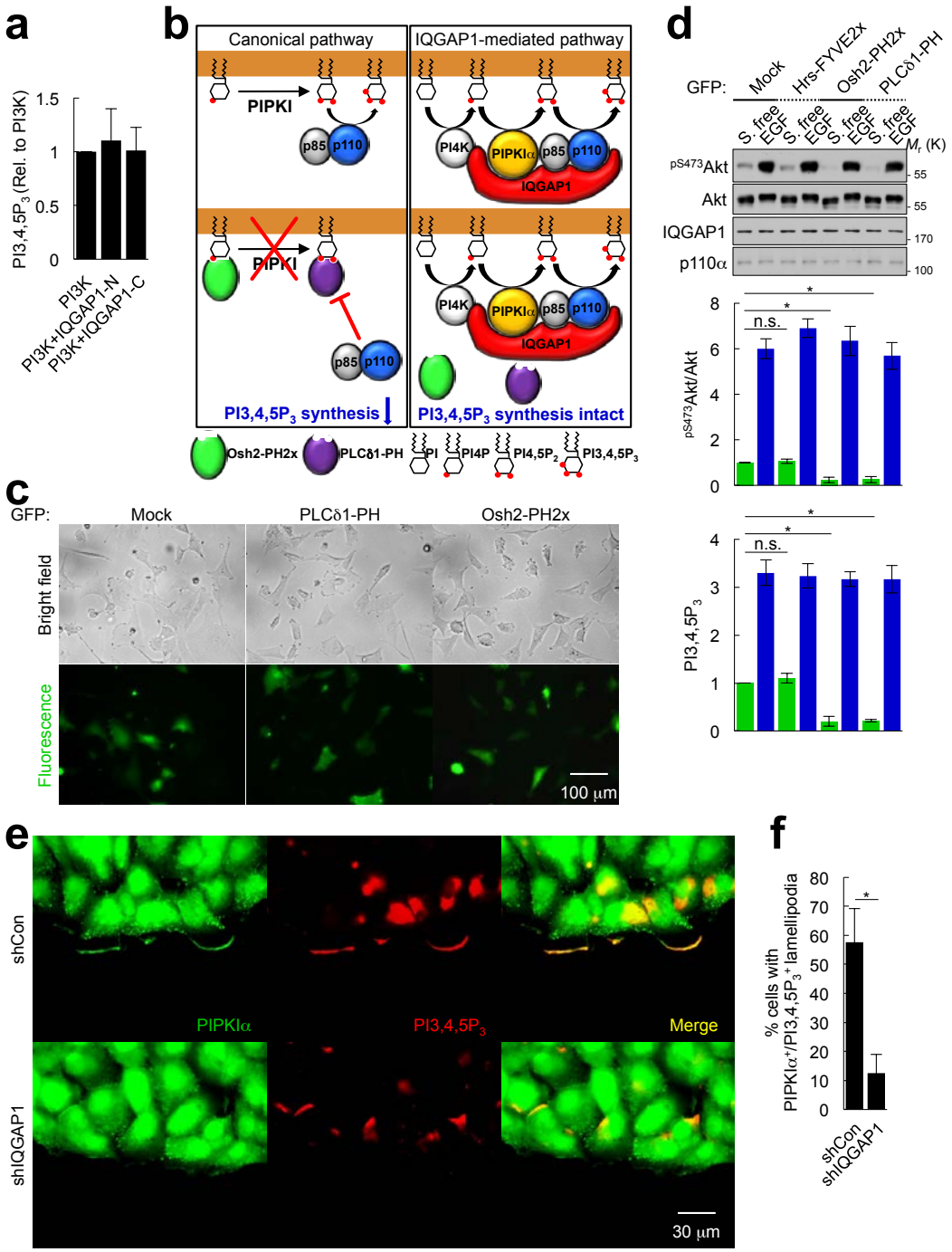


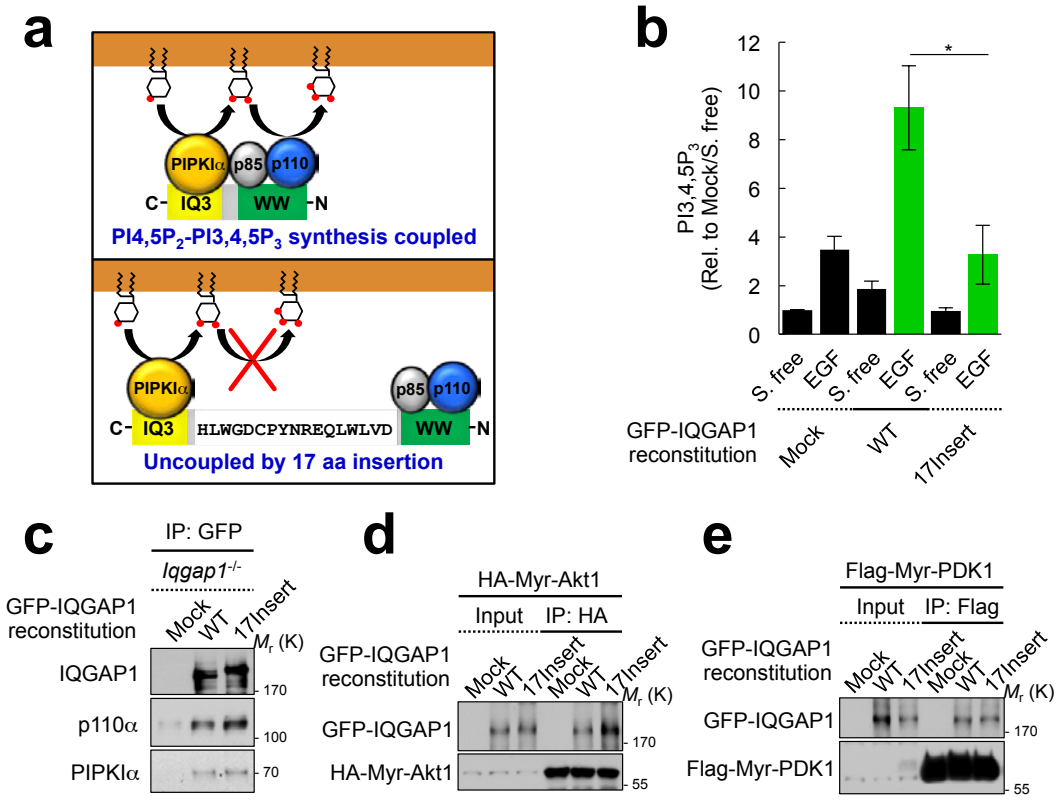
Supplementary Figure 1 PI4KIIIα, PIPKIα and IQGAP1 are required for Akt activation. a, RT-PCR analysis of PIPKIβ mRNA. PIPKIβ mRNA levels were normalized with GAPDH mRNA .The graph is shown as mean ± SD of n=3 independent experiments. Paired Student t-tests were used for statistical analysis (*, p<0.05; **, p<0.01; n.s., not significant). b, c, Indicated proteins were knocked down and/or overexpressed in MDA-MB-231 cells and cell lysates were analyzed by IB. d, Indicated proteins were overexpressed in MDA-MB-231 cells and PI4,5P2 and PI3,4,5P3 contents were analyzed by a competitive ELISA. Paired Student t-tests were used for statistical analysis (*, p<0.05; **, p<0.01; n.s., not significant). e. Wild type or Iqgap1-/- MEFs were overexpressed with PIPKIα and cells were treated with 10 ng/ml EGF for 10 min. Cell lysates were analyzed by IB and the graph is shown as mean ± SD of n=3 independent experiments. Paired Student t-tests were used for statistical analysis (*, p<0.05; **, p<0.01; n.s., not significant). f, Stable Hs578T cells growing in normal culture conditions were harvested PI3P and PI4P were measured by a competitive ELISA (Echelon Biosciences). The graph is shown as mean ± SD of n=4 independent experiments. g, h, MDA-MB-231 cells were transfected with the indicated siRNAs. Akt phosphorylation (i) and cellular PI3,4,5P3 content (j) were measured. The graphs are shown as mean ± SD of n=3 independent experiments. Paired Student t-tests were used for statistical analysis (*, p<0.05; **, p<0.01; n.s., not significant). Source data for a, d, e, f, g, h can be found in Supplementary Table 1. Unprocessed original scans of blots are shown in Supplementary Fig. 7.



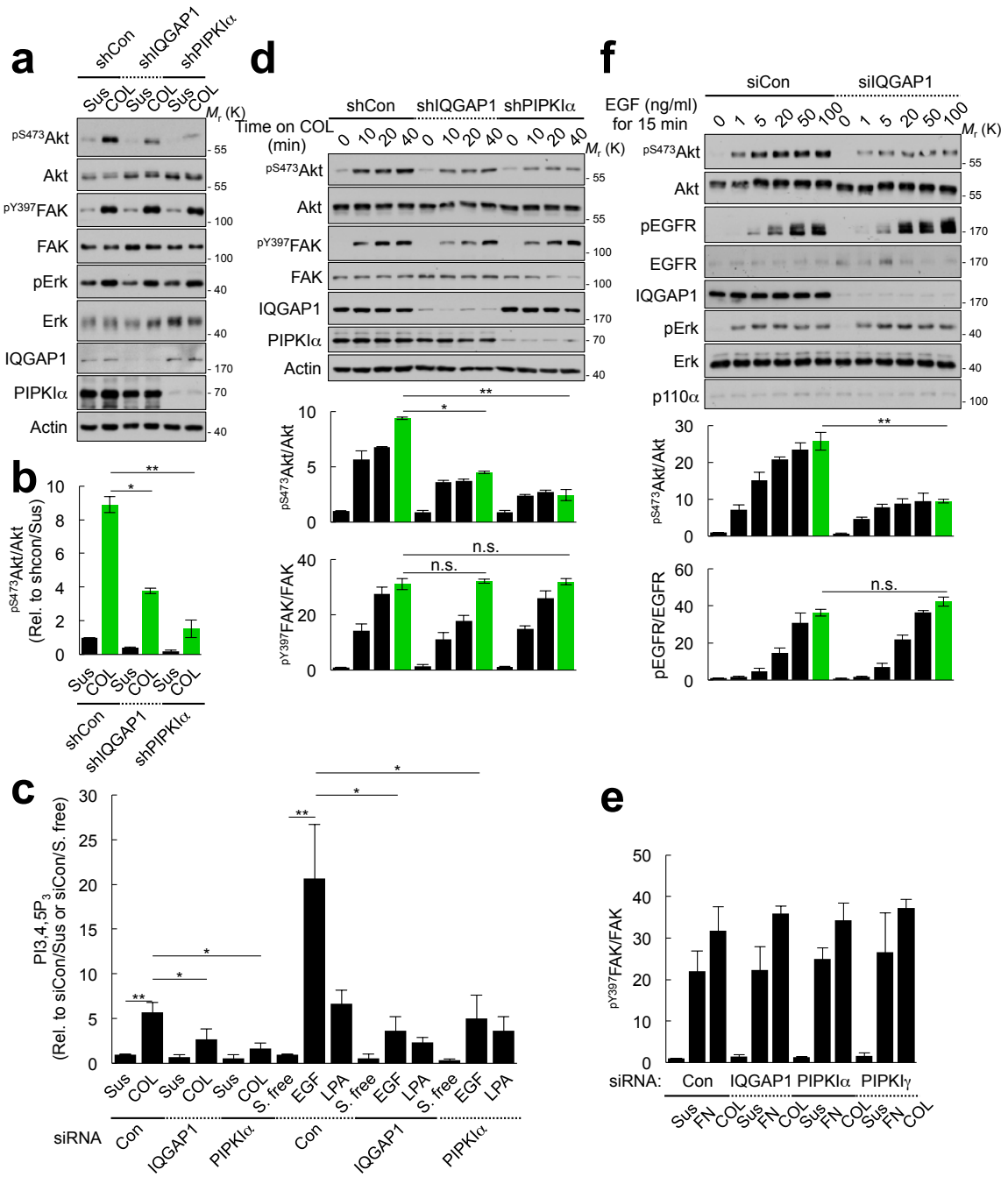
Supplementary Figure 2 PIPKIα and PI3K directly interact on IQGAP1 through the IQ3 and WW motifs. a, Schematic representation of IQGAP1 domains and constructs used in the study. b, 0.1 μM GST-IQGAP1 fragments and PIPKIα immobilized on glutathione beads were incubated with 0.5 μM His-PI3K (His-p110α/His-p85). Associated PI3K subunits were analyzed by IB with an anti-His antibody. IQGAP1-N fragment directly binds to PI3K, whereas neither IQGAP1-C fragment nor PIPKIα binds. c, His-tagged GST alone, GST-WW domain and PIPKIα (0-1 μM) were incubated with untagged 0.1 μM PI3K. PI3K was immunoprecipitated with an anti-p110α antibody and the associated proteins were analyzed by IB with an anti-His antibody. d, The WW domain and IQ motif amino acid sequences. 28 aa from the WW domain and 20 aa (in black) from each IQ motif (IQ1-IQ4) were used in the study. e, 0.1 μM His-PIPKIα was incubated with 0.05 μM GST-WW domain or -IQ motifs immobilized on beads. GST-polypeptides were pulled down and associated PIPKIα was analyzed by immunoblotting. f, g, 0.02 μM PIPKIα and 0.02 μM IQGAP1-N were incubated with 0.1 μM GST-tagged polypeptides. PIPKIα was pulled down and the associated proteins were analyzed by immunoblotting. For g, 0, 0.05 and 0.1 μM GST-IQ3 were used. h, 0.02 μM PI3K (p110α/p85) was incubated with 0.05 μM GST-WW domain or -IQ motifs immobilized on beads. GST-polypeptides were pulled down and associated PI3K subunits were analyzed by immunoblotting. i, j, 0.02 μM PI3K and 0.02 μM IQGAP1-N were incubated with 0.1 μM GST-tagged polypeptides. PI3K was pulled down with and the associated molecules were analyzed by immunoblotting. For j, 0, 0.05 and 0.1 μM GST-WW or IQ3 were used. The experiments described above were performed independently at least four times. Unprocessed original scans of blots are shown in Supplementary Fig. 7.



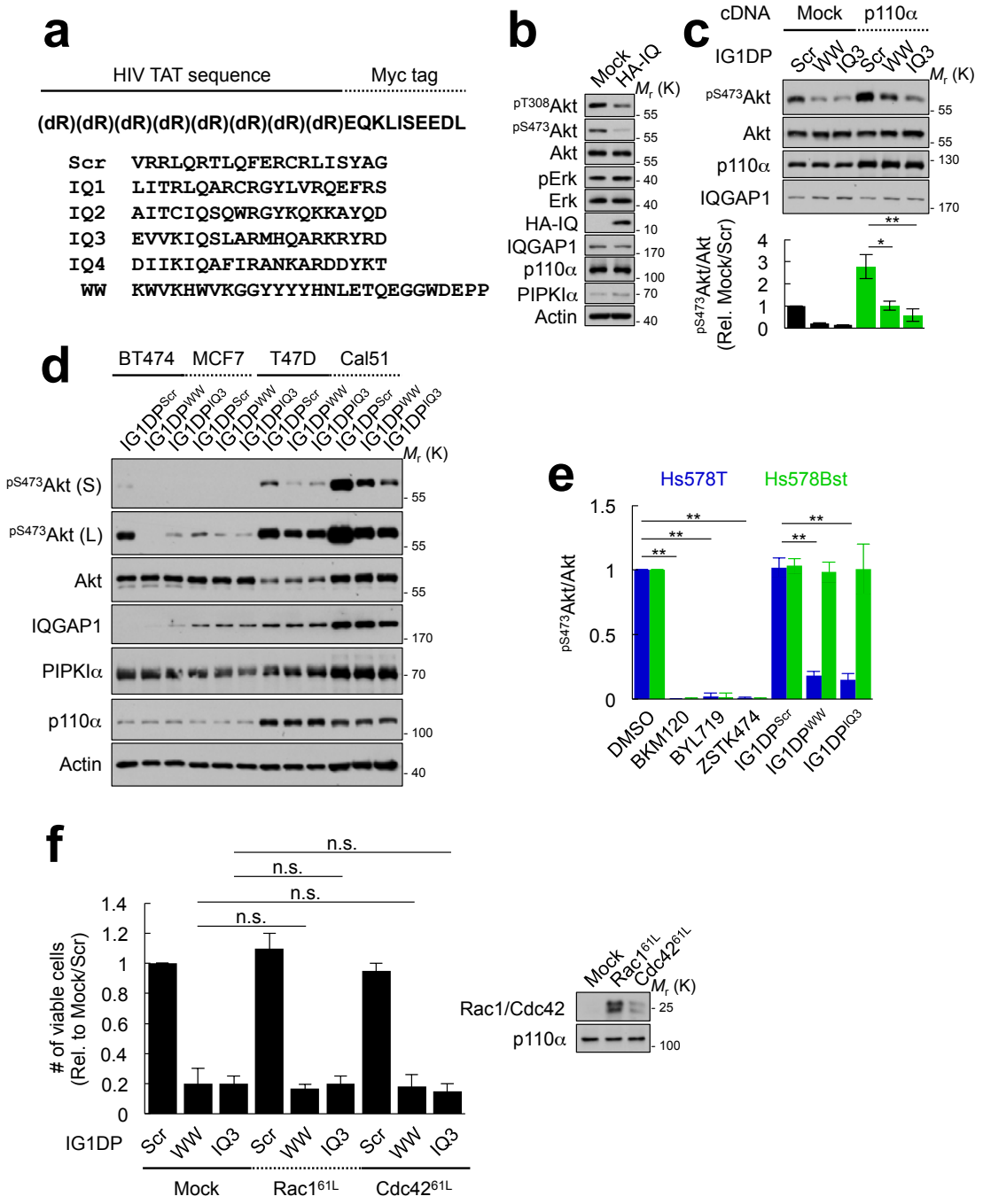
Supplementary Figure 3 PI3,4,5P3 synthesis requires concerted PI4,5P2 generation by PIPKIα nad. a, PI3,4,5P3 generated by PI3K and IQGAP1 fragments from 25 μM liposomes containing 10 molar % of PI4,5P2 was measured. The graph is shown as mean ± SD of n=3 independent experiments. b, Schematic representation of canonical versus IQGAP1-mediated PI3,4,5P3 synthesis pathways. c, The indicated PH domains were stably expressed in Hs578T cells. Cells grown in tissue culture were photographed in bright field and fluorescent channels at 200X magnification. Roughly 70-80% of cells express exogenous proteins. Scale bar, 100 μm. d, Hs578T cells stably expressing the indicated PH domains were treated with 10 ng/ml EGF for 10 min. Cell lysates were analyzed by IB (top) and pS473Akt immunoblots of n=4 independent experiments were quantified (middle). PI3,4,5P3 levels were measured by a competitive ELISA and the graph is shown as mean ± SD of three independent experiments (bottom). Paired Student t-tests were used for statistical analysis (*, $p<0.05$; **, $p<0.01$; n.s., not significant). e, Hs578T cells were stably expressed with shRNA against IQGAP1. Cells expressing non-targeting shRNA were used as a control. Cells were grown to confluence, wounded and fixed 3 h later, followed by immunostaining for PIPKIα and PI3,4,5P3. Cells were photographed at 400X magnification. Scale bar, 100 μm. f, Immunostaining images of e were analyzed and percent of cells (over 100 cells counted for each condition) that are positive for both PIPKIα and PI3,4,5P3 signals at the leading edges were shown in the graph (n= 120 for shCon and 110 for shIQGAP1, mean ± SD of three independent experiments). Unpaired Student t-tests were used for statistical analysis (*, $p<0.05$; **, $p<0.01$; n.s., not significant). The experiments described above were performed independently at least n=3 times. Source data for d, f can be found in Supplementary Table 1. Unprocessed original scans of blots are shown in Supplementary Fig. 7.



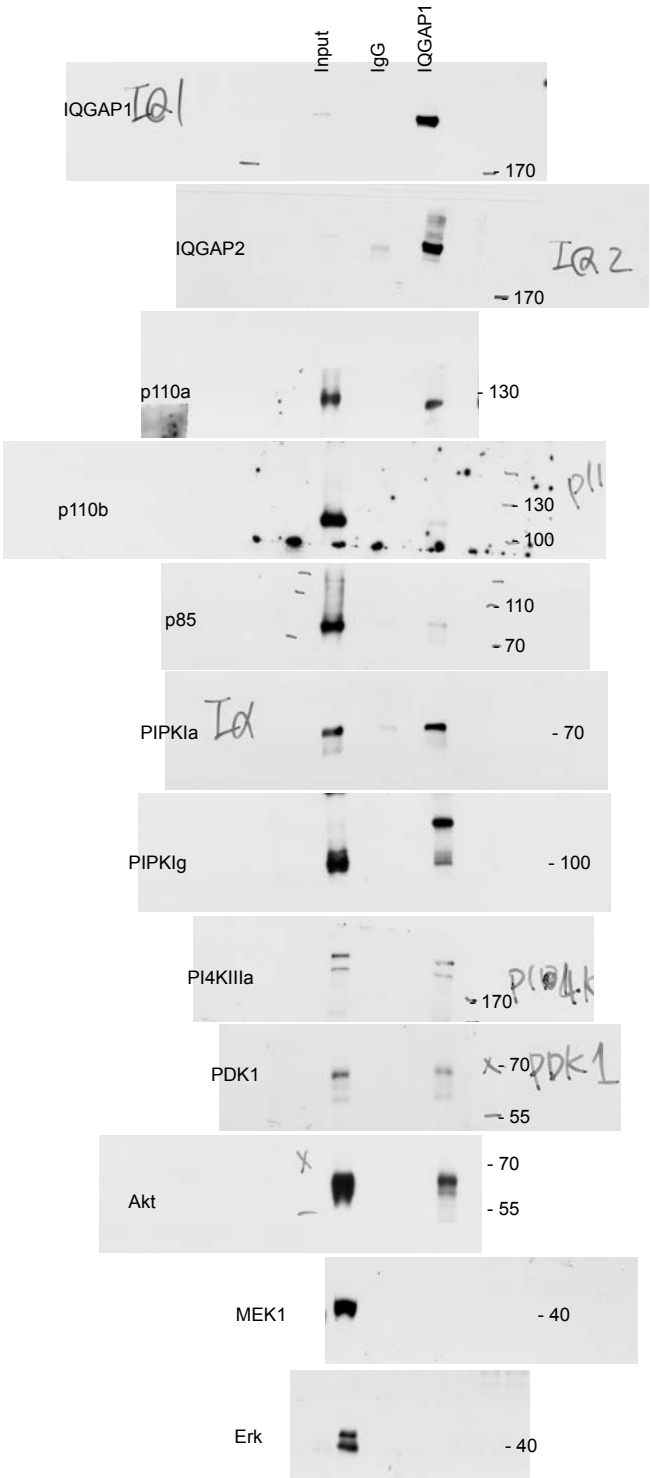
Supplementary Figure 4 Separation of PIPKIα and PI3K binding on IQGAP1 attenuates PI3,4,5P3 synthesis. a, Schematic representation of uncoupling of PI4,5P2 and PI3,4,5P3 synthesis by inserting the 17 aa indicated between the WW and IQ domains. b, Iqgap1 knockout (Iqgap1-/-) mouse embryonic fibroblasts (MEFs) were reconstituted with the indicated GFP-tagged human IQGAP1 constructs. Cells were treated with 10 ng/ml EGF for 15 min and cellular PI3,4,5P3 contents were measured by a competitive ELISA. The graph is shown as mean ± SD of n=3 independent experiments. Unpaired Student t-tests were used for statistical analysis (*, p<0.05; **, p<0.01; n.s., not significant). c, Using cell lysates from the reconstituted MEFs, IQGAP1 proteins were IP'ed with an anti-GFP antibody and associated proteins were analyzed by IB. d, e, The reconstituted MEFs were transfected with constitutively active Akt1 or PDK1 and Akt1 or PDK1 was IP'ed and the associated IQGAP1 proteins were analyzed by IB. The experiments described above were performed independently at least three times. Source data for b can be found in Supplementary Table 1. Unprocessed original scans of blots are shown in Supplementary Fig. 7.



Supplementary Figure 5 Membrane receptor signaling activates the IQGAP1-mediated PI3,4,5P3 synthesis pathway. a, Hs578T cells stably expressing shRNAs against IQGAP1 and PIPKIα were plated on 10 μg/ml type I collagen for 30 min. Cell lysates were analyzed by IB with the indicated antibodies. b, pS473Akt immunoblots were quantified and the graph is shown as mean ± SD of n=3 independent experiments. Paired Student t-tests were used for statistical analysis (*, p<0.05; **, p<0.01; n.s., not significant). c, MDA-MB-231 cells were transfected with the indicated siRNAs for 48 h. Serum starved cells were plated on collagen I-coated dish or treated with 20 ng/ml EGF or 15 μM LPA for 15 min. Lipids were extracted from equal number of cells and analyzed for PI3,4,5P3 content using kits from Echelon Biosciences. The graph is shown as mean ± SD of n=3 independent experiments. Paired Student t-tests were used for statistical analysis (*, p<0.05; **, p<0.01; n.s., not significant). d, Hs578T cells stably expressing indicated shRNAs were plated on 10 μg/ml type I collagen (COL) for the indicated times. Cell lysates analyzed by IB and pS473Akt and pY397FAK immunoblots were quantified and the graph is shown as mean ± SD of n=3 independent experiments. Paired Student t-tests were used for statistical analysis (*, p<0.05; **, p<0.01; n.s., not significant). e, pY397FAK immunoblots in Fig. 4a were quantified and the graph is shown as mean ± SD of n=3 independent experiments. Paired Student t-tests were used for statistical analysis (*, p<0.05; **, p<0.01; n.s., not significant). f, Hs578T cells were transfected with the indicated siRNAs for 24 h. Cells were serum starved for 18 h before treating with 0-100 ng/ml EGF for 15 min. Cell lysates were analyzed by IB for the indicated molecules. pS473Akt and pEGFR immunoblots were quantified and the graph is shown as mean ± SD of three independent experiments. The experiments described above were performed independently at least three times. Source data for b, c, d, e, f can be found in Supplementary Table 1. Unprocessed original scans of blots are shown in Supplementary Fig. 7.



Supplementary Figure 6 The IQGAP1-derived peptides inhibit Akt activation. a, Sequences of cell permeable IG1DPs. b, Empty vector (Mock) and HA-tagged IQ domain alone was stably expressed in Hs578T cells. Cell lysates were analyzed by IB with the indicated antibodies. c, Hs578T cells were transfected with empty vector or p110α subunit of PI3K for 24 h. Then, cells were treated with the indicated 20 μM of IG1DPs for 24 h. Cell lysates were analyzed by IB (top) and pS473Akt immunoblots were quantified and the graph is shown as mean ± SD of three independent experiments (bottom). d, Cells containing PIK3CA mutations were treated with 30 μM IG1DPs for 48 h. Cell lysates were analyzed by IB with the indicated antibodies. e, pS473Akt blots of Fig. 7d were quantified and the graph is shown as mean ± SD of n=3 independent experiments. Paired Student t-tests were used for statistical analysis (*, p<0.05; **, p<0.01; n.s., not significant). f, Hs578T cells were transfected with a constitutively active Rac1 or Cdc42 for 24. Then, cells were treated with 20 μM of the indicated IG1DPs for 48 h. Cell viability and protein expression were measured and the graph is shown as mean ± SD of n=3 independent experiments. Paired Student t-tests were used for statistical analysis (*, p<0.05; **, p<0.01; n.s., not significant). Source data for c, e, f can be found in Supplementary Table 1. Unprocessed original scans of blots are shown in Supplementary Fig. 7.



Supplementary Figure 7 Unprocessed original scans of blots

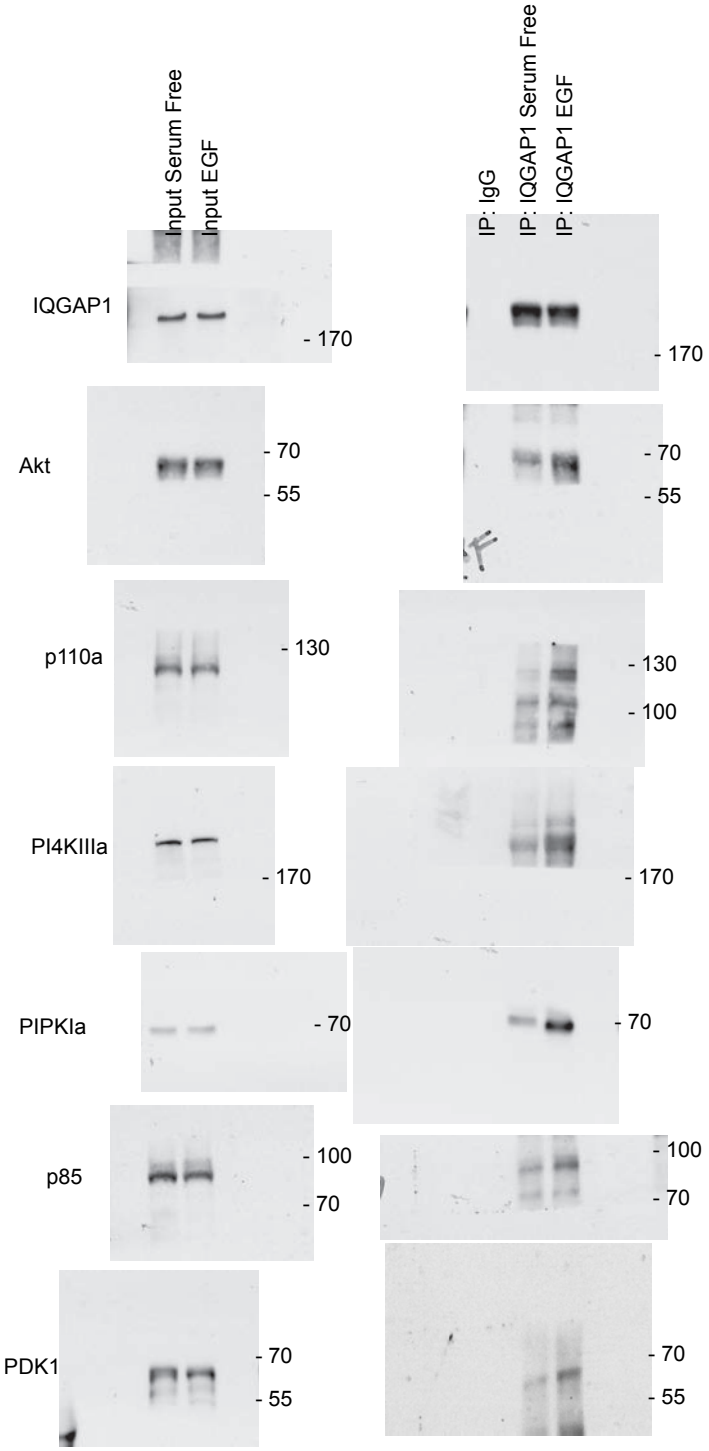


Figure 1b

Supplementary Figure 7 Continued

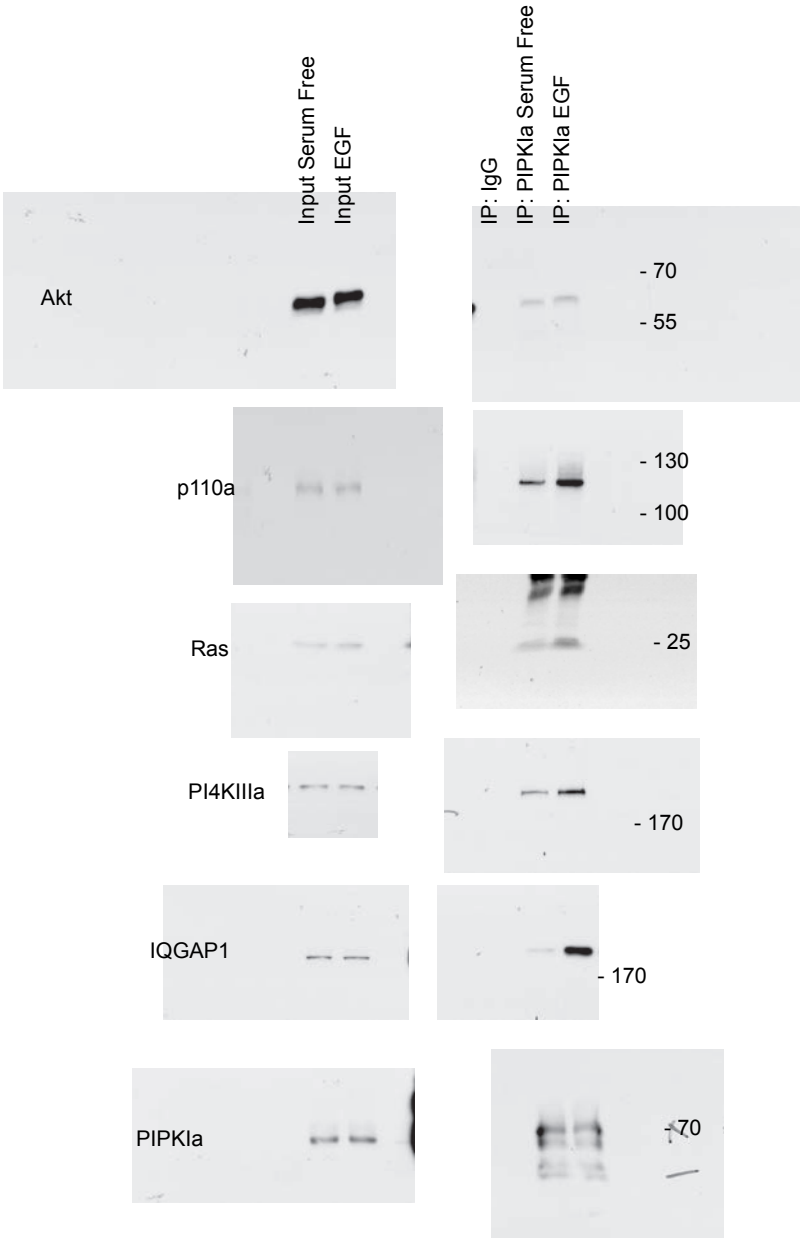


Figure 1c

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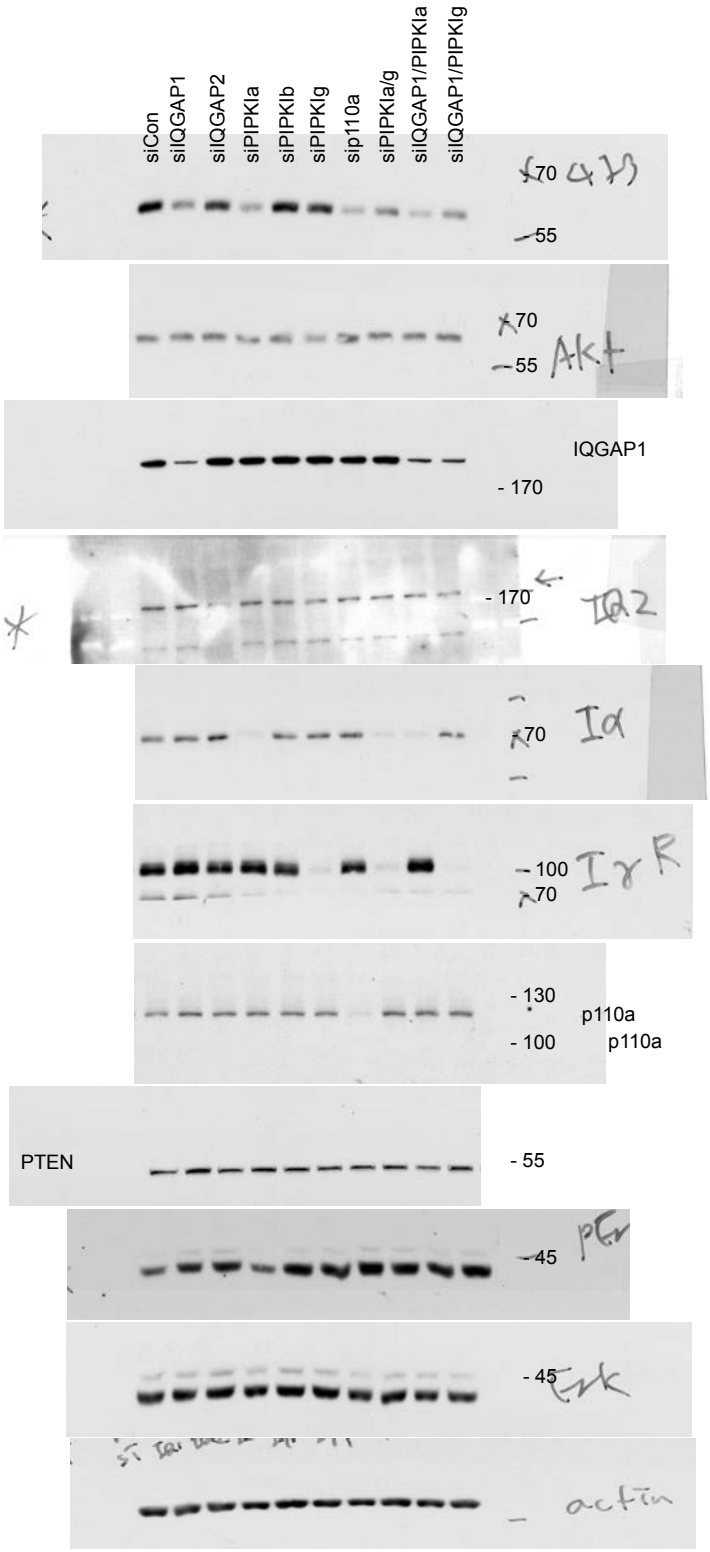

Figure 1d

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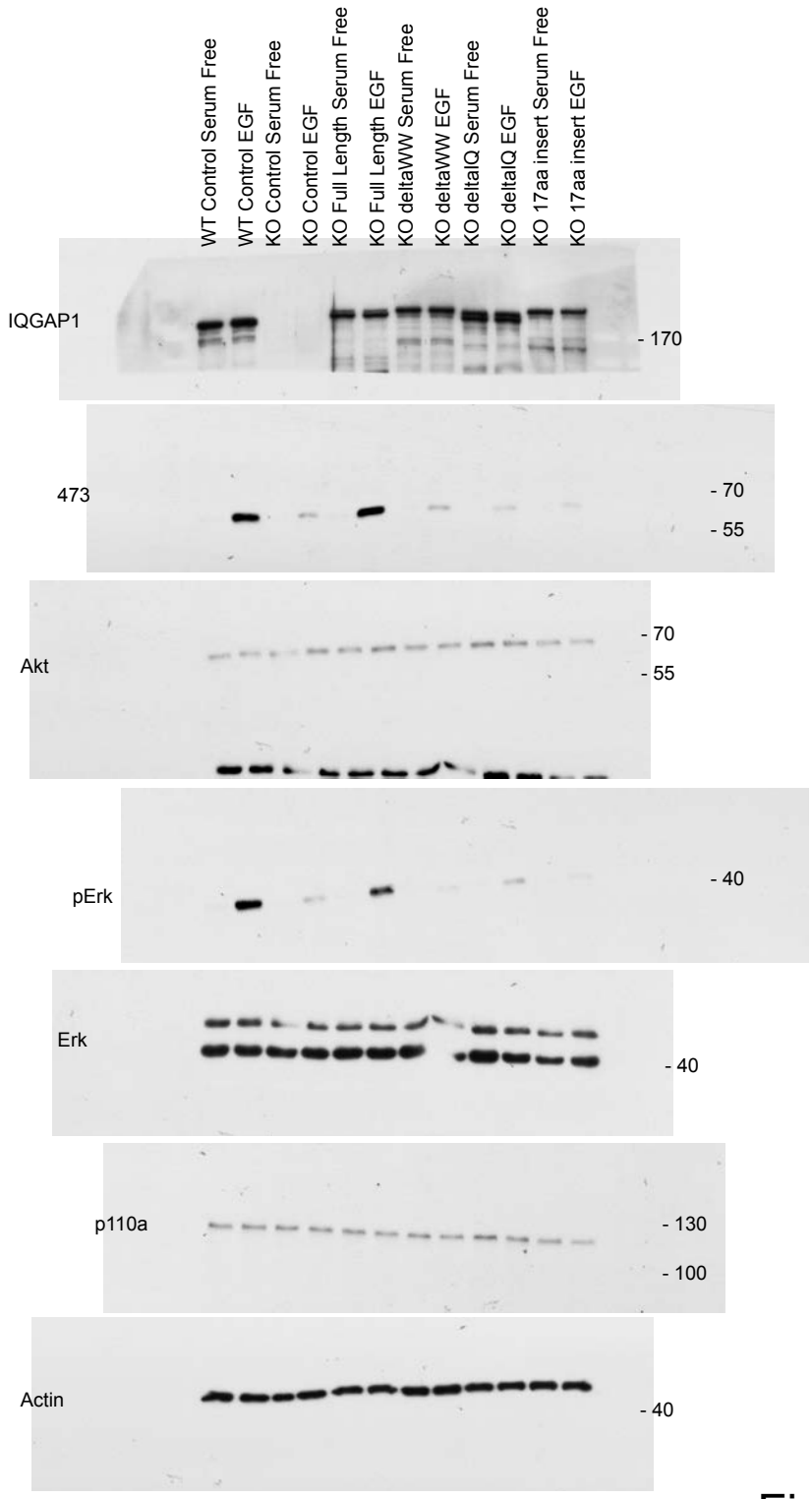

Figure 1e

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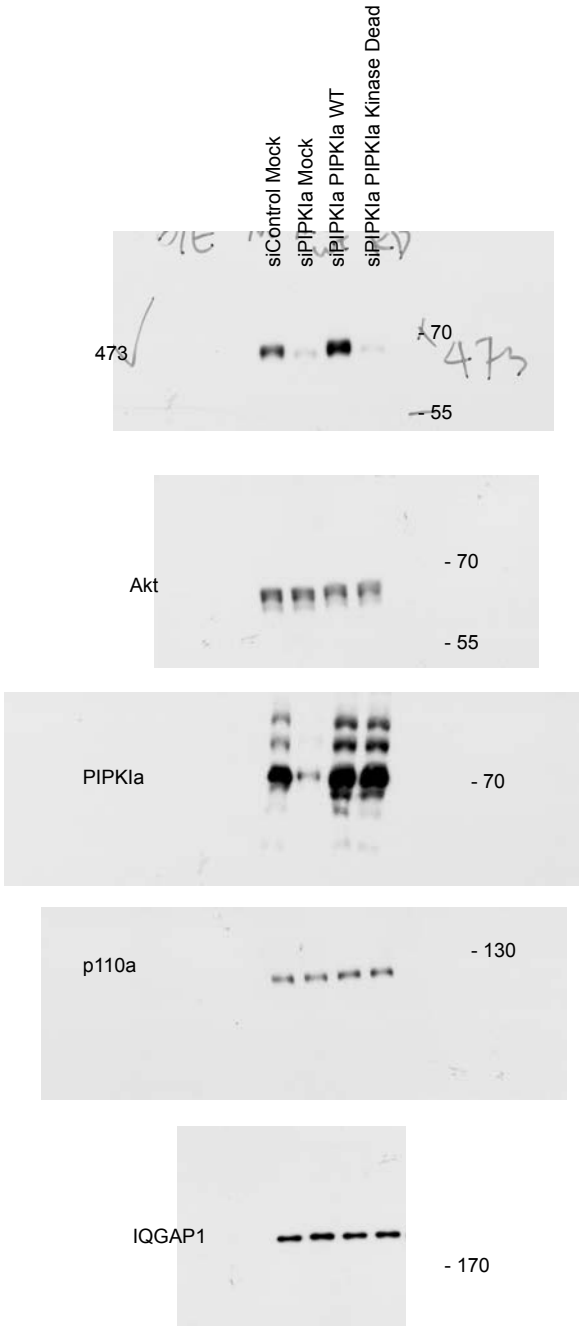

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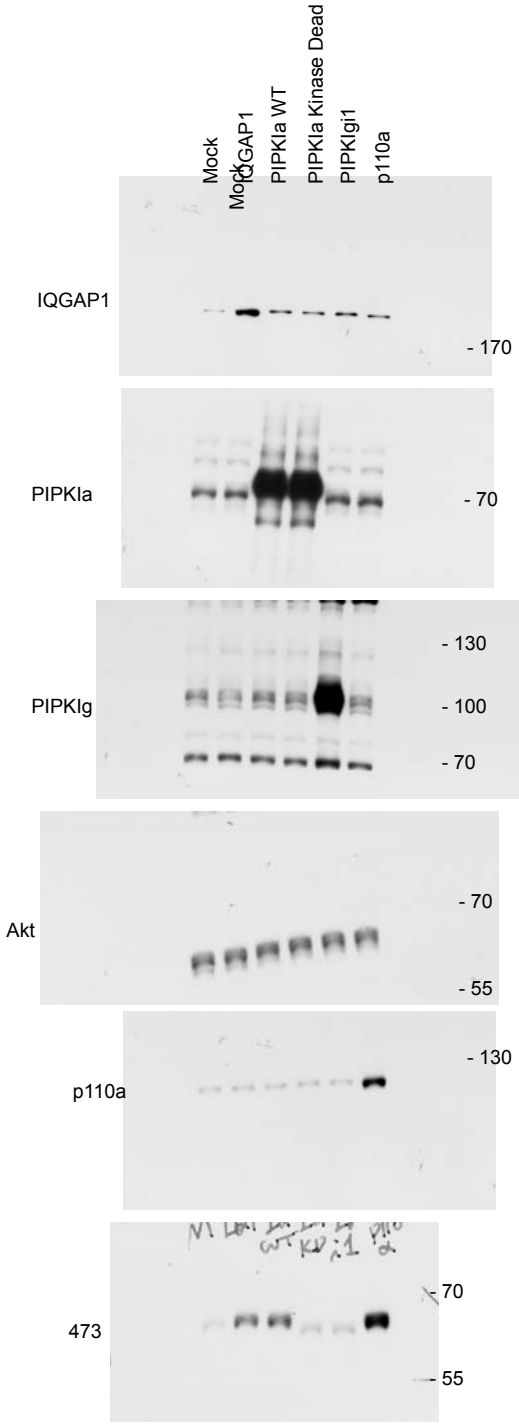

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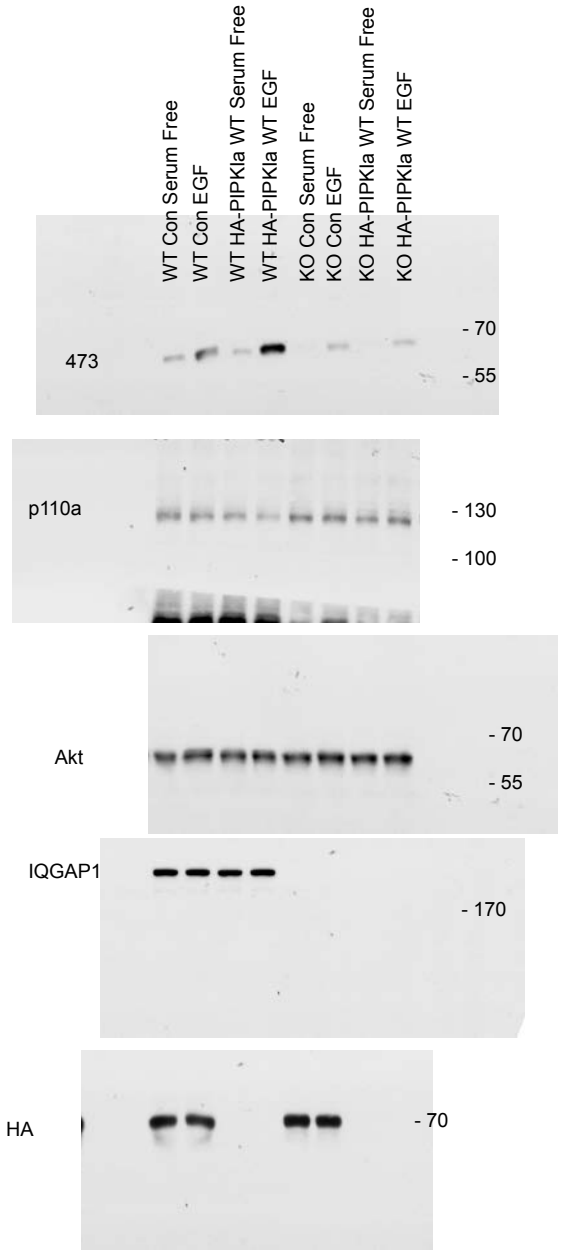

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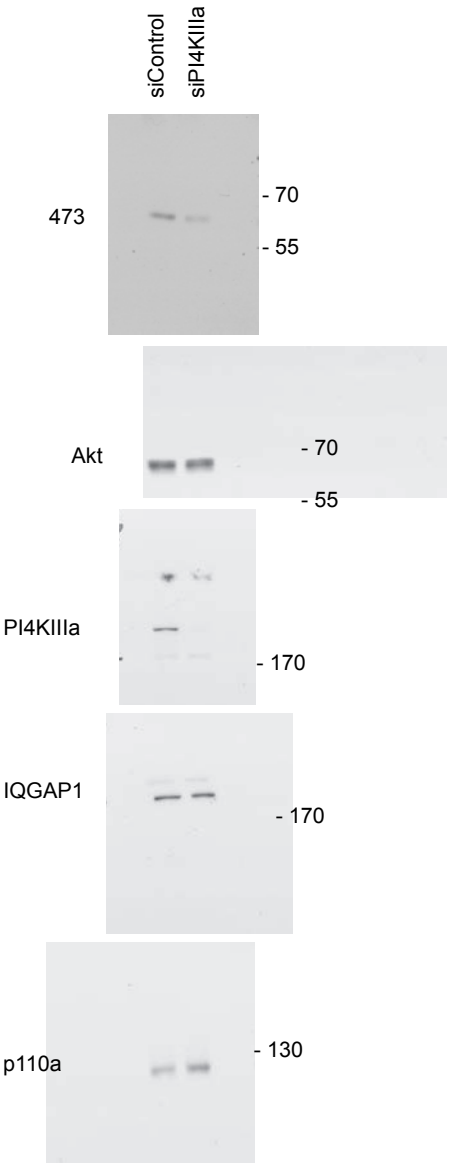

Supplementary Figure 1g

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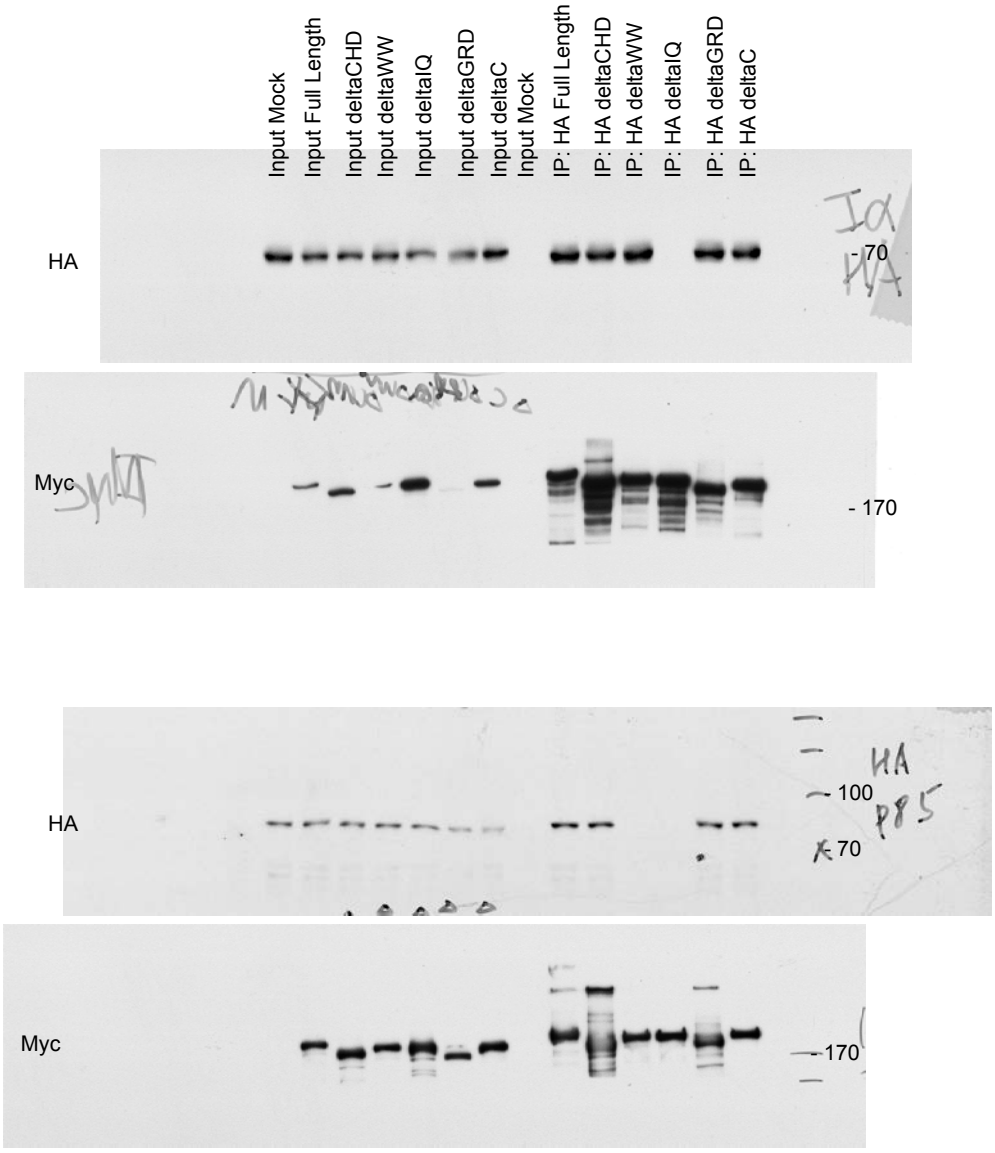

Figure 2a, 2b

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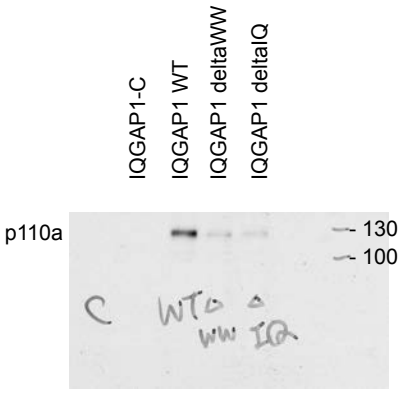

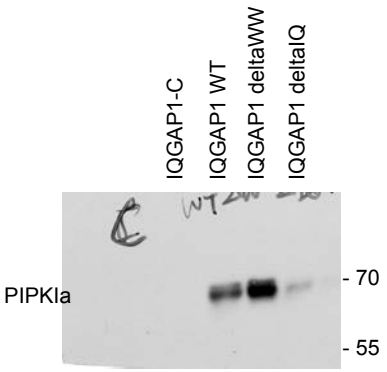

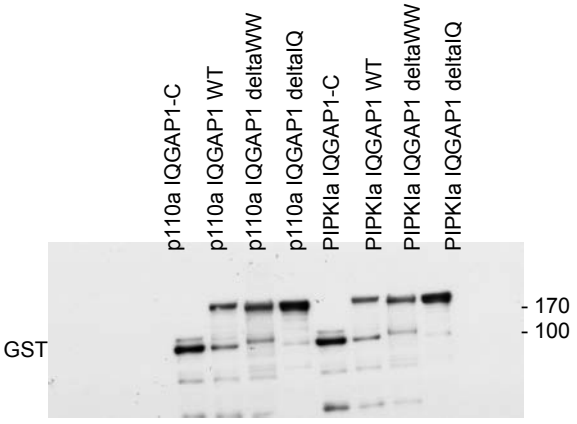

Figure 2c, 2d

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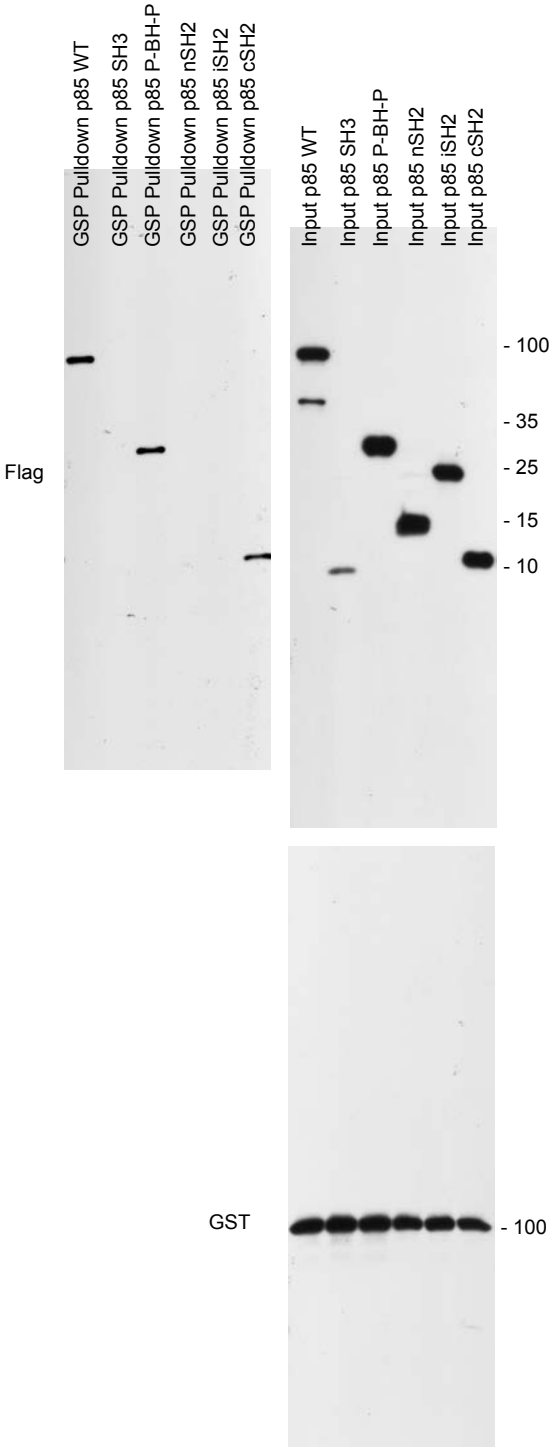

Figure 2e

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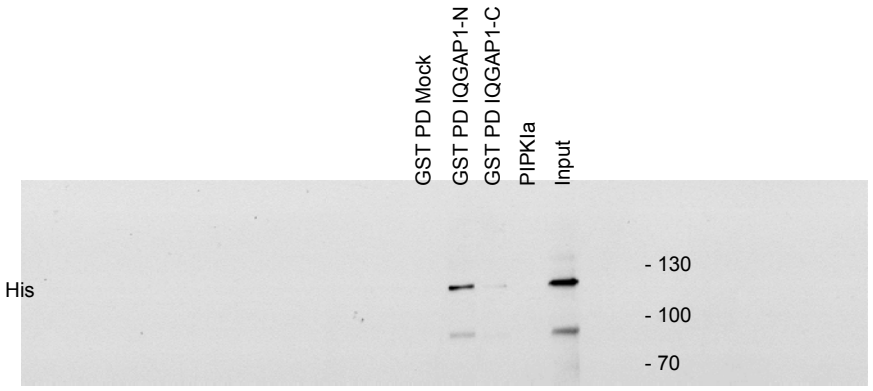

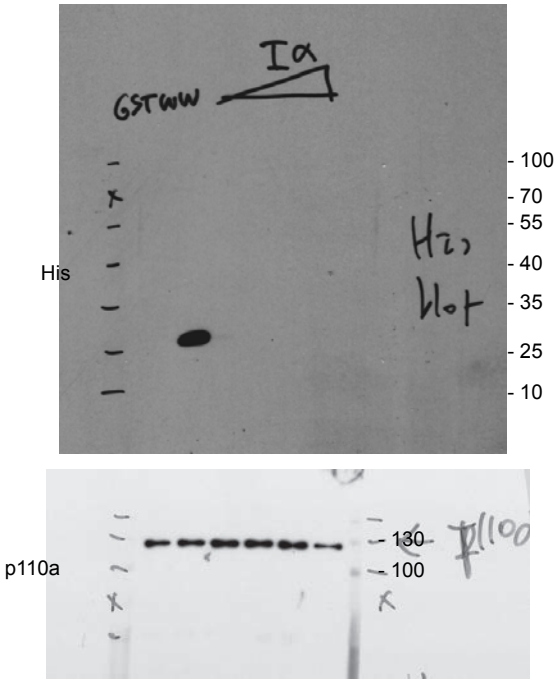

Supplementary Figure 2b, 2c

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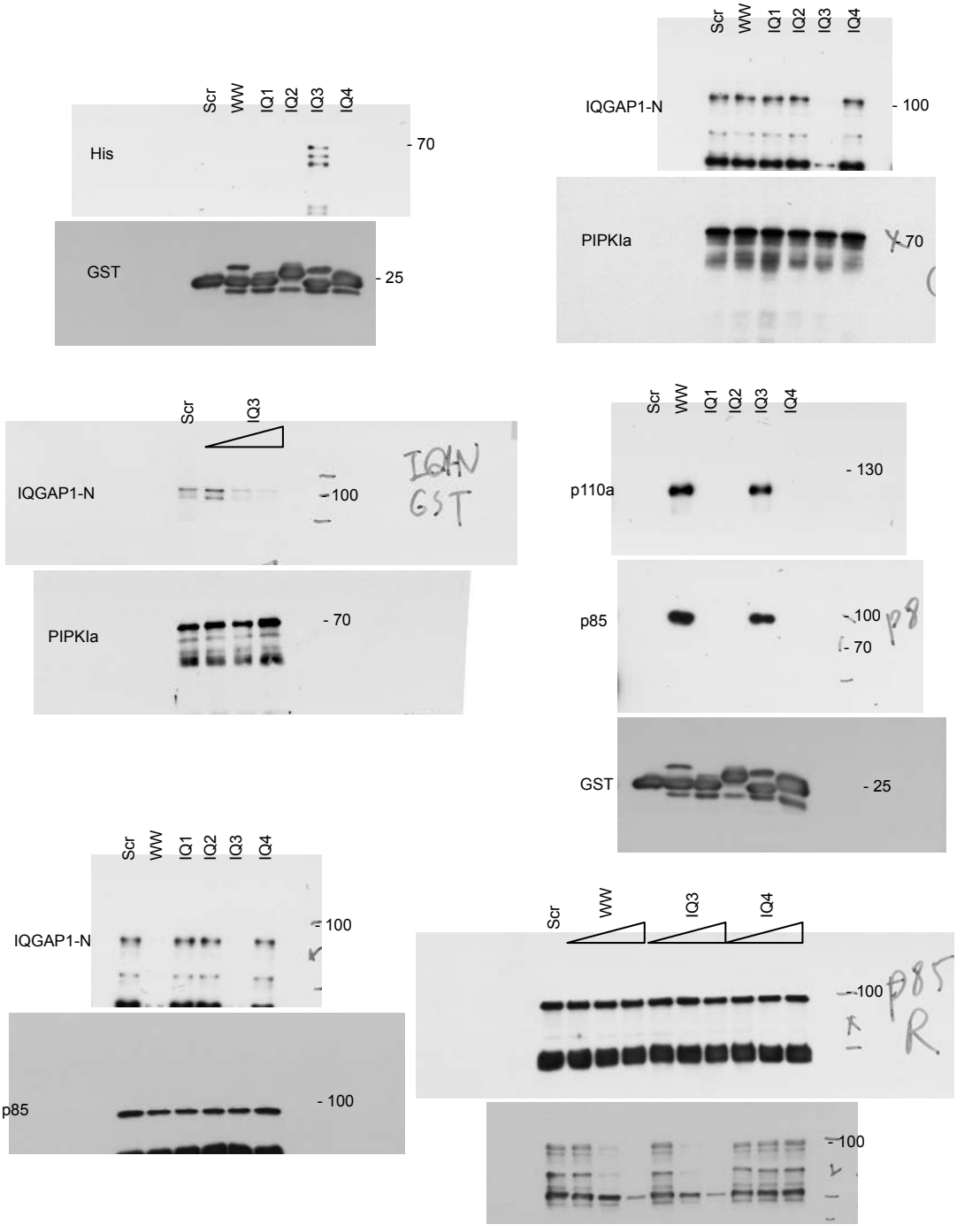

Supplementary Figure 2e-j

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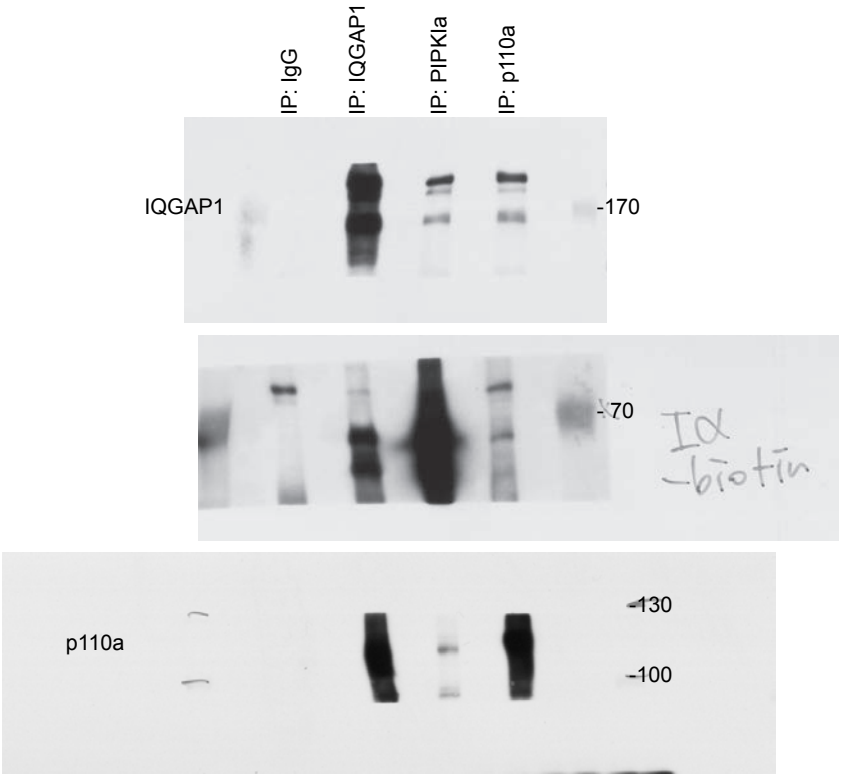

Figure 3a

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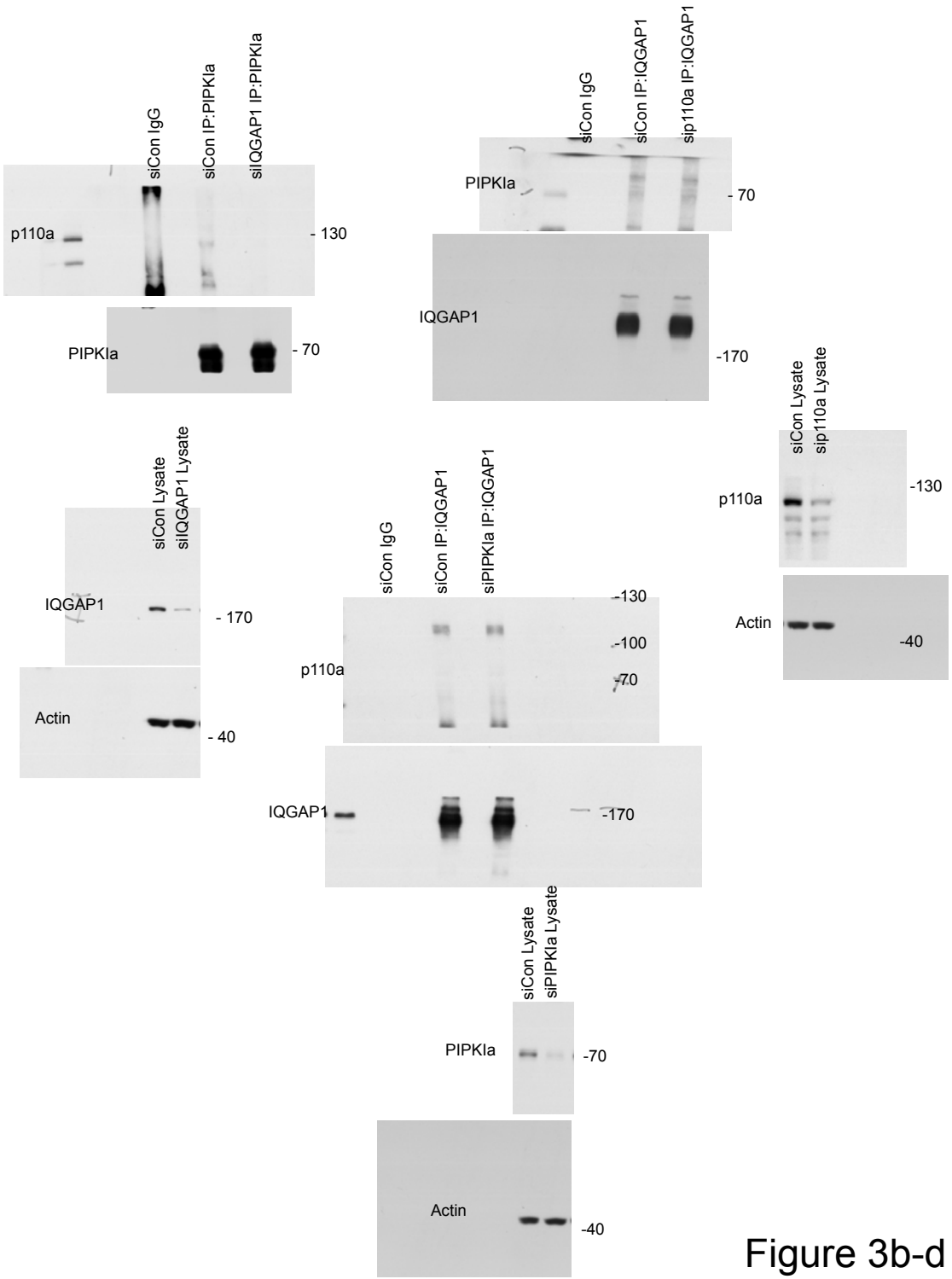

Figure 3b-d

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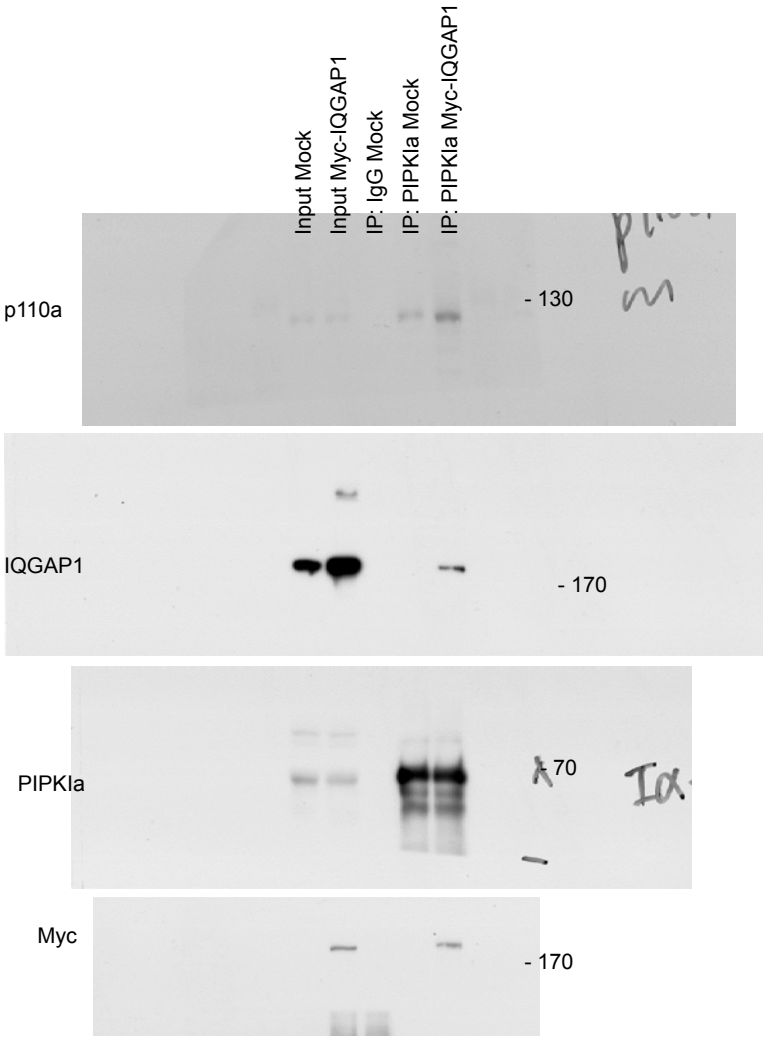

Figure 3e

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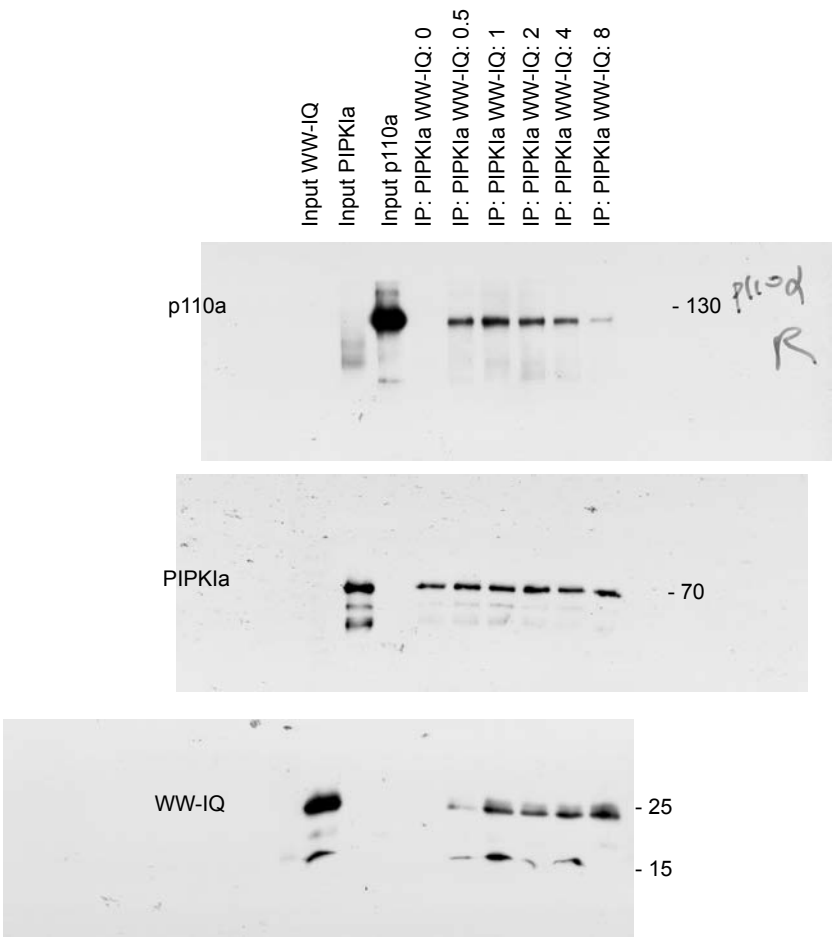

Figure 4b

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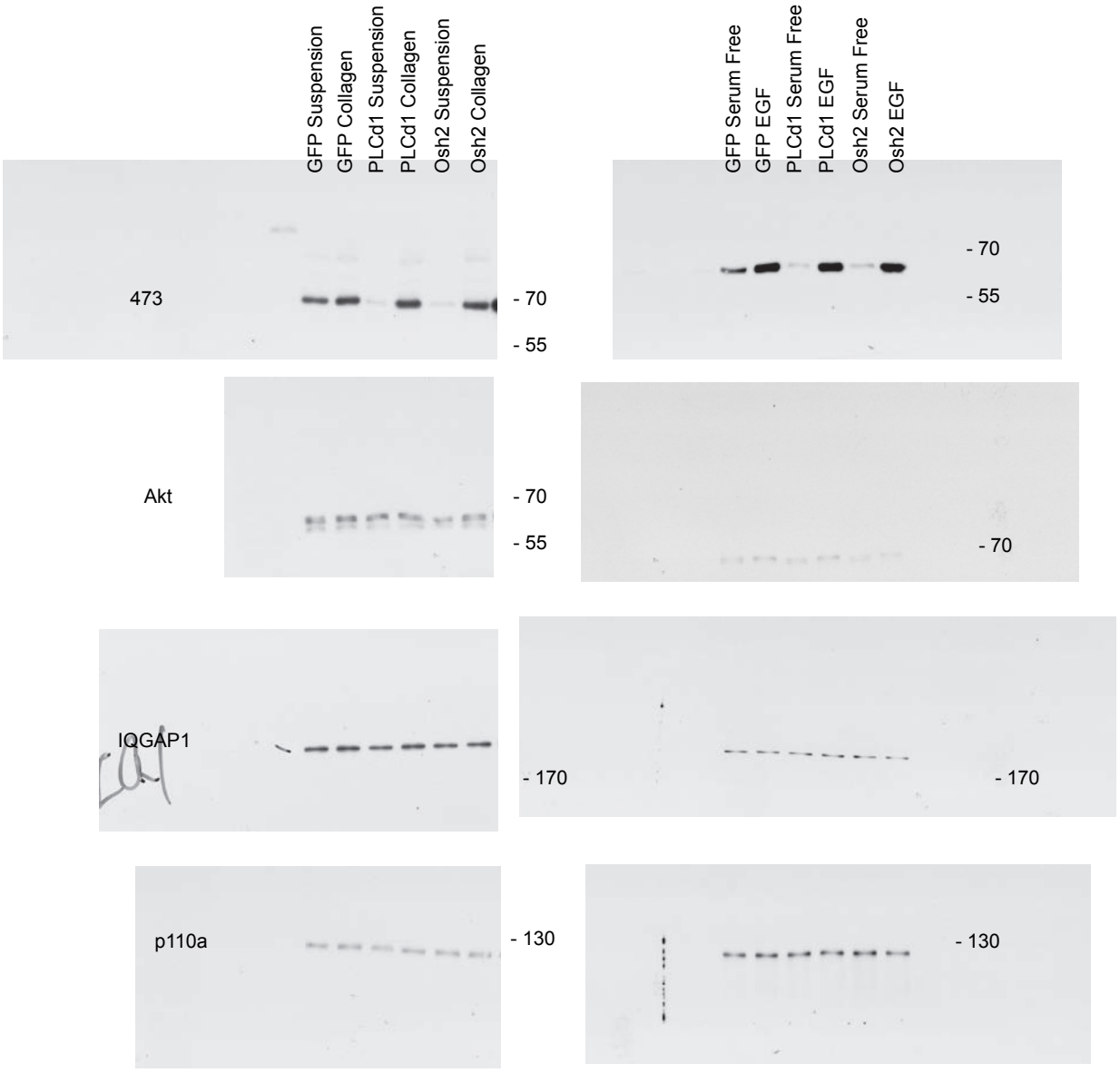

Figure 4f

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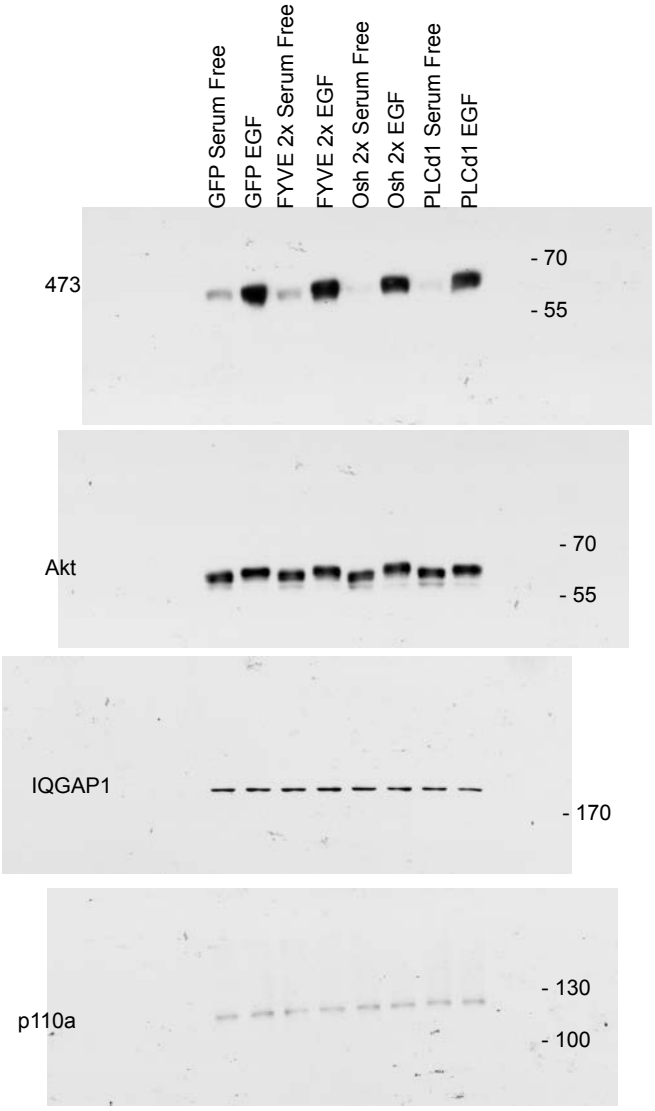

Supplementary Figure 3d

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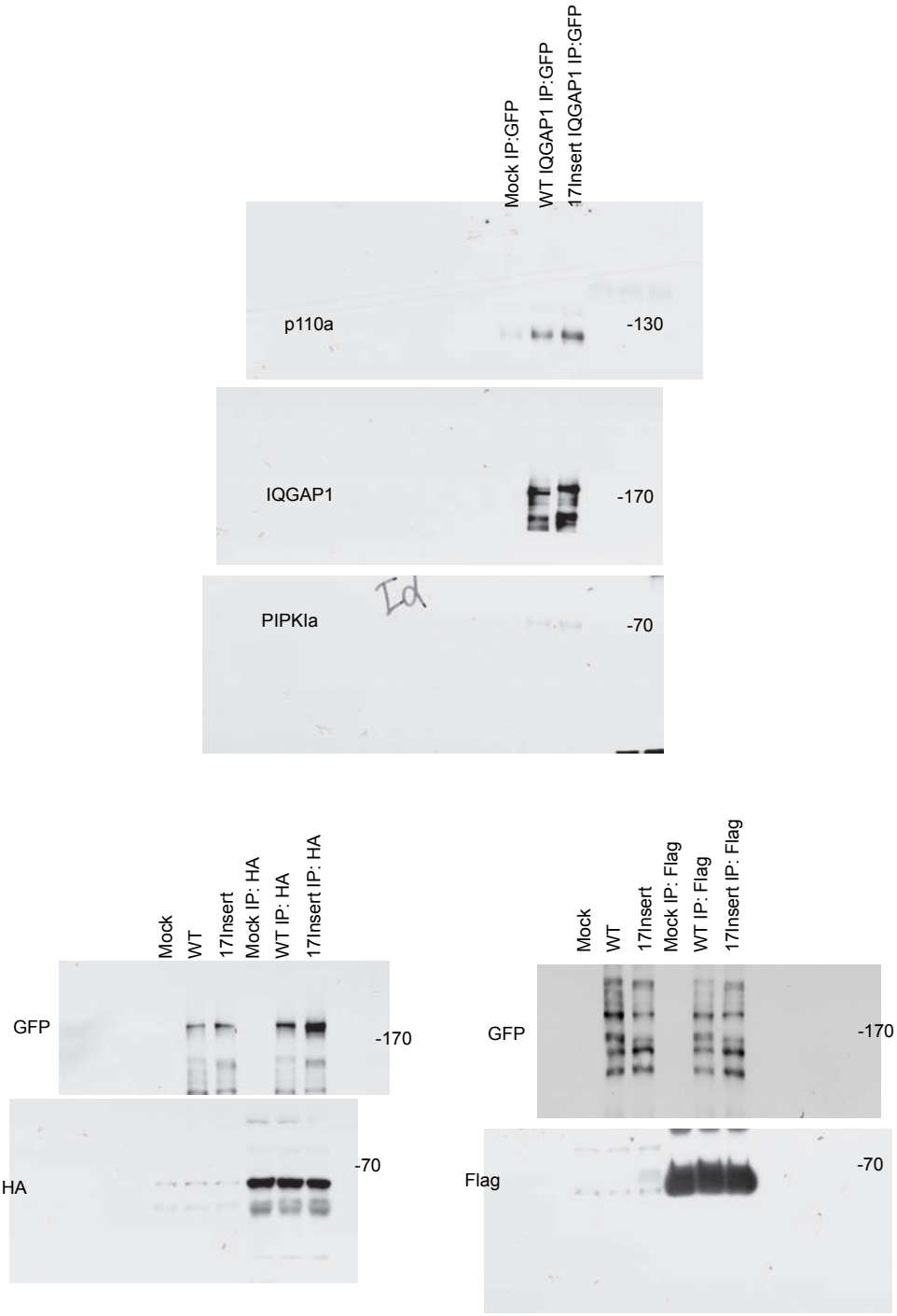

Supplementary Figure 4c-e

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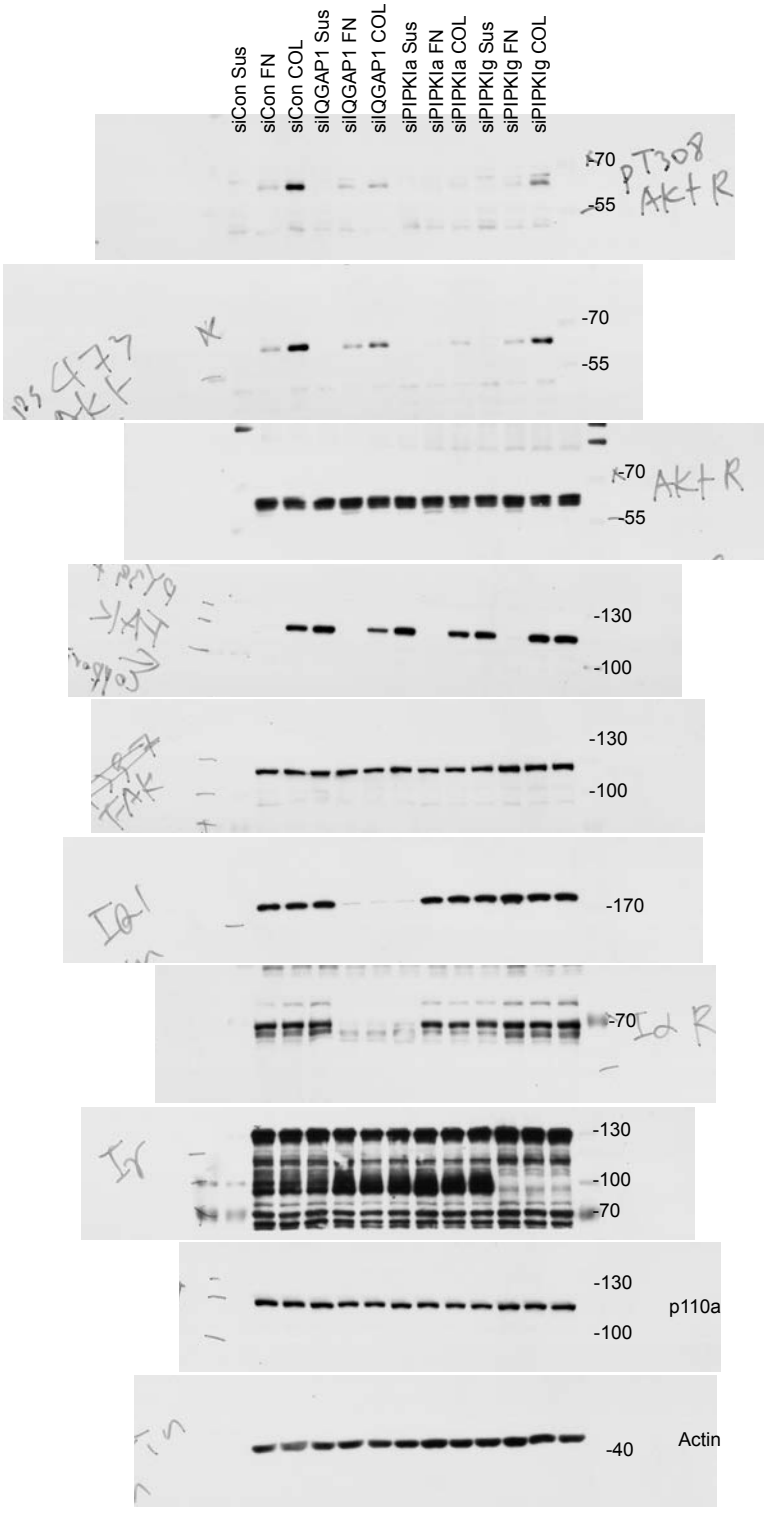

Figure 5a

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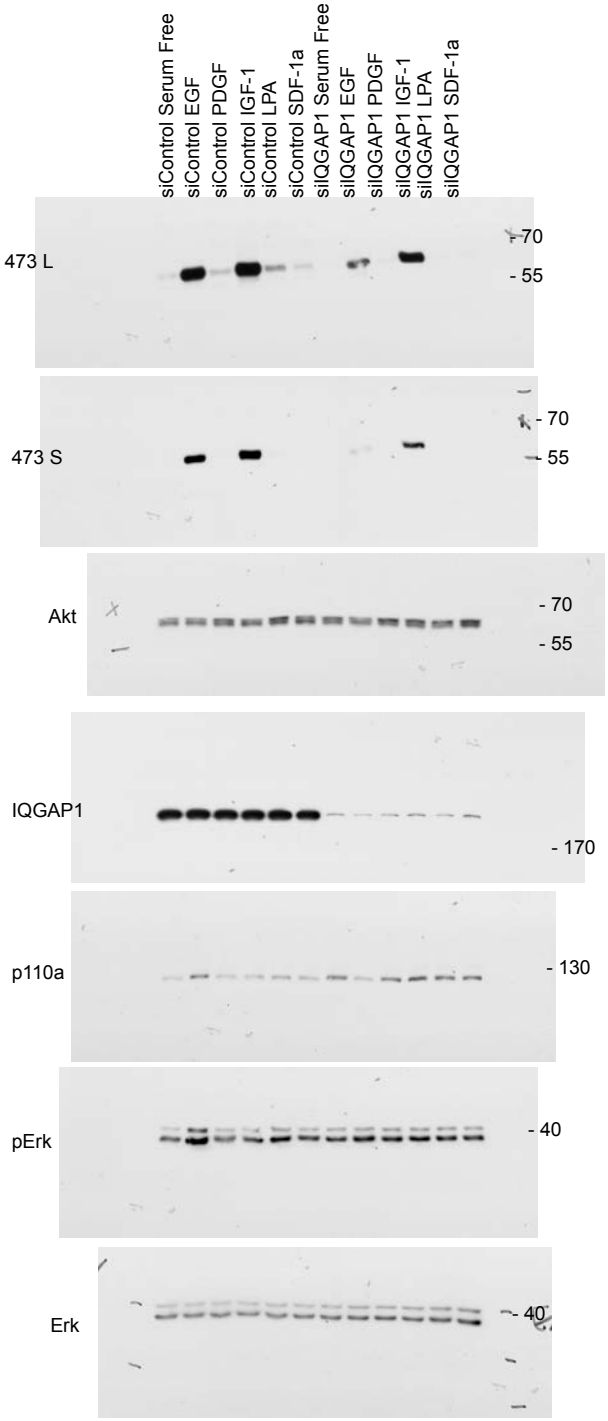

Figure 5c

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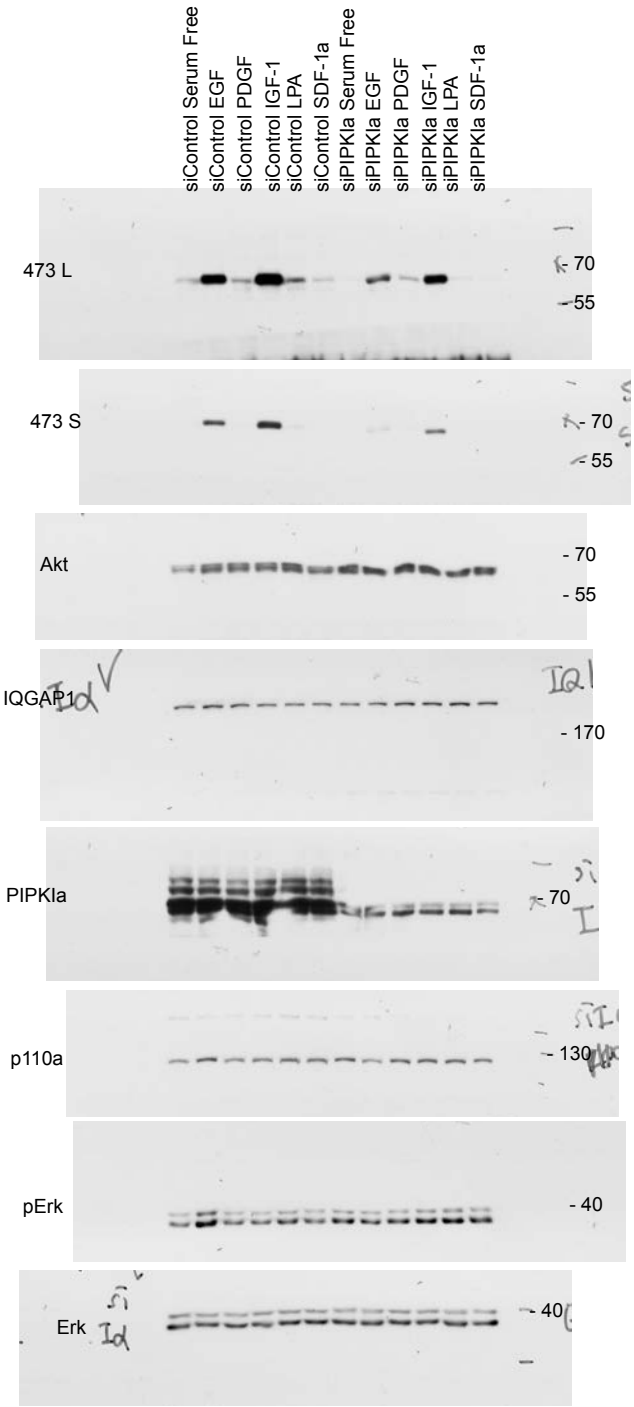

Figure 5d

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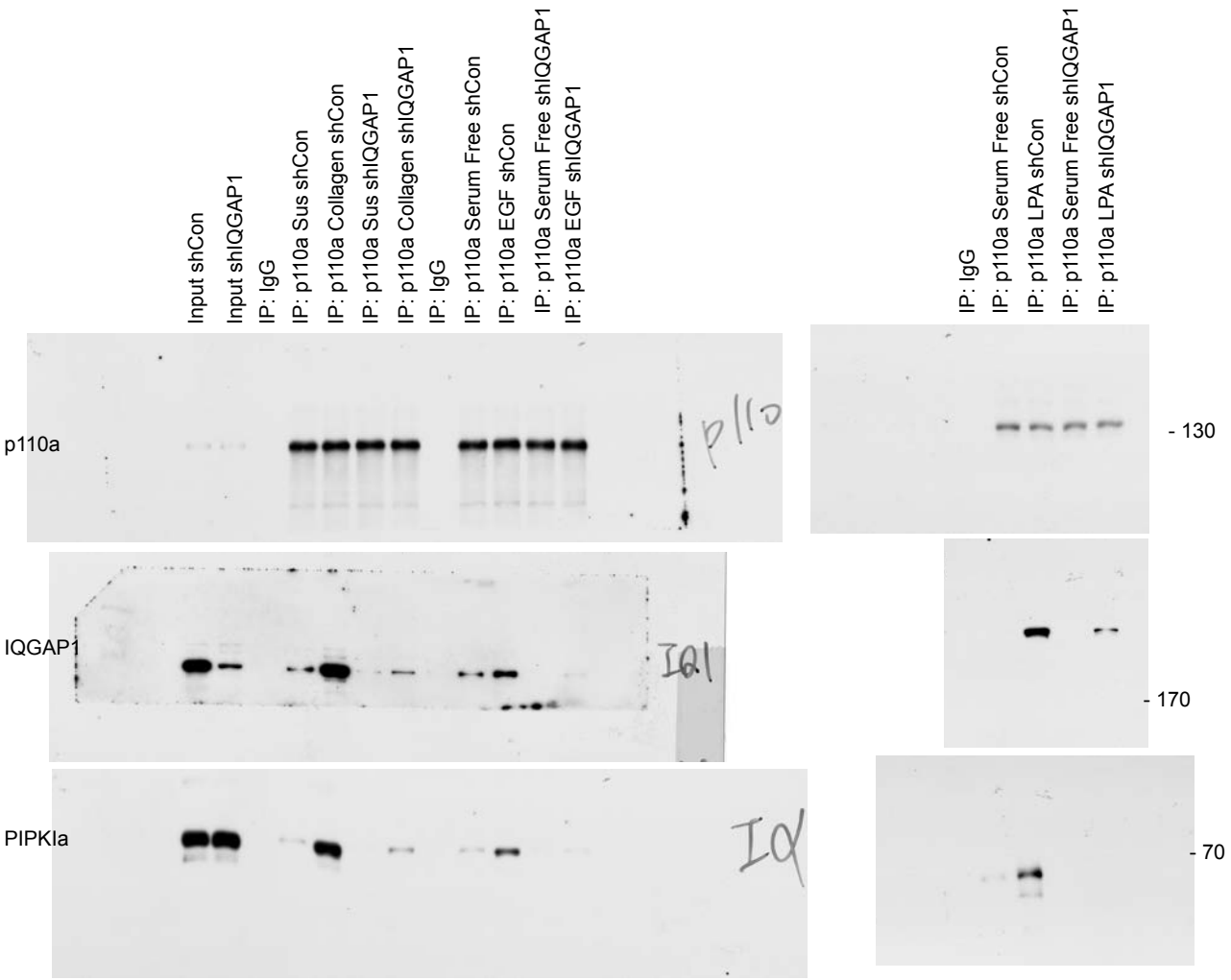
Figure 5f



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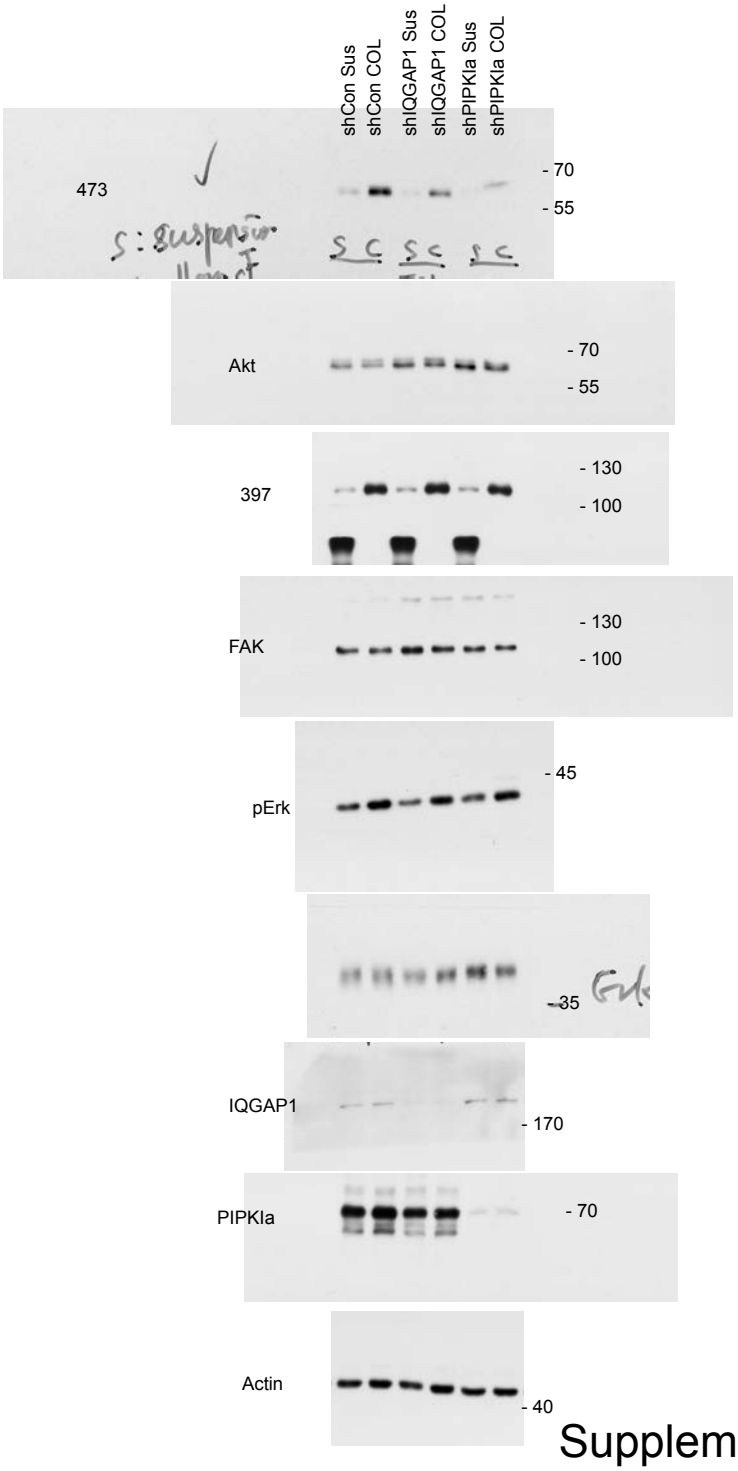

Supplementary Figure 5a

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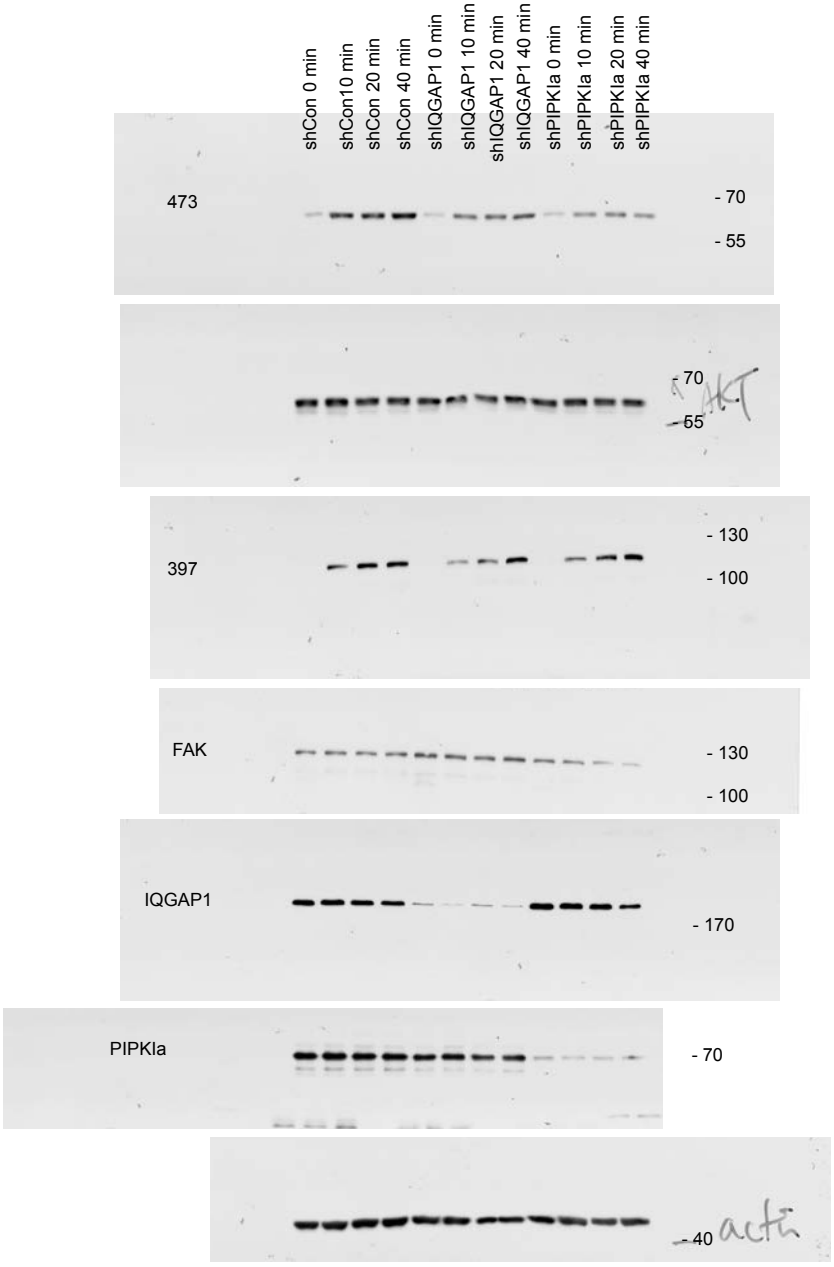

Supplementary Figure 5d

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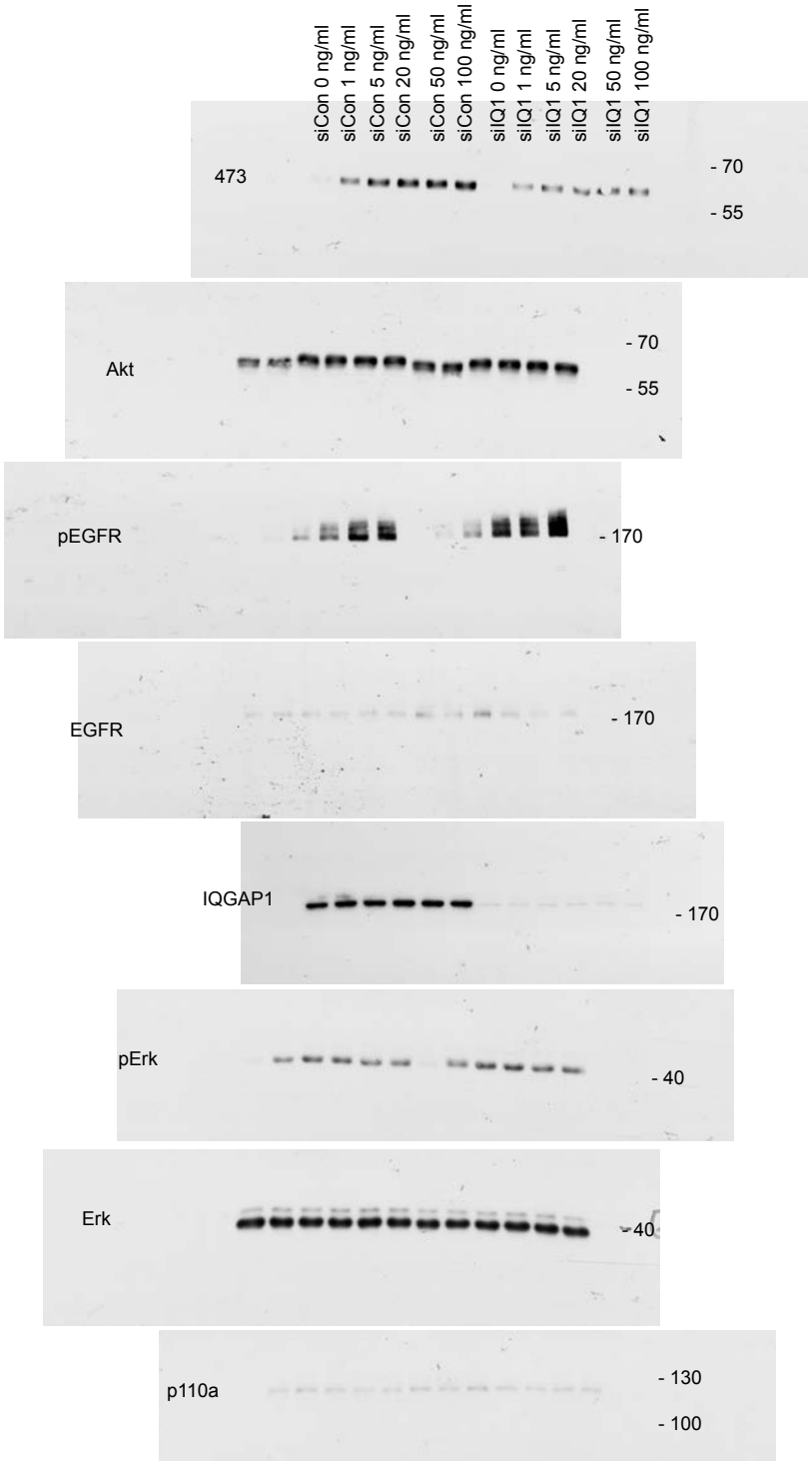

Supplementary Figure 5f

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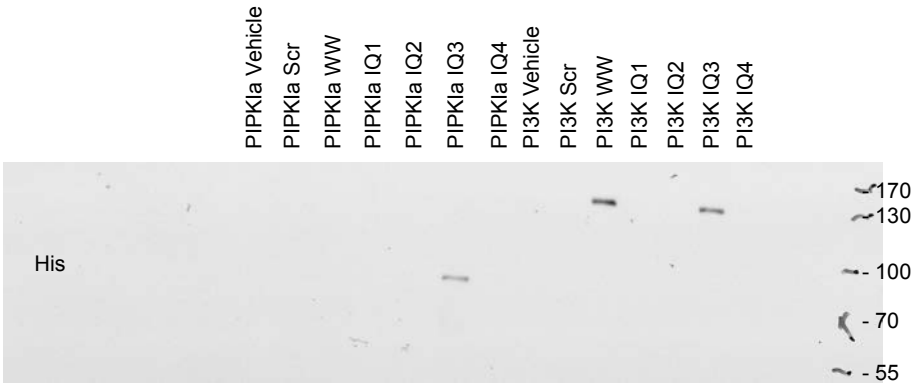


Figure 6a

Supplementary Figure 7 Continued

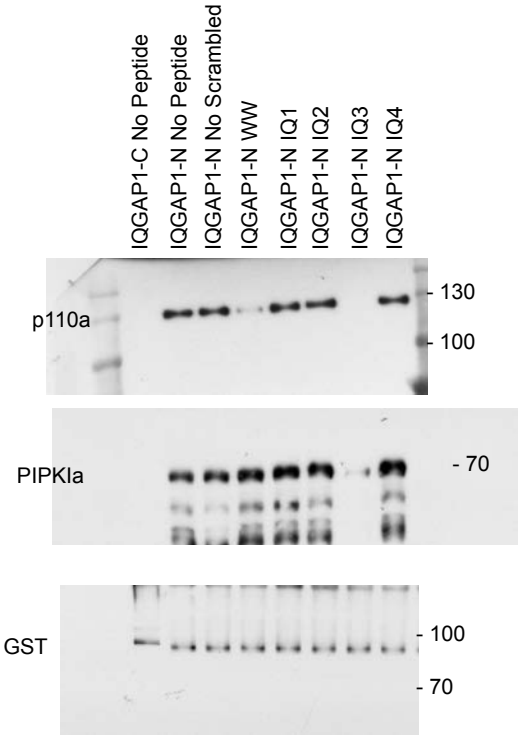


Figure 6b

Supplementary Figure 7 Continued

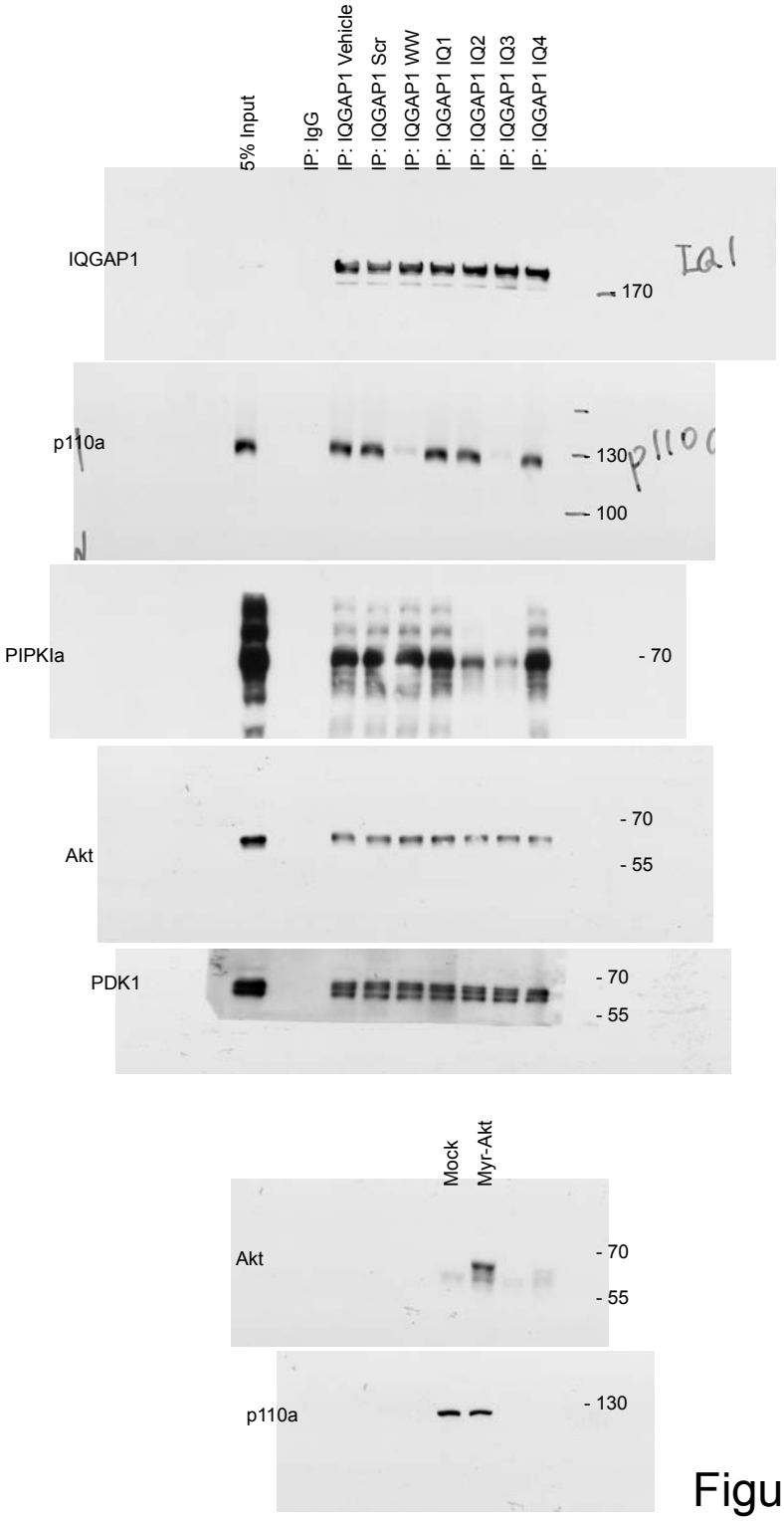
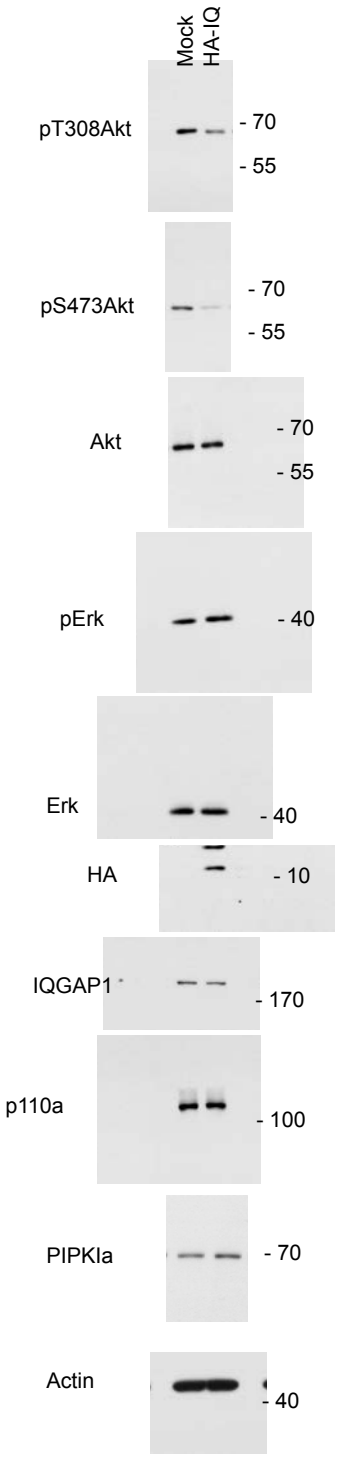


Figure 6d, 6f

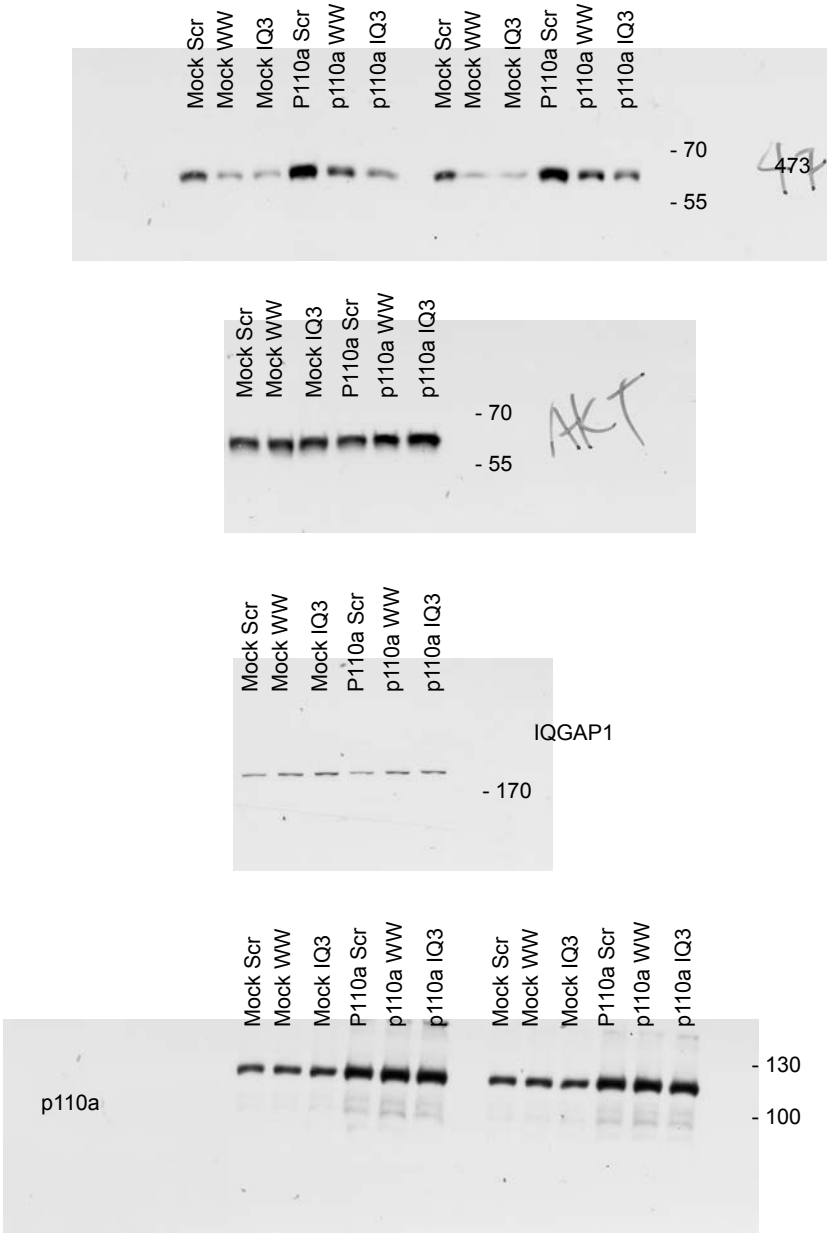
Supplementary Figure 7 Continued



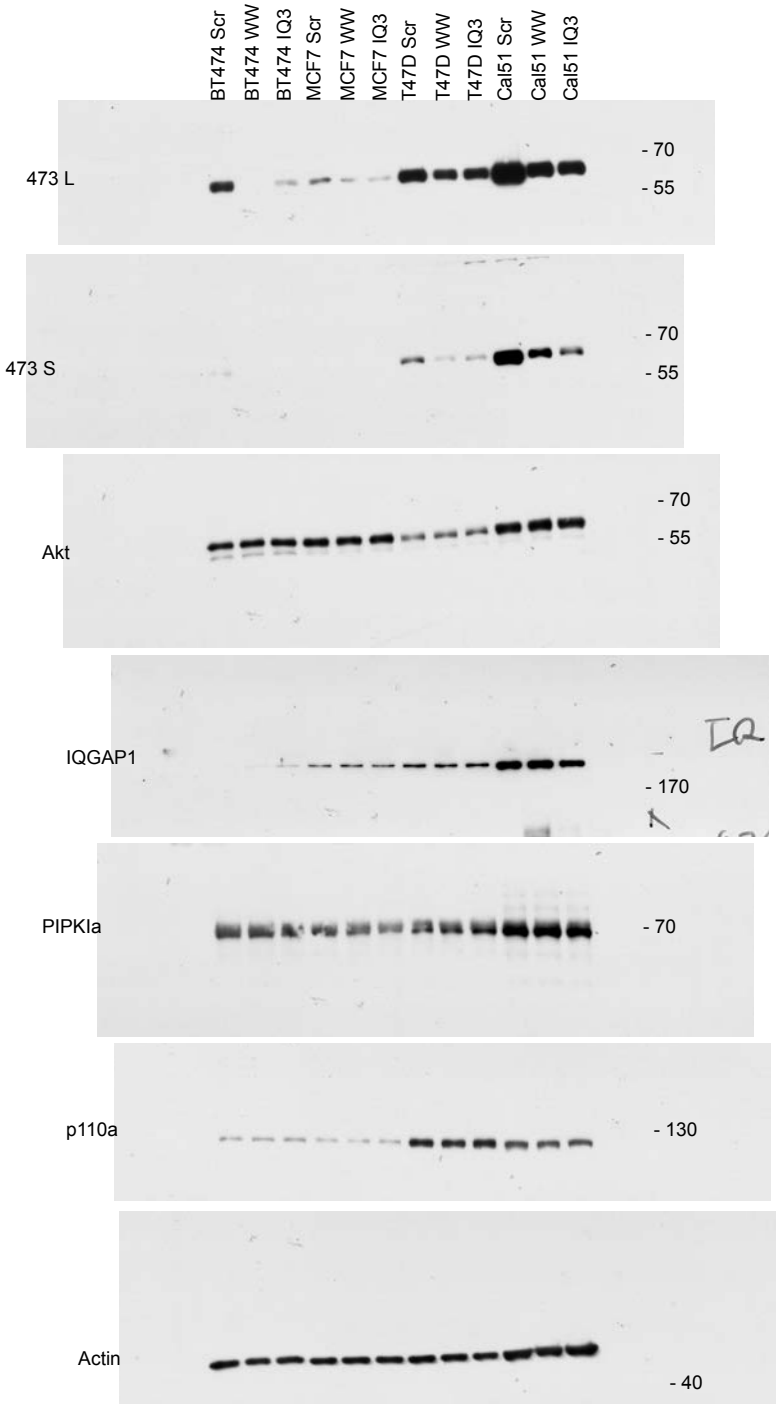
Supplementary Figure 6b

Supplementary Figure 7 Continued

Supplementary Figure 6c

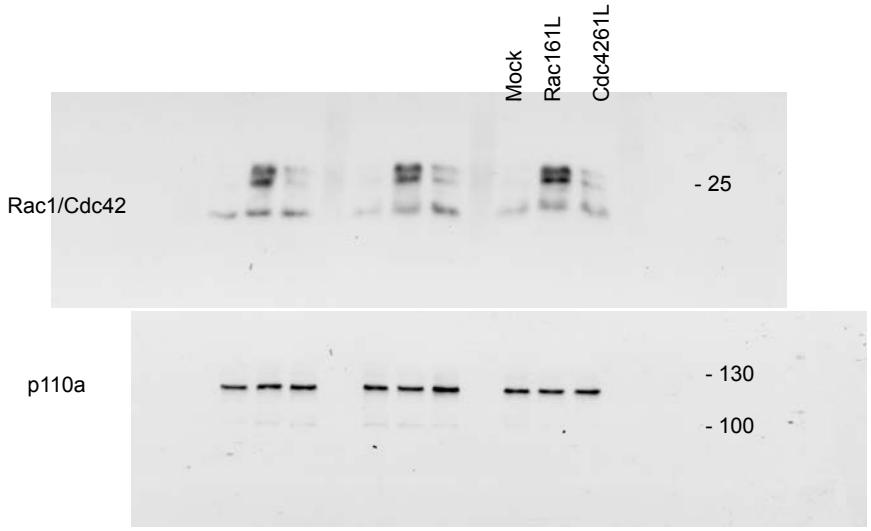


Supplementary Figure 7 Continued



Supplementary Figure 6d

Supplementary Figure 7 Continued



Supplementary Figure 6f

Supplementary Figure 7 Continued

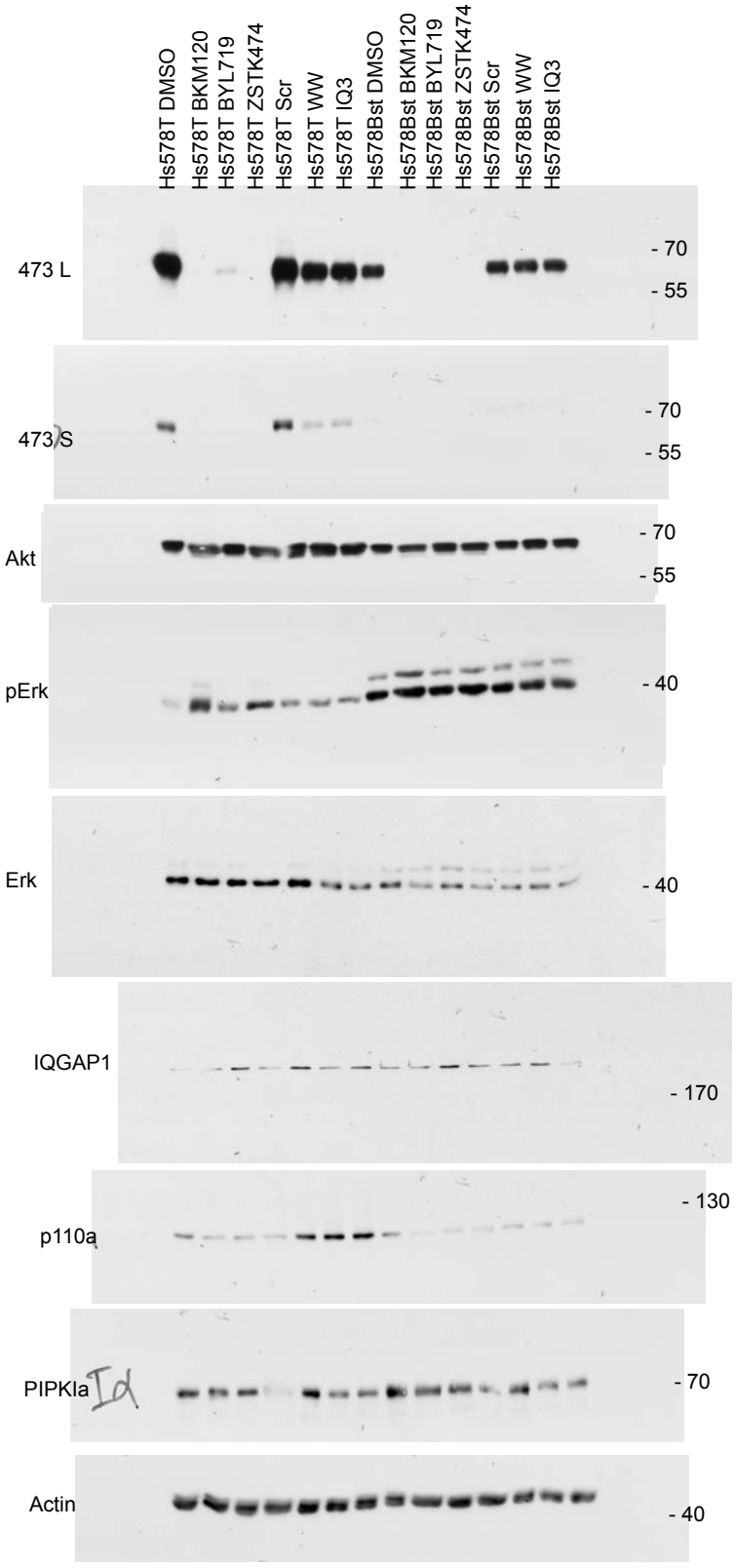


Figure 7d

Supplementary Figure 7 Continued

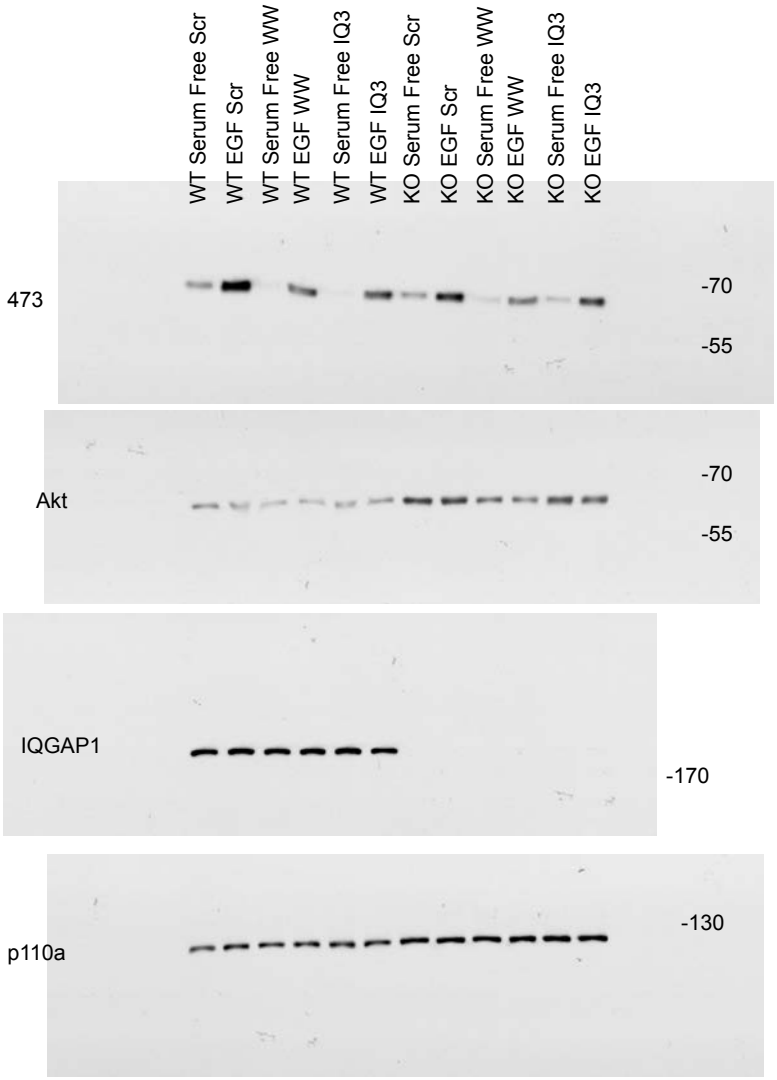


Figure 7e

Supplementary Figure 7 Continued

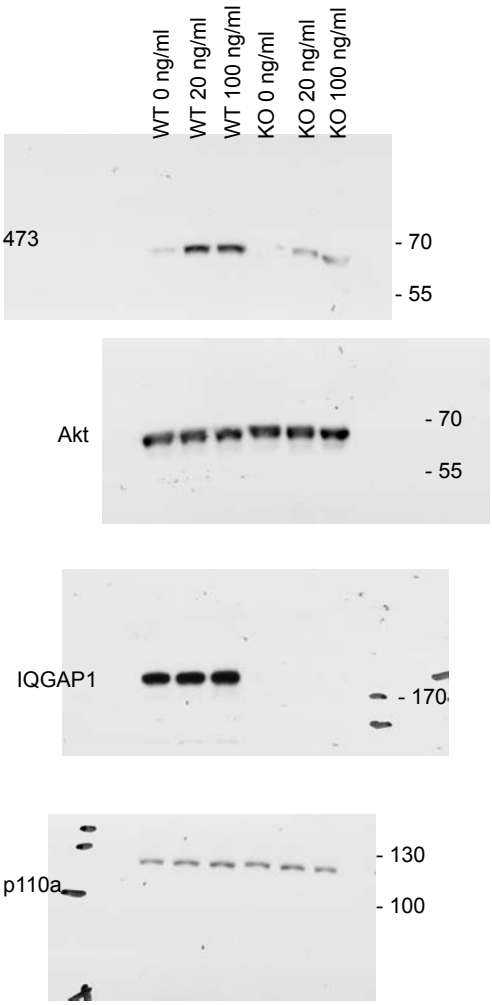


Figure 8a

Supplementary Figure 7 Continued

Supplementary Table Legends

Supplementary Table 1
Statistics Source Data

Supplementary Table 2
Antibody Information