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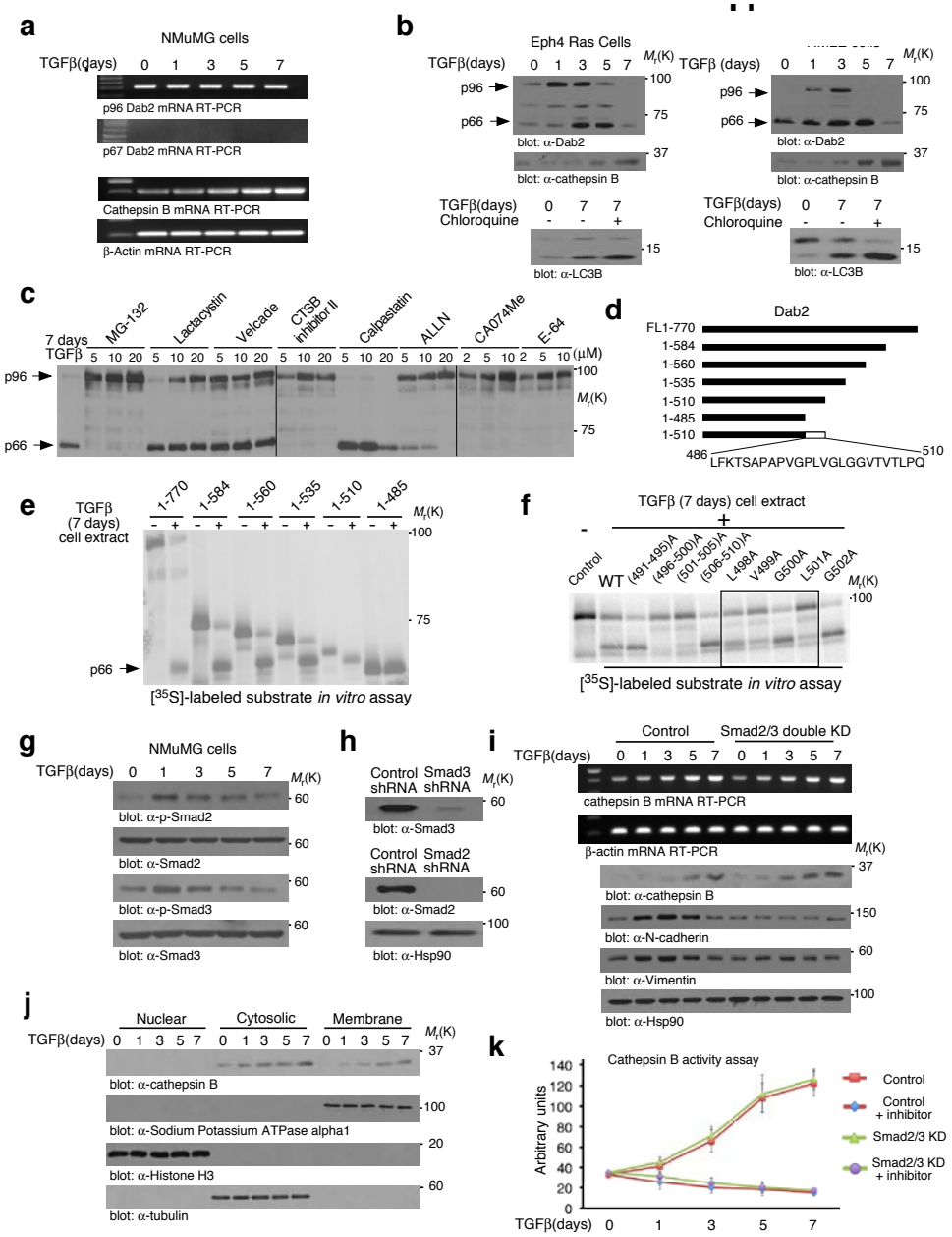
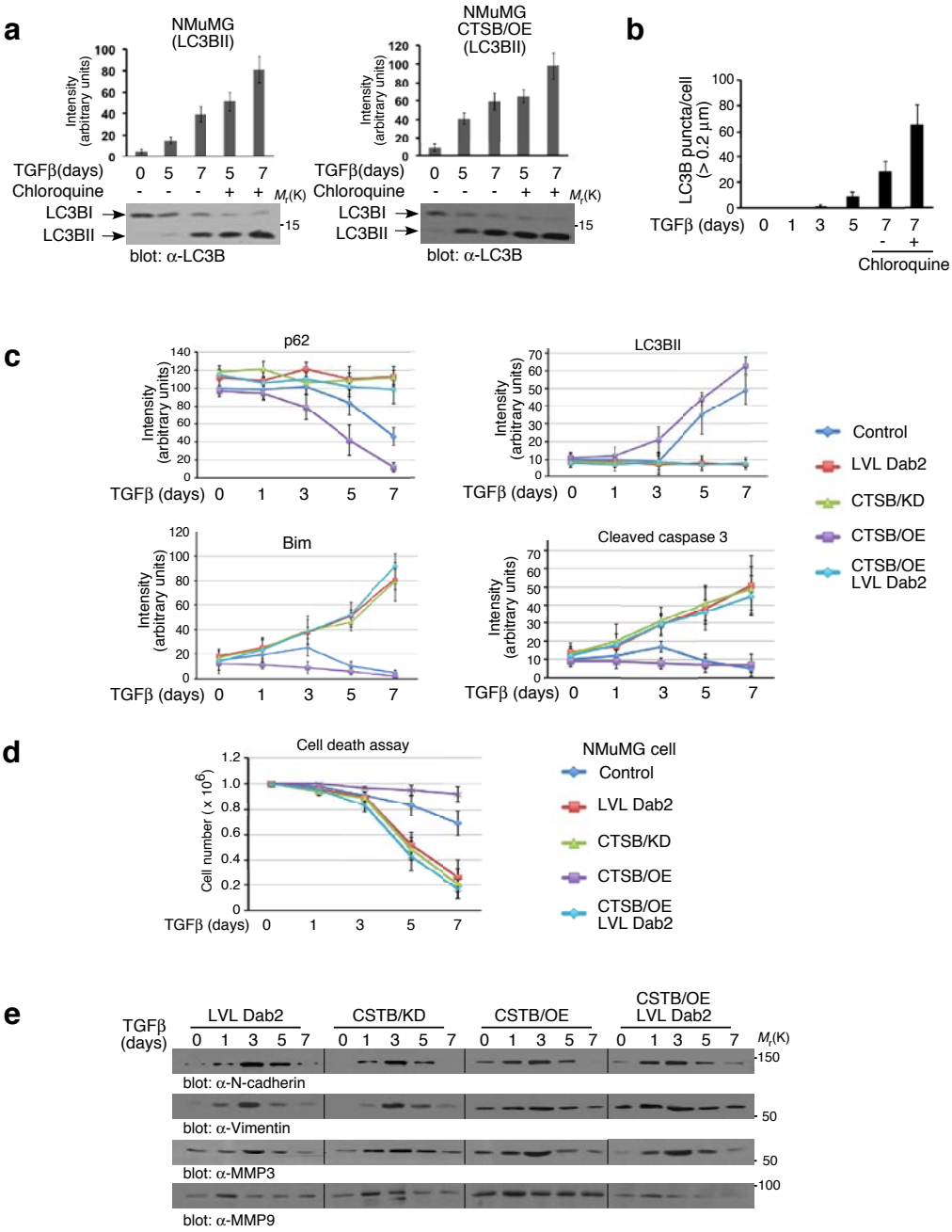
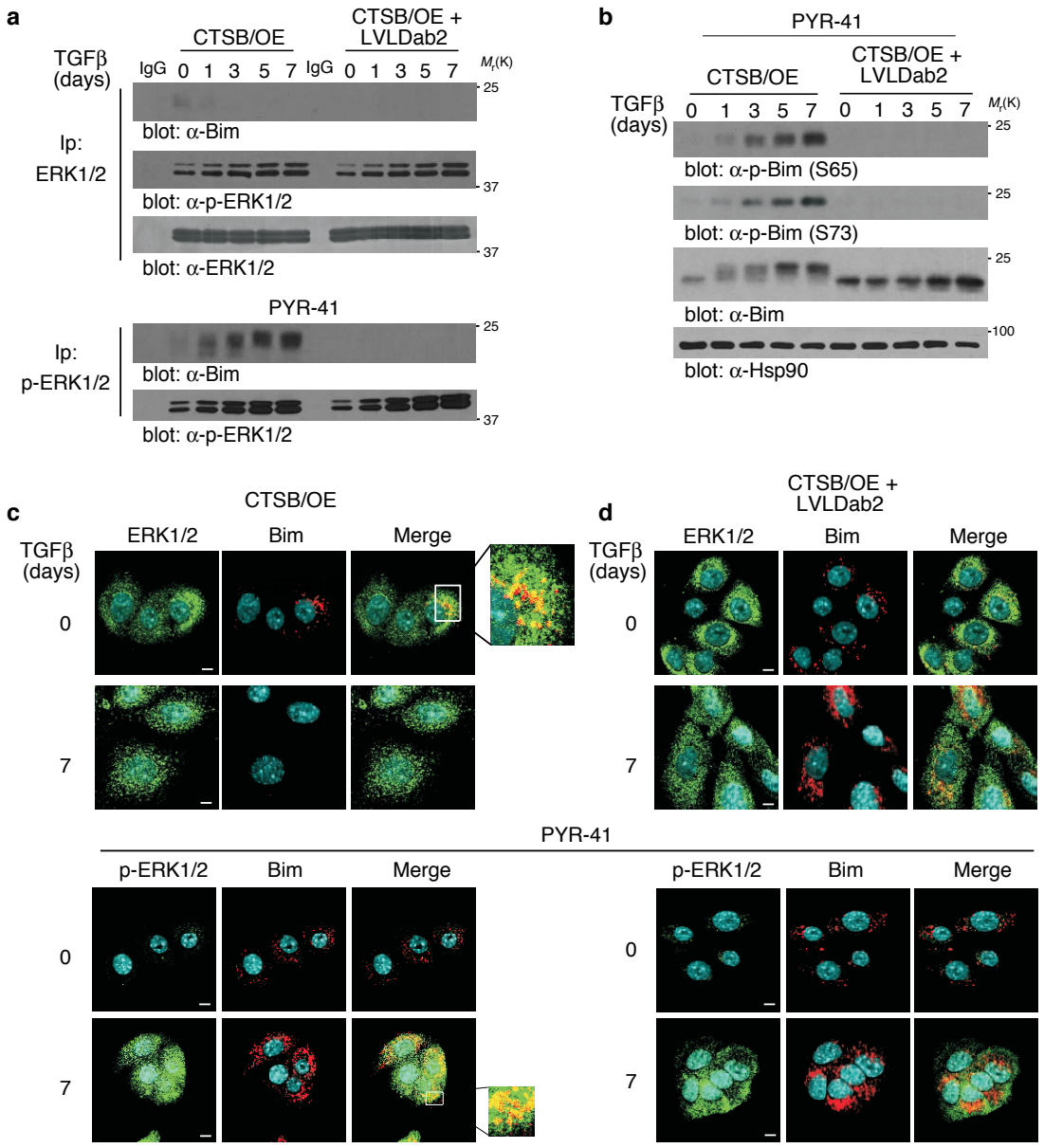


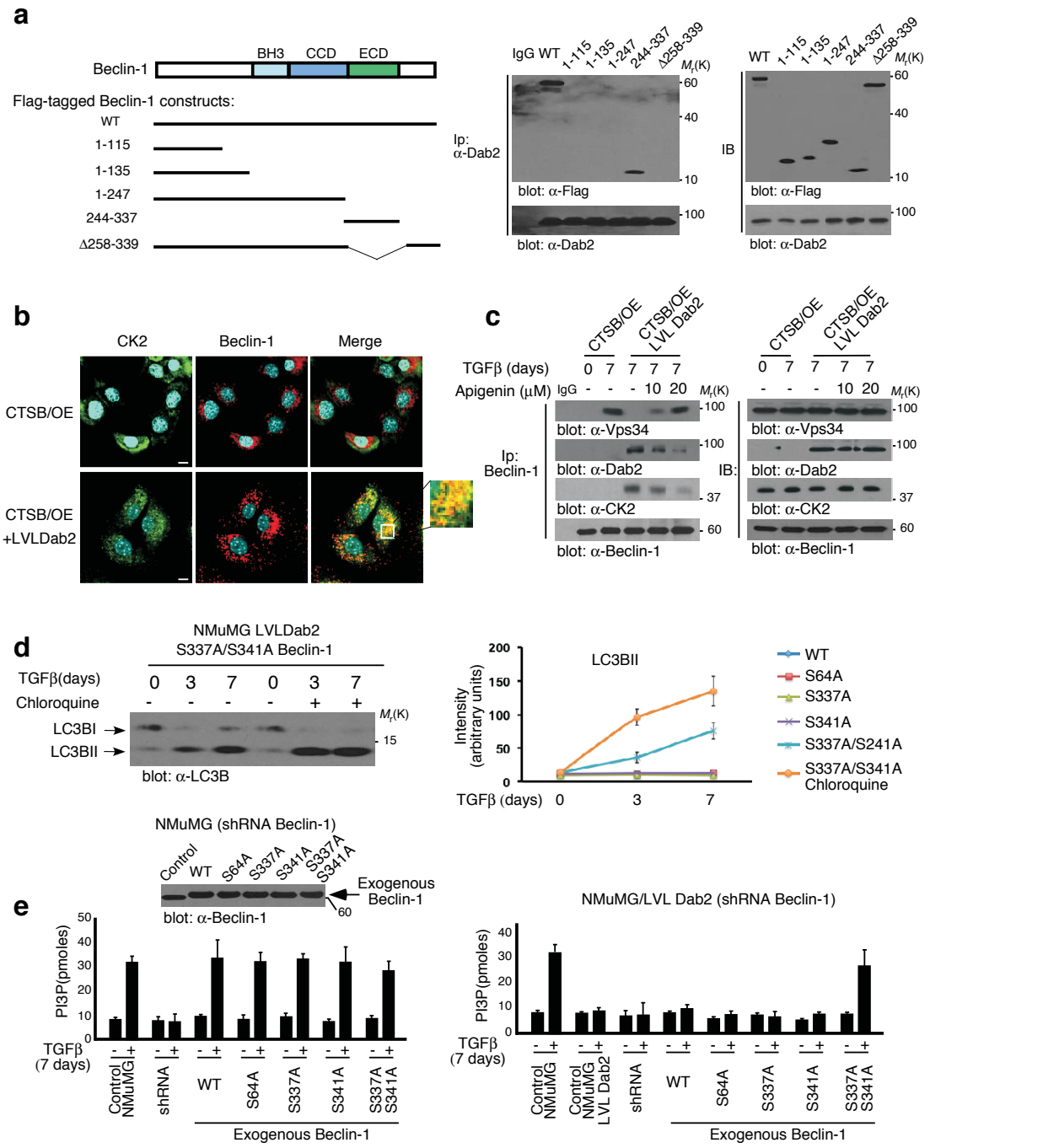
Supplementary Figure 1 Identification of the CTSB cleavage site in Dab2 and the TGFβ signaling pathway for CTSB regulation. (**a**) RT-PCR analysis of p96 Dab2, p67 Dab2, cathepsin B and β-actin mRNA levels in NMuMG cells. **(b)** Eph4 Ras cells and HMLE cells were treated with TGFβ for 7 days and immunoblots were performed to detect the expression of Dab2 and cathepsin B. Meanwhile, LC3B level was compared by immunoblotting between 0 day and 7 days treatment in the presence and absence of chloroquine. (**c**) Inhibitor screen to identify the protease responsible for Dab2 cleavage. Each inhibitor was added to cells at the concentrations indicated for 6 h prior to the preparation of whole cell lysates and immunoblot analysis. **(d)** Schematic of wild-type, full length Dab2, Dab2 mutants and amino acid sequence of peptides 486-510. **(e)** *In vitro* cleavage analysis of [^{35}S]-methionine-labeled p96 Dab2 and Dab2 mutants. (**f**) [^{35}S]-methionine-labeled wild-type and Dab2 mutants shown in (d) were incubated for 1 h at 37ºC with extracts from cells treated with TGFβ for 7 days prior to the preparation of WCLs and autoradiographic analysis of p96 Dab2 cleavage. (**g**) Immunoblot analysis of total and phosphorylated Smad2 and Smad3 following a 7-day TGFβ treatment. (**h**) Immunoblot analysis of total Smad2 and Smad3 following shRNA-mediated silencing. Hsp90 served as a loading control. (**i**) RT-PCR analysis of cathepsin B and β-actin mRNA levels in control and Smad2/3 double-knockdown NMuMG cells (*top panel*), and immunoblot analysis of cathepsin B, N-cadherin, and vimentin (*lower panel*) following a 7-day TGFβ treatment. (**j**) Nuclear, cytosolic and membrane fractions isolated from NMuMG cells treated with TGFβ were subjected to immunoblot analysis to detect the expression of cathepsin B, sodium potassium ATPase alpha1 (membrane fraction marker), histone H3 (nuclear fraction marker) and α-tubulin (cytosolic fraction marker). (**k**) CTSB activity assay *in vitro* using extracts from control and Smad2/3 double knockdown NMuMG cells treated with TGFβ for the times indicated. Data was shown as mean ± s.d., n=3 independent experiments. The experiments were repeated for three times and similar results were observed. Unprocessed blots are available in Supplementary Figure 9.



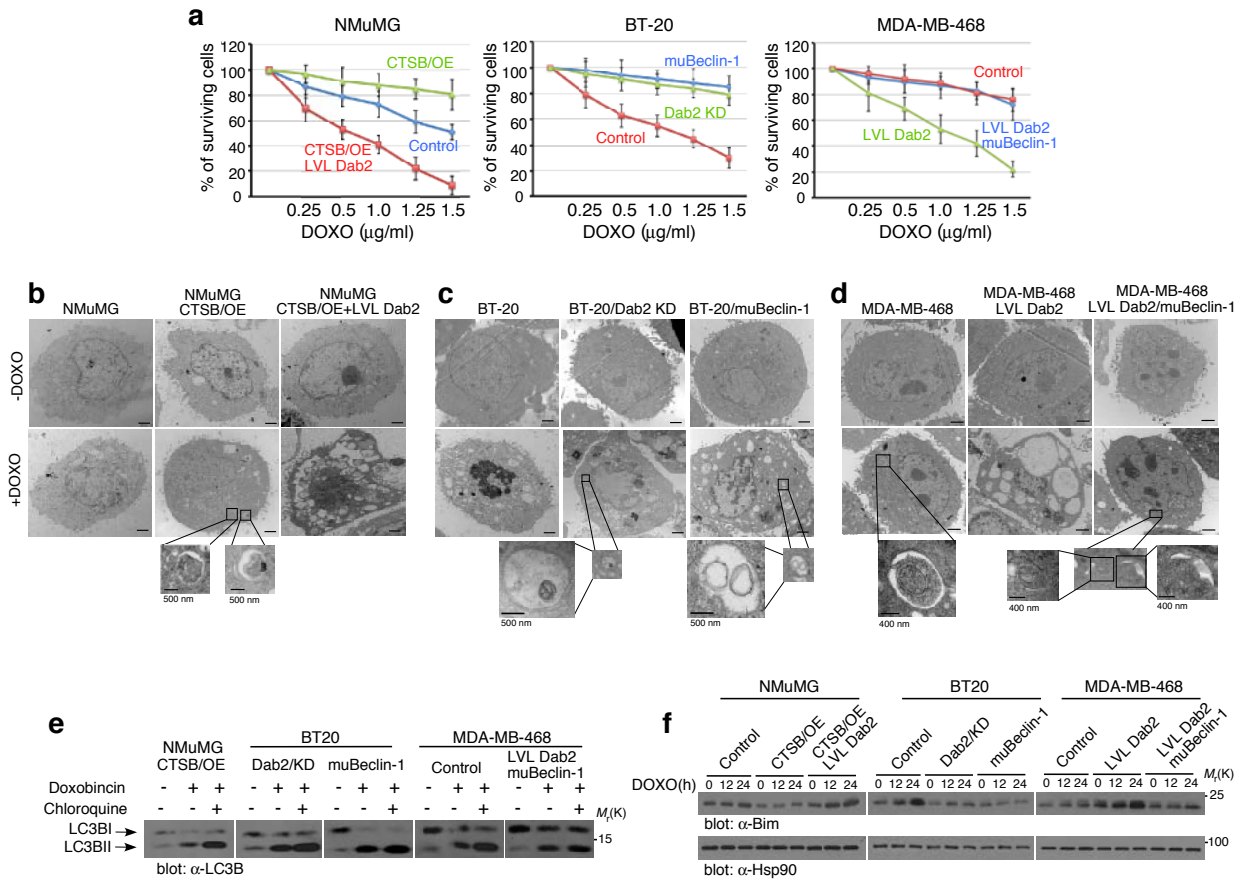
Supplementary Figure 2 Chronic TGFβ treatment results in autophagy and the reversal of EMT. **(a)** NMuMG and NMuMG CTSB/OE cells were treated with TGF-β for 5 and 7 days. Control and 25μm chloroquine solutions were applied to cell culture media 3 h before collecting the cell lysate of each time point. Cell lysates were subjected to immunoblot to detect LC3B expression with a α-LC3B antibody. LC3BII levels were quantified from three independent experiments and shown as mean ± s.d., n=3 independent experiments. **(b)** The GFP-LC3B puncta from top panel of figure 2b was quantified from three independent experiments and shown as mean ± s.d., n=3 independent experiments,. **(c)** The expression of autophagy markers (LC3BII and p62) and apoptosis markers (Bim and cleaved caspase-3) of figure 3b & c was quantified using a STORM scanner and ImageQuant software from Molecular Dynamics (Fairfield, CT, USA). Levels are expressed as relative intensities. The data is presented as the mean ± s.d., n=3 independent experiments**. (d)** Cell death rates were quantified from figure 3d experiments. The data is presented as the mean ± s.d. of three (n = 3) independent experiments. **(e)** Whole cell lysates from Dab2 and CTSB-modulated NMuMG cells treated with TGFβ for the times indicated were subjected to immunoblot analysis to determine the expression of mesenchymal markers and cell invasion markers using their corresponding antibodies. The lines on the western blots demarcate individual blots that were run in parallel. All experiments were repeated for three times and similar results were observed. Unprocessed blots are available in Supplementary Figure 9.



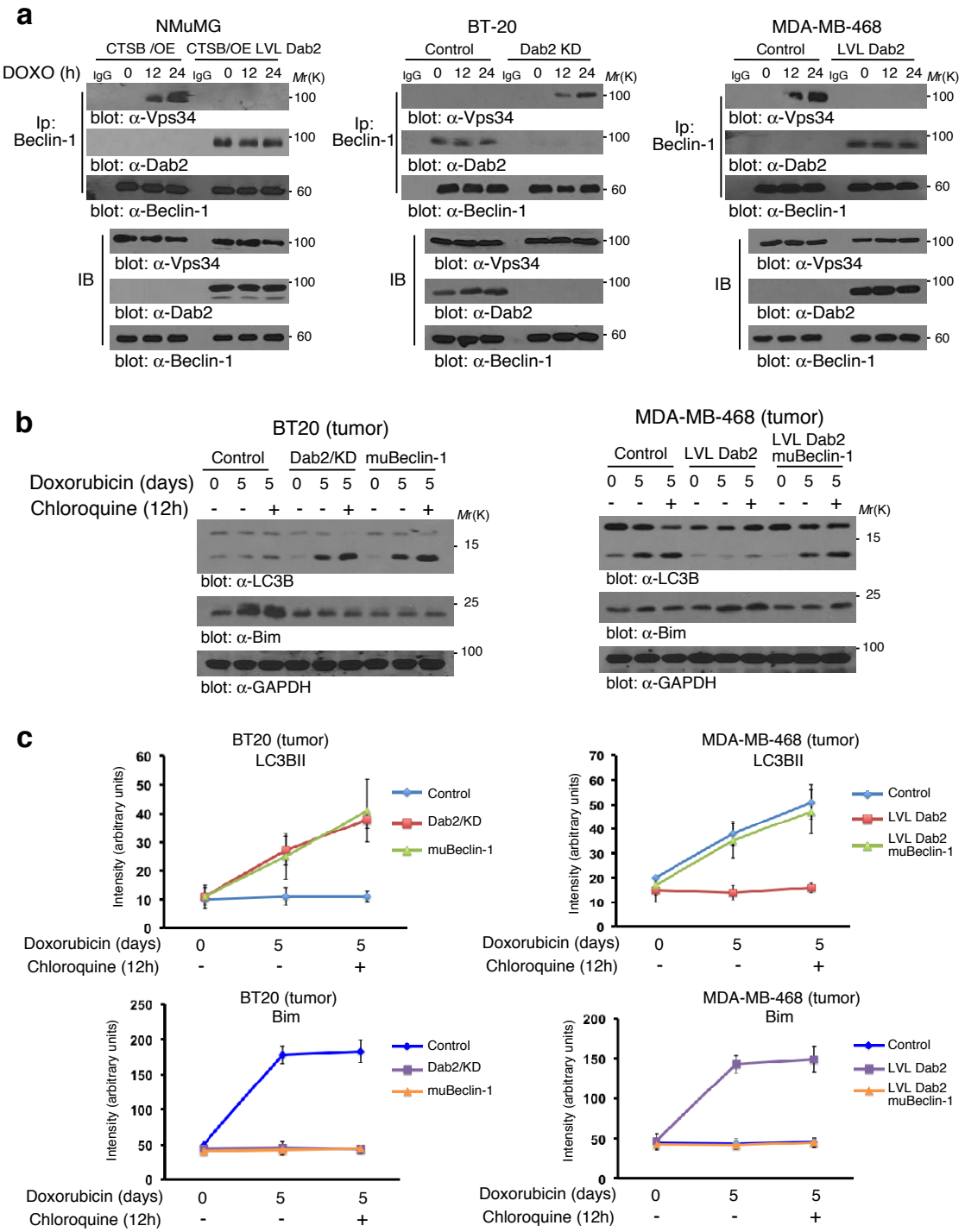
Supplementary Figure 3 Dab2 blocks ERK/Bim interaction and attenuates ERK-mediated Bim phosphorylation and degradation. **(a)** Co-immunoprecipitation analysis using a α-ERK1/2 or α-phospho-ERK1/2 antibody of whole cell lysates from NMuMG/OE ± LVLDab2 cells treated with TGFβ ± PYR-41 for the times indicated. Immunoprecipitated complexes were immunoblotted to detect the expression of p-ERK1/2 and Bim. **(b)** Immunoblotting analysis was applied to detect Bim and p-Bim expression from whole cell lysates of NMuMG/OE ± LVLDab2 cells treated with TGFβ for the times indicated in the presence of 20 μM PYR-41 (applied 4 h before cell lysates were collected). **(c,d)** Immunofluorescence analysis was performed to detect the co-localization between ERK or p-ERK and Bim before and after NMuMG/OE ± LVLDab2 cells were treated for 7 days with TGFβ. Scale bar, 10μm. The experiments were repeated for three times and similar results were observed. Unprocessed blots are available in Supplementary Figure 9.



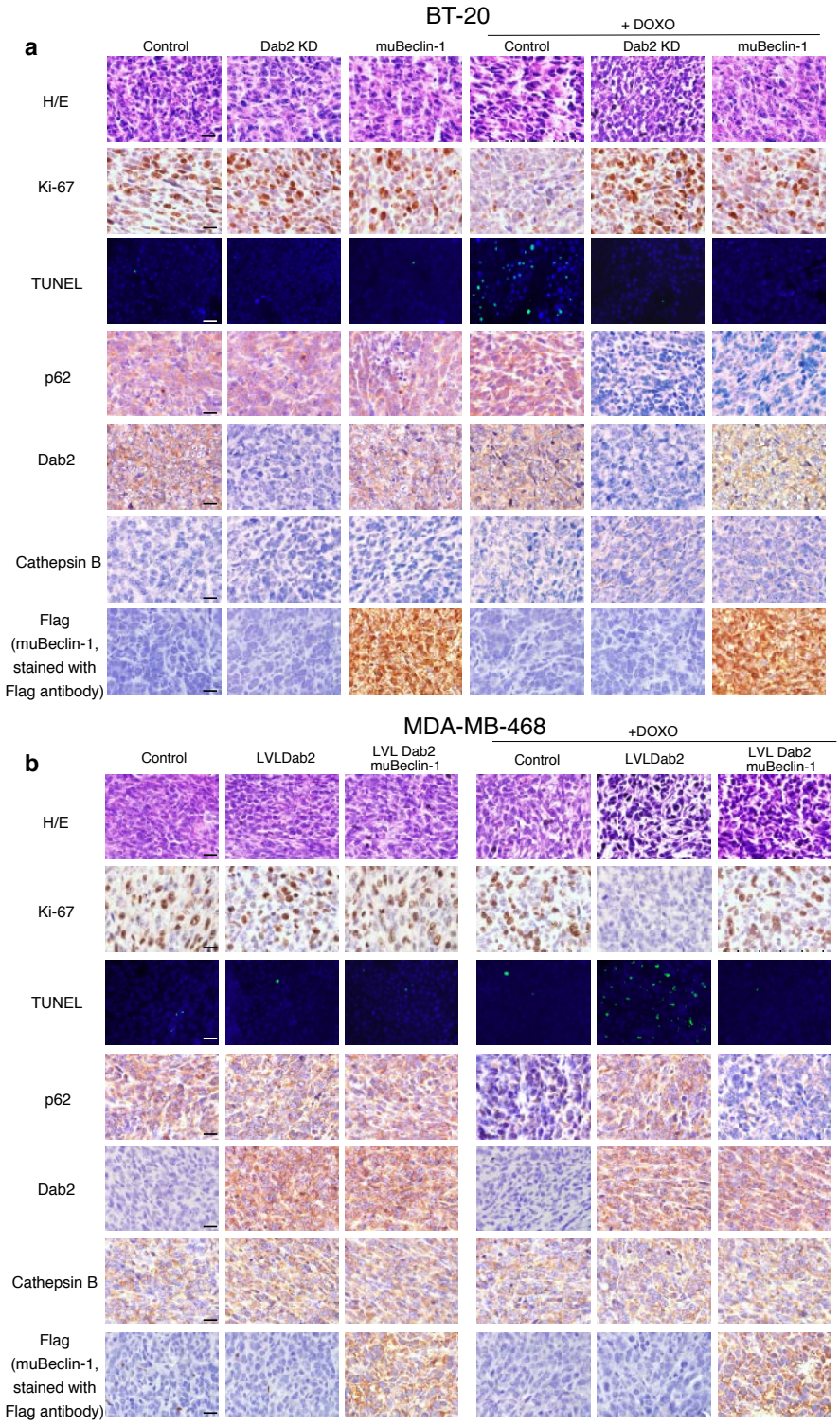
Supplementary Figure 4 Characterization of Beclin-1/Dab2 interactions and followed PI3K induction. **(a)** Mapping the interaction domain between Dab2 and Beclin-1 in NMuMG CTSB/KD cells. Schematic diagram of domains of Beclin-1 and schematic representatives of Flag-tagged wild type Beclin-1 and mutant Beclin-1 constructs employed in co-immunoprecipitation assay (left). Co-immunoprecipitation assays of transfected Flag-tagged wild type Beclin-1 and Beclin-1 mutants with Dab2 (right). **(b)** Immunofluorescence analysis of CK2 and Beclin-1 in NMuMG CTSB/OE cells and NMuMG CTSB/OE+LVLDab2 cells. CK2 is shown as green, and Beclin-1 is shown as red. Colocalization of CK2 and Beclin-1 is depicted as yellow in the panels labeled 'merge'. DAPI was used to stain nuclei. Scale bars, 10 μm. **(c)** Inhibition of CK2 attenuates Dab2's inhibitory effect on Beclin-1/Vps34 interaction. Co-immunoprecipitation analysis using a α-Beclin-1 antibody of whole cell lysates from CTSB/OE and CTSB/OE+LVLDab2 cells treated with TGFβ for the times indicated. Immunoprecipitated complexes were immunoblotted to detect the expression of Vps34, Dab2, CK2, and Beclin-1. Immunoblots (IB) depict expression levels of Vps34, Dab2, and Beclin-1 in whole cell lysates. The CK2 inhibitor apigenin (10 and 20 μM) was added 6 h prior to the preparation of whole cell lysates. **(d)** Analysis of LC3BII turnover in the absence and presence of chloroquine. NMuMG/LVLDab2 cells stably transfected with Beclin-1 S337A/S341A mutant were subjected to TGFβ treatment as indicated. Control and 25μM chloroquine solutions were applied to cell culture media 3 h before collecting the cell lysate of each time point. Cell lysates were subjected to immunoblot to detect LC3B expression with a α-LC3B antibody (left panel), and the quantification of LC3BII (Fig. 6g) is shown in the right panel as mean ± s.d., n=4 independent experiments. **(e)** PI3P levels before and after TGFβ treatment in NMuMG cells (left) and NMuMG/LVLDab2 cells (right) with Beclin-1 knockdown and ectopic expression of WT Beclin-1 and mutant Beclin-1. Data was shown as mean ± s.d., n=4 independent experiments. The experiments were repeated for four times and similar results were observed. Unprocessed blots are available in Supplementary Figure 9.



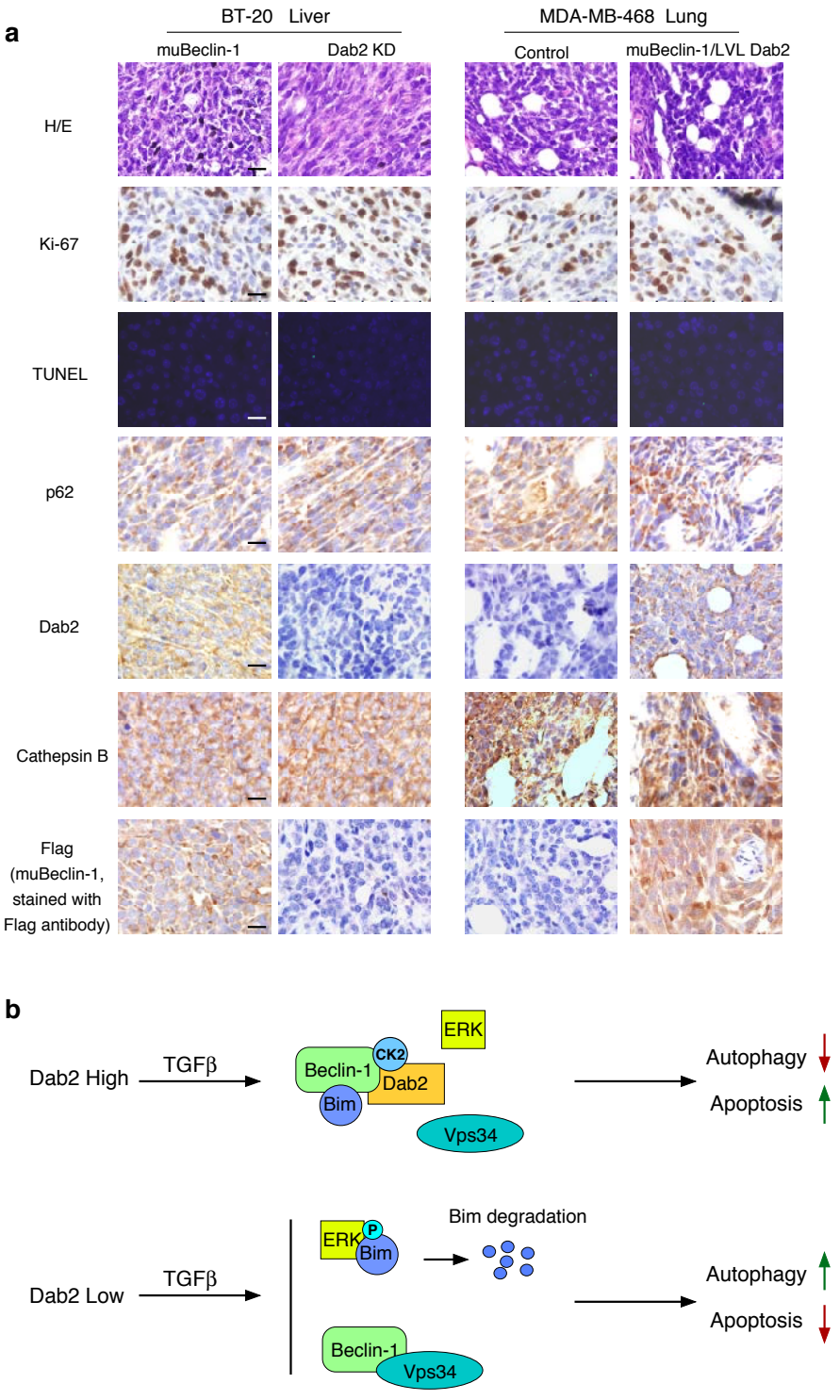
Supplementary Figure 5 Transmission electron microscopy (TEM) analysis. Transmission electron microscopy analysis (TEM) was performed to detect autophagic and apoptotic structures in (**a**) MTT assay depicting the dose dependent effects of doxorubicin (DOXO) on cell survival in NMuMG, BT20, MDA-MB468 cells, and their modified derivatives. Results are shown as means -/+ s.d., n=3 independent experiments **(b)** NMuMG control cells, CTSB/OE cells and CTSB/OE+LVLDab2 cells**,** in **(c)** BT-20 control, Dab2 KD and muBeclin-1 overexpressing cells and in **(d)** MDA-MB-468 control, LVLDab2 and LVLDab2+muBeclin-1 overexpressing cells**,** before and after doxorubicin (DOXO) treatment. Scale bars, 2μm. Frame sections highlight autophagosomes. **(e)** NMuMG, BT20, MDA-MB-468 cells and their transfected derivatives were subjected to DOXO (2 μg/ml) for 24 h treatment. 25μM chloroquine was applied to cell culture media 1 h before cell lysates were collected. Cell lysates were subjected to immunoblotting analysis to detect LC3B expression with α-LC3B antibody. **(f)** NMuMG, BT20, MDA-MB-468 cells and their transfected derivatives were treated with DOXO (2 μg/ml) for 12 h and 24 h. Cell lysates were subjected to immunoblotting analysis to detect Bim expression with α-Bim antibody. Hsp90 served as loading control. The experiments were repeated for three times and similar results were observed. Unprocessed blots are available in Supplementary Figure 9.



Supplementary Figure 6 Dab2 attenuates doxorubicin-induced Beclin-1/Vps34 interactions. **(a)** Immunoprecipitation analysis was performed with a α-Beclin-1 antibody from whole cell lysates of NMuMG/OE ± LVLDab2 cells, BT-20 control cells and Dab2 KD cells, and MDA-MB-468 control ± LVLDab2 cells to detect Beclin-1/Vps34 interactions during DOXO treatment. Immunoprecipitated complexes were immunoblotted to detect the expression of Dab2 and Vps34. **(b)** Immunoblotting analysis of LC3B and Bim in tissue lysates from tumors of Figure 8a with α-LC3B and α-Bim antibodies. Chloroquine was injected 12 h before the extraction of the tumors (i.v., 20 mg/kg) **(c)** Quantification of the LC3B and Bim expression in panel **b.** Data represents the means -/+ s.d., n=3 independent experiments. Unprocessed blots are available in Supplementary Figure 9.



Supplementary Figure 7 Immunohistochemical analysis of primary tumors from BT20, MDA-MB-468 and their transfected derivatives. Representatives of immunohistochemical staining of Hematoxylin and eosin (H&E), Ki-67 TUNEL, p62, Dab2, Cathepsin B and Flag-tagged muBeclin-1 in primary tumors from BT-20 and MDA-MB-468 and their derivatives before and after DOXO treatment to the mice. Scale bar, 25 μm. All experiments were repeated for three times and similar results were observed.



Supplementary Figure 8 The Immunhistochemical analysis of metastases and the working model. **(a)** Immunohistochemical analysis of metastases. Representative immunohistochemical staining of Hematoxylin and eosin (H&E), Ki-67 TUNEL, p62, Dab2, Cathepsin B and Flag-tagged muBeclin-1 of metastatic lesions developed from BT-20 Dab2 KD cells or BT-20 muBeclin-1 overexpressing cells (liver) and from MDA-MB-468 control cells or muBeclin-1/LVLDab2 overexpressing cells (lung). Scale bar, 25 μm. **(b)** Model depicting Dab2's role in mediating autophagy or apoptosis in chronic TGFβ-treated cells. Dab2 inhibits TGFβ-mediated autophagy by attenuating Beclin-1/Vps34 interactions. Dab2 prevents ERK-mediated Bim phosphorylation and degradation and stabilizes TGFβ-induced Bim expression to promote apoptosis. The experiments were repeated for three times and similar results were observed.

Figure 1b

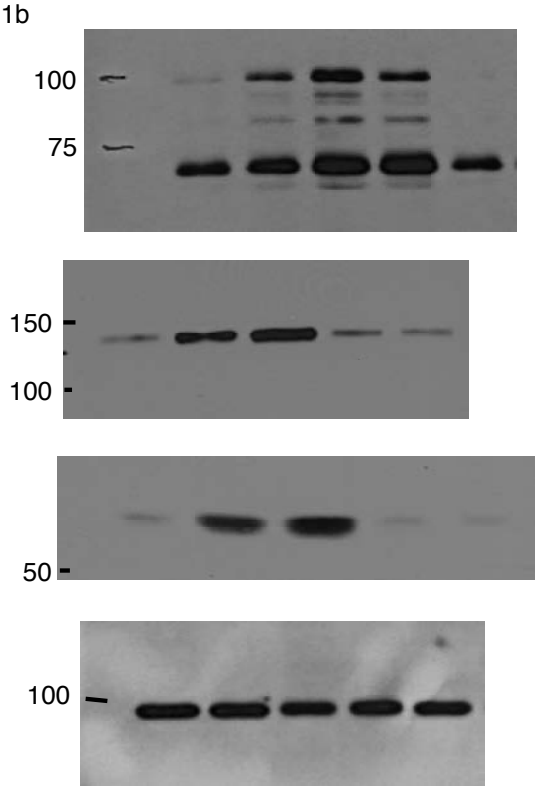
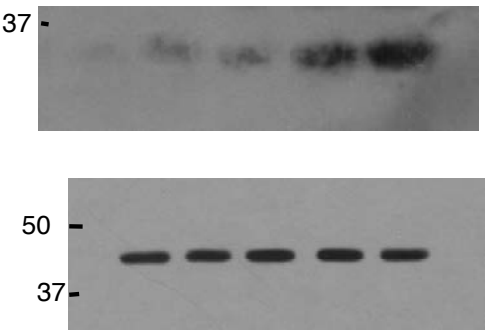


Figure 1f



Supplementary Figure 9 Unprocessed blots. The unprocessed blots of all blots shown in main figures and supplementary figures are included.

Figure 2a

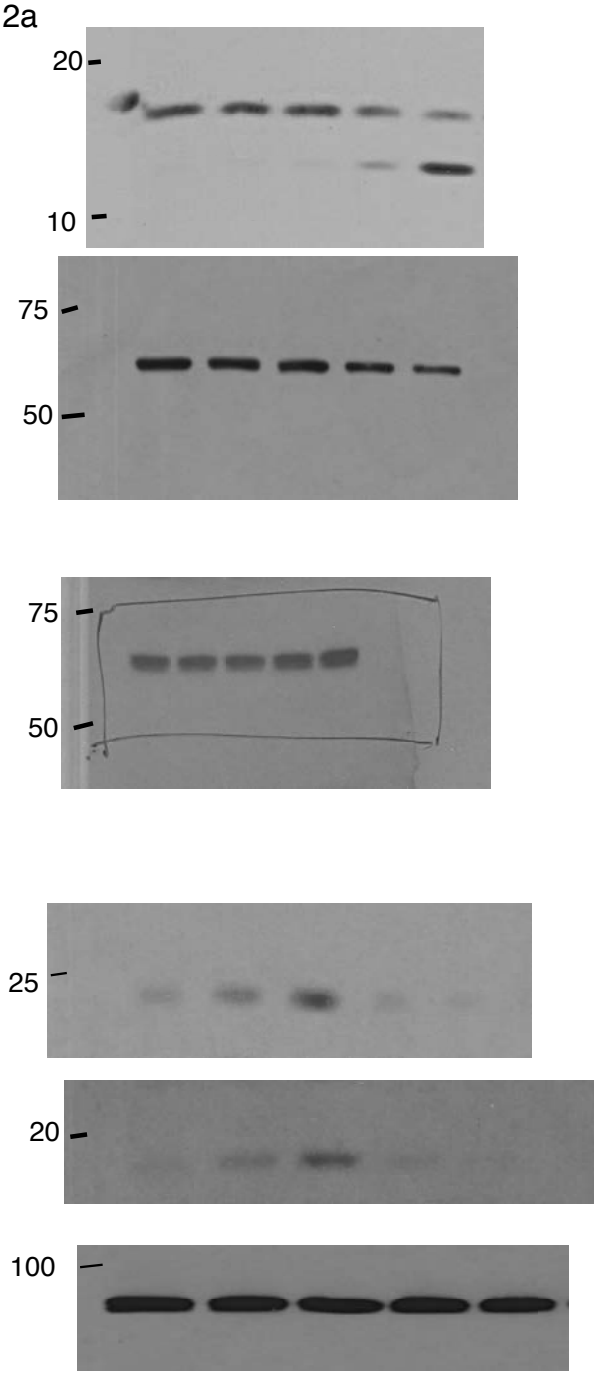
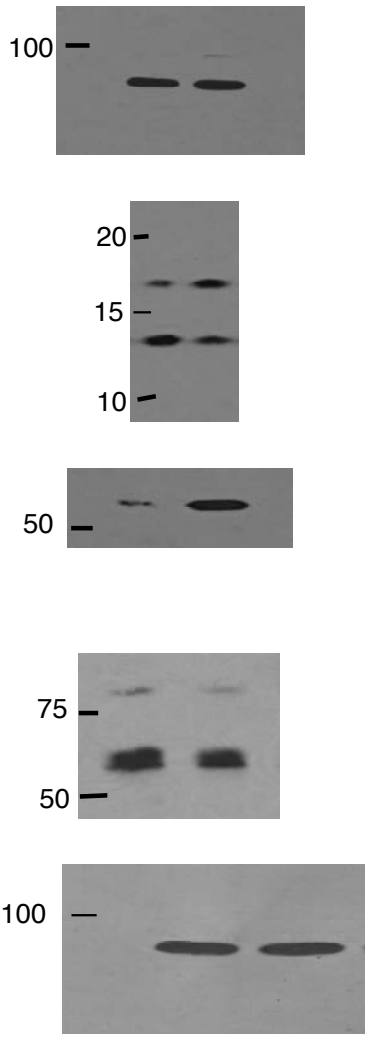
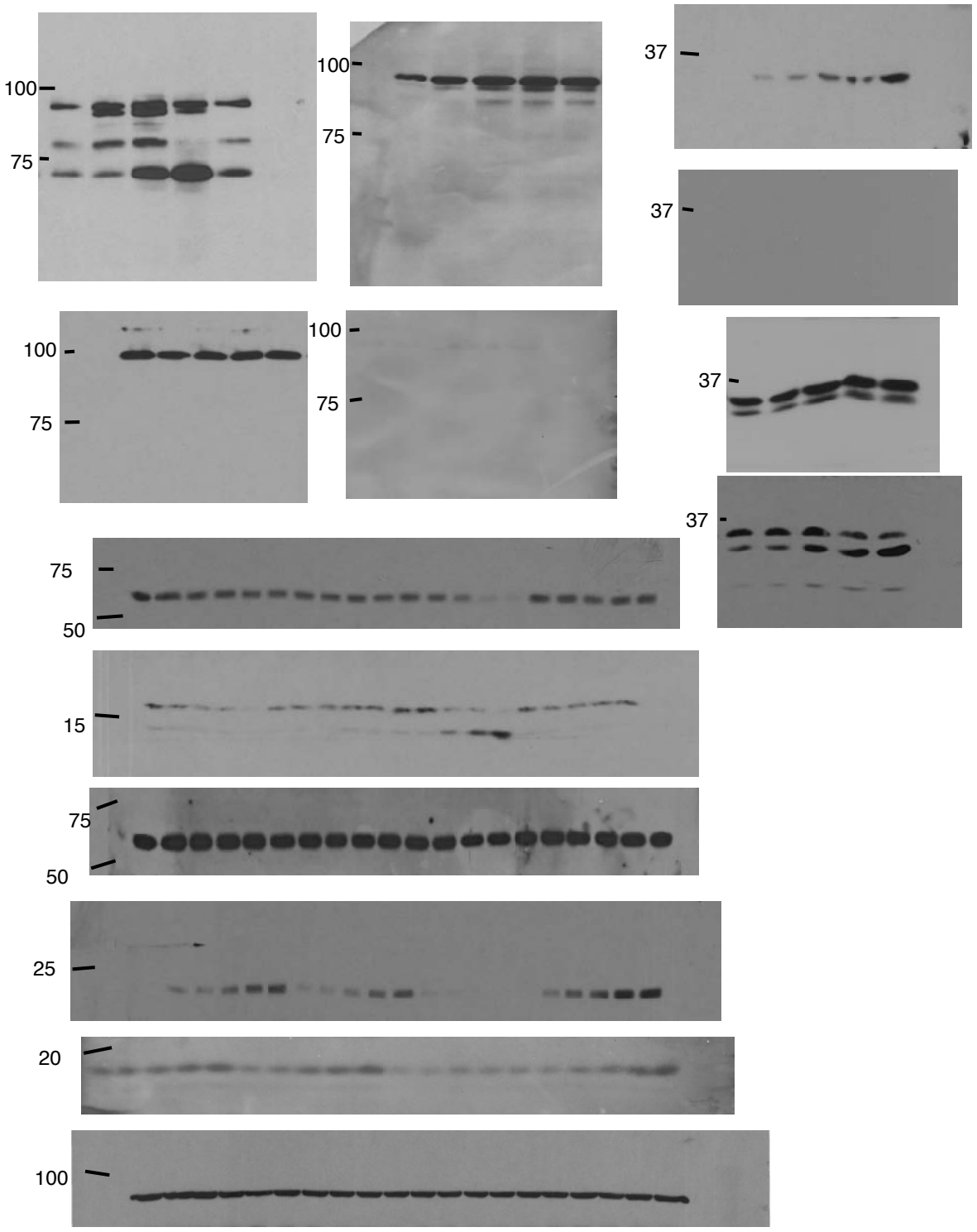


Figure 2d



Supplementary Figure 9 continued

Figure 3



Supplementary Figure 9 continued

Figure 4a

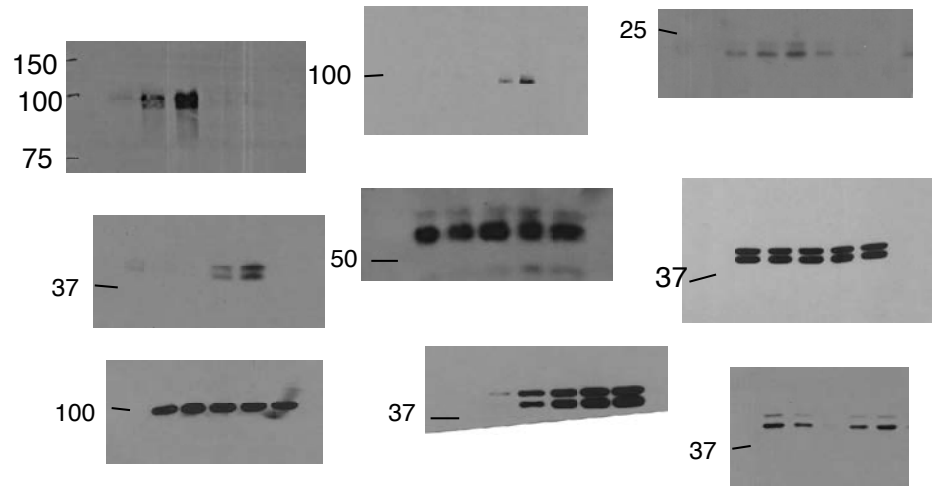


Figure 4c

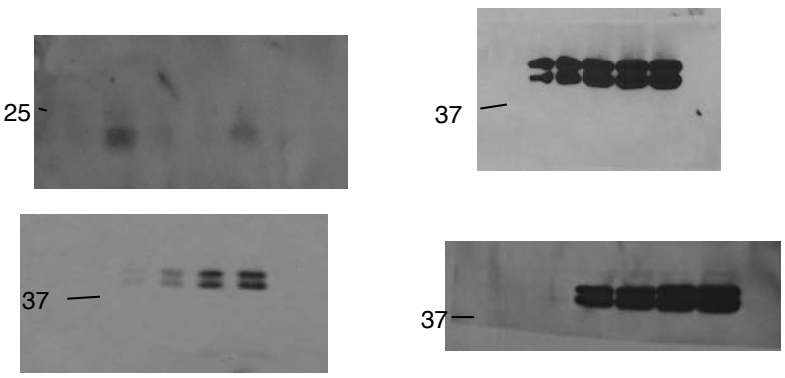
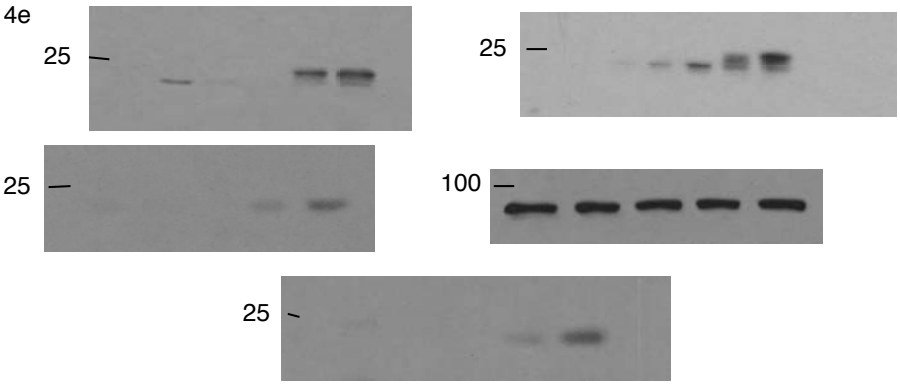


Figure 4e



Supplementary Figure 9 continued

Figure 5a

Figure 5b

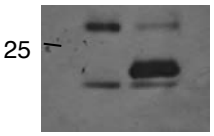
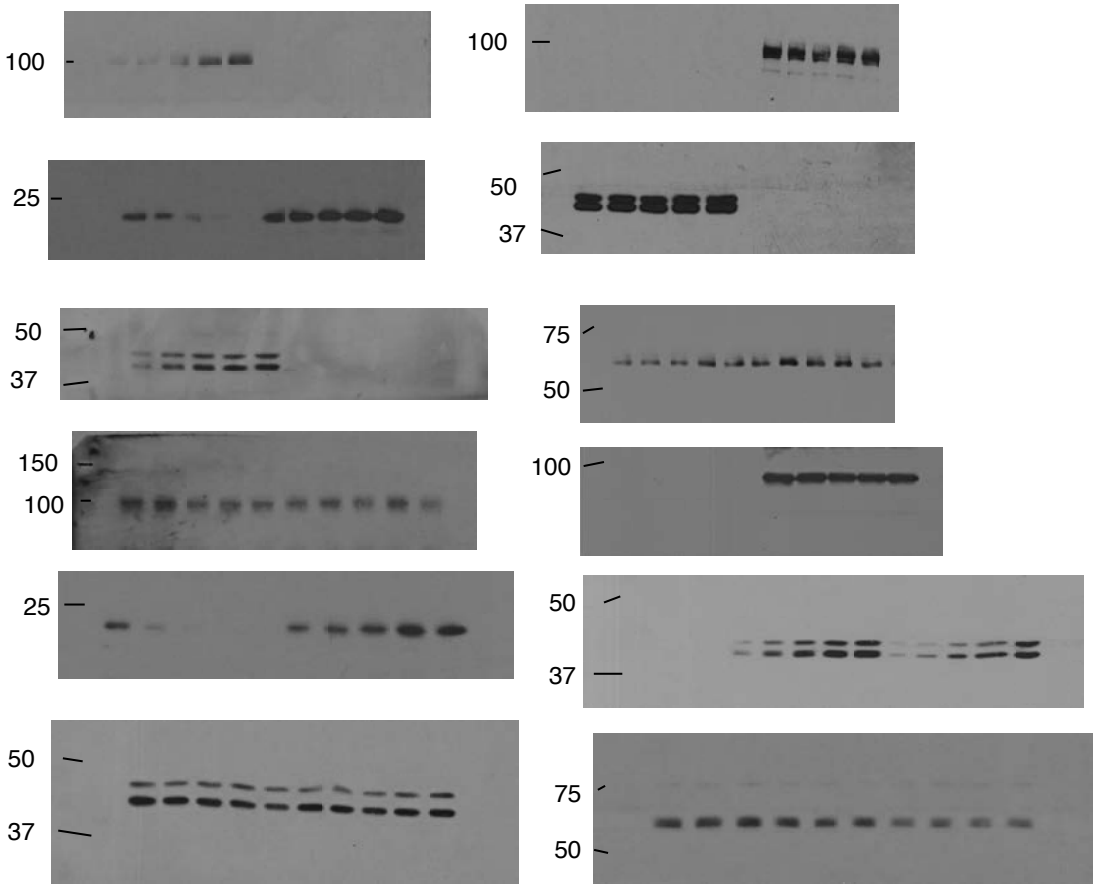


Figure 5c



Supplementary Figure 9 continued

Figure 6b

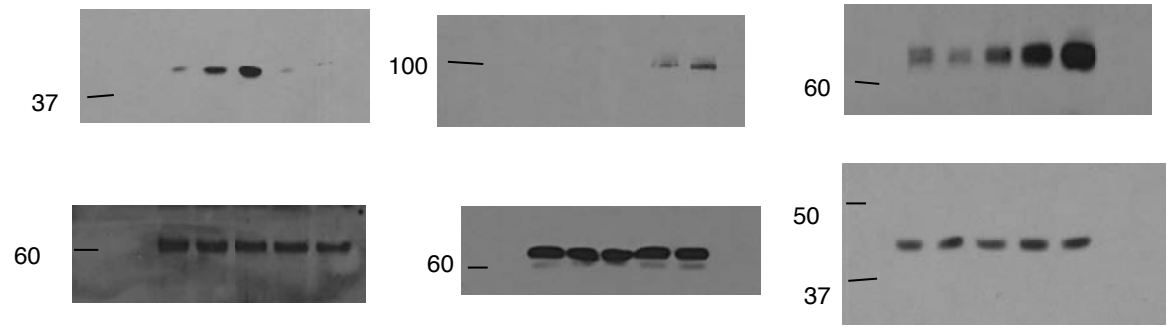


Figure 6c

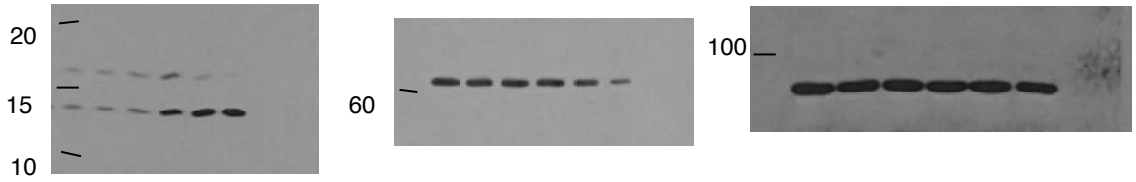


Figure 6d

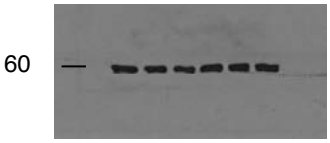
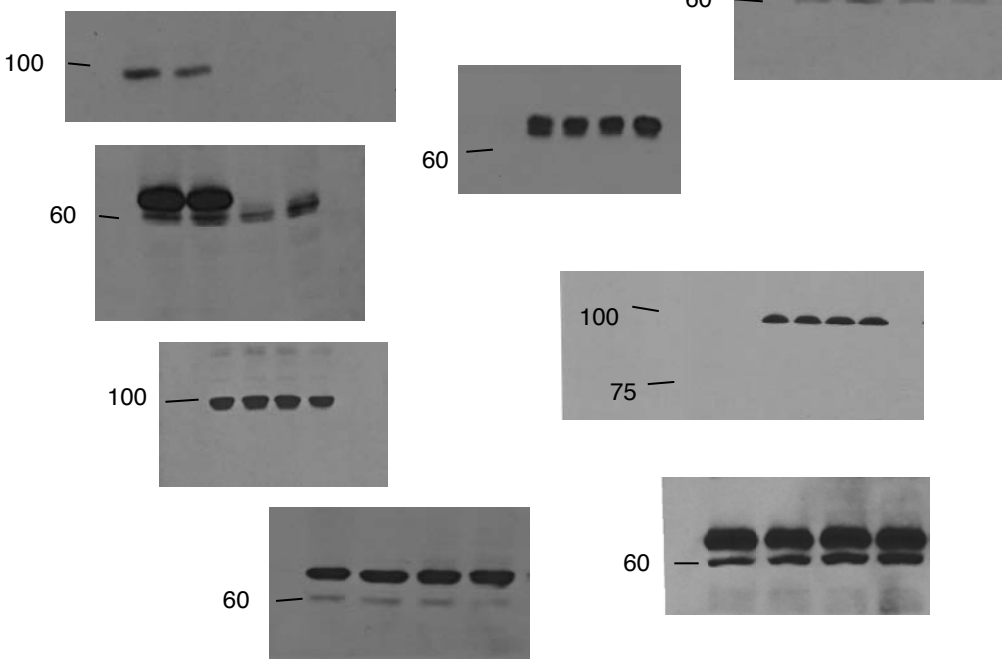


Figure 6e



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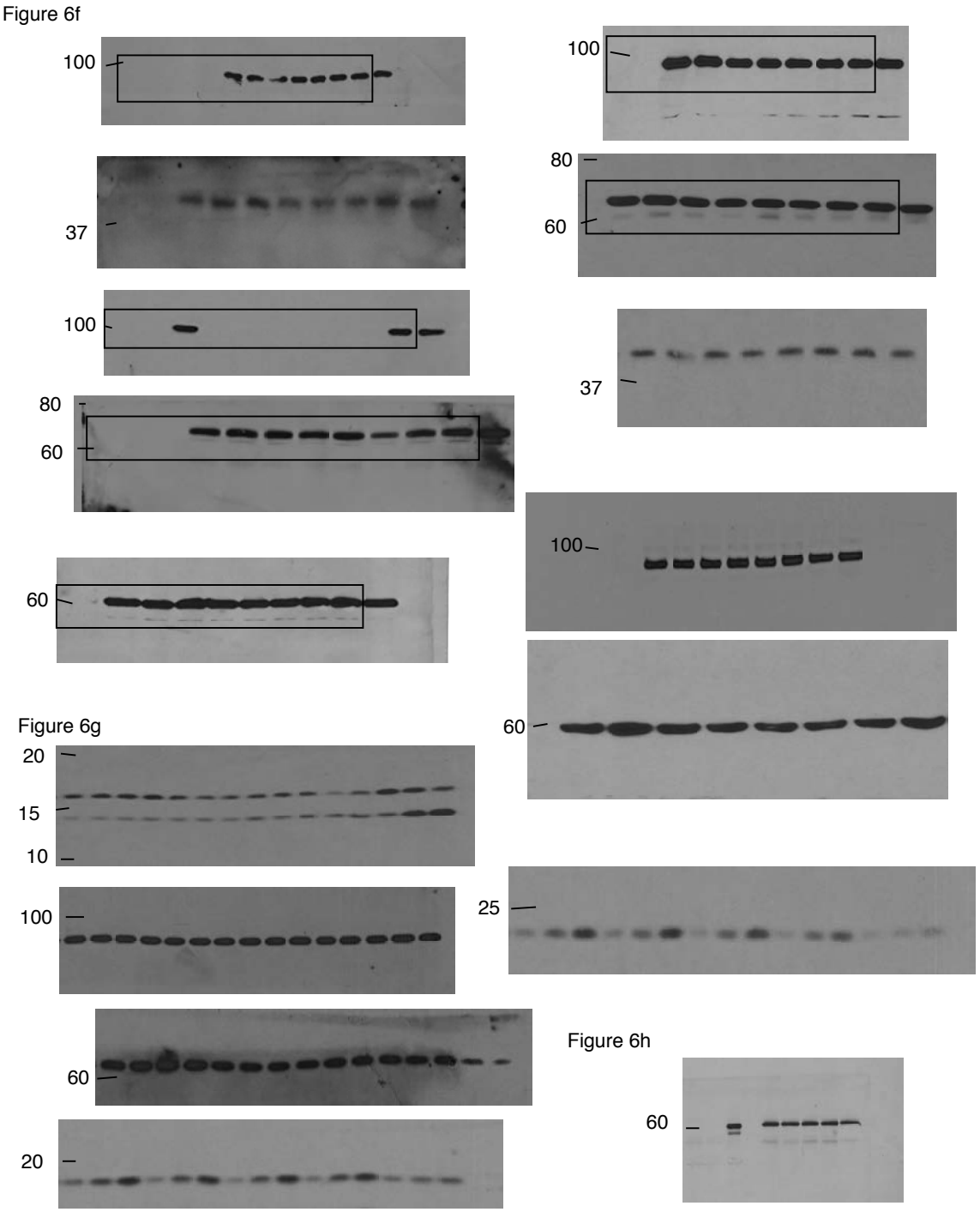
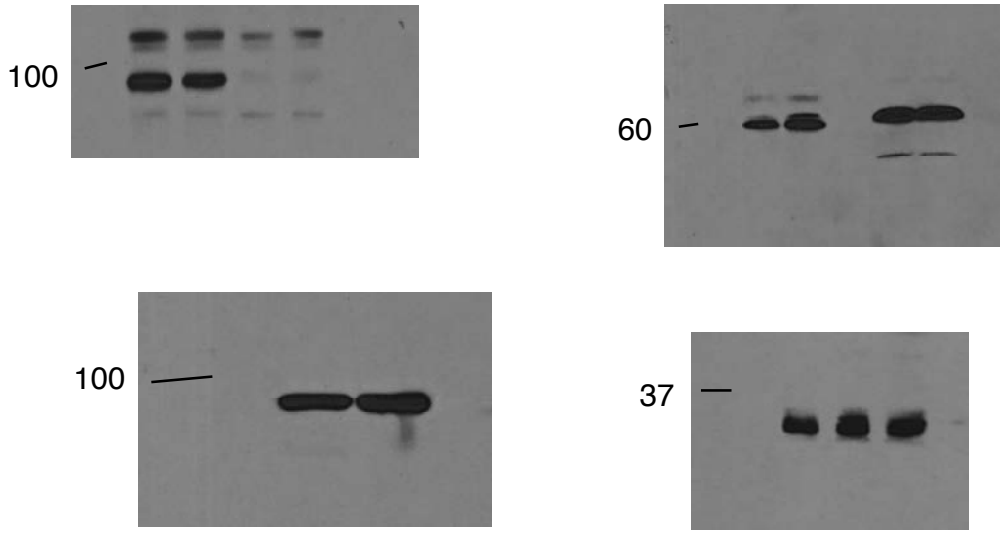


Supplementary Figure 9 continued

Figure 7a



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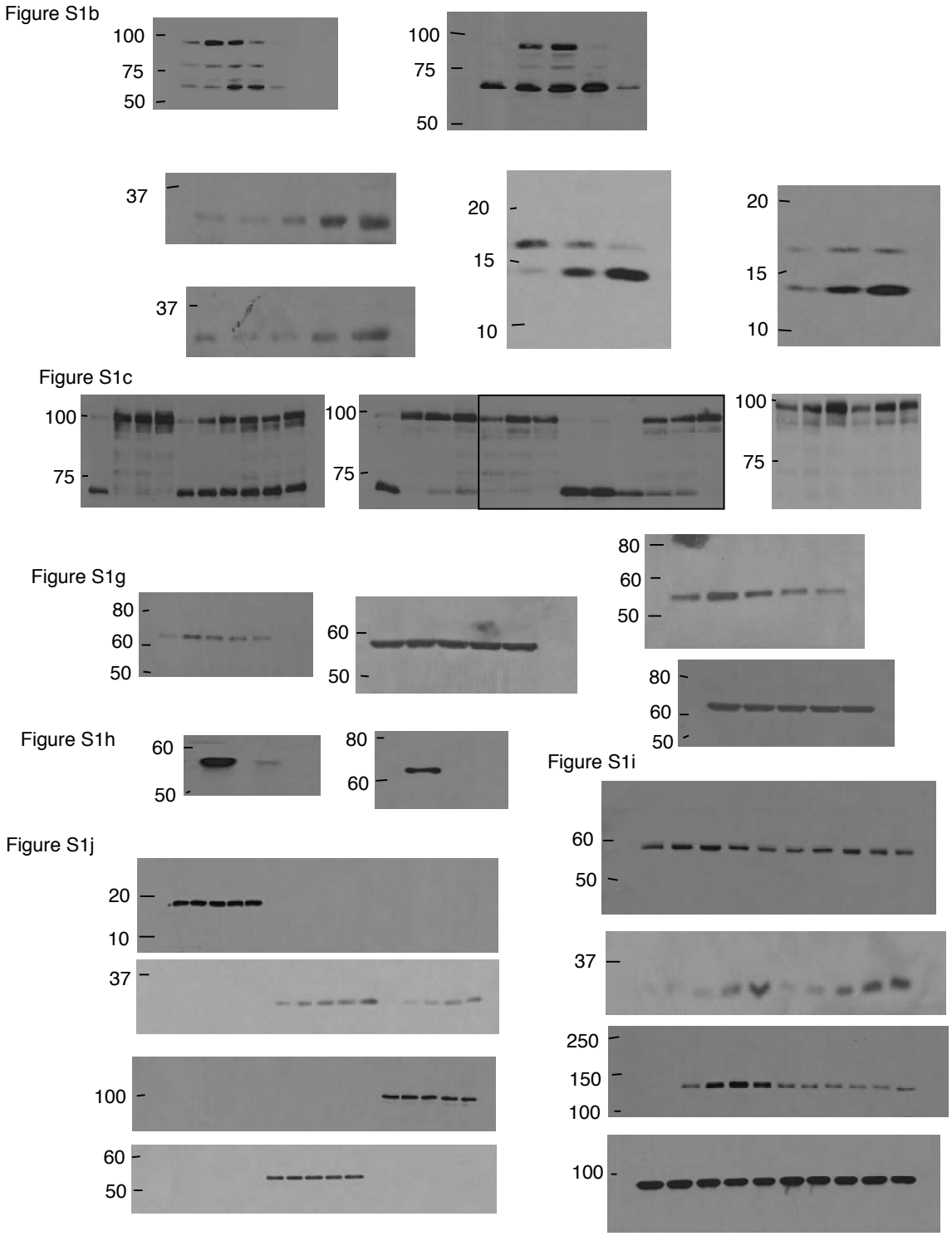

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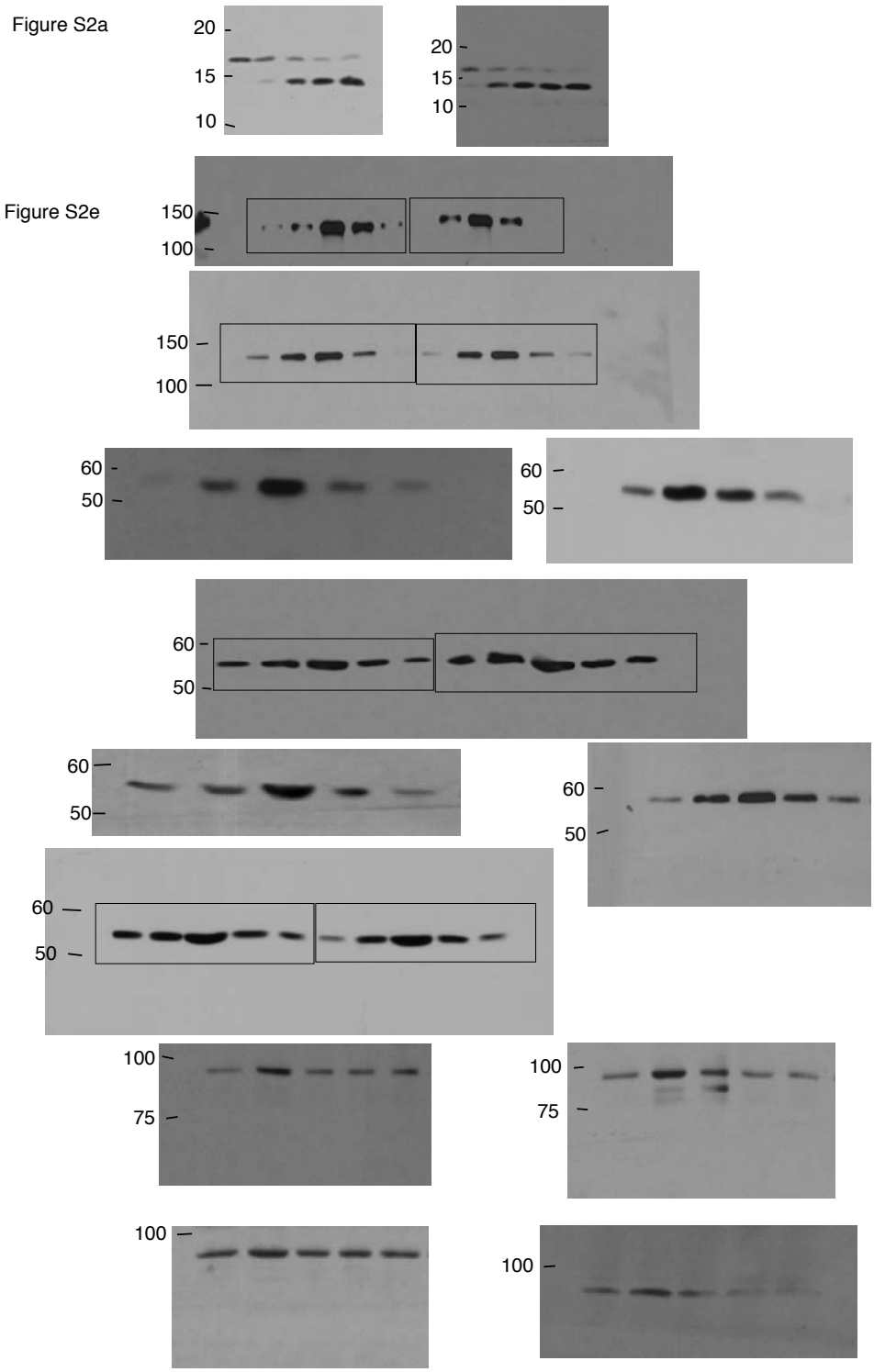

Supplementary Figure 9 continued

Figure S3a

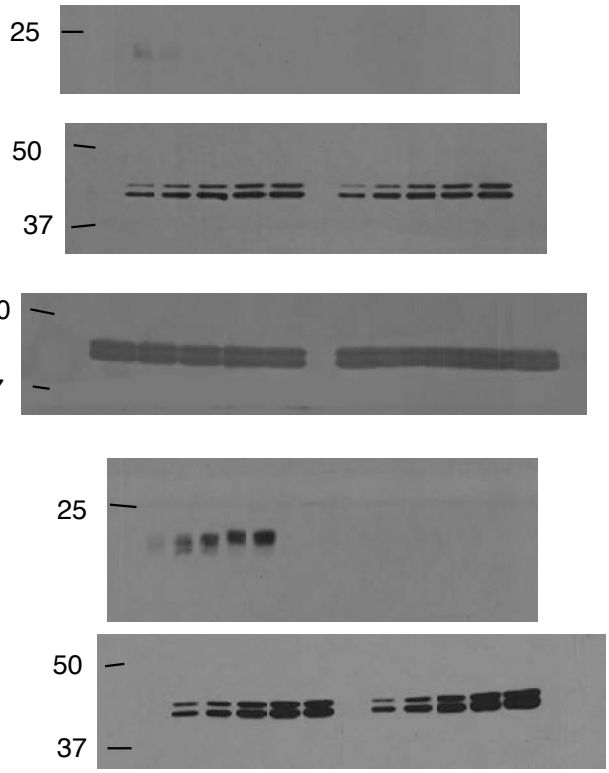

Figure S3b

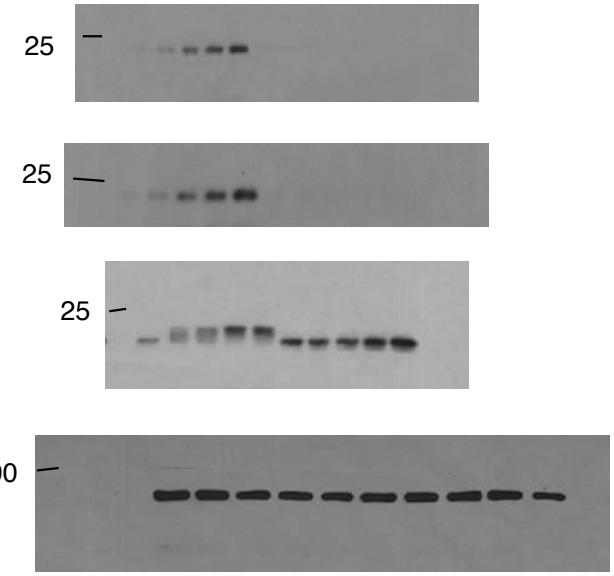

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Figure S4a

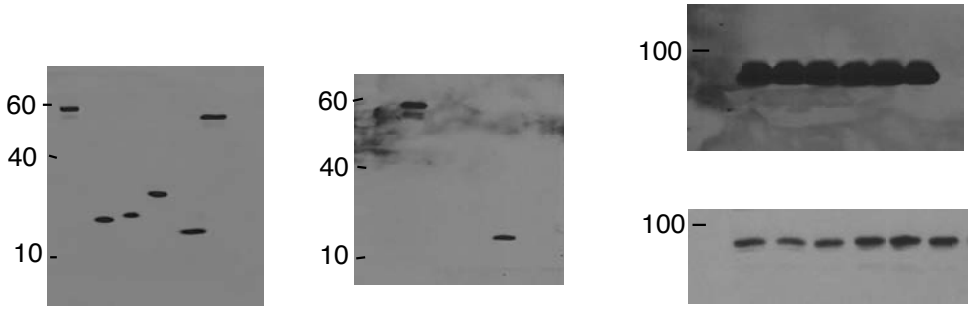

Figure S4c

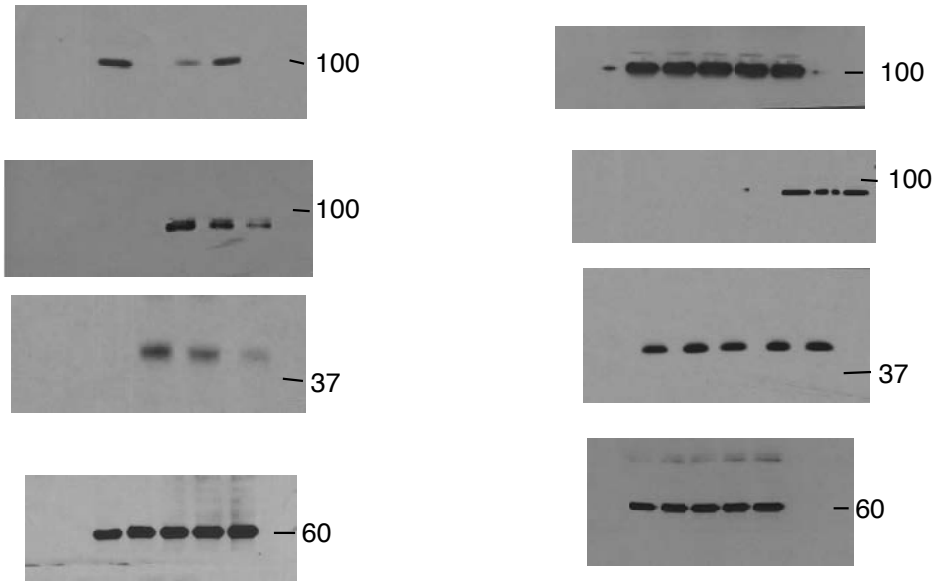

Figure S4d

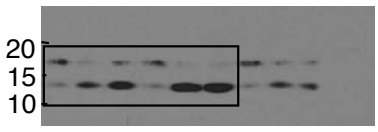

Figure S4e

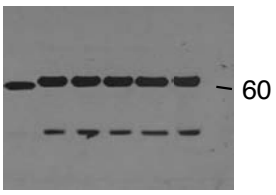

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Figure S5e

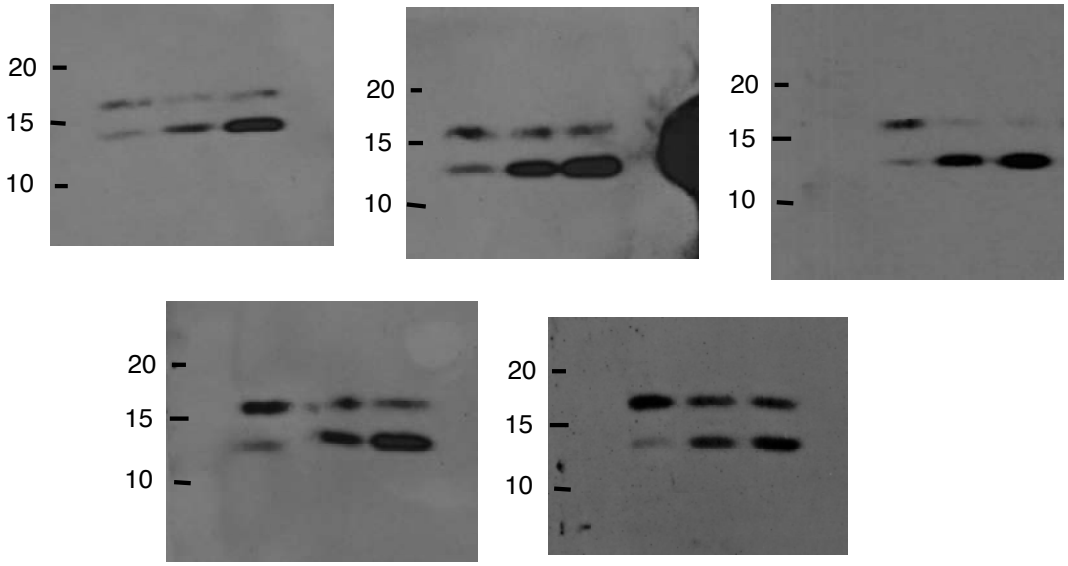

Figure S5f

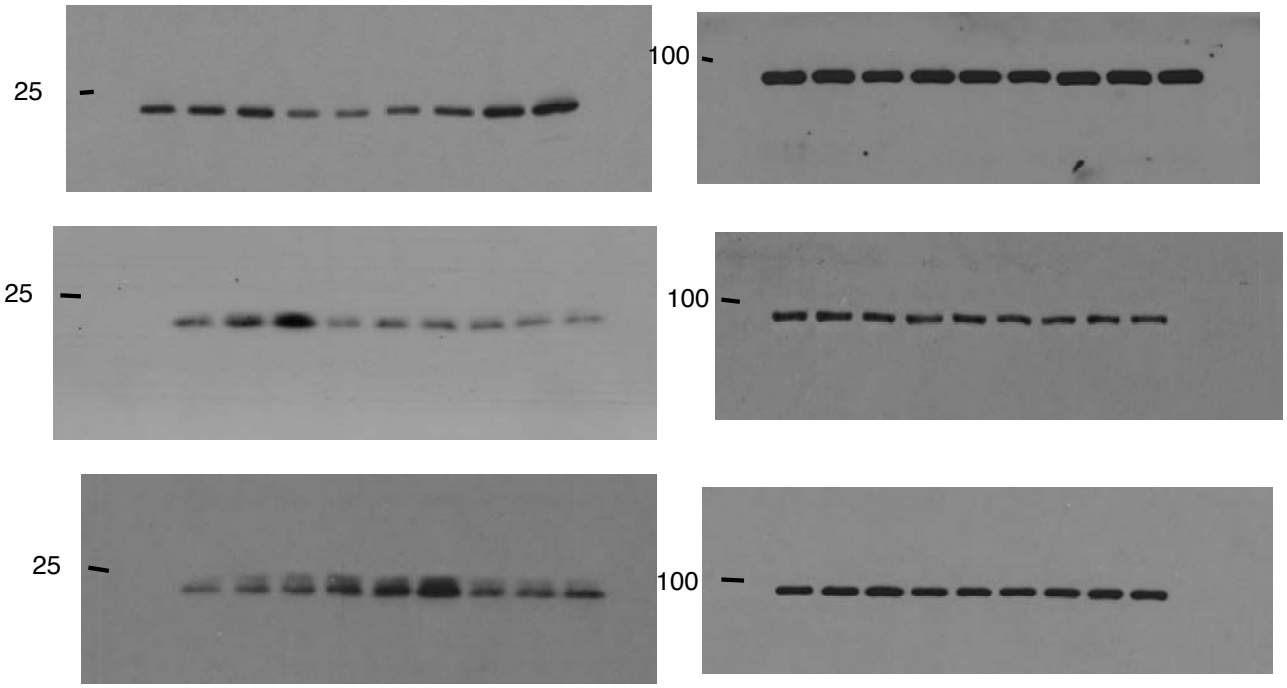

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Figure S6a

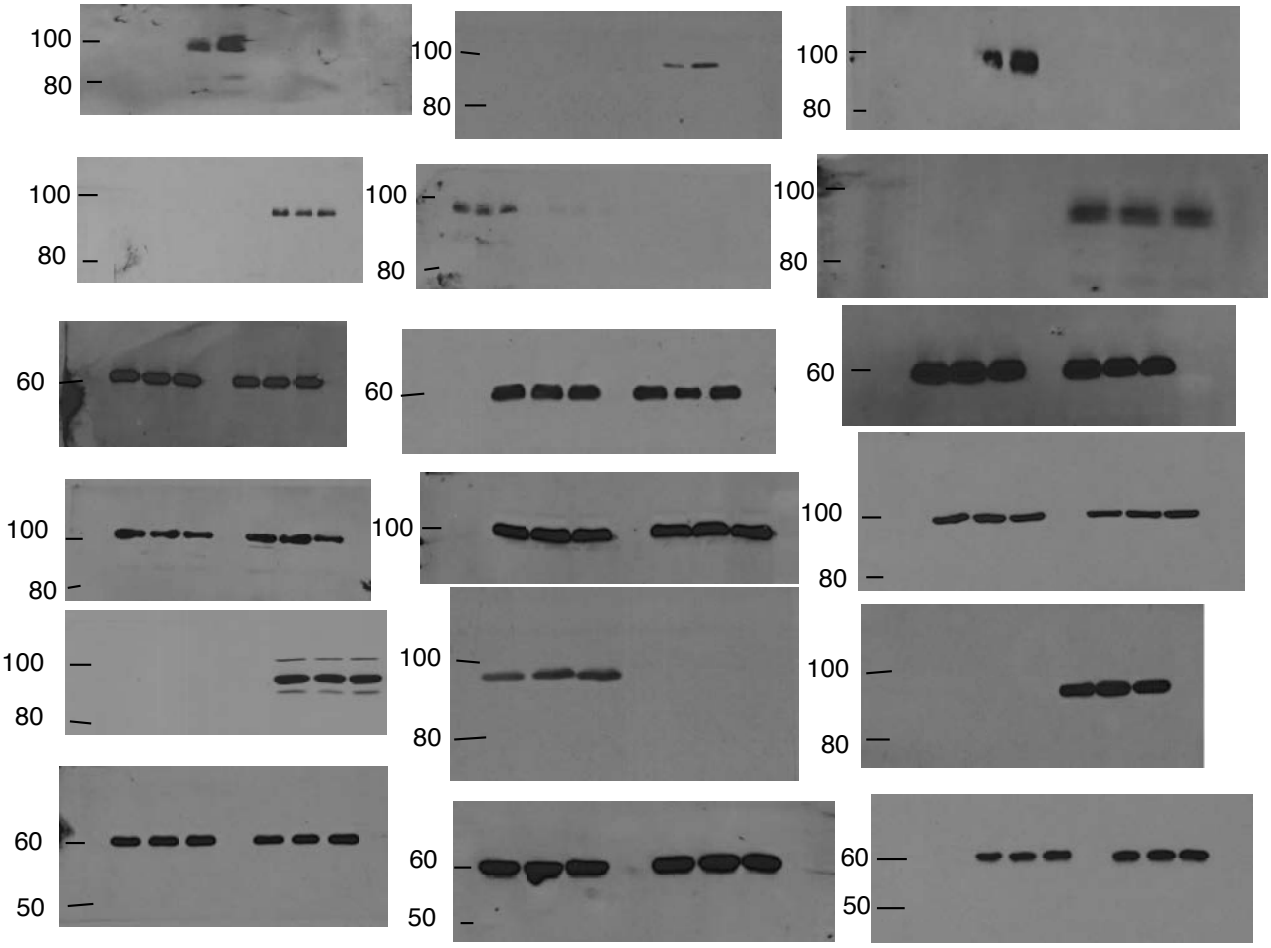
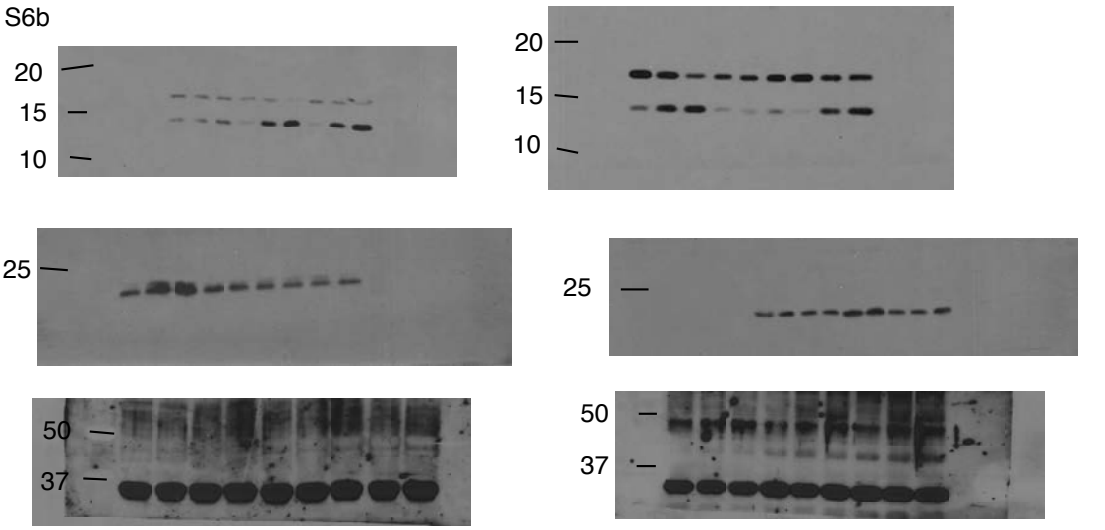


Figure S6b



Supplementary Figure 9 continued

Supplementary Table 1 Statistics source data.