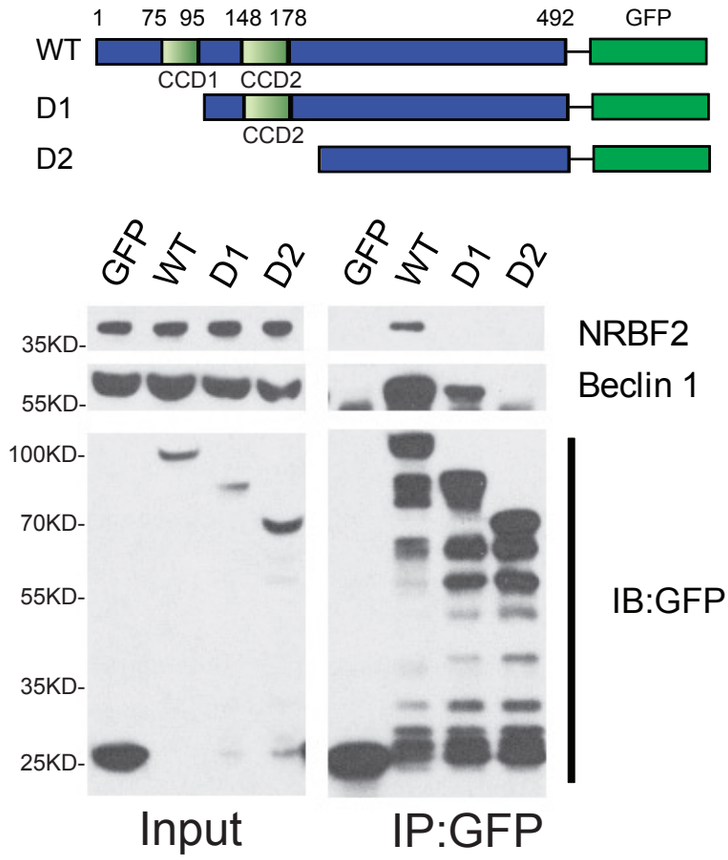
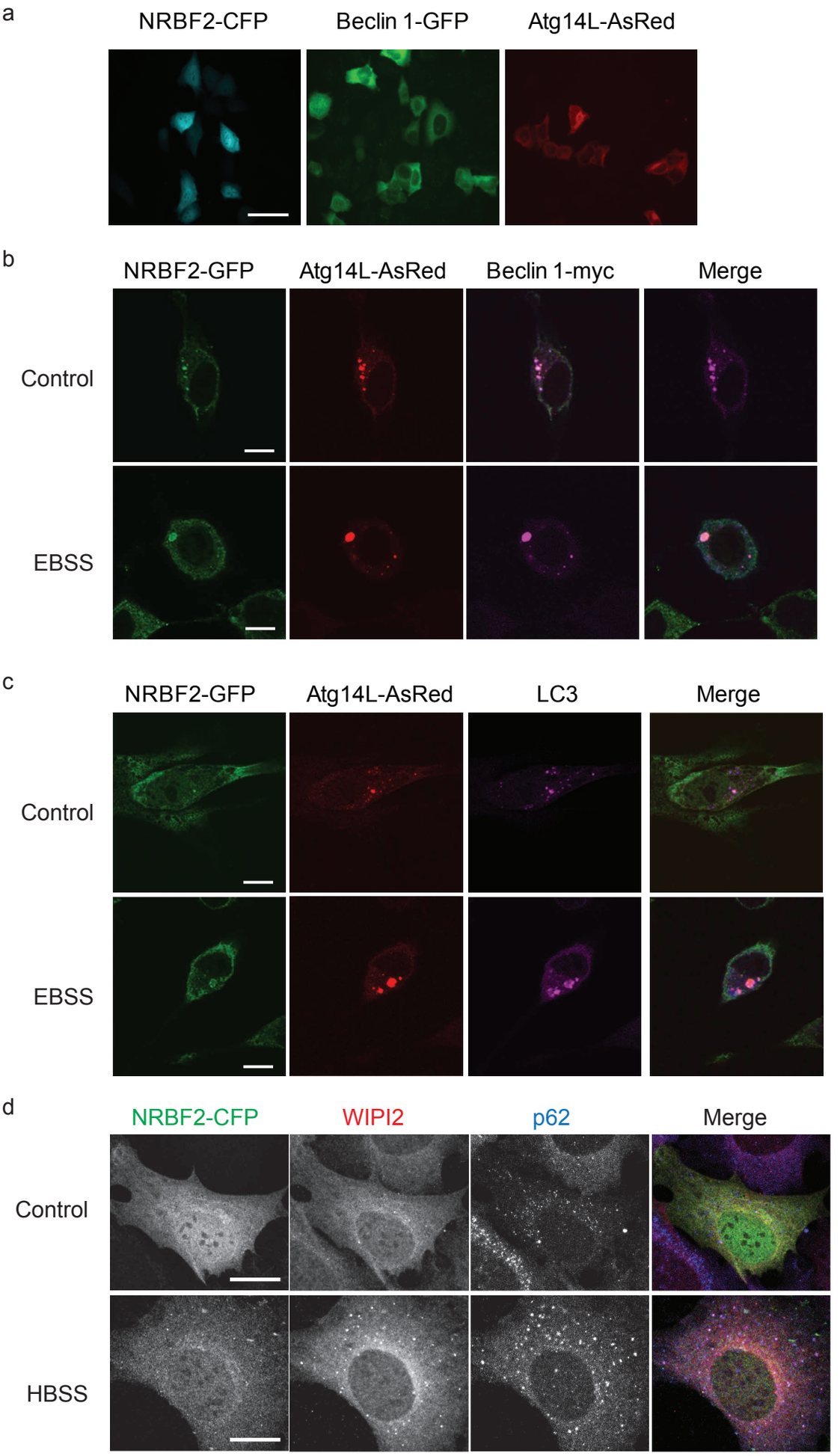


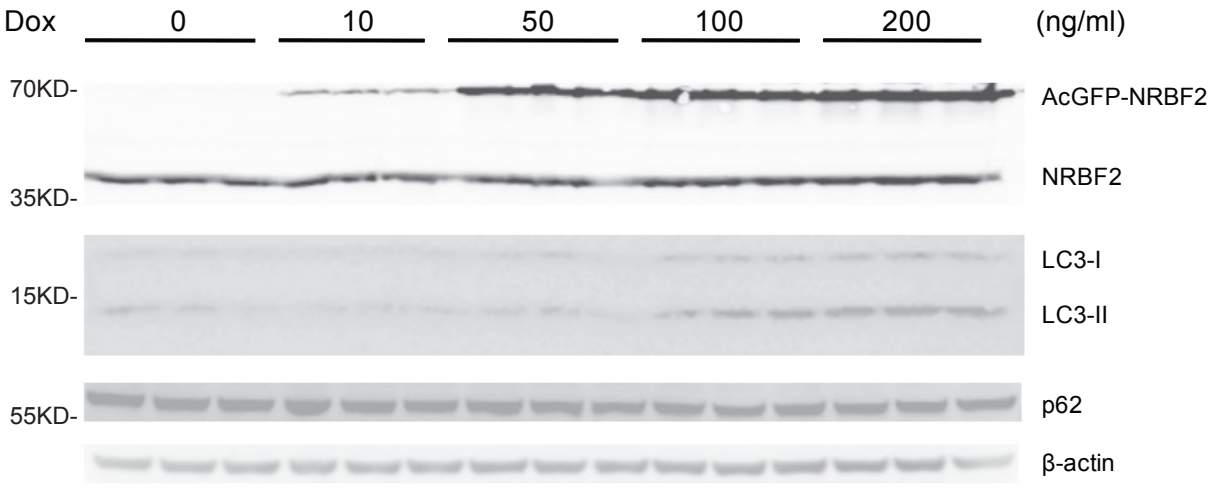
Supplementary Figure 1: Mapping Atg14L domains required for binding to NRBF2

HEK293T cells are transfected with EGFP-tagged Atg14L mutant or WT plasmids. The IP is performed with an anti-GFP antibody, followed by WB with the indicated antibodies. These experiments show that CCD1 domain of Atg14L is associated with NRBF2 interaction.



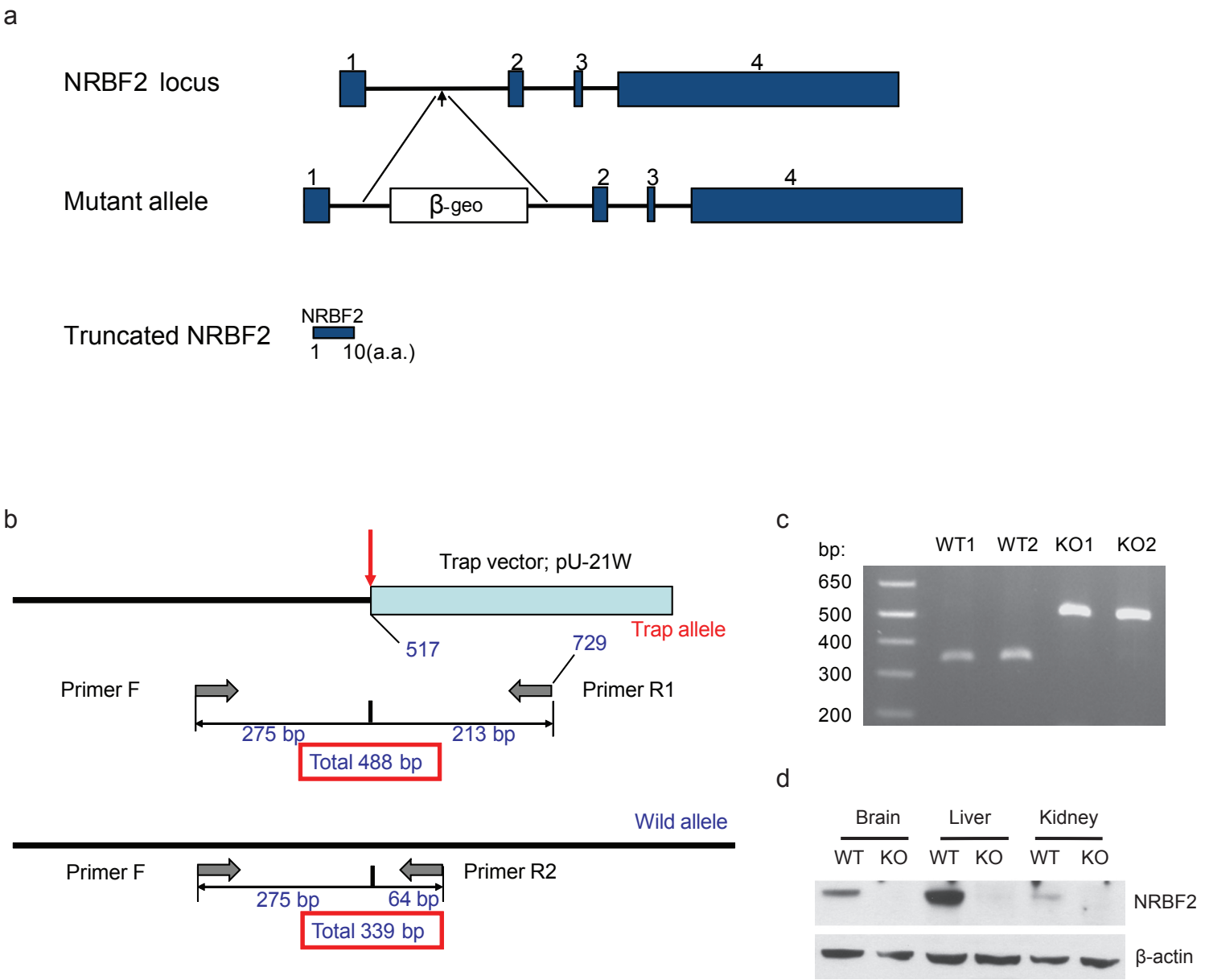
Supplementary Figure 2: NRBF2 form puncta structures and recruit autophagic markers

(a) Beclin 1-EGFP, Atg14L-AsRed, and NRBF2-CFP were transfected into HeLa cells, respectively. NRBF2 is diffused in whole cells when expressed alone. Scale bar =50 μm. (b) NIH3T3 cells stably expressing NRBF2-EGFP were co-transfected with Atg14L-AsRed and Beclin 1-Myc for 24 h, then starved with EBSS for 2 h, and subjected to immunostaining with anti-Myc or LC3 antibodies, respectively. Scale bar =10 μm. (d) NRBF2 KO MEFs were transfected with NRBF2-CFP and starved for 4 h in HBSS. The cells were co-stained with GFP, WIPI2 and p62 antibodies. NRBF2-CFP forms puncta structures under starvation and co-localize with autophagic markers WIPI2 and p62.



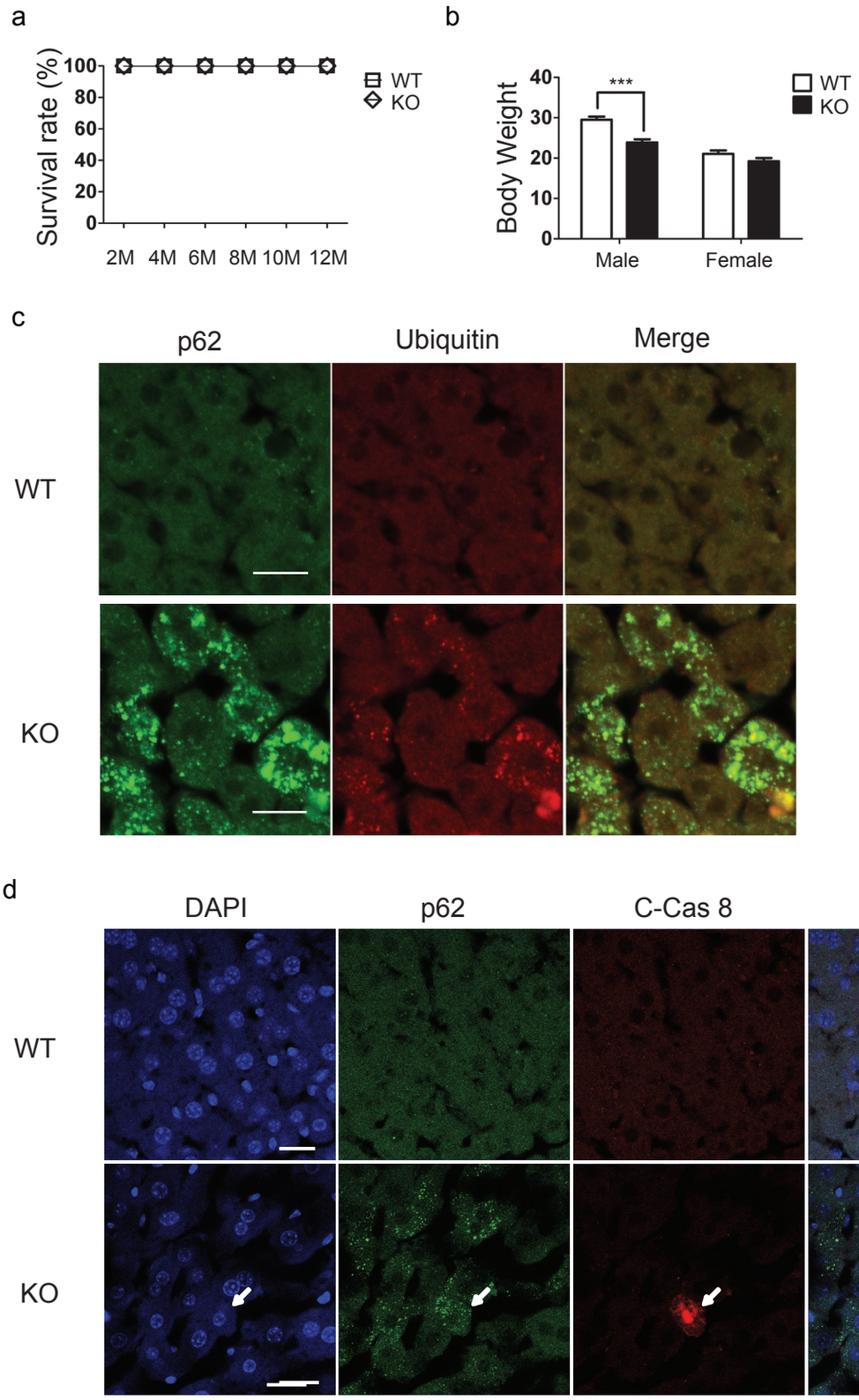
Supplementary Figure 3: Over-expression of NRBF2 up-regulates autophagy

AcGFP-NRBF2 stably expressing HeLa cells are induced with different concentration of doxycycline (Dox) for 24 hours and then subjected to WB analysis. LC3-II level is up-regulated after AcGFP-NRBF2 inducibly expressed.



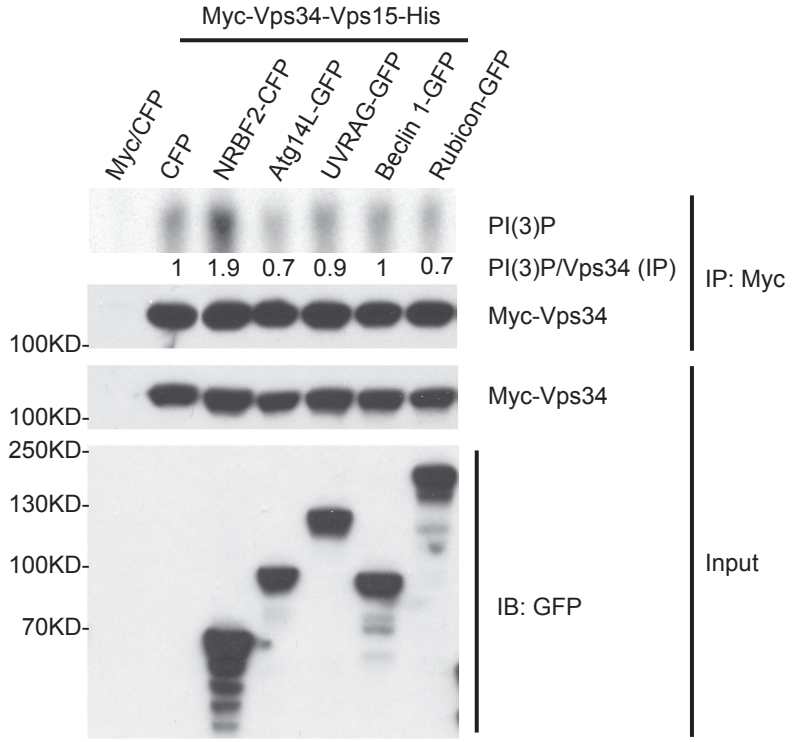
Supplementary Figure 4: NRBF2 knockout strategy

(a) Schematic representation of NRBF2 gene trap strategy. (b) Primers design for genotyping. (c) Validation of NRBF2 gene trap on mouse by genotyping. (d) Validation of NRBF2 protein depletion in the brain, liver and kidney of KO mice.



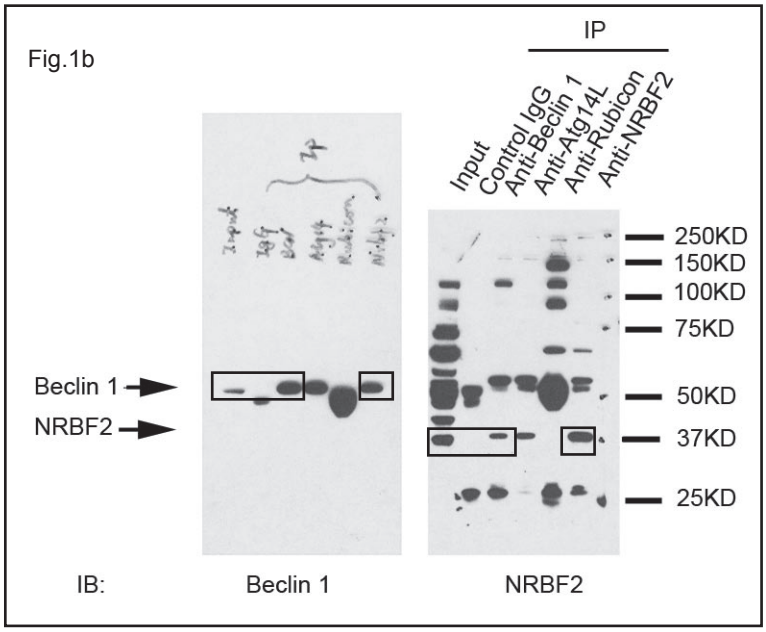
Supplementary Figure 5: Survival, body weight and liver phenotypes of WT and NRBF2 KO mice

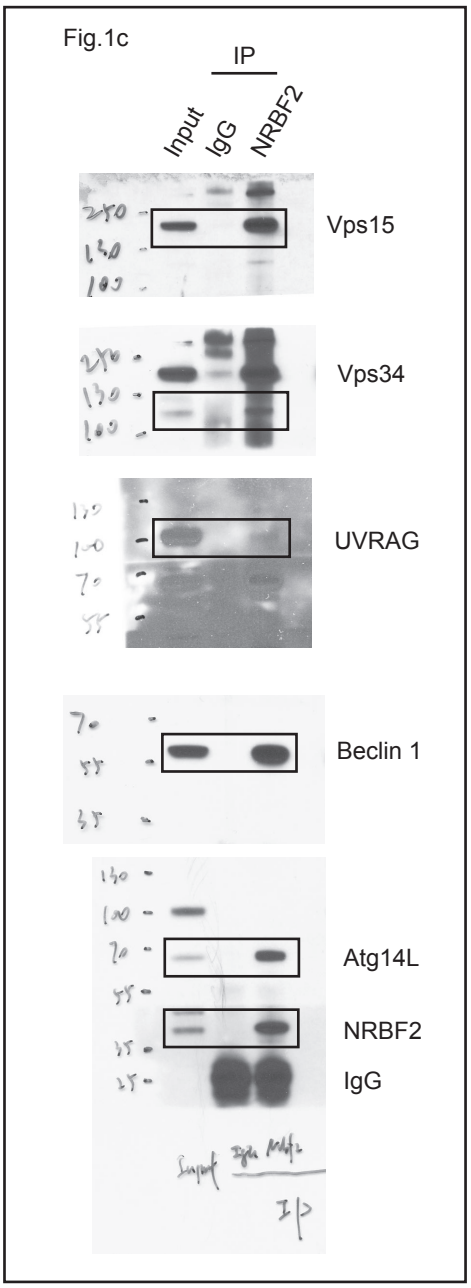
(a) The survival curve of WT and NRBF2 KO mice (for each genotype, n>20). (b) The body weight of adult (4 month old) WT and NRBF2 KO mice (data shown as mean±SEM, P value is indicated on the figure, unpaired Student's t-test. WT, n=15; KO, n=16). (c) Accumulated p62 colocalizes with ubiquitin. Liver frozen sections are double stained with an anti-p62 antibody and an anti-ubiquitin antibody. Scale bar =20 μm. (d) Activation of Capspase-8 (C-Cas 8) in isolated p62 accumulated hepatocyptes. Liver frozen sections are double stained with an anti-p62 antibody and an anti-cleaved Capspase-8 antibody. Scale bar =20 μm.

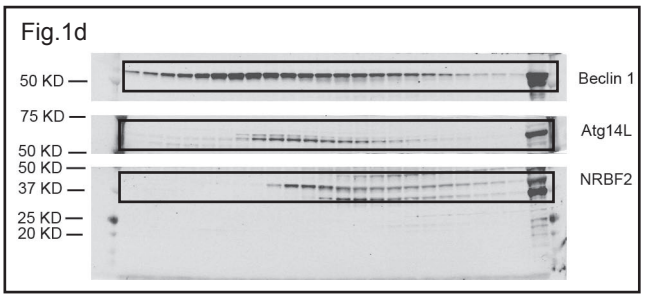


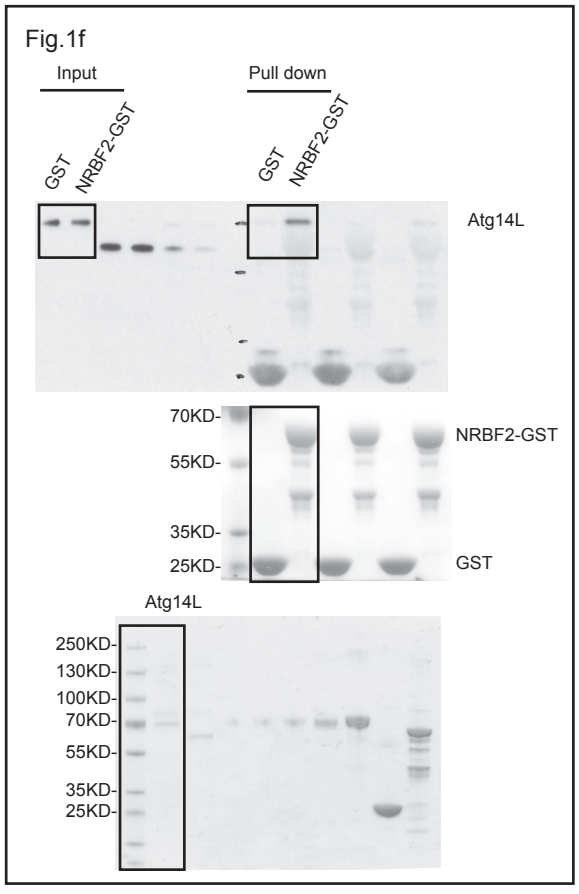
Supplementary Figure 6: Over-expression of NRBF2, but not Atg14L, UVRAG, Beclin 1 or Rubicon, enhances Vps34 activity

HEK293 cells are co-transfected with Myc-Vps3-Vps15-His and CFP, NRBF2-CFP, Atg14L-GFP, UVRAG-GFP, Beclin 1-GFP or Rubicon-GFP. Vps34 is pulled down by an anti-myc antibody and subjected to kinase assay. IP products and inputs are immunoblotted using the antibodies indicated. The numbers indicate quantification of Vps34 kinase activity normalized with immunoprecipitated Vps34.

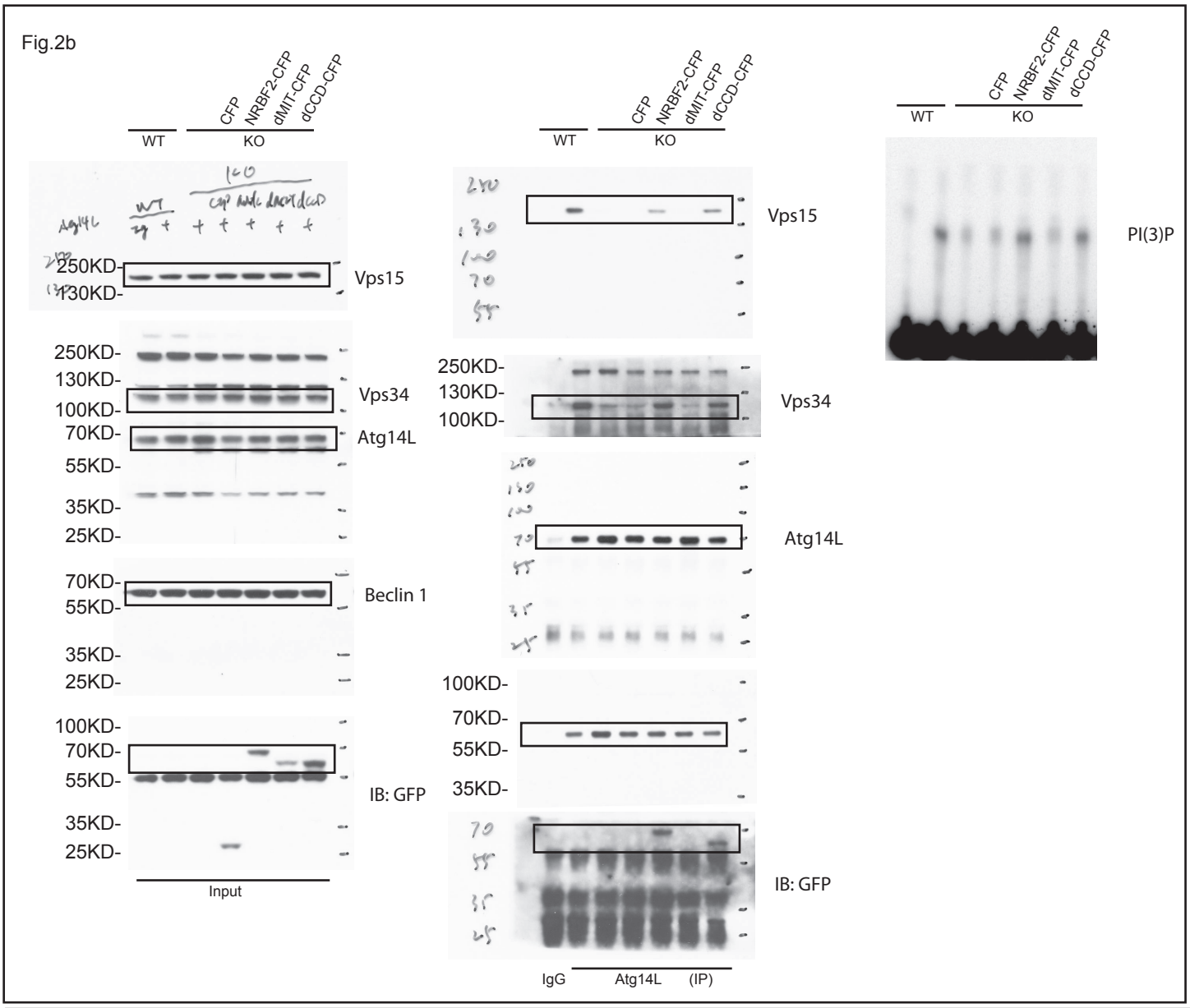


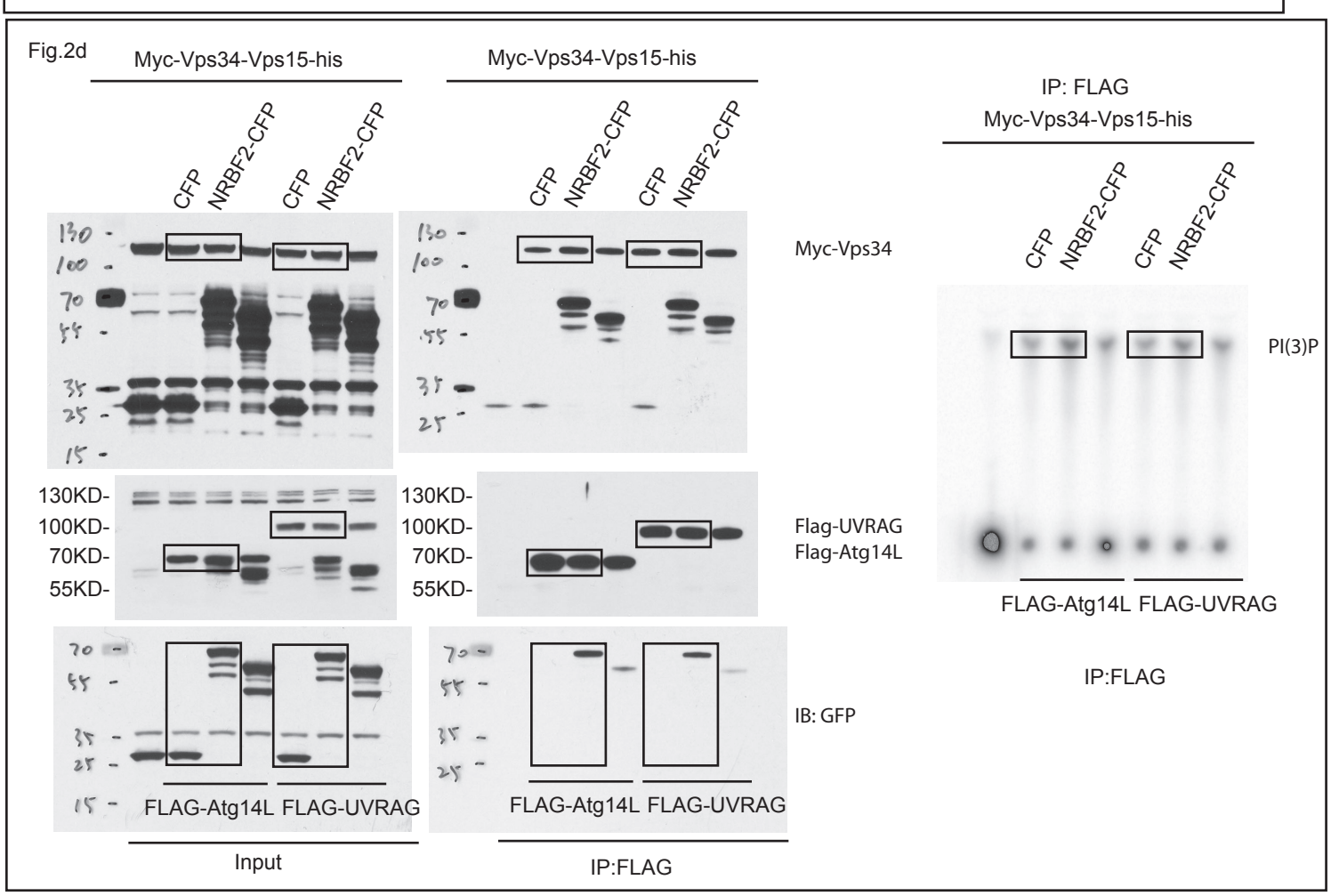




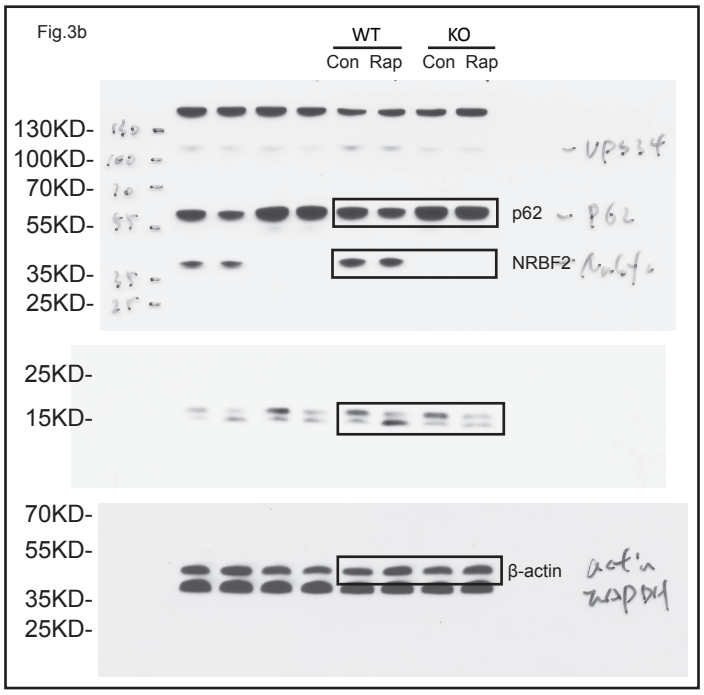


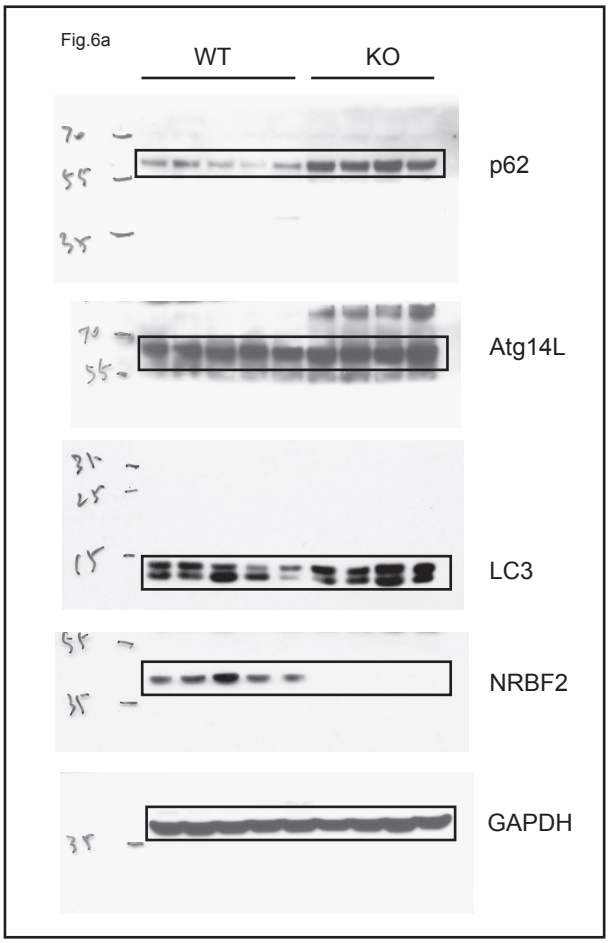
Supplementary Figure 7: full scans of blots in figure 1. To be continued...

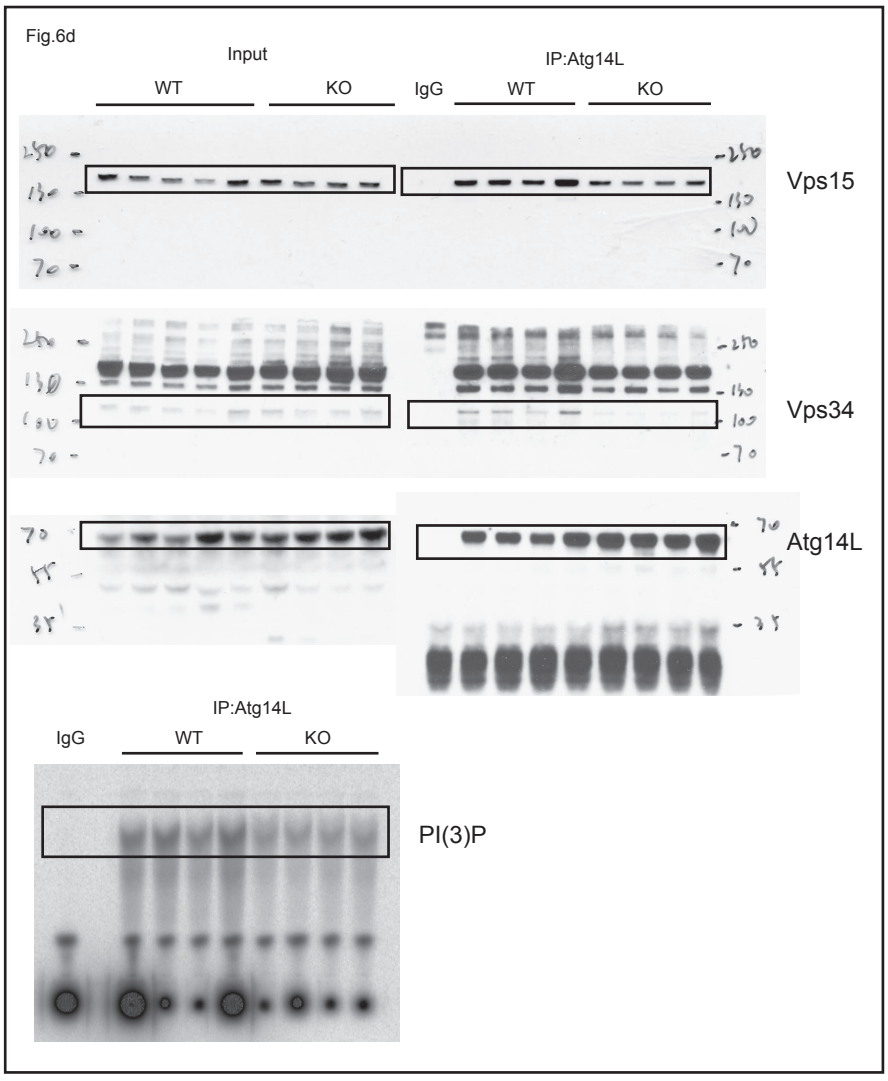




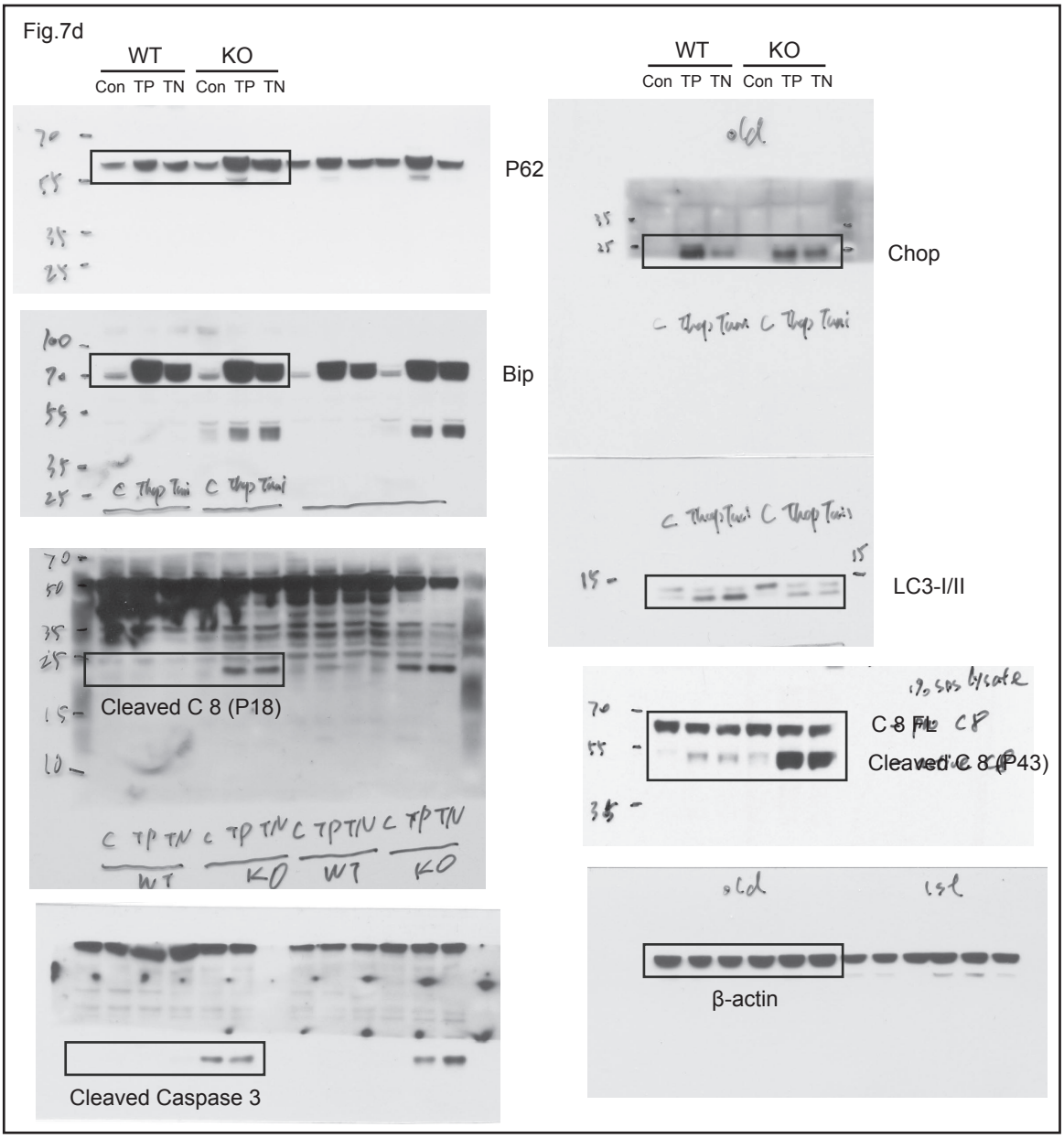
Supplementary Figure 7: full scans of blots in figure 2. To be continued...



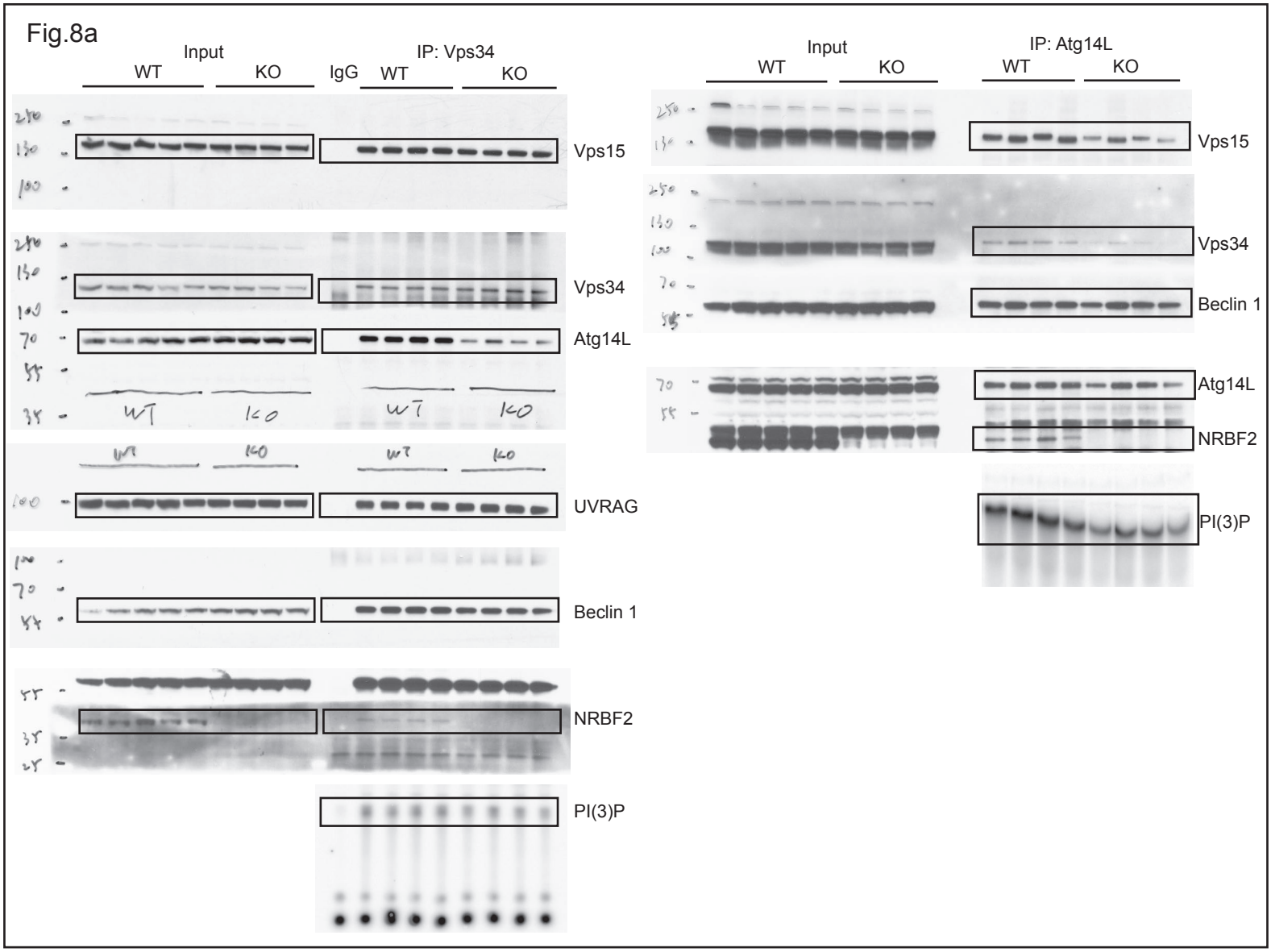


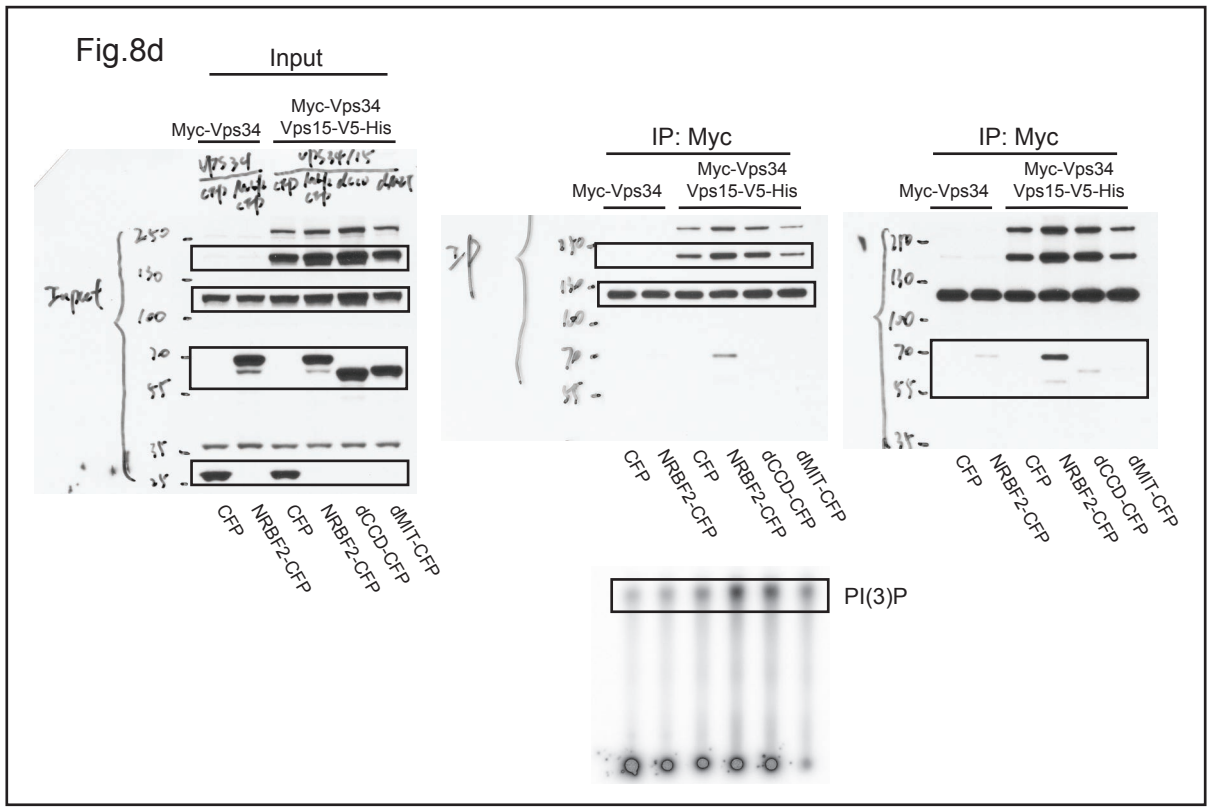


Supplementary Figure 7: full scans of blots in figure 3 and figure 6. To be continued...



Supplementary Figure 7: full scans of blots in figure 7. To be continued...





Supplementary Figure 7: full scans of blots in figure 8