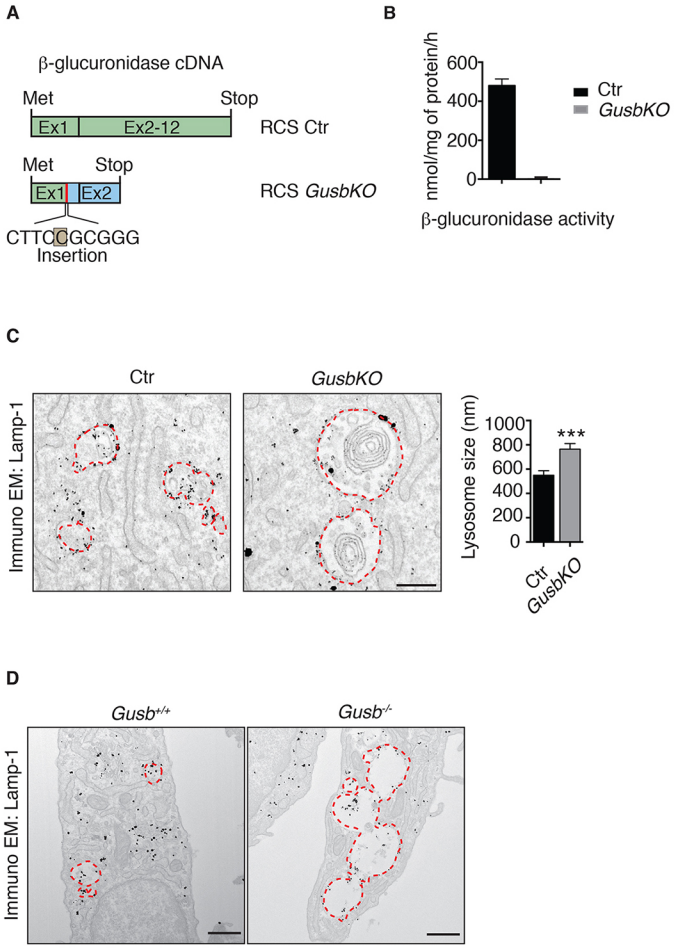
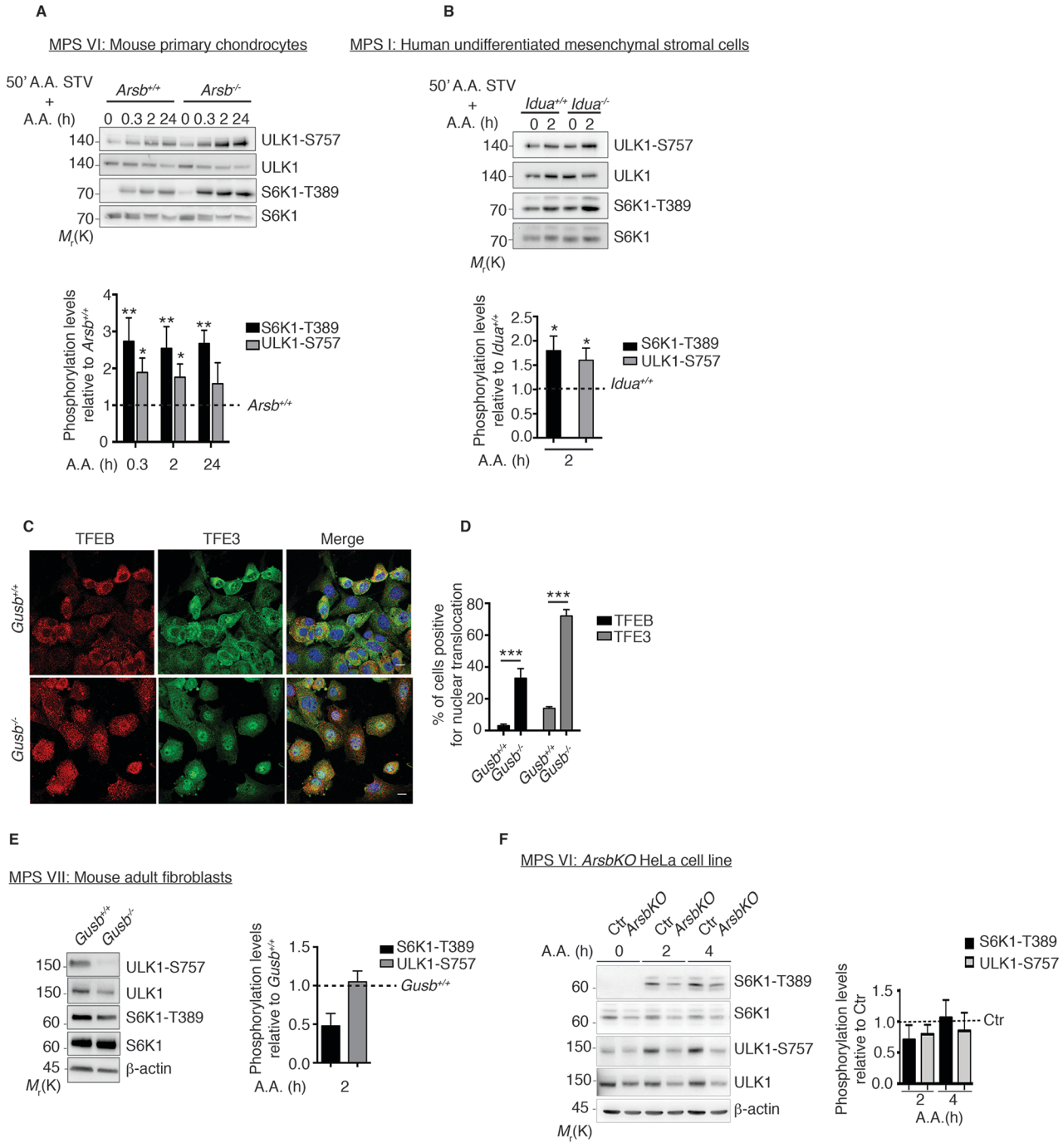
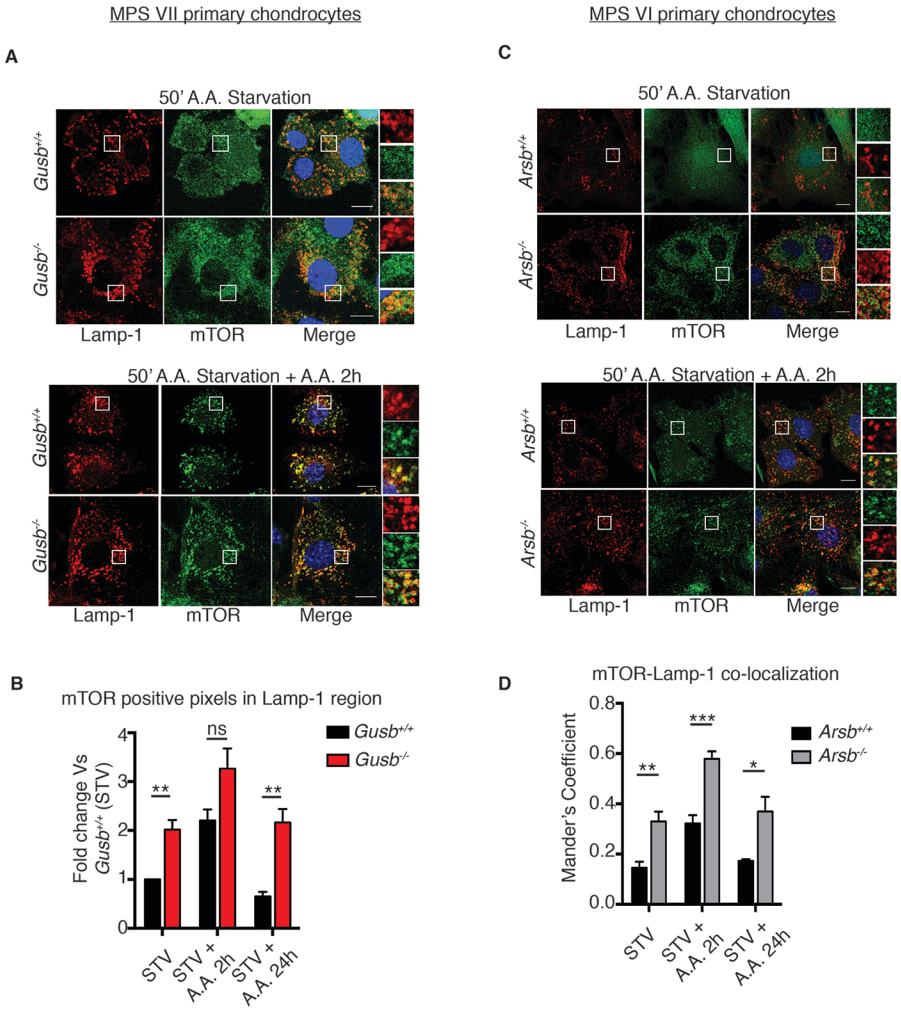


Supplemental Figure 1. Generation and characterization of *GusbKO* RCS clone. (**A**) Schematic representation of genetic mutation found in the *GusbKO* clone: a single base insertion within the first exon causes a frameshift and a premature stop codon within the second exon of the protein. (**B**) Bar graph shows no detectable β-glucuronidase enzymatic activity in *GusbKO* cells. (**C**) Representative images of Lamp-1 Immuno-Electron microscopy (EM) from control (Ctr) and *GusbKO* RCS cells. Scale bar, 500nm. Bar graph displays the lysosome size. N = 20. *** $p \leq 0.0005$; unpaired Student's t-test. (**D**) Representative images of Lamp-1 Immuno-EM from *Gusb*$^{+/+}$ and *Gusb*$^{-/-}$ primary chondrocytes. (**C** and **D**) Red dashed-line highlights lysosome membranes.

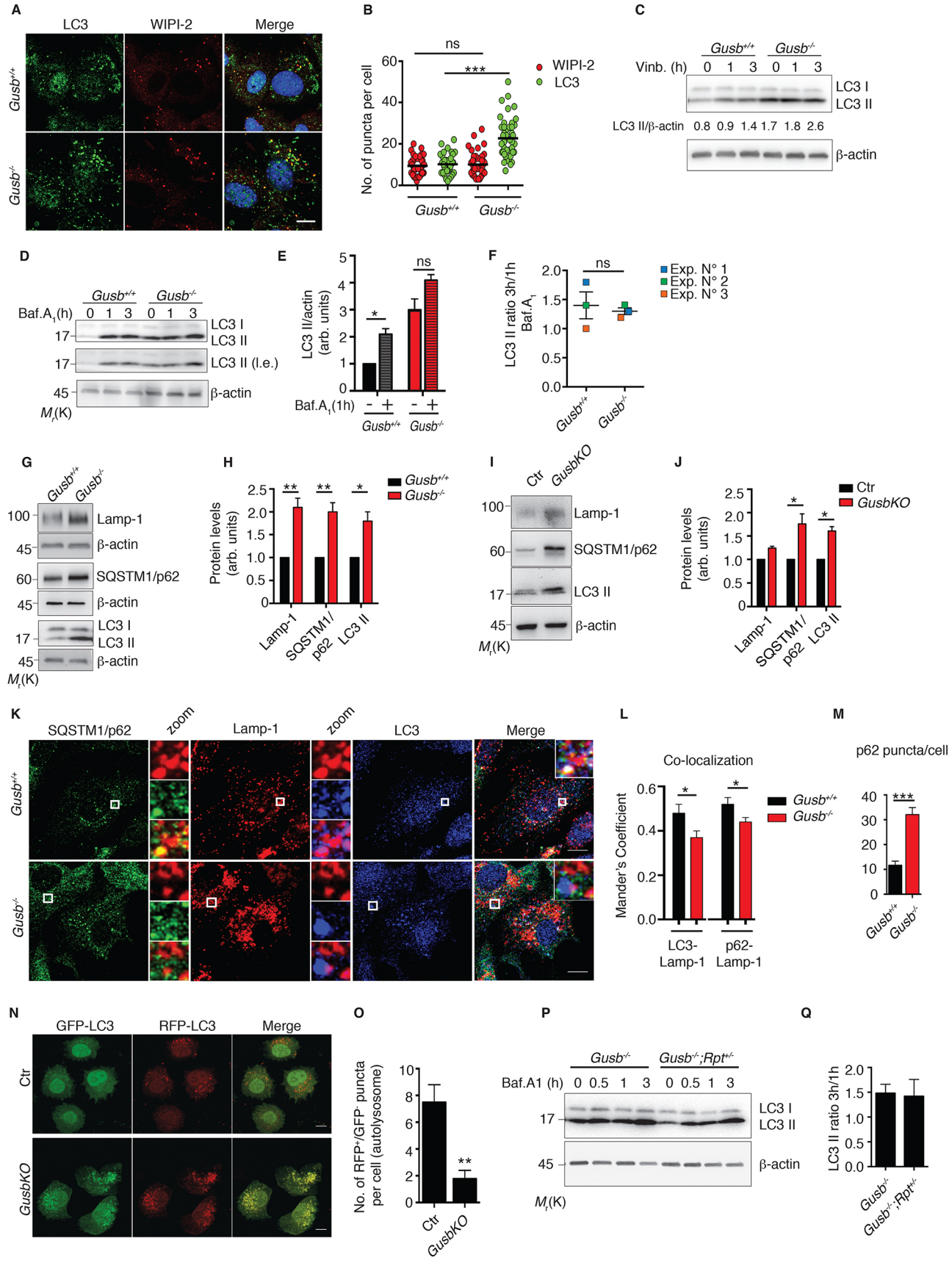


Supplemental Figure 2. Enhanced mTORC1 signaling in MPS chondrocytes. (**A** and **B**) Western blot analysis of mTORC1 signaling upon a time course of amino acid stimulation in (**A**) *Arsb*$^{-/-}$ primary chondrocytes and (**B**) *Idua*$^{-/-}$ undifferentiated human mesenchymal stromal cells. Bar graphs display phosphorylation levels of the indicated mTORC1 substrates (normalized to their total levels). Values are mean + SEM and are expressed as fold increase compared to their corresponding control (represented by dashed line). N = 3 independent experiments for each cell line. (**C**) TFEB/TFE3 nuclear localization in *Gusb*$^{-/-}$ chondrocytes. Immunofluorescence analysis of TFEB and TFE3 nuclear localization in primary chondrocytes. Cells were co-stained with DAPI to define nuclear region. Scale bar, 10µm. (**D**) Bar graph displays quantification of the percentage of cells with nuclear TFEB/TFE3. The data are representative of 3 independent experiments. Values are mean + SEM. N = 90 cells were analyzed. (**E** and **F**) Western blot analysis of mTORC1 signaling after 2h of amino acid stimulation in (**E**) *Gusb*$^{-/-}$ primary mouse fibroblasts and (**F**) *ArsbKO* HeLa cells (2h and 4h). Bar graphs display phosphorylation levels of the indicated mTORC1 substrates (normalized to their total levels). Values are mean + SEM and are expressed as fold increase compared to their corresponding control (represented by dashed line). N = 3 independent experiments for each cell line. *p ≤ 0.05, **p ≤ 0.005; ***p ≤ 0.0005; Student's t-test.

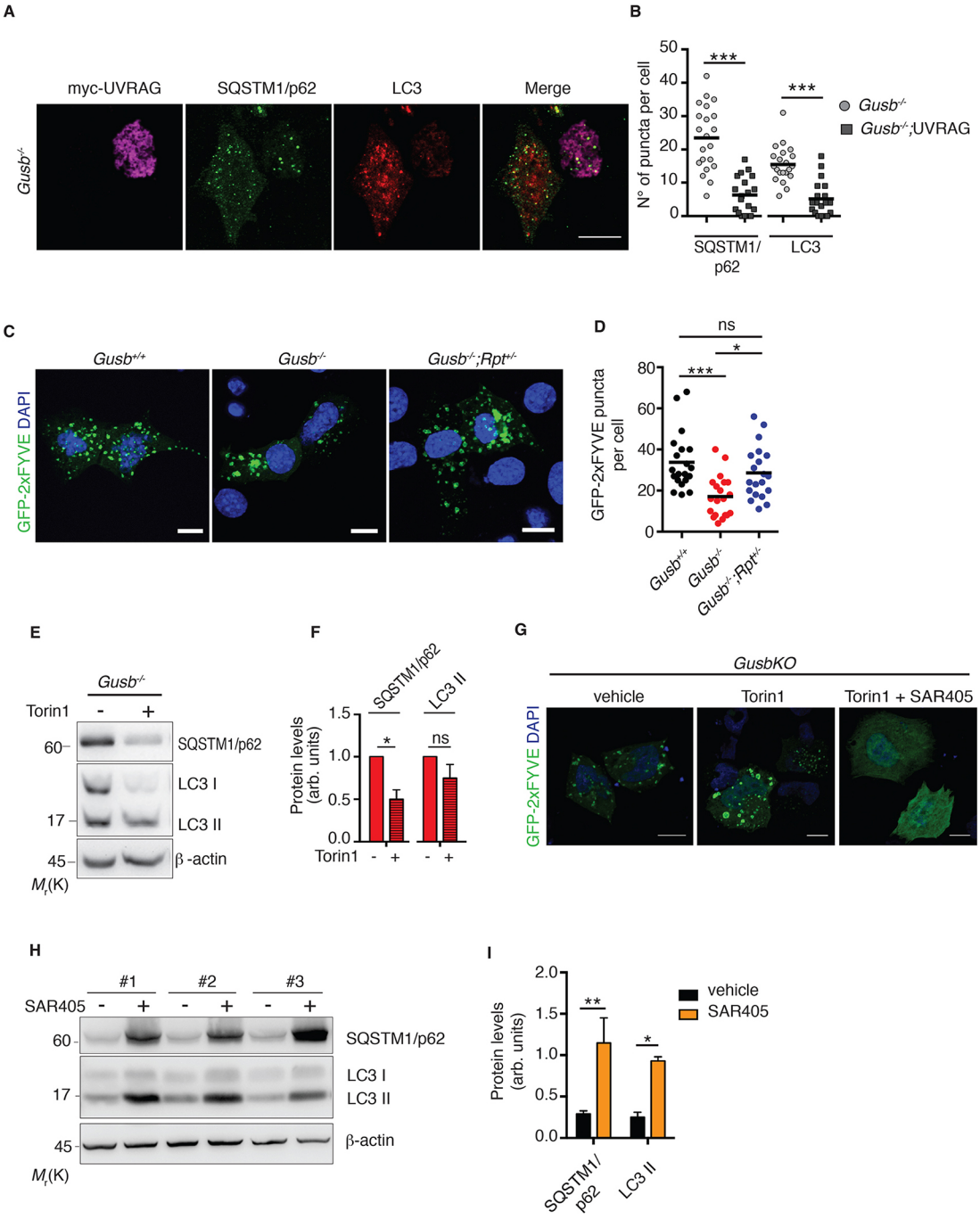


Supplemental Figure 3. Enhanced mTOR association to lysosomes in LSD chondrocytes. *Gusb*$^{-/-}$ (**A** and **B**) and *Arsb*$^{-/-}$ (**C** and **D**) primary chondrocytes were starved for amino acids for 50 min and then stimulated with amino acids for the indicated times. Cells were then processed in an immunofluorescence assay to detect mTOR, Lamp-1, co-stained with DAPI for DNA content, and imaged. The insets show higher magnification and single color channels of the boxed area. Scale bar, 10µm. Representative images of 24h time point are shown in **Figure 1G-H**. Bar graphs (**B** and **D**) display quantitative analysis of mTOR-Lamp-1 co-localization, data are expressed as mean + SEM. N = 3 independent experiments. N = 50 cells were analyzed for each time point. *p ≤ 0.05, **p ≤ 0.005, ***p ≤ 0.0005; unpaired Student's t-test.

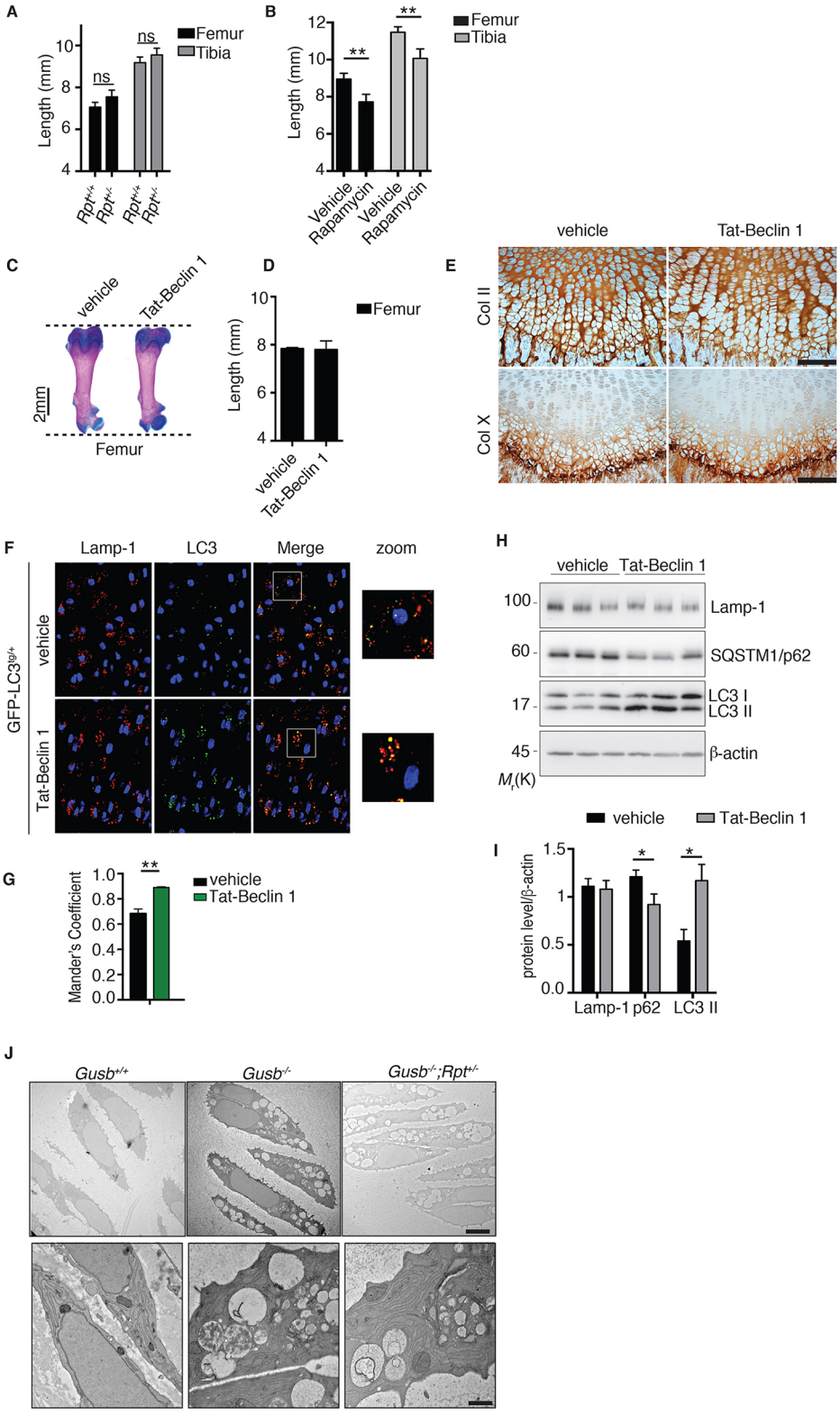
Supplemental Figure 4



Supplemental Figure 4. Autophagy dysfunction in LSD chondrocytes. (**A**) Immunofluorescence of WIPI-2 and LC3 in *Gusb*$^{+/+}$ and *Gusb*$^{-/-}$ primary chondrocytes. (**B**) Bar graph displaying quantization of WIPI-2 and LC3 puncta/cell. N = 3; n = 40 cells were analyzed. (**C**) Western blot analysis of LC3-I/II in presence of Vinblastine (Vinb.; 200nm). The rate of AVs biogenesis was calculated measuring the ratio of LC3-II levels between 3h and 1h of Vinb. treatment, as this eliminates the differences in basal levels (time zero). Numbers show raw values of LC3 II normalized to β-actin. (**D**) Western blot analysis of LC3-I/II in presence of Baf.A_1 (200nm) for the indicated time points (l.e. = low exposure). (**E**) Bar graph displaying LC3II accumulation in presence of Baf.A1 for 1h. Data show LC3-II levels normalized to β-actin and expressed as fold increase of *Gusb*$^{+/+}$-vehicle. N = 3. (**F**) The rate of AVs biogenesis was calculated as in **C**. Dot plot graph displays the ratios obtained from three independent experiments. (**G - J**) Western blot analysis and relative protein quantification of the indicated proteins in primary cultured chondrocytes (**G** and **H**) and RCS cells (**I** and **J**) with the indicated genotypes. β-Actin is a loading control. N = 3. (**K**) Immunofluorescence of Lamp-1, SQSTM1/p62 and LC3 in primary chondrocytes isolated from mice with the indicated genotypes. The insets show higher magnification and single color channels of the boxed area. Scale bar, 10μm. (**L**) Bar graph displays quantification of Lamp-1 co-localization with LC3 and p62. N = 3; n = 35 cells were analyzed. (**M**) Bar graph displays quantification of SQSTM1/p62 puncta per cell. N = 3; n = 35 cells. (**N**) Measure of the rate of delivery of autophagosomes to lysosomes using an RFP-GFP-LC3 protein in Ctr and *GusbKO* cells. Scale bar, 10μm. (**O**) Bar graph displays quantification of RFP-only puncta per cell. N = 3; n = 20 cells analyzed. (**P**) Western blot analysis of LC3-II in presence of Baf.A_1 (200nm) for the indicated time points in primary chondrocytes isolated from *Gusb*$^{-/-}$ and *Gusb*$^{-/-}$;*Rpt*$^{+/-}$ mice. (**Q**) Bar graph displays the rate of AVs biogenesis. N = 3. The data shown are mean values derived from the indicated number of independent experiments. Error bars indicate SEM. P values were calculated by unpaired Student's t-test. *p ≤ 0.05, **p ≤ 0.005, ***p ≤ 0.0005, ns: not significant.



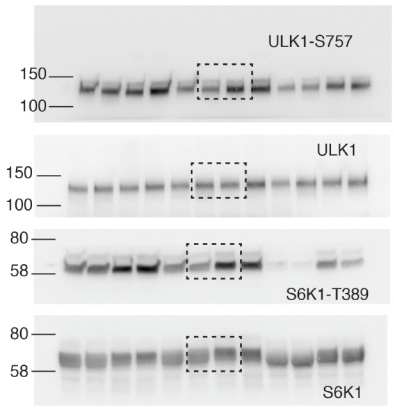
Supplemental Figure 5. mTORC1 inhibits autophagy via Beclin 1-Vps34-UVRAG kinase complex. (**A**) Myc-UVRAG was transiently expressed in *Gusb*$^{-/-}$ primary chondrocytes. Myc expression, LC3 and SQSTM1/p62 were monitored by fluorescence microscope two days post-transfection. Scale bar, 10µm. (**B**) Bar graph displaying quantitative analysis of SQSTM1/p62 and LC3 puncta per cell. Mean value is shown as a horizontal bar. N = 2; n = 20 cells were analyzed. (**C**) GFP-2xFYVE plasmid was transiently transfected in primary chondrocytes isolated from mice with indicated genotypes. GFP fluorescence was monitored by fluorescence microscope 16h post-transfection. Scale bar, 10µm. (**D**) Graph shows quantitative analysis of GFP puncta per cell. N = 25. (**E**) Western blot analysis of P-ULK1, SQSTM1/p62 and LC3 in *Gusb*$^{-/-}$ primary chondrocytes treated with Torin1 (1µM; 6h) or vehicle (DMSO). β-Actin was used as a loading control. (**F**) Bar graph displaying the levels of SQSTM1/p62 and LC3 II after Torin1 treatment. Data are means of protein level (normalized to β-actin) and expressed as fold increase relative to untreated *Gusb*$^{-/-}$. N = 4. (**G**) Representative images of *GusbKO* transfected with GFP-2xFYVE and treated with Torin1 (1µM; 6h) or Torin1 in combination with SAR405 (10µM; 6h). (**H**) Western blot analysis of SQSTM1/p62 and LC3 in RCS cells treated with SAR405 (10 µM, 24h). Blot shows three independent replicates. β-Actin is a loading control. (**I**) Bar graphs displaying proteins levels normalized to β-Actin. The data shown are mean values derived from the indicated number of independent experiments. Error bars indicate SEM. P values in **D** were calculated by ANOVA followed by Tukey's post-hoc test. P values in **B**, **F** and **I** were calculated by paired Student's t-test. *p ≤ 0.05, **p ≤ 0.005, ***p ≤ 0.0005, ns: not significant.



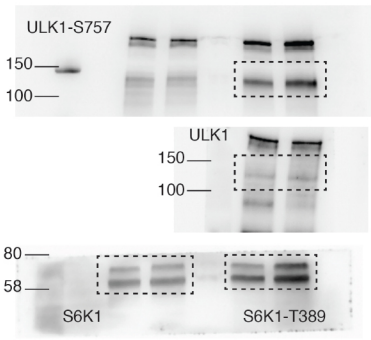
Supplemental Figure 6. Tat-Beclin 1 treatment enhances AV-lysosome fusion in chondrocytes. (**A)** Bar graph shows femur and tibia length of mice with the indicated genotypes at post-natal day 15. N = 9 (*Rpt*$^{+/+}$) and 4 (*Rpt*$^{+/-}$). (**B**) Bar graph shows femur and tibia length of mice injected with rapamycin (0.375 mg/Kg daily for 15 days) or vehicle. N = 4 mice/group. (**C**) Representative images of alcian blue/alizared red staining of femurs from wild-type mice injected with vehicle (PBS) or Tat–Beclin 1 peptide (2 mg/kg, daily for 15 days). (**D**) Bar graph shows quantification of bone length. N = 4. (**E**) Femoral growth plate sections from vehicle or Tat-Beclin 1 treated wild-type mice at P6 (2 mg/kg daily for 6 days) immunostained with Col II and Col X. Nuclei were counterstained with hematoxylin. N = 2 mice/group. Scale bar, 100μm. (**F**) Representative images of GFP–LC3 puncta (autophagosomes) and Lamp-1 (lysosomes) immunostaining in femoral growth plates from GFP–LC3$^{tg/+}$ mice at P6. Tat–Beclin 1 peptide was administered where indicated (2 mg/kg, daily for 6 days). The insets show a higher magnification of co-localization in selected areas. Scale bar, 10μm. (**G**) Bar graph displays quantification of Lamp-1-LC3 co-localization. N = 3 mice/group. (**H**) Western blot analysis of Lamp-1, SQSTM1/p62 and LC3 in femoral growth plates homogenates from wild-type mice. Tat–Beclin 1 peptide was administered where indicated (2 mg/kg, daily for 6 days). (**I**) Bar graph displays amount of proteins relative to β-Actin. N = 3. (**J**) Electron microscopy analysis of growth plates isolated from P6 *Gusb*$^{+/+}$, *Gusb*$^{-/-}$ and *Gusb*$^{-/-}$;*Rpt*$^{+/-}$ mice. Scale bar is 5μm for the upper panels and 1μm for the lower panels. The data shown are mean values derived from the indicated number of independent experiments/mice. Error bars indicate SEM. p values were calculated by unpaired Student's t-test. *p ≤ 0.05, **p ≤ 0.005, ns: not significant.

Bartolomeo et al. 2017 Full unedited gels for Main Figures

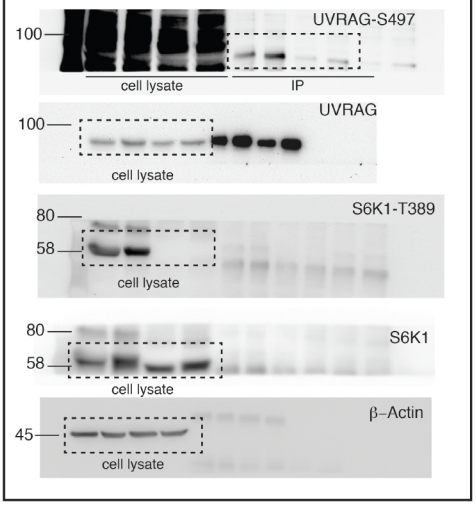
Full unedited gel for Figure 1A



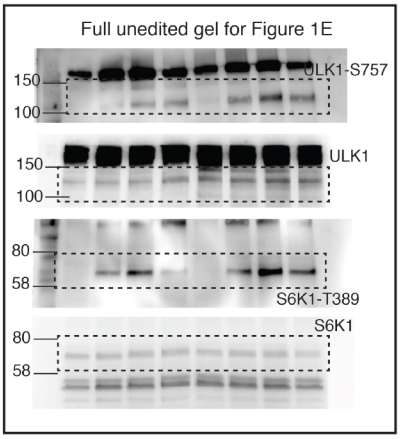
Full unedited gel for Figure 1B



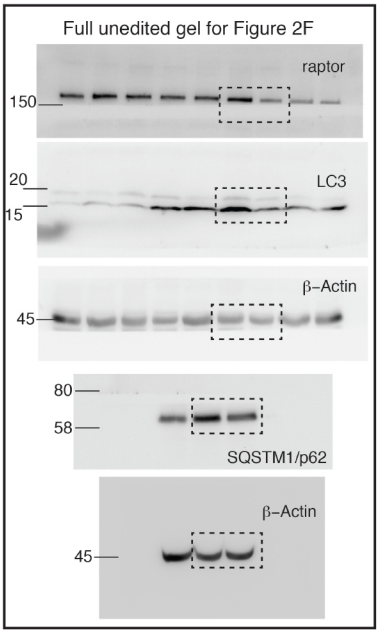
Full unedited gel for Figure 1D



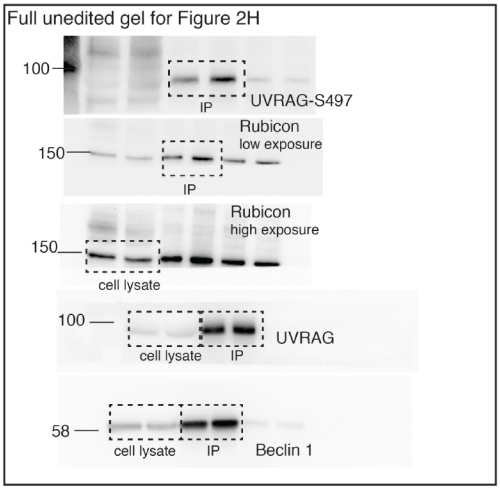
Full unedited gel for Figure 1E



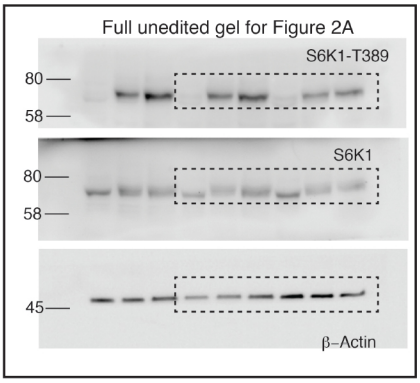
Full unedited gel for Figure 2F



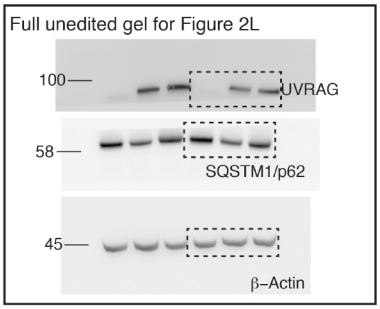
Full unedited gel for Figure 2H



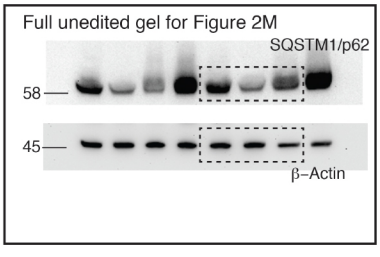
Full unedited gel for Figure 2A



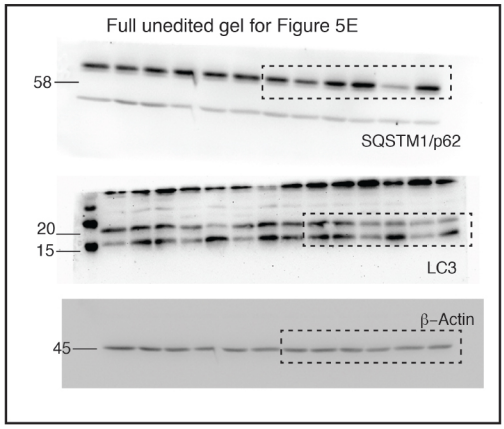
Full unedited gel for Figure 2L



Full unedited gel for Figure 2M

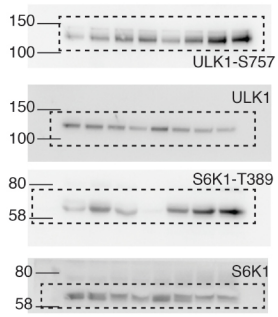


Full unedited gel for Figure 5E

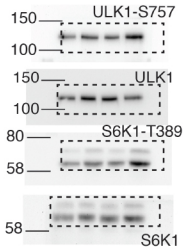


Bartolomeo et al. 2017
Full unedited gels for
Supplemental Figures

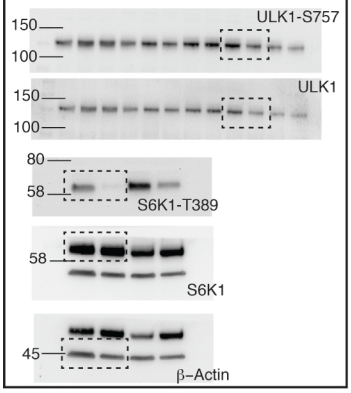
Full unedited gel for Figure 2A



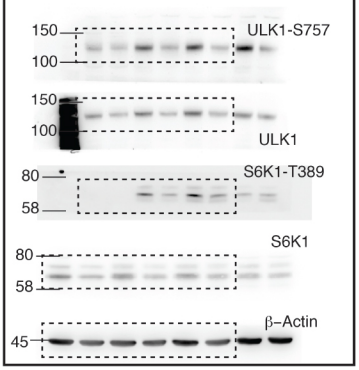
Full unedited gel for Figure 2B



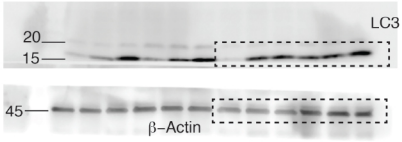
Full unedited gel for Figure 2E



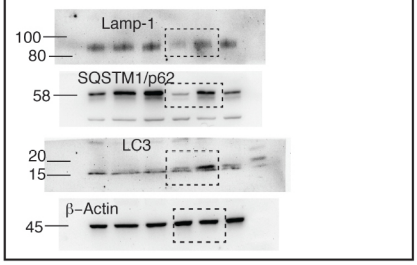
Full unedited gel for Figure 2F



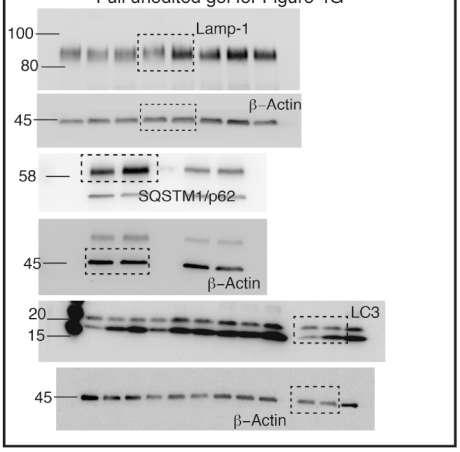
Full unedited gel for Figure 4D



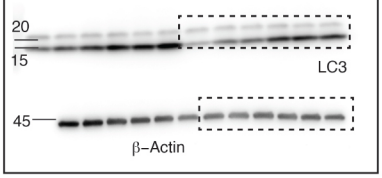
Full unedited gel for Figure 4I



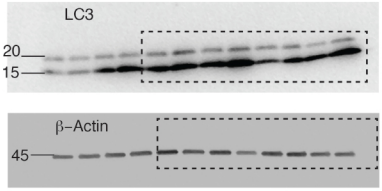
Full unedited gel for Figure 4G



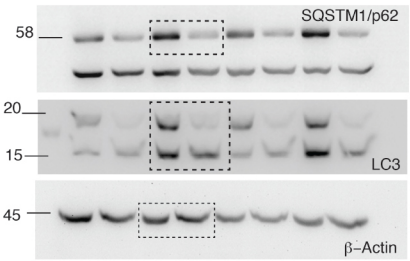
Full unedited gel for Figure 4C



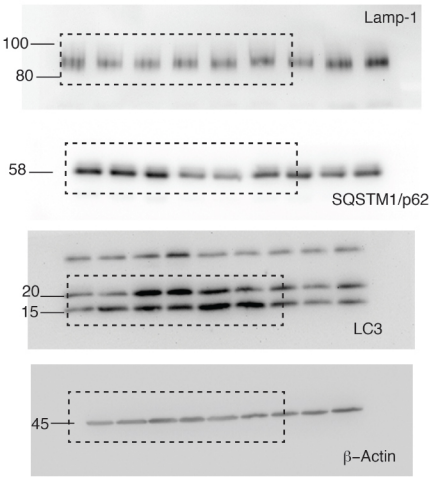
Full unedited gel for Figure 4P



Full unedited gel for Figure 5E



Full unedited gel for Figure 6H



Full unedited gel for Figure 5H