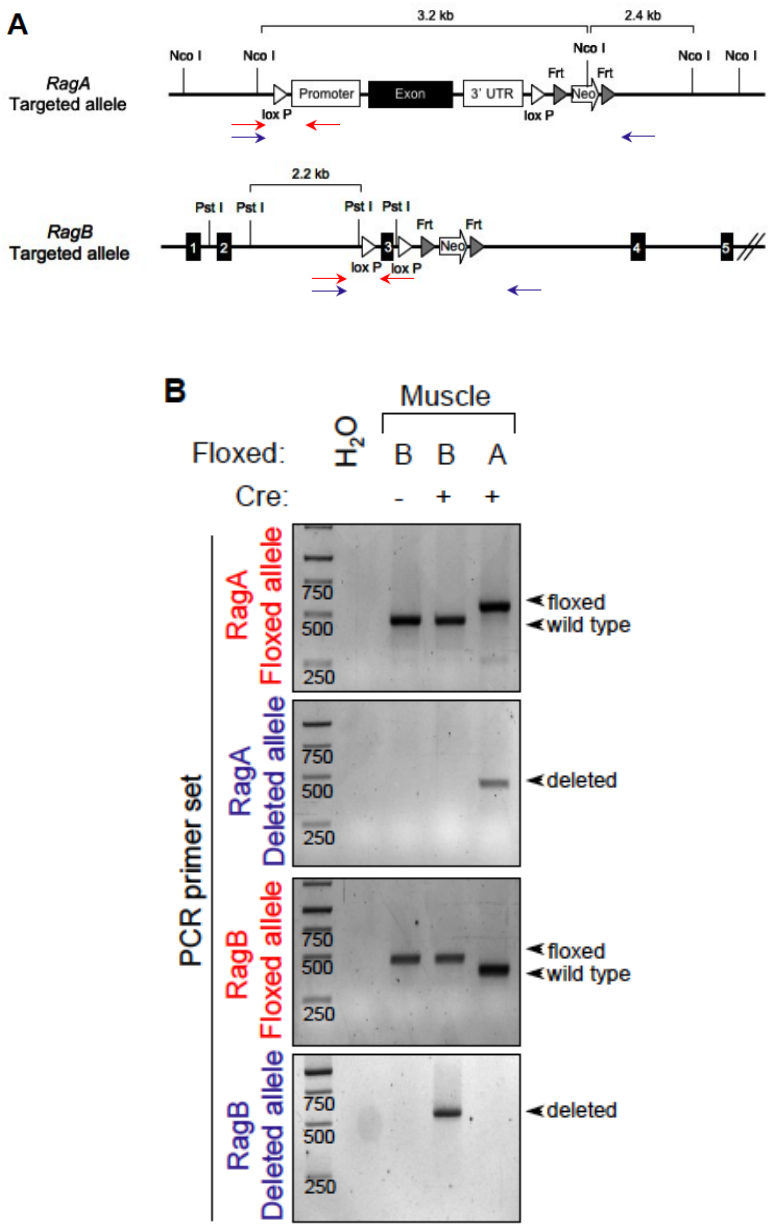
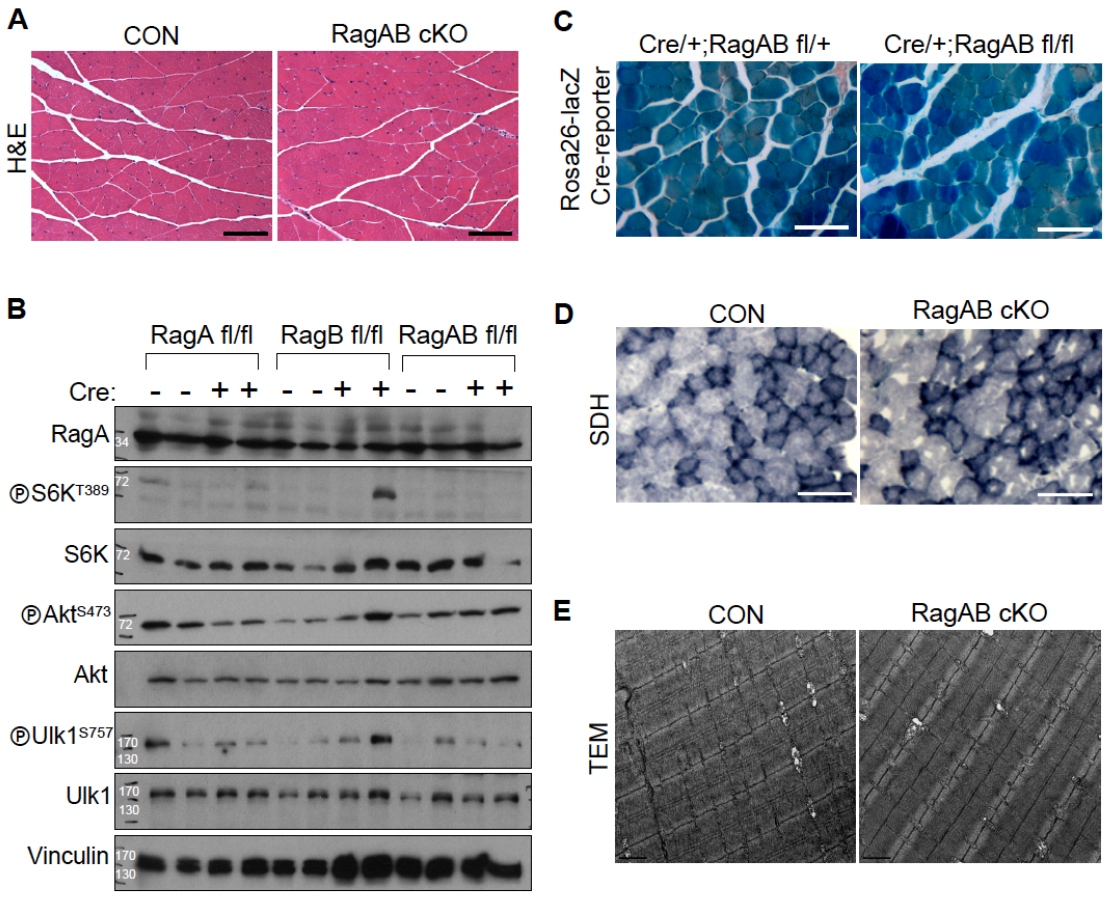
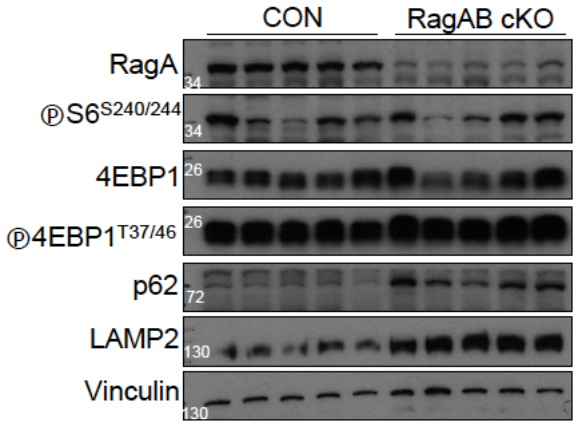


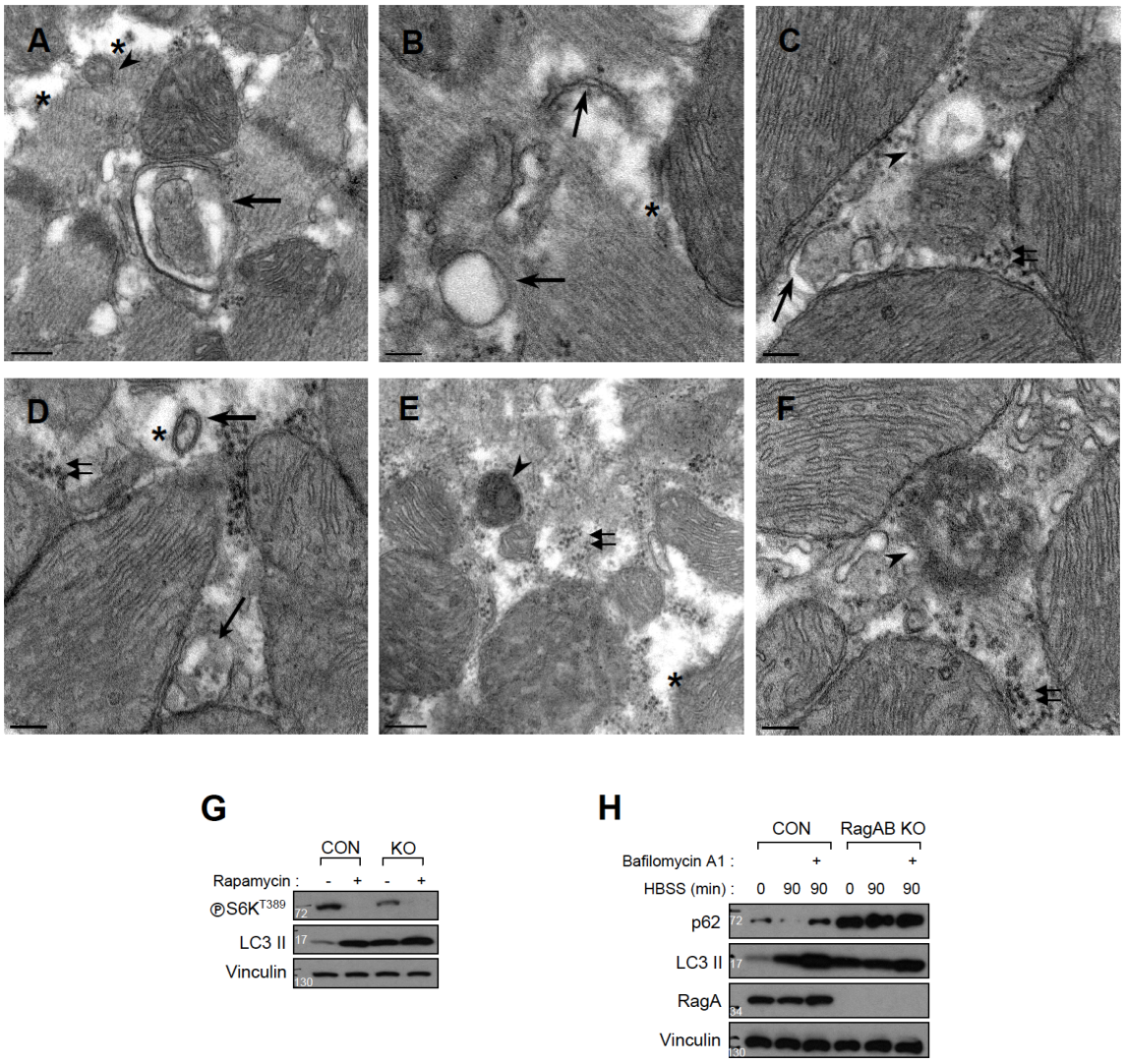
Supplementary Fig. 1. Generation of RagA fl/fl and RagB fl/fl mice.
(A) Depiction of *RagA* and *RagB* targeted alleles. For the targeting of *RagA*, the 5'-promoter, exon, and 3'-UTR sequences were floxed by homologous recombination. For the targeting of *RagB*, the exon 3 of *RagB* sequences were floxed to delete the GTP binding domain after Cre-mediated recombination, which results in prematured translational termination by a frame shift. (B) Confirmation of Cre-mediated deletion of *RagA* and *RagB* floxed alleles. Genomic DNAs from skeletal muscles were prepared, and then wild type, floxed, or deleted alleles were amplified by PCR using specific primer set (denoted as red and blue arrows in (A)). Only Cre-expressing skeletal muscle tissues have deleted alleles.



Supplementary Fig. 2. Skeletal muscles are not affected in *Mck-Cre/+;RagA fl/fl;RagB fl/fl* (RagA/B cKO) mice.
(A) Images of H&E stained gastrocnemius muscles of control and RagA/B cKO mice. Histological analysis using the tissue sections from gastrocnemius muscles showed no obvious difference between RagA/B cKO mice and control littermates. Representative images are shown. Scale bars, 100 μm. (B) Immunoblot analysis of skeletal muscle tissues. Tissue lysates from gastrocnemius muscles of RagA, RagB, RagA/B cKO mice and control littermates were analyzed by immunoblotting using the indicated antibodies. RagA protein levels as well as mTORC1 signaling pathways are not affected in RagA/B cKO mice. (C) Cre-mediated deletion of floxed alleles in skeletal muscles. To determine whether the Cre recombinases are expressed and active in RagA/B cKO skeletal muscles, mice were mated with Rosa26-β-galactosidase (lacZ) Cre reporter mice, and the skeletal muscle tissues were stained for lacZ activity. Both *Mck-Cre/+;RagA fl/+;RagB fl/+* and *Mck-Cre/+;RagA fl/fl;RagB fl/fl* displayed lacZ expression in myocytes, indicating that the Cre recombinases are expressed and functional in these tissues. Representative images of gastrocnemius muscle sections are shown. Scale bars, 50 μm. (D) Composition of oxidative myofibers in control and RagA/B cKO skeletal muscles. Frozen sections of gastrocnemius muscles were stained to detect succinate dehydrogenase (SDH) activity. The composition of slow- and fast-twitch myofiber in gastrocnemius muscles of RagA/B cKO mice is similar to that of control mice. Scale bars, 50 μm. (E) Transmission electron microscopy (TEM) of control and RagA/B cKO skeletal muscles. Examination of ultrastructure of tibialis muscles using TEM showed no difference between control and RagA/B cKO mice. Representative images are shown. Scale bars, 1 μm.



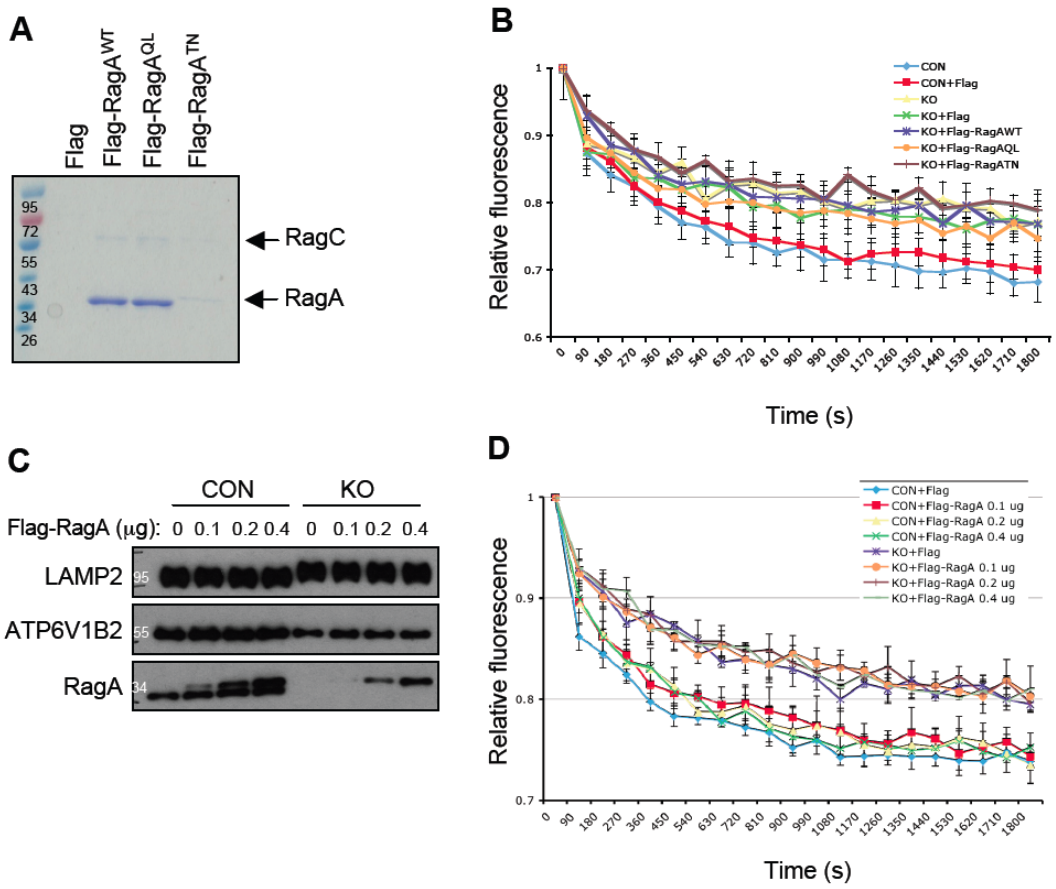
Supplementary Fig. 3. mTORC1 activity is not abnormally increased in RagA/B cKO hearts.
Tissue lysates were prepared from control or RagA/B cKO hearts and analyzed by immunoblotting using the indicated antibodies. The phosphorylation of mTORC1 downstream substrates, S6 or 4EBP1 was not abnormally upregulated in RagA/B cKO hearts compared with controls.



Supplementary Fig. 4. Impaired autophagy flux in RagA/B cKO hearts and RagA/B KO MEFs.

(A-F) TEM images of cardiomyocytes in RagA/B cKO hearts. Autophagosomes and autolysosomes in various stages of autophagy flux are accumulated in RagA/B cKO cardiomyocytes. Arrows and arrowheads indicate AVi (initial autophagic vacuoles) and AVd (late autophagic vacuoles/autolysosomes), respectively. Asterisks show electron-lucent spaces in the cytoplasm. Small double arrows show electron-dense granules. Scale bars, 200 nm.

(G) Increased LC3-II formation in RagA/B KO MEFs by rapamycin treatment. Control or RagA/B KO MEFs were cultured in a nutrient rich medium in the presence or absence of rapamycin (200 nM) for 60 min, and then cell lysates were prepared for immunoblot analysis. Rapamycin treatment further increased LC3-II formation in the RagA/B KO MEFs. (H) Bafilomycin A_1 treatment upon starvation in RagA/B KO MEFs. Autophagy flux was induced by starvation in the presence or absence of bafilomycin A_1 (200 nM), and cell lysates were prepared for immunoblot analysis. Unlike control MEFs, bafilomycin A_1 treatment had little effect on p62 and LC3-II accumulation in the RagA/B KO MEFs.



Supplementary Fig. 5. Lysosomal acidification assay in the presence of immunopurified RagA proteins *in vitro*.
(A) Quantification of immuno-purified RagA proteins by Coomassie blue staining. Flag-tagged RagA wild-type, constitutively active form (QL mutant), or dominant negative form (TN mutant) were co-transfected with RagC wild-type to HEK293 cells, and then cells were incubated in a serum-free medium for 2 hours prior to immunoprecipitation. Co-immunoprecipitated RagA and RagC proteins were resolved by SDS-PAGE and quantified by Coomassie blue staining. Note that the level of $RagA^{TN}$ is significantly lower than other forms of RagA proteins due to instability of the mutant in cells. (B) Lysosomal acidification assay using the purified proteins and lysosome enriched fraction. Lysosomal acidification was measured in the absence or in the presence of purified RagA and RagC proteins as described in Figure 9E. Equal amounts of $RagA^{WT}$ and $RagA^{QL}$ proteins were added to the reaction whereas the amount of $RagA^{TN}$ proteins was adjusted by the amount of co-purified RagC proteins. Each point represents the mean ± S.E of three 30-second intervals from three independent measurements. (C) Addition of different amounts of immuno-purified $RagA^{WT}$ proteins to lysosomal acidification assays. 0, 0.1, 0.2, or 0.4μg of RagA proteins were added to lysosomal fractions. Only 1/100 of the reaction volume was loaded for immunoblotting. (D) Lysosomal acidification assay with different dose of immuno-purified RagA proteins. Each point represents the mean ± S.E of three 30-second intervals from three independent measurements.

Supplementary Fig. 6. Un-cropped Western blot images.

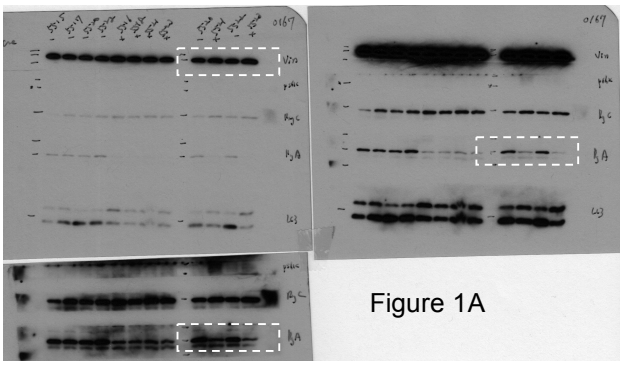


Figure 1A

Figure 3C

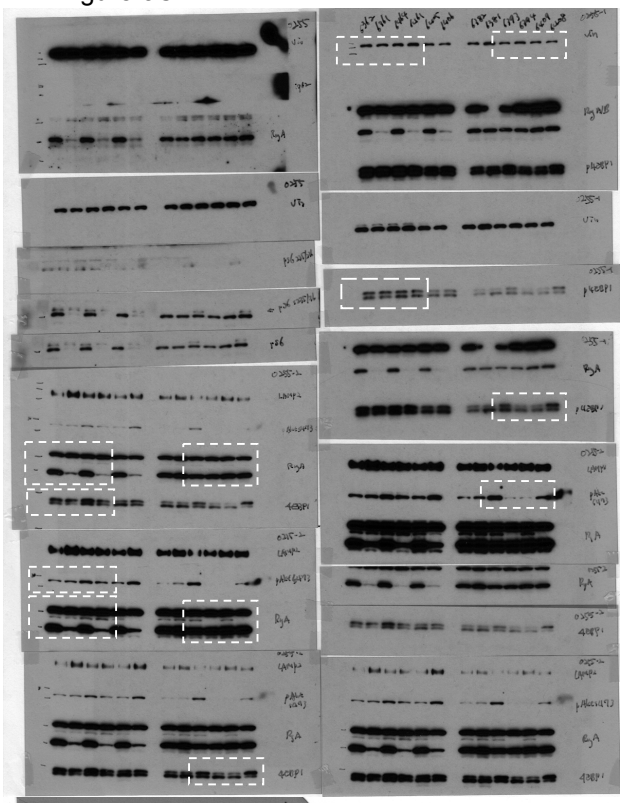


Figure 3C

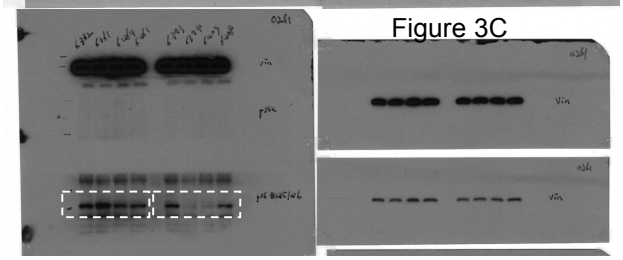


Figure 5F

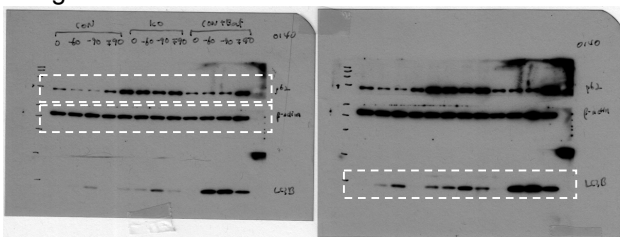


Figure 5A

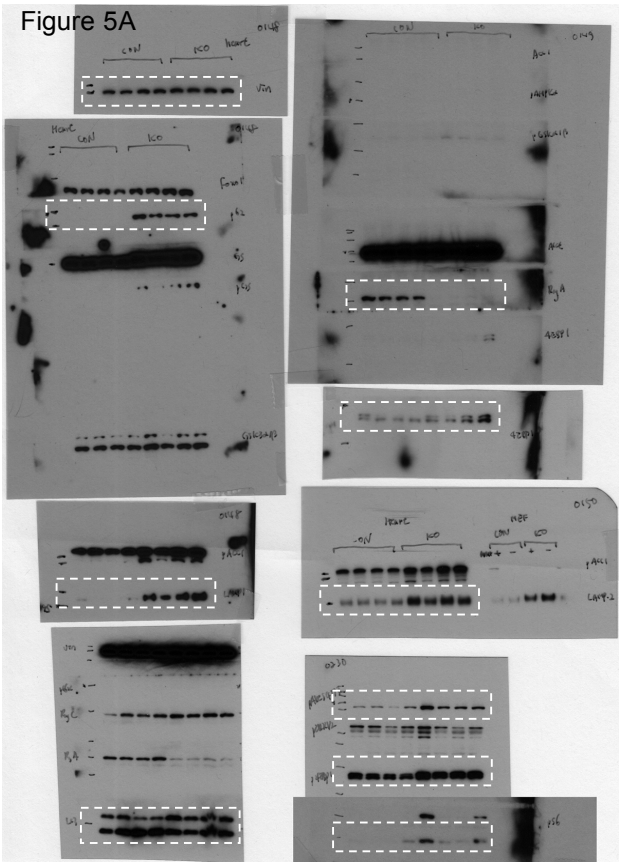
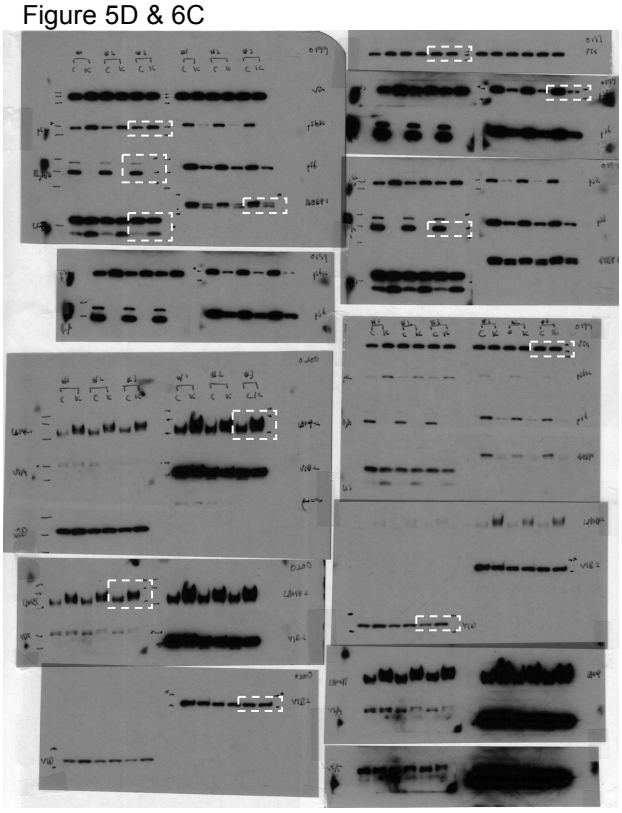


Figure 5D & 6C



Supplementary Fig. 6. Continued.

Figure 6E

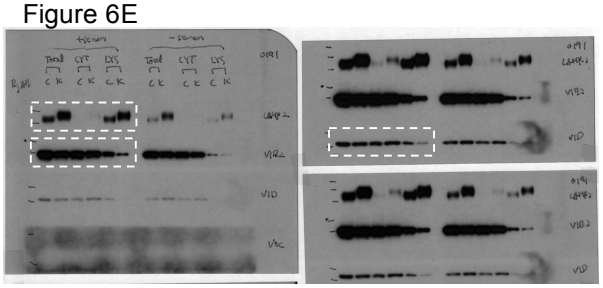


Figure 6F

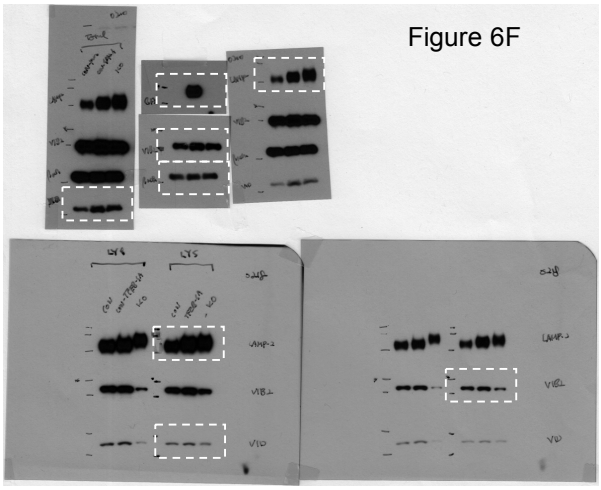


Figure 7A&B

Figure 7C

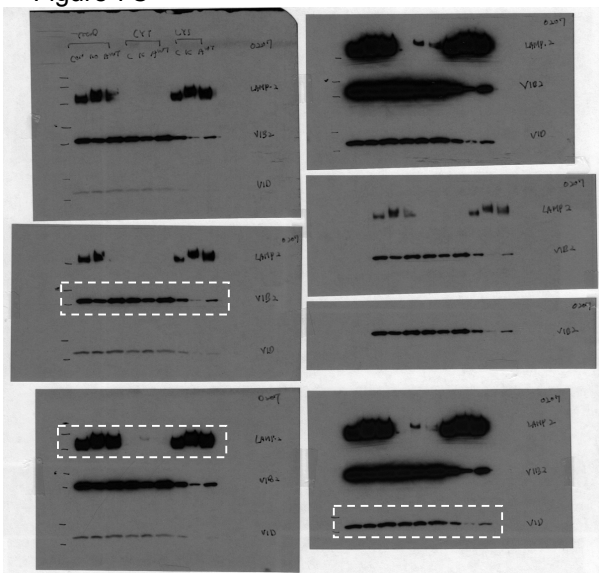
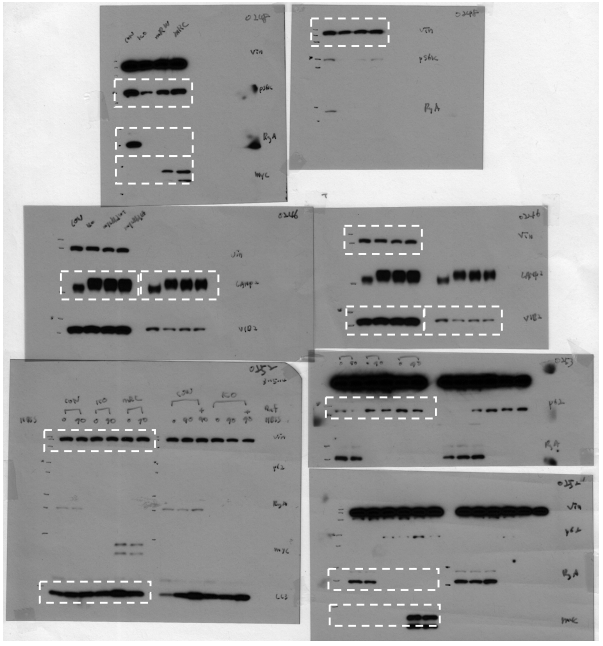


Figure 7D-F



Supplementary Fig. 6. Continued.

Figure 7G

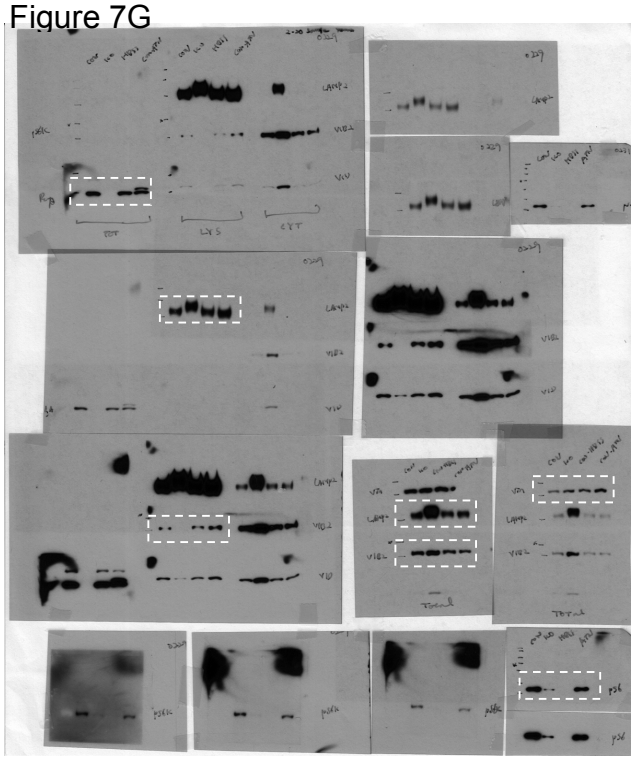


Figure 9B

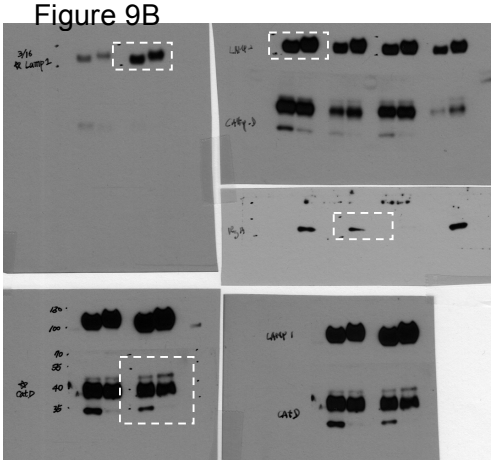


Figure 9C

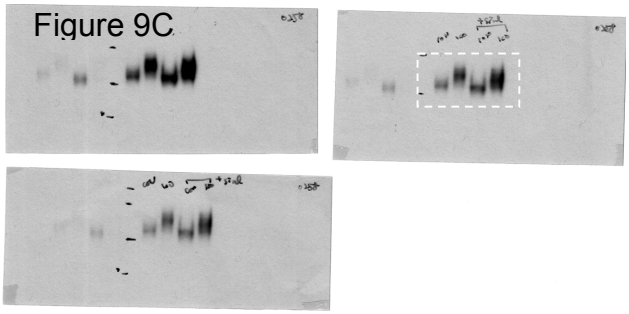


Figure 8B

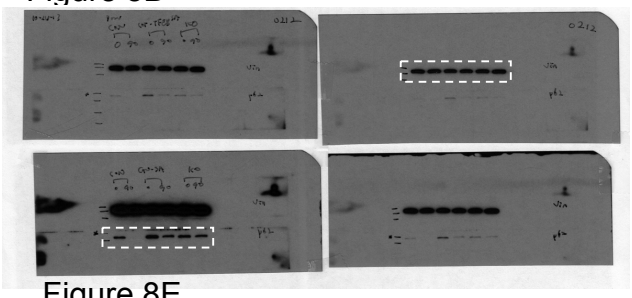


Figure 8E

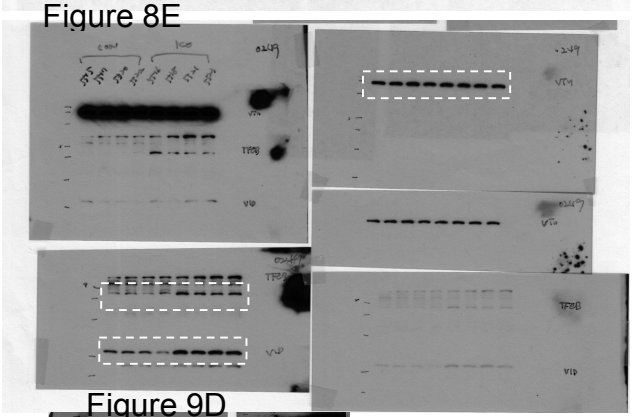


Figure 9D

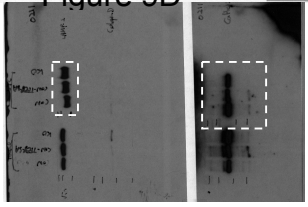
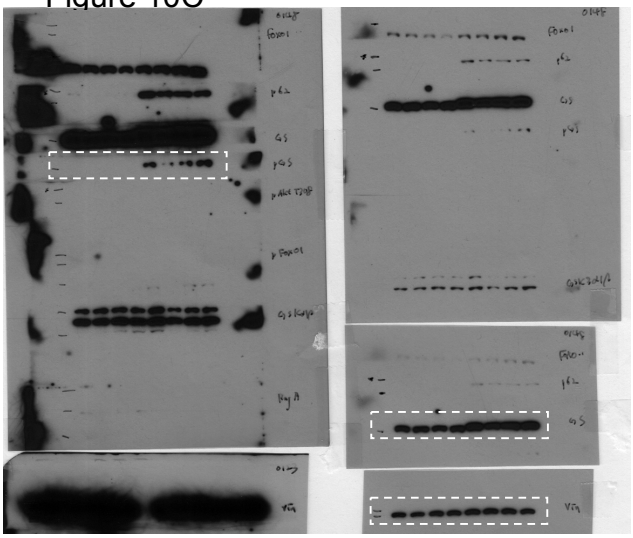


Figure 10C



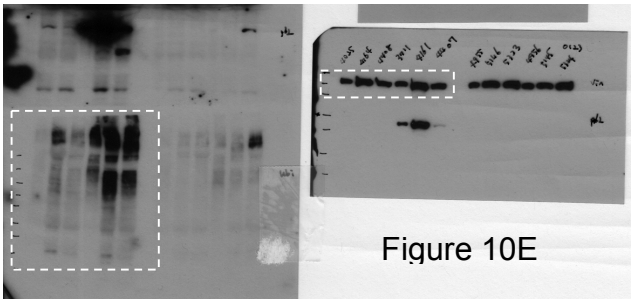


Figure 10E

Supplementary Table 1. List of primers

Target gene	Primer sequence	Application
RagA floxed allele	F: GTTCTCTGGAAGAGCAGTGTGTGT R: CTTTTTACCTCAGCTTGAGAATGTG	Genotyping
RagB floxed allele	F: ATATGCAGAAGGGATGAAATCATGG R: TCCACTTTTACCCATCAACAGGAC	Genotyping
RagA deleted allele	F: GTTCTCTGGAAGAGCAGTGTGTGT R: TATCTGGAAAAGCAGATAGTGCTG	Genotyping
RagB deleted allele	F: GGAAGCAAAGAGACTGTAAACCAT R: CACCTGAACAGTCCTAACTTGTTG	Genotyping
HPRT	F: TCATTATGCCGAGGATTTGGA R: GCACACAGAGGGCCACAAT	qPCR
Nppa	F: GCTTCCAGGCCATATTGGAG R: GGGGGCATGACCTCATCTT	qPCR
Nppb	F: GAGGTCACTCCTATCCTCTGG R: GCCATTTCCTCCGACTTTTCTC	qPCR
Atp2a2	F: GAGAACGCTCACACAAAGACC R: CAATTCGTTGGAGCCCCAT	qPCR
Pln	F: AAAGTGCAATACCTCACTCGC R: GGCATTTCAATAGTGGAGGCTC	qPCR
ATP6V0A1	F: CCGAGGACGAAGTGTTTGACT R: ATCAGCAGGATAGCCACGGT	qPCR
ATP6V0A2	F: TGGTGCAGTTCCGAGACCT R: GCAGGGGAATATCAGCTCTGG	qPCR
ATP6V0C	F: ACTTATCGCTAACTCCCTGACT R: ACACCAGCATCTCCGACGA	qPCR
ATP6V0E	F: GCATACCACGGCCTTACTGT R: TGATAACTCCCCGGTTAGGAC	qPCR
ATP6V1A	F: ACAGAGGAAGCGTGACTTACA R: CACTTGGACCATGCTGAACTT	qPCR
ATP6V1B2	F: ATGCGGGGAATCGTGAACG R: AGGCTGGGATAGGTAGTTCCG	qPCR
ATP6V1C1	F: ACTGAGTTCTGGCTCATATCTGC R: TGGAAGAGACGGCAAGATTATTG	qPCR
ATP6V1E1	F: GAATCAAGCAAGGCTCAAAGTCC R: CGGGTCGTATCTTTTACCACC	qPCR
ATP6V1F	F: GCGGGCAGAGGTAAGCTAATC R: TTAGGGTGGCGGTTCTTGTTT	qPCR
ATP6V1G1	F: CCCAGGCTGAAATTGAACAGT R: TTCTGGAGGACGGTCATCTTC	qPCR
ATP6V1H	F: GGATGCTGCTGTCCCAACTAA R: TCTCTTGCTTGTCCTCGGAAC	qPCR
LAMP-1	F: CAGCACTCTTTGAGGTGAAAAAC R: ACGATCTGAGAACCATTCGCA	qPCR

LAMP-2	F: TGTATTTGGCTAATGGCTCAGC R: TATGGGCACAAGGAAGTTGTC	qPCR
LIMP-2	F: AGAAGGCGGTAGACCAGAC R: GTAGGGGGATTTCTCCTTGGA	qPCR
p62	F: AGGATGGGGACTTGGTTGC R: TCACAGATCACATTGGGGTGC	qPCR
CD63	F: GAAGCAGGCCATTACCCATGA R: TGACTTCACCTGGTCTCTAAACA	qPCR

Supplementary Table 2. List of antibodies

Antibody	Manufacturer (cat#)	Dilution	Application
RagA	Cell Signaling (4357)	1:1000	IB
S6K	Cell Signaling (9202)	1:1000	IB
p-S6K^{T389}	Cell Signaling (9234)	1:1000	IB
p-S6$^{S240/244}$	Cell Signaling (2215)	1:2000/1:100	IB/IHC
Akt	Cell Signaling (9272)	1:1000	IB
p-Akt^{S473}	Cell Signaling (4058)	1:1000	IB
Ulk1	Cell Signaling (8054)	1:1000	IB
p-Ulk1^{S757}	Cell Signaling (6888)	1:1000	IB
Vinculin	Sigma-Aldrich (V9131)	1:10000	IB
4EBP1	Cell Signaling (9452)	1:1000	IB
p62	Progen (GP62-C)	1:1000/1:100	IB/IHC
LC3	Novus (NB100-2220)	1:2000	IB
LC3B	Cell Signaling (2775)	1:1000	IB
LAMP-1	Abcam (ab24170)	1:1000/1:200	IB/IHC
LAMP-2	Abcam (ab13524)	1:1000/1:1000	IB/IF
β-actin	Abcam (ab3280)	1:10000	IB
ATP6V1B2	Abcam (ab73404)	1:500	IB
ATP6V1D	Santa Cruz (sc166218)	1:500	IB
CathepsinD	Santa Cruz (sc6486)	1:500	IB
Glycogen Synthase (GS)	Cell Signaling (3886)	1:1000	IB
p-GS^{S641}	Cell Signaling (3891)	1:1000	IB
TFEB	Abcam (ab113372)	1:1000/1:200	IB/IHC
Ubiquitin	Dako (z0458)	1:3000	IB