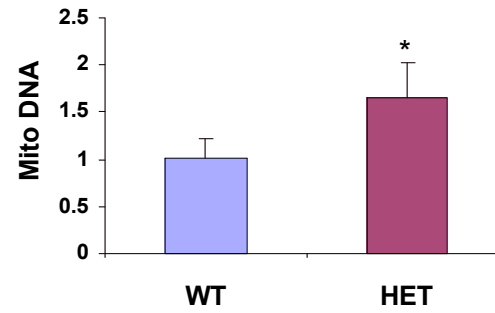
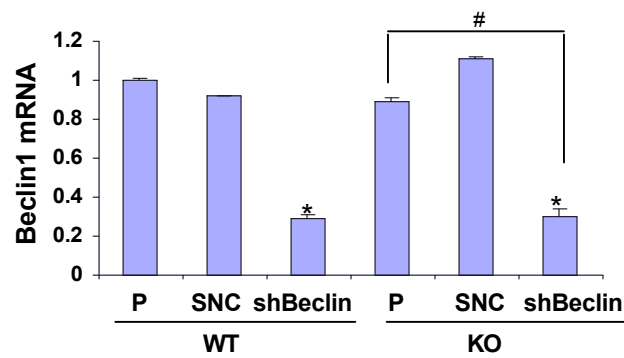


Supplemental Data

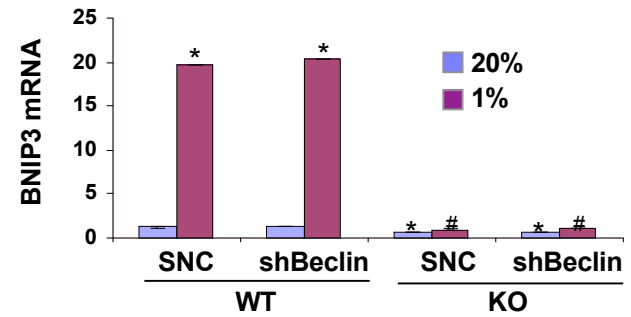
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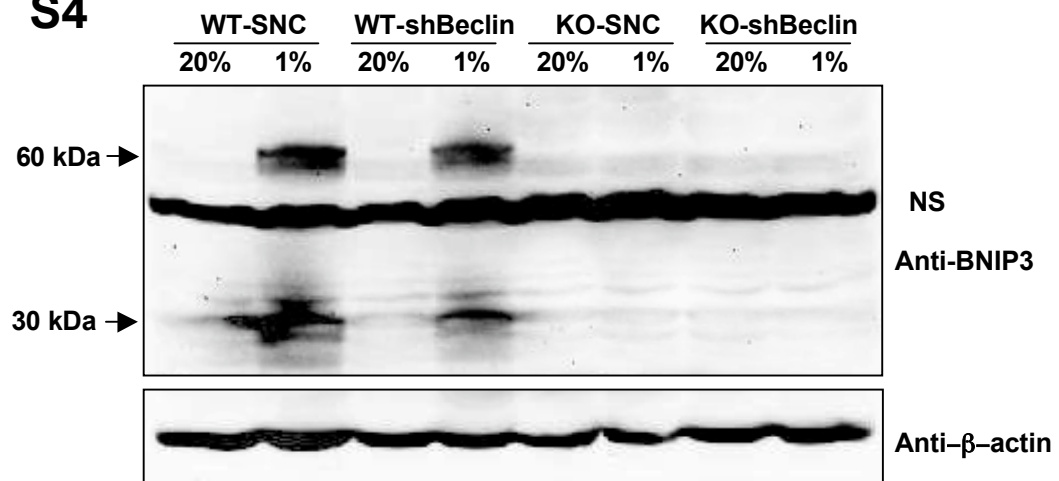
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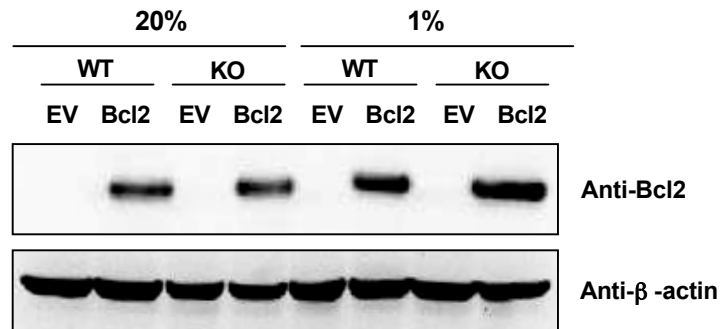
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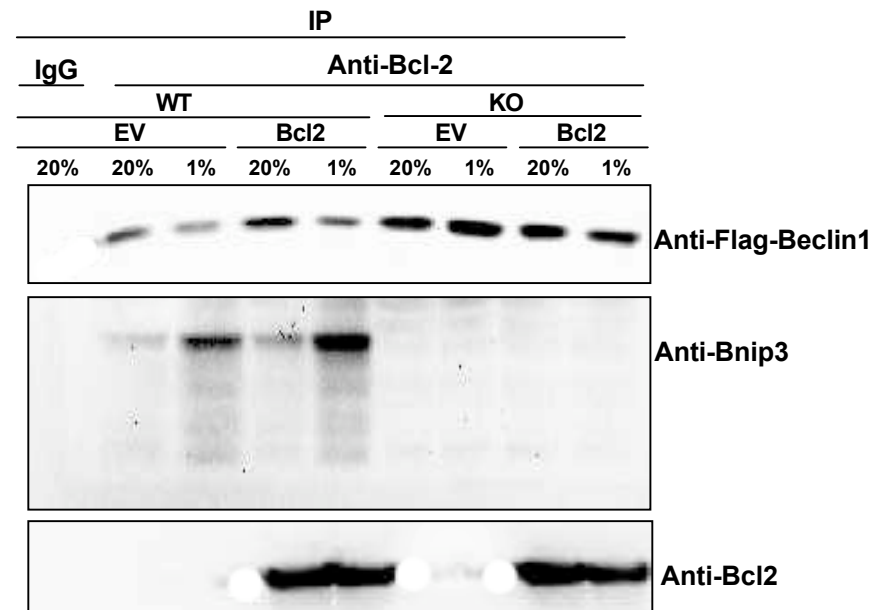
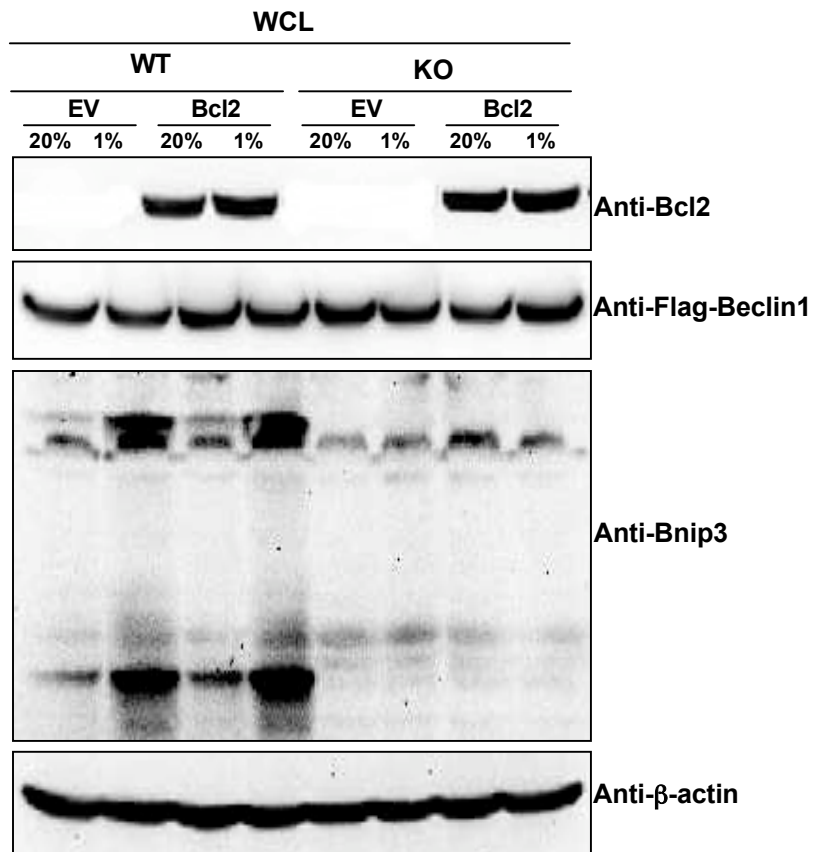
S4



S5



S6



SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Analysis of Mitochondrial DNA in Hearts of WT and HET mice DNA was extracted from the hearts of the same mice that were analyzed in Figure 1G. The mean (\pm SEM) levels of mitochondrial DNA relative to nuclear DNA were determined by quantitative real-time PCR. * $P < 0.05$ by Student's t test compared to WT.

Figure S2. Analysis of Beclin-1 mRNA levels in MEFs with Beclin-1 Knockdown Total RNA was isolated from parental WT and KO MEFs (P) and subclones expressing a scrambled negative control shRNA (SNC) or shRNA targeted against Beclin-1 (shBeclin). Mean (\pm SEM) levels of Beclin-1 mRNA were determined by quantitative real-time RT-PCR. * $P < 0.05$ by Student's t test compared to parental cells; # $P < 0.05$ for indicated comparison.

Figure S3. Analysis of BNIP3 mRNA levels in MEFs with Beclin-1 Knockdown WT and KO MEFs, which were stably transfected with SNC or shBeclin short hairpin RNA, were cultured at 20% or 1% O₂ for 24 h, total RNA was isolated, and mean (\pm SEM) levels of BNIP3 mRNA were determined by quantitative real-time RT-PCR. * $P < 0.05$ by Student's t test compared to WT-SNC at 20% O₂; # $P < 0.05$ by Student's t test compared to WT-SNC at 1% O₂.

Figure S4. Analysis of BNIP3 Protein Levels in MEFs with Beclin-1 Knockdown WT and KO MEFs, which were stably transfected with SNC or shBeclin short hairpin RNA, were cultured at 20% or 1% O₂ for 48 h, and cell lysates were subjected to immunoblot assays using antibodies against BNIP3 and β -actin as a loading control.

Figure S5. Analysis of Bcl2-overexpressing MEFs WT and KO MEFs that were stably transfected with empty lentiviral vector (EV) or a lentivirus encoding Bcl-2 were cultured at 20% or 1% O₂ for 48 h, and cell lysates were subjected to immunoblot assays using antibodies against Bcl2 and β -actin.

Figure S6. Analysis of Bcl2 Interaction with BNIP3 and Beclin-1 WT and KO MEFs that were stably transfected with empty lentiviral vector (EV) or a lentivirus encoding Bcl-2 were transiently transfected with an expression vector encoding FLAG-epitope-tagged Beclin-1 and cultured at 20% or 1% O₂ for 48 h. Whole cell lysates (WCL) were prepared. Aliquots of WCL were analyzed directly by immunoblot assays (left panel) or after immunoprecipitation (IP) with IgG or anti-Bcl2 antibodies (right panel).

Supplemental Table 1. shRNA information

Accession Number	Name	targeted sequence:
NM_009760	Mm Bnip3 sh80	5'-CAG CCT CCG TCT CTA TTT A -3'
	Mm Bnip3 sh82	5'- GCC TCC GTC TCT ATT TAT A -3'
NM_019584	shBeclin1	5'- CAG TTT GGC ACA ATC AAT A -3'

Supplemental Table 2. primer information

Accession No.	Name	Sense Primer	Anti-sense Primer
NC_005089	Mus musculus mitochondrion, ND2	CCCATTCCAATTCTGATTACC	ATGATAGTAGAGTTGAGTAGCG
NM_009760	Mus musculus BNIP3	ACTCAGATTGGATATGGGATTGG	GAGACAGTAACAGAGATGGAAGG
NM_019584	Mus musculus Beclin 1	AATCTAAGGAGTTGCCGTTATAC	CCAGTGTCTTCAATCTTGCC
NM_053069	Mus musculus Atg5	CCTGAAGATGGAGAGAAGAG	GGACAATGCTAATATGAAGAAAG