SUPPLEMENTAL FIGURE LEGENDS

Figure S1, related to Figure 1. Evolutionary tree of Beclin 1 and Beclin 2 protein from multiple species. Sequence alignments of Beclin family members from invertebrates (shown in colored print) cannot clearly be classified as either Beclin 1 or Beclin 2.

Figure S2, related to Figure 2.

(A) Generation of the siRNA non-targetable Beclin 2 mutant (NTm) plasmid. Nucleic acid substitutions in NTm are indicated in red. Beclin 2 levels were detected by western blot analysis in HeLa cells co-transfected with indicated amounts of *beclin 2* siRNA and Flag epitope-tagged Beclin 2 NTm and wild-type (WT) plasmids.

(B) Quantification of relative levels of p62/actin, LC3-II/LC3-I, and total LC3/actin for gels from three independent western blot experiments, including the representative experiment shown in Figure 2B of the main figures. HeLa cells were transfected with indicated siRNA and grown in normal medium or cultured for 3 h in starvation medium prior to western blot analysis. Results represent mean +/- SEM of three independent experiments.

(C) mRNA levels of p62 and LC3 in HeLa cells upon siRNA knockdown of *beclin 1* or *beclin 2* during growth in normal media (starvation "-") or after 3 h amino acid and serum starvation (starvation "+"), detected by semi-quantitative RT-PCR. The level of GAPDH mRNA was used as an internal control.

(D) Quantification of percentage of cells with mitochondrial clearance (mitophagy). Mitophagy was induced by CCCP treatment in HeLa cells co-transfected with a plasmid expressing mCherry-Parkin and the indicated siRNAs. Results represent mean ± SEM of triplicate samples (100-250 cells per sample).

**P*<0.05; **P<0.01; ****P*<0.001; NS, not significant; ANOVA.

Figure S3, related to Figure 3.

(A) Co-immunoprecipitation of endogenous GASP1 with endogenous Beclin 2 in HEK293 cells. WCL, whole cell lysates.

(B) Immunoprecipitation of human Beclin 2 in HEK293 cells treated with non-silencing control (NC) or *beclin 2* siRNA.

Asterix denotes non-specific band.

Figure S4, related to Figure 4.

(A) Schematic illustration of biotin protection degradation assay of GPCRs. "100%" represents total biotinylated GPCR prior to stripping of cell surface biotin; "30’ " and "180’ " represent levels of internalized biotinylated receptor with agonist treatment for 30 min or 180 min, respectively, after stripping; "140'+30' " represents the remaining protected biotinylated receptor from stripping after 140 min agonist treatment followed by 30 min antagonist treatment.

(B) Western blot detection of indicated protein expression in HEK293 cells treated with indicated siRNA or transfected with Myc-cGASP1.

(C) Effects of the lysosomal inhibitor bafilomycin A1 (Baf) on agonist-induced degradation of DOR in HEK293 cells stably expressing Flag-DOR (upper panel).

(D) Effects of the lysosomal inhibitor bafilomycin A1 (Baf) on CXCL12-induced degradation of endogenous CXCR4 in HeLa cells (upper panel), and PDGF-induced degradation of PDGFR in CCD-1064Sk cells (lower panel), after 3 h treatment with indicated agonist.

(E) Effects of indicated siRNAs or cGASP1 overexpression on agonist-induced degradation of endogenous CXCR4 in HeLa cells (left panel) or endogenous PDGFR in CCD-1064Sk cells (right panel), after 3 h treatment with the agonist CXCL12 or PDGF.

(F) Quantification of normalized levels of CXCR4 (left graph) and PDGFR (right graph) at 0 h and 3 h after agonist treatment in cells transfected with indicated siRNA or cGASP

plasmid as shown in (E). Bars represent mean ± SEM of three independent experiments. NS, not significant; two-way ANOVA with Dunnett method.

Figure S5, related to Figure 5.

(A) Western blot detection of ATG14, VPS34, and cGASP1 in HeLa/GFP-LC3 cells transfected with indicated siRNA or cGASP1 plasmid.

(B) Representative images of GFP-LC3 puncta in HeLa/GFP-LC3 cells after co-transfection with indicated siRNA or with cGASP1 plasmid and cultured for 3 h in normal or starvation medium. Arrows denote representative autophagosomes. Scale bar, 20 μm.

(C) Quantification of the GFP-LC3 puncta shown in (B). Results represent mean ± SEM of triplicate samples (~50 cells per sample). Similar results were observed in three independent experiments. ***P<0.001; NS, not significant; ANOVA with Dunnett method.

NC, non-silencing control.

Figure S6, related to Figure 6.

(A) Generation of *beclin 2* knockout mice. Genomic structure of *beclin 2* and *beclin 2* targeting vector.

(B) Southern blot analyses of genomic DNA from *beclin* $2^{+/+}$ and *beclin* $2^{+/-}$ ES cells. Probes indicated in (A) that hybridize to both 5' and 3' regions of the targeting vector were used.

(C) Western blot analysis of Beclin 2 in whole brain lysates of a *beclin* $2^{+/-}$ mouse and a wild-type littermate using an anti-mouse Beclin 2 antibody. Asterisks denote non-specific bands.

(D) Quantification of relative levels of p62/actin, LC3-II/LC3-I, and total LC3/actin for gels from three independent western blot experiments, including the representative

experiment shown in Figure 6C of the main figures. MEFS of indicated genotype were grown in normal medium or cultured for 3 h in starvation medium prior to western blot analysis. Results represent mean +/- SEM of three independent experiments. **P*<0.05; ***P*<0.01; ****P*<0.001; NS, not significant; ANOVA.

(E) Percentages of degradation of ^{3}H-labeled long-lived cellular protein in *beclin* $2^{+/+}$, *beclin* $2^{-/-}$, *beclin* $1^{+/-}$ and *Atg5*$^{-/-}$ MEFs after 2 h starvation. Bars represent mean ± SEM of three independent experiments. ***P*<0.01; ****P*<0.001; ANOVA with Dunnett method.

(F) Western blot detection of p62 and LC3 in whole brain lysates of *beclin* $2^{+/-}$ mice and wild-type littermates. Quantification of the levels of total LC3/actin, LC3-II/LC3-I and p62/actin is shown on the right. Bars represent mean ± SEM of six mice of each genotype. **P*<0.05; ***P*<0.01; ANOVA.

(G) Western blot detection of MOR (mu-opioid receptor) and D2R (dopamine 2 receptor) in whole brain lysates of *beclin* $2^{+/-}$ mice and wild-type littermates. Quantification of the levels of MOR and D2R is shown on the right. Bars represent mean ± SEM of four mice of each genotype. **P*<0.05; NS, not significant; ANOVA.

Figure S7, related to Figure 7.

(A and B) Three-dimensional movement activity of *beclin* $2^{+/-}$ mice and wild-type littermates at the end of 8 weeks of RD (A) or HFD (B) treatment. Results represent mean ± SEM for 5 mice per group. Data shown is for cohort of mice analyzed in Figure 7 of main text. NS, not significant; ANOVA.

(C) Weekly weights of an independent cohort (distinct from that for which data are shown in Figure 7) of 8 week-old *beclin* $2^{+/-}$ and wild-type *beclin* $2^{+/+}$ littermates during 8 weeks of regular diet (RD) or high-fat diet (HFD) treatment. *P*<0.001 in RD conditions and *P*<0.05 in HFD conditions for *beclin* $2^{+/+}$ versus *beclin* $2^{+/-}$ mice; linear mixed effect model.

(D and E) Glucose tolerance tests and insulin tolerance tests performed on the cohort shown in (C) of *beclin 2*$^{+/-}$ mice and wild-type *beclin 2*$^{+/+}$ littermates after 8 weeks RD (D) or HFD (E) treatment. *P* values in each graph represent the difference between two curves; linear mixed effect model. **P*<0.05 for comparison between two groups at indicated time point; ANOVA

For (C-E), results represent mean ± SEM for 6-8 mice per experimental group.

(F-I). Comparison of 12 week-old male RD-fed *beclin 1* heterozygous-deficient mice with wild-type littermates with respect to body weight (F), daily food intake (G), glucose tolerance tests (H) and insulin tolerance tests (I). Results represent mean ± SEM of 4-6 mice per group. For (F-G), NS, not significant; ANOVA. For (H-I), *P* values indicated in graph; linear mixed-effect model.

(J) Glucose tolerance tests and insulin tolerance tests on 16 week-old littermate mice of indicated genotypes fed with RD after 16 days of daily intraperitoneal rimonabant (10 mg kg^{-1}) or vehicle control treatment. *P*=NS; linear mixed effect model. Results represent mean ± SEM for 6-7 mice per group.

Supplemental Figure 1
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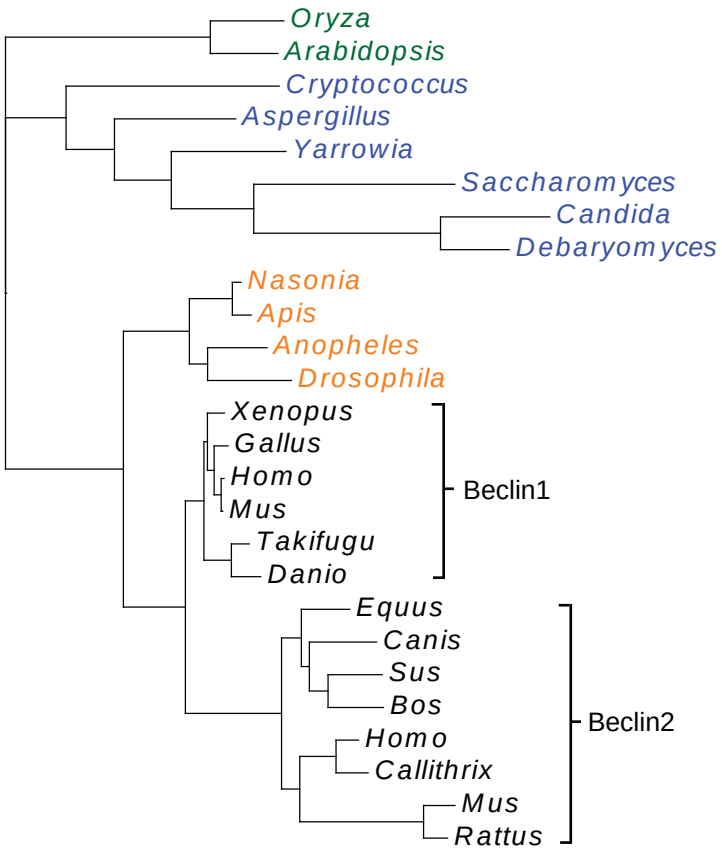
Figure S1
related to Figure 1



Supplemental Figure 2
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Figure S2
related to Figure 2

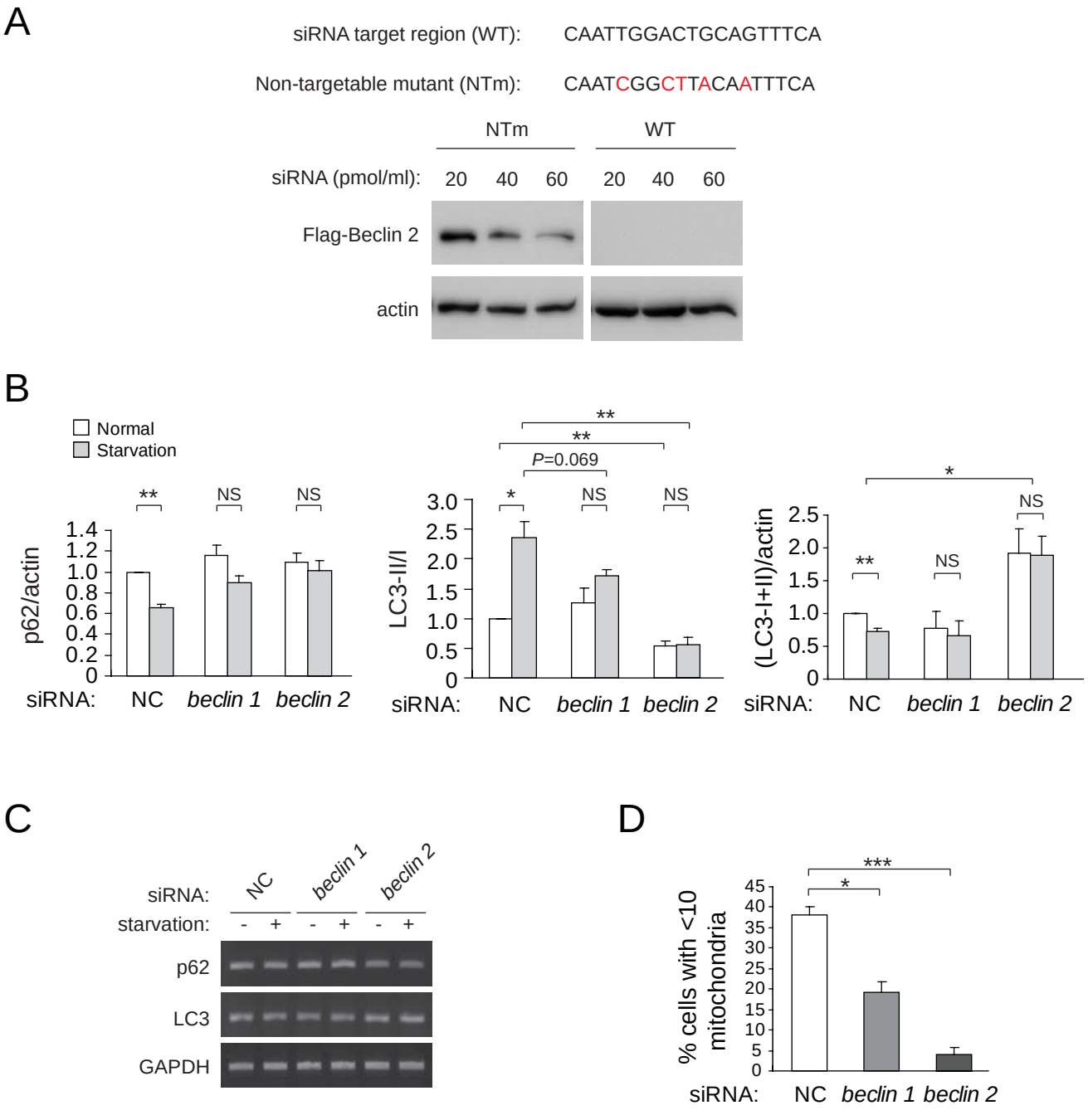

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Figure S3
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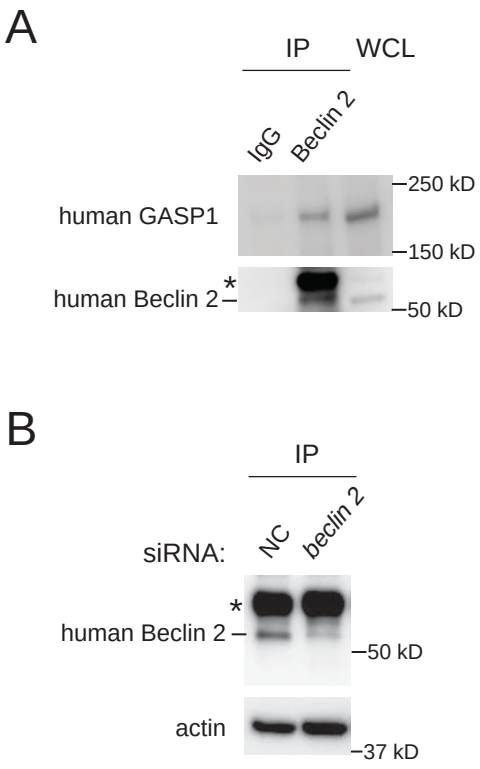

Supplemental Figure 4
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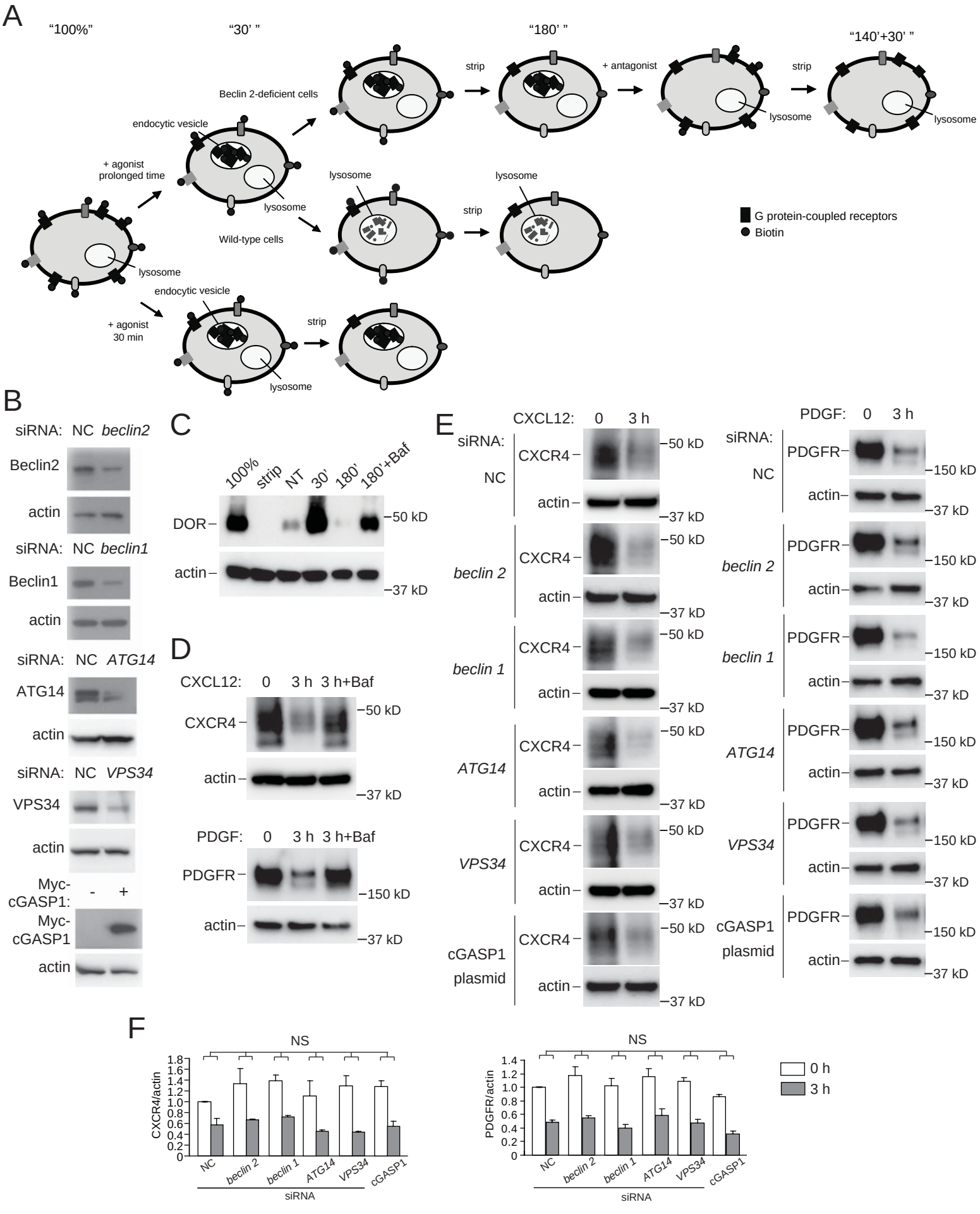
Figure S4
related to Figure 4



Supplemental Figure 5
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Figure S5
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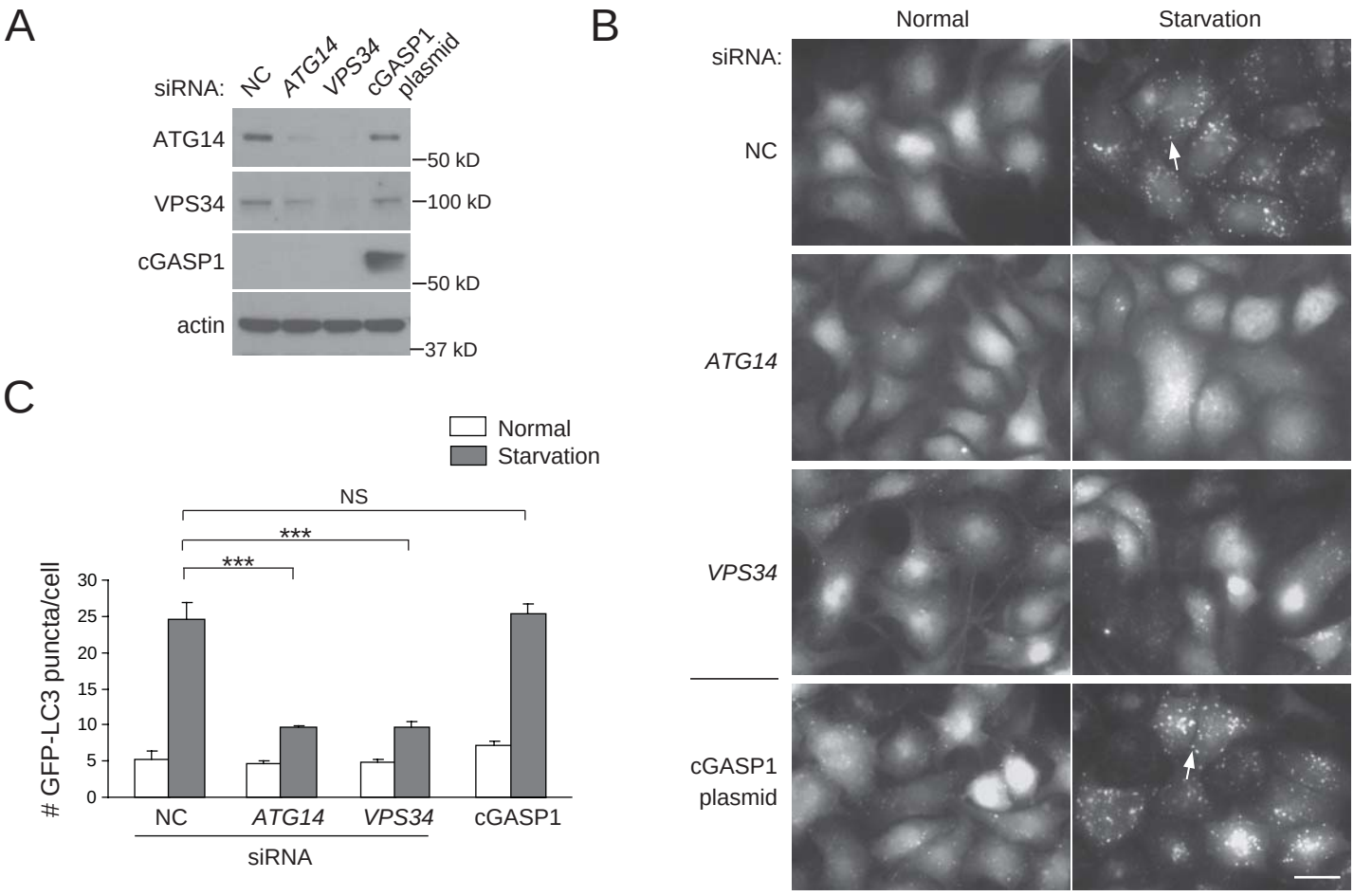

Supplemental Figure 6
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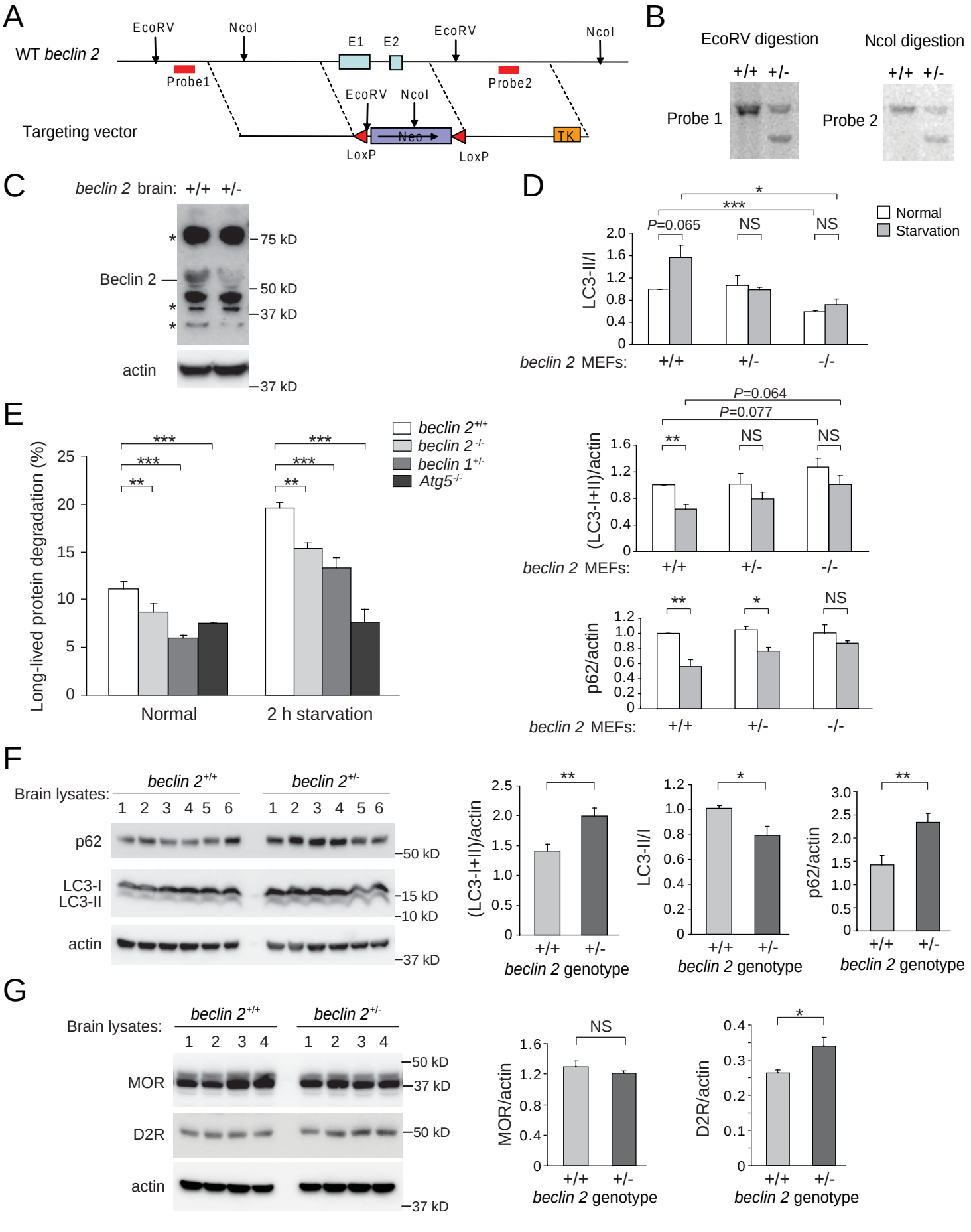
Figure S6
related to Figure 6



Supplemental Figure 7
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Figure S7
related to Figure 7