Figure S1

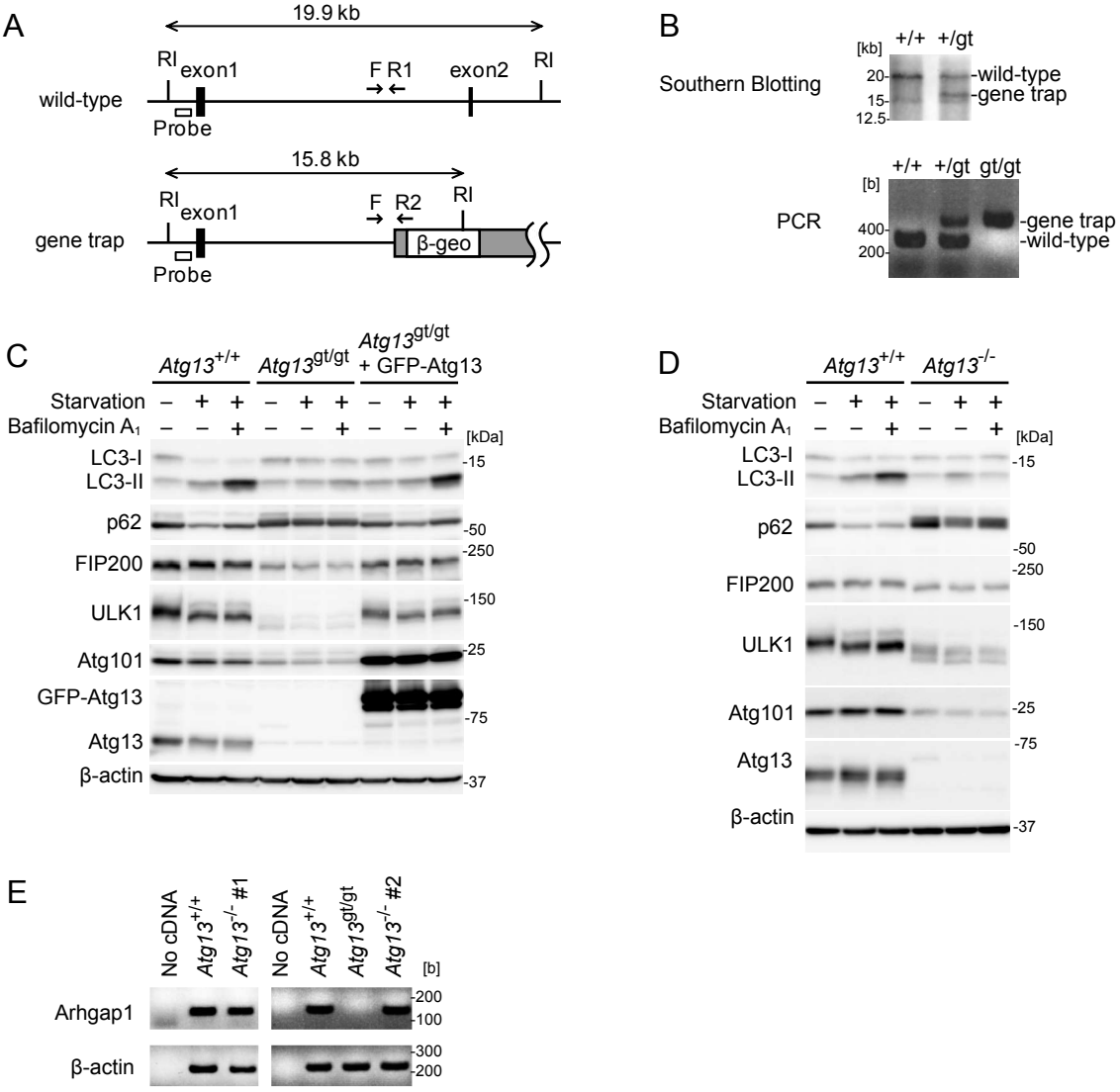

FIG. S1. Evaluation of Atg13-deficient cells

(A) Restriction map of the wild-type and gene trap alleles of the *Atg13* gene. Black boxes indicate exons 1 and 2. The position of the probe used in the Southern blot analysis and the primers (F, R1, and R2) used in the PCR analysis in (B) are indicated by white boxes and arrows, respectively. Restriction enzyme sites for *Eco*RI are indicated as RI. (B) Genotyping by Southern blot analysis (upper panel, genomic DNA of ES cells) and PCR (bottom panel, genomic DNA of MEFs). gt, the gene trap allele. (C) Wild-type MEFs, *Atg13*$^{gt/gt}$ MEFs, and *Atg13*$^{gt/gt}$ MEFs stably expressing GFP-Atg13 were cultured in regular or starvation medium in the presence or absence of 100 nM bafilomycin A_1 for 2 h. (D) Primary cultured wild-type MEFs and *Atg13*$^{-/-}$ MEFs #1 were cultured in regular or starvation medium in the presence or absence of 100 nM bafilomycin A_1 for 2 h. (E) Total RNA from MEFs of the indicated genotypes was reverse-transcribed into cDNA and then subjected to PCR amplification with indicated primers.

Figure S2

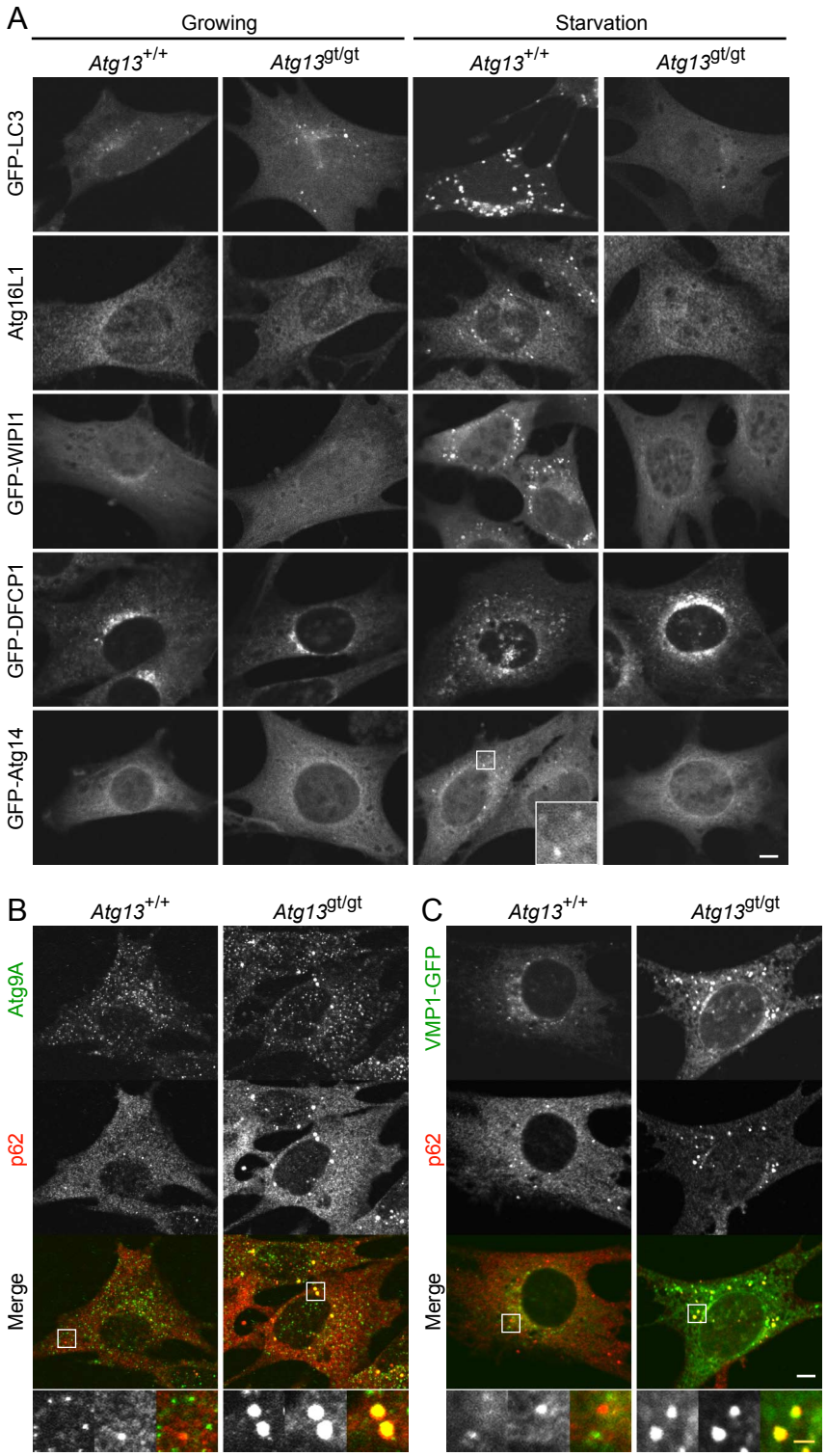

FIG. S2. Impairment of Atg protein recruitment in *Atg13*$^{gt/gt}$ MEFs
(A) Wild-type and *Atg13*$^{gt/gt}$ MEFs stably expressing GFP-LC3, GFP-WIPI1, GFP-DFCP1, or GFP-Atg14 were cultured in regular or starvation medium for 1 h. Cells were fixed and analyzed by fluorescence microscopy. Atg16L1 was immunostained with anti-Atg16L1 antibody. (B) Wild-type and *Atg13*$^{gt/gt}$ MEFs were cultured in regular medium. Cells were fixed and analyzed by immunofluorescence microscopy using anti-Atg9A and anti-p62 antibodies. (C) Wild-type and *Atg13*$^{gt/gt}$ MEFs stably expressing VMP1-GFP were cultured in regular medium. Cells were fixed and analyzed by immunofluorescence microscopy using anti-p62 antibody. Scale bars, 10 μm (white) and 2 μm (yellow).

Figure S3

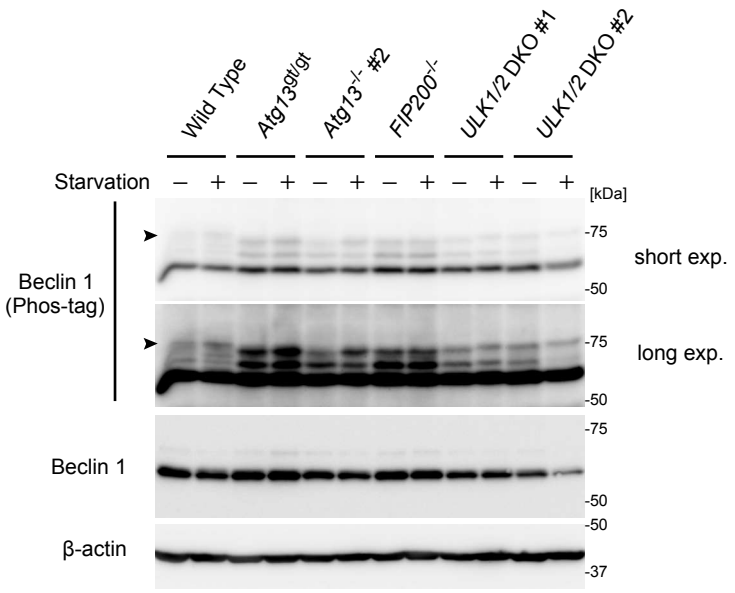

FIG. S3. Impairment of Beclin 1 phosphorylation in Atg13-deficient cells
MEFs of the indicated genotypes were cultured in regular or starvation medium for 2 h. Polyacrylamide gel containing 50 μM Phos-tag was used for Beclin 1 immunoblotting. Arrowheads indicate phosphorylated Beclin 1 which was detected only in wild type MEFs.

Figure S4

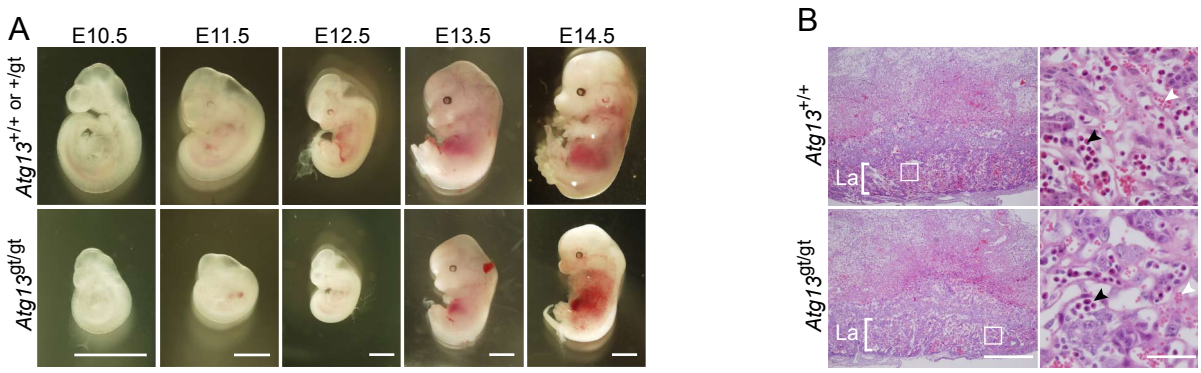

FIG. S4. *Atg13*$^{gt/gt}$ embryos show growth retardation
(A) Control (*Atg13*$^{+/+}$ or *Atg13*$^{+/gt}$) and *Atg13*$^{gt/gt}$ embryos at indicated stages are shown. Scale bars, 2 mm.
(B) Paraffin sections of placentas from wild-type (*Atg13*$^{+/+}$) and *Atg13*$^{gt/gt}$ embryos at E12.5 were subjected to Mayer's hematoxylin and eosin staining. La, labyrinth layer. Black arrowheads, nucleated erythrocytes in the embryonic blood vessels; white arrowheads, non-nucleated maternal erythrocytes. Scale bars, 500 μm (left) and 50 μm (right).

Figure S5

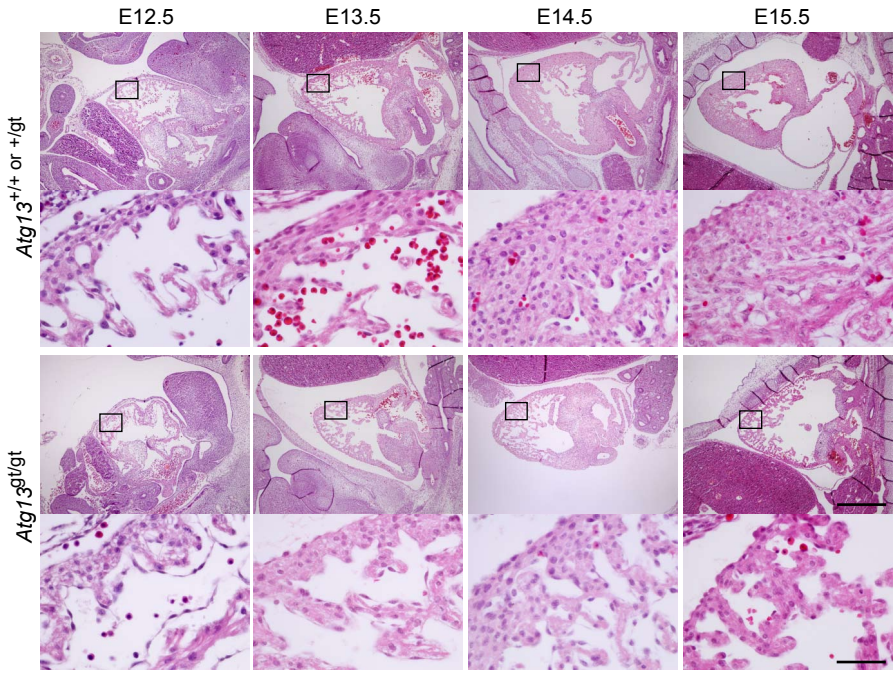

FIG. S5. *Atg13*$^{gt/gt}$ embryos show myocardial growth defect
Sagittal paraffin sections from the heart of control (*Atg13*$^{+/+}$ or *Atg13*$^{+/gt}$) and *Atg13*$^{gt/gt}$ embryos at indicated embryonic stages. Scale bar, 500 μm (upper panels) and 50 μm (lower panels).

Figure S6

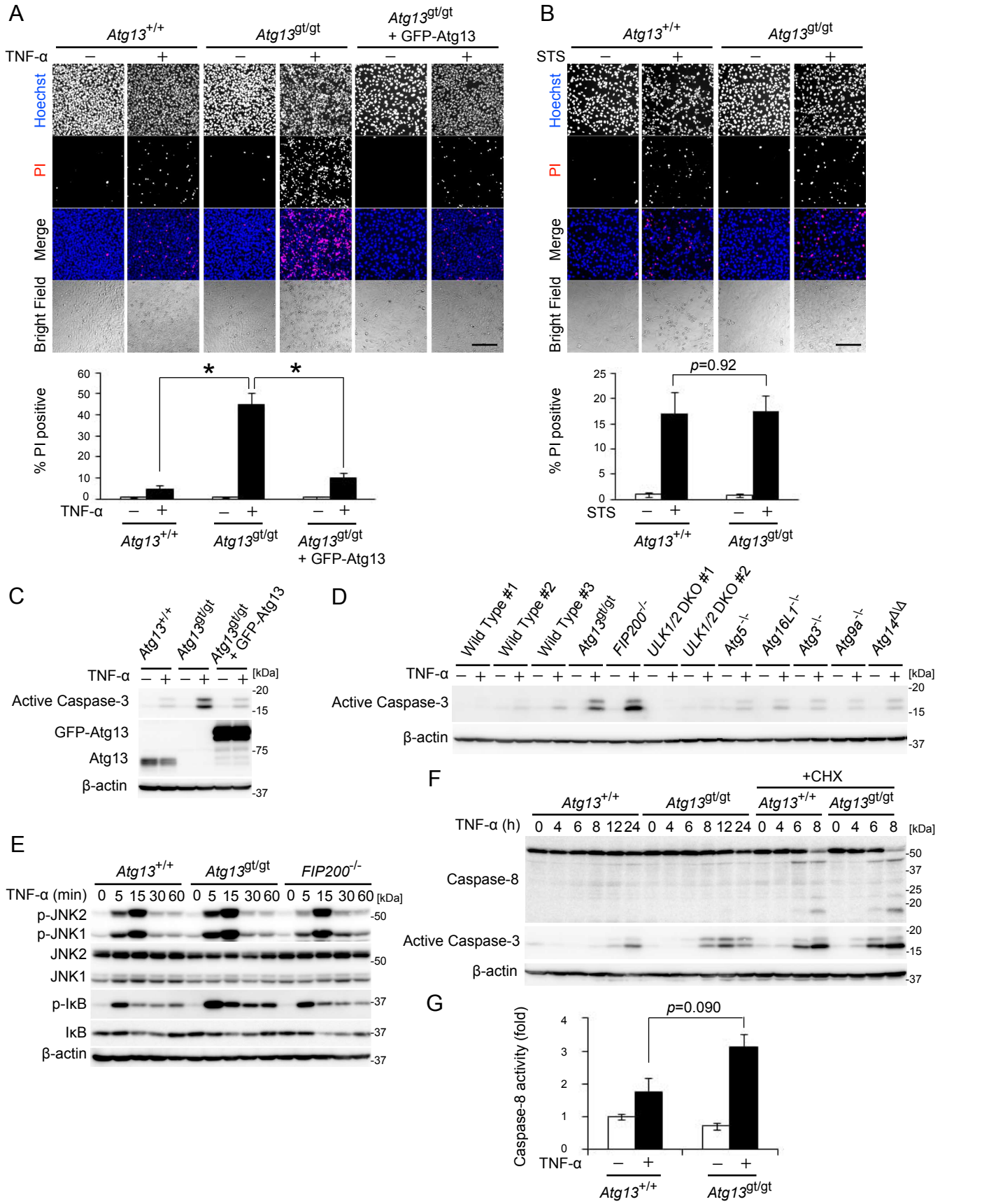

FIG. S6. Atg13 suppresses TNF-α-induced apoptosis independent of autophagy
(A) Wild-type MEFs, *Atg13*$^{gt/gt}$ MEFs, and *Atg13*$^{gt/gt}$ MEFs stably expressing GFP-Atg13 were cultured with or without 20 ng/ml TNF-α for 24 h. Cells were stained with 1 μg/ml Hoechst33342 and propidium iodide (PI) and then analyzedby fluorescence microscopy. Scale bar, 200 μm. The percentage of PI-positive cells of at least 300 cells was determined and the mean ± S.E. from three independent experiments is shown. *, $p < 0.01$, unpaired Student's t test. (B) Wild-type and *Atg13*$^{gt/gt}$ MEFs were cultured with or without 50 ng/ml staurosporine for 24 h and analyzed as in (A). (C) Wild-type MEFs, *Atg13*$^{gt/gt}$ MEFs, and *Atg13*$^{gt/gt}$ MEFs stably expressing GFP-Atg13 were cultured with or without 20 ng/ml TNF-α for 12 h. (D) MEFs of the indicated genotypes were cultured with or without 20 ng/ml TNF-α for 12 h. (E) MEFs of the indicated genotypes were cultured with 20 ng/ml TNF-α for the indicated times. (F) Wild-type and *Atg13*$^{gt/gt}$ MEFs were cultured with 20 ng/ml TNF-α in the presence or absence of 50 μg/ml cycloheximide (CHX) or the indicated times. (G) Wild-type and *Atg13*$^{gt/gt}$ MEFs were cultured with or without 20 ng/ml TNF-α for 12 h. The activity of caspase-8 was measured. Mean ± S.E. (n=3) is shown.

Figure S7

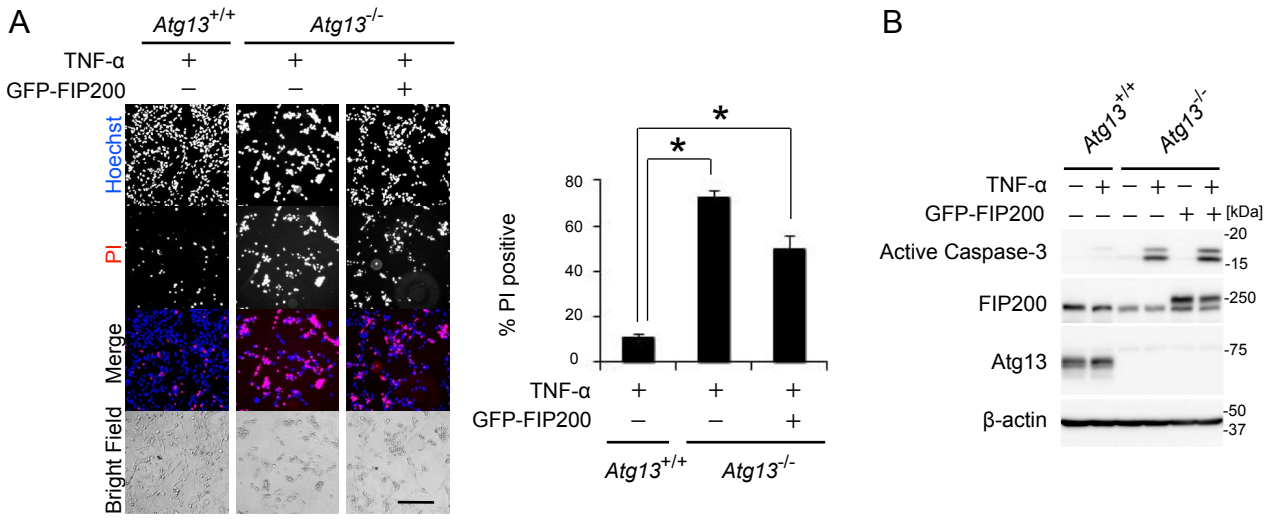

FIG. S7.Exogenous expression of GFP-FIP200 does not restore TNF-a induced apoptosis in *Atg13*$^{-/-}$ MEFs
(A) Wild-type MEFs, *Atg13*$^{-/-}$ MEFs #2, and *Atg13*$^{-/-}$ MEFs #2 stably expressing GFP-FIP200 were cultured with or without 20 ng/ml TNF-α for 24 h. Cells were stained with 1 μg/ml Hoechst33342 and propidium iodide (PI) and then analyzed by fluorescence microscopy. Scale bar, 200 μm. The percentage of PI-positive cells of at least 250 cells was determined and the mean ± S.E. from three independent experiments is shown. *, $p < 0.01$, unpaired Student's t test. (B) These cells cultured with or without 20 ng/ml TNF-α for 12 h were subjected to immunoblotting.

Table S1. The total number of embryos and neonates obtained from mating between *Atg13*$^{+/gt}$ mice

	Genotype			
Stage	+/+	+/gt	gt/gt	Total
E10.5	13	28	12	53
E11.5	0	4	1	5
E12.5	18	42	14 (3)	74
E13.5	21	38	9 (3)	68
E14.5	16	30	10 (6)	56
E15.5	10	41	3	54
E16.5	3	9	0	12
E17.5	6	8	3 (2)	17
E18.5	4	14	1 (1)	19
P0*	16	48	2 (2)	66

( ), the number of dead embryos as judged by the absence of a heartbeat or by signs of resorption

*, neonates were obtained by natural birth or caesarean delivery