Supporting Information

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SI Materials and Methods

Autophagy Measurement. *Staining of Endogenous LC3.* First, 12-μm-thick liver and lung sections of paraffin-embedded WT and *Bat3*$^{-/-}$ E18.5 mouse embryos were deparaffinized, and then subjected to heat-induced epitope retrieval in citrate buffer pH 6.0 at subboiling temperature for 30 min. Endogenous biotin activity was blocked using an avidin/biotin complex system (Vector Laboratories) according to the manufacturer's instructions. Sections were processed using the M.O.M. Basic Immunodetection Kit (Vector Laboratories) and then hybridized at 4 °C with an anti-mouse LC3B primary antibody at a final concentration of 2.5 μg/mL (clone 5F10; Nanotools) overnight. Sections were then incubated with FITC-streptavidin (1:100; (Vector Laboratories) for 30 min. After three washes in phosphate buffer saline (PBS), slides were mounted with Vectashield mounting medium (Vector Laboratories) and analyzed with a Leica DMIRE2 microscope equipped with an oil immersion ×63/1.4 apochromatic objective and a 12-bit Coolsnap FX CCD camera (Princeton Instruments), both controlled by the MetaMorph imaging software (Universal Imaging).

Electron Microscopy. Cells were immersed in a solution of 2.5% glutaraldehyde in Sorensen's buffer (0.1 M, pH 7.4) overnight at 4 °C. After a rinse in Sorensen's buffer, cells were postfixed in a 0.5% osmic acid for 2 h in the dark at room temperature. After two rinses in Sorensen's buffer, the cells were dehydrated in a graded series of ethanol solutions (30–100%). The cells were embedded in EmBed 812 using a Leica EM AMW automated microwave tissue processor for electron microscopy. Thin sections (70 nm, obtained with a Leica-Reichert Ultracut E microtome) were collected at different levels of each block. These sections were counterstained with uranyl acetate and observed with a Hitachi 7100 transmission electron microscope at the Centre de Ressources en Imagerie Cellulaire de Montpellier, Montpellier, France.

GFP-LC3 Assay. When required, cotransfections (peGFP-LC3 to plasmid ratio of 1:3) were performed with Lipofectamine 2000 (Life Technologies). Autophagy was measured by counting the number of GFP-LC3 dots per cell (at least 50–100 cells per condition) using an Axioplan microscope (Carl Zeiss). When required, chloroquine (Sigma-Aldrich) was used at 20 μM for 6 h.

Measurement of Long-Lived Protein Degradation. Cells were incubated for 18 h at 37 °C with 0.2 Ci/mL L-[^{14}C]valine. After three rinses with PBS, cells were incubated for 2 h with complete medium (CM) or Earle's balanced salt solution (EBSS) plus 0.1% BSA and 10 mM cold valine, followed by precipitation in 10% (vol/vol) trichloroacetic acid at 4 °C. The precipitated proteins were separated from soluble radioactivity by centrifugation at 600 × *g* for 10 min. The rate of protein degradation was calculated as acid-soluble radioactivity recovered from both cells and medium.

Staining of Endogenous p300. Cells on coverslips were fixed in 2% paraformaldehyde for 10 min, permeabilized with 0.4% Triton X-100 for 5 min, and then incubated at 37 °C with a primary anti-p300 antibody (N15; 1:200) for 30 min and with a secondary Alexa Fluor 488 goat anti-rabbit antibody (Life Technologies) for 20 min. Slides were observed under an Axioplan II imaging fluorescence microscope with a 63× oil immersion objective and an AxioCam MRm microscope, both controlled by AxioVision software (all from Carl Zeiss). Fluorescence intensity in the cytosol was quantified with Image J.

Western Blot Analysis. The following antibodies were used for Western blot analyses: anti-BAT3 (1:2,000) (1), anti-ATG12 (1:1,000) (Novus Biologicals); anti-LC3 (1:5,000), anti-ATG7 (1:1,000) (Sigma-Aldrich); anti-p53 (1C12, 1:1,000; Cell Signaling Technology); anti-ERK2 (1:2,000), anti-p300 (N15, 1:1,000), anti-ATG5 (1:1,000) (Santa Cruz Biotechnology); anti-acetyl-p53 Lys373 (1:1,000), anti-acetyl-p53 Lys320 (1:1,000) (Upstate Biotechnology); anti-TBP (1:1,000), anti-LAMP2 (1:1,000) (Abcam); and anti-GAPDH (1:1,000) (Thermo Scientific). The appropriate secondary HRP-linked species-specific whole secondary antibodies were used next, and bound antibodies were detected using ECL Plus Western blotting detection reagents (GE Healthcare).

Immunoprecipitation. For detection of acetylated ATG proteins, 2 mg of cell lysates was immunoprecipitated with a rabbit anti-acetylated lysine antibody (1:100) in lysis buffer supplemented with deacetylase inhibitors 50 mM trichostatin A and 500 mM nicotinamide Sigma-Aldrich). This was followed by Western blot analysis using anti-ATG7, anti-ATG5, anti-ATG12, or anti-LC3 antibodies.

Immunoprecipitation. For immunoprecipitation of endogenous p300, cells were lysed in complete lysis buffer (50 mM Hepes pH 7.5, 150 mM NaCl, 1.5 mM $MgCl_2$, 100 mM NaF, 10 mM NaPPi, 1 mM EDTA, 10% glycerol, 1% Triton X-100, and protease inhibitors; Roche Applied Science) at 4 °C for 1 h, followed by overnight immunoprecipitation at 4 °C with a rabbit polyclonal anti-p300 antibody (N15; 1:1,000). Protein A Sepharose beads (GE Healthcare) were added at 4 °C for 2 h, followed by three washes with washing buffer (50 mM Hepes pH 7.5, 150 mM NaCl, 1 mM EDTA, 10% glycerol, and 1% Triton X-100). Immunoprecipitates were separated by SDS/PAGE, and BAT3 or ATG7 was detected by immunoblotting.

Quantitative Real-Time PCR. Real-time PCR was performed in triplicate with SYBR Green Master Mix and the Light Cycler 480 System (Roche Applied Science), with the following cycling parameters: 95 °C for 10 min; 40 cycles of 95 °C for 15 s, 60 °C for 10 s, and 72 °C for 15 s; and then a melting curve protocol. The primers used to amplify *Bat3, p53, Dram1, Sestrin1, Sestrin2, Tigar, Puma*, and *p21* were 5′-TGCTGACAGAGGCAGCTATG-3′ and 5′-AGCCTGGAGGTACTGGTGAA-3′; 5′-GCAACTATGGCTTCCACCTG-3′ and 5′-CAGAGAGCACCGCGACCACG-3′; 5′-ATGCCTAAAACTGCAGCGAT-3′ and 5′-ACACTCCACCATGTTGACCC-3′; 5′-GTGGACCCAGAACGAGATGACGTGGC-3′ and 5′-GACACTGTGGAAGGCAGCTATGTGC-3′; 5′-TCCGAGTGCCATTCCGAGAT-3′ and 5′-TCCGGGTGTAGACCCATCAC-3′; 5′-GAAACCCAGTCTCCGAAAGG-3′ and 5′-CTTGACCGTTATCCGCCAT-3′; 5′-TGTCGATGCTGCTCTTCTTG-3′ and 5′- GTGTGGAGGAGGAGGAGTGG-3′; and 5′-ATCACCAGGATTGGACATGG-3′ and 5′-CGGTGTCAGAGTCTAGGGGA-3′, respectively. mRNA expression levels were normalized versus the expression level of the two housekeeping genes *Mrs9* and *18S*.

Retroviral Infection. Retroviral particles were produced in 293T cells by transfection of gag/pol and env VSV-G and viral vector pSIREN shluc or pSIREN shp300 or pQCXIH CMV/TO DEST BAT3FL or pQCXIH CMV/TO DEST BAT3ΔNLS using JET-PEI (Polyplus Transfection). At 48 h after transfection, cell supernatants containing viral particles were harvested and then added overnight to the culture medium of mouse embryonic fibroblasts (MEFs) with 8 μg/mL polybrene (Sigma-Aldrich).

BAT3 FL and BAT3ΔNLS sequences from PCI-HA BAT3 FL and PCI-HA BAT3ΔNLS were subcloned into pQCXIH CMV/TO DEST using Gateway Cloning Technology (Life Technologies).

BAT3$^{-/-}$ MEFs stably expressing BAT3 FL and BAT3ΔNLS were selected using 100 μg/mL hygromycin B (Sigma-Aldrich). ShRNA directed against mouse p300 mRNA (CCATGTTG-CATTCAACTATAA) or firefly luciferase mRNA as a control were cloned into the retroviral vector RNAi Ready pSIREN (Clontech) following the manufacturer's instructions. shRNA-expressing cells were selected with 2 μg/mL of puromycin (Sigma-Aldrich) at 48 h after infection.

1. Desmots F, Russell HR, Lee Y, Boyd K, McKinnon PJ (2005) The reaper-binding protein scythe modulates apoptosis and proliferation during mammalian development. *Mol Cell Biol* 25(23):10329–10337.

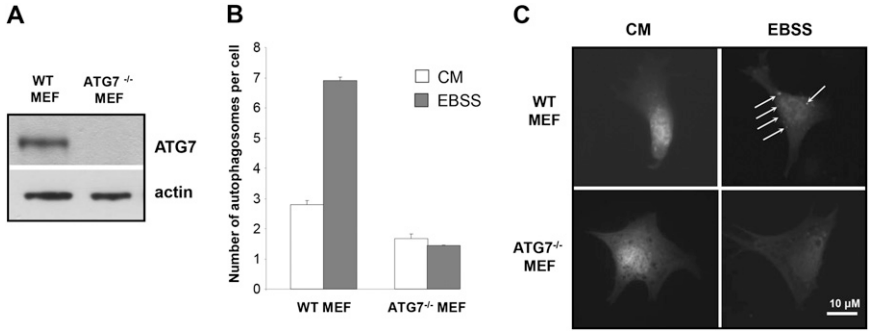

Fig. S1. (*A*) Western blot analysis of ATG7 expression. (*B*) Quantification of autophagosomes per cell in WT and WT and ATG7$^{-/-}$ MEF clones transfected with GFP-LC3. (*C*) Representative images of GFP-LC3 staining. Results are mean (SD) of the number of autophagosomes per cell from three experiments. Arrows indicate GFP-LC3 dots (i.e., autophagosomal structures).

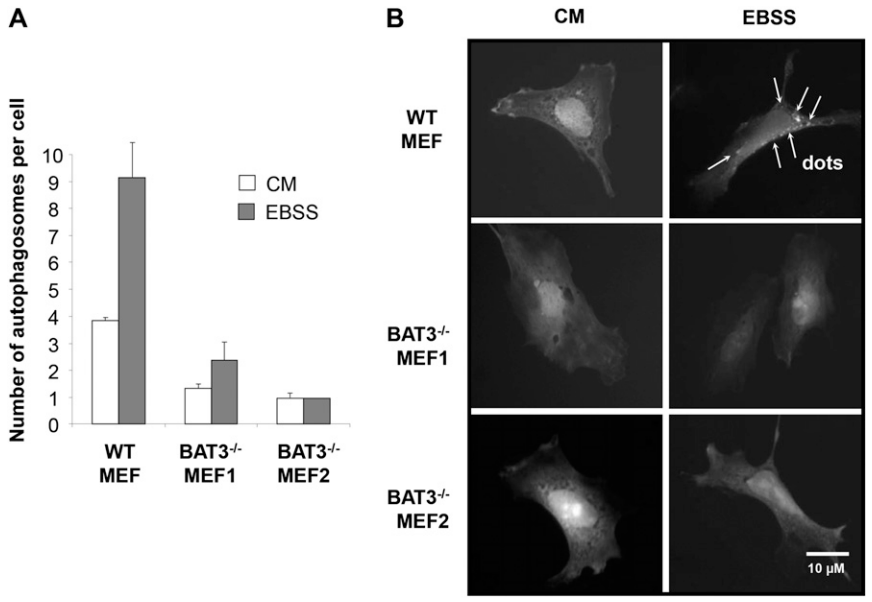

Fig. S2. (*A*) Quantification of autophagosomes per cell in WT and two different *BAT3*$^{-/-}$ MEF clones transfected with GFP-LC3. (*B*) Representative images of GFP-LC3 staining. Results are mean (SD) of the number of autophagosomes per cell from three experiments. Arrows indicate GFP-LC3 dots (i.e., autophagosomal structures).

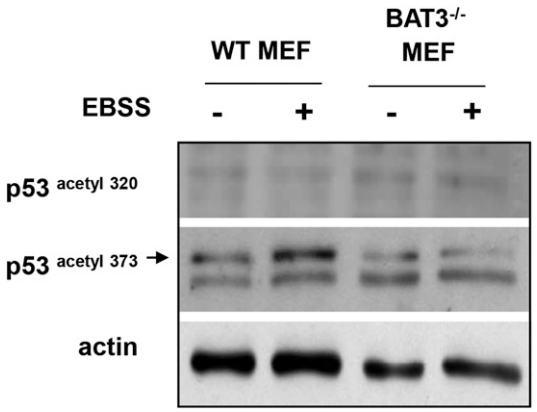

Fig. S3. Western blot analysis of p53 acetylation at lysine 373 and lysine 320 in WT and *BAT3*$^{-/-}$ MEFs in CM (–) or EBSS (+) for 30 min. Cell extracts were immunoblotted using anti-p53 acetylated at lysine 373, anti-p53 acetylated at lysine 320, and anti-actin antibodies.

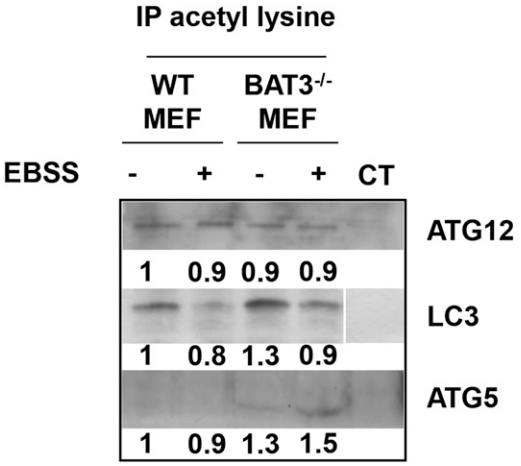

Fig. S4. Western blot analysis of ATG12, LC3, and ATG5 acetylation after immunoprecipitation (IP) of 2 mg of protein lysates using an antibody that recognizes the acetyl-lysine residues in WT and *BAT3*$^{-/-}$ MEFs. Cells were grown in CM (–) or EBSS (+) for 2 h before immunoprecipitation. The densitometric intensity was measured for each condition and compared with that of WT MEFs in CM.

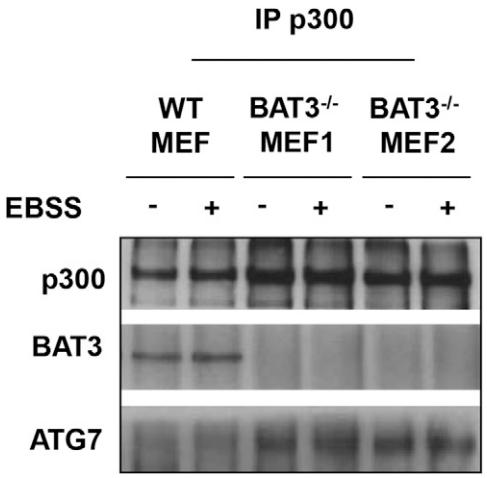

Fig. S5. Coimmunoprecipitation (IP) of ATG7 with an anti-p300 antibody in WT and two different *BAT3*$^{-/-}$. MEF clones in CM (–) or switched to EBSS (+) for 2 h. The control is the same as in Fig. 4*A*.

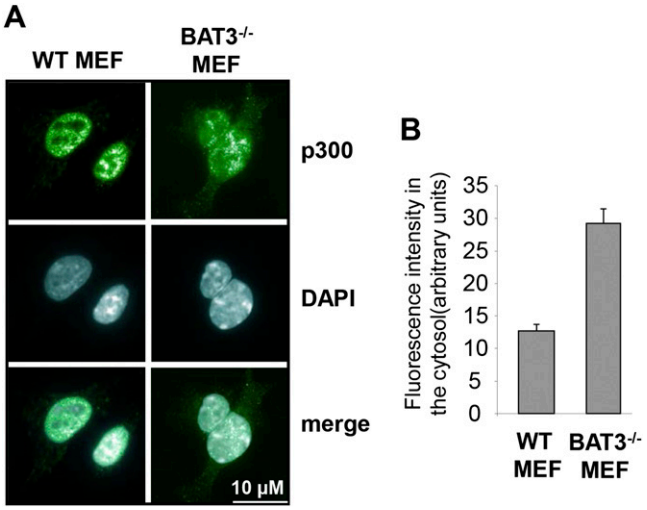

Fig. S6. (*A*) Representative light microscopy images of endogenous p300 in WT and *BAT3*$^{-/-}$ MEFs. Nuclei were visualized with DAPI stain. (*B*) Quantification of the fluorescence intensity of p300 in the cytosol of WT and *BAT3*$^{-/-}$ MEFs. Results are mean (SD) cytosol fluorescence intensity (in arbitrary units) of 100 cells in five independent experiments.