Supplemental Methods

Mice

Polymerase chain reaction (PCR)-based genotyping was performed using the following primer sets: Eμ-*Myc*-positive (5'-AGACGTCAGGTGGCACTTTT-3' and 5'-AGCAAAAACAGGAAGGCAAA-3'), Eμ-*Myc*-negative internal control (5'-CTAGGCCACAGAATTGAAAGATCT-3' and 5'-GTAGGTGGAAATTCTAGCATCATCC-3'), *Bif-1*-positive (5'-CTTAGTGAGCTGTCAGGAGAGC-3' and 5'-AGGTTCTCATGGGAACAGCGAC-3'), *Bif-1*-negative (5'-CTTAGTGAGCTGTCAGGAGAGC-3' and 5'-TCGCCTTCTTGACGAGTTCT-3'). All mice were monitored daily for signs of morbidity and lymphoma development and euthanized when deemed moribund. All animal protocols were approved by the Pennsylvania State University Animal Care and Use committee.

Histological and immunohistochemical analyses

Formalin-fixed tissues were processed in an automated Tissue-Tek VIP processor, paraffin-embedded with a Tissue-Tek TEC embedding station (Sakura Finetek USA) and sectioned at 6 μm thickness. For histopathological analysis, sections were stained with haematoxylin and eosin. Scoring of lymphoblast infiltration of the lung, liver and spleen was determined semiquantitatively based on percent interstitial lymphoblast infiltration of the parenchyma (spleen, liver and lung) as well as distension of the lumina of sinusoids (liver) or alveolar capillaries (lung), consistent with leukemia. All tissues were examined by a diplomate American College of Veterinary Pathologist (T.K.C.) blinded to treatment/genotype/intervention. For immunohistochemistry, sections mounted on charged (plus) slides were deparaffinized and heat-induced antigen retrieval performed in citrate buffer. Endogenous peroxide was blocked with 3% hydrogen peroxide in methanol, and slides were incubated for 1 hour at room temperature with antibodies to cleaved caspase-3 (C-Casp-3, Cell Signaling 9661, 1:1600) or PCNA (Santa Cruz sc-56, 1:2000) followed by biotinylated secondary antibody using a Vector Elite ABC kit and DAB chromogen, counterstained with Mayer's haematoxylin. Specific cytoplasmic (C-Casp-3) or nuclear (PCNA) staining was counted in three random 400x High Power Field (HPF) by a single individual blinded to intervention. All images were obtained with an Olympus BX51 microscope and DP71 digital camera using MicroSuite Basic 2.6 imaging software (Olympus).

Immunoblot analyses

Tissue lysates were prepared in radio-immunoprecipitation assay buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.4, 0.1% SDS, 1% Triton X-100, 1% Deoxycholate, 5 mM EDTA, pH 8.0) containing protease and phosphatase inhibitors using a Tissuemiser homogenizer (Thermo Fisher Scientific). Antibodies used for immunoblot analyses are as follows: mouse monoclonal anti-β-Actin (Sigma A5441), rabbit polyclonal anti-p19ARF (GeneTex GTX 20080), rabbit polyclonal anti-Bax (Santa Cruz sc-493), rabbit monoclonal anti-Bcl-2 (Cell Signaling 2870), mouse monoclonal anti-Bcl-xL (Sigma B9429), mouse monoclonal anti-Bif-1 (Imgenex Img265A), rabbit

polyclonal anti-Cleaved Caspase 3 (Cell Signaling 9661), mouse monoclonal anti-GAPDH (Imgenex IMG-5019A-1), rabbit polyclonal anti-Mcl-1 (Rockland 600-401-394), sheep polyclonal anti-p53 (Calbiochem PC35), guinea pig polyclonal anti-P62 (American Research Products 03-GP62-C) and rabbit polyclonal γ-H2AX (Cell Signaling 2577).

Cell culture and virus production/transduction

SV40 large T antigen-immortalized mouse embryonic fibroblasts (MEFs) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 3 mM fresh L-glutamine, 100 μg/ml streptomycin, 100 units/ml penicillin and 250 ng/ml amphotericin B. The cDNAs encoding myc-Parkin and mCherry-Parkin were obtained from Addgene (#17612, #23956, respectively) and subcloned into the EcoRI-NotI site of pCDH1-MCS1-EF1-puro. The cDNA encoding AcGFP and Bif-1-AcGFP were subcloned into the NheI-NotI site of pCDH1-MCS1-EF1-puro. To generate pCDH1-Mito-mCherry-EF1-puro vector, Mito-DsRed2 cDNA obtained from pDsRed2-Mito (Clontech, #632421) was subcloned into the NheI-NotI site of pCDH1-MCS1-EF1-puro. The cDNA encoding mCherry was amplified by PCR and then subcloned into the BamHI-NotI site of pCDH1-Mito-DsRed2-EF1-puro to substitute DsRed2 for mCherry. The cDNA encoding Mito-mCherry and Bif-1-AcGFP were subcloned into the NheI-NotI site of pCDH1-MCS1-EF1-puro. Recombinant lentiviruses were produced and transduced to targeted MEFs as previously described.[1]

Immunofluorescence microscopy

Cells treated with 30 μM Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) (SIGMA, C2759) for the indicated periods of time were fixed in 4% paraformaldehyde-PBS, permeabilized with 50 μg/ml digitonin for 10 min, incubated with the primary antibodies against Tom20 (Santa Cruz, #sc-11415), Hsp60 (BD Biosciences, #611562), LC3 (MBL International, PM046) and Atg16L (MBL International, PM040) followed by the secondary antibodies conjugated with AlexaFluor 350 or AlexaFluor 488. Images were obtained using an OLYMPUS IX81 deconvolution microscope and analyzed using SlideBook 5.0 software (Intelligent Imaging Innovations).

Supplemental Reference

1. Young MM, Takahashi Y, Khan O, et al. Autophagosomal membrane serves as platform for intracellular death-inducing signaling complex (iDISC)-mediated caspase-8 activation and apoptosis. *The Journal of biological chemistry*. 2012;287(15):12455-12468.

Supplemental Figure Legends

Supplemental Figure 1. *Bif-1* is important for embryogenesis of Eμ-*Myc* transgenic mice. Eμ-*Myc*/*Bif-1*+/- males were crossed with *Bif-1*+/- or *Bif-1*-/- females to generate Eμ-*Myc*/*Bif-1*-/- mice. Gross images of whole uteruses and embryos dissected from pregnant *Bif-1*+/- females at E9.5 (a-e) and E13.5 (f-l) are shown. Note that one of the Eμ-*Myc*/*Bif-1*-/- embryos (shown in a) was already partially resorbed or stopped developing at E9.5. The total number of Eμ-*Myc*/*Bif-1*-/- mice and resorbed embryos obtained from the indicated breeding pairs at the indicated developmental stages are summarized and shown in Supplemental Table 1.

Supplemental Figure 2. *Bif-1* is not required for CCCP-induced Parkin translocation to mitochondria. Representative images at 0 and 6 h time-points in Figure 5B are shown. Arrowheads and arrows represent colocalization of Parkin with Tom20 and Hsp60 and Hsp60-positive and Tom20-negative structures, respectively. The scale bars represent 10 μm.

Supplemental Figure 3. Bif-1 and Parkin are indispensable for CCCP-induced mitophagy in MEFs. (A) myc-Parkin/*Bif-1*-/- MEFs were transduced with lentiviruses encoding Bif-1-AcGFP, pre-stained with MitoTracker CMTMRos and subjected to CCCP treatment for 24 h. Note that cells with restored Bif-1 expression (designated by green dotted lines) regained the capability to degrade mitochondria upon CCCP treatment. (B) The parental *Bif-1*+/+ or *Bif-1*-/- MEFs were treated with 30 μM CCCP for the indicated periods of time were subjected to immunoblot analysis using the indicated antibodies.

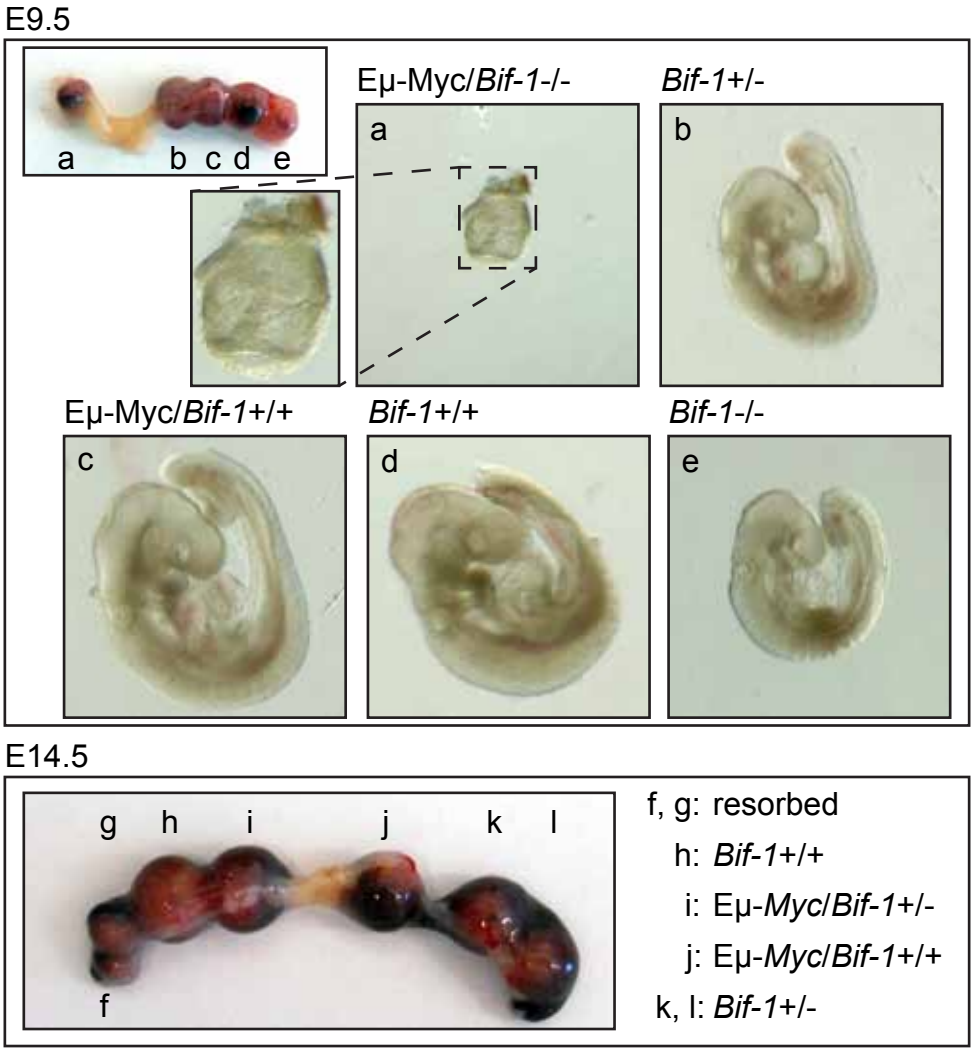
Supplemental Figure 4. Loss of *Bif-1* mitigates p62 downregulation and results in the accumulation of mitochondria under hypoxic and low nutrient conditions. *Bif-1*+/+ and *Bif-1*-/- MEFs were cultured under ischemic condition (HBSS supplemented with 0.5% FBS and 5% DMEM without phenol red, 1% O_2) for the indicated periods of time and subjected to immunoblot analysis using the indicated antibodies.

Supplemental Figure 5. *Bif-1* is dispensable for nascent autophagosome formation and lysosome accumulation in proximity of damaged mitochondria during mitophagy. MEFs stably expressing mCherry-Parkin (A) or MEFs expressing myc-Parkin pre-stained with MitoTracker CMTMRos (B) were treated with 30 μM CCCP for the indicated periods of time and co-immunostained with the indicated antibodies. Magnified images were shown in lower panels. The scale bars represent 10 μm and 1 μm in the magnified images.

Supplemental Figure 6. Bif-1 is dispensable for Parkin-mediated OMM rupture but indispensable for the clearance of damaged mitochondria in response to CCCP treatment. myc-Parkin/*Bif-1*+/+ and myc-Parkin/*Bif-1*-/- MEFs were treated with control DMSO (A) or 30 μM CCCP (B) for 24 h and subjected to electron microscopic analysis. Black arrowheads and arrows in *B* indicate ER membranes and ER-associated IMs,

respectively. Open arrows and double-arrowheads in *B* indicate nascent or Avi-like structures and fragmented mitochondria that were associated with IM/Avi-like structures, respectively. The scale bars represent 1 μm.

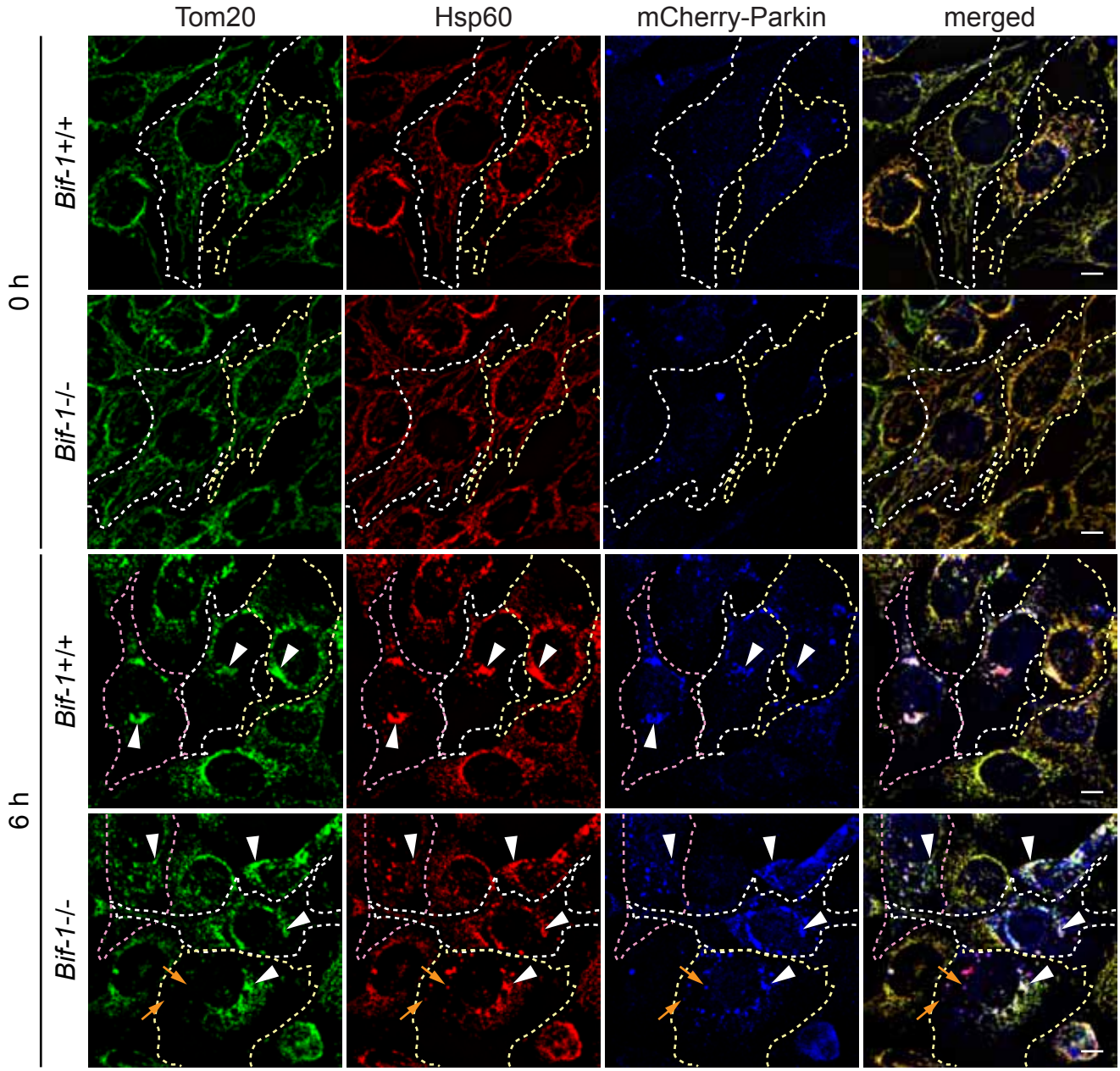
Supplemental Figure 1



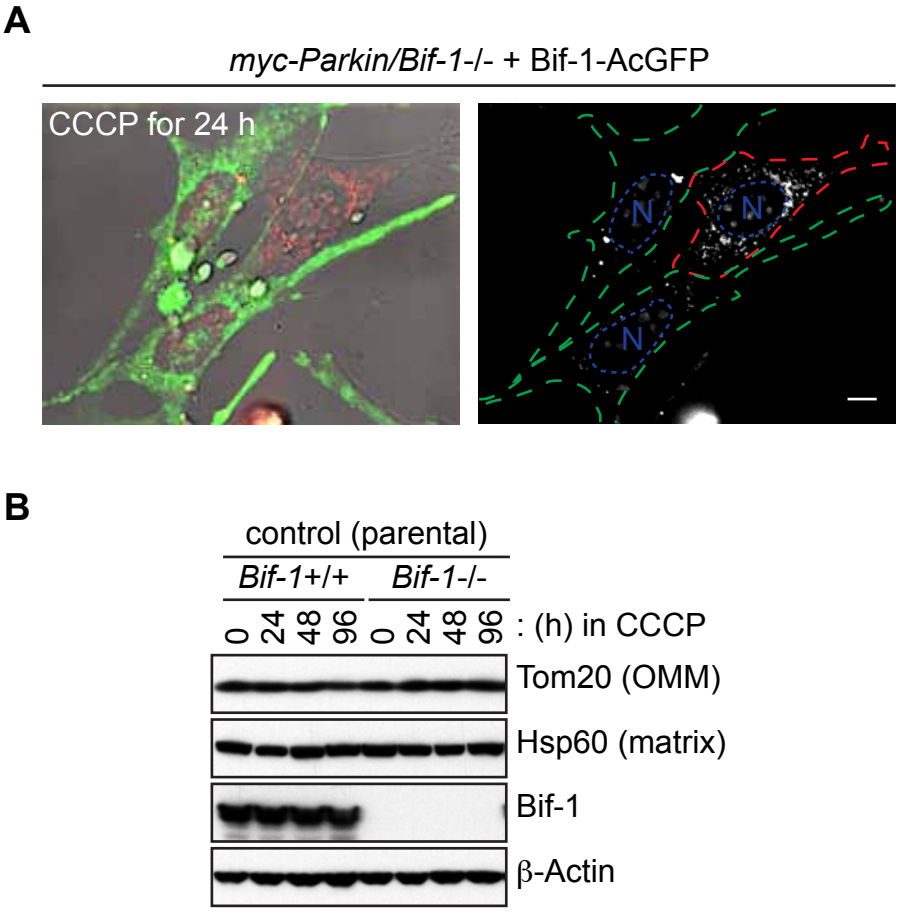
Supplemental Table 1

stage	mating pair (male x female)	total no	no of Eμ-*Myc*/*Bif-1*-/-	expected no of Eμ-*Myc*/*Bif-1*-/-	no of resorbed embryos
E9.5	Eμ-*Myc*/*Bif-1*+/- x *Bif-1*+/-	5	1 (20.0%)	0.6 (12.5%)	0
	Eμ-*Myc*/*Bif-1*+/- x *Bif-1*-/-	20	3 (15.0%)	5.0 (25.0%)	1
E12.5	Eμ-*Myc*/*Bif-1*+/- x *Bif-1*+/-	18	0 (0.0%)	2.3 (12.5%)	4
	Eμ-*Myc*/*Bif-1*+/- x *Bif-1*-/-	11	1 (9.1%)	2.8 (25.0%)	1
E14.5	Eμ-*Myc*/*Bif-1*+/- x *Bif-1*+/-	9	0 (0.0%)	1.1 (12.5%)	2
new born	Eμ-*Myc*/*Bif-1*+/- x *Bif-1*+/-	84	3 (3.6%)	10.5 (12.5%)	N/A
	Eμ-*Myc*/*Bif-1*+/- x *Bif-1*-/-	92	9 (9.8%)	23.0 (25.0%)	N/A
	Eμ-*Myc*/*Bif-1*-/- x *Bif-1*-/-	2	1 (50.0%)	1.0 (50.0%)	N/A

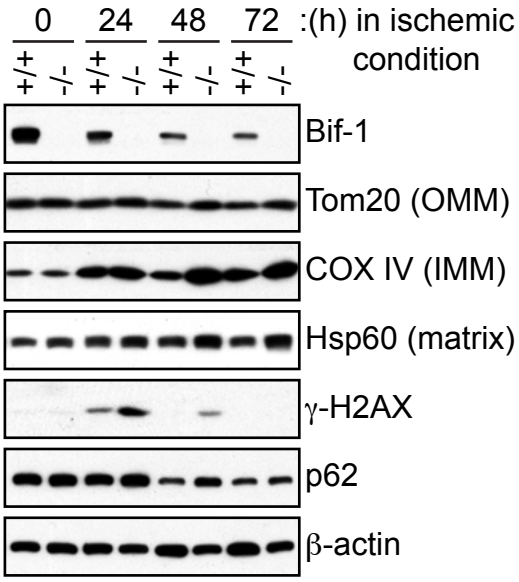
Supplemental Figure 2



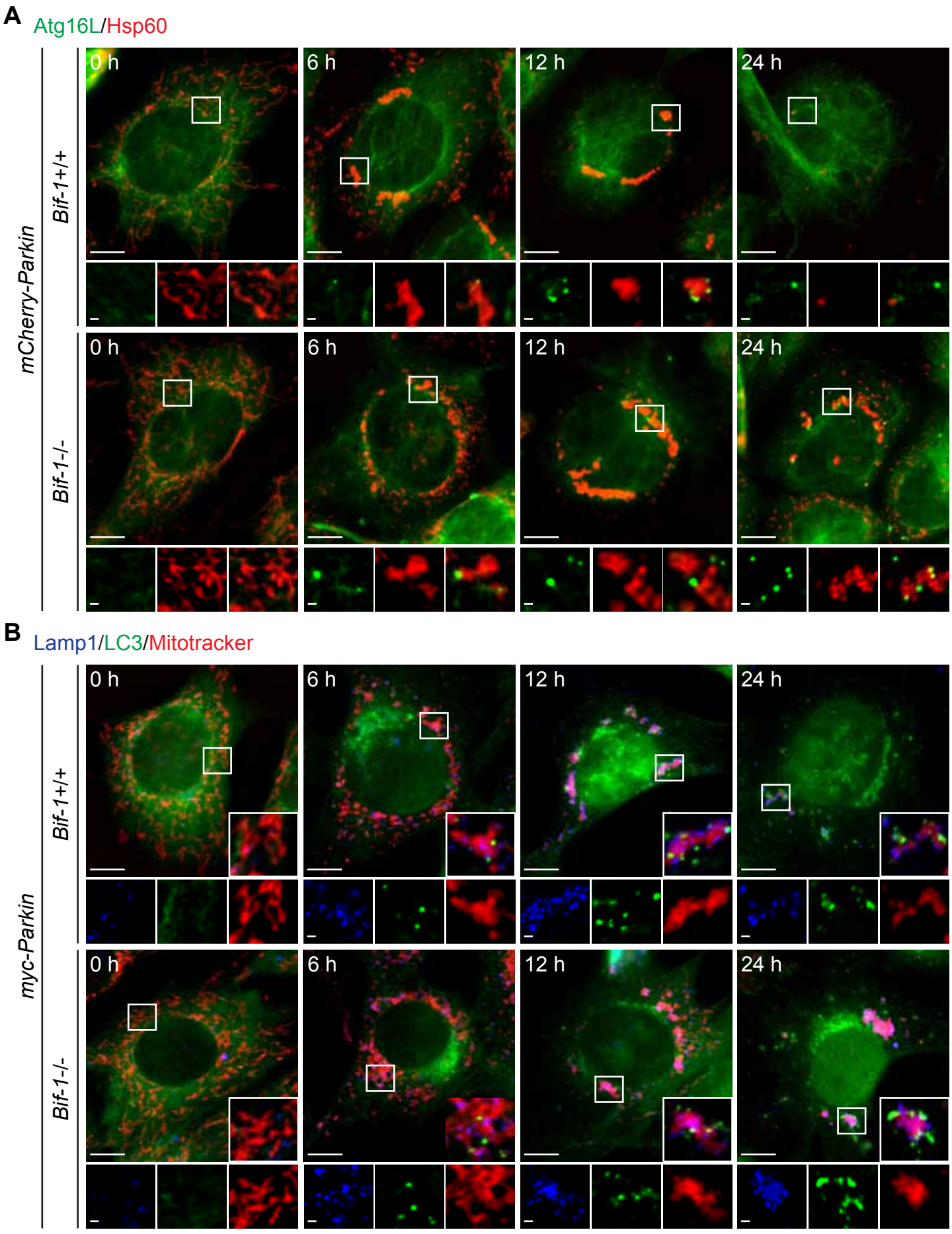
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6