SUPPLEMENTAL MATERIAL

Supplemental Methods:

Experimental animals. WT C57BL/6 mice were conditioned in-house for 5-6 days after arrival with commercial diet and tap water available at will. Mouse strains of cardiac-specific overexpression of Beclin-1 under the α-myosin heavy chain promoter (*Becn1*-Tg)[1] or haploinsufficient for *beclin-1* (*Becn1*$^{+/-}$)[2] were previously developed.

LPS-induced sepsis model. We weighed 8- to 12-week-old male mice to determine the amount of LPS required to achieve indicated doses and administered i.p. in a volume of 100 µl per mouse. Sterile endotoxin-free PBS was used as vehicle control in sham groups. To analyze autophagic flux, bafilomycin A 1 (BafA1; 1.5 mg/kg in PBS) was given i.p. 2 hours before the mice were euthanized. To minimize variability, food was removed 1 hour before BafA1 injection and mice were euthanized at the same time of day to limit circadian variability of autophagy for all experiments. In some experiments, TB-peptide, synthesized according to published sequence[3], was administered i.p. at a dose of 16 mg/kg in 100 µl PBS 30 minutes post LPS challenge.

Preparation of blood serum, tissue lysates, and cellular fractions. When animals were sacrificed, blood was collected using BD vacutainer rapid serum

tubes (BD Diagnostics, Franklin Lakes, NJ) followed by immediate centrifugation at 3,000 g for 15 min at 4°C to isolate serum. The serum preparations were then allocated and stored at -80°C until used. Tissues were harvested, washed in PBS, snap-clamp frozen, and kept at -80°C. Tissue lysates were prepared using CelLyticMT reagent (Sigma-Aldrich, Saint Louis, MO) whereas mitochondrial and cytosolic fractions were separated by differential centrifugation using a mitochondria isolation kit for tissue (Thermo Scientific, Rockford, IL) according to manufacturer's protocols. Protein concentrations were quantified by protein assay kit (BioRad, Hercules, CA, catalog number 500-0122).

Mitochondrial respiratory complex I, II, and III enzyme assays. The activities of mitochondrial complexes were measured using enzyme assay kits according to manufacturer's protocols (Abcam, Cambridge, MA, catalog numbers ab109721 and ab109905). Freshly isolated mitochondrial pellets were resuspended in PBS supplemented with 10% detergent provided in the kits. Protein concentrations of these mitochondrial lysates were estimated and 25 μg (for complex I) or 100 μg (for complex II + III) mitochondrial protein was used per reaction. Enzyme activities were measured spectrophotometricly in triplicate and expressed as changes of absorbance per minute per mg of protein.

Detection of free cytosolic or extracellular mtDNA fragments. In the cytosolic fractions prepared from the heart tissue and culture medium from the primary cardiomyocytes, total DNA was isolated using a DNA extraction kit (Qiagen,

Valencia, CA, catalog number 69506). TaqMan exogenous internal positive control (IPC) DNA (Life Technologies, Carlsbad, CA, catalog number 4308323) was spiked into all samples prior to DNA isolation as a positive control. TaqMan real-time PCR (RPCR) assays were performed using primers against rat mtDNA gene NADH or IPC according to manufacturer's protocol. Samples were tested in triplicates. Results were expressed as a ratio of target gene to IPC (all RPCR reagents and gene assays were from Life Technologies, Carlsbad, CA).

Electron microscopy. Hearts were retrograde perfused (buffer: 4% paraformaldehyde/1% glutaraldehyde/0.1M Na Cacodylate, pH7.4). Small blocks of tissue from the midsection of the left ventricular wall were fixed (buffer: 2.5% glutaraldehyde/0.1M Na Cacodylate, pH7.4). Sections (75-80 nm) were cut using a Leica Ultramicrotome and examined under electron microscopy.

Histology and immunostaining. Fresh heart tissues were fixed in 4% paraformaldehyde, transferred to 18%-10% sucrose in PBS, and embedded in OCT. Samples were sectioned at 8 μm, air-dried and stored at -80°C until used. Frozen slides were then thawed, rehydrated, and subjected to either histological staining with mason trichrome or immunochemistry. For the later, slides were blocked with 3% donkey serum in PBS and stained with a mouse monoclonal antibody against αSMA (Abcam, Cambridge, MA, catalog number ab7817; 1:400), a mouse monoclonal antibody against myeloperoxidase (Hycult Biotech, Plymouth Meeting, PA, catalog number HM1051; 1:50), a rat monoclonal

antibody against Lamp1 (Santa Cruz Biotechnology, Santa Cruz, CA, catalog number sc-1992; 1:300), or a rabbit monoclonal antibody against Mfn2 (Cell Signaling, Danvers, MA, catalog number 9482; 1:100) for 1 hour followed by another 1 hour of incubation with Cy5 labeled goat anti-mouse-IgG (Abcam, Cambridge, MA, catalog number ab97037; 1:500), anti-rabbit-IgG (Jackson ImmunoResearch, West Grove, PA, catalog number 711-606-152; 1:200), or FITC-labeled anti-rat-IgG (Jackson ImmunoResearch, West Grove, PA, catalog number 712-545-153; 1:200) at room temperature. The slides were then examined under a Nikon Eclipse Ti-E inverted microscope at 40x magnification.

Western blot. Prepared SDS-PAGE protein samples were subjected to 4%-20% SDS-PAGE gels, and transferred to PVDF membranes. Membranes were blocked with 5% nonfat milk-PBS at room temperature for 30 minutes and subsequently probed with one of the following antibodies according to experiments: GAPDH, FUNDC1 (Millipore, Billerica, MA, catalog numbers MAB374 and ABC506), LC3, p62, Beclin-1, BNIP3, AMPKα, pAMPKα, pULK1, and mTOR pathway antibodies (Cell Signaling, Danvers, MA, catalog numbers 4108, 5114, 3495, 3769, 2532, 2535, 6888, and 9964), VDAC1, ULK1 (Abcam, Cambridge, MA, catalog number ab14734 and ab128859), Parkin, Ubiquitin (Santa Cruz Biotechnology, Santa Cruz, CA, catalog number sc-30130 and sc-8017), PINK1 (Novus Biologicals, Littleton, CO, catalog number BC100-494) or Cox I (Abcam, Cambridge, MA, catalog number ab110411). The membranes were then rinsed and incubated with corresponding horseradish peroxidase-

conjugated secondary antibodies (Bio-Rad, Hercules, CA, catalog number 170-6515 and 170-6516). Antibody dilutions and incubation time were according to manufacturer's instructions. Membranes were then rinsed and bound antibodies were detected by using SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL, catalog number 34077).

Measurements of cytokines by enzyme-linked immunosorbent assay (ELISA). Cytokine levels in serum or in total heart lysates were measured using Bio-Plex Mouse Cytokine Panel A 6-Plex (Bio-Rad, Hercules, CA, catalog number M6000007NY) according to vendor's instructions. Results were normalized by volume for serum samples and by protein amount for tissue lysates. All samples were tested at least in triplicate.

Echocardiography. Transthoracic echocardiograms were recorded in sedated mice using Visualsonics Vevo 2100 small animal echocardiography machine. Views were taken in planes that approximated the parasternal short-axis view and the apical long-axis view in humans.

Supplemental References:

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2. Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, Rosen J, Eskelinen EL, Mizushima N, Ohsumi Y, Cattoretti G and Levine B. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *The Journal of clinical investigation*. 2003;112:1809-20.
3. Pietrocola F, Pol J, Vacchelli E, Rao S, Enot DP, Baracco EE, Levesque S, Castoldi F, Jacquelot N, Yamazaki T, Senovilla L, Marino G, Aranda F, Durand S, Sica V, Chery A, Lachkar S, Sigl V, Bloy N, Buque A, Falzoni S, Ryffel B, Apetoh L, Di Virgilio F, Madeo F, Maiuri MC, Zitvogel L, Levine B, Penninger JM and Kroemer G. Caloric Restriction Mimetics Enhance Anticancer Immunosurveillance. *Cancer Cell*. 2016;30:147-60.