Supplemental Materials

Materials and Methods

Animal Protocols:

Male 8-10 wk. old FVB/NJ mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were subjected to exercise on a treadmill equipped for simultaneous measurement of VO2 and VCO2 (Columbus Instruments, Columbus, OH), and distance ran and respiratory exchange ratio (RER) were determined using our established protocols [23]. Mice were exercised using two different protocols. First, sub-maximal exercise was performed for 1 h at a speed of 20 m/min with a constant grade of 5°. After 1 h mice were sacrificed and tissues prepared for assessment. Exercise capacity using this protocol was assessed by running mice to exhaustion at a speed of 20m/min at a constant grade of 5° and recording total distance ran. Second, maximal exercise capacity was assessed using a protocol that incrementally increased the degree of elevation by 2° and the speed by 2 m/min every 3 min. Mice were exercised until exhaustion while monitoring VO2 and CO2 levels according to our previously published methodology [23]. RER, a surrogate for reaching anaerobic threshold, was calculated as the ratio VCO2/VO2. Exercise time was determined as the time until exhaustion or 10 seconds while resting on the shock grid. Exercise distance was calculated as the total vertical distance traveled (meters) = Treadmill speed (meters/min) X Percent grade (Sin θ) X Exercise time (min). Work (Joules) was calculated as the body weight (kg) X total vertical distance traveled (meters) X 9.8. Myocardial infarction was induced under isoflurane anesthesia (1.5-3%). A 22-gauge catheter is used to intubate the mouse, which is placed on a Harvard small animal ventilator. A lateral incision between the fourth and fifth ribs is made to open the chest. Myocardial infarction is induced using left anterior descending (LAD) artery ligation, using a 8-0 silk suture passes with a tapered needle underneath the LAD, posterior to the tip of the normally positioned left atrium. The thoracic cavity body wall is then closed with a 7-0 silk suture, and the skin closed with a 6-0 silk suture using an interrupted

pattern. Sham mice underwent the same procedure, with the absence of LAD ligation. Mice were then assessed 24h after surgery. Protein extractions were sampled from the remote zone. Mice were also exposed to 10mg/kg of doxorubicin (NovaPlus, Bedford, OH) or saline control by tail vain injection. After 30min exposure, mice were harvested for protein analysis.

β-Adrenergic Signaling and Mitochondrial Dynamics Modulation:

To assess subtype-specific β-AR signaling, we utilized β2-AR knockout (-/-) mice generated on an FVB background in our laboratory [21]. To assay autophagic flux, mice were treated with Chloroquine (50mg/kg) by intraperitoneal (IP) injection 1hr prior to exercise testing. Chloroquine treated mice were exercise for 1hr at 20m/min and sacrificed immediately after exercise. To assess mitochondrial fission, mice were treated with the Drp1-Fis1 inhibitor P110, provided by Dr. Daria Mochly-Rosen, (0.5 mg/kg) or Mdivi-1 (25 mg/kg) by intraperitoneal injection, followed immediately by exercise testing. Either saline, TAT47-57 peptide (0.35 mg/kg) or 2% DMSO in saline were used as control treatments. To assess the role of β1-AR signaling, mice were treated with the β1-AR antagonist metoprolol (5mg/kg) by IP injection 90 minutes prior to maximal exercise challenge. To assess cardiac specific mitochondrial fission, $Drp1^{loxp/loxp}$ [22] C57B/6NJ mice were provided by Dr. Katsuyoshi Mihara and Dr. Masatoshi Nomura and were crossed onto the *myh6*-MER-Cre-Mer C57B/6NJ mouse line (Jackson Laboratory, Bar Harbor, ME) and gene recombination was induced by administration of tamoxifen (20mg/kg) IP for 5 days. On day 7 mice were used for experimentation. Male aged-matched Drp1+/+Cre+/+ and Drp-/-Cre-/- were treated with tamoxifen and used as controls.

Cell culture and treatment with agonists/antagonists

The HL-1 cardiac muscle and C2C12 skeletal muscle cell line were used for *in vitro* studies, maintained in Claycomb or DMEM media (Sigma-Aldrich, St Louis, MO), respectively, containing 10% FBS, 100g/mL penicillin/streptomycin, 0.1mM norepinephrine and 2mM L-glutamine. For at least one passage before experimentation, HL-1 cells were cultured in norepinephrine free media. When cells reached

100% confluence, they were treated with 10 μM isoproterenol (Sigma-Aldrich, St Louis, MO) (ISO) for 1h. Cells were pre-treated with 300 nM of the subtype specific inhibitors CGP 12177 (Sigma-Aldrich, St Louis, MO) (β1 antagonist) and ICI 118551 (Sigma-Aldrich, St Louis, MO) (β2 antagonist) and 1μM of L748,337 (Cayman Chem, Ann Arbor, MI) (β3 antagonist) and propranolol (Sigma-Aldrich, St Louis, MO) (β1&β2 antagonist) for 30 min before ISO exposure. Cells were also pretreated with 30μM of the mitochondrial division inhibitor Mdivi-1 for 30 min before ISO exposure. After ISO exposure, cells were either washed with PBS or used directly for respiration measurement. C2C12 or isolated adult cardiac myocytes were exposed to 100μM doxorubicin. After exposures, cells were either washed with PBS or used directly for morphology or respiration measurement.

Echocardiography

Serial two-dimensional echocardiographs were performed on anaesthetized mice. Images were acquired with a GE Vivid 7 ultrasound system (GE Healthcare, Milwaukee, WI) with 13-MHz transducer. Left ventricular ejection fraction was quantified by m-mode echocardiography at the level of the papillary muscles. Parasternal long axis view in systole was used to image and calculate cardiac output as HR * SV where SV = LVOT area * LVOT VTI.

Mitochondrial isolation

Mitochondria were isolated from HL-1 cells by first detaching cells with 0.05% Trypsin/EDTA for 10 min, washing with Claycomb media and resuspending cells in a minimal volume of isolation buffer. Cells were then lysed by passing through a 27-gauge syringe (15 strokes). Cell lysates were placed on ice for differential centrifugation. Cardiac mitochondria were isolated from ventricular tissue by first mincing and then homogenizing tissues using a Dounce homogenizer (20 strokes) in isolation buffer (300mM sucrose, 10mM hepes, 2mM EGTA, pH 7.2, 4°C). Tissue lysates were then placed on ice for differential centrifugation. Both cell and cardiac tissue lysates were centrifuged at 500 x g for 10 min and the resulting supernatants were centrifuged at 10,000 x g for 15 min. The mitochondrial pellet was washed once with

isolation buffer and the final pellet resuspended in a minimal volume of isolation buffer and kept on ice for functional assays. If samples were used for Western blot, mitochondria were solubilized in isolation buffer containing 1% Triton X and stored at -80°C. Purity of mitochondrial fractions are confirmed by the absence of the cytosolic protein enolase.

Flow cytometry analysis of mitochondrial size.

Cardiac mitochondria were isolated immediately after exercise as described above. After the 10,000 x g mitochondrial pellet was secured, mitochondria were resuspended in mitochondrial isolation buffer containing 200nM Mitotracker Deep Red (Thermo Ficher Scientific, Waltham, MA). Mitochondria were incubated for 10 minutes at RT and then washed 2X in mitochondrial isolation buffer. After final wash, mitochondria were resuspended in 4% PFA and incubated at RT for 5 minutes. Mitochondria were washed 3X and stored in PBS at 4°C. Mitochondrial suspensions were then analyzed by flow cytometry. Briefly, mitochondria were thresholded by Mitotracker Deep Red fluorescence to exclude non-mitochondrial particles. 10,000 events were recorded and used for analysis. Preliminary assessment of known bead sizes showed that side scatter area (SSA) was the best measure to differentiate particle sizes ranging between 0.2μm-2.0μm (Supplementary Fig. 1) on an LSRII flow cytometer (BD Biosciences, San Jose CA). Side scatter area (SSA) was then plotted against forward scatter area (FSC) to assess mitochondrial size using FlowJo (FlowJo, Ashland, OR).

Myocyte isolation and membrane potential/ROS assessment.

Adult ventricular myocytes were isolated from 3 mo old FVB mice as previously published[3]. Briefly, mice were anesthetized with pentobarbital sodium (100 mg/kg ip). The heart was removed and retrograde perfused at 37^{o}C with a Ca^{2+} free solution (120mM NaCl, 14.7mM KCl, 0.6mM KH_2PO_4, 0.6mM Na_2HPO_4, 1.2mM $MgSO_4$-$7H_2O$, 4.6mM $NaHCO_3$, 10mM Na-HEPES, 30mM taurine, 10mM BDM, 5.5mM glucose) followed by enzymatic digestion with collagenase (Worthington Biochemical Corporation, Lakewood, NJ) (1.5 mg/ml). Ventricles were cut into small pieces and further digested by gently pipetting with plastic

transfer pipettes for 3-5 min. Stop buffer (Ca^{2+} free solution + $CaCl_2$ 12.5μM + 10% bovine calf serum (Hyclone, Logan, UT) was added and the cell suspension was collected in a 15 ml tube and centrifuged at 400 rpm for 3 min. Myocytes were resuspended in stop buffer in increasing $CaCl_2$ concentrations until 1mM was achieved. Experiments were performed with freshly isolated myocytes resuspended in a HEPES-buffered solution (12.5mM $CaCl_2$, 137mM NaCl, 5.4mM KCl, 15mM dextrose, 1.3mM $MgSO_4$, 1.2mM NaH_2PO_4, 20mM HEPES, pH 7.4). Cells were loaded in 96 well plates, then stained with 5μM MitoSox for 10 min to quantify ROS and 10nM TMRM for 30 min to quantify changes in mitochondrial membrane potential. Cells were imaged in a widefield fluorescence microscope (ImageXpress Micro, Molecular Devices, Sunnyvale, CA) and changes in intensity means were quantified using Imaris software.

Mitochondrial respiration assays

Respiration was assessed in isolated mitochondria by measuring O_2 consumption using an Oroboros Oxygraph (Oroboros Instruments, Innsbruck, Austria). Standardized instrumental and chemical calibrations were performed to correct for back-diffusion of O_2 into the chamber from leakage, consumption by the chemical medium and sensor consumption. Measurements were taken from 50 μg of mitochondria or 120,000 isolated cardiac myocytes in suspension (2 mL) gently agitated at 37°C. State 2 respiration (mito only) was assessed with the addition of glutamate (10 mM), malate (2 mM) and succinate (10mM) as the complex I and II substrates, and then State 3 respiration (mito only) was assessed by the addition of ADP (0.5 mM). State 4 (leak) respiration was assessed with the complex V inhibitor oligomyocin (2 μg/mL). Respiration due to reactive oxygen species formation was assessed with Antimycin A (2.5 μM). O_2 flux was measured by Datlab2 software (Oroboros Instruments, Innsbruck, Austria), capable of converting nonlinear changes in the negative time derivative of the oxygen concentration signal.

Mitochondrial respiration was also assessed in plated HL-1 cells by measuring O_2 consumption using the Seahorse XF24^e Analyzer. HL-1 cells were grown on a plastic 24 well plate, cultured with Claycomb media and allowed to grow to confluence. Before experimentation, cells were washed with supplemented XF assay media (Seahorse Bioscience, North Billerica, MA) and allowed to equilibrate for

1 h. Specified cell groups were then pre-treated with the β-blockers CGP 12177 or ICI 118551 (300 nM), immediately placed into the Seahorse XF24^e Analyzer and assessed for O_2 consumption for 30 min. After pre-treatment, cells were then exposed to ISO (10 μM) and O_2 consumption measured for 1 h. Following ISO treatment, ATP production, maximal respiration and non-mitochondrial respiration were assessed by treating cells with oligomycin (1 μM), FCCP (1 μM) and a rotenone (1 μM) / antimycin (1 μM) mixture, respectively. O_2 flux was measured by Wave software (Seahorse Bioscience, North Billerica, MA).

Protein and enzyme assays

Mitochondrial fractions were measured for protein content by Western blot. Commercially available antibodies for Drp1 (Enzo, Farmingdale, NY), Phospho Drp1 ser616 (Cell Signaling, Danvers, MA), Phospho ULK1 Ser555 (Cell Signaling, Danvers, MA), Phospho AMPK Thr172 (Cell Signaling, Danvers, MA), Mfn1 (Abnova, Taipei, Taiwan), Mfn 2 (Abnova, Taipei, Taiwan) and Parkin and PINK1 (Santa Cruz Bioscience, Santa Cruz, CA) were used to identify expression changes in mitochondrial dynamic proteins. VDAC1/Porin (Abcam, Cambridge, MA) was used as a loading control for mitochondrial proteins and α-actinin (Abcam, Cambridge, MA), enolase (Santa Cruz, Santa Cruz, CA) and GAPDH (Advanced Immunochemicals, Long Beach, CA) for cytosolic proteins. 4-HNE (Alpha Diagnostic, San Antonio, TX) was used to assess lipid peroxidation. To ensure faction purity, enolase was used verify that absence of cytosolic proteins in mitochondrial fractions, while Vdac1 was used to verify the absence of mitochondrial proteins in cytosolic fractions.

ATP measurement assays

HL-1 cells were seeded into a 96 well plate at a density of 5,000 cells and used next day for ATP measurements. Cells were treated with ISO (10μM) for 1 hr. After treatments, ATP was measured using a commercially available kit by Abcam (ab65313) according to manufactures instructions.

Microarray

The Stanford mouse transcriptome microarray was used to compare β2-/- and WT mice (Stanford Functional Genomics Facility, Stanford, CA). This microarray includes 43,200 mouse cDNA probes representing ~25,000 unique genes and expressed sequence tags. LV myocardium from nine β2-/- and nine WT mice were divided into three independent pools (3 per pool). Six cDNA arrays, which represented a total of 18 hearts, were analyzed. Previous studies have validated the use of pooling when tissue samples are small[4]. Microarrays were scanned using an Agilent G2565AA scanner, and features extracted and analyzed using SpotReader software (Niles Scientific, Sacramento, CA). Details of microarray analysis are included in statistical analysis, below.

Confocal microscopy

Mitochondrial morphology was assessed in HL-1 cells utilizing the fluorescent probe MitoTracker Red CMXROS (Thermo Ficher Scientific, Waltham MA). Cells were stained with MitoTracker for 30 min and fixed with 3.7% formaldehyde for 15 min. Cells were imaged using a Nikon Spinning Disk Confocal microscope and analyzed by NIS-Elements software. Morphology was quantified using an Image J macro that traces the outlines of mitochondria in confocal images. Images were converted into 32-bit format, inverted, sharpened and with autothreshold. Mitochondrial tracings were determined using a size parameter between 10-100000000 and circularity between 0-1. Confocal images of PBS, ISO, CGP and ICI treated HL-1 cells were assessed using the tracing macro and displayed as outlines. Outlined images were used in the quantitative analysis of mitochondrial morphology.

Electron microscopy

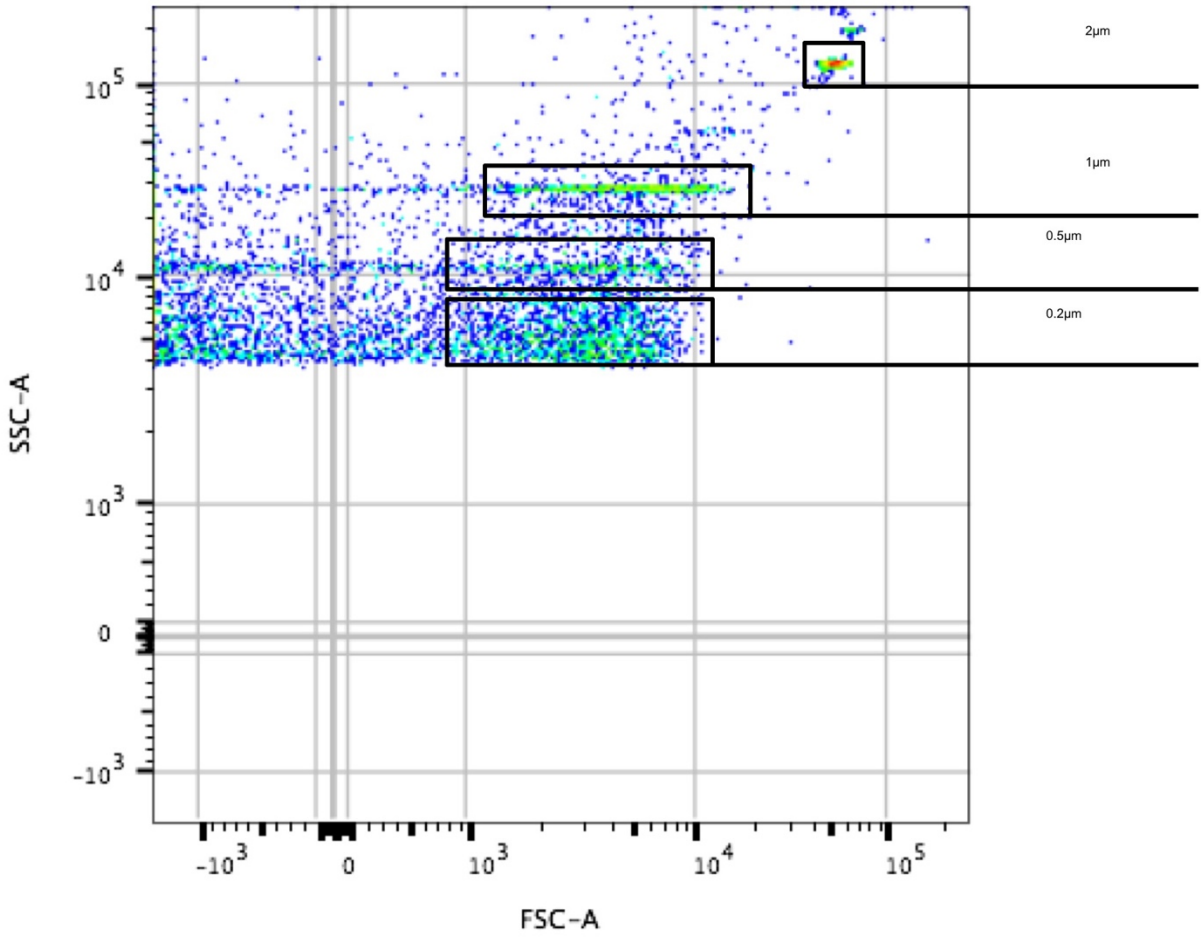
Mitochondrial morphology was assessed by transmission electron microscopy (TEM). LV cardiac tissue was excised from 3-5 age-match mice (7-8 wks) and immediately fixed in a 2% glutaraldehyde 4% paraformaldehyde sodium cacodylate buffer overnight. Samples were then stained with 2% osmium

tetroxide, dehydrated by ethanol series and embedded in an epoxy resin for 24 h. Ultrathin sections (80-150nm) were prepared and interfibrillar mitochondria were imaged at 2000X magnification on a JOEL TEM 1400 (JOEL, Tokyo, Japan) and photographed on a Gatan multiscan 791 digital camera (Gatan, Pleasanton, CA). The operator and analyzer was blinded to experimental condition, quantified mitochondrial number and morphology (number, area, perimeter) using Image J. Briefly, one tissue section was isolated from each of 3-5 mice and imaged by electron microscopy. From each tissue section 10-20 fields were captured, measured and averaged for each mouse. Statistical comparisons were then done between mice resulting in a total n of 3-5.

Statistical analysis

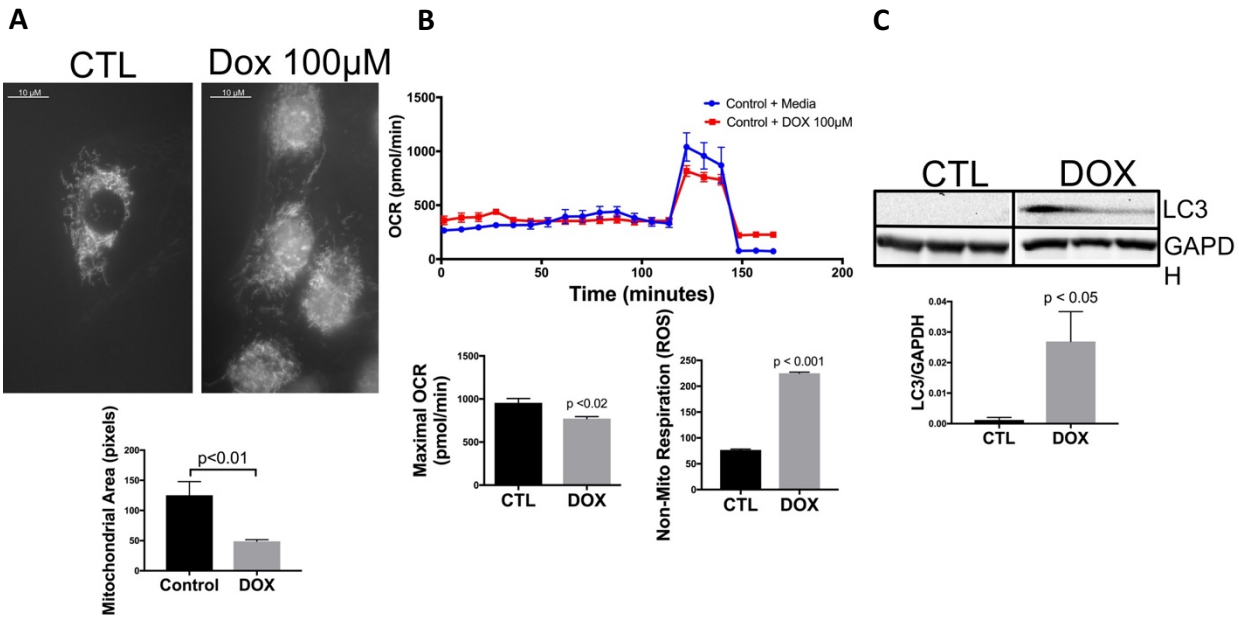
Data are expressed as the mean ± standard error of the mean. Normally distributed data comparing two groups were analyzed by Student's *t* test. Normally distributed data comparing multiple groups were analyzed by ANOVA with multiple comparisons analysis (Tukeys). Non-parametric data comparing two groups were analyzed using the Mann-Whitney U test. Statistical analysis of microarray data was performed using Stanford microarray database software and the Significance Analysis of Microarrays (SAM), Database for Annotation, Visualization, and Integrated Discovery, Hi-Throughput GOMiner and TIGR TM4 software. Multiple comparisons were accounted for by False Discovery Rate (FDR) analysis and genes were considered significantly altered with a p value less than 0.01 by Fisher's exact test. To control for multiple comparisons, we required a false discovery rate of 5%. Microarray data has been submitted to the NCBI Gene Expression Omnibus (GEO) database in MIAME format.

Online Figure I



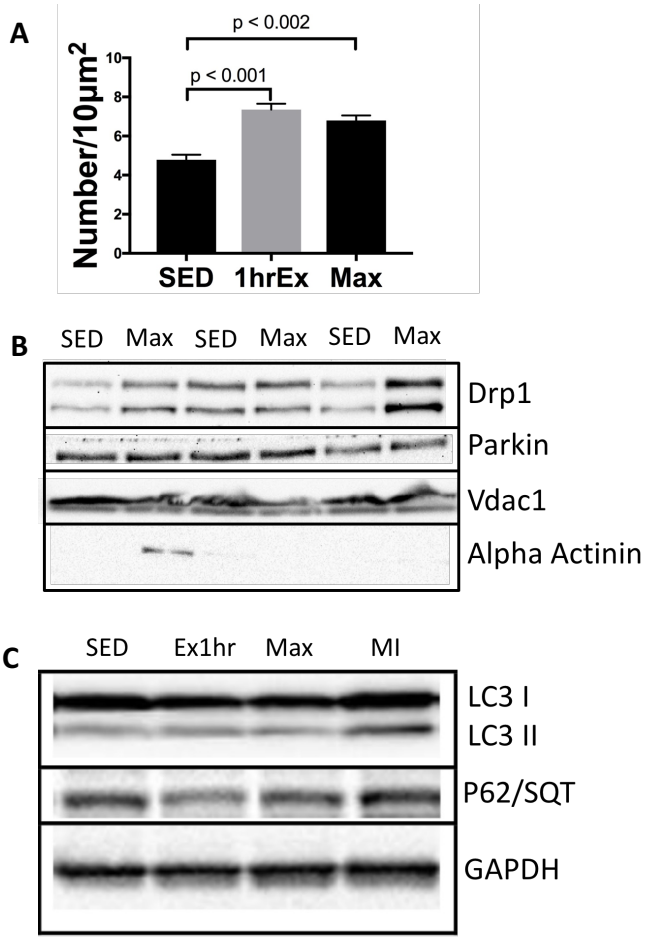
Online Figure I: Beads of known sizes were assessed by flow cytometry. Bead sizes ranged from 0.2µm, 0.5µm, 1µm, and 2µm, which fall within the size range of interfibrillar cardiac mitochondrial. Side scatter area (SSA) was selected to measure mitochondrial size because it was the only measure able to separate different bead sizes below the 1µm range.

Online Figure II



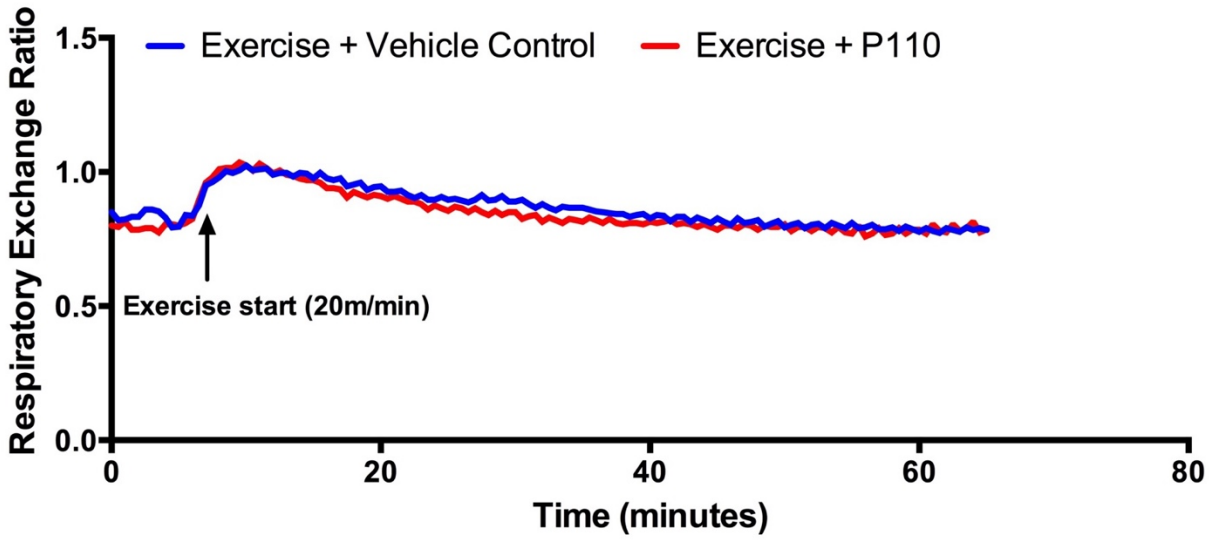
Online Figure II: Doxorubicin exposure induces pathological mitochondrial fragmentation. (A) C1C12 skeletal muscles cells exposed to 100μM of doxorubicin exhibit mitochondrial fragmentation (n = 10-20 cells/group). **(B)** Isolated cardiac myocytes exposed to 100μM of doxorubicin have decreased maximal respiration and increase ROS, as seen by increased non-mitochondrial respiration (n = 5/group). **(C)** Mice exposed to doxorubicin by tail vein injection (10mg/kg) for 30 minutes had increased LC3, indicating autophagy (n=3/group).

Online Figure III



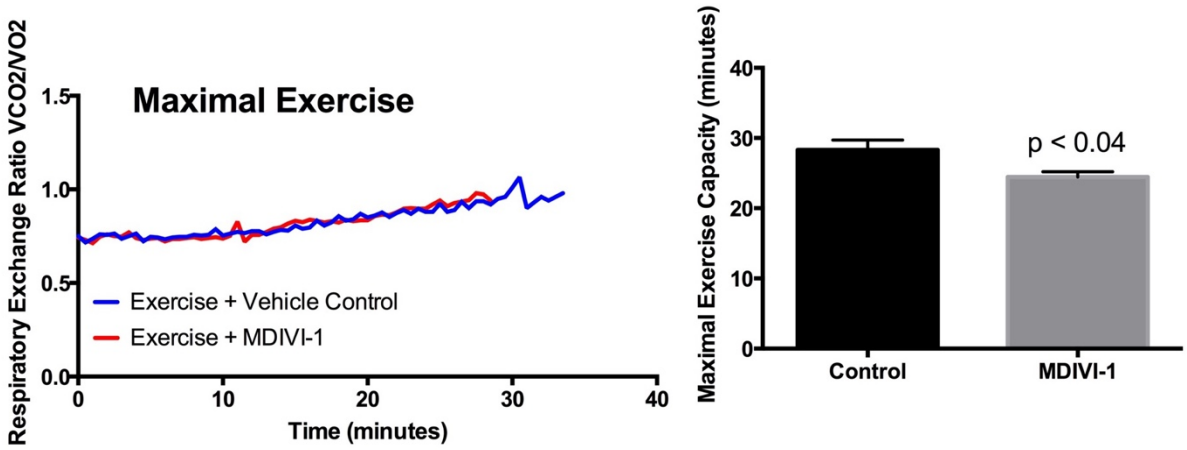
Online Figure III: Maximal and submaximal exercise both induce mitochondrial fragmentation. (A) A comparison of sub-maximal (1hr running at 20m/min) and extreme/maximal (running until exhaustion) exercise show similar mitochondrial fragmentation vs sedentary (n = 4-5/group). **(B)** Mitochondrial fractions from mice subjected to maximal exercise show Drp1 and Parkin translocation to the mitochondrial fraction (n=3/group). **(C)** Whole cell lysates of mice subjected to sub-maximal (Ex1hr) and extreme/maximal exercise (Max) show no change in LC3 or P62 (n=4/group). Mice subjected to 24hr of left anterior descending ligation were used as positive controls (MI).

Online Figure IV



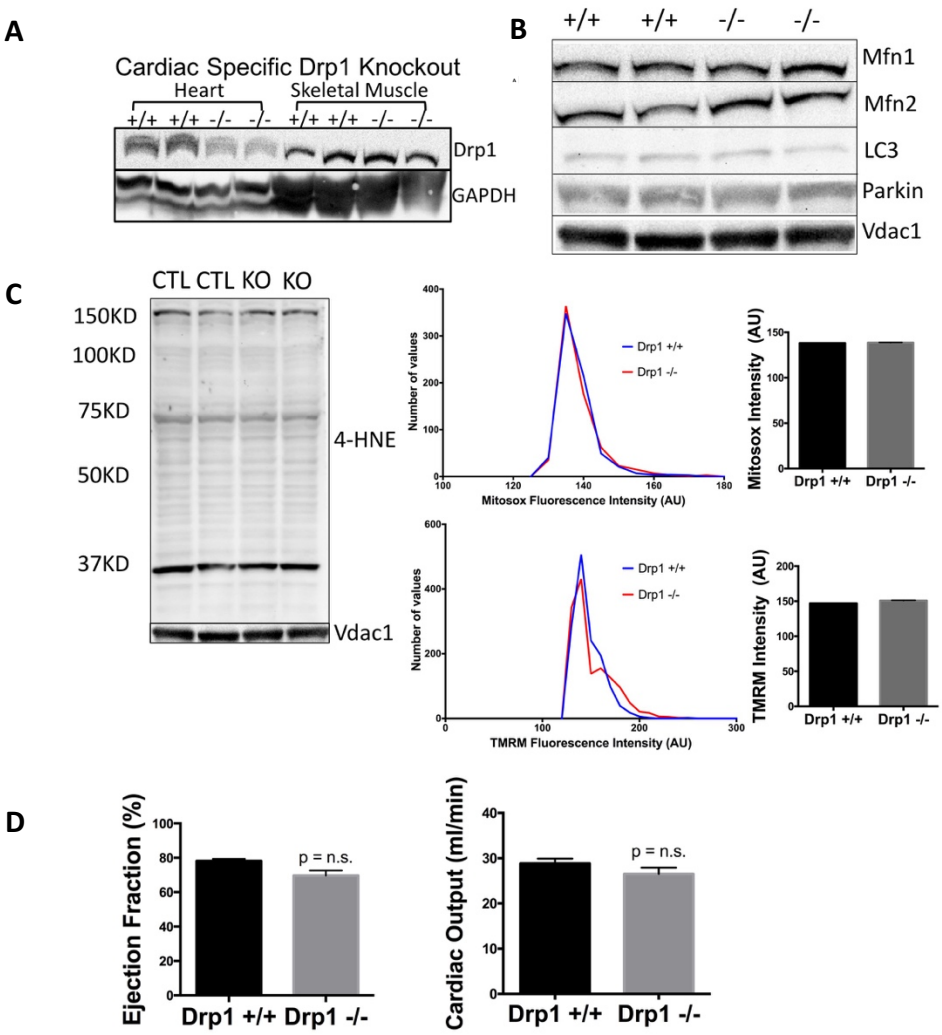
Online Figure IV: The early response to exercise is not affected by Drp1 inhibitor treatment. Mice were treated with control TAT peptide or P110 and placed on a treadmill for 1h at a speed of 20 m/sec. VO2 and CO2 levels were measured and RER (CO2/VO2) was calculated (n=3 per group).

Online Figure V



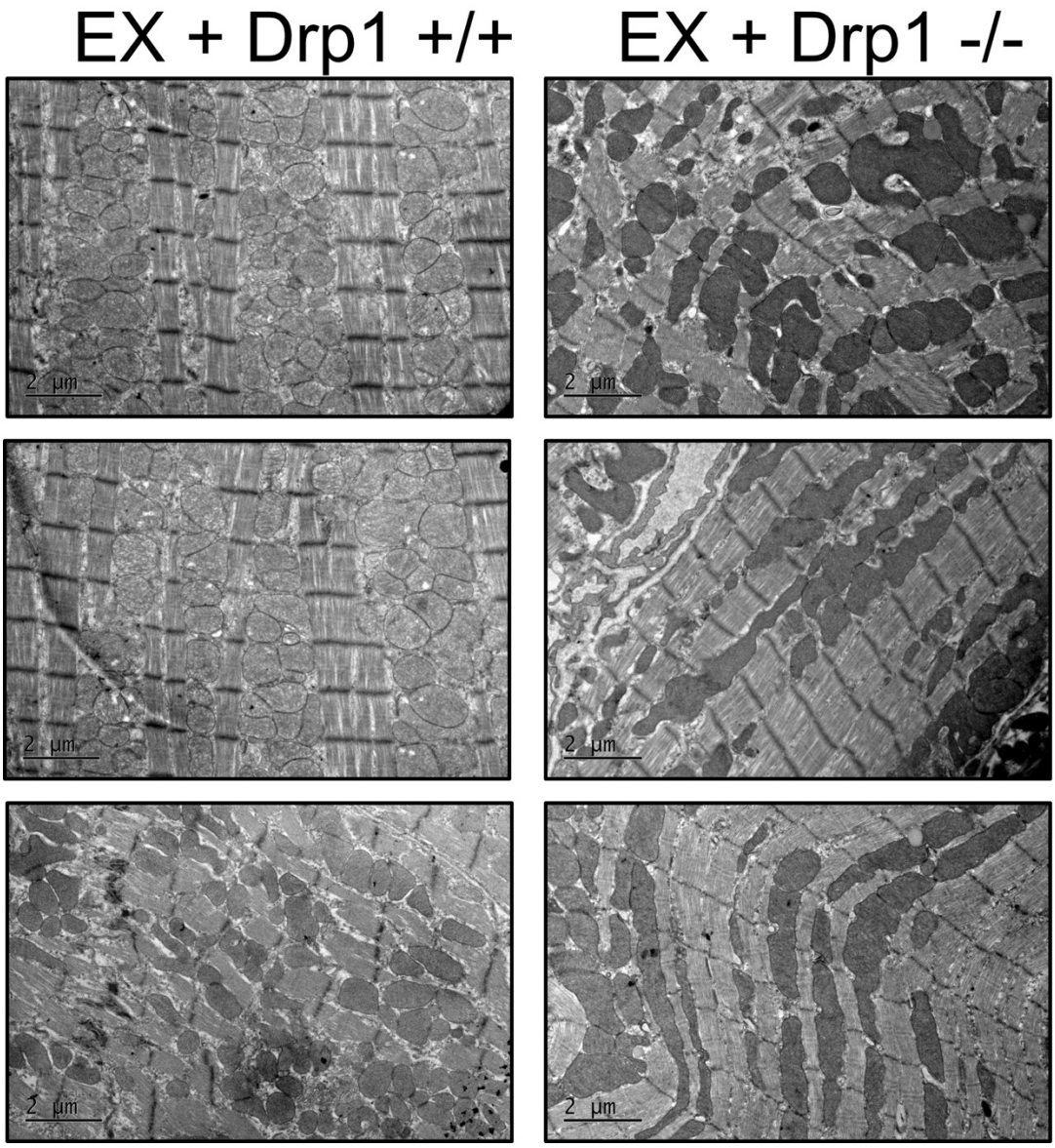
Online Figure V: Similar to P110, the Drp1 GTPase inhibitor, Mdivi-1, also decreases maximal exercise capacity. Mice pretreated with the Mdivi-1 (25 mg/kg i.p.) showed decreased maximal exercise capacity compared to vehicle control (2% DMSO in saline) (n=4/group).

Online Figure VI



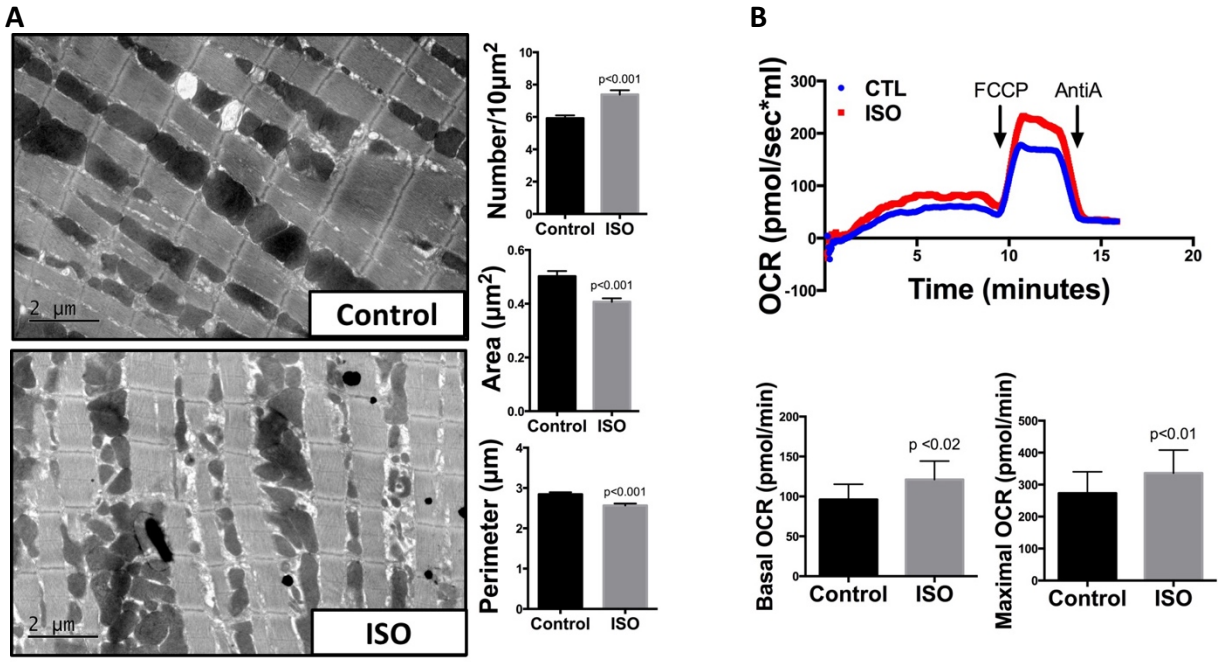
Online Figure VI: Cardiac specific Drp1 knockout results in no change in mitochondrial reactive oxygen species formation and normal cardiac function. (A) Mice were treated with tamoxifen (20mg/kg) for 5 days i.p. and on day 7 mice were used for experimentation. Male aged-matched Drp1+/+Cre+/+ and Drp-/-Cre-/- were treated with tamoxifen and used as controls for all experiments (n=2/group). **(B)** Mfns 1 & 2, LC3 and Parkin remained unchanged in the Drp1 -/- mice (n=3-4/group). **(C)** Reactive oxygen species was assessed by 4-HNE in isolated mitochondria and MitoSox in isolated cardiac myocytes, while membrane potential was assessed by TMRE in isolated cardiac myocytes. (n=3/group). **(D)** Cardiac function (ejection fraction and cardiac output) was assessed by echocardiography (n=3/group).

Online Figure VII



Online Figure VII: Abnormal mitochondria in exercised Drp1-/- mice. Additional images displaying abnormally large, elongated or bizzare-shaped mitochondria in Drp1-/- mice subjected to exercise.

Online Figure VIII



Online Figure VIII: β-AR signaling induces mitochondrial fragmentation and enhanced respiration in isolated cardiac myocytes. **(A)** Isolated cardiac myocytes treated with ISO (10µM) for 1hr were imaged by electron microscopy and showed significant mitochondrial fragmentation with increased number and decreased area and perimeter. **(B)** ISO treatment also increased both basal and maximal mitochondrial function, measured as oxygen consumption rate (OCR) by Oroboros oxygraph normalized to 120,000 cells. (n=4/group).

Online Table I

upregulated in β2 Adrenergic receptor deficient mice compared to WT FVB controls

Gene Name	Symbol	Gene sequence	Genbank ID	Fold Change
Cytochrome p450, family 2, subfamily b, polypeptide 10	cyp2b10	AV054284	NM_009999	2.56595
Prolylcarboxypeptidase (angiotensinase C)	prcp	AV108774	NM_028243	2.38771
Cytochrome b-5	cyb5	AV077886	NM_025797	1.98741
Desmin	des	AV088720	NM_010043	1.62071
Isocitrate dehydrogenase 2 (NADP+), mitochondrial	ldh2	BG068167	NM_173011	1.55985
Histidine triad nucleotide binding protein 3	hint3	AV056903	NM_025798	1.46587
Isocitrate dehydrogenase 2 (NADP+), mitochondrial	ldh2	AV140565	NM_173011	1.44434
Immediate early response 3	ier3	AV104326	NM_133662	1.42981
Nicotinamide phosphoribosyltransferase	nampt	AV108470	NM_021524	1.40950
Alkylation repair homolog 7	alkbh7	BG074929	NM_025538	1.38233
Ubiquitin C	ubc	AV032177	NM_019639	1.37561
Isocitrate dehydrogenase 2 (NADP+), mitochondrial	ldh2	AV089252	NM_173011	1.37240
Clusterin	clu	BG072209	NM_013492	1.36581
Glutathione S-transferase, mu 2	gstm2	BG070501	NM_008183	1.35842
Mitochondrial ribosomal protein L54	mrpl54	AV088357	NM_025317	1.25277
Carnitine palmitoyltransferase 1, muscle	cpt1b	AV006274	NM_009948	1.24645

downregulated in β2 Adrenergic receptor deficient mice compared to WT FVB controls

Gene Name	Symbol	Gene sequence	Genbank ID	Fold Change
Apolipoprotein A-IV	apoa4	AA064294	NM_007468	-3.86802305
L-threonine dehydrogenase	tdh	AV093742	NM_021480	-3.19468405
Chloride channel calcium activated 3	clca3	AV074056	NM_017474	-2.79025642
6-pyruvoyl-tetrahydropterin synthase	pts	AV088699	NM_011220	-2.59571707
Nuclear receptor subfamily 4, group A, member 1	nr4a3	NM_010444	NM_015743	-2.51439491
Jun-B oncogene	junb	AA608298	NM_008416	-2.19959088
ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	atp2a1	AV082969	NM_007504	-2.17112834
Proteasome (prosome, macropain) subunit, alpha type 4	psma4	AV095052	NM_011966	-2.08064583
Mitochondrial ribosomal protein L49	mrpl49	AV011359	NM_026246	-1.76962962
S100 protein, beta polypeptide, neural	s100b	AV031395	NM_009115	-1.71083472
Homer homolog 3	homer3	AV041850	NM_001146153	-1.66958845
Aconitase 1	aco1	AV001502	NM_007386	-1.62250742
Beclin 1, autophagy related	becn1	AV049038	NM_019584	-1.55537928
ATPase, Na+/K+ transporting, beta 3 polypeptide	atp1b1	AV038364	NM_009721	-1.46875229
Mitochondrial ribosomal protein L40	mrpl40	AV093751	NM_010922	-1.43624508
Mitochondrial ribosomal protein L44	mrpl44	BG073050	NM_001081210	-1.35832654
Upregulated during skeletal muscle growth 5	usmg5	AV088589	NM_023211	-1.30340711
Nuclear factor of kappa light chain gene enhancer in B-cells 1, p105	nfkb1	AA388801	NM_008689	-1.2534784
Mitochondrial trans-2-enoyl-coa reductase	mecr	BG064053	NM_025297	-1.22625661

Online Table I: Microarray analysis of gene expression, comparing β2-/- vs WT. 35 mitochondrial-related genes were significantly up- or down-regulated with β2-AR ablation (n=9-10/group). Data were analyzed using the mouse transcriptome array and Stanford microarray database software and Significance Analysis of Microarrays (SAM). Gene ontology (GO) was used to characterize up- or down-regulated genes. Genes were considered significantly altered with a p value less than 0.01 by Fisher's exact test. To control for multiple comparisons, we required an estimated false discovery rate of 5%.

Online Table II

	Physiologic fragmentation (exercise)	Pathologic fragmentation (ischemia)
Drp1	↑	↑
Mfn2	–	↓
ROS	–	↑
Δψm	–	↓
Pink1	↓	↑
Parkin	↓	↑
Respiration	↑	↓

Online Table II: Comparison of physiologic and pathologic fragmentation. Physiological fragmentation is characterized by increased mitochondrial fission, enhanced mitochondrial function, no increase in ROS, preserved Δψm and inhibition of mitophagy. In contrast, pathological fragmentation, also characterized by increased mitochondrial fission, is associated with decreased mitochondrial function, increased ROS, decreased Δψm and activation of mitophagy.

Supplemental References

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4. Kendziorski C, Irizarry RA, Chen K-S, Haag JD, Gould MN. On the utility of pooling biological samples in microarray experiments. *Proc Natl Acad Sci USA*. 2005;102:4252–4257