SUPPLEMENTAL MATERIAL

Detailed Methods

Animals

All animal procedures were approved by the Temple University School of Medicine Institutional Animal Care and Use Committee. Anesthesia was induced and maintained using inhaled isoflurane (Butler Shein Animal Health; Dublin Ohio). For most mouse experiments, wild-type 10-12-week-old male C57BL/6 mice were used (The Jackson Laboratory; Bar Harbor, ME). For fate mapping of endogenous c-kit^{+} cardiac myocytes, constitutive and inducible Kit-Cre-IRES-GFPnls or Kit-MerCreMer knock-in mice were generated as described previously [1] via aggregation of gene-targeted ES cells with 8-cell embryos to generate chimeric mice on a mixed B6.129S4 background. Animals were then backcrossed to C57Bl/6J (Jackson Labs #000664) for a minimum of five generations. Cre-responsive Rosa26-loxP-eGFP reporter mice were generated as described van Berlo [1] by crossing the dual-reporter R26-NZG line (FVB.Cg-Gt(ROSA)26Sortm1(CAG-lacZ,EGFP)Glh/J; Jackson Labs #012429) to FLP-expressing B6.Cg-Tg(Pgk1-FLPo)10Sykr/J (Jackson Labs # 011065). Resulting R26-NG offspring were backcrossed to C57Bl/6J for a minimum of five generations to establish the line and then crossed with Kit-Cre-IRES-GFPnls or Kit-MerCreMer mice to generate experimental animals. To induce Cre-mediated recombination in Kit-MerCreMer x R-GFP mice, animals were fed tamoxifen citrate chow (Teklad TD.130860) at a dose of 40mg/kg bw/d (400mg/kg diet) ad libitum for two weeks. Animals were switched back to normal lab chow for twenty-four hours prior to further procedures.

Animal Numbers

A total of 187 mice were used for this study. This includes wild-type C57BL/6 mice (n=173), kit$^{+/cre}$ x R-GFP transgenic mice (n=6) and their non-transgenic littermates (n=3), and Kit$^{+/MCM}$ x R-GFP (n=5). To determine the maximum tolerated dose of isoproterenol, wild-type C57BL/6 mice (n=21) were used for dose titration, with animal receiving a single subcutaneous dose of 5 (n=5), 200 (n=5), 300 (n=1), 400 (n=1), 500 (n=1), 600 (n=1), 700 (n=1), 800 (n=1) or 1000 (n=5) mg/kg isoproterenol and their survival until 4 weeks after injection was assessed.

Of the wild-type C57BL/6 mice that received vehicle injection (n=39), 14 underwent serial echocardiography and were sacrificed at 1 day (n=4), 1 week (n=5) or 4 weeks (n=5) post-isoproterenol injection and hearts were perfusion-fixed and paraffin-embedded for histology. The body weight, heart weight and tibia length of all animals were recorded at sacrifice. The remaining animals (n=26) were dosed with saline and did not undergo serial echocardiography. Animals (n=9) were sacrificed at 1 day post-ISO injection for troponin I (n=5) measurements and for Western blot analysis of activated caspase 3 (n=4). Animals (n=4) were sacrificed at 1 day post-ISO injection, and hearts were embedded in Optimal Cutting Temperature Compound and frozen in liquid nitrogen for myocyte necrosis analyses using Evan´s blue dye. Animals (n=3) were sacrificed at 1 week post-ISO injection, and hearts were frozen in liquid nitrogen for molecular biology studies. Animals (n=9) were sacrificed at 4 week post-ISO and hearts were either frozen in liquid nitrogen for molecular biology (n=4), enzymatically digested (n=3) for myocyte isolation, or perfusion-fixed and paraffin-embedded for histology (n=2).

Of the wild-type C57BL/6 mice that received an injection of 200 mg/kg isoproterenol (n=45), 15 underwent serial echocardiography and were sacrificed at 1 day (n=5), 1 week (n=5) or 4 weeks (n=5) post-isoproterenol injection and their hearts were perfusion-fixed and paraffin-embedded for histology. The body weight, heart weight and tibia length of all animals were recorded at sacrifice. The remaining animals (n=30) were dosed with saline. Animals (n=9) were sacrificed at 1 day post-ISO injection for troponin I measurements (n=5) and for Western blot analysis of activated caspase 3 (n=4). Animals (n=5) were sacrificed at 1 day post-ISO injection, and hearts were embedded in Optimal Cutting Temperature Compound and frozen in liquid nitrogen for myocyte necrosis analyses using Evan´s blue dye. Animals (n=4) were sacrificed at 1 week post-ISO injection, and hearts were frozen in liquid nitrogen for molecular biology studies. Animals (n=9) were sacrificed at 4 weeks post-ISO and hearts were either frozen in liquid nitrogen for molecular biology (n=4), enzymatically digested (n=3) for myocyte isolation, or perfusion-fixed and paraffin-embedded for histology (n=2). Animals (n=3) underwent continuous ECG monitoring.

Of the wild-type C57BL/6 mice that received injection of 300 mg/kg isoproterenol (n=43), 14 underwent serial echocardiography and were sacrificed at 1 day (n=5), 1 week (n=5) or 4 weeks (n=4) post-

isoproterenol injection and their hearts were perfusion-fixed and paraffin-embedded for histology. The body weight, heart weight and tibia length of all animals were recorded at sacrifice. The remaining animals (n=29) were dosed with saline. Animals (n=8) were sacrificed at 1 day post-ISO injection for troponin I measurements (n=4) and for Western blot analysis of activated caspase 3 (n=4). Animals (n=5) were sacrificed at 1 day post-ISO injection, and hearts were embedded in Optimal Cutting Temperature Compound and frozen in liquid nitrogen for myocyte necrosis analyses using Evan´s blue dye. Animals (n=4) were sacrificed at 1 week post-ISO injection, and hearts were frozen in liquid nitrogen for molecular biology studies. Animals (n=10) were sacrificed at 4 weeks post-ISO and hearts were either frozen in liquid nitrogen for molecular biology (n=4), enzymatically digested (n=4) for myocyte isolation, or perfusion-fixed and paraffin-embedded for histology (n=2). Animals (n=2) underwent continuous ECG monitoring.

The wild-type C57BL/6 mice (n=5) that received vehicle injections twice daily for 6 consecutive days underwent echocardiography at baseline, 7 and 28 days with tissue Doppler imaging (TDI) and strain analysis, and were sacrificed at 28 days post-injections. The hearts were perfusion-fixed and paraffin-embedded for histology. The body weight, heart weight and tibia length of all animals were recorded at sacrifice.

The wild-type C57BL/6 mice (n=9) that received 200mg/kg ISO injections twice daily for 6 consecutive days underwent echocardiography at baseline, 7 and 28 days with tissue Doppler imaging (TDI) and strain analysis, and were sacrificed at 28 days post-injections. The hearts were perfusion-fixed and paraffin-embedded for histology. The body weight, heart weight and tibia length of all animals were recorded at sacrifice

During breeding of the c-kit-GFP transgenic animals, a total of 6 $kit^{+/cre}$ x R-GFP transgenic animals were generated along with 3 non-transgenic littermates. 7 of these animals received 200 mg/kg isoproterenol (4 $kit^{+/cre}$ x R-GFP transgenic and 3 non-transgenic) and underwent serial echocardiography. 2 transgenic mice served as a control and did not receive isoproterenol. All seven animals were sacrificed 4 weeks after injection. 2 of the transgenic $kit^{+/cre}$ x R-GFP transgenic mice were sacrificed at baseline and their hearts were perfusion digested for myocyte isolation to determine the percentage of GFP^+ myocytes present at baseline as previously described[1]. The hearts from the 3 non-transgenic animals and 2 transgenic animals were perfusion-fixed and paraffin-embedded for histology. The hearts of the remaining 2 transgenic females underwent perfusion digestion for myocyte isolation to determine the percentage of GFP^+ myocytes in each heart after isoproterenol injury. The body weight, heart weight and tibia length of all animals were recorded at sacrifice.

$Kit^{+/MCM}$ x R-GFP (n=5) were injected either with 200mg/kg ISO (n=3) or saline (n=2) twice daily for 6 consecutive days and were sacrificed at 28 days (n=5) post-isoproterenol injections and their hearts were cut in half in longitudinal sections. Half were frozen in liquid nitrogen and the other half fixed in formalin and paraffin-embedded for histology. The body weight, heart weight and tibia length of all animals were recorded at sacrifice.

Isoproterenol Dosing

Single-dose isoproterenol (ISO) was administered by subcutaneous injection into the loose skin (Ellison et al., 2013) over the neck. The maximum dose used in their publication was 200 mg/kg for mouse studies. For our study, we used 200mg/kg ISO and a higher dose of 300 mg/kg (as this was the highest tolerable non-lethal dose). Doses greater than or equal to 400 mg/kg were lethal within 30 min of injection. For the chronic ISO experiments, C57BL/6 mice were either injected s.c. with 200mg/kg ISO (n=10) or vehicle (n=5) twice daily for 6 consecutive days. For all experiments, a stock solution of 50 mg/mL isoproterenol (Sigma-Aldrich #I6504; St. Louis, MO) was prepared in sterile 0.9% saline. This solution was aliquoted and stored at -20°C. Dilutions made from thawed stock solution were prepared within 30 minutes of injection in sterile 0.9% saline to achieve concentrations of 200 and 300 mg/kg. All diluted isoproterenol solutions were kept on ice and away from light until injection.

Animal Procedures

EdU Minipump Implantation, Subcutaneous Isoproterenol Injections, Echocardiography and Cardiectomy. Animals were weighed, assigned treatment groups (vehicle, 200 or 300 mg/kg isoproterenol) and sacrifice date (1 day, 1 week or 4 weeks post-isoproterenol injection) prior to the start of experimental procedures to avoid selection bias, and isoproterenol doses were calculated based on the recorded body weights at baseline. Osmotic minipumps (Alzet; Cupertino, CA) were loaded with solution containing 39.0625 mg/mL EdU (Life Technologies, Carlsbad, CA) dissolved in 50/50% mixture of DMSO/ddH_2O. Pumps were prepared as

previously described (Duran et al., 2014), then animals were anesthetized using isoflurane and the pumps were implanted subcutaneously between the two scapulae. The minipumps deliver a continuous infusion of EdU over the course of 1 week, and after 1 week all pumps were removed. Immediately following pump implantation (while the animal was still anesthetized), isoproterenol was injected into a subcutaneous depot just caudal to the site of pump implantation.

Permanent occlusion myocardial infarction was induced in 12-week old male C57BL/6 mice (n=4) (The Jackson Laboratory; Bar Harbor, ME) by ligation of the left anterior descending coronary artery (LAD) as described previously [2]. Anesthesia was induced using 3% isoflurane and maintained at 1% (Abbot Laboratories; Chicago, IL). Mice were intubated after induction of anesthesia and ventilated at a rate of 180-190 breaths per minute and tidal volume of 250 μL. After 24 hours, animals were anesthetized and 1-1.5 mL of arterial blood was collected for troponin I analysis.

All animals underwent serial non-invasive transthoracic echocardiography with tissue Doppler imaging (TDI) and strain analysis under isoflurane anesthesia at 1 day (1d), 3 days (3d), 1 week (1wk), 2 weeks (2wk) and 4 weeks (4wk) after isoproterenol injection as previously described [3-6]. Speckle-tracking based strain analyses were performed on long axis B-mode loops. Extensional strain of soft tissue in a predetermined direction can be defined as the change in length of a segment divided by its original length ([L1-L0]/L0), where strain rate (SR) is the rate of change of this deformation over time ([(L1-L0)/L0] x sec-1) [7]. Diastolic function was determined using ECHO coupled with pulsed Doppler and tissue Doppler imaging (TDI) techniques. From an apical long-axis view, transmitral flow velocities were recorded by setting the sample volume in the mitral orifice close to the tip of the mitral leaflets. Spectral waveforms were analyzed for peak early- and late-diastolic transmitral velocities (E and A waves), E/A ratio and the acceleration time of the transmitral early diastolic peak flow velocity (EAT). EAT correlates well with the invasively measured LV relaxation parameters -dp/dtmin, tau (g) and LVEDP [8]. E′ was the tissue peak velocity obtained from LV posterior wall. Myocardial Performance Index (MPI_{Tei}), a non-invasive Doppler index for the assessment of overall cardiac function, is calculated as the sum of isovolumetric contraction and relaxation time (IVCT and IVRT) divided by the ejection time (ET) [9]. At the assigned date of sacrifice, animals were anesthetized with inhaled isoflurane and cardiectomy was performed and the heart was rinsed and weighed. The aorta was then cannulated and the coronary arteries were cleared by perfusion with 1 mL cold Krebs-Henseleit Buffer. The heart was then arrested in diastole by perfusion with 1 mL of 100 mM cadmium chloride/1 M potassium chloride solution. The hearts were then gravity perfused with 30 mL 10% formalin at mean arterial pressure (100 mmHg). Fixed hearts were immersed overnight in 10% formalin and then stored in 70% ethanol for up to 1 week before being processed and embedded in paraffin wax blocks.

Continuous Electrocardiogram Monitoring

To determine the mechanism of death from high-dose isoproterenol (>400 mg/kg), animals were anesthetized using inhaled isoflurane and monitored with continuous electrocardiogram using leads placed subcutaneously on the ventral surface of both hind limbs and the right forelimb. After anesthetization, continuous ECG (3-lead ECG, ADInstruments) recording was begun using LabChart 7.3.7 (ADInstruments, Colorado Springs, CO), and animals were injected subcutaneously with 200 (n=3), 300 (n=2), 400 (n=3), or 1000 (n=3) mg/kg isoproterenol. In the high-dose groups (400 and 1000 mg/kg isoproterenol), ECG recording was continued until death (after 5min of asystole). Animals dosed with 400 and 1000 mg/kg expired within 25 minutes. Animals dosed with the nonlethal dose of 200 or 300 mg/kg underwent ECG monitoring for 1 hour (long enough to detect lethal arrhythmias at the higher/lethal doses). The animals treated with 200 or 300 mg/kg were sacrificed immediately after ECG recording.

Arterial Cardiac Troponin Analysis

To detect the presence of serum markers of cardiac injury, animals were dosed with vehicle (n=5), or 200 (n=5), or 300 (n=4) mg/kg isoproterenol. Myocardial infarction was induced in C57BL/6 mice (n=4), which served as a positive control. After 24 hours, animals were anesthetized and 1-1.5 mL of arterial blood was collected by inferior vena cava puncture. Blood was placed in lithium carbonate-coated vacutainers, samples were microcentrifuged at 1300 rpm for 10min, and serum samples were sent to Antech Diagnostics (Lake Success, NY) for cardiac troponin I analysis.

Single cell isolation

Mouse cardiac myocytes were isolated as previously described [5, 6, 10, 11]. Briefly, the aorta was cannulated and perfused retrograde to enzymatically digest the heart. When softened, the atria were removed and the

ventricles were mechanically dissociated to obtain single cells. The cell solution was exchanged with a BSA containing solution and allowed to settle. Next, the cells were washed in normal tyrode's solution and then fixed with 4% paraformaldehyde in PBS. Care was taken between solution exchanges to minimize myocyte loss. Following enzymatic digestion, LV myocytes were fixed in paraformaldehyde (4% in PBS, Affymetrix) and nuclei stained with Click-iT EdU Alexa Fluor 488 Imaging Kit (Life Technologies; Eugene, OR) and DAPI (Calbiochem). Total number of myocytes was determined by hemocytometer and Edu^+ myocytes were counted using a fluorescent microscope (Nikon eclipse Ti). Images were collected. Surface area and nuclei distribution were evaluated using ImageJ software. On average ~ 150 myocytes per mouse were analyzed from 10 mice.

Calcium transients and contraction measurements: After isolation, myocytes from transgenic Kit+/Cre x R-GFP mice (n=2) were loaded with 5-10 uM indo-1 in normal tyrode's, as described previously [12]. Myocytes were paced at 1HZ and sarcomere length was recorded with an Ion Optix system. Calcium transients were obtained by indo-1 fluorescence intensity ratio at 410nm to 480 nm. 3-4 GFP+ and GFP- myocytes from 2 mice were used. Myocyte fractional shortening was not different between indo-1 loaded and unloaded cells. A total of 300,000 cells were counted for quantification of $eGFP^+$ fluorescent myocytes.

Myocyte Necrosis Analysis

To quantify the degree of myocyte necrosis, male C57BL/6 mice (n=14) received intraperitoneal injection of 50µl/10g body weight Evans Blue (Sigma-Aldrich; St. Louis, MO). To determine Evan's blue dye (EBD) uptake in cardiomyocytes, mice were injected with EBD (20mg/ml PBS; 50µl/10g body weight). After 24 hours, animals received injection of vehicle (n=4), 200 mg/kg (n=5) or 300 mg/kg (n=5) isoproterenol, and then after 24 hours, animals were sacrificed. Hearts were harvested, embedded in Optimal Cutting Temperature Compound (O.C.T, Tissue-Tek; Sakura Finetek, Torrance, CA), and frozen in liquid nitrogen. Tissues were cut into 7 micron sections and counterstained with wheat germ agglutinin conjugated with fluorescein isothiocyanate (FITC; Sigma-Aldrich; St. Louis, MO) for one hour at room temperature to visualize membranes (green) versus EBD (which fluoresces red). Images were taken on a Nikon Eclipse Ti-S inverted microscope equipped with NIS elements Advanced Research (AR) imaging software (Nikon Instruments Inc.; Melville, NY) to determine the percentage of EBD-positive cardiomyocytes.

Bright Field Histology and Immunohistochemistry

Paraffin-embedded samples fixed at 4 weeks post-injection were stained with Masson's Trichrome (Sigma-Aldrich; St. Louis, MO) for myocyte cross-sectional area and bioquantification of fibrosis. Masson's Trichrome-stained bright field micrographs were acquired using a light microscope (Olympus BX40, Spot Software 5.1.29; Sterling Heights, MI). Myocyte cross-sectional area was measured using NIH Image J software as previously described[13]. A total of 4410 myocytes were counted from 9 animals. Bioquantification of the percent fibrotic area of myocardium was determined using a Nikon microscope (Mellvile, NY) interfaced with an analog camera and a bioquantification software system (Bioquant TCW 98; Nashville, TN). A total of 54 short-axis cross-sections from 9 mice and 24 longitudinal sections from 6 mice were analyzed.

Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) was performed on paraffin-embedded tissues fixed at 24 hours after isoproterenol injection. Samples were then immunostained to quantify the number of apoptotic myocytes. The DeadEnd Fluorometric TUNEL system was used to label and immunostain apoptotic nuclei (Promega #G3250; Madison, WI). Immunostaining for cardiac contractile proteins was performed using mouse IgG anti-sarcomeric tropomyosin (Sigma-Aldrich #T9283; St Louis, MO), and the secondary antibody Rhodamine Red-X donkey anti-mouse IgG (715-295-150) was used to detect IgG anti-sarcomeric tropomyosin. The identification of cardiomyocytes and quantification of Tunel positive/negative cells was done semi-automatically using Nuquantus, a Matlab toolbox for machine learning based detection and quantification of cells of interest in complex fluorescent tissue images[14]. The patterns needed to detect cardiomyocytes were trained in Nuquantus using a reference dataset of mouse and swine cardiomyocytes labeled by an expert histopathologist. These patterns were then used to automatically detect valid cardiomyocytes in the Tunel study dataset. The automatic detection was then checked for quality control by the histopathologist analyst to remove false positive detection errors. A total of 6846 myocyte nuclei were counted for TUNEL analysis.

For immunostaining and EdU detection, the Click-iT EdU Alexa Fluor 488 Imaging Kit (Life Technologies; Eugene, OR) was used along with primary mouse IgM anti-α-sarcomeric actin (Sigma-Aldrich #A2172; St. Louis, MO) with secondary rhodamine red-X donkey anti-Mouse IgM (Jackson

Immunoresearch Laboratories #715-295-020; West Grove, PA) for detection of mouse IgM anti-α-sarcomeric actin. More than 34,000 myocyte nuclei and more than 45,000 non-myocyte nuclei from 9 animals were counted for analysis.

For detection of GFP in paraffin-embedded hearts from transgenic $Kit^{+/Cre}$ x R-GFP mice (n=2) and $Kit^{+/MCM}$ x R-GFP (n=5) mice, sections were stained with primary chicken anti-GFP antibody (Life Technologies # A10262; Eugene, OR) and secondary FITC donkey anti-Chicken IgG (Jackson Immunoresearch Laboratories #703-095-155; West Grove, PA). Immunostaining for cardiac contractile proteins was performed using with primary mouse IgM anti-α-sarcomeric actin (Sigma- Aldrich #A2172; St. Louis, MO) and secondary rhodamine red-X donkey anti-Mouse IgM (Jackson Immunoresearch Laboratories #715-295-020; West Grove, PA) for detection of mouse IgM anti-α-sarcomeric actin. A total of 32 cross-sections from 2 $Kit^{+/Cre}$ x R-GFP mice and 87 longitudinal sections from 5 $Kit^{+/MCM}$ x R-GFP were analyzed for GFP+ cardiomyocytes. Paraffin-embedded hearts from vehicle injected C57BL/6 mice were stained against GFP, which served as control.

For all immunostains, nuclei were labeled with 4',6- diamidino-2-phenylindole (DAPI, Millipore; Billerica, MA). Confocal micrographs of all immunostains were acquired using a Nikon Eclipse T1 confocal microscope (Nikon Inc.; Mellvile, NY).

Real-time polymerase chain reaction (PCR)

Hearts were harvested from vehicle (n=3), 200mg/kg (n=4), and 300mg/kg (n=4) group at 1 week post injection and at 4 week post injection (veh (n=4), 200mg/kg (n=4), 300mg/kg (n=4)). RNA was extracted from mouse hearts with TRIzol Reagent. The RNA was cleaned using the Quick-RNA™ MiniPrep (Zymo Research) clean-up protocol. Reverse transcription (RT) reaction was performed using the SuperScript III first strand synthesis system for RT-PCR (Invitrogen) and oligo-dt primers according to the manufacturer's instructions. Real-time PCR was performed using the Quantifast Sybrgreen PCR kit (Qiagen). Data generated from mouse heart samples were normalized to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression. The following primer sets were used (forward, reverse): GAPDH 5'-ACTGAGCAAGAGAGGCCCTA, 5'-TATGGGGGTCTGGGATGGAA; atrial natriuretic factor (ANF) 5'-GCCCTGAGTGAGCAGACTG, 5'-GGAAGCTGTTGCAGCCTA; brain natriuretic factor (BNP) 5'-CTGCTGGAGCTGATAAGAGA, 5'-AGTCAGAAACTGGAGTCTCC; alpha myosin heavy chain (αMHC) 5'-ACCTACCAGACAGAGGAAGA, 5'-ATTGTGTATTGGCCACAGCG; beta myosin heavy chain (βMHC) 5'-ACCTACCAGACAGAGGAAGA, 5'- TTGCAAAGAGTCCAGGTCTGAG.

Western Analysis

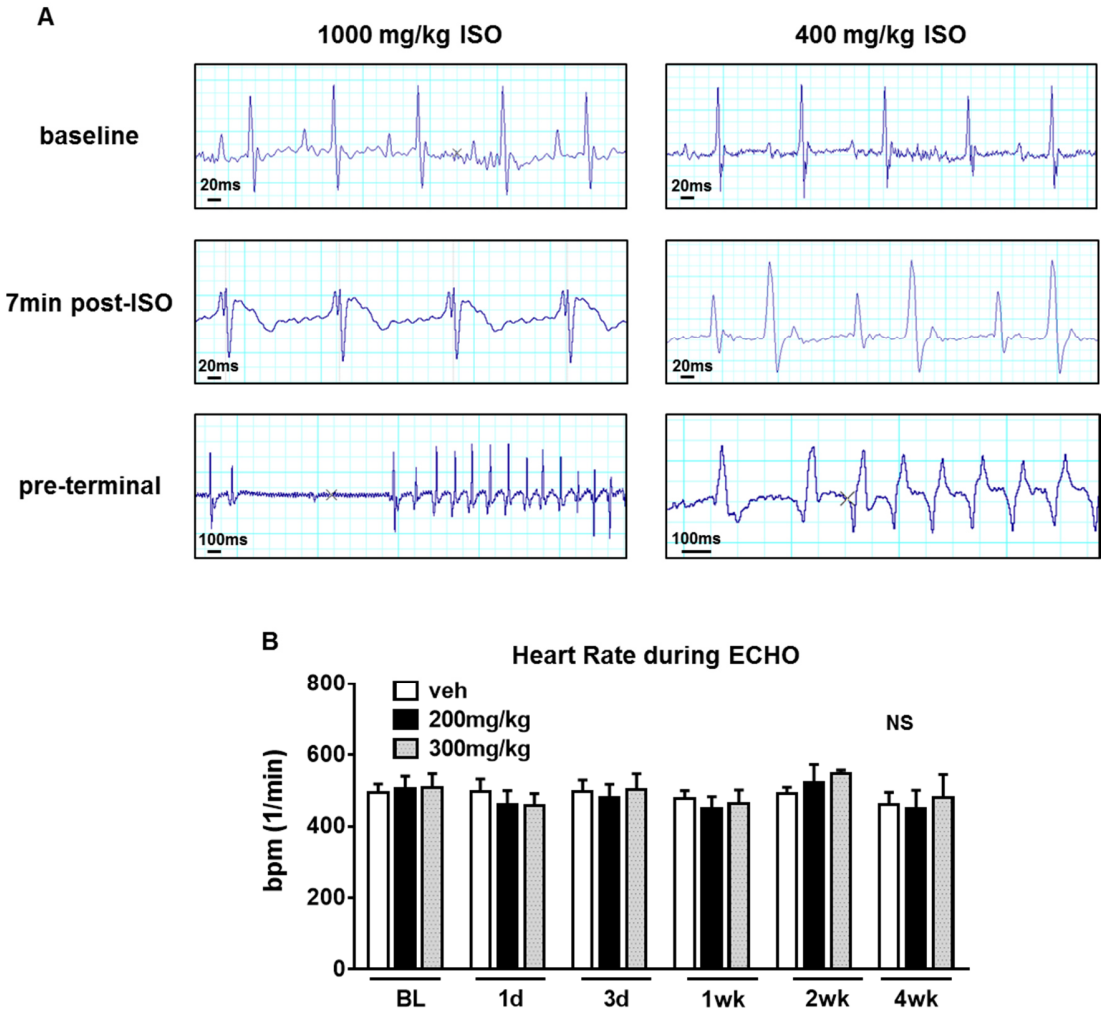
Lysates from heart tissue (n=12) or HEK cells were prepared and analyzed using Western analysis as previously published[15]. The following primary antibodies were used: total caspase 3 (Cell Signaling #9662, Beverly, MA, 1:1000) that detects total and cleaved Caspase 3, activated Caspase 3 (Cell Signaling #9661; Beverly, MA, 1:1000), and glyceraldehyde-3- phosphate dehydrogenase (GAPDH; AbD Serotec #MCA4739G; Kidlington, UK, 1:1000). The following secondary antibodies were used: 800CW Donkey anti-Rabbit (#926-32213) and 680RD Donkey anti-Mouse (#926-68072) purchased from LICOR (Lincoln, NE, 1:10,000).

Statistical Analysis

All data analyses were performed using SAS version 9.3. Data are shown as mean ± SEM. For variables such as ECHO parameters (e.g., ECHO-derived cardiac function and volumes, global and apical longitudinal strain data) that were measured repeatedly over time at baseline (BL) and 1, 3, 7, 14, and 28 days post isoproterenol (ISO) injection, a mixed-effects model approach was employed to study the differences in each variable between three groups (vehicle, 200mg/kg ISO, and 300mg/kg ISO) at each time point as well as the overtime changes at the 5 time points compared to baseline within each of the three groups. This modeling approach takes into account the within-animal correlation among repeated measurements overtime via a flexible structure such as ARH(1) or AR(1) for variance components, affording different magnitude of variability across the three groups. This is especially important in our study as it is often evident that there exists heterogeneity between the three experimental groups. Furthermore, this modeling technique allows us to conveniently test under the unified mixed-effects model framework the 3 pairwise group differences at each timepoint or 5 overtime changes compared to baseline within each of the three groups simultaneously via the Bonferroni or Dunnett-Hsu adjustment, respectively. In addition, to guard against a potentially overinflated type I error rate, $p<=0.05/5=0.01$ was used for between-group comparisons at the 5 post injection timepoints.

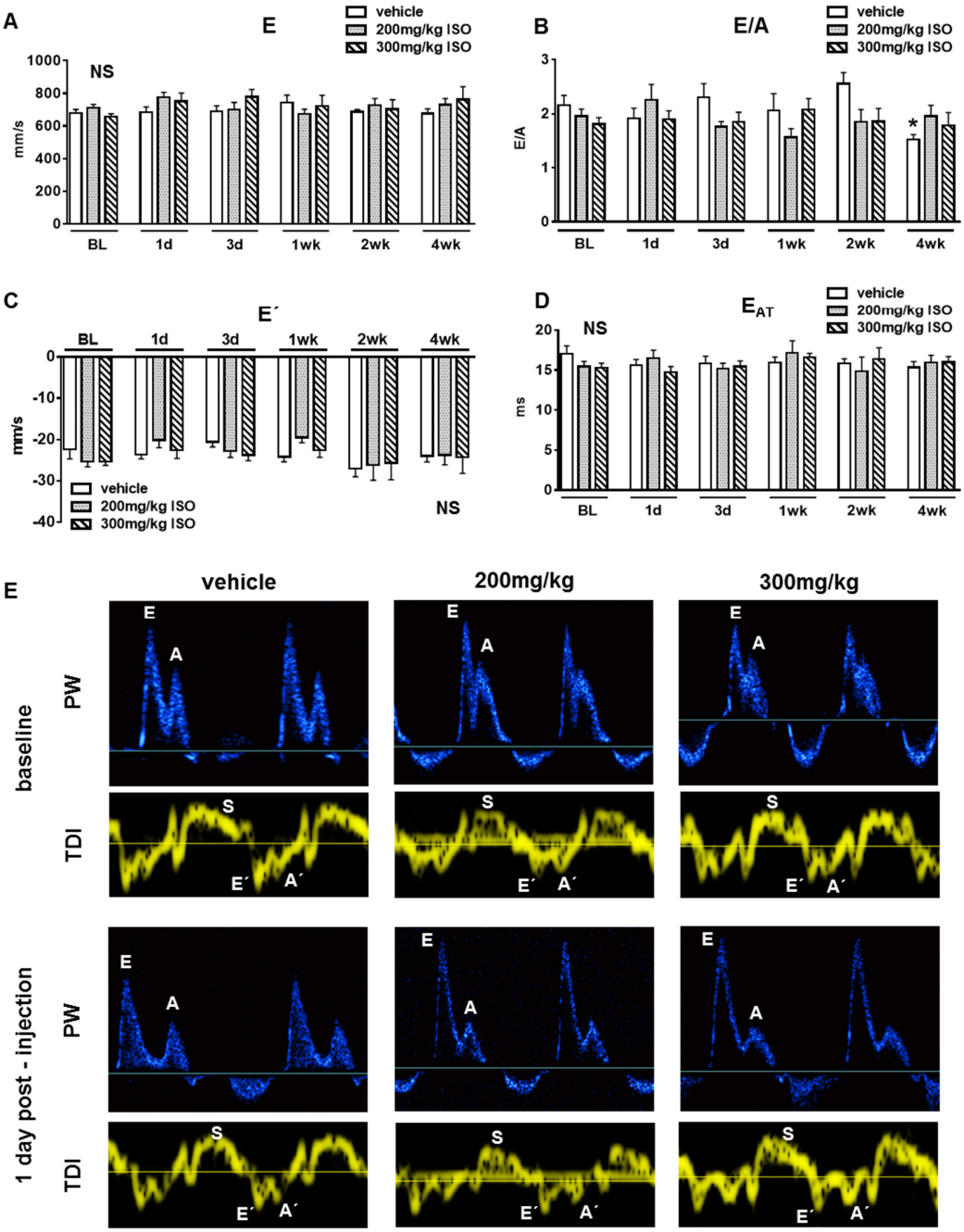
For variables that were measured only once post ISO injection (e.g., the percentages of EBD positive myocytes, TUNEL+ myocyte and non-myocyte nuclei, and Tn-I levels measured at day 1 post-injection), the overall group comparison was tested using the Kruskal-Wallis test or ANOVA F-test, when appropriate, while the pairwise group comparisons were performed using the exact Wilcoxon rank-sum test or unequal variance two-sample t-test. Due to the relatively small group sizes, however, this part of the data analyses was not adjusted for multiplicity.

Supplemental Figures

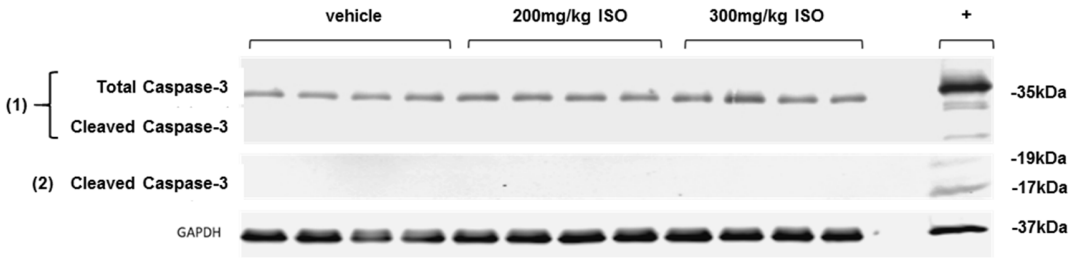


Online Figure I: ECG traces after ISO injection. Related to isoproterenol dosing and heart rate during ECHO

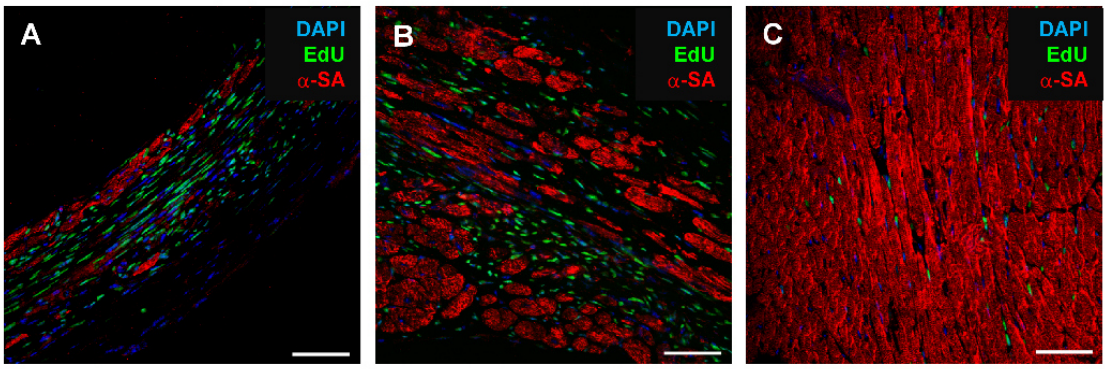
A, Representatives ECG traces after s.c. injection of lethal doses of isoproterenol (1000mg/kg and 400mg/kg isoproterenol). 7 min after injection, ECG showed sinus arrest with a ventricular escape rhythm (1000mg/kg), or with premature ventricular contractions (400mg/kg) which ended with lethal tachyarrhythmias. **B**, Heart rate during echocardiography. Heart rate did not significantly differ between groups at any time point. NS=not significant ($p>0.05$). **B**, Data are shown as mean ± SEM.



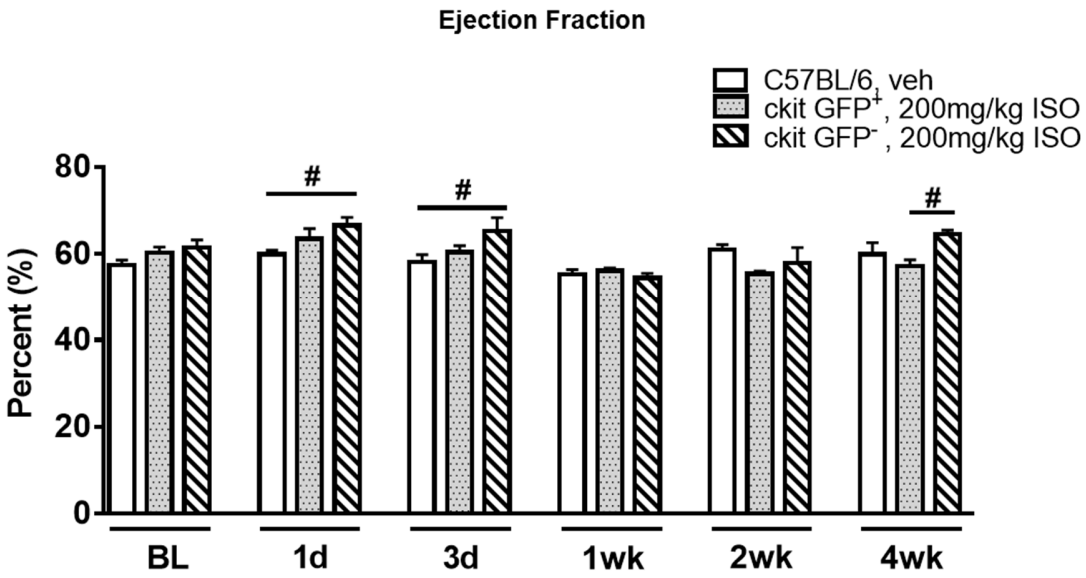
Online Figure II: Diastolic function post-ISO injection. Related to Figure 1
ECHO measurements were performed at baseline (BL), 1, 3, 7, 14, 28 days post-injection (vehicle (n=14), 200mg/kg ISO (n=15) and 300mg/kg ISO (n=14)). **A-D**, Diastolic function was determined using ECHO coupled with pulsed wave Doppler (PW) and tissue Doppler (TDI) techniques. **A-D**, Spectral waveforms were analyzed for peak early- and late-diastolic transmitral velocities (E and A waves), E/A ratio (**B**) and the acceleration time of the transmitral early diastolic peak flow velocity (E_{AT})(**D**). **C**, E´ was the tissue peak velocity obtained from LV posterior wall. There was no difference in diastolic function (E, E/A, E´, E_{AT}) between groups at any time point. *p<0.05 vs BL vehicle. NS=not significant (p>0.05). Data are shown as mean ± SEM. **E**, Representative spectral waveforms at baseline (upper panel) and 1 day post-vehicle, 200mg/kg ISO, or 300mg/kg ISO injection (lower panel). S=systole.



Online Figure III: Western analysis
Measuring total Caspase-3 or activated Caspase-3 after cleavage in heart lysates after treatment with vehicle, 200mg/kg ISO, or 300mg/kg ISO. Two primary antibodies were used: (1) total caspase-3, which detects total and cleaved Caspase-3, and (2) activated Caspase-3. Positive control (+) is lysate from HEK cells treated with 2mmol/L H_2O_2 for 24 hours.

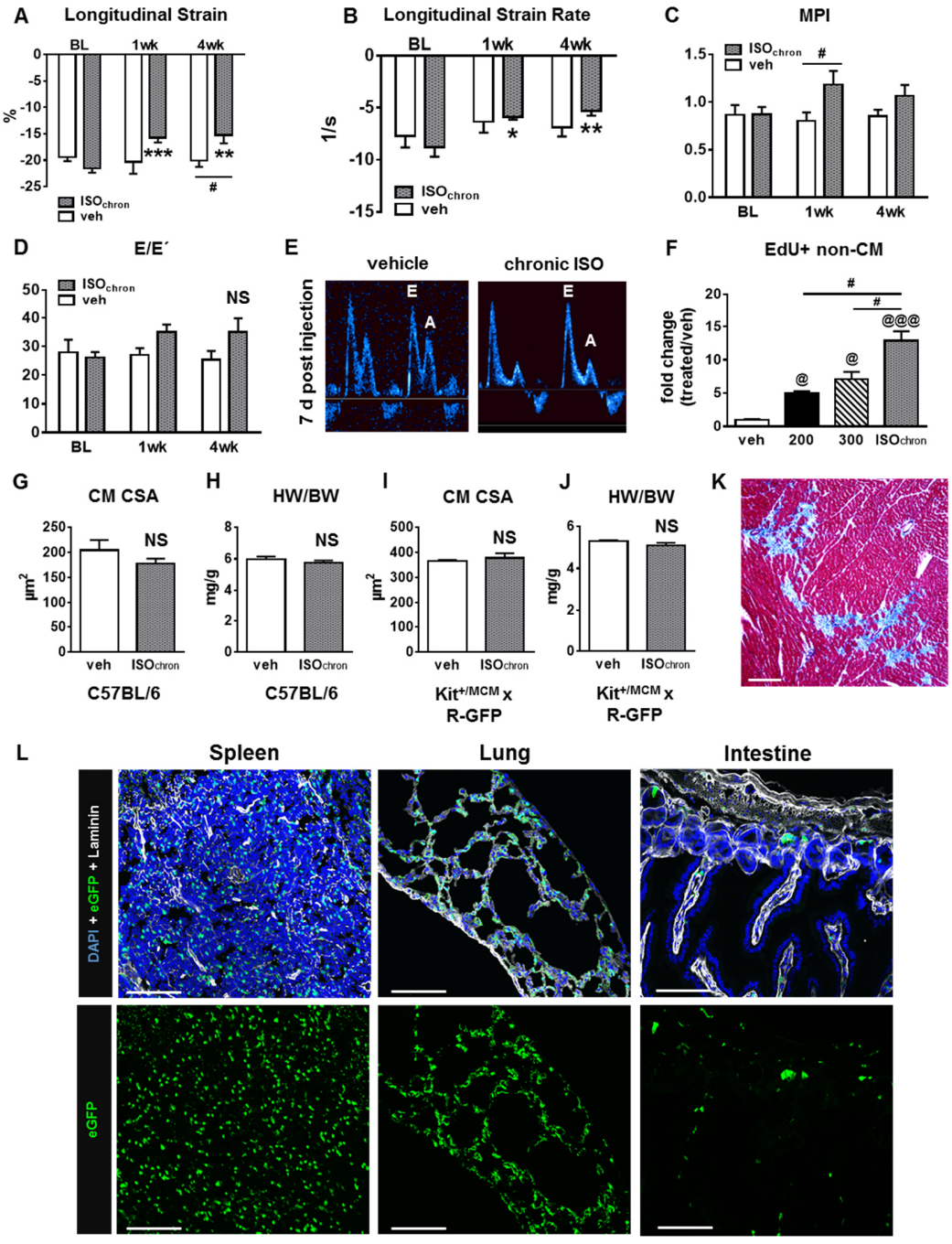


Online Figure IV: EdU^{+} nuclei after myocardial infarction. Related to Figure 5
Representative images of EdU^{+} nuclei in the infarct area (**A**), border zone (**B**), and remote zone (**C**) 4 weeks post-MI induced by permanent occlusion of the LAD. Tissue sections were immunostained against α-sarcomeric actin (red), 5-ethynyl-2'-deoxyuridine (EdU, green), and nuclei labeled with 4',6-diamidino-2-phenylindole (DAPI, blue). A significant number of EdU^{+} non-myocyte nuclei were observed in the injured border zone and infarct area with fewer in the viable tissue of the remote zone. These likely are inflammatory cells that infiltrate into the injured heart after MI. Scale bars=50μm.



Online Figure V: Cardiac function in transgenic kit$^{+/crex}$ x R-GFP mouse post-ISO injection. Related to Figure 7

ECHO measurements were performed at baseline (BL), 1, 3, 7, 14, 28 days post-injection. C57BL/6 mice (n=14) were injected with vehicle, kit$^{+/cre}$ x R-GFP mice (ckit GFP^{+}, n=4) and the non-transgenic littermates (ckit GFP^{-}, n=3) with 200mg/kg isoproterenol (ISO). #$p<0.05$ between groups. Data are shown as mean ± SEM.



Online Figure VI: Cardiac function, heart weight to body weight ratio and myocyte cross-sectional area after chronic ISO administration. Related to Figure 8

A-D, ECHO measurements in C57BL/6 mice were performed at BL (baseline), 1 and 4 weeks post-injections (veh (n=5), ISO_{chron} (n=9)). **A** and **B**, Longitudinal strain and strain rate showed reduced contractility in the ISO group at 1 and 4 weeks post-injection. **C**, The myocardial performance index (MPI), a non-invasive Doppler index for the assessment of overall cardiac function, was significantly increased at 1 week post-ISO injections, indicating an impairment in global cardiac function. **D**, E/E´, a surrogate for left ventricular filling pressures, was slightly increased at 1 and 4 weeks post-ISO injections. **E**, Representative spectral waveforms of peak early- (E) and late-diastolic (A) transmitral velocities in a vehicle and an ISO-treated C57BL/6 mouse. **F**, Dose-dependent fold change increase in

the percentage of EdU^+ non-myocyte nuclei after veh (n=8), single 200mg/kg ISO (n=3), single 300mg/kg ISO (n=3) and multiple (twice daily for 6 days) ISO (n=5) injections in C57BL/6 mice. **G-J**, In C57BL/6 mice, myocyte cross-sectional area (CM CSA) (**G**) and heart weight to body weight ratio (HW/BW) (**H**); in $Kit^{+/MCM}$ x R-GFP mice, CM CSA (**I**) and HW/BW (**J**). **K**, Bright-field micrographs of a Masson´s trichrome-stained tissue section from a $Kit^{+/MCM}$ x R-GFP mouse at 4 weeks post-ISO injection. Scale bar=200µm. **L**, Histological analysis of $eGFP^+$ cells from spleen, lung and intestine from $Kit^{+/MCM}$ × R-GFP mice that were given tamoxifen for two weeks prior to chronic ISO dosing. Nuclei are shown in blue and green shows $eGFP^+$ cells in the expected patterns coincident with known regions of c-kit protein expression. Scale Bars=100µm. *p<0.05, **p<0.01 and ***p<0.001 vs. BL ISO_{chron}, #p<0.05 between groups, @p<0.05 and @@@p< 0.001 vs. veh, NS=not significant (p>0.05). **A-D** and **F-J**, Data are shown as mean ± SEM.

Supplemental References

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Legends for Video files

Online Video I:
Transthoracic ECHO loops at 1 day post-injection of 300mg/kg ISO. Related to Figure 1
Parasternal short axis view showing increased cardiac function.

Online Video II:
Transthoracic ECHO loops at 1 day post-injection of 300mg/kg ISO. Related to Figure 1
Parasternal long axis view showing increased cardiac function.

Online Video III:
Transthoracic ECHO loops at 1 day in a vehicle treated mouse. Related to Figure 1
Parasternal short axis view showing normal cardiac function.

Online Video IV:
Transthoracic ECHO loops at 1 day in a vehicle treated mouse. Related to Figure 1
Parasternal long axis view showing normal cardiac function.