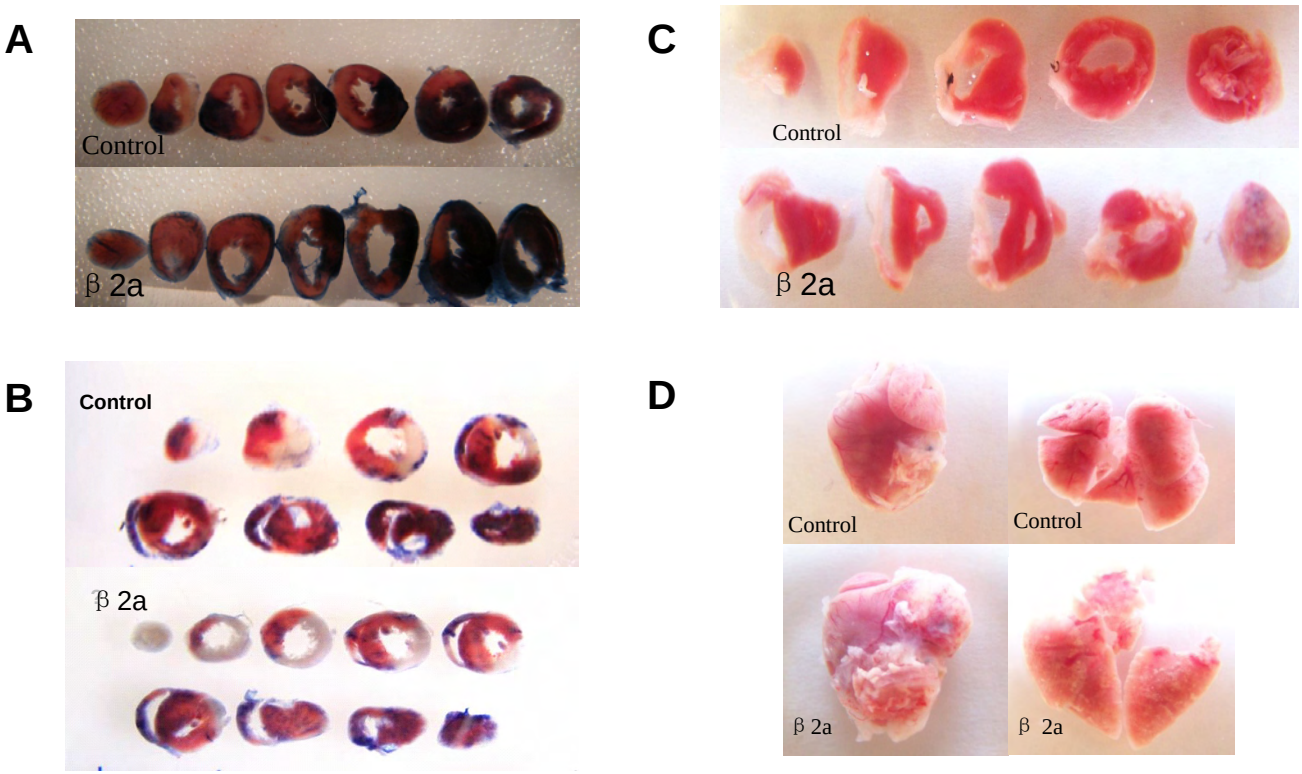
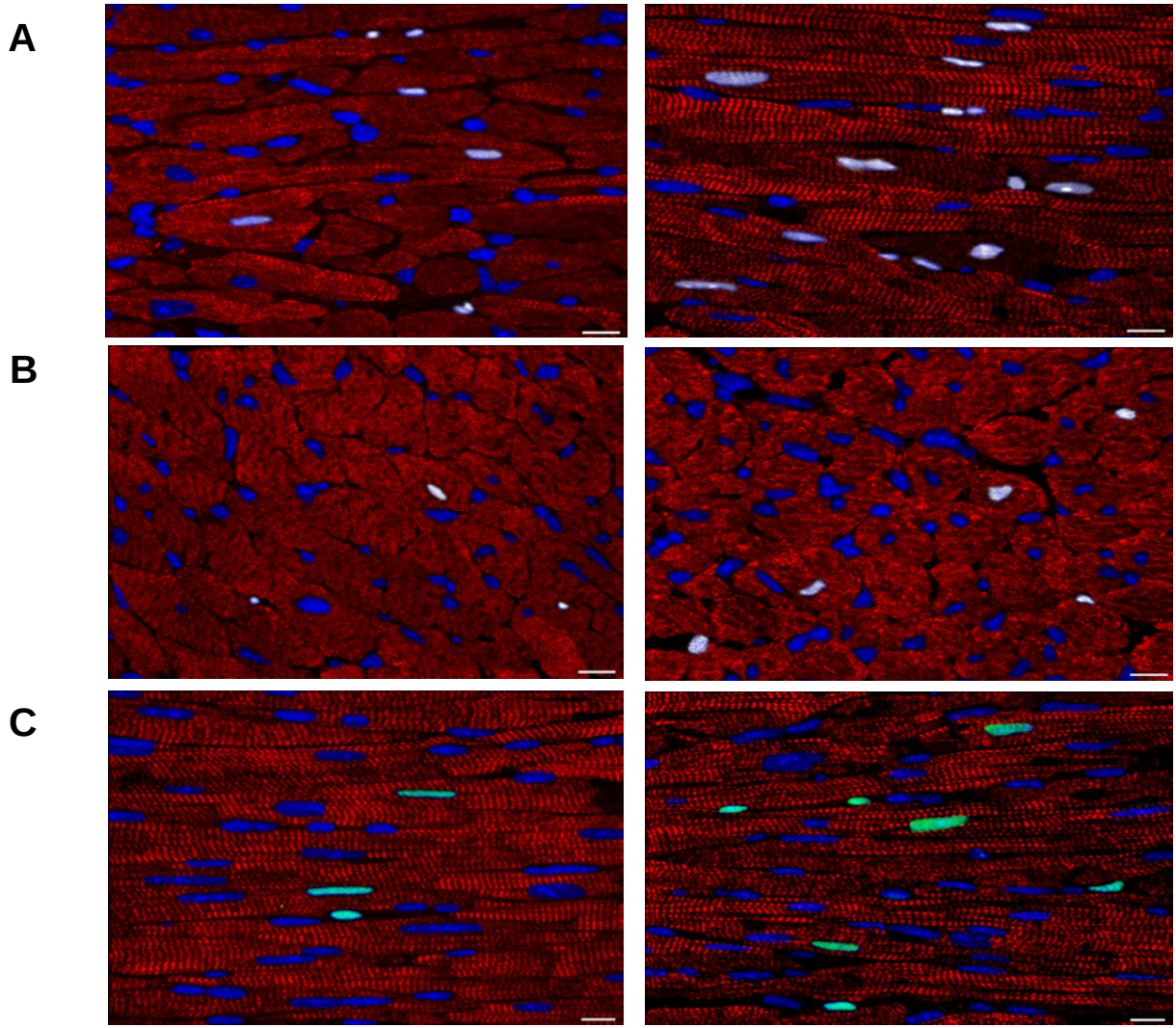
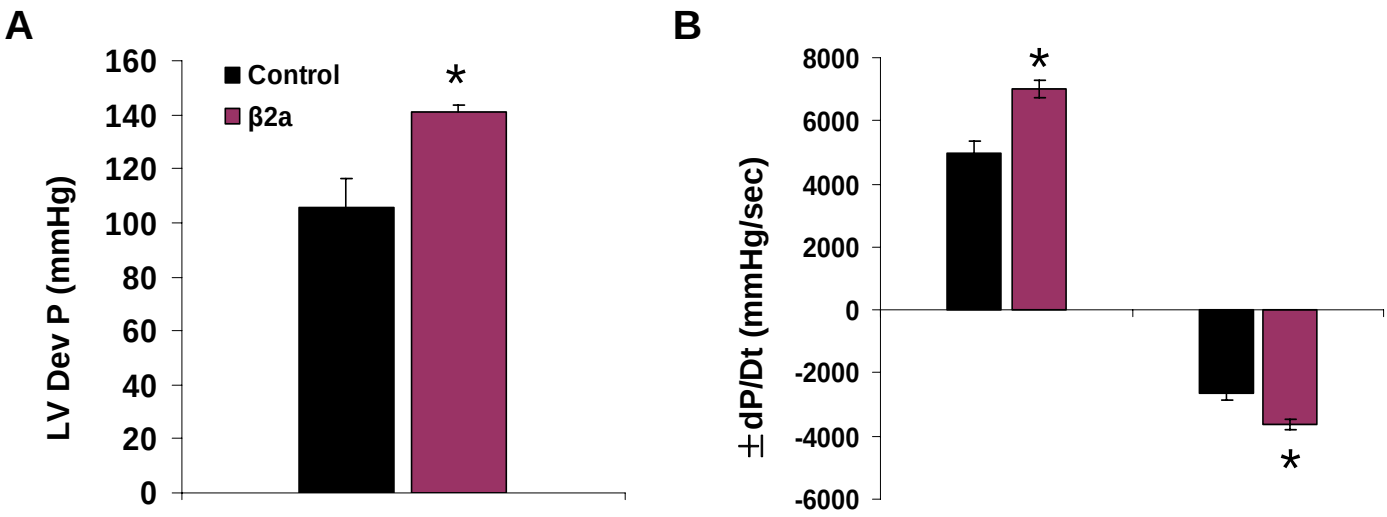
Online Figures



Online Figure I. Area at risk (AAR) and infarction size in hearts subject to I/R or MI. **(A).** Representative images of area at risk (AAR) in cardiac tissue sections stained with Evans blue. (**B).** Infarction area measurement in tissue sections stained with TTC from hearts after 30 minutes ischemia and 24 hours reperfusion. (**C).** Infarct length measurement in cardiac tissue sections stained with TTC from hearts at 6 weeks after MI. (**D).** Representative hearts and lungs from control and β 2a mice at 6 weeks after MI

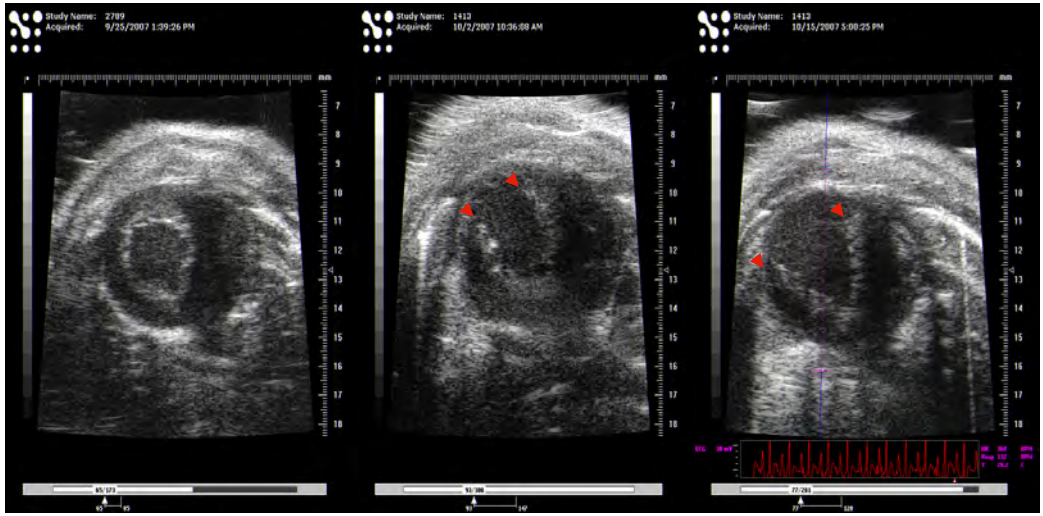


Online Figure II. TUNEL and Ki67 staining in cardiac tissue sections after MI. **A-B**. Representative images of TUNEL+ nuclei in border **(A)** and remote zone **(B)** of myocardium in control and β 2a hearts. TdT is white, alpha-sarcomeric actin is red and DAPI is blue. (**C).** Representative Ki67 staining (shown as green) in tissue sections from control and β 2a hearts. Scale bar = 10um.



Online Figure III. Baseline level of LVDP (**A**) and ±dp/dt (**B**) were greater in β 2a isolated hearts (n=5) versus control (n=8). * $p < 0.05$

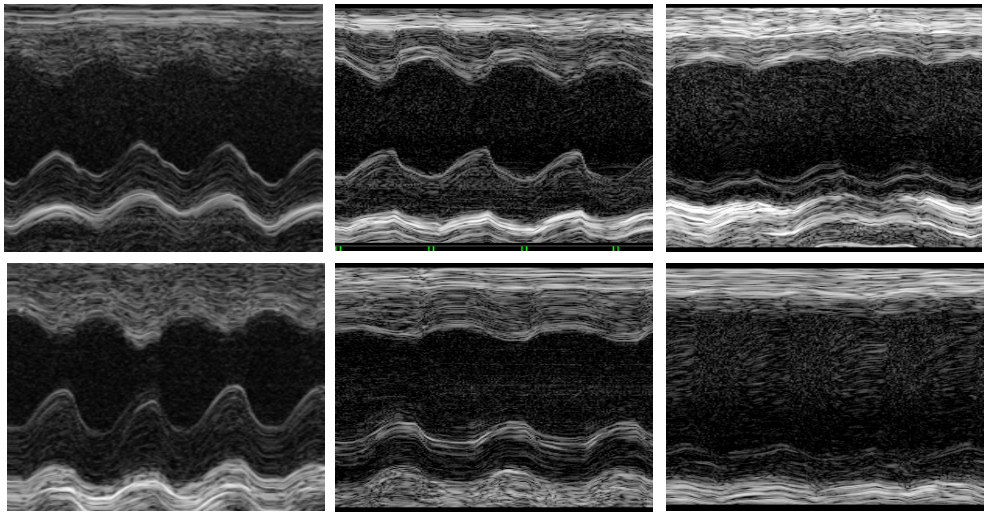
A



β 2a

Pre -MI Post- MI 2 wks Post- MI 4 wks

B

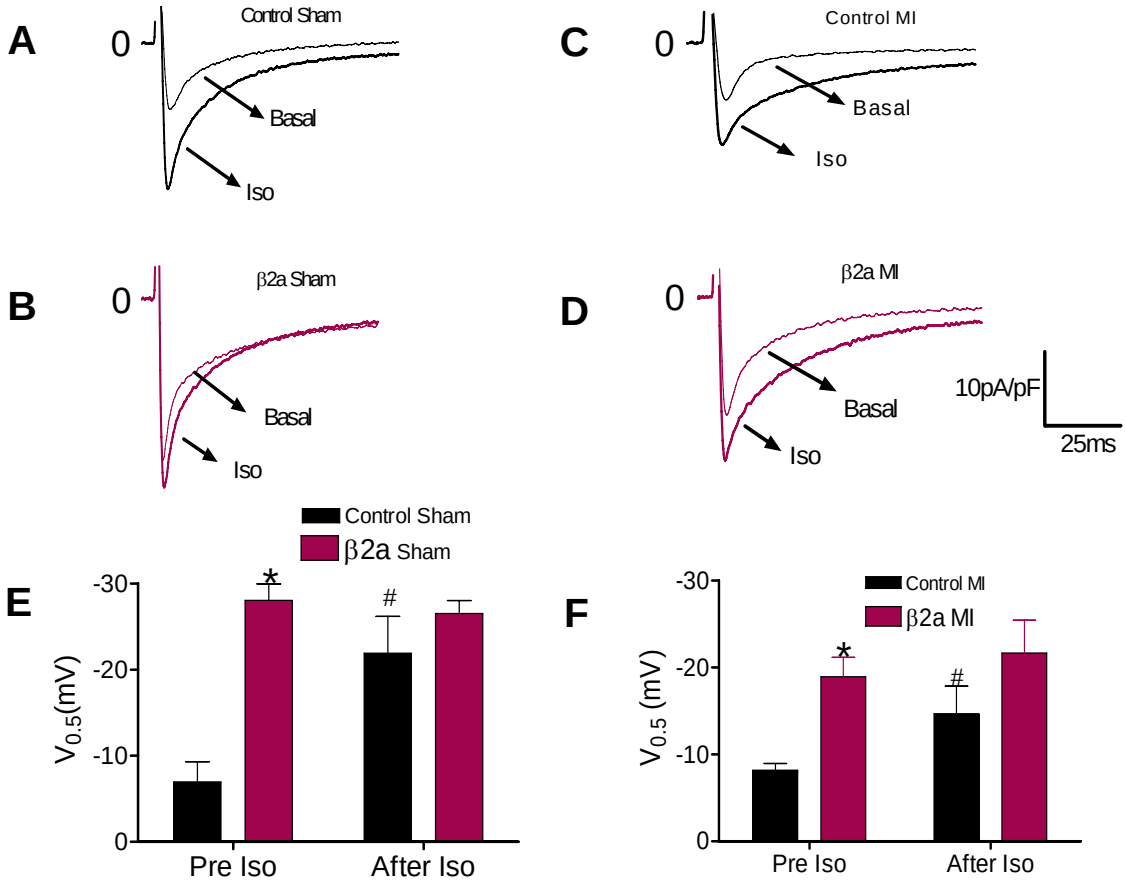


Control

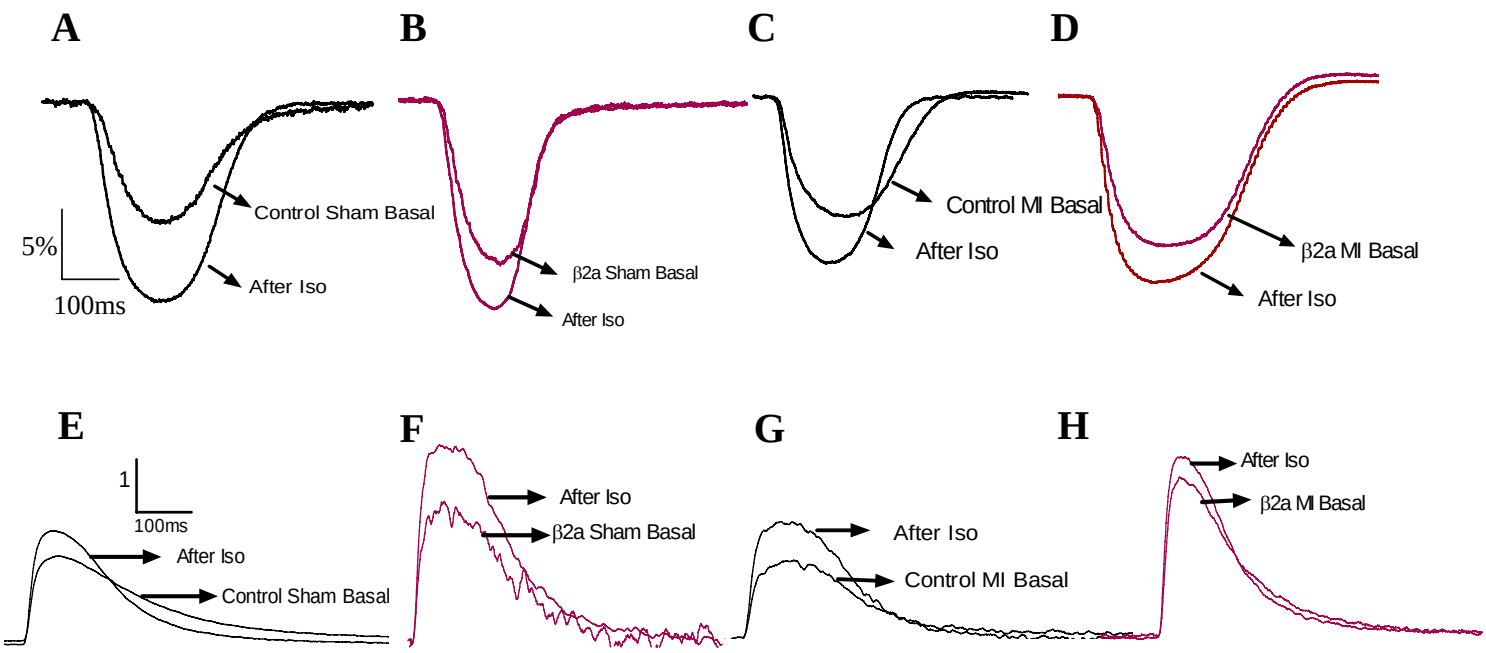
β 2a

Pre -MI Post- MI 2 wks Post- MI 4 wks

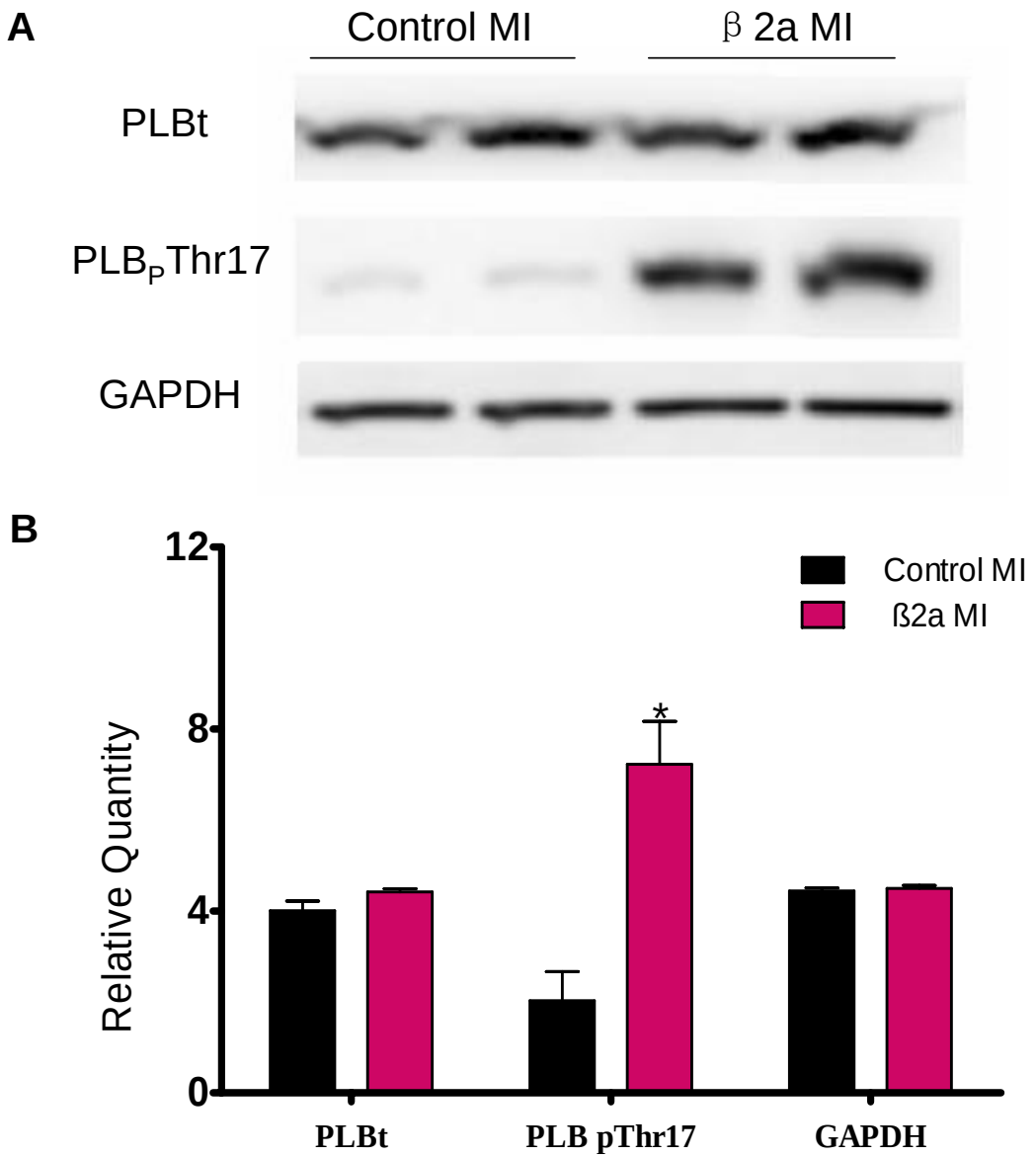
Online Figure IV. (**A).** Representative LV B-mode echocardiographic images in a β 2a mouse before and 2, 4 weeks after MI. The area between the red arrow heads indicate myocardial infarction zone. (**B).** Representative LV M-mode echocardiogrphic images in control and β 2a mice before and 2,4 weeks after MI.



Online Figure V. (**A-B).** Representative recordings of ICa,L before and after the application of Iso in sham control and β 2a VMs. (**C-D).** Representative recordings of ICa,L before and after application of Iso in post-MI control and β 2a VMs. (**E).** Average half-activation potential ($V_{0.5}$) in sham VMs before and after Iso treatment. (**F).** Average half-activation potential ($V_{0.5}$) in post-MI VMs before and after Iso treatment.



Online Figure VI. (**A-D).** Representative recordings of myocyte shortening before and after Iso in sham or post-MI myocytes from control and β 2a hearts. (**E-H).** Representative recordings of $[Ca^{2+}]_i$ transients before and after Iso in sham or post-MI VMs from control and β 2a hearts.



Online Figure VII. Western Blot analysis of PLBt and PLB$_P$Thr17 protein levels in control and β 2a hearts after 3 weeks MI. **(A).** Representative western blot of PLBt and PLB$_P$Thr17 protein from control and β 2a hearts . **(B).** Analysis of abundance of PLBt and PLB$_P$Thr17 protein levels normalized to GAPDH from control (n=3)and β 2a hearts (n=3). * $p < 0.05$.

Detailed Methods:

Animals and Coronary Artery Ligation*:* A transgenic mouse line with cardiac specific, conditional, low level expression of the β2a subunit of the LTCC was used[1]. Experiments were performed following the National Institutes of Health *Guide for the Care and Use of Laboratory Animals,* and all procedures were approved by the Institutional Animal Care and Use Committee at Temple University. Myocardial infarction (MI) surgeries were carried out in mice at the age of 4 months when the β2a gene is fully expressed[1]. Mice were anesthetized with inhalation of 2% isoflurane. A skin incision was made over the left thorax, and the pectoral muscles were retracted to expose the ribs. At the level of the fifth intercostal space, the heart was exposed. A permanent knot or a slipknot (to allow for reperfusion) was made around the left anterior descending coronary artery (LAD) 2–3 mm from its origin with a 6-0 silk suture. After the knot was tied the lung were expanded and muscle and the skin layers were closed. Sham-operated animals were subjected to the same surgical procedures except that the suture was passed under the LAD but was not tied[2]. To induce ischemia/reperfusion injury and determine infarction size the LAD was occluded for 30 min and then the heart was reperfused for 24 hours.

Determination of LV area at risk and infarct size after I/R or MI*:* Area at risk (AAR) was measured by injecting 0.2 ml of 2% Evans blue dye into the right ventricle before the heart was excised. Areas of the heart with normal blood flow stained blue. The stained heart was quickly removed and frozen and placed on dry ice. Then the heart was cut into eight 1.0-mm-thick sections perpendicular to the long axis of the heart. The sections were incubated in PBS at room temperature for 15 min and then digitally photographed. The blue areas (area not at risk, ANAR), and non blue areas (AAR) were measured with the NIH ImageJ software in at least 7 hearts of each group and the percentage of AAR was calculated (AAR/(AAR+ANAR)).

To evaluate infarction size, viable tissue was stained in frozen hearts excised after 24 hours of reperfusion. The sections were incubated in PBS containing 2% triphenyltetrazolium chloride (TTC; Sigma) at room temperature for 15 min and digitally photographed. TTC-negative staining area (infarcted myocardium) was measured and normalized by total myocardial section area. Myocardial infarct size was calculated using normalized infarcted area (TTC-negative staining area) divided by the average AAR determined in other hearts from the same group.

In permanent ischemia studies, only TTC was used to stain viable tissue, and non-stained scar tissue was measured as infarct length, which was expressed as the percentage of total circumference of LV tissue sections.

In-vivo functional analysis *(Echocardiography, ECHO):* ECHO was performed (VisualSonics Vevo 770) as described in our previous studies[3]. Mice were anesthetized with 2% isoflurane initially and then 1% during the ECHO procedure to maintain the heart rate between 400 and 450 bpm. Hearts were viewed in the short-axis between the two papillary muscles and analyzed in M-mode. Parameters (Vevo software) including end-diastolic diameter (EDD), end-systolic diameter (ESD), posterior wall thickness (PWT), and septal wall thickness (SWT) were measured to determine changes in cardiac morphology and function (ejection fraction (EF) and fractional shortening (FS)).

Ex-vivo functional analysis *before and after ischemia (Langendorff):* Hearts were placed on a Langendorff apparatus (ML785B2, ADInstruments, Colorado Springs,Colo), paced at 8.0 Hz, and perfused at a constant pressure of 80 mm Hg (STH pump controller ML175, ADInstruments) with a modified Krebs–Henseleit buffer solution containing (in mmol/L): 2.0 $CaCl_2$, 130 NaCl, 5.4 KCl, 11 glucose, 2 pyruvate, 0.5 $MgCl_2$, 0.5 NaH_2PO_4, and 25 $NaHCO_3$ and aerated with 95% oxygen and 5% carbon dioxide, pH 7.35 to 7.4. The temperature was maintained at 37°C by immersing the heart in a water-heated glassware reservoir containing

preheated KHB. A water-filled balloon was inserted into the left ventricle and adjusted to achieve a left ventricular end-diastolic pressure (LVEDP) of 10 mm Hg. After a 20-min equilibration period, hearts are subjected to 15 min of no flow global ischemia, followed by 30 min of reperfusion. The LVEDP, left ventricular developed pressure (LVDP) (peak systolic pressure minus LVEDP), maximum rate of contraction (+dP/dt), and maximum rate of relaxation (−dP/dt) were recorded continuously by a data acquisition system (Powerlab/8SP, ADInstruments)[4].

***Histology**:* Animals were anesthetized with sodium pentobarbital (120mg/kg BW, intraperitoneal injection) and heparinized intravenously. Hearts were excised, trimmed of excess tissue, weighed, rinsed and perfused with Ca^{2+}-free KHB to to remove blood from blood vessels and then perfused with 10% buffered formalin. The fixed heart tissues were dehydrated, embedded in paraffin, sectioned at 5-μm thickness, and stained with hematoxylin/eosin for cross-sectional area measurement. Myocyte cross-sectional area was measured from sections of non-infarct zone obtained mid-distance between the base and apex. Suitable cross-sections were defined as having nearly circular-to-oval myocyte contour. The perimeter of 100–200 myocytes was traced in sections from 5 regions of the LV of each animal, using NIH Image J software system. The mean area was calculated for all the regions measured in control and β2a heart tissue sections. Masson's trichrome staining was used to evaluate gross morphology, fiber integrity and interstitial fibrosis. Images were acquired using SPOTINSIGHT software (Diagnostic Instruments Ins.). 3 fields were studied from each tissue section per heart and blue area (interstitial fibrosis) were analyzed using Metamorph 6.1 software (Universal Image Corp.). Average blue area percentage was calculated in at least 3 different hearts.

Apoptotic cell death was detected in situ by TUNEL on paraffin sections of mouse hearts. After the TUNEL procedure, DAPI was used for the staining of all nuclei and slides were observed under a fluorescence microscope. The mean number of positive nuclei and DAPI-stained nuclei per 40X field in LV regions was determined by manual counting. A minimum of 5 sections from 5 LV regions (ischemic border zone and remote region of the LV) of each heart were examined for TUNEL-positive cells. Approach from Dr. kajstura lab for TUNEL assay[5]: sections were incubated in a solution containing 5 U TdT, 2.5 mM $CoCl_2$, 0.2 M cacodylate, 25 mM Tris-HCl, 0.25% BSA, and 0.5 nM of biotin-16-dUTP (Roche Biochemicals). dUTP was detected by exposing samples to 5 μg/ml of FITC-avidin dissolved in 4X concentrated saline-sodium citrate buffer containing 0.1% Triton X-100. Myocyte cytoplasm was identified by α-sarcomeric actin antibody. Nuclei were stained with 10 μg/ml of propidium iodide (PI) [6-7]. *Ki67 labeling*: Sections were incubated with Ki67 rabbit polyclonal antibody (Beckman Coulter) and FITC-conjugated anti-rabbit IgG (Sigma). Myocyte cytoplasm was recognized by α-sarcomeric actin antibody and nuclei were identified by propidium iodide. Sections were evaluated by confocal microscopy[5].

Western Blot analysis: *Western blotting:* Cytoplasmic and membrane protein were isolated from ventricular tissue using PBS lysis buffer containing: 0.5% Triton X-100, 5 mM EDTA (pH7.4), phosphatase inhibitors (10 mM NaF and 0.1 mM NaVO4), proteinase inhibitors (10 μg/ml aprotinin, 10 μg/ml leupeptin, 1mM phenylmethylsulfonyl fluoride, 5 μg/ml pepstatin A, 8 μg/ml calpain inhibitor I & II, and 200 μg/ml benzamidine). Cardiac actin was isolated from resulting pellet using PBS lysis buffer containing 2% sodium dodecyl sulfate, SDS (FisherBiotech), 1% IGEPAL CA-630 (Sigma), 0.5 % deoxycholate (Sigma), 5 mM EDTA (pH 7.4), and proteinase inhibitors. Protein abundance and phosphorylation levels in isolated protein were analyzed with Western blot analysis as described previously [8]. Target antigens were probed with the following antibodies: phospholamban (PLB) (Upstate Biotechnology), GAPDH (Serotec) and PT17-PLB (Badrilla).

Caspase Assay: Caspase activity was measured with Caspase assay system (Promega, Madison, WI). In brief, LV lysates were prepared by dounce homogenization in lysis buffer provided with the kit. The lysates were centrifuged at 15,000g for 20 minutes at 4^{o}C, and the supernatants containing 100 µg protein were used for caspase activity assay using specific fluorogenic conjugated substrate MCA-Val-Asp-Gln-Met-Asp-Gly-Trp-Lys-(DNP)-NH2 [9].

Cellular functional analysis*:* Myocytes were isolated from hearts 2-3 weeks after MI to measure cellular fractional shortening (FS), Ca^{2+} transients ($[Ca^{2+}]_i$)and L-type calcium current (I_{CaL}). All experiments were done a 35-37^{o}C, in superfused myocyte chambers mounted on fluorescence-capable microscopes. All myocytes were characterized with the same series of experiments[3, 10-11].
To isolate myocytes[3, 10-11] , mice were anesthetized with sodium pentobarbital (120mg/1 kg BW). The heart was excised, weighed, cannulated and perfused retrogradely on a constant-flow Langendorff apparatus. The heart was digested by retrograde perfusion with normal Tyrode solution containing 180 U/mL collagenase and (in mM): 0.02 $CaCl_2$, 10 glucose, 5 HEPES, 5.4 KCl, 1.2 $MgCl_2$, 150 NaCl, 2 sodium pyruvate, pH 7.4. When the heart was softened, left ventricular tissue was gently minced and myocytes were dissociated by gentle suction with a transfer pipette. Myocytes were filtered and equilibrated in Tyrodes solution with 0.2 mM $CaCl_2$, and 1% bovine serum albumin (BSA) at room temperature. Our initial yield was >90% rod-shaped VMs, and >80% calcium-tolerant, rod-shaped VMS survive by the end of the isolation.

Electrophysiology: I_{CaL} was measured in a sodium-free and potassium-free solution. Isolated myocytes were placed in a chamber mounted on an inverted microscope (Nikon Diaphot) and perfused with 1 mM Ca^{2+} Tyrode solution. Both the inflow solution and the chamber were water-heated to maintain the temperature at 36±1°C. A 4-5 MΩ pipette filled with a Cs^{+}-containing solution [composition in mM: 130 Cs-aspartate, 10 *N*-methyl-Dglucamine (NMDG), 20 tetraethylammonium chloride, 10 HEPES, 2.5 Tris-ATP, 1 $MgCl_2$, and 10 EGTA, pH 7.2] was used to obtain gigaseals. Once a gigaseal was formed, the patch was ruptured and the cell was dialyzed for 10 min. The extracellular bath was then changed to a 2 mM Ca^{2+}-containing Cs^{+} substitution bath solution (composition in mM: 2 4-aminopyridine, 2 $CaCl_2$, 5.4 CsCl, 10 glucose, 5 HEPES, 1.2 $MgCl_2$, and 150 NMDG, pH 7.4 with CsOH). Membrane voltage was controlled by an Axopatch 2B voltage-clamp amplifier and the current was digitized by Digidata 1200 using pClamp8 software (Molecular Devices). Once the signal was converted to digital format, it was stored on a personal computer for off-line analysis with Clampfit 9 (Axon Instruments)[11] .

Contraction and Ca^{2+} transients: Fractional shortening was measured in myocytes incubated with Tyrode solution containing 1 mM Ca^{2+} at rates of 0.5 Hz stimulation with edge detection. $[Ca^{2+}]_i$ were measured in paced myocytes loaded with Fluo-4AM as described in previous studies[12].

***Statistics*:** Data were reported as mean±SEM. When appropriate, paired and unpaired T-test, ANOVA or ANOVA for repeated measures were used to detect significance with SAS 9.0 (SAS Institute Inc.). A *p* value of ≤0.05 was considered significant.

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