Detailed Methods

Mice and Experimental Protocol WT and RyR-S2808A mice were used in these studies. The methods used to generate the RyR-S2808A mouse have been described previously[1]. Experiments were performed based on the National Institutes of Health *Guide for the Care and Use of Laboratory Animals,* and all procedures were approved by the Temple University Committee on Animal Care. Myocardial infarction (MI) surgeries were carried out in mice at the age of 4 months. Mice were anesthetized with 2% isoflurane inhalation. 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 and pumped out through an expanded space between ribs. A permanent knot or slipknot (for reperfusion purpose) was made around the left anterior descending coronary artery (LAD) 2–3 mm from its origin with a 6-0 silk suture. The heart was immediately placed back into the intrathoracic space after the knot was tied,, followed by manual evacuation of pneumothoraces and closure of muscle and the skin suture by means of the previously placed purse-string suture. Sham-operated animals were subjected to the same surgical procedures except that the suture was passed under the LAD but was not tied [2-3].

Determination of Left Ventricle Infarct Size After MI After mouse was anesthetized; heart was quickly removed and frozen in a dish placed on dry ice for a few minutes. Then the heart was quickly sliced into eight 1.2-mm-thick sections perpendicular to the long axis of the heart. 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) and total circumference of section were measured by NIH Image J software. Myocardial infarction size (expressed as percentage) was calculated using infarct length divided by total circumference of tissue sections.

In-vivo Functional Analysis *(Echocardiography, ECHO)* ECHO was performed with VisualSonics Velvo 770 machine which is specifically designed for mice and rats. Mice were anesthetized with 2% isoflurane initially and then 1% during the ECHO procedure. Hearts were viewed in the short-axis between the two papillary muscles and analyzed in M-mode. Parameters were to be measured offline (Velvo software) including end-diastolic diameter (EDD), end-systolic diameter (ESD), posterior wall thickness (PWT), and septal wall thickness (SWT) to determine cardiac morphological changes and ejection fraction (EF), heart rate and fractional shortening (FS).

Histology Animals were anesthetized by sodium pentobarbital (0.1ml/100g) and heparinized intravenously. Hearts were excised, trimmed off excess tissue, weighed, washed and then perfused with 10% buffered formalin. The fixed heart tissues were dehydrated, embedded in paraffin, sectioned at 5-μm thickness, and then perform Masson's Trichrome staning, which was used to evaluate gross morphology, fiber integrity or fibrosis. Images were acquired using SPOTINSIGHT software (Diagnostic Instruments Ins.). 3 fields were captured from each tissue section per heart and blue area were automatically analyzed using Metamorph 6.1 software (Universal Image Corp.). Average blue area percentage was calculated across at least 3 independent hearts.

Cellular Functional Analysis Myocytes were isolated from sham and post-MI animals' hearts at the end of 2-3 weeks to measure cellular fractional shortenting (FS), calcium

transients ($[Ca^{2+}]_i$)and L-type calcium current (I_{CaL}). All experiments were done a 35-37^oC, in superfused myocyte chambers mounted on fluorescence equipped microscopes. All myocytes were characterized with the same series of experiments. The detailed medthods are described as below.

Myocyte Isolation
Mice were anesthetized with sodium pentobarbital (0.1ml/100g). The heart was excised, weighed and cannulated on a constant-flow langendorff apparatus. The heart was digested by retrograde perfusion of normal tyrode solution containing 180 U/mL collagenase and (mM): CaCl2 0.02, glucose 10, HEPES 5, KCl 5.4, MgCl2 1.2, NaCl 150, sodium pyruvate 2, pH 7.4. When the tissue has softened, left ventricular tissue is gently minced, filtered, and equilibrated in Tyrodes solution with 0.2 mM $CaCl_2$, and 1% bovine serum alumin(BSA) at room temperature. Routinely, our initial yield is >90% rod-shaped VMs, and >80% calcium-tolerant, rod-shaped VMS survive by the end of the isolation.

I_{CaL} Measurement 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 1mM calcium-containing 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 2mM calcium -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 2A voltage-clamp amplifier and digitized by Digidata 1322 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 10 (Axon Instruments). The flow of the bathing solution was 2–3 ml/min [4].

Fractional Shortening Magnitude, Ca^{2+} Transients and SR Load Measurements
Fractional Shortening Magnitude and transients were measured simultaneously in myocytes incubated with Tyrodes solution containing 1mM Ca^{2+} at rates of 0.5 and 2 Hz stimulation with an edge detector. $[Ca^{2+}]_i$ were measured in paced myocytes loaded with Fluo-4AM as described in previous studies [5]. Once a steady state had been reached, 10 and 100nM Isoproterenol (Iso) was applied through the perfusion solution. Once a stable effect of Iso had been achieved, at least 20 continuous contractions and Ca^{2+} transients were recorded and averaged for analysis. Ca^{2+} transients were fit with a single-exponential decay function to determine the decay rate. To measure SR Ca^{2+} content, myocytes were paced at 0.5 Hz for 10 consecutive contractions, and 10 mM caffeine was then rapidly applied via a glass pipette close to the myocyte with a Pico spritzer [6-7]. Since caffeine cannot be repetitively applied on the same cell, the SR Ca^{2+} content after 10nM Iso was measured by a caffeine spritz after stable effects of Iso on myocyte contraction and Ca^{2+} transients had been observed.

In vivo hemodynamic measurements Mice were anesthetized with a 2% Isoflurane and the right common carotid artery was isolated and cannulated with 1.4 French micromanometer (Millar Instruments, Houston, TX). LV pressure, LV end-diastolic pressure (LVEDP) and heart rate (HR) were measured by this catheter advanced into

the LV cavity, and data was recorded and analyzed on a PowerLab System (AD Instruments Pty Ltd., Mountain View, CA). These parameters as well as maximal values of the instantaneous first derivative of LV pressure (+dP/dtmax, as a measure of cardiac contraction) and minimum values of the instantaneous first derivative of LV pressure (-dP/dtmin, as a measure of cardiac relaxation) were recorded at baseline and after administration of the β-adrenergic receptor (βAR) agonist, isoproterenol (Iso, 2ug/Kg and 2mg/Kg i.p. injection)

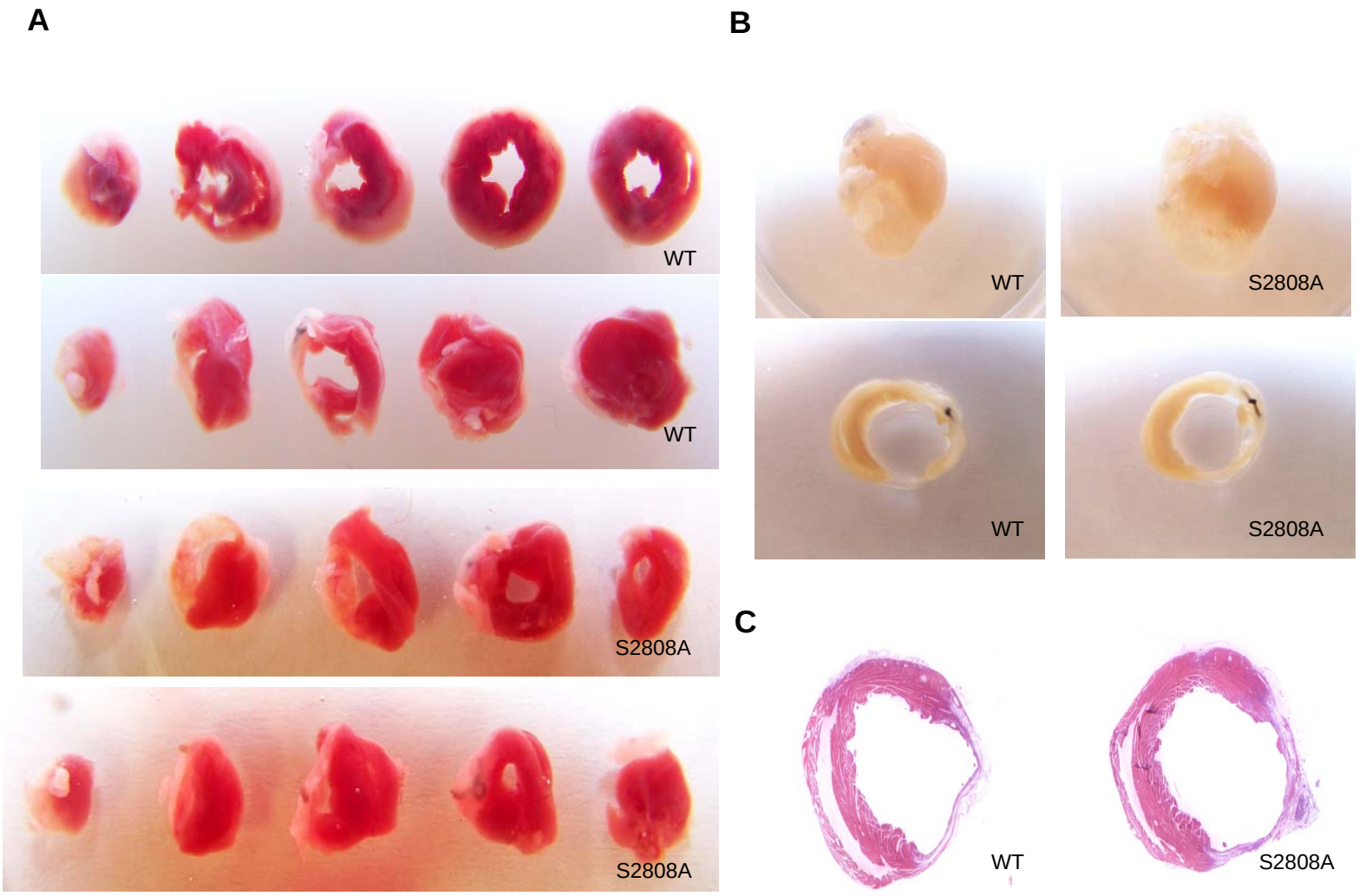
Western Blotting Cytoplasmic and membrane protein were isolated from ventricular tissue using PBS lysis buffer containing: 0.5% Triton X-100, 5 mM EDTA (pH7.4), phosphatase inhibitors (10 mM NaF and 0.1 mM NaVO4), proteinase inhibitors (10 μg/ml aprotinin, 10 μg/ml leupeptin, 1mM phenylmethylsulfonyl 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), RyR (Research Diagnostics), α-sarcomeric actin (Sigma), LTCC-α1C subunit (Chemicon), GAPDH (Serotec), PS2809-RyR, PS16-PLB, and PT17-PLB (Badrilla).

Statistics Data are presented as mean±SEM. Between-group comparisons were performed by using the one way ANOVA or t-test. For all tests, statistical significance was set at $P<0.05$.

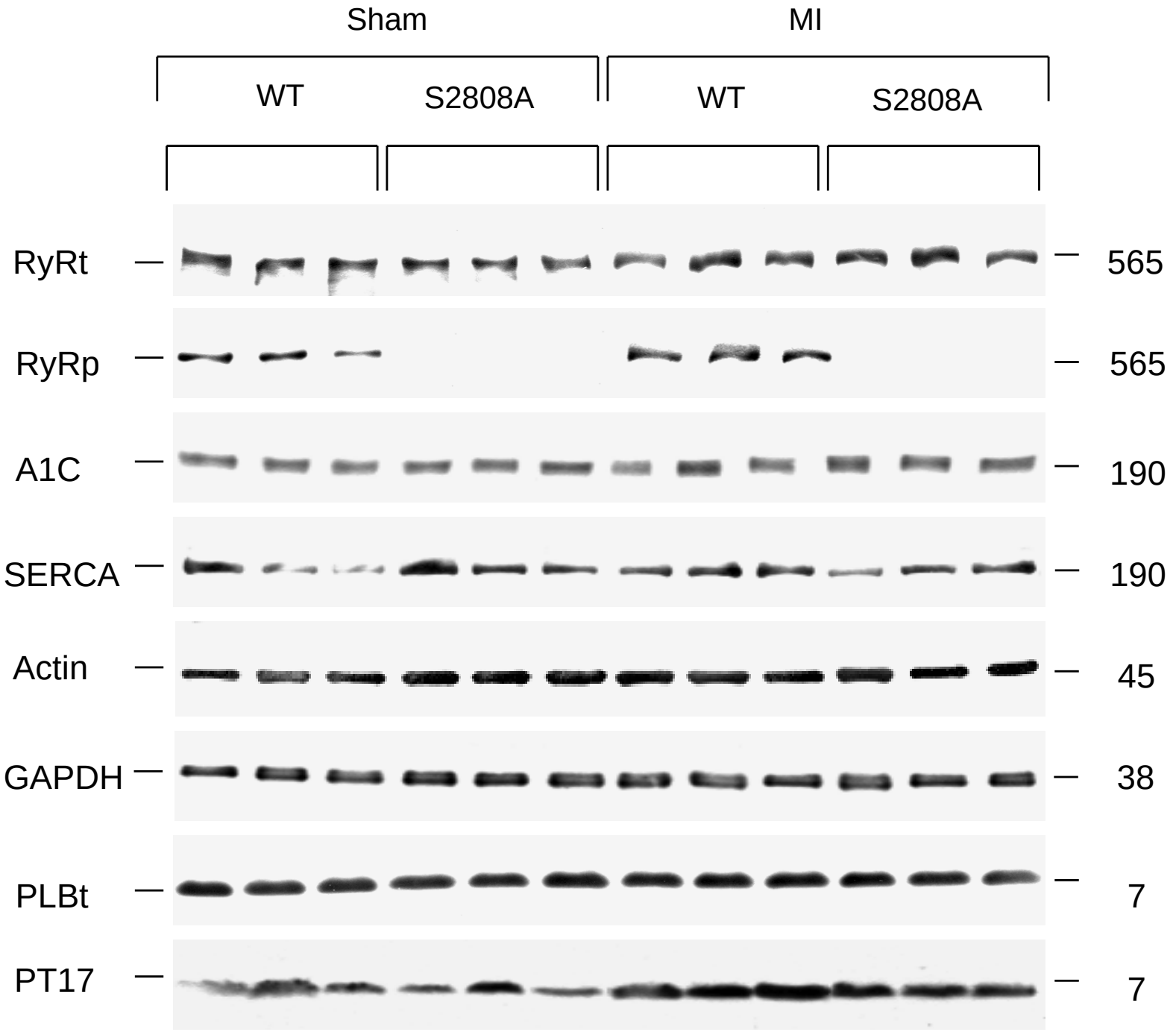
References

1. Benkusky NA, Weber CS, Scherman JA, Farrell EF, Hacker TA, John MC, Powers PA, Valdivia HH. Intact beta-adrenergic response and unmodified progression toward heart failure in mice with genetic ablation of a major protein kinase a phosphorylation site in the cardiac ryanodine receptor. *Circ Res*. 2007;101:819-829
2. Gao E, Boucher M, Chuprun JK, Zhou RH, Eckhart AD, Koch WJ. Darbepoetin alfa, a long-acting erythropoietin analog, offers novel and delayed cardioprotection for the ischemic heart. *Am J Physiol Heart Circ Physiol*. 2007;293:H60-68
3. Gao E, Lei YH, Shang X, Huang ZM, Zuo L, Boucher M, Fan Q, Chuprun JK, Ma XL, Koch WJ. A novel and efficient model of coronary artery ligation and myocardial infarction in the mouse. *Circ Res*. 2010;107:1445-1453
4. Chen X, Zhang X, Harris DM, Piacentino V, 3rd, Berretta RM, Margulies KB, Houser SR. Reduced effects of bay k 8644 on l-type ca2+ current in failing human cardiac myocytes are related to abnormal adrenergic regulation. *Am J Physiol Heart Circ Physiol*. 2008;294:H2257-2267
5. Chen X, Wilson RM, Kubo H, Berretta RM, Harris DM, Zhang X, Jaleel N, MacDonnell SM, Bearzi C, Tillmanns J, Trofimova I, Hosoda T, Mosna F, Cribbs L, Leri A, Kajstura J, Anversa P, Houser SR. Adolescent feline heart contains a population of small, proliferative ventricular myocytes with immature physiological properties. *Circ Res*. 2007;100:536-544
6. Piacentino V, 3rd, Weber CR, Chen X, Weisser-Thomas J, Margulies KB, Bers DM, Houser SR. Cellular basis of abnormal calcium transients of failing human ventricular myocytes. *Circ Res*. 2003;92:651-658
7. Tang M, Zhang X, Li Y, Guan Y, Ai X, Szeto C, Nakayama H, Zhang H, Ge S, Molkentin JD, Houser SR, Chen X. Enhanced basal contractility but reduced excitation-contraction coupling efficiency and beta-adrenergic reserve of hearts with increased cav1.2 activity. *Am J Physiol Heart Circ Physiol*. 2010;299:H519-528
8. Kubo H, Margulies KB, Piacentino V, 3rd, Gaughan JP, Houser SR. Patients with end-stage congestive heart failure treated with beta-adrenergic receptor antagonists have improved ventricular myocyte calcium regulatory protein abundance. *Circulation*. 2001;104:1012-1018

Supplemental Material

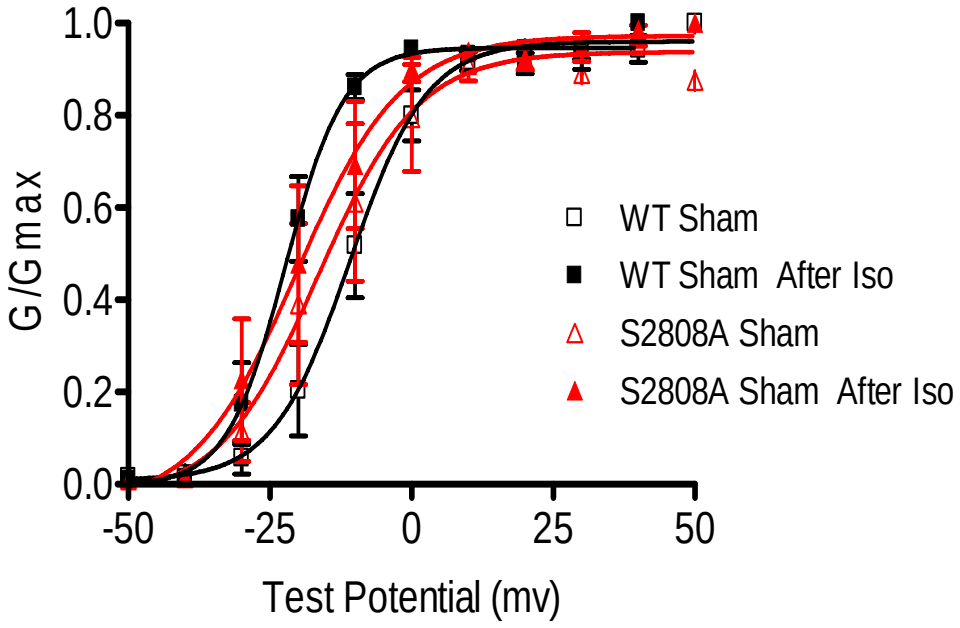


Onlinel Figure I A. Representative images of cardiac tissue sections stained with TTC after 4 weeks MI. **B.** Representative images of fixed hearts and middle cross sections with infarct zone. **C.** Representative cardiac histology tissue sections with H&E staining after 4 weeks MI.

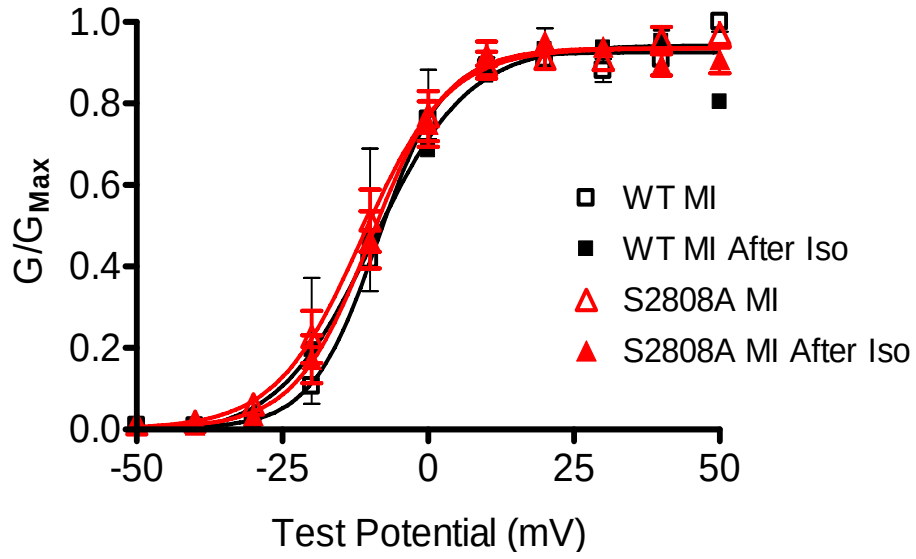


Online Figure II Representative Western blots of Ca^{2+} regulatory proteins in heart tissues from WT and S2808A mice 4 weeks after sham or MI-operated procedure.

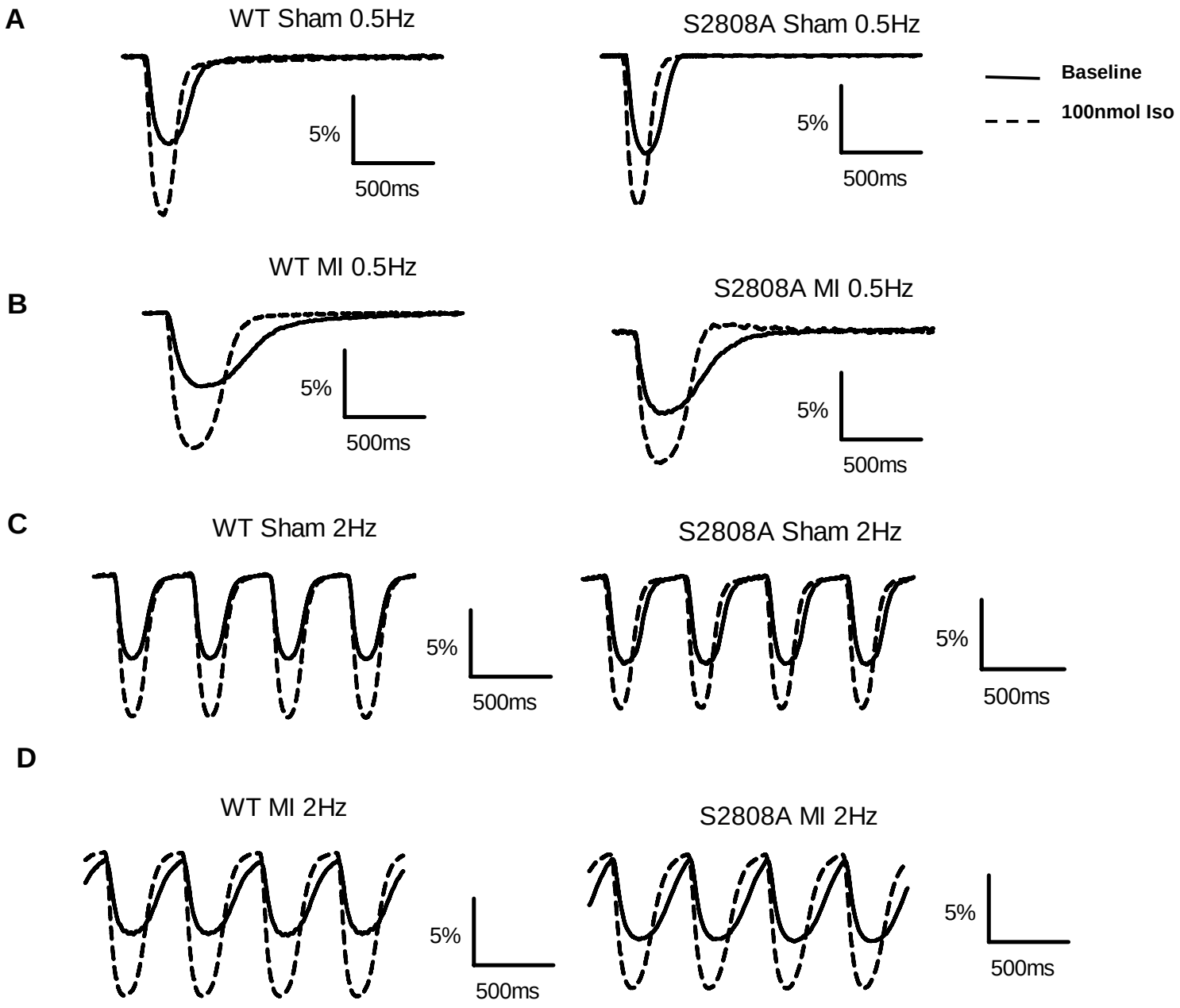
A



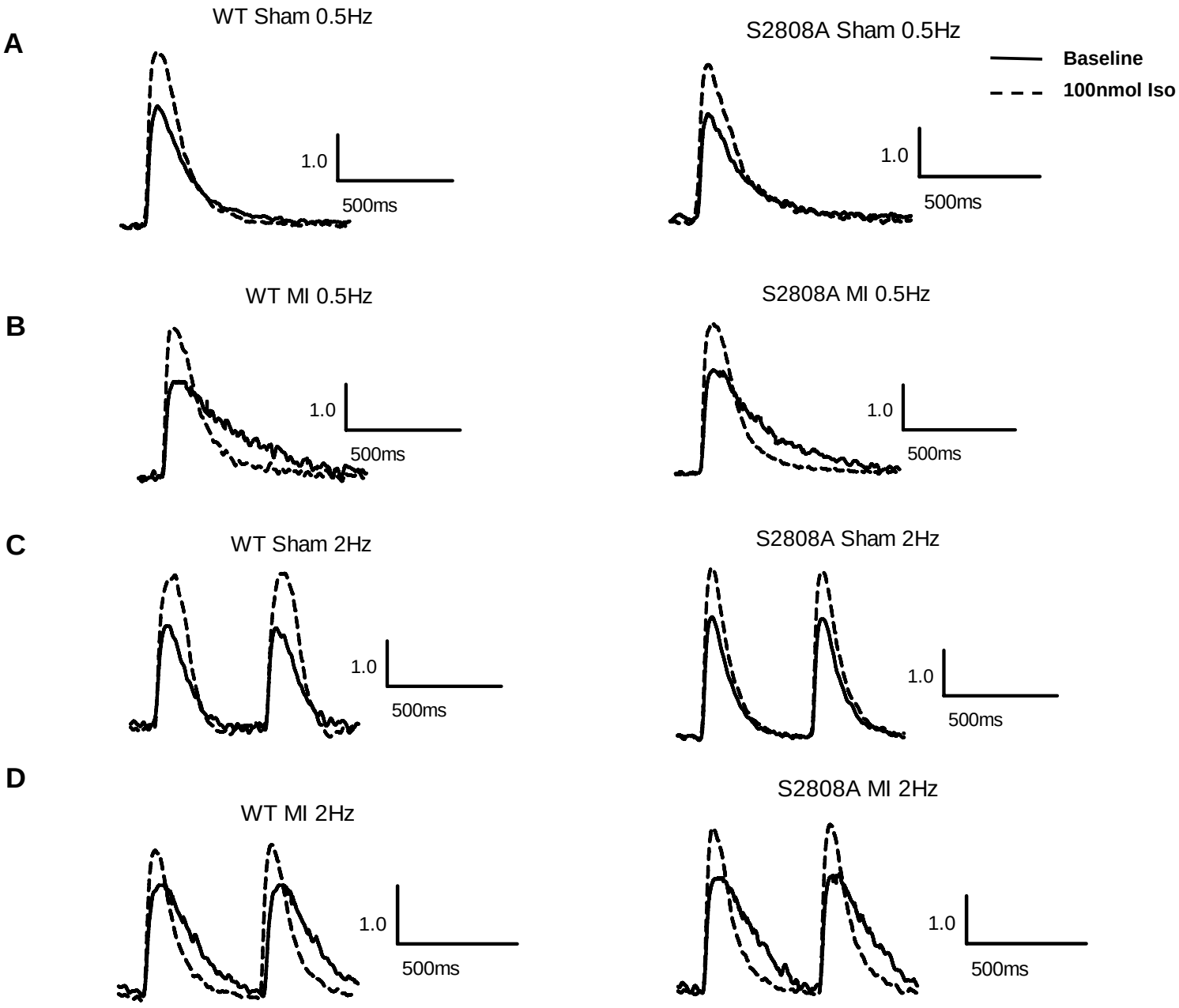
B



Online Figure III **A.** Voltage dependence of $I_{Ca,L}$ activation in sham WT (n=6) and S2808A myocytes (n=5) +/- Iso (1umol/L). **B.** Voltage dependence of $I_{Ca,L}$ activation in post-MI WT (n=6) and S2808A myocytes (n=5) +/- Iso (1umol/L).

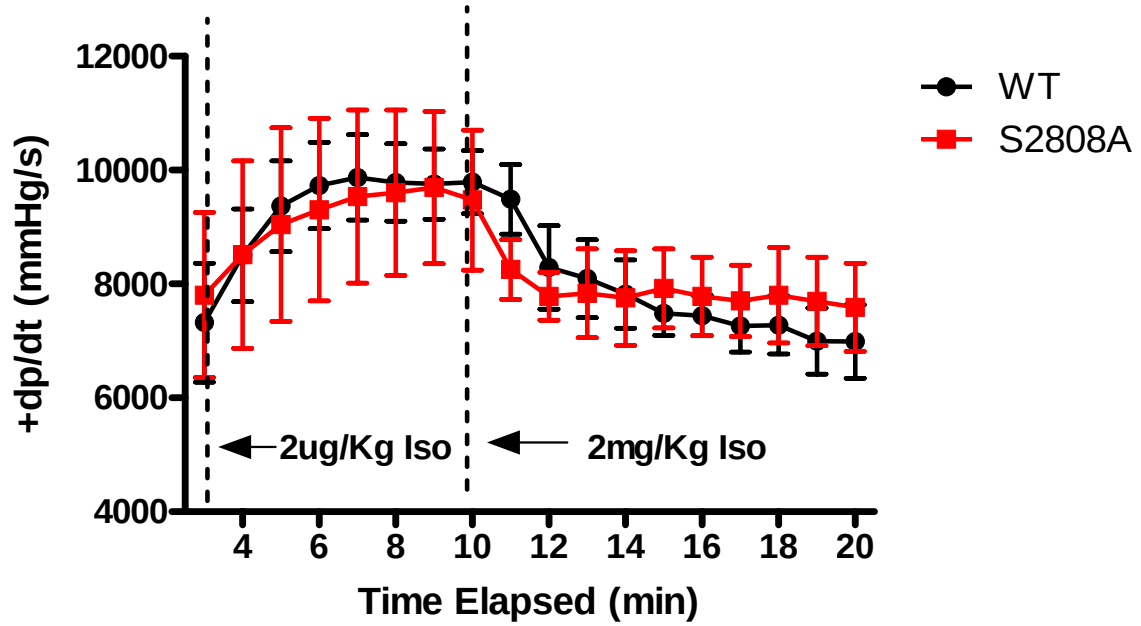


Online Figure IV A-B. Representative traces of sham and post-MI myocyte contractions -/+ Iso (100nmol/L) at 0.5 Hz pacing rate from WT and S2808A hearts. **C-D.** Representative traces of sham and post-MI myocyte contractions -/+ Iso (100nmol/L) at 2 Hz pacing rate from WT and S2808A hearts.

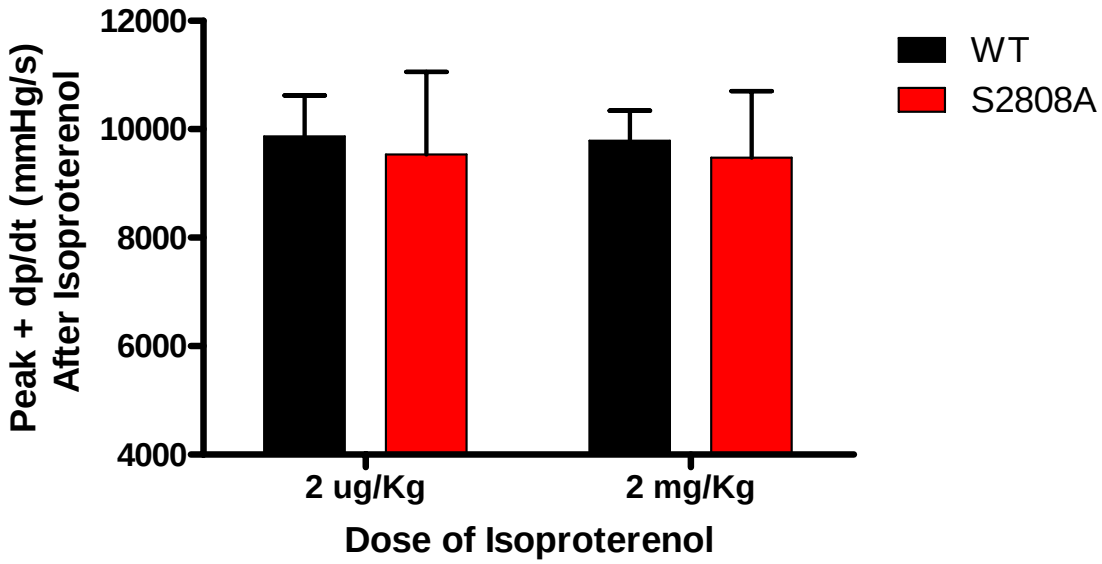


Online Figure V A-B. Representative traces of sham and post-MI myocyte Ca^{2+} transients -/+ Iso (100nmol/L) at 0.5 Hz pacing rate from WT and S2808A hearts. **C-D.** Representative traces of sham and post-MI myocyte Ca^{2+} transients -/+ Iso (100nmol/L) at 2 Hz pacing rate from WT and S2808A hearts.

A



B



Online Figure VI A. Cardiac function (dP/dT) with Isoproterenol treatment (2ug/Kg and 2mg/Kg i.p.injection) was determined by hemodynamics measurement using Miller catheterization. **B.** Peak dP/dT after Iso treatment was assessed in WT and S2808A mice.