## SUPPLEMENTAL INFORMATION

### Supplemental Methods

### Generation of RyR2-S2808D Mice

Murine genomic RyR2 clones were isolated from a 129/SvEvTacfBR λ-phage library (Stratagene, La Jolla, CA) (Supplemental Fig. 1A). A 5.4-kb EcoRI fragment containing exons 53 to 55 was isolated using a 250-bp [$^{32}$P]-labeled RyR2 cDNA probe encoding Ser-2808 and flanking sequences and subcloned into the EcoRI site of pBluescriptSK (Supplemental Fig. 1B). The 3' targeting arm consisting of the 2,463 bp upstream of the EcoRI site was obtained by PCR of murine genomic DNA. After adding SalI sites to both ends of this 2.4-kb fragment, it was cloned into the SalI site of the pACN vector, which contains genes for neomycin resistance, Cre recombinase and a testes-specific promoter (tACE), flanked by loxP sites (Supplemental Fig. 1B). After mutagenesis of Ser-2808 to aspartic acid using a Chameleon Mutagenesis Kit (Stratagene), the EcoRI fragment was cloned into the pACN vector (Supplemental Fig. 1C). The tACE promoter initiates expression of Cre recombinase only during spermatogenesis, resulting in excision of the ACN cassette (Supplemental Fig. 1D). The KpnI linearized targeting vector was electroporated into MM13 mouse embryonic stem (ES) cells. Targeted ES cells were screened by Southern blot analysis using both 5' and 3' external probes to confirm homologous recombination (Supplemental Fig. 1E). Subsequently, a 1,130-bp fragment containing the mutated RyR2 exon was amplified by PCR and then cut with FspI to screen for the S2808D mutation (Supplemental Fig. 1F). Successfully targeted ES cells were injected into C57Bl6 blastocysts, and founders were backcrossed to

C57Bl6 mice. Germline offspring were identified by brown coat color and further verified by Southern blot analyses. Heterozygous males and females were intercrossed to obtain homozygous RyR2-S2808D offspring and backcrossed for more than five generations into the C57Bl6 background. All experiments with animals were approved by Columbia University's Institutional Animal Care and Use Committee. In all cases individuals conducting all experiments were blinded to the genotype and treatment status of the animals.

**Histological Analyses of Infarct Size and Apoptosis**

After hemodynamic analyses, hearts were arrested in diastole by injection of PBS containing 0.5 mM KCl at physiological pressures. Heart tissue was fixed in 10% formalin, cut transversely through the papillary muscles level, and embedded in paraffin. Sections (4 mm) were stained with hematoxylin and eosin, and infarct size was calculated as total infarct circumference divided by total left ventricle circumference. Apoptotic cell death was evaluated using the terminal deoxynucleotidyl transferase enzyme for nick end labeling (TUNEL) method using *i*n *Situ* Cell Death kit (Roche Diagnostics, IN). In brief, three sections were made from each sample, deparaffinized with xylene and washed with ethanol (absolute, 95%, and 70%). Tissues were then treated with proteinase K for 15 min at RT, excess liquids were blotted, 1X equilibrium buffer was applied directly to the specimens, and specimens were placed in a humidified chamber for 5 min at RT. The specimens were then washed twice with PBS, stained with the TUNEL reaction mixture for 60 min at 37°C, washed twice with PBS, and labeled with peroxidase-conjugated goat Ab for 30 min at 37°C. DNA fragmentation was detected by staining with

diaminobenzidine and observed under a microscope (Olympus, Center Valley, PA).

**Heterologous Expression of recombinant RyR2**

Recombinant RyR2 (WT, RyR2-S2808D) were expressed in HEK293 cells using both transient transfection and stable expression. Cells were maintained in minimum Eagle's media containing 10% fetal bovine serum. Three 15 cm dishes (50% confluent) were transfected with 15.0 µg of DNA (pCDNA3.1 containing the RyR2 insert) using the $Ca^{2+}$ phosphate precipitation method. Stable cell lines expressing WT and RyR2-S2808D channels were generated by subcloning the RyR2-S2808D cDNA into A1.2 vector 5' of IRES-GFP sequence. HEK293T cells were co-transfected with the RyR2-S2808D vector and a vector containing puromycin resistance gene. After transfection, the cells were split into selective medium containing G418 (0.5mg/ml) and puromycin (5ug/ml). Clones with the highest expression of RyR2-S2808D were selected. The cells were cultured in selective medium DMEM with 10%FBS, penicillin/streptomycin + G418 (0.5mg/ml) and puromycin (5ug/ml) and were harvested at 95% confluency.

For the inducible cell line, the RyR2 was subcloned into pcDNA4/TO vector between NotI-XhoI restriction sites and the T-REx-CHO line from Invitrogen (cat # R718-07) was used. The cDNA was introduced into the cells using the fugene system, and cell lines with inducible expression of RyR2-S2808D were generated according to the manufacture's (Invitrogen) protocol. Cells were grown in Ham's F12 medium + 10%FBS, with Blasticidin (10ug/ml) and Zeocin (500ug/ml). Cells were induced (when 80-95% confluent) with tetracycline and are harvested 48 hr after

induction.

To analyze the RyR2 from these cells, the cell pellets were resuspended in 0.5 ml of 20 mM HEPES-NaOH, pH 7.5, containing protease inhibitors (complete EDTA-free inhibitors from Roche Molecular Biochemicals), the cells were allowed to swell for 30 min on ice before lysis by 20 strokes of a Dounce homogenizer. Cell homogenates were diluted with an equal volume of ice-cold medium containing 500 mM sucrose and 10 mM HEPES, pH 7.2, and centrifuged at 10,000 × *g* for 15 min. Supernatants were recovered and centrifuged at 100,000 × *g* for 45 min. Pellets were resuspended in the HEPES buffer containing 250 mM sucrose, and the protein concentration of the microsomes was determined by Bradford assay. Aliquots were stored at −80°C. The RyR2 expressed in cells was analyzed by immunoprecipitating the channel from 100 μg of microsomes and analyzing the immunoprecipitate for oxidized RyR as described above.

Supplemental Table 1. *In vivo* WT vs. RyR2-S2808D longitudinal study

| | BW (g) | EF (%) | FS (%) | LVEDD (mm) | LVESD (mm) | HR ($min^{-1}$) | | HW/BW (mg/g) | $dP/dt_{max}$ (mmHg/s) | $dP/dt_{min}$ (mmHg/s) |
|---|---|---|---|---|---|---|---|---|---|---|
| **WT (n=7)** | | | | | | | **WT (n=6)** | | | |
| **6 weeks** | 19.1 ± 1.1 | 78 ± 2 | 41 ± 1 | 3.0 ± 0.2 | 1.8 ± 0.2 | 382 ± 21 | **1 month** | 4.5 ± 0.3 | 7438 ± 561 | -7010 ± 456 |
| **3 months** | 22.0 ± 1.4 | 81 ± 1 | 46 ± 1 | 3.1 ± 0.1 | 1.8 ± 0.1 | 385 ± 22 | **2 months** | 4.7 ± 0.2 | 7874 ± 540 | - 6277 ± 284 |
| **6 months** | 25.3 ± 1.4 | 81 ± 5 | 51 ± 3 | 3.1 ± 0.2 | 1.8 ± 0.3 | 391 ± 18 | **6 months** | 5.0 ± 0.4 | 7975 ± 409 | -6371 ± 273 |
| **12 months** | 30.6 ± 2.0 | 76 ± 2 | 44 ± 2 | 3.5 ± 0.2 | 2.0 ± 0.2 | 350 ± 29 | **12 months** | 5.2 ± 0.1 | 7580 ± 257 | -6241 ± 476 |
| | | | | | | | | | | |
| **RyR2-S2808D (n=10)** | | | | | | | **RyR2-S2808D (n=6)** | | | |
| **6 weeks** | 18.1 ± 0.5 | 82 ± 2 | 45 ± 2 | 2.9 ± 0.1 | 1.7 ± 0.2 | 380 ± 12 | **1 month** | 4.6 ± 0.1 | 7981 ± 626 | -6552 ± 880 |
| **3 months** | 20.4 ± 1.0 | 62 ± 2# | 32 ± 2# | 3.5 ± 1.0* | 2.5 ± 0.1# | 385 ± 13 | **2 months** | 4.7 ± 0.3 | 6818 ± 555 | -4927 ± 576 |
| **6 months** | 24.5 ± 1.0 | 59 ± 2# | 31 ± 1# | 3.6 ± 0.1 | 2.6 ± 0.1* | 374 ± 16 | **6 months** | 5.4 ± 0.4 | 6637 ± 378* | -4801 ± 576* |
| **12 months** | 32.1 ± 2.4 | 50 ± 3# | 25 ± 2# | 4.3 ± 0.1# | 3.3 ± 0.1# | 352 ± 25 | **12 months** | 7.0 ± 0.5* | 5750 ± 505# | -4247 ± 367# |

*, P<0.05 vs. WT; #, P,0.01 vs. WT

Supplemental Table 2. *In vivo* WT vs. RyR2-S2808D chronic Iso study

| | BW (g) | EF (%) | FS (%) | LVEDD (mm) | LVESD (mm) | HR ($min^{-1}$) | $dP/dt_{max}$ (mmHg/s) | $dP/dt_{min}$ (mmHg/s) |
|---|---|---|---|---|---|---|---|---|
| **WT (n=9)** | | | | | | | | |
| **Baseline** | 26.1 ± 1.3 | 82 ± 1 | 44 ± 1 | 3.0 ± 0.1 | 1.7 ± 0.1 | 389 ± 14 | | |
| **1 week** | 26.5 ± 1.0 | 88 ± 1 | 52 ± 2 | 3.4 ± 0.1 | 1.6 ± 0.1 | 436 ± 25 | | |
| **2 week** | 26.5 ± 0.7 | 88 ± 1 | 52 ± 2 | 3.4 ± 0.1 | 1.6 ± 0.1 | 421 ± 19 | | |
| **4 week** | 27.1 ± 0.7 | 64 ± 2 | 31 ± 2 | 3.8 ± 0.1 | 2.7 ± 0.1 | 457 ± 25 | 7177 ± 188 | -5411 ± 407 |
| | | | | | | | | |
| **RyR2-S2808D (n=12)** | | | | | | | | |
| **Baseline** | 24.3 ± 1.7 | 62 ± 2# | 33 ± 1# | 3.5 ± 0.1# | 2.3 ± 0.1# | 392 ± 16 | | |
| **1 week** | 24.5 ± 1.5 | 57 ± 2# | 30 ± 1# | 4.0 ± 0.1# | 3.0 ± 0.1# | 408 ± 13 | | |
| **2 week** | 23.2 ± 1.8 | 57 ± 1# | 30 ± 1# | 4.0 ± 0.1# | 3.0 ± 0.1# | 406 ± 17 | | |
| **4 week** | 22.6 ± 1.6 | 58 ± 3 | 30 ± 2 | 4.1 ± 0.1 | 3.1 ± 0.2 | 418 ± 16 | 6639 ± 355 | -4875 ± 323 |

#, P,0.01 vs. WT

Supplemental Table 3. *In vivo* RyR2-S2808D S107 treatment study

| | BW (g) | EF (%) | FS (%) | LVEDD (mm) | LVESD (mm) | HR ($min^{-1}$) | HW/BW (mg/g) | $dP/dt_{max}$ (mmHg/s) | $dP/dt_{min}$ (mmHg/s) |
|---|---|---|---|---|---|---|---|---|---|
| **Vehicle (n=11)** | | | | | | | | | |
| **Baseline** | 17.9 ± 0.5 | 80 ± 2 | 43 ± 2 | 2.9 ± 0.1 | 1.8 ± 0.8 | 384 ± 12 | | | |
| **4 weeks** | 20.3 ± 1.3 | 63 ± 3 | 31 ± 2 | 3.4 ± 0.1 | 2.6 ± 0.1 | 388 ± 17 | | | |
| **10 weeks** | 24.9 ± 1.4 | 58 ± 2 | 30 ± 1 | 3.6 ± 0.1 | 2.7 ± 0.1 | 387 ± 12 | 5.2 ± 0.3 | 6301 ± 433 | -5509 ± 341 |
| | | | | | | | | | |
| **S107 (n=7)** | | | | | | | | | |
| **Baseline** | 18.8 ± 1.0 | 77 ± 2 | 45 ± 2 | 3.1 ± 0.2 | 1.9 ± 0.2 | 390 ± 11 | | | |
| **4 weeks** | 21.4 ± 1.3 | 71 ± 3 | 37 ± 3 | 3.4 ± 0.2 | 2.4 ± 0.2 | 396 ± 16 | | | |
| **10 weeks** | 25.9 ± 0.6 | 73 ± 2# | 42 ± 2# | 3.6 ± 0.1 | 2.4 ± 0.1 | 391 ± 12 | 4.6 ± 0.1 | 9010 ± 726* | -7103 ± 674 |

*, P<0.05 vs. vehicle; #, P,0.01 vs. vehicle

Supplemental Table 4. *In vivo* WT vs. RyR2-S2808D acute MI study

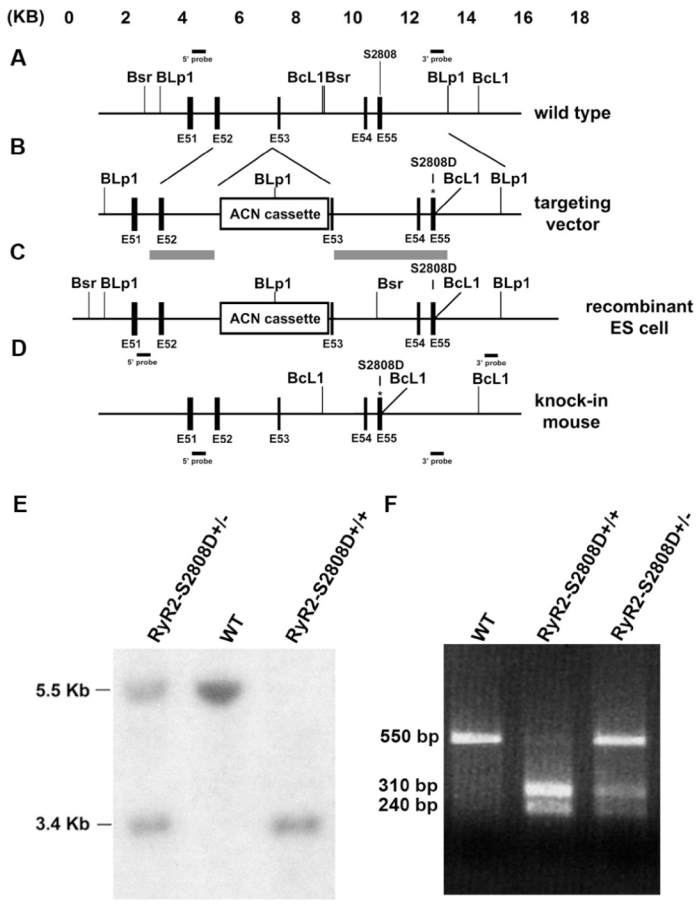
| | BW (g) | EF (%) | FS (%) | LVEDD (mm) | LVESD (mm) | HR ($min^{-1}$) | HW/BW (mg/g) | $dP/dt_{max}$ (mmHg/s) | $dP/dt_{min}$ (mmHg/s) |
|---|---|---|---|---|---|---|---|---|---|
| **WT (n=15)** | | | | | | | | | |
| **Pre-MI** | 23.5 ± 1.0 | 81 ± 1 | 43 ± 1 | 3.0 ± 0.1 | 1.7 ± 0.2 | 403 ± 10 | | | |
| **1 week post-MI** | 23.0 ± 0.4 | 50 ± 2 | 25 ± 1 | 4.2 ± 0.5 | 3.3 ± 0.1 | 401 ± 16 | | | |
| **3 week post-MI** | 23.3 ± 0.8 | 41 ± 2 | 19 ± 1 | 4.6 ± 0.2 | 2.9 ± 0.1 | 392 ± 17 | | | |
| **5 week post-MI** | 23.7 ± 0.7 | 34 ± 2 | 16 ± 1 | 4.9 ± 0.1 | 4.4 ± 0.2 | 393 ± 18 | | | |
| **8 week post-MI** | 25.6 ± 0.8 | 36 ± 2 | 17 ± 1 | 5.4 ± 0.2 | 4.8 ± 0.2 | 387 ± 14 | 8.0 ± 0.4 | 3877 ± 503 | -2918 ± 347 |
| | | | | | | | | | |
| **RyR2-S2808D (n=11)** | | | | | | | | | |
| **Pre-MI** | 24.9 ± 0.6 | 60 ± 1# | 32 ± 1# | 3.6 ± 0.1# | 2.6 ± 0.1# | 400 ± 8 | | | |
| **1 week post-MI** | 24.4 ± 0.7 | 38 ± 2# | 18 ± 1# | 4.7 ± 0.1# | 4.0 ± 0.1# | 382 ± 17 | | | |
| **3 week post-MI** | 24.7 ± 0.8 | 36 ± 3 | 17 ± 2 | 4.9 ± 0.2 | 4.3 ± 0.2 | 390 ± 19 | | | |
| **5 week post-MI** | 24.6 ±0.7 | 32 ± 2 | 15 ± 1 | 5.3 ± 0.1 | 4.8 ± 0.2 | 381 ± 15 | | | |
| **8 week post-MI** | 24.2 ± 0.6 | 30 ± 2 | 14 ± 1 | 5.4 ± 0.2 | 4.9 ± 0.2 | 384 ± 15 | 7.9 ± 0.9 | 4185 ± 357 | -3188 ± 380 |

*, P<0.05 vs. WT; #, P,0.01 vs. WT

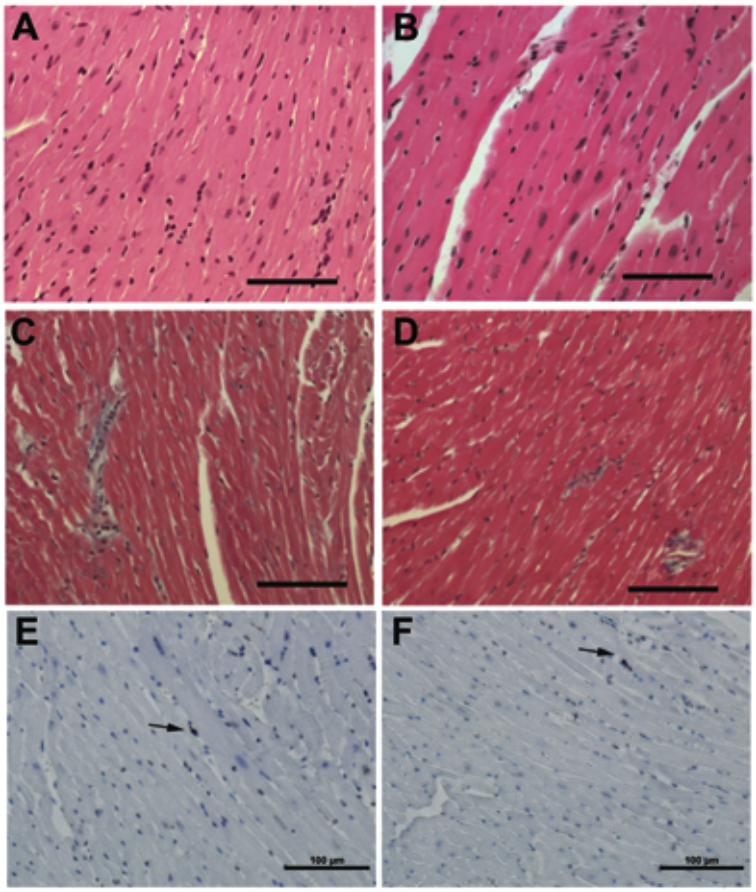
Supplemental Table 5. *In vivo* β-blocker study

| | n | BW (g) | EF (%) | FS (%) | LVEDD (mm) | LVESD (mm) | HR ($min^{-1}$) | HW/BW (mg/g) | $dP/dt_{max}$ (mmHg/s) | $dP/dt_{min}$ (mmHg/s) |
|---|---|---|---|---|---|---|---|---|---|---|
| **WT** | | | | | | | | | | |
| **Vehicle** | 7 | 24.9 ± 1.1 | 32 ± 3 | 13 ± 1 | 5.1 ± 0.1 | 4.6 ± 0.2 | 410 ± 14 | 7.5 ± 1.0 | 3877 ± 502 | -2918 ± 347 |
| **Metoprolol** | 9 | 25.1 ± 0.3 | 42 ± 2* | 21 ± 1* | 5.1 ± 0.2 | 4.2 ± 0.7 | 322 ± 8 | 7.2 ± 1.0 | 5584 ± 258* | -3696 ± 209 |
| **Carvedilol** | 9 | 25.8 ± 1.1 | 43 ± 3* | 21 ± 2* | 5.3 ± 0.2 | 4.4 ± 0.3 | 312 ± 20 | 6.0 ± 0.2 | 5592 ± 562* | -4649 ± 580* |
| **S107** | 9 | 25.7 ± 0.4 | 44 ± 4* | 22 ± 2* | 5.2 ± 0.2 | 4.3 ± 0.3 | 403 ± 9 | 6.3 ± 0.2 | 6420 ± 469* | -5307 ± 391* |
| | | | | | | | | | | |
| **RyR2-S2808D** | | | | | | | | | | |
| **vehicle** | 16 | 24.9 ± 0.8 | 30 ± 2 | 14 ± 1 | 5.7 ± 0.2 | 5.1 ± 0.2 | 393 ± 16 | 8.1 ± 0.7 | 4355 ± 322 | -3116 ± 298 |
| **Metoprolol** | 15 | 26.5 ± 0.9 | 28 ± 2 | 14 ± 1 | 5.6 ± 0.2 | 5.1 ± 0.2 | 331 ± 16 | 7.2 ± 0.4 | 4160 ± 311 | -3284 ± 312 |
| **Metoprolol High dose** | 13 | 26.6 ± 0.8 | 30 ± 2 | 15 ± 1 | 6.3 ± 0.1 | 5.5 ± 0.1 | 331 ± 15 | 8.0 ± 0.4 | 4561 ± 255 | -3253 ± 232 |
| **Carvedilol** | 13 | 25.6 ± 1.0 | 31 ± 1 | 15 ± 1 | 5.8 ± 0.2 | 5.1 ± 0.2 | 333 ± 9 | 7.4 ± 0.3 | 4708 ± 234 | -3664 ± 248 |
| **S107** | 16 | 25.4 ± 0.2 | 47 ± 4* | 24 ± 2* | 4.6 ± 0.2* | 3.8 ± 0.3* | 388 ± 17 | 6.2 ± 0.4* | 6346 ± 349* | -4813 ± 408* |

*, P<0.05 vs. WT



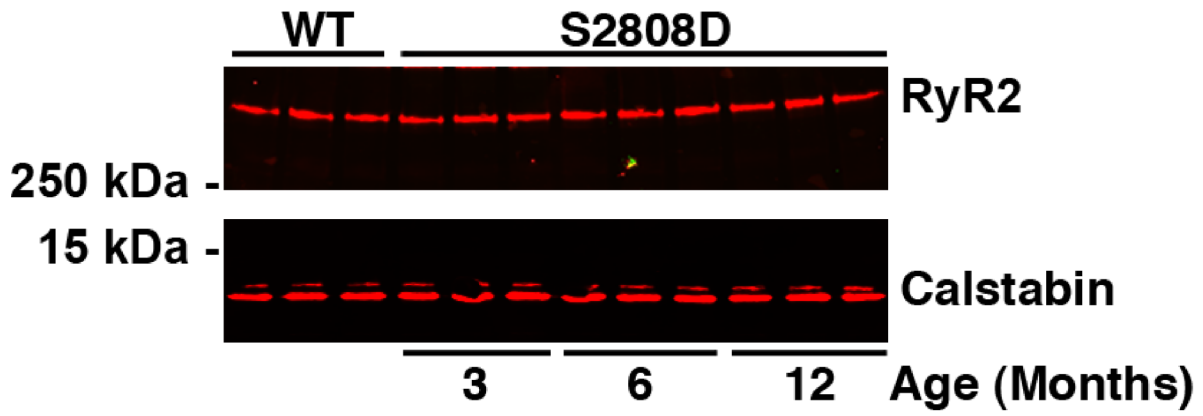

**Supplemental Figure 1. Generation of RyR2-S2808D mice. (A)** The wild-type locus of the murine RyR2 gene containing exons 51–55. (**B**) The targeting construct containing 2.4- and 5.4-kb homologous regions (horizontal gray lines). The S2808D mutation (*) was engineered in exon 55. (**C**) The homologous recombinant mutant allele containing the RyR2-S2808D mutation and the ACN selection marker cassette. (**D**) Final RyR2-S2808D allele after excision of the ACN selection marker. (**E**) Representative Southern blot analysis by using 5' probe and 3' probe. (**F**) Representative PCR analysis of homozygous and heterozygous RyR2-S2808D mice.



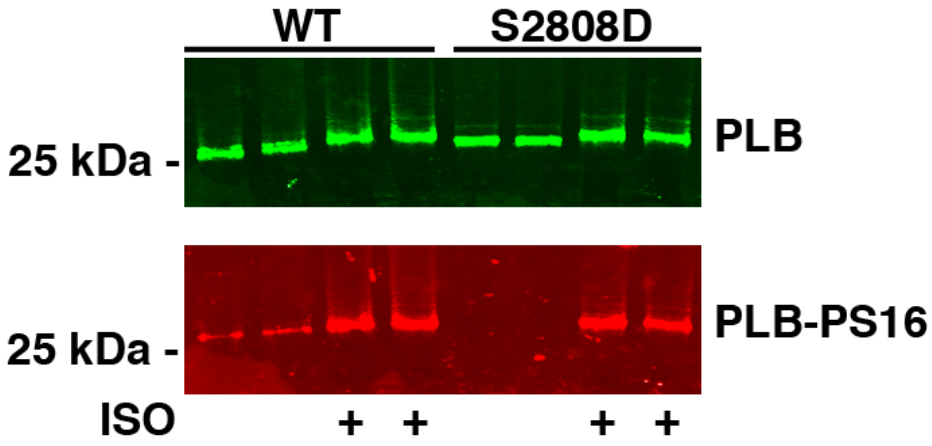

## Supplemental Figure 2

**Representative histology of age matched WT and RyR2-S2808D$^{+/+}$ littermates.** A) WT, B) RyR2-S2808D$^{+/+}$ H&E staining, 50X, bar 100 μm; C) WT, D) RyR2-S2808D$^{+/+}$ Masson's Trichrome staining for fibrosis, 100X, bar 100 μm.

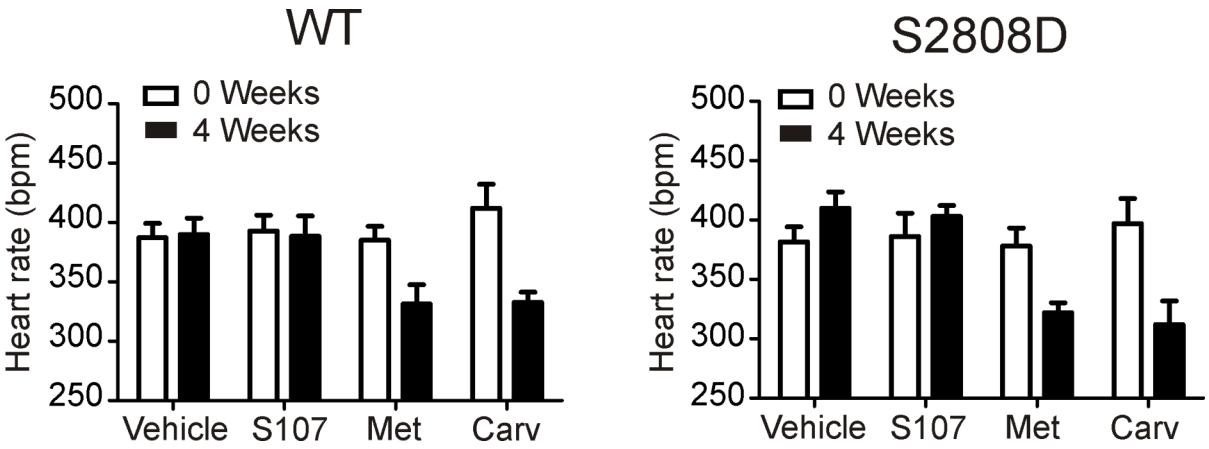
E) WT, F) RyR2-S2808D$^{+/+}$ mice at 12-months of age showed no significant apoptosis comparing with age matched WT littermates. TUNEL assay. Nuclei of cardiomyocytes were stained in blue. Arrows, TUNEL-positive nuclei.




**Supplemental Figure 3. Equivalent RyR2 and calstabin2 levels in WT and RyR2-S2808D mice.** Lysates were prepared from whole hearts from WT and RyR2-S2808D mice. 50 mg of lysates were separated by PAGE and subjected to Western blots using antibodies directed against RyR2 or calstabin.




**Supplemental Figure 4. Intact β-AR signaling in RyR2-S2808D mice.** Iso (1 mg/kg dose; IP) was injected into WT and RyR2-S2808D mice and mice were sacrificed 15 minutes later. Microsomes were prepared and probed for total and phospho-S16 PLB.




**Supplemental Figure 5. Effect of β-AR blockade by metoprolol and carvedilol on heart rate in RyR2-S2808D mice. (A)** Heart rates (bpm) from β-blocker treated mice showing reduced heart rates indicating effective β-AR blockade.

Supplemental Table 6

A

| Mouse | $B_{max}$ (fmol/mg) | $K_d$ (nmol/L) |
|---|---|---|
| WT | 17.5 ± 1.4 | 5.3 ± 0.4 |
| RyR2-S2808D | 15.1 ± 1.4 | 4.4 ± 0.6 |

**Beta adrenergic receptor density in WT and RyR2-S2808D mice.** Crude membrane fractions were prepared from WT and RyR2-S2808D mice and used for receptor binding assays.