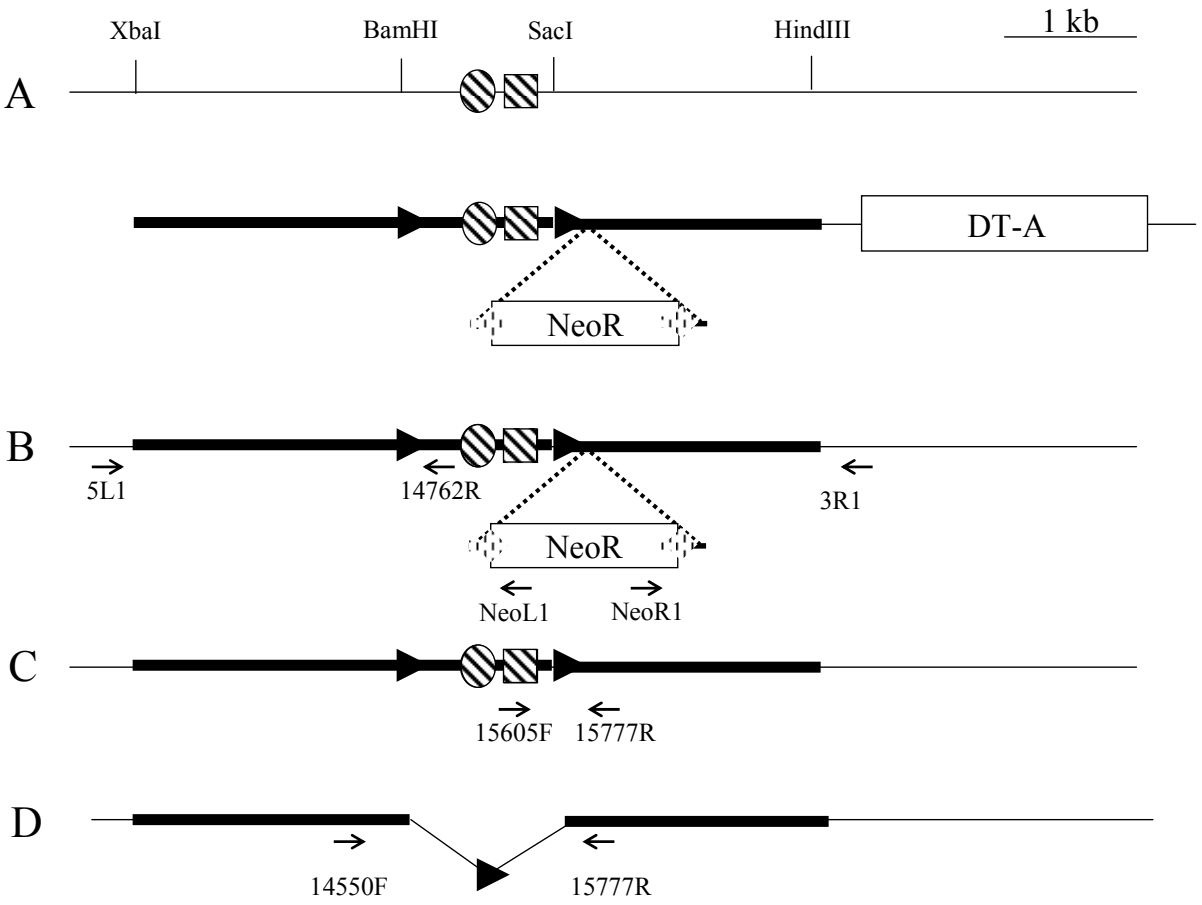
Supplemental Table 1. Echocardiographic and ECG data of 10 week old anesthetized mice.

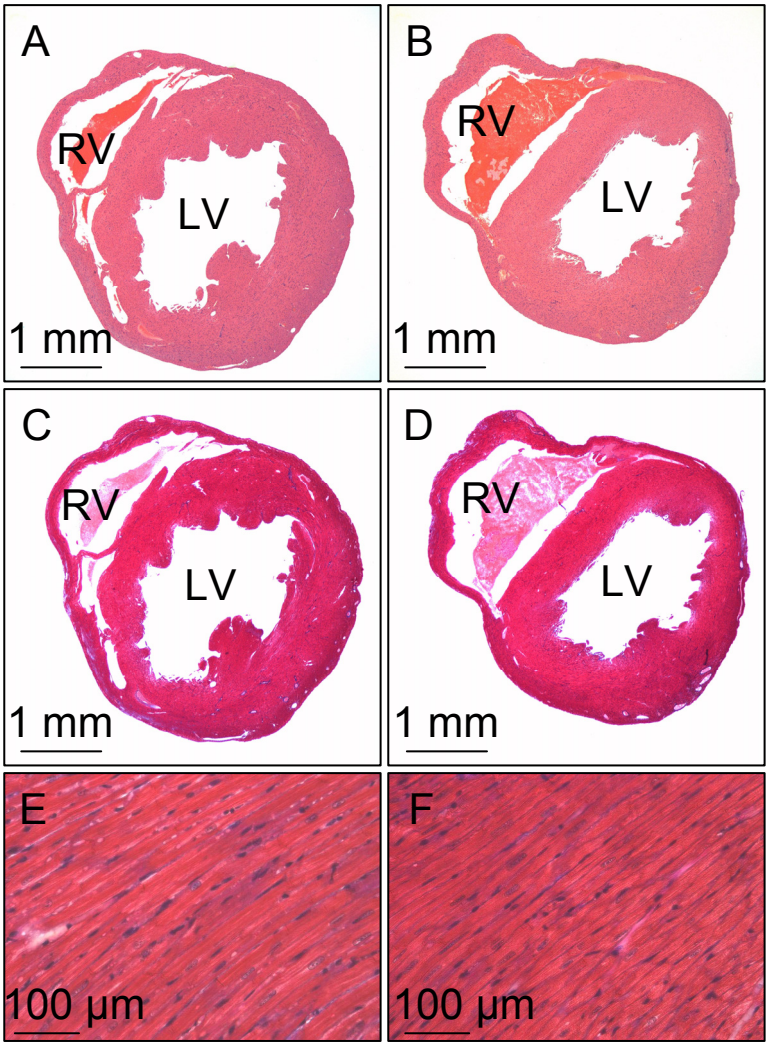
	$Casq2^{+/+}$ (n=5)	**$Casq2^{+/-}$ (n=10)**	**$Casq2^{-/-}$ (n=6)**
HR (beats/min)	474±42	497±24	400±29.4*
IVSD (mm)	0.54±0.02	0.55±0.09	0.64±0.049*
LVPWD (mm)	0.54±0.04	0.57±0.09	0.78±0.12*
LVIDd (mm)	3.6±0.4	3.4±0.3	3.4±0.025
LVIDs (mm)	2.2±0.4	2.2±0.3	2.1±0.025
%FS	38±5.8	37±6.6	38±7.35
PR (ms)	38.9±3.6	37.8±2.4	39.1±3.68
QRS (ms)	10.1±0.8	10.7±1.2	10.5±1.47
QT (ms)	47.4±2.8	47.8±3.3	49.4±2.94
QTc (ms)	41.4±4.4	43.9±3	40.6±2.45

HR, Heart Rate; IVSD, Interventricular Septum Diameter; LVPWD, Left Ventricular Posterior Wall Diameter; LVIDd, LV Internal Dimension in Diastole; LVIDs, LV Internal Dimension in Systole; %FS, percent Fractional Shortening (LVIDs / LVIDd * 100); QTc, heart rate corrected QT interval. *$p<0.05$ compared with *$Casq2^{+/+}$* or *$Casq2^{+/-}$*

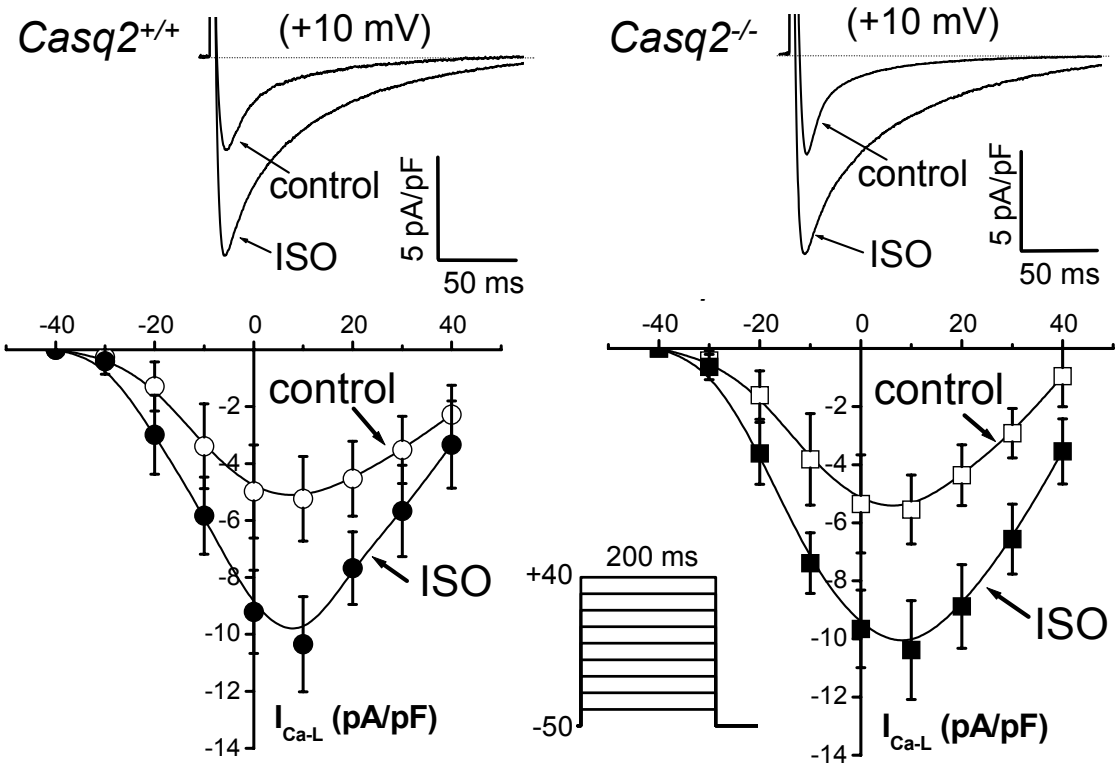


Supplemental Figure 1. Generation and characterization of *Casq2* mutation alleles. The 6 Kb region surrounding *Casq2* Exon 1 (striped square) and the *Casq2* promoter (striped oval) are shown. **(A)** The wild type *Casq2* is shown along with linearized plasmid pKP588 used to generate the *Casq2* mutant alleles. Thickened lines indicate the 5' and 3' homology regions. The *Casq2* promoter and first exon are flanked with *loxP* sites (solid arrowheads) at positions -561 bp and at +538 bp. (All numbering is relative to the major transcriptional start site depicted in Figure 1 of the main text.) Positive selection is via the *NeoR* cassette which is flanked with *Frt* sites (solid diamonds). Negative selection is via the *Diphtheria-toxin A* gene (DT-A). **(B)** The $Casq2^{floxed+NeoR}$ allele was identified using PCR primer pairs 5L1+NeoL1, 5L1+14762R, and 3R1+NeoR1. This allele carries a *NeoR* cassette inserted into intron 1 and two *loxP* sites flanking the *Casq2* promoter and exon 1. $Casq2^{floxed+NeoR}$ cells were injected into wild type C57Bl/6

blastocysts to generate chimeric founders. **(C)** *Casq2*^{floxed} allele was generated by Flp recombinase mediated deletion of the *NeoR* cassette from the *Casq2*$^{floxed+Neo}$. Chimeric founders (see B) were first mated with C567Black6-J females and their progeny then mated with Jackson strain 003946 which carries an insertion of the *Flp recombinase* gene at the *Rosa26* locus. Flp deletion was monitored using primer pair 15605F+15777R. **(D)** *Casq2*$^{-}$ or *Casq2*$^{\Delta Exon1}$ was generated by crossing *Casq2*^{floxed} mice with transgenic animals carrying the *cre recombinase* gene under control of the *EIIa* promoter. Cre recombination was monitored using primer pair 14550F+15777R. The *Casq2*$^{-}$ allele thus replaces 1.1 kb of *Casq2* that carries the *Casq2* promoter and exon 1 with an 80 bp insertion including a single *loxP* element. Exon 1 includes the ATG initiation codon and encodes the first 78 amino acids of the Casq2 peptide**.** *Casq2*$^{-}$ heterozygotes were backcrossed two times with C57Black6-J mice and then interbred to generate all the animals used in this study. Genotypes were determined by amplification with primers 14550F, 15605F, and 15777R to yield bands of 280 and 172 bp, specific to the *Casq2*$^{-}$ and the wild type alleles, respectively. Primer sequences are listed below. 5L1: CCTGGAATCATCTAGCCACCT. 14762R: CGAGGACAGGCACACTCTCCACATGC. NeoL1: CAGCAGCCTCTGTTCCACAT. NeoR1: AGACTGCCTTGGGAAAAGC. 15605F: GGCAGCAGCCTCCTGTATGATAG. 15777R: CCACCTTAAGAGTTTGCCCACAG. 14550F: CACCGGCTTCCCTGCCTCCCACAGC.



Supplemental Figure 2. Representative hearts sections obtained from 10 week old Casq2$^{+/+}$ and Casq2$^{-/-}$ mice. A total of 5 hearts per genotype were examined by an experienced pathologist (B.E.K.) that was blinded to genotype of the hearts. None of the hearts had any evidence for significant fibrosis or myofibrillar disarray, as illustrated on the representative panels below. **(A), (B)** H&E stained hearts from Casq2$^{+/+}$and Casq2$^{-/-}$ mice at a magnification of x2.5, cut at the mid-sagittal level and parallel to the base. **(C), (D)** Masson Trichrome stained hearts of the same Casq2$^{+/+}$ and Casq2$^{-/-}$ mice at 2.5x magnification. **(E), (F)** Higher magnification (x40) of the same Masson Trichrome stained hearts. LV, left ventricle; RV, right ventricle.



Supplemental Figure 3. The current-voltage (I-V) relations for basal (control) and isoproterenol-stimulated (ISO, 1 μmol/l) peak L-type Ca^{2+} currents (I_{Ca-L}) in *Casq2*$^{+/+}$ and *Casq2*$^{-/-}$ myocytes. Data represent averages obtained from 13 (control) and 10 (ISO) *Casq2*$^{+/+}$myocytes, and 14 (control) and 12 (ISO) *Casq2*$^{-/-}$ myocytes. There were not significantly different between *Casq2*$^{+/+}$ and *Casq2*$^{-/-}$ myocytes. Representative I_{Ca-L} traces at +10 mV are shown on the top. Myocyte size (estimated by cell capacitance measurements in pF) was also not statistically different between the groups: 118.8±13.7 pF (n=24) in *Casq2*$^{+/+}$; 124.3±12.2 pF (n=25) in *Casq2*$^{-/-}$; p=0.138.

Methods: L-type Ca currents (I_{Ca-L}) were measured as described (1). Briefly, mouse ventricular myocytes were whole-cell patched in K^+-free solution containing (mmol/l): NaCl, 137; $MgCl_2$, 1; CaCl, 2; Hepes, 10; glucose, 10; pH 7·4. The pipette solution contained (mmol/l): CsCl, 110; TEA-Cl, 20; EGTA, 14;

Hepes, 10; MgATP, 5; glucose, 10; pH 7·2. Na^+ channels were eliminated by adding tetrodotoxin (30 μmol/l) to external solutions and a holding potential of -50 mV. $I_{Ca\text{-}L}$ was elicited with 200-ms depolarization pulses ranging from −50 to 40 mV in 10-mV steps. Where indicated, isoproterenol was used at a concentration of 1 μmol/l.

Reference:

1. Knollmann, B.C., Knollmann-Ritschel, B.E., Weissman, N.J., Jones, L.R., and Morad, M. 2000. Remodelling of ionic currents in hypertrophied and failing hearts of transgenic mice overexpressing calsequestrin. *J Physiol* 525 Pt 2:483-498.