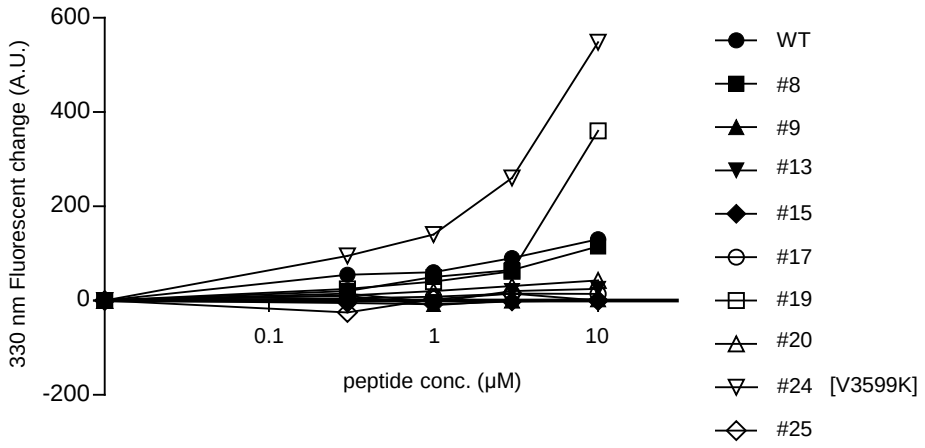
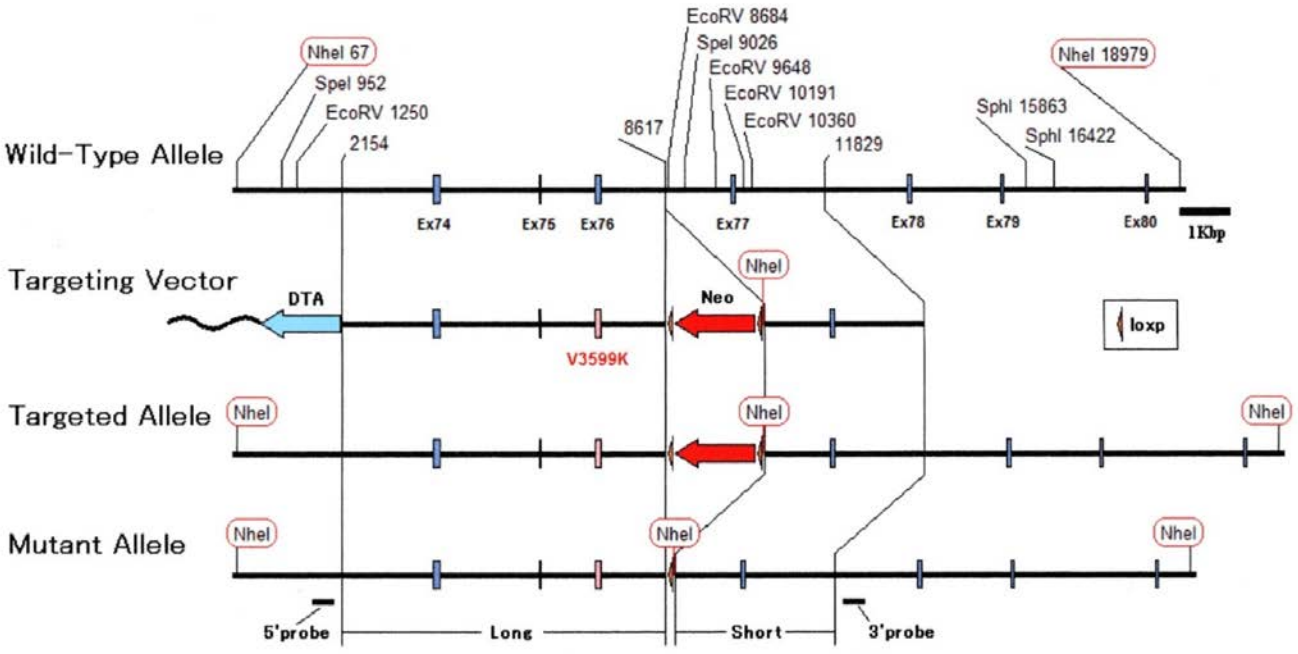
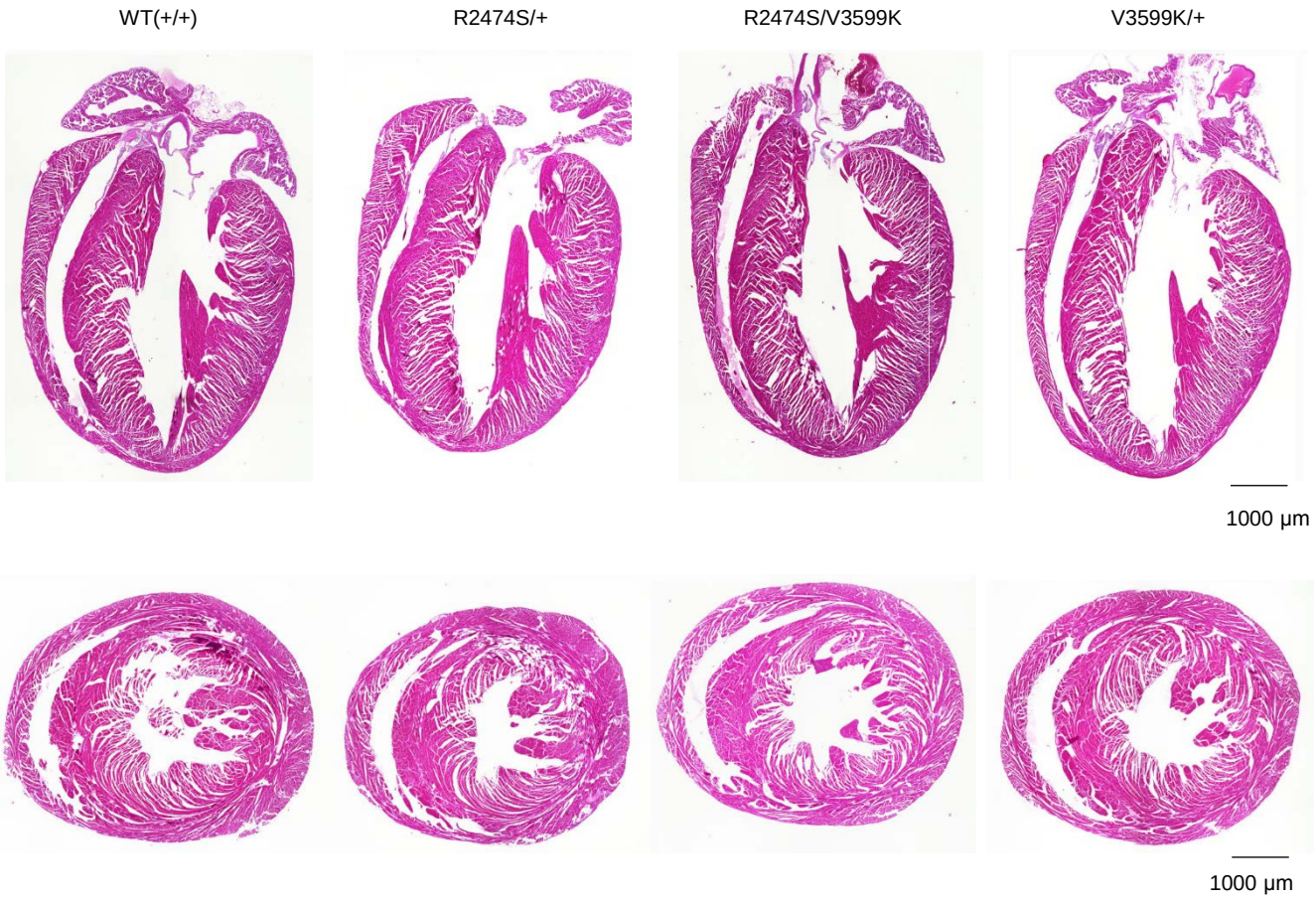


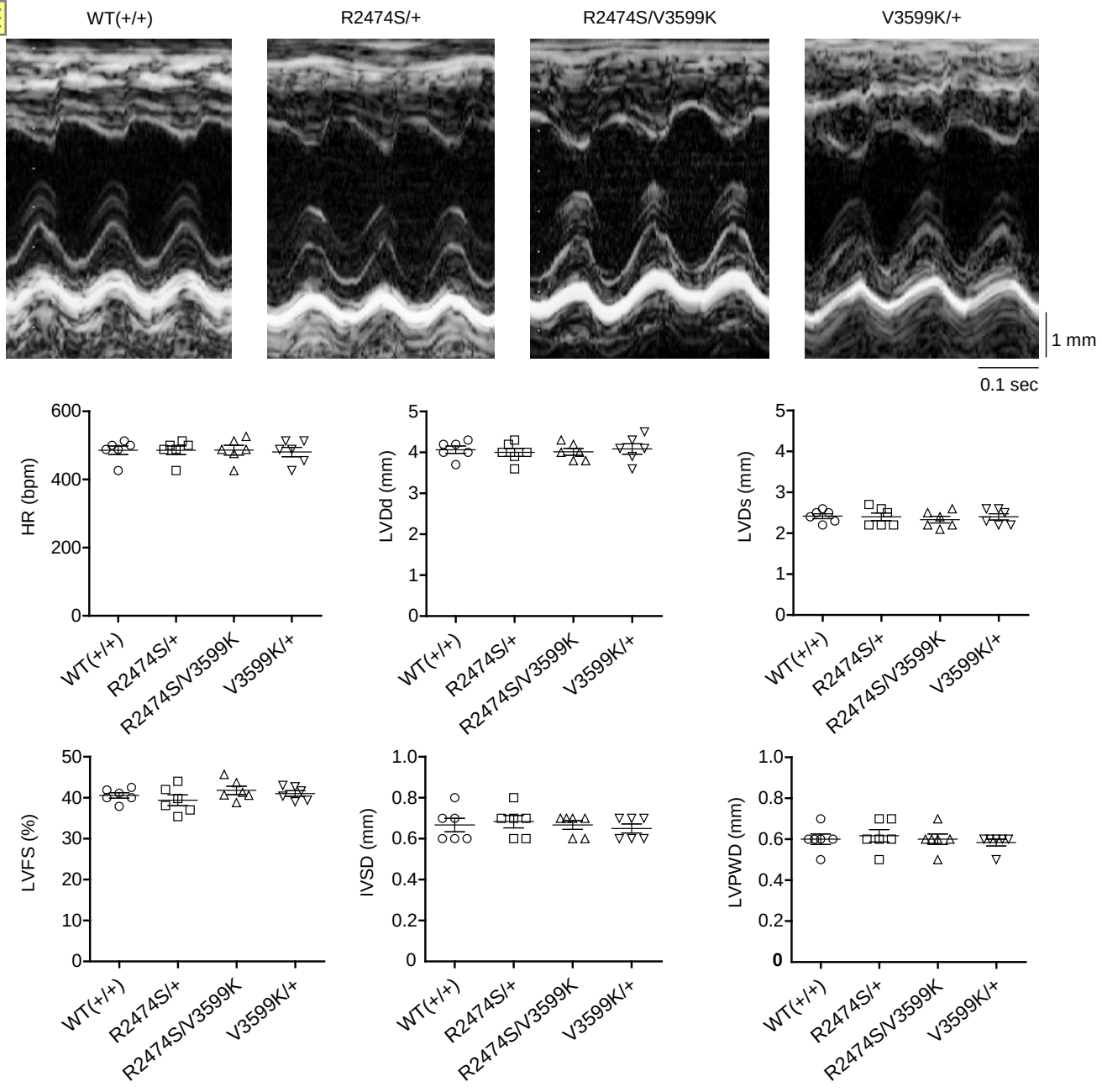
Supplementary Figure 1.
Concentration dependence of CaM-mediated tryptophan fluorescence change upon CaM binding to CaMBPs. The V3599K-CaMBP showed the highest change in CaM-mediated tryptophan fluorescence.



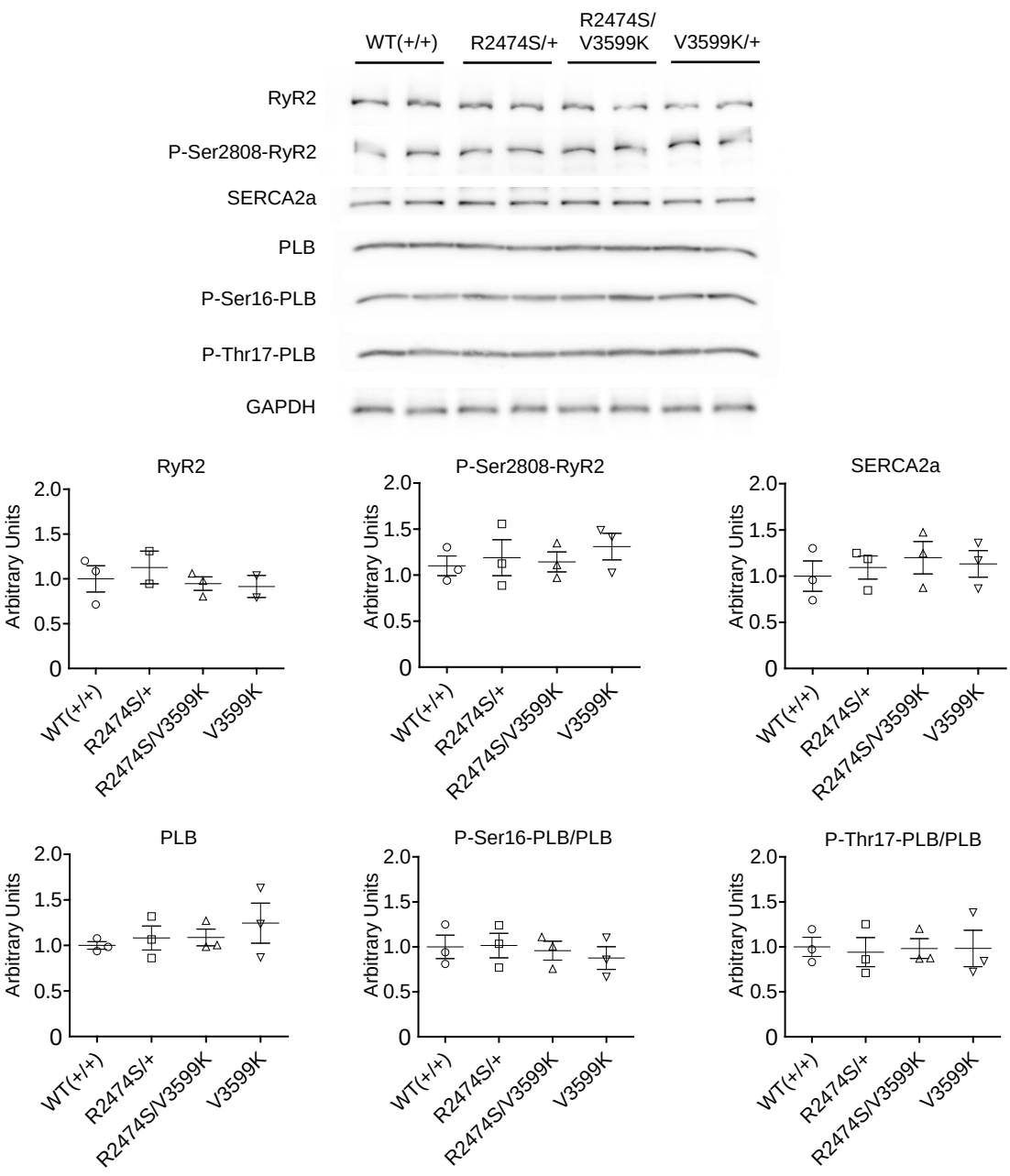
Supplementary Figure 2.
Generation of heterozygous V3599K KI mice. Schematic representation of the genomic structure of the target vector used to generate the V3599K/+ KI mice.



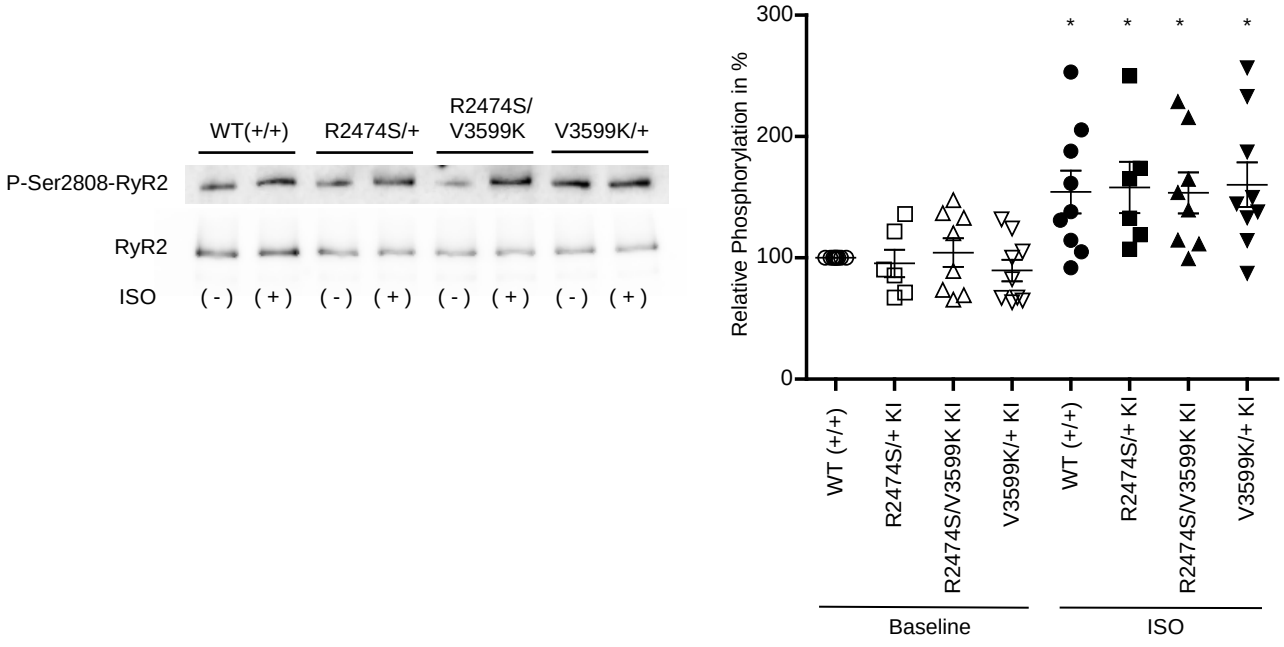
Supplementary Figure 3.
Structural and functional characteristics in WT (+/+), R2474S/+ KI, V3599K/+ KI, and R2474S/V3599K KI mice. Representative images of the long and short axes sections of the mice hearts. Cross-sectional histology of the hearts showed no remarkable difference amongst the mice. Scale bars: 1000μm.



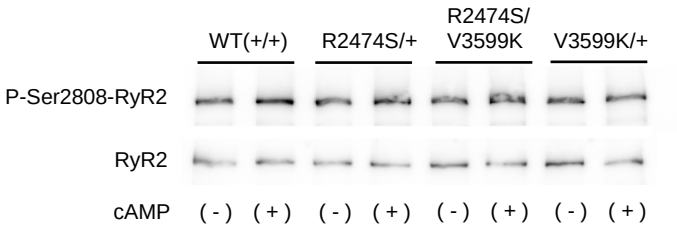
Supplementary Figure 4
Representative images of echocardiography and the summarized data. Echocardiography revealed no functional differences amongst the mice.

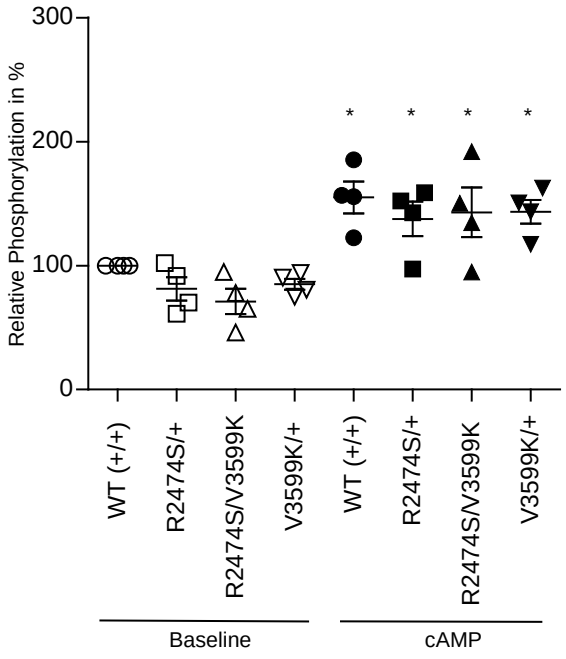


Supplementary Figure 5.
Expression of Ca2+ regulatory SR proteins. (Top) Western blots of various Ca2+ regulatory proteins, RyR2, cardiac ryanodine receptor; P-Ser2808-RyR2, phosphorylated RyR2 at Ser2808; SERCA2a, SR Ca2+-ATPase; PLB, phospholamban; P-Ser16-PLB, phosphorylated PLB at Ser16; P-Thr17-PLB, phosphorylated PLB at Thr17; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. (Bottom) Summarized data of western blotting. Open bar, WT; closed bar, KI. Data are presented as mean ± SEM of 3 hearts.

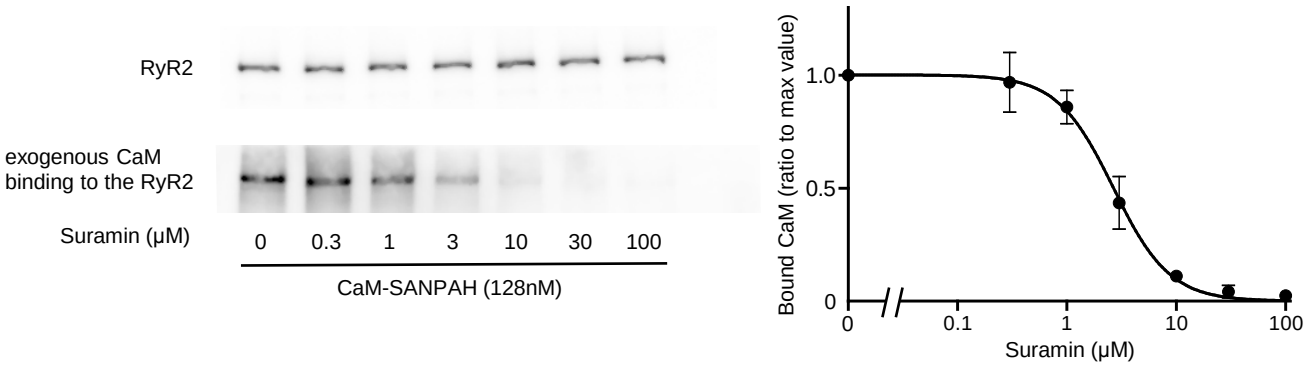


Supplementary Figure 6.
Effect of isoproterenol (ISO) on the phosphorylation level of Ser2808-RyR2 (P-Ser2808-RyR2). In the presence of isoproterenol (10 nM), KI and WT cardiomyocytes were incubated in the lysis buffer, and centrifuged. Then, the supernatant fraction containing crude homogenate was used for the phosphorylation assay. No significant difference was seen in the level of P-Ser2808-RyR2 between WT and a series of KI hearts, regardless of the presence of ISO. Data are presented as mean ± SEM of 4 hearts. *P < 0.05 vs. baseline (Student's t-test)





Supplementary Figure 7
Effect of cAMP on the phosphorylation level of Ser2808-RyR2 (P-Ser2808-RyR2). In the presence of cAMP (1 μM) and okadaic acid (1 μM), KI and WT cardiac homogenates were incubated in a sample solution containing 0.15 M NaCl, 50 mM MOPS, 0.1% CHAPS, at pH 6.8 for 30 min at 22 ° C, followed by western blotting with anti-P-Ser2808-RyR2 antibody. No significant difference was seen in the level of P-Ser2808-RyR2 between WT and a series of KI hearts, regardless of the presence of cAMP. Data are presented as mean ± SEM of 4 hearts. *P < 0.05 vs. baseline (Student's t-test)



Supplementary Figure 8

Inhibitory effect of suramin on the exogenous CaM binding to the RyR2 in WT hearts. Data are presented as mean ± SEM of 3 hearts.