DATA SUPPLEMENTS

Materials and methods

Mouse ventricular cardiomyocytes isolation Mouse ventricular cells were isolated as described before.[1] Mice of 5-6 months were anesthetized by peritoneal injection of urethane (1g/kg). After deep anesthetize was achieved, sternotomy was performed and the heart was quickly excised and cannulated on a Langendorff-perfusion system by aorta. The heart was first perfused with perfusion buffer containing (mM) 10 HEPES, 0.6 Na_2HPO_4, 113 NaCl, 4.7 KCl, 12 $NaHCO_3$, 0.6 KH_2PO_4, 1.2 $MgSO_4$-$7H_2O$, 10 $KHCO_3$, 30 Taurine, 500mM 2,3-Butanedione monoxime, 5.5mM Glucose, pH 7.46 at 37℃ at the speed of 3ml/min. Then the heart was switched to digestion buffer which contained 773.48u/ml Collagenase Type II, 0.14mg/ml Trypsin, and 12.5μM $CaCl_2$. After the heart turning soft, the ventricle was cut off from the heart and cut into pieces in the stopping buffer, which added 10% FBS and 12.5 μM $CaCl_2$ in perfusion buffer. Tissue pieces were gently suspended and the single cell supernatant was transferred to a clean tube. Ca^{2+} was reintroduced to 1.0mM step by step and cells were finally transferred to bath solution containing (mM) 135 NaCl, 4 KCl, 1.0 $CaCl_2$, 1 $MgCl_2$, 10 Hepes, 1.2 NaH_2PO_4, and 10 glucose, pH 7.40 with NaOH.

Confocal Ca^{2+} imaging Ca^{2+} activities, including Ca^{2+} spark, field stimulation-stimulated Ca^{2+} transient, SR Ca^{2+} load, and RyR2-mediated diastolic Ca^{2+} leak, were recorded by the LSM510 Meta inverted confocal microscope (Carl Zeiss) with a 40×/1.2 N.A water immerse objective. Cardiomyocytes were incubated with 10μM Fluo-4 AM, a cell-penetrating Ca^{2+} indicator with Ca^{2+} binding affinity (K_d) of ~335 nM, at 37℃ for 5min. Then cells were washed and kept in fresh bath solution. Binding to Ca^{2+}, Fluo-4 presents an increase in fluorescence, which is excited at the wavelength of 488nm and recorded at wavelength >505nm. Ca^{2+} images were collected by the one-direction line scan of the long axis of cell, at the speed of 3.072ms/line. For β-adrenergic stimulation, 300nM isoproterenol was applied in bath solution.

For Ca^{2+} spark measurement, cardiomyocytes were paced 20 times by 1Hz field stimulation (54V, 2ms duration). After pacing, Ca^{2+} sparks were recorded in quiescent state for 10s. The criterion for the fluorescence amplitude of spark was set at 1.32 (F_{peak}/F_0). Ca^{2+} spark frequency, full duration at half maximum (FDHM), full width at half maximum (FWHM), and half decay time were analyzed by a self-written program based on IDL 5.5.

For Ca^{2+} transient and SR Ca^{2+} load, cardiomyocytes were paced 20 times by 1Hz field stimulation, and 10mM caffeine was perfused to the cell after pacing to deplete SR. Ca^{2+} transient amplitude was defined as the $(F_{peak}-F_0)/F_0$ ($\Delta F/F_0$) of the twitch Ca^{2+} transient, and SR Ca^{2+} load was $\Delta F/F_0$ of caffeine-induced Ca^{2+} transient. During pacing, the Ca^{2+} transient amplitude gradually decreases until SR Ca^{2+} reaches a steady level. We used the averaged peak value of the last 10 Ca^{2+} transients of a train of stimulation to represent the Ca^{2+} transient amplitude.

The RyR2-mediated diastolic Ca^{2+} leak was measured as described before.[2] Briefly, after pacing, the cardiomyocyte was perfused with $0Na^+$-$0Ca^{2+}$ solution containing (mM): 135 LiCl, 4 KCl, 1 $MgCl{\cdot}6H_2O$,10 Hepes, 10 EGTA, 10 Glucose, pH 7.40 with LiOH. As LTCC and NCX of the cardiomyocyte were blocked by $0Na^+$-$0Ca^{2+}$ solution, there was no Ca^{2+} exchange between extracellular and intracellular environment (step 1). After 10s, solution was switched to $0Na^+$-$0Ca^{2+}$

with 1mM RyR2 blocker tetracaine for 30s. Tetracaine blocked diastolic Ca^{2+} leak trough RyR2 so the fluorescence difference between Step 1 and 2 reflected diastolic Ca^{2+} leak (step 2). Finally the cell was perfused with 10mM caffeine to measure the SR Ca^{2+} load (Step 3). To evaluate NCX and SERCA activity, caffeine induced Ca^{2+} transient and twitch Ca^{2+} transient were analyzed by the following method: The $[Ca^{2+}]_i$ (shown as $\Delta F/F_0$) is fitted to the formula $Ca(t) = A + Ca_0 \cdot e^{-\frac{t}{\tau}}$, in which Ca_0 is the apex of Ca^{2+} transient. K, defined as $1/\tau$, is used to describe the decay rate of Ca^{2+} transient. During caffeine perfusion, Ca^{2+} keeps releasing from SR through open RyR2s so the decline of $[Ca^{2+}]_i$ only depends on NCX, thus $K_{caffeine}=K_{NCX}$. In normal twitching, the decay of twitch Ca^{2+} transient depends on both NCX and SERCA, so $K_{SERCA}= K_{twitch}-K_{NCX}$.[3]

For Ca^{2+} imaging with patch clamp, cardiomyocytes were dialyzed with 0.2mM fluo-4 pentapotassium salt via pipette solution. Ca^{2+} activity was recorded by the Olympus IX51 inverted microscopy system with a 40× oil immerse objective. Ca^{2+} images were collected by the one-direction line scan at the speed of 10μs/pixel.

Patch clamp Whole-cell patch clamp experiments were conducted by using an Axopatch 700B and a Digidata 1440A digitizer (Axon Instruments) at room temperature.

For L-type Ca^{2+} current recording, cardiomyocytes were incubated in bath solution plus 30μM TTX and 10mM 4-aminopyridine to inhibit I_{Na} and I_{to}. The pipette solution contains (mM): 110 CsCl, 6 $MgCl_2$, 5 Na_2ATP, 0.3 Na_2GTP, 10 Hepes, and 15 TEA·Cl, pH 7.2 with CsOH. The cell was clamped at -50 mV and depolarized from -50 mV to +70 mV with an increment of 10 mV.

For NCX current recording, cardiomyocytes were bathed in bath solution containing (mM) 130 NaCl, 5 CsCl, 1.2 $MgSO_4$, 1.2 $NaH_2PO_4 \cdot H_2O$, 10 Hepes, 10 Glucose, 0.01 Nifedipine, 1 Ouabain·$8H_2O$, 0.01 Niflumic Acid, 1.0 $CaCl_2$, pH 7.40 with CsOH. Pipette solution contains (mM): 100 CsOH·H_2O, 100 Glutamic Acid, 7.25 Na^+-Hepes, 1 $MgCl_2.6H_2O$, 12.75 Hepes, 2.5 Na_2ATP, 10 EGTA, 6 $CaCl_2.2H_2O$, pH 7.2 with CsOH. Once the whole-cell configuration formed, cardiomyocytes were perfused with bath solution. The cell was depolarized to -40mV to inactivate I_{Na} and go through a ramp from +70mV to -140mV to induce remaining current. Then the perfusion solution was switched to bath solution plus 2.5mM $NiCl_2$ for 30s, and the current recording protocol was repeated. NCX current—a Ni^{2+} sensitive current, was obtained by subtracting the second current from the first one.

For action potential recording, cardiomyocytes were incubated in regular bath solution. The pipette solution contains (mM): 120 K-aspartate, 20 KCl, 1$MgCl_2$, 4 Na_2ATP, 0.1GTP, 10 HEPES, 10 glucose, pH 7.2 with KOH. At current clamp mode, action potential was triggered by the 3-6ms current injection of 400pA at 1Hz for 20 times, and Ca^{2+} transient was recorded by confocal microscopy at the same time. After pacing, the cell was left in quiescent state to observe DADs and Ca^{2+} waves for 10s. To quench Ca^{2+} release and propagation, 10mM EGTA or 10mM 5,5'-dibromo BAPTA tetrapotassium salt was added in pipette solution. When adding BAPTA in pipette solution, K-aspartate was adjusted to 80mM.

Transverse aortic constriction Transverse aortic constriction was conducted as described before.[4] Briefly, mice were anesthetized by 5% isoflurane inhalation at the speed of 0.8-1 L/min, and maintained at anesthetized state by 1.5-2% isoflurane. During surgery, mice were kept ventilated at a respiration rate of 80-120/min, tidal volume 0.15ml. The transverse aorta was dissected, and a piece of 27½ gauge blunt needle was tied to transvers aorta by knots. Then, the needle was removed

promptly to leave a 0.4mm constriction. After surgery, mice were recovered in a clean cage with water and food, being monitored every 5-10 minutes and then returned to animal room. For analgesia, 5mg/kg Carprofen (Rimadyl) was administrated by i.p. injection preemptively and every 24 hours after surgery.

Langendorff perfusion Langendorff perfusion was conducted as described before.[5] Briefly, mouse of 20-24 weeks was sacrificed by cervical dislocation, and the heart was quickly excised and cannulated on a Langendorff-perfusion system by aorta. The heart was perfused with 95% O_2+5% CO_2 gassed Krebs-Henseleit Buffer containing (mM): 118.5 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 11 Glucose, 25 $NaHCO_3$, 1.8 $CaCl_2$. A water-filled balloon connected to the pressure transducer was placed in the left ventricle to record left ventricular pressure. Size of the balloon was adjusted to make the end diastolic pressure under basal condition around 10mmHg. Two electrodes were attached to the atria and apex of the heart to record ECG (AD Instruments, Colorado Springs, CO). After stabilization, ECG and left ventricular pressure of the heart were recorded at basal condition for 10min. Following, the heart was perfused with the KH solution containing 300 nM isoproterenol. ECG and left ventricular pressure under isoproterenol stimulation were recorded for another 10min. Arrhythmic behavior, end diastolic left ventricular pressure, left ventricular developed pressure (maximum systolic pressure-end diastolic pressure), and max dp/dt were analyzed by LabChart 8.

Echocardiography Transthoracic echocardiography (Echo) was performed using a Vevo 2100 system with a 22-55 MHz transducer (MS550D; Visual Sonics), as described previously.[6] Mice were anesthetized by 5% isoflurane inhalation at the speed of 0.8-1 L/min, and maintained at anesthetized state by 1.5-2% isoflurane. Two-dimensionally guided M-mode images of the left ventricle (LV) were acquired at the tip of the papillary muscles. Posterior and anterior wall during systole and diastole, stroke volume, and heart rate were measured. Left ventricular mass was calculated by the formula $[1.05 \times ((\text{Posterior Wall}_{diastole}+\text{Anterior Wall}_{diastole}+\text{LV diameter}_{diastole})^3 - (\text{LV diameter}_{diastole})^3)]$; fractional shortening was calculated by the formula $[(\text{LV diameter}_{diastole} - \text{LV diameter}_{systole})/\text{LV diameter}_{diastole}] \times 100$.

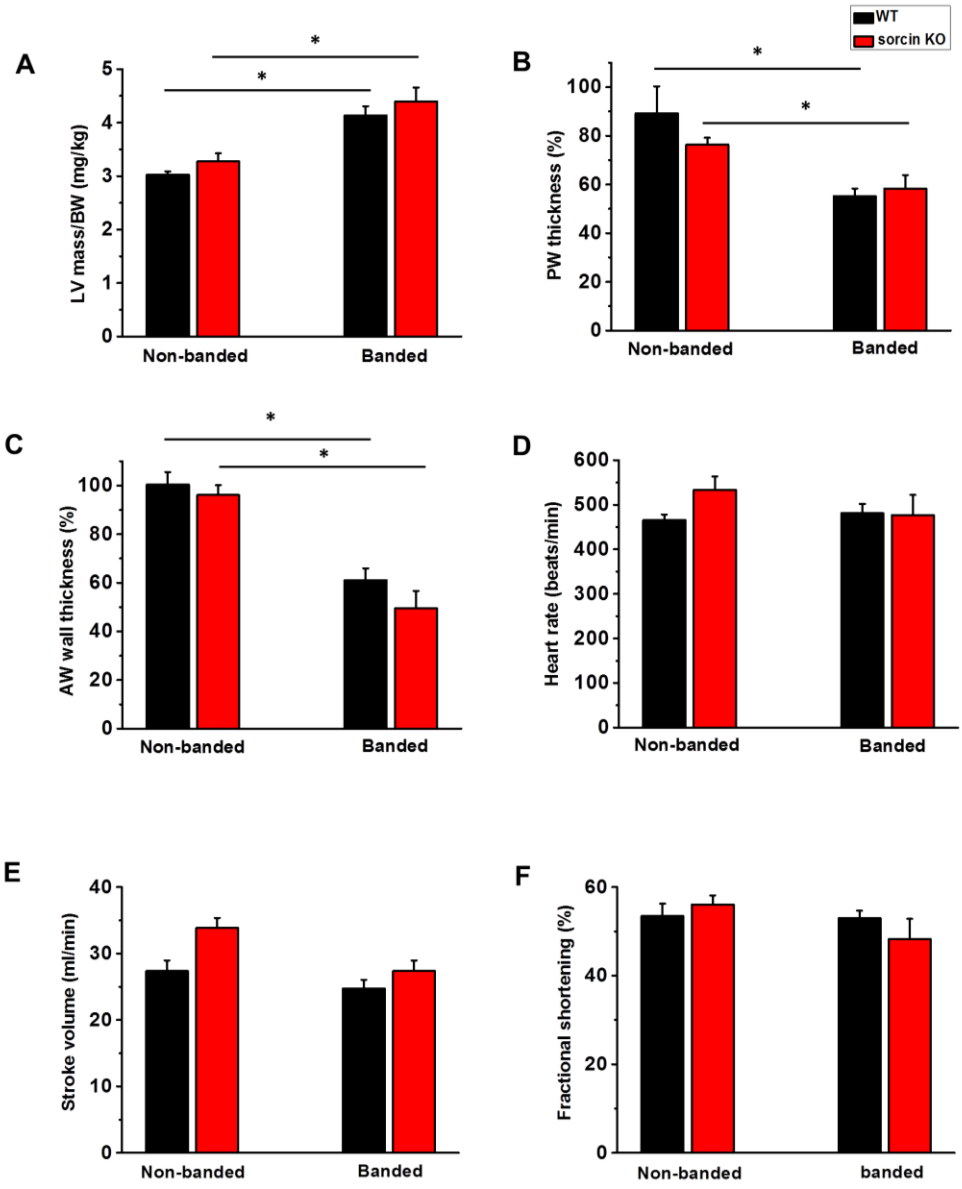
Histological staining Hearts explanted from mice were perfused with PBS and fixed in 10% neutral buffered formalin. Hearts were cut transversely, embedded in paraffin and stained with Masson's trichrome.

REFERENCES

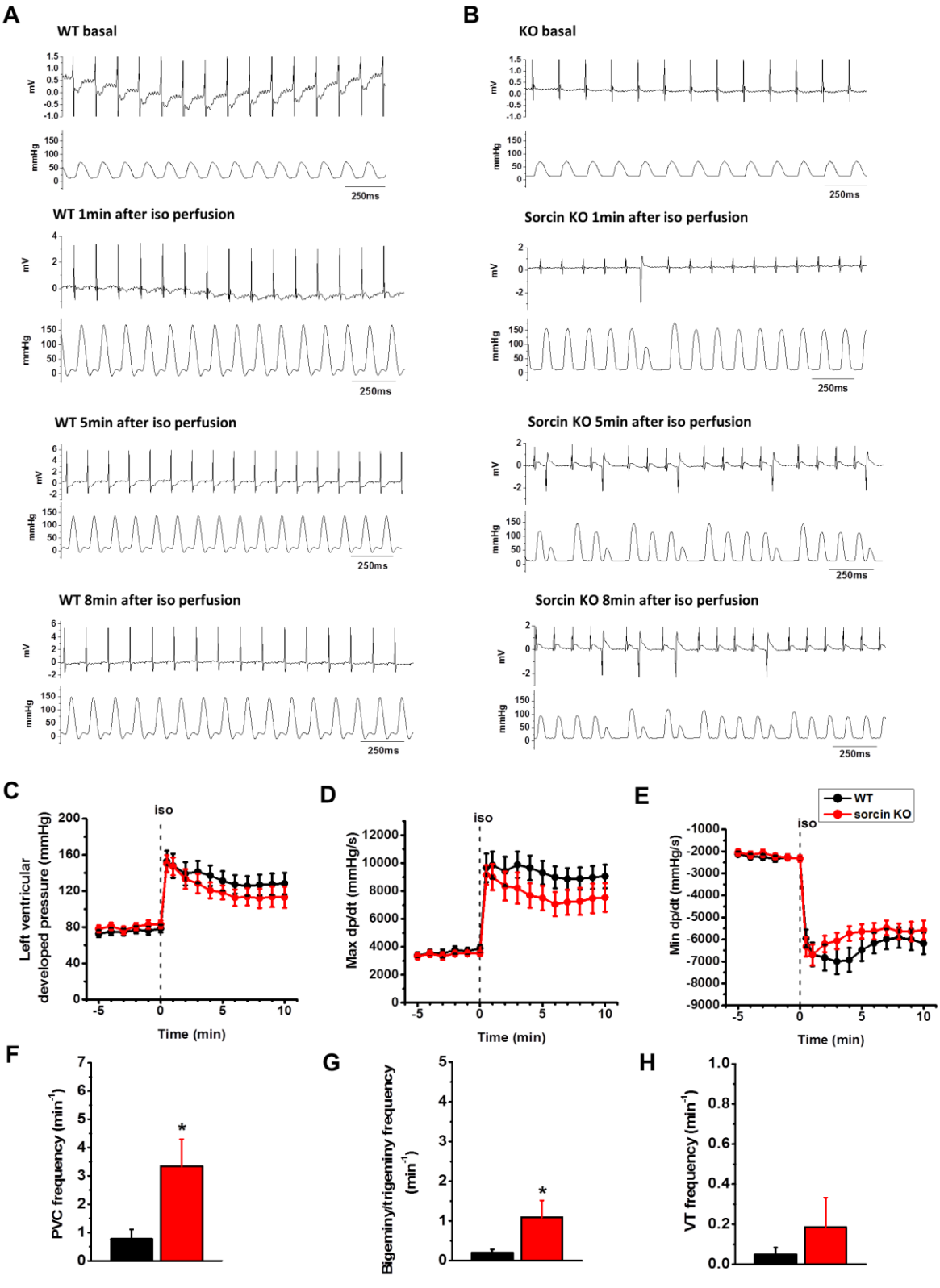
1. Zhao YT, Valdivia CR, Gurrola GB, Powers PP, Willis BC, Moss RL, Jalife J and Valdivia HH. Arrhythmogenesis in a catecholaminergic polymorphic ventricular tachycardia mutation that depresses ryanodine receptor function. *Proc Natl Acad Sci U S A*. 2015;112:E1669-1677.
2. Shannon TR. Quantitative Assessment of the SR Ca2+ Leak-Load Relationship. *Circ Res*. 2002;91:594-600.
3. Rodriguez JS, Velez Rueda JO, Salas M, Becerra R, Di Carlo MN, Said M, Vittone L, Rinaldi G, Portiansky EL, Mundina-Weilenmann C, Palomeque J and Mattiazzi A. Increased Na(+)/Ca(2)(+) exchanger expression/activity constitutes a point of inflection in the progression to heart failure of hypertensive rats. *PLoS One*. 2014;9:e96400.

4. deAlmeida AC, van Oort RJ and Wehrens XH. Transverse aortic constriction in mice. *J Vis Exp*. 2010.
5. Loaiza R, Benkusky NA, Powers PP, Hacker T, Noujaim S, Ackerman MJ, Jalife J and Valdivia HH. Heterogeneity of ryanodine receptor dysfunction in a mouse model of catecholaminergic polymorphic ventricular tachycardia. *Circ Res*. 2013;112:298-308.
6. Benkusky NA, Weber CS, Scherman JA, Farrell EF, Hacker TA, John MC, Powers PA and 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.

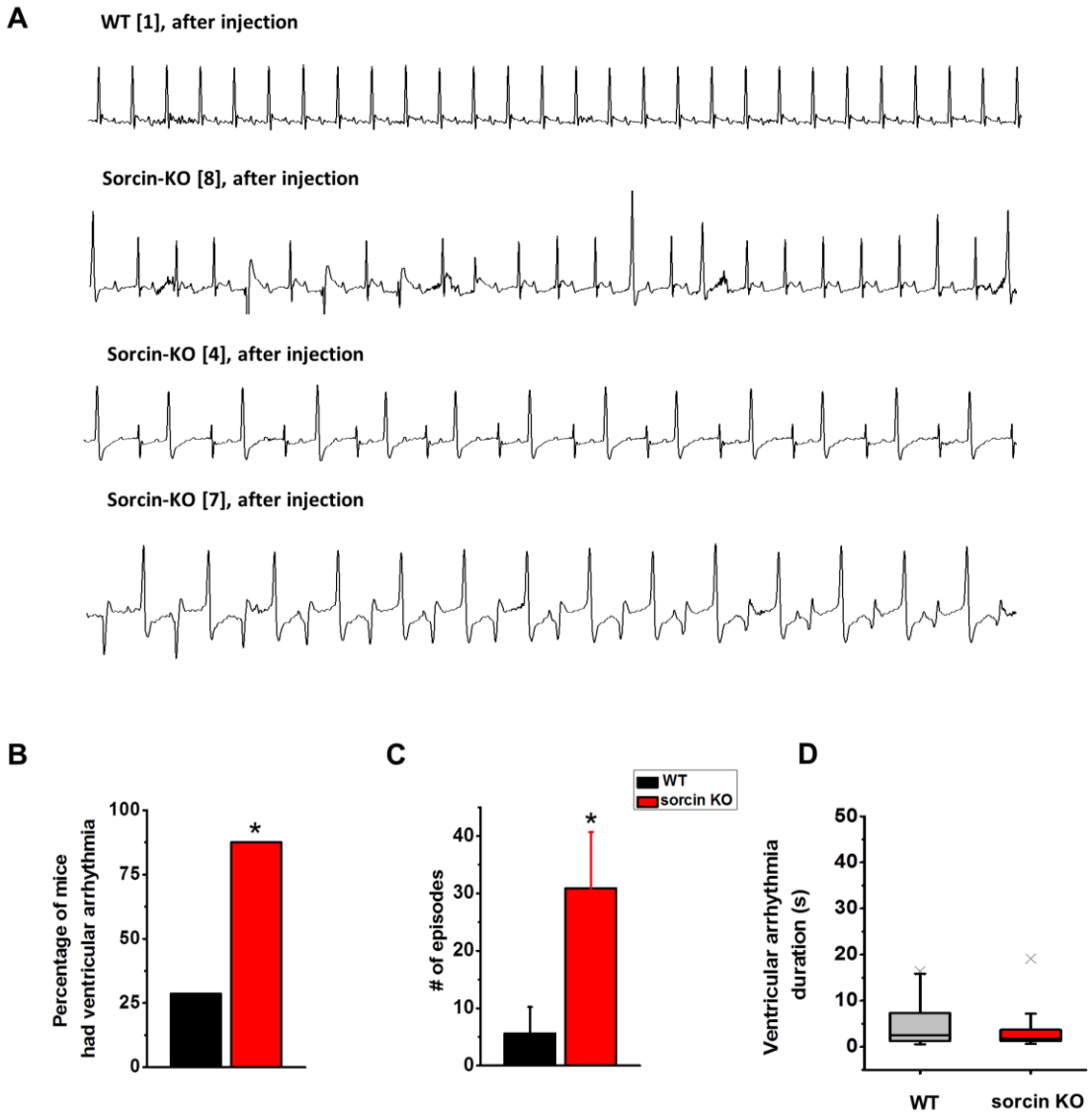
Supplementary figures



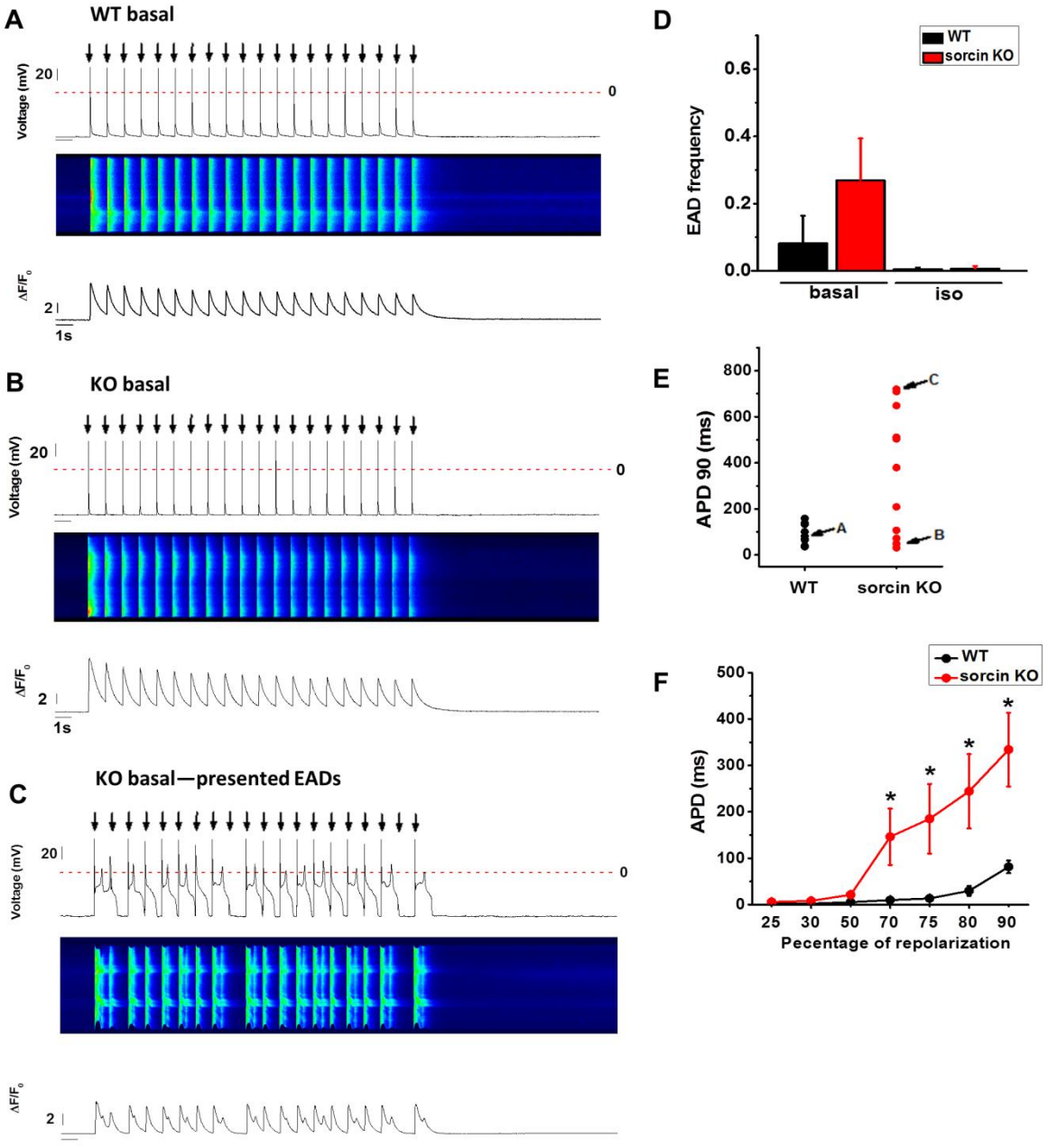
Supplementary Figure 1. Echocardiography measured heart function before (non-banded) and after TAC (banded). (A-F) Echocardiography measured LV mass (A), posterior wall (PW) thickness (B), anterior wall (AW) thickness (C), heart rate (D), stroke volume (E) and fractional shortening (F) before and after TAC. *, $p<0.05$ vs. non-banded hearts


A
WT basal
mV
mmHg
250ms
WT 1min after iso perfusion
WT 5min after iso perfusion
WT 8min after iso perfusion
B
KO basal
Sorcin KO 1min after iso perfusion
Sorcin KO 5min after iso perfusion
Sorcin KO 8min after iso perfusion
C
Left ventricular developed pressure (mmHg)
iso
Time (min)
D
Max dp/dt (mmHg/s)
E
Min dp/dt (mmHg/s)
WT
sorcin KO
F
PVC frequency (min-1)
*
G
Bigeminy/trigeminy frequency (min-1)
H
VT frequency (min-1)

Supplementary Figure 2. Left ventricular pressure and ECG recorded by Langendorff-perfusion. (A-B) Representative ECG and left ventricular pressure of WT (A) and sorcin KO hearts (B) at basal condition and 1, 5, 8 min after isoproterenol perfusion. (C) Left ventricular developed pressure (systolic pressure-end diastolic pressure) of Langendorff-perfused WT and sorcin KO hearts before (-5~0 min) and after (0~10 min) isoproterenol perfusion. (D-E) Systolic (maximum) dp/dt (D) and diastolic (minimum) dp/dt (E) of WT and sorcin KO hearts before and after isoproterenol perfusion. (F) Frequency of premature ventricular contraction (PVC). (G) Frequency of ventricular bigeminy/trigeminy. (H) Frequency of ventricular tachycardia (VT). N=12 in each group. *, $p<0.05$ vs. WT.

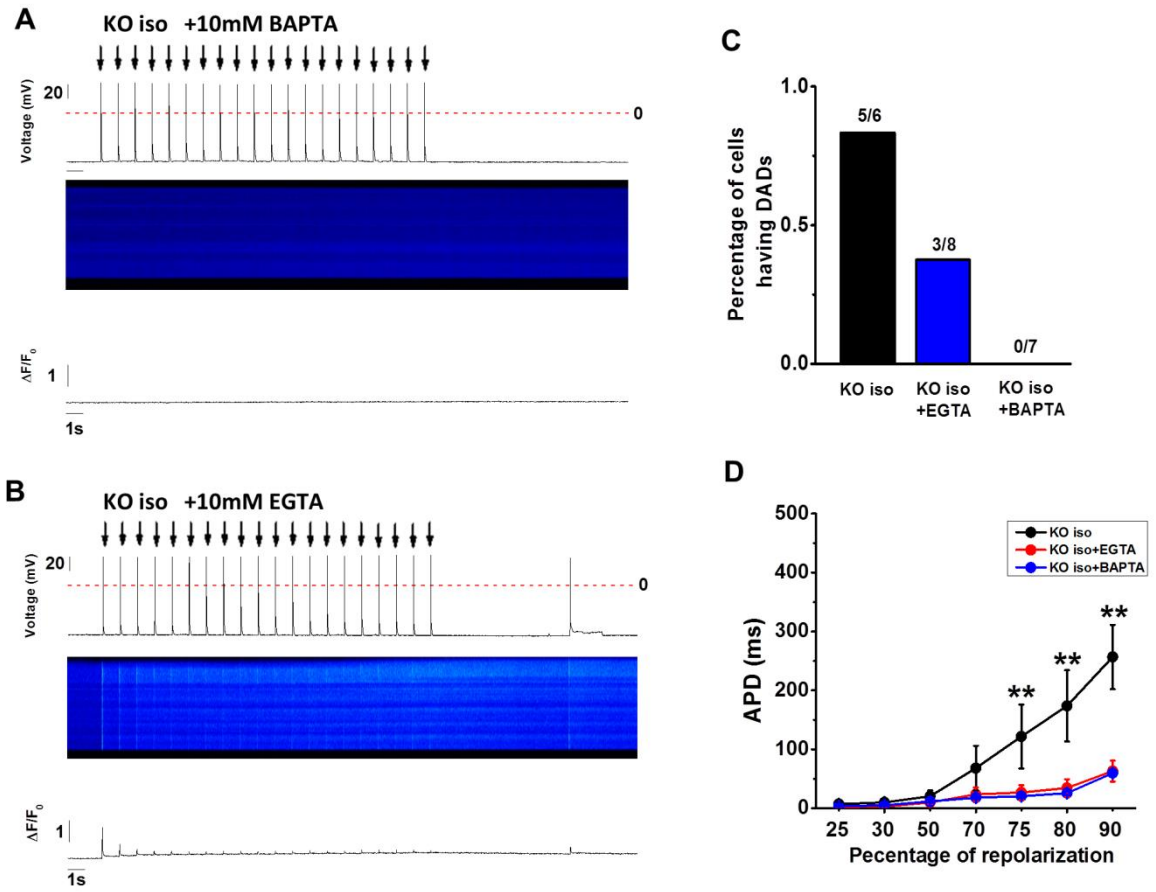


Supplementary Figure 3. ECG recordings of 1-month-old mice that were under stress. (A) ECG recordings of 1-month-old anesthetized mice after the administration of epinephrine (2 mg/kg) + caffeine (120 mg/kg). First trace: representative ECG of a WT mouse after injection; second trace: premature ventricular contractions in a sorcin KO mouse after injection; third trace: bigeminy in a sorcin KO mouse after injection; fourth trace: bidirectional ventricular tachycardia in a sorcin KO mouse after injection. (B) Percentage of mice that had ventricular arrhythmia after injection. (C) Number of ventricular arrhythmia episodes in WT and sorcin KO mice, per arrhythmia cocktail challenge. (D) Duration of arrhythmias in WT and sorcin KO mice. WT N=7, sorcin KO N=8. ×, average value; *, $p<0.05$ vs. WT.



Supplementary Figure 4. Action potential and Ca^{2+} transient under basal condition. (A) A representative recording of action potential and Ca^{2+} transient in a WT cardiomyocyte under basal condition. Upper panel: action potential; arrows indicate the time when the electrical stimulus was applied; middle panel: fluorescent recording of Ca^{2+} transient; under panel: line plot of Ca^{2+} transient. (B) A sorcin KO cardiomyocyte presented normal action potential and Ca^{2+} transients under basal condition. (C) A sorcin KO cardiomyocyte presented prolonged APD and EADs, which were accompanied with spontaneous Ca^{2+} waves between pacing under basal condition. (D) EAD frequency of WT and sorcin KO cardiomyocytes. (E) Scatter plot of APD90 under basal condition. 6 of

12 sorcin KO cells presented APD 90 that was longer than 200ms. Arrows point out APDs of recordings in figure A, B, C. (F) APD at indicated percent of repolarization under basal condition. WT-basal n=11, KO-basal n=12, WT-iso n=10, KO-iso n=14. *, $p<0.05$ vs. WT.



Supplementary Figure 5. Effects of Ca^{2+} chelator on action potential and Ca^{2+} transient. (A) Representative recording of action potential and Ca^{2+} activity in a sorcin KO cardiomyocyte dialyzed with 10 mM BAPTA under isoproterenol stimulation. (B) Representative recording of action potential and Ca^{2+}activity in a sorcin KO cardiomyocyte dialyzed with 10 mM EGTA under isoproterenol stimulation. (C) Percentage of cells that presented DADs. (D) APD at indicated percent of repolarization under isoproterenol stimulation. KO-iso n=6, KO-iso + EGTA n=8, KO-iso + BAPTA n=7 cells. **, p<0.01 vs. KO-iso + EGTA and KO-iso + BAPTA.