SUPPLEMENTAL MATERIAL

Supplemental Methods

***Histology*:** Hearts were fixed in neutral buffered 10% formalin, trimmed along the long axis to show both ventricles and both atria, processed routinely into paraffin, and then sectioned serially at 5 microns. Sections were stained using hematoxylin and eosin (H&E; to evaluate general organ structure and cell characteristics) and Masson's trichrome (to examine the amount of interstitial and perivascular fibrous connective tissue).

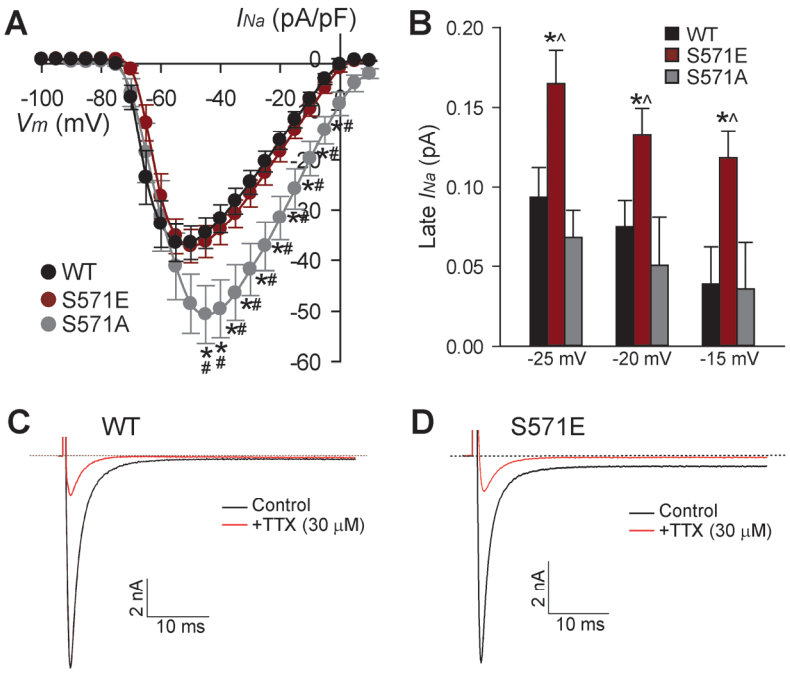
***Biochemistry and immunostaining*:** Equal quantities of ventricular lysates (determined using standard BCA protocols and verified through Ponceau stain of blots) were analyzed by SDS-PAGE and immunoblot, as described.[1-4] Any remaining small differences in loaded protein levels were corrected by normalizing protein levels to GAPDH. Adult cardiomyocytes were isolated, immunostained, and imaged as described previously.[1, 3, 4] Briefly, cells were fixed in 100% ethanol and blocked in PBS containing 0.15% Triton X-100, 3% normal goat serum (Sigma) and 1% BSA (Sigma), and incubated in primary antibody overnight at 4 °C. Cells were then washed, incubated in secondary antibody (Alexa 488, 568) for 2 hours at room temperature, and mounted using Vectashield with DAPI (Vector) and #1 coverslips. Image collection was performed on a Zeiss 780 confocal microscope [Objective W Plan Apochromat 40x/1.0 DIC (Zeiss), pinhole of 1.0 Airy Disc] using Carl Zeiss Imaging software. The following antibodies were used for immunoblotting and immunostaining: β_{IV}-spectrin (N-terminal), $Na_v1.5$ (Alomone or custom[1, 5]), phospho-$Na_v1.5$(S571), CaMKIIδ (Badrilla), phospho-CaMKIIδ (Thermo Scientific), Ankyrin-G (Santa Cruz), N-Cadherin (Invitrogen), and GAPDH (Fitzgerald).

Intracellular Ca^{2+} measurements: Intracellular Ca^{2+} cycling was monitored as described previously.[6] Briefly, cardiomyocytes were loaded with cytosolic Ca^{2+}-sensitive indicators Fluo-4

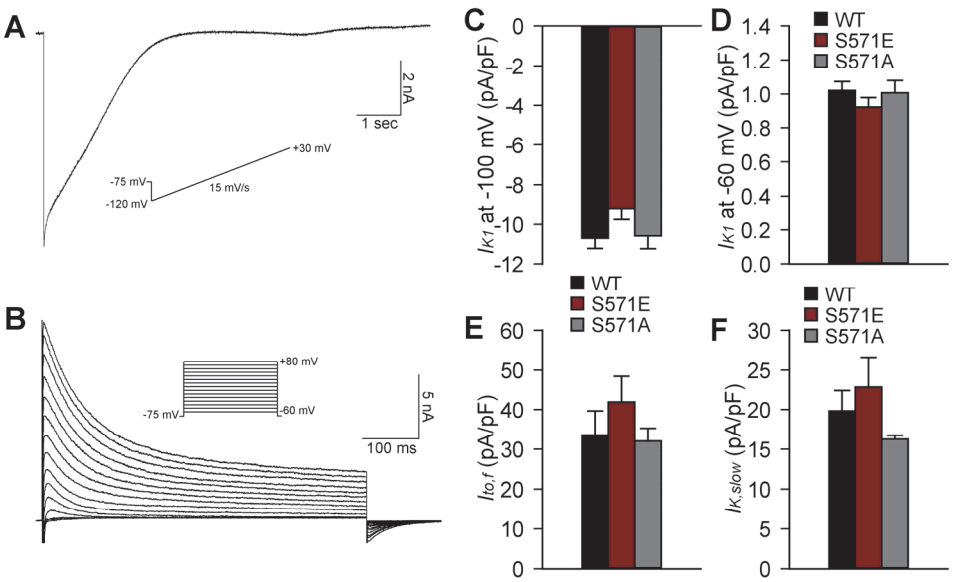
AM. The fluorescent probes were excited with the 488 nm line of an argon laser and emission was collected at 500–600 nm in the line scan mode of Nikon A1 laser scanning confocal microscope. The fluorescence emitted was expressed as F/F_0, where F is the fluorescence at time t and F_0 represents the background signal. Myocytes were paced at 0.3 Hz using extracellular platinum electrodes. Any Ca^{2+} release event (i.e. wave, wavelet) that increased cell-wide fluorescence intensity above 10% of the signal generated by the preceding stimulated Ca^{2+} transient was included in the analysis. Automated Ca^{2+} spark analysis was conducted with SparkMaster. To assess the SR Ca^{2+} load, 20 mM caffeine was applied at the end of the experiments.

***Telemetry*:** Electrocardiogram (ECG) recordings were obtained in awake, unanaesthetized mice using implanted radiotelemeters (DSI, St. Paul, MN, USA) at baseline and following stress protocol, as described.[3] Baseline heart rate analysis was performed by continuously collecting ECG data for 30 minutes on three separate days, and analyzed according to established protocol. For stress tests, mice were exercised to exhaustion on a treadmill and then were injected with epinephrine (2 mg/kg) followed by 90 minutes of continuous recording. A subset of animals was injected with flecainide (20 mg/kg) or ranolazine (20 mg/kg) prior to recording according to established protocol.[7, 8]

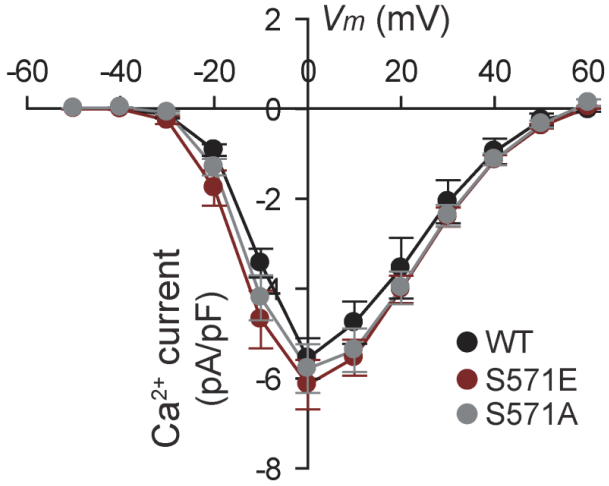
Supplemental Figures



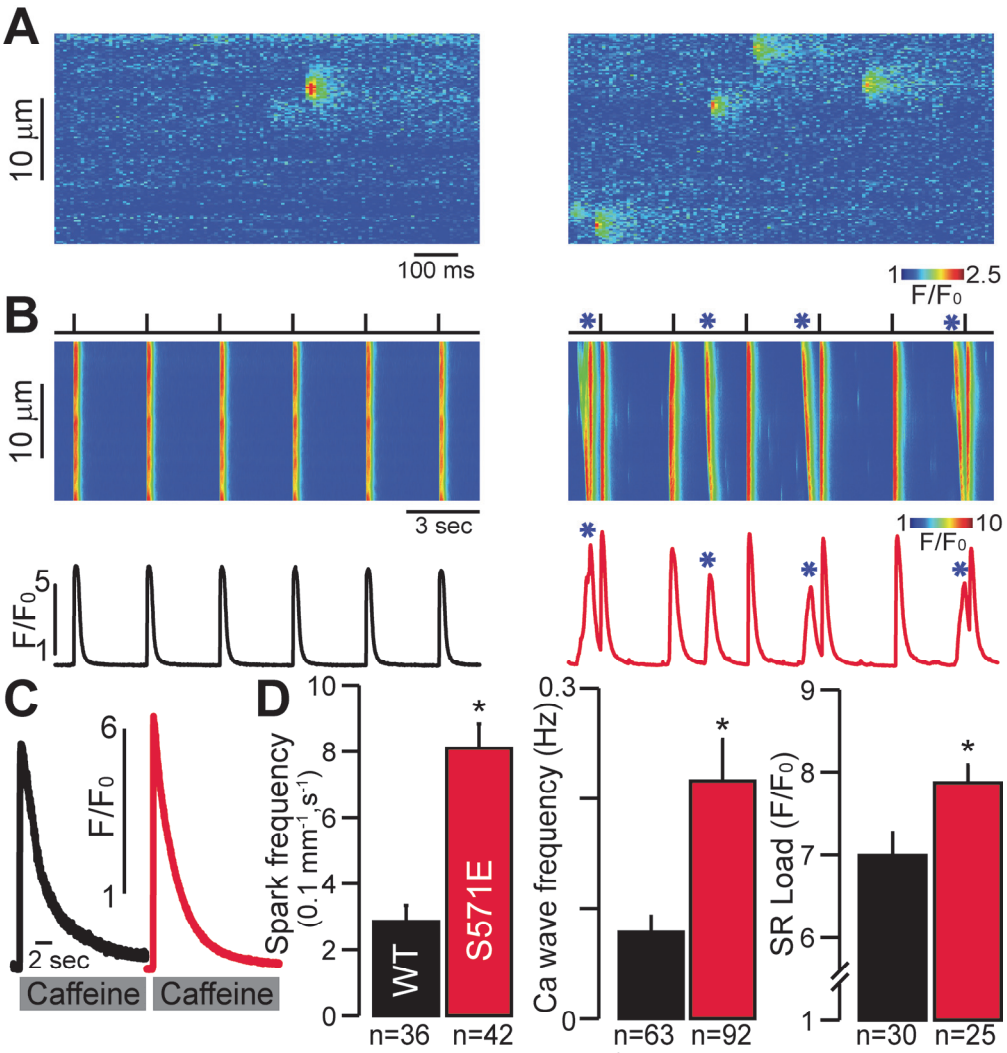
Supplemental Figure 1. **(A)** I_{Na} current-voltage relationship measured in WT, S571E and S571A myocytes at baseline. **(B)** Summary data (mean±SEM) for late Na^+ current amplitude in WT, S571E an S571A myocytes during test pulses to -25 mV, -20 mV or -15 mV (*P<0.05 vs. WT, #P<0.05 vs. S571E, ^P<0.05 vs. S571A, N = 8 for WT, N = 10 for S571E, N = 12 for S571A). **(C-D)** Representative I_{Na} traces from WT and S571E ventricular myocytes at baseline (control) and in the presence of 30 μM tetrodotoxin (TTX).



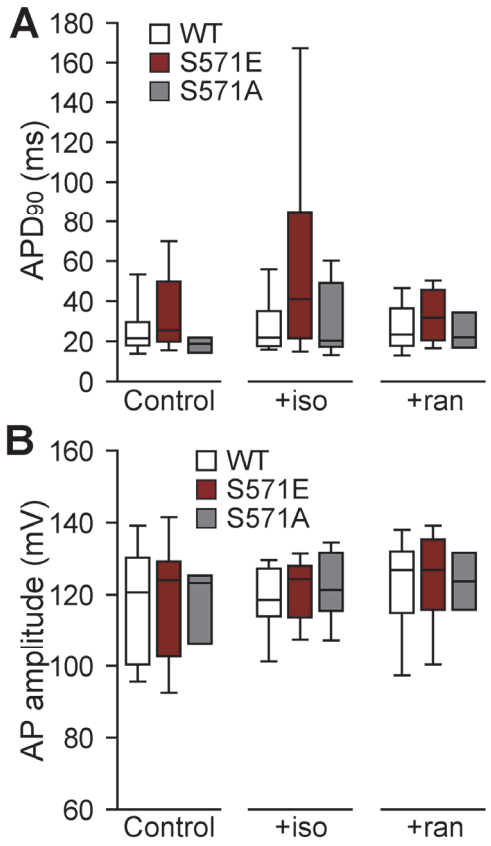
Supplemental Figure 2. K^+ current measurements in WT, S571E and S571A ventricular myocytes. Representative current traces for WT **(A)** inward rectifier K^+ current I_{K1} and **(B)** transient outward K^+ current (fast component) and remaining current after inactivation of I_{to} ($I_{K,slow}$). **(C-F)** Summary data (mean±SEM) for K^+ currents from WT, S571E and S571A (P=NS, N = 10 from two different preparations for WT, N = 11 from two different preparations for S571E, N = 13 from three different preparations for S571A).



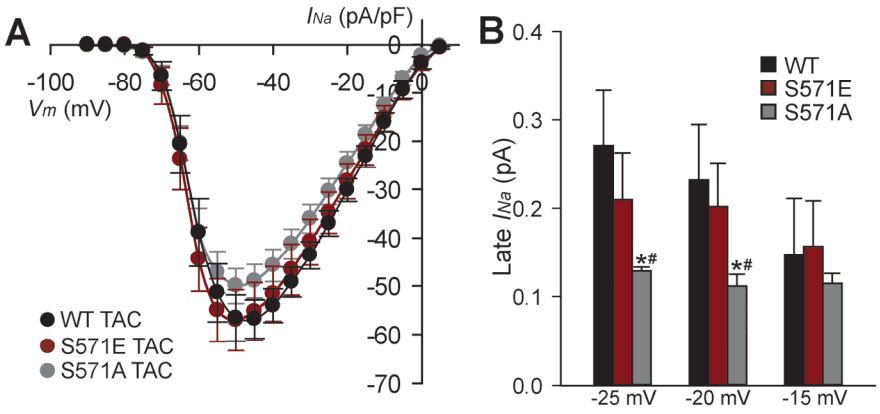
Supplemental Figure 3. Voltage-dependent Ca^{2+} current-voltage relationship from WT, S571E and S571A ventricular myocytes. (P=NS, N = 6 for WT, N = 8 for S571E, N = 11 for S571A from two different preparations for each genotype).



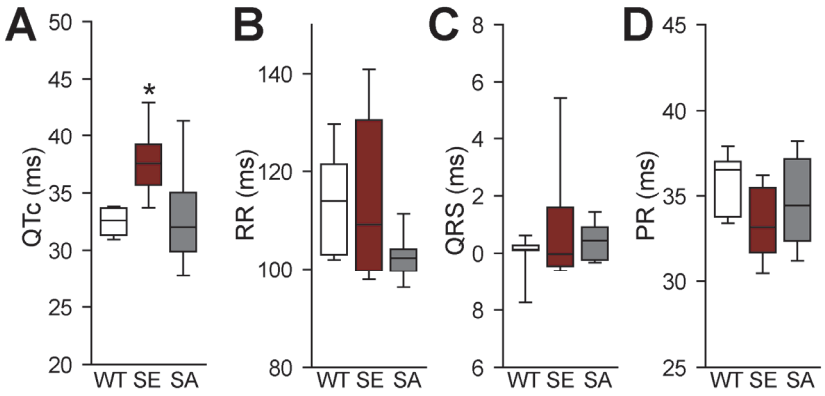
Supplemental Figure 4 – Increased late Na^+ current in S571E myocytes increases diastolic Ca^{2+} release. **(A)** Representative line-scan images of Ca^{2+} sparks and **(B)** Ca^{2+} waves from wild type (WT) and S571E ventricular cardiomyocytes. Cells were treated with isoproterenol (1µM) and paced at 0.3 Hz. Blue asterisks indicate Ca^{2+} waves. **(C)** Representative caffeine-induced (20 mM) Ca^{2+} transients to assess Ca^{2+} load. **(D)** Summary data (mean±SEM) showing increased Ca^{2+} spark frequency, Ca^{2+} wave frequency and SR Ca^{2+} load in S571E myocytes (*P<0.05 compared to WT).



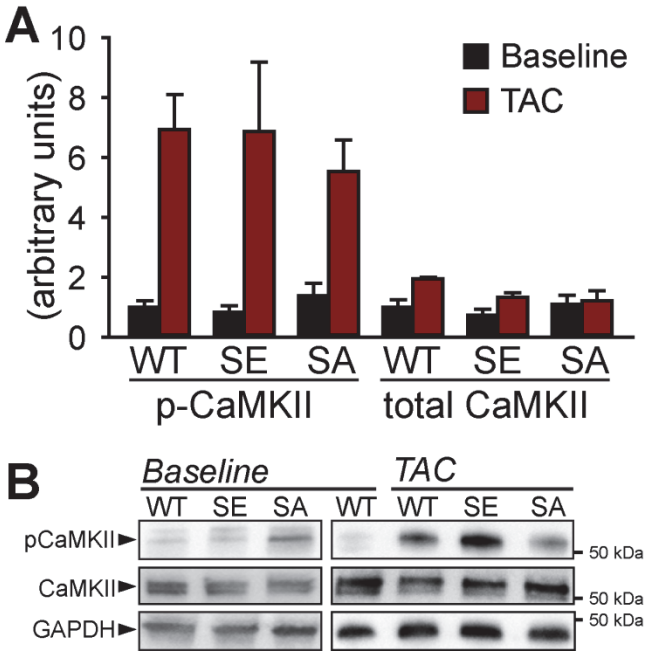
Supplemental Figure 5. Summary data for **(A)** APD at 90% repolarization and **(B)** AP amplitude in WT, S571E and S571A myocytes at 2 Hz pacing at baseline (control), after addition of 10 μM isoproterenol (+iso), followed by 1 μM ranolazine (+ran). AP data were not normally distributed and are shown as median with 25th and 75th percentile (box) and 10th and 90th percentile (whiskers). (P=NS, N = 13 from three different preparations for WT, N = 17 from five preparations for S571E, N = 14 from three preparations for S571A).



Supplemental Figure 6. **(A)** I_{Na} current-voltage relationship measured in WT, S571E and S571A myocytes following 6 weeks of TAC (P = NS; N = 8 from two different preparations for WT, N = 10 from two different preparations for S571E, N = 12 for S571A from three different preparations). **(B)** Summary data (mean±SEM) for late I_{Na} amplitude in WT, S571E and S571A TAC myocytes during test pulses to -25 mV, -20 mV or -15 mV (*P<0.05 vs. WT, #P<0.05 vs. S571E; N = 4 for WT and S571E, N = 6 for S571A from two different preparations).



Supplemental Figure 7. **(A-D)** Summary ECG data showing corrected QT (QTc), RR, QRS, and PR intervals in WT, S571E, and S571A TAC mice (*P<0.05 vs. WT N=6 for WT, N=7 for S571E, N=6 for S571A). Data were not normally distributed and are shown as median with 25th and 75th percentile (box) and 10th and 90th percentile (whiskers).



Supplemental Figure 8. **(A)** Summary densitometry data (mean±SEM) and **(B)** representative immunoblots for phospho- and total CaMKII in WT, S571E, and S571A detergent-soluble heart lysates at baseline and following 6-week TAC. (P=NS, N=3 for WT, S571E and S571A baseline, N = 4 for WT TAC and S571A TAC, N=5 for S571E TAC).

Supplemental References

1. Hund TJ, Koval OM, Li J, Wright PJ, Qian L, Snyder JS, Gudmundsson H, Kline CF, Davidson NP, Cardona N, Rasband MN, Anderson ME, Mohler PJ. A betaIV spectrin/CaMKII signaling complex is essential for membrane excitability in mice. *J Clin Invest*. 2010;120:3508-3519.
2. Hund TJ, Lerner DL, Yamada KA, Schuessler RB, Saffitz JE. Protein kinase Cepsilon mediates salutary effects on electrical coupling induced by ischemic preconditioning. *Heart Rhythm*. 2007;4:1183-1193.
3. Hund TJ, Snyder JS, Wu X, Glynn P, Koval OM, Onal B, Leymaster ND, Unudurthi SD, Curran J, Camardo C, Wright PJ, Binkley PF, Anderson ME, Mohler PJ. betaIV-Spectrin regulates TREK-1 membrane targeting in the heart. *Cardiovasc Res*. 2014;102:166-175.
4. Hund TJ, Wright PJ, Dun W, Snyder JS, Boyden PA, Mohler PJ. Regulation of the ankyrin-B-based targeting pathway following myocardial infarction. *Cardiovasc Res*. 2009;81:742-749.
5. Koval OM, Snyder JS, Wolf RM, Pavlovicz RE, Glynn P, Curran J, Leymaster ND, Dun W, Wright PJ, Cardona N, Qian L, Mitchell CC, Boyden PA, Binkley PF, Li C, Anderson ME, Mohler PJ, Hund TJ. Ca^{2+}/calmodulin-dependent protein kinase II-based regulation of voltage-gated Na^+ channel in cardiac disease. *Circulation*. 2012;126:2084-2094.
6. Radwanski PB, Brunello L, Veeraraghavan R, Ho HT, Lou Q, Makara MA, Belevych AE, Anghelescu M, Priori SG, Volpe P, Hund TJ, Janssen PM, Mohler PJ, Bridge JH, Poelzing S, Gyorke S. Neuronal Na^+ channel blockade suppresses arrhythmogenic diastolic Ca^{2+} release. *Cardiovasc Res*. 2015;106:143-152.
7. Makara MA, Curran J, Little S, Musa H, Polina I, Smith SA, Wright PJ, Unudurthi SD, Snyder JS, Bennett V, Hund TJ, Mohler PJ. Ankyrin-G Coordinates Intercalated Disc Signaling Platform to Regulate Cardiac Excitability In Vivo. *Circ Res*. 2014;115:929-938.
8. Watanabe H. Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. *Nat Med*. 2009;15:380-383.