Supplemental Figures

Fig. 1: Connexin 43 (Cx43) and Nav1.5 colocalize to the same discrete subcellular regions.

Representative confocal micrographs of immunofluorescent (top) and proximaty ligation assay (PLA; bottom) signals from isolated CPVT ventricular myocytes immunolabeled for Cx43 and $Na_v1.5$. Across all images (n = 22) the median number of PLA punctae observed per myocyte was 18.5 ± 8.9 with 90 ± 3% being observed along the cell's periphery.

Fig. 2: β-AR stimulation increases TTX-sensitive nNa_v-mediated persistent I_{Na} in wild type (WT). Representative traces of persistent I_{Na} elicited using the protocol shown in the inset. ISO enhanced persistent I_{Na} in WT cardiomyocytes ($p < 0.001$ Kruskal-Wallis test, n = 13 and 11, respectively, * p=0.006 Wilcoxon rank-sum test). This response to ISO was completely abolished by 100nM TTX (n = 10, # p=0.003 Wilcoxon rank-sum test).

Fig. 3: Effect of various Na^+ channel blockers on peak Na^+ current. Inward Na^+ currents obtained by 200 ms depolarization steps to 0 mV in 5 mV increments at 3 sec intervals. The depolarization step is preceded by a pre-step to -140 mV from holding potential −80 mV. (Right) Corresponding peak I/V relationship for CPVT cardiomyocytes under control conditions and during exposure to riluzole (10 μM), TTX (100 nM), or flecainide (2.5 μM), (n = 14, 8, 12, 14, respectively; $p < 0.05$ Kruskal-Wallis test, $^*p<0.05$ Wilcoxon rank-sum test).

Fig. 4: Effect of TTX-sensitive nNa_v-mediated persistent I_{Na} augmentation and increased SERCA2a expression on SR Ca^{2+} load. (a) Direct augmentation of nNa_v-mediated persistent I_{Na} with β-PMTX (40 μM) in CPVT myocytes exposed to ISO (100 nM) did not have a significant effect on caffeine-induced CaT amplitude (n = 42 for CPVT-ISO, n = 31 for CPVT-ISO+β-PMTX,

p=ns Wilcoxon rank-sum test). CPVT ventricular cardiomyocytes were loaded with Ca^{2+} indicator, Fluo-3 AM. Furthermore, none of the interventions tested had a significant effect on caffeine-induced CaT amplitude (n = 11 for CPVT-ISO+β-PMTX+TTX, n = 15 for CPVT-ISO+β-PMTX+Ril and n = 15 for CPVT-ISO+β-PMTX+Flec, *p*=ns Kruskal-Wallis test). Likewise, CPVT-SERCA myocytes did not evidence higher caffeine-induced CaT amplitude during treatment with β-PMTX relative to the untreated ones (n = 18 and 17, *p*=ns Wilcoxon rank-sum test).Importantly, CPVT-SERCA myocytes evidenced similar caffeine-induced CaT relative to CPVT myocytes treated with ISO. **(b)** Neither intervention, whether pharmacological (KN-93, 1 µM) or genetic (S2814), had a significant effect on caffeine-induced CaT amplitude relative to ISO treated CPVT myocytes (n = 42 for CPVT- ISO, n = 17 for CPVT- ISO -KN93, n = 20 for CPVT2814-ISO and n = 16 for CPVT2814-ISO+Ril, *p*=ns Kruskal-Wallis test).

Fig. 5: Neuronal Na^+ channels and RyR2 colocalize to the same discrete subcellular regions in WT ventricular myocytes. (a) Representative confocal micrographs of ventricular myocytes isolated from WT mice showing fluorescent proximity ligation assay (PLA) signal for RyR2 with different nNa_v isoforms (Na_V1.x). Below each image, are shown the results of digital segmentation with the cell mask in grey and PLA signal in red. **(b)** Plot of average number of PLA punctae per μm^2 (*, *p*=0.002, *p*=0.009 and *p*=1 Wilcoxon rank-sum test between WT and CPVT for Na_V1.1, 1.3 and 1.6, respectively; for WT n = 3165, 53, 2756 punctae from 10, 6 and 8 cells for Nav1.1, 1.3 and 1.6, respectively).

Fig. 6: Effect of Na_v1.6 blockade with 4,9-anhydro-TTX on SR Ca^{2+} load. Na_v1.6 blockade with 4,9-anhydro-TTX (4,9ah-TTX; 300 nM) in CPVT myocytes exposed to ISO (100 nM) did not have a significant effect on caffeine-induced CaT amplitude (n = 19 for CPVT-ISO, n = 28 for CPVT-ISO+4,9ah-TTX, *p*=ns Wilcoxon rank-sum test).

Fig. 7: $Na_v1.6$ silencing. Values are reported as percentage of control (n = 3-4 hearts per group) in **(a)** and **(b)**. Decreased $Na_v1.6$ **(a)** mRNA and **(b)** protein after siRNA treatment, respectively**.**

Fig. 8: Neuronal Na^+ channels and NCX colocalize to the same discrete subcellular regions in CPVT ventricular myocytes. (a) Representative confocal micrographs of ventricular myocytes isolated from CPVT mice showing fluorescent proximity ligation assay (PLA) signal for NCX with different nNa_v isoforms ($Na_V1.x$). Below each image, are shown the results of digital segmentation with the cell mask in grey and PLA signal in red. **(b)** Plot of average number of PLA punctae per μm^2 (*p* = 0.0195 Kruskal-Wallis test; *, *p*=0.005 Wilcoxon rank-sum test for $Na_v1.1$vs. 1.3 and p=0.244 Wilcoxon rank-sum test for $Na_v1.1$vs. 1.6. n = 778, 1969, 1526 punctae from 8, 9 and 10 cells for $Na_v1.1$, 1.3 and 1.6, respectively).