Supplemental Data

Figure S1.
(A-C) Primary ventricular neonatal cardiomyocytes (PVNC) were treated with isoproterenol (Iso; 100uM, 18 hours). Cells stained with TMRM to determine mitochondrial membrane potential or with calcein-AM and $CoCl_2$ to determine mitochondrial permeability transition (A) and relative fluorescence quantified (B). (C) Cells stained with calcein-AM and Eth HD-1 to identify living (green) and necrotic (red) cells, respectively and quantified by calculating the percentage of necrotic cells (Eth HD-1 Positive) and over 200 cells per condition; DMSO used as a control vehicle. (D) PVNCs treated with cyclosporine A (CsA, 1uM), 2-aminoethoxydiphenyl borate (2APB, 2uM), or Ruthenium-360 (Ru-360, 10uM) for 18 hours; DMSO used as a control vehicle. Cells were stained with calcein-AM and $CoCl_2$ and quantified. (E-F) H9c2 cells pre-treated with cyclosporine A (CsA, 1uM, 2 hours) (E) or a caspase inhibitor (zVAD, 50uM, 2 hours) (F) followed by ionomycin treatment (Iono, 2 µM, 18 hours). Cells stained with calcein-AM and Eth HD-1 and quantified; DMSO used as a control vehicle. (G-H) H9c2 cells pre-treated with cyclosporine A (CsA, 1uM, 2 hours) (G) or a caspase inhibitor (zVAD, 50uM, 2 hours) (H) followed by staurosporine treatment (STS, 2uM, 18 hours); DMSO used as a control vehicle. Cell viability assessed by MTT analysis (n=4). Data are expressed as mean +/- SE. * $p<0.05$ compared to control, ** $p<0.05$ compared to treatment, determined by 1-way ANOVA.

Figure S2.
(A) H9c2 cells were transfected with a SR-targeted calcium biosensor, pre-treated with Dantrolene (DAN, 10uM; 2 hours) following treatment with Forskolin (FSK, 10uM) and 3-isobutyl-1-methylxanthine (IBMX, 500uM; FSK-I, 18 hours), or DMSO as a control vehicle. (B-C) H9c2 cells were treated with Forskolin (FSK, 10uM) and 3-isobutyl-1-methylxanthine (IBMX, 500uM; FSK-I, 18 hours), or DMSO as a control vehicle, along with 2-aminoethoxydiphenyl borate (2APB, 2uM). Cells were stained with calcein-AM and cobalt chloride ($CoCl_2$, 5µM) to determine mitochondria permeability transition (B) or TMRM to determine mitochondrial membrane potential (C). (D) Primary ventricular neonatal cardiomyocytes were transduced with a mito-carmine lentivirus (pLenti-Mito-Car-GECO) and treated with isoproterenol (Iso; 100uM, 18 hours) or DMSO as a control vehicle; nuclei were stained with Hoechst. (E) H9c2 cells were transfected with sh-Nix or a scrambled control, following treatment with FSK-I (18 hours), or DMSO as a control vehicle; stained with calcein-AM and Eth HD-1 and quantified. (F) H9c2 cells were transfected with Nix or an empty control vector, and SR-targeted calcium biosensor; following treatment with Dantrolene (DAN; 10uM, 18 hours), and quantified.
(G) 3T3 cells were transfected with wild-type Nix, Nix-MaoB (Nix-mito), or Nix-CytoB5 (Nix-SR). Whole cell, mitochondrial, and ER cellular fractions were subjected to western blot, as indicated, to confirm the subcellular distribution of these engineered constructs.
Data are expressed as mean +/- SE. * $p<0.05$ compared to control, ** $p<0.05$ compared to treatment, determined by 1-way ANOVA.