SUPPLEMENTAL MATERIAL:

Generation of miR-130a overexpression mice

All procedures were approved by and performed in accordance with the University of Chicago Institutional Animal Care and Use Committee. A genomic fragment encoding the miR-130a precursor and flanking region was amplified by PCR using mouse genomic DNA as a template to amplify a 467 bp fragment encoding the miR-130a precursor (miR-130a forward, *5′-CATTGGCGCGCCCCTTTGAGAAGTGTCAAATGATGG* and miR-130a reverse *5′-TGCATTAATTAACTCTGGACAGGTCTACAAAAATGG*. This fragment was cloned into an Asc1 (5') and Pac1 (3') site in the tetracycline responsive vector plasmid. A 2-kb fragment containing Tet operon (TetO), miR-130a precursor, and hGH polyA signal was excised by BamH1 digestion and purified. The TetO–miR-130a gene was injected into the pronuclei of CD-1 embryos and implanted into pseudo-pregnant recipient females by the University of Chicago Transgenics Core. TetO–miR130a founder mice were established and crossed with CD-1 mice and expanded for a minimum of 7 generations on the CD-1 background. Separate strains were maintained for TetO–miR-130a or αMHC-tTA, which expresses heart-specific tTA controlled by the heart-specific αMHC promoter. Non-transgenic littermates (referred to as "control mice") were compared with double-transgenic littermates genotyped αMHC-tTA/TetO–miR-130a (referred to as "αMHC-miR130a mice"). Single-transgenic animals genotyped αMHC-tTA or TetO–miR-130a were also analyzed and compared to both non-transgenic and double transgenic mice.

Electron microscopy

A portion of the left ventricular free-wall from 12-16 weeks off doxycycline αMHC-miR130a (n=3) mice and control littermates (n=3) was removed to 2.5% glutaraldehyde-0.1M Sodium Cacodylate Buffer, pH 7.4. Samples were rinsed in 0.1M Sodium Cacodylate Buffer, pH 7.4 and left overnight at 4°C. Samples were then post-fixed in 1% Osmium Tetroxide-0.1M Sodium Cacodylate Buffer, pH7.4, at 4°C. Following dehydration, samples were treated with a 1:1 mixture of 100% EtOH: Propylene Oxide (PPO); PPO; then 1:1 PPO:EPON, and left in 1:2 PPO: EPON overnight. Samples were embedded in EPON and polymerized at 60° C. Electron microscopy was carried out at the electron microscopy core facility of the University of Chicago. Representative cardiomyocytes at the area of the intercalated disc were photographed.

Fluorescent *in situ* hybridization – Full methods

Adult mouse hearts were harvested and directly frozen in Tissue-Tek OCT compound (Electron Microscopy Sciences, cat. no. 62550-01). Frozen hearts were sectioned at 8-10 μm thickness and used for *in situ* hybridization according to established protocols [16] using specific probes for miR-130a (Exiqon, #38029-04; 7.5 pmol/slide), U6 (Exiqon , # 99002-04; 5 pmol/slide), and scramble-miR (Exiqon, #99004-04; 5 pmol/slide). Heart sections were fixed in 4% phosphate-buffered para-formaldehyde (Electron Microscopy Sciences, cat. no. 15710) and rinsed three times with DEPC-treated H_2O. Next, the slides were incubated in acetylation buffer (500 μl 6 N HCl, 670 μl 1 M triethanolamine, 48.5 ml DEPC-treated H_2O, and 300 μl acetic anhydride) for 5 minutes, and washed. Slides were pre-hybridized in hybridization solution (50% (vol/vol) formamide, 5 × SSC, 500 μg/μl yeast tRNA, 1X Denhardt's solution, H_2O) at 55°C for 30

minutes. Slides were incubated with probe in hybridization buffer for 1 hour at 55°C. Slides were then rinsed three times with 0.1X SSC for 10 minutes each at 59°C and once in 2X SSC for 5 minutes at room temperature. Slides were incubated in 3% H_2O_2 in PBS for 10 minutes, and then rinsed 3 times in TN Buffer (0.1 M Tris–HCl, pH 7.5, 0.15 M NaCl) for 3 minutes each. Next, slides were incubated in Blocking Buffer (0.1 M Tris–HCl, pH 7.5, 0.15 M NaCl, 0.5% (wt/vol) blocking reagent (Roche Applied Sciences, #109676), 0.5% (wt/vol) BSA) for 30 minutes in a humid chamber. Primary antibody to FITC (Dako, cat. no. P5101) was diluted 1:400 in blocking buffer and added to slides in humid chamber for 30 minutes. Slides were then rinsed 3 times in TNT Buffer (0.1 M Tris–HCl, pH 7.5, 0.15 M NaCl, 0.3% (vol/vol) Triton X-100). A 1:50 dilution of FITC-tyramide in amplification buffer supplied in kit was added to slides (TSA Fluorescein plus Evaluation Kit, Perkin Elmer NEL741E001KT) for 8 minutes, after which slides were rinsed 3 times in TNT Buffer in the dark and finally mounted using Prolong Gold with DAPI (Invitrogen-Molecular Probes, #P36931).

Quantitative PCR

Primer sequences for quantitative real-time PCR:

Cx43, forward: 5'-TCCTGGGTACAAGCTGGTCACTGG

Cx43, reverse: 5'-GCTGCTGGCTCTGCTGGAAGG

GAPDH, forward: 5′-TGACAAGCTTCCCATTCTCG

GAPDH, reverse: 5′-GTGAAGGTCGGTGTGAACG.

Electrophysiological studies

After proper anesthetic induction using inhaled isoflurane as described above, a jugular vein cutdown was performed and the vein isolated for direct endovascular access. A 2 french octapolar catheter was advanced into position in the right atrium and ventricle for study. Electrograms were recorded with a BioAmp and Powerlab system with Chart5 software (ADInstruments). Programmed electrical stimulation (PES) studies as described by Gutstein *et al.* [19]. In brief, a 1-cm incision was made in the epigastric region. A custom-designed cardiac stimulating electrode mounted on a micromanipulator (Fine Science Tools; N. Vancouver, BC, Canada) was inserted through the diaphragm directly into contact with the apical surface of the heart. PES was performed with a Programmable Stimulator (model 2352, Medtronic; Minneapolis, MN). Output was set at twice the stimulating threshold in all animals. Pulse width was 1.0 ms for all studies. PES consisted of pacing with a train of twelve beats, followed by a double or triple extrastimulus. This protocol was repeated at pacing cycle lengths of 120, 100, and 80 ms. Double and triple extrastimuli were added at each cycle length of 20, 30, and 40ms, to test for inducible arrhythmias. We used the following criteria to categorize the ventricular arrhythmias: A) Non-sustained VT: 3-30 beats, B) Sustained VT: >30 beats, and C) Cycle Length: <100 ms. [19, 20]

Western Blotting

Murine hearts were harvested and protein lysates were prepared as previously described [14] using an NTEN protein lysis buffer solution (1M Tris pH 8.0, 5M NaCl, Igepal CA-630, 0.5M EDTA pH 8, deionized water, protease inhibitors (Roche), and Laemlli Sample Buffer (Bio-Rad). Lysates were assayed using a BCA assay (Thermo Scientific: Pierce) according to manufacturer's recommendations and measured using a Beckman Coulter spectrometer. Twenty micrograms of whole heart lysate were resolved by 12% SDS-PAGE or a 3-8% Tris-Acetate gel and transferred to a nitrocellulose membrane (Whatman, Piscataway, NJ). The membrane was blocked with Blotto (10 mM Tris (pH 7.5), 140 mM NaCl, 0.05% Tween-20, and 5% instant milk). Antibodies used: Cx43 (3512S, Cell Signaling), phospho-Cx43 (Ser368, 3511, Cell Signaling) Cx40 (AB1726, Millipore), ryanodine receptor (RyR) (34C, Developmental Studies Hybridoma Bank), phospholamban (PLB) (8495, Cell Signaling), phospho-PLB (07-052, Millipore), SERCA2a (2862, Abcam), Amphiphysin 2 (Bin1) (H-100, SC-30099, Santa Cruz), DHPR (MA3-920, Thermo), Junctophilin2 (SC-51313, Santa Cruz), HRP-coupled goat anti-rabbit IgG (Jackson ImmunoResearch), and rabbit anti-gamma-tubulin (T6557, Sigma). The membrane was washed and incubated with a 1:5000 dilution of a HRP-coupled goat anti-rabbit IgG. The membrane was developed using autoradiography and a commercially available kit (ECL-prime, Amersham), and quantitated using Quantity One® (Bio-Rad) software. Membrane was stripped and re-probed with a 1:2000 dilution of a rabbit antibody directed against gamma-tubulin (T6557, Sigma).

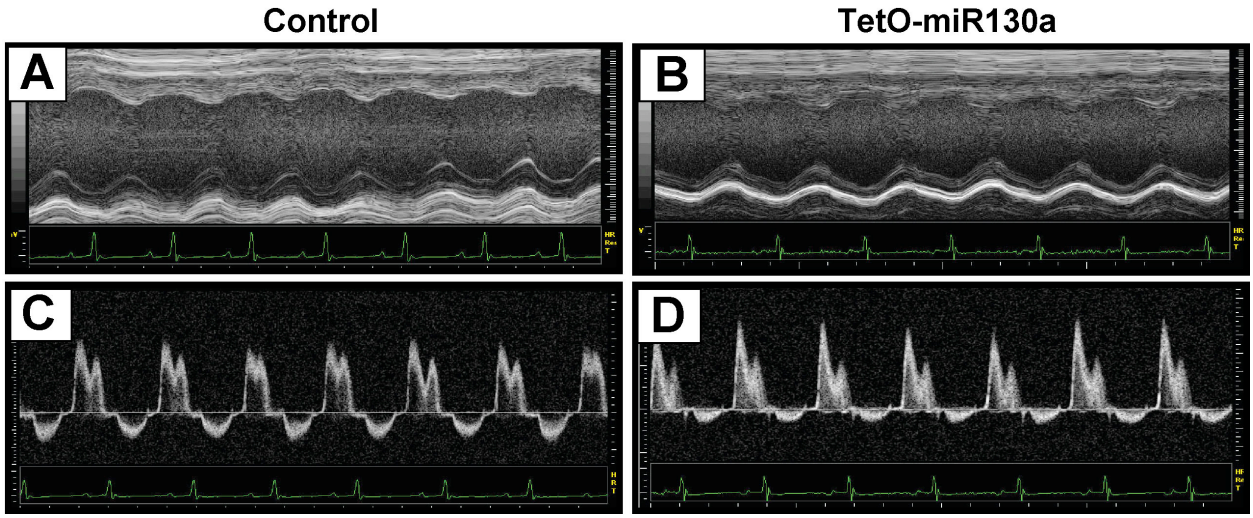

Figure S1. TetO-miR-130a mice maintain preserved LV size and function.

Representative M-mode echocardiography of control vs. Tet0-miR130a mouse taken off doxycycline at 10 weeks after weaning. As demonstrated in Panel (A) and (B), TetO-miR130a mice have preserved left ventricular function and normal sinus rhythm. In panels (C) and (D), 2D-guided pulsed Doppler of the mitral inflow is shown in control and the TetO-mir130a mice respectively. Both display the normal E and A wave pattern in normal sinus rhythm. A total of 10 mice in each group were studied.

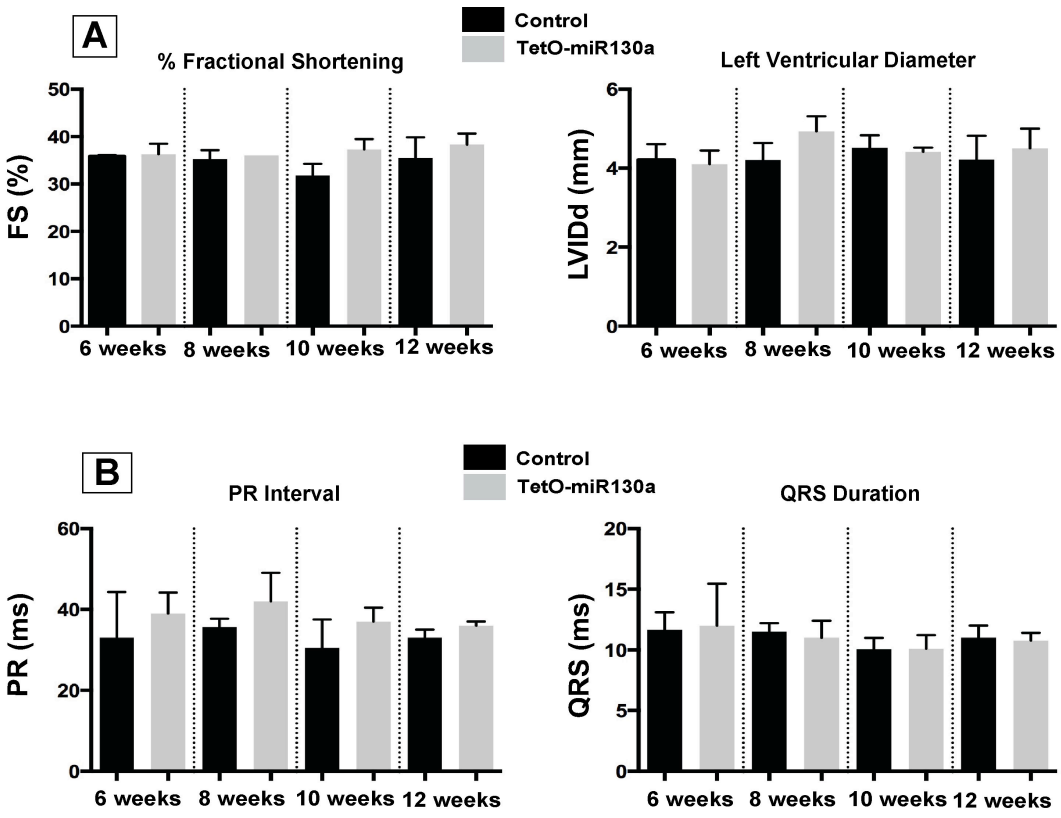

Figure S2. Serial echocardiographic and ECG assessment of littermate control vs TetO-miR-130a mice.

In panel (A), serial assessment of % fractional shortening and LV diameter at 6, 8, 10, and 12 weeks in littermate control vs TetO-miR-130a mice. No statistical differences were seen at any timepoint between the littermate controls and TetO-miR130a mice. In panel (B), serial assessment of the PR interval and the QRS duration at 6, 8, 10, and 12 weeks in littermate control vs TetO-miR-130a mice. Similar to the echocardiograpic parameters, no statistical differences were seen at any timepoint between the littermate controls and TetO-miR130a mice. In each group, n = 5.

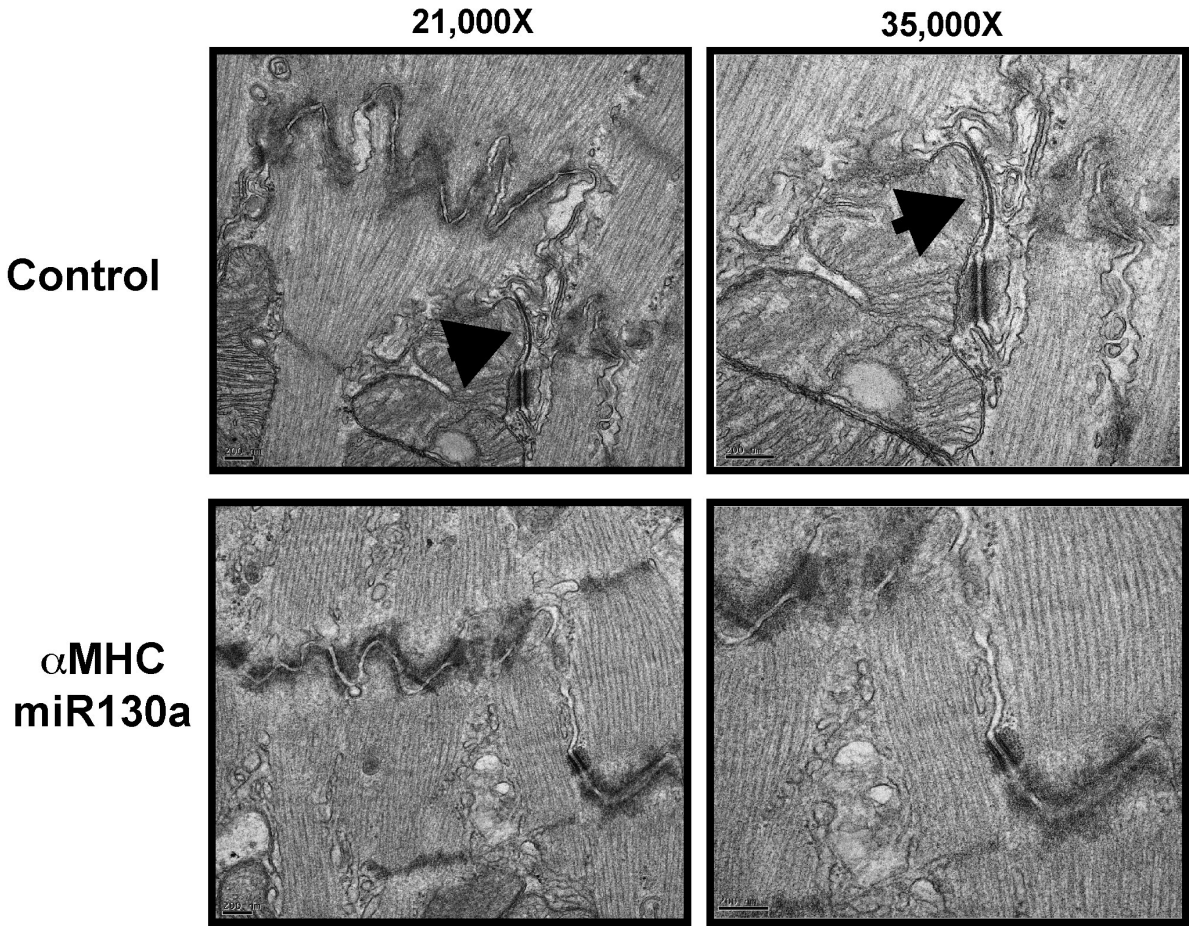

Figure S3. Electron microscopy of the intercalated disc in control and αMHC-miR130a mice 12 weeks off doxycycline.

Control mouse heart shows a gap junction (arrowhead) in close proximity to adherens junctions and desmosomes while a representative intercalated disc in a αMHC-miR130a heart shows no gap junctions. A total of three animals from each group were analyzed.

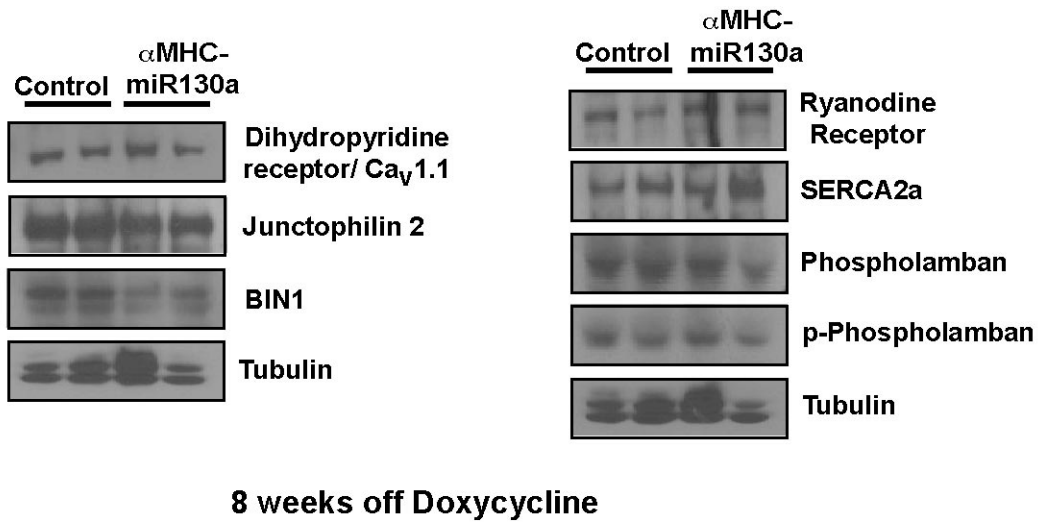

Figure S4. Assessment of proteins important for calcium homeostasis in control and αMHC-miR130a mice.

Western analysis was performed at the 8-week time point in control mice and αMHC-miR130a mice when ventricular arrhythmias were evident without changes in LV function. We performed western analysis on several proteins known to regulate calcium homeostasis including: Cav1.1, BIN1, junctophilin 2 (Left panel) and Ryanodine receptor, Serca2a, phospholamban and phosphorylated-phospholamban (Right panel). Tubulin was used as loading controls. Four animals were studied in each group.