SUPPLEMENTAL MATERIAL

METHODS

ROS Production Assay.

ROS measurement was performed as previous described.[1] Briefly, myocytes were loaded with 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H2DCFDA, Invitrogen). Cells loaded with CM-H2DCFDA were excited at 488 nm using an Argon laser, and emitted fluorescence signals were collected above 500 nm. A time series of 160 cell fluorescence images with 2s interval was recorded for each cell. The laser power was minimized to reduce laser light induced production of ROS.

Western Blotting. Flash frozen mouse ventricles were prepared as previously described.[2] Briefly, mouse ventricles were homogenized in a modified radio-immunoprecipitation buffer containing 10mM Tris and 150mM NaCl at pH 7.4, supplemented with 1% CHAPS, phosphatase and protease inhibitor tablets (Roche), 20mM NaF, and 1mM Na_3VO_4. Samples were sonicated and lysates were clarified by centrifugation. Samples were subjected to electrophoresis on 6%-12% acrylamide gels and electro-transferred onto polyvinylidene difluoride (PVDF) membranes. The following primary antibodies were used to assess protein levels—CaMKII delta (Abcam 181052, 1:1000), oxidized M281/M282-CaMKII (custom antibody, provided by Mark Anderson, 1:1000), GAPDH (MAB-374, Millipore, 1:10,000). Fluorescent secondary antibodies against mouse (Alexa Fluor 680, Invitrogen, 1:10,000) and rabbit (IRDye800, Rockland, 1:10,000) were used in conjunction with the Licor Odyssey imaging system to visualize proteins.

CaMKII Activity Assay. CaMKII activity was assessed as previously described.[3] Heart lysates were made by homogenizing flash-frozen mouse ventricular tissue in 50mM Tris pH 7.5, 100mM NaCl, and 0.1% Triton-X supplemented with Phosstop and cOmplete Mini (containing EDTA)

protease inhibitor tablets (Roche), 20mM NaF, and 1mM Na_3VO_4. Lysates were cleared by centrifugation at 16,000g. CaMKII activity was assessed using the SignaTECT CaMKII activity assay according to the manufacturer's instructions. Briefly, heart lysates were diluted 1:10 in CaMKII dilution buffer containing 0.1mg/ml BSA. The kinase reaction was started by incubating 5ul diluted lysate for 2 minutes at 30° C in buffer containing 0.125mM ATP spiked with [γ-32P]-3000Ci/mmol 10mCi/ml-labeled ATP (Perkin Elmer) in either activation buffer containing Ca^{2+}/CaM and CaMKII synthetic biotinylated substrate, control buffer containing EGTA and CaMKII substrate, or activation buffer with no substrate. The reaction was stopped using termination buffer containing 7.5M guanidine hydrochloride. The reaction was then blotted on SAM^2 biotin capture membranes and washed with 2M NaCl, followed by 2M NaCl and 1% H_3PO_4, and deionized water. The amount of radio-labeled ATP was assessed by spotting 5ul reaction onto the membrane without washing. Counts per minute of ^{32}P were assessed by scintillation counting using the LS6500 Scintillation System (Beckman). Total CaMKII activity was determined by subtracting the activity of the no-substrate control from Ca^{2+}/CaM-activated samples. Ca^{2+}/CaM-independent CaMKII activity was obtained by subtracting the activity of the no-substrate control from that of the samples lacking Ca^{2+}/CaM.

Optical mapping. Mice were anesthetized in an induction chamber with 2% isoflurane mixed with 1.0 L/min 100% O_2 for 15-20mins. 100 units of heparin were injected intraperitoneally. After cervical dislocation, a mid-sternal incision was made and heart was quickly removed and washed in oxygenated (95% O_2, 5% CO_2), cold Tyrode's Solution (128.2mM NaCl, 1.3mM $CaCl_2$, 4.7mM KCl, 1.05mM $MgCl_2$, 1.19mM NaH_2PO_4, 20mM $NaHCO_3$, 11.1mM D-Glucose in deionized water, pH=7.35±0.05). Under a microscope, aorta was dissected rapidly and cannulated to a custom made 21-gauge cannula with a flared tip. 6-0 black-braided silk sutures were used to fix the heart onto the cannula. After cannulation, the heart was retrogradely

perfused with Tyrode's solution, which was heated at 37 Celsius. The perfusion rate was maintained in the range of 2-5 mL/min to keep the aortic pressure between 80 and 120 mmHg. An electrode (Harvard Apparatus, MA, USA) was placed on the surface of right atria to conduct the pacing stimulations, which was generated by PowerLab 26T (AD Instruments, Sydney, Australia). Hearts were loaded with the Blebbistatin (Sigma-Aldrich, B0560-5mg, 50ul of 2.5mg/ml in DMSO) to eliminate motion artifact and stained with voltage sensitive dye RH237 (Invitrogen, S-1109, 20ul of 2.5mg/ml in DMSO). A LED light was used as the light source (excitation wavelength: 530nm). The emitted fluorescence V_m signal was long passed (>700nm, Andover Corporation Optical Filter) and obtained through the MiCAM CMOS camera (SciMedia, CA, USA). Surface ECG (ADInstruments) was recorded via LabChart during experiment. The hearts was paced at 10Hz S1 driving train for 9 pacing spikes followed by the cycle length of a single extra stimulus S2 ranging from 70ms to 40ms, decreasing by 2ms every time. (S1 10Hz S2 70~50ms)

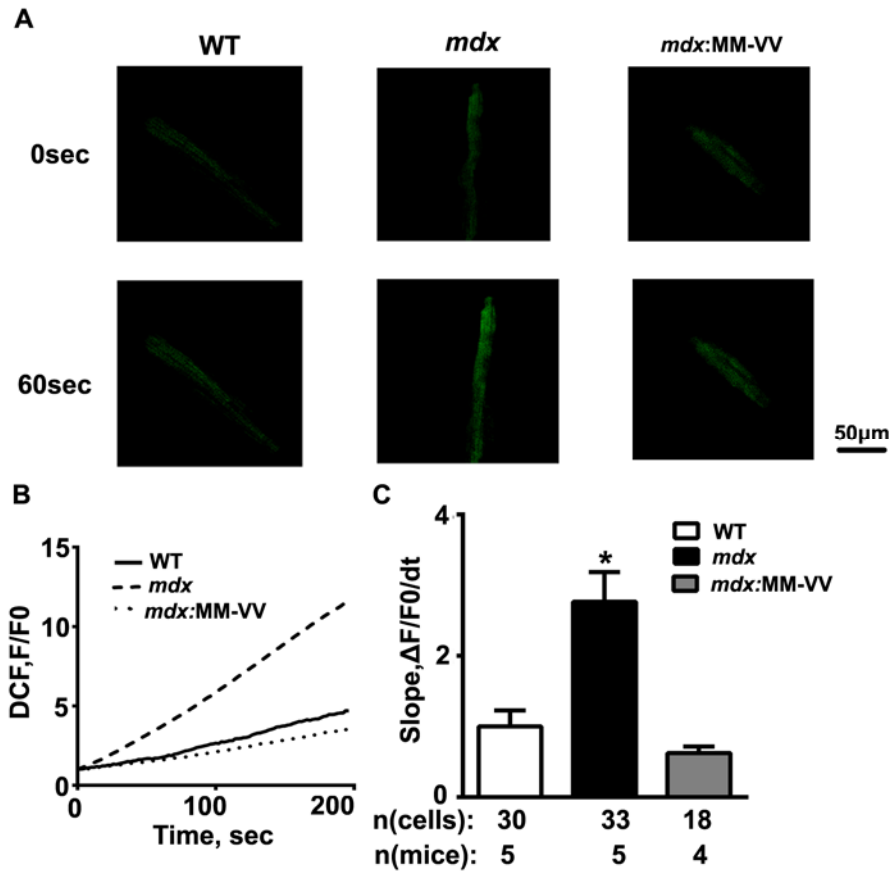
SUPPLEMENTAL REFERENCES

1. Wang Q, Wang W, Wang G, Rodney GG, Wehrens XH. Crosstalk between RyR2 oxidation and phosphorylation contributes to cardiac dysfunction in mice with Duchenne muscular dystrophy. *J Mol Cell Cardiol*. 2015;89:177-84.
2. Reynolds JO, Quick AP, Wang Q, Beavers DL, Philippen LE, Showell J, Barreto-Torres G, Thuerauf DJ, Doroudgar S, Glembotski CC, Wehrens XH. Junctophilin-2 gene therapy rescues heart failure by normalizing RyR2-mediated Ca2+ release. *Int J Cardiol*. 2016;225:371-380.
3. Guilbert A, Lim HJ, Cheng J, Wang Y. CaMKII-dependent myofilament Ca2+ desensitization contributes to the frequency-dependent acceleration of relaxation. *Cell Calcium*. 2015;58:489-99.

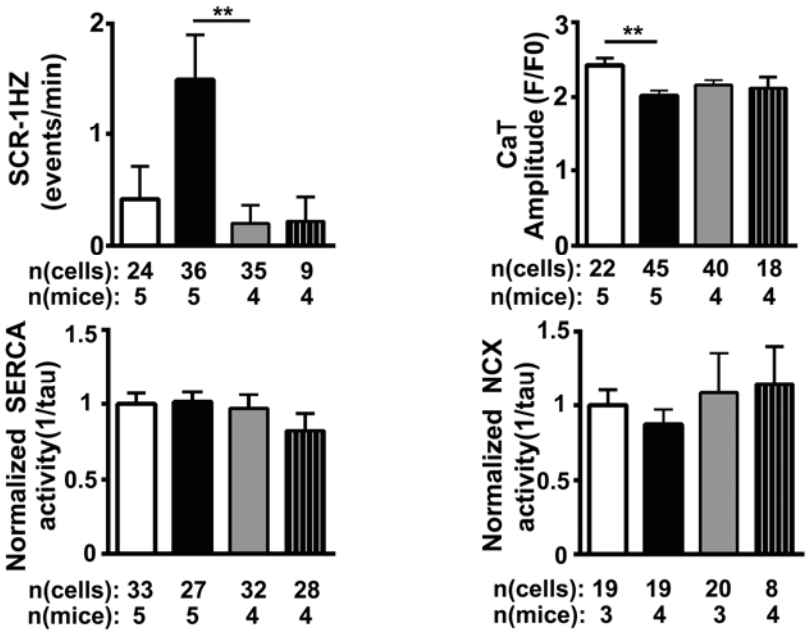
Supplemental Table 1. Unaltered cardiac performance in *mdx* mice at 3 months

Parameter (unit)	**WT (n=6)**	***mdx* (n=6)**	***mdx*:MM-VV (n=4)**
Heart rate (bpm)	478.2 ± 7.5	510.6 ± 19.9	523.2 ± 28.7
Temperature (°C)	36.8 ± 0.25	36.9 ± 0.09	36.2 ± 0.45
Ejection fraction (%)	60.6 ± 6.02	69.8 ± 4.04	68.2 ± 2.54
Fractional shortening (%)	32.6 ± 4.26	39.1 ± 2.92	37.5 ± 2.45
End-systolic diameter (mm)	2.76 ± 0.31	2.33 ± 0.22	2.29 ± 0.25
End-diastolic diameter (mm)	3.93 ± 0.22	3.77 ± 0.17	3.63 ± 0.26

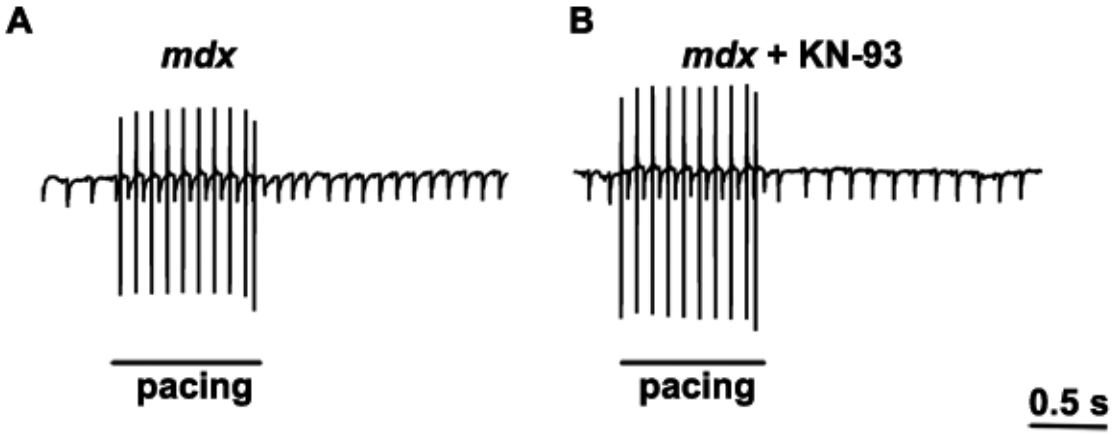
LV function assessment using echocardiography. Data are expressed as mean ± SEM. Bpm = beats per minute.



Supplemental Figure 1. (A) Representative DCF fluorescence images of ROS and **(B)** representative DCF fluorescence intensity tracings in ventricular myocytes isolated from *mdx* mice, WT littermates, and *mdx*:MM-VV mice **(C)** Quantification of the time course slope of fluorescence intensity tracings. *P<0.05 mdx vs. WT, and *mdx* vs. *mdx*:MM-VV.



Supplemental Figure 2. Increased spontaneous calcium waves following 1-Hz pacing in *mdx* mice normalized by inhibition of CaMKII oxidation. (A) Quantification data of spontaneous calcium releases (SCR) also known as Ca^{2+} waves following 1-Hz pacing in WT, *mdx, mdx*:MM-VV, and KN-93-treated *mdx* mice. **(B)** Quantification data of Ca^{2+} transient (CaT) amplitude in WT, *mdx, mdx*:MM-VV, and KN-93-treated *mdx* mice. **(C-D)** Quantification of normalized **(C)** SERCA and **(D)** Na^{+}/Ca^{2+} exchanger (NCX) activity in WT, *mdx mdx*:MM-VV, and, KN-93-treated *mdx* mice. **P<0.01.



Supplemental Figure 3. CaMKII inhibitor KN-93 alleviates ventricular tachycardia in *mdx* mice after pacing. (A) Representative ventricular tachycardia (VT) ECG traces after pacing in *mdx* mice, **(B)** Representative Sinus Rhythm ECG traces after pacing in *mdx* mice with KN-93 treatment.