Supplementary Figure legends

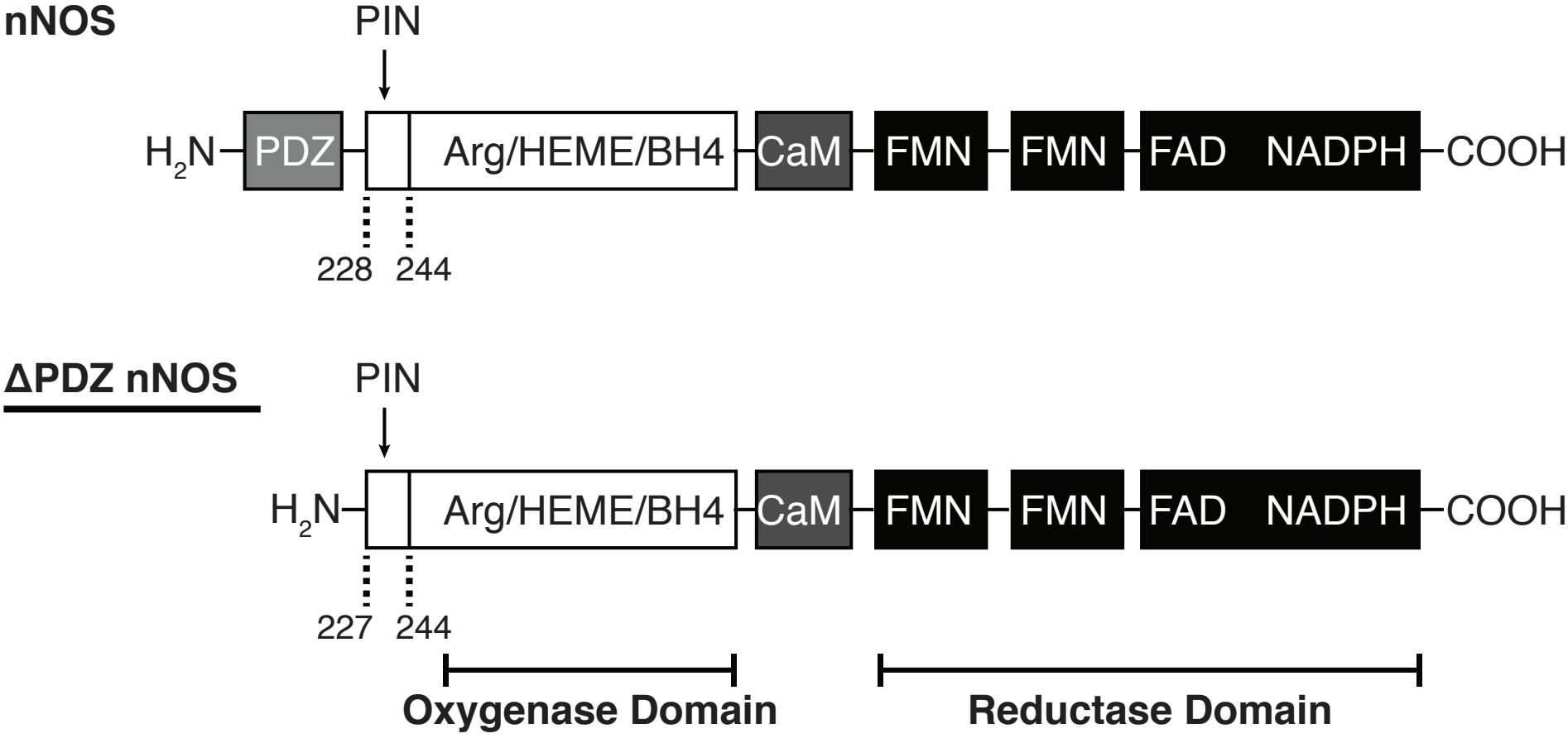
Fig S1. Schematic outline of nNOS, ΔPDZ nNOS and AAV.ΔPDZ nNOS structure. A, Functional domains of full-length nNOS and ΔPDZ nNOS. The PDZ domain is located at the N-terminus of nNOS and it comprises the region of residues 1-226. The region of residues 228-244 is the domain for the protein inhibitor of NOS (PIN). The oxygenase domain binds heme and tetrohydrobiopterin (BH4) while the reductase domain binds flavin adenine mononucleotide (FMN), flavin adenine dinucleotide (FAD), and NADPH. The calmodulin-binding site (CaM) connects the oxygenase domain to the reductase domain. In ΔPDZ nNOS, residues 1-226 were deleted. **B,** Configuration of AAV.ΔPDZ nNOS. The Flag tag is fused to the N-terminus of ΔPDZ nNOS. The expression of Flag-ΔPDZ nNOS is driven by the muscle specific SPc5-12 promoter. The size of the vector genome is about 4.6 kb.

Fig S2. Homogenous cytosolic expression of ΔPDZ nNOS in the cardiomyocytes. Serial sections of AAV.ΔPDZ nNOS-treated mdx heart were examined by double immunofluorescence staining. The panels are (from top to bottom) nNOS and Flag, nNOS and α-actinin, nNOS and phospholamban, and nNOS and cytochrome C. The images from the same row were obtained from the same field but taken with different emission light to view Alex Fluro 488 (nNOS), Fluro 594 (Flag, α-actinin, phospholamban or cytochrome C) and DAPI staining. PLN, phospholamban; Cyt C, cytochrome C. Arrows indicate the same cardiomyocyte in serial sections (scale bar: 50 μm).

Fig S3. Immunoblot analysis of selected calcium regulating proteins. Representative immunoblot results for SERCA2a, RyR2, Calsequestrin and Na^+/K^+ ATPase. GAPDH serves as the loading control.

Fig. S1

A


nNOS
PIN
H2N
PDZ
Arg/HEME/BH4
CaM
FMN
FMN
FAD
NADPH
COOH
228
244
ΔPDZ nNOS
PIN
H2N
Arg/HEME/BH4
CaM
FMN
FMN
FAD
NADPH
COOH
227
244
Oxygenase Domain
Reductase Domain

B

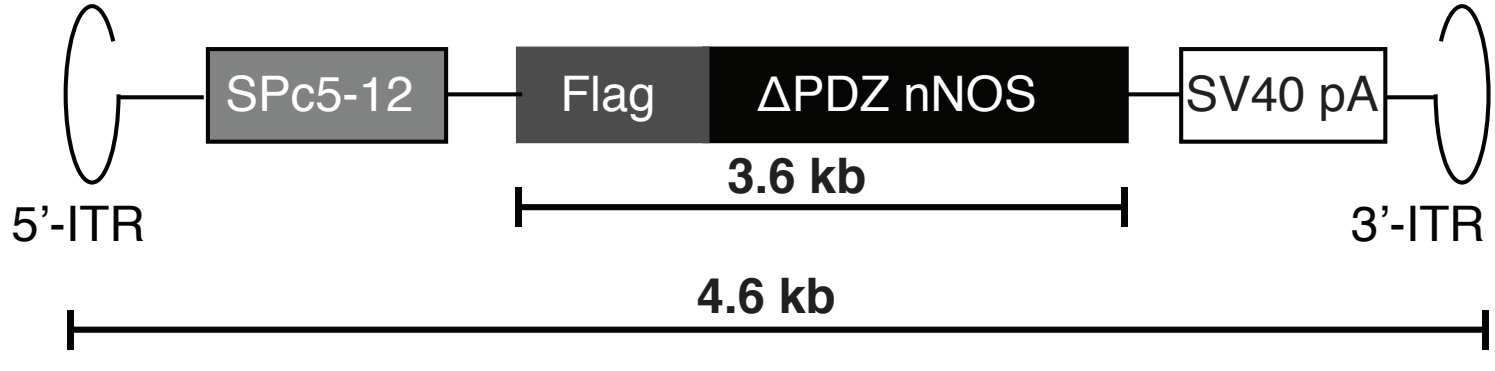

AAV.ΔPDZ nNOS
SPc5-12
Flag
ΔPDZ nNOS
SV40 pA
3.6 kb
5'-ITR
3'-ITR
4.6 kb

Fig. S2

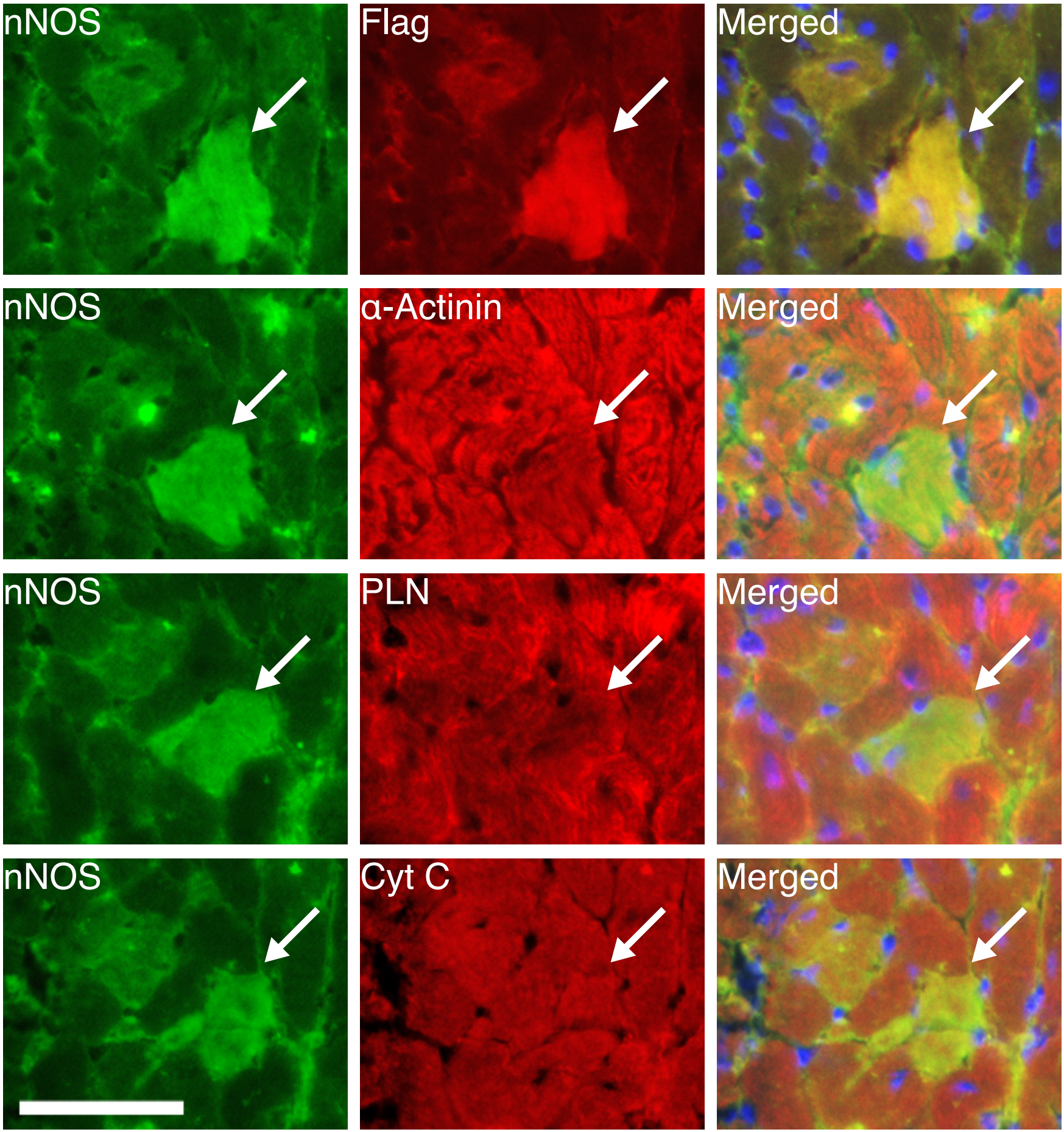

nNOS
Flag
Merged
nNOS
α-Actinin
Merged
nNOS
PLN
Merged
nNOS
Cyt C
Merged

Fig. S3

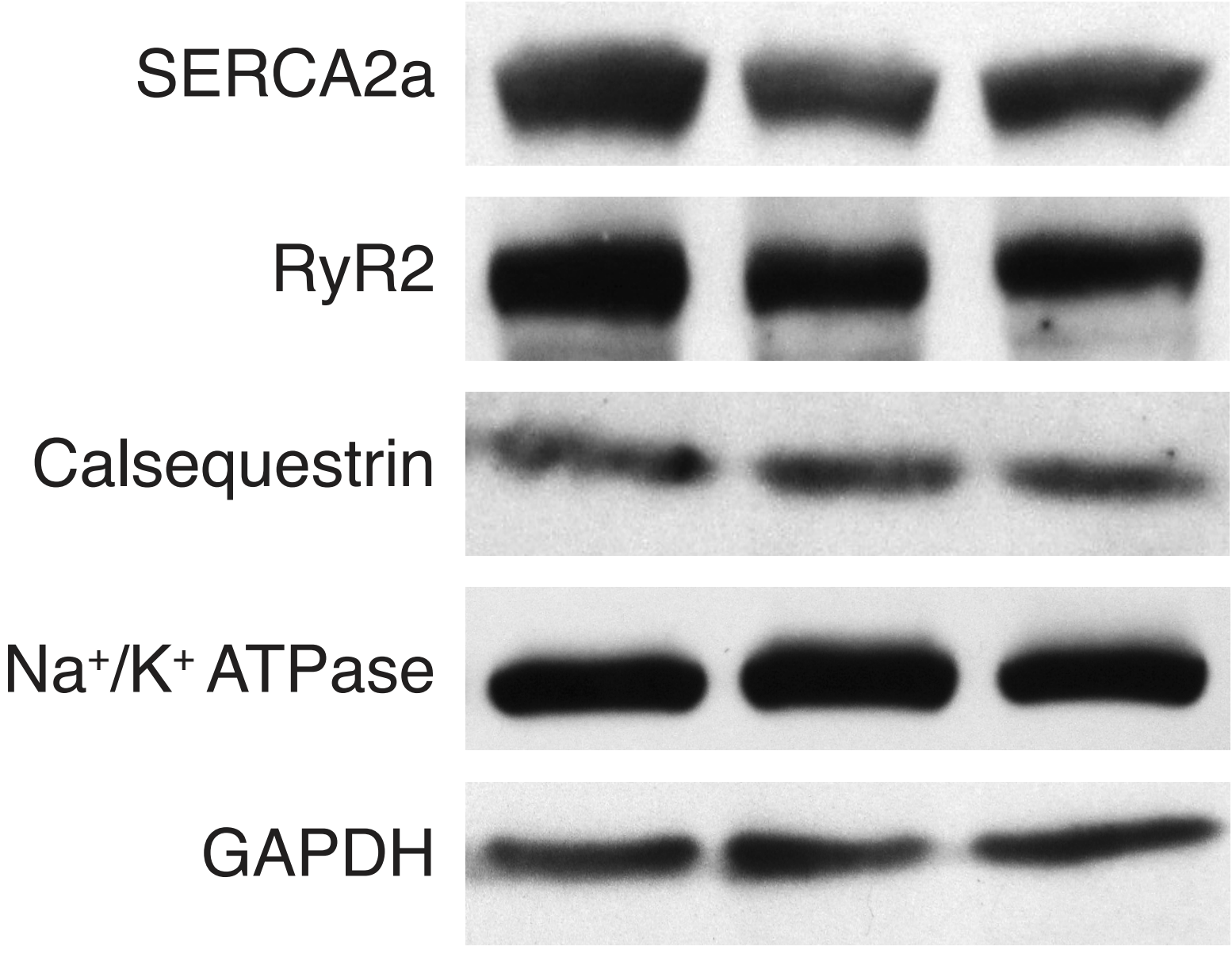


Table S1. ECG data

	BL10 (n=11)	Mdx (n=12)	Mdx.AAV (n=9)	P Value
Age (months)	22±0.7	22±0.5	20.1±0.8	0.1272
HR (BPM)	616.3±7.3	654.6±7.5	592.8±13.04 *	0.0002
PR (ms)	40.22±0.92	33±0.52	38.39±1.11 *	0.0003
QRS (ms)	7.66±0.11	9.28±0.18	9.11±1.03	0.9729
QTc (ms)	21.46±0.39	29.76±1.18	20.54±1.2 *	<0.0001
Q Amp (μV)	-16.24±-3.42	-163.03±-28	-191.2±-64.51	0.8518
C. Index	0.79±0.049	1.63±0.15	0.68±0.044 *	<0.0001

HR, heart rate; BPM, beat per min; QTc, heart rate-corrected QT interval; Q Amp, Q wave amplitude; C. Index, cardiomyopathy index. Asterisk, significantly different from that of untreated mdx group.

Table S2. Results of left ventricular hemodynamic assay.

	BL10 (n=16)	Mdx (n=13)	Mdx.AAV (n=5)	P Value
Age (months)	22±0.7	22±0.5	20.2±1.32	0.3653
ESV (µl)	8.02±0.97	23.43±1.54	20.12±6.4	0.6251
Max Pressure (mmHg)	98.17±1.69	59.04±5.57	71.44±9.29	0.2864
dP/dt Max (kmmHg/sec)	11.4±0.55	4.91±0.64	9.6±1.59 *	0.0027
EDSV (µl)	24.73±1.04	31.16±1.63	33.76±4.24	0.6715
Tau (ms)	7.69±0.45	11.18±1.73	7.67±0.93	0.2568
dP/dt Min (kmmHg/sec)	-10.28±-0.32	-4.5±-0.64	-8.02±-1.43 *	0.007
Ejection Fraction (%)	72.54±2.45	34.65±2.15	51.24±12.04 *	0.0493
Stroke Volume (µl)	18.85±0.72	11.15±0.72	17.28±3.52 *	0.0126
Cardiac Output (ml/min)	10.91±0.51	6.44±0.43	10.72±1.98 *	0.0042

ESV, End systolic volume; EDSV, End diastolic volume. Asterisk, significantly different from that of the untreated mdx group.

Table S3. Antibodies used in immunoblot.

Antigen	Host	Catalog #	Company	Dilution
Akt	Rabbit	9272	Cell Signaling	1:500
α-Tubulin	Mouse	T5168	Sigma	1:3000
Calsequestrin	Rabbit	PA1-913	Thermo	1:1000
Dys-CT	Mouse	NCL-DYS2	Novocastra	1:150
eNOS	Mouse	610296	BD Biosciences	1:500
Flag	Mouse	F1804	Sigma	1:500
GAPDH	Mouse	MAB374	Millipore	1:5000
iNOS	Rabbit	610332	BD Biosciences	1:1000
mTOR	Rabbit	2983	Cell Signaling	1:500
Na+/K+ATPase	Mouse	ab7671	Abcam	1:10000
nNOS-CT	Rabbit	sc-648	Santa Cruz	1:100
nNOS-NT	Rabbit	07-571	Upstate	1:200
p38 α	Rabbit	sc-535	Santa Cruz	1:1000
p70S6K	Rabbit	2708	Cell Signaling	1:500
P-Akt (Ser473)	Rabbit	9271	Cell Signaling	1:1000
P-Akt (Thr308)	Rabbit	2965	Cell Signaling	1:1000

PLN	Mouse	A010-14	Badrilla	1:1000
P-mTOR (Ser2448)	Rabbit	2971	Cell Signaling	1:500
P-p38 (Thr180/Thr182)	Rabbit	9211	Cell signaling	1:200
P-p70S6K (Thr389)	Rabbit	9234	Cell Signaling	1:1000
P-PLN (Ser16)	Rabbit	A010-12	Badrilla	1:2500
RyR2	Mouse	MA3-925	Affinity Bioreagents	1:200
SERCA2a	Rabbit	A010-20	Badrilla	1:5000
Utrophin	Mouse	610896	BD Biosciences	1:200

CT, C terminus; NT, N terminus; Dys, dystrophin; PLN, phospholamban; P, phosphorylated; RyR, ryanodine receptor.