

SUPPLEMENTAL MATERIALS

SUPPLEMENTAL TABLES

	D2.WT	D2.mdx	MDC1	MDC2	MDC3	MDC4	
LV Structure and Function	HR (BPM)	442.73±12.55	405.16±11.12	418.11±15.39	423.23±12.55	396.57±14.09	422.44±14.46
	EDD (mm)	3.44±0.11	3.18±0.15 ^{&}	3.04±0.14 ^{&}	3.46±0.04	3.83±0.09	3.79±0.08
	ESD (mm)	1.68±0.15	1.54±0.14	1.61±0.10	1.68±0.06	2.34±0.15 ^{*#%\$}	2.04±0.13
	EDV (μl)	49.90±3.78	42.00±4.21 ^{&}	37.43±4.27 ^{&}	49.67±1.49	63.61±3.57	61.97±3.03
	ESV (μl)	9.77±2.10	7.66±1.47	7.80±1.20	8.40±0.80	20.10±3.07	14.29±2.13
	EF (%)	82.48±2.70	83.46±2.23	79.25±2.06	83.07±1.58	69.39±3.79 ^{*#}	77.10±3.31
	FS (%)	52.02±3.04	52.46±2.75	47.00±2.08	51.45±1.78	39.26±3.06 ^{*#}	46.25±3.27
	SV (μl)	40.13±2.07	34.34±3.00 ^{&}	29.62±3.39 ^{*\$&}	41.27±1.47	43.51±2.36	47.68±2.92
	CO (ml/min)	17.78±1.05	13.78±1.10	12.04±1.10 ^{*\$&}	17.52±0.90	16.57±1.60	19.95±0.94 [#]
	LV Mass (mg)	183.39±13.995	145.95±13.46	142.53±9.73	146.44±7.03	137.74±10.67	215.65±14.30 ^{#%\$&}
	SAW (mm)	2.10±0.10	1.94±0.13	1.98±0.09	2.06±0.10	1.66±0.08 [*]	2.03±0.09
	DAW (mm)	1.37±0.07	1.30±0.12	1.46±0.10	1.34±0.05	1.15±0.08	1.30±0.07
	SPW (mm)	2.03±0.12	1.87±0.12	1.84±0.07	1.70±0.08	1.49±0.09 ^{*&}	2.13±0.07 ^{\$}
	DPW (mm)	1.54±0.11	1.39±0.15	1.31±0.08	1.18±0.06	1.03±0.07 ^{*&}	1.67±0.11 ^{\$}
	Mitral Inflow	NFT (ms)	73.41±2.01	77.79±2.99	76.90±1.83	67.79±1.47	79.33±2.73
AET (ms)		47.36±1.21	45.70±2.13	47.91±0.80	44.89±0.80	49.88±1.10	45.06±1.60
IVCT (ms)		8.67±0.75	12.59±1.23 [*]	11.80±0.87	9.59±0.71	10.71±0.71	10.63±1.10
IVRT (ms)		17.78±1.28	20.73±1.45	17.51±1.12	14.27±0.82 ^{#&}	19.47±1.17	18.58±0.69
MV E (mm/s)		700.82±23.34	582.98±49.51	789.13±35.81 ^{#&}	772.18±10.48 ^{#&}	715.89±24.87	556.78±44.33
MV A (mm/s)		566.15±20.50	423.34±28.36	240.80±80.93 ^{*#%\$&}	554.40±9.08	506.99±18.76	434.33±29.96
MV E/A		1.25±0.03	1.37±0.07	1.50±0.13	1.40±0.04	1.43±0.08	1.28±0.04
LV MPI IV		0.60±0.05	0.75±0.05 ^{&}	0.61±0.03	0.53±0.03	0.60±0.03	0.65±0.03

Supplemental Table 1: Cardiac function at 6 months of age. HR-heart rate; EDD-end diastolic diameter; ESD-end diastolic diameter; EDV-end diastolic volume; EF-ejection fraction; FS-fractional shortening; SV-stroke volume; CO-cardiac output; SAW-systolic anterior wall; DAW-diastolic anterior wall; SPW-systolic posterior wall; DPW-diastolic posterior wall; NFT-non-filling time; AET-aortic ejection time; IVCT-isovolumic contraction time; IVRT-isovolumic relaxation time; MV E-mitral valve early wave; MV A-mitral valve atrial wave; MPI-myocardial performance index (IVCT + IVRT)/AET; *p<0.05 compared to WT; #p<0.05 compared to untreated D2.mdx; %p<0.05 compared to MDC1; \$p<0.05 compared to MDC2; &p<0.05 compared to MDC3; @p<0.05 compared to MDC4.

	D2.WT	D2.mdx	MDC1	MDC2	MDC3	MDC4		
LV Structure and Function	HR (BPM)	418.83±12.19	405.42±8.87	434.29±18.48	422.19±6.54	417.31±16.89	394.86±15.10	
	EDD (mm)	3.95±0.11	3.16±0.13*	4.10±0.16 [#] \$	3.31±0.13*	3.75±0.10	4.26±0.22 [#] \$	
	ESD (mm)	2.19±0.12	1.41±0.13*	3.04±0.21 [#] \$&	1.61±0.16	2.00±0.20	3.33±0.40 ^{*#} \$&	
	EDV (μl)	69.06±4.2	41.49±3.96*	75.19±6.83 [#] \$	45.25±4.30	60.57±3.84	83.66±10.37 [#] \$	
	ESV (μl)	17.23±2.18	6.49±1.35	37.58±5.98 [#]	8.26±1.74	14.44±3.32	53.37±14.78 ^{*#} \$&	
	EF (%)	76.26±1.89	86.31±2.21	51.44±4.44 ^{*#} \$&	83.18±2.57	77.81±4.06	44.69±9.36 ^{*#} \$&	
	FS (%)	44.99±1.72	56.68±2.88*	26.28±2.75 ^{*#} \$&	52.17±3.41	47.28±4.04	23.80±5.35 ^{*#} \$&	
	SV (μl)	51.83±2.43	35.00±2.96*	37.61±2.56	37.00±2.77*	46.13±1.34	30.29±5.22 ^{*&}	
	CO (ml/min)	21.47±0.88	14.15±1.19*	16.29±1.26	15.60±1.20*	19.16±0.63	12.10±2.26 ^{*&}	
	LV Mass (mg)	173.51±8.35	140.60±13.18	190.54±11.43	138.49±8.21	165.81±11.18	216.60±16.13 [#] \$	
	SAW (mm)	1.99±0.07	2.01±0.09	1.80±0.06	2.07±0.10	1.94±0.05	1.78±0.12	
	DAW (mm)	1.29±0.05	1.28±0.05	1.32±0.05	1.39±0.09	1.39±0.03	1.40±0.50	
	SPW (mm)	1.73±0.05	1.87±0.10	1.52±0.07	1.67±0.06	1.80±0.16	1.54±0.15	
	DPW (mm)	1.20±0.04	1.33±0.10	1.22±0.08	1.15±0.06	1.15±0.09	1.28±0.10	
	Mitral Inflow	NFT (ms)	73.50±2.27	73.60±1.77	74.03±3.00	69.51±1.05	70.46±2.72	77.84±2.66
		AET (ms)	47.25±1.11	43.24±1.12	45.83±2.01	44.57±1.55	42.78±1.63	45.47±1.97
IVCT (ms)		10.17±0.99	11.10±0.74	9.44±0.22	8.35±0.66	8.86±0.87	8.76±1.80	
IVRT (ms)		16.16±0.71	20.30±1.00*	15.97±1.32	16.48±1.18	19.02±0.91	24.43±1.52 ^{*#} \$&@	
MV E (mm/s)		723.97±16.54	590.88±25.41 ^{*%}	773.06±90.47	649.64±27.95	705.68±30.56	559.36±20.02 ^{*%&}	
MV A (mm/s)		518.86±18.18	501.89±21.49	206.46±45.72 ^{*#} \$&@	523.31±24.63	512.44±28.29	502.69±25.70	
MV E/A		1.42±0.05	1.19±0.05	4.83±1.17 ^{*#} \$&@	1.26±0.08	1.39±0.06	1.14±0.05	
LV MPI IV		0.56±0.003	0.73±0.04 ^{*\$}	0.56±0.01	0.57±0.04	0.65±0.03	0.69±0.08	

Supplemental Table 2: Cardiac function at 12 months of age. HR-heart rate; EDD-end diastolic diameter; ESD-end diastolic diameter; EDV-end diastolic volume; EF-ejection fraction; FS-fractional shortening; SV-stroke volume; CO-cardiac output; SAW-systolic anterior wall; DAW-diastolic anterior wall; SPW-systolic posterior wall; DPW-diastolic posterior wall; NFT-non-filling time; AET-aortic ejection time; IVCT-isovolumic contraction time; IVRT-isovolumic relaxation time; MV E-mitral valve early wave; MV A-mitral valve atrial wave; MPI-myocardial performance index (IVCT + IVRT)/AET; *p<0.05 compared to WT; #p<0.05 compared to untreated D2.mdx; %p<0.05 compared to MDC1; \$p<0.05 compared to MDC2; &p<0.05 compared to MDC3; @p<0.05 compared to MDC4.

	D2.WT	D2.mdx	MDC2	MDC3	
LV Structure and Function	HR (BPM)	454.98±10.32	398.53±13.74*	416.89±10.37	423.58±13.48
	EDD (mm)	3.41±0.13	2.76±0.14* ^{&}	4.09±0.14* ^{#&}	3.54±0.14
	ESD (mm)	1.76±0.11	1.09±0.16* ^{&}	2.89±0.23* ^{#&}	1.77±0.16
	EDV (μl)	48.58±4.22	29.38±3.51* ^{&}	74.54±5.86* ^{#&}	53.11±4.67
	ESV (μl)	9.78±1.69	3.44±0.95	34.05±6.23* ^{#&}	10.27±2.28
	EF (%)	80.52±2.04	89.67±2.55	56.53±5.33* ^{#&}	82.00±2.67
	FS (%)	48.51±1.94	61.63±4.66*	29.96±3.61* ^{#&}	50.62±2.76
	SV (μl)	38.80±3.02	25.94±2.86* ^{\$&}	40.49±1.89	42.83±2.90
	CO (ml/min)	17.77±1.60	10.36±1.24* ^{\$&}	16.81±0.65	18.12±1.38
	LV Mass (mg)	150.68±10.19	119.52±9.72* ^{\$&}	175.81±13.76	159.02±6.82
	SAW (mm)	2.01±0.06	2.09±0.09	1.76±0.10 [#]	1.99±0.05
	DAW (mm)	1.31±0.06	1.36±0.05	1.31±0.08	1.33±0.05
	SPW (mm)	1.75±0.07	1.89±0.14	1.45±0.10 [#]	1.84±0.10
	DPW (mm)	1.29±0.07	1.33±0.11	1.12±0.11	1.29±0.08
	Mitral Inflow	NFT (ms)	70.38±2.33	79.93±3.31	76.24±2.72
AET (ms)		42.67±1.40	42.57±1.71	46.07±1.98	42.57±1.67
IVCT (ms)		9.82±1.72	13.11±1.41	10.92±0.76	8.69±0.99
IVRT (ms)		18.45±1.30	24.82±1.55* ^{\$&}	19.49±0.81	19.28±0.91
MV E (mm/s)		665.22±24.52	523.23±15.74* ^{&}	529.39±32.29	687.43±28.77* ^{&}
MV A (mm/s)		503.36±30.73	504.48±28.00	428.33±29.94 ^{&}	538.38±19.70
MV E/A		1.34±0.07	1.06±0.06*	1.25±0.07	1.28±0.04
LV MPI IV		0.67±0.04	0.90±0.06* ^{\$&}	0.67±0.04	0.67±0.05

Supplemental Table 3: Cardiac function at 18 months of age. HR-heart rate; EDD-end diastolic diameter; ESD-end diastolic diameter; EDV-end diastolic volume; EF-ejection fraction; FS-fractional shortening; SV-stroke volume; CO-cardiac output; SAW-systolic anterior wall; DAW-diastolic anterior wall; SPW-systolic posterior wall; DPW-diastolic posterior wall; NFT-non-filling time; AET-aortic ejection time; IVCT-isovolumic contraction time; IVRT-isovolumic relaxation time; MV E-mitral valve early wave; MV A-mitral valve atrial wave; MPI-myocardial performance index (IVCT + IVRT)/AET; *p<0.05 compared to WT; #p<0.05 compared to untreated D2.mdx; \$p<0.05 compared to MDC2; &p<0.05 compared to MDC3.

	D2.WT	D2.mdx	MDC1	MDC2	MDC3	MDC4
RR Interval (ms)	139.19±4.01	151.59±5.58	146.12±5.93	143.87±5.24	150.86±5.14	143.13±6.07
Heart Rate (BPM)	435.36±11.58	400.44±14.06	416.46±16.02	421.55±13.81	400.64±12.35	425.32±16.23
PR Interval (ms)	36.12±0.95	35.84±0.85	35.59±1.66	34.50±0.69	33.72±0.66	33.71±1.13
P Duration (ms)	7.51±0.37	8.01±0.71	9.03±0.56	7.48±0.47	7.39±0.38	8.49±1.16
QRS Interval (ms)	10.37±0.37	10.30±0.50	11.49±0.23	9.75±0.52	10.73±0.69	10.05±0.19
QT Interval (ms)	16.63±0.47	19.51±0.46	22.68±1.85* ^{§&@}	17.75±0.54	16.24±0.69	16.81±0.81
QTc (ms)	44.77±1.44	50.18±0.74	59.86±5.44* ^{§&@}	46.98±1.66	41.81±1.48	44.67±2.32
JT Interval (ms)	6.27±0.60	9.21±0.51	11.63±1.97* ^{&@}	8.00±0.33	5.51±0.42	6.76±0.78
T _{peak} T _{end} Interval (ms)	3.96±0.40	6.31±0.76	7.33±1.75* ^{&}	5.27±0.35	3.86±0.40	4.64±0.64
P Amplitude (V)	117.02±10.37	104.16±8.59	150.75±12.94	138.51±8.97	122.42±16.02	118.91±11.78
Q Amplitude (V)	13.23±3.50	16.31±2.47	-69.43±36.84	10.68±2.54	-25.73±38.18	3.71±4.53
R Amplitude (V)	510.52±39.85	566.08±50.45	324.59±43.83* ^{§&}	502.82±43.06	575.96±58.94	454.29±29.51
S Amplitude (V)	-245.54±53.24	-412.47±121.80	-301.15±80.81	-212.01±67.75	-134.83±75.48	-365.07±65.86
T Amplitude (V)	214.28±26.84	318.25±35.20	170.69±30.02* [#]	291.91±35.43	210.08±37.58	242.43±34.44

Supplemental Table 4: Electrocardiogram measures at 6 months of age. *p<0.05 compared to WT; #p<0.05 compared to untreated D2.mdx; %p<0.05 compared to MDC1; §p<0.05 compared to MDC2; &p<0.05 compared to MDC3; @p<0.05 compared to MDC4.

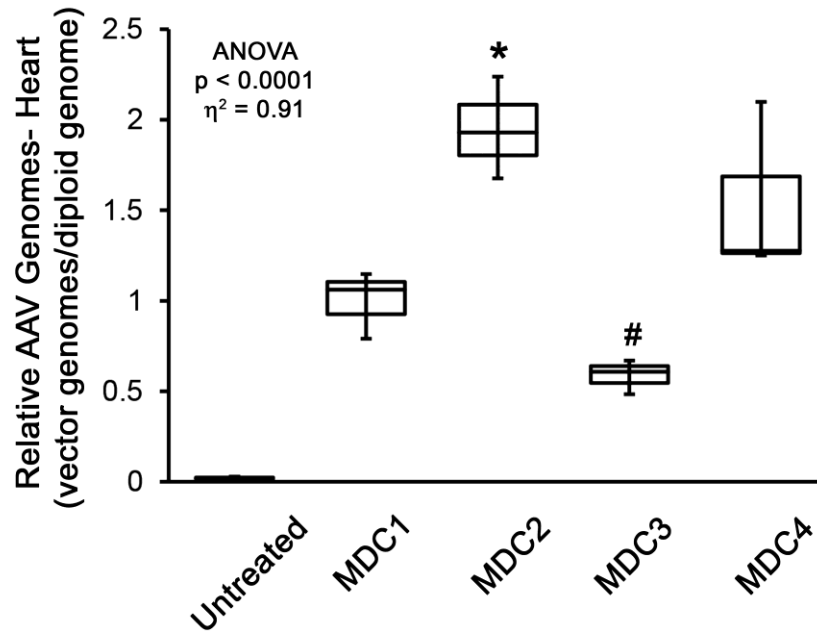
	D2.WT	D2.mdx	MDC1	MDC2	MDC3	MDC4
RR Interval (ms)	139.30±3.99	140.84±3.31	138.53±6.59	148.43±3.52	141.50±5.40	153.38±6.82
Heart Rate (BPM)	432.77±11.83	427.63±10.08	437.28±17.69	405.76±9.34	427.73±16.37	396.16±16.17
PR Interval (ms)	33.10±1.13	34.84±0.62	37.30±1.02	35.68±0.85	32.81±1.42	39.24±3.21
P Duration (ms)	8.08±0.36	7.59±0.63	9.18±0.58	7.14±0.64	7.94±0.40	11.35±1.04* ^{#§&}
QRS Interval (ms)	10.21±0.79	10.24±0.44	11.18±0.22	10.76±0.45	11.66±0.97	12.39±0.88
QT Interval (ms)	16.93±0.46	20.82±0.68*	21.76±0.84*	21.45±0.75*	19.47±0.65	21.45±1.42*
QTc (ms)	45.40±1.00	55.61±2.12*	58.85±3.15*	55.71±1.84*	52.04±2.44	54.81±3.18
JT Interval (ms)	6.72±0.69	10.59±0.65*	10.58±0.73*	10.69±0.56*	7.81±0.49	9.06±0.95
T _{peak} T _{end} Interval (ms)	4.70±0.65	7.06±0.63	8.28±0.76*	7.49±0.88	5.61±0.34	6.54±1.06
P Amplitude (V)	112.74±4.97	103.85±13.64	134.12±10.08	82.09±11.18	108.49±16.63	106.79±14.17
Q Amplitude (V)	15.42±2.84	5.30±8.18	8.37±10.87	-100.42±78.82	-12.67±23.01	7.72±11.28
R Amplitude (V)	496.76±42.21	554.03±36.20	297.97±46.90* [#]	371.15±46.02	492.74±49.77	450.80±52.90
S Amplitude (V)	-61.00±47.21	-551.7875±73.68*	-288.91±106.36	-259.20±60.37	-52.69±95.22*	-293.04±83.45
T Amplitude (V)	228.85±46.46	398.49±36.33* ^{%&@}	133.10±30.08	224.95±41.70	195.89±50.26	113.50±62.78

Supplemental Table 5: Electrocardiogram measures at 12 months of age. *p<0.05 compared to WT; #p<0.05 compared to untreated D2.mdx; %p<0.05 compared to MDC1; §p<0.05 compared to MDC2; &p<0.05 compared to MDC3; @p<0.05 compared to MDC4.

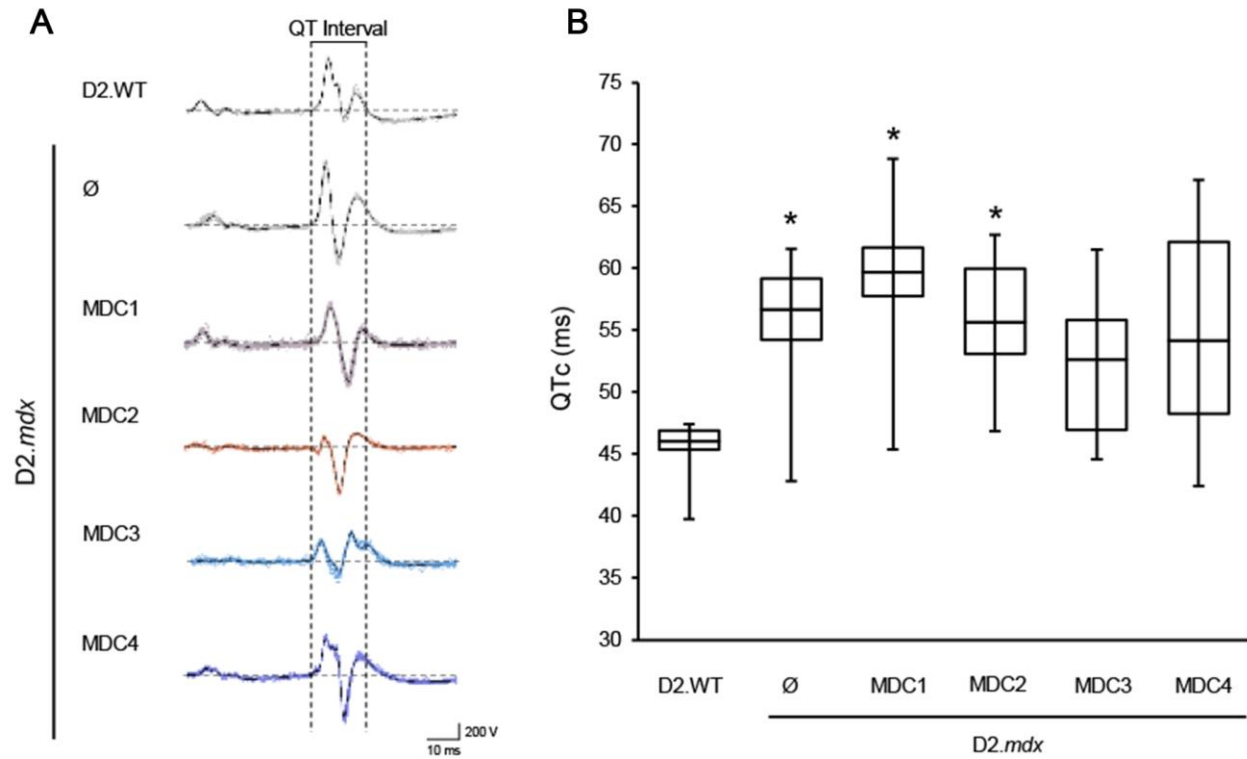
	D2.WT	D2.mdx	MDC2	MDC3
RR Interval (ms)	140.91±4.06	138.92±7.60	144.20±5.91	142.63±3.41
Heart Rate (BPM)	428.46±11.58	441.81±22.76	420.06±16.03	422.28±9.92
PR Interval (ms)	37.03±3.37	34.89±0.98	35.53±1.57	34.13±1.67
P Duration (ms)	8.27±0.32	8.86±0.58	7.91±1.00	6.23±0.69 [#]
QRS Interval (ms)	10.29±0.52	10.52±0.98	11.98±0.62	15.68±3.03 ^{*#}
QT Interval (ms)	19.11±0.69	23.18±1.88	25.61±1.73 [*]	19.74±0.99
QTc (ms)	50.95±1.70	62.21±4.41	67.81±5.09 ^{*&}	52.10±2.75
JT Interval (ms)	8.82±0.51	12.67±1.48	13.63±1.23 ^{&}	6.64±3.14
T_{peak} T_{end} Interval (ms)	5.78±0.42	9.32±1.45	10.09±1.02	4.75±2.09
P Amplitude (V)	99.03±10.19	117.94±15.84	106.68±8.32	31.67±12.08 ^{*#&}
Q Amplitude (V)	20.98±4.67	2.63±8.47	8.27±5.09	-201.49±111.41 ^{*#&}
R Amplitude (V)	492.38±40.47	418.07±54.15	326.87±46.32	640.82±91.55 ^{\$}
S Amplitude (V)	-121.10±93.52	-202.47±82.74	-224.53±64.17	-237.74±80.22
T Amplitude (V)	241.48±28.50	202.77±29.30	165.76±33.13	294.21±90.80

Supplemental Table 6: Electrocardiogram measures at 18 months of age. *p<0.05 compared to WT; #p<0.05 compared to untreated D2.mdx; \$p<0.05 compared to MDC2; &p<0.05 compared to MDC3.

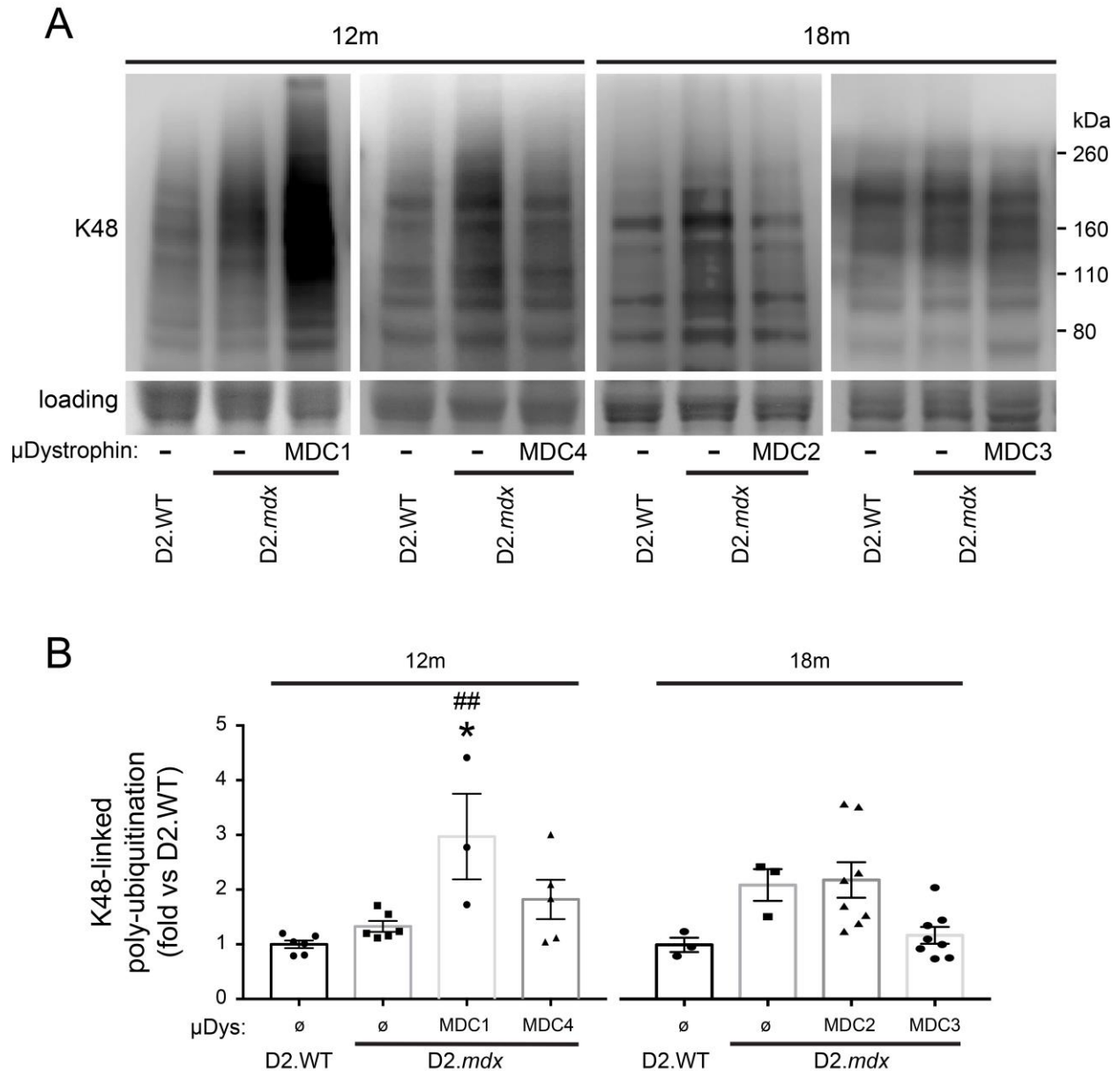
SUPPLEMENTAL FIGURES



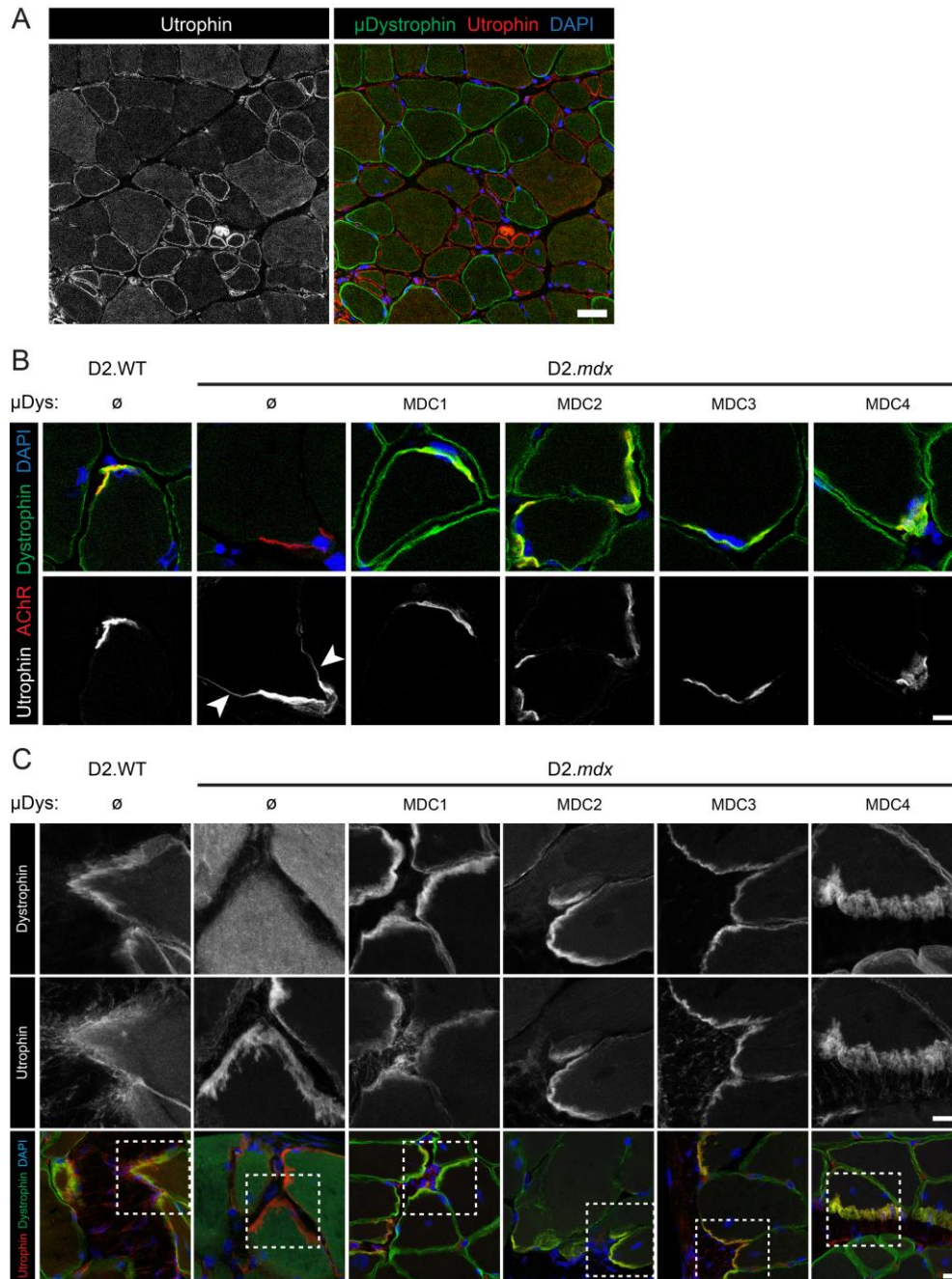
Supplemental Figure 1. AAV vector genomes were quantified in the hearts of untreated D2.*mdx* mice and those treated with 2×10^{14} gc/kg of MDC1, MDC2, MDC3, or MDC4 at 1 month of age using real-time PCR ($n = 3$ for each group). Vector genome content was normalized to murine diploid genomes using the $\Delta\Delta CT$ method and reported relative to MDC1 values. Data were analyzed using one-way ANOVA with Tukey HSD post-hoc tests ($\alpha = 0.05$; effect size reported at η^2 ; * $p < 0.05$ vs. MDC1 values; # $p < 0.05$ vs. MDC2 values) and displayed as box-and-whisker plots (boxes indicate 2nd and 3rd quartiles and error bars represent the minimum and maximum values).



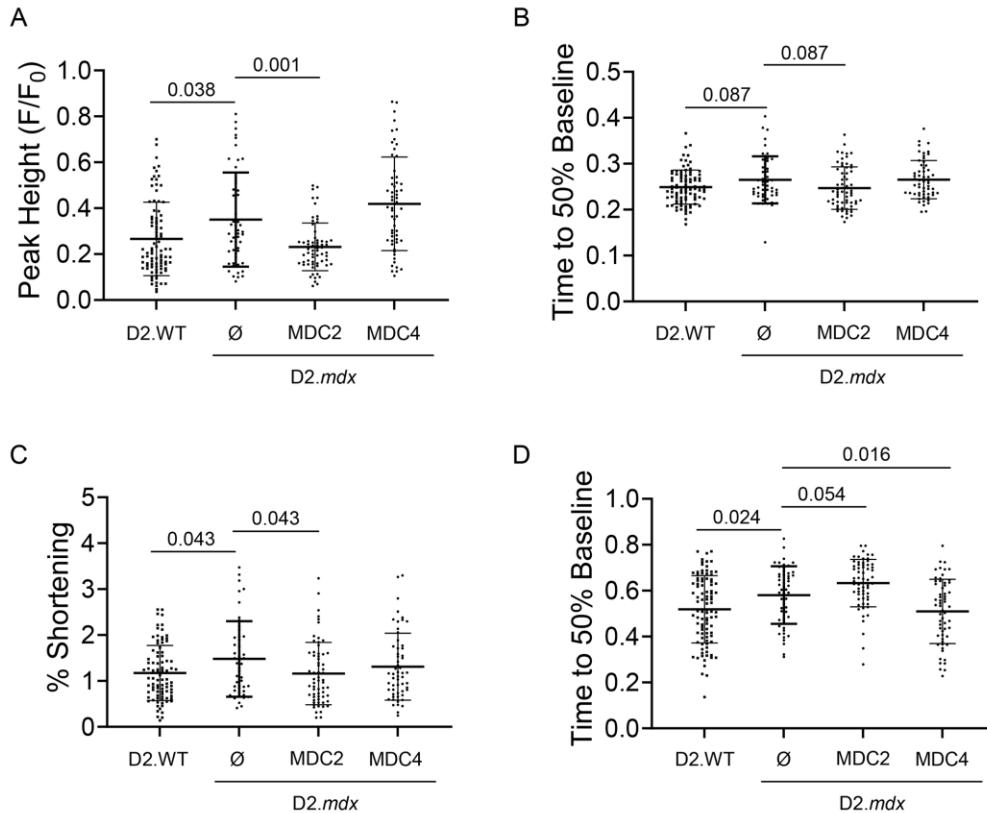
Supplemental Figure 2. Electrocardiogram abnormalities persist with micro-dystrophin treatment. Male *D2.mdx* mice were treated with micro-dystrophin (μ Dys) gene therapy at 1 month of age (mo; refer to **Figure 2A**). **(A)** Representative electrocardiogram tracings from 12-month-old mice reveal prolonged QT intervals in *D2.mdx* mice, even when treated with micro-dystrophin, along with **(B)** QT corrected for heart rate (QTc) quantifications to the right. * $p < 0.05$ vs. age-matched D2.WT values.



Supplemental Figure 3. Impaired protein homeostasis may further contribute to cardiac dysfunction in micro-dystrophin-treated *D2.mdx* hearts. The 5-repeat MDCs examined (MDC2-4) show similar expression in both the muscle, while the 4-repeat MDC1 was expressed at levels several-fold higher in comparison (**Fig 2B**). (**A**) End-point samples of *D2.mdx* hearts treated with micro-dystrophins – MDC1 & MDC4 at 12m and MDC2 & MDC3 at 18m – were analyzed for levels of K48-linked polyubiquitinated proteins compared to hearts of age-matched *D2.WT* and untreated *D2.mdx* mice. (**B**) In hearts of animals treated with micro-dystrophins MDC2 (18m; n=8), MDC3 (12m; n=5) and MDC4 (18m; n=8), no build-up of proteins destined for proteasome-mediated breakdown – evidenced by level of proteins bearing K48-linked polyubiquitin was observed above the levels observed in age matched, untreated *D2.mdx* hearts. A significant (~3-fold) increase in the build-up proteins is observed in lysates of hearts expressing MDC1 (n=3; p<0.01, one-way ANOVA; ## p<0.01 vs *D2.WT*, * p<0.05 vs *D2.mdx*, Tukey post-hoc comparison).



Supplemental Figure 4. Micro-dystrophin overexpression in skeletal muscle fibers displaces utrophin from sarcolemma outside of neuromuscular and myotendinous junctions. (A) Micro-dystrophin and utrophin demonstrate a mutually exclusive pattern of sarcolemmal labeling amongst skeletal muscle fibers in mice treated with MDC4. (B,C) Utrophin distribution is restricted to neuromuscular junctions (NMJs) (B) and (C) myotendinous junctions (MTJs) in myofibers of D2.WT mice. Utrophin labeling extends beyond the postsynaptic membrane (white arrowhead) in D2.mdx skeletal muscle fibers. Utrophin remains concentrated at NMJs (as indicated by co-localization with postsynaptic acetylcholine receptors (AChR, red) or MTJs of muscle fibers overexpressing any of the micro-dystrophins examined. Scale bars: 10 μ m (A-B); 20 μ m (C)



Supplemental Figure 5. MDC2 and MDC4 exert differential impacts on Ca^{2+} transients and contractility in dystrophic cardiomyocytes. Calcium transients were assessed in 12-month-old D2.WT, D2.mdx, and MDC2 and MDC4 treated D2.mdx mice. **(A)** MDC2 normalized peak Ca^{2+} release and MDC4 increased peak Ca^{2+} release. **(B)** Ca^{2+} reuptake kinetics were not significantly different between groups **(C)** MDC4 normalized %shortening in dystrophic cardiomyocytes. **(D)** MDC4 normalized relaxation kinetics and MDC2 increased relaxation kinetics. One-way ANOVA, Holm-Sidak multiple comparisons vs D2.mdx **(B, C)** and Brown-Forsythe and Welch ANOVA vs. D2.mdx **(A, D)**.