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Supplemental Information

Efficacy of Multi-exon Skipping Treatment in Duchenne Muscular Dystrophy Dog Model Neonates

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Supplementary Figures and Table

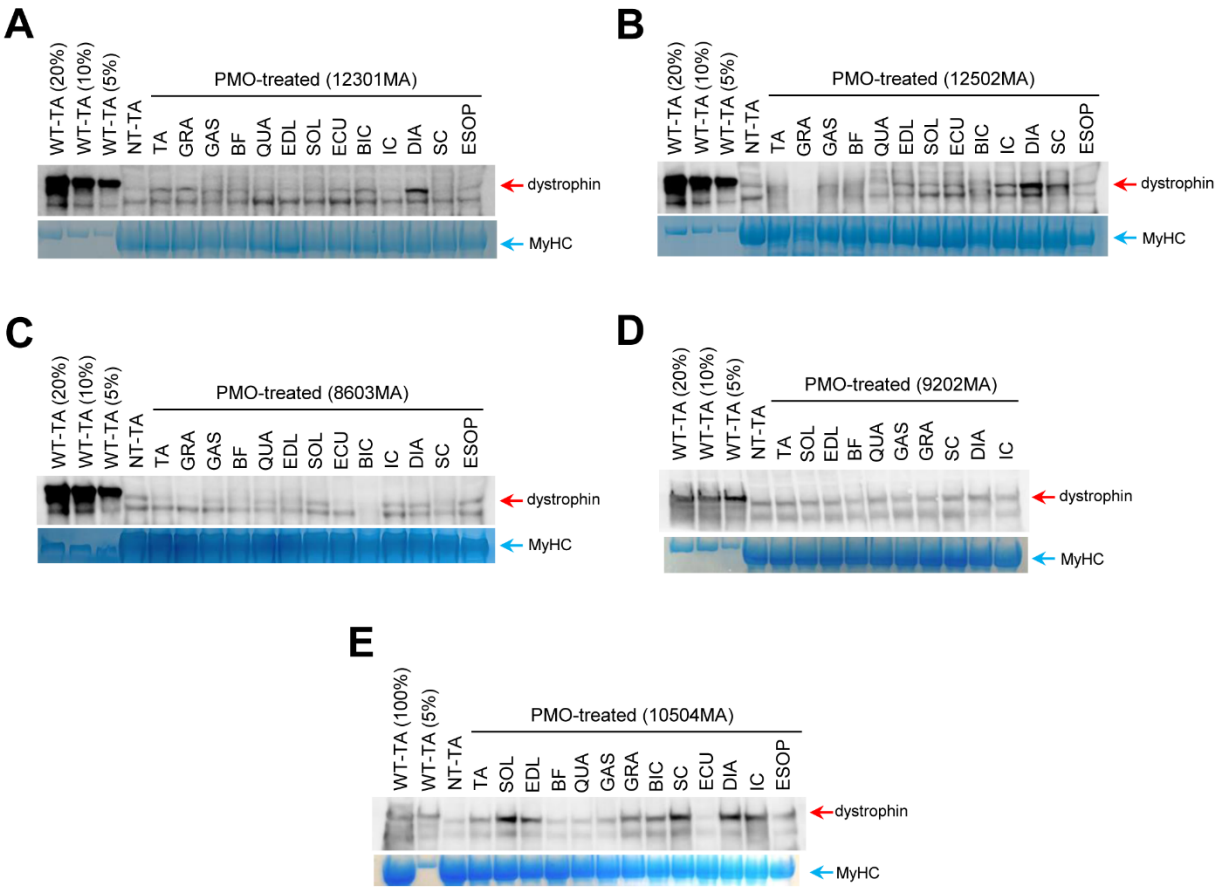

Figure S1. Dystrophin Western blot results for other treated CXMD$_J$ neonatal dogs. Images showing dystrophin protein rescue in (A) 12301MA, (B) 12502MA, (C) 8603MA, (D) 9202MA, and (E) 10504MA. Myosin heavy chain (MyHC) is shown as a loading control. For (A) to (C), 40 µg protein was loaded for non-treated (NT) and treated muscles; for (D) and (E), 60 µg was loaded instead. Wild-type (WT) samples were loaded at the indicated levels, as percentages of the amounts loaded for the treated muscles. Abbreviations: TA, tibialis anterior; GRA, gracilis major; GAS, gastrocnemius; BF, biceps femoris; QUA, quadriceps; EDL, extensor digitorum longus; SOL, soleus; ECU, extensor carpi ulnaris; BIC, biceps brachii; IC, intercostal muscles; DIA, diaphragm; SC, sternocleidomastoid; ESOP, esophagus.

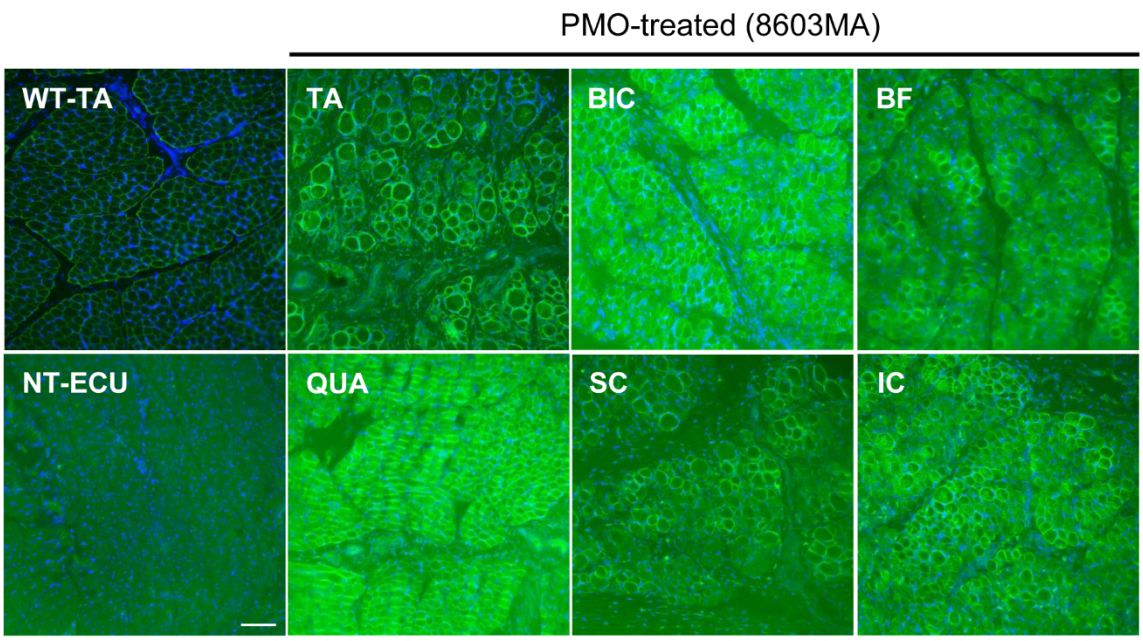

Figure S2. Representative immunohistochemistry images of skeletal muscles from 8603MA, stained using DYS1. Numerous dystrophin-positive fibers (green) can be observed in various skeletal muscles upon treatment; blue: nuclei. Total magnification: 200x; scale bar: 100 μm. Abbreviations: TA, tibialis anterior; ECU, extensor carpi ulnaris; BIC, biceps brachii; BF, biceps femoris; QUA, quadriceps; SC, sternocleidomastoid; IC, intercostal muscles.

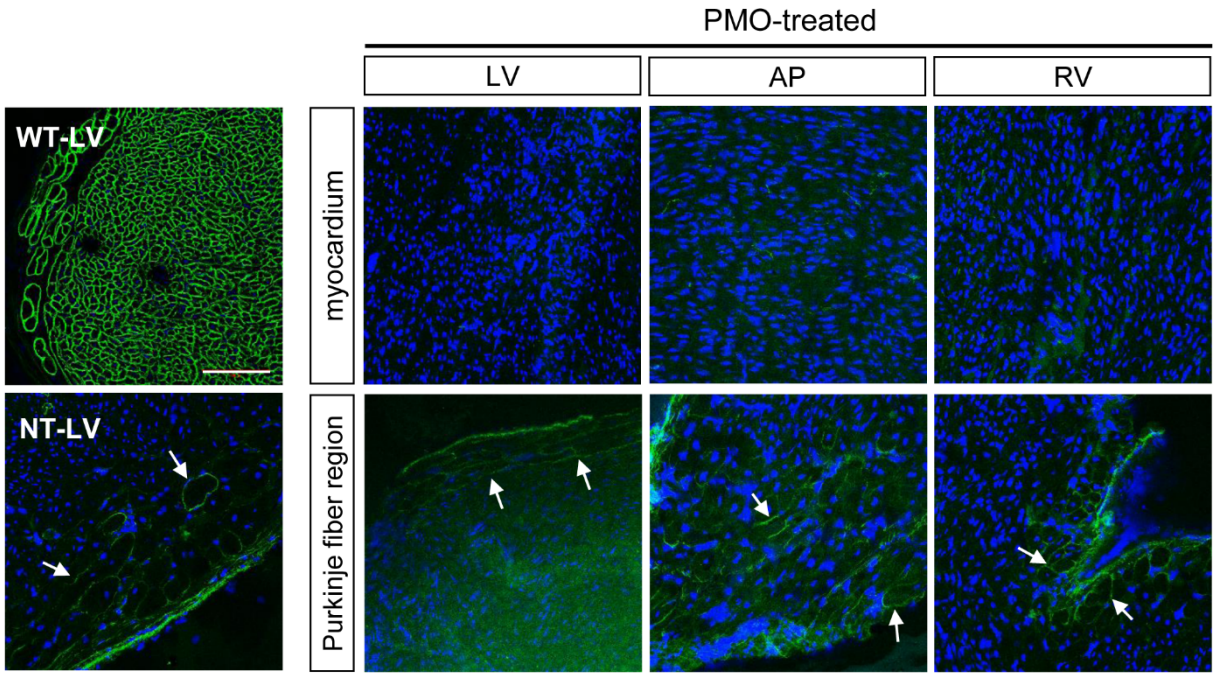

Figure S3. Representative immunohistochemistry images of cardiac muscles detected with DYS2. DYS2 is specific for the C-terminal domain of dystrophin. Dystrophin (green) can be detected in Purkinje fibers, as indicated by the white arrows, but not in the myocardium of treated $CXMD_J$ dog cardiac muscles; blue: nuclei. Total magnification: 200x; scale bar: 100 µm. n = 4 (PMO-treated dogs). Abbreviations: WT, wild-type; NT, non-treated; LV, left ventricle; AP, anterior papillary muscle; RV, right ventricle.

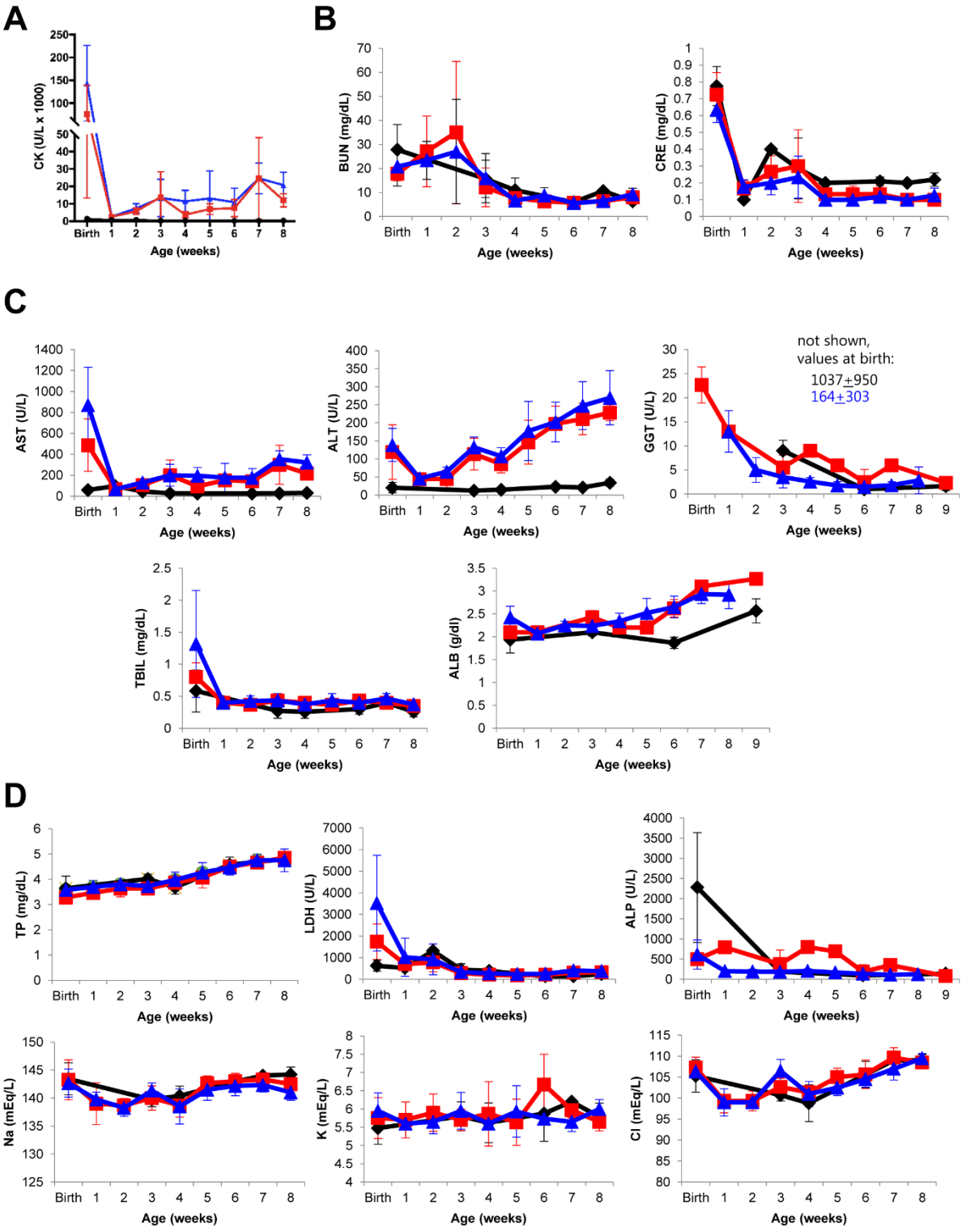

Figure S4. Analysis of serum biomarkers from weekly blood tests in neonatal dogs. The levels of various serum biomarkers were analyzed in weekly samples collected from wild-type

(black), non-treated CXMD$_J$ (red), and PMO-treated CXMD$_J$ (blue) dogs. (A) Creatine kinase (CK) levels. (B) Kidney damage marker levels: blood urea nitrogen (BUN), serum creatinine (CRE). (C) Liver damage marker levels: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total bilirubin (TBIL), serum albumin (ALB). (D) General marker levels: total protein (TP), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), sodium (Na), potassium (K), chloride (Cl). Error bars: S.D. n = 1-12, wild-type; n = 3-4, non-treated; n = 4-6, treated.

Table S1. Comprehensive list of dogs used in the study.

Dog ID*	Treatment group	Functional testing				Molecular analyses**		Histology	ELISA (serum, tissues)	Blood tests
		Grading	15m run	Standing time	Open-mouth width	Skeletal muscles	Cardiac muscles			
8603MA	Treated		X			X	X	X	X	X
9202MA	Treated	X	X	X	X	X		X	X	X
10504MA	Treated	X	X	X	X	X		X	X	X
12301MA	Treated	X	X	X	X	X	X	X	X	X
12303MA	Treated	X	X	X	X	X	X	X	X	X
12502MA	Treated	X	X	X	X	X	X	X	X	X
8609MA	Non-treated		X					X		X
9201MA§	Non-treated									X
12305MA	Non-treated	X	X	X	X	X	X	X		X
12501MA	Non-treated	X	X	X	X	X	X	X		X
11403MA	Non-treated	X	X	X						
402MA	Non-treated					X		X		
2301MA	Non-treated							X		
3701MA	Non-treated			X	X					
5301FA	Non-treated			X	X					
5302FA	Non-treated			X	X					
5303MA	Non-treated			X	X					
5306MA	Non-treated			X	X					
5308FA	Non-treated			X	X					
8106MA	Non-treated			X	X					
11303MA	Non-treated			X	X					
14804MA	Non-treated									X
15001MA	Non-treated									X
15002MA	Non-treated									X
8601MN	Wild-type		X							X
9203MN	Wild-type		X	X	X					
10502MN	Wild-type	X	X	X	X	X	X	X		
12302MN	Wild-type	X	X	X	X	X		X		
12304MN	Wild-type					X		X		
12104MN	Wild-type	X	X	X						
601MN	Wild-type							X		
E09MN	Wild-type							X		
2303MN	Wild-type						X	X		
14003MN	Wild-type									X
14103MN	Wild-type									X
14104MN	Wild-type									X
14304MN	Wild-type									X
14402MN	Wild-type									X
14502MN	Wild-type									X
14504MN	Wild-type									X
14603MN	Wild-type									X
14701MN	Wild-type									X
14702MN	Wild-type									X
14703MN	Wild-type									X
14803MN	Wild-type									X

*the two letters at the end of each ID: the first indicates sex (M/F), the second indicates genotype (N = normal, A = affected, with $CXMD_J$ mutation), **molecular analyses include: RT-PCR, Western blotting, immunohistochemistry, [§]died prior to endpoint.