YMTHE, Volume *25*

Supplemental Information

Systemic AAV8-Mediated Gene Therapy Drives Whole-Body Correction of Myotubular Myopathy in Dogs

David L. Mack, Karine Poulard, Melissa A. Goddard, Virginie Latournerie, Jessica M. Snyder, Robert W. Grange, Matthew R. Elverman, Jérôme Denard, Philippe Veron, Laurine Buscara, Christine Le Bec, Jean-Yves Hogrel, Annie G. Brezovec, Hui Meng, Lin Yang, Fujun Liu, Michael O'Callaghan, Nikhil Gopal, Valerie E. Kelly, Barbara K. Smith, Jennifer L. Strande, Fulvio Mavilio, Alan H. Beggs, Federico Mingozzi, Michael W. Lawlor, Ana Buj-Bello, and Martin K. Childers

SUPPLEMENTAL MATERIAL

Supplemental Movie 1. Representative phenotype of untreated XLMTM dog approaching euthanasia criteria, 4 months of age.

Supplemental Movie 2. Typical play behavior of XLMTM dogs at approximately 4 months of age. The smaller dog was infused with saline at 9 weeks of age; the larger dog received rAAV8-cMTM1 (high dose) at 9 weeks of age. Both dogs are male littermates expressing the p.N155K mutation in *MTM1*.

Supplemental Movie 3. Characteristic motor behavior observed during neurological assessment scoring for a saline-infused and rAAV8-cMTM1-infused XLMTM dog. Both dogs are approximately the same age.

Supplemental Movie 4. Walking gait following a single infusion of saline or rAAV8-cMTM1 at 10 weeks of age. Videos were taken ~7 weeks after infusion of test articles. Dogs walked along an instrumented carpet that captured spatiotemporal data of footfalls. Differences in gait speed and step length can easily be perceived among the treatment groups.

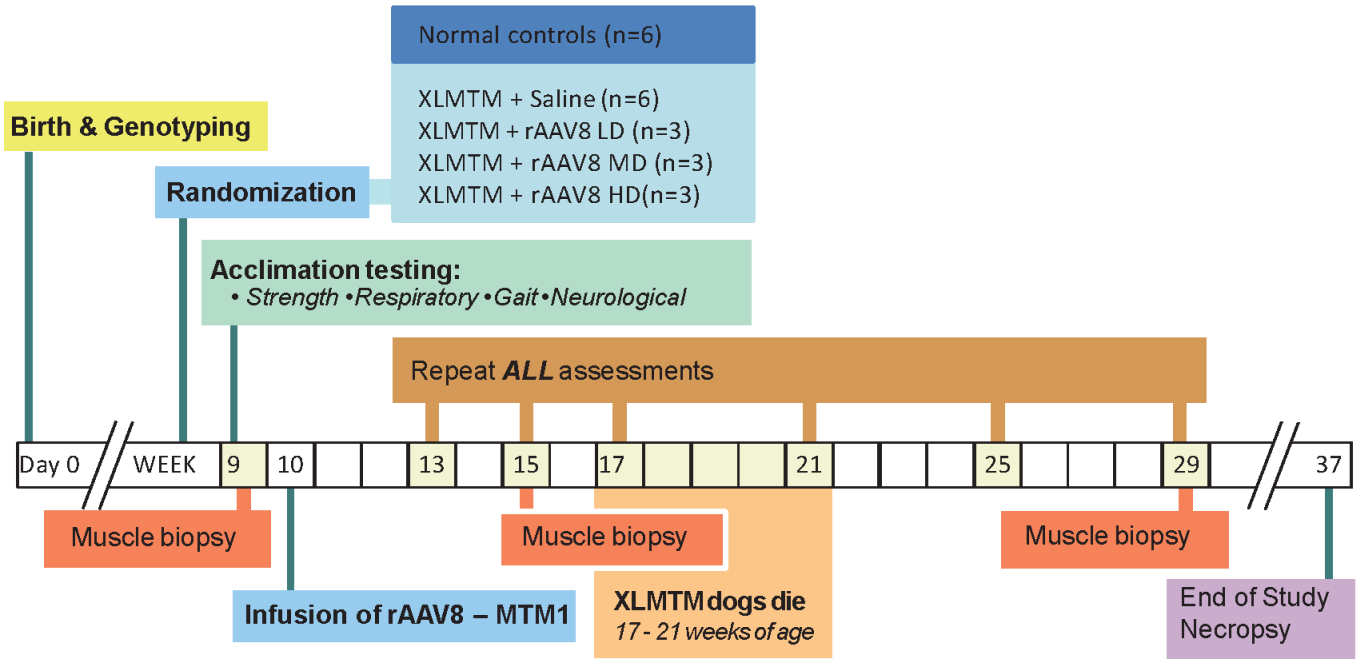
Supplemental Figure S1. Study design and assessment sequence. (A) Overall study design and flow of experimental treatment and assessments. Surviving affected dogs were randomized into treatment groups. Two dogs were excluded from the study due to failure to thrive at birth. Pre-infusion tests preceded and post-infusion tests are listed and described in Methods. (B) Sequence of assessments in an individual dog. Dogs were genotyped shortly after birth. Pre-infusion testing occurred at 9 weeks of age. Dogs were infused with saline or escalating doses rAAV8-cMTM1 at 10 weeks of age followed by repeated tests at the time points illustrated. Muscle biopsies were taken from limb muscles at 9, 15 and 29 weeks of age for analyses of vector copy number, RNA and myotubularin protein. Necropsies were performed at the end of the study in all XLMTM dogs.

Supplemental Figure S2. Representative pathology of varying XLMTM pathology grades. H&E stains were used to evaluate myofiber size and morphology, and NADH stains were used to evaluate mitochondrial localization. Grades were assigned as follows: Grade 0 = no XLMTM pathology, Grade 1 = XLMTM pathology in ≤10% of fibers, Grade 2 = XLMTM pathology in 11–30% of fibers, Grade 3 = XLMTM pathology in 31–60% of fibers, Grade 4 = XLMTM pathology in 61–80% of fibers, Grade 5 = XLMTM pathology in 81–100% of fibers. Bar = 100 μm.

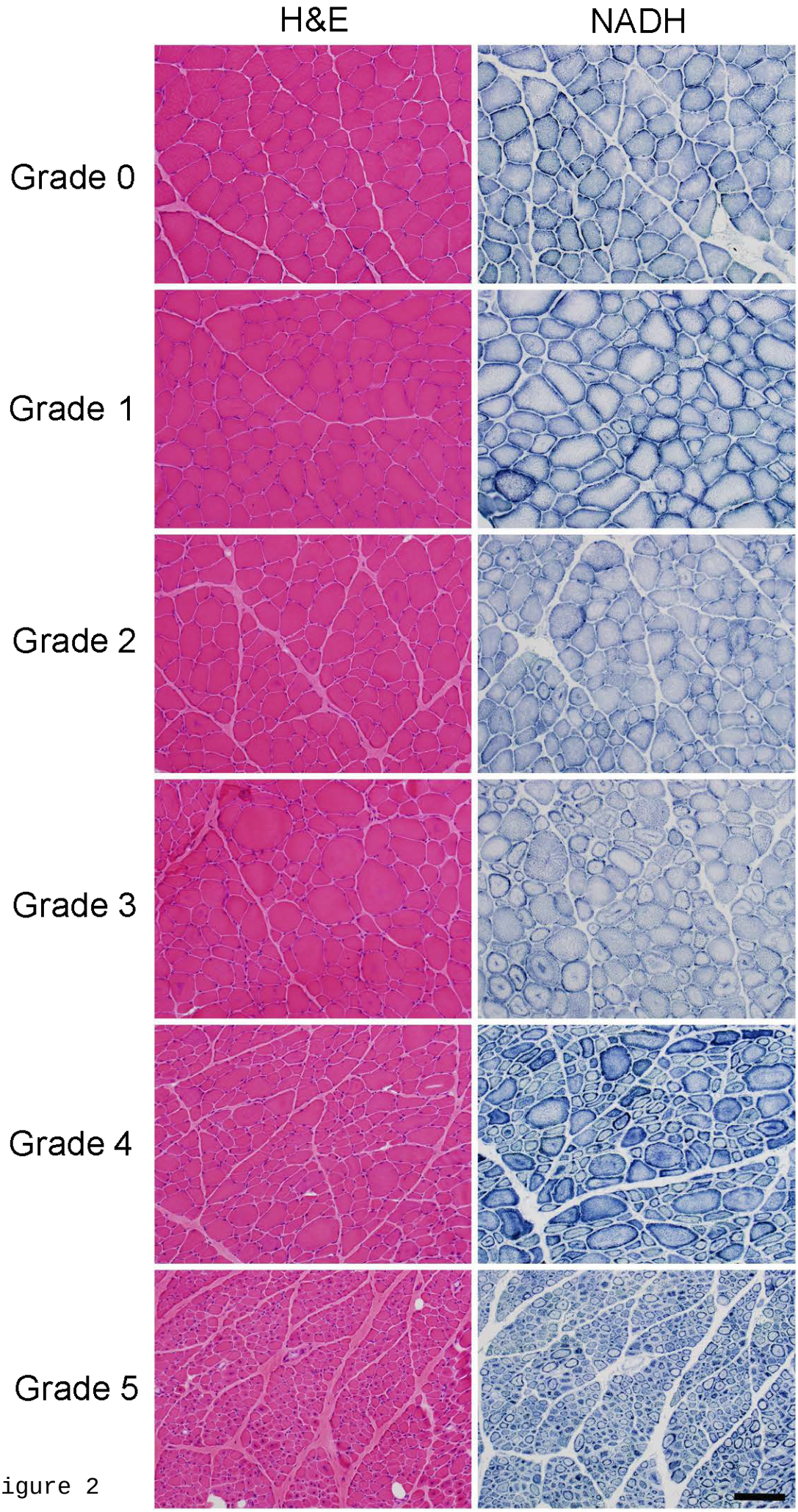
Supplemental Figure S3. AAV8 vector and transgenic protein biodistribution in tissues and organs of XLMTM dogs. (A) Vector genomes per diploid genome (vg/dg) are shown for the three rAAV8-cMTM1 dosing groups as indicated. Tissues were analyzed from XLMTM dogs at the time of necropsy. Legend: TRI bra = triceps brachii; BI bra = biceps brachii; BI fem = biceps femoris; TA = cranial tibialis; GA = gastrocnemius; SOL = soleus; Paravert = paravertebral muscles; Interc = intercostal; Dia = diaphragm; RV = right ventricle; LV = left ventricle. (B) Transgenic myotubularin expression in various tissues from XLMTM dogs following rAAV8-cMTM1 infusion are

expressed relative to WT levels (1) indicated by a horizontal dashed line. Data shown represent mean +/- SD. (C) Representative western immunoblot of myotubularin and the housekeeping gene, GAPDH.

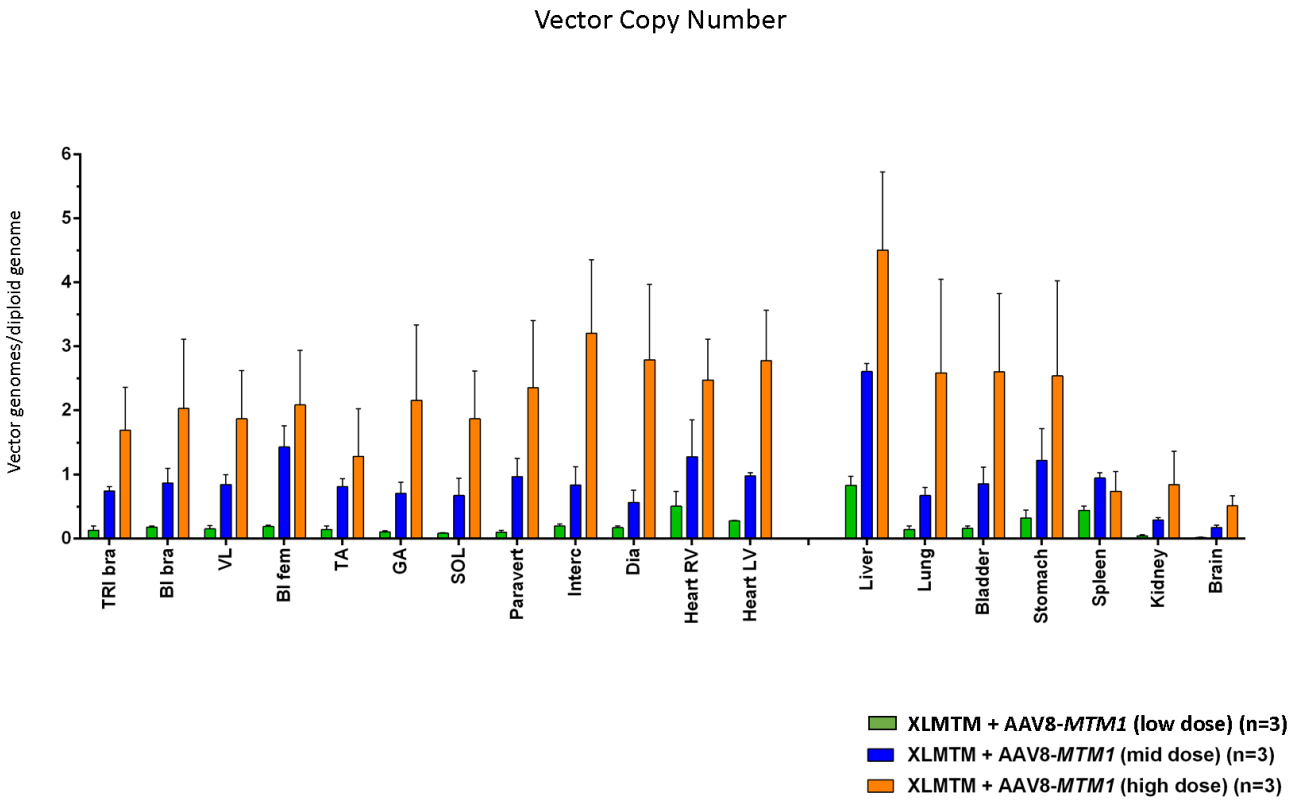
Supplemental Figure S4. rAAV8-cMTM1 does not elicit humoral immune responses to myotubularin. (A) Anti-rAAV8 capsid IgG antibody levels measured by ELISA, values are reported as the sample dilution (1:x) leading to an optical density (OD) above a predetermined binding threshold; (B) IgG antibodies against canine MTM1 transgene product measured by ELISA, results are reported in ng/ml, concentration was determined against a standard curve made of purified canine IgG; (C) Serum neutralizing antibodies against the rAAV8 capsid measured with an *in vitro* transduction inhibition assay; results are reported as the sample dilution (1:x) leading to >50% inhibition of transduction of a reporter vector. Values are reported as average +/- standard deviation.



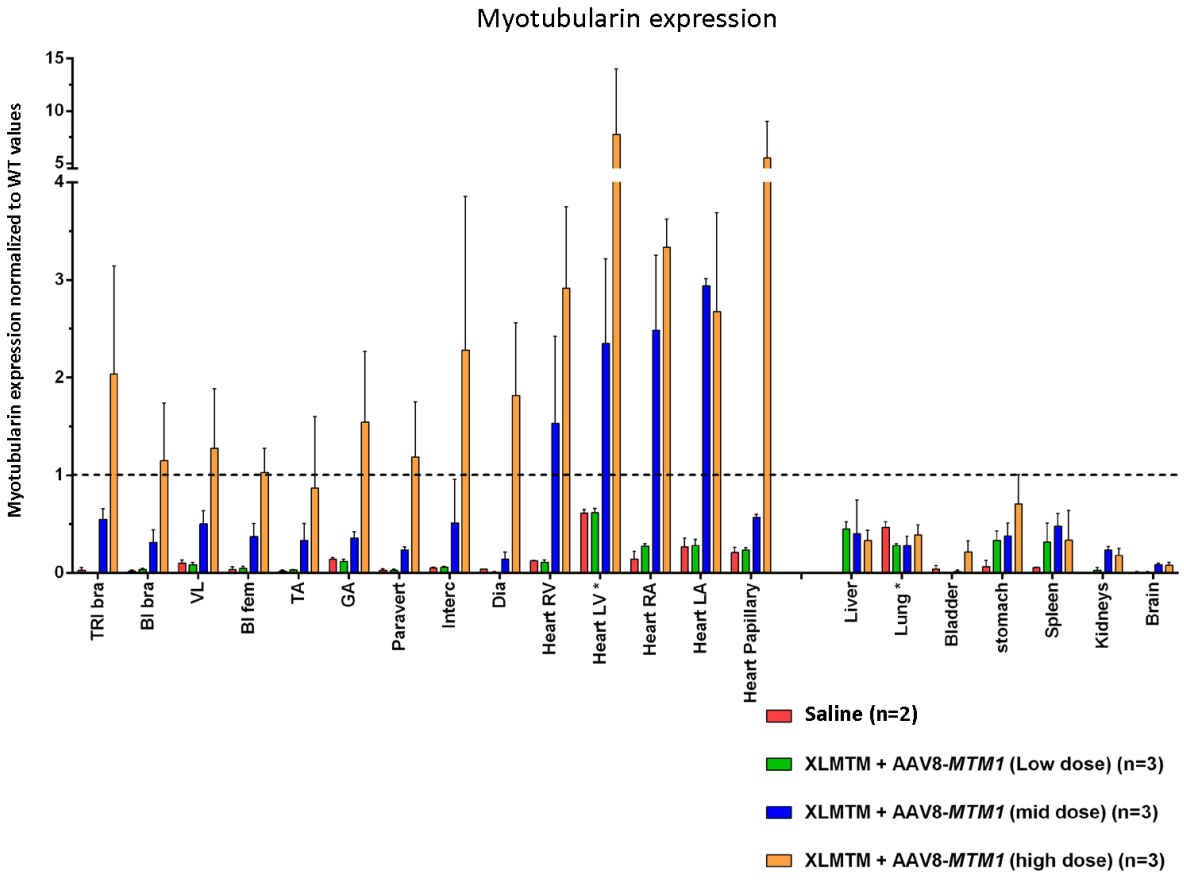
Supplemental Figure 1



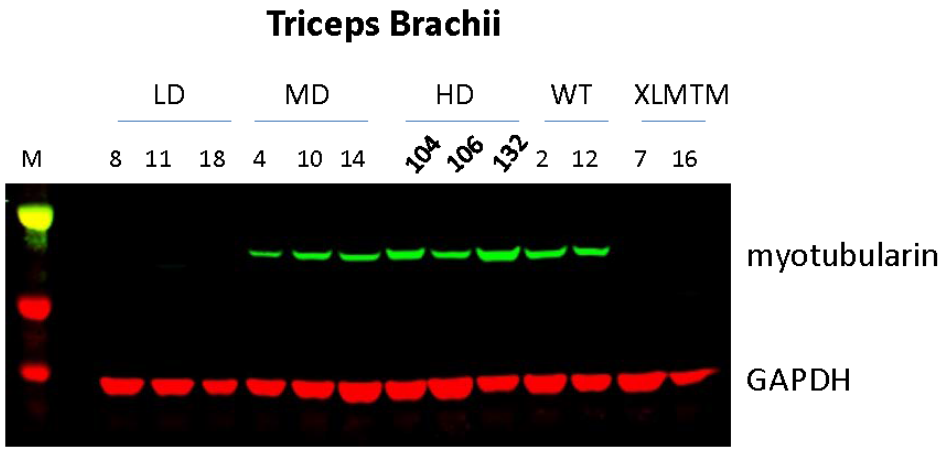
Supplemental Figure 2



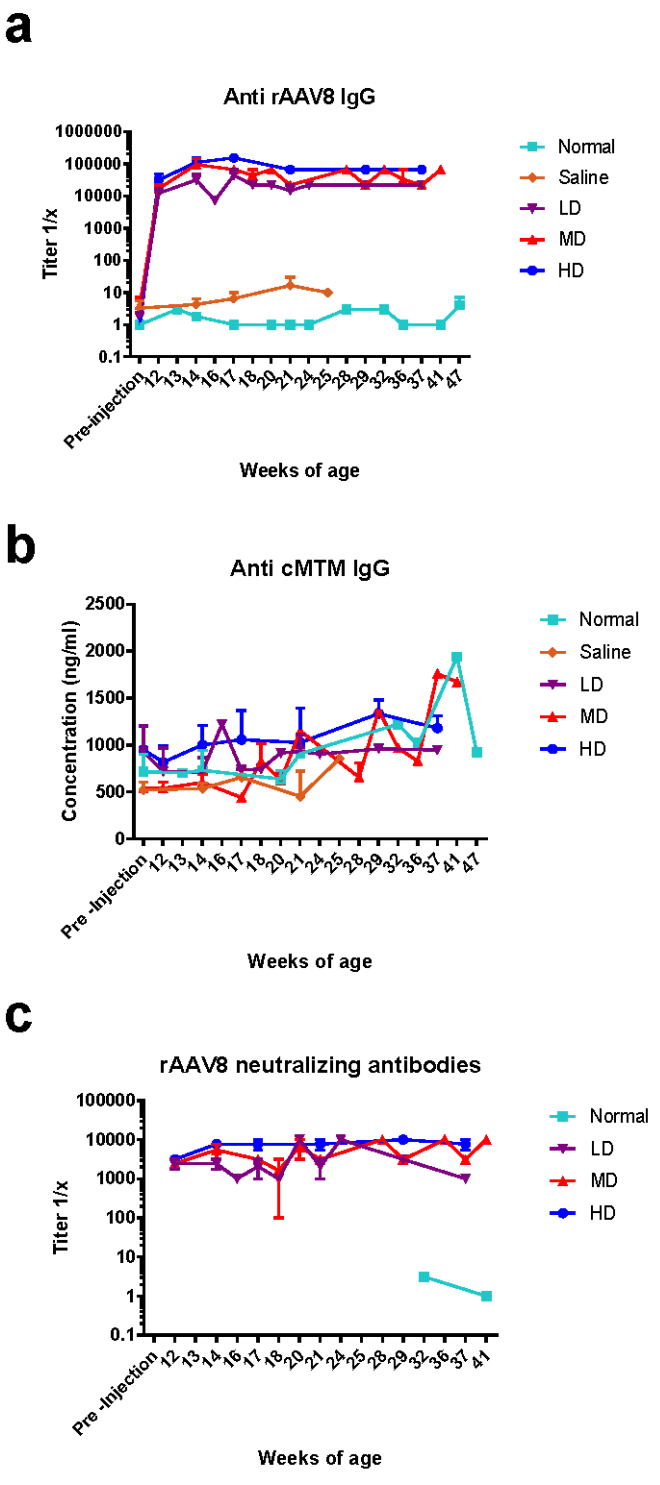
Supplemental Figure 3A



Supplemental Figure 3B



Supplemental Figure 3C



Supplemental Figure 4

Supplemental Table 1. Demographics of study animals.

rAAV8-cMTM1 Dose (vg/kg)	Number of Dogs Males/Females	Number of XLMTM Dogs Males	Females	Number of Normal Control Dogs Males	Females
Saline only	4/6	1	5	3	3
Low Dose $0.3^{E}14$	2/1	2	1	-	-
Mid Dose $2^{E}14$	3/0	3	-	-	-
High Dose $5^{E}14$	3/0	3	-	-	-

Supplemental Table 2: Neurological function

Neurological assessment scores										
Age (weeks)	Normal		Saline		Low Dose		Mid Dose		High Dose	
	Mean ± SEM		Mean ± SEM		Mean ± SEM		Mean ± SEM		Mean ± SEM	
Pre-Infusion	9.8 ±0.2		8.6 ±0.4		7.7 ±0.3		8.3 ±0.3		8.7 ±0.7	
17	10 ±0.0		5.6 ±0.8		6.7 ±0.7		8.7 ±0.3		9.3 ±0.7	
37	10 ±0.0		-		-		8.0 ±0.0		9.3 ±0.7	
Composite reflex scores										
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
Pre-Infusion	1.9 ± 0.1	6	1.4 ± 0.1	5	1.3 ± 0.0	3	1.4 ± 0.1	3	ND	3
13	2.0 ± 0.0	6	1.3 ± 0.1	5	1.3 ± 0.1	3	1.7 ± 0.0	3	1.4 ± 0.1	3
15	1.9 ± 0.1	6	1.2 ± 0.1	5	1.2 ± 0.3	3	1.7 ± 0.1	3	1.9 ± 0.0	3
17	2.0 ± 0.0	6	1.0 ± 0.1	5	1.4 ± 0.1	3	1.8 ± 0.1	3	2.0 ± 0.0	3
21	2.0 ± 0.0	6	0.9 ± 0.3	2	1.1 ± 0.3	3	1.8 ± 0.1	3	2.0 ± 0.0	3
25	2.0 ± 0.0	6	1.2 ± 0.0	1	1.1 ± 0.4	2	1.9 ± 0.1	3	2.0 ± 0.0	3
29	2.0 ± 0.0	6			1.6 ± 0.0	1	1.9 ± 0.1	3	2.0 ± 0.0	3
33	2.1 ± 0.0	6					2.0 ± 0.1	3	2.0 ± 0.0	3
37	2.0 ± 0.1	6					1.9 ± 0.1	2	2.0 ± 0.1	3

Supplemental Table 3: Limb torque (N·m/kg x 10-2)

Forelimb extension torque (N·m/kg x 10^{-2})

Age (weeks)	Normal		Saline		Low Dose		Mid Dose		High Dose	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
Pre-Infusion	2.1 ±0.4	6	1.9 ±0.3	6	1.3 ±0.0	3	1.0 ±0.1	3	2.3 ±0.5	3
13	3.4 ±0.3	6	1.3 ±0.1	5	2.2 ±0.2	3	1.9 ±0.2	3	2.1 ±0.3	3
15	3.1 ±0.3	6	1.1 ±0.2	5	1.2 ±0.4	3	2.0 ±0.3	3	2.1 ±0.2	3
17	3.9 ±0.3	6	1.1 ±0.2	5	1.6 ±0.1	3	2.4 ±0.3	3	3.1 ±0.7	3
21	3.5 ±0.3	6	1.4 ±0.1	2	1.1 ±0.5	3	2.6 ±0.4	3	2.4 ±0.4	3
25	4.4 ±0.3	6	1.0 ±0.0	1	2.1 ±0.0	1	3.0 ±0.7	3	2.9 ±0.6	3
29	4.8 ±0.4	6			2.2 ±0.0	1	2.9 ±0.2	3	3.0 ±0.3	3
33	4.4 ±0.3	6			1.8 ±0.0	1	4.0 ±0.5	3	2.6 ±0.5	3
37	5.1 ±0.2	6			0.5 ±0.0	1	3.9 ±0.6	3	3.3 ±0.4	3

Forelimb flexion torque (N·m/kg x 10^{-2})

Pre-Infusion	9.7 ±0.7	6	11.5 ±1.6	6	12.3 ±2.1	3	9.5 x ±0.6	3	7.6 ±2.3	3
13	11.8 ±1.1	6	11.4 ±0.8	5	13.0 ±2.9	3	13.0 ±0.3	3	10.2 ±0.7	3
15	10.3 ±1.1	6	10.7 ±1.2	5	11.3 ±2.5	3	14.3 ±0.4	3	12.4 ±2.1	3
17	13.2 ±0.8	6	10.3 ±0.8	5	11.8 ±1.6	3	15.0 ±0.6	3	12.8 ±0.1	3
21	15.7 ±2.1	6	19.5 ±0.1	2	11.8 ±2.7	3	13.9 ±0.6	3	12.4 ±0.5	3
25	15.3 ±1.4	6	12.6 ±0.0	1	11.2 ±0.0	1	16.2 ±0.4	3	11.8 ±1.3	3
29	21.9 ±1.8	6			20.6±0.0	1	25.1 ±5.2	3	16.6 ±4.7	3
33	18.1 ±3.1	6			1.7 ±0.0	1	24.1 ±5.1	3	10.8 ±1.9	3
37	22.6 ±3.2	6			6.8 ±0.0	1	23.6 ±4.3	3	14.4 ±1.8	3

Hindlimb extension torque (N·m/kg x 10^{-2})

Pre-infusion	23.0 ±4.0	6	23.3 ±0.7	6	25.6 ±1.8	3	16.9 ±3.6	3	24.9 ±5.3	3
13	29.6 ±5.5	6	24.9 ±5.2	5	27.8 ±8.7	3	17.3 ±1.1	3	35.7 ±6.5	3
15	21.3 ±3.8	6	22.7 ±5.9	5	25.5 ±8.2	3	20.5 ±3.9	3	32.9 ±1.2	3
17	30.5 ±4.4	6	24.1 ±4.1	5	29.1 ±7.1	3	27.9 ±2.3	3	37.1 ±1.1	3
21	39.1 ±6.2	6	19.0 ±3.6	2	23.3 ±6.0	3	39.3 ±3.0	3	35.8 ±3.7	3
25	45.4 ±6.3	6	12.6 ±0.0	1	45.4 ±0.0	1	43.8 ±4.8	3	39.9 ±1.2	3
29	48.1 ±3.9	6			19.7 ±0.0	1	51.8 ±3.0	3	42.6 ±1.4	3
33	54.7 ±3.3	6			27.1 ±0.0	1	52.5 ±3.8	3	43.5 ±3.2	3
37	53.3 ±4.7	6			23.4 ±0.0	1	56.9 ±4.6	3	43.6 ±4.1	3

Hindlimb flexion torque (N·m/kg x 10^{-2})

Pre-Infusion	9.1 ± 1.1	6	6.3 ±0.7	6	3.9 ±0.6	3	4.3 ±1.0	3	4.7 ±1.5	3
13	9.7 ± 1.2	6	5.3 ±1.1	5	6.7 ±2.3	3	6.0 ±1.4	3	9.0 ±1.6	3
15	11.0 ± 0.5	6	5.3 ±1.4	5	7.5 ±1.6	3	7.8 ±0.7	3	9.4 ±0.7	3
17	14.3 ± 1.5	6	5.1 ±1.2	5	5.9 ±2.7	3	12.8 ±2.6	3	11.9 ±1.1	3
21	18.2 ± 1.1	6	4.7 ±0.3	2	7.8 ±2.6	3	14.7 ±2.1	3	11.2 ±0.7	3
25	20.6 ± 1.9	6	5.0 ±0.0	1	16.5±0.0	1	17.6 ±2.6	3	15.1 ±0.6	3
29	22.1 ± 1.7	6			11.6 ±0.0	1	18.0 ±2.5	3	12.9 ±1.6	3
33	22.4 ± 3.9	6			13.6 ±0.0	1	17.9 ±4.0	3	16.9 ±1.4	3
37	23.3 ± 1.5	6			8.3 ±0.0	1	19.8 ±2.6	3	14.9 ±2.7	3

Supplemental Table 4: Walking gait and peak inspiratory flow velocities (mL/s)

Gait speed (cm/s)										
Age (weeks)	Normal		Saline		Low Dose		Mid Dose		High Dose	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
Pre-Infusion	175.8±25.9	6	129.4±45.7	6	135.4±46.4	3	111.6±16.8	3	98.6±16.8	3
13	161.0±15.7	6	122.0±22.3	4	135.5±10.0	3	157.2±25.0	3	119.3±9.0	3
15	165.6±26.1	6	129.9±18.6	4	143.5±10.7	3	158.0±12.6	3	148.5±25.5	3
17	175.6±16.7	6	114.2±1.5	3	124.9±14.9	3	173.0±22.9	3	156.0±19.2	3
21	164.6±16.9	6	125.8±3.5	2	144.2±45.2	3	189.9±26.6	3	156.5±4.9	3
25	178.5±21.1	6			149.0±56.9	2	278.1±8.4	3	172.2±10.9	3
29	177.8±26.8	6			170.4±0.0	1	203.5±16.4	3	178.6±24.8	3
33	183.4±21.9	6			171.6±0.0	1	184.7±11.9	3	193.0±21.0	3
37	201.8±12.0	6					220.6±10.9	3	189.0±10.9	3
Stride length (cm)										
Pre-Infusion	61.6±11.3	6	48.1±11.0	6	51.5±6.5	3	43.1±4.4	3	37.9±6.6	3
13	61.5±6.6	6	52.7±1.6	4	58.5±10.1	3	61.4±6.8	3	50.8±7.7	3
15	66.7±9.9	6	54.0±9.8	4	62.0±10.9	3	65.1±7.7	3	59.5±5.5	3
17	68.5±5.2	6	51.5±6.6	3	58.9±5.5	3	69.4±0.9	3	64.1±0.7	3
21	70.3±9.2	6	55.0±4.9	2	67.0±23.5	3	77.9±8.5	3	67.8±7.1	3
25	75.0±8.8	6			71.6±28.6	2	76.2±7.8	3	73.3±8.0	3
29	74.4±10.0	6			75.1±0.0	1	83.2±9.8	3	76.1±3.3	3
33	75.2±9.4	6			83.2±0.0	1	76.9±8.3	3	78.7±2.0	3
37	81.3±5.5	6					88.1±6.6	3	78.5±5.7	3
Peak inspiratory flow velocities (mL/s)										
Pre-Infusion	296.6±41.1	6	247.5±19.3	6	235.4±41.8	3	245.6±18.8	3	266.1±50.4	3
17	376.5±29.5	6	271.7±31.4	5	278.1±34.1	3	388.6±25.3	3	465.0±77.6	3

Supplemental Table 5: Quantification of pathological finding in canine skeletal muscle

Group/ Timepoint	Pathology score	Average myofiber minFeret diameter	Percent myofibers with mislocalized mitochondria	Percent myofibers with central nuclei	Average number of triads/field
Normal					
10 weeks	0.0±0.0†˚¥	28.3±0.8	0.0±0.0†˚¥	0.1±0.0	5.1±0.6†
17 weeks	0.0±0.0†˚	37.5±2.3¥	0.2±0.2†˚	0.2±0.1	6.4±0.6†˚
25 weeks	0.0±0.0†˚	43.5±1.9	1.1±0.6	2.1±1.2	8.2±0.5˚¥
Autopsy	0.0±0.0†˚	37.8±0.5†	0.0±0.0	0.4±0.2†	7.2±0.7†˚
Saline					
10 weeks	3.2±0.3*	24.7±4.1	67.3±5.9*	2.1±0.9	2.5±0.3
17 weeks	3.5±0.5*	29.4±9.8¥	76.4±4.4*¥	11.4±9.0	2.8±0.5*¥
25 weeks	4.5	40.5	92.7	3.9	5.4±3.6
Autopsy	3.9±0.5*¥	22.0±4.4*˚¥	95.4±2.9*¥	9.1±1.5*˚¥	1.7±0.2*¥
Low dose (LD) rAAV8 – cMTM1					
10 weeks	2.8±0.6*	34.7±4.7	59.0±3.2*	2.9±1.4	3.7±0.6
17 weeks	4.2±0.8*¥	39.6±1.3	39.4±3.2*¥	2.1±0.7	3.1±0.7*¥
25 weeks	3.6±0.6*¥	50.9	13.5	2.4±1.7	3.6±0.6*
Autopsy	3.8±0.3*¥	43.6±5.0†	88.8±5.7*¥	2.9±0.9†	4.4±0.5*¥
Mid dose (MD) rAAV8 – cMTM1					
10 weeks	3.8±0.3*	30.0±0.7	51.0±7.0*	1.7±0.3	3.9±0.4
17 weeks	1.8±0.8†˚	49.6±1.4*†	11.5±4.5†˚	0.8±0.1	5.3±0.7†˚
25 weeks	1.7±0.7†˚	48.7±7.5	3.9±3.9	1.8±1.1	4.1±0.6*
Autopsy	0.8±0.4†˚	52.3±2.4†	7.8±1†˚	1.2±0.5†	8.1±0.7†˚
High dose (HD) rAAV8 – cMTM1					
10 weeks	3.0±0.6*	19.7±2.2˚	56.0±16.5*	3.2±1.5	2.4±0.4
17 weeks	1.3±1.0†˚	39.9±3.9	7.5±5.1†˚	4.7±3.9	7.1±0.5†˚
25 weeks	1.3±1.1†˚	38.6±12.6	12.5±10.2	1.5±1.5	7.8±0.7˚¥
Autopsy	0.8±0.8†˚	45.7±2.8†	1.3±1.3†˚	0.7±0.2†	8.0±0.8†˚

Symbols
* Significantly different from Normal
† Significantly different from Saline
˚ Significantly different from LD
¥ Significantly different from MD
 Significantly different from HD

Supplemental Table 6: Vector copy number biodistribution (Vector genomes/ diploid genome (vg/dg)

	Saline				LD			MD			HD		
Dog Identifier	SSAN 7	SSAN 9	SSAN 13	SSAN 16	SSAN 8	SSAN 11	SSAN 18	SSAN 4	SSAN 10	SSAN 14	SSAN 104	SSAN 106	SSAN 132
Supraspinatus	3.0E-06	2.4E-06	4.2E-06	3.9E-06	2.4E-01	9.9E-02	3.4E-02	5.0E-01	1.2E+00	5.3E-01	2.6E+00	2.8E-01	3.4E+00
Infraspinatus	1.6E-04	2.5E-06	5.6E-05	3.2E-06	1.8E-01	8.8E-02	2.0E-02	4.8E-01	1.3E+00	4.1E-01	4.0E+00	4.5E-01	3.4E+00
Deltoideus	7.6E-04	1.8E-06	4.0E-06	3.3E-06	1.3E-01	2.0E-01	1.2E-01	1.3E+00	5.9E-01	6.2E-01	3.1E+00	6.0E-01	3.1E+00
Triceps brachii	1.8E-04	2.6E-06	3.0E-06	3.6E-06	8.7E-02	2.7E-01	4.2E-02	8.7E-01	7.1E-01	6.5E-01	1.4E+00	7.0E-01	3.0E+00
Anconeus	3.6E-06	2.8E-06	2.7E-06	2.7E-06	7.2E-02	2.5E-01	1.2E-01	2.1E+00	1.0E+00	6.0E-01	3.6E+00	1.3E+00	3.0E+00
Flexor carpi ulnaris	3.0E-04	4.7E-05	5.2E-05	2.7E-06	4.7E-02	1.3E-01	9.0E-02	3.8E-01	7.2E-01	1.0E+00	2.4E+00	6.7E-01	2.6E+00
Ulnaris lateralis	7.4E-05	6.6E-05	1.4E-04	7.4E-05	2.7E-01	2.0E-01	5.9E-02	4.7E-01	1.3E+00	8.5E-01	3.0E+00	8.6E-01	3.1E+00
Ext digit communis	2.3E-06	1.8E-04	3.2E-04	3.1E-06	1.4E-01	1.2E-01	9.0E-02	1.4E+00	9.0E-01	4.9E-01	3.1E+00	6.3E-01	2.4E+00
Ext digit lateralis	3.4E-06	2.7E-06	7.1E-05	8.4E-05	8.6E-02	1.7E-01	6.3E-02	6.2E-01	5.8E-01	5.3E-01	3.0E+00	7.0E-01	1.6E+00
Ext carpi radialis	3.7E-06	8.2E-05	4.0E-05	4.5E-05	1.0E-01	1.6E-01	3.8E-02	2.1E+00	1.1E+00	5.9E-01	2.6E+00	5.0E-01	2.0E+00
Abduct pollicis long	7.1E-06	2.2E-04	3.3E-06	9.8E-05	6.5E-02	8.6E-02	1.3E-01	4.2E-01	1.2E+00	8.2E-01	3.7E+00	4.9E-01	1.6E+00
Axillary LN	5.5E-06	4.1E-06	2.6E-06	2.7E-06	9.1E-03	4.1E-02	4.3E-02	5.6E-01	2.2E-01	2.0E-01	2.3E-01	5.8E-02	5.9E+00
Brachial plexus	2.6E-06	2.5E-06	0.0E+00	3.0E-06	1.8E-01	4.7E-01	4.2E-01	1.9E+00	2.2E+00	2.1E+00	7.6E+00	3.3E+00	4.3E+00
Subscapular LN	3.6E-06	2.4E-06	3.9E-06	2.9E-06	2.5E-02	1.0E-01	1.5E+00	1.2E-01	5.0E-03	5.1E-01	4.0E-01	1.2E-01	1.3E+00
Pectoralis	3.1E-06	2.8E-06	1.4E-04	3.6E-06	3.4E-02	2.0E-01	8.0E-02	2.4E-01	8.5E-01	5.1E-01	1.7E+00	5.0E-01	2.1E+00
Biceps brachii	3.9E-06	2.6E-06	3.3E-06	5.8E-05	1.6E-01	2.2E-01	1.6E-01	4.2E-01	1.2E+00	1.0E+00	4.1E+00	4.1E-01	1.6E+00
Flexor digit superf	2.9E-06	1.1E-04	2.9E-06	2.5E-06	1.4E-01	1.3E-01	1.5E-02	1.0E+00	1.0E+00	4.4E-01	4.1E+00	2.5E-01	1.9E+00
Flexor carpi radialis	5.5E-04	4.4E-05	3.2E-06	3.1E-06	1.4E-01	1.2E-01	6.1E-02	8.5E-01	1.2E+00	9.8E-01	3.8E+00	2.6E-01	2.4E+00
Flexor digit profundus	3.3E-04	6.1E-05	3.5E-06	3.6E-06	6.0E-02	1.3E-01	6.6E-02	3.7E-01	9.5E-01	5.4E-01	4.0E+00	7.1E-01	2.7E+00
Pronator teres	3.0E-06	8.2E-04	1.1E-03	1.0E-04	1.6E-01	1.2E-01	1.3E-01	7.6E-01	1.2E+00	4.2E-01	1.8E+00	6.3E-01	2.7E+00
Soleus	ND	ND	ND	2.7E-06	9.4E-02	ND	7.5E-02	4.4E-01	1.2E+00	3.8E-01	2.6E+00	4.0E-01	2.6E+00
Supinator	5.9E-05	2.9E-06	7.7E-05	1.9E-04	9.4E-02	2.6E-01	3.3E-02	1.0E+00	5.9E-01	2.7E-01	2.8E+00	4.0E-01	2.3E+00
Pronat quadrates	2.2E-04	5.5E-05	3.6E-06	3.4E-06	7.8E-02	2.1E-01	1.3E-01	3.8E-01	1.2E+00	4.6E-01	3.3E+00	6.1E-01	2.9E+00
Inguinal LN	3.0E-06	2.9E-06	3.0E-06	2.4E-06	2.2E-02	1.7E-01	1.0E-01	7.9E-02	1.7E-01	5.7E-01	2.9E-01	8.5E-02	2.1E-01
Skin	1.6E-06	1.2E-03	7.5E-05	3.0E-04	3.2E-01	2.4E-01	3.0E-01	1.9E+00	2.4E+00	1.1E+00	9.7E-01	6.8E-01	4.1E+00
Biceps femoris	3.6E-06	4.1E-05	1.6E-06	2.7E-06	2.3E-01	1.5E-01	1.9E-01	8.2E-01	1.5E+00	1.9E+00	3.6E+00	6.1E-01	2.1E+00
Tibialis cranialis	3.5E-06	1.2E-04	3.0E-06	3.1E-06	1.5E-01	2.4E-01	4.8E-02	9.2E-01	9.5E-01	5.7E-01	2.7E+00	2.4E-01	9.0E-01
Vastus lateralis	5.5E-05	2.5E-06	3.5E-06	6.2E-05	5.8E-02	2.4E-01	1.7E-01	6.3E-01	1.1E+00	7.7E-01	2.5E+00	3.9E-01	2.7E+00
Ext digito longus	3.2E-06	2.4E-06	2.8E-06	3.2E-06	5.2E-02	2.3E-01	1.1E-01	5.1E-01	9.4E-01	3.8E-01	2.3E+00	2.9E-01	1.7E+00
Gastrocnemius	9.4E-05	3.6E-05	1.7E-04	2.5E-06	1.1E-01	1.4E-01	6.1E-02	3.6E-01	8.7E-01	9.0E-01	4.2E+00	9.8E-02	2.2E+00
Paravertebral lumbar	3.9E-06	2.4E-06	3.4E-06	2.6E-06	4.8E-02	1.6E-01	8.8E-02	1.3E+00	1.2E+00	3.9E-01	4.1E+00	4.6E-01	2.5E+00
Rhomboideus cerv	8.2E-04	4.0E-05	1.4E-04	7.7E-05	1.0E-01	2.1E+00	9.0E-02	3.6E-01	8.1E-01	3.3E-01	4.8E+00	3.2E-01	2.4E+00
L thyroid	1.3E-04	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Submandibular LN	8.3E-06	4.3E-06	8.7E-05	4.0E-06	2.1E-02	4.2E-02	3.3E-02	4.4E-02	5.9E-02	2.1E-01	3.3E-01	5.9E-02	2.5E-01
L parotid salivary gland	3.4E-06	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tongue	5.6E-04	1.7E-04	1.6E-04	2.4E-06	1.2E-01	4.7E-01	1.9E-01	7.4E-01	1.5E+00	7.8E-01	2.8E+00	9.1E-01	2.4E+00
Larynx	4.0E-06	2.4E-06	6.6E-05	9.5E-04	1.6E-01	3.2E-01	1.1E-01	1.5E+00	9.0E-01	1.5E+00	5.2E+00	5.7E-01	2.8E+00
Trachea	2.3E-04	3.7E-04	2.8E-06	2.8E-06	2.6E-01	4.1E-01	1.9E-01	1.4E+00	1.5E+00	1.2E+00	6.3E+00	1.7E+00	4.0E+00
Esophagus	3.3E-06	2.5E-06	2.7E-06	2.7E-06	2.5E-01	5.0E-01	2.8E-01	2.5E+00	1.9E+00	1.7E+00	6.9E+00	1.7E+00	2.7E+00
Thymus	3.1E-06	2.7E-06	2.9E-06	2.3E-06	1.4E-01	2.0E-02	1.9E-02	1.8E-02	6.7E-02	8.1E-03	3.1E-02	4.2E-02	ND

Diaphragm	7.1E-05	2.6E-04	1.4E-04	2.7E-06	1.2E-01	2.1E-01	1.8E-01	5.3E-01	9.1E-01	2.6E-01	3.3E+00	5.3E-01	ND
L sciatic nerve	1.8E-04	ND	ND	ND	ND	ND	ND	5.0E+00	1.8E+00	ND	ND	ND	ND
Bladder	2.3E-04	2.5E-06	3.0E-06	2.4E-06	9.1E-02	2.2E-01	1.8E-01	1.1E+00	3.3E-01	1.2E+00	4.6E+00	3.4E-01	2.9E+00
Spleen	2.7E-06	1.3E-04	7.3E-05	2.2E-06	3.1E-01	5.2E-01	5.0E-01	9.6E-01	1.1E+00	8.0E-01	1.3E+00	1.9E-01	7.8E-01
Kidneys	3.6E-06	2.3E-06	3.3E-06	2.4E-06	3.6E-02	2.2E-02	7.4E-02	3.5E-01	3.0E-01	2.3E-01	1.9E+00	1.7E-01	4.8E-01
Pancreas	1.1E-05	8.0E-06	2.4E-04	1.2E-05	6.5E-03	5.1E-02	9.3E-02	1.7E-01	2.2E-01	1.3E-01	7.2E-01	9.6E-02	4.4E-01
Mesenteric LN	2.5E-06	2.5E-06	2.3E-06	2.9E-06	4.3E-02	1.7E-02	5.1E-02	1.0E-01	6.1E-01	1.4E-01	1.7E+00	1.6E-01	3.4E-01
Aorta	2.8E-06	8.3E-05	6.0E-05	5.7E-05	2.5E-01	4.0E-01	5.2E-01	2.9E+00	2.5E+00	9.0E-01	1.4E+01	3.1E+00	4.5E+00
Liver left lobe	6.6E-06	4.2E-06	1.1E-04	4.0E-06	7.6E-01	1.1E+00	6.4E-01	2.1E+00	2.2E+00	3.4E+00	4.2E+00	2.9E+00	9.6E+00
Liver central lobe	5.7E-06	5.3E-06	5.8E-06	2.1E-06	9.3E-01	8.0E-01	4.8E-01	2.5E+00	2.9E+00	2.9E+00	3.9E+00	3.1E+00	6.7E+00
Liver right lobe	1.2E-04	6.3E-06	9.7E-04	3.0E-06	1.1E+00	1.1E+00	5.7E-01	2.7E+00	2.5E+00	2.2E+00	4.7E+00	1.6E+00	3.9E+00
Gall bladder	3.3E-06	9.0E-05	5.2E-05	3.8E-06	1.2E-01	3.7E-01	1.6E-01	1.3E+00	2.4E+00	1.7E+00	6.2E+00	8.0E-01	1.7E+00
Stomach (pyloris)	2.5E-06	3.0E-06	3.3E-06	2.4E-06	5.4E-01	3.3E-01	9.3E-02	1.4E+00	2.0E+00	3.0E-01	5.5E+00	1.2E+00	8.7E-01
Jejunum	5.3E-06	2.8E-06	1.8E-04	2.6E-06	2.0E-02	9.8E-02	3.4E-02	2.3E-01	2.5E-01	2.0E-01	2.5E+00	3.7E-01	1.0E+00
Colon	4.6E-06	3.1E-06	1.4E-04	3.0E-06	3.6E-02	1.7E-01	8.0E-02	2.6E-01	3.5E-01	3.8E-01	1.5E+00	2.0E-01	7.1E-01
Intercostal muscle	3.1E-06	4.4E-05	2.6E-06	8.2E-05	1.7E-01	2.6E-01	1.7E-01	5.5E-01	1.4E+00	5.4E-01	4.9E+00	1.0E+00	3.6E+00
Heart right ventricle	1.6E-04	5.4E-06	2.4E-06	2.5E-06	2.9E-01	9.7E-01	2.6E-01	2.4E+00	1.0E+00	4.2E-01	3.3E+00	1.2E+00	2.9E+00
Heart left ventricle	2.5E-06	3.6E-06	2.4E-06	2.0E-06	2.8E-01	2.9E-01	2.5E-01	1.1E+00	9.7E-01	9.0E-01	3.5E+00	1.2E+00	3.6E+00
Heart right atrium	2.6E-06	3.3E-06	2.5E-06	2.4E-06	7.4E-01	4.4E-01	6.6E-01	2.5E+00	4.0E+00	1.1E+00	5.2E+00	2.7E+00	2.9E+00
Heart left atrium	9.7E-04	5.7E-05	2.5E-06	2.1E-06	5.2E-01	9.0E-01	5.0E-01	2.1E+00	2.5E+00	1.8E+00	3.2E+00	9.0E-01	2.4E+00
Heart papillary	2.5E-06	2.7E-06	2.5E-06	2.5E-06	2.8E-01	4.0E-01	2.9E-01	1.8E+00	1.0E+00	8.3E-01	4.8E+00	1.1E+00	2.3E+00
Lung	5.3E-06	2.6E-06	2.8E-06	2.5E-06	9.1E-02	2.6E-01	8.7E-02	8.8E-01	7.0E-01	4.4E-01	5.1E+00	4.7E-02	2.6E+00
Spinal cord lumbar	3.0E-04	3.0E-06	3.1E-06	3.1E-06	1.2E-02	3.0E-02	6.3E-03	1.0E+00	1.1E-01	4.7E-01	2.2E+00	5.9E-01	5.5E-01
B L hem cerebellum	2.3E-06	2.7E-06	1.4E-04	1.4E-06	3.4E-03	8.0E-03	1.7E-03	1.3E-02	1.9E-02	3.7E-02	1.1E-01	6.0E-02	1.3E-01
B L hem frontal lobe	2.6E-06	3.0E-06	3.4E-04	2.7E-06	2.4E-02	3.0E-02	4.6E-03	1.5E-01	1.3E-01	2.5E-01	8.1E-01	3.0E-01	4.4E-01
B L hem temporal lobe	4.2E-06	9.8E-06	1.6E-06	6.1E-04	1.8E-02	2.7E-02	5.1E-03	1.5E-01	1.6E-01	2.4E-01	6.8E-01	5.8E-01	4.9E-01
B L hem brainstem	2.5E-06	2.1E-06	8.0E-05	1.8E-06	2.9E-02	2.2E-02	5.5E-03	1.2E-01	1.6E-01	1.6E-01	7.5E-01	4.2E-01	6.4E-01
Right adrenal gland	6.4E-06	2.6E-06	3.0E-04	4.5E-06	1.2E-01	8.5E-01	6.0E-01	1.8E+00	2.1E+00	2.4E+00	6.0E+00	3.7E+00	7.1E+00

Abbreviations
B = Brain
L = Left
LN = Lymph node

Supplemental Table 7: mRNA levels in XLMTM canine tissues

	Saline		Low dose			Mid dose			High dose		
Dog Identifier	SSAN 7	SSAN 16	SSAN 8	SSAN 11	SSAN 18	SSAN 4	SSAN 10	SSAN 14	SSAN 104	SSAN 106	SSAN 132
Triceps brachii	1.1E-07	4.0E-08	2.7E-04	2.7E-04	5.0E-04	9.2E-04	3.4E-03	6.5E-03	2.9E-02	2.8E-03	4.0E-02
Biceps brachii	4.8E-08	5.6E-08	4.9E-04	1.6E-04	1.5E-03	1.6E-03	3.9E-03	1.6E-03	4.8E-02	2.8E-03	2.6E-02
Vastus lateralis	4.9E-08	1.1E-07	4.0E-03	4.6E-04	4.7E-04	6.9E-03	5.7E-03	6.5E-03	3.3E-02	1.1E-03	8.5E-02
Biceps femoris	5.5E-08	3.5E-07	4.8E-03	2.7E-04	1.7E-04	1.9E-03	3.1E-03	1.0E-02	6.1E-02	2.2E-02	2.7E-02
Cranial tibialis	5.3E-08	3.7E-08	1.1E-03	1.0E-03	5.0E-03	3.2E-03	8.4E-03	1.0E-02	4.8E-02	5.0E-03	3.1E-02
Gastrocnemius	4.9E-08	4.3E-07	3.7E-04	1.4E-03	3.8E-04	4.5E-03	1.1E-02	7.7E-03	7.0E-02	3.0E-03	5.9E-02
Paravertebral lumbar	7.5E-08	6.2E-08	8.8E-04	3.3E-04	5.0E-03	2.4E-03	1.4E-02	1.6E-03	4.0E-02	3.3E-03	1.3E-02
Intercostal muscle	6.8E-08	6.0E-08	4.9E-04	2.1E-04	3.2E-03	1.3E-03	5.6E-03	2.1E-03	7.7E-02	8.1E-03	1.2E-02
Diaphragm	4.3E-08	8.3E-08	3.6E-04	1.4E-03	3.0E-04	3.8E-03	3.5E-03	9.9E-04	9.6E-02	1.9E-02	1.1E-01
Heart right ventricle	4.9E-08	5.5E-08	3.1E-04	8.9E-04	1.8E-03	1.9E-03	1.4E-02	1.2E-03	8.4E-02	6.2E-03	5.4E-02
Heart left ventricle	1.0E-07	7.2E-08	5.0E-04	6.9E-05	2.2E-03	4.5E-03	7.7E-03	3.0E-03	1.2E-01	8.6E-03	2.5E-01
Heart right atrium	5.2E-08	2.6E-07	6.6E-03	5.2E-04	2.4E-03	7.8E-03	4.5E-03	1.2E-02	6.1E-02	1.3E-02	1.0E-01
Heart left atrium	5.2E-08	5.3E-08	2.0E-03	3.8E-03	2.1E-03	2.2E-02	1.5E-03	3.8E-03	2.7E-02	1.2E-02	4.7E-02
Heart papillary	5.7E-08	5.3E-08	3.9E-03	1.6E-03	2.9E-03	1.1E-02	2.4E-03	4.7E-04	6.9E-02	3.6E-02	1.5E-01
Liver Left lobe	1.1E-07	1.0E-07	2.4E-05	2.4E-04	1.2E-04	7.1E-05	1.0E-04	9.3E-04	6.1E-04	5.7E-04	2.7E-03
Liver central lobe	1.1E-07	2.0E-07	2.1E-05	1.1E-04	6.9E-05	4.5E-04	3.9E-04	8.0E-04	6.1E-04	1.5E-04	1.7E-03
Liver right lobe	1.1E-07	9.9E-08	7.5E-06	8.0E-05	4.0E-05	3.4E-04	6.4E-04	6.4E-04	6.2E-04	3.8E-04	1.9E-03
Lung	5.7E-08	8.8E-08	1.3E-04	1.3E-03	5.7E-04	8.5E-04	2.2E-03	1.3E-04	8.9E-02	1.2E-03	1.7E-02
Bladder	5.9E-08	4.8E-08	2.4E-04	1.4E-04	7.7E-06	2.6E-04	4.6E-04	4.2E-05	6.6E-03	1.1E-03	5.3E-03
Stomach	6.0E-08	1.3E-06	1.3E-04	3.1E-05	1.7E-05	8.2E-05	3.1E-04	2.5E-04	3.7E-02	4.2E-04	1.3E-03
Spleen	2.6E-08	5.2E-08	5.0E-06	1.3E-04	3.4E-05	2.5E-05	2.4E-05	9.1E-06	1.5E-04	1.6E-06	1.6E-05
Kidneys	4.4E-08	3.9E-07	4.6E-05	6.1E-05	3.2E-05	2.4E-05	1.5E-05	5.5E-06	2.2E-04	5.2E-04	5.7E-04
Brain	1.2E-07	6.4E-08	2.0E-05	2.0E-05	1.7E-05	3.7E-04	2.2E-05	3.2E-04	2.2E-03	4.6E-04	2.7E-03

Supplemental Table 8: Myotubularin protein biodistribution in XLMTM dogs after infusion

	Saline		Low dose			Mid dose			High dose		
Dog Identifier	SSAN 7	SSAN 16	SSAN 8	SSAN 11	SSAN 18	SSAN 4	SSAN 10	SSAN 14	SSAN 104	SSAN 106	SSAN 132
Triceps brachii	ND	5.4E-02	3.1E-03	9.1E-03	3.0E-03	3.8E-01	7.5E-01	5.1E-01	1.4E+00	5.0E-01	4.2E+00
Biceps brachii	3.2E-02	3.6E-03	1.9E-02	5.6E-02	3.3E-02	2.4E-01	5.6E-01	1.4E-01	2.1E+00	1.0E-01	1.2E+00
Vastus lateralis	7.4E-02	1.3E-01	1.3E-01	4.9E-02	8.1E-02	6.4E-01	2.4E-01	6.3E-01	1.6E+00	9.1E-02	2.1E+00
Biceps femoris	6.1E-02	6.9E-03	ND	4.7E-02	9.0E-02	1.0E-01	5.7E-01	4.4E-01	1.5E+00	6.0E-01	1.0E+00
Cranial tibialis	1.1E-02	3.0E-02	3.0E-02	2.8E-02	3.8E-02	1.7E-01	6.9E-01	1.4E-01	2.3E+00	8.3E-02	1.8E-01
Gastrocnemius	1.6E-01	1.2E-01	7.8E-02	1.0E-01	1.7E-01	2.4E-01	4.7E-01	3.6E-01	1.6E+00	2.4E-01	2.7E+00
Paravertebral lumbar	4.3E-02	1.4E-02	7.0E-03	4.0E-02	3.7E-02	2.8E-01	2.5E-01	1.7E-01	1.9E+00	6.4E-02	1.6E+00
Intercostal muscle	5.9E-02	4.3E-02	7.4E-02	6.2E-02	3.6E-02	5.6E-02	1.4E+00	8.0E-02	1.0E+00	4.0E-01	5.4E+00
Diaphragm	4.0E-02	3.7E-02	ND	2.6E-02	ND	1.6E-01	2.6E-01	ND	2.4E+00	3.3E-01	2.7E+00
Heart right ventricle	1.2E-01	1.3E-01	7.0E-02	1.2E-01	1.4E-01	3.2E+00	1.1E+00	2.6E-01	3.5E+00	1.3E+00	4.0E+00
Heart left ventricle	5.8E-01	6.5E-01	5.5E-01	6.2E-01	6.9E-01	4.1E+00	1.6E+00	1.3E+00	1.9E+00	1.1E+00	2.0E+01
Heart right atrium	6.2E-02	2.2E-01	2.4E-01	3.3E-01	2.6E-01	3.0E+00	3.5E+00	9.8E-01	3.9E+00	3.0E+00	3.1E+00
Heart left atrium	3.6E-01	1.8E-01	3.2E-01	3.7E-01	1.6E-01	2.9E+00	2.8E+00	3.1E+00	3.6E+00	6.5E-01	3.7E+00
Heart papillary	2.6E-01	1.6E-01	2.1E-01	2.2E-01	2.8E-01	5.2E-01	5.5E-01	6.4E-01	1.3E+01	2.0E+00	2.0E+00
Liver	ND	ND	3.2E-01	5.8E-01	4.5E-01	7.0E-02	4.6E-02	1.1E+00	2.1E-01	5.4E-01	2.5E-01
Lung	4.1E-01	5.2E-01	2.9E-01	2.5E-01	3.1E-01	2.1E-01	1.8E-01	4.7E-01	3.1E-01	2.7E-01	6.0E-01
Bladder	ND	7.8E-02	5.0E-03	ND	2.0E-03	ND	2.2E-03	4.7E-02	1.5E-01	6.4E-02	4.3E-01
Stomach	1.3E-01	ND	1.9E-01	2.9E-01	5.2E-01	2.5E-01	2.5E-01	6.5E-01	1.1E-01	1.0E+00	9.8E-01
Spleen	5.4E-02	5.8E-02	6.8E-01	ND	2.6E-01	3.0E-01	4.0E-01	7.4E-01	4.0E-02	1.7E-02	9.5E-01
Kidneys	ND	ND	8.3E-02	ND	ND	2.9E-01	2.4E-01	1.7E-01	2.7E-01	3.4E-02	2.3E-01
Brain	ND	1.7E-02	5.4E-03	2.0E-02	ND	5.3E-02	9.7E-02	1.1E-01	8.1E-02	3.5E-02	1.3E-01

Supplemental Table 9a: M-Mode Echocardiogram Measurements of Study Animals

SSAN / Gender	Date(s) of Exam	Age (Weeks)	Weight kg	IVSd cm	LVIDd cm	LVPWd cm	LVIDs cm	EDV ml	ESV ml	EF %	% FS
Normal											
1 F	5/28/13	17	9.119	0.5	2.9	0.5	1.8	31	9	71	39
	7/31/13	27	10.869	0.68	2.92	0.51	2.18	32.79	15.85	51.67	25.35
	1/23/14	52	12.619	0.64	3.17	0.5	1.87	39.89	10.67	73.26	41.04
3 F	5/28/13	17	7.266	0.6	2.7	0.5	1.9	27	11	57	29
	7/31/13	27	8.833	0.47	3.03	0.39	2.09	35.95	14.31	60.18	30.94
	1/23/14	52	11.896	0.61	3.23	0.7	1.98	41.9	12.44	70.32	38.64
5 F	5/28/13	18	8.191	0.7	2.9	0.6	2	32	12	61	31
	7/31/13	27	10.152	0.67	2.67	0.75	1.76	26.27	9.15	65.15	34.12
	1/23/14	52	12.472	0.82	3.04	1	1.75	36.03	9.03	74.94	42.38
6 M	7/31/13	18	8.213	0.53	2.37	0.65	1.09	19.52	2.63	86.54	53.79
	10/1/13	27	10.664	0.76	2.8	0.66	1.61	29.62	7.28	75.43	42.58
	1/23/14	43	10.047	0.87	3.27	0.71	1.77	43.09	9.28	78.46	45.9
12 M	1/23/14	38	15.32	0.64	3.36	0.85	2.21	46.19	16.47	64.33	34.15
Saline											
7 F	5/28/13	18	6.712	0.6	2.4	0.6	1.6	20	8	62	32
9 F	5/28/13	18	5.202	0.5	3	0.6	1.9	35	11	68	36
13 F	7/31/13	17	6.7	0.55	2.54	0.59	1.41	23.23	5.17	77.75	44.42
101 F	5/15/14	18	6.258	0.48	2.42	0.48	1.53	20.48	6.4	68.76	36.58
Low Dose											
8 M	7/30/13	18	6.478	0.56	2.34	0.51	1.43	18.91	5.38	71.54	38.71
11 F	5/28/13	18	7.103	0.6	2.4	0.5	1.7	21	9	54	28
	7/31/13	27	6.639	0.52	2.44	0.75	1.27	21.09	3.89	45	48.11
18 M	1/23/14	36	15.563	0.8	3.24	0.78	2.12	42.33	14.84	64.94	34.49
Mid Dose											
10 M	7/31/13	18	9.439	0.59	2.61	0.59	1.26	24.84	3.84	84.54	51.66
	10/1/13	27	11.911	0.74	3.03	0.75	1.75	35.93	9	74.95	42.39
14 M	1/23/14	38	13.076	0.74	2.97	0.69	2.06	34.19	13.71	59.9	30.69
High Dose											
104 M	5/15/14	18	12.498	0.59	2.46	0.65	1.49	21.47	6	72.05	39.28
	7/18/14	27	17.65	0.79	3.14	0.54	2.13	39.04	14.97	61.65	32.04
106 M	5/15/14	18	8.545	0.78	2.29	0.61	1.45	17.96	5.54	69.15	36.75
	7/18/14	27	12.6	0.71	3.01	0.63	2	35.33	12.66	64.17	33.73
132 M	6/25/14	17	12.9	0.6	3.08	0.61	1.9	37.23	11.15	70.06	38.29
	7/18/14	21	15.5	0.72	3.26	0.75	1.84	42.95	10.3	76.01	43.58

Abbreviations:
F = Female
M = Male
SSAN =Study Specific Animal Number
IVSd = interventricular septum thickness at end-diastole
LVIDd = left ventricular internal dimension at end- diastole
LVPWd = left ventricular posterior wall thickness at end-diastole
LVIDs = left ventricular internal dimension at end-systole
EDV = end-diastolic volume of the left ventricle
ESV = end-systolic volume of the left ventricle
EF = ejection fraction
FS = fractional shortening

Supplemental Table 9b: Doppler Echocardiogram Measurements of Study Animals

SSAN / Gender	Date(s) of Exam	Age Weeks	MV E Max vel. m/s	MV A Max vel. m/s	MV E/A	e' m/s	MV E/e'
Normal							
1 F	5/28/13	17	0.69	0.35	1.97	0.1	6.9
	7/31/13	27	0.72	0.47	1.53	0.07	10.29
	1/23/14	52	0.74	0.45	1.64	0.08	9.25
3 F	5/28/13	17	0.54	0.34	1.59	0.06	9
	7/31/13	27	0.86	0.43	2	0.08	10.75
	1/23/14	52	0.74	0.51	1.45	0.08	9.25
5 F	5/28/13	18	0.81	0.48	1.69	0.07	11.57
	7/31/13	27	0.91	0.58	1.57	0.07	13
	1/23/14	52	0.94	0.85	1.11	0.08	11.75
6 M	7/31/13	18	1.16	0.36	3.22	0.08	14.5
	10/1/13	27	0.99	0.48	2.06	0.1	6.6
	1/23/14	43	0.87	0.34	2.56	0.11	7.91
12 M	1/23/14	38	0.74	0.31	2.39	0.14	5.29
Saline							
7 F	5/28/13	18	0.6	0.43	1.4	0.07	8.57
9 F	5/28/13	18	0.775	0.51	1.52	0.08	9.69
13 F	7/31/13	17	0.93	0.53	1.75	0.07	13.29
101 F	5/15/14	18	0.81	0.32	2.53	0.11	7.36
Low Dose							
8 M	7/30/13	18	0.79	0.42	1.88	0.06	13.17
11 F	5/28/13	18	0.82	0.36	2.28	0.07	11.71
	7/31/13	27	0.79	0.43	1.84	0.07	11.29
18 M	1/23/14	36	0.65	0.36	1.81	0.11	5.91
Mid Dose							
10 M	7/31/13	18	0.96	0.45	2.13	0.09	10.67
	10/1/13	27	0.95	0.53	1.79	0.09	8.33
14 M	1/23/14	38	0.76	0.43	1.77	0.2	3.8
High Dose							
104 M	5/15/14	18	1.14	0.29	3.93	0.1	11.4
	7/18/14	27	0.87	0.4	2.18	0.11	7.91
106 M	5/15/14	18	0.77	0.33	2.33	0.1	7.7
	7/18/14	27	0.88	0.54	1.63	0.09	9.78
132 M	6/25/14	17	0.96	0.64	1.5	0.08	12
	7/18/14	21	0.87	0.5	1.74	0.08	10.88

MV E = early filling velocity
MV A = active filling velocity
e' =tissue velocity of the mitral annulus

Supplemental Table 9c: Doppler Echocardiogram Measurements of Study Animals

SSAN / Gender	Date(s) of Exam	Age weeks	AV Max	LVOT Max m/s	LVOT VTI	IVRT ms	PV Max m/s	PV Accl Time	PV ET ms	PAT/ET	TR Max Vel. m/s	TR max PG mm/Hg
Normal												
1 F	5/28/13	17	1.1	0.86	8.82	39.9	0.83	50.35	148.2	0.34	none	
	7/31/13	27	1.3	1.06	11.43	42.8	1.06	98.91	187.4	0.53	2.58	26.62
	1/23/14	52	1.3	1	12.39	38.04	0.99	79.89	206.4	0.39	2.72	29.68
3 F	5/28/13	17	0.7	0.69	7.95	51.3	0.81	79.8	191	0.42	none	
	7/31/13	27	1	0.93	11.55	43.75	0.94	53.26	201.6	0.26	none	
	1/23/14	52	1.1	0.81	8.88	ns	1.94	89.4	182.6	0.49	none	
5 F	5/28/13	18	1.2	0.98	10.73	49.4	ns	ns	ns	ns	none	
	7/31/13	27	1.5	1.15	12.23	40.89	1.29	51.36	204.5	0.25	2.68	28.71
	1/23/14	52	1.2	1.15	14.08	46.65	1.37	66.57	191.2	0.35	2.39	29.68
6 M	7/31/13	18	1.4	0.99	10.95	35.19	1.24	82.74	197.8	0.42	none	
	10/1/13	27	1.5	1.25	13.95	36.14	0.89	79.89	156.9	0.51	none	
	1/23/14	43	1.2	1.11	11.91	39.94	ns	79.89	194	0.41	none	
12 M	1/23/14	38	1.6	1.44	16.4	58.01	ns	87.49	195.9	0.45	2.73	29.81
Saline												
7 F	5/28/13	18	1.2	1.14	7.57	29.45	0.94	58.9	149.2	0.39	none	
9 F	5/28/13	18	0.9	0.76	7.73	42.75	0.96	91.2	171	0.53	none	
13 F	7/31/13	17	1.2	0.9	10.08	ns	0.83	61.82	186.4	0.33	none	
101 F	5/15/14	18	1	0.78	9.15	ns	1.08	75.13	175	0.43	none	
Low Dose												
8 M	7/30/13	18	1	0.81	8.55	37.09	1.38	47.55	180.7	0.26	none	
11 F	5/28/13	18	1.3	1.42	10.45	31.35	1	72.2	173.9	0.42	none	
	7/31/13	27	1.4	1.23	12.2	47.55	ns	ns	ns	ns	none	
18 M	1/23/14	36	1.1	1.07	12.48	47.55	1.21	96.05	180.7	0.53	none	
Mid Dose												
10 M	7/31/13	18	1.5	0.92	10.39	44.7	1.48	72.28	180.7	0.4	none	
	10/1/13	27	1.1	0.84	10.76	39.94	1.48	77.98	174	0.45	none	
14 M	1/23/14	38	1.2	0.96	11.2	43.75	1.1	74.18	175.9	0.42	none	
High Dose												
104 M	5/15/14	18	1.2	0.99	10.48	ns	0.76	84.64	180.7	0.47	none	
	7/18/14	27	1.2	1.13	11.01	ns	1.25	87.49	207.3	0.42	none	
106 M	5/15/14	18	1.3	1.26	12.72	ns	1.33	62.77	170.2	0.37	none	
	7/18/14	27	1.2	1.13	11.01	ns	1.25	87.49	207.3	0.42	none	
132 M	6/25/14	17	1.5	0.95	10.11	48.5	1.42	65.62	156	0.42	none	
	7/18/14	21	1.3	1.19	13.16	ns	1.28	ns	ns	ns	2.4	23.03

ns= not seen

AV max = Aortic valve velocity

LVOT = left ventricular outflow tract velocity

IVRT = isovolumic relaxation time

PV = pulmonic valve

ET = ejection time

TR = tricuspid regurgitation velocity

PG = pressure gradient

ms = millisecond