

SUPPLEMENTARY INFORMATION

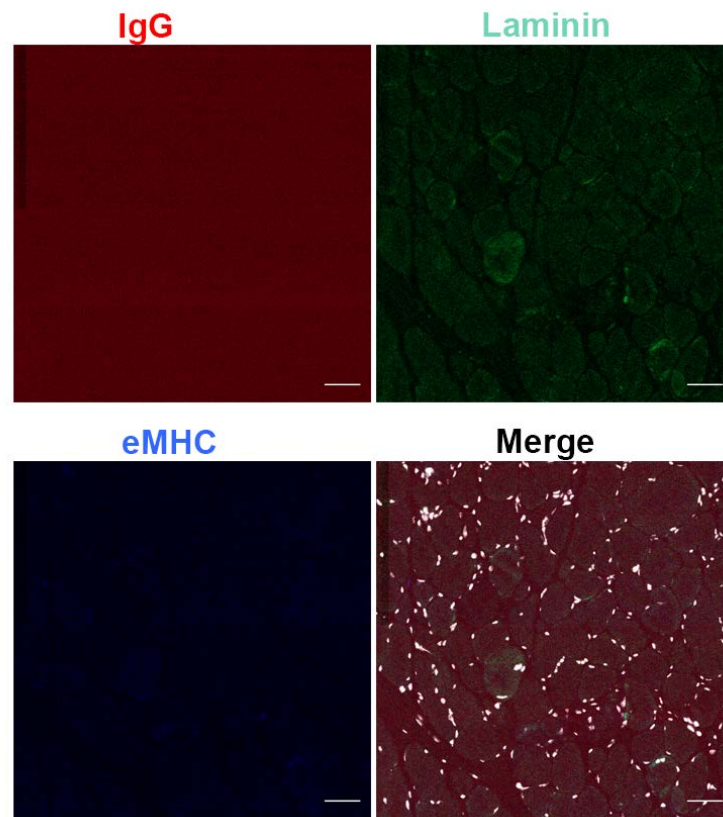
for

Therapeutic Potential of SAT-3247 in a Canine Model of Duchenne Muscular Dystrophy

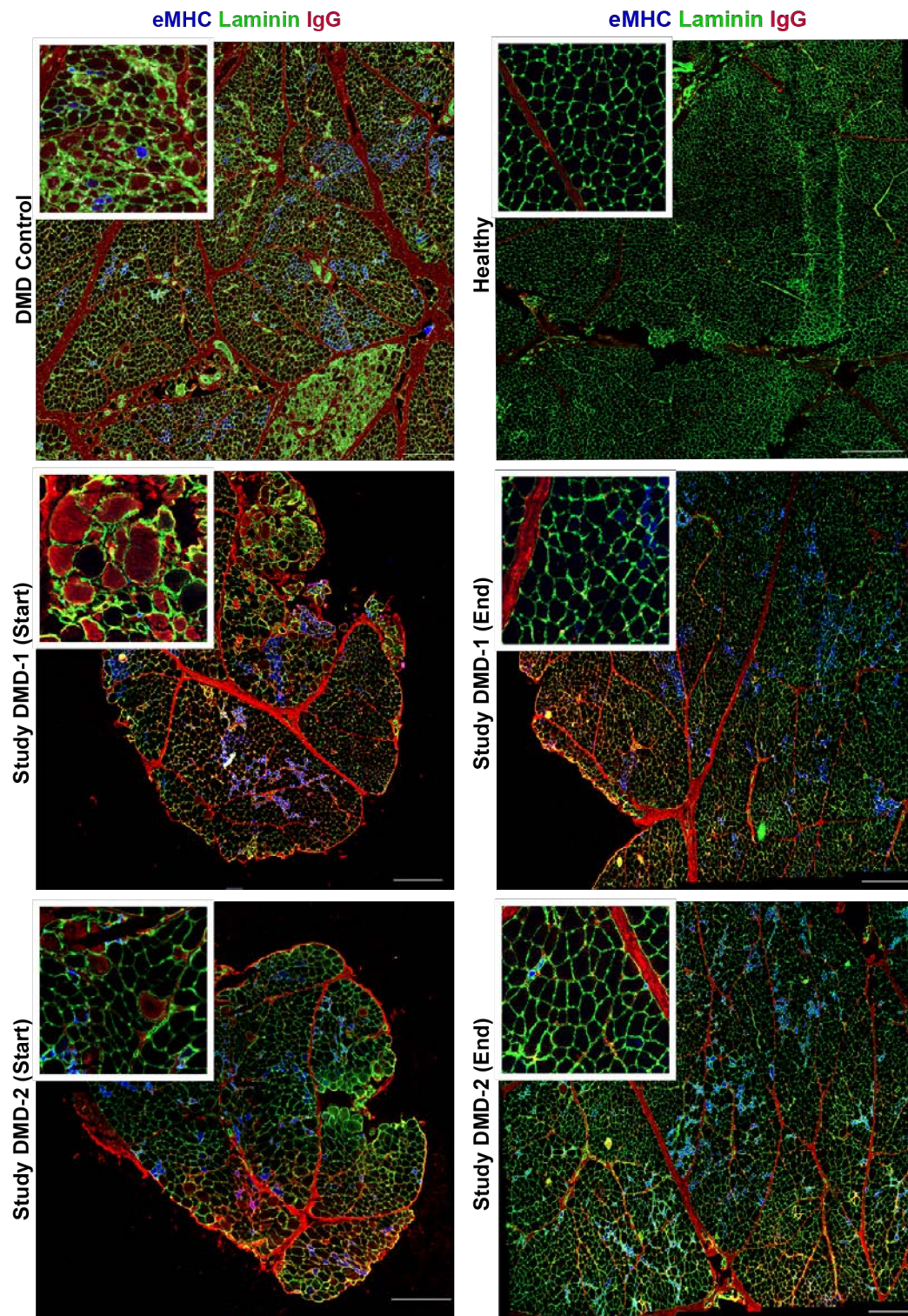
Shanti S. S. Rayagiri, Johnathan K. Smid, Phil Lambert, James Jaquith, Tessa Murray, Ryan Mitchell, Frank Gleeson, Jacob G. Monast, James A. Teixeira, Yongping Yue, Matthew J. Burke, Gang Yao, Dongsheng Duan, and Michael A. Rudnicki

Supplemental Figures 1-7

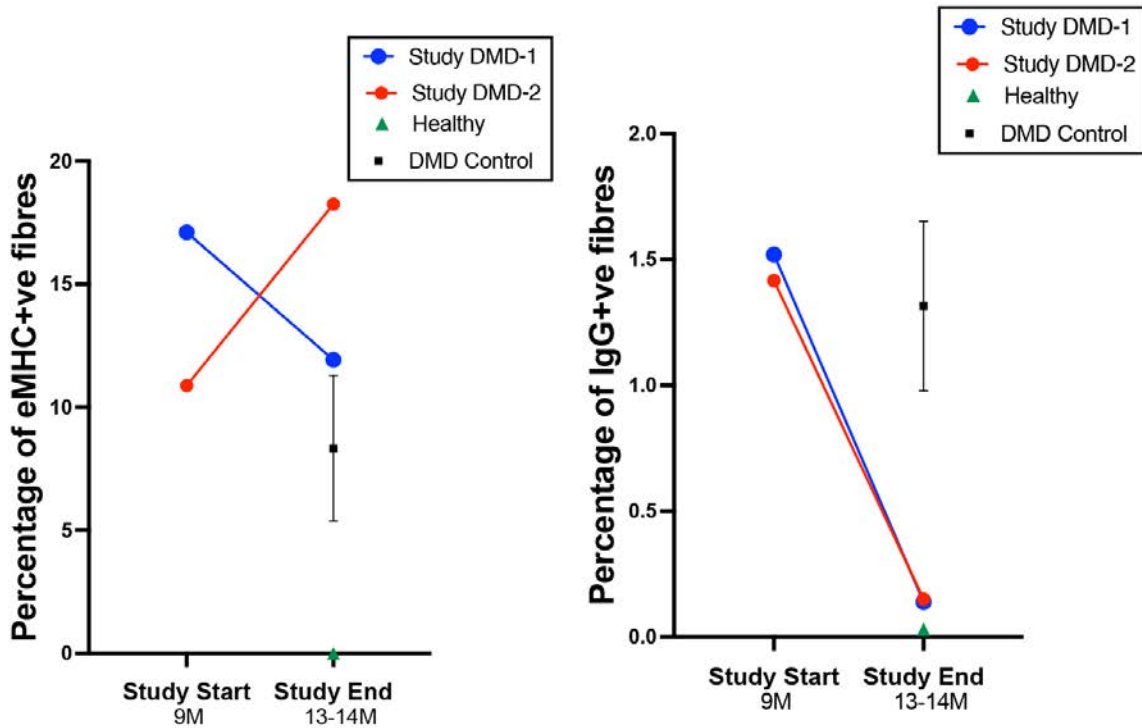
Supplemental Tables 1-2



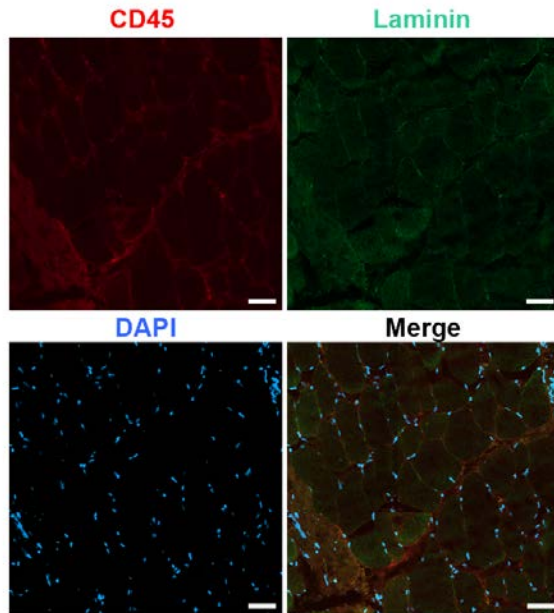
Supplemental Figure 1: Representative image of secondary controls. (a) No signal is observed for IgG (red), Laminin (green) and eMHC (blue). Merge image showing nuclei counterstained with DAPI (white). Scale bar is 50 μ m.



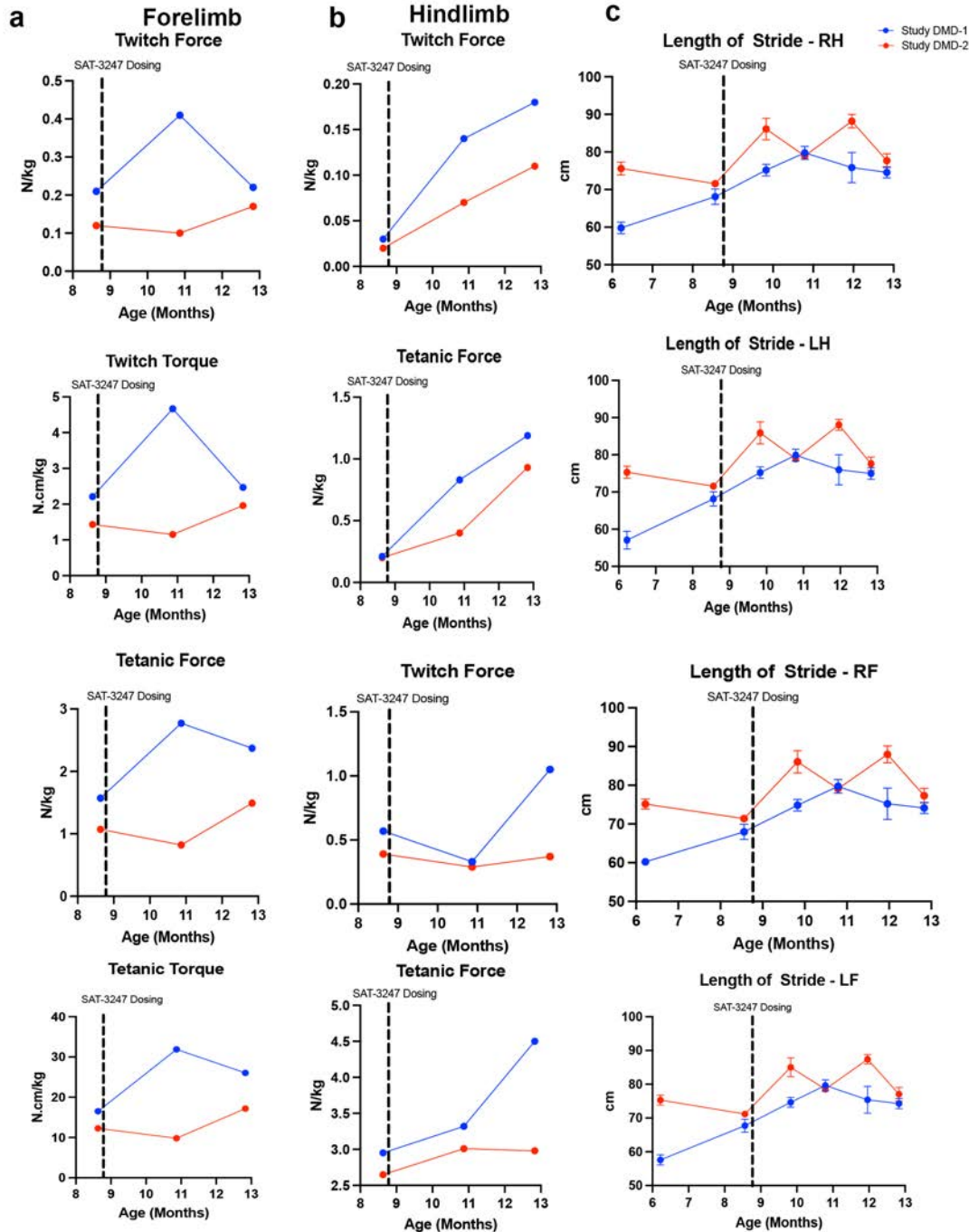
Supplemental Figure 2: Expression of IgG and eMHC in *biceps femoris* muscles:
 Representative immunofluorescence image of *biceps femoris* muscle sections of DMD (untreated), healthy, *Study DMD-1*, *Study DMD-2* stained for IgG (red) and embryonic myosin heavy chain (eMHC, blue). Scale bar is 500 μ m.



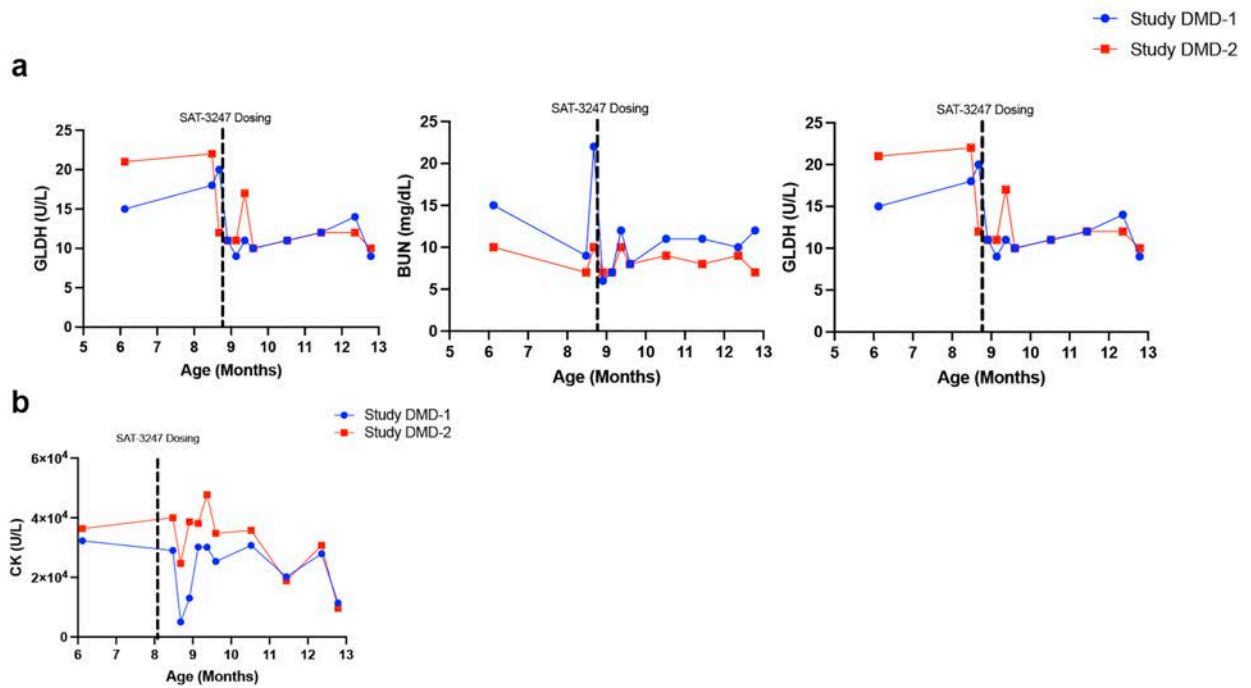
Supplemental Figure 3: Percentage of IgG and eMHC in *biceps femoris* muscles: Percentage of fibers positive for IgG and eMHC indicated for *Study DMD-1* (blue) and *Study DMD-2* (red). Overall eMHC⁺ fibers increased in *Study DMD-2* (red) with a drop in *Study DMD-1* (blue). Dramatic decrease in IgG⁺ fibers observed in both *Study DMD-1* (blue) and *Study DMD-2* (red). Error bars represent SEM.



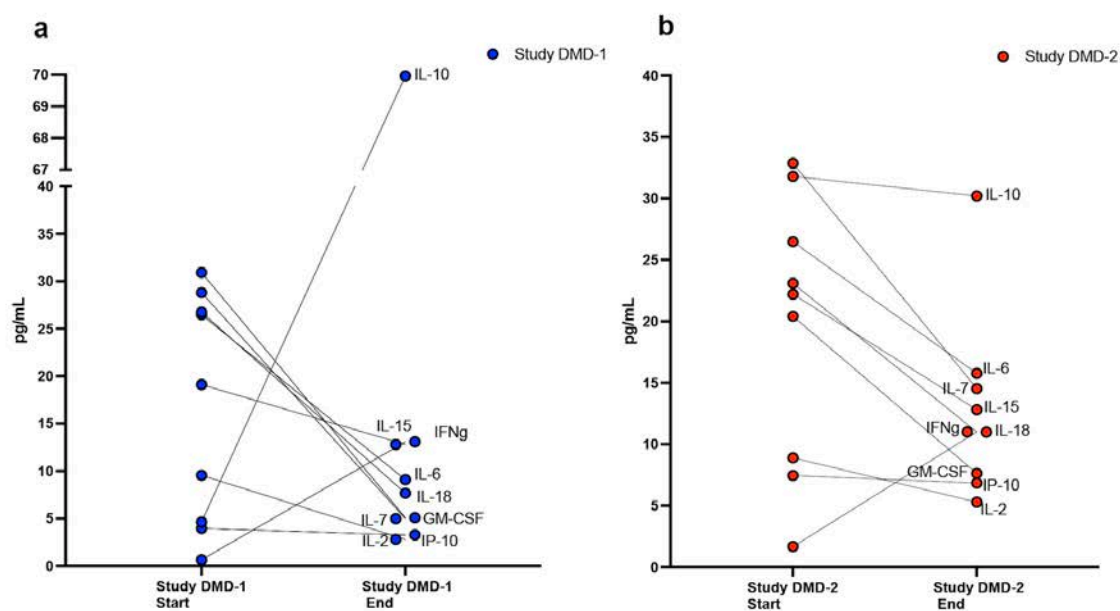
Supplemental Figure 4: Representative image of secondary controls in *biceps femoris* muscles (a) No signal is observed for CD45 (red) and Laminin (green). DAPI was used to stain the nuclei. Scale bar is 50 μ m.



Supplemental Figure 5: Whole limb function: (a) Forelimb flexion contractile properties in *Study DMD-1* (blue) and *Study DMD-2* (red) after SAT-3247 treatment. (b) Hindlimb flexion contractile properties in *Study DMD-1* (blue) and *Study DMD-2* (red) after SAT-3247 treatment. (C) Stride length of *Study DMD-1* (blue) and *Study DMD-2* (red) was maintained before and after dosing with SAT-3247. (LF-Left fore, RF-Right fore, LH-Left hind, RH-Right hind).



Supplemental Figure 6: Clinical pathology: (a) No change in platelet count, liver function (glutaraldehyde dehydrogenase (GLDH)), and kidney function (blood urea nitrogen (BUN)) with SAT-3247 treatment in *Study DMD-1* (blue) and *Study DMD-2* (red). (b) Creatine Kinase remained elevated at 13 months in *Study DMD-1* (blue) and *Study DMD-2* (red).



Supplemental Figure 7: Cytokine profiling: In both *Study DMD-1* (blue) and *Study DMD-2* (red) anti-inflammatory cytokine (IL-10) increased or remained stable by the end of the study. Pro-inflammatory cytokines remained stable or decreased with the SAT-3247 treatment.

	Weightinkgs			Weightinkgs	
Age (Months)	Study DMD-1	Study DMD-2	Age (Months)	Study DMD-1	Study DMD-2
6.2	9.1	12.7	11	10.4	14.5
7.3	10.2	14.5	11	10.7	15.0
8.3	10.7	14.1	11.1	10.7	15.2
8.8	9.1	13.6	11.2	10.7	15.0
8.8	9.1	13.4	11.2	10.7	14.7
8.9	9.1	13.4	11.2	10.4	15.0
8.9	9.3	13.6	11.4	10.4	15.2
9	9.5	13.4	11.4	10.0	14.3
9.1	10.2	13.6	11.4	10.2	14.5
9.1	9.5	14.1	11.5	10.4	14.3
9.1	9.5	13.8	11.6	10.0	14.5
9.3	9.8	13.6	11.6	10.0	14.5
9.3	9.8	14.1	11.7	10.7	15.0
9.3	9.8	13.6	11.7	10.7	15.0
9.4	9.8	13.8	11.8	10.9	15.0
9.5	10.4	14.5	11.9	10.4	15.0
9.5	10.0	13.6	11.9	10.2	14.5
9.6	10.0	13.6	11.9	10.4	14.7
9.6	9.5	13.4	12.1	10.4	15.0
9.7	10.0	13.8	12.1	10.4	14.3
9.8	9.8	13.6	12.1	10.4	14.3
9.8	10.9	13.8	12.2	10.4	14.3
9.8	10.0	13.8	12.3	10.7	14.5
10	10.0	13.8	12.3	10.9	14.7
10	10.2	14.1	12.4	10.2	14.3
10	10.0	13.8	12.4	10.4	14.7
10.1	10.2	14.3	12.5	11.1	15.0
10.2	10.0	14.3	12.6	11.1	15.0
10.2	10.4	14.5	12.6	10.4	14.7
10.3	10.0	14.7	12.6	11.1	14.7
10.3	10.7	14.7	12.8	10.4	14.3
10.4	10.7	14.5	12.8	10.4	14.1
10.5	10.7	15.0	12.8	10.4	14.1
10.5	10.7	14.7	12.9	10.7	14.3
10.5	10.4	15.0	13	10.7	14.3
10.7	10.7	14.5	Min	9.1	12.7
10.7	10.9	15.0	Max	11.1	15.2
10.7	10.7	15.0			
10.8	10.9	15.2			
10.9	9.5	14.7			
10.9	10.4	14.3			

Supplemental Table 1: Body weights in kgs: (a) Body weights documented from 6 months to 13 months. No drop in weight was observed with SAT-3247 treatment, indicating no side effect of the drug.

Electrocardiography reading		
2D	M-Mode	
	Study DMD-1	Study DMD-2
IVSd	8.9mm	9.4mm
LVIDd	30.9mm	31.9mm
LVPWd	7.5mm	8.4mm
NLVIDd	1.463	1.356
LVIDs	19.3mm	22.1mm
%FS	37.70%	30.63%
NLVIDs	0.761	0.766
Ao Diam	1.66cm	1.70cm
LA Diam	2.00cm	2.40cm
LA/Ao	1.2	1.41
Ao/LA	0.83	0.71
LA LAX	30.5mm	31.7mm
NLA LAX	1.465	1.374
LVLd A4C	4.5cm	5.2mm
LVEDV MOD A4C	19.5ml	32.4 ml
EDVI	40.5 ml/m ²	53.2 ml/m ²
LVLs A4C	3.4cm	4.0cm
LVESV MOD A4C	8.3ml	11.9 ml
ESVI	17.4 ml/m ²	19.6 ml/m ²
LVEF MOD A4C	57.16%	63.20%
SV MOD A4C	11.12 ml	20.49 ml
Doppler		
MVE Vel	0.88 m/s	0.96 m/s
MVE/A Ratio	1.94	1.72
MAA Vel	0.46 m/s	0.56 m/s
E' Lat	0.13 m/s	0.15 m/s
A'	0.08 m/s	0.08 m/s
LVOT Vmax	1.22 m/s	1.29 m/s
LVOT maxPG	5.96 mm Hg	6.68 mmHg
IVRT	49.02 m s	68.63 ms
PV Vmax	0.91 m/s	1.21 m/s
PV maxPG	3.28 mm Hg	5.89 mmHg
Prens Vmax	0.84 m/s	-
PRend PG	2.84 mm Hg	-

Supplemental Table 2: Electrocardiogram: Electrocardiography reading for both *Study DMD-1* and *Study DMD-2* at 13 months of age after dosing with SAT-3247. Higher %FS and LVEF, indicating no cardiac involvement at 13 months of age for a DMD model.