Supplemental information

Persistence of exon 2 skipping and dystrophin

expression at 18 months after U7snRNA-

mediated therapy in the Dup2 mouse model

Liubov V. Gushchina, Adrienne J. Bradley, Tatyana A. Vetter, Jacob W. Lay, Natalie L. Rohan, Emma C. Frair, Nicolas Wein, and Kevin M. Flanigan

Supplemental Tables

Table S1. Animals body weight.

Groups	Body weight at necropsy, g	
	n	Mean ± SEM
Dup2-Diluent	9	36.40 ± 1.17
Dup2-ACCA	11	39.66 ± 1.12
C57Bl/6	11	39.74 ± 1.38

Table S2. qPCR quantification of scAAV9.U7.ACCA vector genome biodistribution in WT control and Dup2 treated animals.

Muscles	Groups	Vector Copies /Diploid Genome	
		n	Mean ± SEM
TA	Dup2-Diluent	9	0.00065
	Dup2-ACCA	11	0.38 ± 0.10
	C57Bl/6	11	0.002 ± 0.0004
Heart	Dup2-Diluent	9	0.0006
	Dup2-ACCA	10	0.75 ± 0.18
	C57Bl/6	11	0.00073
Dia	Dup2-Diluent	9	0.003 ± 0.001
	Dup2-ACCA	11	0.65 ± 0.40
	C57Bl/6	10	Undermined
Liver	Dup2-Diluent	9	Undermined
	Dup2-ACCA	11	85.47 ± 27.91
	C57Bl/6	11	Undermined
Brain	Dup2-Diluent	7	Undermined
	Dup2-ACCA	11	0.39 ± 0.09
	C57Bl/6	11	Undermined

Table S3. RT-PCR quantification analysis of exon 2 skipping in WT control and Dup2 treated mice.

Muscles	Groups	n	% Dup2 transcript	% WT transcript	% Del2 transcript	% WT + Del2 (therapeutic transcript)
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
TA	Dup2-Diluent	n = 9	96.81 ± 1.03	3.19 ± 1.03	0.0 ± 0.0	3.19 ± 1.03
	Dup2-ACCA	n = 10	54.02 ± 6.7	23.11 ± 2.15	22.87 ± 5.05	45.98 ± 6.7
	C57Bl/6	n = 10	0.0 ± 0.0	100 ± 0.0	0.0 ± 0.0	100 ± 0.0
Heart	Dup2-Diluent	n = 9	99.57 ± 0.11	0.43 ± 0.11	0.0 ± 0.0	0.43 ± 0.11
	Dup2-ACCA	n = 10	26.56 ± 5.17	36.80 ± 1.76	36.64 ± 4.9	73.44 ± 5.17
	C57Bl/6	n = 11	0.0 ± 0.0	100 ± 0.0	0.0 ± 0.0	100 ± 0.0
Dia	Dup2-Diluent	n = 9	98.78 ± 0.39	1.22 ± 0.39	0 ± 0.0	1.22 ± 0.39
	Dup2-ACCA	n = 11	68.18 ± 4.71	20.13 ± 2.5	11.69 ± 2.44	31.82 ± 4.71
	C57Bl/6	n = 10	0.0 ± 0.0	100 ± 0.0	0.0 ± 0.0	100 ± 0.0

Table S4. Percentage of positive dystrophin fibers (PPDF) and dystrophin intensity in WT control and Dup2 treated mice.

Muscles	Groups	PDPF (%)		Dystrophin Intensity	
		n	Mean ± SEM	n	Mean ± SEM
TA	Dup2-Diluent	9	12.47 ± 1.67	9	6.72 ± 0.3
	Dup2-ACCA	11	51.87 ± 7.15	11	29.59 ± 4.95
	C57Bl/6	9	99.45 ± 0.16	9	102.7 ± 2.81
Heart	Dup2-Diluent	8	1.18 ± 0.19	8	3.7 ± 0.34
	Dup2-ACCA	10	96.50 ± 0.68	10	43.13 ± 1.7
	C57Bl/6	10	99.90 ± 0.04	10	100.8 ± 3.91
Dia	Dup2-Diluent	7	4.03 ± 1.21	7	3.94 ± 0.42
	Dup2-ACCA	11	47.45 ± 2.87	11	25.66 ± 1.96
	C57Bl/6	7	96.33 ± 0.91	7	98.44 ± 1.99

Table S5. Western blot dystrophin quantification analysis in WT control and Dup2 mice treated with diluent or with scAAV9.U7.ACCA vector.

Muscles	Groups	% Dystrophin expression	
		n	Mean ± SEM
TA	Dup2-Diluent	9	13.40 ± 1.49
	Dup2-ACCA	11	41.92 ± 8.93
Heart	Dup2-Diluent	7	3.01 ± 0.31
	Dup2-ACCA	10	65.36 ± 5.12
Dia	Dup2-Diluent	9	5.01 ± 0.43
	Dup2-ACCA	9	17.68 ± 1.89

Table S6. Specific force and eccentric contraction quantification analysis in WT control and Dup2 treated mice.

| Groups	Tibialis anterior						Diaphragm	
	Specific force, mN/mm^2		Eccentric contractions (ECC), % Force decrease		ECC area under the curve (AUC), Absolute Units		Specific force, mN/mm^2	
	n*	Mean ± SEM	n*	Mean ± SEM**	Mean ± SEM		n	Mean ± SEM
Dup2-Diluent	12	147.7 ± 4.23	12	25.75 ± 2.2	429.5 ± 22.61		9	123.0 ± 9.25
Dup2-ACCA	14	168.1 ± 10.05	14	53.24 ± 5.0	619 ± 40.75		11	181.0 ± 13.33
C57Bl/6	15	239.8 ± 10.78	9	87.46 ± 2.8	848.6 ± 17.76		11	246.9 ± 18.99

[*]n corresponds the number of legs that were used for independent TA muscle analysis during tetanic and eccentric contractions.
[**]Force retained is represented as percentage of 1st contraction after 10 cycles of contractions.

Table S7. Histopathological and Morphometric analysis in WT control and Dup2 treated mice.

| Muscles	Groups	CNF (%)		Fiber size (µm)	
		n	Mean ± SEM	n	Mean ± SEM
TA	Dup2-Diluent	9	58.83 ± 2.24	9	43.72 ± 0.74
	Dup2-ACCA	11	65.83 ± 1.79	11	47.71 ± 0.94
	C57Bl/6	9	3.12 ± 0.45	9	48.16 ± 1.26
Dia	Dup2-Diluent	7	27.76 ± 2.64	7	25.50 ± 0.46
	Dup2-ACCA	11	34.79 ± 1.78	11	28.60 ± 0.32
	C57Bl/6	7	12.38 ± 3.45	7	27.93 ± 0.88

Supplemental Figures
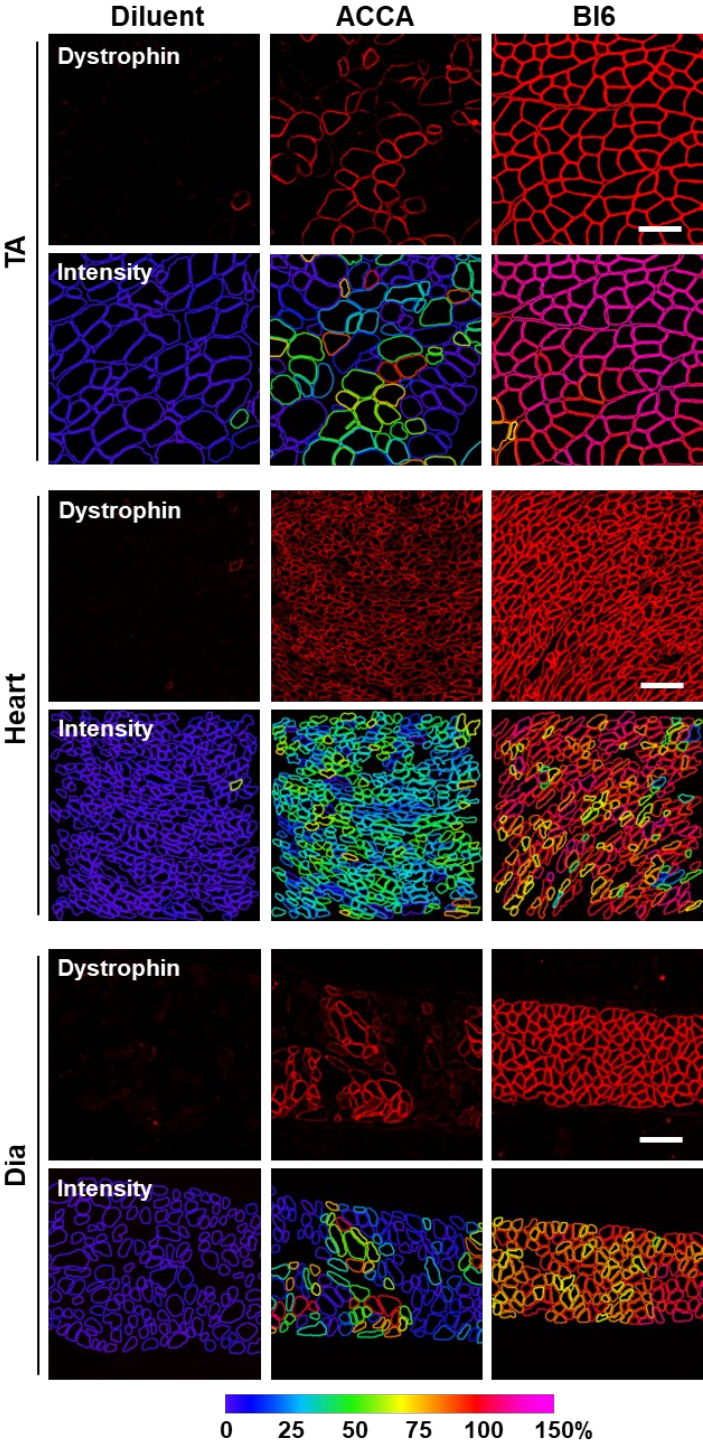
Figure S1. Dystrophin expression in skeletal muscles and heart 18 months post scAAV9.U7.ACCA administration. Representative immunofluorescent images show selected regions of interest (ROI) of left TA (TA), heart and diaphragm (Dia) sections with dystrophin in red displaying localization and intensity of dystrophin signal in Bl6, Dup2-diluent (Diluent) or Dup2-ACCA (ACCA) mice. Color-coded heatmaps of each tissue demonstrate the normalized dystrophin intensity (intensity) for each muscle fiber. Color key in bottom shows intensity heatmaps. Scale bars = 100 μm.
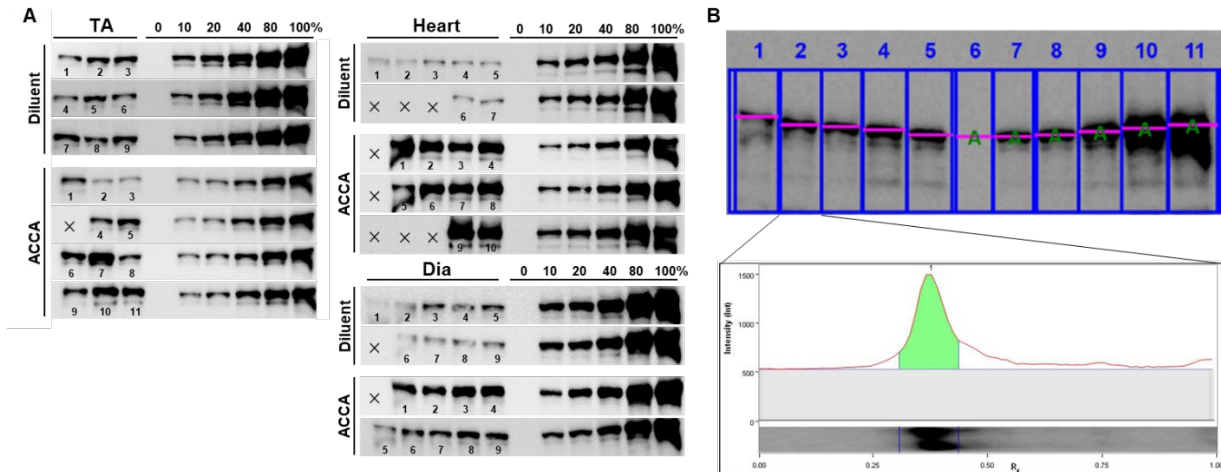
Figure S2. Long-term restoration of full-length dystrophin in Dup2 mice following systemically administered svAAV9.U7.ACCA 18 months post injection. (A) Western blot images of left tibialis anterior (TA) muscles, heart and diaphragm (Dia) showing dystrophin expression in Dup2 mice treated with diluent (Diluent) or scAAV9.U7.ACCA (ACCA) vector 18 months post injection. All tested samples were numbered 1 to 11, and the right six lanes of every blot contain a 6-point standard curve of pooled Bl6 (n = 7-8) lysate diluted in Dup2Del18-41 dystrophin-null muscle lysate, ranging from 0% to 100%. **(B)** Representative WB image of the Dia tissue from the Dup2-ACCA group (top panel) showing dystrophin bands quantification

analysis (bottom panel) using the Image Lab software. The pink lanes represent dystrophin bands that were used to make a 5-point standard curve containing 0%-80% dystrophin (lanes 6-10, A-absolute value) and then to quantify the percentage of dystrophin in the study samples (lanes 1-5). The 6th-point of the standard curve, containing 100% dystrophin, was excluded from the analysis to maintain its linearity of R2 ≥ 0.9. The bottom panel represents the area under the curve of lane 2. For dystrophin quantification analysis, the background in all samples was subtracted with a disk size of 50. https://www.bio-rad.com/en-us/product/image-lab-software?ID=KRE6P5E8Z
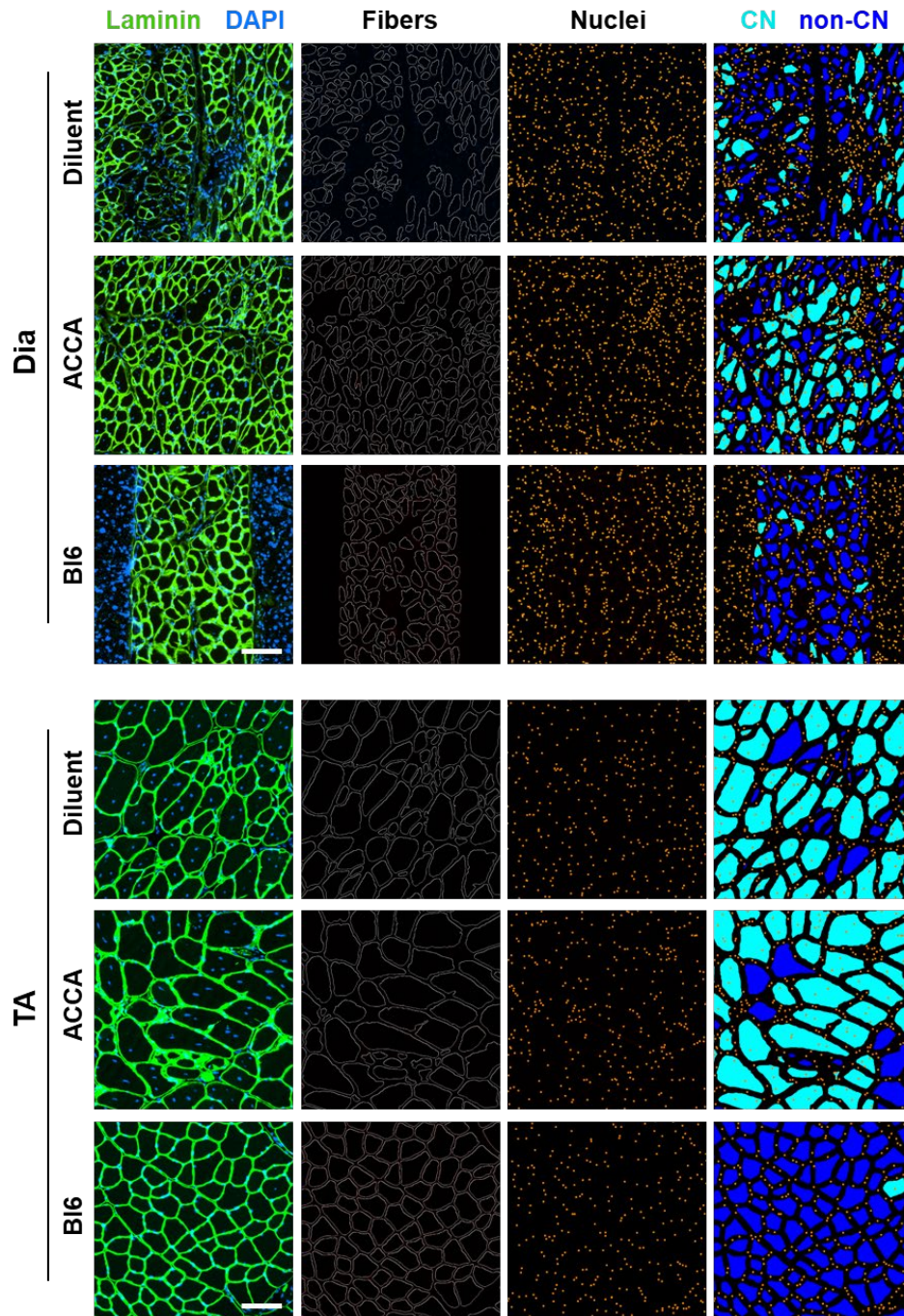
Figure S3. Representative immunofluorescent images show selected regions of interest (ROI) of left side of TA and Dia sections with laminin in green and DAPI in blue that were used for central nucleation (CN) analysis in Bl6, Dup2-diluent (Diluent) or Dup2-ACCA (ACCA) mice. Light teal and dark blue represent CN and non-CN fibers. Scale bar = 100 μm.