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Supplemental Information

Micro-dystrophin AAV Vectors Made by Transient Transfection and Herpesvirus System Are Equally Potent in Treating mdx Mouse Muscle Disease

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Short title: AAV made by TT and HSV methods are equally potent

Supplemental Figure 1

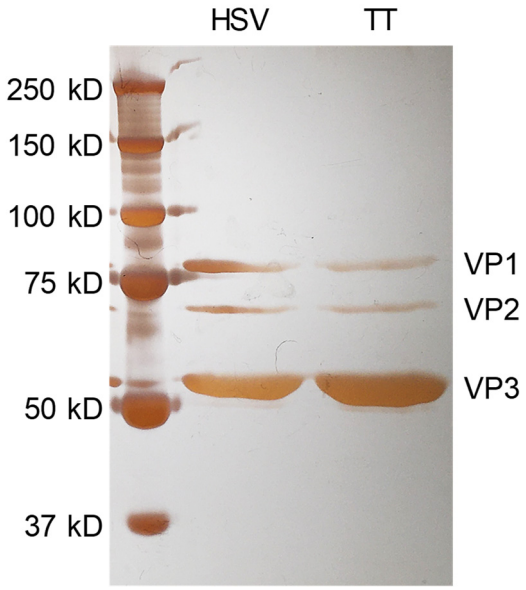
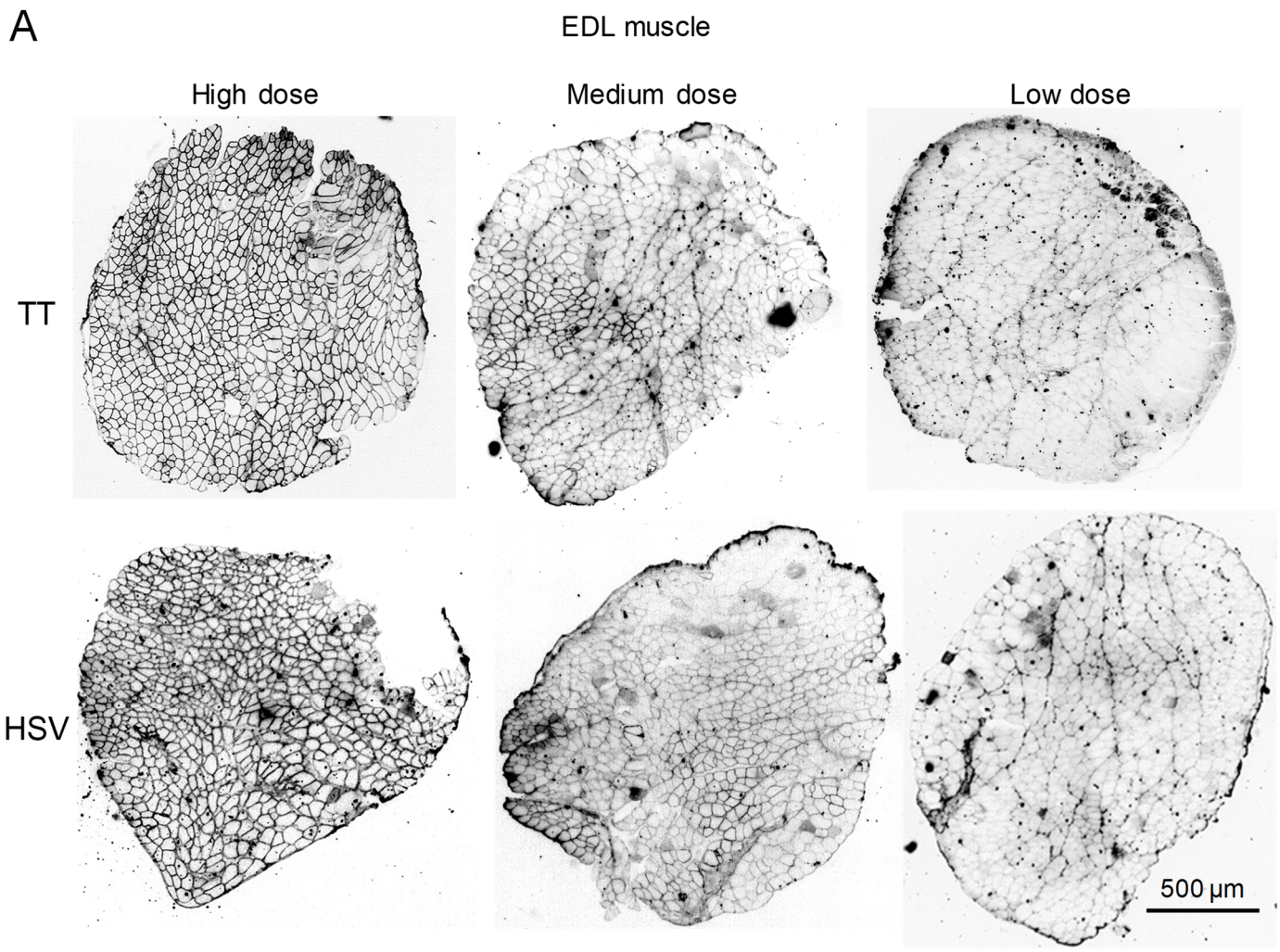
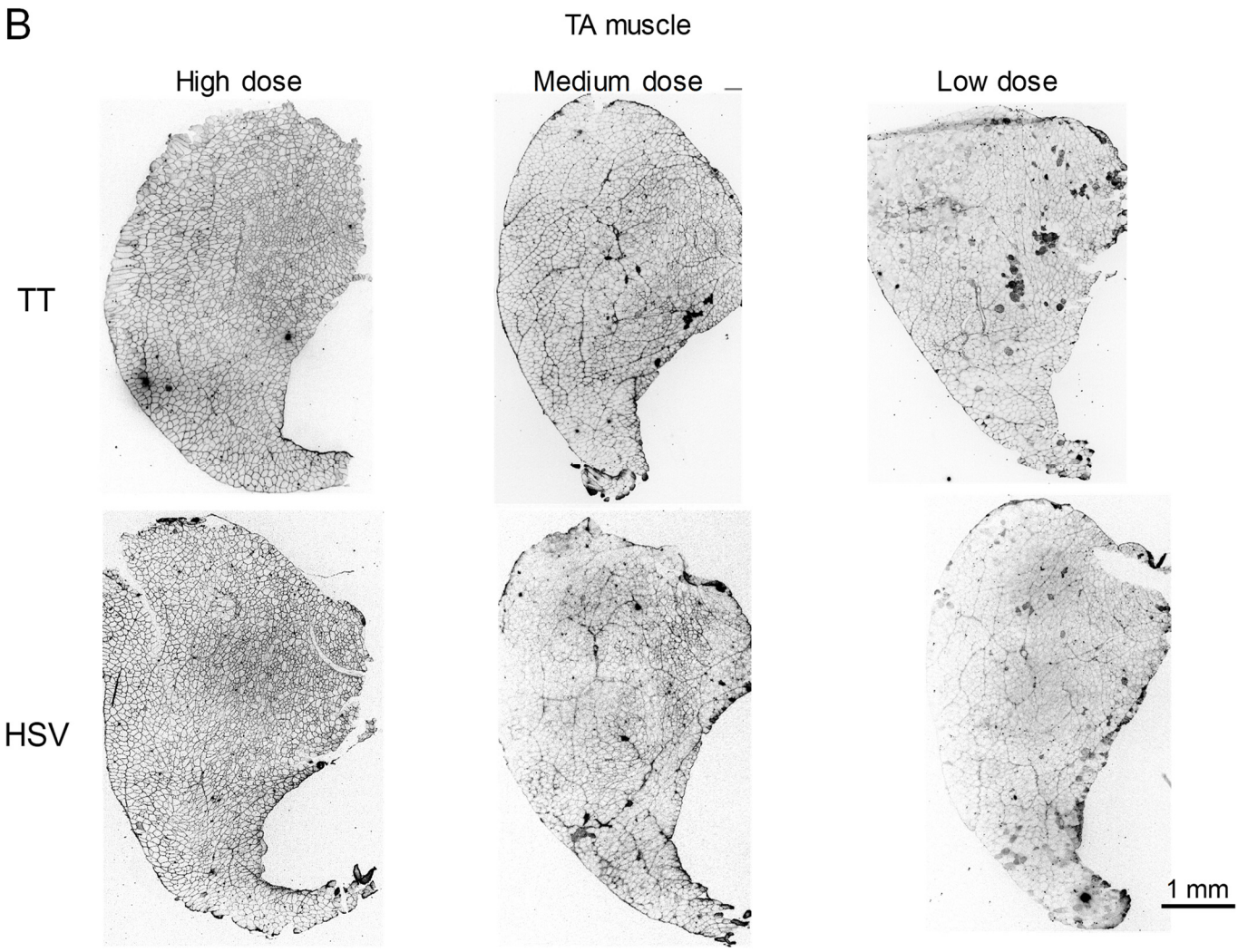


Figure S1. Evaluation of AAV purity by Silver staining. 5 x10^{10} vg particles of purified AAV vectors were loaded in each lane. TT, AAV made with the transient transfection method. HSV, AAV made with the herpes simplex virus system.

Supplemental Figure 2




B
TA muscle
High dose
Medium dose
Low dose
TT
HSV
1 mm

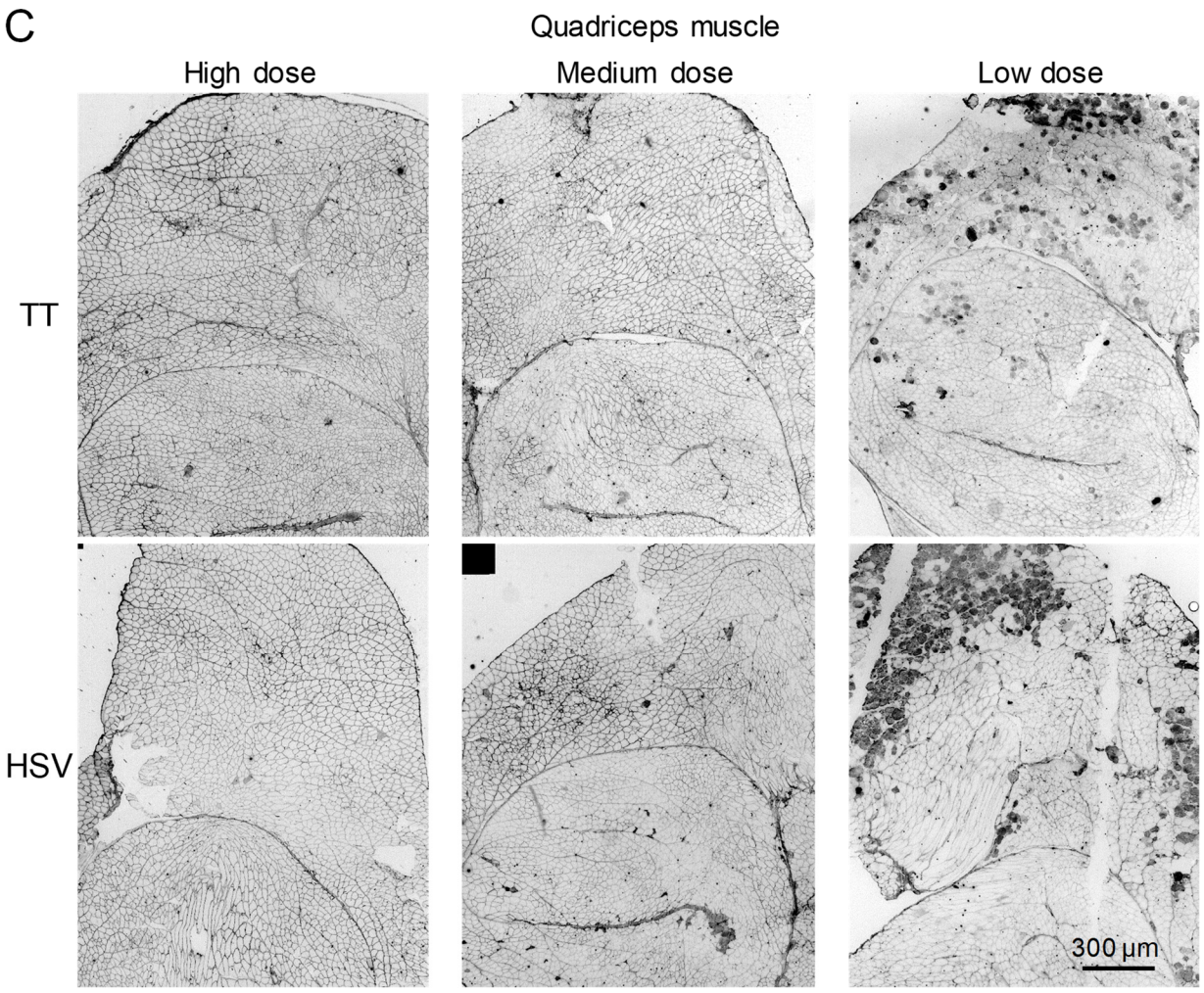


Figure S2. Representative full-view dystrophin immunostaining photomicrographs. A, The extensor digitorum longus. **B,** The tibialis anterior. **C,** The quadriceps. TT, AAV made with the transient transfection method. HSV, AAV made with the herpes simplex virus system.

Supplemental Figure 3

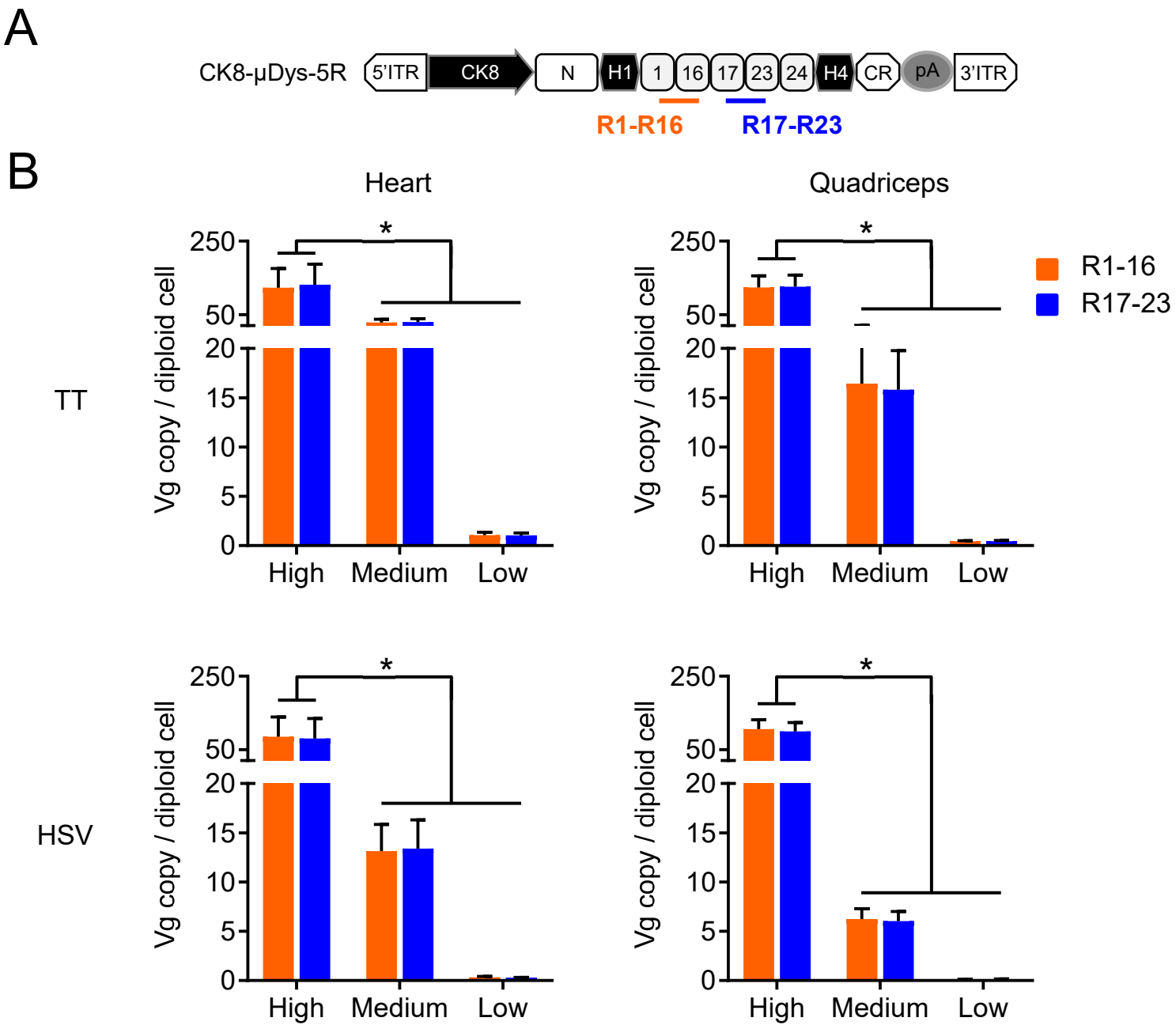


Figure S3. Development of the TaqMan assay for AAV genome copy number quantification in tissues. **A,** Schematic illustration of the AAV micro-dystrophin gene vector. Micro-dystrophin consists of the N-terminal domain, two hinges (H1 and H4), five spectrin-like repeat (R1, R16, R17, R23 and R24) and the cysteine-rich (CR) domain. Micro-dystrophin expression is regulated by the muscle-specific CK8 promoter. Two different TaqMan PCR assays were designed to target two unique junctional regions (R1-R16 and R17-R23) in the micro-dystrophin gene. **B,** Quantification of the AAV genome copy number in the heart and quadriceps from treated mdx4cv mice using the R1-R16 and R17-R23 TaqMan assays. TT, AAV made with the transient transfection method. HSV, AAV made with the herpes simplex virus system. Asterisk (*), significantly different from other groups.

Supplemental Figure 4

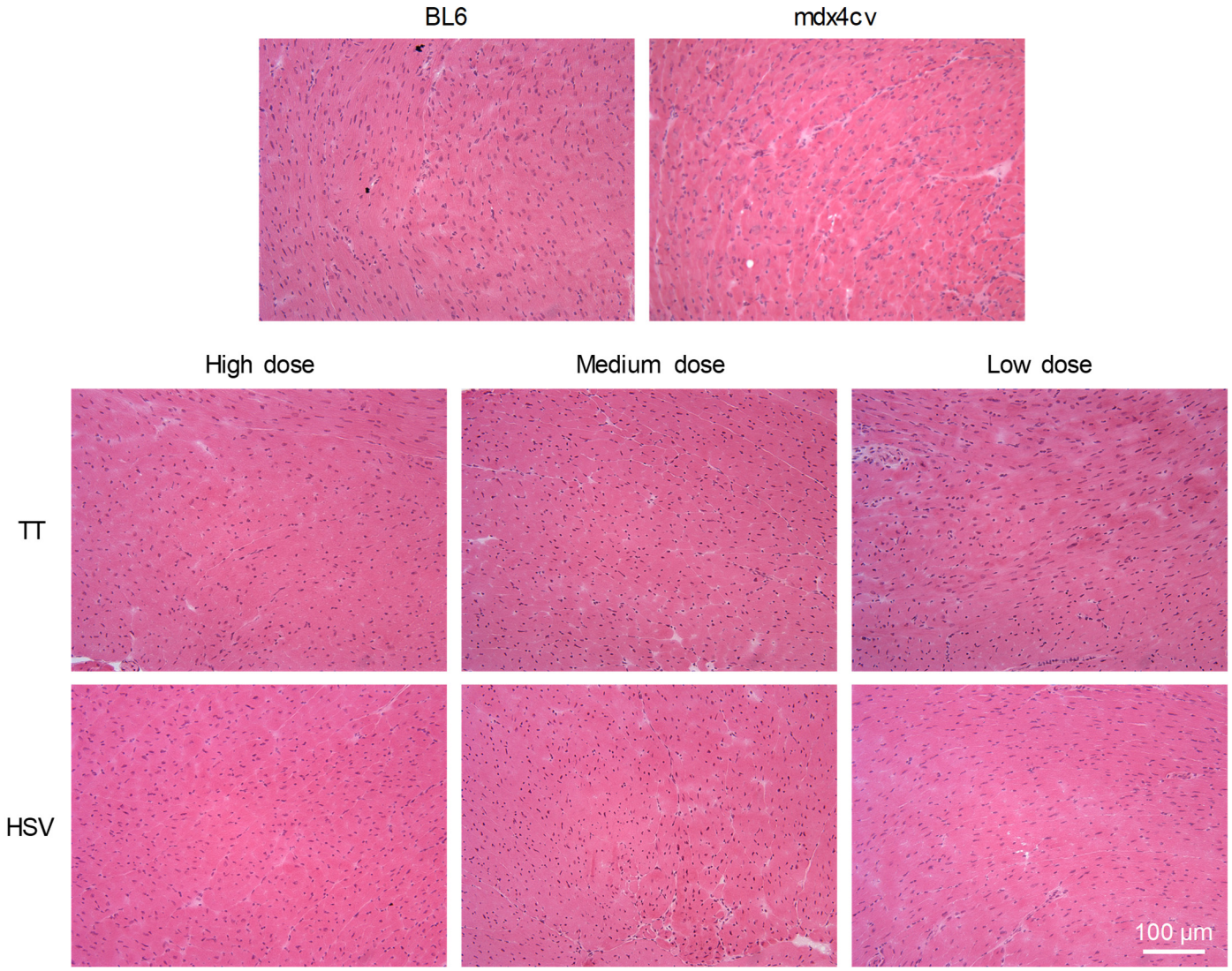


Figure S4. Characterization of the heart histopathology. Representative hematoxylin-eosin staining photomicrographs of the heart from untreated BL6, untreated mdx4cv and AAV injected mdx4cv mice. TT, AAV made with the transient transfection method. HSV, AAV made with the herpes simplex virus system.

Supplementary Table 1. Morphometric properties of the EDL muscle

Strain	Treatment group	AAV production method	EDL weight (mg)	Lo (mm)	CSA (mm^2)	Pt (mN)	Po (mN)
BL6	untreated	N/A	8.68 ± 0.36	13.01 ± 0.11	1.44 ± 0.05	71.16 ± 2.03	350.79 ± 23.77
mdx4cv			18.08 ± 1.46 [a]	13.73 ± 0.12 [a]	2.83 ± 0.21 [a]	77.43 ± 4.91	407.27 ± 6.98
mdx4cv	High	TT	11.50 ± 0.48 [b]	13.16 ± 0.18 [b]	1.88 ± 0.05 [b]	83.76 ± 5.61	402.55 ± 28.85
		HSV	11.28 ± 0.36 [b]	13.12 ± 0.06 [b]	1.85 ± 0.07 [b]	93.31 ± 0.65	418.87 ± 6.41
	Medium	TT	12.59 ± 0.42 [c]	13.11 ± 0.03 [b]	2.07 ± 0.07 [c]	92.26 ± 2.94	423.36 ± 14.52 [a]
		HSV	12.59 ± 0.70 [c]	13.03 ± 0.09 [b]	2.08 ± 0.11 [c]	91.37 ± 4.25	397.80 ± 11.62
	Low	TT	13.78 ± 0.24 [c]	13.14 ± 0.13 [b]	2.26 ± 0.02 [c]	93.91 ± 11.26	398.78 ± 15.50
		HSV	15.32 ± 0.84 [a]	13.35 ± 0.01	2.47 ± 0.13 [a]	110.71 ± 4.40 [c]	456.79 ± 13.32 [a]

a, Significantly different from BL10

b, Significantly different from mdx4cv

c, Significantly different from BL10 and mdx4cv

Data are presented as mean ± standard error of mean

N/A; not applicable; TT, AAV made with the transient transfection method; HSV, AAV made with the herpes simplex virus system