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Supplemental Information

Dystrophin Gene-Editing Stability Is Dependent on Dystrophin Levels in Skeletal but Not Cardiac Muscles

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Supplemental Data:

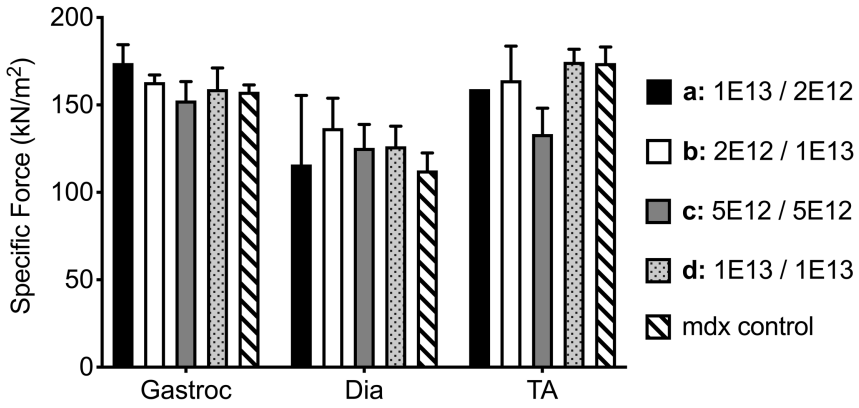

Figure S1: Systemic gene editing to delete the 45 kb genomic region between dystrophin exons 52-53 fails to improve muscle strength at 12 weeks post-treatment. Functional assessment of muscle specific force generation for gastrocnemius (Gastroc), diaphragm (Dia) and tibialis anterior (TA) muscles of treated versus untreated mice. Doses indicated are for the nuclease/target vectors (see Figure 1); 1E13 = $1x10^{13}$ vg per mouse, etc. Replicates (n = x) for each dose and muscle group (Gastroc / Dia / TA) were as follows: **a:** 1E13 / 1E12 (n = 3 / 2 / 1), **b:** 2E12 / 1E13 (n = 3 / 3 / 3), **c:** 5E12 / 5E12 (3 / 3 / 3), **d:** 1E13 / 1E13 (4 / 4 / 4), mdx control (n = 4 / 4 / 4). . None of the vector doses and ratios provided statistically significant improvements in specific force. Statistical significance was determined by two-way ANOVA with Tukey's post hoc test. Values are represented as mean ± SEM.

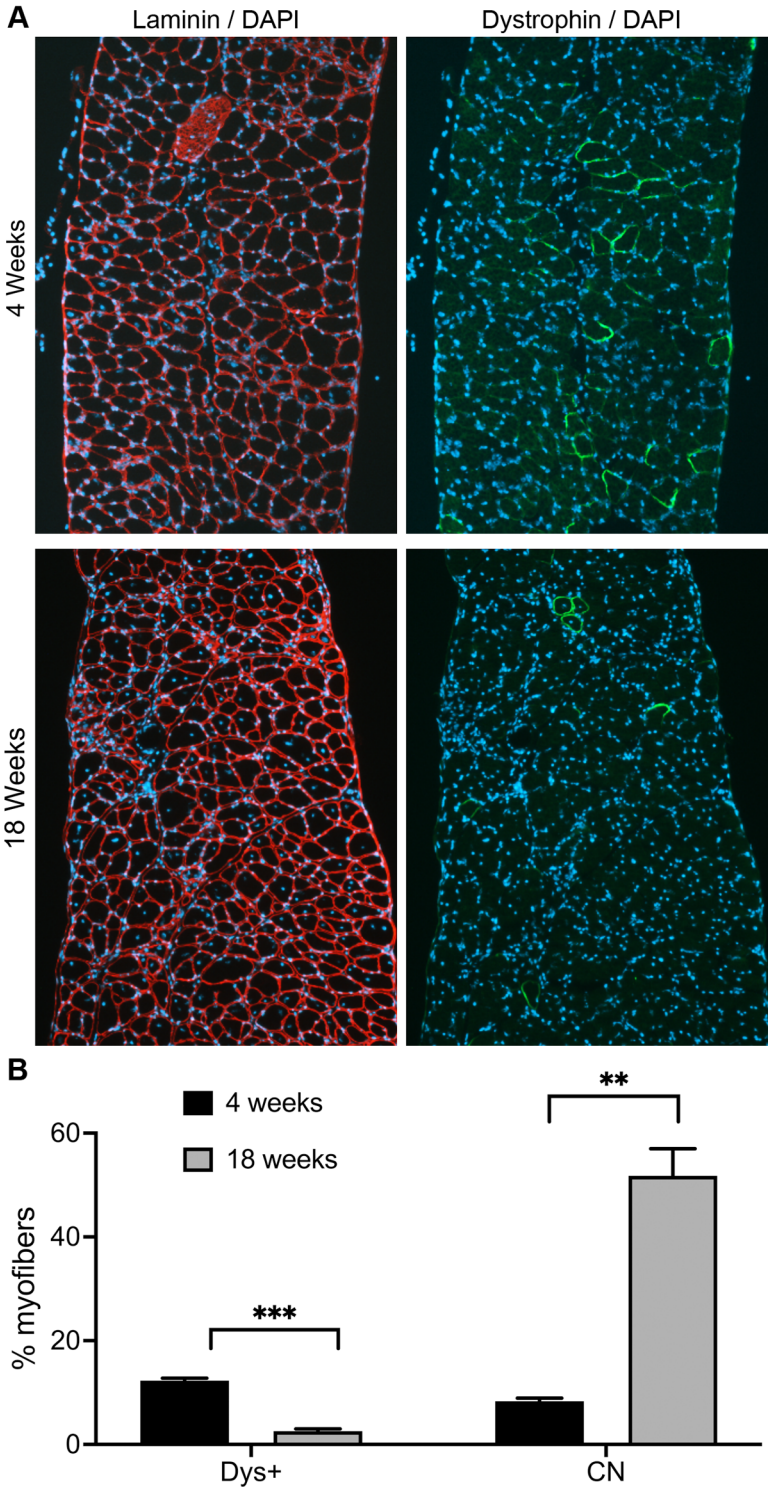

Figure S2: Myofibers of mouse diaphragms exhibit reduced dystrophin expression and increased central nucleation between 4- and 18 weeks post *in vivo* gene editing. A) IF analysis of diaphragm cross-sections from gene-edited mice at 4- (top row) and 18- (bottom row) weeks post-treatment, stained for α_2-Laminin (red) & DAPI (light blue), (left column); and for dystrophin (green) & DAPI (right panels). Images acquired at 100X magnification. **B**) Quantification of percent dystrophin-positive (Dys+), and centrally nucleated (CN) myofibers on mouse diaphragm cross-sections at 4 weeks (n = 2 mice) and 18 weeks (n = 4 mice) post-treatment. Values represent mean ± SEM. Statistical significance was determined using individual Student's t-tests, with statistical significance set to $p<0.05$, (** $p < 0.01$, *** $p < 0.001$).

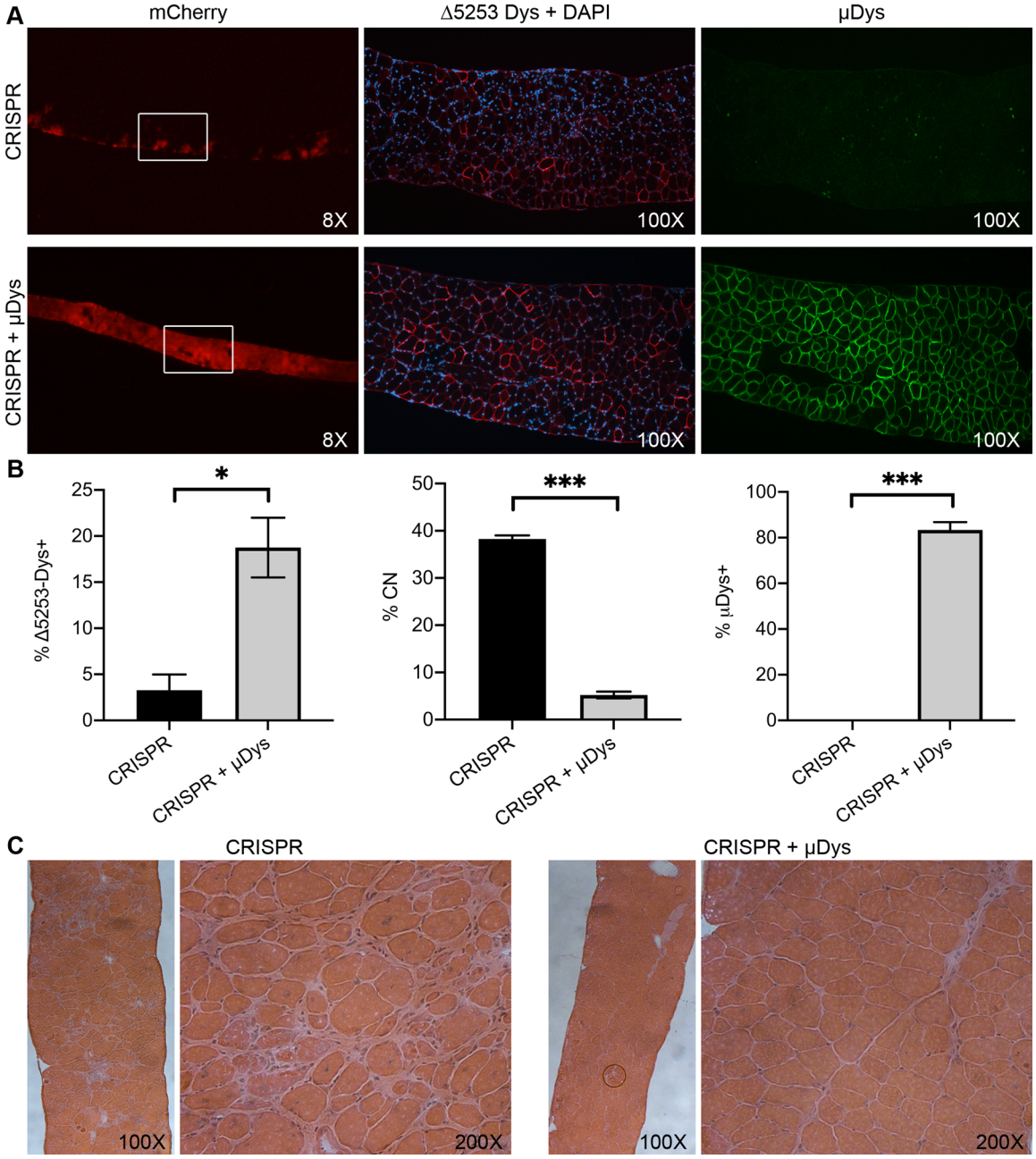

Figure S3: Microdystrophin stabilizes dystrophic skeletal muscle and preserves Δ5253-dystrophin expression.
A) Representative images of diaphragm muscle cross-sections depicting mCherry expression (left panels, acquired at 8X magnification), Δ5253-dystrophin & DAPI (Δ5253-Dys, middle panels) and microdystrophin (μDys, right panels); at 18 weeks post-treatment with CRISPR/Cas9 (CRISPR) or CRISPR/Cas9 with μDys (CRISPR+μDys), acquired at 100X magnification. **B**) Quantification of percent Δ5253-dystrophin positive myofibers (left), centrally nucleated (CN) myofibers (middle), and microdystrophin positive myofibers (μDys^{+}, right): on treated diaphragm cross-sections (n = 3 mice). **C)** Representative diaphragm cross-sections from CRISPR- and CRISPR+μDys treated mice stained with hematoxylin and eosin. Images acquired at 100X (left panels) and 200X (right panels) magnification respectively. Values represent mean ± SEM. Statistical significance was determined using individual Student's t-tests, with statistical significance set to $p<0.05$, (* $p < 0.05$, *** $p < 0.001$).

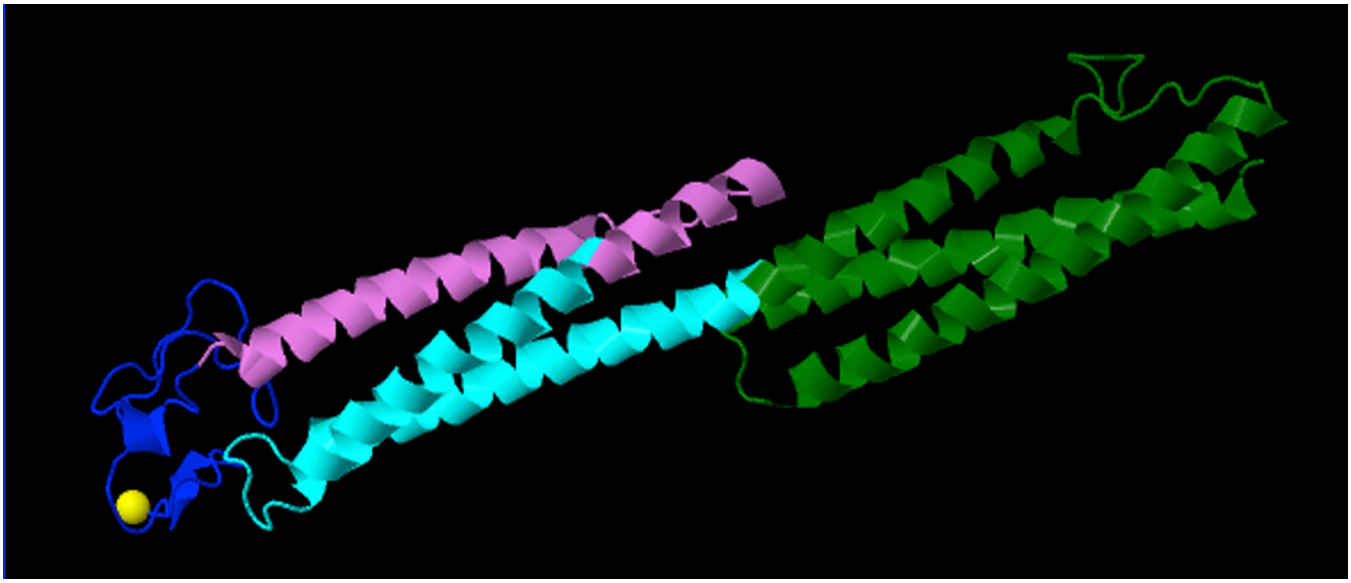

Figure S4: *In silico* model of the region spanning hinge 3 to repeat 22 of Δ5253 dystrophin. Deletion of exons 52-53 is predicted to generate a hybrid repeat 20/21 that preserves the filamentous structure encoded within of the targeted region (blue = hinge 3, Violet = repeat 20, cyan = repeat 21, green = repeat 22), (http://edystrophin.genouest.org).

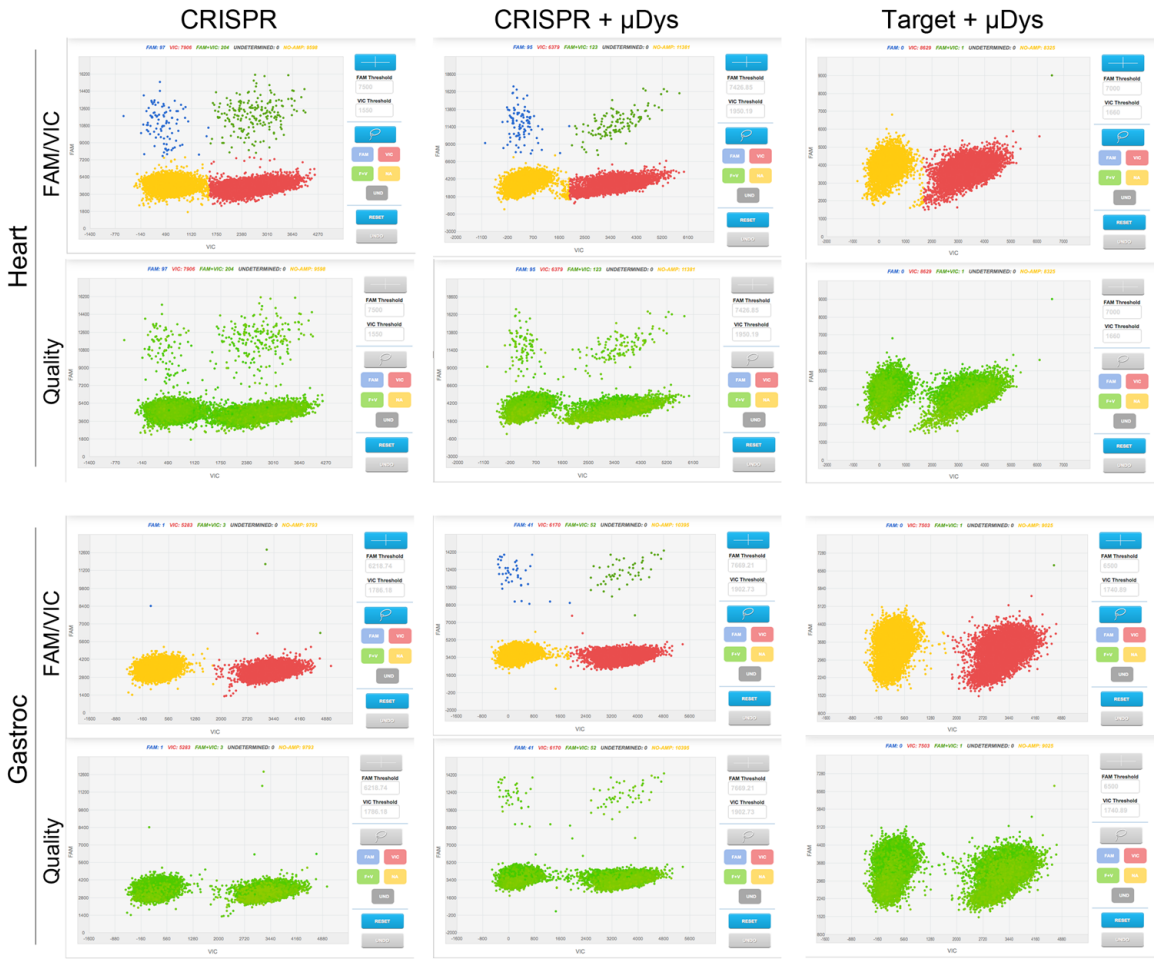

Figure S5: Examples of FAM/VIC calls and data quality determination using Thermo-Fisher's dPCR AnalysisSuite software. Shown are representative FAM/VIC dPCR calls and corresponding quality plots for genomic DNA isolated from Heart (Top) and Gastroc (Bottom) of mice treated with nuclease- and target vectors (CRISPR), CRISPR with microdystrophin co-delivery (CRISPR + µDys), or target vectors only with microdystrophin (Target + µDys).

List of Primers/probes

gRNA oligos

SAgRNA-intron 51	(Forward)	GATACTAGGGTGGCAAATAGA
SAgRNA-intron 51	(Reverse)	TCTATTTGCCACCCTAGTATC
SAgRNA-intron 53	(Forward)	GAGATAAATCCCTGCTTATCAC
SAgRNA-intron 53	(Reverse)	GTGATAAGCAGGGATTTATCTC

PCR primers

(vg)nuclease vector	(Forward)	TGCCCTCATTCTACCACCAC
(vg)nuclease vector	(Reverse)	TCGGTCAGCAGGTTGTAGTC
(vg)Target vector	(Forward)	CACCGATACTAGGGTGGCAAATAGA
(vg)Target vector	(Reverse)	GGGCGTACTTGGCATATGAT
(vg)µDys5 vector	(Forward)	TGCCCTCATTCTACCACCAC
(vg)µDys5 vector	(Reverse)	GCCTTGTTCACGTTGTTCAGG
Δ5253 (intron 51)	(Forward)	CTCATACCCAAAGCTGCTAG
Δ5253 (intron 53)	(Reverse)	ACTGATAACTGATAGCACATTGC

RT-PCR Primers

RTΔ5253 (exon 51)	(Forward)	GCCATCTTCTTTGCTGTTGG
RTΔ5253 (exon 54)	(Reverse)	TCCCGAAGAAGTTTCAGTGC

Digital PCR primers/probes

dPCR-intron 51	(Forward)	ATGAAGTTTTAGAACAAAAATGAGGTAGGT
dPCR-intron 53	(Forward	GAATCAGAAGCATGTCCTTTGC
dPCR-intron 53	(Reverse)	GTGTTCTTAAAAGAATGGTGTGGTG
Δ5253probe (intron 51)	(- strand)	CCACCCTAGTATCTATATTCAATGGCCCAA
HKprobe (intron 53)	(- strand)	TCTCACTTCATAGAGTGCTTGCCTAGC
dRT-exon 51	(Forward)	GCAGACTTCAACCGAGCTTG
dRT-exon 54	(Reverse)	GGTATCATCAGCAGAATAGTCCCG
Δ5253probe (exon 51/54)	(+ strand)	TCATCAAACAGAAGCAGTTGGCCAAAGACC
HKprobe (exon 53)	(- strand)	CTGCAGCTGTTCTTGAACCTCATCCCAC