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Supplementary Materials for

CRISPR-Cas9 corrects Duchenne muscular dystrophy exon 44 deletion mutations in mice and human cells

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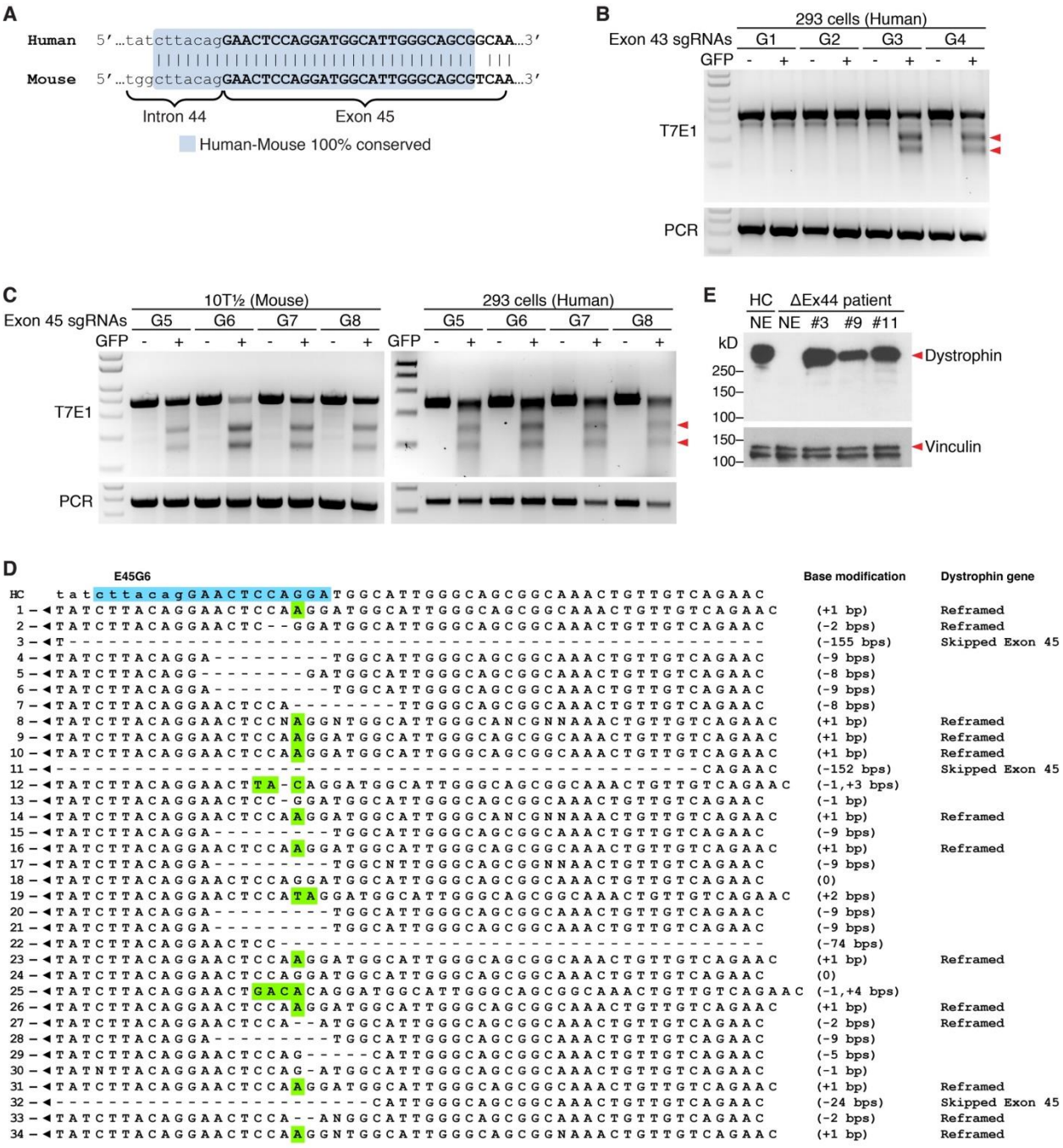

Fig. S1. Analysis of sgRNAs that target the splice acceptor or donor sites for exons 43 and 45. (A) Alignment of human and mouse DNA sequence at the intron-exon junction of exon 45. The conserved region is shaded in light blue. Exon sequence is in bold upper case and intron sequence is in lower case. **(B)** T7E1 assay using human 293 cells transfected with plasmids that express *Sp*Cas9 and exon 43 sgRNA1 (G1), sgRNA2 (G2), sgRNA3 (G3) or sgRNA4 (G4) shows cleavage of the *DMD* locus at the intron-exon junctions of exon 43. Red arrowheads

denote cleavage products. PCR indicates the undigested PCR product. **(C)** T7E1 assay using mouse 10T½ and human 293 cells transfected with plasmids that express *Sp*Cas9 and exon 45 sgRNA5 (G5), sgRNA6 (G6), sgRNA7 (G7) or sgRNA8 (G8) shows cleavage of the *Dmd* locus at the intron-exon junction of exon 45. Red arrowheads denote cleavage products. PCR indicates the undigested PCR product. **(D)** Sequences of the G6 edited 34 single clones. HC is the sequence of the healthy human control. G6 sequence is shaded in blue. Insertions are shaded in green. Base modification and dystrophin gene status are listed on the right. **(E)** Western blot analysis showing restoration of dystrophin expression in three corrected single iPSC clones (clones #3, #9 and #11). Clone #3 and #11 were corrected through exon 45 skipping, and clone #9 was corrected through exon 45 reframing. HC, iPSC-CM from a healthy human control. NE, non-edited. Vinculin is loading control.

A

cagcaatcaggctgaggggggagacagtagatattataatttagtacaaaaaggacattgaaaattgtcttcctgagtcctaggaattatgg
tcactgaacctcaatgatagaagttgtatttacatattgatgactgacataaaagaattggggtagactagaagagaacatgagtctgtgg
gaaggagcaagctgggttagaacaaaggtctgtcagagtcagcatgggaatgaggtagtgttgtaggaggcaattctgcaagcaggtggat
atttagtatcttaactgtagttctgaatcaggaggaaggttgaaactcataaaactgataagacaattttattttgatctgcatgtatttt
aaagttgaacatatgattctcaaataatttttaaaatttaacatgttttaaaatataaaaattacttttaaaattatcacaagggagaa
gatgctaattatcctaagtaattgtctagtactcattttaatcaatgtatatgtttaattttaaaggaaataatacattttgttttaatta
acattctgtataacgttagatattatatctgatagttgaaaatgaaatctacaaatgtaagatactacaaaagtaatacctttgtcagaaa
aaaaaagaatgggtgatgtccacaaaatgtgtcctataaaccacttaccttatttcatccatgttttcaaattatagGCGATTCGACAGAT
CAGTTGAAAAATGGCGACACTTTCATTATGATATGAAGGTATTTAATCAATGGCTGAATGAAGTTGAACAGTTTTTCAAAAAGACACAAAA
TCCTGAAAACTGGGAACATGCTAAATACAAATGGTATCTTAAGgtaagactttgagatttccctttccatgctggcttcatttttcagca
atattttacatattttactactgtaagttcaactaaaaggatgaatggaatatataattcctgactttattgttgctcactgaacatgtta
taaaaatcataacaagtgtgcctgaaaattgtgctatgactgttttgtgaaaggatgagagcatctccctgggcactattggttcatctga
tgaattgtgaagtgtcactgcatctgggatgcaggaaatcatcttatagcaggaaatagtgtggtttaggtctctccccgccctctgtgta
tgtgtgtgtgtgtttccctatgttggaaccagtccagaaggagaagctgtggtatacttagtagtaatgtagttccttcaaatatcca
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gacagactagagg

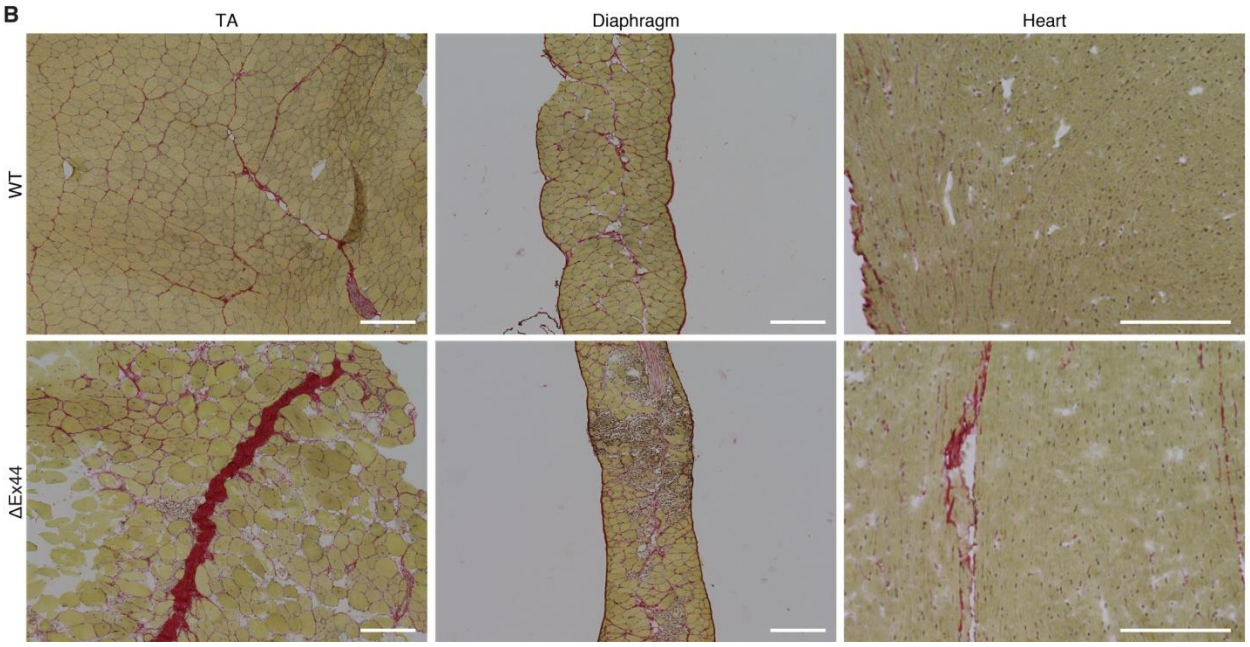


Fig. S2. Characterization of the ΔEx44 mouse line. (A) Genomic sequence of targeted locus of *Dmd* exon 44 and surrounding intron regions. sgRNA sequences are indicated in blue, protospacer adjacent motifs (PAMs) are indicated in red, and genotyping primers are highlighted in yellow. Exon 44 sequence is in bold upper case and intron sequence is in lower case. **(B)** Picrosirius red staining of TA, diaphragm, and heart of WT and ΔEx44 mice. Scale bar is 50 μm.

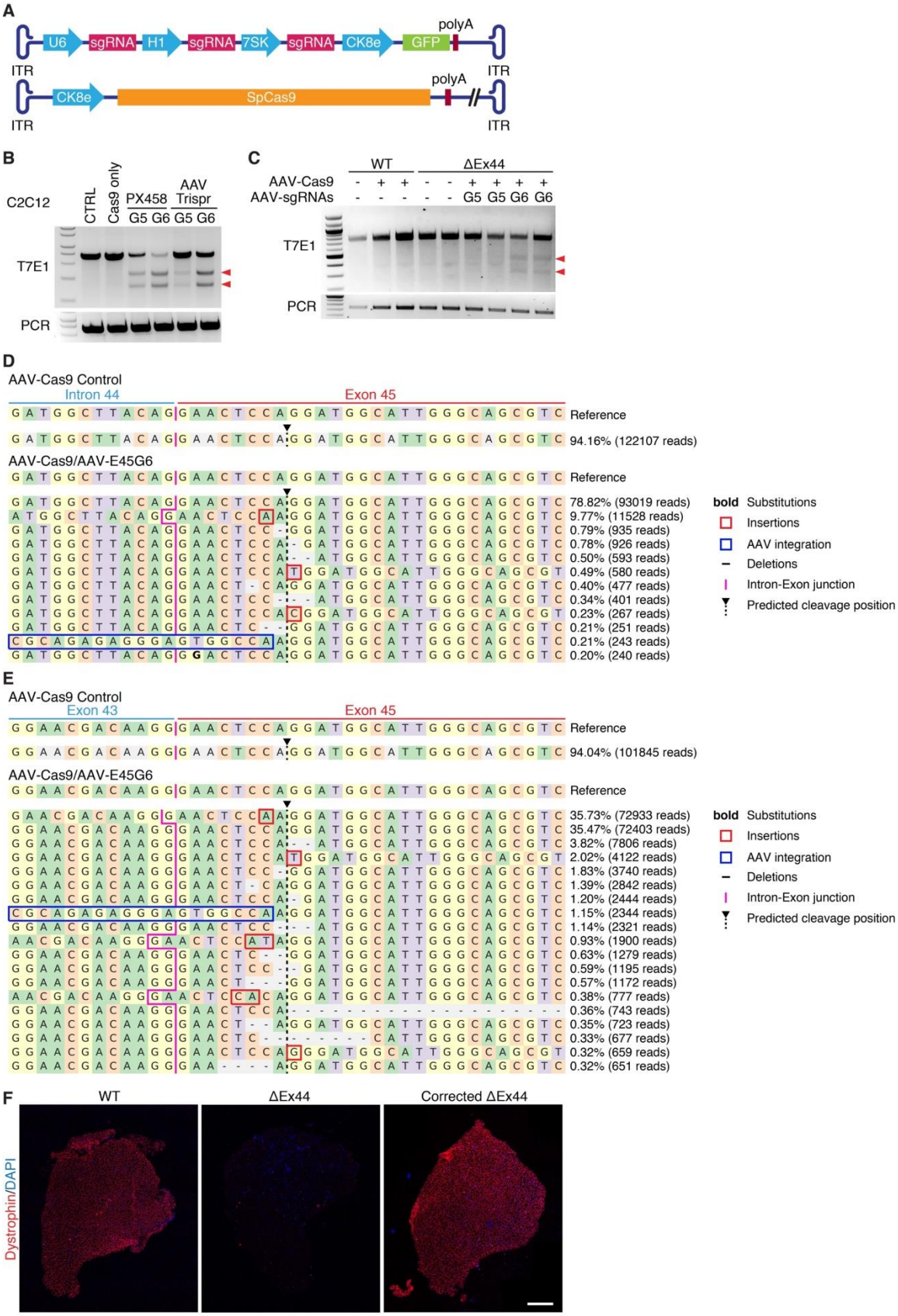
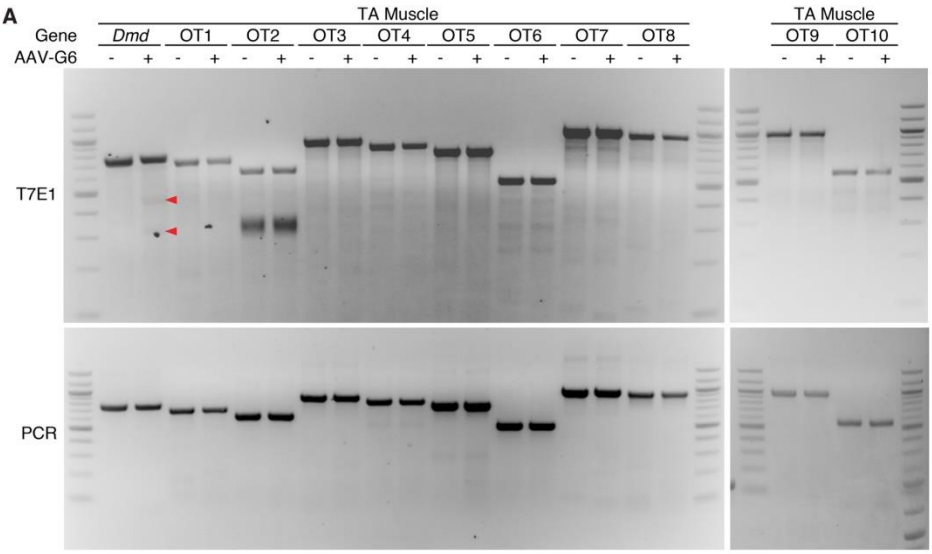

A
U6
sgRNA
H1
sgRNA
7SK
sgRNA
CK8e
GFP
polyA
ITR
ITR
CK8e
SpCas9
polyA
ITR
ITR
B
C2C12
CTRL
Cas9 only
PX458
AAV Trispr
G5 G6 G5 G6
T7E1
PCR
C
WT
ΔEx44
AAV-Cas9
AAV-sgRNAs
G5 G5 G6 G6
T7E1
PCR
D
AAV-Cas9 Control
Intron 44
Exon 45
Reference
94.16% (122107 reads)
AAV-Cas9/AAV-E45G6
Reference
78.82% (93019 reads)
9.77% (11528 reads)
0.79% (935 reads)
0.78% (926 reads)
0.50% (593 reads)
0.49% (580 reads)
0.40% (477 reads)
0.34% (401 reads)
0.23% (267 reads)
0.21% (251 reads)
0.21% (243 reads)
0.20% (240 reads)
bold Substitutions
Insertions
AAV integration
Deletions
Intron-Exon junction
Predicted cleavage position
E
AAV-Cas9 Control
Exon 43
Exon 45
Reference
94.04% (101845 reads)
AAV-Cas9/AAV-E45G6
Reference
35.73% (72933 reads)
35.47% (72403 reads)
3.82% (7806 reads)
2.02% (4122 reads)
1.83% (3740 reads)
1.39% (2842 reads)
1.20% (2444 reads)
1.15% (2344 reads)
1.14% (2321 reads)
0.93% (1900 reads)
0.63% (1279 reads)
0.59% (1195 reads)
0.57% (1172 reads)
0.38% (777 reads)
0.36% (743 reads)
0.35% (723 reads)
0.33% (677 reads)
0.32% (659 reads)
0.32% (651 reads)
bold Substitutions
Insertions
AAV integration
Deletions
Intron-Exon junction
Predicted cleavage position
F
WT
ΔEx44
Corrected ΔEx44
Dystrophin/DAPI

Fig. S3. Intramuscular AAV9 delivery of gene editing components rescues dystrophin expression. (A) Illustration of the AAV construct for CRISPR/Cas9 delivery. The muscle creatine kinase 8 (CK8e) regulatory cassette was used to express *Sp*Cas9 and GFP. The U6, H1, and 7SK RNA polymerase III promoters were used to express sgRNAs. ITR is inverted terminal repeats. **(B)** T7E1 assay shows cleavage of the *Dmd* locus at the intron-exon junction of exon 45 in mouse C2C12 cells with electroporation of G5 or G6 in PX458 or Trispr backbone. Red arrowheads show cleavage products of genome editing. PCR indicates the undigested PCR product. **(C)** T7E1 assay shows cleavage of the *Dmd* locus at the intron-exon junction of exon 45 in TA muscle of corrected ΔEx44 mice. Red arrowheads show cleavage products of genome editing. PCR indicates the undigested PCR product. **(D and E)** On-target (**D**) genomic and (**E**) cDNA amplicon deep sequencing of ΔEx44 DMD, and corrected ΔEx44 DMD mice after 3 weeks of AAV-Cas9 and AAV-G6 intramuscular injection (2.5×10^{10} vg of AAV9-Cas9 and 2.5×10^{10} vg of AAV-G6). Bold represents substitutions, red square is insertions, "-" is deletion. Vertical pink line indicates intron-exon junction in (**D**) and exon-exon junction in (**E**). Black arrowhead points to dotted vertical line representing the predicted cleavage site. **(F)** Dystrophin immunostaining of TA muscle in WT, ΔEx44 DMD, and corrected ΔEx44 DMD mice after 3 weeks of AAV-Cas9 and AAV-G6 intramuscular injection (2.5×10^{10} vg of AAV9-Cas9 and 2.5×10^{10} vg of AAV-G6). Dystrophin is shown in red. Nuclei are marked by DAPI stain in blue. 10X tile scan of the entire TA muscle. Scale bar is 500 μm.

A



B

Selected OT site	Sequence	PAM	Gene	Chromosome	Samples	Total Reads	Unmodified	NHEJ	NHEJ%	AVG
E45G6 On-target	CTTACAGGAACTCCAGGA	TGG	ENSMUSG00000045103	chrX	Δ44-AAV-Cas9 control-1	129677	129273	404	0.31%	0.31%
					Δ44-AAV-Cas9 control-2	118099	117724	375	0.32%	
					Δ44-AAV-Cas9.AAV-E45G6-1	118011	99440	18571	15.74%	14.89%
					Δ44-AAV-Cas9.AAV-E45G6-2	79958	68732	11226	14.04%	
E45G6 OT1	CTCACAGAAACTCCAGGA	AAG	ENSMUSG00000018168	chr11	Δ44-AAV-Cas9 control-1	148291	147900	391	0.26%	0.30%
					Δ44-AAV-Cas9 control-2	140851	140391	460	0.33%	
					Δ44-AAV-Cas9.AAV-E45G6-1	146193	145718	475	0.32%	0.29%
					Δ44-AAV-Cas9.AAV-E45G6-2	141836	141485	351	0.25%	
E45G6 OT2	TTGACAGGAACTCCAGGA	AAG	ENSMUSG00000087615	chr17	Δ44-AAV-Cas9 control-1	195414	195077	337	0.17%	0.21%
					Δ44-AAV-Cas9 control-2	164408	164011	397	0.24%	
					Δ44-AAV-Cas9.AAV-E45G6-1	163644	163237	407	0.25%	0.22%
					Δ44-AAV-Cas9.AAV-E45G6-2	224318	223898	420	0.19%	
E45G6 OT3	CTTCCAGGAACTCCAGCA	CAG	ENSMUSG00000024087	chr17	Δ44-AAV-Cas9 control-1	118944	118753	191	0.16%	0.16%
					Δ44-AAV-Cas9 control-2	137789	137573	216	0.16%	
					Δ44-AAV-Cas9.AAV-E45G6-1	115015	114797	218	0.19%	0.16%
					Δ44-AAV-Cas9.AAV-E45G6-2	115647	115493	154	0.13%	
E45G6 OT4	CTTATAGGAATTCCAGGA	AGG	ENSMUSG00000001123	chr11	Δ44-AAV-Cas9 control-1	112923	112346	577	0.51%	0.50%
					Δ44-AAV-Cas9 control-2	80286	79896	390	0.49%	
					Δ44-AAV-Cas9.AAV-E45G6-1	72867	72514	353	0.48%	0.54%
					Δ44-AAV-Cas9.AAV-E45G6-2	102712	102102	610	0.59%	
E45G6 OT5	GTGACATGAACTCCAGGA	AAG	ENSMUSG00000097736	chr9	Δ44-AAV-Cas9 control-1	132220	132032	188	0.14%	0.15%
					Δ44-AAV-Cas9 control-2	100491	100341	150	0.15%	
					Δ44-AAV-Cas9.AAV-E45G6-1	111366	111173	193	0.17%	0.16%
					Δ44-AAV-Cas9.AAV-E45G6-2	114976	114805	171	0.15%	
E45G6 OT6	TTTCCAGGAATTCCAGGA	AGG	ENSMUSG00000026835	chr2	Δ44-AAV-Cas9 control-1	95824	95395	429	0.45%	0.42%
					Δ44-AAV-Cas9 control-2	103709	103292	417	0.40%	
					Δ44-AAV-Cas9.AAV-E45G6-1	112059	111548	511	0.46%	0.47%
					Δ44-AAV-Cas9.AAV-E45G6-2	128665	128037	628	0.49%	
E45G6 OT7	CTCACCGGAACTCCAGGA	GGG	ENSMUSG00000025650	chr9	Δ44-AAV-Cas9 control-1	131416	130646	770	0.59%	0.61%
					Δ44-AAV-Cas9 control-2	122440	121656	784	0.64%	
					Δ44-AAV-Cas9.AAV-E45G6-1	122052	121559	493	0.40%	0.47%
					Δ44-AAV-Cas9.AAV-E45G6-2	134661	133948	713	0.53%	
E45G6 OT8	ATGACAGAAACTCCAGGA	AAG	ENSMUSG00000056952	chr6	Δ44-AAV-Cas9 control-1	87995	86485	1510	1.72%	1.65%
					Δ44-AAV-Cas9 control-2	130159	128107	2052	1.58%	
					Δ44-AAV-Cas9.AAV-E45G6-1	136626	134574	2052	1.50%	1.48%
					Δ44-AAV-Cas9.AAV-E45G6-2	121227	119468	1759	1.45%	
E45G6 OT9	CTACCAGGAACTCCAGGC	TGG	ENSMUSG00000021275	chr12	Δ44-AAV-Cas9 control-1	123165	122861	304	0.25%	0.60%
					Δ44-AAV-Cas9 control-2	116857	115745	1112	0.95%	
					Δ44-AAV-Cas9.AAV-E45G6-1	135673	135192	481	0.35%	0.39%
					Δ44-AAV-Cas9.AAV-E45G6-2	134603	134030	573	0.43%	
E45G6 OT10	CTTTCAGAGACTCCAGGA	CAG	ENSMUSG00000034731	chr14	Δ44-AAV-Cas9 control-1	89824	89681	143	0.16%	0.19%
					Δ44-AAV-Cas9 control-2	94091	93884	207	0.22%	
					Δ44-AAV-Cas9.AAV-E45G6-1	80995	80807	188	0.23%	0.22%
					Δ44-AAV-Cas9.AAV-E45G6-2	100775	100570	205	0.20%	

C

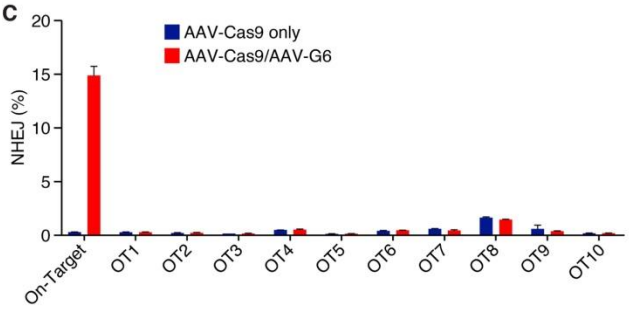


Fig. S4. Analysis of top 10 potential off-target sites. (A) T7E1 analysis of the top 10 predicted off-target (OT) sites of sgRNA-G6 assayed in TA muscle 3 weeks following intramuscular injection of 2.5×10^{10} vg AAV9-Cas9 and 2.5×10^{10} vg AAV-G6. Red arrowheads denote on-target cleavage products. No off-target cleavage products were detected. PCR indicates the undigested PCR product. **(B)** Amplicon genomic deep sequencing analysis on the top 10 predicted off-target sites of G6. Muscle was analyzed 3 weeks following intramuscular injection of 2.5×10^{10} vg AAV9-Cas9 and 2.5×10^{10} vg AAV-G6. Mismatches in the target sequence are highlighted in red. **(C)** Percentage of NHEJ in amplicon genomic deep sequencing analysis on the top 10 predicted off-target sites of G6. Blue indicates AAV-Cas9 only control, and red indicates AAV-Cas9/AAV-G6 injected TA muscle. Data are represented as mean ± SEM.

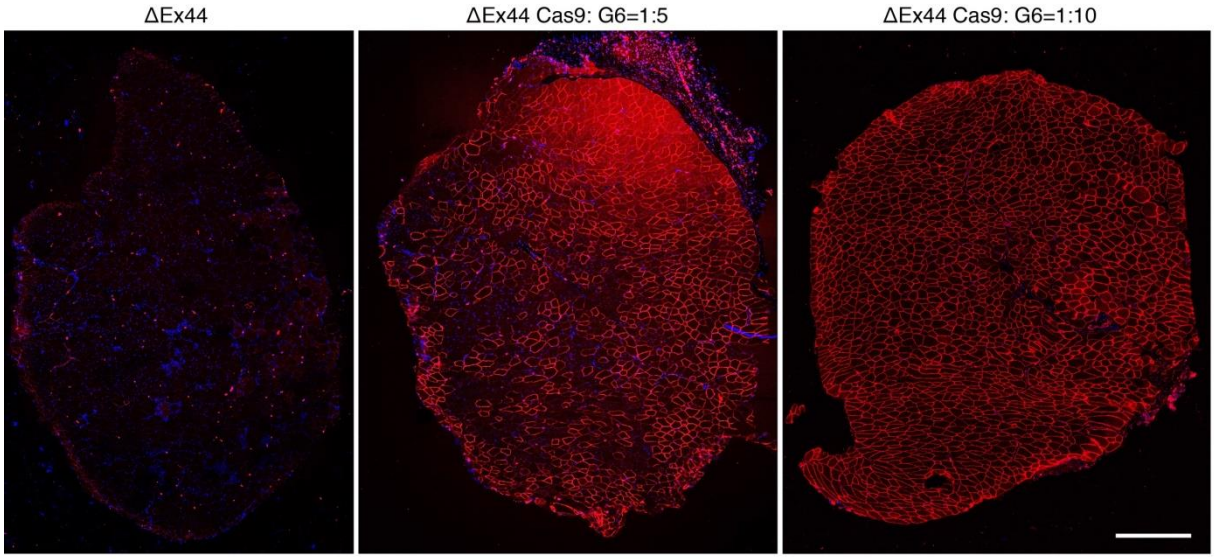


Fig. S5. Correction of ΔEx44 mice by systemic delivery of AAV9 expressing gene editing components. Whole TA muscle scanning of ΔEx44 DMD and corrected ΔEx44 DMD 4 weeks after systemic injection of a 1:5 ratio and 1:10 ratio of AAV-Cas9 to AAV-G6. AAV-Cas9 was administered at 5×10^{13} vg/kg. Dystrophin is shown in red. Nuclei are marked by DAPI stain in blue. 10X tile scan of the entire TA muscle. Scale bar is 500 μm.

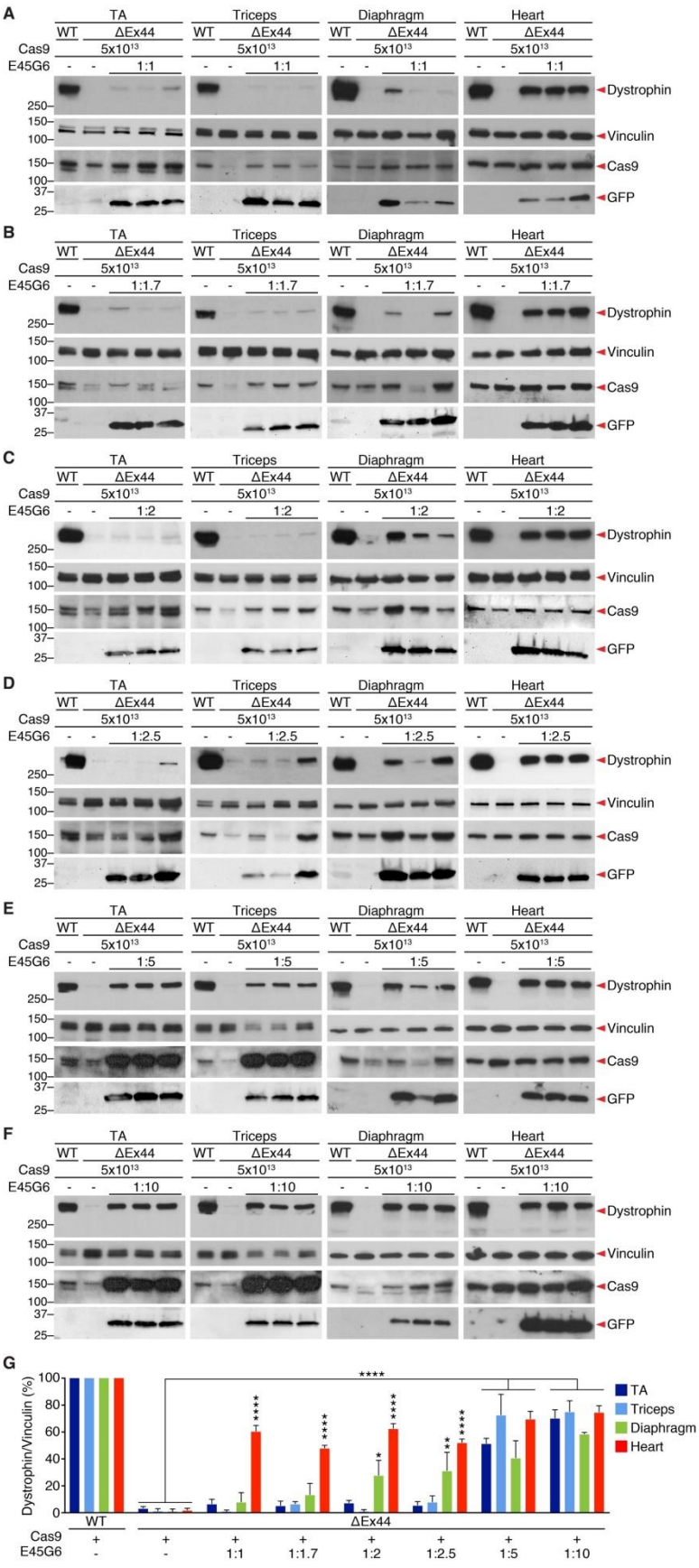
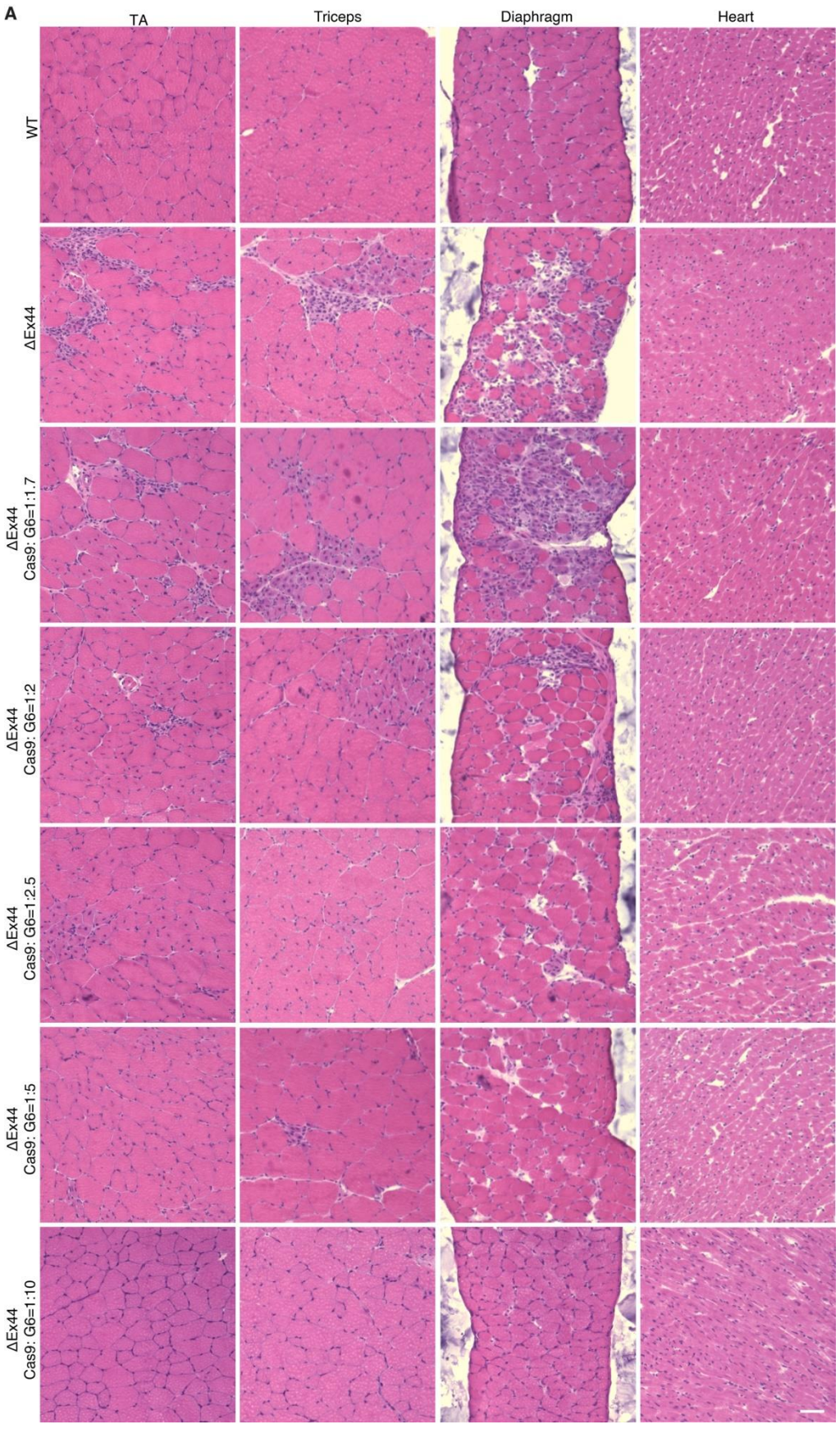
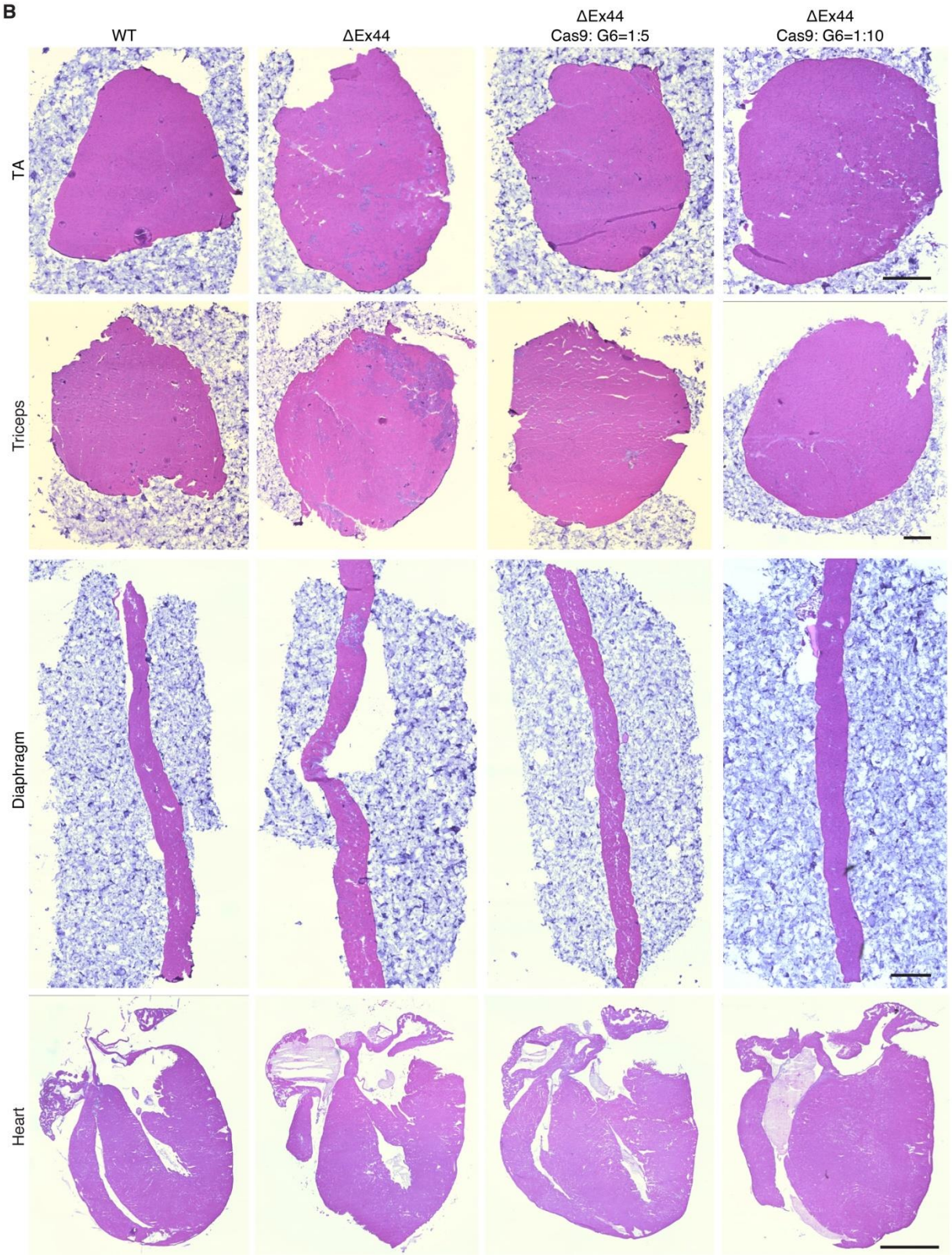

A
TA
Triceps
Diaphragm
Heart
WT
ΔEx44
Cas9
5x10^13
E45G6
1:1
B
1:1.7
C
1:2
D
1:2.5
E
1:5
F
1:10
250–
150–
100–
37–
25–
Dystrophin
Vinculin
Cas9
GFP
G
Dystrophin/Vinculin (%)
100
80
60
40
20
0
****
TA
Triceps
Diaphragm
Heart
WT
ΔEx44
Cas9
E45G6
1:1
1:1.7
1:2
1:2.5
1:5
1:10

Fig. S6. Western blot analysis of corrected ΔEx44 mice by systemic delivery of AAV9 expressing gene editing components. (A)–(F) Western blot analysis of dystrophin, Cas9, and GFP protein expression in TA, triceps, diaphragm, and heart of ΔEx44 mice 4 weeks after systemic delivery of AAV-Cas9 and AAV-G6 at the indicated ratios. AAV-Cas9 was administered at 5×10^{13} vg/kg. Vinculin is loading control. (n= 3). **(G)** Quantification of the Western blot analysis in TA, triceps, diaphragm, and heart. Relative dystrophin intensity was calibrated with vinculin internal control. Data are represented as mean ± SEM. One-way ANOVA was performed followed by Newman-Keuls post hoc test. *P<0.005, **P<0.001, ****P<0.0001 (n=3).


A
TA
Triceps
Diaphragm
Heart
WT
ΔEx44
ΔEx44 Cas9: G6=1:1.7
ΔEx44 Cas9: G6=1:2
ΔEx44 Cas9: G6=1:2.5
ΔEx44 Cas9: G6=1:5
ΔEx44 Cas9: G6=1:10


B
WT
ΔEx44
ΔEx44
Cas9: G6=1:5
ΔEx44
Cas9: G6=1:10
TA
Triceps
Diaphragm
Heart

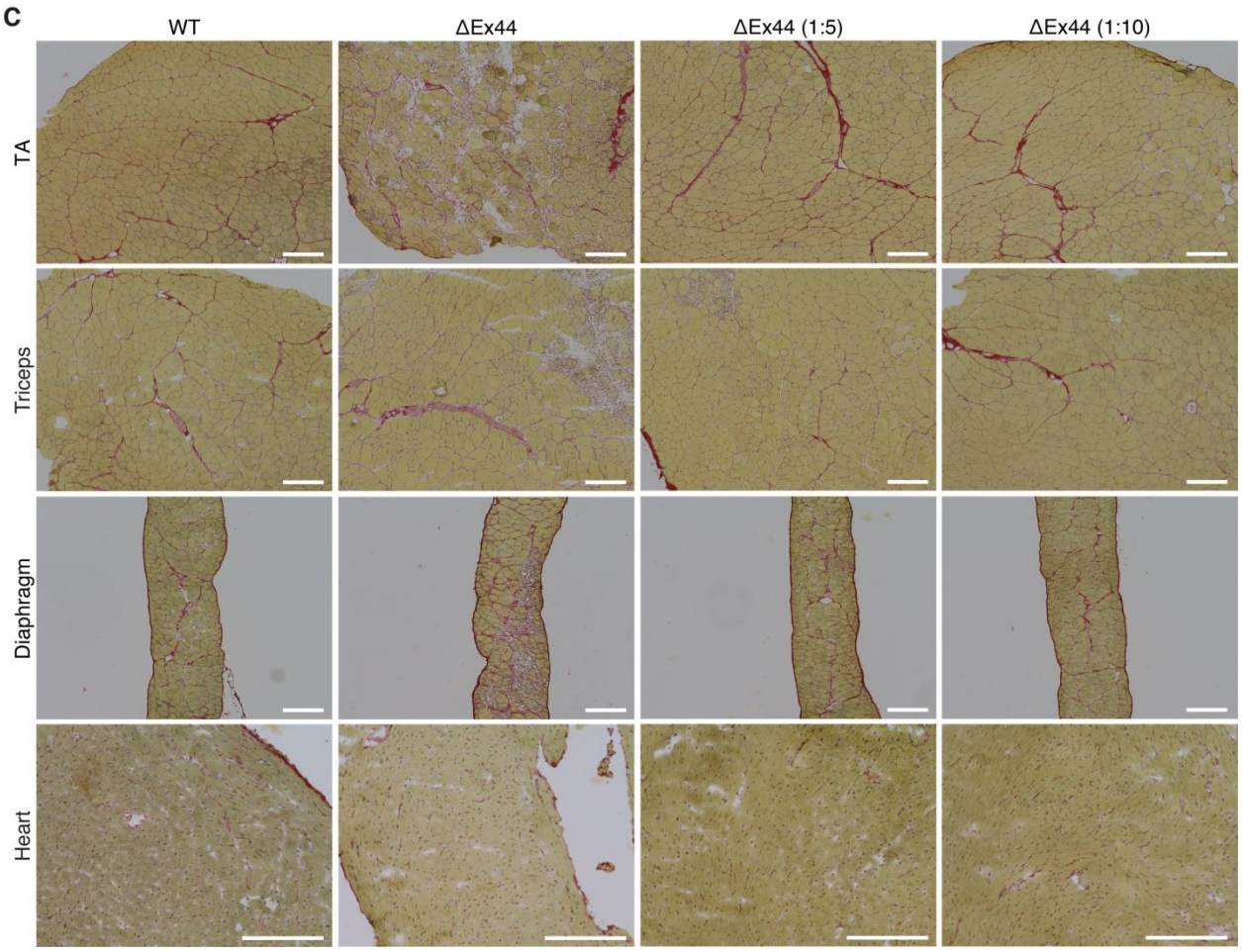


Fig. S7. Histology of ΔEx44 mice after systemic delivery of AAV9 expressing gene editing components. (A) H&E staining of TA, triceps, diaphragm and heart of ΔEx44 mice 4 weeks after systemic delivery of AAV-Cas9 and AAV-G6 at the indicated ratios. AAV-Cas9 was administered at 5×10^{13} vg/kg. Scale bar is 50 μm. **(B)** Whole muscle scanning of TA, triceps, diaphragm and heart of corrected ΔEx44 DMD mice. H&E staining of WT, ΔEx44 DMD and corrected ΔEx44 DMD 4 weeks after systemic injection of a 1:5 ratio and 1:10 ratio of AAV-Cas9 to AAV-G6. AAV-Cas9 was administered at 5×10^{13} vg/kg. Tile scan (4X) of the entire muscle. Scale bar in TA, triceps, diaphragm is 500 μm, in heart is 1.5mm. **(C)** Picrosirius red staining of TA, triceps, diaphragm and heart of ΔEx44 mice 4 weeks after systemic delivery of AAV-Cas9 and AAV-G6 at the indicated ratios. AAV-Cas9 was administered at 5×10^{13} vg/kg. Scale bar is 50 μm.

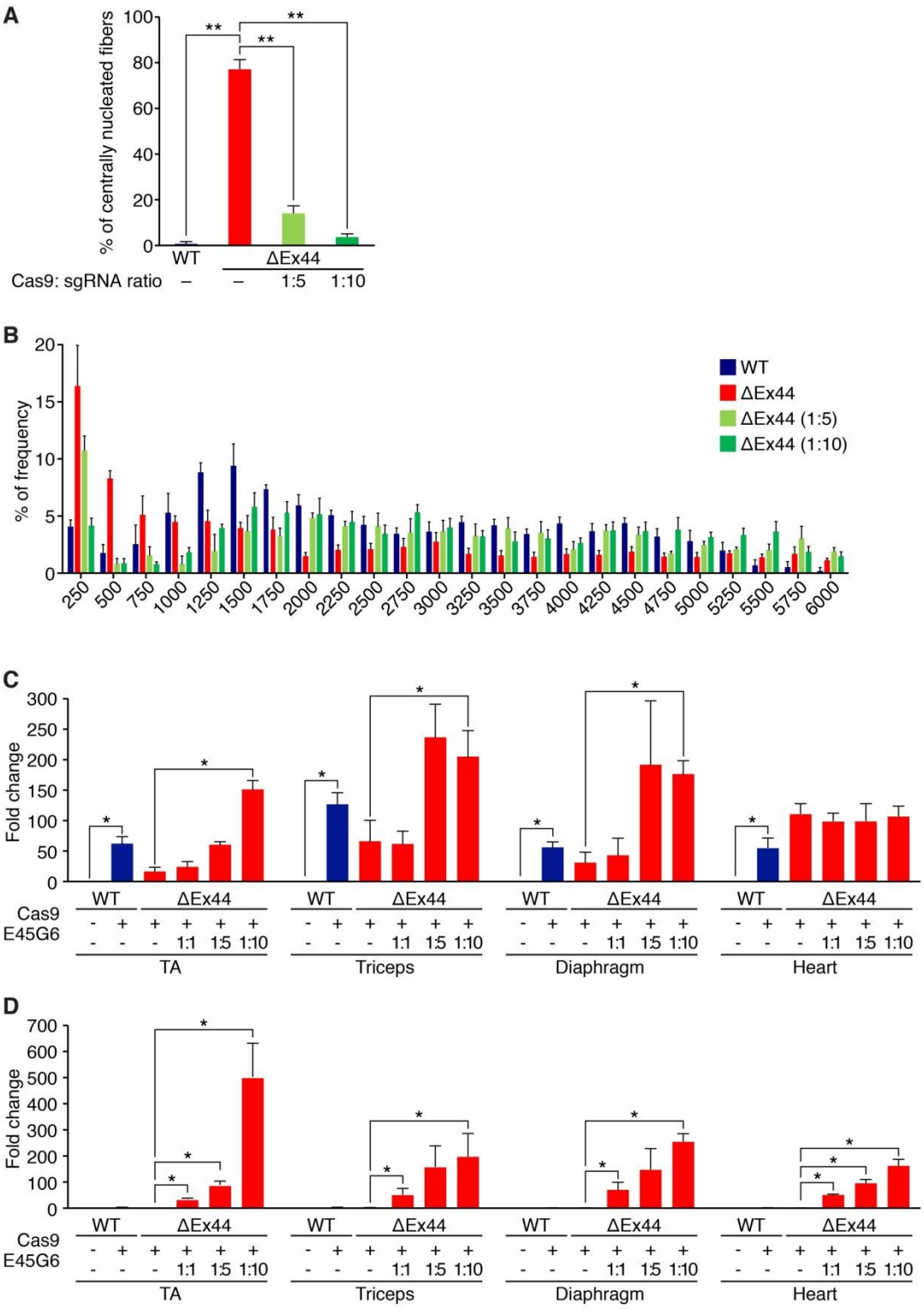

A
% of centrally nucleated fibers
100
80
60
40
20
0
**
**
**
WT
ΔEx44
Cas9: sgRNA ratio – – 1:5 1:10
B
% of frequency
20
15
10
5
0
WT
ΔEx44
ΔEx44 (1:5)
ΔEx44 (1:10)
250 500 750 1000 1250 1500 1750 2000 2250 2500 2750 3000 3250 3500 3750 4000 4250 4500 4750 5000 5250 5500 5750 6000
C
Fold change
300
250
200
150
100
50
0
WT
ΔEx44
Cas9
E45G6
- + + + + +
- - - 1:1 1:5 1:10
TA
Triceps
Diaphragm
Heart
D
Fold change
700
600
500
400
300
200
100
0
WT
ΔEx44
Cas9
E45G6
- + + + + +
- - - 1:1 1:5 1:10
TA
Triceps
Diaphragm
Heart

Fig. S8. Quantification of histological improvement and qPCR analysis of corrected ΔEx44 DMD mice. **(A)** Percentage of centrally nucleated fibers in TA muscle of WT, ΔEx44 DMD control and ΔEx44 DMD-AAV9 treated mice 4 weeks after systemic delivery at indicated ratios. Data are represented as mean ± SEM. One-way ANOVA was performed followed by Newman-Keuls post hoc test. **P<0.001 (n=3). **(B)** Measurement of fiber area of transverse muscle sections. Fiber size is grouped into 250 μm^2 intervals, and represented as the percentage of total fibers in each group. Data are represented as mean ± SEM. One-way ANOVA was performed followed by Newman-Keuls post hoc test. (n=3). **(C)** qPCR analysis of Cas9 mRNA expression in TA, triceps, diaphragm, and heart of ΔEx44 mice 4 weeks after systemic delivery of AAV-Cas9 and AAV-G6 at the indicated ratios. AAV-Cas9 was administered at 5×10^{13} vg/kg. Normalized to 18S ribosomal RNA. Data are represented as mean ± SEM. One-way ANOVA was performed followed by Newman-Keuls post hoc test. *P<0.005. (n= 3). **(D)** qPCR analysis of GFP mRNA expression in TA, triceps, diaphragm, and heart of ΔEx44 mice 4 weeks after systemic delivery of AAV-Cas9 and AAV-G6 at the indicated ratios. AAV-Cas9 was administered at 5×10^{13} vg/kg. Normalized to 18S ribosomal RNA. Data are represented as mean ± SEM. One-way ANOVA was performed followed by Newman-Keuls post hoc test. *P<0.005. (n= 3).

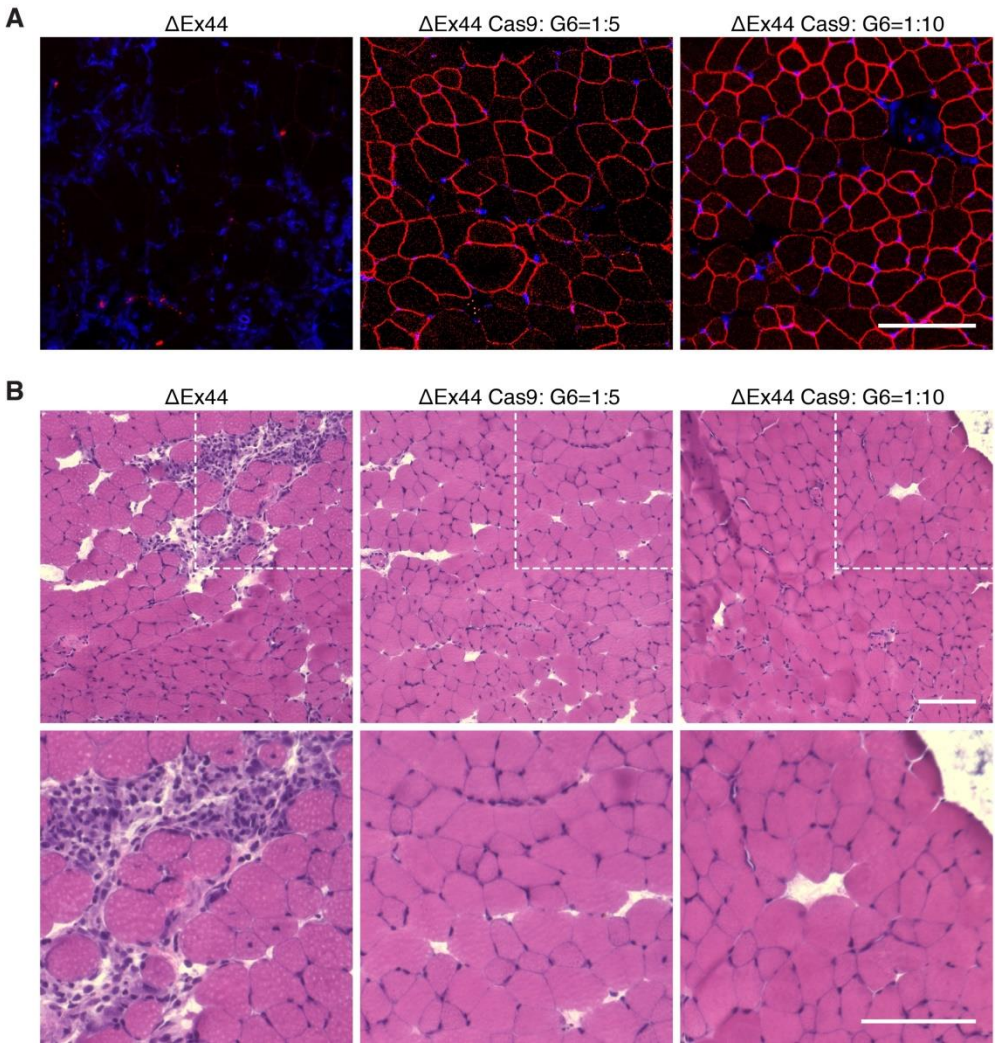


Fig. S9. Histological analysis showing dystrophin restoration in the EDL muscle of corrected ΔEx44 DMD mice. (A) Dystrophin immunostaining of EDL muscle in ΔEx44 DMD and corrected ΔEx44 DMD mice 4 weeks after systemic injection of a 1:5 ratio and 1:10 ratio of AAV-Cas9 to AAV-G6. AAV-Cas9 was administered at 5×10^{13} vg/kg. Dystrophin is shown in red. Nuclei are marked by DAPI stain in blue. Scale bar is 100 μm. **(B)** H&E staining of EDL muscle in ΔEx44 DMD and corrected ΔEx44 DMD mice 4 weeks after systemic injection of a 1:5 ratio and 1:10 ratio of AAV-Cas9 to AAV-G6. AAV-Cas9 was administered at 5×10^{13} vg/kg. Inset box indicates area of magnification shown below. Scale bar is 50 μm.

Table S1. Primer sequences and media components.

Primer sequences for exon targeting and generation of ΔEx44 DMD mouse model		
Purpose of the primers	**ID**	**Sequence (5'-3')**
Primers for sgRNA targeting exon 43	Ex43-gRNA#1-DMD-Top	CACCGTTTTAAAATTTTATATTA
	Ex43-gRNA#1-DMD-Bot	AAACTAATATAAAAATTTTAAAAC
	Ex43-gRNA#2-DMD-Top	CACCGTTTTATATTACAGAATATAA
	Ex43-gRNA#2-DMD-Bot	AAACTTATATTCTGTAATATAAAAC
	Ex43-gRNA#3-DMD-Top	CACCGTATGTGTTACCTACCCTTGT
	Ex43-gRNA#3-DMD-Bot	AAACACAAGGGTAGGTAACACATAC
	Ex43-gRNA#4-DMD-Top	CACCGTACAAGGACCGACAAGGGT
	Ex43-gRNA#4-DMD-Bot	AAACACCCTTGTCGGTCCTTGTAC
Primers for sgRNA targeting exon 45	Ex45-gRNA#5-Top	CACCGCGCTGCCCAATGCCATCCTG
	Ex45-gRNA#5-Bot	AAACCAGGATGGCATTGGGCAGCGC
	Ex45-gRNA#6-Top	CACCGCTTACAGGAACTCCAGGA
	Ex45-gRNA#6-Bot	AAACTCCTGGAGTTCCTGTAAGC
	Ex45-gRNA#7-Top	CACCGAGGAACTCCAGGATGGCATT
	Ex45-gRNA#7-Bot	AAACAATGCCATCCTGGAGTTCCTC
	Ex45-gRNA#8-Top	CACCGCGCTGCCCAATGCCATCC
	Ex45-gRNA#8-Bot	AAACGGATGGCATTGGGCAGCGC
Primers for T7E1 assay	mDmd-T7E1-Ex45-F	CTAACATAAAAGGTGTCTTTCTATC
	mDmd-T7E1-Ex45-R	GGCAATCCCTCATGATTTTTAGCAC
	DMD-T7E1-Ex45-F	GTCTTTCTGTCTTGTATCCTTTGG
	DMD-T7E1-Ex45-R	AATGTTAGTGCCTTTCACCC
Primers for sgRNA targeting Dmd exon 44 to generate the ΔEx44 DMD model	mDmd-In44-2-Top	CACCGGTAGTTCTGAATCAGGAGGA
	mDmd-In44-2-Bot	AAACTCCTCCTGATTCAGAACTACC
	mDmd-In44-6-Top	CACCGTATGTTGGAACCAGTCCAGA
	mDmd-In44-6-Bot	AAACTCTGGACTGGTTCCAACATAC
Primers for in vitro transcription of sgRNA	Exon 44_T7-In44-2-F	GAATTGTAATACGACTCACTATAGGGGTAGTTCTGAATCAGGAGGA
	Exon 44_T7-In44-6-F	GAATTGTAATACGACTCACTATAGGGTATGTTGGAACCAGTCCAGA
	Exon 44_T7-Rv	AAAAGCACCGACTCGGTGCCAC
Primers for genotyping of ΔEx44 DMD model	Geno dE44-F	GCTGAGGGGGAGACAGTAGA
	Geno dE44-R	TCAGAAGGCATTTTGTCAAT

Media for iPSC-CMs differentiation				
Media name	Volume of base media	Base media	Volume of supplement	Supplement
CDM3	500mL	RPMI-1640 (Gibco 11875-093)	5mL	CDM3 Supplement: - 4.224g L-ascorbic acid-2-phosphate - 10g recombinant human albumin - 200mL H_2O
CDM3-C	100mL	CDM3	33.3, 41.6, or 50 µL	4, 5, or 6 µM CHIR99021-HCl (12mM).
CDM3-WNT	100mL	CDM3	20 µL	2 uM WNT-C59 (10mM)
SELECTIVE	500mL	RPMI-1640 -glucose (Gibco 11879-020)	10mL	B27 Supplement (Thermo Fisher Scientific 17504044)
BASAL	500mL	RPMI-1640 (Gibco 11875-093)	10mL	B27 Supplement (Thermo Fisher Scientific 17504044)

Sequence of primers to titer AAV		
Primers for titering of AAV-Trispr	CO388-GFP-F	AGAACGGCATCAAGGTGAAC
	CO389-GFP-R	GAACTCCAGCAGGACCATGT
Primers for titering of AAV-CK8e-Cas9	CO460-spCas9-F	CGGCTTCATCAAGAGACAGC
	CO461-spCas9-R	TTCACTTCCCGGATCAGCTT

Sequence of primers for on and off target genomic amplicon deep sequencing.				
Sites	ID	Sequence (5'->3')	Product (bps)	miSeq-with Adaptor
E45G6 Target	mDmd-Ex45G6-Ontarget-DS-F1	CCCTGAGCTGAAGTGAGAGG	404	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCCTGAGCTGAAGTGAGAGG
	mDmd-Ex45G6-Ontarget-DS-R2	ACCTCTTTCTCCTTTCTGCCAG		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACCTCTTTCTCCTTTCTGCCAG
E45G6 OT1	E45G6OT1-DS-F1-YLM	CTGCCCCAACAAGAGCATTCTAAG	374	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTGCCCCAACAAGAGCATTCTAAG
	E45G6OT1-DS-R1-YLM	AGCCACTGTTTAACTTGCAGTCAC		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGCCACTGTTTAACTTGCAGTCAC
E45G6 OT2	E45G6OT2-DS-F1-YLM	CTTTCCTCCTCCACCCTCACAG	356	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTTCCTCCTCCACCCTCACAG
	E45G6OT2-DS-R1-YLM	TCCTGTTACATGTCCCCGACAC		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTGTTACATGTCCCCGACAC
E45G6 OT3	E45G6OT3-DS-F1-YLM	CTCAGAGAGTCGATGGAACTCCTG	442	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTCAGAGAGTCGATGGAACTCCTG
	E45G6OT3-DS-R1-YLM	TCCTATGGGGTCAATTTCTGCACA		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTATGGGGTCAATTTCTGCACA
E45G6 OT4	E45G6OT4-DS-F1-YLM	GGTTCTCAAAATGCCCTGTTGTGA	487	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGTTCTCAAAATGCCCTGTTGTGA
	E45G6OT4-DS-R1-YLM	TCTCCTGGAGGGGTGAAAGAAAAG		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCTCCTGGAGGGGTGAAAGAAAAG
E45G6 OT5	E45G6OT5-DS-F1-YLM	TGTGGGACTGCTAGAAAGTTTGGA	440	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGTGGGACTGCTAGAAAGTTTGGA
	E45G6OT5-DS-R2-YLM	GATCCCCGCCTGGAGTTTATTAGT		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATCCCCGCCTGGAGTTTATTAGT
E45G6 OT6	E45G6OT6-DS-F2-YLM	TGGACAAAGGAGCAAACAAAAGCT	413	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGGACAAAGGAGCAAACAAAAGCT
	E45G6OT6-DS-R2-YLM	TTTATGGACAGTTGAGGTGCCAGA		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTTATGGACAGTTGAGGTGCCAGA
E45G6 OT7	E45G6OT7-DS-F1-YLM	AAGGGACAGCTCAAAGACCTTCTT	398	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAGGGACAGCTCAAAGACCTTCTT
	E45G6OT7-DS-R1-YLM	ACTTCAAACGCACTGTCACATCAG		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTTCAAACGCACTGTCACATCAG
E45G6 OT8	E45G6OT8-DS-F1-YLM	TCTGAAGAAGCCCTTGGTCATTCA	459	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCTGAAGAAGCCCTTGGTCATTCA
	E45G6OT8-DS-R1-YLM	ATCCTCTACACGTAACAGGAAGCC		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATCCTCTACACGTAACAGGAAGCC
E45G6 OT9	E45G6OT9-DS-F1-YLM	GAAGGCAGTCAAGCAGATTGGATC	414	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAAGGCAGTCAAGCAGATTGGATC
	E45G6OT9-DS-R2-YLM	ACTAGCAGCCTTTGGATGAAGACA		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTAGCAGCCTTTGGATGAAGACA
E45G6 OT10	E45G6OT10-DS-F2-YLM	ATGACGACGACGACAATGTTGATG	445	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGACGACGACGACAATGTTGATG
	E45G6OT10-DS-R2-YLM	CCTCAAAGCCTTCTTGAAGGAAGC		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTCAAAGCCTTCTTGAAGGAAGC

Sequence of primers for on target cDNA amplicon deep sequencing				
RT-Site	ID	Sequence (5'->3')	Product (bps)	miSeq-with Adaptor
E45G6 RT-DS	mDmd-E4346-RT-DS-F1	AGGTGAAAGTACAGGAAGCCGT	370	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGTGAAAGTACAGGAAGCCGT
	mDmd-E4346-RT-DS-R1	CTGCTGCTCATCTCCAAGTGGA		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTGCTGCTCATCTCCAAGTGGA