YMTHE, Volume *28*

Supplemental Information

Correction of Three Prominent Mutations in Mouse and Human Models of Duchenne Muscular Dystrophy by Single-Cut Genome Editing

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Supplemental Information

Supplementary Figures

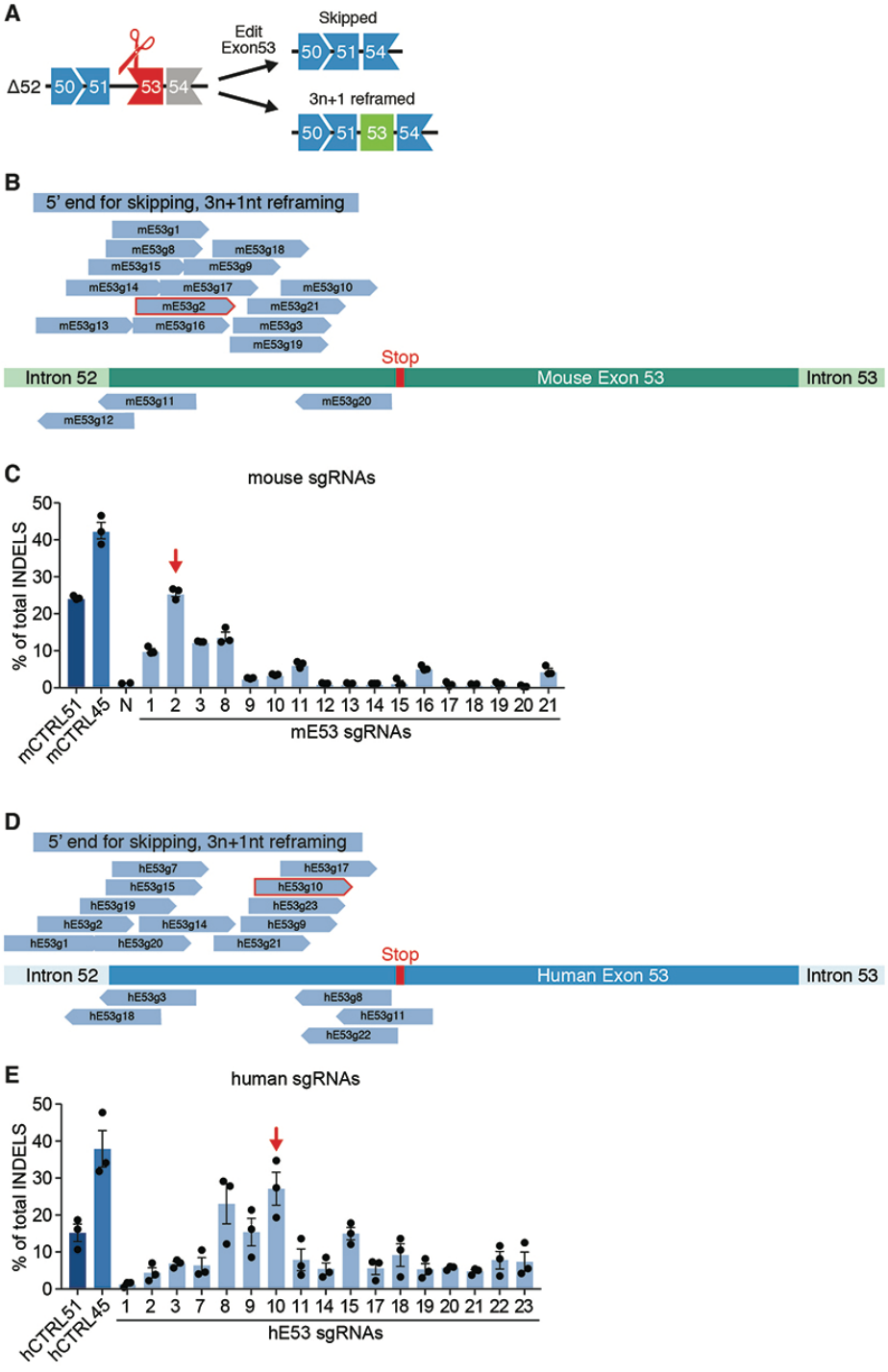

Figure S1. Gene editing strategy and location of mouse and human exon 53 sgRNAs for Δ52 DMD models. (A) Diagram for exon 53 targeting strategy and potential products after editing. Shapes of intron-exon junctions indicate complementarity that maintains the open reading frame upon splicing. (B) Mouse sgRNA location targeting the 5' region of exon 53. The sgRNAs targeting exon 53 before the stop codon in Δ52 mice are candidates for exon skipping or 3n+1 reframing. sgRNA sequences are listed in Table S2. mE53g2, the sgRNA selected for further analyses is bordered in red. (C) Indel analysis of sgRNAs that target exon 53 was performed in N2a mouse cells. Red arrow indicates the most efficient sgRNA which was used for further analyses. mCTRL51 and mCTRL45 are positive validated sgRNA controls targeting mouse exon 51 and exon 45, respectively[18, 19] (n = 3 biological replicates). (D) Human sgRNA location for targeting the 5' region of exon 53. The sgRNAs targeting exon 53 before the stop codon in human Δ52 iPSCs are candidates for exon skipping or 3n+1 reframing. sgRNA sequences are listed in Table S2. hE53h10, the sgRNA selected for further analyses is bordered in red. (E) Indel analysis of sgRNAs that target exon 53 was performed in 293T human cells. Red arrow indicates the most efficient sgRNA used for further analyses. hCTRL51 and hCTRL45 are positive validated sgRNA controls targeting human exon 51 and exon 45, respectively[18, 19] (n = 3 biological replicates). Data are presented as means ± SEM.

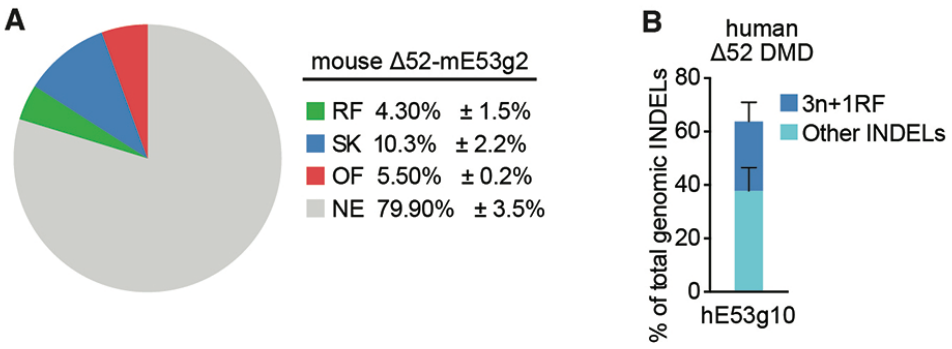

Figure S2. Correction events of mouse and human models after editing of exon 53 in mouse and human Δ52 DMD models. (A) Pie chart showing percentage of events detected in mouse TA muscle at exon 53 after ssAAV-Cas9 and scAAV-mE53g2 treatment using TIDE analysis of the RT-PCR sequences (n=3). RT-PCR products were divided into four groups: Not edited (NE), exon 53 skipped (SK), exon 53 reframed (RF), and out of frame (OF). (B) INDEL genomic analysis of hE53g10 targeting exon 53 in human Δ52 DMD iPSCs (n=3). 3n+1 reframing (RF) events restore the correct open reading frame. Data are presented as means ± SEM

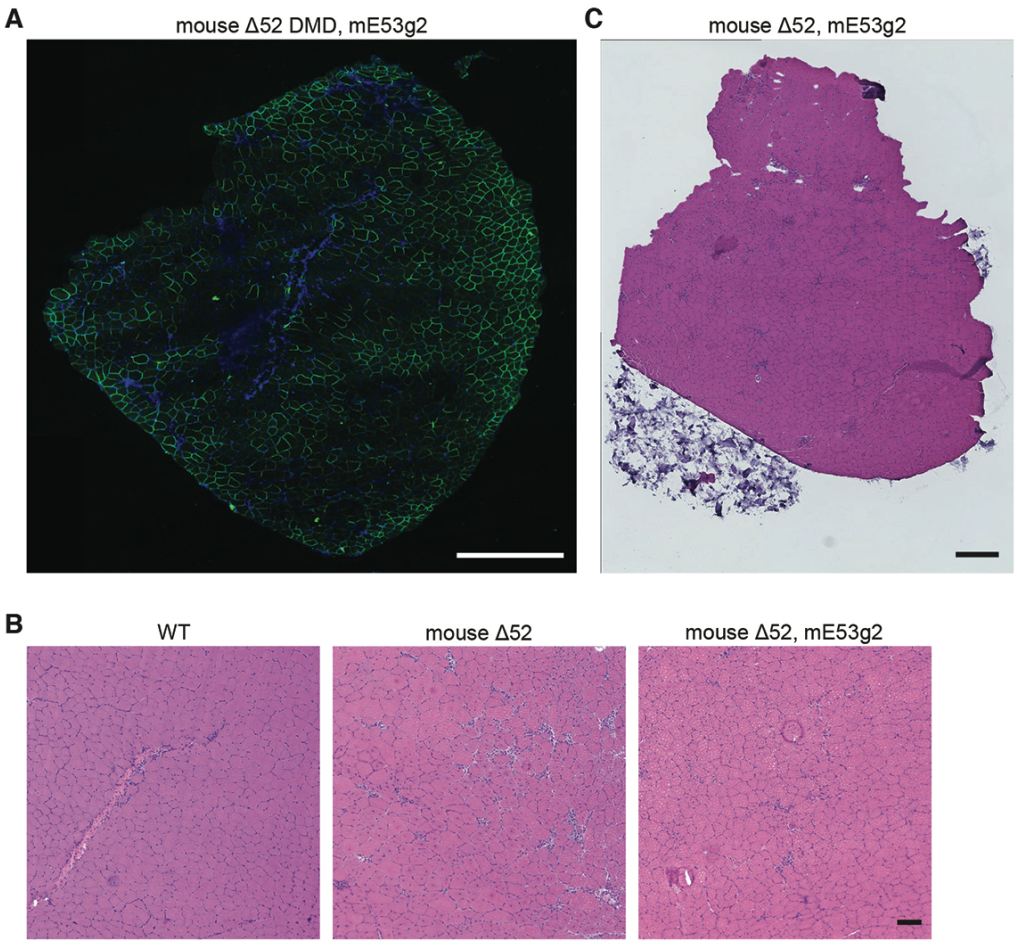

Figure S3. Intramuscular AAV9 delivery of gene editing components rescues dystrophin expression in Δ52 DMD mice. (A) Dystrophin immunohistochemistry of TA muscle in corrected Δ52 DMD mice 3 weeks after ssAAV-Cas9 and scAAV-mE53g2 intramuscular injection (5×10^{10} vg/leg of ssAAV9-Cas9 and 5×10^{10} vg/leg of scAAV-mE53g2). Dystrophin is shown in green. Nuclei are marked by DAPI stain in blue. Tile scan (10X) of the entire TA muscle. Scale bar is 500 μm. (B) H&E staining of TA in WT, Δ52 DMD, and corrected Δ52 DMD mice 3 weeks after ssAAV-Cas9 and scAAV-mE53g2 intramuscular injection (5×10^{10} vg/leg of ssAAV9-Cas9 and 5×10^{10} vg/leg of scAAV-mE53g2). Scale bar is 100 μm. (C) Whole muscle scanning of H&E staining of TA of WT, Δ52 DMD and corrected Δ52. Tile scan (4X) of the entire muscle. Scale bar is 500 μm.

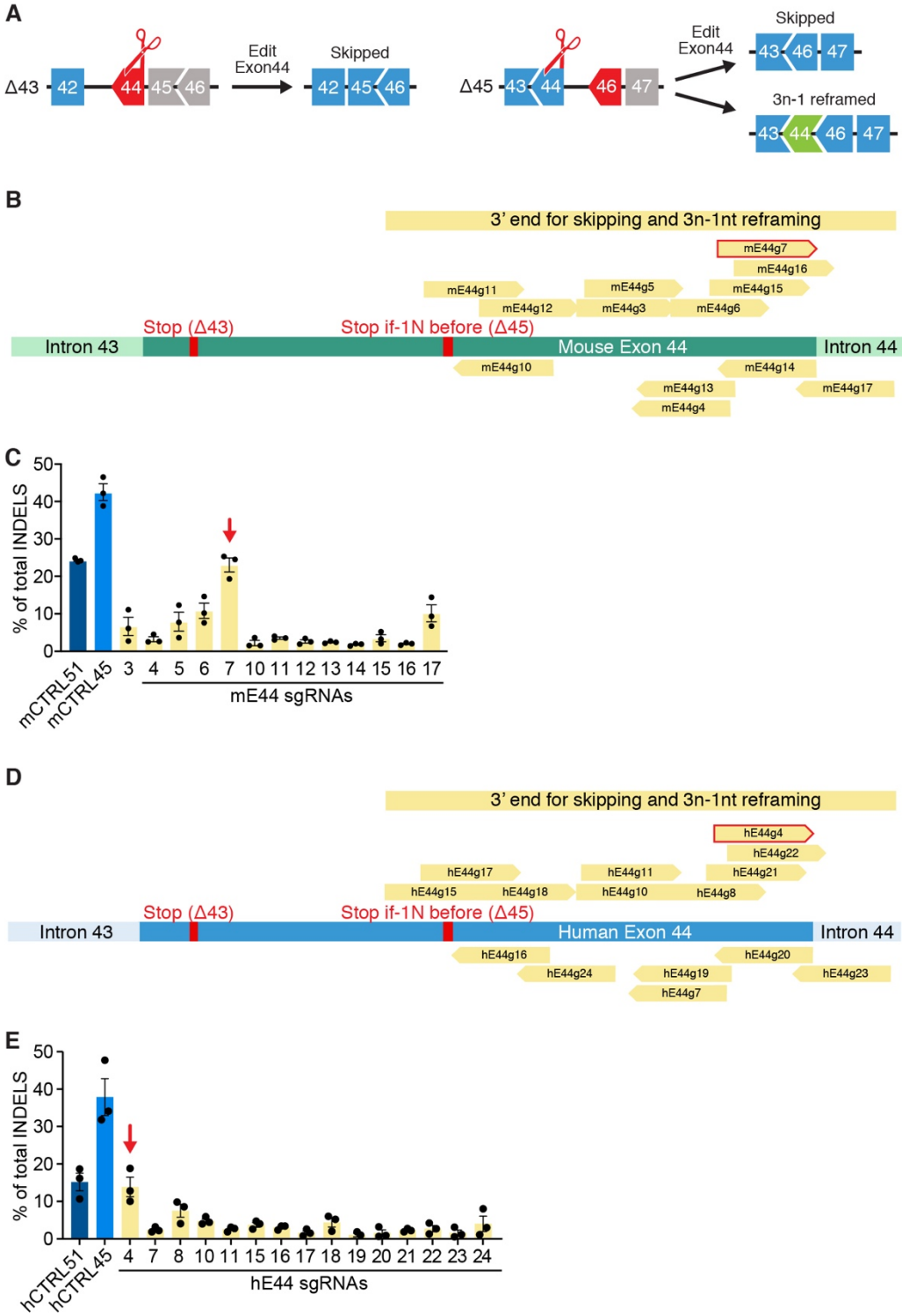
A
Δ43
42
44
45
46
Edit Exon44
Skipped
42
45
46
Δ45
43
44
46
47
Edit Exon44
Skipped
43
46
47
3n-1 reframed
43
44
46
47
B
3' end for skipping and 3n-1nt reframing
mE44g7
mE44g16
mE44g11
mE44g5
mE44g15
mE44g12
mE44g3
mE44g6
Stop (Δ43)
Stop if-1N before (Δ45)
Intron 43
Mouse Exon 44
Intron 44
mE44g10
mE44g14
mE44g13
mE44g17
mE44g4
C
% of total INDELS
50
40
30
20
10
0
mCTRL51
mCTRL45
3
4
5
6
7
10
11
12
13
14
15
16
17
mE44 sgRNAs
D
3' end for skipping and 3n-1nt reframing
hE44g4
hE44g22
hE44g17
hE44g11
hE44g21
hE44g15
hE44g18
hE44g10
hE44g8
Stop (Δ43)
Stop if-1N before (Δ45)
Intron 43
Human Exon 44
Intron 44
hE44g16
hE44g20
hE44g24
hE44g19
hE44g23
hE44g7
E
% of total INDELS
50
40
30
20
10
0
hCTRL51
hCTRL45
4
7
8
10
11
15
16
17
18
19
20
21
22
23
24
hE44 sgRNAs

Figure S4. Gene editing strategy and location of mouse and human exon 44 sgRNAs for Δ43 and Δ45 DMD models. (A) Diagram for exon 44 targeting strategy and potential products after editing in Δ43 and Δ45 DMD models. Shapes of intron-exon junctions indicate complementarity that maintains the open reading frame upon splicing. (B) Mouse sgRNAs targeting the 3' region of exon 44. The sgRNAs targeting exon 44 are candidates for exon skipping (in Δ43 DMD mice) or exon skipping and 3n+1 reframing (in Δ45 DMD). sgRNA sequences are listed in Table S2. mE44g7, the sgRNA selected for further analyses is bordered in red. (C) Indel analysis of sgRNAs that target exon 44 was performed in N2a mouse cells. Red arrow indicates the most efficient sgRNA which was used for further analyses. mCTRL51 and mCTRL45 are positive validated sgRNA controls targeting, respectively, mouse exon 51 and exon 45[18, 19] (n = 3 biological replicates). (D) Human sgRNA location for targeting the 3'region of exon 44. The sgRNAs targeting exon 44 are candidates for exon skipping (in human Δ43 DMD) or exon skipping and 3n+1 reframing (in human Δ45 DMD). sgRNA sequences are listed in Table S2. hE44g4, the sgRNA selected for further analyses is bordered in red. (E) Indel analysis of sgRNAs that target exon 44 was performed in 293T human cells. Red arrow indicates the most efficient sgRNA used for the further analyses. hCTRL51 and hCTRL45 are positive validated sgRNA controls targeting, respectively, human exon 51 and exon 45[18, 19] (n = 3 biological replicates). Data are presented as means ± SEM

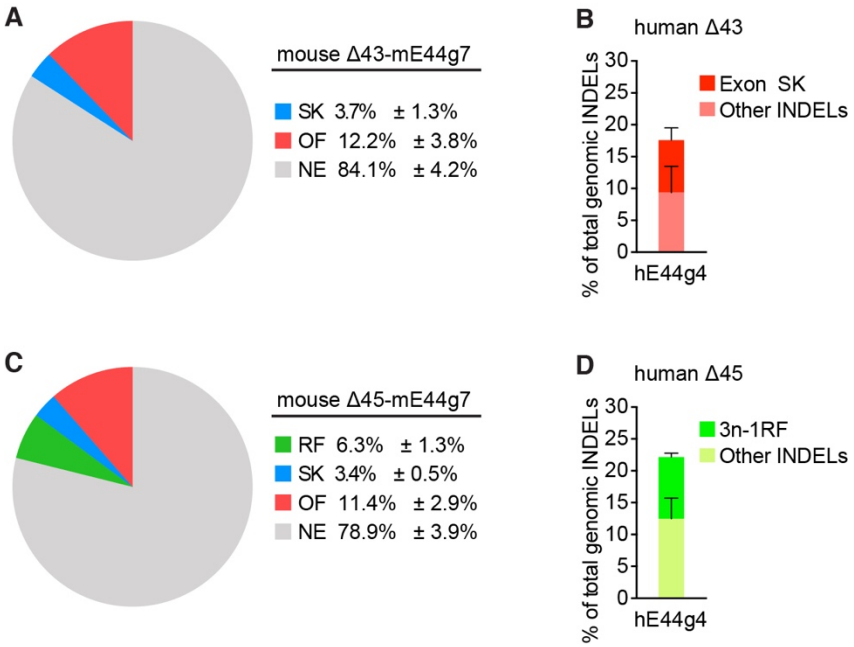

Figure S5. Correction events after gene editing of exon 44 in mouse and human Δ43 and Δ45 DMD models. (A, C) Pie charts showing percentage of events detected in Δ43 and Δ45 DMD mouse TA muscles after ssAAV-Cas9 and scAAV-mE44g7 treatment using TIDE analysis of the RT-PCR sequences (A: n=3; C: n=2). RT-PCR products were divided into four groups: Not edited (NE), exon 44 skipped (SK), exon 44 reframed (RF), and out of frame (OF). (B, D) INDEL genomic analysis of hE44g4 targeting exon 44 in human Δ43 and Δ45 DMD iPSCs (n=3). Exon 44 skipping (SK) restores the correct open reading frame in human Δ43 DMD. 3n-1 reframing (RF) events restore the correct open reading frame in Δ45. Data are presented as means ± SEM

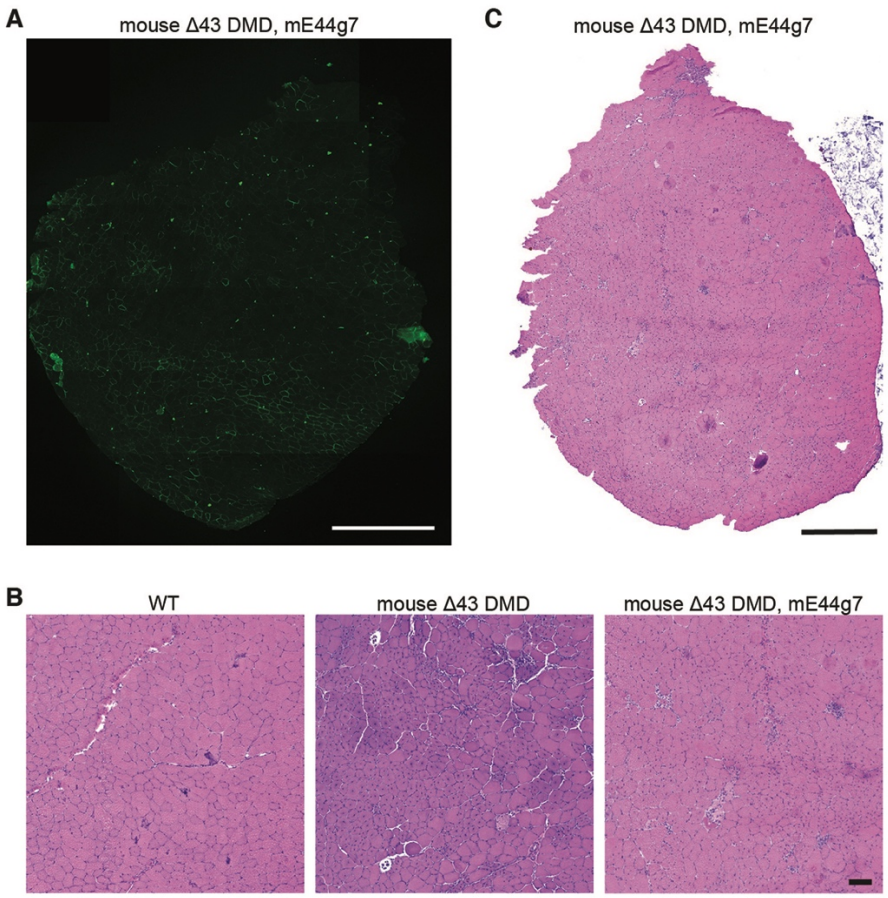

Figure S6. Intramuscular AAV9 delivery of gene editing components restores dystrophin expression in Δ43 DMD mice. (A) Dystrophin immunohistochemistry of TA muscle in corrected Δ43 DMD mice 3 weeks after ssAAV-Cas9 and scAAV-mE44g7 intramuscular injection (5×10^{10} vg/leg of ssAAV9-Cas9 and 5×10^{10} vg/leg of scAAV-mE44g7). Dystrophin is shown in green. Nuclei are marked by DAPI stain in blue. Tile scan (10X) of the entire TA muscle. Scale bar is 500 μm. (B) H&E staining of TA muscles in WT, Δ43 DMD, and corrected Δ43 mice 3 weeks after ssAAV-Cas9 and scAAV-mE44g7 intramuscular injection (5×10^{10} vg/leg of ssAAV9-Cas9 and 5×10^{10} vg/leg of scAAV-mE44g7). Scale bar is 100 μm. (C) Whole muscle scanning of H&E staining of TA of corrected mΔ43. Tile scan (4X) of the entire muscle. Scale bar is 500 μm.

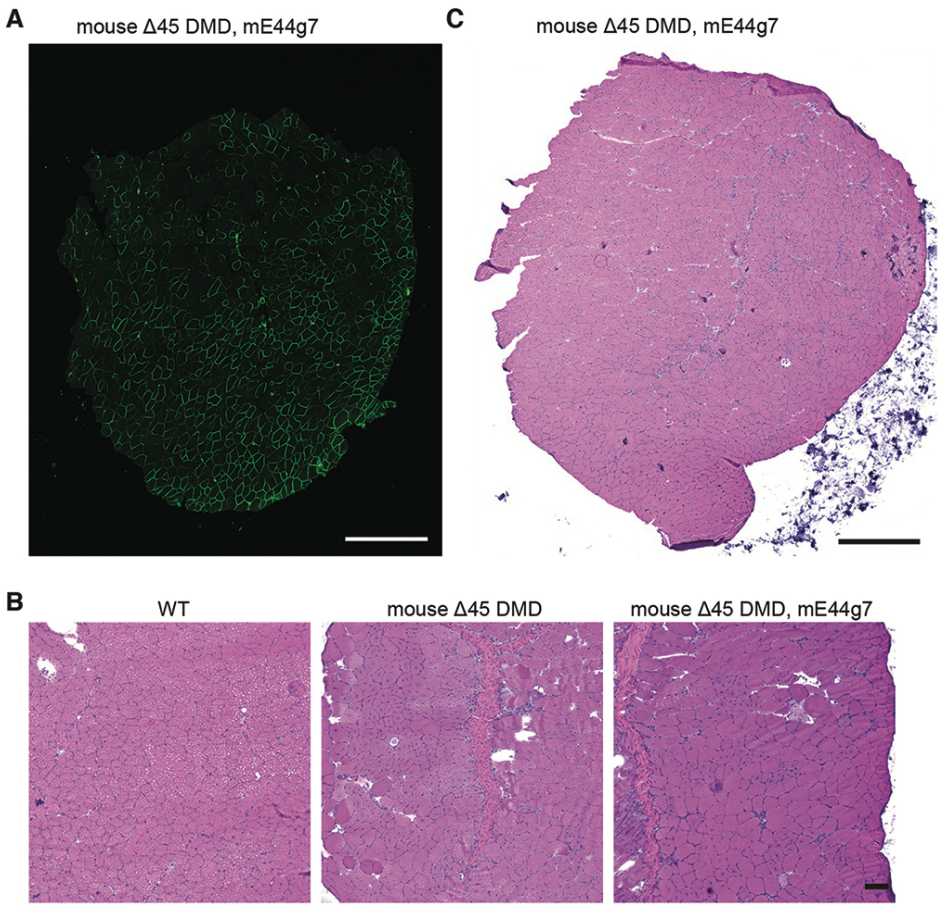

Figure S7. Intramuscular AAV9 delivery of gene editing components restores dystrophin expression in Δ45 DMD mice. (A) Dystrophin immunohistochemistry of TA muscle in corrected Δ45 DMD mice 3 weeks after ssAAV-Cas9 and scAAV-mE44g7 intramuscular injection (5 × 10^{10} vg/leg of ssAAV9-Cas9 and 5 × 10^{10} vg/leg of scAAV-mE44g7). Dystrophin is shown in green. Nuclei are marked by DAPI stain in blue. Tile scan (10X) of the entire TA muscle. Scale bar is 500 μm. (B) H&E staining of TA in WT, Δ45 DMD, and corrected DMD mice 3 weeks after ssAAV-Cas9 and scAAV-mE44g7 intramuscular injection (5 × 10^{10} vg/leg of ssAAV9-Cas9 and 5 × 10^{10} vg/leg of scAAV-mE44g7). Scale bar is 100 μm. (**C**) Whole muscle scanning of H&E staining of TA muscles of corrected Δ45 DMD mice. Tile scan (4X) of the entire muscle. Scale bar is 500 μm.

Supplementary Tables

Table S1. List of sgRNAs used to generate DMD mouse models and primers.

DMD model generation		
Purpose of the primers	**ID**	**Sequence (5'-3')**
Primers for sgRNA targeting Dmd exon 43 to generate the ΔEx43 DMD model	mDmd-Ex43-N2-Top	caccgTTATTAGTACTAACTCAGAA
	mDmd-Ex43-N2-Bottom	aaacTTCTGAGTTAGTACTAATAAc
	mDmd-Ex43-C2-Top	caccgGTAAATATCAACTTCTAAAT
	mDmd-Ex43-C2-Bottom	aaacATTTAGAAGTTGATATTTACc
Primers for sgRNA targeting Dmd exon 45 to generate the ΔEx45 DMD model	mDmd-Exon45-5-G1-top	CACCGaactaatatatccaaatact
	mDmd-Exon45-5-G1-bot	AAACAGTATTTGGATATATTAGTT C
	mDmd-Exon45-3-G4-top	CACCGagtttgtgctaaaaatcatg
	mDmd-Exon45-3-G4-bot	AAACCATGATTTTTAGCACAAACT C
Primers for sgRNA targeting Dmd exon 52 to generate the ΔEx52 DMD model	mDmd-Ex52-N1-Top	CACCGatatatcttaaatgatgtat
	mDmd-Ex52-N1-bottom	AAACATACATCATTTAAGATATATC
	mDmd-Ex52-C3-Top	caccgccaagttaatcaaattgttc
	mDmd-Ex52-C3-Bottom	aaacGAACAATTTGATTAACTTGGc
PCR, RT-PCR and TIDE analysis		
For mouse dE43, skipping exon 44	mDmd-dE43-E4146-RT-R2	CTGCTGCTCATCTCCAAGTG
	mDmd-dE43-E4246-RT-F3	AGTGACGACTGAAGATATGCCT
For mouse dE45, skipping/reframing exon44	mDmd-E4347-RT-F2	AGGTGAAAGTACAGGAAGCCGT
	mDmd-E4347-RT-R2	CTTCTGGCCTTATGGGAGCACT
For mouse dE52, skipping or reframe exon 53	mDmd-E5054-RT-R1	TGACGGAGGTCTTTGGCCAA
	mDmd-E5155-RT-F1	TTGGAGGTACCTGCACTGGC
For human dE43, skipping exon 44	hEx42-RT-F	GCCCTATTAGAAGTGGAACAAC
	hEx46-RT-R	GGTTCAAGTGGGATACTAGC
For human dE45, skipping/reframing exon44	hEx42-RT-F	GCCCTATTAGAAGTGGAACAAC
	hEx46-RT-R	GGTTCAAGTGGGATACTAGC
For human dE52, skipping or reframe exon 53	hEx51-RT-F	GAAACTGCCATCTCCAAACTAGAAA
	hEx54-RT-R	TCATGTGGACTTTTCTGGTATCATC
For editing mouse Dmd exon 44	mE44-T7E1-F1	agggagaagatgctaattatcctaag
	mE44-T7E1-R1	caaaacagtcatagcacaattttcag
For editing mouse Dmd exon 46	mDmd-sE46-T7E1-F2	tcttcacaagccccctctta
	mDmd-sE46-T7E1-R1	Caactggtaggcagtttgcat
For editing mouse Dmd exon 53	mDmd-sE53-T7E1-F2	TGCCCACAAGTAAGTGCTGA
	mDmd-sE53-T7E1-R1	TTGTCTCAAaaccaaccaacc
	hDMD-sE53-T7E1-F1	gggaaatcaggctgatgggt

For editing human exon 53	hDMD-sE53-T7E1-R1	GTCTACTGTTCATTTCAGC
For editing human exon 44	hDMD-sE44-T7E1-F2	GCAGGAAACTATCAGAGTG
	hDMD-sE44-T7E1-R2	ACACCTTGCTGTTACGAT
For editing human exon 46	hDMD-sE46-T7E1-F1	ccaccaaacctggcaaat
	hDMD-sE46-T7E1-R1	GAACTATGAATAACCTAATGGGCAG

Table S2. Sequence of sgRNAs.

Purpose	ID	Sequence	PAM
Human sgRNA targeting exon 53	hE53g1	ATTTATTTTTCCTTTTATTC	TAG
	hE53g2	TTTCCTTTTATTCTAGTTGA	AAG
	hE53g3	TGATTCTGAATTCTTTCAAC	TAG
	hE53g7	TGAAAGAATTCAGAATCAGT	GGG
	hE53g8	ACTGTTGCCTCCGGTTCTGA	AGG
	hE53g9	TACAAGAACACCTTCAGAAC	CGG
	hE53g10	AAGAACACCTTCAGAACCGG	AGG
	hE53g11	TTTCATTCAACTGTTGCCTC	CGG
	hE53g14	AATTCAGAATCAGTGGGATG	AAG
	hE53g15	TTGAAAGAATTCAGAATCAG	TGG
	hE53g17	ACCTTCAGAACCGGAGGCAA	CAG
	hE53g18	AATTCTTTCAActagaataa	AAG
	hE53g19	ttattctagTTGAAAGAATT	CAG
	hE53g20	tagTTGAAAGAATTCAGAAT	CAG
	hE53g21	ATGAAGTACAAGAACACCTT	CAG
	hE53g22	AACTGTTGCCTCCGGTTCTG	AAG
	hE53g23	CAAGAACACCTTCAGAACCG	GAG
Human sgRNA targeting exon 44	hE44g4	TAAATACAAATGGTATCTTA	AGG
	hE44g7	TTAGCATGTTCCCAATTCTC	AGG
	hE44g8	GGGAACATGCTAAATACAAA	TGG
	hE44g10	AGACACAAATTCCTGAGAAT	TGG
	hE44g11	GACACAAATTCCTGAGAATT	GGG
	hE44g15	ATTTAATCAGTGGCTAACAG	AAG
	hE44g16	AGAAACTGTTCAGCTTCTGT	TAG
	hE44g17	AGTGGCTAACAGAAGCTGAA	CAG
	hE44g18	AAGCTGAACAGTTTCTCAGA	AAG
	hE44g19	TTTAGCATGTTCCCAATTCT	CAG
	hE44g20	CTTAAGATACCATTTGTATT	TAG
	hE44g21	CTAAATACAAATGGTATCTT	AAG
	hE44g22	TACAAATGGTATCTTAAGgt	aag
	hE44g23	acaaatcaaagacttacCTT	AAG
	hE44g24	TGTCTTTCTGAGAAACTGTT	CAG
Control sgRNAs	hCTRL1	CACCAGAGTAACAGTCTGAG	TAG
	hCTRL2	ATCTTACAGGAACTCCAGGA	TGG
	mCTRL1	CACTAGAGTAACAGTCTGAC	TGG
	mCTRL2	GGCTTACAGGAACTCCAGGA	TGG
Mouse sgRNA targeting exon 53	mE53g1	TGAAAGAATTCAGATTCAGT	GGG
	mE53g2	AATTCAGATTCAGTGGGATG	AGG

	mE53g3	TTCAAGAACAGCTGCAGAAC	AGG
	mE53g8	TTGAAAGAATTCAGATTCAG	TGG
	mE53g9	AGTGGGATGAGGTTCAAGAA	CAG
	mE53g10	AGCTGCAGAACAGGAGACAA	CAG
	mE53g11	TGAATCTGAATTCTTTCAAC	TGG
	mE53g12	CTTTCAACTGGAATAAAAAT	AAG
	mE53g13	CTTATTTTTATTCCAGTTGA	AAG
	mE53g14	TTATTCCAGTTGAAAGAATT	CAG
	mE53g15	CAGTTGAAAGAATTCAGATT	CAG
	mE53g16	GAATTCAGATTCAGTGGGAT	GAG
	mE53g17	GATTCAGTGGGATGAGGTTC	AAG
	mE53g18	ATGAGGTTCAAGAACAGCTG	CAG
	mE53g19	GTTCAAGAACAGCTGCAGAA	CAG
	mE53g20	AACTGTTGTCTCCTGTTCTG	CAG
	mE53g21	CAAGAACAGCTGCAGAACAG	GAG
Mouse sgRNA targeting exon 44	mE44g3	AGACACAAAATCCTGAAAAC	TGG
	mE44g4	TTAGCATGTTCCCAGTTTTC	AGG
	mE44g5	GACACAAAATCCTGAAAACT	GGG
	mE44g6	GGGAACATGCTAAATACAAA	TGG
	mE44g7	TAAATACAAATGGTATCTTA	AGG
	mE44g10	AAAAACTGTTCAACTTCATT	CAG
	mE44g11	AATGGCTGAATGAAGTTGAA	CAG
	mE44g12	AAGTTGAACAGTTTTTCAAA	AAG
	mE44g13	TTTAGCATGTTCCCAGTTTT	CAG
	mE44g14	CTTAAGATACCATTTGTATT	TAG
	mE44g15	CTAAATACAAATGGTATCTT	AAG
	mE44g16	TACAAATGGTATCTTAAGgt	AAG
	mE44g17	AAATCTCAAAGTCTTACCTT	AAG
sgRNAs with NGG PAM are marked in green and sgRNAs with NAG PAM are marked in black.			