

**YMTHE, Volume 28**

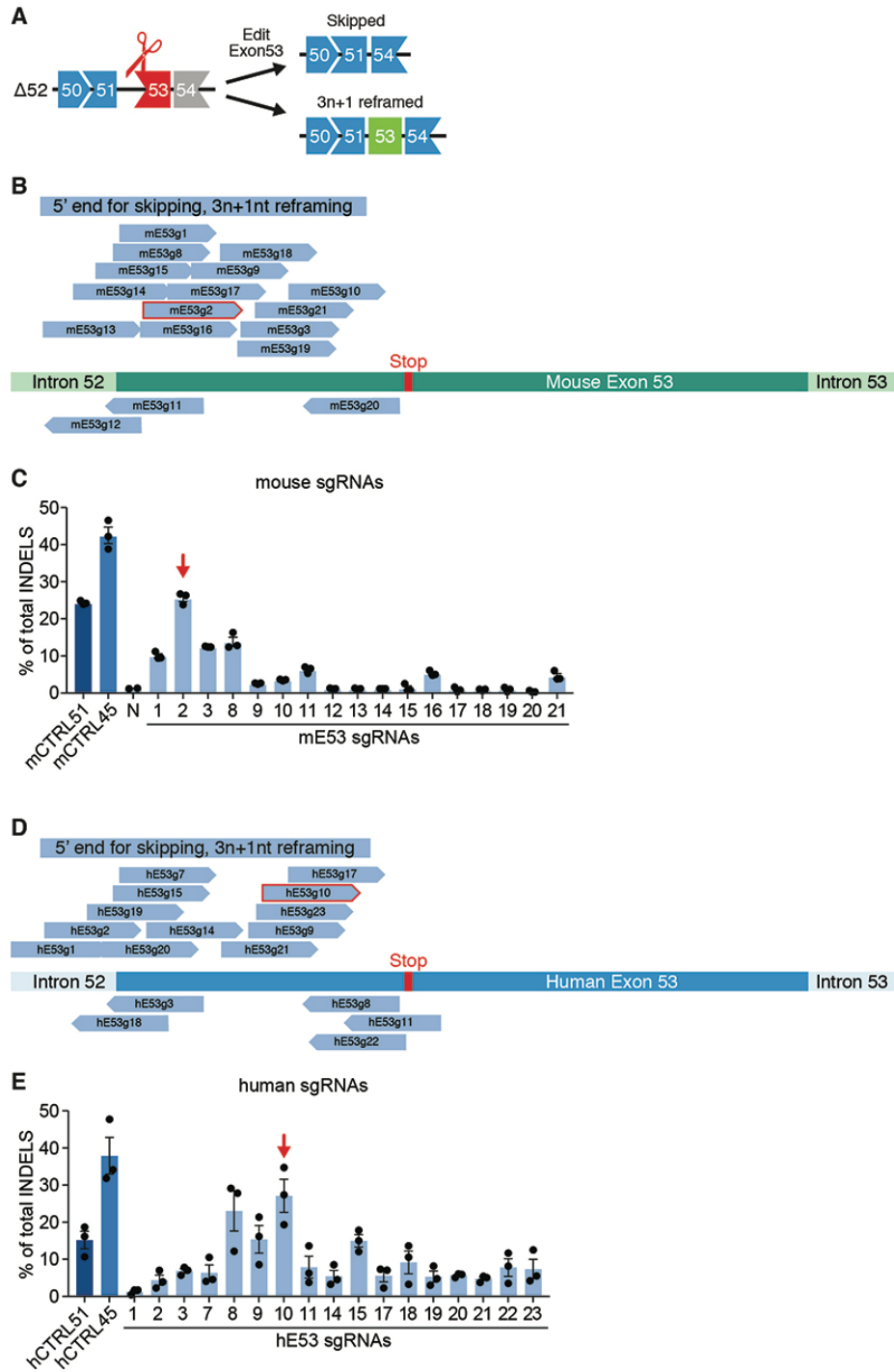
## **Supplemental Information**

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

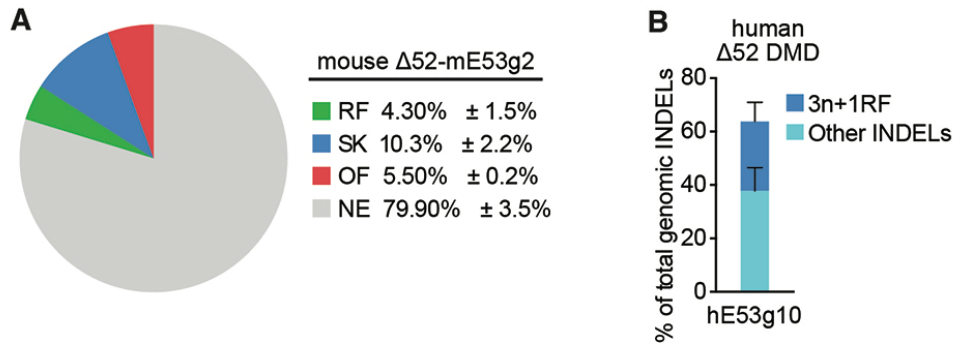
**Yi-Li Min, Francesco Chemello, Hui Li, Cristina Rodriguez-Caycedo, Efrain Sanchez-Ortiz, Alex A. Mireault, John R. McAnally, John M. Shelton, Yu Zhang, Rhonda Bassel-Duby, and Eric N. Olson**

# Supplemental Information

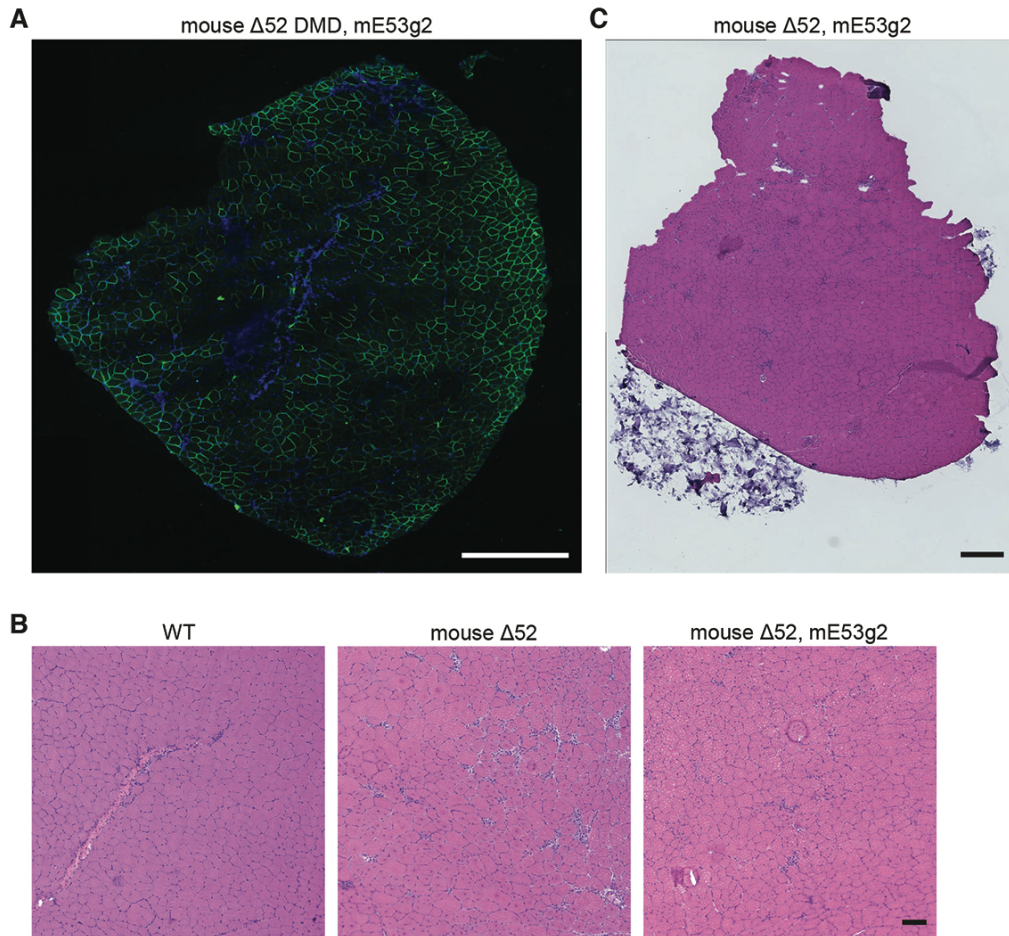
## Supplementary Figures



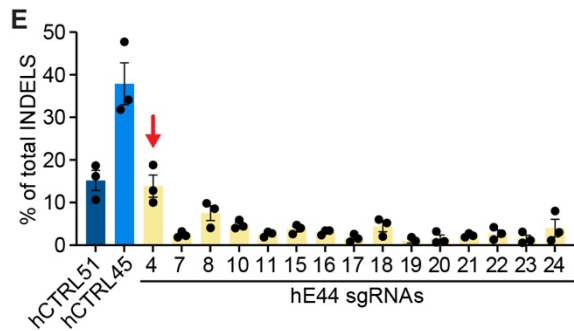
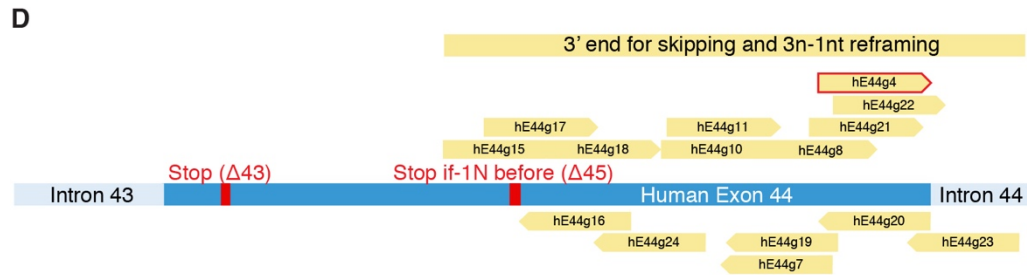
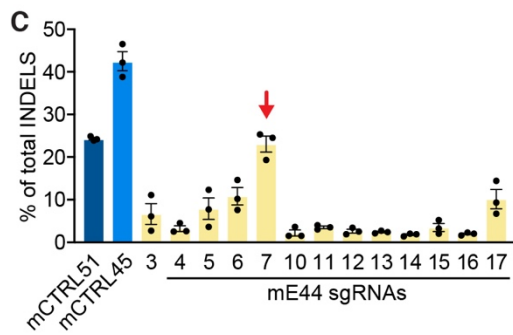
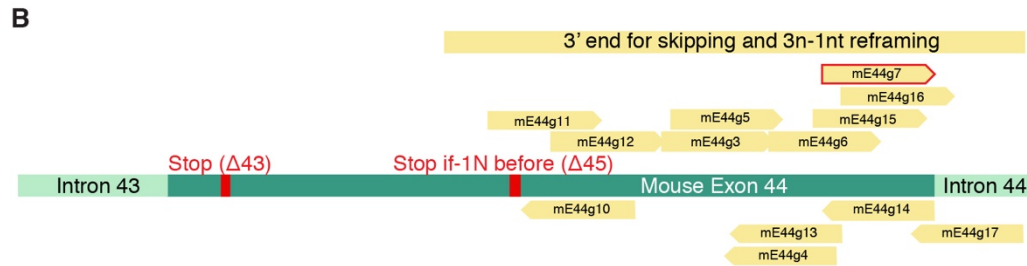
**Figure S1. Gene editing strategy and location of mouse and human exon 53 sgRNAs for  $\Delta 52$  DMD models.** (A) Diagram for exon 53 targeting strategy and potential products after editing. Shapes of intron-exon junctions indicate complementarity that maintains the open reading frame upon splicing. (B) Mouse sgRNA location targeting the 5' region of exon 53. The sgRNAs targeting exon 53 before the stop codon in  $\Delta 52$  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<sup>18, 19</sup> (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  $\Delta 52$  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<sup>18, 19</sup> (n = 3 biological replicates). Data are presented as means  $\pm$  SEM.



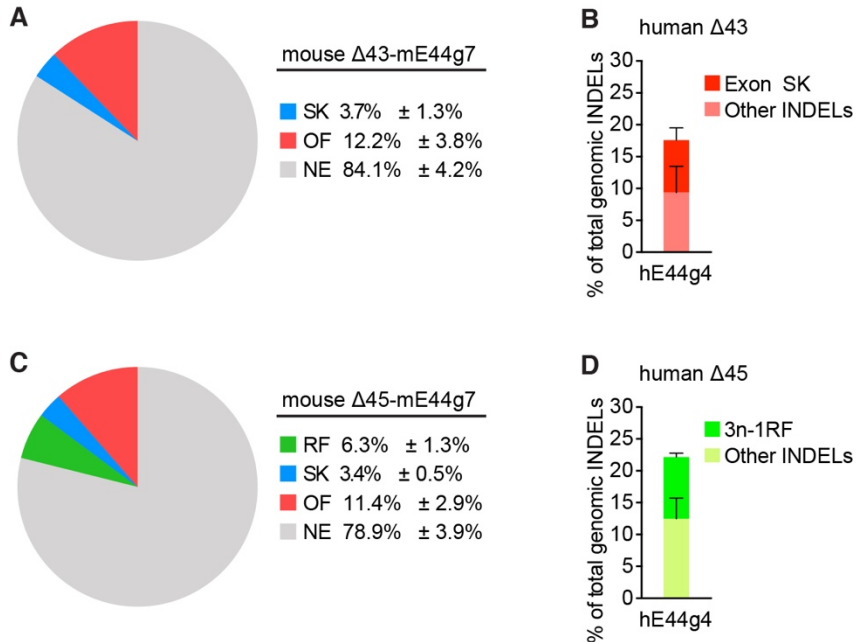
**Figure S2. Correction events of mouse and human models after editing of exon 53 in mouse and human  $\Delta 52$  DMD models.** (A) Pie chart showing percentage of events detected in mouse TA muscle at exon 53 after ssAAV-Cas9 and scAAV-mE53g2 treatment using TIDE analysis of the RT-PCR sequences (n=3). 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  $\Delta 52$  DMD iPSCs (n=3). 3n+1 reframing (RF) events restore the correct open reading frame. Data are presented as means  $\pm$  SEM



**Figure S3. Intramuscular AAV9 delivery of gene editing components rescues dystrophin expression in  $\Delta 52$  DMD mice.** (A) Dystrophin immunohistochemistry of TA muscle in corrected  $\Delta 52$  DMD mice 3 weeks after ssAAV-Cas9 and scAAV-mE53g2 intramuscular injection ( $5 \times 10^{10}$  vg/leg of ssAAV9-Cas9 and  $5 \times 10^{10}$  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  $\mu\text{m}$ . (B) H&E staining of TA in WT,  $\Delta 52$  DMD, and corrected  $\Delta 52$  DMD mice 3 weeks after ssAAV-Cas9 and scAAV-mE53g2 intramuscular injection ( $5 \times 10^{10}$  vg/leg of ssAAV9-Cas9 and  $5 \times 10^{10}$  vg/leg of scAAV-mE53g2). Scale bar is 100  $\mu\text{m}$ . (C) Whole muscle scanning of H&E staining of TA of WT,  $\Delta 52$  DMD and corrected  $\Delta 52$ . Tile scan (4X) of the entire muscle. Scale bar is 500  $\mu\text{m}$ .

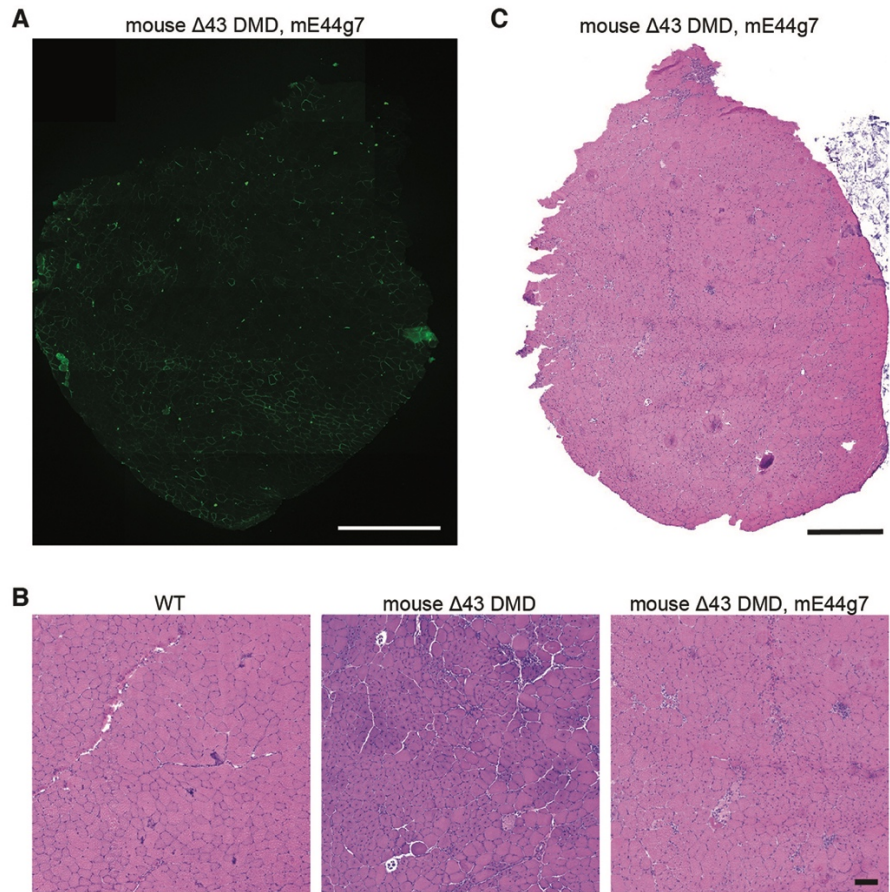


**Figure S4. Gene editing strategy and location of mouse and human exon 44 sgRNAs for  $\Delta 43$  and  $\Delta 45$  DMD models.** (A) Diagram for exon 44 targeting strategy and potential products after editing in  $\Delta 43$  and  $\Delta 45$  DMD models. Shapes of intron-exon junctions indicate complementarity 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  $\Delta 43$  DMD mice) or exon skipping and 3n+1 reframing (in  $\Delta 45$  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<sup>18, 19</sup> (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  $\Delta 43$  DMD) or exon skipping and 3n+1 reframing (in human  $\Delta 45$  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<sup>18, 19</sup> (n = 3 biological replicates). Data are presented as means  $\pm$  SEM

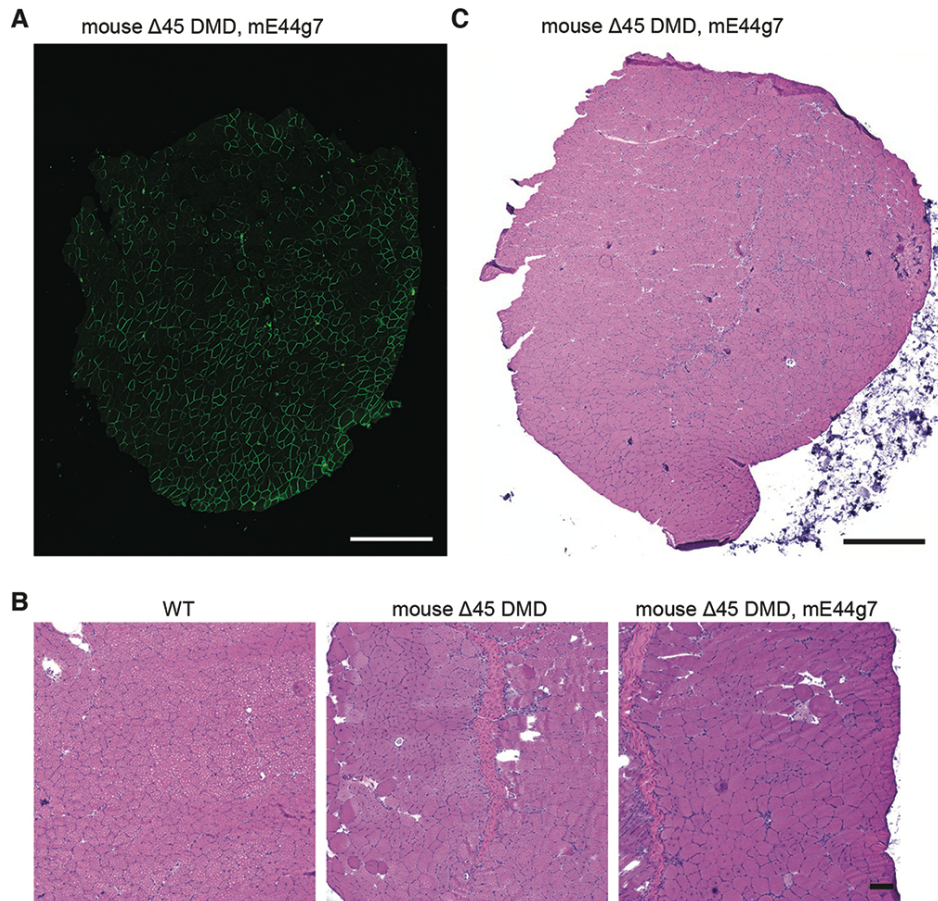


**Figure S5. Correction events after gene editing of exon 44 in mouse and human  $\Delta 43$  and  $\Delta 45$  DMD models.** (A, C) Pie charts showing percentage of events detected in  $\Delta 43$  and  $\Delta 45$  DMD mouse TA muscles after ssAAV-Cas9 and scAAV-mE44g7 treatment using TIDE analysis 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  $\Delta 43$  and  $\Delta 45$  DMD iPSCs (n=3). Exon 44 skipping (SK) restores the correct open reading frame in human  $\Delta 43$  DMD. 3n-1 reframing (RF) events restore the correct open reading frame in  $\Delta 45$ . Data are presented as means  $\pm$  SEM





**Figure S6. Intramuscular AAV9 delivery of gene editing components restores dystrophin expression in  $\Delta 43$  DMD mice.** (A) Dystrophin immunohistochemistry of TA muscle in corrected  $\Delta 43$  DMD mice 3 weeks after ssAAV-Cas9 and scAAV-mE44g7 intramuscular injection ( $5 \times 10^{10}$  vg/leg of ssAAV9-Cas9 and  $5 \times 10^{10}$  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  $\mu\text{m}$ . (B) H&E staining of TA muscles in WT,  $\Delta 43$  DMD, and corrected  $\Delta 43$  mice 3 weeks after ssAAV-Cas9 and scAAV-mE44g7 intramuscular injection ( $5 \times 10^{10}$  vg/leg of ssAAV9-Cas9 and  $5 \times 10^{10}$  vg/leg of scAAV-mE44g7). Scale bar is 100  $\mu\text{m}$ . (C) Whole muscle scanning of H&E staining of TA of corrected m $\Delta 43$ . Tile scan (4X) of the entire muscle. Scale bar is 500  $\mu\text{m}$ .



**Figure S7. Intramuscular AAV9 delivery of gene editing components restores dystrophin expression in  $\Delta 45$  DMD mice.** (A) Dystrophin immunohistochemistry of TA muscle in corrected  $\Delta 45$  DMD mice 3 weeks after ssAAV-Cas9 and scAAV-mE44g7 intramuscular injection ( $5 \times 10^{10}$  vg/leg of ssAAV9-Cas9 and  $5 \times 10^{10}$  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  $\mu$ m. (B) H&E staining of TA in WT,  $\Delta 45$  DMD, and corrected DMD mice 3 weeks after ssAAV-Cas9 and scAAV-mE44g7 intramuscular injection ( $5 \times 10^{10}$  vg/leg of ssAAV9-Cas9 and  $5 \times 10^{10}$  vg/leg of scAAV-mE44g7). Scale bar is 100  $\mu$ m. (C) Whole muscle scanning of H&E staining of TA muscles of corrected  $\Delta 45$  DMD mice. Tile scan (4X) of the entire muscle. Scale bar is 500  $\mu$ m.

## 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 $\Delta$ Ex43 DMD model	mDmd-Ex43-N2-Top	caccgTTATTAGTACTAACTCAGAA
	mDmd-Ex43-N2-Bottom	aaacTTCTGAGTTAGTACTAATAAc
	mDmd-Ex43-C2-Top	caccgGTAAATATCAACTTCTAAAT
	mDmd-Ex43-C2-Bottom	aaacATTTAGAAAGTTGATATTTACc
Primers for sgRNA targeting Dmd exon 45 to generate the $\Delta$ Ex45 DMD model	mDmd-Exon45-5-G1-top	CACCGaactaatatatacctaaataact
	mDmd-Exon45-5-G1-bot	AAACAGTATTTGGATATATTAGTT C
	mDmd-Exon45-3-G4-top	CACCGagtttggtgctaaaaatcatg
	mDmd-Exon45-3-G4-bot	AAACCATGATTTTTTAGCACAAACT C
Primers for sgRNA targeting Dmd exon 52 to generate the $\Delta$ Ex52 DMD model	mDmd-Ex52-N1-Top	CACCGatataatcttaaatgatgtat
	mDmd-Ex52-N1-bottom	AAACATACATCATTTAAGATATATC
	mDmd-Ex52-C3-Top	caccgccaagttaatcaaattggttc
	mDmd-Ex52-C3-Bottom	aaacGAACAATTTGATTAACCTGGc
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	GCCCTATTAGAAGTGAACAAC
	hEx46-RT-R	GGTTCAAGTGGGATACTAGC
For human dE45, skipping/reframing exon44	hEx42-RT-F	GCCCTATTAGAAGTGAACAAC
	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	caaacagtcatagcacaattttcag
For editing mouse Dmd exon 46	mDmd-sE46-T7E1-F2	tcttcacaagccccctctta
	mDmd-sE46-T7E1-R1	Caactggttaggcagtttgcatt
For editing mouse Dmd exon 53	mDmd-sE53-T7E1-F2	TGCCACAAGTAAGTGCTGA
	mDmd-sE53-T7E1-R1	TTGTCTCAAaaccaaccaacc
	hDMD-sE53-T7E1-F1	gggaaatcaggctgatgggt

For editing human exon 53	hDMD-sE53-T7E1-R1	GTCTACTGTTTCATTTTCAGC
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	AATTCTTTCAAActagaataa	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	AGAACTGTTCAGCTTCTGT	TAG
	hE44g17	AGTGGCTAACAGAAGCTGAA	CAG
	hE44g18	AAGCTGAACAGTTTCTCAGA	AAG
	hE44g19	TTTAGCATGTTCCCAATTCT	CAG
	hE44g20	CTTAAGATACCATTTGTATT	TAG
	hE44g21	CTAAATACAAATGGTATCTT	AAG
	hE44g22	TACAAATGGTATCTTAAggt	aag
	hE44g23	acaaatcaaagacttacCTT	AAG
hE44g24	TGTCTTTCTGAGAACTGTT	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	GATTCAGTGGGATGAGGTC	AAG
	mE53g18	ATGAGGTTCAAGAACAGCTG	CAG
	mE53g19	GTTCAAGAACAGCTGCAGAA	CAG
	mE53g20	AACTGTTGTCTCCTGTTCTG	CAG
	mE53g21	CAAGAACAGCTGCAGAACAG	GAG
Mouse sgRNA targeting exon 44	mE44g3	AGACACAAAATCCTGAAAAC	TGG
	mE44g4	TTAGCATGTTCCAGTTTTC	AGG
	mE44g5	GACACAAAATCCTGAAAAC	GGG
	mE44g6	GGGAACATGCTAAATACAAA	TGG
	mE44g7	TAAATACAAATGGTATCTTA	AGG
	mE44g10	AAAACTGTTCAACTTCATT	CAG
	mE44g11	AATGGCTGAATGAAGTTGAA	CAG
	mE44g12	AAGTTGAACAGTTTTTCAA	AAG
	mE44g13	TTTAGCATGTTCCAGTTTT	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.			