

Supplementary Information

In vivo non-invasive monitoring of dystrophin correction in a new Duchenne Muscular Dystrophy reporter mouse

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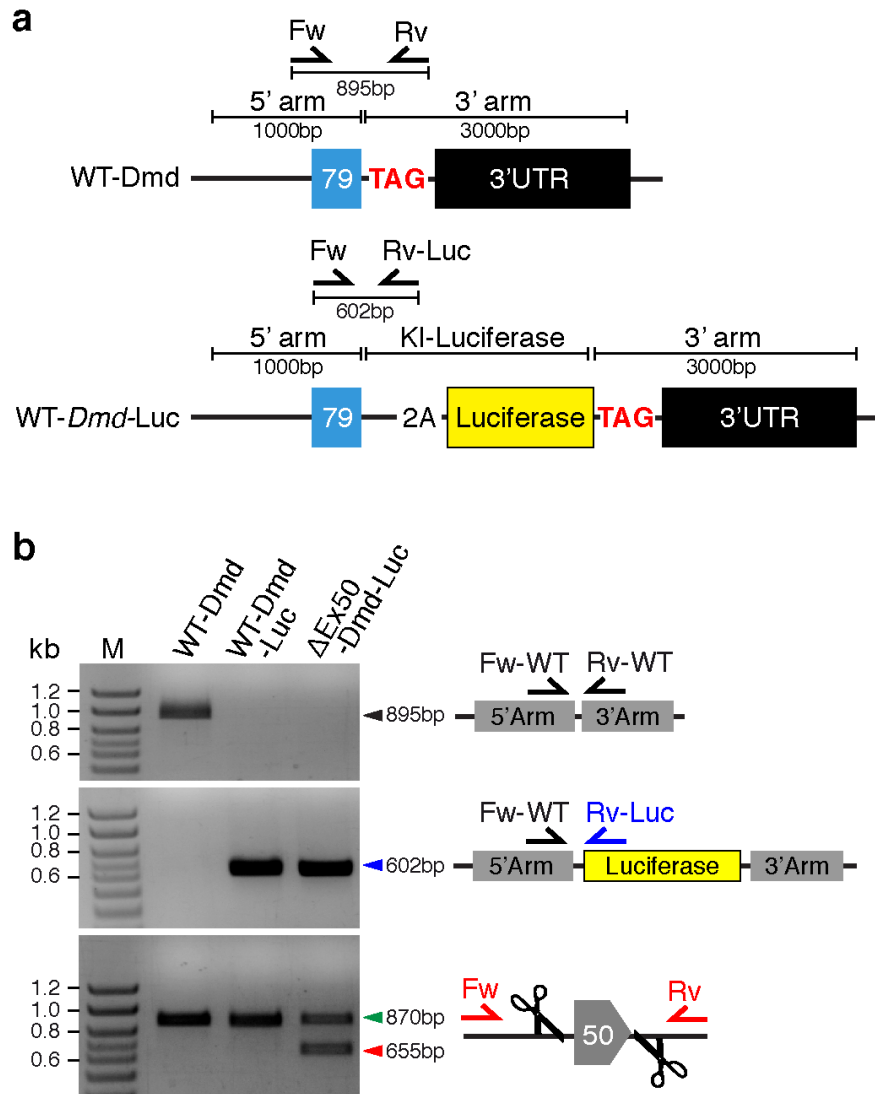
Supplementary Figures

WT-Dmd-Luc	GAAATCACTTTTTACCTGTAATTATTCATGTTTCTCATAAAGAATATACTCAGAAAATGA
ΔEx50-Dmd-Luc	GAAATCACTTTTTACCTGTAATTATTCATGTTTCTCATAAAGAATATACTCAGAAAATGA

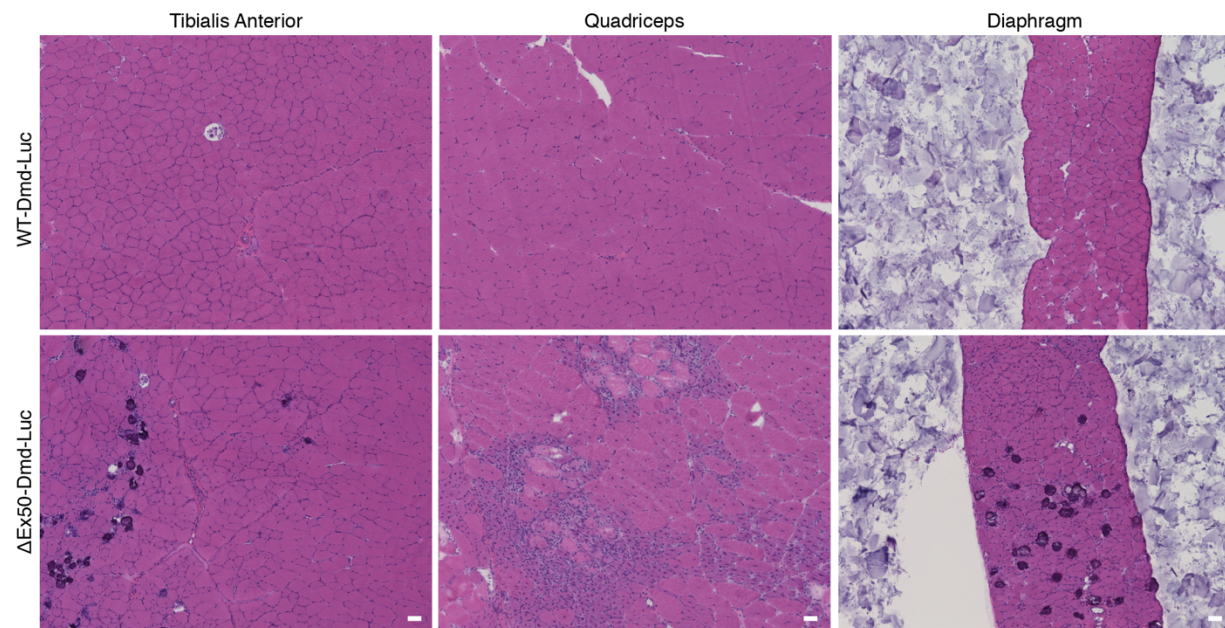
	sgRNA-#1-target sequence PAM
WT-Dmd-Luc	TATTAAATGATGAGTGAAGTTATATGGCAATGTTTGTAATAATCAGTAACACGTATGCT
ΔEx50-Dmd-Luc	TATTAAAAATGATGAGTGAAGTTA-----

WT-Dmd-Luc	TTCCTATTAAAGAGGAAGTTAGAAGATCTGAGGTCTGAGTGGGAGGCTGTAAACCATTTA
ΔEx50-Dmd-Luc	-----
WT-Dmd-Luc	CTTCGGGAGCTGAGGACAAAGCAGCCTGACCGTGCCCTGGACTGAGCACTACTGGAGCC
ΔEx50-Dmd-Luc	-----
	sgRNA-#2-target sequence
WT-Dmd-Luc	TGTAAGTACATTTTGTCCCAGGTGTTCTCTTTTGCTTTAGCTGTTTGTTCAAAAGCGTGG
ΔEx50-Dmd-Luc	-----GG
	**
	PAM
WT-Dmd-Luc	CTGGGAGGTTATCTCTGAGTGAAAGGAAAAATTGGTTTCTGATTCTGCATAAAGGAGTAAA
ΔEx50-Dmd-Luc	CTGGGAGGTTATCTCTGAGTGAAAGGAAAAATTGGTTTCTGATTCTGCATAAAGGAGTAAA

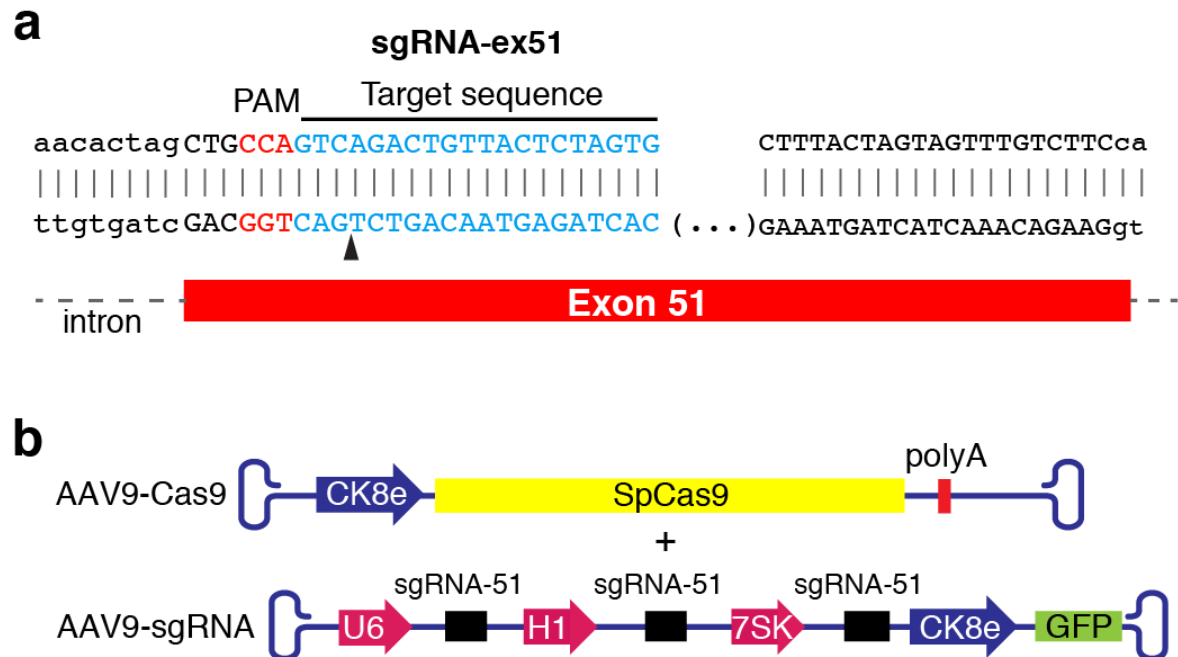
Supplementary Figure 1 ΔEx50-Dmd-Luc mouse model analysis. Genomic sequence of targeted locus of WT-Dmd-Luc (top line) and ΔEx50-Dmd-Luc founder (bottom line) with a 215 base pair deletion that eliminated exon 50 (indicated in color green). sgRNA-#1 and #2 are indicated in blue.



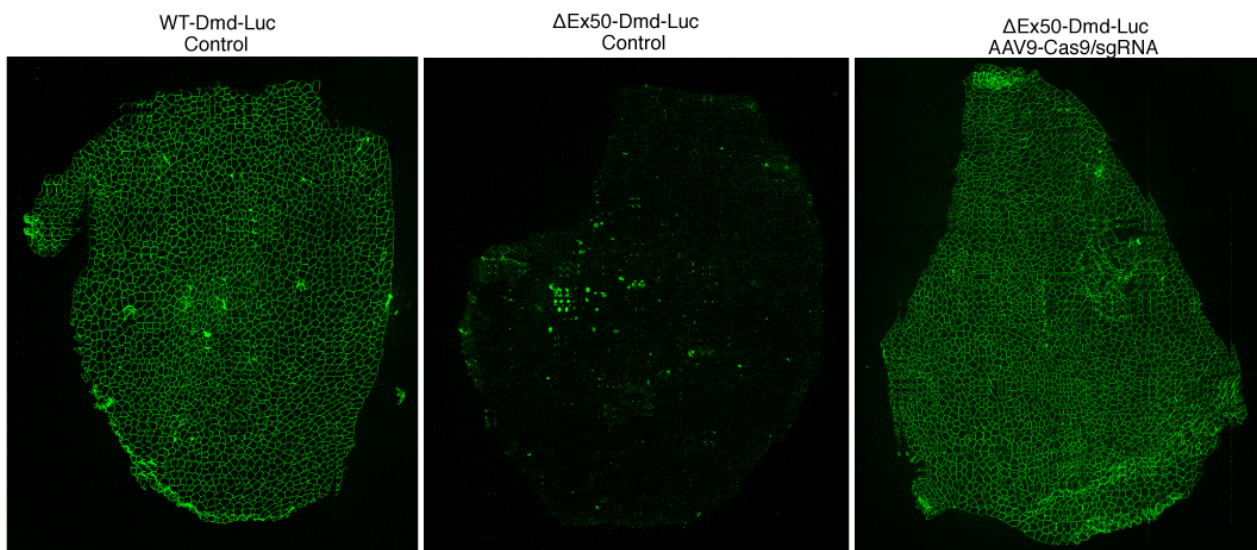
Supplementary Figure 2 Δ Ex50-Dmd-Luc reporter mouse genotyping strategy. (a) Illustration of the WT-Dmd-Luc and WT-Dmd-Luc genes. (b) Genotyping results of Δ Ex50-Dmd-Luc reporter mice. Schematic of genotyping strategy and forward (Fw) and reverse (Rv) primers. The Δ Ex50-Dmd-Luc mouse is the female founder of the colony.



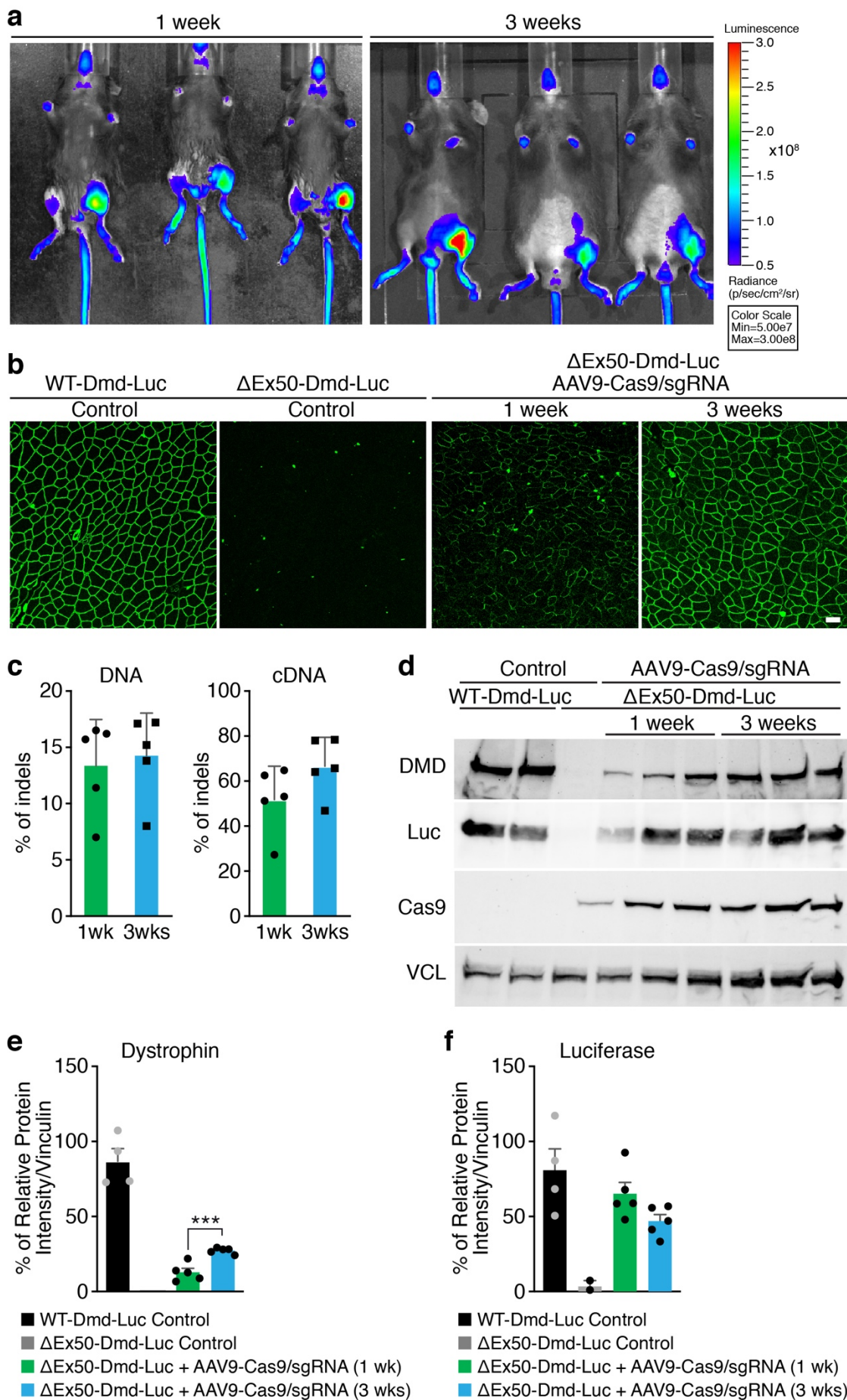
Supplementary Figure 3 Muscle histological analysis of the Δ Ex50-Dmd-Luc mouse model. Hematoxylin and eosin (H&E) staining of tibialis anterior, quadriceps and diaphragm muscles of 6 week old WT-Dmd-Luc and Δ Ex50-Dmd-Luc mice. n=5. Scale bar: 50 μ m.



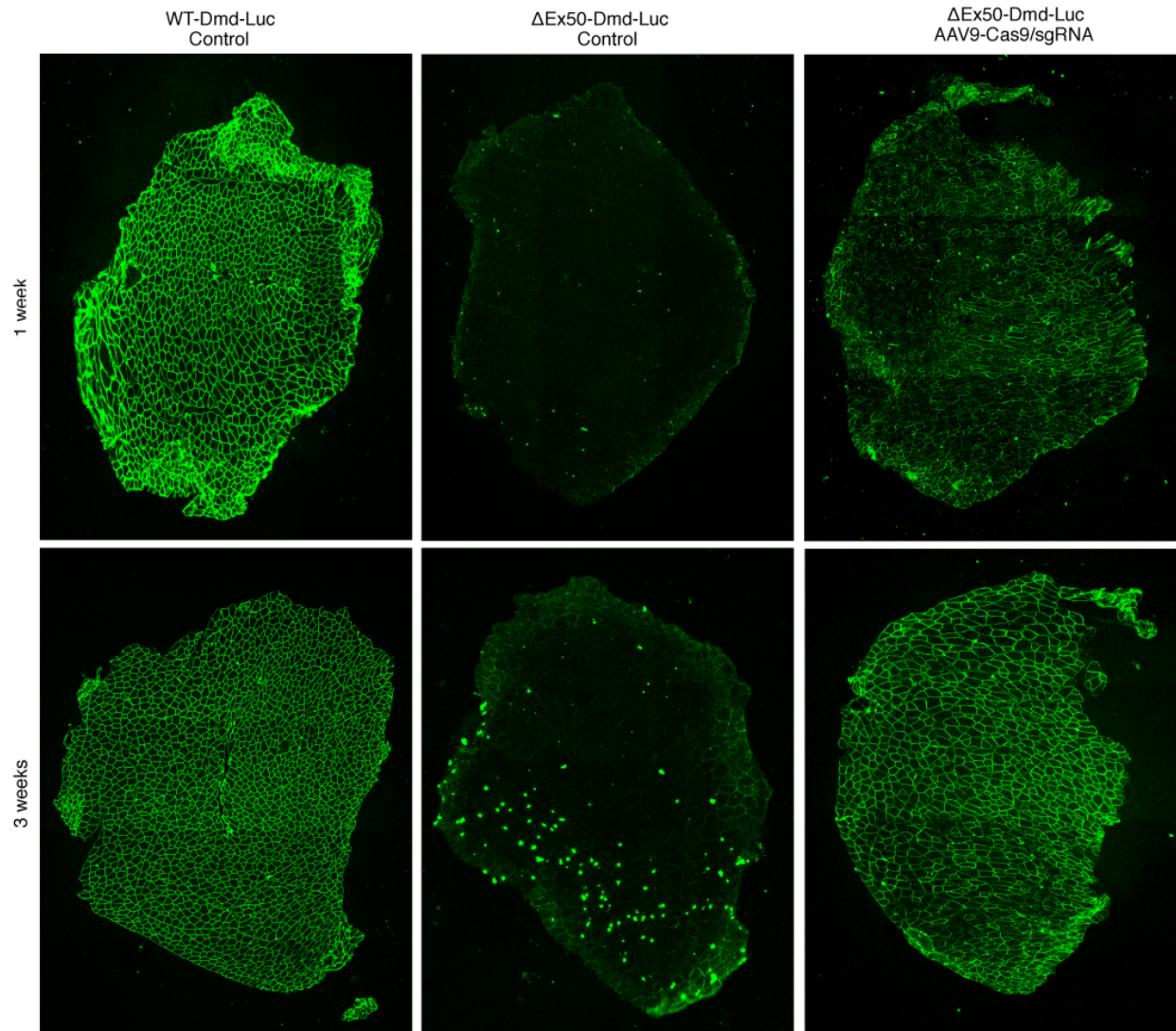
Supplementary Figure 4 Strategy for CRISPR/Cas9-mediated genome editing in Δ Ex50 mice. **(a)** Illustration of sgRNA binding position and sequence for sgRNA-ex51. PAM sequence for sgRNA is indicated in red. Black arrow indicates the cleavage site. **(b)** The muscle creatine kinase 8 (CK8e) promoter was used to express SpCas9. The U6, H1 and 7SK promoters for RNA polymerase III were used to express sgRNAs.



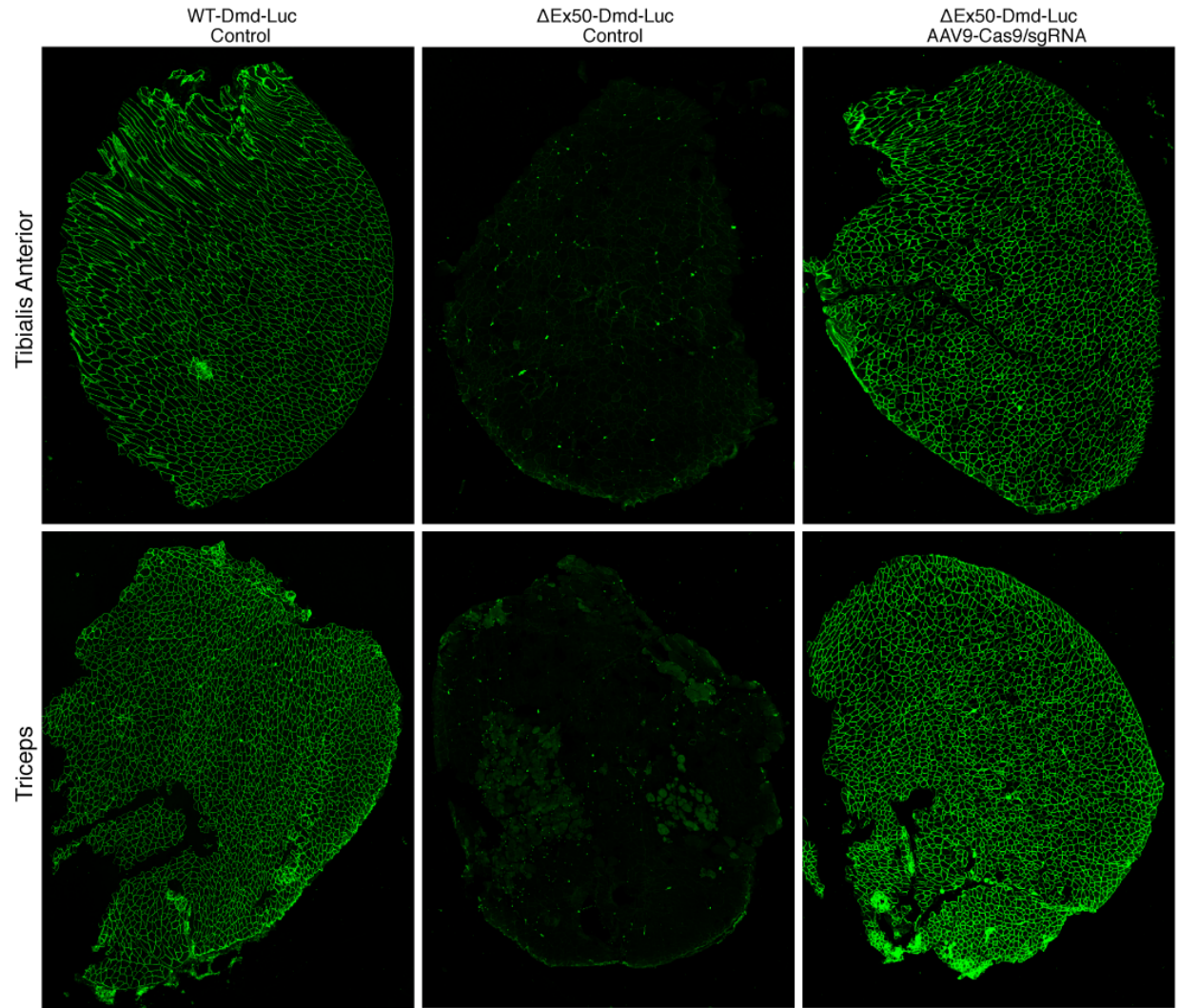
Supplementary Figure 5 Dystrophin immunohistochemistry of entire tibialis anterior muscle of WT-Dmd-Luc mice, control Δ Ex50-Dmd-Luc mice and Δ Ex50-Dmd-Luc mice injected with AAV9-Cas9 and AAV9-sgRNA, 4 weeks after injection.



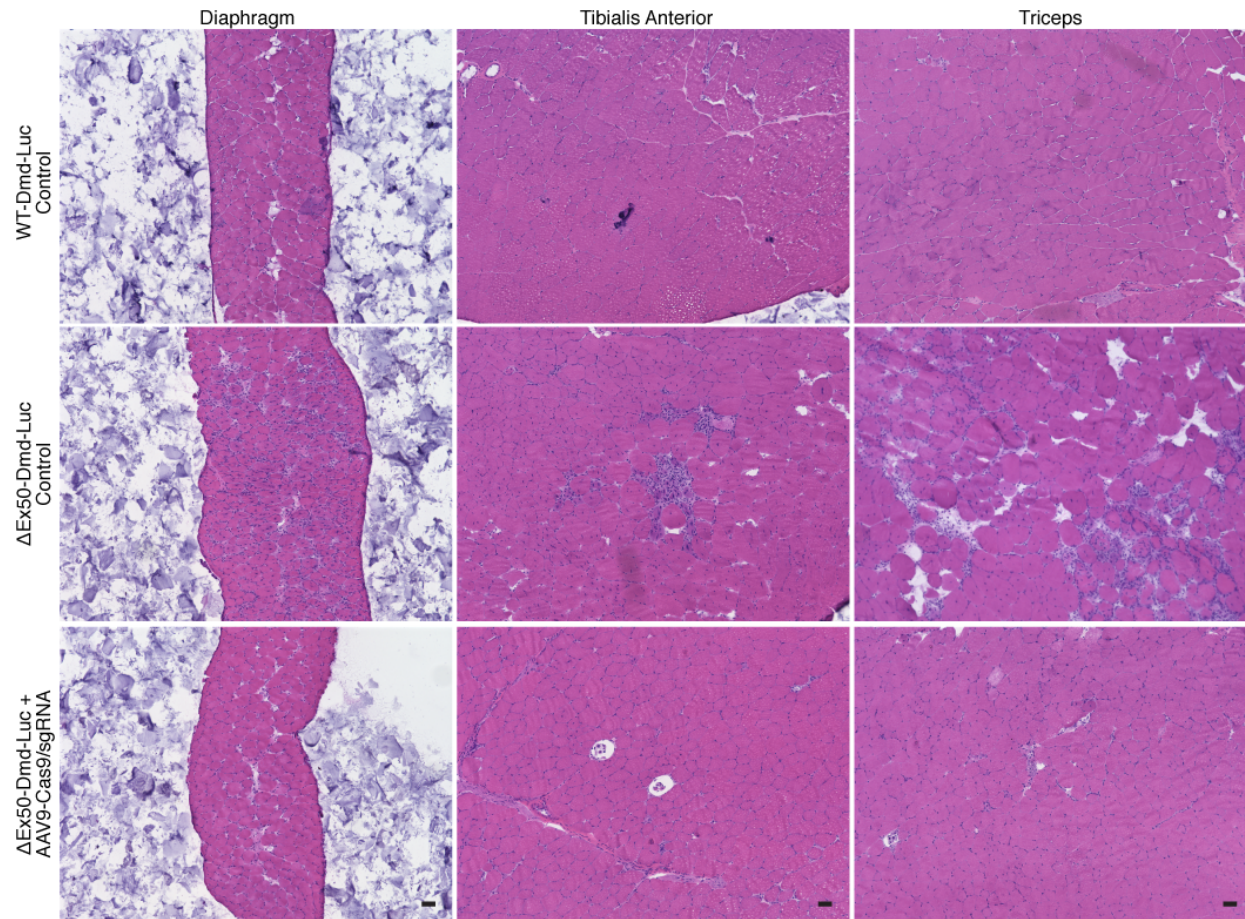
Supplementary Figure 6 Correction of dystrophin expression by intra-muscular injection of AAV9-encoded gene editing components. **(a)** Bioluminescence imaging of Δ Ex50-Dmd-Luc mice injected with AAV9-Cas9 and AAV9-sgRNA 1 week and 3 weeks after injection. **(b)** Dystrophin immunohistochemistry of tibialis anterior muscle of WT-Dmd-Luc mice, control Δ Ex50-Dmd-Luc mice and Δ Ex50-Dmd-Luc mice injected with AAV9-Cas9 and AAV9-sgRNA, 1 week and 3 weeks after injection. **(c)** Percentage of indels detected at exon 51 after AAV9-Cas9 and AAV9-sgRNA-51 treatment using tracking indels by decomposition (TIDE) analysis of genomic DNA and RT-PCR (cDNA) products from tibialis anterior Δ Ex50-Dmd-Luc mice injected with AAV9-Cas9 and AAV9-sgRNA-51 1 week and 3 weeks after injection. **(d)** Western blot analysis of dystrophin (DMD), luciferase (Luc), Cas9 and vinculin (VCL) in tibialis anterior of WT-Dmd-Luc mice, control Δ Ex50-Dmd-Luc mice and Δ Ex50-Dmd-Luc mice injected with AAV9-Cas9 and AAV9-sgRNA. **(e)** Quantification of dystrophin expression from blots after normalization to vinculin. **(f)** Quantification of luciferase expression from blots after normalization to vinculin. Data are represented as mean $\pm$ SEM. n=5. (**P<0.01, ***P<0.0005). Scale bar: 50 μ m.



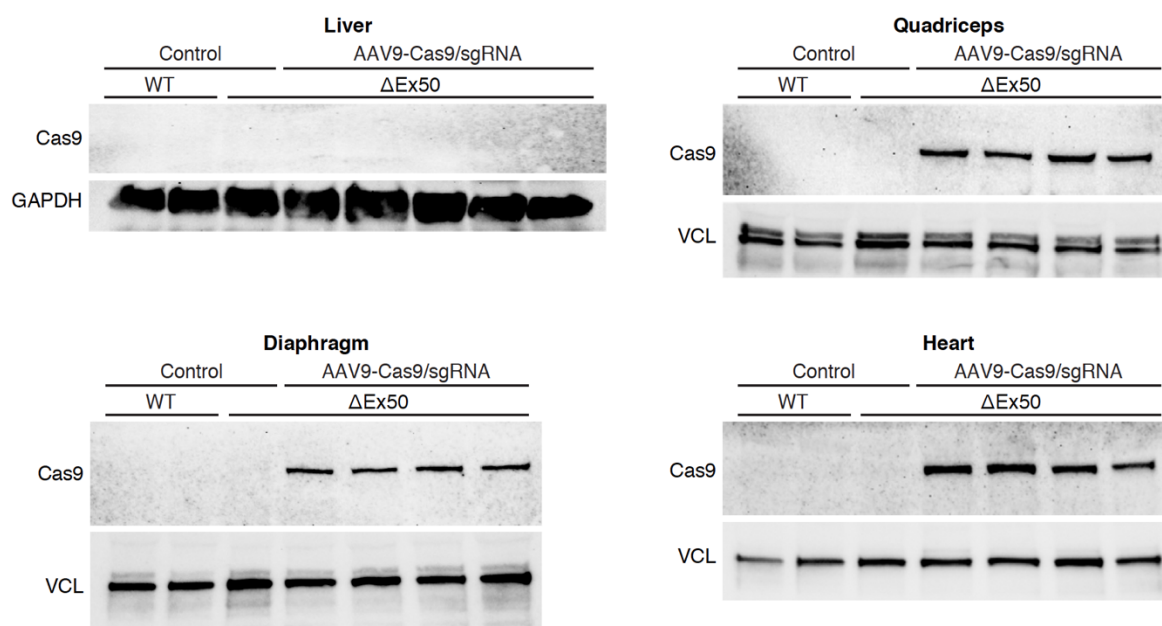
Supplementary Figure 7 Dystrophin immunohistochemistry of entire tibialis anterior muscle of WT-Dmd-Luc mice, control Δ Ex50-Dmd-Luc mice and Δ Ex50-Dmd-Luc mice injected with AAV9-Cas9 and AAV9-sgRNA, 1 week and 3 weeks after injection.



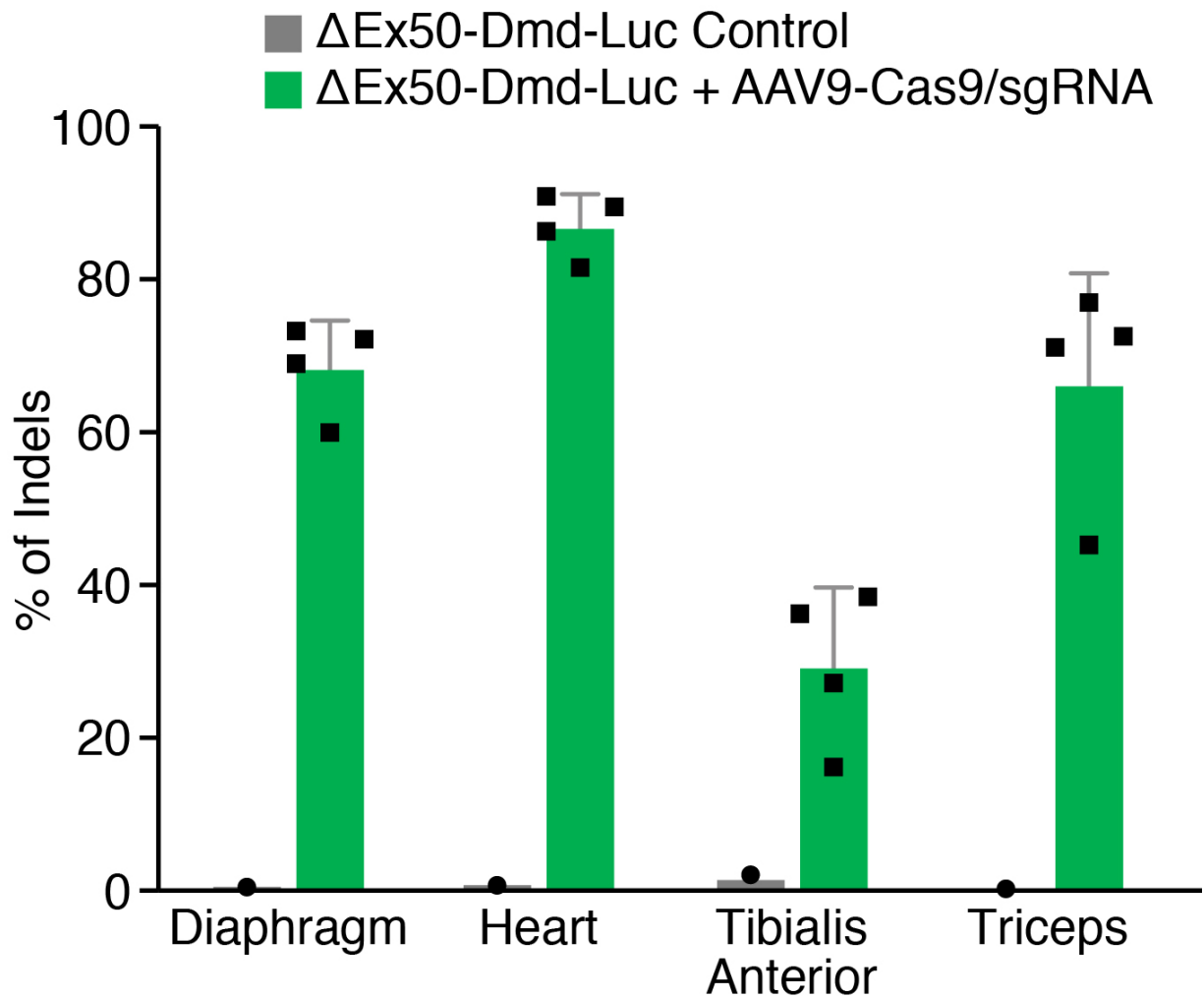
Supplementary Figure 8 Dystrophin immunohistochemistry of entire tibialis anterior and triceps muscles of WT-Dmd-Luc mice, control Δ Ex50-Dmd-Luc mice and Δ Ex50-Dmd-Luc mice injected with AAV9-Cas9 and AAV9-sgRNA, 10 weeks after injection.



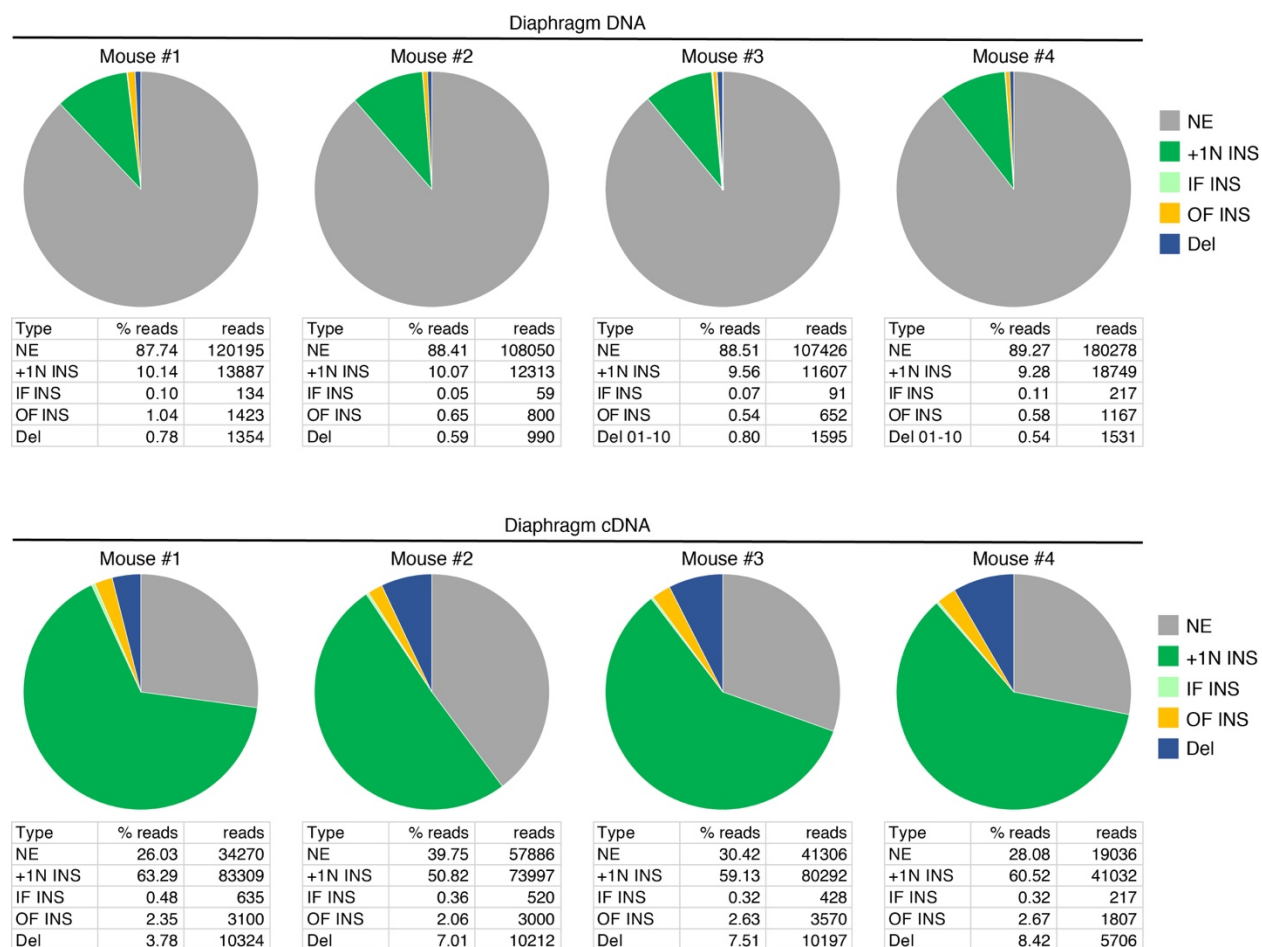
Supplementary Figure 9 Muscle histological analysis of the Δ Ex50-Dmd-Luc mice 10 weeks after systemic injection. Hematoxylin and eosin (H&E) staining of diaphragm, tibialis anterior and triceps muscles. n=4. Scale bar: 50 μ m.



Supplementary Figure 10 Cas9 protein expression 10 weeks following systemic delivery of AAV9-encoded gene editing components. Western blot analysis of Cas9 and vinculin (VCL) in liver, quadriceps, diaphragm and heart of Δ Ex50-Dmd-Luc mice 10 weeks after systemic injection with AAV9-Cas9 and AAV9-sgRNA. n=4.

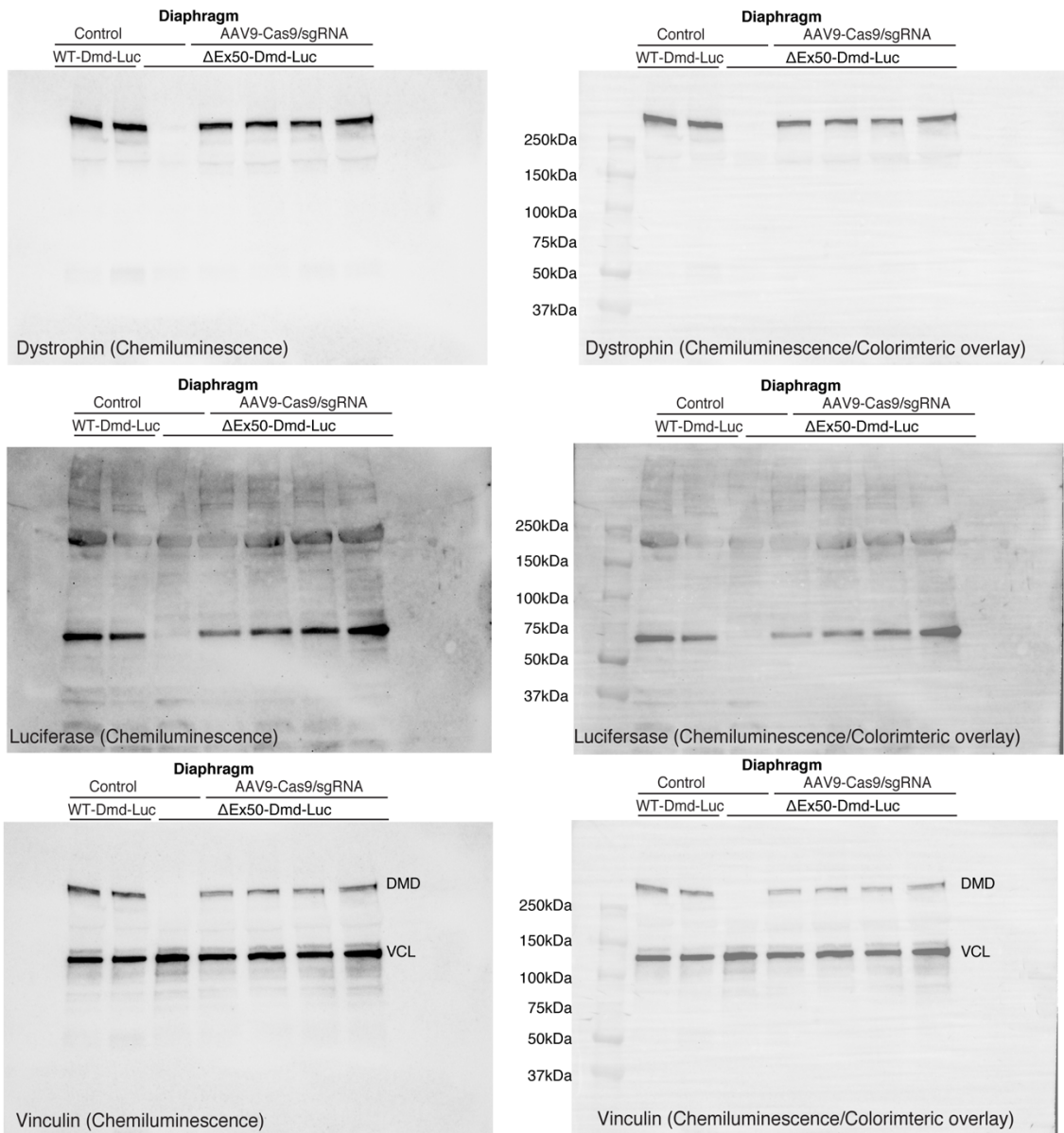


Supplementary Figure 11 In vivo *Dmd* gene editing after systemic delivery of AAV9-Cas9 and AAV9-sgRNA-51. Percentage of indels detected at exon 51 after AAV9-Cas9 and AAV9-sgRNA-51 treatment using tracking indels by decomposition (TIDE) analysis of RT-PCR products from diaphragm, heart, tibialis anterior and triceps muscle samples of untreated ΔEx50-Dmd-Luc mice and ΔEx50-Dmd-Luc mice injected with AAV9-Cas9 and AAV9-sgRNA-51. n=4.

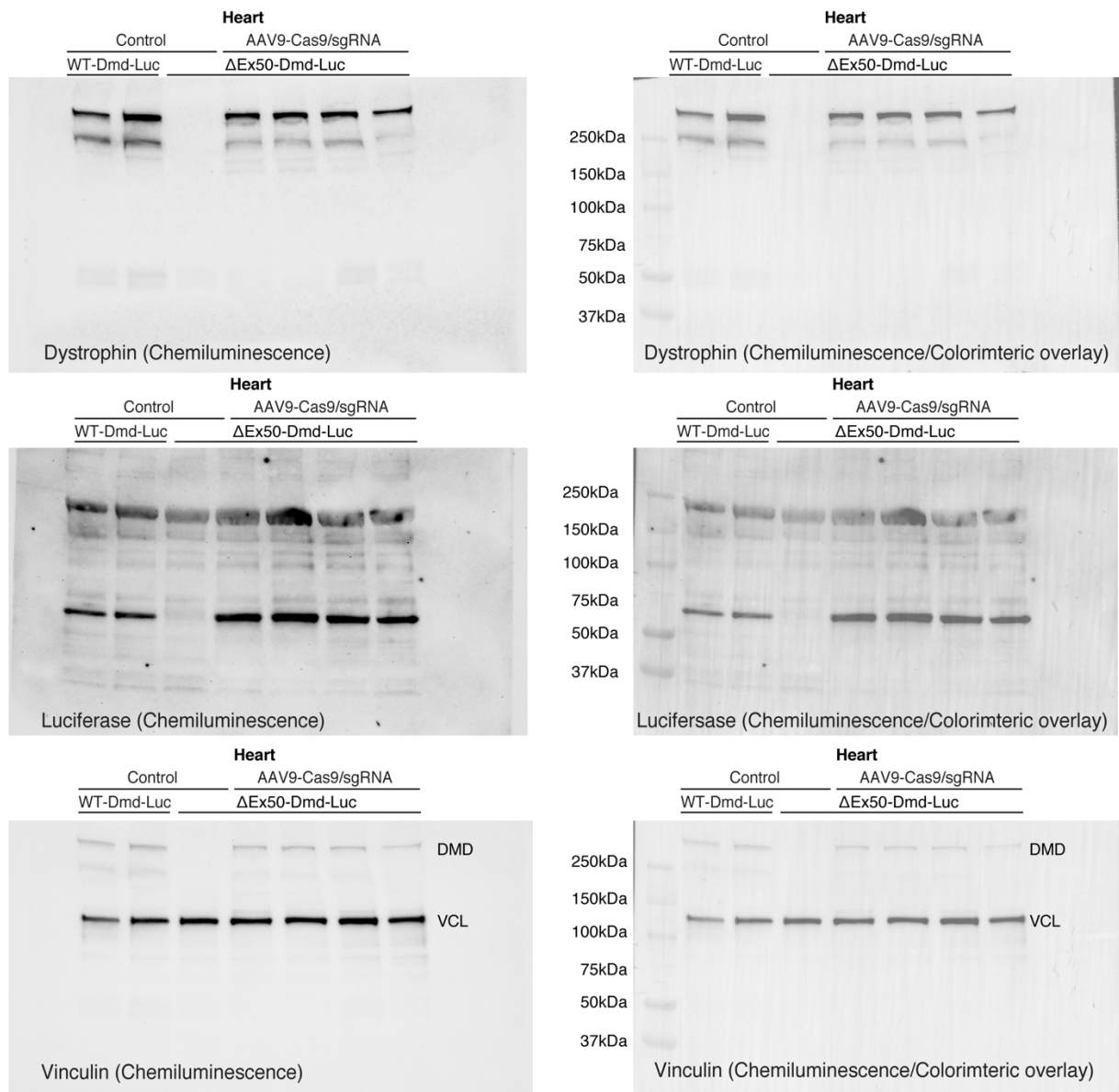


Supplementary Figure 12 In vivo *Dmd* gene editing 10 weeks after systemic delivery of AAV9-Cas9 and AAV9-sgRNA-51. Genomic deep sequencing analysis of DNA from diaphragm muscles of Δ Ex50-Dmd-Luc mice injected with AAV9-Cas9 and AAV9-sgRNA-51 10 weeks after systemic injection (top panel). Genomic PCR amplicons generated across the exon 51 target site were analyzed by deep sequencing. RT-PCR (cDNA) deep sequencing analysis of RNA from diaphragm muscles of Δ Ex50-Dmd-Luc mice injected with AAV9-Cas9 and AAV9-sgRNA-51 10 weeks after systemic injection

(lower panel). RT-PCR with primers for sequences in exons 48 and 53 were subjected to amplicon deep-sequencing. The alignment of reads at the cleavage site was further analyzed and regrouped in non-edited (NE), single nucleotide insertion (+1N INS), in frame insertion (IF INS), out of frame insertion (OF INS) and deletion (DEL) groups. n=4.

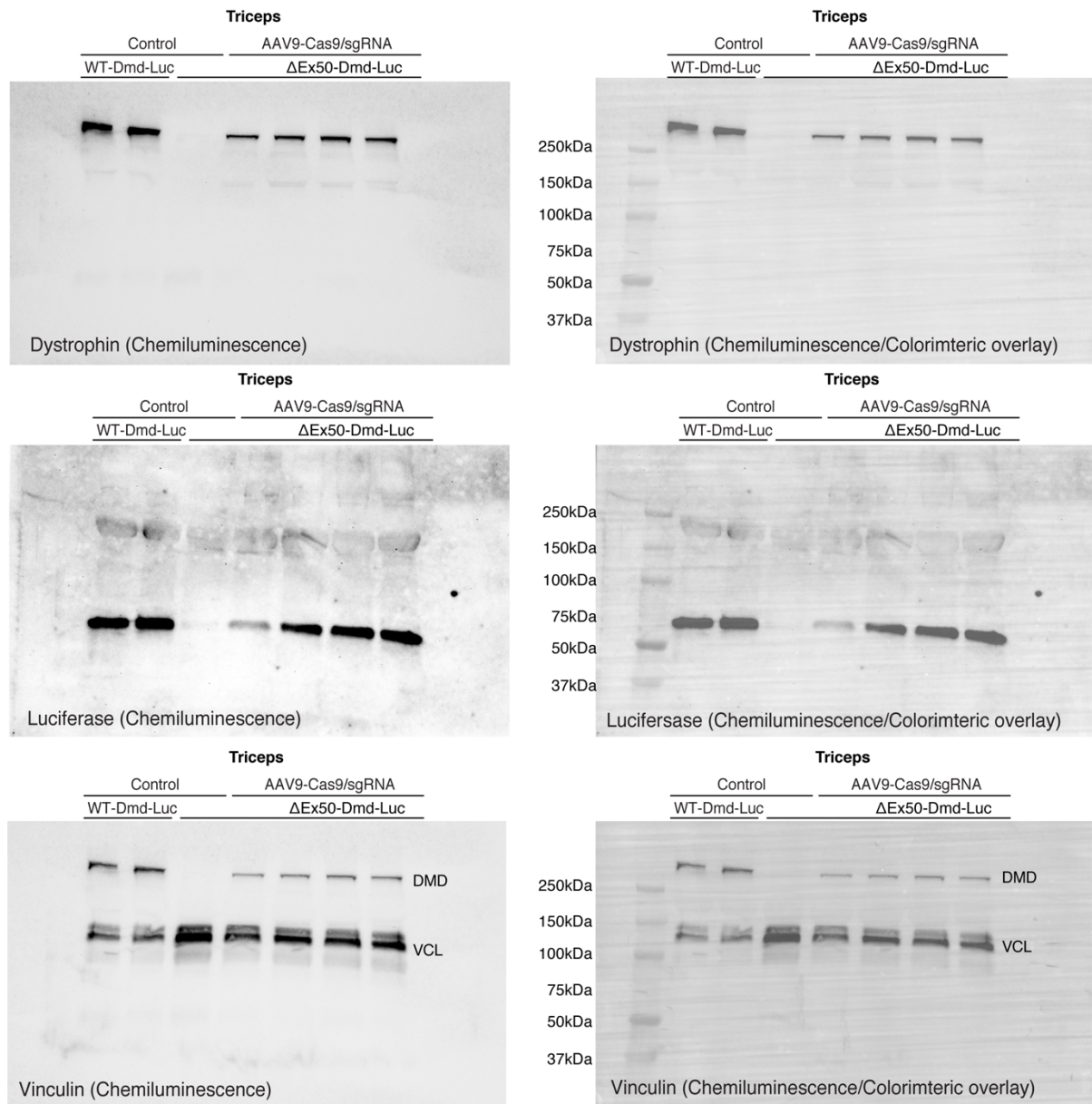


Supplementary Figure 13 Full diaphragm western blot scan corresponding to Figure 4. Dystrophin (DMD) and vinculin (VCL).

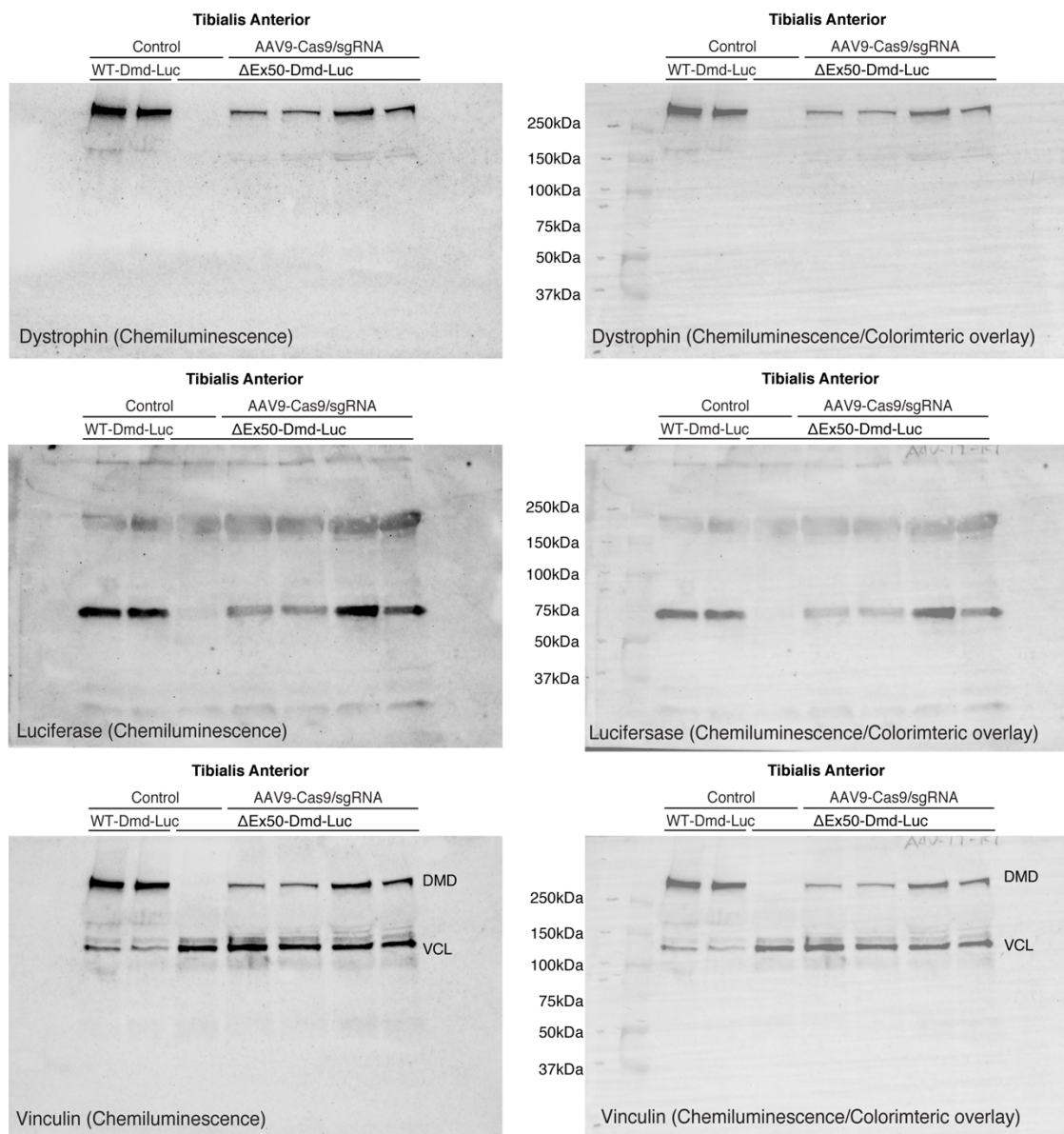


Supplementary Figure 14 Full heart western blot scan corresponding to Figure 4.

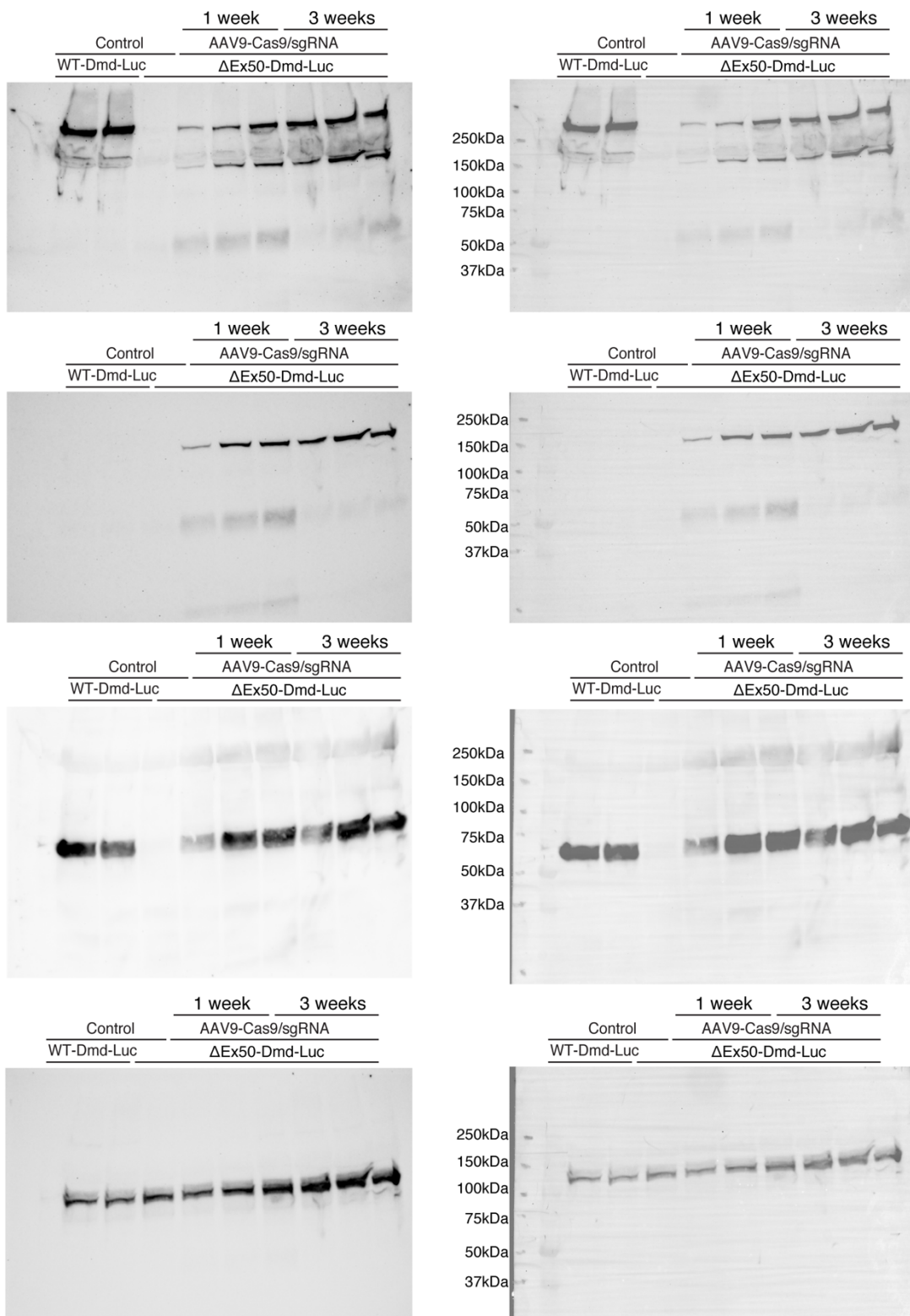
Dystrophin (DMD) and vinculin (VCL).



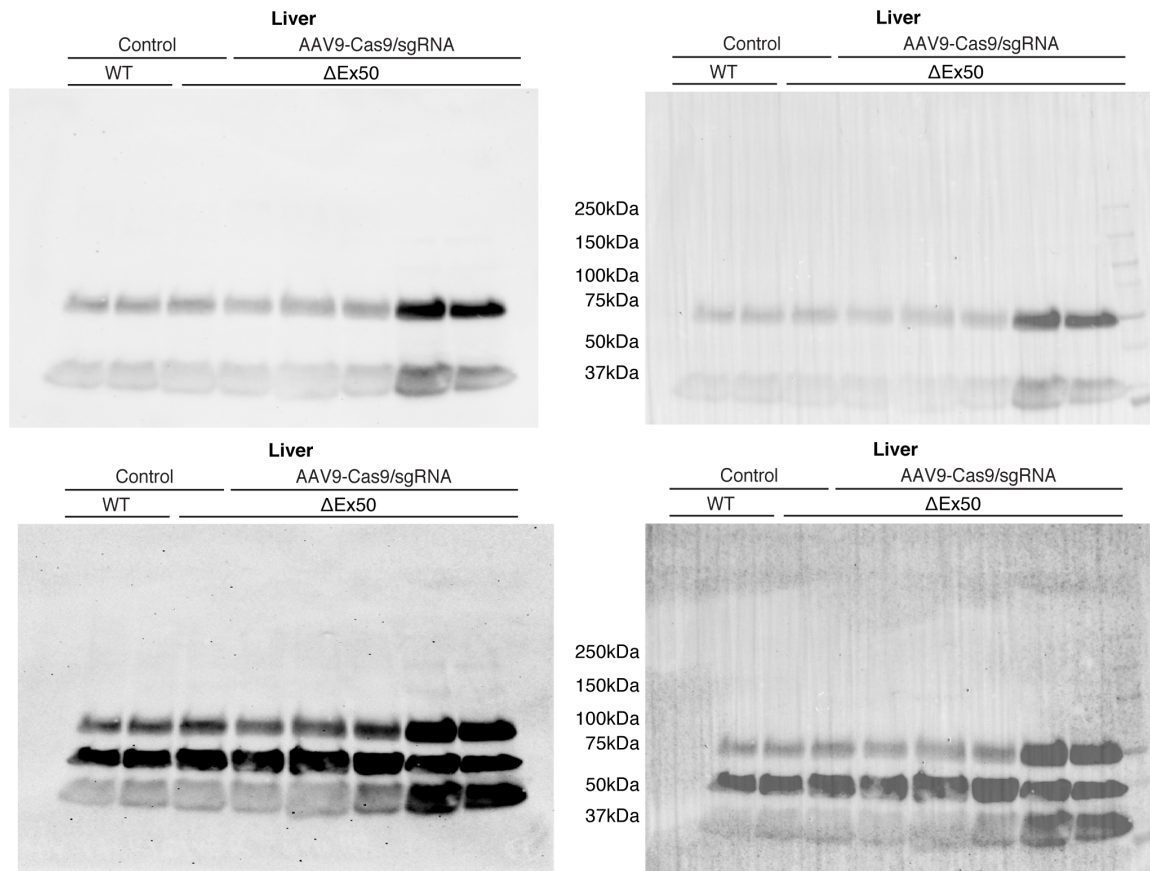
Supplementary Figure 15 Full triceps western blot scan corresponding to Figure 4. Dystrophin (DMD) and vinculin (VCL).



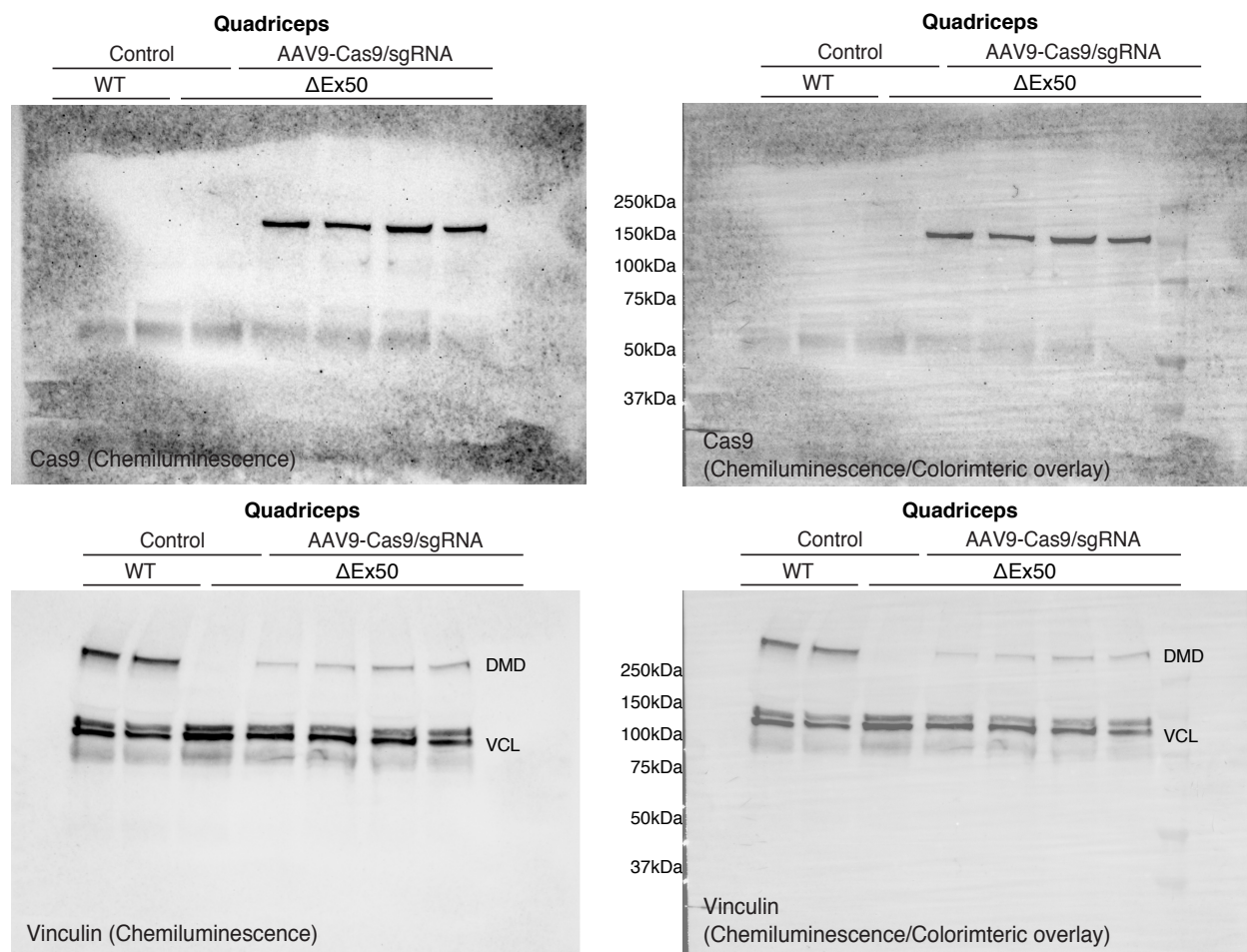
Supplementary Figure 16 Full tibialis anterior western blot scan corresponding to Figure 4. Dystrophin (DMD) and vinculin (VCL).



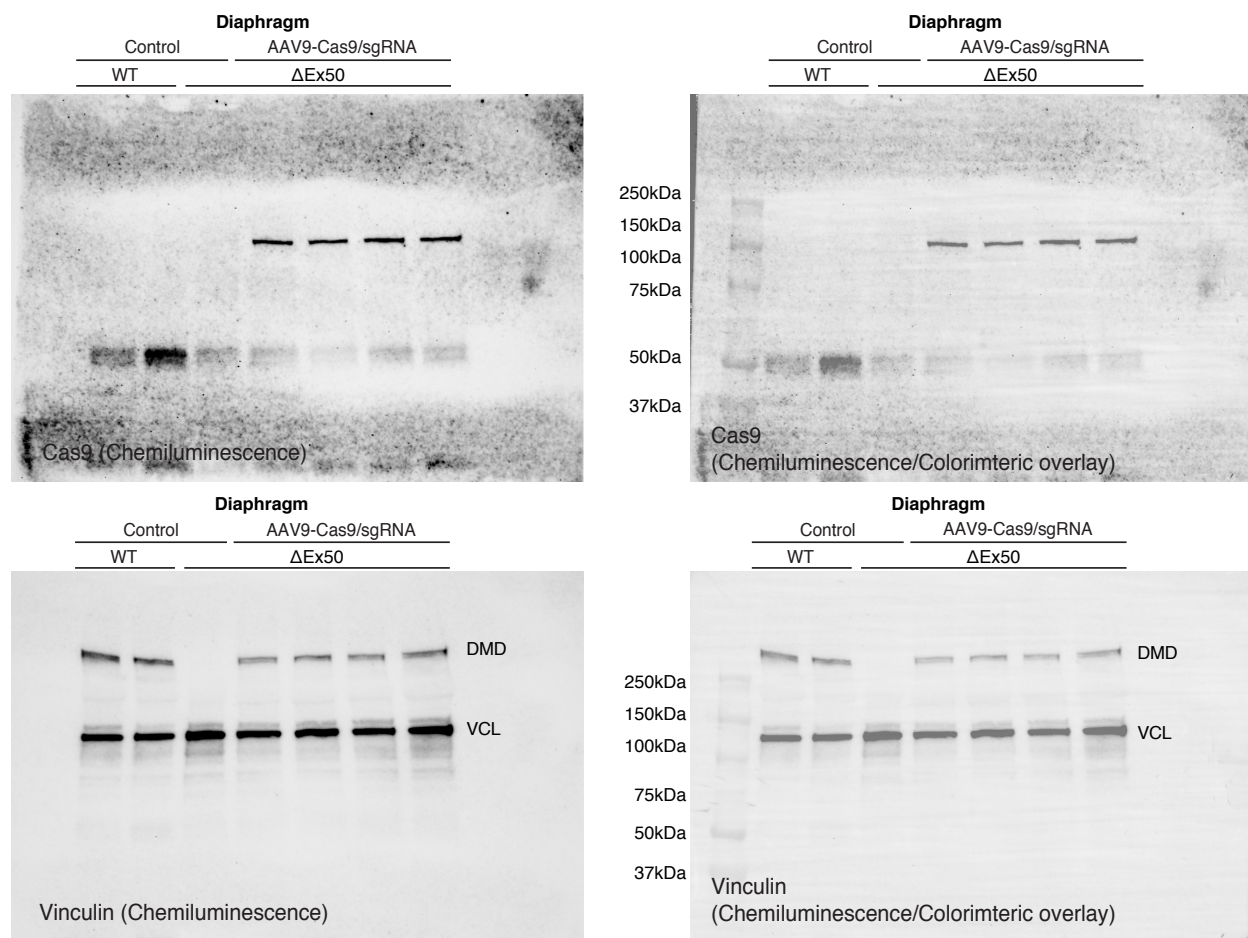
Supplementary Figure 17 Full tibialis anterior western blot scan corresponding to Supplementary Figure 6.



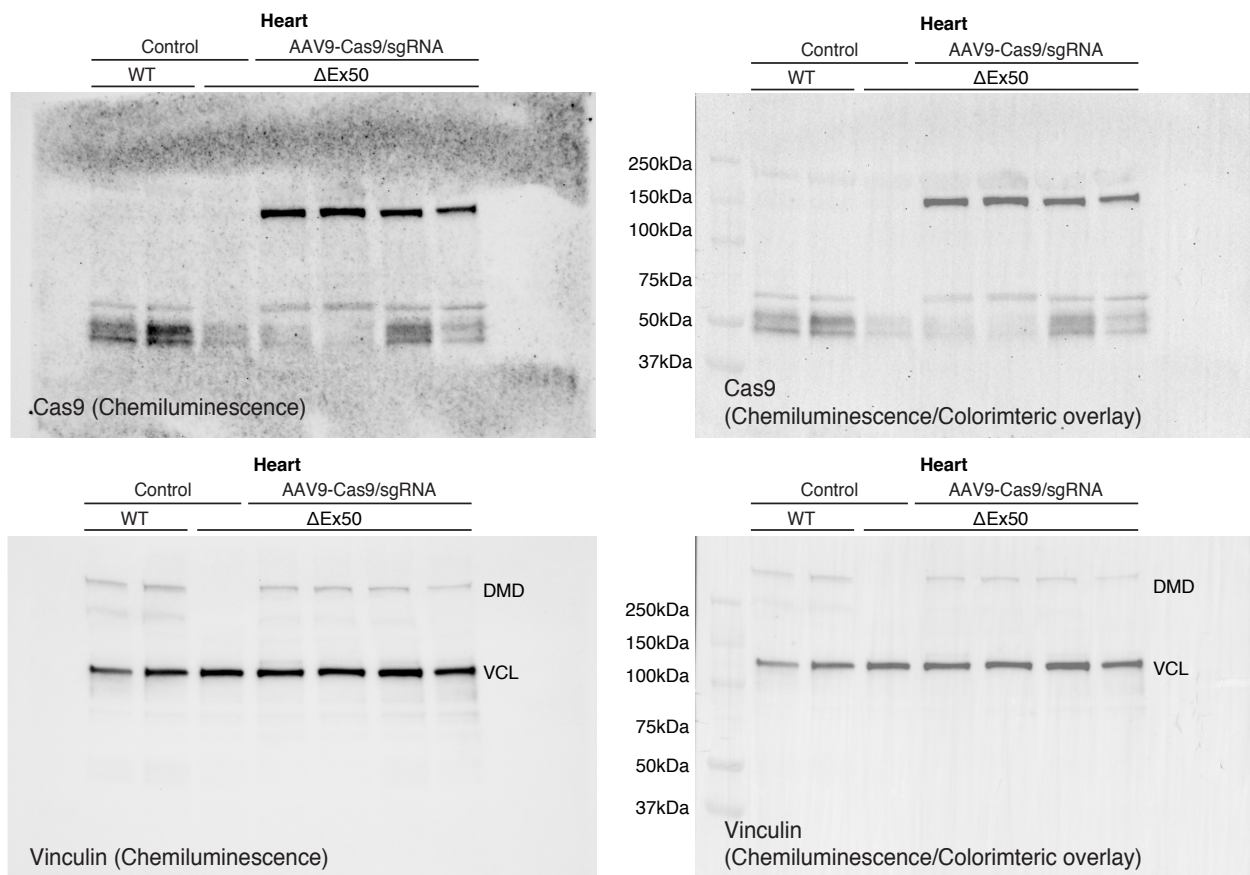
Supplementary Figure 18 Full liver western blot scan corresponding to Supplementary Figure 10.



Supplementary Figure 19 Full quadriceps western blot scan corresponding to Supplementary Figure 10. Dystrophin (DMD) and vinculin (VCL).



Supplementary Figure 20 Full diaphragm western blot scan corresponding to Supplementary Figure 10. Dystrophin (DMD) and vinculin (VCL).



Supplementary Figure 21 Full heart western blot scan corresponding to Supplementary Figure 10. Dystrophin (DMD) and vinculin (VCL).

Supplementary Table 1 Primer sequences.

Experiment	Primer name	Primer sequence (5'-3')
sgRNA for Targeting the end exon 79 for KI	<i>Dmd</i> -Ex79-Top	CACCGAGGCTTCCTACATTGTGTCC
	<i>Dmd</i> -Ex79-Bottom	AAACGGACACAATGTAGGAAGCCTC
sgRNA for exon 50	<i>Dmd</i> exon 50-Top-1	CACCGAATGATGAGTGAAGTTATAT
	<i>Dmd</i> exon 50-Bottom-1	AAACATATAACTTCACTCATCATTC
	<i>Dmd</i> exon 50-Top-2	CACCGGTTTGTTCAAAAGCGTGGCT
	<i>Dmd</i> exon 50-Bottom-2	AAACAGCCACGCTTTGAACAAAC
Genotyping of ΔEx50	Geno-Ex50-F	GGATTGACTGAAATGATGGCCAAGG
	Geno-Ex-50-R	CTGCCACGATTACTCTGCTTCCAG
Genotyping of KI Luciferase	Geno-KLuci-F	AGCAGGCAGAGAAGGTGGTA
	Geno-Kluci-WT-R	GCGTGTGTGTTTGTTTAGGG
	Geno-Kluci-KI-R	GGGCGTATCTCTTCATAGCCTT
sgRNA for exon 51	<i>Dmd</i> -Ex51-Top	CACCG CAC TAG AGT AAC AGT CTG AC
	<i>Dmd</i> -Ex51-Bottom	AAAC GTCAGACTGTACTCTAGTG C
RT-PCR primer flanking exon 48 and exon 53 TIDE	<i>Dmd</i> -Ex48-F	AGGTTCACTTAAAAGATTTTAGGCAG
	<i>Dmd</i> -Ex52-R	TAACATTTCACTCAACTGTTGTCTCC
Primer flanking genomic exon 51 locus TIDE	<i>Dmd</i> -Ex48-F	GATAAATCCTGAAAATCCCCAATA
	<i>Dmd</i> -Ex52-R	TTTCACCCTAACTTTTCATCCCTT
Primers for Mi-seq mouse genomic DNA amplicon deep sequencing	<i>Dmd</i> -Ex51-Mi-seq-F	TCTCGGCAGCGTCAGATGTGTATAAGAGACAGGAAATTTTACCTCAAAGTGTGCTTC
	<i>Dmd</i> -Ex51-Mi-seq-F	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAGGGAAATGGAAAGTGACAATATAC
Primers for Mi-seq Mouse cDNA amplicon deep sequencing	<i>Dmd</i> -Ex51-cDNA-Mi-seq-F	TCTCGGCAGCGTCAGATGTGTATAAGAGACAGGAAATTTATAACCAACCAAGTCAGG
	<i>Dmd</i> -Ex51-cDNA-Mi-seq-R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAATGATTGTTCTAGCTTCTTGATTGC
Primers for Mi-Seq for PCR with Bar Codes	Univ-F	AATGATACGGCGACCAACCGAGATCTACACTCGTCGGCAGCGTC
	BC1-F	CAAGCAGAAGACGGCATACGAGATACATCGGTCTCGTGGGCTCGG
	BC2-F	CAAGCAGAAGACGGCATACGAGATTGGTCA GTCTCGTGGGCTCGG
	BC3-F	CAAGCAGAAGACGGCATACGAGATCACTGTGTCTCGTGGGCTCGG
	BC4-F	CAAGCAGAAGACGGCATACGAGATTGGCGTCTCGTGGGCTCGG
	BC5-F	CAAGCAGAAGACGGCATACGAGATGATCTGTCTCGTGGGCTCGG
	BC6-F	CAAGCAGAAGACGGCATACGAGATTACAAGGTCTCGTGGGCTCGG
	BC7-F	CAAGCAGAAGACGGCATACGAGATCGTGATGTCTCGTGGGCTCGG
	BC8-F	CAAGCAGAAGACGGCATACGAGATGCTAAGTCTCGTGGGCTCGG
	BC9-F	CAAGCAGAAGACGGCATACGAGATCAAGTGTCTCGTGGGCTCGG
	BC10-F	CAAGCAGAAGACGGCATACGAGATAGCTAGGTCTCGTGGGCTCGG
	BC11-F	CAAGCAGAAGACGGCATACGAGATGTCGTCGTCTCGTGGGCTCGG

BC12-F	CAAGCAGAAGACGGCATACGAGAT CGATTA GTCTCGTGGGCTCGG
BC13-F	CAAGCAGAAGACGGCATACGAGAT GAATGA GTCTCGTGGGCTCGG
BC14-F	CAAGCAGAAGACGGCATACGAGAT CTTCGA GTCTCGTGGGCTCGG
BC15-F	CAAGCAGAAGACGGCATACGAGAT CTCTAC GTCTCGTGGGCTCGG
BC16-F	CAAGCAGAAGACGGCATACGAGAT AGGAAT GTCTCGTGGGCTCGG
BC17-F	CAAGCAGAAGACGGCATACGAGAT GCTACC GTCTCGTGGGCTCGG
BC18-F	CAAGCAGAAGACGGCATACGAGAT ATCAGT GTCTCGTGGGCTCGG
BC19-F	CAAGCAGAAGACGGCATACGAGAT TATACT GTCTCGTGGGCTCGG
BC20-F	CAAGCAGAAGACGGCATACGAGAT CAACAA GTCTCGTGGGCTCGG
BC21-F	CAAGCAGAAGACGGCATACGAGAT GTTGTT GTCTCGTGGGCTCGG
BC22-F	CAAGCAGAAGACGGCATACGAGAT TCGGTT GTCTCGTGGGCTCGG
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