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Supplemental Information

CRISPR-Induced Deletion with SaCas9 Restores Dystrophin Expression in Dystrophic Models *In Vitro* and *In Vivo*

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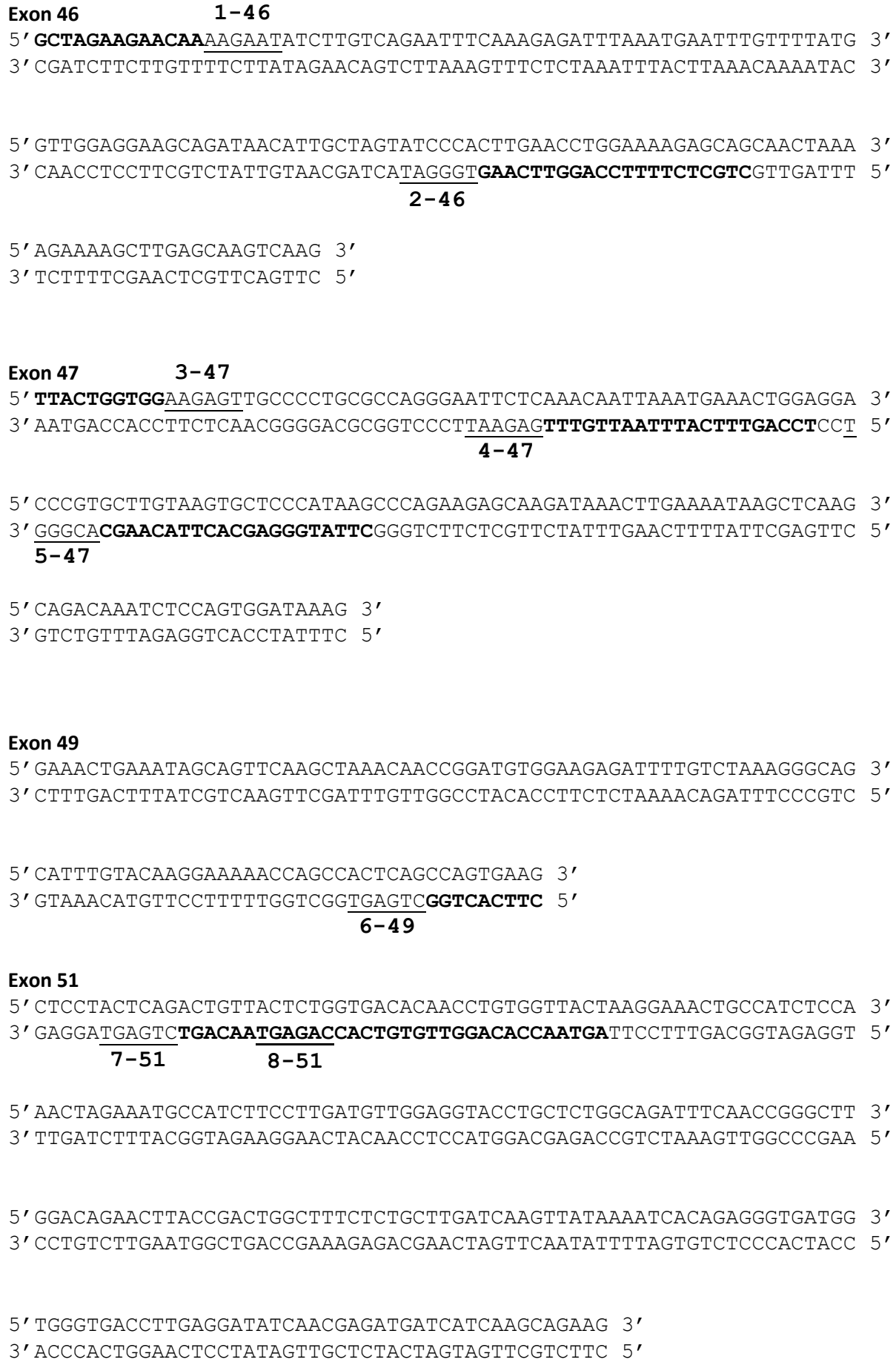
Figure S1A.


Exon 46
1-46
5′GCTAGAAGAACAAAAGAATATCTTGTCAGAATTTCAAAGAGATTTAAATGAATTTGTTTTATG 3′
3′CGATCTTCTTGTTTTCTTATAGAACAGTCTTAAAGTTTCTCTAAATTTACTTAAACAAAATAC 3′
5′GTTGGAGGAAGCAGATAACATTGCTAGTATCCCACTTGAACCTGGAAAAGAGCAGCAACTAAA 3′
3′CAACCTCCTTCGTCTATTGTAACGATCATAGGGTGAACTTGGACCTTTTCTCGTCGTTGATTT 5′
2-46
5′AGAAAAGCTTGAGCAAGTCAAG 3′
3′TCTTTTCGAACTCGTTCAGTTC 5′
Exon 47
3-47
5′TTACTGGTGGAAGAGTTGCCCCTGCGCCAGGGAATTCTCAAACAATTAAATGAAACTGGAGGA 3′
3′AATGACCACCTTCTCAACGGGGACGCGGTCCCTTAAGAGTTTGTTAATTTACTTTGACCTCCT 5′
4-47
5′CCCGTGCTTGTAAGTGCTCCCATAAGCCCAGAAGAGCAAGATAAACTTGAAAATAAGCTCAAG 3′
3′GGGCACGAACATTCACGAGGGTATTCGGGTCTTCTCGTTCTATTTGAACTTTTATTCGAGTTC 5′
5-47
5′CAGACAAATCTCCAGTGGATAAAG 3′
3′GTCTGTTTAGAGGTCACCTATTTC 5′
Exon 49
5′GAAACTGAAATAGCAGTTCAAGCTAAACAACCGGATGTGGAAGAGATTTTGTCTAAAGGGCAG 3′
3′CTTTGACTTTATCGTCAAGTTCGATTTGTTGGCCTACACCTTCTCTAAAACAGATTTCCCGTC 5′
5′CATTTGTACAAGGAAAAACCAGCCACTCAGCCAGTGAAG 3′
3′GTAAACATGTTCCTTTTTGGTCGGTGAGTCGGTCACTTC 5′
6-49
Exon 51
5′CTCCTACTCAGACTGTTACTCTGGTGACACAACCTGTGGTTACTAAGGAAACTGCCATCTCCA 3′
3′GAGGATGAGTCTGACAATGAGACCACTGTGTTGGACACCAATGATTCCTTTGACGGTAGAGGT 5′
7-51
8-51
5′AACTAGAAATGCCATCTTCCTTGATGTTGGAGGTACCTGCTCTGGCAGATTTCAACCGGGCTT 3′
3′TTGATCTTTACGGTAGAAGGAACTACAACCTCCATGGACGAGACCGTCTAAAGTTGGCCCGAA 5′
5′GGACAGAACTTACCGACTGGCTTTCTCTGCTTGATCAAGTTATAAAATCACAGAGGGTGATGG 3′
3′CCTGTCTTGAATGGCTGACCGAAAGAGACGAACTAGTTCAATATTTTAGTGTCTCCCACTACC 5′
5′TGGGTGACCTTGAGGATATCAACGAGATGATCATCAAGCAGAAG 3′
3′ACCCACTGGAACTCCTATAGTTGCTCTACTAGTAGTTCGTCTTC 5′

Exon 52

5′GCAACAATGCAGGATTTGGAACAGAGGCGTCCCCAGTTGGAAGAACTCATTACCGCTGCCCAA 3′
3′CGTTGTTACGTCCTAAACCTTGTCTCCGCAGGGGTCAACCTTCTTGAGTA**ATGGCGACGGGTT** 5′

9-52

10-52

5′AATTTGAAAAACAAGACCAGCAAT**CAAGAGGCTAGAACAATCATT**ACGGATCGAA 3′
3′TTAAACTTTTTGTTCTGGTCGTTAGTTCTCCGATCTTGTTAGTAATGCCTAGCTT 5′

Exon 53

5′TTGAAAGAATTCAGAATCAGTGGGATGAAGTACAAGAACAC**CTTCAGAACCGGAGGCAACAGT** 3′
3′AACTTTCTTAAGTC**TTAGTCACCCTACTTCATGTT**CTTGTGGAAGTCTTGGCCTCCGTTGTCA 5′

11-53

12-53 **13-53**

5′**TGAATGAAATGTTA**AAGGATTCAACACAATGGCTGGAAGCTAAGGAAGAAGCTGAGCAGGTCT 3′
3′ACTTACTTTACAATTTCCTAAGTTGTGTTACCGACCTTCGATTCCTTCTTCGACTCGTCCAGA 5′

14-53

5′TAGGACAGGCCAGA**GCCAAGCTTGAGTCATGGAAG**GAGGGTCCCTATACAGTAGATGCAATCC 3′
3′ATCCTGTCCGGTCTCGGTTCGAACTCAGTACCTTCCTCCCAGGGATATGTCATCTACGTTAGG 5′

15-53

5′AAAAGAAAATCACAGAAACCAAG 3′
3′TT**TTCTTTTAGTGTCTTTGGTTC** 5′

Exon 58 **16-58**

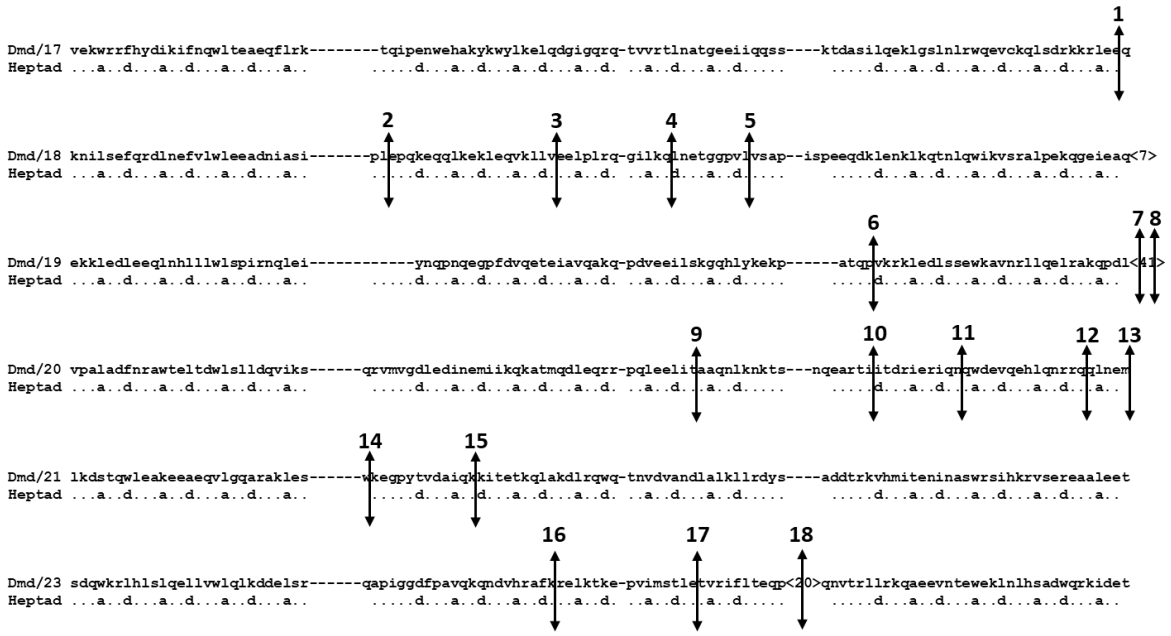
5′**GCCTTCAAG**AGGGAATTGAAAACTAAAGAACCTGTAATCATGAGTACTCTTGAGACTGTACGA 3′
3′CGGAAGTTCTCCCTTAACTTTTGATTTCTTGGACATTAGTACTCATGAGAA**CTCTGACATGCT** 5′

17-58

5′ATATTTCTGACAGAGCAGCCTTTGGAAGGACTAGAGAAACTCTACCAGGAGCCCAGAG 3′
3′**TATAAAGAC**TGTCTCGTCGGAAACCTTCCTGATCTCTTTGAGAT**GGTCCTCGGGTCTC** 5′

18-58

Figure S1B.


1
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Heptad ...a..d...a..d...a..d...a.. .....d...a..d...a..d...a..d. ..a..d...a..d..... .....d...a..d...a..d...a..d...a..
2 3 4 5
Dmd/18 knilsefqrdlnefvlwleeadniasi-------plepqkeqqlkekleqvkllveelplrq-gilkqlnetggpvlvsap--ispeeqdklenklkqtnlqwikvsralpekqgeieaq<7>
Heptad ...a..d...a..d...a..d...a.. .....d...a..d...a..d...a..d. ..a..d...a..d..... .....d...a..d...a..d...a..d...a..
6 7 8
Dmd/19 ekkledleeqlnhlllwlspirnqlei------------ynqpnqegpfdvqeteiavqakq-pdveeilskgqhlykekp------atqpvkrkledlssewkavnrllqelrakqpdl<41>
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9 10 11 12 13
Dmd/20 vpaladfnrawteltdwlslldqviks------qrvmvgdledinemiikqkatmqdleqrr-pqleelitaaqnlknkts---nqeartiitdrieriqnqwdevqehlqnrrqqlnem
Heptad ...a..d...a..d...a..d...a.. .....d...a..d...a..d...a..d. ..a..d...a..d..... .....d...a..d...a..d...a..d...a..
14 15
Dmd/21 lkdstqwleakeeaeqvlgqarakles------wkegpytvdaiqkkitetkqlakdlrqwq-tnvdvandlalkllrdys----addtrkvhmiteninaswrsihkrvsereaaleet
Heptad ...a..d...a..d...a..d...a.. .....d...a..d...a..d...a..d. ..a..d...a..d..... .....d...a..d...a..d...a..d...a..
16 17 18
Dmd/23 sdqwkrlhlslqellvwlqlkddelsr------qapiggdfpavqkqndvhrafkrelktke-pvimstletvriflteqp<20>qnvtrllrkqaeevnteweklnlhsadwqrkidet
Heptad ...a..d...a..d...a..d...a.. .....d...a..d...a..d...a..d. ..a..d...a..d..... .....d...a..d...a..d...a..d...a..

Figure S1C.

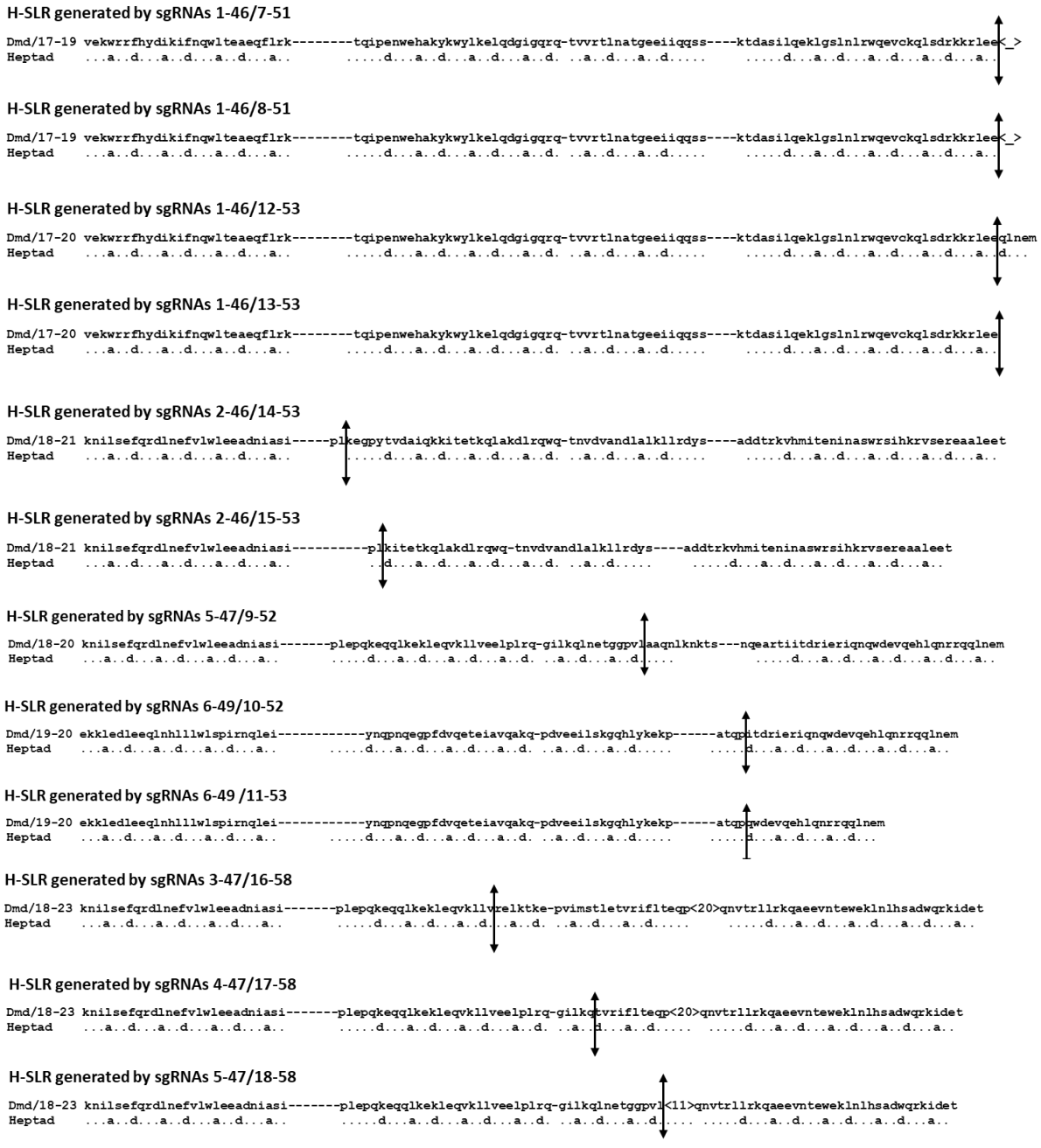


Figure S1. ***In silico*** **identification of sgRNAs for the SaCas9 suitable for the formation of hybrid spectrin-like repeats normally phased.** Potential target sites for SaCas9 sgRNAs were searched in exons 46 to 58 (except exon 50, which was deleted in the DMD patient used for our initial study). There was no SaCas9 PAM suitable for our approach in exons 48, 54, 55, 56 and 57. Panels show the nucleic acid

sequences targeted by the sgRNAs in exons 46, 47, 49, 51, 52, 53 and 58 of the human DMD gene. The exon sequences targeted by the sgRNAs (see table 1.) are in bold and the corresponding PAMs are underlined. Note that intronic sequences are not represented here. Since our strategy relies on the formation of hybrid exons that could result in the formation of a normally conformed spectrin-like repeat, we analysed where, in the corresponding amino-acid sequence, each sgRNA will cut. B) The amino acid sequences of helixes A, B and C are illustrated with the cut sites of each of the possible sgRNA. C) Based on these information, we were able to determine which pairs of sgRNAs could form a hybrid spectrin like repeat with preferably a hydrophobic amino-acid in the positions “a” and “d”.

Figure S2A.

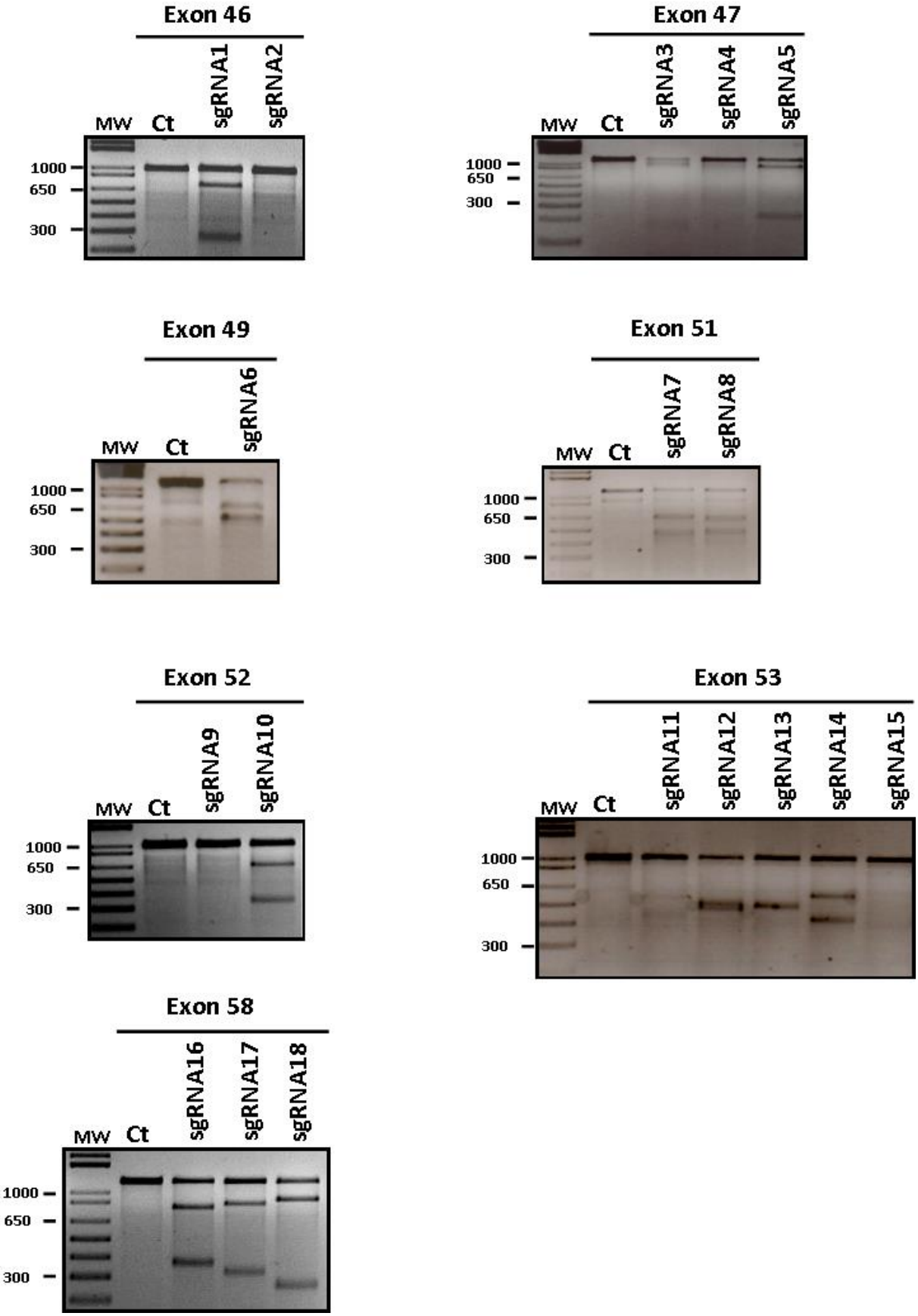

Exon 46
MW
Ct
sgRNA1
sgRNA2
1000
650
300
Exon 47
MW
Ct
sgRNA3
sgRNA4
sgRNA5
1000
650
300
Exon 49
MW
Ct
sgRNA6
1000
650
300
Exon 51
MW
Ct
sgRNA7
sgRNA8
1000
650
300
Exon 52
MW
Ct
sgRNA9
sgRNA10
1000
650
300
Exon 53
MW
Ct
sgRNA11
sgRNA12
sgRNA13
sgRNA14
sgRNA15
1000
650
300
Exon 58
MW
Ct
sgRNA16
sgRNA17
sgRNA18
1000
650
300

Figure S2B.

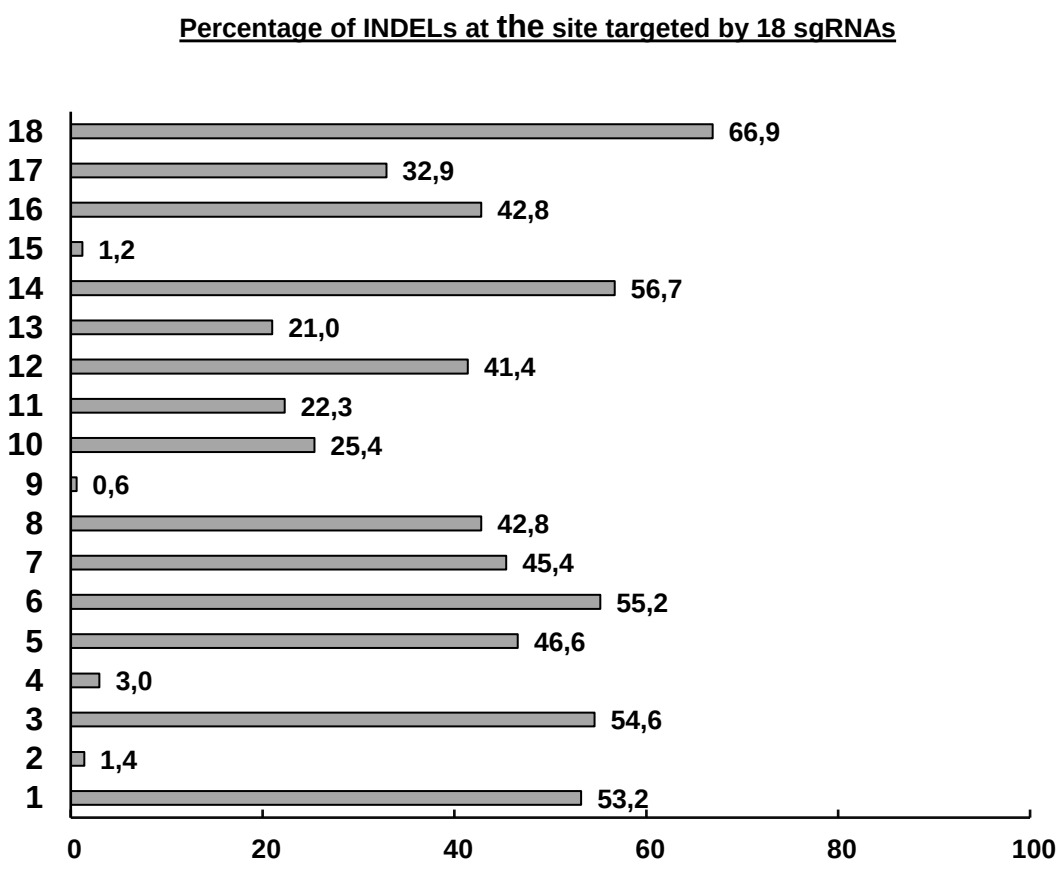


Figure S2. TIDE profiles of INDEL formation generated by individual sgRNA tested in 293T. A plasmid coding for each of the 18 possible SaCas9 sgRNAs was transfected in 293T cells. The targeted region of each sgRNA was PCR amplified. The Surveyor assay (S2A) was performed on genomic DNA extracted 48 h after the transfection. Genomic DNA of non-transfected cells was used as a control (Ct). The sgRNA numbers correspond to the targeted sequences (Table 1). MW: molecular weight marker. Among the tested sgRNAs, sgRNAs 9-52, 11-53 and 15-53 showed no detectable activity under the conditions tested while sgRNAs 1-46, 2-46, 3-47, 4-47, 5-47, 6-49, 7-51, 8-51, 10-52, 12-53, 13-53, 14-53, 16-58, 17-58 and 18-58 exhibited good efficiency as demonstrated by the Surveyor enzyme assay. Next, the presence of INDELs was determined by a TIDE analysis (https://tide-calculator.nki.nl/) (S2B).

We used amplicons from untreated 293T as a normal control sample. The figure illustrates the percentage of INDELs produced by each sgRNA. The sgRNAs 2, 4, 9 and 15 have a very low cutting activity which is in accordance with the Surveyor assay.

Figure S3.

Hybrid exons 46-51

SgRNAs 1-46/7-51

GCTAGAAGAAGTTACTCTGGTGACACAACCTGTGGTTACTAAGGAAACTGCCATCTCCAAACTAGAAATGCCATCTTCCTTGATGTTGGAGGTA
CCTGCTCTGGCAGATTTCAACCGGGCTTGGACAGAACTTACCGACTGGCTTTCTCTGCTTGATCAAGTTATAAAATCACAGAGGGTGATGGTGG
GTGACCTTGAGGATATCAACGAGATGATCATCAAGCAGAAG

SgRNAs 1-46/8-51

GCTAGAAGAAACACAACCTGTGGTTACTAAGGAAACTGCCATCTCCAAACTAGAAATGCCATCTTCCTTGATGTTGGAGGTACCTGCTCTGGCA
GATTTCAACCGGGCTTGGACAGAACTTACCGACTGGCTTTCTCTGCTTGATCAAGTTATAAAATCACAGAGGGTGATGGTGGGTGACCTTGAGG
ATATCAACGAGATGATCATCAAGCAGAAG

Hybrid exons 46-53

SgRNAs 1-46/12-53

GCTAGAAGAACAGTTGAATGAAATGTTAAAGGATTCAACACAATGGCTGGAAGCTAAGGAAGAAGCTGAGCAGGTCTTAGGACAGGCCAGAGCC
AAGCTTGAGTCATGGAAGGAGGGTCCCTATACAGTAGATGCAATCCAAAAGAAAATCACAGAAACCAAG

SgRNAs 1-46/13-53

GCTAGAAGAATTAAAGGATTCAACACAATGGCTGGAAGCTAAGGAAGAAGCTGAGCAGGTCTTAGGACAGGCCAGAGCCAAGCTTGAGTCATGG
AAGGAGGGTCCCTATACAGTAGATGCAATCCAAAAGAAAATCACAGAAACCAAG

Hybrid exon 49-52

SgRNAs 6-49/10-52

GAAACTGAAATAGCAGTTCAAGCTAAACAACCGGATGTGGAAGAGATTTTGTCTAAAGGGCAGCATTTGTACAAGGAAAAACCAGCCACTCAGC
CAATTACGGATCGAA

Hybrid exon 49-53

SgRNAs 6-49/11-53

GAAACTGAAATAGCAGTTCAAGCTAAACAACCGGATGTGGAAGAGATTTTGTCTAAAGGGCAGCATTTGTACAAGGAAAAACCAGCCACTCAGC
CACAGTGGGATGAAGTACAAGAACACCTTCAGAACCGGAGGCAACAGTTGAATGAAATGTTAAAGGATTCAACACAATGGCTGGAAGCTAAGGA
AGAAGCTGAGCAGGTCTTAGGACAGGCCAGAGCCAAGCTTGAGTCATGGAAGGAGGGTCCCTATACAGTAGATGCAATCCAAAAGAAAATCACA
GAAACCAAG

Hybrid exons 47-58

SgRNAs 3-47/16-58

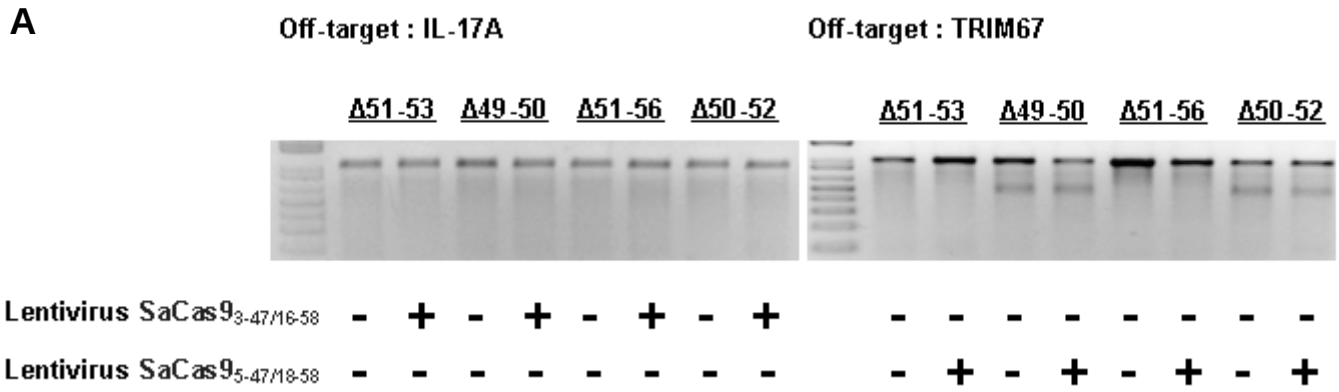
TTACTGGAGAGGGAATTGAAAACTAAAGAACCTGTAATCATGAGTACTCTTGAGACTGTACGAATATTTCTGACAGAGCAGCCTTTGGAAGGAC
TAGAGAAACTCTACCAGGAGCCCAGAG

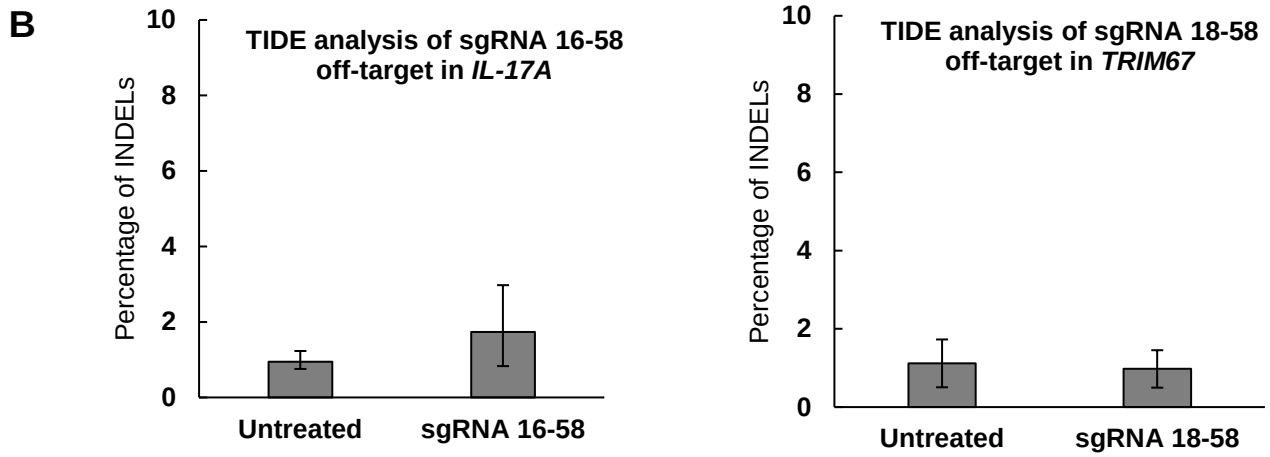
SgRNAs 5-47/18-58

TTACTGGTGGAAGAGTTGCCCCTGCGCCAGGGAATTCTCAAACAATTAAATGAAACTGGAGGACCCGTGCTGGAGCCCAGAG

Figure S3. Nucleotide sequences of the hybrid exons generated by SaCas9 and a pair of sgRNAs. The beginning part of the exon targeted by the first sgRNA of a pair is highlighted in light grey and the remaining part of the exon targeted by the second sgRNA of the pair is highlighted in dark grey. These are nucleotide sequences of the hybrid exons generated during our experiments were exactly as expected.

Figure S4





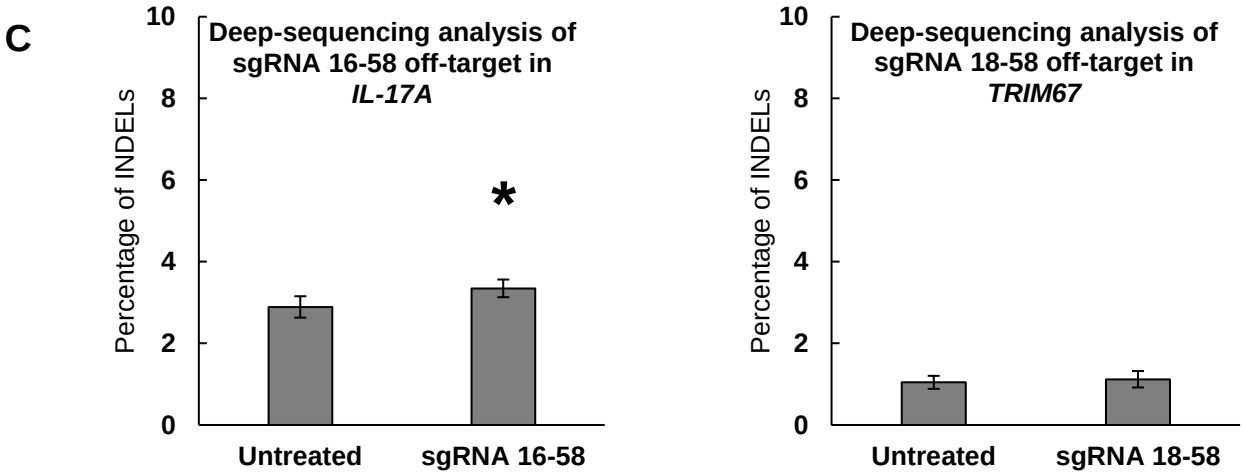


Figure S4. Analysis of the off-targets in the *IL-17A* and *TRIM67* genes. The potential off-targets were analysed by 3 methods: (A) Surveyor assay, (B) TIDE

analysis, (C) targeted deep-sequencing. These analyses were performed on genomic DNA extracted 4 weeks after infection of myoblasts from 4 DMD patients with Lentivirus-SaCas9-sgRNA3-47/16-58 or Lentivirus-SaCas9-sgRNA5-47/18-58. Untreated myoblasts from the same patients were used as controls. For TIDE analysis, the background percentage of INDELs was determined by comparison of untreated samples of the same four DMD patients and T-tests were performed. For deep-sequencing analysis, the background of INDELs was determined from the number of reads which nucleotide sequence differs from the wild-type in untreated samples, and paired T-tests were performed.