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Supplemental Information

Correction of the Exon 2 Duplication in DMD Myoblasts by a Single CRISPR/Cas9 System

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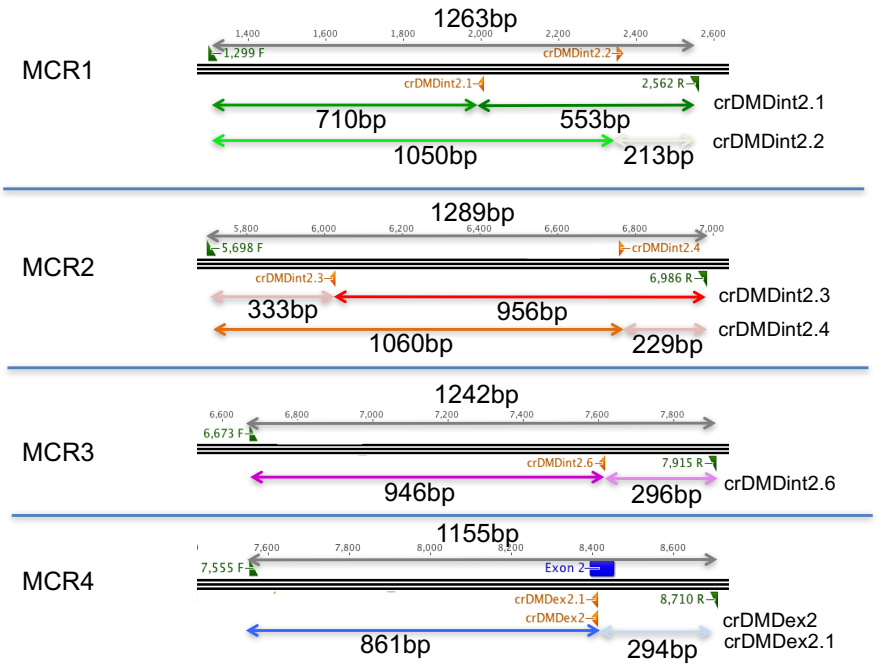
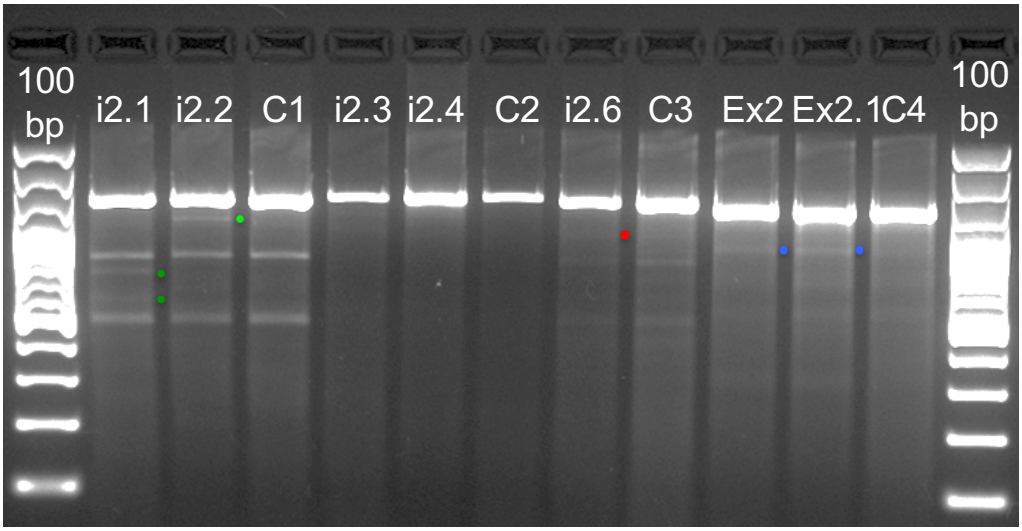
SUPPLEMENTARY FIGURES AND TABLES

a



b



% DNA cleavage	9,23	6,73	nd	nd	nd	nd	6,78	nd	9,72	10,17	nd

Figure S1. Testing gRNAs for NHEJ indel formation in HEK293T cells: (a) schematic of the PCR systems designed to amplify four regions of the minimal common duplicated region (MCR). For each region the intervening gRNAs are shown and predicted fragments generated by the T7E1 treatment are color-coded. (b) T7E1 assay of PCR products from HEK293T cells transfected with MLM3636 gRNA plasmids and spCas9 (JDS246). Coloured Dots indicate the position of T7E1 digestion fragments related to the activity of the gRNAs. Percentage of DNA cleavage is reported below each lane.

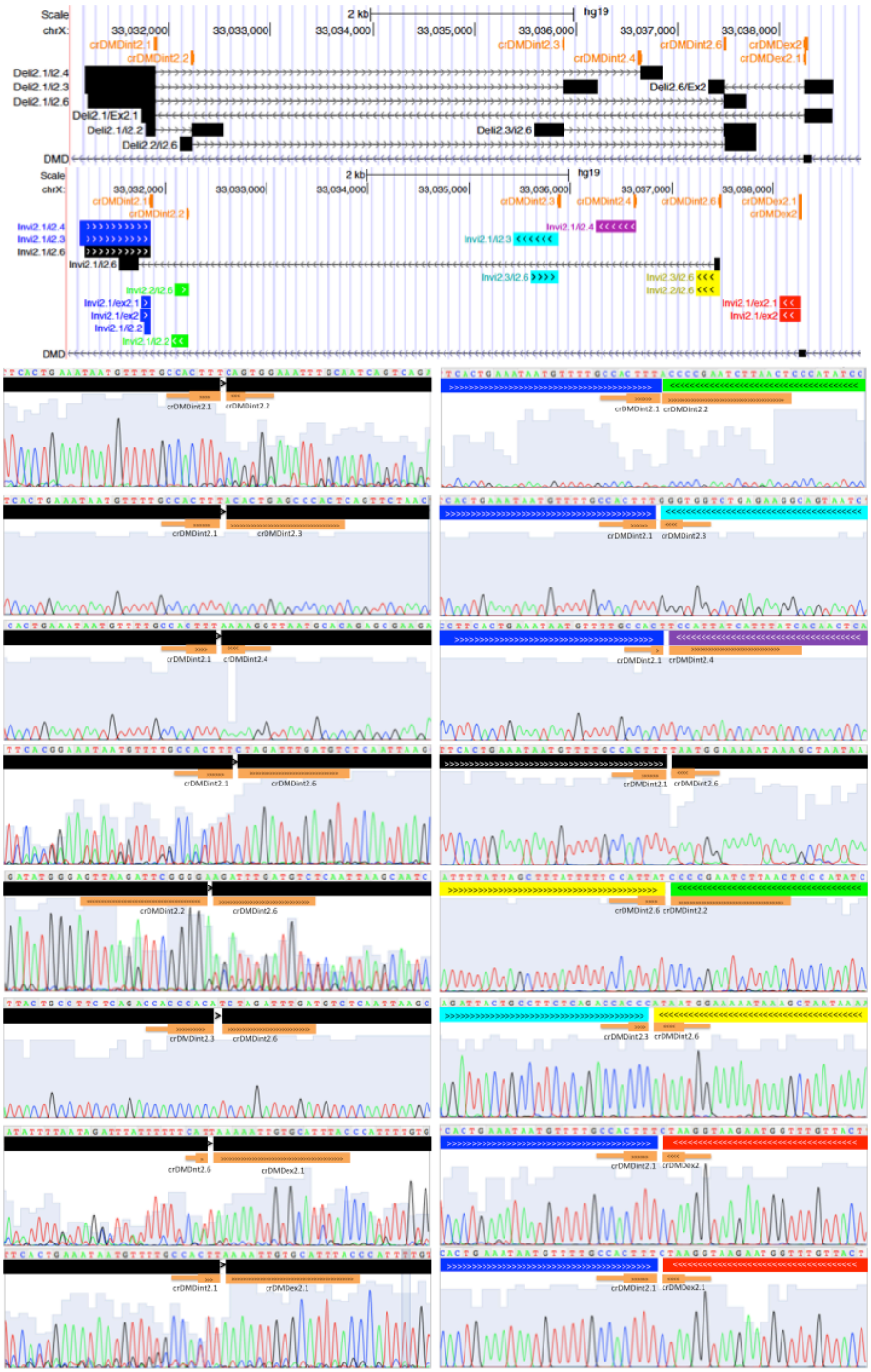


Figure S2: Characterization of deletion and Inversion Breakpoints.
Visualization of deletions and inversions caused by the activity of two gRNAS obtained by loading the resulting sequences (left panels: deletions; right panels: inversions) in UCSC Genome Browser and creating two custom tracks.
The sequences of each gRNA are overlaid over the chromatograms and represented by an orange thick bar for the PAM sequence and arrowed orange bars for the binding sequences. Deletion breakpoints are represented as black arrows between two black bars whereas inversion is shown as colour-coded bars with arrows indicating the original orientation of the sequence.

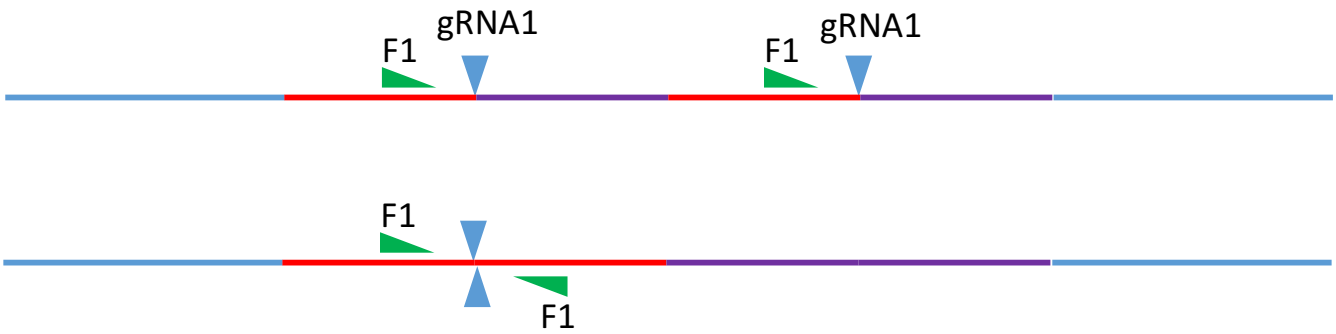


Figure S3: Strategy to amplify inversions of the duplicated region caused by activity of a gRNA with one primer. The primer and the gRNA are represented as green and light blue triangles respectively. The tandem duplication is coloured in red and violet in order to easily recognise the inversion in the scheme.

Deletions **PAM** **gRNA Int2.1**

```
TACCTCCTTCACTGAAATAATGTTTTGCCACTTTCCACTTTGTCCCAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT WT 26.3%(29/110)

TACCTCCTTCACTGAAATAATGTTTTGCCACTT-GTCCCAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT x13
TACCTCCTTCACTGAAATAATGTTTTGCCACTTT--CCCAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT x8
TACCTCCTTCACTGAAATAATGTTTTGCCACT----CCCAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT x3
TACCTCCTTCACTGAAATAATGTTTTGCCACTT-----CAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT x2
TACCTCCTTCACTGAAATAATGTTTTGCCACTTT---CCAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT
TACCTCCTTCACTGAAATAATGTTTTGCCACTTT----CAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT
TACCTCCTTCACTGAAATAATGTTTTGCCACTT---CCCAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT
TACCTCCTTCACTGAAATAATGTTTTGCCACTT-----AGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT
TACCTCCTTCACTGAAATAATGTTTTGCCACTT---------ATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT
TACCTCCTTCACTGAAATAATGTTTTGCCACTTT------------------TTTAAAAACTTCTAAGCTCATTTTAAAT
TACCTCCTTCACTGAAATAATGTTTTGCCACTTTG-----------------------------------ATTTTAAAT
TACCTCCTTCACTGAAATAATGTTTTGCCACTTT---------(133)----------------ATGTTTACTGGGTGTG
                                                                    Deletions:  30.9%(34/110)
```

Insertions

```
TACCTCCTTCACTGAAATAATGTTTTGCCACTTT---GT-CCCAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT WT

TACCTCCTTCACTGAAATAATGTTTTGCCACTTT--TGT-CCCAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT insT  x41
TACCTCCTTCACTGAAATAATGTTTTGCCACTTT-ATGT-CCCAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT insAT x2
TACCTCCTTCACTGAAATAATGTTTTGCCACTTTTATGT-CCCAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT insTAT
TACCTCCTTCACTGAAATAATGTTTTGCCACTTTCTTGT-CCCAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT insCTT
TACCTCCTTCACTGAAATAATGTTTTGCCACTTT---GTGCCCAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT insG
                                                                    Insertions:  41.8%(46/110)
```

Indels

```
TACCTCCTTCACTGAAATAATGTTTTGCCACTTT---C--CCCAGTATCATCCTTTTTTAAAAACTTCTAAGCTCATTTTAAAT insC delGT
                                                                          Indels:   0.01%(1/110)
                                                                **Total Mutations:  72.71%**
```

Potential off-target site #	Sequence	Indels (%)	Chromosome #	Strand	Position	GENE	Mismatch #1	Mismatch #2	Mismatch #3
Site Of Interest	GATGATACTGGGACAAAG	72.71%				DMD, intronic			
Potential Off-Target site # 1	GTTGAACTGGGACAAAG	nd	chr6	-	105368929	none	1:A>T	5:T>A	
Potential Off-Target site # 2	GAAGATACTGGGAGAAAG	nd	chr2	+	217969247	none	2:A>T	13:G>C	
Potential Off-Target site # 3	GATGAGACTGGGAGAAAG	nd	chr12	+	90417929	none	5:G>T	13:G>C	
Potential Off-Target site # 4	GATGACAGTGGGACAAAG	nd	chr2	-	11851832	LPIN1, intronic	5:G>A	7:C>G	
Potential Off-Target site # 6	GGTCACACTGGGACAAAG	nd	chr1	-	162675015	none	1:C>T	3:G>C	5:G>A
Potential Off-Target site # 16	GAACAGACTGGGACAAAG	nd	chr11	-	8381632	none	2:T>A	3:G>C	5:C>A
Potential Off-Target site # 57	GATGATACTGAGACAAAT	nd	chr16	-	87824358	none	10:T>C	17:A>C	
Potential Off-Target site # 58	GATGATACTGGGAGAAAA	nd	chr17	+	60562033	TLK2, intronic	13:G>C	17:A>G	
Potential Off-Target site # 59	GATGATACTGGGAGAAAA	nd	chr7	+	128177242	none	13:G>C	17:A>G	
Potential Off-Target site # 91	GATGATACTGTGATAAAG	nd	chr14	+	66373651	none	10:T>G	13:T>C	
Potential Off-Target site # 92	GATGATAATGGTACAAAG	nd	chr5	-	13605091	none	7:T>G	11:A>C	
Potential Off-Target site # 93	GTTGCCACTGGGACAAAG	nd	chrX	+	48165879	RNLP, intronic	1:T>A	4:C>A	5:C>T
Potential Off-Target site # 99	GACTCTACTGGGACAAAG	nd	chr11	+	23900800	none	2:C>T	3:T>G	4:C>A
Summary: Off by 0 = 1; Off by 1 = 0; Off by 2 = 9; Off by 3 = 143									

Figure S4. On-target and Off-target analysis of the crDMDint2.1: On target activity of the crDMDint2.1 gRNA in myoblasts infected with LV_int2.1 particles. Representative sequences of mutated alleles identified from 110 clonal amplicons. Dashes, deleted bases; red bases, insertions or mutations. Table summary of the on- and off-target analysis based on the ZiFiT software. Only off-targets with two mismatches or three mismatches (red bases) outside the seed gRNA region were tested and are shown.

Name	Forward	Reverse
crDMDex2	ACACCGGTAAATGCACAATTTTCTAG	AAAACTAGAAAATTGTGCATTTACCG
crDMDex2.1	ACACCGTAAATGCACAATTTTCTAG	AAAACTAGAAAATTGTGCATTTACG
crDMDint2.1	ACACCGATGATACTGGGACAAAGG	AAAACCTTTGTCCCAGTATCATCG
crDMDint2.2	ACACCGTTAAGATTCGGGGACAGG	AAAACCTGTCCCCGAATCTTAACG
crDMDint2.3	ACACCGAGTGGGCTCAGTGTGGGG	AAAACCCCACACTGAGCCCACTCG
crDMDint2.4	ACACCGATAAATGATAATGGAAAG	AAAACTTTCCATTATCATTTATCG
crDMDint2.6	ACACCGACATCAAATCTAGATAAG	AAAACTTATCTAGATTTGATGTCG

Table S1: Primers used to clone the DMD gRNAs into MLM3636.
The nucleotides that were removed to clone the gRNAs into the LentiCRISPRv2 are shown in red.

Name	Sequence	Target
1299F	CTGCCTGCTTCTCATAGGACTT	Minimal common region 1
2562R	AACACGGAAGGTTTTGCTGATG	
5698F	AGTTGTATGAATGTGTGTGTGCC	Minimal common region 2
6986R	TGGAATTCTGAAGCAGACAAGAGA	
6673F	AATTGCTCTGAGGCAGTGCTAA	Minimal common region 3
7915R	TGTGCAGGCTTCAATCCATACT	
7555F	TAAAGCCTTGGGTTTAAGGGCC	Minimal common region 4
8710R	GTGATCCGTCCTCACTGGC	
i2.1Offchr5F	CCAGCTGCCGAAGATTCAT	Potential Off-Target site #92 chr5:13605091
i2.1Offchr5R	CATCTCCCAGAAACACAGCA	
i2.1Offchr14F	ATTCAGGTCCATGGAGGCAG	Potential Off-Target site #91 chr14:66373651
i2.1Offchr14R	CTCTTTTCCTTCGCCCTCCC	
i2.1Offchr7F	TCTCAGCTCACTGCAACCTC	Potential Off-Target site #59 chr7:128177242
i2.1Offchr7R	TAGCCAGGATGGTCTCGATC	
i2.1OffChr17F	AGCAATTCTCGTGCCTCAGC	Potential Off-Target site #58 chr17:60562033
i2.1OffChr17R	CACCCACCACCAAGCTCAG	
i2.1Offchr16F	AGCTGCTCTTTGGGGTCTTC	Potential Off-Target site #57 chr16:87824358
i2.1Offchr16R	GCCTTGTGATCCACTCCCAA	
i2.1Offchr2aF	TACCCCAGCAAGGATCCAGA	Potential Off-Target site #04 chr2:11851832
i2.1Offchr2aR	CAGAGCAGTGTGAGAGCAGT	
i2.1Offchr12F	TGGGTGAGGATGGAGAGGAG	Potential Off-Target site #03 chr12:90417929
i2.1Offchr12R	ATCCGGGCCCTATGCTATCT	
i2.1Offchr2bF	TATGGCAGTAGGGGCTGGAT	Potential Off-Target site #02 chr2:217969247
i2.1Offchr2bR	TCTCTCTGGCTTAGACTTCCA	
i2.1Offchr6F	CAGCCTAGGTGACAGAGCAA	Potential Off-Target site #01 chr6:105368929
i2.1Offchr6R	GTTGCACTGAGCCGAAGATG	
i2.1Offchr1F	GAGACCGTGGTTCCTGTAGC	Potential Off-Target site #06 chr1:162675015
i2.1Offchr1R	TAGAGTTGCCCCCTGATCCA	
i2.1OffchrXF	CGACCTGCCTCACATACACT	Potential Off-Target site #93 chrX:48165879
i2.1OffchrXR	TAACCCGAGATCCAGGCATG	
i2.1Offchr11aF	AAGGGCCTGACACTTTCCTG	Potential Off-Target site #16 chr11:8381632
i2.1Offchr11aR	CCTGAACCCAACAAGACCCT	
i2.1Offchr11bF	AGCCAATTTTCCTCACACCA	Potential Off-Target site #99 chr11:23900800
i2.1Offchr11bR	GTAGCTGGGACTACAGGCAC	

Table S2: Primers used for the amplification for the gRNAs on-target and crDMDint2.1 off-target analysis.

Name	Sequence	Name	Sequence	Target
1861F	TCTGTTAAAGCAGGCACATTGC	2194F	GTTTGATGCCAAGAAGGTAGCC	i2.1/i2.2 inversion
		2666R	GTGTCCTGGTTTTCAGTGCATT	i2.1/i2.2 deletion
1861F	TCTGTTAAAGCAGGCACATTGC	5698F	AGTTGTATGAATGTGTGTGTGCC	i2.1/i2.3 inversion
		6154R	GCCTTTCAGCAGAATTCTAGCC	i2.1/i2.3 deletion
1861F	TCTGTTAAAGCAGGCACATTGC	2194F	GTTTGATGCCAAGAAGGTAGCC	i2.1/i2.2 inversion
		2666R	GTGTCCTGGTTTTCAGTGCATT	i2.1/i2.2 deletion
1861F	TCTGTTAAAGCAGGCACATTGC	6673F	AATTGCTCTGAGGCAGTGCTAA	i2.1/i2.4 inversion
		6986R	TGGAATTCTGAAGCAGACAAGAGA	i2.1/i2.4 deletion
1861F	TCTGTTAAAGCAGGCACATTGC	7377F	TCTCCTAGTGGATTGTTTTGGCT	i2.1/i2.6 inversion
		7915R	TGTGCAGGCTTCAATCCATACT	i2.1/i2.6 deletion
1861F	TCTGTTAAAGCAGGCACATTGC	8209F	TTTGCCATATCTTCTGCTGCTT	i2.1/ex2-2.1 inversion
		7915R	TGTGCAGGCTTCAATCCATACT	i2.1/ex2-2.1 deletion
2194F	GTTTGATGCCAAGAAGGTAGCC	7377F	TCTCCTAGTGGATTGTTTTGGCT	i2.2/i2.6 inversion
		7915R	TGTGCAGGCTTCAATCCATACT	i2.2/i2.6 deletion
5698F	AGTTGTATGAATGTGTGTGTGCC	7377F	TCTCCTAGTGGATTGTTTTGGCT	i2.3/i2.6 inversion
		7915R	TGTGCAGGCTTCAATCCATACT	i2.3/i2.6 deletion
6673F	AATTGCTCTGAGGCAGTGCTAA	7377F	TCTCCTAGTGGATTGTTTTGGCT	i2.4/i2.6 inversion
		7915R	TGTGCAGGCTTCAATCCATACT	i2.4/i2.6 deletion
7377F	TCTCCTAGTGGATTGTTTTGGCT	8209F	TTTGCCATATCTTCTGCTGCTT	i2.6/ex2-2.1 inversion
		8710R	GTGATCCGTCCTCACTGGC	i2.6/ex2-2.1 deletion

Table S3: List of primers used to amplify the duplications' and inversions' breakpoints. Primers used to amplify inversions in dup2 myoblasts are underlined in the present table.