

SUPPLEMENTAL INFORMATION

Analysis of CRISPR-SaCas9 Off-Targets

In order to verify whether SaCas9 and sgRNAs led to unspecific cuts in the genome of transduced cells, we analyzed genomic DNA from DM1 edited clones, which were exposed to the action of CRISPR-SaCas9 for several weeks during cell amplification before gDNA extraction (Table S3). In particular, we focused on sgRNAs 4 and 23 and selected 20 potential off-targets having a number of mismatches between 4 and 6 (off-targets 1 to 10 for sgRNA 4 in Table S3A, and 11 to 20 for sgRNA 23 in Table S3B). Most off-target sites were located within intergenic regions or introns, except one, number 17, which was located within an exon. We PCR amplified and sequenced the corresponding off-target genomic regions from four DM1 edited clones carrying the deletion of the expanded CTG repeats (DM1-Delta clones 3, 10, 17, 22) and used a DM1 non-transduced clone (DM1), and a clone transduced only with the sgRNA₄₋₂₃ lentiviral vector (DM1-sgRNA), as controls. Examination of the off-target chromatograms was followed by TIDE analysis, which should result in a cutting efficiency of ~50% if the clonal population harbors indels in one of the two alleles for the selected target. The total cutting efficiency calculated by TIDE using the non-transduced clone control sequence, ranged between 0 and 3.8% in DM1-Delta clones (see Table S3C). These values represent the signal background of each amplified genomic region and similar values were obtained also for the DM1-sgRNA clone. Therefore, we found no off-target indels in all four analyzed DM1-Delta myoblast clones, suggesting that sgRNAs 4 and 23 appeared specific in these cells.

Supplementary Figure Legends

Figure S1. Deletion of *DMPK* CTG Repeat by Nm CRISPR-Cas9. (A) Scheme representing position of sgRNAs (gray arrows) flanking CTG repeat [(CTG)*n*] within the 3' UTR of human *DMPK* exon 15, which are located between the stop codon of the gene (stop) and the polyadenylation signal (pA). (B) Plasmid constructs encoding *Neisseria meningitidis* Cas9 (NmCas9) and the sgRNAs targeting regions upstream (up) and downstream (dw) the CTG repeats. EFS: Elongation Factor1-alpha (EF1- α) gene short promoter; U6: human U6 small nuclear RNA (snRNA) gene promoter; pA: polyadenylation signal. (C) Gel electrophoresis of PCR products using primers F and R from genomic DNA of HeLa cells, non-transfected (NT) or transfected with plasmids expressing NmCas9 and the indicated sgRNAs couples. White and black arrowheads show PCR amplicons without or with the CTG repeats deletion, respectively.

Figure S2. Reduction of foci-containing nuclei in DM1 cells treated with lentiviral CRISPR-SaCas9 vectors. The percentage of DM1 patient-derived muscle line cells without nuclear foci after treatment with indicated MOI of lentiviral vectors SaCas9 and sgRNA couples 4-12A, 8-23 and 8-12A is indicated. Histograms show values obtained from manually counted FISH images of one biological replicate.

Figure S3. Deletion of *DMPK* CTG Repeat Expansion in Heterozygous DMSXL Mice. (A) Gel electrophoresis of PCR products from the genomic *DMPK* 3' UTR of TA muscles 4 weeks after intramuscular administration of rAAV9 vectors. (+) left TA co-injected with equal viral genomes (1:1) of rAAV9-SaCas9 (AAV-Cas9) and rAAV9-sgRNA₄₋₂₃ (AAV-sgRNA) (0.6×10^{11} and 1.0×10^{11} total vg, in 7 DMSXL mice at 3 and 6 weeks of age each (3w, 6w), respectively. (-): right TA injected with PBS as control. Black arrowheads show the band with deleted CTG repeats. (B) Optimized PCR amplification of the genomic *DMPK* 3' UTR revealed products with

undeleted (4527 bp, white arrowheads) or deleted (399 bp, black arrowhead) expanded CTG repeats in PBS (-) and rAAV9 (+) treated TA muscles of two mice.

Figure S4. PCR analysis of the *DMPK* 3'-UTR showing presence of heteroduplex amplicons.

(A-C) Intermediate bands located between PCR amplicons with (upper bands) and without (lower bands) the *DMPK* CTG repeat, obtained in the bulk population of transduced DM1 cells (BULK), and in DM1 clones containing a monoallelic CTG repeat deletion (Delta 17 and 22), were reproduced by using as PCR template a mix of gDNA from DM1 cells not treated (NT) and DM1-Delta clone with biallelic CTG repeat deletion (Delta 10 or 3), or (D) by simple denaturation and renaturation of a mixture of PCR amplicons from gDNA NT and Delta 10, and from NT and Delta 3.

Figure S5. *DMPK* mRNA levels upon effect of the CTG repeat deletion. (A) Expression of the human *DMPK* mRNA *in vitro* in DM1 cells and derivatives clones DM1-Delta 10, 3, 17 and 22 harboring biallelic or monoallelic CTG repeat deletion after treatment with lentiviral vectors SaCas9 and sgRNA 4-23. *DMPK* expression values are normalized for *GAPDH* or *HPRT1*, and represented as fold change relative to control cells from non-affected individual (Ctrl). (B) *DMPK* expression levels *in vivo* in TA muscles of DMSXL mice, 4 weeks after intramuscular injection of rAAV vectors SaCas9 and sgRNA 4-23 (+). *DMPK* values are normalized for the mouse gene *Gapdh*, *Hprt1* or *Tbp*, and represented as fold change relative to the contralateral TA muscle, injected with PBS (-). Data are average of three independent biological replicates in (A) and of 6 animals per group in (B). Statistical analysis by two-tailed Student *t*-test. *: P < 0.05; **: P < 0.01; ns: not significant.

Figure S1.

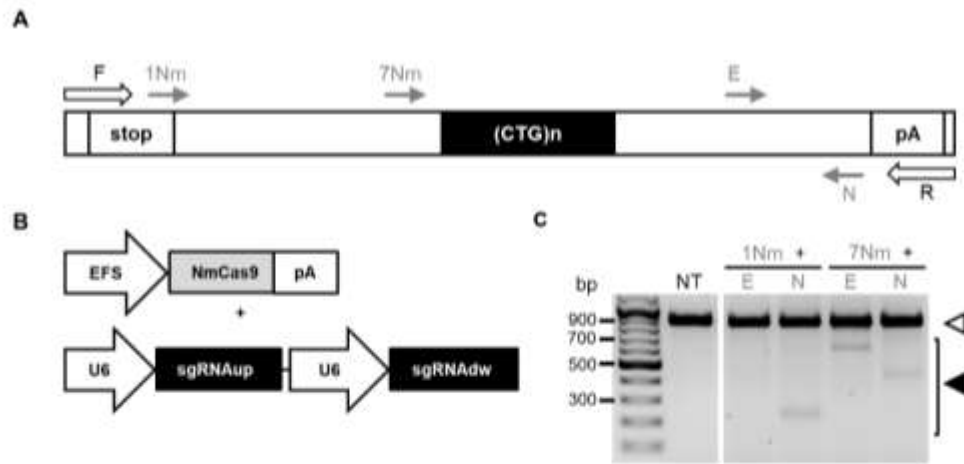


Figure S2.

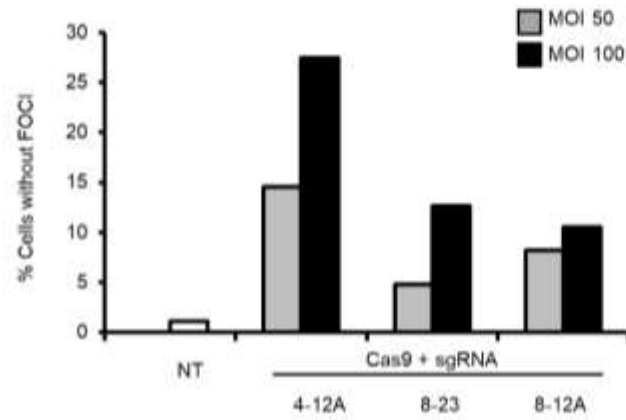


Figure S3.

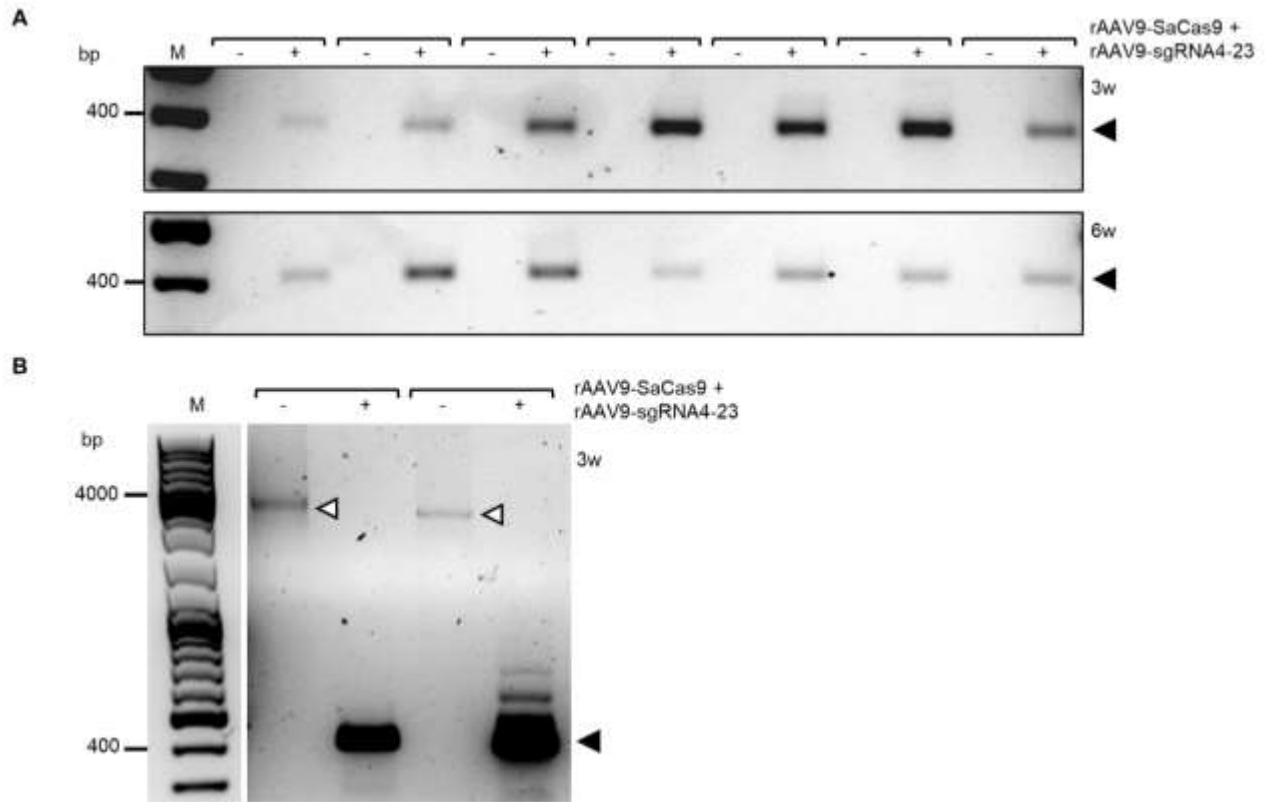


Figure S4.

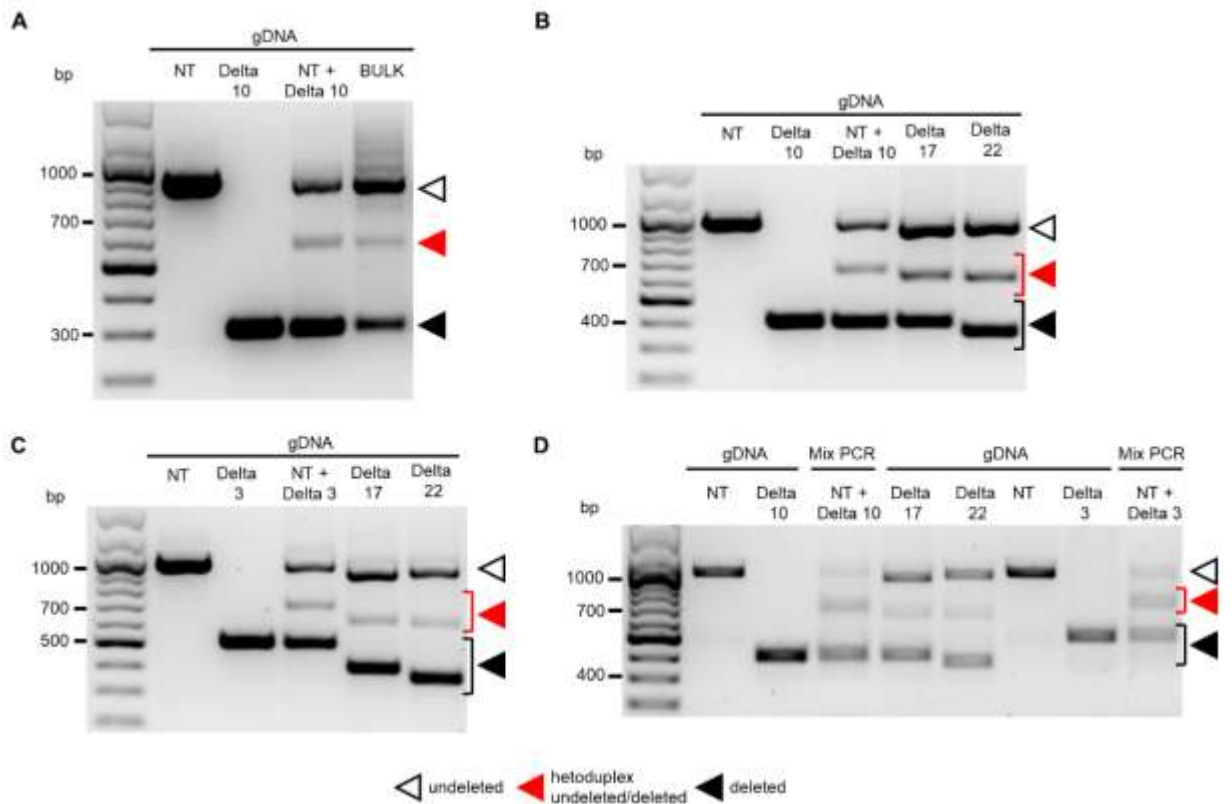
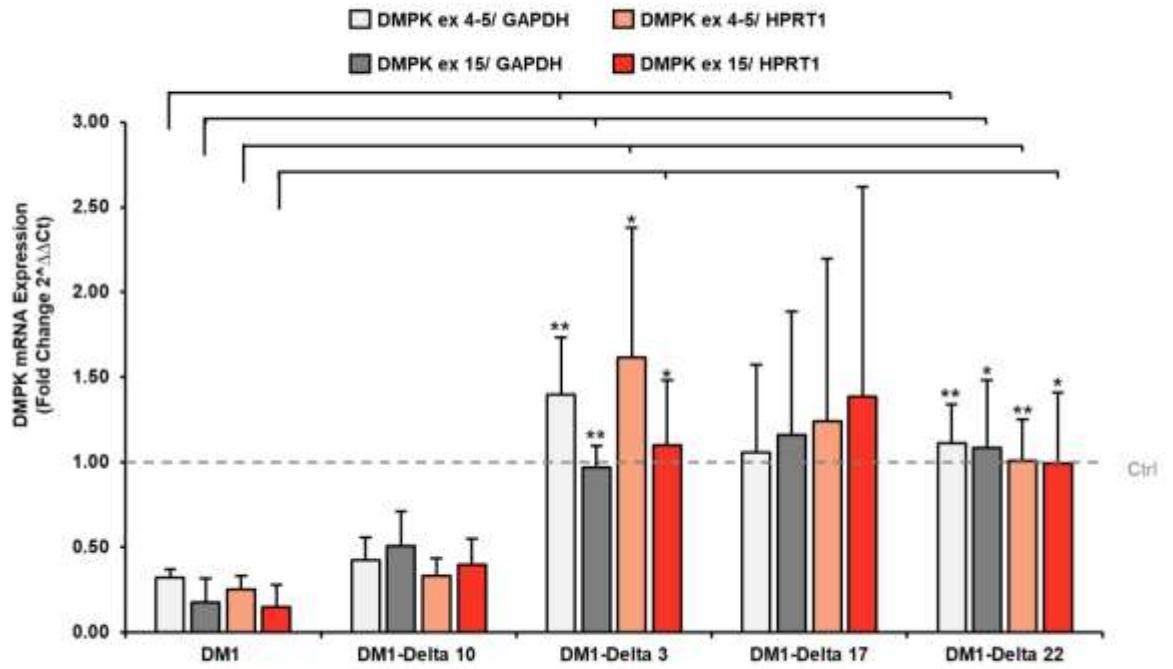


Figure S5.

A



B

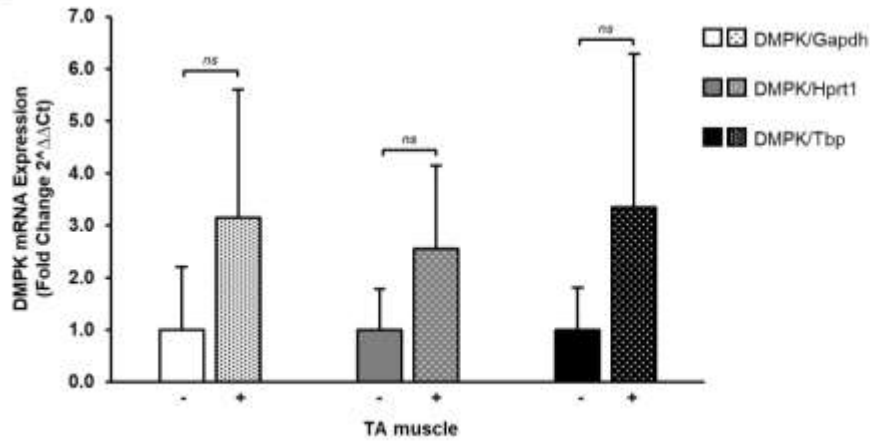


Table S1. Sa sgRNA Sequences, Positions and Cutting Efficiencies

Sa sgRNA protospacer						
sgRNA number	protospacer sequence [5'→3']	Lenght	genomic target sequence (in black) and PAM (in blue) [5'→3']	Genomic position ^a (start-end)	Strand	% cutting by TIDE
1	GCCCCGGAGTCGAAGACAGTTC	22	GCCCCGGAGTCGAAGACAGTTC TAGGGT	45770460 45770487	+	47.4
4	G CAGTTCACAACCGCTCCGAGC	G + 21	CAGTTCACAACCGCTCCGAGC GTGGGT	45770377 45770403	-	42.4
7	GCGGCCGGCGAACGGGGCTCG	21	GCGGCCGGCGAACGGGGCTCG AAGGGT	45770282 45770308	-	43.8
8	GGCTCGAAGGGTCCTTGTAGCC	22	GGCTCGAAGGGTCCTTGTAGCC GGAAT	45770266 45770293	-	42
10	G CGGCCAGGCTGAGGCCCTGAC	G + 21	CGGCCAGGCTGAGGCCCTGAC GTGGAT	45770151 45770177	-	7.8
12A	G CTTTGCGAACCAACGATAGGT	G + 21	CTTTGCGAACCAACGATAGGT GGGGT	45770064 45770090	+	32.5
12B	GCACTTTGCGAACCAACGATAGGT	24	GCACTTTGCGAACCAACGATAGGT GGGGT	45770061 45770090	+	28.8
13A	GGTTTGGCAAAAGCAAATTC	21	GGTTTGGCAAAAGCAAATTC CCGAGT	45769973 45769999	+	36.5
13B	GCGGGTTTGGCAAAAGCAAATTC	24	GCGGGTTTGGCAAAAGCAAATTC CCGAGT	45769970 45769999	+	22.7
15	GGGGGGCGCGGGATCCCCGAAAAA	24	GGGGGGCGCGGGATCCCCGAAAAA GCGGGT	45769946 45769975	+	3.3
17A	GGCTCCGCCCGCTTCGGCGGT	21	GGCTCCGCCCGCTTCGGCGGT TTGGAT	45769887 45769913	-	1.8
17B	GCCGGCTCCGCCCGCTTCGGCGGT	24	GCCGGCTCCGCCCGCTTCGGCGGT TTGGAT	45769887 45769916	-	2
19	G AAAACGTGGATTGGGGTTGTT	G+21	AAAACGTGGATTGGGGTTGTT GGGGT	45769819 45769845	+	1.1
22	GGGGTCTCAGTGCATCCAAAAC	22	GGGGTCTCAGTGCATCCAAAAC GTGGAT	45769802 45769829	+	7.9
23	GACAATAAATACCGAGGAATGT	22	GACAATAAATACCGAGGAATGT CGGGT	45769779 45769806	+	48.3

G = not part of the target sequence, it has been added to optimize the transcription of the sgRNA from U6 promoter

^a Reference genome: Human GRCh38/hg38 (UCSC/Blat)

Table S2. List of Plasmids and Constructs

Name	Description	Ref
pX601-AAV-CMV::NLS-SaCas9-NLS-3xHA-bGHpA;U6::BsaI-sgRNA	AAV plasmid carrying <i>Staphylococcus aureus</i> (Sa) Cas9 under the control of CMV promoter, and one sgRNA expression cassette (U6::BsaI-sgRNA) under the control of human U6 promoter.	MLS42; gift from Feng Zhang (Addgene plasmid # 61591); ¹
pAAV-EFS::NLS-SaCas9-NLS-3xHA-bGHpA;U6::BsaI-sgRNA	Derivative of plasmid MLS42 carrying EFS promoter instead CMV promoter.	MLS43; this study
pAAV-EFS::NLS-SaCas9-NLS-3xHA-bGHpA;U6::BbsI-sgRNA;U6::BsaI-sgRNA	Derivative of plasmid MLS43 carrying a second sgRNA expression cassette (U6::BbsI-sgRNA).	MLS47; this study
pAAV-EFS::NLS-SaCas9-NLS-3xHA-bGHpA;U6::4-sgRNA;U6::23-sgRNA_DMPK	Derivative of plasmid MLS47 carrying sgRNA protospacers 4 into <i>BbsI</i> site and 23 into <i>BsaI</i> site.	MLS93; this study
pCCL-hPGK.GFP	pCCL plasmid harboring eGFP under the control of hPGK promoter.	Gift from Mario Amendola
pCCL-CMV-GFP	Derivative of plasmid MLS87 carrying CMV instead hPGK promoter.	MLS107; this study
pCCL-CMV-SaCas9	Derivative of plasmid MLS107 carrying SaCas9 instead eGFP.	MLS110; this study
pCCL-U6::4-sgRNA;U6::23-sgRNA_DMPK-hPGK.GFP	Derivative of plasmid MLS87 carrying insert U6::4-sgRNA;U6::23-sgRNA-DMPK from plasmid MLS93.	MLS100; this study
pC512-Int-smSVpolyA	AAV plasmid with SPc5-12 promoter, chimeric intron, MCS and small SV40polyA.	MLS1; this study
pAAV-SPc5-12-SaCas9	Derivative of plasmid MLS1 carrying SaCas9 in front of SPc5-12 promoter.	MLS118; this study
pAAV-Desmin-MCS	AAV plasmid carrying Desmin promoter, chimeric intron, MCS and polyA.	Genethon plasmid bank
pBlue-eGFP-KASH	Plasmid carrying synthetically synthesized eGFP-KASH insert (GeneCust) consisting of eGFP coding sequence with a C-terminus KASH peptide.	MLS22; this study
pAAV-Des-eGFP-KASH	Derivative of plasmid ID_772 carrying insert eGFP-KASH from plasmid MLS22.	MLS23; this study
JDS246NMCas9 and U6sgRNANM	Plasmids carrying <i>Neisseria meningitidis</i> (Nm) Cas9 and sgRNA	Gift from Jean-Paul Concordet
pAAV-Des-eGFP-KASH-U6gRNA-NM-1N-DMPK	Derivative of plasmid MLS23 carrying insert U6gRNA-NM-1N-DMPK.	MLS27; this study
pAAV-Des-eGFP-KASH-U6::4-23-sgRNA_DMPK	Derivative of plasmid MLS27 with insert U6::4-23-sgRNA_DMPK (from plasmid MLS93) instead insert U6gRNA-NM-1N-DMPK.	MLS123; this study

Table S3A. Predicted Off-Target Sites of sgRNA 4

Off-target number sgRNA 4	Off-targets				
	position	sequence	strand	N mismatches	location
1	chr11:97774468-97774495	aCAGaaCtCAAaCtCTCCGAGC TTGAGT	+	6	intergenic
2	chr21:20427038-20427065	aCAaaTCACAAaaGCTCtGAGC TTGAGT	-	6	intergenic
3	chr20:46065382-46065409	GCAagTCACAACCG tTCtGAGCCAGGGT	-	4	INTRON 5 gene NCOA5
4	chr17:2435999-2436026	GCAcTcCACaCaGCTCCGAGa CCGAGT	-	5	INTRON 8 gene METTL16
5	chr15:97366952-97366979	GCAcTTCA tAACaGCaCCcAGC ATGAAT	+	5	INTRON gene CTD-2147F2.2
6	chr2:117323541-117323568	tgAGTgCACAACtGCTCtGAt C GAGAGT	-	6	intergenic
7	chr19:42201303-42201330	aCAagTCACAAC CtCTCtGAGC ATGGGT	+	5	INTRON 4 gene DEDD
8	chr19:41888650-41888677	GCAcTTCACA aggGatCCaAGC CAGGGT	-	5	INTRON 3 ARHGEF1
9	chr22:30431446-30431473	GCAagTCACAg CCtCTCCGAGt GTGAGT	+	5	INTRON 8 gene prediction chr22.409
10	chr22:45207691-45207718	GCA GTgCcCAGCCcCTCgGAGC CCGAGT	-	5	INTRON 2 gene KIAA0930

Table S3B. Predicted Off-Target Sites of sgRNA 23

Off-target number sgRNA 23	Off-targets				
	position	sequence	strand	N mismatches	location
11	chr3:82957453-82957480	agCAATAA t TAagGAGGAATGT AAGAAT	-	5	INTRON 1 gene prediction chr3.1029
12	chr18:77715237-77715264	ccCAATAAATACC a AGG t AT a T GAGAGT	+	5	intergenic
13	chr12:78969291-78969318	ccCAATA g ATAC t GAGGAAT g a CTGAAT	+	5	INTRON 2 gene SYT1
14	chr7:115785851-115785878	G t CA a CAAATAC t GAGGAAT t T TAGAGT	+	4	intergenic
15	chr9:100918144-100918171	GA t gATAAATACC a AGGA c TGT GAGGAT	+	4	INTRON 2 gene prediction chr9.1040
16	chr9:110613710-110613737	GAC A tTAAATAC t GAGGA A g G g GTGAAT	+	4	intergenic
17	chr15:39924115-39925142	t tCAATAAATACC t A a GAA a GT TGGAAT	-	5	exon 1 gene RP11-325N19.3
18	chr2:53213301-53213328	G c tAATAAATAC a GAGGAAT G A TAGAAT	-	4	intergenic
19	chr6:27921664-27921691	a c t AATAAATAC a GAGGAAT G g TGGGAT	-	5	intergenic
20	chr13:96983088-96983115	t AT A ATAAA ag CaGAGGAATGT TAGAAT	+	5	intron 1 gene LINC00359

Table S3C. Tide Analysis of Indel Frequencies in Predicted Off-Target Sites

sgRNA	TIDE Indel Frequencies (%)				
N. potential off-target sgRNA 4	DM1-Delta 10	DM1-Delta 3	DM1-Delta 17	DM1-Delta 22	DM1-sgRNA
1	1.7	1.7	0.7	2.8	2.4
2	2.3	1.3	1.7	1.2	1.2
3	1.6	1.0	0.9	2.3	2.9
4	0.6	0.8	0.6	0.4	0.2
5	0.8	1.4	0.2	2.4	2.4
6	0.6	0.2	0.7	2.7	2.0
7	0.7	0.1	0.1	1.0	0.9
8	0.6	0.3	0.8	1.3	1.8
9	1.5	0.5	0.6	1.6	1.0
10	0.3	1.6	0.3	0.2	0.5
23					
11	2.9	2.6	1.7	3.8	3.4
12	0.5	0.4	0.8	0.6	0.8
13	0.0	1.1	0.8	1.3	1.6
14	0.1	0.6	0.9	3.3	3.3
15	1.3	0.7	1.5	2.4	1.4
16	0.8	0.5	0.2	2.1	0.9
17	1.0	0.7	0.0	3.0	1.7
18	1.7	0.4	2.2	0.9	1.2
19	0.5	1.6	0.8	0.7	0.4
20	1.8	1.2	0.5	2.5	2.3

Table S4. List of Primers

Primer Name	Sequence from 5' to 3'	Comment	Reference
Cloning			
F-XhoI-MreI-EFS	CGCTCGAGCGCCGGCGTGAGGCTCCGGTGCCCGTCAGTG G	PCR EFS promoter for plasmid MLS43	MLS63&MLS64; this study
R-XmaI-NruI-EFS	CGCCCGGGTCGCGATCACGACACCTGTGTTCTGGCGGCA AACC		
F-AgeI-SaCas9	GCGACCGGTGCCACCATGGCCCCAAAGAAG	PCR SaCas9 for plasmid MLS110	MLS142&MLS143; this study
R-SaII-SaCas9	CGCGTCGACCTTAAGCGTAATCTGGAACATCGTATGGGT AAGCG		
F-PmeI-SaCas9	GCGGTTTAAACGCCACCATGGCCCCAAAGAAG	PCR SaCas9 for plasmid MLS118	MLS146&MLS147; this study
R-NotI-SaCas9_3xHE	CCGCGGCCGCGGAGCTCTAGGAATTCTTAAGCGTAATC GGAGGTACCTTAAGCAATTGGACATAGTCGTTAAACC		
F-MCS-before-U6SasgRNA	GGAGGTACCTTAAGCAATTGGACATAGTCGTTAAACC	PCR insert U6::4-23-sgRNA_DMPK for plasmid MLS123	MLS163&MLS166; this study
R-PmlI-EndSasgRNA-up	CCTCACGTGTCTGCGGCCGCAAAAATCTCG		
Genomic PCR/Sequencing <i>DMPK</i> 3'-UTR			
F1-DMPK-3UTR	GTTCGCCGTTGTTCTGTCTCG		MLS14; this study
R1-DMPK-3UTR	TCCAGAGCTTTGGGCAGATGG		MLS15; this study
F2-DMPK-3UTR	GTCCAGGAGCCAATCAGAGG		MLS16; this study
R2-DMPK-3UTR	CTAGTCCTCCAGACCTTCG		MLS17; this study
RT-PCR alternative splicing			
F-LDB3	GCAAGACCCTGATGAAGAAGCTC	<i>LDB3</i> exon 11	MLS172 and MLS173; ²
R-LDB3	GACAGAAGGCCGGATGCTG		
F-SERCA1	ATCTTCAAGCTCCGGGCCCT	<i>ATP2A1</i> exon 22	MLS174 and MLS175; ³
R-SERCA1	CAGCTCTGCCTGAAGATGTG		
F-MBNL1	GCTGCCCAATACCAGGTCAAC	<i>MBNL1</i> exon 7	MLS170 and MLS171; ²
R-MBNL1	TGGTGGGAGAAATGCTGTATGC		
F-DMD	TTAGAGGAGGTGATGGAGCA	<i>DMD</i> exon 78	MLS176 and MLS177; ⁴
R-DMD	GATACTAAGGACTCCATCGC		
F-IR-ex10-12	CCAAAGACAGACTCTCAGAT	<i>IR</i> exon 11	MLS178 and MLS179; ⁵
R-IR-ex10-12	AACATCGCCAAGGACCTGC		
F-BIN1	AGAACCTCAATGATGTGCTGG	<i>BIN1</i> exon 11	MLS168 and MLS169; ⁶
R-BIN1	TCGTGTTGACTCTGATCTCGG		

Genomic PCR/Sequencing off-targets			
F-chr11:97774468	CCCTGCCATAACACATACCTAAGTGG	1	MLS207&208; this study
R-chr11:97774468	GCATGCAATTATAGCCTCTGCATTAGCG		
F-chr21:20427038	GAAGATATATATGCATAGCAATATAATATTGCAATTGC	2	MLS209&210; this study
R-chr21:20427038	GGGGCATTGTGATTAAGAAGTCAGAAAGAGAGG		
F-chr20:46065382	GAGAACCTCCCCTGCTAACATCC	3	MLS211&212; this study
R-chr20:46065382	GTTTGTAAGAGGTAAATGGACTTGCTTAAGC		
F-chr17:2435999	CTCAGCATTCTCTGATGGTTGAGATGG	4	MLS213&214; this study
R-chr17:2435999	CTAGAATATCAGGGCCCTCACACC		
F-chr15:97366952	GTCAAAGACAGTTAAGCAGTTCAGTTGAGG	5	MLS215&216; this study
R-chr15:97366952	GGTAAGGGACAGCAGATTTAGAATTCC		
F-chr2:117323541	CCAGAAGCTTCTCATTGCCTTCC	6	MLS217&218; this study
R-chr2:117323541	GACAAAGAGTAGCAACTATAGGAAGACTGAC		
F-chr19:42201303	GCAGCCAACACTTACAGAGTGC	7	MLS219&220; this study
R-chr19:42201303	GAGCCACAGCATGAGACATTCC		
F-chr19:41888650	CACGGTCTGTCTCATCTCTCATC	8	MLS221&222; this study
R-chr19:41888650	GCACTAGCTCGCTGGTTTAATTCG		
F-chr22:30431446	GACAGCCTCCTGCAGGACTG	9	MLS223&224; this study
R-chr22:30431446	GCCAATTCTGCTTTGAAGAATGATGTGC		
F-chr22:45207691	GAGACGGGGTTTCACCATGTTGG	10	MLS225&226; this study
R-chr22:45207691	CTGAGTCCACAGCGCTTTGC		
F-chr3:82957453	CCAGAGCTAATCTGGCTGGTC	11	MLS227&228; this study
R-chr3:82957453	CTAACACAGAAAGTTCTCAATGCCAGC		
F-chr18:77715237	TGCCTGCAGCAAGTTTCAGC	12	MLS229&230; this study
R-chr18:77715237	GTAAACCCACCTCATCTTTAGGAAGC		
F-chr12:78969291	CAGTGACCCTTCTGTATCCACAGG	13	MLS231&232; this study
R-chr12:78969291	CTGCCATGGCCCATAATGGC		
F-chr7:115785851	GCTGTCCATATAAAGGATACTGAGAATGGC	14	MLS233&234; this study
R-chr7:115785851	GCCATGTAACAATGTATACATATATTGAAACATTACAAC G		
F-chr9:100918144	CTTTCAGGTGGGACCAGGAG	15	MLS235&236; this study

R-chr9: 100918144	TACAATAACAAGACCTAAAGCCCTGTTG		
F-chr9:110613710	CACTACATAAAGAAGAGAGCATAAAGGGACC	16	MLS237&238; this study
R-chr9:110613710	GTGTATTATGCTAATGTTGGGTGGTGTATTC		
F-chr15:39925115	GATCTTGGGACTTGTGAGCCTCC	17	MLS239&240; this study
R-chr15:39925115	TCTCAGCATGCTGCTGCTTG		
F-chr2: 53213301	GACTTCTTCTTGCAGTAACTATATACGAAAGGA	18	MLS241&242; this study
R-chr2: 53213301	ACCAACATGGCACACACATACC		
F-chr6:27921664	CTTCTCATTCATCATATTAAGAGGCATCTGATG	19	MLS243&244; this study
R-chr6:27921664	CCTTCTCCACTGAAAGAAATTAGGGC		
F-chr13: 96983088	CTCTGTTAATCTCCTCCCTCATGAGG	20	MLS245&246; this study
R-chr13: 96983088	GAACACTAATTCACCTAGGAAGGTGC		
qRT-PCR DMPK			
F-DMPK-ex4-start	GACGTGTTGGTGAATGGGGA	Human <i>DMPK</i> ex 4-ex 5	MLS249&250; this study
R-DMPK-ex5-start	CCGCCACGTAATACTCCAT		
F-DMPK-ex15-104	GCCAACTCACCGCAGTCT	Human <i>DMPK</i> ex 15	MLS252&253; this study
R-DMPK-ex15-203	GGGCACTCAGTCTTCCAACG		
F-GAPDH	ACCAGGGCTGCTTTAACTCTG	Human <i>GAPDH</i> ex 3-ex 4	MLS258&259; this study
R-GAPDH	ATGGAATTTGCCATGGGTGGAA		
F-HPRT1-ex2	TGCTGAGGATTTGGAAAGGGT	Human <i>HPRT1</i> ex 2-ex 3	MLS264&265; this study
R-HPRT1-ex3	GGGCTACAATGTGATGGCCT		
F-Gapdh	AATGCATCTGCACCACCAAC	Murin <i>Gapdh</i> ex 4-ex 5	MLS272&273; this study
R-Gapdh	GTGGCAGTGATGGCATGGAC		
F-Hprt1-ex8	TGTTGTTGGATATGCCCTTGACT	Murin <i>Hprt1</i> ex 8-ex 9	MLS274&275; this study
R-Hprt1-ex9	TGCAGATTCAAATTGCGCTCA		
F-Tbp	CCTTGTACCCTTACCAATGACT	Murin <i>Tbp</i> ex 3-ex 4	MLS276&277; this study
R-Tbp	CAGCCAAGATTACGGTAGATACA		
Deep Sequencing			
R-DMPK bef CTG	GCTACAAGGACCCTTCGAGC	Nested PCR 1 for target sgRNA 4 in couple with F1-DMPK-3UTR	MLS278; this study
F-DMPK-149up-sgRNA23	CTCCTCACTTGCCTGCTCTC	Nested PCR 1 for target sgRNA 23 in couple with R2-DMPK-3UTR	MLS290; this study
F-nested-DMPK-116-sgRNA4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCCGCGCTCCCTGAACCCTAGAACTGCTTCCG	Nested PCR 2 for amplicon with CTG deletion	MLS286&287; this study
R-nested-DMPK-116-sgRNA23	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACAACGCAAACCGCGGACACTGTGGAGTCC		
R-nested-DMPK-113-sgRNA4	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTACAAGGACCCTTCGAGCCCCGTTCCGG	Nested PCR 2 for target sgRNA 4 in couple with F-nested-DMPK-116-sgRNA4	MLS288; this study
F-nested-DMPK-115-sgRNA23	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCTCCGCCCCTTCGGCGGTTTGGATATTTATTGACC	Nested PCR 2 for target sgRNA 23 in couple with R-nested-DMPK-116-sgRNA23	MLS289; this study

Supplementary References

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