OMTN, Volume *27*

Supplemental information

Time-controlled and muscle-specific CRISPR/Cas9-mediated deletion of CTG-repeat expansion in the *DMPK* gene

Beatrice Cardinali, Claudia Provenzano, Mariapaola Izzo, Christine Voellenkle, Jonathan Battistini, Georgios Strimpakos, Elisabetta Golini, Silvia Mandillo, Ferdinando Scavizzi, Marcello Raspa, Alessandra Perfetti, Denisa Baci, Dejan Lazarevic, Jose Manuel Garcia-Manteiga, Geneviève Gourdon, Fabio Martelli, and Germana Falcone

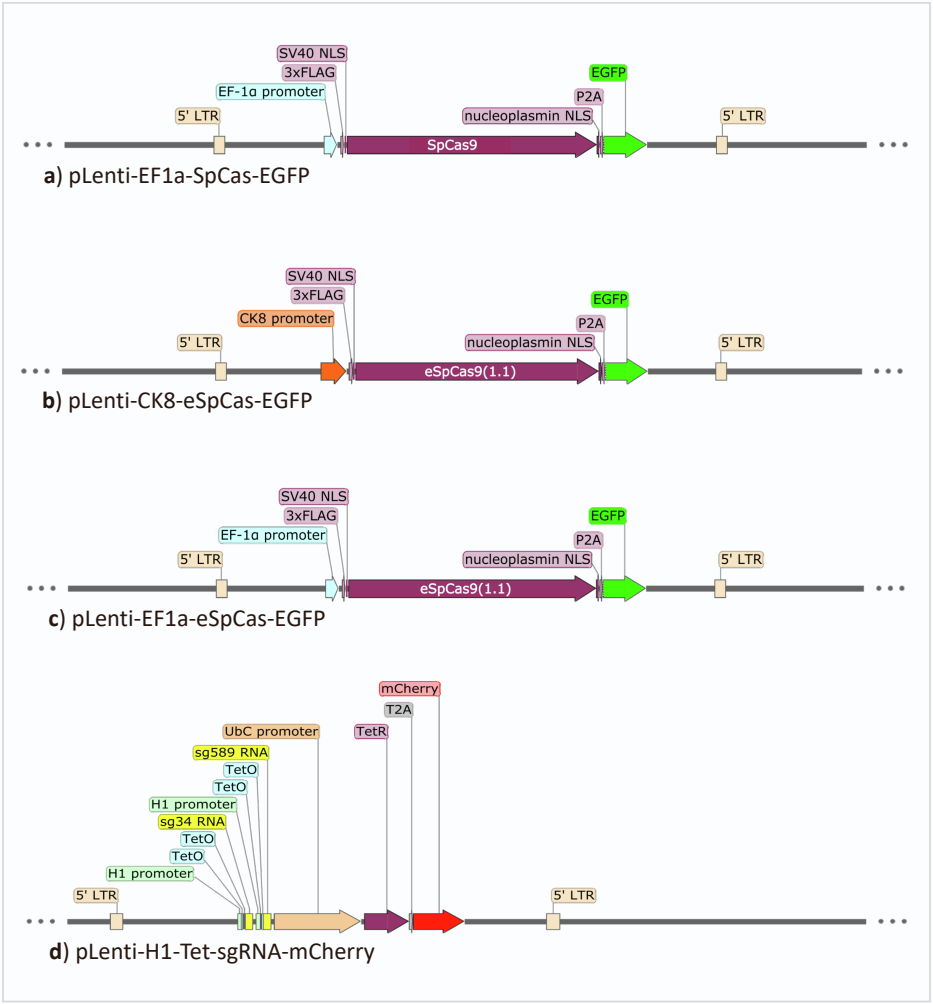
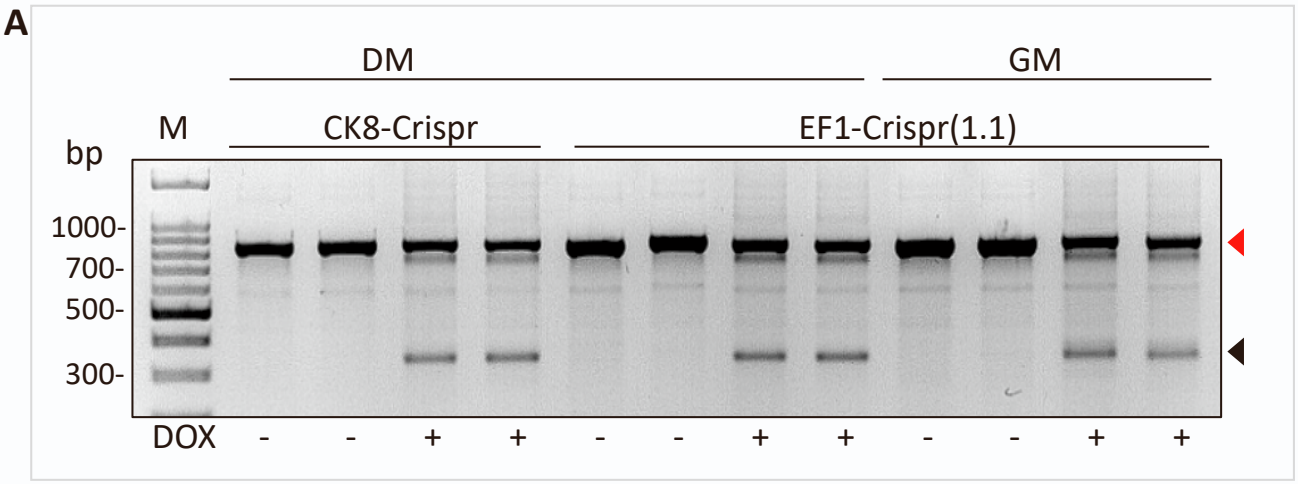
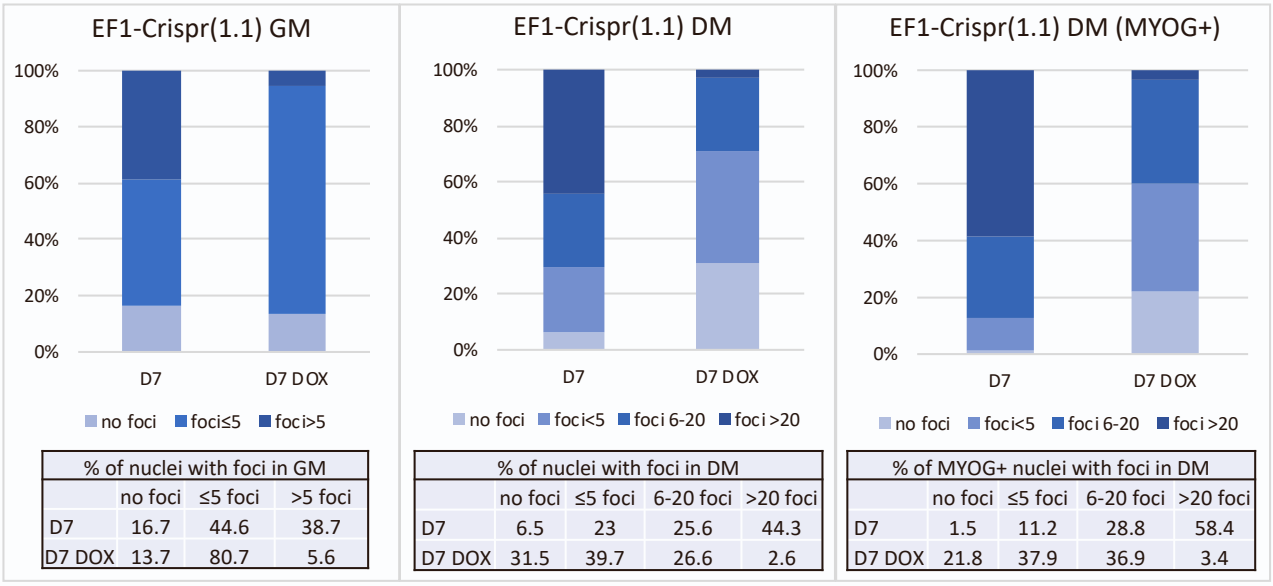


Figure. S1. Schematic representation of lentiviral constructs. LTR, long terminal repeats; EF-1a, elongation factor 1a; SpCas9, Cas9 from *Streptococcus pyogenes (Sp);* eSpCas9(1.1), enhanced specificity Cas9 (1.1) from *Sp*; EGFP, enhanced green fluorescent protein; CK8, creatine kinase 8; TetO, tetracyclin operator; TetR, tetracyclin repressor; UbC, ubiquitin C.

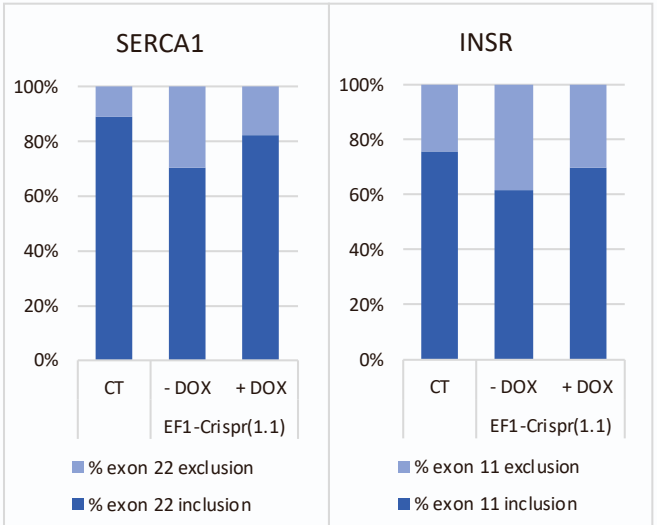




% of nuclei with foci in GM			
	no foci	≤5 foci	>5 foci
D7	16.7	44.6	38.7
D7 DOX	13.7	80.7	5.6

% of nuclei with foci in DM				
	no foci	≤5 foci	6-20 foci	>20 foci
D7	6.5	23	25.6	44.3
D7 DOX	31.5	39.7	26.6	2.6

% of MYOG+ nuclei with foci in DM				
	no foci	≤5 foci	6-20 foci	>20 foci
D7	1.5	11.2	28.8	58.4
D7 DOX	21.8	37.9	36.9	3.4



	% increase of exon inclusion + DOX	P value
SERCA1	12.5 ± 1.1	<0.01
INSR	8.0 ± 1.0	<0.05

Figure S2. DOX-inducible *DMPK* gene editing, foci reduction and splicing improvement in DM1 cells infected with EF1-Crispr(1.1). (A) CK8-Crispr- and EF1-Crispr(1.1)-transduced cells were treated ± DOX for 7 days in growing (GM) and differentiation (DM) medium. Genomic DNA was analysed as described in Figure 2. (B) EF1-Crispr(1.1)-infected cells were treated as described in Figure 3. A representative experiment is shown; at least 300 nuclei were counted for each condition. (C) Quantitative RT-PCR of SERCA1 and INSR in EF1-Crispr(1.1) cells treated as described in Figure 4, using primers specific for each isoform, normalised to the total amount of SERCA1 and INSR transcripts, respectively. The histogram represents the percentage of exon inclusion and exclusion for SERCA1 and INSR transcripts ± DOX, compared to the control cells (CT). For each transcript the percentage of increase of exon inclusion + DOX is shown in the table (mean ± S.E., n=3).

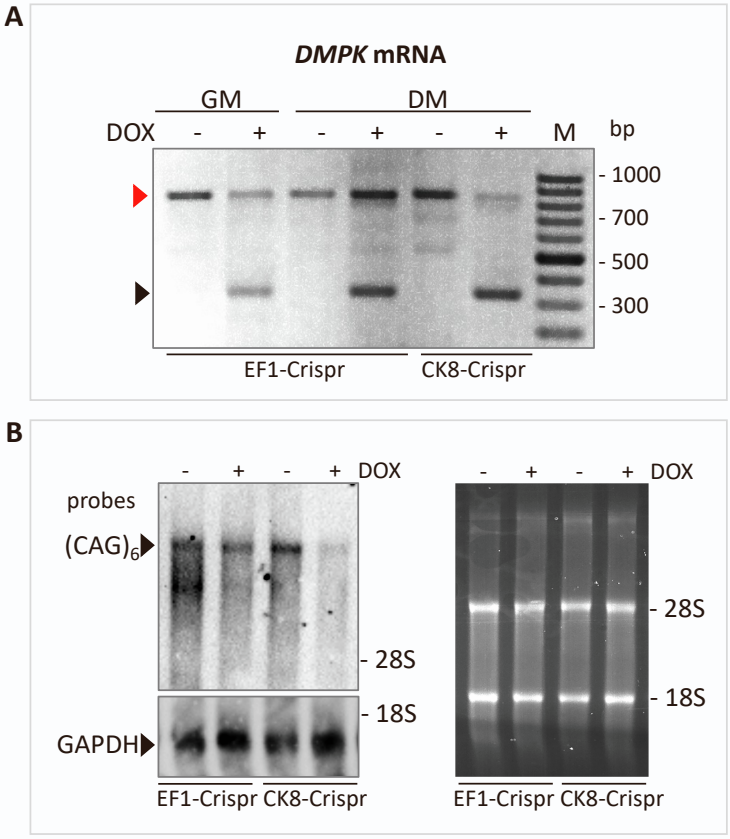


Figure S3. Expression of *DMPK* in DM1-derived proliferating myoblasts and differentiated myotubes following DOX treatment. (A) RT-PCR analysis of *DMPK* mRNA from EF1-Crispr and CK8-Crispr myogenic cells, cultured in growing medium (GM) or induced to differentiate in differentiation medium (DM) for 2 days and then treated with DOX in GM/DM for 7 days. The black triangle indicates the expected CTG-deleted product, amplified using primers binding upstream (DMPK upF) and downstream (DMPK dwR) of CTG expansion; the red triangle indicates undeleted wt transcript. (B) Polyadenylated RNA from EF1-Crispr and CK8-Crispr cells grown in GM ± DOX and shifted in DM for 1 day was analysed by Northern blot and hybridised with the probes indicated on the left. The *DMPK* mutated transcript is shown in the top panel and the *GAPDH* transcript in the bottom panel. In-gel RNA staining of polyadenylated and residual 18S and 28 ribosomal RNAs is shown on the right panel as a proof of RNA integrity.

A

EF1-Crispr_GM	No DeDup		DeDup	
	# reads (x1000)	% modified reads	# reads (x1000)	% modified reads
double cut	99.2	93.7	8.3	93.9
single cut sg34	108.1	56.5	37.6	56.2
single cut sg589	168.6	13.5	55.3	13.7

B

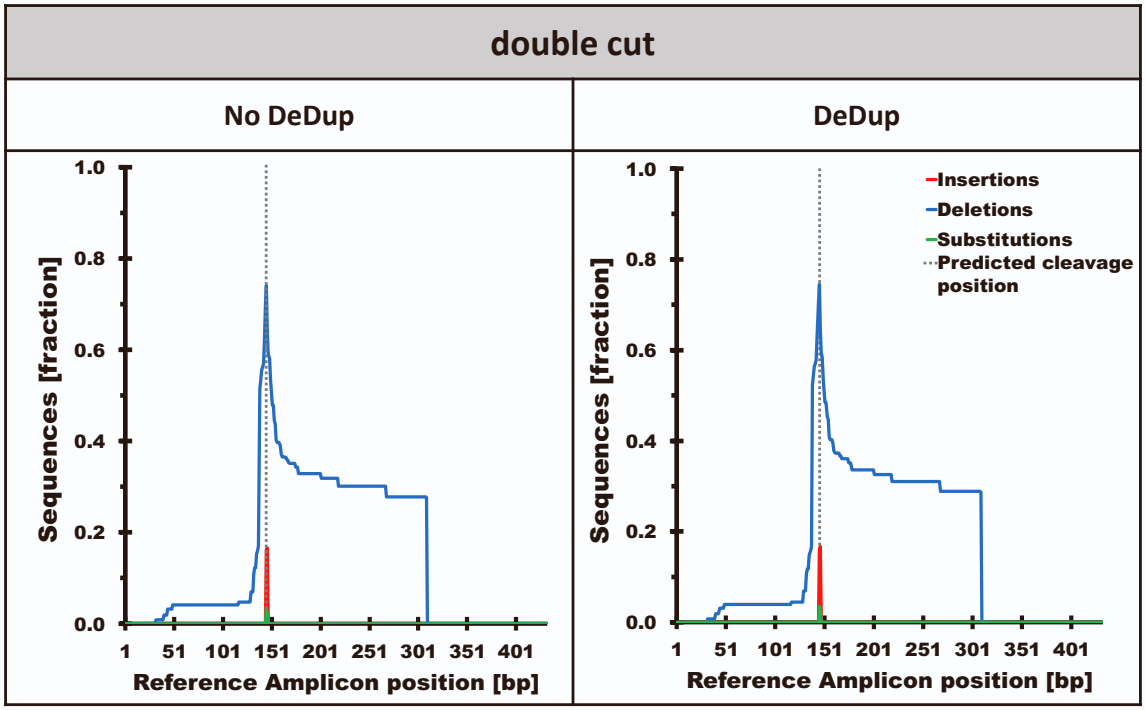


Figure S4. Similar results of on-target analysis before and after deduplication. UMIs were added to each DNA fragment before amplicon production and subsequently exploited in the down-stream analysis to identify and remove read duplicates produced during PCR. Fractions of insertions, deletions and substitutions identified in EF1-Crisp-treated cells in GM are shown before (NoDeDup) and after deduplication (DeDup). (A) Number of reads aligning to the investigated region are shown together with percentage of modified reads before and after deduplication for each possible editing activity. (B) Representative amplicon sequencing analysis before and after deduplication performed for on-target double cut event. Representative single cut on-target analyses before and after deduplication are shown in figure S5.

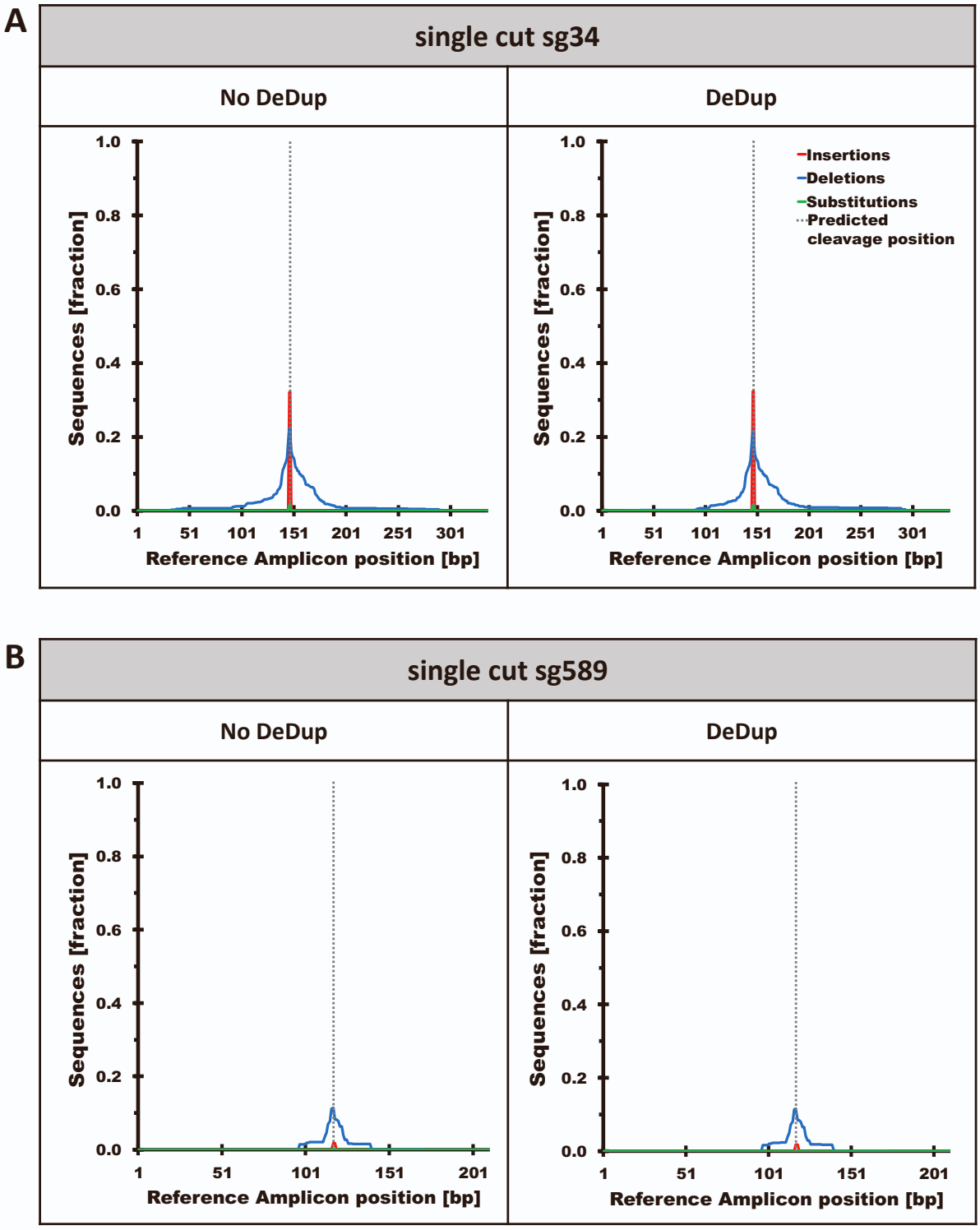


Figure S5. Single cut on-target analysis before and after deduplication. UMIs were added to each DNA fragment before amplicon production and subsequently exploited in the down-stream analysis to identify and remove read duplicates produced during PCR. Fractions of insertions, deletions and substitutions identified in EF1-Crisp-treated cells in GM are shown before (NoDeDup) and after deduplication (DeDup). Comparison was performed for single cuts of either (A) sg34 (single cut sg34) or (B) sg589 (single cut sg589). Representative samples are shown.

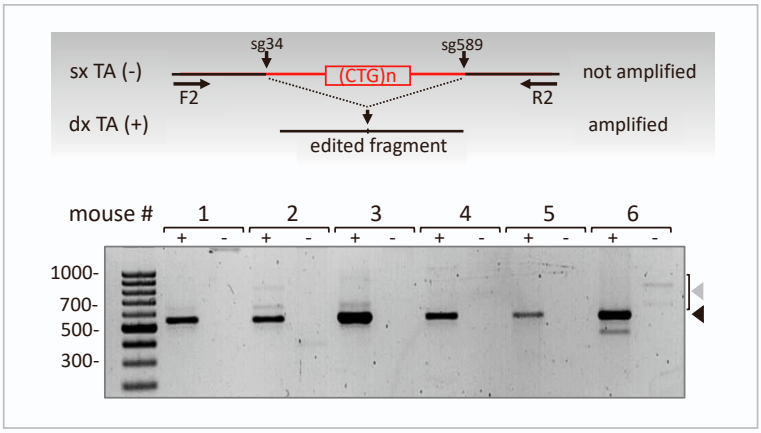


Figure S6. DOX-inducible DMPK gene editing in tibialis anterior muscles of DMSXL homozygous mice. Editing of genomic DNA extracted from TA muscles of DMSXL homozygous mice, injected with the AAV vectors (dx TA, +) or with saline solution (sx TA, -) and fed with DOX diet for two weeks. Primers DMPK F2 (F2) and DMPK R2 (R2) were used for PCR amplification. In the diagram the expected outcomes in AAV-injected or contralateral TA muscles are indicated. The black triangle indicates the expected CTG-deleted products, the gray triangle indicates non-specific PCR products.

Table S1. Sg34 and sg589 potential off-targets identified by at least 2 prediction algorithms, ranked decreasingly by number of intersections (Excel file)

Table S2. Investigation of sequence variations in potential sg34 and sg589 off-targets (Excel file)

Table S3. Primers used for amplicon production for on- and off-target analysis by amplicon deep sequencing (Excel file)

Table S4. Primers used for PCR analysis of genomic DNA and RNA

PCR Primers			
DMPK upF	TGTTCGCCGTTGTTCTGTCTC	DMPK dwR	CAGAGCTTTGGGCAGATGGAG
DMPK F2	GTCCCAGGAGCCAATCAGAGG	DMPK R2	CTAGCTCCTCCCAGACCTTCG
hINSR ex11 F	CCAAAGACAGACTCTCAGAT	hINSR ex11 R	AACATCGCCAAGGGACCTGC
hSERCA1 ex22 F	ATCTTCAAGCTCCGGGCCCT	hSERCA1 ex22	CAGCTCTGCCTGAAGATGTG
qPCR Primers			
Cas9 F	CGGCACAGCATCAAGAAGAA	Cas9 R	TCTTCTGGCGGTTCTCTTCA
DMPK up R	GCATTCCCGGCTACAAGGAC	DMPK dw1 R	CAGTGCATCCAAAACGTG
DMPK ref F	CTGGGTGTATTCGCCTATG	DMPK ref R	CGTGCTCACCTTGTAGTG
GAPDH univ F	CACCATCTTCCAGGAGCGAG	GAPDH univ F	CCTTCTCCATGGTGGTGAAGAC
hrpL23 F	TCCGGATTTCCTTGGGTCTT	hrpL23 R	TGTTCAGCCGTCCCTTGATC
INSR com F	ATCGACTGGTCCCGTATCCT	INSR com R	AGATGGTCGGGCAAACTTTCT
INSR excl 11 F	TTTTCGTCCCCAGGCCATC	INSR excl 11 R	ACCAGCGACTCCTTGTTCAC
INSR incl 11 F	CGAATGCTGCTCCTGTCCAA	INSR incl 11 R	GCCTGAAGAGGTTTTTCTGGG
SERCA com F	GGTGATCCGCCAGCTAATGA	SERCA com R	GGGCACCCTTGACAAACATC
SERCA excl 22 F	GGAACTACCTAGAGGATCCAGAAGA	SERCA excl 22 R	AGCTCTGCCTGAAGATGTGTC
SERCA incl 22 F	CAGTGGCTCATGGTCCTCAA	SERCA incl 22 R	GGGGAACAGTTATCCCTCTAGGT
sg34RNA F	GGCACTCAGTCTTCCAACG	sgRNAcom R	CGACTCGGTGCCACTTTT
sg589RNA F	ATATCCAAACCGCCGAAG	sgRNAcom R	CGACTCGGTGCCACTTTT
ddPCR Primers			
ddPCR.Ref.DMPK F	CTGGGTGTATTCGCCTATG	ddPCR.Ref.DMPK R	CGTGCTCACCTTGTAGTG
dd589F	CTTTCTTGTGCATGACGCCC	dd589R	CTGTAGCCTGTCAGCGAGTC
ddDelF	GCGCTCCCTGAACCCTAGAA	ddDelR	TCCAAAACGTGGATTGGGGT