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Supplemental Information

CRISPR/Cas9-Mediated Deletion of CTG Expansions Recovers Normal Phenotype in Myogenic Cells Derived from Myotonic Dystrophy 1 Patients

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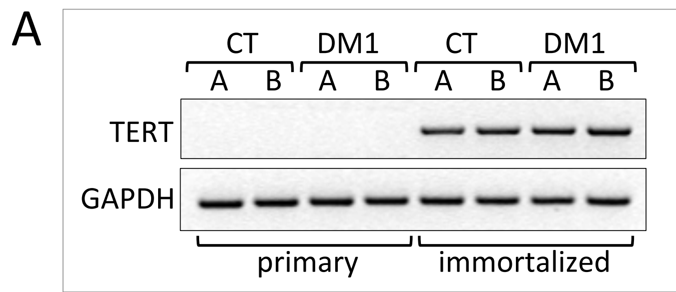


Figure S1. Immortalization and MYOD1-dependent differentiation of human DM1 and Control fibroblasts. (**A**) Semiquantitative RT-PCR analysis of *TERT* (TERT) and *GAPDH* (GAPDH) transcripts in primary fibroblasts derived from control (CT, A and B) and DM1 (DM1, A and B) patients before (primary) and after transduction with *TERT*-expressing retrovirus (immortalized). (**B**) Immunofluorescence analysis of control (CT-A and CT-B) and DM1 patient-derived cell lines (DM1-A and DM1-B) allowed to differentiate for 5 days and stained with antibodies anti-mouse MYOD1 and anti-myosin (MHC), and Hoechst dye (scale bar 20 μm). Staining of MYOD1-ER alone or merged with MHC is shown in the absence and in the presence of β-estradiol. Note change of MYOD1-ER from cytoplasmic to nuclear localization upon hormone induction.

A

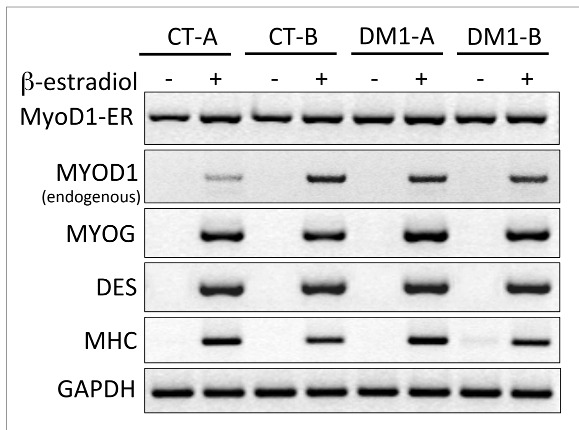
CT-A CT-B DM1-A DM1-B

- β-estradiol

+ β-estradiol

	Fusion index (%)	MHC⁺ cells (%)
CT-A	32 ± 5	61 ± 6
CT-B	39 ± 11	72 ± 5
DM1-A	44 ± 5	59 ± 5
DM1-B	28 ± 8	57 ± 3

B



C

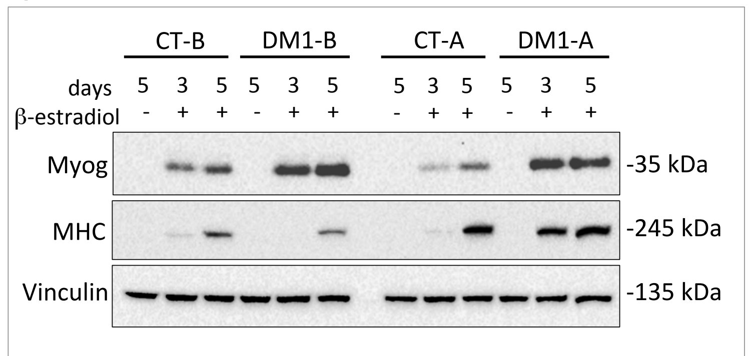


Figure S2. Differentiation of immortalized human DM1 and Control myogenic cells. Immortalized myogenic cells derived from DM1 patients and control individuals were cultured in differentiation medium with or without β-estradiol addition. (**A**) Immunofluorescence analysis of control (CT-A and CT-B) and DM1 patient-derived cell lines (DM1-A and DM1-B) allowed to differentiate for 5 days and stained with anti-MHC antibody and Hoechst dye (scale bar 50 µm). The fusion index and the percentage of MHC positive cells is shown in the table below (average ± standard error, n ≥ 3). The fusion index is calculated as the percentage of nuclei in MHC positive myotubes (containing ≥ 2 nuclei) over the total number of cells. At least 300 cell nuclei were counted for each experiment. (**B**) Semiquantitative RT-PCR analysis of *MyoD1-ER* (MyoD1-ER) and muscle specific endogenous *MYOD1* (MYOD1), *MYOG* (MYOG), *DESMIN* (DES) and *MYHC* (MHC) mRNAs in control and DM1 cell lines following induction to differentiation for 5 days. *GAPDH* transcript (GAPDH) was analyzed as control. (**C**) Western blot analysis of the muscle specific proteins myogenin (Myog) and myosin (MHC), and constitutively expressed vinculin (Vinculin) in control and DM1 cell lines without or with β-estradiol addition for 3 and 5 days.

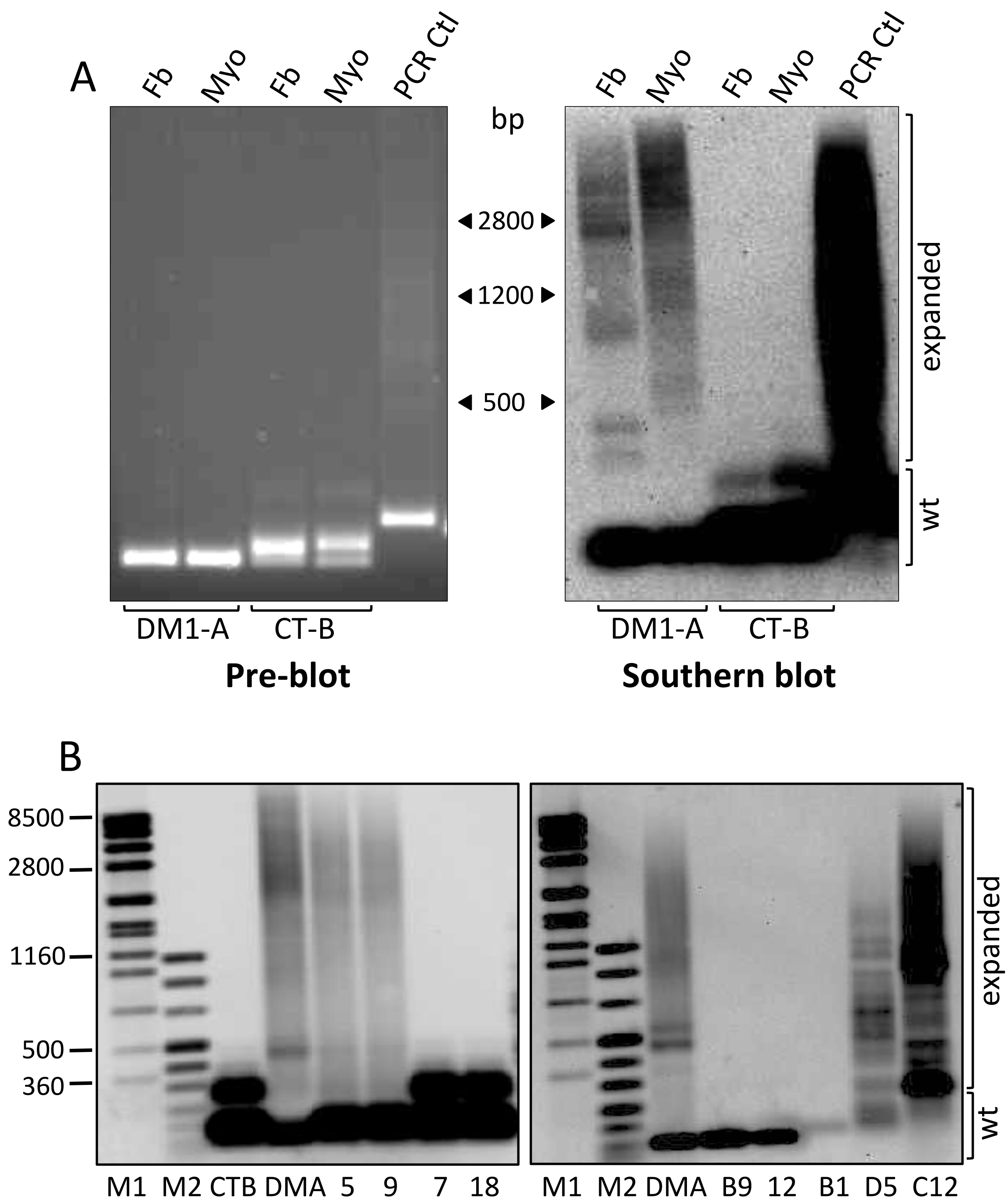
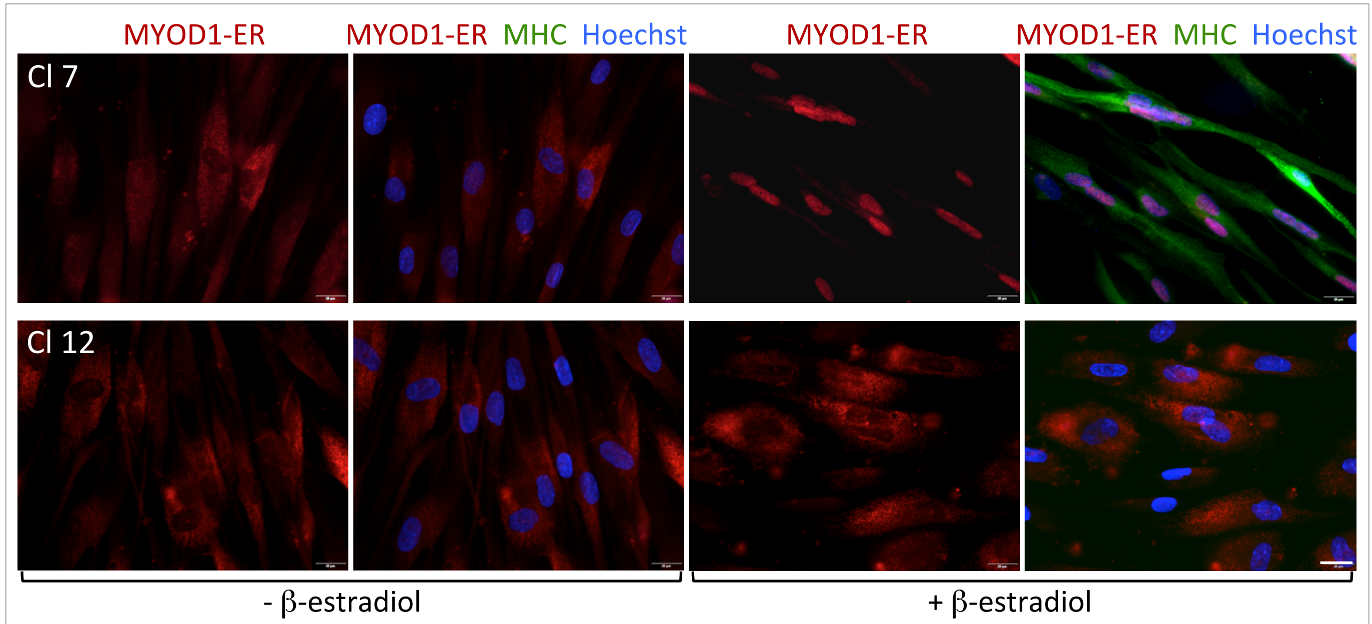


Figure S3. Analysis of CTG repeats in DM1-A and CT-B cells and in CRISPR/Cas9-edited clones. (**A**) Genomic DNAs from DM1-A and CT-B primary fibroblasts (Fb) and immortalized myogenic cells (Myo) were analyzed by long-PCR of CTG expansions followed by Southern blot with a 5'DIG-labeled $(CTG)_{10}$ probe. PCR positive controls were loaded on the gel (PCR Ctl). Note that only bands corresponding to amplification of the wt alleles are visible on the gel before blotting (pre-blot). (**B**) Genomic DNAs from CT-B and DM1-A cells and CRISPR/Cas9-treated clones (5, 9, 7, 18, B9, 12, B1, D5 and C12) were analyzed as described in **A**. Bands corresponding to wt alleles and to alleles with expansions of multiple sizes can be visualized. Molecular weight markers are indicated: 500 bp correspond to about 120 triplets, 1200 to about 340 triplets and 2800 to about 890 triplets.



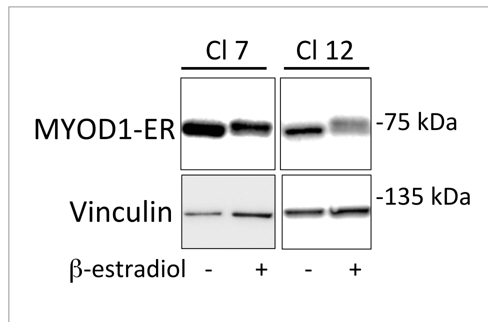


Figure S4. Defective MYOD1-ER nuclear translocation impairs differentiation of CRISPR/Cas9-edited clone 12. (**A**) Immunofluorescence analysis of CRISPR/Cas9-edited clone 7 and clone 12 allowed to differentiate for 5 days and stained with antibodies to mouse MYOD1 and to myosin (MHC), and Hoechst dye (scale bar 20 μm). Staining of MYOD1-ER alone or merged with MHC is shown in the absence and in the presence of β-estradiol. Note that translocation of MYOD1-ER from cytoplasm to nucleus does not occur in clone 12 upon hormone induction. (**B**) Western blot analysis of MYOD1-ER and constitutively expressed vinculin (Vinculin) in clone 7 and clone 12 without or with β-estradiol addition for 5 days.

Table S1. Potential genomic off-target sites for sgRNAs 34 and 589

GUIDE	TARGET	SEQUENCE	PAM	SCORE	CHR	Strand	GENE
sg34	**ON-Target**	GGGCACTCAGTCTTCCAACG	GGG	**0**	**19**	**+**	**DMPK**
	sg34_OT1	GGGC^CTGTGTCTTCCAACG	GGG	1.32	2	+	-
	sg34_OT2	TGGGACTCAGT^TTCCAACG	TGG	1.6	2	+	TNS1
	sg34_OT3	GGGCATTCAGACTT^CAACG	AGG	3.32	2	-	None
	sg34_OT4	GGACACTCAGTCTTCC^ACG	GGG	3.66	14	+	LOC105370681
	sg34_OT5	GGACACTCAGGCTTCC^ACG	TGG	4.36	16	-	LOC105371392
	sg34_OT6	AGGCATTCAGTCTTCCAA^G	TGG	5.84	1	+	SLC44A3-AS1
	sg34_OT7	GGGCGCTCAG^CTTCCAAGG	TGG	6.4	5	+	ZDHHC11
sg589	**ON-Target**	AAATATCCAAACCGCCGAAG	CGG	**0**	**19**	**+**	**DMPK**
	sg589_OT1	GAATATCAAAACCACCGAAG	GGG	1.69	20	+	None
	sg589_OT2	AAAT^TCCAAACAGCCGAAG	TGG	1.8	2	-	CNTNAP5
	sg589_OT3	AAATAACCAAACC^CAGAAG	AGG	4.32	3	-	TF
	sg589_OT4	AAATATCCATACTGCCCAAG	GGG	4.6	1	+	None
	sg589_OT5	AAATATCCAAACCTCC^AAG	GGG	4.81	16	+	CDH8
	sg589_OT6	AAGTATCCAAACCCCCGATG	TGG	6.45	1	-	IFI16
	sg589_OT7	AAATATCCAAACTGCCAGCAG	TGG	8.8	5	-	LINC00992

Table S2. List of primers used for PCR and cloning

RT- PCR Primers		
Name	**Forward (F)**	**Reverse (R)**
hGAPDH	AAACCTTCCTCAGCTATGCCC	TGACGCGCAGGAAAAATGTG
hMYOD1	AGCACTACAGCGGCGACTCC	GCGACTCAGAAGGCACGTCC
hMYOG	CCCTGAAGAGAAGCACCCTG	CAGATGATCCCCTGGGTTGG
hDESM	GAGGACCGATTTGCCAGTGA	GATGGGGAGATTGATCCGGC
hMYH1	TCCAAAGCCAAGGGAAACCT	CCCCTCGAGAGCTGTGAAAC
mMyoD	CCGTGTTTCGACTCACCAGA	CATTCACTTTGCTCAGGCGG
hINSR ex11	CCAAAGACAGACTCTCAGAT	AACATCGCCAAGGGACCTGC
hSERCA1 ex22	ATCTTCAAGCTCCGGGCCCT	CAGCTCTGCCTGAAGATGTG
Off targets PCR Primers		
Name	**Forward (F)**	**Reverse (R)**
sg34_OT1	TCATAGCTTGAATCATACCATCCAG	TCTGAGACGCACTTTTAACGC
sg34_OT2	TTGTGGTGGTCCCAAGCAAT	CCTGGGGAGGTCTAAGGACA
sg34_OT3	ATTCTGGACAACGTCCACCC	GGATTCAAGCCTCTGGGGAC
sg34_OT4	ACAGATGCCAGGATGTGTGG	GCGTGGCACATAGCAACTTC
sg34_OT5	TCTGTCCCGAGGGATGGATT	CTCCTCCCTCACACCTCTCA
sg34_OT6	AGTTCCTTCCCTTGTGCTCT	AGAGCAGCCAGAGATCCTCA
sg34_OT7	CTCAAACACACCTGCACACC	TGAAGGTGCATGTATGGGGG
sg589_OT1	CCCCTCCAGTCTCCTCAACT	GGGGAAATGGAGACCAGGTG
sg589_OT2	AGATCCCAACACTTTGACACA	TCCAACTCATGAAAACGGAGA
sg589_OT3	ACAAAGCAAGCAGTAGGTTAGC	CAAACGAGAGCTTTGCCCATTG
sg589_OT4	AGCCTACGATGAGATCACTGA	ATGTGTGGATTTATGTCTGGGT
sg589_OT5	AATAAGTGTATGCAGCCCATGC	TTCAGAGCAGAAGTTCCTGGAAAG
sg589_OT6	TTCCTGAATAATAAATCCCCCAGT	ACTTTCCCACCCCTGTGTTG
sg589_OT7	ACCACGTTCTTACAGATCAAACA	GGGGAAAGTGCTCTGTGGTA
DMPK PCR Primers		
Name	**Forward (F)**	**Reverse (R)**
up	TGTTCGCCGTTGTTCTGTCTC	GCATTCCCGGCTACAAGGAC
dw	GGATCACAGACCATTTCTTTCTTTC	CAGAGCTTTGGGCAGATGGAG
in	AACGGGGCTCGAAGGGTCCTTGTAGC	CTTCCCAGGCCTGCAGTTTGCCCATC
DNA oligonucleotides for cloning of sgRNAs		
Name	**Forward (F)**	**Reverse (R)**
sgRNA 34	Phosphate_GGGCACTCAGTCTTCCAACGGTTTC AGAGCTATGCTGGAAACAGCATAGCAAGTTG AAATAAGGCTAGTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGCTTTTTC	Phosphate_TCGAGAAAAAGCACCGACTCGGTGCCAC TTTTTCAAGTTGATAACGGACTAGCCTTATTTCAAC TTGCTATGCTGTTTCCAGCATAGCTCTGAAACCGTT GGAAGACTGAGTGCCC
sgRNA 101	Phosphate_GCGGAGACCCACGCTCGGAGGTTTC AGAGCTATGCTGGAAACAGCATAGCAAGTTG AAATAAGGCTAGTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGCTTTTTC	Phosphate_TCGAGAAAAAGCACCGACTCGGTGCCAC TTTTTCAAGTTGATAACGGACTAGCCTTATTTCAAC TTGCTATGCTGTTTCCAGCATAGCTCTGAAACCTCC GAGCGTGGGTCTCCGC
sgRNA 384	Phosphate_GGTGCGTGGAGGATGGAACAGTTTC AGAGCTATGCTGGAAACAGCATAGCAAGTTG AAATAAGGCTAGTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGCTTTTTC	Phosphate_TCGAGAAAAAGCACCGACTCGGTGCCAC TTTTTCAAGTTGATAACGGACTAGCCTTATTTCAAC TTGCTATGCTGTTTCCAGCATAGCTCTGAAACTGTT CCATCCTCCACGCACC
sgRNA 589	Phosphate_AAATATCCAAACCGCCGAAGGTTTC AGAGCTATGCTGGAAACAGCATAGCAAGTTG AAATAAGGCTAGTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGCTTTTTC	Phosphate_TCGAGAAAAAGCACCGACTCGGTGCCAC TTTTTCAAGTTGATAACGGACTAGCCTTATTTCAAC TTGCTATGCTGTTTCCAGCATAGCTCTGAAACCTTC GGCGGTTTGGATATTT