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Supplemental information

MATR3 is an endogenous inhibitor of DUX4 in FSHD muscular dystrophy

Valeria Runfola, Roberto Giambruno, Claudia Caronni, Maria Pannese, Annapaola Andolfo, and Davide Gabellini

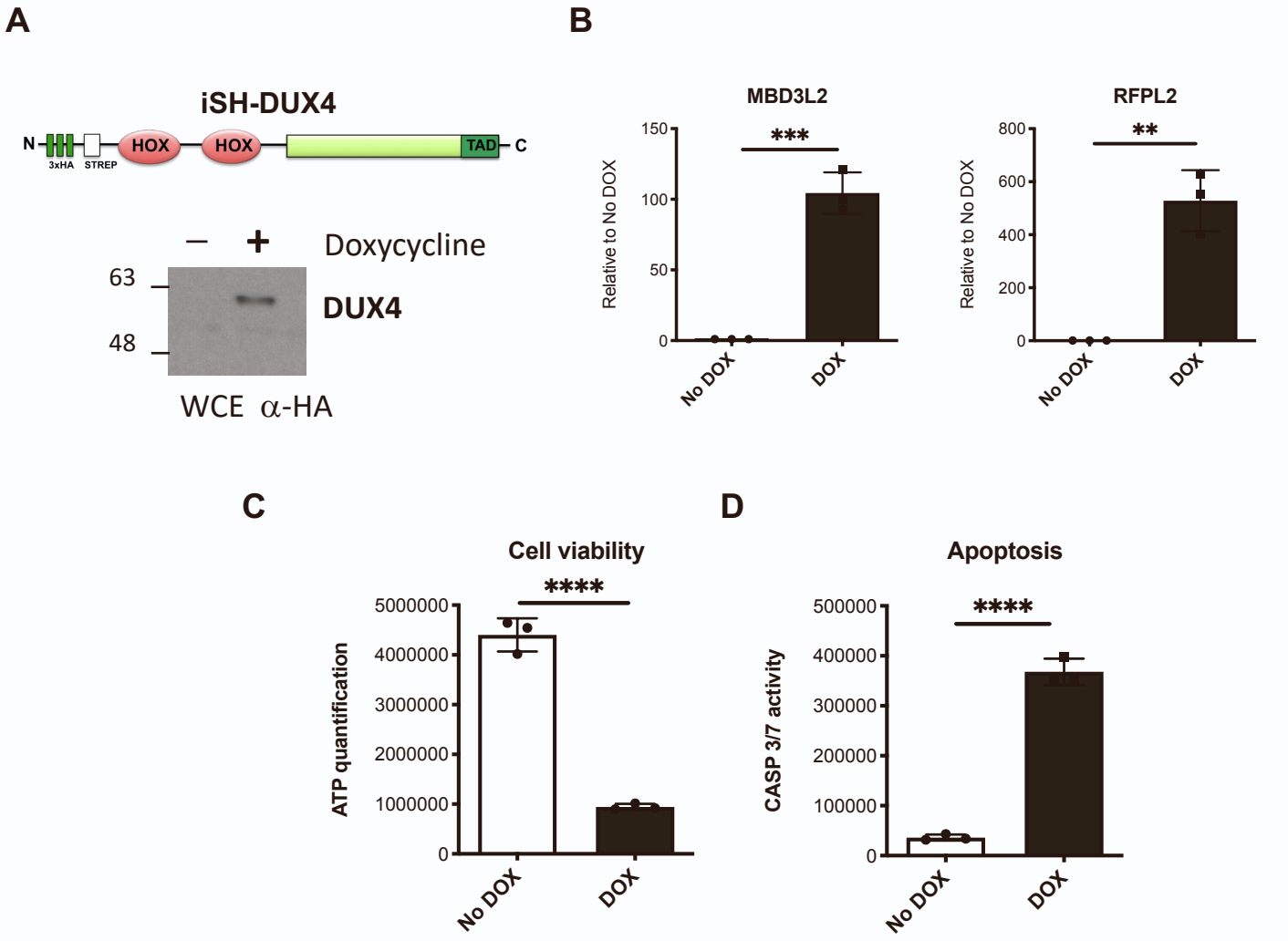


Fig. S1. Characterization of iSH-DUX4 HEK293 cells. (Related to Figure 1). **A.** Top: scheme of the inducible DUX4 protein expressed by iSH-DUX4 HEK293 cells, with 3x HA and 1x STREP N-terminal tags, fused to *DUX4* ORF, composed of 2 homeoboxes (HOX) at the N-terminus and a transactivation domain (TAD) at the C-terminus. Bottom: Immunoblotting with anti-HA antibody performed on whole-cell extracts (WCE) from iSH-DUX4 cells after 4 h of doxycycline administration. **B.** RT-qPCR showing the expression of the indicated DUX4 target genes, performed in HEK293 iSH-DUX4 cells, after 8 h of doxycycline administration. Values are expressed relative to non-induced cells (No DOX) (unpaired Student's t test, **p<0.01; ***p<0.001, n=3). **C.** Cell viability assay performed in HEK293 iSH-DUX4 cells after 24 h of doxycycline administration (unpaired Student's t test, ****p<0.0001, n=3). **D.** Caspase 3/7 activity assay performed in HEK293 iSH-DUX4 cells after 24 h of doxycycline administration (unpaired Student's t test, ****p<0.0001, n=3).

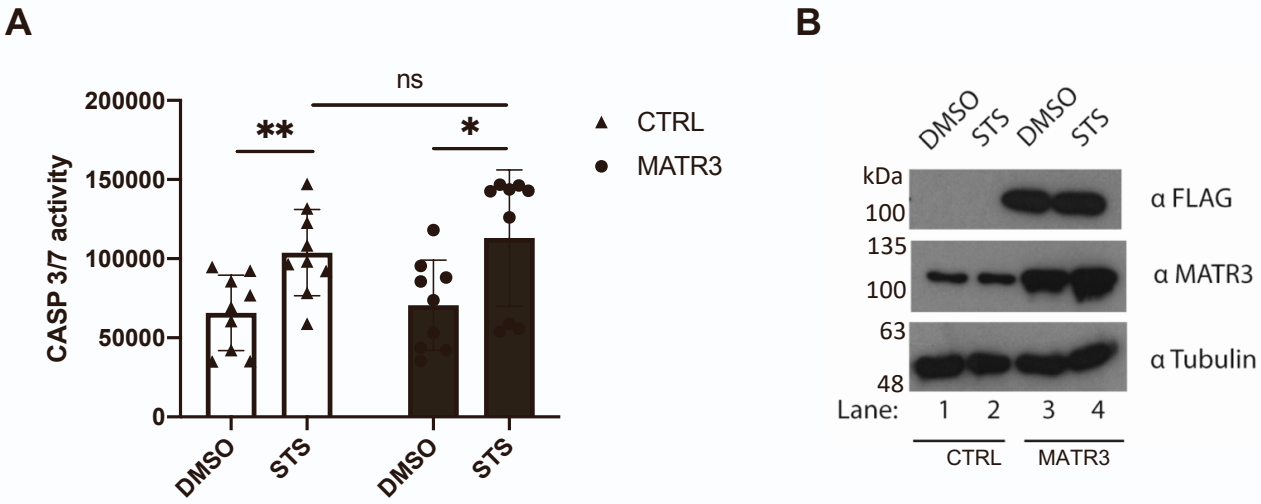
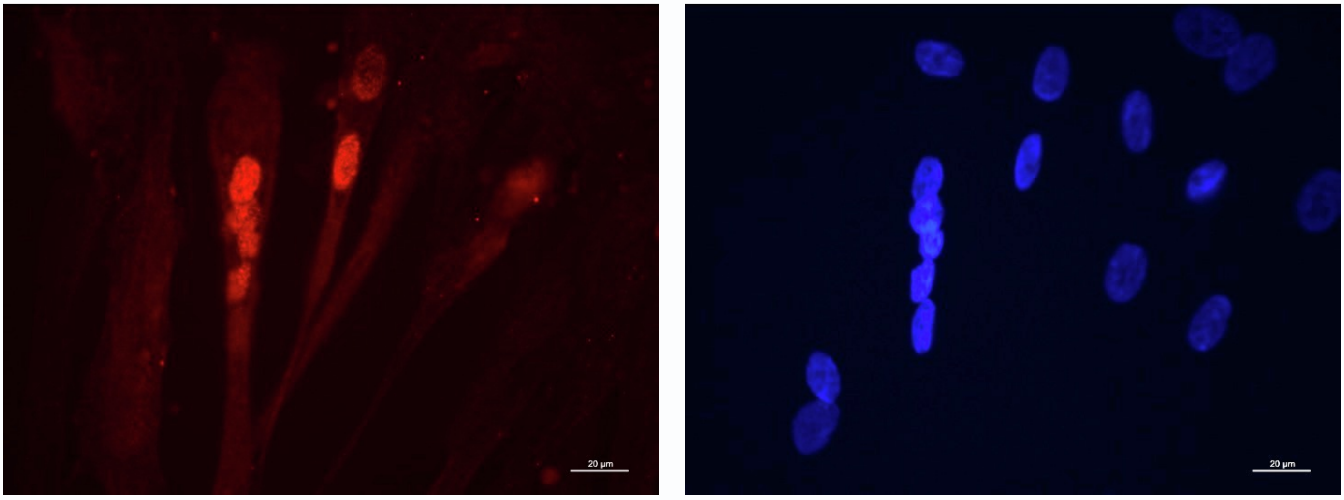


Fig. S2. MATR3 does not protect from Staurosporine-induced apoptosis. (Related to Figure 1). **A.** Caspase 3/7 activity assay performed in HEK293 cells transfected with empty vector (CTRL) or MATR3, and treated 24 h post-transfection with Staurosporine or DMSO (as negative control) for 6 h (unpaired Student's t test, *p<0.05, **p<0.01, n=9). **B**. Immunoblotting with anti-FLAG (recognizing transfected MATR3), anti-MATR3 (recognizing endogenous as well as transfected MATR3) and anti-tubulin (as loading control) on total proteins extracts from HEK293 cells transfected with empty vector (CTRL, lanes 1 and 2) or MATR3 (lanes 3 and 4) and treated with DMSO (lanes 1 and 3) or Staurosporine (lanes 2 and 4).



α-DUX4 Hoechst

Fig. S3. Endogenous DUX4 is expressed only in a fraction of FSHD myonuclei. (Related to Figure 2). Representative immunofluorescence of DUX4 (red, left) performed with anti-DUX4 E5-5 antibody in differentiated primary FSHD muscle cells. Hoechst 33342 was used to stain nuclei (blue, right). Scalebar: 20 µm

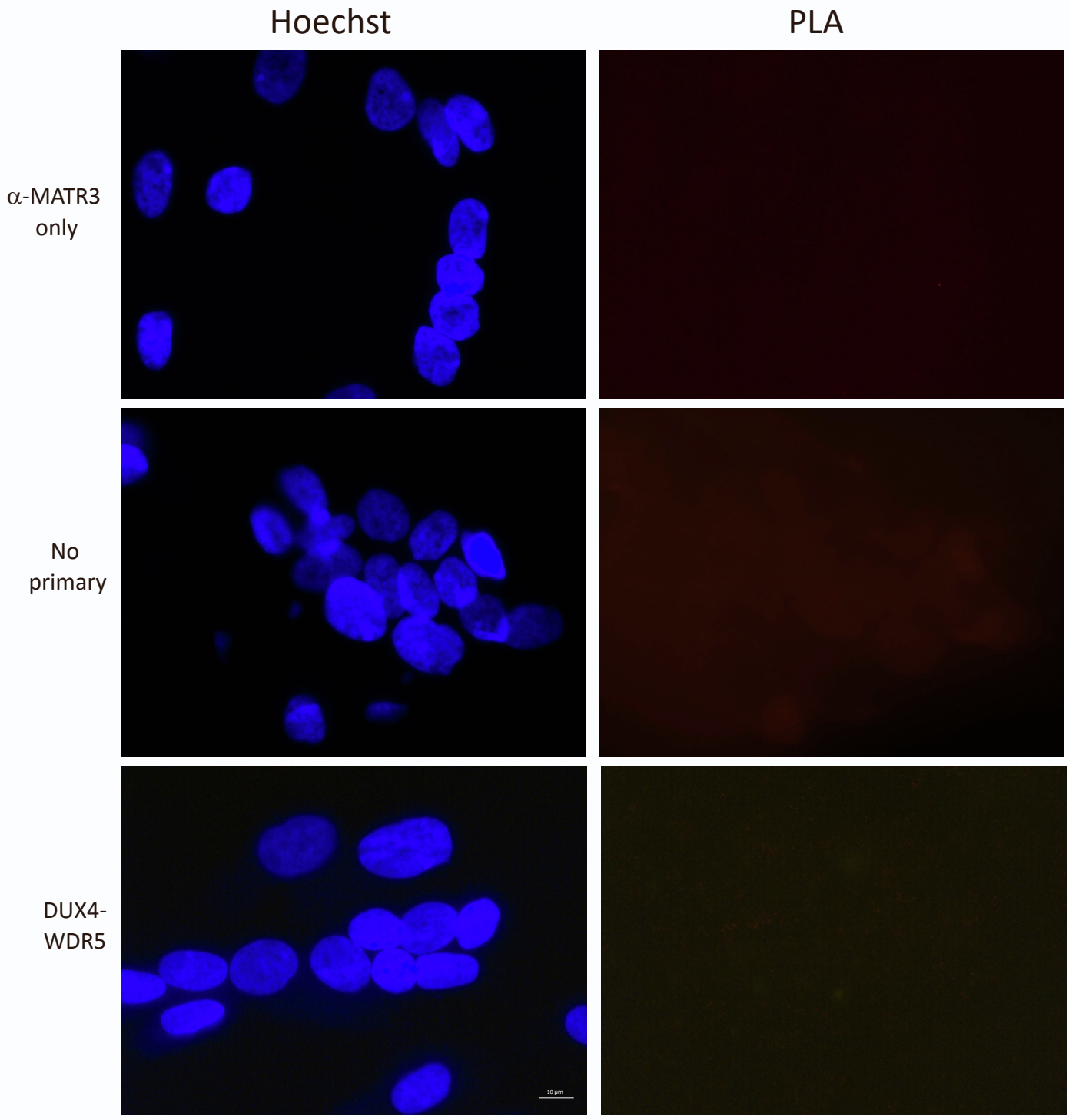


Fig. S4. PLA signal is specific for the DUX4-MATR3 interaction. (Related to Figure 2). Representative pictures of proximity ligation assay (PLA) performed in terminally differentiated primary FSHD muscle cells with only one primary antibody (anti-MATR3, top), without any primary antibody (middle) or with primary antibodies against DUX4 and WDR5, a protein that does not interact with DUX4 (bottom), as negative controls, to assess the specificity of the interaction between endogenous MATR3 and DUX4 shown in Fig. 2B. DAPI was used to stain nuclei (blue, left). Scalebar: 10 µm.

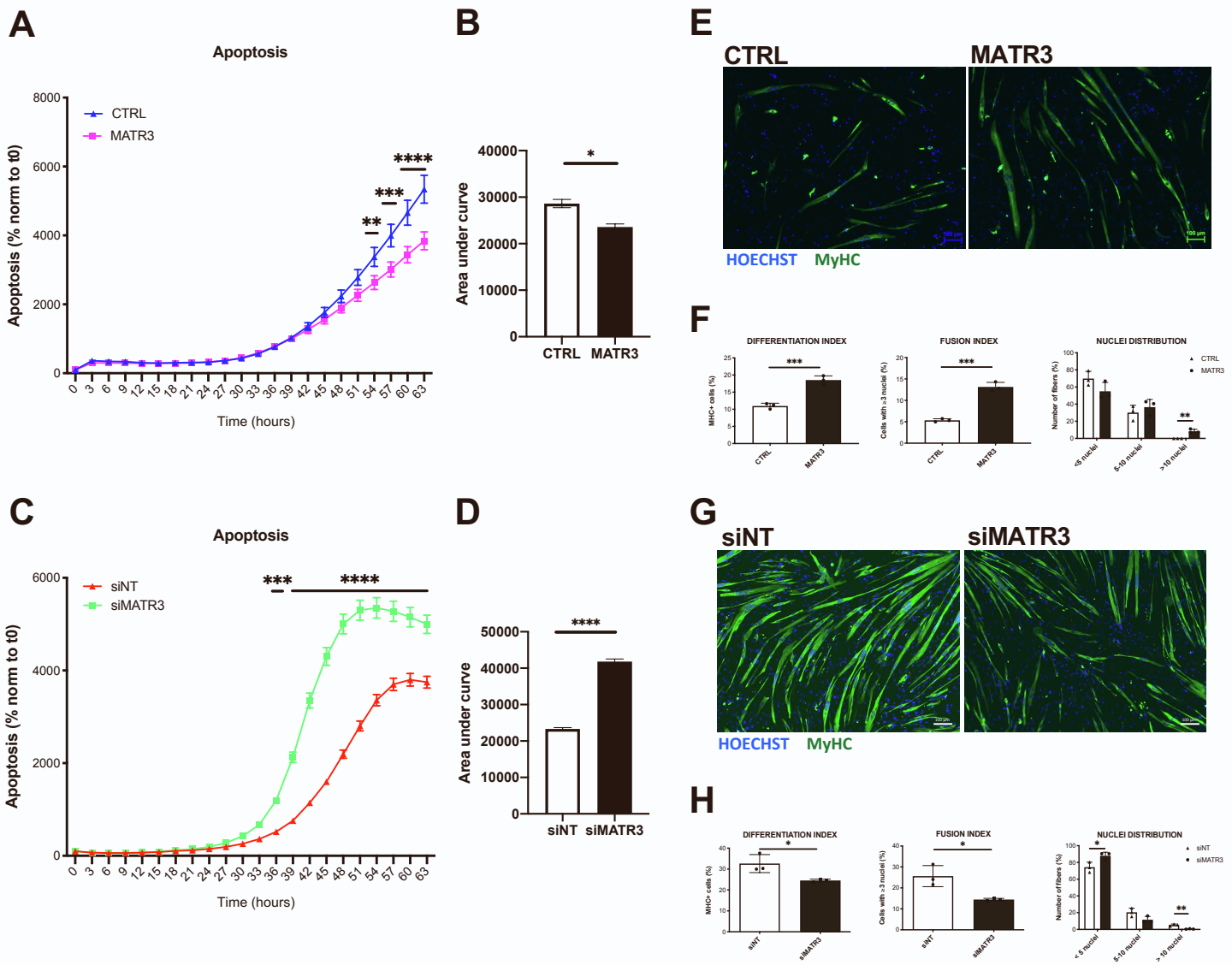


Fig. S5. MATR3 overexpression rescues viability and myogenic differentiation of FSHD muscle cells. (Related to Figure 3). **A.** Apoptotic assays on primary FSHD muscle cells (from a different patient than the one used in Fig. 3A) transduced with control (CTRL) or *MATR3* lentiviruses. Data are reported as percentage (%) of green-fluorescent apoptotic cells normalized to time 0 (Two-Way ANOVA, **p<0.01, ***p<0.001, ****p<0.0001 n=3). **B.** Quantification of the area under the curve in A (unpaired Student's t test, *p<0.05). **C.** Apoptotic assays on primary FSHD muscle cells (from a different patient than the one used in Fig. 3C) transfected with control (si*NT*) or *MATR3* (si*MATR3*) siRNAs. Acquisition, quantification and data representation like in A. (Two-Way ANOVA, ***p<0.001, ****p<0.0001, n=3). **D.** Quantification of the area under the curve in C (unpaired Student's t test, ****p<0.0001). **E.** Representative images of Myosin Heavy-chain (MyHC, green) and Nuclei (Hoechst 33342, blue) staining performed on differentiated primary FSHD muscle cells (from a different patient than the one used in Fig. 3E) transduced with control (CTRL) or *MATR3* lentiviruses. Scalebar: 100 µm. **F.** Quantification of differentiation index, fusion index, and nuclei distribution in primary FSHD muscle cells treated as in E (unpaired Student's t test, **p<0.01, ***p≤0.001, n=3). **G.** Representative images of Myosin Heavy-chain (MyHC, green) and Nuclei (Hoechst 33342, blue) staining performed on differentiated primary FSHD muscle cells (from a different patient than the one used in Fig. 3G) transfected with control (si*NT*) or *MATR3* (si*MATR3*) siRNAs. Scalebar: 100 µm. **H.** Quantification of differentiation index, fusion index, and nuclei distribution in primary FSHD muscle cells treated as in G (unpaired Student's t test, *p<0.05, **p<0.01, n=3).

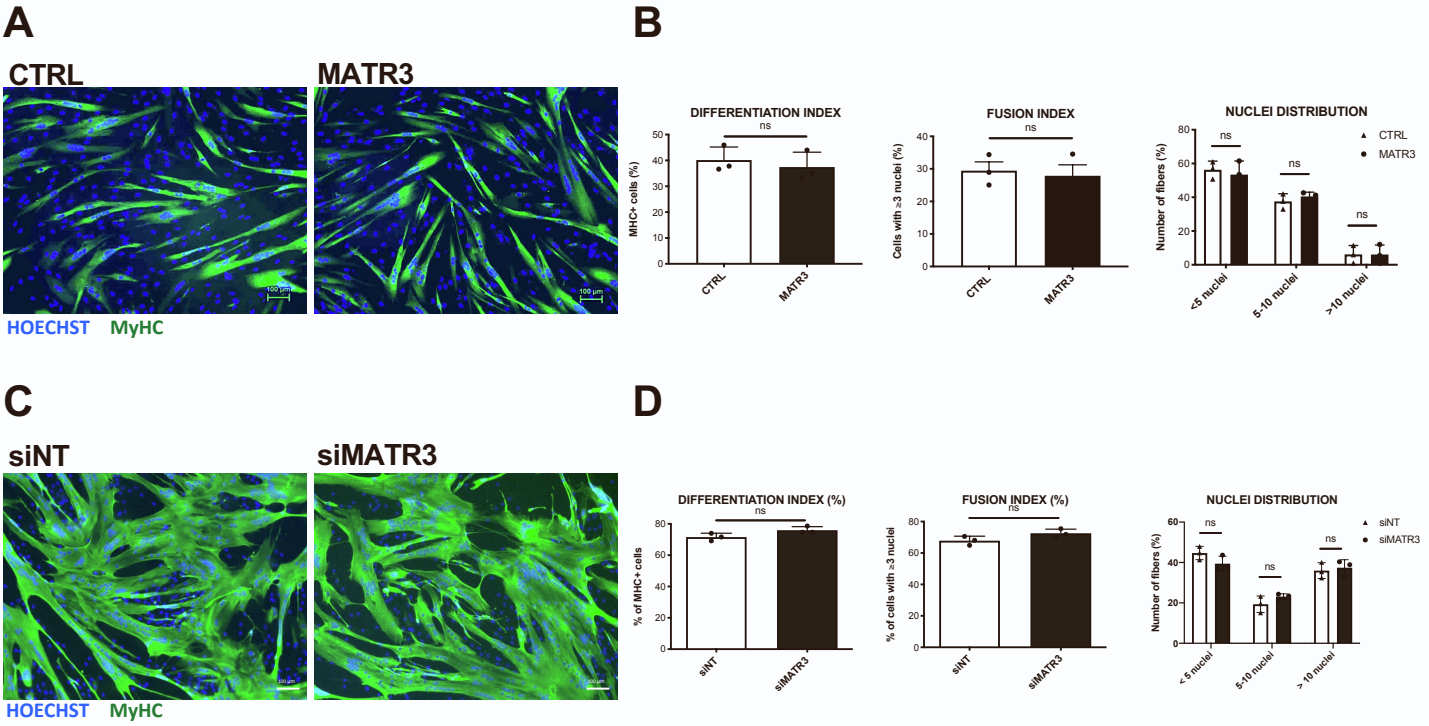


Fig. S6. **MATR3 manipulation does not affect differentiation of healthy primary muscle cells.** (Related to Figure 3). **A.** Representative images of Myosin Heavy-chain (MyHC, green) and Nuclei (Hoechst 33342, blue) staining performed on differentiated healthy primary muscle cells transduced with control (CTRL) or *MATR3* lentiviruses. Scalebar: 100 µm. **B.** Quantification of differentiation index, fusion index, and nuclei distribution in healthy primary muscle cells treated as in A (unpaired Student's t test, n=3). **C.** Representative images of Myosin Heavy-chain (MyHC, green) and Nuclei (Hoechst 33342, blue) staining performed on differentiated healthy primary muscle cells transfected with control (si*NT*) or *MATR3* (si*MATR3*) siRNAs. Scalebar: 100 µm **D.** Quantification of differentiation index, fusion index, and nuclei distribution in primary healthy muscle cells treated as in C (unpaired Student's t test, n=3).

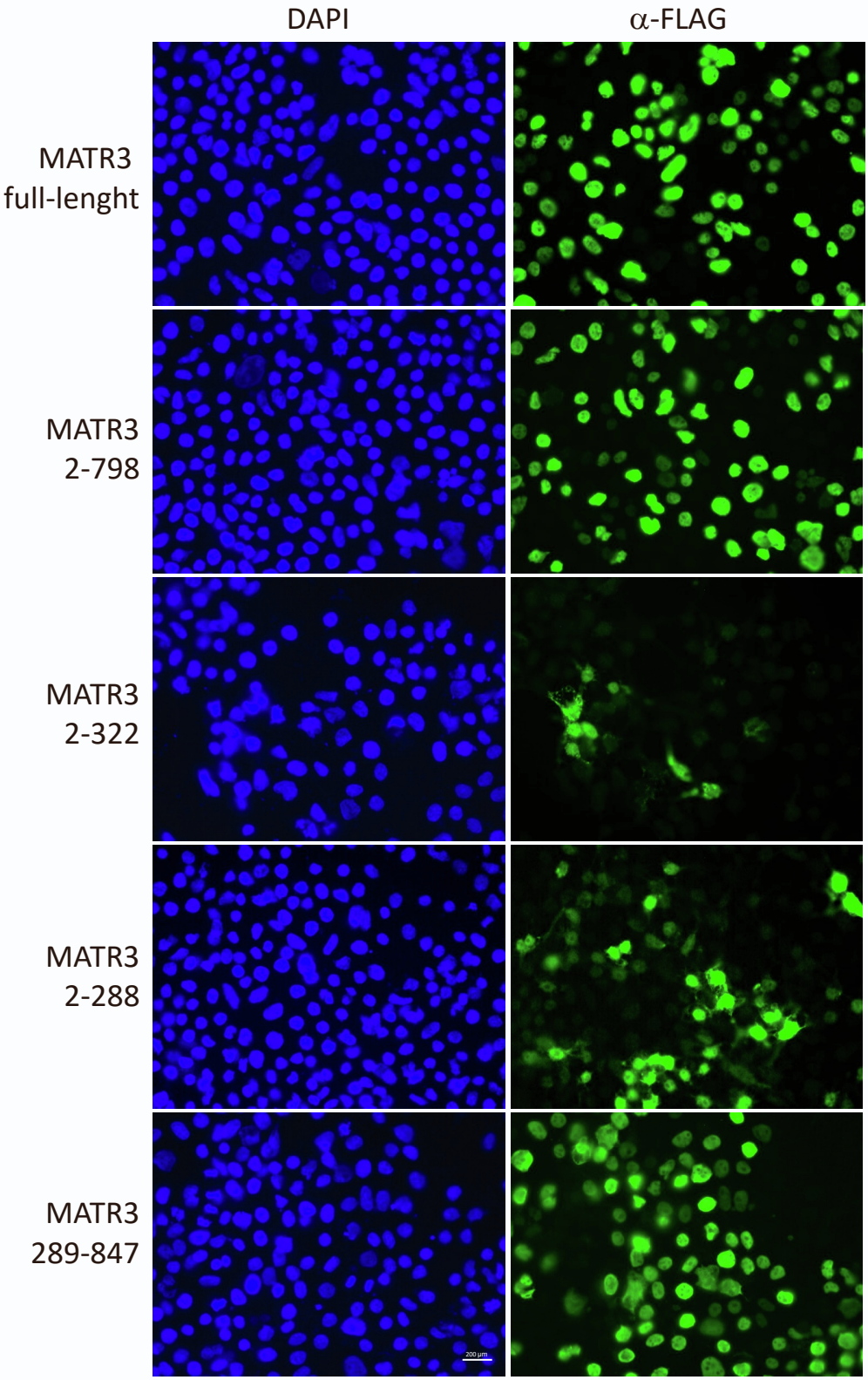


Fig. S7. **MATR3 deletion mutants maintain the nuclear localization.** (Related to Figure 4). Representative immunofluorescence of FLAG-tagged MATR3 mutants (green, right) performed with anti-FLAG antibody in HEK293 cells transfected with the MATR3 constructs indicated on the left. DAPI was used to stain nuclei (blue, left). Scalebar: 200 μm

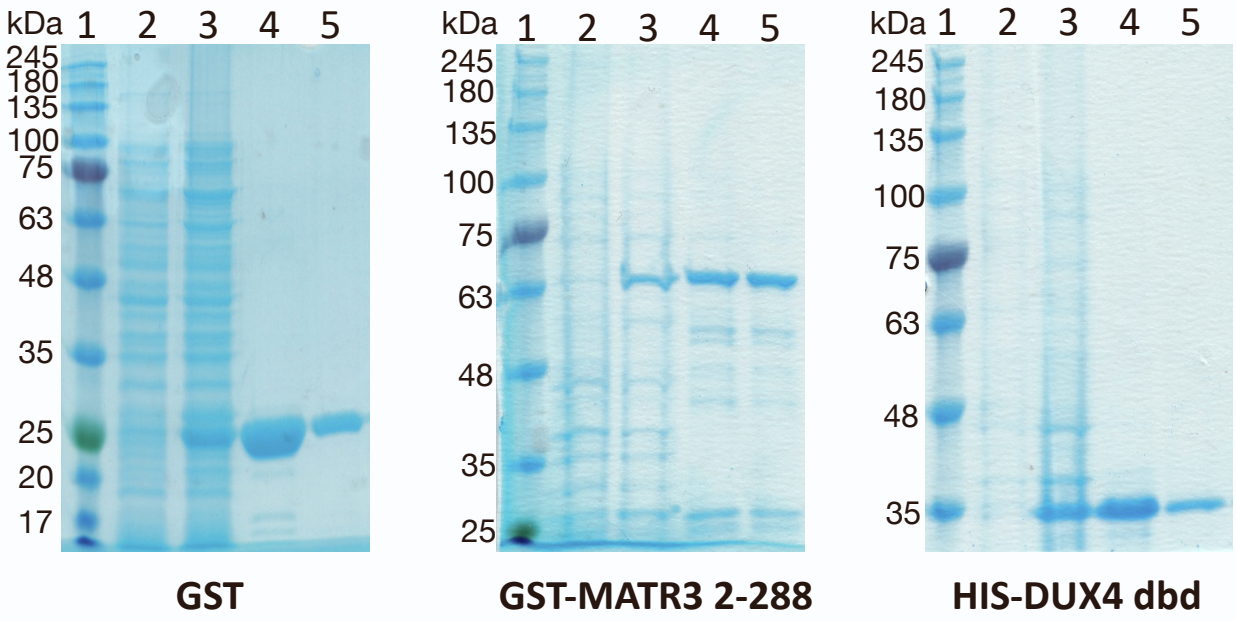


Fig. S8. Purification of recombinant DUX4 dbd and MATR3 $_{2\text{-}288}$. (Related to Figure 4). Coomassie staining showing the purification steps towards the production of recombinant GST (used as control, left), GST-fused MATR3 $_{2\text{-}288}$ (GST-MATR3 2-288, middle) and HIS-fused DUX4 DNA-binding domain (HIS-DUX4 dbd, right). Loading order is the following: molecular weight marker (lane 1), bacteria before induction (lane 2), bacteria after induction (lane 3), protein attached to beads (lane 4), purified protein (lane 5).

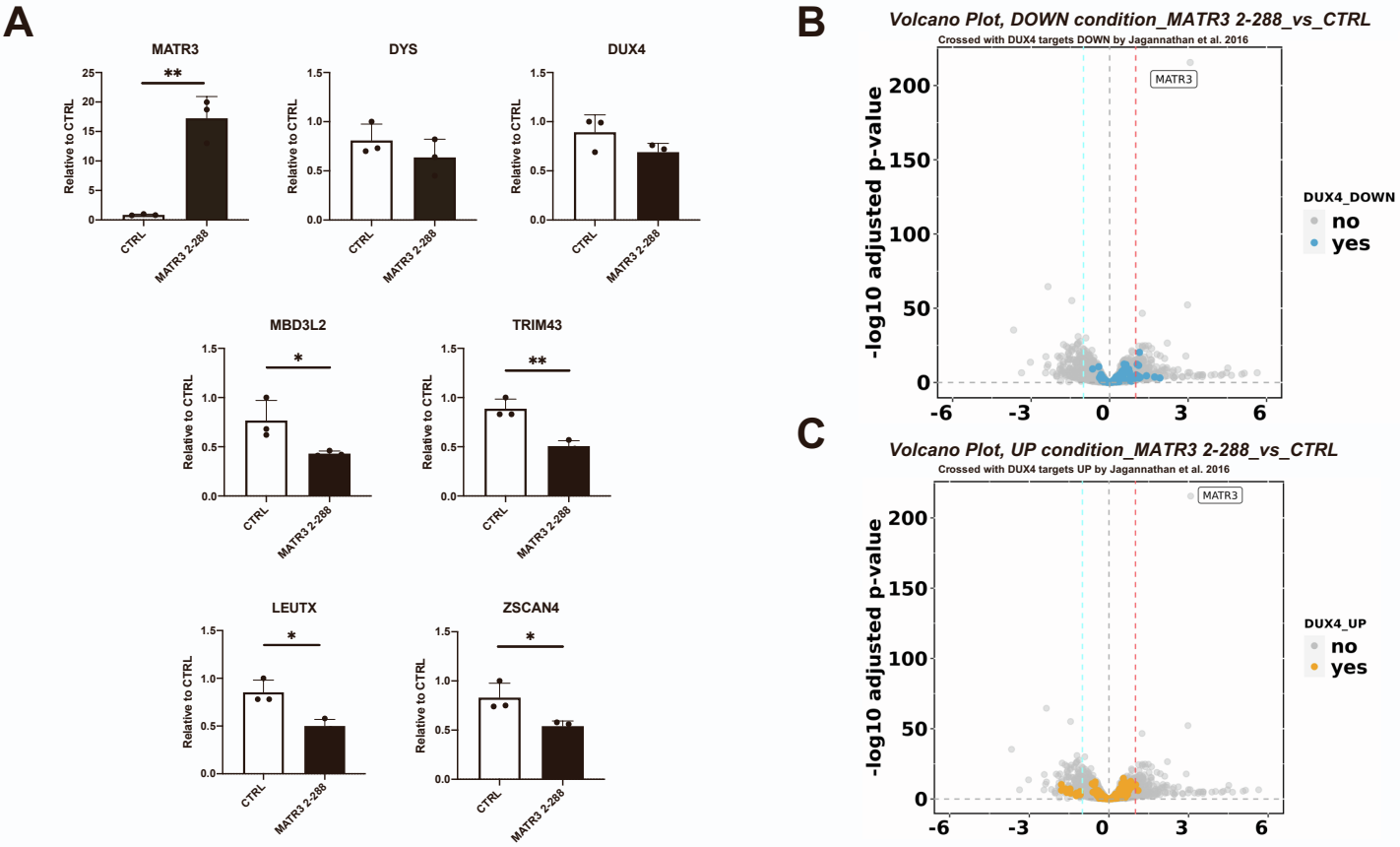


Fig. S9. MATR3 $_{2\text{-}288}$ blocks DUX4-dependent gene expression. (Related to Figure 5). **A.** RT-qPCR for the indicated genes performed on RNA from differentiated FSHD primary muscle cells (from a different patient than the one used in Fig. 5A) transduced with a control (CTRL) or *MATR3* $_{2\text{-}288}$ lentiviruses. Data are represented as relative to CTRL (unpaired Student's t test, *p<0.05, **p<0.01, n=3). **B.** Volcano plot (same as Fig. 5C) showing no significantly downregulated genes by MATR3 $_{2\text{-}288}$ overexpression among the lists of DUX4 down regulated targets from Jagannathan et al. 2016, filtered for absolute values of |log2 FC| > 1. **C.** Volcano plot (same as Fig. 5B) showing no significantly upregulated genes by MATR3 $_{2\text{-}288}$ overexpression among the lists of DUX4 upregulated targets from Jagannathan et al. 2016, filtered for absolute values of |log2 FC| > 1.

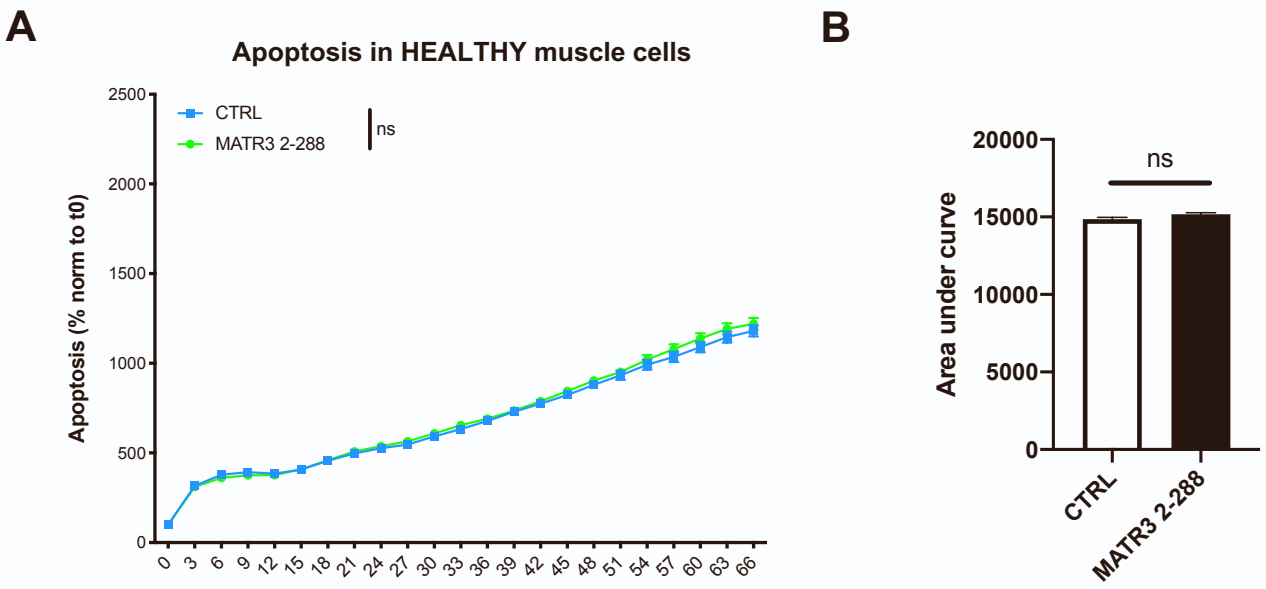


Fig. S10. MATR3 $_{2\text{-}288}$ does not affect cell viability of healthy primary muscle cells. (Related to Figure 5). **A.** Live-cell, real-time, caspase 3/7 apoptotic assays on healthy primary muscle cells transduced with control (CTRL) or *MATR3* $_{2\text{-}288}$ lentiviruses. Live-cell imaging was performed by IncuCyte S3 Imager system and quantified using the IncuCyte software. Data are reported as percentage (%) of green-fluorescent apoptotic cells normalized to time 0 (Two-Way ANOVA, n=3). **B.** Quantification of the area under the curve in A (unpaired Student's t test).

Table S2. List of primers used for cloning

Related to Figures 1-5.

Target gene	Sequence of Fw primer	Sequence of Rv primer
FLAG MATR3 2-288	CATGGACTCTTACCGAAGTAATATCCCCATCTGTGCTCT	AGAGCACAGATGGGGATATTACTTCGGTAAGAGTCCATG
FLAG MATR3 2-322	CGTCGATGCCAGCTTCTTTAAGAAATCTACCCAGAATGG	CCATTCTGGGTAGATTTCTTAAAGAAGCTGGCATCGACG
FLAG MATR3 2-798	GACTATGTGATACCTTAAACAGGGTTTTACTGTAAGCTG	CAGCTTACAGTAAAACCCTGTTTAAGGTATCACATAGTC
FLAG MATR3 289-end	AAAGGATCCTATCCCCATCTGTGCTCTATATGTG	AAACTCGAGTTAAGTTTCCTTCTTCTG
DUX4 full-length (attB)	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCGCCCTCCCGACACCCTCGGAC	GGGGACCACTTTGTACAA GAAAGCTGGGTCTAAAGCTCCTCCAGCAGAGCC
DUX4 dbd (attB)	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCGCCCTCCCGACACCCTCGGAC	GGGGACCACTTTGTACAAGAAAGCTGGGTCTAGACCTGCGCGGGCGCCC

Table S3. List of siRNA

Related to Figures 1-3.

Target	Species	Description	Sequence/Catalogue number
MATR3	Human	ON-TARGETplus SMARTpool - Dharmacon	FE5L017382000005
ILF2	Human	ON-TARGETplus SMARTpool - Dharmacon	FE5L017599000005
PRKDC	Human	ON-TARGETplus SMARTpool - Dharmacon	FE5L005030000005
RUVBL1	Human	ON-TARGETplus SMARTpool - Dharmacon	FE5L008312000005
C1QBP	Human	ON-TARGETplus SMARTpool - Dharmacon	FE5L011225010005
CDC23	Human	ON-TARGETplus SMARTpool - Dharmacon	FE5L009523000005
CDC27	Human	ON-TARGETplus SMARTpool - Dharmacon	FE5L003229000005
SMARCC2	Human	ON-TARGETplus SMARTpool - Dharmacon	FE5L008977000005
ANAPC7	Human	ON-TARGETplus SMARTpool - Dharmacon	FE5L021035000005
SLC25A5	Human	ON-TARGETplus SMARTpool - Dharmacon	FE5L007486020005
DUX4	Human	Stealth siRNA	CCGAGCCTTTGAGAAGGATCGCTTT

Table S4. List of primers used for RT-qPCR

Related to Figures 1, 2 and 5.

Target gene	Sequence of Fw primer	Sequence of Rv primer
GAPDH	TCAAGAAGGTGGTGAAGCAGG	ACCAGGAAATGAGCTTGACAAA
MATR3	ATCAATGGAGCAAGTCACAGTC	TGCAACATGAATGGATCACCC
MATR3 2-288	TACACGGGAGCCACCATACA	CAGGACCACGTCCCATTCTC
ILF2	CTCAGACTCTCGTCCGAATCC	CAGAAGCAAGATAGCTGGCATC
PRKDC	GAGAAGGCGGCTTACCTGAG	CGAAGGCCCGCTTTAAGAGA
RUVBL1	GGCATGTGGCGTCATAGTAGA	CACGGAGTTAGCTCTGTGACT
C1QBP	CGTGTGCTGGGCTCCTC	AAAGCTTTGTCTCCGTCGGT
CDC23	CTGCGAGTACCTCCATGGTC	AGAGAGAAAGCCAACTCCGC
CDC27	TGCTGACGTGTTTCTTGTCC	TTGCACTGCCTTTCATTCTG
SMARCC2	CCGTGACCCAGTTCGACAAC	CGGCAGTTTAGTGAGCGGT
ANAPC7	GCTTTTCGAGTCAGTGCTGC	GGGGAGAATAACTCAGGGTTG
SLC25A5	TTGATTTTGCCCGTACCCGT	GGATCCGGAAGCATTCCCTT
DUX4 (overexpressed	GCGCAACCTCTCCTAGAAAC	AGCAGAGCCCGGTATTCTTC
DUX4 (endogenous)	CCCAGGTACCAGCAGACC	TCCAGGAGATGTAACTCTAATCCA
DUX4 dual (endogenous)	CTTCCGTGAAATTCTGGCTGAATG	TTTTTTTTTTTTTTTTTCTATAGGATCCACAGG
MBD3L2	GCGTTCACCTCTTTTCCAAG	GCCATGTGGATTTCTCGTTT
RFPL2	CCCACATCAAGGAACTGGAG	TGTTGGCATCCAAGGTCATA
TRIM43	ACCCATCACTGGACTGGTGT	CACATCCTCAAAGAGCCTGA
TRIM48	GGAGCTGTGTTTTGGTGACCT	GTAGTTCATGCAGATGGGGCA
LEUTX	GGGAAACTGGCTTCAAAGCTA	TGATGGCCGTGTCTGCATT
ZSCAN4	TGGAAATCAAGTGGCAAAAA	CTGCATGTGGACGTGGAC
DYSTROPHIN	AGCAAGAGCACAACAATTTGG	CCCTGTTCGTCCCGTATCA

Table S6. Human muscle cells information.
Related to Figures 2, 3 and 5.

	Demographics			DNA Results: Deletion Size/Haplotype												Diagnostic Status and muscle sample			
					Allele 4_1			Allele 4_2			Allele 10_1			Allele 10_2						
Sample ID	Subject ID#	Sex	Age(s) at Time of Collection(s)	# of Pre-served 4qA Units	Del Size (Kb)	A/B	SSLP	Del Size (Kb)	A/B	SSLP	Del Size (Kb)	A/B	SSLP	Del Size (Kb)	A/B	SSLP	FSHD1	Unaffected	Muscle biopsied	Cultures: Myoblast
T2082	2082	F	46	7	27	A	161	80	A	163	60	A	166	250	A	166	X		QUAD	X
T2349	2349	F	29	2	12	A	161	69	B	163	28	A	166	43	A	166	X		QUAD	X
T230	2401	M	58	12	42	A	161	74	B	168	34	A	166	50	A	166		X	QUAD	X
T135	2081	F	42	18	65	B	163	250	A	161	95	A	166	100	A	166		X	QUAD	X
T216	2316	M	34	6	24	A	161	36H2	A	168	56	A	166	82(10/4)	A	176	X		QUAD	X
T188	2061	F	54	8	30	A	161	110	B	168	65	A	166	95	A	166	X		QUAD	X