

Table S1: sgRNAs targeting the FSHD locus

sgRNA	target	*19-nt sequence + PAM (NGG)	score**	Enrich [^]	OT(12) [#]	OT(19) ^{##}
1	p13-E11	TACCACAGACAGCCA <u>ACTG</u> GGG	0.61	6.9	18	0
2	p13-E11	TTCACCCAGAACAGTAACT GGG	0.60	1.6	27	0
3	<i>DUX4</i> E1	CACCCGGGCAA <u>AAAGCCGG</u> GAGG	0.61	2.5	8	1 (Y)
4	<i>DUX4</i> E1	CTGGAAGCACCCCTCAGCG AGG	0.85	2.4	9	3 (14/18/Y)
5	<i>DUX4</i> E1	CTGGAGGAGCTTTAGGACG CGG	0.65	2.2	14	6 (14/18/20/22/Y)
6	<i>DUX4</i> prom	CTCGCTCTGGTCTTCTACG TGG	0.72	2.0	4	0
7	<i>DUX4</i> prom	CCGTCCGTGAAATTC <u>CGGC</u> CGG	0.76	1.7	10	1 (20)
8	<i>DUX4</i> prom	TCCGACAGCACCC <u>TCCCCG</u> CGG	0.79	2.6	19	3 (3/14/Y)
9	<i>DUX4</i> E3	CTCCCTTGCACGTCAGCC <u>G</u> GGG	0.84	3.4	7	2 (14/18)
10	<i>DUX4</i> E3	GAATTTCA <u>CGGA</u> GAACA AGGG	0.76	1.8	5	0
11	<i>DUX4</i> E3	ATCTTCTATAGGATCCAC AGGG	0.88	2.0	9	0

*CpGs are underlined

**sgRNA Designer score (<http://www.broadinstitute.org/rnai/public/analysis-tools/sgrna-design>).

[^]Relative enrichment of dCas9-VP64-HA (Addgene plasmid #50918) at each target region measured by chromatin immunoprecipitation (ChIP) using HA antibodies normalized to mouse IgG.

[#]Number of off-target (non-Ch 4/10) matches to 12-nt seed sequence + NGG in human genomic database (<https://blast.ncbi.nlm.nih.gov>).

^{##}Number of off-target (non-Ch 4/10) matches to 19-nt sequence + NGG in human genomic database (<https://blast.ncbi.nlm.nih.gov>) (chromosomes in parentheses).

All off-target matches except those underlined are in D4Z4 homologous repeat sequences ^{1,2}.

Table S2: Sequences of oligonucleotide primers (5' to 3')

qRT-PCR primers (human):

DUX4-fl-F: GCTCTGCTGGAGGAGCTTTAGGA³
DUX4-fl-R: GCAGGTCTGCWGGTACCTGG³
MyHC1-F: TGGAGGCCAGGGTTCGTGAA³
MyHC1-R: ATTGTTCCCTCCGCTTCTTCAGC³
FRG1-F: TCTACAGAGACGTAGGCTGTCA⁴
FRG1-R: CTTGAGCACGAGCTTGGTAG⁴
FRG2-F: GGGAAAACCTGCAGGAAAA⁵
FRG2-R: CTGGACAGTTCCTGCTGTGT⁵
TRIM43-F: ACCCATCACTGGACTGGTGT⁶
TRIM43-R: CACATCCTCAAAGAGCCTGA⁶
ZSCAN4-F: TGGAAATCAAGTGGCAAAAA⁶
ZSCAN4-R: CTGCATGTGGACGTGGAC⁶
MBD3L2-F: GCGTTCACCTCTTTTCCAAG⁶
MBD3L2-R: GCCATGTGGATTTCTCGTTT⁶
Jumonji-F: AAGAAAAAGCCTCGAAAGTG
Jumonji-R: AGAGCACACTCCAGACAGAA
KLF14-F: TGCAACGTGTATATCATCCT
KLF14-R: ACACCAGAGTCCTTTGAGAC
UBR4-F: GGAGTCTGTGGCAACTGTGGAGAGAATG⁷
UBR4-R: CCGGTCTTCTTCATTCTCAATGGGATCCACT⁷

ChIP primers:

OT-F: GAATGTGGACACGGTAAAGA (intergenic region on Ch. 8, ref NC_018919.2)
OT-R: TAGGTTTGACTGCCAATGAC (intergenic region on Ch. 8, ref NC_018919.2)
p13-E11-F: TGGGCATTTTCTCATTAGCC
p13-E11-R: CTGGAGCAGAGATGACCACA
DUX4-exon1-F: GACACCCTCGGACAGCAC
DUX4-exon1-R: GTACGGGTTCCGCTCAAAG
DUX4-intron1-F: CTCAGCGAGGAAGAATACCG
DUX4-intron1-R: AGTCTCTCACCGGGCCTAGA
DUX4-exon3-F: CTGACGTGCAAGGGAGCT
DUX4-exon3-R: CAGGTTTGCCTAGACAGCG

Supplementary References

1. Zeng, W, Chen, YY, Newkirk, DA, Wu, B, Balog, J, Kong, X *et al.*, (2014). Genetic and epigenetic characteristics of FSHD-associated 4q and 10q D4Z4 that are distinct from non-4q/10q D4Z4 homologs. *Hum Mutat* **35**: 998-1010.
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3. Himeda, CL, Debarnot, C, Homma, S, Beermann, ML, Miller, JB, Jones, PL *et al.*, (2014). Myogenic enhancers regulate expression of the facioscapulohumeral muscular dystrophy associated DUX4 gene. *Mol Cell Biol* **34**: 1942-1955.
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