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Supplemental Information

***DUX4* Transcript Knockdown with Antisense 2'-O-Methoxyethyl Gapmers for the Treatment of Facioscapulohumeral Muscular Dystrophy**

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Supplemental Figures

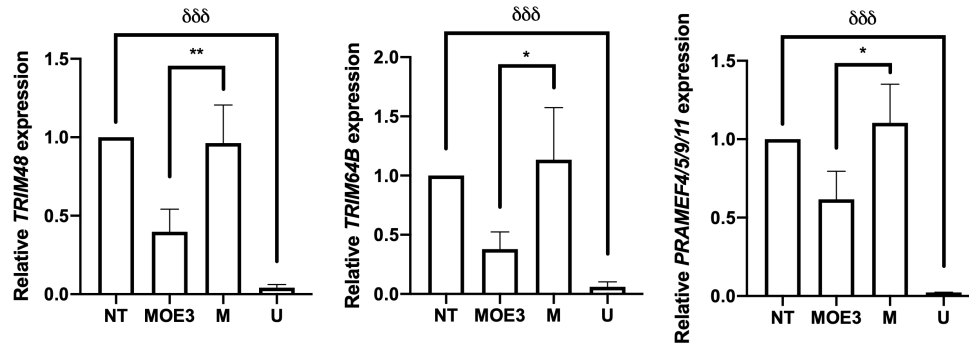


Figure S1. qPCR analysis of *TRIM48*, *TRIM64B*, and *PRAMEF4/5/9/11* expression (see also Figure 2). Relative expression levels of *TRIM48*, *TRIM64B*, and *PRAMEF4/5/9/11*, all significantly up-regulated FSHD-associated genes identified from our RNA sequencing analysis, were found to be significantly reduced by MOE3 treatment compared to mock gaper-treated (M) controls. NT, non-treated; U, FSHD-unaffected/healthy. Error bars: S.D., n=3. * $p < 0.05$, ** $p < 0.005$ vs M, one-way ANOVA with Dunnett's test. $\delta\delta\delta$ $p < 0.0005$, unpaired, two-tailed t -test.

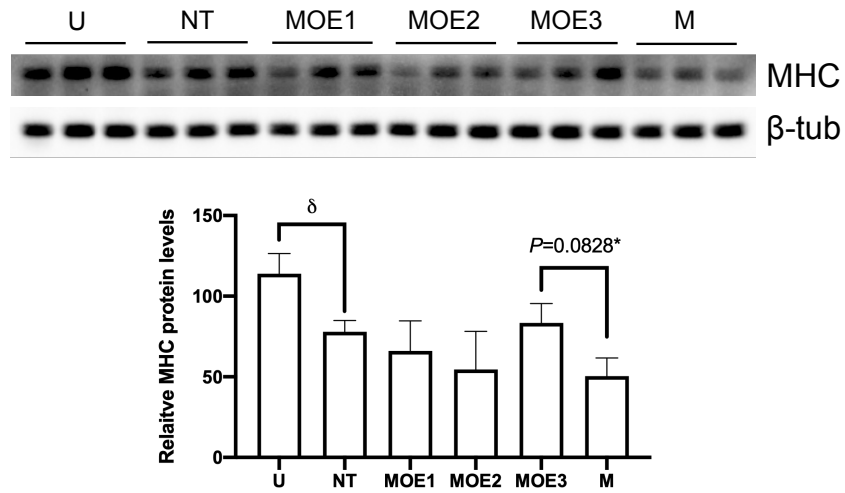


Figure S2. Western blot analysis of myosin heavy chain protein levels (see also Figure 3). Myosin heavy chain (MHC) protein levels were detected via Western blot, with β -tubulin (β -tub) as the loading control. Protein samples (12 μ g) were extracted from healthy WS234 myotubes (U), non-treated FSHD WS229 myotubes (NT), 2'-MOE gapmer-treated WS229 myotubes (MOE1-MOE3), and mock 2'-MOE gapmer-treated WS229 myotubes (M). Top: image of the visualized Western blot, bottom: quantification of MHC protein levels normalized to β -tub and calculated relative to one replicate from the healthy myotube samples. Error bars: S.D., n=3. *one-way ANOVA with Dunnett's test vs M. δ $p < 0.05$, unpaired, two-tailed t -test.

Supplemental Tables

Table S1. Information on the 94 FSHD signature genes obtained from RNA sequencing analysis.

Gene*	Transcript ID	Non-treated FSHD vs Healthy**			Treated FSHD vs Non-treated FSHD**		
		log2FC	adj. p-value	up/down?	log2FC	adj. p-value	significantly restored by treatment?
<i>HSPA1B a</i>	ENST00000391555	12.81	1.60E-25	up	0.18	0.05558362	No
<i>MBD3L3</i>	ENST00000333843	10.23	6.80E-16	up	-0.66	0.150525844	No
<i>PSMB9 a</i>	ENST00000453059	8.16	1.31E-09	up	-0.79	0.320090145	No
<i>PSMB9 b</i>	ENST00000434471	8.16	1.31E-09	up	-0.79	0.320090145	No
<i>PSMB9 c</i>	ENST00000427870	8.16	1.31E-09	up	-0.79	0.320090145	No
<i>TRIM51</i>	ENST00000449290	7.89	6.11E-09	up	-1.18	0.036749033	Yes
<i>KDM4E</i>	ENST00000450979	7.61	2.42E-08	up	-0.51	0.670007042	No
<i>PRAMEF17</i>	ENST00000376098	7.59	4.53E-08	up	-0.12	0.990219585	No
<i>PFKFB3</i>	ENST00000536985	7.58	4.68E-07	up	-2.63	0.58814826	No
<i>AMOT a</i>	ENST00000371959	7.38	2.26E-06	up	-0.13	0.992445062	No
<i>PRAMEF9</i>	ENST00000415919	7.38	6.87E-07	up	-1.12	0.457011498	No
<i>TFIP11</i>	ENST00000619735	7.37	0.011892526	up	0.49	0.983196789	No
<i>STIL a</i>	ENST00000337817	7.30	0.012435153	up	-0.61	0.982576204	No
<i>KHDC1L a</i>	ENST00000471312	7.12	8.45E-07	up	0.48	0.781306098	No
<i>ZNF280A a</i>	ENST00000302097	7.09	6.64E-07	up	-0.05	0.995731523	No
<i>ZNF280A b</i>	ENST00000620282	7.09	6.64E-07	up	-0.05	0.995731523	No
<i>PRAMEF10</i>	ENST00000235347	6.87	4.31E-06	up	-0.36	0.943642771	No
<i>AMACR</i>	ENST00000506639	6.71	0.034837731	up	-0.85	0.978289808	No
<i>KHDC1L b</i>	ENST00000370388	6.48	3.58E-29	up	-0.20	0.821838147	No
<i>LEUTX a</i>	ENST00000396841	6.36	1.06E-07	up	-0.65	0.30504829	No
<i>LEUTX b</i>	ENST00000629267	6.36	1.06E-07	up	-0.65	0.30504829	No
<i>PRAMEF22</i>	ENST00000616664	6.36	3.43E-05	up	0.14	0.991303373	No
<i>AMOT b</i>	ENST00000304758	6.21	6.02E-05	up	0.18	0.987440238	No
<i>TRIM43</i>	ENST00000272395	6.19	1.02E-15	up	-1.31	0.00068713	Yes
<i>PRAMEF4</i>	ENST00000235349	6.05	2.74E-05	up	-0.50	0.768132302	No
<i>TRIM48</i>	ENST00000417545	5.96	0.000173991	up	-1.72	0.249308715	No
<i>SLC34A2 a</i>	ENST00000382051	5.88	4.53E-17	up	-0.73	3.34E-05	Yes
<i>ZSCAN4</i>	ENST00000612521	5.86	5.07E-16	up	-1.15	0.00014191	Yes
<i>PRAMEF2</i>	ENST00000240189	5.76	1.04E-06	up	-1.52	0.133552181	No
<i>SLC34A2 b</i>	ENST00000513204	5.75	3.45E-06	up	-0.44	0.747265402	No
<i>PRAMEF19</i>	ENST00000376101	5.60	0.000249056	up	0.25	0.982576204	No
<i>PRAMEF5 a</i>	ENST00000622421	5.45	1.85E-08	up	-0.73	0.475006079	No
<i>PRAMEF5 b</i>	ENST00000621481	5.45	1.85E-08	up	-0.73	0.475006079	No
<i>PRAMEF1</i>	ENST00000332296	5.36	1.14E-07	up	-1.51	8.51E-05	Yes
<i>TRIM43B</i>	ENST00000432468	5.28	3.97E-11	up	-1.64	0.001063378	Yes
<i>PRAMEF12</i>	ENST00000357726	5.26	5.65E-07	up	-0.80	0.345957318	No
<i>MBD3L2</i>	ENST00000381393	5.21	4.98E-10	up	-0.37	0.576811781	No

Table S1 (cont'd.)

Gene*	Transcript ID	Non-treated FSHD vs Healthy**			Treated FSHD vs Non-treated FSHD**		
		log2FC	adj. p-value	up/down?	log2FC	adj. p-value	significantly restored by treatment?
<i>TRIM49C</i>	ENST00000448984	5.21	0.000369479	up	-0.56	0.876161011	No
<i>THOC5 a</i>	ENST00000488052	5.18	8.26E-07	up	-0.69	0.495486875	No
<i>OLFMI</i>	ENST00000252854	4.81	8.15E-05	up	0.12	0.988946093	No
<i>HSPA1B b</i>	ENST00000391548	4.78	4.55E-70	up	-0.02	0.991152883	No
<i>TRIM49</i>	ENST00000329758	4.78	0.000403045	up	-1.55	0.187848853	No
<i>TRIM49B</i>	ENST00000332682	4.56	1.13E-07	up	-0.73	0.30431758	No
<i>PRAMEF11</i>	ENST00000619922	4.40	0.010586296	up	-0.13	0.992833412	No
<i>CENPA a</i>	ENST00000475662	4.40	0.000326357	up	-1.17	0.84823361	No
<i>GPR37</i>	ENST00000303921	4.39	1.19E-08	up	0.14	0.981222213	No
<i>HSPA1A a</i>	ENST00000441618	4.31	0.000662654	up	-0.39	0.18443152	No
<i>SERPINF2</i>	ENST00000382061	4.07	6.14E-05	up	0.38	0.891316067	No
<i>ZNF296</i>	ENST00000303809	4.02	0.004702231	up	-0.35	0.969612411	No
<i>RFPL4B</i>	ENST00000441065	3.87	8.47E-08	up	-1.72	1.44E-06	Yes
<i>PRAMEF8</i>	ENST00000357367	3.78	0.019759829	up	-3.05	0.035391996	Yes
<i>TRIM53AP</i>	ENST00000532014	3.75	0.001015777	up	-0.70	0.597602437	No
<i>CCNA1 a</i>	ENST00000255465	3.31	3.94E-19	up	-0.37	0.463772143	No
<i>CCNA1 b</i>	ENST00000625767	3.27	2.05E-16	up	-0.45	0.09064676	No
<i>PTP4A3 a</i>	ENST00000520105	2.99	3.12E-13	up	0.43	0.166532775	No
<i>RIPK4</i>	ENST00000332512	2.93	6.86E-10	up	-0.03	0.995355899	No
<i>ACKR4</i>	ENST00000249887	2.90	0.000808967	up	-0.89	0.226235022	No
<i>PPP2R2B</i>	ENST00000394411	2.87	0.004828947	up	-1.23	0.845669111	No
<i>THOC5 b</i>	ENST00000484924	2.78	3.42E-05	up	-0.35	0.909720201	No
<i>JUP a</i>	ENST00000449889	2.59	3.54E-07	up	0.21	0.957590214	No
<i>TRIM64B</i>	ENST00000329862	2.51	0.02102623	up	-1.45	0.077007169	No
<i>C1QTNF3</i>	ENST00000231338	2.41	3.24E-51	up	-0.12	0.916619331	No
<i>HPGD</i>	ENST00000296522	2.37	0.04900649	up	0.12	0.992774603	No
<i>JUP b</i>	ENST00000591690	2.30	8.74E-07	up	0.00	0.999964077	No
<i>PTP4A3 b</i>	ENST00000521578	2.29	2.12E-25	up	0.58	0.002408606	No
<i>PNMA2</i>	ENST00000522362	2.15	1.87E-28	up	0.03	0.991303373	No
<i>SLAH1</i>	ENST00000356721	2.06	0.004347022	up	0.05	0.99534489	No
<i>THOC5 c</i>	ENST00000443089	2.05	1.24E-17	up	-0.20	0.772030789	No
<i>TRIL</i>	ENST00000539664	2.01	0.001074742	up	0.04	0.994199094	No
<i>CCR4</i>	ENST00000330953	-2.11	0.005067901	down	-0.53	0.971783714	No
<i>MELK a</i>	ENST00000298048	-2.12	0.000252848	down	0.40	0.94825751	No
<i>MTFR2 a</i>	ENST00000420702	-2.28	0.010004786	down	1.43	0.630429768	No
<i>GOLGA6A</i>	ENST00000290438	-2.34	5.35E-09	down	0.50	0.791090752	No
<i>BORA</i>	ENST00000377815	-2.38	0.009935466	down	0.05	0.999371732	No

Table S1 (cont'd.)

Gene*	Transcript ID	Non-treated FSHD vs Healthy**			Treated FSHD vs Non-treated FSHD**		
		log2FC	adj. p-value	up/down?	log2FC	adj. p-value	significantly restored by treatment?
<i>MTFR2 b</i>	ENST00000451457	-2.56	0.036193589	down	-2.42	0.866364671	No
<i>GOLGA6C</i>	ENST00000300576	-2.63	3.60E-05	down	-0.79	0.98271602	No
<i>MAP7D2</i>	ENST00000379643	-3.08	2.21E-10	down	0.14	0.992774603	No
<i>MELK b</i>	ENST00000626154	-3.38	0.003594245	down	-5.38	0.478787787	No
<i>ODC1</i>	ENST00000446285	-3.74	0.0101774	down	3.37	0.667785883	No
<i>CDC20 a</i>	ENST00000372462	-3.75	0.000246158	down	-0.34	0.992833412	No
<i>SCG5 a</i>	ENST00000475752	-3.81	2.45E-38	down	-0.71	0.776061124	No
<i>CENPA b</i>	ENST00000233505	-3.83	0.012455446	down	-4.13	0.862519595	No
<i>UBE2C a</i>	ENST00000356455	-3.83	5.08E-57	down	-0.24	0.944545174	No
<i>CDC20 b</i>	ENST00000310955	-3.98	1.25E-87	down	-0.63	0.263401187	No
<i>SCG5 b</i>	ENST00000498607	-3.99	1.17E-07	down	-1.28	0.967200594	No
<i>UBE2C b</i>	ENST00000372568	-4.33	5.29E-07	down	1.31	0.943676285	No
<i>CD248</i>	ENST00000311330	-4.62	2.42E-275	down	-0.08	0.987440238	No
<i>SYT7</i>	ENST00000542836	-4.71	1.31E-05	down	2.44	0.868797513	No
<i>DNER</i>	ENST00000341772	-4.91	1.32E-88	down	-0.11	0.991213343	No
<i>HSPA1B c</i>	ENST00000445736	-5.32	0.002037533	down	0.24	0.997320359	No
<i>CENPA c</i>	ENST00000335756	-5.48	2.08E-06	down	2.20	0.716243485	No
<i>HSPA1B d</i>	ENST00000450744	-6.14	5.68E-05	down	1.80	0.6047912	No
<i>STIL b</i>	ENST00000447475	-6.84	0.025544257	down	6.90	5.84E-05	Yes
<i>SPATA33</i>	ENST00000579310	-7.19	7.01E-06	down	5.94	0.294771871	No

*letters after the underscore are arbitrary identifiers of different transcripts from the same gene

**this group served as reference for the comparison

Table S2. Validation of some FSHD signature genes from RNA sequencing analysis

Transcript	Status in FSHD	Significantly restored by treatment in RNA-seq?	Validated by qPCR? (status, figure)
<i>TRIM51</i>	up-regulated	yes	not validated
<i>TRIM43</i>	up-regulated	yes	yes (restored, Fig. 1C)
<i>SLC34A2_a</i>	up-regulated	yes	not validated
<i>ZSCAN4</i>	up-regulated	yes	yes (restored, Fig. 1C)
<i>PRAMEF1</i>	up-regulated	yes	not validated
<i>TRIM43B</i>	up-regulated	yes	not validated
<i>RFPL4B</i>	up-regulated	yes	not validated
<i>PRAMEF8</i>	up-regulated	yes	not validated
<i>MBD3L2</i>	up-regulated	no	yes (restored, Fig. 1C)
<i>TRIM48</i>	up-regulated	no	yes (restored, Fig. S1)
<i>TRIM64B</i>	up-regulated	no	yes (restored, Fig. S1)
<i>PRAMEF4/5/9/11</i>	up-regulated	no	yes (restored, Fig. S1)
<i>STIL_b</i>	down-regulated	yes	not validated

Table S3. Characteristics of the immortalized human muscle cells used in this study.

Cell ID	Disease Status	Sex	Age at biopsy	EcoRI/BlnI allele size (4q haplotype)
WS229	FSHD	Male	66 y/o	>112 kb (B) / 28 kb (A)
WS234	Healthy	Female	60 y/o	>145 kb (B) / 107 kb (B)

Table S4. Primers used for qPCR evaluation of gene expression in this study.

Gene	Forward (F) and Reverse (R) primers, 5' to 3'
<i>DUX4</i>	F: CCCAGGTACCAGCAGACC R: TCCAGGAGATGTA ACTCTAATCCA
<i>GAPDH</i>	F: GCAAATCCATGGCACCGT R: AGGGATCTCGCTCCTGGAA
<i>BANF1</i>	F: TGACAAGGCCTATGTTGTCC R: CACAAGTGTCTTTCAGCCATTC
<i>SSR4</i>	F: GCAGGCACCTATGAGGTTAG R: CTCGTTATTCCTCTGAGCCTTC
<i>FARP1</i>	F: GACTGCCGAGCCGCTTT R: TCTTGAGTTCGTGCAGCTTCTG
<i>ZBTB7B</i>	F: AACTGCCTCGCCACAT R: CAGCTTGTCGTTCTGCTGGT
<i>TRIM48</i>	F: TATGGAGAGGAGGGACTCTTTAG R: CTACATGGTTGGTAGGTCTTGG
<i>PRAMEF4/5/9/11</i>	F: CCAGAGCAGAAGAAGGAGATTG R: TGGCCTTCGAGGAAAGAAAC