

Figure S1: Myofusion Index was Not Impacted by Apabetalone, JQ1, or Losmapimod Treatment

Myofusion Index (MI), defined as the percentage of nuclei within myosin heavy chain (MHC) positive multinucleated cells, was determined following 144h of pre-differentiation and 72h of treatment. MHC⁺ and MHC⁻ nuclei counts for 3 distinct imaging fields were pooled for each sample. Bars show mean \pm SEM of three samples, significance calculated by one-way ANOVA with Dunnett's post-testing for multiple comparisons.

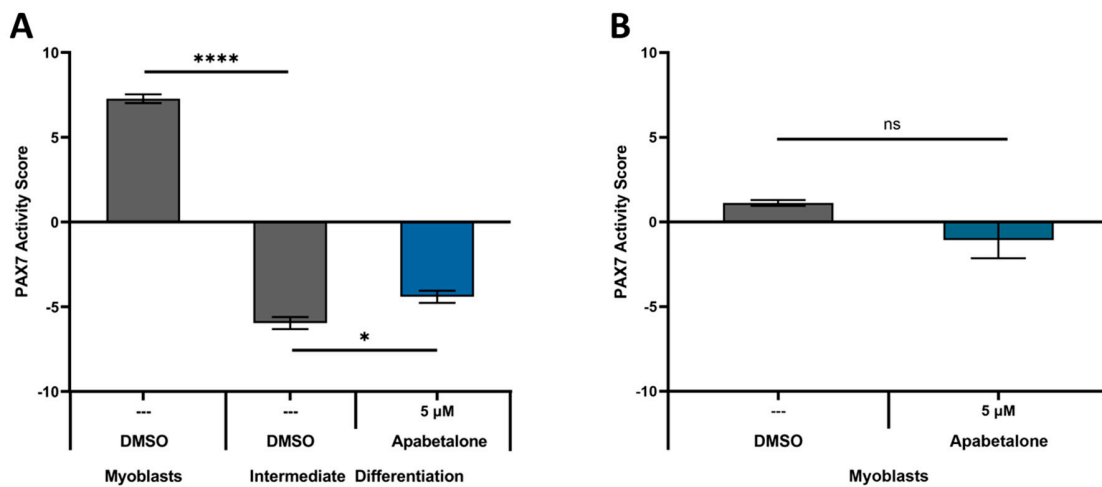


Figure S2: Apabetalone Treatment Impacts on PAX7 Activity Score Depend on Stage of Differentiation

Composite biomarkers of and PAX7 activity, derived and validated by Banerji et al. [19,44], were used to evaluate treatment impact on undifferentiated (A) pHSMCs, and intermediate differentiation (B) based on RNA-seq data. Intermediate samples were allowed to differentiate for three days prior to treatment and all samples were treated for one day. Bars show mean \pm SEM of three technical replicates, and statistical significance was assessed by one-way ANOVA with Dunnett's post-testing for intermediate samples and by unpaired Student's t-test for undifferentiated samples.

Table S1: Donor Details and Source of pHSMCs

Cell ID	Donor 1 (GM17869)	Donor 2 (GM17940)
Source	NIGMS Human Genetic Cell Repository	NIGMS Human Genetic Cell Repository
Genotype	FSHD1	FSHD1
Clinically Affected	Yes	Yes
D4Z4 Repeats – 4q35	5 / 31	3 / 33
D4Z4 Repeats – 10q26	5 / 39	15 / 26
Age at Sampling	21	13
Sex	Female	Male
Cell Type	Skeletal myoblast	Skeletal myoblast

Table S2: List of TaqMan Assay IDs Used for qRT-PCR Evaluation of pHSMCs

Gene Name	Symbol	TaqMan Assay ID
Zinc Finger and SCAN Domain-Containing Protein 4	<i>ZSCAN4</i>	Hs00537549_m1
Methyl-CpG Binding Domain Protein 3 Like Protein 2	<i>MBD3L2</i>	Hs00544743_m1
Myosin Heavy Chain 2	<i>MYH2</i>	Hs01126511_m1
Paired Box 7	<i>PAX7</i>	Hs00242962_m1
Myogenin	<i>MYOG</i>	Hs01072232_m1
Cyclophilin A	<i>PPIA</i>	Hs99999904_m1

Table S3: Impact of Differentiation and Treatment on Terminal Differentiation Markers by RNA-seq in Donor 1 Cells

Terminal Skeletal Muscle Differential Markers (Chal 2017 [48])		Differentiation		Apabetalone						JQ1		Losmapimod	
		Vehicle		1 μ M		5 μ M		25 μ M		0.1 μ M		10 μ M	
		FC	padj	FC	padj	FC	padj	FC	padj	FC	padj	FC	padj
Muscle Regulatory Factors													
Myogenic Differentiation 1	MYOD1	1.21	8E-03	1.37	2E-04	1.36	5E-05	1.35	3E-05	1.31	3E-04	0.99	0.95
Myogenin	MYOG	40.76	<1E-308	1.31	4E-03	1.21	0.04	0.91	0.34	1.09	0.40	1.02	0.85
Myogenic Factor 6	MYF6	2.18	1E-09	0.88	0.46	0.54	9E-06	0.33	3E-16	0.50	1E-07	1.13	0.49
Nuclear Factor I X	NFIX	1.02	0.79	0.98	0.79	1.16	0.02	1.27	2E-05	1.34	7E-08	1.40	9E-10
Primary Myogenesis Markers													
Myosin Heavy Chain 3	MYH3	39.30	<1E-308	1.22	5E-04	1.35	2E-10	1.13	0.01	1.37	1E-11	0.76	5E-09
Myosin Heavy Chain 7	MYH7	33.27	<1E-308	1.34	2E-03	1.58	1E-08	1.35	1E-04	1.60	1E-09	4.21	4E-86
Myosin Heavy Chain 8	MYH8	36.04	<1E-308	1.30	2E-05	1.60	3E-19	1.34	4E-08	1.81	2E-31	1.03	0.69
Myosin Light Chain 1	MYL1	42.26	<1E-308	1.13	0.18	1.00	0.98	0.58	2E-17	1.00	0.99	0.84	0.02
Actin Alpha Skeletal Muscle 1	ACTA1	42.32	<1E-308	1.16	0.18	1.08	0.52	0.85	0.09	1.05	0.70	0.71	1E-04
Actin Alpha Cardiac Muscle 1	ACTC1	17.55	<1E-308	1.43	1E-05	1.56	4E-10	1.43	2E-07	1.56	2E-10	0.92	0.43
Cholinergic Receptor Nicotinic Gamma Subunit	CHRNA3	43.18	<1E-308	0.98	0.84	1.20	0.06	1.15	0.14	1.10	0.40	0.83	0.04
Calcium Voltage-Gated Channel Subunit Alpha1 H	CACNA1H	44.87	2E-212	0.97	0.74	0.82	0.01	0.29	1E-78	0.55	3E-20	0.40	7E-44
Solute Carrier Family 2 Member 1	SLC2A1	0.17	<1E-308	1.13	0.05	1.18	2E-03	1.44	1E-16	1.32	2E-09	0.82	4E-05
Solute Carrier Family 2 Member 4	SLC2A4	26.70	2E-35	1.43	0.01	1.36	0.02	0.47	7E-10	0.79	0.09	1.17	0.31
Secondary Myogenesis Markers													
Myosin Heavy Chain 1	MYH1	29.30	6E-306	1.33	2E-06	1.41	1E-10	0.82	4E-04	1.19	2E-03	0.41	2E-62
Myosin Heavy Chain 2	MYH2	41.12	<1E-308	0.97	0.78	0.88	0.12	0.44	6E-38	0.50	7E-26	1.22	0.01
Myosin Heavy Chain 4	MYH4	27.22	3E-05	0.79	nr	1.07	0.90	0.86	0.73	0.93	0.89	1.07	0.90
Myosin Light Chain 3	MYL3	23.41	3E-08	1.18	nr	1.25	0.58	0.42	5E-03	0.60	0.12	0.70	0.34
Actin Alpha Skeletal Muscle 1	ACTA1	42.32	<1E-308	1.16	0.18	1.08	0.52	0.85	0.09	1.05	0.70	0.71	1E-04

Cholinergic Receptor Nicotinic Epsilon Subunit	CHRNE	0.60	0.03	0.82	nr	0.86	0.71	0.87	0.70	0.84	0.63	0.79	0.50
Calcium Voltage-Gated Channel Subunit Alpha1 S	CACNA1S	41.65	<1E-308	1.02	0.78	1.01	0.93	0.56	7E-38	0.77	4E-08	0.75	2E-09
Calcium Voltage-Gated Channel Subunit Alpha1 C	CACNA1C	0.74	0.05	0.83	0.38	0.90	0.69	1.21	0.31	1.28	0.17	1.10	0.71
Solute Carrier Family 2 Member 4	SLC2A4	26.70	2E-35	1.43	0.01	1.36	0.02	0.47	7E-10	0.79	0.09	1.17	0.31

Terminal differentiation markers reported in Chal et al. [48]. CACNA1F, CACNA1D, CACNA1G, CACNA1I, SLC2A2 excluded from analysis due to low transcript abundance. nr = not reported in DESeq2 differential expression analysis. FC = fold change, padj = Benjamini-Hochberg adjusted p-value. Significantly differentially expressed markers highlighted in bold (padj <0.05).

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Table S4: Impact of Differentiation and Treatment on Muscle Function Associated Markers by RNA-seq in Donor 1 Cells

Neuromuscular Junction and Dyetrophin-associated Complex Markers		Differentiation		Apabetalone						JQ1		Losmapimod	
		Vehicle		1 μ M		5 μ M		25 μ M		0.1 μ M		10 μ M	
		FC	padj	FC	padj	FC	padj	FC	padj	FC	padj	FC	padj
Defined Neuromuscular Junction Function (Rodríguez Cruz 2020 [49])													
Agrin	AGRN	1.49	2E-14	1.12	0.10	1.30	3E-06	1.47	3E-13	1.37	5E-09	1.03	0.70
Cholinergic Receptor Nicotinic Alpha Subunit	CHRNA1	10.46	<1E-308	1.02	0.76	0.82	1E-07	0.48	5E-103	0.67	2E-32	1.34	7E-17
Cholinergic Receptor Nicotinic Beta Subunit	CHRNA1	5.89	<1E-308	1.01	0.93	0.95	0.36	0.83	2E-06	0.81	6E-08	1.13	3E-03
Cholinergic Receptor Nicotinic Gamma Subunit	CHRNA1	43.18	<1E-308	0.98	0.84	1.20	0.06	1.15	1E-01	1.10	4E-01	0.83	4E-02
Cholinergic Receptor Nicotinic Delta Subunit	CHRNA1	36.78	<1E-308	1.08	0.28	1.06	0.43	1.07	0.32	1.10	0.14	0.66	1E-15
Cholinergic Receptor Nicotinic Epsilon Subunit	CHRNA1	0.60	0.03	0.82	nr	0.86	0.71	0.87	0.70	0.84	0.63	0.79	0.50
Docking Protein 7	DOK7	2.56	3E-38	1.06	0.61	0.88	0.18	0.67	4E-08	0.71	1E-05	1.03	0.84
LDL Receptor Related Protein 4	LRP4	8.22	5E-112	0.87	0.27	0.88	0.32	0.63	1E-06	0.70	3E-04	0.62	9E-07
Microtubule Actin Crosslinking Factor 1	MACF1	2.33	2E-14	0.82	0.20	0.84	0.26	0.82	0.14	0.89	0.46	0.70	0.01
Muscle Associated Receptor Tyrosine Kinase	MUSK	8.33	5E-58	0.72	0.05	0.68	0.01	0.36	1E-15	0.51	4E-07	1.04	0.86
Plectin	PLEC	0.74	5E-06	0.99	0.95	1.15	0.10	1.37	3E-06	1.30	2E-04	0.96	0.70
Receptor Associated Protein Of The Synapse	RAPSN	2.69	1E-44	1.06	0.59	0.85	0.09	0.52	2E-19	0.67	8E-08	1.10	0.32
Sodium Voltage-Gated Channel Alpha Subunit 4	SCN4A	47.01	<1E-308	1.04	0.69	1.02	0.87	0.76	2E-06	0.78	4E-05	1.24	8E-05
ALG2 Alpha-1,3/1,6-Mannosyltransferase	ALG2	0.63	1E-24	0.98	0.82	1.07	0.35	1.23	4E-05	1.09	0.15	0.78	4E-06
ALG14 UDP-N-Acetylglucosaminyltransferase Subunit	ALG14	0.64	1E-05	0.93	0.66	0.77	0.06	0.78	0.05	0.72	8E-03	0.88	0.39
Dolichyl-Phosphate N-Acetylglucosaminophosphotransferase 1	DPAGT1	0.69	4E-10	1.07	0.46	1.03	0.78	1.02	0.88	0.97	0.77	0.94	0.51

Glutamine--Fructose-6-Phosphate Transaminase 1	GFPT1	0.75	4E-06	0.90	0.22	1.02	0.90	1.12	0.12	1.10	0.22	0.85	0.03
GDP-Mannose Pyrophosphorylase B	GMPPB	0.55	2E-17	1.05	0.64	1.06	0.62	0.93	0.44	0.99	0.94	0.95	0.65
Solute Carrier Family 25 Member 1	SLC25A1	0.44	5E-17	1.34	0.03	1.47	7E-04	2.09	2E-13	1.68	8E-07	1.14	0.35
Dystrophin-associated Complex (Gao 2015 [50])													
Dystroglycan 1	DAG1	1.57	5E-22	1.03	0.64	1.23	1E-04	1.50	7E-18	1.45	1E-14	0.86	8E-03
Dystrophin	DMD	11.60	5E-96	0.81	0.21	0.98	0.94	0.95	0.81	1.02	0.94	0.71	0.01
Dystrobrevin Alpha	DTNA	6.06	2E-185	1.01	0.90	0.84	7E-03	0.53	5E-30	0.78	3E-05	1.09	0.22
Dystrobrevin Beta	DTNB	0.99	0.97	0.90	0.57	1.13	0.51	1.66	2E-05	1.19	0.28	1.29	0.08
Laminin Subunit Alpha 2	LAMA2	2.06	4E-14	0.87	0.33	1.02	0.93	1.26	0.03	1.24	0.06	0.84	0.15
Sarcoglycan Alpha	SGCA	4.32	4E-89	1.19	0.08	1.02	0.88	0.75	3E-04	0.81	0.02	1.28	3E-03
Sarcoglycan Beta	SGCB	0.93	0.26	0.96	0.68	1.13	0.10	1.42	3E-09	1.31	1E-05	0.96	0.62
Sarcoglycan Gamma	SGCG	43.16	2E-123	0.98	0.86	0.71	4E-05	0.26	6E-60	0.48	2E-20	1.53	5E-08
Sarcoglycan Delta	SGCD	8.73	2E-169	0.67	1E-05	0.49	1E-19	0.23	2E-82	0.38	7E-36	0.63	2E-08
Syntrophin Alpha 1	SNTA1	0.64	3E-10	1.18	0.09	1.11	0.33	1.16	0.07	1.14	0.15	1.06	0.63
Syntrophin Beta 1	SNTB1	4.06	8E-86	0.85	0.09	0.79	4E-03	0.58	2E-13	0.71	7E-06	1.10	0.36
Syntrophin Beta 2	SNTB2	0.30	3E-97	1.03	0.77	1.26	1E-03	2.12	9E-37	1.83	4E-23	1.08	0.39
Sarcospan	SSPN	11.01	<1E-308	0.92	0.31	0.78	4E-05	0.52	1E-31	0.71	2E-09	0.91	0.23
Utrophin	UTRN	0.77	0.18	0.71	0.16	0.79	0.35	1.01	0.97	1.03	0.94	0.80	0.37

Neuromuscular Junction Function markers reported in Rodríguez Cruz et al. [49] and dystrophin-associated complex marker from Gao et al. [50]. NOS1, SNTG1, and SNTG2 excluded from analysis due to low transcript abundance. nr = not reported in DESeq2 differential expression analysis. FC = fold change, padj = Benjamini-Hochberg adjusted p-value. Significantly differentially expressed markers highlighted in bold (padj <0.05).

Table S5: Composition and Derivation of Composite FSHD Transcriptomic Biomarkers

Source of Gene List	Target	Target-associated Genes	Composite Readout	Method	Derivation Model	Reference
Geng (2012)	DUX4	165 upregulated	Mean of z-score normalized gene expression data	Microarray	DUX4 transduced myocytes	[8]
Yao (2014)	DUX4	213 upregulated	Mean of z-score normalized gene expression data	RNA-seq	DUX4 transduced myocytes, primary muscle cells, and muscle tissue biopsies	[43]
Choi (2016)	DUX4	212 upregulated	Mean of z-score normalized gene expression data	RNA-seq	DUX4 transduced myocytes	[14]
Banerji (2017)	PAX7	311 upregulated 290 downregulated	t-statistic of z-score normalized gene expression data	Microarray	PAX7 transduced myocytes	[19]