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Bioengineered Model of Human LGMD2B Skeletal Muscle Reveals Roles of Intracellular Calcium Overload in Contractile and Metabolic Dysfunction in Dysferlinopathy

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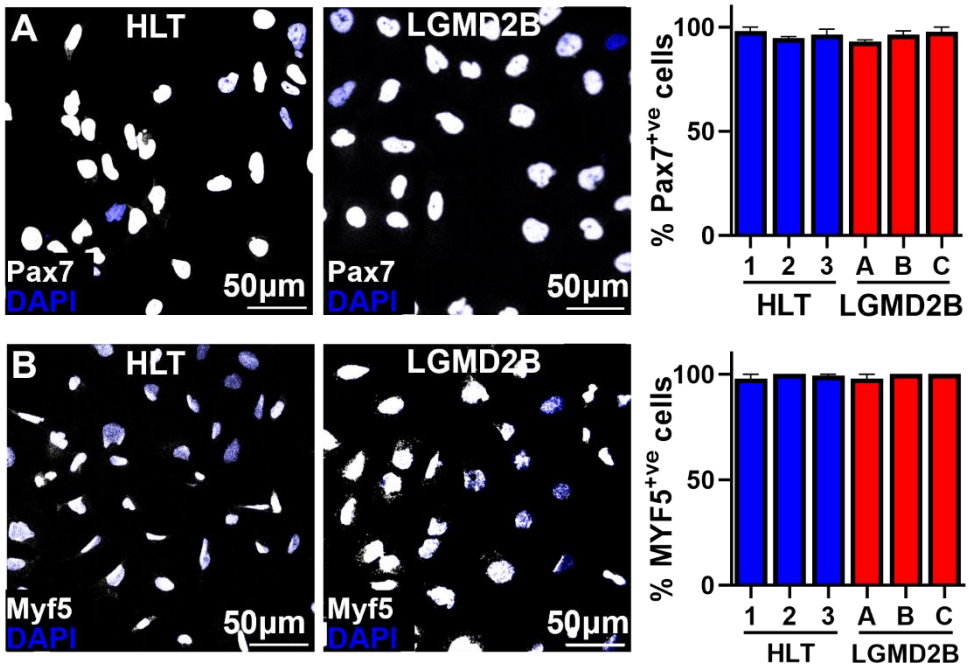

Figure S1. Myogenic regulatory factor expression in healthy and LGMD2B iMPCs. Representative images and quantifications of passage 2 iMPCs stained for (**A**) Pax7 and (**B**) Myf5 (N = 1 differentiation and n = 3 replicates per hiPSC line).

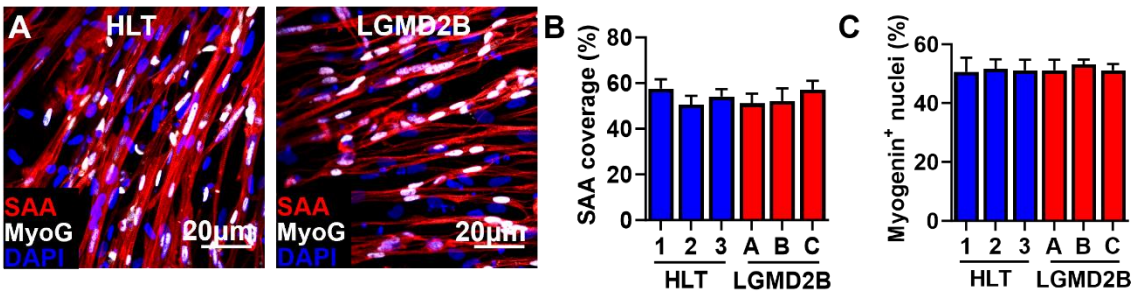

Figure S2. Two-dimensional myogenic differentiation of healthy and LGMD2B iMPCs. Healthy and LGMD2B iMPCs were differentiated for 8 days in traditional two-dimensional culture. (**A**) Representative images of cultures stained for sarcomeric alpha-actinin (SAA),

myogenin (MyoG), and DAPI. (**B-C**) Quantifications of (**B**) SAA area and (**C**) MyoG-positive nuclei (N = 1 differentiation and n = 3 replicates per hiPSC line).

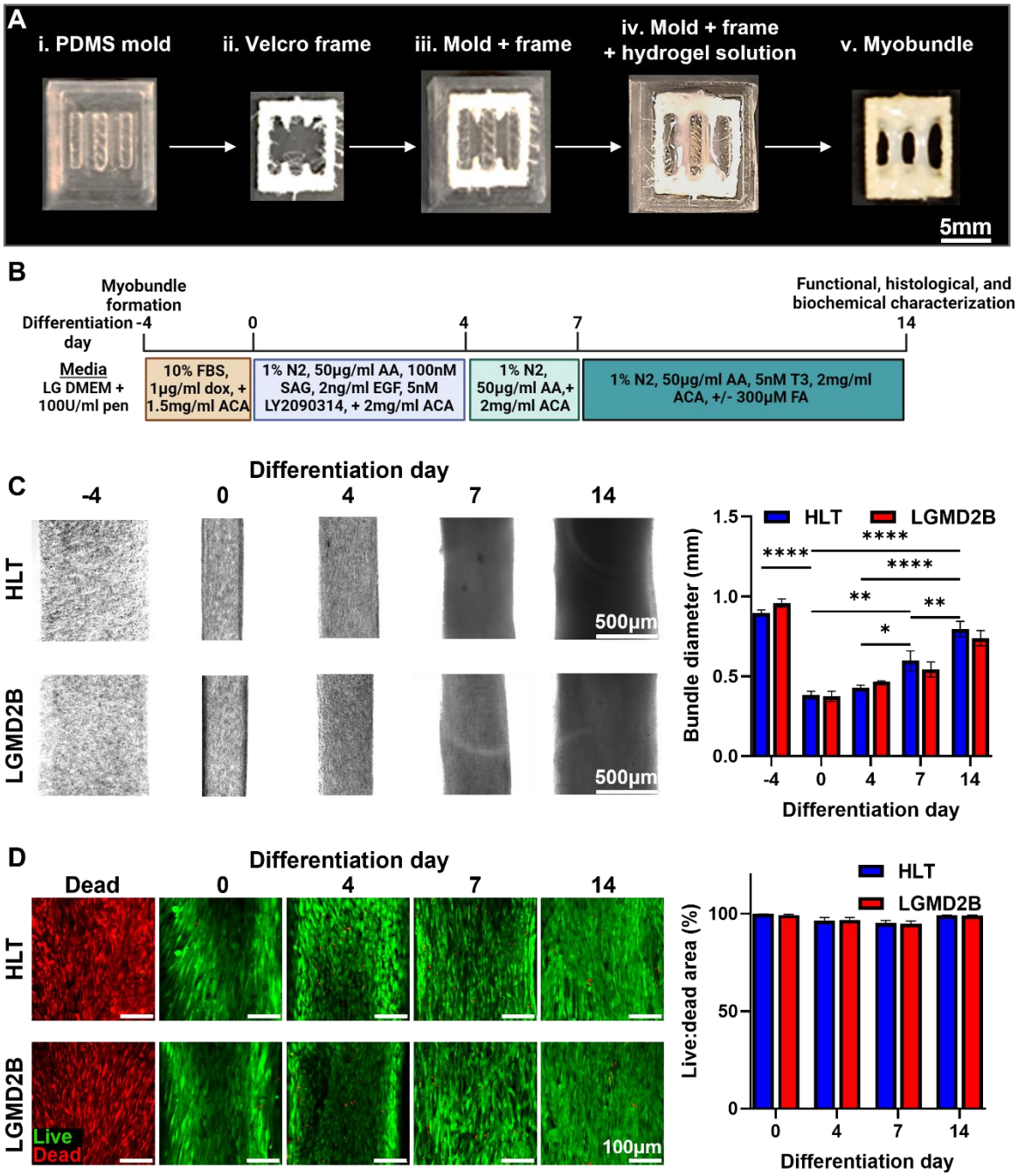

Figure S3. Myobundle formation and culture. (**A**) Representative images of myobundle fabrication process. (**B**) Schematic depicting media composition used during myobundle culture. (**C**) Representative brightfield images and quantification of myobundle width over culture duration (N = 1 differentiation and 1 hiPSC line, n = 7-8 replicates per line) (**D**) Representative live/dead images and corresponding quantification (N = 1 & 1 hiPSC line, n = 6). LG, low glucose; pen, penicillin; dox, doxycycline; FBS, fetal bovine serum; ACA, aminocaproic acid; AA, ascorbic acid-2-phosphate; SAG, smoothened agonist; EGF, epidermal growth factor; LY2090314, GSK3β inhibitor; T3, triiodothyronine; FA, fatty acid. *P<0.05, **P<0.01, ****P<0.0001

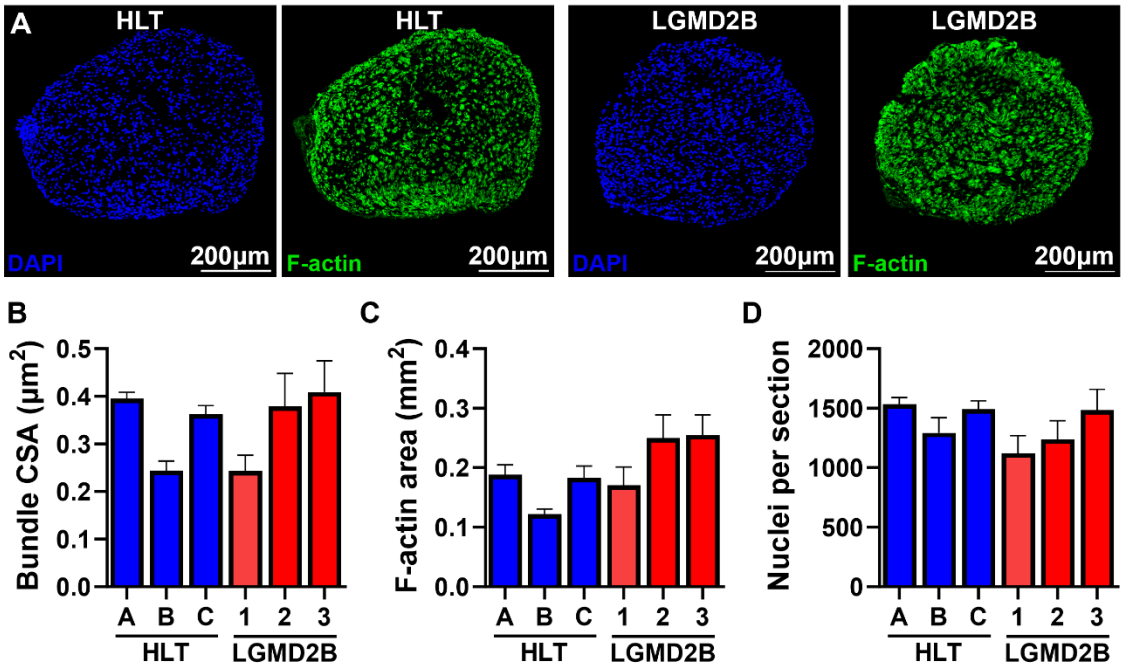

Figure S4. Cross-sectional analysis of 2-wk healthy and LGMD2B myobundles. (**A**) Representative images of 2-wk myobundle cross-sections stained for filamentous actin (F-actin) and DAPI. (**B-C**) Quantifications of (**B**) entire myobundle cross-sectional area (CSA), (**C**) F-actin^{+} area, and (**D**) nuclei per cross-section (N = 1 differentiation and n = 6-8 myobundles per hiPSC line).

A

GEO ID	AGE	SEX	SEVERITY
GSM2934843	12	F	Mild
GSM2934836	NP	F	Mild
GSM2934838	16	M	Mild
GSM2934840	19	F	Mild
GSM2934842	20	M	Mild
GSM2934837	28	M	Moderate
GSM2934839	31	F	Severe
GSM2934841	NP	F	Severe

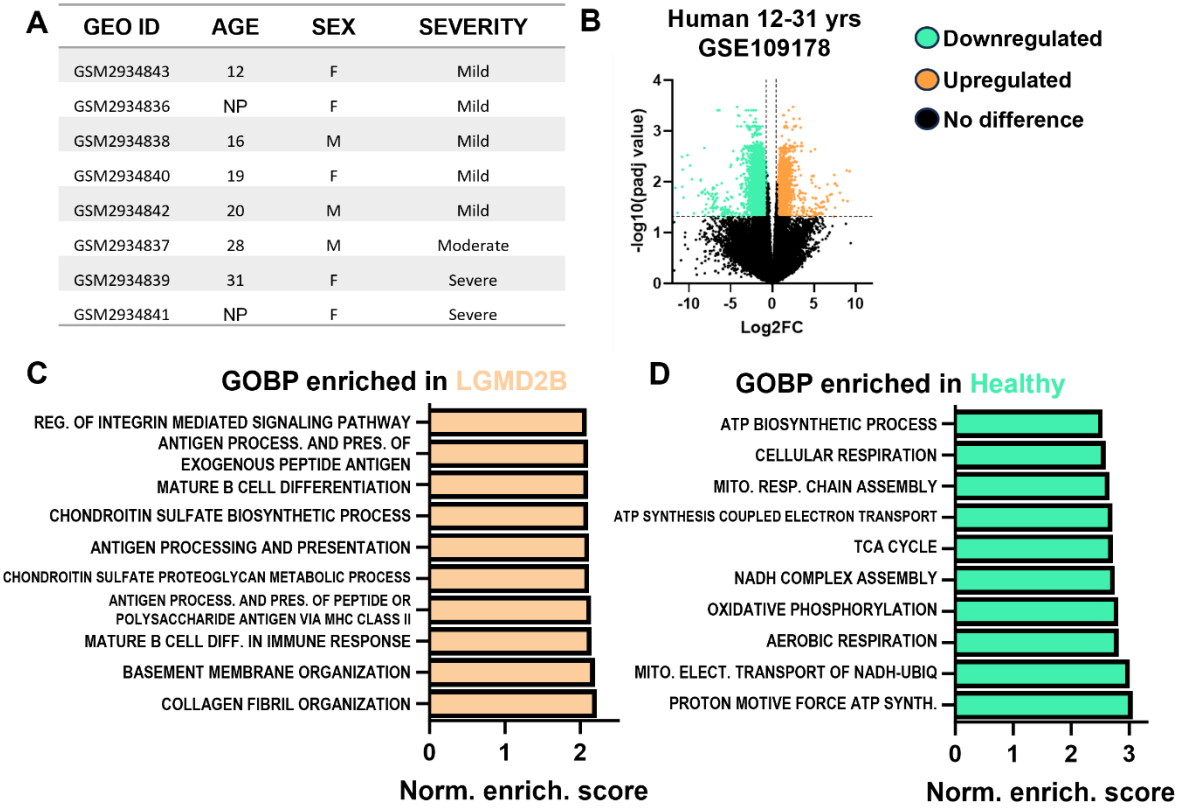

Figure S5. Microarray analysis of human LGMD2B muscle samples. (**A**) LGMD2B patient information (NP = not provided). (**B**) Volcano plot of probes detected in GSE109178. (**C-D**) Top 10 differentially regulated gene ontology biological processes (GOBP) in (**C**) LGMD2B and (**D**) healthy donors.

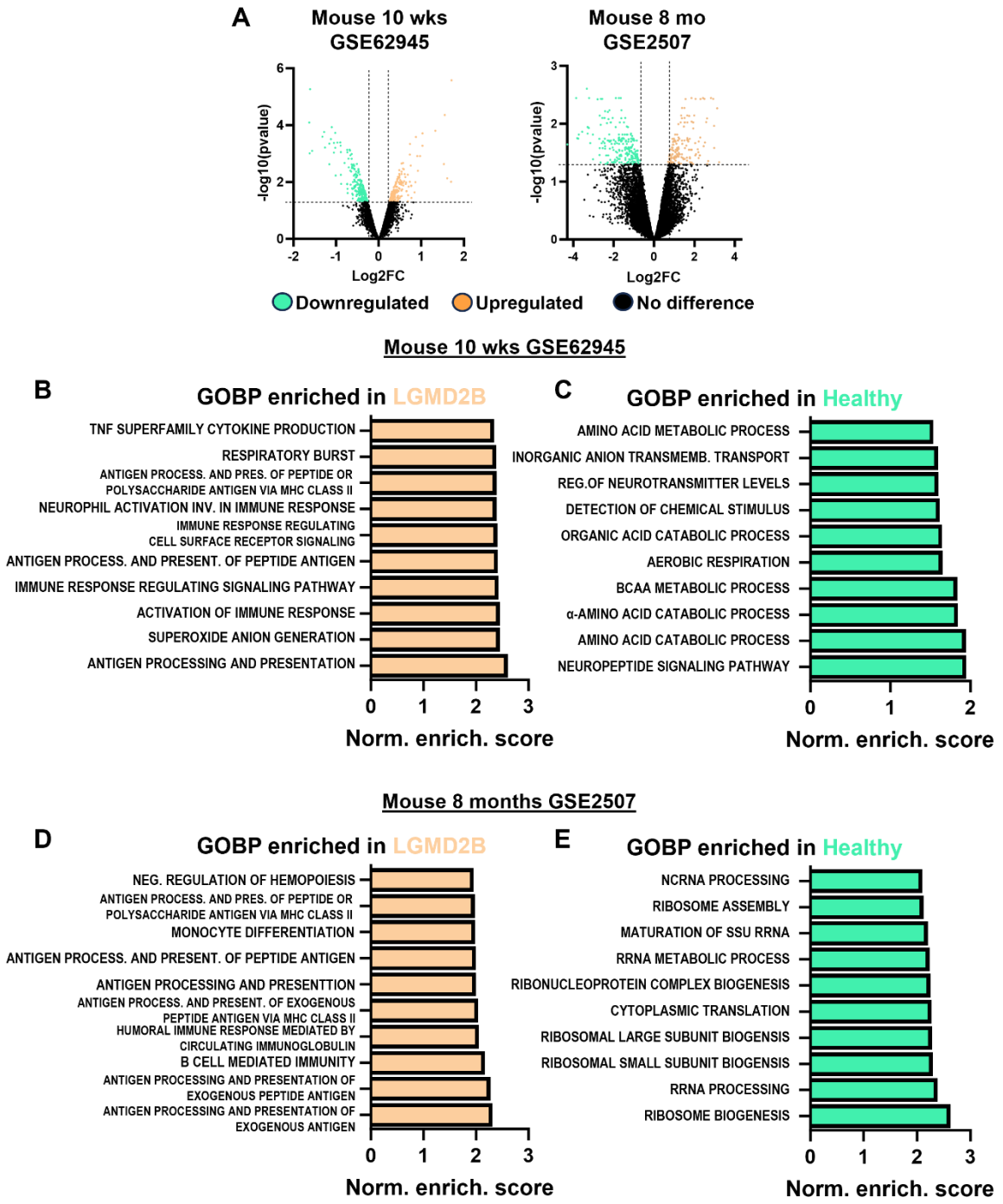

Figure S6. Microarray analysis of presymptomatic and symptomatic mouse LGMD2B muscle samples. (**A**) Volcano plots of probes detected in healthy and presymptomatic (10 weeks, GSE62945) and symptomatic (8 months, GSE2507) LGMD2B mouse muscle. (**B-E**) Top 10 differentially regulated gene ontology biological processes (GOBP) in (**B**) presymptomatic LGMD2B and (**C**) healthy 10-week mice, and (**D**) symptomatic LGMD2B and (**E**) healthy 8-month mice.

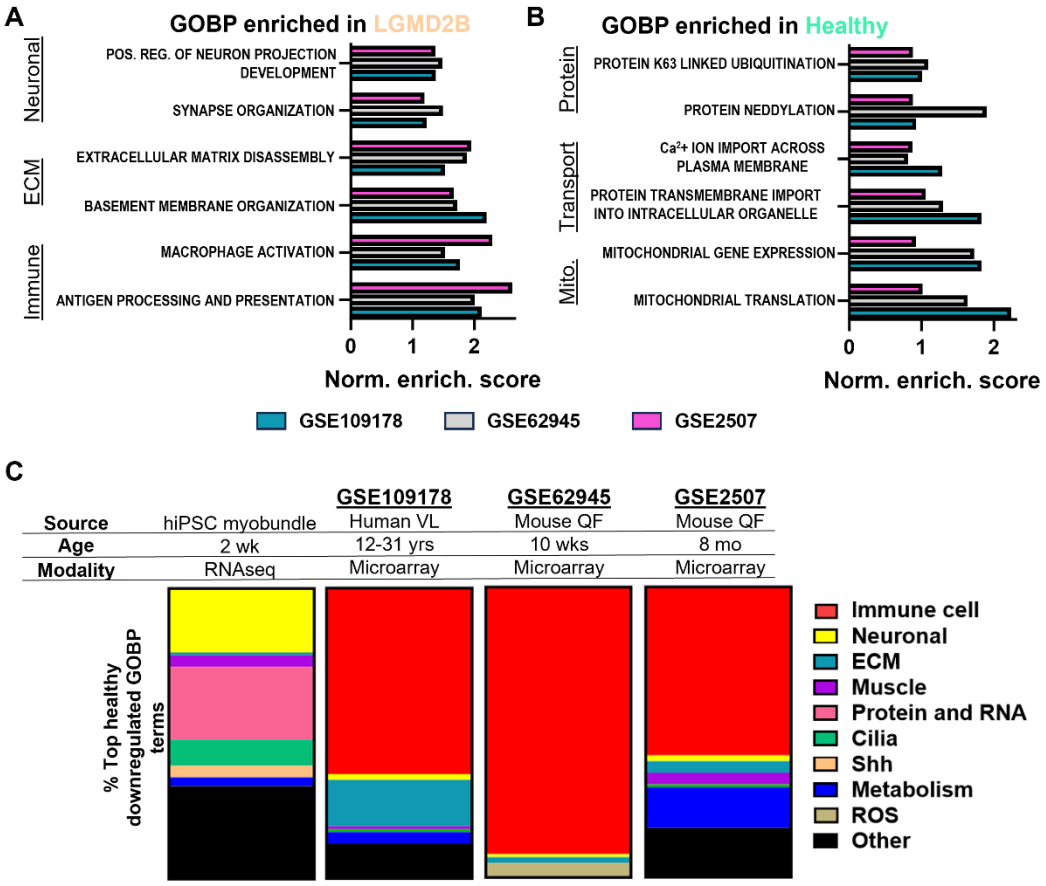

Figure S7. Gene ontology analysis of human and mouse myobundle and microarray data. (**A,B**) Representative gene ontology (GO) terms significantly enriched in (**A**) LGMD2B and (**B**) healthy human and mouse DNA microarray datasets. (**C**) Comparison of GOBP terms grouped by biological function upregulated in LGMD2B vs. healthy muscle in myobundle RNA-seq and native human and mouse microarray datasets.

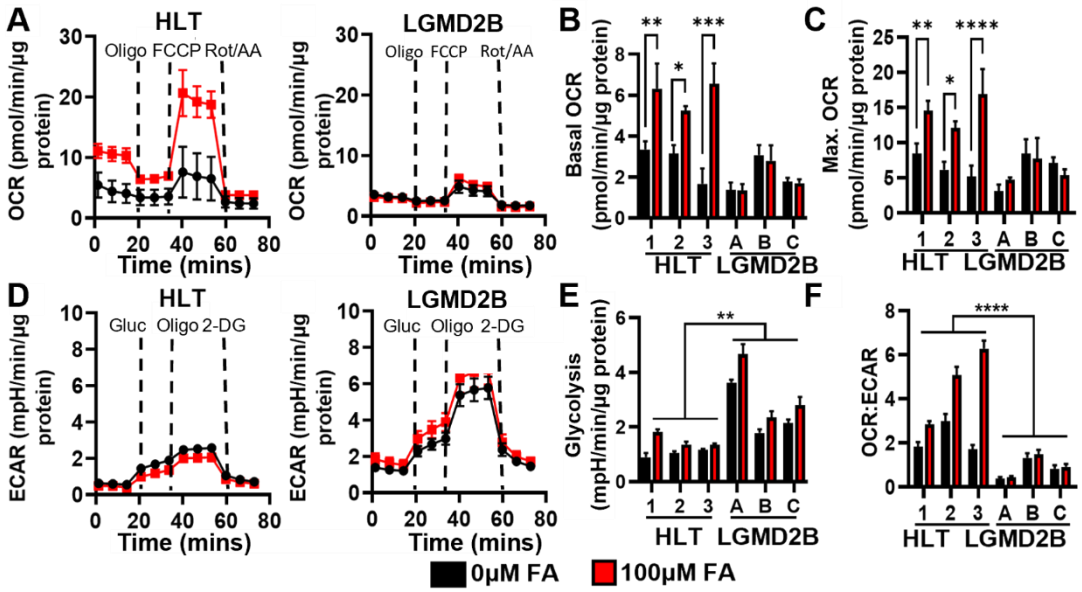

Figure S8. Cellular bioenergetics in healthy and LGMD2B myotubes. (**A**) Representative oxygen consumption rate (OCR) traces of healthy and LGMD2B myotubes cultured in 2D monolayers for 8 days and treated with 0 µM or 100 µM FA during the last 4 days of culture.

(**B,C**) Quantified (**C**) basal and (**D**) maximal OCR (N=1 differentiation, n=8-12 wells per group). (**D**) Representative extracellular acidification rate (ECAR) traces of healthy and LGMD2B myotubes cultured in 2D monolayers for 8 days and treated with 0 μM or 100 μM FA during the last 4 days of culture. (**E,F**) Quantified (**E**) basal ECAR and (**F**) OCR:ECAR ratio (N=1, n=8-12). *P<0.05, **p<0.01, ****P<0.001.

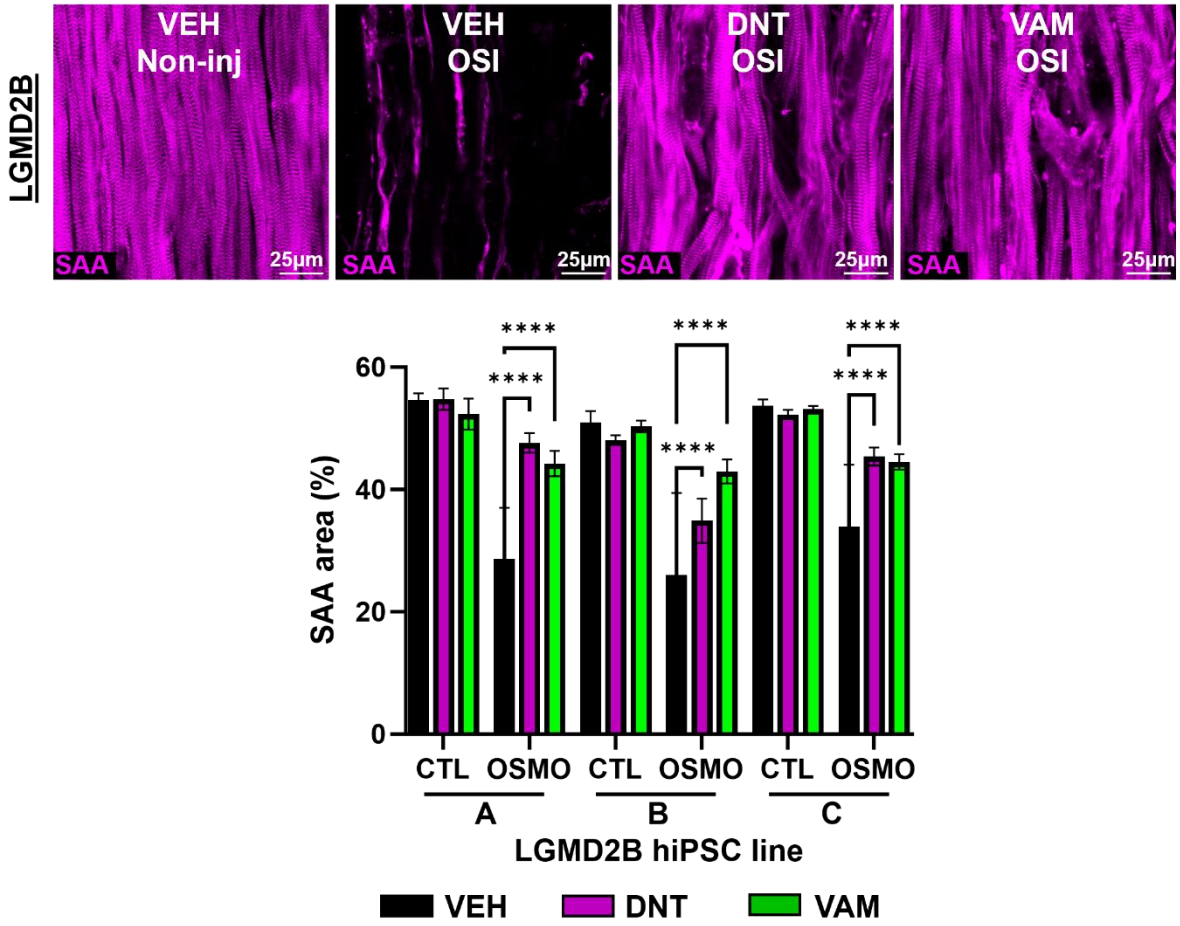

Figure S9. Membrane repair in dantrolene- and vamorolone-treated LGMD2B myobundles. LGMD2B myobundles were treated for 1 week with vehicle (VEH), dantrolene (DNT), or vamorolone (VAM) before undergoing osmotic shock injury (OSI). Representative whole-myobundle images of non-injured (non-inj) and OSI myobundles stained for sarcomeric alpha-actinin (SAA) with corresponding quantification of SAA^+ area (N = 1 differentiation and n = 3 bundles per hiPSC line). ****P<0.001 vs. VEH.

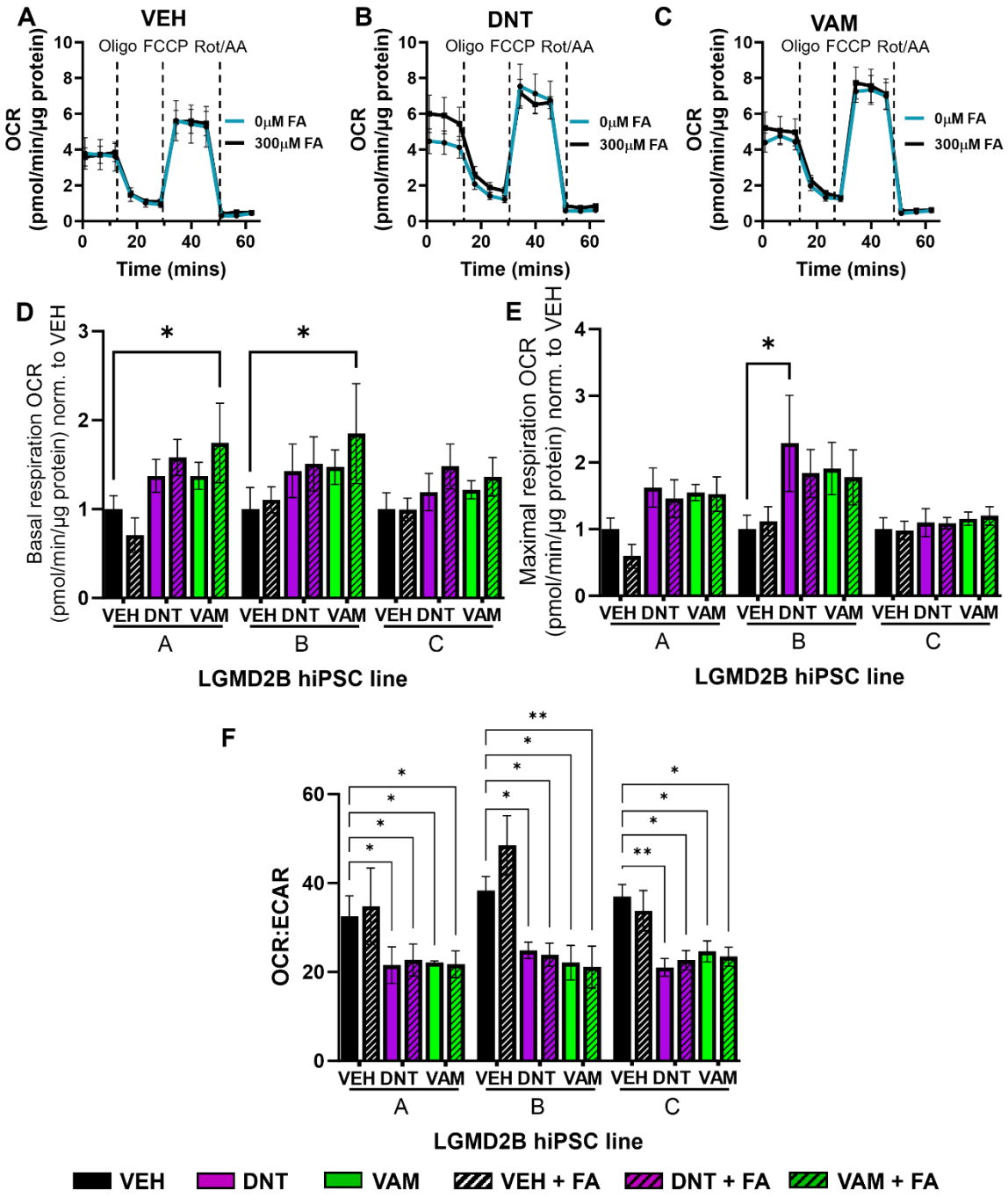

Figure S10. Cellular bioenergetics of dantrolene and vamorolone treated LGMD2B myobundles. (**A-C**) Representative seahorse oxygen consumption rate (OCR) traces in (**A**) vehicle (VEH), (**B**) dantrolene (DNT), and (**C**) vamorolone (VAM) treated LGMD2B myobundles with or without 300 µM fatty acids (FA). Corresponding quantifications of (**D**) basal respiration, (**E**) maximal respiration, and (**F**) OCR:ECAR (n = 5-10 myobundles per group). *P<0.05, **P<0.01 vs. VEH.

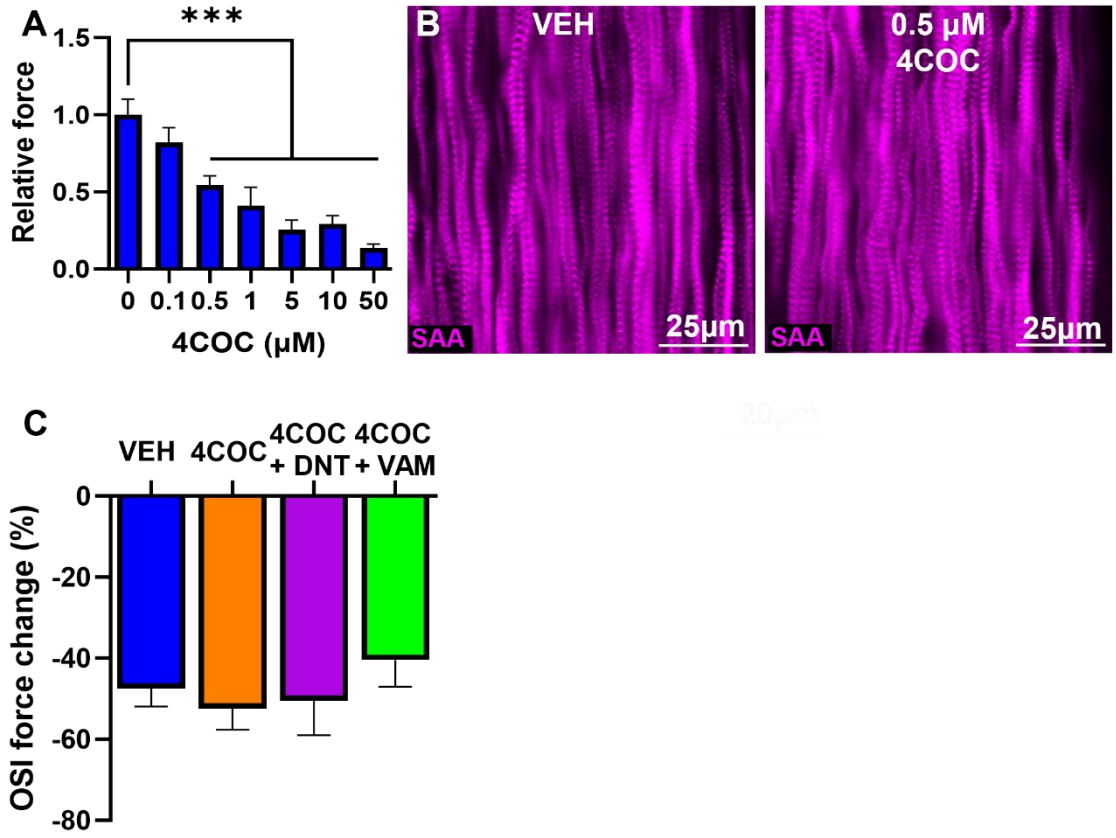

Figure S11. Effects of pharmacologically induced RyR leak on healthy myobundles. (**A**) Dose-dependent effect of 4COC on myobundle force generation (N = 1, n=3-4 per group). (**B**) Representative staining of sarcomeric alpha actinin (SAA) in vehicle (VEH) and 0.5 μM 4COC treated tissues. (**C**) Quantification of force change following OSI injury (N = 1 differentiation and n = 4-5 myobundles per group).

Media and solutions	Formulation
Neutralizing media	Low glucose DMEM supplemented with DNaseI (20 μg/mL, Sigma) and 50% FBS (Hyclone)
Sorting solution	E6 media supplemented with Y27632 (10 μM, Tocris), Doxycycline (1 μg/mL, Sigma), DNaseI (20 μg/mL, Sigma), 1% penicillin/streptomycin (Thermo)
Collecting solution	FBS (Hyclone) supplemented with Y27632 (10 μM, Tocris), Doxycycline (1 μg/mL, Sigma), 1% penicillin/streptomycin (Thermo)
Expansion media	Low glucose DMEM (Thermo) supplemented with 10% FBS (Hyclone), hEGF (10 μg/mL), Dexamethasone (0.4 μg/mL) Doxycycline (1 μg/mL, Sigma), 1% penicillin/streptomycin (Thermo)
Differentiation media 1	Low glucose DMEM (Thermo) supplemented with 1x N2 supplement (Thermo), penicillin G (100 unit/mL, Sigma), 2 mg/ml aminocaproic acid (Sigma), 0.1 μM smoothened agonist (Cayman chemical), EGF (2 ng/ml, Peprotech), LY209314 (Cayman chemical)
Differentiation media 2	Low glucose DMEM (Thermo) supplemented with 1x N2 supplement (Thermo), penicillin G (100 unit/mL, Sigma), 2 mg/ml aminocaproic acid (Sigma),
Differentiation media 3	Low glucose DMEM (Thermo) supplemented with 1x N2

	supplement (Thermo), penicillin G (100 unit/mL, Sigma), 2 mg/ml aminocaproic acid (Sigma), 5 nM T3 (Sigma), 200 µM carnitine, and 10 ng/ml biotin.
2D seahorse media	Seahorse XF DMEM, 5 mM glucose, 4 mM glutamine, 1 mM pyruvate, 0.1% ITS-X, 200 uM carnitine, and 300 uM fatty acids (1:1:1 oleic, linoleic, and palmitic acid)
3D seahorse media	Phys AA MEM (Thermo), 5 mM glucose, 2 mM glutamine, 0.2 mM pyruvate, 0.1% ITS-X, 200 µM carnitine, and 300 µM fatty acids (1:1:1 oleic, linoleic, and palmitic acid)
Cell/hydrogel mixture	12.5 million cells/mL in expansion media, bovine fibrinogen (4 mg/mL, Sigma), growth factor reduced matrigel (20% v/v, Corning), thrombin (0.2 U, Sigma)

Table S1. Cell culture media and solutions

Antibody	Application	Dilution	Company	Product No.
Dysferlin	IF	1:200	Abcam	Ab124684
Dysferlin	WB	1:500	Leica	NCL-Hamlet-2
Fast myosin heavy chain	IF	1:200	DSHB	F59
Myosin heavy chain	WB	1:1000	DSHB	MF20
Sarcomeric alpha actinin	IF, WB	IF (1:200) WB (1:1000)	Sigma	A7732
Myomesin 2	IF	1:200	DSHB	mMaC myomesin B4
Titin	IF	1:200	DSHB	9D10
Pax7	IF	1:200	DSHB	Pax7
Myf5	IF	1:200	SCBT	Sc-302
Myogenin	IF	1:200	Abcam	Ab124800
RyR1	WB	1:1000	Thermo	MA3-925
DHPRa	WB	1:500	DSHB	IIC12D4
F-actin 488	IF	1:400	Thermo	A12379
DAPI	IF	1:400	Sigma	D1306
LipidSpot 610	IF	1:500	Biotium	70069

Table S2. Antibody information

Movie S1. Representative 2 week healthy and LGMD2B myobundle calcium transients.