Table S1. Sequences of the oligonucleotide used in this study.

Primer used for sequencing for DMD gene sequencing and ssODN production			
TARGET	PRIMER NAME	SEQUENCE	PRODUCT LENGHT
DMD GENE	DMD_ex48AF	TGTGTAAGGTGTGTGTTTTGAAA	396
	DMD_ex48AR	AGGAGATAACCACAGCAGCA	
	DMD_ex48BF	AAGCTCAAATAAAAGACCTTGGG	555
	DMD_ex48BR	CACCCTCAGTATTCTCCACCA	
	DMD_ex48CF	AACATCAGGGGTAAGTGGGA	394
	DMD_ex48CR	CAGTAACAAATATGATGCAGGCA	
	DMD1fosfo_48BR	5'PHOSPHO-CACCCTCAGTATTCTCCACCA	
DMD EXON 47-53 ON cDNA	DMD1_47F	TCTCCAGTGGATAAAGGTTTCC	806 wt 595 DMD1
	DMD1_53R	GGTGTTCTTGTACCTCATCCCA	
Primer used for sequencing off target regions			
TARGET	PRIMER NAME	SEQUENCE	PRODUCT LENGHT
ADAMTS20	ADAMTS20_F	CTGCTATGCCTTCAACCAACA	172
	ADAMTS20_R	GTGTGCGTGATTGGTCTTGT	
FTO	FTO_F	TGTACAACAAGCATCTGAGATTG	210
	FTO_R	CCCCATTCTCCCTGTAGACA	
L3MBTL3	L3MBTL3_F	GGAAACTTCACCAATGCCTAGT	216
	L3MBTL3_R	CCGAAAGACATTATGACCCAGAG	
LIMCH1	LIMCH1_F	TGGCACCAAAATGACCCTTT	210
	LIMCH1_R	ACCTGTGGTCTCTGAGTCAC	
MMP15	MMP15_F	GGTACGAGTGAAAGCCAACC	156
	MMP15_R	CTGGGAAAGGAGGTCTGAGG	
PRRT3	PRRT3_F	AGCTCCTCTCACCCGCTG	163
	PRRT3_R	TCTCATTTTCCTCTTTGTGGCA	
RP1-65P5.1	RP1-65P5.1_F	AGCACAACAAATGACCAGCA	164
	RP1-65P5.1_R	AGGTAAGGATGCTATGGGGC	
SCARB1	SCARB1_F	CACTCTCAGACACCACCTCC	151
	SCARB1_R	CACACAGCCACATGAGGAAT	
CANCNA1B	CACNA1B_F	GACCTTTTCAGCATCCATAGAGA	336
	CACNA1B_R	GGAGTGGAAATGAGACCCTCT	
CLEC16A	CLEC16A_F	TCAAGAAAGGTGAGGTCAGGG	150
	CLEC16A_R	CAGCCTTCCCACTCCTCAAG	
IL1RAP	IL1RAP_F	GTGATATGTCTGTTTTGGGCCT	157
	IL1RAP_R	GCATTTCAAAGCACCTGAGAG	
RP11-522M21.3	RP11-522M21.3_F	TCTAACTCAGTCTCCAGGTGG	160
	RP11-522M21.3_R	TTGTTATGGTGAGGGTTGGC	
SCP2	SCP2_F	GCCAAAAGAAACAGCCAAACC	181
	SCP2_R	ACTGAGCACTTACTACACTAGGT	
STK3	STK3_F	TGAAAACCAACCTGAAGATTCCA	166
	STK3_R	CCTGGCCGATTACCTTACTTTC	
TAF1B	TAF1B_F	TGGACCCATCAAAATTATTGGCT	168
	TAF1B_R	GAGGTGTGTTACTGACTTCAAGA	
AC009234.1-NRXN1	AC009234.1_F	TGTCAAAACAGTGTTGCCTTT	425
	AC009234.1_R	TGCTTGCACGTAGAACAACT	
AC079163.1-AC079248.1	AC079163.1_F	ATCGTCCACAGTCCACAGAG	367
	AC079163.1_R	CGGGAAGTTGAAGCAGGAGA	
ADH5P2-RP11-339A11.1	339A11.1_F	GCTCTTGATTTTGCCTGGTCT	483
	339A11.1_R	AGGTCACAAGTGTCTCCTGA	
BAI3-AL358133.1	BAI3_F	ACTACGCTCAAGTAAATCAAAGC	593
	BAI3_R	CATCTCTCCCTTATGTGCAATGT	
CSRP2BP-PET117/CSRP2BP	CSRP2BP_F	TCCTGGGAAAACTTGAGATGGA	223
	CSRP2BP_R	TTGTTGAACCAGGTCCCCAT	
FECHP1-KRT8P18	KRT8P18_F	TGTGCTCTGCTTGCTAAACA	400
	KRT8P18_R	CCCAGCACTGACTCTCCTAT	
IQCJ-SCHIP1-IQCJ-SCHIP1/IQCJ	SCHIP1_IQCJ_F	ATGGAGGGAAGTCTTCAGCC	395
	SCHIP1_IQCJ_R	CCAATGAGACTAGCCCCTCC	
LINC00460-RPL35P9	RPL35P9_F	GGGGCTTCTTGAAAATCTTCCT	264
	RPL35P9_R	CATGCCTTTTCTACTTTCAACCA	

Primer used for sequencing off target regions (continues)			
TARGET	PRIMER NAME	SEQUENCE	PRODUCT LENGHT
MXRA5P1-RPS24P1	RPS24P1_F	TCTGCTTTCTCTTCTGTTGATGA	299
	RPS24P1_R	GCATCCTTATTTATGAGACCCCT	
RNA5SP336-NELL1	NELL1_F	TGATGGGTTTGTGTAGGAGGG	298
	NELL1_R	TCAGGTTGAGGCATTAACTTCA	
RP11-183E24.2-CTD-2544M6.1	CTD-2544M6.1_F	CATCAGCAGCCTACCCCTG	296
	CTD-2544M6.1_R	TCAGCGACAAAATAGGGAAGC	
RP11-197P3.1-C9orf40	C9orf40_F	ACATTGTGCCTCTCCCTGTC	229
	C9orf40_R	ATGCTTCCTGTATACCCTGTG	
RP11-354I10.1-OFCC1	OFCC1_F	GGTGGTATGTGACTTTTGGGG	284
	OFCC1_R	ACCATCCTCCCATCTCAGAC	
RP11-371F15.3-RP11-577B7.1	RP11-577B7.1_F	AAGGCACCCACCACTTTG	208
	RP11-577B7.1_R	TCAAATTCTGCTGAGTCACGG	
RP11-481N16.1-RP11-47P18.1	RP11-47P18.1_F	AGCCCAGAAGATTTCATTGCC	458
	RP11-47P18.1_R	ACTGACAGACACCTCACTCA	
RP11-545H22.1-AC006552.1	AC006552.1_F	GGTTCCAAGGATTTCTGGAGC	234
	AC006552.1_R	CTTCCCCTCCCTGAGATGC	
RP11-756H20.1-CTC-394G3.1	CTC-394G3.1_F	GTCTGTAGTCTCTGCATCCCA	299
	CTC-394G3.1_R	CTTCCCCAGTTTCATTCAGCA	
snoU13-RP5-1168A5.1	RP5-1168A5.1_F	ACTGGCTGTGTCTTACTCAAA	374
	RP5-1168A5.1_R	GTAAGCAGCAACAACTTGATACA	
TBC1D5-AC090960.1	AC090960.1_F	GTTGTTGGACACGTTGGGAA	209
	AC090960.1_R	TCAGATTACGGAGGATAGGAGT	
TECTA-RP11-775A1.2	RP11-775A1.2 F	AGTCACTTAACATGGAGCCCT	509
	RP11-775A1.2 R	TGGCATTCAGAAACATCAGCA	
TMEM200A-Y_RNA	Y_RNA_F	GGTGCTGCCTTTACTTGACT	196
	Y_RNA_R	GGTCAGAGGGTCAGAAGTCA	
Primer used for qRT-PCR			
TARGET	PRIMER NAME	SEQUENCE	PRODUCT LENGHT
COL1A1	COL1A1_F	CCCCTGGAAAGAATGGAGATG	148
	COL1A1_R	TCCAAACCACTGAAACCTCTG	
ACTA2	ACTA2_F	TACTGCTGAGCGTGAGATTG	108
	ACTA2_R	TTCTCAAGGGAGGATGAGGA	
TGFβ1	TGFβ1_F	AAGTGGACATCAACGGGTTC	218
	TGFβ1_R	GTCCTTGCGGAAGTCAATGT	
DMD EXON 13-17 ON cDNA	DMD_13F	ACTGCTGCTTTGGAAGAACA	499
	DMD_17R	CCCTTGTGGTCACCGTAGTT	
GAPDH	GAPDH_F	ATGTTCGTCATGGGTGTGAA	173
	GAPDH_R	GTCTTCTGGGTGGCAGTGAT	

Table S2. Sequence of the DMD insert of the pUCIDT-AMP

INSERT SEQUENCE OF THE pUCIDT-AMP
TGTGTAAGGTGTGTGTTTTGAAATTCCAAAAAGGTATTAGTTTCTTTAAAGCAAAGAATTTTTGTAGCAGGTTAATGAATAATTTT GAATACATTGGTTAAATCCCAACATGTAATATATGTAAATAATCAATATTATGCTGCTAAAATAACACAATTCAGTAAGATTCTGT AATATTTCATGATAAATAACTTTTGAAAATATATTTTTAAACATTTTGGCTTATGCCTTGAGAATTATTTACCTTTTTAAAATGTA TTTTCCTTTCAGGTTTCCAGAGCTTTACCTGAGAAACAAGGAGAAATTGAAGCTCAAATAAAAGACCTTGGGCAGCTTGAAAAAAA GCTTGAAGACCTTGAAGAGCAGTTAAATCATCTGCTGCTGTGGTTATCTCCTATTAGGAATCAGTTGGAAATTTATAACCAACCAA ACCAAGAAGGACCATTTGACGTTAAGGAAACTGAAATAGCAGTTCAAGCTAAACAACCGGATGTGGAAGAGATTTTGTCTAAAGGG CAGCATTTGTACAAGGAAAAACCAGCCACTCAGCCAGTGAAGAGGAAGTTAGAAGATCTGAGCTCTGAGTGGAAGGCGGTAAACCG TTTACTTCAAGAGCTGAGGGCAAAGCAGCCTGACCTAGCTCCTGGACTGACCACTATTGGAGCCTGTAGGGAACTTTTTGCTTTAA ATATTTTTGTCTTTTTTAAGAAAAATGGCAATATCACTGAATTTTCTCATTTGGTATCATTATTAAAGACAAAATATTACTTGTTA AAGTGTGGTAAGGAAGACTTTATTCAGGATAACCACAATAGGCACAGGGACCACTGCAATGGAGTATTACAGGAGGTTGGATAGAG AGAGATTGGGCTCAACTCTAAATACAGCACAGTGGAAGTAGGAATTTATAGCCAAGGAGCAGTGTAGGAGTCAGTAGATGGAAAAT TATTAAGAGGAAACATCAGGGGTAAGTGGGATTCTGGCTAAACCAACCTCACAGGATTCTTGCTGAAGATAGGCCAGGGTTATCTT ATCAGACAACCCTTGGGGAATGGTGGAGAATACTGAGGGTGATCAGGTACCAAGGATAAAGGG

Table S3. Composition of LI-BPEL medium.

Component	Volume for 500 mL
Iscove's Modified Dulbecco's Medium (IMDM) (Thermo Fisher Scientific, cat# 21056-023)	215 mL
Ham's F-12 Nutrient Mix (Thermo Fisher Scientific, cat# 31765-027)	215 mL
Protein-free Hybridoma Medium-II (PFHMII) (Thermo Fisher Scientific, cat# 12040-077)	25 mL
Poly(Vinyl Alcohol) (PVA) 5% wt/vol in distilled water (Sigma-Aldrich cat# P8136)	12.5 mL
Bovine Serum Albumin (BSA) 10% wt/vol in IMDM (Sigma-Aldrich, cat# A3311)	12.5 mL
Chemically defined lipid concentrate (CDLC) 100× (Thermo Fisher Scientific, cat# 11905-031)	5 mL
Insulin-transferrin-selenium-ethanolamine (ITS-X) (Thermo Fisher Scientific, cat# 51599-056)	0.5 mL
α-Monothioglycerol 1.3% in IMDM (Sigma-Aldrich, cat# M6145)	1.5 mL
L-ascorbic acid 2-phosphate 5 mg/mL in distilled water (Sigma-Aldrich, cat# A8960)	5 mL
Glutamax (Thermo Fisher Scientific, cat# 35050-038)	5 mL
Penicillin–streptomycin (Euroclone, Cat# ECB3001D)	2.5 mL
Phenol red (Sigma-Aldrich, cat# P0290)	1 mL

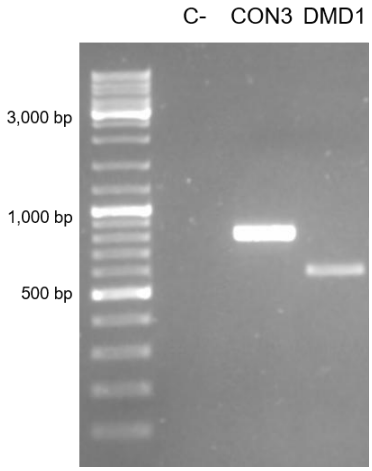
Table S4. Antibodies used for immunofluorescence experiments.

Antibodies	Dilution	Source	Identifier
Rabbit anti-OCT4	1:250	Abcam	Cat# ab19857 RRID: AB_445175
Rabbit anti-SOX17	1:300	Cell Signaling Inc.	Cat# 81778; RRID: AB_2650582
Mouse anti-FOXA2	1:200	Abcam	Cat# ab60721; RRID: AB_941632
Mouse anti-cardiac troponin T (cTnT)	1:200	Thermo Fisher Scientific	Cat# MA- 512960; RRID: AB_11000742
Mouse anti-NESTIN	1:150	Abcam	Cat# ab22035; RRID: AB_446723
Rabbit anti-PAX6	1:300	BioLegend	Cat# PRB-278P; RRID: AB_291612
Rabbit anti-Dystrophin	1:150	Abcam	Cat# ab15277; RRID: AB_301813;
Rabbit anti-COL1A1 (E8F4L) XP	1:200	Cell Signaling Inc.	Cat# 72026; RRID:AB_2904565
Mouse anti-α-Actinin (Sarcomeric)	1:200	Sigma-Aldrich	Cat# A7811 RRID: AB_476766

Table S5. Characteristics of the gRNA selected for CRISPR experiments.

Guide Sequence + PAM	MIT Specificity Score	CFD Spec. score	Predicted Efficiency		Off-targets for 0-1-2-3-4 mismatches	Off target positions
			Doench '16	Mor.-Mateos		
GACCATTTGACGTTCAGGTA *GGG*	74	95	47	44	0 - 0 - 0 - 5 - 31	3:intergenic:RP11-756H20.1-CTC-394G3.1; 3:intron:RP11-522M21.3; 3:intergenic:CSRP2BP-PET117/CSRP2BP; 3:intron:LIMCH1; 3:intergenic:TECTA-RP11-775A1.2; 4:intron:ADAMTS20; 4:intergenic:AC079163.1-AC079248.1; 4:intron:IL1RAP; 4:intron:L3MBTL3; 4:intergenic:AC009234.1-NRXN1; 4:intron:FTO; 4:intergenic:RP11-197P3.1-C9orf40; 4:intergenic:RP11-354I10.1-OFCC1; 4:intergenic:LINC00460-RPL35P9; 4:intron:TAF1B; 4:intergenic:FECHP1-KRT8P18; 4:exon:SCARB1; 4:intergenic:RP11-371F15.3-RP11-577B7.1; 4:intron:STK3; 4:intergenic:TMEM200A-Y_RNA; 4:intron:CLEC16A; 4:intergenic:snoU13-RP5-1168A5.1; 4:intergenic:TBC1D5-AC090960.1; 4:intron:RP1-65P5.1; 4:intron:SCP2; 4:intergenic:RNA5SP336-NELL1; 4:intergenic:IQCJ-SCHIP1-IQCJ-SCHIP1/IQCJ; 4:intron:PRRT3; 4:intergenic:RP11-183E24.2-CTD-2544M6.1; 4:intron:CACNA1B; 4:intergenic:ADH5P2-RP11-339A11.1; 4:intergenic:RP11-481N16.1-RP11-47P18.1; 4:exon:MMP15; 4:intergenic:BAI3-AL358133.1; 4:intergenic:RP11-545H22.1-AC006552.1; 4:intergenic:MXRA5P1-RPS24P1.

A



B

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WT      1 AAGGTTTCCAGAGCTTTACCTGAGAAACAAGGAGAAATTGAAGCTCAAAT  50
          ||||||||||||||||||||||||||||||||||||||||||||||||||
DMD1    1 AAGGTTTCCAGAGCTTTACCTGAGAAACAAGGAGAAATTGAAGCTCAAAT  50

WT     51 AAAAGACCTTGGGCAGCTTGAAAAAAAGCTTGAAGACCTTGAAGAGCAGT 100
          ||||||||||||||||||||||||||||||||||||||||||||||||||
DMD1   51 AAAAGACCTTGGGCAGCTTGAAAAAAAGCTTGAAGACCTTGAAGAGCAGT 100

WT    101 TAAATCATCTGCTGCTGTGGTTATCTCCTATTAGGAATCAGTTGGAAATT 150
          ||||||||||||||||||||||||||||||||||||||||||||||||||
DMD1  101 TAAATCATCTGCTGCTGTGGTTATCTCCTATTAGGAATCAGTTGGAAATT 150

WT    151 TATAACCAACCAAACCAAGAAGGACCATTTGACGTTAAGGAAACTGAAAT 200
          |||||||||||||||||||||||||||||||||||.||
DMD1  151 TATAACCAACCAAACCAAGAAGGACCATTTGACGTTCAG----------- 189

WT    201 AGCAGTTCAAGCTAAACAACCGGATGTGGAAGAGATTTTGTCTAAAGGGC 250

DMD1  190 ---------------------------------------------------  189

WT    251 AGCATTTGTACAAGGAAAAACCAGCCACTCAGCCAGTGAAGAGGAAGTTA 300

DMD1  190 ---------------------------------------------------  189

WT    301 GAAGATCTGAGCTCTGAGTGGAAGGCGGTAAACCGTTTACTTCAAGAGCT 350

DMD1  190 ---------------------------------------------------  189

WT    351 GAGGGCAAAGCAGCCTGACCTAGCTCCTGGACTGACCACTATTGGAGCCT 400

DMD1  190 ---------------------------------------------------  189

WT    401 CTCCTACTCAGACTGTTACTCTGGTGACACAACCTGTGGTTACTAAGGAA 450
          ||||||||||||||||||||||||||||||||||||||||||||||||||
DMD1  190 CTCCTACTCAGACTGTTACTCTGGTGACACAACCTGTGGTTACTAAGGAA 239

WT    451 ACTGCCATCTCCAAACTAGAAATGCCATCTTCCTTGATGTTGGAGGTACC 500
          ||||||||||||||||||||||||||||||||||||||||||||||||||
DMD1  240 ACTGCCATCTCCAAACTAGAAATGCCATCTTCCTTGATGTTGGAGGTACC 289
```

C

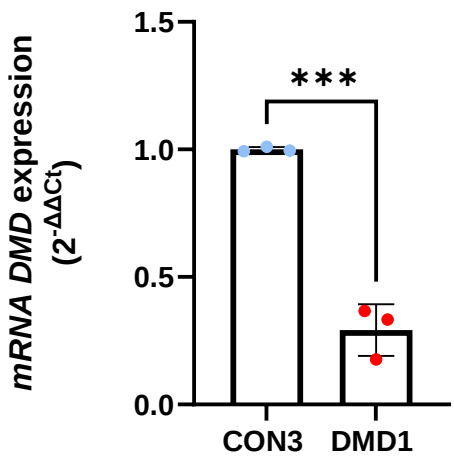

Figure S1. Analyses of splicing between exon 48 and exon 51 of *DMD* mRNA. (A) Representative agarose gel image of exons 47-53 PCR product on cDNA obtained from healthy control (CON3) and DMD1. **(B)** Alignment of wild type (WT) and DMD1 mRNA sequence obtained from Sanger sequencing results. **(C)** *DMD* mRNA level determined in total RNA extracted from CON3 and DMD1 iPSC-CMs. Expression data were normalized to *GAPDH*, and relative mRNA levels were determined using the $2^{-\Delta\Delta Ct}$ method. (n = 3 biological replicates; data are expressed as mean ± SEM. Two-tailed Student's t-tests; ****P < 0.001*).

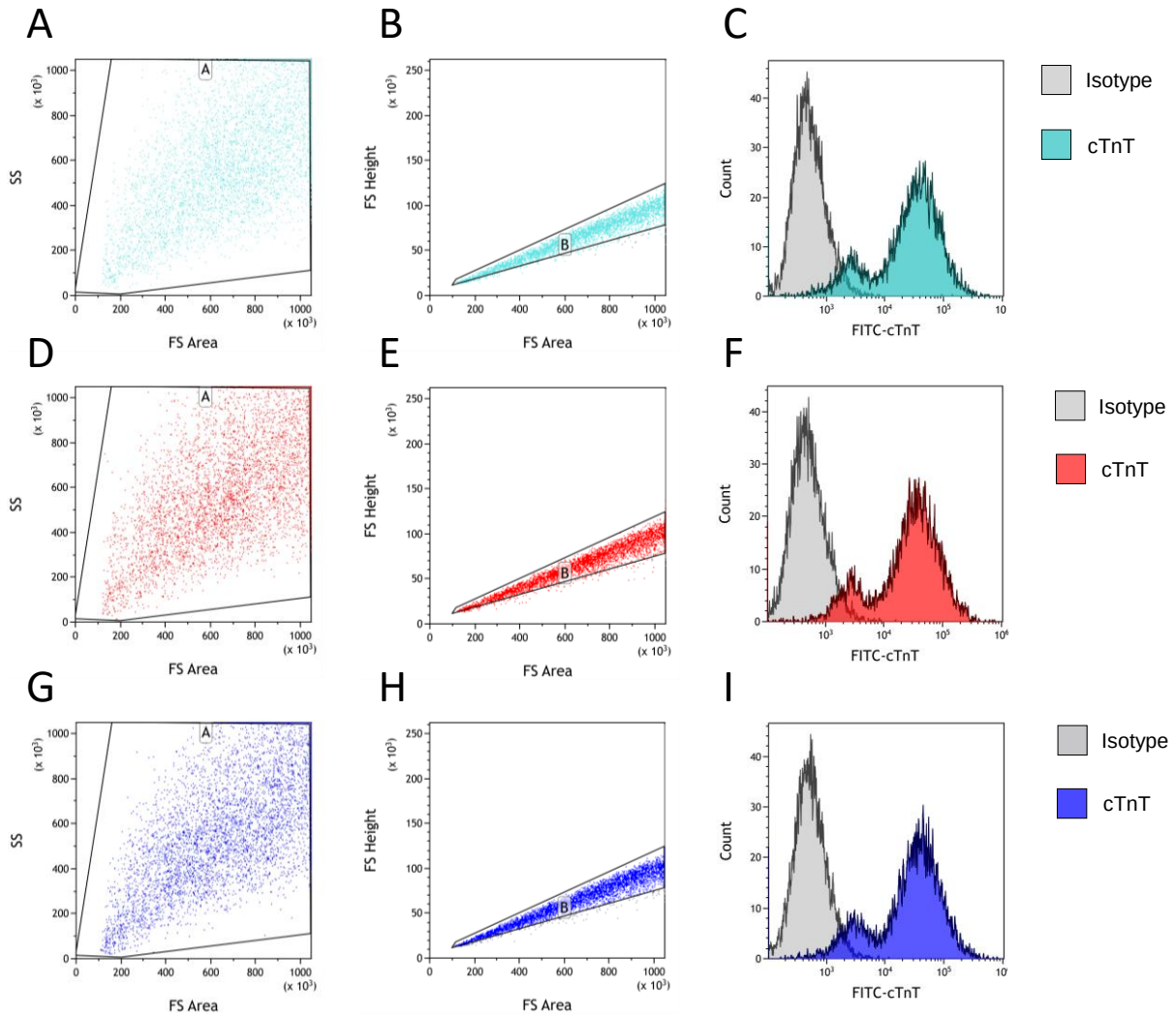

Figure S2. FACS analyses of iPSC-CMs using anti-cTnT atibody. Gating strategy and representative FACS analysis histogram showing that a similar percentages of (**A-C**) control (CON3; light blue), (**D-F**) DMD1 (red, middle) and (**G-I**) ISO-50 (blue, down) are positive for the cardiomyocyte marker troponin T. (**A**,**B**,**D**,**E**,**G**,**H**) Gating strategy used

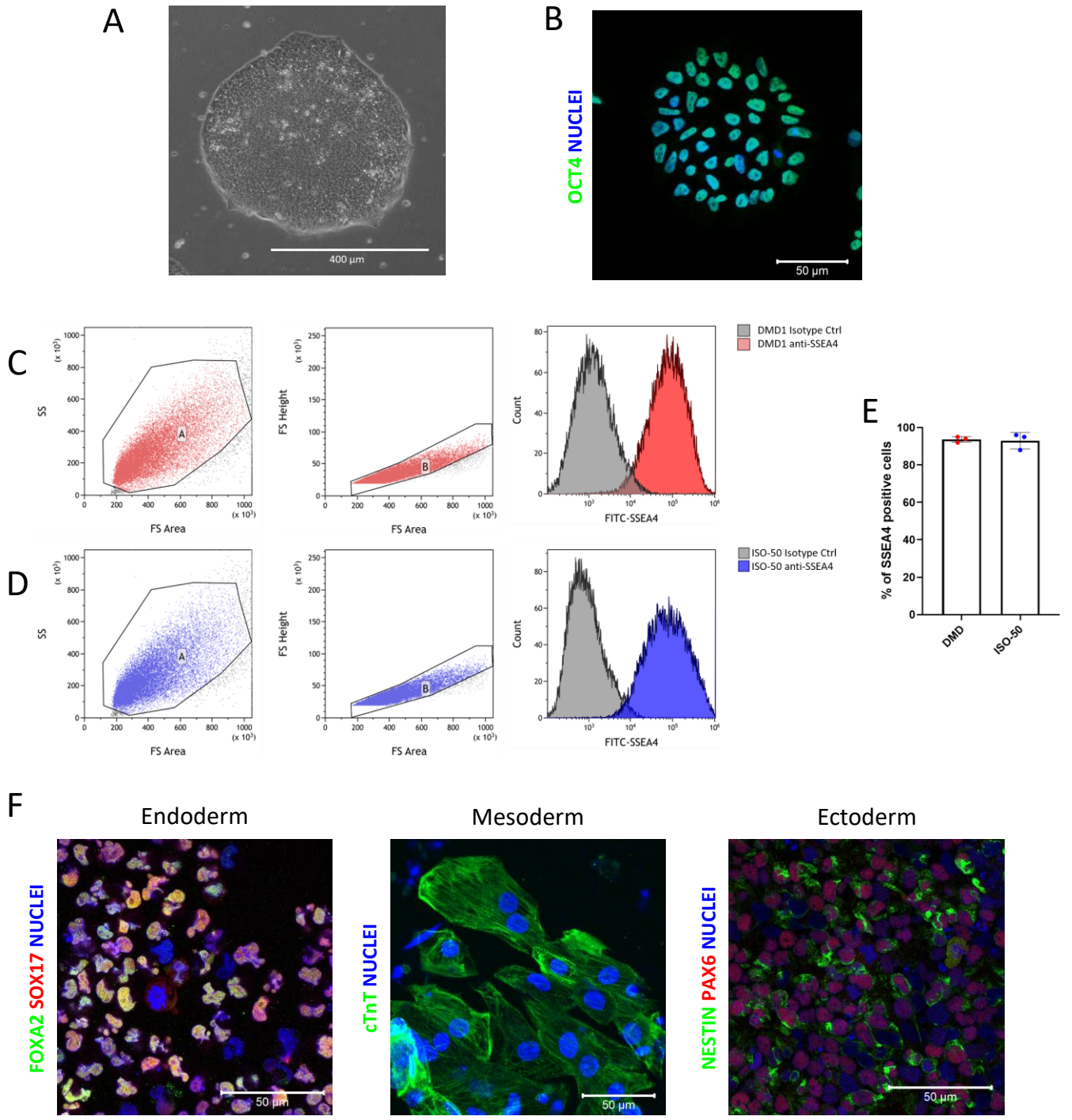

Figure S3. Pluripotency characterization of ISO-50 edited iPSCs. (A) Transmitted light images showing the pluripotent cell morphology. **(B)** Immunofluorescence image showing in green the expression of OCT4**. (C-D)** Gating strategy and **r**epresentative FACS analysis histogram showing that large percentages of ISO-50 cells expressed the pluripotency marker SSEA4 at similarly to original DMD1 cells, as indicated by **(E)** the summary data graph (values are expressed as mean ± SEM, Student's t test, n = 3). **(F)** *In vitro* trilineage differentiation assay highlighted that ISO-50 was capable of differentiation into all three germ layers.

ADAMTS20

```
DMD1     1 AGGTTCTGGCCATACCTGAATGTTAAATGGCACCTGCTACAATGCTAAAA   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 AGGTTCTGGCCATACCTGAATGTTAAATGGCACCTGCTACAATGCTAAAA   50

DMD1    51 ATAAATGTATAAATATGATAGATGGACTCATTGTAYACCATGTCACTACA  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 ATAAATGTATAAATATGATAGATGGACTCATTGTAYACCATGTCACTACA  100

DMD1   101 AGACCAATCACG  112
           ||||||||||||
ISO-50 101 AGACCAATCACG  112
```

CLEC16A

```
DMD1     1 GAGAGCTGGACCGTGTGAAGTTCAGGCAGGGCAGGTGGCATGAATGGGGT   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 GAGAGCTGGACCGTGTGAAGTTCAGGCAGGGCAGGTGGCATGAATGGGGT   50

DMD1    51 CCAGGGCTGGCTGTGGTGAATGCATTCGCTTGAGGAGTGGGAAGGCTGAA  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 CCAGGGCTGGCTGTGGTGAATGCATTCGCTTGAGGAGTGGGAAGGCTGAA  100

DMD1   101 T  101
           |
ISO-50 101 T  101
```

L3MBTL3

```
DMD1     1 GTCTCAGCGCCAGCTGTAAGAACAGGGAGGTGACTAAGTGGCAGTGCAGA   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 GTCTCAGCGCCAGCTGTAAGAACAGGGAGGTGACTAAGTGGCAGTGCAGA   50

DMD1    51 GGGACATGGTAGGGCTTCCCATAACTGAGCAGTTTCCCTACCTGAACTTC  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 GGGACATGGTAGGGCTTCCCATAACTGAGCAGTTTCCCTACCTGAACTTC  100

DMD1   101 TAATGATGTCTGAAATTTACAGAGAATTATTCCTCTGGGTCAA  143
           |||||||||||||||||||||||||||||||||||||||||||
ISO-50 101 TAATGATGTCTGAAATTTACAGAGAATTATTCCTCTGGGTCAA  143
```

LIMCH1

```
DMD1     1 GCCACCCTGGCCCTACCTGACCGTCCAATGTGCCTTGCCTGTCCCTGCCA   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 GCCACCCTGGCCCTACCTGACCGTCCAATGTGCCTTGCCTGTCCCTGCCA   50

DMD1    51 CAGGGCCTTTGCAGTGGCCGCTCCAGGCAGCATTCCTCATGTGGCTGTGT  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 CAGGGCCTTTGCAGTGGCCGCTCCAGGCAGCATTCCTCATGTGGCTGTGT  100

DMD1   101 GAAT  104
           ||||
ISO-50 101 GAAT  104
```

CACNA1B

```
DMD1     1 ATGGGGATTTATAGTAATGAATTTTTTGATATGAAATATCTTTGCAATCT   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 ATGGGGATTTATAGTAATGAATTTTTTGATATGAAATATCTTTGCAATCT   50

DMD1    51 TAGAATAAATCCAAGTGGTTATTATATTTATTTTGTAATATGCCATTGGA  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 TAGAATAAATCCAAGTGGTTATTATATTTATTTTGTAATATGCCATTGGA  100

DMD1   101 TTCTCTTTAACATTTTTTGTAGGATTTTTGGATTGATATTTTTAAGCGAG  150
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50 101 TTCTCTTTAACATTTTTTGTAGGATTTTTGGATTGATATTTTTAAGCGAG  150

DMD1   151 ATGTGTTCTGTAGCTTTCTTTTGTCACATTTGAGGATCATAAGGTTTAAA  200
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50 151 ATGTGTTCTGTAGCTTTCTTTTGTCACATTTGAGGATCATAAGGTTTAAA  200

DMD1   201 TATCAATGACATAGAGTTATCTATTGCTTAACTTCACAGCTCCATTCCTG  250
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50 201 TATCAATGACATAGAGTTATCTATTGCTTAACTTCACAGCTCCATTCCTG  250

DMD1   251 AAAATCAAATGTTCTAAAAGAGGGTCTCT  279
           |||||||||||||||||||||||||||||
ISO-50 251 AAAATCAAATGTTCTAAAAGAGGGTCTCT  279
```

MMP15

```
DMD1     1 ATGGGGATTTATAGTAATGAATTTTTTGATATGAAATATCTTTGCAATCT   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 ATGGGGATTTATAGTAATGAATTTTTTGATATGAAATATCTTTGCAATCT   50

DMD1    51 TAGAATAAATCCAAGTGGTTATTATATTTATTTTGTAATATGCCATTGGA  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 TAGAATAAATCCAAGTGGTTATTATATTTATTTTGTAATATGCCATTGGA  100

DMD1   101 TTCTCTTTAACATTTTTTGTAGGATTTTTGGATTGATATTTTTAAGCGAG  150
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50 101 TTCTCTTTAACATTTTTTGTAGGATTTTTGGATTGATATTTTTAAGCGAG  150

DMD1   151 ATGTGTTCTGTAGCTTTCTTTTGTCACATTTGAGGATCATAAGGTTTAAA  200
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50 151 ATGTGTTCTGTAGCTTTCTTTTGTCACATTTGAGGATCATAAGGTTTAAA  200

DMD1   201 TATCAATGACATAGAGTTATCTATTGCTTAACTTCACAGCTCCATTCCTG  250
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50 201 TATCAATGACATAGAGTTATCTATTGCTTAACTTCACAGCTCCATTCCTG  250

DMD1   251 AAAATCAAATGTTCTAAAAGAGGGTCTCT  279
           |||||||||||||||||||||||||||||
ISO-50 251 AAAATCAAATGTTCTAAAAGAGGGTCTCT  279
```

PRRT3

```
DMD1     1 TTGCTGCTCTCTCTGAATGACAAAGCAGTCATCACCTTACCTGACCCTCC   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 TTGCTGCTCTCTCTGAATGACAAAGCAGTCATCACCTTACCTGACCCTCC   50

DMD1    51 TATGGTCCTCCTAATCCCTCAGGATTGAGTCACAATCAACCTCATTTGCC  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 TATGGTCCTCCTAATCCCTCAGGATTGAGTCACAATCAACCTCATTTGCC  100

DMD1   101 ACAAAGAGGAAAATGAGAAAA  121
           |||||||||||||||||||||
ISO-50 101 ACAAAGAGGAAAATGAGAAAA  121
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RP1-65P5

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DMD1     1 CAAAACAGACGGTGGCAATAAGACACCAAACTATAAACAAGACCTGAGGC   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 CAAAACAGACGGTGGCAATAAGACACCAAACTATAAACAAGACCTGAGGC   50

DMD1    51 CATACAAGGCAAGGGTTAACTCACGCCTGCGGGCCATCAGTTTTGCCCCA  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 CATACAAGGCAAGGGTTAACTCACGCCTGCGGGCCATCAGTTTTGCCCCA  100

DMD1   101 TAGC    104
           ||||
ISO-50 101 TAGC    104
```

SCARB1

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DMD1     1 GCCACCCTGGCCCTACCTGACCGTCCAATGTGCCTTGCCTGTCCCTGCCA   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 GCCACCCTGGCCCTACCTGACCGTCCAATGTGCCTTGCCTGTCCCTGCCA   50

DMD1    51 CAGGGCCTTTGCAGTGGCCGCTCCAGGCAGCATTCCTCATGTGGCTGTGT  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 CAGGGCCTTTGCAGTGGCCGCTCCAGGCAGCATTCCTCATGTGGCTGTGT  100

DMD1   101 GAAT  104
           ||||
ISO-50 101 GAAT  104
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RP11-522M21.3

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DMD1     1 TCACATGGTTGATCCTTCTCTTGCAACCAGCTTGGACCATATGACTATCA   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 TCACATGGTTGATCCTTCTCTTGCAACCAGCTTGGACCATATGACTATCA   50

DMD1    51 GGTATGGCCCATCCTACCCTAAGATCTTATGTGGCCAACCCTCACCATAA  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 GGTATGGCCCATCCTACCCTAAGATCTTATGTGGCCAACCCTCACCATAA  100

DMD1   101 CAAAAT  106
           ||||||
ISO-50 101 CAAAAT  106
```

SCP2

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DMD1     1 GAGGGTATATACCTTCCCTGAGAGTCCAATGGTCTTATCTAAATATGATG   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 GAGGGTATATACCTTCCCTGAGAGTCCAATGGTCTTATCTAAATATGATG   50

DMD1    51 AGAATAATTTCTATCCATAGAACTGTTGTGGGGATTAAATGAGATAAGGA  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 AGAATAATTTCTATCCATAGAACTGTTGTGGGGATTAAATGAGATAAGGA  100

DMD1   101 AGATAGAGCACCTAGTGTAGTAAGTGCTCAGTT  133
           |||||||||||||||||||||||||||||||
ISO-50 101 AGATAGAGCACCTAGTGTAGTAAGTGCTCAGTT  133
```

TAF1B

```
DMD1     1 ATCAAAATTATTGGCTTTTGAGACAATTCGAGACCCTTTCTGGGAAGGAA   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 ATCAAAATTATTGGCTTTTGAGACAATTCGAGACCCTTTCTGGGAAGGAA   50

DMD1    51 TTGGGTTAGTTAGCAGAGGTGGGGAGACTTTGTGACATTCAGGTAAGGGA  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 TTGGGTTAGTTAGCAGAGGTGGGGAGACTTTGTGACATTCAGGTAAGGGA  100

DMD1   101 GAAG  104
           ||||
ISO-50 101 GAAG  104
```

STK3

```
DMD1     1 TGCAAATAAGAGGCAGCAGCATTCATGAACAAAGGAAAGCTGCAGGAACA   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 TGCAAATAAGAGGCAGCAGCATTCATGAACAAAGGAAAGCTGCAGGAACA   50

DMD1    51 TTTGAGATACAGGTAAGGGTCACCCAGCTTCACTTAACTGTGAAAGTAAG  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 TTTGAGATACAGGTAAGGGTCACCCAGCTTCACTTAACTGTGAAAGTAAG  100

DMD1   101 GTAATCGGCCAGGAA  115
           |||||||||||||||
ISO-50 101 GTAATCGGCCAGGAA  115
```

FTO

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DMD1     1 AGCAGTAATTCTTGTTTATATTGTCAAGAACCTTTCCACTGAGCACAAGC  50
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 AGCAGTAATTCTTGTTTATATTGTCAAGAACCTTTCCACTGAGCACAAGC  50

DMD1    51 GCACCATACTTGAAGGTCAAATGCTTAACCCTTCTGCATGGTTACAATGA  100
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 GCACCATACTTGAAGGTCAAATGCTTAACCCTTCTGCATGGTTACAATGA  100

DMD1   101 AAATGGACCCTTTTGTTTTCATTAATGTATACCCCAGCCATGTCTACAGG  150
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50 101 AAATGGACCCTTTTGTTTTCATTAATGTATACCCCAGCCATGTCTACAGG  150

DMD1   151 GAGAATGGGGA    161
           ||||||||||||
ISO-50 151 GAGAATGGGGA    161
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IL1RAP

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DMD1     1 CAATGGCGTCACATTGCCCAAGTGATGTGCTCCTTCCTCCCTCCTCTCTA   50
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
ISO-50   1 CAATGGCGTCACATTGCCCAAGTGATGTGCTCCTTCCTCCCTCCTCTCTA   50

DMD1    51 CTCCGCTTCCTTCTCTACTCCCTACTCTCAGGTGCTTTGAAATGC    95
           |||||||||||||||||||||||||||||||||||||||||||||
ISO-50  51 CTCCGCTTCCTTCTCTACTCCCTACTCTCAGGTGCTTTGAAATGC    95
```

Figure S4. Sequencing results of predicted off-target regions in ISO-50. Alignment of original DMD1 and edited ISO-50 sequence obtained from Sanger sequencing results of 15 predicted off-target sites.

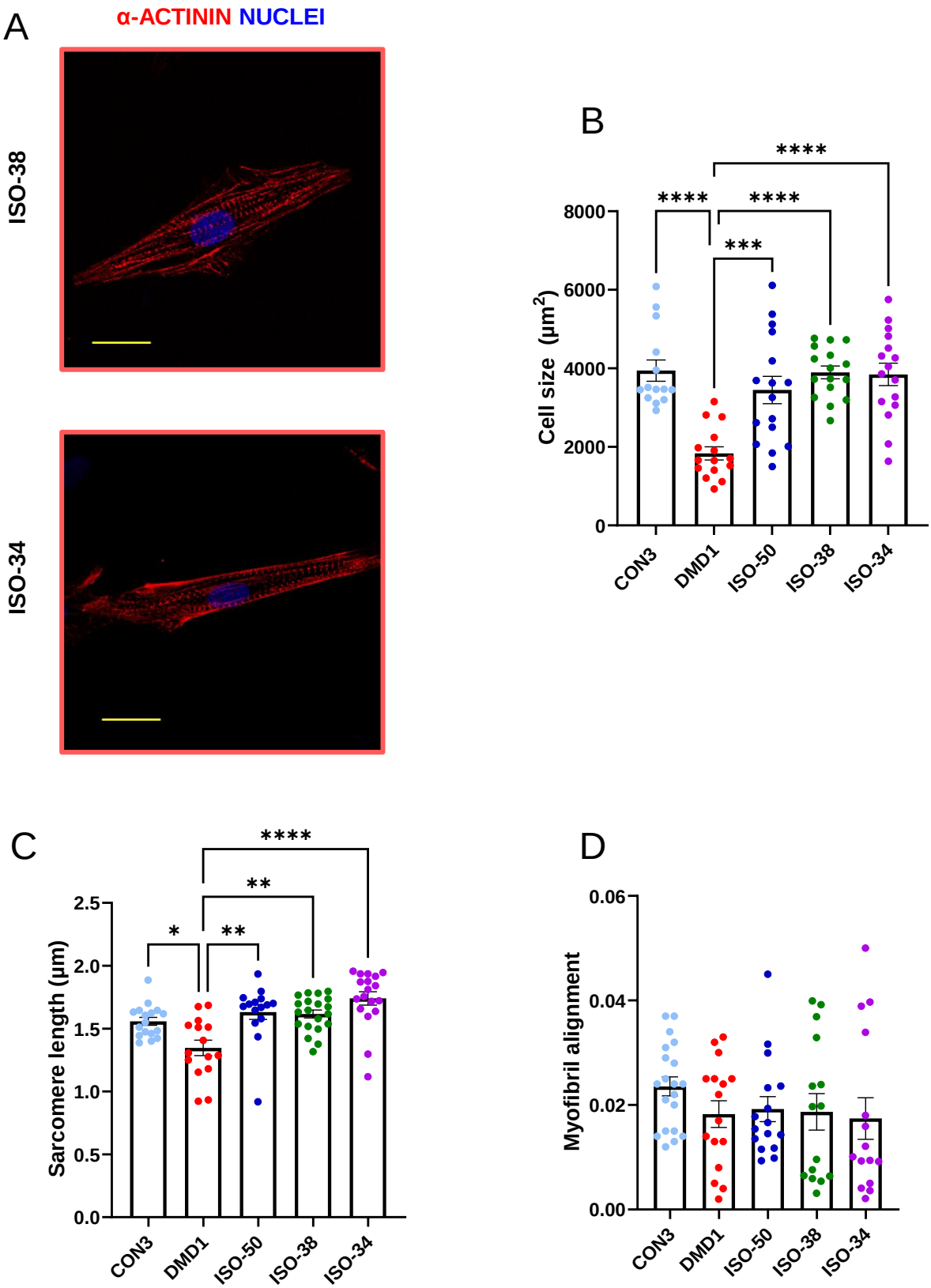

Figure S5. Analysis of cell morphology. (A) Representative ISO-38 and ISO-34 iPSC-CMs stained for α-actinin (red) and nuclei (blue) from which **(B)** cell size, **(C)** sarcomere length, and **(D)** myofibril alignment measurements were measured, the latter assessed by FFT-based analysis of Z-band organization and angular distribution. For comparison, data from the cell lines shown in the main figures are also included, demonstrating a consistent trend across the three isogenic lines. Magnification is 63X and the scale bar indicates 20 μm. (n = 3 biological replicates; One-way ANOVA and Tukey's post-test).

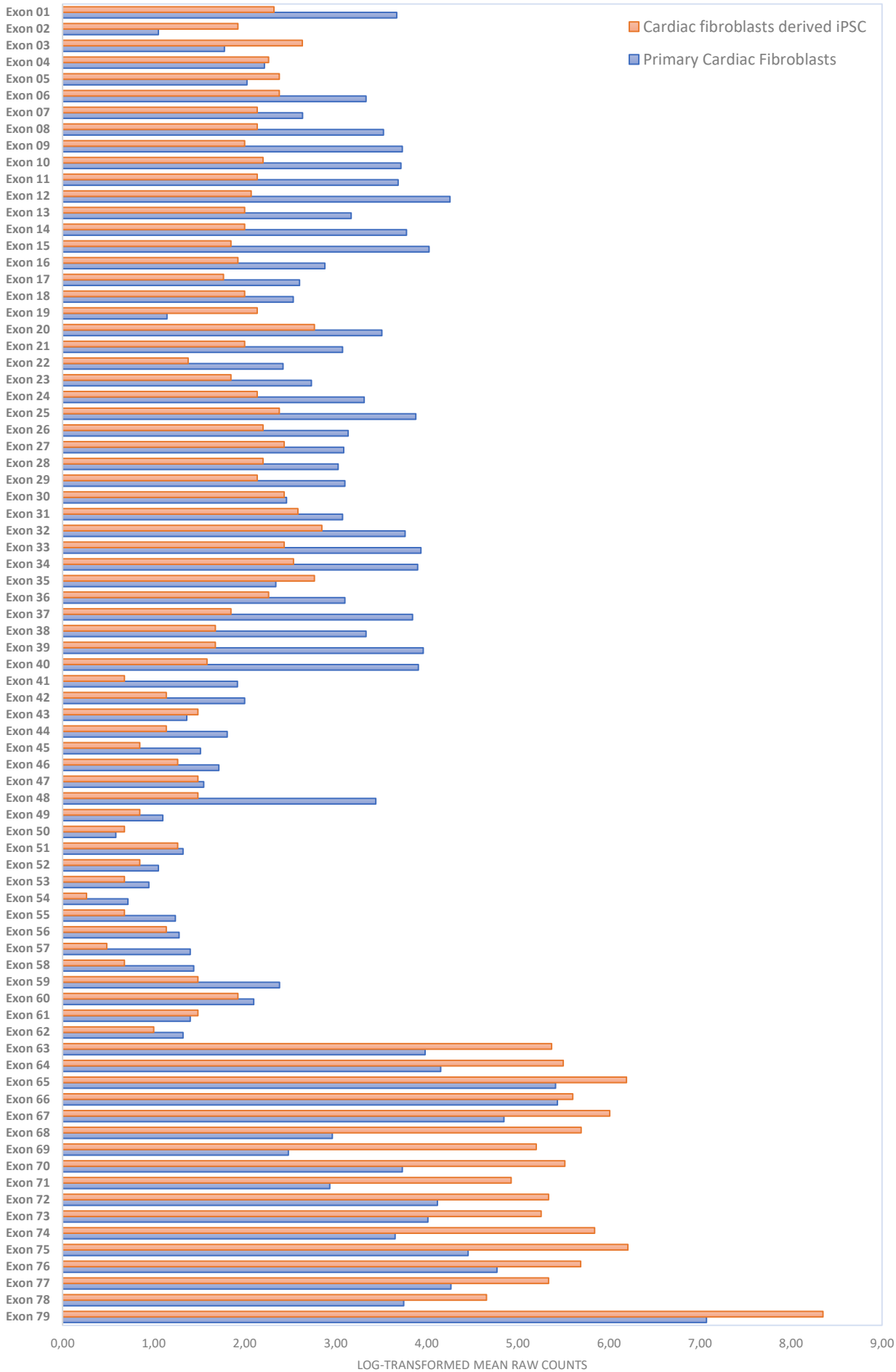

Figure S6. Expression of *DMD* exons in primary and iPSC-derived human cardiac fibroblasts. RNA-seq analysis showed detectable expression of all *DMD* exons in primary human cardiac fibroblasts isolated from 14 different subjects and iPSC-derived cardiac fibroblasts generated from 5 iPSCs. Exons 1–29, which are unique to the full-length Dp427 transcript, were detected in both primary and iPSC-derived cardiac fibroblasts. Values represent log-transformed mean raw counts.

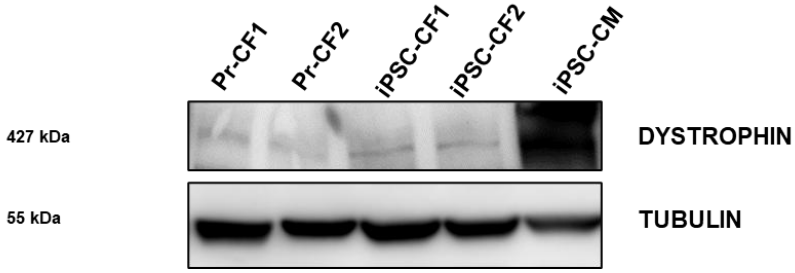

Figure S7. Analysis of full-lenght dystrophin expression in cardiac fibroblasts. Selected images of WB analysis of proteins extracted from primary cardiac fibroblasts (Pr-CF; isolated from two different subjects) and iPSC-CF (protein obtained from two independent CF differentiation process: CF1 and CF2), hybridized with specific antibody for dystrophin. Proteins extracted from iPSC-CM were used as positive control for dystrophin expression. Immunostaining of the housekeeping Tubulin is shown for normalization.

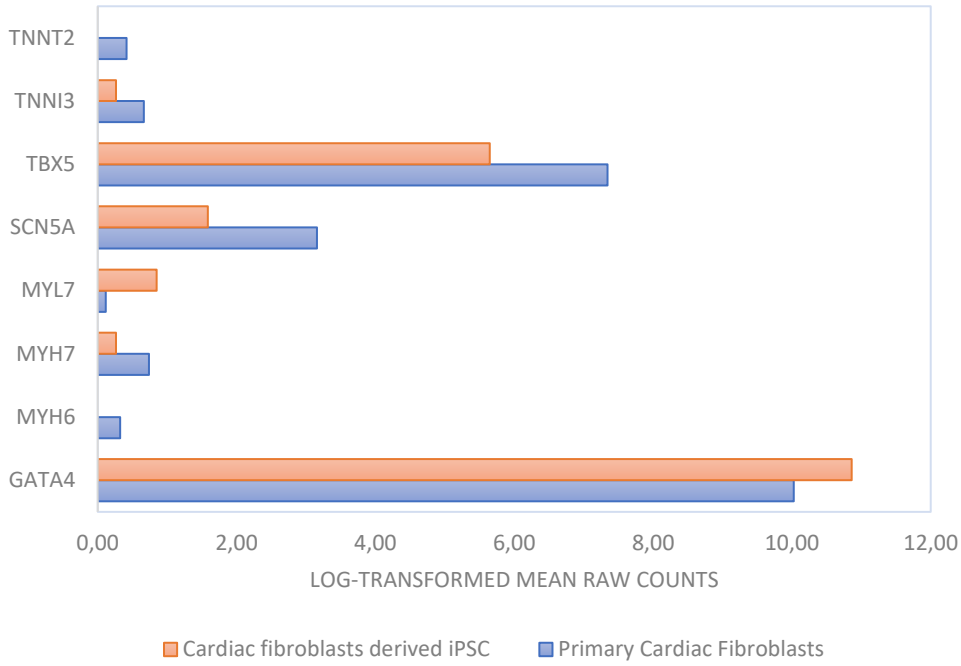

Figure S8. Expression of cardiomyocyte-associated genes in primary and iPSC-derived human cardiac fibroblasts. RNA-seq analysis showed comparable expression of selected cardiomyocyte-associated genes in primary human cardiac fibroblasts isolated from 14 different subjects and iPSC-derived cardiac fibroblasts generated from 5 independent iPSC lines. Cardiomyocyte-specific structural and functional genes, including *MYH6*, *MYH7*, *MYL7*, *SCN5A*, *TNNI3*, and *TNNT2*, showed low expression in both groups, supporting the absence of significant myogenic characteristics in iPSC-derived cardiac fibroblasts. Values represent log-transformed mean raw counts.

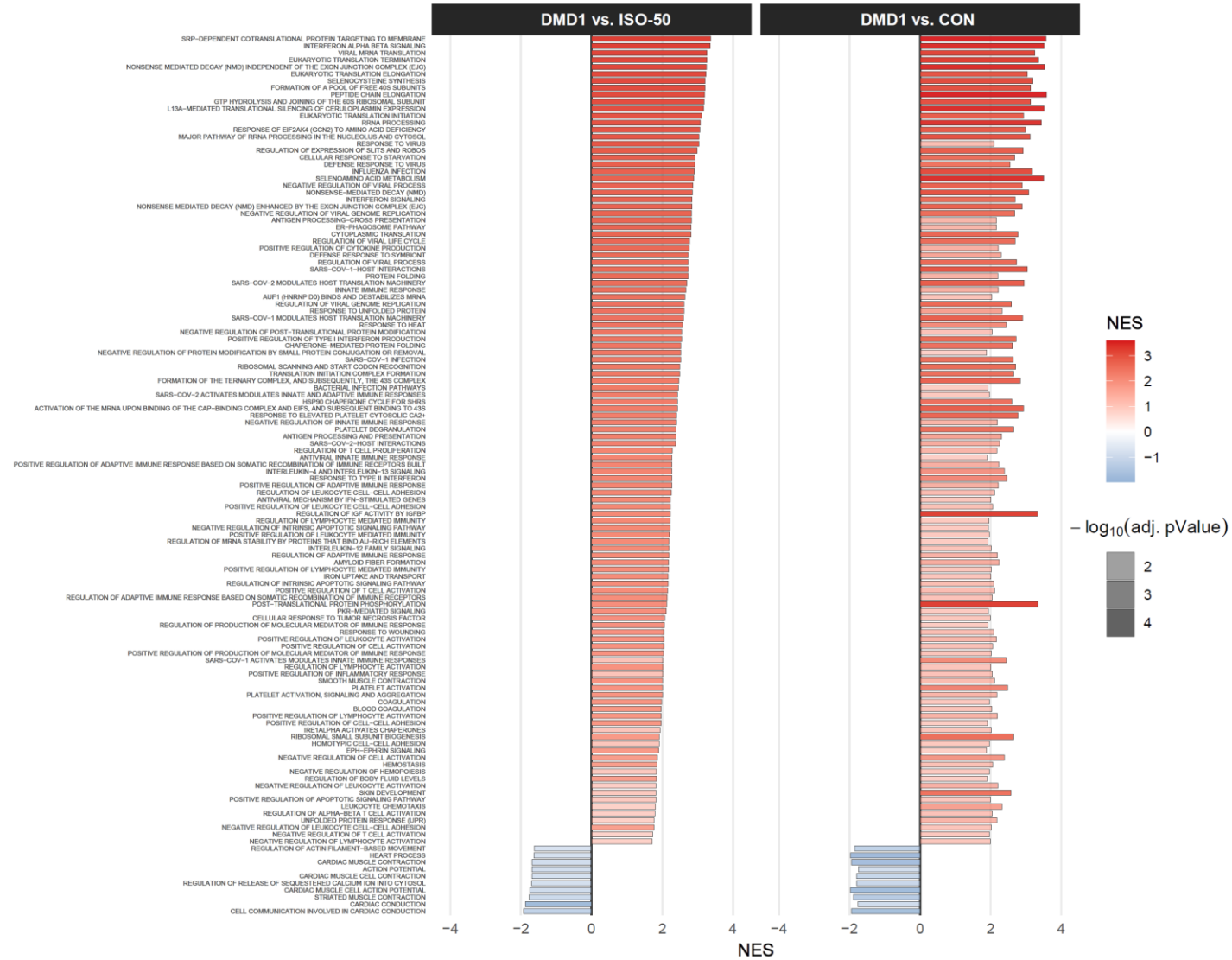

Figure S9. Pathway enrichment landscape in DMD1 CMs. Tornado plot displaying normalized enrichment scores (NES) of significantly enriched pathways in cardiomyocytes from DMD1 vs. ISO-50 and DMD1 vs. CON comparisons for cardiomyocytes. Positive NES values indicate pathways enriched in DMD1, whereas negative NES values indicate pathways enriched in the corresponding control conditions. Color encodes NES direction and magnitude, and shading reflects enrichment significance as −log10 adjusted p-value.

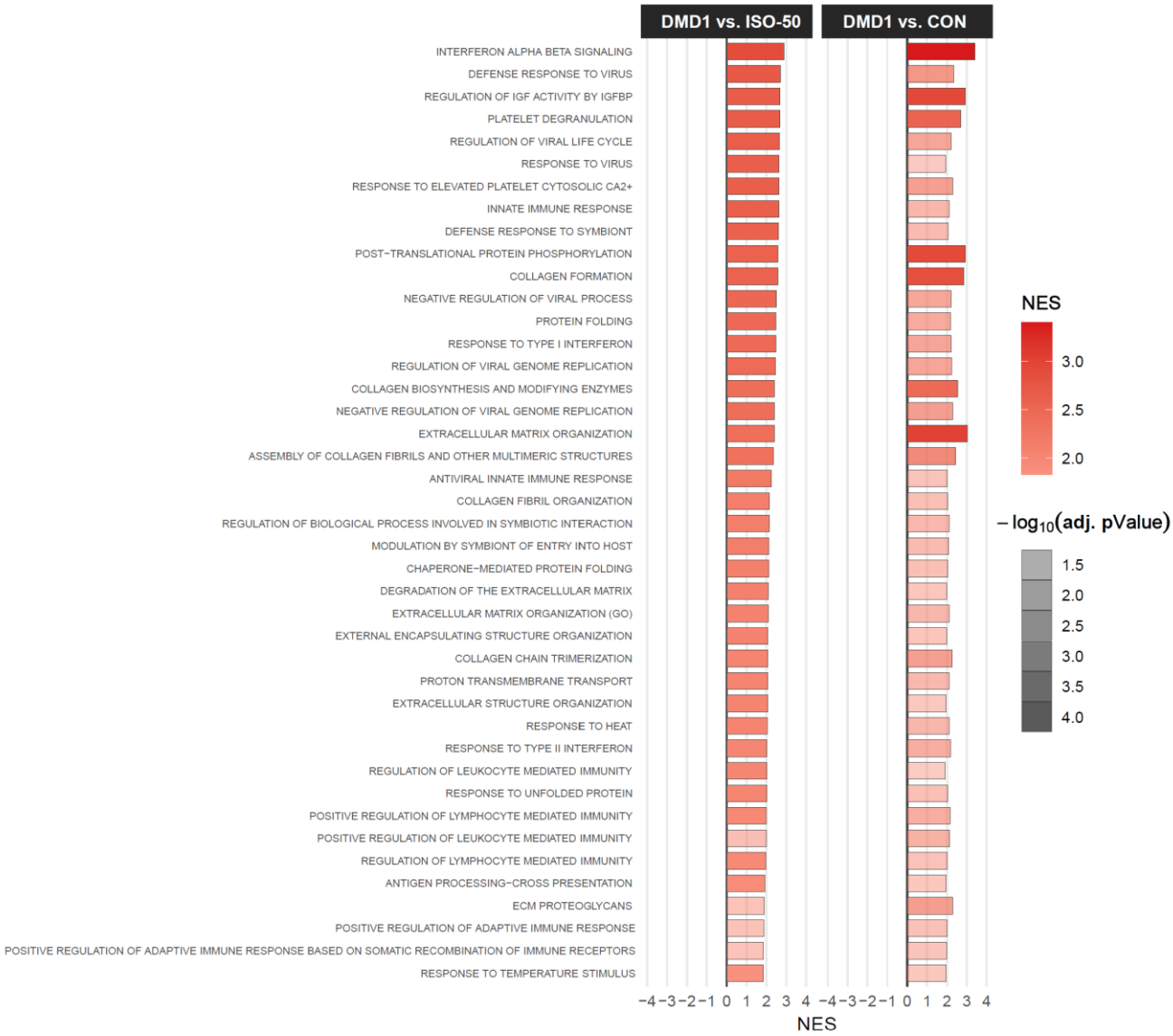

Figure S10. Pathway enrichment landscape in DMD1 CFs. Tornado plot displaying normalized enrichment scores (NES) of significantly enriched pathways in fibroblasts from DMD1 vs. ISO-50 and DMD1 vs. CON comparisons for cardiomyocytes. Positive NES values indicate pathways enriched in DMD1, whereas negative NES values indicate pathways enriched in the corresponding control conditions. Color encodes NES direction and magnitude, and shading reflects enrichment significance as −log10 adjusted p-value.