SUPPLEMENTARY FILE

DP71 supports early homeostatic adaptation in DP427-deficient muscle cells

Short title: DP71 supports early adaptation in DP427-deficient muscle cells

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Supplementary Methods

Cell culture

Mouse C2C12 myoblasts and human HEK293 and HeLa cells were obtained from ATCC, whereas human primary myoblasts (HSkM) were obtained from Thermo Fisher (A12555), and mouse HL-1 and human AC16 cardiomyocytes were obtained from Sigma-Aldrich (SCC065 and SCC109, respectively). *DMD* knockout HeLa and HEK293 cell lines used in this study were generated and characterized as previously described [13].

C2C12, HeLa, and HEK293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Biowest, LO102-500) supplemented with 10% foetal bovine serum (FBS; Genos, FBS-HI-12A) and 1x antibiotic-antimycotic reagents (AA; Sigma Aldrich, A5955). HSkM cells were cultured in Nutrient Mixture F-10 Ham Medium (Thermo Fisher, 11550043) supplemented with 20% FBS, 10 µg/µl epidermal growth factor (EGF; Sigma Aldrich, E9644), 1 mg/µl dexamethasone (Sigma Aldrich, D4902), and 1x AA. HL-1 cells were cultured in Claycomb Basal Medium supplemented with 10% HL-1-qualified FBS (Sigma Aldrich, TMS-016-B) and 0.1 mM NE (Sigma Aldrich, A7257). AC16 cells were cultured in DMEM/F12 medium (Sigma Aldrich, DF-041-B) supplemented with 12.5% FBS. Both media for cardiomyocytes were enriched with 2 mM L-glutamine (Cell Lab, BE17-605E) and 1x AA. Additionally, the HL-1 cells were cultured on wells covered with 0.01% gelatin solution (Sigma Aldrich, G9391), and 5 µg/mL fibronectin (Sigma Aldrich, F1141) and maintained at high confluency. C2C12 and HSkM cells were cultured at low density to maintain an undifferentiated state. For experiments on differentiation toward myofibres, C2C12 and HSkM cells were seeded on cell culture plates covered with 0.1% gelatin (Sigma Aldrich) to reach high confluence (>80%) and then cultured in starving medium (DMEM (Capricorn Scientific, DMEM-HA) supplemented with 2% horse serum and 1x AA). All the cells were grown at 37°C in a humidified incubator containing 5% CO2.

siRNA transfection

Cell transfection with Silencer Select siRNA (Thermo Fisher) was performed with Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher, 13778150) according to the manufacturer's protocol. The final concentration of each siRNA was 25 nM (in the case of double siRNA transfection, the final concentration of negative control siRNA was 50 nM). For the experiments involving proliferating cells, the siRNA was added 24 h (HeLa, HEK293) or 4 h (HSkM) after passage, and the material was collected 48 h after transfection. For the differentiation of HSkM, siRNA was added 4 h after passage, and after another 48 h of proliferation, differentiation was induced. Two different si*DP427* molecules were tested (si*DP427.1,* si*DP427.2*), and for further analysis, si*DP427.1* was chosen. The siRNAs used in this study are listed in Supplementary Table S1.

Actinomycin D treatment

HeLa cells transfected with control siRNA (siCtrl) or siRNA targeting *UTRN* transcripts (si*UTRN*) were cultured in 12-well plates. At 120 h post-transfection, actinomycin D (Sigma Aldrich/Merck, A1410),

which was prepared in DMSO, was added directly to the culture medium to a final concentration of 5 µg/mL to inhibit transcription. The cells were harvested at 0, 2, 4, 8, and 12 h following actinomycin D treatment for total RNA isolation. RT-qPCRs were then performed to quantify *DP71* mRNA levels in relation to *RPLP0* levels via *DP71*/*RPLP0*-specific primers (Supplementary Table S1).

Plasmid preparation

The procedures for constructing the EF1α-GFP-FLAG-DP71 splice variants are illustrated in Supplementary information. Briefly, the insert was amplified via high-fidelity polymerase (Thermo Fisher, F530S) with primers containing additional nucleotides for restriction enzyme sites, following the manufacturer's protocol. Both the plasmid and the insert were digested overnight at 37°C with Fast Digest restriction enzymes (Thermo Fisher). After electrophoresis on a 1% agarose gel, the target band was separated according to the manufacturer's protocol (GeneJET Gel Extraction Kit, Thermo Fisher, K0722), and both fragments were ligated for 1 h at room temperature using T4 DNA ligase (Thermo Fisher, EL0011). The plasmid with the insert was then introduced into *E. coli* cells via the heat shock method. The bacterial cells were first thawed on ice for approximately 20 min for heat shock. Next, the ligation mixture was added to the bacterial cells, which were then incubated for 30 min on ice. After incubation, the cells were heat-shocked for 1 min at 42°C, followed by a 2-min incubation on ice. Then, 700 µl of LB medium preheated to 37°C was added to the cells (without antibiotics). The culture was shaken for 1 h at 37°C and 300 rpm. Afterward, the cells were centrifuged for 5 min at 500 x g, and the pellet was resuspended in 50 µl of LB medium. Finally, the cells were spread onto a labelled LB agar plate (LB Agar, LabEmpire, LBL406.1) containing the appropriate antibiotic (carbenicillin disodium salt (Sigma Aldrich, C1389) (as an ampicillin substitution) or kanamycin (Sigma Aldrich, B5264)). These plasmids were tested by restriction enzymes and confirmed by electrophoresis, Sanger sequencing, and Western blotting after transfection into the HeLa cell line.

To validate the response of the *DP71* promoter upon silencing of the *UTRN* gene, the approximately 1 kbp human *DP71* promoter sequence was amplified from HeLa genomic DNA via PCR with primers containing *Kpn*I and *Nhe*I restriction sites. The amplified fragment was then cloned and inserted into the pGL4.10[luc2] vector (Promega, E6651) to serve as the promoter for the *luc2* gene encoding firefly luciferase via the *Kpn*I and *Nhe*I restriction sites. The pGL4.75n[hRluc/CMV] plasmid (Promega, E6931) containing the Renilla luciferase sequence, served as the control reporter for normalization of firefly luciferase activity. The primers used in this study are listed in Supplementary Table S1.

Plasmid transfection

Cell transfection with vectors was performed via FuGene HD or ViaFect (Promega, E2311, E4982, respectively) for the HeLa and HEK293 cell lines and via TransfeX (ATCC, ATCC-ACS-4005) for the LHCN-M2 cells, maintaining a 2:1 ratio of transfection reagent to DNA. For the 6-well plates, 5 µL of transfection reagent was used; for the 24-well plates, 2.5 µL was used; and for the 96-well plates, 0.16 µL was used. The plasmid and the transfection reagent were added to Opti-MEM, mixed by pipetting, and incubated for 30 min (FuGene HD), 20 min (ViaFect), or 15 min (TransfeX). The DNA/transfection reagent mixture was then added to the cells. For proliferating cells, transfection was performed 24 h

after seeding. For C2C12 cell differentiation, transfection was performed 24 h after seeding. After 48 h, the medium was replaced with starvation medium, and the cells were allowed to differentiate for 6 days.

Alternative splicing analysis

PCRs were performed with GoTaq Polymerase (Promega, M7845) according to the manufacturer's protocol. Electrophoresis was performed on a 2% agarose gel, and the results were detected on an InGenius3 (SyNGENE). The analysis of the bands was performed with the use of ImageJ software. The primers used in this study are listed in Supplementary Table S1.

Subcellular protein fractionation

Protein extraction was performed with a Subcellular Protein Fractionation Kit for cultured cells (Thermo Fisher, 78840) according to the manufacturer's protocol. The experiment was performed in three biological repeats for each condition. Subcellular fractions were confirmed by Western blotting by the presence of proteins specific to each targeted fraction. The antibodies used in this study are listed in Supplementary Table S2 and the immunoblots are shown in Supplementary Original Data.

Imaging, immunoblotting and RT-qPCR

The experimental procedures were carried out as previously described [13]. Clontech human cDNA libraries were used to evaluate the expression patterns of *DMD* and *UTRN* isoforms and *DP71* alternative splicing across human foetal and adult tissues. The primers and antibodies used in this study are listed in Supplementary Tables S1 and S2. The uncropped Western blot images are provided in Supplementary Original Data.

Cell viability and proliferation

Cell viability/proliferation was determined via the RealTime-Glo™ MT Cell Viability Assay (Promega, G9711). The cells were plated on a Nunc™ MicroWell™ 96-well plate (Thermo Fisher) at a density of 7000 cells. Cell proliferation was monitored every 12 h on a Tecan SPARK plate reader with luminescence. The detailed protocols can be found in Szwec *et al.* [13].

Mitochondrial content, oxidative stress, apoptosis, intracellular calcium levels, and plasma membrane staining

The experimental procedures were carried out as previously described [13].

***DP71* promoter activity**

To assess *DP71* promoter activity, 7×10^3 HeLa cells per well were cultured in a 96-well plate and allowed to adhere for 24 h before transfection. The cells were then cotransfected with 50 ng of the pGL4.10[luc2]-*DP71* promoter construct and 2 ng of pGL4.75 using FuGENE HD Transfection Reagent. In parallel, 25 nM siRNA targeting *UTRN* mRNA or negative control siRNA was delivered via Lipofectamine RNAiMAX according to the manufacturer’s instructions. The luciferase activity was

measured 48 h after cotransfection via the Dual-Glo Luciferase Assay System (Promega, E2980). Luminescence was recorded with a Tecan SPARK microplate reader, and firefly luciferase signals driven by the *DP71* promoter were normalized to Renilla luciferase activity.

Supplementary Tables

Table S1: A list of primers and siRNAs used in the study.

Target	Species	Forward	Reverse
RT-PCR			
RPLP0	Human	CGTCCTCGTGGAAGTGACAT	CTTGGAGCCCACATTGTCTG
DMD	Human	GGACCAGCACAACCTCAAGCA	TCCTCCCTGTTCGTCCCGTATC
DP427	Human	CTGCACTAGGCTGAATGGGAA	CTCCGCCAGGAATGTTTTCAG
DP140	Human	TGGGGACCGAAAGAGGTTTT	CAGGCTTCCCAATTTTTCCTG
DP116	Human	TCAGTGCTGTAGTGCCCGGT	CAGGACTGCATCATCGGAACCT
DP71	Human	GCCATGAGGGAACAGCTCAA	TTGCTTGAGGTTGTGCTGGT
DP40	Human	GAACACCCTCCTTCACTCCC	AAGTGACGTGGGAAAGTGGC
UTRN	Human	AAACTCCTCAGGCAGCACAA	GAGGCATCTGGATCAAGCGA
UP395	Human	GTTCCAGAGCTCAACACTGC	TGCCCTGTCAAAACAACCTTTC
UP140	Human	CACAATACTTGGGTGGATTCATTGC	TGCCTGGGAGAGTTTCTGCAC
UP113	Human	ACTTAAAATGATGGCTGTGGTGAGA	TCTGCCTGTTTCCGTCAATGCT
UP71	Human	GCATTGGGTTTCTGAATGGTTGA	CCAAGGGATTGAAAGAGTTCGG
MYOD	Human	CGCAACGCCATCCGCTAT	TGTAGTCCATCATGCCGTCG
MYOG	Human	TGCCATCCAGTACATCGAGC	CAGATGATCCCCTGGGTTGG
MYH1	Human	AGGTCGCATCTCTACGCCA	CGCTCCCTTTCAGACTTTCG
FOXO1	Human	GAGGGTTAGTGAGCAGGTTACA	ACTGCTTCTCTCAGTTCCTGC
DRAM1	Human	TCAACCCCTTCCTCCCGTA	TCGTGGCTGCACCAAGAAA
RYR1	Human	GGGTGAAGTTCCTGGTCTCAG	TCTCATCCTCATCGCCCTCT
STAC3	Human	TTTTTCCCCAGCACCACCAT	GTCCTCAAAGGGGAGGGTTC
FOS	Human	GGGGCAAGGTGGAACAGTTA	AGTTGGTCTGTCTCCGCTTG
MYOM3	Human	ACCCTGGATGAGATCTGGCT	GCATCCTCAAAAGCTTGCCC
Rplp0	Mouse	GCTTTGGGCATCACCACGAA	GTTGCGGACACCCTCCAGAA

Dp427	Mouse	CTCCACCCTCAGCACAAGAG	TCATGCCAACATGCCCAAAC
Dp260	Mouse	ACAACAGATTGCAACAACTGAGA	AATTCCTGGGCAGACTGGAT
Dp140	Mouse	GCATTGCTGACTGTTCTGAGC	CCCAGTTGCATTCAGTGTTCTG
Dp116	Mouse	TTTCTTAGGTTTGCTATGCAACAGG	CAGGGGTGCTTCATCCGAAC
Dp71	Mouse	ATGAGGGAACAGCTCAAAGG	TGCAGCTGACAGGCTCAAGA
Up395	Mouse	CACTGGCAGGTGAAGGATGT	TGAGGACGTTGACTTGACTGT
Up140	Mouse	AGGCCCTCTTCCAGTTCCTA	AAGACATTATAGCCCTACCCCG
Up113	Mouse	TCATCCAGTCGATGCTGAAG	CAAAGGATGGCAGTTTCCTC
Up71	Mouse	AAGAGCTCATTTTAGGATGAT	CTAGATTTCTGAGCGATCC
PCR			
DP71 (PCR1)	Human	AAAACCATGCGGGAATCAGG	AAAACCATGCGGGAATCAGG
DMD ex70,76 (PCR2)	Human	TTGCGAAGCATCCCCGAAT	ACTGCTGTCGGACCTCTGTA
DMD ex76,79 (PCR3)	Human	GGTTGGCAGTCAAACTTCGG	TCCATCGCTCTGCCCAAATC
Dmd ex69,75 (PCR2)	Mouse	CATGGTAGAGTATTGCACTCCG	GGAGGAGATGGCAGTGGAGAC
Dmd ex77,79 (PCR3)	Mouse	TCTTCTGAGTCCTCCCCAGG	CTCCATCGCTCTGCCCAAAT
DP71 promoter	Human	GTAAGGTACCTGGACAAAGAAGGGCAGTGA	CGTTGCTAGCCGAGTGGAGGAGTGAGCTTC

Name	**Species**	**Sequence**
siRNA		
DMD/*Dmd* (s501299)	Human, mouse	AAACAAAUUUCGAACCAAAtt
DP427.1	Human	GGAUAACCCAAAUCAGAUUtt

DP427.2	Human	GGAGGGAACUACAUGAAGAtt
DP71	Human	GCUCACUCCUCCACUCGUAtt
UTRN (s553059)	Human	GGAAUAUUGUAUACCUACAtt

Table S2: A list of antibodies used in the study.

Antibody	Species	Concentration		Manufacturer
		WB	IF	
Dystrophin (+78)	Rabbit	1:1000		Abcam (ab15277)
Dystrophin	Rabbit	1:2000	1:200	Proteintech (12715-1-AP)
Dystrophin(Mandra1)	Mouse	1:1000		Abcam (ab7164)
Lamin B1	Rabbit	1:1000	1:500	Abcam (ab16048)
Myosin (B-5)	Mouse		1:50	Santa Cruz Biotechnology (sc-376157)
FLAG	Rabbit	1:2000	1:100	Abcam (ab1162)
α-Tubulin (DM1A)	Mouse	1:2000	1:500	Abcam (ab7291)
Desmin (Clone D33)	Mouse	1:750		Perlan (M076029-2)
GAPDH	Mouse	1:1000		Proteintech (60004-1-Ig)
RPLP0	Rabbit	1:2000		Abcam (ab192866)
β-Dystroglycan(43DAG 1/8D5)	Mouse	1:1000	1:50	Abcam (ab49515)
α-syntrophin	Mouse	1:2000	1:20	Abcam (ab11425)
anti-mouse IgG (Fab specific)–peroxidase antibody	Goat	1:10 000		Sigma–Aldrich (A9917)

anti-rabbit IgG	Goat	1:10 000		Abcam (ab97051)
Rhodamine (TRITC) anti-mouse	Donkey		1:1000	Jackson Immunoresearch (715-025-150)
Alexa 488 anti-rabbit IgG H&L	Donkey		1:400	Abcam (ab150073)
Alexa 594, streptavidin			1:500	Molecular Probes

Supplementary Figures

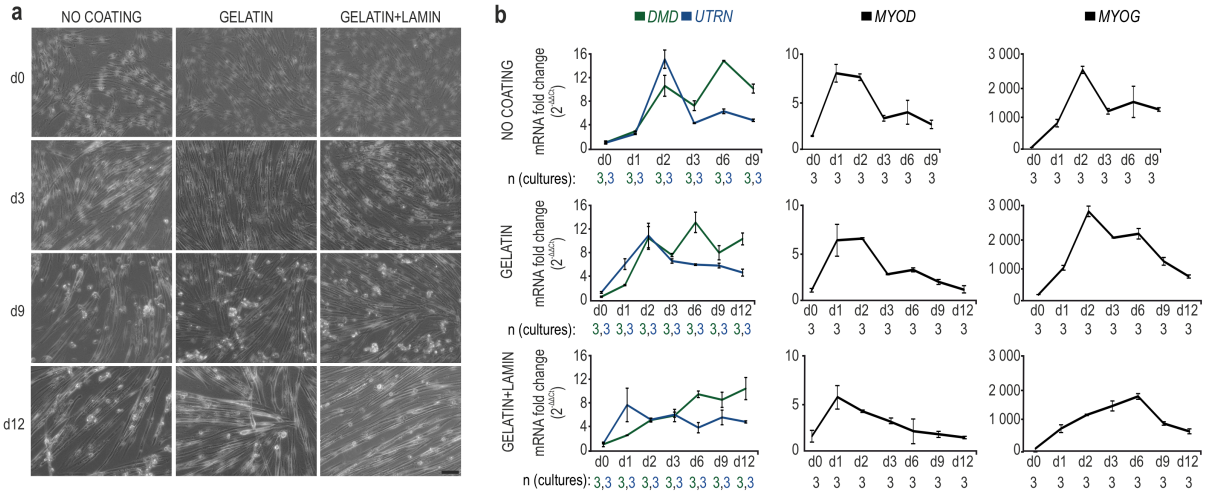


Figure S1: HSkM cells exhibit similar differentiation patterns regardless of the coating type.

(a) Live images of HSkM cells at different myogenic time points, cultured on various substrate coatings (no coating, gelatin alone or gelatin and laminin). Scale bar: 50 µm.

(b) RT-qPCR analyses of the *DMD* and *UTRN* genes as well as differentiation markers (*MYOD, MYOG*) in HSkM cells after differentiation induction on plates without coating or coated with gelatin or gelatin/laminin. The data are presented as mean ± SD. n, number of biological replicates.

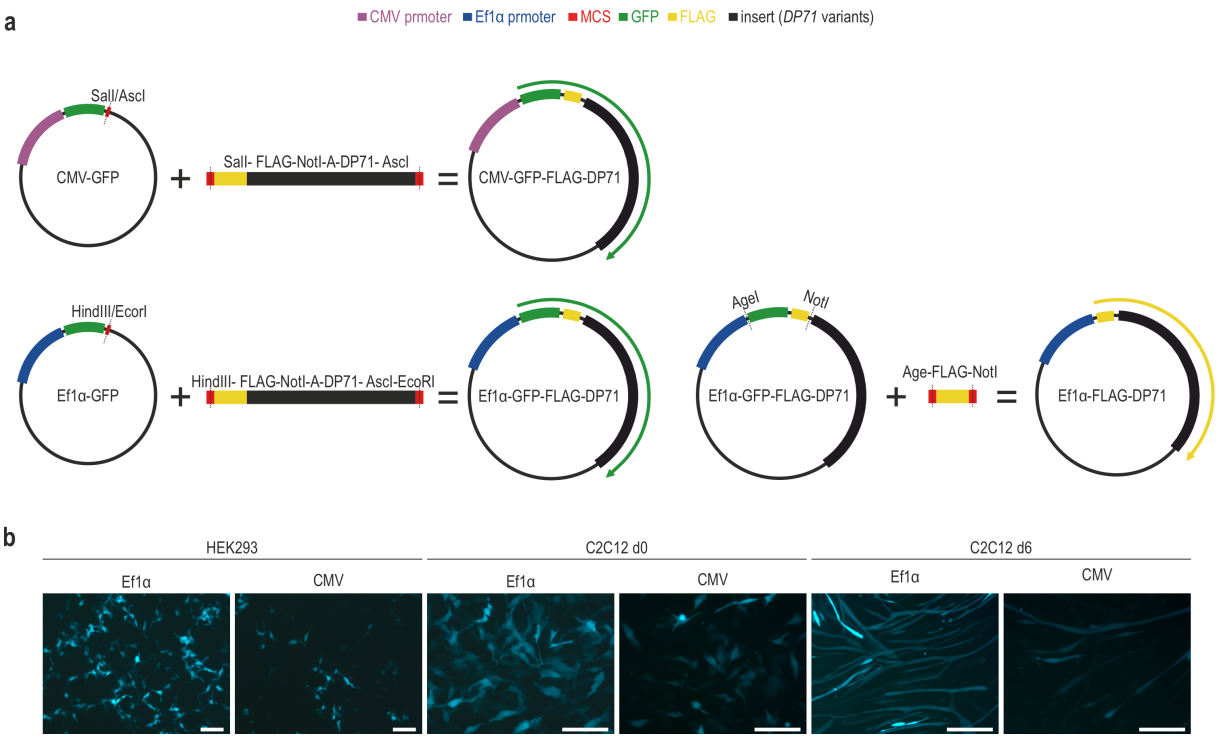


Figure S2: Preparation of the CMV and EF1α plasmids.

(a) Graphic representation of cloning strategies and preparation of CMV/EF1α-FLAG-DP71 vectors w/o GFP.

(**b)** Comparison of CMV and αEF1 promoter activities in HEK293 and C2C12 cells following delivery of the CMV-GFP-FLAG and αEF1-GFP-FLAG vectors. Scale bar: 50 µm.

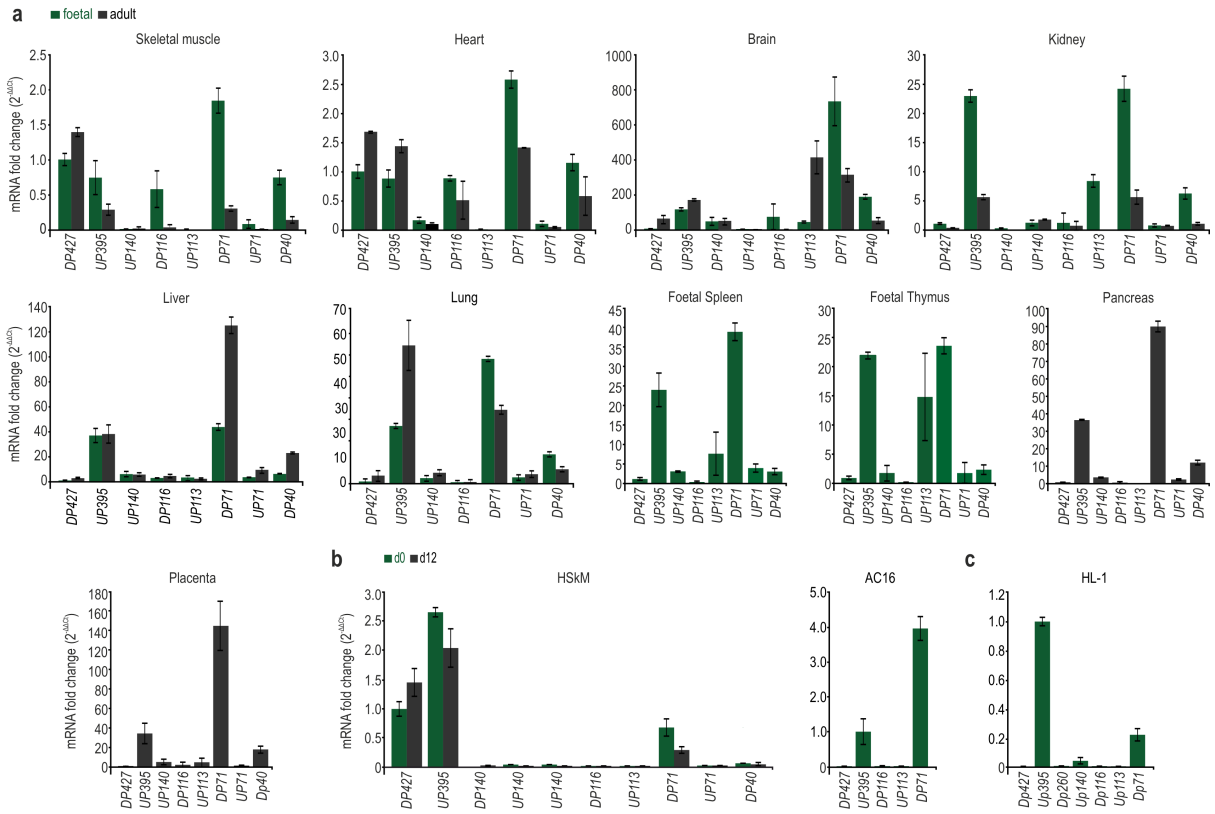


Figure S3: *DMD* and *UTRN* genes exhibit varying expression patterns depending on tissue type and developmental stage.

(a) RT-qPCR analyses of *DMD* and *UTRN* gene expression in foetal and adult tissues (skeletal muscle, heart, brain, kidney, liver, and lung), foetal tissues (spleen and thymus), and adult samples (pancreas, placenta). The mRNA level was normalized to the *DP427* level in foetal skeletal muscle tissue.

(b, c) RT-qPCR analyses of *DMD/UTRN* expression in human proliferating (d0) and differentiated (d12) skeletal muscle cells (HSkM) and proliferating cardiomyocytes (AC16) (**b**) and proliferating mouse-derived (HL-1) cardiomyocytes (**c**).

The data are presented as mean ± SD.

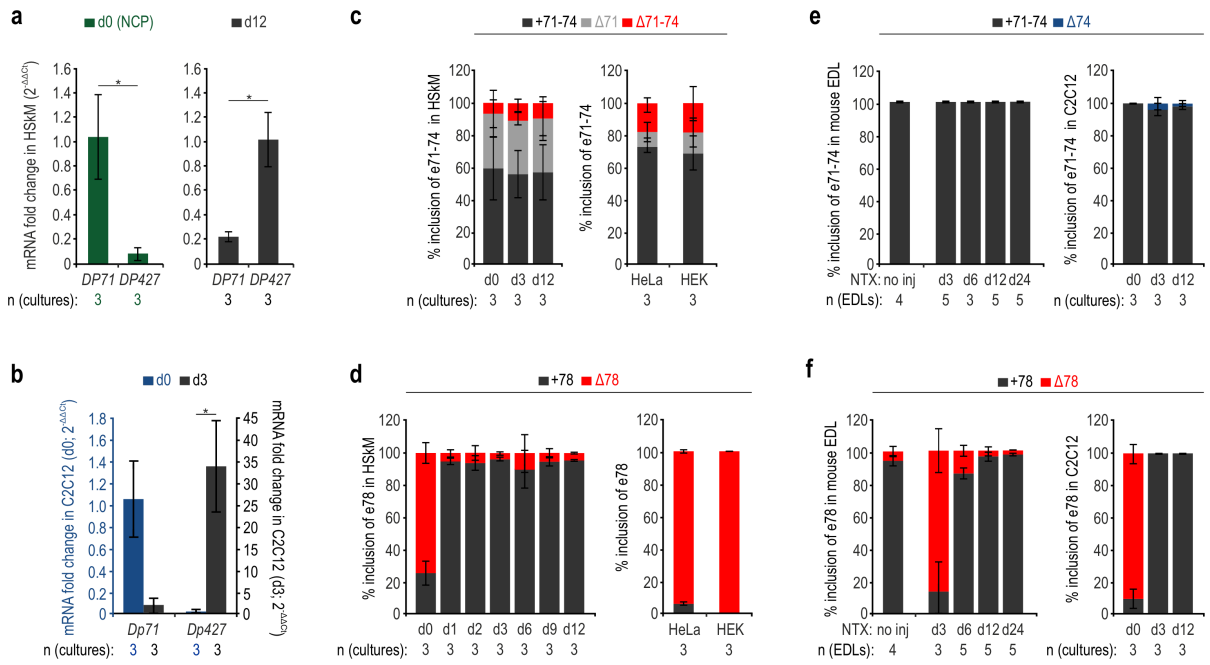


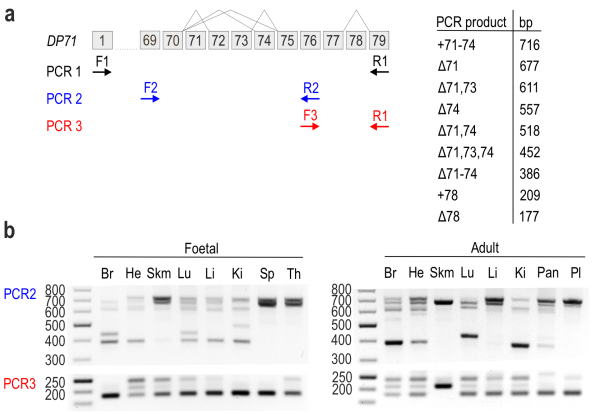
Figure S4: *DP71* shows more complex expression patterns in human than in murine cell models.

(a, b) RT-qPCR analyses of *DP71* and *DP427* mRNAs in human HSkM (**a**) and murine C2C12 (**b**) cells at different timepoints during myogenic differentiation. Proliferating HSkM cells were isolated from nonconfluent plates (NCP) to prevent differentiation initiation.

(c, d) Quantitative analyses of exon 71–74 (**b**) and 78 (**e**) inclusion in human cells (HSkM, HeLa and HEK293).

(e, f) Quantitative analyses of exon 71–74 (**c**) and 78 (**f**) inclusion in murine models, noninjected and NTX-treated EDLs and C2C12 cells.

The data are presented as mean ± SD. n, number of biological replicates. *, $p < 0.05$ (two-tailed unpaired t–test).



c

Tissue	+71-74	Δ71	Δ71, 73	Δ74	Δ71, 74	Δ71, 73,74	Δ71-74	+78	Δ78
Brain	A	F A	F A	A		F	F A	A	F A
Heart	F A	F A	F A				F A	F A	F A
Skeletal muscle	F	F A	F A	A			F	F A	F
Lung	F A	F A	F A			F A	F	F A	F A
Liver	F A	F A	F A				F A	F A	F A
Kidney	F A		F A		F		F A	A	F A
Spleen	F	F							F
Thymus	F	F						F	F
Pancreas	A	A	A				A	A	A
Placenta	A	A						A	A

Figure S5: Differentiation stage- and tissue-dependent splicing diversity of *DP71* transcripts.

(a) Schematic illustration of the PCR primers used for whole *DP71* isolation (F1/R1) and detection of splice variants targeting exons 71–74 (F2/R2) and exon 78 (F3/R1) exclusion, with corresponding table of product sizes.

(b) The corresponding PCR results display the amplified products in foetal and adult tissues: brain, Br; heart, Hr; skeletal muscle, Skm; lung, Lu; liver, Li; kidney, Ki; spleen, Sp, thymus, Th; pancreas, Pa; placenta, Pl. The hybrid duplex (~240 bp) is a result of the hybridization of two products, +78 and Δ78.

(c) Summary of *DP71* isoform transcript levels across different tissues with their distribution at both the foetal (F) and adult (A) stages.

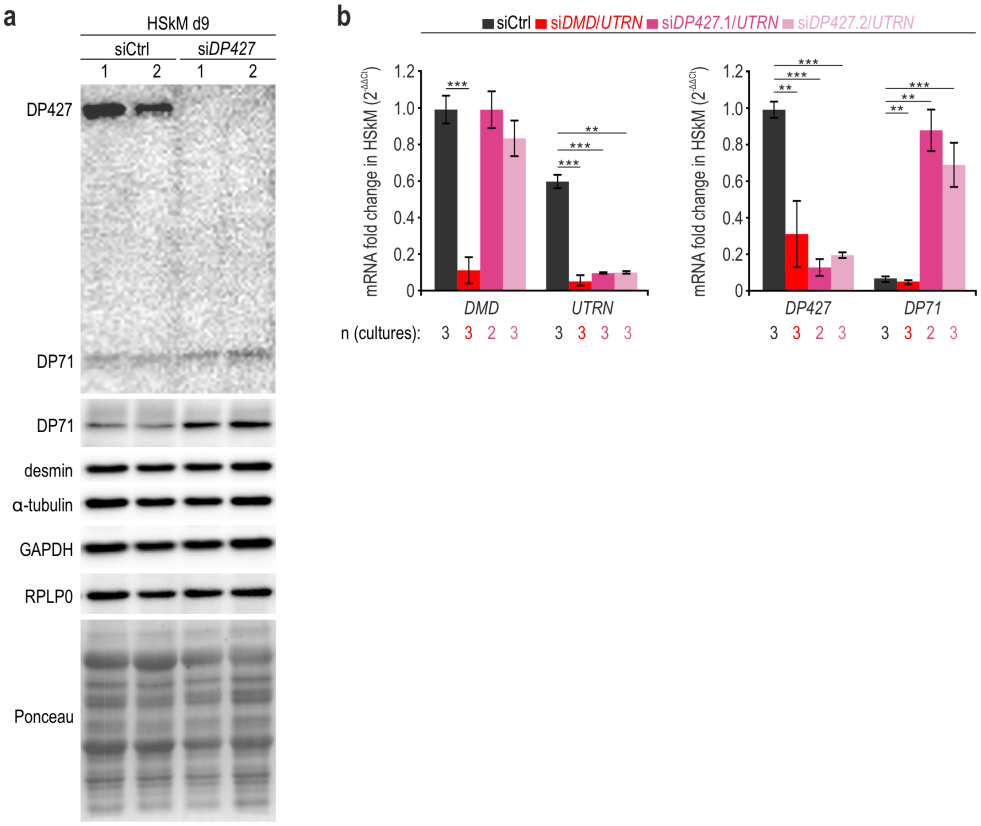


Figure S6: DP71 elevated levels following depletion of DP427.

(a) Immunoblot analyses of siRNA-treated HSkM myotubes at day 9 of differentiation. Uncropped membranes are provided in Supplementary Original Data.

(b) RT-qPCR analyses of *DMD/UTRN* and *DP427/DP71* isoforms in the HSkM cell line on day 9 of differentiation after siRNA treatment. Note that two different siRNAs targeting *DP427* resulted in a similar increase in *DP71*.

The data are presented as mean ± SD. n, number of biological replicates. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (two-tailed unpaired t–test).

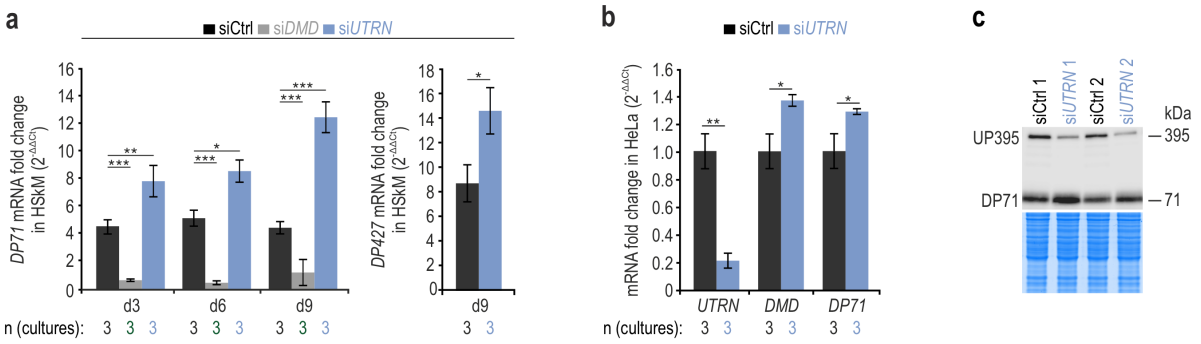


Figure S7: *DP71* and *DP427* expression increases in cells deficient in utrophin (UP395).

(a) RT-qPCR analysis of *DP71* and *DP427* expression during HSkM differentiation following *DMD* or *UTRN* silencing.

(b, **c)** RT-qPCR (**b**) and immunoblot (**c**) analyses in HeLa cells following siRNA treatment. DMD knockout lysate was used as a negative control (Supplementary Original Data). Coomassie-stained polyacrylamide gels were used to normalize the protein content.

The data are presented as mean ± SD. n, number of biological replicates. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (two-tailed unpaired t–test).

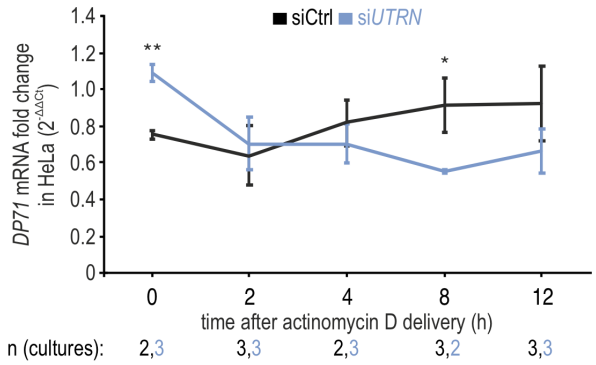


Figure S8: Utrophin deficiency does not lead to increased stability of *DP71* transcripts.

Time course of *DP71* mRNA decay (relative to *RPLP0* levels) in HeLa cells after transcriptional inhibition with actinomycin D, comparing siCtrl and si*UTRN* conditions.

The data are presented as mean ± SD. n, number of biological replicates. *, $p < 0.05$; **, $p < 0.01$ (two-tailed unpaired t–test).

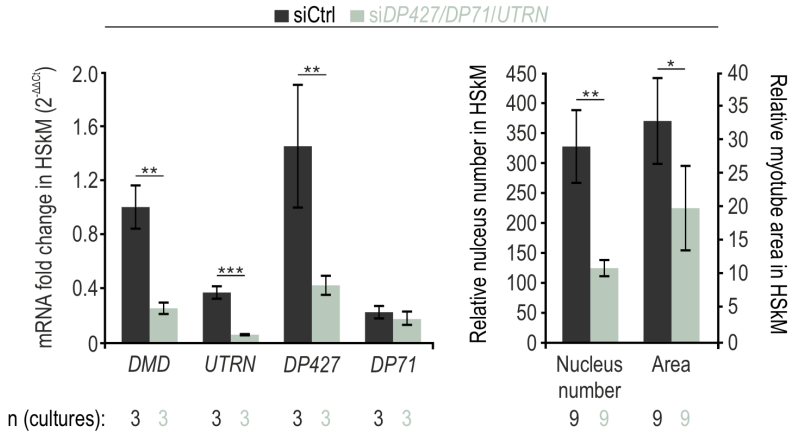


Figure S9: Depletion of DP71 in DP427-deficient myotubes results in a more severe phenotype.

Quantification of fibre plate coverage, and analysis of the number of nuclei per area following delivery of either siCtrl or si*DP427*/*DP71*/*UTRN* siRNAs in HSkM cells at day 9 of differentiation. RT-qPCR analyses confirmed the efficient silencing of the corresponding mRNAs.

The data are presented as mean ± SD. n, number of biological replicates. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (two-tailed unpaired t–test).

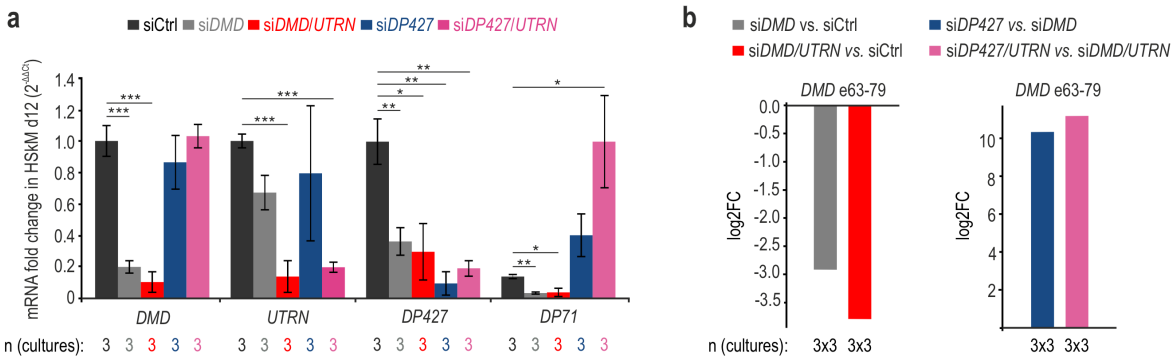


Figure S10: Validation of siRNA knockdown efficiency before and after RNA sequencing.

(a) RT-qPCR analyses of *DMD/UTRN* and *DP427/DP71* isoforms in HSkM cells on day 12 of differentiation after siRNA treatment.

(b) RNA sequencing analysis of *DMD* exons 63–79 levels in HSkM cells on day 12 of differentiation following siRNA treatment. Note the downregulation of *DP71*/*DP427* mRNAs after si*DMD* delivery and the upregulation of *DP71* levels after si*DP427* administration.

The data are presented as mean ± SD. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (two-tailed unpaired t–test).

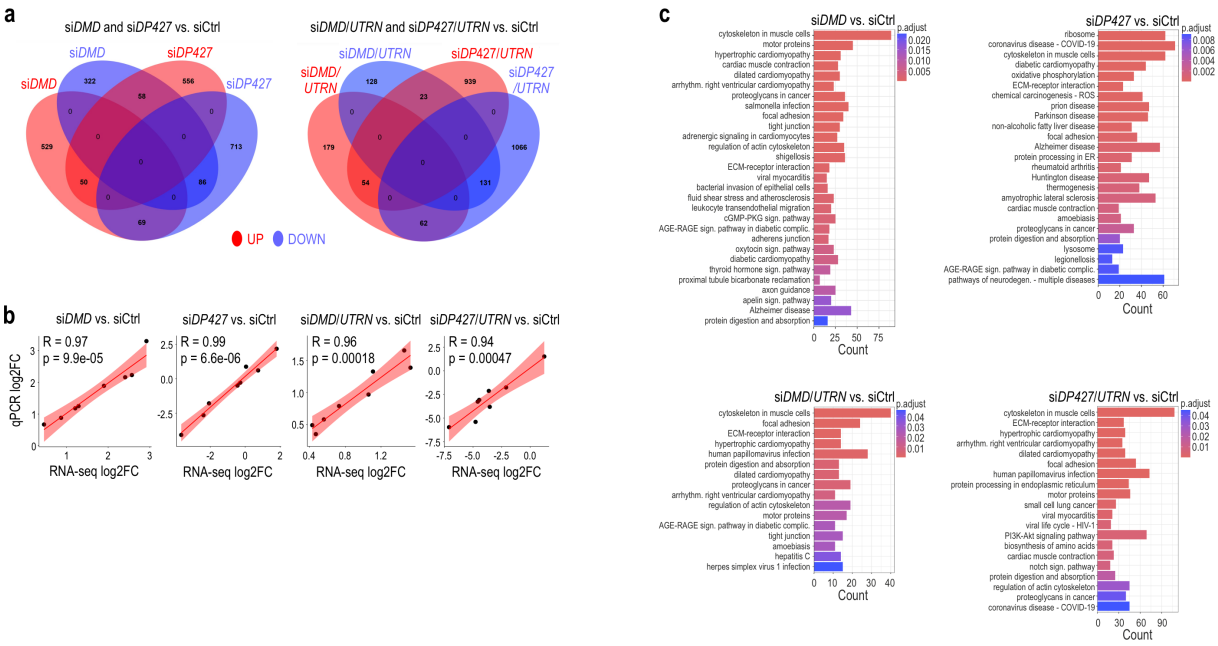


Figure S11: DP71-dependent transcriptional responses distinguish DP427-deficient from complete dystrophin-deficient myofibres.

(a, **d)** Numbers of upregulated (red) and downregulated (blue) genes in differentiated (d12) HSkM myotubes following siRNA treatment. Venn diagrams show shared and unique gene sets. Differential expression was determined using Wald test with Benjamini–Hochberg correction (FDR ≤ 0.05).

(b) Pearson correlation between RNA-seq and RT-qPCR data.

(c) KEGG pathway enrichment analysis of differentially expressed genes. Bar plots show significantly enriched pathways ranked by gene count, with colour indicating adjusted p value.

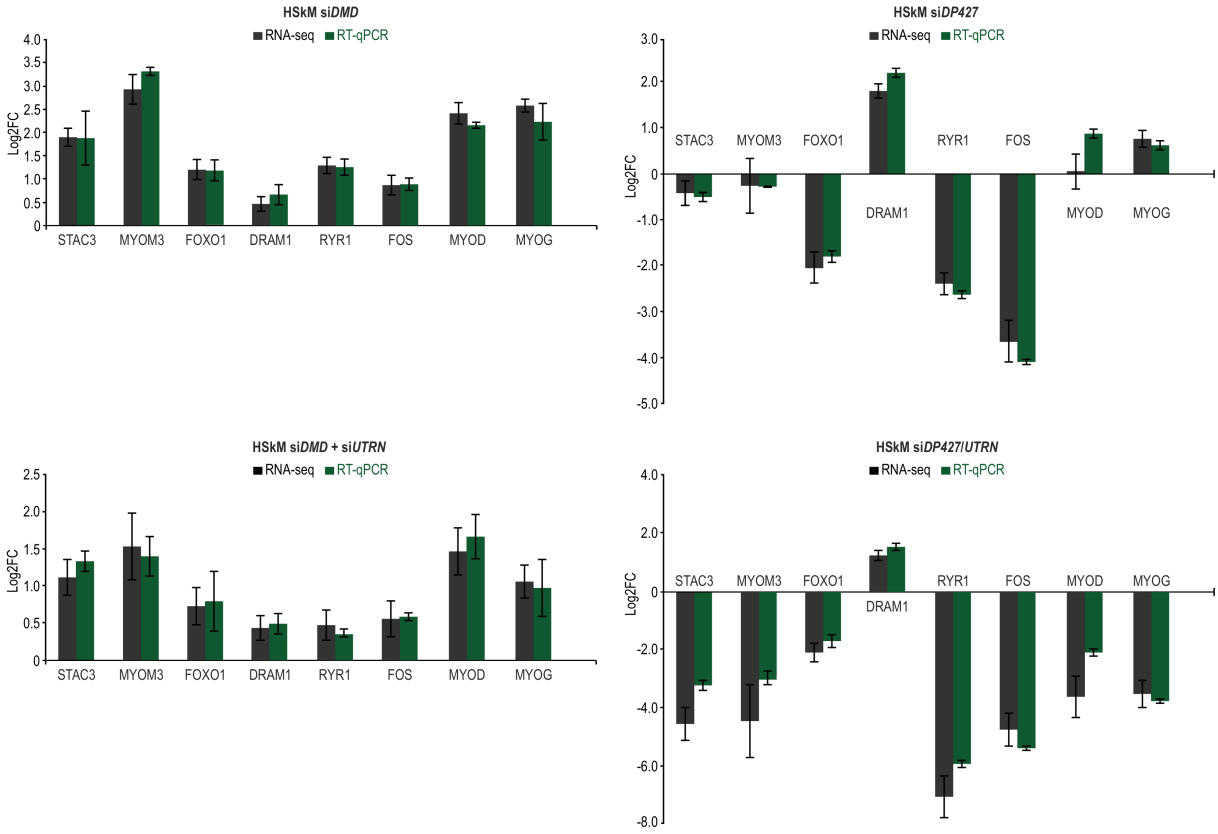


Figure S12: Validation of the RNA sequencing results.

Gene expression changes in si*DMD*, si*DP427*, si*DMD* + si*UTRN* and si*DP427* + si*UTRN* HSkM myotubes, comparing log2FC values obtained from RNA sequencing with those measured by RT-qPCR relative to their respective controls.

The data are presented as mean ± SD.

Supplementary Original Data

Fig. 4b

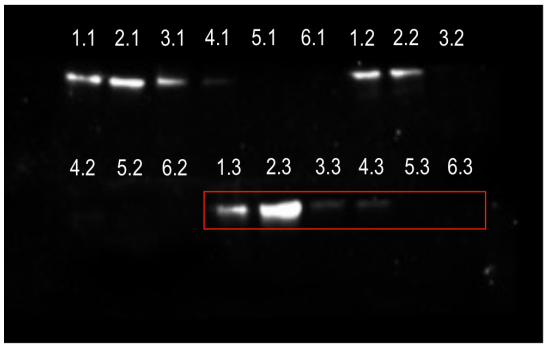
1 - total protein extract; 2 - cytoplasmic; 3 - membrane; 4 - soluble nuclear; 5 - chromatin bound; 6 - cytoskeletal

1.1 - 6.1 - biological repeat #1
1.2 - 6.2 - biological repeat #2
1.3 - 6.3 - biological repeat #3

Red frames mark immunoblot fragments shown in Figure 4b

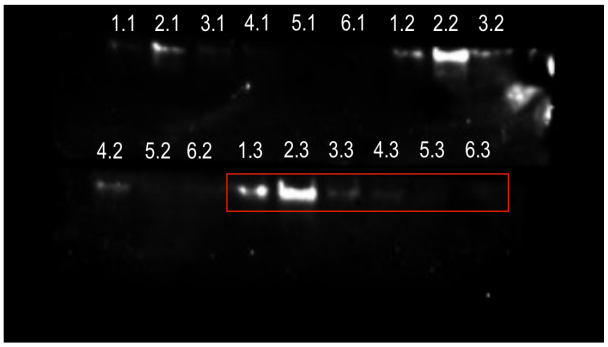
HeLa wt

GAPDH

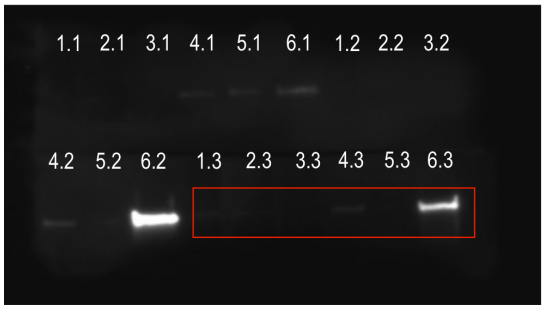


HeLa *DMD* ko

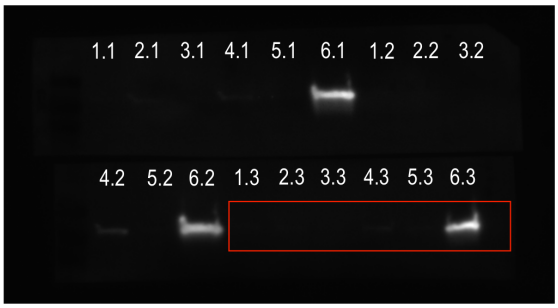
GAPDH



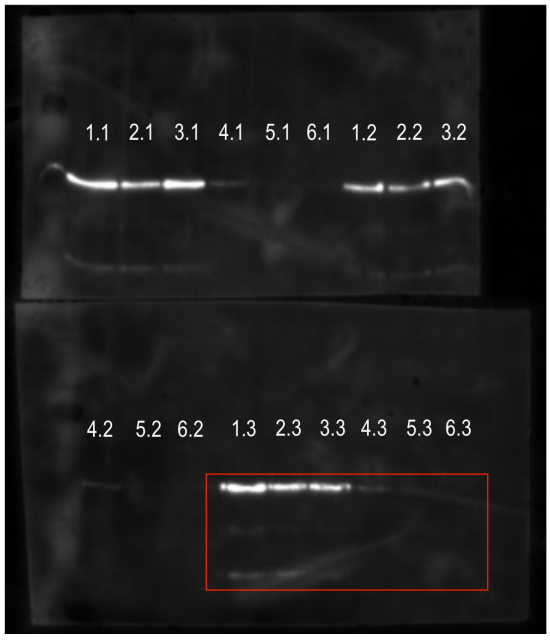
lamin B



lamin B



β-dystroglycan



β-dystroglycan

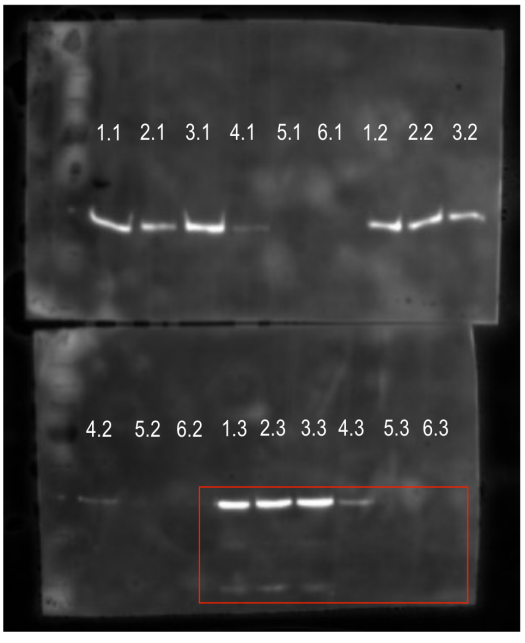


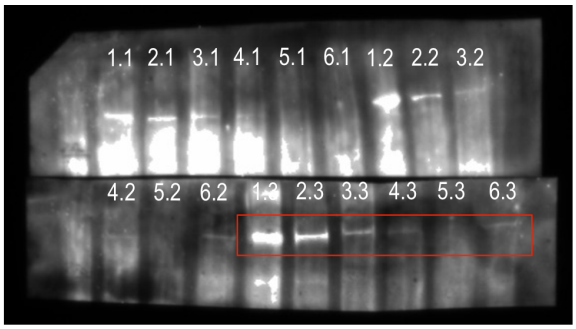
Fig. 4b

1 - total protein extract; 2 - cytoplasmic; 3 - membrane; 4 - soluble nuclear; 5 - chromatin bound; 6 - cytoskeletal

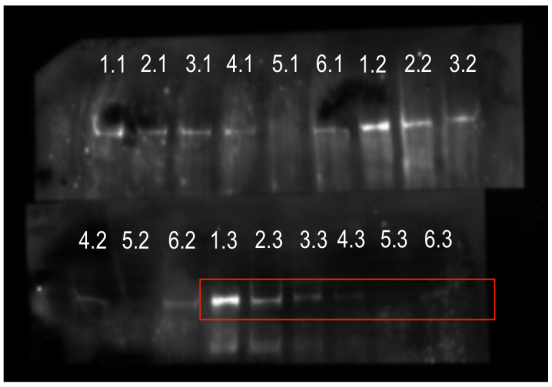
1.1 - 6.1 - biological repeat #1
1.2 - 6.2 - biological repeat #2
1.3 - 6.3 - biological repeat #3

Red frames mark immunoblot fragments shown in Figure 4b

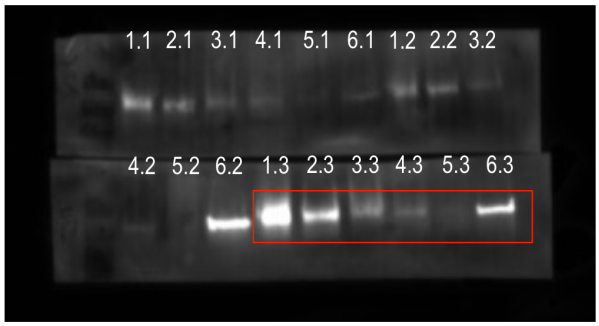
HeLa wt
UP395



HeLa *DMD* ko
UP395



DP71



DP71 +e78

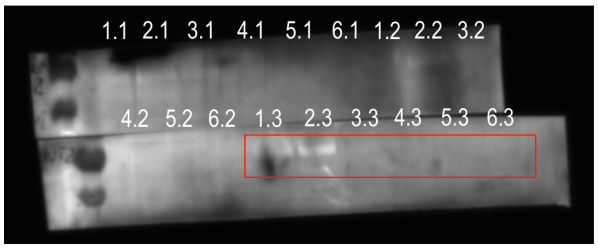
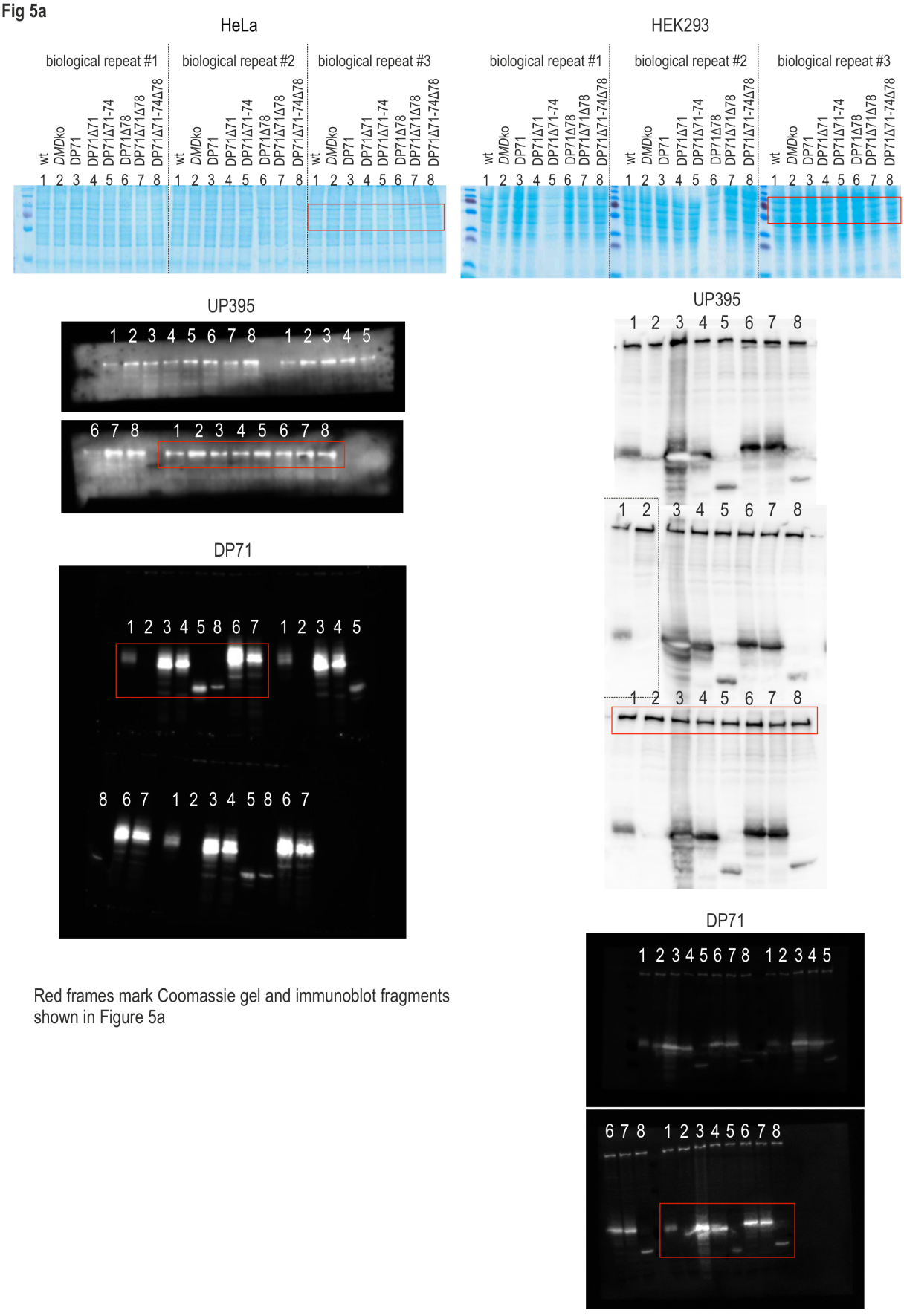


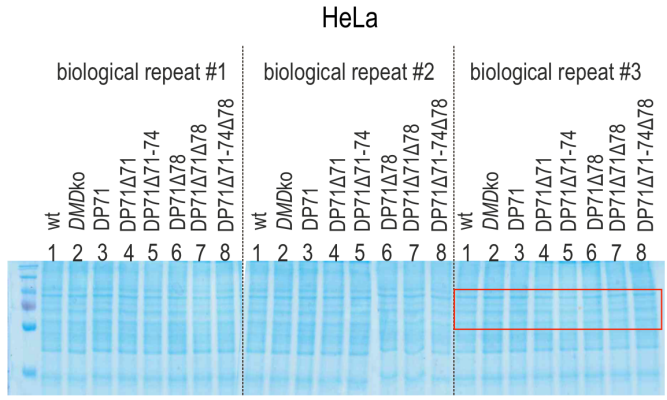
Fig 5a

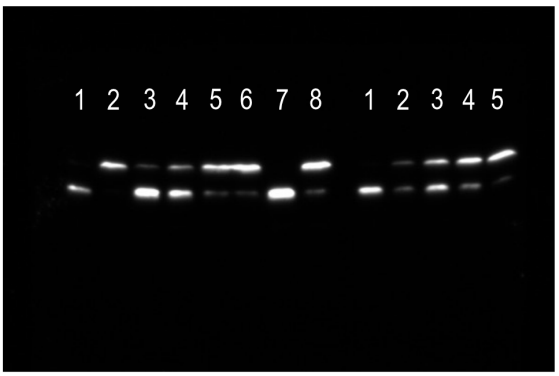


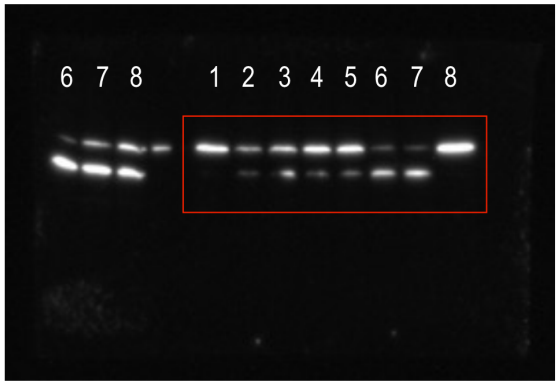
Red frames mark Coomassie gel and immunoblot fragments shown in Figure 5a

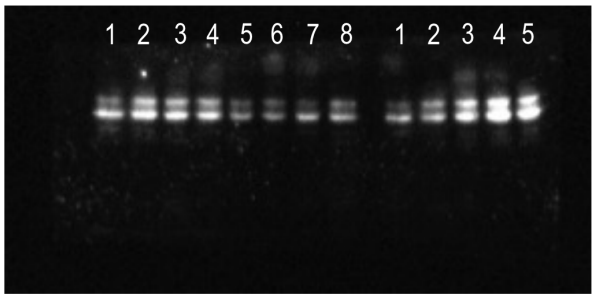
Fig 5b

Red frames mark immunoblot fragments shown in Figure 5b









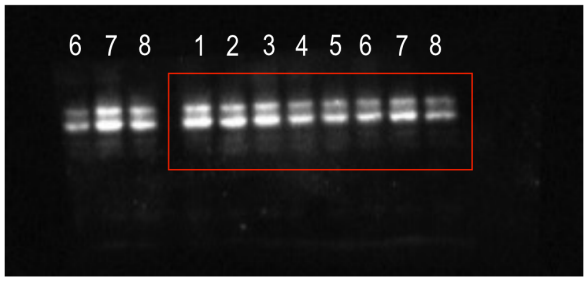


Fig. S6a 1, 2, 3 - biological repeats

Red frames mark immunoblot and Ponceau-stained membrane fragments shown in Figure S6a

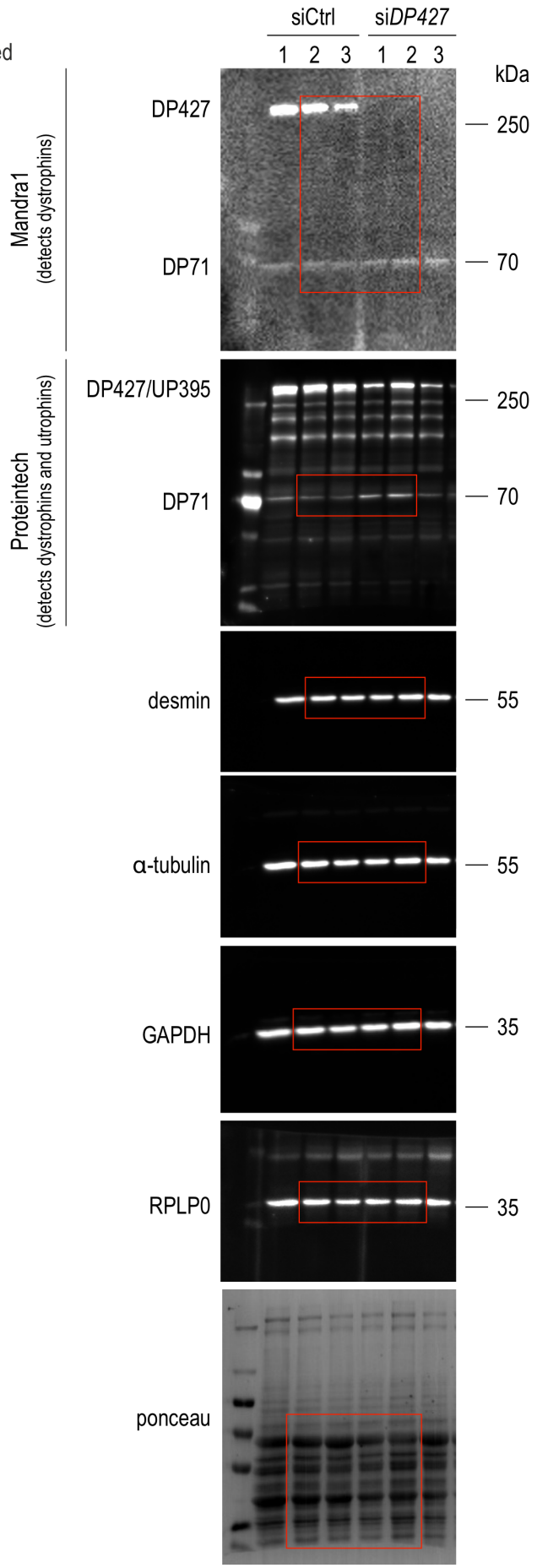


Fig. S7c

1, 2, 3 - biological repeats

Red frames mark immunoblot and Coomassie-stained gel fragments shown in Figure S7c