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Corresponding author(s): Nathalie ESCANDE-BEILLARD

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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	■	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	■	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	■	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
■	☐	A description of all covariates tested
☐	■	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	■	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	■	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
■	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
■	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
■	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection: For Data collection, no software was used.

Data analysis: Whole exome sequencing was performed using Ion PI chip (Life Technologies) in the Ion Proton Instrument (Life Technologies) followed by exome library preparation via Ion OneTouchSystem (Life Technologies). The Torrent Mapping Alignment Program (TMAP) from the Torrent Suite was then used to align the sequence reads to the human reference genome (GRCh37/hg19 Assembly). The variant calling was performed by the Torrent Variant Caller plugin (v.5.0.2). Finally, the annotated variants with their genes, genomic position, coverage and quality score were analyzed by the Clinical Reporter (Fabric Genomics). Following variant annotation, they were checked and filtered to retain those variants with low allele frequency (< 0.5%) in public genomic databases including Genome Aggregation Database (https://gnomad.broadinstitute.org/), the Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) and the BRAVO/TOPmed database (https://bravo.sph.umich.edu/freeze8/hg38/). Besides, potential pathogenicity of the variants was predicted using online tools Polyphen2, SIFT, MutationTaster, M-CAP, FATHMM-MKL, Mutation assessor, CADD and DANN.

For RNA-sequencing, Illumina BaseSpace (Illumina Inc., San Diego, CA) was used for bioinformatics analysis of the raw fastq files. Briefly, raw fasta files were aligned to genome version hg19 via the STAR aligner using paired-end reads option, with default settings. Obtained bam files were then employed as inputs for DESeq2 to obtain counts data and to find differentially expressed genes. Splicing analyses were performed with rMATS 4.1.2 turbo software, using the mentioned bam files as input and enabling the “variable read length option”.Sashimi plots were visualized via “rmats2sashimiplot” package. IDEP.951, a web-based tool for RNA-Seq analyses was used for further visualization and statistical analyses.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data analysis

The protein 3D structure predictions were generated by I-TASSER and visualized by PyMOL software, version 2.4.1. To analyze coiled-coil interfaces, Socket2 software was utilized. GraphPad Prism 9 was used to statistically analyze the rest of data.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The RNA sequencing datasets generated during the current study are available in the Gene Expression Omnibus repository, accession number GSE232712, available online at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE232712. Due to privacy restrictions, full set of DNA sequencing is not available, however specific data can be requested to the corresponding author. The data generated in this study are available within the article and its supplementary data files, and source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	There are 18 patients in this study including 10 males and 8 females. We also included 3 unaffected controls (1 male and 2 females). Consent has been obtained for sharing of individual-level data. The samples are selected according to their genotype. No pre-selection was made based on age, gender and origin.
Reporting on race, ethnicity, or other socially relevant groupings	There is not any socially relevant grouping. Samples were allocated into experimental groups according to their genotype.
Population characteristics	This study involves the participation of male and female individuals of all ages presenting with muscular dystrophy with homozygous or compound heterozygous variants of unknown significance (VUS) in *SNUPN* gene. No pre-selection was made according to age, gender or origin. For reference, all detailed demographic information for the individuals is included in Supplementary data 1.
Recruitment	The convenient sampling technique was used to recruit the participants. The inclusion criteria involved all the patients with Muscular dystrophy and have *SNUPN* VUS. There was no exclusion criteria. All patients who were found to fulfill the inclusion criteria by the collaborating physicians and centers were offered to participate in this study. Parents (legal representatives) and patients who agreed to participate, signed the informed consent form and were recruited accordingly.
Ethics oversight Note that full information on the approval of the study protocol must also be provided in the manuscript.	Written informed consent was obtained from the individuals/their legally authorized guardians for the use of their clinical and genetic information and available biological samples, as well as for the publication of patients' images (Fig.1 and Supplementary Fig.1) and the used data in this project in accordance with local ethical review boards in Turkey, Italy, Switzerland, Iraq, Iran, Macedonia, Colombia, Roumania and Guatemala. The study protocol was also approved by Koç university hospital Institutional Review Board (Koç University 2015.120.IRB2.047).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

■ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical method was used to predetermine sample size. For experiments involving patients samples, sample size was based on the availability of the specimens and patients' data. Cell based assays were all performed in technical and biological triplicates to ensure reproducibility. Immunofluorescence studies were conducted for each protein of interest, wherein the cell count in five randomly selected areas for each sample image was used for measurements. This approach was performed in triplicate for each sample and each protein under study, resulting in a sufficient number of cells to provide statistically valid measurements.
Data exclusions	No data was excluded.
Replication	All experiments were replicated at least 3 times.
Randomization	Samples were allocated into experimental groups according to their genotype. No experiments were done in this study that needed to be randomized.
Blinding	The investigators were not blinded to group allocation during data analysis as our studies did not involve therapeutic interventions. Samples were grouped and analyzed by their genotype. All the differences between patients and control samples were measured using objective methods, which eliminate the risk of measurement bias by the study team. We made conscious effort to avoid bias and the reproducibility of the results was confirmed by independent replicates.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	■ Antibodies
☐	■ Eukaryotic cell lines
■	☐ Palaeontology and archaeology
■	☐ Animals and other organisms
■	☐ Clinical data
■	☐ Dual use research of concern
■	☐ Plants

Methods

n/a	Involved in the study
■	☐ ChIP-seq
■	☐ Flow cytometry
■	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Antibody	Host	Class	Assay and dilution	Company	Clone #	Catalog #
SPN1	Rabbit	Polyclonal	Immunoblot (1/1000) IF (cell: 1/50; tissue: 1/100)	Proteintech	-	15358-1-AP
SNRPB	Mouse	Monoclonal	Immunoblot (1/100) IF (1/50)	Invitrogen	Y12	MA5-13449
COILIN	Mouse	Monoclonal	Immunoblot (1/500) IF (1/100)	Abcam	IH10	ab87913
SMN	Rabbit	Polyclonal	IF (1/25)	Proteintech	-	11708-1-AP
α-SARCOGLYCAN	Mouse	Monoclonal	IF (tissue: 1/50)	Leica	AD1/20A6	NCL-L-a-SARC
β-DYSTROGLYCAN	Mouse	Monoclonal	Immunoblot (1/1000)	Leica	43DAG1/8D5	NCL-b-DG
β-TUBULIN	Mouse	Monoclonal	IF (1/100)	Sigma	TUB2.1	T5201
VIMENTIN	Rabbit	Monoclonal	IF (1/100)	Abcam	EPR3776	ab92547
VINCULIN	Mouse	Monoclonal	IF (1/100)	Abcam	VIN-54	ab130007
COLLAGEN-IV	Rabbit	Polyclonal	Immunoblot (1/2500) IF (cell: 1/100; tissue: 1/400)	Abcam	-	ab6586
FIBRILLARIN	Rabbit	Polyclonal	IF (1/200)	Abcam	-	ab5821
H2A	Rabbit	Polyclonal	Immunoblot (1/1000)	Cell Signaling	-	2595
GAPDH	Mouse	Monoclonal	Immunoblot (1/500)	Santa Cruz	0411	47724
MYC	Mouse	Monoclonal	Immunoblot (1/1000)	Sigma	4A6	05-724
FLAG	Rabbit	Polyclonal	Immunoblot (1/1000)	Cell Signaling	-	2368
DESMIN	Mouse	Monoclonal	IHC - IF (2.5 µg/ml)	Millipore	DE-B-5	Mab3430
ACTIN	Mouse	Monoclonal	IF (1/50)	Dako	HHF35	M0635
αβ-CRYSTALLIN	Mouse	Monoclonal	IF (1/100)	Leica	G2JF	Ncl-abcrys-512

Validation

SPN1 western blot from human cells: "HeLa cells were subjected to SDS PAGE followed by western blot with 15358-1-AP (SNUPN antibody) at dilution of 1:2000 incubated at room temperature for 1.5 hours.
SPN1 immunofluorescence: "Immunofluorescent analysis of MCF-7 cells, using SNUPN antibody 15358-1-AP at 1:25 dilution and Rhodamine-labeled goat anti-rabbit IgG (red). Immunofluorescent analysis of HeLa cells using 15358-1-AP (SNUPN antibody) at dilution of 1:25 and Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L)."
SNRPB western blot for human cells: "This Antibody was verified by Knockdown to ensure that the antibody binds to the antigen stated" "Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HeLa cells were transfected with SNRPB siRNA and decrease in signal intensity was observed in western blot application using anti-SNRPB siRNA"
SNRPB immunofluorescence: "This Antibody was verified by Knockdown to ensure that the antibody binds to the antigen stated." "Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HeLa cells were transfected with SNRPB siRNA and reduction of specific signal was observed in immunofluorescence application using SNRPB Mouse Monoclonal antibody"
COILIN western blot from human cells: This antibody is stated to be validated by KO cells for the target protein by the manufacturer. "Validated in ICC, IP, Flow Cyt (Intra), WB, IHC-P and tested in Human samples."
COILIN immunofluorescence: This antibody is stated to be validated by KO cells for the target protein by the manufacturer. "Validated in ICC, IP, Flow Cyt (Intra), WB, IHC-P and tested in Human samples."
SMN immunofluorescence: "Immunofluorescent analysis of (4% PFA) fixed HepG2 cells using SMN antibody (11708-1-AP) at dilution of 1:800 and CoraLite®488-Conjugated AffiniPure Goat Anti-Rabbit IgG(H+L), CL594-Phalloidin (red)." has been provided as an image by the manufacturer.

α-SARCOGLYCAN immunofluorescence: Manufacturer states that the antibody is regulated for use *in vitro* diagnostic purposes for muscle pathologies. IF staining using the stated antibody has been performed in a published article previously by Justel *et al.* (2023) DOI:10.1136/jmg-2022-109132
β-DYSTROGLYCAN western blot from human cells: Manufacturer states that the antibody is regulated for use *in vitro* diagnostic purposes for muscle pathologies. "Clone 43DAG1/8D5 has been used in immunohistochemical and immunoblotting studies of more than 930 patients to identify a deficiency of the 43 kD dystrophin-associated glycoprotein, beta-dystroglycan"
β-TUBULIN immunofluorescence: "SH-SY-5Y cells were fixed and permeabilized with methanol followed by methanol:acetone. Fixed cells were stained with 2 µg/mL Monoclonal Anti-ß-Tubulin, Clone: TUB 2.1 (Cat. No. T5201). The antibody was developed using Goat Anti-Mouse IgG, Cy3 conjugate. Cells were counterstained with DAPI (blue) to stain nuclei."
VIMENTIN immunofluorescence: This antibody is stated to be validated by KO cells for the target protein by the manufacturer. "This product gave a positive signal in HeLa (VIM knockout HeLa cells were used as a negative control) fixed with 4% formaldehyde (10 min) and 100% methanol (5 min)."
VINCULIN immunofluorescence: "Immunocytochemistry/Immunofluorescent analysis of human mammary cancer cells labelling Vinculin with ab130007 at 2µg/ml, followed by a DyLight®488 Conjugated Goat Anti-Mouse secondary antibody at 1/100 dilution. DAPI was used as a nuclear counterstain." has been provided as an image by the manufacturer.
COLLAGEN-IV western blot from human cells: WB using the stated antibody in human cells has been performed in a published article previously by Jiang *et al.* (2016) DOI: 10.3892/etm.2016.3497
COLLAGEN-IV immunofluorescence: The antibody has been cited in 3 published articles for IF staining of human cells by Gouveia *et al.* (2017) DOI: 10.1016/j.biomaterials.2016.12.023, by Surolia *et al.* (2017) DOI: 10.1172/jci.insight.91377 and by Patrikoski *et al.* (2013) DOI: 10.1155/2017/6909163
FIBRILLARIN immunofluorescence: "ab5821 staining Fibrillarin in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab5821 at 0.1µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control." has been provided as an image by the manufacturer.
H2A western blot from human cells: Western blot image for the antibody has been provided by the manufacturer. "Western blot analysis of extracts from 293 cells ..., using … or Histone H2A.X Antibody (lower)."
GAPDH western blot from human cells: "GAPDH Antibody (0411): sc-47724. Fluorescent western blot analysis of GAPDH expression in MCF7, HeLa, C32, Jurkat, Hep G2 and BJAB whole cell lysates." "Western blot analysis of GAPDH expression in U-87 MG and SK-BR-3 (B) whole cell lysates." has been provided as an image by the manufacturer.
MYC western Blot from human cells: Manufacturer states "Anti-Myc Tag, clone 4A6, is a mouse monoclonal antibody that is validated for use in ChIP, IC, IF, IP and WB for the detection of Myc Tag…"
FLAG western blot from human cells: "Western blot analysis of extracts from COS or 293T cells, mock transfected (lane 1) or expressing DYKDDDDK-tagged p73 (lane2), DYKDDDDK-tagged FKHRL1 (lane 3) or truncated DYKDDDDK-tagged huntingtin (lane 4), using DYKDDDDK Tag Antibody." has been provided as an image by the manufacturer.
DESMIN immunohistochemistry: This antibody was stated to be validated by the manufacturer. "DE-B-5 validated for use in WB, IH, IH(P), IH(P)." "The antibody reacts with desmin from human, pig, rat and taod. In tissue sections thisantibody is used to stain skeletal, cardiac, visceral, and some vascular smooth muscle cells. Cell lines such as RD (ATCC CCL 136) and hamster BHK-21 are positive (1)."
DESMIN immunofluorescence: This antibody was stated to be validated by the manufacturer. "DE-B-5 validated for use in WB, IH, IH(P), IH(P)." "The antibody reacts with desmin from human, pig, rat and taod. In tissue sections this
antibody is used to stain skeletal, cardiac, visceral, and some vascular smooth muscle cells. Cell lines such as RD (ATCC CCL 136) and hamster BHK-21 are positive (1)."
ACTIN immunofluorescence: IF staining using the stated antibody has been performed in 2 published articles previously by Campos *et al.* (2013) DOI: 10.1016/j.jsbmb.2012.08.002 and Nitschke *et al.* (2013) DOI: 10.1038/s12276-018-0163-5
αB-CRYSTALLIN immunofluorescence: IF staining using the stated antibody has been performed in a published article previously

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Primary fibroblasts derived from patients and controls: 1) Patient F1-II:1 (Female) 2) Patient F3-II:4 (male) 3) Patient F4-II:2 (male) 4) Patient F4-II:3 (Female) 5) Patient F10-II:1 (Female) 6) Control WT1 (Male) 7) Control WT2 (Female) 8) Control WT3 (Female) Commercial cell lines: 1) Hela Cells (ECACC, Catalog #93021013, PRID:CVCL_0030) 2) HEK293T Cells (ATCC, Catalog#CRL_3216, PRID:CVCL_0063)
Authentication	None of the cell lines used were authenticated.
Mycoplasma contamination	We confirm that all cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	None of the cell lines used are listed as commonly misidentified in the ICLAC database.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	Not applicabale
Study protocol	Not applicabale
Data collection	Not applicabale
Outcomes	Not applicabale