

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Zeiss ZEN software v3.8 was used for epifluorescence, H&E, and Masson's trichrome image; Leica Leica SP8 Lightning confocal microscope (DMI8, Leica Microsystems, Wetzlar, Germany) equipped with Leica LAS X software (version 1.4.5.27713, Leica Microsystems, Wetzlar, Germany) was used to collect confocal fluorescence images; ChemiDoc XRS+ system was used to collect Western blot data; DMA v5.501 was used to collect muscle contractility data.
Data analysis	Graphpad prism 10.1.1 was used to analyze the data. Adobe Illustrator 28.3 was used to assemble figures. Western blots were imaged and quantified using Image Lab 6.1 software. ImageJ 1.53a was used to analysis fiber size and CNF quantification.

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

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](#)

All data generated or analyzed during this study are included within the article and its Supplementary Information files. 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

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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](https://www.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	Sample size is estimated with G-power software 3.1 by the significance level (0.05), effect size (based on the data in our previous studies) and a given power (usually 0.8). We also conducted power analysis after the experiment and found enough power value (>0.8) in each statistical analysis.
Data exclusions	No data exclusion
Replication	All the experimental findings were repeated with a minimum of three independent experiments (or animals). All attempts for replication were successful.
Randomization	Mice and cells were assigned randomly into experimental groups and processed in an arbitrary order.
Blinding	All the enrolled mice or subsequent samples were labeled only with mouse ID numbers without genotype or type of treatment information. Genotype or treatment type were decoded after the data acquisition and quantification analysis were complete. The culture studies (transfection, protein isolation) were carried out identically through standard procedures that should not bias outcomes.

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

Methods

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

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

Antibodies

Antibodies used

Mouse anti-MANHINGE1B (10F9) Developmental Studies Hybridoma Bank WB (1:100);
 Mouse anti-MANHINGE1C (5D12) Developmental Studies Hybridoma Bank WB (1:100);
 Mouse anti-MANEX50 (6A9) Developmental Studies Hybridoma Bank WB (1:100);
 Mouse anti- α -dystroglycan (IIH6 C4) Developmental Studies Hybridoma Bank IF (1:10);
 Mouse anti- β -dystroglycan Santa Cruz Biotechnology #sc-33702 IF (1:50);
 Mouse anti- α -sarcoglycan Abcam #ab234589 IF (1:100);
 Mouse anti- β -sarcoglycan Santa Cruz Biotechnology #sc-14176 IF (1:50);
 Mouse anti-nNOS Santa Cruz Biotechnology #sc-5302 IF (1:50);
 Mouse anti- α -dystrobrevin Becton Dickinson and Company #610766 IF (1:100);
 Mouse anti-p44/42 MAPK (Erk1/2) Cell Signaling Technology #4696 WB (1:1000);
 Rabbit anti-Phospho-p44/42 MAPK (Erk1/2) Cell Signaling Technology #9101 WB (1:500);
 Rabbit anti-MURC Abcam #ab121647 IF (1:100);
 Rabbit anti-DMD/8773R NeoBiotechnologies WB (1:200);
 Rabbit anti-Dystrophin Abcam #ab15277 WB (1:1000);
 Rabbit anti-GAPDH Cell Signaling Technology #2118S WB (1:2000);
 Rat anti-laminin- α 2 Enzo Life Sciences #ALX-804-190-C100 IF (1:100);
 Goat anti-Mouse IgG (H+L) Alexa Fluor'M 594 Invitrogen #A-11032 IF(1:400);
 Goat anti-Rat IgG (H+L) Alexa Fluor'M 488 Invitrogen #A-11006 IF(1:400);
 Goat anti-Rabbit IgG (H+L) Alexa Fluor Texas Red Invitrogen #T2767 IF(1:400);
 Goat anti-Mouse IgM (H+L) Alexa Fluor'M 568 Invitrogen #A21043 IF(1:400);
 Secondary HRP-conjugated goat anti-mouse Cell Signaling Technology#7076 WB (1:4000);
 Secondary HRP-conjugated goat anti-rabbit Cell Signaling Technology# 7074 WB (1:4000).

Validation

All antibodies are available on the manufacturer's websites as listed below. The antibodies have been validated by the manufacturers. No additional validation was carried out.

1. Mouse anti-MANHINGE1B (10F9) Developmental Studies Hybridoma Bank

The species: Canine, Human

Application: WB

Website: <https://dshb.biology.uiowa.edu/MANHINGE1B-clone-10F9>

2. Mouse anti-MANHINGE1C (5D12) Developmental Studies Hybridoma Bank

The species: Canine, Human

Application: WB

Website: <https://dshb.biology.uiowa.edu/MANHINGE1C-5D12>

3. Mouse anti-MANEX50 (6A9) Developmental Studies Hybridoma Bank

The species: Human

Application: WB

Website: <https://dshb.biology.uiowa.edu/MANEX50-6A9>

4. Mouse anti- α -dystroglycan (IIH6 C4) Developmental Studies Hybridoma Bank

The species: Mus musculus (House mouse), Homo sapiens (Human)

Application: WB, IHC, IF, IHC-IF, FC/FACS, IHC-Frozen

Website: <https://dshb.biology.uiowa.edu/IIH6-C4>

5. Mouse anti- β -dystroglycan Santa Cruz Biotechnology #sc-33702

The species: mouse, rat, human

Application: WB, IP, IF and IHC(P)

Website: <https://www.scbt.com/p/beta-dystroglycan-antibody-4f7?requestFrom=search>

6. Mouse anti- α -sarcoglycan Abcam #ab234589

The species: Mouse, Rat, Human

Application: IHC-Fr

Website: <https://www.abcam.com/products/primary-antibodies/alpha-sarcoglycan-antibody-ivd3-a9-ab234589.html>

7. Mouse anti- β -sarcoglycan Santa Cruz Biotechnology #sc-14176

The species: mouse, rat and human

Application: WB, IF, ELISA

Website: [extension://bfdogplmndidlpjfhiojckpakdkjkkil/pdf/viewer.html?file=https%3A%2F%2Fdatasheets.scbt.com%2Fsc-14176.pdf](https://www.bfdogplmndidlpjfhiojckpakdkjkkil/pdf/viewer.html?file=https%3A%2F%2Fdatasheets.scbt.com%2Fsc-14176.pdf)

8. Mouse anti-nNOS Santa Cruz Biotechnology #sc-5302

The species: mouse, rat and human

Application: WB, IP, IF and ELISA

Website: <https://www.scbt.com/p/nos1-antibody-a-11?requestFrom=search>

9. Mouse anti- α -dystrobrevin Becton Dickinson and Company #610766

The species: Rat, Mouse, Rabbit

Application: WB, IHC, IF

Website: <https://www.bdbiosciences.com/en-us/products/reagents/microscopy-imaging-reagents/immunohistochemistry-reagents/purified-mouse-anti-dystrobrevin.610766>

10. Mouse anti-p44/42 MAPK (Erk1/2) Cell Signaling Technology #4696

The species: H M R Mk Mi Z B Pg

Application: WB, IF, IHC

Website: <https://www.cellsignal.com/products/primary-antibodies/p44-42-mapk-erk1-2-l34f12-mouse-mab/4696>

11. Rabbit anti-Phospho-p44/42 MAPK (Erk1/2) Cell Signaling Technology #9101

The species: H M R Hm Mk Mi Dm Z B Pg Ce

Application: WB, IF, IHC, F

Website: <https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-antibody/91011>

12. Rabbit anti-MURC Abcam #ab121647 IF (1:100)

The species: Human

Application: ICC/IF, IHC-P

Website: <https://www.abcam.com/products/primary-antibodies/murc-antibody-ab121647.html>

13. Rabbit anti-DMD/8773R NeoBiotechnologies

The species: Human

Application: IF, IHC, WB

Website: <https://www.neobiotechnologies.com/product/dystrophin-dmd-marker-of-duchenne-and-becker-muscular-dystrophy-9/>

14. Rabbit anti-Dystrophin Abcam #ab15277

The species: Mouse, Human

Application: IHC-Fr, IHC-P

Website: <https://www.abcam.com/products/primary-antibodies/dystrophin-antibody-ab15277.html>

15. Rabbit anti-GAPDH Cell Signaling Technology #2118S

The species: H M R Mk B Pg

Application: WB, IF, IHC

Website: <https://www.cellsignal.com/products/primary-antibodies/gapdh-14c10-rabbit-mab/2118>

16. Rat anti-laminin- α 2 Enzo Life Sciences #ALX-804-190-C100

The species: Human, Mouse

Application: ELISA, IHC, IP

Website: <https://www.fishersci.com/shop/products/laminin-2-a-2-chain-4h8-2-100/NC1425153>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

HEK293 cell line was obtained from the American Type Culture Collection (ATCC).

Authentication

HEK293 cell line was not authenticated.

Mycoplasma contamination

HEK293 cells were tested for mycoplasma contamination by PCR.

Commonly misidentified lines
(See [ICLAC](#) register)

To the best of our knowledge, no misidentified cell lines have been used in this study.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

The C57BL/6J and mdx4cv (B6Ros.Cg-Dmd mdx4cv/J) mice purchased from the Jackson Laboratory and housed at Indiana University Laboratory Animal Resource Center following animal use guidelines. The mice from 3-12 weeks of age were used for experiments. All mice were maintained under standard conditions of constant temperature ($72 \pm 4^\circ\text{F}$), humidity (relative, 30–70%), in a specific

pathogen-free facility and exposed to a 12-h light/dark cycle.

Wild animals

This study did not involve wild animals.

Reporting on sex

This study used male mice.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight

The animal experiments were ethically reviewed by the Animal Care, Use and Review Committee of Indiana University and carried out in accordance with animal use guidelines.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A