nature portfolio

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# 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 (*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<br>*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<br>*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 software packages used in analysis of raw sequencing data and statistics, see below or Methods section. |
| Data analysis | The single nuclei RNA sequencing data of all samples were demultiplexed using bcl2fastq (version 2.20) and aligned using cellranger count (version 5.0). Data analysis was carried out on R (version 4.0.3) using Seurat (version 4.0.0).<br>Bulk-RNA sequencing data was aligned using Salmon (version 1.1.0) and analyzed on R (version 4.1.2) using DESeq2.<br><br>Other software used for data anlysis included:<br>GraphPad Prism 9<br>Microsoft Excel 2016 |

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

Source data are provided with this paper. Raw single nuclei RNA-seq and bulk RNA-seq sequencing data are deposited on NCBI Gene Expression Omnibus (GEO) under the accession number GSE267913 (SubSeries GSE267910 and GSE267909). Processed single nuclei RNA-seq data is accessible for download from CZ CELLxGENE.

## 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 | *Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.*<br>*Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.*<br>*Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.* |
| Reporting on race, ethnicity, or other socially relevant groupings | *Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).*<br>*Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)*<br>*Please provide details about how you controlled for confounding variables in your analyses.* |
| Population characteristics | *Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."* |
| Recruitment | *Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.* |
| Ethics oversight | *Identify the organization(s) that approved the study protocol.* |

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

# Life sciences study design

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

| | |
|---|---|
| Sample size | Sample size was chosen depending on the technique and previous experience (Castets et al., 2019, Nature Communications)(Petrany et al., 2020, Nature Communications)(Chemello et al., 2020, PNAS). No power calculations were performed to choose group size. |
| Data exclusions | No data were excluded from our analyses. |
| Replication | Individual replicates are shown on each graph and stated in the figure legends. |
| Randomization | For each experimental group, animals were randomly selected from a group of mice within the same genotype and age.<br>For cell culture analysis, random fields were imaged for all groups. |
| Blinding | As the same person was conducting most experiments, the investigator was not always blinded. The investigator was not blinded for group allocation for experiments, in which sample order mattered (e.g. Western blot, qPCR). For imaging and analysis the investigator was blinded or |

another scientist was asked to image and quantify.

# 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 |
|---|---|
| [ ] | [x] Antibodies |
| [ ] | [x] Eukaryotic cell lines |
| [x] | [ ] Palaeontology and archaeology |
| [ ] | [x] Animals and other organisms |
| [x] | [ ] Clinical data |
| [x] | [ ] Dual use research of concern |
| [x] | [ ] Plants |

## Methods

| n/a | Involved in the study |
|---|---|
| [x] | [ ] ChIP-seq |
| [x] | [ ] Flow cytometry |
| [x] | [ ] MRI-based neuroimaging |

## Antibodies

Antibodies used

Immunostaining:
Laminin α4 (1:400, Gift from P. D. Yurchenco), Neurofilament (1:15000, N4142, Sigma), Synaptic vesicle protein 2 (1:300, SV2, DSHB), MuSK (1:8000, 194T, Prof. Markus A. Rüegg), Agrin (1:2000, C-Term 204, Prof. Markus A. Rüegg), FLAG (1:500, F1804, Sigma), Desmin (1:200, ab15200, Abcam), Laminin (1:500, L9393, Sigma), Synaptophysin (1:300, MA5-14532, Invitrogen), GLUT4 (1:300, ab33780, Abcam), GRP78 (1:300, ab21685, Abcam), Lamp1 (1:300, D2D11, Cell Signaling), TOM20 (1:300, D8T4N, Cell Signaling), GM130 (1:3200, D6B1, Cell Signaling)

Western Blot:
FLAG (1:1000, F1804, Sigma), alpha-Tubulin (1:800, ab15246, Abcam), Na/K ATPase (1:1000, 3010, Cell Signaling), GM130 (1:1000, D6B1, Cell Signaling), MuSK (1:40000, 194T, Prof. Markus A. Rüegg), GAPDH (1:2000, 14C10, Cell Signaling), TGOLN2 (1:1000, AHP500G, Biorad), TPST2 (1:1000, GTX46685, GeneTex)

Validation

Laminin α4: https://www.jci.org/articles/view/90854,
Neurofilament: https://www.sigmaaldrich.com/CH/en/product/sigma/n4142,
Synaptic vesicle protein 2: https://dshb.biology.uiowa.edu/SV2,
MuSK: https://doi.org/10.1016/j.expneurol.2011.04.018,
Agrin: https://www.sciencedirect.com/science/article/pii/S0960896603000361,
FLAG: https://www.sigmaaldrich.com/CH/en/product/sigma/f1804,
Desmin: https://www.abcam.com/en-us/products/primary-antibodies/desmin-antibody-cytoskeleton-marker-ab15200,
Laminin: https://www.sigmaaldrich.com/CH/en/product/sigma/l9393,
Synaptophysin: https://www.thermofisher.com/antibody/product/Synaptophysin-Antibody-clone-SP11-Monoclonal/MA5-14532,
GLUT4: https://www.abcam.com/en-us/products/primary-antibodies/glucose-transporter-glut4-antibody-ab33780,
GRP78: https://www.abcam.com/en-us/products/primary-antibodies/grp78-bip-antibody-ab21685,
Lamp1: https://www.cellsignal.com/products/primary-antibodies/lamp1-d2d11-xp-rabbit-mab/9091,
TOM20: https://www.cellsignal.com/products/primary-antibodies/tom20-d8t4n-rabbit-mab/42406,
GM130: https://www.cellsignal.com/products/primary-antibodies/gm130-d6b1-xp-rabbit-mab/12480,
alpha-Tubulin: https://www.abcam.com/en-us/products/primary-antibodies/alpha-tubulin-antibody-microtubule-marker-ab15246,
Na/K ATPase: https://www.cellsignal.com/products/primary-antibodies/na-k-atpase-antibody/3010,
GAPDH: https://www.cellsignal.com/products/primary-antibodies/gapdh-14c10-rabbit-mab/2118,
TGOLN2: https://www.bio-rad-antibodies.com/polyclonal/human-tgn46-antibody-ahp500.html?f=purified,
TPST2: https://www.genetex.com/Product/Detail/TPST2-antibody-Internal/GTX46685

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | HEK293T cells from ATCC (CRL-3216)<br>HeLa cells from ATCC (ATCC CCL-2) |
| Authentication | Cells were not authenticated once received by the manufacturer. |
| Mycoplasma contamination | Cells were not tested for Mycoplasma contamination. |
| Commonly misidentified lines (See ICLAC register) | None. |

## 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 | Mice were bred on a C57BL/6JRj background and kept at the local animal facility (Biozentrum, Basel) under a 12-hour light to dark cycle, with ad libitum access to standard laboratory chow and water. All mice used in the experiments were 2-14 months of age. |
| Wild animals | This study did not involve wild animals. |
| Reporting on sex | This study included male and female mice. Sex was not considered in this study. |
| Field-collected samples | This study did not include field-collected samples |
| Ethics oversight | All animal studies were performed in accordance with the law and guidelines of the Swiss authorities and were approved by the veterinary commission of canton Basel-Stadt (Schlachthofstrasse 55, 4056 Basel, Switzerland). |

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

## Plants

| | |
|---|---|
| Seed stocks | *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.* |
| Novel plant genotypes | *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.* |
| Authentication | *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.* |