

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	Proteomics: Easy-nLCTM 1200 coupled to a Q-Exactive HF-X mass spectrometer and operated in data-independent acquisition (DIA) mode for MS1 followed by DIA scan. Metabolomics: XCalibur (Thermo Fisher Scientific) Imaging: Opera Phenix High-Content Screening System (Revvity) or Olympus FV3000R confocal microscope (Olympus) or Incucyte (Sartorius) RT-qPCR: QuantStudio 12K (Applied Biosystems) Patch-clamp: MultiClamp 700B Amplifier (Molecular Device) RNAseq: Illumina HiSeq 2500 (Illumina) AlphaLISA: EnVision (Revvity) ELISA: Tecan Spark (Tecan)
Data analysis	Proteomics: Spectronaut (version 17.1.221229.55965, Biognosys) and a search of the DIA data was done against a Human UniProt FASTA file. Pathway analysis was performed using Ingenuity Pathway Analysis (Qiagen) and STRING. Metabolomics: Skyline software, and visualized using Python/R scripts publicly available. Image analysis: Harmony High-Content Imaging and Analysis Software (Revvity), ImageJ, Incucyte NeuroTrack Module (Sartorius) Western blot: Image J Graph plotting and statistical analysis: Prism (GraphPad)

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 supporting the findings of this study are available within the paper and its supplementary information files. Any other data required can be made available upon request to the corresponding author. The proteomics data generated in this study has been deposited to the ProteomeXchange Consortium via PRIDE partner repository using the dataset identifier: PXD062725.

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	Both TDP-43WT and TDP-43ΔNLS iPSCs were generated from human male XCL-1 cells (XCell Science)
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable
Population characteristics	Not applicable
Recruitment	Not applicable
Ethics oversight	Not applicable

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 sizes in this study were determined based on established practices reported in the relevant literature. Specifically, we referenced previous studies that explored similar experimental conditions to inform our sample size selection. No formal sample size calculation was conducted. Sample size for each experiment is indicated in the figure legend.
Data exclusions	For most experiments, no data were excluded from the analysis. Data were excluded only when samples were lost during the staining procedure or when no analyzable data were acquired during the analysis.
Replication	Reproducibility of the data was tested by the replication of the same established protocol performed by more than two different researchers in different experimental time point resulting in similar data.
Randomization	Randomization was performed when cells were assigned to experimental groups.
Blinding	Blinding was not possible in this study due to the nature of the experimental design. Specifically, the characteristics of the treatment groups were such that the investigators were aware of the group allocation during both data collection and analysis. While this limitation may introduce bias, we employed rigorous methods throughout the study to minimize any potential influence on data collection and interpretation. For example, most data was processed in a high-throughput and automated method. For non-automated experiments, all samples and data were processed in a consistent manner by different researchers.

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

Full list of primary antibodies can be found in Supplementary Table 1 and Supplementary Table 2

Antibodies for IHC Staining:

GFAP - Cell Signalling, 3670
 ChAT - Synaptic systems, 297015
 HB9/MNX1 - Novus Biologicals, NBP2-24691
 Islet-1 - R&D System, AF1837
 TDP-43 - Proteintech, 10782-2-AP
 pTDP-43 (Ser409/410) - Proteintech, 22309-1-AP
 G3BP1 - Abcam, ab56474
 Tubulin - Abcam, ab6161
 Neurofilament - Millipore, MAB5262
 PGP9.5 - Genetex, GTX109637
 STMN2 - Novus Biologicals, nbp1-49461
 ALDH1L1 - Abcam, ab190298
 Synaptophysin - Abcam, ab8049
 NeuN 1:100 Cell Signaling Technology, 24307S
 vGlut1 1:500 Millipore, AB5905
 Tyrosine Hydrolase 1:500 Abcam, ab137869
 GAD67 1:100 Millipore, MAB5406
 S100 β 1:300 Abcam, ab52642

Antibodies for Western Blotting:

TDP-43 - Proteintech, 10782-2-AP
 TDP-43 [3H8] - Abcam Ab104223
 pTDP-43 (Ser409/410) - Proteintech, 22309-1-AP
 STMN2 - Proteintech, 10586-1-AP
 GAPDH - Santa Cruz, sc-47724
 Goat anti-Rabbit IgG (H+L) HRP 1:10 000 Invitrogen, 31460
 Goat anti-Mouse IgG (H+L) HRP 1:10 000 Invitrogen, 31430

Validation

All antibodies were procured from vendors and each antibody's validity is specified by the manufacturer.

Eukaryotic cell lines

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

Cell line source(s)

Both TDP-43WT and TDP-43 Δ NLS iPSCs were generated from human male XCL-1 cells (XCell Science) at MilliporeSigma. Subsequently, spinal motor neurons were differentiated using these iPSCs at BrainXell, Inc.

Authentication

Both iPSCs were karyotyped.

Mycoplasma contamination

All cells used in this study were tested negative for mycoplasma contamination.

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

None of the cell lines utilized in this study are listed as commonly misidentified according to the ICLAC database.

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable