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 *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	Plasmid design – SnapGene (V4); Imaging – Metamorph (V7); Western blot: ImageStudio (V5).
Data analysis	Proteomics - Proteome Discoverer (V2); Image analysis – Fiji (2019 - 2024), custom Jython scripts for Fiji, Imaris (V9 and V10), Labkit (plugin for Imaris), ilastik (V1), Trackmate (V7); Statistics – custom scripts in Python (V2 and V3) or R (V4), clusterProfiler (R package, V4).

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

MS proteomics data have been deposited to the Japan ProteOme STandard Repository (jPOSTrepo) under identifier 9443. Plasmids generated in this study and their maps and sequences will be submitted to Addgene and also available on request.

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	Not applicable.
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

Life sciences study design

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

Sample size	The sample sizes were stated for each figure. Usually, for experiments using RPE-1/HEK293T cell lines, 3 replicate experiments were performed on different days with > 15 cells imaged in each experiment; for experiments using hESC/hiPSC-derived motor neurons (iMNs), 3 ALS patient and 3 non-ALS (control) individuals were included, with > 30 iMNs imaged for each individual; for experiments using postmortem brain biopsies, the (ALS-affected) motor cortices and (control) occipital cortices of 3 ALS patients were analyzed, with > 30 neurons imaged for each.
Data exclusions	No data exclusion was performed.
Replication	For experiments using RPE-1/HEK293T cell lines, 3 replicate experiments were performed on different days; for experiments using hESC/hiPSC-derived motor neurons (iMNs), 3 ALS patient and 3 non-ALS (control) individuals were included; for experiments using postmortem brain biopsies, the (ALS-affected) motor cortices and (control) occipital cortices of 3 ALS patients were analyzed.
Randomization	Wherever possible, experiments of control and different treatment groups were performed together. Alternatively, the grouping of samples was random.
Blinding	Analysis of ALS patient postmortem sections was performed in double-blinded manner. Briefly, the identities of these samples were blinded by Lyle Ostrow Lab and then stained and imaged by Rong Li Lab.

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	Antibodies used in this study are listed in Table S2.
Validation	TDP43, G3BP and CRT antibodies (Abcam 3H8, Abcam EPR13986(B), Abcam EPR3924) were Knock-out (KO) validated by manufacturers. SEC16A antibody (Sigma HPA005684) was validated by Human Protein Atlas (HPA) based on subcellular localization in immunofluorescence (IF) staining, correlation between stain/band intensities and RNA expression, and protein array of 384 different antigens including SEC16A. SEC24 antibody (Sigma HPA040196) was validated by manufacturers and HPA based on subcellular localization in IF, protein array result, and correlation with RNA expression.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	RPE-1 cell line (female) was acquired from a freezer stock in the lab, passaged from what was originally purchased from ATCC. HEK293T cell line (female) was a kind gift from B.C. Low Lab. BJ hiPSC (male) was obtained from National Center for Global Health (Japan), GM23720 hiPSC (female) was obtained from the NIGMS Human Genetic Cell Repository, H9 hESC (female) was purchased from Corning, and ALS patient hiPSCs of NDS00268 (female), NDS00269 (male), NDS00270 (male) were obtained from NINDS Human Cell and Data Repository.
Authentication	RPE-1 and HEK293T cell lines were limited to within 20 passages with continuous surveillance on cell morphology. RPE-1 was additionally tested for Hygromycin B and puromycin resistance. The induction of motor neurons from stem cells were performed according to previous publications (Hor et al., 2018, Hor et al., 2021) and validated by immunofluorescence staining of motor neuron markers NFM and ChAT.
Mycoplasma contamination	Mycoplasma tests were performed quarterly using MycoAlert PLUS Mycoplasma Detection Kit (Lonza) or EZ-PCR-Mycoplasma Test Kit (Biological Industries) to ensure the absence of Mycoplasma.
Commonly misidentified lines (See ICLAC register)	Not applicable.

Plants

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