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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	Holotomography: TomoStudio version 3.3.9, AFM: SPMControl Software (version 6; JPK Instruments AG), LLSM: The custom-built LLSM was developed under a non-disclosure agreement, and the data collection methods are fully described in the original paper (Science 346 (6208), 1257998, 2014)
Data analysis	SPR: Biacore T200 evaluation Software, Holotomography: TomoStudio version 3.3.9, AFM: Data Processing (version 6.4.21; JPK Instruments – Bruker Nano GmbH), ImageJ ver. 1.53, Imaris ver. 9.8.2, TEM: Graphic Converter ver 12

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 included in the main text and Supplementary Information. Source data underlying the figures and tables are available from the corresponding author upon reasonable 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	*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.* *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.* *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).* *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.)* *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	No formal statistical method was used to predetermine sample size. Sample sizes were selected in accordance with commonly used practices in related studies, and all experiments were performed with at least three independent biological replicates ($n \geq 3$).
Data exclusions	No data were excluded from the analyses.
Replication	All experiments were independently replicated at least three times with similar results.
Randomization	Randomization was not applicable, as no animal or human subjects were used and all samples were treated under the same experimental conditions.
Blinding	Blinding was not applicable, as the experiments involved cultured cells and purified substances, and all samples were handled under uniform experimental conditions.

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

Anti-FLAG-M2-HRP (Cat. No. A8592, Sigma-Aldrich, western blot and ELISA,1:5000)
Anti-HA-HRP (Cat. No. 12013819001, Roche, western blot and ELISA, 1:1000).
Anti-GFP antibody was produced using the plasmid encoding the N86/38.1R clone (Addgene plasmid #114492). The expression vector was transiently transfected into Expi293F cells, and the antibody was purified from the culture supernatant using Protein A affinity chromatography.
Anti-TDP-43 antibody was generated based on the 41D1 clone described in US Patent No. 9,587,014 B2. Expression vectors encoding the variable region sequences were synthesized and transiently transfected into Expi293F cells. The antibody was purified from culture supernatants using Protein A affinity chromatography.
Anti-pAkt (Ser473) antibody (clone C7) was generated in-house using a plasmid kindly provided by Dr. Nakakido (University of Tokyo). The variable regions were grafted onto a human IgG1 framework, and the antibody was expressed in Expi293F cells and purified via Protein A affinity chromatography.

Secondary antibody
HRP-conjugated anti-mouse-IgG antibody (31410, ,ThermoFisher Scientific, 1:10000)
HRP-conjugated anti-mouse-IgG antibody (31430, ThermoFisher Scientific, 1:10000)
Anti-mouse IgG-Alexa488 (A-11001, ThermoFisher Scientific, immunofluorescence, 1 :400)
Anti-mouse IgG-Alexa568 (A-11004, ThermoFisher Scientific, immunofluorescence, 1 :400)
Anti-human IgG-Alexa405 (A48275, ThermoFisher Scientific, immunofluorescence, 1 :400)

Validation

Anti-FLAG-HRP and anti-HA-HRP have been validated by the manufacturers for use in western blotting and immunodetection. In addition to the manufacturer's validation, we confirmed their specificity in our experimental system by western blotting and ELISA. In both assays, the antibodies detected tagged proteins with high specificity, yielding signals at the expected molecular weight (WB) or in a tag-dependent manner (ELISA), and showed no detectable signal in untagged controls.
The specificity of the 41D1 anti-TDP-43 antibody was validated by ELISA and immunofluorescence. In ELISA, the antibody specifically bound to purified TDP-43 protein, with negligible signal for unrelated control proteins. Immunofluorescence analysis using HT1080 cells expressing TDP-43-EYFP demonstrated clear nuclear localization consistent with the known subcellular distribution of endogenous TDP-43.
The specificity of the anti-GFP antibody was validated by ELISA and immunofluorescence. In ELISA, the antibody specifically bound to EGFP with minimal cross-reactivity to unrelated proteins. Immunofluorescence analysis in HeLa cells expressing HRas-EGFP showed a clear plasma membrane localization pattern, consistent with the known subcellular distribution of HRas fusion proteins. No signal was observed in non-transfected control cells.
The specificity of the anti-pAkt (Ser473) antibody was assessed by western blotting and immunofluorescence. In both assays, the antibody failed to demonstrate selective binding to phosphorylated Akt. No distinct signal was detected in stimulated cells where pAkt is expected to be upregulated, indicating that the antibody lacked sufficient specificity under the tested conditions.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HeLa cells, ECACC Cat# 93021013
HT1080 cells, JCRB Cat# JCRB9113
Expi293F cells, ThermoFisher Scientific

Authentication

Cell lines were not authenticated by STR profiling, but their morphology and growth characteristics were routinely monitored.

Mycoplasma contamination

Mycoplasma contamination was routinely monitored by Hoechst 33342 staining without the use of commercial detection kits, and no contamination was observed.

Commonly misidentified lines (See ICLAC register)	HeLa cells, which are listed among commonly misidentified cell lines by ICLAC, were used in this study. They were obtained from ECACC.

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.*