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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	Nikon microscopes coupled with Metamorph® or Leica microscopes coupled with Leica Application Software LAS AF 2.4 (Leica Application Suite).were used for image acquisition.
Data analysis	ImageJ version 1.52n was used for all image analysis and processing. For focal adhesion density and TFM, a custom macro was used and calculations were done as described in the materials and methods. ImageJ plugin for TFM was downloaded from https://sites.google.com/site/qingzongtseng/tfm. For mass spectrometry data analysis, SequestHF through proteome discoverer (version 2.2), myProMS 45 v3.6, MassChroQ version 2.2 softwares were used. Graphpad Prism 6 was used for data representation and statistical analyses.

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The data supporting the findings of this study are available within the manuscript. All the raw data generated during this study are available from the corresponding author on reasonable request. The mass spectrometry data has been uploaded on the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD015871 (username: reviewer89842@ebi.ac.uk; password: EC4DSdRf (Check materials and methods on details of how to access the data).

Unmodified westernblots of figures 1, 4 and 5, as well as extended data Figure 1, 2 and 3 can be found in the corresponding source data files.

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 size was limited by experimental constraints. The authors however estimated that the size was sufficiently high to represent faithfully the total population by considering the dispersion of values and their density around the most frequent value. In all cases, a minimum of three independent experiments were carried out to confirm the reproducibility of the data
Data exclusions	No data were excluded
Replication	All experiments were reproduced independently at least 3 times (unless otherwise stated), starting with primary cells produced from different animals.
Randomization	All biochemical samples were analyzed. For cell analysis, cells were randomly chosen and imaged without any bias.
Blinding	Data acquisition was performed blindly. Researchers were unblinded for data analysis. Automatic quantitative measurements by the relevant softwares and devices did not require blinding.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	For Immunofluorescence: Antibody anti-Acetylated Tubulin (1:10000; clone 6-11B-1, mouse monoclonal, T-6793 Sigma Aldrich or 1:200; clone 6-11B-1, ab24610, Abcam), anti-Poly-Glu Tubulin (1:1000; AbC0101, ValBiotech), anti-α-Tubulin (1:1000; MCA77G, rat, Biorad), anti-Paxillin (1:500; 610051, mouse monoclonal, lot 5246880; BD; and ab32084, rabbit monoclonal, clone Y133 and lot GR215998-1; Abcam), anti-Talin (1:1000; T3287, mouse monoclonal, clone 8D4, lot 035M4805V; Sigma-Aldrich), anti-GEF-H1 (1:100; ab155785, Abcam), Alexa fluor 647 Phalloidin (1:2000; 176759, lot GR278180-3; Abcam or 1:300; Thermo Fisher Scientific), anti-Vimentin (1:200; V6630, mouse monoclonal lot 10M4831; Sigma-Aldrich), anti-pMLC (1:1000; 3675S, S19, mouse, Cell signalling), anti-Myosin IIA (1:1000; non-muscle, M8064, rabbit polyclonal, Sigma-Aldrich), anti-Vinculin (1:400; V9131, Sigma-Aldrich). Secondary antibodies were Alexa Fluor 488 donkey anti–rabbit (711-545-152), Rhodamine (TRITC) donkey anti–rabbit (711-025-152), Alexa Fluor 647 donkey anti–rabbit (711-695-152), Alexa Fluor 488 donkey anti–mouse (715-545-151), Rhodamine (TRITC) donkey anti–mouse (715-025-151), Alexa Fluor 647 donkey anti-rat (711-605-152), and Alexa Fluor 488 donkey anti–rat (712-545-153); all from Jackson ImmunoResearch. For Western Blots : Antibody anti-Acetylated Tubulin (1:10000; clone 6-11B-1, mouse monoclonal, T-6793 Sigma Aldrich), anti-αTAT1 (1:400; HPA046816, rabbit, Atlas Antibodies), anti-Poly-Glu Tubulin (1:1000; AbC0101, ValBiotech), anti-α-Tubulin (1:1000; MCA77G, rat, Biorad), anti-β1 integrin (ab52971, Abcam), anti-Talin (1:1000; T3287, mouse monoclonal, clone 8D4, lot 035M4805V; Sigma-Aldrich), anti-GEF-H1 (1:100; ab155785, Abcam), anti-GAPDH (MAB374, lot 2689153 Millipore). Secondary HRP antibodies were all purchased from Jackson ImmunoResearch.
Validation	Anibodies were validated by the manufacturer. We verified that the antibodies showed proteins of the correct size by westernblotting. Potential anibody crossreaction, and antibody validity with rat proteins was evaluated by using rat and human cells in parallel and by testing samples obtained from cells in which the protein of interest had been depleted or overexpressed

in a tagged version.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293T (ATCC #CRL-11268TM
Authentication	The cell line was not authenticated after purchase
Mycoplasma contamination	Cell lines regularly tested negative for Mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Primary astrocytes were obtained from E17 OFA rat embryos
Wild animals	No wild animals were used in this study
Field-collected samples	No field collected samples were used in this study
Ethics oversight	Use of these animals is in compliance with ethical regulations and has been approved from the Prefecture de Police and Direction departementale des services veterinaires de Paris.

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