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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	ZEN software for ZEISS microscope (Apotome, Light Sheet, Spinning Disk), LAS software for confocal Leica microscope, MetaMorph for volume and mass analysis (Molecular Devices)
Data analysis	SEQUEST (https://proteomicsresource.washington.edu/protocols06/sequest.php), Skyline (https://skyline.ms/project/home/software/Skyline/begin.view), R (http://www.r-project.org/foundation/), Fiji (https://fiji.sc/), PRISM 6 for Windows (Version 6.01), custom made MatLab program (MathWorks) .

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

All data are available from the corresponding author upon reasonable request. The information and requests for resources and materials should be directed and will be fulfilled by Dr. Mario Pende (mario.pende@inserm.fr). The source data underlying all figures in the main text of the manuscript are provided as a Source Data file. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD014832. Project Name: S6K1 Phosphoproteome in Tsc1-null mouse kidney iMCD3 cells. Reviewer account details: Username: reviewer37436@ebi.ac.uk; Password: e5EWNpQl

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 statistical method was used to predetermine sample size. Experiments were independently repeated at least twice as indicated, and mean and the standard error from the mean were calculated. In studies with animals, the animal numbers were chosen to reflect the expected magnitude of response taking into account the variability observed in previous experiments.
Data exclusions	No data were excluded from the analyses.
Replication	Experiments were carried out in biological and/or technical replicates as indicated in the results part (text and figure legends). The reproducibility of the experimental findings were verified by performing additional independent experiments (at least two) or by having several technical replicates (as described in the figure legends). All attempts at replication were confirmed to be successful.
Randomization	Randomization was not relevant in this study as there was no comparison of cohorts.
Blinding	Experiments were performed in non-blinded manner.

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 immunoblotting: anti-Rictor (#2114), anti- phospho Rictor Thr1135 (#3806), anti-DNAJC2 (#12844), anti-phospho DNAJC2 (Ser47) (#12397), anti-CAD (#93925S), anti- phospho CAD (Ser1859) (#12662), anti-S6K1 (#2708), anti phospho S6K1 (Thr389) (#9234), anti-phospho Akt (#9271), anti-phospho RPS6 (Ser240/244) (#2215), anti-eIF4B (#3592), anti-phospho eIF4B (Ser422) (#3591), anti-phospho 4E-BP1 (Thr37/46) (#2855), anti-Afadin (for cells) (#13531), anti-phospho Afadin (Ser1795) (#5485) were purchased from Cell Signaling Technology; anti-TSC1 (#sc- 377386) was purchased from Santa Cruz Biotechnology, Inc; anti-β actin (#A5441), anti- α Tubulin (#T5168) and anti-FLAG M2® (1/1000; #F1804) were purchased from Sigma-Aldrich. For immunofluorescence, immunohistochemistry and immunocytofluorescence: anti-Afadin (1/500; #A0224), anti-BrdU (1/200; #BMC9318), anti-γ-Tubulin (1/500; #T5326), anti-α-catenin (1/200; #C2081) and Phalloidin-TRITC (1/500; #P1951) were purchased from Sigma-Aldrich; anti-phospho Histone H3 (Ser10) (1/400; #ab5176) and anti-NuMA (1/200; #ab109262) were purchased from Abcam; rhodamine labeled Dolichos Biflorus Agglutinin (DBA) (1/200; #RL-1032) was purchased from Vector Laboratories; anti-Arl13b (1/400; #17711-1-AP) was purchased from Proteintech; anti-LGN (1/200; #ABT174), anti-PAR3 (1/200; #07-330) and anti-β-catenin (1/200; #06-734) were purchased from Merk Millipore; anti-aPKC (1/100; #sc-17781), anti-Scribble (1/200; #sc-28737) and anti ZO-1 (1/200; #sc-33725) from Santa Cruz Biotechnology, Inc; anti-FGFR1OP (FOP) (1/200; #H00011116-M01) was from Abnova; anti E-cadherin (1/400; # 13-1900) was purchased from Thermo Scientific; anti-Ezrin (1/200; P81) and anti-Integrin-β1 (1/200; P5D2) were purchased by DSHB. For immunofluorescence after clearing: anti-phospho Histone H3 (Ser10) (1/200); anti AQP2-Alexa Fluor ® 488 conjugated (1/200; # sc-515770) was purchased from Santa Cruz Biotechnology, Inc; FITC-labelled Wheat germ agglutinin (WGA) (1/100; #FL-1021) was purchased from Vector Laboratories; TO-PRO™-3 Iodide (1/1000; #T3605) from Thermo Fisher Scientific.
Validation	Validation statements for all the antibodies used in the study are available at the websites of the respective commercial providers.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	mIMCD3 cell line, acquired from ATCC
Authentication	mIMCD3 cell line was obtained from original source and was not further authenticated
Mycoplasma contamination	mIMCD3 cells were routinely bi-weekly tested for potential contaminations with mycoplasma using commercial PCR Mycoplasma Detection Kit (ABM, #G-238). All tests were negative.
Commonly misidentified lines (See ICLAC register)	Does not apply.

Animals and other organisms

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

Laboratory animals	TSC1fl/fl (#005680) and Ksp-Cre (#012237) mice were obtained from The Jackson Laboratory. The generation and genotyping of S6k1-mutant and S6k1/S6k2-double mutant mice are described in Pende et al., 2004. S6k1-mutant mice were first crossed with TSC1fl/fl mice and then with Ksp-Cre mice. CAGGCre-ER (#004682) mice were obtained from The Jackson Laboratory and crossed with TSC1fl/fl mice as described in Liang et al., 2014. Mice of both sexes and of the indicated ages were used for the experimentation. All animals used in the study were fed ad libitumstandard chow diet (Teklad global protein diet; 20% protein, 75% carbohydrate, 5% fat) and kept under 12h/12h (8am/8pm) light on/off cycle. All animal studies were performed by authorized users in compliance with ethical regulations for animal testing and research.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected in the field.
Ethics oversight	The study was approved by the Direction Départementale des Services Vétérinaires, Préfecture de Police, Paris, France (authorization number 75-1313) and the ethical committee of Paris Descartes University.

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