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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.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Image J with FIJI
Data analysis	GraphPad Prism

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 upon request. 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 that support the findings of this study are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	Sample sizes for all experiments are reported in Supplementary Table 2. No statistical methods were used to predetermine sample sizes. Our sample sizes are consistent with what is generally used in the field.
Data exclusions	No data were excluded.
Replication	Experiments were reliably reproduced using independent samples (cultures or spheroids) from separate differentiations or passages. Exceptions to this were the following figure panels for which replication experiments were not attempted: Fig. 2m, Fig. 4m-o, Fig. S1c-l, Fig. S4f-h, Fig. S5j, Fig. S6a-c & f-i, Fig. S7d, Fig. S8f & g.
Randomization	Spheroids were randomly assigned to rapamycin or vehicle treatment groups.
Blinding	The investigator was blind to genotype during the image analysis of cortical spheroids with constitutive mutations. The investigator was not blind during the analysis of TSC2-/c;LSL-tdTom cells and spheroids since the mutant cells are labeled with a tdTomato reporter.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	The hESC and hiPSC lines we generated will be made available to researchers upon reasonable request.

Antibodies

Antibodies used

Antibody - Host species - Company - Catalog # - Western blot dilution - ICC/IHC dilution
4E-BP1 - rabbit - Cell Signaling - 9644 - 1:1000 - n/a
phospho-4E-BP1 (Ser65) - rabbit - Cell Signaling - 9451 - 1:1000 - n/a
AKT - rabbit - Cell Signaling - 4691 - 1:1000 - n/a
phospho-AKT (Ser473) - rabbit - Cell Signaling - 4060 - 1:1000 - n/a
B-Actin - mouse - Sigma - a1972 - 1:15000 - n/a
CAMK2A - mouse - Cell Signaling - 50049 - n/a - 1:300
CD44 - mouse - Cell Signaling - 3570 - n/a - 1:400
Doublecortin (DCX) - rabbit - Cell Signaling - 4604 - n/a - 1:2000
EAAT1 - rabbit - Cell Signaling - 5684 - n/a - 1:100
GFAP - rabbit - Fisher - 180063 - 1:100 - 1:100
GFP - chicken - AbCam - ab13970 - n/a - 1:5000
GLUA1 - rabbit - Millipore - AB1504 - 1:800 - n/a
HuC/HuD - mouse - Fisher - A21271 - n/a - 1:500
Ki-67 - mouse - Cell Signaling - 9449T -n/a - 1:500

MAP2 - chicken - AbCam - ab5392 - n/a - 1:5000
MAP2 - mouse - AbCam - ab11267 - 1:2000 - n/a
NANOG - goat - R & D Systems - AF1997 - n/a - 1:40
Nestin - mouse - Covance - MMS-570P-100 - n/a - 1:1000
NeuN - mouse - Millipore - MAB377 - n/a - 1:1000
OCT4 - rabbit - AbCam - ab19857 - 1:1000 - 1:500
PAX6 - rabbit - Anaspec - PRB-278P-100 - n/a - 1:300
RFP - rabbit - Rockland - RL600-401-379 - n/a - 1:2000
S100B - rabbit - Abcam - ab868 - 1:200 - 1:300
S6 - rabbit - Cell Signaling - 2317S - 1:1000 - n/a
phospho-S6 (Ser240/244) - rabbit - Cell Signaling - 5364S - 1:2000 - 1:1000
Neurofilament (SMI-311) - mouse - Biolegend - 837301 - n/a - 1:1000
SOX2 - rabbit - ThermoFisher - 48-1400 - n/a - 1:150
phospho-STAT3 (Ser727) - rabbit - Cell Signaling - 94994 - 1:1000 - n/a
phospho-STAT3 (Tyr705) - rabbit - Cell Signaling - 9145 - 1:800 - n/a
STAT3 - mouse - Cell Signaling - 9139 - 1:1000 - n/a
TBC1D7 - rabbit - Cell Signaling - 14949 - 1:1000 - n/a
Beta III Tubulin (Tuj1) - chicken - Millipore - AB9354 - n/a - 1:2000
TSC1 - rabbit - Cell Signaling - 6935S - 1:1000 - n/a
TSC2 - rabbit - Cell Signaling - 4308 - 1:1000 - n/a
Vimentin - mouse - EMD Millipore - MAB3400 - n/a - 1:1000

Validation

Antibody validation was performed by the manufacturer, we used antibodies that have been validated for each assay (i.e. western blot or immunostaining).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

WIBR3 hESCs were obtained from Dr. Rudolf Jaenisch's lab, BJ fibroblasts were purchased from ATCC, TSC patient cells were obtained from the Coriell Institute.

Authentication

WIBR3 hESCs were authenticated as described in Lengner et al 2010. TSC patient cells were authenticated by qPCR. All stem cell lines were assessed for pluriotency by marker staining and genomic integrity was confirmed by array comparative genomic hybridization (aCGH) analysis.

Mycoplasma contamination

All cell lines were tested monthly for mycoplasma contamination and were negative.

Commonly misidentified lines
(See ICLAC register)

None of the cell lines used are listed in the ICLAC database.

Animals and other organisms

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

Laboratory animals

The teratoma formation assay was performed using six-month old male NOD-SCID mice (26-30g) obtained from Taconic. Mouse embryonic fibroblasts (MEFs) were isolated from E13.5 male and female CD-1 (Charles River) or DR-4 mice (Jackson Laboratories strain #003208).

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.