

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                                                                                                                                                                                                                                                                                      |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                     |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                                      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), 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 | Cell Ranger 6.1.2, Olympus FV31S-SW 2.3.2.169, Revvity Harmony 5.2, Image Lab 6.1.0                                                                                                                                                                                                                                                                                           |
| Data analysis   | Seurat 5.1.0<br>Ashlar ( <a href="https://github.com/jmuhlich/ashlar/tree/rotation-correction/">https://github.com/jmuhlich/ashlar/tree/rotation-correction/</a> )<br>Stardist 0.9.1<br>DESeq2 1.46.0<br>Single Cell Pathway Analysis 1.6.1<br>Cocoa 0.4.0<br>DittoSeq 1.18.0<br>EnhancedVolcano 1.20.0<br>VoxHunt 1.0.1<br>PyImageJ 1.8.0<br>Napari 0.6.6<br>Kallisto 0.48.0 |

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](#)

Code capsules demonstrating the image UMAP and image binarization pipelines on sample data are available on Code Ocean (Binary Quantification DOI: 10.24433/CO.4440783.v1; Image UMAP DOI: 10.24433/CO.2878862.v1). Raw sequencing data and processed datasets have been deposited in the NCBI Gene Expression Omnibus under accessions GSE341690 (Bulk RNA-seq) and GSE341692 (single cell RNA-seq). The fetal brain tissue atlas was obtained from the Linnarsson Lab (<https://github.com/linnarsson-lab/developing-human-brain>).

## 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                                        | Stem cell lines include lines derived from biologically male and female individuals.                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Reporting on race, ethnicity, or other socially relevant groupings | Self-reported ethnicity information was collected for patients undergoing tuber resection surgery, but was not used to draw any other socially relevant conclusions.                                                                                                                                                                                                                                                                                                                                                           |
| Population characteristics                                         | The sex, ethnicity, year of birth, year of surgery, and identified mutation of each patient are described in Supplementary Table 9.                                                                                                                                                                                                                                                                                                                                                                                            |
| Recruitment                                                        | Patients were recruited on the basis of clinical criteria, including a diagnosis of tuberous sclerosis complex and medically intractable seizures.                                                                                                                                                                                                                                                                                                                                                                             |
| Ethics oversight                                                   | Surgically resected cortical tuber tissue was collected under Stanford University Institutional Review Board protocol IRB-12625 ("The Neuroscience Brain Bank: Collection of Neurosurgical Tissue for Research"). Informed consent was obtained from the caregivers and legal guardians of all participants. Derivation, maintenance and differentiation of human pluripotent stem cell lines was approved by the University of California, Berkeley Stem Cell Research Oversight (SCRO) Committee under protocol 2014-10-029. |

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](https://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 sample size calculation was performed. Sample sizes were determined based on pilot experiments and prior publications in the field.                                                                                                                                                                                                                                  |
| Data exclusions | No data were excluded from this study.                                                                                                                                                                                                                                                                                                                                  |
| Replication     | Results were replicated using multiple organoid differentiation batches and multiple different genetic backgrounds (stem cell lines). Batches and replication schemes for each experiment are described in Supplementary Table 10.                                                                                                                                      |
| Randomization   | Randomization was used for the rapamycin and Torin experiments. Culture wells were randomly assigned to different treatments. For in vitro cultures, all comparisons were made within isogenic controls, so group membership was determined by genotype, rather than allocation. For human tuber data, comparisons were made between cell populations within the tuber. |
| Blinding        | Experimenters were not blind to genotype during experiments, however analysis pipelines were automated and the same settings were applied to all 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                                     |
|-------------------------------------|-----------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies            |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                           |

## Methods

| n/a                                 | Involved in the study                           |
|-------------------------------------|-------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

## Antibodies

### Antibodies used

Antibodies used are described in Supplemental Table 13.

### Validation

Antibodies were validated according to manufacturer's statements and citations. Validation data is available on the vendors' websites.

rabbit TSC1 Cell Signaling 6935 D43E2 <https://www.cellsignal.com/products/primary-antibodies/hamartin-tsc1-d43e2-rabbit-monoclonal-antibody/6935>

rabbit TSC2 Cell Signaling 4308 D93F12 <https://www.cellsignal.com/products/primary-antibodies/tuberin-tsc2-d93f12-rabbit-monoclonal-antibody/4308>

rabbit p-Akt(S473) Cell Signaling 4060 D9E <https://www.cellsignal.com/products/primary-antibodies/phospho-akt-ser473-d9e-rabbit-monoclonal-antibody/4060>

rabbit Akt Cell Signaling 4691 C67E7 <https://www.cellsignal.com/products/primary-antibodies/akt-pan-c67e7-rabbit-monoclonal-antibody/4691>

rabbit p-S6(S240/244) Cell Signaling 5364 D68F8 <https://www.cellsignal.com/products/primary-antibodies/phospho-s6-ribosomal-protein-ser240-244-d68f8-rabbit-monoclonal-antibody/5364>

rabbit S6 Cell Signaling 2217 5G10 <https://www.cellsignal.com/products/primary-antibodies/s6-ribosomal-protein-5g10-rabbit-monoclonal-antibody/2217>

rabbit p-4EBP1(S65) Cell Signaling 9451 Polyclonal <https://www.cellsignal.com/products/primary-antibodies/phospho-4e-bp1-ser65-antibody/9451>

rabbit p-4EBP1(T37) Cell Signaling 2855 236B4 <https://www.cellsignal.com/products/primary-antibodies/phospho-4e-bp1-thr37-46-236b4-rabbit-monoclonal-antibody/2855>

rabbit 4EBP1 Cell Signaling 9644 53H11 <https://www.cellsignal.com/products/primary-antibodies/4e-bp1-53h11-rabbit-monoclonal-antibody/9644>

mouse b-actin Millipore Sigma a1978 AC-15 <https://www.sigmaaldrich.com/US/en/product/sigma/a1978>

rabbit S100B Abcam ab52642 EP1576Y <https://www.abcam.com/en-us/products/primary-antibodies/s100-beta-antibody-ep1576y-astrocyte-marker-ab52642>

mouse GFAP Cell Signaling 3670 GA5 <https://www.cellsignal.com/products/primary-antibodies/gfap-ga5-mouse-monoclonal-antibody/3670>

rabbit Clusterin Abcam ab69644 Polyclonal <https://www.abcam.com/en-us/products/primary-antibodies/clusterin-antibody-ab69644>

rabbit CRYAB Cell Signaling 45844 D6S9E <https://www.cellsignal.com/products/primary-antibodies/cryab-d6s9e-rabbit-monoclonal-antibody/45844>

rabbit EAAT1 Cell Signaling 5684 D44E2 <https://www.cellsignal.com/products/primary-antibodies/eaat1-d44e2-rabbit-monoclonal-antibody/5684>

rabbit Cx43 Cell Signaling 3512 Polyclonal <https://www.cellsignal.com/products/primary-antibodies/connexin-43-antibody/3512>

rabbit Kir4.1 Alomone APC-035 Not Given <https://www.alomone.com/products/apc-035-anti-k-sub-ir-sub-4-1-kcnj10-antibody>

goat GAD1 R&D AF2086 Polyclonal [https://www.rndsystems.com/products/human-mouse-rat-gad1-gad67-antibody\\_af2086](https://www.rndsystems.com/products/human-mouse-rat-gad1-gad67-antibody_af2086)

goat EGFR R&D AF231 Polyclonal [https://www.rndsystems.com/products/human-egfr-antibody\\_af231](https://www.rndsystems.com/products/human-egfr-antibody_af231)

goat PROX1 R&D AF2727 Polyclonal [https://www.rndsystems.com/products/human-prox1-antibody\\_af2727](https://www.rndsystems.com/products/human-prox1-antibody_af2727)

chicken GFAP Abcam ab4674 Polyclonal <https://www.abcam.com/en-us/products/primary-antibodies/gfap-antibody-astrocyte-marker-ab4674>

chicken MAP2 Abcam ab5392 Polyclonal <https://www.abcam.com/en-us/products/primary-antibodies/map2-antibody-neuronal-marker-ab5392>

rat CTIP2 Biolegend 650601 25B6 <https://www.biolegend.com/en-us/products/purified-anti-bcl11b-antibody-7248>

mouse HuC/D Thermo Fisher A21271 16A11 <https://www.thermofisher.com/antibody/product/HuC-HuD-Antibody-clone-16A11-Monoclonal/A-21271>

mouse NeuN Millipore Sigma MAB377 A60 <https://www.sigmaaldrich.com/US/en/product/mm/mab377>

mouse S100B Millipore Sigma S2532 SH-B1 <https://www.sigmaaldrich.com/US/en/product/sigma/s2532>

mouse Vimentin Millipore Sigma MAB3400 V9 <https://www.sigmaaldrich.com/US/en/product/mm/mab3400>

mouse Nestin Invitrogen 14-9843-82 10C2 <https://www.thermofisher.com/antibody/product/Nestin-Antibody-clone-10C2-Monoclonal/14-9843-82>

mouse p62 Abcam ab56416 2C11 <https://www.abcam.com/en-us/products/primary-antibodies/sqstm1-p62-antibody-2c11-bsa-and-azide-free-ab56416>

rabbit EDNRB Abcam ab117529 Polyclonal <https://www.abcam.com/en-us/products/primary-antibodies/endothelin-b-receptor-et-b-antibody-ab117529>

rabbit APOE Thermo Fisher 701241 16H22L18 <https://www.thermofisher.com/antibody/product/APOE-Antibody-clone-16H22L18-Recombinant-Monoclonal/701241>  
 rabbit CRYAB Abcam ab76467 EPR2752 <https://www.abcam.com/en-us/products/primary-antibodies/alpha-b-crystallin-antibody-epr2752-ab76467>  
 rabbit AQP4 Invitrogen PA5-36521 Polyclonal <https://www.thermofisher.com/antibody/product/Aquaporin-4-Antibody-Polyclonal/PA5-36521>  
 rabbit PTGDS Millipore Sigma ABN253 Polyclonal <https://www.sigmaaldrich.com/US/en/product/mm/abn253>  
 goat SOX9 R&D AF3075 Polyclonal [https://www.rndsystems.com/products/human-sox9-antibody\\_af3075](https://www.rndsystems.com/products/human-sox9-antibody_af3075)  
 chicken RFP Rockland 600-901-379 Polyclonal <https://www.rockland.com/categories/primary-antibodies/rfp-antibody-600-901-379/>  
 mouse SCGN Invitrogen MA5-31337 CL0271 <https://www.thermofisher.com/antibody/product/SCGN-Antibody-clone-CL0271-Monoclonal/MA5-31337>  
 mouse SMI 311 Biolegend 837801 SMI 311 <https://www.biolegend.com/en-us/products/anti-neurofilament-marker-pan-neuronal-cocktail-11132>  
 goat NANOG R&D AF1997 Polyclonal [https://www.rndsystems.com/products/human-nanog-antibody\\_af1997](https://www.rndsystems.com/products/human-nanog-antibody_af1997)  
 rabbit Oct4 Abcam ab19857 Polyclonal <https://www.abcam.com/en-us/products/primary-antibodies/oct4-antibody-ab19857>  
 mouse Ki67 Cell Signaling 9449T 8D5 <https://www.cellsignal.com/products/primary-antibodies/ki-67-8d5-mouse-monoclonal-antibody/9449>  
 rabbit HOPX Millipore Sigma HPA030180 Polyclonal <https://www.sigmaaldrich.com/US/en/product/sigma/hpa030180>  
 chicken GFP Abcam ab13970 Polyclonal <https://www.abcam.com/en-us/products/primary-antibodies/gfp-antibody-ab13970>  
 mouse CD44 Cell Signaling 3570 156-3C11 <https://www.cellsignal.com/products/primary-antibodies/cd44-156-3c11-mouse-monoclonal-antibody/3570>

## Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

WiBR3 hESCs were received from Dr. Rudolf Jaenisch's laboratory.  
 BJ Fibroblasts were received from ATCC and reprogrammed in Dr. Dirk Hockemeyer's laboratory.  
 8858 and 8119 iPSC lines were received from Dr. Sergiu Pasca's laboratory.

Authentication

Stem cell lines were authenticated by array comparative genomic hybridization and PCR genotyping.

Mycoplasma contamination

All cell lines were tested and determined to be negative for mycoplasma.

Commonly misidentified lines  
 (See [ICLAC](#) register)

No commonly misidentified cell lines were used in this study.

## Plants

Seed stocks

No seed stocks were used in this study.

Novel plant genotypes

No plants were used in this study.

Authentication

No plants were used in this study.