nature portfolio

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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	Microscopy images were acquired using Leica LAS AF software (Leica Microsystems) and Keyence BZ-X710/810 microscope software (Keyence). Micro-CT images were collected using the CT Lab In-vivo 90 acquisition system.
Data analysis	Image processing and quantification were performed using ImageJ (NIH). Statistical analyses were conducted using GraphPad Prism 8.0 (GraphPad Software). Micro-CT reconstructions were analyzed using AVIZO 9.4.0 (Thermo Fisher Scientific). Single-cell RNA-seq data were analyzed using the Seurat (v4.0) and CellChat packages in R. Bulk RNA-seq data were analyzed using Partek Flow (Partek Inc.) with read alignment performed using STAR (v2.6.1d). Brain volume calculations based on the DevCCF atlas were performed using custom Python scripts, which are available at Zenodo (https://doi.org/10.5281/zenodo.19664792).

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

Single-cell and bulk RNA-seq data analyzed in this study are available from the FaceBase data repository (facebase.org) under accession numbers: FB00000970 (https://doi.org/10.25550/3TYP), FB00001013 (https://doi.org/10.25550/1-4TSP), FB00000971 (https://doi.org/10.25550/3V0E), and FB00000902 (https://doi.org/10.25550/TJY). Source data are provided with this paper.

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	Not applicable. This study did not involve human participants.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable. This study did not involve human participants.
Population characteristics	Not applicable. This study did not involve human participants.
Recruitment	Not applicable. This study did not involve human participants.
Ethics oversight	Not applicable. This study did not involve human participants.

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 statistical methods were used to predetermine sample size. Sample sizes were selected based on previous studies in the field and our prior experience with similar experimental systems. The exact sample size (N) for each experiment is indicated in the corresponding figure legends.
Data exclusions	No data were excluded from the analyses.
Replication	All experiments were independently repeated at least three times with similar results. For in vivo experiments, each data point represents an individual mouse.
Randomization	The experiments were not randomized.
Blinding	Investigators were not blinded to group allocation during experiments or outcome assessment.

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

The following primary antibodies were used in this study: anti-Ki67 (Cell Signaling Technology, #9129, 1:100), anti-p-SMAD2 (Cell Signaling Technology, #3108, 1:100-1:1000), anti-TGFβ1 (Santa Cruz Biotechnology, #sc-1303483, 1:50), anti-TGFβ2 (Santa Cruz Biotechnology, #sc-374658, 1:50), anti-TGFβ3 (Santa Cruz Biotechnology, #sc-166833, 1:50), anti-decorin (R&D Systems, #AF1060, 1:20), anti-SP7 (Abcam, #ab209484, 1:200), and anti-RBP1 (Proteintech, #22683-1-AP, 1:100). Alexa Fluor-conjugated secondary antibodies were used for immunofluorescence staining at a dilution of 1:200, including goat anti-rabbit IgG (H+L) cross-adsorbed, Alexa Fluor™ 488 (Thermo Fisher Scientific, #A-11008), goat anti-mouse IgG (H+L) cross-adsorbed, Alexa Fluor™ 568 (Thermo Fisher Scientific, #A-11004), and donkey anti-goat IgG (H+L) cross-adsorbed, Alexa Fluor™ 647 (Thermo Fisher Scientific, #A-21447). Anti-rabbit HRP-conjugated secondary antibodies (Cell Signaling Technology, #7074) were used for western blotting.

Validation

All antibodies used in this study were commercially available and validated by the manufacturers for the indicated applications. Information on antibody specificity and validation was obtained from the manufacturers' datasheets and previously published studies.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

All experiments were performed using laboratory mice maintained on a C57BL/6J background. The following strains were used: Gli1-CreERT2 (JAX #007913), ROSA26-LSL-tdTomato (JAX #007905), ROSA26-LSL-ZsGreen1 (JAX #007906), Wnt1-Cre (JAX no. 003829), Tgfbr2fl/fl (JAX #012603), and Twist1+/− mice. Mice were housed under standard laboratory conditions with a 12 h light/12 h dark cycle at 22-24°C and 40-60% humidity, with food and water available ad libitum. Neonatal and juvenile mice at the indicated developmental stages (e.g., PN3.5, PN3W, PN4W) were used depending on the experimental design.

Wild animals

This study did not involve wild animals.

Reporting on sex

Both male and female mice were used in this study. Sex was not considered as a biological variable because no sex-specific differences were expected for the analyses performed.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Southern California and were conducted in accordance with institutional and federal guidelines for animal care and use.

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

Plants

Seed stocks

This study did not involve plant materials or seed stocks.

Novel plant genotypes

This study did not involve plant genotypes.

Authentication

Not applicable. No plant materials were used in this study.