

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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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 <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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	Image acquisition: Zeiss Zen software (version 3.7). RT-qPCR: Step One Plus software (version 2.3)
Data analysis	MATLAB version 9.14.02489007 (R2023a), custom-made codes are available in the link provided in the main text. Imaris software (version 9.1.0; Bitplane). Tissue Analyzer software. ImageJ/Fiji software was used to perform some image analysis and pre-processing, custom-made codes are available in the link provided in the main text. GraphPad Prism 9 software was used for graph generation and for statistical analysis.

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](#)

Source data are provided with this paper. The source data underlying Figs. 1 to 7 and Supplementary Figs. 1 to 9 are provided as Source Data files. Other data can be made available upon request to the corresponding authors.

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

Use the terms *sex* (biological attribute) and *gender* (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

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://www.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 define sample size. A number of N independent experiments as indicated in the figure captions were performed, with multiple n replicates per experiment. Sample size was chosen based on previous publications in the field:
<https://doi.org/10.1038/s41598-019-46497-x>
<https://doi.org/10.1038/s41556-021-00699-6>

Data exclusions

No data were excluded from the study.

Replication

Experiments were performed at several times. Replication of the data was performed at different days, with different organoid batches and by different people.

Randomization

We did not use randomization.

Blinding

We did not use blinding. Blinding was unnecessary due to the nature of the data collection and analysis, which mostly relied on automated pipelines using custom-made codes. Thus, reducing subjective biases.

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 is a list of the primary antibodies used and their respective dilutions: mouse anti-cytokeratin 20, 1:100 (Dako, cat. no. M701901); goat anti-GFP, 1:100 (Abcam, cat. no. ab6673); rabbit anti-GFP, 1:100 (Life Technologies, cat. no. A-11122); rabbit anti-Ki67, 1:100 (Abcam, cat. no. ab16667); mouse anti-Ki67, 1:100 (BD Biosciences, cat. no. 550609); rabbit anti-laminin, 1:200 (Abcam, cat. no. ab11575); rabbit anti-lysozyme, 1:100 (Dako, cat. no. A0099); rabbit anti-Wnt3a, 1:200 (Abcam, cat. no. ab219412); mouse anti-Wnt3a, 1:200 (Abcam, cat. no. ab169175).

The secondary antibodies used were: donkey anti-goat Alexa Fluor 488 (Thermo Fisher Scientific, cat. no. A-11055); donkey anti-goat Alexa Fluor 488 (Jackson ImmunoResearch, cat. no. 705-545-003); donkey anti-mouse Alexa Fluor 488 (Thermo Fisher Scientific, cat. no. A-21202); donkey anti-mouse Alexa Fluor 488 (Jackson ImmunoResearch, cat. no. 715-545-150); donkey anti-rabbit Alexa Fluor 488 (Thermo Fisher Scientific, cat. no. A-21206); donkey anti-rabbit Alexa Fluor 488 (Jackson ImmunoResearch, cat. no. 711-545-152); donkey anti-goat Alexa Fluor 647 (Thermo Fisher Scientific, cat. no. A-21447); donkey anti-mouse Alexa Fluor 647 (Thermo Fisher Scientific, cat. no. A-31571); donkey anti-rabbit Alexa Fluor 647 (Thermo Fisher Scientific, cat. no. A-31573); donkey anti-rabbit Alexa Fluor 647 (Jackson ImmunoResearch, cat. no. 711-605-152). To label F-actin, phalloidin rhodamine (Cytoskeleton cat. no. PHDR1) was used at 3,5:500. 4',6-diamidino-2-phenylindole (DAPI) (1:1000) (Thermo Fisher Scientific, cat. no. D1306) was used to label nuclei.

Validation

All the antibodies were used for immunofluorescence. They were all validated by their respective manufacturers and by previous studies. Previous validations and citations can be found by using the following RRID numbers or DOI reference:

mouse anti-cytokeratin 20, M701901 https://www.antibodyregistry.org/AB_2133718

goat anti-GFP, ab6673 https://www.antibodyregistry.org/AB_305643

rabbit anti-GFP, A-11122 https://www.antibodyregistry.org/AB_221569

rabbit anti-Ki67, ab16667 https://www.antibodyregistry.org/AB_302459

mouse anti-Ki67, 550609 https://www.antibodyregistry.org/AB_393778

rabbit anti-laminin, ab11575 https://www.antibodyregistry.org/AB_298179

rabbit anti-lysozyme, A0099

rabbit anti-Wnt3a, ab219412 https://www.antibodyregistry.org/AB_2924383

mouse anti-Wnt3a, ab169175 10.1186/s12860-018-0152-5, 10.1590/1414-431X20186997

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

Organoids were cultured from Lgr5-EGFP-IRES-creERT2 male and female mice (3-6 month old) (WT) and from Apcfl/fl-Villin-CreERT2 male and female mice (3-6 month old) (Apc^{-/-}).

Wild animals

This study did not involve wild animals.

Reporting on sex

Organoids were obtained from male and female mice and no sex-specific differences were observed.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight

Animal experimentation was approved by the Animal care and Use Committee of Barcelona Science Park (CEEAA-PCB) and the Catalan government and performed by their relevant guidelines and regulations.

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.