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	Confocal imaging was acquired using a Leica TCS SP5 II or SPE confocal microscope and Leica Application Suite, Advanced Fluorescence software version LAS AF 2.7.9723. Light microscopy imaging was acquired using a Leica ICC50 W microscope and Leica Application Suite version LAS EZ 3.4.0. Organoid and fibroblast image was acquired using an EVOS-FL Cell imaging system.
Data analysis	Image J/Fiji (version 2.1.0/1.52n), Adobe Photoshop (version 19.0), QuPath sofware version 0.1.2

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

Data supporting this protocol are available in previous publications

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 sample size calculations were performed as no statistical analysis was performed in this study
Data exclusions	No data was excluded
Replication	The figures display representative images of experiments which were replicated at least 3 times by 2-3 researchers.
Randomization	Randomisation was not required for this study as it is a protocol intended for others to follow
Blinding	No blinding was performed for this study as it is a protocol intended for others to follow

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
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Alkaline Phosphatase, Vector Substrate kit IV BCIP/NBT SK-5400, Collagen-1 rabbit Abcam ab34710, 1:200, ECadherin rabbit Proteintech 20874-1-AP (1:1500), Ki67 rabbit Abcam ab15580 (1:1000), Lysozyme rabbit Dako A0099 (1:2000), Na+K+ATPase rabbit Abcam ab198367 (1:100), SOX9 rabbit Sigma-Aldrich (1:250), Sucrase Isomaltase mouse Santa Cruz sc-393424 (1:100), UEA-1 (Rhodamine conjugated) Vector Laboratories RL-1062 (1:200), Villin mouse Santa Cruz SC-58897 1:50, Vimentin mouse Abcam ab92547 (1:500). The following Alexa Fluor secondary antibodies were used: anti-mouse 568 Goat Invitrogen A-11031 1:1000, anti-rabbit-488 Goat Invitrogen A-11031 1:1000. The following HRP conjugated secondary antibodies were used: OmniMap anti-rabbit HRP Roche 760-4311, OmniMap anti-mouse HRP Roche 760-4310, goat anti-mouse HRP P0447 Dako (1:100).
Validation	The antibodies listed are widely used commercially available antibodies and are validated by the companies with publications cited on the relevant company websites. Several antibodies were further validated "in house" with human sample controls.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The organoid lines used were derived from endoscopic small intestinal tissue biopsies. Informed consent was given and ethical approval for the use of human tissue obtained from the Bloomsbury (London) NRES committee (REC reference 04-Q0508-79)
Authentication	No authentication was performed
Mycoplasma contamination	All organoid and fibroblast lines used in this study were assessed for mycoplasma and tested negative; both during expansion

	and following injection/seeding into scaffolds.
Commonly misidentified lines (See ICLAC register)	No misidentified lines were used

Human research participants

Policy information about studies involving human research participants

Population characteristics	Organoids, fibroblasts and scaffolds derived from human tissue were included in this protocol. This tissue was obtained from paediatric patients undergoing either endoscopy or surgery following informed consent.
Recruitment	Relevant patients listed for either endoscopy or surgery were identified by the clinical team, separate from the laboratory research team. The patients and their parents or guardians were approached by a member of the clinical team. After obtaining informed consent, tissue was collected and transferred to the research team where it was processed for organoid and fibroblast isolation and decellularisation for scaffold.
Ethics oversight	Ethical approval for the use of human tissue was obtained from the Bloomsbury (London) NRES committee (REC references 04-Q0508-79 and 18-EE-1050). The committee was constituted in accordance with the Governance Arrangements for Research Ethics Committees and complied fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

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