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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 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	LAS X
Data analysis	LAS X, MetaMorph, IMARIS, AutoQuant (X3.0.1)

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 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

Data presented in these following figures are available from the cited original references; Figs. 1, “Cytodifferentiation”, 3b, and 3c2; Figs. 1, “Intestinal physiology”, “Disease modeling”, 3c, and 3e5; Figs. 1, “Host-microbe co-culture” and 3d3; Figs. 3e and 3f12; Fig. 4c4. All the other data were generated for this article. Any raw data is available for research purposes from the corresponding author upon request. Intestinal organoid lines used in this paper are available from the corresponding author upon request, under a material transfer agreement between institutions.

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	We have repeated the protocol at least 10 times for each cell line and culture format that we used in the study
Data exclusions	No data were excluded.
Replication	To verify the reproducibility, we observed the establishment of 3D intestinal epithelial layers in each trial. Successful completion of the protocol results in more than 90% of reproducibility.
Randomization	Randomization is not required for this study.
Blinding	Blinding is not required for this study because any epithelial cell source can be used to establish a 3D epithelial structure.

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	Rabbit anti-ZO-1 polyclonal antibody (Thermo Fisher Scientific, Cat. No. 61-7300, https://scicrunch.org/resolver/RRID: AB_2533938), Rabbit anti-LGR5 polyclonal antibody (Abcam, Cat. No. ab75732, https://scicrunch.org/resolver/RRID: AB_1310281), Mouse anti-MUC2 monoclonal antibody (Santa Cruz Biotechnology, Cat. No. sc-515032, https://scicrunch.org/resolver/RRID: AB_2815005), Goat anti-mouse IgG DyLight 488 (Abcam, Cat. No. ab96871, https://scicrunch.org/resolver/RRID: AB_10680543), Goat anti-rabbit IgG Alexa Fluor 555 (Abcam, Cat. No. ab150078, https://scicrunch.org/resolver/RRID: AB_2722519)
Validation	Used antibodies were validated by manufacturer and via immunofluorescence imaging in the laboratory.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Caco-2 human colon cancer cells (Harvard Digestive Disease Center), Human intestinal organoids (established in the laboratory), Cultrex R-spondin1-producing 293T cells (Trevigen, Cat. No. 3710-001-K), Noggin-producing 293T cells (Digestive Diseases Center at Baylor College of Medicine)
Authentication	No authentication was performed.
Mycoplasma contamination	Mycoplasma contamination testing was routinely performed on all cell lines.
Commonly misidentified lines (See ICLAC register)	N/A