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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	The Olympus IX73 fluorescence microscope with cellSens (2.3) was used to take phase-contrast or fluorescence images. Confocal images were taken by Zeiss LSM880 microscope with Zeiss Zen 3. The CFX384 Real-Time System (BioRad) with CFX Maestro (1.1) was used to collect qRT-PCR data. Flow cytometry analysis was performed using BD FACSVerse (BD Biosciences) and data analysis was performed using FlowJo (V10) software. scRNA-seq were processed using Cellranger (7.1.0, 10x Genomics) and analyzed in R (4.2.2) or Python (3.10.0).

Data analysis	All statistical analyses were performed using GraphPad Prism (version 10.1.2, GraphPad software). Data are presented as means ± standard deviation (SD). Two-tailed unpaired t-test, one-way ANOVA with Dunnett's multiple comparisons test, one-way ANOVA with Tukey's multiple comparisons test, two-way ANOVA analysis with Sidak's multiple comparisons test and two-way ANOVA with Dunnett's multiple comparisons test were used to determine the statistical significance. The significance levels are ****P<0.0001; ***P < 0.001; **P<0.01; *P<0.05. For all analyses, p < 0.05 was considered statistically significant. The specific statistical test used and the number of experiments performed for each analysis are indicated in the corresponding figure legend. Normal distribution was assumed for all analyses, and no data points were excluded. All experiments were performed three or more times independently under identical or similar conditions. Zeiss ZEN 3 and imageJ software were used for confocal images analysis. Microsoft excel was used for analysis of qRT-PCR or chemical screening data. FlowJo software was used for the analysis of flow cytometry data. SnapGene software was used for visualize and document cloning and PCR. The generated filtered cell UMI count matrix was imported into Seurat (4.3.0) for analysis in R. Sequence data were normalized, scaled, and variance stabilized using SCTransform (V2) in Seurat. Differentially expressed genes between cell types were identified using FindMarkers in Seurat. The annotated full dataset and secretory subset were further analyzed in Python using Scanpy (1.9.3). Trajectory analysis was performed using Partition-based Graph Abstraction (PAGA) in Scanpy (tl.paga). RNA velocity analysis on the full dataset and secretory subset was performed using Dynamo (1.2.0). The PROGENy R package (1.17.3) was used for signaling pathway activity analysis. Detailed analysis methods of scRNA-seq was provided in methods section.

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

The main data supporting the results in this study are available within the paper and its Supplementary Information. Source data are provided as a Source Data file. The raw sequence data for scRNA-seq reported in this paper have been deposited in the Genome Sequence Archive58 in National Genomics Data Center59 (NGDC), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences, under accession numbers: HRA004691 (https://ngdc.cncb.ac.cn/gsa-human/browse/HRA004691) and HRA009421 (https://ngdc.cncb.ac.cn/gsa-human/browse/HRA009421). The raw fastq data are available under restricted access for academic use only, in accordance with regulations on human genetic resources management by the Minister of Science and Technology. Access can be obtained by registering with the GSA database website (https://ngdc.cncb.ac.cn/gsa-human/) and submitting an application to the Data Access Committee (DAC) via the GSA-Human System. The typical response time for access requests is approximately 2 weeks. Additional scRNA-seq datasets for ES, IF, Crypt, and IL22 condition cultured human organoids, as well as in vivo intestinal epithelial cells, are available through published accessions GSE189423 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE189423), GSE119969 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE119969),GSE125970 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE125970), GSE185224 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE185224), and E-MTAB-9543 (https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-9543).

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	The sex and gender were not considered in study design.
Reporting on race, ethnicity, or other socially relevant groupings	The race, ethnicity, and other socially relevant groupings were not considered in study design.
Population characteristics	25-70 years-old patients with healthy small intestine tissues.
Recruitment	Human healthy intestine tissues were obtained with written consent from patients at Shanghai East Hospital.
Ethics oversight	Small intestine tissue biopsies used in this study were obtained from patients who provided written informed consent under the ethical committee of Shanghai East Hospital.

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 sizes. For initially optimizing organoid culture methods, we collected 12 small intestinal tissue samples. This sample size was sufficient to test the stability of the culture method and exclude the impact caused by individual differences. For all other experiments, sample sizes were determined based on standard practices in the field and our previous experience with similar experimental setups. These sample sizes were sufficient to detect biologically meaningful differences between groups, as evidenced by the statistical significance of our results. The exact sample size for each experiment is indicated in the corresponding figure legends.
Data exclusions	No data were excluded.
Replication	Since we included detailed methods and sources of all reagents and protocols for experiments in the manuscript, human small intestinal organoid culture can be easily reproduced.
Randomization	Samples were randomly selected prior to immunostaining, qRT-PCR analysis, flow cytometry, proliferation assay, and single-cell RNA-sequencing. The allocation of samples is not relevant to the experimental results.
Blinding	No formal blinding was employed in this study. Since all experiments were conducted in accordance with standardized protocols and blinding would have no impact on the experimental results, unbiased experimental procedures and data analysis were carried out as far as possible.

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	Rabbit anti-Lysozyme (1:500, Invitrogen), mouse anti-Mucin 2 (1:100, Santa Cruz), mouse anti-Chr-A (1:100, Santa Cruz), mouse anti-α-defensin 5 (1:100, Santa Cruz), rabbit anti-OLFM4 (1:200, Cell Signaling Technology), rat anti-Somatostatin (1:200, Millipore), mouse anti-Glucagon (1:200, Santa Cruz), mouse anti-GP2 (1:500, MBL), rabbit anti-E-cadherin (1:500, Proteintech), mouse anti-ALPI (1:50, Santa Cruz). Donkey anti-Mouse IgG (H+L), Alexa Fluor™ Plus 488 (1:1000, Invitrogen), Donkey anti-Rabbit IgG (H+L), Alexa Fluor™ Plus 488 (1:1000, Invitrogen), Donkey anti-Mouse IgG (H+L), Alexa Fluor™ Plus 555 (1:1000, Invitrogen), Donkey anti-Rabbit IgG (H+L), Alexa Fluor™ Plus 555 (1:1000, Invitrogen), Goat anti-Rat IgG (H+L), Alexa Fluor™ 555 (1:1000, Invitrogen), Donkey anti-Rabbit IgG (H+L), Alexa Fluor™ Plus 647 (1:1000, Invitrogen), Donkey anti-Mouse IgG (H+L), Alexa Fluor™ 647 (1:1000, Invitrogen). Mouse anti-MBD2/3 (1:500, Santa Cruz), rabbit anti-GAPDH (1:1000, Cell Signaling Technology).
Validation	Lysozyme (Invitrogen,Cat#PA5-16668, RRID:AB_10984852), Mucin 2 (Santa Cruz,Cat#sc-7314, RRID:AB_627970), Chr-A (Santa Cruz,Cat#sc-393941, RRID:AB_2801371), α-defensin 5 (Santa Cruz,Cat#sc-53997, RRID:AB_2091709), OLFM4(Cell Signaling Technology,Cat#14369, RRID:AB_2798465), Somatostatin (Millipore,Cat#MAB354, RRID:AB_2255365), Glucagon (Santa Cruz,Cat#sc-514592, RRID:AB_2629431), GP2(MBL, Cat#D277-3, RRID:AB_10598500), E-cadherin(Proteintech, Cat#20874-1-AP, RRID:AB_10697811), ALPI (Santa Cruz, Cat#sc-271431, RRID:AB_10649489). MBD2/3 (Santa Cruz, Cat#sc-271562, RRID:AB_10659107), GAPDH (Cell Signaling Technology, Cat#5174T, RRID:AB_10622025). Donkey anti-Mouse IgG (H+L), Alexa Fluor™ Plus 488 (Invitrogen,Cat#A32766, RRID:AB_2762823), Donkey anti-Rabbit IgG (H+L), Alexa Fluor™ Plus 488 (Invitrogen,Cat#A32790, RRID:AB_2762833), Donkey anti-Mouse IgG (H+L), Alexa Fluor™ Plus 555 (Invitrogen,Cat#A32773, RRID:AB_2762848), Donkey anti-Rabbit IgG (H+L), Alexa Fluor™ Plus 555 (Invitrogen,Cat#A32794, RRID:AB_2762834), Goat anti-Rat IgG (H+L), Alexa Fluor™ 555 (Invitrogen,Cat#A-21434, RRID:AB_2535855), Donkey anti-Rabbit IgG (H+L), Alexa Fluor™ Plus 647 (Invitrogen,Cat#A32795, RRID:AB_2762835), Donkey anti-Mouse IgG (H+L), Alexa Fluor™ 647 (Invitrogen,Cat#A-31571, RRID:AB_162542).

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Organoids were dissociated from the BME with TrypLE and dissociated into single cells. Cells were washed with ice-cold DPBS and filtered into fluorescence-activated cell sorting (FACS) tubes through a 40 µm cell strainer (Falcon).
Instrument	BD FACSVerse (BD Biosciences)
Software	FlowJo software was used to analyse the obtained data.
Cell population abundance	All the cells in culture plates were dissociated, stained with and without primary antibodies, and sorted.
Gating strategy	Gating was defined to remove debris and doublet cells using FSC and SSC. The boundary of positive and negative populations was determined by the fluorescence intensity of cells.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.