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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection.
Data analysis	Data were analyzed by GraphPad Prism 5.0. Kaplan–Meier survival analysis was performed by SPSS 20.0. All flow cytometry data were analyzed with FlowJo 10 (Treestar). Adobe Photoshop CC 14.0 and ImageJ 1.48 were used for Figure presentation. One-tailed unpaired Student's t-test was performed using Excel 2010. No computer code was used.

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/reviewers upon request. 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

Microarray data have been deposited in the NCBI GEO under accession number GSE107959. The main data supporting the findings of this study are available within the article and its Supplementary files. All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For Western blotting, Northern blotting experiments and real-time qPCR in this study, at least 3 mice each group were analyzed at least 3 separate experiments. For Lgr5 staining, 200 fields of 6 mice each group were analyzed at least 3 individual experiments, and the sample size is acceptable for the field. For Ascl2 and Olfm4, 20 fields of 6 mice each group were analyzed. All experiments were conducted with multiple available biological replicates and based on previous experience with specific experimental setup; no statistical method was used to determine sample size. The sample size is acceptable for the field.
Data exclusions	No data were excluded from the anaylses. For Fig.1-6 of our manuscript, we used littermates with the same age and gender for each group. We excluded the mice 5 g thinner than other littermates before any treatment or analysis.
Replication	At least 3 mice were used for Western blotting and realtime PCR, and 6 mice were used for intestinal observation. The samples were selected randomly and investigators were blinded to group allocation. We also reproduced the key results by independent individuals. All the results can be reproduced.
Randomization	The animals were grouped by the same age and gender, and were randomly allocated to experimental groups other than 5 g thinner than other littermates.
Blinding	For all animal and human research participants, the investigator was blinded to the genotype of mice and participant characteristics during experiments.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

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

Antibodies

Antibodies used	We show antibodies used in this study in methods section “antibodies and regents”. Anti-GFP (cat. no. ab183735, 1:300), anti-Ki67 (cat. no. ab15580, 1:1000) and anti-Digoxin (cat. no. ab51949, 1:500) antibodies were obtained from Abcam. Anti-Olfm4 (cat. no.14369, 1:300), anti-EPCAM (cat. no. 14452, 1:200) and anti-E-Cadherin (cat. 8437S, 1:500) antibodies were from Cell Signaling Technology. Anti-Krt20 (cat. no. 17329-1-AP, 1:300), anti-BrdU (cat. no. 66241-1-Ig, 1:500), anti-Acta2 (cat. no.

14395-1-AP, 1:200), anti-Rbbp4 (cat. no. 20364-1-AP, 1:500), anti- Axin2 (cat. no. 20540-1-AP, 1:500) and anti-Lgr4 (cat. no. 20150-1-AP, 1:200) antibodies were purchased from Proteintech. Anti-Bptf (cat. no. SAB5300139, 1:200) was from Sigma. Anti-Snf2l (cat. no. MABE366, 1:300) was from Millipore. Anti-c-Myc (cat. no. sc-70469, 1:1000) was purchased from Santa Cruz. Anti-Lgr5 (cat. no. GT11312, 1:300) and anti-Ehf (cat. GTX49008, 1:200) antibodies were from Genetex. Alexa-594, Alexa-488, and Alexa-647-conjugated anti-rabbit and anti-mouse secondary antibodies were purchased from Invitrogen. HRP-conjugated secondary antibodies were from Sungene Biotech.

Validation

All the antibodies were validated with preliminary experiments. We examined primary antibodies according to manuals, and got similar results with validation results on manufacturer's website or relevant citations.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Unless specifically stated, for all relevant experiments, all the mice we used were C57BL/6 background, female, and 8-12 week old. Specifically, 3-month old mice were used for phenotype observation, and 60 days old (postnatal 60 days, P60) mice were used for lineage tracing. We complied the ethical regulations of the ethical committee of Institute of Biophysics, Chinese Academy of Sciences and complied the ARRIVE guidelines. The study is compliant with all relevant ethical regulations regarding animal research.

Wild animals

No wild animals were used in this study.

Field-collected samples

This study did not involve sample collected from the field.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Human intestine and colorectal cancer samples were collected from PLA General Hospital (Beijing, China). Both male and female patients were used.

Recruitment

Human resected tissues were obtained from the Department of Hepatobiliary Surgery, PLA General Hospital (Beijing, China) with informed consents, and approved by the Institutional Review Board of the Institute of Biophysics, Chinese Academy of Sciences. The study is compliant with all relevant ethical regulations regarding research involving human participants. The participant were collected randomly, without potential self-selection bias or other biases. Samples were ranked according to receiving time.

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

For ISC enrichment, intestines were excised, dissected and cleaned with PBS. Then intestines were cut longitudinally, washed with PBS, and crypt samples were separated according to standard manual. For ISC isolation, crypts were digested into single cells, followed by FACS enrichment. GFPhigh cells were intestinal stem cells.
For competitive organoid formation, 1×104 ISCs from 60-day-old Lgr5GFP;Rosa26lsl-lacZ (LRlacZ) mice were mixed with 1×104 LRYFP;lncGata6-/- ISCs, and incubated in organoid medium containing EGF, Rspo1 and Noggin (ERN medium) for organoid formation. 2 weeks later, organoids were collected for single cell preparation , stained with APC-conjugated anti-β-galactosidase antibody, and analyzed by FACS for β-galactosidase and YFP.

Instrument

BD FACSCalibur was used for detection and BD FACSAria IIIu for enrichment.

Software

All flow cytometry data were analyzed with FlowJo 10 (Treestar).

Cell population abundance

For Lgr5GFP mice, Lgr5+ cells were grouped separately from Lgr5- cells, and can be detected/analyzed directly. WT mice can also serve as negative controls. About 100000 cells were obtained from a mice.

Gating strategy

For ISC analysis, GFP+ cells from Lgr5GFP mice were detected or enriched directly.

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