

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	FACS sorted intestinal stem cells and their progenitors were partitioned into Gel Beads in Emulsion (GEMs) in the Chromium instrument, Data analysis where cell lysis,b arcoding,a nd reverse transcription of RNA occurred,f ollowed by amplification,t agmentation and 5' adaptor attachment. Libraries were sequenced on an Illumina NextSeq 500. BD FACS Diva software was used for flow analysis. GitHub repository (https://github.com/KochInstitute-Bioinformatics/Imada_FastingCancer/tree/master) is available for this study.
Data analysis	For single cell (sc) RNAseq analysis,t he cellranger (version 3.1.0,I Ox Genomics) application mkfastq was used to deconvolute raw fastq sequences and the application count was used to collapse UM ls,m ap to the mm10 mouse genome assembly and prepare count matrices. The cellranger filtered_feature_bc_matrix output data were imported into Seurat (version 3.2.0.) The vst method was used to identify the top 2000 most variable gene and dimensionality reduction was done using UMAP using 30 principal components. Initial examination of UMAP plots identified a subtle systemic difference between samples that were accounted for by dataset integration. Cell clusters were assigned to cell type by examining the expression of individual genes or ssGSEA projections. Pre-ranked GSEA was run using GSEA version4 .1 using custom gene sets derived from Biton et al.,(PMID: 30392957). The human G2M and S cell cycle gene lists provided by the Seurat package were mapped to mouse orthologs using Mouse Genome Informatics orthology data for cell cycle analysis. Single sample gene set enrichment analysis with Biton 1/11/111 gene set (PMID: 30392957) was performed using the escape version 1.0.0 running under R version4 .0.3. FlowJo Version 10 was used for the analysis of the flow data.

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](#)

GSE192482 for scRNAseq data.

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

N/A

Reporting on race, ethnicity, or other socially relevant groupings

N/A

Population characteristics

N/A

Recruitment

N/A

Ethics oversight

N/A

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 methods were used to predetermine sample size. Sample sizes were based on those used in previous and preliminary studies from our lab.

Data exclusions

No data were excluded from the analyses.

Replication

The reported data reflects at least two independent experiments, and one representative data is shown.

Randomization

Samples (i.e. allocation of sex and age-matched animals to groups) were randomized wherever possible

Blinding

Blinding was not performed in this study due to the low likelihood of author's bias in the final readouts. Analysis of the metabolite data was done automatically. The authors also followed specific and fixed criteria in analyzing the organoid cultures and histology data according to existing protocols in the lab (used in previous publications).

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

IHC:

Rabbit polyclonal anti-RFP (1:500, Rockland, 600-401-379), Rabbit monoclonal anti-Phospho H3 (1:500, Cell Signaling Technologies, 3377), Rabbit monoclonal anti-Phospho S6 ribosomal protein (1:500, Cell Signaling Technologies, 4858), , Rabbit monoclonal anti-Olfm4 (1:10,000, Cell Signaling Technologies, 39141), Mouse monoclonal anti- β -catenin (1:100, BD Biosciences, 610164), Rat monoclonal anti-BrdU (1:2000, Abcam 6326), Rabbit polyclonal anti-Lysozyme (1:2000, Thermo Fisher, RB372-A1), Rabbit-monoclonal anti-Cleaved Caspase 3 (1:500, Cell Signaling Technologies, 9664)

Western Blot

Mouse monoclonal anti-Actin (clone C4, 1:1000, Millipore sigma MAB1501), Rabbit monoclonal anti-S6 ribosomal protein (1:500, CST #2217), Rabbit monoclonal anti-Phospho-S6 ribosomal protein (1:500, CST #4858), Rabbit monoclonal anti-4E-BP1 (1:1000, CST #9644), Rabbit monoclonal anti-Phospho-4E-BP1 (1:500, CST #2855), Rabbit monoclonal anti-Phosphor-Akt (Ser473) (1:500, CST #4060), Rabbit monoclonal anti-Akt (pan) (1:1000, CST #4691), Rabbit monoclonal anti-Hypusine (1:1000, Millipore sigma #ABS1064), Mouse monoclonal anti-elf5A (1:1000, BD Biosciences #611977), Mouse monoclonal anti-Puromycin (clone 12D10, 1:10,000, Millipore sigma MABE343), Rabbit polyclonal anti-OAT (1:1000, Abcam #ab137679, Rabbit polyclonal anti-DHPS (1:1000, Abcam #ab190266).

Flow cytometry:

CD45-PE (1:500, eBioscience, 12-0451-83), CD31-PE (1:500, Biolegend, 102514), Ter119-PE (1:500, Biolegend, 116208), CD24-Pacific Blue (1:300, Biolegend, 101820), CD117-APC/Cy7 (1:300, Biolegend, 105826), Epcam-APC (1:300, eBioscience, 17-5791-82).

Validation

All antibodies for IHC, western blot and flow cytometry were well-recognized clones in the field and validated by the manufacturers.

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

Both male and female mice were used at the ages of 8-16 weeks.

Mouse strains:

C57BL/6J, Lgr5-eGFP-IRES-CreERT2, Apcfl/fl; Lgr5-eGFP-IRES-CreERT2, Lgr5-CreERT2; tdTomatoLSL, Rosa26-LSL-lacZ, Apcfl/fl; Villin-CreERT2, Rpl24Bst/+, Apcfl/fl Rpl24Bst/+; Lgr5-eGFP-IRES-CreERT2, Raptorfl/fl; Villin-CreERT2, Tsc1fl/fl; Villin-CreERT2, Odc1fl/fl; Villin-CreERT2, Apcfl/fl Tsc1fl/fl; Lgr5-eGFP-IRES-CreERT2, Atoh1fl/fl; Villin-CreERT2

Wild animals

No wild animals were used in this study

Reporting on sex

Both male and females mice were used in this study

Field-collected samples

This study did not involve field-collected samples

Ethics oversight

Animal experiments were in accordance with Committee on Animal Care at MIT

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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	Following small intestinal crypt isolation, crypt suspensions were pelleted (300 g, 5 min, 4°C) and dissociated with TrypLE (GIBCO 12604039) . Dissociated single cells were treated with the following antibody cocktail for flow cytometry analysis: CD45-PE (eBioscience, 12-0451-83), CD31-PE (Biolegend, 102514), Ter-119PE (Biolegend, 116208), CD24-Pacific Blue (Biolegend, 101820), CD117-APC/Cy7 (Biolegend, 105826), and EpCAM-APC (eBioscience, 17-5791-82). 7AAD (Invitrogen, A1310) was used as a viability dye to exclude dead cells from the analysis.
Instrument	BD FACS ARIA
Software	BD FACSDIVA, Flowjov10
Cell population abundance	For Lgr5-GFP positive population including intestinal stem cells (ISCs) and progenitors, ~10%, ISCs (GFP high) <5%.
Gating strategy	To visualize all populations: FSC-A/SSC-A. For doublet exclusion: FSC-W/FSC-A and SSC-W/SSC-A. To obtain live cells: 7-AAD negative population was chosen. To obtain epithelial cells: Epcam+, CD31-, Ter119-, CD45-. Stem cells and their progenitors: Since we used Lgr5-eGFP reporter strain, we used high GFP as a proxy for intestinal stem cells (ISCs) and low GFP expression as a proxy for ISC progenitors. To increase stringency of sorting, we additionally chose CD24- population. To summarize, ISCs: Lgr5-eGFP(high)/EpCAM+/CD24-/CD31-/Ter119-/CD45-/7AAD- population. ISC progenitors: Lgr5-eGFP(low)/EpCAM+/CD24-/CD31-/Ter119-/CD45-/7AAD-.

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