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	No software was used
Data analysis	Softwares FastQC v0.11.5. BSMAP v2.89 R v3.4.4 MethylKit v1.4.1 ChAMP R package v2.32.0 IPA Ingenuity Pathway Analysis v45868156 TopHat v2.1.0 HTSeq-Count 0.11.2 DAVID database v6.8 DESeq2 R package v1.20.0 Prism 8 V. 8.4.2 PyroMark Q48 Autoprep Software version 2.4.2 (Qiagen) PyroMark Assay Design Software 2.0 (Qiagen)

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

All raw sequencing data reported in this paper are deposited in the GEO database under the following accession numbers:
RRBS Crypts: GSE129712
WGBS LGR5: GSE129767
RNAseq Single Crypts: GSE271646
DNA methylation data of PDX are deposited in GSE208713.

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	not applicable
Reporting on race, ethnicity, or other socially relevant groupings	not applicable
Population characteristics	not applicable
Recruitment	not applicable
Ethics oversight	not applicable

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	Sample size was not predetermined. The experiments were done with n > 3 biological replicates. Sample sizes were selected on reaserch-based common standards and on the minimum number of samples allowing statistic (BMJ 2009;338:a3166).
Data exclusions	No data were excluded from the analysis.
Replication	All the experiments were successfully replicated at least 3 times also for experiments where only one representative image or result is shown.
Randomization	Biological samples were randomly selected for the experiments by using simple randomization methods.
Blinding	No blind experiments were performed.

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: Primary antibodies used for western blotting were rabbit polyclonal Ferroportin/SLC40A1 antibody (#NBP1-21502 from Novus Biologicals, 1:250); mouse monoclonal CD71/TFRC/Transferrin Receptor antibody (#65882 from Santa Cruz, 1:2000), mouse monoclonal anti-β Actin (#A5316 from Sigma-Aldrich, 1:20000), rabbit polyclonal anti-GAPDH antibody (#sc-25778 from Santa Cruz, 1:3000). The antibodies used for ChIP were rabbit polyclonal TET1 antibody [N1] (#GTX125888, GeneTex), rabbit monoclonal TET2 (D9K3E) antibody (#92529, Cell Signaling), rabbit polyclonal anti-TET3 antibody (#ABE290, Merck), normal rabbit IgG polyclonal antibody (#12-370, Millipore). 5 µg of antibody was used for each ChIP experiment.

Validation: Validation of antibodies was done by the manufacturer.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): Lenti-X 293T Cell Line: purchased from Takara Bio, cat. no. 632180.

Authentication: Done by the manufacturer.

Mycoplasma contamination: The cells has been regularly tested and found to be free of Mycoplasma contamination.

Commonly misidentified lines (See ICLAC register): None.

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: mouse lines:
Lgr5-ki-e-GFP-creER
Rosa26-lsl-Confettiki/+;Villin-creERT2tg/+
Tet2/3fl/fl and Tet2/3fl/fl VillinCre
C57BL6/J
We used young (2-4 months old) and old (18-26 months old) male and female mice.

Wild animals: The study does not involve wild animals.

Reporting on sex: We used young (2-4 months old) and old (18-26 months old) male and female mice.

Field-collected samples: The study does not field-collected samples

Ethics oversight: All animal experiments were conducted according to protocols approved by the state government of Thuringia (licenses number: TG/J-0002858/A; TG/J-0003616/A; TG/J-0003681/A; FLI-18–016; O_FN_18–20 and FLI-18–005).

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

Plants

Seed stocks	not applicable
Novel plant genotypes	not applicable
Authentication	not applicable

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 the Lgr5-eGFP ISCs isolation, freshly isolated crypts were dissociated in complete TrypLE media (10 mM Tris-HCl pH 7.5, 5 mM CaCl2, 2.5 mM MgCl2, 20 µM Y27632 and 1 mg/mL DNAase I) for 30 min in a water-bath at 37°C with brief vortexing every 10 min. The single-cell suspension was then passed through a 40 µm cell strainer and centrifuged at 800xg for 5 minutes at 4°C. Cell pellet was resuspended in 3 ml FACS staining medium (FSM) containing PBS supplemented with 2% Fetal Bovine Serum, 2.5 mM EDTA, 10 µM Y27632 and DAPI (1:1000).
Instrument	FACS Aria II, FACS ARIA III (BD Biosciences)
Software	FlowJo 10.7.1. PRISM v.7.
Cell population abundance	not applicable
Gating strategy	GFP+ cells were sorted to isolate intestinal stem cells as previously reported (10.1016/j.ejcb.2022.151282).

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