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	Chromium Next GEM Single Cell Multigroup ATAC + Gene Expression Sequencing using 10x Genomics.
Data analysis	Analysed mainly using R (4.2.2) and Python (3.7.16)

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 sequencing data have been uploaded to China National Center for Bioinformation (CNCB) Genome Sequence Archive (GSA) database under the accession number: PRJCA030631.

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	9,722 LGR5+ high quality mouse intestinal cells for sequencing
Data exclusions	no data were excluded from the analyses
Replication	Verification was performed using published human jejunal single-cell transcriptome data(9 samples).
Randomization	The experimental design involved the use of healthy adult mice, devoid of any sub-groupings, for the execution of the sequencing process. The control group comprised normal small intestinal organoids, while the experimental groups consisted of small intestinal organoids that had undergone a Foxa3 knockout.
Blinding	The investigators were blended to group allocation during data collection and/or analysis.

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	Purified anti-CD324 (E-Cadherin) (Biolegend;Cat #: 866701) Keratin 20 (D9Z1Z) XP® Rabbit mAb (Cell Signaling Technology;Cat #: 13063S)

MMP-7 (D4H5) XP® Rabbit mAb (Cell Signaling Technology;Cat #: 3801S)
Fluor™594 Donkey Anti-Rabbit IgG(H+L) (YEASEN;Cat #: 34212ES60)
Fluor™647 Donkey Anti-Mouse IgG(H+L) (YEASEN;Cat #: 34113ES60)
Foxa3 protein antibody (Santa Cruz, sc-74424)

Validation

All antibodies were validated by the manufacturer or have been used in previous publications.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	ATCC
Authentication	Cell lines not specifically authenticated
Mycoplasma contamination	Cells tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	NA

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	the Lgr5-EGFP-IRES-CreERT2 mouse(12 weeks old, male)
Wild animals	the study did not involve wild animals
Reporting on sex	male
Field-collected samples	the study did not involve samples collected from field
Ethics oversight	the Animal Care and Use Committee of Fudan University

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

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	https://ngdc.cncb.ac.cn/bioproject/browse/PRJCA030631
Files in database submission	raw files and peak files
Genome browser session (e.g. UCSC)	no longer applicable

Methodology

Replicates	two samll instestinal organoids

Sequencing depth	Clean data is over 10G for each sample.
Antibodies	the Foxa3 protein antibody (Santa Cruz,sc-74424)
Peak calling parameters	Genome index is based on mm10,p-value is 1e-5 and g is 2.00e-9.
Data quality	We performed ChIP-seq peak calling using the MACS2 pipeline on both samples. The analysis incorporated background correction, and peaks were identified with an FDR q-value threshold of 0.05. Sample 1 yielded 1558 significant Foxa3 binding sites, while Sample 2 identified 2386 significant Foxa3 binding sites.
Software	The sequencing data were mapped to the mm10 genome using Bowtie2. Peak calling was then performed using MACS2.

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 Lgr5+ cell enrichment, intestines of Lgr5-GFP mice were excised, dissected and cleaned with cold PBS. Then intestines were cut longitudinally and washed twice with cold PBS. Villi were carefully scraped off with operating scalpel. The remaining part was cut into small pieces (5 mm) and incubated in 10 mM EDTA in PBS for 40 min on ice. After removal of EDTA, the small pieces were vigorously suspended using a 10-ml pipette with cold PBS. The supernatant, which enriched in crypts, was passed through 70 µm cell strainer (BD) and centrifuged at 600 rpm for 3 min. The pure crypts obtained were used for single Lgr5-GFP cells purification.
Instrument	BD Fortessa flow cytometer
Software	BD Diva Software
Cell population abundance	Lgr5-GFP cells were sorted directly.
Gating strategy	WT mice serve as negative controls and GFP+ cells from Lgr5-GFP mice were gated.

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