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Corresponding author(s): Moshe Biton, Ramnik Xavier and Aviv Regev

☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

We analyzed over 50,000 single epithelial cells to gain maximum power to detect heterogeneity, and to detect shifts in cell proportions using single cell RNA-seq. We used 'how many cell' tool to estimate the probability of detecting cell types, see p.4.

2. Data exclusions

Describe any data exclusions.

No animals were excluded. Low quality, immune cells and doublets were filtered out computationally, see p. 31-32.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All mouse experiments were repeated at least twice in which we analyzed single cells from 2 mice per group at a single time, most experiments had n=4 mice in total.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

All mouse models (control; C57bl/6J, Lgr5-EGFP-IRES-CreERT2 and Gfi1b-eGFP) that were used in this study were 7-10 weeks old littermates, which assigned randomly or by genotype to groups (p. 23).

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

We preformed an unbiased analysis to all datasets from different mouse models on a single cell resolution.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	We used available softwares and pipelines to analyze single cell data. All methods are found in the Method section in 'Computational Analysis' p. 28-38.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	No unique materials were used in this study. All materials are found in the Method section.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	All antibodies in this study are found in Methods, p. 27-28. in brief: rabbit anti-DCLK1 (1:200, Abcam GR245168- 1), rat anti-CD45 (1:100, Biolegend 30-F11, 103101), rabbit anti-RELMbeta (1:200, Peprotech 500-p215) mouse anti-E-cadherin (1:100, BD Biosciences 610181), rat anti-Lysozyme (Dako, A0099), anti-mouse CD31-PE (1:500, e-Bioscience, 12-0311-81), anti-mouse TER-119 (1:500, e-Bioscience, 12-5921-81) anti-mouse CD326 (EpCAM)-APC-780 (1:300, e-Bioscience, 47-5791-82) anti-mouse CD45-PB (1:300, Biolegend 30-F11, 103125) anti-mouse CD24a-APC (1:300, e-Bioscience, 17-0242-82)

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	N/A
b. Describe the method of cell line authentication used.	N/A
c. Report whether the cell lines were tested for mycoplasma contamination.	N/A
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	N/A

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	mouse, C57bl/J6, males or females, the sex was consistent with the experiment, 7-10 weeks of age.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	N/A

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

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

☒ 2. 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).

☐ 3. All plots are contour plots with outliers or pseudocolor plots.

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

Methodological details

5. Describe the sample preparation.	All cells isolated from mouse small intestine as described in material and methods, pages 23-25.
6. Identify the instrument used for data collection.	Data was collected on a Beckman Coulter MoFlo Astrios EQ cell sorter.
7. Describe the software used to collect and analyze the flow cytometry data.	Acquisition was performed with Summit v6.1, and analysis was performed with FlowJo v10.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Post-sorting we performed single-cell RNA-seq to verify purity of relevant cell populations. See Method section p. 25-26. and Figures 1a,b for cell proportions post-sort.
9. Describe the gating strategy used.	The preliminary gating for the starting population used FSC1-Area vs SSC1-Area. Singlets were then gated using SSC1-Area vs SSC1-Width and live/ dead was gated using 7AAD-Area vs SSC1-Area. Positive and negative boundaries were determined based on gating of a fluorescence minus one (FMO) control.

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