nature portfolio

Corresponding author(s): Hugo Snippert

Last updated by author(s): Jun 30, 2025

# 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<br>*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<br>*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 Celesta (for quantification of organoid knock-in fluorescent populations) BD FACS DIVA v9.2<br>• FACSaria III (for sorting cells for scRNA-seq): BD FACS DIVA v.8.0.1<br>• Leica SP8 (for imaging organoids): LAS X v3.5.7.23225<br>• Nikon A1R MP (for imaging organoids): NIS elements<br>• Olympus VS200 slide scanner (for imaging RNAscope and immunofluorescence slides) |
| Data analysis | scRNA-seq:<br>• nf-core scrnaseq pipeline v2.4.0<br>• STARsolo v2.7.10b<br>• STARsolo v2.7.10b<br>• Seurat v5.0.1<br>• scVelo v0.3.2<br>• ClusterProfiler v4.8.3<br><br>Bulk RNA-seq<br>• DESeq2 v1.40.2 |

• ClusterProfiler v4.8.3
• Enrichplot v1.20.3

Flow cytometry:
• Floreada v8/6/24
• DIVA version v9.2
• flowCore v2.14.0
• CytoExploreR v1.1.0

Microscopy:
• FIJI ImageJ 2.16.0/1.54g
• OrganoidTracker v2.0
• QuPath v0.5.1

General:
• Rstudio 2024.12.1+563
• R v4.2.0
• Excel 16.95
• ggplot2 v3.5.1
• ggpubr v0.6.0

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

Organoid and primary tissue single-cell RNA sequencing data presented in this study are publicly available and can be accessed at GSE276950.

## 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 | *Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.*<br>*Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.*<br>*Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.* |
| Reporting on race, ethnicity, or other socially relevant groupings | *Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).*<br>*Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)*<br>*Please provide details about how you controlled for confounding variables in your analyses.* |
| Population characteristics | *Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."* |
| Recruitment | *Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.* |
| Ethics oversight | *Identify the organization(s) that approved the study protocol.* |

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 | There was no statistical calculation of sample size upfront. Sample sizes of experiments are indicated throughout the manuscript. |
| Data exclusions | In scRNA-seq, cells that did not pass QC were excluded (as described in methods section of the manuscript). |
| Replication | All experiments were performed in multiple independent replicates, as described in the figure legends and Methods section. |
| Randomization | Mice and organoids used in experiments were randomly assigned to an experimental group. |
| Blinding | In the experiments, no blinding of researchers for organoid identity, mouse identity or treatment was performed, to avoid sample swaps and as is according to common practice in the field. |

# 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 | The following antibodies were used for immunofluorescence: anti-mouse CD45 (Biolegend 103102); anti-mouse Folr1 (R&D Systems AF6936-SP); anti-mouse b-catenin (Sigma C2206); anti-muouse DCLK1 (Abcam ab37994); anti-GFP (Aves GFP-1020); anti-mouse DCLK1 (Abcam EPR6085) and anti-GFP (Abcam EPR14104)<br>The following antibodies were used for flow cytometry: APC anti-mouse EpCAM (Biolegend 118213) and AF700 anti-mouse CD45 (Biolegend 103127)<br>The following antibodies were used for live-microscopy: APC anti-mouse CD24 (ThermoFischer 17-0242-80) |
| Validation | All antibodies were validated by the manufacturer. Additional validation was performed by confirming the expected localization and expression patterns. |

# Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | All organoids used were generated in this study as described in the methods. |
| Authentication | Organoids were subjected to genotyping by PCR of genomic knock-in loci and phenotypically profiled by flow cytometry, microscopy and scRNA-seq. This way, the organoids could be authenticated based on their specific phenotypic and genetic properties. |
| Mycoplasma contamination | Organoid lines were routinely tested for mycoplasma and results were always negative. |

| | |
|---|---|
| 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 | Chat-eGFP transgenic mice (B6) were obtained from the Jackson Laboratory and housed at the Hubrecht institute and Netherlands Cancer Institute mouse facilities under specific pathogen-free (SPF) conditions. |
| Wild animals | *Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.* |
| Reporting on sex | For all experiments, adult mice at least 6 weeks of age, of either sex, were used. Sex is not expected to have an influence on the results of this study and was therefore not considered in the experiments. |
| Field-collected samples | *For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.* |
| Ethics oversight | Animal experiments were approved by the IvD HI-KNAW and Netherlands Cancer Institute animal welfare committees and conducted according to relevant guidelines and regulations. |

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

## Plants

| | |
|---|---|
| Seed stocks | *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.* |
| Novel plant genotypes | *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.* |
| Authentication | *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.* |

## 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 isolation of tuft cells from mouse tissue, small intestines were excised, rinsed with PBS and incubated for 10 min in PBS containing 100 uM DTT. Next, the small intestines were cut into smaller fragments, transferred into DPBS containing 5mM EDTA and spun on a carousel for 40 min at 4 °C. Organoids were extracted from Matrigel with cold advanced DMEM. To make single-cell suspensions, tissue fragments or organoids were trypsinized for 5 min at 37 °C with TypLE containing 10 uM Y-27632 Rho-kinase inhibitor to inhibit anoikis. Trypsinized single-cell preparations were washed with advanced DMEM, filtered (40 um cell strainer) and resuspended in advanced DMEM containing 10 uM Y-27632. In case of immunofluorescence, single-cell suspensions were stained for 30 min on ice. Cells were stained briefly with DAPI prior to flow cytometry. |
| Instrument | For flow cytometry: BD FACSCelesta<br>For sorting for scRNA-seq: BD FACSAria III |
| Software | Data collection: |

• FACS Celesta: BD FACS DIVA v9.2
• FACS ARIA III: BD FACS DIVA v.8.0.1

Data analysis:
• Floreada v8/6/24
• DIVA version v9.2
• flowCore v2.14.0
• CytoExploreR v1.1.0

Cell population abundance

Fluorescent and negative cell populations from organoids and GFP+ cells from Chat-GFP mice were subjected to scRNA-seq after sorting, which confirmed identities of fluorescent cells as tuft cells. Moreover, the index sorting allowed us to confirm the expression of Chat-GFP and mScarlet (tuft subtype-specific knock-ins) in the appropriate clusters, as shown in Figure 3 and Supplementary Figure 5..

Gating strategy

SSC-A/FSC-A was used to select cells, FSC-H/FSC-A was used to select singlets. DAPI staining was used to mark dying cells. Single live cells (DAPI-) were gated in the BV421 channel and GFP and mScarlet-I fluorescence were measured in the FITC-A and PE-A channels, respectively. Gates were set based on negative control samples.

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