nature portfolio

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Last updated by author(s): Sep 26, 2024

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	The sequenced cDNA libraries from 10x Genomics were alligned to the mouse reference genome (mm39) using cellranger (v6.1.2, 10x Genomics). Count matrices were then processed in R (v.4.1.1 (2021-08-10)). SoupX (v.1.6.1) was used to filter out ambient RNA from the sequencing runs, and Seurat (v.4.1.1) was used to process objects for further analysis. Further data quality measures were calculated using DoubletFinder (v.2.0.3). To correct for any batch defects between sample runs, we used the harmony (v.0.1.0). To calculate pseudotime for inferring differentiation trajectories, the phateR package (v.1.0.7) (https://github.com/scottgigante/seurat/tree/patch/add-PHATE-again) embedded the cell clusters for further pseudotime value calculations made by Monocle3 (v.1.2.7). All code generated for data analysis can be found on GitHub (https://github.com/coulterr24/MouseTE_scRNA).

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

The raw sequencing data generated in this study have been deposited in the Gene Expression Omnibus (GEO) database under accession code GSE252786 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE252786]. All processed Seurat objects for scRNA-seq analysis are available in the Dryad repository at https://doi.org/10.5061/dryad.4mw6m90hm [https://doi.org/10.5061/dryad.4mw6m90hm]. All data generated in this study are provided in the Source Data file. The remaining data are available within the Article, Supplementary Information, or Source Data file.

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.* *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.* *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).* *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.)* *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	Samples sizes were chose based on our previous studies in the same experimental settings.
Data exclusions	No data were excluded.
Replication	All experiments were replicated at least two times.
Randomization	All experiments were performed on animals of identical sex and age.
Blinding	The phenotype of all mice was evaluated before confirmatory of their genotype.

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
[ ]	[x] Antibodies
[x]	[ ] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

All antibodies and their origin are described in Supplementary Table 7 of the manuscript:

List of antibodies used for immunostaining*

Antigen conjugation, Antibody source, catalogue number, Clone, Lot. number, Host Retrieval, Dilution

Acetylated α-tubulin Sigma-Aldrich, T7451 6-11B-1 103M4772V Mouse Citrate 1:200 (IF#)
CD140a Biolegend, 135910 APA5 B376160 Rat (MACS)
CD31 Biolegend, 102503 MEC13.3 B379519 Rat (MACS)
CD45 Biolegend, 103103 30-F11 B394828 Rat (MACS)
FAM183B Invitrogen,PA5-71109 PC& ZE4342976 Rabbit 1:150 (IF)
FoxJ1 Novus Biologicals, AF3619-SP PC XVG022308 Goat 1:800 (IF)
Ki67 Thermo Fisher,14-5698-82 SolA-15 2496198 Rat Citrate 1:4000 (IHC†)
OVGP1 Abcam, Ab118590 PC 1078217-2 Rabbit None Citrate 1:600 (IF) 1:2000-8000 (IHC), 1:800 (IF)
P16 Abcam, Ab241543 PABLO-33B - Rat Citrate 1:500 (IHC)
P73 Abcam, Ab40658 EP436Y 1014031-3 Rabbit Citrate 1:200 (IF)
Pax8 Proteintech, 60145-4-Ig 4H7B3 10003229 Mouse Citrate 1:5000-10000 (IHC), 1:100 (IF)
Pax8 Proteintech, 10336-1-AP PC - Rabbit Citrate 1:4000 (IHC), 1:400 (IF)
RFP/tdTomato Rockland Immunochemical, 200-101-379 PC 50005 Goat 1:100 (IF)
RFP/tdTomato Rockland Immunochemical, 600-401-379S PC 48776 Rabbit 1:4000 (IHC), 1:250 (IF)
Slc1a3 Novus Biologicals, NB100-1869 PC D112656-5 Rabbit 1:1000 (IHC), 1:500 (IF)
Slc1a3/EAAT1 R&D Systems, AF6048 PC CAGI032408A Sheep Citrate 1:75 (IF)
Slc1a3 Novus Biologicals, NB-100-1869B PC D160350 Rabbit (MACS)
TER-119 Biolegend, 116203 TER-119 B378223 Rat (MACS)
Upk1α Proteintech, 25275-1-AP PC - Rabbit Citrate 1:4000 (IHC), 1:250 (IF)
Wilm's Tumor Protein 1 Abcam, Ab267377 EPR-23963 - Rabbit Citrate 1:500 (IHC)
Anti-goat IgG, Alexa Fluor 594 Invitrogen, A11058 PC 2185074 Donkey 1:200
Anti-mouse IgG, biotinylated Vector Labs, BA-9200-1.5 PC ZH0819 Goat 1:200
Anti-mouse IgG, Alexa Fluor 488 Thermo Fisher, A-11001 PC 2714439 Goat 1:200
Anti-rabbit IgG, biotinylated Vector Labs, BA-1000-1.5 PC ZG0122 Goat 1:200
Anti-rabbit IgG, Alexa Fluor 488 Invitrogen, A21206 PC 2072687 Donkey 1:200
Anti-rabbit IgG, Alexa Fluor 594 Thermo Fisher, A-21207 PC 2747441 Donkey 1:200
Anti-rat IgG, biotinylated Vector Labs, BA-4000-1.5 PC ZJ1101 Rabbit 1:200
Anti-sheep IgG, Alexa Fluor 594 Invitrogen, A11016 PC 2260924 Donkey 1:200

*All antibodies were validated by manufacturers.
#IF, Immunofluorescence
$MACS, Magnetic activated cell sorting, all dilutions were 0.5µg of antibody per million cells
&PC: Polyclonal
†IHC, Immunohistochemistry (ABC eliite method)

Validation

All antibodies were validated by manufacturers, followed by confirmatory analysis on known samples in our laboratory.

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	Mice of similar age on FVB/N and BL6/J background were used in this study. Specifically, our study includes Tg(Slc1a3-cre/ERT)1Nat/J (Slc1a3-CreERT, Stock number 012586), Tg(Pax8-rtTA2S*M2)1Koes/J (Pax8-rtTA, stock number 007176), Tg(tetO-Cre)1Jaw/J (Tre-Cre, stock number 006234), Tg(KRT5-cre/ERT2)2Ipc/Jeldj (K5-Cre-ERT2, Stock number 018394), Gt(ROSA)26Sortm9(CAG-tdTomato)Hze (Rosa-loxp-stop-loxp-tdTomato/Ai9, Stock number 007909), and C57BL6 (Stock number 000664) mice obtained from The Jackson Laboratory (Bar Harbor, ME, USA). Trp53loxP/loxP and Rb1loxP/loxP mice, which have Trp53 and Rb1 genes flanked by loxP alleles, respectively, were a gift from Dr. Anton Berns.
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	Only female mice were used in all experiments because our study focuses on the female reproductive tract.
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	All animal experiments were approved by the Cornell University Institutional Laboratory Animal Use and Care Committee

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	Mouse uterine tubes were harvested, minced and digested into single cell suspension as described in the methods section
Instrument	Sony MA-900
Software	Data was acquired using Sony MA-900 complementary software. Analysis was performed using Flowjo.
Cell population abundance	On average, SLC1A3+ cells made up 1.44% of the cell population. Post FACS, organoids were visualized under a microscope to check for tdTomato+
Gating strategy	Slc1a3-CreERT negative litter mates were used to gate negative controls. After gating single cells, sytox blue was used to gate for live or dead populations. From the live cell population, tdtomato+ cells were determined by using Slc1a3-CreERT negative litter mates (Supplemental Figure 13).

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