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	scRNA-seq data were downloaded from GEO datasets GSM6213387, GSM5492035, and GSM5952176. All image quantification data were obtained using Image J software version 1.54e (32-bit Java 1.8.0_241). Flow cytometry data were obtained using FACS Diva software (BD Biosciences; version 9.0).
Data analysis	Data analysis was conducted using the Seurat package as shown in the published code. All statistical analyses were performed using Prism version 8.0 (GraphPad). Two-tailed Student t-tests were performed to calculate P values, with P<0.05 considered statistically significant. Error bars indicate ± SD or SEM and are described within individual figure legends. Code used to analyze scRNA-seq data was deposited at Zenodo [https://zenodo.org/records/17087174].

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

Source data for quantitative assays (e.g., graphs) in Figs. 1–7 and Supplementary Figs. 1, 3, 4, and 6 are provided with the paper in the Source Data file. Other data supporting the findings of this study are available within the paper and its supplementary information files, and they are also available from the corresponding author upon request.

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	No human research participants are included in this study.
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	No power analyses were performed to determine sample size. At least 3 independent biological replicates were performed for all experiments. The number of biological replicates was determined based on the minimum number required to perform statistical analyses.
Data exclusions	No data were excluded.
Replication	For immunofluorescence, organoid, and qRT-PCR assays, at least three independent experiments were performed, and within each experiment multiple uteri (n≥3) were used. The mouse model used and the time points for sample collection in each experiment are described in figure legends, as well as sample sizes used. Each dot on graphs represents an individual, independent measurement from one mouse; for every experimental condition, at least three mice (n≥3) were used.
Randomization	Samples were allocated into experimental groups by genotype or treatment condition.
Blinding	No specific methods were used for blinding in this study, although virtually all experiments were performed by multiple authors with virtually identical results.

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

The following antibodies were used for immunofluorescence: rabbit anti-Nestin (1:1000, BioLegend #PRB-315C); rat anti-PECAM1 (1:250, BD Pharmingen #553370); rabbit anti-ERG (1:250, Abcam #ab92513); rat anti-CK8 (1:500, EMD Milipore #MABT329); rabbit anti-CSPG4 (1:500, Millipore-Sigma #AB5320); rat anti-CDH1 (1:500, Invitrogen #13-1900); rabbit anti-ESR1 (1:200, Santa Cruz #sc-542); rabbit anti-PGR (1:200, Cell Signaling #8757); rabbit anti-NOTCH3 (1:1000, Cell Signaling #5276T); rabbit anti-Ki67 (1:300, Thermo RM-9106-S); rabbit anti-RBPJ (1:1000, Cell Signaling Technology #5314); rat anti-Ki67 (1:500, Thermo 14-5698-80); and goat anti-DLL4 (1:100, R&D Systems #AF389). The following antibodies were used for flow cytometry: rabbit anti-Nestin (1:1000, BioLegend #PRB-315C); Alexa Fluor 488 donkey anti-rabbit IgG (H+L) (1: 500, Thermo Fisher #A21206); and Alexa Fluor 700 anti-CD326 (EpCAM) (1:100, BioLegend #118240).

Validation

Commercially available antibodies have provided validation statements on their respective websites. We have performed our own validation and control experiments to ensure specificity of antibodies for immunofluorescence, such as: omitting primary antibody; staining tissues known to lack antigen (either knockout tissue or tissue not containing cell/protein of interest); and costaining with other antibodies to assess cell-type specificity.

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

Mus musculus (mouse) were used. Specific strains used were: CBF:H2B-Venus [Tg(Cp-HIST1H2BB/Venus)47Hadj; JAX stock #020942]; Rosa-Tomato [B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze; JAX stock #007914]; Nestin-CreER [Tg(Nes-cre/ERT2,-ALPP)1Sbk]; and Rbpj-flox (Rbpjtm1Hon). Adult females (at least 2 months old; P60 or older) were used for harvesting of uterine tissues.

Wild animals

No wild animals were used in this study.

Reporting on sex

We focused specifically on uterine function and homeostasis, so findings only applied to one sex (female).

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

Mice were housed in accordance with National Institutes of Health guidelines, and experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Cincinnati Children's Hospital Medical Center (animal experimental protocol numbers IACUC2021-0016 and IACUC2024-0039).

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

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A

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	Uteri were cut open and dissociated in 5mL Hanks' Balanced Salt Solution (HBSS) containing 6 mg/mL dispase (Thermo Fisher, Waltham, MA) and 1% (w/v) trypsin (Thermo Fisher, Waltham, MA) at 4°C for 45 minutes, room temperature for 20 minutes, and 37°C for 5 minutes to remove luminal epithelial cells. The remaining endometrial tissue pieces were incubated in 5mL HBSS containing 0.15 mg/mL collagenase IV at 37°C for 20 minutes, and the supernatant was filtered through a 70 µm cell strainer to collect stromal cells. Filtered cells were centrifuged at 350 g for 10 minutes. Cells were subsequently fixed with 1% paraformaldehyde (PFA) for 10 minutes at 4°C. After fixation, cells were washed with PBS and incubated with anti-Nestin (1:2000, BioLegend, #PRB-315C, San Diego, CA) or Alexa Fluor 700 anti-CD326 (EpCAM) (1:100, BioLegend, #118240, San Diego, CA) primary antibodies for 1 hour at 4°C. Following primary antibody incubation, cells stained with anti-Nestin antibody were further incubated with Alexa Fluor 488 donkey anti-rabbit IgG (H+L) (#A21206, Life Technologies, Carlsbad, CA) for 30 minutes at 4°C.
Instrument	SONY MA900 Multi-Application Cell Sorter
Software	FACS Diva software (BD Biosciences; version 9.0)
Cell population abundance	The percentage of cell populations was provided.
Gating strategy	To ensure accurate multicolor flow cytometry analysis, fluorescence compensation is first applied to correct for spectral overlap between fluorophores. Following compensation, forward scatter (FSC-A) and side scatter (SSC-A) are used for selecting the main cell population. FSC-A versus FSC-H is used to eliminate doublets; live and single cells are gated for cell culture and organoid formation. $Tomato^+$ cells are gated using a 7-AAD negative Tomato positive plot. For $Venus^+$ cells, a plot of 7-AAD negative GFP positive is used. In the EpCAM vs Tomato plot, $Tomato^+$ cells are gated based on the expression in the EpCAM versus Tomato plot, distinguishing $EpCAM^-$ and $EpCAM^+$ subsets. In the Nestin vs FSC-A plot, $Nestin^+$ cell population is identified with the $Nestin^+$ gate defined in the high-fluorescence region on the x-axis.

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