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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	AxioVision v4.8.2; Leica Applications Suite v4.9; Nikon A1R two-photon; MicroXCT-200; Scanco vivaCT 40; BD FACSDiva v8.0.1; Chromium Controller (10X Genomics, 120263); HiSeq4000 (Illumina, SY-401-4001); Cell Ranger (10X Genomics, v2.0.0).
Data analysis	FlowJo v9.9; Prism v7.0h; Excel v14.7.7; Photoshop CC2015.5; Illustrator CC2015.3; Avizo Light 9.1.1; ImageJ2; R version 3.4.3 (2017-11-30), Python – Jupyter Notebook; https://github.com/AllonKleinLab/SPRING/; https://github.com/theislab/scanpy; https://github.com/dgrun/FateID; GSEA Desktop v3.0. Custom code was developed to perform cell segmentation analysis (Figure 5). Custom code used open source libraries FunImageJ (version 0.2.5) and Mosaic toolsuite (version 1.0.8). Both libraries are based upon ImageJ, but were invoked directly through FunImageJ which is the key library for ensuring reproducibility of this study.

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Single-cell RNA sequencing data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession code GSE131204. Source data for figures have been provided as Supplementary Table 11. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	In most cases we assumed a minimum of 4 mice would be required to recognize differences between conditions, based upon published data in this system and in other contexts. To determine the steady state (Supplementary figure 1) 3 mice/developmental stage were analyzed. A minimum number of animals were used to conform with NIH guidelines.
Data exclusions	For the H2B dilution experiment, one mouse from the 1 day and one mouse from the 21 days chase were excluded due to very low of GFP coverage, probably due to error in Dox injection.
Replication	All experiments were replicated at least twice with similar findings, except 1) single cell RNAseq (due to prohibitive costs). 2) Modelling proliferation dynamics using H2B-GFP incisor (A cohort of 20 mice was used for FACS-sorting cells assay)
Randomization	Samples were randomly assigned.
Blinding	Investigators were not blinded to group allocation because treatments and data collection were performed by the same people.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	The following primary antibodies were used: Ameloblastin (sc50534, Santa Cruz biotechnology, clone J2612, various lot #s); rat anti-BrdU antibody (ab6326, Abcam, clone BU1/75(ICR1), various lot #s); mouse BrdU antibody (B35128, Invitrogen, clone MO-BU-1, various lot #s); GFP (GFP-1020, Aves, Chicken IgY, various lot #s; ab13790, Abcam, clone GR3190550, various lot #s); NICD (Cleaved Notch1, 4147 (Val1744/D3B8), Cell signaling, various lot #s); RFP (600-401-379, Rockland, various lot #s). The following secondary antibodies were used (1:500 dilution each): goat anti-rat IgG Alexa fluor 488 (Invitrogen; A-11006, AB_2534074), goat anti-rabbit IgG Alexa fluor 568 (Invitrogen; A-11011, AB_143157), goat anti-mouse IgG Alexa fluor 488

(Invitrogen; A-11001, AB_2534069), goat anti-rabbit IgG Alexa fluor 633 (Invitrogen; A-21070, AB_2535731), rabbit anti-rat IgG Alexa fluor 488 (Thermo Fisher Scientific; AB_2535796), and biotinylated goat anti-rabbit IgG (Vector Laboratories; AB_2313606).

EdU was detected using Click-iT Plus EdU Assay Kit (ThermoFisher, C10640).

Validation

Validation available from peer-reviewed publications: Ameloblastin (MacDougall, M., et al. 2000, Cloning characterization and immunolocalization of human Ameloblastin Eur. J. Oral. Sci. 108 303-310).

Validation statements available from manufacturers, and state the species and application for which each antibody has been validated:
- rat BrdU (Abcam #ab6326, https://www.abcam.com/BrdU-antibody-BU175-ICR1-ab6326.pdf)
- mouse BrdU (Invitrogen #B35128, https://www.thermofisher.com/order/genome-database/generatePdf?productName=BrdU&assayType=PRANT&detailed=true&productId=B35128)
- GFP (Aves #GFP-1020, http://www.aveslab.com/wp-content/uploads/GFP-10201.pdf)
- GFP (Abcam #ab13790, http://www.abcam.com/gfp-antibody-ab13970.html)
- NICD (Cell signaling #4147, https://media.cellsignal.com/pdf/4147.pdf)
- RFP (Rockland #600-401-379, https://rockland-inc.com/store/Antibodies-to-GFP-and-Antibodies-to-RFP-600-401-379-O4L_24299.aspx)
- Click-iT Plus EdU Assay Kit (ThermoFisher #C10640, https://assets.thermofisher.com/TFS-Assets/LSG/manuals/mp10637.pdf).

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Male mice aged 8–12 weeks were used for all experiments, except those analyzing mice at various age to determine the steady state (Supplementary Figure 1). The following mouse lines were used: Wild-type (C57BL/6J, Jax: 000664); Gli1CreER [Gli1tm3(cre/ERT2)Alj, Jax: 007913], Sox2CreER [Sox2tm1(cre/ERT2)Hoch, Jax: 017593]; Bmi1CreER [Bmi1tm1(cre/ESR1)Mrc, Jax: 010531]; Lrig1CreER [Lrig1tm1.1(cre/ERT2)Rjc, Jax: 018418]; Notch1CreER [Notch1tm5(cre/ERT2)Rko, Jax: 027235]; K14Cre [Tg(KRT14-cre)1Amc, MGI: 2445832]; R26RmT/mG [Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo, Jax: 007576]; R26RlacZ [Gt(ROSA)26Sortm1Sor, Jax: 003474]; R26RtdTomato [Gt(ROSA)26Sortm14(CAG-tdTomato)Hze, Jax: 007914]; Rag1–/– (Rag1tm1Mom; Jax: 002216); TetOff-H2B-GFP [Tg(tetO-HIST1H2BJ/GFP)47Efu, Jax: 005104]; K5tTA [Tg(KRT5-tTA)1216Glk, MGI: 3575755]; TetOn-H2B-GFP [Gt(ROSA)26Sortm1(rtTA*M2)Jae::Col1a1tm7(tetO-HIST1H2BJ/GFP)Jae, Jax: 016836]; rtTA [Gt(ROSA)26Sortm1(rtTA*M2)Jae::Col1a1tm1(tetO-cre)Haho, Jax: 021025]; CBF1:H2B-Venus [Tg(Cp-HIST1H2BB/Venus)47Hadj, Jax: 020942]. Rosa-DTA [B6.129P2-Gt(ROSA)26Sortm1(DTA)Lky/J, Jax: 009669].

Wild animals

The study did not involve Wild Animals.

Field-collected samples

The study did not involve Field-Collected Samples.

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

Wild-type (C57BL/6J, Jax: 000664) mice were used as a source of incisor epithelial cells for single cells RNA sequencing (Figures 1,3,6). TetOn-H2B-EGFP mice were used as the source for incisor epithelial cells for the label dilution experiment (Figure 5).

Proximal incisors (roughly 3 mm in length) were first severed away using a pair of scissors after removal of the surrounding jaw bones. The bulbous portion, as well as the lateral wing-shaped epithelium, and the surrounding mesenchyme were subsequently dissected from the rest of the incisor and collected in cold HBSS (pH 8.0) and digested enzymatically at 37°C in collagenase type II at concentration 2 mg/ml (Gibco/BRL type II in DMEM) for 30 min, followed by collagenase inactivation with 2% FBS. The digested tissue was mechanically disrupted using 1 ml pipette tip and centrifuged at 400 x g for 3 min. Cells were resuspended in HBSS (pH 8.0) containing 2% FBS.

FACS plots are provided in Supplementary Figure 3.

Instrument

BD FACSAria2 SORP

Software

BD FACSDiva v8.0.1; FlowJo v10

Cell population abundance

In all cases, sorted cells were checked for purity by running a fraction of the sorted material on a cytomtery, including fresh DAPI to exclude dead cells. Prior to determination of purity, events were gated for FSC x SSC to exclude bubbles and debris in the

sample collection tube, and only DAPI(lo) events were considered. Sorted samples generally had a purity >80%. Samples that were obviously outliers for the frequency of DAPI+ events or of obviously low purity were removed from downstream analysis, on a case-by-case basis.

Gating strategy

Single cell RNAseq sorting:
CELLS: x-axis:FSC-Area (linear scale); y: SSC-Area (log scale)
SINGLETS: x-axis: FSC-Area; y-axis: FSC-Width
SINGLETS: x-axis:SSC-Area; y-axis: SSC-Width
LIVE CELLS: x-axis: FSC-Area; y-axis: DAPI (DAPI- cells were gated)
EPCAM-APC+ CELLS: x-axis SSC-Area; y-axis: EPCAM-APC (EPCAM-APC+ cells were gated and collected)

label dilution experiments:
CELLS: x-axis:FSC-Area (linear scale); y: SSC-Area (log scale)
SINGLETS: x-axis: FSC-Area; y-axis: FSC-Width
SINGLETS: x-axis:SSC-Area; y-axis: SSC-Width
LIVE CELLS: x-axis: FSC-Area; y-axis: DAPI (DAPI- cells were gated)
EPCAM-APC+ CELLS: x-axis SSC-Area; y-axis: EPCAM-APC (EPCAM-APC+ cells were gated)
Analysis of GFP: histogram of GFP signal of EPCAM-APC+ cells (epithelial cells were analyzed for GFP signal)

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