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Reporting Summary

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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						
	X 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.						
\boxtimes	A description of all covariates tested						
	igtarrow A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons						
	A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (e.g. confidence intervals)						
\boxtimes	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>						
\ge	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings						
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes						
\boxtimes	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), 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	ImSpector v5.0222.0 (LaVision) was used to collect two-photon imaging data.						
Data analysis	Fiji (ImageJ v1.51s, NIH), Imaris v9.1.2 (BitPlane) and Prism 6 (GraphPad) were used to analyze the data.						

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

Statistics source data for Fig. 3c and Supplementary Fig. 1b have been provided as Supplementary Table 1. All the data that support the findings of this study are available from the corresponding author upon 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 <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>

Life sciences study design

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

Sample size	The samples sizes were chosen based on previous studies with similar methodologies.
Data exclusions	Hair follicles without enough resolution were excluded from the analyses.
Replication	All attempts at replication were successful.
Randomization	Mice from experimental and control groups were randomly selected for live imaging experiments.
Blinding	No blinding was used since the control and experimental mice can be distinguished by the distinct cell behaviors or fluorescent reporters.

Reporting for specific materials, systems and methods

Materials & experimental systems **Methods** n/a Involved in the study n/a Involved in the study Unique biological materials \boxtimes ChIP-seq \boxtimes Antibodies Flow cytometry \boxtimes Eukaryotic cell lines MRI-based neuroimaging \times \times Palaeontology Animals and other organisms Human research participants Antibodies

Antibodies used	Guinea pig anti-K31 (polyclonal, 1:50, Progen, GP-hHa1), rabbit anti-GATA3 (monoclonal [EPR16651], 1:250, Abcam, ab199428), chicken anti-GFP (polyclonal, 1:1000, Abcam, ab13970), rabbit anti-Ki67 (polyclonal, 1:200, Abcam, ab15580), rat anti-BrdU (monoclonal [BU1/75 (ICR1)], 1:200, Abcam, ab6326) were used in this study
Validation	Antibody validation information can be found on manufacturers' website. anti-K31 (https://www.progen.com/anti-acidic-hair-keratin-k31-guinea-pig-polyclonal-serum.html), anti-GATA3 (http://www.abcam.com/gta3-antibody-epr16651-chip-grade-ab199428.html), anti-GFP (http://www.abcam.com/gfp-antibody-ab13970.html), anti-Ki67 (http://www.abcam.com/ki67-antibody-ab15580.html), anti-BrdU (http://www.abcam.com/brdu-antibody-bu175-icr1-ab6326.html). For mouse hair follicle-specific staining, anti-K31 and anti-GATA3 have been validated before (Yang, Cell, 2017; Brown, Nature, 2017). anti-GFP, anti-Ki67 and anti-BrdU are routinely used in the lab (Deschene, Science, 2014; Brown, Nature, 2017).

Animals and other organisms

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

Laboratory animals

K14-H2BGFP, pTRE-H2BGFP (Tumbar et al., 2004), K14-actinGFP (Vaezi et al., 2002), Lef1-RFP (Rendl et al., 2005) and K14-Cre (Vasioukhin et al., 1999) mice were obtained from E. Fuchs. β-cateninflox(Ex3) (Harada et al., 1999) mice were obtained from M.Taketo. K14-H2BmCherry (Mesa et al., 2015) and K14-H2BPAmCherry (Rompolas et al., 2016) mice were generated in the lab and described previously. Lgr5-CreER (Barker et al., 2007), Hopx-CreER (Takeda et al., 2013), Shh-CreER (Harfe et al., 2004), R26flox-STOP-tdTomato (Madisen et al., 2010), R26-flox-STOP-tTA (Wang et al., 2008), tetO-Cdkn1b (Pruitt et al., 2013), TCF-H2BGFP (Ferrer-Vaquer et al., 2010) and mTmG were obtained from The Jackson Laboratory. pTRE-dNβCatGFP was generated by the Yale Transgenic Facility. Mice were bred to a mixed albino background. Mice between postnatal day 20 to 35 were used for experiments. Both genders were analyzed.

	Wild	animals	
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The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.