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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.

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	Intravital imaging was permormed on an Olympus FVMPE-RS multiphoton with FluoView software https://www.olympus-lifescience.com/en/laser-scanning/fvmpe-rs/ 3D confocal imaging was performed on Zeiss 880 microscope with Zen black software https://www.zeiss.com/microscopy/int/products/microscope-software.html
Data analysis	Imaris versions 8.2, 8.4 or 9.5 (Bitplane) https://imaris.oxinst.com/ Prism 8 (GraphPad) https://www.graphpad.com/scientific-software/prism/ FIJI/ImageJ https://imagej.nih.gov/ij/ ZEN Black (Zeiss) https://www.zeiss.com/microscopy/int/products/microscope-software.html Adobe After Affects CC 2018 https://www.adobe.com/ Adobe Media Encoder CC 2018 https://www.adobe.com/

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. 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

Data in Figure 5a and Figure 5b is from Dawson et al., 2020, Nature Cell Biology https://doi.org/10.1038/s41556-020-0505-0. The source data for Figure 6e is included in a source data file. All other data are too large to include in the paper but are available from the authors upon reasonable request.

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	Sample size was chosen to ensure the reproducibility of observations. Sample sizes were not predetermined by statistical analysis.
Data exclusions	No data were excluded, except for datasets where resolution was too low to clearly see cell behaviour.
Replication	All experimental findings were reliably reproduced. Most experiments were performed on at least three biological replicates in separate experiments. Exact n, indicating biological replicates, is stated in figure legends.
Randomization	Experiments were not randomised. Transgenic and floxed mice were studied on the basis of their genotype. Covariant control is not relevant as all mice were inbred, co-housed and compared with litter-mates or mice of identical strains.
Blinding	Investigators were not blinded during experiments or analysis of outcome. This was not possible due to the need for mice to be genotyped prior to experiments to ensure fluorescent reporter expression.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

MHCII Alexa Fluor 647 (rat, clone M5/114, Biolegend, Cat# 107617, 1/100)

CD45 APC (rat , clone 30-F11, BioLegend, Cat# 103112)

GFP (chicken polyclonal, Abcam, Cat# ab13970, 1/200)

RFP (rabbit, polyclonal, Rockland Cat# 600-401-379)

K8/18 (rat, clone TROMA-1, DSHB, Cat# TROMA-I)

anti-rabbit Alexa Fluor 555 (Invitrogen, Cat# A-31572, 1/400)

anti-chicken Alexa Fluor 488 (Invitrogen, Cat# A-11039, 1/400)

anti-rat Alexa Fluor 647 (Invitrogen, Cat# A21247, 1/400)

Validation

All antibodies used in this study have been validated and information and references can be found on the manufacturers website given below:

MHCII Alexa Fluor 647 (rat, clone M5/114, Biolegend, Cat# 107617, 1/100); https://www.biolegend.com/en-gb/products/alexa-fluor-647-anti-mouse-i-a-i-e-antibody-3135

GFP (chicken polyclonal, Abcam, Cat# ab13970, 1/200);https://www.abcam.com/gfp-antibody-ab13970.html

RFP (rabbit, polyclonal, Rockland Cat# 600-401-379); https://rockland-inc.com/store/Antibodies-to-GFP-and-Antibodies-to-RFP-600-401-379-O4L_24299.aspx

K8/18 (rat, clone TROMA-1, DSHB, Cat# TROMA-I); https://dshb.biology.uiowa.edu/TROMA-I

anti-rabbit Alexa Fluor 555 (Invitrogen, Cat# A-31572, 1/400); https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31572

anti-chicken Alexa Fluor 488 (Invitrogen, Cat# A-11039, 1/400); https://www.thermofisher.com/antibody/product/Goat-anti-Chicken-IgY-H-L-Secondary-Antibody-Polyclonal/A-11039

anti-rat Alexa Fluor 647 (Invitrogen, Cat# A21247, 1/400); https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21247

Animals and other organisms

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

Laboratory animals	Krt5-rtTA-IRES-GFP (K5) and Elf5-rtTA-IRES-GFP (Elf5-GFP) mice (Rios and Fu, 2014 PMID: 24463516) were generated at the Walter and Eliza Hall Institute of Medical Research. B6.Cg-Tg(tetO-cre) Cat# 006234 (TetCre) and B6.129P2(Cg)-Cx3cr1tm1Litt/J Cat# 005582 (Cx3cr1-GFP) mice were acquired from the Jackson Laboratory. Pr-Cre mice (Soyal, 2005 PMID: 15682389) were provided by John Lydon, Baylor College of Medicine, Unites States of America. R26R-Confetti (Confetti) (Snippert, 2010 PMID: 20887898) mice were provided by Hans Clevers, Hubrecht Institute, The Netherlands. Kaede mice (Tomura, 2008 PMID: 18663225) were provided by Yoshihiro Miwa, University of Tsukuba, Japan. All mice were C57BL/6 background, except for K5/TetCre/Confetti mice (mixed C57BL/6, FVB/N) and Elf5-GFP mice (FVB/N).
Wild animals	NA
Field-collected samples	NA
Ethics oversight	All mice were bred and maintained at the Walter and Eliza Hall Institute animal facility according to institutional guidelines. All experiments were approved by the Walter and Eliza Hall Institute Animal Ethics Committee.

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