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 | LabView2014 was used to synchronously control the laser power, photon counting , galvo mirror and motorized stage.

Data analysis | The raw data of confocal microscopy was processed by a Gaussian filter in ImageJ (1.53c).
3D rendering and multi-color fluorescence image merging were also performed in ImageJ (1.53c) or ImarisViewer 9.6.0 (Oxford Instruments).
The FWHM was measured in Origin 9.0.
The standard deviation and mean were calculated by Origin 9.0.
The PSF was calculated in Matlab (R2019b).

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 are provided with this paper. All data that support the findings of this study are presented in the main text and the Supplementary Information.

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 sizes were provided in the figure legends for each experiment. Sample sizes were chosen on the basis of previous publications (Nature Methods 2017, 14, 388–390; Nature Methods, 2018, 15,789–792; Nature Nanotechnology 2009, 4, 773–780; Nature Methods 2019, 16, 545–552).
Data exclusions	No data were excluded from the analyses.
Replication	All experiments were carried out at least 3 times successfully.
Randomization	Mice were randomly selected from cages, divided into groups for the studies.
Blinding	The investigators were blinded to group allocation during data collection and/or analysis

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 and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	1. Purified anti-mouse/human PNAd antibody (BioLegend; Clone: MECA-79; Cat. #.: 120802; Dilution: 100 ug/ 200 uL) 2. Purified anti-mouse CD169 antibody (BioLegend; Clone: 3D6.112; Cat. #.: 142402; Dilution: 50 ug/ 50 uL) 3. DRAQ7 (BioLegend; Cat. #.: 424001; Dilution: 1:100) 4. Anti-mouse CD3 antibody (BioXCell; Clone: KT3; Cat. #.: BE0261; Dilution: 200 ug/ 200 uL) The conjugation procedure can be found in Methods.
Validation	The antibodies used in this study were commercially available. There are publications using these antibodies. For purified anti-mouse/human PNAd antibody (Clone: MECA-79): (1) Thomas SN, et al. 2012. J. Immunol. 189:2181 (2) Hirakawa J, et al. 2010. J. Biol. Chem. 285:40864 For purified anti-mouse CD169 antibody (Clone: 3D6.112) : (1) Barral P, et al. 2010. Nat. Immunol. 11:303 (2) Klass M, et al. 2012. J. Immunol. 189:2414 For DRAQ7 (# 424001) (1) Pavesi A, et al. 2016. Sci Rep. 6: 26584 (2) Garvey C, et al. 2016. Sci Rep. 6:29752 For Anti-mouse CD3 antibody (Clone: KT3) (1) Reuter, A., et al. (2015). J Immunol 194(6): 2696-2705 (2) Sathe, P., et al. (2014). Immunity 41(1): 104-115.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	3-week-old BALB/c female mice were purchased from Charles River. The animals were housed on a 12 h: 12 h light: dark cycle (temperature: 20–25 °C, humidity: 50–65 %) in the Stanford University's Veterinary Service Center (VSC), and fed with food and water ad libitum as appropriate.
Wild animals	No wild animals were used in this study.
Field-collected samples	No field-collected samples were used.
Ethics oversight	All procedures performed on the mice were approved by Stanford University's Institutional Animal Care and Use Committee (IACUC). All experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The laboratory animal care program at Stanford is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

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