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 | |
|---|---|---|
| X | ☐ | The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| X | ☐ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| X | ☐ | The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| X | ☐ | A description of all covariates tested |
| X | ☐ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| X | ☐ | 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) |
| X | ☐ | For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| X | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| X | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| X | ☐ | 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 | *Code and software versions and descriptions used to collect the data in this study are listed in the section "Data processing and analysis tools/software" of the article* |
| Data analysis | *Code and software versions and descriptions used to analyze the data in this study are listed in the section "Data processing and analysis tools/software" of the article.* |

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

*The data shown in this study including all the raw imaging scans are available from the corresponding author upon 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 | *not applicable* |
| Data exclusions | *Animals that were negative for the expression of the fluorescent proteins after genotyping were excluded from the study.* |
| Replication | *The data shown in the study which are obtained by the described protocol were replicated at least 3 times and by at least 3 different operators.* |
| Randomization | *not applicable* |
| Blinding | *not applicable* |

# Behavioural & social sciences study design

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

| | |
|---|---|
| Study description | |
| Research sample | |
| Sampling strategy | |
| Data collection | |
| Timing | |
| Data exclusions | |
| Non-participation | |
| Randomization | |

# Ecological, evolutionary & environmental sciences study design

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

| | |
|---|---|
| Study description | |
| Research sample | |

Research sample

Sampling strategy

Data collection

Timing and spatial scale

Data exclusions

Reproducibility

Randomization

Blinding

Did the study involve field work? ☐ Yes ☐ No

## Field work, collection and transport

Field conditions

Location

Access & import/export

Disturbance

# 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: *All nanobodies used in this study are shown in Table 1 with their RRID that includes all relevant information. Used nanobodies are*

Validation: *tested at least 3 times and by 3 different operators. A protocol used to validate the nanobodies are described in Box 1 of the article.*

## Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Authentication

Mycoplasma contamination

Commonly misidentified lines
(See ICLAC register)

## Palaeontology and Archaeology

Specimen provenance

Specimen deposition

Dating methods

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

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

## Animals and other organisms

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

Laboratory animals

*Young mixed gender mice of the following lines are used in this study:Thy1-GFPM37 (and Thy1-Y FPH), CX 3CR1GFPI+ (B6.129 P-Cx3cr1tm1LittlJ ; Jackson Laboratory strain code 00558 228 ), Prox1-EGFP (Tg(Prox1-EGFP)KY 221GsatIMmucd; Mutant Mouse Resource and Research Centers strain code 031006-UCD) and PDGFRb-EGFP (Mouse Genome Informatics strain code 4847 307 ), C57 BLI 6 mouse transplanted with murine syngeneic R254 pancreatic cancer cells expressing eGFP for 38 days, an adult Emx1-Cre x RΦ GT. mice64 ,65 inj ected with EnvA-pseudotyped G-deleted rabies virus expressing GFP (SADB19 66) in the neocortex.*

Wild animals

*This study does not involve wild animals*

Field-collected samples

*This study does not involve Field-collected samples*

Ethics oversight

*Animal experiments followed European directive 2010I63IEU for animal research and were approved by the Institutional Animal Care and Use Committees (IACUC) of Technische Universität München and the ethical review board of the government of Upper Bavaria (Regierung von Oberbayern, Munich, Germany) and UK Home office. Experiments were conformed to Institutional guidelines in Klinikum der Universität München/Ludwig Maximilian University of Munich).*

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

## Human research participants

Population characteristics

Policy information about studies involving human research participants

Recruitment

Ethics oversight

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

## Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

Study protocol

Data collection

Outcomes

# Dual use research of concern

Policy information about dual use research of concern

## Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

| No | Yes | |
|---|---|---|
| x | ☐ | Public health |
| x | ☐ | National security |
| x | ☐ | Crops and/or livestock |
| x | ☐ | Ecosystems |
| x | ☐ | Any other significant area |

## Experiments of concern

Does the work involve any of these experiments of concern:

| No | Yes | |
|---|---|---|
| x | ☐ | Demonstrate how to render a vaccine ineffective |
| x | ☐ | Confer resistance to therapeutically useful antibiotics or antiviral agents |
| x | ☐ | Enhance the virulence of a pathogen or render a nonpathogen virulent |
| x | ☐ | Increase transmissibility of a pathogen |
| x | ☐ | Alter the host range of a pathogen |
| x | ☐ | Enable evasion of diagnostic/detection modalities |
| x | ☐ | Enable the weaponization of a biological agent or toxin |
| x | ☐ | Any other potentially harmful combination of experiments and agents |

# ChIP-seq

## Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
*May remain private before publication.*

Files in database submission

Genome browser session
(e.g. UCSC)

## Methodology

Replicates

Sequencing depth

Antibodies

Peak calling parameters

Data quality

Software

## 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

Instrument

Software

Cell population abundance

Gating strategy

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

## Magnetic resonance imaging

### Experimental design

Design type

Design specifications

Behavioral performance measures

### Acquisition

Imaging type(s)

Field strength

Sequence & imaging parameters

Area of acquisition

Diffusion MRI ☐ Used ☐ Not used

### Preprocessing

Preprocessing software

Normalization

Normalization template

Noise and artifact removal

Volume censoring

## Statistical modeling & inference

Model type and settings

Effect(s) tested

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference
(See Eklund et al. 2016)

Correction

## Models & analysis

| n/a | Involved in the study |
|---|---|
| ☐ | ☐ Functional and/or effective connectivity |
| ☐ | ☐ Graph analysis |
| ☐ | ☐ Multivariate modeling or predictive analysis |

Functional and/or effective connectivity

Graph analysis

Multivariate modeling and predictive analysis