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	Confocal imaging was conducted with Zeiss LSM 880 laser scanning confocal microscope and Zen software (v3.1). Calcium imaging: GECIquant (v1.0), ImageJ (v1.54f), and Clampfit (v10.7) were used to analyze calcium signals. FACS-sorting was performed using a Becton Dickinson FACSaria II with FACSDiva software. RNA-seq was performed on Illumina Nextseq system suite. Images in schematics were created using Biorender.com.
Data analysis	ImageJ (v1.54f) was used for confocal image analysis. Clampfit (10.7) was used for electrophysiological data analysis. The following were used for calcium image analysis: GECIquant, ImageJ (v1.54f), Clampfit (10.7). The following were used for RNA-seq and bioinformatic analyses: RNA-seq data was processed using fastQC (v0.11.7), MultiQC (v1.6), STAR (v2.5.0a), HOMER (v4.10), and DESeq2 (v1.30.1). Graphpad Prism (v10) was used for all statistical analysis.

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 RNA-seq data are available at GEO with accession ID GSE254016. The analyzed RNA-seq data are provided as Extended data Excel file 1.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

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	No formal estimates of statistical power were done. Sample sizes were based on previous publications using similar techniques. Animal numbers were minimized to conform to ethical guidelines while maintaining adequate numbers to derive accurate measurements.
Data exclusions	There were no data exclusions.
Replication	All attempts at replication were successful. Mice from multiple litters were used to attain at least 3 biological replicates per group for each measurement.
Randomization	Mice were randomly allocated to groups when possible. Where dependent on genotype, mice from multiple litters were randomly selected to be used.
Blinding	Investigators were blinded to group allocation during experimentation and data 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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

The following primary antibodies were used: rat anti-c-Fos (1:5000; Synaptic Systems, 226 017), guinea pig anti-Cre (1:1000; Synaptic Systems, 257 004), chicken anti-GFAP (1:1000; abcam, ab4674), rabbit anti-GFAP (1:1000; DAKO, Z0334), rabbit anti-HA (1:500; Roche, 11867423001), rabbit anti-NeuN (1:2000, Millipore, MABN140), rabbit anti-NFIA (1:500; Sigma, HPA006111), rabbit anti-RFP (1:500; Rockland, 600-401-379), and rabbit anti-Sox9 (1:500; Millipore, AB5535). The following secondary antibodies were used: Alexa Fluor 488-conjugated donkey anti-chicken (1:500; Jackson ImmunoResearch, 703-545-155), Alexa Fluor 647-conjugated donkey anti-chicken (1:500; Jackson ImmunoResearch, 703-605-155), Alexa Fluor 594-conjugated donkey anti-guinea pig (1:500; Jackson ImmunoResearch, 706-585-148), Alexa Fluor 488-conjugated donkey anti-rabbit (1:500; Jackson ImmunoResearch, 711-545-152), Alexa Fluor 594-conjugated donkey anti-rabbit (1:500; Jackson ImmunoResearch, 711-585-152), Alexa Fluor 647-conjugated donkey anti-rabbit (1:500; Jackson ImmunoResearch, 711-605-152), Alexa Fluor 488-conjugated donkey anti-rat (1:500; Jackson ImmunoResearch, 712-545-153), Alexa Fluor 594-conjugated donkey anti-rat (1:500; Jackson ImmunoResearch, 712-585-153), Alexa Fluor 647-conjugated donkey anti-rat (1:500; Jackson ImmunoResearch, 712-605-153).

Validation

Antibodies were validated by manufacturers and used in previously published work. Appropriate primary antibody dilutions were determined by testing multiple dilutions on sample mouse brain tissue. Secondary antibody only controls were used to confirm lack of signal in the absence of primary antibodies.
Rat anti-c-Fos Synaptic Systems 226 017: Manufacturer states this is a monoclonal antibody validated for use in immunostaining mouse tissue. Clone number 108B5H5. Immunogen is a synthetic peptide corresponding to residues near the amino terminus of rat c-Fos (UniProt Id: P12841). Previously used in PMID: 36289226.
Guinea pig anti-Cre recombinase Synaptic Systems 257 004: Manufacturer states this is a polyclonal antibody validated for use in immunostaining mouse tissue. Immunogen is full length recombinant Cre-recombinase from Bacteriophage P1 (UniProt Id: P06956). Previously used in PMID: 32991831.
Chicken polyclonal anti-GFAP Abcam ab4674: Manufacturer states immunogen is recombinant full length protein corresponding to Human GFAP. Isotype 1 expressed in and purified from E. coli. Validated for immunohistochemistry in mouse brain tissue. Used in PMID: 37393623.
Rabbit polyclonal anti-GFAP Dako Z0334: Manufacturer states immunogen is GFAP isolated from cow spinal cord. The antibody has been solid-phase absorbed with human and cow serum proteins. In crossed immunoelectrophoresis using 50 µL antibody per cm2 gel area, no reaction with 2 µL human plasma and 2 µL cow serum is observed. The antibody shows one distinct precipitate (GFAP) with cow brain extract. GFAP shows 90-95% homology between species, and, as demonstrated by immunohistochemistry, the antibody reacts strongly with human GFAP. Used for immunohistochemistry in mouse brain tissue in PMID: 33910014.
Rabbit anti-HA Roche 11867423001: Manufacturer states this antibody is a monoclonal antibody to the HA-peptide (clone 3F10). Recognizes the HA peptide sequence (YPYDVPDYA), derived from the influenza hemagglutinin protein (immunogen is amino acids 98-106). Validated for use in immunocytochemistry.
Rabbit monoclonal anti-NeuN Millipore MABN140: Manufacturer states validated for use in immunohistochemistry in mouse brain tissue. Used in PMID: 35177618.
rabbit anti-NFIA Sigma HPA006111: Sequence of immunogen: LKSVEDEMDSPGEEPFYTGQGRSPGSGSQSSGWHEVEPGMPSPTTLKKSEKSGFSSPSPSQTSSLGTAFTQHHRPVITGPRASPHATPSTLHFPTSPIIQQ. Validated for use in immunohistochemistry. Previously used in PMID: 32320644.
Rabbit anti-RFP Rockland 600-401-379: Manufacturer states the immunogen is a Red Fluorescent Protein fusion protein corresponding to the full-length amino acid sequence (234aa) derived from the mushroom anemone Discosoma. This product was prepared from monospecific antiserum by immunoaffinity chromatography using Red Fluorescent Protein (Discosoma) coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities. Validated for immunostaining.
Rabbit anti-Sox9 Millipore AB5535: Manufacturer states the immunogen is a KLH-conjugated linear peptide corresponding to the C-terminal sequence of human Sox9. Validated for use in immunohistochemistry. Previously used in PMID: 32320644.
All secondary antibodies (Jackson Immunoresearch) were purified from antisera by immunoaffinity chromatography using antigens coupled to agarose beads. These antibodies display minimal cross reactivity to off-target species.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

The following transgenic mouse strains were used: Fos-flox (037115-JAX), Rosa-CAG-LSL-tdTomato (Ai14; Jackson Laboratory, 007914), Rosa-CAG-FSF-tdTomato (Ai65F; Jackson Laboratory, 032864), Aldh1l1-CreER (Jackson Laboratory, 029655), Aldh1l1-GFP (RRID:MMRRC_011015-UCD), and NFIA-flox. All mice were on a C57/b6 background.

Wild animals	No wild animals were used in this study.
Reporting on sex	Mice of both sexes were used. Data was not stratified nor analyzed based on sex.
Field-collected samples	No field-collected samples were used.
Ethics oversight	Experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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	Mice were perfused with cold saline and hippocampi were dissected on ice. Dissected tissue was dissociated as previously described (PMID: 28166219).
Instrument	BD FACSAria II
Software	BD FACSDiva software
Cell population abundance	Enrichment of astrocytes and de-enrichement of neurons were confirmed by qPCR of cDNA ibraries prepared from sorted samples.
Gating strategy	Singlet gating was done based on forward and side scatter. Singlet events were assessed for GFP and tdTomato fluorescence. Non-fluorescent brain tissue was used as a negative control to set the double negative population gate. We also performed single color controls to establish ideal voltage, potential compensation, and gating. The GPF signal was detected with a 488nm laser, through a 505nm long pass filter, and 530/30 detector. tdTomato signal was detected with 561nm laser, through a 570 long pass filter, and 585/51 detector.

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