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.

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
- [x] Confirmed
  - 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.
  - 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 statistics 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
  - 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 | Lickometer (Dialog Instruments), Leica Application Suite X 3.5.5.19976 and 10x Genomics Cell Ranger 3.0.1. were used for data collection. |
| Data analysis   | Custom R code, Custom Python 3 code and GraphPad Prism 8 were used to analyze data. Code is available from the corresponding author upon reasonable request. ScRNA-seq data were processed in R 3.5.1 using the following analysis and data visualization packages: Seurat v.3.0.3.9019, ggplot2 3.2.0, viridis 0.5.1., scales 1.0.0., spatstat 1.62-2, Matrix 1.2-14, dplyr 0.8.3, cowplot 1.0.0, psych 1.9.12.31. |

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

The behavioral and histology data that support the findings are available from the corresponding author upon reasonable request. Raw and fully processed scRNA-seq data is available at the NCBI Gene Expression Omnibus (GEO accession # GSE154048).

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 nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size
No statistics to determine sample size were used. Sample size is similar to recent papers in behavioural neuroscience (Augustine, V. et al. Nature 555, 204-209, 2018 and Lee, S. et al. Nature 568, 93-97, 2019)

Data exclusions
For behavioral experiments, all data were included after verification of virus expression in the target brain area. For single cell RNA-seq experiments common thresholding to remove doublets and unhealthy cells was implemented.

Replication
We verified all experiments and analyses by replicating them by more than two lab members. All of the replication attempts were successful.

Randomization
No randomization was used for behavioral assays. All solutions were presented in an alternate fashion. In our pilot studies, we randomized the order and found no difference in the results.

Blinding
No blinding was used for data collection. All data were analyzed using an automated software that calculates preference to each solution. Thus, randomization is less relevant in this study.

Reporting for specific materials, systems and methods

Materials & experimental systems
n/a
- Involved in the study
- ☑ Unique biological materials
- ☑ Antibodies
- ☑ Eukaryotic cell lines
- ☑ Palaeontology
- ☑ Animals and other organisms
- ☑ Human research participants

Methods
n/a
- Involved in the study
- ☑ ChIP-seq
- ☑ Flow cytometry
- ☑ MRI-based neuroimaging

Antibodies

Antibodies used
- rabbit anti-c-Fos (1:500, Cell Signaling, #2250), chicken anti-GFP (1:1000, Abcam, ab13970, Lot# GR3190550-1), rabbit anti-ETV1 (1:500, Abcam, ab81086), sheep anti-Foxp2 (1:2000, R&D systems, AF5647, Lot# CCUB0109061), and rat anti-mCherry (1:500, Invitrogen, 16D7). For secondary antibodies we used donkey-anti-rabbit-Cy3 (711-165-152, Lot# 144186), donkey-anti-rabbit-A488 (711-545-152, Lot# 147626), donkey-anti-chicken-A488 (703-545-155, Lot# 147805), donkey-anti-sheep-A488 (713-545-147, Lot# 144916), donkey-anti-rat-Cy3 (712-165-150, Lot# 146156). All secondary antibodies were used at 1:500 dilution and were obtained from Jackson Immunoresearch.

Validation
All antibodies were purchased from vendors in the USA. These products are normally quality controlled at the company.
Nevertheless, we characterized and validated their signals in the brain regions known to express these genes prior to experiments.

### Animals and other organisms

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

<table>
<thead>
<tr>
<th>Category</th>
<th>Details</th>
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<tbody>
<tr>
<td>Laboratory animals</td>
<td>The following mice were purchased from the Jackson Laboratory: C57BL/6J, stock number 00064; Ai14, stock number 007914; Ai3, stock number 007903. Rxp1-P2A-Cre line was prepared in J. Ngai lab (UC Berkeley). Trap2 mice were a gift from Liqun Luo (Stanford University). PDYN-Cre mice were provided by B. Lowell (Harvard Medical School) and M. Krashes (NIH).</td>
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<tr>
<td>Wild animals</td>
<td>No wild animals were used in the study.</td>
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<tr>
<td>Field-collected samples</td>
<td>No field collected samples were used in the study.</td>
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