natureresearch

Corresponding author(s): Robert E., Campbell

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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main

Statistical parameters

| text | text, or Methods section). | | | | | |
|-------------|---|--|--|--|--|--|
| n/a | onfirmed | | | | | |
| | 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 | | | | | |
| \boxtimes | 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. | | | | | |
| \boxtimes | A description of all covariates tested | | | | | |
| \boxtimes | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons | | | | | |
| | A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (e.g. confidence intervals) | | | | | |
| \boxtimes | For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable</i> . | | | | | |
| \boxtimes | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings | | | | | |
| \boxtimes | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes | | | | | |
| \boxtimes | Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), 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 Molecular Devices MetaMorph and MetaFluor 7.7, NIS-Elements AR software, LabView, Andor Solis 4.21, OLYMPUS FLUOView 3000

Graphpad Prism 6.0 and 7.0, Origin9, Microsoft Excel, Clampfit 10.7, MatLab2017b, ImageJ, MetaFluor 7.7

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

Data analysis

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

One page 15 of the Supplementary Material we state: "Data Availability. Gene sequence data will be deposited in GenBank with accession codes that are TBD. Plasmids will be distributed via Addgene according to the terms of the Uniform Biological Material Transfer Agreement. Source data for Figs. 1-2, and Supplementary Figs. 5-7, 9, 11 will be included in the final version of the paper."

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 <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>

Life sciences study design

| All studies must dis | sclose on these points even when the disclosure is negative. |
|----------------------|--|
| Sample size | On page 9 of Supplementary Material we state: "No statistical methods were used to estimate sample size for animal studies throughout. " As note Dell et al ILAR. J (2002) and recommended by the NIH, "In experiments based on the success or failure of a desired goal, the number of animals required is difficult to estimate" As noted in the aforementioned paper, "The number of animals required is usually estimated by experience instead of by a formal statistical calculation, although the procedures will be terminated [when the goal is achieved]." |
| | On page 15 of the Supplementary Material we include the following statement: "Statistical analysis. All data are expressed as mean ± s.d or mean ± s.e.m, as specified in figure legends. Box plots with notches19 are used for Figs. 1g, 2d, and Supplementary Figs. 7b, 12a and 13c. In these plots, the narrow part of notch is the median; the top and bottom of the notch is the 95% confidence interval of the median; the horizontal line is the mean; the top and bottom horizontal lines are the 25% and 75% percentiles for the data; and the whiskers extend 1.5× the interquartile range from the 25th and 75th percentiles. Sample sizes (n) are listed with each experiment. No samples were excluded from analysis and all experiments were reproducible. No randomization or blinding was used." |
| Data exclusions | P31. The P0 pups were screened for corresponding fluorescence, negative pups were excluded for further experiments. |
| Replication | P33. All attempts at replication were successful. |
| Randomization | P33. No randomization or blinding was used |
| Blinding | P33. No randomization or blinding was used |
| | |

Reporting for specific materials, systems and methods

| erials & experimental systems | Methods | |
|-------------------------------|--|--|
| Involved in the study | n/a | Involved in the study |
| Unique biological materials | \ge | ChIP-seq |
| Antibodies | \ge | Flow cytometry |
| Eukaryotic cell lines | \ge | MRI-based neuroimaging |
| Palaeontology | | • |
| Animals and other organisms | | |
| Human research participants | | |
| | Unique biological materials Antibodies Eukaryotic cell lines Palaeontology Animals and other organisms | Involved in the study n/a Unique biological materials Image: State |

Eukaryotic cell lines

| Policy information about <u>cell lines</u> | |
|---|--|
| Cell line source(s) | HeLa (ATCC), HEK293FT (Thermo Fisher Scientific), MIN6 (Miyazaki laboratory, Osaka University) |
| Authentication | Cell lines were not authenticated |
| Mycoplasma contamination | MIN6 cells were tested weekly for Mycoplasma using DNA staining. Other cell lines were not tested. |
| Commonly misidentified lines (See <u>ICLAC</u> register) | HEK293FT cells were used for production of lentivirus due to the following advantages: fast-growing, high transfection efficientcy and tolerance of high levels of proteins. |

Animals and other organisms

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

Laboratory animals Pages 4 and 5 of Supplementary material. We state: "Animal care. For experiments performed at Massachusetts Institute of

| Laboratory animals | Technology (MIT), all methods for animal care and use were approved by the MIT Committee on Animal Care and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Four time pregnant Swiss Webster mice (Taconic) were used for this study, as were five C57BL/6 mice (Taconic), ages 4–12 weeks. Mice were used without regard to gender. For experiments performed at Technical University of Munich, all animal in vivo experimentation was done in full compliance with the institutional guidelines of the Institute for Biological and Medical Imaging and with approval from the Government District of Upper Bavaria. A total of 9 mice were used for these experiments: three female FOXN1 nude mice that were injected with the NIR-GECO1 virus; three female Black6 (C57BL/6J) mice transgenically expressing GCaMP6s; and three more mice (two female FOXN1 and one female Black6) that were injected with PBS as negative controls. All experiments at University of Alberta for obtaining the cortical neurons were approved by the University of Alberta Animal Care and Use Committee and carried out in compliance with guidelines of the Canadian Council for Animal Care and the Society for Neuroscience's Policies on the Use of Animals and Humans in Neuroscience Research. For experiments at HHMI Janelia Research Campus, all surgical and experimental procedures were in accordance with protocols approved by the HHMI Janelia Research Campus Institutional Animal Care and Use Committee and Institutional Biosafety Committee." |
|-------------------------|---|
| Wild animals | The study did not involve wild animals. |
| Field-collected samples | The study did not involve sample collected from the field. |