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# 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.

## 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<br>*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<br>*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 | AMT Capture Engine version 7.00 was used for electron microscopy data collection, cellSense version 1.16 was used for bright-field light microscopy data collection, and ZEN verison 2.3 SP1 was used for confocal fluorescence light microscopy data collection. |
| Data analysis | Fiji/ImageJ version 2.0.0-rc-68/1.52h was used for all image analysis except TrakEM2 and 3D Viewer. Fiji/ImageJ version 2.0.0-rc-43/1.51h was used for TrakEM2 and 3D Viewer. |

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. 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 authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files.

# 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

# Life sciences study design

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

| | |
|---|---|
| Sample size | No statistical methods were used to pre-determine sample sizes but our sample sizes were similar to those reported in previous studies. |
| Data exclusions | Micrographs were excluded from analysis if they were out-of-focus, had inappropriate background correction, or had debris or other artifacts obscuring the field of view. These exclusion criteria are conventional and comparable to other electron microscopy studies. |
| Replication | Most experiments were replicated for 1-5 times after the initial experiments. All replications were successful over the course of data collection. |
| Randomization | Samples were not randomized because there was no quantitative comparison between experimental and control groups. |
| Blinding | Investigators were not blinded to genotype/conditions because blinding is not relevant to the determination of best sample preparation parameters of the new technique. |

# 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 |
| ☐ | ☒ Animals and other organisms |
| ☒ | ☐ Human research participants |
| ☒ | ☐ Clinical data |

## Methods

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

## Antibodies

| | |
|---|---|
| Antibodies used | Rabbit anti-DsRed polyclonal (1:500, Takara Bio, 632496); mouse anti-NeuN, clone A60 (1:1000, Millipore, MAB377); goat anti-Rabbit IgG (H+L) highly cross-adsorbed, Alexa 546 (1:500, Thermo Fisher, A-11035); goat anti-Mouse IgG1 cross-adsorbed, Alexa 488 (1:500, Thermo Fisher, A-21121). |
| Validation | Rabbit anti-DsRed polyclonal was validated with Western blot by the manufacturer: hhttps://www.takarabio.com/assets/documents/Certificate%20of%20Analysis/632496-101717.pdf. Mouse anti-NeuN, clone A60 was validated with immunohistochemistry by the manufacturer: http://www.emdmillipore.com/US/en/product/Anti-NeuN-Antibody-clone-A60,MM_NF-MAB377?bd=1#anchor_COA. Goat anti-Rabbit IgG (H+L) highly cross-adsorbed, Alexa 546 was validated with immunocytochemistry by the manufacturer: https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11035. Goat anti-Mouse IgG1 cross-adsorbed, Alexa 488 was validated with immunocytochemistry by the manufacturer: https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG1-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21121. |

## Eukaryotic cell lines

Policy information about cell lines

| | |
|---|---|
| Cell line source(s) | HEK293T cells were obtained from ATCC. 129S4/SvJae ES cells (J1) were provided by the Boston Children's Hospital Mouse Gene Manipulation Core Facility. |
| Authentication | No authentication was performed. |
| Mycoplasma contamination | Cell lines were not tested for mycoplasma contamination. |
| Commonly misidentified lines (See ICLAC register) | HEK293T cells and 129S4/SvJae ES cells (J1) are not commonly misidentified lines. |

## Animals and other organisms

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

| | |
|---|---|
| Laboratory animals | Mus musculus of mixed strains, age and sex were used in the study. Age ranged from P21 to P35 (median P21). |
| Wild animals | The study did not involve wild animals. |
| Field-collected samples | The study did not involve field-collected samples. |
| Ethics oversight | All experiments using animals were approved by the Institutional Animal Care and Use Committee at Harvard Medical School. |

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