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
☐ 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 possible.
☐ 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: Custom written software in LabView 2016

Data analysis: Custom written software in Matlab 2018b

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 data that support the findings of this study are available from the corresponding author S.W.H. upon reasonable 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-list.pdf
Life sciences study design

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

**Sample size**
No sample size calculation was done. For each sample more than 4 cells from at least two coverslips were imaged. For Nup96 experiments, the coverslips were taken from different cell culture passage numbers and preparations for covering biological variations of the samples. The measurements resulted in low statistical variation.

**Data exclusions**
Only data that were obviously failing (like wrong microscope configuration or wrong manual focusing) were excluded. The exclusion criteria were pre-established.

**Replication**
All replications were successful.

**Randomization**
Samples were not randomized. Our experimental workflow did not allow/need randomization.

**Blinding**
No blinding was done. Our experimental workflow did not allow/need blinding.

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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

<table>
<thead>
<tr>
<th>Materials &amp; experimental systems</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>☑ Antibodies</td>
<td>☑ ChiP-seq</td>
</tr>
<tr>
<td>☑ Eukaryotic cell lines</td>
<td>☑ Flow cytometry</td>
</tr>
<tr>
<td>☑ Palaeontology</td>
<td>☑ MRI-based neuroimaging</td>
</tr>
<tr>
<td>☑ Animals and other organisms</td>
<td></td>
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<tr>
<td>☑ Human research participants</td>
<td></td>
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<tr>
<td>☑ Clinical data</td>
<td></td>
</tr>
</tbody>
</table>

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Eukaryotic cell lines

Policy information about [cell lines](#)

<table>
<thead>
<tr>
<th>Cell line source(s)</th>
<th>ATCC-HTB-96, Lot # 61074667. The cells can be obtained via Cell Line Services (CLS, clsgmbh.de, Nup96-SNAP #300444, Nup96-m Maple #300461).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Authentication</td>
<td>Cell lines were not further authenticated.</td>
</tr>
<tr>
<td>Mycoplasma contamination</td>
<td>Cell lines have been tested and are negative for mycoplasma contamination.</td>
</tr>
<tr>
<td>Commonly misidentified lines (See <a href="#">ICLAC register</a>)</td>
<td>No commonly misidentified cell lines were used.</td>
</tr>
</tbody>
</table>

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Animals and other organisms

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research

<table>
<thead>
<tr>
<th>Laboratory animals</th>
<th>PSD-95-HaloTag knock-in mice (C57Bl/6j background) were kindly provided by Seth G. N. Grent (Edinburgh University, United Kingdom) and bred in-house for several generations.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild animals</td>
<td>No wild animals were involved.</td>
</tr>
<tr>
<td>Field-collected samples</td>
<td>No samples collected from the field were used.</td>
</tr>
</tbody>
</table>

Ethics oversight

Cultures of hippocampal neurons were prepared in accordance with the Animal Welfare Law of the Federal Republic of Germany (Tierschutzgesetz der Bundesrepublik Deutschland, TierSchG) and the regulation about animals used in experiments (Tierschutzversuchsverordnung). Sacrificing of animals is not an experiment on animals according to TierSchG, so that no specific authorization or notification is required.

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