

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 & References](#) 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

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☒

The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement

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A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

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☒

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.

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A description of all covariates tested

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A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons

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☐

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)

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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.

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For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

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For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

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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

Software used (commercial): Nikon Elements, Zeiss Zen

Data analysis

Software used: Nikon N-STORM, Fiji/ImageJ. Custom Fiji/ImageJ code for data analysis is available at <https://github.com/deterrier/tab-repositories>.

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

Raw data from the data presented in the Figures of this manuscript are available from Figshare at the following link: <https://figshare.com/s/7d4262cd6d922add64ac>

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/hr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size

No sample-size calculations were performed. Number of replicates and individual data points are similar or above standards in the field.

Data exclusions

No data exclusion was performed

Replication

Fluorescence microscopy experiments were replicated independently (different neuronal cultures) at least 3 times. Extensive observation of electron microscopy samples was performed to ensure representative results. See Tables S1 and S2 of the manuscript for more details.

Randomization

No randomization was performed.

Blinding

No blinding was performed but quantification was realized using semi-automatic tracing scripts that minimize user variability and potential bias.

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

Methods

n/a

Involvement in the study

☐

☒

Antibodies

☒

☐

chIP-seq

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Flow cytometry

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MRI-based neuroimaging

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Animals and other organisms

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Human research participants

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Clinical data

Antibodies

Antibodies used

Rabbit polyclonal anti βIV-spectrin antibody (against residues 2237-2256 of human βIV-spectrin, 1:800 dilution for immunofluorescence IF, 1:20 for immunogold IG) was a gift from Matthew Rasband (Baylor College of Medicine, Austin, TX). Mouse monoclonal anti βIII-spectrin (against residues 2101-2189 of human βIII-spectrin, 1:100 for IF, 1:20 for IG) was from BD Biosciences (#612563). Chicken anti-map2 antibody was from abcam (#ab5392, 1:1000 for IF). Rabbit polyclonal anti-Alexa Fluor 488 antibody was from Thermo Fisher (A11094, 1:20 for IG). Mouse monoclonal antibodies anti ankym G (clone 106/65 and 106/36, 1:300 for IF) were from NeuroLab. Rabbit polyclonal anti-480-10a ankiG (residues 2735-2935 of rat 480-10a ankiG, 1:300 for IF, 1:100 for IG) was a gift from Yann Bennett (Duke University, Durham, NC). Rabbit anti phospho-Mycosin Light Chain 2 Thr18/Ser19 (pMLC) was from Cell Signaling Technologies (#9674, 1:50 for IF, 1:20 for IG).

Validation

Each antibody has been previously used in previously published studies, including our lab and is extensively validated.

Animals and other organisms

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

Laboratory animals

Neuronal culture were made from embryonic Wistar rats (both sexes).

Wild animals

No wild animals used.

Field-collected samples

No field-collected samples used.

Ethics oversight

The use of Wistar rats followed the guidelines established by the European Animal Care and Use Committee (B6/609/CEE) and was approved by the local ethics committee (agreement D13-055-8).

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