nature research

Corresponding author(s): Naoko Mizuno

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	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	For EM data collection - EPU (from FEI) For Cryo -Tomography,Data was collected using Serial -EM (http://bio3d.colorado.edu/SerialEM/). For Immunoflourescence,Cells were imaged on a Zeiss Axio Imager.M2 microscope with 20x or 40x objectives using the Zeiss Zen software. For DNA-PAINT imaging was carried out on an inverted Nikon Eclipse Ti microscope (Nikon Instruments) with the Perfect Focus System, applying an objective-type TIRF configuration with an oil-immersion objective (Apo SR TIRF 100x, NA 1.49, Oil).
Data analysis	For light microscopy - Zeiss Zen and Fiji V 1.0 (https://fiji.sc/) was used. For DNA-PAINT in addition Picasso software suite (https://github.com/jungmannlab/picasso) and the SOAX software (https://www.nature.com/articles/srep09081) was used. For electron microscopy - SPIDER(https://spider.wadsworth.org/), RELION (https://www2.mrc-lmb.cam.ac.uk/relion/), Frealign(grigoriefflab.janelia.org/frealign), customized software for microtubule analysis (PMID: 26424086) and for tomographic reconstruction IMOD (bio3d.colorado.edu/imod/) was used. Prism 7 and Microsoft Excel 15.35 were used for statistical analysis and graphical data presentation (https://www.graphpad.com/).

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

Cryo-EM structure of the protein complex is available through EMDB (or PDB) with the accession code: EMD-4188.
Additional tomographic images could be found in figshare.

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	For neuron experiments, primary neuron preps were performed from independent 3 mice and the neurons were grown on independent glass slides. The sample sizes were determined by counting ALL neurons on the entire slides. For TIRF analysis, triplicate experiments were performed for each conditions, and microtubules and asters (objects of interest) were counted from the entire view of the CCD camera. For cryo-EM analysis, sample sizes were determined by the number of the images acquired by given available time slots. Typically it would be an over-night session. For microtubule morphological analysis, sample sizes were determined by the number of images recorded. Typically > 50 images are recorded. In each image, ~10 microtubules were observed.
Data exclusions	There is no data excluded.
Replication	All attempts of replication of the biological experiments were successful. For quantification triplicates datasets (biologically and technical replicates) are collected.
Randomization	Randomization was not performed as all the data were included in the large data analysis.
Blinding	Neuron analysis were done both automatically as well as manually. For the manual analysis, investigators were blinded.

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	The information about dilution and manufacturer are mention in Supplementary Table 2
Validation	Validations are based on the datasheet from the manufacturer.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Mouse embryonic fibroblasts as described in Austen et al (DOI: 10.1038/ncb3268).
Authentication	No further authentication .
Mycoplasma contamination	Original cells are not mycoplasma contaminated.
Commonly misidentified lines (See ICLAC register)	Cells are not listed in the database of commonly misidentified cell lines.

Animals and other organisms

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

Laboratory animals	17.5 days embryo from C57Bl6/N mouse strain was used for the preparation of hippocampus neuron.
Wild animals	This study did not involve any wild animals.
Field-collected samples	This study did not involve any field-collected samples.