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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 *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 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	Data presented were either taken in a typical imaging facility on a Leica STED SP8 microscope (BIOP, EPFL), acquired on a custom-built standard microscope using Micromanager 1.4 or kindly provided by Prof. T. Huser (Bielefeld)-SIM figure 3, Prof. M. Sauer (Wurzburg) SIM figure 3, Dr. H. Deschout (EPFL Lausanne) SOFI figure 4 and Dr. J. Schmied and Prof. P. Tinnefeld (GATTAquant and LMU) for DNA-Origami nanoruler data..
Data analysis	The algorithm is implemented in MATLAB R2017b (Mathworks) and ImageJ 1.52. All the codes are made available on Github.

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

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors

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-flat.pdf

Life sciences study design

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

Sample size	we were not performing a biological study, so sample sizes were chosen to estimate the precision by calculating the standard deviation based on the number of images that we could acquire in reasonable experimental time
Data exclusions	no data was excluded unless it contained clear sample preparation artifacts in the case of STED microscopy (air bubble)
Replication	all attempts at replication were successful
Randomization	data was not split into different groups, we always used all images of a set of experiments
Blinding	data was not split into different groups

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	anti alpha-tubulin antibody (dilution 1:150, clone [DM1a] mouse monoclonal, ab7291, Lot GR310199-6, Abcam); anti alpha-tubulin antibody (dilution 1:100-1:200, clone B-5-1-2 mouse monoclonal ascites fluid, T5168 , Lot 047M4760V, Sigma-Aldrich) ; donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (0.005 mg ml-1, provided by histology core facility (EPFL), A-31571, Invitrogen); donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (0.005 mg ml-1 , A-21203, Lot 1820027, Invitrogen); donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (dilution 1:100, labeled with Abberior Flip 565 and purified as described in Materials and Methods, A16019, Lot 28-167-092613, Invitrogen), goat anti-mouse IgG Abberior Star 635P (0.005-0.01 mg ml-1, 2-0002-007-5, Lot 11042018Hp, Abberior), Anti-mouse IgG-Atto594 antibody produced in goat (0.01 mg ml-1, 76085, LotBCBW2434, SigmaAldrich), Atto490LS strepatavidin (0.01 mg ml-1, AD 490LS-61, Lot SA02S25, Atto-tec), Biotin-SP (long spacer) AffiniPure Donkey Anti-Mouse IgG (H +L) (dilution 1:200, provided by histology core facility (EPFL), 715-065-150, Jackson ImmunoResearch)
Validation	anti alpha-tubulin antibody (clone [DM1a] mouse monoclonal, ab7291, Abcam) used in HeLa cells, reactive for Homo sapiens (Human) according to manufacturer; anti alpha-tubulin antibody (clone B-5-1-2 mouse monoclonal ascites fluid, T5168 , Sigma-Aldrich) used in COS-7 cells, reactive for African green monkey according to manufacturer

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HeLa cells are from ATCC, COS-7 cells were a kind gift of the Manley lab (EPFL), MEF cells used in ref 29 were provided by Dr Luca Scorrano and U2OS cells used in ref 26 were cell line ACC785 from DSMZ
Authentication	none of the cell lines used were authenticated

Mycoplasma contamination	cell lines were not tested for Mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	no commonly misidentified cell lines were used