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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 our Editorial Policies 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	No collected data, all data were captured by our own structured illumination microscopy (SIM), spinning-disc confocal-based SIM (SD-SIM), stimulated emission depletion (STED) microscope, repetitive optical selective exposure (ROSE) microscope, and miniaturized two-photon microscope (MTPM), in which the details can be found in the Methods section. The DNA origami was designed using the caDNAno v2.2.0.
Data analysis	The version of sparse deconvolution software used in this manuscript (accompanied with example data and user manual) is available as Supplementary Software. The readily usable executable binary files for Windows (.EXE) and Mac (.APP) operating systems are at https://github.com/WeisongZhao/Sparse-SIM/releases/tag/v1.0.3. Moreover, the updating version of sparse deconvolution software can be found at https://github.com/WeisongZhao/Sparse-SIM. The adaptive median filter has been written as an ImageJ plug-in and can be found at https://github.com/WeisongZhao/AdaptiveMedian.imagej. All data processing was achieved using MATLAB 2017b and ImageJ v1.53g (and plugins: NanoJ-SQUIRREL, ClearVolume, PureDenoise, Adaptive_Median_Filter). All the Figures were prepared with MATLAB 2017b, ImageJ v1.53g, Microsoft Visio 2013, and OriginPro 2017, and videos were all produced with our self-written MATLAB library, which is available at https://github.com/WeisongZhao/img2vid.

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 and 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 images from Fig. 1, Fig. 4a, 4m, 4j, 6e, and Extended Data Fig. 6; and several templates for processing different types of structures along with example datasets can be found at https://github.com/WeisongZhao/Sparse-SIM/releases/tag/v1.0.3 or https://doi.org/10.5281/zenodo.5079743. All other data that support the findings of this study are available from the corresponding author on 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-flat.pdf

Life sciences study design

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

Sample size	The sample size (n) of each experiment is provided in the figure/table legends in the main manuscript and supplementary information files.
Data exclusions	No data was excluded from the analysis.
Replication	The number of repetitions for each experiment is provided in the figure/table legends in the main manuscript and supplementary information files.
Randomization	Samples were randomly assigned by independent persons.
Blinding	Data acquisition and analysis were being blinded to the experimental 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 and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Commercial primary antibodies: monoclonal rabbit anti-alpha-tubulin, EP1332Y, Abcam (ab52866); monoclonal mouse anti-Nup107, (Mab414), Abcam (ab24609); monoclonal mouse anti-Tom20, Tom20 (29), BD Biosciences (612278); monoclonal rat anti-tubulin, YL1/2, Abcam (ab6160). Commercial secondary antibodies: goat anti-rabbit secondary antibody, Alexa Fluor 488, Thermo (A-11070); goat anti-mouse secondary antibody, Alexa Fluor 594, Abcam (ab150120); goat anti-rat secondary antibody, Alexa Fluor 647, Abcam (ab150167).
Validation	The antibodies have been extensively used in previous studies, and our staining pattern was compared to existing literature and images published online. The details of these antibodies and their uses can be found in the Methods section. These labels include: monoclonal rabbit anti-alpha-tubulin, EP1332Y, Abcam (ab52866), https://doi.org/10.1038/s41592-020-01005-2; monoclonal mouse anti-Nup107, Mab414, Abcam (ab24609), https://doi.org/10.1128/mSphere.00709-19;

monoclonal mouse anti-Tom20, Tom20 (29), BD Biosciences (612278), https://doi.org/10.1038/s41467-021-22279-w;
monoclonal rat anti-tubulin, YL1/2, Abcam (ab6160), https://doi.org/10.7554/eLife.61669;

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	INS 1 cells were kindly provided by Dr. Ian Sweet, University of Washington Islet Core Facility (Sigma, SCC207) COS-7 cells were kindly provided by Professor Heping Cheng, Peking University (ATCC, CRL-1651) HeLa cells were kindly provided by Professor Heping Cheng, Peking University (ATCC, CCL-2)
Authentication	Cell lines were not authenticated
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No cell line listed by ICLAC was used.

Animals and other organisms

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

Laboratory animals	Animals were housed with 12-h light/dark cycle at 22 °C and free access to food and water. A male 3-5 months old Thy-1-GFP transgenic (C57BL6J) mouse was used.
Wild animals	We did not use any wild animals.
Field-collected samples	We did not use any field-collected samples.
Ethics oversight	All procedures were approved by the Animal Use and Care Committee of the Peking University(IACUC, No. IMM-chenLY) and complied with the standards of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International), including the animal breeding and experimental manipulation.

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