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Reporting Summary

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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	Super-resolution image series were acquired using Elyra Zen edition (Zeiss).
Data analysis	Single particle image series were processed using the PALM image reconstruction module of Elyra Zen edition (Zeiss). Trajectory generation fidelity was verified using ICY 1.9.5 and Imaris 8.4.1 (Bitplane) software. ImageJ 1.0 was used to render structured illumination image series. Matlab 9 (Mathworks) was used for trajectories analysis, as described in methods. Graph recosntruction from SPTs uses the DBSCAN algorithm implemented in scikit-learn obtained through the Anaconda distribution (Anaconda Inc.) v.4.3.8. Junction positions were determined and extracted from fSIM images using the AnalyzeSkeleton plugin from ImageJ 1.51 and junction trajectories were reconstructed with ICY 1.9.5.FLIM images were analysed using Backer and Hickl SPCI 7. Custom code generated for single particle tracking analysis and visualisation, as well as for ER network analysis of SIM images, can be obtained from Zenodo database along with experimental raw data examples, DOI: 10.5281/zenodo.1317630 and DOI: 10.5281/zenodo.1318129, respectively.

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

Source image-series data for Fig. 1e, Supplementary Fig. 2 and Fig 4e, f have been provided as supplementary video1, 2 and 8 - 10 respectively; and statistical information for Fig. 2 and supplementary Fig. 2 in supplementary table 1. All other data supporting the findings of this study are available from the corresponding authors on reasonable request. Custom code generated for single particle tracking analysis and visualisation as well as for SIM ER network analysis of SIM images can be obtained from Zenodo database along with experimental raw data examples, DOI: 10.5281/zenodo.1317630 and DOI: 10.5281/zenodo.1318129 respectively.

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	No statistical method was used to determine sample size. The sample size was sufficient to yield clear statistical significance. Maximal number of trajectories (tens of thousands) that can be handled using the available computational tool was sampled from the live cell time series.
Data exclusions	Cells with clear morphological appearance of necrosis/apoptosis in eye examination using light microscopy were excluded. For quality control purposes trajectories with less then three detection points were excluded from analysis, as described in methods.
Replication	All attempts at replication noted in figures were successful. All the reagents used are indicated, and the methods, including original techniques developed for the purpose of the current study, are described in detail.
Randomization	Cell for microscopy measurements were randomly selected from sample cultures in independent repeats.
Blinding	Mathematical analysis of most of the single particle trajectories data was performed by an investigator who was blind to the type of cell and organelle marker used in the analysed cells.

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK 293, COS-7and SH-SY5Y cells used in the present study were from ATCC.
Authentication	Cell authentication was based on morphological criteria, in case of SHS5Y5 cells their ability to undergo differentiation into neurons was observed.
Mycoplasma contamination	Cells were periodically tested for mycoplasma contamination and found negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.