

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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)
<input type="checkbox"/>	<input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted <i>Give P values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input type="checkbox"/>	<input checked="" type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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 was acquired using Leica Application Suite Advanced Fluorescence 2.7.3.9723 and NIS Elements 5.02.03
Data analysis	Custom code written in Matlab2017b and 2019b, Python 3.7.6, icy 1.9.5.1, Graphpad Prism 8 and Excel 2016 was used in this study. Mass spectrometry analysis was performed using MaxQuant 1.5.3.8 or 1.6.0.16.

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

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD015308. Image data are available from the corresponding author on reasonable 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 largest possible numbers of experiments were performed, taking into account the high number of target proteins (>100), and is well within the range of typical super-resolution imaging experiments. No formal sample size calculation was performed, as this would require an estimate of the effect size between two conditions, whereas we are only investigated a single condition here.
Data exclusions	No data were excluded
Replication	In general 3 independent experiments were performed, with several hundred synapses investigated per protein. All replications were successful. For a detailed list see Supplementary Table 4, for an example analysis please see Extended Data Figure 6.
Randomization	Not relevant for this manuscript, as only 1 experimental group was investigated.
Blinding	Blinding was not relevant to the study, because no conditions were compared, only 1 experimental group was investigated.

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	Please refer to the relevant part of the Methods, describing at length all antibodies
Validation	<p>ADAM22 mouse 500 Novus Biologicals NBP2-22425 Validated in 21</p> <p>Akt (pan) rabbit 400 Cell Signaling 4691 Validated in 37</p> <p>α/β-SNAP mouse 100 Jahn Laboratory 77.2 Validated in 23</p> <p>α-internexin rabbit 500 LSBio LS-B10413 Clear observation of internexin filaments, as expected from the literature. Low signal outside of filaments. Shown in Supplementary Data File 1.</p> <p>AP180 rabbit 100 Synaptic Systems 155 003 K.O. validated</p> <p>Ankyrin-G mouse 500 Neuromab AB_10673030, clone N106/36 Validated in 115</p> <p>APP mouse 100 Millipore MAB-348 Validated in 23</p> <p>Arc rabbit 1000 Synaptic Systems 156 003 K.O. validated</p> <p>β-2-spectrin mouse 100 BDBiosciences 612562 Validated in 14</p> <p>β-tubulin llama 100 Self-made See 38 Validated in 38</p>

β -3-Tubulin rabbit 500 Cell Signaling 5568 Validated in 39

BDNF rabbit 100 Biorbyt orb38809 Manufacturer, available at antibodypedia.com

Calbindin-D28K rabbit 500 Synaptic Systems 214 002 Control Antigen

Calcineurin A rabbit 1000 Synaptic Systems 387 002 Control Antigen

Calmodulin rabbit 100 Abcam ab45689 Validated in 23

Calreticulin rabbit 200 Cell Signaling 12238 Validated in 14

Calretinin rabbit 250 Novus Biologicals NBP1-88220 K.D. validated

CaMKII (alpha subunit, phosphorylated form) mouse 500 Abnova MAB6627 Validated in 40

CAPS1 rabbit 500 Abcam ab69797 Validated in 23

Cav1.3 rabbit 50 Alomone Labs ACC-311 Control Antigen

Cav2.1 rabbit 500 Synaptic Systems 152 203 K.O. validated

CDC42 rabbit 100 Thermo Scientific PA1-092 K.D. validated 41

Chromogranin A rabbit 500 Synaptic Systems 259 003 K.O. validated

Chromogranin B rabbit 500 Synaptic Systems 259 103 K.O. validated

Chromogranin C rabbit 250 Abcam ab12241 Validated in 42

Clathrin heavy chain mouse 100 BD Biosciences 610499 Validated in 23

Clathrin light chain mouse 1000 Synaptic Systems 113 011 Validated in 23

Cortactin mouse 500 Synaptic Systems 313 111 Validated in 43

DLGAP1 rabbit 50 Novus Biologicals NBP1-76911 Validated in 21

Dopamine receptor 1 rabbit 1000 Abcam ab40653 Control antigen

Dopamine receptor 2 rabbit 500 Merck AB5084P K.O. validated in 44

Drebrin1 mouse 100 Novus Biologicals NB100-1951 Validated in 45

Dynamin 1/2/3 mouse 100 BDBiosciences 610245 Validated in 23

ERp72 rabbit 100 Cell Signaling 5033 Validated in 21

GAD65 mouse 500 Synaptic Systems 198 111 Control antigen

GFAP mouse 500 Synaptic Systems 173 011 Control antigen

GluK1 rabbit 100 Alomone AGC-008 Control antigen

GluN1 mouse 1000 Synaptic Systems 114 011 Validated in 46

GluN2A mouse 100 NeuroMab 75-288 Validated in 21

GluN2B mouse 100 NeuroMab 75-101 Validated in 21

GluR1 rabbit 500 Synaptic Systems 182 003 K.O. validated

GluR2 rabbit 100 Alomone Labs AGC-005 Control antigen

GluR3 mouse 100 Invitrogen 32-0400 Validated in 21

GRIP1/2 rabbit 100 Synaptic Systems 151 003 Control antigen

Homer1 mouse 500 Synaptic Systems 160 011 Control antigen

Homer1 rabbit 500 Synaptic Systems 160 003 Control antigen

Homer2 rabbit 500 Synaptic Systems 160 203 Validated in 47

Homer3 rabbit 250 Synaptic Systems 160 303 Control antigen

HSC70 mouse 100 Santa Cruz sc-7298 Control antigen

Iba1 guinea pig 500 Synaptic Systems 234 004 Control antigen

IGF-1 Receptor rabbit 300 Cell Signaling 3027 Validated in 21,48

KCNJ2 rabbit 100 Novus Biologicals NBP1-95482 Validated in 21

Kv1.1 rabbit 100 Thermo Scientific PA5-19593 Validated in 21

Kv2.1 rabbit 500 Synaptic Systems 231 002 Control antigen

LNGFR rabbit 1000 Cell Signaling 8238 Validated in 49

m-AChR-1 rabbit 100 Novus Biologicals NBP1-87466 Validated in 50

MAP2 rabbit 1000 Synaptic Systems 188 002 Control antigen

mGluR1 α rabbit 250 Abcam ab51314 Validated in 51

mGluR2 rabbit 100 Abcam ab150387 Validated in 52

mGluR5 rabbit 100 Abcam ab76316 Validated in 53

myosin5a rabbit 200 Sigma-Aldrich M5062 Validated in 21

Na β 1 rabbit 50 Alomone Labs ASC-041 Control antigen

Na⁺/K⁺ ATPase mouse 1000 Thermo Scientific MA3-915 Validated in 21

Nav1.1 rabbit 100 Merck 06-811 Validated in 54

Nav1.3 rabbit 250 Alomone Labs ASC-004 Control antigen

Neurofilament H rabbit 1000 LSBio LS-C143052 Clear observation of filaments, as expected from the

literature. Shown in Supplementary Data File 1. Similar pattern to Neurofilament L. Similar STED fluorescence pattern to multiple previous STED publications 55,56

Neurofilament L rabbit 500 Synaptic Systems 171 002 Validated in 14

nNOS rabbit 100 Thermo Scientific PA1-033 Validated in 21

NSF rabbit 500 Synaptic Systems 123 002 Validated in 23

Olig2 rabbit 500 Synaptic Systems 292 003 Presence only in subset of cells with oligodendrocyte morphology. Missing in all other cells. Data analyzed in Extended Data Fig. 2.

Parvalbumin rabbit 500 Swant PV25 K.O. validated

PSD93 rabbit 300 Invitrogen 34-4700 Validated in 57

PSD95 rabbit 100 Cell Signaling 3450 Validated in 14

Rab11 rabbit 100 Cell Signaling 3539 Validated in 21

Rab3 mouse 100 BD Biosciences 610379 Validated in 23

Rab4 mouse 100 BD Biosciences 610888 Validated in 58

Rab5 mouse 100 Jahn Laboratory cl. 621.3 Validated in 21

Rab7 rabbit 100 Cell Signaling 9367 Validated in 21

Rab9 rabbit 100 Cell Signaling 5118 Validated in 59–61

Rapsyn rabbit 100 Atlas Antibodies HPA039475 Validated in 21

Ribosomal protein L7a rabbit 100 Cell Signaling 2403 Validated in 62

Ribosomal protein S3 rabbit 50 Cell Signaling 9538 Validated in 63

Ribosomal protein S6 rabbit 100 Cell Signaling 2217 Validated in 64

Sec22b rabbit 100 Synaptic Systems 186 003 K.D. validated

Septin7 rabbit 50 Atlas Antibodies HPA029524 Validated in 21

Shank1 rabbit 500 Synaptic Systems 162 013 K.O. validated

Shank2 rabbit 500 Synaptic Systems 162 202 K.O. validated

Shank3 rabbit 500 Synaptic Systems 162 302 K.O. validated

SMI310 mouse 200 Abcam 24570 Validated in 65

SNAP29 rabbit 500 Synaptic Systems 111 302 K.O. validated

SNAP47 rabbit 200 Synaptic Systems 111 403 Validated in 21

SNAP23 rabbit 100 Synaptic Systems 111 202 K.O. validated

SNAP25 mouse 100 Synaptic Systems 111 011 K.O. validated

Synaptophysin guinea pig 1000 Synaptic Systems 101 004 Control antigen

Synaptotagmin4 rabbit 1000 Synaptic Systems 105 143 K.O. validated

Synaptotagmin5/9 rabbit 100 Synaptic Systems 105 053 K.O. validated

Synaptotagmin7 rabbit 250 Synaptic Systems 105 173 K.O. validated

SynGAP1 rabbit 1000 Thermo Scientific PA1-046 Validated in 21

Syntaxin1 mouse 200 Synaptic Systems 110 011 Control antigen

Syntaxin13 mouse 100 Jahn Laboratory cl. 151.1 Validated in 23

Syntaxin16 rabbit 100 Synaptic Systems 110 162 Control antigen

Syntaxin2 rabbit 100 Synaptic Systems 110 022 Control antigen

Syntaxin3 rabbit 100 Synaptic Systems 110 033 Control antigen

Syntaxin4 rabbit 100 Synaptic Systems 110 042 Control antigen

Syntaxin5 rabbit 100 Synaptic Systems 110 053 K.D. validated

Syntaxin6 rabbit 100 Cell Signaling 2869 Validated in 21

Syntaxin8 rabbit 100 Synaptic Systems 110 083 Control antigen

TGN38 rabbit 100 Sigma-Aldrich T9826 Validated in 66

TOM20 mouse 200 Sigma-Aldrich WH0009804M1 Validated in 21

Transferrin Receptor rabbit 100 Abcam ab84036 Validated in 67

TrkB rabbit 500 Abcam ab33655 Validated in 68

vAChT rabbit 100 Synaptic Systems 139 103 K.O. validated

Vamp1 rabbit 500 Synaptic Systems 104 002 Control antigen

Vamp2 mouse 1000 Synaptic Systems 104 211 K.O. validated

VAMP7 rabbit 100 Abcam ab68776 Validated in 21

Vti1a mouse 100 BDBiosciences 611220 Validated in 23

Animals and other organisms

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

Laboratory animals

Rattus norvegicus, Wistar, both sexes, E18-P0

Wild animals

None

Field-collected samples

None

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