# nature portfolio

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## **Reporting Summary**

Nature Portfolio 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 Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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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
	$\square$ 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. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
$\boxtimes$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\boxtimes$	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\boxtimes$	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

### Software and code

Policy information about availability of computer code

Data collection

Animal behavioral data was collected by FreezeFrame version 4 software. IHC and IF images were acquired by ZEN 2012 software and SlideBook version 6.0. Western blot data was collected by Image studio version 5.0 (Licor). Quantitative PCR data was acquired by RotorGene Q software version 2.3.5.

Data analysis

All analysis pipeline are available on GitHub at https://github.com/Qiongyi/lncRNA\_nucleus\_synapse. Image analysis was performed using the Huygen Professional version 15.05, ImageJ version 1.54f, FigureJ version 1.36 and Imaris version 10.0.1. All statistical analysis was performed by Graphpad Prism version 9. Single molecule data was analyzed by Meta-Morph Microscopy Automation and Image Analysis version 7.7.8, PalmTracer version 2.0.4.1778, SharpVisu version 1.3 and NASTIC version 1. Neurons recordings was analyzed by pClamp version 10.5 and Matlab version R2021b. Softwares used for sequencing data analysis are Cutapt version 1.17, HISAT2 version 2.1.0, SAMtools version 1.8, StringTie version 2.1.4, Ballgown version 2.22.0 and SUPPA2 version 2.3. Isoforms expression was plotted and visualized by IsoVis version 1.1.1 (https://isomix.org). Mass spectrometry data was analyzed by Protein Pilot version 5.0 and Analyst version 1.7.

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Protein network analysis was performed using the STRING protein query database version 11.5 (https://string-db.org). All sequencing data are available on GitHub at https://github.com/Qiongyi/IncRNA\_nucleus\_synapse. The sequencing data generated in this study have been deposited in the NCBI Gene Expression Omnibus database under the accession code GSE207149. Proteomics data generated in this study have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD046479. All image data generated in this study are available at Figshare (https://doi.org/10.6084/m9.figshare.24431452). All data generated in this study are provided in the Supplementary information or Source Data file. Source data are provided with this paper (Source Data.xlsx). The raw data that support the findings of this study are also available from the corresponding authors upon reasonable request.

## Research involving human participants, their data, or biological material

	ut studies with <u>human participants or human data</u> . See also policy information about <u>sex, gender (identity/presentation),</u> and <u>race, ethnicity and racism</u> .	
Reporting on sex and		
Reporting on race, e other socially relevan	hnicity, or N/A	
Population character	istics N/A	
Recruitment	N/A	
Ethics oversight	N/A	
Note that full information	on the approval of the study protocol must also be provided in the manuscript.	
Please select the one be Life sciences For a reference copy of the d	fic reporting  elow that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.  Behavioural & social sciences	
All studies must disclos	e on these points even when the disclosure is negative.	
Sample size Sa	mple size was chosen based on those reported in previous publications (PMID: 30778148; PMID: 32367065).	
	We excluded animals if they did not reach freezing thresholds of 40%. We also excluded data points from animals that had misplaced cannulas or inefficient viral spread.	
Replication	Ve successfully reproduced all biological replicates.	
	Animals were randomly assigned to groups using Random.org following fear conditioning to ensure each groups has a balanced freezing scores.	
	ring behavioral test, data was blinded captured and analyzed by full automatic analysis software. RT-qPCR, Single-molecule imaging,	

# 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 & experime	ntal systems	Methods
n/a   Involved in the study	,	n/a Involved in the study
Antibodies		ChIP-seq
Eukaryotic cell lines		Flow cytometry
Palaeontology and a	rchaeology	MRI-based neuroimaging
Animals and other o	rganisms	
Clinical data		
Dual use research of	concern	
∑ Plants		
Antibodies		
		his and formation like on a fillion Anti Constitution I /4 2000 Pertiated CAU 457424AD Lat
Antibodies used  Validation	00073969), Anti-Psd95 (1:2 GR3376678-6), Anti-b-actin #ab32127, Lot: GR3270277 study for IF and brain tissue Abcam Cat# ab6556, Lot: GCat# ab18258, Lot: GR3258 Antibody (2 ug, rabbit, Prot (2 ug, Cell Signaling Cat#27 Cat#926-32211, Lot: C6081 antibodies used for IF and bCat#A11039, Lot: 1599396) Alexa Fluor 546 (1:500, The Anti-Caprin1 antibody was 32341095. Anti-FLAG was validation statement was list-Anti-PSD95. As stated on A-Anti-G3bp2. As stated on A-Anti-G3bp2. As stated on A-Anti-HDAC2. As stated on A-Anti-Map2. As stated on A-Anti-GFP.	this study for western blot are as follows: Anti-Caprin1 Antibody (1:2000, Proteintech Cat# 151121AP, Lot: 000, Abcam Cat# ab18258, Lot: GR3258736-3), Anti-G3bp2 (1:2000, Abcam Cat# ab86135, Lot: (1:1000, Cell Signaling Cat# 3700, Lot: 8, Clone: 8H10D10), Anti-Synaptophysin (1:20,000, Abcam -1, Clone: YE269), Anti-HDAC2 (1:1000, Cell Signaling Cat #2540S, Lot: 1). Primary antibodies used in this estaining are as follows: Anti-Map2 (1:2000, Abcam Cat# ab5392, Lot: GR3450786-1), Anti-GFP (1:2000, R3459456-1), Anti-Sv2a (1:200, Abcam Cat# ab32942, Lot: GR3250909-1), Anti-Psd95 (1:2000, Abcam Cat# ab32942, Lot: GR3250909-1), Anti-Psd95 (1:2000, Abcam Cat# 151121AP, Lot: 00073969), Anti-FLAG (2 ug, Sigma Cat# F7425, Lot: 0000131574); Anti-IgG 29, Lot: G). Secondary antibodies used for western blot are Anti-Rabbit IRDye 800CW (1:15,000, Li-COR 6-02) and Anti-Mouse IRDye 800CW (1:15,000, Li-COR cat#926-32212, Lot: C80108-05). Secondary orian tissue staining are as follows: Anti-Chicken Alexa Fluor 488 (1:2000, Thermo Fisher Scientific p, Anti-Rabbit Alexa Fluor 647 (1:1000, Thermo Fisher Scientific Cat#A21245, Lot: 1837984) and Anti-Rabbit rmo Fisher Scientific Cat#A11035, Lot: 1904467).  validated in previous publication: PMID: 31272829. Anti-Sv2a was validated in previous publication: PMID: alidated in previous publication: PMID: 31272829. Anti-Sv2a was validated by others and the sted on the manufacture's website: blocam website: Suitable for: ICC/IF, WB, IPC-P, IF-IC, FC-FP ted on Abcam website: Applications: WB, IF-IC blocam website: Suitable for: ICC/IF, WB, IPC-P, IF-IC, FC-FP ted on Abcam website: Suitable for: WB, IHC-P, WB, IHC-P, IF-IC, FC-FP ted on Abcam website: Suitable for: ICC, IHC-P, WB am website: Suitable for: IHC-P, Electron Microscopy, ICC, IP, Flow Cyt, IHC-Fr, WB IPC-PCR assays as negative control to ensure enrichment and as stated on Cell Signaling website:
Eukaryotic cell line	es	
Policy information about <u>ce</u>		er in Research
Cell line source(s)	HEK-293T cell line,	ATCC Cat# CRL-11268
Authentication	None of the cell line	es used were authenticated.
Mycoplasma contamination All cell lines tested r		negative for mycoplasma contamination.
Commonly misidentified I (See <u>ICLAC</u> register)	ines No commonly misic	dentified cell lines were used in this study
Animals and othe	r research organ	isms
Policy information about <u>studies involving animals</u> ; <u>ARRIVE guidelines</u> recommended for reporting animal research, and <u>Sex and Gender in</u> <u>Research</u>		
Laboratory animals		were purchased from Animal Resources Centre, Australia. Animals were maintained on a 12 h light/dark ree C and a relative humidity between 30-70%.
Wild animals	The study did not involve w	ild animals.
Reporting on sex	Findings apply to only male	mice.

The study did not involve samples collected from the field.

Field-collected samples

All testing took place during the light phase in red-light-illuminated testing rooms following protocols approved by the Institutional Animal Care and Use Committee of the University of Queensland.

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