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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.

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	No software was used.
Data analysis	Excel, ImageJ, ZEN 2 (blue edition), Python2.7 and Graphpad Prism 5.0 softwares were used.

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

All the datasets we used are publicly available datasets generated by other groups with accession codes indicated in the paper. All the raw data associated with all the figures and supplementary figures are available upon request. There is no restrictions on data availability.

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	Sample size was reported in the 'Statistical analysis' section of the Methods and figure legends. The sample size for quantifying the number of neurons and single-mRNA molecules was based on previous publications for biological experiments as well as the property of our data, i.e. clear separation between excitatory neurons and inhibitory neurons. For single-nucleu RNA-seq datasets, there are 3227 single nuclei and 19,550 single nuclei in each dataset, which are enough based on previous single-nucleus RNA-seq analysis.
Data exclusions	In order to avoid biases in the analysis and reduce the amount of noise, the bottom 5% low quality samples (samples with less reads across the transcriptome) in DroNc-Seq dataset were discarded as they were considered to have been damaged during the experimental procedure.
Replication	In order to ensure our experimental findings can be reliably reproduced, we include appropriate number of animals, sections, and neurons as listed in the figure legends. Further, three independent experiments were repeated with similar results. Also, the staining has been repeated by three persons in the lab independently. In order to ensure our experimental findings can be reliably reproduced, we include appropriate number of animals, sections, and neurons as listed in the figure legends. Further, three independent experiments were repeated with similar results. Also, the staining has been repeated by three persons in the lab independently.
Randomization	The experimental groups were allocated by the animal genotype, human case information, and the treatment. The covariants of age, gender, culture days, and vehicle were controlled as the same between experimental groups. Further, the sections, coverslips and neurons within each group were selected randomly. 10 images per sm-FISH stained section was randomly taken from the superficial layers of the entorhinal cortex or BA9 region. 60 excitatory and 60 inhibitory neurons from 3 human non-AD and AD sections were randomly selected for comparing the mean intensity of BAG3. Also, 5 excitatory and 5 inhibitory neurons from each coverslip (n = 11 coverslips each group) were randomly taken for comparing the endogenous 12E8 tau+ punta in neurites. Additionally, 20 images per coverslip at 1,024 × 1,024 resolution were taken randomly from all the orientations of the coverslip for the tau seeding experiment. The number of TBR1+ EX and GAD1+ IN neurons with tau inclusions were quantified blindly.
Blinding	The experimenter was blind to the experimental groups when they performed the immunostaining and sm-FISH as well as the counting of neurons and the single-mRNA molecules, and the comparison of the mean intensity of BAG3.

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	The Tau RD-P301S-YFP lentivirus and DS9 clone cell lines were provided by Dr. Marc Diamond. The BAG3 shRNA and the shRNA-resistant BAG3 were provided by Dr. Gail Johnson. All the brain tissue were provided by New York Brain Bank and Banner Sun Health Research Institute Brain and Body Donation Program.

Antibodies

Antibodies used

Human conformation-dependent tau (MC1, 1:750) and human/murine phospho-tau pSer396/ Ser404 (PHF1, 1:500) monoclonal antibodies were provided by Dr. Peter Davies. Mouse anti-phosphorylated tau Ser262 and/or Ser356 (12E8, 1:2000) antibody is a kind gift from Dr. Philip Dolan. Human/murine phospho-tau pSer202/Thr205 (AT8, Cat# MN1020, 1:500) and pThr212/Ser214 (AT100, Cat# MN1060, 1:500) monoclonal antibodies, rabbit anti-phospho-tau pSer422 (pS422, Cat# 44-764G, 1:250) and parvalbumin (PVALB, Cat# PA5-18389, 1:1000) polyclonal antibodies, Alexa Fluor dye-labeled cross-absorbed goat and donkey secondary antibodies (Cat# A-11029, A-11037, A-11007, A-11058, and A-21202, 1:1000) were purchased from Thermo Fisher Scientific. Rabbit anti-TBR1 (Cat# ab31940, 1:250) and SATB2 (Cat# ab92446, 1:250) polyclonal antibodies were purchased from Abcam. Rat anti-somatostatin (SST) (Cat# MAB354, 1:100) and mouse anti-NeuN (Cat# MAB377, 1:250) monoclonal antibody and goat anti-GAD1 (Cat# AF2086, 1:100) polyclonal antibody were purchased from Millipore and R&D Systems, respectively. Rabbit anti-calretinin (CALB2) (Cat# 7697, 1:1000), IBA-1 (Cat# 019-19741, 1:500), and GFAP (Cat# G9269, 1:2500) polyclonal antibodies were purchased from Swant, Wako and Sigma-Aldrich, respectively. Rabbit anti-TBR1 (Cat# 20932-1-AP, 1:250) and rabbit anti-BAG3 (Cat# 10599-1-AP, 1:100) polyclonal antibody were purchased from Proteintech Group.

Validation

All of them have been used in previous publications and validated by the manufacturers or research scientists.
MC1: validated in Neurobiol Aging. 2000 Sep-Oct;21(5):719-27; https://www.alzforum.org/antibodies/tau-mc1
PHF1: validated in https://www.alzforum.org/antibodies/tau-phos-ser396ser404-phf-1
12E8: validated in https://www.alzforum.org/antibodies/tau-12e8
AT8: validated in https://www.thermofisher.com/antibody/product/Phospho-Tau-Ser202-Thr205-Antibody-clone-AT8-Monoclonal/MN1020
AT100: validated in https://www.thermofisher.com/antibody/product/Phospho-Tau-Thr212-Ser214-Antibody-clone-AT100-Monoclonal/MN1060
pS422: validated in https://www.thermofisher.com/antibody/product/Phospho-Tau-Ser422-Antibody-Polyclonal/44-764G
PVALB: validated in https://www.thermofisher.com/antibody/product/Parvalbumin-Antibody-Polyclonal/PA5-18389
TBR1: validated in http://www.ptglab.com/Products/TBR1-Antibody-20932-1-AP.htm#validation
SATB2: validated in https://www.abcam.com/satb2-antibody-epncir130a-ab92446.html
SST: validated in http://www.emdmillipore.com/US/en/product/Anti-Somatostatin-Antibody-clone-YC7,MM_NF-MAB354#overview
NeuN: validated in http://www.emdmillipore.com/US/en/product/Anti-NeuN-Antibody-clone-A60,MM_NF-MAB377
GAD1: validated in https://www.rndsystems.com/products/human-mouse-rat-gad1-gad67-antibody_af2086
calretinin : validated in https://www.swant.com/?p=products&c=1.2
IBA-1: validated in http://www.wako-chem.co.jp/english/labchem/product/life/Antilba1/index.htm
GFAP: validated in https://www.sigmaaldrich.com/catalog/product/sigma/g9269?lang=en&region=US
BAG3: validated in https://www.ptglab.com/products/BAG3-Antibody-10599-1-AP.htm#validation

Animals and other organisms

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

Laboratory animals

The species, strain, sex and age of the laboratory animals used in this study have been clearly written in the paper. The F1 mouse offspring (both males and females at 22 and 30+ months old, strain FVB/N:C57BL/6) were used as experimental animals. All animals were maintained on a 12 hr light/dark cycle with food and water provided ad libitum. All animal experiments were performed in accordance with national guidelines (National Institutes of Health) and approved by the Institutional Animal Care and Use Committee of Columbia University. Primary cortical neuron culture were prepared from and embryonic day 16-18 embryos from C57BL/6 mice.

Wild animals

This study does not include wild animals.

Field-collected samples

This study does not include the samples collected from the field.