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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	Fiji (ImageJ 64 bits 1.51s) was used to analyze confocal images
Data analysis	GraphPad Prism 5 was used to perform statistical tests

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

Data reported in this study are tabulated in the main text and Extended Data Figures and Tables. The data that support the findings of this study are available from the corresponding author (Dr. Llorens-Martín) upon request. All requests for raw and analyzed data will be promptly reviewed by the Center for Networked

Biomedical Research on neurodegenerative diseases (CIBERNED) to verify whether the request is subject to any intellectual property or confidentiality obligations. Any data and materials that can be shared will be released via a Material Transfer Agreement.

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 not predicted. They were chosen based on previous literature. We have used a collection of human samples composed by 13 healthy control individuals and 45 Alzheimer´s disease patients. Pilot experiments provided an estimate of effect size, and indicated the appropriateness of sample sizes chosen.
Data exclusions	No data were excluded
Replication	Experimental findings were reproduced in at least three independent experiments. All replications were successful.
Randomization	Subjects were classified on the basis of neurological and neuropathological examination. Specifically, Braak-Tau stage was used for classification purposes.
Blinding	Investigators were blinded to group allocation when processing the tissue, performing cell counts or during confocal image acquisition.

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

Antibodies

Antibodies used	The following primary antibodies have been used in immunofluorescence: Goat anti-Doublecortin (DCX), Santa Cruz Biotechnology, RRID:AB_2088494, 1:1000; Mouse anti-Doublecortin (DCX), Santa Cruz Biotechnology, RRID:AB_10610966, 1:50; Rabbit anti-Doublecortin (DCX), kindly provided by Dr. Gleeson (University of California), 1:200; Rabbit anti-Doublecortin (DCX), Atlas Antibodies, RRID:AB_2674950, 1:50; Mouse anti-PSA-NCAM, Millipore, RRID:AB_9521, 1:1000; Rabbit anti-Calretinin, Swant, RRID:AB_261971, 1:500; Rabbit anti-Calbindin, Swant, RRID:AB_272122, 1:500; Rabbit anti-Phospho-Histone3, RRID:AB_310177, 1:500; Rabbit anti-Prox1, Reliatech, RRID:AB_10013821, 1:500; Rabbit anti-NeuN, Millipore, RRID:AB_10807945, 1:1000; Mouse anti-4R-Tau, Millipore, RRID:AB_310014, 1:500; Rabbit anti-total Tau, Synaptic Systems, RRID:AB_114719, 1:500; Mouse anti-BetaIII Tubulin, Promega, RRID:AB_430874, 1:1000; Mouse anti-GFAP, Millipore, RRID:AB_2294571, 1:1000; Rabbit anti-Phospho-Tau(S396), Thermo Fisher, RRID:AB_253374, 1:500; Mouse anti-Beta-Amyloid, Covance, RRID:AB_2564682, 1:1000. The following secondary antibodies were used to detect the binding of primary antibodies: Donkey Alexa-555 anti-goat, Thermo Fisher, A-21432, 1:1000; Donkey Alexa-488 anti-goat, Thermo Fisher, A-110552, 1:1000; Donkey Alexa-488 anti-rabbit, Thermo Fisher, A-21206, 1:1000; Donkey Alexa-647 anti-rabbit, Thermo Fisher, A-31573, 1:1000; Donkey Alexa-555 anti-rabbit, Thermo Fisher, A-31572, 1:1000; Donkey Alexa-488 anti-mouse, Thermo Fisher, A-21202, 1:1000; Donkey Alexa-647 anti-mouse, Thermo Fisher, A-31571, 1:1000; Donkey Alexa-555 anti-mouse, Thermo Fisher, A-31570, 1:1000.
Validation	- Goat anti-Doublecortin (DCX), Santa Cruz Biotechnology, RRID: AB_2088494: Validated by manufacturer to detect DCX of mouse, rat, human, and avian origin by WB, IP, IF, IHC, and ELISA. We further validated this antibody by pre-adsorption with a

specific blocking peptide (Abcam, Cat # ab19804). This validation was performed by dot blot and IHC.

- Mouse anti-Doublecortin (DCX), Santa Cruz Biotechnology, RRID:AB_10610966: Validated by manufacturer to detect DCX of mouse, rat, and human origin by WB, IP, IF, IHC, and ELISA.

- Rabbit anti-Doublecortin (DCX), kindly provided by Dr. Gleeson (University of California): Validated by Dr. Gleeson´s lab to detect DCX of mouse and human origin.

- Rabbit anti-Doublecortin (DCX), Atlas Antibodies, RRID:AB_2674950: Validated by manufacturer to detect DCX of human origin by IHC.

- Mouse anti-PSA-NCAM, Millipore, RRID:AB_9521: Validated by manufacturer to detect PSA-NCAM of human, rat, and mouse origin by ICC, IHC, RIA, and WB.

- Rabbit anti-Calretinin, Swant, RRID:AB_261971: Validated by manufacturer to detect Calretinin of human, monkey, rat, mouse, guinea pig, chicken, and fish origin by WB and IHC.

- Rabbit anti-Calbindin, Swant, RRID:AB_272122: Validated by manufacturer to detect Calbindin of human, monkey, rat, mouse, chicken, and fish origin by WB and IHC.

- Rabbit anti-Phospho-Histone3, RRID:AB_310177: Validated by manufacturer to detect phosphorylated histone 3 of mouse and human origin by ICC, IP, and WB.

- Rabbit anti-Prox1, Reliatech, RRID:AB_10013821: Validated by manufacturer to detect Prox1 of human origin by IF and WB.

- Rabbit anti-NeuN, Millipore, RRID:AB_10807945: Validated by manufacturer to detect NeuN of human, mouse, rat, and snail origin by ICC, IHC, IF, and WB.

- Mouse anti-4R-Tau, Millipore, RRID:AB_3100: Validated by manufacturer to detect 4R-Tau of human, rabbit, bovine, and mouse origin by IHC and WB.

- Rabbit anti-total Tau, Synaptic Systems, RRID:AB_114719: Validated by manufacturer to detect Tau of rat, mouse, and human origin by WB, IP, ICC, and IHC.

- Mouse anti-Beta-III-Tubulin, Promega, RRID:AB_430874: Validated by manufacturer to detect Beta-III-Tubulin of most mammalian species, specifically rat and human, by ICC, IHC, and WB.

- Mouse anti-GFAP, Millipore, RRID:AB_2294571: Validated by manufacturer to detect GFAP of chicken, human, porcine, and rat origin by IP, IHC, and WB.

- Rabbit anti-Phospho-Tau(S396), Thermo Fisher, RRID:AB_253374: Validated by manufacturer to detect phosphorylated Tau (S396) of human, mouse, and rat origin by WB, ICC, IF, and ELISA.

- Mouse anti-Beta-Amyloid, Covance, RRID:AB_2564682: Validated by manufacturer to detect Beta-Amyloid of human origin by WB, IHC, and direct ELISA.

Secondary antibodies were validated by manufacturer and have been extensively validated in the literature.

All the primary and secondary antibodies have been validated in our experiments by performing appropriate control tests in the absence of secondary and primary antibodies respectively.

Human research participants

Policy information about studies involving human research participants

Population characteristics	A total number of 58 subjects were included in the present study. Extended Data Figures 1 and 7 include detailed epidemiological data of these subjects, including gender and age. Statistically significant differences were found only between the age of Controls and Braak-Tau stage VI patients (Dunn´s Multiple comparison test (p = 0.045) for Kruskal-Wallis test). The population included 23 female and 35 male individuals. Tau phosphorylation (AT100 epitope) in the anterior hippocampus, prefrontal, parietal and temporal associative isocortex, and in the primary visual cortex was quantified at the neuropathology unit of the Banco de Tejidos CIEN to determine Braak-Tau stage, following previously described protocols. It should be noted that all the neurologically healthy individuals included in this work were at Braak-Tau 0 stage. Moreover, medical records were carefully examinated to determine the neurological status of the subjects. In all cases, brain tissue donation, processing, and use for research were in compliance with published protocols, which include the obtaining of informed consent for brain tissue donation from living donors, and the approval of the whole donation process by the local Ethical Committee.
Recruitment	Subjects were recruited on the basis of the brain tissue donation program coordinated by the neuropathology unit of the Banco de Tejidos CIEN. There are no potential self-selection bias that may be likely to impact results. Written informed consent was

obtained from all donnors, and the whole donation process was approved by the Ethical Committee of the Banco de Tejidos CIEN.