nature portfolio

Corresponding author(s): Sreeganga S. Chandra

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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 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
☐	☒ 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. *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

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	Noldus Ethovision XT 15 software for Open Field Test, Novel Object Recognition, and Fear Conditioning. Olympus VS200 ASW version 3.4.1 software for capturing fluorescence images
Data analysis	We used Graph Pad Prism 10 software to analyze behavior, histology, and Western blotting data. For snRNA-seq analysis, pre-mRNA reference was obtained from 10X Genomics and CellRanger version 3.1.0. was used to match the snRNA-seq data with pre-mRNA data. Seurat (version 4.2.0) single cell analysis R package was used for processing the snRNA-seq data. Differential expression analysis for snRNA-seq data was performed using the Wilcoxon Rank Sum test using the function FindMarkers of the Seurat package (4.1.0) in R. Adjustment was made for differences in sex, batch and read depth with MAST. Gene-set and protein enrichment analysis was performed using the function enrichGO from the R package clusterProfiler in Bioconductor (3.14). Genes known to function in the synapse were chosen as defined by the SynGO consortium (https://syngoportal.org).

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

The raw data for snRNAseq analysis are available on NCBI GEO GSE283187 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE283187).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Not Applicable
Reporting on race, ethnicity, or other socially relevant groupings	Not Applicable
Population characteristics	Not Applicable
Recruitment	Not Applicable
Ethics oversight	Not Applicable

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

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 number of animals was determined by power calculation (Software G*Power) using the Standard Deviation (SD) and effect size (d) of each assay estimated from preliminary results, with alpha set at 0.05 and beta between 0.1 and 0.15. We determined that n=4-6 was required for western blotting and histology, while n=6–12 was necessary for behavioral experiments.
Data exclusions	No data were excluded
Replication	To ensure replication, all experiments were conducted independently on separate cohorts of animals under identical conditions. Rigorous standard operating procedures (SOPs) were followed consistently for all protocols, and biological replicates were used in all conditions. Number of animals was determined by power calculation to ensure replicability, and results were cross-validated using complementary techniques. This approach minimized technical and biological variability, enhancing the reliability and reproducibility of our findings.
Randomization	Animals were allocated to groups randomly.
Blinding	All experiments and data analyses were conducted with the experimenter blinded to the experimental groups. Human interference was minimized wherever possible by automating processes, including image analysis and snRNA-seq analysis, to ensure objectivity and reproducibility.

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
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

1. Mouse Anti-α-Synuclein, 1:500 dilution for IHC, 1:1000 dilution for Western, BD Biosciences (610786), AB_398107
2. Guinea Pig Anti-NeuN, 1:500 dilution, Sigma-Aldrich (ABN90P), AB_2341095
3. Rabbit Anti-α-synuclein (phospho S129), 1:800 dilution for IHC, 1:1000 dilution for Western, Abcam (ab51253), AB_869973
4. Rabbit Anti-Iba1, 1:300 dilution, Wako Chemicals (019-19741), AB_839504
5. Guinea Pig Anti-GFAP, 1:400 dilution, Synaptic Systems (173004), AB_1064116
6. Mouse Anti-CD68, 1:400 dilution, Invitrogen (MA1-80557), AB_929627
7. Rabbit Anti-Endophilin 1, 1:200 dilution, Synaptic Systems (159002), AB_887757
8. Mouse Anti-PIP2, 1:200 dilution, Invitrogen (MA3-500), AB_568690
9. Mouse anti-β-actin, 1:1000 for Western, GeneTex (GTX629630), AB_2728646

Validation

Antibody validation involved a thorough literature review before selection to ensure suitability. Specificity was confirmed through staining in control tissues, verifying expected molecular weights via Western blotting, and assessing staining patterns using controls like primary antibody omission. Validation was further supported using genetically modified models to confirm signal specificity.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Mus musculus, C57BL/6J strain, consisting of 3-month-old and 12-month-old mice
Wild animals	Study did not involve wild animals
Reporting on sex	All experiments were sex-balanced. In this study, no significant male/female differences were observed in the mice.
Field-collected samples	Study did not involve field-collected samples
Ethics oversight	All animal protocols were approved by the Yale University Institutional Animal Care and Use Committee in accordance with National Institute of Health guidelines.

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

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable