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 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	Immunoblots were imaged using a LI-COR Odyssey CLx 4.0 (Licor). EM images were collected using a FEI Tecnai transmission electron microscope at an 80 kV accelerating voltage, and digital images were acquired with an AMT NanoSprint15 MK2 camera (Advanced Microscope Techniques). For proteomic analyses, the SDS gel for assessing protein content for proteomics was imaged by ChemiDoc (BioRad), the high pH reverse phase fractionation chromatography was carried out using a Dionex UltiMate 3000 HPLC system with the built-in micro fraction collection option in its autosampler and UV detection (Thermo Scientific, Sunnyvale, CA), the nanoLC-MS/MS analysis was carried out using an Orbitrap Eclipse (Thermo-Fisher Scientific, San Jose, CA) mass spectrometer equipped with a nanospray Flex Ion Source coupled with the UltiMate 3000 RSLCnano (Dionex, Sunnyvale, CA), raw MS data files for all the fractions were acquired using a real-time search (RTS) synchronous precursor selection (SPS) MS3 workflow, and all data were acquired under Xcalibur 4.3 operation software in Orbitrap Eclipse (Thermo Fisher Scientific). For lipidomic analyses, lipids were analyzed by LC-MS/MS on an Accucore C30 column (2.1 mm id x 150mm) under both positive and negative ion modes.
Data analysis	Immunoblot images were analyzed using ImageStudioLite 4.0 (LI-COR). EM images were analyzed using ImageJ 1.52p (NIH).For proteomic analyses, proteins separated by SDS-PAGE for assessing protein content were quantified using Image Lab 6.1 (Bio-Rad), all raw MS spectra were processed and searched using the Sequest HT search engine within the Proteome Discoverer 2.5 (PD2.5, Thermo), raw data files were searched and processed using LipidSearch 5.1 (Thermo Scientific), for data processing and heat map generation, obtained data were loaded into the R environment (Version 2023.06.0+421), and for data visualization, gplots and heatmap.2 packages were used. For the pathway analysis of lipidomic datasets, BioPAN was used. Power analysis was performed using G*Power 3.1.9.7. Statistical analyses were performed using Prism 7 Software (GraphPad).

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 mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE263 partner repository with the dataset identifier PXD048771. Lipidomics data are available at the NIH Common Fund's National Metabolomics Data Repository (NMDR) website, the Metabolomics Workbench, https://www.metabolomicsworkbench.org where it has been assigned Study ID ST003052 (DOI: 10.21228/M80H83). The electron microscopy images analyzed during the current study are not publicly available due to their use in a separate study but are available from the corresponding author upon request. Source data are provided with this paper.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	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.

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	Sample sizes were chosen based on preliminary experiments or similar studies performed in the past. For sample size estimation, an a priori power analysis was conducted using G*Power (version 3.1.9.7).239 Behavior: Based on previous motor behavioral data,23 the effect size was 0.50 using Cohen's criteria. With a significance criterion of α = 0.05 and power = 0.80, the minimal sample size needed with this effects size is n = 6 for two-way ANOVA. Immunoblotting of brain homogenates: Based on immunoblotting data from total brain homogenates,70 the effect size was 0.9 using Cohen's criteria. With a significance criterion of α = 0.05 and power = 0.80, the minimal sample size needed with this effects size in n = 3 for t-test and n = 3 for two-way ANOVA. Immunoblotting of SVs and synaptosomes: Based on immunoblotting data from synaptic vesicle and synaptosome isolations from prior experiments in the laboratory (unpublished data), the effect size was 0.50 using Cohen's criteria. With a significance criterion of α = 0.05 and power = 0.80, the minimal sample size needed with this effects size is n = 5 for two-way ANOVA. Proteinase K digestion experiments: Based on immunoblotting data from brain homogenates treated with proteinase K from prior experiments in the lab (unpublished data), the effect size was 0.9 using Cohen's criteria. With a significance criteria of α = 0.05 and power = 0.80, the minimal sample size needed with this effects size is n = 3 for two-way ANOVA. Sample sizes for the EM data were based on preliminary experiments in the lab (unpublished data) and discussion with the core director. The proteomic and lipidomic sample sizes were chosen in discussion with experts at the Metabolomics and Proteomics Core.
Data exclusions	No samples or animals were excluded from the analysis, except for EM analyses where outliers were identified using Prism 7 (GraphPad).
Replication	For quantification of immunoblots, 3-9 mice per genotype and per age were analyzed. For analysis of synapses by electron microscopy, 30-40 images per mouse/genotype/age were taken blinded to the groups by the EM Core Facility (Yale) and were analyzed in blind as well, with n = 3-5 mice per group. For lipidomic analysis, 6 mice per genotype and per age were analyzed. For proteomic analyses, 2 out of 6 mice per genotype and per age were combined to result in n = 3 per genotype and age.
Randomization	Mice were randomly allocated to the various age groups.
Blinding	Analysis of proteomic and lipidomic profiles was performed blind to the groups by the Proteomics and Metabolomics Core (Cornell University). For analysis of synapse by electron microscopy, perfusions were done by investigator 1, dissections by investigator 2 who was

blinded to the groups, 30-40 images per mouse/genotype/age were taken blinded to the groups by the EM Core Facility (Yale) and were analyzed in blind by a fourth investigator as well.

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
[ ]	[x] Antibodies
[x]	[ ] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[x]	[ ] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

αSyn (BD Biosciences Cat# 610787, RRID:AB_398108, 1:500), βSyn (Santa Cruz Biotechnology Cat# sc-136452, RRID:AB_10609953, 1:500), γSyn (Pacific Immunologies, peptide antibody generated against the C-terminal 24 residues, 1:500), AP1B1 (Proteintech Cat# 16932-1-AP, RRID:AB_2058204, 1:500), ATP8A1 (Proteintech Cat# 21565-1-AP, RRID:AB_10734587, 1:500), Bin1 (Santa Cruz Biotechnology Cat# sc-23918, RRID:AB_667901, 1:500), GAPDH (Santa Cruz Biotechnology Cat# sc-365062, RRID:AB_10847862, 1:500), Munc18-1 (BD Biosciences Cat# 610337, RRID:AB_397727, 1:500), NeuN (Millipore Cat# ABN78, RRID:AB_10807945, 1:500), PICALM (Cell Signaling Technology Cat# 26765, RRID:AB_3073648, 1:500), rab27B (Synaptic Systems Cat# 168 103, RRID:AB_887767, 1:500), pS129-αSyn (FUJIFILM Wako Pure Chemical Corporation Cat# 014-20281, RRID:AB_516843, 1:500; Abcam Cat# ab51253, RRID:AB_869973, 1:500), SNAP-25 (Abcam Cat# ab24737, RRID:AB_448273; 1:500), SV2 (DSHB Cat# SV2, RRID:AB_2315387, undiluted hybridoma supernatant produced in-house; SV2 was deposited to the DSHB by Buckley, K.M.), syntaxin-1 (Santa Cruz Biotechnology Cat# sc-12736, RRID:AB_2271330, 1:500), synaptophysin 1 (Santa Cruz Biotechnology Cat# sc-17750, RRID:AB_628311, 1:500), synaptotagmin 1 (Santa Cruz Biotechnology Cat# sc-136480, RRID:AB_10611453, 1:500), synapsin I and II (E028, Südhof lab), Tuj1 (Santa Cruz Biotechnology Cat# sc-80005, RRID:AB_2210816, 1:500), vAChT (Synaptic Systems Cat# 139 103, RRID:AB_887864, 1:500), VAMP2 (Santa Cruz Cat# 139 103, RRID:AB_887864, 1:500), vGaT (Santa Cruz Biotechnology Cat# sc-393373, RRID:AB_2801273, 1:500), vGluT1 (Santa Cruz Biotechnology Cat# sc-377425, RRID:AB_2687960, 1:500), vGluT2 (Synaptic Systems Cat# 135 402, RRID:AB_2187539, 1:500), vGluT3 (Synaptic Systems Cat# 135 203, RRID:AB_887886, 1:500), vMAT2 (Santa Cruz Biotechnology Cat# sc-374079, RRID:AB_10917928, 1:500).

Validation

Antibodies were validated either by the vendor (using knockout animals/cells/samples), by us using recombinant proteins (other publications), by knockout samples in this study (alpha-synuclein knockout animals), by other studies using these antibodies, or by observing the correct molecular mass.

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

All animal procedures including husbandry and experimental procedures were performed according to NIH guidelines and approved by the Institutional Animal Care and Use Committee at Weill Cornell Medicine (Animal Welfare Federal Wide Assurance No: A3290-01; protocol number 2014-0016).

The αSynBAC mice86 were obtained from JAX (Strain #: 023837). These mice carry a transgene containing the human SNCA gene and a knockout of the mouse Snca gene. Mice were bred as non-carrier of the transgene and homozygotes for mouse Snca knockout. Due to the nature of the cross, C57BL/6J mice were used as WT controls. αSynA53T mice were obtained from JAX (Strain #: 008135) and were bred as αSynA53T to WT. αSynL61 mice78 were obtained from the Przedborski lab. Similar numbers of male and female mice were used in this study except for the αSynL61 mice which were all male (see Source Data file), but our study is not sufficiently powered to enable sex-separated data analysis. Mice were group-housed with same sex littermates with a 12 h light/dark cycle in a temperature-controlled room with free access to water and food. Mice were anesthetized using isoflurane, followed by cervical dislocation and brain extraction (for biochemical studies) or by transcardiac perfusion (for EM studies).

Wild animals

N/A

Reporting on sex

Similar numbers of male and female mice were used in this study except for the αSynL61 mice which were all male (see Source data file), but our study is not sufficiently powered to enable sex-separated data analysis.

Field-collected samples

N/A

Ethics oversight	All animal procedures were performed according to NIH guidelines and approved by the Committee on Animal Care at Weill Cornell Medicine.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A