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	N/A
Data analysis	FlowJo V10, Li-COR Image Studio Studio Lite, ImageJ, MaxQuant V2.0.3.0, Syline-daily V 22.2.1.351, GraphPad Prism, Image Lab 6.1.0

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

Data is available from the corresponding author upon request.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	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	No statistical method was used to determine sample size. All experiments were performed with at least three biological replicates.
Data exclusions	No data was excluded.
Replication	All experimental results were replicated at least three times, and the results were reproducible.
Randomization	Samples were randomized whenever possible. Randomization was not applied to cell culture experiments.
Blinding	Investigators were not blinded to the identity of the samples, but findings were replicated by multiple investigators. Mass spectrometry experiments were performed blinded to the investigator who did not know the identity of the samples prior to data analysis.

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

Methods

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

Antibodies

Antibodies used	anti-syn BD Science Cat 610787, anti-myc Proteintech Cat 60003-2-IG, anti-GAPDH Proteintech, anti Rabbit IgG LiCor Cat 926-68071, Goat anti-mouse IgG LiCOR Cat 926-32210, anti-pSyn Abcam MJFR13, anti-tau Sigma T49
Validation	https://www.bdbiosciences.com/en-us/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-synuclein.610787 https://www.ptglab.com/products/MYC-Antibody-60003-2-Ig.htm https://www.ptglab.com/products/GAPDH-Antibody-60004-1-Ig.htm

https://www.licor.com/bio/reagents/irdye-680rd-goat-anti-rabbit-igg-secondary-antibody
https://www.licor.com/bio/reagents/irdye-800cw-goat-anti-mouse-igg-secondary-antibody
https://www.abcam.com/alpha-synuclein-phospho-s129-antibody-mjf-r13-8-8-ab168381.html
https://www.sigmaaldrich.com/US/en/product/mm/mabn827

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Primary hippocampal neurons are from embryonic-18 CD-1 mice. HEK293T aSyn biosensor cells were obtained from Tritia Yamasaki
Authentication	The cell lines were not authenticated
Mycoplasma contamination	The cell lines were not tested for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	HEK293T biosensor cells were obtained from Tritia Yamasaki. Cells post-treatment were harvested with 0.05 % trypsin. Cells were pelleted and resuspended in 4% paraformaldehyde for fixation. Cells were then pelleted again and resuspended in MACSQuant Flow Running buffer for analysis.
Instrument	MACSQuant YVB
Software	FlowJo 10.5.3
Cell population abundance	At least 10,000 cells were analyzed for each experiment
Gating strategy	SSC-A/FCS-A gate was drawn to select intact cells after paraformaldehyde fixation. Then FSC-H/FCS-A gate was drawn to define single cells and exclude cell doublets. In FRET-A and CFP-A bivariate plot, a polygon gate was drawn in control cells such that the FRET-negative and -positive populations are separated.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.