nature portfolio

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Last updated by author(s): 06/16/2026

# 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<br>*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<br>*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

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| Data collection | Zeiss LSM 880 and Nikon A1Rsi SIM Confocal are used for confocal imaging. A PharosFX plus Molecular Imager (Bio-Rad) was used for western blot imaging. nCounter Prep Station is used for probes immobilization in the sample cartridge. The cartridge is then analyzed by the nCounter Digital Analyzer. BD LSR II Flow Cytometer is used for flow cytometry. A FEI Tecnai Spirit Twin Transmission Electron Microscope is used for electron microscopy imaging. |
| Data analysis | Zeiss ZEN 3.0 blue edition, Imaris 10.0.1 and FIJI-ImageJ 1.54i software were used for images analysis. GraphPad Prism 9.5.1 was used for statistical analysis. FlowJo™ v10.6.1 was used for flow cytometry analysis. nSolver 4.0 was used for Nanostring data analysis. |

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 GSE number for the nano string data is GSE245929. The information on human samples is listed in Supplementary Table 1. The datasets generated and/or analyzed in the current study are within the paper or Supplementary Information. 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.

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| Reporting on sex and gender | Human postmortem hippocampal tissue from AD patients and healthy controls was obtained from the NIH NeuroBioBank. Each group included two males and two females. |
| Reporting on race, ethnicity, or other socially relevant groupings | All the brain samples from the same race (white). |
| Population characteristics | The ages of the patients ranged from 55 to 66 years, with an average of 62 for the healthy controls and 63 for the AD group. All AD patients experienced clinical onset of Alzheimer's disease before the age of 65 and were diagnosed with early-onset Alzheimer's disease accompanied by cerebral amyloid angiopathy, while no clinical brain pathology was found for the healthy controls based on the information from the NIH NeuroBioBank. |
| Recruitment | N/A |
| Ethics oversight | All samples were obtained from NIH NeuroBioBank and were de-identified before use. |

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.

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| Sample size | For animal experiments, we initially used n = 6 per AD and WT group to confirm and characterize the novel structure (mitochondrial plaques, MPs) in our APP/PS1/mt-Keima mouse model. As the the phenotype of MP appearance in AD are significant and stable, we use n =3/age/genotype for time-course experiment, which gave reliable results with reasonable variations. For in vitro cell experiments, n = 3 independent wells. 3 independent biological experiments were done.<br>For human postmortem hippocampal tissue, we requested 4 individuals in both AD and healthy control groups from the NIH NeuroBioBank, with 2 males and 2 females in each group. |
| Data exclusions | We used a threshold of 100 µm3 to characterize a single mitochondria-enriched object as a MP in 3D images. As stated in the methods, the cerebrovascular status is highly enriched with mitochondria; thus, their reconstructed 3D structures based on mitochondria would likely show a single object surpassing a threshold size while devoid of typical MP characteristics. Though MPs may also potentially develop in the vascular, single mitochondrial objects with sizes over 100 um3 but in a vascular shape were not considered as typical MPs in the present study. |
| Replication | The number of biological replicates for each experiment on animal and human samples is listed in the corresponding figure legend. All in vitro cell experiments were repeated 3 times independently. |
| Randomization | In the present study, we did not have any treatment for mice. The AD and WT mice are chosen randomly after considering their genotype and age.<br>The imaging region of mouse brain were randomly choose unless indicated specifically for certain regions, like the dentate regions, CA1, CA2 and CA3.<br>The human AD postmortem hippocampal tissue are chosen based on the standard with diagnosed early-onset Alzheimer's disease and cerebral amyloid angiopathy. The healthy control are with no clinical brain diagnosis, but age, race and sex matched to the chosen AD samples. All the human samples are from the same repository (Miami). |

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| Blinding | The investigators were blinded to mouse genotypes during ex vivo/in vitro imaging and 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 |
| ☒ | ☐ Plants |

## Methods

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

## Antibodies

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| Antibodies used | CTSD (Proteintech, 21327-1-AP), LAMP1 (Abcam, ab24170), Rab 9 (Proteintech, 11420-1-AP), GAPDH (Cell Signaling, #2118), GFAP (DAKO, Z033401-2), Amyloid (Cell Signaling, 2454s), NeuN (Proteintech, 66836-1-Ig), MAP2 (cell signaling, #4542), phosphorylated NF-H (Biolegend, 801602), Iba-1(FUJIFILM, 019-19741), APP (Proteintech, 10524-1-AP), APP (Proteintech, 60342-1-Ig), VDAC2 (Proteintech, 11663-1-AP),LAMP2 (Proteintech, 66301-1-Ig),β-Amyloid (D3D2N) (Cell Signaling, 15126), MAP2 (Thermofisher Scientific, PA1-16751), PINK1(Cell Signaling, #6946),PARKIN (Proteintech, 14060-1-AP), BNIP3 (Cell Signaling #44060S), Nix (Cell Signaling, #12396S), α-tubulin (Proteintech, 66031-1-Ig), HSP60 (Proteintech, 15282-1-AP), VDAC1 (Abcam, ab186321)<br>The applied secondary antibodies include: ab175652, ab150171, ab175660, ab150113, ab 175471. |
| Validation | All the antibodies were validated for use in mouse/human tissues based on previous publications. The detailed antibody validation profiles are available in the website of designated companies. |

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

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| Cell line source(s) | SH-SY5Y cell from ATCC |
| Authentication | The cells were newly bought from ATCC and had not been further authenticated after arrival. |
| Mycoplasma contamination | The cells were newly bought from ATCC and were not tested for mycoplasma contamination. |
| Commonly misidentified lines<br>(See ICLAC register) | N/A |

## Animals and other research organisms

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

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| Laboratory animals | Wild type mt-Keima mice (FVBNCrl.129(Cg)-Igs2tm1(CAG-mt-Keima)) were crossed with APP/PS1 double transgenic mice expressing a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9). Mt-Keima mice were backcrossed 5 times onto the 2xAD background. 5XFAD mouse strain contains five mutations within amyloid precursor protein and presenilin-1 genes identified in human familial Alzheimer's disease. This strain, denoted as B6. Cg-Tg (APPSwFlLon, PSEN1*M146L*L286V)6799Vas/Mmjax, RRID: MMRRC_034848-JAX, was obtained from the Mutant Mouse Resource and Research Center at The Jackson Laboratory. Hemizygous male carriers were crossed with C57BL/6J females to generate experimental mice. All mice were maintained on a standard NIH diet (T.2018SX.15 Global 18% Protein Extruded Rodent Diet, sterilizable) with food and water available ad libitum. Animals were maintained on a 12-h light/dark cycle, with all testing performed during the light cycle. The animals were group housed, if possible. |
| Wild animals | N/A |
| Reporting on sex | Both female and males are included in the experiments. However, no sex-based analysis were performed due to the lack of enough number of single sex mice or human samples. |
| Field-collected samples | N/A |

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| Ethics oversight | All animal experiments were performed using protocols approved by the institutional animal care and use committee of the NIA, 361-OSD-2023. |

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

## Plants

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

## 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

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| Sample preparation | To detect mitophagy events produced in 24h, SH-SY5Y cells receiving different treatments were stained with Mtphagy Dye from Dojindo 24h before measurement on flow cytometry. Briefly, cells were washed with FBS-free medium twice before staining with 100 nmol/l Mtphagy dye in FBS-free medium in the incubator for 30 min. The Mtphagy dye containing medium was moved and cells were washed with FBS-free medium twice before being replaced with medium containing the appropriate treatments. At 24h time point, cells are collected in PBS and mitophagy events was measured directly by flow cytometry. Lysosomal degradation ability was measured using the Lysosomal Intracellular Activity Assay Kit, ab234622, and by following the manufacturer's protocol. Notably, the cells in the positive control group were treated with DMEM/F12 medium containing 0.5% FBS and 100 nM Bafilomycin A1 for 1h before incubation with a self-quenched substrate mix. To detect lysosomal pH changes, SH-SY5Y cells receiving different treatments were trypsinized and incubated with an FBS-free medium containing 1 uM LysoSensor™ Green DND-189 (ThermoFisher Scientific, L7535) at 37 °C with 5% CO2 for 30min. Stained cells were kept on ice before measurement. |
| Instrument | BD LSR II Flow Cytometer |
| Software | FlowJo™ v10.6.1 |
| Cell population abundance | For 24h time-point data, 10,000 cells/sample were collected. After gating to remove cell dead/debris and doublet, around 6,000 cells/samples were used for data analysis in mitophagy detection; around 8,000 cells/sample were used for LysoSensor staining and Lysosomal Intracellular Activity detection .<br>For 4D time-point data, 10,000 cells/sample were collected. After gating to remove cell dead/debris and doublet, around 6,000 cells/samples were used for data analysis in mitophagy detection; around 8,500 cells /sample were used for LysoSensor staining and 7,000 cells/sample were used for Lysosomal Intracellular Activity detection.<br>For 10 D time-point data, 10,000 cells/sample were collected. After gating to remove cell dead/debris and doublet, around 6000/samples were used for data analysis in mitophagy detection; around 7,000 cells /sample were used for LysoSensor staining and Lysosomal Intracellular Activity detection. |
| Gating strategy | All flow cytometry samples were stained with single dye. For each experiment and staining, a negative control without any staining was added to help define the negative population. The preliminary FSC/SSC gating of the start population is 10,000 cells/sample. Dead/debris and doublet were gated out based on FSC and SSC. |

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