

## 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 | <p>Tomahito API v1 (This API is freely available, although an API license from Thermo is necessary for installation on instrument.) Yu et al., 2020 Ref.35; <a href="https://gygi.hms.harvard.edu/software.html">https://gygi.hms.harvard.edu/software.html</a></p> <p>Orbitrap Eclipse Tribrid Mass Spectrometer (Cat#FSN04-10000) with FAIMS Pro Interface (#FMS02-10001) - Thermo Fisher Scientific</p> <p>Orbitrap Fusion Lumos Tribrid MS (Cat#QLAAEGAAPFADBMBHQ) with or without FAIMS Pro Interface (#FMS02-10001) - Thermo Fisher Scientific</p> <p>Orbitrap Fusion Tribrid MS (Cat#QLAAEGAAPFADBMBXC) with FAIMS Pro Interface (#FMS02-10001) - Thermo Fisher Scientific</p>  |
| Data analysis   | <p>Quantification of targeted proteomics data: Skyline v20.2 MacLean et al., 2010 Ref. 79 ; <a href="https://skyline.ms/project/home/begin.view?">https://skyline.ms/project/home/begin.view?</a></p> <p>Annotation of lipidomics spectra: LipiDex Spectrum Annotator This study <a href="https://github.com/coongroup/LipiDexSpectrumAnnotator">https://github.com/coongroup/LipiDexSpectrumAnnotator</a></p> <p>Lipidomics identification software: Compound Discoverer 2.1 Thermo Fisher Scientific <a href="https://www.thermofisher.com/us/en/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-software/multi-omics-data-analysis/compound-discoverer-software.html">https://www.thermofisher.com/us/en/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-software/multi-omics-data-analysis/compound-discoverer-software.html</a></p> <p>Lipidomics analysis: LipiDex v1.1 Hutchins et al., 2018 Ref. 50 <a href="https://github.com/coongroup/LipiDex">https://github.com/coongroup/LipiDex</a></p> <p>Programming language: R 3.6.3 R Core Team <a href="https://www.r-project.org/">https://www.r-project.org/</a></p> <p>Analysis of cells visualized by light microscopy: MetaMorph v7.10 Molecular Devices <a href="https://www.moleculardevices.com/products/cellular-imaging-systems/acquisition-and-analysis-software/metamorph-microscopy#">https://www.moleculardevices.com/products/cellular-imaging-systems/acquisition-and-analysis-software/metamorph-microscopy#</a></p> <p>Visualization of immunoblots: ImageLab v6.0.1 Biorad <a href="https://www.bio-rad.com/en-us/product/image-lab-software?ID=KRE6P5E8Z&amp;source_wt=imagelabsoftware_surl">https://www.bio-rad.com/en-us/product/image-lab-software?ID=KRE6P5E8Z&amp;source_wt=imagelabsoftware_surl</a></p> <p>Quantification of images: Fiji ImageJ and SciJava projects Ref.78 <a href="https://imagej.net/software/fiji/">https://imagej.net/software/fiji/</a></p> <p>Peptide identification from mass spectrometry data: Comet release 2019.01; Eng et al 2013 Ref. 61; <a href="http://comet-ms.sourceforge.net/">http://comet-ms.sourceforge.net/</a></p> <p>Statistical analysis and graphing of data: Prism v9 GraphPad <a href="https://www.graphpad.com/scientificsoftware/prism/">https://www.graphpad.com/scientificsoftware/prism/</a></p> |

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](#)

All MS raw files have been deposited in MassIVE with the identifiers MSV000088132 (proteomics) [<https://doi.org/doi:10.25345/C5RN99>] and MSV000088048 (lipidomics) [<https://doi.org/doi:10.25345/C5MC31>]. Annotated lipid spectra are in Supplementary Data File 15. Uncropped images can be found in Source Data 1. Source data for individual plots can be found in Source Data 2. The searching database for proteomics was constructed from Swiss-Prot human database (released on Jun 17, 2020 at UniProt, [[https://ftp.uniprot.org/pub/databases/uniprot/previous\\_major\\_releases/release-2020\\_06/](https://ftp.uniprot.org/pub/databases/uniprot/previous_major_releases/release-2020_06/)])

## Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender

Population characteristics

Recruitment

Ethics oversight

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](https://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

Data exclusions

Replication

Randomization

Blinding

## 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 &amp; experimental systems

## Methods

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Antibodies

## Antibodies used

anti-EEA1 (C45B10) rabbit mAb Cell Signaling Technology 3288; RRID:AB\_2096811  
 anti-RAB5 (C8B1) rabbit mAb Cell Signaling Technology 3547; RRID:AB\_2300649  
 anti-PSEN1 (D39D1) rabbit mAb Cell Signaling Technology 5643; RRID:AB10706356\_  
 anti-PSEN2/AD5 (EP1515Y) rabbit mAb Abcam ab51249; RRID:AB\_882202  
 anti-LAMP1 (D2D11) rabbit mAb Cell Signaling Technology 9091; RRID:AB\_2687579  
 anti-LAMP2 (D5C2P) rabbit mAb Cell Signaling Technology 49067; RRID:AB\_2799349  
 anti-TMEM192 rabbit pAb Proteintech 28263-1-AP; RRID:AB\_2881099  
 anti-HA Biogen 901513; RRID:AB\_2565335  
 anti-HA (6E2) mouse mAb Cell Signaling Technology 2367; RRID:AB\_10691311  
 anti-FLAG M2 mouse mAb Sigma-Aldrich F1804; RRID:AB\_262044  
 anti-ZO-1 rabbit pAb Proteintech 21773-1-AP; RRID:AB\_10733242  
 anti-Golga1 rabbit pAb Proteintech 12640-1-AP; RRID:AB\_2115315  
 anti-Calreticulin rabbit pAb Proteintech 10292-1-AP; RRID:AB\_513777  
 anti-RAB11 (D4F5) rabbit mAb Cell Signaling Technology 5589; RRID:AB\_10693925  
 anti-Lamin A/C (4C11) mouse mAb Cell Signaling Technology 4777; RRID:AB\_10545756  
 anti-VDAC1/Porin rabbit pAb Proteintech 55259-1-AP; RRID:AB\_10837225  
 anti-RAB7 (D95F2) rabbit mAb Proteintech 9367  
 anti-DYKDDDDK tag, mouse mAb (FG4R) Thermo Fisher Scientific MA1-91878; RRID:AB\_1957945  
 anti-GAPDH (D16H11) XP rabbit mAb Cell Signaling Technology 5174; RRID:AB\_10622025  
 anti-APP CTF (C1/6.1) mouse mAb BioLegend 802801; RRID:AB\_2564648  
 anti-APP A4 (22C11) mouse mAb Sigma MAB348  
 anti-TFRC/CD71 (D7G9X) Cell Signaling Technology 13113; RRID:AB\_2715594  
 anti-Transferrin Abcam Ab82411; RRID:AB\_1659060  
 anti-ATF4/CREB (B-3) Santa Cruz Biotechnology SC-390063; RRID:AB\_2810998  
 anti-PEX19 rabbit pAb Proteintech 14713-1-AP; RRID:AB\_2162265  
 anti-CD71/TFR1 (D7G9X) rabbit mAb Cell Signaling Technology 13113; RRID:AB\_2715594  
 anti-HSP90 (3F11C1) mouse mAb Proteintech 60318-1-Ig; RRID:AB\_2881429  
 anti-BACE1 (D10E5) rabbit mAb Cell Signaling Technology 5606; RRID:AB\_1903900  
 IRDye 680RD Goat anti-Rabbit IgG secondary antibody Li-Cor 926-68071; RRID:AB\_10956166  
 IRDye 680RD Goat anti-Mouse IgG secondary antibody Li-Cor 926-68070; RRID:AB\_10956588  
 IRDye 800CW Goat anti-Rabbit IgG secondary antibody Li-Cor 926-32211; RRID:AB\_621843  
 IRDye 800CW Goat anti-Mouse IgG secondary antibody Li-Cor 926-32210; RRID:AB\_621842  
 Goat anti-Rabbit IgG, HRP-linked antibody Cell Signaling Technology 7474P2  
 Goat anti-Rabbit IgG HRP conjugate Bio-Rad 1706515; RRID:AB\_11125142  
 Goat anti-Mouse IgG HRP conjugate Bio-Rad 1706516; RRID:AB\_11125547  
 Alexa Fluor 594 Goat anti-Rabbit IgG (H+L) cross-adsorbed secondary antibody Thermo Fisher Scientific A-11012; RRID:AB\_2534079  
 Alexa Fluor 488 Goat anti-Mouse IgG (H+L) highly cross-adsorbed secondary antibody Thermo Fisher Scientific A-11029; RRID:AB\_2534088

\* Dilution factor for each antibody is described in the Supplementary Table 14.

## Validation

Specificity (human) for antibodies against APP, CTF, and A4 were validated using cells that lack APP (created by CRISPR-Cas9 gene deletion). Similarly, PSEN1, PSN2 and BACE1 antibodies were validated using cells lacking the respective genes. All antibodies were commercially obtained, and validation was based on the datasheets from the manufacturer as well as the extensive usage in the field.

## Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

## Cell line source(s)

Human: HEK293 ATCC CRL-1573; RRID:CVCL\_0045.  
 293\_L and 293\_EL cells were derived from HEK293 using CRISPR-Cas9 gene editing and lentiviral transduction.

## Authentication

Karyotyping (GTG-banded karyotype) of the original HEK293 was performed by Brigham and Women's Hospital CytoGenomics Core Laboratory.

## Mycoplasma contamination

All cell lines were found to be free of mycoplasma using Mycoplasma Plus PCR assay kit (Agilent).

Commonly misidentified lines  
(See [ICLAC](#) register)

none