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	Data analysis and visualization: GraphPad Prism v10.0 Proteomics: Spectronaut v18.2 (Biognosys) directDIA+ workflow (library-free); Spectronaut HTRMS Converter; timsControl/Window Editor (Bruker) for DIA-PASEF window optimization. Image analysis / microscopy: Fiji/ImageJ; Imaris; ONI Nanoimager acquisition/analysis software (for single-EV super-resolution imaging), as applicable to the imaging platform.
Data analysis	Data are presented as mean ± SEM unless otherwise indicated. Statistical analyses were performed in GraphPad Prism v10.0. Raw data distributions were assessed for normality. Depending on the experiment, statistical testing included Wilcoxon–Mann–Whitney tests, ROC analysis, or ANOVA with multiple-comparison tests as specified in the figure legends. Statistical significance was set at $p < 0.05$. In functional enrichment analyses, p-values were adjusted for multiple comparisons using Benjamini–Hochberg FDR correction where indicated. Proteomics DIA-PASEF datasets were analyzed using Spectronaut v18.2 directDIA+ (library-free), with identification against a reviewed Human Swiss-Prot FASTA (downloaded 08/30/2024) using Spectronaut's Pulsar search engine and standard settings described in Methods. Proteins identified in at least one of the three technical DIA injections were retained for downstream analysis. Comparative proteomic analysis between isogenic APOE3 and APOE4 iMGs and their derived EVs was performed using MetaboAnalyst v6.0. The study used paired/isogenic comparisons to emphasize consistent genotype-dependent effects across matched lines; nominal p-values are reported for exploratory proteomic comparisons as described in the manuscript limitations.

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 data supporting the findings of this study are available within the paper and its Supplementary Information. The information of human iPSC lines are provided in Supplementary Table 1. The exported proteomics datasets are provided in Supplementary Table 2 (Cell) and 9 (EVs). The raw data files from mass spectrometry were deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD074177 and are available at the following URL: http://www.ebi.ac.uk/pride.

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	This study did not recruit new human participants. Experiments were performed using previously derived, de-identified human iPSC lines. Donor sex/gender information was available for all three donors used: two male donors and one female donor (Supplementary Table S1). The study was not designed or powered to test sex/gender as a biological variable.
Reporting on race, ethnicity, or other socially relevant groupings	No new participant recruitment occurred. Donor ethnicity information was available for all three donors and was reported as Caucasian (Supplementary Table S1). Analyses were not stratified by ethnicity because the study was not designed or powered for such comparisons and donor diversity was limited in the available isogenic lines.
Population characteristics	Biological material consisted of four individual donors from whom iPSC lines were previously derived, three of them contributing an isogenic APOE pair (APOE3/3 and APOE4/4 hiPSC lines) and one of them bearing MAPT P301L mutation from FTDP-17. Donor characteristics available from the source documentation are: Donor count: 3. Sex/gender: 2 male, 1 female. Ethnicity: Caucasian (all donors). Disease status: 1 Alzheimer's disease donor; 2 control donors; 3 FTDP-17 donor. Age at skin biopsy / PBMC collection: reported as 80 years (AD donor), 76 years (control donor) and 50 years (FTDP-17 donor); age was not provided for one control donor (Supplementary Table S1). Additional provenance (cell source and reprogramming source, karyotype) is provided in Supplementary Table S1.
Recruitment	N/A
Ethics oversight	This work used established, de-identified human iPSC lines obtained from internal and external sources. The originating institutions/providers obtained appropriate informed consent and ethics/IRB approvals for derivation and distribution of these lines according to their policies. No identifiable private information was accessed by the authors.

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 based on available isogenic iPSC line pairs and standard practice for mechanistic iPSC-based studies. The study used three independent isogenic APOE3/3 vs APOE4/4 iPSC line pairs as biological replicates. For each assay, replicate structure (independent differentiations/experiments and technical replicates such as duplicate wells) is described in the Methods and/or figure legends.
Data exclusions	No data points were excluded unless they failed predefined technical QC criteria (e.g., assay failure, instrument QC, or proteomics identification/quantification QC thresholds as defined by the software/workflow).
Replication	Key findings were reproduced across independent isogenic line pairs and replicated experiments. Many assays included technical replication (e.g., duplicate wells) and independent experimental repeats as stated in Methods (e.g., ELISAs performed with duplicate wells and repeated in independent experiments). Proteomics conclusions were supported by orthogonal single-EV validation (nano-flow cytometry; super-resolution imaging).
Randomization	For in vitro experiments, samples were processed in parallel under standardized conditions. Where applicable, sample processing/acquisition order was counterbalanced or handled in a consistent manner to minimize batch effects; statistical comparisons were primarily paired within isogenic line pairs to control for background variation.

Blinding	Blinding was routinely performed for in vitro treatment assignments. Objective quantitative readouts (automated instrumentation/software quantification for proteomics, imaging, and electrophysiology) were used to minimize bias.

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
[ ]	[ ] Palaeontology and archaeology
[ ]	[ ] Animals and other organisms
[ ]	[ ] Clinical data
[ ]	[ ] Dual use research of concern
[ ]	[ ] Plants

Methods

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

Antibodies

Antibodies used	Primary antibodies used in this study (supplier; catalog #; dilution; application as described): anti-TREM2 (R&D Systems; AF1828; 1:1000; immunostaining), anti-PU.1 (Cell Signaling Technology; 2258S; 1:1000; immunostaining), anti-IBA1 (Wako; 019-19741; 1:500; immunostaining), anti-MAP2 (Thermo Fisher Scientific; 13-1500; 1:200; immunostaining), anti-Ac-Caspase-3 (Cell Signaling Technology; 9661S; 1:1000; immunostaining), anti-phospho-tau AT8 (Thermo Fisher Scientific; MN1020; immunostaining), anti-PAX6 (R&D Systems; AF8150; 1:1000; immunostaining), anti-SOX2 (R&D Systems; reported in Methods; 1:1000; immunostaining), anti-NESTIN (R&D Systems; MAB1259; 1:1000; immunostaining), anti-TBR2 (Abcam; ab23345; 1:200; immunostaining), anti-TBR1 (Abcam; ab31940; 1:200; immunostaining), anti-SATB2 (Abcam; ab92446; 1:200; immunostaining), anti-CTIP2 (Abcam; ab18465; 1:200; immunostaining), anti-GFAP (Agilent; Z033429; 1:700; immunostaining), anti-NeuN (Chemicon/Millipore; MAB377B; 1:50; immunostaining), anti-NF-κB p65 (Santa Cruz Biotechnology; sc-8008 for immunostaining; sc-372 for western blot; 1:20 and 1:100 respectively), anti-phospho NF-κB p65 (1:1000, 3033S, Cell Signaling Technology; western blot),anti-3R Tau (1:1000, 05-803, Millipore; western blot), anti-4R Tau (1:1000, 823701, BioLegend; western blot), anti-phospho-Tau (Ser202, Thr205) (AT8; 1:1000, MN1020B, Invitrogen; western blot), anti-total Tau (1:2000, A0024, Dako; western blot) anti-β-actin (Santa Cruz Biotechnology; sc-130065; 1:1000; western blot), fluorescent conjugates for single-EV imaging/analysis: anti-PSMA1-FITC antibody (Abbexa, Cat# abx317148), anti-PSMB5-CL488 (Proteintech; CL488-67959; 1:1000), anti-CD9-APC (1:100, Cat# 341648, BD biosciences), anti-human CD81-APC (BioLegend; 349509; 1:100), anti-human CD63-APC (Thermo Fisher Scientific; A15712; 1:100). Pluripotency characterization used the Human Pluripotent Stem Cell Marker Antibody Panel (R&D Systems; SC008; OCT3/4, NANOG, alkaline phosphatase, SSEA-4) per manufacturer protocol. Secondary antibodies included species-matched Alexa Fluor secondaries (Life Technologies; catalog # not specified in manuscript) and HRP-linked anti-mouse IgG secondary for western blot (Cell Signaling Technology; 7076S).
Validation	All antibodies were commercially sourced and used according to manufacturer-recommended applications and dilutions as described in the Methods. Primary antibodies for cell identity/state markers (e.g., TREM2, PU.1, IBA1, MAP2, GFAP, NeuN, PAX6, SOX2, NESTIN, TBR1/2, SATB2, CTIP2) were validated through expected cell-type-specific localization/patterns in the corresponding differentiated cultures (hiPSCs, iMGs, and iNs) and consistent signal across independent differentiations/lines. The AT8 monoclonal antibody (MN1020) is widely used for phosphorylated tau detection and was applied to neuronal cultures as described. For western blot, NF-κB p65 and β-actin antibodies produced bands at expected molecular weight and were consistently validated according to the manufacturer's websites, with β-actin serving as a loading control. For EV/single-EV analyses, conjugated antibodies (PSMA1-FITC, PSMB5-CL488 and APC-conjugated CD9/CD63/CD81) were used to identify EV subpopulations based on established EV markers and co-localization/marker enrichment patterns and validated according to the manufacturer's protocols.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Human iPSC lines: Three pairs of isogenic APOE3/3 and APOE4/4 hiPSC lines. One isogenic pair from ALSTEM (Catalog# iPS26 and iPS16). Two additional isogenic pairs from University of California, Irvine Alzheimer's Disease Research Center (UCI ADRC) (see Table S1; referenced as Tcw et al., 2022). One MAPT P301L line from Mayo Clinic.
Authentication	APOE genotype confirmed by Sanger sequencing (primers provided in Methods). G-banding karyotyping performed by WiCell Cytogenetics to confirm normal chromosomes. Pluripotency confirmed using a marker antibody panel (OCT3/4, NANOG, alkaline phosphatase, SSEA-4) following manufacturer protocols.
Mycoplasma contamination	All lines were authenticated and confirmed mycoplasma-free.
Commonly misidentified lines (See ICLAC register)	N/A

Palaeontology and Archaeology

Specimen provenance	N/A
Specimen deposition	*Indicate where the specimens have been deposited to permit free access by other researchers.*
Dating methods	*If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

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

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	N/A
Wild animals	*Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Reporting on sex	*Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.*
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	N/A
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☒	☐	Public health
☒	☐	National security
☒	☐	Crops and/or livestock
☒	☐	Ecosystems
☒	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☒	☐	Demonstrate how to render a vaccine ineffective
☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks: N/A

Novel plant genotypes: *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*

Authentication: *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
May remain private before publication.
N/A

Files in database submission: *Provide a list of all files available in the database submission.*

Genome browser session
(e.g. UCSC)
Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates: *Describe the experimental replicates, specifying number, type and replicate agreement.*

Sequencing depth: *Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*

Antibodies: *Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*

Peak calling parameters: *Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*

Data quality: *Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*

Software | *Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

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	Nano-flow cytometry was performed on extracellular vesicles (EVs) collected from conditioned media of human iPSC-derived microglia (iMGs). EVs were isolated from conditioned medium using sequential centrifugation followed by size-exclusion chromatography (as previously described; see Methods and Fig. 4A context). For NanoFCM labeling, EV samples were diluted to 5 × 10^9 particles/mL in dfPBS. Each staining reaction contained 2 µL EV sample, 0.5 µL of pre-diluted (1:20) PSMA1-FITC antibody (Abbexa, Cat# abx317148), and 0.3 µL of 0.1% Triton X-100. Samples were vortexed for 5 s and incubated overnight at 4°C in the dark. After incubation, 1 µL of labeled sample was further diluted 1:30 in dfPBS before acquisition. Quality control included QC beads (S16M-Exo), size standards, and blanks, following the manufacturer's protocol.
Instrument	Flow NanoAnalyzer (NanoFCM Inc.).
Software	Data acquisition and analysis were performed using the instrument manufacturer's NanoFCM acquisition/analysis software (Flow NanoAnalyzer platform). EV subpopulations were quantified using the auto-threshold function provided by the instrument software.
Cell population abundance	Not applicable to cell populations (this study used NanoFCM for EV particle analysis). EV readouts included EV size distribution and fluorescence-labeled EV subpopulations, quantified as particle counts/concentration and/or proportions based on fluorescence thresholding. Samples were standardized by dilution to a defined particle concentration (5 × 10^9 particles/mL) prior to labeling and acquisition.
Gating strategy	NanoFCM analysis used manufacturer QC beads, size standards, and blanks to define measurement/thresholding conditions. EV subpopulations were quantified using the instrument software's auto-threshold function (i.e., threshold-based classification of fluorescence-positive events above background/noise). Size distribution was assessed using the NanoFCM size standards per manufacturer guidance. No figure exemplifying the gating strategy is available for NanoFCM.

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

Magnetic resonance imaging

Experimental design

Design type	N/A
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	*Specify in Tesla*
Sequence & imaging parameters	*Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software — *Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*

Normalization — *If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*

Normalization template — *Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*

Noise and artifact removal — *Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

Volume censoring — *Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings — *Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*

Effect(s) tested — *Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference — *Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*

(See Eklund et al. 2016)

Correction — *Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity — *Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*

Graph analysis — *Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*

Multivariate modeling and predictive analysis — *Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*