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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	The raw BCL files were demultiplexed and aligned by Cellranger (v.3.1.0) against the human genome database (hg19, Ensembl 87). Raw count matrices were imported in R (v4.1.3) for data analysis. Flow cytometry data was collected using the MACSQuantify™ Tyto® Software

Data analysis	Cellranger v5.0.1, Cellranger v7.1.0. [http://griffan.github.io/VerifyBamID/] https://github.com/aertslab/popscle_helper_tools http://ftp.1000genomes.ebi.ac.uk [https://github.com/statgen/popscle] Scanpy 1.7.2 Harmony v0.1.0 in R v4.1.2 SCope vsn-pipelines [https://doi.org/10.5281/zenodo.3703108] ggplot2 (v 3.3.5) in R v4.1.2, Seurat (v4.1.0) under R version 4.1.2, Seurat v5.1.0. in R version 4.4.0 DESEQ2 in Seurat v4.1.0 Findmarkers 3 GO terms (GO:0005764, GO:0006954 and GO:006906, axon: 9606) and 1 pathway in KEGG (M189). hdWGCNA package (v0.1.1.9005) (SetupForWGCNA, MetacellsByGroups, NormalizeMetacells, ConstructNetwork) g:OSt functionality of gProfiler via the gprofiler-official python package (v1.0.0). MiloR v2.0.0 in R version 4.4.1. ggplot2 v3.5.1 CellChat v1.5.0 in R v4.1.2.

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

Raw sequencing data of human postmortem samples have been deposited in the European Genome-phenome Archive (EGA) under and with data accession no. EGAS00001006711 (to access the data itself under restricted access). Requests for accessing raw sequencing data will be reviewed by the VIB data access committee (dac@vib.be). Any data shared will be released via a Data Transfer Agreement that will include the necessary conditions to guarantee protection of personal data (according to European GDPR law). Further information and requests for resources and reagents should be directed to and will be fulfilled by Pegah Masrori (pegah.masrori@kuleuven.be) and Philip Van Damme (philip.vandamme@kuleuven.be). An online platform for user-friendly access and visualization of the data shown in this study is available and can be accessed through https://mancusolab.bioinf.be/c9orf72-als-microglia.html. The sequencing data of iPSC-derived human microglia in vitro and in vivo experiments is accessible at Gene Expression Omnibus (GEO) under the accession code detailed in the manuscript. The datasets from Gerrits et al. can be found at GSE16312. 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	For single nuclei RNA sequencing on brain and spinal cord samples we used 15 brain samples (central cortex) and 15 spinal cord samples, from: - control individuals (patients that died from other diseases, without history of neurological disorder and consented to undergo brain and/or spinal cord autopsy). - 5 patients with sporadic ALS (without mutation in SOD1, C9orf72, TARDBP or FUS) - 5 patients with ALS due to a C9orf72 mutation - ratio male/female 4:1
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	The samples from C9orf72 and sALS ranged 46-75 years old
Recruitment	The brain and spinal cord samples from were obtained from the Department of Pathology at University Hospitals Leuven (UZ Leuven), the Netherlands Brain Bank (NBB), Netherlands Institute for Neuroscience (NIN), Amsterdam and Hospital Clinic de Barcelona (HCB) and the Centre d'nvestgiacions Biomédiques August Pi I Sunyer (IDIBAPS).
Ethics oversight	The postmortem study protocol was reviewed and approved by the UZ Leuven NBB and HCB-IDIBAPS under approval numbers S65097, S59292, S60803. All human material has been collected from donors for or from whom a written informed consent for a brain autopsy and the use of the material and clinical information for research purposes had been obtained by the UZ Leuven.

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	To estimate the number of samples needed per experimental group for siingle nuclei sequencing experiments we used the power calculation POWSC tool. The following assumptions were made: 1) The smallest proportion of cell types we will study represents 5% of the sequenced cells. 2) The aim is to pick up changes of a logfold change of 0.5 with a false discovery rate of 0.1 and at least 40 reads coverage. 3) With a power of 80%. 4) Typically 5,000-6,000 nuclei per sample were sequenced. Using these assumptions, the power calculations show that we should sequence 26,000 cells per condition, which corresponds to between 4.3 and 5.2 samples per condition. For the in vitro experiments with iPSC-microglia and xenograft models, the sample size was calculated based on our previous studies (Mancuso et al., Nature Neuroscience 2024 and 2025)
Data exclusions	No data was excluded from analysis.
Replication	Multiple postmortem human samples were pooled together based on the disease of the patient or control. The RIN value of individual samples was determined multiple times to assess the viability to perform snRNAseq. We replicated the main findings from the postmortem data using orthogonal approaches, including iPSC-microglia in vitro systems, and xenograft models. For in vitro studies, at least 3 independent replicates were performed (the exact number is depicted in each figure legend). For the xenograft experiments, the exact number of mice per group are reported in the figure legends and methods section
Randomization	Postmortem samples were assigned to one of three groups based on the disease of the patient or controls. In vitro experiments as well as mouse experiments were randomized, and in all independent replicates there was an even representation of all experimental groups.
Blinding	The experimenters were blind when performing the analysis of the data

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

Antibodies used

Flow cytometry:
Mouse FcR blocker (1:10, Miltenyi, Cat#130-092-575)
PE-Pan-CD11b (1:50, Miltenyi, Cat#130-113-806)
BV421-mCD45 (1:500, BD Biosciences, Cat#563890)
APC-hCD45 (1:50, BD Biosciences, Cat#555485)
Viability dye (1:2000, eFluor 780, Thermo Fisher Scientific, Cat#65-0865-14)
TotalSeqTM-A cell hashing antibodies (1:500, Biolegend)

Histology:
Anti-Iba1 (Abcam, ab5076, 1:500, goat).
Anti-Cathepsin D (Abcam, ab75852, 1:500, rabbit)
Anti-Lamp1 (DSHB, ab2296838, 1:200, mouse)
Anti-Rab7 (ThermoFisher, PA5-52369, rabbit, 1:100)
Anti-EEA1 (SYSY, 237002, rabbit, 1:100)
Donkey anti-goat (Thermofisher, A32758, 1:1000)

	Donkey anti-rabbit (Thermofisher, A31573, 1:1000 and Biotium, 20802, 1:500) Donkey anti-mouse (Biotium, 20015, 1:500)
Validation	PE-Pan-CD11b: validated in splenocytes from BALB/c mice were stained with CD11b antibodies or with the corresponding REA Control antibodies. APC-hCD45: validated on human peripheral blood lymphocytes, stained with either APC Mouse IgG1, or κ isotype control. BV421-mCD45: validated on mouse splenic leucocytes preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody and then stained with either BD Horizon™ BV421 Rat IgG2b or κ Isotype Control. Anti-Iba1: validated on human spleen tissue, human lymph node, human and lateral prefrontal cortex. Anti-Cathepsin D: validated on mouse brain tissue lysate and human tissues. Rabbit monoclonal IgG (ab172730) used as negative control. Anti-Lamp1: validated on human tissue, epitope mapping confirmed. Anti-Rab7: validated with siRNA-mediated knockdown to confirm specificity in cell lines (e.g., A□431 cells showing loss of signal upon Rab7 silencing) Anti-EEA1: Validated for human, mouse, and rat; tested negative in zebrafish.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Name of line, Genotype, Source, Citation. BIONi010-C, Unedited Control, Bioneer, EBiSC RRID:CVCL_II81. BIONi010-C, C9orf72-KO, VIB-Center for Molecular Neurology, N/A C9-HRE, C9-1, University of Edinburgh, DOI: 10.1038/s41467-017-02729-0 C9-ISO, C9-1Δ, University of Edinburgh, DOI: 10.1038/s41467-017-02729-0 C9-HRE, C9-2, University of Edinburgh, DOI: 10.1038/s41467-017-02729-0 C9-ISO, C9-2Δ, University of Edinburgh, DOI: 10.1038/s41467-017-02729-0
Authentication	All the cell lines were authenticated by regular assessment of pluripotency as well as chromosomal duplications.
Mycoplasma contamination	All the lines used were regularly tested negative for mycoplams
Commonly misidentified lines (See ICLAC register)	None of the cell lines used in this study is known to be cross-contaminated or otherwise misidentified, and is not listed in the Register of Misidentified Cell Lines from ICLAC

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	Rag2-/- IL2rg-/- hCSF1-KI
Wild animals	No wild animals were used.
Reporting on sex	Experimental groups were balanced in terms of the sex of the mice
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	Animal experiments were approved by the local Ethical Committee of Laboratory Animals of the University of Antwerp (government license LA1100470 ECD project number 2021-13, following local and EU guidelines.

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

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	Mic were sacrificed with an overdose of sodium pentobarbital and immediately perfused with ice-cold 1x DPBS (Gibco, Cat#14190-144) supplemented with 5U of heparin (LEO). After perfusion, 1 hemisphere of each mouse brain without cerebellum and olfactory bulbs was placed in FACS buffer (1x DPBS, 2% FCS and 2 mM EDTA) + 5 µM Actinomycin D (ActD, Sigma, Cat#A1410-5MG) for transcriptomics. Brains were mechanically and enzymatically dissociated using Miltenyi neural tissue dissociation kit P (Miltenyi, Cat#130-092-628) supplemented with 5 µM ActD. Next, samples were passed through a 70 µm strainer (BD2 Falcon), washed in 10 ml of ice-cold FACS buffer + 5 µM ActD and spun at 300g for 15 minutes at 4°C. Note that 5 µM ActD was kept during collection and enzymatic dissociation of the tissue to prevent artificial activation of human microglia during the procedure as previously reported12. ActD was removed from the myelin removal step to prevent toxicity derived from long-term exposure. Following dissociation, myelin was removed by resuspending pelleted cells into 30% isotonic Percoll (GE Healthcare, Cat#17-5445-02) and centrifuging at 300g for 15 minutes at 4°C. Accumulating layers of myelin and cellular debris were discarded and Fc receptors were blocked in FcR blocking solution (1:10, Miltenyi, Cat#130-092-575) in cold FACS buffer for 10 minutes at 4°C. Next, cells were washed in 5 ml of FACS buffer and pelleted cells were incubated with the following antibodies: PE-Pan-CD11b (1:50, Miltenyi, Cat#130-113-806), BV421-mCD45 (1:500, BD Biosciences, Cat#563890), APC-hCD45 (1:50, BD Biosciences, Cat#555485), TotalSeqTM-A cell hashing antibodies (1:500, Biolegend) and viability dye (1:2000, eFluor 780, Thermo Fisher Scientific, Cat#65-0865-14) in cold FACS buffer during 30 minutes at 4°C. After incubation, cells were washed, and the pellet was resuspended in 500 µl of FACS buffer and passed through 35 µm strainer prior sorting.
Instrument	MACSQuant Tyto
Software	MACSQuantify™ Tyto® Software 1.0 and FlowJo v10
Cell population abundance	Both mouse host (CD11b+ mCD45+) and human transplanted microglia (CD11b+ hCD45+) are two clear distinct populations. This study did not aim to quantify these populations by flow cytometry, but rather purify them for downstream trannscriptomic and epigenomic analyses.
Gating strategy	BSC and SSC were used to filter debri and doublet discrimination. e780 (Thermo Fisher) was used as a viability marker. All analyses were performed on viable singlets.

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