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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	no software was used
Data analysis	CellRanger (version 3.0.0) software from 10x Genomics was used to preprocess the data (i.e. deduplication, mapping to GRCh38 genome, read counting according to the pre-mRNA reference from 10x Genomics). ABACUS, was used to count exonic- and intronic-mapping reads separately which were used for barcode filtering. The single-cell analysis R package Seurat (3.0.3.9029) was used for quality control, dataset integration, dimensionality reduction, clustering and differential gene expression analyses. Cell-cell interaction analysis was performed with CellChat (Jin et al. 2020). Gene ontology analyses were performed with the R package clusterProfiler (v3.12.0) and/or Metascape (Zhou et al. 2019). Visualizations were made with the R packages ggplot2 (3.1.1) and gplots (3.0.1.1). Quantification of immunohistochemical staining were performed with ImageJ (2.0.0) and/or QuPath (v0.2.3). Analysis of multiplexed IHC was performed with nForm advanced image analysis software (PerkinElmer) and image segmentation was done in Nis Elements (Nikon). FANS data was analyzed with Kaluza Analysis Software (v2.1). Inform advanced image software (v2.0) was used for IF quantification.

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 data reported in this study are available through Gene Expression Omnibus at https://www.ncbi.nlm.nih.gov/geo with accession number GSE163122.

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 power calculations were performed prior to data collection. We included 42 samples from frontal, temporal and occipital cortex of 7 CTR and 13 FTD-GRN donors that were age- and sex-matched.
Data exclusions	For snRNAseq, barcodes with < 250 intronic reads, <100 exonic reads and less intronic reads than exonic reads were removed from the dataset as these contain ambient RNA or cellular RNA. Given the high quality of the dataset, no strict quality control thresholds were used. A low number of nuclei with a mitochondrial content > 5% were removed from the analysis.
Replication	We validated our findings at the protein level with immunohistochemical stainings on tissue from the contralateral hemisphere of all donors. We performed RT-qPCR on total brain tissue RNA from the same tissue block as used for nuclei isolation.
Randomization	We isolated nuclei in batches of 6 samples per day, obtained from two donors (i.e. all region's of 1 donor in the same batch). Each day we performed nuclei isolation of 1 FTD-GRN and 1 CTR donor to avoid confounding batch effects.
Blinding	During sample collection the researchers were blinded for the diagnosis of the donors and degree of pathology.

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
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

FACS:
NeuN-AF647 (RBFOX3/NeuN 1B7 AF647 mouse mAb, Novus Biologicals, NBP1-92694AF647)
Olig2-AF488 (Anti-Olig2 clone 211F1.1 AF488 mouse mAb, Merck Millipore, MABN50A4)
IHC:
TDP43 (anti-pTDP-43, CAC-TIP-PTD-M01, Cosmo Bio)
IBA1 (anti-Iba1, 019-19741, Wako)
GFAP (anti-GFAP, Z0334, Dako)
GFAP (anti-GFAP, 14-9892-82, eBioscience)

Claudin5 (anti-CLDN5, 4C3C2, Thermo Fisher)
NEUN (anti-NeuN, Mab377, EMD Millipore)
PDGFRB (anti-PDGFRB, Ab32570, Abcam)
Laminin (Laminin, NB300-144, Novus Biologicals)
Aquaporin4 (anti-AQP4, AB3594, Merck Millipore
ULEX (anti-UEA-1, B-1065, Vector labs)
WDR49 (anti-WDR49, Human Protein Atlas, HPA036226)
Fibronectin (anti-fibronectin, AB2033, Merck Millipore)

Validation

NeuN: Validated for Flow Cytometry. Validated for Human
Olig2: Predicted to react with human based on 100% sequence homology
TDP43: Validated for IHC. Validated for Human.
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IBA1: Validated for IHC. Validated for Human.
GFAP: Validated for IHC. Validated for Human.
CLDN5:Validated for IHC. Validated for Human.
NEUN: Validated for IHC. Validated for Human.
PDGFRB: Validated for IHC. Validated for Human.
Laminin: Validated for IHC. Validated for Human.
AQP4: Validated for IHC. Validated for Human.
ULEX: Validated for IHC.
WDR49 alidated for IHC, Validated for Human
Fibronectin Validated for IHC, Validated for Human

Human research participants

Policy information about studies involving human research participants

Population characteristics	Frozen postmortem brain tissue of 20 human donors. 12 donors were diagnosed with FTD-GRN. 7 donors were clinical controls.
Recruitment	FTD samples were selected based on GRN-mutation. CTR samples were age- and sex-matched to the FTD-GRN donors. Brain tissue was selected and provided by the Netherlands Brain Bank and NeuroCEB Neuropathology network .
Ethics oversight	University Medical Center Groningen

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

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	Frozen brain tissue was cryosectioned and 30-40 sections of 40 um per sample were collected in a sucrose lysis buffer containing Triton-X100. The suspension was pipetted on top of a dense sucrose layer and 1.5 hours of ultracentrifugation was performed. The pelleted nuclei were resuspended in PBS and incubated with anti-NeuN-AF647 and anti-Olig2-AF488 for 45 minutes and 5 minutes with DAPI.
Instrument	MoFlo Astrios (BeckmanCoulter).
Software	Flow cytometry data was analyzed with Kaluza Analysis Version 2.1.
Cell population abundance	We enriched for microglia nuclei by depleting other abundant CNS cell types. Nuclei were stained with anti-NeuN and anti-Olig2 antibodies. Of each sample, ~40% of the nuclei were Olig2+, ~40% of the nuclei were NeuN+ and ~10% of the nuclei were negative for both markers. The DAPI+NeuN-Olig2- nuclei were loaded on a 10x Genomics Chromium system to perform single-nucleus RNA sequencing. Subsequent clustering analysis of the resulting sequencing data showed clear segregation of microglia, astrocytes and vasculature subtypes, as discussed in the manuscript.
Gating strategy	Nuclei were sorted on a MoFlo Astrios (BeckmanCoulter) us ing a 70 um Nozzle, as shown in Figure S1 of the manuscript. The blue (488 nm) laser was used for measuring Forward Scatter (FSC), Side Scatter (SSC), Olig2-AF488 (513/26) and 2 parameters to measure autofluorescence (576/21 and 795/21). DAPI (450/50) was excited using a violet (405 nm) laser and NeuN-AF647 (660/20) was excited by a red (640 nm) laser. First, a threshold was set on SSC and sorting gates were applied on FSC/SSC to

remove mainly big aggregates. Then, the FSC Height/SSC Width plot was used to select single nuclei by pulse processing. The FSC/DAPI plot was used to remove all other debris from the sample by using DAPI as a DNA stain to select the nuclei. By using the 795/70 and 576/21 (auto) fluorescence we could remove even further all high fluorescent nuclei assuming those were likely to be aggregates that passed through all previous gates. In the final plot the microglia-enriched nuclei population was selected by using NeuN-AF674 and Olig2-AF488 to deplete neurons and oligodendrocytes. The most negative corner of the NeuN-Olig2-population was sorted.

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