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Reporting Summary

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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	bwa version 0.6.2-r126 for sequence alignment. http://bio-bwa.sourceforge.net/ A custom Perl script for the extraction of mRNA counts based on unique molecular identifiers. Summit software version6.3.0.16900 for data acquisition
Data analysis	STAR (version 2.5.2b) for sequence mapping/alignment to the mouse genome: https://github.com/alexdobin/STAR Gencode M11 available from https://www.gencodegenes.org/ featurecounts (version 1.5.1) for determination of gene count, available from http://subread.sourceforge.net/ RaceID/StemID for clustering and other single cell data analysis available from https://github.com/dgrun/StemID Data presentation (tsne plots, heatmaps): R (version 3.4.3), ggplot2 (version 2.2.1) and gplots (vesion 3.0.1) were used available from https://cran.r-project.org/ (R), https://www.bioconductor.org/ (ggplot2, qplots) https://academic.oup.com/bioinformatics/article/32/18/2847/1743594 (Heatmap) Canonical correlation analysis was available from https://www.nature.com/articles/nbt.4096 FlowJo software (v. X.0.7) for post-acquisition analysis of FACS data

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Raw data for mouse and human single cell RNA-sequencing are available from the following accession numbers: GSE120629 (mouse), GSE120747 (mouse) and GSE124335 (human).

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 statistical methods were used to predetermine sample sizes. We ensured that they were similar to those generally employed in the field.
Data exclusions	Samples, for single-cell sequencing related to Fig.1-5, that failed during library preparation or had very low counts (less than 5000 reads mapped to mouse genes and human cells with a minimal total transcript count of less than 1500) were excluded. Also samples with no detectable Hexb (less than 10 counts) were excluded. No other exclusions were made. From the human samples non-microglial cells were excluded if they expressed a cell-type specific gene signature other than microglia (e.g. t cells, monocytes, oligodendrocytes).
Replication	To ensure the reproducibility of the experimental findings for single-cell RNA-seq, 121-178 microglia cells from 3-4 individual mice for each sample group, 1180 cells from healthy human brain regions of 5 patients, and 422 cells from the brains of 5 MS patients, were used in this study. For the quantification shown in Fig. 2d, 2g, 3f and g, Extended Data Fig. 2b, Extended Data Fig. 3d, Extended Data Fig. 5d, f and h, we have used more than 100 cells from 3-4 individual mice for each sample group. All attempts at replication were successfully done.
Randomization	For all experiments, mice used were randomly allocated into each experimental group by TM, CB and PK.
Blinding	Blinding was not possible because we needed to know how many cells were properly sorted from each genotype of mice during sample collection and for 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
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	For FACS sorting of mouse samples: anti-CD11b antibody (Brilliant Violet 421, M1/70, BioLegend); anti-CD45 antibody (PE/Cy7, 30-F11, BD Biosciences); anti-Ly6C antibody (PerCP/Cy5.5, AL-21, BD Biosciences); anti-Ly6G antibody (PE, 1A8, BD Biosciences); anti-CD206 antibody (APC, C068C2, BioLegend). For FACS sorting of human samples: anti-CD45 (clone HI30, BD Bioscience, Heidelberg, Germany; Cat# 555485) anti-CD11b (clone M1/70, eBioscience, San Diego, USA; Cat# 101237) anti-CD3 (clone SP34-2, Alexa Flour® 405 1:500, BD Bioscience, Heidelberg, Germany; Cat# 551916) anti-CD19 (clone SJ25C1, BioLegend, San Diego, USA; Cat# 363003)

and anti-CD20 (clone 2H7, BioLegend, San Diego, USA; Cat# 302311)
For histological analysis of mouse sections: anti-Cystatin C (Cst3) antibody (AF1238, R&D systems); anti-Iba1 antibody (ab178846, Abcam); anti-NeuN antibody (MAB377, Millipore); anti-APC antibody (OB80, Millipore); anti-Aldh1l1 antibody (ab87117, Abcam); anti-APOE antibody (AB947, Millipore); anti-CTSB antibody(ab58802, Abcam);anti-SPARC antibody (IC942G, R&D Systems);anti-TMEM119 antibody (ab209064, abcam); anti-SPP1 antibody (ab8448, abcam); anti-CD74 antibody (In1/CD74, BioLedend);
For human sections; anti-Iba1 antibody (ab178846, Abcam; ab139590, Abcam; NB100-1028, Novus Biologicals); anti-SPP1 antibody (HPA027541, Sigma); anti-CD74 antibody (ab9514, abcam); anti-CTSD antibody (ab6313, abcam); anti-MRP14 antibody (T-1026, BMA Biomedicals; LS-B12844, LSBio); Alexa Flour® 405 (1:500, ab175651, abcam); Alexa Flour® 488 (1:500, A-21206, A32814, A-21202, Thermo Fisher Scientific); Alexa Flour® 568 (1:500, A-10042, A-11057, Thermo Fisher Scientific); Alexa Fluor® 647 (1:500, A-31573, A32849, A-31571, Thermo Fisher Scientific).

Validation

All antibodies used were validated for use in FACS or histological analysis with mouse or human samples, which are shown on the website provided by respective companies.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Mouse (CD1 females at the age of 3 weeks and 16 weeks, CD1 embryos at the age of embryonic E16.5 days)
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	The ethics board of the universities of Freiburg and Göttingen, Charité Universitätsmedizin Berlin approved of all experiments.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	All information for human material is provided in Supplementary Table 1.
Recruitment	Patients were prospectively recruited.
Ethics oversight	The institutional review boards of the universities of Freiburg and Essen approved of all experiments and informed consent was obtained from the human subjects prior to inclusion in the study.

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	For mouse, after transcardial perfusion with PBS, brains were roughly minced and homogenized with a potter in HBSS containing 15 mM HEPES buffer and 0.54 % Glucose. Whole-brain homogenate was separated by 70/37/30 % layered Percoll gradient centrifugation at 800 g for 30 min at 4 °C (no brake). The CNS macrophages containing interphase was then collected and washed once with PBS containing 2% FCS and 10mM EDTA before staining. Cells were stained with primary antibodies directed against CD11b (M1/70, BioLegend), CD45 (30-F11, BD Biosciences), Ly6C (AL-21, BD Biosciences) and Ly6G (1A8, BD Biosciences) for 20 min, and CD206 (C068C2, BioLegend) for 45 min at 4 °C. For human, brain blocks were roughly minced and homogenized with a potter in HBSS containing 15 mM HEPES buffer and 0.54 % Glucose. Whole-brain homogenate was separated by 37 % Percoll centrifugation at 800 g for 30 min at 4 °C (no brake). The pelet containing CNS macrophages was then collected and washed once with PBS containing 2% FCS and 10mM EDTA before staining. Cells were stained with primary antibodies directed against CD45 (, BD Biosciences) for 20 min at 4 °C.
Instrument	MoFlo Astrios (Beckman Coulter) for sorting
Software	Data were acquired with Summit software version6.3.0.16900 (Becton Dickinson). Postacquisition analysis was performed using FlowJo software, version X.0.7

Cell population abundance	We have confirmed at a single cell level that the sorted cells express well-known microglial genes for mouse samples. For human samples, more than 80 of all single cells were Dapi-negative. Microglia consisted of more than 80 % of lineage-negative(CD3, CD19, CD20) CD45-positive cells.
Gating strategy	We have shown the gating strategy for mouse samples that we used in this study in Supplementary Fig. 1. CNS cells were gated for G1 and G2 (singlets), followed by being gated for living cell (G3, fixable viability dye), CD45intCD11b+ (G4), Ly6C-Ly6G- (G5), and CD206- (G6). For human samples, single cells were obtained and Dapi-negative live cells were selected. Cells were FACS-sorted into 384-wells based on positivity for CD45 and lineage negativity (CD3, CD19, CD20).

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