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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	qPCR data were collected on the StepOnePlus™ Real-Time PCR System (ThermoFisher Scientific). Flow cytometry data were acquired on a BD LSRII and ARIAII sorter (BD Biosciences) with BD FACSDiva™ v8 software . The complete blood cell counts were collected with an automated blood count analyzer (Hemavet, Drew Scientific Inc). Images were acquired with an Axioplan II epifluorescence microscope (Zeiss) equipped with Plan-Neofluar objectives (10 × 0.3 NA, 20 × 0.5 NA, or 40 × 0.75 NA) or all-in-one BZ-X700 fluorescence microscope (Keyence), Fluoview FV 1000 (Olympus) confocal microscope and Fluoview software v3.1b with Olympus 40X and 0.8 NA water-immersion lens as described9, or Aperio Versa scanner (Leica) with Aperio Imagescope 12.4 and 1.25X, 10X, and 20X lenses. Microglia HTS was imaged using an INCell Analyzer 2000 (GE Healthcare) equipped with a 10x objective and excitation/emission filter pairs 350 nm/455 nm and 579 nm/ 624 nm.
Data analysis	GraphPad Prism v 7.03 (GraphPad Software, Inc.) was used to prepare graphs and to perform statistical analyses. Microglia HTS analysis: Images were analyzed with the GEHC IN-Cell Developer Toolbox version 1.9. Flow cytometry data were analyzed using FlowJo version 10.6.2 software (Tree Star, Inc.). R and R packages: R version 3.5.2; bindrcpp_0.2.2; dplyr_0.8.3; Seurat_3.1.1; Matrix_1.2-14; cowplot_1.0.0; ggplot2_2.2.1; RColorBrewer_1.1.-2; Tophat_2.0.13; edgeR_3.24.2; Hopach_2.42.0 For bulk RNA-seq, functional enrichment analysis of a data set with 2,145 DEGs in ROS+ versus MHC II+ microglia was performed in Cytoscape v3.3 using BiNGO plugin and GO annotations downloaded on 8-Dec-2016. Filtering for GO term results with <2000 genes, 592 Biological Process terms with a corrected P-value <0.05 were found. GO term enrichment was also performed on the exclusive subsets of ROS-expressed genes (1,613) and MHC II-expressed genes (924), resulting in 58 and 783 filtered Biological Process terms, respectively. From these GO term results, 6 were selected that represented processes of interest, maximum specificity (i.e., low total gene membership) and exclusive significance (i.e., non-overlapping terms). Interaction networks were constructed for each set of DEGs associated with these 6 terms using GeneMANIA v3.4.1. Default co-expression and physical interaction sources were selected from the

network construction; the option to add a number of related genes was set to zero; all other settings were default values. The interaction data from GeneMANIA was imported into Cytoscape for visualization and data overlay. Co-expression clusters were defined as the largest connected set of either up- or down-regulated genes and extracted as subnetworks

For scRNA-seq, reads were aligned to the mm10 assembly using the Cellranger count v2.0.1 (10x Genomics) and all downstream clustering analysis, visualisation, and differential gene expression analysis was performed using the Seurat v3.4.2 R Tool kit as described in Methods.

The statistical significance of the changes in the mean clinical score for each day of the EAE experiment was estimated using permutation tests . The corresponding P values were estimated using 1000 permutations. In each permutation, mice were randomly permuted.

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

The scRNA-seq and bulk RNA-seq data files are available in the Gene Expression Omnibus database under SuperSeries accession code GSE146295.

GGT pathway model will be available at WikiPathways: https://www.wikipathways.org/instance/WP4466. All data generated and analyzed in this study are available within the paper. Any additional data can be made available from the corresponding author upon reasonable request.

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 size, but our sample sizes are similar to those reported previously for pharmacologic treatments in EAE models (Adams et al., J Exp Med 204, 571-582 (2007); Ryu et al., Nat Immunol., 19, 1212-1223 (2018); and RNAseq experiments (Bagadia et al., Nat Immunol 20, 1174-1185 (2019); Zemmour et al., Nat Immunol 19, 291-301 (2018))

Data exclusions

No samples or animals were excluded from the analysis.

The R toolkit Seurat was used for quality control (QC) processing, graph-based clustering, visualizations, and differential gene expression analyses of scRNA-seq data and performed in R version 3.4.2. Cellranger Aggr aggregated dataset of 9,079 cells were filtered with QC parameters 200 – 5,000 nFeature_RNA per cell, > 5% and > 25% mitochondrial and ribosomal genes, respectively. The percent of mitochondrial (percent.mito) and ribosomal (percent.ribo) genes were regressed out. All remaining variable genes were used for downstream analyses, including immediate early response genes induced by cell isolation procedure24. Following QC, 17,814 genes across 8,701 single cells were analyzed with Seurat version 3 default parameters unless otherwise stated Extended Data Fig. 2c,d). To determine statistically significant principal components (PC), JackStraw was performed with num.replicate = 100. For clustering analysis, FindNeighbors and FindClusters were used with the first 20 significant PC and a resolution = 0.8, respectively. For subclustering analysis, monocyte/macrophage or microglia clusters in Fig 1h, were separately re-clustered by running through the Seurat pipeline as described above. FindClusters was implemented at a resolution = 0.4 for monocyte/macrophages or resolution = 0.3 for microglia subcluster analysis. Differentially expressed genes (DEGs) for each cluster was determined by FindAllMarkers with parameters min.pct, 0.25; log2fc.threshold, 0.25 using Wilcox statistical test. Genes that met the above criteria with adjusted P-value < 0.05 (Benjamini-Hochberg correction) were used for functional enrichment analysis. GO enrichment analysis of DEGs was performed in DAVID and Metascape bioinformatic resources.

Replication

The number of experimental repeats is detailed at the bottom of each legend for each figure. All attempts at replication following the protocols described in the methods were successful. Since microglia activation can be influenced by the culture conditions, FBS was batch-tested with three QC criteria: high cell yield, no effect on morphologic activation at baseline, and response to LPS-induced morphologic activation by at least 50%. M-CSF was also batch-tested for effects on baseline gene expression and cell differentiation. Microglia activation assays in the presence of small molecule inhibitors performed at the UCSF Small Molecule Discovery Center (SMDC) were replicated at the Gladstone Institutes with similar results.

Randomization

Animals were randomly assigned to experimental groups at the beginning of experiments. The randomization of animal studies is stated in the Method section.

Blinding

For EAE studies and histopathological experiments, the researchers were blinded as to the mouse treatment conditions. Mice were divided into experimental groups in an unbiased manner. The mice were randomized and coded to assign groups or collect data for animal

experiments. All imaging and quantification experiments were performed in a blinded manner.

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

Flow cytometry: Cells were stained with CD3 (17A2, Biolegend), CD4 (GK1.5, Biolegend), CD8 (53-6.7, Biolegend), CD62L (MEL-14, Biolegend), CD44 (1M7, Biolegend), CD25 (3C7, Biolegend), CD11b (M1/70, Biolegend), CD45 (30-F11, Biolegend), MHCII (M5/114.15.2, Biolegend), IL-17A (TC11-18H10.1, eBioscience), IFN-γ (XMG1.2, eBioscience), Foxp3 (FJK-16S, eBioscience), or GGT1 (1:100; ab55138, Abcam). Antibodies were used at 1:300 dilution unless otherwise stated in the Methods.

Immunohistochemistry: Tissue sections were stained with mouse anti-gp91 (CYBB, 1:200; 53, BD Biosciences), mouse anti-iNOS (1:500, 610329, BD Biosciences), rabbit anti-Iba-1 (1:1000; 019-19741, Wako), rat anti-MHCII (1:300; M5/114.15.2, Thermo Fisher Scientific), rabbit-coagulation factor X (F10; NBP1-33320, NOVUS), mouse anti-CLEC4E (1:700; AT16E3, abcam), mouse anti-neurofilament H non-phosphorylated (1:100; SMI-32, BioLegend), mouse anti-myelin basic protein (1:100; SMI-99, BioLegend), rabbit polyclonal anti-GGT1 (1:100; SAB2701966, Sigma), goat polyclonal anti-4HNE (1:200; ab46544, Abcam), or rabbit polyclonal anti-TH (1:2000; P40101, Pel Freez)

Validation

All antibodies used in this study are from commercial sources and have been validated by the vendors and previous studies done by our lab or other labs. Antibodies CD3 (17A2, Biolegend), CD4 (GK1.5, Biolegend), CD8 (53-6.7, Biolegend), CD11b (M1/70, Biolegend), CD45 (30-F11, Biolegend), MHCII (M5/114.15.2, Biolegend), IL-17A (TC11-18H10.1, eBioscience), IFN-γ (XMG1.2, eBioscience), Foxp3 (FJK-16S, eBioscience), mouse anti-iNOS, rabbit anti-Iba-1, rat anti-MHCII , mouse anti-neurofilament H non-phosphorylated SMI-32, mouse anti-myelin basic protein, were previously validated in our publications Adams et al., J Exp Med 204, 571-582 (2007); Ryu et al., Nat Commun 6, (2015); Ryu et al., Nat Immunol., 19, 1212-1223 (2018); Petersen et al., Neuron 96, 1003-1012 (2017). Validation data are available on the manufacturer's website. CD62L (MEL-14, Biolegend), CD44 (1M7, Biolegend), CD25 (3C7, Biolegend) were validated in mouse splenocytes by the manufacture. Mouse anti-CLEC4E and rabbit anti-GGT1 were validated by immunohistochemistry in colorectral cancer and xenograft tissue by the manufacture; and mouse anti-gp91 was validated by immunohistochemistry in mouse macrophages by the manufacture. We validated antibodies in spinal cord tissue from healthy and EAE mice using species-specific secondary antibody controls. Appropriate antibody dilutions were performed based on preliminary experiments and intensity of fluorescent signals.

Animals and other organisms

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

Laboratory animals

SJL/J, NOD, C57BL/6, and Ggt1dwg/dwg mice were purchased from The Jackson Laboratory, and Sprague-Dawley rat P0 litters were purchased from Charles River Laboratories. Ccr2RFP/RFP mice on C57BL/6 background (provided by I. F. Charo, Gladstone Institutes) were crossed with Cx3cr1GFP/GFP mice58 to generate Cx3cr1GFP/+Ccr2RFP/+ mice.

Wild animals

The study did not involve wild animals

Field-collected samples

The study did not involve samples collected from the field

Ethics oversight

Mice were housed under IACUC guidelines in a temperature and humidity-controlled facility with 12 h light–12 h dark cycle and ad libitum feeding. All animal protocols were approved by the Committee of Animal Research at the University of California, San Francisco, and were in accordance with the National Institutes of Health guidelines.

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	Sample preparation for Tox-seq assay is described in detail in the Methods of the manuscript. Primary splenocytes and spinal cord leukocytes were harvested and processed for flow cytometric analysis as described in Methods. Extended Data Fig 5: Cultured BMDMs were incubated with Sytox blue live/dead stain and processed for flow cytometric analysis; and Extended Data Fig 7: Cells were harvested, stained for surface antigens, fixed and then immediately analyzed by LSR II (BD Biosciences).
Instrument	ARIAII & LSR II (BD)
Software	BD FACSDiva v8 and FlowJo software v10
Cell population abundance	When cells were sorted or enriched, the purity was confirmed by flow cytometry and routinely >95 %.
Gating strategy	Extended Data Fig 1: single cells were gated by FSC-A/SSC-A and then doublet discrimination was performed first by FSC-H/FSC-W followed by SSC-H/SSC-W. Next, live sytox- CD11b+ cells were gated for, then cells were sorted based on ROS- and ROS+. Extended Data Fig 4: single cells were gated by FSC-A/SSC-A and then doublet discrimination was performed first by FSC-H/FSC-W followed by SSC-H/SSC-W. Next, CD45hiCD11b+ cells and CD45loCD11b+ cells were gated on, followed by sorting for ROS-MHCII-, ROS-MHCII+, ROS+MHCII-, ROS+MHCII+. Extended Data Fig 5c: single cells were gated by FSC-A/SSC-A and then doublet discrimination was performed first by FSC-H/FSC-W, then CD45+ cells were gated on followed by live CD45+ Sytox blue- and dead CD45+ Sytox blue+. Extended Data Fig 5e: single cells were gated by FSC-A/SSC-A and then doublet discrimination was performed first by FSC-H/FSC-W, then CD45+ cells were gated on followed by CD45 and CD11b expressing populations

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