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	ZEN software (ZEISS Image acquisition software, v2.3), ZEISS Atlas 5 software (v5.2.3.1)
Data analysis	Image J (NIH, v1.52g), GraphPad Prism (v7.0), TopHat (v2.1.1), StringTie (v1.3.5), EdgeR (v3.32.1), Cutadapt (v1.16), Bowtie2 (v2.3.4), MACS2 (v2.2.7.1), ˜eatureCounts (v2.0.0), BWA-mem (0.7.17-r1188), covNorm (v1.0.0), Taiji (v1.3.0), CellOracle (v0.18.0), cellranger(v3.0.2), Seurat (v3.1.5)

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 raw and processed data generated in this study have been deposited in the GEO database under accession number GSE158329 [https://www.ncbi.nlm.nih.gov/

geo/query/acc.cgi?acc=GSE158329]. The single-cell transcriptome data of developing mouse cortice were available at the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under accession PRJNA637987 [https://www.ncbi.nlm.nih.gov/sra?term=PRJNA637987] or at http://mousebrain.org/development/.

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	
Reporting on race, ethnicity, or other socially relevant groupings	
Population characteristics	
Recruitment	
Ethics oversight	

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 chosen as standard in the field or depend on the availability of datasets.
Data exclusions	Low-quality cells were excluded for single-cell data analyses. For public scRNA-seq datasets, other cell types were discarded for further analysis, except for astrocyte lineage and glutamatergic neuronal lineage.
Replication	Experiments were repeated and yielded reproducible results. Experimental autoimmune encephalomyelitis and imaging experiments were performed independently at least three times. RNA-seq, ATAC-seq, HiCAR, and snRNA-seq were conducted using two independent cohorts of animals, with each cohort processed separately.
Randomization	Mice were randomly assigned to experimental or control groups with matched age and sex, whenever possible.
Blinding	During the experiment and analysis, all experiments were performed blindly.

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

rabbit anti-S100β (Abcam, ab52642, 1:500)
chicken anti-GFAP (Aves Labs, GFAP, 1:500)
rabbit anti-GR (Cell signaling Technology, 12041S, 1:500)
rabbit anti-IBA1 (WAKO, 019-19741, 1:500)
chicken anti-GFP (Aves Labs, GFP-1020, 1:1000)
rat anti-CD45 (BD, 550539, 1:500)
rat anti-CD4 (Biolegend, 100506, 1:100).
rabbit anti-GR (Thermo Fisher, PA1-511A, 2ug used for ChIP-seq)
rabbit c-JUN (Cell Signaling Technology, #9165, 2ug used for ChIP-seq)

Alexa fluorophore-conjugated secondary antibodies (Alexa Fluor 647) (Jackson, 703-605-155, 1:500)
Alexa fluorophore-conjugated secondary antibodies (Alexa Fluor 488) (Jackson, 703-545-155, 1:1000)
Alexa fluorophore-conjugated secondary antibodies (Alexa Fluor 488) (Invitrogen, A-21206, 1:1000)
Alexa fluorophore-conjugated secondary antibodies (Alexa Fluor 488) (abcam, ab150153, 1:1000)
Alexa fluorophore-conjugated secondary antibodies (Alexa Fluor 594) (Invitrogen, A-21209, 1:1000)
Alexa fluorophore-conjugated secondary antibodies (Alexa Fluor 594) (Invitrogen, A-21207, 1:1000)
Alexa fluorophore-conjugated secondary antibodies (Alexa Fluor 405) (abcam, ab175670, 1:500)
anti-mouse CD11b (BD, 553311, 1:100)
PerCP/Cyanine5.5 anti-mouse Ly-6G (BioLegend, 127615, 1:100)
APC/Cyanine7 anti-mouse CD11c (BioLegend, 117323, 1:100)
APC/Cyanine7 anti-mouse CD4 (BioLegend, 100525, 1:100)
PE/Cyanine7 anti-mouse CD8a (BioLegend, 100721, 1:100)
PE anti-mouse Foxp3 (BioLegend, 126403, 1:100)
BV510 anti-mouse IFN-γ (BioLegend, 505841, 1:100)
BV421 anti-mouse IL17A (BD, 566286, 1:100)

Validation

chicken anti-GFAP (Aves Labs) : supported by several references including PubMed: 30355486
Rabbit anti-S100b (Abcam) : supported by several references including PubMed: 30478038
rabbit anti-GR (Cell signaling) : supported by several references including PubMed: 37527657
rabbit anti-GR (Thermo Fisher, for ChIP-seq) : supported by several references including PubMed: 36640364
rabbit anti-IBA1 (Wako) : supported by several references including PubMed: 24270812
chicken anti-GFP (Aves labs) : supported by several references including PubMed: 30808661
rat anti-CD4 (Biolegend) : supported by several references including PubMed: 34297917
rat anti-CD45 (BD) : supported by several references including PubMed: 36470947
rabbit c-JUN (Cell Signaling Technology) : supported by several references including PubMed: 33627422
anti-mouse CD11b (BD) : supported by several references including PubMed: 38748792
PerCP/Cyanine5.5 anti-mouse Ly-6G (BioLegend) : supported by several references including PubMed: 35849045, 36928411
APC/Cyanine7 anti-mouse CD11c (BioLegend) : supported by several references including PubMed: 16985170
APC/Cyanine7 anti-mouse CD4 (BioLegend) : supported by several references including PubMed: 34719426
PE/Cyanine7 anti-mouse CD8a (BioLegend) : supported by several references including PubMed: 36385524
PE anti-mouse Foxp3 (BioLegend) : supported by several references including PubMed: 36914891
BV510 anti-mouse IFN-γ (BioLegend) : supported by several references including PubMed: 28778586
BV421 anti-mouse IL17A (BD) : supported by several references including PubMed: 30454647

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

Aldh1l1-EGFP mice generated by the GENSAT project were obtained from the Barres laboratory. FVB-Tg(Aldh1l1-cre/ERT2)1Khakh/J mice and B6.Cg-Nr3c1tm1.1Jda/J mice were obtained from the Jackson Laboratory.
The Aldh1l1-gfp;FVB/N mice were used for multi-omics analyses of developmental stages (E16.5, E18.5, P1, P3, P7, P17, P30) and WT;FVB/N littermates were used for FACS sorting as a negative control.
Tg(Aldh1l1-cre/ERT2)1Khakh/J mice were crossed with B6.Cg-Nr3c1tm1.1Jda/J mice to generate astrocyte-specific Nr3c1 conditional knockout (AGR cKO) mice. For confirming GR expression, the mice were sacrificed at P5, P7, P17, and P53. For imaging and EAE experiments, the 9-13 weeks mice were used.
All mice were housed in specific pathogen-free (SPF) conditions, in a temperature-controlled (20-24C) and humidity-controlled (40-60%) environment, under a 12h light-dark cycle with free access to chow and water.
During astrocyte collection at developmental stages, embryonic mice were obtained from pregnant females. Pregnant female mice were anesthetized with 2% isoflurane, and the abdomen was surgically opened to collect embryos. The cortex of each embryonic pup was prepared by decapitation. After surgery, the pregnant females were euthanized by carbon dioxide (CO_2) inhalation. P0-P3 pups were placed on ice for approximately 10 minutes for anesthesia. P17 and adult mice were euthanized by CO_2 inhalation before cortical tissue collection.

Wild animals

No wild animals were used in this study

Reporting on sex	To minimize variability in disease onset and severity, only female mice were used for EAE induction. For other experiments without EAE induction, both sexes of mice were used.
Field-collected samples	No field-collected samples were used in this study
Ethics oversight	All experimental procedures performed in the study were approved by the Institutional Animal Care and Use Committee (IACUC, KA2024-074) of the Korea Advanced Institute of Science and Technology (KAIST).

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

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
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.*	All raw and processed data are publicly avaiable in GEO repository (GSE158329, yxunmuaibbqtrwd).
Files in database submission	NR3C1 ChIP-seq fastq files cJUN ChIP-seq fastq files input fastq files peak calling results
Genome browser session (e.g. UCSC)	No longer applicable

Methodology

Replicates	NR3C1 and cJUN ChIP-seq were performed with two biological replicates.
Sequencing depth	NR3C1 replicate 1: 75,654,335 NR3C1 replicate 2: 65,556,660 cJUN replicate 1: 95,389,130 cJUN replicate 2: 68,836,907 input: 184,317,107
Antibodies	NR3C1 : Invitrogen, PA1-511A,LOT: ZF400272 JUN : Cell signaling technology, 9165 (60A8), LOT: 13
Peak calling parameters	peak calling was performed using MACS2 (v2.2.7.1) with default parameter except q<0.01
Data quality	Data quality was checked by enrichment of ChIP-seq signal compared to input control through genome browser. Also, we checked the FRiP scores of each data. NR3C1_rep1=11.6, NR3C1_rep2=12.1, JUN1_rep1=20.1, JUN1_rep2=28.5.
Software	For the data processing, sequencing reads were aligned to the mouse reference genome (GRCm38.p6) using BWA-mem. Low-quality alignments (MAPQ < 10) were removed, and PCR duplicates were eliminated using Picard (v2.6.0). The signal-enriched peaks were called using MACS2 v2.2.7.1 (-q 1e-2).

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	11weeks mouse brain, spinal cord, and spleen are used for experiment. Spleens are firstly dissected before perfusion, and brain and spinal cord are obtained after perfusion with DPBS. Each tissue is prepared separately, and used right after preparation.
Instrument	BD FACSCanto™ II
Software	FlowJo v10.10.0
Cell population abundance	Brains and spinal cords were separately stained and gated, and the total cell numbers from both tissues were combined to represent the CNS cell count for each mouse. An equal number of 50,000 cells was initially acquired from each sample, and downstream gating was performed using FlowJo software. Sample quality check was also done using FlowJo software. Results are presented as total number of gated cells per mouse.
Gating strategy	Initial gating was performed on FSC-A vs. SSC-A to select sells, followed by FSC-A vs. FSC-H to gate singlets. CD4+ T cells were identified based on CD45+CD3+CD4+ expression. Subsets were further defined by intracellular staining for Foxp3, IL-17A, and IFN-γ. Fluorescence compensation was performed using single color stained beads, and gating boundaries between positive and negative populations were determined using unstained brain and spinal cord tissue samples. Fluorescence minus one (FMO) controls were also used to define gates for intracellular markers.

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