

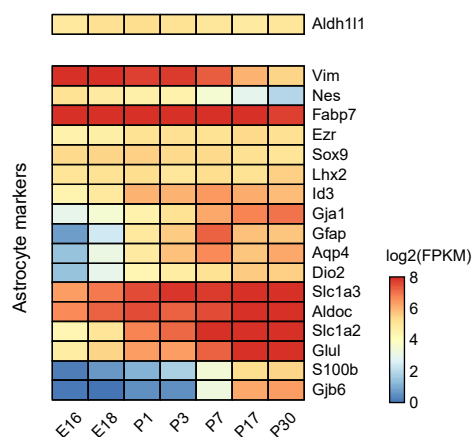
NR3C1-mediated epigenetic regulation suppresses astrocytic immune responses in mice

Park et al.

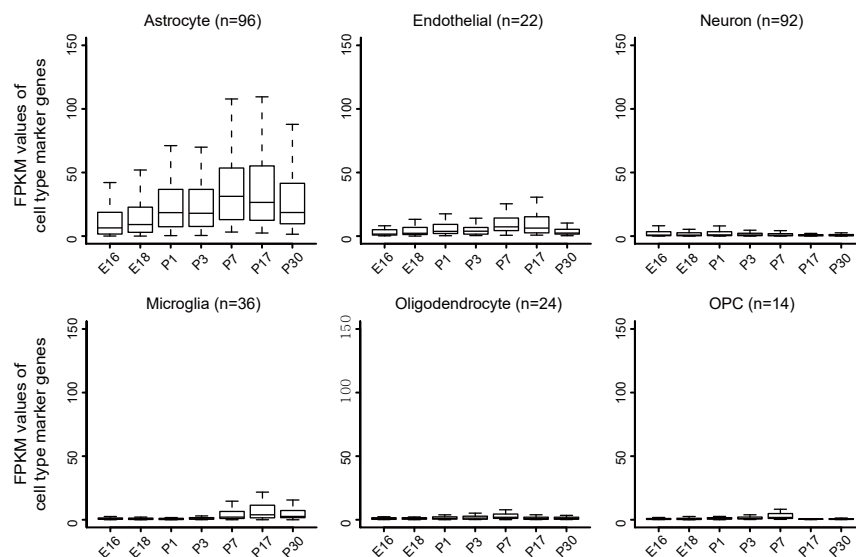
Supplementary Materials

Supplementary Figure 1

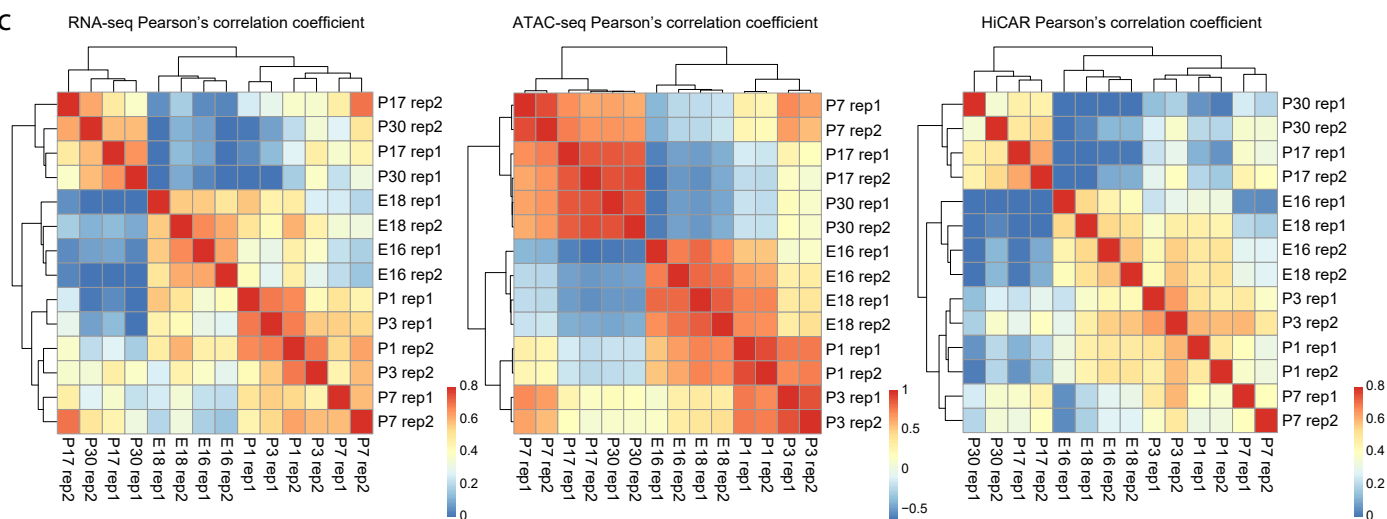
a



b



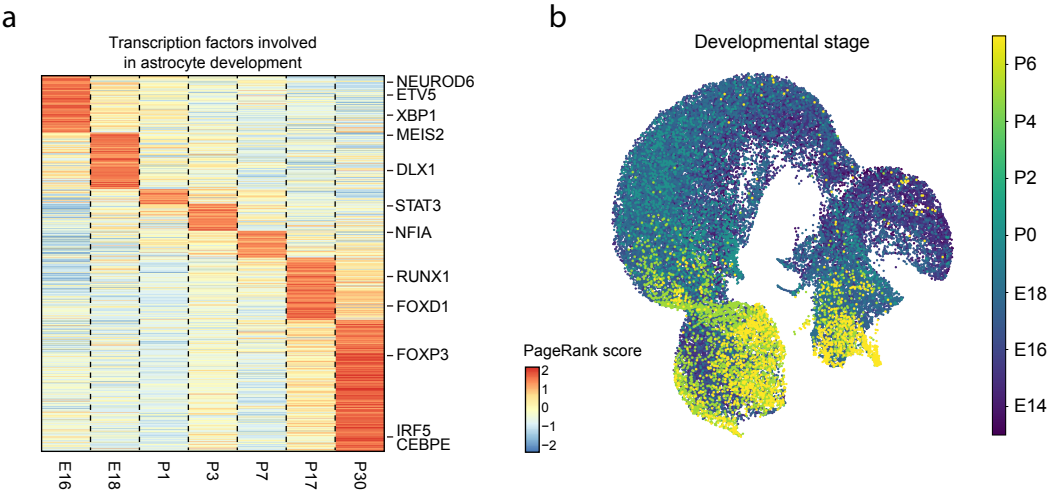
c



Supplementary Figure 1. Transcriptomic and epigenomic characteristics of *Aldh1l1*⁺ astrocytes

a, Heatmap showing dynamic expression patterns of astrocyte marker genes throughout astrocyte development. **b**, Boxplots representing the expression levels of known marker genes of various brain cell types, including astrocytes, endothelial cells, neurons, microglia, oligodendrocytes, and OPCs. The box indicates the interquartile range (IQR), and the whiskers represent the highest and lowest points within $1.5 \times \text{IQR}$. **c**, Hierarchical clustering analysis performed based on Euclidean distances of Pearson's correlation coefficients utilizing RNA expression (left), chromatin accessibility (middle), and chromatin interaction (right).

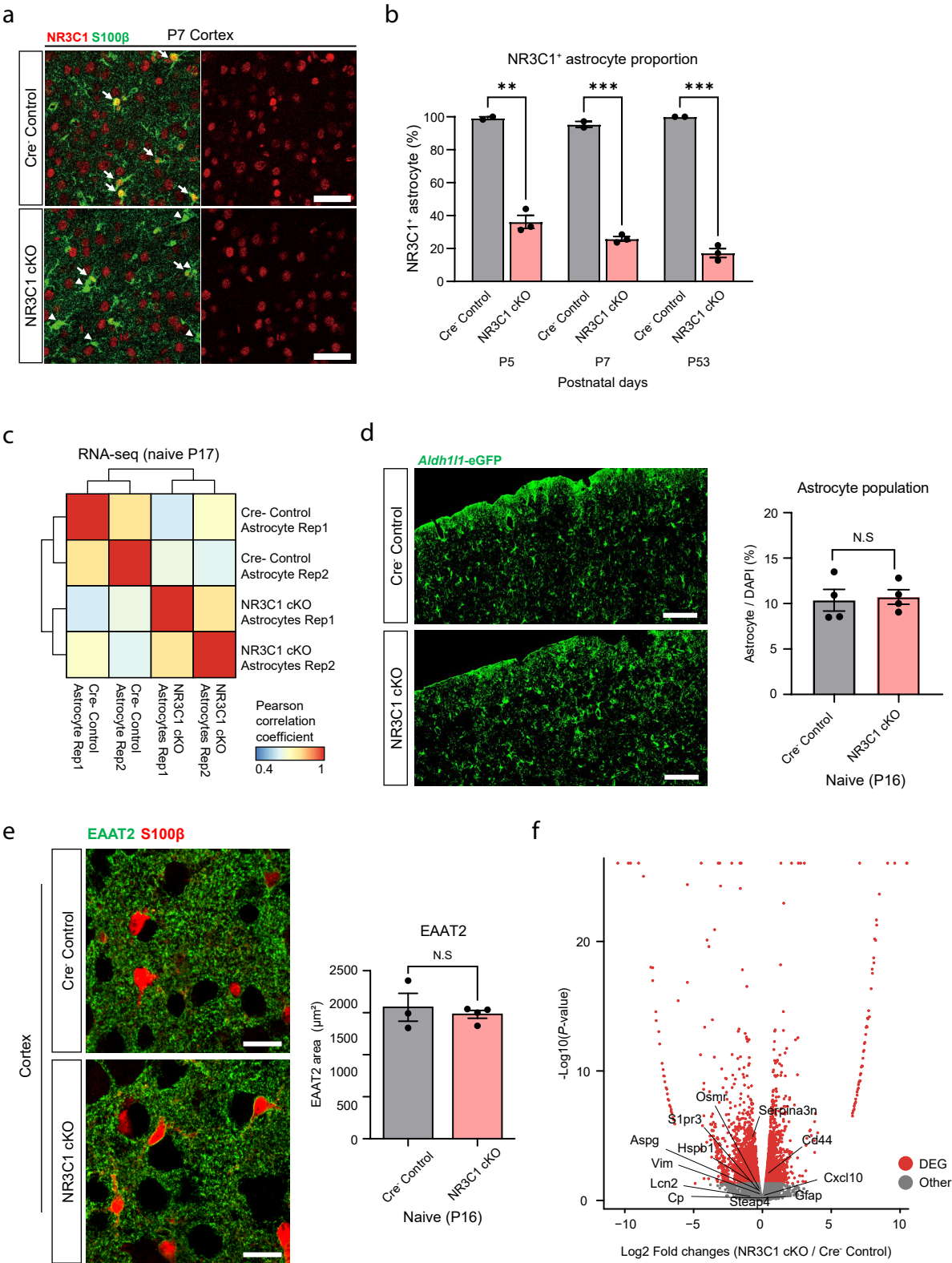
Supplementary Figure 2



Supplementary Figure 2. Identification of TF candidates during astrocyte development

a, Heatmap showing the scaled activity scores of TFs during each time point. **b**, UMAP embedding of public scRNA-seq datasets. The color indicates the developmental stages of each cell's origin.

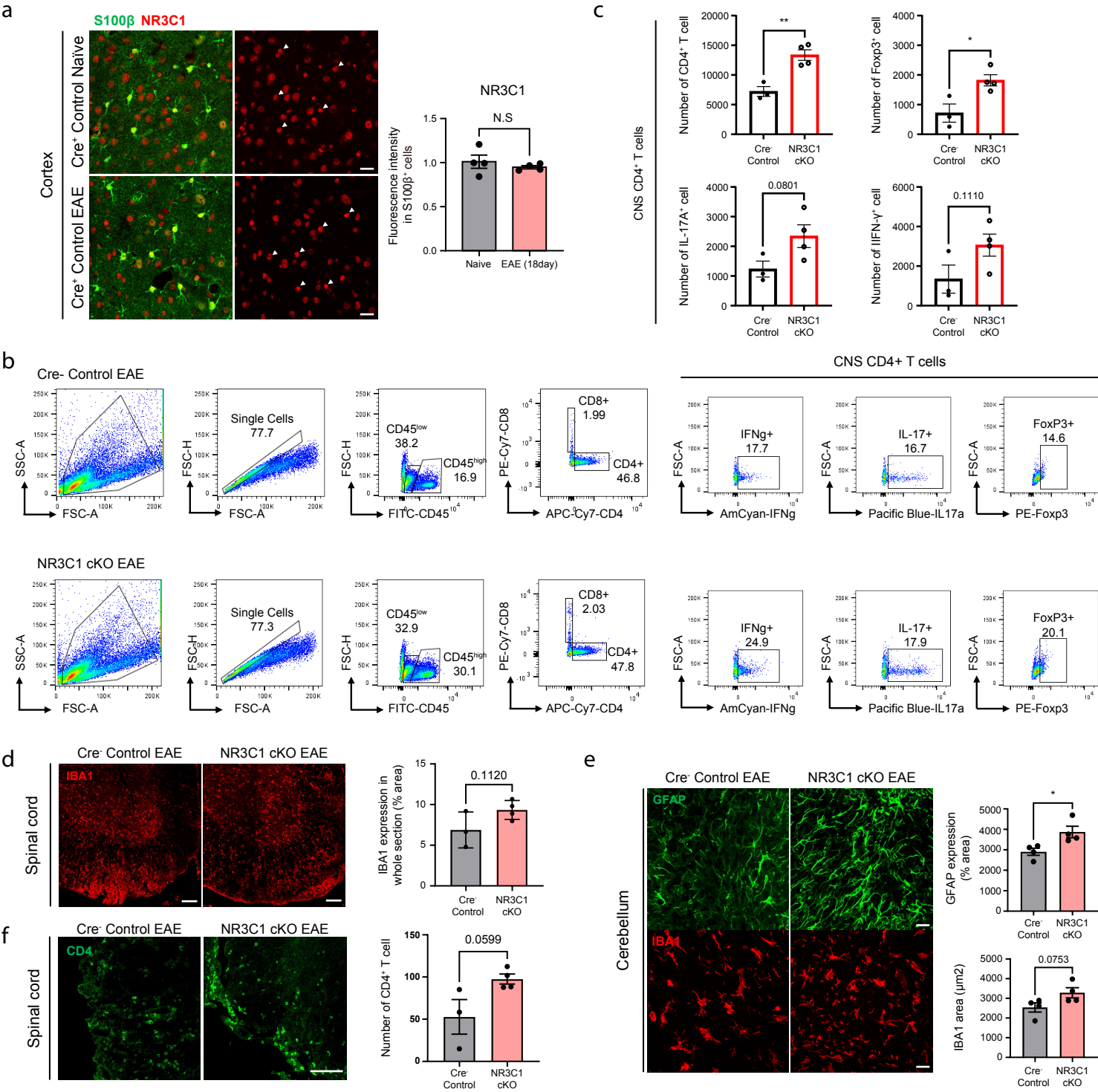
Supplementary Figure 3



Supplementary Figure 3. Phenotypic analysis of NR3C1 cKO mice

a, Representative confocal z-stack images showing NR3C1 expression (red) in astrocytes (S100 β , green) from NR3C1 cKO mice. White arrows indicate NR3C1 positive astrocytes and arrowheads indicate NR3C1 negative astrocytes. Scale bars, 40 μ m. **b**, Bar graphs showing the percentage of NR3C1 expressing astrocytes. (Mice were used with $n = 2$ for Cre⁻ control and $n = 3$ for NR3C1 cKO in each time point). Data are shown as mean $\pm$ SEM and are analyzed by unpaired t-test ($P = 1.16 \times 10^{-3}$ for P5, $P = 7.70 \times 10^{-5}$ for P7, $P = 1.62 \times 10^{-4}$ for P53). **c**, Hierarchical clustering analysis performed based on Euclidean distances of Pearson's correlation coefficients using RNA expression of Cre⁻ control and NR3C1 cKO astrocytes at P17 under naïve conditions. **d,e**, Representative confocal images of *Aldh1l1-eGFP* (**d**, green) and EAAT2 (**e**, green), alongside S100 β (**e**, red) in the cortex of Cre⁻ control and NR3C1 cKO mice at P16. Bar graphs showing the number of astrocytes (**d**, $P=0.810$) and EAAT2 expression (**e**, $P=0.600$) in cortex. Mice were used with $n=4$ and 4 in (**d**), $n=3$ and 4 in (**e**) for Cre⁻ control and NR3C1 cKO mice, respectively. Data are shown as mean $\pm$ SEM and are analyzed by unpaired t-test. Scale bars, 100 μ m (**d**) and 20 μ m (**e**). **f**, Volcano plot showing DEGs between Cre⁻ control and NR3C1 cKO astrocytes under naïve conditions. Each dot indicates individual gene, colored by red when a gene is significant DEG. Known marker genes of reactive astrocytes were exemplified.

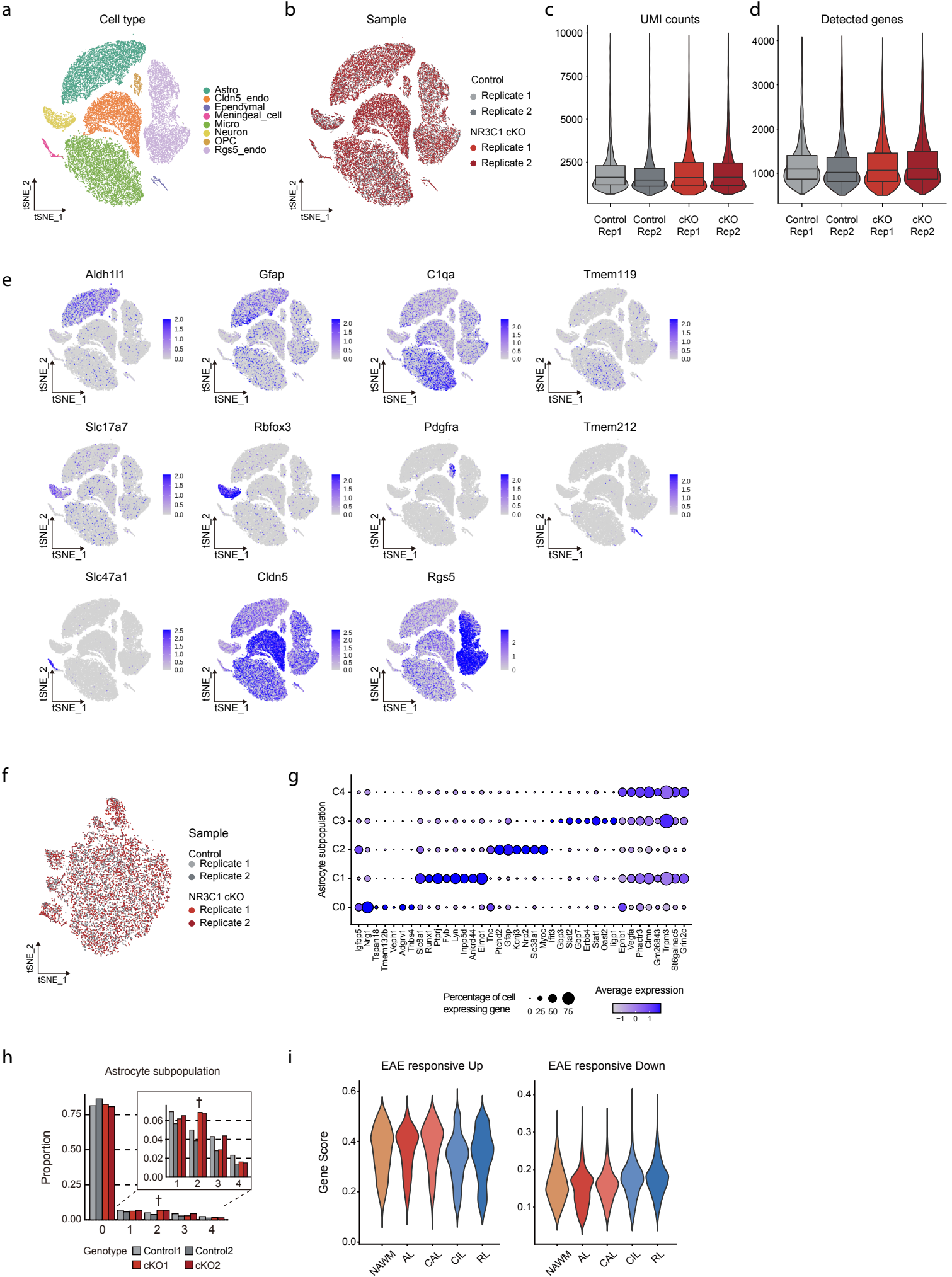
Supplementary Figure 4



Supplementary Figure 4. Exacerbated immune responses at the peak of EAE across CNS regions in NR3C1 cKO mice

a, Representative confocal images of NR3C1 expression under naïve conditions (upper) and at the peak of EAE (bottom). Bar graphs showing normalized NR3C1 expression levels (right). $n=4$ for naïve and $n=4$ for EAE. Scale bars, $20\mu\text{m}$. **b**, FACS sorting schematic for CD4^+ T cell and its subsets. **c**, Bar graphs showing the number of CD4^+ T cell ($P = 4.60 \times 10^{-3}$) and its subsets (Foxp3^+ , $P = 2.26 \times 10^{-2}$; IL-17A^+ , $P = 8.01 \times 10^{-2}$; $\text{IFN-}\gamma^+$, $P = 0.111$). Cell counts represent the combined total from the spinal cord and brain. $n=3$ for Cre^- control and $n=4$ for NR3C1 cKO mice. **d**, Representative confocal images for IBA1 expression in the spinal cord from Cre^- control and NR3C1 cKO mice (left). Bar graphs showing the area of IBA1 expression (right). $n=3$ for Cre^- control, $n=4$ NR3C1 cKO mice. Scale bars, $100\mu\text{m}$. **e**, Representative confocal images for GFAP and IBA1 expression in the cerebellum from Cre^- control (upper) and NR3C1 cKO mice (bottom). Bar graphs showing the area of GFAP ($P = 2.61 \times 10^{-2}$) and IBA1 expression ($P = 7.53 \times 10^{-2}$) (right). $n=4$ for Cre^- control and $n=4$ for NR3C1 cKO mice. Scale bars, $20\mu\text{m}$. **f**, Representative confocal images for CD4^+ T cell infiltration in the spinal cord from Cre^- control and NR3C1 cKO mice (left). Bar graphs showing the number of infiltrated CD4^+ T cell (right). $n=3$ for Cre^- control, $n=4$ NR3C1 cKO mice. Data are shown as mean $\pm$ SEM and are analyzed by unpaired t-test. Scale bars, $50\mu\text{m}$.

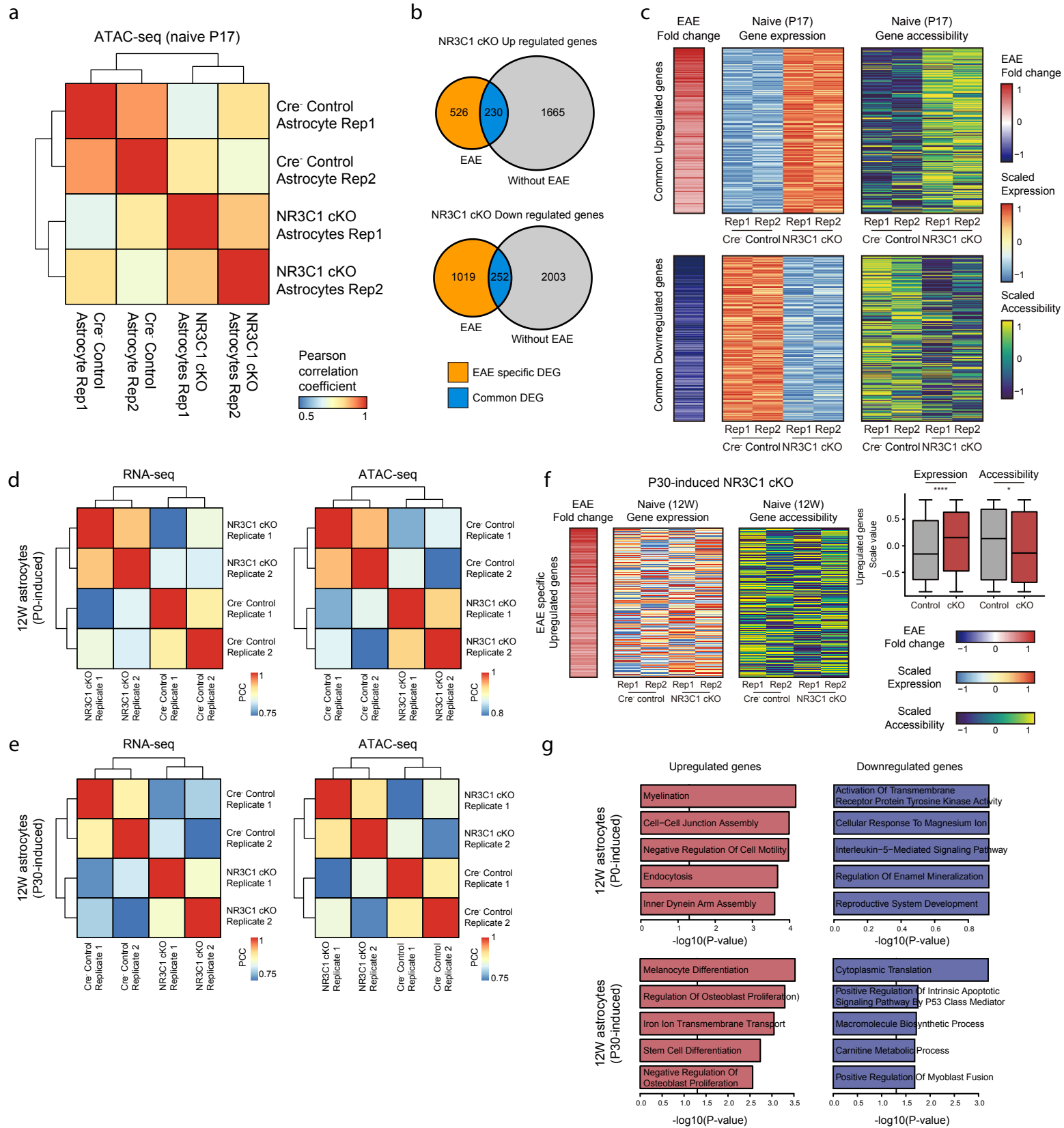
Supplementary Figure 5



Supplementary Figure 5. Characterization of astrocyte at the peak of EAE in NR3C1 cKO mice

a,b t-SNE projections of nuclei colored by annotated cell type information (**a**) or sample information (**b**). **c,d** Violin plots showing the number of unique molecular identifiers (UMIs, **c**) and detected genes (**d**) per nucleus. The box indicates the IQR, and the whiskers represent the highest and lowest points within $1.5 \times \text{IQR}$. **e**, t-SNE projections illustrating the gene expression of cell type markers (astrocytes, *Aldh1l1* and *Gfap*; microglia, *C1qa* and *Tmem119*; neurons, *Slc17a7*; OPCs, *Pdgfra*; ependymal cells, *Tmem212*; meningeal cells, *Slc47a1*; endothelial cells, *Cldn5* and *Rgs5*). **f**, t-SNE projections of astrocyte subpopulation colored by sample information. **g**, Dot plots showing the expression patterns of representative marker genes of astrocyte subpopulations. Dot size indicates the percentages of cell expressing a representative gene. Color represents average expression level of genes in astrocyte subpopulations. **h**, Bar plots showing the proportion of each subpopulation in astrocytes. **i**, Violin plots showing expression levels of DEGs at the EAE peak in various brain lesions of multiple sclerosis patients (NAWM, normal-appearing white matter; AL, active lesion; CAL, chronic active lesion; CIL, chronic inactive lesion; RL, remyelinated lesion). Source data are provided as a Source Data file.

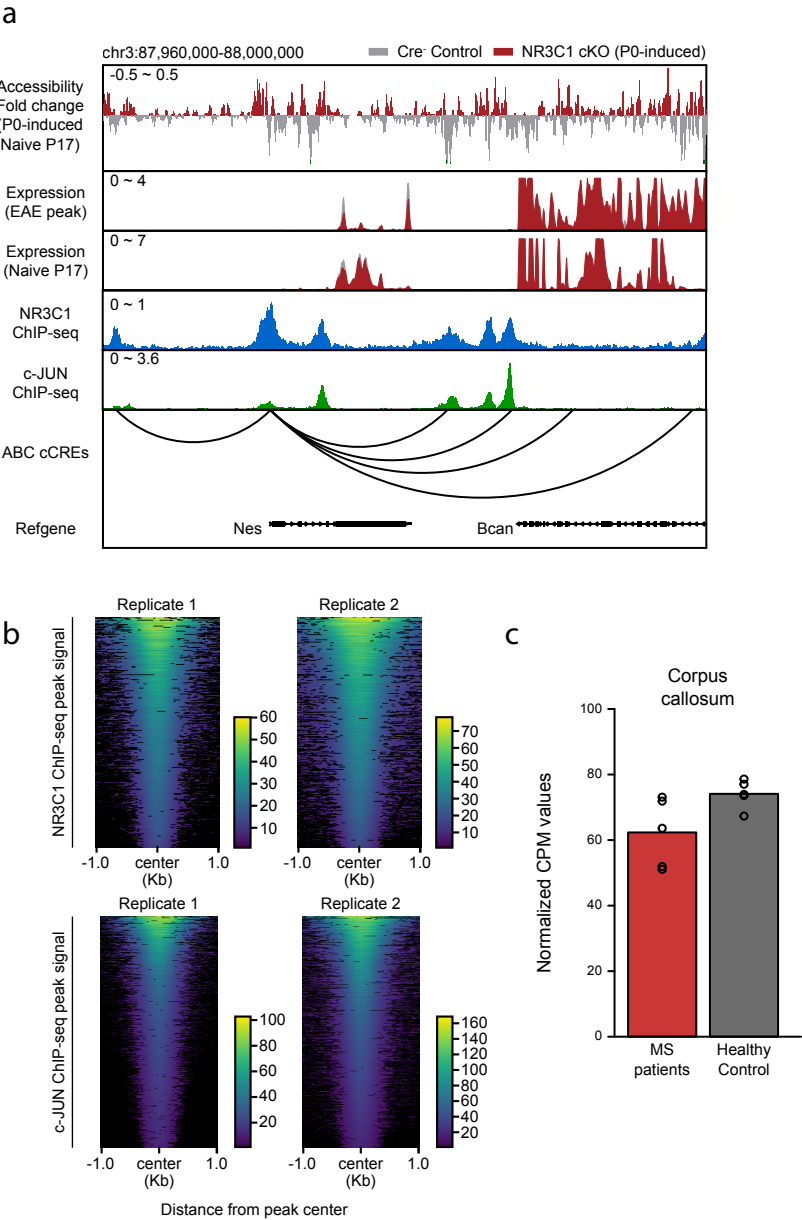
Supplementary Figure 6



Supplementary Figure 6. Characteristics of epigenetically predisposed cCREs for EAE-responsive genes

a, Hierarchical clustering analysis performed based on Euclidean distances of Pearson's correlation coefficients using chromatin accessibility of Cre⁻ control and NR3C1 cKO astrocytes at P17 under naïve conditions. **b**, Venn diagram of DEGs between Cre⁻ control and NR3C1 cKO astrocytes with or without EAE induction. EAE specific NR3C1 DEGs (used in Fig. 6a) were colored by orange. The common NR3C1 DEGs (used in Supplementary Fig. 6c) were colored by blue. **c**, Heatmap showing epigenetic priming state of common DEGs. Expression fold changes at EAE peak, expression levels at naïve, and accessibility of gene body in naïve P17 astrocytes were shown. **d,e**, Hierarchical clustering analysis performed based on Euclidean distances of Pearson's correlation coefficients using gene expression (left) and chromatin accessibility (right) of Cre⁻ control and NR3C1 cKO astrocytes from 12W naïve mice following P0-induced (**d**) and P30-induced (**e**) NR3C1 cKO. **f**, Heatmap showing gene expression and gene body accessibility of EAE-specific upregulated genes. Expression levels and accessibility of gene body in astrocytes from 12W naïve mice following P30-induced NR3C1 cKO were shown (left). Boxplots showing the gene expression and chromatin accessibility of EAE-specific upregulated genes (right). Two-sided Kolmogorov Smirnov test, expression $P = 2.50 \times 10^{-4}$, accessibility $P = 3.85 \times 10^{-2}$. The box indicates the IQR, and the whiskers represent the highest and lowest points within $1.5 \times \text{IQR}$. **g**, Top 5 enriched gene ontology of upregulated or downregulated DEGs in astrocytes from 12W naïve mice following P0-induced (top) or P30-induced (bottom) NR3C1 cKO.

Supplementary Figure 7



Supplementary Figure 7. Involvement of transcription factors in the regulation of cCREs linked to EAE-responsive genes

a, Genome browser snapshots of representative EAE responsive downregulated gene (*Nes*). **b**, Heatmap showing NR3C1 and c-JUN ChIP-seq signals on their peaks (NR3C1, n=25,111; c-JUN, n=47,071). **c**, Bar graphs showing the normalized expression of NR3C1 in corpus callosum of MS patients and age-matched healthy control (two-sided Kolmogorov-Smirnov test, $P = 0.079$). Source data are provided as a Source Data file.