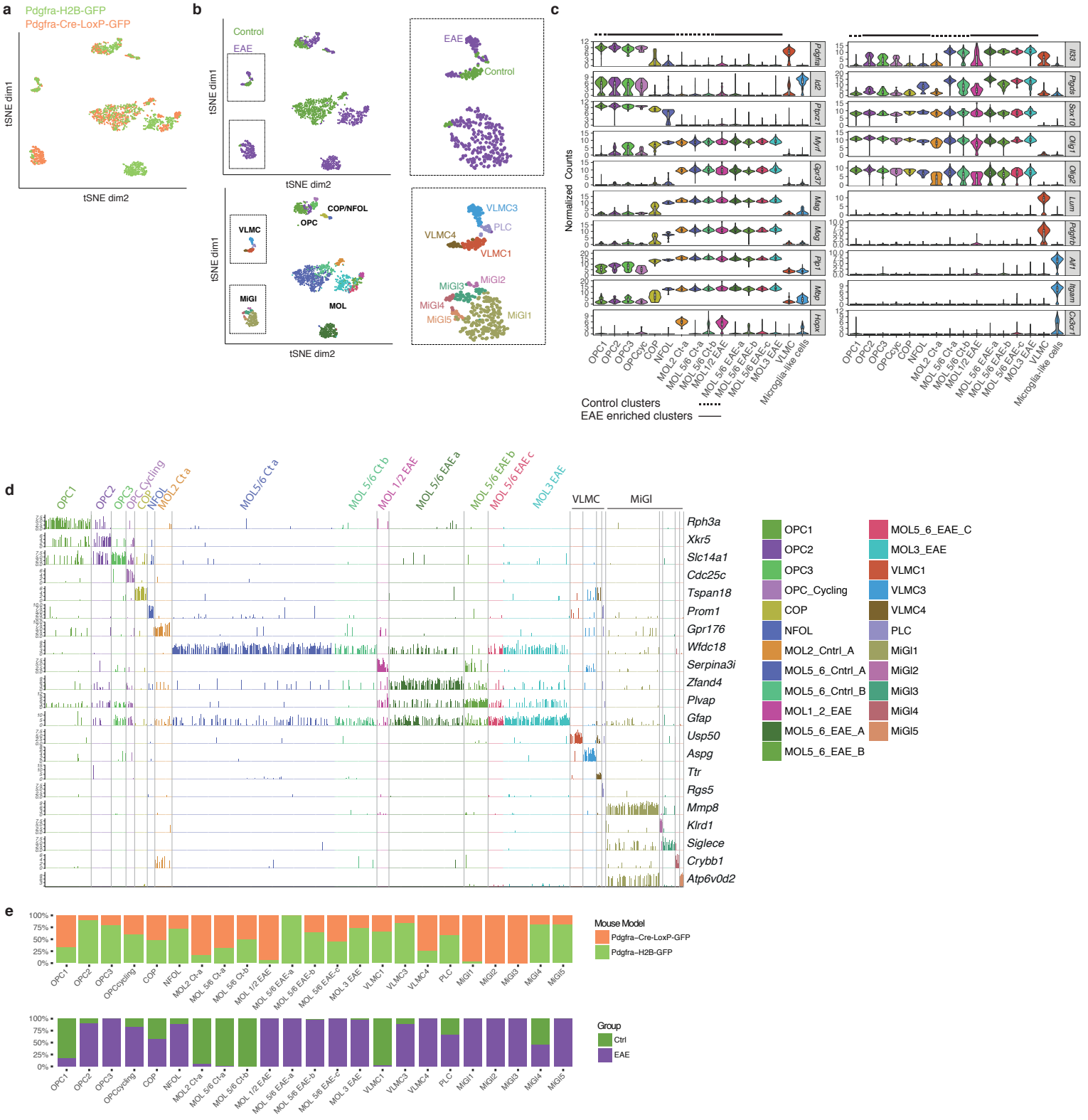
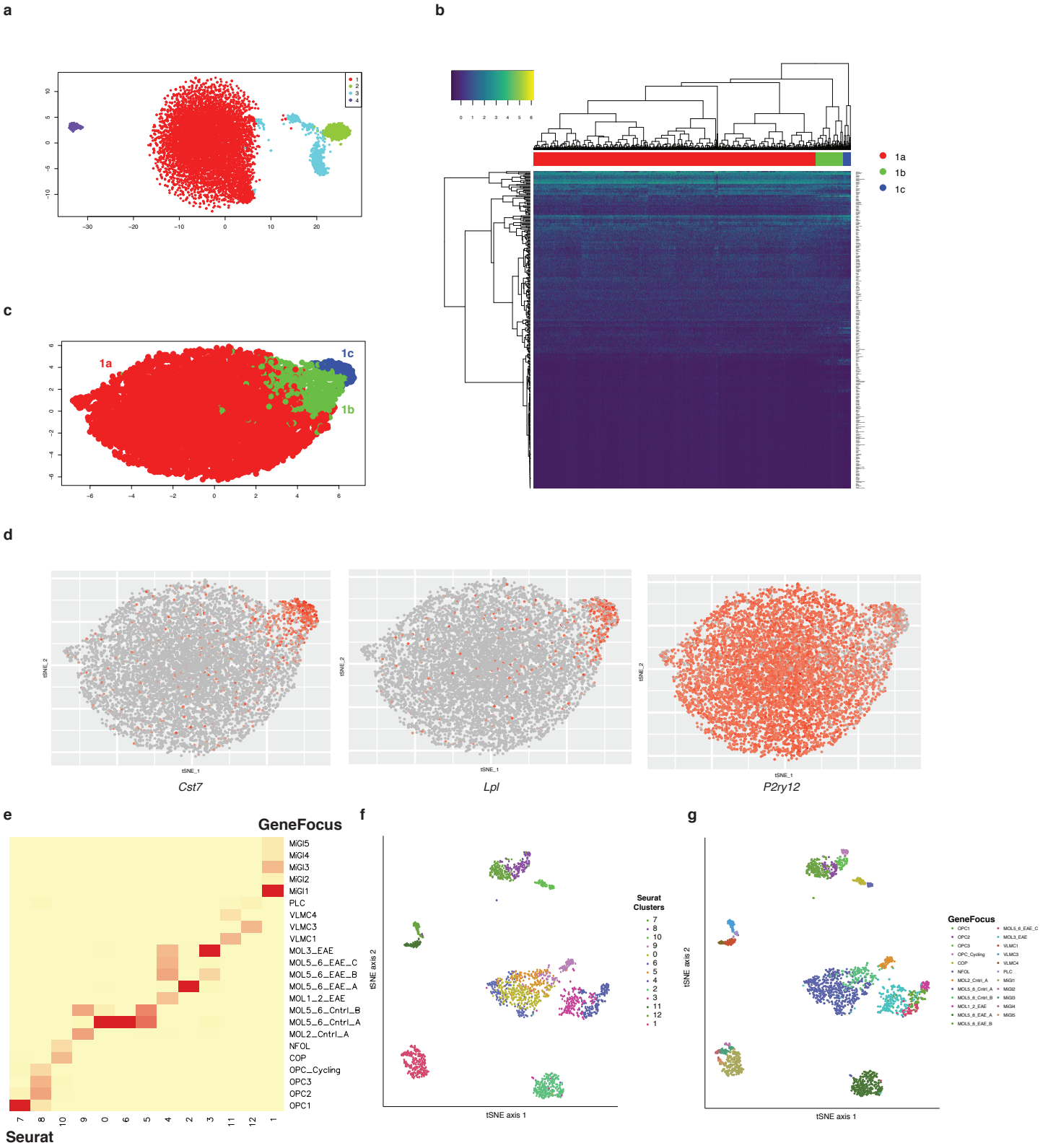
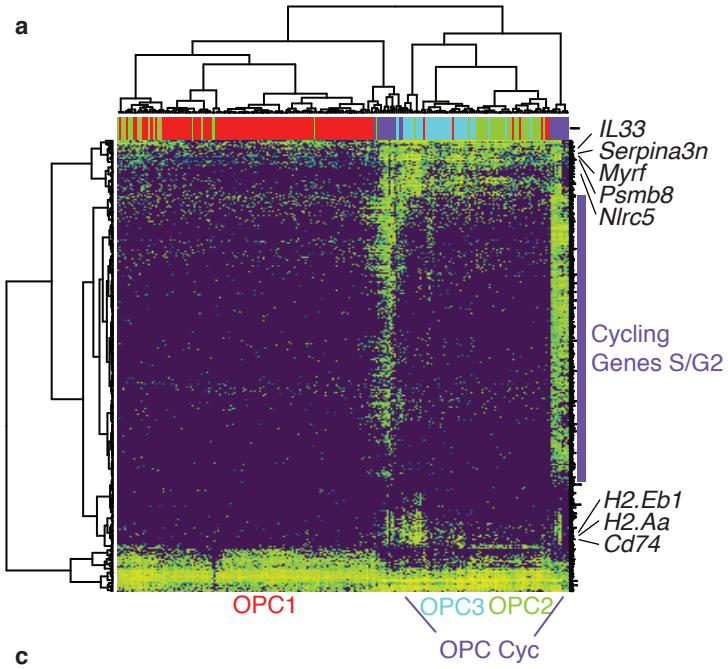
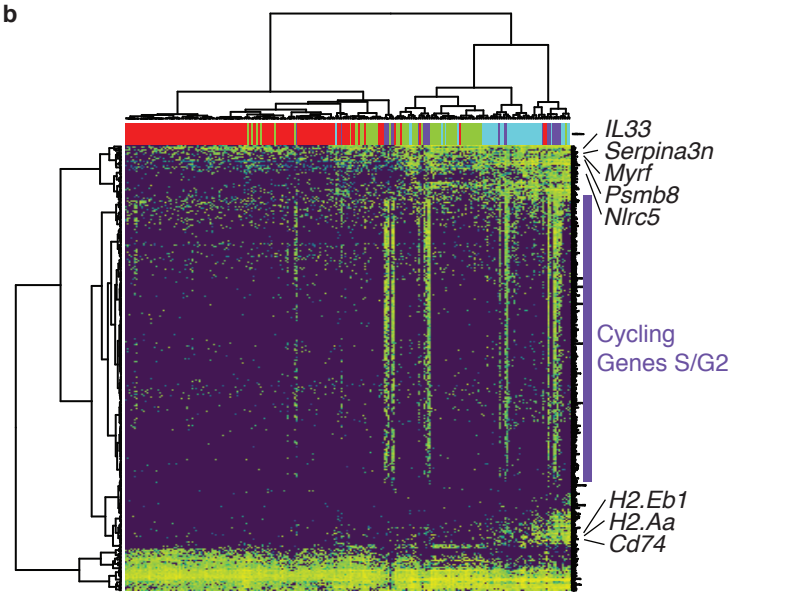


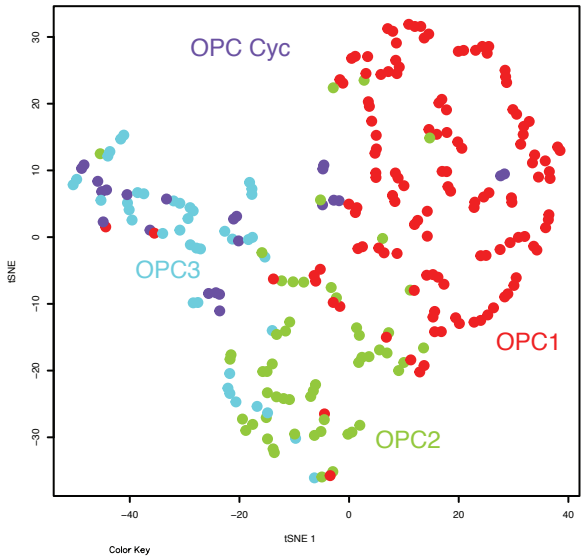
Supplementary Figure 1 | Single cell RNA-sequencing analysis of OL lineage cells in response to EAE uncovers new disease-specific populations and markers. a, t-SNE plot showing cells isolated from the spinal cords derived from the two transgenic mouse lines used in this study, Pdgfra-H2B-GFP and Pdfra-Cre-LoxP-GFP (n=4 biologically independent mouse spinal cord samples per condition). **b,** t-SNE plots representing control and EAE derived cells, highlighting the four clusters derived from vascular and leptomeningeal cells (VLMC1,3,4) and pericyte like cells (PLC) and the five clusters derived from microglia-like cells (MiGL1-5) (n=4 biologically independent mouse spinal cord samples per condition). **c,** Violin plots depicting the expression of canonical markers of OPC1, OPC2, OPC3, OPCcycling, COPs, NFOLs, MOL2 Ct-a, MOL5/6 Ct-a, MOL5/6 Ct-b, MOL1/2 EAE, MOL5/6 EAE-a, MOL5/6 EAE-b, MOL5/6 EAE-c and MOL3 EAE, derived from CFA controls and EAE mice (n=4 biologically independent mouse spinal cord samples per condition); VLMCs and microglia-like cells in all cells collected both from controls and EAE. Violin plots are centered around the median with interquartile ranges and shape represents cell distribution. **d,** Bar plots representing markers for all cell populations and the respective clusters analysed in this study, including OPCs, MOLs, VLMCs and microglia-like cells. **e,** Representation of the percentage of cells derived from the two transgenic mouse lines, Pdgfra-H2B-GFP and Pdgfra-Cre-LoxP-GFP, or from controls and EAE mice, in all cell clusters obtained in this study.

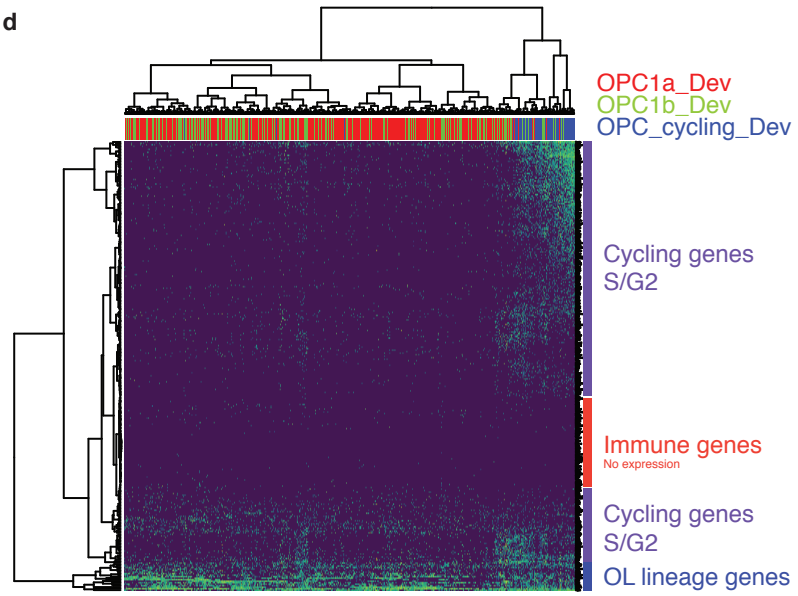


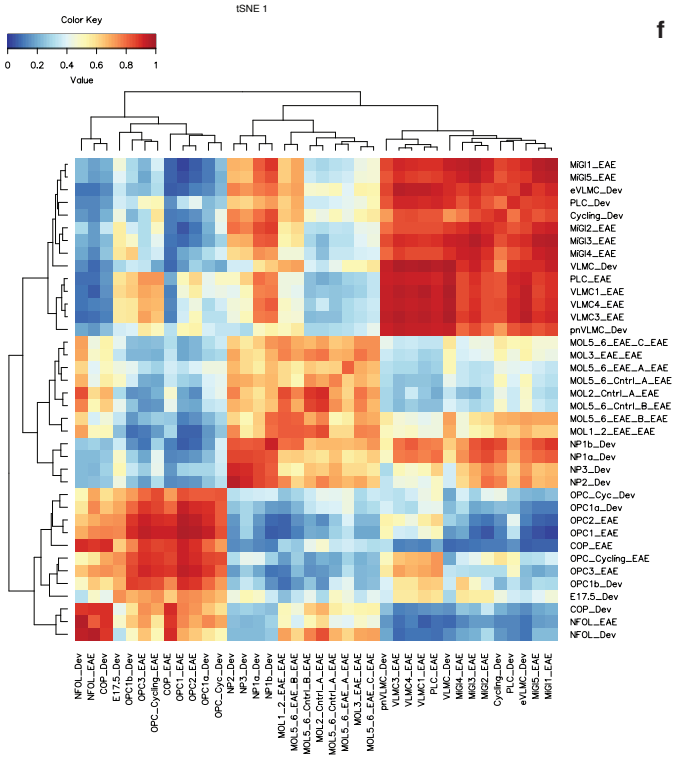
Supplementary Figure 2 | Identification of disease-associated microglia (DAM) by applying GeneFocus clustering to a Alzheimer disease mouse model dataset from Keren-Shaul et al. (ref 31), and cluster comparison between GeneFocus and Seurat Louvain clustering. **a,** tSNE of 8861 cells depicting the Level 1 clusters (1-4) from Keren-Shaul et al.(ref 41), as defined by the GeneFocus pipeline. **b,** Heatmap of 7036 cells showing spatially filtered genes of the three subclusters (1a, 1b, 1c) of the microglial cluster (1). Gene expression values are log transformed counts. **c,** tSNE of 7036 cells depicting the final clustering of the microglial cluster. **d,** Expression values of 7036 cells colored in a tSNE for major marker genes of disease associated clusters (*Cst7* and *Lpl*) and microglia (*P2ry12*). **e**, Heatmap depicting a frequency table comparing the GeneFocus clustering with the Seurat Louvain clustering. **f,g,** tSNE plots colored by the Seurat louvain clustering and GeneFocus respectively. (n=4 biologically independent mouse spinal cord samples per condition, depicting 1765 cells).







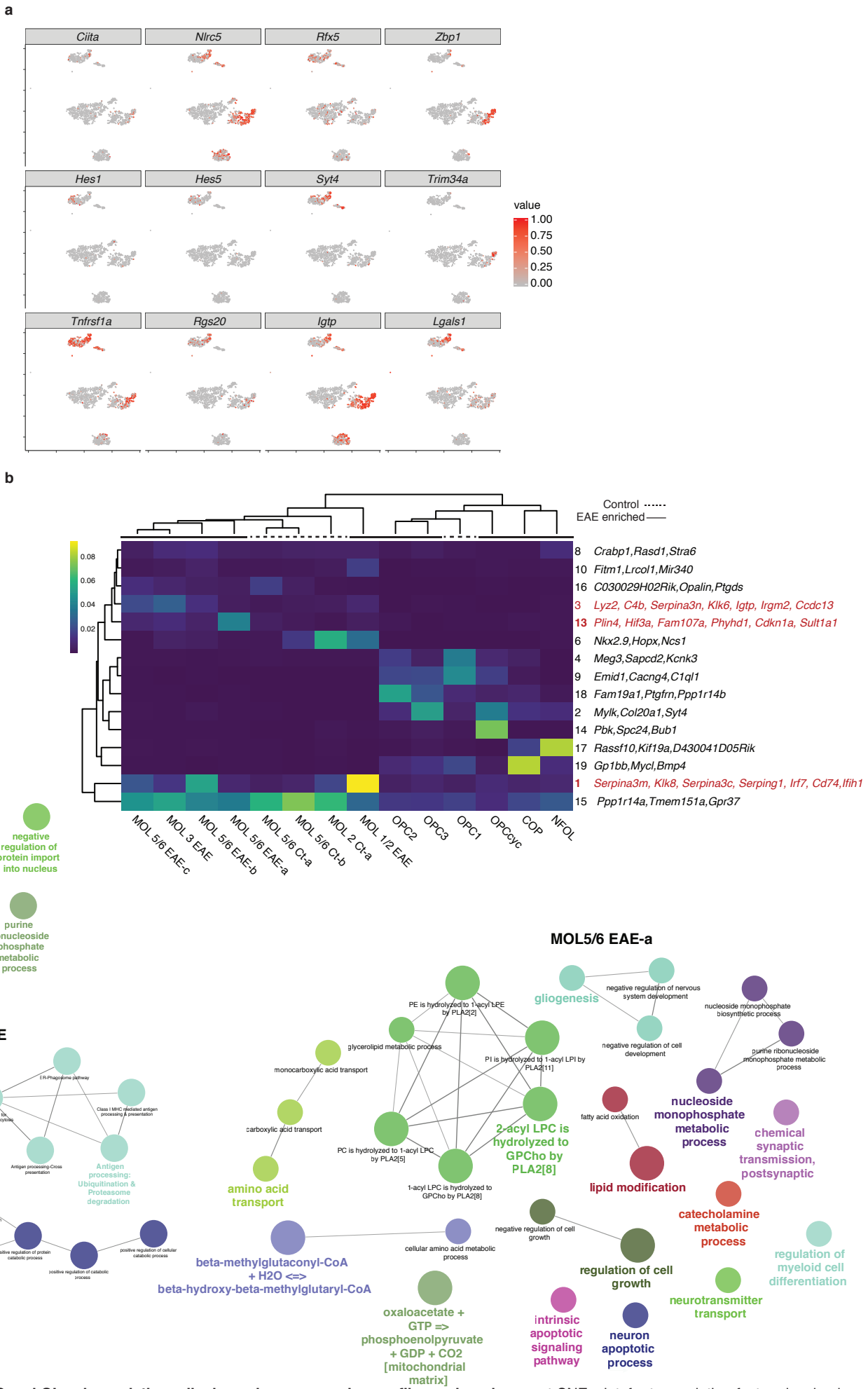




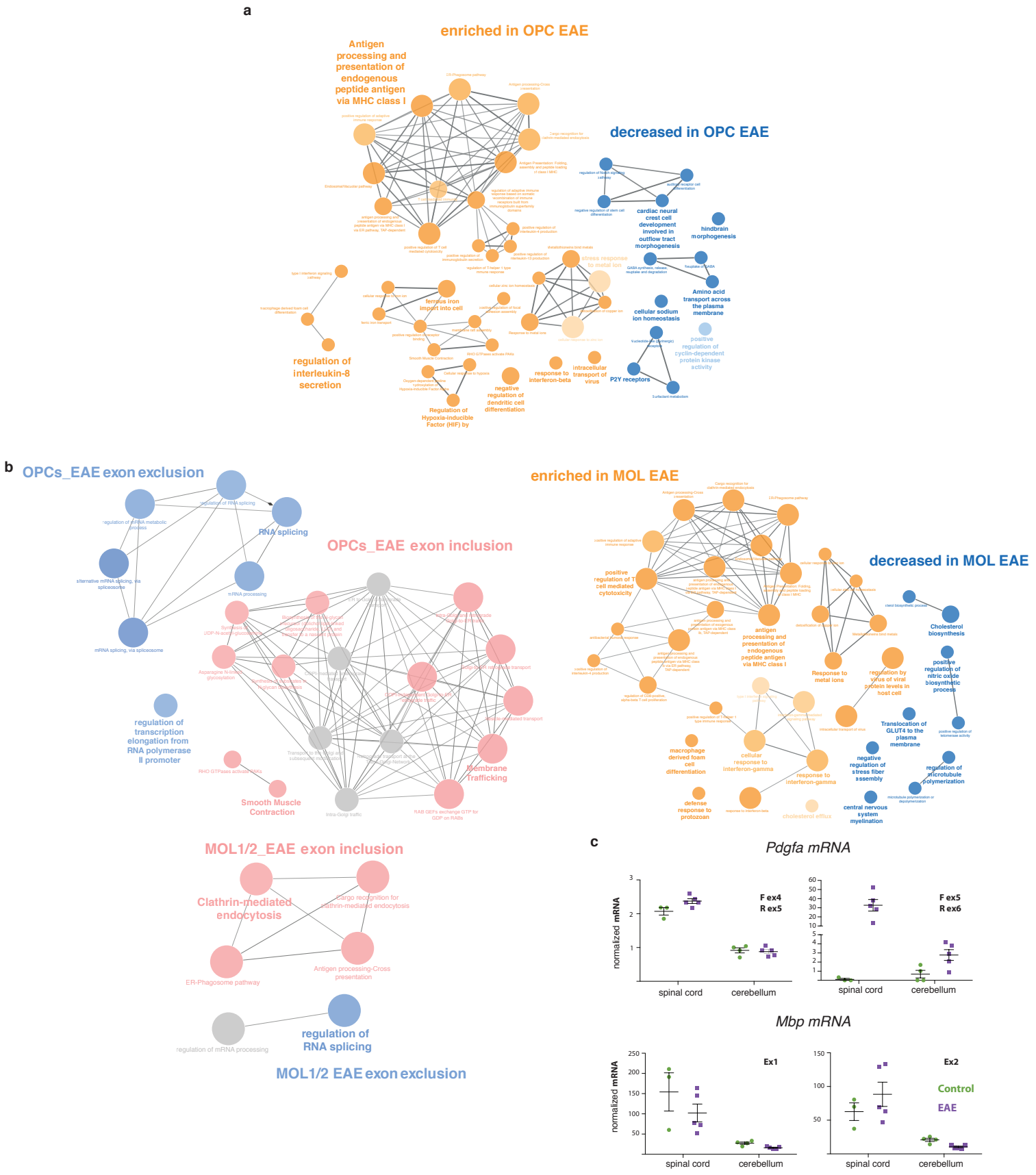
f

Celltype_1	Celltype_2	Mean AUROC	Match_type
VLMC_Dev	VLMC3_EAE	0.98	Reciprocal_top_hit
VLMC_Dev	VLMC1_EAE	0.97	Above_0.9
NFOL_Dev	NFOL_EAE	0.97	Reciprocal_top_hit
VLMC_Dev	VLMC4_EAE	0.97	Above_0.9
VLMC3_EAE	eVLMC_Dev	0.95	Above_0.9
VLMC_Dev	PLC_EAE	0.95	Above_0.9
VLMC3_EAE	pnVLMC_Dev	0.94	Above_0.9
VLMC_Dev	MiGl1_EAE	0.93	Above_0.9
OPC1a_Dev	OPC1_EAE	0.93	Reciprocal_top_hit
VLMC_Dev	MiGl3_EAE	0.92	Above_0.9
VLMC3_EAE	PLC_Dev	0.92	Above_0.9
NFOL_Dev	COP_EAE	0.91	Above_0.9
OPC1a_Dev	OPC2_EAE	0.90	Above_0.9
OPC1_EAE	OPC1b_Dev	0.90	Above_0.9
VLMC_Dev	MiGl5_EAE	0.90	Above_0.9
COP_EAE	COP_Dev	0.90	Above_0.9

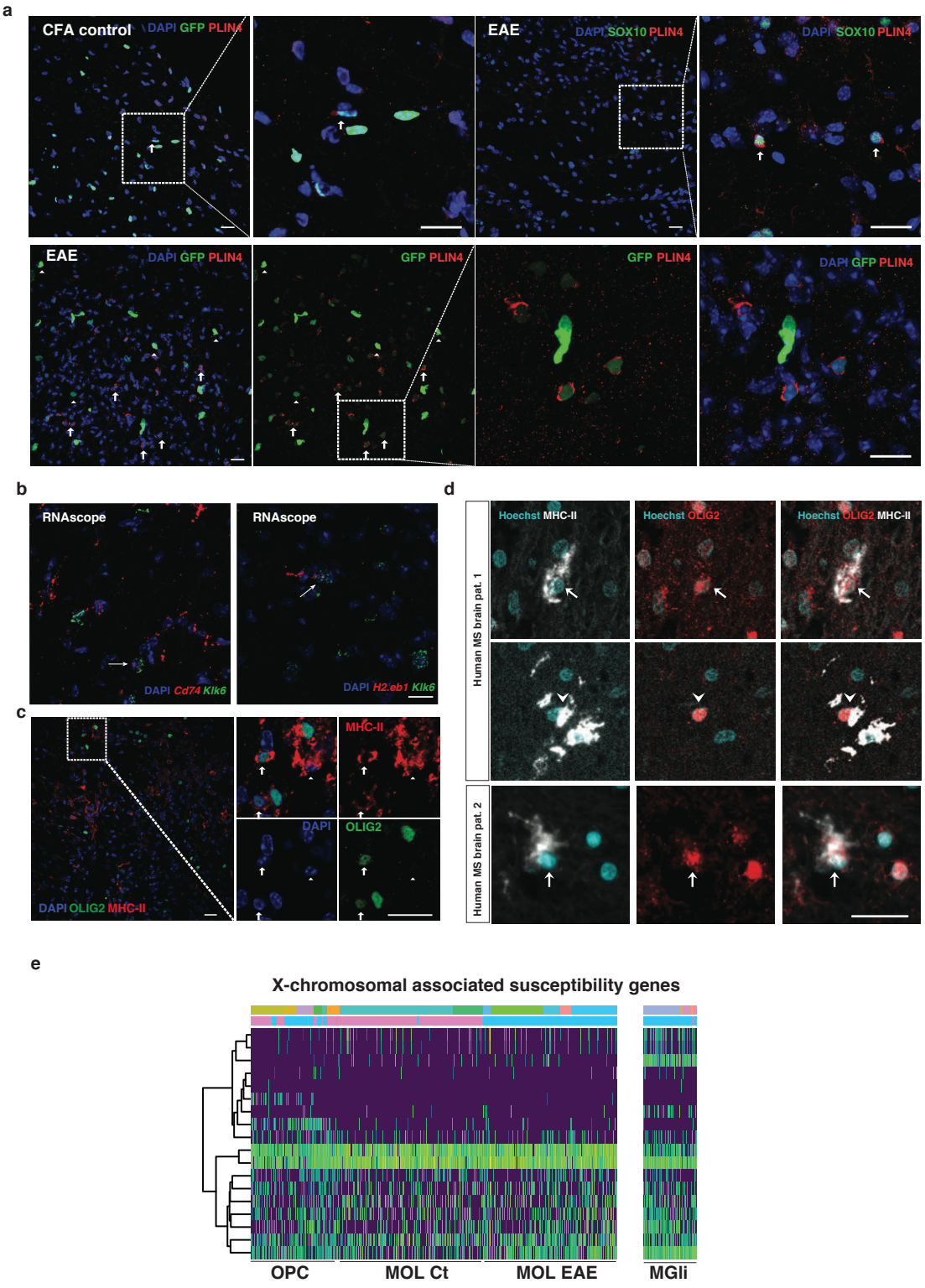
Supplementary Figure 3 I In-depth analysis of OPC clusters using GeneFocus. **a**, Heatmap showing the expression of a spatially correlating GeneFocus derived geneset specific for hierarchically ordered OPCs (248 cells). **b**, Heatmap using a distance matrix with the cell cycle gene-component removed. **c**, tSNE showing the intermingling of the cycling OPCs when the cycling gene component is removed (248 cells). **d**, P7 and adult OPCs from normal mice (ref 3), showing lack of expression of EAE specific genes. **e-f**, Comparisons of cell types between current datasets and datasets from ref.3



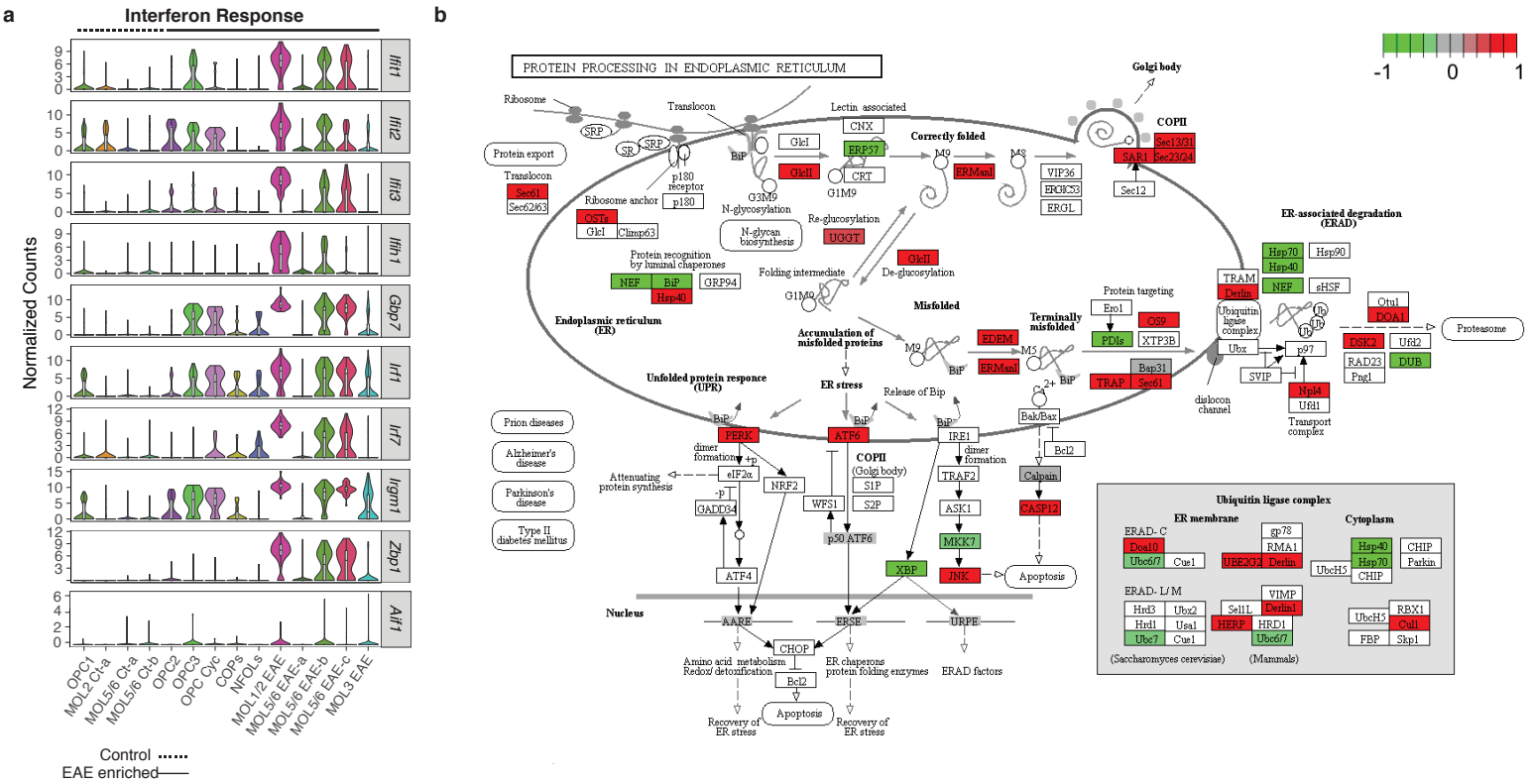
Supplementary Figure 4 | OPC and OL subpopulations display unique expression profiles and markers. a, t-SNE plot for transcription factors involved in MHC-II genes activation, *Ciita*, *Nlrc5*, *Rfx5* and *Zbp1*, OPC maintenance/ differentiation inhibition, *Hes1* and *Hes5*, and other genes enriched in specific populations in EAE (n=4 biologically independent mouse spinal cord samples per condition, CFA control or EAE; total number of cells is 1452) **b,** Gene modules enriched in the different OL lineage cells populations showing major gene trends underlying the data. Three or more genes representative of the modules are shown on the side of the module number. **c**, Gene ontology and REACTOME pathway analysis for the most significantly differentially expressed genes for OPC2 EAE, OPC3 EAE, MOL1/2 EAE and MOL5/6 EAE-a (n=4 biologically independent mouse spinal cord samples per condition, CFA control or EAE; Analyses were performed with Cytoscape and p <0.05; p values were calculated with Bonferroni two-sided hipergeometric test).



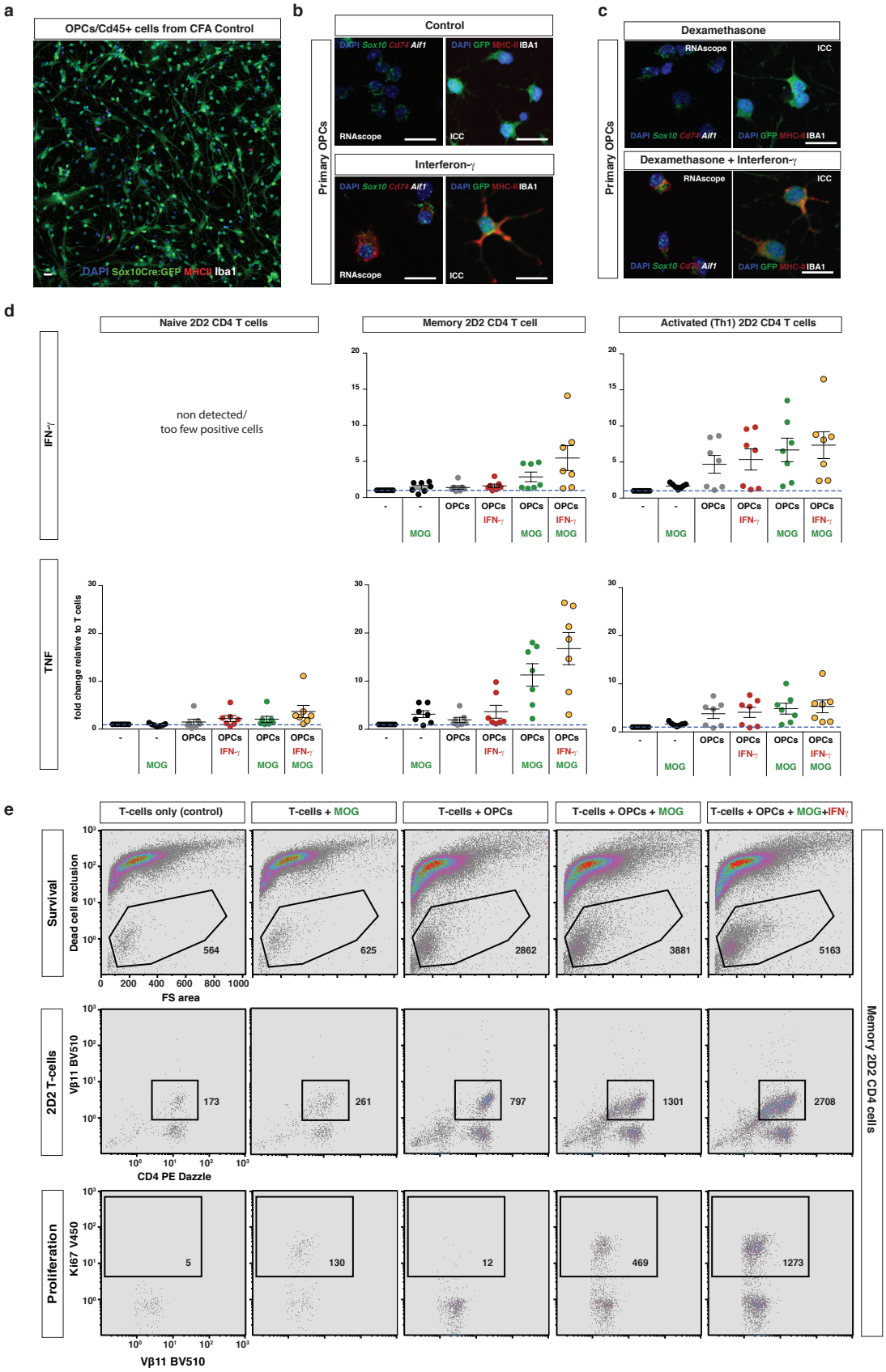
Supplementary Figure 5 | OPCs and MOLs display distinct expression profile and alternative splicing in response to EAE. a,Gene ontology and REACTOME pathway analysis for the most significantly differentially expressed genes for OPC EAE and MOLs EAE.(n=4 biologically independent mouse spinal cord samples per condition, CFA control or EAE; Analyses were performed with Cytoscape and p <0.05; p values were calculated with Bonferroni two-sided hipergeometric test). **b,** Gene ontology and REACTOME pathway analysis for the genes of most significantly alternatively spliced exons for OPCs EAE and MOL1/2 EAE, when compared to their respective controls. Both exon exclusion and inclusion outcome are represented, in blue and red, respectively (n=4 biologically independent mouse spinal cord samples per condition, CFA control or EAE; Analyses were performed with Cytoscape and p <0.05; p values were calculated with Bonferroni two-sided hipergeometric test).. **c,** qRT-PCRs on bulk spinal cords and cerebellum of EAE and control mice for different isoforms of *Pdgfa* (exon 4 and 5 –regular isoform; exon 5 and 6 –alternative spliced isoform) and *Mbp* (isoforms including either exon 1 or exon 2). CFA n=3 independent biological samples per condition; data represented as mean ± s.e.m., EAE n=5, independent biological samples per condition; data represented as mean ± s.e.m.



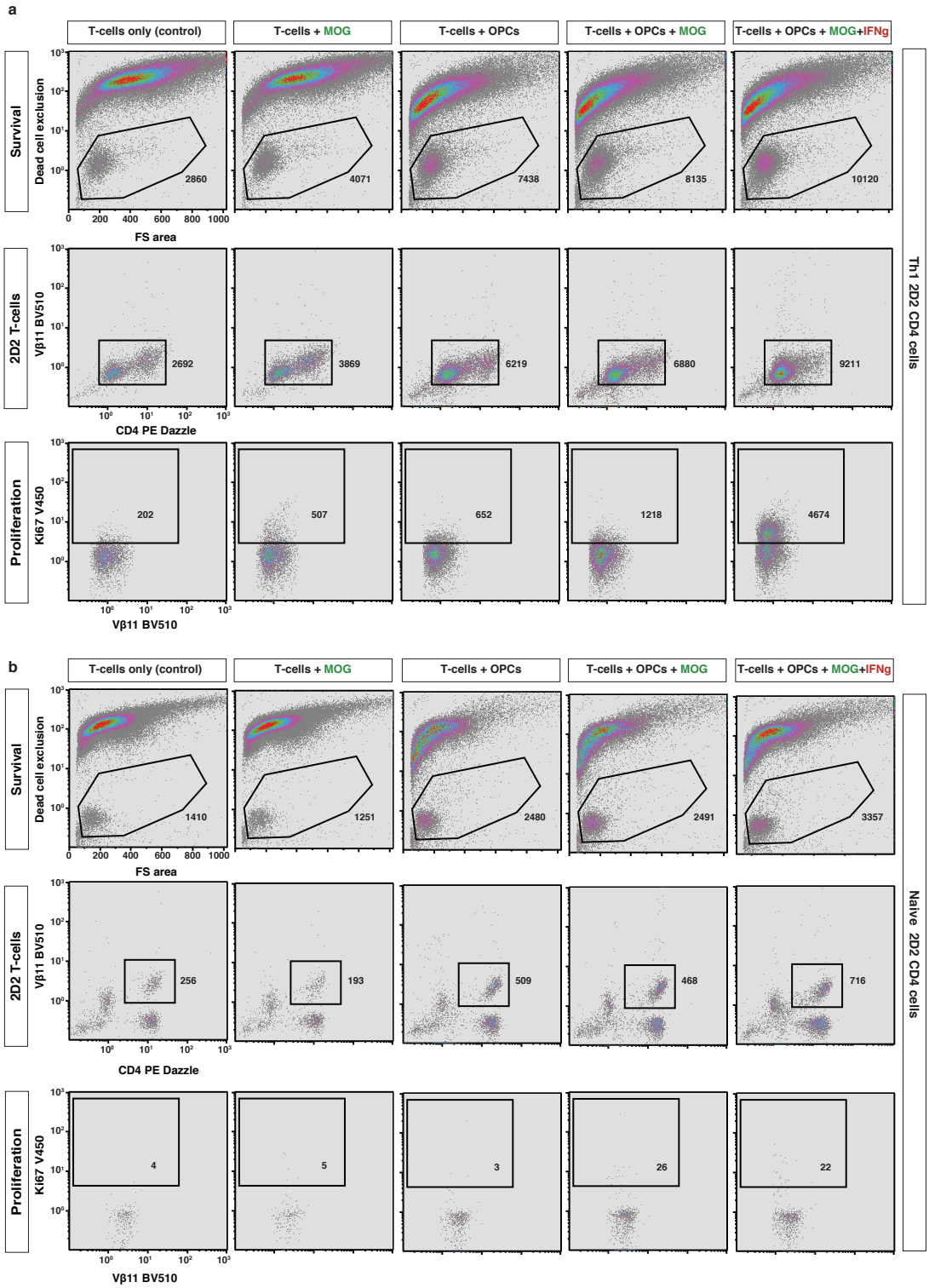
Supplementary Figure 6 | OPC and OL subpopulations display unique expression profiles and markers in EAE and MS. a, Immunohistochemistry showing low-GFP expressing cells (from Pdgfra-H2B-GFP mice) or SOX10 (from wild type EAE mice), co-expressing PLIN4 protein in EAE derived spinal cords, indicating the presence of the MOL5/6 EAE-a. This population is small or rarely found in CFA control mice. Representative images, n=4 biologically independent mouse spinal cord samples for EAE and n=2 biologically independent mouse spinal cord samples for CFA controls; scale bars - 20µm. **b,** RNAscope representing a spinal cord from EAE mice marked with probes for MHC-II, *Cd74*, *H2-eb1* and MOL2, *Klk6*. Arrows show OL lineage cells expressing MHC-II double positive cells. n=2 biologically independent mouse spinal cord samples; scale bars - 20µm. **c,** Immunohistochemistry showing OL lineage cells (positive for OLIG2) co-expressing MHC-II protein in EAE derived spinal cords. Representative images, n=2 biologically independent mouse spinal cord samples, scale bars - 20µm. **d**, Immunohistochemistry in human samples from two MS patients representing OLIG2 positive OLs enwrapped by MHC-II processes (arrowhead) and OLs expressing MHC-II (arrows). Representative images, n=2 biologically independent human brain samples; scale bars represent 25 µm. **e,** Expression-based heat map of X-chromossomal associated susceptibility genes for the MS (ref. 1) in the microglia and OL lineage cells analysed in this study.



Supplementary Figure 7 | **Expression of interferon response and endoplasmic reticulum stress genes in OL lineage cell populations. a,** Violin plots representing several interferon-mediated inducible genes enriched in the OL lineage cell populations OPC1, MOL2 Ct-a, MOL5/6 Ct-a, MOL5/6 Ct-b, OPC2, OPC3, OPCcycling, COPs, NFOLs, MOL1/2 EAE, MOL5/6 EAE-a, MOL5/6 EAE-b, MOL5/6 EAE-c and MOL3 EAE, derived from CFA controls and EAE mice. (n=4 biologically independent mouse spinal cord samples per condition). Violin plots are centered around the median with interquartile ranges and shape represents cell distribution. **b,** KEGG Pathway visualization of genes found differentially expressed between EAE and control oligodendrocyte populations in the protein processing in endoplasmic reticulum category (FDR=0.05). Red is upregulation in EAE and green downregulation in EAE (n=4 biologically independent mouse spinal cord samples per condition).



Supplementary Figure 8 | Induction of MHC class II expression in the OL lineage by EAE-derived CD45+ cells or treatment with interferon-gamma; OPCs regulate expression of cytokines in T-cells. a, Immunohistochemistry showing OL lineage cells (positive for SOX10), lacking the expression of MHC-II protein and the microglia marker IBA, upon coculture with CD45+ cells from CFA control mice. Representative images, n=2 independent experiments; scale bars - 20µm. **b,c,** OPCs, derived either from WT or Sox10-Cre-RCE mice, cultured with interferon-γ, or with interferon-γ and dexamethasone for 72h, but not dexamethasome alone, express MHC-II as represented by *Cd74/Sox10* and MHC-II/GFP double staining in RNAscope ISH and Immunocytochemistry, respectively. The microglia marker IBA1/Aif1 was not observed in these cells. Representative images, n=3 independent experiments per condition; scale bars - 20µm. **d,** Graph plots obtained from FACS analysis of naïve, memory and activated Th1 2D2 CD4 T cells after 72 hours of co-culture with OPCs. Expression of cytokines (interferon-γ and TNF-α) was assessed. Plots represent the averages of the assessed values for the different conditions divided by the values of the control (T cells only) for fold change differences (n=7 independent experiments; data represented as mean ± s.e.m). **e,** FACS plots of memory 2D2 CD4 cells after 72 hours co-coculture with OPCs. General survival (dead cell exclusion), survival of MOG responsive T-cells (V β11+CD4+) and proliferation (Ki67+CD4) was assessed. Representative FACS plots, n=3 biological replicates of one of the seven independent experiments performed. Numbers represent total numbers of cells in each gate.



Supplementary Figure 9 | OPCs regulate T-cells survival and proliferation. a,b, FACS analysis of activated Th1 (a) and naïve (b) 2D2 CD4 cells after 72 hours co-coculture with OPCs. General survival (dead cell exclusion), survival of MOG responsive T-cells (Vβ11+CD4+) and proliferation (Ki67+CD4) was assessed. Representative FACS plots, n=3 biological replicates of one of the seven independent experiments performed. Numbers represent total numbers of cells in each gate.