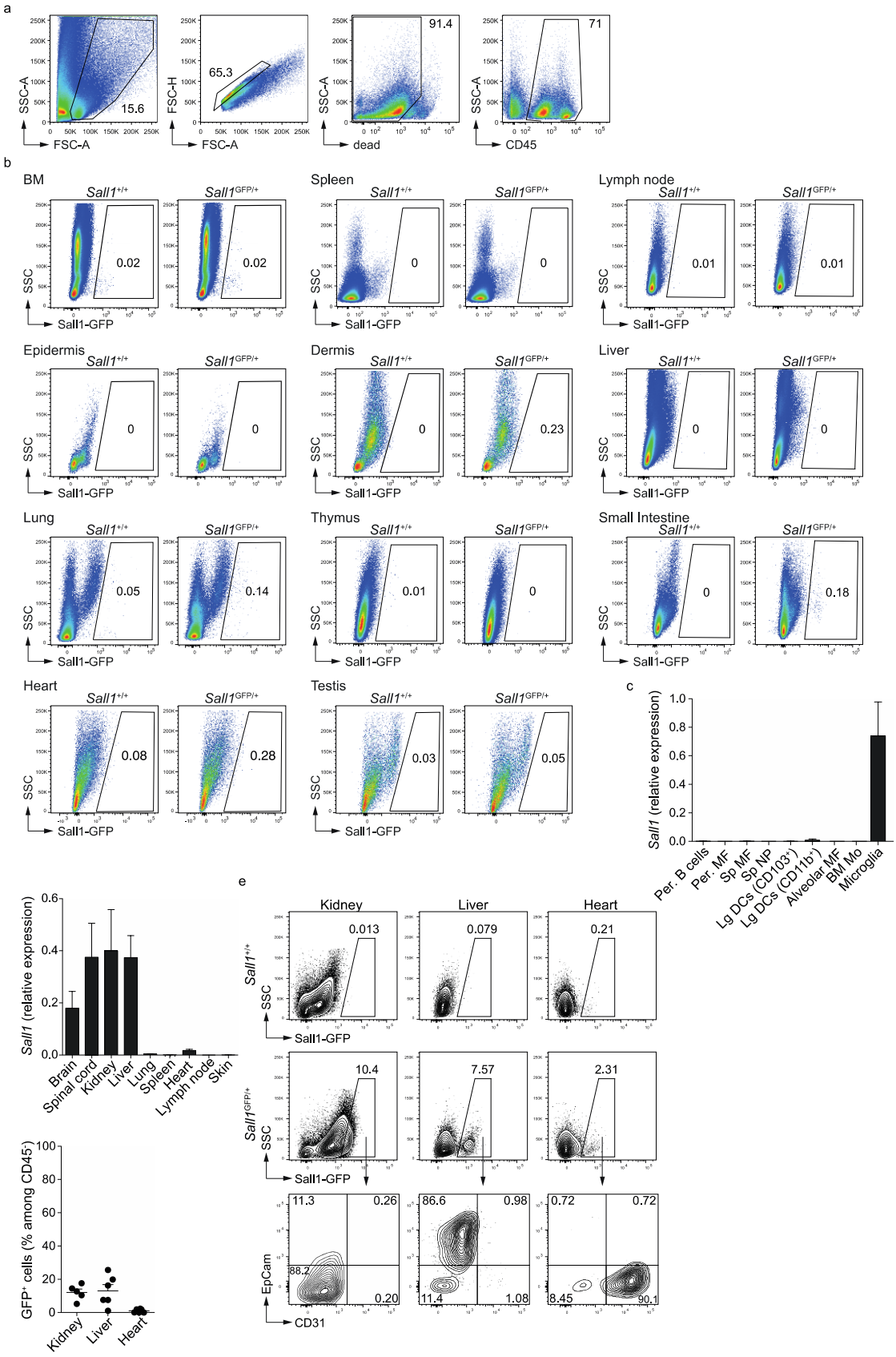
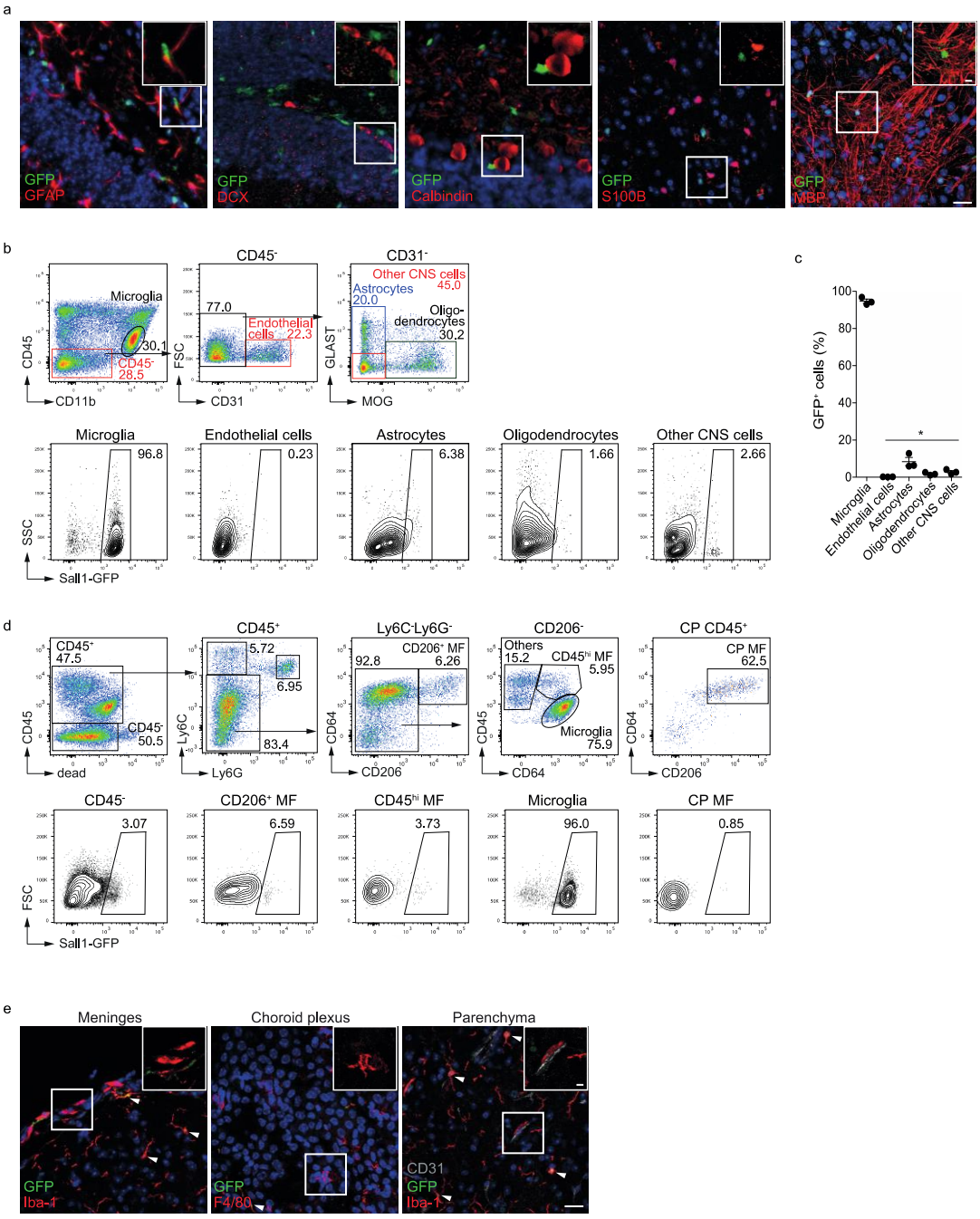


Nature Immunology: doi:10.1038/ni.3585

Supplementary Figure 1

Sall1 expression is restricted to microglia within the hematopoietic system.

(**a**) Flow cytometry plots show representative pre-gating strategy for $CD45^+$ cells (shown are CNS cells). (**b**) Flow cytometry analysis of GFP (Sall1) expression in different organs of *Sall1*$^{GFP/+}$ and *Sall1*$^{+/+}$ (control) mice (pre-gated on $CD45^+$ cells as in **a**. (**c**) qPCR analysis of *Sall1* mRNA in sorted cell populations derived from WT mice; results were normalized to *Pol2* expression. Alveolar MFs: $CD45^+$Siglec-F^+CD11c^+, Lung $CD11b^+$ DCs: $CD45^+$Siglec-$F^-CD11c^+MHCII^+CD11b^+$, Lung $CD103^+$ DCs: $CD45^+$Siglec-$F^-CD11c^+MHCII^+CD103^+$, SP MF (spleen macrophages): $F4/80^{hi}CD11b^+$, SP NPs (spleen neutrophils): $Ly6G^+SSC^{hi}$, BM Mo (BM monocytes): $Lin^-CD11b^+Ly6C^+CD115^+$, microglia: $CD45^{lo}Ly6C^-Ly6G^-CD11b^+F4/80^+$, Per. B cells (peritoneal B cells): $B220^+$, Per. (peritoneal) MFs: $CD115^+CD11b^+F4/80^+$. (**d**) qPCR analysis of *Sall1* mRNA in total tissue lysates of different organs; results were normalized to *Pol2* expression. (**e**) Representative flow cytometry plots of kidney, liver and heart of *Sall1*$^{GFP/+}$ mice (pre-gated on $CD45^-$ cells). (**f**) Quantification of results in **e**, presented as frequency of GFP^+ (Sall1) cells; each symbol represents an individual mouse; small horizontal lines indicate the mean (± s.e.m.). Data are representative of 2-4 mice per genotype, 2 experiments (**b**); 2 samples per population pooled from 2-3 WT mice, 2 experiments (**c**; mean ± s.e.m.); 13 (spleen), 12 (brain), 11 (kidney, liver), 9 (spinal cord), 7 (lung, heart), 4 (skin), 3 (lymph node) WT mice, 2-5 experiments, 1 experiment (lymph node) (**d**; mean ± s.e.m.); 6 (liver), 5 (kidney, heart) *Sall1*$^{GFP/+}$ mice, 2 experiments (**e,f**).

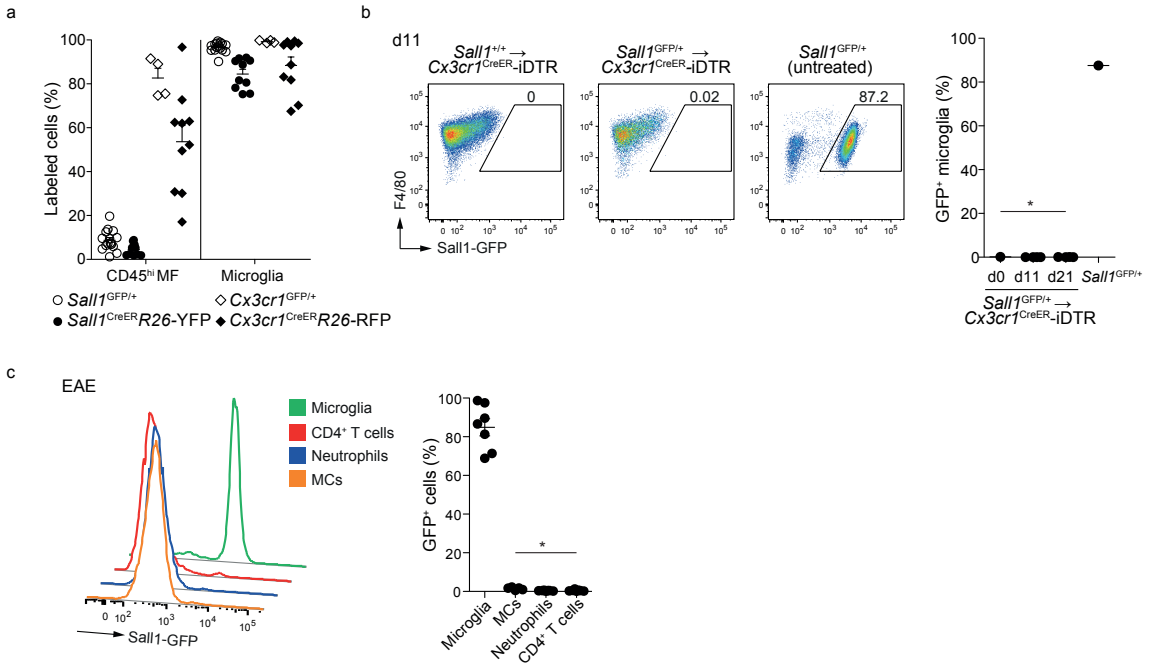


Supplementary Figure 2

Sall1 expression is specific to resident microglia within the adult CNS.

(**a**) IHC of brain sections of *Sall1*$^{GFP/+}$ mice, showing GFP (green), DAPI (blue), and GFAP (radial-glia-like stem cells), DCX (neuroblasts), Calbindin (Purkinje neurons), S100B (astrocytes) or MBP (oligodendrocytes) (red); insets (without DAPI; top right) are enlargements of the outlined areas in the main images. Scale bars, 20 μm (main image) or 5 μm (insets). (**b**) Gating strategy of non-hematopoietic (CD45^{-}) CNS-resident cells and representative flow cytometry plots for their GFP expression in *Sall1*$^{GFP/+}$ mice. (**c**) Quantification of results in **b**, presented as frequency of GFP^{+} cells. Each symbol represents an individual mouse; small horizontal lines indicate the mean (± s.e.m.). *P < 0.0001 (one-way ANOVA). (**d**) Gating strategy of CNS-resident myeloid cells and separately isolated choroid plexus (CP) cells. Representative flow cytometry plots display the percentage of GFP^{+} cells in *Sall1*$^{GFP/+}$ mice. MF:

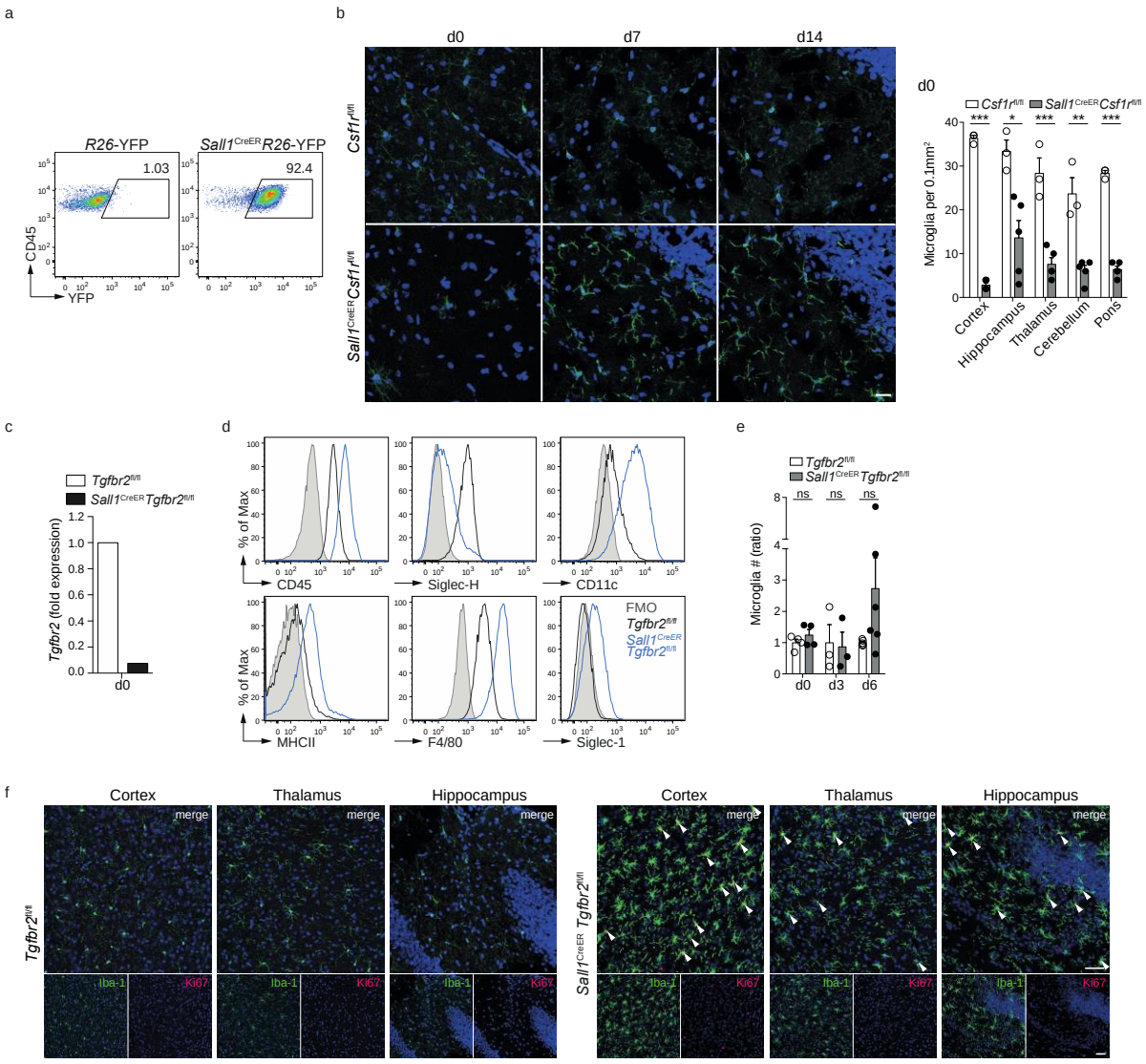
Macrophage. (**e**) IHC of brain sections of *Sall1*$^{GFP/+}$ mice, showing GFP (green), DAPI (blue), Iba-1 or F4/80 (microglia and CNS-MF) (red) and CD31 (endothelial cells) (gray); arrowheads indicate Iba-1 and GFP or F4/80 and GFP double-positive microglia; insets (without DAPI; top right) are enlargements of the outlined areas in the main images. Scale bars, 20 μm (main image) or 5 μm (insets). Data are representative of 2-3 mice per staining, 2 experiments (**a**); 3 mice, 1 experiment (**b,c**); 6 mice, 2 experiments (**d**); 2-3 mice per staining, 2 experiments (**e**).



Supplementary Figure 3

CNS-infiltrating myeloid cells and BM-derived microglia and/or macrophages do not express *Sall1*.

(**a**) Frequency of GFP$^+$ microglia and CD45^{hi} MF of *Sall1*$^{GFP/+}$ and *Cx3cr1*$^{GFP/+}$ reporter mice and of YFP$^+$ or RFP$^+$ microglia and CD45^{hi} MFs in tamoxifen treated *Sall1*^{CreER}*R26*-YFP or *Cx3cr1*^{CreER}*R26*-RFP mice. Microglia: CD45^{lo}CD11b$^+$F4/80$^+$Ly6C$^-$Ly6G$^-$, CD45^{hi} MFs: CD45^{hi}CD11b$^+$F4/80$^+$Ly6C$^-$Ly6G$^-$. (**b**) Flow cytometry analysis and quantification of the frequency of GFP$^+$ microglia in tamoxifen- and diphtheria toxin-treated *Sall1*$^{+/+}$(CD45.1)→*Cx3cr1*^{CreER}-iDTR(CD45.2) or *Sall1*$^{GFP/+}$(CD45.1)→*Cx3cr1*^{CreER}-iDTR(CD45.2) BM chimeras on day 0, 11 and 21 after treatment (pre-gated on CD11b$^+$F4/80$^+$CD45.1^{lo}Ly6C$^-$Ly6G$^-$ cells) or in untreated *Sall1*$^{GFP/+}$ (control) mice. (**c**) Representative histograms and quantification of GFP expression in monocyte-derived cells (MCs) (gated on CD45^{hi}CD11b$^+$CD11c$^+$MHCII$^+$Ly6C$^+$), neutrophils (gated on CD45^{hi}CD11b$^+$ Ly6G$^+$), CD4$^+$ T cells (gated on CD45^{hi}CD11b$^-$CD4$^+$) and microglia (gated on CD45^{lo}Ly6C$^-$Ly6G$^-$CD11b$^+$) in *Sall1*$^{GFP/+}$ mice at peak disease of MOG$_{35-55}$/CFA-induced EAE. Each symbol (**a-c**) represents an individual mouse; small horizontal lines (**a-c**) indicate the mean (± s.e.m.). *P < 0.0001 (one-way ANOVA). Data are representative of 15 (*Sall1*$^{GFP/+}$), 10 (*Sall1*^{CreER}*R26*-YFP, *Cx3cr1*^{CreER}*R26*-RFP), 4 (*Cx3cr1*$^{GFP/+}$) mice, at least 2 experiments (**a**); 4 mice (d11, d21), 2 experiments and 1 mouse (d0, untreated *Sall1*$^{GFP/+}$) (**b**); 7 (microglia), 5 (MCs, Neutrophils, CD4$^+$ T cells) mice, 3 experiments (**c**).

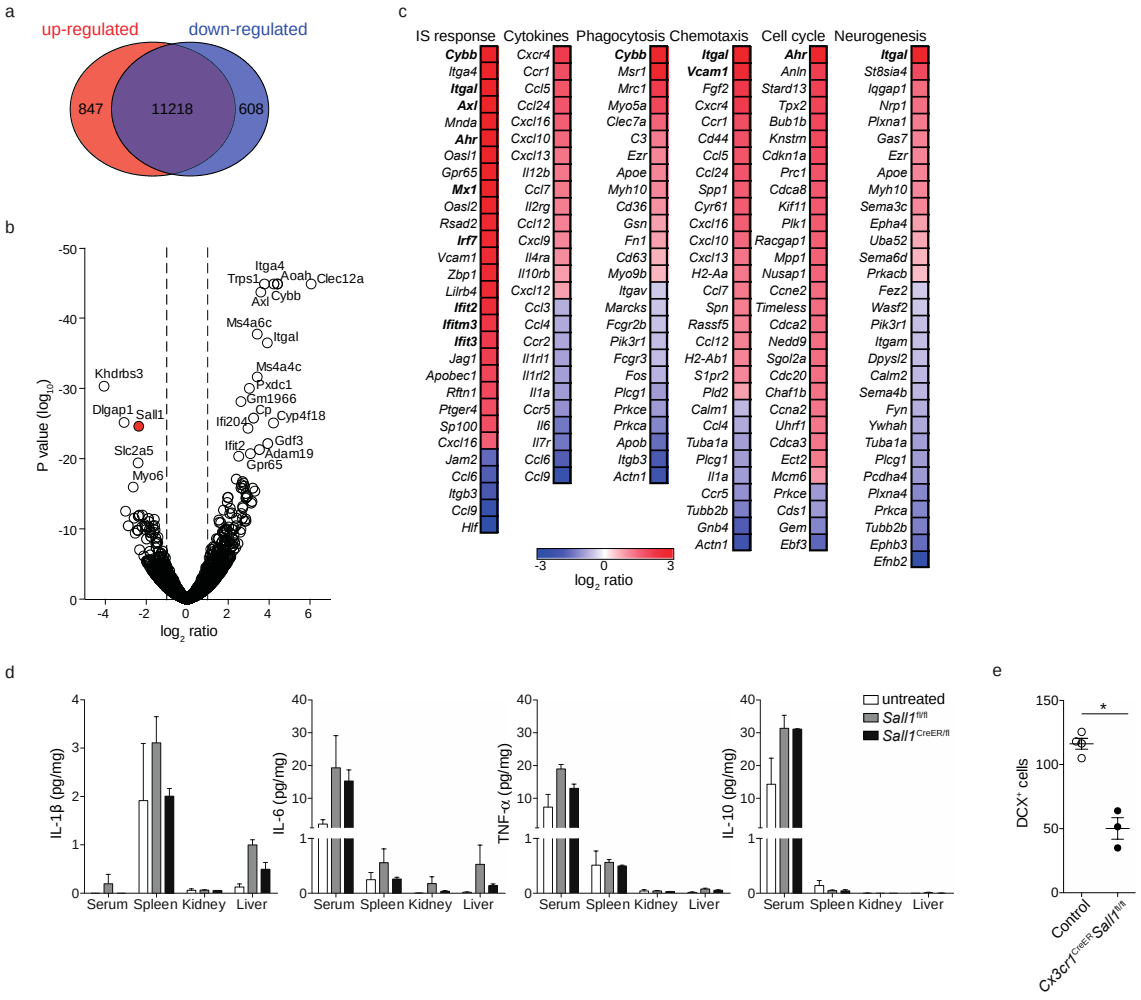


Supplementary Figure 4

Microglia-specific targeting utilizing *Sall1*^{CreER} mice.

(**a**) Flow cytometry analysis of *Sall1*^{CreER}*R26*-YFP mice and *R26*-YFP (control) littermates on day 3 after 5 consecutive days of tamoxifen treatment, showing the frequency of YFP^{+} microglia (pre-gated on CD45^{lo}Ly6G^{-}Ly6C^{-}CD11b^{+}). (**b**) IHC of cortical brain sections of *Sall1*^{CreER}*Csf1r*$^{fl/fl}$ and *Csf1r*$^{fl/fl}$ mice at day 0, 7 and 14 after 5 consecutive days of tamoxifen treatment, showing Iba-1 (microglia) (green) and DAPI (blue). Scale bar, 20 µm. Quantification shows microglia counts in different brain areas at day 0 after tamoxifen treatment. (**c**) qPCR analysis of *Tgfbr2* mRNA in microglia sorted from *Sall1*^{CreER}*Tgfbr2*$^{fl/fl}$ and *Tgfbr2*$^{fl/fl}$ mice on day 0 after three consecutive days of tamoxifen treatment; results were normalized to *Pol2* expression. (**d**) Histograms display the expression of different surface markers vs. FMO on microglia of *Sall1*^{CreER}*Tgfbr2*$^{fl/fl}$ and *Tgfbr2*$^{fl/fl}$ mice as in **c** on day 6 after tamoxifen treatment. (**e**) Quantification of microglia numbers in *Sall1*^{CreER}*Tgfbr2*$^{fl/fl}$ and *Tgfbr2*$^{fl/fl}$ mice as in **c** on day 0, 3 and 6 after tamoxifen treatment. Numbers are displayed as ratios to control (*Tgfbr2*$^{fl/fl}$) mice. (**f**) IHC of brain sections from *Sall1*^{CreER}*Tgfbr2*$^{fl/fl}$ and *Tgfbr2*$^{fl/fl}$ mice as in **c** analyzed on day 3 after tamoxifen treatment, showing Iba-1 (green), Ki67 (red) and DAPI (blue). Arrowheads indicate Iba-1 and Ki67 double-positive cells. Scale bar, 50 µm. Each symbol (**b,e**) represents an individual mouse. ns = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (unpaired *t*-test). Data are representative of 10 mice, 5 experiments (**a**); 3-5 sections of 2 mice, 2 experiments (**b**; mean ± s.e.m.); 1 pooled sample of 3-4 mice per genotype (**c**); 2-5 mice per genotype, at least 2

experiments (**d**); 4 (d0), 3 (d3), 5-6 (d6) mice, 2 experiments (**e**; mean ± s.e.m.); 1-2 mice per genotype (**f**).



Supplementary Figure 5

Gene expression profile of *Sall1*-deficient microglia.

(**a-c**) Gene expression analysis of microglia sorted from *Sall1*$^{CreER/fl}$ and *Sall1*$^{fl/fl}$ mice on day 1 after 5 times of tamoxifen treatment every second day as described in **Figure 4**. (**a**) Venn diagram of differentially expressed genes. (**b**) Volcano plot showing log_2 ratios vs. p values (log_{10}) of all 12,673 detected genes. Genes with highest significance values are annotated. (**c**) Expression (log_2 ratio) of *Sall1*-regulated genes clustered according to their indicated GO-pathways; IS, Immune system; (bold indicates genes discussed in Results). (**d**) Multiplex immunoassays show levels (pg/mg) of IL-1, IL-6, TNF-α and IL-10 in serum and whole tissue lysates of spleen, kidney and liver of untreated (control) mice and *Sall1*$^{CreER/fl}$ and *Sall1*$^{fl/fl}$ mice at day 6 after start of tamoxifen treatment. (**e**) Graph displays cell counts of DCX$^+$ neuroblasts in hippocampal brain sections of tamoxifen treated *Cx3cr1*^{CreER}*Sall1*$^{fl/fl}$ and Cre$^-$ (control) littermates; each symbol represents an individual mouse; small horizontal lines indicate the mean (± s.e.m.). * p = 0.0006 (unpaired *t*-test). Data are representative of 3-5 mice pooled per genotype and biological replicate, 3 experiments (**a-c**); 3 (*Sall1*$^{CreER/fl}$, *Sall1*$^{fl/fl}$), 2 (untreated) mice, 1 experiment (**d**; mean ± s.e.m.); 3 (*Cx3cr1*^{CreER}*Sall1*$^{fl/fl}$), 4 (control) mice, 2 experiments (**e**).