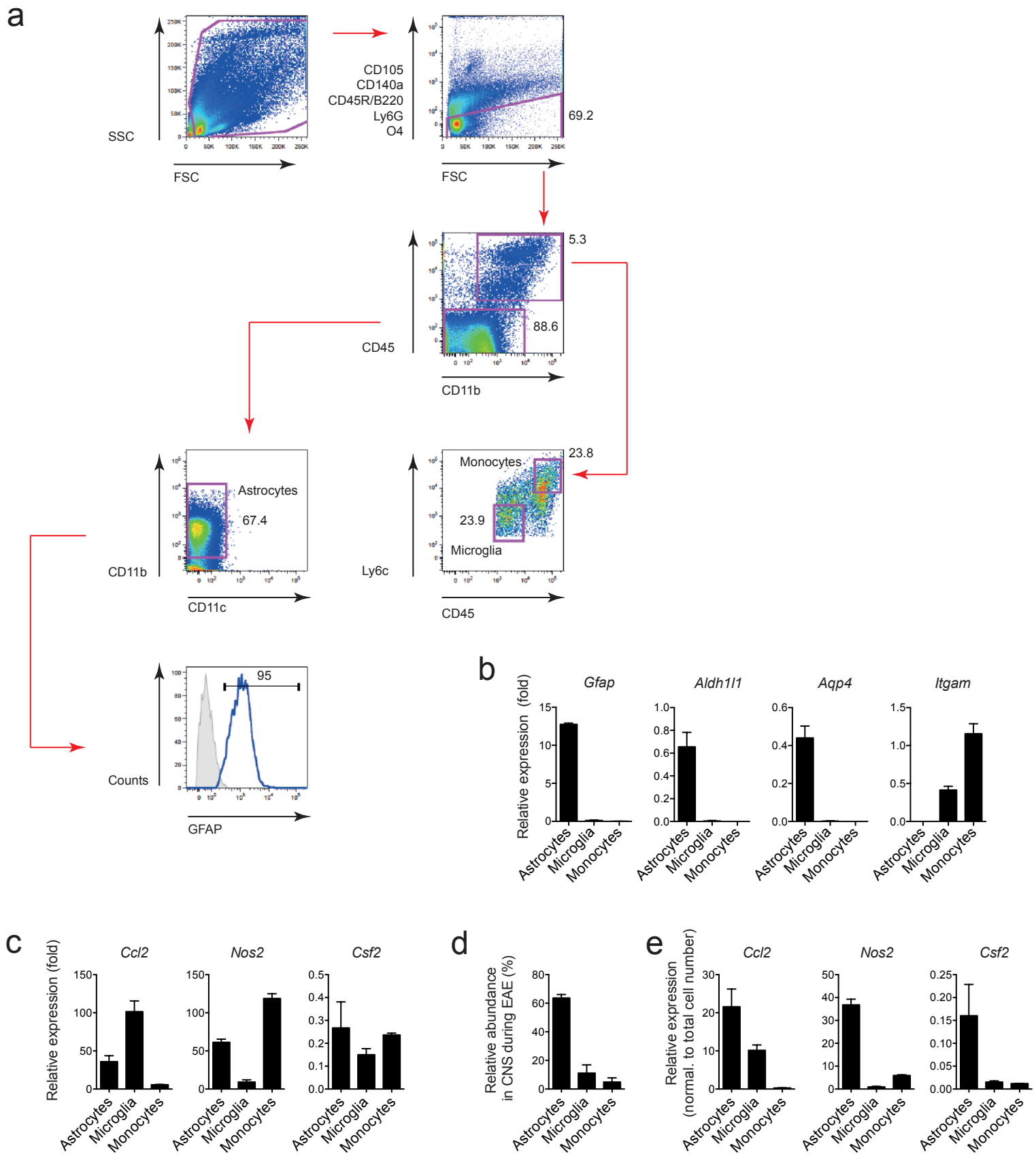
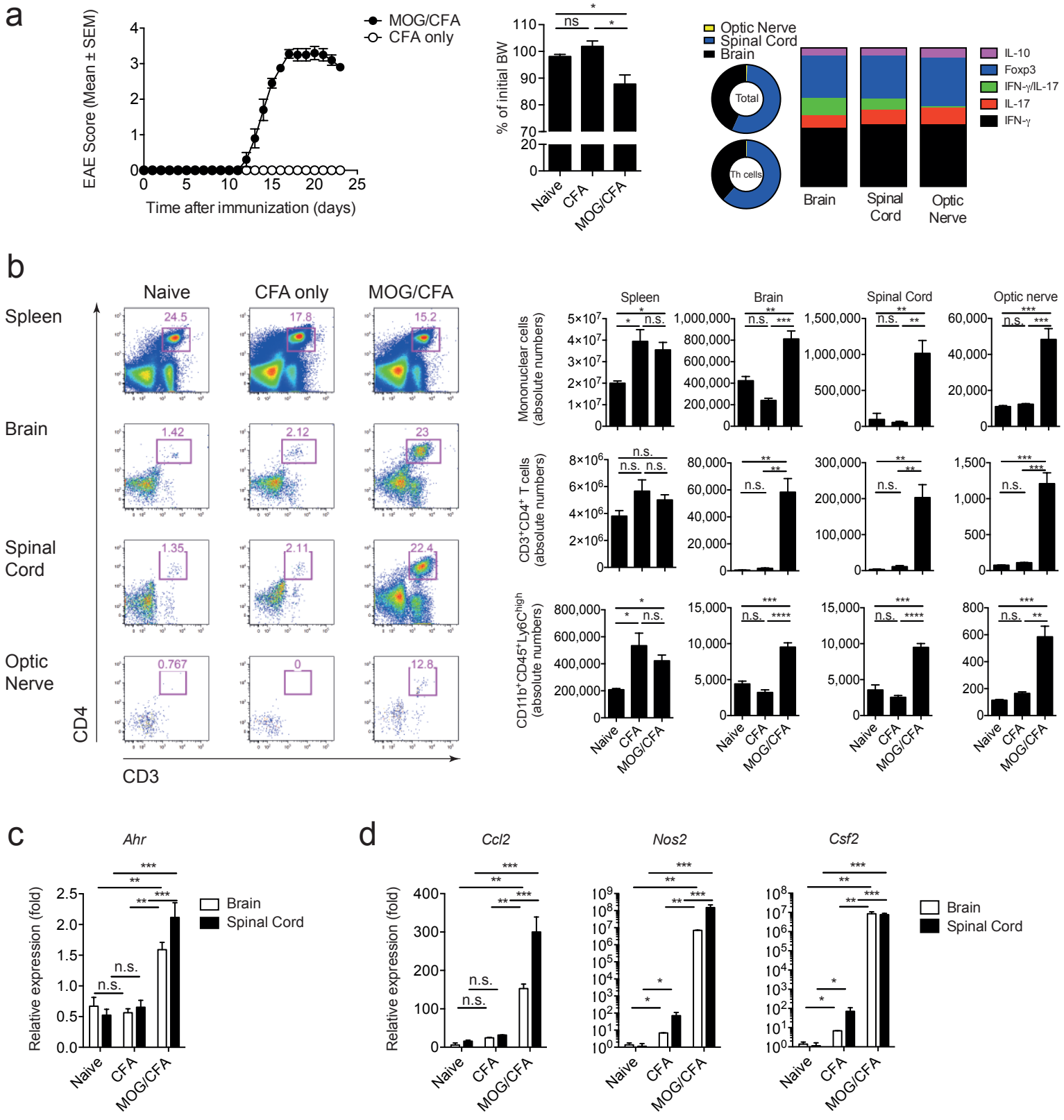
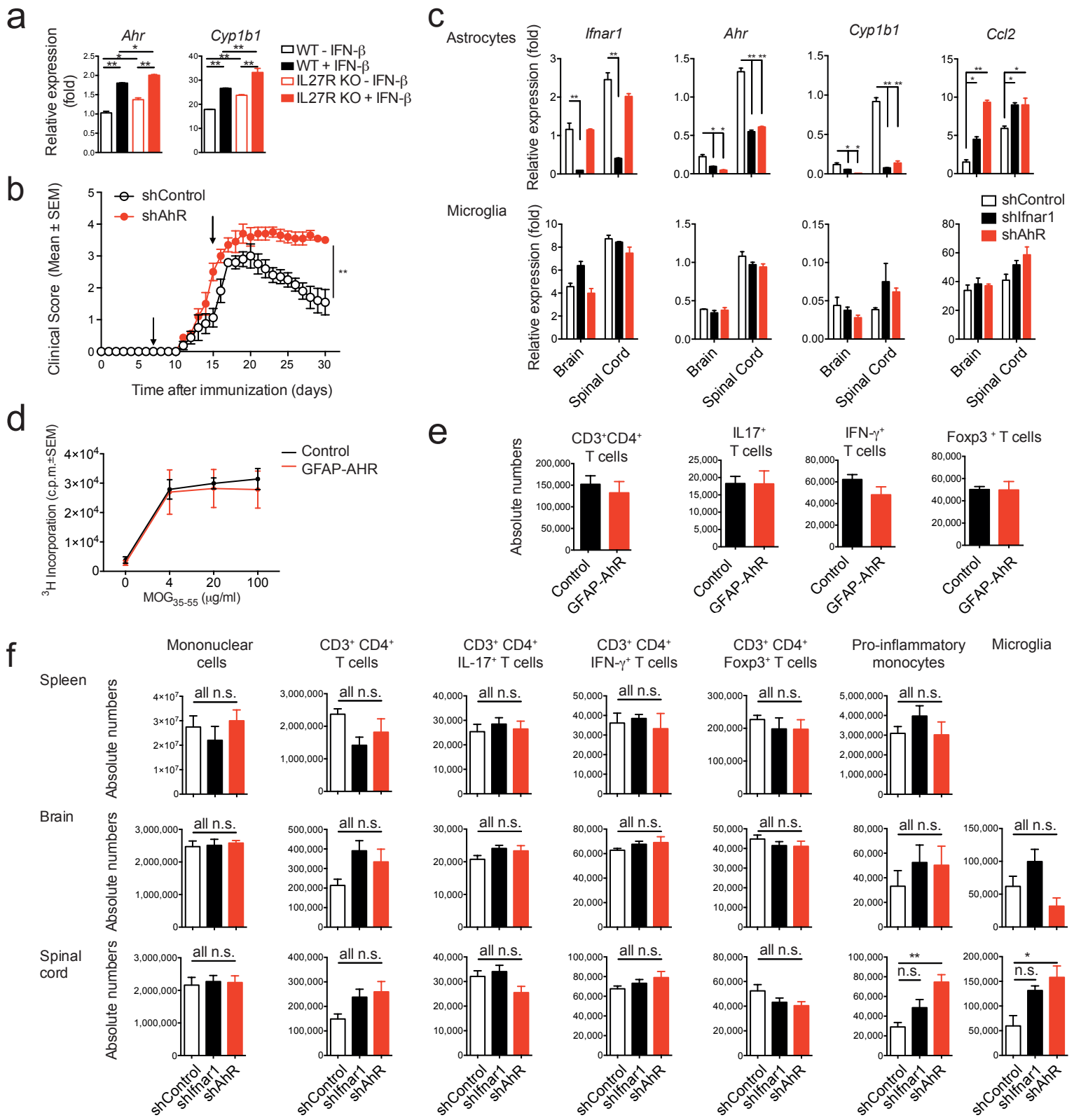


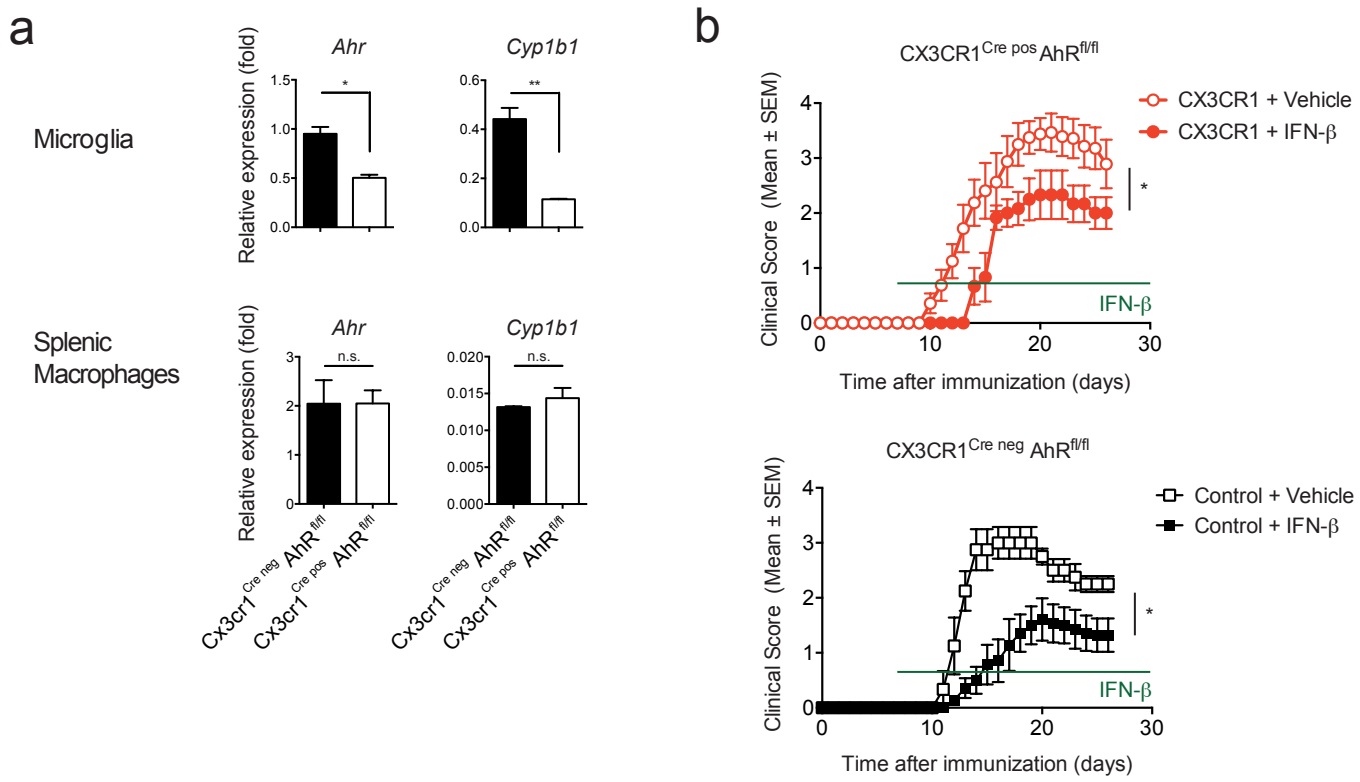
Supplementary Figure 1. FACS sorting, purity and effector molecule production of CNS cell populations during EAE. (**a**) FACS gating strategy for the purification of astrocytes, monocytes and microglia from mononuclear CNS cell suspensions. Purity of sorted astrocyte population was higher than 95% as determined by intracellular staining for glial fibrillary acidic protein (GFAP). Numbers indicate percentages. Representative for $n > 10$ experiments. (**b,c**) Relative expression of indicated genes in sorted cell populations from peak disease EAE mice ($n = 3$; representative of three independent experiments; normalized to Monocytes *Itgam*). (**d**) Relative abundance of indicated cell type in CNS of peak disease EAE mice ($n = 3$; representative of two independent experiments). (**e**) Relative expression of *Ccl2*, *Nos2*, and *Csf2* in indicated cell types as normalized to the total abundance of each cell type in mouse EAE brain ($n = 3$; representative of two independent experiments).



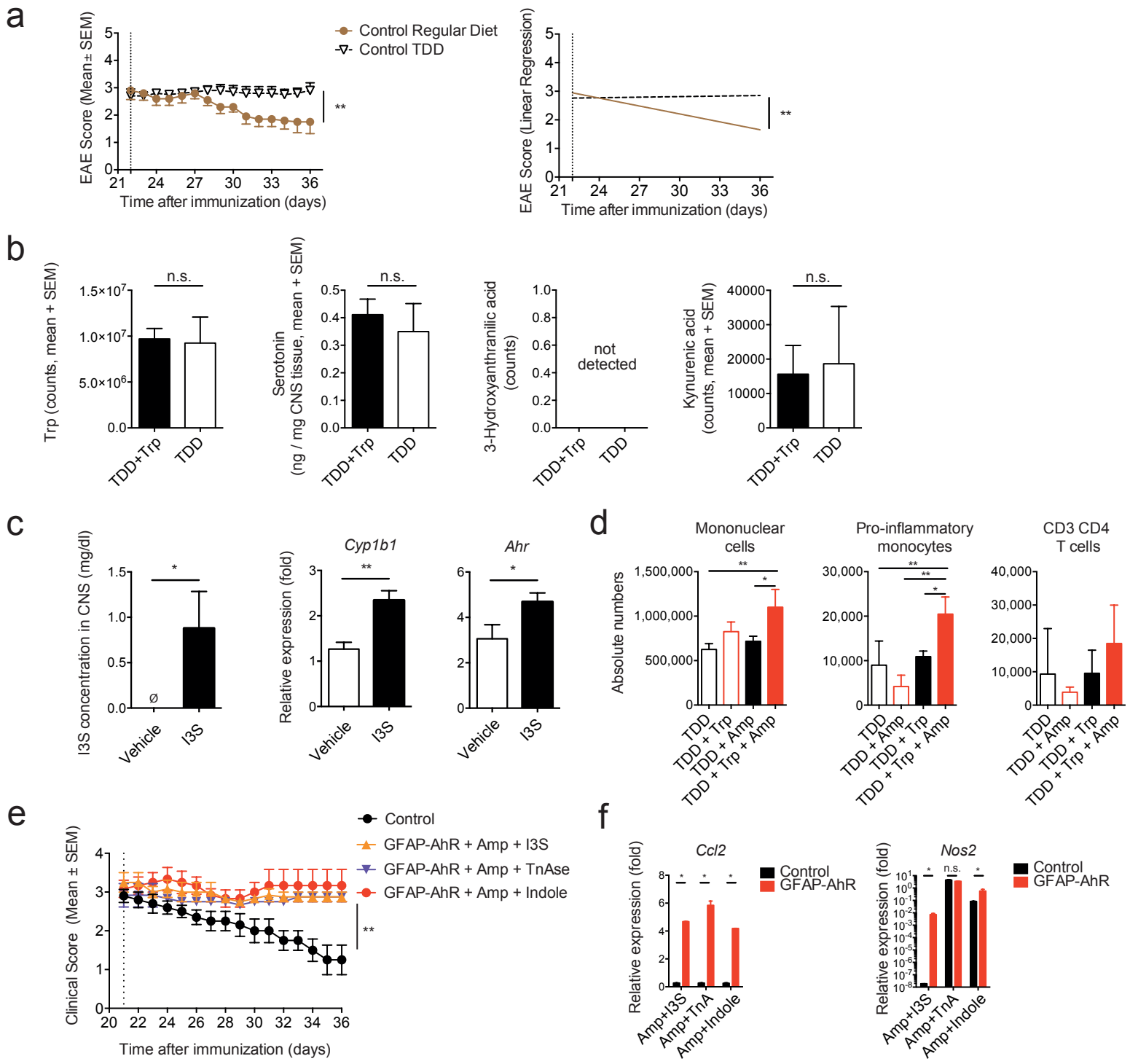
Supplementary Figure 2. Effects of adjuvant on CNS immune cell infiltration and pro-inflammatory molecule production. (**a**) Left: Clinical Score of mice immunized with CFA only or MOG_{35-55}/CFA immunized mice (n = 5, mean + s.e.m.). Middle: Percentage of initial body weight (BW) at day 23 after EAE induction (means + s.e.m., n = 5, one-way ANOVA followed by Tukey's post-hoc test). Right: relative fractions of total mononuclear and T cells (pie charts), as well as T cell cytokine distribution (parts of whole diagram) in brain, spinal cord, and optic nerve during EAE (d23). Representative out of two independent experiments with n = 5 mice each. (**b**) Left: Spleen, brain, spinal cord, and optic nerve infiltrating mononuclear cells were isolated separately and stained for CD3 and CD4. Numbers in dot plots indicate percentages in live cell gate (representative out of five independent experiments). Right: quantification of absolute cell numbers of mononuclear cells, T cells, and pro-inflammatory monocytes in spleen, brain, spinal cord, and optic nerve at peak disease (day 23; means + s.e.m, n = 5, one-way ANOVA followed by Tukey's post-hoc test). (**c,d**) qPCR for indicated transcripts from astrocytes sorted separately from brain and spinal cord at day 23 after immunization (n = 3, means + s.e.m., one-way ANOVA followed by Tukey's post-hoc test; normalized to Brain naïve *Ccl2*). Significance levels: * P<0.05; ** P<0.01; *** P<0.001. n.s. not statistically significant.



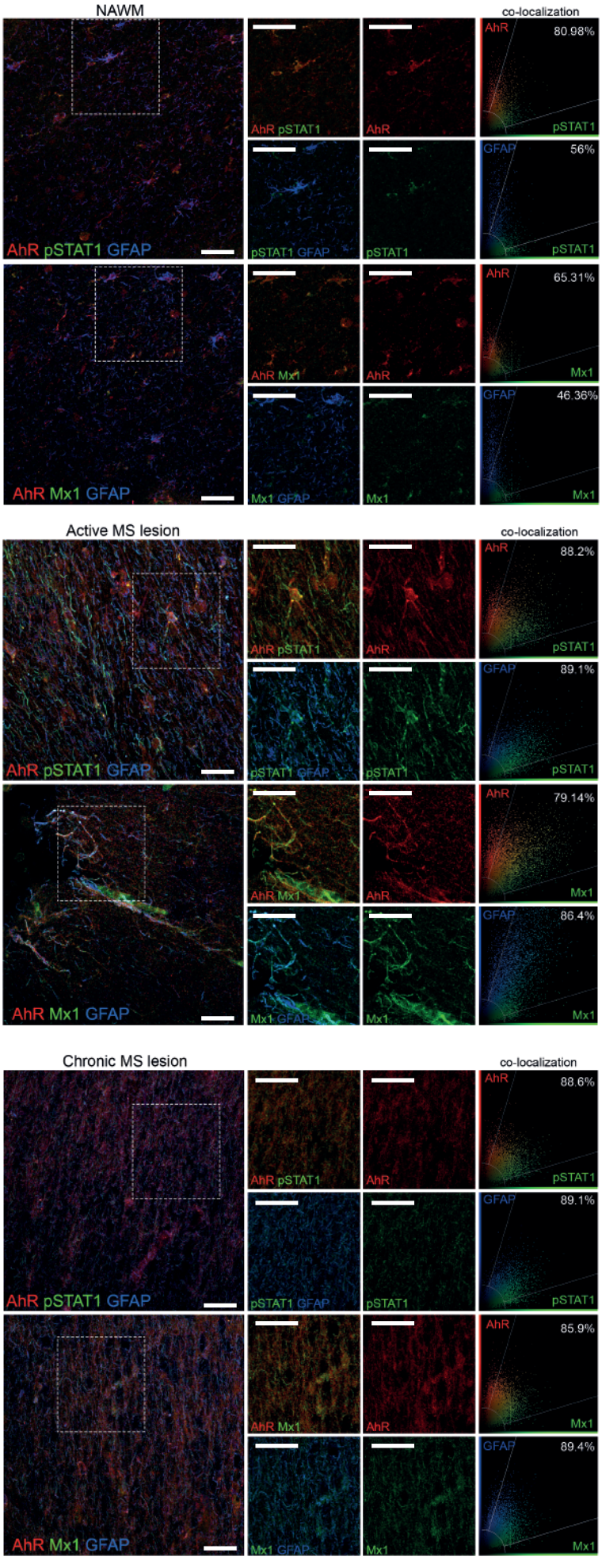
Supplementary Figure 3. IFNAR1 and AhR signaling in astrocytes modulate EAE.
(**a**) WT or IL27R KO astrocytes were treated with IFN-β or vehicle and indicated mRNAs were measured by qPCR (n = 3, representative of two independent experiments; one-way ANOVA followed by Tukey's multiple comparisons test; normalized to WT – IFN-β *Ahr*). (**b**) Clinical scores of shControl or shAhR injected mice after intracerebroventricular injection on day 7 and day 15 (mean ± SEM representative out of two independent experiments with n = 10 mice per group; Two-way ANOVA). (**c**) qPCR of indicated genes in astrocytes and microglia FACS-sorted separately from brains and spinal cords of shControl, shIfnar1, and shAhR injected mice (n = 3 per group, representative of two independent experiments, one-way ANOVA followed by Tukey's multiple comparisons test; normalized to Brain shControl *Ifnar1*). (**d**) Proliferation assay of splenocytes isolated from control or GFAP-AhR mice at peak of disease (n = 3; representative of three independent experiments). (**e**) FACS analysis of absolute numbers of CNS infiltrating immune cells at peak of disease (CD3+CD4+ T cells, CD3+ CD4+ IFN-γ- IL17+ Th17 cells, CD3+ CD4+ IL-17+ Ifn-γ+ Th1 cells, CD3+ CD4+ IFN-γ- IL-17- Foxp3+ Treg cells). n = 5 per group, representative of 5 independent experiments, no significant difference as determined by Student's t-test. (**f**) Quantification of CD3+ CD4+ T cells, pro-inflammatory monocytes, and microglia of indicated cell populations in shControl, shIfnar1, and shAhR injected mice at day 30 after disease induction (n = 5 per group, representative of two independent experiments, one-way ANOVA, Tukey's multiple comparisons test). Significance levels: * P<0.05; ** P<0.01; n.s. not statistically significant.



Supplementary Figure 4. AhR expressed in microglia does not mediate the protecitve effects of intranasal IFN-β. (***a***) CX3CR1^{CreERT2}AhR$^{fl/fl}$ or Cre negative littermates were injected with tamoxifen. Microglia and splenic macrophages were FACS-sorted 30 days after tamoxifen injection and relative expression of *Ahr* and *Cyp1b1* determined by qPCR (n = 2, representative of two independent experiments; one-way ANOVA followed by Tukey's post-hoc test; normalized to control microglia *Ahr*). (**b**) 30 days after Cre induction, CX3CR1^{CreERT2}AhR$^{fl/fl}$ or Cre negative littermates were immunized with MOG_{35-55}/CFA. Treatment with intranasal IFN-β was initiated daily at day 7 after disease induction. Clinical scores (mean ± SEM; representative out of two independent experiments with n = 4 mice per group; two-way ANOVA). Signifance levels: *P<0.05, **P<0.01, n.s. not statistically significant.

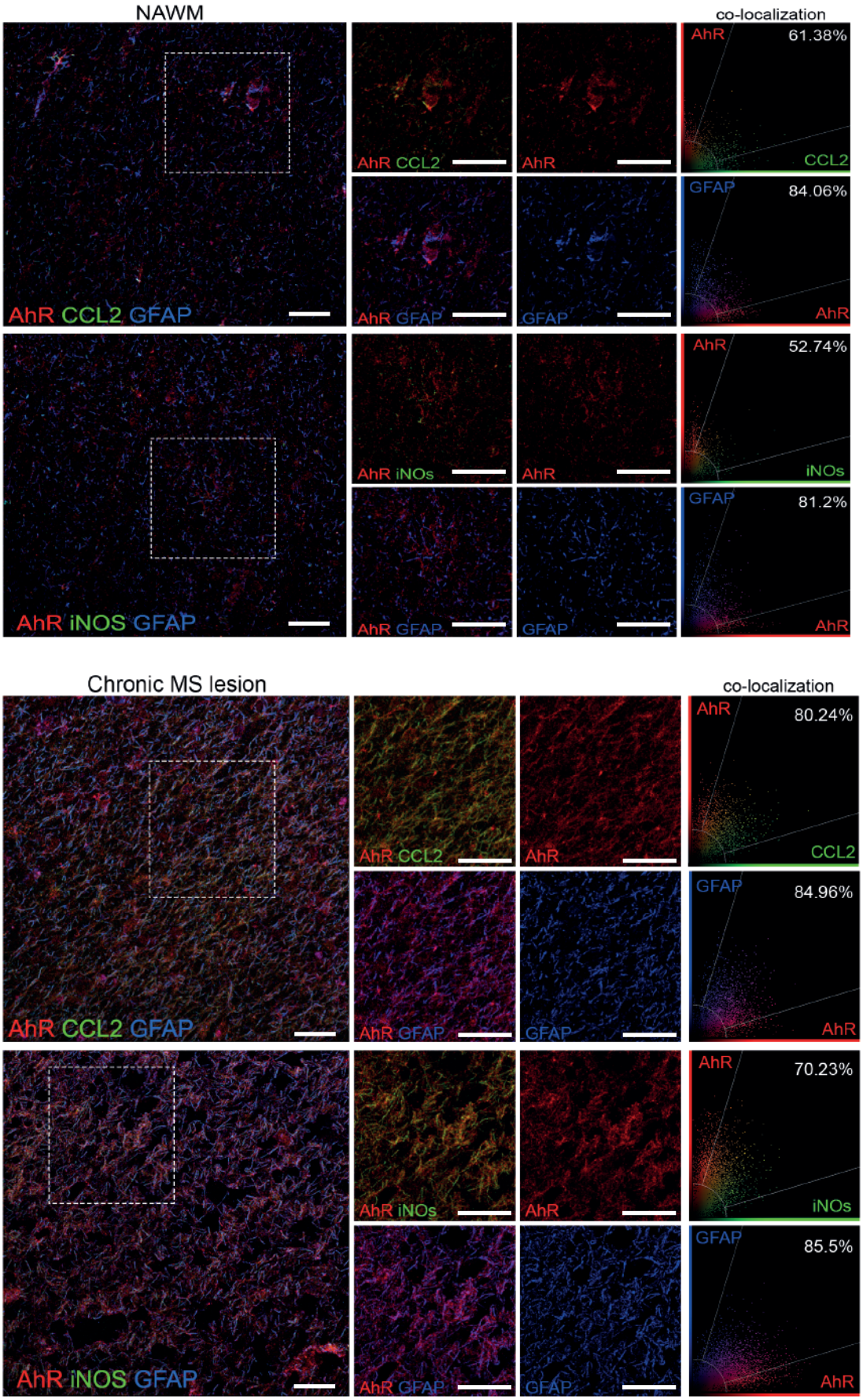


Supplementary Figure 5. Effects of TDD on EAE, CNS metabolites and astrocyte activation.
(**a**) Control animals were treated as indicated starting on day 22 after EAE induction (TDD: Tryptophan depleted diet; Trp: Tryptophan) Clinical scores (left) and linear regression (right) (mean ± s.e.m. in left graph; linear regression in right diagram; representative out of two independent experiments with *n* = 10 mice per group; two-way ANOVA). (**b**) Trp, Serotonin, 3-Hydroxyanthranilic acid, and Kynurenic acid levels in the CNS of EAE mice after 14 days of TDD or control diet as measured by mass spectrometry (Trp, 3-Hydroxyanthranilic acid, Kynurenic acid) or ELISA (Serotonin); *n* = 5 per group, mean + SEM, Student's *t*-test. (**c**) Naive WT mice were treated with daily intraperitoneal injections of I3S for three days. Thereafter, mice were sacrificed and perfused extensively. CNS was isolated, minced and sonicated. After removal of cellular debris I3S levels were determined by Indican ELISA (left). (I3S levels normalized to CNS tissue weight; representative of two independent experiments, *n* = 3, Student's *t*-test). Astrocytes from I3S or contrl treated mice were FACS-sorted, and relative RNA abundances of *Cyp1b1* and *Ahr* were determined by qPCR (representative of two independent experiments, *n* = 3; Student's *t*-test; normalized to Vehicle *Cyp1b1*). (**d**) Quantification of absolute cell numbers of indicated populations in antibiotics or diet treated experiments (representative of two independent experiments, *n* = 5; one-way ANOVA followed by Tukey's post-hoc test). (**e**) Control or GFAP-AhR animals were treated as indicated starting on day 22 after EAE induction. Clinical scores (mean ± s.e.m., representative out of two independent experiments with *n* = 5 mice per group; two-way ANOVA). (**f**) Treatment groups from **e** were sacrificed at day 36, astrocytes were FACS-sorted and total RNA subjected to qPCR for *Ccl2* and *Nos2*. (representative out of two independent experiments with *n* = 5 mice per group; one-way ANOVA followed by Tukey's post-hoc test; normalized to GFAP-AhR Amp+Indole). Significance levels: * $P<0.05$, ** $P<0.01$, *** $P<0.001$, n.s. not statistically significant, ø not detected).



Supplementary Figure 6. IFN-I signaling is activated in MS tissue.

Immunofluorescence staining of human white matter brain tissue of NAWM, active and chronic MS lesions for AhR (red), pSTAT1 or Mx1 (green), and GFAP (blue). The co-expression of AhR with Mx1 and pSTAT1 is shown in the colocalization scatter graphs (right panels). Data shown are representative of *n* = 10 fields per tissues type from two distinct MS brains. Scale bar: 20 µm.



Supplementary Figure 7. CCL2, iNOS and AhR colocalize in GFAP+ astrocytes in MS lesions.
Immunofluorescence staining of human white matter brain tissue from NAWM and chronic MS lesions for AhR (red), CCL2 (green, top), iNOS (green, bottom) and GFAP (blue). The co-expression of AhR with CCL2 and iNOs is shown in the colocalization scatter graphs (right panels). Data shown are representative of *n* = 12 fields per tissues type from three distinct MS brains.
Scale bar: 20 μm.

Name of gene set	Gene set	Overlap	p-value
Signal transduction	1634	151	6.4×10^{-20}
Interferome Type I Interferon responsive genes	988	271	5.2×10^{-19}
Reactome Interferon Signaling	159	41	2.1×10^{-18}
Interferon-beta response up	102	32	1.6×10^{-16}
Interferon-beta1 targets	95	27	1.8×10^{-15}
IkB-Kinase - NF-kB Cascade	114	28	3.1×10^{-14}
Positive regulation of cell proliferation	149	24	2.0×10^{-8}
Regulation of cellular metabolic processes	787	64	6.4×10^{-7}
Cytokine activity	113	18	1.4×10^{-6}
Cell migration	96	16	2.9×10^{-6}
Chemokine activity	42	10	8.1×10^{-6}
Inflammatory response	129	17	3.6×10^{-5}

Supplementary Table 1. Ingenuity pathway analysis of the transcriptional profile of astrocytes in EAE or healthy mice.

Nanostring array						
Ahr	*Chi3l3*	*Fgf2*	*Il18*	*Mbp*	*Sirt2*	*Traf3ip2*
Arg1	*Ciita*	*Fos*	*Il18r1*	*Mcam*	*Sirt3*	*Tubb5*
Arnt	*Cltc*	*Fosb*	*Il18rap*	*Megf10*	*Sirt4*	*Tyro3*
Axl	*Cntf*	*Fosl1*	*Il1b*	*Meig1*	*Sirt5*	*Vcam1*
B2m	*Csf1*	*Fosl2*	*Il2*	*Mertk*	*Sirt6*	*Vegfa*
B4galt6	*Csf2*	*Foxj1*	*Il21*	*Mmp11*	*Sirt7*	*Vim*
Bach1	*Csf3*	*Gapdh*	*Il21r*	*Mmp12*	*Slc1a2*	*Traf3ip2*
Bag3	*Cspg4*	*Glul*	*Il23a*	*Mmp2*	*Socs3*	*Tubb5*
Bcan	*Ctla4*	*Glycam1*	*Il27*	*Mmp3*	*Sox8*	*Tyro3*
Bcl2	*Cx3cl1*	*Gusb*	*Il27ra*	*Mmp9*	*Spp1*	*Vcam1*
Bcl3	*Cx3cr1*	*H2 -Aa*	*Il33*	*Cd206*	*Sra1*	*Vegfa*
Beclin1	*Cxcl10*	*H2 -Ab1*	*Il4*	*Msx1*	*Stat1*	*Vim*
Ccl1	*Cxcl11*	*H2 -Ea*	*Il4ra*	*Msx2*	*Stat2*	
Ccl17	*Cxcl12*	*Helz2*	*Il5*	*cMyc*	*Stat3*	
Ccl19	*Cxcl13*	*Hif1a*	*Il6*	*Ncan*	*Stat4*	
Ccl2	*Cxcl14*	*Hmox1*	*Il7r*	*Nfe2l2*	*Stat5a*	
Ccl20	*Cxcl15*	*Hprt1*	*Irf1*	*Nfil3*	*Stat6*	
Ccl3	*Cxcl16*	*Hsp90*	*Irf2*	*Ngf*	*Tbk1*	
Ccl4	*Cxcl2*	*Icam1*	*Irf3*	*Nos2*	*Tgfb1*	
Ccl5	*Cxcl3*	*Ifih1*	*Irf 4*	*Nqo1*	*Tgfb2*	
Ccl7	*Cxcl9*	*Ifnar1*	*Irf5*	*Nr1d1*	*Tgfb3*	
Ccl8	*Cxcl8*	*Ifnb1*	*Irf6*	*Cd73*	*Timp1*	
Ccr2	*Cxcr4*	*Ifng*	*Irf7*	*Ntf3*	*Tiparp*	
Ccr5	*Cyp1a1*	*Igf1*	*Irf8*	*Ntf4*	*Tlr1*	
Cd14	*Cyp1b1*	*Il10*	*Irf9*	*Pdgfa*	*Tlr11*	
Cd163	*Ddx58*	*Il10ra*	*Itga7*	*Pdgfb*	*Tlr12*	
Cd209	*Dhx58*	*Il11*	*Itgam*	*Pgk1*	*Tlr2*	
Cd24a	*Ebi3*	*Il12a*	*Itgax*	*Ptgs1*	*Tlr3*	
Cd36	*Emr1*	*Il12b*	*Keap1*	*Ptgs2*	*Tlr4*	
Cd38	*Entpd1*	*Il12rb1*	*Lif*	*Rela*	*Tlr5*	
Cd40	*Era*	*Il12rb2*	*Lifr*	*Relb*	*Tlr6*	
Cd80	*Esrra*	*Il13*	*Ly6c1*	*Retnla*	*Tlr7*	
Cd83	*Esrrb*	*Il15*	*Ly6g*	*Runx1*	*Tlr8*	
Cd86	*Fas*	*Il15ra*	*Maf*	*Sele*	*Tlr9*	
Brunol4	*Fasl*	*Il17ra*	*Marco*	*Sirt1*	*TNF*	

Supplementary Table 2. Custom-made NanoString nCounter code set.

Pro-inflammatory gene cluster				
Ccl4	*Cd83*	*H2-ab*	*Il15*	*Il23a*
Ccl5	*Cd86*	*H2-Ea*	*Il15ra*	*Irf5*
Ccl8	*Cxcl9*	*Marco*	*Il12a*	*Nos2*
Ccl19	*Cxcl10*	*Icam*	*Il12b*	*Ptgs2*
Ccl20	*Cxcl11*	*Il1b*	*Il12ra*	*Socs3*
Cd40	*Cxcl13*	*Il6*	*Il18*	*Stat1*
Cd80	*H2-Aa*	*Il7r*	*Il18r*	*Tnf*

Supplementary Table 3. Pro-inflammatory gene cluster used for Nanostring Analyses.

	Control	GFAP-AhR	p
Onset (day)	12.18 ± 0.1822	10.96 ± 0.4598	0.0054
Peak (score)	3.413 ± 0.07984	3.761 ± 0.1024	0.0099
End (score)	1.75 ± 0.4257	3.25 ± 0.5379	0.027
Mortality	0/40	1/23	n.s.

Supplementary Table 4. EAE in GFAP-AhR deficient and Control mice. n.s. not statistically significant.