

Supplementary Figure 1. FACS sorting and purity of CNS cell populations during EAE.

(A-B) Representative dot-plots depicting FACS strategy for the purification of astrocytes and microglia from EAE mice. Astrocytes were sorted as ACSA-2⁺ cells following exclusion of contaminating hematopoietic cells (CD45⁺), microglia/macrophages and hematopoietic cells (CD11b⁺/CD45⁺), oligodendrocytes (O1⁺), oligodendrocyte progenitors (A2B5⁺) and endothelial cells (CD31⁺) grouped in a dump channel. *Right:* The purity of sorted astrocyte population by FACS analysis of the astrocytic marker GLAST. (B) Microglial cells were sorted as CD11b positive cells with low CD45 expression (CD11b⁺/CD45^{low}). (C) qPCR analysis of astrocyte (*Aldh1l1*, *Aqp4*, *Atp1b2*, *Gfap*, *Slc1a3*) and microglia (*Gpr34*, *Itgam*, *P2ry12*, *Tmem119*) enriched genes in both cell types purified at acute EAE disease ($n = 5-6$ mice; normalized to astrocytic *Gfap*).

Supplementary Figure 2. Cell-population analysis during EAE. (A) Clinical score of the diseased animals used in the study ($n = 19$ mice; 2 independent EAE experiments).

Astrocytes and microglia were sorted from control and immunized mice at the presymptomatic (7-8 dpi), acute (14-17 dpi) and recovery (28-31 dpi). (B) Neurological score of the diseased mice used for FACS analysis at each time-point ($n = 6$ mice). *** $p < 0.001$; one-way ANOVA followed by Newman-Keuls tests. (C) Relative abundance of CD11b⁺CD45^{high} and CD11b⁻CD45^{high} peripheral immune cell populations in CNS single-cell suspensions from control and EAE mice at presymptomatic, acute and recovery phases of the disease ($n = 6-8$ mice per condition). *** $p < 0.001$ referred to CD11b⁺ cells, Student's *t*-tests.