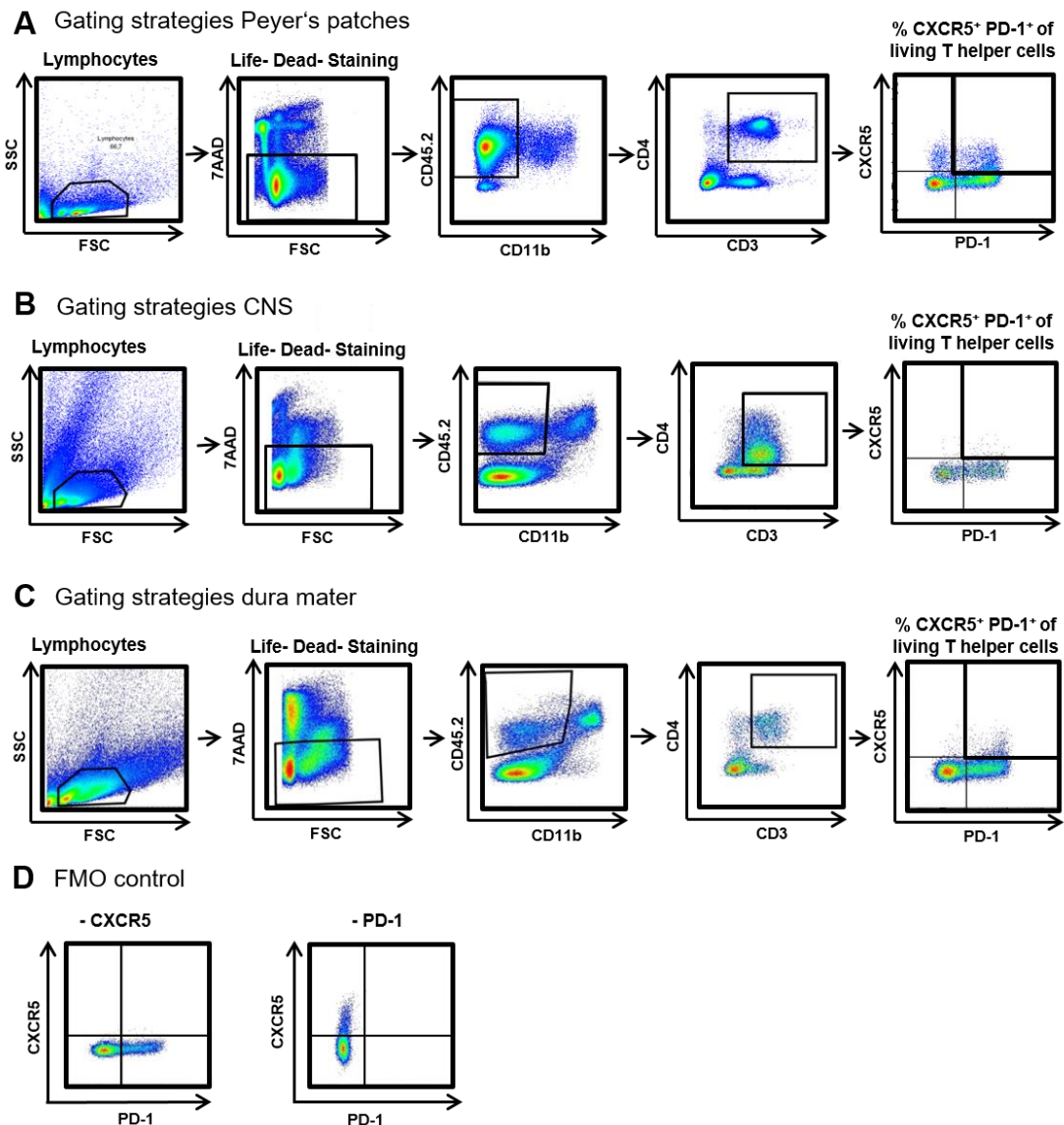


The frequency of follicular T helper cells differs in acute and chronic neuroinflammation

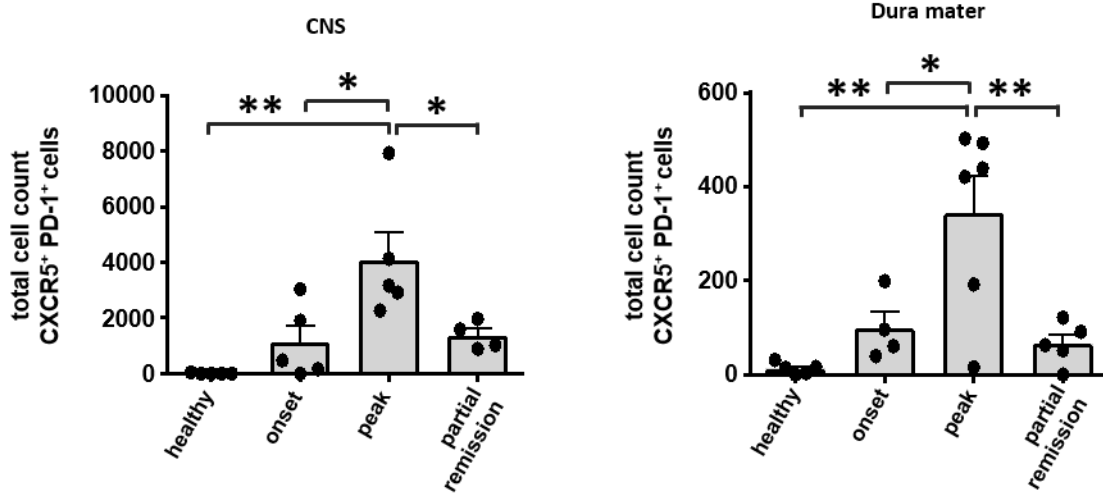
Adalie Baniahmad, Katharina Birkner, Johanna Görg, Julia Loos, Frauke Zipp, Beatrice Wasser, and Stefan Bittner

Supplemental Material

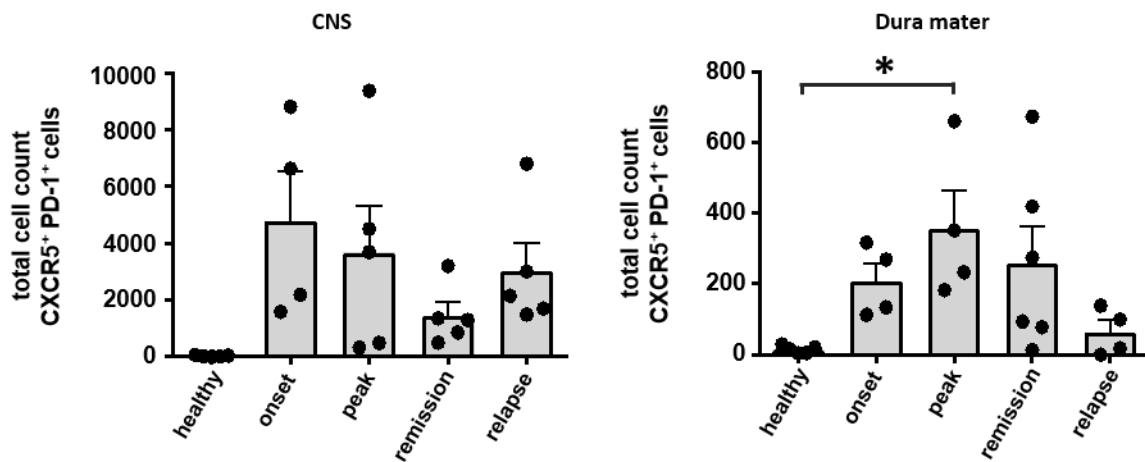


Supplemental Figure 1: Gating strategies of Tfh cells in flow cytometry analysis. Single cell suspensions of Peyer's patches (**A**), CNS (**B**) and dura mater (**C**) of EAE-diseased SJL/J and C57BL/6 mice were prepared and analyzed by FACS. Live-dead-staining of the lymphocytes was performed with 7-aminoactinomycin (7AAD). The inclusion of leucocytes and exclusion of monocytes followed, gating on the CD45⁺ and CD11b⁻ population. T helper cells were then defined as CD4⁺ CD3⁺ leucocytes. Finally, our target population of Tfh cells was characterized as the percentage of CXCR5⁺ PD-1⁺ cells of living T-helper cells. Additionally, Fluorescence Minus One (FMO) control for the relevant marker CXCR5 and PD-1 was performed (**D**).

A C57B6/J total cell count



B SJL/J total cell count



Supplemental Figure 3: Absolute Tfh cell count in health and disease. EAE was induced in C57BL/6 mice via MOG₃₅₋₅₅ peptide immunization (**A**) or in SJL/J mice via PLP₁₃₉₋₁₅₁ peptide immunization (**B**). In addition, non-immunized (healthy) mice were analyzed. Absolute numbers of living Tfh cells (CD45+CD11b-CD4+CXCR5+PD-1+) were compared between the defined disease stages (n=4-5) in the CNS (left panel) and dura mater (right panel). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. * p<0.05; ** p< 0.01.

Supplemental Table 1: Characteristics of Th17 cell adoptive transfer EAE.

Characteristics of adoptive EAE: Th17-skewed cells transferred into Rag2^{-/-} mice	
sex of mice (f/m)	20/3
total mice included	23
day of onset (mean + range)	19.5 [18-22] days
day of peak (mean + range)	26 [24-30] days
time between onset and peak (mean + range)	6.5 [3-12] days
maximum score (mean + range)	3.25 [3-4]