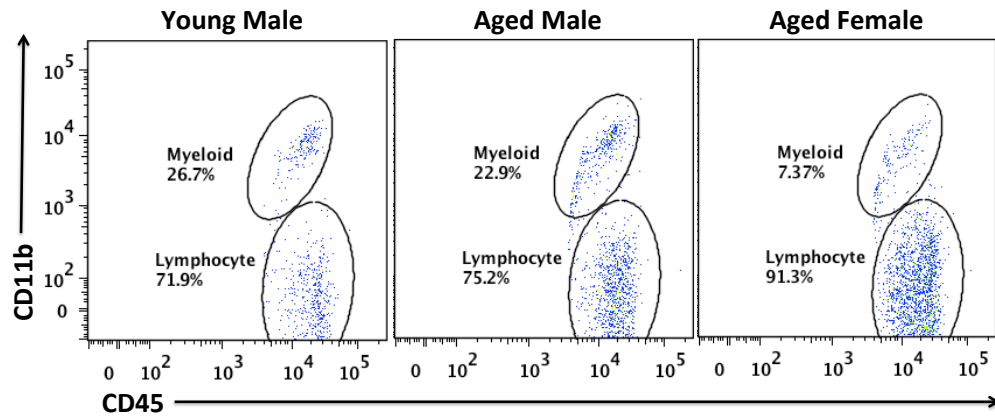


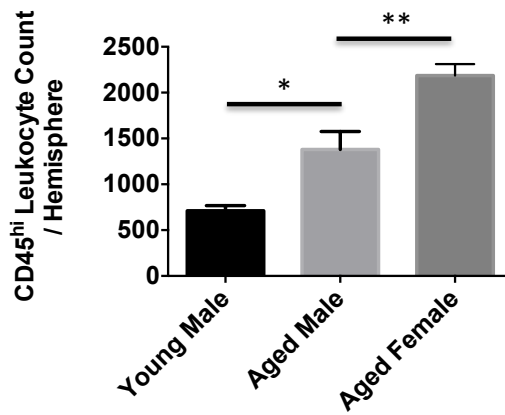
Supplementary Figure 1. Flow cytometry gating strategy and identification of regional and sex differences in CNS CD45^{hi} leukocyte counts of C57BL/6J mice with age.

A representative series of dot plots illustrating the gating strategy used in this study (A). The initial leukocyte gate is based on the typical scatter properties for spenocytes. Subsequently, singlets were selected for to eliminate doublets and cell clumping. CASE dye exclusion is used to identify living cells. CD45^{int}CD11b⁺ microglia represent the resident macrophages, whereas the CD45^{hi} leukocyte population is bone marrow-derived and not native to the CNS. CD45^{hi}CD11b⁺ represent the peripheral myeloid population. CD45^{hi}CD11b-negative cells are putative lymphocyte populations. The lymphocyte population is generally composed of CD19⁺MHCII⁺ B cells and CD3⁺CD4⁺ and CD3⁺CD8⁺ T cells. FMO controls were used to determine positive gating. The absolute number of CD45^{hi} peripheral leukocytes in young and aged brain hemispheres and spinal cords normalized to tissue weight (B; N=10-15/group). Comparison between CD45^{hi} leukocyte subset counts of aged male and female brains (C; N=12-13/group). Error bars show mean SEM. Abbreviation: SSC side scatter intensity, FSC forward side scatter, CASE carboxylic acid succinimidyl ester, n.s. not significant, mg milligram, SEM standard error of mean. *p<0.05; **p<0.01; ***p<0.001

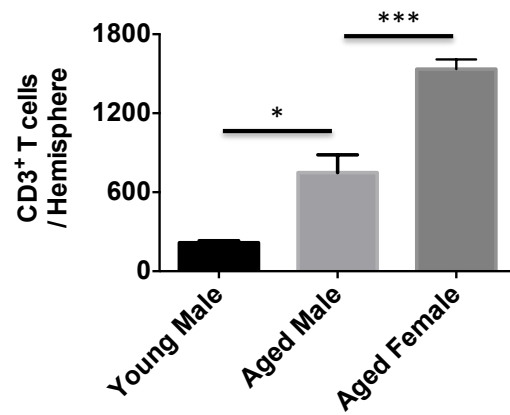
A



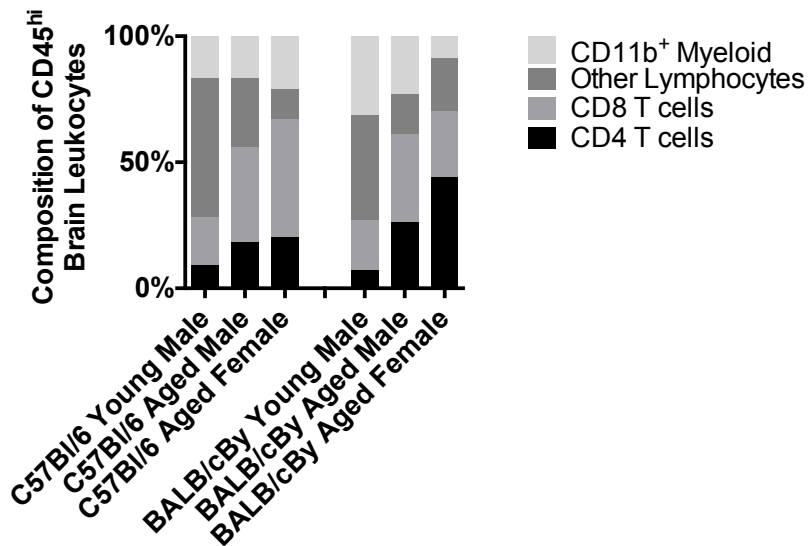
B



C



D



Supplementary Figure 2. CNS recruitment of T cells in the BALB/cBy mouse strain with age.

A representative dot plot showing relative frequencies of CD45^{hi} myeloid (CD11b⁺) and lymphocyte (CD11b⁻) populations in the normal brain of young male, aged male, and aged female BALB/cBy mice (A). The total number of CD45^{hi} peripheral leukocytes (B) and CD3⁺ T cell counts (C) in young and aged brain hemispheres of BALB/cBy mice were quantified (N=5-7/group). Age, sex, and strain-related differences in CD45^{hi} brain leukocyte composition (D). Error bars show mean SEM. Abbreviation: SEM standard error of mean. *p<0.05; **p<0.01; ***p<0.001