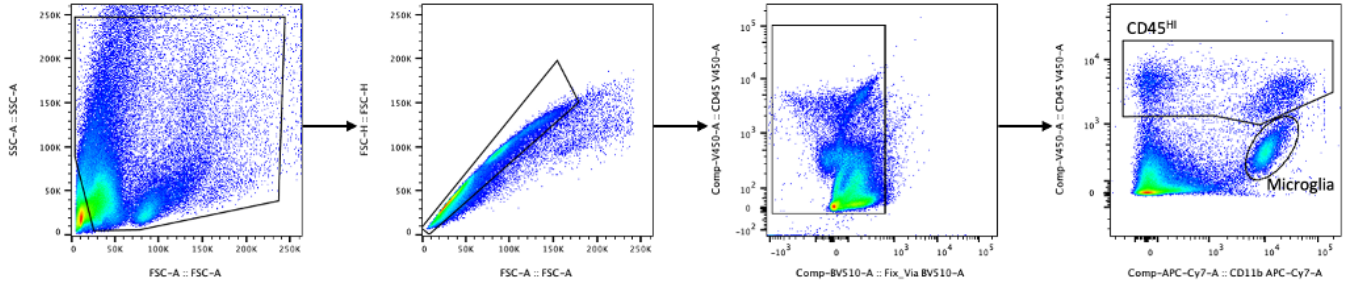


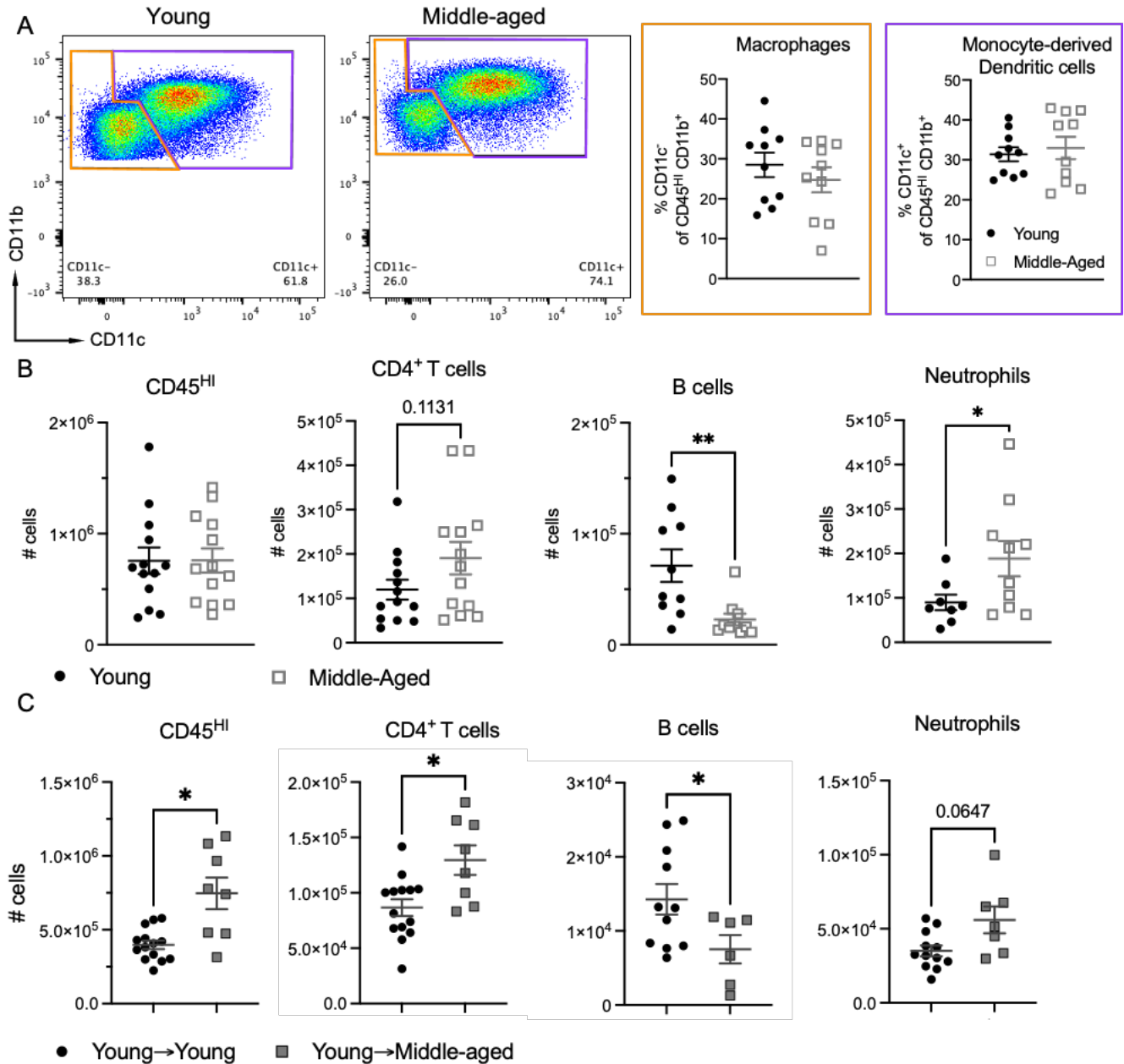
1

2 **Supplemental Figure 1. The age of MOG-primed donor CD4+ T cells does not impact the clinical course of**  
 3 **adoptively transferred EAE.** MOG/CFA primed lymph node cells from young adult (8-12 week old) or middle-  
 4 age (M.A., 40-44 week old) mice were cultured with MOG<sub>35-55</sub> peptide and Th17 polarizing factors. The cells were  
 5 harvested 96 hours later. CD4+ T cells were purified and injected into naïve, syngeneic young adult mice (n=10  
 6 mice/ group). **(A)** Mean clinical scores of mice in each group (left) and areas under the curve (AUC) of individual  
 7 mice (right). **(B)** Peak clinical scores for individual mice. **(C)** Disease incidence in each group over time. **(E)**  
 8 Percent of surviving mice in each group over time. **(E)** Mean change in weight (shown as percent of baseline  
 9 weight) of mice in each group throughout the clinical course (left), and AUCs of individual mice (right). **(F)** Clinical  
 10 courses of young (n=70) and middle-aged (n=43) adoptive transfer recipients that survived throughout the  
 11 duration of the study. Data are pooled from 4 independent experiments. **(G)** Clinical courses of young and  
 12 middle-aged adoptive transfer recipients, separated by sex. n=23-35/ group. Data were pooled from 2  
 13 independent experiments. Curves in **F** and **G** were compared using a mixed effects model. Error bars indicate  
 14 mean  $\pm$  SEM. \*\*\*\*p < 0.0001.



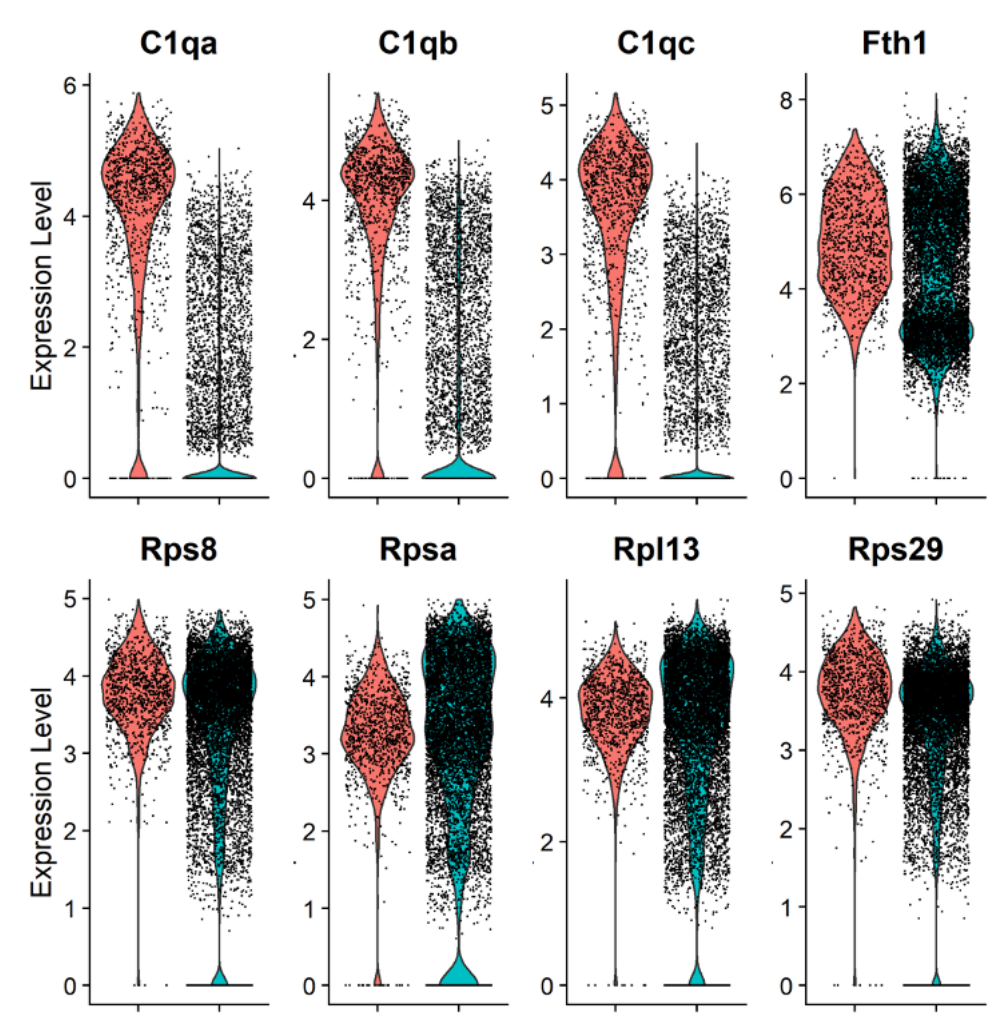
15

16 **Supplemental Figure 2. Representative gating strategy for flow cytometric analysis and FACS isolation.**  
 17 Single cells were gated via forward scatter (FSC) and side scatter (SSC), and dead cells were excluded using a  
 18 fixable viability dye. Microglia were identified as CD45<sup>int</sup>CD11b<sup>+</sup>, infiltrating myeloid cells as CD45<sup>hi</sup>CD11b<sup>+</sup>, and  
 19 infiltrating lymphocytes as CD45<sup>hi</sup>CD11b<sup>-</sup>.



20

21 **Supplemental Figure 3. The frequencies of monocyte-derived dendritic cells and macrophages and total**  
 22 **number of CNS infiltrates of young and middle-aged adoptive transfer recipients, and chimeric mice, at**  
 23 **peak EAE.** CNS inflammatory cells were harvested from the spinal cords of young and middle-aged mice at day  
 24 10 adoptive post-transfer. **(A)** Representative dot plots of CNS infiltrating myeloid cells (left panels). Frequencies  
 25 of macrophages (CD11b<sup>+</sup> Ly6G<sup>-</sup> CD11c<sup>-</sup>) and monocyte-derived dendritic cells (CD11b<sup>+</sup> Ly6G<sup>-</sup> CD11c<sup>+</sup>) among  
 26 CD45<sup>HI</sup> mononuclear cells isolated from the spinal cords of individual adoptive transfer recipients (right panels).  
 27 **(B, C)** Total numbers of CD45<sup>HI</sup> infiltrating immune cells, CD4<sup>+</sup> T cells, donor (CD45.1<sup>+</sup>) CD4<sup>+</sup> T cells, B cells, and  
 28 neutrophils per spinal cord harvested from non-chimeric mice **(B)**, and young→young and young→middle-aged  
 29 bone marrow chimeras **(C)**, on day 10 postTh17 cell transfer. Each symbol represents a data point generated  
 30 from a single mouse. Statistical significance was determined using the unpaired 2-tailed Student's *t* test. \**p* < 0.05,  
 31 \*\**p* < 0.01.



32

33 **Supplemental Figure 4. Microglia from middle-aged mice with EAE express high levels of genes identified**  
 34 **in microglia located in the rims of chronic active pMS lesions.** CD45<sup>+</sup> mononuclear cells were FACS sorted  
 35 from middle-aged mice with EAE on day 10 post-cell transfer and subjected to scRNAseq. Selected differentially  
 36 expressed genes in microglia (red) compared with all other cells (blue), shown as violin pots. Numbers on the y-  
 37 axis correspond to the z score for each gene.