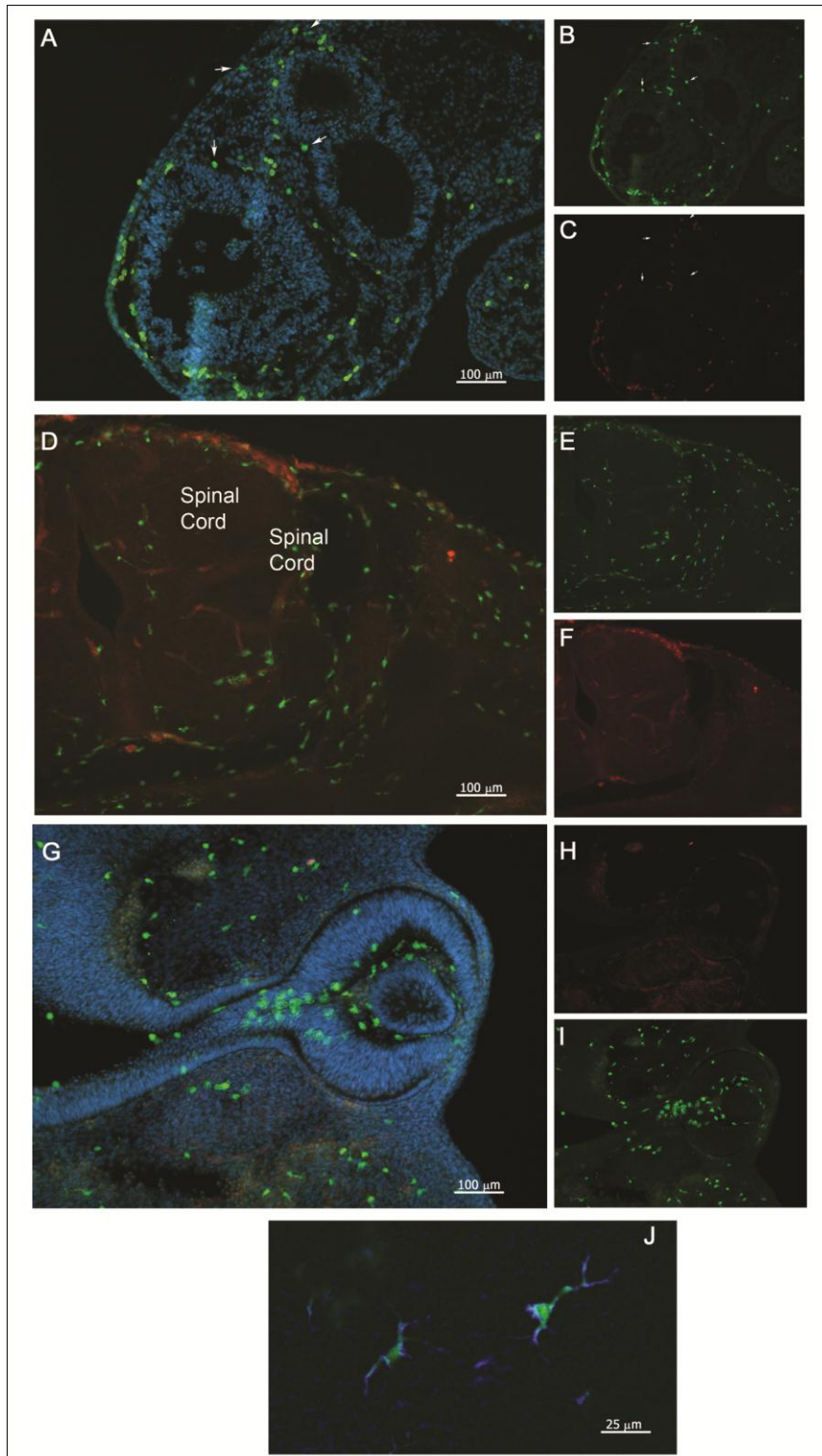
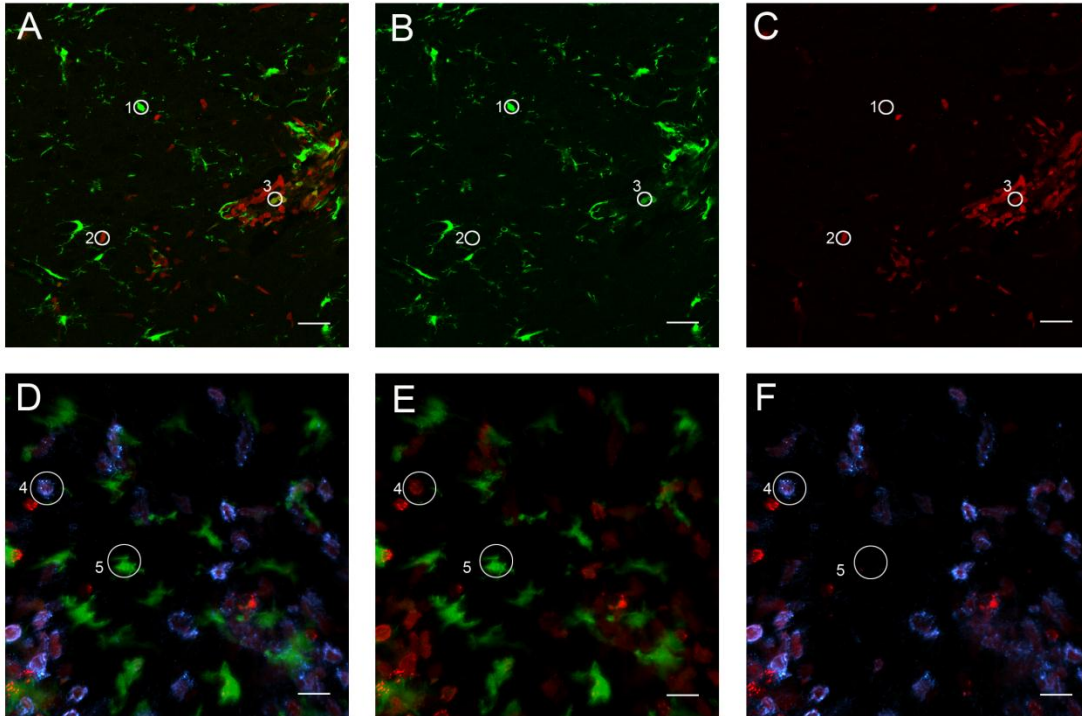


Supplementary Figure 1. *Cx3cr1*^{+GFP} positive myeloid precursors are visualized within yolk sac at E9.5 stage of development. Yolk sacs were dissected and evaluated for the presence of CX3CR1 and CCR2 expressing cells (A, merged image). Single positive CX3CR1 cells (B, green), are prominent and CCR2 expression was detected in double positive precursors (C, red). Crossection of a representative embryo at stage E9.5 (C) shows the presence of double positive cells (D, merged image). Single positive CX3CR1 cells (E, green), are detected on the neural tube (arrow) and most cells appear as double positive (D, and F, red).

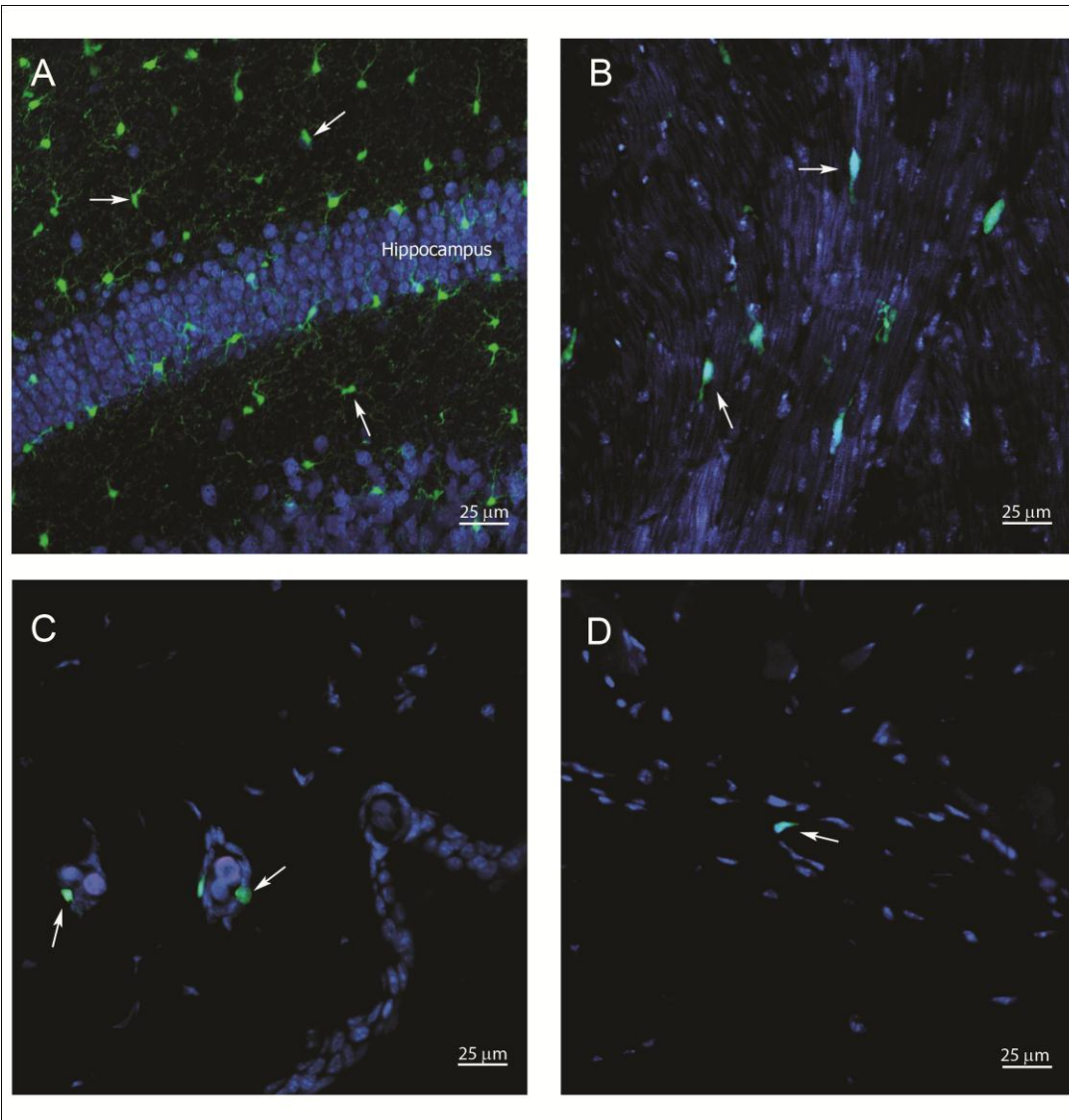


Supplementary Figure 2. *Cx3cr1*^{+/*GFP*} positive myeloid precursors rapidly colonize CNS tissues. Sagittal sections at E10.5 revealed a rapid expansion of CX3CR1+ cells (A, merged

image; B, CX3CR1-green; C, CCR2-red), colonizing the developing cortex (A-C), spinal cord (D-F) and optic vesicles (G-I). These precursors exhibit features of microglia, as evident by their branched morphology, IB4-binding and immunoreactivity to IBA-1 (J, merged image, CX3CR1-GFP/CCR2-Red and IBA staining in blue). In sections A, and G, nuclei visualized with DAPI, CX3CR1-GFP channel shown in B, E and H and CCR2-Red in C, F, and I.



Supplementary Figure 3. Confocal analyses of monocyte subsets in brain lesions of EAE $CX3CR1^{+/GFP} CCR2^{+/RFP}$ mice at peak disease. Merged CX3CR1-GFP (green) and CCR2-RFP (red) image (A) shows activated microglial cells (representative cell “1”), and classical infiltrating monocytes (cell “2”) as $CX3CR1^{neg} / CCR2^{hi}$, and cell “3” $CX3CR1^{lo} / CCR2^{hi}$ are visualized in (B) CX3CR1-GFP channel and (C) CCR2-RFP channel. (D-F) Sections were obtained after staining with the 7-4 antibody. (D) Merged images of red/CCR2, green/CX3CR1 and blue/7-4. (E) Red/CCR2 and green/CX3CR1 and (F) blue/7-4 and red/CCR2. The majority of $CCR2^{+}$ cells are $7-4^{+}$ and CX3CR1 negative, further supporting that they are classical infiltrating monocytes (representative cell “4”) and also abundant in the lesion are activated microglia (cell “5”). Scale bar (C-H), 25 μ m.



Supplementary Figure 4. CCR2-RFP is not detected in resident CNS, skin or cardiac monocytes. Confocal images from (A) Brain, (B) heart and (C, D) skin tissues show lack of CCR2-RFP expression in resident long-lived monocyte populations. All microglial cells appear CX3CR1^{GFP} positive (A, arrows) and neurons stained with anti-Neu antibodies are visualized in blue. CX3CR1^{GFP} positive myeloid cells were also visualized in heart (B, arrows) and skin (C, D). Nuclei visualized with DAPI is shown in blue in (B-D).