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Supplementary Information for

CD11c^{+}CD88^{+}CD317^{+} myeloid cells are critical mediators of persistent CNS autoimmunity

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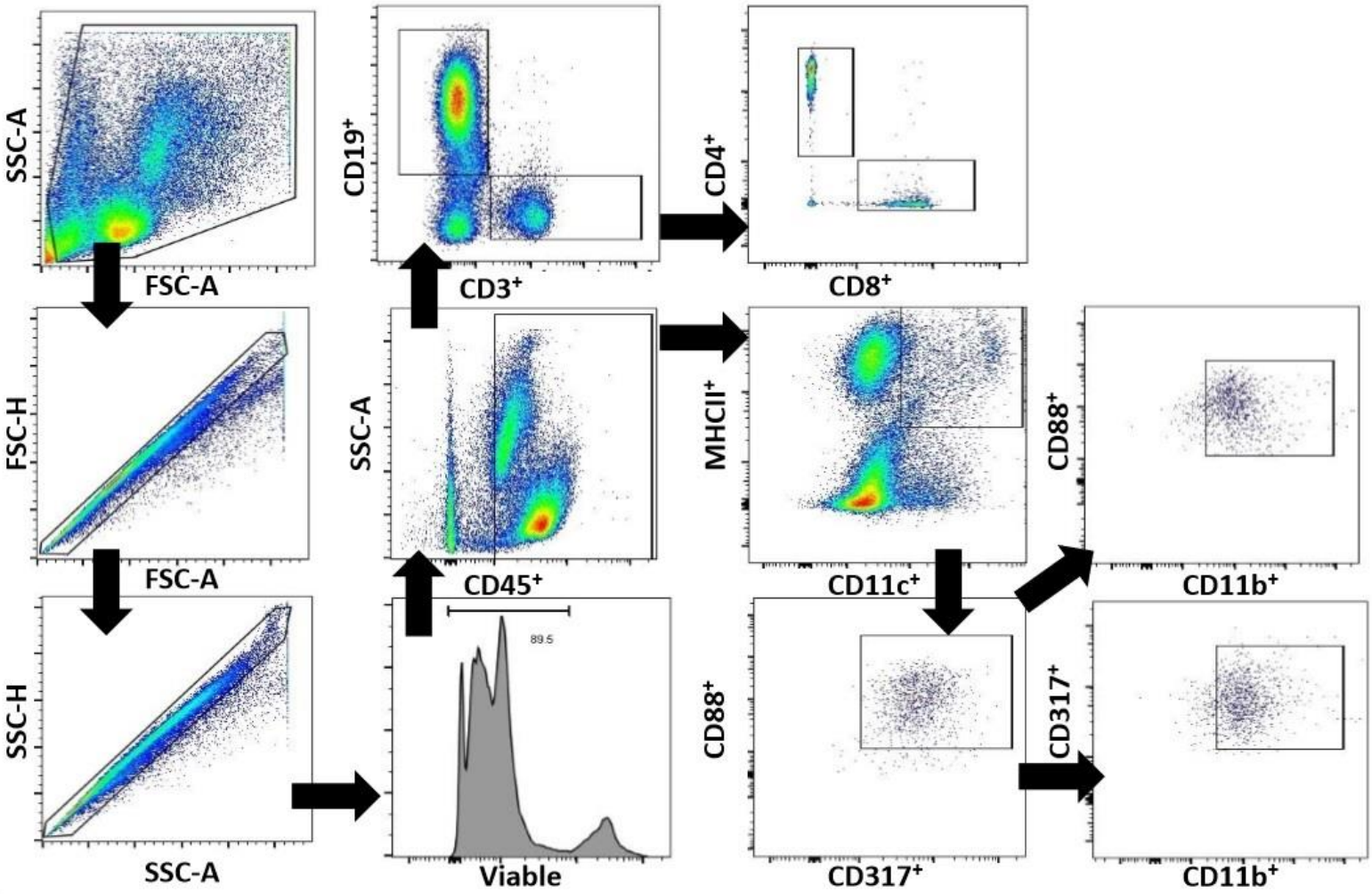
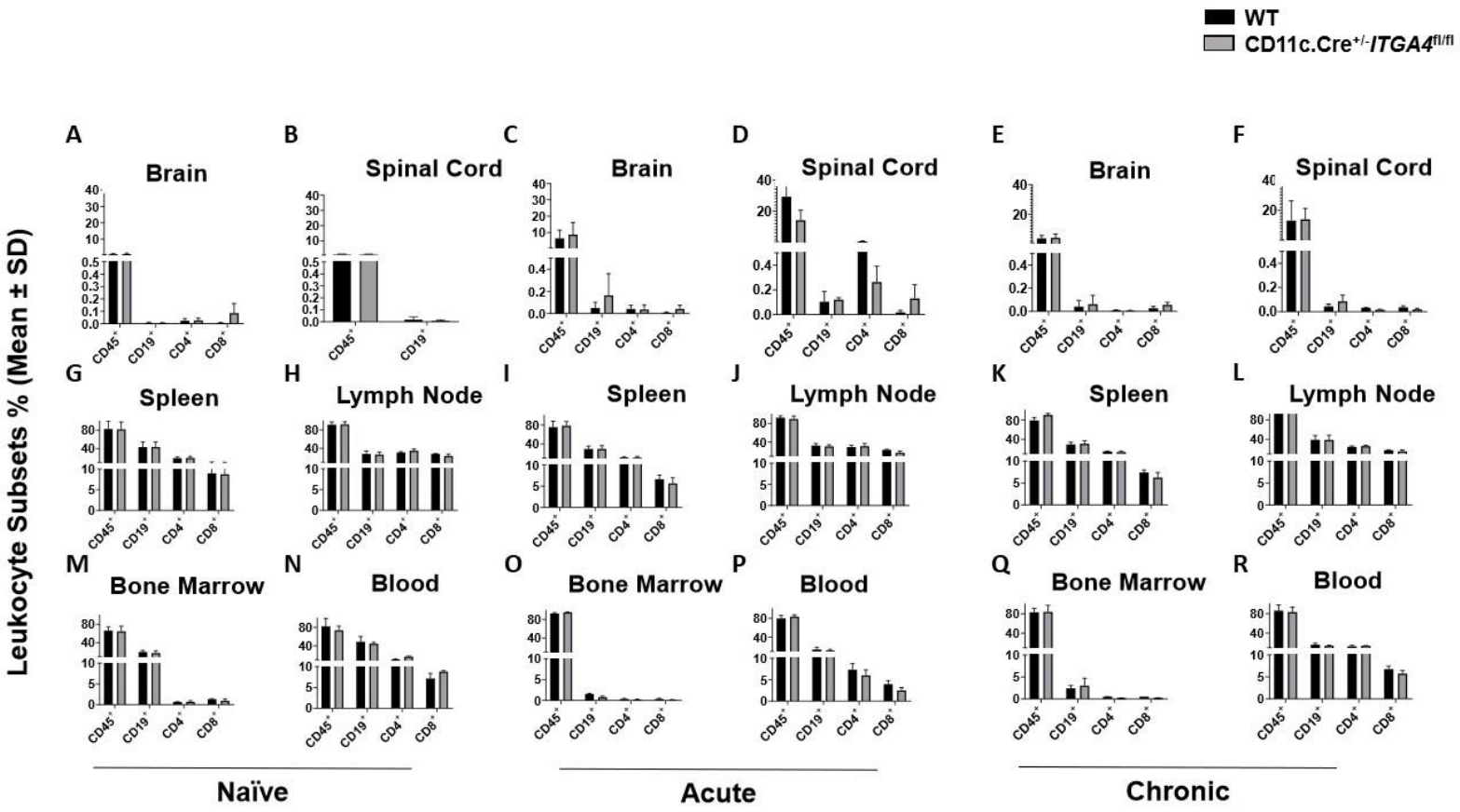
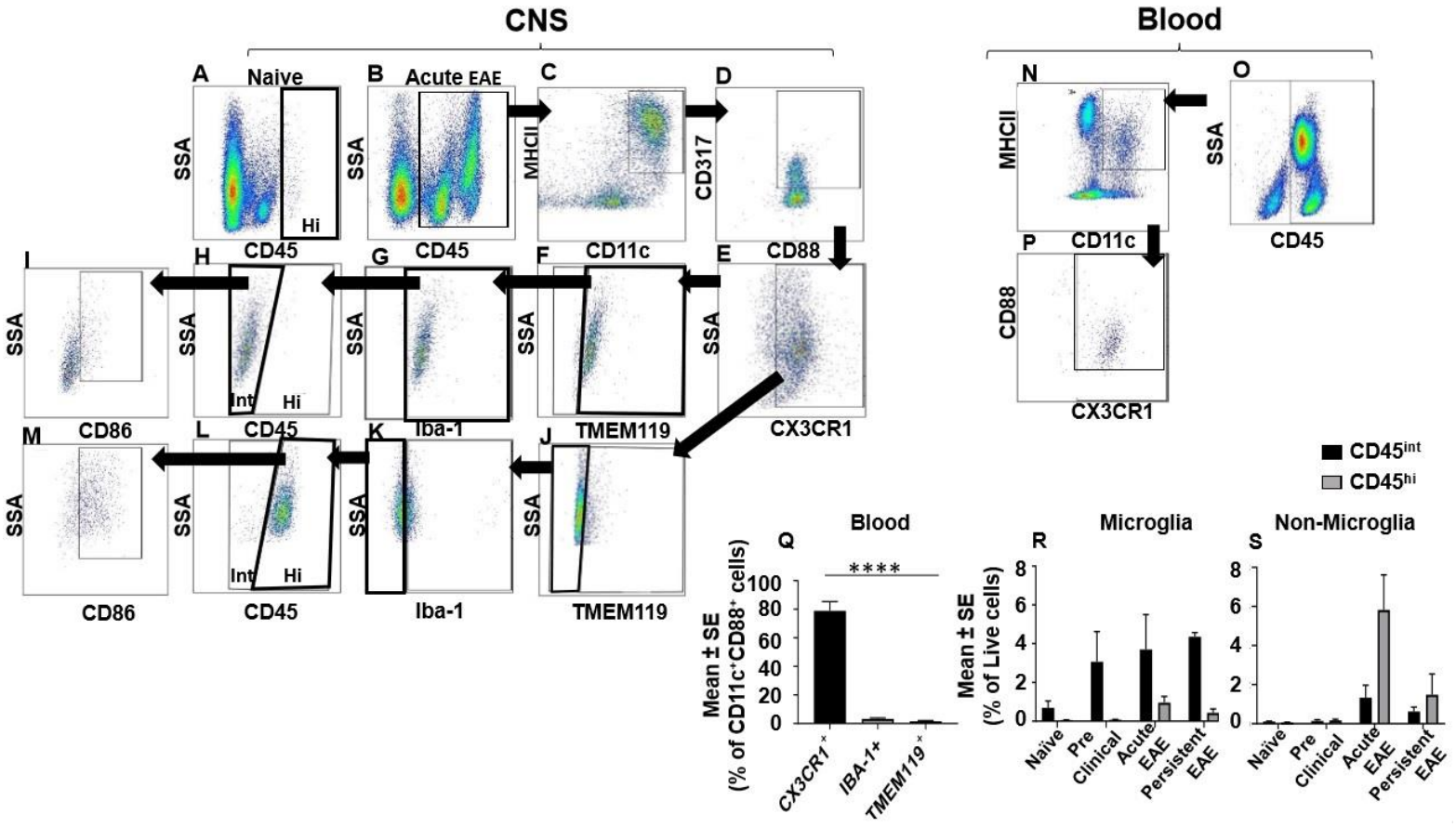


Supplementary Figure 1. Flow cytometry study gating strategy. Cells were gated according to morphology side scatter (SSC-A) vs forward scatter (FSC-A). Doublets were excluded (FSC-A vs FSC-H and SSC-A vs SSC-H). Live cells were selected using the viability dye. Flow cytometry density plots showing gating strategy used to identify $CD11c^{+}CD88^{+}CD317^{+}$ cells ($CD45^{+}MHCII^{+}D11c^{+}CD88^{+}CD317^{+}$), B cells ($CD45^{+}CD3^{-}CD19^{+}$), $CD4^{+}$ T cells ($CD45^{+}CD3^{+}CD4^{+}$) and $CD8^{+}$ T cells ($CD45^{+}CD3^{+}CD8^{+}$). Each sample contains a minimum of 50 x 10^{3} live events.



Supplementary Figure 2. The composition of leukocytes outside the CD11c^{+} lineage is not altered in CD11c.Cre$^{+/-}$*ITGA4*$^{fl/fl}$ mice. The mean ± standard deviation (SD) of leukocyte subsets (%) from total 50 x 10^3 recorded viable cells in different compartments, including (**A-F**) brain and spinal cord, (**G-L**) spleen and lymph node and (**M-R**) bone marrow and blood, is presented during naïve, acute and persistent clinical actively-induced experimental autoimmune encephalomyelitis (EAE) in CD11c.Cre$^{+/-}$*ITGA4*$^{fl/fl}$ mice or C57BL/6 wild type (WT) controls (N =6 experimental animals per group; data show pooled analysis of all study cohorts). There was no difference in frequency of cell types including CD45^{+}, CD19^{+}, CD4^{+} and CD8^{+} cells between the two groups during different stages of the disease (P value > 0.05).



Supplementary Figure 3: Characterization of CD11c^{+}CD88^{+}CD317^{+} cells and microglia using *ex vivo* flow cytometry. (**A**) CD45^{hi} cells were not abundantly present in naïve CNS tissue. (**B**) From CD45^{+} cells, (**C**) CD11c^{+}MHCII^{+} and (**D**) CD88^{+}CD317^{+} cells were selected. Next, (**E**) CX3CR1^{+} cells were gated for, and selected for (**F**) expression of TMEM119^{+} or (**J**) the absent expression of TMEM119^{-}. (**G**) Iba-1^{+} cells were selected from TMEM119^{+}cells. (**H, R**) The majority of CX3CR1^{+}TMEM119^{+}Iba-1^{+} cells defined as parenchymal microglia localized in the CD45^{int} gate. (**K**) Iba-1^{-} cells were selected from TMEM119^{-}cells. (**L, S**) The majority of CX3CR1^{+}TMEM119^{-}Iba-1^{-} cells defined as non-microglia localized in the CD45^{hi} gate. (**I**) FewerCD45^{int} microglia expressed CD86 compared to (**M**) CD45^{hi} non-microglia. (**O, N**) CD45^{+}CD11c^{+}CD88^{+} cells in the blood were mostly (**P**) CX3CR1^{+}, but (**Q**) TMEM-119^{-} and Iba-1^{-}.