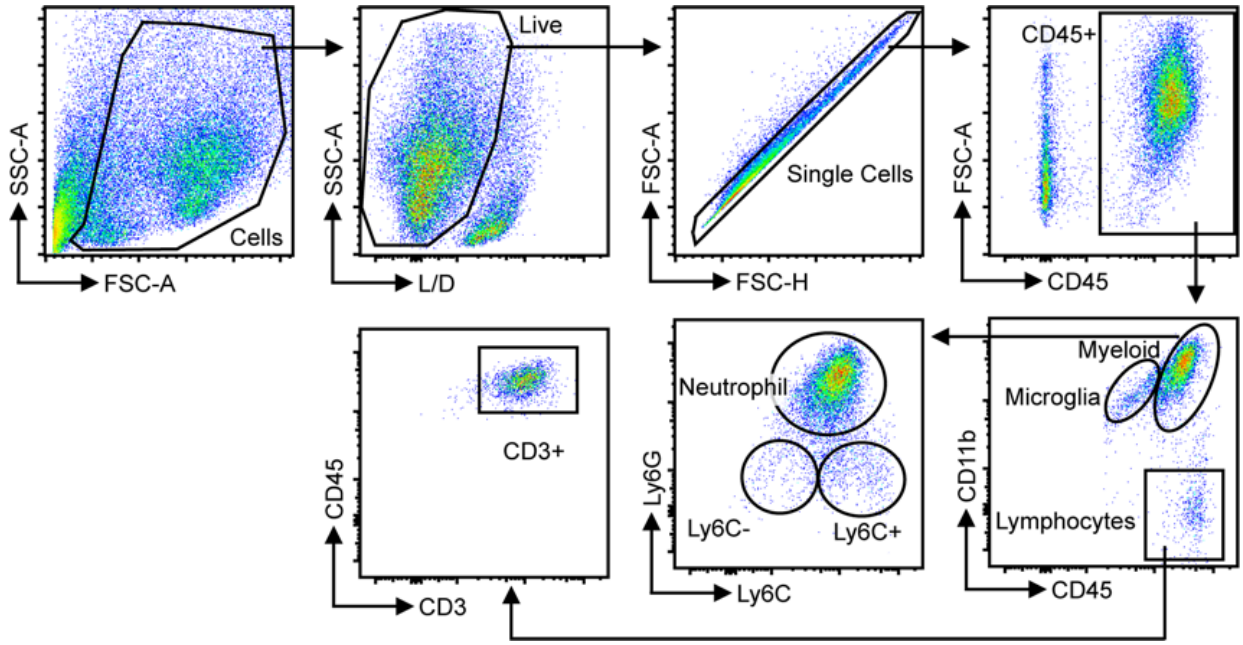
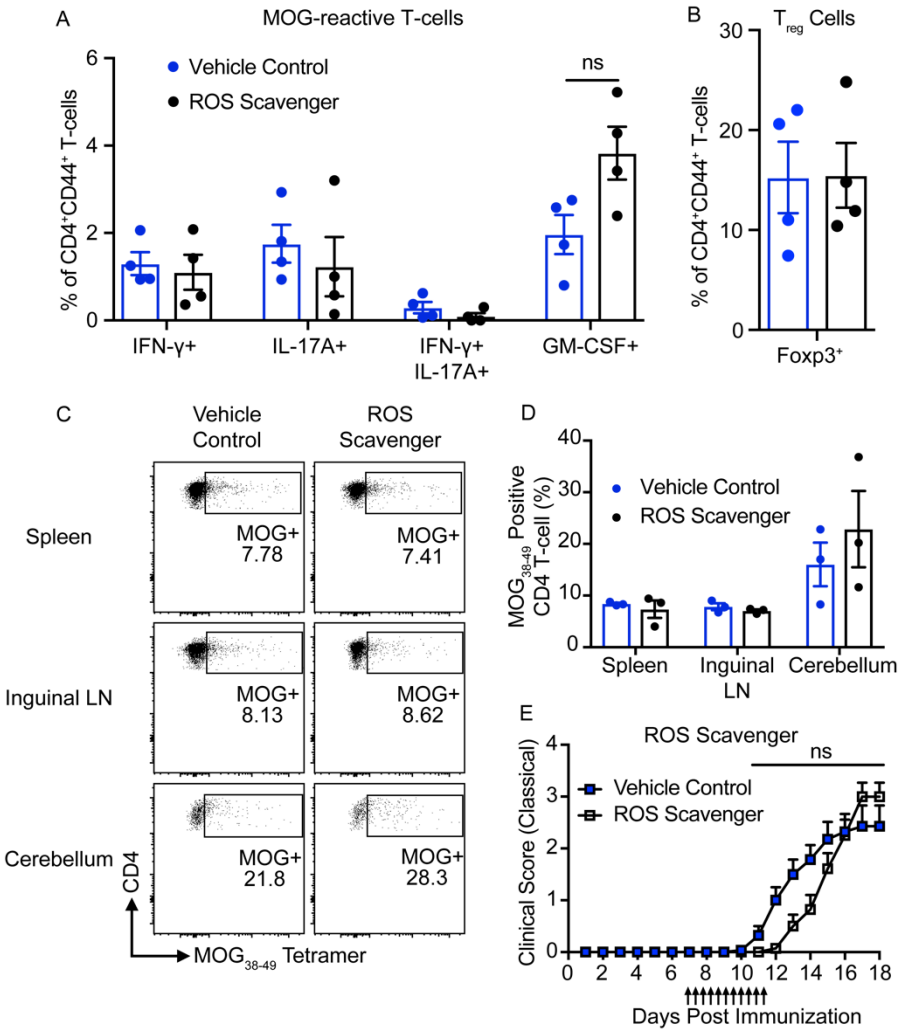
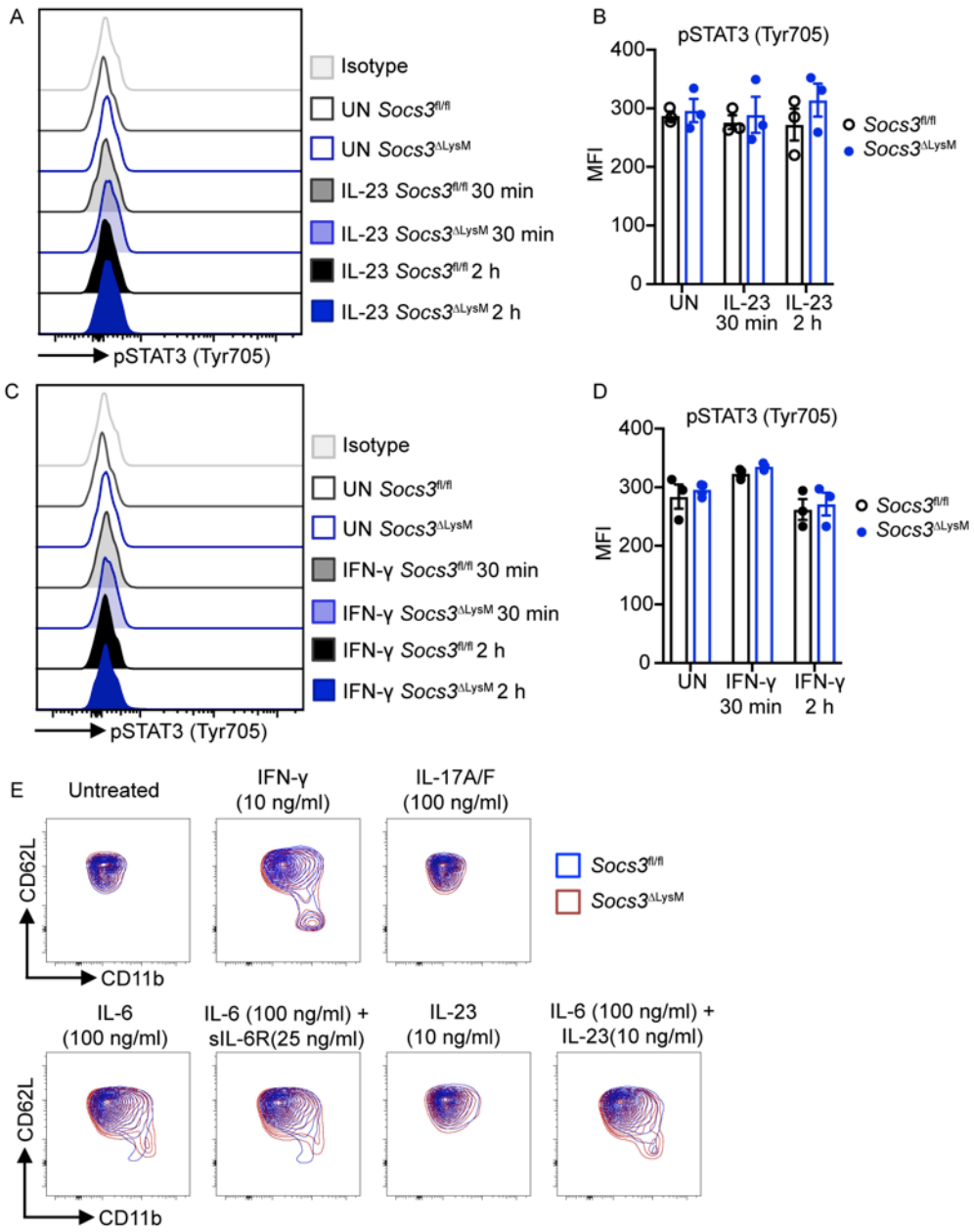


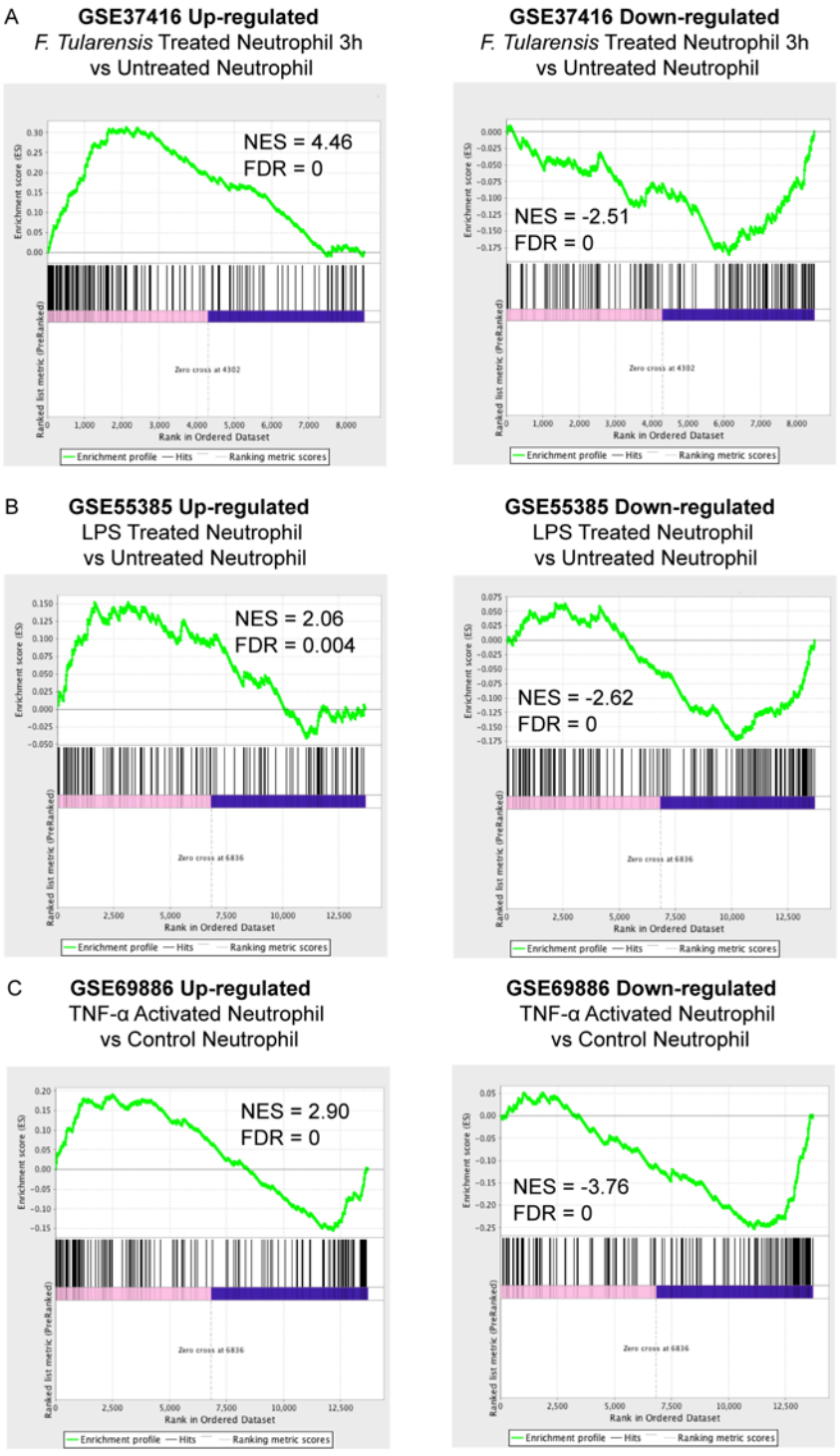

**Supplemental Figure 1. Gating Strategy for CNS Infiltrating Cells.** EAE was induced and at the indicated times, mice were perfused and CNS tissues isolated. Infiltrating immune cells were then isolated by 30%/70% Percoll gradient centrifugation. Subsets of immune cells were gated as follows: Microglia ($CD45^{lo}CD11b^{+}$); Neutrophils ($CD45^{+}CD11b^{+}Ly6C^{lo}Ly6G^{+}$); $Ly6C^{+}$ Monocytic Cells ($CD45^{+}CD11b^{+}Ly6C^{+}Ly6G^{-}$); $Ly6C^{-}$ Monocytic Cells ($CD45^{+}CD11b^{+}Ly6C^{-}$ Ly6G); and CD3+ T-cells ($CD45^{+}CD11b^{-}CD3^{+}$).



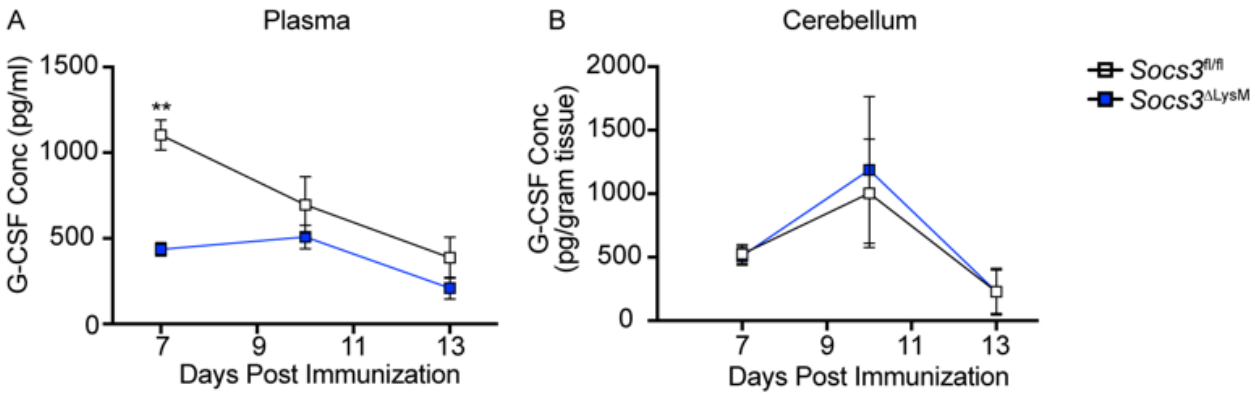

**Supplemental Figure 2. ROS Scavenger Treatment Does Not Affect T-cell Priming or Classical EAE.** EAE was induced in *Socs3*$^{\Delta LysM}$ mice. Beginning at day 7, ROS scavenger cocktail (FeTPPS 20 mg/kg, PBN 50 mg/kg and EUK134 15 mg/kg) was administered i.p. twice per day for 5 days. **(A, B)** At day 13, mice were sacrificed, and immune cells isolated from inguinal lymph nodes. Cells were then stimulated with $MOG_{35-55}$ (20 µg/ml) for 24 h and T-cell subsets were determined by intracellular staining followed by flow cytometry analysis. Cells were gated on $CD45^+CD3^+CD4^+CD44^{hi}$ (n= 4). (**C, D**) At day 13, immune cells were isolated from the indicated tissues and stained with $MOG_{38-49}$ tetramer antibody (n = 3). (**C**) Representative dot plot and (**D**) statistical analysis of tetramer positive CD4+ T-cells (n = 3). Cells were gated on $CD45^+CD3^+CD4^+$. **(E)** Classical EAE score of mice treated with ROS scavenger cocktail (n = 14) or vehicle control (n = 14). Mice that did not develop EAE (classical or atypical) were excluded. ns = not significant by two-tailed Student's t test **(A)** or Mann-Whitney rank sum test **(E)**.



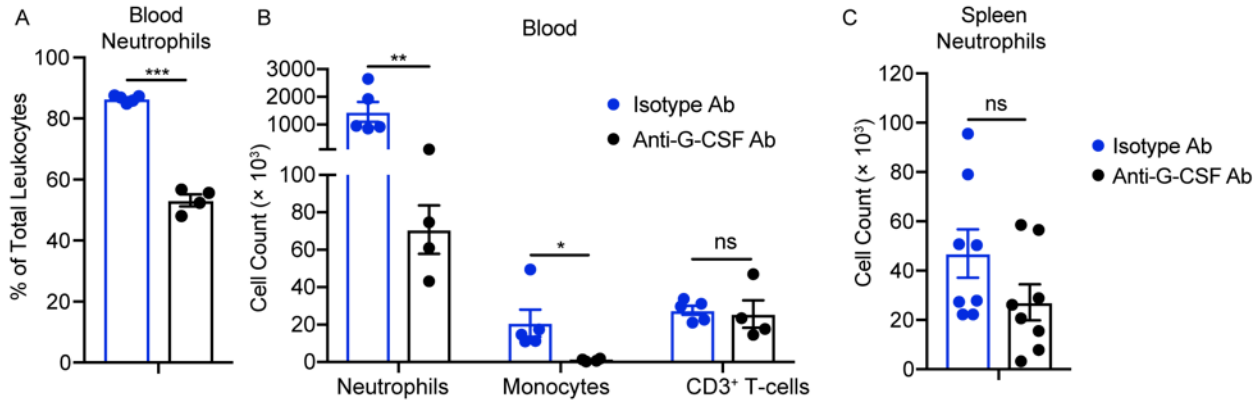

**Supplemental Figure 3. Response of *Socs3*-deficient Neutrophils to Other Cytokines.** Bone marrow neutrophils were isolated from *Socs3*$^{fl/fl}$ or *Socs3*$^{\Delta LysM}$ mice. **(A, B)** Neutrophils were stimulated with IL-23 (10 ng/ml) for the indicated times, followed by intracellular staining for pSTAT3 (Y705) (n = 3). **(C, D)** Neutrophils were stimulated with IFN-γ (10 ng/ml) for the indicated times, followed by intracellular staining for pSTAT3 (Y705) (n = 3). **(E)** Neutrophils were stimulated with the indicated cytokines for 8 h, and expression of surface markers was analyzed. Plots are representative of three independent experiments. All flow cytometry results were gated on live, single CD45$^{+}$CD11b$^{+}$Ly6G$^{+}$ neutrophils.



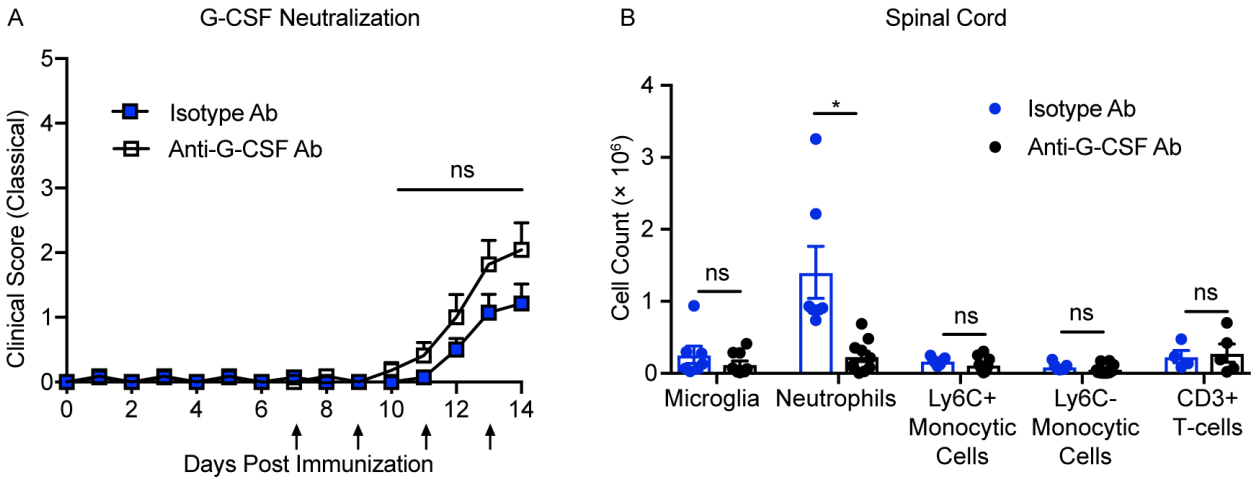

**Supplemental Figure 4. Gene Set Enrichment Analysis Examining Gene Sets Derived from Previously Reported Activated Neutrophil Gene Sets.** Differentially expressed genes (*Socs3*$^{\Delta LysM}$ G-CSF vs *Socs3*$^{fl/fl}$ G-CSF) were ranked based on the adjusted *p* value and Log2 fold change. GSEA was performed using the following previously reported data sets. Top 200 up-regulated (left) and down-regulated (right) genes from the data sets were used, respectively. **(A)** GSE37416. **(B)** GSE55385. **(C)** GSE69886. NES, normalized enrichment score; FDR, false discovery rate.




**Supplemental Figure 5. G-CSF Levels in *Socs3*$^{fl/fl}$ and *Socs3*$^{\Delta LysM}$ Mice with EAE.** EAE was induced in *Socs3*$^{fl/fl}$ and *Socs3*$^{\Delta LysM}$ mice. **(A)** On days 7, 10, and 13, plasma samples were collected (n = 5-9) and **(B)** Cerebellar tissues were homogenized and supernatants collected. G-CSF levels were determined by ELISA (n = 3-5). ** $p < 0.01$ by two-tailed Student's t test.




**Supplemental Figure 6. G-CSF Neutralization Reduces Circulating Neutrophils. (A-C)** EAE was induced in *Socs3*$^{\Delta LysM}$ mice, and from day 7, anti-G-CSF Ab (20 µg/mouse) or isotype control Ab (20 µg/mouse) were administered i.p. every other day. Mice were sacrificed on day 14 for analysis. **(A)** Percentage of neutrophils in the blood of isotype and anti-G-CSF Ab-treated mice (n = 4-5). **(B)** Frequencies of neutrophils, monocytes and CD3$^+$ T-cells in the blood of isotype Ab and anti-G-CSF Ab-treated mice (n = 4-5). **(C)** Frequencies of neutrophils from the spleen of isotype Ab and anti-G-CSF Ab-treated mice (n = 8). *$p$ < 0.05, **$p$ < 0.01, ***$p$ < 0.001, ns = not significant by two-tailed Student's t test.




**Supplemental Figure 7. G-CSF Neutralization Does Not Affect Classical EAE.** EAE was induced in *Socs3*$^{\Delta LysM}$ mice, and from day 7, anti-G-CSF Ab (20 µg/mouse) or isotype control Ab (20 µg/mouse) were administered i.p. every other day. **(A)** Classical EAE scores of mice treated with anti-G-CSF Ab (n = 11) or isotype control Ab (n = 14). **(B)** At days 13-14, mice were sacrificed and immune cells from the spinal cord were isolated by Percoll gradient, and the frequencies of Microglia ($CD45^{lo}CD11b^{+}$); Neutrophils ($CD45^{+}CD11b^{+}Ly6C^{lo}Ly6G^{+}$); $Ly6C^{+}$ Monocytic Cells ($CD45^{+}CD11b^{+}Ly6C^{+}Ly6G^{-}$); $Ly6C^{-}$ Monocytic Cells ($CD45^{+}CD11b^{+}Ly6C^{-}Ly6G^{-}$); and CD3+ T-cells ($CD45^{+}CD11b^{-}CD3^{+}$) were determined (n = 4-7). *$p$ < 0.05, ns = not significant by Mann-Whitney rank sum test **(A)** or two-tailed Student's t test **(B)**.