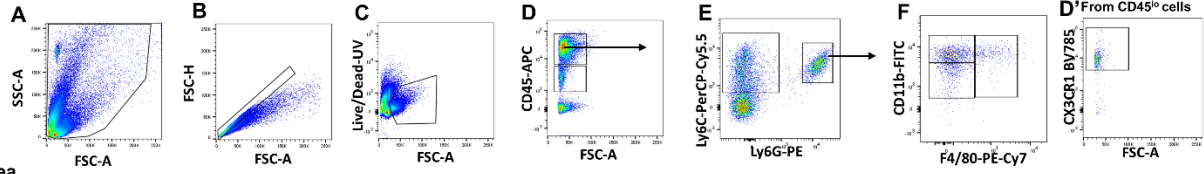
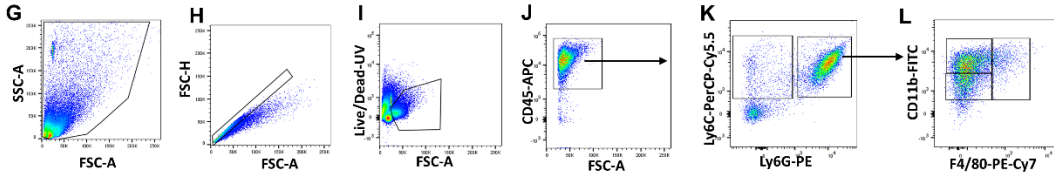


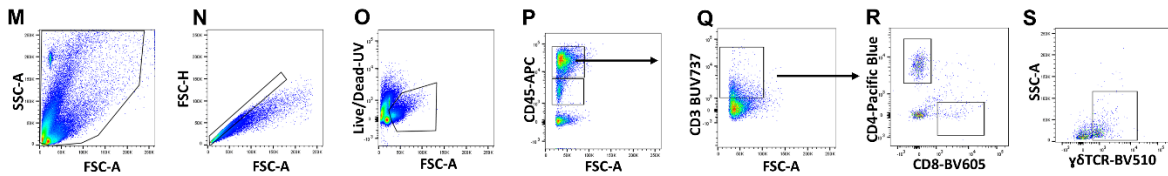
Brain Innate



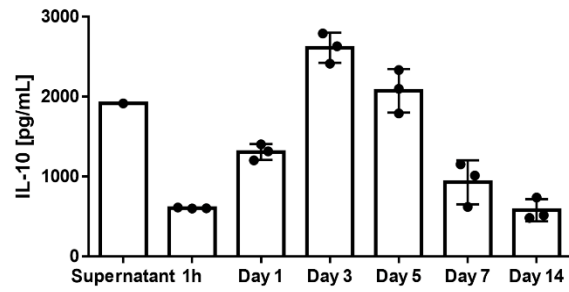
Galea



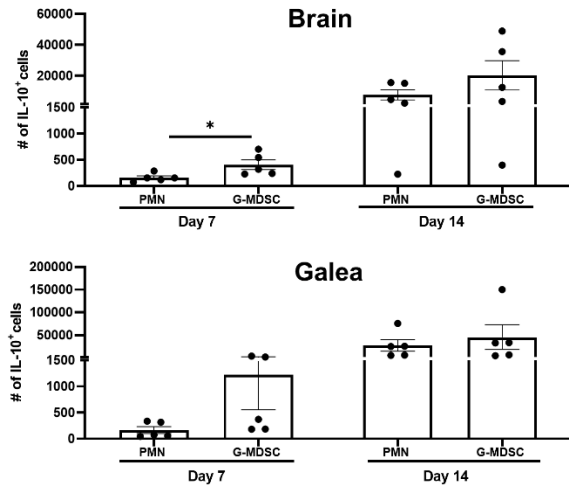
Brain Adaptive



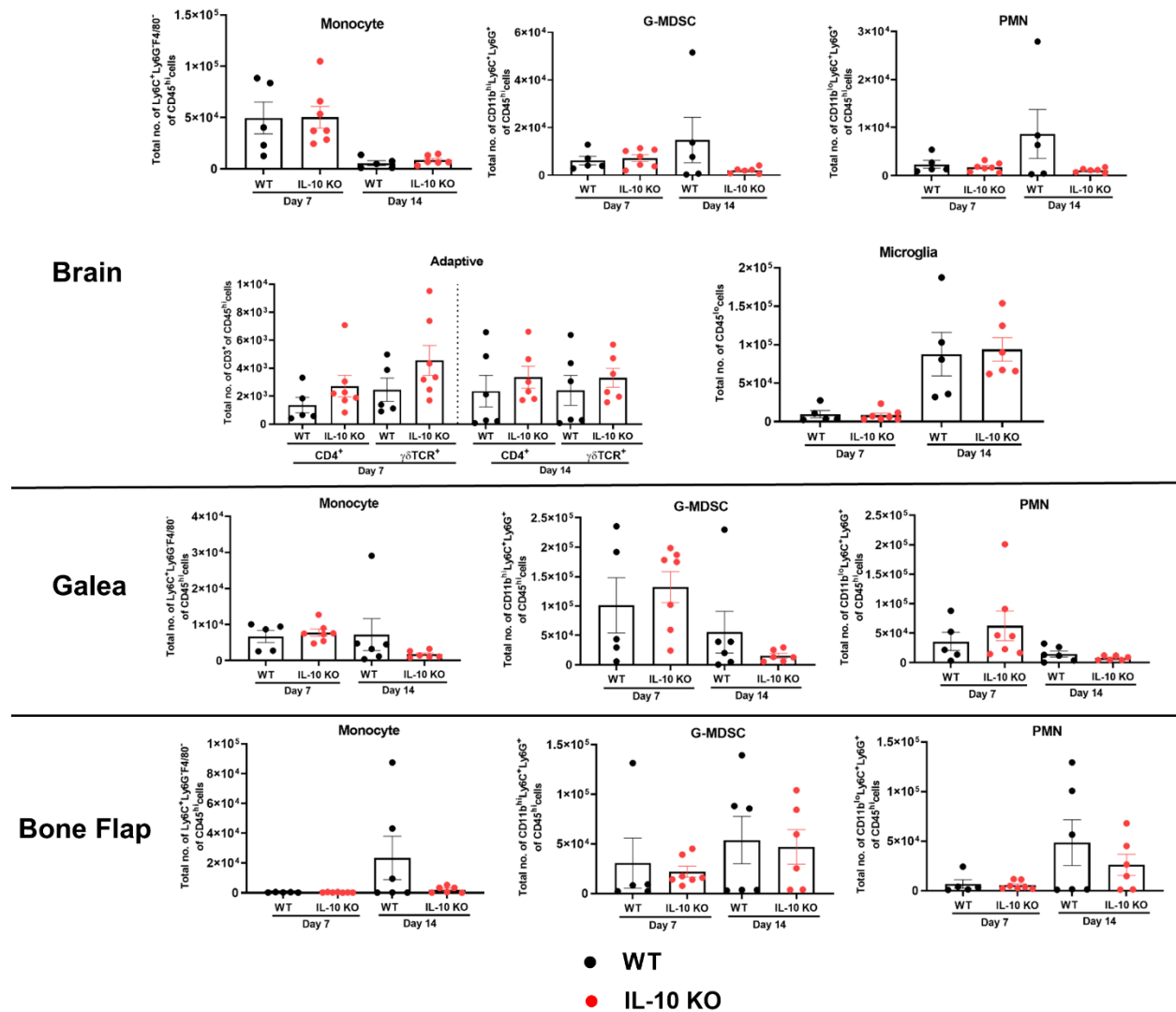
Additional file 1: Fig. S1. Gating strategy to quantify immune populations in the brain and galea following *S. aureus* craniotomy infection. From the (A, G, M) total events, (B, H, N) single cells were gated using FSC-A vs. FSC-H, followed by (C, I, O) exclusion of dead cells. For the innate immune panel, (D, J) live, CD45^{high} leukocytes were separated into (E, K) Ly6G⁻ Ly6C⁺ monocytes vs. Ly6G⁺ Ly6C⁺ cells, which were further identified as (F, L) G-MDSCs (CD11b^{high} Ly6G⁺ Ly6C⁺ F4/80⁻) and neutrophils (CD11b^{low} Ly6G⁺ Ly6C⁺ F4/80⁻) based on CD11b and F4/80 expression. Microglia were defined as (D') CD45^{low} vs. FSC and CX3CR1⁺. Adaptive immune cells were subjected to the same initial steps (M-O) as described for the innate immune panel, where (P) live, CD45^{high} leukocytes were separated into (Q) CD3⁺ cells that were gated on (R) CD4⁺ and CD8⁺ T cells and (S) γδ T cells (γδ TcR⁺).



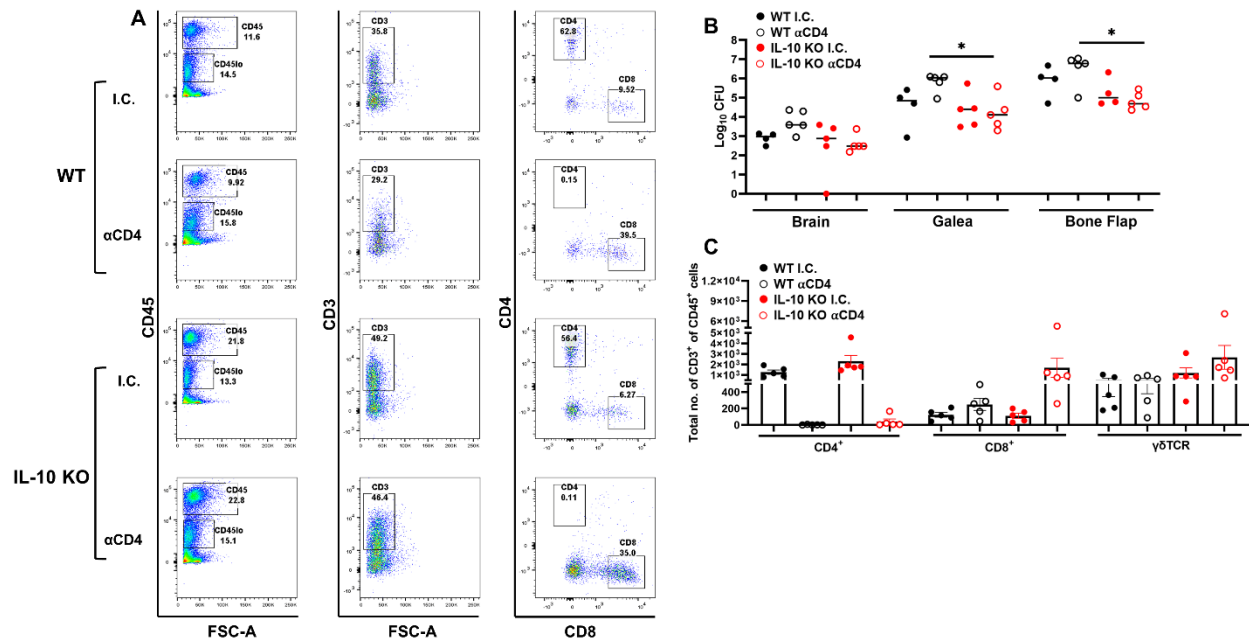
Additional file 1: Fig. S2. Characterization of IL-10 containing microparticles. Release kinetics of IL-10 loaded poly(lactide-co-glycolide) (PLGA) microparticles (n=3 per time point) over a 14-day period *in vitro* vs. non-encapsulated cytokine in the supernatant (n=1) following microparticle synthesis.



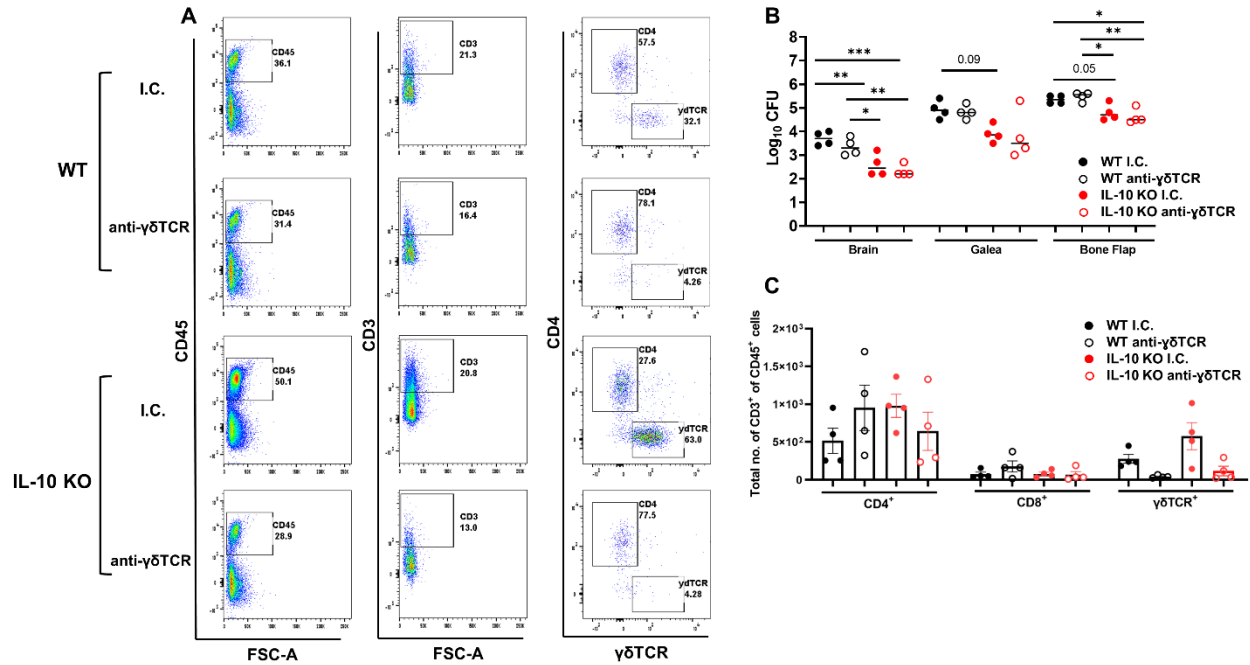
Additional file 1: Fig. S3. Absolute numbers of IL-10 producing G-MDSCs and PMNs during *S. aureus* craniotomy infection. Wild type mice (n=5/group) were sacrificed at days 7 and 14 following *S. aureus* craniotomy infection, whereupon the absolute numbers of IL-10⁺ granulocytic myeloid-derived suppressor cells (G-MDSCs) and neutrophils (PMNs) in the brain and galea were determined using counting beads (*, $p < 0.05$; unpaired Student's *t*-test).



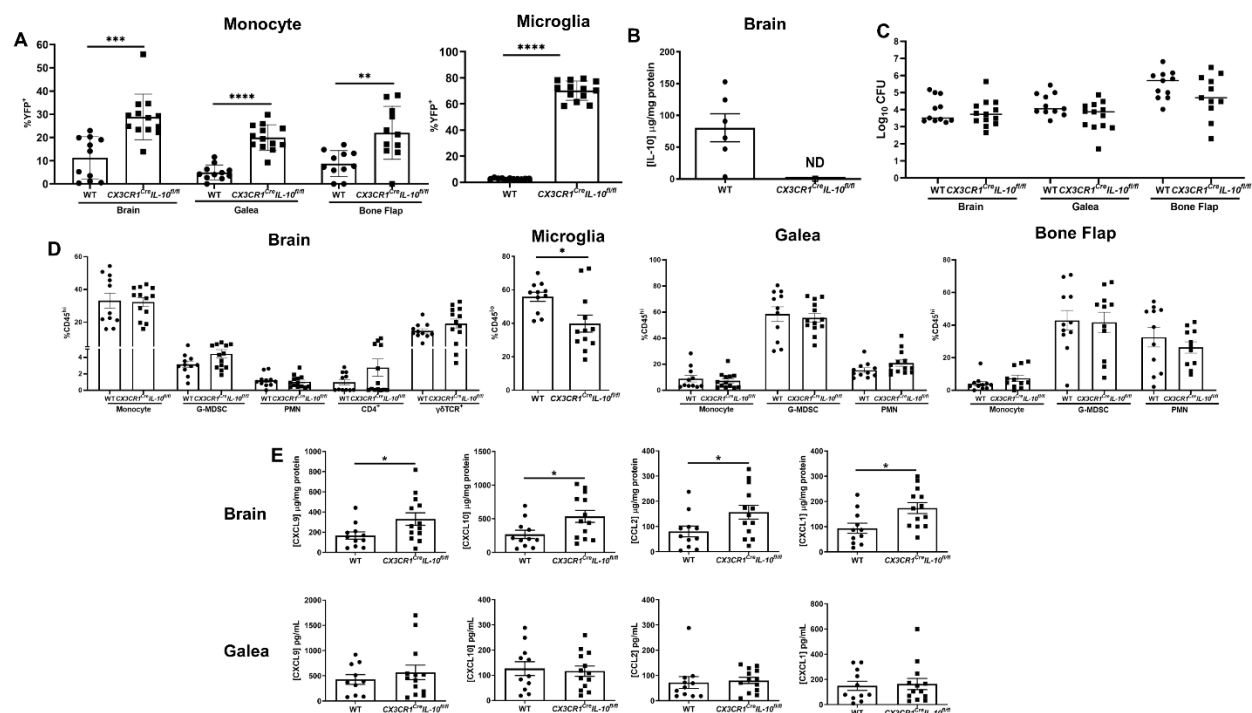
Additional file 1: Fig. S4. Absolute numbers of immune cell infiltrates in WT and IL-10 KO mice during craniotomy infection. Wild type (WT; n=5-6) and IL-10 knockout (KO; n=6-7) mice were sacrificed at days 7 and 14 following *S. aureus* craniotomy infection, whereupon the absolute numbers of resident microglia and leukocytes in the brain, galea, and bone flap were determined using counting beads.



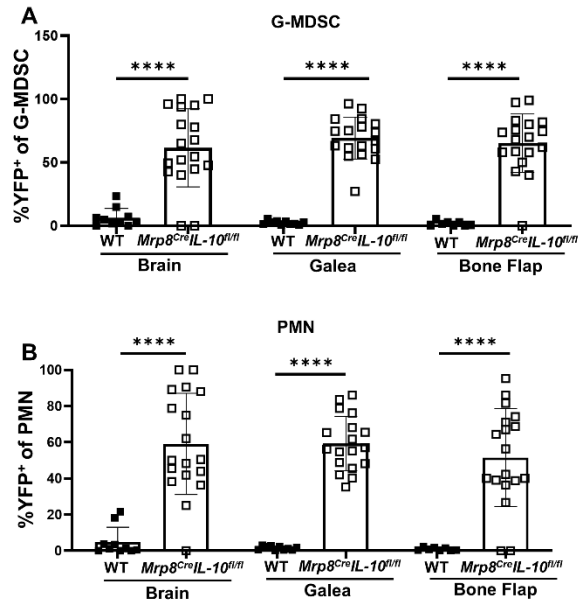
Additional file 1: Fig. S5. CD4⁺ T cells are not critical for *S. aureus* containment during craniotomy infection in IL-10 knockout mice. Wild type (WT; n=4) and IL-10 knockout (KO; n=5) animals received anti-CD4 or isotype-matched control antibody (I.C.) beginning 3 days prior to *S. aureus* infection and every fourth day until sacrifice at day 14 post-infection, whereupon **(A)** CD4⁺ T cell depletion in the brain and **(B)** bacterial burden in various CNS compartments were assessed. **(C)** Absolute number of leukocytes infiltrating the brain was quantified using counting beads via flow cytometry. Results are presented as the total number of CD4⁺, CD8⁺ or γδ T cells gated from live CD3⁺CD45⁺ cells (mean ± SEM). Significant differences were determined using a One-way ANOVA with Tukey's correction and are denoted by asterisks (*, $p < 0.05$).



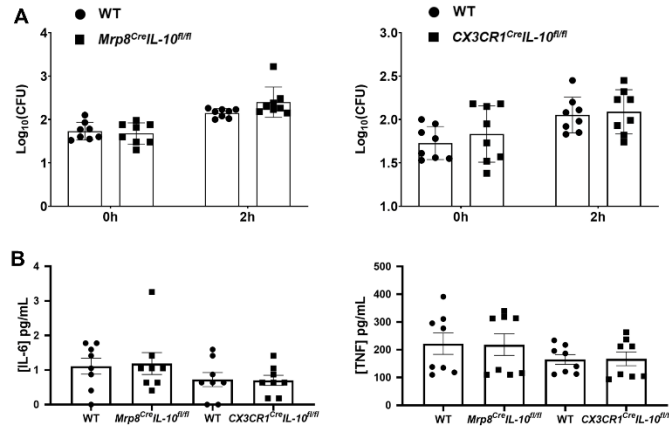
Additional file 1: Fig. S6. $\gamma\delta$ T cells do not influence bacterial growth during *S. aureus* craniotomy infection. Wild type (WT) and IL-10 knockout (KO) animals (n=4/group) received anti- $\gamma\delta$ TCR or isotype-matched control antibody (I.C.) beginning two days prior to *S. aureus* infection and every third day until sacrifice at day 14 post-infection, whereupon (A) $\gamma\delta$ T cell depletion and (B) bacterial burden was assessed. (C) Absolute number of leukocytes infiltrating the brain was quantified using counting beads via flow cytometry. Results are presented as the total number of CD4⁺, CD8⁺ or $\gamma\delta$ T cells gated from live CD3⁺CD45⁺ cells (mean $\pm$ SEM). Significant differences were determined using a One-way ANOVA with Tukey's correction and are denoted by asterisks (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).



Additional file 1: Fig. S7. IL-10 deletion in monocytes and microglia has minimal effects on *S. aureus* craniotomy infection. (A) Percentage of YFP⁺ microglia as well as monocytes in the brain, galea, and bone flap from CX3CR1^{Cre}IL-10^{fl/fl} mice (n=12-13) and WT littermates (n=11-13) at day 14 following *S. aureus* craniotomy infection. (B) Quantification of IL-10 levels in the brain by ELISA at day 14 post-infection (n=6 WT and 7 Cre). (C) Bacterial burden and (D) resident microglia and leukocyte infiltrates in the brain, galea, and bone flap, and (E) chemokine expression (n=11 WT and 13 Cre), was determined at day 14 after infection (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; unpaired Student's *t*-test). ND, not detected.



Additional file 1: Fig .S8. Characterization of IL-10 deletion from G-MDSCs and PMNs in *Mrp8^{Cre}IL-10^{fl/fl}* mice. Percentage of (A) YFP⁺ granulocytic myeloid-derived suppressor cells (G-MDSCs) and (B) neutrophils (PMNs) in the brain, galea, and bone flap of *Mrp8^{Cre}IL-10^{fl/fl}* mice (n=11-18) and WT littermates (n=8-15) at day 14 following *S. aureus* craniotomy infection ($p < 0.0001$; unpaired Student's *t*-test).



Additional file 1: Fig. S9. *S. aureus* intracellular burden and cytokine production are not affected in PMNs from *Mrp8^{Cre}IL-10^{fl/fl}* or *CX3CR1^{Cre}IL-10^{fl/fl}* mice. PMNs isolated from *Mrp8^{Cre}IL-10^{fl/fl}* or *CX3CR1^{Cre}IL-10^{fl/fl}* animals were exposed to live *S. aureus* USA300 LAC for 2 h at an MOI of 10:1 (bacteria:cell) to evaluate **(A)** *S. aureus* survival by gentamicin protection assays and **(B)** cytokine production by cytometric bead array (n=8 biological replicates/group combined from two independent experiments).