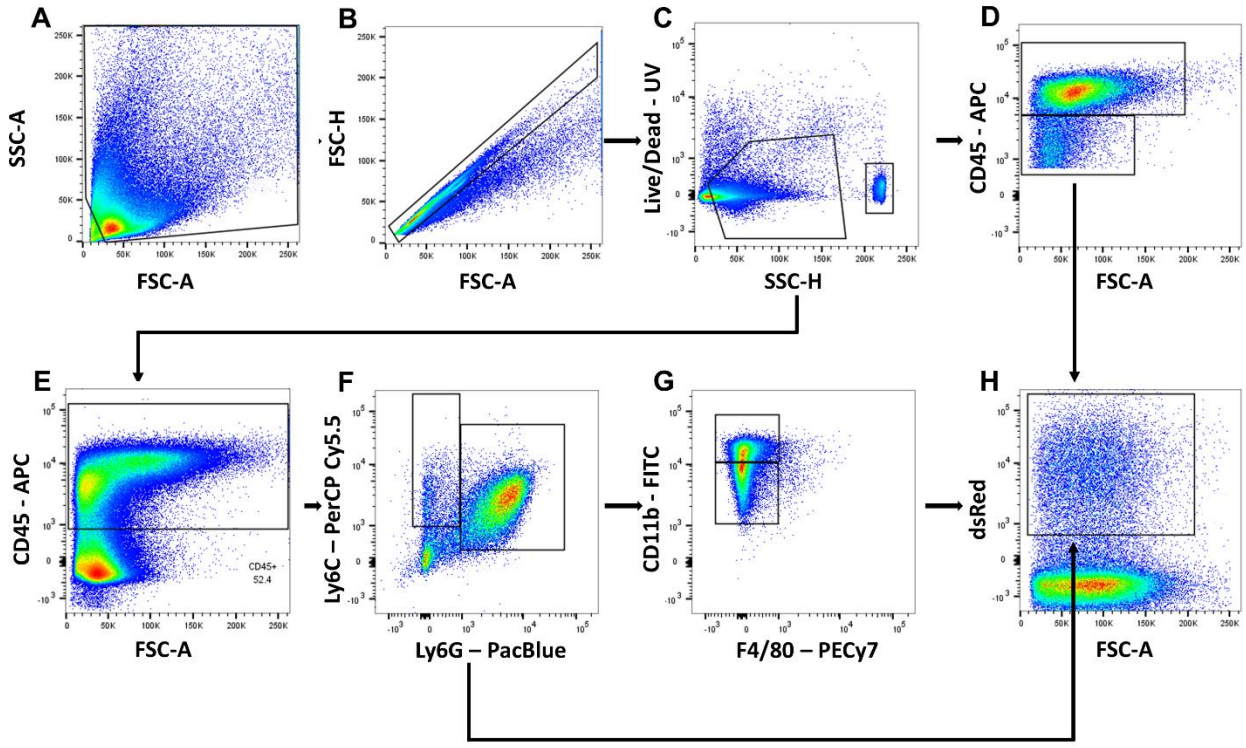
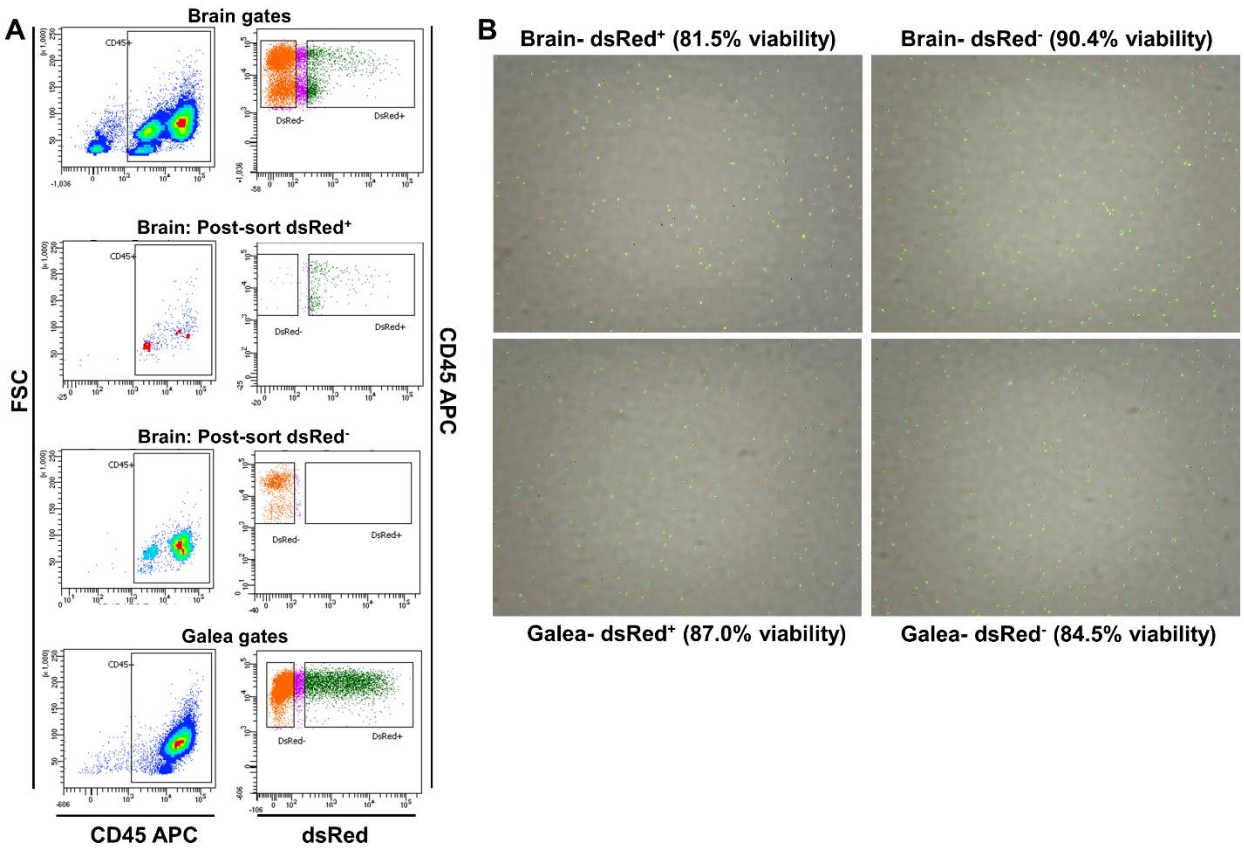
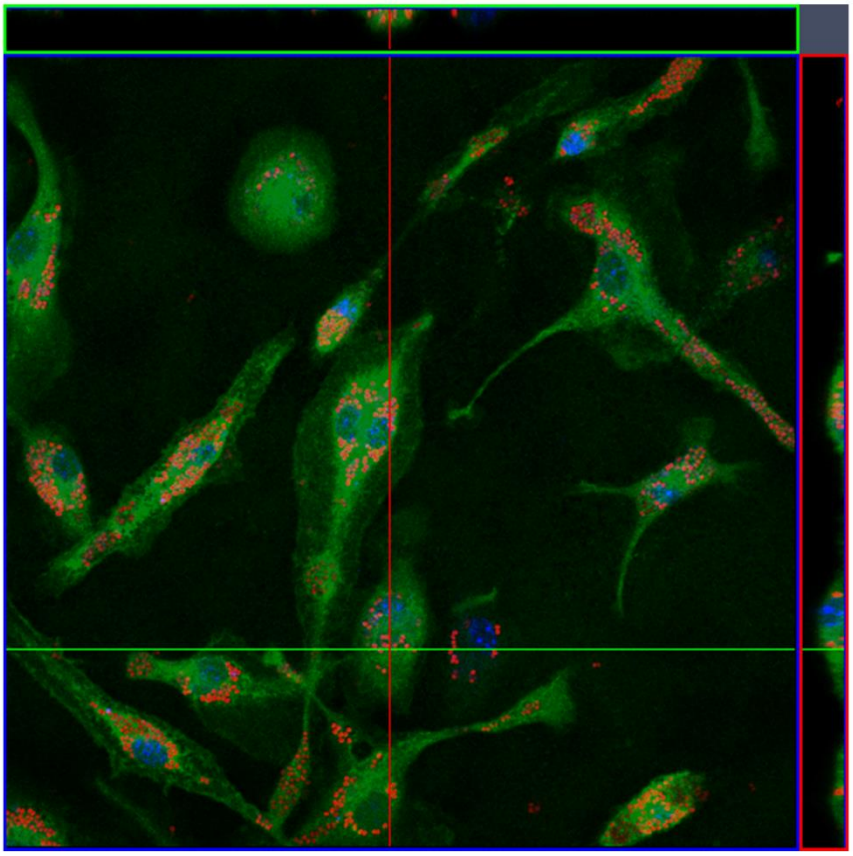


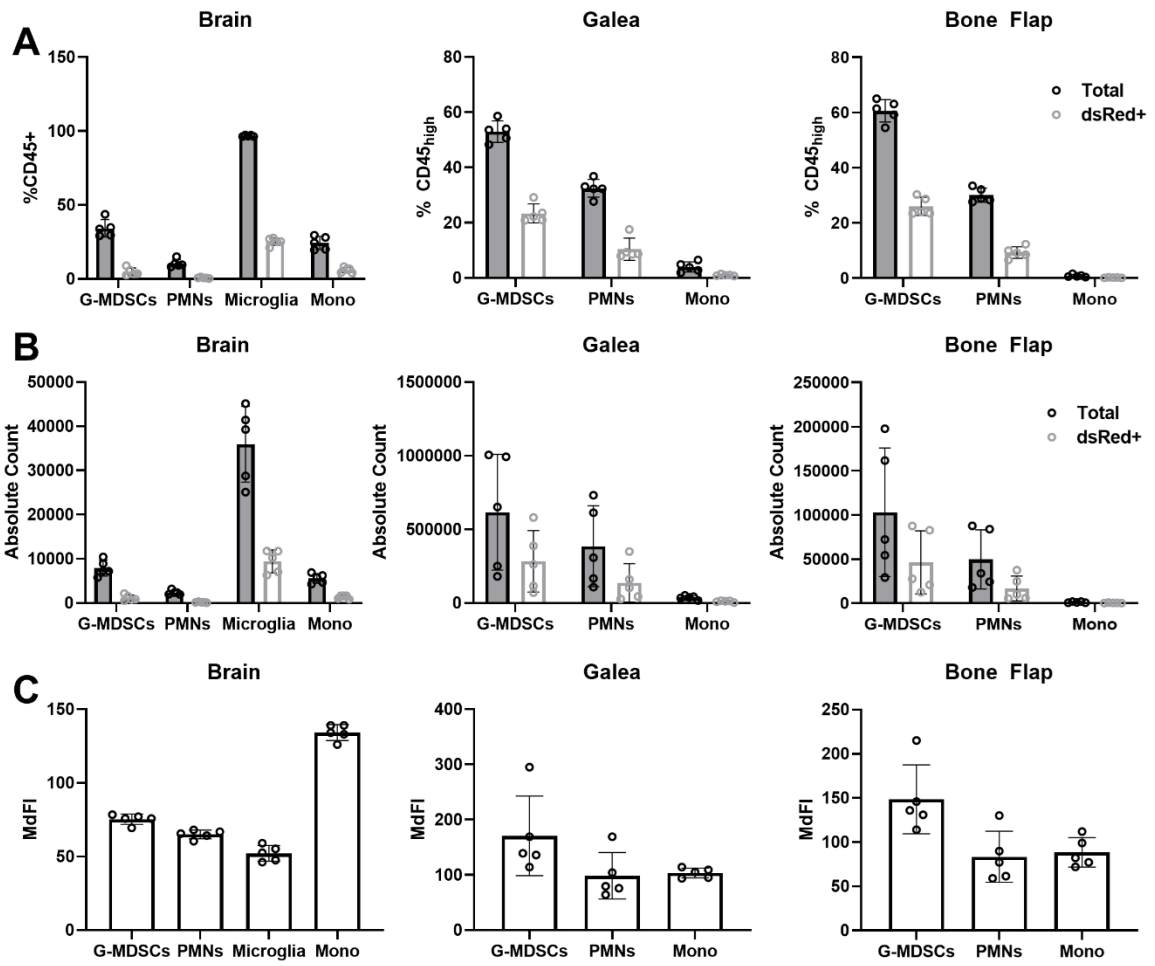
Supplemental Figure 1. Gating strategy to quantify immune populations and dsRed signal in the brain, galea, and bone flap following *S. aureus* craniotomy infection. From the (**A**) total events, (**B**) single cells were gated using FSC-A vs. FSC-H, followed by (**C**) exclusion of dead cells using SSC-H vs. Live/Dead UV and AccuCount beads (right gate). (**D**) Live cells were gated into $CD45^{high}$ leukocytes and $CD45^{low}$ microglia. (**E**) $CD45^{high}$ leukocytes were separated into (**F**) $Ly6C^{+}Ly6G^{-}$ monocytes and $Ly6G^{+}Ly6C^{+}$ cells, which were further classified as (**G**) G-MDSCs ($CD11b^{high}Ly6G^{+}Ly6C^{+}F4/80^{-}$) and neutrophils ($CD11b^{low}Ly6G^{+}Ly6C^{+}F4/80^{-}$) based on differential CD11b expression. (**H**) *S. aureus*-$dsRed^{+}$ microglia, monocytes, G-MDSCs, and neutrophils were then identified.



Supplemental Figure 2. FACS parameters for acquisition of CD45$^+$ dsRed$^+$ and dsRed$^-$ cells from the brain and galea for scRNA-seq and post-sort viability. C57BL/6J mice (n=25) were sacrificed at day 3 following craniotomy infection with a *S. aureus*-dsRed strain, whereupon dsRed$^+$ and dsRed$^-$ cells were sorted from the total CD45$^+$ population by FACS. (**A**) Gates used to capture CD45$^+$ dsRed$^+$ and dsRed$^-$ cells from the brain and galea and (**B**) post-sort viability and debris analysis, where green and red depict live vs. dead cells, respectively.



Supplemental Figure 3. *S. aureus*-dsRed is efficiently phagocytosed by macrophages. Primary macrophages were labeled with CellTracker Deep Red (pseudocolored green) and Hoechst 33342 (blue) prior to live *S. aureus*-dsRed challenge at a MOI of 10:1 (bacteria:macrophage). After a 2 h incubation period phagocytosis was assessed by confocal microscopy (40x magnification).



Supplemental Figure 4. Leukocyte phagocytic activity is influenced by the local tissue environment during *S. aureus* craniotomy infection. C57BL/6J mice (n=5) were sacrificed at day 1 following craniotomy infection with a *S. aureus*-dsRed strain, whereupon microglia and leukocyte infiltrates in the brain, galea, and bone flap were quantified by flow cytometry. (**A**) Percentages and (**B**) absolute counts of $CD45^+$ cells in the galea, brain, and bone flap expressed as the total number of cells and those that had phagocytosed *S. aureus* ($dsRed^+$). (**C**) Median fluorescence intensity (MdFI) of $dsRed^+$ phagocytic cells.