

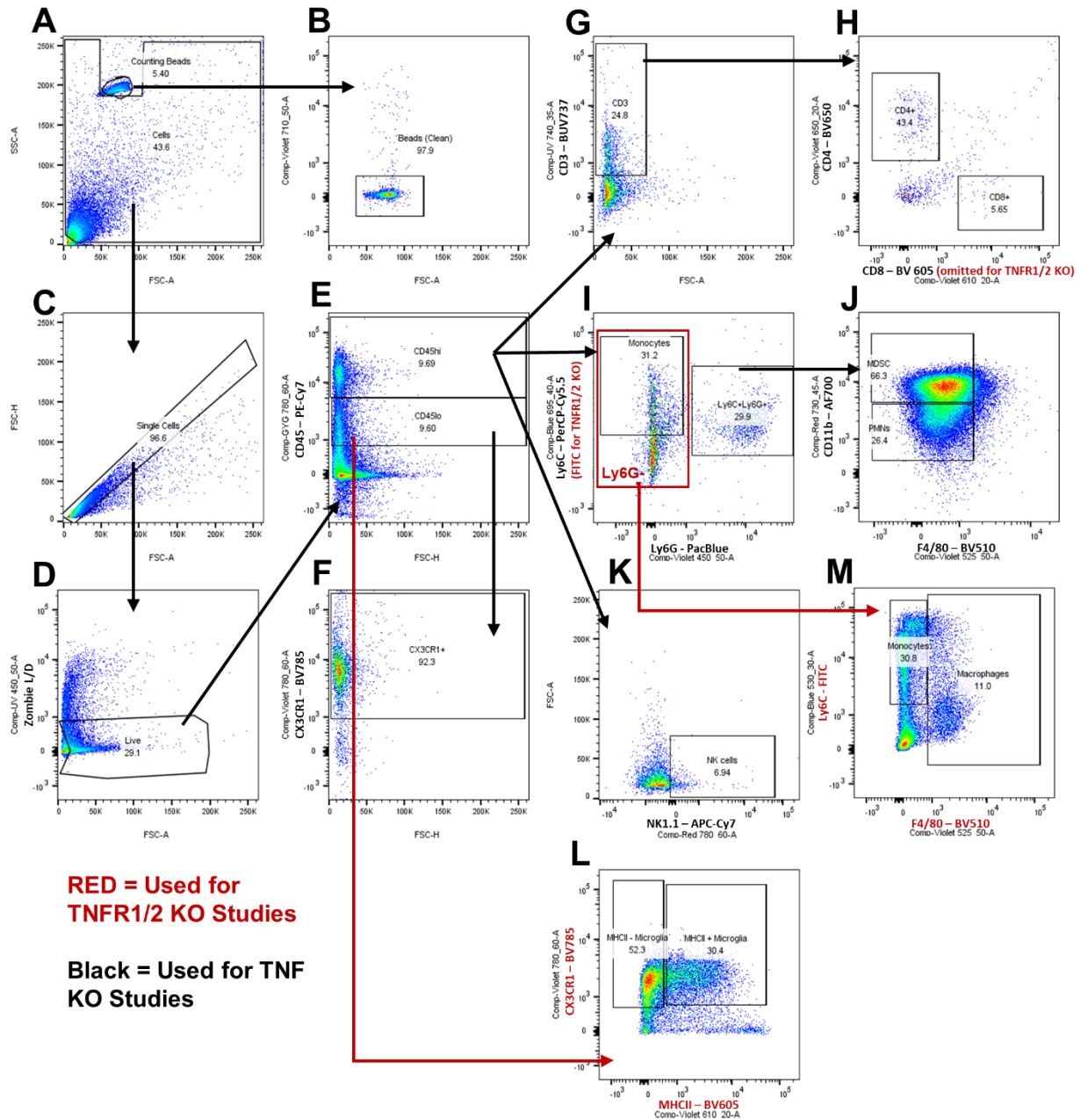


Figure S1. Gating strategies for *in vivo* flow cytometry experiments. From total events (A), counting beads were excluded (B) to quantify total cell counts and the remaining events were gated on FSC and SSC to isolate single (C) and subsequently live cells (D). Immune cells were then divided into CD45^{low} and CD45^{high} populations (E). CD45^{low} cells (E) were further separated into microglia based on CXCR1 (F) for TNF KO studies and segregated further by

CXCR1/MHCII microglia (**L**) for TNFR1 and 2 KO experiments. CD45^{high} cells (**E**) were gated on CD3 (**G**) and CD4/CD8 (**H**) to characterize T cells, Ly6C and Ly6G to separate granulocytes from monocytes (**I**), and NK1.1 to identify NK cells (**K**). Ly6G⁺ cells (**I**) were further separated into neutrophils and G-MDSCs based on CD11b and F4/80 levels (**J**). Ly6G⁻ cells were separated into monocytes and macrophages by Ly6C and F4/80 expression (**M**).

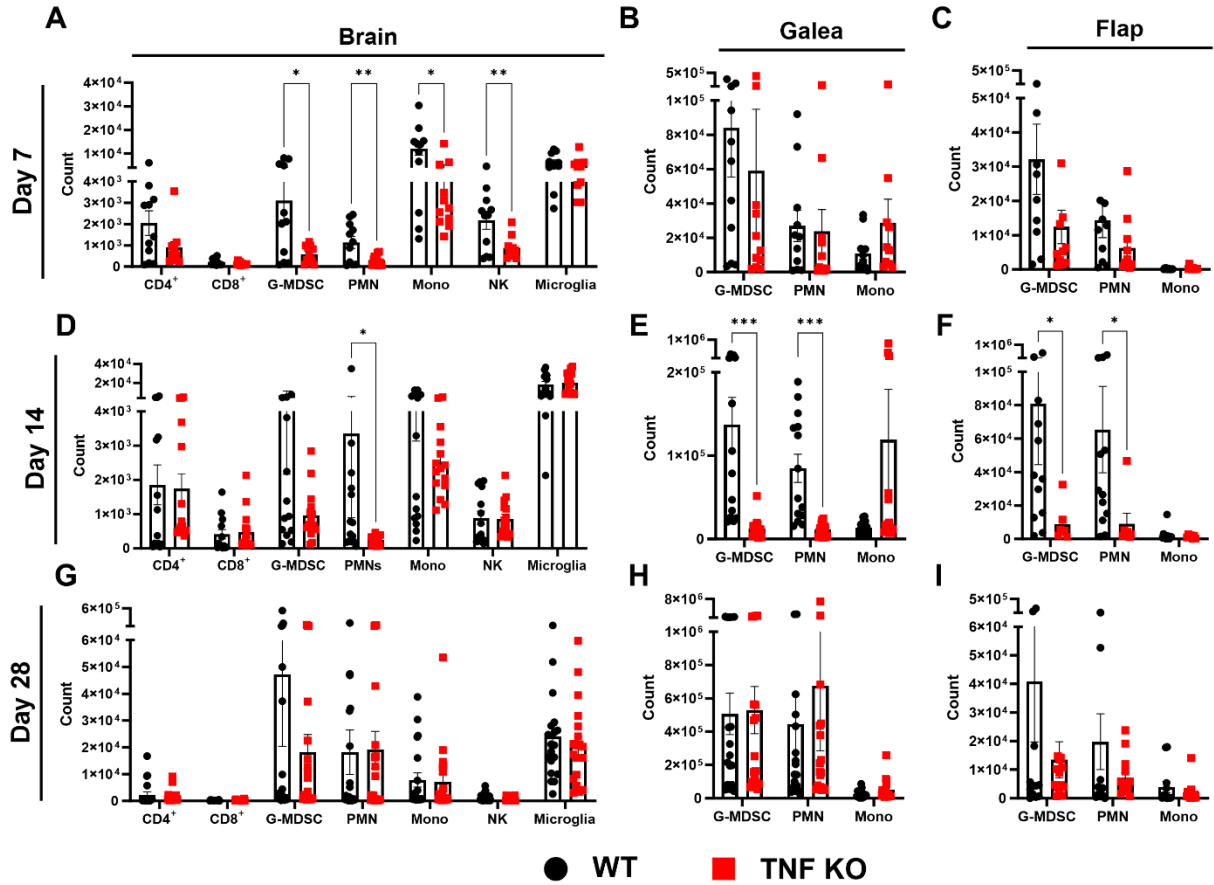


Figure S2. Absolute cell counts of immune populations in TNF KO mice during *S. aureus* craniotomy infection. TNF knockout (KO; n=7-35) and wild type (WT; n=11-35) mice were subjected to craniotomy infection and sacrificed at days 7 (**A-C**), 14 (**D-F**), and 28 (**G-I**) post-infection, whereupon absolute counts of immune populations were quantified in the brain (**A, D, G**), galea (**B, E, H**), and bone flap (**C, F, I**) using counting beads. Data was pooled from 2-5 independent experiments (mean $\pm$ SEM) and analyzed by multiple unpaired t-test. Mono, monocyte; *, $p > 0.05$; **, $p > 0.01$; ***, $p > 0.001$.

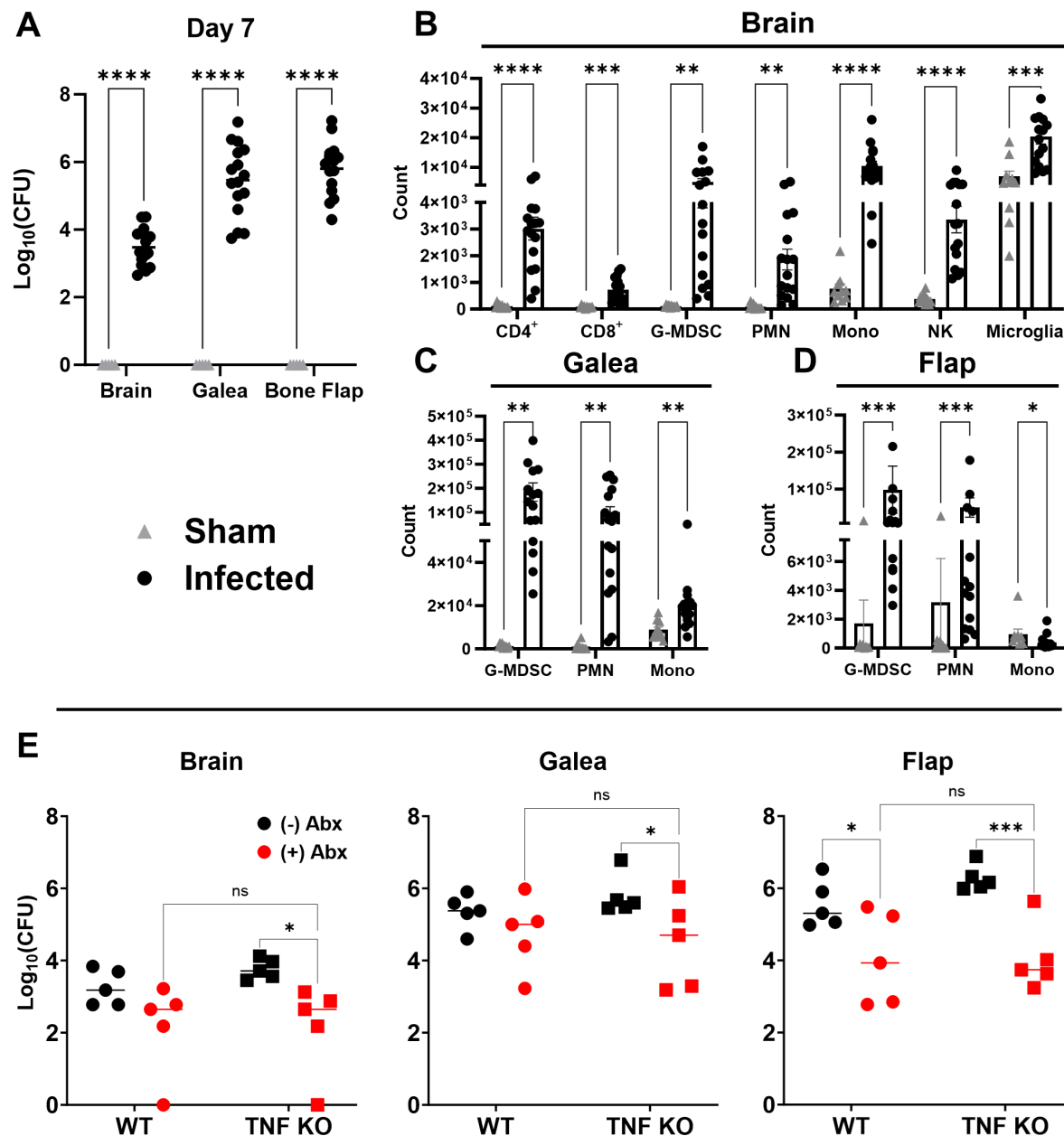


Figure S3. Craniotomy infection actively elicits leukocyte recruitment and the lack of TNF involvement on bacterial burden is not altered with systemic antibiotics. (A-D) Wild type (WT; n=15) mice were subjected to either sterile (sham) craniotomy or *S. aureus* infection and sacrificed at day 7 post-surgery, whereupon bacterial burden (**A**) and immune populations (**B-D**) were assessed as absolute counts. (**E**) WT and TNF KO mice were subjected to craniotomy infection $\pm$ daily antibiotic administration beginning at day 3 post-infection (n=5/group). Animals

were sacrificed at day 7 post-infection, whereupon bacterial burden in the brain, galea, and bone flap was assessed. Data analyzed by (**A**) 2-way ANOVA and (**B-D**) multiple unpaired t-test. Mono, monocyte; NK, NK cell; Abx, antibiotics; *, $p>0.05$; **, $p>0.01$; ***, $p>0.001$; ****, $p>0.0001$; ns, not significant.

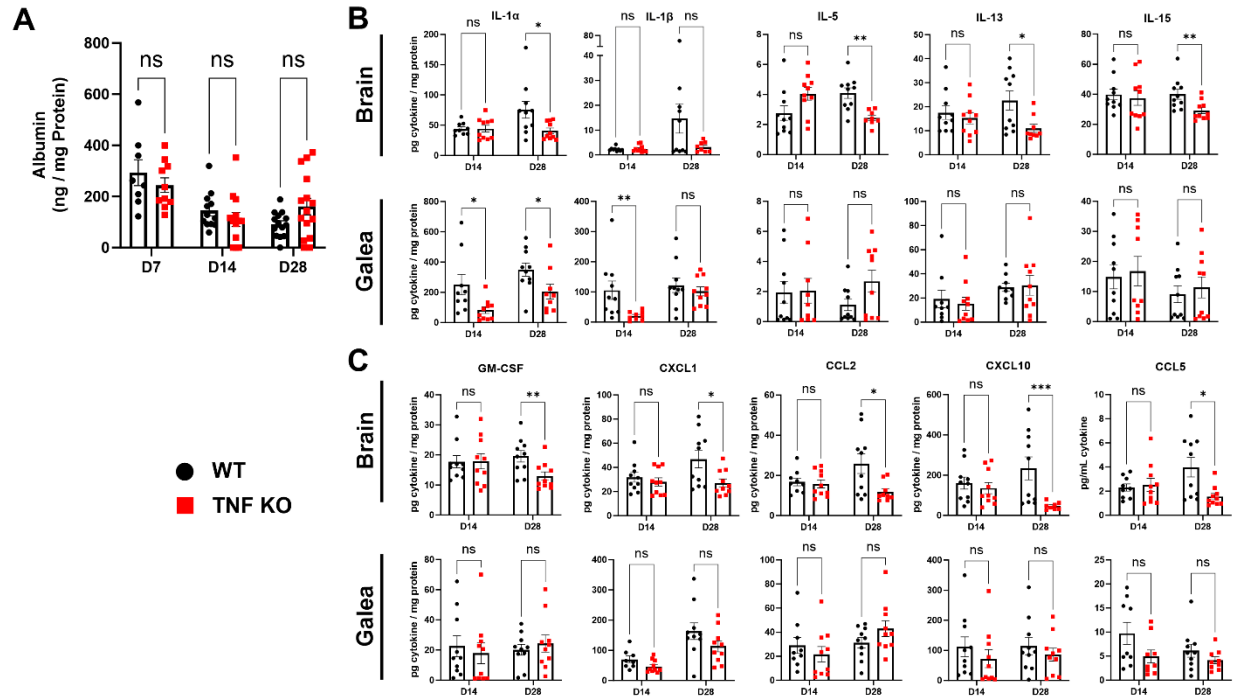


Figure S4. Blood-brain barrier permeability during craniotomy infection is not altered following TNF loss. TNF knockout (KO; n=10) and wild type (WT; n=10) mice were subjected to craniotomy infection and sacrificed at the indicated timepoints, whereupon **(A)** albumin levels were quantified in brain supernatants, and cytokines **(B)** and chemokines **(C)** were quantified in galea and brain homogenates. All measurements were normalized to total protein levels and pooled from 2 independent experiments (mean $\pm$ SEM). Data were analyzed by two-tailed Student's t-test **(A)** or non-parametric multiple Mann-Whitney test **(B)**. *, $p > 0.05$; **, $p > 0.01$; ns, not significant.

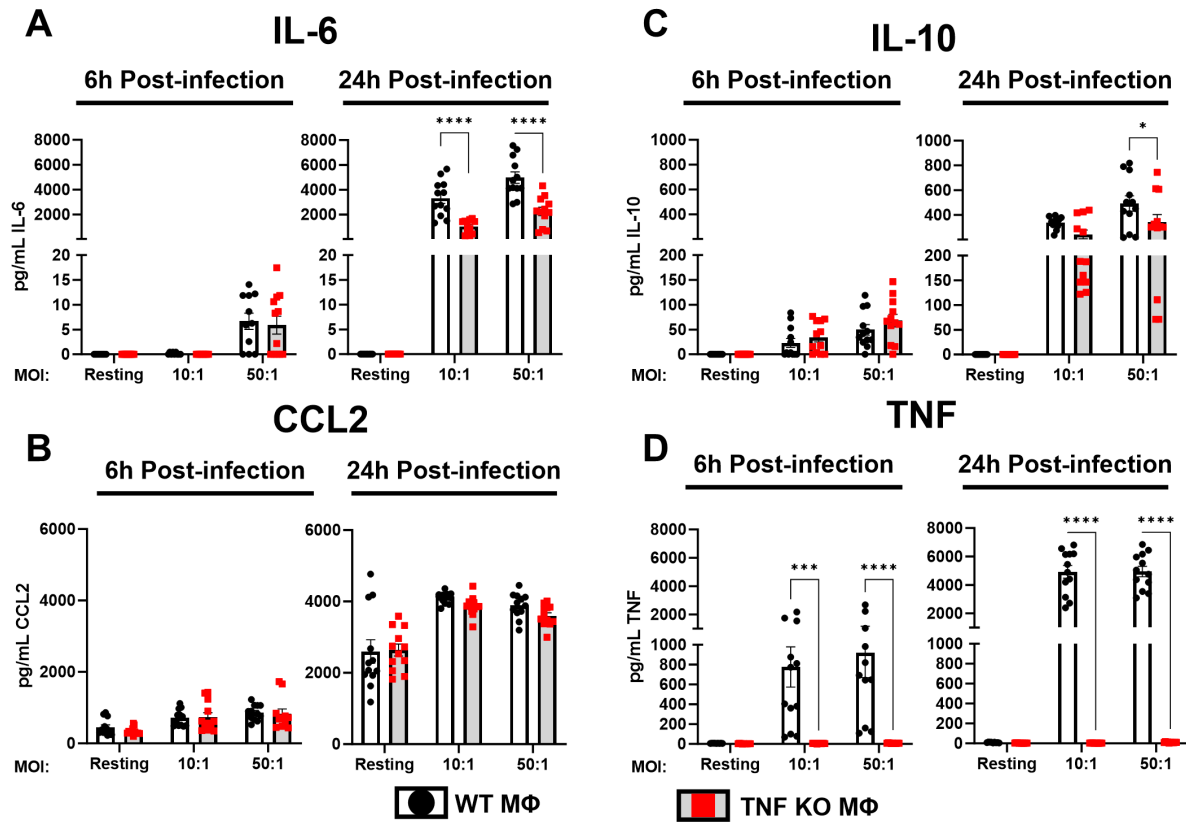


Figure S5. TNF deficiency attenuates macrophage IL-6 production following *S. aureus* exposure. Primary mouse macrophages (MΦs) were generated from wild type (WT; n=12) or TNF knockout (KO; n=12) mice and challenged with live *S. aureus* at a MOI of 10:1 or 50:1 for 1 h before elimination of extracellular bacteria by gentamicin treatment. Following incubation for the indicated duration, cell-conditioned supernatants were collected and assessed for (A) IL-6, (B) CCL2, (C) IL-10, and (D) TNF. Data combined from 3 independent experiments and analyzed by two-way ANOVA. *, $p > 0.05$; ***, $p > 0.001$; ****, $p > 0.0001$.

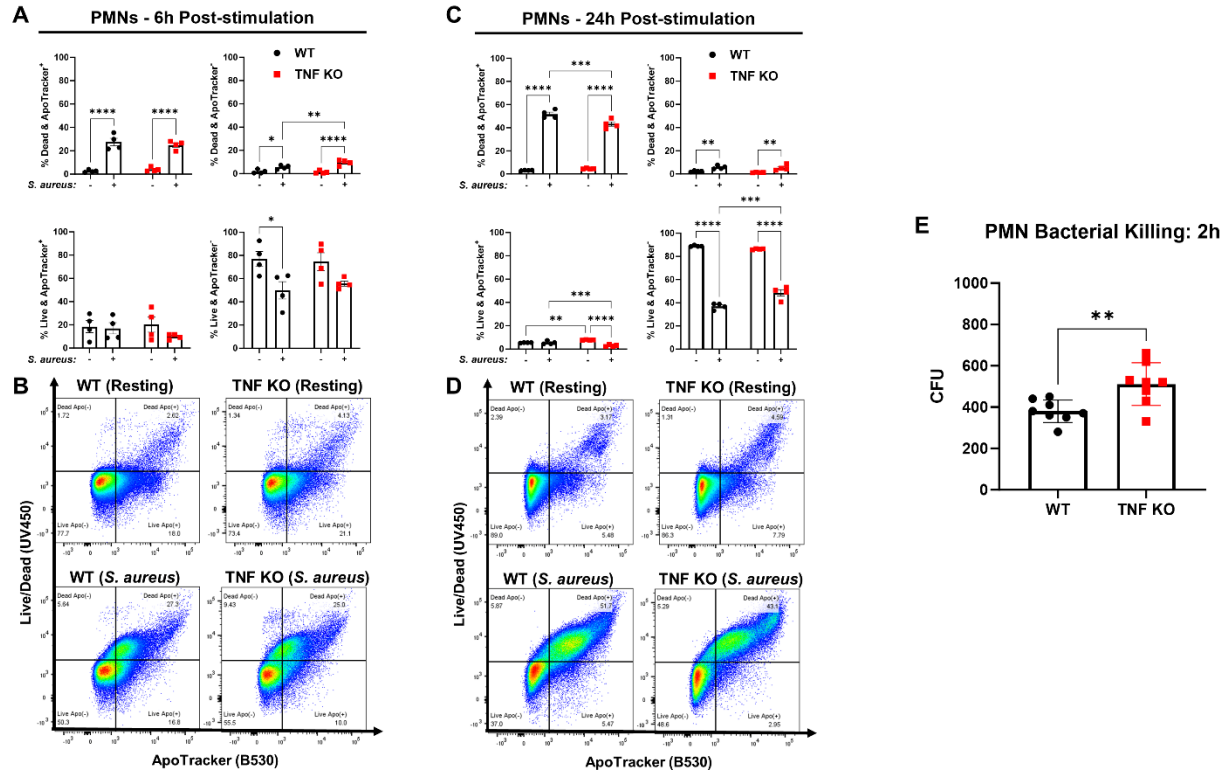


Figure S6. Role of TNF on PMN viability, apoptosis, and bactericidal activity in response to *S. aureus*. (A-D) Primary neutrophils (PMNs) from TNF knockout (KO; n=5) and wild type (WT; n=5) mice were challenged with live *S. aureus* at an MOI of 10:1 for 1h before elimination of extracellular bacteria with gentamicin. Following incubation for 6 (A-B) or 24 h (C-D), PMN viability and apoptosis was assessed by two-way ANOVA (A, C) with representative flow plots shown (B, D). (E) Primary PMNs from TNF KO (n=8) and WT (n=8) mice were stimulated with live *S. aureus* at an MOI of 10:1, whereupon bacterial killing was assessed at 2 h post-infection via gentamicin protection assay. Data analyzed by two-tailed t-test. *, $p>0.05$; **, $p>0.01$; ***, $p>0.001$; ****, $p>0.0001$.

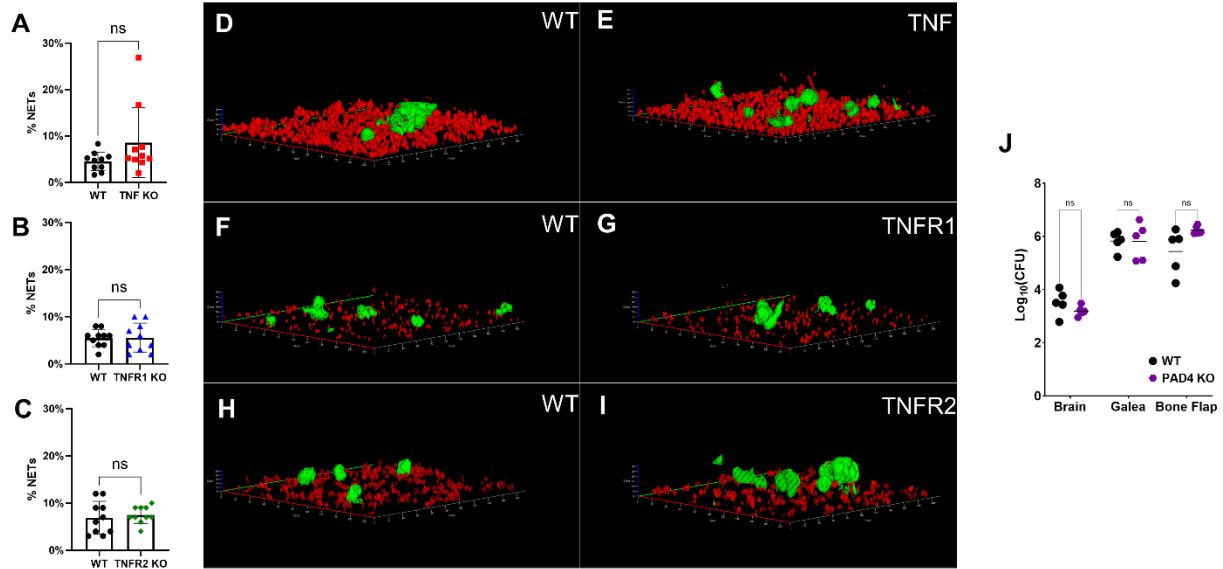


Figure S7. TNF does not affect NET formation in response to *S. aureus*. Primary PMNs from TNF (**A, E**), TNFR1 (**B, G**), and TNFR2 (**C, I**) knockout (KO; n=10) or wild type (WT; n=10) mice (**D, F, H**), were challenged with live *S. aureus*-dsRED (red) at a MOI of 10:1 for 1 h, and subsequently stained with Sytox Green (Green). NETs were quantified by the percentage of total cells in each 40x field of view (**A-C**). Representative Z-stack images from each condition are also shown (**D-I**). (**J**) WT and PAD4 KO mice (n=5/group) were subjected to *S. aureus* craniotomy infection and sacrificed at day 7 post-infection, whereupon bacterial burden was assessed from the brain, galea, and bone flap. Data analyzed by two-tailed Student's t-test (**A-C**) or 2-way ANOVA (**J**). ns, not significant.

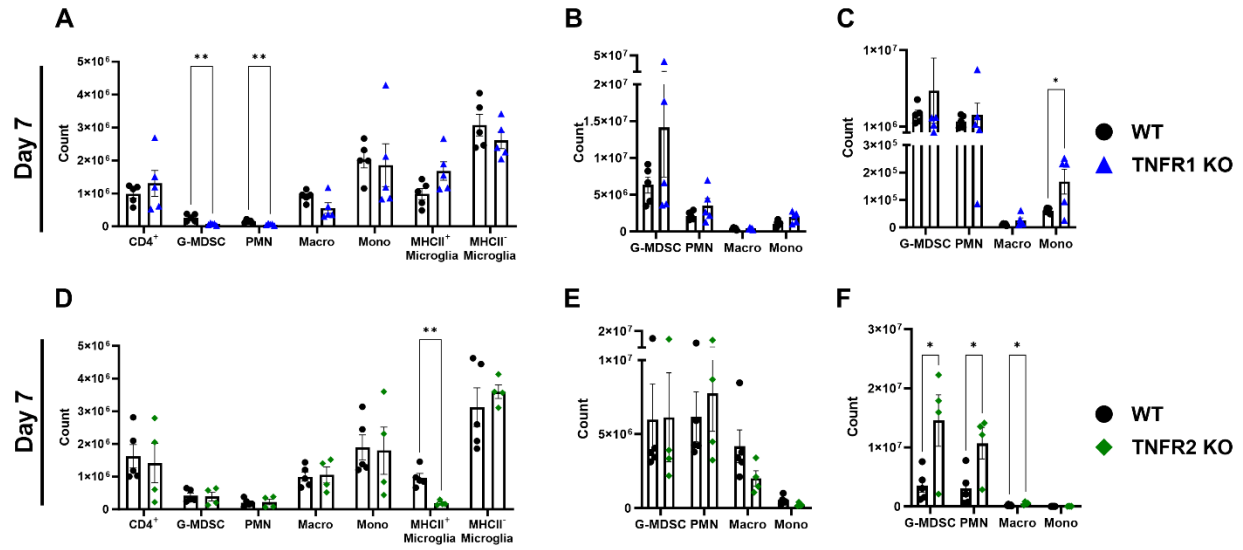


Figure S8. Absolute cell counts of immune populations in TNFR1 or TNFR2 KO mice during *S. aureus* craniotomy infection. TNFR1 (A-C) or TNFR2 (D-F) knockout (KO; n=4-5) and wild type (WT; n=5) mice were subjected to craniotomy infection and sacrificed at day 7 post-infection, whereupon absolute counts of immune populations in the brain (A, D), galea (B, E), and bone flap (C, F) were quantified using counting beads. Data was analyzed by multiple unpaired t-test (mean ± SEM). Macro, macrophage; Mono, monocyte; *, $p > 0.05$; **, $p > 0.01$.