Supplemental Figure 1: Development of Stat4\textsuperscript{fl/fl}LysM\textsuperscript{cre}, and Stat4\textsuperscript{fl/fl}S100A8\textsuperscript{cre} mice. (A) Schematic map of the Stat4 locus showing the insertedloxP sites flanking the third exon and the FRT-flanked neo cassette before (top) and after (bottom) Flp-mediated deletion. (B) Schematic of the targeted Stat4 locus following Cre-mediated deletion. The third exon was chosen for targeting because removal causes a nonsense frameshift following splicing of the remaining exons. (C) STAT4 expression in T cells, peritoneal macrophages, and BM neutrophils isolated from WT, Stat4\textsuperscript{fl/fl}LysM\textsuperscript{cre}, and Stat4\textsuperscript{fl/fl}S100A8\textsuperscript{cre} mice. (D) Naïve CD4 T cells from WT, Stat4\textsuperscript{−/−}, and Stat4\textsuperscript{fl/fl}LysM\textsuperscript{cre} mice were differentiated under Th1 conditions for 5 days, then activated with PMA/ionomycin for 6 hours, and stained with intracellular cytokine Abs. (E) Representative FACS plots for CD11b\textsuperscript{+}Gr-1\textsuperscript{+} neutrophils in the BM and the spleen of WT, Stat4\textsuperscript{−/−}, and Stat4\textsuperscript{fl/fl}LysM\textsuperscript{cre} mice. (F) Number of neutrophils in the spleen and peritoneal blood of WT, Stat4\textsuperscript{−/−}, and Stat4\textsuperscript{fl/fl}LysM\textsuperscript{cre} mice. (G-H) Bone marrow derived macrophages (BMDM) from WT, Stat4\textsuperscript{−/−}, and Stat4\textsuperscript{fl/fl}LysM\textsuperscript{cre} mice were activated with HK-MRSA and IL-12. (G) Representative FACS plot for pSTAT4. (H) Cytokine and chemokine levels by qPCR. Data are average±SEM. * p<0.05, **p<0.01.
Supplemental Figure 2. STAT4-deficiency has no impact on neutrophil apoptosis and phagocytosis. (A-B) Neutrophils were incubated with or without IL12 (40 ng/ml) in complete RPMI media. After different time points (0.5-12 hrs), neutrophil were collected, stained with Annexin V and 7-AAD, and analyzed by FACS (n=2/per group). Representative FACS plot and averages for live cells are shown. (C-D) WT and Stat4⁻/⁻ bone marrow neutrophils were incubated with fluorescein-labeled K-12 E. coli or MRSA. After 30 and 90 min, neutrophils were collected, washed, non-phagocytosed bioparticles were quenched with trypan blue, and fluorescein positive cells were detected using FACS. Data are average±SEM.