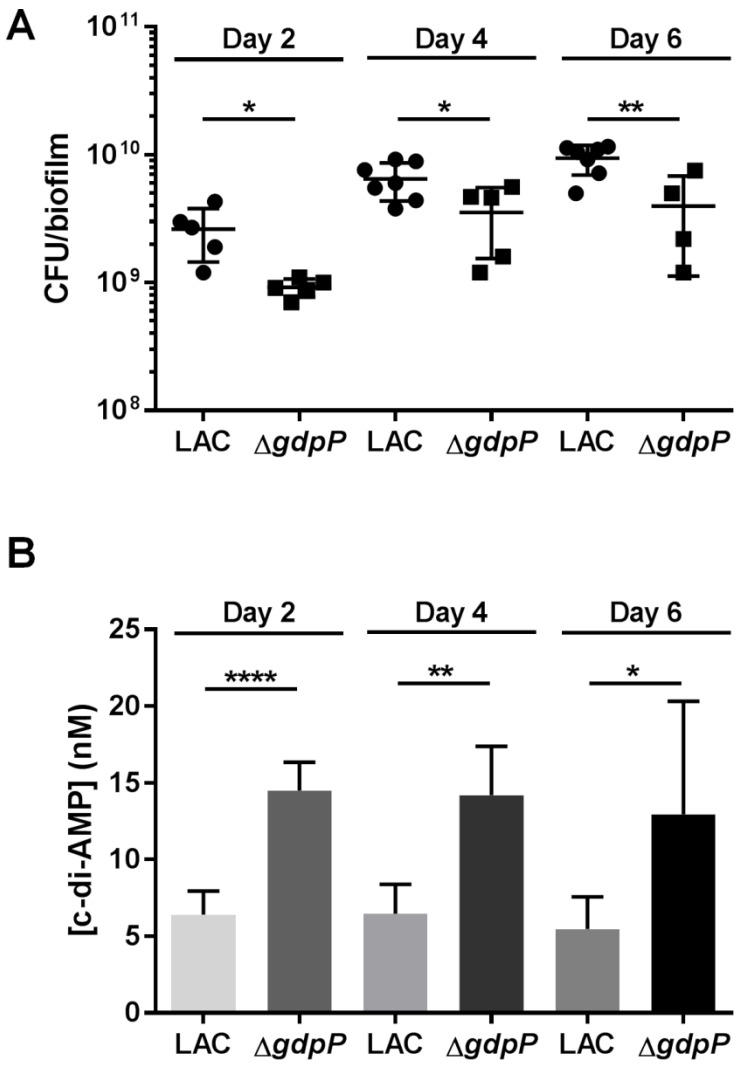
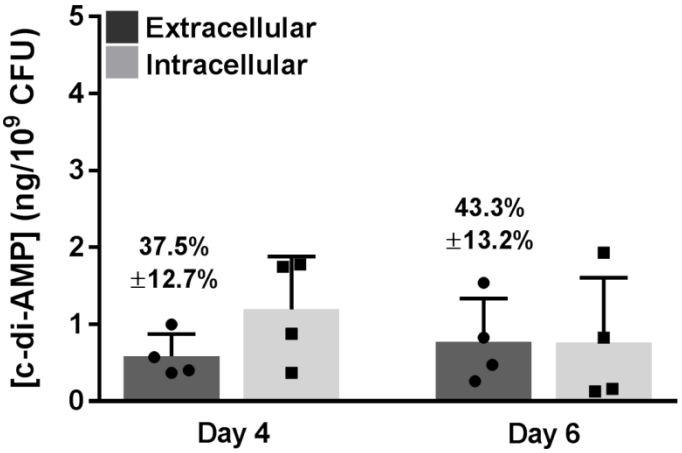
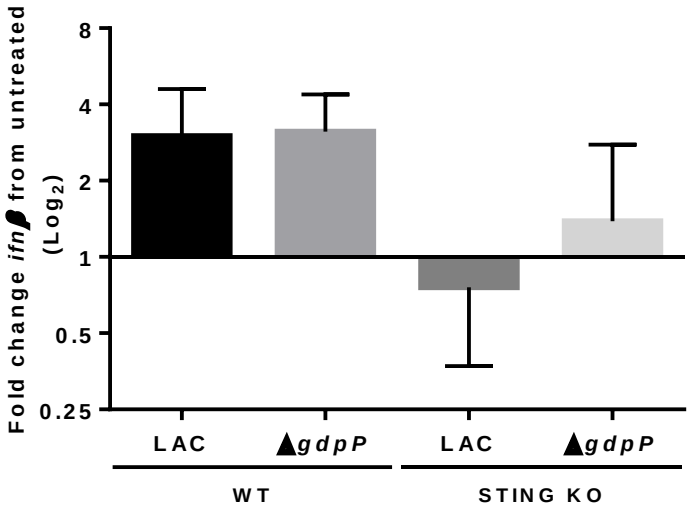


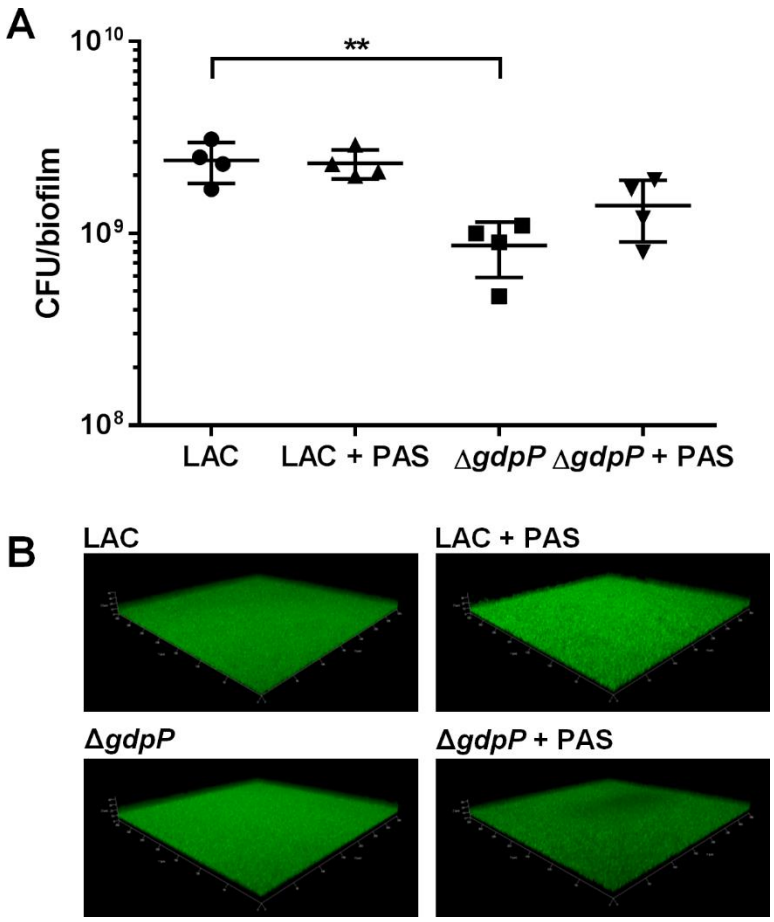
Supplemental Figure 1. Enumeration of viable bacteria and extracellular [c-di-AMP] from *S. aureus* biofilms. (A) Bacterial viability in wild-type LAC and Δ*gdpP* static biofilms grown in RPMI + 1% casamino acids for 2, 4, and 6 days. (B) Calculated concentrations of extracellular c-di-AMP from WT and Δ*gdpP* static biofilms from Figure 2 in the main text. Error bars indicate standard deviation of the average from at least two independent experiments. Statistical analysis was performed using an unpaired t-test between groups. *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$.



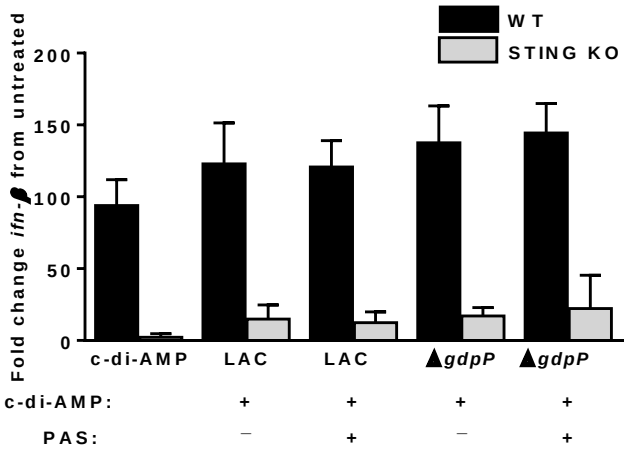
Supplemental Figure 2. The *S. aureus mdrM* homologues are not a major route of extracellular c-di-AMP release during biofilm growth. c-di-AMP levels were quantified in extracellular (biofilm-conditioned medium) and intracellular fractions from static biofilms of a triple *mdrM* homologue mutant (CMG8) using UPLC-MS/MS (refer to Figure 2 for c-di-AMP levels from wild type *S. aureus* LAC). Viable bacteria were quantified and c-di-AMP values plotted per 10^9 CFU for normalization. The percentage of total c-di-AMP detected in biofilm-conditioned medium after four and six days of biofilm growth are indicated. Error bars indicate standard deviation of the average from at least two independent experiments with individual data points plotted.



Supplemental Figure 3. LAC *and* Δ*gdpP* planktonic culture supernatants are not major inducers of *ifn-β* expression. WT and STING KO BMDMs were treated for 3 h with WT LAC and Δ*gdpP* culture supernatants from overnight (16h) planktonic cultures grown in RPMI-1640 + 1% casamino acids. RNA was isolated and analyzed for changes in *ifn-β* expression by qRT-PCR. Gene expression levels were normalized to the housekeeping gene GAPDH and are presented as the fold-induction ($2^{-\Delta\Delta Ct}$) value relative to untreated BMDMs. Results depict averages from two independent experiments with standard deviation shown.



Supplemental Figure 4. PAS treatment has no effect on biofilm formation or viability. Day 6 wild-type and Δ*gdpP* biofilms subjected to 50 µg/mL PAS treatment for 48 h were assessed for (**A**) viability and (**B**) biofilm formation by confocal microscopy following Syto9 staining. The average CFU/biofilm (with standard deviations indicated) and representative images from two independent experiments are shown. Statistical analysis was performed using a one-way ANOVA method with Tukey post-hoc analysis. **, $p < 0.01$.



Supplemental Figure 5. PAS does not affect macrophage responsiveness to c-di-AMP. WT and STING KO BMDMs were exposed for 3 h to biofilm-conditioned medium from WT LAC or Δ*gdpP* biofilms following the addition of exogenous c-di-AMP (1 µM). RNA was isolated and analyzed for changes in *ifn-β* expression by qRT-PCR. Gene expression levels were normalized to the housekeeping gene GAPDH and are presented as the fold-induction ($2^{-\Delta\Delta Ct}$) value relative to untreated BMDMs. Results depict averages from three independent experiments with standard deviation shown.