Persister formation in *Staphylococcus aureus* is associated with ATP depletion

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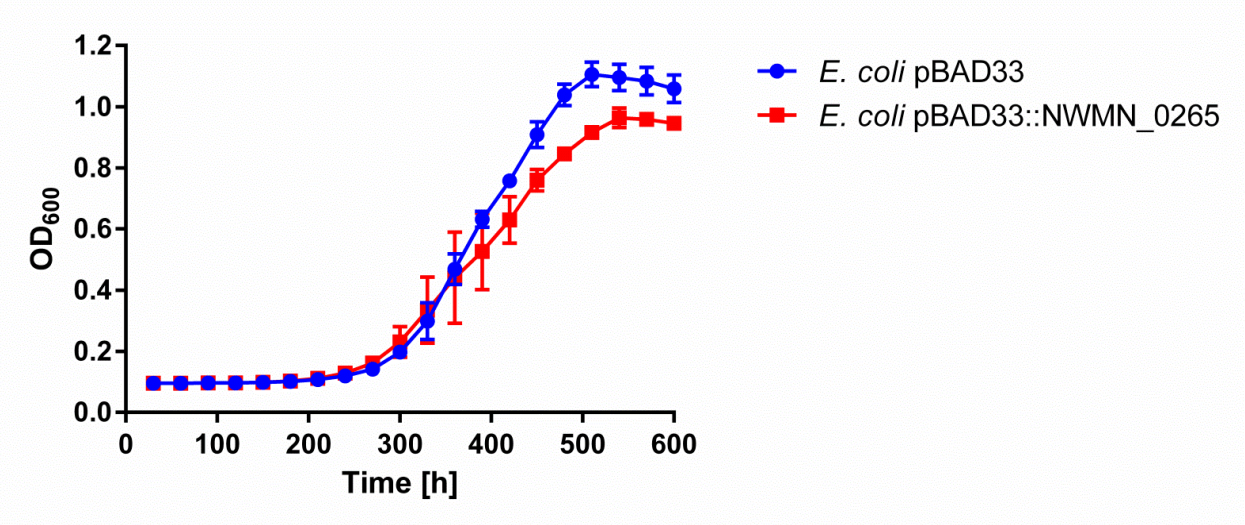
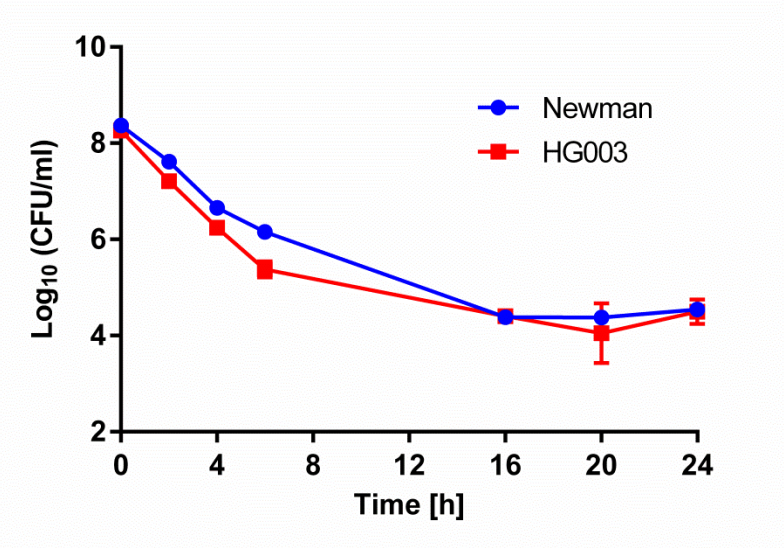
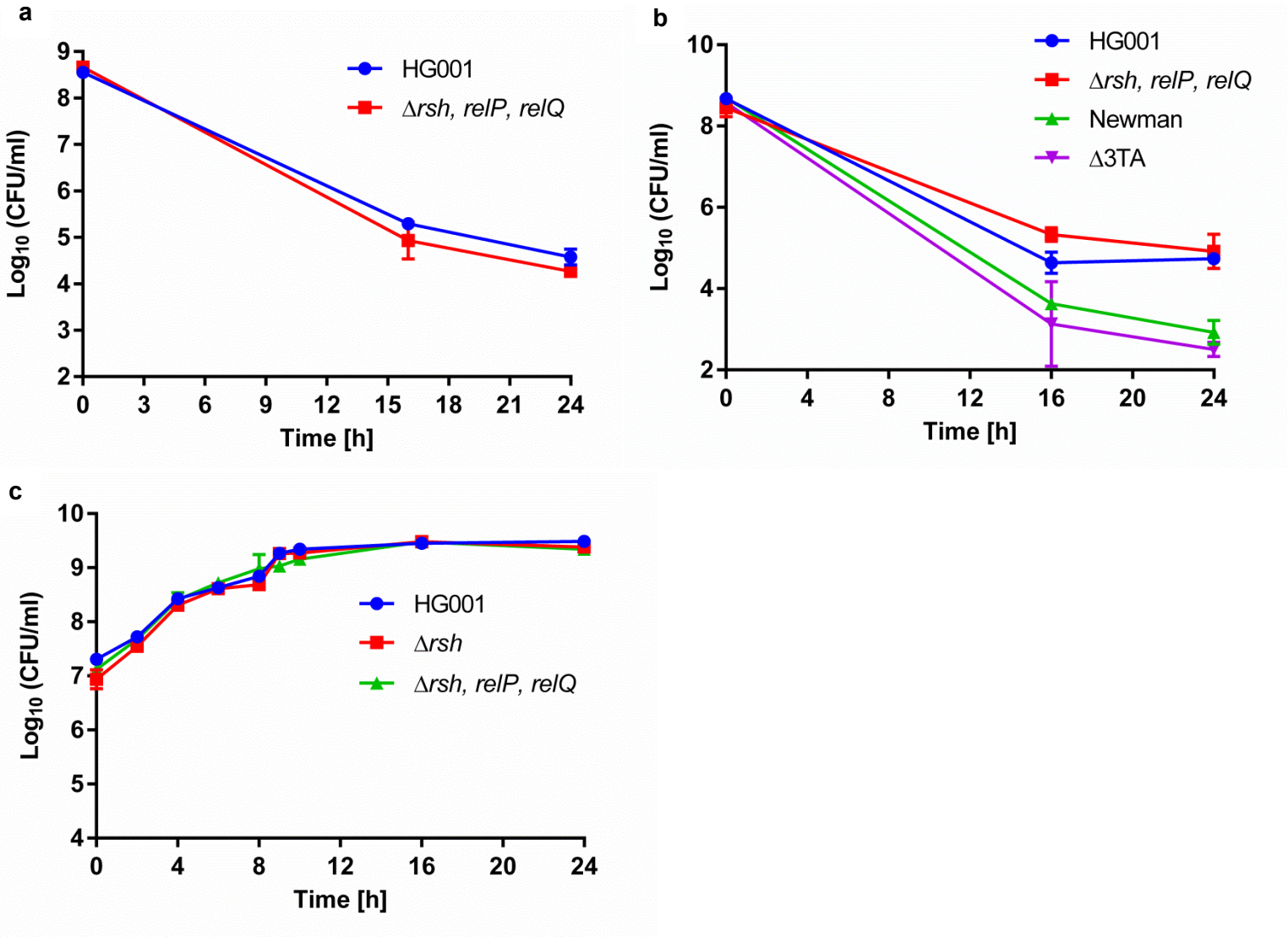
Supplementary Information



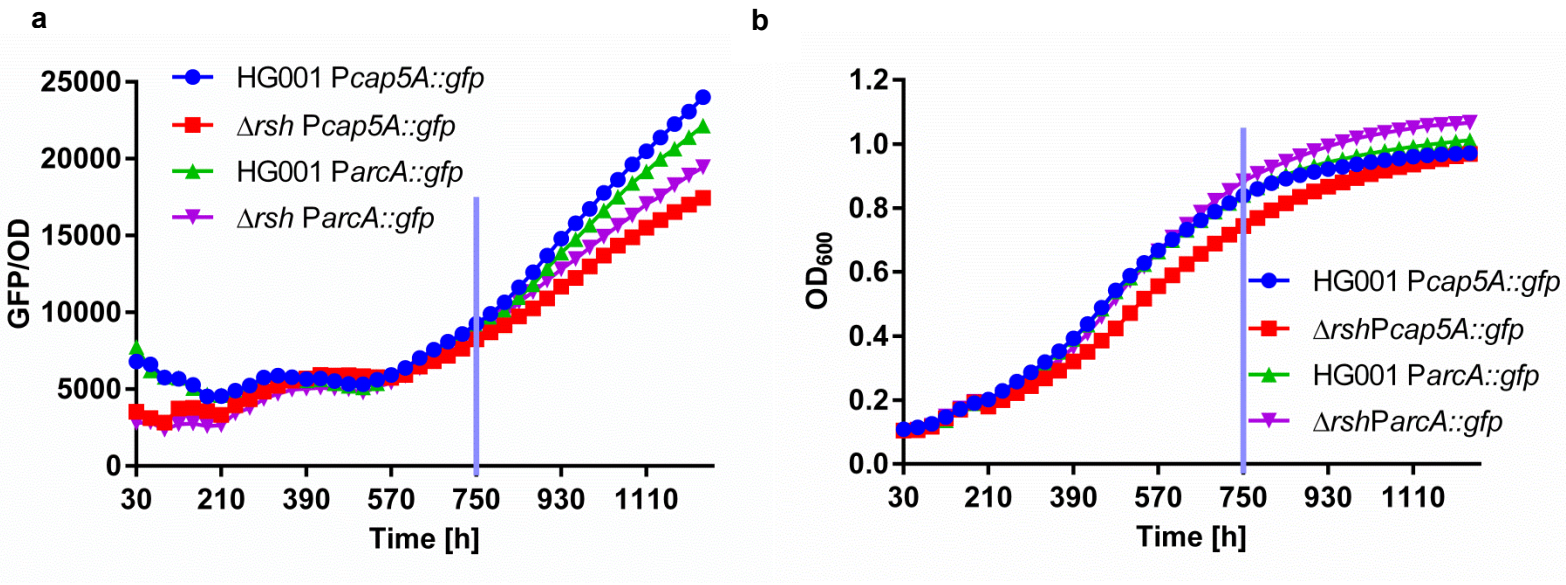
Supplementary Figure 1. Overexpression of the hypothetical phage associated toxin has no effect on growth of *E. coli*. The gene encoding the hypothetical toxin, *NWMN_0265* from *S. aureus* Newman was overexpressed *in E. coli* MG1655 in vector pBAD33. The toxin was cloned downstream of an arabinose inducible promoter and grown in the presence of 0.2% inducer and growth was compared to that of an empty vector control at 37°C in Luria Bertoni (LB) broth. Data represent biological triplicates. Error bars represent standard deviation.



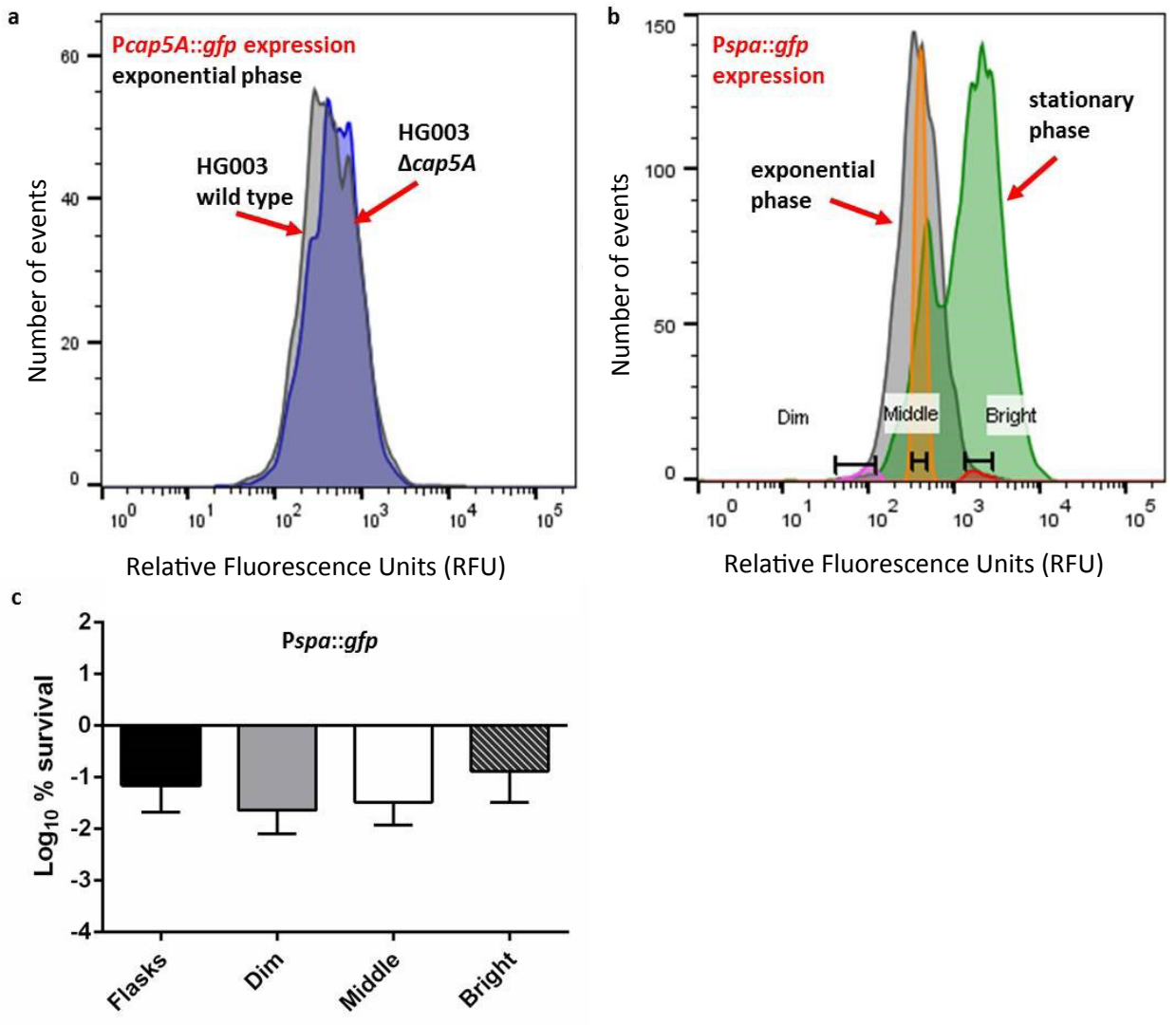
Supplementary Figure 2. Identification of biphasic killing and the persister plateau. HG003 and Newman were grown to mid-exponential phase and challenged with 4 µg/ml of ciprofloxacin. At regular intervals, an aliquot was removed and centrifuged, resuspended in PBS and aliquots of serial dilutions were plated on MH agar plates. Data is representative of 3 independent experiments and error bars represent standard deviation.



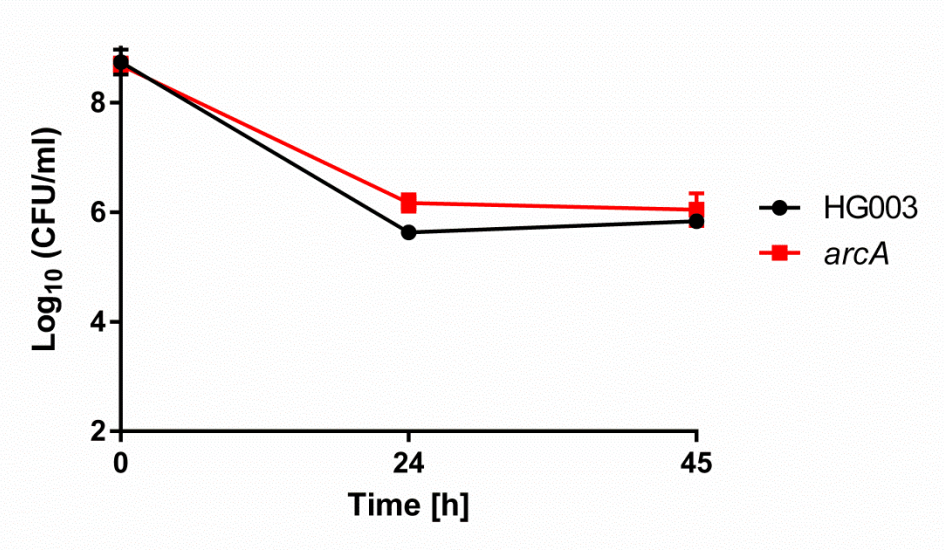
Supplementary Figure 3. Persisters and entrance into stationary phase in a ppGpp negative strain. a, Mutation of all 3 ppGpp synthases, *rsh*$_{syn}$, *relP* and *relQ* did not have an effect on survival in the presence of ciprofloxacin at 10 x MIC. **b**, Neither ppGpp (Δ*rsh*$_{syn}$, Δ*relP* and Δ*relQ*) nor toxin-antitoxin modules (Δ*mazEF*, Δ*axe1-txe1* and Δ*axe1-txe2*) have an effect on survival in the presence of gentamicin at 10 x MIC. **c**, Growth was not effected by mutation of ppGpp synthase, *rsh*$_{syn}$ or triple mutation of *rsh*$_{syn}$, *relP* and *relQ* in MHB at 37°C, aeration at 225 rpm. Data is an average of 3 biological replicates and error bars represent standard deviation.



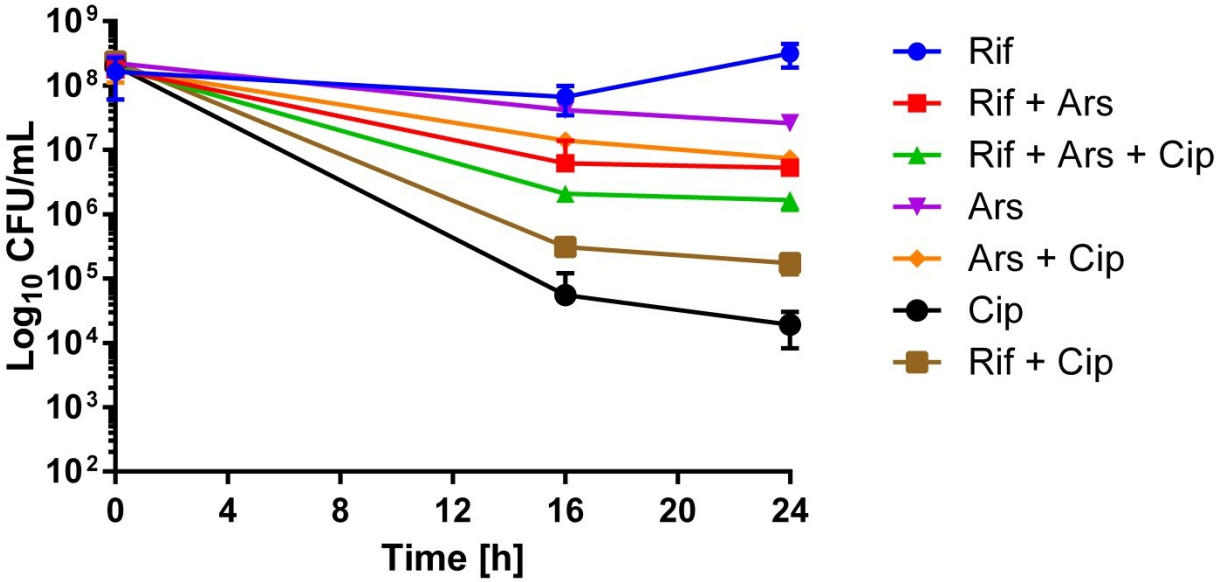
Supplementary Figure 4. Promoter activity in a *rsh*$_{syn}$ mutant background. a, Promoters of *cap5A* and *arcA* are specifically induced upon the onset of stationary phase with GFP expression increasing as **b,** growth ceases at the onset of stationary phase. Expression of P*cap5A* and P*arcA* is not affected by mutation of *rsh*$_{syn}$. The blue line represents an estimation of the entrance to stationary phase state. Data is an average of 3 biological replicates.



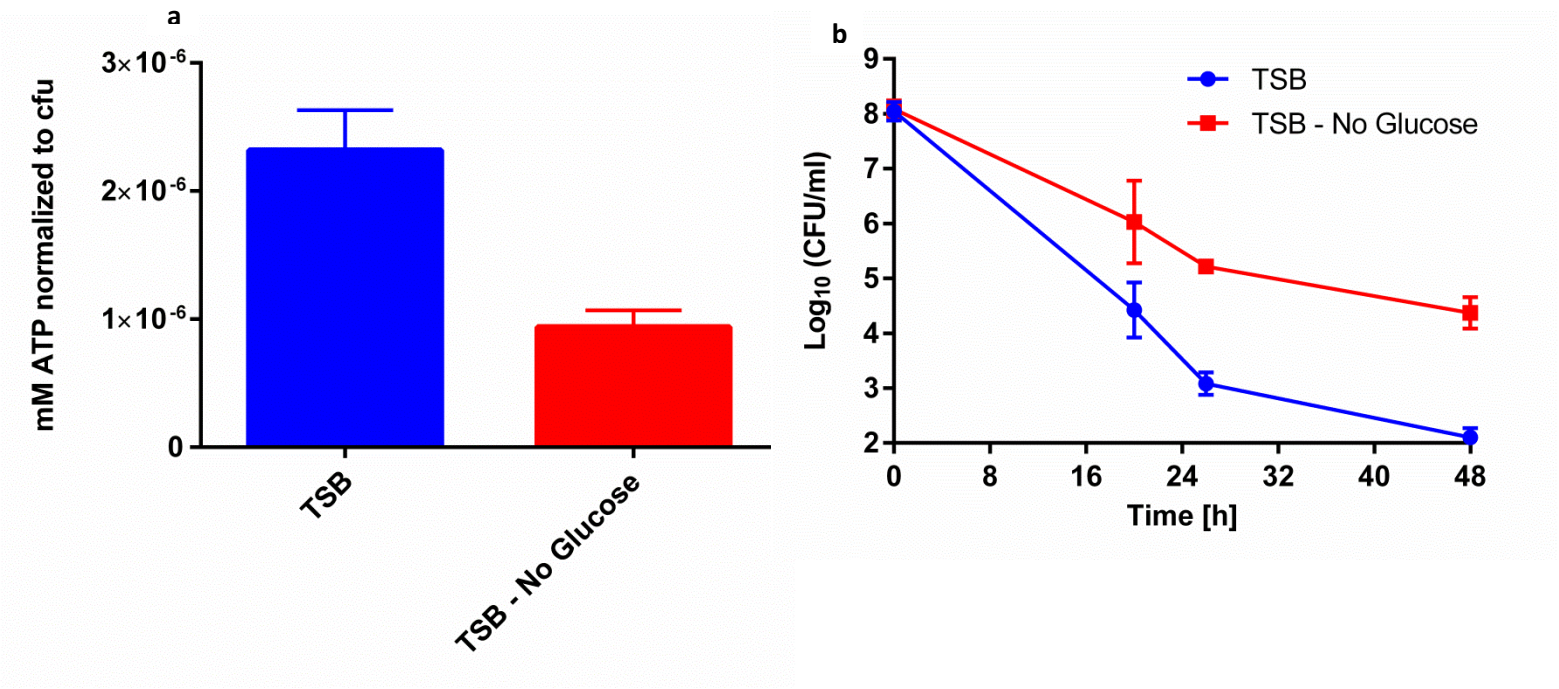
Supplementary Figure 5. P*cap5A* activity is not affected by mutation of *cap5A* and cells expressing P*spa::gfp* in exponential phase are not enriched for persisters. a, P*cap5A*::*gfp* expression in HG003 wild type (grey peak) and the *cap5A* mutant (blue peak). Strains were grown to mid-exponential phase and analyzed by FACS. **b,** *S. aureus* HG003 P*spa*::*gfp* expression in exponential phase following ciprofloxacin treatment (grey peak) and stationary phase (green peak) measured by FACS. Exponential phase cells treated with ciprofloxacin were gated to *dim* (purple peak), *middle* (orange peak) and *bright* (red peak) expression of GFP. **c,** Survivors from each population were sorted onto MHA plates and enumerated following incubation overnight at 37ºC. a and b are representative experiments. c is the average of 3 biological replicates and error bars represent standard deviation.



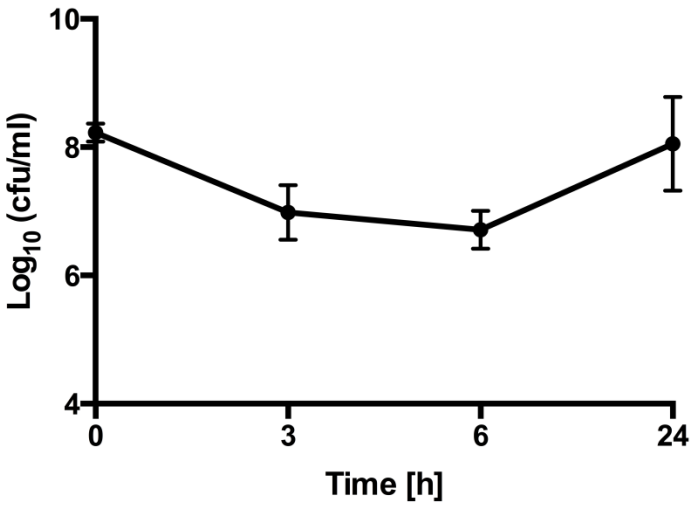
Supplementary Figure 6. Mutation of *arcA* does not have an impact on persister formation in *S. aureus* HG003. Wild-type and Δ*arcA* cells were grown to mid-exponential phase and challenged with 10 x MIC of ciprofloxacin. Cfus were recorded at 24 and 48 hours. Data is an average of 3 biological replicates and error bars represent standard deviation.



Supplementary Figure 7. Arsenate protects against killing by ciprofloxacin. Killing by ciprofloxacin (4.0 µg/ml) in the presence of rifampicin (0.01 µg/ml) and/or arsenic acid (1 mM). Rifampicin was added where indicated 15 minutes before the start of the experiment. Arsenate was added, where indicated 30 minutes before the start of the experiment. Ciprofloxacin was added where indicated at t = 0. Survivors were enumerated after 16 and 24h exposure. Data is averaged from 3 biological replicates and error bars represent standard deviation.



Supplementary Figure 8. **Increased ATP results in fewer persisters. a,** ATP levels were measured after 3 hours of growth in TSB and TSB without glucose. **b,** Survival of cells in TSB and TSB without glucose after treatment with ciprofloxacin at 10 x MIC. Results are the average of 3 biological replicates and error bars represent standard deviation.



Supplementary Figure 9. Rifampicin resistance emerges in exponential phase. *S. aureus* HG003 was grown to mid-exponential phase and rifampicin was added at t=0 to 10 x MIC (0.4 µg/ml). Cfus were counted at various timepoints. After an initial decline, the culture rebounded over 24 hours due to a high frequency of resistance. Results are an average of 3 biological replicates and error bars represent standard deviations.