

**Figure S1. CCCP induction of *IrgAB* promoter activity.** *S. aureus* UAMS-1 (panels A and B) and its *lytS* mutant derivative, KB999 (panels C and D), each containing the *Irg::gfp* promoter fusion plasmid, were grown to mid-exponential phase and treated with CCCP, a depolarizing agent previously shown to induce *IrgAB* transcription. GFP positive cells were visualized by confocal laser scanning microscopy as described in Materials and Methods. Scale bar represents 5  $\mu\text{m}$ .

**Figure S2. Analysis of *cid* and *Irg* expression during static biofilm growth.** *S. aureus* UAMS-1 cells containing: (A) *cid::gfp* promoter fusion in UAMS-1 [UAMS-1(pEM81)], (B) *ldh::gfp* promoter fusion in UAMS-1 [UAMS-1(pEM87)], (C) *cid::gfp* promoter fusion in the *cidR* mutant [KB1090(pEM81)], and (D) *Irg::gfp* promoter fusion in UAMS-1 [UAMS-1(pEM80)]. Three-dimensional images of the biofilms were generated using confocal laser scanning microscopy as described in the Materials and Methods. Images are representative of three independent experiments. Scale bar represents 40  $\mu\text{m}$ .

**Figure S3. Temporal analysis *ldh* expression during biofilm development.** *S. aureus* cells containing the *ldh::gfp* reporter plasmid were inoculated into a BioFlux microfluidics system and allowed to form a biofilm within a flow-shear environment at a flow rate of 64  $\mu\text{l/h}$  for a total of 18 h. Bright-field and epifluorescence microscopic images were collected at 5-min intervals using 200 $\times$  magnification. The images presented were taken from the complete set of 217 images (see supplementary video S1 for a video compilation of these images) spanning 12.5-18 h and illustrate typical tower development and GFP expression observed in multiple experiments. For complete video compilation of this experiment, see Video S2. Scale bar represents 50  $\mu\text{m}$ .