

Supplemental Figure 1. LIdR is not a major effector of *IIdA* **expression.***IIdA* promoter activity in liquid cultures of WT and $\Delta IIdR$ grown in MOPS medium containing 40 mM L-lactate. Values are representative of three biological replicates and standard deviation is shown.



Supplemental Figure 2. Activators and inhibitors of *IIdA* and *IIdPDE* expression identified by screening with plate PM-1 (Biolog, Inc.). Left: Schematic of constructs in the dual-reporter strain used in the screens. Conditions for activator and inhibitor screens are shown. Center and right: Raw fluorescence curves, growth curves, and chemical structures of compounds identified in the screen for small molecules affecting *IIdA* (center) or *IIdP* (right) promoter activity. Two lines are shown per condition, representing two independent experiments. Results for activators are plotted in green, those for inhibitors are plotted in red, and those for control wells are plotted in gray.



Supplemental Figure 3. Effect of various α-HB concentrations on *IIdA* **expression. Activity of the** *IIdA* **promoter at α-HB concentrations ranging from 10 \muM to 30 mM (pink data points), with the L-lactate titration from Figure 1C provided for comparison (yellow data points). Cultures of the P_{***IIdA***}-***Iux* **reporter strain were grown shaking in a 96-well plate at 37°C for 24 hours in a base medium of MOPS containing 20 mM succinate. (A) Each value shown represents the maximum luminescence produced during growth in the indicated condition. (B) Values from A were normalized to the maximum luminescence value produced in the most-stimulatory concentration. Values shown for each concentration are averages of two biological replicates and error bars represent standard deviation.**



Supplemental Figure 4. Liquid culture growth is limited in MOPS medium without added ferrous sulfate. (A) Growth (optical density at 500 nm) of WT in liquid MOPS medium containing 40 mM L-lactate and either 0 or 3.5 μ M of added iron. (B) *IIdA* (top) and *IIdP* (bottom) promoter activity in liquid cultures of WT grown in MOPS medium containing 40 mM L-lactate with ferrous sulfate added (3.5 μ M) or no iron added (0 μ M). Values are representative of three biological replicates and standard deviation is shown. Arrows indicate the time point represented in Figure 4C.



Supplemental Figure 5. *IIdA* expression is unaffected by the addition of increased ferrous sulfate to the medium. *IIdA* promoter activity in liquid cultures of WT grown in MOPS medium containing 40 mM L-lactate and either 3.5 or 10 μ M of added iron. Values are representative of three biological replicates and standard deviation is shown.



Supplemental Figure 6. Spatial patterning of fluorescence in biofilms of the dual-reporter strain is recapitulated in mixed biofilms containing single-reporter strains. (A) Fluorescence images of a thin-section from a biofilm inoculated using an equal mixture of the P_{IIdP} -gfp and P_{IIdA} -mScarlet reporter strains. *mScarlet* fluorescence is shown in yellow and gfp fluorescence is shown in cyan. (B) Fluorescence images of a thin-section from a biofilm inoculated using an equal mixture of P_{IIdP} -mScarlet and P_{IIdA} -gfp reporter strains. *mScarlet* fluorescence is shown in cyan and gfp fluorescence is shown in yellow. Biofilms were grown on MOPS medium containing 20 mM succinate and 10 mM L-lactate. Images are representative of three biological replicates.



Supplemental Figure 7. Expression of the *glcDEFG* **operon is induced by added glycolate.** *glcD* promoter activity in liquid cultures of WT grown in MOPS medium containing 20 mM succinate (blue data points) or the same medium with glycolate added at 10 mM (green data points). Values are representative of three biological replicates and standard deviation is shown.



Supplemental Figure 8. Intracellular burden of P. aeruginosa complemented strains in macrophages. Recovered CFUs of *P. aeruginosa* WT and indicated complemented strains in RAW264.7 macrophages 3 hours post-infection and subjected to the gentamicin protection assay. Each dot represents one replicate and error bars represent standard deviation, *p<0.05, ns = not significant.