Supplemental Figures

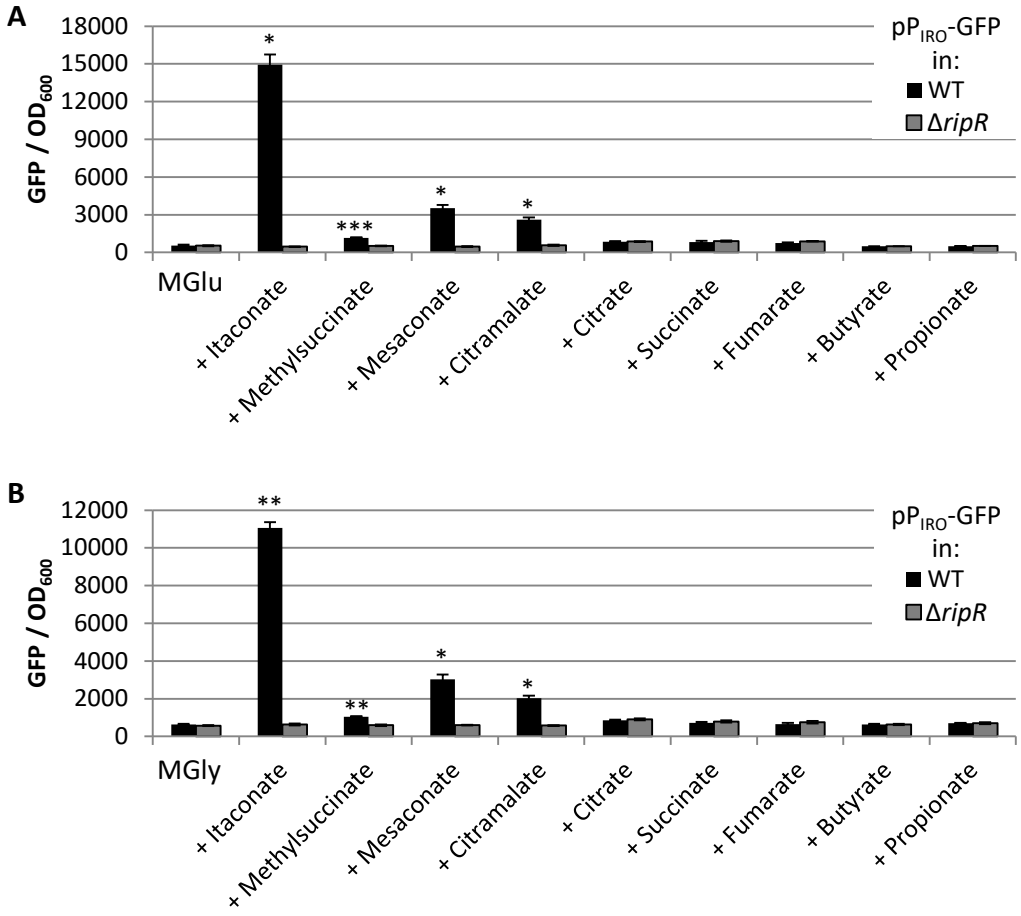

Figure S1: Itaconate induction of P_{IRO} occurs in minimal media. Expression of P_{IRO}-sfGFP in wild-type (WT) or *ripR* knockout (Δ*ripR*) *Salmonella* as in Figure 1 except grown in MOPS minimal media with 0.2% Glucose (**A**; MGlu) or Glycerol (**B**; MGly) instead of LB. Data show GFP fluorescence normalized to OD_{600} after 16h of growth and are the average of at least three biological replicates. Error bars show one standard deviation. pP_{IRO}, plasmid-borne transcriptional fusion of P_{IRO} to sfGFP. A Games-Howell ANOVA was conducted comparing WT to Δ*ripR* for each added metabolite. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

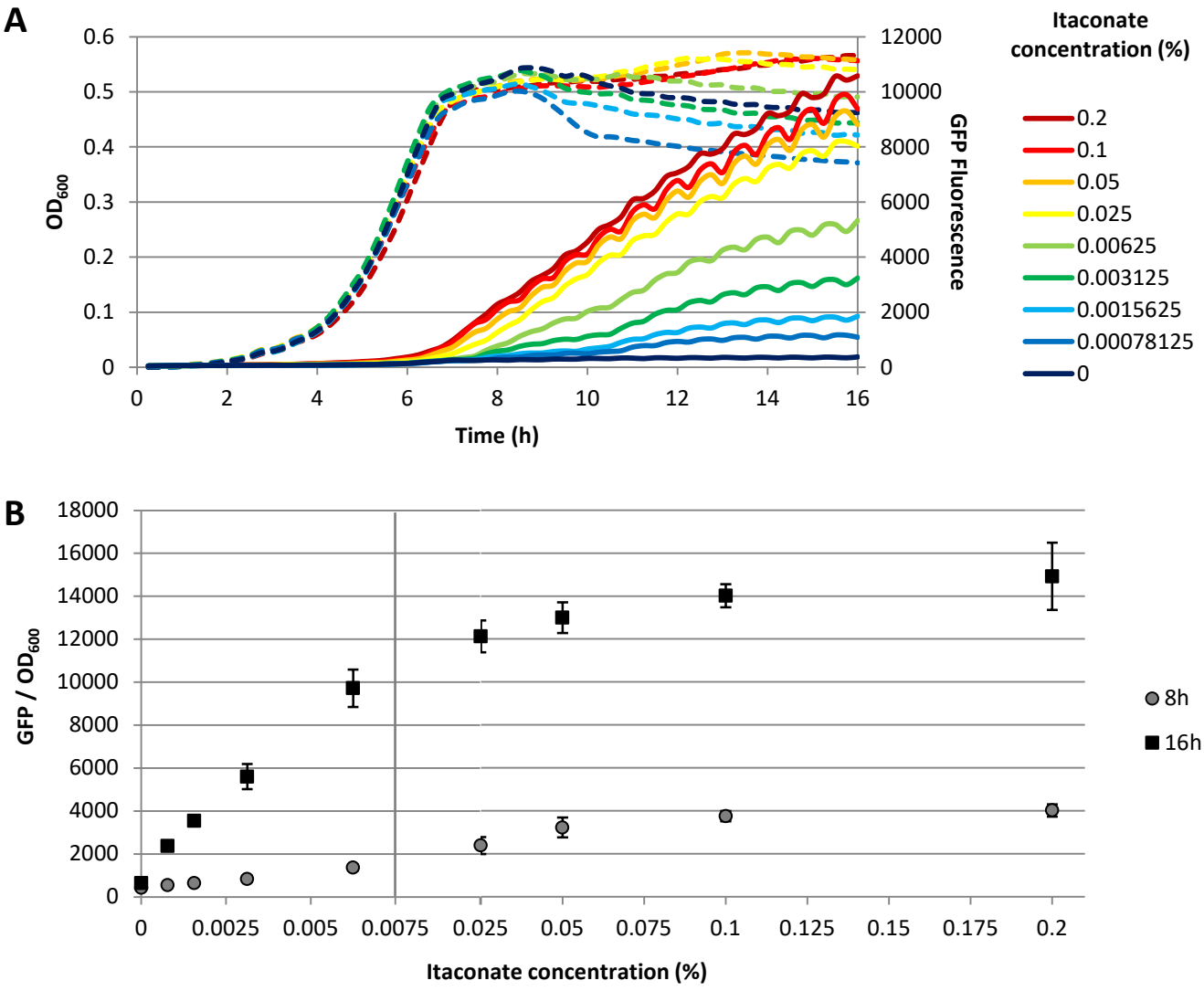

Figure S2: P_{IRO} is induced by itaconate in a dose-dependent manner. A) Example replicate showing *Salmonella* optical density (dashed lines) and P_{IRO}-sfGFP fluorescence (solid lines) for a range of itaconate concentrations across 16h of growth. All conditions were in MOPS minimal media with 0.2% glucose as a carbon source and indicated concentration of itaconate (%, g/100ml). **B)** Average P_{IRO}-sfGFP fluorescence (normalized to OD_{600}) at 8h or 16h across two biological replicates. Error bars indicate one standard deviation. The x-axis changes scale at 0.0075% to show lower concentrations more clearly.

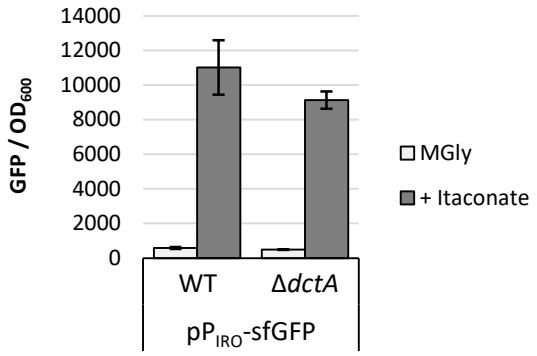

Figure S3: DctA is not required for the response to itaconate. Expression of P_{IRO}-sfGFP in wild-type (WT) or *dctA* knockout (Δ*dctA*) *Salmonella*. Figure shows GFP fluorescence normalized to optical density at 600nm (OD_{600}) after 16h of growth in MOPS minimal media with 0.2% glycerol as the carbon source. Data are the average of at least two biological replicates and error bars show one standard deviation. pP_{IRO}-sfGFP*,* plasmid-borne transcriptional fusion of P_{IRO} to sfGFP.

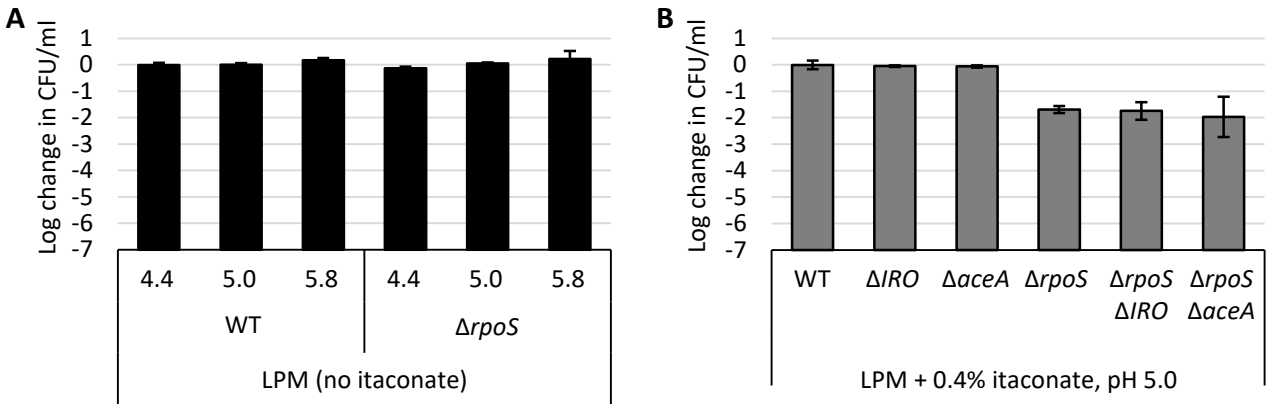

Figure S4: pH 4.4 without itaconate was not bactericidal. A) Survival (relative to 0h time point) of wild-type (WT) or *rpoS* mutant *Salmonella* after 3h in LPM media adjusted to pH 4.4, 5.0, or 5.8 as indicated. **B)** Survival of *Salmonella aceA* and itaconate response operon (IRO) mutants in LPM + itaconate media at pH 5.0. All data are the average of at least three biological replicates and error bars show one standard deviation.

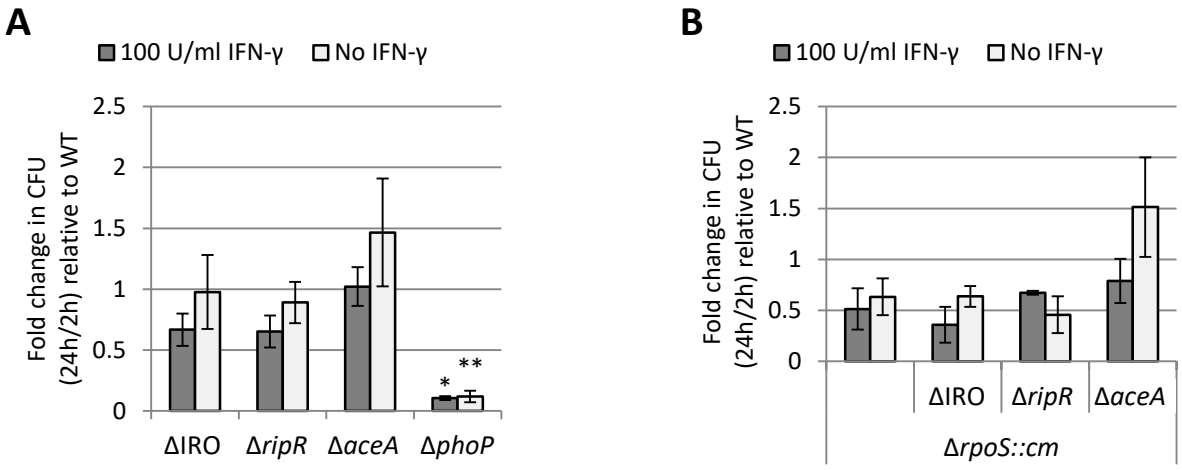

Figure S5: Survival of *Salmonella* mutants in J774 macrophage with and without IFN-γ stimulation relative to wild-type. The fold change in CFU recovered (24h relative to 2h post-infection) is shown and data from each replicate was normalized to the 24h/2h ratio obtained for wild-type *Salmonella*. Columns show the average of three biological replicates and error bars show one standard error of the mean. Since samples were normalized to wild-type in each replicate, a one-way ANOVA with Sidak's multiple comparison test was conducted comparing each strain to the Δ*aceA* strain (A) or to the Δ*rpoS* strain (B). Only the Δ*phoP* mutant was found to have a $p < 0.05$. *, $p<0.05$; **, $p < 0.01$.

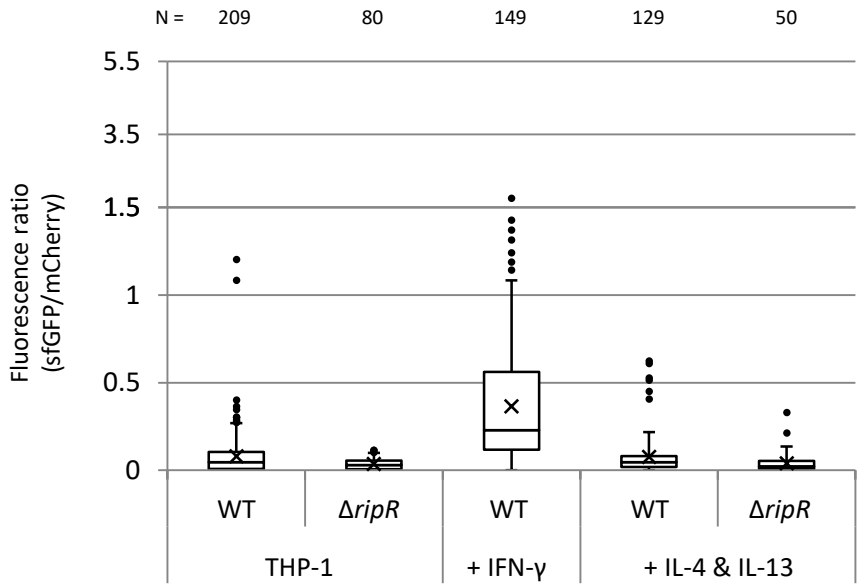

Figure S6: P_{IRO} shows little-to-no activation in THP-1 macrophage stimulated with IL-4 and IL-13. As in Figure 4, expression of P_{IRO}-sfGFP in intracellular wild-type (WT) or *ripR* knockout (Δ*ripR*) *Salmonella* containing the pICM-P_{IRO} plasmid (constitutive mCherry expression). THP-1 macrophage were pre-treated with no stimulant, human IFN-γ, or IL-4 and IL-13 and infected with *Salmonella* for 8h. Data for WT *Salmonella* in THP-1 with no treatment or IFN-γ are shown in Figure 4 and are shown again here for comparison. As in Figure 4, data shows relative fluorescence quantification of individual bacterial particles. Number of bacteria quantified is indicated above and totalled from at least three biological replicates. Boxes indicate first and third quartiles; central line, median; X, mean; whiskers, 95th percentile; dots, all non-zero data points outside 95th percentile. The y-axis changes scale at 1.5 to better show outliers.