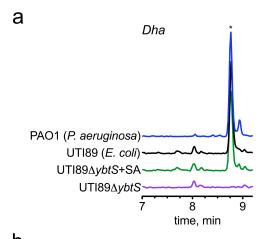
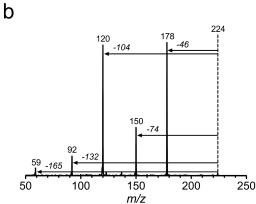
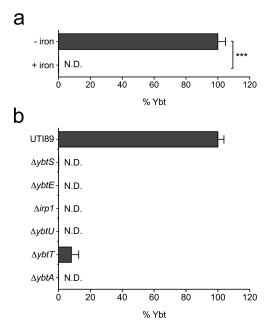
#### SUPPLEMENTAL FIGURES





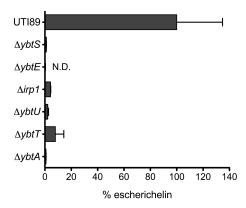
# Supplemental Figure 1. The molecular feature with m/z 224 is dihydroaeruginoic acid (Dha)

(a) UTI89 $\Delta ybtS$ , and P. aeruginosa strain PAO1 were grown under iron-restricted conditions and the supernatants were analyzed by positive ion LC-MS. Extracted ion chromatograms show that the molecular feature with m/z 224 found in UTI89 co-elutes with Dha from a pyochelin-producing strain of P. aeruginosa. It is absent when the salicylate synthase (YbtS) is deleted from the genome and restored when UTI89 $\Delta ybtS$  is chemically complemented with salicylate in the medium. (b) MS/MS spectrum of the molecular feature with m/z 224 in PAO1 supernatants, which is consistent with fragments observed in a previous study of Dha (31).



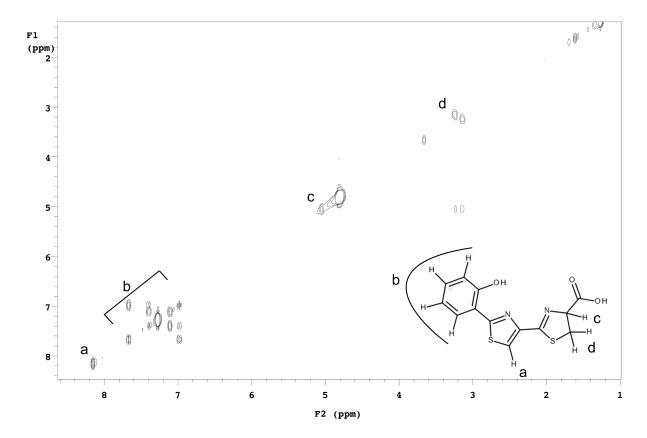
# Supplemental Figure 2. Extracellular Ybt levels are repressed in the presence of iron and abolished in strains lacking biosynthetic genes and the transcription factor YbtA

Fe(III)-Ybt levels in culture supernatants were quantified by LC-MS/MS relative to a  $^{13}$ C-internal standard. (a) UTI89 grown in complete M63 medium with or without 16.2 mg/L FeCl<sub>3</sub>. Mean of n = 3 replicates with standard deviation plotted, unpaired t-test \*\*\*p < 0.001. (b) Isogenic mutants of UTI89 grown in iron restricted minimal medium. Mean of n = 4 replicates with standard deviation plotted.



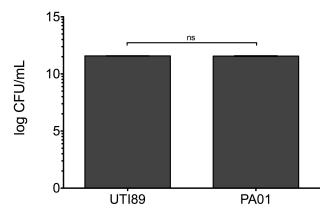
# Supplemental Figure 3. Intracellular escherichelin levels mimic the extracellular escherichelin levels in *Yersinia* HPI mutants

Escherichelin levels in cellular extracts were quantified by LC-MS/MS relative to a  $^{13}$ C-escherichelin internal standard. Mean of n = 3 replicates with standard deviation plotted.



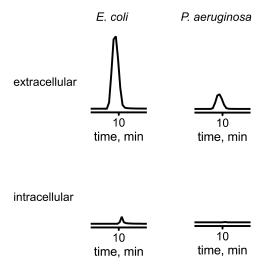
**Supplemental Figure 4. Characterization of purified escherichelin by** <sup>1</sup>**H-NMR** <sup>1</sup>**H-COSY of purified escherichelin and the assignments of the proton chemical shifts on the escherichelin structure (inset).** 

### Supplemental Figure 5. Density functional theory (DFT) calculation of escherichelin bound to Cu(II) The simulation predicts a stable complex in solution with square planar geometry.



Supplemental Figure 6. *E. coli* UTI89 and *P. aeruginosa* PA01 grow equivalently in iron-restricted medium

UTI89 and PA01 were grown for 18 hours in complete M63 medium supplemented with 1% casamino acids and bacterial growth was assessed by quantifying colony forming units per mL (CFU/mL). Mean of n = 3 with standard deviation plotted. p = 0.56, unpaired t-test.



## Supplemental Figure 7. Intracellular escherichelin levels mimic the extracellular escherichelin levels in *E. coli* and *P. aeruginosa*

LC-MS/MS chromatograms are displayed for extracellular escherichelin (top) and intracellular escherichelin (bottom) from *E. coli* (UTI89, left) and *P. aeruginosa* (PA01, right) supernatants after growth in iron-restricted medium. Chromatograms are identically scaled. The extracellular escherichelin data is reproduced for comparison from Figure 4.