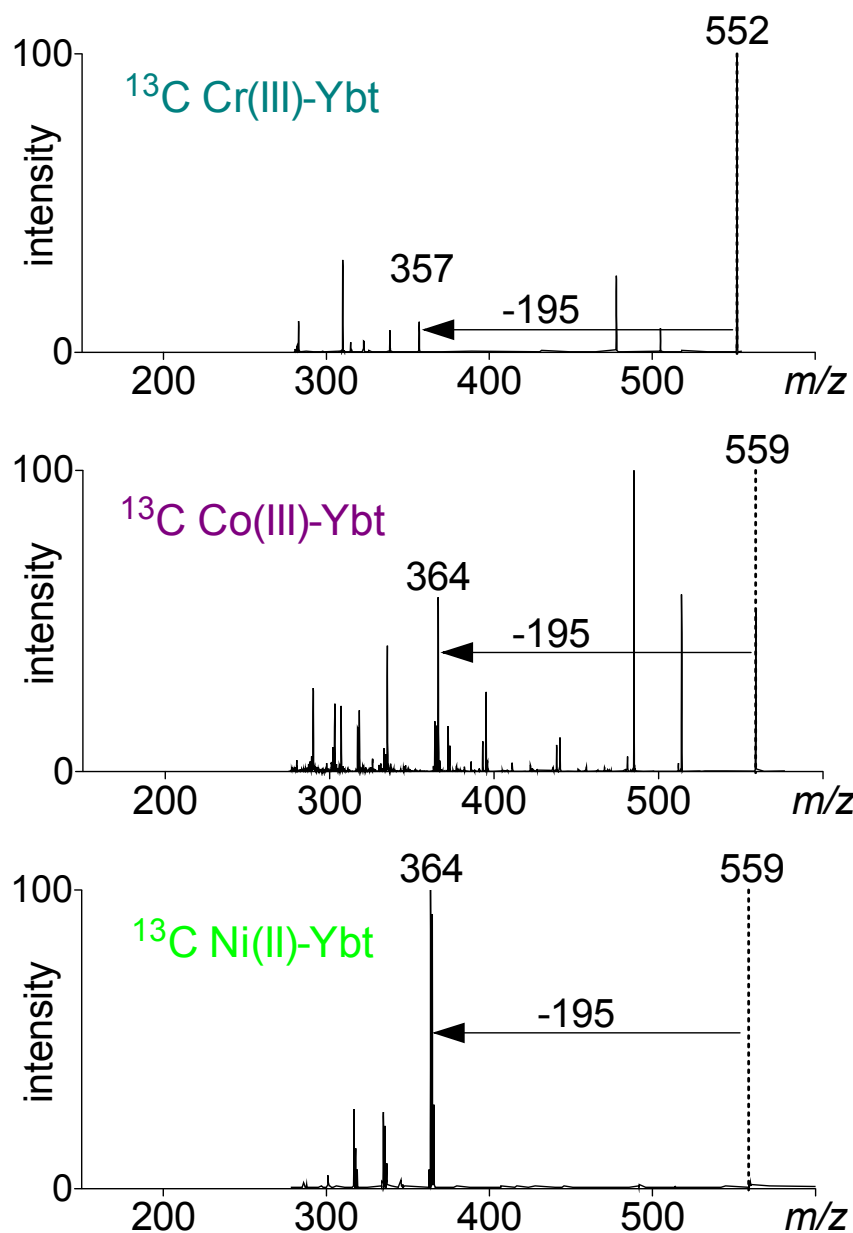


1

2 **Supplementary Figure 1. Fe(III)-Ybt and apo-Ybt levels do not decrease in presence of**
3 **excess zinc ions.** HPLC-purified Fe(III)-Ybt and apo-Ybt were separately incubated with
4 excess zinc ions. Samples were eluted on C18 silica columns to remove unbound zinc ions and
5 ¹³C-labeled Fe(III)-Ybt was added just prior to elution to normalize for column retention. Fe(III)-
6 Ybt and apo-Ybt levels were measured using MRM detection method. Relative levels were
7 calculated using MRM peak areas normalized with ¹³C-labeled internal standard. **(A)** Fe(III)-Ybt
8 and **(B)** apo-Ybt levels did not change despite mixing with zinc ions in molar excess. Results are
9 shown as mean ± s.d.; *n*=3.

10



11

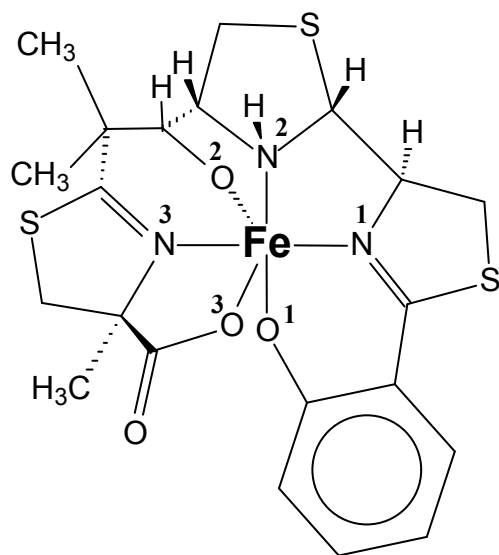
12 **Supplementary Figure 2. Structural confirmation of metal-Ybt complexes by stable**

13 **isotope labeling.** MS/MS of new ^{13}C -labeled metal-Ybt complexes revealed a shifted dominant

14 MS/MS neutral loss of 195 mass units, corresponding to loss of a fragment containing eight

15 carbons as previously observed for Fe(III)-Ybt and Cu(II)-Ybt.

Neutral Ferric Yersiniabactin Complex, calculated



Bond	Bond Lengths	
	Calculated	Crystal Structure(A)
Fe-O(1)	1.913	1.920
Fe-O(2)	1.902	1.899
Fe-O(3)	2.007	2.078
Fe-N(1)	2.084	2.084
Fe-N(2)	2.322	2.255
Fe-N(3)	2.164	2.130

Mean absolute deviation: 0.030

RMS deviation: 0.042

In Angstroms

16

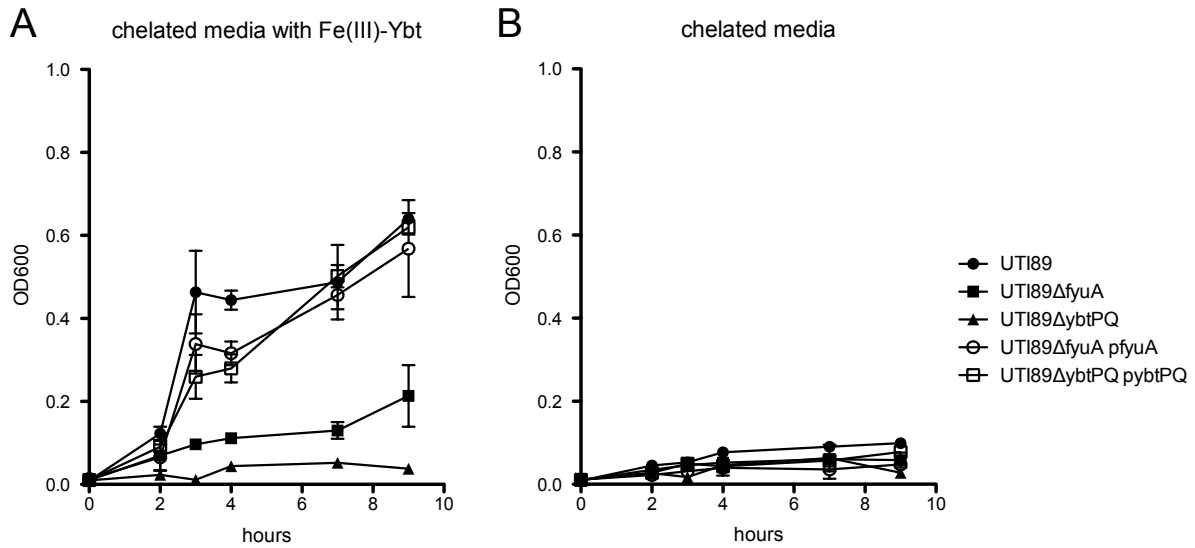
17 **Supplementary Figure 3. Comparison of neutral Fe(III)-Ybt complexes.** Figure and Table

18 show comparison of ligand bond lengths to the ferric ion between as calculated (**Theoretical**

19 **Calculations** under **Materials and Methods**) to the published crystal structure²⁰ (structure A of

20 four in unit cell) of neutral Fe(III)-Ybt.

21



22

23 **Supplementary Figure 4. FyuA and YbtPQ are necessary for Fe(III)-Ybt-dependent growth**

24 **in UPEC. (A)** Fe(III)-Ybt-dependent growth is limited in UTI89 mutants lacking FyuA and YbtPQ.

25 In this condition, Fe(III)-Ybt is added to a rich media in which bioavailable ferric ions are

26 chelated with EDDHA. Plasmid-complemented mutants show restored growth with exogenous

27 Fe(III)-Ybt. **(B)** UTI89 strains are indistinguishable in the absence of Fe(III)-Ybt in chelated

28 media.

29