Supporting Information

Cupric yersiniabactin is a virulence-associated superoxide dismutase mimic.

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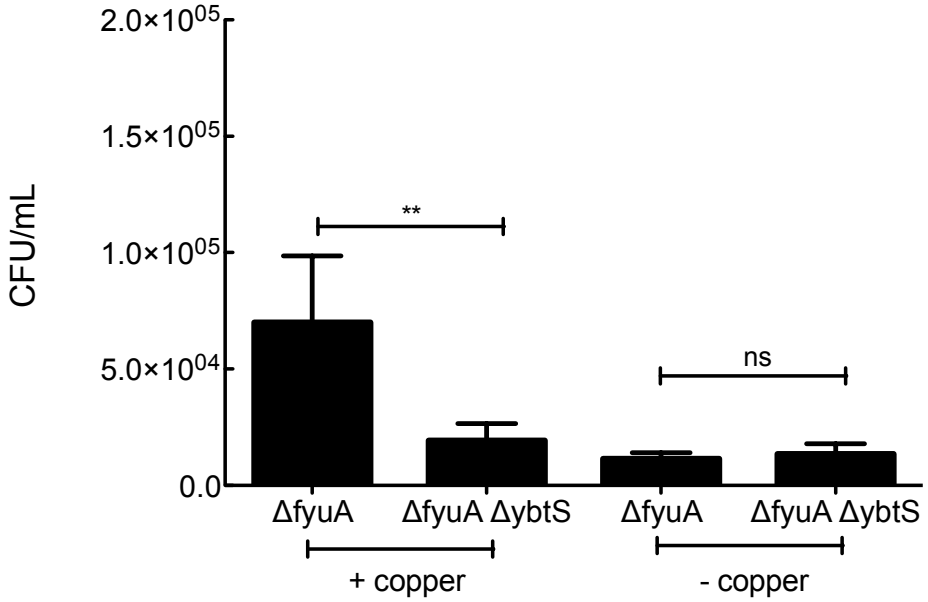
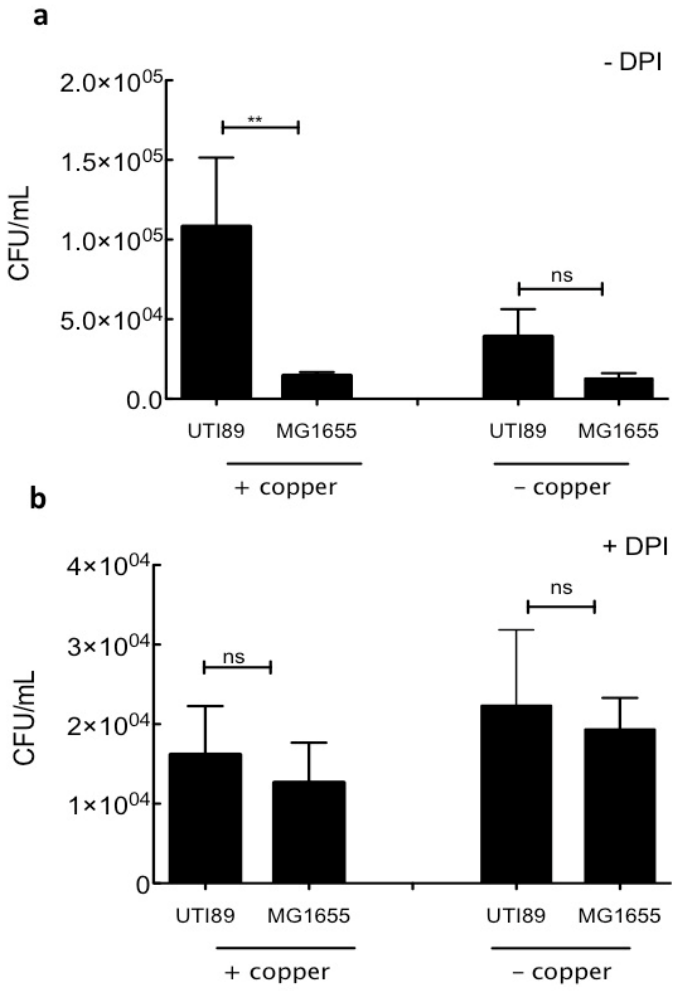
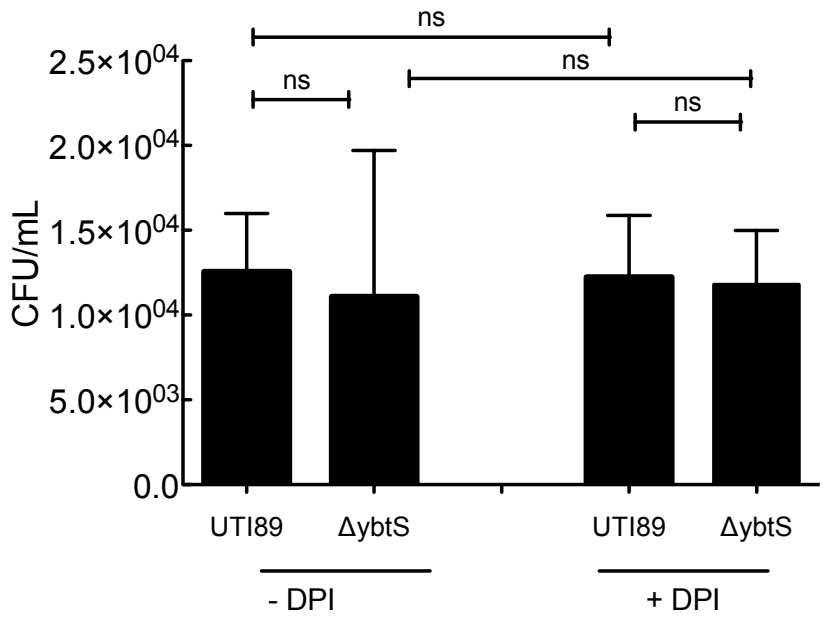
Supplementary Figures



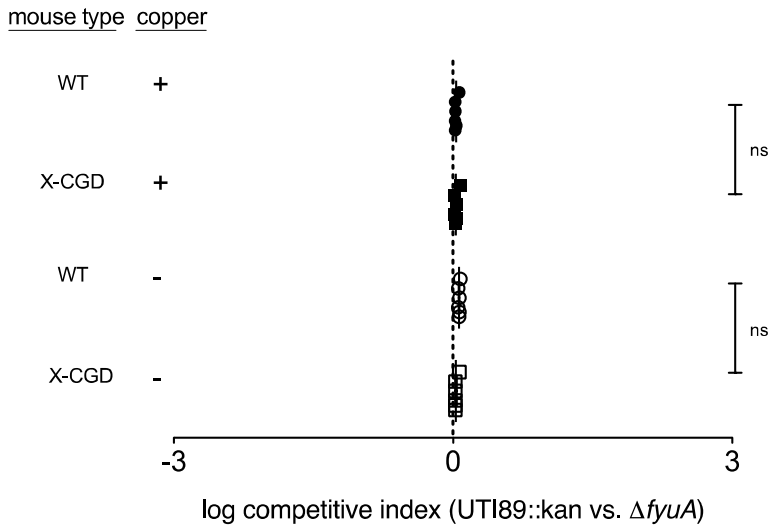
Supplementary Figure S1. Ybt import is not required for uropathogen survival within copper-replete RAW264.7 macrophages. RAW264.7 macrophages were infected with the uropathogen UTI89, and isogenic Δ*fyuA* or Δ*fyuA*Δ*ybtS* mutants following supplementation with or without 20 μM copper sulfate. Intracellular bacterial survival after 1 hour was expressed as a difference from initial internalized *E. coli*. Similar to UTI89, the yersiniabactin expressing mutant Δ*fyuA* was significantly (p=0.001, t-test) more viable than the isogenic, yersiniabactin-deficient Δ*fyuA*Δ*ybtS* mutant.



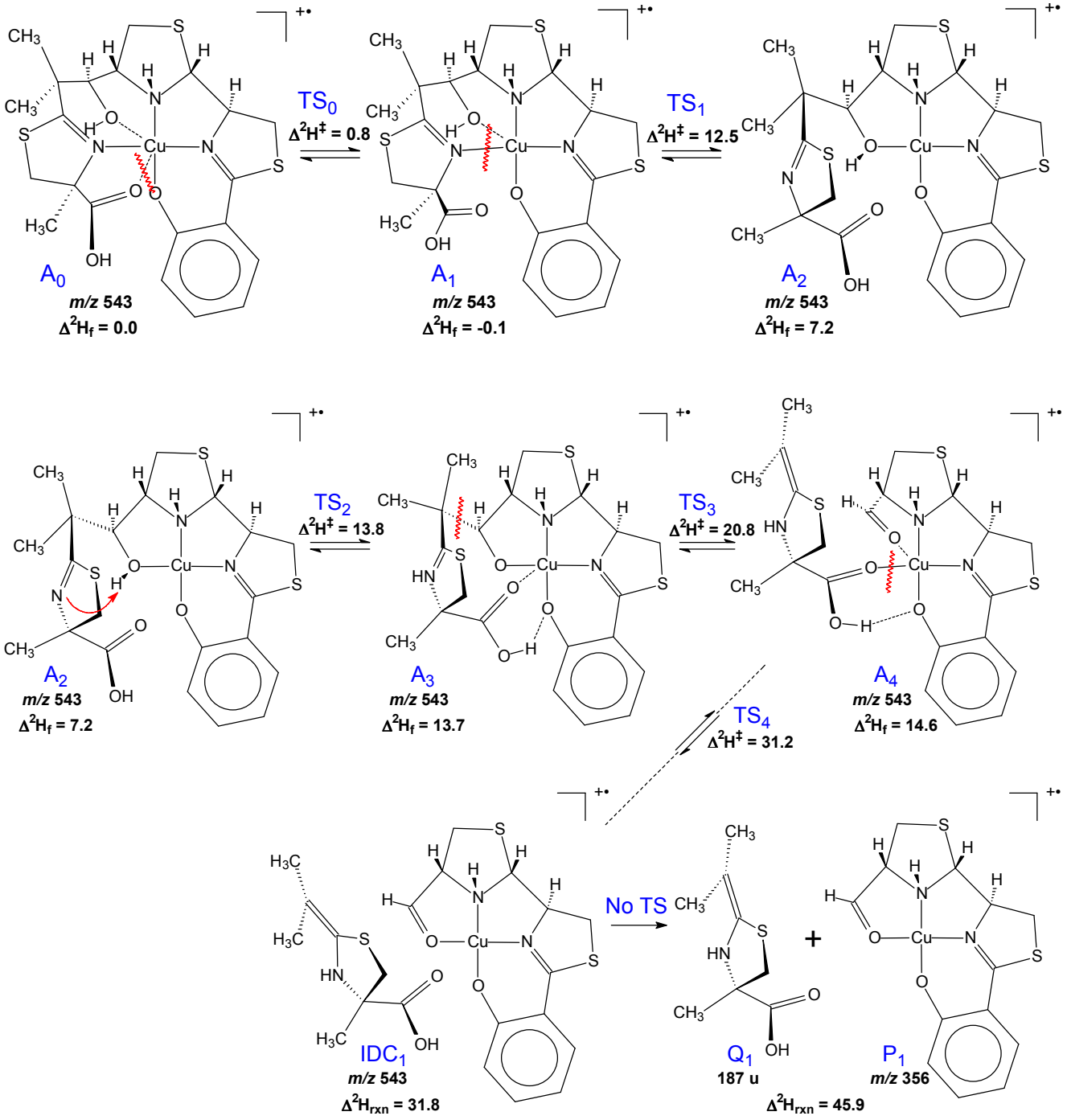
Supplementary Figure S2. The commensal strain MG1655 cannot modulate copper-dependent bactericidal activity of RAW 264.7 cells. RAW264.7 macrophages were treated with 20 μM diphenyleneiodonium chloride (DPI) in the presence or absence of 20 μm copper prior to exposure to *E. coli*. Survival of MG1655 one hour post incubation in RAW264.7 cells is indicated. The survival phenotype is not observed for the commensal strain, suggesting that Ybt expression predominantly contributes to the survival phenotype observed in UTI89. Both UTI89 and MG1655 are equally susceptible to the bactericidal activity of DPI treated RAW264.7 cells regardless of copper treatment. These results were combined from four independent experiments.



Supplementary Figure S3. Ybt expression does not promote pathogen survival in RAW264.7 cells without copper treatment. RAW264.7 macrophages were infected for one hour in the presence or absence of 20 μM DPI without copper supplementation. DPI treatment did not restore Cu(II)-Ybt dependent survival advantage in the absence of copper. These results were combined from four independent experiments.

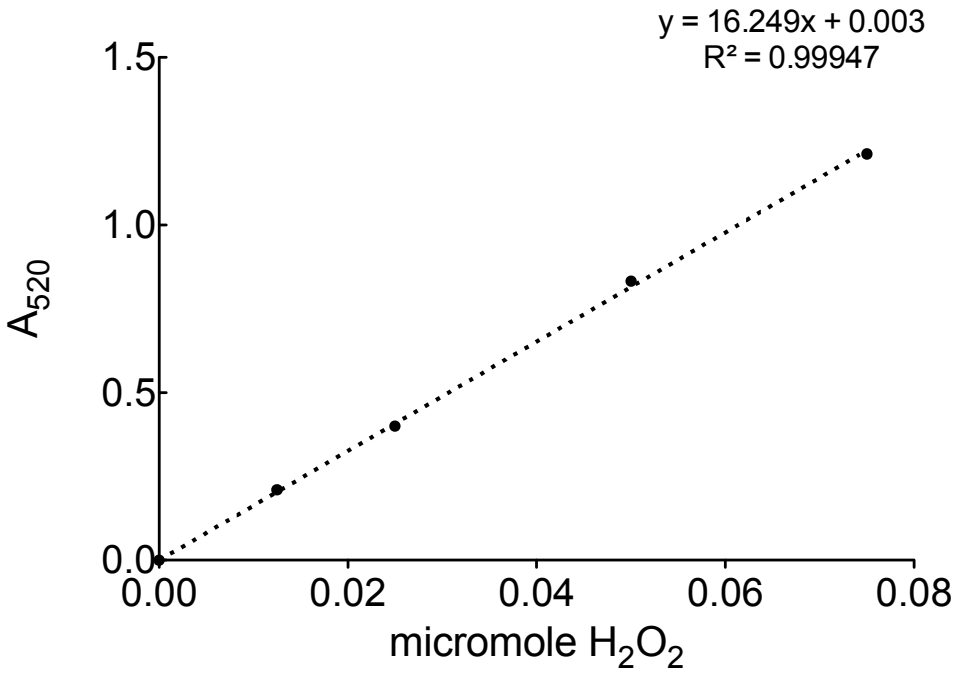


Supplemental Figure S4. Ybt synthesis, not import, contributes to UTI89 survival advantage in phagocytes. Survival of UTI89Δ*ybtS* and UTI89Δ*fyuA* following competitive co-infection with wild type UTI89 was assessed in resident peritoneal macrophages from wild type and X-CGD (gp91$^{phox-/-}$) mice. CIs determined using the yersiniabactin uptake mutant UTI89Δ*fyuA* revealed CIs of zero with both cell types under all conditions, indicating that yersiniabactin biosynthesis, not uptake, primarily contribute to the UTI89Δ*ybtS* survival phenotype.



Supplemental Figure S5. Proposed CID fragmentation mechanism. Ybt fragmentation pattern is consistent with the rearrangement of the C13-C14 bond and loss of the third, carboxylated thiazoline

ring. Cu(II)-Ybt complexes feature a core that is essentially square planar comprised of lone pairs from the salicylate O (ring 1) and from the N of rings 2 and 3 and another pair from ring 4 N or secondary OH. In addition to the square-planar core, there are long-bonded and interactions in some species with the Cu(II) ion; these bonds along with hydrogen bonds are shown by undirected dashes. (Relative calculated enthalpies of minima and transition states are given in kcal/mol.)



Supplementary Figure S6. Calibration curve for the determination of hydrogen peroxide concentrations. Equation of the line and regression from linearity are indicated.

Table 1: UTI89 mutant strains used in this study

strain	gene function	Reference
UTI89	Wild type *E. coli*	(*1*)
UTI89::kan	kanamycin resistance	This paper
UTI89Δ*ybtS*	salicylate synthase, yersiniabactin biosynthesis	(*2*)
UTI89Δ*fyuA*	yersiniabactin transport	(*2*)
UTI89Δ*fyuA* Δ*ybtS*	salicylate synthase, yersiniabactin biosynthesis, yersiniabactin transport	(*2*)
UTI89Δ*entB*	isochorismate lyase, catecholate siderophore (enterobactin/salmochelin) biosynthesis	(*2*)

References

1. Hunstad, D. A., Justice, S. S., Hung, C. S., Lauer, S. R., and Hultgren, S. J. (2005) Suppression of bladder epithelial cytokine responses by uropathogenic Escherichia coli, *Infect Immun 73*, 3999-4006.
2. Chaturvedi, K. S., Hung, C. S., Crowley, J. R., Stapleton, A. E., and Henderson, J. P. (2012) The siderophore yersiniabactin binds copper to protect pathogens during infection, *Nat Chem Biol*.