SUPPLEMENTAL DATA

Leveraging Copper Import by Yersiniabactin Siderophore System for Targeted PET Imaging of Bacteria

Nabil A. Siddiqui¹, Hailey A. Houson², Nitin S. Kamble¹, Jose R. Blanco², Robert E. O'Donnell³, Daniel J. Hassett⁴, Suzanne E. Lapi² & Nalinikanth Kotagiri^{1*}

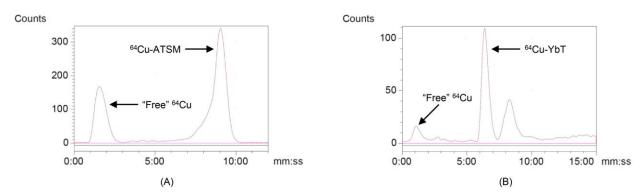
¹Division of Pharmaceutical Sciences, James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, Ohio, USA

²Division of Advanced Medical Imaging Research, Department of Radiology and Chemistry, University of Alabama at Birmingham, Birmingham, Alabama, USA

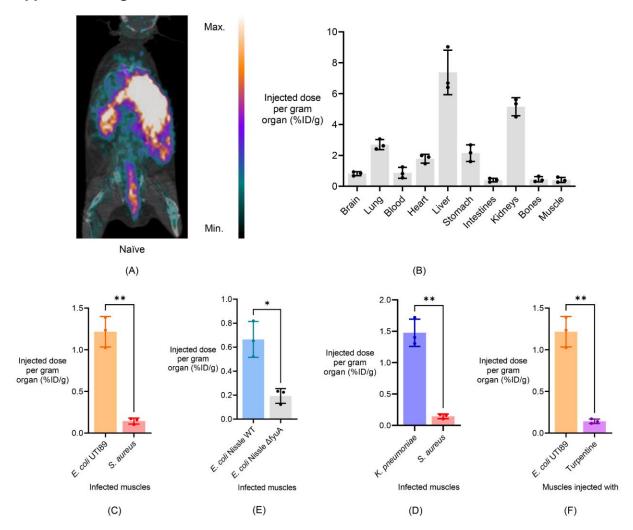
³Department of Internal Medicine, Heart, Lung and Vascular Institute, University of Cincinnati, Cincinnati, Ohio, USA

⁴Department of Molecular Genetics, Biochemistry and Microbiology, College of Medicine, University of Cincinnati, Cincinnati, Ohio, USA

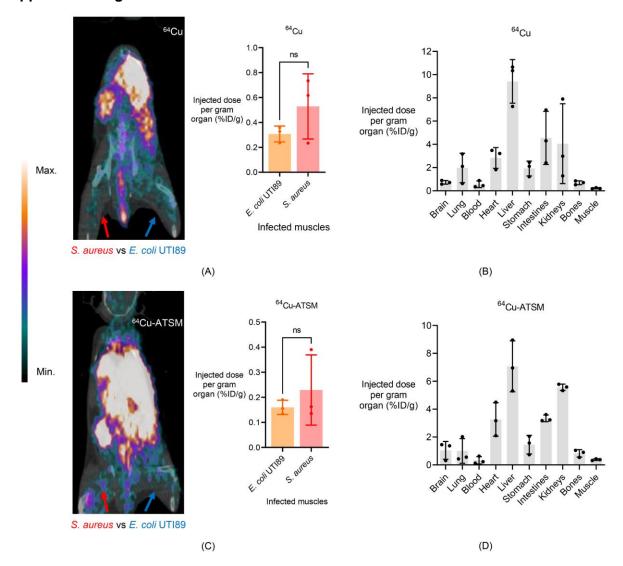
*Corresponding author.



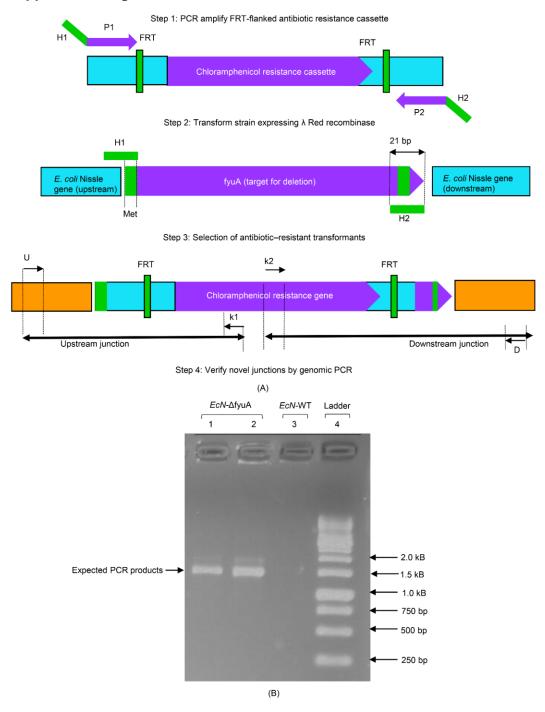
Supplemental Figure 1. HPLC analyses. Radio-chromatograms of (**A**) freshly prepared ⁶⁴Cu-ATSM and (**B**) ⁶⁴Cu-YbT in mouse serum 4 hrs after incubation.



Supplemental Figure 2. *In vivo* profile of ⁶⁴Cu-YbT. (A) PET/CT image and (B) ex vivo biodistribution in major organs of naïve mice. ⁶⁴Cu-YbT accumulation (t = 24 hrs) in mice muscles injected with: (C) *E. coli* UTI89 and *S. aureus* (D) *E. coli* Nissle wild-type (WT) and *E. coli* Nissle FyuA knock-out mutant ($\Delta fyuA$) (E) *K. pneumoniae* and *S. aureus* and (F) *E. coli* UTI89 and turpentine. Note: arrows indicate sites of bacterial injection. Data presented as mean \pm s.d. (n = 3) analyzed by Welch's t-test; *P < 0.05, **P < 0.01.



Supplemental Figure 3. Control ⁶⁴Cu-based probes lack bacterial specificity. PET/CT images with muscle uptakes and biodistribution in major organs of (**A** and **B**) Unchelated ⁶⁴Cu and (**C** and **D**) ⁶⁴Cu-ATSM 24 hrs post-administration of probes. Note: arrows indicate sites of bacterial injection. Data presented as mean \pm s.d. (n = 3) analyzed by Welch's t-test; ns: not significant.



Supplemental Figure 4. (**A**) Schematic of knock-out mutant (KO) generation using λ Red Recombinase method. (**B**) Colony PCR products of *E. coli* Nissle FyuA KO mutant (*EcN*-Δ*fyuA*, lanes 1 and 2) and *E. coli* Nissle wild-type (*EcN*-WT, lane 3) were run on 1% agarose gel alongside a 1 kB DNA ladder.