A Systematic Approach for Developing Bacteria-Specific Imaging Tracers

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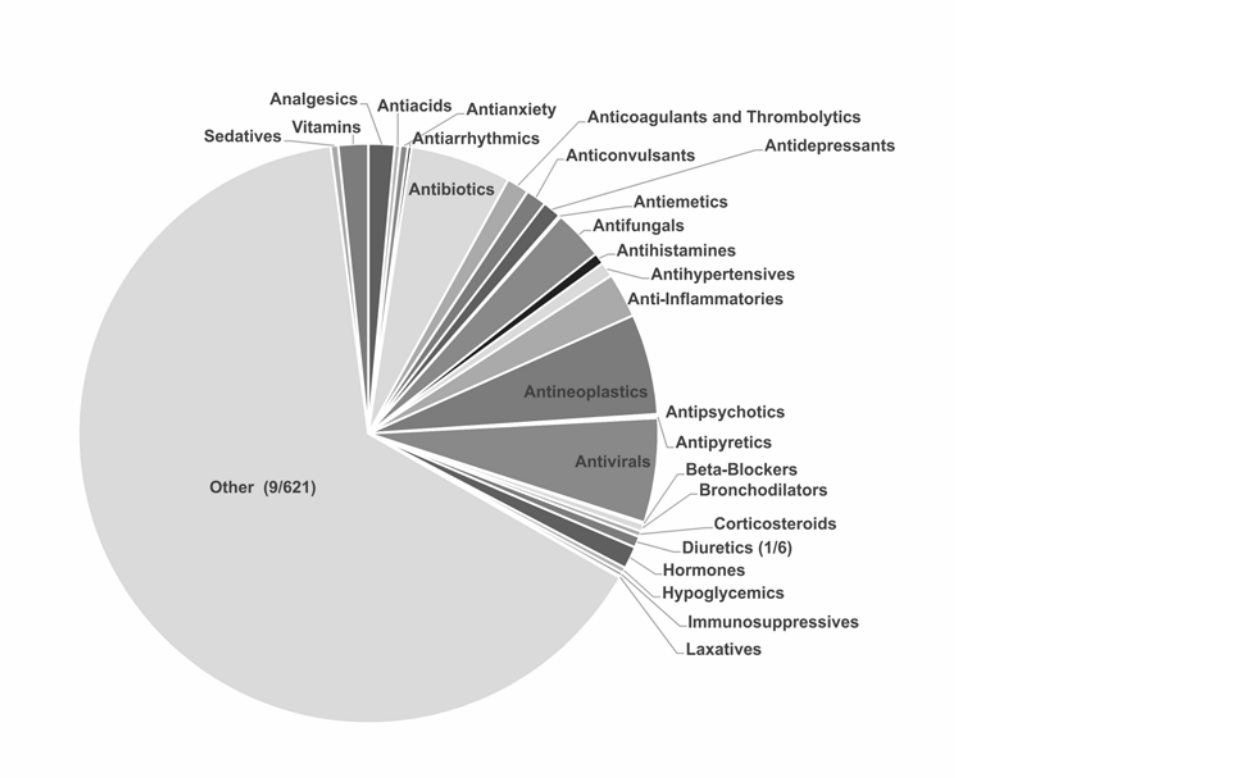
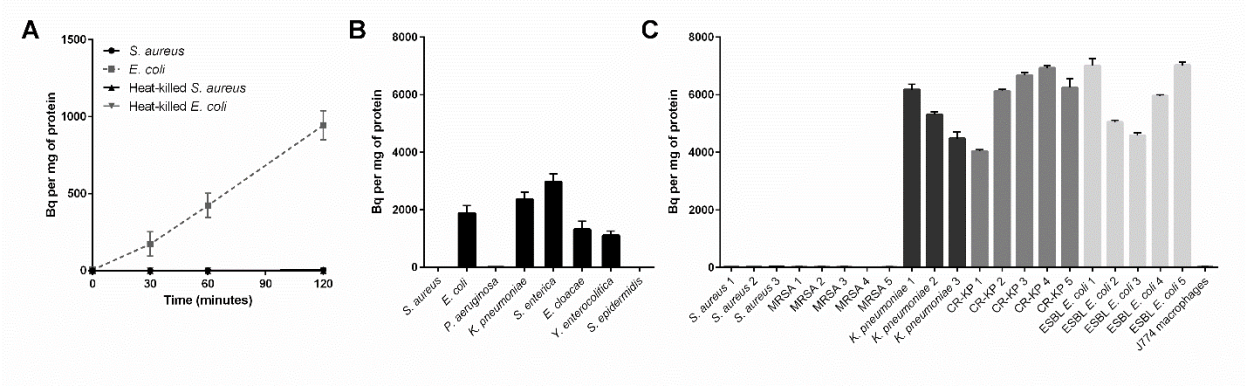
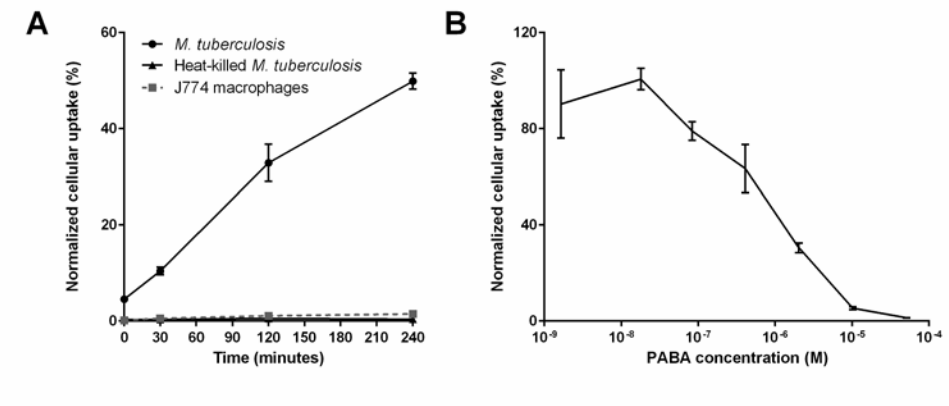


Supplemental Figure 1. Pharmacological classification of compounds screened.

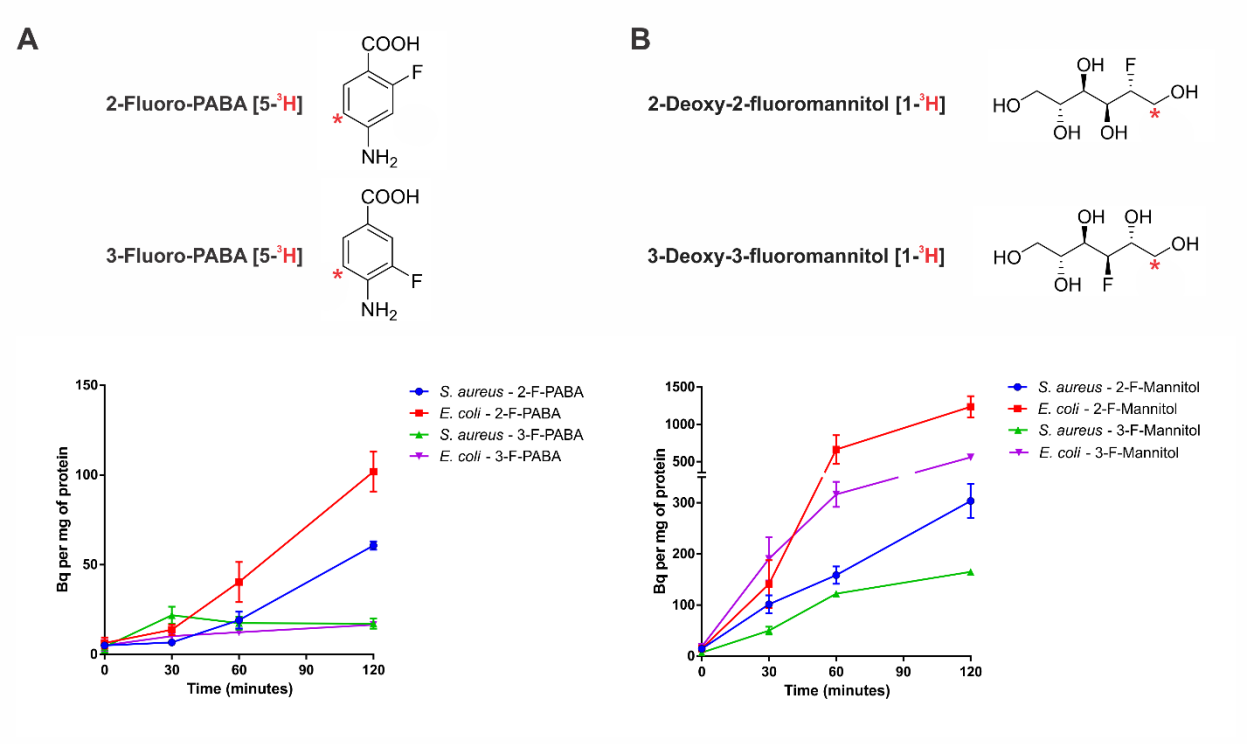


Supplemental Figure 2. Bacterial uptake of ^{18}F-FDS. (A) Uptake by *S. aureus*, *E. coli* and the corresponding heat killed controls over 120 minutes. Uptake at 120 minutes in an extended panel of bacteria (B), and by drug-susceptible and drug-resistant clinical isolates (C), compared to mammalian cells (J774 murine macrophages). Labels indicate methicillin resistant *S. aureus* (MRSA), carbapenem-resistant *K. pneumoniae* (CR-KP), and extended-spectrum beta-lactamase producing *E. coli* (ESBL *E. coli*). Mean and standard deviations are shown.

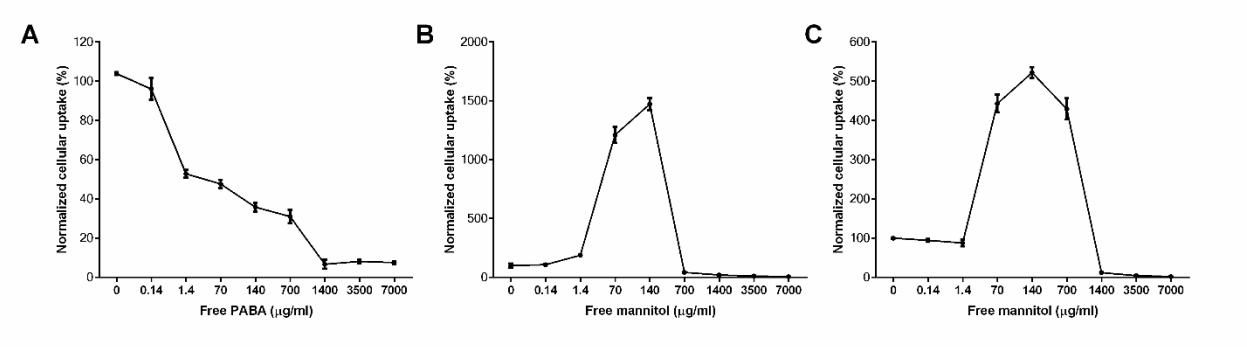


Supplemental Figure 3. Selective and specific accumulation of ^{3}H-PABA by *Mycobacterium tuberculosis*. (A) Uptake by live, heat killed bacteria and mammalian cells (J774 murine macrophages). (B) Competition of ^{3}H-PABA uptake with increasing concentrations of unlabeled

parent molecule (normalized to uptake without unlabeled parent molecule). The incubation period was 120 minutes for the competition assay. Mean and standard deviations are shown.

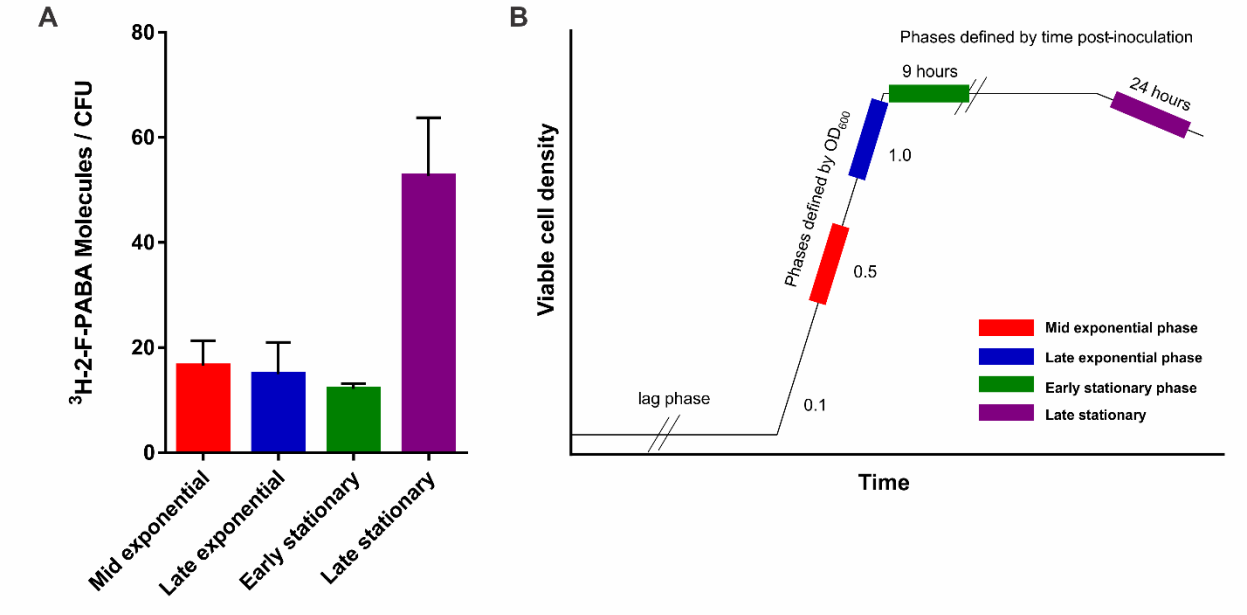


Supplemental Figure 4. *In vitro* uptake of 2- and 3-fluoro-analogs. (A) *In vitro* uptake of 2-fluoro-PABA [5-^{3}H] (2-F-PABA) and 3-fluoro-PABA [5-^{3}H] (3-F-PABA) was tested in *S. aureus* and *E. coli.* (B) *In vitro* uptake by *S. aureus* and *E. coli* of 2-deoxy-2-fluoro-D-mannitol [1-^{3}H] (2-F-mannitol) and 3-deoxy-3-fluoro-D-mannitol [1-^{3}H] (3-F-mannitol). The incubation period was 120 minutes for all experiments. Mean and standard deviations are shown.



Supplementary Figure 5. Specific accumulation of ^{3}H-2-F-PABA and ^{3}H-2-F-mannitol.

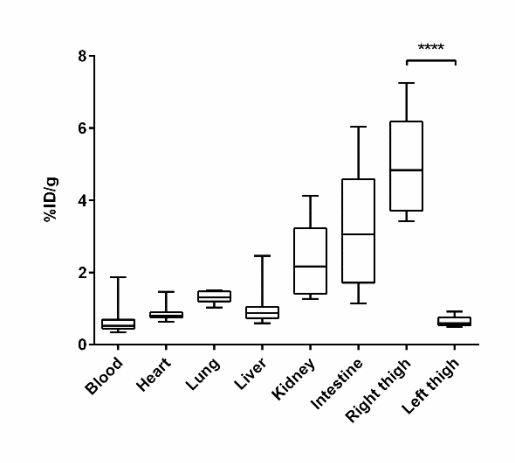
Competition of ^{3}H-2-F-PABA (A), ^{3}H-2-F-mannitol in *S. aureus* (B) and ^{3}H-2-F-mannitol in *E. coli* (C) with increasing concentrations of unlabeled parent molecule (normalized to uptake without unlabeled parent molecule). The incubation period was 120 minutes for all experiments. Mean and standard deviations are shown.



Supplementary Figure 6. Effect of bacterial growth phase on the uptake of ^{3}H-2-F-PABA.

(A) Uptake of ^{3}H-2-F-PABA in *S. aureus* in different growth phases measured 10 minutes after adding the tracer to the bacterial culture. (B) Growth phase was determined by absorbance at 600

nm during the exponential growth phase and by time after inoculation for the stationary phase. Mean and standard deviations are shown. CFU = colony forming units.



Supplementary Figure 7. Tissue biodistribution of ^{18}F-FDS in a murine *E. coli* myositis model. Mice were inoculated with live *E. coli* in the right thigh and a 10-fold higher burden of heat-killed bacteria in the left thigh (sterile inflammation). The tracer was injected intravenously and accumulation in tissues measured using gamma counting from surgically resected tissues. N = 8 animals. **** $P < 0.0001$ (Mann-Whitney U test, two-tail). Median, interquartile, and range are shown.