

## Supplemental Material

### Histidine utilization is a critical determinant of *Acinetobacter* pathogenesis

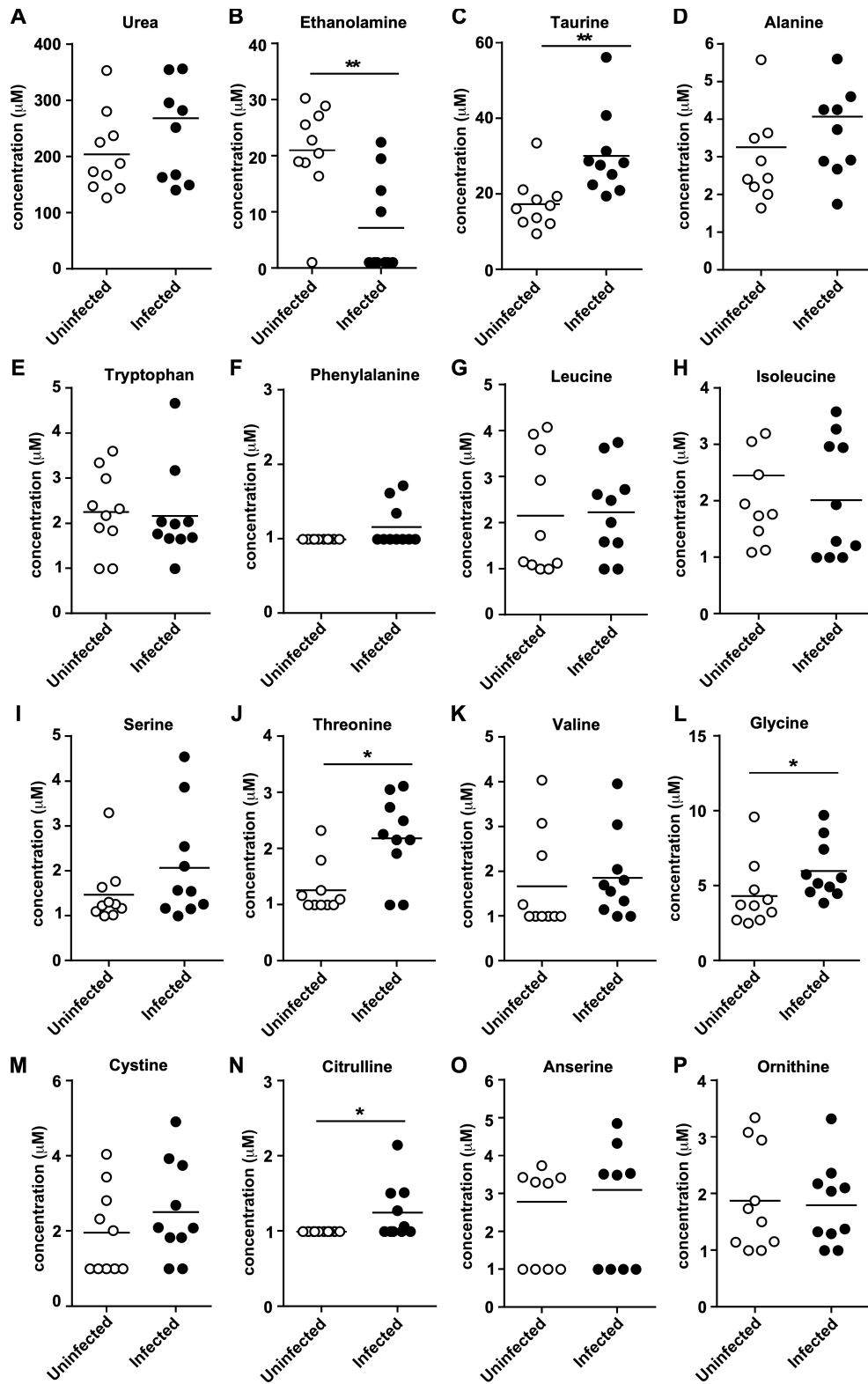
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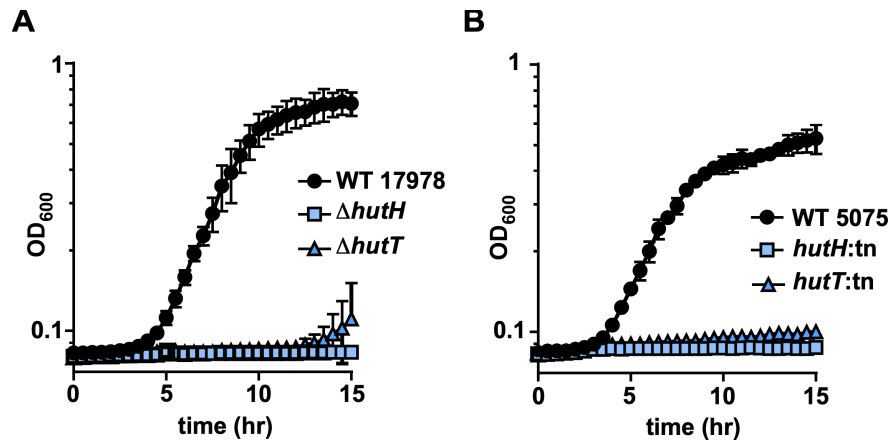
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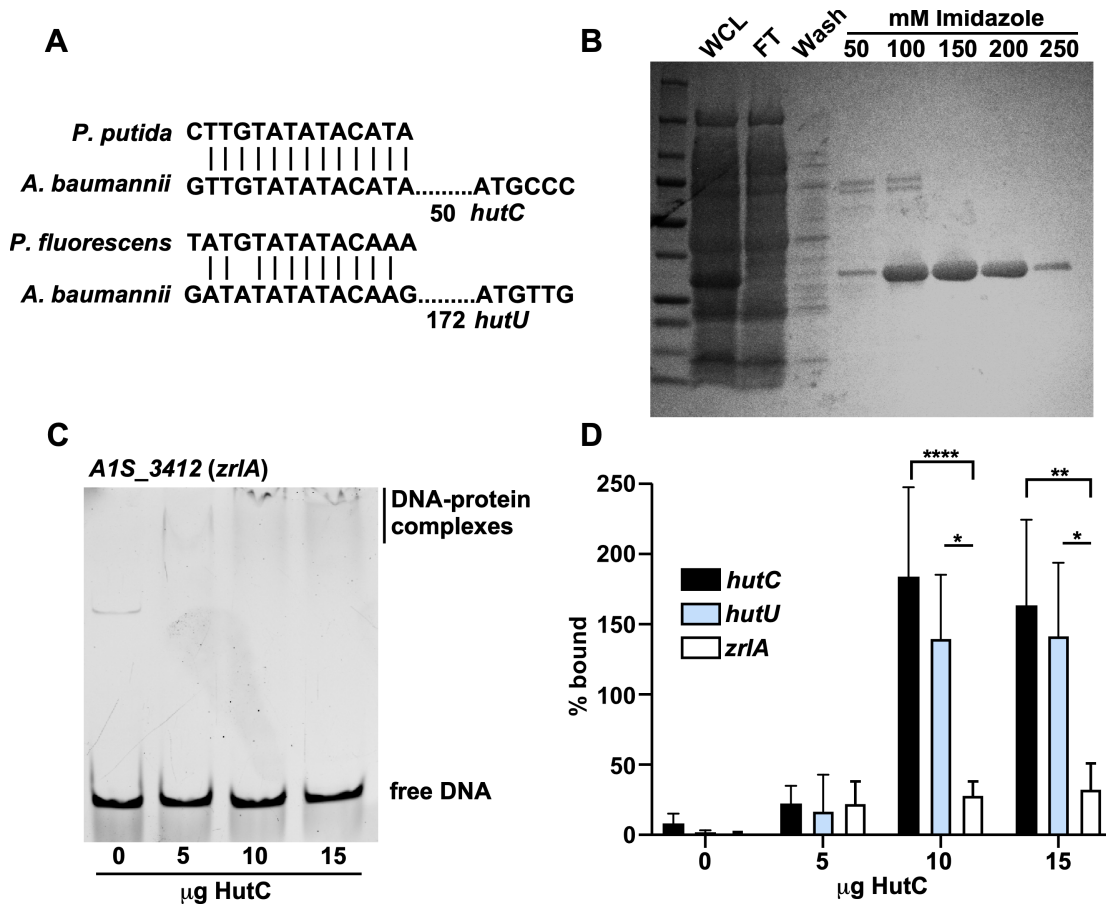
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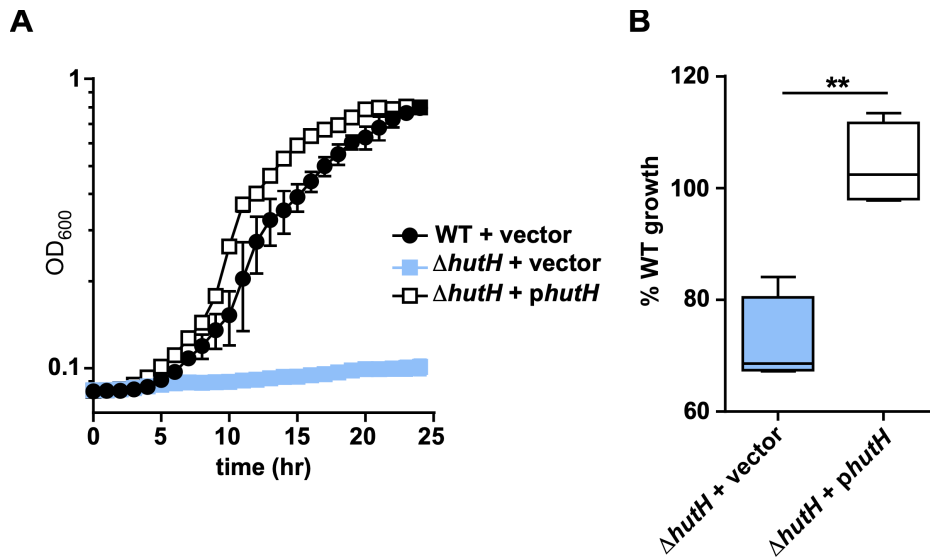
**Figure S1.** A variety of metabolites are detectable within the murine lung. A-P) Bronchial alveolar lavage fluid (BALF) was collected at 36 hours post infection from mice and compared to uninfected counterparts following metabolite analysis for the indicated molecule. \*  $p < 0.05$ , \*\*  $p < 0.01$  as determined by Mann-Whitney U test,  $n = 10$  mice per group.



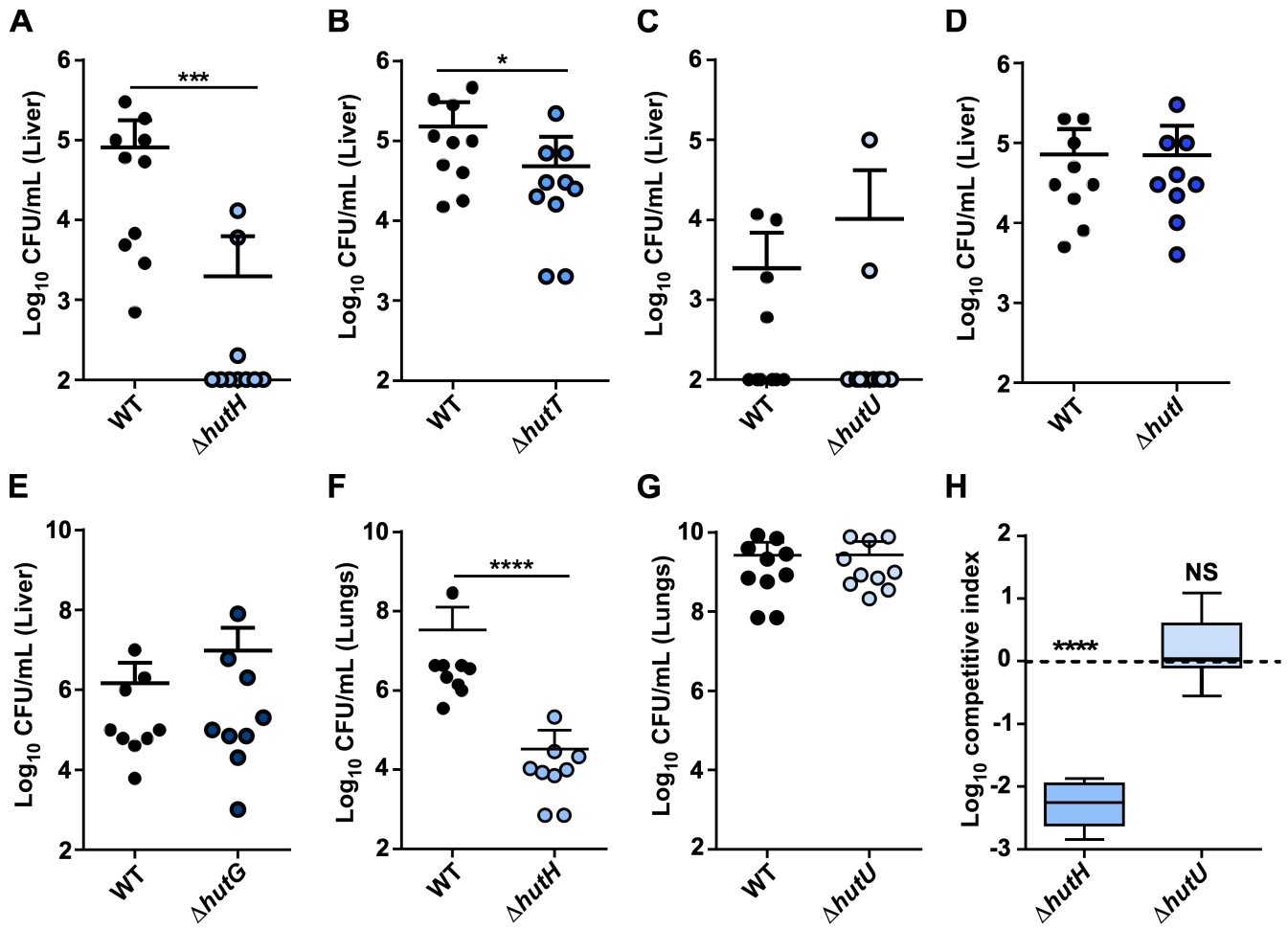
**Figure S2.** The Hut system requires a histidine transporter and enzymatic activity. A) Representative growth curve monitored by OD<sub>600</sub> of WT *A. baumannii* 17978,  $\Delta hutH$ , and  $\Delta hutT$  in M9 minimal media with histidine as the sole carbon source. B) Representative growth curve monitored by OD<sub>600</sub> of WT *A. baumannii* 5075,  $hutH:tn$ , and  $hutT:tn$  in M9 minimal media with histidine as the sole carbon source.



**Figure S3.** HutC regulates the *hut* system. A) Alignments between HutC binding sites in the promoter regions of *Pseudomonas putida hutC* and *P. fluorescens hutF* compared to *A. baumannii hutC* and *hutU*. Exact matches are denoted by vertical lines. B) Purification scheme for recombinant HutC from *E. coli*. Abbreviations: WCL = whole cell lysate, FT = flow through. C) EMSA performed with recombinant HutC and a DNA probe targeting the intergenic region of *AIS\_3412 (zrlA)*. D) Densitometry analysis of relative HutC-DNA complexes formed with *hutC*, *hutU*, and *zrlA* probes. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*\*  $p < 0.0001$ , as determined by one-way ANOVA with Tukey multiple comparisons test from three independent experiments.



**Figure S4.** The Hut system promotes histidine energy generation. A) Representative growth monitored by OD<sub>600</sub> of WT *A. baumannii* 17978 + pWH1266 (vector),  $\Delta hutH$  + pWH1266 (vector), or  $\Delta hutH$  + pWH $hutH$  in M9 minimal media with histidine as the sole carbon and nitrogen source. B) Percent growth relative to WT at 24 hours for  $\Delta hutH$  + pWH1266 or  $\Delta hutH$  + pWH $hutH$  with fumarate as a carbon source and histidine as the sole nitrogen source. \*\*  $p < 0.01$  as determined by Student's  $t$  test and combined from two independent experiments.



**Figure S5.** Components of the Hut system are important for *A. baumannii* pathogenesis. Eight to 10-week-old mice were competitively infected intranasally with a 1:1 mixture of WT and mutant *A. baumannii*. Lung burdens are reported in Figure 5. A) Bacterial burdens of WT and  $\Delta hutH$  strains recovered at 36 hours post infection (hpi) in the liver. B) Bacterial burdens of WT and  $\Delta hutT$  strains recovered at 36 hpi in the liver. C) Bacterial burdens of WT and  $\Delta hutU$  strains recovered at 36 hpi in the liver. D) Bacterial burdens of WT and  $\Delta hutI$  strains recovered at 36 hpi in the liver. E) Bacterial burdens of WT and  $\Delta hutG$  strains recovered at 36 hpi in the liver. F) Bacterial burdens from a competitive infection between WT and  $\Delta hutH$  strains recovered in the lungs at 36 hpi from  $CP^{-/-}$  mice. G) Bacterial burdens from a competitive infection between WT and  $\Delta hutU$  strains recovered in the lungs at 36 hpi from  $CP^{-/-}$  mice. \*  $p < 0.05$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$  as determined by Mann Whitney U-test with Dunnett's multiple comparisons,  $n = 9-10$  mice per group. H) Competitive index ( $[\text{input mutant/WT}]/[\text{output mutant/WT}]$ ) for the indicated mutants in the lungs of  $CP^{-/-}$  mice. \*\*\*\*  $p < 0.0001$  as determined by Student's  $t$  test against a value of 1.