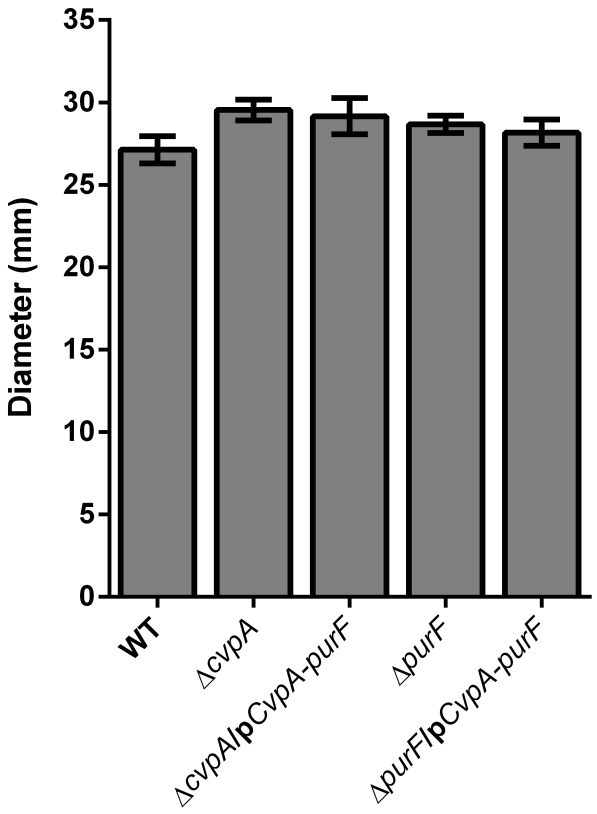

Figure S1. Loss of purine biosynthesis does not alter bacterial mobility. Swimming motility of UTI89 strains was evaluated using soft agar motility assays. Graph depicts the average motility diameter ± standard error of the mean for each strain. Bacterial motilities were recorded in triplicate plates in a minimum of three independent biological replicate experiments.

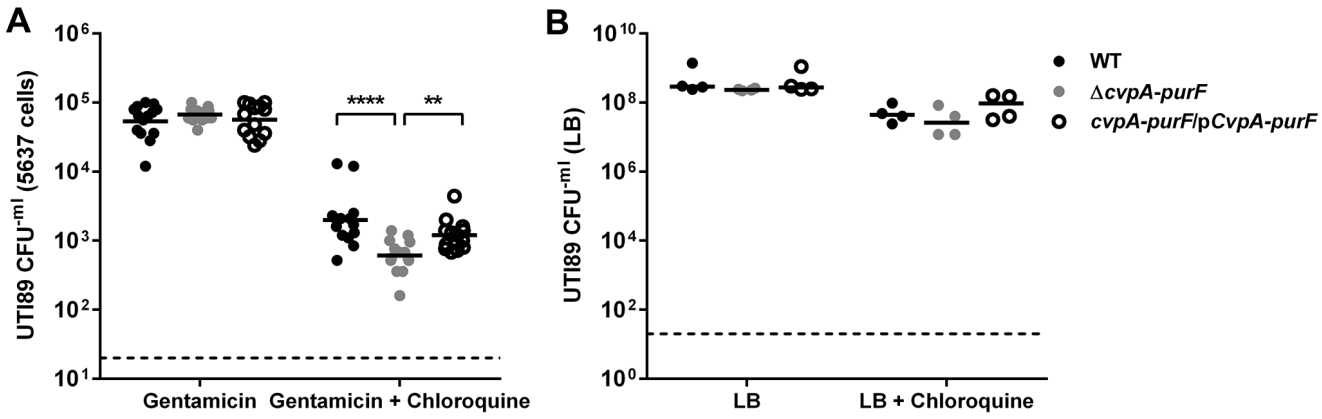

Figure S2. Chloroquine eliminates UPEC from the endocytic vacuole. (**A**) 5637 bladder epithelial cells were co-cultured with the indicted UTI89 strain at an MOI of 20 for 2 h prior to treatment with gentamicin or gentamicin and chloroquine. Intracellular bacterial titers were enumerated by disruption of 5637 cell monolayers and plating of 10-fold serial dilutions. Graph depicts the recovered colony forming units for each strain. Lines in **A** represent the geometric mean of each distribution. Gentamicin and chloroquine resistance assays were performed on at least six wells per replicate in a minimum of two independent biological replicate experiments. (**B**) UTI89 strains were aerobically grown in LB, and were diluted to an OD_{600} of 0.3 in sterile PBS. Normalized cultures were treated with chloroquine for 90 min (LB + chloroquine), or left untreated (LB), and surviving CFUs were enumerating by plating 10-fold serial dilutions. Graph depicts the surviving CFU for each strain in four biological replicate experiments. Lines in **B** depict the median of each distribution. *p* values in A were calculated by Mann-Whitney t-test. ****, $p<0.0001$; **, $p<0.01$.

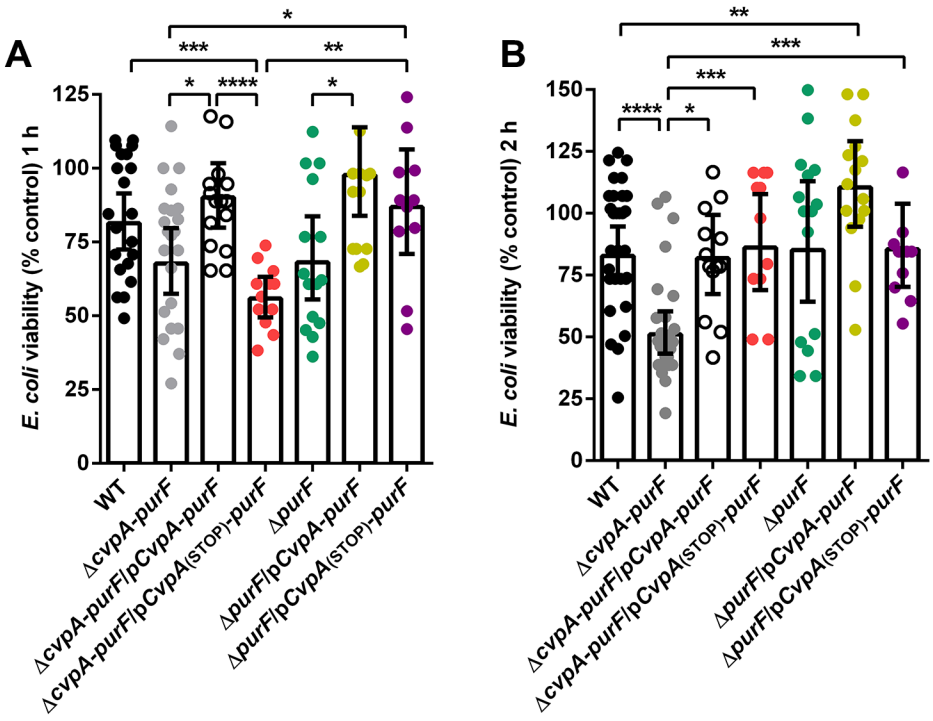

Figure S3. CvpA promotes UPEC resistance to phagocytic clearance. (**A**) Survival of UPEC deficient in *cvpA-purF* or CvpA co-cultured with polymorphonuclear neutrophils (PMNs) for 1 h. (**B**) Recovery of surviving UTI89 strains co-cultured with PMNs for 2 h. Values represent the geometric mean (bars) and 95% confidence intervals (whiskers) of each distribution. Dots represent individual measurements of UPEC survival (recovered CFUs) in at least three independent experiments with a minimum of 4 individual wells per experiment. *p* values were calculated by Mann-Whitney t-test. ****, $p<0.0001$; ***, $p<0.001$; **, $p<0.01$.

Supplemental Experimental Procedures

Motility assays. Motility assays were performed as previously described (1). Briefly, overnight cultures grown in LB at 37°C with shaking were used to inoculate soft agar (0.25% LB agar–0.001% 2,3,5-triphenyltetrazolium chloride). Motility diameters for each strain was recorded after incubation at 37°C for 7 h. For each strain, a minimum of three biological replicates consisting of triplicate plates was performed for each experiment.

Neutrophil killing assay. Polymorphonuclear neutrophils (PMNs) were elicited by injection of 1 ml sterile casein solution into the peritoneal cavity of C57Bl/6 mice (2). At 24 h post-injection, a second dose of casein solution was administered. The animal was euthanized at 3 h post-injection, and the abdominal skin was sterilized and retracted to expose the intact peritoneal wall. Neutrophils were collected from the peritoneal cavity by performing 2 peritoneal cavity washes with 5 ml of sterile PBS. Cells recovered from the peritoneal cavity using this methodology yield 90-95% PMNs based on flow cytometry utilizing markers $CD11b^{+}Ly6g^{+}$ (data not shown). The pooled peritoneal fluid was centrifuged for 10 min a 200 x g, and red blood cells were lysed using Ammonium-Chloride-Potassium (ACK) lysing buffer (Gibco). Peritoneal exudates were then washed 3 times, re-suspended in Dulbecco's Modified Eagle Medium/Ham's F-12 supplemented with 5% FBS and PMNs were enumerated. PMNs were constituted at 5×10^5 cells/ml and 1 ml aliquots were distributed to each well of a 12-well cell culture plate. Plates were incubated for 1 h at 37°C in 5% CO_2 prior to infection. UTI89 strains were grown as described for bacterial adherence and invasion assays. Bacterial cultures were normalized in sterile PBS to an OD_{600} of 0.5, and neutrophils (100,000 per well) were infected at an MOI of 30.

In parallel, triplicate wells containing Ham's F-12 medium alone were inoculated with an equivalent volume of UTI89 strain to serve as a control. At the indicated time points, Triton X-100 was added to a final concentration of 0.1% to each well, and ten-fold serial dilutions were plated on solid LB to enumerate CFUs. UPEC survival in the presence of PMNs was calculated as a percentage of CFUs obtained from control (no PMN) wells. Neutrophil killing assays were repeated at least three times with six replicate wells of each strain per independent experiment.

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1. **Hadjifrangiskou M, Gu AP, Pinkner JS, Kostakioti M, Zhang EW, Greene SE, Hultgren SJ.** 2012. Transposon mutagenesis identifies uropathogenic *Escherichia coli* biofilm factors. J Bacteriol **194:**6195-6205.
2. **Luo Y, Dorf ME.** 2001. Isolation of mouse neutrophils. Curr Protoc Immunol **Chapter 3:**Unit 3 20.