Figure S1: Deletion of *ubil* leads to reduced pmf across the inner membrane, increasing tolerance to certain antibiotics. (A) Proton motive force (pmf) differences between UTI89 and UTI89Δubil were measured using flow cytometry and the oxocarbo cyanine dye, DiOC$_6$. The left panel indicates the background dye incorporation of depolarizing controls, in which 30 mM of the proton ionophore carbonyl cyanide m-
chlorophenylhydrazone (CCCP) was added to abolish the membrane potential prior to addition of DiOC₂. Membrane-associated DiOC₂ emits green fluorescence and it is readily detectable in all bacteria. The presence of membrane potential across the inner membrane facilitates the import of DiOC₂ into the cytosol, where fluorescence emission shifts to red. Panel on the right indicate DiOC₂ internalization by WT UTI89 and UTI89Δubi₁. Results shown are representative of three independent experiments. (B) Panels on the left indicate growth of WT UTI89 or UTI89Δubi₁ in the presence of antibiotics that require energy-dependent transport (gentamycin, GM; streptomycin, S). The graph on the right depicts the quantitation of the zones of clearance determined for WT UTI89 and UTI89Δubi₁ from 5 biologically independent experiments with 3 technical replicates each. Statistical analyses were performed using unpaired students t-test, with ***, p<0.0001 and **, p<0.001
Figure S2: Standard curve for ATP quantification. Graph depicts corresponding luminescence values from known concentrations of ATP disodium salt hydrate. This standard curve allows luminescence values from ATP in bacterial samples to be matched to the amount of ATP present, resulting in a quantitative amount of ATP. The luminescence values fell between $10^3 - 10^4$ in ATP experiments, which correspond to the linear region of $10^{-10} - 10^{-11}$ moles of ATP.
Figure S3: Deletion of *ubil* reduces bacterial surface pili levels independent of the *fim* promoter orientation. Graph depicts the enumeration of bacteria with zero, low (1-20), moderate (21-50), or abundant (>50) numbers of pili in the WT UTI89 (black bars), UTI89*Δubil* (white bars), UTI89_LON (blue bars), and UTI89*Δubil*_LON after aerobic
growth for 2 x 24 h in static LB media. Pili images were obtained from TEM. The person enumerating bacteria was blinded to the identity of samples. The graph depicts the average from enumerating bacteria from three independent experiments. Statistical analysis was performed by two-tailed Student’s t-test with P<0.05 considered significant
Figure S4: Metabolic profiling indicates a stall at the fumarate to malate step in the TCA cycle. (A) Graphical representation of the TCA cycle with identified block points.
Table indicates the phenotypes gained by the *ubil* mutant during Biolog profiling, compared to the WT parent. During Biolog analyses, the strains are seeded in micro-titer plates that carry a single carbon source per well. The ability of each strain to grow in the presence of each carbon source is recorded at 15-minute intervals and a growth difference is averaged at the end of a 24 h run. “Phenotypes gained” indicate the carbon sources on which the mutant had a growth advantage over the WT. “Phenotypes lost” indicate the carbon sources on which the mutant had a growth disadvantage compared to the WT. The table shown depicts values averaged from three independent plates run per strain.