

Supplemental Table 1. MICs of CTX-M-14  $\beta$ -lactamase and mutant enzymes.

$\beta$ -lactamase	benzylpenicillin ( $\mu\text{g/mL}$ )	cephalothin ( $\mu\text{g/mL}$ )	cefotaxime ( $\mu\text{g/mL}$ )
CTX-M-14 (wt*)	>256	>256	12
S237A	>256	>256	0.38
R276A	>256	>256	2
R276N	>256	>256	1.5
S237A:R276A	>256	>256	0.5
S237A:R276N	>256	24	0.125
<i>E. coli</i> RB791	16	12	0.064

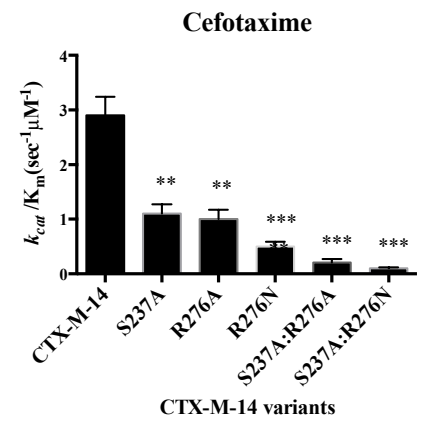
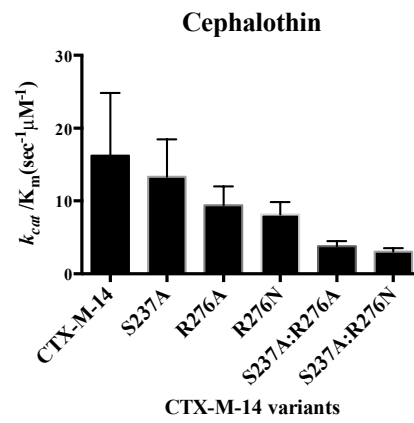
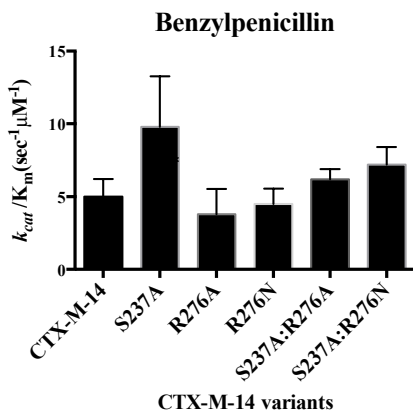
\*CTX-M-14 and mutants used in this study are on the pTP123 vector

## SUPPLEMENTAL FIGURE LEGENDS

**Supp. Figure 1. Catalytic efficiency of CTX-M-14  $\beta$ -lactamase and mutant enzymes.** The catalytic efficiency ( $k_{cat}/K_m$ ) of the enzyme is plotted on the y-axis and the CTX-M-14 variants are plotted on the x-axis. The catalytic efficiency is shown for three different substrates moving from left to right: benzylpenicillin, cephalothin and cefotaxime. Significant changes between CTX-M-14 and the variants are denoted as '\*\*' (p-value<0.005) and '\*\*\*' (p-value<0.0005) as determined by an unpaired T-test.

**Supp. Figure 2. Fluorescence scan of CTX-M-14  $\beta$ -lactamase and mutant enzymes.** Normalized intensity is plotted on the y-axis and wavelength (nm) is plotted on the x-axis. The fluorescence scan for CTX-M-14 (WT) is shown as red dots, the S237A variant is shown as blue triangles and S237A:R276A variant is shown as boxes. All proteins tested exhibited maximum intensity between 333 nm and 335 nm.

Supplemental Figure 1.



Supplemental Figure 2.

