

Expanded View Figures

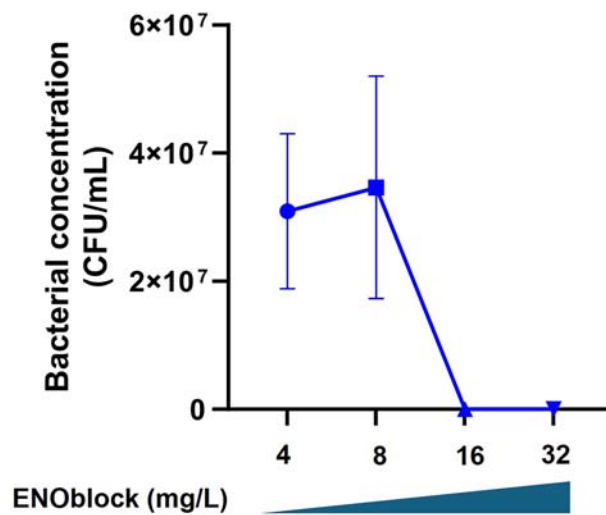


Figure EV1. ENOblock selective pressure.

A. baumannii Ab ATCC 17978 concentrations after incubation with increasing concentrations of ENOblock.

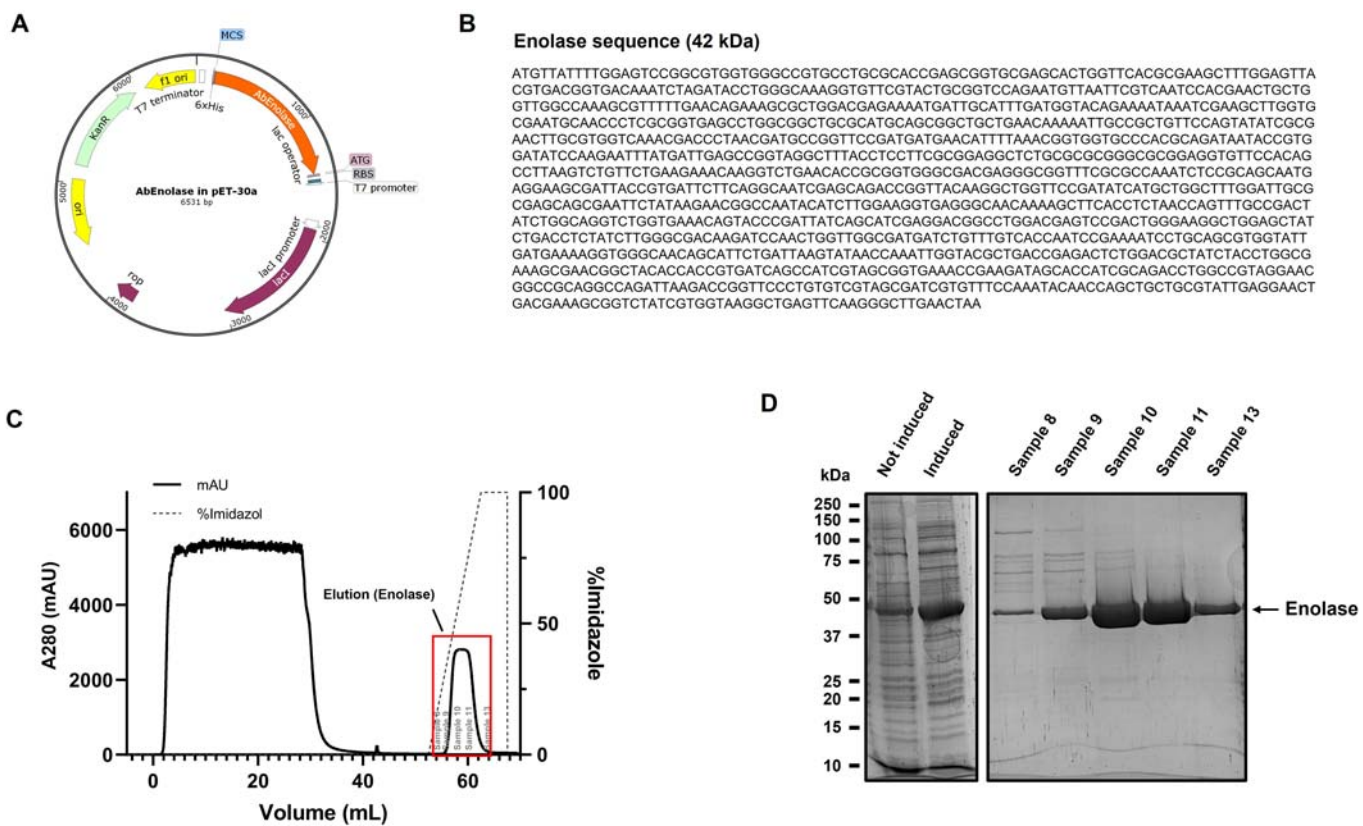


Figure EV2. Construction and characterization of *A.baumannii* enolase.

(A) Illustration of the recombinant expression vector of enolase. (B) The sequence of the enolase. (C) Purification of enolase using the Histrap FF column. (D) SDS-PAGE gel analysis of the purified enolase.

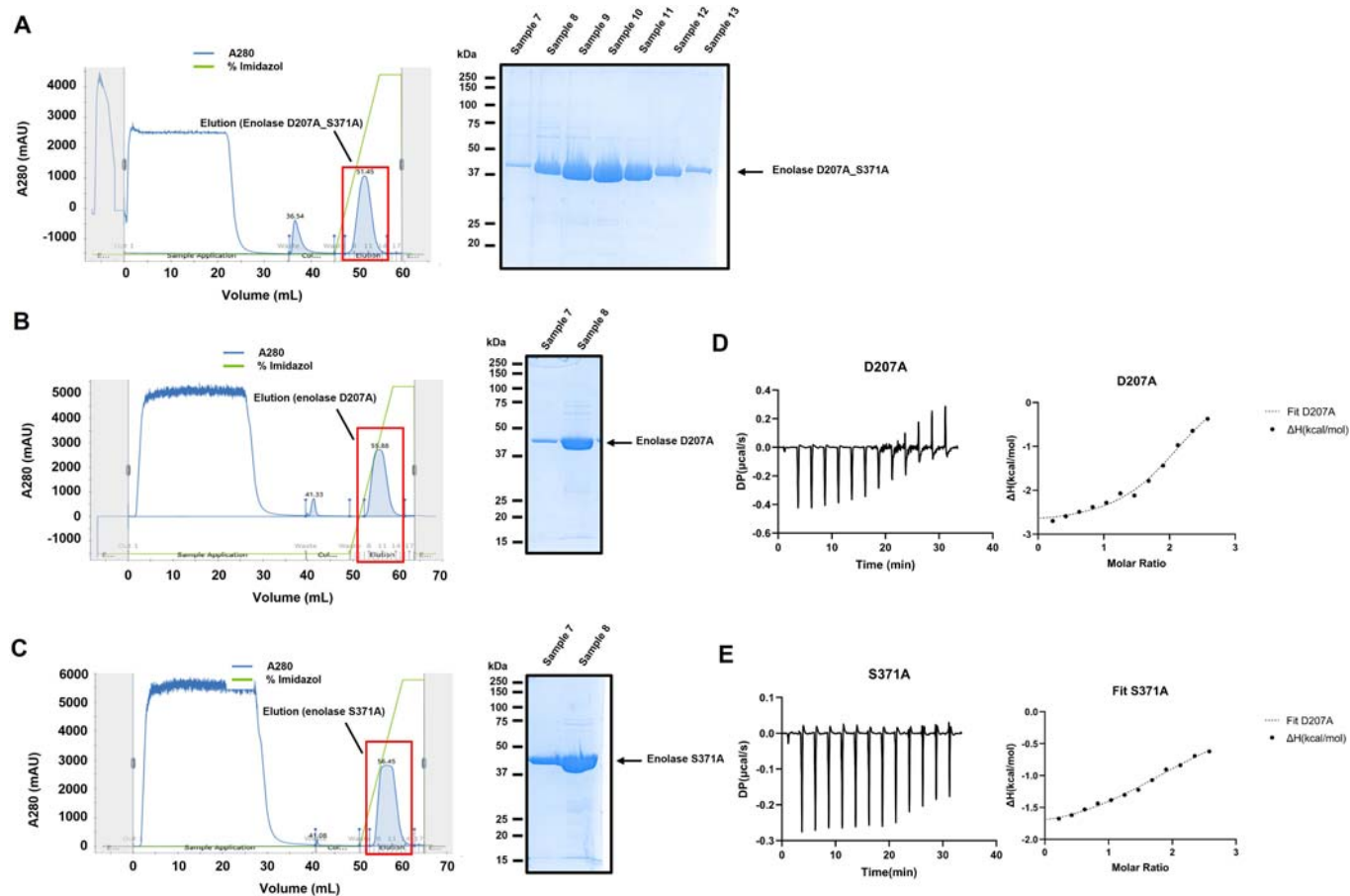


Figure EV3. Construction and characterization of *A. baumannii* enolase mutants.

(A–C) Purification and SDS-PAGE gel analysis of the purified enolase D207A_S371, enolase D207A and enolase S371 using the Histrap FF column. (E, F) Isothermal titration calorimetry (ITC) titrations with integrated fitted heat plots of ENOblock binding with enolase (D207A) or enolase (S371A).

Q14

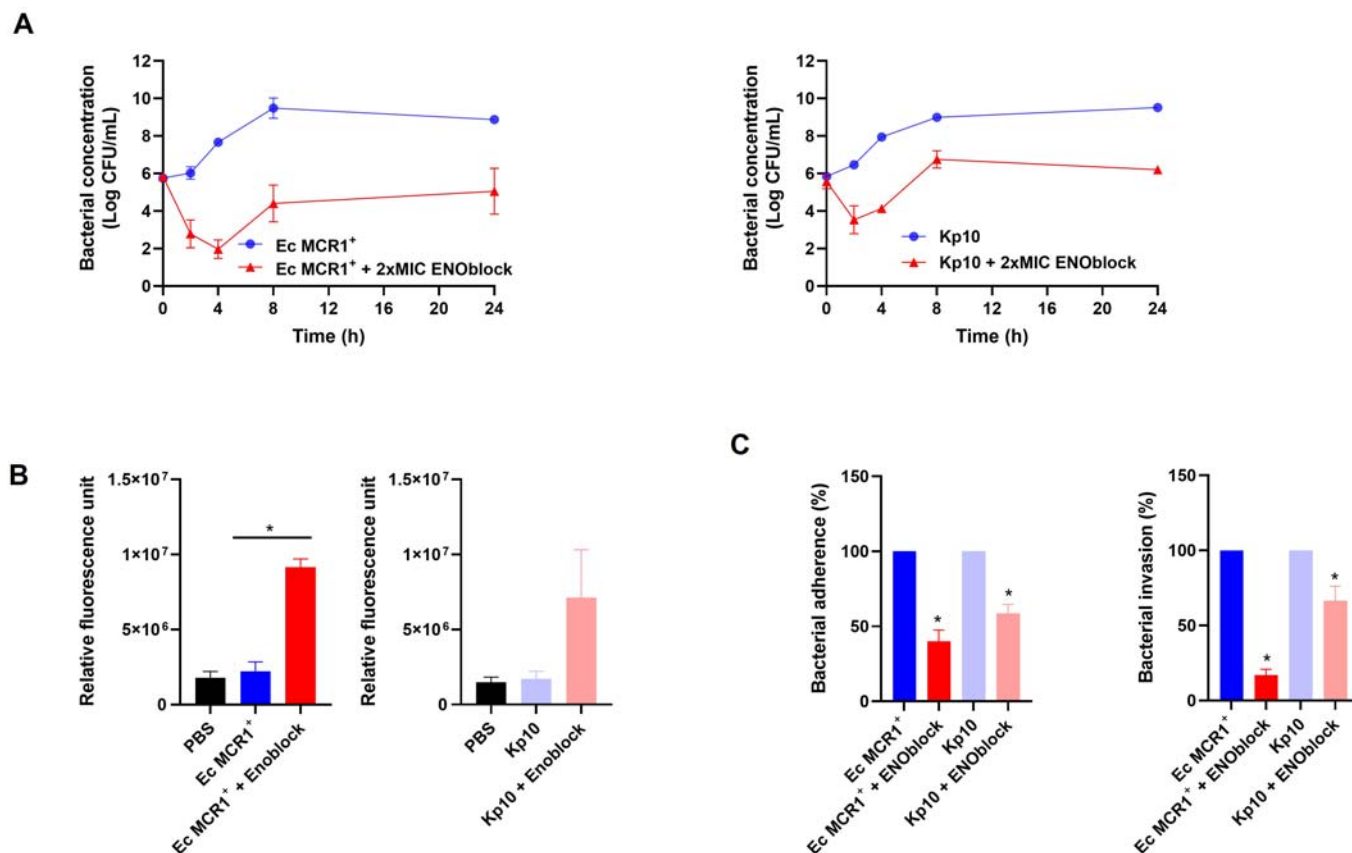


Figure EV4. ENOblock is active against *E. coli* and *K. pneumoniae*.

(A) Time-kill curves of *E. coli* Ec MCR1⁺ and Kp10 strains in the presence of 2xMIC ENOblock for 24 h. (B) Membrane permeabilization of *E. coli* Ec MCR1⁺ and Kp10 strains in the presence of 0.5xMIC ENOblock, incubated for 10 min, was quantified by Typhon Scanner. Data are represented as mean $\pm$ SEM from three independent replicates and experiments. * $P = 0.001$: Ec MCR1⁺ vs Ec MCR1⁺ + ENOblock (two-tailed Student's *t* test). (C) Analysis of *E. coli* Ec MCR1⁺ and Kp10 strains adhesion into HeLa cells with (1xMIC) and without ENOblock treatment. The data are presented as means $\pm$ SEM, * $P = 0.015$: Ec MCR1⁺ vs Ec MCR1⁺ + ENOblock and * $P = 0.02$: Kp10 vs Kp10 + ENOblock (two-tailed Student's *t* test). (D) Analysis of *E. coli* Ec MCR1⁺ and Kp10 strains invasion into HeLa cells with (1xMIC) and without ENOblock treatment. The data are presented as means $\pm$ SEM, * $P = 0.002$: Ec MCR1⁺ vs Ec MCR1⁺ + ENOblock and * $P = 0.037$: Kp10 vs Kp10 + ENOblock treatment (two-tailed Student's *t* test).

Q15

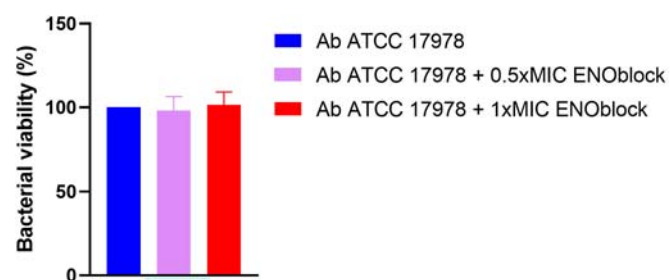


Figure EV5. Bacterial viability after incubation of ATCC with 0.5 and 1xMIC during 30 min with ENOblock.