

Table S1 | *E. coli* strains utilized in this study

<i>E. coli</i> strain	Relevant characteristics and references	Niclosamide MIC ($\mu\text{g}\cdot\text{mL}^{-1}$)
Wild-type BW25113	$\Delta(\text{araD-araB})567$, $\Delta\text{lacZ4787}>::\text{rrnB-3}$, λ^- , <i>rph-1</i> , $\Delta(\text{rhaD-rhaB})568$, <i>hsdR514</i> [16738554]	>32
ΔacrA	Deletion of AcrA membrane fusion protein of resistance-nodulation-division family (1)	>32
ΔacrB	Deletion of AcrB transporter of resistance-nodulation-division family (1)	>32
ΔacrZ	Deletion of AcrZ accessory protein of resistance-nodulation-division family (1)	>32
ΔemrA	Deletion of EmrA membrane fusion protein of major facilitator superfamily (1)	>32
ΔemrB	Deletion of EmrB transporter of major facilitator superfamily (1)	>32
ΔmacA	Deletion of MacA membrane fusion protein of ATP-binding cassette family (1)	>32
ΔmacB	Deletion of MacB transporter of ATP-binding cassette family (1)	>32
ΔtolC	Deletion of TolC outer membrane factor, interacts with multiple efflux families (1)	0.5
ΔmdtP	Deletion of MdtP outer membrane factor, interacts with multiple efflux families (1)	>32
$\Delta 7\text{NR}$	Deletion of seven azo- and nitro-reductase enzymes (AzoR, MdaB, NemA, NfsA, NfsB, YcaK, YieF) from <i>E. coli</i> K-12 W3110 (2)	>32
$\Delta 7\text{NRtolC}$	$\Delta 7\text{NR}$ with additional deletion of the TolC outer membrane factor (2)	0.016

(1) Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. 2006. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol Syst Biol* 2:2006.0008.

(2) Copp JN, Williams EM, Rich MH, Patterson AV, Smaill JB, Ackerley DF. 2014. Toward a high-throughput screening platform for directed evolution of enzymes that activate genotoxic prodrugs. *Protein Eng Des Sel* 27:399–403.