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Supplementary Materials for

Ampicillin-controlled glucose metabolism manipulates the transition from tolerance to resistance in bacteria

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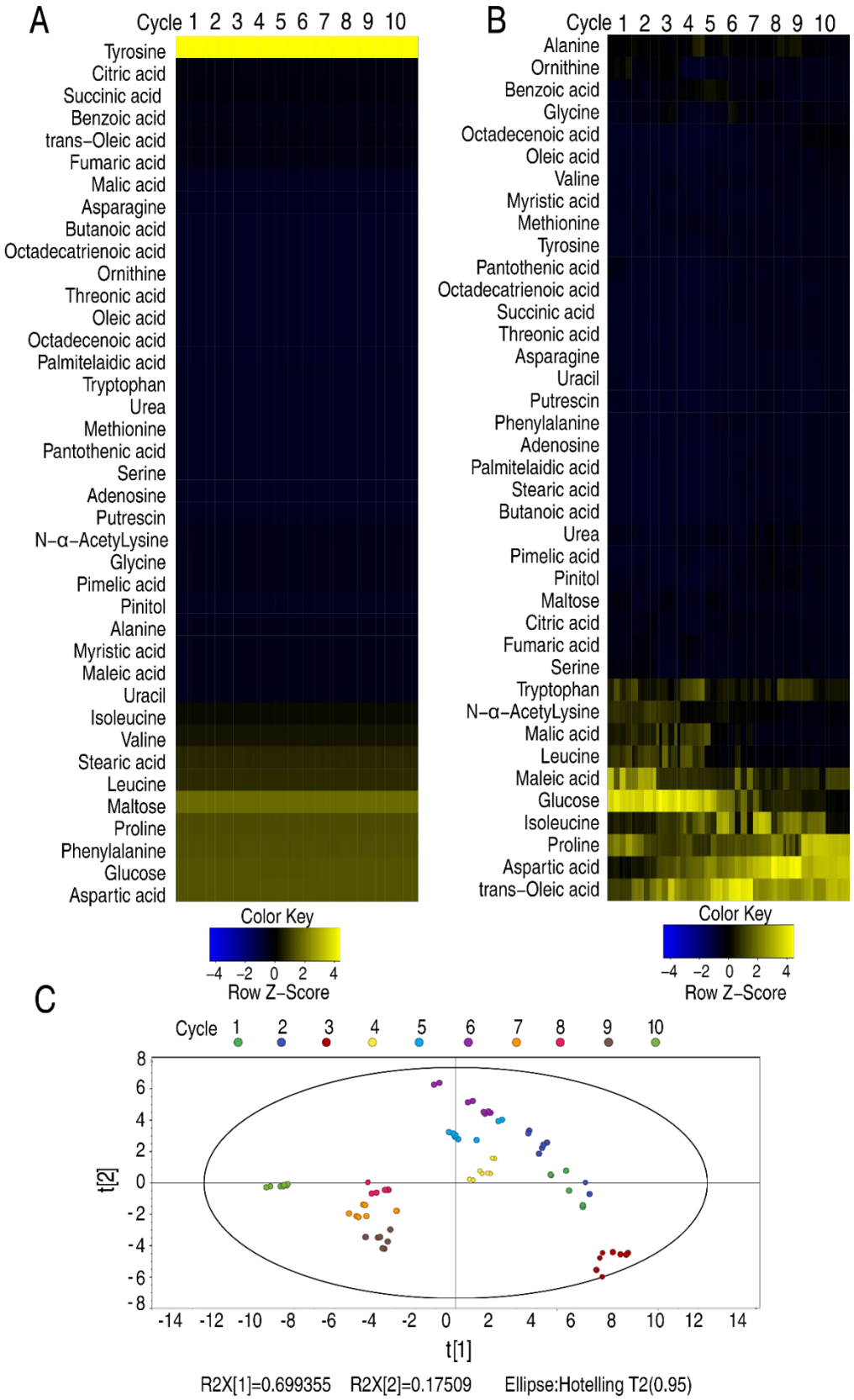

Fig. S1 Metabolic profile of bacterial cycles of daily intermittent exposure to ampicillin. A and **B,** Heatmaps for control and test groups, respectively. **C,** PCA analysis of metabolomic profiling of samples from daily intermittent exposure to ampicillin. Each dot represents the technique replicates in the plot.

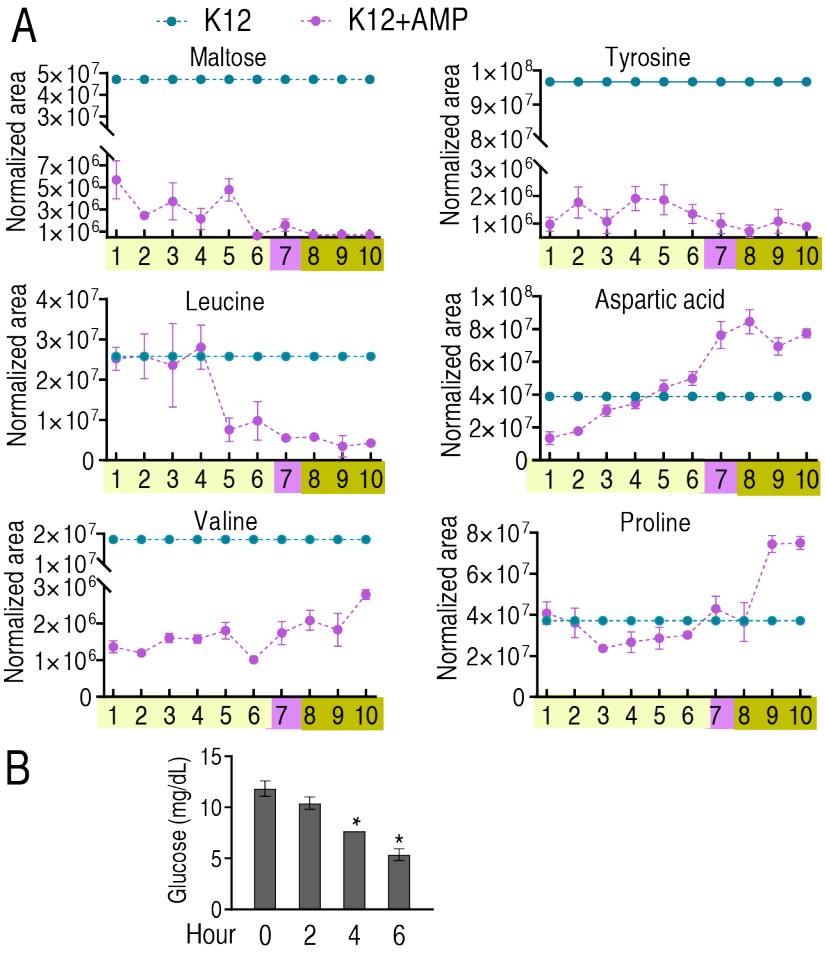

Fig. S2 Abundance of putative biomarkers as a function of increasing tolerance/resistance to ampicillin, as shown in (Fig. 1A) and glucose in LB medium and with culture period (B). Results are displayed as mean ± SEM, and statistically significant differences are identified by Kruskal–Wallis followed by Dunn's multiple comparison post hoc test. *, $P < 0.05$.

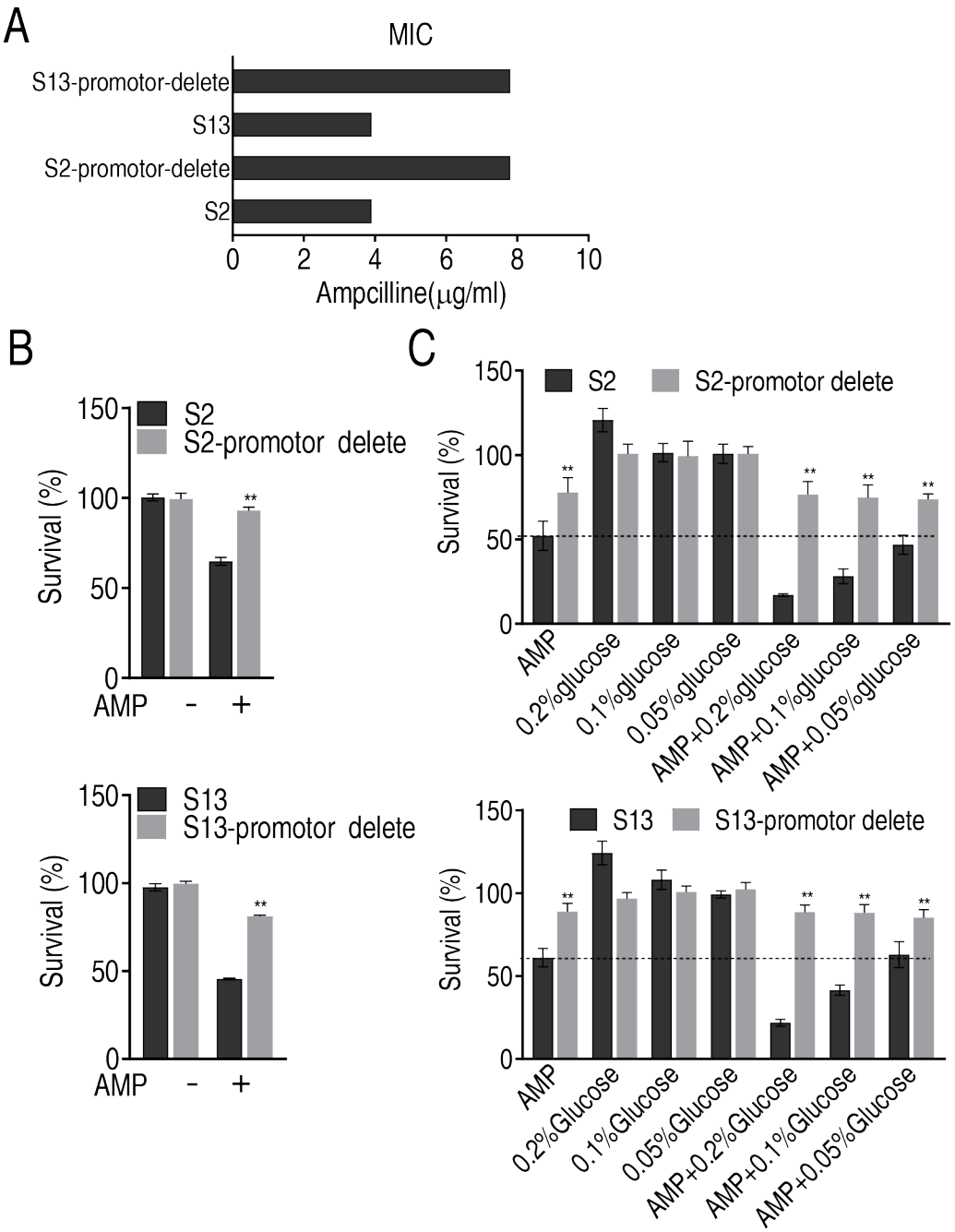

Fig. S3 MIC (A) and viability (B and C) of clinically isolated antibiotic-resistant *E. coli* strains with loss of promoter. B, Percent survival of bacteria inoculated by 1:1000 in LB medium with 2 μg/mL ampicillin and cultured for 6 h. **C**, Percent survival of bacteria in M9 medium in the presence of the indicated glucose plus 2 μg/mL ampicillin. Results are displayed as mean ± SEM, and statistically significant differences are identified by Kruskal–Wallis followed by Dunn's multiple comparison post hoc test. **, $P < 0.01$.

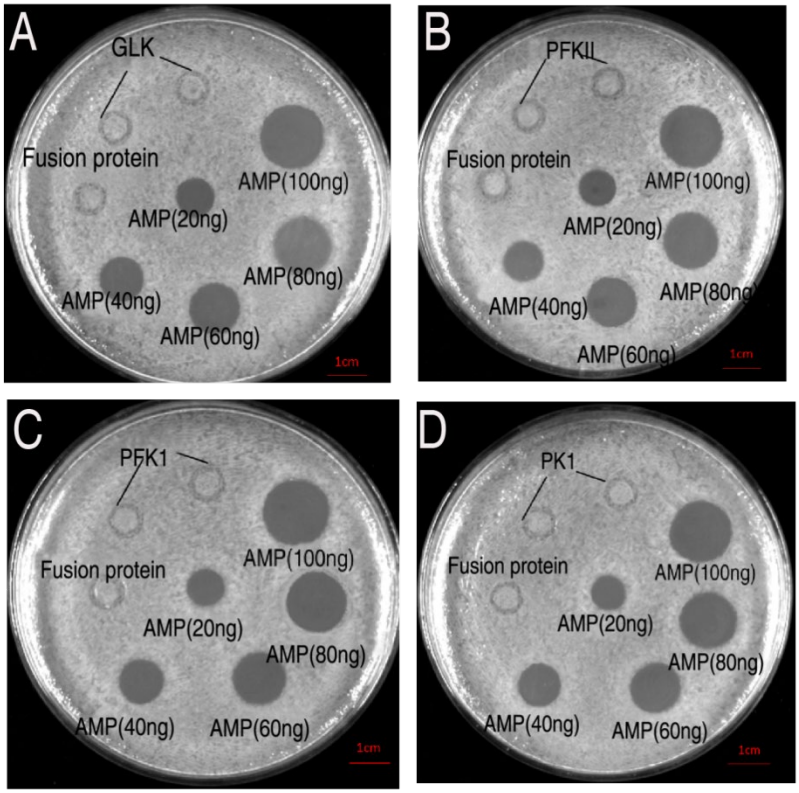

Fig. S4 Oxford cup test for ampicillin-binding capability of GLK (A), PFKII (B), PFK1 (C) and PK1 (D). Purified recombinant proteins (GLK, PFK1, PFKII, and PK1) were separately mixed with ampicillin and then proteins were precipitated by acetone. The precipitated proteins were diluted and used for the test. Fusion protein was used as negative control.

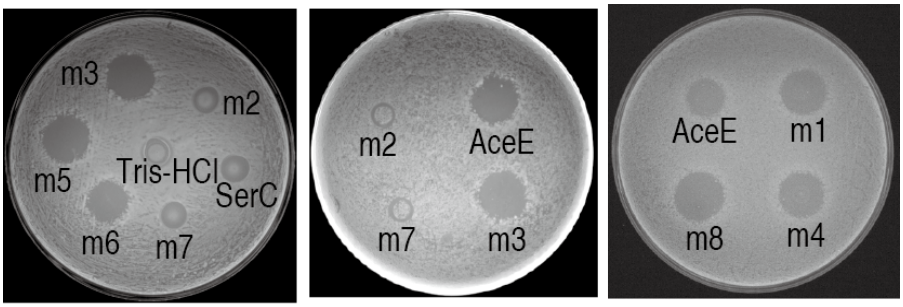

Fig. S5 Oxford cup test for ampicillin-binding capability of single amino point-mutated AceE. G232A (m1), G232S (m2), G232M (m3), N259Q (m4), C260S (m5), C264N (m6), R266A (m7), D266A (m8). SerC, phosphoserine/phosphohydroxythreonine aminotransferase, is used as a recombinant protein control.

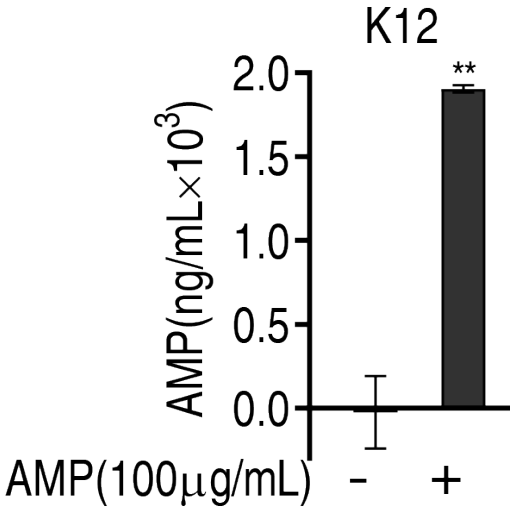

Fig. S6 Intracellular ampicillin concentration in the presence or absence of 100 μg/mL ampicillin. Results are displayed as mean ± SEM, and statistically significant differences are identified by Kruskal–Wallis followed by Dunn's multiple comparison post hoc test. **, $P < 0.01$.

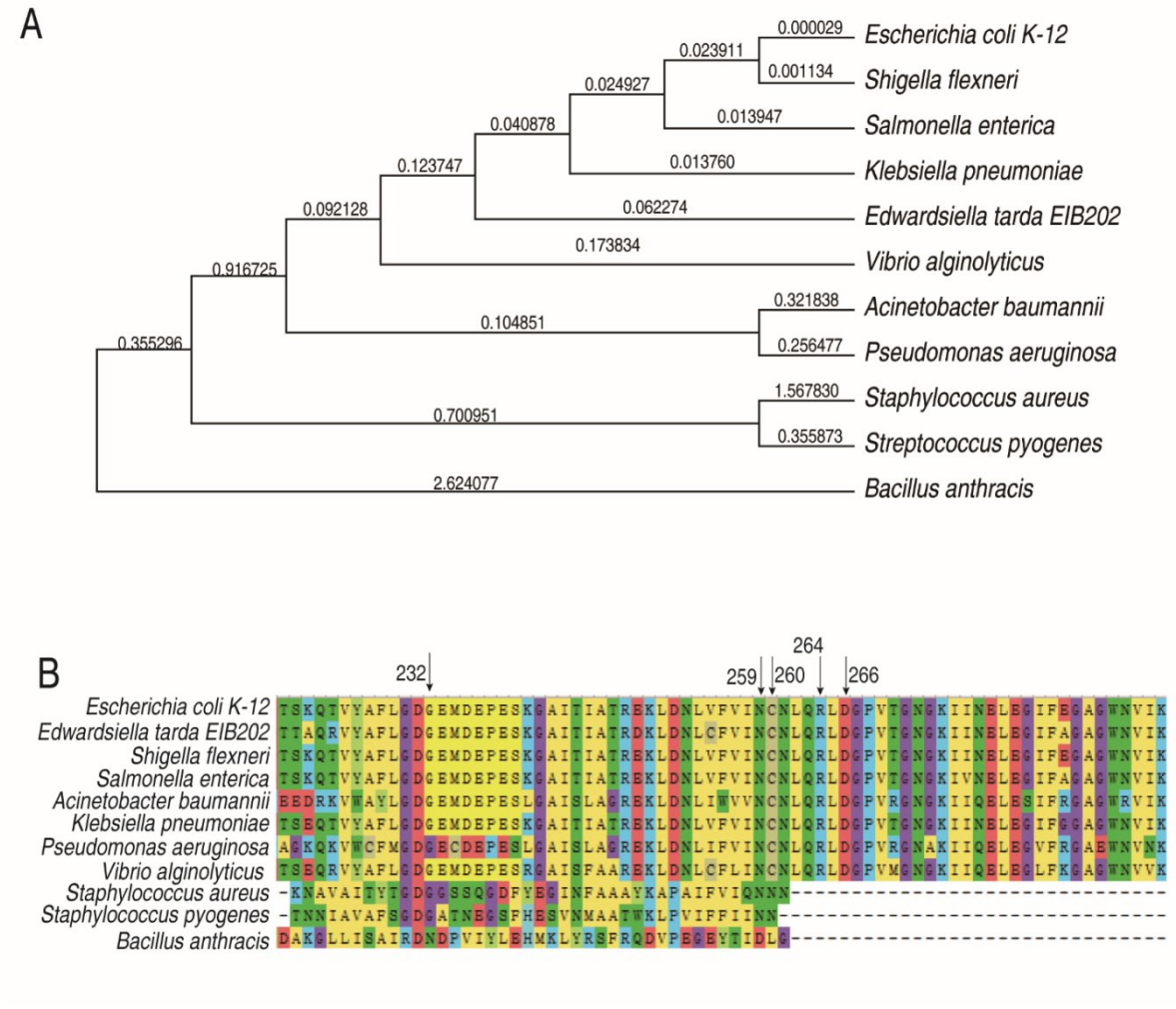

Fig. S7 Blast analysis of the putative binding pocket in AceE positions 229-269 as primers. (A) Phylogenetic tree. Branch length values are indicated above the branch. The phylogenetic tree was constructed by using the ML method with 100 bootstraps (MEGA version 11, www.megasoftware.net). (**B**) Blast analysis of the putative binding pocket. The indicated amino acid sequences of AceE was aligned by ClustaIW. Genome accession number, PRJNA909844

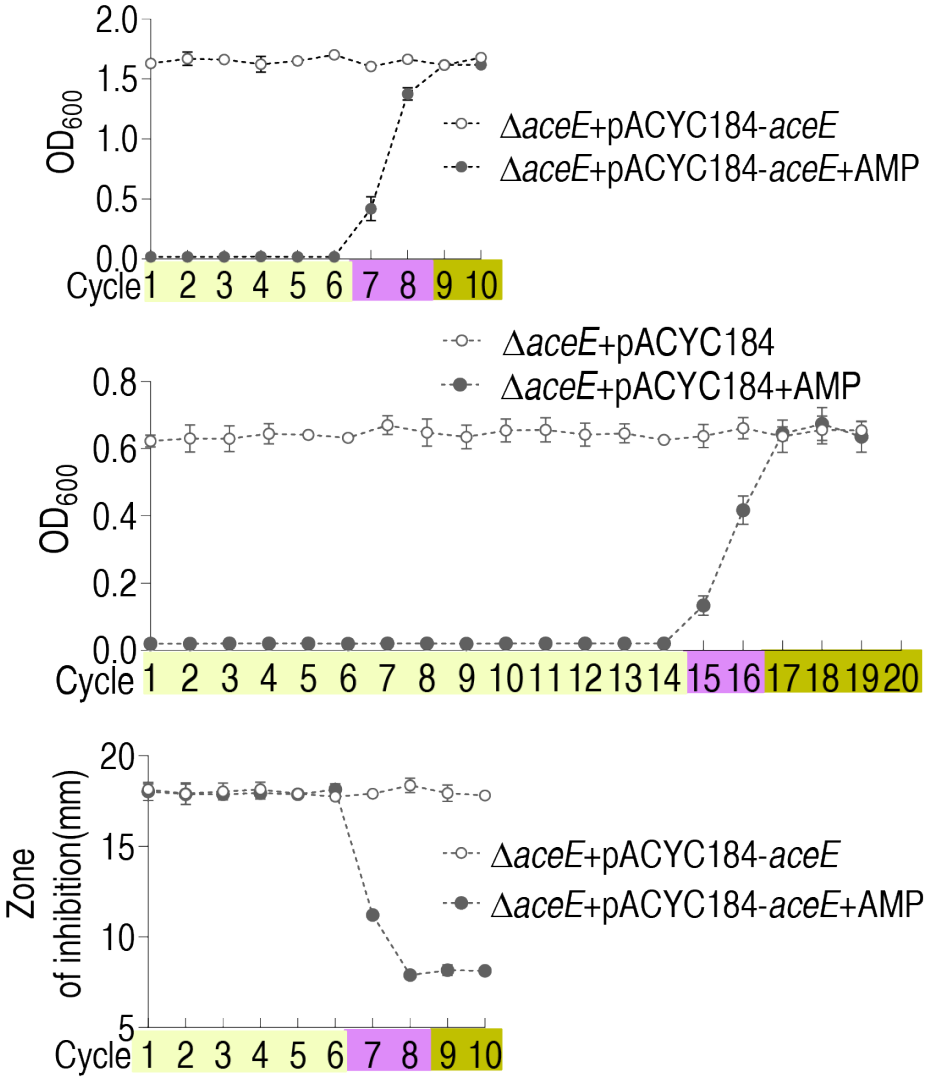

Fig. S8 Growth/viability of Δ*aceE*-pACYC184 cells (upper) and Δ*aceE*-pACYC184-*aceE* cells (middle) and MIC of Δ*aceE*-pACYC184-*aceE* cells (lower) were estimated from OD_{600} and zone, respectively, after indicated number of cycles of exposure to ampicillin. Bacteria are treated using 100 μg/mL ampicillin for 4.5 h and then grown at 37 ^{0}C for 16h. Δ*aceE*-pACYC184 + AMP, with ampicillin; Δ*aceE*-pACYC184, without ampicillin.

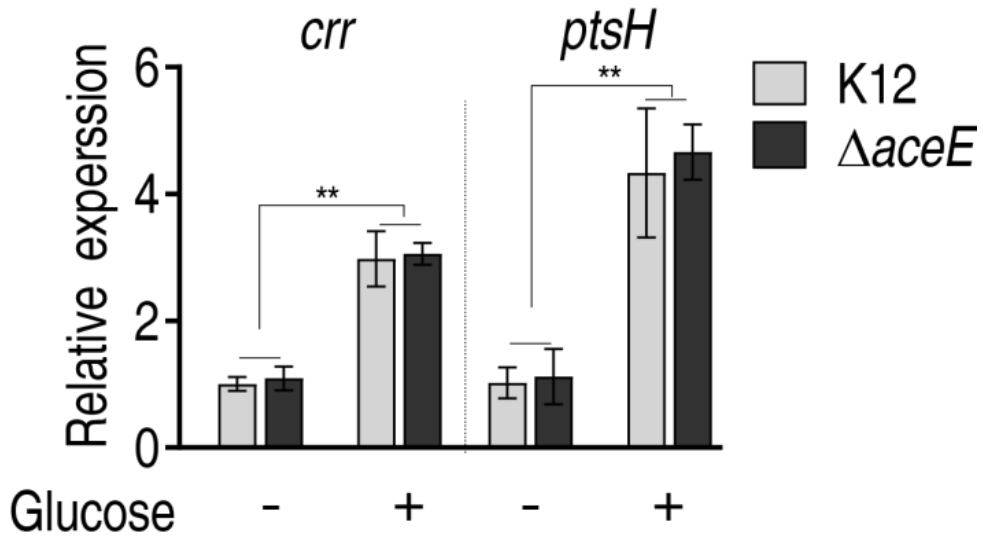

Fig. S9 qRT-PCR for expression of *crr* and *pstH* in the presence or absence of glucose in K12 and *aceE*-deleted mutant.

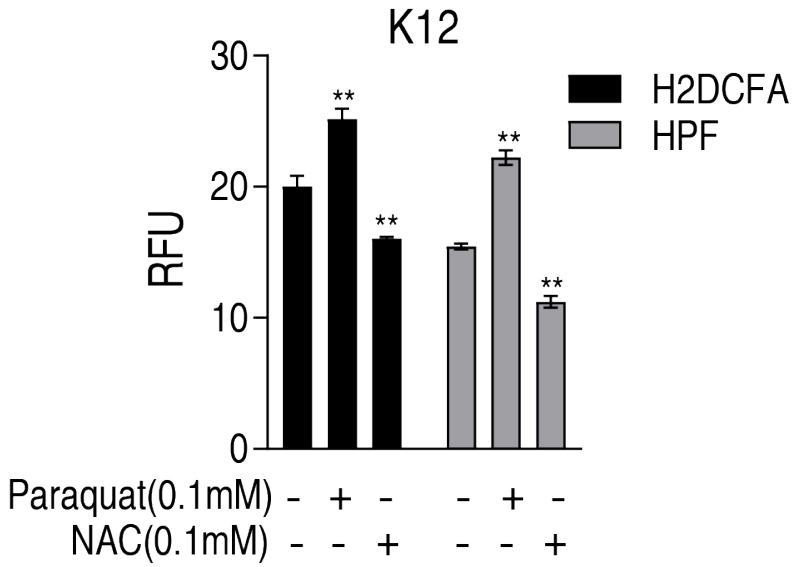

Fig. S10 Evaluation of ROS-reactive dyes H2DCFA and HPF via promoter and inhibitor in K12. Promoter paraquat and inhibitor N-acetylcysteine were used positive and negative controls to ascertain the two probes. Correspondingly, RFU of H2DCFA and HPF was promoted and inhibited by paraquat and N-acetylcysteine. Results are displayed as mean ± SEM, and statistically significant differences are identified by Kruskal–Wallis followed by Dunn's multiple comparison post hoc test. **, $P < 0.01$.

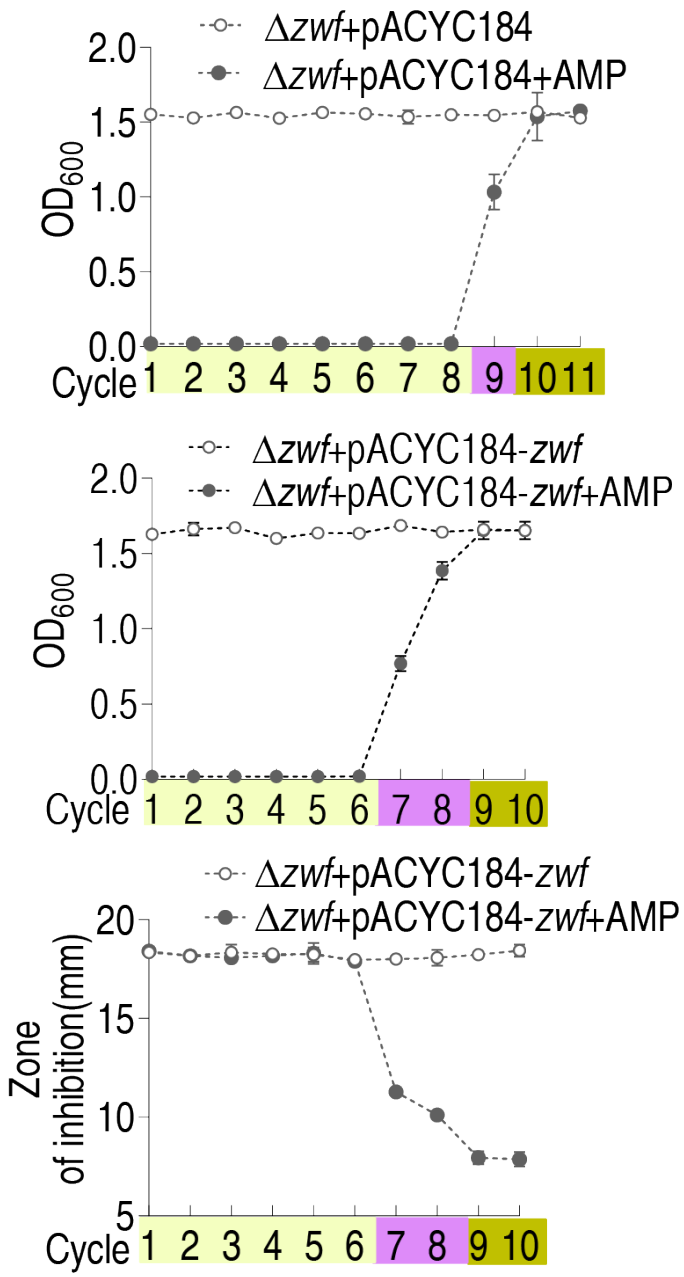

Fig. S11 Growth/viability of Δ*zwf*-pACYC184 cells (upper) and Δ*zwf*-pACYC184-*zwf* cells (middle) and MIC of Δ*zwf*-pACYC184-*zwf* cells (lower) were estimated from OD_{600} and zone, respectively, after indicated number of cycles of exposure to ampicillin. Bacteria are treated using 100 μg/mL ampicillin for 4.5 h and then grown at 37 ^{0}C for 16h. Δ*zwf*-pACYC184 + AMP, with ampicillin; Δ*zwf*-pACYC184, without ampicillin.

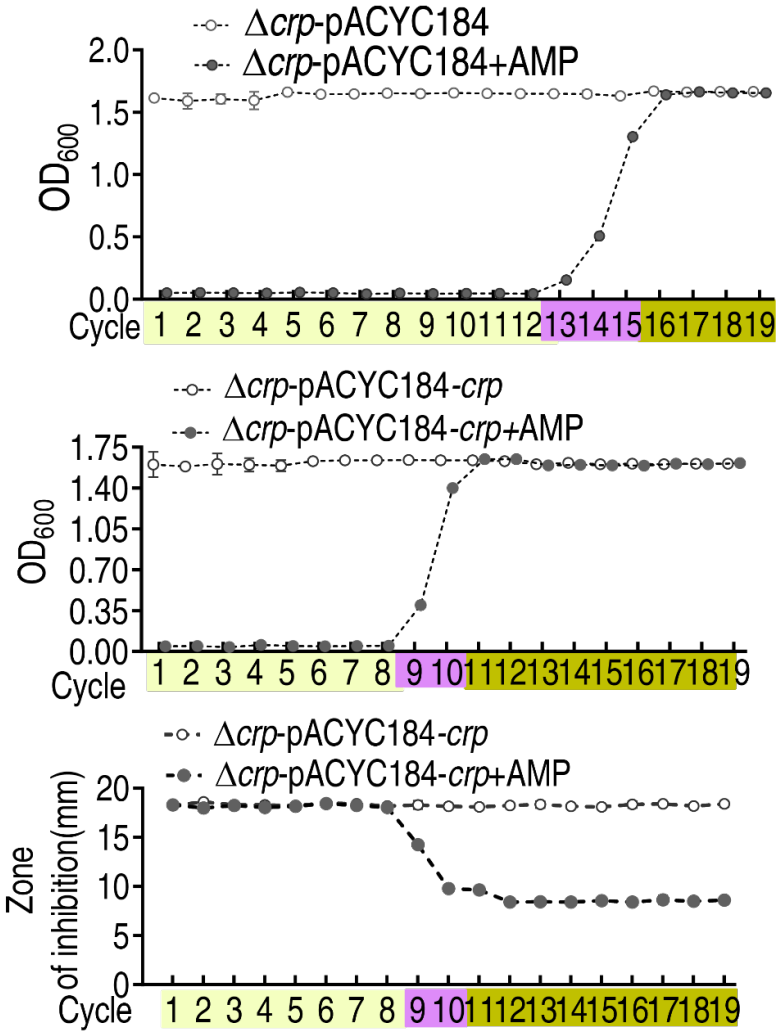

Fig. S12 Growth/viability of Δ*crp*-pACYC184 cells (upper) and Δ*crp*-pACYC184-*crp* cells (middle) and MIC of Δ*crp*-pACYC184-*crp* cells (lower) were estimated from OD_{600} and zone, respectively, after indicated number of cycles of exposure to ampicillin. Bacteria are treated using 100 μg/mL ampicillin for 4.5 h and then grown at 37 ^{0}C for 16h. Δ*crp*-pACYC184 + AMP, with ampicillin; Δ*crp*-pACYC184, without ampicillin.

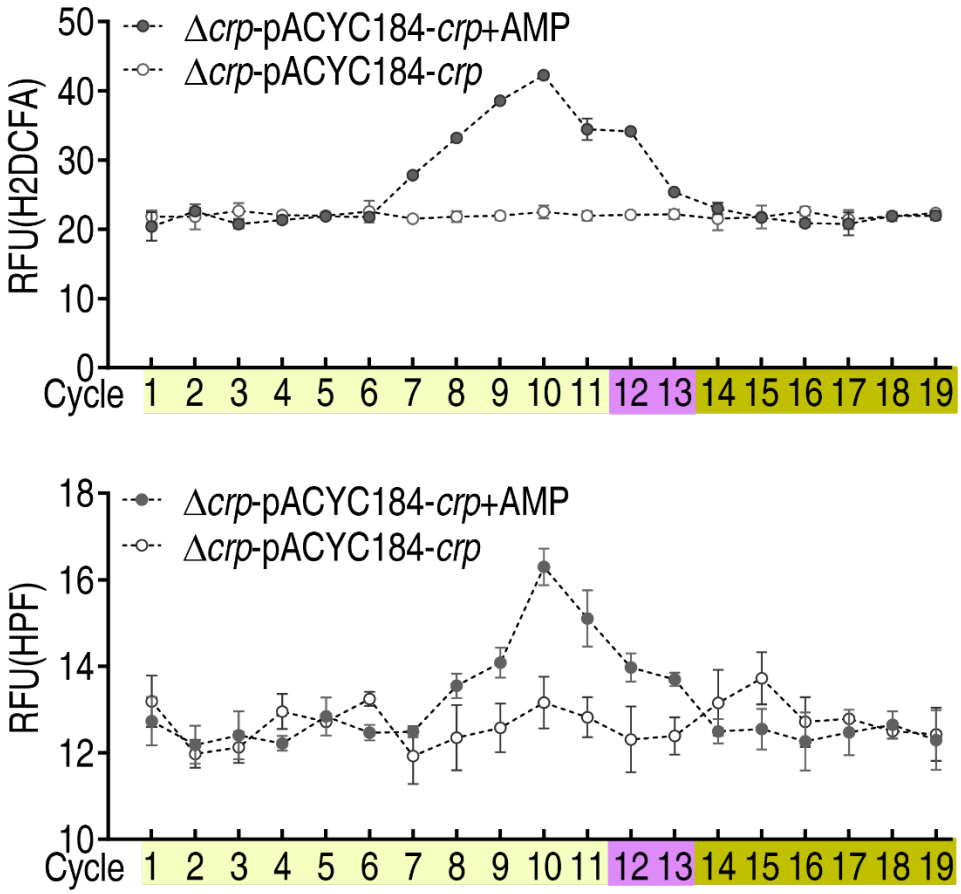

Fig. S13 ROS of Δ*crp* plus vector control in the presence or absence of ampicillin. Bacteria are treated using 100 μg/mL ampicillin for 4.5 h and then grown at 37 ^{0}C for 16h. Δ*crp*-pACYC184 + AMP, with ampicillin; Δ*crp*-pACYC184, without ampicillin.

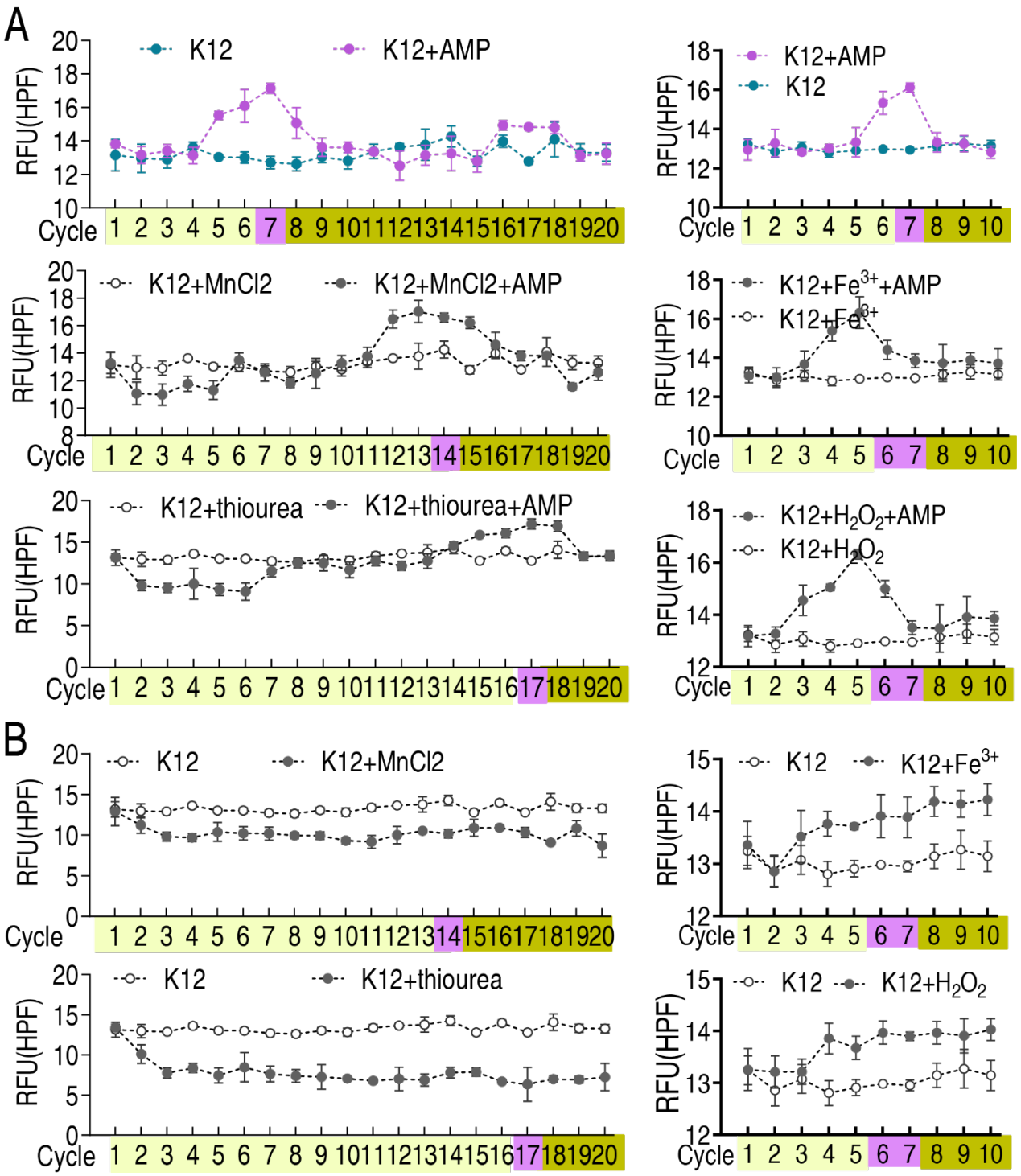

Fig. S14. ROS of K12 exposed to $MnCl_2$, thiourea (A), $FeCl_3$ or H_2O_2 (B) in the presence or absence of ampicillin. Bacteria are treated using 100 μg/mL ampicillin for 4.5 h and then grown at 37 ^{0}C for 16h.

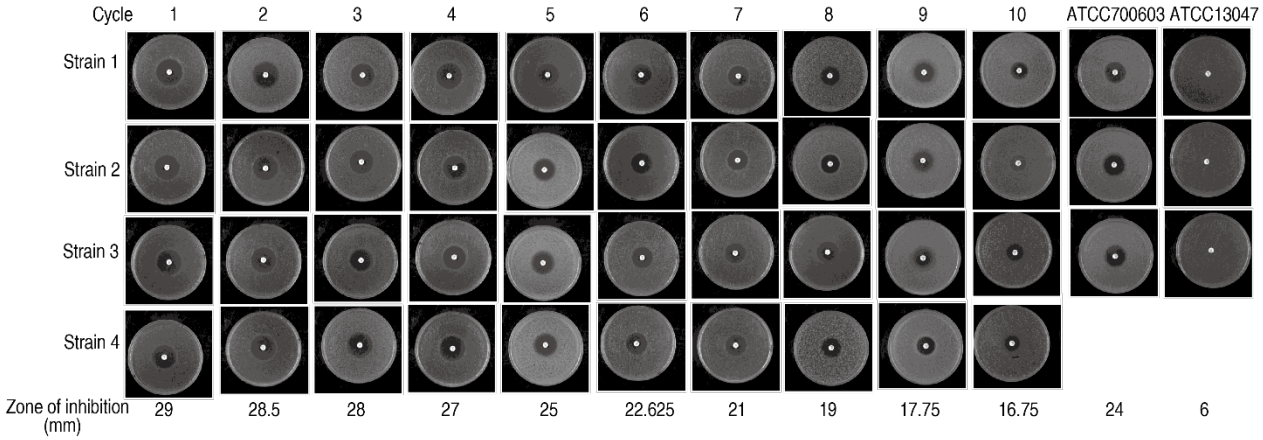

Fig. S15 Activity of AmpC in the evolutionary cycle using susceptibility test disks (Oxoid), according to 2010 CLSI guidelines. Bacteria cultured in 0.625 μg/mL ampicillin at 37 °C for 16 h were adjusted to a 0.5 McFarland standard. These bacteria were uniformly inoculated with Mueller-Hinton agar and then a 30 μg cefoxitin disk was placed at the center of the disk. After overnight incubation at 37 °C, zone of inhibition was detected. Zone of inhibition 18 was defined as a positive threshold. *Klebsiella pneumoniae* ATCC700603 and *Enterobacter cloacae* ATCC13047 were used as negative and positive controls, respectively.

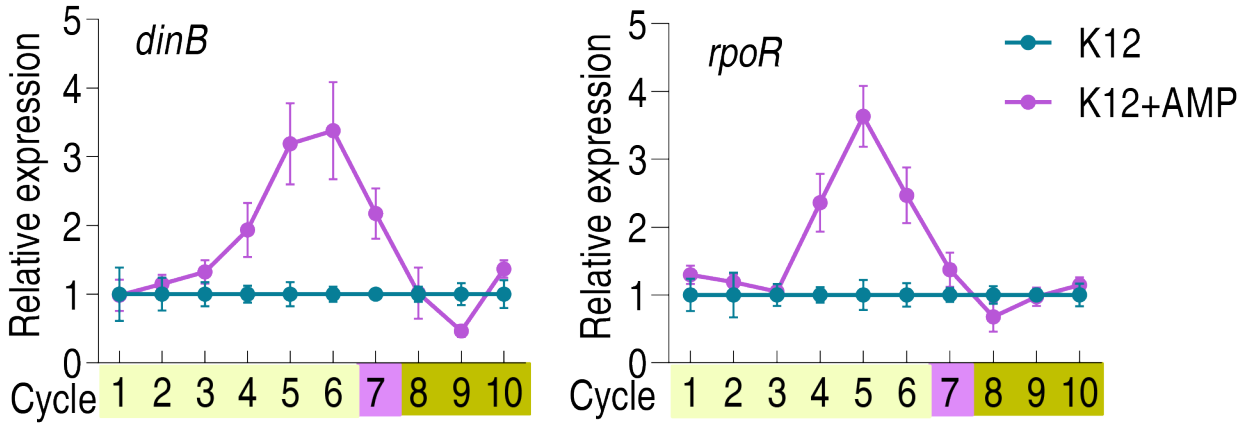

Fig. S16 qRT-PCR for expression of *rpoS* and *dinB* in the evolutionary cycle.

A

Site	Number sample	Variantion_type	Gene	Gene_ID	Mutation_c	Mutation_p	Presence	Proportion
190570	1	missense_variant	*dxr*	BW25113_0173	563G>C	Gly188Ala	2	1/20
442807	1	LOF	*cyoE*	BW25113_0428	--	--	9	1/20
444022	1	missense_variant	*cyoC*	BW25113_0430	95A>G	Tyr32Cys	4	1/20
444023	1	missense_variant	*cyoC*	BW25113_0430	94T>C	Tyr32His	4	1/20
444026	1	missense_variant	*cyoC*	BW25113_0430	91A>T	Ile31Phe	4	1/20
494641	1	synonymous_variant	*aes*	BW25113_0476	789G>A	Pro263Pro	2	1/20
550208	5	missense_variant	*cysS*	BW25113_0526	142G>A	Asp48Asn	6	5/20
1368466	179	synonymous_variant	*ycjP*	BW25113_1312	120G>A	Lys40Lys	all	full
1613605	1	missense_variant	*marR*	BW25113_1530	229C>A	Arg77Ser	6	1/20
1649891	1	missense_variant	*ynfA*	BW25113_1582	40T>A	Cys14Ser	4	1/20
2236298	1	missense_variant	*yeiB*	BW25113_2152	149G>T	Trp50Leu	4	1/20
2922492	2	missense_variant	*sdaC*	BW25113_2796	905C>T	Ala302Val	5	2/20
3493210	1	upstream_gene_variant	*frlA*	BW25113_3370	-59C>T	--	9	1/20
3521411	1	missense_variant	*yrfF*	BW25113_3398	1584C>A	Asn528Lys	4	1/20
3528780	1	missense_variant	*envZ*	BW25113_3404	448A>C	Thr150Pro	8	1/20
3528809	42	missense_variant	*envZ*	BW25113_3404	419C>T	Ser140Leu	7, 8, 9	3/20, 19/20, 20/20
3529300	6	missense_variant	*ompR*	BW25113_3405	644A>G	Asp215Gly	7	6/20
3621421	1	missense_variant	*rbbA*	BW25113_3486	1478A>T	Glu493Val	4	1/20
3751903	1	missense_variant	*selB*	BW25113_3590	1319C>T	Pro440Leu	1	1/20
3846511	1	ncRNA_variant	*istR*	BW25113_4616	--	--	4	1/20
3921357	1	synonymous_variant	*asnA*	BW25113_3744	843G>A	Ala281Ala	6	1/20
3965390	1	conservative_inframe_insertion	*wecC*	BW25113_3787	778_783dupCCTGGC	Pro260_Gly261dup	7	1/20
4010877	1	missense_variant	*rmuC*	BW25113_3832	185G>A	Cys62Tyr	3	1/19
4094336	1	missense_variant	*cpxA*	BW25113_3911	568G>A	Ala190Thr	8	1/20
4094834	1	conservative_inframe_deletion	*cpxA*	BW25113_3911	64_69delATGTTG	Met22_Leu23del	8	1/20
4431404	1	missense_variant	*msrA*	BW25113_4219	590G>A	Gly197Asp	5	1/20
4544972	1	missense_variant	*uxuR*	BW25113_4324	580C>A	Arg194Ser	1	1/20

B

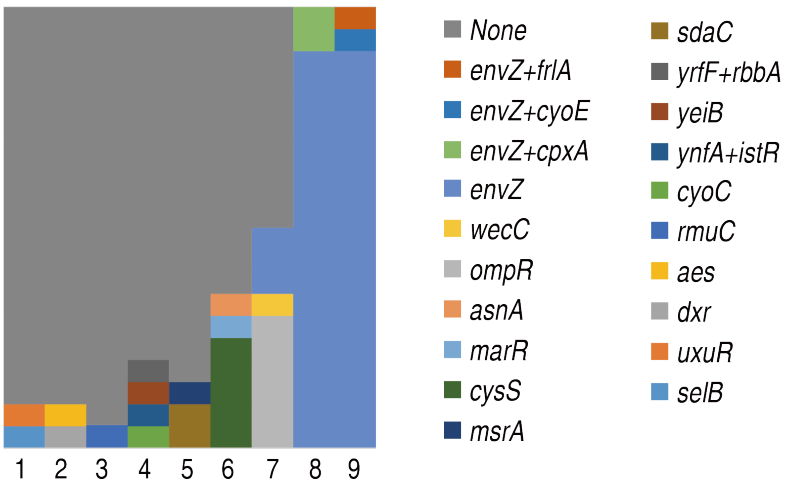

Fig. S17 Whole genome sequencing and bioinformatics analysis. *E. coli* BW25113 cells with and without ampicillin were cycled for 9 cycles. A total of 20 clones with ampicillin and 3 clones without ampicillin each cycle were collected. Whole genome was sequenced by Biomarker Technologies (Beijing, China) with Illumina platforms, yielding 206 data sets (one absent in third cycle with ampicillin). A total 27 mutations involved in 23 genes were found. Among these

mutations, mutation of *ycjP* (site1368466) was determined in every sample, which was estimated to be a difference site in the primary bacteria relative to the reference genome itself, so this site was excluded in the subsequent analysis. The other 26 mutations involved in the other 22 genes were identified only in samples with ampicillin. Specifically, only mutations in *envZ* (working for two-component system) were detected continuously in the 7th, 8th, and 9th cycles. Mutations in the other genes were detected only in one cycle and not in the others. *envZ* had 3 mutations in 20 clones in the 7^{th} cycle and all of the 20 clones in the 8^{th} and 9^{th} cycles had mutations, among which one clone in the 8^{th} cycle had mutation at 3528780, and the other clones had mutations at 3528809. In addition to *envZ* mutations, *envZ* + *cyoE* (the 9^{th} cycle; *cyoE* works for electron transport chain), *envZ* + *frlA* (the 9^{th} cycle; *frlA* works for fructose lysine transporter) and *envZ* + *cpxA* (the 8^{th} cycle; *cpxA* works for two-component system) appeared simultaneously in the 8-9^{th} cycles. (A) The mutant genes and species. (B) Graph showing these mutated genes related to the evolutionary cycle.

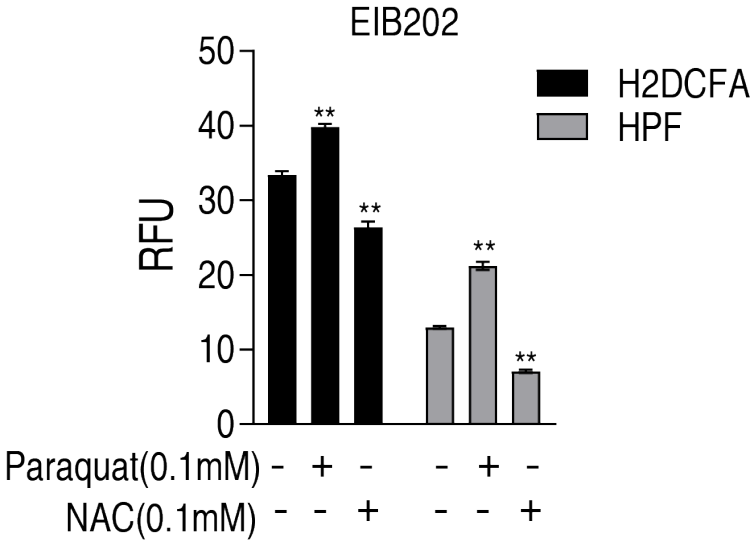

Fig. S18 Evaluation of ROS-reactive dyes H2DCFA and HPF in via promoter and inhibitor *E. tarda*. Promoter and inhibitor N-acetylcysteine were used positive and negative controls to ascertain the two probes. Correspondingly, RFU of H2DCFA and HPF was promoted and inhibited by paraquat and N-acetylcysteine. Results are displayed as mean ± SEM, and statistically significant differences are identified by Kruskal–Wallis followed by Dunn's multiple comparison post hoc test. **, $P < 0.01$.

Table S1 MIC list on cells of the evolutionary cycle, measured by the Oxford cup method

K12

Strain(Cycle)	MIC	Strain(Cycle)	MIC	Strain(Cycle)	MIC	Strain(Cycle)	MIC
K12(1)	18	K12+AMP(1)	18.1	K12+glucose(1)	20.1	K12+glucose+AMP(1)	20
K12(2)	18.2	K12+AMP(2)	17.9	K12+glucose(2)	19.9	K12+glucose+AMP(2)	20.1
K12(3)	18	K12+AMP(3)	18.1	K12+glucose(3)	20	K12+glucose+AMP(3)	19.9
K12(4)	17.9	K12+AMP(4)	18	K12+glucose(4)	20.1	K12+glucose+AMP(4)	20.4
K12(5)	18	K12+AMP(5)	18	K12+glucose(5)	20	K12+glucose+AMP(5)	20
K12(6)	17.9	K12+AMP(6)	18	K12+glucose(6)	20.1	K12+glucose+AMP(6)	20.1
K12(7)	18.1	K12+AMP(7)	9.9	K12+glucose(7)	20.1	K12+glucose+AMP(7)	20.1
K12(8)	18	K12+AMP(8)	8.4	K12+glucose(8)	20.1	K12+glucose+AMP(8)	20.1
K12(9)	17.9	K12+AMP(9)	8.5	K12+glucose(9)	20.3	K12+glucose+AMP(9)	20.1
K12(10)	18	K12+AMP(10)	8.5	K12+glucose(10)	19.9	K12+glucose+AMP(10)	19.7
△*aceE*(1)	16.6	△*aceE*+AMP(1)	16.5	K12+glucose(11)	19.9	K12+glucose+AMP(11)	20.1
△*aceE*(2)	16.3	△*aceE*+AMP(2)	16.8	K12+glucose(12)	20.2	K12+glucose+AMP(12)	13.6
△*aceE*(3)	16.5	△*aceE*+AMP(3)	16.5	K12+glucose(13)	20.1	K12+glucose+AMP(13)	8.7
△*aceE*(4)	16.3	△*aceE*+AMP(4)	16.6	K12+glucose(14)	19.9	K12+glucose+AMP(14)	8.5
△*aceE*(5)	16.7	△*aceE*+AMP(5)	16.7	K12+glucose(15)	20.1	K12+glucose+AMP(15)	8.3
△*aceE*(6)	16.5	△*aceE*+AMP(6)	16.4	K12+H_2O_2(1)	18.2	K12+H_2O_2+AMP(1)	17.8
△*aceE*(7)	16.5	△*aceE*+AMP(7)	16.6	K12+H_2O_2(2)	17.8	K12+H_2O_2+AMP(2)	18.1
△*aceE*(8)	16.6	△*aceE*+AMP(8)	16.4	K12+H_2O_2(3)	18.1	K12+H_2O_2+AMP(3)	18
△*aceE*(9)	16.5	△*aceE*+AMP(9)	16.5	K12+H_2O_2(4)	18	K12+H_2O_2+AMP(4)	17.9
△*aceE*(10)	16.4	△*aceE*+AMP(10)	16.7	K12+H_2O_2(5)	17.8	K12+H_2O_2+AMP(5)	18
△*aceE*(11)	16.7	△*aceE*+AMP(11)	16.9	K12+H_2O_2(6)	17.9	K12+H_2O_2+AMP(6)	12.2
△*aceE*(12)	16.7	△*aceE*+AMP(12)	16.5	K12+H_2O_2(7)	17.8	K12+H_2O_2+AMP(7)	8.6
△*aceE*(13)	16.6	△a*ceE*+AMP(13)	16.4	K12+H_2O_2(8)	17.8	K12+H_2O_2+AMP(8)	7.9
△*aceE*(14)	16.5	△*aceE*+AMP(14)	16.5	K12+H_2O_2(9)	18.1	K12+H_2O_2+AMP(9)	8.2
△*aceE*(15)	16.6	△*aceE*+AMP(15)	16.8	K12+H_2O_2(10)	17.9	K12+H_2O_2+AMP(10)	7.8
△a*ceE*(16)	16.5	△*aceE*+AMP(16)	10.2	K12+H_2O_2(11)	18.3	K12+H_2O_2+AMP(11)	8.1
△*aceE*(17)	16.5	△*aceE*+AMP(17)	8.6	K12+Fe^{3+}(1)	17.7	K12+Fe^{3+}+AMP(1)	18.1
△*aceE*(18)	16.5	△*aceE*+AMP(18)	8.6	K12+Fe^{3+}(2)	18	K12+Fe^{3+}+AMP(2)	17.9
△*aceE*(19)	16.4	△*aceE*+AMP(19)	8.5	K12+Fe^{3+}(3)	18	K12+Fe^{3+}+AMP(3)	18.1
△*crr*(1)	16.7	△*crr*+AMP(1)	16.7	K12+Fe^{3+}(4)	18.2	K12+Fe^{3+}+AMP(4)	18.3
△*crr*(2)	16.7	△*crr*+AMP(2)	16.3	K12+Fe^{3+}(5)	18	K12+Fe^{3+}+AMP(5)	18.3
△*crr*(3)	16.7	△*crr*+AMP(3)	16.9	K12+Fe^{3+}(6)	18.1	K12+Fe^{3+}+AMP(6)	13.1
△*crr*(4)	16.6	△*crr*+AMP(4)	16.6	K12+Fe^{3+}(7)	17.8	K12+Fe^{3+}+AMP(7)	8.4
△*crr*(5)	16.5	△*crr*+AMP(5)	11.3	K12+Fe^{3+}(8)	18.1	K12+Fe^{3+}+AMP(8)	7.9
△*crr*(6)	16.4	△*crr*+AMP(6)	8.4	K12+Fe^{3+}(9)	18.2	K12+Fe^{3+}+AMP(9)	8.3
△*crr*(7)	16.6	△*crr*+AMP(7)	8.5	K12+Fe^{3+}(10)	18.2	K12+Fe^{3+}+AMP(10)	7.8
△*ptsH*(1)	16.9	△*ptsH*+AMP(1)	17	K12+Fe^{3+}(11)	17.8	K12+Fe^{3+}+AMP(11)	8.2
△*ptsH*(2)	16.7	△*ptsH*+AMP(2)	16.5	K12+$MnCl_2$(1)	18.1	K12+$MnCl_2$+AMP(1)	17.8
△*ptsH*(3)	16.6	△*ptsH*+AMP(3)	16.4	K12+$MnCl_2$(2)	17.9	K12+$MnCl_2$+AMP(2)	18.2
△*ptsH*(4)	16.7	△*ptsH*+AMP(4)	16.5	K12+$MnCl_2$(3)	18.3	K12+$MnCl_2$+AMP(3)	18.1
△*ptsH*(5)	16.8	△*ptsH*+AMP(5)	11.1	K12+$MnCl_2$(4)	18	K12+$MnCl_2$+AMP(4)	17.7
△*ptsH*(6)	16.6	△*ptsH*+AMP(6)	9.3	K12+$MnCl_2$(5)	17.9	K12+$MnCl_2$+AMP(5)	18.1
△*ptsH*(7)	16.4	△*ptsH*+AMP(7)	8.3	K12+$MnCl_2$(6)	18	K12+$MnCl_2$+AMP(6)	18

△*zwf*(1)	17.1	△*zwf*+AMP(1)	17.2	K12+$MnCl_2$(7)	17.9	K12+$MnCl_2$+AMP(7)	18.3
△*zwf*(2)	17	△*zwf*+AMP(2)	17.1	K12+$MnCl_2$(8)	17.7	K12+$MnCl_2$+AMP(8)	17.9
△*zwf*(3)	17.1	△*zwf*+AMP(3)	17.1	K12+$MnCl_2$(9)	17.8	K12+$MnCl_2$+AMP(9)	18.1
△*zwf*(4)	17.2	△*zwf*+AMP(4)	17.2	K12+$MnCl_2$(10)	18.3	K12+$MnCl_2$+AMP(10)	17.9
△*zwf*(5)	17.2	△*zwf*+AMP(5)	17.2	K12+$MnCl_2$(11)	18.2	K12+$MnCl_2$+AMP(11)	17.9
△*zwf*(6)	17.2	△*zwf*+AMP(6)	17.2	K12+$MnCl_2$(12)	17.7	K12+$MnCl_2$+AMP(12)	18.1
△*zwf*(7)	17.1	△*zwf*+AMP(7)	17.1	K12+$MnCl_2$(13)	18	K12+$MnCl_2$+AMP(13)	14.8
△*zwf*(8)	17.2	△*zwf*+AMP(8)	17.2	K12+$MnCl_2$(14)	18.2	K12+$MnCl_2$+AMP(14)	13.2
△*zwf*(9)	17	△*zwf*+AMP(9)	13.8	K12+$MnCl_2$(15)	18.1	K12+$MnCl_2$+AMP(15)	12.8
△*zwf*(10)	17.1	△*zwf*+AMP(10)	8.5	K12+$MnCl_2$(16)	17.9	K12+$MnCl_2$+AMP(16)	10.9
△*zwf*(11)	17.1	△*zwf*+AMP(11)	8.3	K12+$MnCl_2$(17)	18	K12+$MnCl_2$+AMP(17)	8.6
△*sodB*(1)	18	△*sodB*+AMP(1)	18.1	K12+$MnCl_2$(18)	18	K12+$MnCl_2$+AMP(18)	8.5
△*sodB*(2)	18.1	△*sodB*+AMP(2)	18.1	K12+$MnCl_2$(19)	17.9	K12+$MnCl_2$+AMP(19)	8.5
△*sodB*(3)	18.1	△*sodB*+AMP(3)	18.1	K12+$MnCl_2$(20)	18	K12+$MnCl_2$+AMP(20)	8.5
△*sodB*(4)	18	△*sodB*+AMP(4)	18.1	K12+Thiourea(1)	18	K12+Thiourea+AMP(1)	17.9
△*sodB*(5)	18	△*sodB*+AMP(5)	12	K12+Thiourea(2)	18.1	K12+Thiourea+AMP(2)	18
△*sodB*(6)	18	△*sodB*+AMP(6)	8.5	K12+Thiourea(3)	17.9	K12+Thiourea+AMP(3)	18.2
△*sodB*(7)	18.2	△*sodB*+AMP(7)	8.2	K12+Thiourea(4)	17.9	K12+Thiourea+AMP(4)	18.2
△*sodB*(8)	18.1	△*sodB*+AMP(8)	8.2	K12+Thiourea(5)	18	K12+Thiourea+AMP(5)	18
△*crp*(1)	17	△*crp*+AMP(1)	16.8	K12+Thiourea(6)	18	K12+Thiourea+AMP(6)	17.9
△*crp*(2)	16.8	△*crp*+AMP(2)	17.1	K12+Thiourea(7)	18.1	K12+Thiourea+AMP(7)	18.1
△*crp*(3)	17.2	△*crp*+AMP(3)	16.9	K12+Thiourea(8)	18.2	K12+Thiourea+AMP(8)	18
△*crp*(4)	17	△*crp*+AMP(4)	16.9	K12+Thiourea(9)	18	K12+Thiourea+AMP(9)	18.1
△*crp*(5)	17	△*crp*+AMP(5)	17.2	K12+Thiourea(10)	18	K12+Thiourea+AMP(10)	17.9
△*crp*(6)	17.1	△*crp*+AMP(6)	16.9	K12+Thiourea(11)	17.9	K12+Thiourea+AMP(11)	18.1
△*crp*(7)	16.8	△*crp*+AMP(7)	17.1	K12+Thiourea(12)	18	K12+Thiourea+AMP(12)	18.1
△*crp*(8)	17.1	△*crp*+AMP(8)	17.1	K12+Thiourea(13)	18	K12+Thiourea+AMP(13)	18.1
△*crp*(9)	16.9	△*crp*+AMP(9)	17	K12+Thiourea(14)	18.2	K12+Thiourea+AMP(14)	17.9
△*crp*(10)	17	△*crp*+AMP(10)	17.1	K12+Thiourea(15)	18.1	K12+Thiourea+AMP(15)	17.8
△*crp*(11)	16.9	△*crp*+AMP(11)	17.1	K12+Thiourea(16)	18.1	K12+Thiourea+AMP(16)	18
△*crp*(12)	17.3	△*crp*+AMP(12)	14.9	K12+Thiourea(17)	17.9	K12+Thiourea+AMP(17)	13.8
△*crp*(13)	17	△*crp*+AMP(13)	9.6	K12+Thiourea(18)	17.9	K12+Thiourea+AMP(18)	8.6
△*crp*(14)	17.1	△*crp*+AMP(14)	8.6	K12+Thiourea(19)	17.8	K12+Thiourea+AMP(19)	8.6
△*crp*(15)	16.9	△*crp*+AMP(15)	8.5	K12+Thiourea(20)	17.7	K12+Thiourea+AMP(20)	8.5
△*crp*(16)	16.8	△*crp*+AMP(16)	8.5	△*crp*-pACYC184-*crp*(1)	18.3	△*crp*-pACYC184-*crp*+AMP(1)	18.3
△*crp*(17)	17.1	△*crp*+AMP(17)	8.5	△*crp*-pACYC184-*crp*(2)	18.6	△*crp*-pACYC184-*crp*+AMP(2)	18
△*crp*(18)	17.2	△*crp*+AMP(18)	8.4	△*crp*-pACYC184-*crp*(3)	18.3	△*crp*-pACYC184-*crp*+AMP(3)	18.3
△*crp*(19)	17	△*crp*+AMP(19)	8.7	△*crp*-pACYC184-*crp*(4)	18.3	△*crp*-pACYC184-*crp*+AMP(4)	18.1
				△*crp*-pACYC184-*crp*(5)	18.3	△*crp*-pACYC184-*crp*+AMP(5)	18.2
				△*crp*-pACYC184-*crp*(6)	18.5	△*crp*-pACYC184-*crp*+AMP(6)	18.5
				△*crp*-pACYC184-*crp*(7)	18.4	△*crp*-pACYC184-*crp*+AMP(7)	18.3
				△*crp*-pACYC184-*crp*(8)	18.2	△*crp*-pACYC184-*crp*+AMP(8)	18.1
				△*crp*-pACYC184-*crp*(9)	18.3	△*crp*-pACYC184-*crp*+AMP(9)	14.3
				△*crp*-pACYC184-*crp*(10)	18.2	△*crp*-pACYC184-*crp*+AMP(10)	9.8
				△*crp*-pACYC184-*crp*(11)	18.1	△*crp*-pACYC184-*crp*+AMP(11)	9.7
				△*crp*-pACYC184-*crp*(12)	18.3	△*crp*-pACYC184-*crp*+AMP(12)	8.4
				△*crp*-pACYC184-*crp*(13)	18.4	△*crp*-pACYC184-*crp*+AMP(13)	8.5

	△*crp*-pACYC184-*crp*(14)	18.2	△*crp*-pACYC184-*crp*+AMP(14)	8.4
	△*crp*-pACYC184-*crp*(15)	18.1	△*crp*-pACYC184-*crp*+AMP(15)	8.6
	△*crp*-pACYC184-*crp*(16)	18.4	△*crp*-pACYC184-*crp*+AMP(16)	8.4
	△*crp*-pACYC184-*crp*(17)	18.4	△*crp*-pACYC184-*crp*+AMP(17)	8.7
	△*crp*-pACYC184-*crp*(18)	18.2	△*crp*-pACYC184-*crp*+AMP(18)	8.5
	△*crp*-pACYC184-*crp*(19)	18.4	△*crp*-pACYC184-*crp*+AMP(19)	8.6

EIB202

Strain(Cycle)	MIC	Strain(Cycle)	MIC	Strain(Cycle)	MIC	Strain(Cycle)	MIC
EIB202(1)	17.5	EIB202+AMP(1)	17.6	△*ptsH*(1)	17.9	△*ptsH*+AMP(1)	18.1
EIB202(2)	17.8	EIB203+AMP(2)	17.4	△*ptsH*(2)	17.9	△*ptsH*+AMP(2)	17.7
EIB202(3)	17.5	EIB204+AMP(3)	17.6	△*ptsH*(3)	17.8	△*ptsH*+AMP(3)	17.9
EIB202(4)	17.6	EIB205+AMP(4)	17.6	△*ptsH*(4)	17.7	△*ptsH*+AMP(4)	17.8
EIB202(5)	17.5	EIB206+AMP(5)	17.6	△*ptsH*(5)	17.9	△*ptsH*+AMP(5)	17.8
EIB202(6)	17.7	EIB207+AMP(6)	17.5	△*ptsH*(6)	17.6	△*ptsH*+AMP(6)	17.9
EIB202(7)	17.1	EIB208+AMP(7)	17.5	△*ptsH*(7)	18	△*ptsH*+AMP(7)	13.8
EIB202(8)	17.3	EIB209+AMP(8)	17.3	△*ptsH*(8)	17.6	△*ptsH*+AMP(8)	9.2
EIB202(9)	17.5	EIB210+AMP(9)	9.5	△*ptsH*(9)	17.8	△p*tsH*+AMP(9)	8.8
EIB202(10)	17.5	EIB211+AMP(10)	8.2	△*crr*(1)	17.2	△*crr*+AMP(1)	17
EIB202(11)	17.4	EIB212+AMP(11)	8.1	△*crr*(2)	16.7	△crr+AMP(2)	17
EIB202(12)	17.4	EIB213+AMP(12)	8.4	△*crr*(3)	17.1	△*crr*+AMP(3)	16.9
				△*crr*(4)	17.1	△*crr*+AMP(4)	17.1
				△*crr*(5)	17	△*crr*+AMP(5)	16.9
				△*crr*(6)	17.1	△*crr*+AMP(6)	11.3
				△*crr*(7)	16.8	△*crr*+AMP(7)	9.6
				△*crr*(8)	17.1	△*crr*+AMP(8)	9

S2

Strain(Cycle)	MIC	Strain(Cycle)	MIC	Strain(Cycle)	MIC	Strain(Cycle)	MIC
S2(1)	17.6	S2+AMP(1)	17.4	S2 *pts* promotor delete(1)	16.6	S2 *pts* promotor delete+AMP(1)	16.5
S2(2)	17.5	S3+AMP(2)	17.9	S2 *pts* promotor delete(2)	16.5	S2 *pts* promotor delete+AMP(2)	16.6
S2(3)	18.1	S4+AMP(3)	17.6	S2 *pts* promotor delete(3)	16.8	S2 *pts* promotor delete+AMP(3)	16.8
S2(4)	17.9	S5+AMP(4)	18	S2 *pts* promotor delete(4)	16.8	S2 *pts* promotor delete+AMP(4)	16.8
S2(5)	18.1	S6+AMP(5)	17.9	S2 *pts* promotor delete(5)	16.8	S2 *pts* promotor delete+AMP(5)	14.6

S2(6)	17.9	S7+AMP(6)	17.6	S2 *pts* promotor delete(6)	16.7	S2 *pts* promotor delete+AMP(6)	14.2
S2(7)	18	S8+AMP(7)	17.9	S2 *pts* promotor delete(7)	16.7	S2 *pts* promotor delete+AMP(7)	13.6
S2(8)	18.2	S9+AMP(8)	17.3	S2 *pts* promotor delete(8)	16.9	S2 *pts* promotor delete+AMP(8)	13
S2(9)	17.2	S10+AMP(9)	16.1	S2 *pts* promotor delete(9)	16.6	S2 *pts* promotor delete+AMP(9)	13.8
S2(10)	17.6	S11+AMP(10)	14.9	S2 *pts* promotor delete(10)	16.8	S2 *pts* promotor delete+AMP(10)	13.5
S2(11)	17.7	S12+AMP(11)	13.8	S2 *pts* promotor delete(11)	16.3	S2 *pts* promotor delete+AMP(11)	13.2
S2(12)	17.9	S13+AMP(12)	13.8	S2 *pts* promotor delete(12)	16.4	S2 *pts* promotor delete+AMP(12)	13.3

Table S2 Primer for *pts* promoter and its mutation

Primer	Primer sequence（5'→3'）
promoter-F	tgccagcttgttaaaaatg
promoter-R	tgtatttccccaacttataggt
P0a-F1	ggcaagaccgatcttatctc
P0a-R1	cgcatcaaaataatCagtctgg
P0a-F2	tttaaccagactGattattttg
P0a-R2	gtcacagtaatttcagaagt
P1a-F1	ctgatggaagaagaaggtat
P1a-R1	gtacgtTattaaacattatgc
P1a-F2	gcataatgtttaatAacgtac
P1a-R2	agtttaaccagatgttcaac
P1b-F1	ggcgttgaaactacaagaag
P1b-R1	ctgacgtttAgtacgtcatt
P1b-F2	gtttaatgacgtacTaaacgt
P1b-R2	acaacggtaccttgagtcag
pts-F	acgtacgaaacgtcagcggtcaacacccgcc
pts-R	ggcgggtgttgaccgctgacgtttcgtacgt
1-F	Gcgtacgaaacgtcagcggtcaacacccgcc
1-R	ggcgggtgttgaccgctgacgtttcgtacgC
3-F	acTtacgaaacgtcagcggtcaacacccgcc
3-R	ggcgggtgttgaccgctgacgtttcgtaAgt
5-F	acgtCcgaaacgtcagcggtcaacacccgcc
5-R	ggcgggtgttgaccgctgacgtttcgGacgt
7-F	acgtacTaaacgtcagcggtcaacacccgcc
7-R	ggcgggtgttgaccgctgacgtttAgtacgt
9-F	acgtacgaCacgtcagcggtcaacacccgcc
9-R	ggcgggtgttgaccgctgacgtGtcgtacgt
11-F	acgtacgaaaTgtcagcggtcaacacccgcc
11-R	ggcgggtgttgaccgctgacAtttcgtacgt
12-F	acgtacgaaacAtcagcggtcaacacccgcc
12-R	ggcgggtgttgaccgctgaTgtttcgtacgt
13-F	acgtacgaaacgCcagcggtcaacacccgcc
13-R	ggcgggtgttgaccgctgGcgtttcgtacgt
15-F	acgtacgaaacgtcCgcggtcaacacccgcc
15-R	ggcgggtgttgaccgcGgacgtttcgtacgt
17-F	acgtacgaaacgtcagTggtcaacacccgcc
17-R	ggcgggtgttgaccActgacgtttcgtacgt
all-mut-F	gatccatccgttccagcggtcaacacccgcc
all-mut-R	ggcgggtgttgaccgctggaacggatggatc

Note: Capital letter indicates mutated point.

Table S3 Primer for qRT-PCR.

K12

Gene		Primer sequence（5'→3'）	Gene		Primer sequence（5'→3'）
16s DNA	F	catgccgcgtgtatgaagaa	*crr*	F	ataccggaactattgagatc
	R	cgggtaacgtcaatgagcaaa		R	atcagattcgatagagaatg
trxA	F	attcacctgactgacgacagttttg	*ampC*	F	cagcccgtcacacagcaaac
	R	gccagggttttgatcgatgttc		R	ccagcagtgtaggttgcgagatg
ahpC	F	gtcttcttcttctacccggctgac	*bolA*	F	tagaagaaaaattaagggcggcg
	R	cgccgatcatcgcatatttg		R	ggtagtagagagttcctccgctaaagtac
bcp	F	tctacccgaaagccatgaccc	*ompR*	F	tagtcagagcaacccgatgcc
	R	acctggtggtcctcatcagacag		R	ttaccgcctcttcctgtgacg
gor	F	cgggcgtggaatacggtatt	*crp*	F	aaaagcggaaacgctgtactacatc
	R	aatcatcgggtcgaagctgc		R	cgaaatttcagccacttcacagg
yfcG	F	cggaatttttgcgcatttcg	*cpdA*	F	ggatcaatcctctgcggcct
	R	gcctacctgccagaataaccactg		R	aacacctggctatccagcaacag
sodB	F	acctgattaaaggtaccgcgtttg	*cyaA*	F	ggcgaagattgtggctccac
	R	caaaagatgcggcgatagcttc		R	cgagatccagccattcatttgg
sodC	F	tagggcagtcaattggtagcgtc	*pykA*	F	aaaagtactggaagttcagggcatg
	R	ttttgtggatcaagatgcccg		R	gggaaggagacagccaggtaatcta
oxyR	F	taccgtgctgcgtgaggtga	*pykF*	F	accgccattgaaggtaacaaagtt
	R	tgggtctgtgcttcatgcagat		R	cttacgaataaaggaagcagcaaca
uvrD	F	tggcagaaggtgtatcaggcg	*ribB*	F	tgaagaccgtgaaaacgaaggtg
	R	tccacgcgtactgaatgttgttg		R	taaaaccggtgccataggcg
dam	F	gtactgatgagtacgtacaggccgc	*ribF*	F	gcgggaaaaactgcgttacc
	R	ccgcgcagattgtaacgaca		R	aaatcgccttcacgaccagc
dpoI	F	gtgccgccagaactgatcatc	*tktB*	F	cgcagtttaaccagccagacc
	R	gagcttcgctgccattgttttc		R	cgtcggtaaaccagccttctgt
xth	F	gctgaccaaagagacgccga	*tktA*	F	aagtgggttacaccgctggtg
	R	cgtttgagttcggtttccaggtag		R	atgccttccatcatgcagcc
nfo	F	ccccacgacagttatctgattaacc	*zwf*	F	tcatgaaagaaaccattgatgaagg
	R	gattcggcaatacgcgcaag		R	tgcaaattgcgccaaaagtg
recJ	F	gcagcggaagcgatcattaac	*gnd*	F	tgaaaggtccttctattatgcctgg
	R	cgcgaccagatccagcagtt		R	gttcttcgttggtgaggttcagg
alkA	F	agaacctgttgccgcagagtg	*glk*	F	tgcgcatctggtccatgt
	R	cgctcaccagttggcctaaaa		R	aaattcaccagcccaggg
mug	F	aaggtgatatatcaggccgggttt	*pfkA*	F	ctgactacactatcggtttcttcact
	R	ttcaatcagcttacgcccgc		R	accacaacgaattcacagcc
tag	F	gaagggcagcaggctggatt	*pfkB*	F	tggtcataagcggaagcct
	R	gcccgcgcattaccaataat		R	ccagcgcactgagttctttt
recC	F	aattgctggagcgcgaagac	*aceE*	F	gttgagcgtgctcagtatct
	R	tgcttctcccagcccttcaac		R	cacggaagaagtggttaaag
ssB	F	cgctggctacttccgaatcc	*ptsG*	F	gtggcttcctgttcaaaatg
	R	tctgtggtgtagcgatcctgacc		R	tccccagaagaatacagattg
mutL	F	tatcagttcggtttcccgcc	*ptsI*	F	atcatagccctgattaaaga
	R	aatgcggcggatgatctcat		R	ctcaggtcgataatcttcag
mutY	F	cgctcgacgaagttctccact	*ptsH*	F	agaagctaagggcttcactt
	R	cccagagaaagcgagagaatcg		R	tctgcggagatagtcacaac
mutH	F	caagattttgctgctctgggc	*rpoS*	F	agctgaacgtttacctgcga
	R	ctcgccttcaaccggtatcc		R	tgtccagcaacgctttttcg
mutS	F	cgacgcgtcgtaatctggaa	*dinB*	F	gcaaagtctcagcggcaaaa

	R	ctgcaatgcgccaatagtttg	R gattttcgcaaccgttcgct

EIB202

Gene		Primer sequence（5’→3’）
16s DNA	F	ctggaactgagacacggtcc
	R	ggtgcttcttctgcgggtaa
ptsG	F	tgatgctgccggtatcagtc
	R	gcttggaagcaatctcttcc
ptsI	F	aaggcgcttcttctgaaaga
	R	tgctcaagctcttcatcttc
ptsH	F	caaagcattcgcttctgaca
	R	ggcgttctgctcatcttcac
crr	F	atcggtaaaatctttgaaact
	R	atcagctccttgatctcatc

Table S4 Primers for *aceE* and its point mutation

Primer	Primer sequence（5'→3'）
aceE-F	gccgagctc atgtcagaacgtttcccaaatgacg
aceE-R	ccgctcgagttacgccagacgcgggttaactttatc
m1-F	gttcctcggtgac GCTgaaatggacgaaccggaatcc
m1-R	ggttcgtccatttcAGCgtcaccgaggaacgcgtaa
m2-F	gttcctcggtgacTCTgaaatggacgaaccggaatcc
m2-R	ggttcgtccatttcAGAgtcaccgaggaacgcgtaa
m3-F	gttcctcggtgacATGgaaatggacgaaccggaatcc
m3-R	ggttcgtccatttcCATgtcaccgaggaacgcgtaa
m4-F	tggtcttcgttatcCAGtgtaacctgcagcgtcttgacg
m4-R	gctgcaggttaca CTGgataacgaagaccaggttatc
m5-F	tcttcgt tatcaacAGTaacctgcagcgtcttgacg
m5-R	agacgctgcaggtt ACTgttgataacgaagaccaggttatc
m6-F	tcttcgt tatcaacAATaacctgcagcgtcttgacg
m6-R	agacgctgcaggtt ATTgttgataacgaagaccaggttatc
m7-F	actgtaacctgcagGCActtgacggcccggtcaccggt
m7-R	accgggccgtcaag TGCctgcaggttacagttgataacg
m8-F	acctgcagc gtctt GCCggcccggtcaccggtaacg
m8-R	ccggtgaccgggcc GGCaagacgctgcaggttacagttg

Note: Capital letter indicates mutated point.