Supplementary Information

A broadly applicable, stress-mediated bacterial death pathway regulated by the phosphotransferase system (PTS) and the cAMP-Crp cascade

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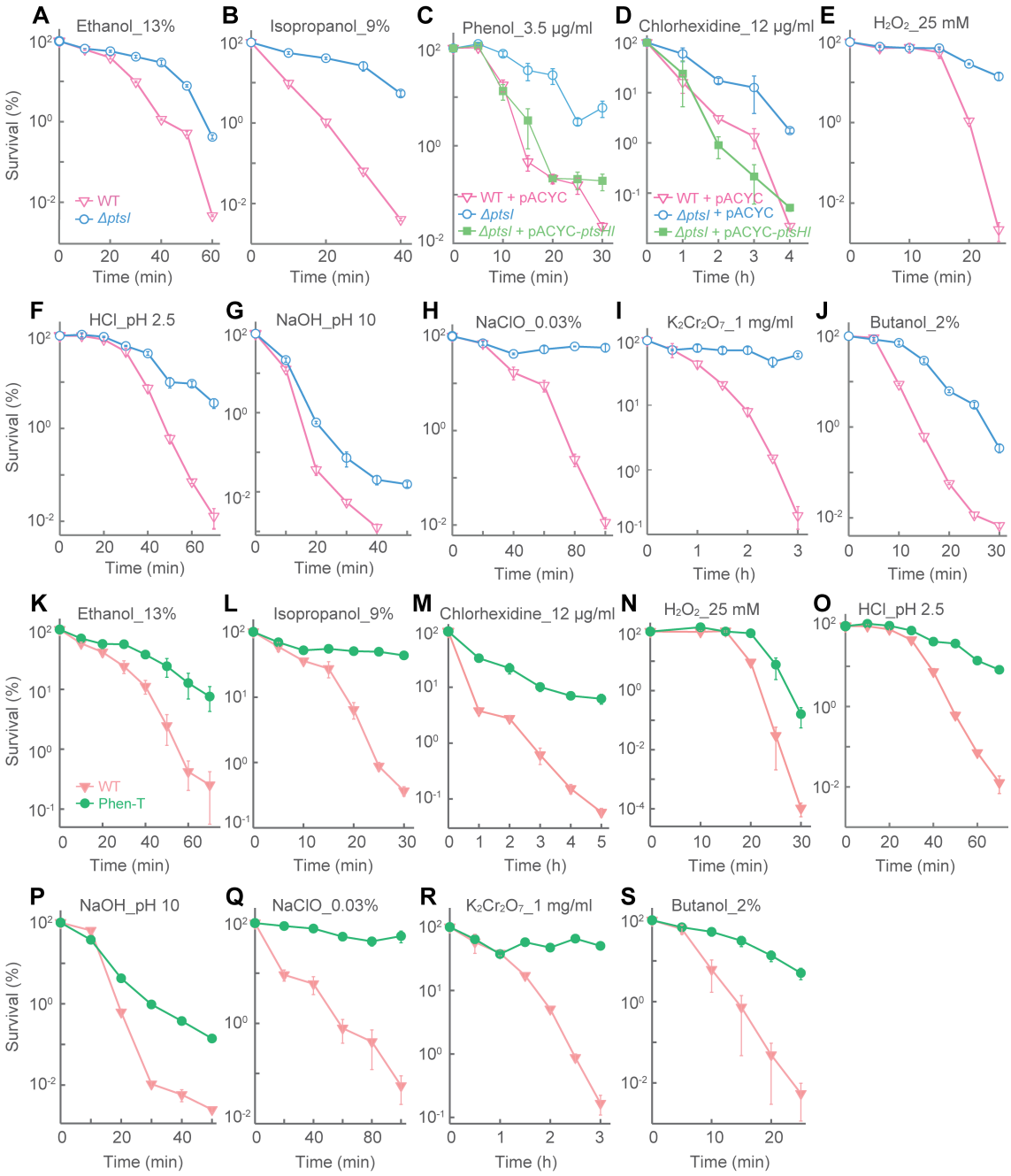
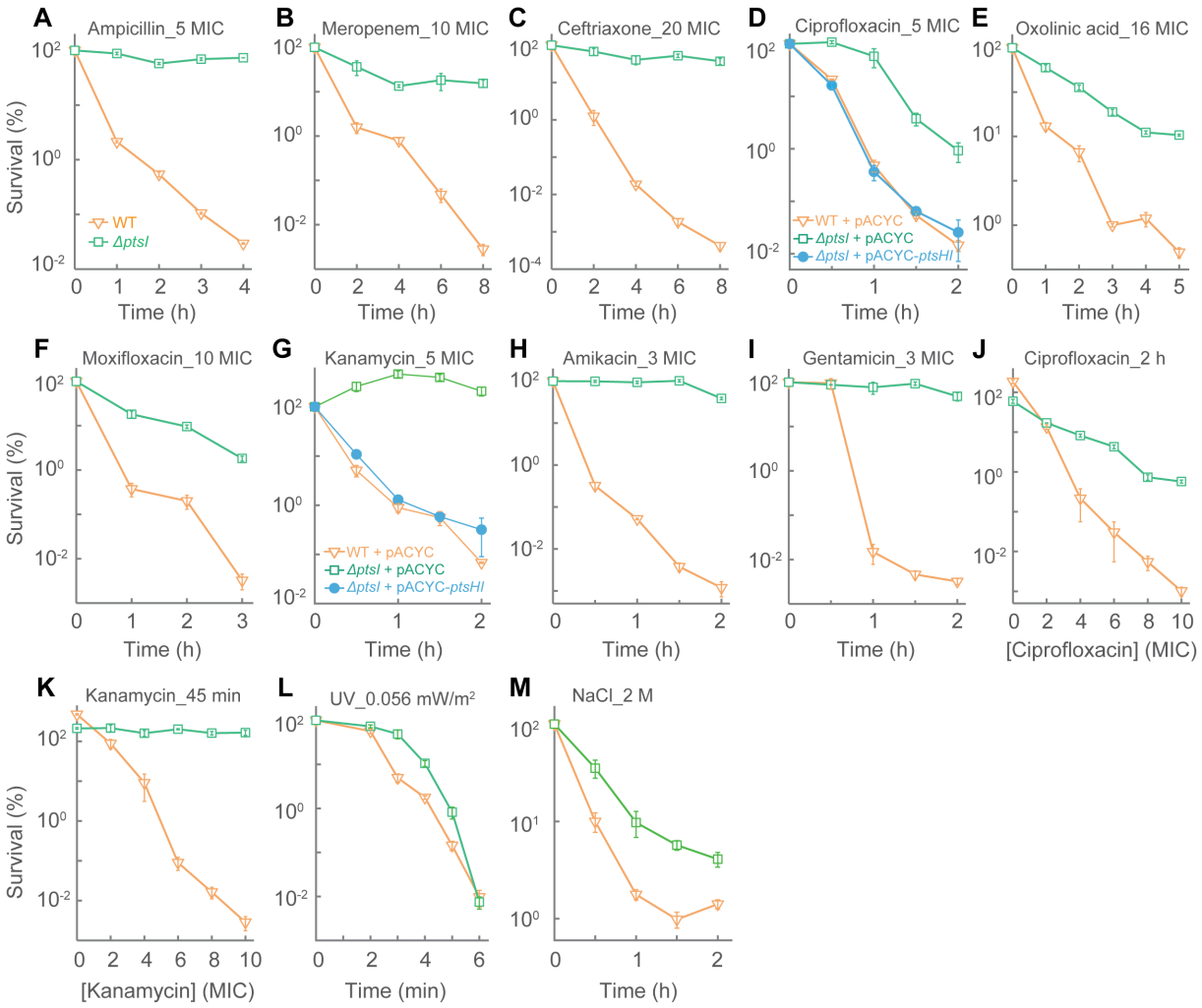
Supplementary Figures

Supplementary Tables

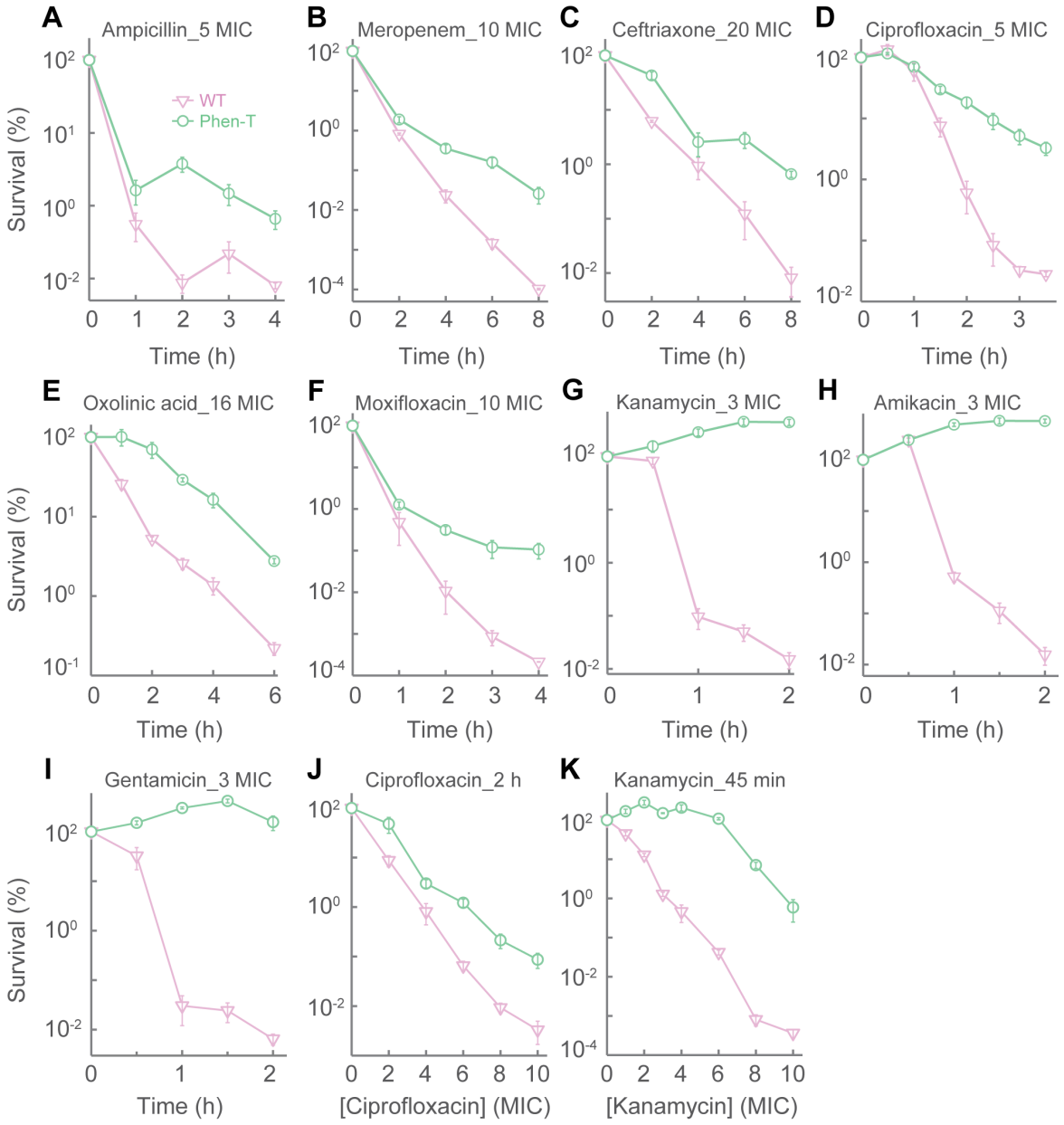
Supplementary References



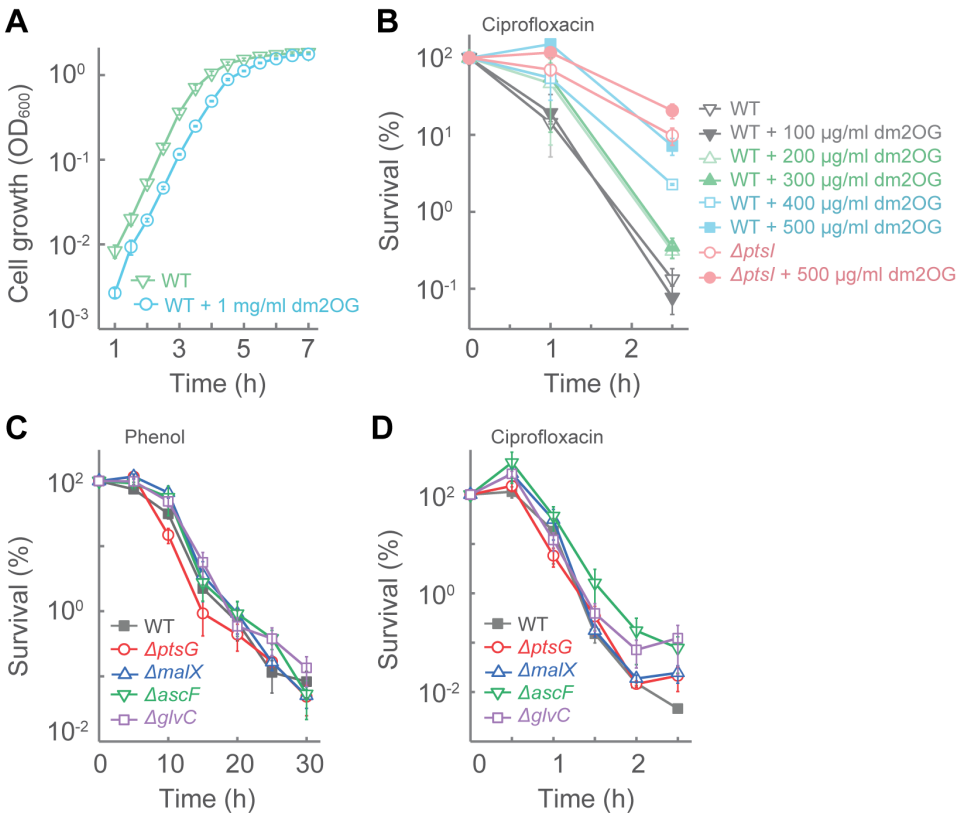
Supplementary Fig. S1. A deficiency in *ptsI* confers pan-tolerance to diverse disinfectants. *A-J*: Exponentially growing cultures of wild type (WT) and *ΔptsI* mutant were treated with the indicated disinfectants at the indicated concentrations for the indicated times. Samples were then washed by centrifugation and resuspension, diluted, and plated on LB agar for CFU determination. Percent survival was calculated relative to samples taken at the time of disinfectant addition. Complementation was performed by expression of a plasmid-borne wild-type *ptsHI* operon (pACYC184-*ptsHI*) for two representative disinfectants, phenol and chlorhexidine. ***K-S***: Same as *A-J*, but the Phen-T mutant rather than *ΔptsI* was used. Data indicate average of 3 biological replicates; error bars indicate SEM. The data show that a deficiency in the PTS reduces killing by a variety of lethal disinfectants and that the *ΔptsI* and Phen-T mutants behave in a similar way. Tolerance is established by little change occurring in MIC (Supplementary Tables 1 and 3).



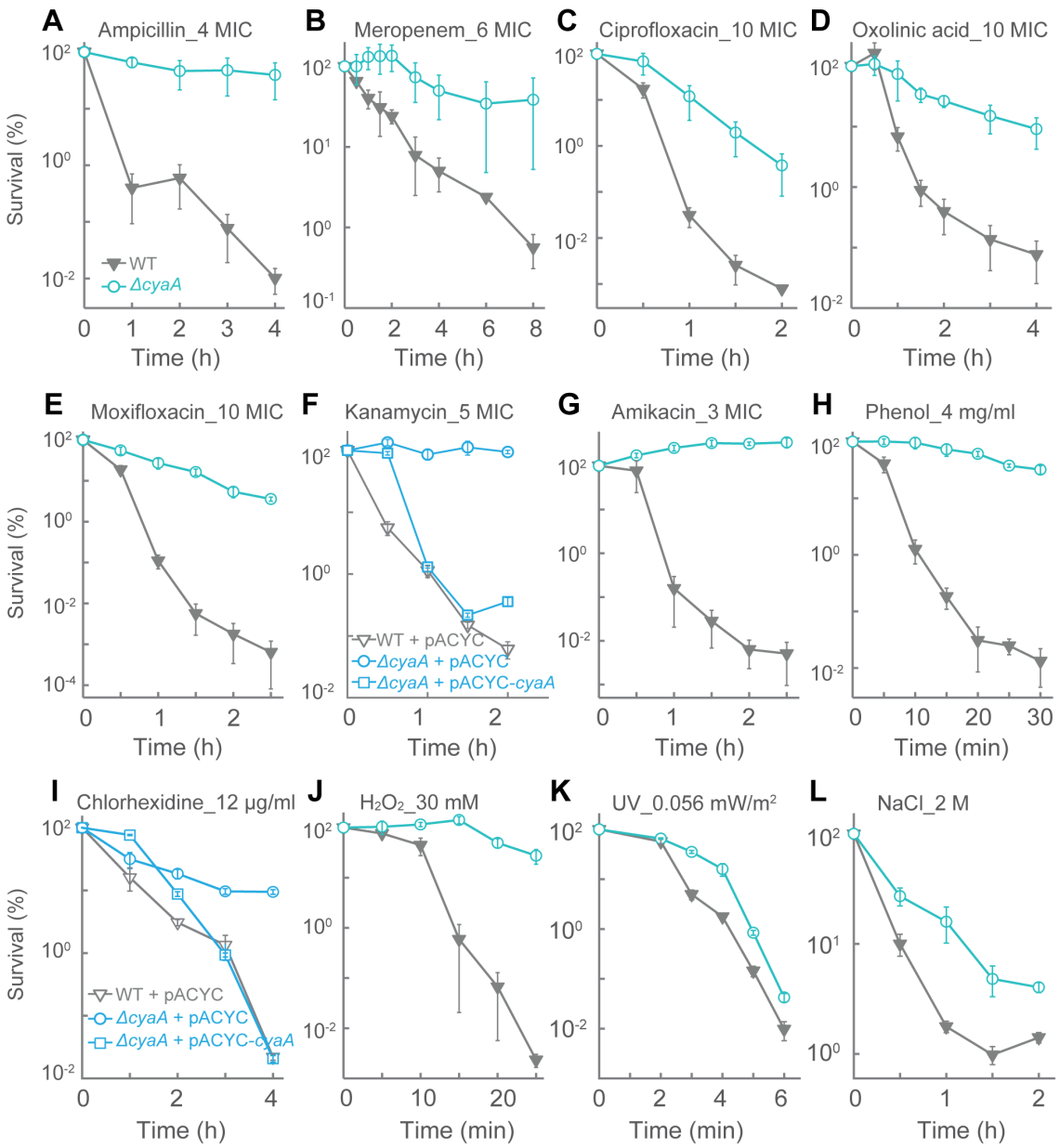
Supplementary Fig. S2. A deficiency in *ptsI* confers pan-tolerance to diverse antimicrobials and environmental stressors. Exponentially growing cultures of *E. coli* wild type (WT) and *ΔptsI* mutant were treated with the indicated antimicrobials or environmental stressors (UV or high osmolarity) at the indicated concentrations (intensity) for the indicated times after which samples were washed, diluted, and plated on LB agar for viability determination. Percent survival was calculated relative to samples taken at the time of stressor addition. Complementation was performed by expression of a plasmid-borne, wild-type *ptsHI* operon (pACYC184-*ptsHI*) for two representative antimicrobials, ciprofloxacin and kanamycin. Data indicate an average of 3 biological replicates; error bars indicate SEM. The data show that a deficiency in the PTS reduces killing by a variety of lethal antimicrobials and environmental stressors. Tolerance is established by little change occurring in MIC (Supplementary Tables 1 and 3).



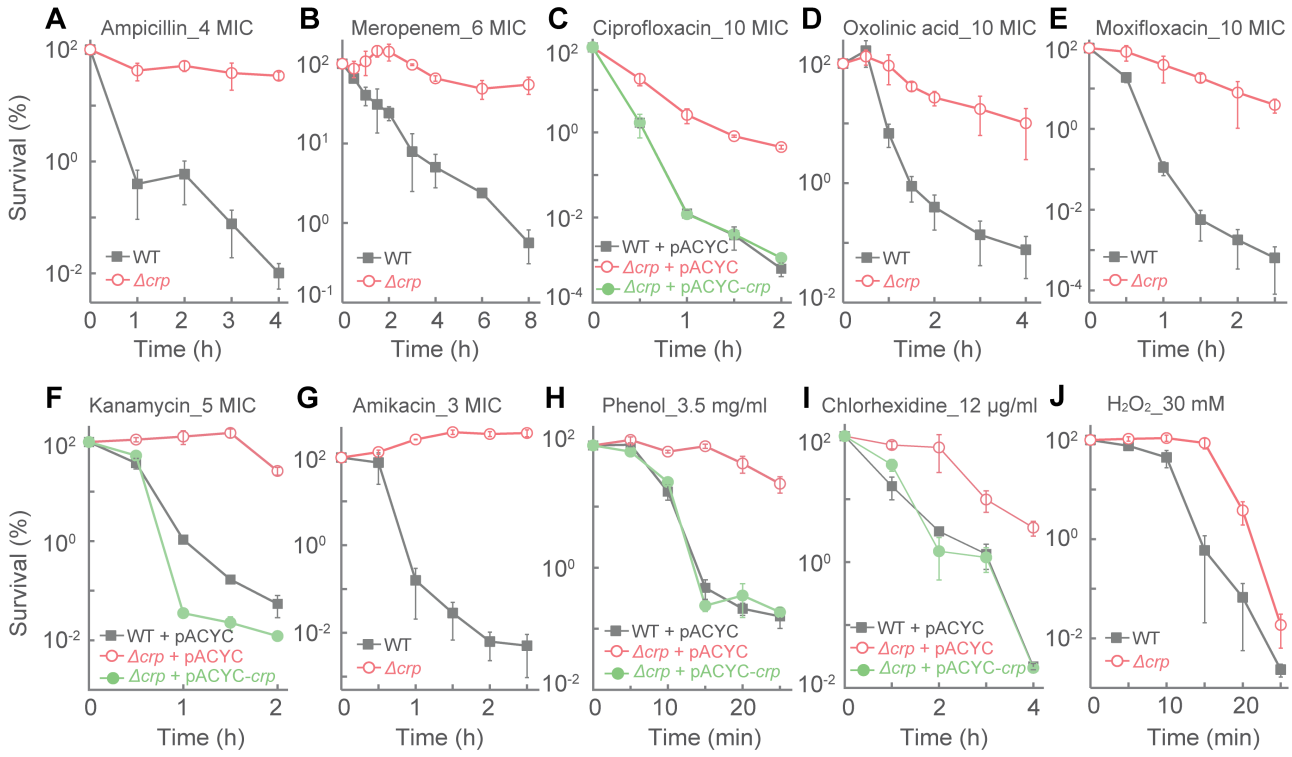
Supplementary Fig. S3. The Phen-T mutant exhibits pan-tolerance to diverse antimicrobials. Exponentially growing cultures of wild type (WT) and the Phen-T mutant cells were treated with the indicated antimicrobials at the indicated concentrations for the indicated times after which samples were washed, diluted, and plated on LB agar for CFU determination. Percent survival was determined relative to samples taken at the time of antimicrobial addition. Data indicate averages of 3 biological replicates; error bars indicate SEM. The data show that a deficiency in the PTS (in the Phen-T mutant) reduces killing by a variety of lethal antimicrobials. Tolerance is established by little change occurring in MIC (Supplementary Tables 1 and 3).



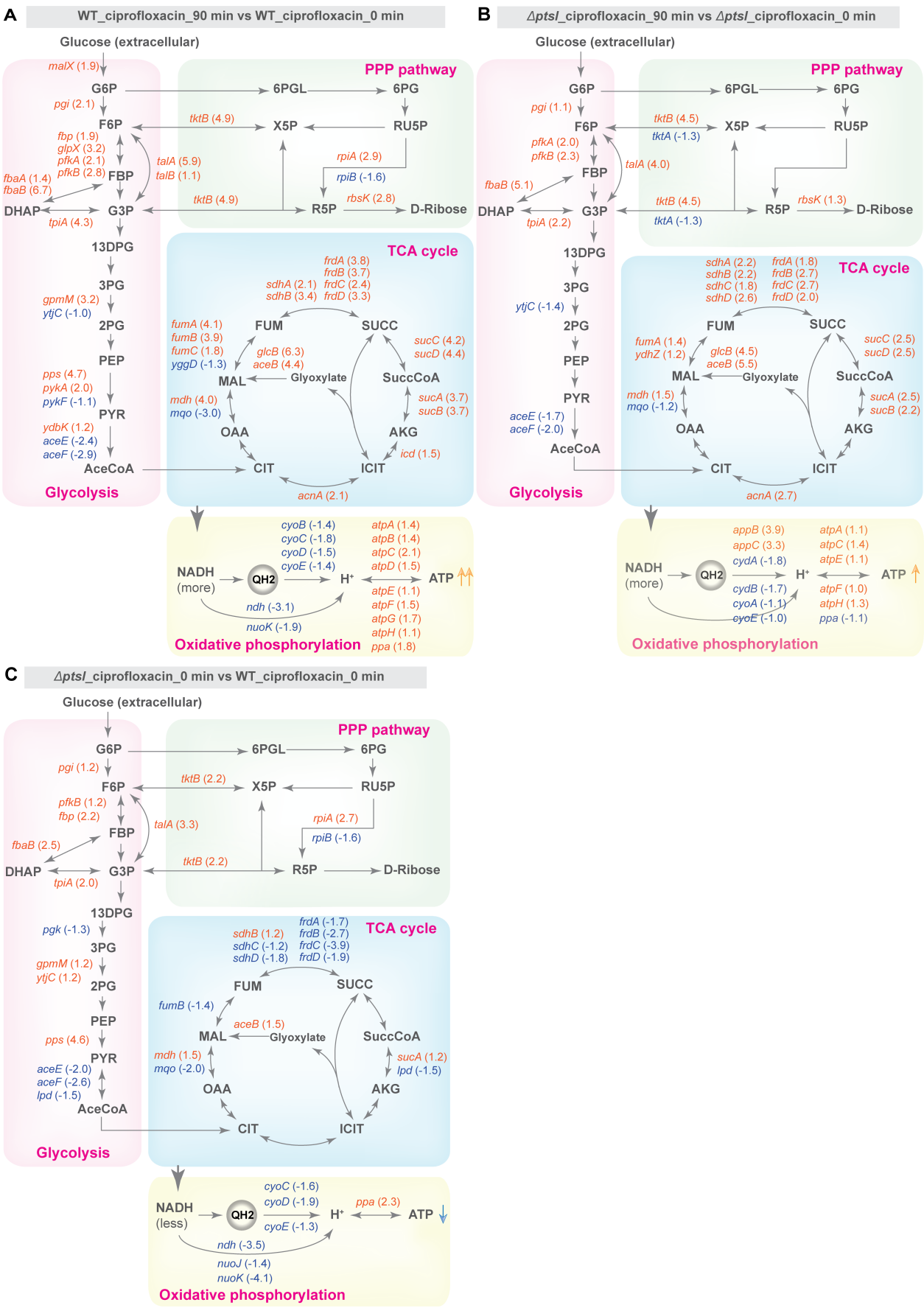
Supplementary Fig. S4. Effect of dimethyl-2-oxoglutarate and deficiencies in genes encoding transporters downstream of Crr (EIIA) of the PTS on killing by phenol and ciprofloxacin. (***A***) Effect of dimethyl-2-oxoglutarate (dm2OG) on bacterial growth. Overnight wild-type cultures (WT) were diluted 2,000-fold into fresh LB medium containing or lacking 1 mg/ml dm2OG. They were then grown aerobically at 37 °C with shaking at 200 rpm for the indicated times after which turbidity (OD_{600}) was measured. (***B***) Protective effect of dimethyl-2-oxoglutarate on ciprofloxacin-mediated killing. Exponentially growing cultures of wild-type and *ΔptsI*-mutant *E. coli* were pre-treated with/without the indicated concentrations of dm2OG for 40 min, and then ciprofloxacin was added to 5 x MIC. At the indicated times, samples were taken, washed, diluted, and plated on LB agar for CFU determination. (***C***) Effect of deficiencies in genes encoding transporters downstream of the Crr (EIIA) phosphorelay on killing by phenol. Exponentially growing cultures of *E. coli* wild type (WT), *ΔptsG*, *ΔmalX*, *ΔascF*, and *ΔglvC* mutants were treated with 3.5 mg/ml phenol for the indicated times after which samples were washed, diluted, and plated on LB agar for viability determination. Survival was calculated relative to samples taken at the time of phenol addition. (***D***) Same as in *c*, but with ciprofloxacin (5 x MIC) rather than phenol. Data points were from an average of 3 biological replicates; error bars indicate SEM. The data show that a known inhibitor of PtsI has no effect on *E. coli* growth rate but interferes with stress-mediated killing. Moreover, genes involved in downstream (e.g., after *crr*) carbohydrate phosphorylation and transport are not involved in the death pathway.



Supplementary Fig. S5. A deficiency in *cyaA* confers pan-tolerance to diverse lethal agents. Exponentially growing cultures of *E. coli* wild-type (WT) and *ΔcyaA*-mutant cells were treated with the indicated antimicrobials, disinfectants, ultra-violet irradiation, or high osmolarity at the indicated concentrations (intensity) for the indicated times after which samples were washed, diluted, and plated on LB agar for viability determination. Percent survival was calculated using samples taken at the time of stressor addition as reference points. Complementation was performed by expression of plasmid-borne, wild-type *cyaA* (pACYC184-*cyaA*) for two representative stressors, kanamycin and chlorhexidine. Data indicate average of 3 biological replicates; error bars indicate SEM. These data demonstrate the involvement of adenyl cyclase in pan-tolerance and the death process common to diverse stressors.



Supplementary Fig. S6. A deficiency in *crp* confers pan-tolerance to diverse lethal agents. Exponentially growing cultures of *E. coli* wild type (WT) and a *Δcrp* mutant were treated with the indicated antimicrobials and disinfectants at the indicated concentrations for the indicated times after which samples were washed, diluted, and plated on LB agar for viability determination. Percent survival was calculated using samples taken at the time of stressor addition. Complementation was performed by expression of a plasmid-borne, wild-type *crp* (pACYC184-*crp*) for 4 representative stressors, ciprofloxacin, kanamycin, phenol, and chlorhexidine. Data points were determined from an average of 3 biological replicates; error bars indicate SEM. These data show that Crp is involved in the stress-mediated death pathway common to diverse stressors.


A
WT_ciprofloxacin_90 min vs WT_ciprofloxacin_0 min
B
Δptsl_ciprofloxacin_90 min vs Δptsl_ciprofloxacin_0 min
C
Δptsl_ciprofloxacin_0 min vs WT_ciprofloxacin_0 min
Glucose (extracellular)
PPP pathway
TCA cycle
Glycolysis
Oxidative phosphorylation
G6P
6PGL
6PG
F6P
X5P
RU5P
FBP
DHAP
G3P
R5P
D-Ribose
13DPG
3PG
2PG
PEP
PYR
AceCoA
CIT
ICIT
AKG
SuccCoA
SUCC
FUM
MAL
Glyoxylate
OAA
NADH
(more)
(less)
QH2
H+
ATP
malX (1.9)
pgi (2.1)
fbp (1.9)
glpX (3.2)
pfkA (2.1)
pfkB (2.8)
fbaA (1.4)
fbaB (6.7)
tpiA (4.3)
talA (5.9)
talB (1.1)
tktB (4.9)
rpiA (2.9)
rpiB (-1.6)
rbsK (2.8)
gpmM (3.2)
ytjC (-1.0)
pps (4.7)
pykA (2.0)
pykF (-1.1)
ydbK (1.2)
aceE (-2.4)
aceF (-2.9)
frdA (3.8)
frdB (3.7)
sdhA (2.1)
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frdC (2.4)
frdD (3.3)
fumA (4.1)
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fumC (1.8)
yggD (-1.3)
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mdh (4.0)
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sucB (3.7)
icd (1.5)
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cyoC (-1.8)
cyoD (-1.5)
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atpA (1.4)
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ppa (1.8)
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frdB (2.7)
frdC (2.7)
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cyoD (-1.9)
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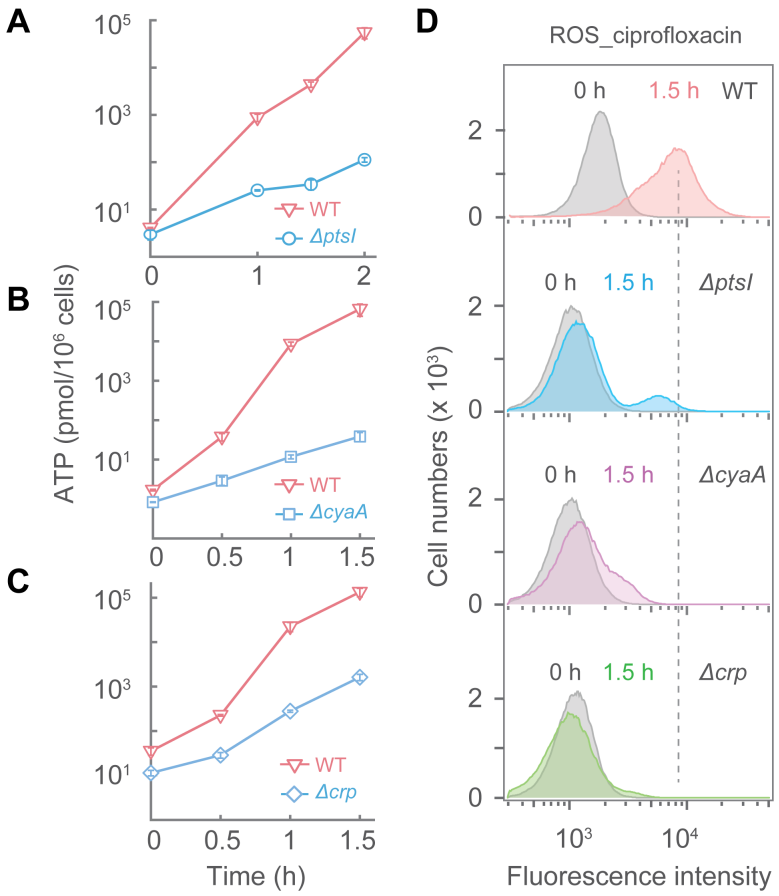
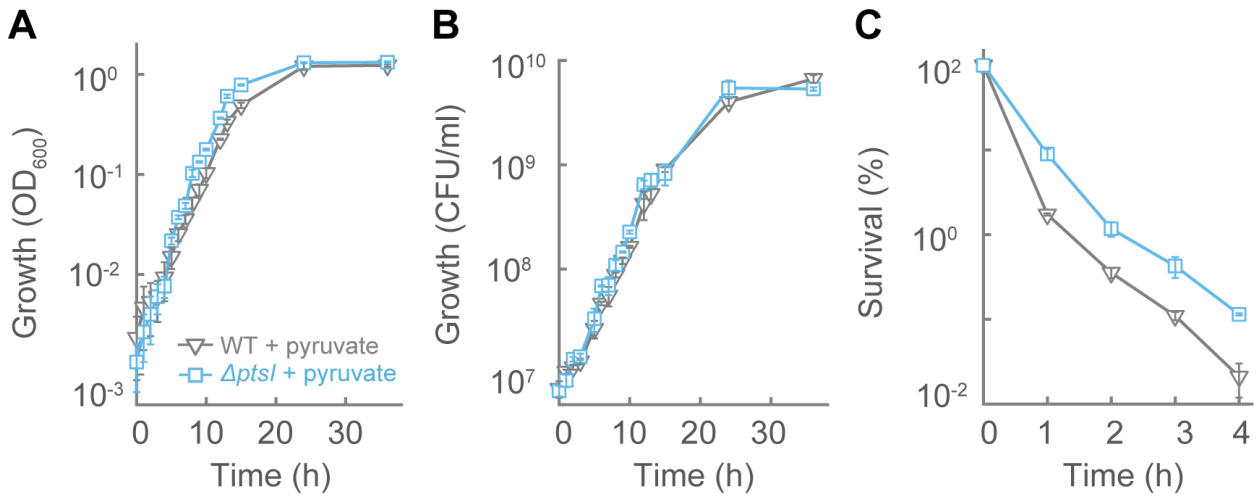
Supplementary Fig. S7. Effect of ciprofloxacin treatment and the *ptsI* mutation on transcription of genes involved in the TCA cycle, oxidative phosphorylation, glycolysis, and the pentose phosphate pathway (PPP). Since Fig. 5***A*** of the main text does not show the effect of ciprofloxacin on the two strains relative to untreated cells, exponentially growing cultures of wild type (WT) and the *ΔptsI* mutant were treated with 5 x MIC ciprofloxacin for 0 or 90 min after which samples were taken for RNA-seq analysis. Genes up-regulated (orange) and down-regulated (blue) due to ciprofloxacin treatment are labeled as $\log_2$-fold changes in parenthesis following each gene name. Genes involved in glycolysis, PPP, TCA, and oxidative phosphorylation are labeled at left, upper right, lower right, and the lowest corners using shaded rectangles and bold pink font. (***A***) Treatment of wild-type cells with ciprofloxacin caused a dramatic increase in the expression of genes involved in the TCA cycle and oxidative phosphorylation; a moderate increase of genes involved in the early steps of glycolysis and in some steps of PPP was seen. (***B***) A deficiency in *ptsI* allowed only a moderate increase in the expression of genes involved in the TCA cycle, PPP, and early steps of glycolysis following exposure to ciprofloxacin. Thus, Fig. 5a in the main text resulted from the combined effect of ciprofloxacin treatment and the *ptsI* mutation. (***C***) A deficiency in *ptsI* caused down-regulation of genes involved in the TCA cycle and oxidative phosphorylation but up-regulation of genes involved in early steps of glycolysis and in the PPP in the absence of ciprofloxacin. Overall, the data in this figure show that ciprofloxacin exposure stimulates transcription of many genes in the TCA cycle, oxidative phosphorylation, glycolysis, and the PPP with both wild-type and the *ptsI* mutant cells, but such stimulation is weaker with the *ptsI* mutant than with wild-type cells, leading to an overall net suppression of these genes by the *ptsI* mutation, as shown in the main text Fig. 5a. Moreover, the *ptsI* mutation also moderately suppressed the expression of a similar set of genes in the absence of stress, indicating that the PtsI defect preconditions cells to cope with lethal stress.



Supplementary Fig. S8. Suppression of ciprofloxacin-induced intracellular ATP and ROS surges by deficiencies in *ptsI*, *cyaA*, or *crp*. (***A-C***) A deficiency in *ptsI*, *cyaA*, or *crp* lowers ciprofloxacin-stimulated intracellular ATP increases. Wild-type, *ptsI*, *cyaA*, and *crp* mutant cells were treated with 5 x MIC ciprofloxacin for the indicated times after which intracellular ATP levels were determined and expressed as pmol/10^6 cells. (***D***) Deficiencies in *ptsI*, *cyaA*, or *crp* suppress ciprofloxacin-stimulated intracellular ROS accumulation. Wild-type, *ptsI*, *cyaA*, and *crp* mutant cells were pre-treated with carboxy-H2DCFDA for 20 min and then treated with 5 x MIC ciprofloxacin for the indicated times before samples were subjected to flow cytometry for analysis of intracellular ROS levels. Data are averages of 3 biological replicates; error bars indicate SEM.



Supplementary Fig. S9. Effect of a deficiency in *ptsI* on bacterial growth and protection from ciprofloxacin-mediated killing in M9-pyruvate medium. (***A***, ***B***) Growth curves of wild-type and *ΔptsI* mutant cells grown in M9-pyruvate medium. Wild-type and *ΔptsI* mutant cells were first grown overnight in M9-pyruvate (0.4%) medium and then diluted by 200-fold into fresh M9-pyruvate medium for determination of growth curve by measurement of optical density at 600 nm (***A***) or viable count (***B***) after growing serially diluted samples overnight on LB agar. (***C***) Protection from ciprofloxacin-mediated killing by a deficiency in *ptsI*. Exponentially growing cultures of *E. coli* wild type and *ΔptsI* mutant were treated with 5 x MIC ciprofloxacin for the indicated times prior to measurement of survival. Data are averages of 3 biological replicates; error bars indicate SEM.

Supplementary Tables

Table S1. Bacterial strains and MIC for ciprofloxacin and phenol used in this study

Strain information			MIC	
Strain number	Relevant genotype	Source	Ciprofloxacin (μg/ml)	Phenol (mg/ml)
1	WT	*E. coli* BW25113	0.02	1.5
2	*ΔptsI*	This work, constructed by CRISPR-cas9	0.02	1.5
3[a]	*ΔptsI*::*kan*, kan^R	Keio collection, JW2409	ND	ND
4	*ΔptsI*-FRT	This work, by kanamycin marker excision	ND	ND
5	Phen-T, *ptsI* I393S, dgc*M* G228A	This work, by enrichment with phenol	0.02	1.5
6	Phen-T:: *ptsI* ^{WT}	This work, constructed by CRISPR-cas9	0.02	1.5
7	*ptsI* I393S	This work, constructed by CRISPR-cas9	0.02	1.5
8	*dgcM* G228A	This work, constructed by CRISPR-cas9	0.02	1.5
9	*ptsI* H189Q	This work, constructed by CRISPR-cas9	0.02	1.5
10	*ΔptsH*::*kan*, kan^R	Keio collection, JW2408	ND	ND
11	*ΔptsH*	This work, by kanamycin marker excision	ND	ND
12	*pts*H H15Q	This work, constructed by CRISPR-cas9	0.02	1.5
13	*Δcrr*::*kan*, kan^R	Keio collection, JW2410	ND	ND
14	*Δcrr*	This work, by kanamycin marker excision	ND	ND
15	*crr* H91Q	This work, constructed by CRISPR-cas9	0.02	1.5
16	*ΔcyaA*::*kan*, kan^R	Keio collection, JW3778	ND	ND
17	*ΔcyaA*	This work, by kanamycin marker excision	0.02	1.5
18	*ΔkatG*::*kan-ΔkatE*, kan^R	This work, by P1-mediated transduction from *ΔkatG* into *ΔkatE*	0.02	1.5
19	*cyaA854(del)-crp*-1004*	Coli Genetic stock Center CGSC#: 7040	ND	ND
20[b]	*crp**	This work, constructed by CRISPR-cas9	0.015	1.5
21	*ΔptsI-crp**	This work, constructed by CRISPR-cas9	0.0075	1.5
22	WT + pACYC184, CM^R	Wild-type strain transformed with pACYC184	0.02	1.5
23	Phen-T + pACYC184, CM^R	Phen-T transformed with pACYC184	0.02	1.5
24	Phen-T+ pACYC184-*ptsHI*, CM^R	Phen-T transformed with pACYC184-*ptsHI*	0.02	1.5
25	*ΔptsI* + pACYC184, CM^R	*ΔptsI* strain transformed with pACYC184	0.02	1.5
26	*ΔptsI* + pACYC184-*ptsHI*, CM^R	*ΔptsI* strain transformed with pACYC184-*ptsHI*	0.02	1.5
27	*ΔcyaA* + pACYC184, CM^R	*ΔcyaA* strain transformed with pACYC184	0.02	1.5
28	*ΔcyaA* + pACYC184-*cyaA*, CM^R	*ΔcyaA* transformed with pACYC184-*cyaA*	0.02	1.5
29	*Δcrp* + pACYC184, CM^R	*Δcrp* strain transformed with pACYC184	0.02	1.5
30	*Δcrp* + pACYC184-*crp*, CM^R	*Δcrp* strain transformed with pACYC184-*crp*	0.02	1.5
31	*ΔdgcM*::*kan*, kan^R	Keio collection, JW5206	0.02	1.5
32	*ΔdgcM*	This work, by kanamycin marker excision	ND	ND
33	*Δcrp*::*kan*, kan^R	Keio collection, JW3320	ND	ND
34	*Δcrp*	This work, by kanamycin marker excision	0.02	1.5

35	*ptsG*::*Kan*, kan^{R}	Keio Collection, JW1087	0.02	1.5
36	*malX*::*Kan*, kan^{R}	Keio Collection, JW1613	0.02	1.5
37	*ascF*::*Kan*, kan^{R}	Keio Collection, JW2685	0.02	1.5
38	*glvC*::*Kan*, kan^{R}	Keio Collection, JW3660	0.02	1.5

[a]The Keio collection *ptsI* mutant was found to have an unexpected frame-shift mutation in *cyaA* (Indel 1436-1442) in present work.

[b]The *crp** mutant was derived from strain 19 and contained Ile112Leu, Thr127Ile, and Ala144Thr mutations (1).

Kan: kanamycin; CM: chloramphenicol; ND: Not determined

Table S2. Properties of tolerant mutants obtained by phenol or antimicrobial enrichment.

Stressor (s) used for enrichment	Mutant name	Gene name	Base change	Amino acid (AA) change	Gene description
Phenol	Phen-T	*ptsI* *dgcM*	T1178G G683C	Ile393Ser Gly228Ala	Phosphoenolpyruvate-protein phosphotransferase PtsI (EI) Diguanylate cyclase DgcM
Amp, Cip, Kan[a]	Mut 1	*cyaA*	T900A	Asp300Glu	Adenylate cyclase
Amp, Cip, Kan[a]	Mut 2	*ptsI*	C802T	Gln268Stop	Phosphoenolpyruvate-protein phosphotransferase PtsI (EI)
Amp, Cip, Kan[a]	Mut 3	*ptsI*	Δ1120-1201[b]	27 AA deletion	
Amp, Cip, Kan[a]	Mut 4	*ptsI*	C103T	Gln35Stop	

[a]Order of sequential antimicrobial challenge: Amp (ampicillin), Cip (ciprofloxacin), and Kan (kanamycin), each for two rounds at 5 MIC.

[b]Deletion of 81 bases.

Table S3. Minimal Inhibitory Concentrations (MIC) of compounds tested

Chemical	MIC values for different strains				
	WT	*ΔptsI*	Phen-T	*ΔcyaA*	*Δcrp*
Kanamycin	4 µg/ml	6 µg/ml	8 µg/ml	12 µg/ml	12 µg/ml
Gentamicin	2 µg/ml	4 µg/ml	3 µg/ml	ND[a]	ND
Amikacin	3 µg/ml	6 µg/ml	6 µg/ml	6 µg/ml	6 µg/ml
Oxolinic acid	0.3 µg/ml	0.3 µg/ml	0.4 µg/ml	0.6 µg/ml	0.6 µg/ml
Moxifloxacin	0.04 µg/ml	0.04 µg/ml	0.06 µg/ml	0.08 µg/ml	0.08 µg/ml
Ampicillin	4 µg/ml	4 µg/ml	3 µg/ml	4 µg/ml	4 µg/ml
Meropenem	0.03 µg/ml	0.03 µg/ml	0.03 µg/ml	0.048 µg/ml	0.048 µg/ml
Ceftriaxone	0.03 µg/ml	0.03 µg/ml	0.03 µg/ml	ND	ND
dm2OG[b]	10 mg/ml	5 mg/ml	ND	ND	ND
Cipro + 200 µg/ml dm2OG	0.02 µg/ml	ND	ND	ND	ND
Cipro + 300 µg/ml dm2OG	0.03 µg/ml	ND	ND	ND	ND
Cipro + 400 µg/ml dm2OG	0.03 µg/ml	ND	ND	ND	ND
Cipro + 500 µg/ml dm2OG	0.04 µg/ml	0.04 µg/ml	ND	ND	ND
Cipro + 1000 µg/ml dm2OG	0.08 µg/ml	0.08 µg/ml	ND	ND	ND
Cipro + 8 mM cAMP	0.02 µg/ml	0.02 µg/ml	ND	ND	ND
Phenol + 200 µg/ml dm2OG	1.5 mg/ml	1.5 mg/ml	ND	ND	ND
Phenol + 300 µg/ml dm2OG	1.5 mg/ml	1.5 mg/ml	ND	ND	ND
Phenol+ 400 µg/ml dm2OG	1.5 mg/ml	1.5 mg/ml	ND	ND	ND
Phenol + 500 µg/ml dm2OG	1.5 mg/ml	1.5 mg/ml	ND	ND	ND
Phenol + 1000 µg/ml dm2OG	1.5 mg/ml	1.5 mg/ml	ND	ND	ND
Phenol + 8 mM cAMP	1.5 mg/ml	1.5 mg/ml	ND	ND	ND
DMSO[d]	30%	ND	ND	ND	ND
Phenol + 5% DMSO	1.5 mg/ml	ND	ND	ND	ND
Cipro + 5% DMSO	0.02 µg/ml	ND	ND	ND	ND
Chlorhexidine	0.48 µg/ml	0.48 µg/ml	0.48 µg/ml	0.48 µg/ml	ND
Ethanol	5%	5%	5%	5%	ND
Isopropanol	4%	4%	4%	ND	ND
1-butanol	1%	1%	1%	ND	ND
Potassium dichromate	32 µg/ml	32 µg/ml	32 µg/ml	ND	ND
Sodium hypochlorite	0.026%	0.026%	0.026%	ND	ND

[a]ND: not determined
[b]dm2OG: dimethyl-2-oxoglutarate
[c]Cipro: ciprofloxacin
[d]DMSO: dimethyl sulfoxide

Table S4. Primers and plasmids used in the study.

Primer[a]	**Description**	**Sequence of primers (5′-3′)**[b]
1. *ptsI*-check-F	Upstream primer for *ΔptsI*, Phen-T::*ptsI*^{wt} confirmation	CCGTTGTGACTATCTCCG
2. *ptsI*-check-R	Same as 1 but downstream reverse primer	AAACCCATGATCTTCTCCT
3. *ptsI*-sgRNA-F	Preparation of *ptsI*-sgRNA template for cloning into pTargetF for CRISPR-based mutant construction	AAT**ACTAGT**GACGTAAACCTGATTATTT AGTTTTAGAGCTAGAAATAG (*SpeI*)
4. *ptsI*-sgRNA-R	Same as 3 but downstream reverse primer	CTCAAAAAAAGCACCGACTCGG
5. *ptsI*-up-F	Preparation of upstream homologous arm for CRISPR-based *ptsI* knockout	CCGAGTCGGTGCTTTTTTTGAGTGACGTA CGAAACGTCAGCG
6. *ptsI*-up-R	Same as 5 but downstream reverse primer	CTGCCAGAGTGTACTGCCATAACCCTACC TTACTTG
7. *ptsI*-down-F	Preparation of downstream homologous arm for CRISPR-based *ptsI* knockout	GCAGTACACTCTGGCAGTTGA
8. *ptsI*-down-R	Same as 7 but downstream reverse primer	CCC**AAGCTT**CTTCTCCTAAGCAG (*HindIII*)
9. *dgcm*-check-F	Upstream primer for *ΔdgcM* confirmation	CCAGCATCCAGGCAAATA
10. *dgcm*-check-R	Downstream primer for *ΔdgcM* confirmation	GCGTGTTACGTCGCACTA
11. *ptsI* I393S-F	Amplification of *ptsI* (I393S) homologous template from Phen-T genome for CRISPR-based allelic exchange	CACTAAACCTATAAGTTGGGG
12. *ptsI* I393S-R	Same as 11 but downstream reverse primer	AACAGTTCAACGCCGCTATCAG
13. *ptsI* I393S-check-F	Upstream primer for confirming *ptsI* (I393S) mutation	TGGACTGTATTGCGCTCTTCGT
14. *ptsI* I393S-check-R	Downstream primer for confirming *ptsI* (I393S) mutation	AACAGTTCAACGCCGCTATCAG
15. Phen-T::*ptsI*^{WT}-sgRNA-F1	Preparation of sgRNA for restoring wild-type *ptsI* in Phen-T mutant by CRISPR-based knock-in	GG**ACTAGT**CTTCAACAGAGATGCTCATC GTTTTAGAGCTAGAAATAGC (*SpeI*)
16. Phen-T::*ptsI*^{WT}-sgRNA-R1	Same as 15 but downstream reverse primer	CTCAAAAAAAGCACCGACTCGG
17. Phen-T::*ptsI*^{WT}-F2	Amplifying upstream fragment for construction of wild-type *ptsI* homologous template for restoring Phen-T back to wild-type *ptsI* by CRISPR	CCGAGTCGGTGCTTTTTTTGAGCTGTATC GTACTGAGTTCCTGTT
18. Phen-T::*ptsI*^{WT}-R2	Same as 17 but downstream reverse primer	CTTCAACAGAGATGATCATCGGAAAC
19. Phen-T::*ptsI*^{WT}-F3	Amplifying downstream fragment for construction of wild-type *ptsI* homologous template for restoring Phen-T back to wild-type *ptsI* by CRISPR	GTTTCCGATGATCATCTCTGTTGAAG
20. Phen-T::*ptsI*^{WT}-R3	Same as 19 but downstream reverse primer	AA**CTGCAG**AGCATCAATAACTTGC (*PstI*)
21. *dgcM*-G228A-F	Amplifying *dgcM* (G228A) from Phen-T as homologous template for constructing *dgcM* (G228A) mutant by CRISPR targeting of FRT sequence after kanamycin marker removal of the Keio library *DdgcM* mutant	TGGAGTTTCGGCGGGAAGGGTT
22. *dgcM*-G228A-R	Same as 21 but downstream reverse primer	TTAAGGTGGAGCCAACAGGGA
23. *dgcM*-G228A check-F	Sequencing of *dgcM* (G228A) for mutation confirmation	TAACCAACGTGCGCCGCAGCGTAT

24. *ptsH* H15Q-F1	Amplifying upstream fragment for construction of *ptsH* (H15Q) homologous template for CRISPR-based construction of *ptsH* (H15Q) mutant	GAAGAAGCGATTTCTACCGCGCGT
25. *ptsH* H15Q-R1	Same as 24 but downstream reverse primer	GTCTGCAGACCGTTCGGAGCGGTA
26. *ptsH* H15Q-F2	Amplifying downstream fragment for construction of *ptsH* (H15Q) homologous template for CRISPR-based construction of *ptsH* (H15Q) mutant	AACGGTCTGCAGACCCGCCCTG
27. *ptsH* H15Q-R2	Same as 26 but downstream reverse primer	CCTGAATGGCGCTCAGGTCGA
28. *ptsH* H15Q-check-F	Amplifying, sequencing *ptsH* (H15Q) fragment for mutation confirmation	CGCTGGTTTTATCCCGGCTAACC
29. *ptsH* H15Q-check-R	Same as 28 but downstream reverse primer	CGCGTCGGTGATGAAACCCA
30. *ptsI* H189Q-F1	Amplifying upstream fragment for construction of *ptsI* (H189Q) homologous template for CRISPR-based construction of *ptsI* (H189Q) mutant	AATGACGTACGAAACGTCAGCG
31. *ptsI* H189Q-R1	Same as 30 but downstream reverse primer	CCATGATAGAGGTCTGGGAAGTAC
32. *ptsI* H189Q-F2	Amplifying downstream fragment for construction of *ptsI* (H189Q) homologous template for CRISPR-based construction of *ptsI* (H189Q) mutant	GTACTTCCCAGACCTCTATCATGGC
33. *ptsI* H189Q-R2	Same as 32 but downstream reverse primer	CGTCCATGTTGGAGATAACAACCGG
34. *ptsI* H189Q-check-F	Amplifying, sequencing *ptsI* (H189Q) fragment for mutation confirmation	AAACTTTCGCCCCTCCTGGCATTG
35. *ptsI* H189Q-check-R	Same as 34 but downstream reverse primer	GTTACGCTACCGGACAGTTTG
36. *crr* H91Q-F1	Amplifying upstream fragment for construction of *crr* (H91Q) homologous template for CRISPR-based construction of *crr* (H91Q) mutant	CGGCACCAATGATTTAACGCAG
37. *crr* H91Q-R1	Same as 36 but downstream reverse primer	GTGTCGATACCGAACTGGACGA
38. *crr* H91Q-F2	Amplifying downstream fragment for construction of *crr* (H91Q) homologous template for CRISPR-based construction of *crr* (H91Q) mutant	ACTGTTCGTCCAGTTCGGTATCGACA
39. *crr* H91Q-R2	Same as 38 but downstream reverse primer	GGAATTACCCCCAATATCTTTGAG
40. *crr* H91Q-check-F	Amplifying, sequencing *crr* (H91Q) fragment for mutation confirmation	CAACAATTGCACGTCATTTAGCC
41. *crr* H91Q-check-R	Same as 40 but downstream reverse primer	GGGAATTACCCCCAATATCT
42. pACYC184-*ptsHI*-F	Amplifying wild-type *ptsHI* operon for constructing recombinant pACYC184-*ptsHI* for complementation	AAGC**TCTAGA**CGCCAGGCTAGACTT (*XbaI*)
43. pACYC184-*ptsHI*-R	Same as 42 but downstream reverse primer	GCATGC**GTCGAC**TTAGCAGATTGTTT (*SalI*)
44. *crp**-F	Amplifying *crp** mutant allele from strain 19 for constructing *crp** mutant in strain 1 (WT) using CRISPR	CGCGCTTGCATTTTTGCTACTCC
45. *crp**-R	Same as 44 but downstream reverse primer	GCAAAAAGGGCAGGGGAACATC

46. *crp**-check-F	Amplifying, sequencing *crp** fragment from *ΔptsI-crp** and *crp** mutants for *crp** mutation confirmation	ACTTCACAATCGACCACATCCTG
47. *crp**-check-R	Same as 46 but downstream reverse primer	GTTAAACAATCCGTACCAGAGAG
48. pACYC184-*cyaA*-F	Amplifying wild-type *cyaA* for constructing recombinant pACYC184-*cyaA* for complementation	GC**TCTAGA**GAATTCAACCGGCACATCTTTC (*XbaI*)
49. pACYC184-*cyaA*-R	Same as 48 but downstream reverse primer	ACGC**GTCGAC**TCACGAAAAATATTGCTGTAA (*SalI*)
50. pACYC184-*crp*-F	Amplifying wild-type *crp* for constructing recombinant pACYC184-*crp* for complementation	GC**TCTAGA**ATCTGATGACCAGAGGCGGA (XbaI)
51. pACYC184-*crp*-R	Same as 50 but downstream reverse primer	ACGC**GTCGAC**TTAACGAGTGCCGTAAACGACGA (*SalI*)
plasmid	**Characteristics**[b]	**Source or reference**
pACYC184	Cm^R, tet^R, *E. coli* plasmid vector	(2, 3)
pACYC184-*ptsHI*	*ptsH-ptsI* operon with native promoter cloned into pACYC184	This study
pACYC184-*cyaA*	*cyaA* with native promoter cloned into pACYC184	This study
pACYC184-*crp*	*crp* with native promoter cloned into pACYC184	This study
pCas9	Kan^R, *rep*A101^{ts}, *lac*Iq-Ptrc fragment, sg-RNA-pMBI, λ-Red recombinase gene (exo bet gam paraB)	(4)
pTargetF	aadA ($spec^R$), pMB1, pj23119 (synthetic promoter)-N20-sgRNA	(4)
pTargetF-sgRNA-FRT	$spec^R$, sgRNA with an N20 sequence targeting the *FRT* region of marker-less Keio library mutant.	This study
PTargetF-sgRNA-*ptsI*	$spec^R$, sgRNA with an N20 sequence for targeting the partial *ptsI* fragment from wildtype, with the homologous fragment of *ptsI*	This study
PTargetF-sgRNA-*ptsI*$^{Phen\text{-}T}$	$spec^R$, sgRNA with an N20 sequence targeting the mutated *ptsI* sequence from Phen-T, with the homologous fragment of *ptsI*^{Wt} (wild type sequence)	This study
pCP20	Plasmid containing yeast FLP recombinase for antibiotic marker removal; Amp^R, Cm^R	(5, 6)

[a]Capital letters in primer names indicate forward (F) and reverse (R) primers for PCR amplification; check indicates primers used for mutation confirmation; up and down indicate primers used for PCR amplification of the upstream and the downstream homologous fragment of the targeted gene for mutant construction.

[b]Bold, underlined bases indicate restriction enzyme recognition sites (listed in parenthesis) used for recombinant plasmid construction

[c]Antibiotic resistance marker: Cm^R, chloramphenicol; Tet^R, tetracycline; $Spec^R$, spectinomycin; Amp^R, ampicillin

Supplementary References

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