

Supplemental Materials

1 **FILE S1** Quantification of intracellular TMP via LC-MS.

2 TMP analysis was detected using an HPLC system (Ultimate 3000 HPLC, Thermo Fisher,
3 CA, USA) coupled to an AB Sciex 4500 triple-quadruple mass spectrometer (LC-MS/MS).
4 Samples were filtered and centrifuged at 10,000 g for 5 min, and these supernatants were
5 further diluted with purified water (1:1, v/v). The injector temperature was set to 20°C, and
6 the column was maintained at 40°C. The separation was performed on a column Synchronis
7 C18 (1.7 µm, 2.1 mm × 100 mm, Thermo Fisher) after injection of a 5 µL sample aliquot.
8 The gradient elution, with 0.1% (v/v) formic acid in methanol (A) and 0.1% (v/v) formic
9 acid in water (B) at a flow rate of 0.3 mL/min, was optimized as follows: 0–5 min (90–30%
10 B), hold for 3 min (30% B), 8.01 min (90% B), and equilibrate for 2 min (90% B). The
11 MS/MS was operated in the multiple reaction monitoring (MRM) mode. The LC-MS/MS
12 parameters of TMP are provided in Table S8. The retention time of TMP was 5.32 min.
13 Quantification was conducted using a standard calibration curve consisting of a
14 concentration gradient (0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10 ng mL⁻¹) with the deviation of
15 every point from the regression line being less than 20% from that of its theoretical value.
16 The regression coefficients (r^2) of the calibration curve for the target analyte were higher
17 than 0.99. The TMP concentration was finally normalized to the OD600, the reading of
18 which was taken from the original bacterial culture.

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20 **TABLE S1** TMP MICs in Enterohemorrhagic *E. coli* (EHEC) and *E. coli* BW25113 and
 21 MG1655 strains.

Strains	MIC for TMP ($\mu\text{g}/\text{mL}$)	Fold change
EHEC O157:H7	0.64	—
EHEC O157:H7:: <i>Kan</i>	0.64	—
EHEC O157:H7 Δ <i>gshA</i> :: <i>Kan</i>	2.56	4
EHEC O157:H7 Δ <i>gshB</i> :: <i>Kan</i>	2.56	4
EHEC O157:H7 Δ <i>grxA</i>	1.28	2
EHEC O157:H7 Δ <i>cydD</i> :: <i>Kan</i>	5.12	8
<i>E. coli</i> ATCC 25922	2.56	—
<i>E. coli</i> ATCC 25922 Δ <i>gshA</i>	20.48	8
<i>E. coli</i> ATCC 25922 Δ <i>gshB</i>	10.24	4
<i>E. coli</i> ATCC 25922 Δ <i>grxA</i>	5.12	2
<i>E. coli</i> ATCC 25922 Δ <i>cydD</i>	10.24	4
<i>E. coli</i> BW25113	0.32	—
<i>E. coli</i> BW25113 Δ <i>gshA</i>	1.28	4
<i>E. coli</i> BW25113 Δ <i>gshB</i>	1.28	4
<i>E. coli</i> BW25113 Δ <i>grxA</i>	0.64	2
<i>E. coli</i> BW25113 Δ <i>cydD</i>	1.28	4
<i>E. coli</i> MG1655	0.32	—
<i>E. coli</i> MG1655 Δ <i>gshA</i>	1.28	4
<i>E. coli</i> MG1655 Δ <i>gshB</i>	1.28	4
<i>E. coli</i> MG1655 Δ <i>grxA</i>	0.32	—
<i>E. coli</i> MG1655 Δ <i>cydD</i>	1.28	4

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24 **TABLE S2** Susceptibilities of *E. coli* W3110 Δ *cydD* and complement strain to multiple
 25 antimicrobial drugs with different IPTG concentration.

Strains	IPTG(μ m)	MIC ^a (μ g/mL) of							
		TMP	K	Neo	Gen	Spe	SM	Cm	RIF
<i>E. coli</i> W3110		0.32	5	5	2	20	8	5	8
<i>E. coli</i> W3110 Δ <i>cydD</i>	0	1.28	40	40	16	40	32	10	16
<i>E. coli</i> W3110 Δ <i>cydD</i> pCA24N:: <i>cydD</i>		0.32	10	10	8	40	16	—	16
<i>E. coli</i> W3110		0.32	5	5	2	20	8	5	8
<i>E. coli</i> W3110 Δ <i>cydD</i>	2.5	1.28	40	40	16	40	32	10	16
<i>E. coli</i> W3110 Δ <i>cydD</i> pCA24N:: <i>cydD</i>		0.32	10	10	8	40	16	—	16
<i>E. coli</i> W3110		0.32	5	5	2	20	8	5	8
<i>E. coli</i> W3110 Δ <i>cydD</i>	5	1.28	40	40	16	40	32	10	16
<i>E. coli</i> W3110 Δ <i>cydD</i> pCA24N:: <i>cydD</i>		0.32	20	20	8	40	16	—	16

26 ^aTMP, trimethoprim; K, kanamycin; Neo, neomycin; Gen, gentamicin; Spe,
 27 spectinomycin; SM, streptomycin; Cm, chloramphenicol; RIF, rifampin.

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29 **TABLE S3** Comparative analysis for target gene mutations in NCBI pathogen list.

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31 **TABLE S4** GSH content in *E. coli* W3110, $\Delta grxA$, $\Delta cydD$, and $\Delta gshA$.

Strains	GSH _{in} ($\mu\text{m}/\text{OD}_{600}$)	GSH _{per} ($\mu\text{m}/\text{OD}_{600}$)
<i>E. coli</i> W3110	24.32 \pm 0.81 (1.0)	9.98 \pm 0.44 (1.0)
<i>E. coli</i> W3110 $\Delta grxA$	26.49 \pm 0.89 (1.09)	3.98 \pm 0.52 (0.4)
<i>E. coli</i> W3110 $\Delta cydD$	23.00 \pm 0.98 (0.95)	6.31 \pm 0.80 (0.63)
<i>E. coli</i> W3110 $\Delta gshA$	0	0

32 Data are presented the mean \pm SD and set as the ratio between the values in mutants and wild-
 33 type strain in parentheses.

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37 **TABLE S5** The effects of exogenous GSH treatments on TMP sensitivity in different
 38 strains.

Strains	MIC for TMP (mg/L)	
	No addition	2 mM GSH
<i>E. coli</i> W3110	0.32	0.32
<i>E. coli</i> W3110 $\Delta cydD$	1.28	0.32
<i>E. coli</i> W3110 $\Delta grxA$	1.28	0.64
<i>E. coli</i> W3110 $\Delta gshA$	1.28	0.64
<i>E. coli</i> W3110 $\Delta gshB$	1.28	0.64

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40 **TABLE S6** The gene expression levels of *acrAB*, *tolC*, and *soxS* in different strains.

Strains	<i>acrA</i> ^a		<i>acrB</i>		<i>tolC</i>		<i>soxS</i>	
	FC ^b	R ^c	FC ^b	R ^c	FC ^b	R ^c	FC ^b	R ^c
<i>E. coli</i> W3110	1	–	1	–	1	–	1	–
<i>E. coli</i> W3110 Δ <i>grxA</i>	3.20	UP	6.29	UP	2.28	UP	5.05	UP
<i>E. coli</i> W3110 Δ <i>cydD</i>	1.55	UP	2.69	UP	2.97	UP	3.12	UP
<i>E. coli</i> W3110 Δ <i>cpxR</i>	0.72	Down	0.71	Down	0.63	Down	0.71	Down
<i>E. coli</i> W3110 Δ <i>cydD</i> Δ <i>cpxR</i>	0.56	Down	0.51	Down	0.31	Down	0.37	Down

41 X^a means the Different gene names; FC^b means the fold change of gene expression ($2^{-\Delta\Delta C_t}$)
 42 $\Delta\Delta C_t$) which normalized by the *E. coli* W3110; R^c means the regulation of gene expression
 43 which was up-regulated or down-regulated.

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45 **TABLE S7** The Relative concentration levels of TMP accumulated within different *E.*
 46 *coli* strains.

Strains	TMP (ng/ml/OD ₆₀₀)
<i>E. coli</i> W3110	7.48 ± 0.19 (1.0)
<i>E. coli</i> W3110 Δ <i>cydD</i>	4.87 ± 0.17 (0.65)
<i>E. coli</i> W3110 Δ <i>cydD</i> Δ <i>acrA</i>	8.09 ± 0.03 (1.08)
<i>E. coli</i> W3110 Δ <i>cydD</i> Δ <i>tolC</i>	8.22 ± 0.25 (1.10)
<i>E. coli</i> W3110 Δ <i>acrA</i>	8.93 ± 0.05 (1.19)
<i>E. coli</i> W3110 Δ <i>acrB</i>	9.06 ± 0.30 (1.21)
<i>E. coli</i> W3110 Δ <i>tolC</i>	9.02 ± 0.06 (1.21)

47 Data are presented the mean ± SD and set as the ratio between the values in mutants and wild-
 48 type strain in parentheses.

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51 **TABLE S8** LC-MS/MS parameters of individual TMP

Compound	Q1 (m/z)	Q3 (m/z)	DP (V)	EP (V)	CE (ev)	CXP (V)
TMP	291.000	261.000	110.0	4.5	32.0	12.0
	291.000	123.000	110.0	4.5	32.0	9.0

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55 **TABLE S9** Plasmids, strains and primers used in this study.

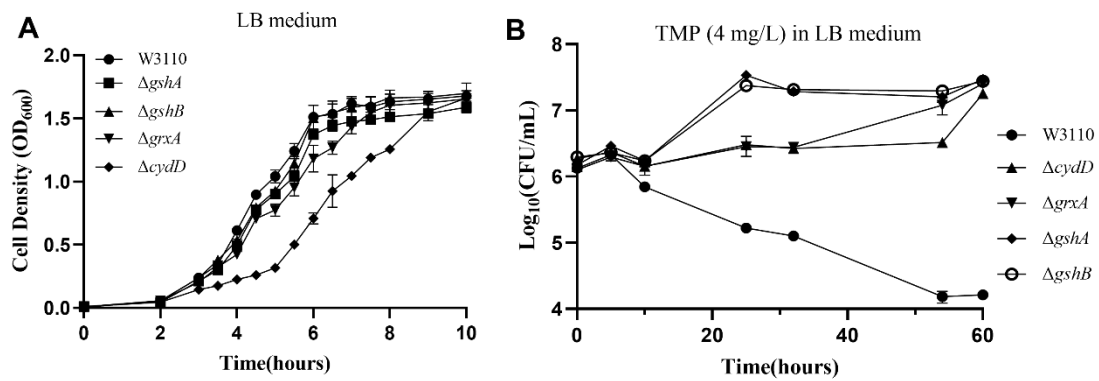
Category	Name	Relevant Features/Sequences	Source
Strains			
	<i>E. coli</i> W3110		Lab stock
	<i>E. coli</i> W3110 ΔX	<i>X</i> gene was deleted in <i>E. coli</i> W3110	This study
	<i>E. coli</i> W3110 $\Delta cydD\Delta X$	<i>X</i> gene was deleted in <i>E. coli</i> W3110 $\Delta cydD$	This study
	<i>E. coli</i> W3110 $\Delta grxA\Delta X$	<i>X</i> gene was deleted in <i>E. coli</i> W3110 $\Delta grxA$	This study
	<i>E. coli</i> W3110 $\Delta gshA\Delta X$	<i>X</i> gene was deleted in <i>E. coli</i> W3110 $\Delta gshA$	This study
	<i>E. coli</i> W3110 $\Delta gshB\Delta X$	<i>X</i> gene was deleted in <i>E. coli</i> W3110 $\Delta gshB$	This study
	<i>E. coli</i> BW25113		Lab stock
	<i>E. coli</i> BW25113 ΔX	<i>X</i> gene was deleted in <i>E. coli</i> BW25113	This study
	<i>E. coli</i> MG1655		Lab stock
	<i>E. coli</i> MG1655 ΔX	<i>X</i> gene was deleted in <i>E. coli</i> MG1655	This study
Primers			
	gshA ko-F	5- TTGATCCCGGACGTATCACAGGCGCTGGCCTGGCTGGAAAAACA TCCTCAATATGAATATCCTCCTTAG-3	This study
	gshA ko-R	5- TCAGGCGTGTTTTTCAGCCACACCGCAAACGGTTCGGTATCAGC GGCTTGTAGGCTGGAGCTGCTTCG-3	This study
	gshB ko-F	5- ATGATCAAGCTCGGCATCGTGATGGACCCATCGCAAACATCAA CATCAAATATGAATATCCTCCTTAG-3	This study
	gshB ko-R	5- TACTGCTGCTGTAAACGTGCTTCGATGGCATCCATTAACATTCC GGTGATGTAGGCTGGAGCTGCTTCG-3	This study
	grxA ko-F	5- ATGCAAACCGTTATTTTGGTCGTTTCGGGTTGCCCTTACTGTGTGC GTGCATATGAATATCCTCCTTAG-3	This study
	grxA ko-R	5- TCAGGCGTCCAGATTTTCTTTCACCCATGCAGCAAAATCGGTATA GCCGCTGTAGGCTGGAGCTGCTTCG-3	This study
	cydD ko-F	5- ATGAATAAATCTCGTCAAAAAGAGTTAACCCGCTGGTTAAAAACA GCAAAGATATGAATATCCTCCTTAG-3	This study
	cydD ko-R	5- TAAATCTCCTCTGACGATGGGCCAGTAATGTGGCGAATGGGCC ACCAGTGTAGGCTGGAGCTGCTTCG-3	This study
	gsiA ko-F	5- TTGCCACACAGTGATGAACTTGATGCCGGTAATGTGCTGGCGGTT GAAAAATATGAATATCCTCCTTAG-3	This study
	gsiA ko-R	5- TTATCTACGCATGAATGCGTATTCTGATTGTGGTTGTGCGACGTA ATGCCTGTAGGCTGGAGCTGCTTCG-3	This study
	gsiB ko-F	5- ATGGCAAGAGCTGTACACCGTAGTGGGTTAGTGGCGCTGGGCAT TGCGACATATGAATATCCTCCTTAG-3	This study
	gsiB ko-R	5- TTATTGCAAATCCGCGTCTTCAAAGCTGAAGCCGGTGTCTGGCAT GATCCTGTAGGCTGGAGCTGCTTCG-3	This study
	gsiAB ko-F	5- TTGCCACACAGTGATGAACTTGATGCCGGTAATGTGCTGGCGGTT GAAAAATATGAATATCCTCCTTAG-3	This study
	gsiAB ko-R	5- TTATTGCAAATCCGCGTCTTCAAAGCTGAAGCCGGTGTCTGGCAT GATCCTGTAGGCTGGAGCTGCTTCG-3	This study
	gsiC ko-F	5- ATGCTTAATTACGTTATCAAACGCTTACTGGGGTTGATTCCGACG CTGTTATATGAATATCCTCCTTAG-3	This study
	gsiC ko-R	5- TTACTTGTACCTGATAGCCGGGTTAATGGCGGCGTAAAGCACATC CACCATGTAGGCTGGAGCTGCTTCG-3	This study
	gsiD ko-F	5- ATGCGACTATTTAACTGGCGACGTCAGGCGGTGTTAAACGCCATG CCTACTATATGAATATCCTCCTTAG-3	This study
	gsiD ko-R	5- CTATCCTTTAATTTTCGGATCCAGCGCATCGCGTAAACCATCGCC CAACATGTAGGCTGGAGCTGCTTCG-3	This study

ggt ko-F	5- ATGATAAAACCGACGTTTTACGCCGGGTGGCCATTGCTGCTCTG CTCTCATATGAATATCCTCCTTAG-3	This study
ggt ko-R	5- TTAGTACCCCGCCGTTAAATCATCCACCGAGCGCGGGTCGGATGCT GCCGTTGTAGGCTGGAGCTGCTTCG-3	This study
acrA ko-F	5- ATGAACAAAAACAGAGGGTTTACGCCTCTGGCGGTCTGTTCTGAT GCTCTCATATGAATATCCTCCTTAG-3	This study
acrA ko-R	5- TTAAGACTTGGACTGTTTACGGCTGAGCACCGCTTGGCGGCTTGCTG GTTATTGTAGGCTGGAGCTGCTTCG-3	This study
acrB ko-F	5- ATGCCTAATTTCTTTATCGATCGCCCATTGCGTGGGTGATCG CCATATATGAATATCCTCCTTAG-3	This study
acrB ko-R	5- TCAATGATGATCGACAGTATGGCTGTGCTCGATATCTTCATTCTT GCGGCTGTAGGCTGGAGCTGCTTCG-3	This study
tolC ko-F	5- ATGAAGAAATTGCTCCCCATTCTTATCGGCCTGAGCCTTTCTGGG TTCAGATATGAATATCCTCCTTAG-3	This study
tolC ko-R	5- TCAGTTACGGAAGGGTTATGACCGTTACTGGTGGTAGTGCCTGCT GGATGTGTAGGCTGGAGCTGCTTCG-3	This study
cpxA ko-F	5- ATGATAGGCAGCTTAACCGCGCGCATCTTCGCCATCTTCTGGCTG ACGCTATATGAATATCCTCCTTAG-3	This study
cpxA ko-R	5- TTAACTCCGCTTATACAGCGGCAACCAAATCACCAGCCGTAAACCT GCCCATGTAGGCTGGAGCTGCTTCG-3	This study
cpXR ko-F	5- ATGAATAAAATCCTGTTAGTTGATGATGACCGAGAGCTGACTTCC CTATTATATGAATATCCTCCTTAG-3	This study
cpXR ko-R	5- TCATGAAGCAGAAACCATCAGATAGCCGCGACCACGCAAGGTTT TAAACCTGTAGGCTGGAGCTGCTTCG-3	This study
soxS ko-F	5- ATGTCCCATCAGAAAATTATTCAGGATCTTATCGCATGGATTGAC GAGCAATATGAATATCCTCCTTAG-3	This study
soxS ko-R	5- TTACAGGCGGTGGCGATAATCGCTGGGAGTGGCGATCAAACCTGCC GACGGATGTAGGCTGGAGCTGCTTCG-3	This study
gshA d-F	5-CTATGGTGCAGTCACGCTATTA-3	This study
gshA d-R	5-GGCATCACTTCTTTGTTCCGGCA-3	This study
gshB d-F	5-ATGAAGGACTGAAACTGAATCT-3	This study
gshB d-R	5-CCAGAATCCCTTCAATTTTGGAG-3	This study
grxA d-F	5-ATGGCTCAGGCAGCGACAGGGA-3	This study
grxA d-R	5-GCTTGCTATTTTTCATTCTTCC-3	This study
cydD d-F	5-AGTCCGGTATTCATGGTAATGC-3	This study
cydD d-R	5-ACTTACCAGACGTTCAAAATAG-3	This study
gsiA d-F	5-GGCACGGGCGAAGTCTTCATCC-3	This study
gsiA d-R	5-TTATCCAGACCGAACAGCCCT-3	This study
gsiB d-F	5-GATGTATCTCGGGCAAATTGTT-3	This study
gsiB d-R	5-GCTGGCAATCTCATCGGCAACC-3	This study
gsiAB d-F	5-CTGCGAGCGGGTAGTAATGGAA-3	This study
gsiAB d-R	5-GCTGCTGACGCACCAGTTCTAT-3	This study
gsiC d-F	5-ACTTCTGGCTCAGGCACTGAA-3	This study
gsiC d-R	5-AAAATGTCACGCCCCAGTGAAT-3	This study
gsiD d-F	5-GAGGGCGAAAGGGGTGAGCGAA-3	This study
gsiD d-R	5-GTTAATGATGAAAAAAGACCA-3	This study
ggt d-F	5-AGTGCCGTAAGAATCAGAAGTCG-3	This study
ggt d-R	5-CCGCTGGAAGAATATCTACAGGAT-3	This study
acrA d-F	5-GCCCAGCGAGGTGGATGATACCCCC-3	This study
acrA d-R	5-GGTACCCGTGGAGTCACTGTAGAG-3	This study
acrB d-F	5-GCGGATGACAAAGTGAAACCC-3	This study
acrB d-R	5-GGTGACACTAATACCAGGATTGCTC-3	This study
tolC d-F	5-ATGTAACGGGCAGGTTGTCTGG-3	This study
tolC d-R	5-CGATGCGTGGCGTATGGATTT-3	This study
cpxA d-F	5-GCATCTGGGTGAGGTTTCC-3	This study
cpxA d-R	5-TGACGATCAATATCACCGTTACTCT-3	This study

cpxR d-F	5-TCGAACATATGGCTCTGCGTACTGC-3	This study
cpxR d-R	5-CTGTCCTGGCGGTGCCCACTTATCA-3	This study
soxS d-F	5-GTCGCCAGCGGAATGCCAATAC-3	This study
soxS d-R	5-AACCTTAAATCATTGAATGTCG-3	This study
gapA-RT-F	5-ACTGACTGGTATGGCGTTCC-3	This study
gapA-RT-R	5-GCAGCTTTTCCAGACGAAC-3	This study
acrA-RT-F	5-TGGCGCGGATGACAAAG-3	This study
acrA-RT-R	5-CCAATAGCCTGGCTTGCAA-3	This study
acrB-RT-F	5-TGGTGACCGCAACGGTACT-3	This study
acrB-RT-R	5-GGCGAACCACCACAAAGAAT-3	This study
tolC-RT-F	5-AAAACGTTGCACCGCAAAC-3	This study
tolC-RT-R	5-GGCGCATAACCATCAGCAAT-3	This study
soxS-RT-F	5-GGTGACGCATCAGACGCTTGG-3	This study
soxS-RT-R	5-CGAGACATAACCCAGGTCCATTGC-3	This study

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59 **FIG. S1** Growth curves (A) and time-kill curves (B) of different *E. coli* strains in LB
 60 medium. Data represent mean \pm standard deviation (SD) of the results from three
 61 independent experiments.