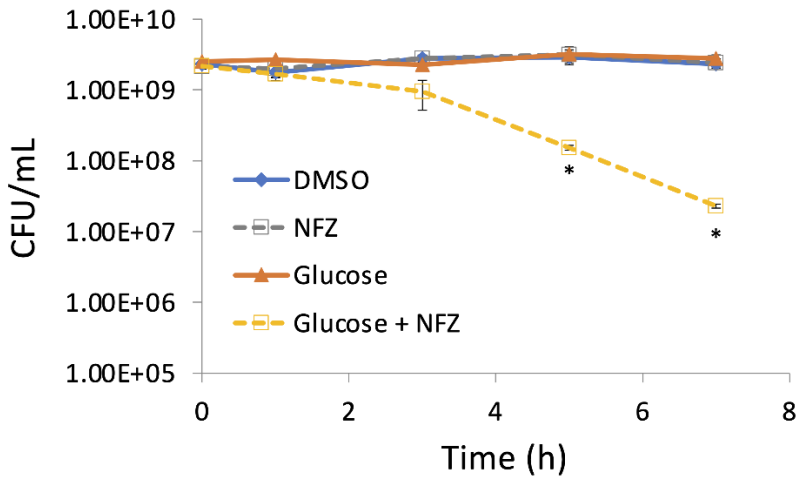
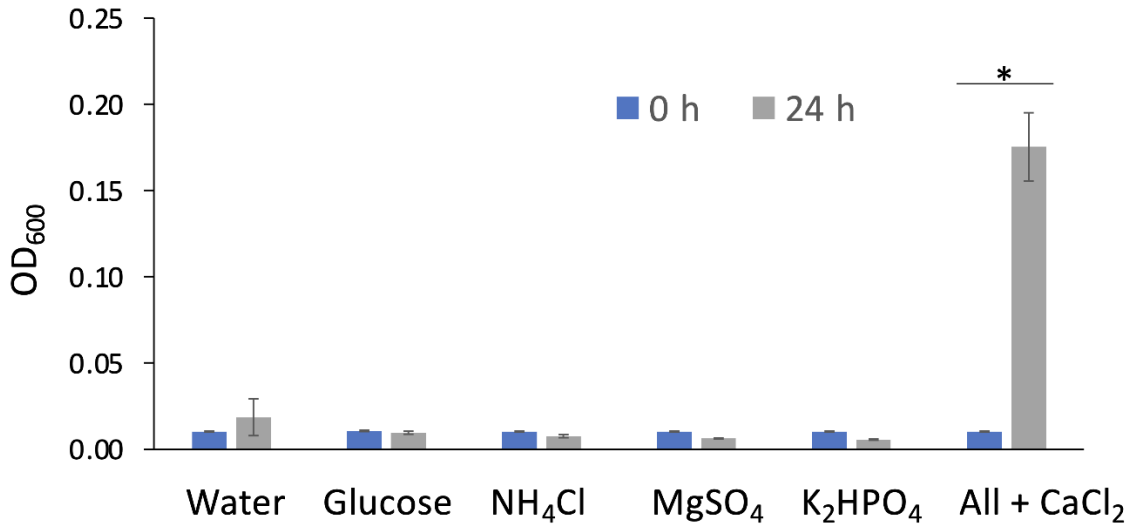
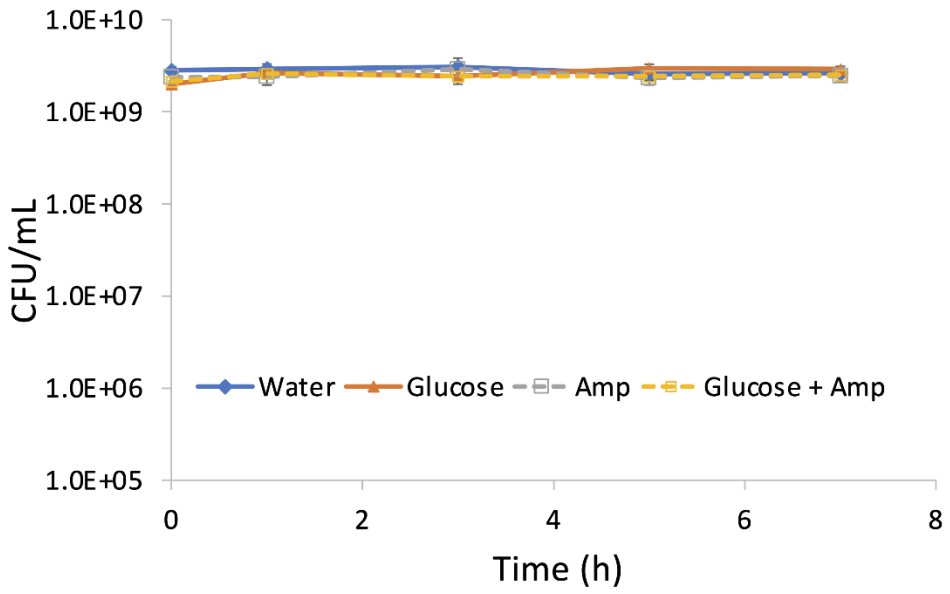


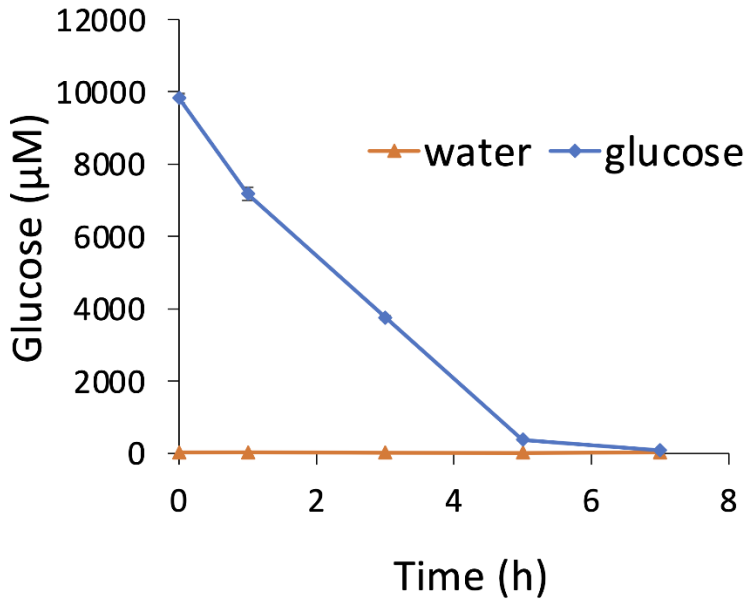
Supplementary Figure 1. Glucose-mediated re-sensitization of non-growing *E. coli* to nitrofurazone. Overnight cultures were treated with 250 µg/mL NFZ, 10 mM glucose, NFZ supplemented with glucose or DMSO (solvent for NFZ). CFUs per mL were monitored at the indicated time points. * $p < 0.05$ (t-test), comparing NFZ with NFZ + glucose. Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



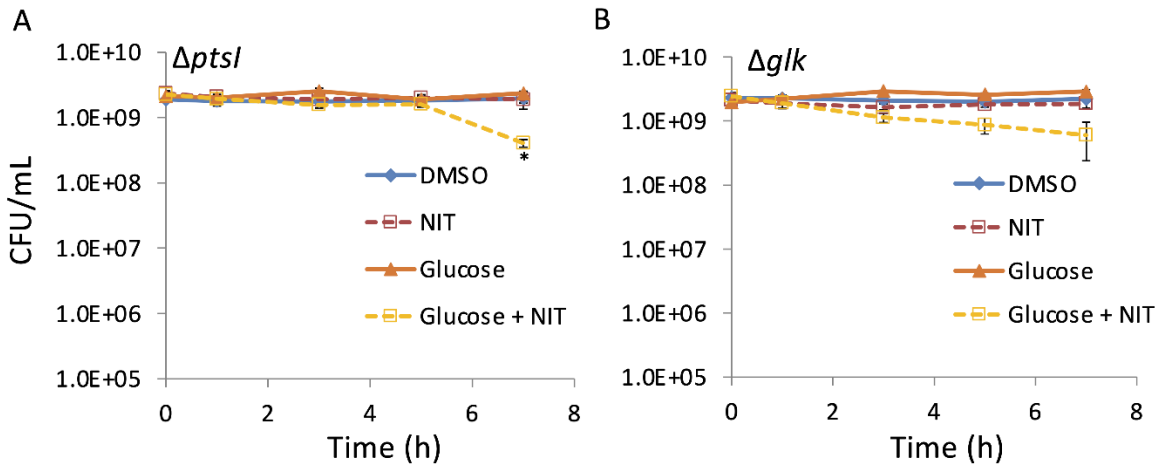
Supplementary Figure 2. Confirmation of non-growing conditions. Stationary-phase cultures in MOPS + glucose were washed with saline, diluted to OD_{600} ~ 0.01 in saline and further incubated for 1 h before addition of water, 10 mM glucose, 9.5 mM NH_4Cl, 0.286 mM $MgSO_4$, or 1.32 mM K_2HPO_4. One test tube received all the chemicals plus 0.1 mM $CaCl_2$ (growth control). Following treatments, cultures were incubated for 23 h. OD_{600} was determined immediately after dilution and 24 h after treatments. * $p < 0.05$ (t-test), comparing 0 with 24 h. Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



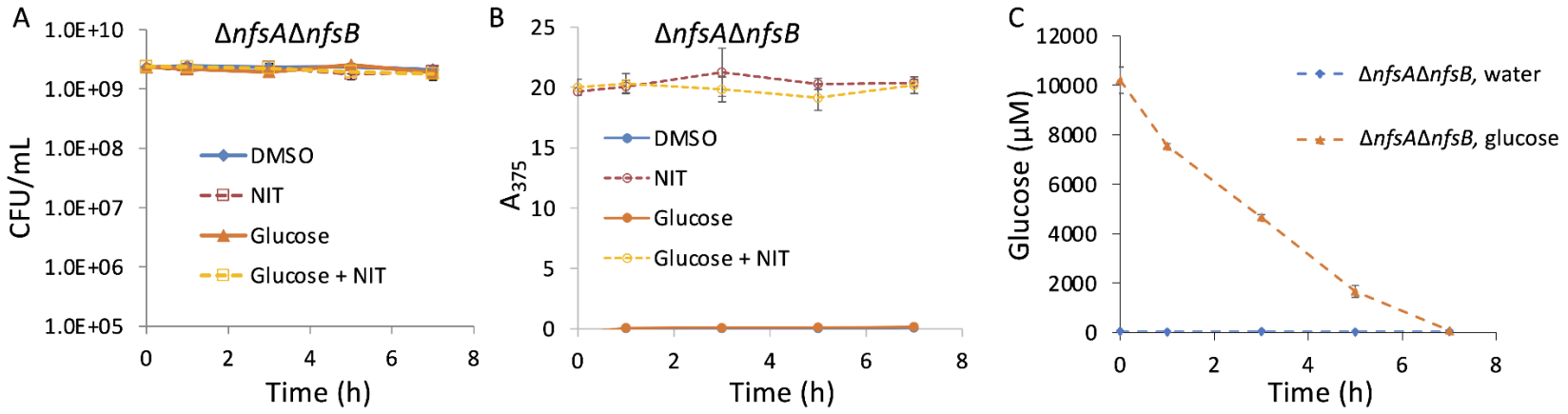
Supplementary Figure 3. Ampicillin challenge assay. Cultures of *E. coli* were grown to stationary phase in MOPS + glucose and treated with water (control), 200 µg/mL ampicillin, 10 mM glucose, or ampicillin plus glucose. CFUs per mL were determined at the indicated time points. No significant killing was observed by ampicillin treatment with or without glucose supplementation when compared with the water-treated control. Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



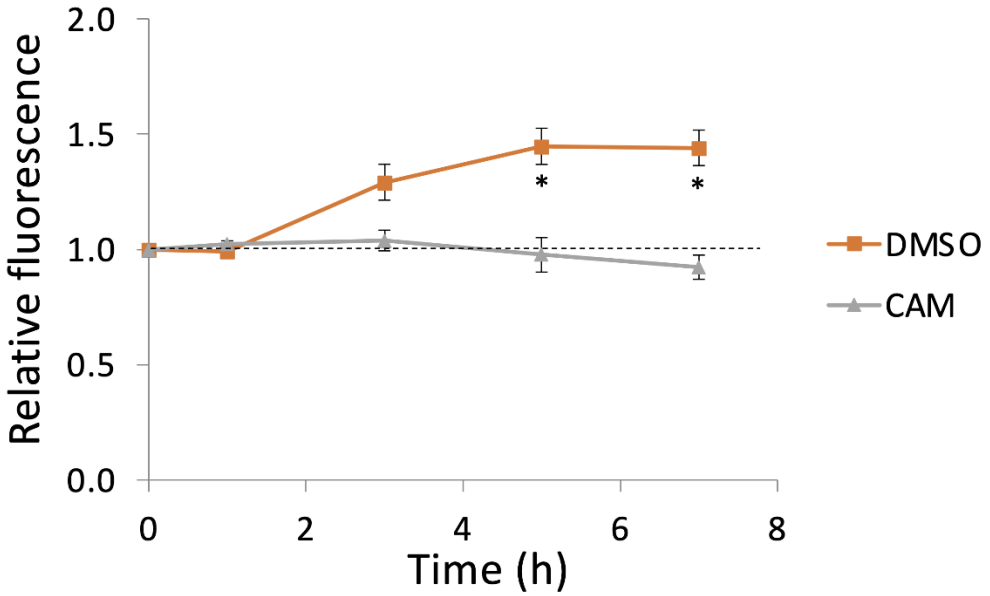
Supplementary Figure 4. Glucose consumption by wild-type *E. coli*. Overnight cultures were treated with glucose or water. Supernatant was collected for determination of glucose concentration immediately after addition of glucose or water and after 1, 3, 5, and 7 h of incubation. Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



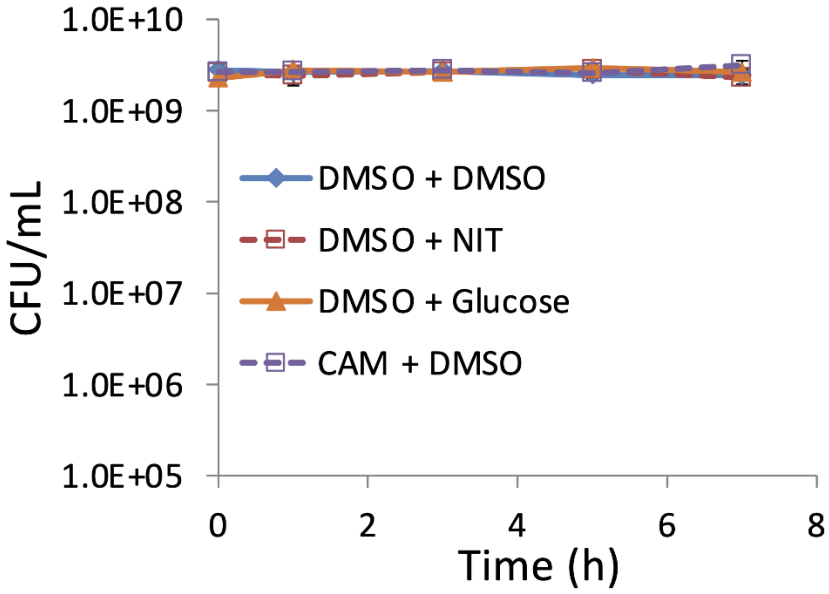
Supplementary Figure 5. Assessment of glucose-mediated NIT re-sensitization in Δ*ptsI* and Δ*glk*. Stationary-phase cultures of Δ*ptsI* (A) or Δ*glk* (B) were treated with DMSO, 250 µg/mL NIT, 10 mM glucose or NIT supplemented with glucose for 7 h. At the indicated time points aliquots were taken for CFUs per mL determination. * $p < 0.05$ (t-test), comparing NIT versus Glucose + NIT at t = 7 h. Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



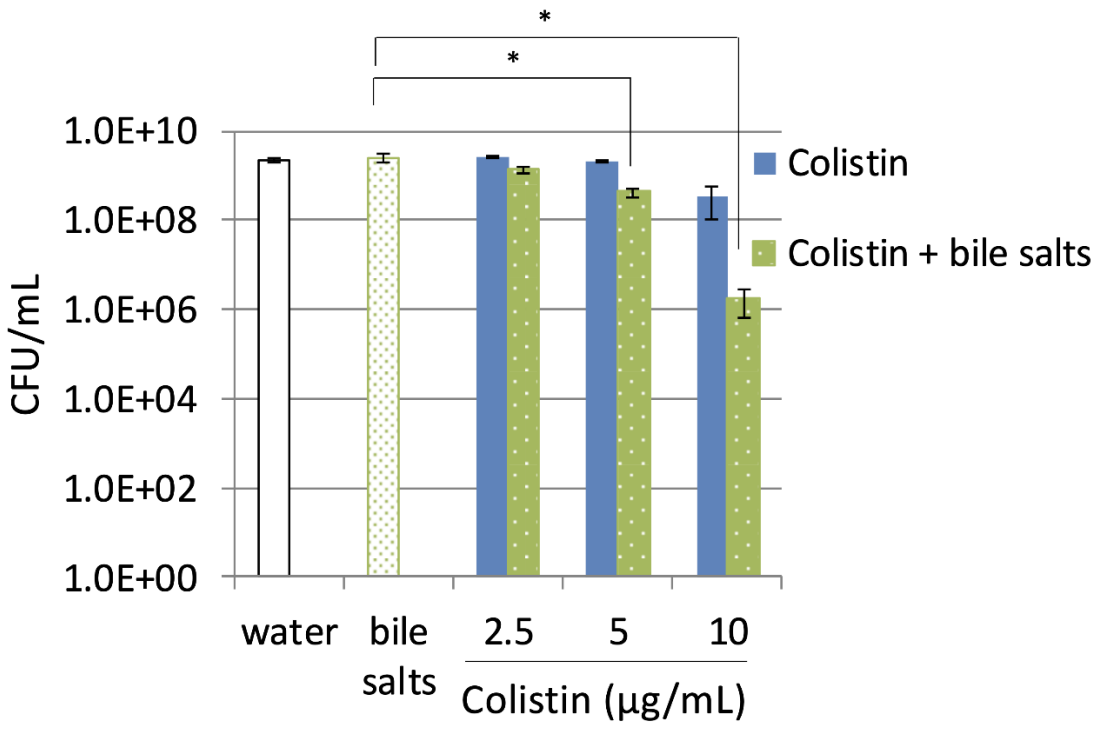
Supplementary Figure 6. NIT activation requires NfsA and NfsB during non-growing conditions. An *E. coli* strain where *nfsA* and *nfsB* had been deleted (Δ*nfsA*Δ*nfsB*) was grown to stationary phase and treated with DMSO, 250 µg/mL NIT, 10 mM glucose or NIT supplemented with glucose for 7 h. At the indicated time points aliquots of the cultures were taken for CFUs per mL determination (A), absorbance measurements at 375 nm (B), or glucose concentration measurements (C). Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



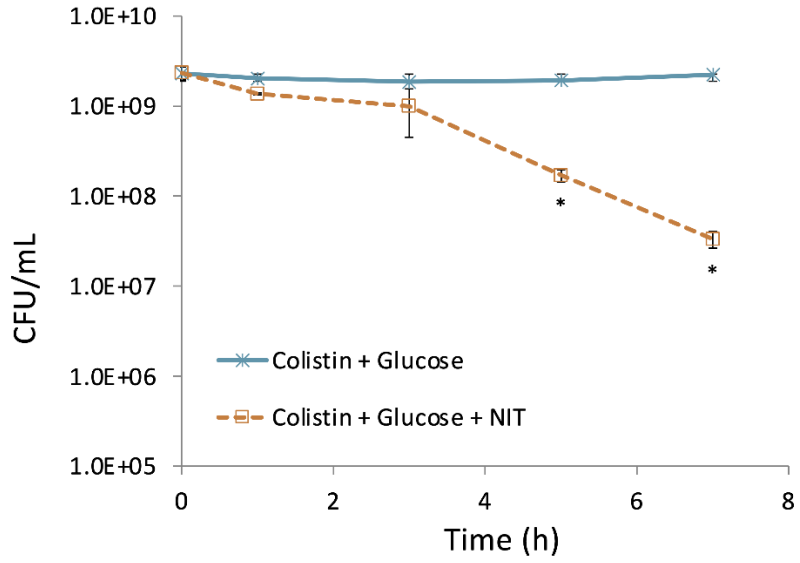
Supplementary Figure 7. Determination of experimental conditions for inhibition of protein synthesis during stationary phase. A culture of wild-type *E. coli* strain carrying pQE-80L-*gfp* (IPTG-inducible GFP) was grown to stationary phase for 19 h (t = 19 h). At t = 19 h, DMSO (solvent for CAM) or 100 µg/mL CAM was added, and cultures further incubated for 1 h. At t = 20 h, 1 mM IPTG was added to both cultures. Green fluorescence was monitored at the indicated time points. Data are presented relative to the green fluorescence signal at the time of IPTG addition (t = 0). * $p < 0.05$ (t-test), comparing values to that of 1, which represents the absence of change with respect to the initial time point. Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



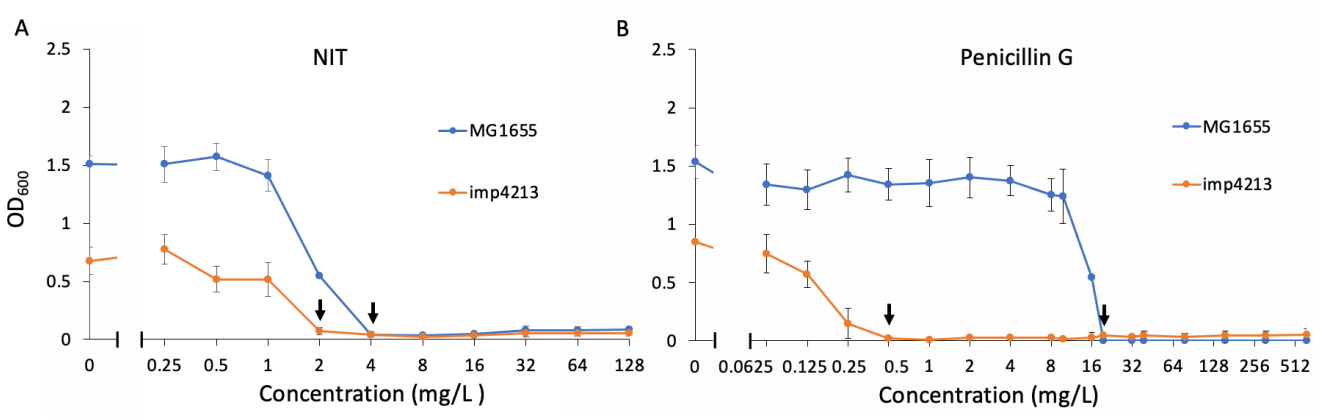
Supplementary Figure 8. Pre-treatment with DMSO does not impact NIT or glucose treatments. Stationary-phase cultures of *E. coli* were treated with DMSO (solvent for CAM) or CAM and incubated for 1 hour. After 1 h incubation, cultures were treated with DMSO (solvent for NIT), 250 µg/mL NIT or 10 mM glucose. CFUs per mL were determined at the indicated time points. Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



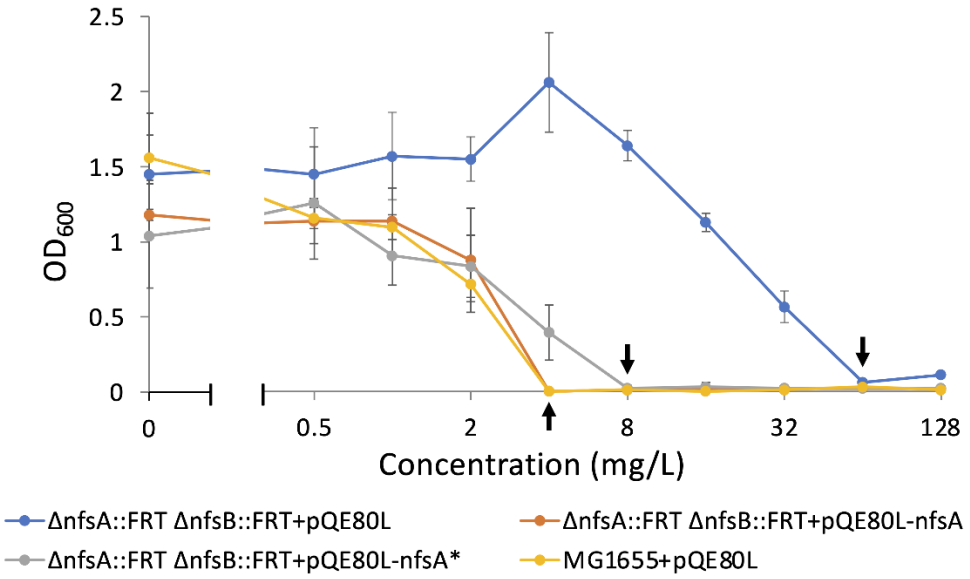
Supplementary Figure 9. Determination of colistin concentration necessary to permeate stationary-phase *E. coli* membranes. Stationary-phase cultures of *E. coli* were divided into eight test tubes before treatment with water, 1.5 mg/mL bile salts, or different concentrations of colistin (2.5, 5, and 10 µg/mL) in the absence or presence of 1.5 mg/mL bile salts. Cultures were incubated for 7 h. At t = 7 h, samples were processed to measure CFUs per mL and plated onto LB agar plates. * $p < 0.05$ (t-test), comparing bile salts with colistin + bile salts. Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



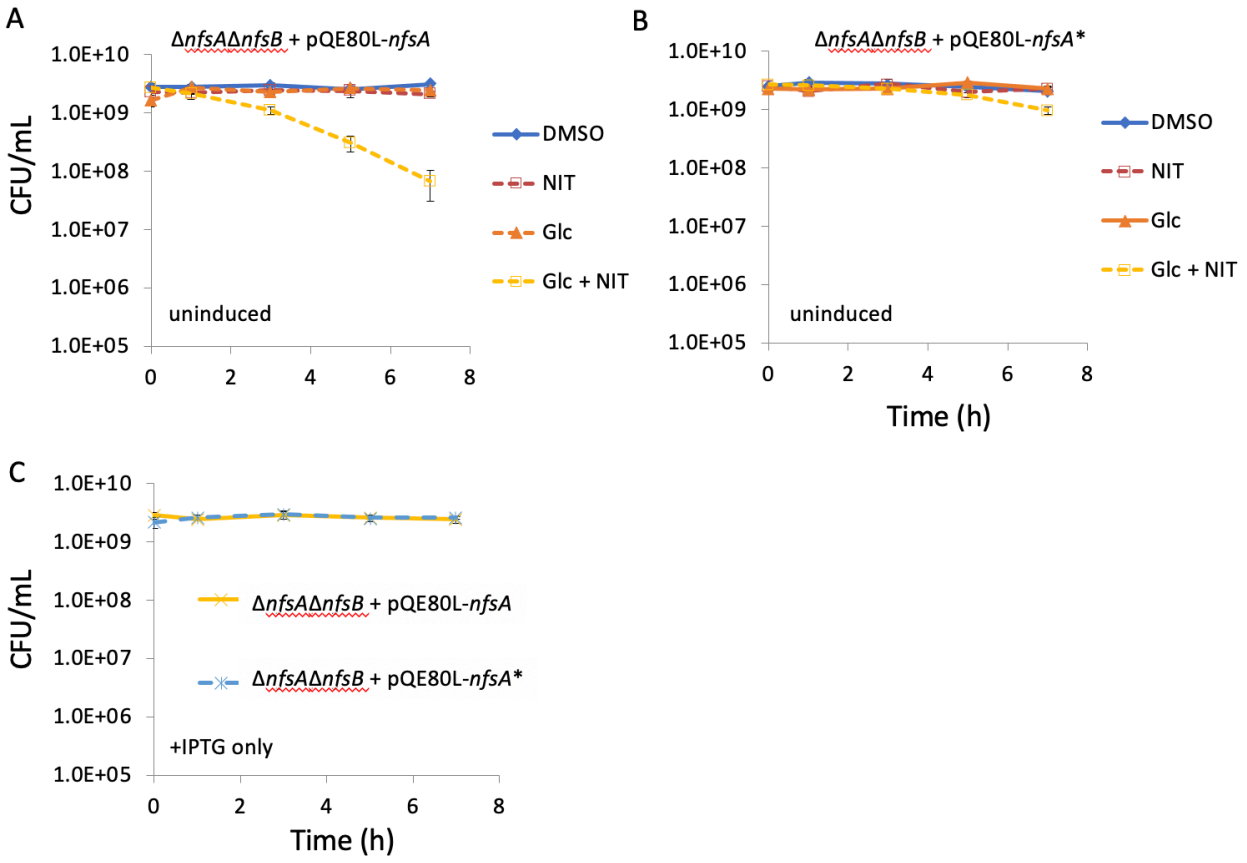
Supplementary Figure 10. Additional controls for membrane permeabilization experiments. Stationary-phase cultures of *E. coli* were grown the same way as described for Figure 6A and treated with 5 μg/mL colistin plus glucose or colistin plus glucose and NIT. * $p < 0.05$ (t-test), comparing Colistin + Glucose with Colistin + Glucose + NIT. Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



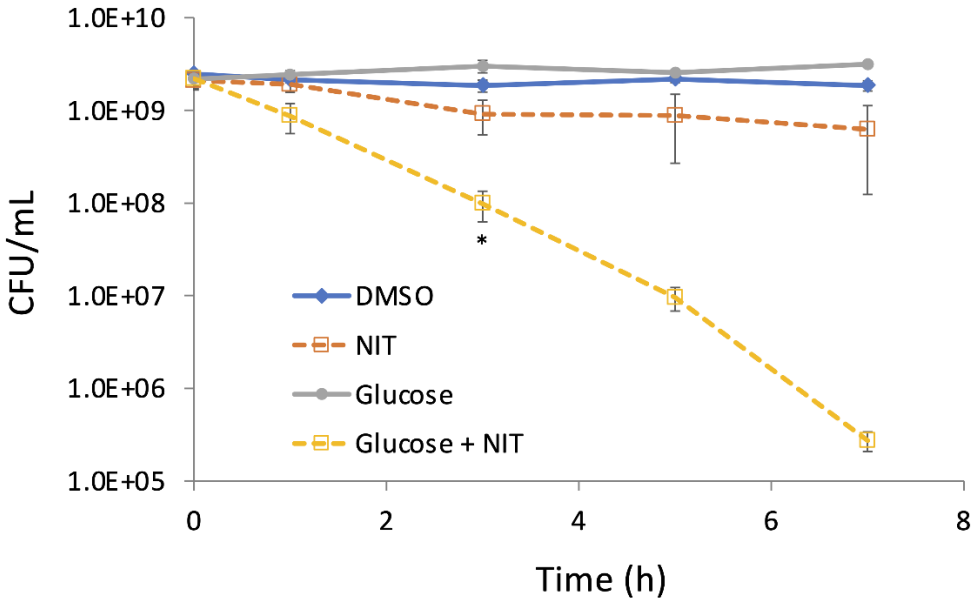
Supplementary Figure 11. Minimum inhibitory concentrations of *imp4213* and MG1655 strains. MICs were determined by the microdilution method as described in the Materials and Methods. Optical density (OD_{600}) measurements were carried out after incubation for 16-18 h with NIT or penicillin G. MICs are indicated (arrowhead). Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



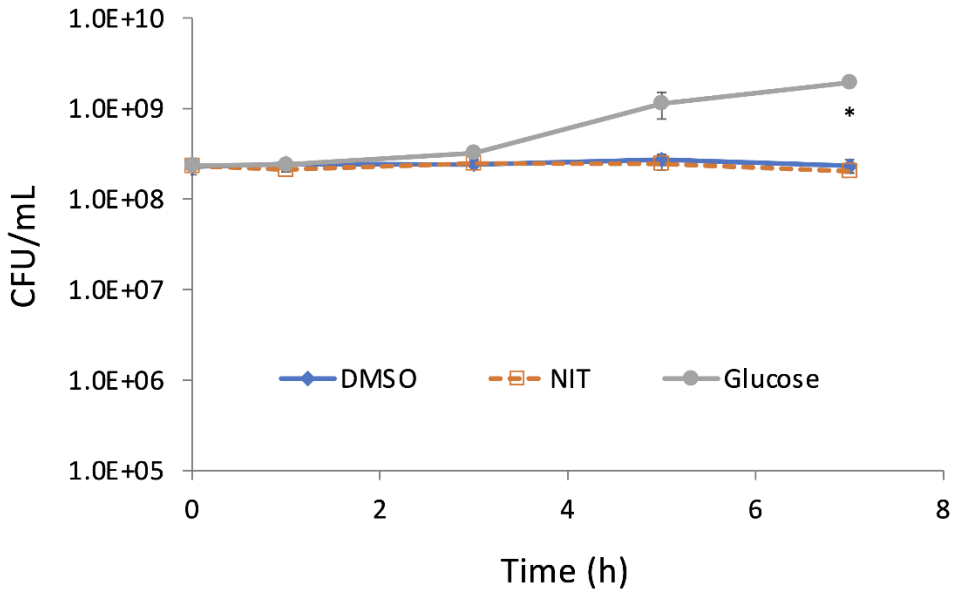
Supplementary Figure 12. Minimum inhibitory concentrations of Δ*nfsA*Δ*nfsB* strains expressing NfsA, NfsA*, or empty vector. MICs were determined by the microdilution method as described in the Materials and Methods. Optical density (OD_{600}) measurements were carried out after incubation for 16-18 h with NIT. MICs are indicated (arrowhead). Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



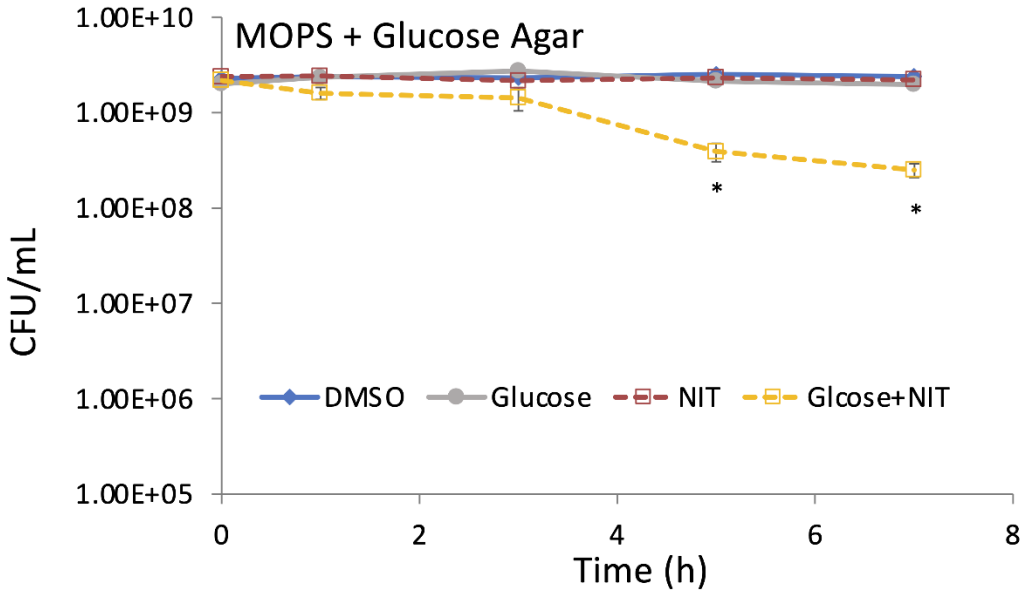
Supplementary Figure 13. Controls for NfsA and NfsA* expression experiments. Stationary-phase cultures of Δ*nfsA*Δ*nfsB* carrying either wild-type NfsA (pQE-80L-*nfsA*) (A) or a mutant NfsA protein (pQE-80L-*nfsA**) with decreased NAPDH binding affinity (B) were treated with DMSO, 250 µg/mL NIT, 10 mM glucose, or NIT supplemented with glucose. In (C), stationary-phase cultures of Δ*nfsA*Δ*nfsB* carrying either pQE-80L-*nfsA* or pQE-80L-*nfsA** were treated with 1 mM IPTG. CFUs per mL were determined at the indicated time points. Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



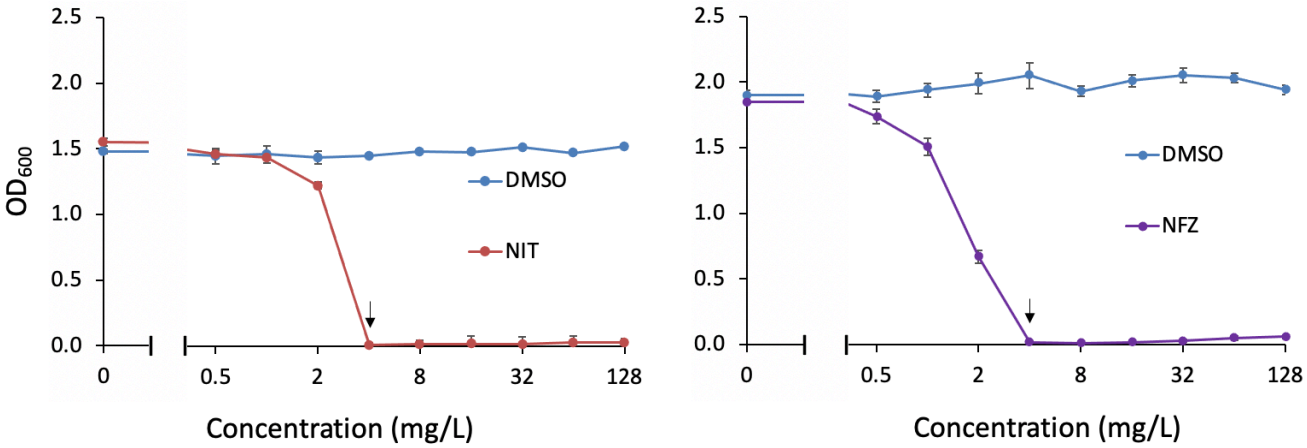
Supplementary Figure 14. Metabolite-mediated potentiation of NIT with stationary-phase cultures grown in LB for 24h. Stationary-phase cultures of *E. coli* were grown in LB for 24h and treated with DMSO, 250 µg/mL NIT, 10 mM glucose with or without NIT. CFUs per mL were monitored at indicated time points. * $p < 0.05$ (t-test), comparing NIT with Glucose + NIT. Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



Supplementary Figure 15. Stationary-phase cultures grown in AUM treated with 10 mM glucose resulted in growth. Cultures of *E. coli* were grown to stationary phase in AUM and treated with DMSO, 250 µg/mL NIT, or 10 mM glucose. CFUs per mL were monitored at indicated time points. * $p < 0.05$ (t-test), comparing DMSO with Glucose. Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.



Supplementary Figure 16. Re-sensitization of non-growing *E. coli* to NIT evaluated on MOPS + glucose agar plates. Stationary-phase cultures were processed identically as in Figure 2 except plating was on MOPS + glucose agar, rather than LB + agar. * $p < 0.05$ (t-test), comparing NIT with Glucose + NIT.



Supplementary Figure 17. Minimum inhibitory concentrations of NIT and NFZ. MICs were determined by the microdilution method. Optical density (OD_{600}) measurements were carried out after incubation for 16-18 h with NIT, NFZ, or DMSO, the solvent for NIT and NFZ. MICs are indicated (arrowhead). For a definition of the MIC, see Materials and Methods. Data represent at least three biological replicates. Each data point was denoted as mean ± s.e.

Supplementary Table 1. Bacterial strains and plasmids

Strain	**Relevant genotype**	**Source**
MG1655	F-, λ-, *ilvG-*, *rfb-50, rph-1*	ATCC 700926 (1)
MG1655Δ*ptsI*	Δ*ptsI*::FRT	P1 transduction of Keio mutant (2), followed by curing of the kanamycin resistance cassette
MG1655Δ*ptsI*Δ*glk*	Δ*ptsI*::FRTΔ*glk*::kan	PCR-based (3) *glk* inactivation in MG1655Δ*ptsI*
MG1655Δ*glk*	Δ*glk*::kan	P1 transduction of Keio mutant (2)
MG1655Δ*nfsA*Δ*nfsB*	Δ*nfsA*::FRTΔ*nfsB*::FRT	Sequential P1 transduction of Keio mutants (2) with step-wise curing of the kanamycin resistance cassette
CFT073 [WAM2267]	Genome sequenced strain. GenBank: AE014075.1	ATCC 700928. *rpoS* was sequenced and as indicated in the Genbank sequence, a 5 bp duplication of GTAGA starting at nucleotide 432 of the open reading frame was detected, which produced a frame-shift in the protein.
UTI89	UPEC, cystitis isolate (O18:K1:H7) GenBank: CP000243.1	(4, 5)
imp4213	MG1655, *lptD4213*	(6)
Plasmid	**Description**	**Source**
pQE-80L-kan	pQE-80L variant conferring kanamycin resistance	(7)
pQE-80L-*gfp*	*gfp* in pQE-80L-kan	(7)
pCP20	pCP20, repA101(ts), Amp^R and CAM^R	(3)
pQE-80L	pQE-80L, Amp^R	Qiagen
pQE-80L-*nfsA*	NfsA cloned into pQE-80L	This study
pQE-80L-*nfsA**	NfsA* (R203A) cloned into pQE-80L	This study

Supplementary Table 2. DNA oligonucleotides

Primers used to generate *nfsA* and *nfsA expression plasmids**		
Primer name	**Sequence**	**Description**
nfsA_EcoRI	GCGCGGGAATTCCACGTTCAGAAAGAGAAAAAGATAATG	Used in conjunction with nfsA_HindIII to clone *nfsA* into pQE-80L
nfsA_HindIII	GCGCGGAAGCTTTTAGCGCGTCGCCCAACCCT	Used in conjunction with nfsA_EcoRI to clone *nfsA* into pQE-80L
sdm_NfsAR203A_Fwd	TTACCTCACCGCAGGCAGCAATAATCGCC	Used in conjunction with sdm_NfsA_R203A_Rev to substitute R203 to an A in pQE-80L-*nfsA* to generate pQE-80L-*nfsA**
sdm_NfsA_R203A_Rev	TATTCCGCCAGTTGCTCG	Used in conjunction with sdm_NfsA_R203A_Fwd to substitute R203 to an A in pQE-80L-*nfsA* to generate pQE-80L-*nfsA**
Primers used in chromosomal perturbations		
Primer name	**Sequence**	**Description**
KanR_Rev	ATGATGGATACTTTCTCGGCAGGAG	Used in conjunction with gene external primer to confirm chromosomal location of kanamycin cassette
nfsA_Ext_Fwd	ACTTTGCAGAGGGAAGCGTA	Used in conjunction with KanR_Rev to confirm chromosomal location of kanamycin cassette
nfsA_Ext_Rev	CATGGAACGCAATTTGTCAC	Used in conjunction with nfsA_Ext_Fwd to confirm excision of kan cassette
nfsA_Int_Fwd	GTGTCGTTGATACGGCAATG	Used in conjunction with nfsA_Int_Rev to rule out gene duplication after deletion of *nfsA* gene
nfsA_Int_Rev	CCAGCGGTTGATAGCTGTTT	Used in conjunction with nfsA_Int_Fwd to rule out gene duplication after deletion of *nfsA* gene
nfsB_Ext_Fwd	CCAGAGACCAGGTGGGAATA	Used in conjunction with nfsB_Ext_Rev to confirm excision of kan cassette
nfsB_Ext_Rev	CCAAGCATTAAGCCAGGGTA	Used in conjunction with KanR_Rev to confirm chromosomal location of kanamycin cassette

Primers used in chromosomal perturbations (continuation)		
Primer name	**Sequence**	**Description**
nfsB_Int_Fwd	ACCTGTTTTGCCATCCACTC	Used in conjunction with nfsB_Int_Rev to rule out gene duplication after deletion of *nfsB* gene
nfsB_Int_Rev	GCTGCCGGTAATTACGTGTT	Used in conjunction with nfsB_Int_Fwd to rule out gene duplication after deletion of *nfsB* gene
glk_Ext_Rev	AGGGGTGAATCCTGGGCTAT	Used in conjunction with KanR_Rev to confirm chromosomal location of kanamycin cassette
glk_Int_Fwd	TAAATGTGCACCGGAACCGA	Used in conjunction with glk_Int_Rev to rule out gene duplication after deletion of *glk* gene
glk_Int_Rev	GCAGAACCGGTCGAAGGTAA	Used in conjunction with glk_Int_Fwd to rule out gene duplication after deletion of *glk* gene
ptsI_Ext_Fwd	AGACGAGCAGAAAGCGGTTG	Used in conjunction with KanR_Rev to confirm chromosomal location of kanamycin cassette
ptsI_Int_Fwd	ATTTCAGGCATTTTAGCATCC	Used in conjunction with ptsI_Int_Rev to rule out gene duplication after deletion of *ptsI* gene
ptsI_Int_Rev	GATCGCGCAGGATCTCTCTA	Used in conjunction with ptsI_Int_Fwd to rule out gene duplication after deletion of *ptsI* gene
Primers used to sequence *nfsA*/*nfsA in pQE-80L**		
Primer name	**Sequence**	**Description**
pQE-80L_Seq_Fwd	TATTTGCTTTGTGAGCGGATA	For sequencing of cloned *nfsA*/*nfsA** into pQE-80L
pQE-80L_Seq_Rev	CTGAAAATCTCGCCAAGCTA	For sequencing of cloned *nfsA/nfsA** into pQE-80L

Supplementary Table 3. Artificial Urine Recipe

	MW (g/mol)	Molarity (mM)/	Quantity (g) for 1 L
CH_4N_2O Urea	60.06	170	10.21
$C_5H_4N_4O_3$ Uric acid	168.11	0.4	0.067
$C_4H_7N_3O$ Creatinine	113.12	7	0.79
$C_6H_8O_7.H_2O$ Citric Acid monohydrate	210.15	2	0.42
NaCl	58.44	90	5.26
NH_4Cl	53.491	25	1.34
$CaCl_2.2H_2O$	147.02	2.5	0.37
$MgSO_4.7H_2O$	246.48	2	0.49
$NaHCO_3$	84.01	25	2.10
$Na_2SO_4.10H_2O$	322.2	10	3.22
KH_2PO_4	136.09	7	0.95
K_2HPO_4	174.18	7	1.22
$FeSO_4.\ 7H_2O$	278.01	0.005	0.0014
(90%) DL-Lactic acid	90.08	1.1	0.11
Peptone		-	1.00
Yeast extract		-	0.005

Peptone, yeast extract, NaCl, $Na_2SO_4{\cdot}10H_2O$, KH_2PO_4, K_2HPO_4 were dissolved in about 700 mL ddH_2O and autoclaved. Afterwards, the remainder of the components were added, and the pH was adjusted to 6.0 and final volume to 1 L. The final solution was then sterilized by filtering with a 0.22 μm filter.

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