# Supplementary Materials

## Elevated expression of toxin TisB protects persister cells against ciprofloxacin but enhances susceptibility to mitomycin C

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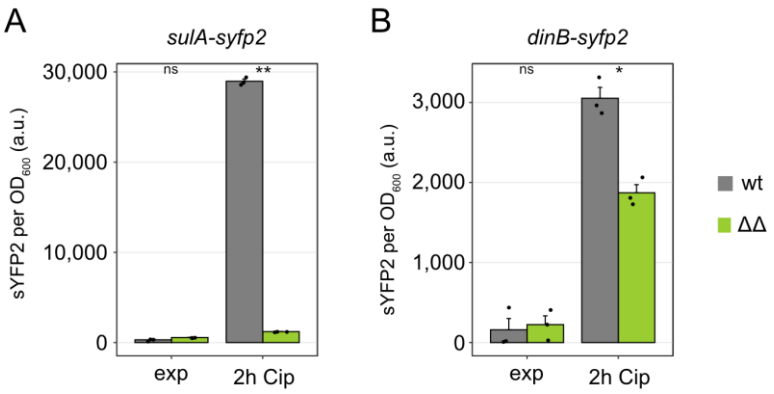


**Figure S1.** *Induction of SOS genes upon ciprofloxacin.*

sYFP2 fluorescence from chromosomal *sulA-syfp2* (A) and *dinB-syfp2* (B) fusions was monitored in a microplate reader. Reporter strains were analyzed during exponential phase (exp, $OD_{600}$ of ~0.4) and after two hours of Cip treatment (0.1 µg $mL^{-1}$; 10x MIC). Fluorescence measurements were background-corrected and normalized to $OD_{600}$. Bars represent the mean (± SEM; n=3). A pairwise *t*-test was performed to compare wt and ΔΔ (ns: not significant, * $p<0.05$, ** $p<0.01$). (wt: wild type MG1655; ΔΔ: Δ1-41 Δ*istR*).

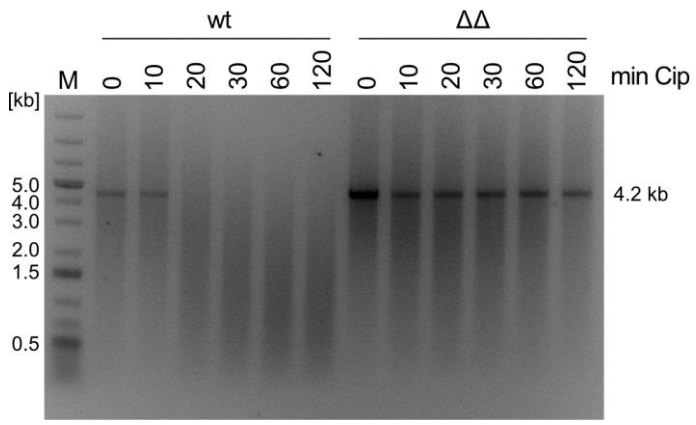


**Figure S2.** *Time course experiment for DNA damage analysis.*

Linearized plasmid DNA was analyzed on an agarose gel. Plasmid DNA was extracted from cultures in exponential phase ($OD_{600}$ of ~0.4) and at several time points of Cip treatment (1 µg $mL^{-1}$; 100x MIC). A marker (M) in kb is shown on the left-hand side. (wt: wild type MG1655; ΔΔ: Δ1-41 Δ*istR*).

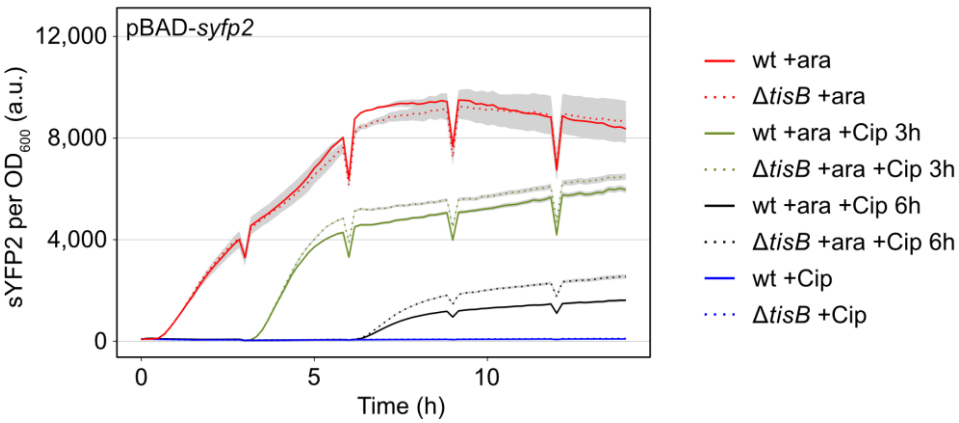


**Figure S3.** *sYFP2 expression upon prolonged ciprofloxacin treatment.*

sYFP2 fluorescence from plasmid pBAD-*syfp2* was monitored in a microplate reader. sYFP2 expression was induced by 0.2% L-Ara (+ara) at the indicated time points during Cip treatment (0.1 µg $mL^{-1}$; 10x MIC). Treatments with L-ara or Cip alone served as controls. Fluorescence measurements were normalized to the corresponding $OD_{600}$ measurements. Data represents the mean (colored lines) and SEM (grey ribbons; n=3). (wt: wild type MG1655; Δ*tisB*: *tisB* deletion strain).

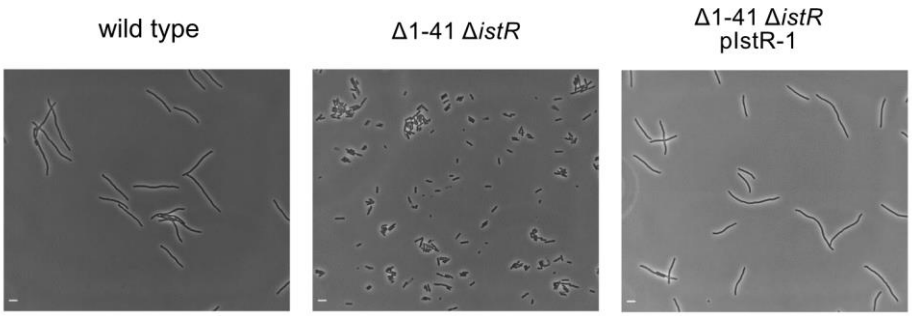


**Figure S4.** *Cell filamentation upon ciprofloxacin.*

Differential interference contrast (DIC) microscopy images of strains after two hours of Cip treatment (0.1 µg $mL^{-1}$; 10x MIC). pIstR-1 is an overexpression plasmid for RNA antitoxin IstR-1. Scale bars indicate 5 µm.

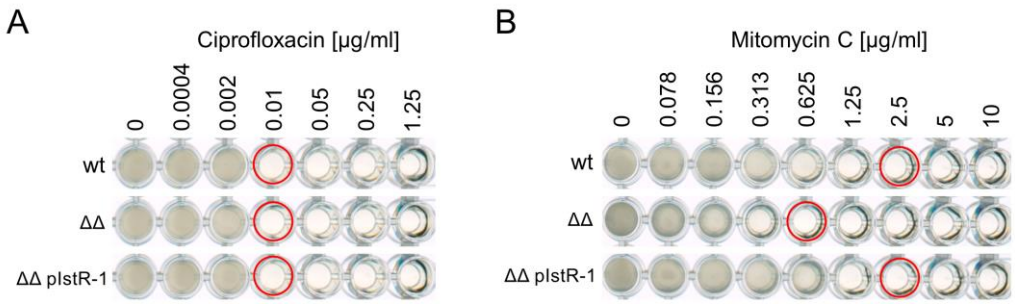


**Figure S5.** *MIC determination for ciprofloxacin and mitomycin C.*

Stationary-phase cultures were diluted 1:1,000 in fresh LB medium, and 135 µl were transferred to each well of a 96-well microplate. Subsequently, 15 µl of antibiotics were added to reach the final concentrations as indicated. Cells were cultivated at 37°C and 180 rpm for ~24 hours. The minimum inhibitory concentration (MIC) is reflected by the first well (red circle) in which cell growth was below 10% of the control (0 µg/ml), as assessed by $OD_{600}$ measurements in a microplate reader. (wt: wild type MG1655; ΔΔ: Δ1-41 Δ*istR*; ΔΔ pIstR-1: Δ1-41 Δ*istR* with IstR-1 overexpression plasmid).

## Table S1. Strains and plasmids used in this study.

| Strain | Relevant features | Source/Reference |
|---|---|---|
| MG1655 | K-12 $F^-$ $\lambda^-$ | Lab strain |
| B047 | Δ*tisAB::frt* in MG1655 | [1] |
| B133 | Scarless deletion of +1-41 region of *tisB* 5'-UTR and deletion of *istR* (Δ1-41 Δ*istR::frt-kan-frt*) | [2] |
| B076 | MG1655 *sulA-syfp2-cat* | [2] |
| GC-40 | MG1655 *dinB-syfp2-cat* | This study |
| NES3 | Δ1-41 Δ*istR::frt-kan-frt sulA-syfp2-cat* (P1 from B076 to B133) | This study |
| NES5 | Δ1-41 Δ*istR::frt-kan-frt dinB-syfp2-cat* (P1 from GC-40 to B133) | This study |
| GC-100 | Deletion of *recB* with *cat* (Δ*recB::cat*) in MG1655 | This study |
| GC-102 | Deletion of *ruvAB* with *cat* (Δ*ruvAB::cat*) in MG1655 | This study |
| GC-103 | MG1655 Δ*recB::cat* (P1 from GC-100 to MG1655) | This study |
| GC-106 | Δ1-41 Δ*istR::frt-kan-frt* Δ*recB::cat* (P1 from GC-100 to B133) | This study |
| GC-105 | MG1655 Δ*ruvAB::cat* (P1 from GC-102 to MG1655) | This study |
| DE-157 | Δ1-41 Δ*istR::frt-kan-frt* Δ*ruvAB::cat* (P1 from GC-102 to B133) | This study |
| DE-222 | Δ1-41 Δ*istR::frt-kan-frt* with *3xFLAG-tisB* | [3] |

| Plasmid | Relevant features | Source/Reference |
|---|---|---|
| pSIM5 | λ red expression vector, pSC101 *ori*, $repA^{ts}$, $Tet^R$ | [4] |
| pJV3H22 | IstR-1 transcribed from $P_L$ promoter in pZE12-luc, ColE1 *ori*, $Amp^R$ | [5] |
| pBAD-*syfp2* | pBAD with *syfp2* under control of $P_{BAD}$, $Amp^R$ | This study |
| pBAD+42 | pBAD with *tisB* from +42 to +354, $Amp^R$ | [6] |
| pBAD3xFlag | pBAD with *tisB* from +42 to +354, 3xFLAG tag inserted, $Amp^R$ | [6] |

**Table S2. Oligodeoxyribonucleotides used in this study.**

| Name | Sequence (5' to 3') | Description |
|---|---|---|
| recB-KO-1 | GCAGCAAACAATGCCCCTGATGAGTGAAAAGAATGAG TGAGCTCATATGAATATCCTCCTTAG | chr. Deletion of *recB* with *cat* |
| recB-KO-2 | TGTGCTCCACAGCTTCCAGTAATTGCTTTTGCAATTTCAT CTAGAGCTAACTAACTTGTAGGCTG | chr. Deletion of *recB* with *cat* |
| recB-scr-1 | CCTGAAGGCTGGAAAGTGTG | screening of chr. *recB* deletion |
| recB-scr-2 | ACATCCAGCGGGCGTAG | screening of chr. *recB* deletion |
| ruvAB-KO-1 | CATACAGCATTATCTTTGATTCATTACGCAGGAGCGTC ATGCTCATATGAATATCCTCCTTAG | chr. Deletion of *ruvAB* with *cat* |
| ruvAB-Ko-2 | TATTGCCAGTGCCGGATGCGGCGCGAGCGACCAATCCG ACGCCTTTGAGTGAGCTGATAC | chr. Deletion of *ruvAB* with *cat* |
| ruvAB-scr-1 | TCGCTGGATATCTATCCAGC | screening of chr. *ruvAB* deletion |
| ruvAB-scr-2 | CGAACAGCGTCGCATCAG | screening of chr. *ruvAB* deletion |
| dinB-yfp-1 | TGACCCGCAAATGGAAAGACAACTGGTGCTGGGATTA TGACCGAATTCAGAGAAAGAGGAG | *dinB-syfp2* fusion with *cat* |
| dinB-yfp-2 | CTCATAATAATGCACACCAGAATATACATAATAGTATAC ACTAGAGCTAACTAACTTGTAGGCTG | *dinB-syfp2* fusion with *cat* |
| dinB-yfp-scr1 | CAGCAAACCACCCAGGAG | screening of *dinB-syfp2* fusion |
| dinB-yfp-scr2 | GAACCGCAACCGGTTGATC | screening of *dinB-syfp2* fusion |
| sulA_yfp_out | CAACTCTGGTTAACACCGC | screening of *sulA-syfp2* fusion |
| sYFP2_out | CGCGTCTTGTAGTTACCG | screening of *sulA-syfp2* fusion |
| topo-fw-Hind | TATAAAGCTTTGTTTTGGCGGATGAGAGA | pBAD amplification, HindIII |
| topo-rev-Eco | TATAGAATTCTGGAGAAACAGTAGAGAGTTG | pBAD amplification, EcoRI |
| syfp2-for-Eco | TATAGAATTCAGAGAAAGAGGAGAAATAC | *syfp2* amplification, EcoRI |
| syfp2-rev-Hind | TATAAAGCTTATTATTTATACAGCTCATCC | *syfp2* amplification, HindIII |
| yfp-1-alt | TGAGGGTGAAGGTGACGCAA | qRT-PCR against *syfp2* |
| yfp-2-alt | AACGCGCGAAACACTGAACG | qRT-PCR against *syfp2* |
| cysG-1-new | CGGACCGTGTTTTCGTCG | qRT-PCR against *cysG* |
| cysG-2-new | ACGGATCGCCACCTTTCA | qRT-PCR against *cysG* |
| hcaT-1 | ATCCGTCCGACGATTCAG | qRT-PCR against *hcaT* |
| hcaT-2 | CCGTAATAGGCCGCATGT | qRT-PCR against *hcaT* |

## Supporting references


1. Dörr, T.; Vulic, M.; Lewis, K. Ciprofloxacin causes persister formation by inducing the TisB toxin in *Escherichia coli*. *PLoS Biol* **2010**, *8*, e1000317.
2. Berghoff, B.A.; Hoekzema, M.; Aulbach, L.; Wagner, E.G.H. Two regulatory RNA elements affect TisB-dependent depolarization and persister formation. *Mol. Microbiol.* **2017**, *103*, 1020–1033.
3. Edelmann, D.; Oberpaul, M.; Schäberle, T.F.; Berghoff, B.A. Post-transcriptional deregulation of the *tisB/istR-1* toxin–antitoxin system promotes SOS-independent persister formation in *Escherichia coli*. *Environ. Microbiol. Rep.* **2021**, *13*, 159–168.
4. Datta, S.; Costantino, N.; Court, D.L. A set of recombineering plasmids for gram-negative bacteria. *Gene* **2006**, *379*, 109–115.
5. Vogel, J.; Argaman, L.; Wagner, E.G.H.; Altuvia, S. The small RNA IstR inhibits synthesis of an SOS-induced toxic peptide. *Curr. Biol.* **2004**, *14*, 2271–2276.
6. Unoson, C.; Wagner, E.G.H. A small SOS-induced toxin is targeted against the inner membrane in *Escherichia coli*. *Mol. Microbiol.* **2008**, *70*, 258–270.