Supporting Information

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SI Materials and Methods

Media, Antibiotics. Cells were grown in LB or Mops glucose medium supplemented with all amino acids at 37 °C with shaking. When required, the medium was supplemented with 25 μg/mL kanamycin, 10 μg/mL tetracyclin, 50 μg/mL ampicillin, or 50 μg/mL chloramphenicol. Expression of protein from plasmids carrying the P_{BAD} promoter was induced by 0.2% arabinose and repressed by 0.2% glucose. Expression of protein from plasmids carrying P_{tet} was induced by aTc (50 ng/mL).

Construction of Plasmids. In Table S1, SD8 indicates a distance of eight nucleotides between the Shine–Dalgarno and the start codon. The start codons used are also given. Oligonucleotides used to construct the plasmids are described in Table S2.

pEG5 (pBAD33 SD8 gtg hipA). The *hipA* gene was amplified with primers OEG58 and OEG89. The PCR product was digested by XbaI and SphI and ligated with pBAD33 digested with the same enzymes. The resulting plasmid contains the *hipA* gene with an efficient SD sequence downstream of the P_{BAD} promoter.

pBbS2K-hipA. The *hipA* gene was amplified with primers GB081210-hipA-BB.F and GB081210-hipA-BB.R. The PCR product was digested by BglII and XhoI and ligated with pBbS2K digested with the same enzymes. The resulting plasmid contains the *hipA* gene with its own SD sequence downstream of the P_{tet} promoter.

pBbS2K-relE. The *relE* gene was amplified with primers OEG122 and OEG123. The PCR product was digested by BglII and XhoI and ligated with pBbS2K digested with the same enzymes. The resulting plasmid contains the *relE* gene with an optimized SD sequence downstream of the P_{tet} promoter.

pBbS2K-mazF. The *mazF* gene was amplified with primers OEG124 and OEG125. The PCR product was digested by BglII and XhoI and ligated with pBbS2K digested with the same enzymes. The resulting plasmid contains the *mazF* gene with an optimized SD sequence downstream of the P_{tet} promoter.

pBbS2K-yafO. The *yafO* gene was amplified with primers OEG128 and OEG129. The PCR product was digested by BglII and BamHI and ligated with pBbS2K digested with the same enzymes. The resulting plasmid contains the *yafO* gene with an optimized SD sequence downstream of the P_{tet} promoter.

pEG6 (pBAD33::SDopt::chpB). The *chpB* gene was amplified with primers OEG137 and OEG138. The PCR product was digested by XbaI and SphI and ligated with pBAD33 digested with the same enzymes. The resulting plasmid contains the *chpB* gene with an efficient SD sequence downstream of the P_{BAD} promoter.

pEG7 (pBAD33::SDopt::hicA). The *hicA* gene was amplified with primers OEG139 and OEG140. The PCR product was digested by XbaI and SphI and ligated with pBAD33 digested with the same enzymes. The resulting plasmid contains the *hicA* gene with an efficient SD sequence downstream of the P_{BAD} promoter.

pEG8 (pBAD33::SDopt::yhaV). The *yhaV* gene was amplified with primers OEG143 and OEG144. The PCR product was digested by XbaI and SphI and ligated with pBAD33 digested with the same enzymes. The resulting plasmid contains the *yhaV* gene with an efficient SD sequence downstream of the P_{BAD} promoter.

pKP3057 (pBAD33::SDopt::yoeB). The *yoeB* gene was amplified with primers yoeB5′ and yoeB3′. The PCR product was digested by BanHI and EcoRI and ligated with pLitmus28 digested with the same enzymes. The resulting plasmid has been digested with StuI and NsiI and cloned in the pBAD33 previously digested with HincII and PstI. The resulting plasmid contains the *yoeB* gene with an efficient SD sequence downstream of the P_{BAD} promoter.

Microfluidic and Flow Cell Chamber. Time-lapse experiments were done in a microfluidic chamber as previously described (1). Briefly, the cells were placed immediately on a coverslip and covered with a 0.9 mm thin layer of agarose pad [0.9 × 25 × 10 0mm, 0.5% low melting agarose + 1% agarose in 1 × M9 medium 10% LB (vol/vol)] and left to dry gently to absorb the cells onto the agar substrate. The chamber was then mounted by sealing the coverslip with a sticky-slide I (0.8 μm Luer) chamber (ibidi R). Flow perfusion was performed by gravity at a relatively constant rate (5 μL/s) or manually by injection with syringes.

1. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S (2004) Bacterial persistence as a phenotypic switch. *Science* 305(5690):1622–1625.

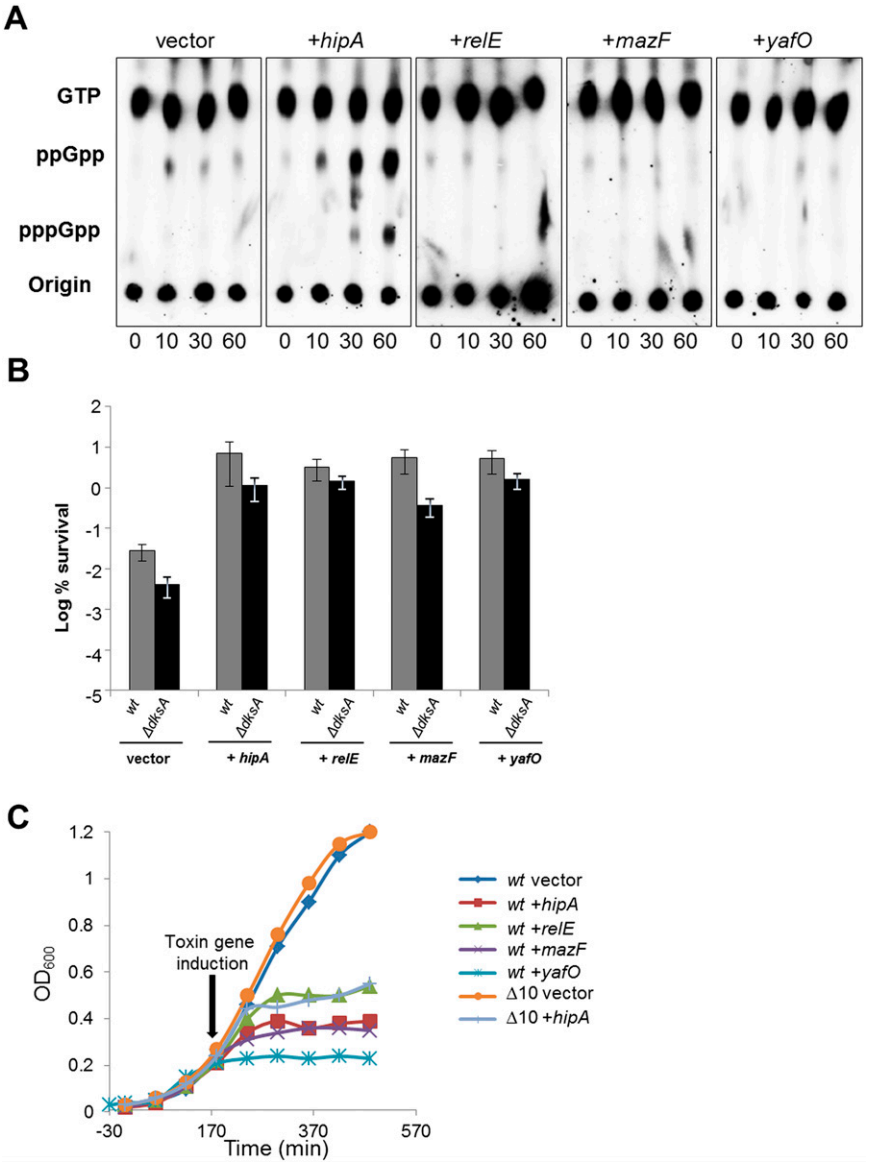


Fig. S1. HipA mediates persistence independently of DksA. (*A*) Accumulation of (p)ppGpp following ectopic expression of toxins. MG1655 carrying either *hipA*, *relE*, *mazF*, or *yafO* on pBAD33 was grown exponentially in low phosphate Mops minimal medium (*Materials and Methods*). Representative autoradiograph of the TLC plates is shown (related to Fig. 2*A*). (*B*) Exponentially growing cells of MG1655 (gray bars) and isogenic deletion strain Δ*dksA* (black bars) overexpressing either *hipA*, *relE*, *mazF*, or *yafO* from pBAD33 were exposed to 2 μg/mL of ciprofloxacin (for details, see *Materials and Methods*). Percentage of survival after 4 h of antibiotic treatment was compared with that of the control strains carrying the pBAD33 vector plasmid (log scale). Error bars indicate the SDs on the averages of at least three independent experiments. (*C*) Growth curves of MG1655 or Δ*10TA* strains containing the plasmids indicated. Overnight cultures were diluted 100-fold in fresh Mops glucose medium (supplemented as described in *Materials and Methods*) with kanamycin (25 μg/mL) and incubated at 37 °C. The arrow indicates that toxins were induced (*hipA*, *relE*, *mazF*, *yafO*) at OD_{600} 0.2, by the addition of 50 ng/mL of aTc.

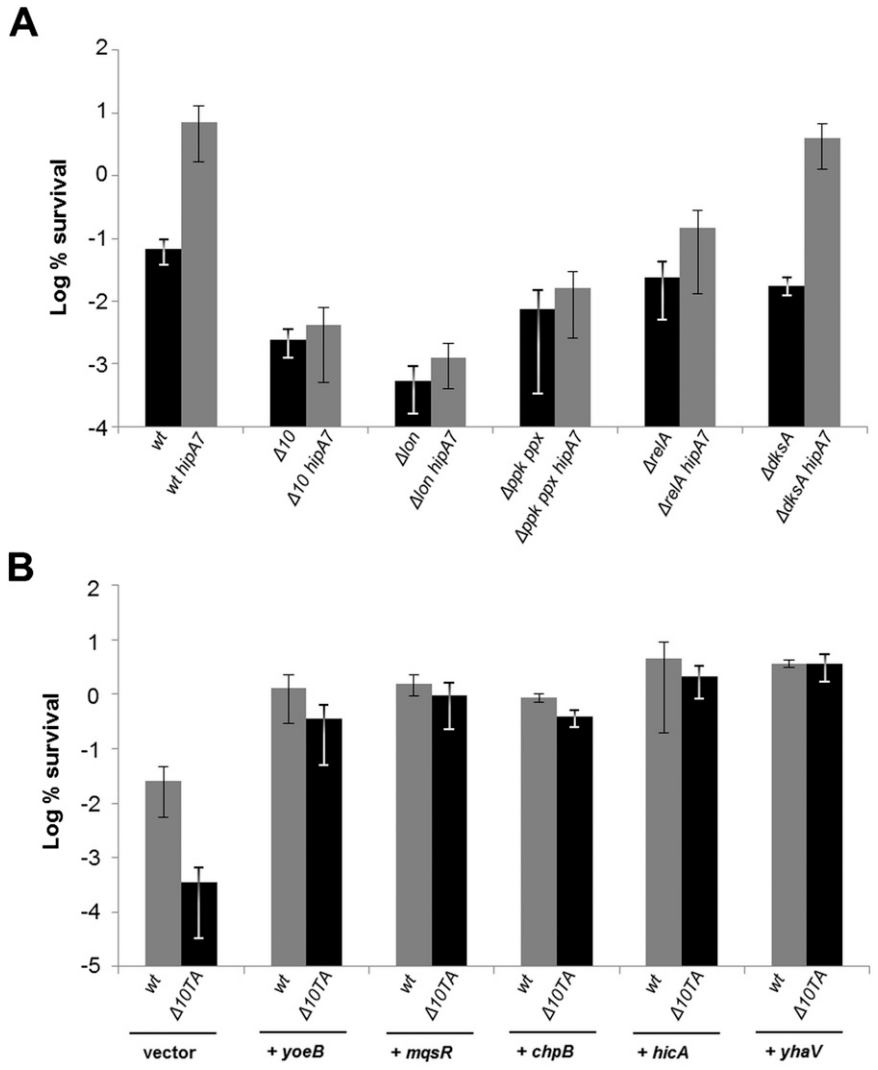


Fig. S2. High persistence mediated by *hipA7* depends on (p)ppGpp, Poly(P), Lon, and the other type II TA loci. (*A*) Exponentially growing cells of MG1655 and isogenic deletion strains Δ*10TA*, Δ*(ppk ppx)*, and Δ*lon* exposed to 1 μg/mL of ciprofloxacin (black bars) (for details, see *Materials and Methods*). Percentage of survival after 4 h of antibiotic treatment was compared with that of the control strains carrying the chromosomal *hipA7* allele (gray bars) (log scale). (*B*) Exponentially growing cells of MG1655 (gray bars) and Δ*10TA* strains (black bars) carrying TA-encoded toxin genes *yoeB*, *mqsR*, *chpB*, *hicA*, or *yhaV* on pBAD33 induced for 30 min were exposed to 2 μg/mL of ciprofloxacin (for details, see *Materials and Methods*). Percentage of survival after 4 h of antibiotic treatment was compared with that of the control strains carrying the pBAD33 vector plasmid (log scale). Error bars indicate the SDs on the averages of at least three independent experiments.

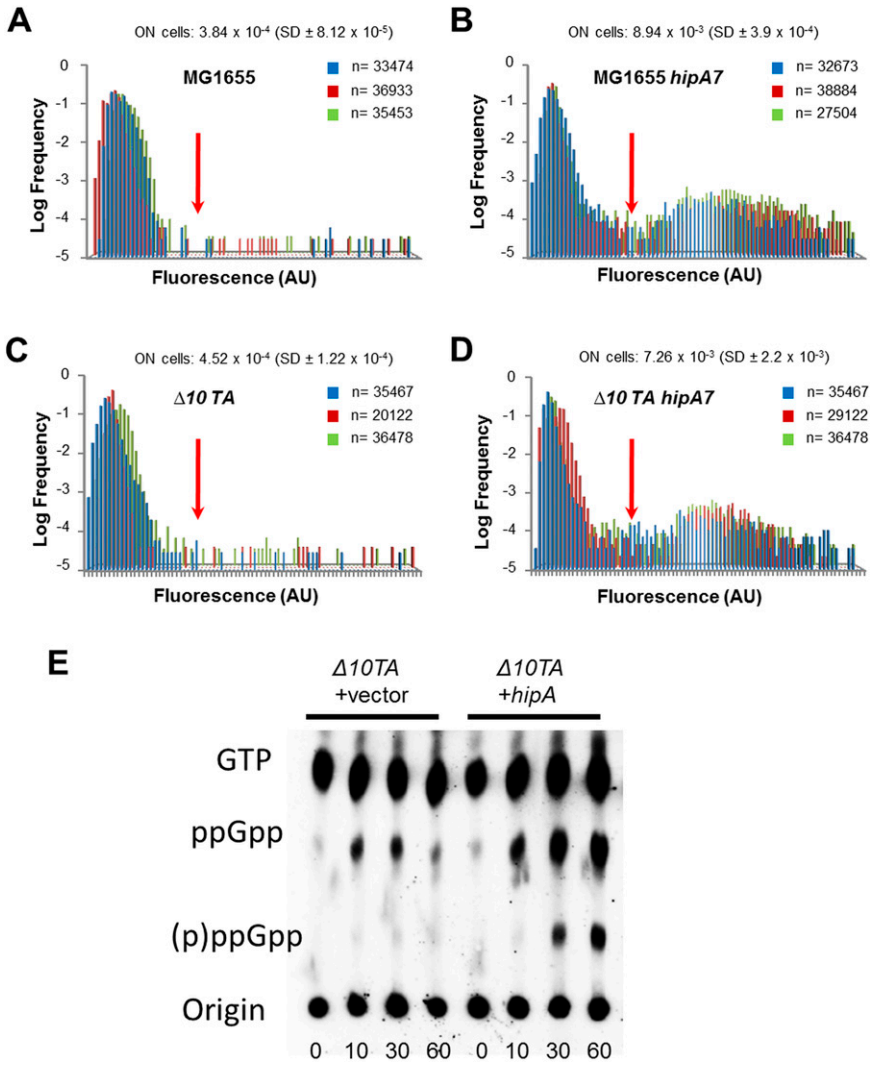


Fig. S3. *hipA7* increased the frequency of (p)ppGpp level in single cells irrespectively of the presence of the 10 TA loci. (*A–D*) Statistical distribution of the fluorescence level of individual cells from (*A*) MG1655 *rpoS::mCherry*, (*B*) MG1655 *hipA7 rpoS::mCherry*, (C) Δ*10TA rpoS::mCherry*, and (*D*) Δ*10TA hipA7 rpoS::mCherry*, collected in exponential culture and analyzed by fluorescence microscopy. Panels show the fluorescence distribution as a histogram consisting of 80 repartition bins (log scale). The colors are three individual experiments including a total of around 10,000 cells for each strain (n, the number of cells analyzed in each set). Arrows point to the threshold applied to discriminate ON cells from OFF cells (frequency and SD of the ON cells are given on the top of each panel). (*E*) (p)ppGpp synthesis measurement following *hipA* overexpression. Cells of MG1655 and Δ*10TA* strains overexpressing *hipA* were grown in Mops minimum medium (as described in *Materials and Methods*). Samples were collected before and 10 min, 30 min, and 1 h after toxin induction and separated by TLC (*Materials and Methods*). Representative autoradiograph of the TLC plates is shown.

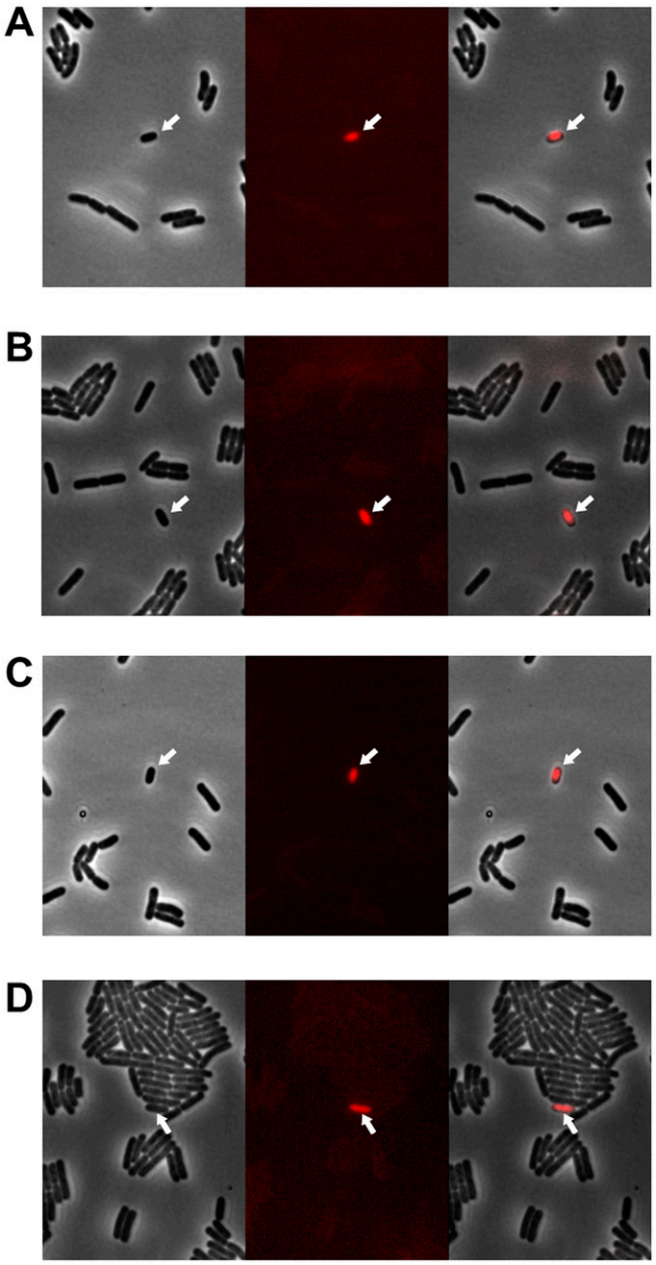


Fig. S4. (p)ppGpp level mediated by activated HipA triggers a nongrowing state but is not enough to induce persistence in the absence of the other 10 TA loci. (*A*–*D*) Separate phase contrast and fluorescence of time 0 image of Fig. 5 *A*–*D* (respectively related also to Movies S1, S2, S4, and S5). From left to right, phase contrast, RpoS-mCherry fluorescence, and overlay.

Table S1. Strains and plasmids used in this work

Strains/plasmids	Genotype	Source
Strains		
MG1655	Wild-type *E.coli*	(1)
JW0141	BW25113 Δ*dksA::Kan*	(2)
JW0429	BW25113 Δ*lon::Kan*	(2)
Δ*dksA*	MG1655 Δ*dkSA::kan*	P1 JW0141 × MG1655
Δ*10*	MG1655 Δ*chpAK* Δ*chpB* Δ*relBE* Δ*yefM/yoeB* Δ*dinJ/yafQ* Δ*ygjNM* Δ*prlF/yhaV* Δ*yafNO* Δ*ygiUT* Δ*hicAB*	(3)
Δ*lon*	MG1655 Δ*lon::tet*	(4)
Δ*ppKppX*	MG1655 Δ*ppk ppx*::*kan*	(5)
CF1693 *(ΔrelAspoT)*	MG1655 Δ*relA251::kan* Δ*spoT207*::*cat*	(6)
Δ*relA*	MG1655 Δ*relA251::kan*	P1 CF1693 × MG1655
Δ*lon (Kan)*	MG1655 Δ*lon::kan*	P1 JW0429 × MG1655
RpoS-Mcherry	MG1655 *rpoS::mcherry-frt-aphA-frt*	(7)
Δ*10* RpoS-Mcherry	MG1655 Δ*10 rpoS*::mcherry-*frt-aphA-frt*	P1 RpoS-Mcherry × Δ*10*
HM21	AT984 *zde264*::Tn*10 dapA6*	(8)
HM22	AT984 *hipA7 zde264*::Tn*10 dapA6*	(8)
RpoS-Mcherry	MG1655 *rpoS::mcherry-frt-aphA-frt zde264*::Tn*10 dapA6*	P1 HM21 × RpoS-Mcherry
RpoS-Mcherry *hipA7*	MG1655 *rpoS::mcherry-frt-aphA-frt hipA7 zde264*::Tn*10 dapA6*	P1 HM22 × RpoS-Mcherry
Δ*10* RpoS-Mcherry	MG1655 Δ*10 rpoS*::mcherry-*frt-aphA-frt zde264*::Tn*10 dapA6*	P1 HM21 × Δ*10* RpoS-Mcherry
Δ*10* RpoS-Mcherry *hipA7*	MG1655 Δ*10 rpoS*::mcherry-*frt-aphA-frt hipA7 zde264*::Tn*10 dapA6*	P1 HM22 × Δ*10* RpoS-Mcherry
MG1655 *zde*	MG1655 *zde264*::Tn*10 dapA6*	P1 HM21 × MG1655
Δ*relA zde*	MG1655 Δ*relA251::aphA zde264*::Tn*10 dapA6*	P1 HM21 × Δ*relA*
Δ*dksA zde*	MG1655 Δ*dkSA::kan zde264*::Tn*10 dapA6*	P1 HM21 × Δ*dksA*
Δ*lon zde*	MG1655 Δ*lon::tet zde264*::Tn*10 dapA6*	P1 HM21 × Δ*lon*
Δ*ppKppX zde*	MG1655 Δ*ppk ppx*::*kan zde264*::Tn*10 dapA6*	P1 HM21 × Δ*ppKppX*
Δ*10 zde*	MG1655 Δ*10 zde264*::Tn*10 dapA6*	P1 HM21 × Δ*10*
hipA7	MG1655 *hipA7 zde264*::Tn*10 dapA6*	P1 HM22 × MG1655
Δ*relA hipA7*	MG1655 Δ*relA251::aphA hipA7 zde264*::Tn*10 dapA6*	P1 HM22 × Δ*relA*
Δ*dksA hipA7*	MG1655 Δ*dkSA::kan hipA7 zde264*::Tn*10 dapA6*	P1 HM22 × Δ*dksA*
Δ*lon hipA7*	MG1655 Δ*lon::tet hipA7 zde264*::Tn*10 dapA6*	P1 HM22 × Δ*lon*
Δ*10 hipA7*	MG1655 Δ*10 zde264*::Tn*10 dapA6*	P1 HM22 × Δ*10*
Δ*ppKppX hipA7*	MG1655 Δ*ppk ppx*::*kan hipA7 zde264*::Tn*10 dapA6*	P1 HM22 × Δ*ppKppX*
Plasmids		
pBAD33	p15, *cat*, *araC*, P_{BAD} promoter	(9)
pEG5	pBAD33 P_{BAD}:: SD8 *gtg hipA*	This work
pKP3035	pBAD33 P_{BAD}::*relE*	(10)
pMCD3326	pBAD33 P_{BAD}::SDopt::*mazF*	(11)
pMCD3306	pBAD33 P_{BAD}::SD::*yafO*	(12)
pBbS2K		Addgene
pBb-*hipA*	pBbS2K P_{tet}::*hipA*	This work
pBb-*relE*	pBbS2K P_{tet}::*relE*	This work
pBb-*mazF*	pBbS2K P_{tet}::*mazF*	This work
pBb-*yafO*	pBbS2K P_{tet}::*yafO*	This work
pLitmus28	pUC *bla* pT7	New England Biolabs
pEG6	pBAD33 P_{BAD}::SDopt::*chpB*	This work
pEG7	pBAD33 P_{BAD}::SDopt::*hicA*	This work
pEG8	pBAD33 P_{BAD}::SDopt::*yhaV*	This work
pMCD3312	pBAD33 P_{BAD}::SDopt::*mqsR*	(12)
pKP3057	pBAD33 P_{BAD}::SDopt::*yoeB*	This work

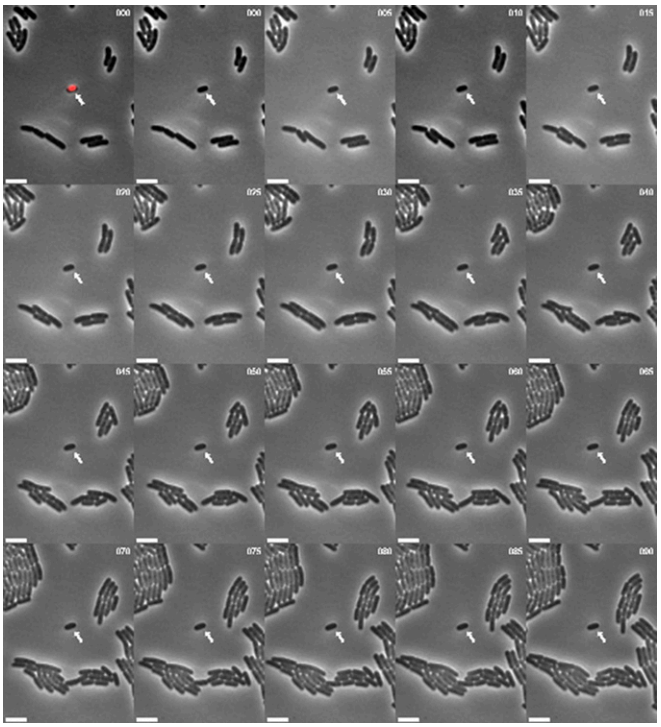
1. Guyer MS, Reed RR, Steitz JA, Low KB (1981) Identification of a sex-factor-affinity site in E. coli as gamma delta. *Cold Spring Harb Symp Quant Biol* 45(Pt 1):135–140.
2. Baba T, et al. (2006) Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: The Keio collection. *Mol Syst Biol* 2:0008.
3. Maisonneuve E, Shakespeare LJ, Jørgensen MG, Gerdes K (2011) Bacterial persistence by RNA endonucleases. *Proc Natl Acad Sci USA* 108(32):13206–13211.
4. Winther KS, Gerdes K (2009) Ectopic production of VapCs from Enterobacteria inhibits translation and trans-activates YoeB mRNA interferase. *Mol Microbiol* 72(4):918–930.
5. Kuroda A, Kornberg A (1997) Polyphosphate kinase as a nucleoside diphosphate kinase in Escherichia coli and Pseudomonas aeruginosa. *Proc Natl Acad Sci USA* 94(2):439–442.
6. Xiao H, et al. (1991) Residual guanosine 3′,5′-bispyrophosphate synthetic activity of relA null mutants can be eliminated by spoT null mutations. *J Biol Chem* 266(9):5980–5990.
7. Maisonneuve E, Castro-Camargo M, Gerdes K (2013) (p)ppGpp controls bacterial persistence by stochastic induction of toxin-antitoxin activity. *Cell* 154(5):1140–1150.
8. Moyed HS, Bertrand KP (1983) hipA, a newly recognized gene of Escherichia coli K-12 that affects frequency of persistence after inhibition of murein synthesis. *J Bacteriol* 155(2): 768–775.
9. Guzman LM, Belin D, Carson MJ, Beckwith J (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J Bacteriol* 177(14): 4121–4130.
10. Pedersen K, Christensen SK, Gerdes K (2002) Rapid induction and reversal of a bacteriostatic condition by controlled expression of toxins and antitoxins. *Mol Microbiol* 45(2):501–510.
11. Christensen-Dalsgaard M, Gerdes K (2008) Translation affects YoeB and MazF messenger RNA interferase activities by different mechanisms. *Nucleic Acids Res* 36(20):6472–6481.
12. Christensen-Dalsgaard M, Jørgensen MG, Gerdes K (2010) Three new RelE-homologous mRNA interferases of Escherichia coli differentially induced by environmental stresses. *Mol Microbiol* 75(2):333–348.

Table S2. DNA oligonucleotides used in this work

Primers	Sequence	Source
OEG58	cccccctctagagtcgactaaaggaaaaaaaaa**gtg**cctaaacttgtcacttggat	(1)
OEG89	cccccgcatgctcacttactaccgtattctcggc	(1)
GB081210-hipA-BB.F	ttcaaaagatctcaacagaacagcaaaatctggagtgg	(2)
GB081210-hipA-BB.R	tccttactcgagttaggatcctcacttactaccgtattctcggcttaac	(2)
OEG122	ttcaaaagatctaaaataaggaggaaaaaaaaa**atg**gcgtattttctggattttg	This work
OEG123	tccttactcgagtcagagaatgcgtttgacc	This work
OEG124	ttcaaaagatctaaaataaggaggaaaaaaaaa**atg**gtaagccgatacgtacc	This work
OEG125	tccttactcgagctacccaatcagtacgttaattttgg	This work
OEG128	ttcaaaagatctaaaataaggaggaaaaaaaaa**atg**cgggtattcaaaacaaaac	This work
OEG129	tccttaggatcctcaaaaacgcatgcgaaacgct	This work
yoeB5′	gggggatccataaggagttttataa**atg**aaactaatctggtctgagg	This work
yoeB3′	ggggaattcgttcaataatgataacgacatgc	This work
OEG137	cccccctctagaaaaataaggaggaaaaaaaaa**atg**gtaaagaaaagtgaatttga	This work
OEG138	cccccgcatgcttattccaccaccgcctgcaagc	This work
OEG139	cccccctctagaaaaataaggaggaaaaaaaaa**gtg**aaacaaagcgagttcagacg	This work
OEG140	cccccgcatgcttaactcaaaccgagttgtttca	This work
OEG143	cccccctctagaaaaataaggaggaaaaaaaaa**atg**gattttccacaaagggttaa	This work
OEG144	cccccgcatgctcaatgggtttcttctgtttctc	This work

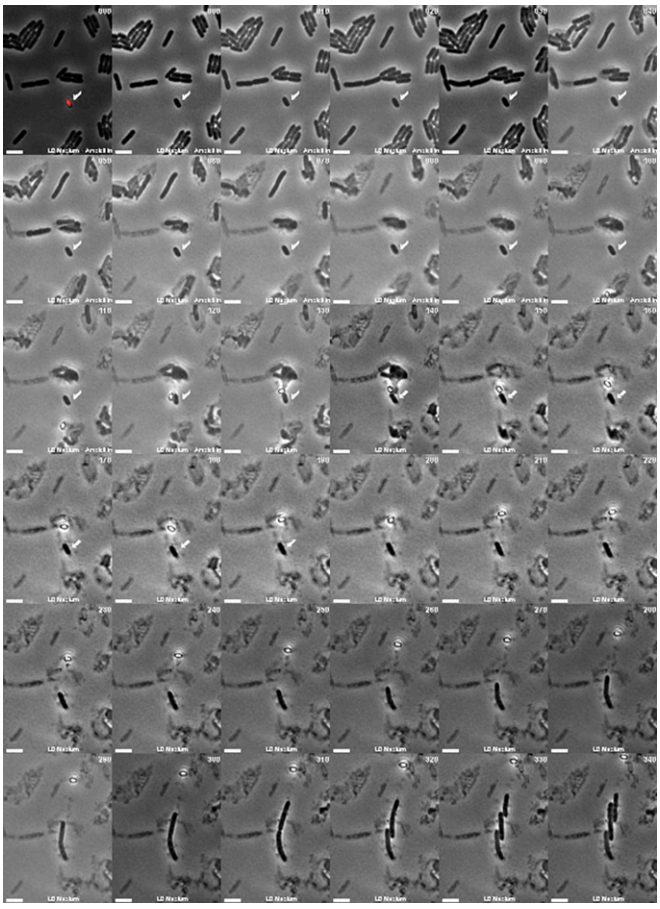
Bold, start codon; underlined, restriction site.

1. Germain E, Castro-Roa D, Zenkin N, Gerdes K (2013) Molecular mechanism of bacterial persistence by HipA. *Mol Cell* 52(2):248–254.
2. Bokinsky G, et al. (2013) HipA-triggered growth arrest and β-lactam tolerance in Escherichia coli are mediated by RelA-dependent ppGpp synthesis. *J Bacteriol* 195(14):3173–3182.



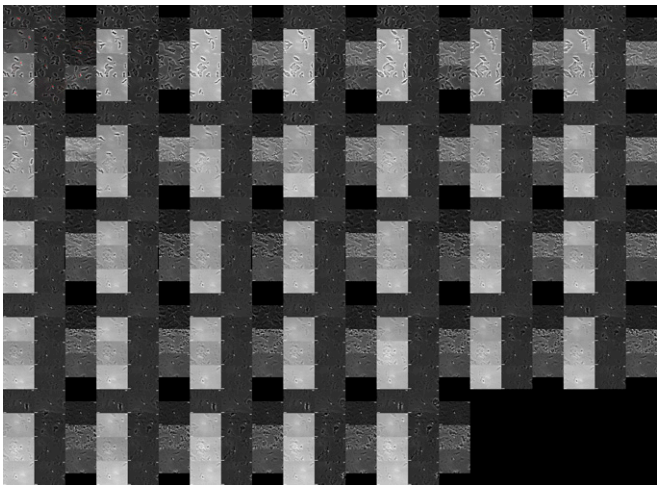
Movie S1. Time-lapse series showing cells of MG1655 *rpoS::mCherry* from which the panels in Fig. 4*A* were obtained. Bacteria from an exponential culture were introduced into a microfluidic device and subjected to growth upon injection of M9 medium supplemented with 10% (vol/vol) LB medium. The first image is the overlay of phase contrast and fluorescence acquisition showing the initial level of RpoS-mCherry level at time 0. Phase contrast images were then acquired automatically every 5 min for 90 min. (Scale bar, 4 μm.)

Movie S1



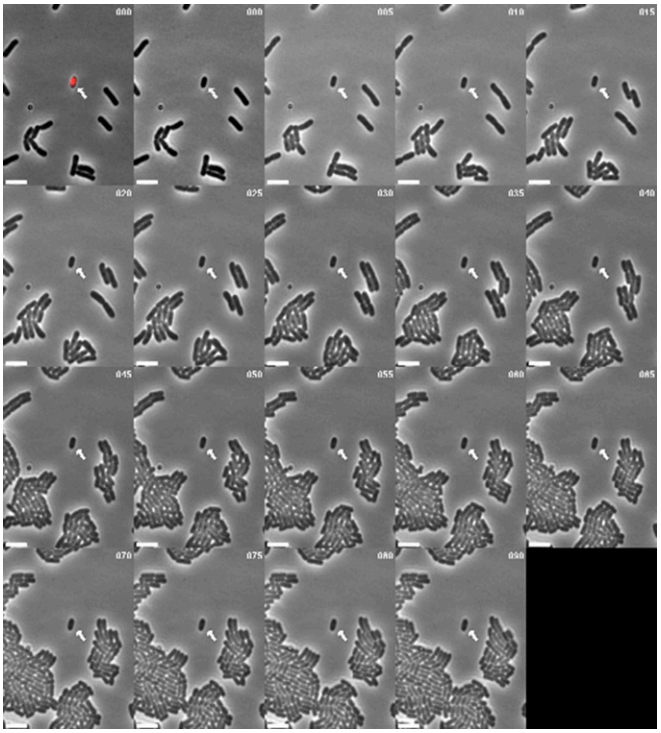
Movie S2. Time-lapse series showing growing cells of MG1655 *rpoS::mCherry* from which the panels in Fig. 4*B* were obtained. Bacteria from an exponential culture were introduced into a microfluidic device and subjected to growth upon injection of M9 medium supplemented with 10% (vol/vol) LB medium. The first image is the overlay of phase contrast and fluorescence acquisition showing the initial level of RpoS-mCherry at time 0. Phase contrast images were then acquired automatically every 10 min for more than 5 h. At the indicated time points and for the given period, 500 μg/mL of ampicillin was injected. (Scale bar, 4 μm.)

Movie S2



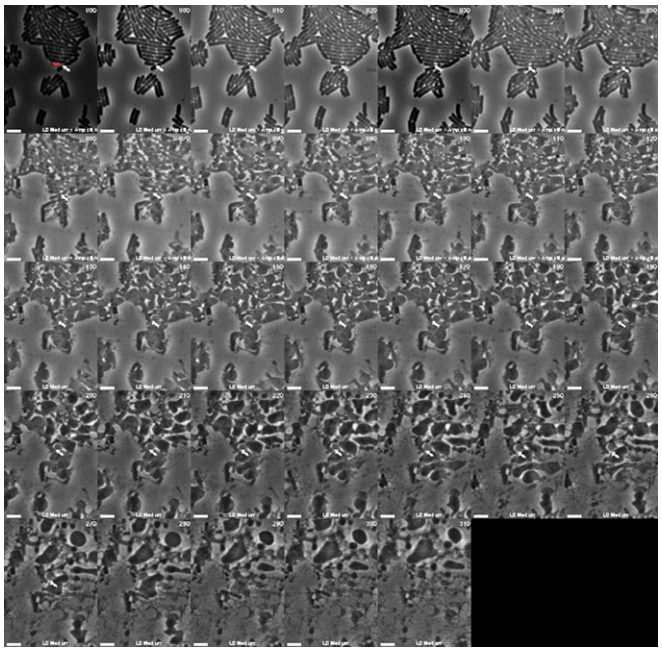
Movie S3. Time-lapse series showing cells of MG1655 *rpoS::mCherry* upon ampicillin treatment. The movie shows a combination of several independent experiments and reveals the behavior of the 12 individual cells having a high RpoS-mCherry level. Bacteria from an exponential culture were introduced in the microfluidic device and subjected to growth upon injection of M9 medium supplemented with 10% (vol/vol) LB medium. The first image is the overlay of phase contrast and fluorescence acquisition showing the initial level of RpoS-mCherry level at time 0. Then 500 μg/mL of ampicillin was injected, and phase contrast images were acquired automatically every 10 min and presented at the indicated time. (Scale bar, 4 μm.)

Movie S3



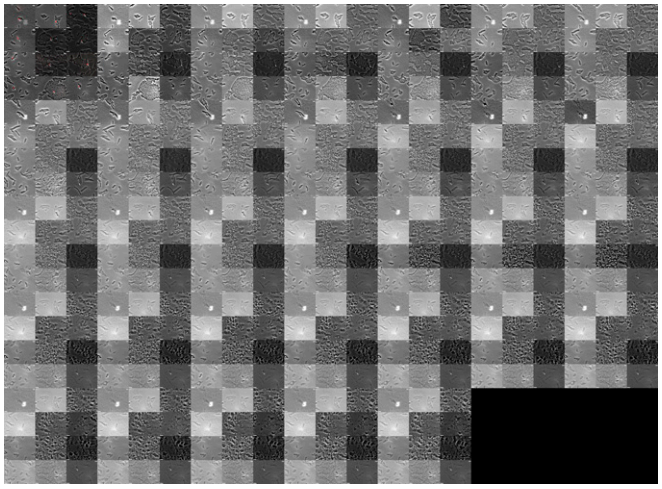
Movie S4. Time-lapse series showing cells of *Δ10TA rpoS::mCherry* carrying the *hipA7* allele from which the panels in Fig. 4*C* were obtained. Bacteria from an exponential culture were introduced into a microfluidic device and subjected to growth upon injection of M9 medium supplemented with 10% (vol/vol) LB medium. The first image is the overlay of phase contrast and fluorescence acquisition showing the initial level of RpoS-mCherry level at time 0. Phase contrast images were then acquired automatically every 5 min for 90 min. (Scale bar, 4 μm.)

Movie S4



Movie S5. Time-lapse series showing growing cells of *Δ10TA rpoS::mCherry* carrying the *hipA7* allele from which the panels in Fig. 4*D* were obtained. Bacteria from an exponential culture were introduced into a microfluidic device and subjected to growth upon injection of M9 medium supplemented with 10% (vol/vol) LB medium. The first image is the overlay of phase contrast and fluorescence acquisition showing the initial level of RpoS-mCherry at time 0. Phase contrast images were then acquired automatically every 10 min for more than 5 h. At the indicated time points and for the given period, 500 μg/mL of ampicillin was injected. (Scale bar, 4 μm.)

Movie S5



Movie S6. Time-lapse series showing cells of *Δ10TA rpoS::mCherry* carrying the *hipA7* allele upon ampicillin treatment. The movie shows a combination of several independent experiments and reveals the behavior of the 12 individual cells having a high RpoS-mCherry level. Bacteria from an exponential culture were introduced in the microfluidic device and subjected to growth upon injection of M9 medium supplemented with 10% (vol/vol) LB medium. The first image is the overlay of phase contrast and fluorescence acquisition showing the initial level of RpoS-mCherry level at time 0. Then 500 μg/mL of ampicillin was injected, and phase contrast images were acquired automatically every 10 min and presented at the indicated time. (Scale bar, 4 μm.)

Movie S6