Supporting information for:

Pervasive transcription enhances the accessibility of H-NS-silenced promoters and generates bistability in *Salmonella* virulence gene expression

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Supplementary Materials and Methods

RT-qPCR. Amplification reactions were set up in 384-well plates by mixing serial dilutions of each reverse-transcribed sample with the appropriate primer pairs (each primer used at a 0.25 µM final concentration) and the LightCycler 480 SYBR Green I Master Mix (Roche Applied Science). Real-time qPCR was carried out in a LightCycler 480 Instrument (Roche) with the following program: activation: 95°C for 5 min; amplification (40 cycles): 95°C for 10 sec; 55°C for 20 sec; 72°C for 20 sec; melting curve: 95°C for 30 sec; 65°C for 30 sec (ramp 0.06 °C/sec, 10 acquisitions/°C). Target-to-reference transcript ratios and relative transcript levels were calculated with the Pflaffl method (1).

Template switching Reverse Transcription. Total RNA (1 µg) was combined with 1 µl of a mixture of up to four gene-specific primers, 5 µM each (including AI41 (*hilD*), AI48 (*prgH*) and AJ33 (*ompA*)), and 1 µl of 10 mM dNTPs in a 6 µl final volume. After a 5 min treatment at 70°C (in a Thermocycler), samples were quickly cooled on ice. Each sample were then mixed with 2.5 µl of Template Switching Buffer (4x), 0.5 µl of 75 µl of Template Switching Oligonucleotide (TSO) and 1 µl of Template Switching RT Enzyme Mix in a final volume of 10 µl. Reverse transcription was carried out for 90 min at 42°C, followed by a 5 min incubation at 85°C. The 42 nucleotide-long TSO carries three 3'-terminal riboguanosines that hybridize to the polycytosine overhang (typically 3 or 4 Cs) added by the RT enzyme when it reaches the 5' end of the RNA. This allows the RT enzyme to switch template and copy the TSO.

CHIP-Seq analysis. Following two washes with TBS buffer**,** Formaldehyde-treated cells were resuspended in 0.5 ml of Lysis buffer (50mM Tris pH 7.4, 150mM NaCl, 1mM EDTA supplemented with cOmplete™ Mini Protease Inhibitor cocktail (Sigma-Aldrich). Then, 3 µl of Ready-Lyse™ Lysozyme solution, 25,000 – 40,000 U/µl (Lucigen R1804M) were added and incubated on ice for 10 min, followed by the addition of 25 µl of 25% Triton and 450 µl of Lysis buffer. Cells were disrupted in a Covaris 220 focused ultrasonicator (Peak Incidence = 140W; Duty Factor = 5; Cycles per burst = 200). This step was followed by 10 min centrifugation at 16 000 rcf at 4°C. After removing aliquots for "Input" controls, supernatants (0.9 ml) were treated overnight with Anti-Flag M2 affinity Gel (Sigma A2220, 40 µl of slurry per sample) on a gyratory wheel at 8°C. Next day, beads were washed in the cold with 500 µl of TBS plus 0.05% Tween 20 for 10 minutes, followed by four TBS washes, of 10 minute each. The elution of 3xFLAG-H-NS DNA complex from the beads was accomplished as follows: 3xFLAG peptide solution (prepared according to Sigma Aldrich instructions) was diluted with TBS (3 µl of 3xFLAG peptide concentrated solution per 100 µl of TBS) and an aliquot of 2.5 times volume (relative to beads) of the peptide-TBS solution was added on top of the beads. Tubes were incubated for 1 hour on the gyratory wheel at 8°C. Samples were centrifuged briefly and supernatant transferred to a clean tube. Inputs as well as samples were treated with Proteinase K (2.5 µl of a 2 mg/ml solution) at 65°C

overnight. DNA was cleaned using Qiagen Mini-elute reaction clean up kit. DNA fragments were end-repaired and dA-tailed (NEB#E7595), Illumina TruSeq adapters were ligated (NEB#E6040) and libraries were amplified with Kapa Hifi polymerase (Kapa Biosystem #KK2103). Final libraries quality was assessed on an Agilent Bioanalyzer 2100, using an Agilent High Sensitivity DNA Kit. Libraries were pooled in equimolar proportions and sequenced on a Paired-End 2x75 bp run, on an Illumina NextSeq500 instrument. Output files were demultiplexed and adapters were removed. Only reads longer than 10 pb were kept for analysis. The raw data from all CHIP-Seq experiments were deposited into ArrayExpress under the accession number E-MTAB-11386.

Measurement of ß-galactosidase activity. Static overnight cultures were diluted 1:200 in 2 ml of LB (with or without ARA) and grown with shaking at 37°C to early or mid-stationary phase. Bacteria were harvested from 1 ml aliquots and resuspended in 1 ml of PBS. β-galactosidase activity was assayed in toluene-permeabilized cells as described by Miller (2) and is expressed in Miller units. Measurements were repeated three times, each time using duplicate cultures originating from independent colonies.

Statistics and Reproducibility. All data described in this paper originate from three or more independent experiments, with one of more measurements performed on each replicate of the experiment. Statistical significance was calculated as specified in the legends to the figures. In pairwise comparisons of expression levels, significance was generally determined by unpaired two-tailed Student's t-tests with Welch's correction for unequal variances. In flow cytometry experiments, significance was determined performing one-way ANOVA with Dunnett's multiple comparisons test. All statistical analyses were done GraphPad Prism 9 software. P values were included in the figures (using the asterisk symbol) or specified in the figure legends.

Bioinformatic analyses. Demultiplexing of raw data from the Illumina sequencer was performed with the bcl2fastq2 V2.2.18.12 program and adapters were trimmed with Cutadapt1.15. The reads from the CHIP-Seq experiments were mapped on the genomes of *Salmonella enterica* serovar Typhimurium strain LT2 (wt) and the SPI-1-modified variant MA14358 with BWA 0.6.2-r126. Bedgraph files were generated from aligned Bam files using bedtools genomecov. Coverage track (number of reads per base) was converted to the BigWig format using the bedGraphToBigWig command line utility from UCSC. Read depth was calculated using the bedcov tool of the Samtools suite. Bam and Bigwig files were visualized with Integrative Genome Viewer (IGV) (3). In the processing of the RACE-Seq data, the reads containing the TSO were filtered and kept thanks to grep command in the SeqKit package. These reads were then trimmed with the PRINSEQ tool to remove 33 bp from the 5' end (the amplified portion of the TSO except the terminal 3Gs).

References

1. M. W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29**, e45 (2001).
2. J. H. Miller, *A Short Course in Bacterial Genetics. A Laboratory Manual and Handbook for Escherichia coli and Related Bacteria.* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1992).
3. J. T. Robinson *et al.*, Integrative genomics viewer. *Nat Biotechnol* **29**, 24-26 (2011).

SI Appendix Fig. S1

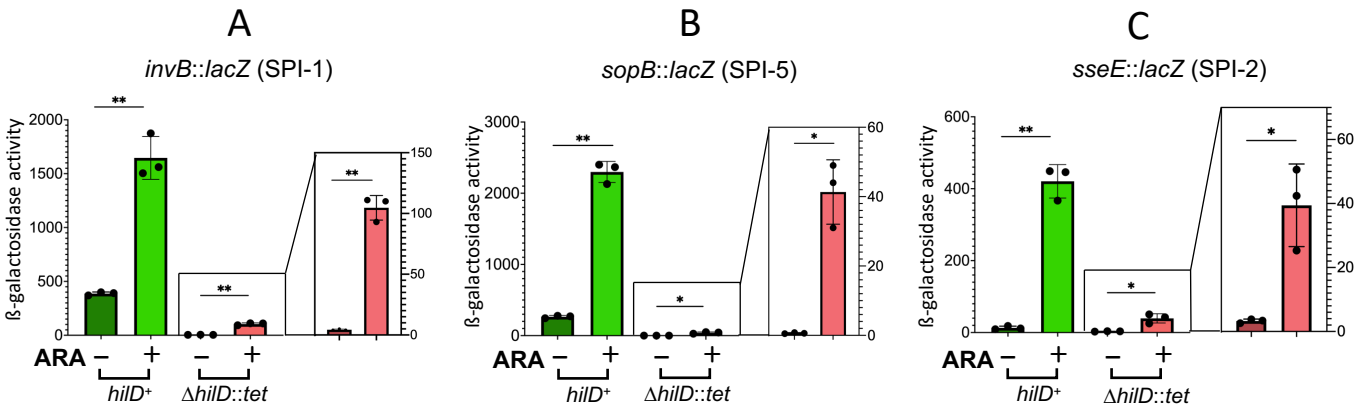

Fig. S1. Upregulation of SPI genes in NusG-depleted cells is largely HilD-mediated. Strains carrying translational *lacZ* gene fusions to SPI genes in the ARA-inducible NusG depletion background were grown at 37°C in LB (with or without ARA) for 4.5 hours (strains with *invB-lacZ* and *sopB-lacZ*) or for 6 hours (strain with *sseE-lacZ*). At this time, OD_{600} readings ranged between 1.1 and 2.1 (4.5 h) or between 1.9 and 3.3 (6 h) depending on the strain genotype and the presence or absence of ARA. Cells were assayed for ß-galactosidase activity as described in Methods. Activity is expressed in Miller units. (*A*) Strains MA13397 (*hilD*+) and MA13971 (Δ*hilD*::*tet*) carrying *invB-lacZ* (SPI-1). (*B*) Strains MA13398 (*hilD*+) and MA13972 (Δ*hilD*::*tet*) carrying *sopB-lacZ* (SPI-5). (*C*) Strains MA13409 (*hilD*+) and MA13973 (Δ*hilD*::*tet*) carrying *sseE-lacZ* (SPI-2). The results shown represent the means of three independent determinations (with error bars indicating SD) each carried out in duplicate. Significance was determined by unpaired two-tailed Student's t-tests with Welch's correction for unequal variances (*, $P \leq 0.05$; **, $P \leq 0.01$).

SI Appendix Fig. S2

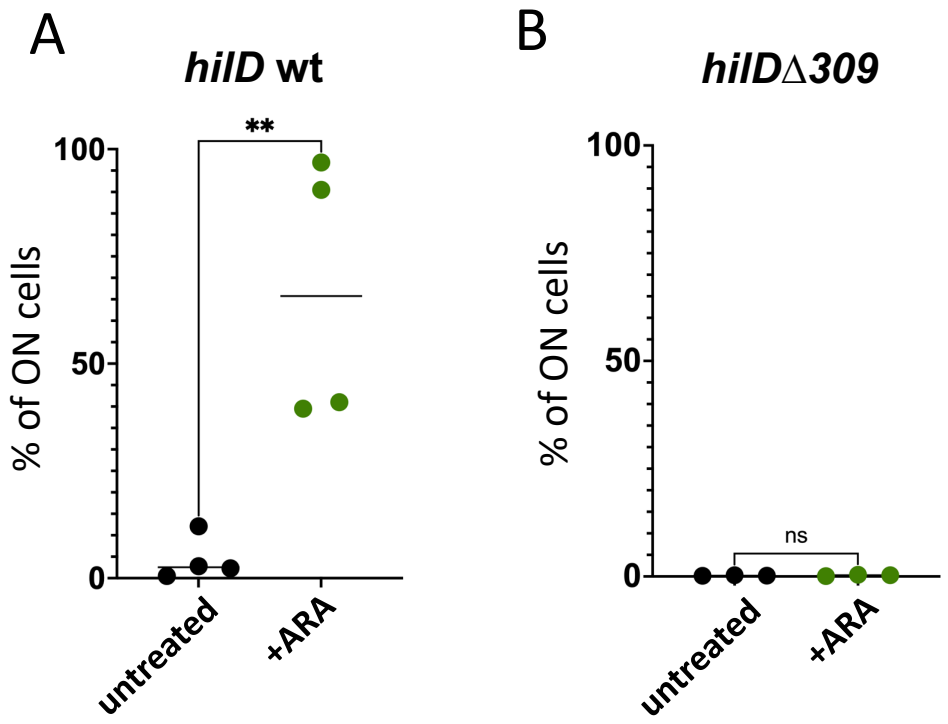

Fig. S2. NusG depletion enhances HilD-dependent SPI-1 bistability. Flow cytometry analysis of strains MA14302 (*hilD* wt) (*A*) and MA14561 (*hilDΔ309*) (*B*). Cells were grown at 37°C in LB with or without ARA added to an OD_{600} of ~0.8. GFP fluorescence intensity was measured, the number of cells analysed as 100,000, and the sizes of the SPI-1^{ON} subpopulations are shown as percentages. The plot represents the results of four (*A*) and three (*B*) independent experiments. Statistical significance was determined by the unpaired two-tailed Student's t-test (ns, $P > 0.05$; **, $P \leq 0.01$).

SI Appendix Fig. S3

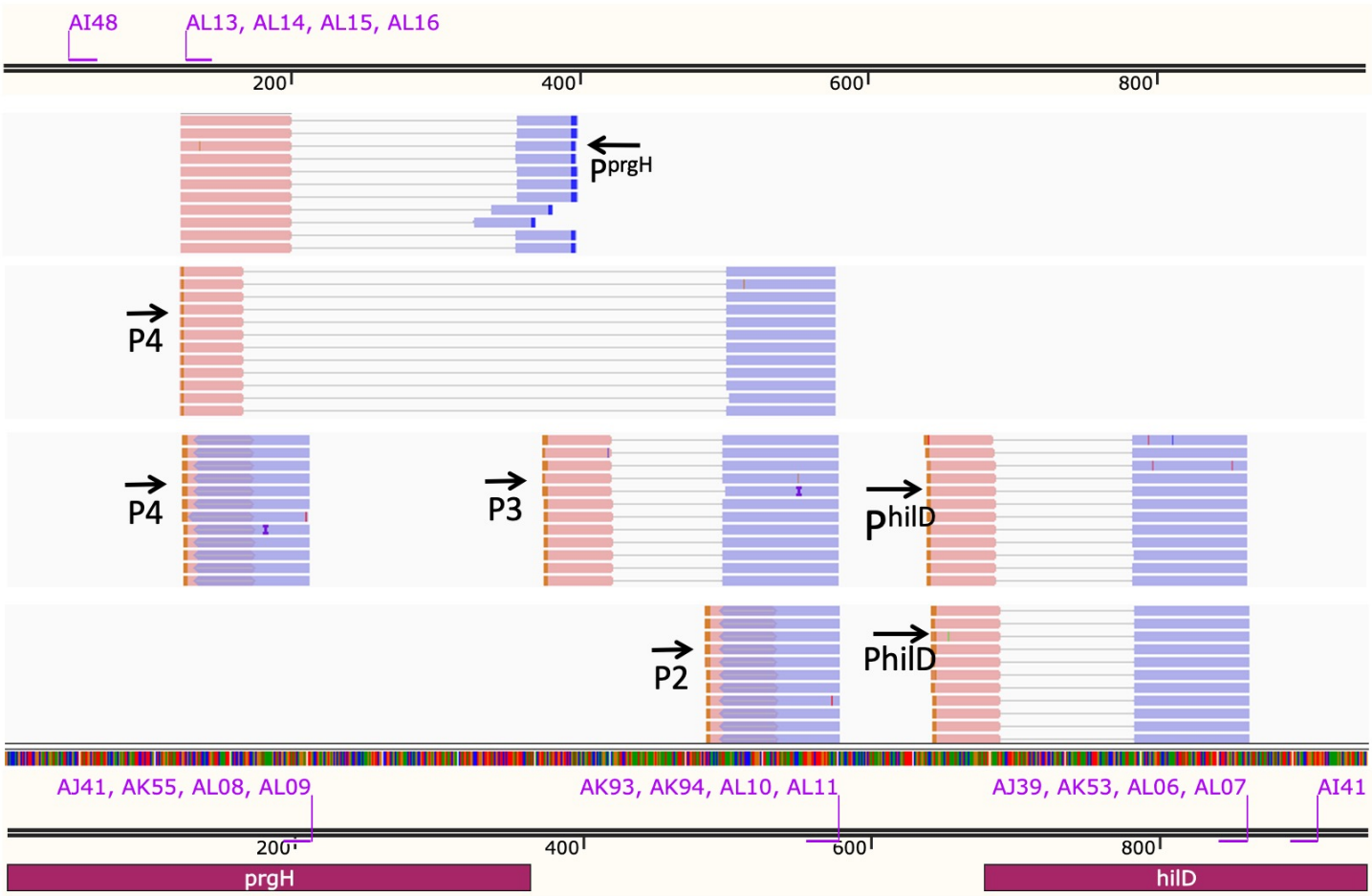

Fig. S3. Schematic representation of the primer combinations used for 5' RACE Seq. The positions of RT primers (AI41 and AI48) and reverse PCR primers are shown relative to representative reads generated by high-throughput sequencing (Bam files visualized with Integrative genome viewer, IGV).

SI Appendix Fig. S4

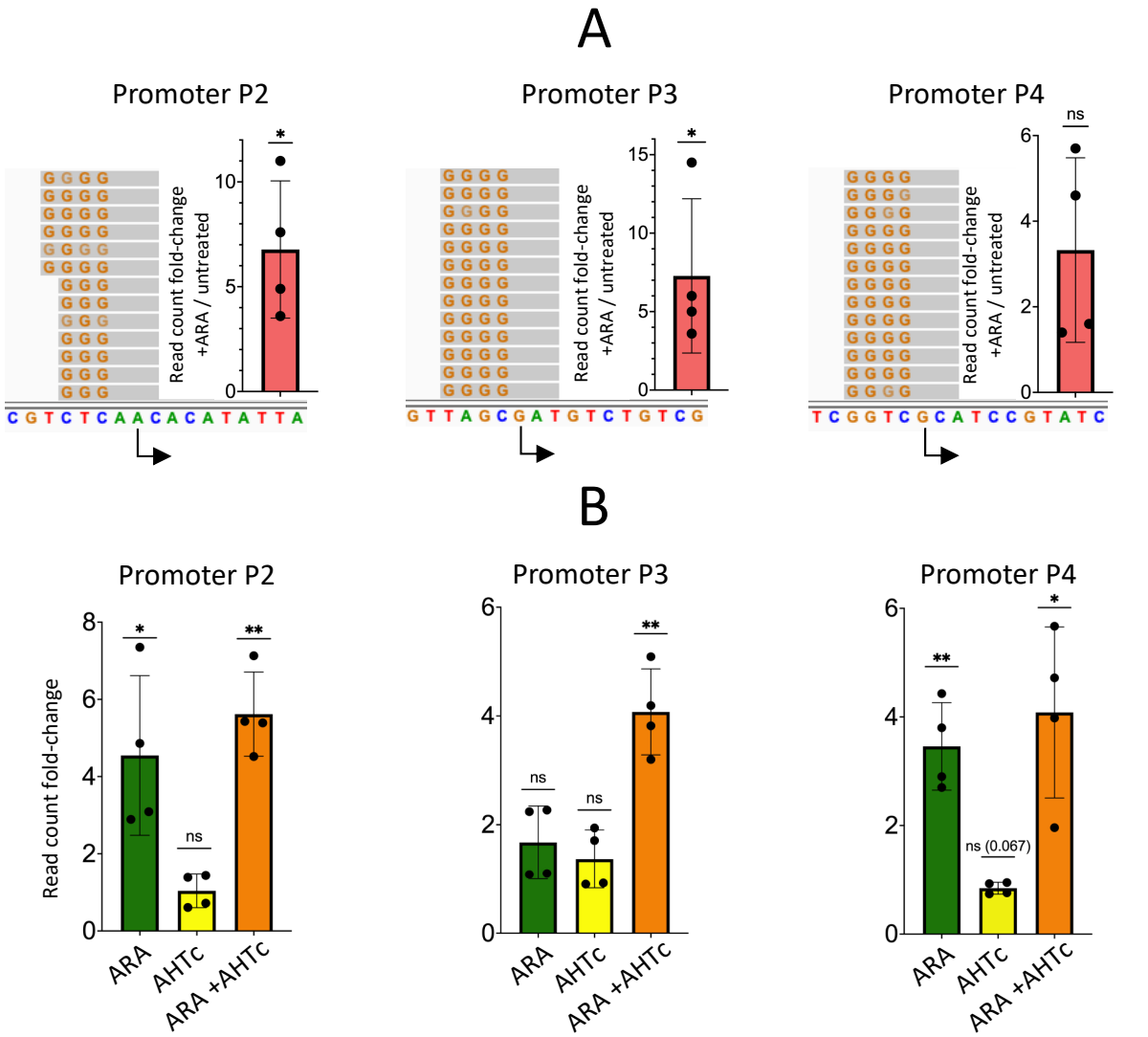

Fig. S4. 5' RACE-Seq analysis of *hilD* secondary promoters and the P^{tetA} promoter. RNA from strains MA14302 (*A*) and MA14358 (*B* and *C*) was reverse-transcribed in the presence of a template-switching oligonucleotide (TSO). The resulting cDNA was subjected to semi-quantitative PCR amplification (primers shown in *SI Appendix,* Fig. S3) and high-throughput sequencing. Read counts were normalized to those measured at the *ompA* promoter. The results shown represent the ratios between the normalized counts from treated and untreated cells. Results originate from three or four independent experiments (with error bars representing SD). Significance was determined by unpaired two-tailed Student's t tests with Welch's correction (ns, P> 0.05; *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001).

SI Appendix Fig. S5

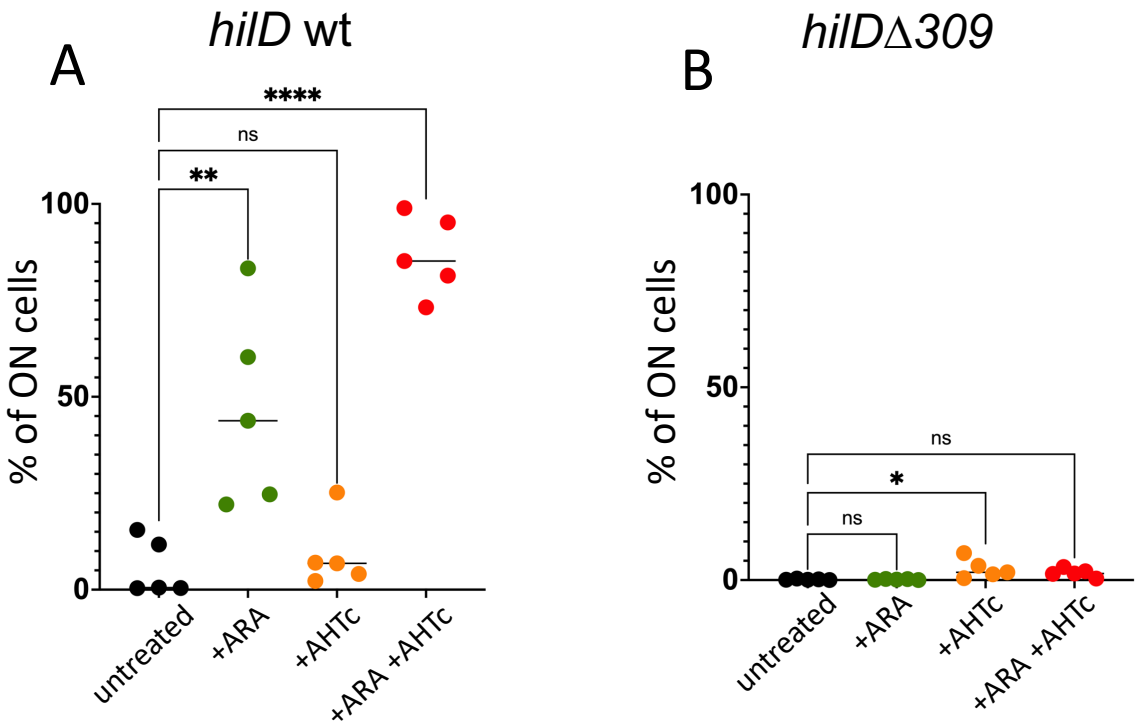

Fig. S5. Overlapping transcription promotes HilD-dependent bistability of SPI-1 expression. Flow cytometry analysis of strains MA14358 (*tetR*-P^{tetA} *hilD*$^{+}$) (*A*) and MA14569 (*tetR*-P^{tetA} *hilDΔ309*) (*B*). Cells were grown at 37°C in plain LB or in LB supplemented with either ARA or AHTc or both. GFP fluorescence intensity was measured, the number of cells analyzed as 100,000 and the sizes of the SPI-1^{ON} subpopulations are shown as percentages. The plot represents the results from five independent experiments. Statistical significance was determined by one-way ANOVA with Dunnett's multiple comparisons test (ns, P> 0.05; *, P ≤ 0.05; ***, P ≤ 0.001; ****, P ≤ 0.0001).

SI Appendix Fig. S6

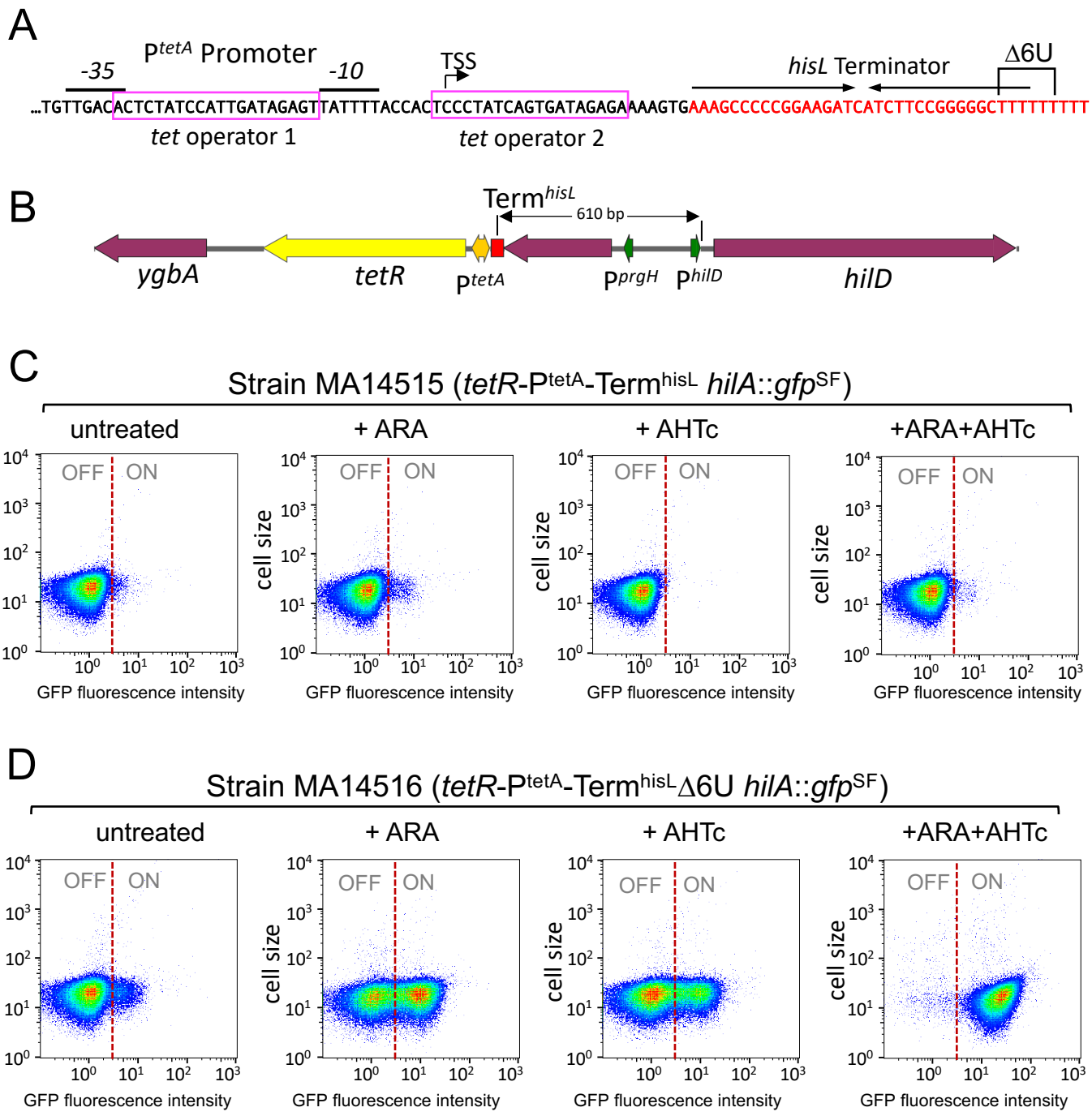

Fig. S6. Rho-independent transcription terminator abolishes the effects of NusG depletion on SPI-1 bistability. (*A*) Sequence of the P^{tetA}-$Term^{hisL}$ fusion in strains MA14515 (wt terminator) and MA14516 (Δ6U mutant), respectively. (*B*) Schematic diagram showing $Term^{hisL}$ position relative to P^{tetA} and P^{hilD} (secondary *hilD* promoters not shown). (*C*) and (*D*) Representative flow cytometry analysis of cells from strains MA14515 (*C*) and MA14516 (*D*). See legend in next page for details.

SI Appendix Fig. S6 continued

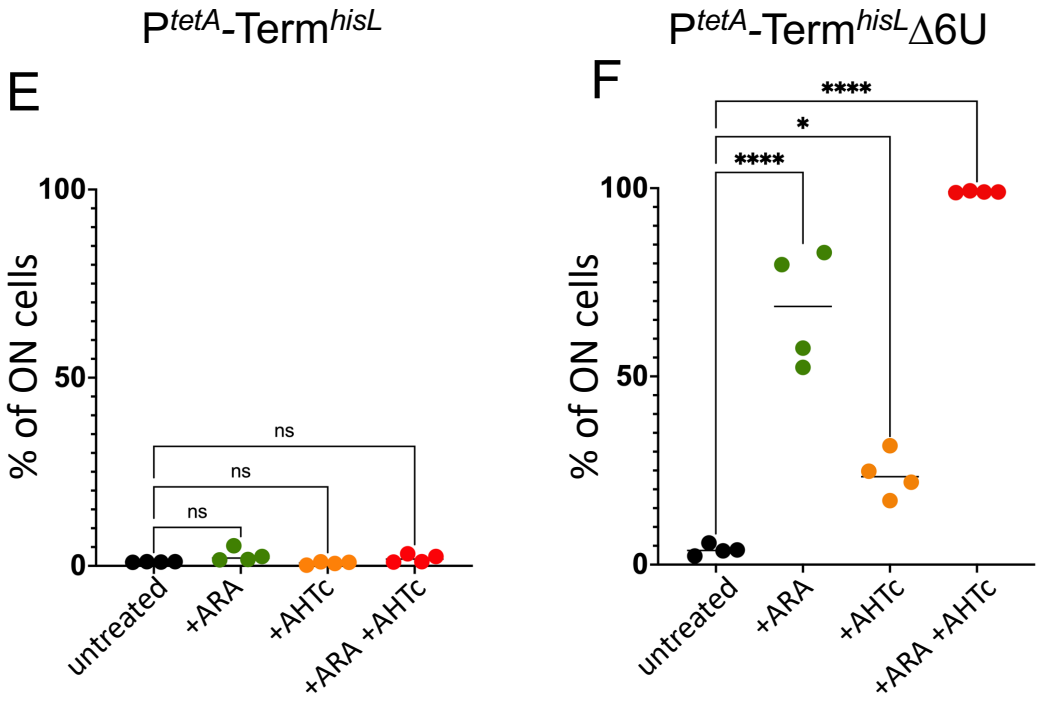

Fig. S6 continued. Rho-independent transcription terminator abolishes the effects of NusG depletion on SPI-1 bistability. Flow cytometry analysis of strains MA14515 (wt terminator) (*E*) and MA14516 (Δ6U mutant) (*F*), respectively. Cells were grown at 37°C in plain LB or in LB supplemented with either ARA or AHTc or both. GFP fluorescence intensity was measured, the number of cells analyzed as 100,000 and the sizes of the SPI-1^{ON} subpopulations are shown as percentages. The plot represents the results from four independent experiments. Statistical significance was determined by one-way ANOVA with Dunnett's multiple comparisons test (ns, P> 0.05; *, P ≤ 0.05; ***, P ≤ 0.001; ****, P ≤ 0.0001).

Table S1

Supplementary Table 1. Salmonella enterica serovar Typhimurium strains used in this work

Strain	Genotype	Source or reference
MA3409	Δ[Gifsy-1]	https://pubmed.ncbi.nlm.nih.gov/11902718/
MA12603	Δ[Gifsy-1] hilD-tetR-P^tet-ccdB-cat	this work
MA12604	Δ[Gifsy-1] hilD-3xFLAG	this work
MA12996	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA	https://pubmed.ncbi.nlm.nih.gov/31589608/
MA13397	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA invB-lacZY_kan	https://pubmed.ncbi.nlm.nih.gov/31589608/
MA13398	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA sopB-lacZY_kan	https://pubmed.ncbi.nlm.nih.gov/31589608/
MA13409	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA sseE-lacZY_kan	https://pubmed.ncbi.nlm.nih.gov/31589608/
MA13748	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA hns-3xFLAG_FRT-kan-FRT	this work
MA13971	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA invB-lacZY_kan ΔhilD::tetRA	this work
MA13972	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA sopB-lacZY_kan ΔhilD::tetRA	https://pubmed.ncbi.nlm.nih.gov/31589608/
MA13973	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA sseE-lacZY_kan ΔhilD::tetRA	https://pubmed.ncbi.nlm.nih.gov/31589608/
MA14067	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA hilAΔK28::FRT-kan-FRT	this work
MA14082	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA hilA::gfpSF ΔK28_kan	this work
MA14115	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA ΔhilA338::gfpSF	this work
MA14122	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA ΔinvB1::gfpSF	this work
MA14271	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA Δ[hisGDCBHAFIE]::P^Tac-mCherry_FRT-kan-FRT	this work
MA14275	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA Δ[hisGDCBHAFIE]::P^Tac-mCherry_FRT	this work
MA14302	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA Δ[hisGDCBHAFIE]::P^Tac-mCherry hilA::gfpSF ΔK28_kan	this work
MA14315	Δ[Gifsy-1] Δ[hisGDCBHAFIE]::P^Tac-mCherry hilA::gfpSF ΔK28_kan	this work
MA14332	Δ[Gifsy-1] Δ[hisGDCBHAFIE]::P^Tac-mCherry Δ[sitA-orgA]::tetR-P^tet-ccdB-cat hilA::gfpSF ΔK28_kan	this work
MA14339	Δ[Gifsy-1] Δ[hisGDCBHAFIE]::P^Tac-mCherry Δ[sitA-prgH332]::tetR-P^tet hilA::gfpSF ΔK28_kan	this work
MA14345	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA orgB::tetR-P^tet-ccdB-kan	this work
MA14358	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA Δ[hisGDCBHAFIE]::P^Tac-mCherry Δ[sitA-prgH332]::tetR-P^tet hilA::gfpSF ΔK28_kan	this work
MA14363	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA hilD-3xFLAG	this work
MA14505	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA Δ[hisGDCBHAFIE]::P^Tac-mCherry Δ[sitA-prgH332]::tetR-P^tet hilD-3xFLAG hilA::gfpSF ΔK28_kan	this work
MA14513	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA Δ[sitA-prgH332]::tetR-P^tet hilA::gfpSF ΔK28_kan hns-3xFLAG_FRT	this work
MA14515	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA Δ[hisGDCBHAFIE]::P^Tac-mCherry Δ[sitA-prgH332]::tetR-P^tet-Term^hikL hilA::gfpSF ΔK28_kan	this work
MA14516	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA Δ[hisGDCBHAFIE]::P^Tac-mCherry Δ[sitA-prgH332]::tetR-P^tet-Term^hikLΔ6U hilA::gfpSF ΔK28_kan	this work
MA14558	Δ[Gifsy-1] Δ[hisGDCBHAFIE]::P^Tac-mCherry hilA::gfpSF ΔK28 hilDΔ309::tetR-P^tet-ccdB-cat	this work
MA14560	Δ[Gifsy-1] Δ[hisGDCBHAFIE]::P^Tac-mCherry hilDΔ309 hilA::gfpSF ΔK28_kan	this work
MA14561	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA Δ[hisGDCBHAFIE]::P^Tac-mCherry hilDΔ309 hilA::gfpSF ΔK28_kan	this work
MA14569	Δ[Gifsy-1] Δ[Gifsy-2] Δ[araBAD]::gtgR-P^G2R-nusG-cat Δ[nusG]::aadA Δ[hisGDCBHAFIE]::P^Tac-mCherry Δ[sitA-prgH332]::tetR-P^tet hilDΔ309 hilA::gfpSF ΔK28_kan	this work

Table S2

Supplementary Table 2. DNA oligonucleotides used in this work

Name	Sequence	Use
ppY88	GTGACTGACAATTCATACTATAAATGCGAAATGAAAAAAGCGCTTGACAATTAATCATCGGCTCG	PCR forward primer in λ-red construction (reverse primer is AA30)
ppZ13	TTTAAAACTACGCCATCGACATTCATAAAAATGGCGAACCATTAAGACCCACTTTCACATT	PCR forward primer in λ-red construction
ppZ14	TTTTAATAAAAATCTTTACTTAAGTGACAGATACAAAAAATGCTTACGCCCCGCCCTG	PCR reverse primer in λ-red construction
ppZ45	TTTAAAACTACGCCATCGACATTCATAAAAATGGCGAACCATGACTACAAAGACCATGACGGTGA	PCR forward primer in λ-red construction
ppZ46	TTAATAAAAATCTTTACTTAAGTGACAGATACAAAAAATGTTATTTATCGTCGTCATCTTTGTAGTC	PCR reverse primer in λ-red construction
ppW78	GAAGAACAAGGTAAGCAACTGGAAGATTTCCTGATCAAGGAAGACTACAAAGACCATGACGG	PCR forward primer in λ-red construction
ppW79	AGCGGCGGGATTTTAAGCATCCAGGAAGTAAATTATTCCCATATGAATATCCTCCTTAG	PCR reverse primer in λ-red construction
AA30	CGTAGCTATAAGCCAAAAGCCTTGTAAGCTTCTTTGCCGGATGGTGTAGGCTGGAGCTGCTT	PCR reverse primer in λ-red construction
AE12	GCAAAGGCTATATTCGATGATTAATTAACCACATTGTTGCGAGTTAAGACCCACTTTCACATTTA	PCR forward primer in λ-red construction (reverse primer is AH30)
AE45	GTCATCAGCGTCCTGCCGCAGATAACTTACAGAAATTAAAATCACTTAAGACCCACTTTCACATTTA	PCR forward primer in λ-red construction
AE46	CATTGAATGAAGTAGGACGTGCTATCATAACCACATTTTAATGCCTAAGCACTTGTCTCCTGTTTA	PCR reverse primer in λ-red construction
AF19	GATAGGCGTACATCCGCAAGGTCTTTGCCTCTTCTTCCGCCTCTCTTAAGACCCACTTTCACATTTA	PCR forward primer in λ-red construction (reverse primer is AG26)
AF26	CCGATGGCAAAGCAAAAGTTTAAAATCAACATCGGAGCGGCAGCTGTAGGCTGGAGCTGCTTC	PCR reverse primer in λ-red construction (forward primer is AF93)
AF93	GTACGGACAGGGCTATCGGTTTAATCGTCCGGTCGTAGTGGTGTCGATCCGTCGACCTGCAGTTC	PCR forward primer in λ-red construction
AG21	GTACGGACAGGGCTATCGGTTTAATCGTCCGGTCGTAGTGGTGGCTTCTAAAGGTGAAGAACTGTTC	PCR forward primer in λ-red construction (reverse primer is AG39)
AG26	CTCTATTGAAAGATTAGAACAGCAGGCGCATCAACGGGCTAAGCGGGTCATTGGCATGTTCAA	PCR reverse primer in λ-red construction
AG39	CTGCATCTGAAAAGGAAGTATCGCCAATGTATGAGTCGTAGGTTGCTGTAGGCTGGAGCTGCTTC	PCR reverse primer in λ-red construction
AG46	GTTATAAAAACAATATAAGGGCTTAATTAAGGAAAAGATCTATGGCTTCTAAAGGTGAAGAACTG	PCR forward primer in λ-red construction (reverse primer is AG49)
AG49	GGCCAGATATTGCAGTAAACGAGGTGTTTTCATCTCATTAGCGACCTGTAGGCTGGAGCTGCTTC	PCR reverse primer in λ-red construction
AH30	GGCGGCAAATGAGTTAATACTGGCGGCATGGCGGCTTAAGAACGGTCATTGGCATGTTCAA	PCR reverse primer in λ-red construction
AH38	CATTGATAGAGTTATTTTACCACTCCCTATCAGTGATAGAGAAAAGTGGATAAGCAATTCAC	PCR forward primer in λ-red construction
AH39	CGGTGCAATTAAATACGCCAATACAGGTCGGTGAATTGCTTATCCACTTTTCTC	PCR reverse primer in λ-red construction
AI27	CATTGATAGAGTTATTTTACCACTCCCTATCAGTGATAGAGAAAAGTGAAAGCCCCCGGAAGATCATCTTCCGG	PCR forward primer in λ-red construction
AI28	CGGTGCAATTAAATACGCCAATACAGGTCGGTGAATTGCTTATCAAAAAAAAAGCCCCCGGAAGATGATCTTCCGG	PCR reverse primer in λ-red construction
AI39	GCTAATCATTGCAAGCAGTGGTATCAACGCAGAGTACAT**rGrGrG**	Template-switching oligo (TSO)
AI41	CAACATCCCAGGTTCGTCAC	RT primer (hilD)
AI48	ACCGACCTGTATTGGCGTAT	RT primer (prgH)
AI50	CGCATCCGTATCCACCTGG	qPCR forward primer (prgH)
AI51	GACAGGCCGAACACTCTTTG	qPCR reverse primer (prgH)
AI57	GTGTGTTGGCAATGGTCTGA	qPCR reverse primer (hilD CHIP, forward primer is AL12)
AI62	TGCCGCAGATAACTTACAGAA	qPCR forward primer (hilD)
AI63	GTCAGTTTACCGCTCCGAAA	qPCR reverse primer (hilD)
AJ32	CGTTGGAGATATTCATGGCGT	qPCR forward primer (ompA)
AJ33	ACCAGTCGTAGCCCATTTCA	RT primer (ompA)
AJ37	GAGACCAGCCCAGTTTAGCA	qPCR reverse primer (ompA)
AJ38	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCATTGCAAGCAGTGGTATCAAC	Illumina sequencing universal forward primer (anneals to TSO compleme
AJ39	CAAGCAGAAGACGGCATACGAGAAAGGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCAGTTTACCGCTCCGAAA	Illumina sequencing reverse primer (hilD primary promoter)
AJ40	CAAGCAGAAGACGGCATACGAGAAAGGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGTCCTGTGCGGTAATCT	Illumina sequencing reverse primer (hilD P3 promoter)
AJ41	CAAGCAGAAGACGGCATACGAGAAAGGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTCCCTGATATACCTGCCG	Illumina sequencing reverse primer (hilD P4 promoter and P^tet promote
AK19	GAGTCTTCTGGCTCGCAGTA	qPCR forward primer (katN CHIP)
AK20	CTCTGGCAAGTTTAGGAAGAGC	qPCR reverse primer (katN CHIP)
AK53	CAAGCAGAAGACGGCATACGAGAATAGTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCAGTTTACCGCTCCGAAA	Illumina sequencing reverse primer (hilD primary promoter)
AK54	CAAGCAGAAGACGGCATACGAGAATAGTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGTCCTGTGCGGTAATCT	Illumina sequencing reverse primer (hilD P3 promoter)
AK55	CAAGCAGAAGACGGCATACGAGAATAGTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTCCCTGATATACCTGCCG	Illumina sequencing reverse primer (hilD P4 promoter and P^tet promote
AK93	CAAGCAGAAGACGGCATACGAGAAAGGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATTGTTACACCAAAAGAACCCA	Illumina sequencing reverse primer (hilD P2 and P3 promoters)
AK94	CAAGCAGAAGACGGCATACGAGAATAGTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATTGTTACACCAAAAGAACCCA	Illumina sequencing reverse primer (hilD P2 and P3 promoters)
AL01	CAAGCAGAAGACGGCATACGAGAAAGGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGACCAGCCCAGTTTAGCA	Illumina sequencing reverse primer (ompA promoter)
AL02	CAAGCAGAAGACGGCATACGAGAATAGTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGACCAGCCCAGTTTAGCA	Illumina sequencing reverse primer (ompA promoter)
AL06	CAAGCAGAAGACGGCATACGAGAAGTCGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCAGTTTACCGCTCCGAAA	Illumina sequencing reverse primer (hilD primary promoter)
AL07	CAAGCAGAAGACGGCATACGAGAACTTCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCAGTTTACCGCTCCGAAA	Illumina sequencing reverse primer (hilD primary promoter)
AL08	CAAGCAGAAGACGGCATACGAGAAGTCGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTCCCTGATATACCTGCCG	Illumina sequencing reverse primer (hilD P4 promoter and P^tet promote
AL09	CAAGCAGAAGACGGCATACGAGAACTTCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTCCCTGATATACCTGCCG	Illumina sequencing reverse primer (hilD P4 promoter and P^tet promote
AL13	CAAGCAGAAGACGGCATACGAGAAAGGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCATCCGTATCCACCTGG	Illumina sequencing reverse primer (prgH promoter)
AL14	CAAGCAGAAGACGGCATACGAGAATAGTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCATCCGTATCCACCTGG	Illumina sequencing reverse primer (prgH promoter)
AL15	CAAGCAGAAGACGGCATACGAGAAGTCGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCATCCGTATCCACCTGG	Illumina sequencing reverse primer (prgH promoter)
AL16	CAAGCAGAAGACGGCATACGAGAACTTCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCATCCGTATCCACCTGG	Illumina sequencing reverse primer (prgH promoter)
AL17	CAAGCAGAAGACGGCATACGAGAAGTCGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGACCAGCCCAGTTTAGCA	Illumina sequencing reverse primer (ompA promoter)
AL18	CAAGCAGAAGACGGCATACGAGAACTTCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGACCAGCCCAGTTTAGCA	Illumina sequencing reverse primer (ompA promoter)
AL21	TTAGTACTAACGGTCAGGTTGAG	RT primer (hilA)
AM24	CAAGCAGAAGACGGCATACGAGAAAGGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGGCTCGGGTTTATATGTT	Illumina sequencing reverse primer (hilA promoter)
AM25	CAAGCAGAAGACGGCATACGAGAATAGTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGGCTCGGGTTTATATGTT	Illumina sequencing reverse primer (hilA promoter)
AM26	CAAGCAGAAGACGGCATACGAGAAGTCGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGGCTCGGGTTTATATGTT	Illumina sequencing reverse primer (hilA promoter)
AM27	CAAGCAGAAGACGGCATACGAGAACTTCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGGCTCGGGTTTATATGTT	Illumina sequencing reverse primer (hilA promoter)
AL85	CAAGCAGAAGACGGCATACGAGAAGTCGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGTCCTGTGCGGTAATCT	Illumina sequencing reverse primer (hilD P3 promoter)
AL86	CAAGCAGAAGACGGCATACGAGAACTTCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGTCCTGTGCGGTAATCT	Illumina sequencing reverse primer (hilD P3 promoter)
AM38	TATAACCTTCCGCCCCGTAC	qPCR forward primer (prgH antisense)
AM39	CAGAGCCCGTCAATTTGTCG	qPCR reverse primer (prgH antisense)

Red lettering denotes sequences annealing to chromosomal DNA or cDNA in λ red recombineering experiments and in 5'RACE-Seq analysis, respectively. Colored hexameric sequences correspond to indexes used in Illumina sequencing.

Table S3

Supplementary Table 3. Relevant alleles constructed by λ red recombineering			
Allele	**Primer pair**	**template**	**Notes**
hilD-tetR-P^tet-ccdB-cat	ppZ13 - ppZ14	pNNB5	AHTc-inducible ccdB on the 3' side of hilD. Step 1 in the construction of markerless hilD-3xFLAG
hilD-3xFLAG	ppZ45 - ppZ46	pSUB11	
hns-3xFLAG_FRT-kan-FRT	ppW78- ppW79	pSUB11	
ΔhilD::tetRA	AE45 - AE46	pTn5-tetRA-SH1	
hilAΔK28::FRT-kan-FRT	AF93 - AF26	pKD13	FRT-kan-FRT insertion removing a 28266 bp SPI-1 segment from position 338 of hilA to the end of STM2906. Step 1 in the construction of hilA::gfpSF ΔK28_kan
ΔhilA338::gfpSF	AG21 - AG39	pNCM2	
ΔinvB1::gfpSF	AG46 - AG49	pNCM2	
Δ[hisGDCBHAFIE]::P^Tac-mCherry_FRT-kan-FRT	ppY88 - AA30	pNCM4	
Δ[sitA-orgA]::tetR-P^tet-ccdB-cat	AE12 - AH30	pNNB5	Step 1 in the construction of Δ[sitA-prgH332]::tetR-P^tet
Δ[sitA-prgH332]::tetR-P^tet	AH38 - AH39	Fill-in	Primers anneal to each other
Δ[sitA-prgH332]::tetR-P^tet-Term^hisL	AI27 - AI28	Fill-in	Primers anneal to each other
orgB::tetR-P^tet-ccdB-kan	AF19 - AG26	pNNB7	Step 1 for moving hilD-3xFLAG in the NusG depletable background
hilDΔ309::tetR-P^tet-ccdB-cat	AL78 - AL79	pNNB5	Step 1 in the construction of markerless hilD309
hilDΔ309	AL81 - AL82	Fill-in	Primers anneal to each other

Table S4

Supplementary Table 4. Primers used in qPCR experiments

Fig. 2a			RT primer	qPCR primer pair
	gene of interest	hilD	AI41	AI62 - AI63
	reference gene	ompA	AJ33	AJ32 - AJ37
Fig. 2b			RT primer	qPCR primer pair
	gene of interest	prgH	AI48	AI50 - AI51
	reference gene	ompA	AJ33	AJ32 - AJ37
Fig. 2c			qPCR primer pair	
	gene of interest	prgH-hilD IG	AL12 - AI57	
	reference gene	katN	AK19 - AK20	
Fig. 2f			RT primer	qPCR-1 primer pair
	gene of interest	hilD	AI41	AI62 - AI63
	reference gene	ompA	AJ33	AJ32 - AJ37
				qPCR-2 primer pair
	gene of interest	prgH antisense	AI41	AM38 - AM39
	reference gene	ompA	AJ33	AJ32 - AJ37
Fig. 2g			RT primer	qPCR-1 primer pair
	gene of interest	prgH	AI48	AI50 - AI51
	reference gene	ompA	AJ33	AJ32 - AJ37
				qPCR-2 primer pair
	gene of interest	hilD antisense	AI48	AI62 - AI63
	reference gene	ompA	AJ33	AJ32 - AJ37
Fig. 3b			RT primer	qPCR primer pair
	gene of interest	hilD	AI41	AI62 - AI63
	reference gene	ompA	AJ33	AJ32 - AJ37
Fig. 3c			RT primer	qPCR primer pair
	gene of interest	prgH	AI48	AI50 - AI51
	reference gene	ompA	AJ33	AJ32 - AJ37
Fig. 3f			qPCR primer pair	
	gene of interest	prgH-hilD IG	AL12 - AI57	
	reference gene	katN	AK19 - AK20	