Supplemental information

Supplemental figure legends

Figure S1. Purified PhoP-His6, His6-H-NS and His6-SlyA proteins used in the *in vitro* experiments.
Shown are Coomassie-blue-stained gels after SDS-PAGE of 3 μg of purified His-tagged proteins.

Figure S2. The *slyA* gene is dispensable for transcription of the ancestral PhoP-regulated *pagP*, *rstA*, and *slyB* genes.
A-C. Transcript levels corresponding to the *pagP* (A), *rstA* (B) and *slyB* (C) genes as determined by quantitative real-time PCR in wild-type (14028s), *phoPQ* (EG15598) and *slyA* (EG14078) cells grown in N-minimal medium containing 10 mM (H) or 10 μM (L) $MgCl_2$. Shown are the mean values and standard deviations of at least three independent experiments.
Quantitative real-time PCR was performed as described in the main text. Primers 4487 (5'-CAGTCTCTGCGGCGGATAAA-3') and 4488 (5'-AGGCTGTCGCCACGTTTCT-3'); 4125 (5'-GCATGACGATATGTCGCGATT-3') and 4126 (5'-AGCGAGGTGAGCAGGACAA-3'); 4493 (5'-CAAGTTCAGAATGTAACGTACGGTACT-3') and 4494 (5'-GAATCATCACCGCCCTGAAT-3') were used to quantify the *pagP*, *rstA*, and *slyB* transcripts, respectively.

Figure S3. Point mutations in the PhoP binding site of the *pagC* promoter region abolish PhoP-dependent *pagC* transcription *in vitro*.
Run-off *in vitro* transcription assays with linear templates corresponding to the wild-type *pagC* promoter region (-178 to +122 nt with respect to the transcription start site) (top) or an equivalent DNA fragment harboring point mutations in the PhoP binding site (Fig. 3A) (bottom), RNA polymerase and increasing amounts of the PhoP protein (0, 1, 2, 3, and 4 μM). The *pagC* transcript (lower band) is indicated by an arrow. The upper band in both panels corresponds to a ~150 nt run-off transcript resulting from spurious transcription going in the reverse orientation.

Figure S4. PhoP and SlyA counteract H-NS-promoted repression.
Single round *in vitro* transcription assay with supercoiled plasmid template harboring the *ugtL* promoter region (-180 to +125 nt with respect to the transcription start site). Reactions contained RNA polymerase alone or in combination with PhoP (2 μM), H-NS (1.25 μM), and SlyA (0.3 μM) as indicated on top of the figure.
The plasmid template for *in vitro* transcription was constructed as follows: A fragment corresponding to the *ugtL* promoter region was PCR amplified with primers 7359 (5'-CGGAATTCCTCCTGGCCATGAAATATG-3') and 7360 (5'-AACTGCAGCCGTCAGCTAATCGTTACAA-3') and genomic DNA from wild-type *Salmonella* as template and cloned into the EcoRI and PstI sites of pSA508 (S1).

Figure S5. H-NS and SlyA can bind simultaneously to the *pagC* promoter region.
Gel-shift analysis of a DNA fragment corresponding to the *pagC* promoter region (-178 to +122 nt with respect to the transcription start site) with increasing amounts of SlyA and H-NS proteins. Gel-shifts were carried out as described in the main text. After electrophoresis, gels were dried and analyzed with a Fuji phosphorimager.

Figure S6. Mutation in a shared SlyA/H-NS binding site does not affect SlyA binding to other sites of the *pagC* promoter region.

Gel-shift analysis of a DNA fragment corresponding to the *pagC* promoter region (-178 to +122 nt with respect to the transcription start site) containing wild-type sequence (top) or harboring a 12 nt deletion in a SlyA/H-NS binding site (bottom) with increasing amounts of SlyA protein. Gel-shifts were carried out as described in the main text. After electrophoresis, gels were dried and analyzed with a Fuji phosphorimager.

Reference

S1. Lewis, D.E. (2003) *Methods Enzymol*. **370**, 618-645.

Table S1. Primers used in this study.

Primer number	Sequence	Gene
Construction of Salmonella strains		
7885	5'CAATGACGCCAGGAAAGGGAATACTTTCTCCAGAACATAAGTG TAGGCTGGAGCTGCTTC-3'	
7886	5'-GCTAAAAGTAGTCGTTCGGCACCATTGTAGATAAATAACACAT ATGAATATCCTCCTTAG-3'	
7977	5'-ATAAGAGAGATATATCATTCCAAAAAAATAATAACTAACGCA AATATTGAACACGCG-3'	
2896	5'-AAAGGCGTTAGTATCGGCCTGTGC-3'	
7978	5'-TATTTTTTTGGAATGATATATCTCTCTTATCACAGGTGATATTT ATGTTGG-3'	
7984	5'-GCTAAAAGTAGTCGTTCGGCACCATTGTAGATAAATAACACT CTAATGCGCTGTTAATCACT-3'	
7888	5'-TAGAATCAACACCACAATTCCAACATAAATATCACCTGTGCT AAGCACTTGTCTCCTGTT-3'	
8293	5'-TAGAATCAACACCACAATTCCAACATAAATATCACCTGTGTT TAGAGAGAATTTACATTCCAAAAAAATAATAACTGAACACGCGA TAAAAAAGTC-3'	
7068	5'-GCTCAACAAACCACCCCAATATAAGTTTGAGATTACTACAGT GTAGGCTGGAGCTGCTTC-3'	
7069	5'-TCCCGCCAGCGGCGGGATTTTAAGCATCCAGGAAGTAAATCA TATGAATATCCTCCTTAG-3'	
Plasmid construction		
7239	5’-CGGCATATGCACCACCACCACCACCACAGCGAAGCACTTAAA ATTCTG-3’	*hns*
7252	5’-CGCAAGCTTATTCCTTGATCAGGAAATCTTCC-3’	*hns*
7070	5’-CGGCATATGCACCACCACCACCACCACAAATTGGAATCGCCA CTAGG-3’	*slyA*
7065	5’-CGCAAGCTTTCAATCGTGAGAGTGCAATT-3’	*slyA*
Quantification of transcripts by real-time PCR		
6684	5’-TAAAGCGTGCTAAGCATCATCG-3’	*pagC*
6685	5’-TTATATGCCATCGCAGGCG-3’	*pagC*
6492	5’-CGGGTCTGTTGAGCCTGAAG-3’	*pagC*
6493	5’-TAGGCTGGCCCAACCATTAA-3’	*pagC*
7108	5’-CGATTAGCTGACGGCTTTGTTT-3’	*ugtL*
7114	5’-GATTTCTTCATTTTGAGCCTCCTC-3’	*ugtL*
6494	5’-TACGGTATCATCGCAGGGTTT-3’	*ugtL*
6495	5’-TTTGGACAAGCGCTGAACCT-3’	*ugtL*
4443	5’-TAATTGCCACAAAACTTATG-3’	*mgtA*
4446	5’-TCGCGGGAGAGGGGTGGGTT-3’	*mgtA*
6970	5’-CCAGCAGCCGCGGTAAT-3’	*rrs*
6971	5’-TTTACGCCCAGTAATTCCGATT-3’	*rrs*
Quantification of ChIP DNA by real-time PCR		
4149	5’-ACCGTGGCACAAATGATGCT-3’	*rpoD*
4150	5’-TCGGCAATCGCCTTATCTG-3’	*rpoD*
5852	5’-GTGAGCCGGTTTTGCATC-3’	*mgtA*
5853	5’-CTCCGGTAAGTAAATAATTTGCG-3’	*mgtA*
7857	5’-ATCGCGTGTTCAATATTTGC-3’	*pagC*

7858	5’-TCAACACCACAATTCCAACA-3’	*pagC*
7855	5’-TTGCTCAACCGTGTAGAAATTG-3’	*ugtL*
7856	5’-CTGCCCTACCGCTAAACATC-3’	*ugtL*
Templates for in vitro *transcription, DNase I footprinting, and EMSA*		
7756	5’-CAACGAAGAGTTAACCACTC-3’	*pagC*
7758	5’-GACGCTCCATCCGCAATACGG-3’	*pagC*
7193	5’-CCTCCTGGCCATGAAATATG-3’	*ugtL*
7194	5’-GCCGTCAGCTAATCGTTACAA-3’	*ugtL*

Figure S1

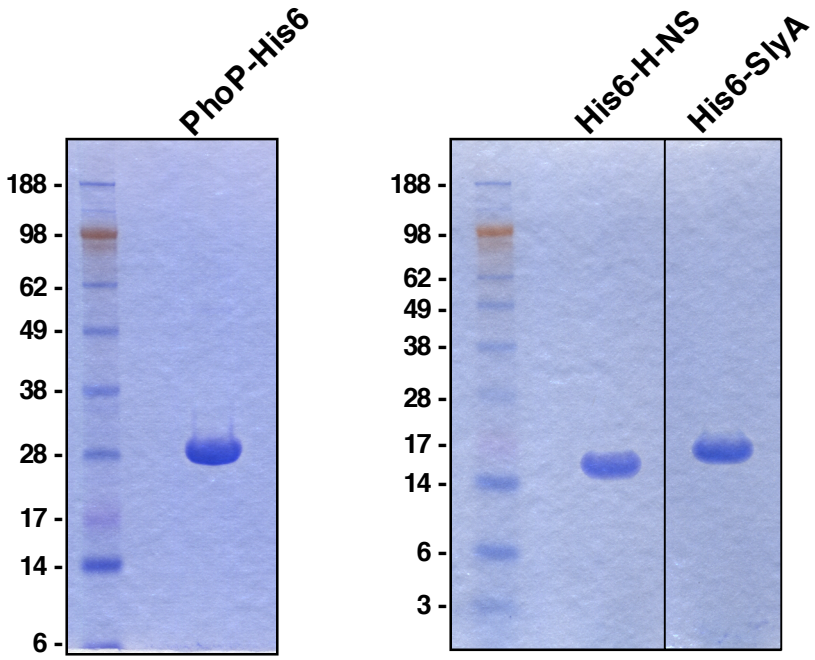
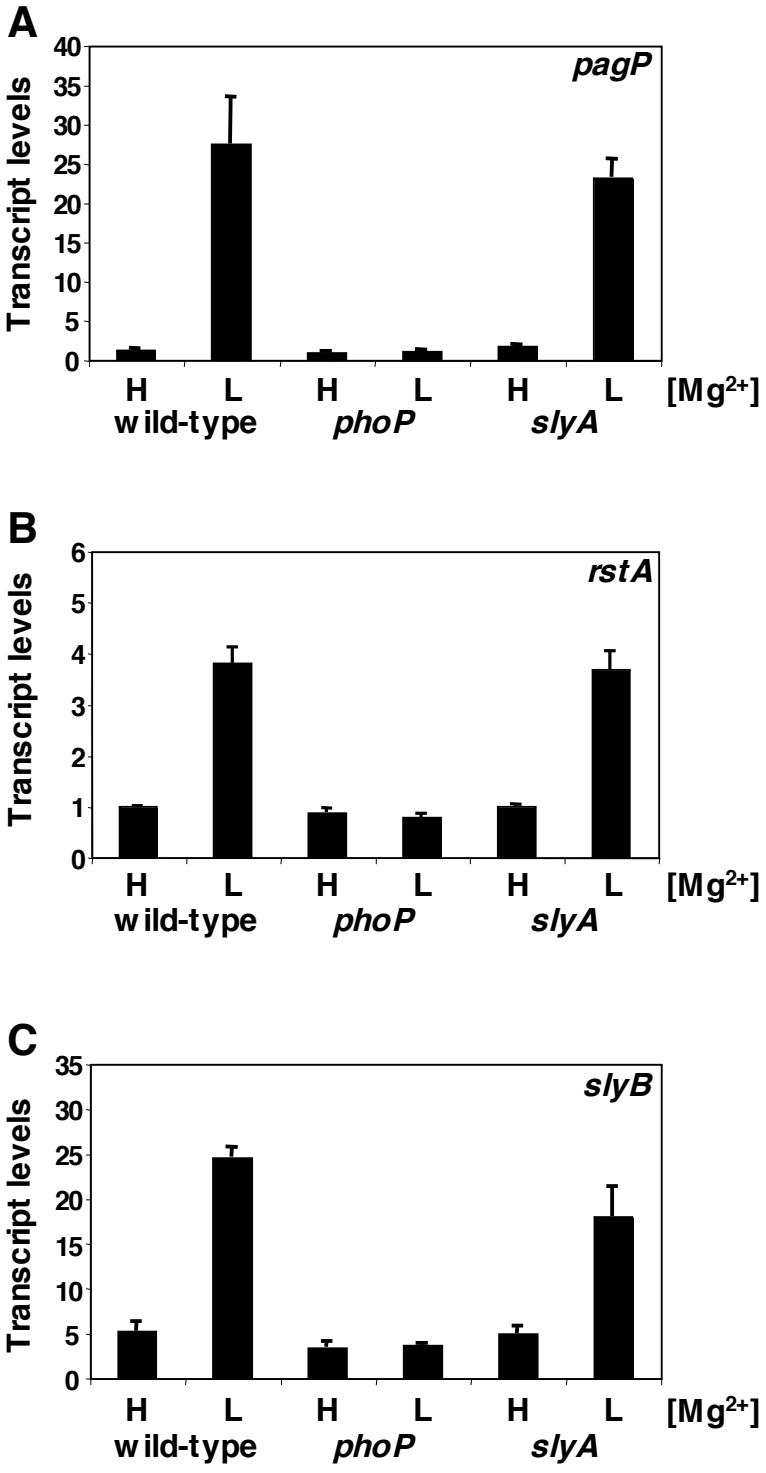


Figure S2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pagP
Transcript levels
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35
30
25
20
15
10
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B
rstA
Transcript levels
6
5
4
3
2
1
0
H L H L H L [Mg2+]
wild-type phoP slyA
C
slyB
Transcript levels
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wild-type phoP slyA

Figure S3

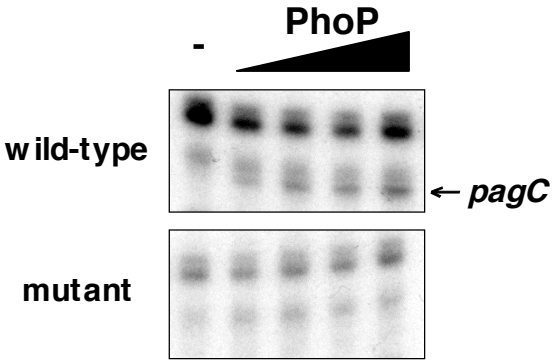


Figure S4

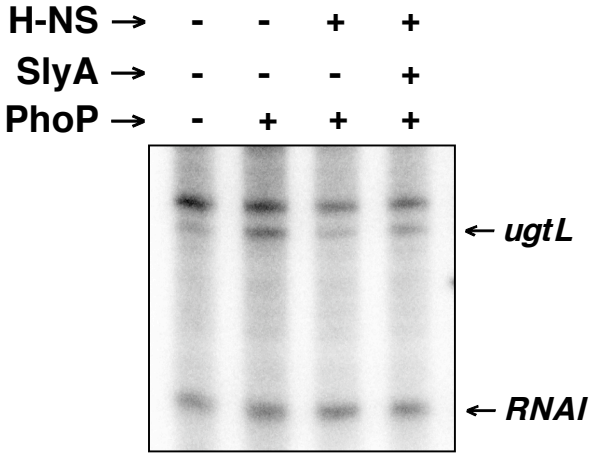
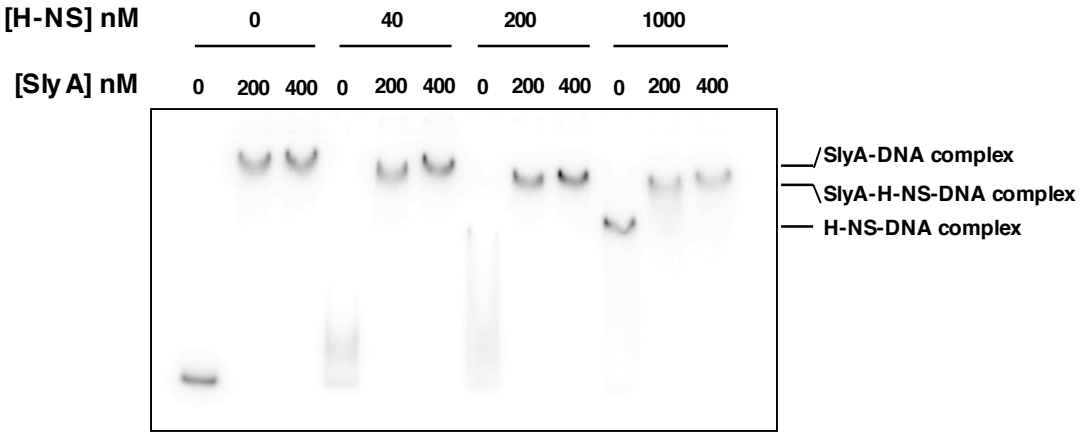


Figure S5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200
1000
[Sly A] nM
0 200 400 0 200 400 0 200 400 0 200 400
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H-NS-DNA complex

Figure S6