Supplementary Information

Cyclic di-GMP rescues H-NS-mediated silencing of bacterial type VI secretion systems

Shuyu Li[1], Qinmeng Liu[1], Chongyi Duan[1], Jialin Li[1], Hengxi Sun[1], Lei Xu[1], Qiao Yang[2], Yao Wang[1], Xihui Shen[1]*, Lei Zhang[1]*

[1]State Key Laboratory of Crop Stress Biology for Arid Areas, Shaanxi Key Laboratory of Agricultural and Environmental Microbiology, College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, China.

[2]ABI Group, College of Marine Science and Technology, Zhejiang Ocean University, Zhoushan 316021, China.

[3]Donghai Laboratory, Zhoushan 316021, China.

*For correspondence:

Xihui Shen (xihuishen@nwsuaf.edu.cn);

Lei Zhang (zhanglei0075@nwsuaf.edu.cn).

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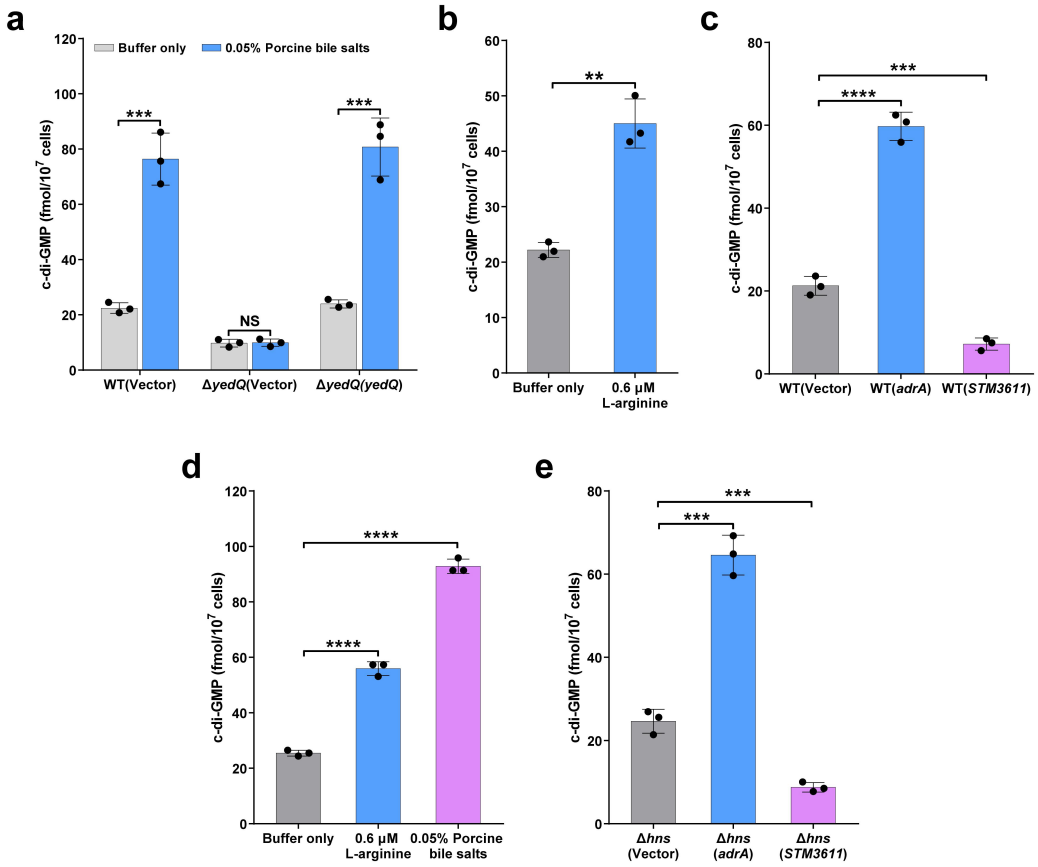
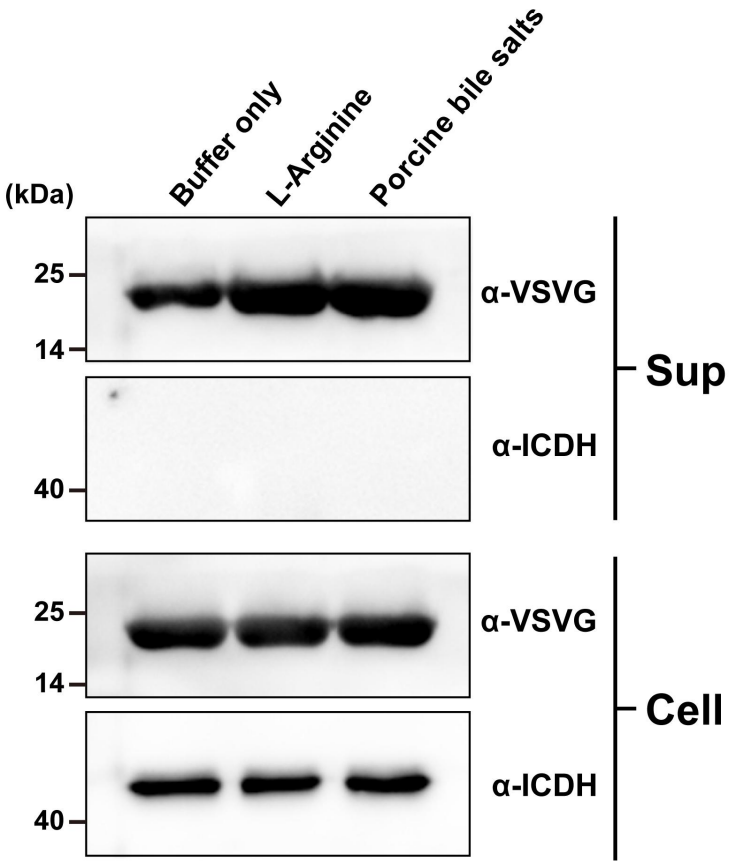
Supplementary Figures



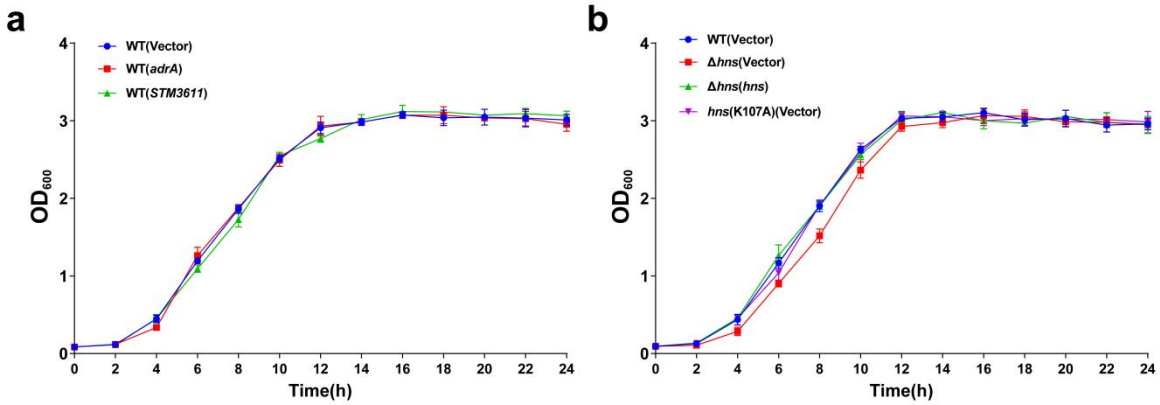
Supplementary Fig. 1 Intracellular c-di-GMP levels in *S.* Typhimurium are changed by stimulation with L-arginine and bile salts, or overexpression of *adrA* and *STM3611.*

a, An increase in intracellular c-di-GMP concentrations in response to bile salts requires YedQ. **b,** L-Arginine stimulates an increase in intracellular c-di-GMP concentrations in the wild type. **c,** Intracellular c-di-GMP levels in the wild type were modulated by overexpression of *adrA* or *STM3611*. **d,** L-Arginine and bile salts stimulate an increase in intracellular c-di-GMP concentrations in the Δ*hns* mutant. **e,** Intracellular c-di-GMP levels in the Δ*hns* mutant were modulated by overexpression of *adrA* or *STM3611*. Data shown are mean ± SD of three biological replicates. Statistical significance was calculated using the Student's *t* test. ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001; NS, not significant.



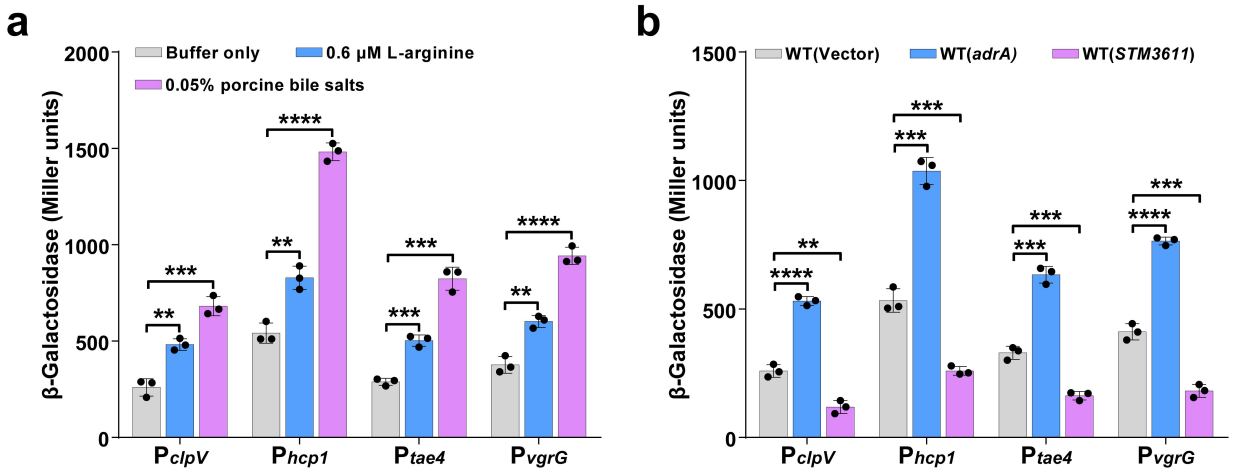
Supplementary Fig. 2. L-Arginine and bile salts stimulate Hcp1 secretion in *S.* Typhimurium SL1344.

S. Typhimurium SL1344 carrying a plasmid expressing Hcp1-VSVG were induced by 0.6 μM L-arginine, 0.05% bile salts or a buffer control, and then Hcp1-VSVG in cell pellet (Cell) and concentrated supernatant (Sup) was analyzed by western blot. ICDH was used as a loading control. Data shown were one representative from three independent experiments with similar results.



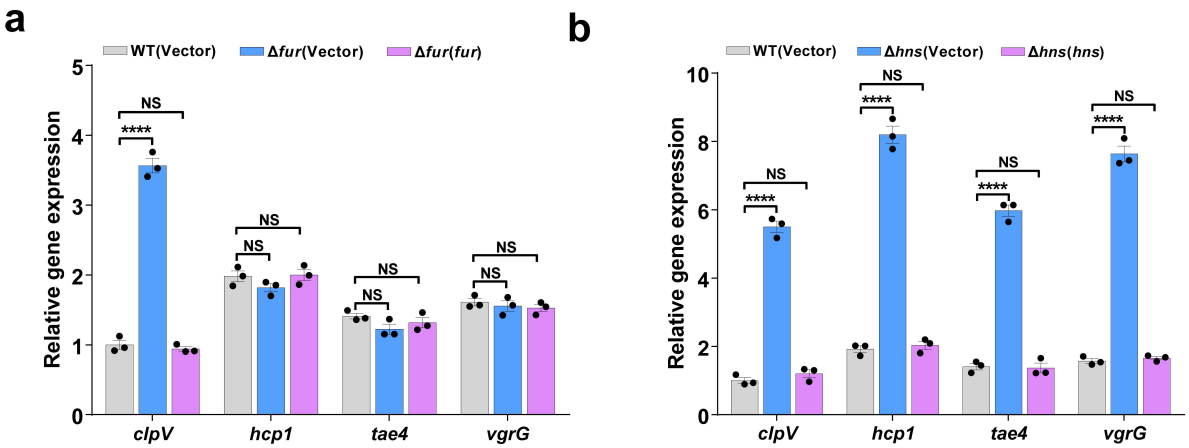
Supplementary Fig. 3. Growth curves of *S*. Typhimurium strains.

a, b, *S*. Typhimurium strains were grown in LB medium with shaking at 37 °C. 0.5 mM IPTG was added as an inducer at the time of inoculation. At each time point during growth, cell density was recorded by measuring the optical density at 600 nm. Data are mean ± SD of three biological replicates.



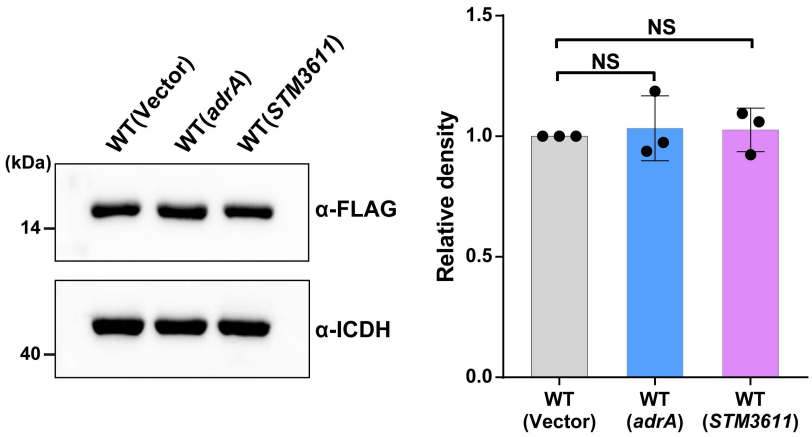
Supplementary Fig. 4. Elevated c-di-GMP levels induce the promoter activities of T6SS genes in *S.* Typhimurium SL1344.

a, The promoter activities of *clpV*, *hcp1*, *tae4* and *vgrG* in the wild-type strain were induced by L-arginine and bile salts. **b,** The promoter activities of the T6SS genes in the wild-type strain were upregulated by overexpressing *adrA*, but downregulated by overexpressing *STM3611*. Data shown are mean ± SD of three biological replicates. Statistical significance was calculated using the Student's *t* test. ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.



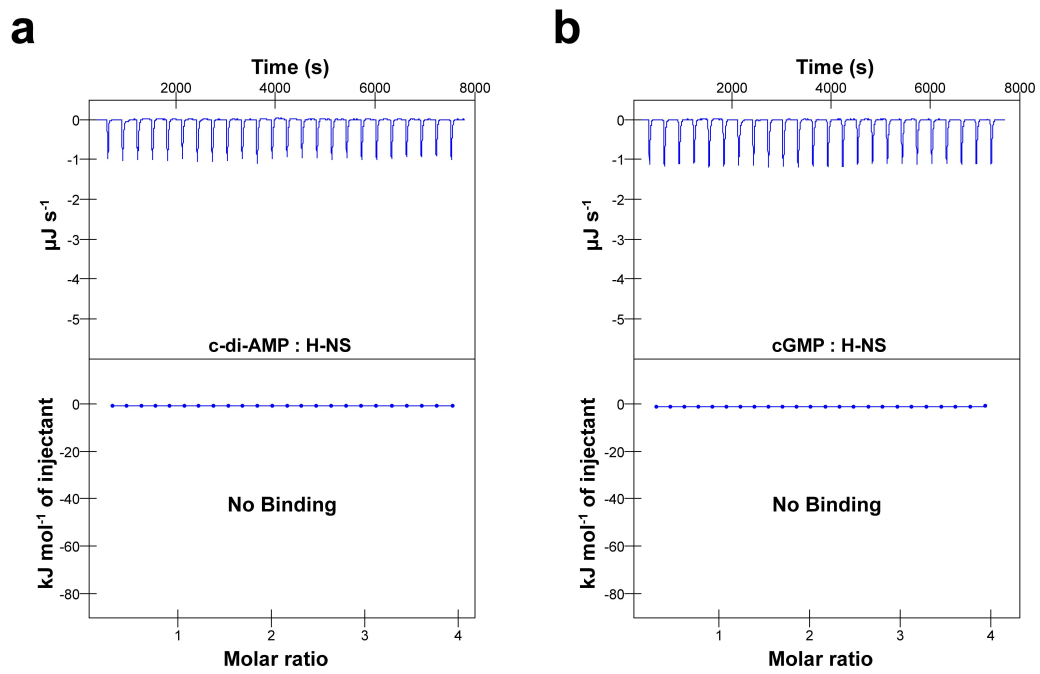
Supplementary Fig. 5. T6SS gene expression in *S.* Typhimurium wild type, mutants, and the corresponding complemented strains.

a, qRT-PCR analysis of T6SS gene expression in *S.* Typhimurium wild type, the Δ*fur* mutant and its complemented strain. **b,** qRT-PCR analysis of T6SS gene expression in *S.* Typhimurium wild type, the Δ*hns* mutant and its complemented strain. Data are mean ± SD of three biological replicates. ****$P < 0.0001$; NS, not significant (Student's *t* test).



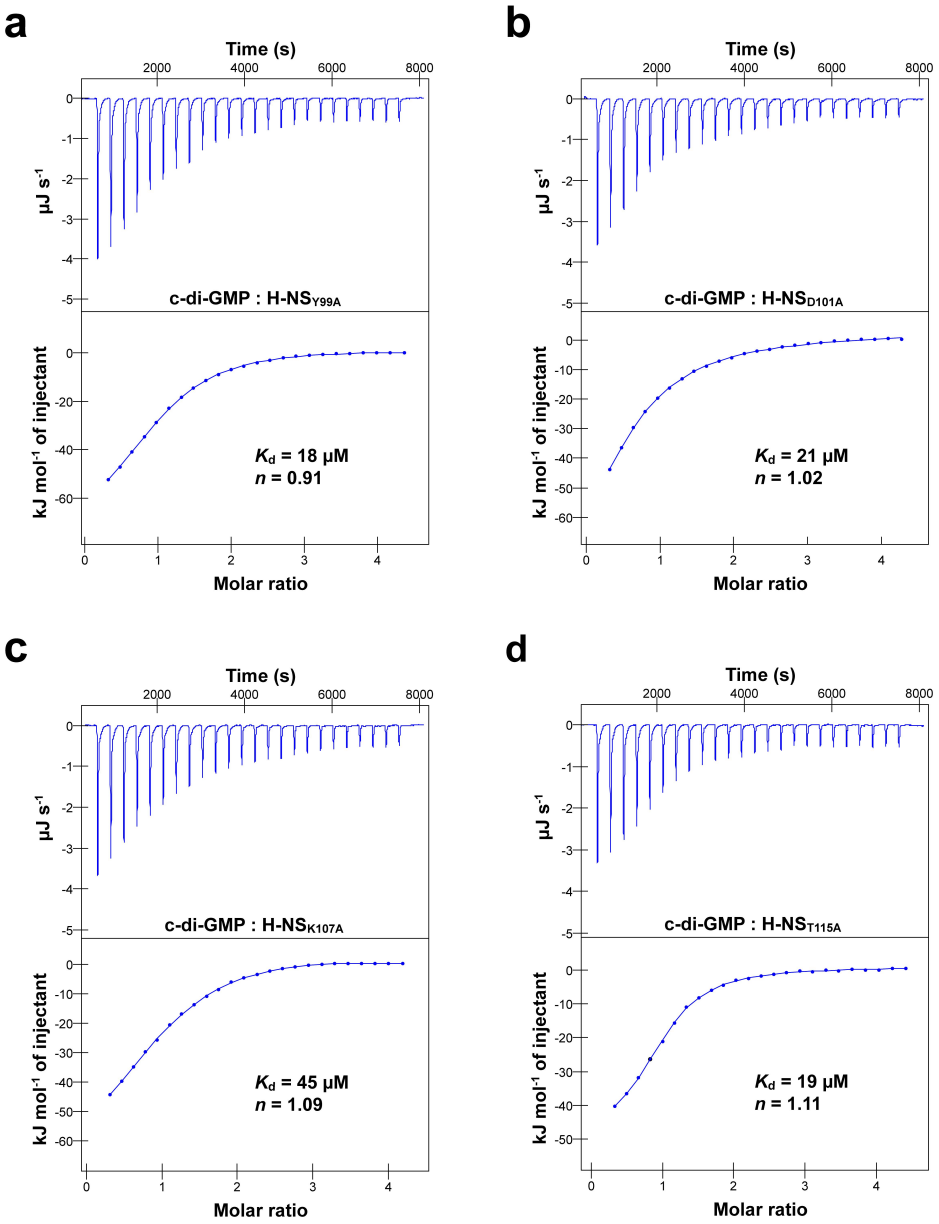
Supplementary Fig. 6. The production of H-NS in *S.* Typhimurium wild type is not affected by overexpression of *adrA* or *STM3611*.

The plasmid pBBR1MCS1 and its derivatives harboring *adrA* or *STM3611* were separately transformed into the wild-type strain of *S.* Typhimurium expressing *in situ* tagged FLAG–H-NS, and the production of H-NS in cells were assessed by Western blot analysis with anti-FLAG antibody. The blots shown are representative of three independent experiments with similar results. The band intensities were quantified by scanning densitometry using ImageJ (NIH, USA), normalized to intracellular ICDH, and presented as values relative to that of the wild type without gene overexpression (mean ± SD; *n* = 3 independent experiments). NS, not significant (Student's *t* test).



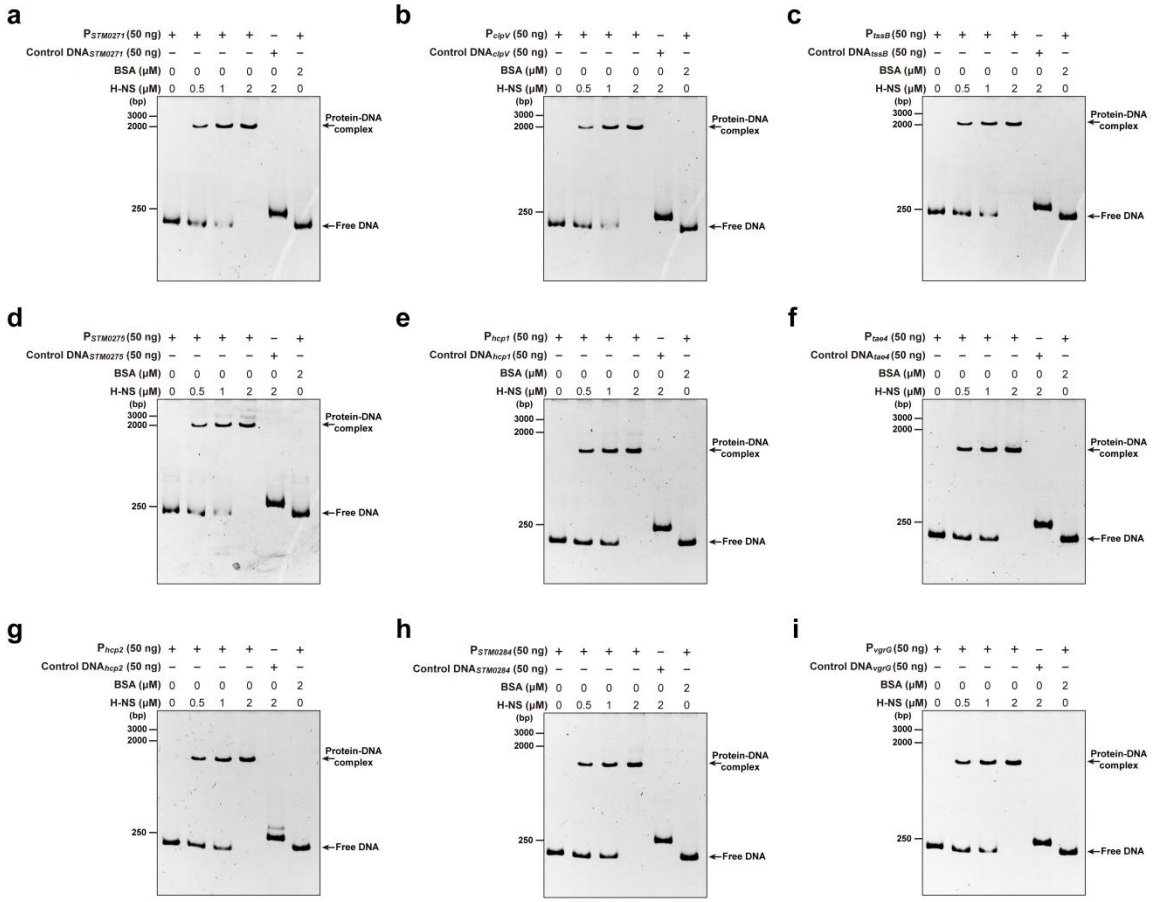
Supplementary Fig. 7. Isotherms representing binding of the H-NS protein of *S.* Typhimurium with c-di-AMP or cGMP, as measured by ITC.

a, b, ITC data and plots of injected heat for injections of 100 µM c-di-AMP (**a**) or cGMP (**b**) into the sample cell containing 10 µM H-NS are shown in the upper and lower plots, respectively. The heats of ligand dilution were subtracted and the corrected data were fit to a one-site binding model by using the NanoAnalyze software. Isotherms shown are one representative of three independent experiments with similar results.



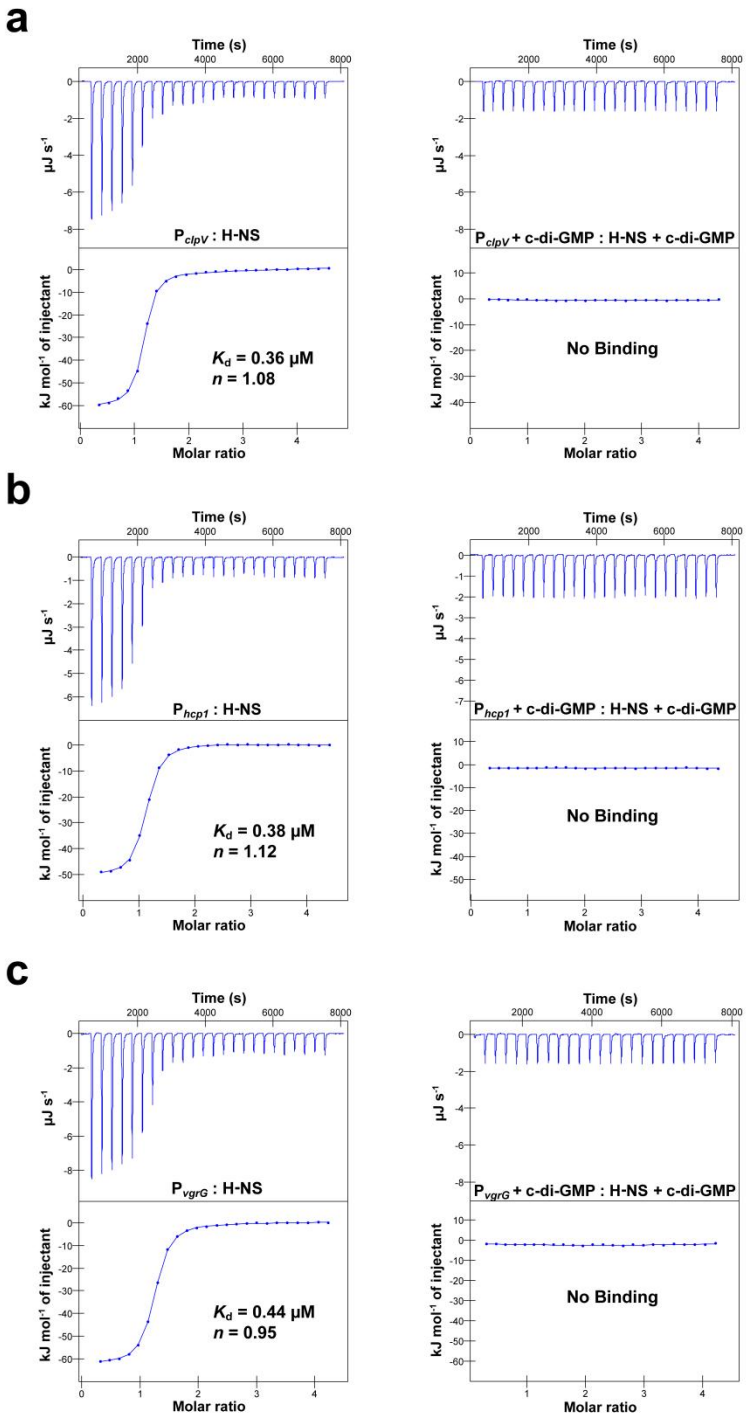
Supplementary Fig. 8. Four mutants of H-NS show lower binding affinity to c-di-GMP compared with the wild type.

a-d, The binding affinity was evaluated by ITC analysis. ITC data and plots of injected heat for injections of 100 µM c-di-GMP into the sample cell containing 10 µM Y99A (**a**), D101A (**b**), K107A (**c**) or T115A (**d**) mutants of H-NS are shown in the upper and lower plots, respectively. The binding curves were corrected for the dilution effects in the final analysis. Data shown are one representative of three independent experiments with similar results. The K_d and complex stoichiometry (*n*) values are indicated.



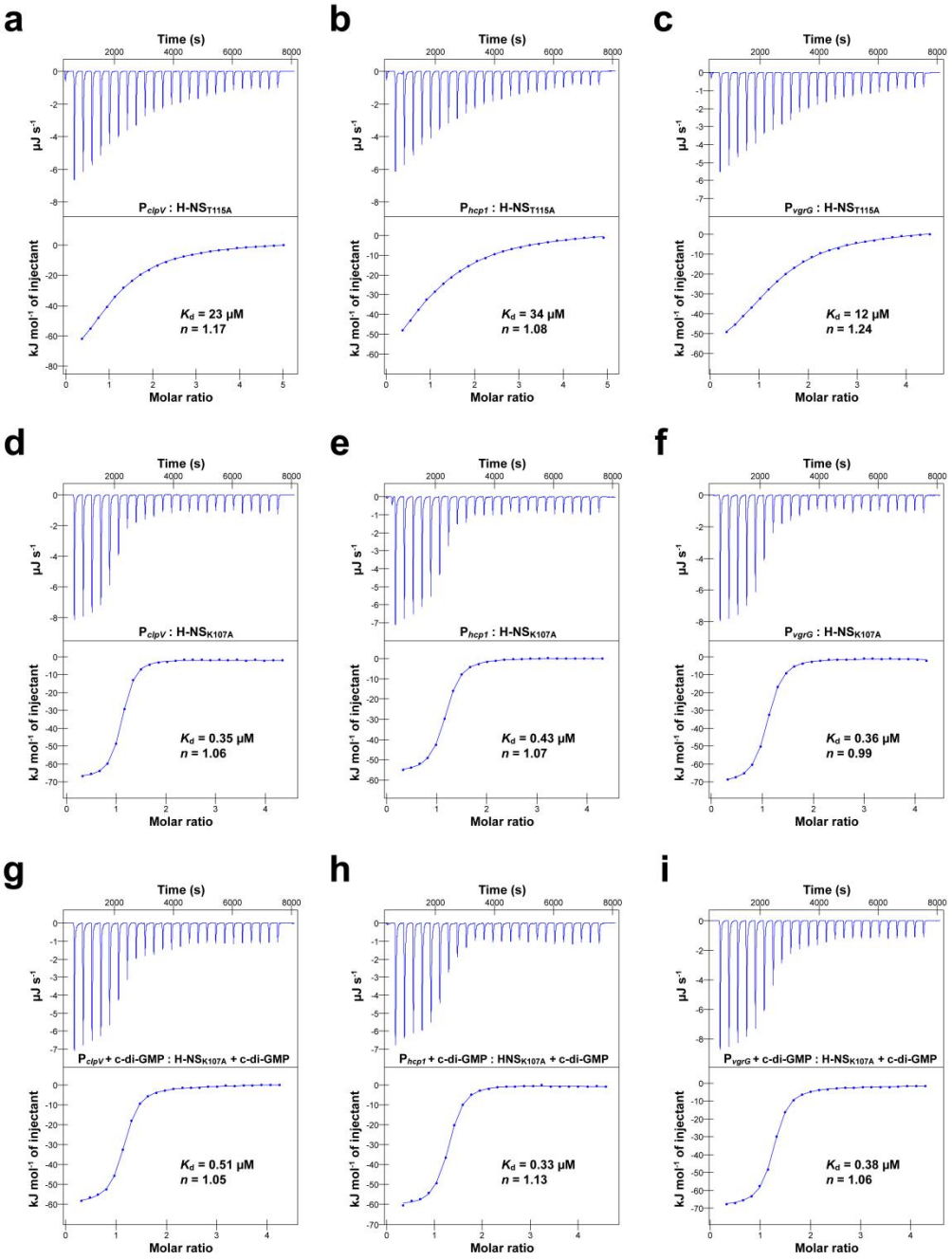
Supplementary Fig. 9. EMSAs demonstrating specificity of H-NS binding to promoters of T6SS genes.

a-i, 50 ng of $P_{STM0271}$ (**a**), P_{clpV} (**b**), P_{tssB} (**c**), $P_{STM0275}$ (**d**), P_{hcp1} (**e**), P_{tae4} (**f**), P_{hcp2} (**g**), $P_{STM0284}$ (**h**) or P_{vgrG} (**i**) DNA (the average AT content of each promoter sequence is above 62%) was incubated with H-NS (the concentrations are noted in the panel) in a 20-μl reaction system. DNA fragments amplified from the coding regions of the corresponding genes were used as DNA negative controls (the average AT content of each control sequence is below 44%) and BSA was used as the protein control. Gels shown are one representative of three independent experiments with similar results.



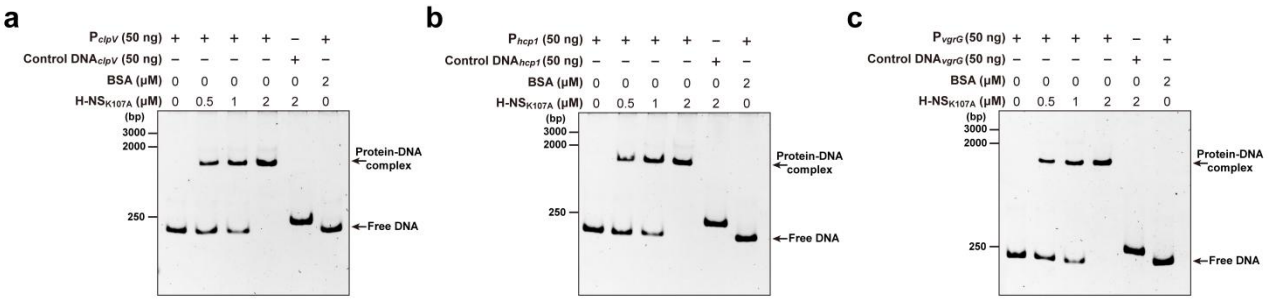
Supplementary Fig. 10. ITC analysis of H-NS binding to promoters of *clpV*, *hcp1* and *vgrG* in the absence or presence of c-di-GMP.

a-c, ITC data and plots of injected heat for injections of 100 µM promoter sequences of *clpV* (**a**), *hcp1* (**b**) and *vgrG* (**c**) into the sample cell containing 10 µM H-NS in the absence or presence of 50 µM c-di-GMP are shown in the upper and lower plots, respectively. ITC Data shown are one representative from three independent experiments with similar results. The K_d and complex stoichiometry (*n*) values are indicated.



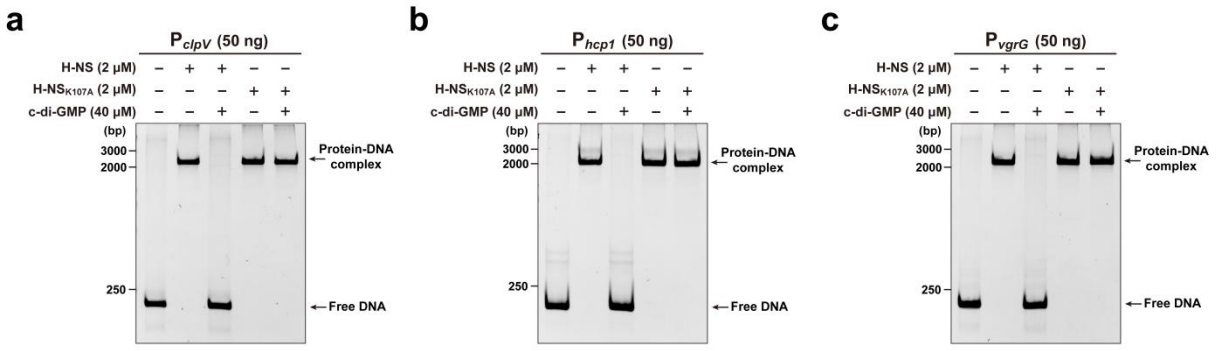
Supplementary Fig. 11. ITC analysis of the binding of variants of H-NS with the T6SS promoters in the presence and absence of c-di-GMP.

a-i, ITC data and plots of injected heat for injections of 100 μM promoter regions of *clpV* (**a, d, g**), *hcp1* (**b, e, h**), and *vgrG* (**c, f, i**) into the sample cell containing 10 μM H-NS$_{T115A}$ (**a-c**) or H-NS$_{K107A}$ (**d-i**) in the absence (**a-f**) or presence (**g-i**) of 50 μM c-di-GMP are shown in the upper and lower plots, respectively. The binding curves were corrected for the dilution effects and fit to a one-site binding model. Isotherms shown are one representative of three independent experiments with similar results. K_d and complex stoichiometry (n) are indicated.



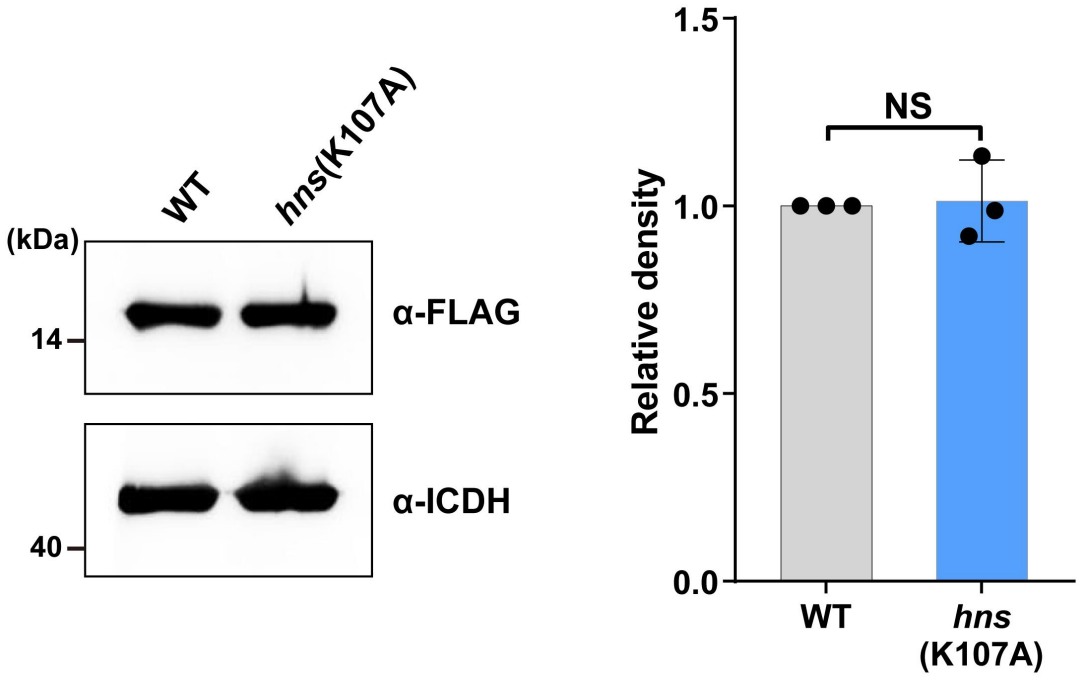
Supplementary Fig. 12. EMSAs demonstrating specificity of H-NS_{K107A} binding to promoters of T6SS genes.

a-c, 50 ng of P_{clpV} (**a**), P_{hcp1} (**b**) or P_{vgrG} (**c**) DNA was incubated with H-NS_{K107A} (the concentrations are noted in the panel) in a 20-μl reaction system. DNA fragments amplified from the coding regions of the corresponding genes were used as DNA negative controls and BSA was used as the protein control. Gels shown are one representative of three independent experiments with similar results.



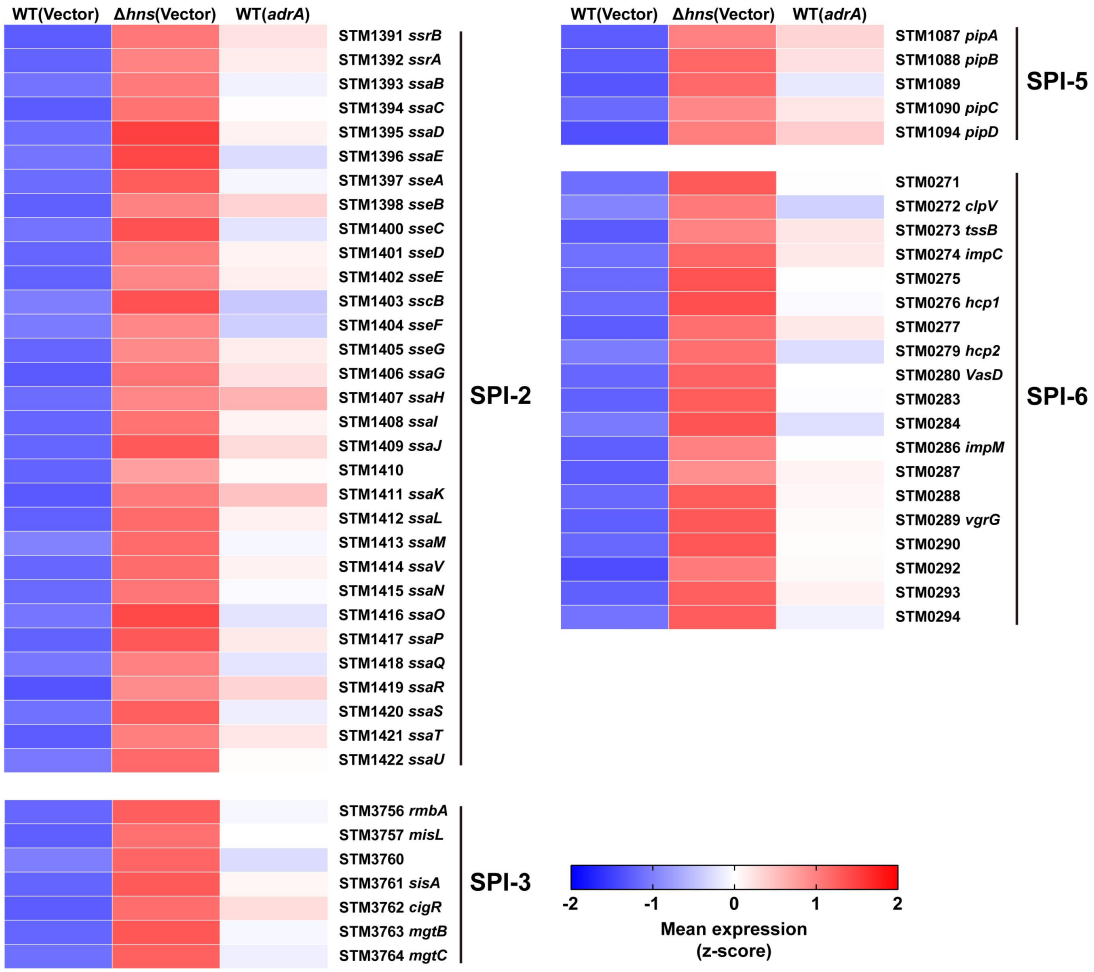
Supplementary Fig. 13. EMSAs for binding of H-NS or H-NS$_{K107A}$ to promoters of T6SS genes in the absence or presence of nucleotides.

a-c, 50 ng of P$_{clpV}$ (**a**), P$_{hcp1}$ (**b**) or P$_{vgrG}$ (**c**) DNA was incubated with H-NS or H-NS$_{K107A}$ in a 20-μl reaction system. c-di-GMP was supplemented at the indicated concentrations for competitive binding analysis. Gels shown are one representative of three independent experiments with similar results.

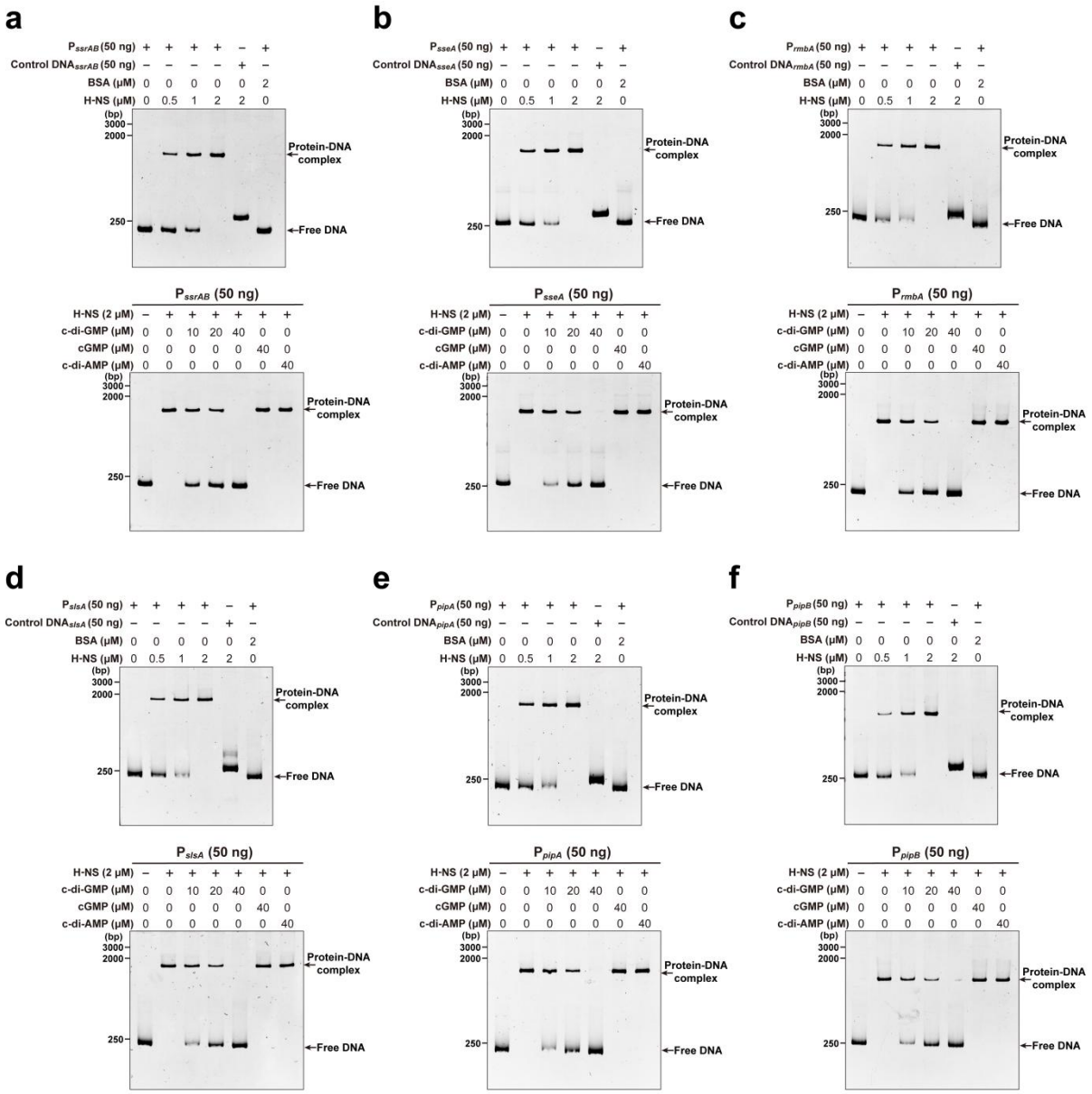


Supplementary Fig. 14. The K107A substitution in H-NS did not affect the abundance of the protein in *S.* Typhimurium.

Western blot analysis of intracellular accumulation of H-NS and H-NS_{K107A} in the wild type and the *hns*(K107A) mutant with a FLAG tag fused to the N terminus of the *hns/hns*(K107A) gene in the chromosome. The blots shown are representative of three independent experiments with similar results. The band intensities were quantified by scanning densitometry using ImageJ (NIH, USA), normalized to intracellular ICDH, and presented as values relative to that of the wild type (mean ± SD; *n* = 3 independent experiments). NS, not significant (Student's *t* test).

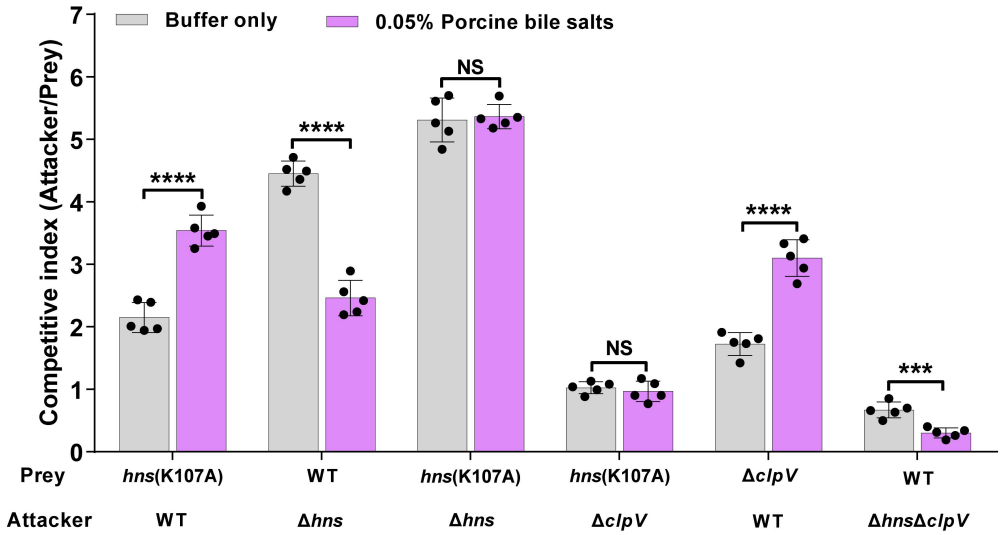


Supplementary Fig. 15. Heatmap showing z-scores of normalized, $\log_2$-transformed and scaled expression of genes within SPIs upregulated in the Δ*hns* mutant without gene overexpression or the wild type overexpressing *adrA* compared to the wild type without gene overexpression in RNA-Seq data (*n* = 3 biological replicates). Red represents higher abundance, and blue represents lower abundance.



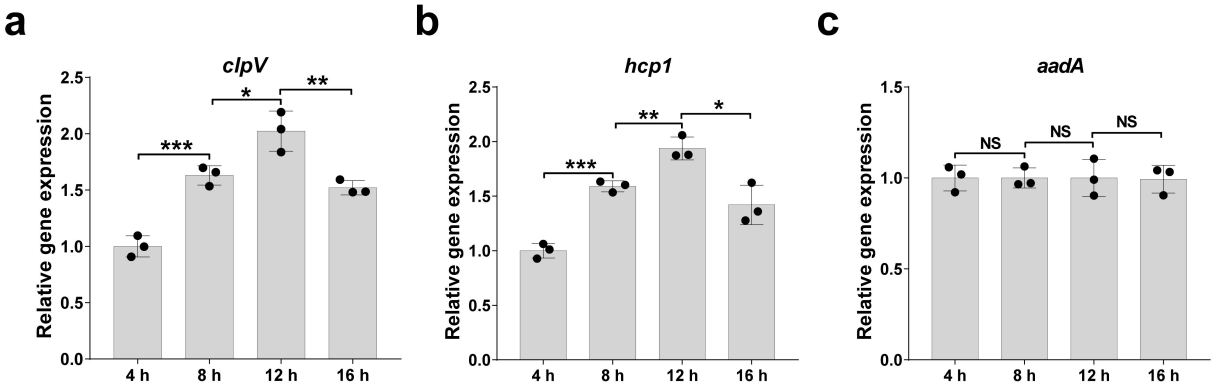
Supplementary Fig. 16. Specific binding of H-NS to the promoters of the target genes or operons within SPI-2, SPI-3 and SPI-5 is inhibited by c-di-GMP.

a-f, EMSA assays show that H-NS specifically binds to the promoter sequences of *ssrAB* (**a**), *sseA* (**b**), *rmbA* (**c**), *slsA* (**d**), *pipA* (**e**) or *pipB* (**f**) (top), and the binding is impaired by c-di-GMP in a dose-dependent fashion (bottom). DNA fragments amplified from the coding regions of the corresponding genes or operons were used as DNA negative controls and BSA was used as the protein control (**a-f**, top). The average AT content of each promoter sequence is above 56%, while the average AT content of each DNA negative control is below 45%. Gels shown are one representative of three independent experiments with similar results.



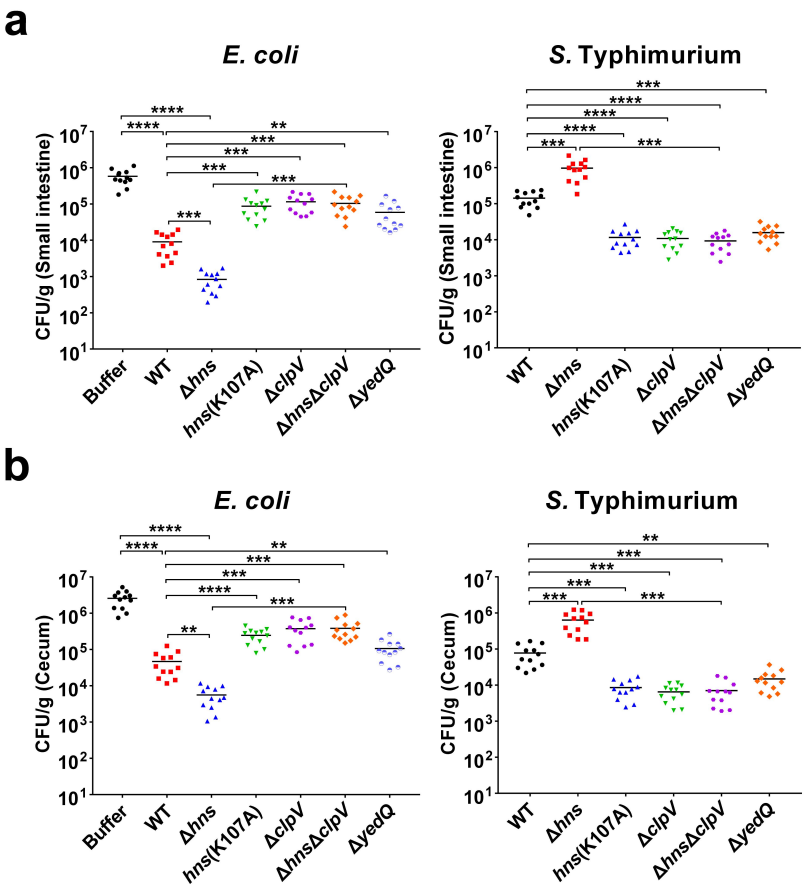
Supplementary Fig. 17. Bile salts increase the competitive ability of the wild-type strain, but not Δ*clpV* or Δ*hns*, against the *hns*(K107A) mutant.

The attacker carrying pBBR1MCS1 (chloramphenicol-resistant) and prey carrying pKT100 (kanamycin-resistant) *S*. Typhimurium strains were mixed 1:1 and grown for 48 h on LB agar supplemented with 0.05% porcine bile salts at 37 °C. Competitive indexes were calculated based on CFU counts (mean ± SD; *n* = 5 biological replicates). Statistical significance was determined using the Student's *t* test. ***$P < 0.001$; ****$P < 0.0001$; NS, not significant.



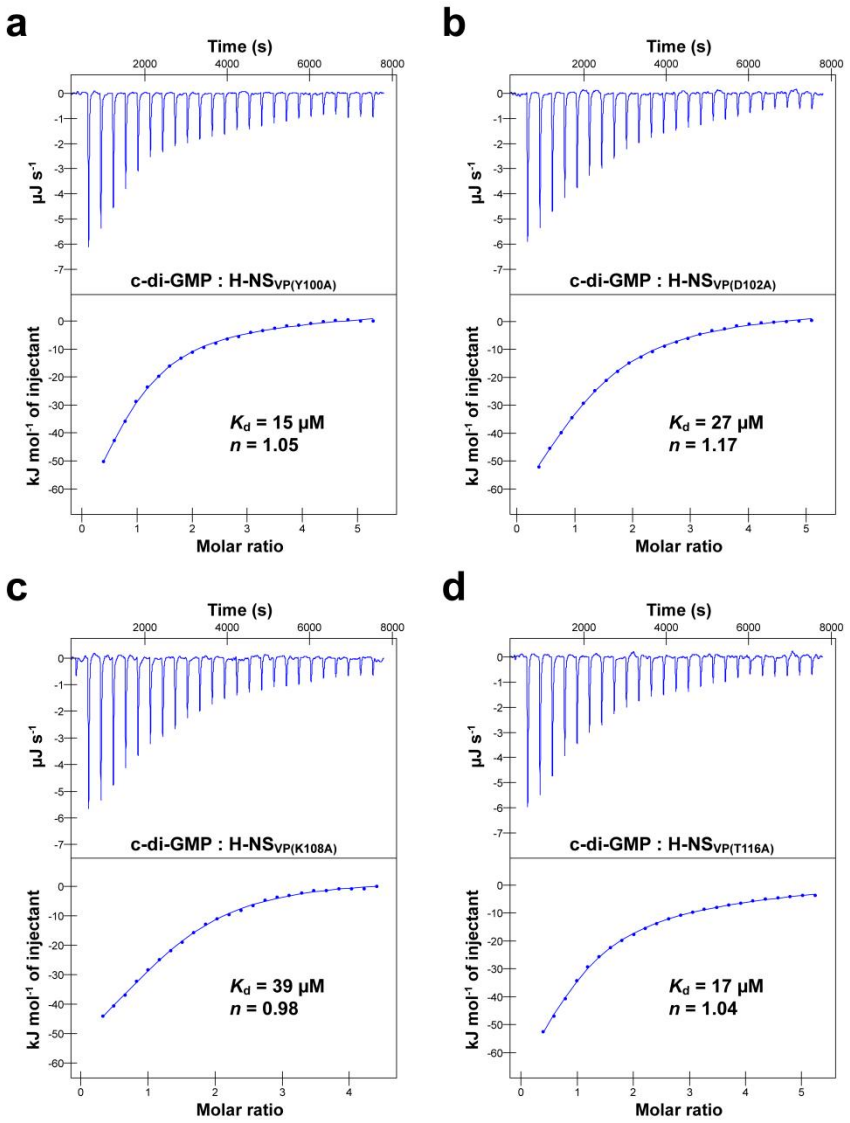
Supplementary Fig. 18. mRNA levels of the *clpV*, *hcp1* and *aadA* genes in *S.* Typhimurium wild type during its growth period.

a-c, *S*. Typhimurium SL1344 was grown in LB medium with shaking at 37 °C. After incubation for 4, 8, 12, 16 h, cultures were harvested for total RNA extraction. Expression of the *clpV* (**a**), *hcp1* (**b**) and *aadA* (**c**) genes was normalized to 16S rRNA and reported as fold change relative to that at 4 h. Data are mean ± SD of three biological replicates. Statistical significance was evaluated by two-tailed unpaired Student's *t* test. **P* < 0.05, ***P* < 0.01;****P* < 0.001; NS, not significant.



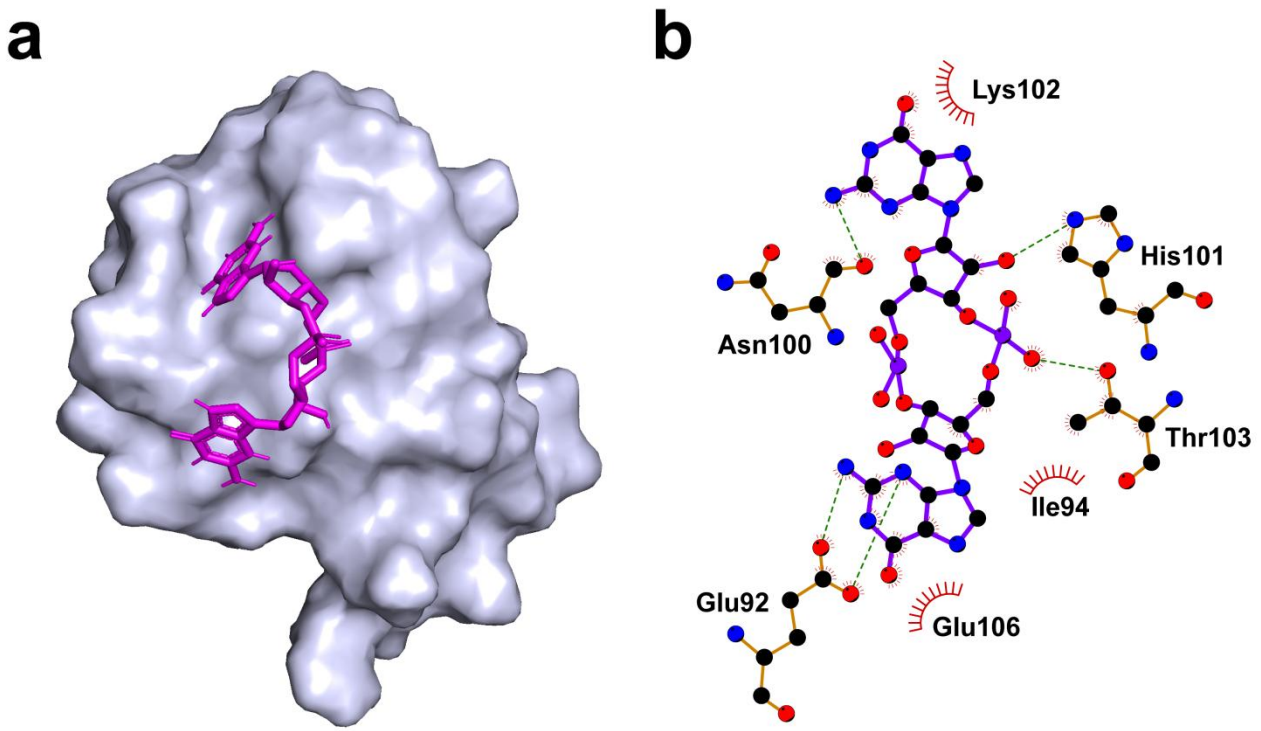
Supplementary Fig. 19. c-di-GMP–mediated derepression of the T6SS via targeting H-NS enhances the ability of *S.* Typhimurium to outcompete *E. coli* within the host gut.

a, b, Streptomycin-treated mice were infected orally with 5 × 10^8 CFU of *E. coli* for 24 h and then challenged with 5 × 10^8 CFU of one *S.* Typhimurium strain or a buffer control. Mice were sacrificed 48 h after the challenge, and surviving *E. coli* and *S.* Typhimurium in the small intestine (**a**) and cecum (**b**) were determined by CFU enumeration. Horizontal lines in *A* and *B* represent the geometric mean value for each group of mice (*n* = 12 mice per group). Statistical significance was evaluated by two-tailed Mann–Whitney *U* test in *A* and *B*. ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.



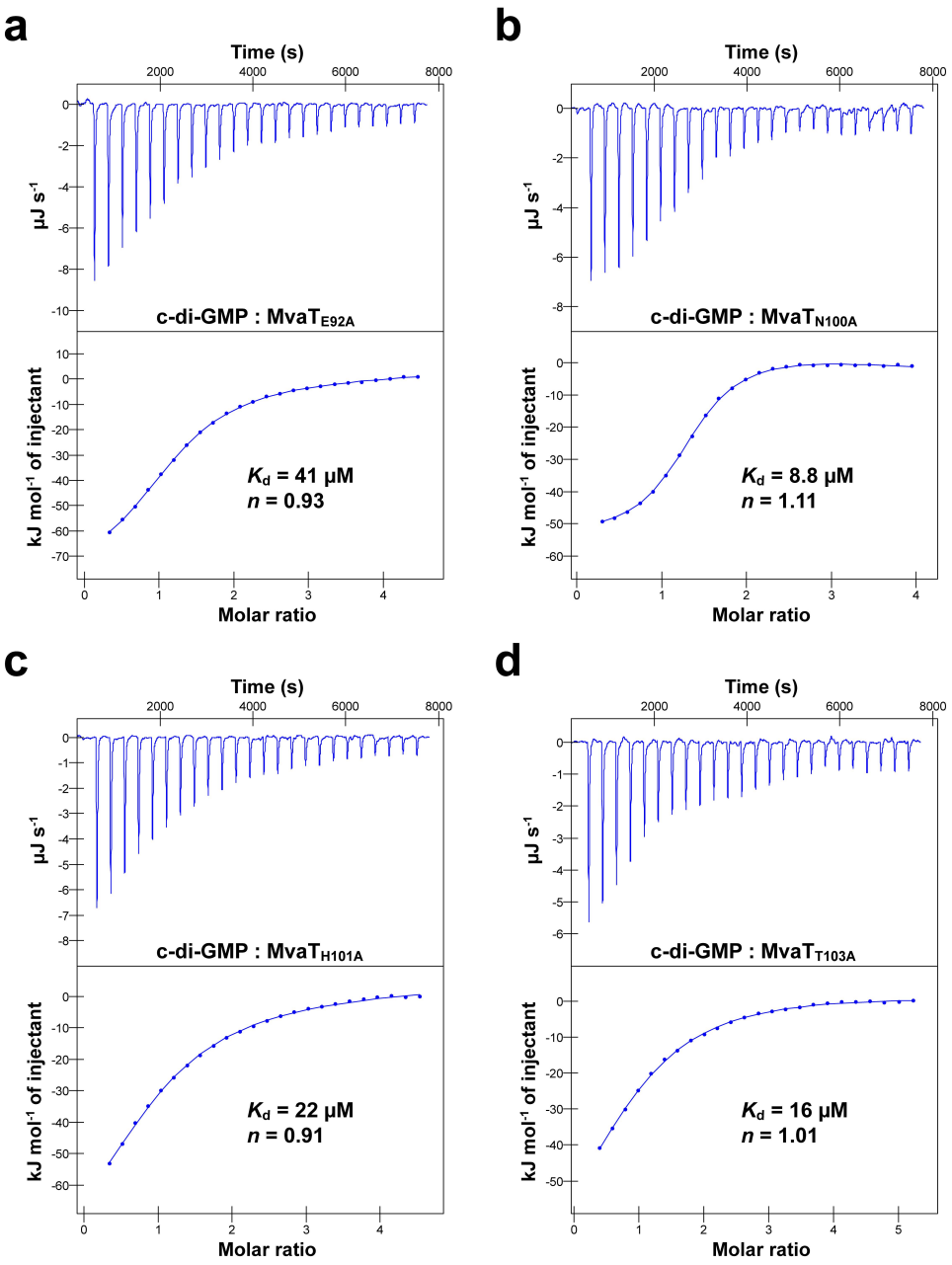
Supplementary Fig. 20. ITC analysis of the binding of variants of H-NS$_{VP}$ with c-di-GMP.

a-d, ITC data and plots of injected heat for injections of 100 µM c-di-GMP into the sample cell containing 10 µM Y100A (**a**), D102A (**b**), K108A (**c**) or T116A (**d**) mutants of H-NS$_{VP}$ are shown in the upper and lower plots, respectively. The binding curves were corrected for the dilution effects and fit to a one-site binding model. Isotherms shown are one representative of three independent experiments with similar results. K_d and complex stoichiometry (*n*) are presented.



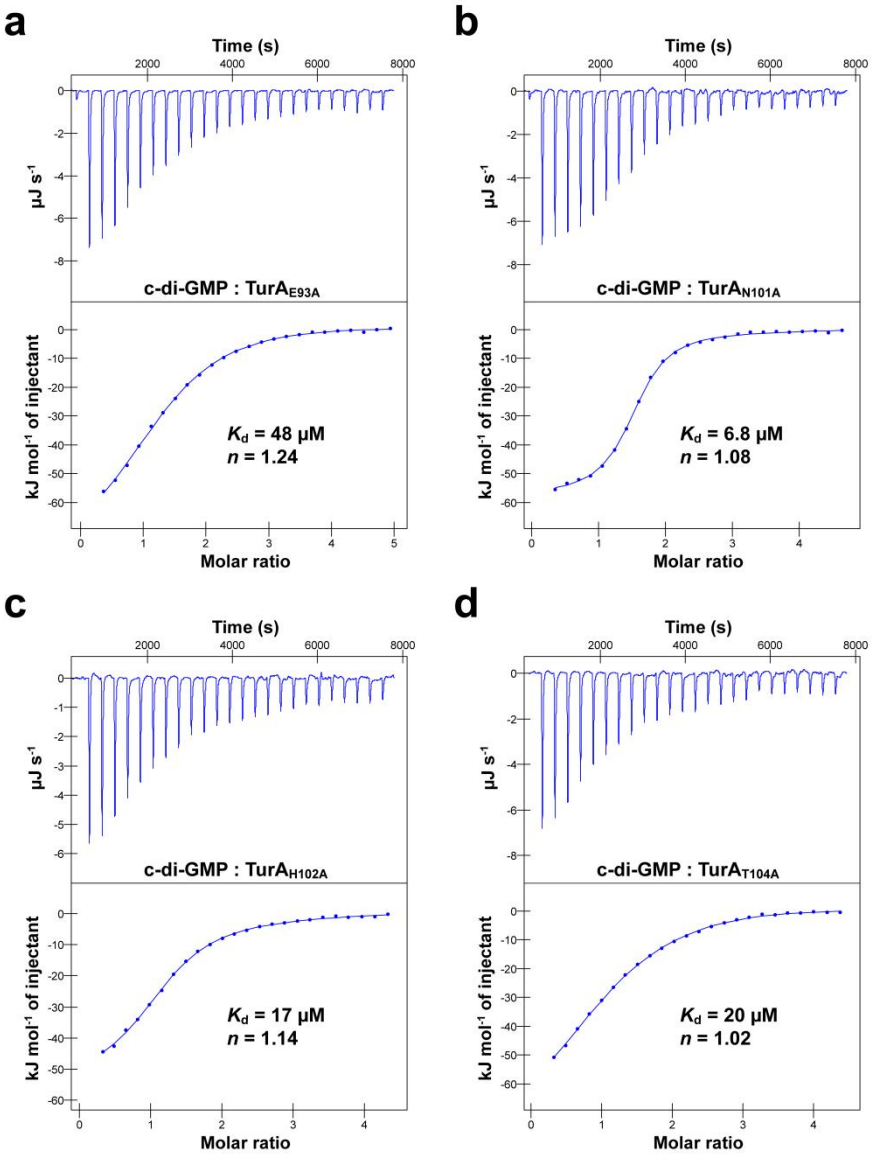
Supplementary Fig. 21. Docking analysis of the C-terminal domain of MvaT with c-di-GMP.

a, Surface representation of the structural model of the C-terminal domain of MvaT in complex with c-di-GMP. c-di-GMP is shown as purple sticks. **b,** Schematic of the predicted contacts between c-di-GMP and the C-terminal domain of MvaT. Potential hydrogen bonds are indicated as green dashed lines.



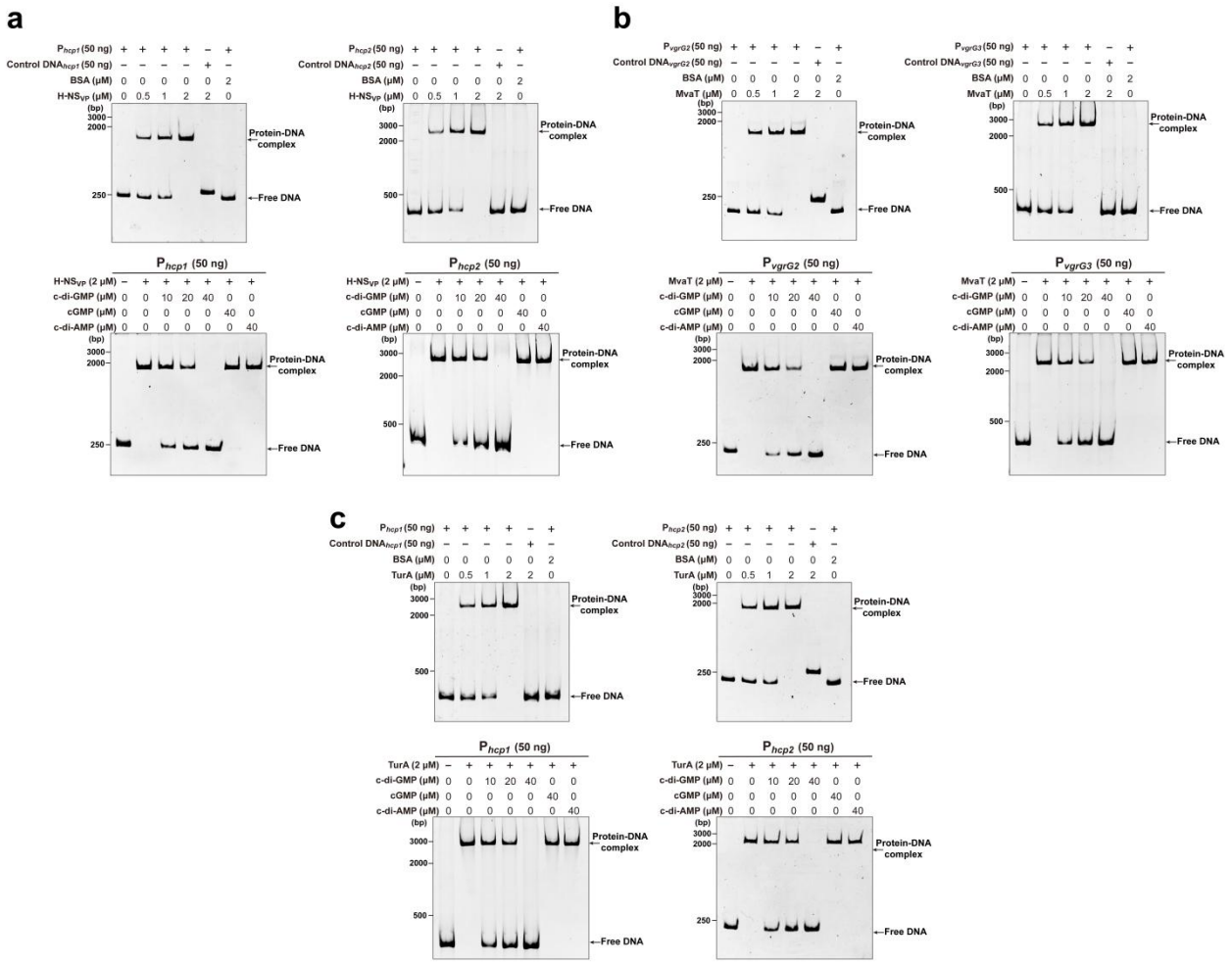
Supplementary Fig. 22. ITC analysis of the binding of variants of MvaT with c-di-GMP.

a-d, ITC data and plots of injected heat for injections of 100 µM c-di-GMP into the sample cell containing 10 µM E92A (**a**), N100A (**b**), H101A (**c**) or T103A (**d**) mutants of MvaT are shown in the upper and lower plots, respectively. The binding curves were corrected for the dilution effects and fit to a one-site binding model. Isotherms shown are one representative of three independent experiments with similar results. K_d and complex stoichiometry (*n*) are indicated.



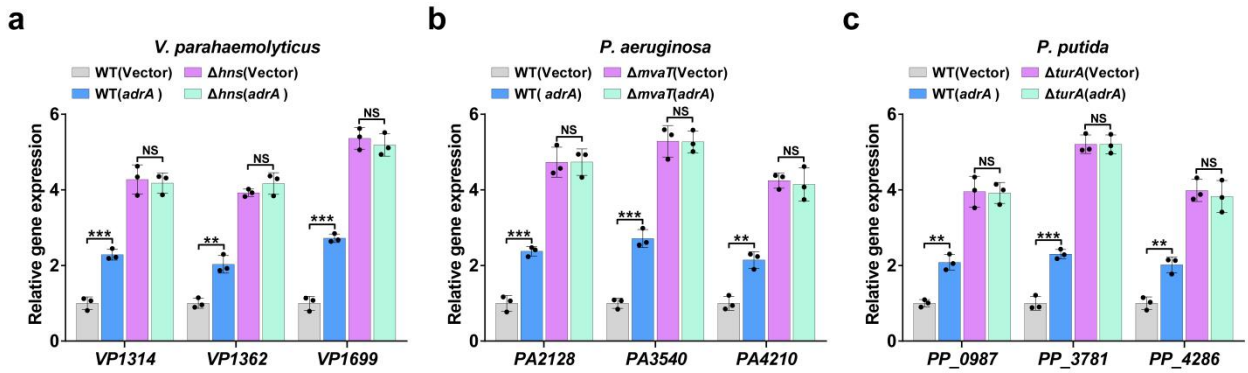
Supplementary Fig. 23. ITC analysis of the binding of variants of TurA with c-di-GMP.

a-d, ITC data and plots of injected heat for injections of 100 µM c-di-GMP into the sample cell containing 10 µM E93A (**a**), N101A (**b**), H102A (**c**) or T104A (**d**) mutants of TurA are shown in the upper and lower plots, respectively. The binding curves were corrected for the dilution effects and fit to a one-site binding model. Isotherms shown are one representative of three independent experiments with similar results. K_d and complex stoichiometry (n) are indicated.



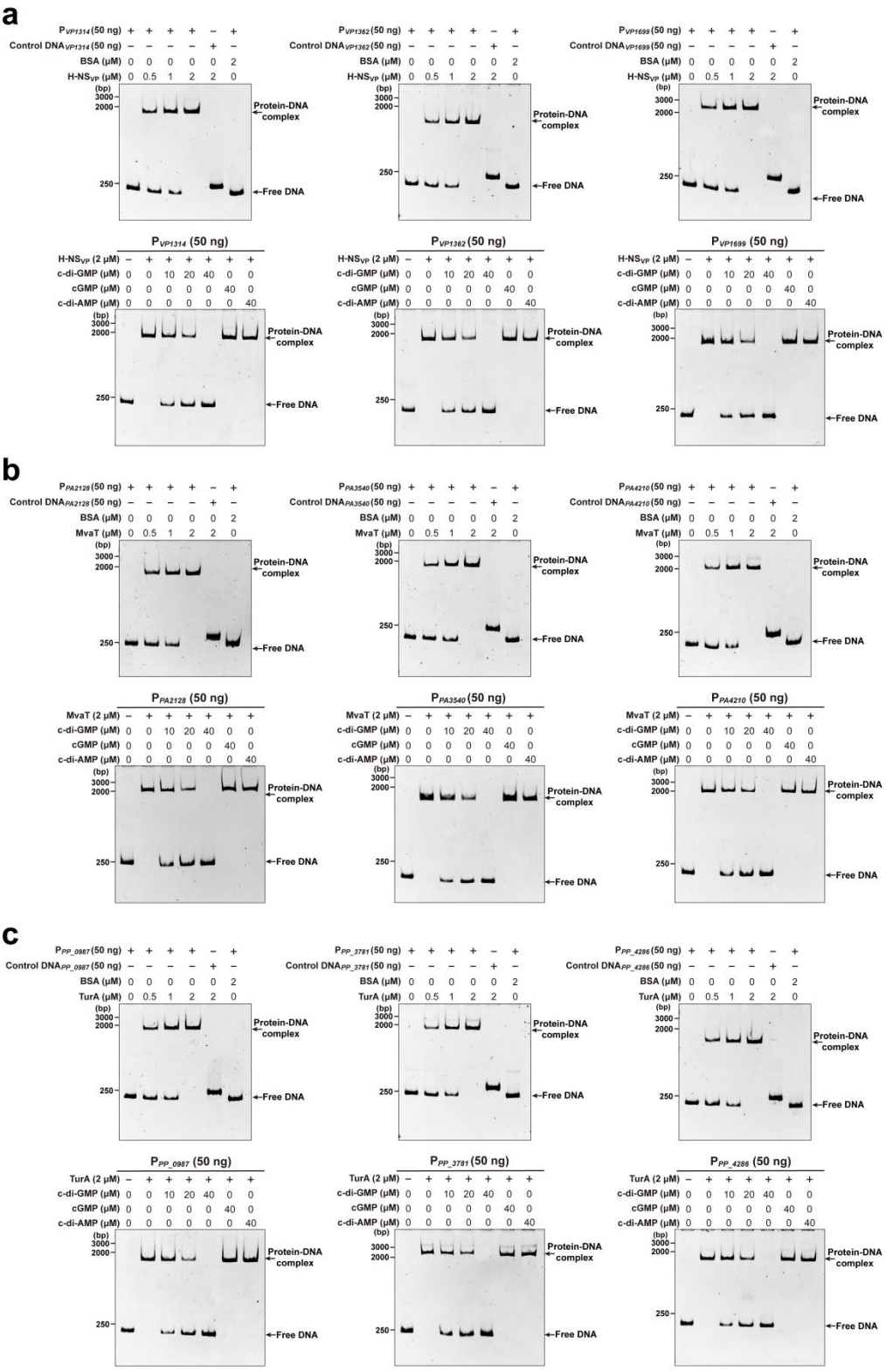
Supplementary Fig. 24. Specific binding of H-NS$_{VP}$, MvaT and TurA to promoters of T6SS genes is inhibited by c-di-GMP.

a-c, EMSA assays show that H-NS$_{VP}$ (**a**), MvaT (**b**) and TurA (**c**) specifically binds to the promoter sequences of *hcp1* (*VP1393*) and *hcp2* (*VPA1027*) from *V. parahaemolyticus* (**a**), *vgrG2* (*PA1511*) and *vgrG3* (*PA2373*) from *P. aeruginosa* (**b**), and *hcp1* (*PP_2615*) and *hcp2* (*PP_4082*) from *P. putida* (**c**) (top), and the binding is impaired by c-di-GMP in a dose-dependent fashion (**a-c**, bottom). DNA fragments amplified from the coding regions of the corresponding genes were used as DNA negative controls and BSA was used as the protein control (**a-c**, top). The average AT content of each promoter sequence is above 54%, while the average AT content of each DNA negative control is below 45%. Gels shown are one representative of three independent experiments with similar results.



Supplementary Fig. 25. H-NS$_{VP}$–, MvaT- and TurA-repressed genes are significantly upregulated following ectopic expression of *adrA* in the wild type, but not in the H-NS– or H-NS-like–deficient mutant.

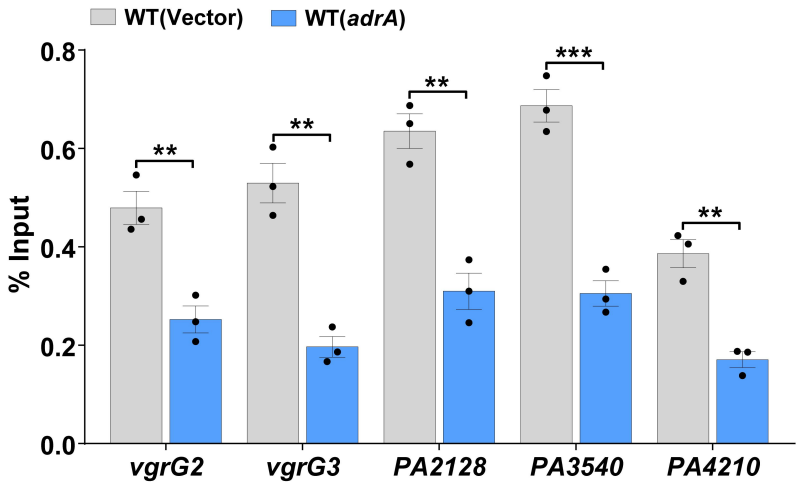
a-c, qRT-PCR analysis of gene expression in the wild-type strain and the H-NS– or H-NS-like–deficient mutant of *V. parahaemolyticus* (**a**)*, P. aeruginosa* (**b**) and *P. putida* (**c**) with or without expression of a plasmid-derived copy of *adrA.* Expression was normalized to 16S rRNA and reported as values relative to that of the wild type without *adrA* expression. Data shown in **a**, **b** and **c** are mean ± SD of three biological replicates. *P* values were determined using the two-tailed unpaired Student's *t* test. ***P* < 0.01; ****P* < 0.001; NS, not significant.



Supplementary Fig. 26. Specific binding of H-NS$_{VP}$, MvaT and TurA to the promoters of the target genes is inhibited by c-di-GMP.

a-c, EMSA assays show that H-NS$_{VP}$ (**a**), MvaT (**b**) and TurA (**c**) specifically binds to the promoter sequences of their target genes (**a-c,** top), and the binding is impaired by c-di-GMP in a dose-dependent fashion (**a-c,** bottom). DNA fragments amplified from the

coding regions of the corresponding genes were used as DNA negative controls and BSA was used as the protein control (**a-c,** top). The average AT content of each promoter sequence is above 55%, while the average AT content of each DNA negative control is below 45%. Gels shown are one representative of three independent experiments with similar results.



Supplementary Fig. 27. Ectopic expression of *adrA* in *P. aeruginosa* significantly reduces the occupancy of FLAG-MvaT at the promoter regions of the target genes. ChIP-qPCR quantifying binding of FLAG-MvaT at the promoters of the indicated genes in the wild-type strain of *P. aeruginosa* with or without expression of a plasmid-derived copy of *adrA*. The ChIP-qPCR signals were normalized to their respective DNA inputs. Data are mean ± SD of three biological replicates. *P* values were determined using the two-tailed unpaired Student's *t* test. $^{**}P < 0.01$; $^{***}P < 0.001$.

Fig. 1a

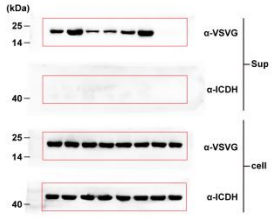


Fig. 1b

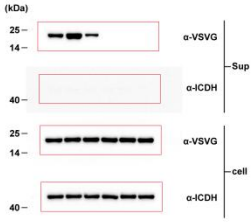


Fig. 3a

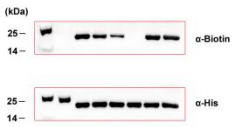


Fig. 4a

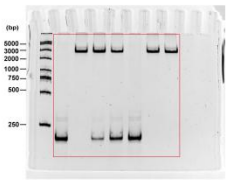


Fig. 4b

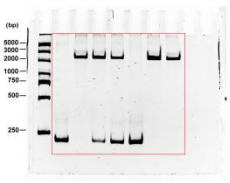


Fig. 4c

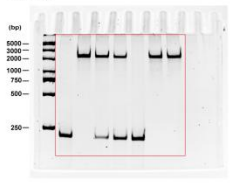


Fig. 4d

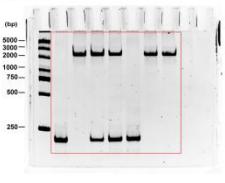


Fig. 8a

Fig. S2

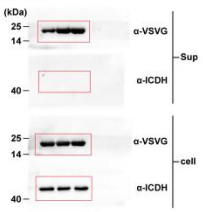


Fig. S6

Fig. S9a

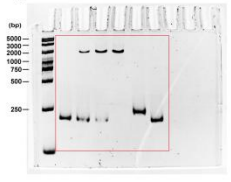


Fig. S9b

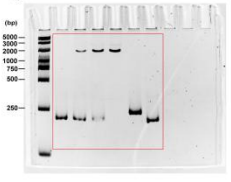


Fig. S9c

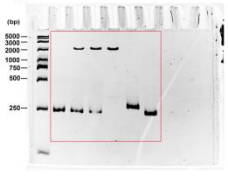


Fig. S9d

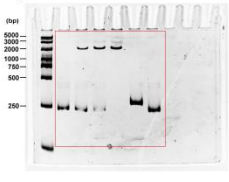


Fig. S9e

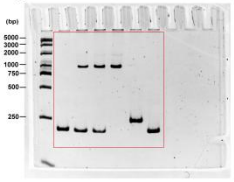


Fig. S9f

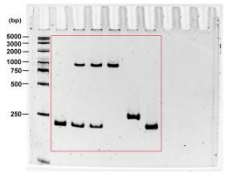


Fig. S9g

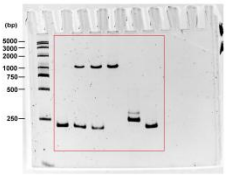


Fig. S9h

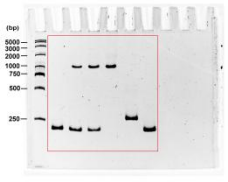


Fig. S9i

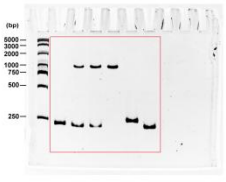


Fig. S12a

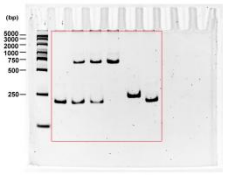


Fig. S12b

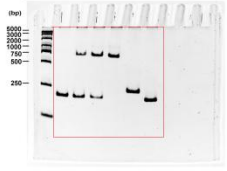


Fig. S12c

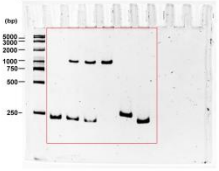


Fig. S13a

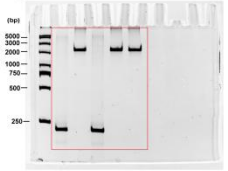


Fig. S13b

Fig. S13c

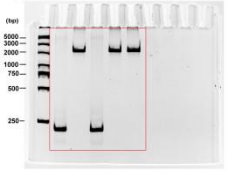


Fig. S14

Fig. S16a-1

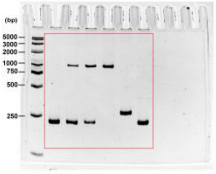


Fig. S16a-2

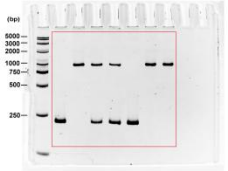


Fig. S16b-1

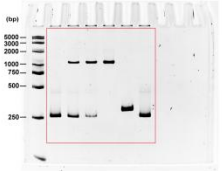


Fig. S16b-2

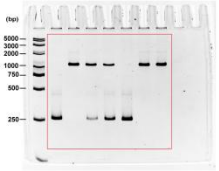


Fig. S16c-1

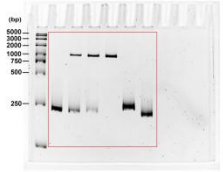
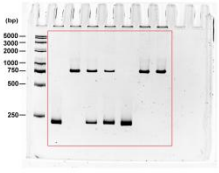
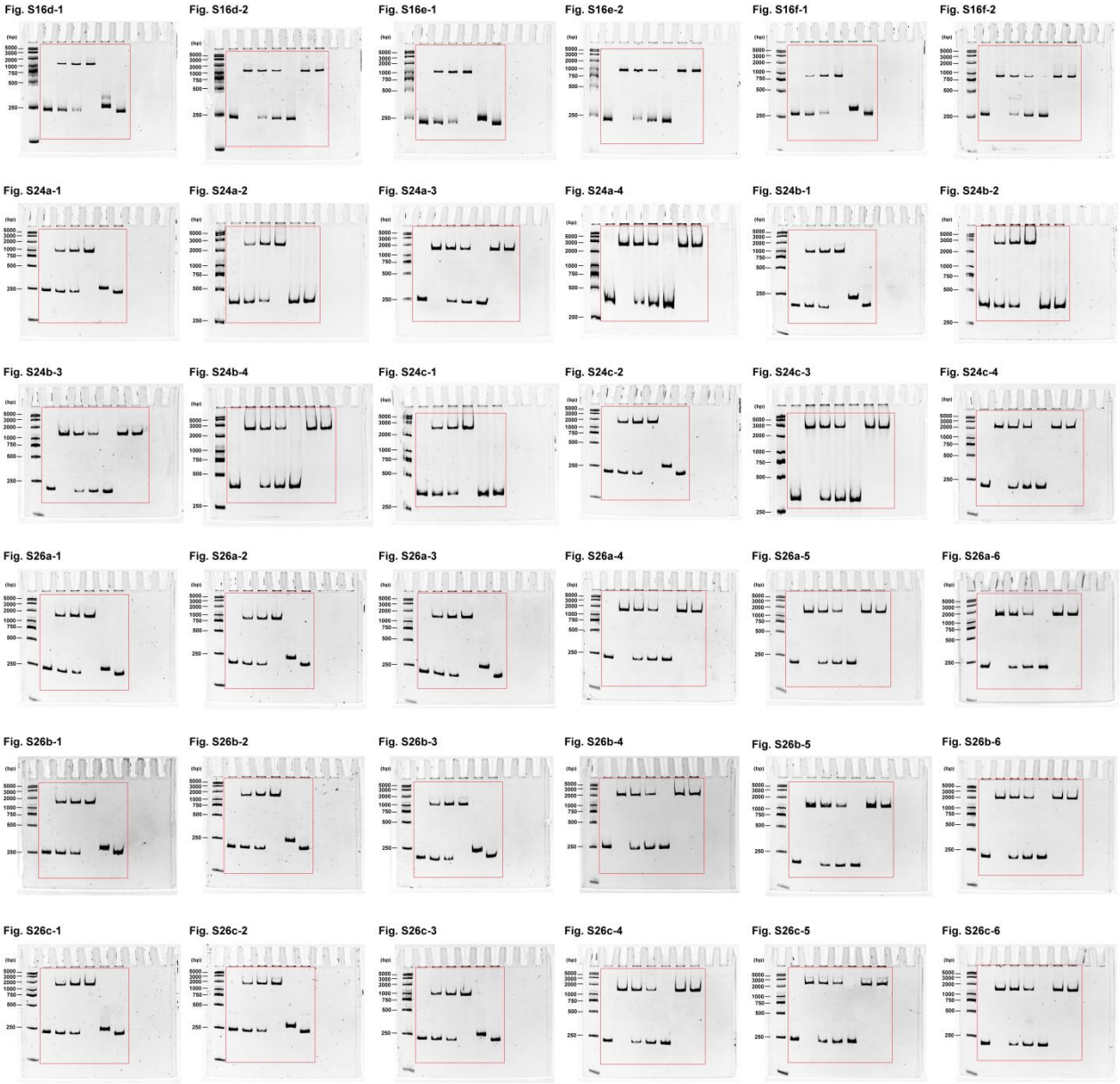


Fig. S16c-2





Supplementary Fig. 28. Uncropped versions of Fig. 1a, b, 3a, 4a-d, 8a, S2, S6, S9a-i, S12a-c, S13a-c, S14, S16a-f, S24a-c and S26a-c.

Supplementary Tables

Table S1. Bacterial strains and plasmids used in this study.

Strains and plasmids	Relevant characteristics*	Source
Strains		
***S.* Typhimurium**		
SL1344	Wild-type	Laboratory stock
Δ*yedQ*	*yedQ* deletion mutant in SL1344	1
Δ*fur*	*fur* deletion mutant in SL1344	This study
Δ*hns*	*hns* deletion mutant in SL1344	This study
Δ*clpV*	*clpV* double deletion mutant in SL1344	This study
Δ*hns*Δ*clpV*	*hns*/*clpV* double deletion mutant in SL1344	This study
hns(K107A)	SL1344 Wild-type strain with the K107A mutation *of hns*	This study
FLAG-*hns*	SL1344 Wild-type strain expressing in *situ* tagged FLAG–H-NS	This study
FLAG-hns_{K107A}	SL1344 Wild-type strain expressing in *situ* tagged FLAG–H-NS_{K107A}	This study
E. coli		
BL21(DE3)	Host for expression vector pET-28a	Novagen
TG1	Host for cloning	Stratagene
S17-1λpir	λ-pir lysogen of S17-1, F^- *thi pro hsdR* [RP4-2 Tc::Mu Km::Tn7 (Tp Sm)]	2
P. aeruginosa		
PAO1	Wild-type	Laboratory stock
Δ*mvaT*	*mvaT* deletion mutant in PAO1	This study
FLAG-*mvaT*	PAO1 Wild-type strain expressing in *situ* tagged FLAG-MvaT	This study
P. putida		
KT2440	Wild-type	Laboratory stock
Δ*turA*	*turA* deletion mutant in KT2440	This study
V. parahaemolyticus		
V. parahaemolyticus RIMD 2210633	Wild-type	Laboratory stock
Δhns_{vp}	*hns* deletion mutant in *V. parahaemolyticus* RIMD 2210633	This study
Plasmids		
pCas	Crispr-Cas9 system plasmid used for in-frame deletion, Km^r	3
pTargetF1	pTargetF with the spectinomycin resistance gene replaced by a chloramphenicol resistance gene, Cm^r	4
pTargetF1-Δ*yedQ*	pTargetF1 derivative for *yedQ* deletion in SL1344	This study
pTargetF1-Δ*fur*	pTargetF1 derivative for *fur* deletion in SL1344	This study
pTargetF1-Δ*hns*	pTargetF1 derivative for *hns* deletion in SL1344	This study
pTargetF1-Δ*clpV*	pTargetF1 derivative for *clpV* deletion in SL1344	This study

pTargetF1-*hns*(K107A)	pTargetF1 derivative for the K107A mutation *of hns* in SL1344	This study
pTargetF1–FLAG-*hns*	pTargetF1 derivative for in *situ* tagged FLAG–H-NS and FLAG–H-NS$_{K107A}$ in SL1344	This study
pK18*mobsacB*	*sacB*-based gene replacement vector; Km^r	5
pK18-Δ*mvaT*	pK18 derivative for *mvaT* deletion in PAO1	This study
pK18-Δ*turA*	pK18 derivative for *turA* deletion in KT2440	This study
pK18–FLAG-*mvaT*	pK18 derivative for in *situ* tagged FLAG-MvaT in PAO1	This study
pDM4	Suicide vector, *mob*RK2, *ori*R6K, *pir*, *sacB*, Cm^r	6
pDM4-Δ*hns$_{vp}$*	pDM4 derivative for *hns* deletion in *V. parahaemolyticus* RIMD 2210633	This study
pDM4-P$_{clpV}$::lacZ	pDM4 derivative for *clpV* promoter fusion	This study
pDM4-P$_{hcp1}$::lacZ	pDM4 derivative for *hcp1* promoter fusion	This study
pDM4-P$_{tae4}$::lacZ	pDM4 derivative for *tae4* promoter fusion	This study
pDM4-P$_{vgrG}$::lacZ	pDM4 derivative for *vgrG* promoter fusion	This study
pKT100	Cloning vector, p15A replicon, Km^r	7
pKT100-*hcp1*-VSVG	pKT100 expressing *hcp1* with a C-terminal VSVG tag	This study
pBBR1MCS1	Cloning vector containing REP, Cm^r	8
pBBR1MCS1-*hns*	*hns* cloned into pBBR1MCS1 for complementation	This study
pBBR1MCS1-*fur*	*fur* cloned into pBBR1MCS1 for complementation	This study
pBBR1MCS1-*adrA*	pBBR1MCS1 expressing *adrA*	This study
pBBR1MCS1-*STM3611*	pBBR1MCS1 expressing *STM3611*	This study
pBBR1MCS1-*yedQ*	pBBR1MCS1 expressing *yedQ*	This study
pBBR1MCS5	Cloning vector containing REP, Gm^r	8
pBBR1MCS5-*adrA*	pBBR1MCS5 expressing *adrA*	This study
pET-28a	Expression vector with N-terminal hexahistidine affinity tag, Km^r	Novagen
pET28a-*ycgR*	pET-28a expressing *ycgR*	This study
pET28a-*invF*	pET-28a expressing *invF*	1
pET28a-*hns*	pET-28a expressing *hns*	This study
pET28a-*hns$_{Ctd}$*	pET-28a expressing *hns$_{Ctd}$*	This study
pET28a-*hns*^{Y99A}	pET-28a expressing *hns*^{Y99A}	This study
pET28a-*hns*^{D101A}	pET-28a expressing *hns*^{D101A}	This study
pET28a-*hns*^{K107A}	pET-28a expressing *hns*^{K107A}	This study
pET28a-*hns*^{T115A}	pET-28a expressing *hns*^{T115A}	This study
pET28a-*mvaT*	pET-28a expressing *mvaT*	This study
pET28a-*turA*	pET-28a expressing *turA*	This study
pET28a-*hns$_{vp}$*	pET-28a expressing *hns$_{vp}$*	This study

***Km^r, Cm^r and Gm^r represent resistance to kanamycin, chloramphenicol and gentamicin, respectively.**

Table S2. Primers used in this study.

Primiers	5'-3' sequence*	
Δ*fur*-sg20-F	AGCTAGCTCAGTCCTAGGTATAATACTAGTCATCTTATC TGCCTTGATTGGTTTTAGAGCTAGAAATAGC	To generate pTargetF1-Δ*fur*
Δ*fur*-sg20-R	CCGAGTCGGTGCTTTTTTTGAAGCCACGATTGTGGGTC A	
Δ*fur*-up-F	TGCGGTATTGTTGTCAGTCA	
Δ*fur*-up-R	AAATCTTCCAGTTGCTTACCTTAAGTGCTTCGCTCATTG TAGTA	
Δ*fur*-down-F	TGACTGACAACAATACCGCATCACAGCCTCTATCTTTAC GG	
Δ*fur*-down-R	GGTAATAGATCTAAGCTTCTGCAGGTCGACCGGACATC GGCATAGTTTT	
Δ*hns*-sg20-F	AGCTAGCTCAGTCCTAGGTATAATACTAGTAAGCGCTG CTGCTGCTGAAGGTTTTAGAGCTAGAAATAGC	To generate pTargetF1-Δ*hns*
Δ*hns*-sg20-R	GGGTGATGATAAGCATTGTTGTTCAAAAAAAGCACCGA CTCGG	
Δ*hns*-up-F	CCGAGTCGGTGCTTTTTTTGAACAACAATGCTTATCATC ACCC	
Δ*hns*-up-R	AAATCTTCCAGTTGCTTACCTTAAGTGCTTCGCTCATTG TAGTA	
Δ*hns*-down-F	TACTACAATGAGCGAAGCACTTAAGGTAAGCAACTGGA AGATTT	
Δ*hns*-down-R	GGTAATAGATCTAAGCTTCTGCAGGTCGACTTGTCGGG CGTTATGTGT	
Δ*clpV*-sg20-F	AGCTAGCTCAGTCCTAGGTATAATACTAGTTGTTGCAGA TTGCCGAACCGGTTTTAGAGCTAGAAATAGC	To generate pTargetF1-Δ*clpV*
Δ*clpV*-sg20-R	GGGTGATGATAAGCATTGTTGTTCAAAAAAAGCACCGA CTCGG	
Δ*clpV*-up-F	CCGAGTCGGTGCTTTTTTTGAACTGCGACTGTATTTTCC GTA	
Δ*clpV*-up-R	GTCGTGGCAGAATGTGCTGGGATTAGAGCGTAGTTTGC	
Δ*clpV*-down-F	GCAAACTACGCTCTAATCCCAGCACATTCTGCCACGAC	
Δ*clpV*-down-R	GGTAATAGATCTAAGCTTCTGCAGGTCGACTCAGCGTA TTCGGCACAG	
hns(K107A)-sg20-F	AGCTAGCTCAGTCCTAGGTATAATACTAGTTGTATCCGC TTCTGTTCGCCGTTTTAGAGCTAGAAATAGC	To generate pTargetF1-*hns*(K107A)
hns(K107A)-sg20-R	GCCAAAAAGTAAAGTCACCAGTTCAAAAAAAGCACCGA CTCGG	
hns(K107A)-up-F	CCGAGTCGGTGCTTTTTTTGAACTGGTGACTTTACTTTT TGGC	
hns(K107A)-up-R	TATCCGCAGACTTTAGCCATCGCCTGTTCAACCGCT	
hns(K107A)-down-F	AGCGGTTGAACAGGCGATGGCTAAAGTCTGCGGATA	
hns(K107A)-down-R	GGTAATAGATCTAAGCTTCTGCAGGTCGACAAGTGATT	

	GCTGAGCCGAT	
FLAG-*hns*-sg20-F	AGCTAGCTCAGTCCTAGGTATAATACTAGTCATCCGTACT CTTCGTGCGCGTTTTAGAGCTAGAAATAGC	
FLAG-*hns*-sg20-R	TGCAATGAGATCGTATTTCATCTTCAAAAAAAGCACCGAC TCGG	
FLAG-*hns*-up-F	CCGAGTCGGTGCTTTTTTTGAATGCAATGAGATCGTATTT CATC	To generate
FLAG-*hns*-up-R	CTTATCGTCGTCCTTGTAATCCATTGTAGTAATCTCAAAC TTATA	pTargetF1-FLAG-*hns*
FLAG-*hns*-down-F	ATGGATTACAAGGACGACGATAAGATGAGCGAAGCACTT AAAATTCT	
FLAG-*hns*-down-R	GGTAATAGATCTAAGCTTCTGCAGGTCGACTTAGCGGCA GCCATGCTATTCA	
Δ*mvaT*-up-F	GGAAACAGCTATGACCATGATTACGAATTCCAGGGAAT CTCGCAGTCCAGTT	
Δ*mvaT*-up-R	CCCACTTGGCTTTCCACTCTTTCGCTCCTGAAGTTCCTT GATGG	To generate
Δ*mvaT*-down-F	CCATCAAGGAACTTCAGGAGCGAAAGAGTGGAAAGCCA AGTGGG	pK18-Δ*mvaT*
Δ*mvaT*-down-R	GCATGCCTGCAGGTCGACTCTAGAGGATCCGATGAGTG CCAGCAAAAAGCC	
Δ*turA*-up-F	GGAAACAGCTATGACCATGATTACGAATTCAGGTCGTT GTGGTTGGAAATCA	
Δ*turA*-up-R	GTCCAGCAGGGTTGCCCACGGGCCTGAAGTTCCTTGAT	To generate
Δ*turA*-down-F	ATCAAGGAACTTCAGGCCCGTGGGCAACCCTGCTGGAC	pK18-Δ*turA*
Δ*turA*-down-R	GCATGCCTGCAGGTCGACTCTAGAGGATCCGGAAAGTG CTGAAGTTTTGCGT	
FLAG-MvaT-up-F	GGAAACAGCTATGACCATGATTACGAATTCATGCAGTC GGCGCCATGTT	
FLAG-MvaT-up-R	CTTATCGTCGTCCTTGTAATCCATGTCAGGTACCTTGTCT GTGCTGAGT	To generate
FLAG-MvaT-down-F	ATGGATTACAAGGACGACGATAAGATGTCCCTGATCAAC GAATATCGC	pK18-FLAG-*mvaT*
FLAG-MvaT-down-R	GCATGCCTGCAGGTCGACTCTAGAGGATCCAGATCCAG GTCAACCGTTGCCC	
Δhns_{vp}-up-F	AGTACGCGTCACTAGTGGGGCCCTTCTAGAGCAGTGTT GTTCCTTTCAGA	
Δhns_{vp}-up-R	CTAGTGATTTACCTGCGTCAAAGGCTACGGATATTTAGA AGTG	To generate
Δhns_{vp}-down-F	CACTTCTAAATATCCGTAGCCTTTGACGCAGGTAAATCA CTAG	pDM4-Δhns_{vp}
Δhns_{vp}-down-R	GAGAGCTCAGGTTACCCGCATGCAAGATCTGCCTGAAG CAACAATGAAA	
P$_{clpV}$::*lacZ*-F	ACGCGTCGACATGTTGCTCAAAAAGCGGAA	To generate

P_{clpV}::*lacZ*-R	CTAGTCTAGAAGTTTCCATGTGTTATGCCTTGT	pDM4-P_{clpV}::lacZ
P_{hcp1}::*lacZ*-F	ACGCGTCGACTCGCTATTGGGTTTCGTGTA	To generate
P_{hcp1}::*lacZ*-R	CTAGTCTAGAGTCATAAGCCATTTTTATATCCTTA	pDM4-P_{hcp1}::lacZ
P_{tae4}::*lacZ*-F	ACGCGTCGACGATTGTCGCCGTGGTTTC	To generate
P_{tae4}::*lacZ*-R	CTAGTCTAGATCTGTTCATTTTGCTTAACCTCTA	pDM4-P_{tae4}::lacZ
P_{vgrG}::*lacZ*-F	ACGCGTCGACTGGTGAAAAGACATTTACGGTT	To generate
P_{vgrG}::*lacZ*-R	CTAGTCTAGATACAAAACTCATGGTAGTGACTCCT	pDM4-P_{vgrG}::lacZ
hcp1-F	GACGACAAGCTTACTAGTCTGCAGGGATCCATGGCTTA TGACATTTTTTTGAA	To generate pKT100-*hcp1*-VSVG
hcp1-VSVG-R	ATCTTAGTTACTTAGGTACCCGGGGTCGACTTATTTTCC TAATCTATTCATTTCAATATCTGTATAAATTTCTTTGTTGG CCTTGAA	
C*hns*-F	GACGGTATCGATAAGCTTGATATCGAATTCATGAGCGAA GCACTTAAAATTCT	To generate pBBR1MCS1-*hns*
C*hns*-R	GGTGGCGGCCGCTCTAGAACTAGTGGATCCTTATTCCT TGATCAGGAAATCTTC	
C*fur*-F	GACGGTATCGATAAGCTTGATATCGAATTCATGACTGAC AACAATACCGCA	To generate pBBR1MCS1-*fur*
C*fur*-R	GGTGGCGGCCGCTCTAGAACTAGTGGATCCTTATTTAG TCGCGTCATCGTG	
adrA-F	GACGGTATCGATAAGCTTGATATCGAATTCATGTTCCCA AAAATAATGAATGAT	To generate pBBR1MCS1-*adrA* *and* pBBR1MCS5-*adrA*
adrA-R	GGTGGCGGCCGCTCTAGAACTAGTGGATCCTCATGCC GCCACTTCG	
STM3611-F	GACGGTATCGATAAGCTTGATATCGAATTCATGATAAAG CAGGTTATCCAGC	To generate pBBR1MCS1-*STM3611* *and* pBBR1MCS5-*STM3611*
STM3611-R	GGTGGCGGCCGCTCTAGAACTAGTGGATCCTTACAGG GTCAGAATCACCTCT	
ycgR-F	ACTGGTGGACAGCAAATGGGTCGCGGATCCGTGAGTG GTTACAATGAGCAGTTCC	To generate pET28a-*ycgR*
ycgR-R	GTGGTGGTGCTCGAGTGCGGCCGCAAGCTTTTATTCTC GCACTTTATTCGCTCTT	
hns-F	ACTGGTGGACAGCAAATGGGTCGCGGATCCATGAGCG AAGCACTTAAAATTCT	To generate pET28a-*hns*
hns-R	GTGGTGGTGCTCGAGTGCGGCCGCAAGCTTTTATTCCT TGATCAGGAAATCTTC	
hns_{Ctd}-F	ACTGGTGGACAGCAAATGGGTCGCGGATCCATGGCAG CTCGTCCGGCTAA	To generate pET28a-hns_{Ctd}
hns_{Ctd}-R	GTGGTGGTGCTCGAGTGCGGCCGCAAGCTTTTATTCCT TGATCAGGAAATCTTC	
hns^{Y99A}-up-F	TGATAAAATGTGACCTGACTCCTA	To generate pET28a-hns^{Y99A}
hns^{Y99A}-up-R	TAGTTTCACCGTTTTCGTCAACAGCGCTATATTTAGCCG GAC	
hns^{Y99A}-down-F	GTCCGGCTAAATATAGCGCTGTTGACGAAAACGGTGAA	

	ACTA	
hns^{Y99A}-down-R	ATTGTCGGGCGTTATGTGTT	
hns^{D101A}-up-F	CGGTGAGTATCCCCCCTG	To generate pET28a-*hns*^{D101A}
hns^{D101A}-up-R	CAGGTTTTAGTTTCACCGTTTTCGGCAACATAGCTATATTTAGCCG	
hns^{D101A}-down-F	CGGCTAAATATAGCTATGTTGCCGAAAACGGTGAAACTAAAACCTG	
hns^{D101A}-down-R	ATTGTCGGGCGTTATGTGTT	
hns^{K107A}-up-F	CAGACGGTGAGTATCCCCC	To generate pET28a-*hns*^{K107A}
hns^{K107A}-up-R	CCCTGGCCAGTCCAGGTTGCAGTTTCACCGTTTTCG	
hns^{K107A}-down-F	CGAAAACGGTGAAACTGCAACCTGGACTGGCCAGGG	
hns^{K107A}-down-R	AAAAACCAAAAGCGGATGTC	
hns^{T115A}-up-F	TCTTTTTTGTGCGGTGCCT	To generate pET28a-*hns*^{T115A}
hns^{T115A}-up-R	TGCTTTTTTGATTACAGCCGGTGCACGACCCTGGCCA	
hns^{T115A}-down-F	TGGCCAGGGTCGTGCACCGGCTGTAATCAAAAAAGCA	
hns^{T115A}-down-R	TGGTTGGCGTGGTTGAAAA	
mvaT-F	ACTGGTGGACAGCAAATGGGTCGCGGATCCATGTCCCTGATCAACGAATATC	To generate pET28a-*mvaT*
mvaT-R	GTGGTGGTGCTCGAGTGCGGCCGCAAGCTTTTAGCCGAGCAGGGTGG	
turA-F	ACTGGTGGACAGCAAATGGGTCGCGGATCCATGTCCCTGATCAACGAGTACCGC	To generate pET28a-*turA*
turA-R	GTGGTGGTGCTCGAGTGCGGCCGCAAGCTTTCAGTCCAGCAGGGTTGCCC	
hns$_{vp}$-F	ACTGGTGGACAGCAAATGGGTCGCGGATCCATGTCAGAGCTGACTAAAACACTT	To generate pET28a-*hns*$_{vp}$
hns$_{vp}$-R	GTGGTGGTGCTCGAGTGCGGCCGCAAGCTTTTAGATTAGGAAATCGTCTAGTGAT	
16S-F	GAAGGTGTTGTGGTTAATA	qRT-PCR
16S-R	AGTAATTCCGATTAACGCTT	
clpV-RT-F	AAAAAATGGGGATTCAGTC	qRT-PCR
clpV-RT-R	CCTGCCACTCCCTTAGC	
hcp1-RT-F	GGATGACAAACACAAAAATGAAA	qRT-PCR
hcp1-RT-R	ACAATCAGGTCGGTGAAGGTA	
tae4-RT-F	ATTCCGTCGCTGATGTG	qRT-PCR
tae4-RT-R	TCAGGCTTGCCCATAGTA	
vgrG-RT-F	AAGAGTGAAGGCGAGATGCT	qRT-PCR
vgrG-RT-R	AAACGGGTGGAAAAGGTAAA	
aadA-RT-F	GCGGTCTGAAGCCAAACA	qRT-PCR
aadA-RT-R	GGTTCATAAATCCCCTGACAAAT	
16S$_{PA}$-RT-F	GGTGGTTCAGCAAGTTGGATGT	qRT-PCR
16S$_{PA}$-RT-R	AATCCTGTTTGCTCCCCACG	
vgrG2$_{PA}$-RT-F	CTGGGCAGGTGCCGATTG	qRT-PCR

vgrG2$_{PA}$-RT-R	GATGGTGCTGAGGATCTTGTGG	
vgrG3$_{PA}$-RT-F	CCAGCAACAACCGGGTGTT	qRT-PCR
vgrG3$_{PA}$-RT-R	TGGGTGAAGAACCAGAAGATGC	
PA2128-RT-F	ACCGCCTTCACCCTGCAAC	qRT-PCR
PA2128-RT-R	CGACGCTATCGAGCAATTGC	
PA3540-RT-F	ATGCTCGGTTCGTTGATGC	qRT-PCR
PA3540-RT-R	CTCGATGTATTCCTTGTTGGCC	
PA4210-RT-F	TGGACCACGGAAAGCGGCGAACC	qRT-PCR
PA4210-RT-R	CGTCGCACTCGACCCAGAAGT	
16S$_{PP}$-RT-F	GTTACCGACAGAATAAGCACCG	qRT-PCR
16S$_{PP}$-RT-R	GCCACTGGTGTTCCTTCCTAT	
hcp1$_{PP}$-RT-F	ATTCTGGTTCAGGAAATCAAGCAT	qRT-PCR
hcp1$_{PP}$-RT-R	TGCTCTTGTTTGCCTTCCGA	
hcp2$_{PP}$-RT-F	ACTACGCCCACTGACCCCC	qRT-PCR
hcp2$_{PP}$-RT-R	TTCGAGCGTGGTGGTGAAAA	
PP_0987-RT-F	TACTCGGTAGGCGGTGGTTTCGT	qRT-PCR
PP_0987-RT-R	CGGGTTTCTGCTTCTGGGCG	
PP_3781-RT-F	TGCTCATTCTGCGACCTTGCC	qRT-PCR
PP_3781-RT-R	GCGTTCCACCTCCGAAGTAGAGA	
PP_4286-RT-F	ACACCCGCCGCCTGGTGA	qRT-PCR
PP_4286-RT-R	AGAACTGCTCGCCGCAGTTGAAG	
16S$_{VP}$-RT-F	CAACCCTTATCCTTGTTTGCCA	qRT-PCR
16S$_{VP}$-RT-R	TACGACGCACTTTTTGGGATTC	
hcp1$_{VP}$-RT-F	TTGATCACGTGCTAACTGTTCC	qRT-PCR
hcp1$_{VP}$-RT-R	TAGTAGTGCTCTTGCTTGCCTT	
hcp2$_{VP}$-RT-F	GATTGGAGTGTTGGTCGTGAAA	qRT-PCR
hcp2$_{VP}$-RT-R	CAACTTTTTCGCCCTGCTTAG	
VP1314-RT-F	ATCACGCAACAAGTCGATCA	qRT-PCR
VP1314-RT-R	GCAGCAGAATATCCTCAAGC	
VP1362-RT-F	TTTCATCTCTTCCCTTATCTTT	qRT-PCR
VP1362-RT-R	TATGTAGTCCGCATCTCGTT	
VP1699-RT-F	CGGGCTTGTTTTCTTTTGG	qRT-PCR
VP1699-RT-R	AGCGGTCTGAGGTTCGGT	
ssrB-RT-F	TCCTTGATCTTAGTCTACCTGG	qRT-PCR
ssrB-RT-R	CTGCTTTTTAAAACATAGCCAT	
sseA-RT-F	TAAGGTGAGTCAACAGCTTGC	qRT-PCR
sseA-RT-R	TTGTTTTTCCTGACGGTATCT	
rmbA-RT-F	TGACAGCGCAGTACGGGT	qRT-PCR
rmbA-RT-R	CGTCGAAGATTCGGGAAA	
misL-RT-F	CAGCATCCTCCTCCAGAAC	qRT-PCR
misL-RT-R	TCGCCCGCCATAGTAATA	

slsA-RT-F	CTGGCTAAGATCGCCACCTT	qRT-PCR
slsA-RT-R	CGCTTTTACCGCTTGTACAAAA	
pipA-RT-F	ACTTCAAACTTTCAGAAGGCAG	qRT-PCR
pipA-RT-R	GGTATATTCGACAACAGGGC	
pipB-RT-F	AGTAAACATAATATACAACAGCCT	qRT-PCR
pipB-RT-R	AACACAAATTTGCACCG	
clpV-ChIP-F	TCTGTATCATTGTTTTGTTTCGATA	ChIP-qPCR
clpV-ChIP-R	AGTTTCCATGTGTTATGCCTTG	
hcp-ChIP-F	AATAACTAAATACATTATCGGTAC	ChIP-qPCR
hcp-ChIP-R	ATATCCTTATTAAGTTTTACTTAAAAT	
tae4-ChIP-F	GATTCGCTACTTGACCCCTAA	ChIP-qPCR
tae4-ChIP-R	TTTGCTTAACCTCTAAAAATTCTG	
vgrG-ChIP-F	TATTTTCATGGCTCTTGTCAGCAAT	ChIP-qPCR
vgrG-ChIP-R	GGTAGTGACTCCTTTAATACCGGAT	
ssrAB-ChIP-F	TAATTGTAGTCATCGACTGGGTTAT	ChIP-qPCR
ssrAB-ChIP-R	AATGCTTCCCTCCAGTTGC	
sseA-ChIP-F	TAGTACGTGAGGTTTGACTCGC	ChIP-qPCR
sseA-ChIP-R	TCCCCTCCATATACACGATAGA	
rmbA-ChIP-F	TGTCATGGTTCCTTAAAGCTGTATT	ChIP-qPCR
rmbA-ChIP-R	CCTGAACGACTCCTGTCGATG	
slsA-ChIP-F	CTGTCATCAGCTTAAAAACGG	ChIP-qPCR
slsA-ChIP-R	TTAATTCATTCCTTGTTGTTGCCTT	
pipA-ChIP-F	GCCCCTAAGTTTCATTATAAATA	ChIP-qPCR
pipA-ChIP-R	AACTTCTTATTTCCTGACTACAATT	
pipB-ChIP-F	CTACTCATACTCAACCAAAGCTCTA	ChIP-qPCR
pipB-ChIP-R	TTTGATTCCTTCTTATGGAAGTG	
vgrG2$_{PA}$-ChIP-F	AATGTCCGCTACTTCGCGAAAT	ChIP-qPCR
vgrG2$_{PA}$-ChIP-R	GGATCGTTCCTTGTTCCTGGC	
vgrG3$_{PA}$-ChIP-F	GCTGGAGTTCCTCGACAATAC	ChIP-qPCR
vgrG3$_{PA}$-ChIP-R	TAGTGTGCCTTGTCATGCCAGC	
PA2128-ChIP-F	GGTTCGCACCACTGATTATCGATTT	ChIP-qPCR
PA2128-ChIP-R	GCCTATGTTCCTTGTGTGAGGCAAT	
PA3540-ChIP-F	TCTGGATCGGGGCTCGTAGTA	ChIP-qPCR
PA3540-ChIP-R	GCATTCACCTCGATTGTTTGT	
PA4210-ChIP-F	GAATATCGCTCCCTTACCGAGCGAC	ChIP-qPCR
PA4210-ChIP-R	GCCGCCTCCGAGAGGGCTC	
P$_{STM0271}$-EMSA-F	AATAATATTTCAAAAAAGACATCTATGC	EMSA
P$_{STM0271}$-EMSA-R	ATGTCTTGTTTCATTATGTCCCTTA	
P$_{STM0271}$-EMSA-CK-F	ATTACGGAAAATACAGTCGCAG	To generate fragment of negative control for EMSA
P$_{STM0271}$-EMSA-CK-R	GCATACGGTGATGAGGTTCC	
P$_{clpV}$-EMSA-F	TTTTTATCTTGCTGTTCATTGATTA	EMSA

P_{clpV}-EMSA-R	TTCCATGTGTTATGCCTTGTG	
P_{clpV}-EMSA-CK-F	TGTTTGACAAGGGTGGGATG	To generate fragment of
P_{clpV}-EMSA-CK-R	CAGCGGCAGGTAAGGGAT	negative control for EMSA
P_{tssB}-EMSA-F	CTGATTGGCTACATCGAACA	EMSA
P_{tssB}-EMSA-R	TTTCCTTTTTAGTCAACTGGTTGC	
P_{tssB}-EMSA-CK-F	TTACGGTTCCGAGAAAAAAAT	To generate fragment of
P_{tssB}-EMSA-CK-R	AACTGGTTCAGGGCGTCC	negative control for EMSA
$P_{STM0275}$-EMSA-F	TATATATTTTGTGAATGTTTAAGCGAG	EMSA
$P_{STM0275}$-EMSA-R	TGGGCTTATGGTCATAATATGTATT	
$P_{STM0275}$-EMSA-CK-F	TGAGCCTGAAGTCTGATGTCC	To generate fragment of
$P_{STM0275}$-EMSA-CK-R	TTGCGTCAACCATATCTGTTC	negative control for EMSA
P_{hcp1}-EMSA-F	TATCGGAAGTTATGAAGCATTATTT	EMSA
P_{hcp1}-EMSA-R	TGTCATAAGCCATTTTTATATCCTTA	
P_{hcp1}-EMSA-CK-F	TCAATGGATGACAAACACAAAA	To generate fragment of
P_{hcp1}-EMSA-CK-R	CAATCAGGTCGGTGAAGGTAT	negative control for EMSA
P_{tae4}-EMSA-F	CACTGGGTGGTTATGTGCGGTAG	EMSA
P_{tae4}-EMSA-R	ATTCTGTGTAGGTCATTAATTCT	
P_{tae4}-EMSA-CK-F	AGCCCGTAACTCTCCGTATG	To generate fragment of
P_{tae4}-EMSA-CK-R	AATAAGTGGCACTGATCTGAACA	negative control for EMSA
P_{hcp2}-EMSA-F	TTTATCCATGACAGGGAATTAAAT	EMSA
P_{hcp2}-EMSA-R	AATGTCATAAGACATATCTACTATCCTTT	
P_{hcp2}-EMSA-CK-F	CATTACATCGATCGCGCC	To generate fragment of
P_{hcp2}-EMSA-CK-R	CCACCCTGCTGGTTCTGC	negative control for EMSA
$P_{STM0284}$-EMSA-F	AACTTATCATAAATAAATTAATAGAGT	EMSA
$P_{STM0284}$-EMSA-R	TATCTGAACTGGCAGCGATAACA	
$P_{STM0284}$-EMSA-CK-F	AAGGATAAAACAAAGGTAGAGATTC	To generate fragment of
$P_{STM0284}$-EMSA-CK-R	ATCTTACAGAACACTCATCAGCAT	negative control for EMSA
P_{vgrG}-EMSA-F	TATTGTTATGTTCATTTCCTTATTTA	EMSA
P_{vgrG}-EMSA-R	CAAAACTCATGGTAGTGACTCC	
P_{vgrG}-EMSA-CK-F	TATTGTTATGTTCATTTCCTTATTTA	To generate fragment of
P_{vgrG}-EMSA-CK-R	CAAAACTCATGGTAGTGACTCC	negative control for EMSA
$P_{vgrG2\text{-}PA}$-EMSA-F	CTACATGCACAACTGCCAGGACC	EMSA
$P_{vgrG2\text{-}PA}$-EMSA-R	ATTTTCGGCTCGACGGAGAA	
$P_{vgrG2\text{-}PA}$-EMSA-CK-F	AAACTCGCGTTCGATGTCGT	To generate fragment of
$P_{vgrG2\text{-}PA}$-EMSA-CK-F	TTCCAGCAGCAGTTCGTA	negative control for EMSA
$P_{vgrG3\text{-}PA}$-EMSA-F	CGCCATCTCAAATAAAAAGGAAA	EMSA
$P_{vgrG3\text{-}PA}$-EMSA-R	GCCCGAAGGAATGATGAATAAC	
$P_{vgrG3\text{-}PA}$-EMSA-CK-F	TGCTGTTGCGCCCCTGGCTC	To generate fragment of
$P_{vgrG3\text{-}PA}$-EMSA-CK-R	CCACTTGCGGGCCGTTGGGAA	negative control for EMSA
P_{PA2128}-EMSA-F	CATTACACGGATGACGGTTTTTATT	EMSA
P_{PA2128}-EMSA-R	CAGTGGTGCGAACCACCGATATA	

P_{PA2128}-EMSA-CK-F	ACGAGCATTTCACCACCCTGTTC	To generate fragment of negative control for EMSA
P_{PA2128}-EMSA-CK-R	TAACGCAGCGTGTAGGTCACGAC	
P_{PA3540}-EMSA-F	CCCTCGCAGAGAAAACATCCTA	EMSA
P_{PA3540}-EMSA-R	TTTCAGGTGGGTTTCAATAACGG	
P_{PA3540}-EMSA-CK-F	GTCATGAAGTCATTGGTGTGGA	To generate fragment of negative control for EMSA
P_{PA3540}-EMSA-CK-R	TCTCGATGTAGCCCAGGTCCA	
P_{PA4210}-EMSA-F	GAGCGTTCTGTGTTTTATGCAATCC	EMSA
P_{PA4210}-EMSA-R	TATGTTCGCCTGTCGTTTCCAATT	
P_{PA4210}-EMSA-CK-F	GTTACAGCGGCACAGCCTGTTCGT	To generate fragment of negative control for EMSA
P_{PA4210}-EMSA-CK-R	GTTCTCGCAATAGCCCTGCGGAT	
$P_{hcp1\text{-}PP}$-EMSA-F	GGCGAAGAAACACTCAACGCATTG	EMSA
$P_{hcp1\text{-}PP}$-EMSA-R	GTGGTGCTGTGACACGGTTGGCTAT	
$P_{hcp1\text{-}PP}$-EMSA-CK-F	GTTCAGGAAATCAAGCATGAGGTGAC	To generate fragment of negative control for EMSA
$P_{hcp1\text{-}PP}$-EMSA-CK-R	TGTCTTTGTCGAGTGCGTGGGGA	
$P_{hcp2\text{-}PP}$-EMSA-F	TGGCGGTGGCCTACGGGATTTA	EMSA
$P_{hcp2\text{-}PP}$-EMSA-R	AGTTACTCCTGCGTCCATGTGGGTA	
$P_{hcp2\text{-}PP}$-EMSA-CK-F	GCCAACACCCGCTTACATCAAGA	To generate fragment of negative control for EMSA
$P_{hcp2\text{-}PP}$-EMSA-CK-R	TCGCCGGTGGCAAGTGCCTGA	
P_{PP_0987}-EMSA-F	AGGCAAGTACTGGCCGCCGG	EMSA
P_{PP_0987}-EMSA-R	ATTGAGCCCTGTAGGCGC	
P_{PP_0987}-EMSA-CK-F	TGTCGGTGAGCCAGGTGATGCTG	To generate fragment of negative control for EMSA
P_{PP_0987}-EMSA-CK-R	AAGGCGTAGAGGTTGACCCAGTCG	
P_{PP_3781}-EMSA-F	GAGGAACACCTGCTCGTATATCAGC	EMSA
P_{PP_3781}-EMSA-R	CAACTATATCAAAAATGTATTGCCTGTGA	
P_{PP_3781}-EMSA-CK-F	TCTCTACTTCGGAGGTGGAACGCC	To generate fragment of negative control for EMSA
P_{PP_3781}-EMSA-CK-R	CCAGATCGAAATGCTCCAGTGTGG	
P_{PP_4286}-EMSA-F	AAGACATTCCATCCAAGGATTGTACA	EMSA
P_{PP_4286}-EMSA-R	AATCTTTTGTAAGCCTGTTTTTCCG	
P_{PP_4286}-EMSA-CK-F	CCTGATCGGTTACGGCAACAAC	To generate fragment of negative control for EMSA
P_{PP_4286}-EMSA-CK-R	AACAGCTTCAGCAGGCGCCATA	
$P_{hcp1\text{-}VP}$-EMSA-F	AGTGGGAATATTTATCACAAG	EMSA
$P_{hcp1\text{-}VP}$-EMSA-R	AAAGTAGAGCAAAAACAATGA	
$P_{hcp1\text{-}VP}$-EMSA-CK-F	GTGAAACTCAAGGTCACAT	To generate fragment of negative control for EMSA
$P_{hcp1\text{-}VP}$-EMSA-CK-R	TAGAAACGAATCAAACACTCA	
$P_{hcp2\text{-}VP}$-EMSA-F	ATCAGAGACACGCACTCCACCAC	EMSA
$P_{hcp2\text{-}VP}$-EMSA-R	CTATATCTAGGCTACCGCTTAAA	
$P_{hcp2\text{-}VP}$-EMSA-CK-F	GGCGCTAATTTTGGTCAATATCT	To generate fragment of negative control for EMSA
$P_{hcp2\text{-}VP}$-EMSA-CK-R	TTACTCCTTGGTTAATCGCAA	
P_{VP1314}-EMSA-F	GTTTTTTACCCATACTTTGGCTTA	EMSA
P_{VP1314}-EMSA-R	TCTACGCTTTCACAAATGTGTTTTA	
P_{VP1314}-EMSA-CK-F	AAGCCACAGCGTAACAAACAAAT	To generate fragment of

P*VP1314*-EMSA-CK-R	TGGATGGAAAGTCGCCACTTG	negative control for EMSA
P*VP1362*-EMSA-F	GTTCGAAAAAACCATCTTCT	EMSA
P*VP1362*-EMSA-R	GGAGTCTTATCCCGACTCTC	
P*VP1362*-EMSA-CK-F	GTAGCAAGCGCATCACTCTT	To generate fragment of
P*VP1362*-EMSA-CK-R	AGTCACCATCGTGGTCAGAACG	negative control for EMSA
P*VP1699*-EMSA-F	GCGCTATCTGAAAATAAAACAACT	EMSA
P*VP1699*-EMSA-R	CTCTCGTGTGTAAAAGAGAAACTG	
P*VP1699*-EMSA-CK-F	ACCACAGGGTGGGAATTATCA	To generate fragment of
P*VP1699*-EMSA-CK-R	ATAGAGGACTTCGCCCCCTTGA	negative control for EMSA
P*ssrAB*-EMSA-F	TACAATATCAGGATGCTGTCTACA	EMSA
P*ssrAB*-EMSA-R	TAATCGATGGTGTTATCATTAGG	
P*ssrAB*-EMSA-CK-F	AACCCATGCCGGGTTTTACT	To generate fragment of
P*ssrAB*-EMSA-CK-R	ATCCATCATGCAGCGTTACATT	negative control for EMSA
P*sseA*-EMSA-F	AACATTATTTATTATCGTTACCATAAC	EMSA
P*sseA*-EMSA-R	GCTATTTTACTTGCCATTTTGA	
P*sseA*-EMSA-CK-F	GCTGCGTTTAGTGAATATCGT	To generate fragment of
P*sseA*-EMSA-CK-R	TATCTCCACCGGGGCTT	negative control for EMSA
P*rmbA*-EMSA-F	ATAGTGACGAAGGCAGCAGA	EMSA
P*rmbA*-EMSA-R	AAACTCCTGGAAAGACCTAAAAT	
P*rmbA*-EMSA-CK-F	GCGGTTTTATCTTTGTTGAT	To generate fragment of
P*rmbA*-EMSA-CK-R	TTCGTCCACGTTTACTGTTG	negative control for EMSA
P*slsA*-EMSA-F	TTTCAATCAATAATCCTCTC	EMSA
P*slsA*-EMSA-R	TGATAGTCACTATCAGTGTTAAAGTCA	
P*slsA*-EMSA-CK-F	GGGTACAAGGTATTTGCGG	To generate fragment of
P*slsA*-EMSA-CK-R	AATCCAGCAGTTCATTATTTTT	negative control for EMSA
P*pipA*-EMSA-F	AGGTAGTCAACATACCCCTACTC	EMSA
P*pipA*-EMSA-R	CATACAGGTAACGCTATGATTCA	
P*pipA*-EMSA-CK-F	ACAGATTAATACCTCAAAGCGG	To generate fragment of
P*pipA*-EMSA-CK-R	GGATAGTTCATCGTAGCATTCG	negative control for EMSA
P*pipB*-EMSA-F	TTTATTCAATGATGATGAAGCGT	EMSA
P*pipB*-EMSA-R	TTCTTATGGAAGTGAGCCGAC	
P*pipB*-EMSA-CK-F	TTTGGAATGTATGTCGAATGTTA	To generate fragment of
P*pipB*-EMSA-CK-R	CAAGTCAGGTCTGCGTGAGT	negative control for EMSA

***Underlined sites indicate restriction enzyme cutting sites added for cloning.**

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