Supporting information

Supplemental Figure 1

Number of hook-basal-body (HBB) complexes as analyzed by C-ring-GFP microscopy and hook immunostaining.

(A) Number of C-ring complexes as analyzed by FliG-GFP microscopy. A strain harboring a functional FliG-GFP fusion as well as the *flhDC* operon under arabinose control was grown in the presence or absence of arabinose and analyzed by fluorescence microscopy. Upper panel: distribution of C-rings per cell and exemplary images of cells expressing FliG-GFP in the absence of arabinose (wildtype *flhDC* levels). Non-linear fitting of the Gaussian distribution was employed (red line) and the average number of C-rings per cell is 5.2 ± 1.3 (n = 117). Lower panel: distribution of C-rings per cell and exemplary images of cells expressing FliG-GFP in the presence of arabinose (increased *flhDC* levels). Non-linear fitting of the Gaussian distribution was employed (red line) and the average number of C-rings per cell is 7.8 ± 2.2 (n = 115). Scale bar is 2 μM.

(B) Number of C-ring complexes as analyzed by FliM-GFP microscopy. A strain harboring a functional FliM-GFP fusion as well as several *flhD* promoter mutations was analyzed by fluorescence microscopy. Upper panel: distribution of C-rings per cell and exemplary images of a strain expressing FliM-GFP with a wildtype *flhD* promoter. Non-linear fitting of the Gaussian distribution was employed (red line) and the average number of C-rings per cell is 5.6 ± 2.2 (n = 266). Middle panel: distribution of C-rings per cell and exemplary images of a strain expressing

FliM-GFP with a perfect -10 box of the P1 *flhD* promoter. Non-linear fitting of the Gaussian distribution was employed (red line) and the average number of C-rings per cell is 8.0 ± 2.0 (n = 138). Lower panel: distribution of C-rings per cell and exemplary images of a strain expressing FliM-GFP with a perfect -10 box of the P1 + P4 *flhD* promoter. Non-linear fitting of the Gaussian distribution was employed (red line) and the average number of C-rings per cell is 8.9 ± 2.2 (n = 197). Scale bar is 2 μM.

(C) Number of assembled hook-basal-body (HBB) complexes as analyzed by hook immunostaining. A strain harboring a functional FlgE::3xHA mutation as well as *flhDC* under arabinose control was grown in the presence or absence of arabinose and analyzed by fluorescence microscopy. Upper panel: exemplary images of cells with immunostained hooks in the absence of arabinose (wildtype *flhDC* levels). Lower panel: exemplary images of cells with immunostained hooks in the presence of arabinose (increased *flhDC* levels). Green: HBB complexes (FlgE::3xHA tag) labeled with anti-hemagglutinin antibodies coupled to Alexa Fluor 488. Red: cell membrane stained with FM-64. Blue: DNA stained with Hoechst. Scale bar is 2 μM.

Supplemental Figure 2

Schematic of the *flhD* promoter region. The DNA sequence of the *flhD* promoter region of *S. enterica* and the six described transcriptional start sites (Yanagihara *et al*., 1999) of the *flhD* promoter are displayed. A red box annotates the wildtype -10 box sequence, whereas the black box annotates the respective consensus -10 box mutation. A black arrow displays the transcriptional start site (+1) of the respective promoter.

Supporting Table 1

Salmonella enterica **serovar** ***typhimurium*** **strains used and constructed in this study.**

Strains	**Relevant characteristics**	**Source or reference[a]**
TH437	LT2 Wild-type for motility and chemotaxis	J. Roth
TH7420	*fliM5978*::GFP*mut2*	lab stock
TH9857	*ecnR*::T-POP *ecnR3*::Mu*d*J	C. E. Wozniak
TH9949	*flgE6554*::*bla* Δ*flgBC6557*	(Lee & Hughes, 2006)
TH10068	*ecnR3*::Mu*d*J	(Wozniak *et al.*, 2008)
TH12470	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7392*	
TH12731	Δ*flgBC6557 flgE6569*::*bla* Δ*fliP7457*	
TH14156	Δ*araBAD1007::flhD*$^{+}$*C*$^{+}$	
TH14525	*flgE7742::3x*HA (HA-tag after AA241)	F. F. V. Chevance
TH14680	Δ*flgBC6557 flgE6569*::*bla* P*flhDC7460* (-38G:A from AUG) *STM1911*::Tn*10d*Tc Δ*fliG7388*	
TH14681	Δ*flgBC6557 flgE6569*::*bla* P*flhDC7461* (-152C:T from AUG) *STM1911*::Tn*10d*Tc Δ*fliMN7392*	
TH14683	Δ*flgBC6557 flgE6569*::*bla zec-3521*::Tn*10d*Cm Δ*fliMN7392 fliA7463* (Ap^{5R})	
TH14684	Δ*flgBC6557 flgE6569*::*bla zec-3521*::Tn*10d*Cm Δ*fliMN7392 fliA7464*	
TH14781	P*flhDC7776* (P1 -10 TATAAT promoter)	C. E. Wozniak

	fliM5978::GFP*mut2*	
TH14782	P*flhDC7777* (P5 -10 TATAAT promoter) *fliM5978*::GFP*mut2*	C. E. Wozniak
TH14815	P*flhDC7790* (P3 -10 TATAAT) *fliM5978*::GFP*mut2*	C. E. Wozniak
TH14902	Δ*araBAD1007*::*flhD*$^+$*C*$^+$ Δ*flgBC6557 flgE6569*::*bla*	
TH14903	Δ*araBAD1007*::*flhD*$^+$*C*$^+$ Δ*flgBC6557 flgE6569*::*bla* Δ*fliF7387*	
TH14905	Δ*araBAD1007*::*flhD*$^+$*C*$^+$ Δ*flgBC6557 flgE6569*::*bla* Δ*fliHIJ7398*	
TH14906	Δ*araBAD1007*::*flhD*$^+$*C*$^+$ Δ*flgBC6557 flgE6569*::*bla* Δ*fliG7402* Δ*fliMN7392*	
TH14909	Δ*araBAD1007*::*flhD*$^+$*C*$^+$ Δ*flgBC6557 flgE6569*::*bla* Δ*fliMN7392 fliG7780*::GFP*mut2*	
TH14924	P*flhDC7793* (P1 + P4 -10 TATAAT) *fliM5978*::GFP*mut2*	
TH14980	P*flhDC7797* (P2 -10 TATAAT) *fliM5978*::GFP*mut2*	C. E. Wozniak
TH14981	P*flhDC7798* (P6 -10 TATAAT*) fliM5978*::GFP*mut2*	C. E. Wozniak
TH15184	*flgE7742*::3xHA Δ*araBAD1007*::*flhD*$^+$*C*$^+$	
TH15413	P*flhD7776* (P1 -10 TATAAT) *flhC5213*::Mu*d*J *fliM5978*::GFP*mut2*	
TH15414	*PflhD7793* (P1 + P4 -10 TATAAT *flhC5213*::Mu*d*J *fliM5978*::GFP*mut2*	
TH15415	P*flhD7797* (P2 -10 TATAAT) *flhC5213*::Mu*d*J	

	fliM5978::GFP*mut2*	
TH15434	P*flhDC7793* (P1 + P4 -10 TATAAT) Δ*flgBC6557* *flgE6569*::*bla* Δ*fliP7457*	
TH15461	*fliL5100*::Mu*d*J *fliD7879*::TPOP	
TH15462	*fliL5100*::Mu*d*J *lrhA2*::TPOP	
TH15463	*fliL5100*::Mu*d*J *STM1856-1*::TPOP	
TH15464	*fliL5100*::Mu*d*J *slyA1*::TPOP	
TH15466	*fliL5100*::Mu*d*J *ddg/yfdZ1*::TPOP	
TH15467	*fliL5100*::Mu*d*J *STM2011-2*::TPOP	
TH15468	*fliL5100*::Mu*d*J *fliD7881*::TPOP	
TH15469	*fliL5100*::Mu*d*J *ecnR6*::TPOP	
TH15470	*fliL5100*::Mu*d*J *STM1856-2*::TPOP	
TH15471	*fliL5100*::Mu*d*J *ecnR7*::TPOP	
TH15496	*ecnR3*::Mu*d*J *ecnR7*::TPOP	
TH15497	Δ*araBAD1007*::*flhD*$^{+}$*C*$^{+}$ *flgE6569*::*bla* Δ*flgBC6557* Δ*fliG7388*	
TH15498	Δ*araBAD1007::flhD*$^{+}$*C*$^{+}$ *flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7392*	
TH15567	*flgE7742*::3xHA P*flhDC7776*	
TH15568	*flgE7742*::3xHA P*flhDC7793*	
TH15569	*flgE7742*::3xHA P*flhDC7797*	
TH15589	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7392 lrhA1*	
TH15590	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7393 lrhA2*	

TH15591	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7394 lrhA3*	
TH15592	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7395 lrhA4*	
TH15593	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7396 lrhA5*	
TH15594	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7397 ecnR6*	
TH15595	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7398 ecnR7*	
TH15596	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7399 slyA1*	
TH15597	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7400 rcsB131*	
TH15598	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7401 rcsB132*	
TH15599	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7402 yojN253*	
TH15600	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7406 clpP71*	
TH15601	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7403 ydiV254*	
TH15602	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7404 ydiV255*	
TH15603	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7405 ydiV256*	
TH15604	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7423 STM1856-1*	
TH15605	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7424 STM1856-2*	
TH15606	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7425 STM2011-1*	
TH15607	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7426 STM2011-2*	
TH15608	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7427 rfbP1*	
TH15609	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7428 pgtE1*	
TH15610	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7429 ddg/yfdZ1*	
TH15611	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7430 pykF1*	
TH15612	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7431 garL1*	
TH15613	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7432 yieP1*	

TH15614	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7433 hpaX1*	
TH15615	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7410 flhDC7872*	
TH15616	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7411 flhDC7873*	
TH15617	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7412 flhDC7874*	
TH15618	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7413 flhDC7875*	
TH15619	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7407 fliA7876*	
TH15620	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7408 fliA7877*	
TH15621	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7409 fliA7878*	
TH15622	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7418 fliD7879*	
TH15623	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7419 fliD7880*	
TH15624	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7420 fliD7881*	
TH15625	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7421 fliD7882*	
TH15756	*flgE6569*::*bla* Δ*flgBC6557* Δ*fliMN7421* Δ*fliT5758*::FCF	
TH15885	*flhC5213*::Mu*d*J Δ*araBAD1049*::*slyA*$^{+}$ (D97E and A98P compared to the published *S.t*. LT2 genome sequence)	
TH15886	*flhC5213*::Mu*d*J Δ*araBAD1049*::*lrhA*$^{+}$	
TH15939	Δ*araBAD956::fliA*$^{+}$ Δ*flgM5628*::FRT Δ*fliA5647*::FRT *flhC5213*::Mu*d*J	

[a] Strains for which no source or reference is given were constructed for this study.

References

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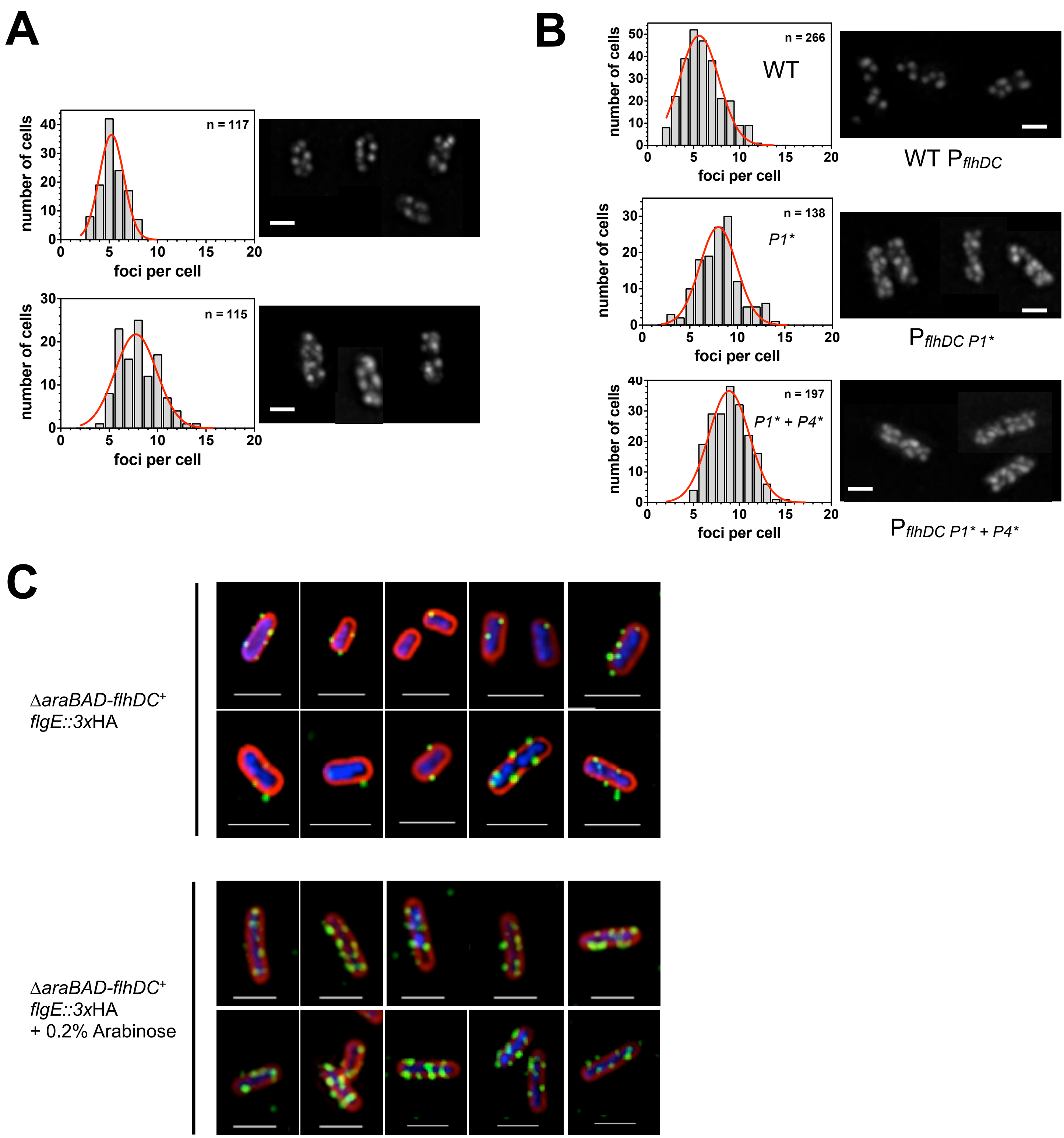
Wozniak, C. E., C. Lee & K. T. Hughes, (2008) T-POP array identifies EcnR and PefI-SrgD as novel regulators of flagellar gene expression. *Journal of Bacteriology*.

Yanagihara, S., S. Iyoda, K. Ohnishi, T. Iino & K. Kutsukake, (1999) Structure and transcriptional control of the flagellar master operon of Salmonella typhimurium. *Genes Genet Syst* **74**: 105-111.

Supplemental Figure 1



Supplemental Figure 2

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       TATAAT     P5 →
GAGGGCGTATGCTGTGACGAGATTAATTAATAACGTTTAATATTTAAGCTACTGTTTACT  < 60
         10        20        30        40        50

AAAGGTAAAATAAAAGCGTTTCTAAAATAGAAATAATAGCCTGTTATCTATTATCCTGGC  < 120
         70        80        90        100       110

GTTATTTTAACAGAGAGAAACAAAAAAGAATTTGGTGTTGACGTACCCCTATTCAGCAGT  < 180
         130       140       150       160       170

 TATAAT     P4 →
GTGGAATAGAAAAAGTGAACATTAGGTTATTAATTAAACAAAGTAAAAGCCATGCTGATG  < 240
         190       200       210       220       230

    TATAAT     P3 → TATAAT     P6 →      TATAAT     P2 →
GTTTTATCGTAAGTATTCCGTTAAAATATGTGATCTGCATCACATATTTTCTAAAATCGC  < 300
         250       260       270       280       290

                                   TATAAT     P1 →
CGTCCCGCTCCGTTGTATGTCACGAAGCTGACGAGTAGAGTTGCGTCGAATTAGGAAAAA  < 360
         310       320       330       340       350

TCTTAGGCATTTGTAAAAATTGATGTAAACGTGTAAGGCGAATCTCAGTGGGAGGCTGCG  < 420
         370       380       390       400       410

TTATACGTCACAATGTCCATAATGTCTGAGCGCTGCTATGCATTTGACCTTTTTGCTTCT  < 480
         430       440       450       460       470

TTTACCGGGCCTTCCCGGCGACATCACGGGGTGCGGCTACGTCGCACAAAAATAAAGTTG  < 540
         490       500       510       520       530

GTTATTCTGGATG  < 553
         550
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