### Supplemental Table 1: Salmonella enterica serovar Typhimurium LT2 and ATCC

### 14028s (ST14028) strains used in this study.

Strains &			
Plasmids	Relevant characteristics	Reference or source	
	LT2 $\Delta araBAD1065::hilD^+$ rflM3::MudJ		
EM3	ΔinvH-sprB::FCF	This study	
	LT2 $\Delta araBAD1065::hilD^+$ flhC5213::MudJ		
EM4	ΔinvH-sprB::FCF	This study	
	LT2 $\Delta araBAD1065::hilD^+$ rflM3::MudJ		
EM20	ΔinvH-sprB::FCF rtsB::TPOP	This study	
<b>F1</b> (42)	$LT2  \Delta araBAD1065::hilD+ rtsB::T-POP$		
EM43	$flhC5213::MudJ \Delta invH-sprB (\Delta spi-1)$	This study	
	L12 DaraBAD1065::hilD rflM3::MudJ		
EM50	AfthDC7002EDT	This study	
ENIJU	$\frac{\Delta g m D C 7902 \Gamma K I}{I T 2 A ara P A D 025 t at P A flb C 5213 Mu dL}$		
EM07	$L12$ $\Delta u r u D A D 925 le i KA jui C 5215 Muu J\Delta i m H s n r B \cdots E C E$	This study	
EN197	$\Delta m m s p r D C \Gamma$	This study	
	$14028c AaraBAD 1065 \cdots hilD^+ flbC5213 \cdots Mudl$		
EM640	$140288 \Delta arabAD1005naD jinc 5215viuas$	This study	
LIVI040	14028s AgraBAD1005. FRT flbC5213. MudI		
EM665	140203 201 0DAD 10051 K1 juie 5215viuus	This study	
Linous	14028s AaraBAD1065::hilD <sup>+</sup> flhC5213::MudI	This study	
	AinvH-sprB::FCF		
EM667		This study	
	14028s ДагаBAD1005::FRT flhC5213::MudJ		
	ΔinvH-sprB::FCF		
EM674		This study	
EM706	LT2 P(flhDC)8093 (PflhDC-luxCDBAE-Km-		
	$PflhDC+) \Delta araBAD1005::FRT$	This study	
EM707	LT2 P( <i>flhDC</i> )8124 (P <i>flhDC</i> P1+ (-10 of		
	P2,P3,P4,P5,P6 changed to GTTGGT)-		
	<i>luxCDBAE</i> -Km-PflhDC <sup>+</sup> ) ∆araBAD::FRT	This study	
EM708	L12 P(flhDC) 8125 (PflhDC P2+ (-10 of D1 P2 P4 P5 P6 P2))		
	P1,P3,P4,P5,P6 changed to GTTGGT)-		
EN/700	$IUXCDBAE-KM-PfinDC^{*}) \Delta araBAD1005::FK1$	This study	
EM/09	$L12 P(flnDC) \otimes 126 (PflnDC P3+ (-10 of P1 P2 P4 P5 P6 - channel to CTTCCT)$		
	P1, P2, P4, P3, P0 changed to $G11GG1$ )- hurCDBAEKm DflhDC+) AgraBAD1005EDT	This study	
EM710	I T2 P(flhDC) 8127 (DflhDC P4 + (10 of		
	P1 P2 P3 P5 P6 changed to GTTGGT)		
	$lurCDBAF_Km_PflhDC^+$ ) AgraBAD1005FRT	This study	
EM711	LT2 P(fhDC) 8128 (PfhDC P5+ (-10 of		
	P1 P2 P3 P4 P6 changed to GTTGGT)-		
	$ uxCDBAE-Km-PflhDC^+\rangle \Delta araBAD1005::FRT$		
	ΔinvH-sprB::FCF	This study	
EM712	LT2 P(flhDC)8129 (PflhDC P6+ (-10 of		
	P1,P2,P3,P4,P5 changed to GTTGGT)-	This study	

	<i>luxCDBAE</i> -Km- <i>PflhDC</i> <sup>+</sup> ) ΔaraBAD1005::FRT		
EM713	LT2 P(flhDC)8093 (PflhDC-luxCDBAE-Km-		
	$PflhDC^+$ ) $\Delta araBAD1065:: hilD^+$	This study	
EM714	LT2 P(flhDC)8124 (PflhDC P1+ (-10 of		
	P2,P3,P4,P5,P6 changed to GTTGGT)-		
	$luxCDBAE$ -Km-PflhDC <sup>+</sup> ) $\Delta araBAD1065$ ::		
	$hilD^+$	This study	
EM715	LT2 P(flhDC)8125 (PflhDC P2+ (-10 of		
	P1,P3,P4,P5,P6 changed to GTTGGT)-		
	$luxCDBAE$ -Km-PflhDC <sup>+</sup> ) $\Delta araBAD1065$ ::		
	hilD <sup>+</sup>	This study	
EM716	LT2 P(flhDC)8126 (PflhDC P3+ (-10 of		
	P1,P2,P4,P5,P6 changed to GTTGGT)-		
	$luxCDBAE$ -Km-PflhDC <sup>+</sup> ) $\Delta araBAD1065$ ::		
	$hilD^+$	This study	
EM717	LT2 P( <i>flhDC</i> )8127 (P <i>flhDC</i> P4+ (-10 of		
	P1,P2,P3,P5,P6 changed to GTTGGT)-		
	$luxCDBAE$ -Km-PflhDC <sup>+</sup> ) $\Delta araBAD1065$ ::		
	$hilD^+$	This study	
EM718	LT2 P( <i>flhDC</i> )8128 (P <i>flhDC</i> P5+ (-10 of		
	P1,P2,P3,P4,P6 changed to GTTGGT)-		
	$luxCDBAE$ -Km-PflhDC <sup>+</sup> ) $\Delta araBAD1065::$		
	hilD <sup>+</sup>	This study	
EM719	LT2 P( <i>flhDC</i> )8129 (P <i>flhDC</i> P6+ (-10 of		
	P1,P2,P3,P4,P5 changed to GTTGGT)-		
	$luxCDBAE$ -Km-P $flhDC^+$ )		
	$\Delta araBAD1065::hilD^+$	This study	
	LT2 P(flhDC)8093 (PflhDC-luxCDBAE-Km-		
	$PflhDC+)$ $\Delta araBAD1005::FRT \Delta invH-$		
EM/34	sprB::FCF	This study	
	L12 P(fhDC) 8124 (PfhDC PI+ (-10 of PI))		
	P2,P3,P4,P5,P6 changed to $GTIGGI$ -		
EN/725	<i>luxCDBAE</i> -Km-PfinDC <sup>*</sup> ) <i>DaraBAD</i> ::FK1	This star lar	
EM/35	$\Delta INVH-sprB::FCF$	This study	
	$L12 P(finDC) \otimes 125 (PfinDC P2+ (-10 of P1 P2 P4 P5 P( -10 of P1 P2 P4 P5 P( -10 of P2 P4 P5 P( -10 of P2 P4 P5 P( -10 of P2 P4 P5 $		
	P1,P3,P4,P3,P6 changed to $G11GG1$ )-		
EM736	$M_{A}CDDAE-KIII-FJIIIDC) \Delta arabAD 1005FK1$	This study	
Elvi730	$\frac{\Delta (h)(H-sp)DPCP}{IT2 D(flhDC) 8126 (DflhDC D2 + (10 of))}$		
	$P_1 P_2 P_4 P_5 P_6$ changed to $P_3 P_4 P_5 P_6$		
	$lurCDBAF_Km_PflhDC^+$ ) AaraBAD1005ERT		
FM737	AinvH_sprR::FCF	This study	
LINITST	$I T2 P(flhDC) \otimes 127 (PflhDC) \otimes 127$	This study	
	P1.P2.P3.P5.P6 changed to GTTGGT)		
	$luxCDBAF-Km-PflhDC^+$ ) $AaraBAD1005\cdots$ FRT		
EM738	AinvH-sprB::FCF	This study	
	LT2 P(flhDC)8128 (PflhDC P5+ (-10 of		
	P1.P2.P3.P4.P6 changed to GTTGGT)		
	<i>luxCDBAE</i> -Km-PflhDC <sup>+</sup> ) AaraBAD1005::FRT		
EM739	$\Delta invH-sprB::FCF$	This study	

	LT2 P( <i>flhDC</i> )8129 (P <i>flhDC</i> P6+ (-10 of	
	P1,P2,P3,P4,P5 changed to G11GG1)- $lurCDBAFKm PflbDC^+$ AaraBAD1005EBT	
EM740	ΔinvH-sprB::FCF	This study
	LT2 P(flhDC)8093 (PflhDC-luxCDBAE-Km-	
	$PflhDC^+$ ) $\Delta araBAD1065:: hilD^+$	
EM741	ΔinvH-sprB::FCF	This study
	L12 $P(fhDC)$ 8124 ( $PfhDC$ P1+ (-10 of P2 P2 P4 P5 P6 shoreed to CTTCCT)	
	$P_2, P_3, P_4, P_3, P_6$ changed to $OIIOOI)$ - $lurCDBAF_Km_PflhDC^+$ $AaraBAD1065$ .	
	hilD <sup>+</sup> <i>AinvH-sprB</i> ::FCF	
EM742	1 -	This study
	LT2 P(flhDC)8125 (PflhDC P2+ (-10 of	
	P1,P3,P4,P5,P6 changed to GTTGGT)-	
	$luxCDBAE-Km-PflhDC^{+})$ $\Delta araBAD1065::$	
FM743	nud Диллн-sprb.:FCF	This study
	LT2 P( <i>flhDC</i> )8126 (P <i>flhDC</i> P3+ (-10 of	This study
	P1,P2,P4,P5,P6 changed to GTTGGT)-	
	$luxCDBAE$ -Km-PflhDC <sup>+</sup> ) $\Delta araBAD1065$ ::	
	<i>hilD</i> <sup>+</sup> <i>∆invH-sprB</i> ::FCF	
EM744	$\mathbf{L} = \mathbf{D} \left( \frac{\partial \mathbf{D}}{\partial t} \mathbf{D} \right) \mathbf{D} \left( \mathbf{D} \right) \mathbf{D} \left$	This study
	$L12 P(finDC) \otimes 127 (PfinDC P4+ (-10 of P1 P2 P3 P5 P6 changed to CTTGGT)$	
	$luxCDBAE-Km-PflhDC^+$ $AaraBAD1065::$	
	hilD <sup>+</sup> ∆invH-sprB::FCF	
EM745	-	This study
	LT2 P( <i>flhDC</i> )8128 (P <i>flhDC</i> P5+ (-10 of	
	P1,P2,P3,P4,P6 changed to GTTGGT)-	
	$uxCDBAE-KM-PJInDC^{+}) \qquad \Delta araBAD1005::$ $hilD^{+} AimuH snrB:ECE$	
EM746		This study
	LT2 P( <i>flhDC</i> )8129 (P <i>flhDC</i> P6+ (-10 of	
	P1,P2,P3,P4,P5 changed to GTTGGT)-	
	<i>luxCDBAE</i> -Km-P <i>flhDC</i> <sup>+</sup> )	
	ΔaraBAD1065::hilD <sup>+</sup> ΔinvH-sprB::FCF	This step he
EN1/4/	$\mathbf{L} \mathbf{T} 2 = \mathbf{A} \mathbf{z} \mathbf{z} \mathbf{z} \mathbf{B} \mathbf{A} \mathbf{D} 1 0 4 5 \dots \mathbf{L} \mathbf{i} \mathbf{D}^{\dagger} = \mathbf{f} \mathbf{i} \mathbf{D} 5 0 0 1 \dots \mathbf{M} \mathbf{n} \mathbf{d} \mathbf{L}$	I his study
	$L12 \Delta araBAD1005::nuD JIJB5001::MudJ Ahin-5718::FRT PflhDC5451::Tn10dTc[del-$	
EM801	$\Delta hin-5718$ ::FRT PflhDC5451::Tn10dTc[del- 25]	This study
EM801	$\Delta hin-5718::FRT PflhDC5451::Tn10dTc[del-25] LT2 \Delta araBAD1065::hilD+ fliL5100::MudJ$	This study
EM801 EM802	$ \begin{array}{c} \Delta hin-5718::FRT  PflhDC5451::Tn10dTc[del-25] \\ LT2  \Delta araBAD1065::hilD^+  fliL5100::MudJ \\ PflhDC5451::Tn10dTc[del-25] \end{array} $	This study This study
EM801 EM802	$ \begin{array}{c} \Delta hin-5718::FRT  PflhDC5451::Tn10dTc[del-25] \\ LT2  \Delta araBAD1065::hilD^+  fliL5100::MudJ \\ PflhDC5451::Tn10dTc[del-25] \\ LT2  \Delta araBAD1065::hilD^+  flhC5213::MudJ \\ Defined a finite fin$	This study This study
EM801 EM802 EM804	$ \begin{array}{c} \Delta hin-5718::FRT  PflhDC5451::Tn10dTc[del-25] \\ LT2  \Delta araBAD1065::hilD^+  fliL5100::MudJ \\ PflhDC5451::Tn10dTc[del-25] \\ LT2  \Delta araBAD1065::hilD^+  flhC5213::MudJ \\ PflhDC5451::Tn10dTc[del-25] \\ LT2  \Delta araBAD1065::hilD^+  flhC5213::MudJ \\ PflhDC5451::Tn10dTc[del-25] \\ \end{array} $	This study This study This study
EM801 EM802 EM804	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	This study This study This study
EM801 EM802 EM804 EM827	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	This study This study This study This study
EM801 EM802 EM804 EM827	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	This study This study This study This study
EM801 EM802 EM804 EM827 EM858	L12       ΔaraBAD1005::httD $jijB5001::MtdJ$ Δhin-5718::FRT $PflhDC5451::Tn10dTc[del-25]$ LT2       ΔaraBAD1065::hilD <sup>+</sup> $fliL5100::MudJ$ $PflhDC5451::Tn10dTc[del-25]$ LT2       ΔaraBAD1065::hilD <sup>+</sup> $flhC5213::MudJ$ $PflhDC5451::Tn10dTc[del-25]$ LT2       ΔaraBAD1005::FRT $flhC5213::MudJ$ $PflhDC5451::Tn10dTc[del-25]$ LT2       ΔaraBAD1005::FRT $flhC::MudJ$ $PflhDC5451::Tn10dTc[del-25]$ LT2       ΔaraBAD1005::FRT $flhC::MudJ$ $PflhDC5451::Tn10dTc[del-25]$ $flhC5213::MudJ$	This study This study This study This study This study
EM801 EM802 EM804 EM827 EM858	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	This study This study This study This study This study This study

	<i>fliL5100</i> ::Mu <i>d</i> J	
	LT2 $\Delta araBAD1182::hilD\Delta HTH$	
	<i>P(flhDC)5451</i> ::Tn10 <i>d</i> Tc[ <i>del</i> -25]	
EM869	<i>fljB5001</i> ::MudJ <i>Δhin-5718</i> ::FRT	This study
	LT2 ΔaraBAD1182::hilDΔHTH	
EM885	<i>fljB5001</i> ::MudJ <i>Δhin-5718</i> ::FCF	This study
	LT2 $\Delta araBAD1182::hilD\Delta HTH$	
EM886	<i>flhC5213</i> ::Mu <i>d</i> J	This study
	LT2 $\Delta araBAD1182::hilD\Delta HTH$	
E) (007	<i>fliL5100</i> ::Mu <i>d</i> J	
EM887		This study
EM027	L12 ΔaraBAD1005::FR1 rtsB::1-POP flbC5212Ma dL Aim H ampDuECE (A mi 1)	This study
EM937	$JINC5215::MUAJ \Delta INVH-SprB::FCF (\Delta spi-1)$	This study
EM1009	L12 DaraBAD1183::hilA fijB5001::Muaj	This study
EMI010	LT2 ΔaraBAD1005::FRT ftjB5001::MudJ	This study
EM1011	LT2 $\Delta araBAD1183::hilA^+ flhC5213::MudJ$	This study
EM1018	LT2 <i>DaraBAD1005</i> ::FRT <i>fliL5100</i> ::MudJ	This study
EM1019	LT2 ΔaraBAD1183::hilA <sup>+</sup> fliL5100::MudJ	This study
EM1048	LT2 P( <i>flhDC</i> )8093 (P <i>flhDC-luxCDBAE</i> -Km-	
<b>EN</b> (10,40)	$PflhDC^+$ ) $\Delta araBAD1065:: hilD^+$	This study
EM1049	L12 P(flhDC) 8124 (PflhDC P1+ (-10 of P2 P2 P4 P5 PC))	
	P2,P3,P4,P5,P6 changed to $GIIGGI)$ -	
	$uxCDBAE-Km-PfinDC^{+}$ $\Delta araBAD1109::$	This study
EM1050	$\frac{flsD}{LT2} = \frac{D(flbDC) \otimes 125}{D(flbDC} = \frac{D2}{L} + \frac{flbDC}{LT2} + flb$	
ENITOSO	P1 P3 P4 P5 P6 changed to $GTTGGT$	
	$lurCDBAF_Km_PflhDC^+$ $AaraBAD1109$ .	
	$rtsB^+$	This study
EM1051	LT2 $P(flhDC)$ 8126 ( $PflhDC$ P3+ (-10 of	
	P1,P2,P4,P5,P6 changed to GTTGGT)-	
	$luxCDBAE$ -Km-PflhDC <sup>+</sup> ) $\Delta araBAD1109::$	
	rtsB <sup>+</sup>	This study
EM1052	LT2 P(flhDC)8127 (PflhDC P4+ (-10 of	
	P1,P2,P3,P5,P6 changed to GTTGGT)-	
	$luxCDBAE$ -Km-PflhDC <sup>+</sup> ) $\Delta araBAD1109$ ::	
	rtsB <sup>+</sup>	This study
EM1053	LT2 P( <i>flhDC</i> )8128 (P <i>flhDC</i> P5+ (-10 of	
	P1,P2,P3,P4,P6 changed to GTTGGT)-	
	$luxCDBAE$ -Km-PflhDC <sup>+</sup> ) $\Delta araBAD1109::$	
EN41054	rtsB	This study
EM1054	$L12 P(finDC) \otimes 129 (PfinDC PO+ (-10 OF))$	
	$h_{\mu\nu}CDRAF Km PflbDC^{+}$ $AaraBAD1100^{-1}$	
	$rtsB^+$	This study
	LT2 pJS28(Ap <sup>R</sup> P22-9 <sup>+</sup> )/F'114 <sup>ts</sup> Lac <sup>+</sup> $77f_{-}$	- Ins stary
	20::Tn10[tetA::MudPl(TcS)	
TH3923	3823::Tn10dTc[del-25]/leuA414 hsdSB Fels2	Lab collection
TH6701	LT2 ΔaraBAD925::tetRA (ΔaraBAD(aa1-73))	Lab collection
	LT2 $\Delta araBAD1005::FCF$	
TH13659	<i>PflhDC5451::</i> Tn10 <i>d</i> Tc[ <i>del</i> -25]	Lab collection

	<i>flhC5213::</i> Mu <i>d</i> J	
TH13751	LT2 ДагаBAD1005::FCF flhC5213::MudJ	Lab collection
TH13752	LT2 ДагаBAD1005::FCF fliL5100::MudJ	Lab collection
	LT2 ΔaraBAD1005::FCF	
	<i>PflhDC5451</i> ::Tn10dTc[del-25]	
TH13919	fliL5100::MudJ	Lab collection
	LT2 ΔaraBAD1005::FCF fljB5001::MudJ	
TH14571	Δhin-5718::FRT	Lab collection
	LT2 ΔaraBAD1005::FCF	
	<i>PflhDC5451::</i> Tn <i>10d</i> Tc[ <i>del-</i> 25]	
TH14845	<i>fljB5001</i> ::MudJ <i>∆hin</i> -5718::FRT	Lab collection
TH16339	LT2 $\Delta araBAD1065::hilD^+$	This study
TH16385	LT2 ∆araBAD1065::hilD <sup>+</sup> fliL5100::MudJ	This study
TH16386	LT2 ДагаBAD1065::hilD+ flhC5213::MudJ	This study
	LT2 $\Delta araBAD1065::hilD^+$ fljB5001::MudJ	
TH16423	Δhin-5718::FRT	This study

### Supplemental Table 2: Oligonucleotide sequences used for quantitative real time PCR

analysis of gene expression in Salmonella.

Primer name	5'-3' sequence
flhDC-fw	GTAGGCAGCTTTGCGTGTAG
flhDC-rv	TCCAGCAGTTGTGGAATAATATCG
gmk-fw	TTGCAGAAATGAGCCATTACGCCG
gmk-rv	GACGTTCAGCGCGAATGATGGTTT
gyrB-fw	CTGCTCAAAGAGCTGGTGTATCA
gyrB-rv	AGCGCGTTACAGTCTGCTCAT
rflM-fw	TCTCAACGATGCCTTACCCGAACA
rflM-rv	GCAAGCTCATGTAAAGGCGTGTGT
rpoB-fw	CAACCTGTTCGTACGTATCGAC
rpoB-rv	CAGCTCCATCTGCAGTTTGTTG
rpoD-fw	CAACAGTATGCGCGTGATGAT
rpoD-rv	CGACGCAGAGCTTCATGATC

#### Supplemental Figure S1: HilD increases rflM expression via flhDC

**A**. Quantitative real-time PCR comparing *rflM* mRNA levels of strain TH6701 ( $P_{ara}$ ::*tetRA*) and TH16339 ( $P_{ara}$ ::*hilD*<sup>+</sup>) upon induction by arabinose. The mRNA levels were analyzed from at least three independent biological samples. Biological replicates are shown as individual data points (diamonds) in all figures.

**B**. Relative *rflM* expression analyzed in a  $\beta$ -galactosidase assay using an *rflM-lac* reporter system described above. *rflM* gene expression was analyzed in EM3 ( $\Delta araBAD$ ::*hilD*<sup>+</sup>  $\Delta invH$ -*sprB*::FCF *rflM*::MudJ, labeled 2), EM20 ( $\Delta araBAD$ ::*hilD*<sup>+</sup> *rtsB*::TPOP  $\Delta invH$ -*sprB*::FCF *rflM*::MudJ, labeled 3), EM50 ( $\Delta araBAD$ ::*hilD*<sup>+</sup> *rtsB*::TPOP  $\Delta flhDC$   $\Delta invH$ -*sprB*::FCF *rflM*::MudJ, labeled 4). *hilD* transcription was induced by the addition of 0.2% arabinose. Expression was normalized to the wild-type control EM126 ( $\Delta araBAD$ ::*tetRA*  $\Delta invH$ -*sprB*::FCF *rflM*::MudJ, labeled 1). Six independent biological replicates were tested and error bars represent the standard error of the mean.

#### Supplemental Figure S2: Effect of HilA on flagellar gene expression.

The effect of overproduced *hilA* under the control of the *araBAD* promoter on the expression of the flagellar genes *flhC* (class 1), *fliL* (class 2) and *fljB* (class 3) in a  $\beta$ -galactosidase assay. Expression of HilA was induced by addition of 0.2% arabinose in the following strains: (i) *flhC* (class 1): EM517 (P<sub>ara</sub>::FRT *flhC*::MudJ), EM1011(P<sub>ara</sub>::*hilA*<sup>+</sup> *flhC*::MudJ); (ii) *fliL* (class 2): EM1018 (P<sub>ara</sub>::FRT *fliL*::MudJ), EM1019 (P<sub>ara</sub>::*hilA*<sup>+</sup> *fliL*::MudJ); and (iii) *fljB* (class 3): EM1010 (P<sub>ara</sub>::FRT *fljB*::MudJ), EM1009 (P<sub>ara</sub>::*hilA*<sup>+</sup> *fljB*::MudJ). Biological replicates are shown as individual data points (diamonds).

Supplemental Figure S3: HilD and RtsB act on different promoters to activate and repress *flhDC* operon transcription

A. RtsB and HilD were simultaneously induced by addition of arabinose and anhydrotetracycline (left panel); HilD was induced by addition of arabinose (middle panel); or RtsB was induced by addition of anhydrotetracycline (right panel). Expression of *flhD-lac* was monitored in a  $\beta$ -galactosidase assay. A (+\*) indicates the presence of the *rtsB* gene, however *rtsAB* is likely not expressed due to the absence of its activator HilD. Strains used were EM827 ( $\Delta araBAD$ ::FRT *flhC*::MudJ  $\Delta invH$ -sprB::FCF), EM937 ( $\Delta araBAD$ ::FRT *flhC*::MudJ  $\Delta invH$ -sprB::FCF); EM43 ( $\Delta araBAD$ ::hilD<sup>+</sup> *flhC*::MudJ  $\Delta invH$ -sprB::FCF); EM43 ( $\Delta araBAD$ ::hilD<sup>+</sup> *flhC*::MudJ  $\Delta invH$ -sprB::FCF); EM43 ( $\Delta araBAD$ ::hilD<sup>+</sup> *flhC*::MudJ  $\Delta invH$ -sprB::FCF).

**B**. *flhDC* transcription from individual *flhDC* promoters under conditions when HilD or RtsB were overproduced was analyzed using a (*luxDCABE*-Km)-*flhDC* promoter fusion. As outlined in detail in Figure 1E, the entire *flhDC* promoter region with GTTGGT -10 box mutations of five of the six known transcriptional *flhDC* start sites was fused to a *luxCDBAE*-kanamycin cassette. Luminescence is shown relative to  $P_{ara}$ ::FRT *P*<sub>*fhDC*</sub>(P1-6)-*luxCDBAE*-Km-P<sub>*fhD+C+*</sub>. Strains used were EM706-712 ( $P_{ara}$ ::FRT), EM713-719 ( $P_{ara}$ ::*hilD*<sup>+</sup>) and EM1048-1054 ( $P_{ara}$ ::*rtsB*<sup>+</sup>). Error bars represent the standard error of the mean. Data were analyzed by the Student's *t* test. Stars indicate the gene expression levels that differed significantly with \*\* (P<0.01).

# **Supplemental Figure S1**



# **Supplemental Figure S2**



## **Supplemental Figure S3**





Β