Supplemental Table 1: *Salmonella enterica serovar* Typhimurium LT2 and ATCC 14028s (ST14028) strains used in this study.

Strains & Plasmids	Relevant characteristics	Reference or source
EM3	LT2 Δ*araBAD1065::hilD*$^+$ *rflM3*::Mu*dJ* Δ*invH-sprB*::FCF	This study
EM4	LT2 Δ*araBAD1065::hilD*$^+$ *flhC5213*::Mu*dJ* Δ*invH-sprB*::FCF	This study
EM20	LT2 Δ*araBAD1065::hilD*$^+$ *rflM3*::Mu*dJ* Δ*invH-sprB*::FCF *rtsB*::TPOP	This study
EM43	LT2 Δ*araBAD1065*::*hilD*+ *rtsB*::T-POP *flhC5213*::Mu*dJ* Δ*invH-sprB* (Δ*spi-1*)	This study
EM50	LT2 Δ*araBAD1065::hilD*$^+$ *rflM3*::Mu*dJ* Δ*invH-sprB*::FCF *rtsB*::TPOP Δ*flhDC7902*::FRT	This study
EM97	LT2 Δ*araBAD925*::*tetRA* *flhC5213*::Mu*dJ* Δ*invH-sprB*::FCF	This study
EM517	LT2 Δ*araBAD1005*::FRT *flhC5213*::Mu*dJ*	This study
EM640	14028s Δ*araBAD1065::hilD*$^+$ *flhC5213*::Mu*dJ*	This study
EM665	14028s Δ*araBAD1005*::FRT *flhC5213*::Mu*dJ*	This study
EM667	14028s Δ*araBAD1065::hilD*$^+$ *flhC5213*::Mu*dJ* Δ*invH-sprB*::FCF	This study
EM674	14028s Δ*araBAD1005*::FRT *flhC5213*::Mu*dJ* Δ*invH-sprB*::FCF	This study
EM706	LT2 P(*flhDC*)8093 (P*flhDC-luxCDBAE*-Km-P*flhDC*+) Δ*araBAD*1005::FRT	This study
EM707	LT2 P(*flhDC*)8124 (P*flhDC* P1+ (-10 of P2,P3,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) Δ*araBAD*::FRT	This study
EM708	LT2 P(*flhDC*)8125 (P*flhDC* P2+ (-10 of P1,P3,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) Δ*araBAD*1005::FRT	This study
EM709	LT2 P(*flhDC*)8126 (P*flhDC* P3+ (-10 of P1,P2,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) Δ*araBAD*1005::FRT	This study
EM710	LT2 P(*flhDC*)8127 (P*flhDC* P4+ (-10 of P1,P2,P3,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) Δ*araBAD*1005::FRT	This study
EM711	LT2 P(*flhDC*)8128 (P*flhDC* P5+ (-10 of P1,P2,P3,P4,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) Δ*araBAD*1005::FRT Δ*invH-sprB*::FCF	This study
EM712	LT2 P(*flhDC*)8129 (P*flhDC* P6+ (-10 of P1,P2,P3,P4,P5 changed to GTTGGT)-	This study

	luxCDBAE-Km-*PflhDC*$^+$) *ΔaraBAD*1005::FRT	
EM713	LT2 P(*flhDC*)8093 (P*flhDC-luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1065:: *hilD*$^+$	This study
EM714	LT2 P(*flhDC*)8124 (P*flhDC* P1+ (-10 of P2,P3,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1065:: *hilD*$^+$	This study
EM715	LT2 P(*flhDC*)8125 (P*flhDC* P2+ (-10 of P1,P3,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1065:: *hilD*$^+$	This study
EM716	LT2 P(*flhDC*)8126 (P*flhDC* P3+ (-10 of P1,P2,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1065:: *hilD*$^+$	This study
EM717	LT2 P(*flhDC*)8127 (P*flhDC* P4+ (-10 of P1,P2,P3,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1065:: *hilD*$^+$	This study
EM718	LT2 P(*flhDC*)8128 (P*flhDC* P5+ (-10 of P1,P2,P3,P4,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1065:: *hilD*$^+$	This study
EM719	LT2 P(*flhDC*)8129 (P*flhDC* P6+ (-10 of P1,P2,P3,P4,P5 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1065::*hilD*$^+$	This study
EM734	LT2 P(*flhDC*)8093 (P*flhDC-luxCDBAE*-Km-P*flhDC*+) *ΔaraBAD*1005::FRT *ΔinvH-sprB*::FCF	This study
EM735	LT2 P(*flhDC*)8124 (P*flhDC* P1+ (-10 of P2,P3,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*::FRT *ΔinvH-sprB*::FCF	This study
EM736	LT2 P(*flhDC*)8125 (P*flhDC* P2+ (-10 of P1,P3,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1005::FRT *ΔinvH-sprB*::FCF	This study
EM737	LT2 P(*flhDC*)8126 (P*flhDC* P3+ (-10 of P1,P2,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1005::FRT *ΔinvH-sprB*::FCF	This study
EM738	LT2 P(*flhDC*)8127 (P*flhDC* P4+ (-10 of P1,P2,P3,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1005::FRT *ΔinvH-sprB*::FCF	This study
EM739	LT2 P(*flhDC*)8128 (P*flhDC* P5+ (-10 of P1,P2,P3,P4,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1005::FRT *ΔinvH-sprB*::FCF	This study

EM740	LT2 P(*flhDC*)8129 (P*flhDC* P6+ (-10 of P1,P2,P3,P4,P5 changed to GTTGGT)-*luxCDBAE*-Km-*PflhDC*$^{+}$) *ΔaraBAD*1005::FRT *ΔinvH-sprB*::FCF	This study
EM741	LT2 P(*flhDC*)8093 (P*flhDC-luxCDBAE*-Km-P*flhDC*$^{+}$) *ΔaraBAD*1065:: *hilD*$^{+}$ *ΔinvH-sprB*::FCF	This study
EM742	LT2 P(*flhDC*)8124 (P*flhDC* P1+ (-10 of P2,P3,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^{+}$) *ΔaraBAD*1065:: *hilD*$^{+}$ *ΔinvH-sprB*::FCF	This study
EM743	LT2 P(*flhDC*)8125 (P*flhDC* P2+ (-10 of P1,P3,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^{+}$) *ΔaraBAD*1065:: *hilD*$^{+}$ *ΔinvH-sprB*::FCF	This study
EM744	LT2 P(*flhDC*)8126 (P*flhDC* P3+ (-10 of P1,P2,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^{+}$) *ΔaraBAD*1065:: *hilD*$^{+}$ *ΔinvH-sprB*::FCF	This study
EM745	LT2 P(*flhDC*)8127 (P*flhDC* P4+ (-10 of P1,P2,P3,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^{+}$) *ΔaraBAD*1065:: *hilD*$^{+}$ *ΔinvH-sprB*::FCF	This study
EM746	LT2 P(*flhDC*)8128 (P*flhDC* P5+ (-10 of P1,P2,P3,P4,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^{+}$) *ΔaraBAD*1065:: *hilD*$^{+}$ *ΔinvH-sprB*::FCF	This study
EM747	LT2 P(*flhDC*)8129 (P*flhDC* P6+ (-10 of P1,P2,P3,P4,P5 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^{+}$) *ΔaraBAD*1065::*hilD*$^{+}$ *ΔinvH-sprB*::FCF	This study
EM801	LT2 *ΔaraBAD1065::hilD*$^{+}$ *fljB5001*::Mu*d*J Δ*hin-5718*::FRT *PflhDC5451*::Tn*10d*Tc[*del*-25]	This study
EM802	LT2 Δ*araBAD1065::hilD*$^{+}$ *fliL5100*::Mu*d*J *PflhDC5451*::Tn*10d*Tc[*del*-25]	This study
EM804	LT2 Δ*araBAD1065::hilD*$^{+}$ *flhC5213*::Mu*d*J *PflhDC5451*::Tn*10d*Tc[*del*-25]	This study
EM827	LT2 Δ*araBAD1005*::FRT *flhC*::Mu*d*J Δ*invH-sprB*::FCF (Δspi-1)	This study
EM858	LT2 Δ*araBAD1182*::*hilD*ΔHTH *PflhDC5451*::Tn*10d*Tc[*del*-25] *flhC5213*::Mu*d*J	This study
EM868	LT2 Δ*araBAD1182::hilD*ΔHTH *P(flhDC)5451*::Tn*10d*Tc[*del*-25]	This study

	fliL5100::Mu*d*J	
EM869	LT2 Δ*araBAD1182::hilD*ΔHTH *P(flhDC)5451*::Tn*10d*Tc[*del*-25] *fljB5001*::Mu*d*J Δ*hin-5718*::FRT	This study
EM885	LT2 Δ*araBAD1182::hilD*ΔHTH *fljB5001*::Mu*d*J Δ*hin-5718*::FCF	This study
EM886	LT2 Δ*araBAD1182*::*hilD*ΔHTH *flhC5213*::Mu*d*J	This study
EM887	LT2 Δ*araBAD1182*::*hilD*ΔHTH *fliL5100*::Mu*d*J	This study
EM937	LT2 Δ*araBAD1005*::FRT *rtsB*::T-POP *flhC5213*::Mu*d*J *ΔinvH-sprB*::FCF (Δspi-1)	This study
EM1009	LT2 Δ*araBAD1183::hilA*$^+$ *fljB5001*::Mu*d*J	This study
EM1010	LT2 Δ*araBAD1005*::FRT *fljB5001*::Mu*d*J	This study
EM1011	LT2 Δ*araBAD1183::hilA*$^+$ *flhC5213*::Mu*d*J	This study
EM1018	LT2 Δ*araBAD1005*::FRT *fliL5100*::Mu*d*J	This study
EM1019	LT2 Δ*araBAD1183*::*hilA*$^+$ *fliL5100*::Mu*d*J	This study
EM1048	LT2 P(*flhDC*)8093 (P*flhDC-luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1065:: *hilD*$^+$	This study
EM1049	LT2 P(*flhDC*)8124 (P*flhDC* P1+ (-10 of P2,P3,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1109:: *rtsB*$^+$	This study
EM1050	LT2 P(*flhDC*)8125 (P*flhDC* P2+ (-10 of P1,P3,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1109:: *rtsB*$^+$	This study
EM1051	LT2 P(*flhDC*)8126 (P*flhDC* P3+ (-10 of P1,P2,P4,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1109:: *rtsB*$^+$	This study
EM1052	LT2 P(*flhDC*)8127 (P*flhDC* P4+ (-10 of P1,P2,P3,P5,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1109:: *rtsB*$^+$	This study
EM1053	LT2 P(*flhDC*)8128 (P*flhDC* P5+ (-10 of P1,P2,P3,P4,P6 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1109:: *rtsB*$^+$	This study
EM1054	LT2 P(*flhDC*)8129 (P*flhDC* P6+ (-10 of P1,P2,P3,P4,P5 changed to GTTGGT)-*luxCDBAE*-Km-P*flhDC*$^+$) *ΔaraBAD*1109:: *rtsB*$^+$	This study
TH3923	LT2 pJS28(Ap^R P22-9$^+$)/F'114^{ts} Lac$^+$ *zzf-20*::Tn*10*[*tetA*::MudP](Tc^S) *zzf-3823*::Tn*10d*Tc[*del-25*]/*leuA414 hsdSB Fels2*$^-$	Lab collection
TH6701	LT2 Δ*araBAD925*::*tetRA* (*ΔaraBAD*(aa1-73))	Lab collection
TH13659	LT2 *ΔaraBAD1005::FCF PflhDC5451::*Tn*10d*Tc[*del*-25]	Lab collection

	*flhC5213::*Mu*dJ*	
TH13751	LT2 *ΔaraBAD1005*::FCF *flhC5213::*Mu*dJ*	Lab collection
TH13752	LT2 *ΔaraBAD1005*::FCF *fliL5100*::Mu*dJ*	Lab collection
TH13919	LT2 *ΔaraBAD1005*::FCF *PflhDC5451::*Tn*10d*Tc[*del*-25] *fliL5100::MudJ*	Lab collection
TH14571	LT2 *ΔaraBAD1005*::FCF f*ljB5001*::Mu*dJ* *Δhin-5718*::FRT	Lab collection
TH14845	LT2 *ΔaraBAD1005*::FCF *PflhDC5451::*Tn*10d*Tc[*del*-25] *fljB5001*::Mu*dJ* *Δhin*-5718::FRT	Lab collection
TH16339	LT2 *ΔaraBAD1065::hilD*$^+$	This study
TH16385	LT2 *ΔaraBAD1065::hilD*$^+$ *fliL5100*::Mu*dJ*	This study
TH16386	LT2 *ΔaraBAD1065*::*hilD*$^+$ *flhC5213*::Mu*dJ*	This study
TH16423	LT2 *ΔaraBAD1065::hilD*$^+$ f*ljB5001*::Mu*dJ* *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*$^+$) 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 β-galactosidase assay using an *rflM-lac* reporter system described above. *rflM* gene expression was analyzed in EM3 (Δ*araBAD*::*hilD*$^+$ Δ*invH-sprB*::FCF *rflM*::Mu*d*J, labeled 2), EM20 (Δ*araBAD*::*hilD*$^+$ *rtsB*::TPOP Δ*invH-sprB*::FCF *rflM*::Mu*d*J, labeled 3), EM50 (Δ*araBAD*::*hilD*$^+$ *rtsB*::TPOP Δ*flhDC* Δ*invH-sprB*::FCF *rflM*::Mu*d*J, labeled 4). *hilD* transcription was induced by the addition of 0.2% arabinose. Expression was normalized to the wild-type control EM126 (Δ*araBAD*::*tetRA* Δ*invH-sprB*::FCF *rflM*::Mu*d*J, 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.

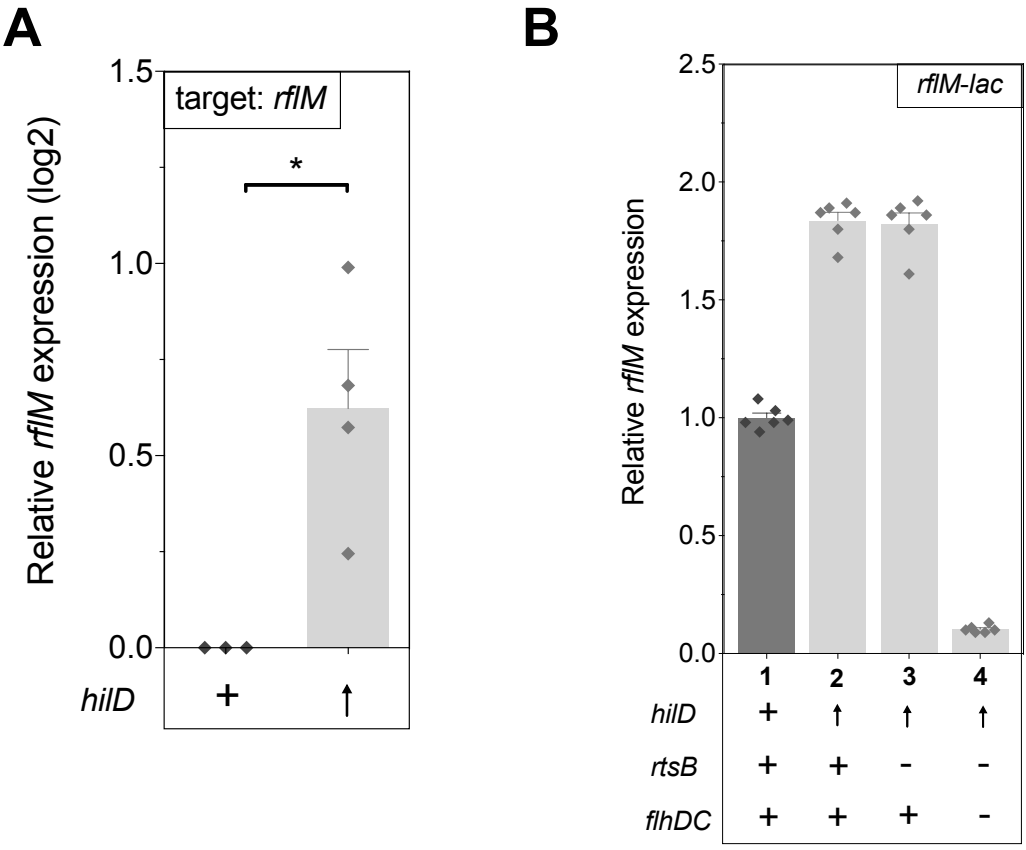
The effect of overproduced *hilA* under the control of the *araBAD* promoter on the expression of the flagellar genes *flhC* (class 1), *fliL* (class 2) and *fljB* (class 3) in a β-galactosidase assay. Expression of HilA was induced by addition of 0.2% arabinose in the following strains: (i) *flhC* (class 1): EM517 (P_{ara}::FRT *flhC*::Mu*d*J), EM1011(P_{ara}::*hilA*$^+$ *flhC*::Mu*d*J); (ii) *fliL* (class 2): EM1018 (P_{ara}::FRT *fliL*::Mu*d*J), EM1019 (P_{ara}::*hilA*$^+$ *fliL*::Mu*d*J); and (iii) *fljB* (class 3): EM1010 (P_{ara}::FRT *fljB*::Mu*d*J), EM1009 (P_{ara}::*hilA*$^+$ *fljB*::Mu*d*J). 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

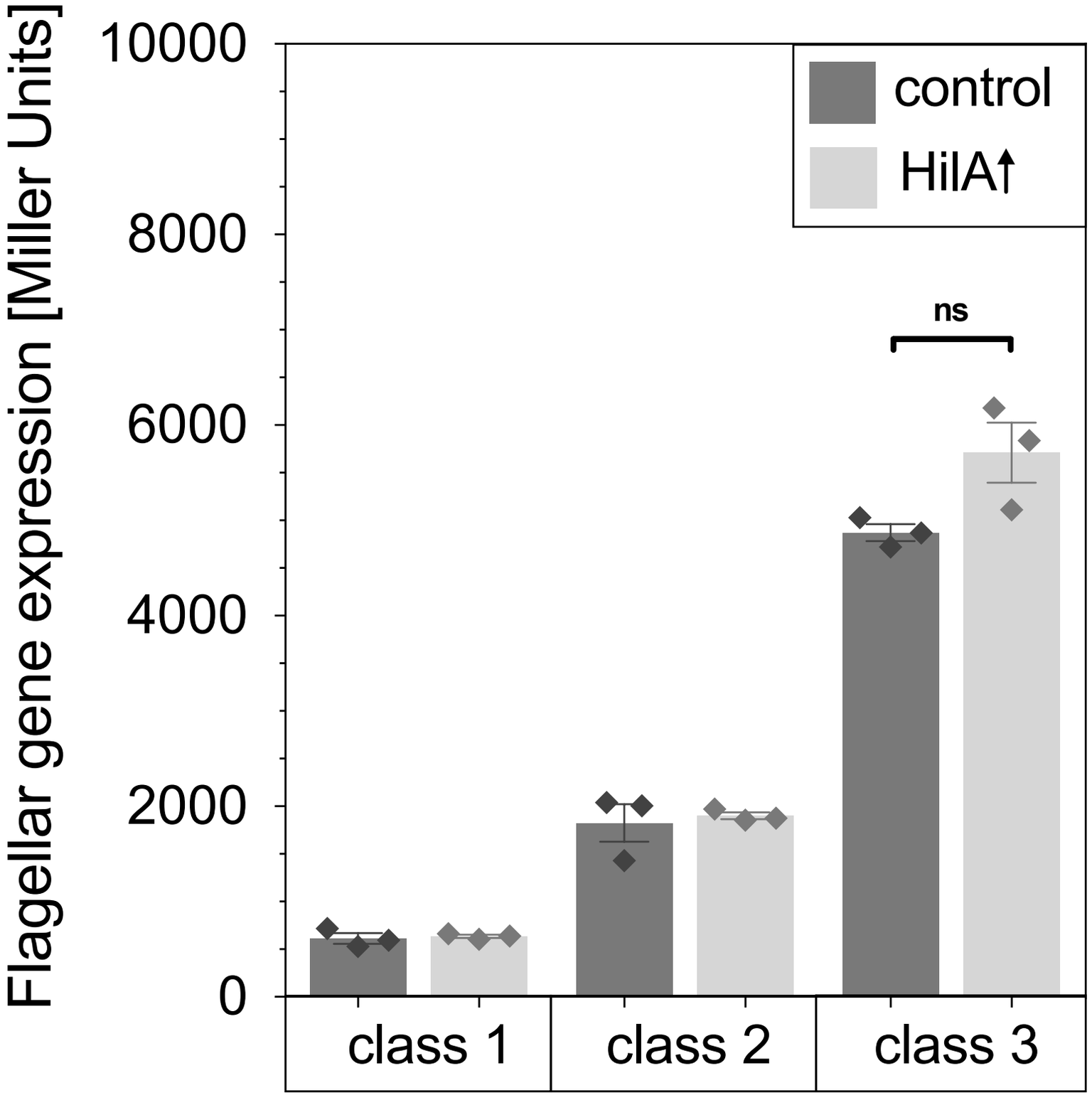
A. RtsB and HilD were simultaneously induced by addition of arabinose and anhydrotetracycline (left panel); HilD was induced by addition of arabinose (middle panel); or RtsB was induced by addition of anhydrotetracycline (right panel). Expression of *flhD-lac* was monitored in a β-galactosidase assay. 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 (Δ*araBAD*::FRT *flhC*::Mu*d*J Δ *invH-sprB*::FCF), EM937 (Δ*araBAD*::FRT *flhC*::Mu*d*J Δ *invH-sprB*::FCF *rtsB*::T-POP); EM4 (Δ*araBAD*::*hilD*$^+$ *flhC*::Mu*d*J Δ *invH-sprB*::FCF); EM43 (Δ*araBAD*::*hilD*$^+$ *flhC*::Mu*d*J Δ *invH-sprB*::FCF *rtsB*::T-POP). Biological replicates are shown as individual data points (diamonds) in all figures.

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_{flhDC}(P1-6)-*luxCDBAE*-Km-$P_{flhD+C+}$. Strains used were EM706-712 (P_{ara}::FRT), EM713-719 (P_{ara}::*hilD*$^+$) and EM1048-1054 (P_{ara}::*rtsB*$^+$). 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