

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 <i>araBAD1065::hilD</i> ⁺ <i>rflM3::MudJ</i> Δ <i>invH-sprB::FCF</i>	This study
EM4	LT2 Δ <i>araBAD1065::hilD</i> ⁺ <i>flhC5213::MudJ</i> Δ <i>invH-sprB::FCF</i>	This study
EM20	LT2 Δ <i>araBAD1065::hilD</i> ⁺ <i>rflM3::MudJ</i> Δ <i>invH-sprB::FCF</i> <i>rtsB::TPOP</i>	This study
EM43	LT2 Δ <i>araBAD1065::hilD</i> ⁺ <i>rtsB::T-POP</i> <i>flhC5213::MudJ</i> Δ <i>invH-sprB</i> (Δ <i>spi-1</i>)	This study
EM50	LT2 Δ <i>araBAD1065::hilD</i> ⁺ <i>rflM3::MudJ</i> Δ <i>invH-sprB::FCF</i> <i>rtsB::TPOP</i> Δ <i>flhDC7902::FRT</i>	This study
EM97	LT2 Δ <i>araBAD925::tetRA</i> <i>flhC5213::MudJ</i> Δ <i>invH-sprB::FCF</i>	This study
EM517	LT2 Δ <i>araBAD1005::FRT</i> <i>flhC5213::MudJ</i>	This study
EM640	14028s Δ <i>araBAD1065::hilD</i> ⁺ <i>flhC5213::MudJ</i>	This study
EM665	14028s Δ <i>araBAD1005::FRT</i> <i>flhC5213::MudJ</i>	This study
EM667	14028s Δ <i>araBAD1065::hilD</i> ⁺ <i>flhC5213::MudJ</i> Δ <i>invH-sprB::FCF</i>	This study
EM674	14028s Δ <i>araBAD1005::FRT</i> <i>flhC5213::MudJ</i> Δ <i>invH-sprB::FCF</i>	This study
EM706	LT2 <i>P(flhDC)8093</i> (<i>PflhDC-luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD1005::FRT</i>	This study
EM707	LT2 <i>P(flhDC)8124</i> (<i>PflhDC</i> P1 ⁺ (-10 of P2,P3,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD::FRT</i>	This study
EM708	LT2 <i>P(flhDC)8125</i> (<i>PflhDC</i> P2 ⁺ (-10 of P1,P3,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD1005::FRT</i>	This study
EM709	LT2 <i>P(flhDC)8126</i> (<i>PflhDC</i> P3 ⁺ (-10 of P1,P2,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD1005::FRT</i>	This study
EM710	LT2 <i>P(flhDC)8127</i> (<i>PflhDC</i> P4 ⁺ (-10 of P1,P2,P3,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD1005::FRT</i>	This study
EM711	LT2 <i>P(flhDC)8128</i> (<i>PflhDC</i> P5 ⁺ (-10 of P1,P2,P3,P4,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD1005::FRT</i> Δ <i>invH-sprB::FCF</i>	This study
EM712	LT2 <i>P(flhDC)8129</i> (<i>PflhDC</i> P6 ⁺ (-10 of P1,P2,P3,P4,P5 changed to GTTGGT)-	This study

	<i>luxCDBAE-Km-PflhDC⁺</i> Δ <i>araBAD1005::FRT</i>	
EM713	LT2 <i>P(flhDC)8093</i> (<i>PflhDC-luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1065::hilD⁺</i>	This study
EM714	LT2 <i>P(flhDC)8124</i> (<i>PflhDC</i> P1+ (-10 of P2,P3,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1065::hilD⁺</i>	This study
EM715	LT2 <i>P(flhDC)8125</i> (<i>PflhDC</i> P2+ (-10 of P1,P3,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1065::hilD⁺</i>	This study
EM716	LT2 <i>P(flhDC)8126</i> (<i>PflhDC</i> P3+ (-10 of P1,P2,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1065::hilD⁺</i>	This study
EM717	LT2 <i>P(flhDC)8127</i> (<i>PflhDC</i> P4+ (-10 of P1,P2,P3,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1065::hilD⁺</i>	This study
EM718	LT2 <i>P(flhDC)8128</i> (<i>PflhDC</i> P5+ (-10 of P1,P2,P3,P4,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1065::hilD⁺</i>	This study
EM719	LT2 <i>P(flhDC)8129</i> (<i>PflhDC</i> P6+ (-10 of P1,P2,P3,P4,P5 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1065::hilD⁺</i>	This study
EM734	LT2 <i>P(flhDC)8093</i> (<i>PflhDC-luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1005::FRT</i> Δ <i>invH-sprB::FCF</i>	This study
EM735	LT2 <i>P(flhDC)8124</i> (<i>PflhDC</i> P1+ (-10 of P2,P3,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD::FRT</i> Δ <i>invH-sprB::FCF</i>	This study
EM736	LT2 <i>P(flhDC)8125</i> (<i>PflhDC</i> P2+ (-10 of P1,P3,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1005::FRT</i> Δ <i>invH-sprB::FCF</i>	This study
EM737	LT2 <i>P(flhDC)8126</i> (<i>PflhDC</i> P3+ (-10 of P1,P2,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1005::FRT</i> Δ <i>invH-sprB::FCF</i>	This study
EM738	LT2 <i>P(flhDC)8127</i> (<i>PflhDC</i> P4+ (-10 of P1,P2,P3,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1005::FRT</i> Δ <i>invH-sprB::FCF</i>	This study
EM739	LT2 <i>P(flhDC)8128</i> (<i>PflhDC</i> P5+ (-10 of P1,P2,P3,P4,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1005::FRT</i> Δ <i>invH-sprB::FCF</i>	This study

EM740	LT2 P(<i>flhDC</i>)8129 (<i>PflhDC</i> P6+ (-10 of P1,P2,P3,P4,P5 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD1005::FRT</i> Δ <i>invH-sprB::FCF</i>	This study
EM741	LT2 P(<i>flhDC</i>)8093 (<i>PflhDC-luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD1065::hilD</i> ⁺ Δ <i>invH-sprB::FCF</i>	This study
EM742	LT2 P(<i>flhDC</i>)8124 (<i>PflhDC</i> P1+ (-10 of P2,P3,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD1065::hilD</i> ⁺ Δ <i>invH-sprB::FCF</i>	This study
EM743	LT2 P(<i>flhDC</i>)8125 (<i>PflhDC</i> P2+ (-10 of P1,P3,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD1065::hilD</i> ⁺ Δ <i>invH-sprB::FCF</i>	This study
EM744	LT2 P(<i>flhDC</i>)8126 (<i>PflhDC</i> P3+ (-10 of P1,P2,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD1065::hilD</i> ⁺ Δ <i>invH-sprB::FCF</i>	This study
EM745	LT2 P(<i>flhDC</i>)8127 (<i>PflhDC</i> P4+ (-10 of P1,P2,P3,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD1065::hilD</i> ⁺ Δ <i>invH-sprB::FCF</i>	This study
EM746	LT2 P(<i>flhDC</i>)8128 (<i>PflhDC</i> P5+ (-10 of P1,P2,P3,P4,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD1065::hilD</i> ⁺ Δ <i>invH-sprB::FCF</i>	This study
EM747	LT2 P(<i>flhDC</i>)8129 (<i>PflhDC</i> P6+ (-10 of P1,P2,P3,P4,P5 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC</i> ⁺) Δ <i>araBAD1065::hilD</i> ⁺ Δ <i>invH-sprB::FCF</i>	This study
EM801	LT2 Δ <i>araBAD1065::hilD</i> ⁺ <i>fljB5001::MudJ</i> Δ <i>hin-5718::FRT</i> <i>PflhDC5451::Tn10dTc[del-25]</i>	This study
EM802	LT2 Δ <i>araBAD1065::hilD</i> ⁺ <i>fliL5100::MudJ</i> <i>PflhDC5451::Tn10dTc[del-25]</i>	This study
EM804	LT2 Δ <i>araBAD1065::hilD</i> ⁺ <i>flhC5213::MudJ</i> <i>PflhDC5451::Tn10dTc[del-25]</i>	This study
EM827	LT2 Δ <i>araBAD1005::FRT flhC::MudJ</i> Δ <i>invH-sprB::FCF</i> (Δ <i>spi-1</i>)	This study
EM858	LT2 Δ <i>araBAD1182::hilD</i> Δ <i>HTH</i> <i>PflhDC5451::Tn10dTc[del-25]</i> <i>flhC5213::MudJ</i>	This study
EM868	LT2 Δ <i>araBAD1182::hilD</i> Δ <i>HTH</i> <i>P(flhDC)5451::Tn10dTc[del-25]</i>	This study

	<i>fliL5100::MudJ</i>	
EM869	LT2 Δ <i>araBAD1182::hilD ΔHTH <i>P(flhDC)5451::Tn10dTc[del-25]</i> <i>fljB5001::MudJ</i> Δ<i>hin-5718::FRT</i></i>	This study
EM885	LT2 Δ <i>araBAD1182::hilD ΔHTH <i>fljB5001::MudJ</i> Δ<i>hin-5718::FCF</i></i>	This study
EM886	LT2 Δ <i>araBAD1182::hilD ΔHTH <i>flhC5213::MudJ</i></i>	This study
EM887	LT2 Δ <i>araBAD1182::hilD ΔHTH <i>fliL5100::MudJ</i></i>	This study
EM937	LT2 Δ <i>araBAD1005::FRT</i> <i>rtsB::T-POP</i> <i>flhC5213::MudJ</i> Δ <i>invH-sprB::FCF</i> (Δ <i>spi-1</i>)	This study
EM1009	LT2 Δ <i>araBAD1183::hila⁺</i> <i>fljB5001::MudJ</i>	This study
EM1010	LT2 Δ <i>araBAD1005::FRT</i> <i>fljB5001::MudJ</i>	This study
EM1011	LT2 Δ <i>araBAD1183::hila⁺</i> <i>flhC5213::MudJ</i>	This study
EM1018	LT2 Δ <i>araBAD1005::FRT</i> <i>fliL5100::MudJ</i>	This study
EM1019	LT2 Δ <i>araBAD1183::hila⁺</i> <i>fliL5100::MudJ</i>	This study
EM1048	LT2 <i>P(flhDC)8093</i> (<i>PflhDC-luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1065::hilD⁺</i>	This study
EM1049	LT2 <i>P(flhDC)8124</i> (<i>PflhDC</i> P1+ (-10 of P2,P3,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1109::</i> <i>rtsB⁺</i>	This study
EM1050	LT2 <i>P(flhDC)8125</i> (<i>PflhDC</i> P2+ (-10 of P1,P3,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1109::</i> <i>rtsB⁺</i>	This study
EM1051	LT2 <i>P(flhDC)8126</i> (<i>PflhDC</i> P3+ (-10 of P1,P2,P4,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1109::</i> <i>rtsB⁺</i>	This study
EM1052	LT2 <i>P(flhDC)8127</i> (<i>PflhDC</i> P4+ (-10 of P1,P2,P3,P5,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1109::</i> <i>rtsB⁺</i>	This study
EM1053	LT2 <i>P(flhDC)8128</i> (<i>PflhDC</i> P5+ (-10 of P1,P2,P3,P4,P6 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1109::</i> <i>rtsB⁺</i>	This study
EM1054	LT2 <i>P(flhDC)8129</i> (<i>PflhDC</i> P6+ (-10 of P1,P2,P3,P4,P5 changed to GTTGGT)- <i>luxCDBAE-Km-PflhDC⁺</i>) Δ <i>araBAD1109::</i> <i>rtsB⁺</i>	This study
TH3923	LT2 pJS28(Ap ^R P22-9 ⁺)/F'114 ^{ts} Lac ⁺ <i>zzf-</i> <i>20::Tn10[tetA::MudP](Tc^S)</i> <i>zzf-</i> <i>3823::Tn10dTc[del-25]/leuA414 hsdSB Fels2⁻</i>	Lab collection
TH6701	LT2 Δ <i>araBAD925::tetRA</i> (Δ <i>araBAD(aa1-73)</i>)	Lab collection
TH13659	LT2 Δ <i>araBAD1005::FCF</i> <i>PflhDC5451::Tn10dTc[del-25]</i>	Lab collection

	<i>flhC5213::MudJ</i>	
TH13751	LT2 Δ <i>araBAD1005::FCF flhC5213::MudJ</i>	Lab collection
TH13752	LT2 Δ <i>araBAD1005::FCF fliL5100::MudJ</i>	Lab collection
TH13919	LT2 Δ <i>araBAD1005::FCF PflhDC5451::Tn10dTc[del-25] fliL5100::MudJ</i>	Lab collection
TH14571	LT2 Δ <i>araBAD1005::FCF fljB5001::MudJ Δhin-5718::FRT</i>	Lab collection
TH14845	LT2 Δ <i>araBAD1005::FCF PflhDC5451::Tn10dTc[del-25] fljB5001::MudJ Δhin-5718::FRT</i>	Lab collection
TH16339	LT2 Δ <i>araBAD1065::hilD⁺</i>	This study
TH16385	LT2 Δ <i>araBAD1065::hilD⁺ fliL5100::MudJ</i>	This study
TH16386	LT2 Δ <i>araBAD1065::hilD⁺ flhC5213::MudJ</i>	This study
TH16423	LT2 Δ <i>araBAD1065::hilD⁺ fljB5001::MudJ Δhin-5718::FRT</i>	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	GACG TTCAGCGCAATGATGGTTT
gyrB-fw	CTGCTCAAAGAGCTGGTGTATCA
gyrB-rv	AGCGCGTTACAGTCTGCTCAT
rflM-fw	TCTCAACGATGCCTTACCCGAACA
rflM-rv	GCAAGCTCATGTAAAGGCGTGTGT
rpoB-fw	CAACCTGTTTCGTACGTATCGAC
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 ($\Delta araBAD::hilD^+ \Delta invH-sprB::FCF rflM::MudJ$, labeled 2), EM20 ($\Delta araBAD::hilD^+ rtsB::TPOP \Delta invH-sprB::FCF rflM::MudJ$, labeled 3), EM50 ($\Delta araBAD::hilD^+ rtsB::TPOP \Delta flhDC \Delta invH-sprB::FCF rflM::MudJ$, 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 β -galactosidase assay. Expression of HilA was induced by addition of 0.2% arabinose in the following strains: (i) *flhC* (class 1): EM517 ($P_{ara}::FRT flhC::MudJ$), EM1011 ($P_{ara}::hilA^+ flhC::MudJ$); (ii) *fliL* (class 2): EM1018 ($P_{ara}::FRT fliL::MudJ$), EM1019 ($P_{ara}::hilA^+ fliL::MudJ$); and (iii) *fljB* (class 3): EM1010 ($P_{ara}::FRT fljB::MudJ$), EM1009 ($P_{ara}::hilA^+ fljB::MudJ$). Biological replicates are shown as individual data points (diamonds).

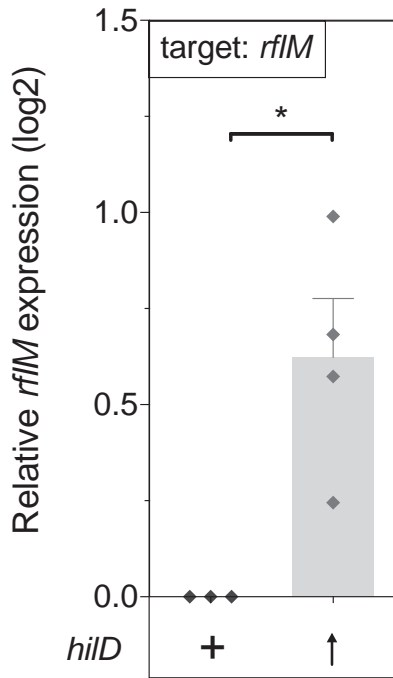
Supplemental Figure S3: HilD and RtsB act on different promoters to activate and repress *flhDC* operon transcription

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 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 ($\Delta araBAD::FRT$ *flhC::MudJ* $\Delta invH-sprB::FCF$), EM937 ($\Delta araBAD::FRT$ *flhC::MudJ* $\Delta invH-sprB::FCF$ *rtsB::T-POP*); EM4 ($\Delta araBAD::hilD^+$ *flhC::MudJ* $\Delta invH-sprB::FCF$); EM43 ($\Delta araBAD::hilD^+$ *flhC::MudJ* $\Delta invH-sprB::FCF$ *rtsB::T-POP*). Biological replicates are shown as individual data points (diamonds) in all figures.

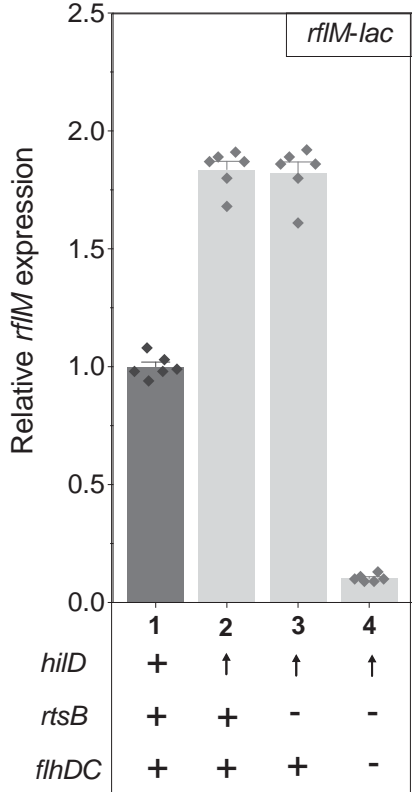
B. *flhDC* transcription from individual *flhDC* promoters under conditions when HilD or RtsB were overproduced was analyzed using a (*luxDCABE*-Km)-*flhDC* promoter fusion. As outlined in detail in Figure 1E, the entire *flhDC* promoter region with GTTGGT -10 box mutations of five of the six known transcriptional *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

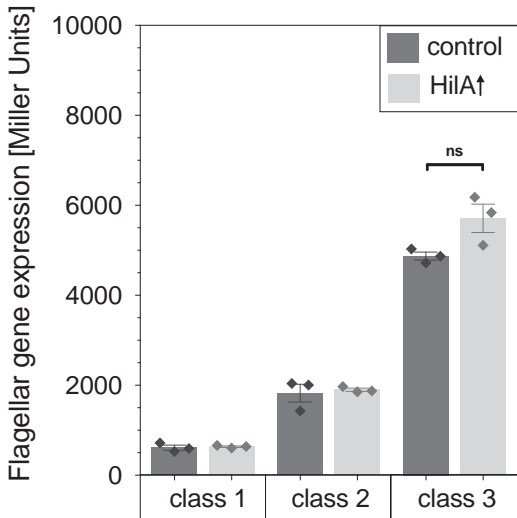
A



B

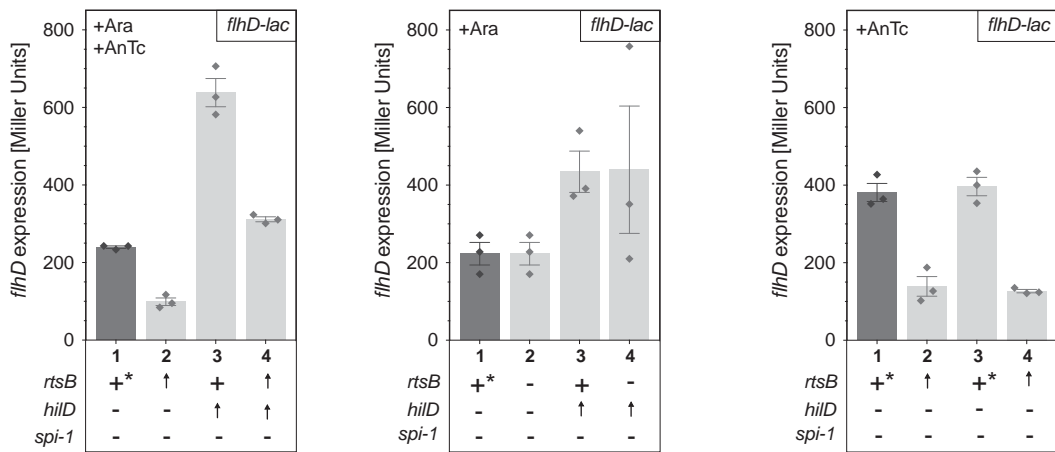


Supplemental Figure S2



Supplemental Figure S3

A



B

