Table S1: P_{hag}-*lacZ* β-galactosidase assays[a]

Genotype	P_{hag}-*lacZ* β-gal specific activity (MU)	Strain
wild type	168 ± 33	DS793
swrA swrB	16 ± 2	DS3789
swrA swrB degU::Tn	175 ± 21	DS3442
swrA swrB ΔdegU	168 ± 21	DS3794
swrA swrB ΔdegU (P_{degU}-*degU*)	164 ± 29	DS5643
swrA swrB ΔdegU (P_{degS}-*degU*)	155 ± 28	DS5502
swrA swrB ΔdegU ($P_{degS}P_{degU}$-*degU*)	20 ± 1	DS5503
swrA swrB ΔdegU ($P_{degS}P_{degU}$-*degU*^{D56A})	132 ± 11	DS6563

[a]Mean B-galactosidase activity of three replicates ± standard deviation.

Table S2: P_{hag}-*lacZ* and P_{flgM}-*lacZ* β-galactosidase assays[a]

Genotype	P_{hag}-*lacZ* β-gal specific activity (MU)	Strain	P_{flgM}-*lacZ* β-gal specific activity (MU)	Strain
wild type	124 ± 6	DS793	37 ± 1	DS811
ΔswrA ΔswrB	6 ± 1	DS3789	2 ± 1	DS3792
ΔswrA ΔswrB ΔdegU	122 ± 8	DS3794	3 ± 1	DS3797
ΔdegU	81 ± 9	DS3654	3 ± 1	DS3658
ΔswrA ΔswrB ΔflgM	307 ± 4	DS6385	133 ± 13	DS6386
ΔflgM	224 ± 14	DS4752	91 ± 14	DS4754
ΔswrA ΔswrB ΔflgM ΔdegU	313 ± 48	DS6408	15 ± 5	DS6409
ΔflgM ΔdegU	170 ± 5	DS6859	37 ± 1	DS6860

[a]Mean B-galactosidase activity of three replicates ± standard deviation.

Table S3: Plasmids

Plasmid	Genotype	
pAH25	*amyE::spec amp*	(Blair et al., 2008)
pCC1	*amyE::P_{flhO}-lacZ cat amp*	
pDG268	*amyE::lacZ cat amp*	(Antoniewski et al,. 1990)
pDG1664	*thrC::erm spec amp*	(Guérout-Fleury et al., 1996)
pDP242	*ΩΔswrB mls amp*	
pDP365	*amyE ::$P_{flgM}^{\Delta degsite1}$-flgM cat amp*	
pET28a	*P_{T7} 6-His kan*	
pJH24	*ΩΔdegU mls amp*	
pKB17	*amyE ::P_{motA}-lacZ cat amp*	
pLC1	*thrC::P_{degU}-degU mls amp*	
pLC2	*thrC::P_{degS}-degU mls amp*	
pLC126	*amyE ::P_{lytF}-lacZ cat amp*	
pMF35	*amyE::gfp cat amp*	(Fujita and Losick, 2002)
pMarA	TnYLB-1 *amp mls ori^{BsTs}*	
pMiniMAD	*ori^{BsTs} amp mls*	(Patrick and Kearns, 2008)
pNW43	*P_{T7} DegU-His$_6$ kan*	(Verhamme et al., 2007)
pRC2	*amyE ::P_{flgM}-flgM cat amp*	
pRC7	*amyE ::$P_{flgM}^{\Delta degsite2}$-flgM cat amp*	
pRC9	*amyE ::$P_{flgM}^{\Delta degsite1\Delta degsite2}$-flgM cat amp*	
pYH5	*thrC::$P_{degS}P_{degU}$-degU mls amp*	
pYH6	*thrC::$P_{degS}P_{degU}$-deg^{D56E} mls amp*	
pYH7	*thrC::$P_{degS}P_{degU}$-deg^{D56A} mls amp*	
pYH8	*P_{T7} DegS-His$_6$ kan*	
pYH9	*amyE ::P_{flgM}-GFP spec amp*	

Table S4: Primers[a]

Primer	Sequence
346	TCATGTATTCATAGCCTTCAGCCTT
596	AGGAGAAGCTTAAGACCGATGGCCCTTGATGACC
597	CTCCTGGATCCGACCTGCCTAGTAAAAGGCAAGT
598	AGGAGCCATGGGAACAATAATACAAGGAGGCGTGG
599	AGGAGGAATTCGGCGCAAGTATCCTTCAGCCTG
600	CTCCTCCATGGGTCACGGCGTTGTCATATATTTTTATT
740	ATACCTACGCCTCGTTTAGAATTC
741	CTCCTGTCGACCCATAATAGTGTTGACATGTTTTTCA
766	GGGAATCATTTGAAGGTTGGT
749	TTAGAGTTATTAATGGAATTGCTGANNNNNNNNNN
798	AGGAGGAATTCGATTCACTGAGCATGTTCTTTAAAC
799	CTCCTGGATCCGCTTGTCTATGGTTAATATCGGTTTT
839	AGGAGGTCGACGAAGAGCTCGTAAACAGTGAGGTC
840	CTCCTGGATCCTCCGCCTTCAGCAGCAAGATTTTTA
917	AGGAGGAATTCCTACGTAGAAACGATACGCGGAC
1109	AGGAGGGTACCAGTTTATACCCGCCAAGCCCTAC
1110	TCCTCGGATCCCATAATTTCCCTCCGTCACGGCG
1111	AGGAGGGATCCGTTCCGTTATCTCTTTGACTATG
1112	CTCCTGTCGACACTCAATTTCTTCACGGTAAGTCTC
1227	CCATCCATGGGCAATAAAACAAAGATGGATTCCAAAGTGCTG
1228	AAGAAGCTTAAGAGATAACGGAACCTTAATCATAATAAATGTCCC
1251	AGGAGGAATTCATTTCCATTCGCGGACGGGACATG
1252	CTCCTGGATCCATTTCCCACCTCTAAAAATGGCATTT
1375	GGGAGAACTGGCTAATTGTCCGA
1409	GCACCGCTTGCTTATCATAATT
1411	GCGCTTTAAGCTGCGCAAT
1664	GATGTTGTGATCATGGAAATCAATATGCCAAACG
1665	CGTTTGGCATATTGATTTCCATGATCACAACATC
1666	GATGTTGTGATCATGGCAATCAATATGCCAAACG
1667	CGTTTGGCATATTGATTGCCATGATCACAACATC
1771	CTCCTGGATCCAATGATTTTGCATTTGCTGTTTCTTTT
1782	CTATATGCTTATTGTAAGAAATAACAGG
1812	/5IRD800/GGAGAAAAAACAGAAATTCTGCTATTTT
1931	/5IRD700/TGTCAGGCGTGTATTAAGGAAGAAG
1981	/5IRD700/GTCGTTATTTCGTTCATTATAAGGAATT
2009	TGCAGAACTTGCCTTCTCTAAT
2010	AGTTTAGGGAATCAGCTGTT
2092	TTACTATTTTCTTTCTCGATTTTCAGAGC
2220	TCCTAATTTGGCATACCCTTGT
2221	CTCGCTTTCCGTTGCAGTCTT
2256	AGGAGCTCGAGACATAAGGAGGAACTACTATGAGTAA
2257	TCTGATCGGATCCTTATTTGTATAGTTCATCCATG
2258	AGGAGGCATGCGTCAGGCGTGTATTAAGGAAGAA
2259	TCCTCCTCGAGCTTTCCGTTGCAGTCTTTAAACAA
2365	AGGAGGAATTCAGATCACTCATCTTCCTAATTTGGC
2366	CTCCTGGATCCCAAAGTCGCTTCAATTGTTCAATAA
2475	CTCCTCTCGAGGTTCAAATGATCCATTTGATCC
2476	AGGAGCTCGAGCTGAAAATCGAGAAAGAAAATAGT
2644	CTCCTCTCGAGGTGTCTTTTTTACTATTTTCTTTC
2645	AGGAGCTCGAGCAAAAACAGCTGATTCCCTAAA
2786	CTCCTGTCGACTTTTTACTATTTTCTTTC
2787	AGGAGGTCGACCAGCTGATTCCCTAAA

[a]/5IRD800/ and /5IRD700/ indicate 5’ labeled tags for infra-red detection.

Figure Legends for Supplemental Figures

Figure S1: Primer extension mapping the 5'-end of the *B. subtilis flgM* transcript. Total RNA was isolated from exponentially growing strain 3610 and primer extension mapping was performed as described in Experimental procedures. The bottom lane shows the single transcript end detected for *flgM* transcript using probe 1409. The four upper lanes show the sequence ladder used to pinpoint the likely *flgM* transcription start, which is indicated in the sequence at the top as an emboldened "a". See text for additional details.

Figure S2: Analysis of phosphorylation of purified DegS-His_6 histidine kinase and DegU-His_6 response regulator. A) SDS-PAGE of DegU-His_6 and DegS-His_6 purified as described in Experimental procedures and stained with Coomassie Brilliant Blue dye. Molecular mass markers are indicated in the left lane. B) Autophosphorylation of DegS-His_6 to DegS-His_6~P (left six lanes) and phosphoryltransfer from DegS-His_6-P to DegU (right 5 lanes) were carried out as described in Experimental procedures. The autophosphorylation reaction was stopped at the times indicated and samples were analyzed by SDS-PAGE. DegS-His_6 autophosphorylation continued over the course of the reaction. For the combined phosphoryltransfer reaction, DegS-His_6-P was allowed to form for 10 minutes (time = 0, lane 7) before DegU was added. The phosphoryltransfer reaction was stopped at the times indicated and samples were analyzed by SDS-PAGE. Phosphoryltransfer to DegU appeared to occur rapidly, and the accumulation of DegU~P

is indicative of a very low phosphatase activity by DegS (see Gutu et el., 2010). See text for additional details.

Figure S3. The primary sequences of FlgM and σ^{28}/σ^{D} from *S. typhimurium* and *B. subtilis* are divergent. The protein sequences from *S. typhimurium* (Sty) and *B. subtilis* (Bsu) were aligned by CLUSTALW and presented by BOXSHADE.

Figure S4. Mutation of DegU has modest effects on the expression of a reporter for *fla/che* operon expression. A) Electrophoretic mobility shift experiments of the $P_{D3}P_{fla/che}$ promoter region. The left hand series of panels includes an increasing concentration of DegU protein. The right hand series of panels includes an increasing concentration of DegU-P protein that was phosphorylated by DegS and ATP. Concentrations of DegU are listed across the top in μM. Open triangles indicate the position of the unbound fragment; closed triangles indicate the position of the shifted fragment. B) Map of the promoter region for the *fla/che* operon (top) and map of the $P_{D-3}P_{fla/che}$-*lacZ* reporter construct (bottom). C) Expression of $P_{D3}P_{fla/che}$-*lacZ* in the 3610 genetic background expressed as Miller units. The following strains were used to generate this figure: wild type (DS791), *swrA swrB* (DS3790), *swrA swrB degU* (DS3795), and *degU* (DS3713). Columns are the average of six replicas; error bars are standard deviations. D) Expression of $P_{D-3}P_{fla/che}$-*lacZ* in the 168 laboratory strain genetic background expressed as Miller units. The 168 strain is a natural *swrA* mutant, and the *swrA* gene was complemented to compare $P_{D-3}P_{fla/che}$-*lacZ* expression to the 3610 background. The following strains were used to generate this figure: *swrA*$^{+}$ (DS7198),

and *swrA*$^{+}$ *degU* (DS7202). Columns are the average of six replicas; error bars are standard deviations.

Supplemental References:

Blair, K. M., Turner, L., Winkelman, J.T., Berg, H.C., and Kearns, D.B. (2008) A molecular clutch disables flagella in the *Bacillus subtilis* biofilm. *Science* **320**:1636-1638.

Chen, R., Guttenplan, S.B., Blair, K.M., and Kearns, D.B. (2009) Role of the σ^D-dependent autolysins in *Bacillus subtilis* population heterogeneity. *J Bacteriol* **191**:5775-5784.

Fujita, M., and Losick, R. (2002) An investigation into the compartmentalization of the sporulation transcription factor E in *Bacillus subtilis*. *Mol Microbiol* **43**:27-38.

Guerout-Fleury, A.-M., Frandsen, N., and Stragier, P. (1996) Plasmids for ectopic integration in *Bacillus subtilis*. *Gene* **180**:75-61.

Gutu, A.D., Wayne, K.J, Sham, L-T, Winkler, M.E. (2010) Kinetic Characterization of the $WalRK_{Spn}$ (VicRK) Two-Component System of *Streptococcus pneumoniae*: Dependence of $WalK_{Spn}$ (VicK) Phosphatase Activity on Its PAS Domain. *Journal of Bacteriology*, in press.

Patrick, J.E., and Kearns, D.B. (2008) MinJ (YvjD) is a topological determinant of cell division in *Bacillus subtilis*. *Mol Microbiol* **70**: 1166-1179.

Verhamme, D.T., Kiley, T.B., and Stanley-Wall, N.R. (2007) DegU co-ordinates multicellular behavior exhibited by *Bacillus subtilis*. *Mol Microbiol* **65**: 554-568.

Figure S1

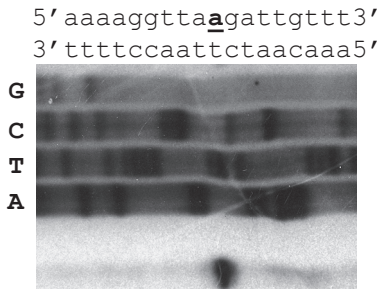

Figure S2

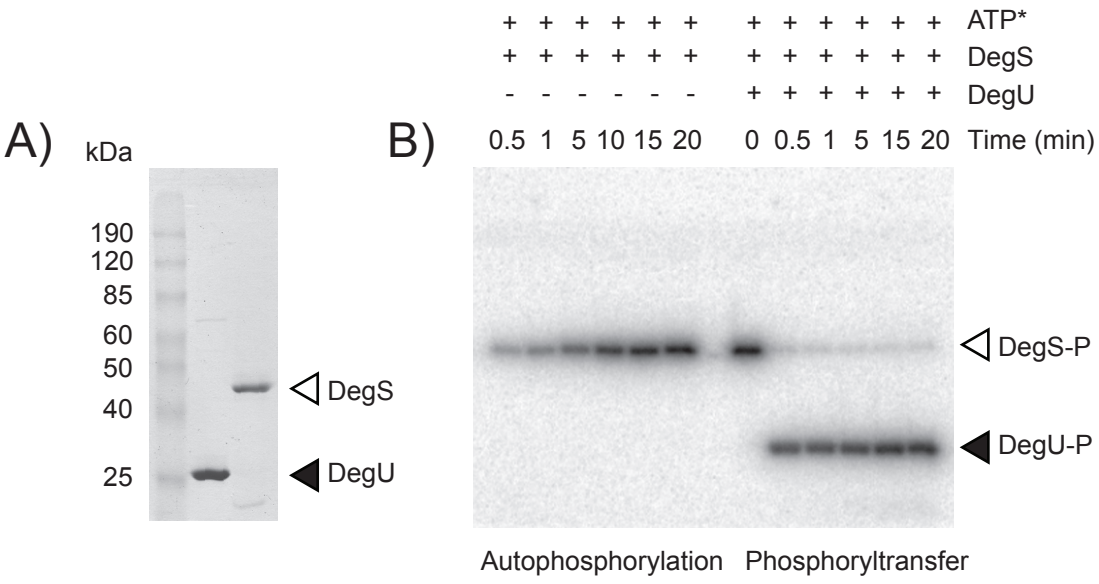
A)
kDa
190
120
85
60
50
40
25
DegS
DegU
B)
+ + + + + + + + + + + + ATP*
+ + + + + + + + + + + + DegS
- - - - - - + + + + + + DegU
0.5 1 5 10 15 20 0 0.5 1 5 15 20 Time (min)
DegS-P
DegU-P
Autophosphorylation
Phosphoryltransfer

Figure S3

A)

```
Sty_FlgM     1 MSIDRTSPLKPVSTVQTRETSDTPVQKTRQEKTSAATSASVTLSDAQAKLMQPGVSDINMERVEALKTAIRNGELKMDTG
Bsu_FlgM     1 MKLNQFG-TQSVNPYQK-NYDKQAVQKTVAQPQ---DKIEISSQAKEMQHASDAVTGSRQEKIAQLKAQIENGSYKVDAN

Sty_FlgM    81 KIADSLIREAQSYLQSK
Bsu_FlgM    76 HIAKNMINFYKKQ----
```

B)

```
Sty_σ28      1 MNSLYTAEGVMDK-----------HSLWQRYVPLVRHEALRLQVRLPASVELDDLLQAGGIGLLNAVDRYDALQGTAFTT
Bsu_σD       1 MQSLNYEDQVLWTRWKEWKDPKAGDDLMRRYMPLVTYHVGRISVGLPKSVHKDDLMSLGMLGLYDALEKFDPSRDLKFDT

Sty_σ28     70 YAVQRIRGAMLDELRSRDWVPRSVRRNAREVAQAMGQLEQELGRNATETEVAERLGIPVAEYRQMLLDTNNSQLFSYDEW
Bsu_σD      81 YASFRIRGAIIDGLRKEDWLPRTSREKTKKVEAAIEKLEQRYLRNVSPAEIAEELGMTVQDVVSTMNEGFFANLLSIDEK

Sty_σ28    150 R--EEHGDSIELVTEEHQQENPLHQLLEGDLRQRVMDAIESLPEREQLVLTLYYQEELNLKEIGAVLEVGESRVSQLHSQ
Bsu_σD     161 LHDQDDGENIQVMIRDDKNVPPEEKIMKDELIAQLAEKIHELSEKEQLVVSLFYKEELTLTEIGQVLNLSTSRISQIHSK

Sty_σ28    228 AIKRLRTKLGKL--
Bsu_σD     241 ALFKLKNLLEKVIQ
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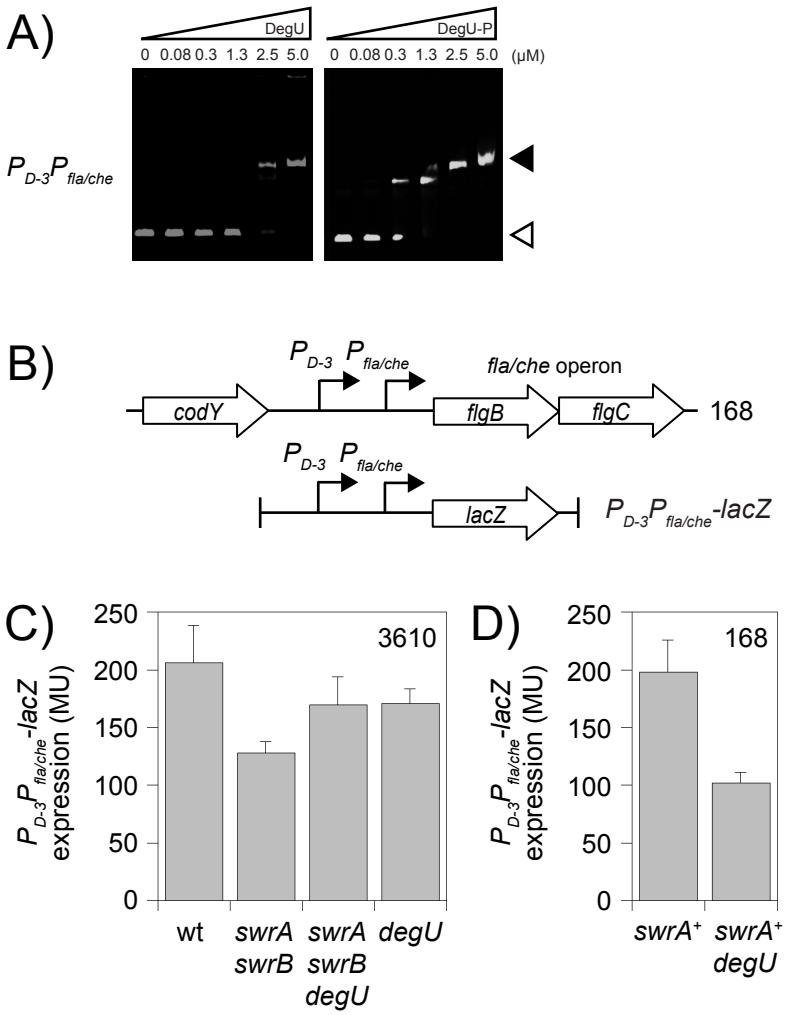
Figure S4


A)
DegU
DegU-P
0 0.08 0.3 1.3 2.5 5.0
0 0.08 0.3 1.3 2.5 5.0 (μM)
$P_{D-3}P_{fla/che}$
B)
P_{D-3} $P_{fla/che}$
fla/che operon
codY
flgB
flgC
168
P_{D-3} $P_{fla/che}$
lacZ
$P_{D-3}P_{fla/che}$-lacZ
C)
3610
$P_{D-3}P_{fla/che}$-lacZ expression (MU)
250
200
150
100
50
0
wt
swrA swrB
swrA swrB degU
degU
D)
168
$P_{D-3}P_{fla/che}$-lacZ expression (MU)
250
200
150
100
50
0
swrA+
swrA+ degU