1 Supplementary Tables

2

3 Table S1: List of strains

Strains	Relevant genotype/properties	Source/construction
B.subtilis 168	trpC2	(Anagnostopoulos and Spizizen, 1961)
BUG1	$trpC2 \Delta clpP::spec$	(Gerth et al., 2004)
BNM103	$trpC2 \Delta clpP::spec$	BUG1 \rightarrow B. subtilis 168
BNM104	$trpC2 \Delta clpP::spec$	Suppressor mutant obtained from BNM103
QBP418	$PY79 \ \Delta clpC::tet$	(Pan et al., 2001)
BNM105	$trpC2 \Delta clpC::tet$	QBP418 \rightarrow B. subtilis 168
BMM103	$trpC2 \Delta clpE::spec$	(Miethke et al., 2006)
BNM106	$trpC2 \Delta clpE::spec$	BMM103 \rightarrow B. subtilis 168
BEK90	trpC2 ∆clpX∷kan	(Gerth et al., 2004)
BNM107	trpC2 ∆clpX::kan	BEK90 \rightarrow B. subtilis 168
ORB3834	trpC2 pheA1 ∆spx::kan	(Nakano et al., 2001)
BNM109	$trpC2 \Delta clpX::tet$	This work
BNM111	trpC2 Δspx::kan	ORB3834 \rightarrow B. subtilis 168
ORB3838	$trpC2 pheA1 \Delta spx::kan \Delta clpX::spec$	(Nakano et al., 2001)
BNM112	Δ <i>spx::kan</i> Δ <i>clpX::spec</i>	ORB3838 \rightarrow B. subtilis 168
BNM126	trpC2 ∆hag	pMAD <i>hag</i> \rightarrow <i>B. subtilis</i> 168
NRS1499	NCIB 3610 ∆degSU::spec	N. Stanley-Wall, unpublished
BNM138	$trpC2 \Delta degSU::spec$	NRS1499 \rightarrow <i>B. subtilis</i> 168

BNM140	$trpC2 \Delta degSU::spec \Delta clpC::tet$	BNM105/BNM138 \rightarrow B. subtilis 168
BNM142	$trpC2 \Delta degSU::spec \Delta clpX::kan$	BNM107/BNM138 \rightarrow B. subtilis 168
PS258	$trpC2 \Delta codY::erm$	(Brinsmade and Sonenshein, 2011)
BNM143	trpC2 ∆codY::erm	$PS258 \rightarrow B.$ subtilis 168
BNM147	$trpC2 \Delta codY::erm \Delta clpX::kan$	BNM107 → BNM143
BNM149	$trpC2 \Delta comK$	$pMADcomK \rightarrow B.$ subtilis 168
BNM150	$trpC2 \Delta comK \Delta clpC::tet$	BNM105 → BNM149
DS791	NCIB3610 amyE::PflgB–lacZ cat	(Kearns and Losick, 2005)
BNM301	trpC2 amyE::PflgB–lacZ cat	DS791 \rightarrow B. subtilis 168
BNM302	<i>trpC2 amyE::PflgB–lacZ cat</i> $\Delta clpP::spec$	BNM103 → BNM301
BNM303	<i>trpC2 amyE::PflgB–lacZ cat</i> <i>\DeltaclpC::tet</i>	BNM105 → BNM301
BNM305	<i>trpC2 amyE::PflgB–lacZ cat</i> <i>\DeltaclpX::kan</i>	BNM107 → BNM301
BNM306	trpC2 amyE::PflgB–lacZ cat \[\Delta degSU::spec \]	BNM138 → BNM301
BNM307	trpC2 amyE::PflgB–lacZ cat	BNM111 → BNM301
BNM308	trpC2 amyE::PflgB–lacZ cat	BNM112 \rightarrow BNM301
BNM309	trpC2 amyE::PflgB–lacZ cat \[\lambda codY::erm \]	BNM143 → BNM301
DS793	NCIB3610 amyE::Phag-lacZ cat	(Kearns and Losick, 2005)
BNM328	trpC2 amyE::Phag–lacZ cat	DS793 \rightarrow B. subtilis 168
BNM329	<i>trpC2 amyE::Phag–lacZ cat</i> Δ <i>clpP::spec</i>	BNM103 \rightarrow BNM328
BNM330	<i>trpC2 amyE::Phag–lacZ cat</i> <i>\DeltaclpC::tet</i>	BNM105 → BNM328
BNM332	<i>trpC2 amyE::Phag–lacZ cat</i> Δ <i>clpX::kan</i>	BNM107 → BNM328

BNM333	trpC2 amyE::Phag-lacZ cat ∆degSU::spec	BNM138 → BNM328
BNM334	<i>trpC2 amyE::Phag–lacZ cat</i> Δ <i>spx::kan</i>	BNM111 → BNM328
BNM335	$trpC2 amyE::Phag-lacZ cat \Delta spx::kan \Delta clpX::spec$	BNM112 \rightarrow BNM328
BNM336	trpC2 amyE::Phag-lacZ cat $\Delta codY::erm$	BNM143 \rightarrow BNM328
BNM338	trpC2 amyE::Phag-lacZ cat $\Delta degSU::spec \Delta clpC::tet$	BNM138 → BNM328
BNM339	trpC2 amyE::Phag-lacZ cat $\Delta degSU::spec \Delta clpX::kan$	BNM138/BNM107 → BNM328
BNM341	$trpC2 amyE::PflgB-lacZ cat \Delta degSU::spec \Delta clpC::tet$	BNM138/BNM105 → BNM301
BNM343	trpC2 amyE::Phag-lacZ cat $\Delta codY::erm \Delta clpX::kan$	BNM107 → BNM336
BNM346	trpC2 amyE::flgB152p-lacZ cat	$pflgB152 \rightarrow B.$ subtilis 168
BNM347	trpC2 amyE::flgB152p−lacZ cat ∆degSU::spec	BNM138 \rightarrow BNM346
BNM348	<i>trpC2 amyE::flgB152p–lacZ cat</i> Δ <i>clpC::tet</i>	BNM105 \rightarrow BNM346
BNM349	$trpC2 amyE::flgB152p-lacZ cat \Delta degSU::spec \Delta clpC::tet$	BNM105/BNM138 → BNM346
BNM350	$trpC2 \Delta spx::kan$ $amyE::P_{hyperspank(Hy)}$ -spx spec	pMMN521 \rightarrow <i>B</i> . <i>subtilis</i> 168 (Nakano et al., 2003)
BNM351	$trpC2 \Delta spx::kan$ $amyE::P_{hyperspank(Hy)}$ -spx ^{DD} spec	$pSN56 \rightarrow BNM111$ (Nakano et al., 2003)
BNM421	$trpC2 \Delta hag amyE::P_{xyl}-hag1 cat$	$pXhag1 \rightarrow BNM126$
BNM422	$trpC2 \Delta hag amyE::P_{xyl}-hag1 cat \Delta clpP::spec$	BNM103 \rightarrow BNM421
BNM423	$trpC2 \Delta hag amyE::P_{xyl}-hag1 cat \Delta clpC::tet$	BNM105 \rightarrow BNM421
BNM424	$trpC2 \Delta hag amyE::P_{xyl}-hag1 cat \Delta clpE::spec$	BNM106 \rightarrow BNM421
BNM425	$trpC2 \Delta hag amyE::P_{xyl}-hag1 cat \Delta clpX::kan$	BNM107 → BNM421
BNM426	$trpC2 \Delta hag amyE::P_{xyt}-hag4 cat$	$pXhag4 \rightarrow BNM126$

BNM427	$trpC2 \Delta hag amyE::P_{xyl}-hag4 cat \Delta clpP::spec$	BNM103 \rightarrow BNM426
BNM428	$trpC2 \Delta hag amyE::P_{xyl}-hag4 cat \Delta clpC::tet$	BNM105 \rightarrow BNM426
BNM429	$trpC2 \Delta hag amyE::P_{xyl}-hag4 cat \Delta clpE::spec$	BNM106 \rightarrow BNM426
BNM430	$trpC2 \Delta hag amyE::P_{xyl}-hag4 cat \Delta clpX::kan$	BNM107 → BNM426
BNM436	$trpC2 \Delta hag amyE::P_{xyl}-hag4 cat \Delta spx::kan$	BNM111 → BNM426
BNM437	$trpC2 \Delta hag amyE::P_{xyl}-hag4 cat \Delta spx::kan \Delta clpX::spec$	BNM112 → BNM426
BNM810	<i>trpC2 amyE::P_{hyperspank(Hy)}-spx^{DD}</i> <i>spec</i>	$pSN56 \rightarrow BNM101$ (Nakano et al., 2003)
BNM834	<i>trpC2 amyE::PflgB–lacZ cat</i> ΔcodY::erm ΔclpX::kan	BNM107 \rightarrow BNM309
BNM840	$trpC2 \Delta codY::erm \Delta clpX::kan$	BNM107 \rightarrow BNM143
BNM844	$trpC2 amyE::P_{hyperspank(Hy)}-spx^{DD}$ $spec \Delta codY::erm$	BNM143 \rightarrow BNM810
LUW272	$trpC2 \Delta yjbH::spec$	(Rogstam et al., 2007)
BNM855	trpC2 ∆yjbH∷spec	LUW272 → BNM101
BNM857	$trpC2 \Delta yjbH::spec \Delta spx::kan$	BNM855 → BNM111
ABH282	PY79 ywrK::Tn917::amyE::cat	pAH120 \rightarrow <i>B. subtilis</i> PY79 (Camp and Losick, 2009)
BNM860	trpC2 ywrK::Tn917::amyE::cat	ABH282 → BNM101
BNM866	trpC2 ywrK::Tn917::amyE:: P _{hyperspank(Hy)} -spx ^{DD} spec	pSN56 → BNM860
BNM878	trpC2 ywrK::Tn917::amyE:: P _{hyperspank(Hy)} -spx ^{DD} spec amyE::PflgB-lacZ cat	BNM301 → BNM866
BNM1001	trpC2 ywrK::Tn917::amyE:: P _{hyperspank(Hy)} -spx ^{DD} spec amyE::Phag-lacZ cat	BNM328 \rightarrow BNM866
$swrA^+ de \overline{g}Q^+$	<i>trpC2 sacA::swrA cat thrC::degQ kan</i>	Gift of Nicola Stanley-Wall Jörn Hoßmann, Diploma thesis
BNM1266	$trpC2 \ sacA::swrA \ cat \ thrC::degQ$ $kan \ \Delta clpC::tet$	BNM105 \rightarrow swrA ⁺ degQ ⁺

BNM1268	trpC2 sacA::swrA cat thrC::degQ kan AclpP::spec	BNM103 \rightarrow swrA ⁺ degQ ⁺
BNM1270	$trpC2 \ sacA::swrA \ cat \ thrC::degQ$ $kan \Delta clpX::tet$	BNM109 \rightarrow swrA ⁺ degQ ⁺
DK1042	NCIB3610 comIQ12L	BGSC (Lab of Dan Kearns)
BHS122	NCIB3610 comIQ12L \DeltaclpC::tet	BNM105→ DK1042
BHS123	NCIB3610 <i>comI-Q12L \DeltaclpX::kan</i>	BNM107→ DK1042
<i>E.coli</i> XL1- blue competent cells	recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F proAB lac1 ^q ZM15 Tn10 (Tet ^r)]	Stratagene
E.coli FI1202	lacI ^q lacL8 gln5::Tn5 1202	(Fiedler and Weiss, 1995)

Table S2: List of plasmids

Plasmid	Construction	Reference
pQE60	C-terminal His ₆ -tag fusion	Qiagen
pQE60-hag	hag in NcoI/BamHI of pQE60	This work
pQE60– <i>spx</i>	<i>spx</i> in <i>NcoI/Bam</i> HI of pQE60	This work
pDG268	Vector for transcriptional <i>lacZ</i> fusion	(Antoniewski et al., 1990)
pflgB152	<i>flgB152</i> fragment in <i>Eco</i> RI/ <i>Bam</i> HI of pDG268	This work
pMAD	Vector for markerless deletions	(Arnaud et al., 2004)
pMAD-hag	<i>hag</i> flanking regions in <i>Bam</i> HI/ <i>Nco</i> I of pMAD	(Blair et al., 2008)
pMAD– <i>comK</i>	<i>comK</i> flanking regions in <i>Bam</i> HI/ <i>Nco</i> I of pMAD	This work
pMMN521	$P_{hyperspank(Hy)}$ -spx for amyE insertion	(Nakano et al., 2003)
pSN56	$P_{hyperspank(Hy)}$ -spx ^{DD} for amyE insertion	(Nakano et al., 2003)
рХ	Expression from P _{xyl} in <i>amyE</i>	(Kim et al., 1996)
pX-hag1	hag1 fragment in BamHI of pX	This work

	pX–hag4	<i>hag4</i> fragment in <i>Bam</i> HI of pX	This work
6 7	L	1	
, 8 0	References		
9 10 11	Anagnostopoulos, C., and Spizizen, J. (1961). Requirements for Transformation in <i>Bacillus subtilis. J. Bacteriol.</i> 81, 741–746.		
12 13 14	Antoniewski, C., Savelli, B., and Stragier, P. (1990). The <i>spoIIJ</i> gene, which regulates early developmental steps in <i>Bacillus subtilis</i> , belongs to a class of environmentally responsive genes. <i>J. Bacteriol.</i> 172, 86–93.		
15 16 17	Arnaud, M., Chastanet, A., and Débarbouillé, M. (2004). New vector for efficient allelic replacement in naturally nontransformable, low-GC-content, gram-positive bacteria. <i>Appl. Environ. Microbiol.</i> 70, 6887–6891. doi:10.1128/AEM.70.11.6887-6891.2004.		
18 19 20	Blair, K. M., Turner, disables fla doi:10.112	L., Winkelman, J. T., Berg, H. C., and Kearns, D. F gella in the <i>Bacillus subtilis</i> biofilm. <i>Science</i> 320 6/science.1157877.	3. (2008). A molecular clutch), 1636–1638.
21 22 23	Brinsmade, S. R., an direct gene 5648. doi:1	d Sonenshein, A. L. (2011). Dissecting complex tic evidence for CodY activation by guanine nuc .0.1128/JB.05510-11.	metabolic integration provides cleotides. <i>J. Bacteriol.</i> 193, 5637–
24 25 26	Camp, A. H., and Los factor durin doi:10.110	sick, R. (2009). A feeding tube model for activat ng sporulation in <i>Bacillus subtilis. Genes Dev.</i> 23 1/gad.1781709.	ion of a cell-specific transcription , 1014–1024.
27 28 29	Fiedler, U., and Wei PhoB: phos 3705.	ss, V. (1995). A common switch in activation of sphorylation induces dimerization of the receive	the response regulators NtrC and er modules. <i>EMBO J.</i> 14, 3696–
30 31	Gerth, U., Kirstein, J regulation	., Mostertz, J., Waldminghaus, T., Miethke, M., Ko of Clp protein content in <i>Bacillus subtilis. J. Bact</i>	ock, H., et al. (2004). Fine-tuning in <i>teriol.</i> 186, 179–191.
32 33	Kearns, D. B., and Lo <i>Genes Dev.</i>	osick, R. (2005). Cell population heterogeneity o 19, 3083–3094. doi:10.1101/gad.1373905.	during growth of <i>Bacillus subtilis</i> .
34 35	Kim, L., Mogk, A., an its applicat	nd Schumann, W. (1996). A xylose-inducible Bad ion. Gene 181, 71–76.	cillus subtilis integration vector and
36 37 38	Miethke, M., Hecker degradatio 06.	r, M., and Gerth, U. (2006). Involvement of <i>Bacili</i> n and protein quality control. <i>J. Bacteriol.</i> 188, 4	<i>lus subtilis</i> ClpE in CtsR 4610–4619. doi:10.1128/JB.00287-
39 40 41	Nakano, M. M., Haja in ClpX- an 42, 383–39	rizadeh, F., Zhu, Y., and Zuber, P. (2001). Loss-o d ClpP-independent competence development o 4.	of-function mutations in <i>yjbD</i> result of <i>Bacillus subtilis. Mol. Microbiol.</i>
42 43 44	Nakano, S., Küster-S transcripti Natl. Acad.	Schöck, E., Grossman, A. D., and Zuber, P. (2003) onal control is induced by thiol-specific oxidativ <i>Sci. U. S. A.</i> 100, 13603–13608. doi:10.1073/pn). Spx-dependent global ve stress in <i>Bacillus subtilis. Proc.</i> as.2235180100.

- Pan, Q., Garsin, D. A., and Losick, R. (2001). Self-reinforcing activation of a cell-specific transcription
 factor by proteolysis of an anti-sigma factor in *B. subtilis. Mol. Cell* 8, 873–883.
- 47 Rogstam, A., Larsson, J. T., Kjelgaard, P., and von Wachenfeldt, C. (2007). Mechanisms of adaptation to
 48 nitrosative stress in *Bacillus subtilis. J. Bacteriol.* 189, 3063–3071. doi:10.1128/JB.01782-06.

Primer	Sequence
PhagQE60–for	catg ccatgg tgagaattaaccacaatattgcagcg
PhagQE60-rev	gg ggatcc acgtaataattgaagtacgttttgc
spxpQE60-for	ggagtgaagat ccatgg ttacac
spxpQE60-rev	gatacgat ggatcc gtttgccaaacgc
hag1-for	ccc ggatcc atgagaattaaccacaatattgc
hag1-rev	ccc ggatcc ttaacgtaataattgaagtacgttttgc
hag4-for	ccc ggatcc tgtagccgggaggaggcgca
hag4-rev	cccc ggatcc agaccctggcaacgccaagg
flgB152–for	g gaattc ttgctgaccgtgtcggcattac
flgB152-rev	cg ggatcc gcgatattattagttatgac
flgB (-209 to -6)-for	ggaattcattttgcatttttcttcaaaaag
flgB (-209 to -6)-rev	cgggatcctattgtaagaaataacaggc
flgB (-106 to 98)-for	ggaattcctaacaatctaggactttatac
flgB (-106 to 98)-rev	cgggatcccaagattttgtatcgt
flgB (-1 to 203)-for	ggaattctatagttttacaattctcgac
flgB (-1 to 203)-rev	cgggatccaattttggaaagagactttttttg
hag-probe-for	attgaaagttgttgatgaag
clpXtetP1	agtgacattccctgaggag
clpXtetP2	gaacaacctgcattgcaagatctttcaccccttaatcttg
clpXtetP3	ttgatcctttttttataacaggaattcagataagcacaaacct
	cctg
clpXtetP4	tttggcattctcgtggtcgcg

52 Table S3: List of primers

63 Supplementary Figure Legends

64

65 Fig S1 in vivo stability of Hag

66 *B. subtilis* wild type cells were grown in Belitsky minimal medium and pulse labeled 67 with 25 μ Ci ³⁵S-methionine for 10 minutes. Samples were TCA-precipitated and 68 immunoprecipitation was performed with Hag-specific antibodies and protein A-coated 69 magnetic beads. Samples were separated by SDS-PAGE (12.5 %) and subjected to 70 autoradiography.

71

Fig S2 Northern blot analysis of flagellar transcripts in wild type and *clp* mutant cells.

74 Northern blot using total RNA of the wild type and strains BNM126 (Δhag), BNM103 $(\Delta clpP)$, BNM105 ($\Delta clpC$), BNM106 ($\Delta clpE$) and BNM107 ($\Delta clpX$) grown to OD₆₀₀ 1.0 75 at 37 °C in LB medium. 200 ng total RNA were separated by gel electrophoresis and 76 blotted on nylon membranes. The RNA was hybridized to a digoxygenin (DIG)-labeled 77 78 DNA probe against hag, and developed using anti-digoxygenin antibodies conjugated to alkaline phosphatase. Antibodies were detected using CDP-Star (Roche Applied 79 Sciences). M: 100 ng DIG-labeled RNA Molecular Weight Marker III (Roche Applied 80 Sciences). 81

82

83 Fig S3 Uncoupling *hag* expression from flagellar regulation.

- (A) Hag Western blot analysis of strain BNM421 (WT), BNM422 ($\Delta clpP$), BNM423 84 $(\Delta clpC)$, BNM424 ($\Delta clpE$) and BNM425 ($\Delta clpX$), carrying a xylose-inducible copy of 85 hag at the amyE locus. Cells were grown to OD 1.0 at 37 °C in LB medium with 2 % w/v 86 xylose (+) or in the absence of xylose (-). Cells were lysed by lysozyme treatment and 87 boiling in SDS sample buffer (see Materials and Methods). (B) Same as A for strains 88 BNM426 (WT), BNM427 ($\Delta clpP$), BNM428 ($\Delta clpC$), BNM429 ($\Delta clpE$) and BNM430 89 90 $(\Delta clp X)$, in which the hag coding region along with the untranslated regions is expressed under the control of the P_{xvl} promoter. (C) Motility assay of strains BNM426 (WT), 91 BNM427 ($\Delta clpP$), BNM428 ($\Delta clpC$), BNM429 ($\Delta clpE$) and BNM430 ($\Delta clpX$) in the 92 absence (left) or presence (right) of 2 % w/v xylose. (D) Hag Western blots of strains 93 BNM426 (WT), BNM430 ($\Delta clpX$), BNM436 (Δspx) and BNM437 ($\Delta spx \Delta clpX$), induced 94 95 with 2 % w/v xylose.
- 96

97 Fig S4 *clpC* affects swimming motility and Hag levels via *comK*.

(A) Motility assay of *B. subtilis* wild type and $\Delta clpC::tet$ (BNM105), $\Delta comK$ (BNM149), and $\Delta clpC::tet \Delta comK$ (BNM150). (B) Cells of the indicated strains (as in A) were grown to OD₆₀₀ 1.0 at 37 °C. Cell lysates were analyzed by SDS–PAGE and Western blotting using anti–Hag antibodies.

102

103 Fig S5 DegU levels in wild type and *clp* mutant cells.

104 The wild type and strains BNM103 ($\Delta clpP$), BNM105 ($\Delta clpC$), BNM106 ($\Delta clpE$), 105 BNM107 ($\Delta clpX$) and BNM138 ($\Delta degSU$) were grown in LB medium at 37 °C to T-1

- 106 (one hour prior to entry into stationary phase, A), T0 (time of entry into stationary phase,
- 107 B), T2 (C), T3 (D) and T4 (E), protoplasts were prepared and lysed (see Materials &

Methods). Lysates were separated by SDS–PAGE and subjected to Western blot analysis using polyclonal DegU antibodies (upper panels) or HtpG antibodies as a loading control (lower panels). Band intensities were normalized by calculating the ratio between the DegU signal and the HtpG signal and subsequently normalized to wild type levels of each time point.

113

114 Fig S6 The influence of *clpX* acts on motility independently of *degU*.

115 (A) Motility assay of the wild type and strains BNM107 ($\Delta clpX$), BNM138 ($\Delta degSU$), 116 and BNM140 ($\Delta degSU \ \Delta clpX$). (B) β -galactosidase assay of the indicated strains 117 carrying a *Phag-lacZ* fusion. Circles: wild type 168, triangles: $\Delta clpX$::*kan* (BNM305), 118 diamonds: $\Delta degSU$::*spec* (BNM333), inverted triangles: $\Delta degSU$::*spec* $\Delta clpX$::*kan* 119 (BNM339). Results from one representative experiment are depicted.

120

121 Fig S7 Clp proteases influence motility independently of *codY*.

122 (A) CodY Western blot of lysates from the wild type and strains BNM103 ($\Delta clpP$), BNM105 ($\Delta clpC$), BNM106 ($\Delta clpE$) and BNM107 ($\Delta clpX$) grown to OD₆₀₀ 1.0 at 37 °C 123 in LB medium. (B) Motility assay of the wild type and strains BNM107 ($\Delta clpX::kan$), 124 BNM143 ($\Delta codY$::erm) and BNM147 ($\Delta codY$::erm $\Delta clpX$::kan). (C) Hag Western blot 125 analysis, strains as in (A). (D) β -galactosidase assays of strains carrying a *PflgB*-lacZ 126 fusion. $\Delta clpX$: kan (BNM305, triangles), $\Delta codY$: erm (BNM309, diamonds) and 127 $\Delta codY$::erm $\Delta clpX$::kan (BNM834, inverted triangles). Results from one representative 128 experiment are shown. (E) Same as (D) for the *Phag-lacZ* fusion. Circles: wild type 168 129 (BNM328), triangles: ΔcodY::erm (BNM336) diamonds: ΔclpX::kan (BNM332), 130 inverted triangles: $\Delta codY$::*erm* $\Delta clpX$::*kan* (BNM343). 131

132

133 **FigG S8 A** *clpP* suppressor mutant restores motility and Hag levels.

134 (A) Growth curves in LB medium at 37 °C of the *B. subtilis* wild type (circles), freshly 135 transformed $\Delta clpP$::spec mutant (BNM103, squares) and from a $\Delta clpP$::spec suppressor 136 mutant (BNM104, triangles). (B) Motility assay of the indicated strains (as in A). (C) 137 Hag Western blot of the wild type and strain BNM104 ($\Delta clpP$ sup, upper panel) and Spx 138 Western blot of the wild type, strain BNM104 ($\Delta clpP$ sup), and strain BNM103 139 ($\Delta clpP$::spec).

140

141 Fig S9 Production of Spx^{DD} reduces *hag* mRNA levels and Hag protein levels.

142 (A) Northern blot analysis of samples from strain BNM351 ($\Delta spx::kan amyE::P_{Hy}$ -143 spx^{DD}) induced with 1 mM IPTG as indicated. Blots were hybridized with DIG-labeled 144 *hag* probe. (B) Western blot analysis of samples from strains BNM350 ($\Delta spx::kan$ 145 $amyE::P_{Hy}$ -spx) and BNM351 ($\Delta spx::kan amyE::P_{Hy}$ -spx^{DD}) induced with 1 mM IPTG 146 using Spx and Hag antibodies.

147

148 Fig S10 YjbH influences swimming motility and Hag levels.

- (A) Motility assay of the wild type and strains BNM111 (Δspx), BNM855 ($\Delta yjbH$) and
- 150 BNM857 ($\Delta spx \Delta yjbH$). (B) Hag Western blot analysis. Strains as in (A).
- 151

Fig S11 Electrophoretic mobility shift assay using purified Spx and *fla/che* promoter DNA fragments.

154 Gelshift experiments using different *PflgB* fragments (A: -209 to -6, B: -106 to 98, C: -1 155 to 203 relative to the *flgB* transcription start site) and purified Spx–His₆ (0, 1.25, 2.5 or 156 5μ M) were performed in the presence of poly–d–IC under oxidizing (1 mM diamide, 157 left) and reducing conditions (5 mM DTT, right).

158

159 Fig S12 Influence of *clpC and clpX* mutations on swimming motility of *B. subtilis*

160 strains carrying the $swrA^+$ and $degQ^+$ alleles. (A) Swimming plate with the indicated

161 complemented *B. subtilis* 168 strains. (The plate was inoculated with 3 µl of a growing

162 culture (OD₆₀₀ of 1) and incubated for 7 h at 37 °C and o/n at room temperature) (B) A

- 163 Western Blot (with anti Hag and anti HtpG serum) of extracts prepared from indicated *B*.
- 164 *subtilis* strains (OD₆₀₀ 0,9-1,1) 5 μg total protein was loaded. (C) Swimming plate of the

wild type NCIB 3610 strain and the respective *clpC* and *clpX* mutant.

166

167

168

169