Supplemental Information.

FlgN function in flagellar based motility by *Bacillus subtilis*

Lynne S. Cairns [1], Victoria L. Marlow [1], Taryn B. Kiley [1], Christopher Birchall [2], Adam Ostrowski [1], Phillip D. Aldridge [2] and Nicola R. Stanley-Wall [1]

[1.] Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Dundee, UK DD1 5EH

[2.] Centre for Bacterial Cell Biology, Medical Sciences New Building, Newcastle University, Richardson Road, Newcastle upon Tyne, UK

*Running Title: The function of FlgN in *Bacillus subtilis*.

To whom correspondence should be addressed:

Nicola R. Stanley-Wall

Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Dundee DD1 5EH.

Email: n.r.stanleywall@dundee.ac.uk Tel: +44(0)1382 385136; Fax: +44(0)1382 388216;

Figure S1 Amino Acids Identified in Mass Spectrometry Identification of Hag. The protein sequence of Hag is shown with amino acids from peptide sequences which were identified by mass spectrometry analysis in the identification of Hag in whole cell lysates highlighted in bold.

Figure S2 Western Blot Analysis of FlgE. Western blot analysis of cellular (including assembled flagella) fractions of the wild-type (NCIB3610), Δ*flgE* (NRS4042), Δ*hag* (DS1677), Δ*fliD* (NRS4041), Δ*flgN* (NRS3570) and Δ*flgK-flgL* (NRS4060) probed with the α-FlgE antibody.

Figure S3 Characterisation of His-tagged FlgK Strains and Enrichment of Hook-Basal Bodies. (A) swimming expansion assay of wild-type (NCIB3610), Δ*flgK-flgL* (NRS4060), His-*flgK* (NRS4812) and *flgK*-His (NRS4801). Photographs were taken after 24 hours incubation at room temperature. **(B)** Coomassie stained SDS-PAGE gel analysis of flagellar hook-basal body proteins enriched from the wild-type strain (NCIB3610). Mass spectrometry analysis of this sample is detailed in Table S4.

Table S1 Strains used in this study.

Strain	Relevant Genotype [a]	Source [b]
NCIB3610	Prototroph	B.G.S.C.
168	*trpC2*	(1)
NRS1314	3610 *degU::pBL204 (cml)*	(2)
DS1677	3610 *Δhag*	Kind gift D. Kearns
NRS1467	3610 *amyE::Phy-spank-gfp-lacI (spc)*	(2)
NRS2544	3610 *ΔptkA*	(3)
NRS3070	168 *sacA::Phag-yfp (kan)*	pNW546 →168
NRS3076	3610 *sacA::Phag-yfp (kan)*	SPP1 3070 →3610
NRS3256	168 *amyE::*$P_{hy\text{-}spank}$*-flgN-lacI (spc)*	pNW380 →3610
NRS3570	3610 *ΔflgN*	pNW399 →3610
NRS3571	3610 *flgN* $Y^{49}A$	pNW801 →3610
NRS3578	3610 *ΔflgN+ amyE::*$P_{hy\text{-}spank}$*-flgN-lacI (spc)*	SPP1 3256 →NRS3570
NRS3708	3610 *ΔflgN + sacA::*P_{hag}*-yfp (kan)*	SPP1 3070 →NRS3570
NRS3713	3610 *ΔflgN + sacA::*P_{hag}*-yfp (kan) + amyE::*$P_{hy\text{-}spank}$*-flgN-lacI (spc)*	SPP1 3256 →NRS3708
NRS3718	3610 *ΔflgN hag* $T^{209}C$	pNW1010 →NRS3570
NRS3719	3610 *hag* $T^{209}C$	pNW1010 →3610
NRS3724	3610 *flgN* $R^{49}E$	pNW1012 →3610
NRS3735	168 *amyE::*$P_{hy\text{-}spank}$*-flgKL-lacI (spc)*	pNW1017 →168
NRS4017	3610 *flgN* $R^{60}E$	pNW1028 →3610
NRS4041	3610 Δ*fliD*	pNW1034 →3610
NRS4042	3610 Δ*flgE*	pNW1036 →3610
NRS4043	3610 *ΔflgN+ amyE::*$P_{hy\text{-}spank}$*-flgKL-lacI (spc)*	SPP1 3735 →NRS3570
NRS4060	3610 *ΔflgKL*	pNW1042 →3610
NRS4063	3610 *flgN* $R^{60}A$	pNW1027 →3610
NRS4064	3610 *ΔflgKL + amyE::*$P_{hy\text{-}spank}$*-flgKL-lacI (spc)*	SPP1 3735 → NRS4060
NRS4071	3610 *ΔflgKL+ sacA::*$P_{hag\text{-}}$*yfp (kan)*	pNW1042 →NRS4060
NRS4078	3610 *ΔflgKL + sacA::*P_{hag}*-yfp (kan) + amyE::*$P_{hy\text{-}spank}$*-flgKL-lacI (spc)*	SPP1 3735 → NRS4078
NRS4794	168 *amyE::*P_{hag}*-lacZ* translational *(cml)*	pNW1069 →168
NRS4795	3610 *amyE::*P_{hag}*-lacZ* translational *(cml)*	SPP1 4794 →3610
NRS4796	3610 *ΔflgN + amyE::*P_{hag}*-lacZ* translational *(cml)*	SPP1 4794 →3570
NRS4798	3610 *ΔflgE + amyE::*P_{hag}*-lacZ* translational *(cml)*	SPP1 4794 →4042
NRS4799	3610 *ΔflgKL + amyE::*P_{hag}*-lacZ* translational *(cml)*	SPP1 4794 →4060
NRS4801	3610 *His-flgK*	pNW1063 →3610
NRS4812	3610 *flgK-His*	pNW1065 →3610

[a] Drug resistance cassettes are indicated as follows: *kan*, kanamycin resistance; *mls*, lincomycin/erythromycin resistance; *cml*, chloramphenicol resistance; and *spc*, spectinomycin resistance.

[b] The direction of strain construction is indicated with DNA or phage (SPP1) (→) recipient strain. B.S.G.C. is the *Bacillus* genetic stock centre.

45 **Table S2. Plasmids used in this study.**

Plasmid	Relevant Genotype [a]	Source
pDR111	*amyE* integration plasmid (spc)	(4)
pBL132	*cml*^{R} cassette in pUC19	(5)
pBL165	*amyE* integration plasmid $P_{spac\text{-}hy}$-*gfp mut2*	(5)
pDR183	*lacA integration plasmid*	(5)
pKM3a	*yfp amyE* integration plasmid	Kind gift D. Rudner
pSAC-KAN	*sacA* integration plasmid	(6)
pEHISGFPTEV	Protein expression vector	(7)
pDG1728	*lacZ* reporter fusion plasmid, *amyE* integration	(8)
pMAD	In-frame markerless mutation plasmid	(9)
pNW380	*flgN* coding region in pDR111	This study
pNW398	*flgN* in pUC19	This study
pNW399	Δ*flgN* in pMAD	This study
pNW546	P_{hag}-*yfp* in pSAC-KAN	This study
pNW642	*sigA* in pEHISGFPTEV	This study
pNW700a	P_{hag} in pKM3a	This study
pNW800	*flgN* Y^{49}A in pUC19	This study
pNW801	*flgN* Y^{49}A in pMAD	This study
pNW1006	*hag* in pBL132	This study
pNW1009	*hag* T^{209}C in pBL132	This study
pNW1010	*hag* T^{209}C in pMAD	This study
pNW1011	*flgN* Y^{49}E in pUC19	This study
pNW1012	*flgN* Y^{49}E in pMAD	This study
pNW1017	*flgKL* coding region in pDR111	This study
pNW1025	*flgN* R^{60}A in pUC19	This study
pNW1026	*flgN* R^{60}E in pUC19	This study
pNW1027	*flgN* R^{60}A in pMAD	This study
pNW1028	*flgN* R^{60}E in pMAD	This study
pNW1034	Δ*fliD* in pMAD	This study
pNW1036	Δ*flgE* in pMAD	This study
pNW1042	Δ*flgKL* in pMAD	This study
pNW1063	His-flgK in pMAD	This study
pNW1065	*flgK*-His in pMAD	This study
pNW1069	P_{hag} in pDG1728	This study

46

47 [a] Drug resistance cassettes are indicated as follows: *cml*, chloramphenicol resistance and *spc*,
48 spectinomycin resistance.

Table S3 Primers used in this study.

Primer	Target	Sequence [a]	Position [b]
DEN5	*rRNA*	TCACGRCACGAGCTGACGAC	Internal 16S rRNA
DEN7	*rRNA*	ACTCCTACGGGAGGCAGC	Internal 16S rRNA
NSW652	*hag*	CCGGAATTCGAATTGACGCCCCAAAGCATATTG	-272→-248
NSW653	*hag*	GGCAAGCTTCTGAATATGTTGTTAAGGCACGTC	-38→-14
NSW860	*sigA*	GCATCCATGGCTGATAAACAAACCCACG	+5→+22
NSW861	*sigA*	GCATCTCGAGTTATTCAAGGAAATCTTTC	+1095→+1116
NSW936	*flgN*	GCATGGATCCATC AGG CAG AAG CGA ATT CAG	-588→-558
NSW937	*flgN*	GCATTCTAGATAATTGCCTTCGCTGACATGG	+92→+123
NSW938	*flgN*	GCATTCTAGACTGTTTGAT TCAAAAGCTTAGCAGAAAG	+453→+490
NSW939	*flgN*	GCATGTCGACCAACACAGTTGTATTTAGCT TGC	+1104→1137
NSW940	*flgN*	GCAT GTCGACAAGCAATAAAAAAGGAGAAAGCCC	-34→-1
NSW941	*flgN*	GCATGCATGCAATTCCTTTCTGCTAAGCTTTTGAATC	+469→+505
NSW942	*flgN*	ACAAAAGAGCAAAAA**GCA**ATTCAAGCAATCACG	+130→+162
NSW943	*flgN*	CGTGATTGCTTGAAT**TGC**TTTTTGCTCTTTTGT	+130→+162
NSW1401	*hag*	GCATGGATCCGCGATCTCTGAAAA	+39→+52
NSW1402	*hag*	CGATGGATCCTCGTTTATATCGACTAAGT	+1103→1121
NSW1403	*hag*	GCAGATAATGCAGCAGAT**TG**TGCTGATATCGGTTTCGATG C	+107→+147
NSW1404	*hag*	GCATCGAAACCGATATCAGC**AC**AATCTGCTGCATTATCTGC	+107→+147
NSW1436	*flgN*	ACAAAAGAGCAAAAA**GAA**ATTCAAGCAATCACG	+130→+162
NSW1437	*flgN*	CGTGATTGCTTGAAT**TTC**TTTTTGCTCTTTTGT	+130→+162
NSW1438	*yvyF*	GAAAACCGGCAATCAACTTTGAGC	+133→+156
NSW1439	*yvyF*	CTCGTGGTTCAAATGATCCATTTGATC	+322→+348
NSW1440	*flgM*	ATCAATCAATTTGGAACACAATCCG	+7→+31
NSW1441	*flgM*	GTCTACTTTGTATGACCCGTTTTC	+196→+216
NSW1442	*flgN*	GCC GGC AAAACAAAAGAGCTTTCT	+97→+120
NSW1443	*flgN*	TCCGAGAACTTGAGAAAGAGATTCGTA	+283→+309
NSW1444	*flgK*	TACAACAAATCAGGCGGGAATGCA	+682→+705
NSW1445	*flgK*	AGACTCTATAAACCCTAAAAGGGATCCC	+894→+921
NSW1446	*flgL*	CAAGCGATTGGCGTAGAGGTAAA	+322→+344
NSW1447	*flgL*	GCCTCCAAAAGCTGACTTTGGATC	+526→+549
NSW1459	*yviE*	CTGCTCATTTTTAAGCTGGC	+64→+83
NSW1462	*flgK*	GCATGTCGACTTAGCAGAAAGGAATT	-22→-6
NSW1463	*flgL*	GACTGCATGCTTACTTTAAAAAGTCAAT	+880→+897
NSW1464	*flgN*	CAGACAGAAGATGAC**GCG**ATCAAAACAACTTCG	+163→+193
NSW1465	*flgN*	CGAAGTTGTTTTGAT**CGC**GTCATCTTCTGTCTG	+163→+193
NSW1466	*flgN*	CAGACAGAAGATGAC**GAG**ATCAAAACAACTTCG	+163→+193
NSW1467	*flgN*	CGAAGTTGTTTTGAT**CTC**GTCATCTTCTGTCTG	+163→+193
NSW1670	*hag*	GCAGGATCCAAGAAGAACAAATCATTCTTTTTGAAAG	-766→-727
NSW1671	*hag*	TTGCGGTCGACGTGGTTAATTCTCATTGTTTTGTTCCT	-12→+25

[a] Underlined sequences indicate endonuclease restriction cut sites. Bold sequences represent base pairs mutated by site-directed mutagenesis.

[b] Position of primer is indicated in relation to the translational start site (noted as +1) of the named gene.

Table S4. Mass spectrometry analysis of top 12 proteins identified from hook-basal body enrichment of NCIB3610. The top 12 proteins from the NCIB3610 sample are presented as identified by their Mascot protein score.

Protein Rank	Protein Description	Mascot Protein Score	Coverage (%)	Unique Peptides	No. Peptides
1	flagellin [Bacillus subtilis subsp. subtilis str. 168]	13717.54	96.71	38	45
2	flagellar basal body rod protein FlgG [Bacillus subtilis subsp. subtilis str. 168]	6251.24	96.97	27	27
3	elongation factor Tu [Bacillus subtilis subsp. subtilis str. 168]	6107.34	92.42	38	38
4	surfactin synthetase [Bacillus subtilis subsp. subtilis str. 168	3582.23	34.80	84	104
5	surfactin synthetase [Bacillus subtilis subsp. subtilis str. 168]	3267.57	38.44	77	97
6	flagellar MS-ring protein [Bacillus subtilis subsp. subtilis str. 168]	3254.30	70.34	32	32
7	flagellar hook-associated protein FlgK [Bacillus subtilis subsp. subtilis str. 168	2784.52	73.77	27	27
8	putative flagellin [Bacillus subtilis subsp. subtilis str. 168]	2249.41	48.75	1	11
9	glyceraldehyde-3-phosphate dehydrogenase [Bacillus subtilis subsp. subtilis str. 168	1957.55	54.63	15	15
10	30S ribosomal protein S2 [Bacillus subtilis subsp. subtilis str. 168]	1776.43	84.55	26	26
11	flagellar capping protein [Bacillus subtilis subsp. subtilis str. 168]	1765.60	63.25	29	29
12	flagellar hook-associated protein FlgL [Bacillus subtilis subsp. subtilis str. 168]	1693.66	67.45	16	16

Plasmid Construction.

Construction of Plasmid pNW546. Plasmid pNW546 was constructed to assay *hag* transcription using YFP as a reporter. The promoter region of *hag* was PCR amplified from *B. subtilis* 3610 chromosomal DNA using primers NSW652 and NSW653 and ligated into pKM3a using EcoRI and HindIII restriction sites to create pNW700a. pNW700a was digested with EcoRI and BamHI to release the P*hag-yfp* fragment which was ligated into pSAC-KAN (6), to allow for integration at the non-essential *sacA* locus.

Construction of Plasmid pNW1017. Plasmid pNW1017, used to introduce the *flgK* and *flgL* coding regions under the control of the IPTG-inducible promoter $P_{hy\text{-}spank}$ at the non-essential *amyE* locus, is a derivative of pDR111 (4). The coding regions of *flgK* and *flgL* (including ribosome binding site) and the intergenic region were amplified from *B. subtilis* 3610 chromosomal DNA with primers NSW1462 and NSW1463. The PCR product was digested SalI/SphI and cloned into pDR111 cut the same. Plasmid pNW380 for *flgN* was constructed in an identical manner using primers NSW940 and NSW941.

Construction of Plasmid pNW1069. Plasmid pNW1069 was constructed to introduce a *hag'-'lacZ* translational reporter fusion at the non-essential *amyE* locus. The *hag* promoter, the *hag* 5'UTR and the 5' end of the *hag* open reading frame (10) were amplified by PCR with the primers NSW1670 and NSW1671 from *B. subtilis* 3610 chromosomal DNA. The PCR product was digested BamHI/SalI and cloned into the BamHI and SalI sites of pDG1728 (8).

Construction of Plasmid pNW1034. Plasmid pNW1034 was constructed to introduce an in-frame markerless deletion of *fliD* to the chromosome. A region of approximately 1000 bp, including 500bp upstream of *fliD*, the first two codons and final two codons of *fliD*, and 500 bp downstream of *fliD* was synthesised and cloned into a pUC cloning plasmid by Dundee Cell Products (http://www.dundeecellproducts.com/). The Δ*fliD* region was excised from the pUC plasmid with EcoRI and BamHI and cloned into the EcoRI and BamHI sites of pMAD (9). Plasmid pNW1036 and pNW1042 were constructed in a similar manner. Synthesised sequences are detailed below. Restriction sites are underlined and the first two codons and final two codons of each gene deleted are highlighted in bold.

Construction of pNW1063. Plasmid pNW1063 was constructed to introduce a poly-Histidine epitope tag at the N-terminus of *flgK*. A region of approximately 1000 bp including 500bp upstream of the *flgK* transcriptional start site, the *flgK* start codon, a region encoding 10 Histidine residues and 500bp downstream of the *flgK* transcriptional start site was synthesised and cloned into a pUC cloning

plasmid by GenScript (http://www.genscript.com/). The His-*flgK* region was excised from the pUC plasmid with BamHI and cloned into the BamHI site of pMAD (9). pNW1065 was constructed in a similar manner. Synthesised sequences are detailed below. Restriction sites are underlined and His tag regions highlighted in bold underline.

Regions of DNA synthesised for cloning into pMAD.

Δ*fliD* used to construct pNW1034

GAATTCACAAGAAATCTAAAACAGAAGATTTTTTTCCAAAAATATGTGTAATCTTATCTCGACTTAGT
CGATATAAACGATAGATTGGGGCATAGGGGATGATCAATTGAACATTGAAAGGCTCACTACGTTACAA
CCTGTTTGGGATCGTTATGATACTCAAATACATAATCAGAAAGATAATGATAACGAGGTTCCTGTTCA
TCAAGTTTCATATACCAATCTTGCTGAAATGGTGGGGGAAATGAACAAGCTTTTGGAACCTTCGCAAG
TTCATCTGAAGTTCGAGCTTCATGACAAGTTAAATGAATACTATGTAAAGGTAATAGAGGACTCTACA
AATGAAGTGATCCGCGAAATTCCACCAAAACGGTGGCTTGATTTTTATGCGGCTATGACTGAATTTCT
TGGGTTATTTGTAGATGAAAAAAAGTAGAATAGGAGTGGTTTGAG**ATGGTCCAATAA**ATGTAATTTGG
AGGATGACACATGGCGATCCAAAATCCATATACAGCCTATCAGCAAAATTCAGTGAATACGGCTACAC
CCGGGGAGCTGACGCTTATGCTGTATAATGGCTGTTTGAAATTTATAAGACTTGCCGCTCAGGCCATT
GAGAATGATGATATGGAACGTAAAAATGAAAATCTGATTAAAGCGCAAAATATTATTCAGGAATTAAA
TTTTACACTTAACCGTAACATAGAGCTTTCCGCTTCTATGGGTGCGATGTACGATTATATGTATCGCA
GATTGGTACAGGCAAATATCAAAAATGATACGGGCATGCTGGCTGAGGTTGAAGGTTATGTAACAGAT
TTTCGCGATGCTTGGAAACAAGCCATTCAAAGTGAGCGGAAGGACCGGCACGGATCAGGCGGGATCGC
ATGAATAATATAGATCAACTATACACTGAGACGAAGAGTATGCTGTCACACATACAAAATACGCCGGA
AAGCGATGAACTTTTAAAGCAAATTGAAGACTTTGTGGCTACACGGTCTGAACTGATTCAGGAGATAT
CTCTGCCGCTTTCAGGATCC

Δ*flgE* used to construct pNW1036

GAATTCATGACTTCTATAAGTTCAGAATATAAACTGCCTGAAAAAACGAACACTGTGTCGACGAACAA
CAGCAGCTTGGGGAAAGACGAGTTTTTAAAAATATTAATGACTCAAGTTCAAAACCAAGATCCGCTTA
ACCCGATTGACGATAAAGAATTTATCAGCCAGATGGCGACTTTTTCAAGCTTGGAGCAAATGATGAAT
CTGAATACGACAATGACTCAATTCGTTGAAAACCAAGATCCGTTTACAACGTATGTTGATTGGATGGG
AAAAGAAGTATCTTGGACTGATGGTAAAAGTGCAACAGATAAAACAGGCACAGTAAGCTCTGTTAAAC
ATTTTAAAGGAAATTATTATCTCGTTCTTGATGATGGGACCGAGATCAGTCCTGCGAATGTCATGTCT
GTGGGACAATCATCTAAATAAAAACATCTGGGGGAATATATT**ATGTTACGTTAA**GGAGGGAGGGGAGG
CGAGTAATCGCTTCTCCGCTGTTTTATGATTAAAGTAACCCGTTTGAACGGGCAGCCCTTTACACTGA
ATGCGCTATTTATTGAACAGATTGAATGTTTTCCGGATACTACAATTACTCTGTCAAATGGTAAGAAG
TTTGTAGTAAAAGAAGATGAAGAAGCTGTTCTGGAAAAGATCGCAGCTTTCTACCGAAAAATACAAAT
ATTTGCAATGGATCAAGGAATAGAGGAACCGGAATGAAGAAAAAGTTAATGATCATATTACTAATTAT
TCTTATCGTAATTGGTGCTCTCGGGGCGGCGGCTTATTTTGTTTTAGGCGGAAAGTCCGAAAAAAGTG
AAGCGAAAAAAAGTATTGATGAAATCGTTGCGTCTTCTGTTGATGTAGAAGAGATCACAACAAATTTA
AAGTCTGATAACATTATCCGTCTTGCTATTAAACTTGAAACTGATTCTGATAAATCAAAAGAAGAACT
TGAGAAACGTGATTTCCAAGTGAAAGACGCTGTTATATCACTGCTGGCTGATACGAATGCTGGGATCC

Δ*flgKL* used to construct pNW1042

GGATCCTGTCAGCGAAGGCAATTATTGAACAATTGAAGCGACTTTGCGTTCTGCATGAGCACCTGCTC
ACGCTGTCTGAAGAAAAGACGGAAGCGCTCAAAGCCGGCAAAACAAAAGAGCTTTCTAACATTTTGAC
AAAAGAGCAAAAATATATTCAAGCAATCACGCAGACAGAAGATGACCGGATCAAAACAACTTCGGCCT

TTCTCGGATATAGCGAAAATAATACTATTTCCGCATGTATCGCCAAAACCTCAGGCAGTGAAAAGGAA
GAGCTGGAACAACTATACGAATCTCTTTCTCAAGTTCTCGGACGTCTGAAAAAAGTAAATGAGATGAA
TAGGCAGCTGACAAGAGACGCGCTGCAATTCATCTCTATTTCGTACGATATGCTGGTTCCTAAGGAAA
ATAACTTCAATTACAGCAAATCAATTAAAGCTGAGCTGCCGAAAAGTAGCAAAATGAAACTGTTTGAT
TCAAAAGCTTAGCAGAAAGGAATTCAGAAA**ATGACAAAGTAA**GCGGCTCTTAGGAGTTCGCTTTTTTT
ATAGTTCAGGAGGTAGAGTGATGCAGATTCCCAGATTGATTATGCATAGTGTTCAAGGAAAAATTGGT
TTAACAACGACGCCTGCCAGCTTAAAAATGGAGCAGCCTCAAGCTGATCTAGAGATCGAACAGCCGAG
TGCGGAAATGGAAATATCGGTGACACCTGGAAAACTCACGATTGACCAGACACAAGCATGGGAAGAAT
TAGACAGAAAGCATGTTTTCAAGAGAATTGAAGAAGCCGCCCAACAAGGGCATGAGGATGTAATGGAG
GGAATAGCACGCACTGCAGAAGAAGGCGACGAGCTTATGAAGATTGAAAATAAGGGGAACCCAATCGC
TTCACAAGCAAGGAGGAACTCTGAAATGCACCAAATTCAATTAGGCGAAAATTATGCTCCTTCTCTTT
CGAGGGTGAAAATACAATATACTCCGTCACAGCTTGATGTGCAGATTACGCCGCGAAAGCCTGGGATC
C

His-flgK used to construct pNW1063

GGATCCCCATGTCAGCGAAGGCAATTATTGAACAATTGAAGCGACTTTGCGTTCTGCATGAGCACCTG
CTCACGCTGTCTGAAGAAAAGACGGAAGCGCTCAAAGCCGGCAAAACAAAAGAGCTTTCTAACATTTT
GACAAAAGAGCAAAAATATATTCAAGCAATCACGCAGACAGAAGATGACCGGATCAAAACAACTTCGG
CCTTTCTCGGATATAGCGAAAATAATACTATTTCCGCATGTATCGCCAAAACCTCAGGCAGTGAAAAG
GAAGAGCTGGAACAACTATACGAATCTCTTTCTCAAGTTCTCGGACGTCTGAAAAAAGTAAATGAGAT
GAATAGGCAGCTGACAAGAGACGCGCTGCAATTCATCTCTATTTCGTACGATATGCTGGTTCCTAAGG
AAAATAACTTCAATTACAGCAAATCAATTAAAGCTGAGCTGCCGAAAAGTAGCAAAATGAAACTGTTT
GATTCAAAAGCTTAGCAGAAAGGAATTCAGAAAATG**CATCACCATCACCATCACCATCACCATCAC**AC
ATCTACCTTTATGGGGCTTGAAACTGCAAGGCGGGCGTTAAGCGCTCAGCAGGCAGCGTTAAGCACTA
CTGCAAATAACGTGGCAAATGCCAATACTGATGGTTATACAAGACAGCGGGTCTCATTGGAGGCAACT
GACTATTTCCCTGCTGTATCTAAAAATGCAGAAAAAACAGCGGGACAAATGGGTACGGGCGTTCAAGG
AAAATCAGTTGAGAGAATAAGAGATATCTTTCTTGACTACCAATACCGTCTTCAAAACAACAGTGCCG
GATACTATGACACGAAGGCAAAAGCGCTGTCCCAAATGGAAGGCGTTTTAAATGAAACGGATGACAGC
GGCTTGAACAGTGTGCTCAATTCGTTTTGGAATTCCCTGCAGGAATTATCGAATAATACAAATGAAGA
AAGTGCACGTTCTGTTGTTGCTCGAAAAGGACAAGCTGTAGCTGAAACGTTTAATTATATTTCTGAAT
CACTTACAAATGTCCGGATCC

flgK-His used to construct pNW1065

GAATTCGAAGTGCACAGAAATGGTGTGACCAAGAGCGGTGAACAAGGCGGAGACTTTTTTGATTTTAC
TGGCGGTGAAACTGAACCTGCCAAGGGCGCGGCGGGCAAGATCAAAGTGGCTGACAGCATAATAGATT
CAAAAGGCGCAAACATTGCTTTCTCACTGACTGGCGCAGCCAACGATAACGCAAATGCTACAAAATTA
GCAAATGTTTTAACCGGTAAAATAACCATTAACGGTAAAGAAACTAGTGTTTTAGATTATTATGCGGG
TCTGATTGGCGAGCTAGGGATCGAAGCTCAAGAGGCTAATCGACTGGCGTCTAATACAGAAACACAGC
TGAATGATGCTGACATAAACCGTCAGCAAATGAGCGCAGTTTCTTTAGACGAAGAAATGACGAATATG
ATTCAATTCCAACACGCATACAATGCAGCTGCAAGAATGGTGACTTTACAAGACGAATTGCTTGATAA
AGTGATCAACGGCATGGGTGTTGGAGGAAGGGGAGCAGGA**CATCACCATCACCATCACCATCACCATC**
ACTAGTGGGTGTTGGAGGAAGGGTAGAGTCACATGAGAGTAACACAAGGCATGATACAGCAAAACTCA
CTGAGATATATCGGTTCAAGCTACTCGAAGCTGGATAAACTCCAGTCGCAGATTTCTTCAGGAAAAAA
AATCTCAAAAGCTTCCGACGATCCTGTAGTAGCAATGAAAAGCTTAAAGTATAATACGCAACTGTCTC
AAGTGCAGCAGTACAAAAGCAATGCTTCTCAAGCCTTTACCTGGCTCGAAAACACAGAAACAAACATT
ACAGAAGGAATTGACATCTTGTCAAAGGTCAGAGAATTAGCGGTTGAAGCTCAAAATGATACAAACGG
CGAGCCGGAGCGGCAAGCGATTGGCGTAGAGGTAAAGCAGTTAAAGGAACAGCTTTTAAATATTGCGA

ATACACAAGTGAACGGCAGATATATCTTTAATGGCACAAATTCAGATAAGCCTCCGGTTACAGATAAC
GGAGACGGAACTTATACGGATCC

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Figure S1

MR**INHNIAALNTLNRL**SSNNSASQKNMEKLSSGLRINR**AGDDAAGLAISEK**MRGQIRGLEMASK**NSQDGISLIQTA**
EGALTETHAILQRVRELVVQAGNTGTQDKATDLQSIQDEISALTDEIDGISNRTEFNGK**KLLDGTYKVDTATPANQ**
KNLVFQIGANATQQISVNIEDMGADALGIKEADGSIAALHSVNDLDVTKFADNAADTADIGFDAQLKVVDEAINQV
SSQRAK**LGAVQNRLEHTINNLSASGENLTAAESR**IRDVDMAKEMSEFTK**NNILSQASQAMLAQANQQPQNVLQLLR**

215 Figure S2.

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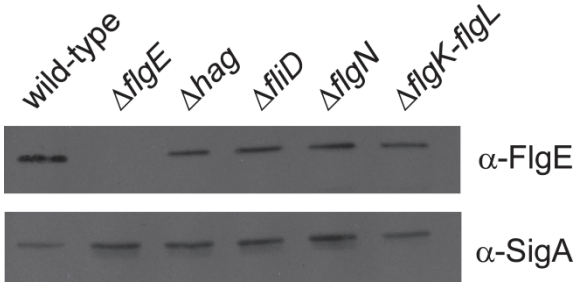
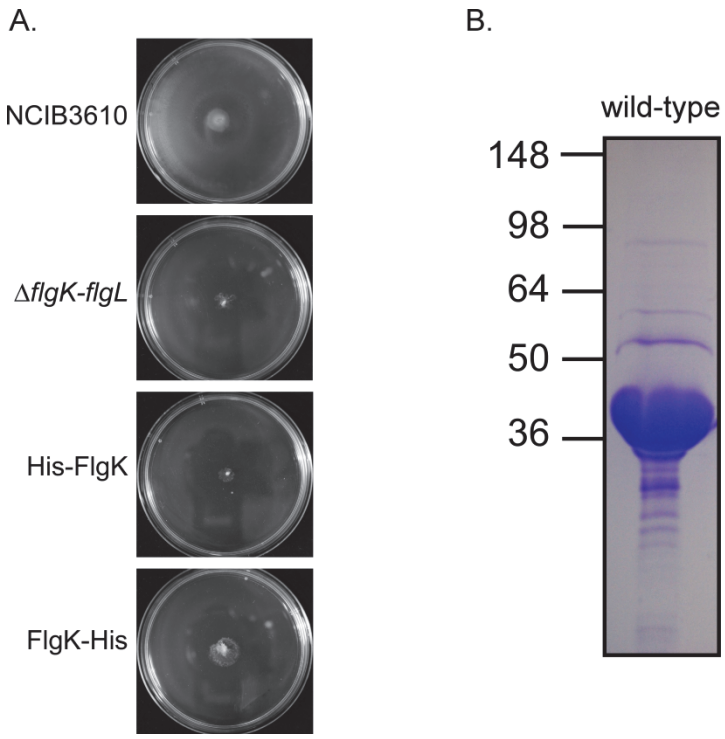


217

218 Figure S3



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