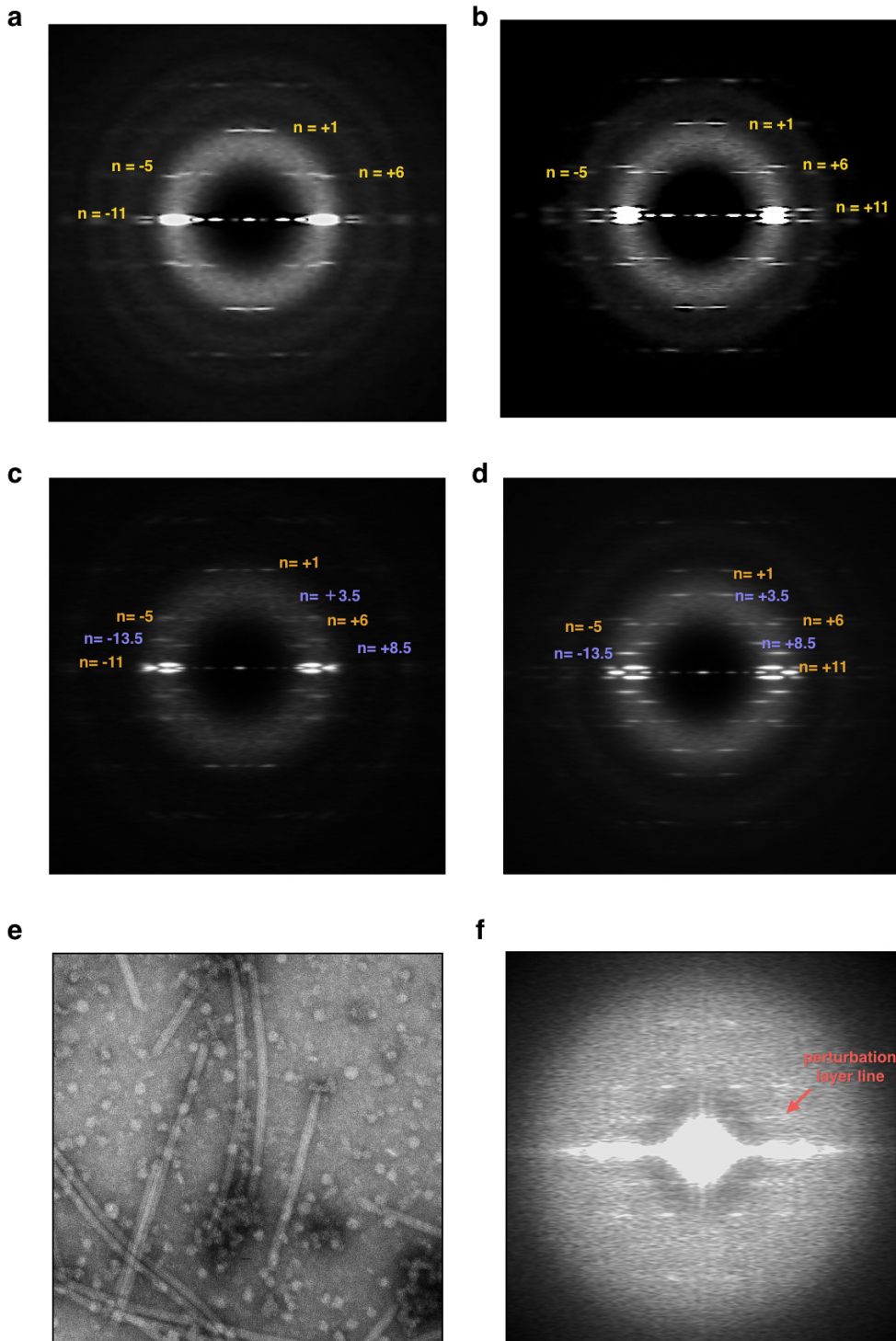
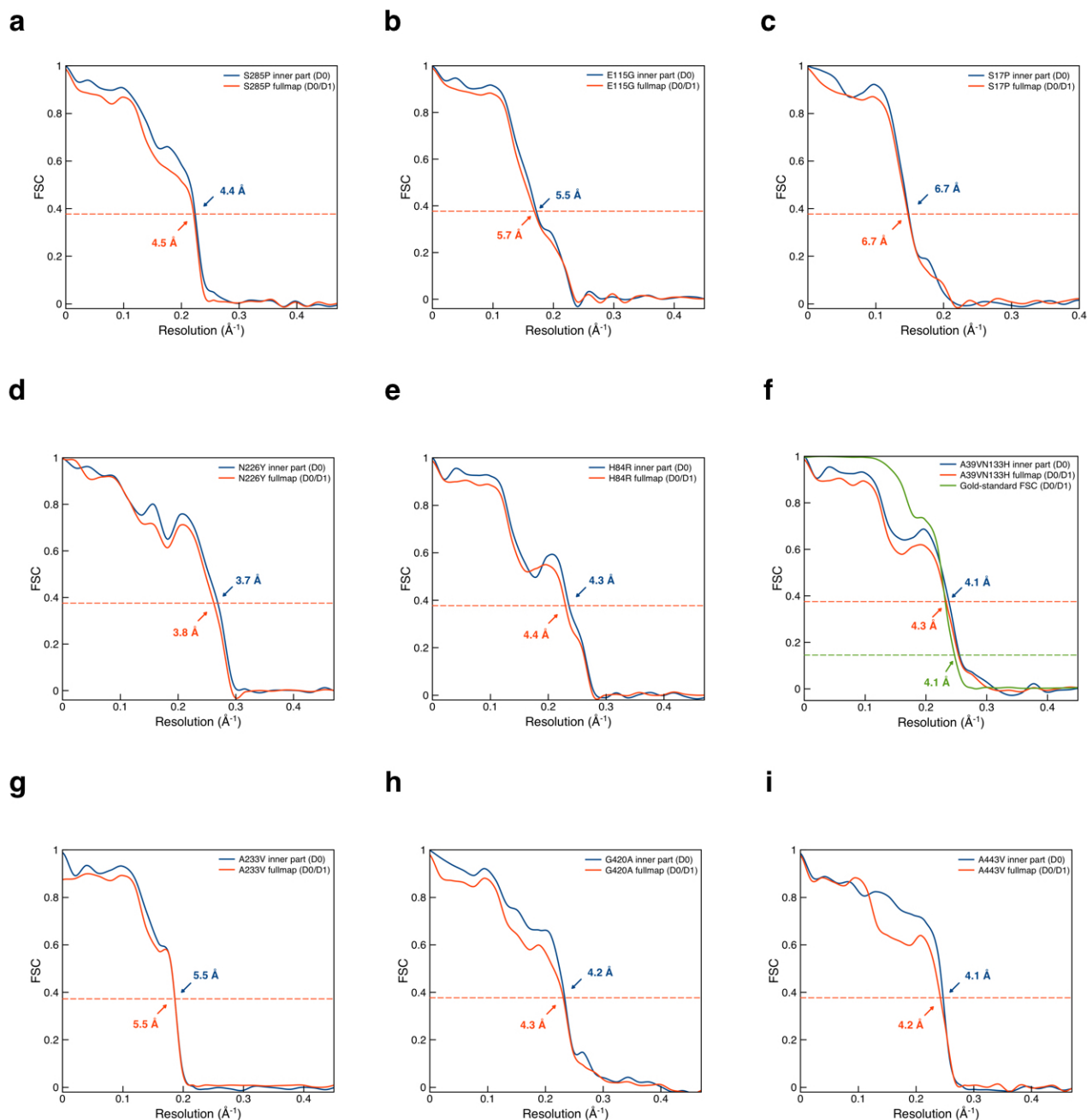
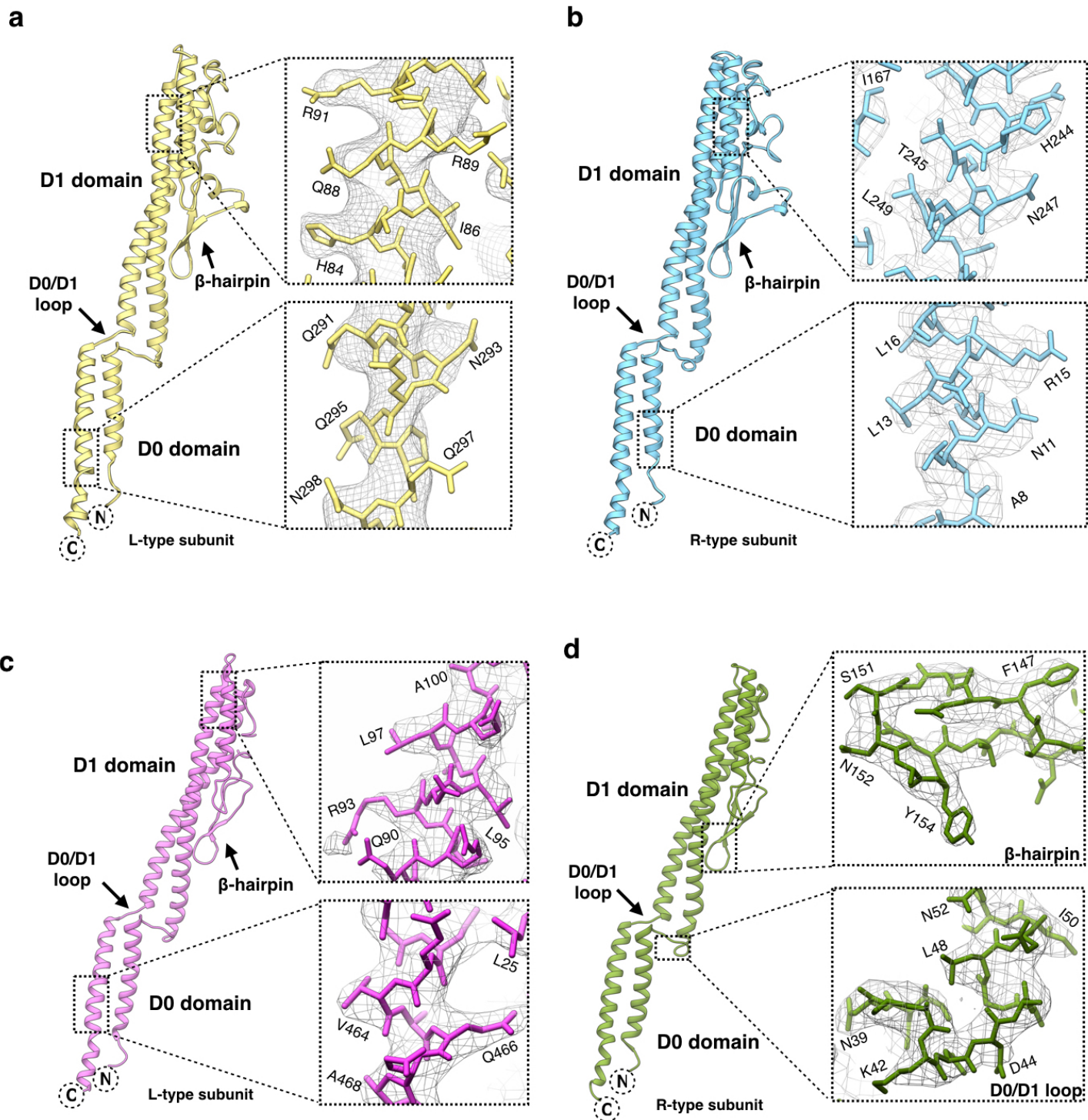




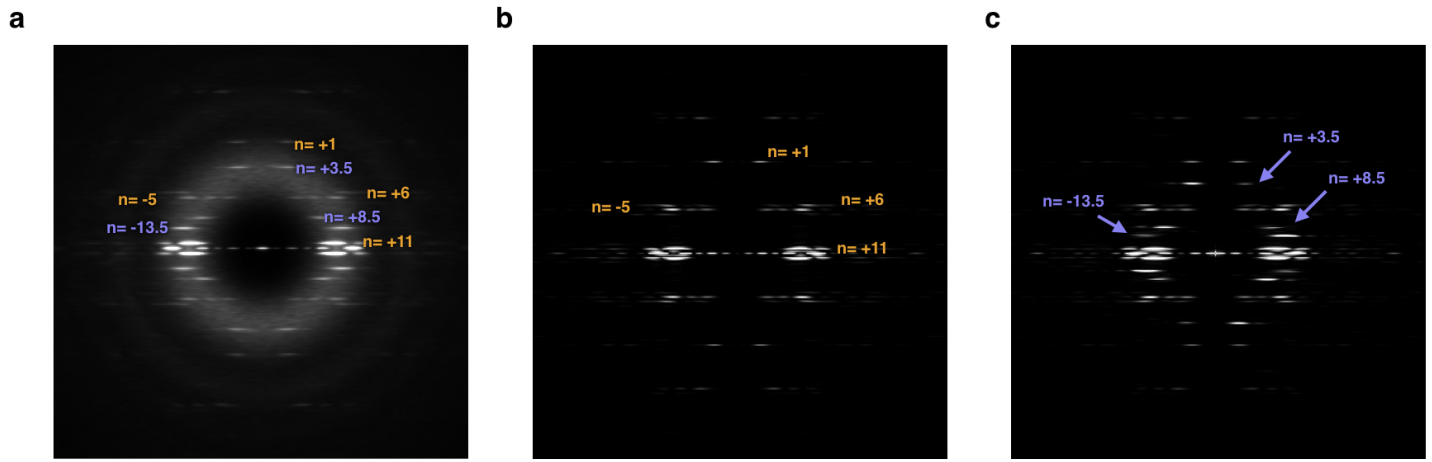
Supplementary Figure 1. Power spectra of flagellar filaments. The power spectra of raw image stack of L- (a) and R-type (b) straight filaments of *B. subtilis*. The power spectra of raw image stack of L- (c) and R-type (d) straight filaments of *P. aeruginosa*. (e) An image of negatively stained wild-type flagellar filaments of *P. aeruginosa*, and the corresponding averaged power spectrum from these filaments showing that the non-helical perturbation observed in mutants also exists in the wild-type.



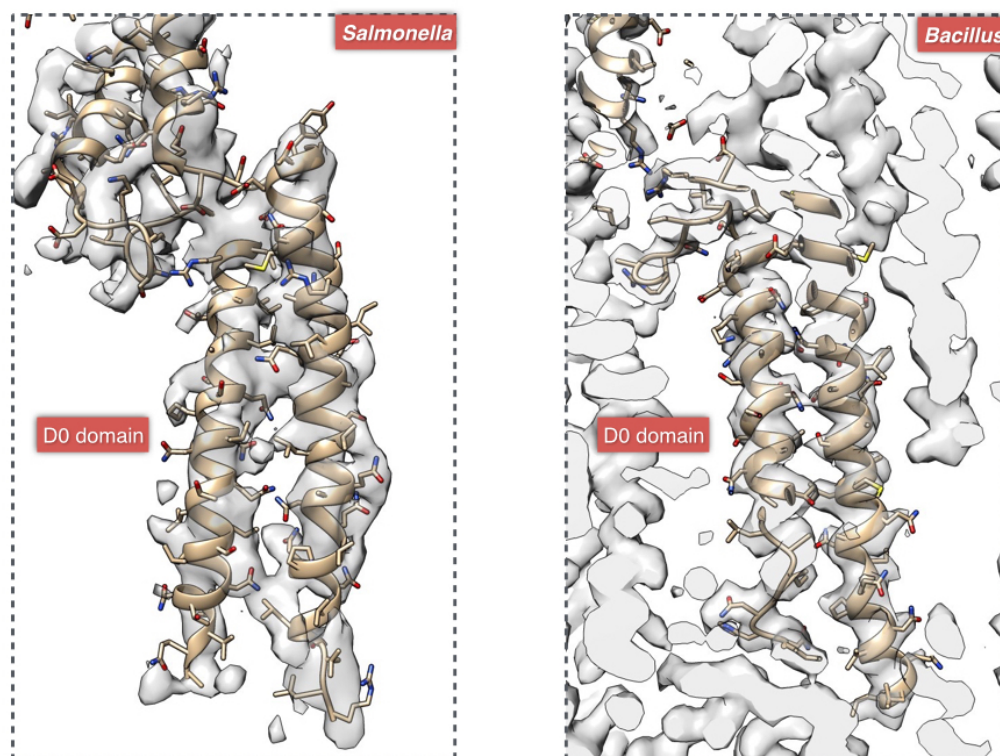
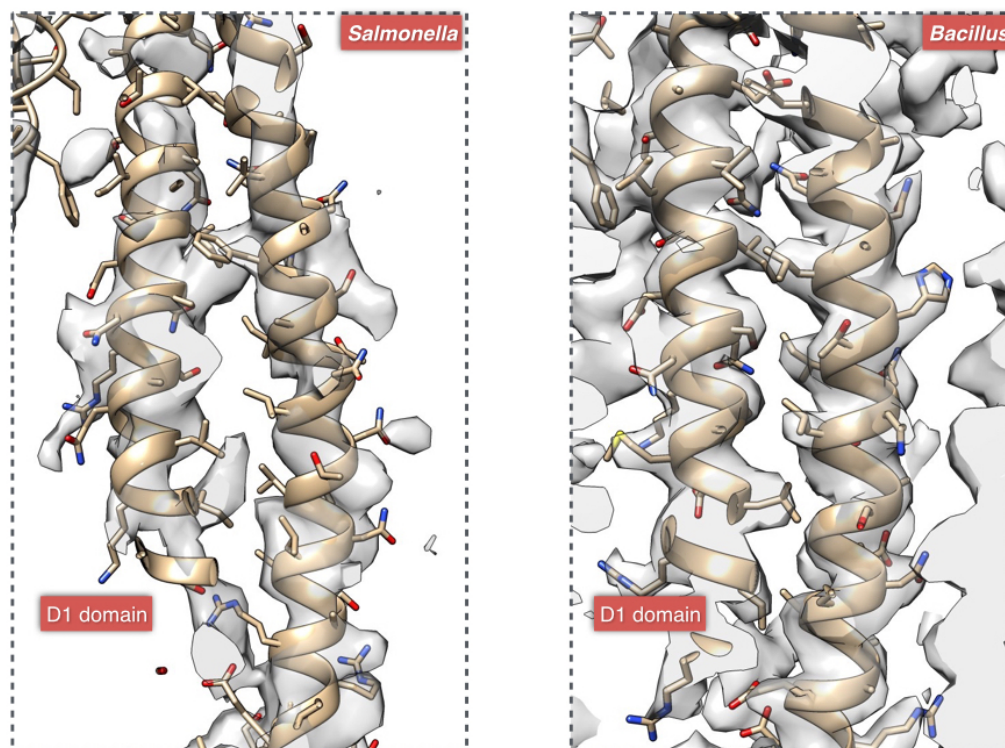
Supplementary Figure 2. Resolution of the flagellar filament structures of *B. subtilis* (a-g) and *P. aeruginosa* (h, i), derived from Fourier Shell Correlation (FSC) calculation. The red and blue lines are FSC of the full map and the D0 core, respectively, calculated between the refined atomic model and the map. The resolution in these plots is estimated using $FSC=0.38$, which is $\sqrt{0.143}$. A “gold standard” FSC between two half maps was also calculated for the A39VN133H dataset (f, green line) yielding a resolution of 4.1 Å at $FSC=0.143$. This suggests that the model:map FSC curves provide a similar estimate of resolution as the more conventional map:map FSC, and if anything are more conservative.



Supplementary Figure 3 The structure of a single flagellin subunit and its corresponding map density in *B. subtilis* and *P. aeruginosa*. **(a)** L-type subunit (S285P) of *B. subtilis* **(b)** R-type subunit (N226Y) of *B. subtilis* **(c)** L-type subunit (G420A) of *P. aeruginosa* **(d)** R-type subunit (A443V) of *P. aeruginosa*



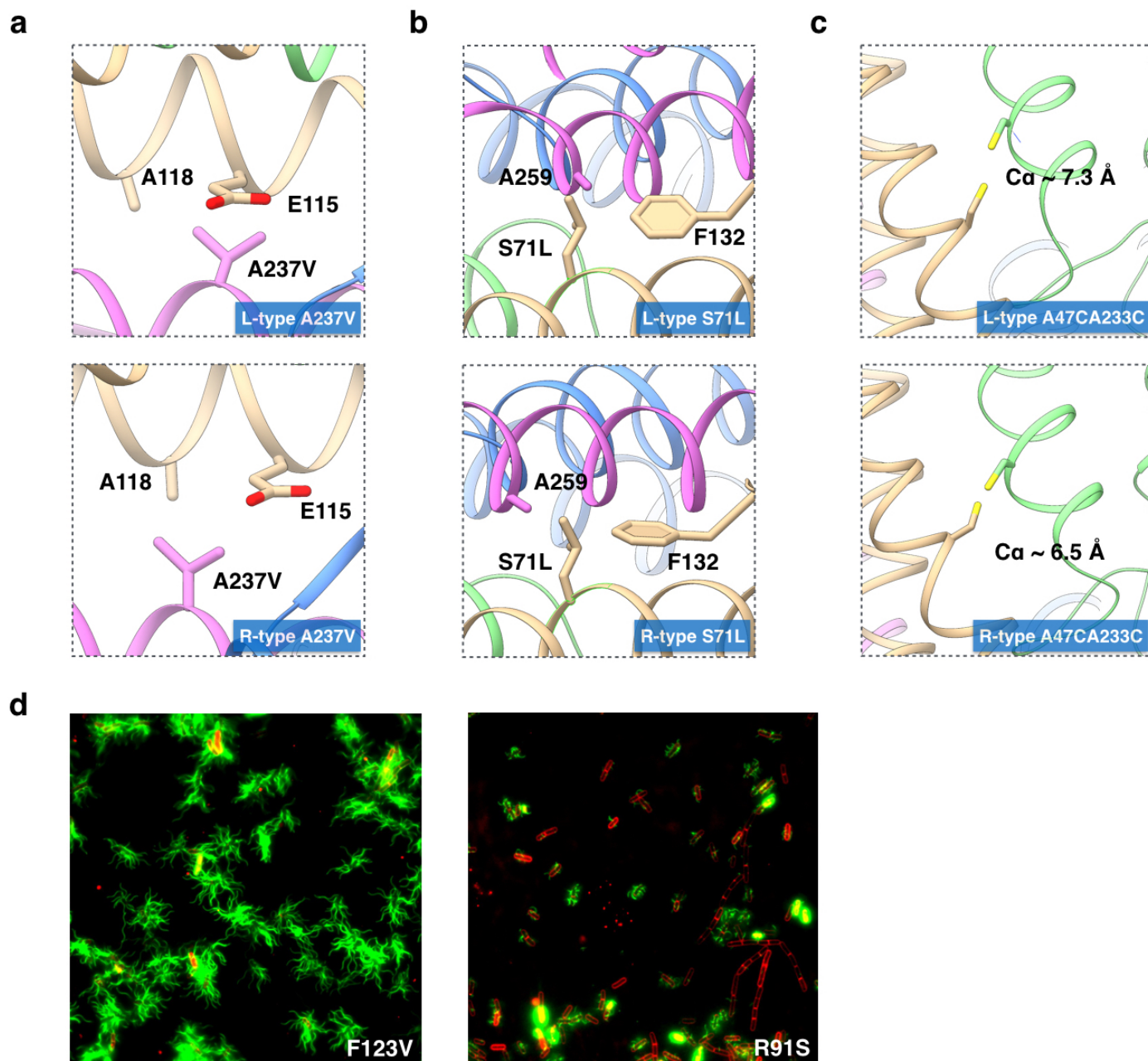
Supplementary Figure 4 power spectra of *P. aeruginosa* filaments. (a) The power spectrum of raw filament images of *P. aeruginosa*. (b) The power spectrum of the reconstructed volume projection, considering one subunit as the asymmetrical unit (D0 and D1 domains) (c) The power spectrum of the reconstructed volume projection, considering 22 subunits as the asymmetrical unit (D2 and D3 domains)

a**b**

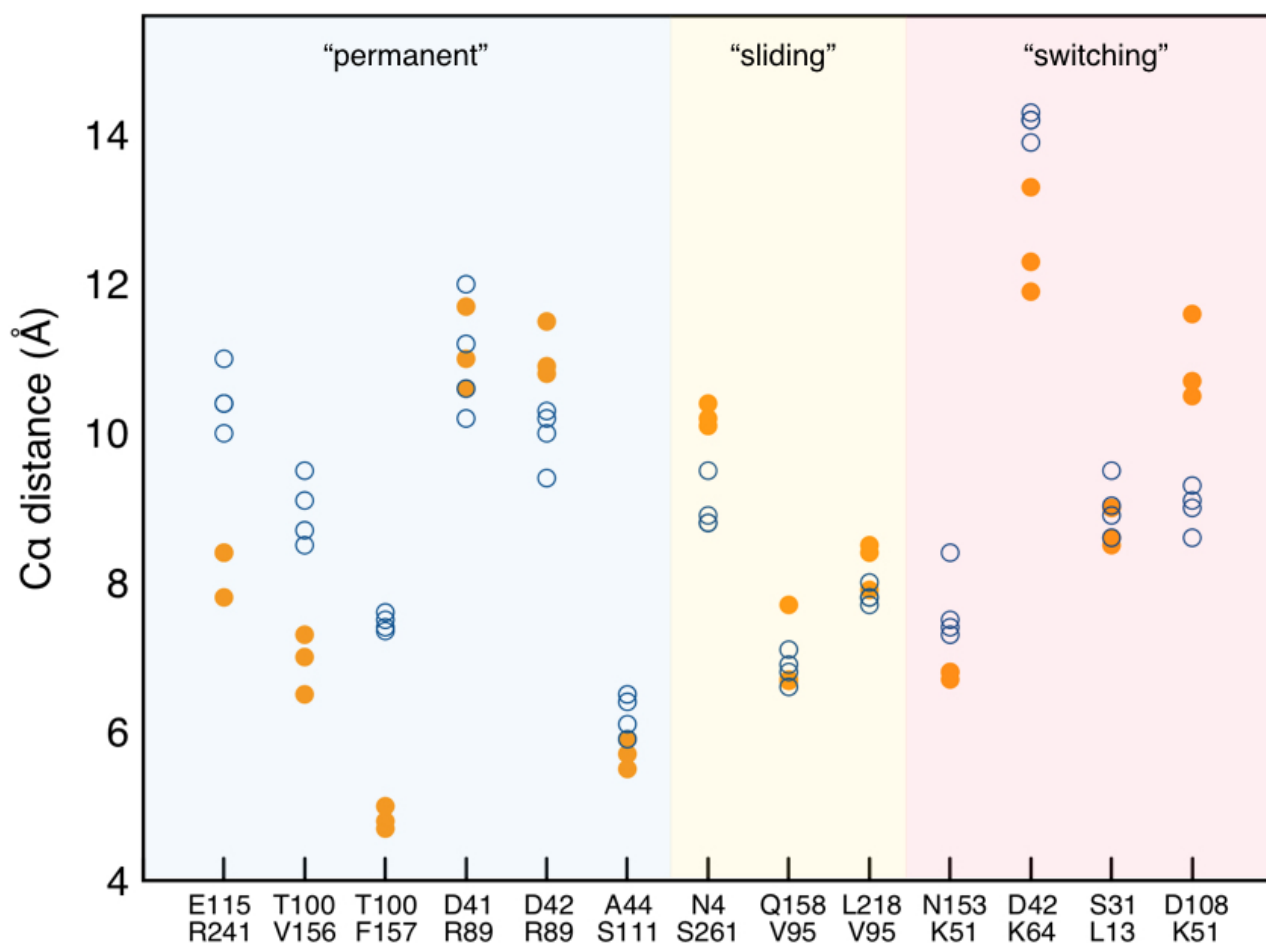
Supplementary Figure 5. The density maps comparison between *Salmonella* (EMD-1641) and *Bacillus* (N226Y). Corresponding cryo-EM map of D0 and D1 domains with similar threshold are shown in (a) and (b). The atomic models were aligned to the maps.

<i>Bacillus subtilis</i>	1	MR--	INHNIAALNTLNRLSSNSASQKNMEKLSGLRLNRAGDDAAGLAISEKMRGQIRGLEMASKNSQDGLSLIQT	75
<i>Clostridium tetani</i>	1	MI--	INHNLNAMNAHRQMGINIGNAGKATEKLSGLRLNRAGDDAAGLAISEKMRGQIRGLNQASRNAQDGLSLIQT	75
<i>Listeria monocytogenes</i>	1	MK--	VNTNIIISLKTQEYLRKNNEGMTQAQERLASGKRLNSSLDDAAGLAVVTRMNVKSTGLDAASKNSSMGIDLLQT	75
<i>Streptococcus pneumoniae</i>	1	MR--	IGTNVLSMNAQSLYENEKRMNVAMEHLATGKKLNHASDNPANVAIVTRMYARASGMRVAIRNNEDAISMLRT	75
<i>E. coli</i>	1	MAQV	INTNSLSLITQNNINKNQSALESSIERLSSGLRLNSAKDDAAGQAIANRFTSNIKGLTQAARNANDGISVAQT	77
<i>Helicobacter pylori</i>	1	MSFR	INTNIAALTSHAVGVQNNRDLSSSLERLSSGLRLNKAADSSGMAIADSLRSQSANLQGAIRNANDAIMVMQT	77
<i>Salmonella enterica</i>	1	MAQV	INTNSLSLITQNNLNKSQSLGTALERLSSGLRLNSAKDDAAGQAIANRFTANIKGLTQASRNANDGISIAQT	77
<i>Pseudomonas aeruginosa</i>	1	MALT	VNTNIIASLNTQRNLNASSNDLNTSLQRLTTGYRINSAKDDAAGLQISNRLSNQISGLNVATRNANDGISLAQT	77
<i>Bacillus subtilis</i>	76	AEGAL	TETHAILQRVRELVQAGNTGTQDKATDLQSIQDEISALTDEIDGISNRTEFNGKKLLDGYTKVDTATPANQ	152
<i>Clostridium tetani</i>	76	AEGAL	NETHAILHRMKELTVQAANDTNVT--VDKDNLQLEIKELQSEINRIASQTOFNTKTLLNGSLSK-----	142
<i>Listeria monocytogenes</i>	76	ADSAL	SSMSSIQRMRQLAVQSSNGSFSD--EDRKQYTAFFGSLIKELDHVADTTNNYNIKLLDQTATGA-----A	144
<i>Streptococcus pneumoniae</i>	76	AEAAL	QTVTNILQRMFDLAVQSANGTNSN--KNRDLLSKEFQSLIEQSGYIGEMTEFNDLSVFDGQNRP-----	142
<i>E. coli</i>	78	TEGAL	SEINNNLQRVRELTQATTGTNSE--SDLSSIQDEIKSRLEDIDRVSGQTQFNGVNVLAKNGS-----	143
<i>Helicobacter pylori</i>	78	ADKAM	DEQIKILDTIKTKAVQAAQDGGTLE--ESRRALQSDIQRLLEELDNIAINTSFNGQQMLSGSFS-----	143
<i>Salmonella enterica</i>	78	TEGAL	NEINNNLQRVRELAVQSANGTNSQ--SDLDSIQAEITQRLNEIDRVSGQTQFNGVKVLAQDNT-----	143
<i>Pseudomonas aeruginosa</i>	78	AEGAL	QQSTNIIQRIRDLALQVSANGNSD--ADRAALQKEVAAQQAELTRISDTTTEGGRKLLDGSFGT-----	144
<i>Bacillus subtilis</i>	153	KNLVFQ	IGANATQQISVNI EDMGADALG I KEA---DG-----	186
<i>Clostridium tetani</i>	143	SALTFO	IGANAGQTTLTIGDMTTSGLKLASI---NI-----	176
<i>Listeria monocytogenes</i>	145	TQVSIQ	ASDKANDLINDLFNAGKLSAGT--ITL---GSGS-----T-VA--GY-----	185
<i>Streptococcus pneumoniae</i>	143	--ITLD	--DIGHTINMTKHIPPS-----	161
<i>E. coli</i>	144	--MKIQ	VGANDNQTTIDLKQIDAKTLGLDGFVS---KNNDTVTTSA-P-VT--AFGATTTNNIKLT-----GITL	205
<i>Helicobacter pylori</i>	144	--NKEFQ	IGAYSNTTVKASIGSTSSDKIGHVRMETSSFSGEGMLASAAQNLTVEGLNFKQ---VNGVNDYKIE TVRI	216
<i>Salmonella enterica</i>	144	--LTIQ	VGANDGETTIDDLKQINSQTLGLDRTLNV---QQKYKVSDTAAT-VT--GYADTT---IALD-----NSTF	203
<i>Pseudomonas aeruginosa</i>	145	--TSFQ	VGSNAYETIDISLQNASATIGSYQVGS---NGAGTVASVAGT-ATASGIASGT---VNLV-----GGGQ	206
<i>Bacillus subtilis</i>	187	-----	-----S I-----AALHSVN-----	195
<i>Clostridium tetani</i>	177	-----	-----GG-----	178
<i>Listeria monocytogenes</i>	-----	-----	-----	-----
<i>Streptococcus pneumoniae</i>	206	ST----	EAATDTGGTNPASIEGV--YTDNGNDYYAK--ITGGDNDG-KY-YA-----VTVAN-DGTVTM	258
<i>E. coli</i>	217	ST----	SAGTGIGALSE-IINRFSNTLGVRASY--NVMATGGTPVQSGTVRELTINGVEIGTVNDVHKND--A----	280
<i>Helicobacter pylori</i>	204	-K----	ASATGLGGTDQ-KIDGDLKFDDTTGKYKAVTVTGGTGKD-GY-YE-----VSVDKTNGETVL	259
<i>Salmonella enterica</i>	207	VKNIIA	IAGDSAKAIAE-KMDGAIPNLSARARTVFTADVSGVTGGS-LN-FD-----TVGS--NTVSL	265
<i>Pseudomonas aeruginosa</i>	-----	-----	-----	-----
<i>Bacillus subtilis</i>	196	-----	-----DLDV-----TKFADNA-----	206
<i>Clostridium tetani</i>	-----	-----	-----	-----
<i>Listeria monocytogenes</i>	-----	-----	-----	-----
<i>Streptococcus pneumoniae</i>	259	ATGATANAT	-----VTD-----ANTTKATTITSGGTPVQIDNTAGSATANLGAVS--LVKL--ODSKGNDTD	316
<i>E. coli</i>	281	-----	-----DGRLTNAINSVKDRT-----GVEASLDIQGRINLHLSIDGRAISVHAASA--S	325
<i>Helicobacter pylori</i>	260	AGGATSP	LTGGLPATATEDVKNVQVANADLT-----EAKAALTAAGVTGTAS--VVKMSYTDNNGKTID	321
<i>Salmonella enterica</i>	266	AGV-----	-----TSTQDLADQLNSNSSKL-----GITASINDKGVLTTISATGENVKFGAQTGTATA	318
<i>Pseudomonas aeruginosa</i>	-----	-----	-----	-----
<i>Bacillus subtilis</i>	207	-----	-----A-----	207
<i>Clostridium tetani</i>	179	-----	-----A-----	179
<i>Listeria monocytogenes</i>	-----	-----	-----	-----
<i>Streptococcus pneumoniae</i>	162	-----	-----PTQ-----	164
<i>E. coli</i>	317	TYAL-----	-----KDTNGNL-----YAADVNETTGAHSVKTITYTDSGGAASSPTAV	359
<i>Helicobacter pylori</i>	326	G-QVFG	GGGNFAGISGTQHAVIGRLTLTRTDARDIIVSGVNFSHVGFHSAQGVAEYTVNLRVIRGIFDANVA-----	395
<i>Salmonella enterica</i>	322	G-GL-----	-----AVKVGDD-----YYSATQNKDGSISINTTKYATDDGTSTKL-N	362
<i>Pseudomonas aeruginosa</i>	319	G-QVA--	--VKVQGS DGKFEAAAKNVVAAG-----TAATTTITVGYVQLSPTAYSVSGTGTQ----	371
<i>Bacillus subtilis</i>	208	-----	-----DTADIGFDAQLKVVDEAIINQVSSQRAKLGAVQNRRL	242
<i>Clostridium tetani</i>	180	-----	-----KGA-ASISSQLKTIIDTALNEVSKERAKLGANQNRRL	213
<i>Listeria monocytogenes</i>	186	-----	-----SALSADA-DSSQATEAIDELINNI SNGRALLGAGMSRL	224
<i>Streptococcus pneumoniae</i>	165	-----	-----HDIKISTE-QEARTAIRKIEEALQNVSLHRADLGAMINRL	203
<i>E. coli</i>	360	KLGGDD	GKTEVVDDIGKTYDSADLNGGNLQTGLTAGGEALTAVAN-GKTTDPLKALDDAIASVDKFRSSLGAVQNRRL	435
<i>Helicobacter pylori</i>	396	-----	-----SAAGANANGAQAETNSQG--IGAGVTSL-KGAMI VMDMAD SARTQLDKIRSDMGSVQMEL	452
<i>Salmonella enterica</i>	363	KLGGAD	GKTEVVSIGGKTYAASKAEGHNFAQ-----PDLAEEAA--TTTENPLQKIDALAAQVDTLRLSDLGAVQNRRL	433
<i>Pseudomonas aeruginosa</i>	372	-----	-----ASQVFGNASAAQKSS-VASVDISTA-DGAQNAIAVVDNALAAIDAOHADLGAVQNRRL	426
<i>Bacillus subtilis</i>	243	EHTIN	NLSASGENLTAAESRIIRDVDMAKEMSEFTKNNILSQASQAMLAQANQQPQNVLQLL-R	304
<i>Clostridium tetani</i>	214	EHTI	ANVNNASENLQAAESRVRDMDMAKEMMNFSSKNNILQQAQAMLAQANQAPQILQLL-R	275
<i>Listeria monocytogenes</i>	225	SYNVSN	VNNQSIATKASASSIEDADMAAEMSEMTKYKLLTQTSISMLSQANQTPQMLTQLINS	287
<i>Streptococcus pneumoniae</i>	204	QFNI	ENLNQNSMALTDAA SLIEDADMAQEMSDFLKFKLLTEVALSMVSOANQIQMWSKLLQS	266
<i>E. coli</i>	436	DSAVT	NLNNTTTLNSEAQSRIRQDADYATEVSNMSKAQTIQQAGNSVLAKANQVPPQVLSLLQG	498
<i>Helicobacter pylori</i>	453	VTTI	NNISVTQVNVKAAESQIRQDVFAEESANFSKYNILAQSGSFAMAAQANAVQNNVLRLL-Q	514
<i>Salmonella enterica</i>	434	NSAIT	NLGNTVNNLTSAARSRIEDSDYATEVSNMSRAQILQQAGTSVLAAQANQVPPQNVLSLL-R	495
<i>Pseudomonas aeruginosa</i>	427	KNTID	NLTNISENATNARSRIKDTDFAAETAALSKNQVLLQAGTAIALAQANQLPQAVLSLL-R	488

Supplementary Figure 6. Alignments of the flagellin amino acid sequence from four Gram-positive bacteria and four Gram-negative bacteria. This alignment contains full sequences with D2 and D3 domains. Dark blue indicates higher sequence identities among species and light blue indicates intermediate sequence identities among species.



Supplementary Figure 7. Predicted mutation sites in *B. subtilis*. (a-c) Straight filaments S285P and N226Y structures were used as the modeling template, and the resulting interfaces are shown in (a) A237V (b) S71L and (c) A47CA233C. (d) Fluorescence images of three mutant filaments: F123V and R91S



Supplementary Figure 8. Mapping three types of interactions in *Salmonella* onto seven *Bacillus* mutants.

Three types of interactions were described in *Salmonella*, called “permanent”, “sliding” and “switching” interactions (Kitao et al., 2006), and many *Salmonella* residues are involved in those interactions. The corresponding residues in *Bacillus* can be identified and mapped based on the sequence alignment. Here we are showing the Ca distances of the corresponding *Bacillus* residues that belong to those three types of interactions. The orange solid circles are the three L-type straight mutants (E115G, S17P and S285P), and the blue empty circles are the four R-type straight mutants (H84R, N226Y, A233V, and A39VN133H).

Supplementary Table 1: Subunit-subunit interfacial area along different helices

Mutation site(s)	5-start (Å ²)	11-start (Å ²)	6-start (Å ²)	16-start (Å ²)
<i>B. subtilis</i>				
S285P	1850	1954	552	238
E115G	1823	1810	485	237
S17P	1893	1833	574	265
N226Y	1819	1985	683	278
A39VN133H	1673	1874	596	275
H84R	1671	1924	516	330
A233V	1779	1867	571	271
<i>P. aeruginosa</i>				
G420A	1800	1630	495	211
A443V	1758	1922	542	286

Supplementary Table 2 Strains and mutations

Strain	Genotype
3610	Wild type
DS1677	<i>Δhag</i> (Kearns and Losick, 2005)
DS1919	<i>Δhag amyE::P_{hag}-hag^{T209C} spec</i> (Blair <i>et al.</i> , 2008)
DK29	<i>P_{fla/che}ΩPhyspank fla/che operon kan</i> (Guttenplan <i>et al.</i> , 2013)
DK620	[PY79] <i>Δhag sfp⁰ swrA^{fs}</i>
DK1343	<i>Δhag amyE::P_{hag}-hag^{S17PT209C} spec</i>
DK1362	<i>Δhag amyE::P_{hag}-hag^{H84RT209C} spec</i>
DK2750	<i>Δhag amyE::P_{hag}-hag^{T209CN226Y} spec</i>
DK2751	<i>Δhag amyE::P_{hag}-hag^{A39VNI33HT209C} spec</i>
DK2790	<i>comI^{Q12L} Δhag</i>
DK2956	<i>Δhag amyE::P_{hag}-hag^{S285PT209C} spec</i>
DK2977	<i>Δhag amyE::P_{hag}-hag^{E115GT209C} spec</i>
DK3400	<i>Δhag amyE::P_{hag}-hag^{A233VT209C} spec</i>
DK3965	<i>Δhag amyE::P_{hag}-hag^{H84RT209C} spec P_{fla/che}ΩPhyspank fla/che operon kan</i>
DK4152	<i>Δhag amyE::P_{hag}-hag^{S17PT209C} spec P_{fla/che}ΩPhyspank fla/che operon kan</i>
DK4153	<i>Δhag amyE::P_{hag}-hag^{E115GT209C} spec P_{fla/che}ΩPhyspank fla/che operon kan</i>
DK4229	<i>Δhag amyE::P_{hag}-hag^{T209CN226Y} spec P_{fla/che}ΩPhyspank fla/che operon kan</i>
DK4230	<i>Δhag amyE::P_{hag}-hag^{A39VNI33HT209C} spec P_{fla/che}ΩPhyspank fla/che operon kan</i>
DK4324	<i>Δhag amyE::P_{hag}-hag^{A233VT209C} spec P_{fla/che}ΩPhyspank fla/che operon kan</i>
DK4399	<i>Δhag amyE::P_{hag}-hag^{S285PT209C} spec P_{fla/che}ΩPhyspank fla/che operon kan</i>
DK4859	<i>comI^{Q12L} Δhag aprE::P_{hag}-hag^{S31PT209C} kan</i>
DK4860	<i>comI^{Q12L} Δhag aprE::P_{hag}-hag^{S71LT209C} kan</i>
DK4864	<i>comI^{Q12L} Δhag aprE::P_{hag}-hag^{T209CA237V} kan</i>
DK4879	<i>comI^{Q12L} Δhag aprE::P_{hag}-hag^{T209CF123V} kan</i>
DK4884	<i>comI^{Q12L} Δhag aprE::P_{hag}-hag^{R91ST209C} kan</i>
DK4894	<i>comI^{Q12L} Δhag aprE::P_{hag}-hag^{A47CT209CA233C} kan</i>
PY79	<i>sfp⁰ swrA^{fs}</i>

Supplementary Table 3 List of used primers

Primers	Genotype
953	GGAGTGTCAAGAATGTTTGCAAAAC
1008	TGCAGCCGCTGAAGAATATGGCA
1009	TGCCGTATTGATAGAGAXAGAGGA
1115	AGGAGGAATTCTTTAGAAATCGCACCTTAGCTGGTG
1116	CTCCTCTCGAGGCCAGAACCAGCAGCGGAGCCAGCGGAACCTGATCCCGAAGCGACTG CTTCG
1117	AGGAGGAATTCCCTTACCTGATTGATATGCTGTAATG
1118	CTCCTCTCGAGGCCAGAACCAGCAGCGGAGCCAGCGGAACCATCCTTTTCTTTAAGCTG ACGCTCC
3251	CTCACTAAAGGGAACAAAAGCTGG
3459	GCTAATACGACTCACTATAGGGTAATGATGTAGCCGGGAGGA
4439	CGTTTAGAAAGCACTGCAACA
4440	CGCCATCGTAAAGATTAACGT
4893	TACCACGATGGCTGCCACTA
4894	GTACTTGCCACCACATCATAA
4895	TGTTGCAGTGCTTTCTAAACGCGCTTATCCAGCGATGTGAT
4897	ACGTTAATCTTTACGATGGCGACGACTCACTATAGGGCGAATTG
4932	CTTTTGTTCCCTTTAGTGAGCTGGCAACGCCAAGGTCTTTTTTAAAA
5518	GAAACTTCCTTCAGGTCTTCGCATCAAC
5519	GAAGGAAGTTTCTCCATGTTCTTTTG
5520	GAATCCTTCTTATCCAAACAGCTGAG
5521	GATAAGAAGGATTCCGTCTTGAGAG
5522	GTTAGTGAGCTAGTTGTTCAAGCTGG
5523	CTCACTAACACGTTGAAGGATCGCATG
5524	GAAGTCAATGGTAAGAAATTGCTCG
5525	CATTGACTTCTGTACGATTTGAATAC
5526	GGTGTGGTACAAAATCGTCTAGAGCAC
5527	GTACCACACCAAGCTTAGCACGTTGAG
5528	CTTTGCATCTCTGAAAAAATGAGAGG
5529	GATGCAAAGACCTGCTGCGTCATCTC
5530	CGTTGCAAGCTTGGTGCGGTACAAAATC
5531	GCTTGCAACGTTGAGAAGAACTTGG

Reference

Kitao, A., Yonekura, K., Maki-Yonekura, S., Samatey, F.A., Imada, K., Namba, K., and Go, N. (2006). Switch interactions control energy frustration and multiple flagellar filament structures. *Proc Natl Acad Sci U S A* *103*, 4894-4899.