

## 1 SUPPLEMENTAL FIGURE LEGENDS

2

3 **FIG. S1.** Amino acid sequence alignment. Amino acid sequence alignment of  
4 the cytoplasmic loop regions of PomA (A) and the C-terminal regions of FliG  
5 (B) with residues mutated in this study. Residues shown in black boxes are  
6 residues that are conserved and are involved in torque generation of the  
7 H<sup>+</sup>-driven flagellar motor in *E. coli* as described previously (14). Residues  
8 shown in gray boxes are additional residues mutated in this study. Numbers  
9 over the arrowheads indicate the residue numbers of PomA or FliG of *V.*  
10 *alginolyticus* mutated in this study. The multiple sequence alignment was  
11 carried out by the Clustal Omega application at the European Bioinformatics  
12 Institute website (<http://www.ebi.ac.uk/Tools/msa/clustalo/>). *Va*, *V.*  
13 *alginolyticus*; *So*, *S. oneidensis*; *Bs*, *B. subtilis*; *Ec*, *E. coli*; *Tm*, *T. maritima*; *Aa*,  
14 *A. aeolicus*. MotP, Na<sup>+</sup>/K<sup>+</sup>-driven stator protein in *B. subtilis*; LafT, H<sup>+</sup>-driven  
15 stator protein in lateral flagella.

16

17 **FIG. S2.** Complementation of the *pomAB fliG* triple-mutant strain by wild-type  
18 *pomAB* and *fliG* genes on plasmids and detection of PomA and FliG proteins  
19 with mutations. (A) NMB301,  $\Delta pomAB\Delta fliG$  strain, was transformed with two  
20 plasmids, pSU41 (vector) or pYA303 (encoding the *pomAB* genes), and  
21 pMMB206 (vector) or pNT1 (encoding the *fliG* gene), and cells were inoculated  
22 in soft-agar plates and incubated at 30°C for 8 hr. (B) Expression of mutant  
23 PomA proteins. NMB301 cells harboring two plasmids, pSU41 or pYA303  
24 encoding wild-type or mutant *pomA* with wild-type *pomB*, and pNT1 encoding  
25 the wild-type *fliG*, were grown at 30°C for 4 hr, and whole-cell extracts were  
26 analyzed by immunoblotting with the anti-PomA antibody. (C) Expression of  
27 the mutant FliG proteins. NMB301 cells harboring two plasmids, pYA303  
28 encoding wild-type *pomAB*, and pMMB206 or pNT1 encoding the wild-type or  
29 the mutant *fliG*, were grown at 30°C for 4 hr, and whole-cell extracts were

30 analyzed by immunoblotting with the anti-FliG antibody.

31

32 **FIG. S3.** Motilities of *pomA/fliG* combined mutants in soft-agar plates (i).

33 Swimming abilities were observed in soft-agar plates of *pomA/fliG* combined  
34 mutants where highly conserved charged residues were substituted by single  
35 uncharged residues. Cells were incubated on VPG-soft-agar plates at 30°C for  
36 4.5 hr.

37

38 **FIG. S4.** Motilities of *pomA/fliG* combined mutants in soft-agar plates (ii).

39 Swimming abilities were observed in soft-agar plates of *pomA/fliG* combined  
40 mutants where charged residues were substituted by charge-reversed residues.  
41 Cells were incubated on VPG-soft-agar plates at 30°C for 5 hr.

42

43 **FIG. S5.** Motilities of *pomA/fliG* combined mutants in soft-agar plates (iii).

44 Swimming abilities were observed in soft-agar plates of *pomA/fliG* combined  
45 mutants where charged residues were substituted by charge-reversed residues.  
46 Cells were incubated on VPG-soft-agar plates at 30°C for 6 hr.

47

(A)

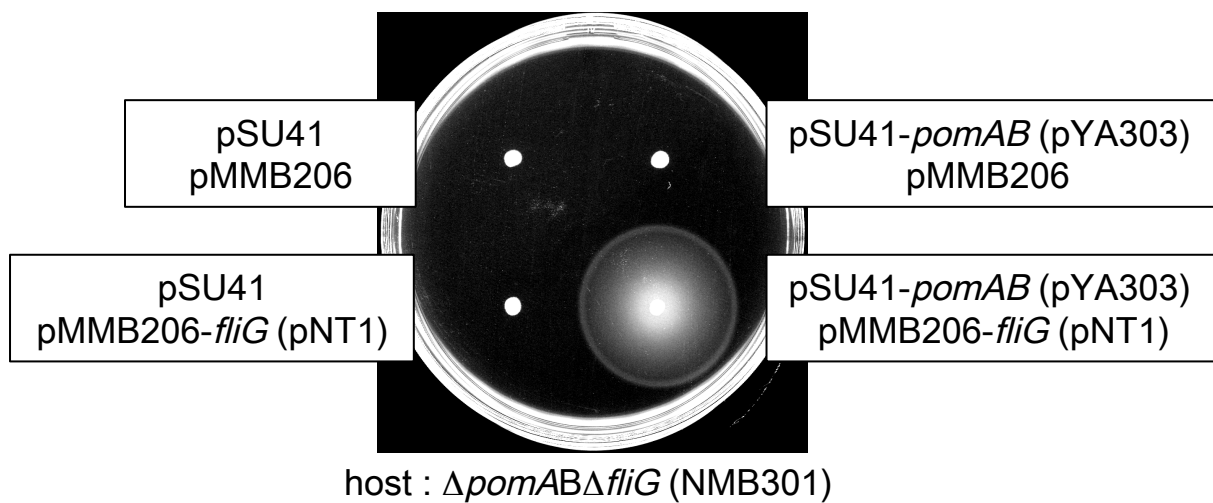
<i>Va PomA</i>	56	FGATK IAGKAFMF - KAD - - EPEDL IAK I VEMADAAR	<b>R</b>	KGGF
<i>So PomA</i>	56	LGAVK IAAKAF I F - KID - - RPEDL IEQSVTMADAAR	<b>R</b>	KGGF
<i>Bs MotP</i>	62	KKAPSVL KQAF I R - QED - - NVKDLVKTFVSLSDHAR	<b>R</b>	KHGL
<i>Ec MotA</i>	55	KGTLKALPLLFRRSKYTKAMYMDLLALLYRLMAKS	<b>R</b>	QMGM
<i>Va LafT</i>	55	KEMRRQVPAT I KGPTEEHEYYMELMALLNLLLETAR	<b>R</b>	NRGY
<i>So MotA</i>	54	I ASMKRFKWL I FPL - RT - - DLNERAEFL I E I AGDVR	<b>R</b>	KGGL
<i>Bs MotA</i>	57	KKVPTLFRVLFKENKQL - - TIEEL I PMFSEWAQLAR	<b>R</b>	REGL
		96 97 99		
<i>Va PomA</i>	93	LAL <b>E</b> E - M - <b>E</b> INN - - - - - - - - - - - - - - - TFMQKGI DLL		
<i>So PomA</i>	93	LAL <b>E</b> E - A - Q ISN - - - - - - - - - - - - - - - SFMQKAVDML		
<i>Bs MotP</i>	99	LSL <b>D</b> DQARE I KD - - - - - - - - - - - - - - - PFLKKGLLLA		
<i>Ec MotA</i>	95	FSL <b>E</b> RDI - ENPRESE I FASYPRILADSVMLDF IVDYLRLI		
<i>Va LafT</i>	95	KFL <b>D</b> SHI - ESPEQSSMFLAYPTIAEDHRL I SFI TDNLRLM		
<i>So MotA</i>	91	LSI <b>E</b> DKI - DQIDD - - - - - - - - - - - - - - - PFLHKGLELL		
<i>Bs MotA</i>	95	LAL <b>E</b> AS I - EDVDD - - - - - - - - - - - - - - - AFLKNGLSMA		
		128 133~136		
<i>Va PomA</i>	114	VDGHD - ADVVRAALK <b>K</b> D IALT <b>D</b> ERHTQGTGVFRAFGD		148
<i>So PomA</i>	114	VDGHD - GEVVRAALE <b>K</b> D ILT <b>D</b> EDRHRIGIA I FRAFAD		148
<i>Bs MotP</i>	122	IDGWD - EET IRLVMDSE I AAMEERHRKGRRVFEKAGE		156
<i>Ec MotA</i>	135	ISGHMNTFE I EALMDEE I ETHESEAEVPANSLALVGD		170
<i>Va LafT</i>	135	AMGQMSPHELEGLLEQE I EA IQNEMLLPSRSLQRTAE		170
<i>So MotA</i>	114	VDGYE - KDN IVE I LEKE I EFEQHG I EQTVKVYEAMGG		148
<i>Bs MotA</i>	118	VDGQS - AEF I RDIMTEE I VEAME <b>D</b> DRHQAGAA I FTQAGT		152

(B)

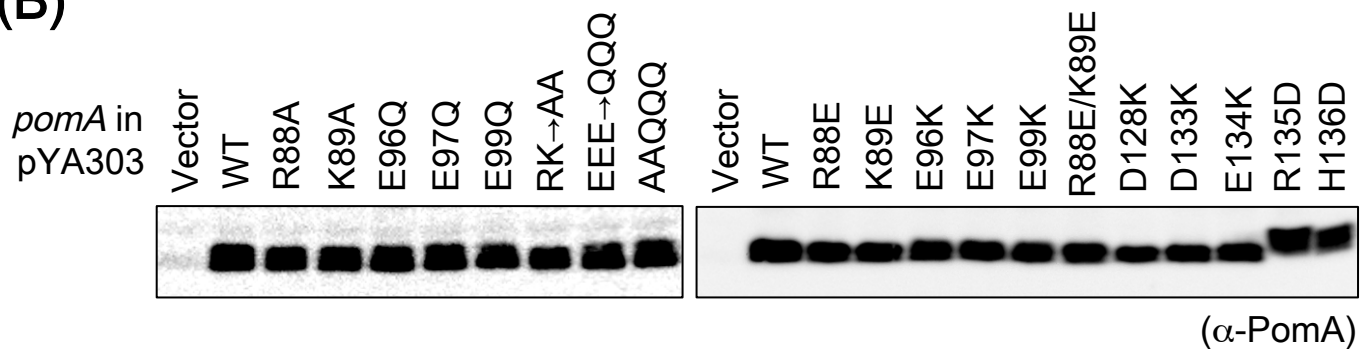
<i>Va FliG</i>	214	GGLKAAAE I MNYLDNNVEGLLMEQ I RDQDEDMATQ I QDLM		
<i>So FliG</i>	210	GGLKAAAN I MNYLDTGVESQLMETMRE TDEEMAQQ I QDLM		
<i>Bs FliG</i>	198	GG I EAVVEVLNGVDRGTEKT I LDSLE I QDPDLAEE I KCRM		
<i>Ec FliG</i>	194	GGVRTAAE I I NLMKTQQEEAV I TAVRE FDGE LAQK I I DEM		
<i>Tm FliG</i>	196	GG I DTAAE I MNLDRTTEKK I MDKLVQENPE LADE I RRRM		
<i>Aa FliG</i>	197	EGTAVAAE LNTLDKETREL I LQS I GQEDPLLEERI REKM		
		284		
<i>Va FliG</i>	254	FVFENLVEVDDQGI QKLLRDVPQDVLQKAL	<b>K</b>	KGADDS LREK
<i>So FliG</i>	250	FVFENL I DVDDRGI QTLLREVQQDVLMKAL	<b>K</b>	KGADDQLKDK
<i>Bs FliG</i>	238	FVFEDI VTLDNRA I QRVI RDVENDDL LLSL	<b>K</b>	KVASEEVEKE I
<i>Ec FliG</i>	234	FLFENLVDDRS I QRLLQEVDSESLL I AL	<b>K</b>	KGAEQP LREK
<i>Tm FliG</i>	236	FVFEDI LKLDPRS I QLVLREVDTRDLALAL	<b>K</b>	KGASDE LKEK
<i>Aa FliG</i>	237	FTFEDI RKLSDRDI I E I LKVVDKNTLM I ALLGAPEDI KQK		
		300 301 304 308 309 311 317		
<i>Va FliG</i>	294	V FKNMS <b>K</b> RAA <b>E</b> MMR <b>D</b> D I <b>E</b> AMPPV <b>R</b> VADVEAAQKE I LA I AR		
<i>So FliG</i>	290	I LGNMS <b>K</b> RAA <b>E</b> LLR <b>D</b> DL <b>E</b> AMGP I R I SEVE I AQKE I LSI AR		
<i>Bs FliG</i>	278	V FNNMSQRMV E T FKEE M E FMGPVRLKDVEEAQSR I VS I VR		
<i>Ec FliG</i>	274	FLRNMSQRAA D I LRDDL ANRGPVRLS QVENEQA I LL I VR		
<i>Tm FliG</i>	276	I FKNMS <b>K</b> RAAALL <b>K</b> DELE YMGPVRLKDVEEAQK I I NI I R		
<i>Aa FliG</i>	277	FLSNMS <b>K</b> RAAKLFL <b>E</b> DM <b>E</b> ALGPV <b>K</b> KSE I EKAQRQVVNI I R		
<i>Va FliG</i>	334	RMADAGELMLSGGA - DEFL -		351
<i>So FliG</i>	330	RLSDSGE IMLGGGGGDEFL -		348
<i>Bs FliG</i>	318	KLEEAGE I V I ARGGGDD I I V		337
<i>Ec FliG</i>	314	RLAETGEMV I GSGE - DTYY -		321
<i>Tm FliG</i>	316	RLEEAGE I V I ARGGGEEL I M		335
<i>Aa FliG</i>	317	KMI DEGK I E I GD - - - - -		328

Fig. S1. Takekawa *et al.*

(A)



(B)



(C)

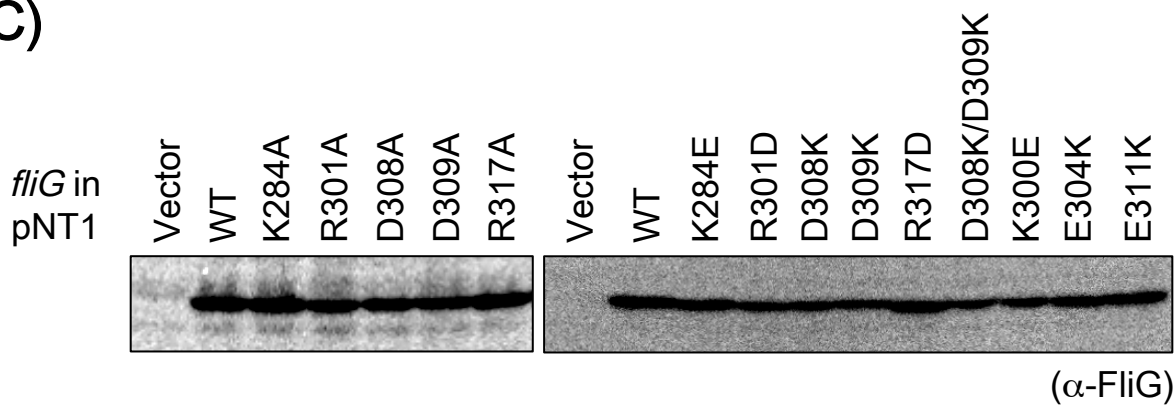


Fig. S2. Takekawa *et al.*

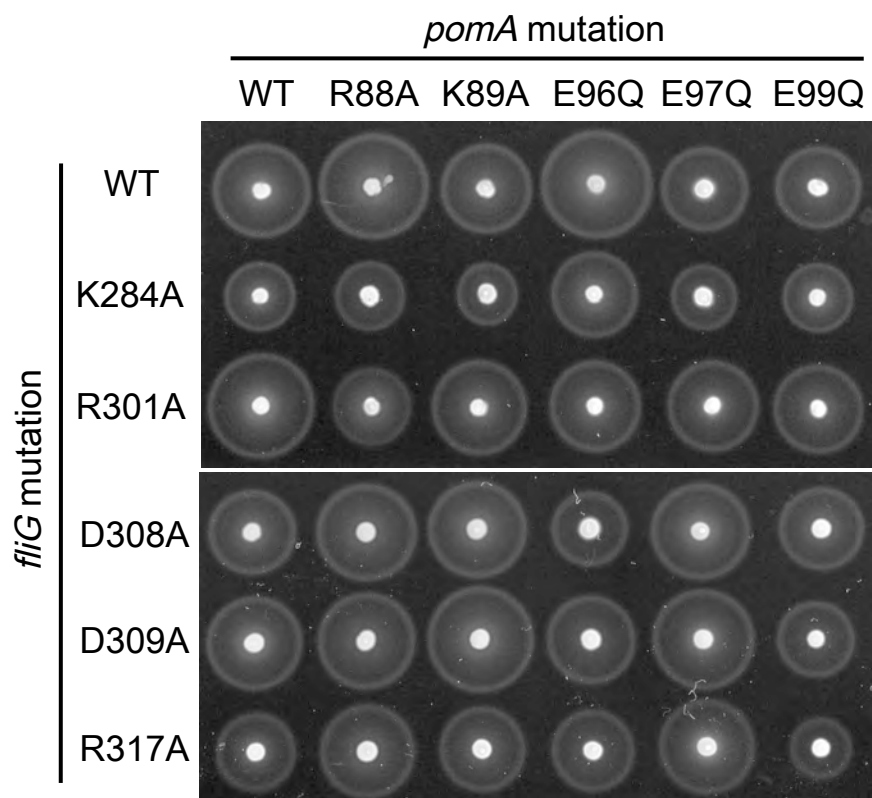


Fig. S3. Takekawa *et al.*

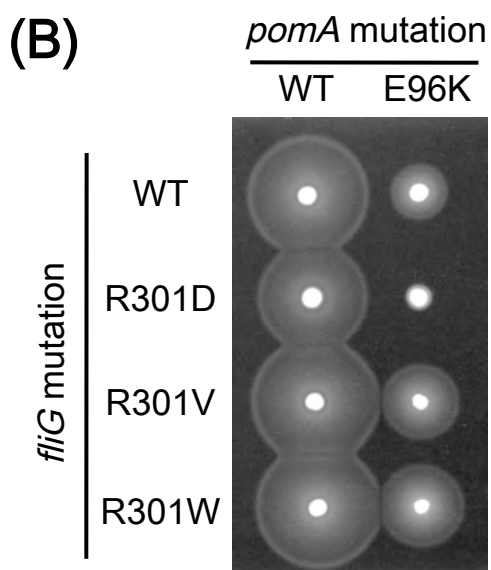
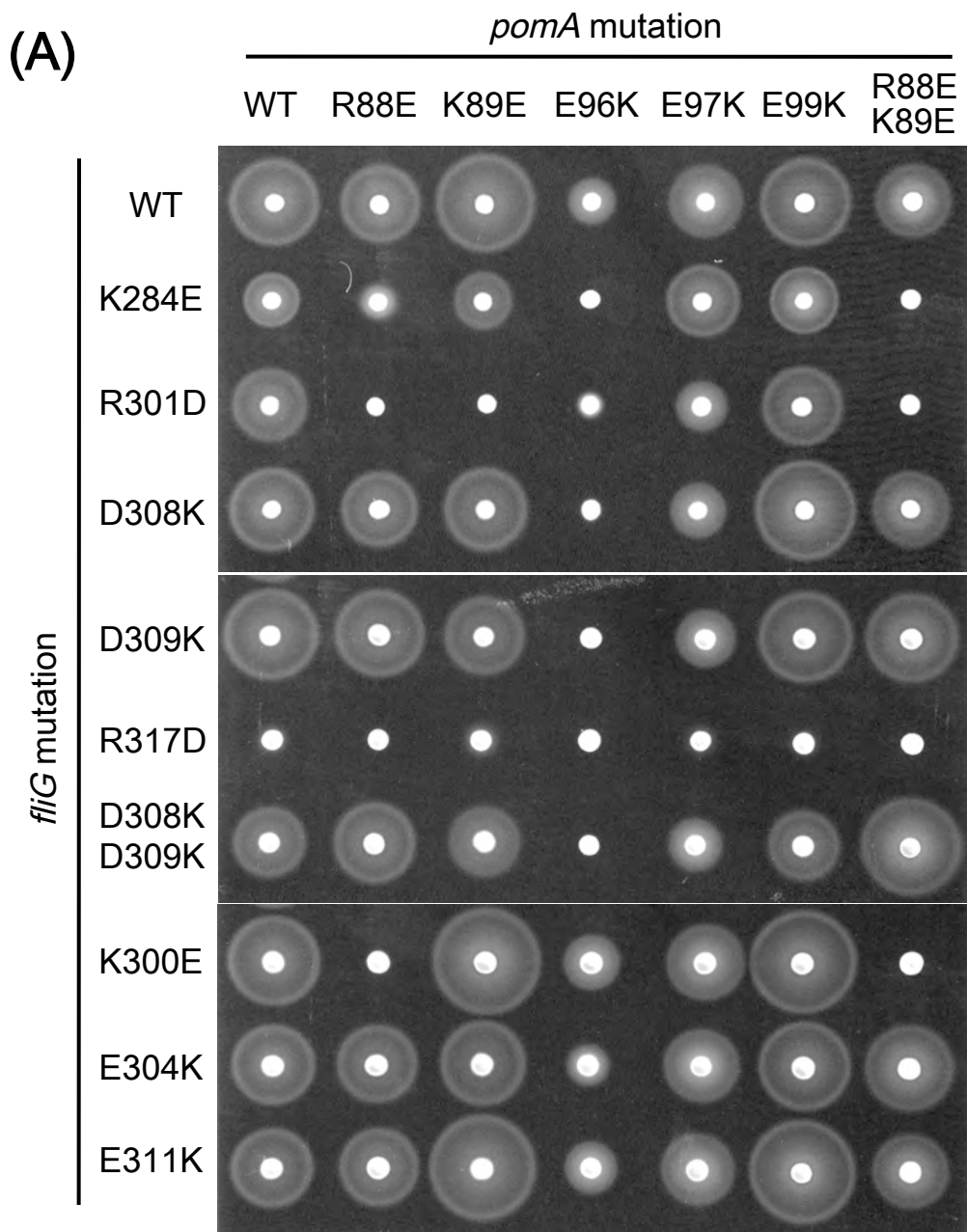


Fig. S4. Takekawa *et al.*

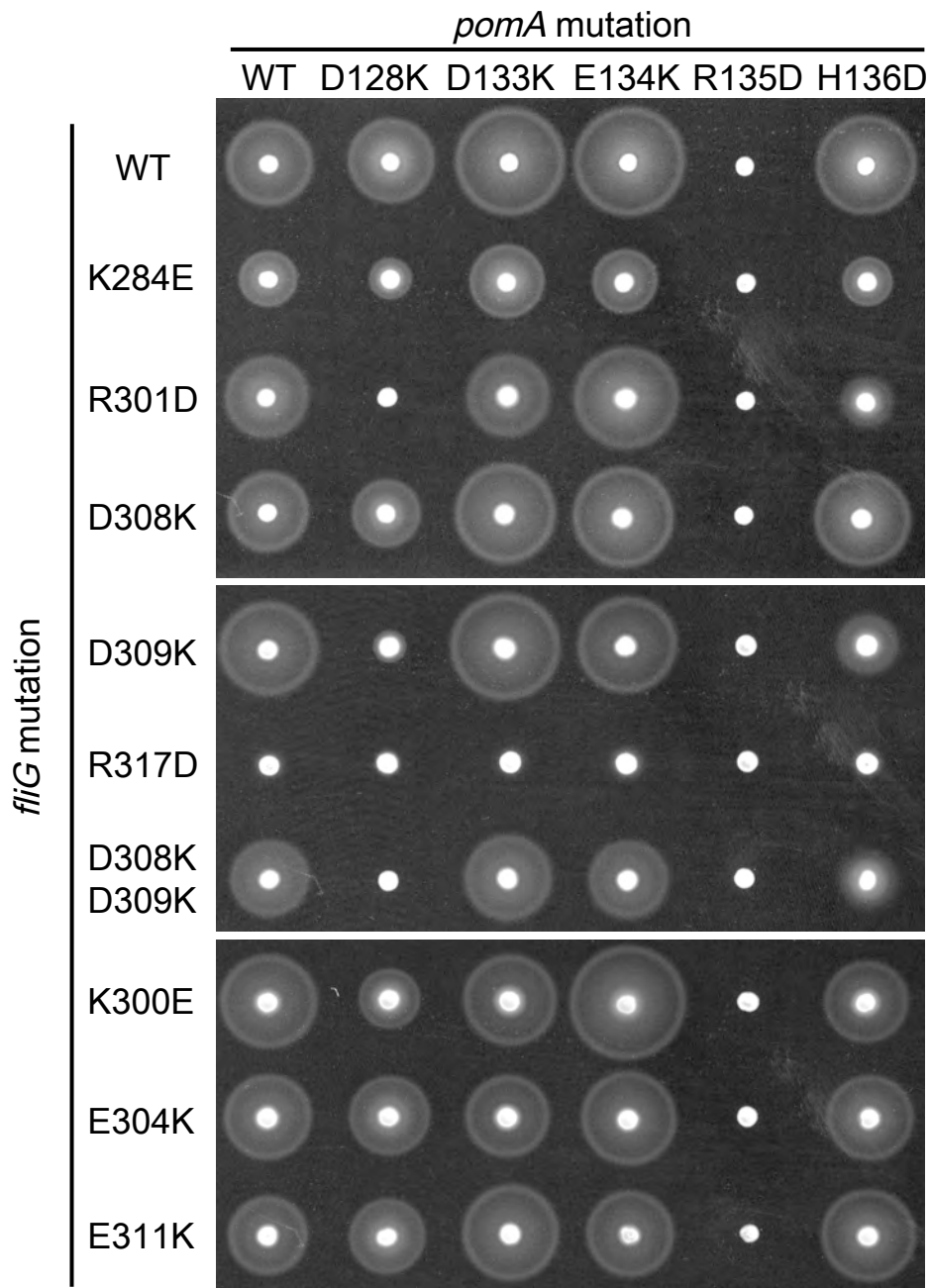


Fig. S5. Takekawa *et al.*