Supplemental Information For:

Structure of a proton-powered motor that drives protein transport and gliding motility

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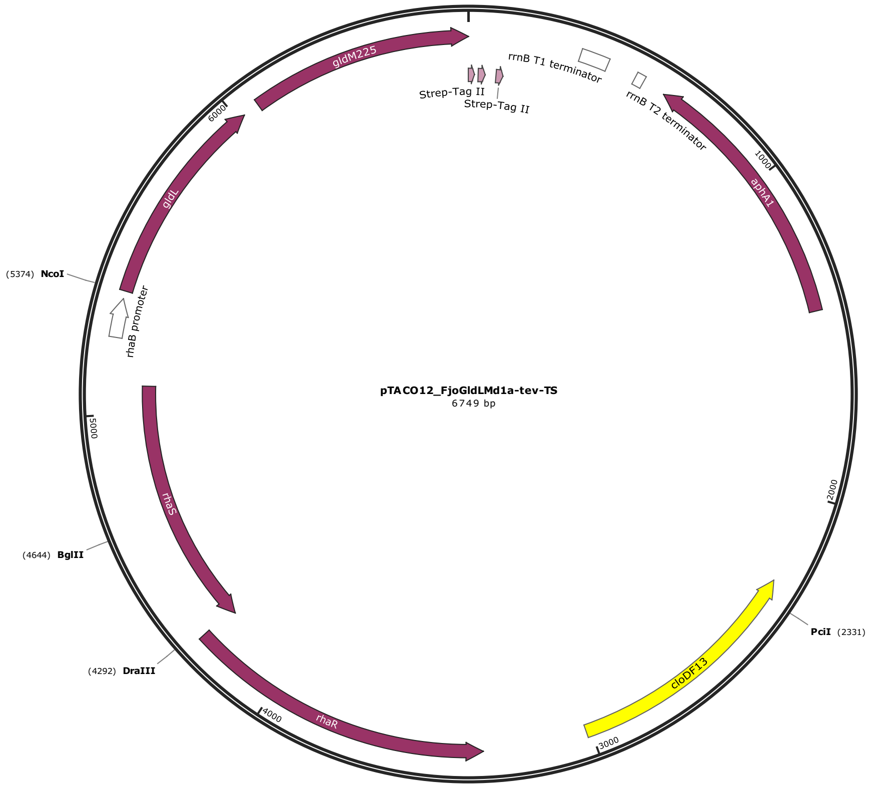

Supplementary Figure 1. Map of plasmid used for over-expression of GldLM’ (see methods).

Strain	Genotype	Reference
E. coli		
S17-1	*pro, res*$^-$ *hsdR17 (rK*$^-$ *mK*$^+$*) recA*$^-$, *RP4-2-Tc::Mu-Km::Tn7*, Tp^r	(Simon et al., 1983)
BL21 Star^{TM} (DE3)	*F*$^-$ *ompT hsdS*$_B$ *(r*$_B$*-, m*$_B$*-) gal dcm rne131 (DE3)*	Invitrogen
F. johnsoniae		
UW101		(McBride and Braun, 2004)
Fl_004	UW101 Δ*sprA*	(Lauber et al., 2018)
Fl_030	UW101 Δ*porV*	(Lauber et al., 2018)
Fl_082	UW101 Δ*gldL*	This study
Rhj_006	UW101 Δ*gldM*	This study
Rhj_017	UW101 *gldL*$_{N10A}$	This study
Rhj_018	UW101 *gldL*$_{Y13A}$	This study
Rhj_024	UW101 *gldL*$_{Y13F}$	This study
Rhj_019	UW101 *gldL*$_{K27A}$	This study
Rhj_011	UW101 *gldL*$_{H30A}$	This study
Rhj_013	UW101 *gldL*$_{S43A}$	This study
Rhj_020	UW101 *gldL*$_{T48A}$	This study
Rhj_021	UW101 *gldL*$_{E49A}$	This study
Rhj_022	UW101 *gldL*$_{E49Q}$	This study
Rhj_025	UW101 *gldL*$_{E49D}$	This study
Rhj_023	UW101 *gldL*$_{S56A}$	This study
Rhj_035	UW101 *gldM*$_{R9A}$	This study
Rhj_032	UW101 *gldM*$_{R9K}$	This study
Rhj_029	UW101 *gldM*$_{R9E}$	This study
Rhj_012	UW101 *gldM*$_{Q10A}$	This study

Rhj_030	UW101 *gldM*$_{K11A}$	This study
Rhj_016	UW101 *gldM*$_{N14A}$	This study
Rhj_014	UW101 *gldM*$_{Y17A}$	This study
Rhj_015	UW101 *gldM*$_{Y17F}$	This study
Rhj_028	UW101 *gldM*$_{N27A}$	This study
Ak_73	UW101 Δ*porV halo-sprB*	This study
Ak_203	AK_73 Δ*gldM*	This study
Ak_205	AK_73 Δ*gldL*	This study
Ak_196	AK_73 *gldL*$_{Y13A}$	This study
Ak_197	AK_73 *gldL*$_{H30A}$	This study
Ak_198	AK_73 *gldL*$_{K27A}$	This study
Ak_199	AK_73 *gldM*$_{Y17F}$	This study
Ak_289	Ak_73 *gldM*$_{R9K}$	This study

Table S1 Bacterial strains used in this study.

Plasmid	Description[a]	Reference
pGEM-T	General cloning vector; Ap^r	Promega
pWALDO-sfGFPd	pET28(a+) derived expression vector fusing a TEV cleavage site, superfolder GFP, and a His$_8$ tag to the C-terminus of the expressed protein; Kan^r	(Drew et al., 2006)
pCDFDuet-1	Co-expression of two *orfs* under the control of T7*lac* promoters; *ori* CDF, Sm^r	Novagen
pETDuet-1	Co-expression of two *orfs* under the control of T7*lac* promoters; *ori* ColE1, Ap^r	Novagen
pT12_ SpaPQR^{3xFLAG}	Encodes *Salmonella enterica* serovar Typhimurium *SpaPQR* operon with a C-terminal 3xFLAG tag on SpaR under the control of the *E. coli rhaB* promoter; *ori* cloDF13, Kan^r	(Dietsche et al., 2016)
pYT313	*sacB*-containing mobilizable suicide vector; Ap^r (Em^r)	(Zhu et al., 2017)
pYT354	*sacB*-containing mobilizable suicide vector; Ap^r (Em^r)	(Zhu et al., 2017)
pRHJ006	pETDuet-1 *porL-(tev)-gfp-8xHis porM-twinstrep*	This study
pRHJ007	pT12 *gldL gldM*(1-225)-*twinstrep*	This study
pRHJ008	pT12 *gldL gldM*(1-232)-*twinstrep*	This study
pRHJ011	Suicide plasmid used to delete *gldM*; 2.7-kbp upstream and 2.5-kbp downstream of *gldM* in pYT354	This study
pRHJ012	pGEM-T *gldL*	This study
pRHJ013	pGEM-T *gldM*	This study
pRHJ036	Suicide plasmid used to introduce the N10A codon change into *gldL*; *gldL(N10A)* in pYT354	This study
pRHJ037	Suicide plasmid used to introduce the Y13A codon change into *gldL*; *gldL(Y13A)* in pYT354	This study
pRHJ038	Suicide plasmid used to introduce the Y13F codon change into *gldL*; *gldL(Y13F)* in pYT354	This study
pRHJ039	Suicide plasmid used to introduce the K27A codon change into *gldL*; *gldL(K27A)* in pYT354	This study
pRHJ040	Suicide plasmid used to introduce the H30A codon change into *gldL*; *gldL(H30A)* in pYT354	This study
pRHJ041	Suicide plasmid used to introduce the S43A codon change into *gldL*; *gldL(S43A)* in pYT354	This study
pRHJ042	Suicide plasmid used to introduce the T48A codon change into *gldL*; *gldL(T48A)* in pYT354	This study
pRHJ043	Suicide plasmid used to introduce the E49A codon change into *gldL*; *gldL(E49A)* in pYT354	This study
pRHJ044	Suicide plasmid used to introduce the E49Q codon change into *gldL*; *gldL(E49Q)* in pYT354	This study
pRHJ045	Suicide plasmid used to introduce the E49D codon change into *gldL*; *gldL(E49D)* in pYT354	This study
pRHJ046	Suicide plasmid used to introduce the S56A codon change into *gldL*; *gldL(S56A)* in pYT354	This study
pRHJ047	Suicide plasmid used to introduce the R9A codon change into *gldM*; *gldM(R9A)* in pYT354	This study
pRHJ048	Suicide plasmid used to introduce the R9K codon change into *gldM*; *gldM(R9K)* in pYT354	This study
pRHJ049	Suicide plasmid used to introduce the R9E codon change into *gldM*; *gldM(R9E)* in pYT354	This study
pRHJ050	Suicide plasmid used to introduce the Q10A codon change into *gldM*; *gldM(Q10A)* in pYT354	This study
pRHJ051	Suicide plasmid used to introduce the K11A codon change into *gldM*; *gldM(K11A)* in pYT354	This study
pRHJ052	Suicide plasmid used to introduce the N14A codon change into *gldM*; *gldM(N14A)* in pYT354	This study
pRHJ053	Suicide plasmid used to introduce the Y17A codon change into *gldM*; *gldM(Y17A)* in pYT354	This study
pRHJ054	Suicide plasmid t used to introduce the Y17F codon change into *gldM*; *gldM(Y17F)* in pYT354	This study

pRHJ055	Suicide plasmid used to introduce the N27A codon change into *gldM*; *gldM(N27A)* in pYT354	This study
pAK021	Suicide plasmid used to insert a HaloTag domain after the signal peptide of SprB	This study
pFL43	Expresses the cytoplasmic domain of GldL; pWALDO-sfGFPd *gldL(66-215)*	This study
pFL44	Expresses the periplasmic domain of GldM; pWALDO-sfGFPd *gldM(78-513)*	This study
pFL80	Suicide plasmid used to delete *porV* ; 2.5-kbp upstream and 2.5-kbp downstream of *porV* in pYT313	(Lauber et al., 2018)
pFL89	Suicide plasmid used to delete *gldL*; 2.6-kbp upstream and 2.5-kbp downstream of *gldL* in pYT313	This study
pPG01	Expresses the first 50 amino acids of RemA fused to mCherry and the C-terminal 97 amino acids of RemA; pCP-remA_{us}-mch-$\text{CTD97}_{\text{remA}}$	This study
pPG02	Expresses the first 50 amino acids of RemA fused to mCherry and the C-terminal 97 amino acids of RemA with the K1432A point mutation; pCP-remA_{us}-mch-$\text{CTD97(K1432A)}_{\text{remA}}$	This study

Table S2 Plasmids used in this study.

[a] Selection markers functional in *F. johnsoniae* are in brackets.

Primer	Sequence 5'-3'	Used in the construction of
FL125	AAAAAAAACATATGGAGGATGAATTAGATTGGACTCTAGTTTACCCAG	pFL43, NdeI site is underlined
FL126	AAAAATTTGGATCCTCCTTTGTTACTCATTGCAGAAAGCATACCACC	pFL43, BamHI site is underlined
FL128	AAAAAAAAACATATGGCTCACAAAGTTCAGGCTGCTTCAAAAG	pFL44, NdeI site is underlined
FL129	AAAAAAAAGGATCCTTGTATTTCGTAAATTACCGGAGCAGTTCTTGG	pFL44, BamHI site is underlined
FL309	GCAGCGGAAAAATTCGGGGACGATTTAGAGCAAATGGTAAATATGG	pFL89
FL310	ATTGCAGAAAGCATACCACCAGCGAAATTCATAACTTTTTTACTTAATAATGCCAT	pFL89
FL311	GTAAAAAAGTTATGAATTTCGCTGGTGGTATGCTTTCTGCAATGAGTAAC	pFL89
FL312	GACCATGATTACGCCAAGCTTGCAGTACTTCACGTGCATTTGG	pFL89
FL313	CCCAAATGCACGTGAAGTACTGCAAGCTTGGCGTAATCATGGTCATAG	pFL89
FL314	CCATTTGCTCTAAATCGTCCCCGAATTTTTCCGCTGCATAACC	pFL89
AK39	tggctccaagcgctcatatgagaaattaaattttgcgagtaggaggca	pAK021
AK37	Actcgcaaaatttaatttctcatatgagcgcttggagccac	pAK021
AK40	TTACGCCAAGCTTGCATGCcctcttcaggcaaaacaagcgact	pAK021
AK41	cgcttgttttgcctgaagaggGCATGCAAGCTTGGCG	pAK021
AK59	TGACCCCGAAGCAGGGTTATaatatcatctaatgtcaagtcatttccaaaatcaacagc	pAK021
AK60	AGATTTCCGGTGAGCTctttttaattcaaagtttgataaaaacttgaaggaaaatatctttctaaaagc	pAK021
AK61	ttatcaaactttgaattaaaaaagAGCTCACCGGAAATCTCCA	pAK021
AK62	acttgacattagatgatattATAACCCTGCTTCGGGGTCA	pAK021
RHJ025	TGGATCGGGAGGTTCAGCGTGGAGCCACCCGCAGTTCGAGAAATAACTCGAGTCTGGTAAAGAAACC	pRHJ006, twinstrep tag sequence is underlined
RHJ026	CCGCCAGAACCTCCACCTTTTTCGAACTGCGGGTGGCTCCAAGCGCTGGTACCGACGTCAGCGAT	pRHJ006, twinstrep tag sequence is underlined
RHJ046	CCAGAGTCGGCCAAGGAACCGGCAGTT	pRHJ006, mutated residues are underlined
RHJ047	CCAGAGTCGGCCAAGGAACCGGCAGTT	pRHJ006, mutated residues are underlined.
RHJ051	GCGCGCCCATGGGTCATTATAGAAGATACAAG	pRHJ006, NcoI site underlined
RHJ052	AAAAAAGGATCCTAAGGGTGAGCTGCC	pRHJ006, BamHI site underlined
RHJ057	GCACGTCATATGGCAGTAGGTTCTAATGG	pRHJ006, NdeI site underlined

RHJ058	AACCAAGGTACCGTTCACAATTACTTCAATGGC	pRHJ006, KpnI site underlined
RHJ110	AAAAAAGGATCCTCCGCTGGAAGAAGAGGAAC	pRHJ011, BamHI site underlined
RHJ111	AAAAAAGTCGACCTGTCTAGGGGTTAATTTTCCTCC	sequencing
RHJ112	AAAAAAGTCGACACCAAGAACTGCTCCGGTAATTTAC	sequencing
RHJ113	AAAAAAGGTACCACAAGCCTCCTGCAATTCTCGAAG	pRHJ011, KpnI site underlined
RHJ114	CAGTTGAGGATGAATTAGATTGG	screening for *gldM* mutations and sequencing
RHJ115	TAGAAGGTCGACCTCCAGCGATAGAAACAATAGC	screening for *gldM* mutations and sequencing
RHJ117	AAAAAAGTCGACCATAACTTTTTTACTTAATAATGCCAT	sequencing
RHJ118	GATGAGTATTATGCTGAAGTAATGG	sequencing
RHJ123	AAAAAAGGTACCAGAAGCAGCAGCAACTG	sequencing
RHJ124	AAAAAAGGATCCTTTACCTAATGCACTGTTGTAAACG	sequencing
RHJ144	GTCGACCATATGGGAGAGCTCC	pRHJ010
RHJ145	GCATGCGACGTCGGGCC	pRHJ010
RHJ146	CCCGACGTCGCATGCGGCTGAGCCTGTATTTCGAGATG	pRHJ012
RHJ147	TCTCCCATATGGTCGACACAAGCCTCCTGCAATTCTCG	pRHJ010 and pRHJ013
RHJ148	CCCGACGTCGCATGCTCCGCTGGAAGAAGAGGAACTTGTG	pRHJ010 and pRHJ013
RHJ149	TCTCCCATATGGTCGACCAGTACTTCACGTGCATTTGGGAAG	pRHJ012
RHJ154	CTCAAGTAACAGGAGGCGG	screening for *gldL* mutations and sequencing
RHJ155	AAGAAATGTCTATGGCAGCTGC	sequencing
RHJ156	GCTTCAGCAGCTTTTTGATC	screening for *gldL* mutations
RHJ157	AGTCCGATCTGAGCAGGAG	sequencing
RHJ160	AGTTCTTGGTCTGTCTAGGGGTTAATTTTCCTCCTGC	pRHJ010
RHJ161	CCTAGACAGACCAAGAACTGCTCCGGTAATT	pRHJ010 and sequencing
RHJ162	GAAAACCTGTACTTCCAGG	pRHJ007, pRHJ008, pRHJ009
RHJ163	GGTGAATTCCTCCTGAATTTC	pRHJ007, pRHJ008, pRHJ009
RHJ164	AAATTCAGGAGGAATTCACCATGGCATTATTAAGTAAAAAAGTTATGAATTT	pRHJ007
RHJ165	CCTGGAAGTACAGGTTTTCTTTACCTAATGCACTGTTGTAAAC	pRHJ007
RHJ166	AAATTCAGGAGGAATTCACCATGGCATTATTAAGTAAAAAAGTTATGAATTT	pRHJ008
RHJ167	CCTGGAAGTACAGGTTTTCAGAAGCAGCAGCAACT	pRHJ008
RHJ168	AAATTCAGGAGGAATTCACCATGGGTCATTATAGAAGATACAAGAAC	pRHJ009

RHJ169	CCTGGAAGTACAGGTTTTCCTTTACCAGATCGGCAAGT	pRHJ009
RHJ174	CGGCTGTGGTAAGTCAGG	sequencing
RHJ175	CAGCACTTCCAATTTCTTTCG	sequencing
RHJ269	ACCTGATGGCTCTGGTTTTCATCGCAATG	*gldM(Y17A)* mutations, mutation site is underlined
RHJ270	AAACCAGAGCCATCAGGTTAATCATCTTCTG	*gldM(Y17A)* mutations, mutation site is underlined
RHJ271	TAGCAATGGCCGTATCAAAAGAAGTTATTTCTGC	*gldM(N27A)* mutations, mutation site is underlined
RHJ272	TTGATACGGCCATTGCTAACATTGCGATG	*gldM(N27A)* mutations, mutation site is underlined
RHJ273	CTTTATTCGCAATTACTCACTTTGAGATTGGG	*gldL(K27A)* mutations, mutation site is underlined
RHJ274	TGAGTAATTGCGAATAAAGCTCCAACGATTACC	*gldL(K27A)* mutations, mutation site is underlined
RHJ275	AAATTACTGCCTTTGAGATTGGGCCATTAAC	*gldL(H30A)* mutations, mutation site is underlined
RHJ276	ATCTCAAAGGCAGTAATTTTGAATAAAGCTCCAAC	*gldL(H30A)* mutations, mutation site is underlined
RHJ277	TTTTGACTGCGGCATTAATCTTTGCGTTATCTG	*gldL(E49A*) mutations, mutation site is underlined
RHJ278	ATTAATGCCGCAGTCAAAAGACCGATAGAAAG	*gldL(E49A*) mutations, mutation site is underlined
RHJ279	GTCTTTTGGCTGAGGCATTAATCTTTGCG	*gldL(T48A)* mutations, mutation site is underlined
RHJ280	TGCCTCAGCCAAAAGACCGATAGAAAGCATC	*gldL(T48A)* mutations, mutation site is underlined
RHJ281	GTCTACACGAACCCTTTGG	sequencing
RHJ310	AAGTTATGGCTTTCGCTTATGGTATGGGAG	*gldL(N10A)*mutations, mutation site is underlined
RHJ311	AAGCGAAAGCCATAACTTTTTTACTTAATAATGCCATAATAATTC	*gldL(N10A)*mutations, mutation site is underlined
RHJ312	ATTTCGCTGCTGGTATGGGAGCGGC	*gldL(Y13A)* mutations, mutation site is underlined
RHJ313	CCATACCAGCAGCGAAATTCATAACTTTTTTACTTAATAATG	*gldL(Y13A)* mutations, mutation site is underlined
RHJ314	TGATGCTTGCTATCGGTCTTTTGACTGAGG	*gldL(S43A)* mutations, mutation site is underlined
RHJ315	GACCGATAGCAAGCATCACTGTCCCTG	*gldL(S43A)* mutations, mutation site is underlined

RHJ316	TTGCGTTAGCTGCTTTTGAACCAGTTGAG	*gldL(S56A)* mutations, mutation site is underlined
RHJ317	AAAAGCAGCTAACGCAAAGATTAATGCCTC	*gldL(S56A)* mutations, mutation site is underlined
RHJ318	CCTGATGTTTCTGGTTTTCATCGCAATG	*gldM(Y17F)* mutations, mutation site is underlined
RHJ319	AAACCAGAAACATCAGGTTAATCATCTTCTG	*gldM(Y17F)* mutations, mutation site is underlined
RHJ320	CCCCTAGAGCGAAGATGATTAACCTGATGTATCTG	*gldM(Q10A)* mutations, mutation site is underlined
RHJ321	TCATCTTCGCTCTAGGGGTTAATTTTCCTCCTG	*gldM(Q10A)* mutations, mutation site is underlined
RHJ322	TTTTGACTCAGGCATTAATCTTTGCGTTATC	*gldL(E49Q)* mutations, mutation site is underlined
RHJ323	TAATGCCTGAGTCAAAAGACCGATAGAAAGC	*gldL(E49Q)* mutations, mutation site is underlined
RHJ341	CGGCCGCTCTAGAACTAGTGGATCCGCTGAGCCTGTATTTCGAG	suicide vectors for *gldL* mutations
RHJ342	TACCGGGCCCCCCCTCGAGGTCGACCAGTACTTCACG	suicide vectors for *gldL* mutations
RHJ343	CGGCCGCTCTAGAACTAGTGGATCCCGCTGGAAGAAGAGG	suicide vectors for *gldM* mutations
RHJ344	TACCGGGCCCCCCCTCGAGGTCGACCAAGCCTCCTGCAATTC	suicide vectors for *gldM* mutations
RHJ345	CCTGACTTACCACAGCCG	sequencing
RHJ346	CGTCATTTTTGATCCGCGG	verifying *F. johnsoniae* co-integrants
RHJ347	CTCCGGTAAACATTACGGC	verifying *F. johnsoniae* co-integrants
RHJ348	TTTCGCTTTTGGTATGGGAGCGGC	*gldL(Y13F)* mutations, mutation site is underlined
RHJ349	CCATACCAAAAGCGAAATTCATAACTTTTTTACTTAATAATG	*gldL(Y13F)* mutations, mutation site is underlined
RHJ350	TTGACTGATGCATTAATCTTTGCGTTATCTG	*gldL(E49D)* mutations, mutation site is underlined
RHJ351	ATTAATGCATCAGTCAAAAGACCGATAGAAAG	*gldL(E49D)* mutations, mutation site is underlined
RHJ352	TAACCCCTGCACAGAAGATGATTAACCTGATGTATC	*gldM(R9A)* mutations, mutation site is underlined
RHJ353	TCTTCTGTGCAGGGGTTAATTTTCCTCCTG	*gldM(R9A)* mutations, mutation site is underlined
RHJ354	AACCCCTAAACAGAAGATGATTAACCTGATG	*gldM(R9K)* mutations, mutation site is underlined
RHJ355	TCTTCTGTTTAGGGGTTAATTTTCCTCCTG	*gldM(R9K)* mutations, mutation site is underlined

RHJ356	TAACCCCTGAACAGAAGATGATTAACCTGATG	*gldM(R9E)* mutations, mutation site is underlined
RHJ357	TCTTCTGTTCAGGGGTTAATTTTCCTCCTG	*gldM(R9E)* mutations, mutation site is underlined
RHJ358	CTAGACAGGCGATGATTAACCTGATGTATCTGGTTTTC	*gldM(K11A)* mutations, mutation site is underlined
RHJ359	TTAATCATCGCCTGTCTAGGGGTTAATTTTCC	*gldM(K11A)* mutations, mutation site is underlined
RHJ360	TACCGGGCCCCCCCTCGAGGTCGACCAGTACTTCACGTGCATTTG	suicide vectors for *gldM mutations*
PG001	AGCTAGTCTAGAACGTTCCTGATAGGCACAAAAATG	pCP-remA$_{us}$-mch-CTD97$_{remA}$, XbaI iste is underlined
PG002	AGCTAGACTAGTGCCATTAGTTGGCATTCCAGG	pCP-remA$_{us}$-mch-CTD97$_{remA}$, SpeI site is underlined
PG003	CGTTACTAGTATGGTGAGCAAGGGCGAGGAGGATAAC	pCP-remA$_{us}$-mch-CTD97$_{remA}$, SpeI site is underlined
PG004	GACAGAGCTCCTTGTACAGCTCGTCCATGCC	pCP-remA$_{us}$-mch-CTD97$_{remA}$, SacI site is underlined
PG005	GACAGAGCTCGATCGTTTTGCACTTCGTTACAC	pCP-remA$_{us}$-mch-CTD97$_{remA}$, SacI site is underlined
PG006	GGACGTCGACGTCTTACTTGGCAAATGGATTTTT	pCP-remA$_{us}$-mch-CTD97$_{remA}$, SalI site is underlined
PG007	GCCCAGATAACCAGAGCAGTGATCTTTAAATAG	pCP-remA$_{us}$-mch-CTD97$_{remA(K1433A)}$, mutation site is underlined
PG008	CTATTTAAAGATCACTGCTCTGGTTATCTGGGC	pCP-remA$_{us}$-mch-CTD97$_{remA(K1433A)}$, mutation site is underlined

Table S3. Oligonucleotides used in this study.

Supplemental Video1: Mutant strains gliding on glass. Wild type, $gldL_{Y13A}$, $gldL_{Y13F}$, $gldL_{K27A}$, $gldL_{H30A}$, $gldL_{E49D}$, $gldM_{R9K}$, $gldM_{Y17A}$ and $gldM_{Y17F}$ cells are shown.

Supplemental Video 2: Fluorophore-labelled SprB adhesin moving on the surface of a wild type cell.

Supplemental Video 3: Fluorophore-labelled SprB adhesin moving on the surface of a $gldL_{Y13A}$ cell.

Supplemental Video 4: Fluorophore-labelled SprB adhesin moving on the surface of a $gldL_{K27A}$ cell.

Supplemental Video 5: Fluorophore-labelled SprB adhesin moving on the surface of a $gldL_{H30A}$ cell.

Supplemental Video 6: Fluorophore-labelled SprB adhesin moving on the surface of a $gldM_{R9K}$ cell.

Supplemental Video 7: Fluorophore-labelled SprB adhesin moving on the surface of a $gldM_{Y17F}$ cell.

Supplemental Video 8: Animation of a rotary model of GldLM mechanism.