Supplementary to **‘The Nucleotide-Dependent Interaction of FlaH and FlaI is Essential for Assembly and Function of the Archaellum Motor**

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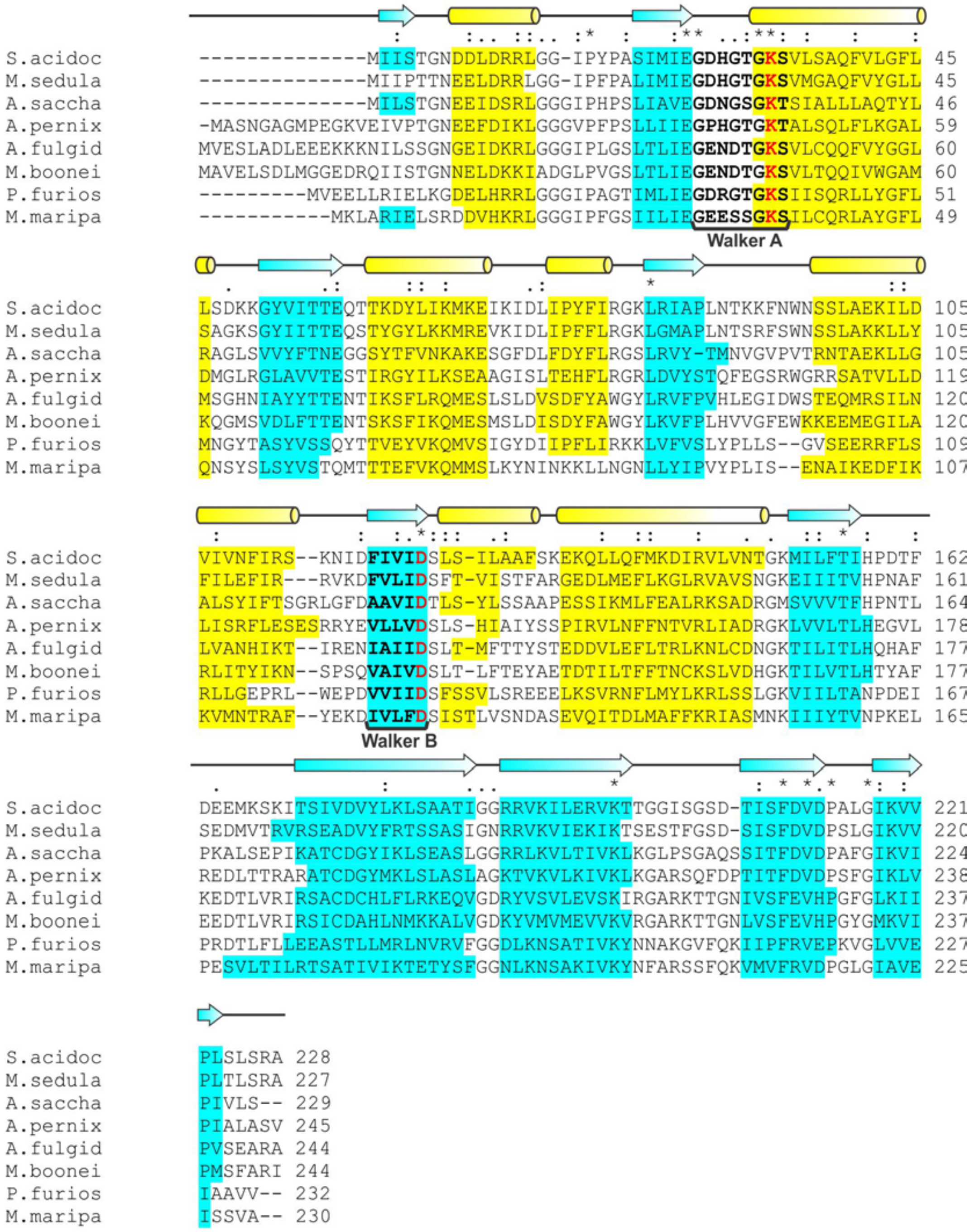

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                            :     :.. ::.. :*  :   :** ..:**: :   :    :
S.acidoc    --------------MIISTGNDDLDRRLGG-IPYPASIMIEGDHGTGKSVLSAQFVLGFL 45
M.sedula    --------------MIIPTTNEELDRRLGG-IPFPALIMIEGDHGTGKSVMGAQFVYGLL 45
A.saccha    --------------MILSTGNEEIDSRLGGGIPHPSLIAVEGDNGSGKTSIALLLAQTYL 46
A.pernix    -MASNGAGMPEGKVEIVPTGNEEFDIKLGGGVPFPSLLIIEGPHGTGKTALSQLFLKGAL 59
A.fulgid    MVESLADLEEEKKKNILSSGNGEIDKRLGGGIPLGSLTLIEGENDTGKSVLCQQFVYGGL 60
M.boonei    MAVELSDLMGGEDRQIISTGNNELDKKIADGLPVGSLTLIEGENDTGKSVLTQQIVWGAM 60
P.furios    ---------MVEELLRIELKGDELHRRLGGGIPAGTIMLIEGDRGTGKSIISQRLLYGFL 51
M.maripa    -----------MKLARIELSRDDVHKRLGGGIPFGSIILIEGEESSGKSILCQRLAYGFL 49
                                                     Walker A

              .       .:     :: :      .:        *                   ::
S.acidoc    LSDKKGYVITTEQTTKDYLIKMKEIKIDLIPYFIRGKLRIAPLNTKKFNWNSSLAEKILD 105
M.sedula    SAGKSGYIITTEQSTYGYLKKMREVKIDLIPFFLRGKLGMAPLNTSRFSWNSSLAKKLLY 105
A.saccha    RAGLSVVYFTNEGGSYTFVNKAKESGFDLFDYFLRGSLRVY-TMNVGVPVTRNTAEKLLG 105
A.pernix    DMGLRGLAVVTESTIRGYILKSEAAGISLTEHFLRGRLDVYSTQFEGSRWGRRSATVLLD 119
A.fulgid    MSGHNIAYYTTENTIKSFLRQMESLSLDVSDFYAWGYLRVFPVHLEGIDWSTEQMRSILN 120
M.boonei    KQGMSVDLFTTENTSKSFIKQMESMSLDISDFYAWGYLKVFPLHVVGFEWKKEEMEGILA 120
P.furios    MNGYTASYVSSQYTTVEYVKQMVSIGYDIIPFLIRKKLVFVSLYPLLS--GVSEERRFLS 109
M.maripa    QNSYSLSYVSTQMTTTEFVKQMMSLKYNINKKLLNGNLLYIPVYPLIS--ENAIKEDFIK 107

             :           :  :.*:::  .:       :  ::   :   . .  :: * :   :
S.acidoc    VIVNFIRS--KNIDFIVIDSLS-ILAAFSKEKQLLQFMKDIRVLVNTGKMILFTIHPDTF 162
M.sedula    FILEFIR---RVKDFVLIDSFT-VISTFARGEDLMEFLKGLRVAVSNGKEIIITVHPNAF 161
A.saccha    ALSYIFTSGRLGFDAAVIDTLS-YLSSAAPESSIKMLFEALRKSADRGMSVVVTFHPNTL 164
A.pernix    LISRFLESESRRYEVLLVDSLS-HIAIYSSPIRVLNFFNTVRLIADRGKLVVLTLHEGVL 178
A.fulgid    LVANHIKT--IRENIAIIDSLT-MFTTYSTEDDVLEFLTRLKNLCDNGKTILITLHQHAF 177
M.boonei    RLITYIKN--SPSQVAIVDSLT-LFTEYAETDTILTFFTNCKSLVDHGKTILVTLHTYAF 177
P.furios    RLLGEPRL--WEPDVVIIDSFSSVLSREEELKSVRNFLMYLKRLSSLGKVIILTANPDEI 167
M.maripa    KVMNTRAF--YEKDIVLFDSISTLVSNDASEVQITDLMAFFKRIASMNKIIIYTVNPKEL 165
                          Walker B

             .             :      ...         *           : * *.*  *: :
S.acidoc    DEEMKSKITSIVDVYLKLSAATIGGRRVKILERVKTTGGISGSD-TISFDVDPALGIKVV 221
M.sedula    SEDMVTRVRSEADVYFRTSSASIGNRRVKVIEKIKTSESTFGSD-SISFDVDPSLGIKVV 220
A.saccha    PKALSEPIKATCDGYIKLSEASLGGRRLKVLTIVKLKGLPSGAQSSITFDVDPAFGIKVI 224
A.pernix    REDLTTRARATCDGYMKLSLASLAGKTVKVLKIVKLKGARSQFDPTITFDVDPSFGIKLV 238
A.fulgid    KEDTLVRIRSACDCHLFLRKEQVGDRYVSVLEVSKIRGARKTTGNIVSFEVHPGFGLKII 237
M.boonei    EEDTLVRIRSICDAHLNMKKALVGDKYVMVMEVVKVRGARKTTGNLVSFEVHPGYGMKVI 237
P.furios    PRDTLFLLEEASTLLMRLNVRVFGGDLKNSATIVKYNNAKGVFQKIIPFRVEPKVGLVVE 227
M.maripa    PESVLTILRTSATIVIKTETYSFGGNLKNSAKIVKYNFARSSFQKVMVFRVDPGLGIAVE 225

S.acidoc    PLSLSRA 228
M.sedula    PLTLSRA 227
A.saccha    PIVLS-- 229
A.pernix    PIALASV 245
A.fulgid    PVSEARA 244
M.boonei    PMSFARI 244
P.furios    IAAVV-- 232
M.maripa    ISSVA-- 230
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Figure S1 Multiple sequence alignment and secondary structure prediction of FlaH homologues from different archaeal species. *Sulfolobus acidocaldarius* FlaH homologues were identified with BLAST analysis (1) and aligned using ClustalW (2). A secondary structure prediction for all aligned FlaH homologues was performed using PSIPRED (3). The structural predictions were consistent for all the homologues: predicted α-helices are highlighted in yellow, β-sheets in cyan. The identified Walker motifs are marked with a bold font and the highly conserved lysine in the Walker A motif and the aspartic acid in the Walker B box are highlighted in red. (Strains: *Metallosphaera sedula*, *Acidilobus saccharovorans*, *Aeropyrum pernix*, *Archaeoglobus fulgidus*, *Methanoregula boonei*, *Pyrococcus furiosus*, *Methanococcus maripaludis*)

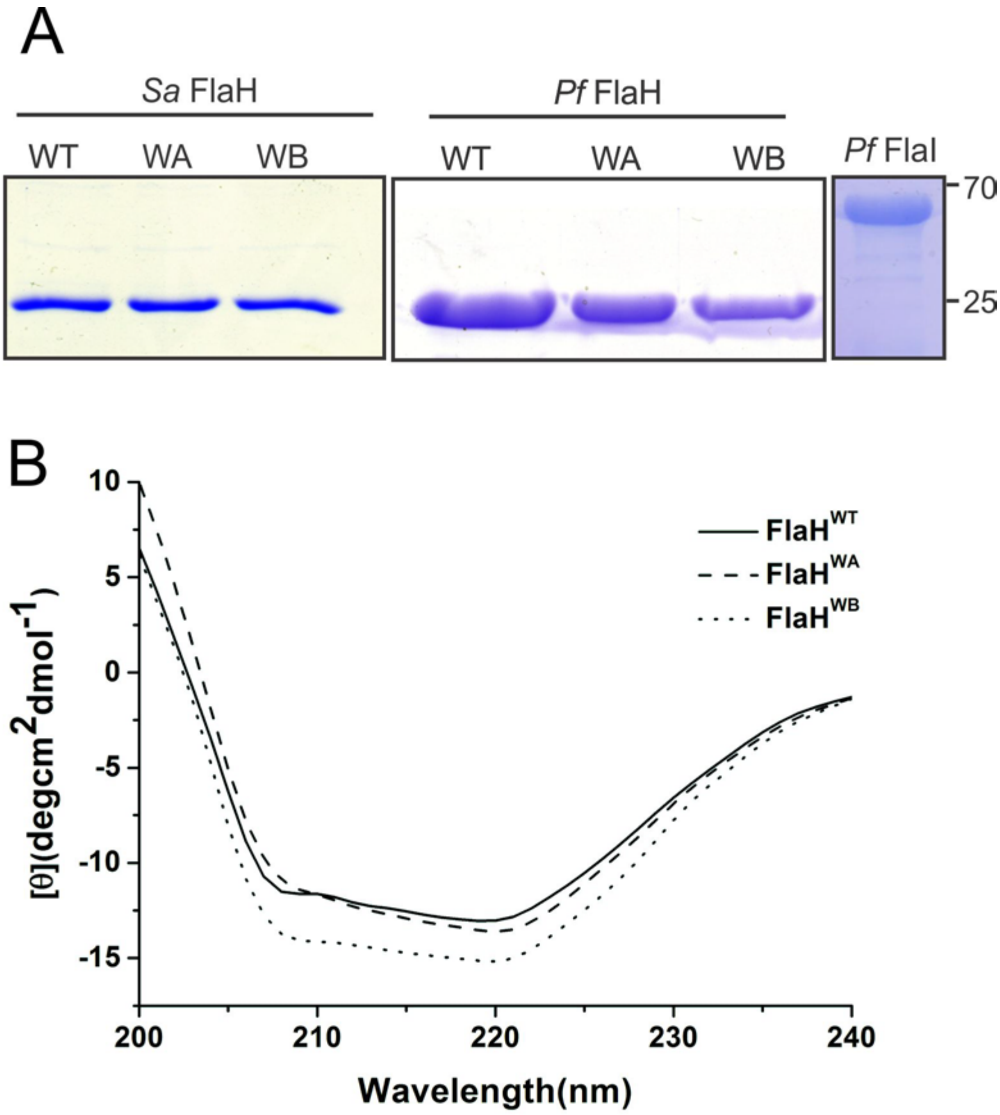

Figure S2 Purified *Sa*FlaH and *Pf*FlaH and their Walker A and B mutants and CD analysis on the PfFlaH and mutants (A) Coomassie stained SDS-PAGE of purified FlaH variants (FlaH^{WT}, FlaH^{WA}, FlaH^{WB}). Molecular masses are indicated in kDa. (B) Far-UV CD spectra (200-240 nm) of FlaH^{WT}, FlaH^{WA} and FlaH^{WB} (4.5-5.5 µM) in 10mM HEPES pH 7.2, 100 mM NaCl at room temperature in a Jasco J-810 spectro polarimeter. Experiments were conducted in 1 mm path length cells. Far-UV spectra of the tested proteins are presented as the smoothed mean of three accumulations. Characteristic CD spectral profiles having two minima at 222 nm and 208 nm indicate FlaH wildtype as well as the mutants are able to fold properly.

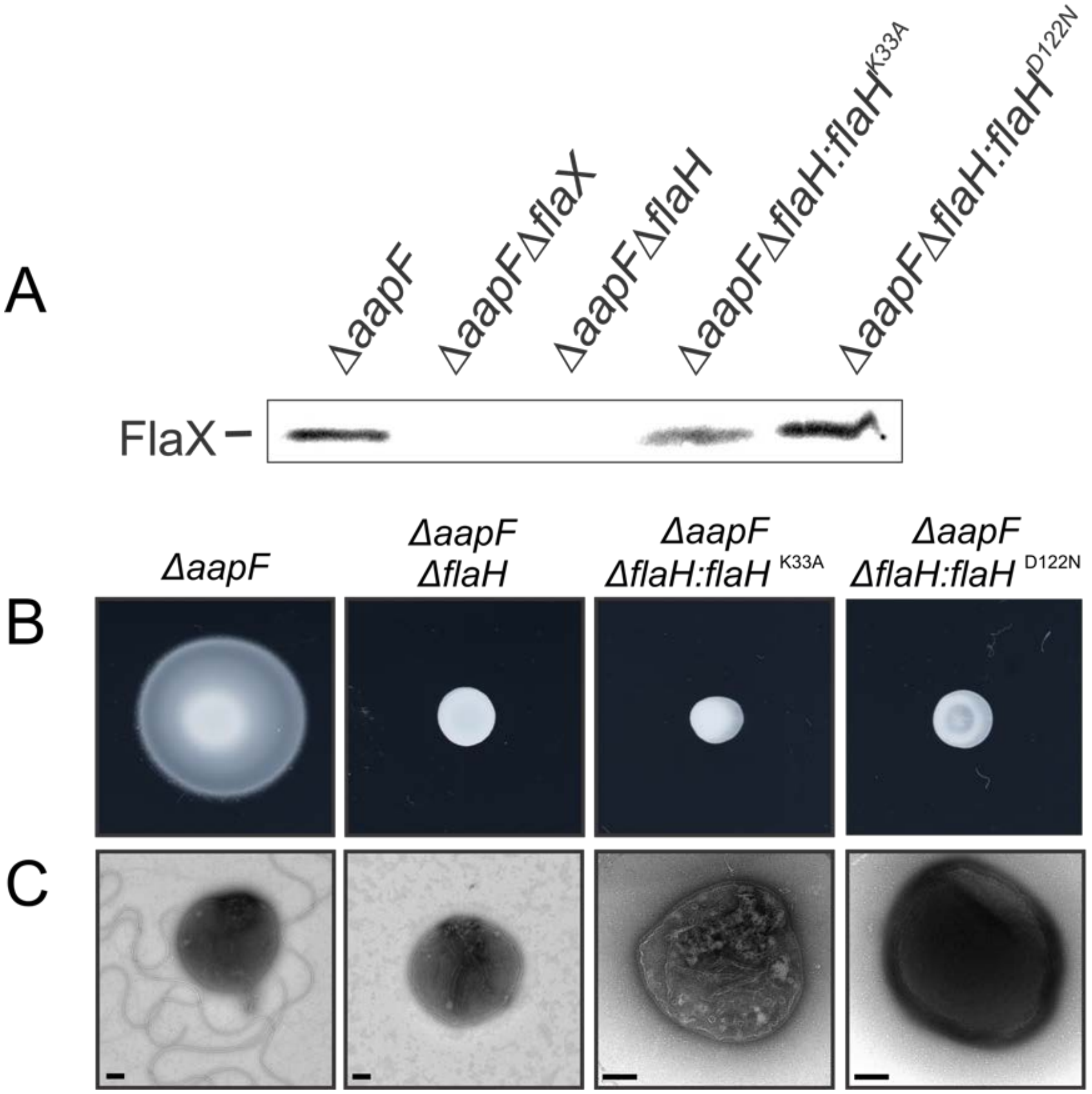

Figure S3 ATP binding to FlaH is essential for archaellation. (A) Membranes isolated from the indicated *S. acidocaldarius* strains were separated on SDS-PAGE, blotted to PVDF membranes and Western Blot analysis was performed using anti-FlaX antibodies. (B) Motility assay on semi solid swarm plates using the indicated *S. acidocaldarius* strains. (C) Electron micrographs of negatively stained cells of the indicated *S. acidocaldarius* strains (Scale bar: 200 nm).

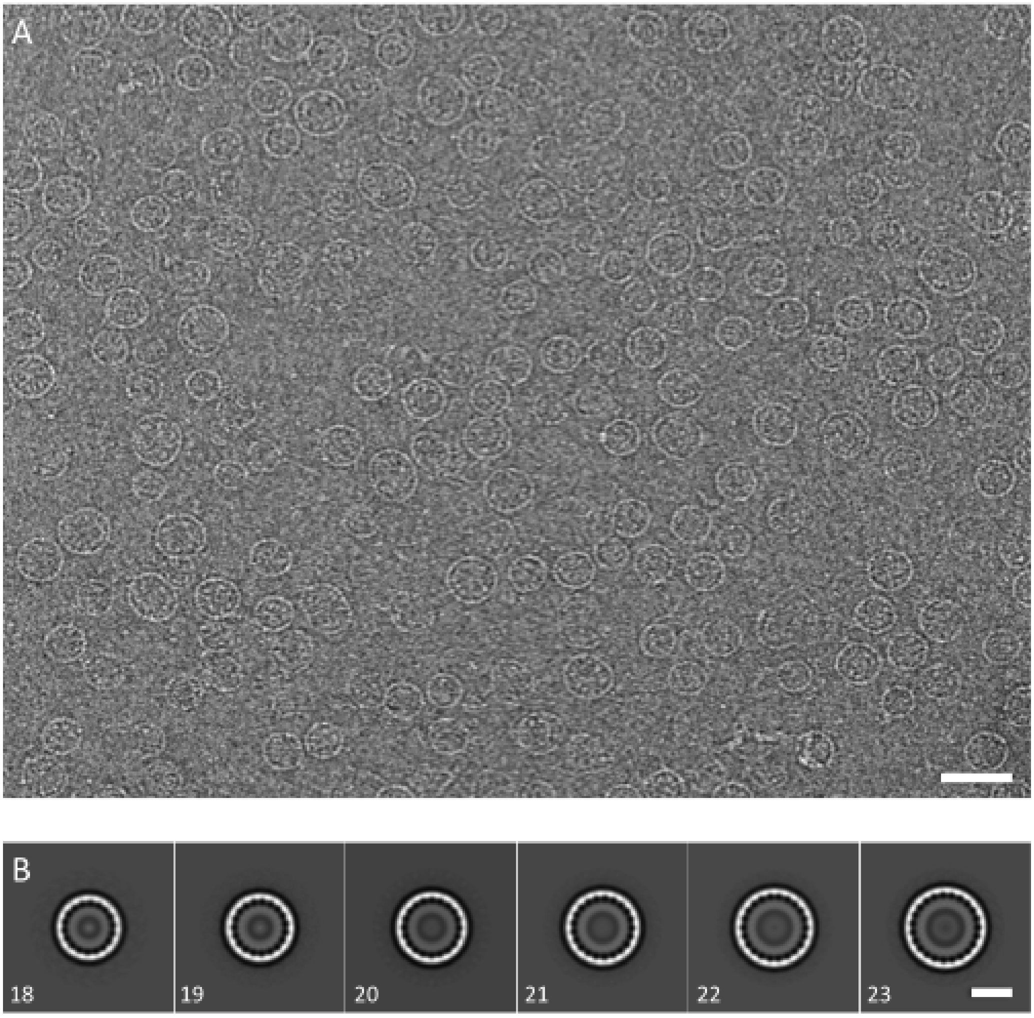

Figure S4 Cryo-electron microscopy and image processing of FlaX/FlaH complexes. (A) A typical micrograph of the complex at 3 μm defocus. (B) Class averages after multireference alignment in order of increasing size with rotational symmetry applied from 18 (*left*) to 23 (*right*). The number of particles per class follows a Gaussian distribution with a maximum at 20. A diffuse ring is visible inside the *Sa*FlaX ring, showing that *Sa*FlaH is not present with the same stoichiometry as *Sa*FlaX. The scale bar is 50 nm in A and 20 nm in B.

Table S1. Strains used in present study

Strains	**Relevant characteristics**	**Source**
E.coli		
DH5α	l2 f80d/lacZ DM15 D(lacZYA-argF)U169 recA1 endA1 hsdR17 (rK2 mK1) supE44 thi-1 gyrA relA1	Gibco
Bl21(DE3)-RIL	B F-ompThsdS(rB − mB −) dcm + Tetr E. coli gal λ (DE3) endAHte [argUileYleuWCam^r]	Stratagene
ER1821	F$^-$*glnV44 e14*$^-$*(McrA*$^-$*) rfbD1? relA1? endA1 spoT1? thi-1* Δ*(mcrC-mrr)114::IS10*	NEB
S.acidocaldarius		
DSM639	Wild-type *Sulfolobus acidocaldarius*	DSMZ
MW001	DSM 639 Δ*pyrE*	(17)
MW156	MW001 Δ*aapF* (*Saci_2318*)	(4)
MW455	MW156 Δ*flaH* (*Saci_1174*)	(4)
MW466	MW156 *flaH* K33A	This study
MW467	MW156 *flaH* D122N	This study
P. furiosus		
DSM3638	Wild-type *Pyrococcus furiosus*	DSMZ

Table S2 Plasmids used in present study

Plasmids	Relevant characteristics	Source
pETDuet-1	Amp^r ,Cam^r , expression plasmid containing replicon ColE1 (pBR322) and two MCS (MCS1 and MCS2)	Novagen
pUC57	Amp^r, *E. coli* cloning vector	
pSVA251	Amp^r ,Cam^r , pETDuet-1 carrying untagged *flaI* in MCS2 using restriction sites NdeI-XhoI	(Ghosh et al., 2011a)
pSVA284	Amp^r codon optimized *flaH (sflaH*) in pUC57	
pSVA293	Amp^r, Cam^r, pETDuet-1 carrying C-terminal His$_6$ tagged s*flaH* in MCS2 using restriction sites NdeI-XhoI	This study
pSVA406	Amp^r, gene targeting plasmid, pGEM-T Easy backbone, pyrEF cassette of S.solfataricus	(Wagner et al., 2012)
pSVA1911	Del 37 FlaX (*flaXc*) gene in pSA4 using restriction sites NcoI-BamHI	(Banerjee et al., 2012a)
pSVA1934	Amp^r, Cam^r, pETDuet-1 Carrying N-terminal His$_6$ tagged *flaXcn*(37th-127th amino acid FlaX) in MCS1 using restriction sites EcoRI-HindIII	(Banerjee et al., 2012a)
pSVA1935	Amp^r, Cam^r, pETDuet-1 Carrying N-terminal His$_6$ tagged *flaXcc1*(87th-250th amino acid FlaX) in MCS1 using restriction sites EcoRI-HindIII	(Banerjee et al., 2012a)
pSVA2100	Amp^r, Cam^r, pETDuet-1 carrying N-terminal His$_6$ tagged s*flaH* in MCS1 using restriction sites EcoRI-HindIII	This study
pSVA2108	Amp^r, Cam^r, pETDuet-1 carrying N-terminal Strep tagged s*flaH* in MCS2 using restriction sites NdeI-XhoI	This study
pSVA2113	Amp^r, *flaH* K33A cloned into pSVA406 with ApaI-PstI	This study
pSVA2124	Amp^r, *flaH* D122N cloned into pSVA406 with ApaI-PstI	This study
pSVA2126	Amp^r, *flaH* cloned into pSVA406 with ApaI-PstI	This study
pSVA2130	Amp^r, Cam^r, pETDuet-1 carrying N-terminal His$_6$ tagged s*flaH* K33A in MCS1 using restriction sites EcoRI-HindIII	This study
pSVA2131	Amp^r, Cam^r, pETDuet-1 carrying N-terminal His$_6$ tagged s*flaH* D122N in MCS1 using restriction sites EcoRI-HindIII	This study

pSVA2167	Amp^{r}, Cam^{r}, pETDuet-1 carrying N-terminal His$_{6}$ tagged *PfflaH* in MCS1 using restriction sites BamHI-NotI	This study
pSVA2176	Amp^{r}, Cam^{r}, pETDuet-1 carrying N-terminal His$_{6}$ tagged *PfflaH* K53A in MCS1 using restriction sites BamHI-NotI	This study
pSVA2178	Amp^{r}, Cam^{r}, pETDuet-1 carrying N-terminal His$_{6}$ tagged *PfflaH* D140A in MCS1 using restriction sites BamHI-NotI	This study
pSVA3116	Amp^{r}, Cam^{r}, pETDuet-1 carrying N-terminal His$_{6}$ tagged *PfflaI* in MCS1 using restriction sites EcoRI-PstI	This study
pSVA3135	C-terminal HA tagged *FlaH Wt* gene in pSVA1450 backbone using NcoI-EagI	This study
pSVA3138	C-terminal HA tagged *FlaH*^{K33A} gene in pSVA1450 backbone using NcoI-EagI	This study
pSVA3139	C-terminal HA tagged *FlaH*^{D122N} gene in pSVA1450 backbone using NcoI-EagI	This study

Table S3. Primer details

Primers	Sequence and characteristics	Source
771	5'-GGGGGCATATGATTATCAGCACCGGCAACG-3'; Forward primer for *sflaH* containing an NdeI restriction site (underlined)	This study
772	5'GGGGGCTCGAGTTAATGATGATGATGATGATGTGCGCGAGACAGGC-3'; Reverse primer for *sflaH* containing a XhoI restriction site (underlined)	This study
786	5'-GGGGGCTCGAGTTATGCGCGAGACAGGC-3'; Reverse primer for *sflaH* containing a XhoI restriction site (underlined)	This study
2168	5'GGGGGCATATGTGGTCCCATCCTCAATTTGAAAAGATTATCAGCACCGGCAACG-3'; Forward primer for *sflaH* containing an NdeI restriction site (underlined)	This study
3612	5'-CCCCCCGAATTCAATGATTATCAGCACCGGC-3'; Forward primer for *sflaH* containing a EcoRI restriction site (underlined)	This study
3613	5'-GGGGGGAAGCTTTTATGCGCGAGACAGGC-3'; Reverse primer for *sflaH* containing a HindIII restriction site (underlined)	This study
3614	5'-CCCGGGGGGCCCGGGGGAAACAACGATCTC-3'; Forward primer for *flaH* down-stream containing a ApaI restriction site (underlined)	This study
3615	5'-CACTCGCACCCGTGCCATGATCTCC-3'; Forward primer for *flaH*K33A site directed mutagenesis	This study

3616	5’-GCGAGTGTGTTATCTGCTCAATTCG-3’; Reverse primer for *flaH*K33A site directed mutagenesis	This study
3617	5’-CCCCCCCTGCAGCCATCATAGAGGATAATGTTCC-3’; Reverse primer for *flaH* up-stream containing a PstI restriction site (underlined)	This study
3620	5'-GGGGGGCCATGGGCCTATGATTATAAGTACCGG-3'; Forward Primer for *flaH* up-stream containing a NcoI restriction site(underlined)	This study
3627	5’-GACAAACTATTGATTACGATGAAGTCTATATTT-3’; Forward primer for *flaH* D122N site directed mutagenesis	This study
3628	5’-GTAATCAATAGTTTGTCAATACTAGCTGCTTTCTC-3’; Reverse primer for *flaH* D122N site directed mutagenesis	This study
3632	5’-GATCATGGCACGGGTGCAAGTGTGCTGAGCG-3’; Forward primer for s*flaH* K33A site directed mutagenesis	This study
3633	5’-CGCTCAGCACACTTGCACCCGTGCCATGATC-3’; Reverse primer for s*flaH* K33A site directed mutagenesis	This study
3634	5’-CGATTTCATTGTTATCAATAGTCTGAGCATTCTG-3’; Forward primer for s*flaH* D122N site directed mutagenesis	This study
3635	5’-CAGAATGCTCAGACTATTGATAACAATGAAATC-3’; Reverse primer for s*flaH* D122N site directed mutagenesis	This study
3681	5’-CCCCCCGGATCCGATGGTCGAGGAACTCTTAAGG-3’; Forward primer for *PfFlaH* containing a BamHI restriction site(underlined)	This study
3682	5’-CCCCCCGCGGCCGCTCACACGACTGCAGCAATCTC-3’; Reverse primer for *PfFlaH* containing a NotI restriction site(underlined)	This study
3699	5’-CTGGGATATTATGGACGCCCCAGTACCCCTGTCTCCTTC-3’; Reverse primer for *PfFlaH* K53A site directed mutagenesis	This study
6000	5’-GGAGACAGGGGTACTGGGGCGTCCATAATATCCCAGAGAC-3’; Forward primer for *PfFlaH* K53A site directed mutagenesis	This study
6003	5’-GCACGGATGAAAATGAAGCAATGATTACAACATCGGGC-3’; Reverse primer for *PfFlaH* D140A site directed mutagenesis	This study
6004	5’-GCCCGATGTTGTAATCATTGCTTCATTTTCATCCGTGC-3’; Forward primer for *PfFlaH* D140A site directed mutagenesis	This study
5109	5’-GGGGAATTCGATGGCGGAAGTTATGTCAC-3’; Forward primer for *PfFlaI* containing a EcoRI restriction site(underlined)	This study
5110	5’-GGGCTGCAGTCAGATTCTGAAGCTTAGTC-3’; Reverse primer for *PfFlaI* containing a PstI restriction site(underlined)	This study

5117	5'-GGCACGGCCGTTAAGCGTAATCTGGAACATCGTATGGGTATGCCCTCGATAAGGATAATGG-3'; Reverse primer for HA tag FlaH containing a EagI restriction site(underlined)	This study

References

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3. Jones DT (1999) Protein secondary structure prediction based on position-specific scoring matrices. *J Mol Biol* 292(2):195–202.