Supporting Information

A localized multimeric anchor attaches the *Caulobacter* holdfast to the cell pole.

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Supporting Experimental Procedures

Generation of deletion, overexpression and Y2H constructs. For deletion of the *hfa hfs*, and *rsaA* genes, two ~500 bp fragments were cloned into pNPTS138. For the *hfa* genes, the upstream fragment was digested with HindIII and BamHI and the downstream fragment was digested with *Bam*HI and *Eco*RI. For the *rsaA* deletion, the upstream fragment was digested with HindIII and EcoRI and the downstream fragment was digested with EcoRI and SalI. For the *hfsDAB* deletion, the upstream fragment was digested with BamHI and XhoI and the downstream fragment was digested with XhoI and HindIII. Chromosomal DNA was isolated from transconjugants that were screened for the correct gene deletion by PCR and sequenced to confirm the deletion.

The *hfaA* deletion (YB4250) was created using the hfaAupF and hfaAupR primers to generate the upstream fragment and the hfaAdwnF and hfaAdwnR primers to generate the downstream fragment (Table S3). The *hfaB* deletion (YB4251) was created using the hfaBupF, hfaBupR, hfaBdwnF2, and hfaBdwnR primers (Table S3). The *hfaD* deletion (YB4252) was created using the hfaDupF, hfaDupR, hfaDdwnF, and hfaDdwnR primers (Table S3). The *hfaA*, *hfaD* double deletion was created by conjugating the *hfaD* deletion plasmid into the *hfaA* (YB4250) deletion mutant and subsequent selection for sucrose resistance. The *hfsDAB* deletion (YB991) was created using primers HfsBBamup, HfsBXhoup, HfsBXhoe, HfsBHine2 (Table S3). The *rsaA* deletion was generated using primers FHindIIIrsaA, RecoRIrsaA, FmEcoRIrsaA, RsalIrsaA (Table S3).

The *hfa* gene deletions were complemented with pMR20 plasmids containing the respective *hfa* gene cloned downstream of a 200 bp DNA fragment that contains the *hfa* promoter. The HfaPrf and HfaPrR primers (Table S3) were used to amplify the *hfa* promoter and

the following primer pairs were used to amplify the *hfaB* and *hfaD*: HfaBNde, HfaBendPst, HfaDNde, and HfaDendPst (Table S3). The *hfa* promoter was digested with BclI and NdeI, and *hfaB* and *hfaD* were digested with NdeI and *Pst*I. The *hfa* promoter and either *hfaB* or *hfaD* DNA fragments were ligated into pMR20 digested with BglI and PstI. *hfaA* and the *hfa* promoter were amplified together using primers HfaPrf and HfaAendPst (Table S3) and digested with BclI and PstI and ligated into pMR20. The *hfa* promoter, *hfaA* and *hfaD* were amplified together using primers HfaPrF and HfaDendPst using chromosomal DNA from the *hfaB* deletion (YB4251) as a template. The *hfaA*, *hfaD* PCR product was digested with Bc*l*I and Ps*t*I and ligated into pMR20. The complementation constructs were then transformed into *E. coli* DH5α and S17-1 and subsequently conjugated into *C. crescentus*.

Overexpression constructs of HfaA, HfaB and HfaD were generated by isolating PCR fragments that contained each *hfa* gene, digesting with EcoRI and HindIII, cloning each *hfa* gene into pUJ142 (Meisenzahl *et al.*, 1997) to put control of gene expression under a xylose inducible promoter and add a C-terminal M2 epitope tag (DYKDDDK). The overexpression plasmids were cloned into *E. coli* DH5α and S17-1 and subsequently mated into *C. crescentus* CB15, CB15 Δ*hfaA* (YB4250), CB15Δ*hfaB* (YB4251) or CB15 Δ*hfaD* (YB4252). *hfaA* was isolated as an ~500bp PCR fragment from YB2578 DNA using hfaAEcoATG and pJMM2TagR primers (Table S3). A ~1200 bp DNA fragment containing *hfaB* was generated using primers hfaBEcoATG and pJMM2TagR (Table S3) from YB2580 DNA. A ~1300 bp DNA fragment containing *hfaD* was isolated using primers hfaDEcoATG and pJMM2TagR (Table S3) from YB2579 DNA.

hfaA fused to the M2 epitope tag (YB2578) was generated by cloning a PCR fragment using primers HfaA215PstI and HfaAend2 (Table S3) that encompassed the *hfaA* gene and the

hfa promoter. The 698 bp fragment was digested with PstI and BamHI and cloned into pJM23, transformed into *E. coli* DH5α and S17-1 containing the helper plasmid pLVC9 and then mated into *Caulobacter*. pJM23 does not replicate in *Caulobacter* resulting in the integration of any constructs into the chromosome by homologous recombination. *hfaA* is merodiploid in this strain.

The HfaBmcherry construct was generated by cloning a PCR fragment containing the *hfa* promoter, *hfaA* and *hfaB* using primer HfaPrNdeF and HfaBKpnend. The 2-kb PCR fragment was digested with NdeI and KpnI and cloned into pCHYC-1 (Thanbichler *et al.*, 2007). The HfaBmcherry construct in the *hfaA* mutant was generated by amplifying a PCR fragment from CB15 Δ*hfaA* containing the *hfa* promoter and *hfaB* using the primers HfaPrNdeF and HfaBKpnend. A 1.5-kb PCR product was digested with NdeI and KpnI and cloned into pCHYC-1. Both plasmids were transformed into *E. coli* Alpha-select (Bioline USA, Taunton, MA and then *E. coli* SM10. The plasmids were then conjugated into *C. crescentus*. pCHYC-1 is a non-replicating plasmid and integrates into the chromosome. *hfaB* is a merodiploid in these strains.

Yeast two-hybrid (Y2H) analysis was performed using the Matchmaker GAL4 Yeast Two-Hybrid System 3 (Clontech, protocol #PT3247-1). Clones of *hfaA, hfaB and hfaD* were constructed without signal sequences. Each of the *hfa* genes were amplified using primers hfaAEcoF6970, hfaABamR7358, HfaAendPst2, hfaBEcoF7385, hfaBBamR8372, hfaBPstR8372, hfaDEcoF8327, hfaDBamR9536, and hfaDPstR9536 (Table S3); digested, and ligated into the bait (pGBKT7) and prey (pGADT7) vectors, using EcoRI, PstI and BamHI restriction sites. Ligation reactions were transformed into *E. coli* DH5α and constructs were confirmed by sequencing. Plasmids were purified from six *hfa* transformants, along with the positive (pGBKT7-p53, pGADT7-T) and negative (pGBKT7-Lam) Y2H control plasmids. Bait-

and-prey plasmid pairs of the desired combinations were co-transformed into yeast reporter strain AH109 using the PEG/LiAc method (Clontech protocol # 3204-1) (Gietz & Schiestl, 2007). Co-transformants were plated on –Leu/-Trp SD agar to select for the presence of the bait and prey vectors. The presence of both bait and prey vectors in the co-transformants was confirmed by yeast colony PCR (Walhout & Vidal, 2001) and sequencing.

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Supporting Figure Legends

Figure S1. Coverslip, short-term adherence and biofilm assays of a Δ*hfaC* mutant. A) Coverslip assay of *hfaC* mutant. Panels A, C and E are phase microscopy of bound cells. Panels B, D and F are fluorescence microscopy of holdfast bound with WGA-FITC. Panels A and B are CB15; C and D are NA1000 and E and F are CB15 Δ*hfaC*. B) Short-term binding and biofilm assay. Graph of relative short-term and biofilm formation. White bars represent the short-term binding data (45 min) and black bars represent the biofilm assay (24 h time point). Coverslips below the graph are from a representative assay at the 24 h time point stained with crystal violet.

Figure S2. Cover-slip binding assay and lectin labeling showing holdfast shedding in *hfa* deletions complemented with M2-tagged constructs. Panels A, C, E, G, I, K, M and O are phase contrast images of cells. Panels B, D, F, H, J, L, N and P are fluorescence images of holdfast stained with WGA-lectin. All images are representative areas of the glass cover-slip that were submerged in a culture of each strain, washed, stained, and imaged. (A, B) CB15/pUJ142 (positive control); (C, D) NA1000 /pUJ142 (negative control); (E, F) CB15 Δ*hfaA*/pUJ142; (G, H) CB15 Δ*hfaA*/pUJhfaAM2; (I, J) CB15 Δ*hfaB*/pUJ142; (K, L) CB15 Δ*hfaB*/pUJhfaBM2; (M, N) CB15 Δ*hfaD*/pUJ142; (O, P) CB15 Δ*hfaD*/pUJhfaDM2.

Movie S1. Timelapse movie of HfaBmCherry localization during the *C. crescentus* cell cycle. HfaBmCherry was visualized in cells placed on an M2G agarose pad at 30ºC using epifluorescence microscopy and DIC imaging. This time series is in 10 min intervals for a total of 6h and 50 min. Here we see a predivisional cell just before division, which gives rise to a new swarmer cell. The new swarmer is then followed through the cell cycle back to a predivisional

cell just before cell division. HfaBmCherry localization can be seen at both the tip of the stalk and the swarmer pole in the predivisional cell. After cell division, HfaB is maintained at the swarmer pole during the swarmer to stalked transition. HfaB is then pushed out at the tip of the stalk as it is elongated in the stalked cells and early predivisional cells. Finally, HfaB localization can be seen again at both the new swarmer pole and the stalked pole in the late predivisional cell.

Figure S3. Western blot of HfaBmCherry in CB15, *hfsDAB*, *podJ*, and *hfaA* mutants and localization of HfaB-mCherry in an *hfaA* mutant. OM fractions were isolated from each strain and 7 μg of protein from each OM fraction were examined by Western blot. Western blots were probed with anti-dsRed antibody, which binds to mCherry. (A) Western blot Samples: CB15 *hfaB*::pCHYC-1hfaB; CB15 Δ*hfsDAB hfaB*::pCHYC-1hfaB; CB15 Δ*podJ hfaB:*:pCHYC-1hfaB. (B) Western blot Samples: CB15 *hfaB*::pCHYC-1hfaB; CB15 Δ*hfaA hfaB*::pCHYC-1hfaB. (C) Localization of HfaB-mCherry in CB15 and an *hfaA* mutant.

Figure S4. Immunofluorescence localization of HfaA-M2 and HfaD-M2 in CB15 Δ*hfsDAB* and CB15 Δ*podJ*. Panel A, CB15 HfaA-M2; Panel B, CB15 HfaD-M2; Panel C, CB15 Δ*hfsDAB* HfaA-M2; Panel D, CB15 Δ*hfsDAB* HfaD-M2; Panel E, CB15 Δ*podJ* HfaA-M2 and Panel F, CB15 Δ*podJ* HfaDM2.

Figure S5. Western blot of Hfa proteins in *hfsDAB* and *podJ* mutants. For each strain, OMP were isolated and 10 μg were examined by Western Blot with anti-M2-HRP antibody.

Figure S6. HfaA has amino acid similarity to curlin proteins. HfaA, CsgA, and AgfA CLUSTALW alignment. High identity is shown with an asterisk (*) in the consensus. High similarity is shown with a semicolon in the consensus (:) and low similarity is shown with one dot (.) in the consensus. The aa of the signal sequences are in light grey. The conserved alanine at the signal sequence cleavage site is underlined and in black. The aa of the 22-aa secretion targeting sequence of CsgA is shown in dark grey. P28307, *E. coli* CsgA; P0A1E7, *Salmonella enteritidis* AgfA; AAC14298, *C. crescentus* CB15

Figure S7. HfaA alignment in closely related prostehcatebacteria and HfaA predicted secondary structure. (A) Clustal alignment of HfaA from *C. crescentus* strain CB15 (AAC14298), *Caulobacter sp.* strain K31 (ABZ72853), *Asticcacaulis biprosthecum* (xxxxxxxx, submission of genome sequence in progress), *Asticcacaulis excentricus* (ZP04772105), *Brevundimonas diminuta* (xxxxxxxx, submission of genome sequence in progress), *Maricaulis maris* (ABI65376), and *Oceanicaulis alexandrii* (ZP00958005). High identity is shown with an asterisk (*) in the consensus. High similarity is shown with a semicolon in the consensus (:) and low similarity is shown with one dot (.) in the consensus. The predicted signal sequence cleavage site is indicated with an arrow. Predicted AGGRESCAN and TANGO aggregation domains for HfaA are indicated by gray and black lines, respectively, above the sequence.

Figure S8. Secondary structure prediction of HfaA. PSI-pred was used to predict the secondary structure of HfaA. The predicted aggregation/amyloid domains are indicated above the structure region with a black line for TANGO and a gray line for AGGRESCAN. Arrows indicate extended strand structure and cylinders indicate alpha-helical structure.

Figure S9. Transcription of *hfa* promoter in *hfa, hfsDAB* and *podJ* mutants. Transcription was measured using β-galactosidase assays of *hfa* promoter transcriptional fusions. A) Transcription of the *hfa* promoter in each of the *hfa* deletions. B) Transcription of the *hfa* promoter in the *hfsDAB* and *podJ* mutants.

Figure S10. Secondary structure prediction of CsgG. PSI-pred was used to predict the secondary structure of CsgG. Arrows indicate extended strand structure and cylinders indicate alpha-helical structure.

Figure S11. Secondary structure prediction of HfaB. PSI-pred was used to predict the secondary structure of CsgB. Arrows indicate extended strand structure and cylinders indicate alpha-helical structure.

TABLE S1. Complementation of *hfa* mutants by *hfa*-M2 gene fusions.

Strain	Lectin Binding[a] (mean±SE)	Cell adherence[b] (mean±SE)
CB15/pUJ142	41.0±4.7	99.9±0.005
NA1000/pUJ142	0.2±0.2	9.6±1.9
CB15 *ΔhfaA*/pUJ142	23.6±6.4	77.5±5.7
CB15 *ΔhfaB*/pUJ142	2.4±0.3	41.6±1.2
CB15 *ΔhfaD*/pUJ142	46.9±14.8	72.7±1.5
CB15 *ΔhfaA*/pUJ142hfaAM2	64.6±16.4	111.6±8.5
CB15 *ΔhfaB*/pUJ142hfaBM2	73.1±19.1	75.1±2.6
CB15 *ΔhfaD*/pUJ142hfaDM2	77.7±11.1	95.5±5.3

[a] Percent of predivisional cells with polar WGA-lectin binding

[b] Cell adherence to polystyrene measured by short-term binding assay

TABLE S2. Bacterial strains

Strain	Derivation/phenotype/genotype	Reference
Caulobacter crescentus		
CB15	Wildtype	(Poindexter, 1964)
NA1000	*syn-1000*, previously called CB15N, holdfast deficient	(Evinger & Agabian, 1977)
YB2412	CB15 *mcpA*::pRMC22	this study
YB2578	CB15 *hfaA*::pJM23*hfaA*	this study
YB2579	CB15 *hfaD*::pJM23*hfaD*	(Cole *et al*., 2003)
YB2580	CB15 *hfaB*::pJM21*hfaB*	(Cole et al., 2003)
YB991	CB15 Δ*rsaA*	this study
YB2138	CB15 Δ*podJ*; same as YB3079	(Hinz *et al*., 2003)
YB2551	CB15/placPHfaA	(Cole et al., 2003)
YB2851	CB15 Δ*hfsD, hfsA, hfsB*	this study
YB3073	CB15/plac290	(Cole et al., 2003)
YB4250	CB15 Δ*hfaA*	this study
YB4251	CB15 Δ*hfaB*	this study
YB4252	CB15 Δ*hfaD*	this study
YB4258	YB4250/pMR20Phfa-hfaA	this study
YB5618	YB4250/pUJ142hfaAM2	this study
YB4259	YB4251/pMR20Phfa-hfaB	this study
YB4270	YB4251/pUJ142hfaAM2	this study
YB5619	YB4251/pUJ142hfaBM2	this study
YB4271	YB4251/pUJ142hfaDM2	this study
YB4260	YB4252/pMR20Phfa-hfaD	this study
YB5620	YB4252/pUJ142hfaDM2	this study
YB4227	YB991 *hfaA*::pJM23*hfaA*	this study
YB4228	YB991 *hfaB*::pJM21*hfaB*	this study
YB4229	YB991 *hfaD*::pJM23*hfaD*	this study
YB4248	YB2138 *hfaA*::pJM23hfaA	this study
YB4249	YB2138 *hfaD*::pJM23hfaD	this study
YB5625	YB2851 *hfaA*::pJM23hfaA	this study
YB5626	YB2851 *hfaD*::pJM23hfaD	this study
YB4284	CB15 *hfaB*::pCHYChfaAB	this study
YB5616	YB2138 *hfaB*::pCHYChfaAB	this study
YB5617	YB2851 *hfaB*::pCHYChfaAB	this study
YB5631	YB4250/placPHfaA	this study
YB5632	YB4251/placPHfaA	this study
YB5633	YB4252/placPHfaA	this study
YB5634	YB2138/placPHafA	this study
YB5637	YB4250 hfaB::pCHYChfaB	this study
YB5957	YB2851/placPHfaA	this study
E. coli		
DH5αF'	Φ80 dLacZΔM15 Δ(*lacZYA-argF*)*U169 endA1 recA1 hsdR17* (r^-m^+)*deoR thi-1 supE44 gyrA96 relA1*	(Liss, 1987)
S17-1	*E. coli* 294::RP4-2 (Tc::Mu)(Km::Tn7)	(Simon *et al*., 1983)
SM10	*thi*-1 *thr leu tonA supE recA*::RP4-2 Tc::Mu, Km^R	(Simon et al., 1983)
Alpha-Select	F- *deoR endA1 recA*1 *rel*A1 *gyrA*96 *hsdR*17(r_k^-, m_k^+) *supE*44 *thi*-1 *phoA* Δ(*lacZYA argF*)U169 ϕ80*lacZ*ΔM15 λ-	Bioline
Saccharomyces cerevisiae		
AH109	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, LYS2::$GAL1_{UAS}$-$GAL1_{TATA}$-HIS3, $GAL2_{UAS}$-$GAL2_{TATA}$	(James *et al*., 1996)

	-ADE2, URA3::MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ	
Plasmids		
pMR20	shuttle plasmid for *E. coli* and *Caulobacter*, Tc^R	(Roberts *et al*., 1996)
pLVC9	conjugation helper plasmid carrying a ColEI *mob,* Tc^R	(G. Warren, unpublished)
pUJ142	Derivative of pBBR1MCS, contains a xylose inducible promoter for expression, Cm^R	(U. Jenal, unpublished)
pJM21 and pJM23	ColE1 ori, Kan^R, oriT vectors with the M2 epitope in different reading frames with respect to the polylinker.	(Alley *et al*., 1993)
pNPTS138	plasmid for generation of deletions using SacB, Km^R	(MRK Alley, unpublished)
plac290	lacZ transcriptional fusion vector, TcR, IncP-1 replicon, mob+	(Gober & Shapiro, 1992)
pRCM22	full-length McpA-M2 on a replicating vector, Tc^R	(Alley et al., 1993)
pCHYC-1	integrating plasmid for generation of mCherry fusion, Sp^R	(Thanbichler et al., 2007)
pJM21HfaB	1.7 kb PCR product from oligos HfaA215Pst and HfaBend cut with PstI and BamHI and cloned into pJM21 cut with BamHI and PstI	(Cole et al., 2003)
pJM23HfaA	698 bp PCR product from oligos HfaA215Pst and HfaAend2 cut with PstI and BamHI and cloned into pJM23 cut with BamHI and PstI	this study
pJM23HfaD	A 350-bp PCR product from oligos HfaDmid and HfaDend cut with EcoRI and BamHI and cloned into pJM23 cut with EcoRI and BamHI.	(Cole et al., 2003)
pMR20Phfa-hfaA	A 710 bp PCR product from oligos HhfaPrF and HfaAendPst cut with BclI and PstI cloned into pMR20 cut with BamHI and PstI.	(Cole et al., 2003)
pMR20Phfa-hfaB	A 253-bp PCR product from oligos HfaAPrF and HfaAPrR cut with BclI and NdeI and a 1000-bp PCR product from oligos HfaBNde and HfaBendPst cut with NdeI and PstI cloned into pMR20 cut with BamHI and PstI.	this study
pMR20Phfa-hfaD	A 253-bp PCR product from oligos HfaAPrF and HfaAPrR cut with BclI and NdeI and a 1286-bp PCR product from oligos HfaDNde and HfaDendPst cut with Nd*e*I and Ps*t*I cloned into pMR20 cut with BamHI and PstI.	(Cole et al., 2003)
pUJ142hfaAM2	A 450-bp PCR product from oligos HfaAEcoATG and pJM-M2tagR cut with EcoRI and HindIII and cloned into pUJ142 cut with EcoRI and HindIII.	this study
pUJ142hfaBM2	A 1.2 kb PCR product from oligos HfaBEcoATG and pJM-M2tagR cut with *Eco*RI and *Hin*dIII and cloned into pUJ142 cut with EcoRI and HindIII.	this study
pUJ142hfaDM2	A 1.3 kb PCR product from oligos HfaDEcoATG and pJM-M2tagR cut with EcoRI and HindIII and cloned into pUJ142 cut with EcoRI and HindIII.	this study
pNPTS138ΔhfaA	A 459 bp PCR product upstream of *hfaA* from oligos FhfaupA and RhfaupA cut with HindIII and BamHI a 513 bp PCR product downstream of *hfaA* from oligos FmhfadownA and RmhfadownA and cut with BamHI and EcoRI were cloned into the HindIII and EcoRI restriction sites of pNPTS138.	this study
pNPTS138ΔhfaB	A 456 bp PCR product upstream of *hfa*B from oligos FhfaBup and RhfaBup2 cut with HindIII and BamHI and a 485 bp PCR product downstream of *hfaB* from oligos FhfaBdwn2 and RmhfadownB and cut with BamHI and EcoRI were cloned into the HindIII and Ec*o*RI restriction sites of pNPTS138.	this study
pNPTS138ΔhfaD	A 485 bp PCR product upstream of *hfaD* from oligos FhfaDup and RhfaDup2 cut with HindIII and BamHI and a	this study

	477 bp PCR product downstream of *hfaD* from oligos FmhfadownD and RmhfadownD and cut with BamHI and EcoRI were cloned into the HindIII and EcoRI restriction sites of pNPTS138.	
pNPTS138ΔrsaA	A 471 bp PCR product upstream of *rsaA* from oligos HindIIIrsaA and REcoRIrsaA cut with HindIII and EcoRI and a 530 bp PCR product downstream of *rsaA* from oligos FmEcoRIrsaA and RmSalIrsaA and cut with EcoRI and SalI were cloned into the HindIII and SalI restriction sites of pNPTS138.	this study
pNPTShfsDB	A 500 bp product upstream of *hfsD* from oligos hfsDBamup and hfsDXhoup cut with BamHI and XhoI and a 500 bp0 PCR product downstream of *hfsB* using primers hfsBHine2 and And hfsBXhoe2 and cut with XhoI and HindIII were cloned into pNPTS138 using restriction sites BamHI and HindIII.	this study
pCHYChfaAB	A 2 kb PCR product from oligos HfaPrNdeF and HfaBKpnend that contains the *hfa* promoter, *hfaA*, and *hfaB* and cut with NdeI and KpnI and cloned into the NdeI and KpnI restriction sites of pCHYC-1.	this study
pCHYChfaB	A 1.1 kb PCR product isolated from the CB15 Δ*hfaA* using oligos HfaPrNdeF and HfaBKpnend that contains the hfa promoter and hfaB and cut with NdeI and KpnI was cloned into the NdeI and KpnI restriction sites of pCHYC-1	this study
pGBKT7-53	encodes fusions between the GAL4 DNA-binding domain and murine p53; interacts with large T-antigen in Y2H assay	(Li & Fields, 1993)
pGADT7-T	encodes fusion between the GAL4 activation domain and SV40 large T-antigen; interacts with p53 in Y2H assay	(Iwabuchi *et al*., 1993)
pGBKT7-Lam	encodes a fusion between the GAL4 DNA-binding domain and human lamin-C and provides a control for fortuitous interaction between an unrelated protein and either the pGADT7-T control or other protein to be tested. Lamin-C neither forms complexes nor interacts with most other proteins	(Bartel *et al*., 1993) (Ye & Worman, 1995)
pGBKT7-hfaA	A 365-bp PCR product of *hfaA* from oligos hfaAEcoF6970 and hfaABamR7358 cut with EcoRI and BamHI and ligated into pGBKT7 cut with EcoRI and BamHI	this study
pGADT7-hfaA	A 365-bp PCR product of *hfaA* from oligos hfaAEcoF6970 and hfaAendPst2 cut with EcoRI and PstI and ligated into pGADT7 cut with EcoRI and PstI	this study
pGBKT7-hfaB	A 959-bp PCR product of *hfaB* from oligos hfaBEcoF7385 and hfaBBamR8372 cut with EcoRI and BamHI and ligated into pGBKT7	this study
pGADT7-hfaB	A 959-bp PCR product of *hfaB* from oligos hfaBEcoF7385 and hfaBPstR8372 cut with EcoRI and PstI and ligated into pGADT7 cut with EcoRI and PstI	this study
pGBKT7-hfaD	A 1183-bp PCR product of *hfaD* from oligos hfaDEcoF8327 and hfaDBamR9536 cut with EcoRI and BamHI and ligated into pGBKT7 cut with EcoRI and BamHI.	this study
pGADT7-hfaD	A 1183-bp PCR product of *hfaD* from oligos hfaDEcoF8327 and hfaDPstI9536 cut with EcoRI and PstI and ligated into pGADT7 cut with EcoRI and PstI.	this study
pSP64-hfaAbait	A 500-bp PCR product of *hfaA* fused to c-myc epitope amplified from pGBKT7-hfaA template using oligos GBKHindIIIF and GBKXbaIR and cut with HindIII and XbaI and ligated into pSP64polyA cut with HindIII and XbaI.	this study
pSP64-hfaDbait	A 1300-bp PCR product of *hfaD* fused to c-myc epitope	this study

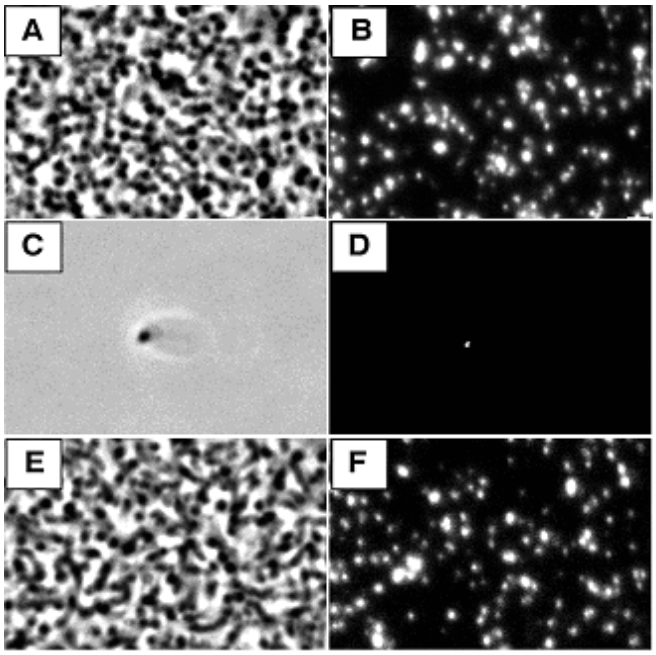
	amplified from pGBKT7-hfaD template using oligos GBKHindIIIF and GBKXbaIR and cut with HindIII and XbaI and ligated into pSP64polyA cut with HindIII and XbaI.	
pSP64-Lam	A750-bp PCR product of Lamin-C fused to c-myc epitope amplified from pGBKT7-Lam using oligos GBKXbaF and GBKXmaR and cut with XbaI and XmaI and ligated into pSP64polyA cut with XbaI and XmaI.	this study
pSP64-hfaAprey	A 500-bp PCR product of *hfaA* fused to HA-epitope amplified from pGADT7-hfaA template using oligos GADHindIIIF and GADXbaIR and cut with *Hin*dIII and *Xba*I and ligated into pSP64polyA cut with HindIII and XbaI.	this study
pSP64-hfaDprey	A 1300-bp PCR product of *hfaD* fused to HA-epitope amplified from pGADT7-hfaD template using oligos GADHindIIIF and GADXbaIR and cut with HindIII and XbaI and ligated into pSP64polyA cut with HindIII and XbaI.	this study

TABLE S3. DNA Primers

Primer	Sequence 5' to 3'[a]	Source
HfaAPrF	CAGCAGAAGGTGCGGAATGATCACGGCCGC	CB15
HfaAPrR	CCGATGCCAGGCCATATGAGGCTCCGCGAC	CB15
HfaPrNdeF	CAGCAGAAGGTGCCATATGATCACGGCCGC	CB15
HfaAendPst	GGACCGCGCCTGCAGGCTTGACCATCATTT	CB15
HfaAEcoATG	GTCGCGGAGGAATTCATGGCCTGGCATCGG	CB15
FhfaupA	GCGATCACGAAGCTTACGAGGTAG	CB15
RhfaupA	CCCACGCGTGGATCCGTTCCGATG	CB15
FmhfadownA	CGTCGTGGAAGGATCCGTTCCGATG	CB15
RmhfadownA	GGCGTCAATGAATTCCGAGCGGAT	CB15
HfaA215Pst	GCAGCAGAAGGTCTGCAGTGATCACGGCCG	CB15
HfaAend2	TTGTGCGCTTGACCGGATCCTGGGAGTACCGCCCT	CB15
HfaBNde	GCGGTACTCCCATATGATGGTCAAGCG	CB15
HfaBendPst	CTCGGCGTCGTCCTCCAGCTAGTAGCGACC	CB15
HfaBKpnend	GGCGTCGTCGTCGGTACCGTAGCGACC	CB15
FhfaBup	CGATGGTCGAAGCTTCCGTCACGC	CB15
RhfaBup2	GCAGGCGCTGAGGGATCCGGTGGCCAG	CB15
FhfaBdown2	GCCTTCACGCCGGGATCCAACAATCTGGGA	CB15
RmHfadownB	CACGTTGGCGAATTCCGACTGGCT	CB15
HfaBEcoATG	GGTACGAATTCATGATGGTCAAGCGCACAG	CB15
FhfaupD	GGCATCACCAAGCTTAACTACAAC	CB15
RhfaDup2	CGGGCTCGGCGTGGATCCCTGGCTAGTAGC	CB15
FmHfadownD	GCCAGCTTCGGATCCAACGCGCCC	CB15
RmHfadownD	GCGGCCTGGGAATTCTAGTCCTGA	CB15
HfaDNde	TGGGAACGACATATGCGCAGACCCGCG	CB15
HfaDendPst	TCGCAGCGCCTGCAGGGCCCGGGACTTGCAG	CB15
HfaDEcoATG	ACAACAATCTGGGAAGAATTCATGCGCAGACCC	CB15
pJM-M2tagR	CACCTAGATCCTTTAAGCTTTTACTTGTCGTCGTC	pJM23
FHindIIIrsaA	GACCTCCAGAAGCTTGGCCCAGTC	CB15
RecoRIrsaA	CGCAGTCACGAATTCGGCCGTCGT	CB15
FmEcoRIrsaA	TTCGCCACCGAATTCCTGACGCTA	CB15
RsalIrsaA	GCCGTCGAAGTCGAGACCGCC	CB15
HfsDBamup	CTGGCCACGGATCCCAACGACC	CB15
HfsDXhoup	CATCTCGACCTCGAGATCCACCAT	CB15
HfsBHine2	CATCCATAGCCAAGCTTAGGCGCCGGGA	CB15
HfsBXhoe2	CAGCCTTCCTGCTCGAGATCCTGCCGTG	CB15
GADForPCR	CGTATAACGCGTTTGGAATCACTACAGGGATG	pGADT7
GADRevPCR	CGATGCACAGTTGAAGTGAACTTGCGG	pGADT7
GBKForPCR	GGAGACTGATATGCCTCTAACATTGAGACAGC	pGBKT7
GBKRevPCR	GTAGAGGTGTGGTCAATAAGAGCGACC	pGBKT7
hfaAEcoF6970	GGCGTCGCCGAATTCCAATCGATGTCG	CB15
hfaABamR7358	CTGTGCGCTGGATCCTCATTTCGGAGT	CB15
hfaBEcoF7385	GCGGCGCTCGAATTCTGCGGCAGCACG	CB15
hfaBBamR8372	CTCGGCGTCGTCGGATCCCTAGTAGCG	CB15
hfaDEcoF8327	CGTGATGGAATTCGGGGTCGCTACTAGCCA	CB15
hfaDBamR9536	CGTCGAGGGCCCGGATCCTCAGTTCCC	CB15
hfaAendPst2	CTGTGCGCTGCAGCATCATTTCGGAGTACC	CB15
hfaBPstR8372	CTCGGCGTCGTCCTGCAGCTAGTAGCG	CB15
hfaDPstR9536	CGTCGAGGGCCCCTGCAGTCAGTTCCC	CB15
GBKHindIIIF	GGAATTTGTAATAAGCTTCACTAT	pGBKT7
GBKXbaIR	GCAAAAAACCCCTCTAGACCCGTTT	pGBKT7
GADHindIIIF	GATCTTTAATAAGCTTCACTATAGGGCG	pGADT7
GADXbaIR	GTTGAAGTGATCTAGAGGGGTTTTT	pGADT7

GBKXbaF	GTATCGCCGGAATCTAGAATACGACTC	pGBKT7
GBKXmaR	GAAATTCGCCCGGGATTAGCTTGGCT	pGBKT7

A.


A
B
C
D
E
F

B.

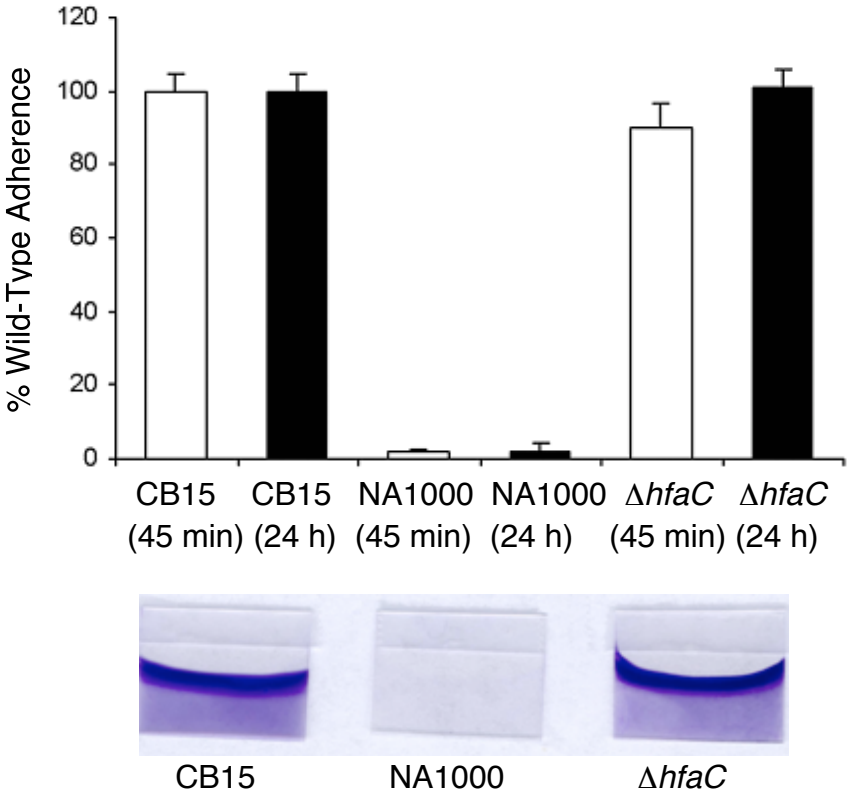

% Wild-Tvpe Adherence
120
100
80
60
40
20
0
CB15 (45 min)
CB15 (24 h)
NA1000 (45 min)
NA1000 (24 h)
ΔhfaC (45 min)
ΔhfaC (24 h)
CB15
NA1000
ΔhfaC

Fig. S1

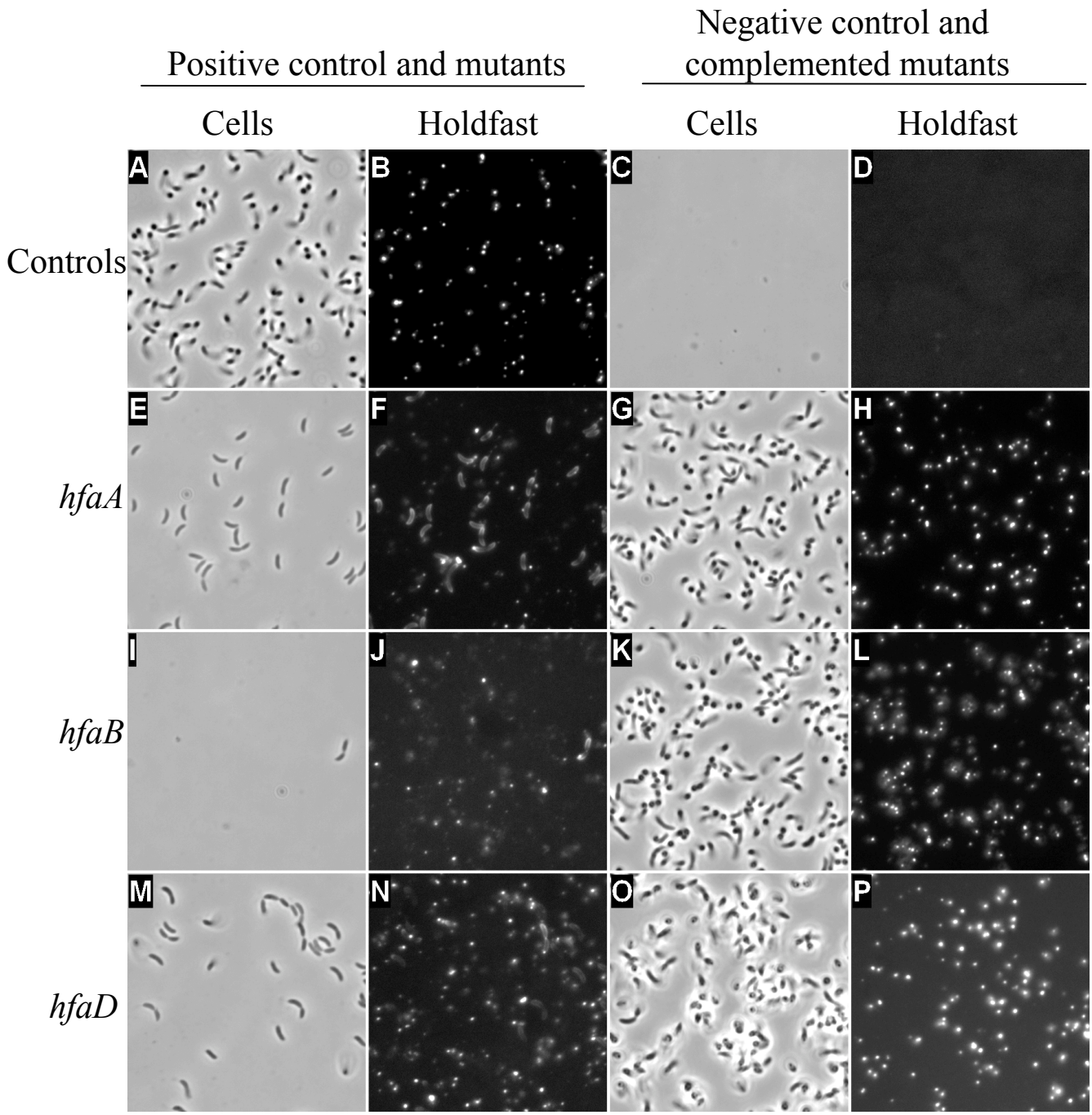

Positive control and mutants
Negative control and complemented mutants
Cells
Holdfast
Cells
Holdfast
Controls
hfaA
hfaB
hfaD
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P

Fig. S2

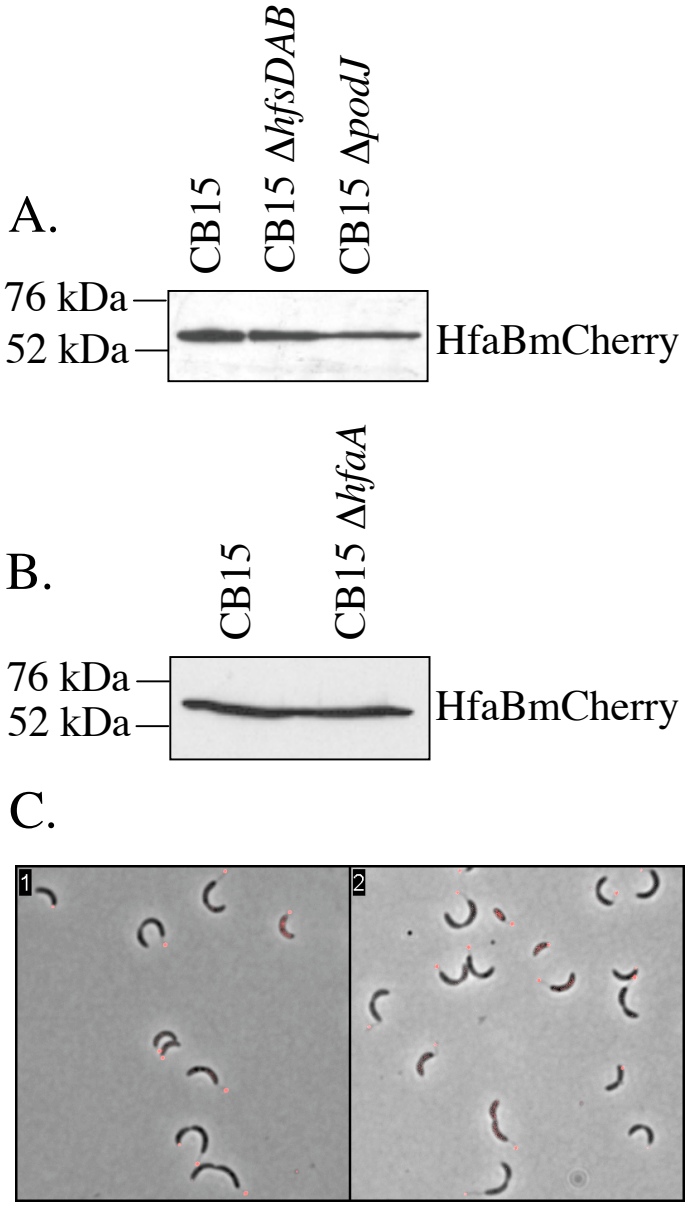

A.
CB15
CB15 ΔhfsDAB
CB15 ΔpodJ
76 kDa
52 kDa
HfaBmCherry
B.
CB15
CB15 ΔhfaA
76 kDa
52 kDa
HfaBmCherry
C.
1
2

Fig. S3

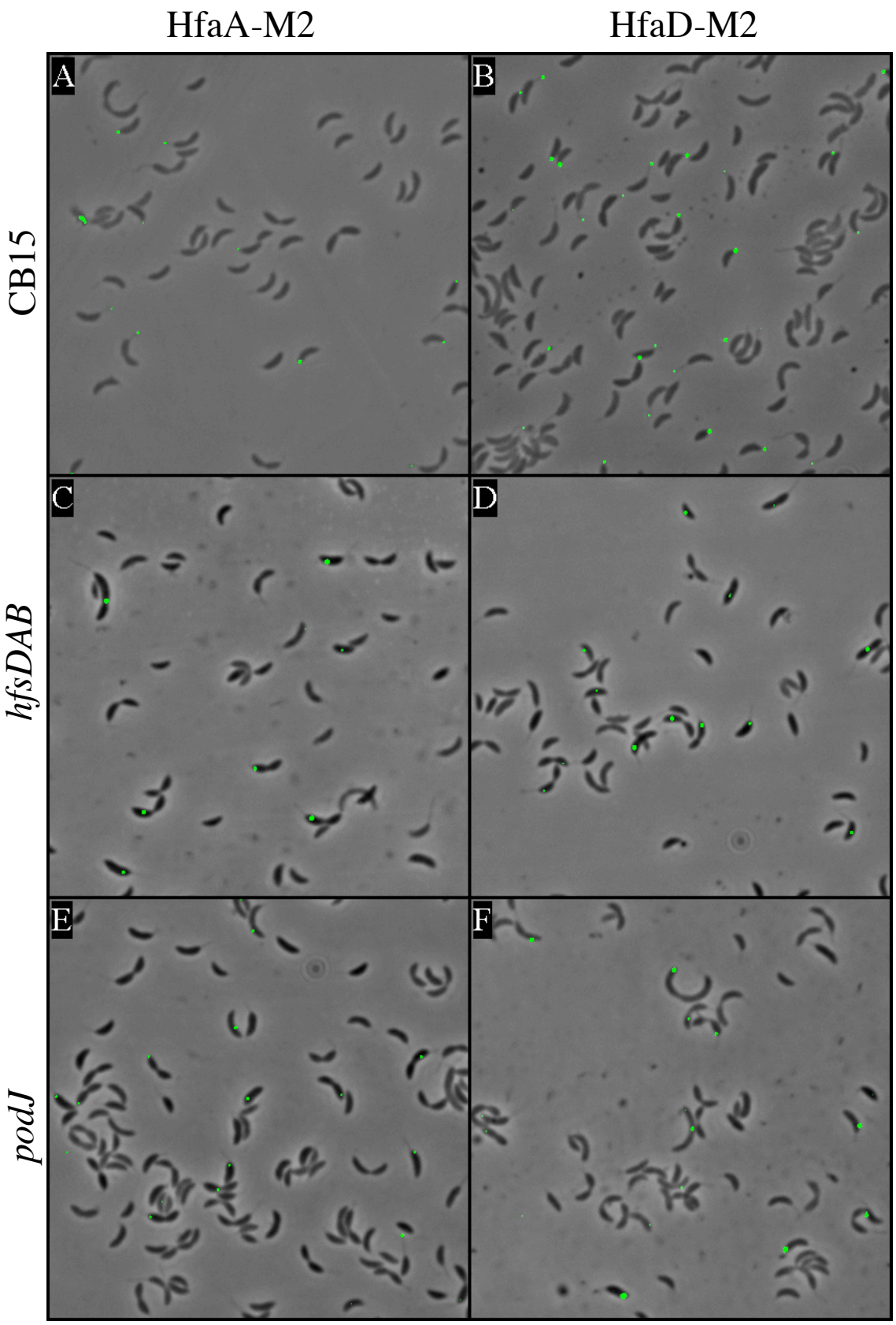


Fig. S4

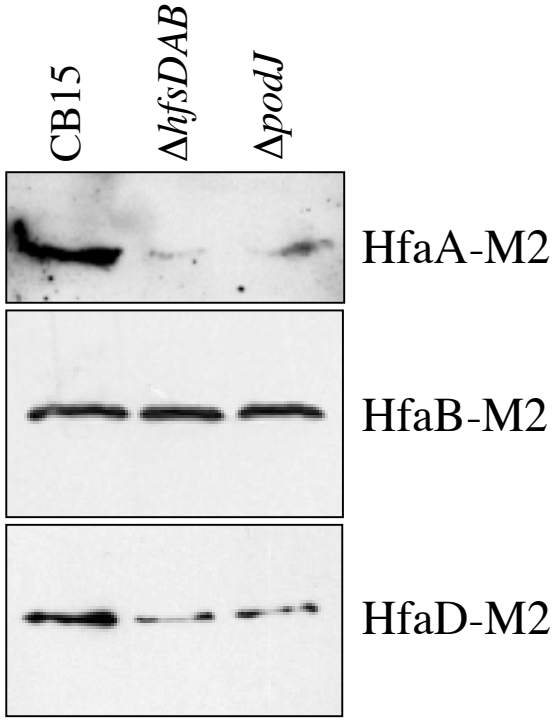


Fig. S5

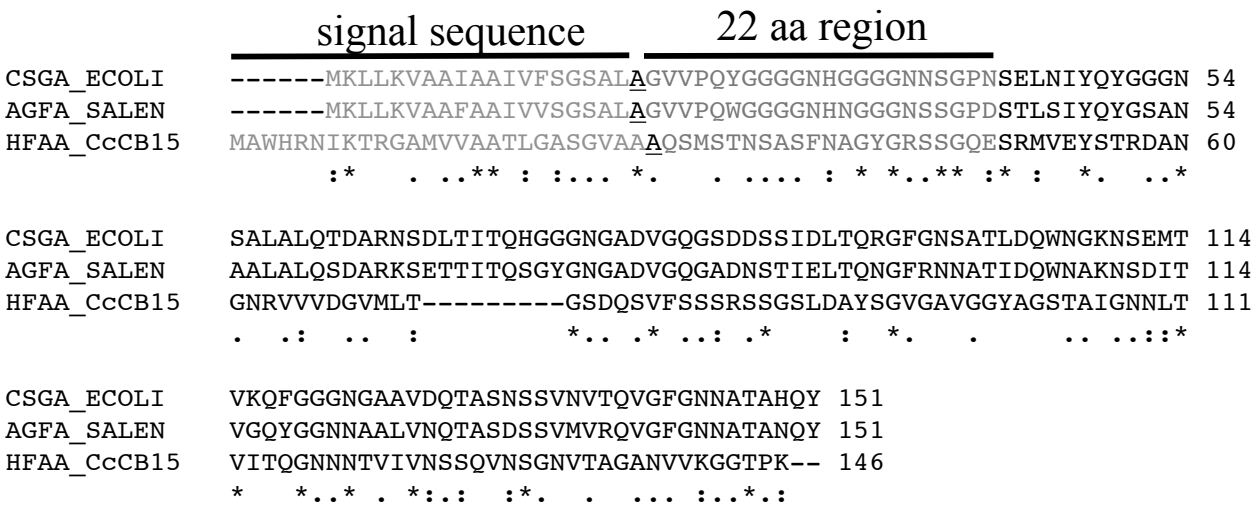
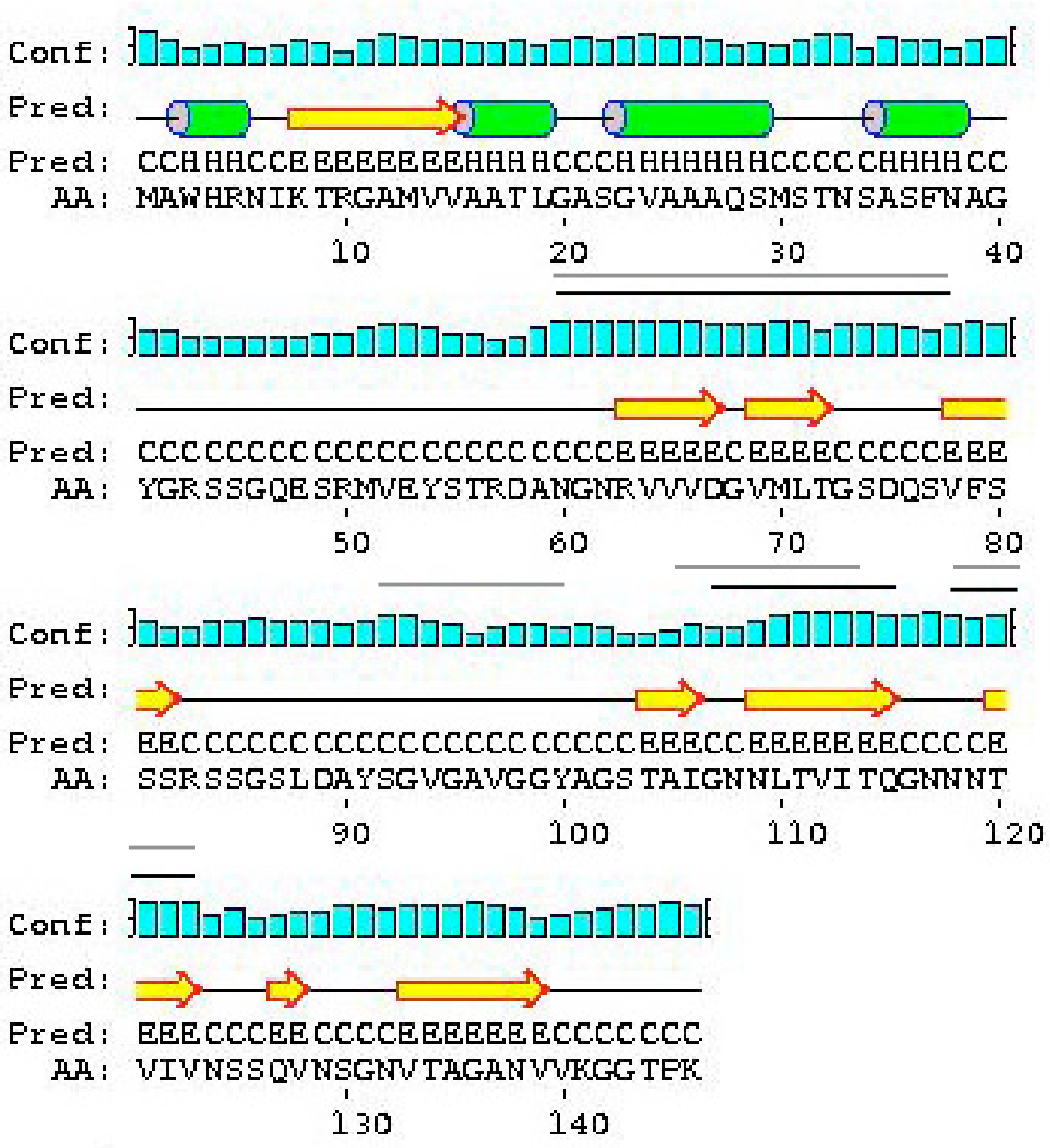

signal sequence
22 aa region
CSGA_ECOLI ------MKLLKVAAIAAIVFSGSALAGVVPQYGGGGNHGGGGNNSGPNSELNIYQYGGGN 54
AGFA_SALEN ------MKLLKVAAFAAIVVSGSALAGVVPQWGGGGNHNGGGNSSGPDSTLSIYQYGSAN 54
HFAA_CcCB15 MAWHRNIKTRGAMVVAATLGASGVAAAQSMSTNSASFNAGYGRSSGQESRMVEYSTRDAN 60
:* . ..** : :... *. . .... : * *..** :* : *. ..*
CSGA_ECOLI SALALQTDARNSDLTITQHGGGNGADVGQGSDDSSIDLTQRGFGNSATLDQWNGKNSEMT 114
AGFA_SALEN AALALQSDARKSETTITQSGYGNGADVGQGADNSTIELTQNGFRNNATIDQWNAKNSDIT 114
HFAA_CcCB15 GNRVVVDGVMLT---------GSDQSVFSSSRSSGSLDAYSGVGAVGGYAGSTAIGNNLT 111
. .: .. : *.. .* ..: .* : *. . .. ..::*
CSGA_ECOLI VKQFGGGNGAAVDQTASNSSVNVTQVGFGNNATAHQY 151
AGFA_SALEN VGQYGGNNAALVNQTASDSSVMVRQVGFGNNATANQY 151
HFAA_CcCB15 VITQGNNNTVIVNSSQVNSGNVTAGANVVKGGTPK-- 146
* *..* . *:.: :*. . ... :..*.:

Fig. S6


Signal Sequence
CB15 -MAWHRNI------KTRGAMVVA--AVTLGASGVAAAQSMSTN-SASFNA 40
Caulobacter sp. K31 MAAYEKKLPGNDRSRTIGAAVLTLLATSLALPGLTHAQTLDSN-SASFNA 49
A. biprosthecum -MSLTKAA-----VLALAGIALV------ASP--ALAQNMSTK-KAAFET 35
A. excentricus -MAFNKIA-----AATAGLVVLT------GAP--ALAQTMSTT-SSGFET 35
B. diminuta -MAVRRKI------LAGGGLALA----VIAAP--ALAQTTGSGGMASFQN 37
M. maris -MGHLVNIAARRRLPTSDQQTDD-------PEEAAMPIASFALAAAQMAT 42
O. alexandrii -MTRQLKV-----WLCATAAAIF-------AASSAMAQSTAAN-PSEWNR 36
CB15 GYGRSSGQESRMVEYSTRDANGNRVVVDGVMLTGSDQSVFSSSRSSGSLD 90
Caulobacter sp. K31 GYGRVAGSENHVVEYSTRDANGNRVIVDGVMLTGADQSVYSSSHSSGSLD 99
A. biprosthecum GYGIGRNQMQHGVDPSTRDANGNRVLLDGSILTGSDQSVFSYSKTLGAGD 85
A. excentricus GYGRTRGQEERAIDPSTRDANGNRVLLDGVIVTGGDQSVYSKSMTYGAGD 85
B. diminuta GYGGARQSVTTAQTGSTRDQNGNRLIVDGIIQAG--ASAYS-AQSGGVSQ 84
M. maris PVSLDTGFAVNQAIGFQKDAQGNRVVLSGTSQLSAGTSGGG---TQSRLM 89
O. alexandrii PYGQAYGSENQAYVGAR--VGGNRVVLNGIIQTGVGVSAQASALTQSATG 84
CB15 AYSGVGAVGGY---AGSTAIGNNLTVITQGNNNTVIVNSSQVNSGNVTA- 136
Caulobacter sp. K31 SYTGVGSLGGY---GSSTAIGNNLTVITQGNNNTVIVNSNQINNGAITA- 145
A. biprosthecum TYAGAGGRGG------ATAIGNNLQVIVNGNHNTVIVHSNQVNNGNVTAN 129
A. excentricus SYSGAGAVGG------ATAIGNNLSVVVNGNYNTVIVNSTQTNNGNVTAT 129
B. diminuta TWSGSGNASGGSAIGGSTAIGNNLNVVVQGSHNTVIVNSRQTNTGNVSA- 133
M. maris PSWGVTATTS-----QATAIGNLVSVTITGNNNTVQLDTTQINLGNQTAI 134
O. alexandrii VGLNSQSQSGLFTSAGASAIGNQLNVVVNGNYNTVVINNRQTNTGDITAH 134
CB15 -GANVVKGGTPK------------ 147
Caulobacter sp. K31 -GTNVGKSGNGQ------------ 156
A. biprosthecum NGSSSATAPTGTTTNDITGQVN-F 152
A. excentricus NGNQTATGATGT---DITGNLNGF 150
B. diminuta -RTDLTGTLTGF------------ 144
M. maris VGELPNVSGN-------------- 144
O. alexandrii AGATANTQSSQGEAANDR------ 152

Fig. S7



Conf: confidence of prediction

Pred: predicted secondary structure

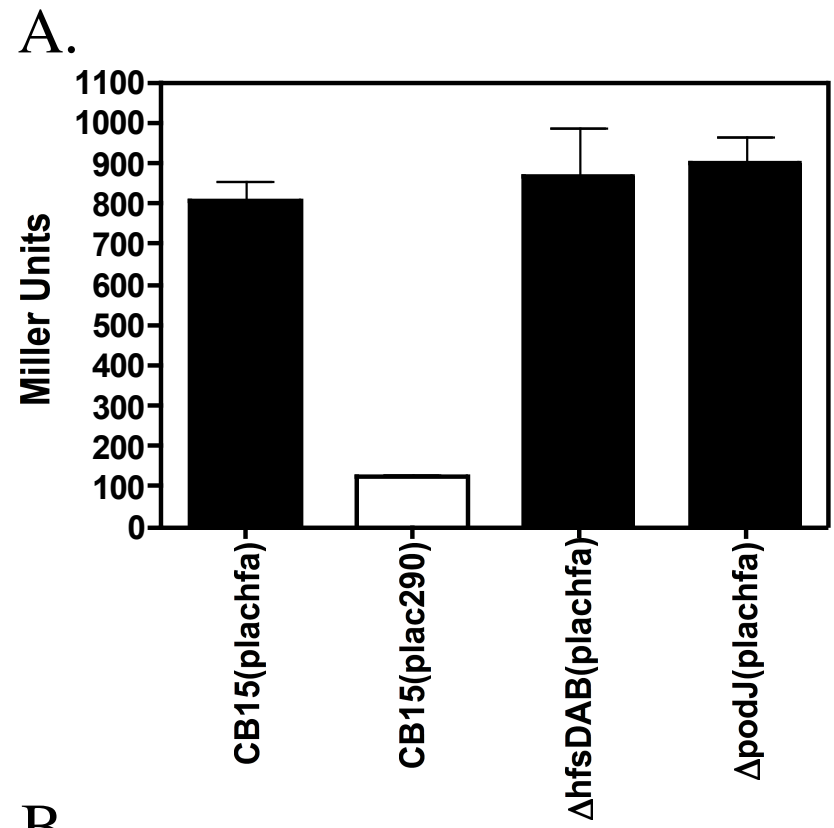
AA: target amino acid sequence

β-strand

α-helix

Random coil

Fig. S8


A.
Miller Units
1100
1000
900
800
700
600
500
400
300
200
100
0
CB15(plachfa)
CB15(plac290)
ΔhfsDAB(plachfa)
ΔpodJ(plachfa)

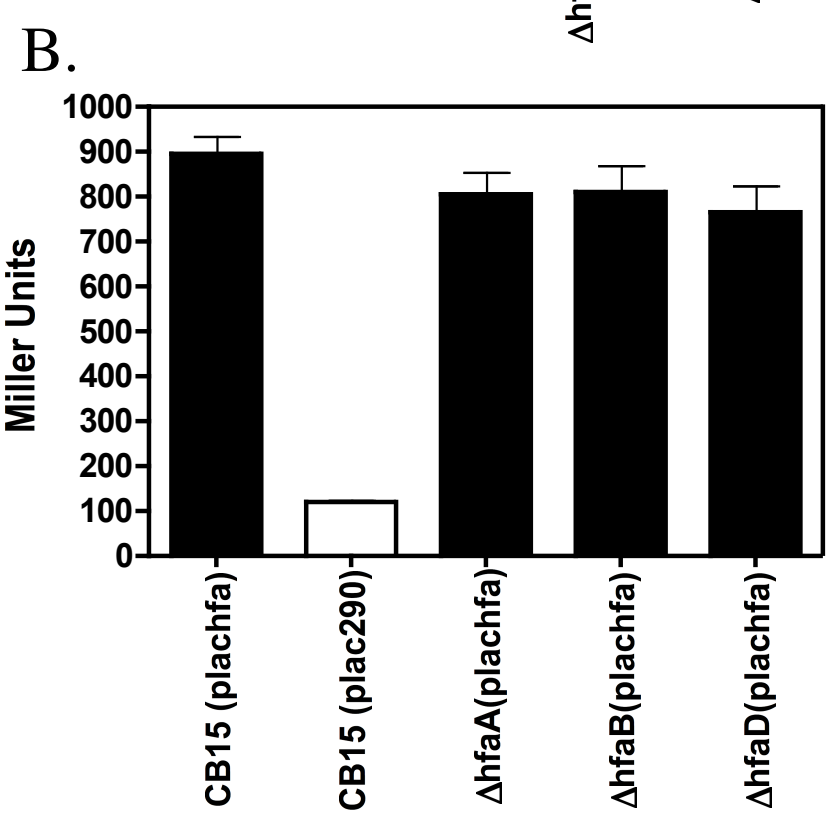
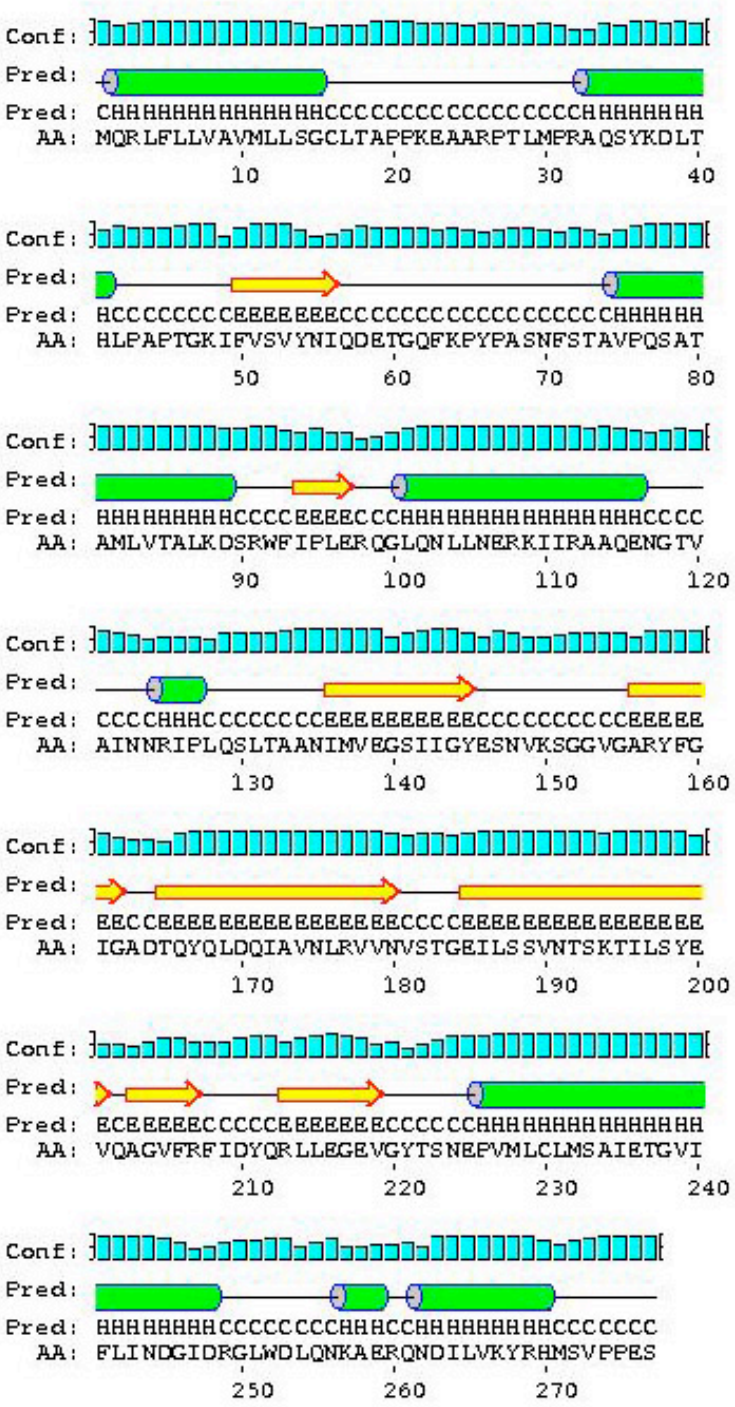

B.
Miller Units
1000
900
800
700
600
500
400
300
200
100
0
CB15 (plachfa)
CB15 (plac290)
ΔhfaA(plachfa)
ΔhfaB(plachfa)
ΔhfaD(plachfa)

Fig. S9



Conf: confidence of prediction

Pred: predicted secondary structure

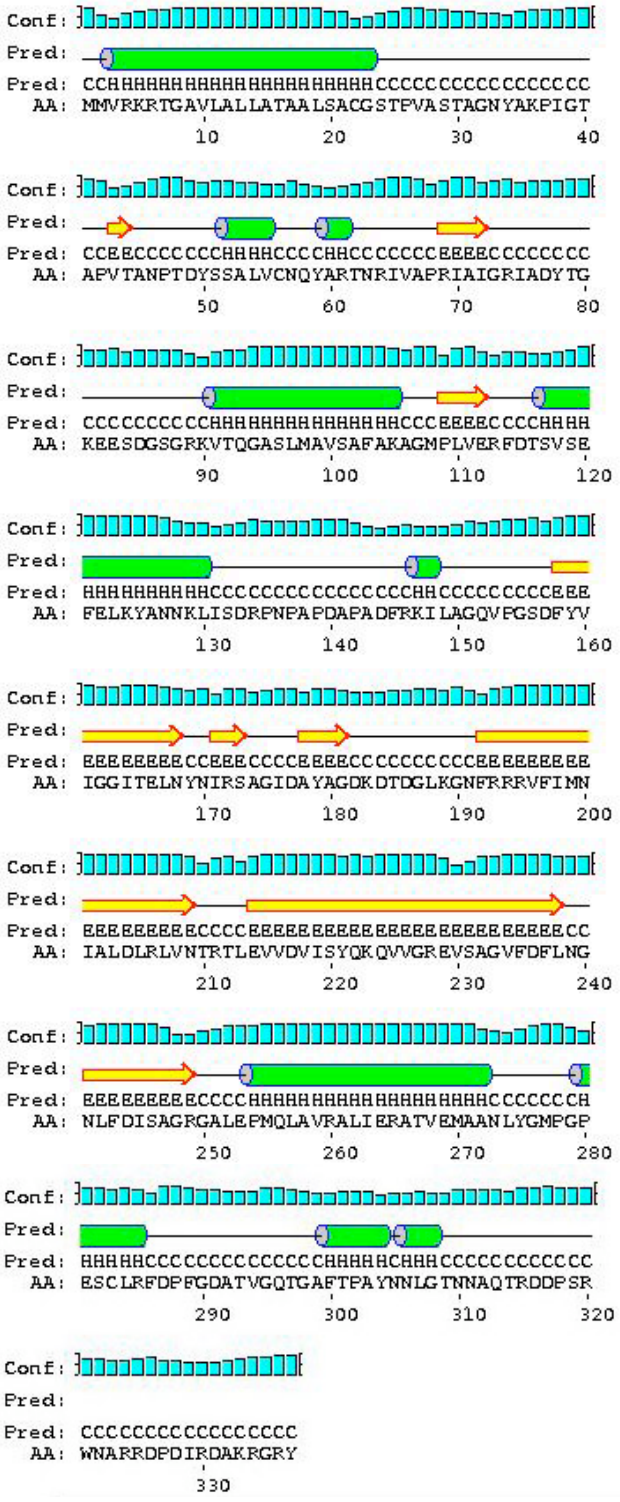
AA: target amino acid sequence

β-strand

α-helix

Random coil

Fig. S10



Conf: confidence of prediction

Pred: predicted secondary structure

AA: target amino acid sequence

β-strand

α-helix

Random coil

Fig. S11