SUPPORTING INFORMATION

Feedback regulation of *Caulobacter crescentus* holdfast synthesis by flagellum assembly via the holdfast inhibitor HfiA.

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TABLE S1: Bacterial strains and plasmids used in this study

Strain	Description or construction	Source or reference
***E. coli* strains**		
YB4026	S17-1 / pNPTS138Δ*flgE*	This study
YB4030	S17-1 / pNPTS138Δ*pilA*	This study
YB4648	S17-1 / pNPTS138Δ*motA*	This study
YB4029	S17-1 / pNPTS138Δ*motB*	This study
FC1268	TOP10 / pNPTS138Δ*flgH*	This study
UJ278	DH10B / pNPTS138*divK* Δ*pleD*	(Aldridge *et al.*, 2003)
YB5673	α Select / pNPST139 *fljK*T176C	This study
YB6566	α Select / pNPST139 *fljK*T103C	This study
***Caulobacter* strains**		
YB135 [1]	CB15 WT	(Poindexter, 1964)
YB4037	CB15 Δ*mot*B	This study
YB4800	CB15 Δ*mot*A	This study
YB4036	CB15 Δ*flg*E	This study
YB4251	CB15 Δ*hfaB*	(Hardy *et al.*, 2010)
FC1365	CB15 Δ*hfiA*	(Fiebig *et al.*, 2014)
YB5283	CB15 Δ*hfiA* Δ*flgE*	This study
YB4038	CB15 Δ*pilA*	This study
YB5086	CB15 Δ*pilA* Δ*flgE*	This study
YB2857	CB15 Δ*hfs*DAB	(Hardy *et al.*, 2010)
FC1365	CB15 Δ*hfiA*	(Fiebig *et al.*, 2014)
YB5284	CB15 Δ*hfiA* Δ*flgE*	This study
YB7356	CB15 Δ*pleD*	This study
YB7358	CB15 Δ*pleD* Δ*flgE*	This study
YB8944	CB15 WT pMT630	This study
YB8947	CB15 Δ*motB* pMT630	This study
YB8948	CB15 Δ*motB* pMT630-*motB*	This study
YB8949	CB15 Δ*pleD* pMT630	This study
YB8950	CB15 Δ*pleD* pMT630-*pleD*	This study
YB8951	CB15 WT pMR10	This study
YB8945	CB15 Δ*flgE* pMR10	This study
YB8946	CB15 Δ*flgE* pMR10-*flgE*	This study
FC1399	CB15 *xyl::*pMT680-*hfiA*	(Fiebig *et al.*, 2014)
YB7984	CB15 Δ*mot*B *xyl::*pMT680-*hfiA*	This study
YB7985	CB15 Δ*flg*E *xyl::*pMT680-*hfiA*	This study
YB8937	CB15 pMT335	This study
YB8938	CB15 Δ*flg*E pMT335	This study
YB8939	CB15 Δ*motB* pMT335	This study
YB8940	CB15 pBV-*PA5295*	This study
YB8941	CB15 Δ*flg*E pBV-*PA5295*	This study
YB8942	CB15 Δ*motB* pBV-*PA5295*	This study
UJ5100	CB15 cdG0	(Abel *et al.*, 2013)
UJ8732	CB15 rcdG0	(Sprecher *et al.*, 2017)
YB8243	CB15 / pRKlac290	This study
FC1949	CB15 / pRKlac290-P*hfiA* (pAF427)	(Fiebig *et al.*, 2014)
FC1951	CB15 *xyl::*pMT585-*lovR vanR::*pMT528-*lovK* / pRKlac290-P*hfiA*	(Fiebig *et al.*, 2014)
YB8820	CB15 Δ*mot*B / pRKlac290-P*hfiA*	This study

YB8819	CB15 Δ*flg*E / pRKlac290-P*hfiA*	This study
YB7986	CB15 Δ*ple*D / pRKlac290-P*hfiA*	This study
YB7987	CB15 Δ*ple*D Δ*flg*E / pRKlac290-P*hfiA*	This study
ACC147	CB15 Δ*flg*H / pRKlac290-P*hfiA*	This study
YB8952	CB15 pMT335 / pRKlac290-P*hfiA*	This study
YB8953	CB15 Δ*flgE* pBV-*PA5295* / pRKlac290-P*hfiA*	This study
YB8954	CB15 Δ*motB* pBV-*PA5295* / pRKlac290-P*hfiA*	This study
YB8955	CB15 rcdG0 / pRKlac290-P*hfiA*	This study
SC1131	CB15 *fliM*::Tn5	(Ely & Ely, 1989)
FC1266	CB15 Δ*flgH*	This study
FC764[1]	NA1000 *hfsA*$^+$ WT	(Marks *et al.*, 2010)
YB6375	NA1000 *hfsA*$^+$ Δ*flgE*	This study
YB7377	NA1000 *hfsA*$^+$ Δ*motB*	This study
YB8826	NA1000 *hfsA*$^+$ Δ*pleD*	This study
YB6562	NA1000 *hfsA*$^+$ FljKT103C	This study
YB6564	NA1000 *hfsA*$^+$ FljKT176C	This study
Plasmids		
pNPTS138	Litmus 38 derivative, *ori*T *sac*B Kan^r	M.R.K Alley
pNPTS139	Litmus 39 derivative, *ori*T *sac*B Kan^r	M.R.K Alley
pNPTS*mot*A	pNPTS138 containing 480 bp fragments upstream and downstream of *mot*A	This study
pNPTS*mot*B	pNPTS138 containing 480 bp fragments upstream and downstream of *mot*B	This study
pNPTS*flg*E	pNPTS138 containing 480 bp fragments upstream and downstream of *flg*E	This study
pNPTS*pil*A	pNPTS138 containing 480 bp fragments upstream and downstream of *pil*A	This study
pPA24	pNPTS138*divK* Δ*pleD*	(Aldridge & Jenal, 1999)
pNPTS*fljK*T103C	pNPTS139 containing 753-bp of *fljK* with a T to C point mutation at 103 bp from the start codon	This study
pNPTS*fljK*T176C	pNPTS139 containing 753-bp of *fljK* with a T to C point mutation at 176 bp from the start codon	This study
pMT630	Low copy replicating plasmid, vanillate inducible (Kn)	(Thanbichler *et al.*, 2007)
pMT630-*motB*	*motB* under control of the vanillate promoter in pMT630	This study
pMT630-*pleD*	*pleD* under control of the vanillate promoter in pMT630	This study
pMT335	High copy replicating plasmid, vanillate inducible (Gm)	(Thanbichler *et al.*, 2007)
pBV-*PA5295*	*PA5295* under control of the vanillate promoter in pMT335	(Duerig *et al.*, 2009)
pMR10	Mid copy replicating plasmid, IPTG inducible (constitutive in *C. crescentus*) (Kn)	(Roberts *et al.*, 1996)
pMR10-*flgE*	*flgE* under control of the *lac* promoter in pMR10	This study
pRKlac290	plasmid for *lacZ* transcriptional fusions (Tet)	(Gober & Shapiro, 1992)
pAF427	pRKlac290-P*hfiA*	(Fiebig *et al.*, 2014)
pMT680	Integrating plasmid at the xylose locus (Chlor)	(Thanbichler *et al.*, 2007)
pMT680-*hfiA*	*hfiA* under control of the xylose promoter in pMT680, integration at the xylose locus	(Fiebig *et al.*, 2014)

[1] These two strains and their Δ*flgE* and Δ*motB* derivatives have the same adhesion phenotypes.

TABLE S2: PCR primers used in this study

Primer	Sequence (5' – 3')	Function
Primers used for in-frame deletions		
5'motA_U -484	CAGGAACAGCAC**gcatgc**CACCATCGGCGG	5' region for deletion of *motA*
3'motA_U 24	AACGATGCCGAT**ctgca**GAACATAGTGTG	
5'motA_D 765	GCCCAAATCTCG**ctgcag**ATCGGTCGCGGC	3' region for deletion of *motA*
3'motA_D +378	GGCGGCCTCGGC**aagctt**GGCCGCTTCGGC	
5'motB_U -484	CGGCTTGGCCGC**gcatgc**CGGGCGCGTCGA	5' region for deletion of *motB*
3'motB_U 24	CGGCTAGTTCGC**ctgcag**CCGCCATCGTGCT	
5'motB_D 873	CCCGTACTGCCC**ctgcag**CTGTGACCGTTC	3' region for deletion of *motB*
3'motB_D +484	CTCGGCCATGCC**aagctt**GGCGTGGCGGGT	
5'flgE_U -486	CCAGCCCAGCGC**gcatgc**CTCGCTGAAGGT	5' region for deletion of *flgE*
3' flgE _U 24	GAGCATGGCGCT**ctgcag**GCTCATGACTGA	
5' flgE _D 1752	GAACTCTTGAAT**ctgcag**CGCTAATCATAG	3' region for deletion of *flgE*
3' flgE _D +454	AGGGCGGTCACA**aagctt**CACCCTACC	
FlgH UP F	a**gaattc**GTCTGGACCAACGCCAAC	5' region for deletion of *flgH*
FlgH UP R	cgagaacttcATAGCAGGACGACGCATGAT	
FlgH DN F	cctgctatGAAGTTCTCGCCCTTCTAGGC	3' region for deletion of *flgH*
FlgH DN R	a**GGATCC**GCTTGAGATCGCCTGACTTT	
5'pilA_U -486	GAACCGGCGAGG**gcatgc**TACGAGGCCTGA	5' region for deletion of *pilA*
3'pilA_U 24	GAAGCGCGTGAC**ctgcag**GGTCATGACTTG	
5'pilA_D 156	GTTTCGACGGCG**ctgcag**ACCTAAGCCACT	3' region for deletion of *pilA*
3'pilA_D +486	GTACAGCATGAA**aagctt**GGCCGCCGGCCA	
Primers used for complementation strains constructs		
flgE-3HindIII_F	ACCCTCGTCCCC**aagctt**TGTAGGAATTCA	Cloning of *flgE* in pMR10
flgE+3KpnI_R	AACTCAAGAATT**ggtacc**TGTTAAGCCCTA	
motB-9NdeI_F	GAAACG**catatg**GCGGTCAACAGCGAACAGC	Cloning of *motB* in pMT630
motB+18KpnI_R	CACGT**ggtacc**AGAACGGTCACAGGCCGTCG	
pleD-9NdeI_F	GAAACG**catatg**AGCGCCCGGATCCTCGTCG	Cloning of *pleD* in pMT630
pleD+18KpnI_R	CCACGT**ggtacc**CGCTGCTCAGGCGGCCTTG	
Primers used for cysteine knock-in strains construction		
fljK5'	GGCAC**gaattc**CGAGCTGACGACCGTTCAG	*fljK* sequencing
fljK3	CAGGCTCA**ggatcc**AGGACGAAGACTGGTTG	
fljKT176C+	GACGACCACCTCGtgcTTCACCACGGCCGC	*fljK* T176C point mutation
fljKT176C-	GCGGCCGTGGTGAAgcaCGAGGTGGTCGTC	
fljKT103C+	GGCCGCTTCCGACtgcTCGCTGAACACCGC	*fljK* T103C point mutation
fljKT103C-	GCGGTGTTCAGCGAgcaGTCGGAAGCGGCC	
M13 reverse	AACAGCTATGACCATG	pSKII plasmid sequencing
T7 promoter	TAATACGACTCACTATAGGG	

Uppercase matches genome sequence. Lower case bases are either restriction sites (bold), overlap with the opposite fragment, or point mutations in the sequence.

SUPPLEMENTARY MOVIE LEGEND

Movie S1. The FljK-cys mutation does not affect motility. Flagellar filaments were labeled using AF488-mal and visualized by fluorescence microscopy in real-time using a PDMS flow device. RAM capture imaging was used to capture cell swimming at a rate of 9.8 frames per second.

Movie S2. Comparison of rotating and non-rotating flagellar filaments in liquid medium. Flagellar filaments were labeled using AF488-mal and visualized by fluorescence microscopy in real-time using a PDMS flow device. RAM capture imaging was used to capture flagellum rotation at a rate of 5 frames per second. The white arrows indicate non-rotating flagella. The flagellum on the right of the screen is stationary for the duration of the video, while the flagellum on the left is rotating until the appearance of the white arrow.

Movie S3. Obstructed flagellum filament under a PYE agarose pad. Cells were imaged under PYE 0.8% agarose pads using phase contrast microscopy, and flagella and holdfasts were stained using AF488-mal and AF594-WGA, respectively. The first panel shows phase contrast of a predivisional cell, the second panel shows a flagellum and a holdfast labeled with AF488-mal, the third panel shows a holdfast labeled with AF594-WGA, and the fourth panel is an overlay of all three channels. Time-lapse images were taken every 2 min. The flagellum remains non-motile for the duration of the experiment. Cell division occurs at 14 min, and subsequent holdfast synthesis at 24 min, 10 min after cell division has occurred.

SUPPLEMENTARY REFERENCES

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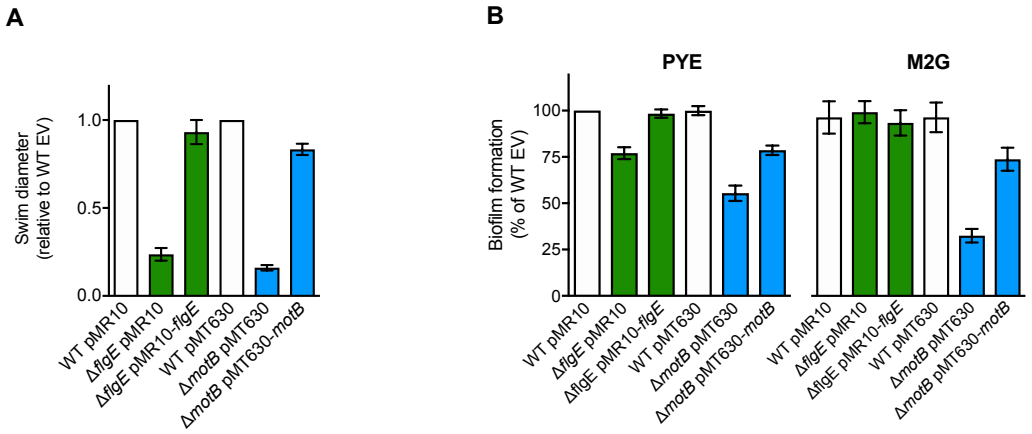

Fig. S1: (A) Motility in low percentage agar plates. 0.3% agar PYE plate containing 5 µg/ml kanamycin for pMR10 plasmids and 5 µg/ml kanamycin + 50 mM vanillate for pMT630 plasmids. Results are expressed as the swim diameter relative to CB15 WT empty vector. (B) Biofilm formation in 24-well plates (after 16 h incubation at 30˚C) by *C. crescentus* CB15 WT empty vector (pMR10 or pMT630), Δ*flgE,* and Δ*motB* mutant complemented strains in PYE and M2G 5 µg/ml kanamycin for pMR10 plasmids and 5 µg/ml kanamycin + 50 mM vanillate for pMT630 plasmids. Results are expressed as a percentage of WT empty vector biofilm formation. Error bars represent the SEM of at least three independent replicates.

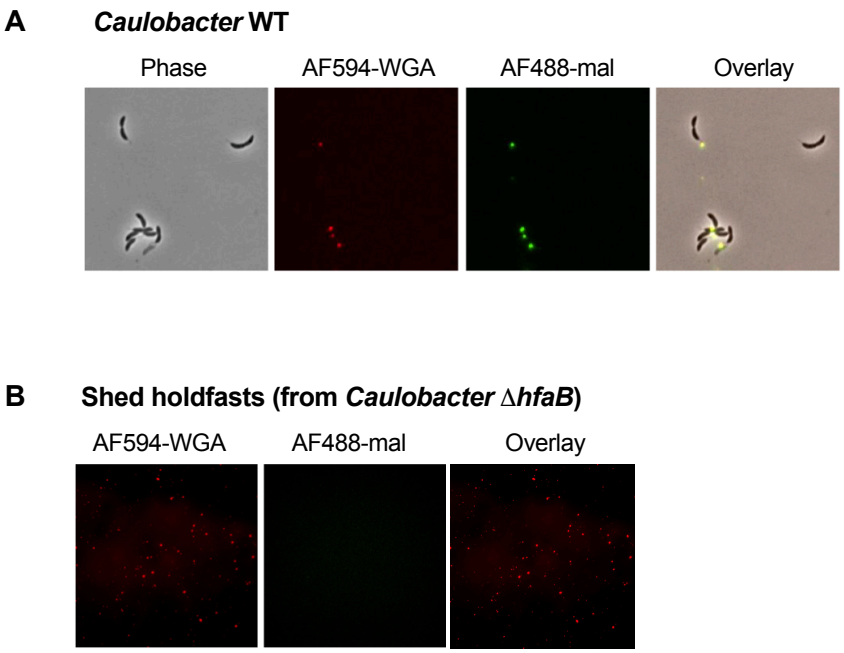

Fig. S2: Staining of *C. crescentus* WT (A, top panels) and purified holdfasts (B, bottom panels) using AF488-mal and AF594-WGA.

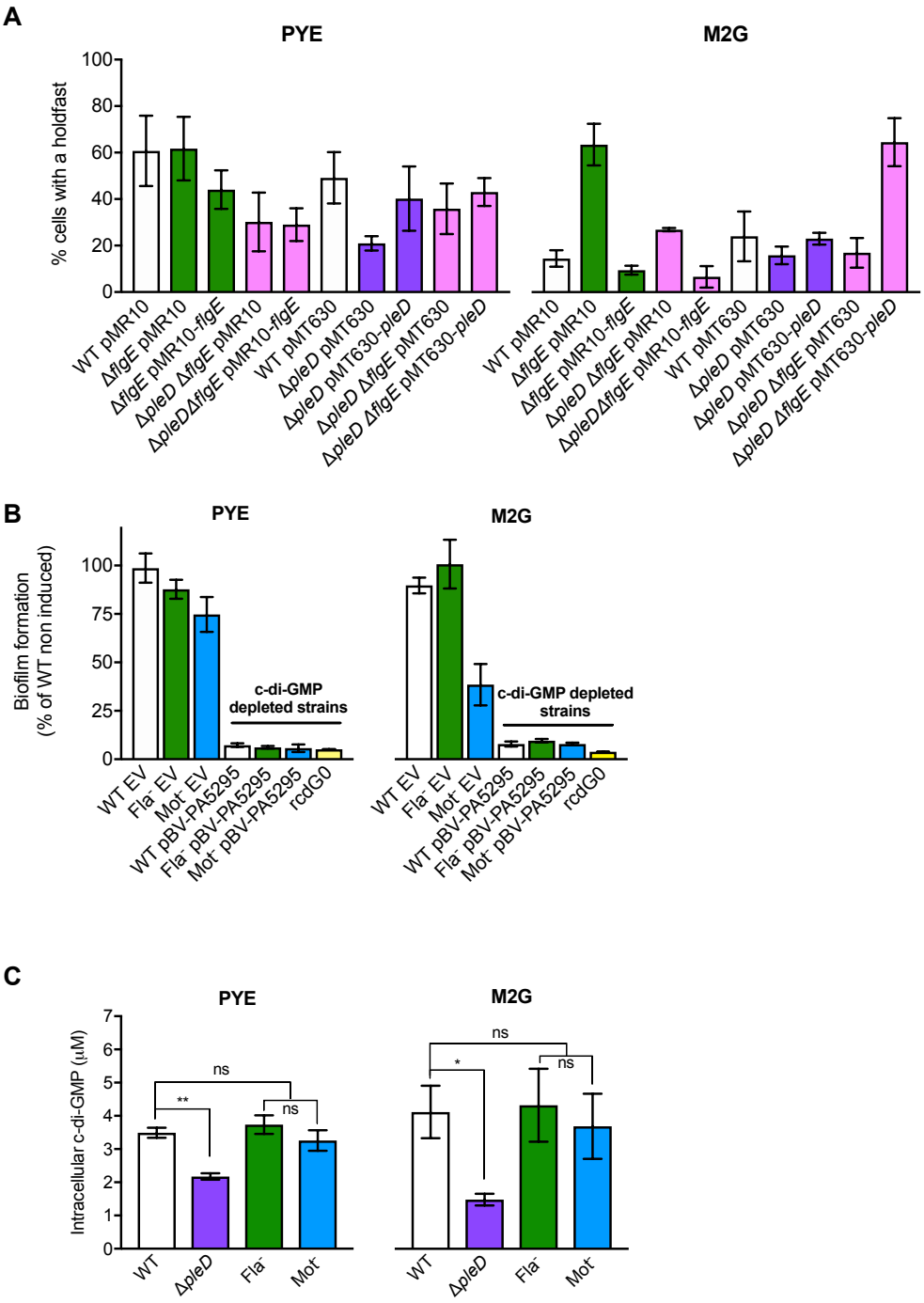

Fig. S3: (A) Quantification of cells harboring a holdfast in mixed populations grown to exponential phase. Cells are grown in PYE or M2G medium containing 5 µg/ml kanamycin. Results are expressed as the average of three independent replicates (more than 300 cells per replicate); the error bars represent the SEM. (B) Biofilm formation in 24-well plates by cells overexpressing a phosphodiesterase (PA5295 from *P. aeruginosa*). *C. crescentus* CB15 WT, Δ*flgE,* and Δ*motB* mutants harboring the pMT335 empty vector (EV) or the PDE overexpressing vector (pBV-PA5295) were grown in PYE (left) or M2G (right) containing 15 µg/ml gentamycin + 50 mM vanillate. Results are expressed as a percentage of WT empty vector biofilm formation. Error bars represent the SEM of three independent replicates. (C) Intracellular c-di-GMP quantification for the indicated strains and conditions. Error bars represent the SEM of at least 4 independent replicates. Statistical comparisons are calculated using Student's unpaired t-tests. ** $P < 0.01$; * $P < 0.1$; ns = not significant.

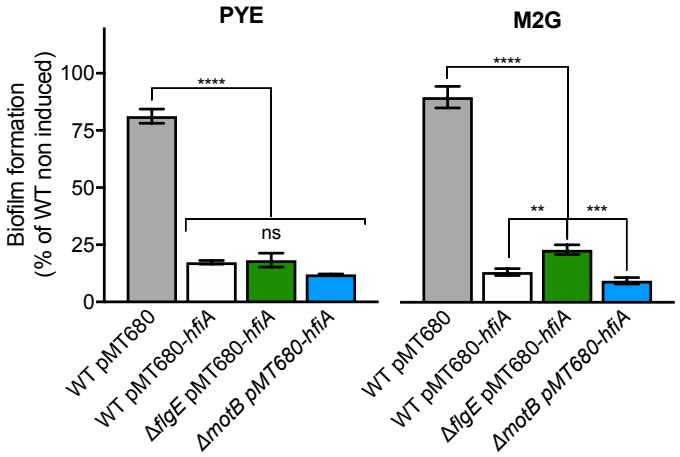

Fig. S4: Static biofilm formation in 24-well plates by *C. crescentus* CB15 WT, Δ*flgE* and Δ*motB* expressing *hfiA* ectopictly under the control of the *xylX* promoter. The results are expressed as the percentage of inhibition of the induced cultures (by addition of 0.3% xylose overnight) compared to the non-induced ones. The error bars represent the SEM of independent duplicates run in triplicates. Statistical comparisons are calculated using Student's unpaired t-tests. **** $P < 0.0001$; *** $P < 0.001$; ** $P < 0.01$; ns = not significant.