Biophysical Journal, Volume *119*

Supplemental Information

AimB Is a Small Protein Regulator of Cell Size and MreB Assembly

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Supplementary methods

Strain construction

Xylose-inducible expression of conserved hypothetical proteins. Cloning of the entry-vector set of *C. crescentus* open reading frames (ORFs) was described previously (1). Plasmid pZG241, which contains a xylose-inducible promoter upstream of a Gateway cloning cassette, was used as the destination vector (2). The 224 ORFs corresponding to conserved hypothetical proteins were inserted into pZG241 using a previously described *in vivo* LR reaction (1, 3).

Chromosomal expression of *mreB-GFP*^{SW}. The gene encoding msfGFP was cloned at the site of a loop-domain of MreB using Gibson assembly. The pNPTS-138 plasmid backbone was PCR amplified using primers pNPTS-F/R, the *mreB* upstream and downstream chromosomal DNA fragments were amplified using primers MreB-up/down-F/R, and *msfGFP* was amplified using msfGFP-F/R. The four fragments were joined using Gibson assembly. The resulting plasmid (pZG1534) was transformed into *C. crescentus*. Individual colonies were grown overnight in PYE without antibiotic selection and streaked onto PYE-3% sucrose plates to select for bacteria that lost the *sacB* cassette. Individual colonies were screened for MreB fluorescence, resulting in strain ZG1511.

Chromosomal expression of *aimB-FLAG*. The *aimB* upstream and downstream chromosomal DNA fragments were amplified using primers EK582/1150 and EK585/1151, respectively, and cloned into the HindIII/EcoRI site of pNPTS-138. The resulting plasmid (pEK391) was transformed into *C. crescentus*. Individual colonies were grown overnight in PYE without

antibiotic selection and streaked onto PYE-3% sucrose plates to select for bacteria that lost the *sacB* cassette. Individual colonies were screened by PCR using primers EKS212/S260, resulting in strain EK399.

AimB overexpression strains. To generate *C. crescentus* AimB overexpression strains, the *aimB* gene was amplified using primers aimB-F/R and inserted into the NdeI/EcoRI site of pBXMCS-2 (plasmid pZG825). A FLAG-tag was added to the C-terminus of AimB by inverse-PCR with primers aimB-FLAG-F/R (plasmid pZG826). These plasmids were electroporated into NA1000 or *mreB::mreB-GFP*^{SW} to generate strains ZG870, ZG871, and EK25. To generate low-copy *E. coli* AimB overexpression strains, *aimB-FLAG* was PCR-amplified from pZG826 using primers EK809/810 (Ptet) or EK813/814 (Plac). Plasmid backbones were amplified with primers EK807/808 (pBbS2k, Ptet) or EK811/812 (pTrc99a, Plac). The resulting PCR fragments were ligated by Gibson assembly, yielding plasmids pEK188 (Plac) and pEK189 (Ptet); the plasmids were transformed into ZG1516 (*mreB::mreB-GFP*^{SW}) to generate strains EK191 and EK192. For high-copy expression, Ptet-*aimB-FLAG* was amplified from pEK189 using primers EK840/841 and plasmid pUC19 was amplified with primers EK838/839. The PCR products were ligated by Gibson assembly and the resulting plasmid (pEK199) was transformed into ZG1516 to yield strain EK200. High-copy expression of Jann_2546-FLAG was achieved by PCR-amplifying Jann_2546-FLAG from plasmid EK796 using primers EK1175/1176 and ligating the PCR product into the NdeI/EcoRI site of pBXMCS-2. The resulting plasmid (EK397) was transformed into NA1000 to yield strain EK398.

Supplemental References

1. Werner, J. N., E. Y. Chen, J. M. Guberman, A. R. Zippilli, J. J. Irgon, and Z. Gitai. 2009. Quantitative genome-scale analysis of protein localization in an asymmetric bacterium. PNAS 106(19):7858-7863.
2. Ingerson-Mahar, M., A. Briegel, J. N. Werner, G. J. Jensen, and Z. Gitai. 2010. The metabolic enzyme CTP synthase forms cytoskeletal filaments. Nat Cell Biol 12(8):739-746.
3. Werner, J. N., and Z. Gitai. 2010. High-throughput screening of bacterial protein localization. Methods Enzymol 471:185-204.
4. Evinger, M., and N. Agabian. 1977. Envelope-associated nucleoid from *Caulobacter crescentus* stalked and swarmer cells. J Bacteriol 132(1):294-301.
5. Irnov, I., Z. Wang, N. D. Jannetty, J. A. Bustamante, K. Y. Rhee, and C. Jacobs-Wagner. 2017. Crosstalk between the tricarboxylic acid cycle and peptidoglycan synthesis in *Caulobacter crescentus* through the homeostatic control of alpha-ketoglutarate. PLoS Genet 13(8):e1006978.
6. Dye, N. A., Z. Pincus, I. C. Fisher, L. Shapiro, and J. A. Theriot. 2011. Mutations in the nucleotide binding pocket of MreB can alter cell curvature and polar morphology in *Caulobacter*. Mol Microbiol 81(2):368-394.
7. Simon, R., U. Priefer, and A. Pühler. 1983. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in Gram negative bacteria. Bio/Technology 1(9):784-791.

8. Ursell, T. S., J. Nguyen, R. D. Monds, A. Colavin, G. Billings, N. Ouzounov, Z. Gitai, J. W. Shaevitz, and K. C. Huang. 2014. Rod-like bacterial shape is maintained by feedback between cell curvature and cytoskeletal localization. PNAS 111(11):E1025-1034.

9. Thanbichler, M., A. A. Iniesta, and L. Shapiro. 2007. A comprehensive set of plasmids for vanillate- and xylose-inducible gene expression in *Caulobacter crescentus*. Nucleic Acids Res 35(20):e137.

10. Amann, E., B. Ochs, and K. J. Abel. 1988. Tightly regulated tac promoter vectors useful for the expression of unfused and fused proteins in *Escherichia coli*. Gene 69(2):301-315.

11. Lee, T. S., R. A. Krupa, F. Zhang, M. Hajimorad, W. J. Holtz, N. Prasad, S. K. Lee, and J. D. Keasling. 2011. BglBrick vectors and datasheets: A synthetic biology platform for gene expression. J Biol Eng 5:12.

12. Norrander, J., T. Kempe, and J. Messing. 1983. Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene 26(1):101-106.

13. Chin, J. W., A. B. Martin, D. S. King, L. Wang, and P. G. Schultz. 2002. Addition of a photocrosslinking amino acid to the genetic code of *Escherichia coli*. Proc Natl Acad Sci U S A 99(17):11020-11024.

14. Cox, R. S., 3rd, M. J. Dunlop, and M. B. Elowitz. 2010. A synthetic three-color scaffold for monitoring genetic regulation and noise. J Biol Eng 4:10.

15. Madeira, F., Y. M. Park, J. Lee, N. Buso, T. Gur, N. Madhusoodanan, P. Basutkar, A. R. N. Tivey, S. C. Potter, R. D. Finn, and R. Lopez. 2019. The EMBL-EBI search and sequence analysis tools APIs in 2019. Nucleic Acids Res.

89 **Table S1: Strains used in this study.**

90

C. crescentus			
Strain	**Genotype**	**Construction**	**Source**
NA1000	Synchronizable variant of wild-type *C. crescentus* strain CB15		(4)
ZG949	Pxyl-*aimB* (Gateway)	*In vivo* Gateway cloning of conserved hypothetical genes	This study
ZG1511	*mreB::mreB-GFP*^{SW}	Transformation of NA1000 with pZG1534 followed by sucrose selection	This study
ZG870	pBXMCS-Pxyl-*aimB*	Transformation of NA1000 with pZG825	This study
ZG871	pBXMCS-Pxyl-*aimB*-FLAG	Transformation of NA1000 with pZG826	This study
EK25	*mreB::mreB-GFP*^{SW}; pBXMCS-Pxyl-*aimB*-FLAG	Transformation of ZG1511 with pZG826	This study
ZG883	pBXMCS-2	Transformation of NA1000 with pBXMCS-2	This study

CJW5939	Δ*vanA::*pV-dcas9hum-RBSmut1 with plasmid psgRNA-base		(5)
EK335	Δ*vanA::*pV-dcas9hum-RBSmut1 with plasmid psgRNA-*aimB*	Transformation of CJW6270 with pEK334	This study
CJW6270	Δ*vanA::*pV-dcas9hum-RBSmut1		(5)
JAT684	*PmreB::T167A-mreB*		(6)
JAT669	*PmreB::L23P-mreB*		(6)
JAT692	*PmreB::D192G-mreB*		(6)
JAT699	*PmreB::V324A-mreB*		(6)
ZG896	*PmreB::L23P-mreB*; pBXMCS-AimB	Transformation of JAT669 with pZG825	This study
ZG897	*PmreB::T167A-mreB*; pBXMCS-AimB	Transformation of JAT684 with pZG825	This study
ZG898	*PmreB::D192G-mreB*; pBXMCS-AimB	Transformation of JAT692 with pZG825	This study
ZG899	*PmreB::V324A-mreB*; pBXMCS-AimB	Transformation of JAT699 with pZG825	This study
ZG917	*PmreB::A171V-mreB*	AimB-overexpression suppressor screen	This study

ZG918	*PmreB::K236T-mreB*	AimB-overexpression suppressor screen	This study
ZG920	*PmreB::T277A-mreB*	AimB-overexpression suppressor screen	This study
ZG921	*PmreB::I290M-mreB*	AimB-overexpression suppressor screen	This study
ZG922	*PmreB::M74I-mreB*	AimB-overexpression suppressor screen	This study
ZG923	*PmreB::A20G-mreB*	AimB-overexpression suppressor screen	This study
ZG924	*PmreB::N21G-mreB*	AimB-overexpression suppressor screen	This study
ZG925	*PmreB::V170A-mreB*	AimB-overexpression suppressor screen	This study
ZG926	*PmreB::T168A-mreB*	AimB-overexpression suppressor screen	This study
ZG928	*PmreB::A325T-mreB*	AimB-overexpression suppressor screen	This study
EK398	pBXMCS-*jann_2546-FLAG*	Transformation of NA1000 with pEK397	This study
EK399	*aimB::aimB-FLAG*	Transformation of NA1000 with pEK391 followed by sucrose selection	This study

E. coli			
Strain	**Genotype**	**Construction**	**Source**
S17-1	λ–pir cloning strain, $Spec^R$		(7)
XL1-Blue	Cloning strain, Tet^R		Agilent Technologies
ZG1516	*mreB::mreB-GFP*^{SW}		(8)
EK191	*mreB::mreB-GFP*^{SW}; Plac-*aimB*-FLAG	Transformation of ZG1516 with pEK188	This study
EK192	*mreB::mreB-GFP*^{SW}; Ptet-*aimB*-FLAG (low copy)	Transformation of ZG1516 with pEK189	This study
EK200	*mreB::mreB-GFP*^{SW}; Ptet-*aimB*-FLAG (high copy)	Transformation of ZG1516 with pEK199	This study
NO36	Δ*mreB* (MC4100)		Lab collection
EK85	Δ*mreB*; pEVOL-pBpF	Transformation of NO36 with pEVOL-pBpF	This study
MreBXL-con	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*/Para-*aimB*	Transformation of EK85 with pMreBXL-con	This study
MreBXL-1	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{K58}/Para-*aimB*	Transformation of EK85 with pMreBXL-1	This study
MreBXL-2	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{L61}/Para-*aimB*	Transformation of EK85 with pMreBXL-2	This study

MreBXL-3	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{G62}/Para-*aimB*	Transformation of EK85 with pMreBXL-3	This study
MreBXL-4	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{P65}/Para-*aimB*	Transformation of EK85 with pMreBXL-4	This study
MreBXL-5	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{E69}/Para-*aimB*	Transformation of EK85 with pMreBXL-5	This study
MreBXL-6	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{A70}/Para-*aimB*	Transformation of EK85 with pMreBXL-6	This study
MreBXL-7	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{R75}/Para-*aimB*	Transformation of EK85 with pMreBXL-7	This study
MreBXL-8	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{E83}/Para-*aimB*	Transformation of EK85 with pMreBXL-8	This study
MreBXL-9	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{F102}/Para-*aimB*	Transformation of EK85 with pMreBXL-9	This study
MreBXL-10	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{T116}/Para-*aimB*	Transformation of EK85 with pMreBXL-10	This study
MreBXL-11	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{G149}/Para-*aimB*	Transformation of EK85 with pMreBXL-11	This study
MreBXL-12	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{G166}/Para-*aimB*	Transformation of EK85 with pMreBXL-12	This study
MreBXL-13	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{T167}/Para-*aimB*	Transformation of EK85 with pMreBXL-13	This study

MreBXL-14	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{R185}/Para-*aimB*	Transformation of EK85 with pMreBXL-14	This study
MreBXL-15	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{E193}/Para-*aimB*	Transformation of EK85 with pMreBXL-15	This study
MreBXL-16	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{I196}/Para-*aimB*	Transformation of EK85 with pMreBXL-16	This study
MreBXL-17	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{R200}/Para-*aimB*	Transformation of EK85 with pMreBXL-17	This study
MreBXL-18	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{H202}/Para-*aimB*	Transformation of EK85 with pMreBXL-18	This study
MreBXL-19	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{E209}/Para-*aimB*	Transformation of EK85 with pMreBXL-19	This study
MreBXL-20	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{K217}/Para-*aimB*	Transformation of EK85 with pMreBXL-20	This study
MreBXL-21	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{L240}/Para-*aimB*	Transformation of EK85 with pMreBXL-21	This study
MreBXL-22	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{A260}/Para-*aimB*	Transformation of EK85 with pMreBXL-22	This study
MreBXL-23	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{A276}/Para-*aimB*	Transformation of EK85 with pMreBXL-23	This study
MreBXL-24	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{T277}/Para-*aimB*	Transformation of EK85 with pMreBXL-24	This study

MreBXL-25	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{D287}/Para-*aimB*	Transformation of EK85 with pMreBXL-25	This study
MreBXL-26	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{K339}/Para-*aimB*	Transformation of EK85 with pMreBXL-26	This study
EK210	Δ*mreB*; pEVOL-pBpF; Plac-*mreB*^{R185}/Para-*aimB*-FLAG	Transformation of EK85 with pMreBXL-14-FLAG	This study

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92 **Table S2: Plasmids used in this study.**

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Name	Description	Source
pZG241	Gateway destination vector based on pJS14 modified to include a xylose-inducible promoter, Tet^R	(2)
pNPTS-138	*sacB* expressing plasmid used for allelic exchange in *C. crescentus*, Kan^R	M.R.K. Alley, unpublished
pZG1534	pNPTS-138 based plasmid for replacing the chromosomal *C. crescentus mreB* with *mreB-GFP*^{SW}, Kan^R	This study
pBXMCS-2	*C. crescentus* high-copy plasmid with a xylose-inducible promoter, Kan^R	(9)
pZG825	pBXMCS-2-based plasmid for *aimB* expression, Kan^R	This study
pZG826	pBXMCS-2-based plasmid for *aimB-FLAG* expression, Kan^R	This study
psgRNA-Base	sgRNA cloning vector for CRISPRi, Kan^R	(5)
pEK334	*aimB* sgRNA expression plasmid, Kan^R	This study
pTrc99a	Ptac containing expression vector used for IPTG induced overexpression in *E. coli*, $Carb^R$	(10)
pEK188	pTrc99a-based plasmid for *aimB*-FLAG expression, $Carb^R$	This study

pBbS2k	Ptet containing low-copy expression vector used for anhydro-tetracycline induced overexpression in *E. coli*, Kan^R	(11)
pEK189	pBbS2k-based plasmid for *aimB*-FLAG expression, Kan^R	This study
pUC19	Plac containing high-copy expression vector used for IPTG induced overexpression in *E. coli*, $Carb^R$	(12)
pEK199	pUC19-based plasmid with Plac replaced with Ptet-*aimB*-FLAG, $Carb^R$	This study
pEVOL-pBpF	Plasmid encoding a tRNA synthetase/tRNA pair for *in vivo* incorporation of p-benzoyl-l-phenylalanine in *E. coli* amber codons (Addgene #31190), Cm^R	(13)
pZS2-123	Cloning plasmid with 3 promoters to drive independent expression of CFP, YFP, and mCherry (Addgene #26598), Kan^R	(14)
pEK70	pZS2-123 derived plasmid with CFP cassette removed and mCherry replaced with *aimB*, Kan^R	This study
pMreBXL series	pEK70-derived plasmids with YFP replaced by *mreB* containing the respective amber codon mutation; *mreB* mutants synthesized by Genscript (Table S1), Kan^R	This study
pMreBXL-14-FLAG	pMreBXL-14-based plasmid with an AimB C-terminal FLAG tag, Kan^R	This study

pEK796	Codon optimized *jann_2546-FLAG* cloned into pUC57; synthesized by Genscript, $Carb^R$	This study
pEK397	pBXMCS-2 derived plasmid for xylose-inducible expression of Jann_2546-FLAG, Kan^R	This study
pEK391	pNPTS-138 based plasmid for replacing the chromosomal *C. crescentus aimB* with *aimB-FLAG*, Kan^R	This study

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Table S3: Primers used in this study.

Name	Sequence
Cloning primers	
pNPTS-F	CTCTGCAGGATATCTGGATC
pNPTS-R	CTAGTGAGTCGTATTACGTAG
mreB-up-F	cgtaatacgactcactagTGTTCAAGGAACGCCTGACCCCTTTGCAGGTGGTC
mreB-up-R	gagccagaGCCGTCGGCCGGCGCGCG
msfGFP-F	cgacggcTCTGGCTCGAGCAGTAAAGGTGAAGAAC
msfGFP-R	gaccttcGCCCGGCGCGCCAGATTT
mreB-down-F	cgccgggcGAAGGTCTGTCGATCGACG
mreB-down-R	ccagatatcctgcagagAAGCTTGGGATTGGGCCC
aimB-F	tactcatATGACCACCTTCGACGAACG
aimB-R	tactgaattcTTACTCAGACTTGATCTGCTCGCG
aimB-FLAG-F	gacgacgacaagTAAGCGCTCGGAGCGTCG
aimB-FLAG-R	gtccttgtagtcCTCAGACTTGATCTGCTCGCG
EK582	tactaagcttGTACTCGCTGATCCGGTTGT
EK585	tactgaattcGGACAGTACTACGGCCATCC
EK644	TAGAACTTCCGAGAAGTTCA
EK645	GGTGGTTTGTTTGCCGGATC
EK646	TAATAACCAGGCATCAAATAAAACGAAAGGC
EK647	GGCAGGTGCTCCTTCTTAAAGTT
EK648	tatttgatgcctggttattattaCTCAGACTTGATCTGCTCGC
EK649	agaaggagcacctgccatgACCACCTTCGACGAACG
EK679	TAATAATAACCAGGCATCAAATAAAACGAAAGG
EK680	cttgtcatcgtcatccttgtagtcCTCAGACTTGATCTGCTCGCG
EK807	ATGTATATCTCCTTCTTAAAAGATCTTTTGAATTCTTTTC

EK808	GGATCCAAACTCGAGTAAG
EK809	tttaagaaggagatatacatATGACCACCTTCGACGAAC
EK810	actcgagtttggatccTTACTTGTCGTCGTCGTC
EK811	GGTCTGTTTCCTGTGTGAAATTG
EK812	GGATCCTCTAGAGTCGACC
EK813	acacaggaaacagaccATGACCACCTTCGACGAAC
EK814	cgactctagaggatccTTACTTGTCGTCGTCGTC
EK838	CGAGCTCGAATTCACTGGCC
EK839	CCTGCAGGCATGCAAGCTTG
EK840	agtgaattcgagctcgTTAAGACCCACTTTCACATTTAAG
EK841	ttgcatgcctgcaggTATAAACGCAGAAAGGCC
EK1003	tagtgGCGTTCGTCGAAGGTGGTCA
EK1004	aaacTGACCACCTTCGACGAACGCc
EK1150	gtcgtcgtcGTCCTTGTAGTCCTCAGACTTGATCTGCTCGCGG
EK1151	tacaaggacGACGACGACAAGTAAGCGCTCGGAGCGTCG
EK1175	tactcatATGAGCACCTTCGACGACCG
EK1176	tactgaattcTTACTTGTCGTCGTCGTCCTTGT
EKS212	CGGATGAAGTGGTTCTGGAC
EKS260	TTACTTGTCGTCGTCGTCCTTGTAG

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QPCR primers	*Forward*	*Reverse*
rpoD	CTCTATGCGATCAACAAGCG	ATAGGCCTTGAGGAACTCGC
aimB	ACGTGCTGCGCAAGGTCT	CCAGCAGCTCGGCCATTT

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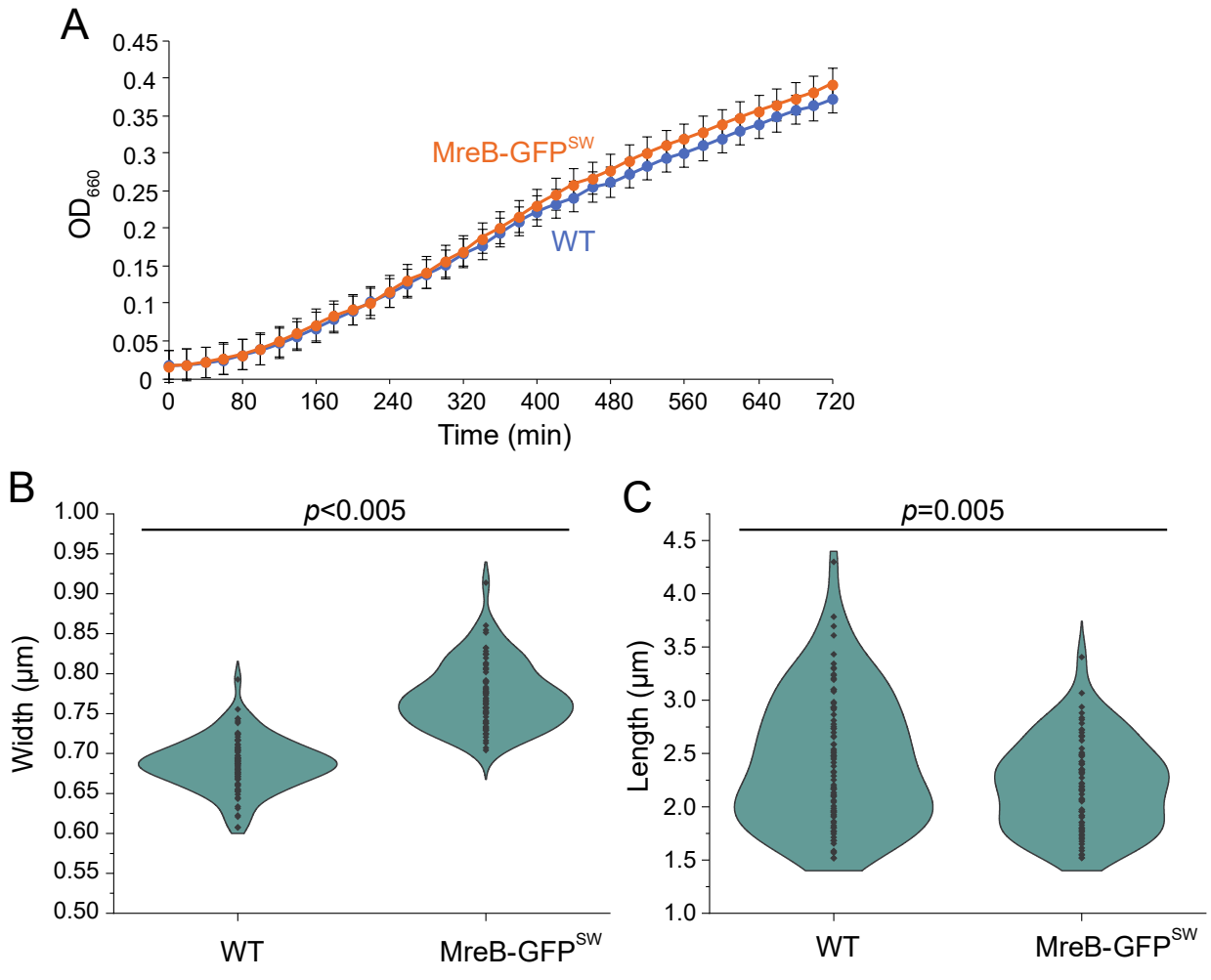
Table S4: MD simulation systems in this study.

Name	Structure source	Ligand	Atoms	Simulation time (ns)	Replicates
*Cc*MreB	PDB ID: 4CZM	ATP, Mg^{2+}	84,000	100	1
*Ec*MreB	PDB ID: 4CZM (homology model)	ATP, Mg^{2+}	84,000	100	1
*Cc*MreB - AimB	PDB ID: 4CZM; PDB ID: 2KZC (homology model)	ATP, Mg^{2+}, AimB	89,000	100	2
*Ec*MreB - AimB	PDB ID: 4CZM (homology model); PDB ID: 2KZC (homology model)	ATP, Mg^{2+}, AimB	89,000	100	2
*Cc*MreB - Jann_2546	PDB ID: 4CZM; PDB ID: 2KZC	ATP, Mg^{2+}, Jann_2546	89,000	100	2

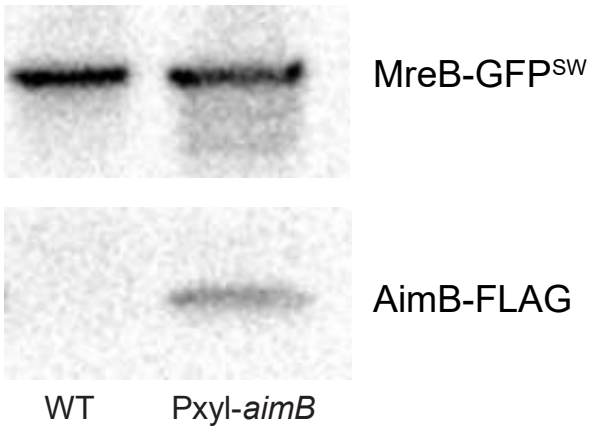
Supplemental Figures



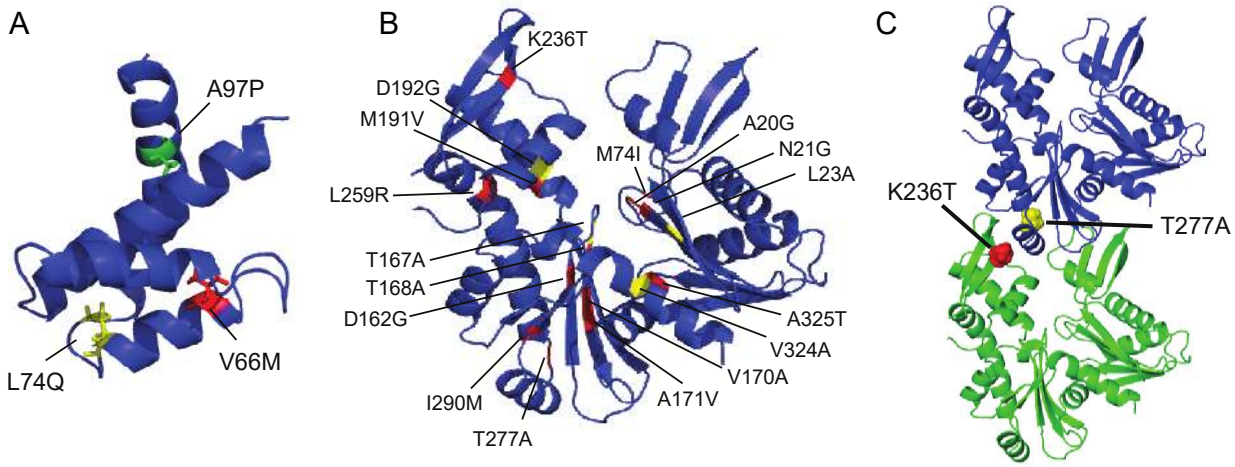
Supplemental Figure 1: MreB-GFP^{SW} minimally perturbs *C. crescentus* growth and shape.

A) The growth of wild-type and MreB-GFP^{SW} cells as measured by optical density was virtually identical. Error bars are the standard error of the mean (n=3).

B,C) Wild-type and MreB-GFP^{SW} cells were imaged by phase microscopy for quantification of cell width (B) and length (C) (n>70 cells per strain; p-values: two-tailed t-test). The MreB sandwich fusion caused a small increase in cell width.

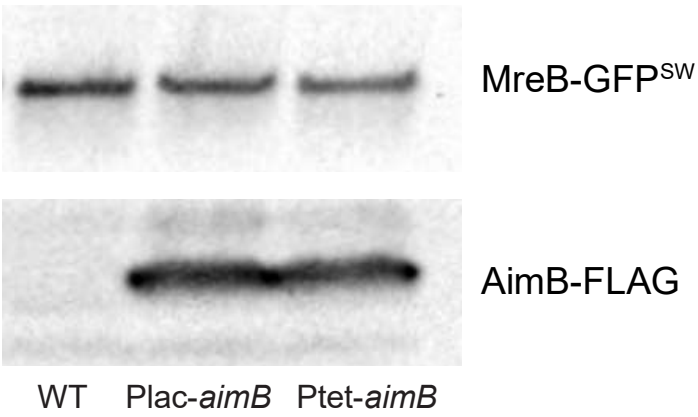


Supplemental Figure 2: Relative expression levels of MreB and AimB in *C. crescentus*. AimB-FLAG and MreB-GFP^{SW} expression were assayed by immunoblotting. Samples were normalized by OD. AimB-FLAG expression was induced in *C. crescentus* with 0.3% xylose for 9 h. Cultures were back-diluted 1:100 after 4.5 h to keep cells in log-phase.



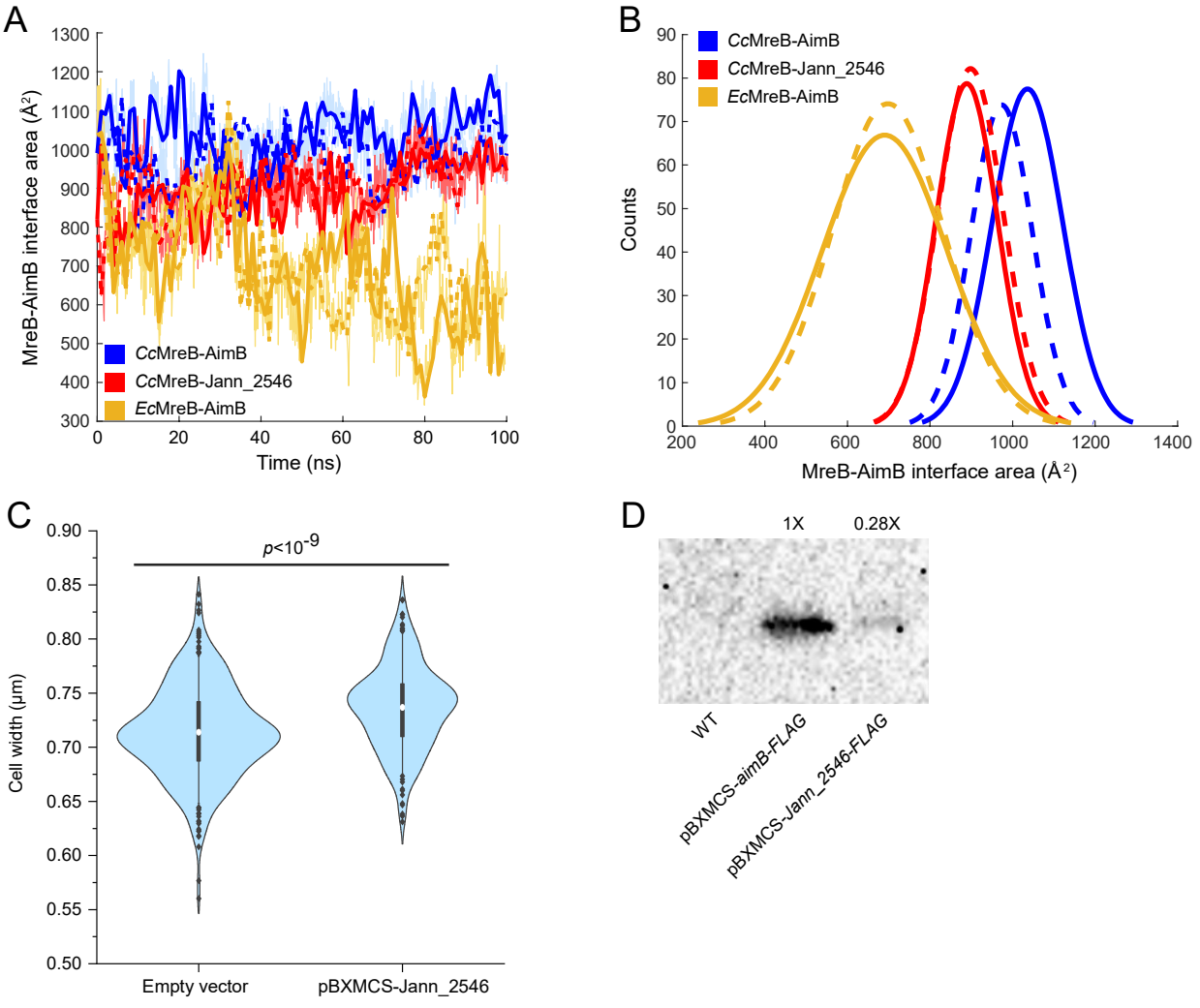
Supplemental Figure 3: Identification of AimB-overexpression suppressor mutants.

(A-C) The locations of the identified AimB-overexpression suppressor mutants are mapped to structures for AimB (A), MreB (B), and the MreB longitudinal polymerization interface (C). In (C), two interacting MreB monomers are colored by dark blue (top) and green (bottom), respectively.



Supplemental Figure 4: Relative expression levels of MreB and AimB in *E. coli*.

AimB-FLAG and MreB-GFP^{SW} expression were assayed by immunoblotting. Samples were normalized by OD. AimB-FLAG expression was induced in *E. coli* with 1 mM IPTG (Plac) or 100 ng/mL aTc (Ptet) for 9 h. Cultures were back-diluted 1:100 after 4.5 h to keep cells in log-phase.



Supplemental Figure 5: Justification of the AimB homology model.

A) The interfacial area between MreB and AimB or Jann_2546 showed that the docked heterodimer of *Cc*MreB and either AimB homologue remained stable throughout 100 ns of MD simulation, while the interfacial area of AimB docking to *Ec*MreB decreased over time.

B) The distribution of interfacial areas over the course of the molecular dynamics simulations in (A) demonstrated that Jann_2546 interacts with *Cc*MreB in a manner similar to AimB.

C) Overexpression of Jann_2546 for 24 h resulted in a significant increase (4%) in cell width compared to cells harboring the empty expression plasmid (*n*>230 cells per strain; *p*-values, two-tailed t-test). White circles represent the mean of each sample.

D) AimB and Jann_2546 were induced from the same plasmid backbone with 0.3% xylose for 8 h and assessed by Western blot. Image quantification indicated ~70% lower expression of Jann_2546.

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145 **Supplemental Figure 6: Comparison of AimB binding cleft in *C. crescentus* and *E. coli*.**

146 MreB residues that approach within 5 Å of AimB in our molecular dynamics

147 simulations (Figure 4F) are highlighted in orange. The protein sequences of *Cc*MreB

148 and *Ec*MreB were aligned using Clustal Omega (15) and the interacting residues are

149 highlighted.

Supplemental Movies

Movie S1: Molecular dynamics simulation of *Cc*MreB-AimB interactions. The interactions between AimB and *Cc*MreB were simulated over 100 ns using molecular dynamics (see Methods). AimB remained stably associated with *Cc*MreB.

Movie S2: Molecular dynamics simulation of *Ec*MreB-AimB interactions. The interactions between AimB and *Ec*MreB were simulated over 100 ns using molecular dynamics (see Methods). AimB did not remain stably associated with *Ec*MreB.