**The small protein MbiA interacts with MreB and modulates cell shape in *Caulobacter crescentus***

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# Supplemental Information

## Strain and Plasmid Construction

Strains

- ZG472: pVGN-mreB was integrated into CB15N chromosome.
- ZG498: pVGN-mreB was integrated into LS4275 chromosome.
- ZG690: pVRN-mbiA was integrated into CB15N chromosome.
- ZG691: *Pvan::mCherry-mbiA* was transduced from ZG690 into MT114, selecting for $Tet^R$.
- ZG692: pVRN-mbiA was integrated into LS3814.
- ZG693: pVRN-mbiA was integrated into YB1585.
- ZG694: pmbiA-FLAG was integrated into CB15N.
- ZG695: *Pvan::ftsZ-CFP* was transduced from EG056 into ZG733, selecting for $Strep/Spec^R$.
- ZG696: *mreBG311A* mutation was isolated in the screen for MbiA-resistant mutants. pNPTS-mreB was then integrated near the *mreB* locus of the original mutant, using its $Kan^R$ marker to transduce the mutation into CB15N. pNPTS-mreB insert was cured out of the strain by counterselection for sucrose resistance and screen for $Kan^S$ colonies. The resulting transductants were confirmed to contain G311A mutation by PCR and sequencing.
- ZG697: *mreBR307C* mutation was also isolated in the screen for MbiA-resistant mutants, and transductants of R307C into a clean genetic background were obtained with the same method as described above for ZG696.
- ZG698 and ZG699: pXGFP4-mreBG311A and pXGFP4-mreBR307C plasmids were integrated into ZG696 and ZG697, respectively.
- ZG700 and ZG701: *Pvan::mCherry-mbiA* was transduced from ZG690 into ZG698 and ZG699, respectively.
- ZG702: pXCFP-RodZ was integrated into CB15N chromosome.

- ZG703: *Pvan::ftsZ-yfp* was transduced from MT196 into ZG702, selecting for Kan$^R$.

- ZG704 and ZG705: first, pXCFP-RodZ was integrated into ZG696 and ZG697, respectively, and then *Pvan::ftsZ-yfp* from MT196 was transduced into the resulting integrants.

- ZG706: *rodZ::Himar1* was transduced from CJW2537 into ZG472, selecting for Kan$^R$.

- ZG707: pXRN-mreBQ26P was integrated into the chromosome of CJW1715.

- ZG708: pNPTS-mbiA was integrated into CB15N chromosome followed by counterselection for sucrose resistance and a screen for the loss of Kan$^R$. The resulting Suc$^R$ Kan$^S$ colonies were then screened by PCR for *mbiA* deletion.

- ZG709: pXGFP4-mreB was integrated into ZG708.

- ZG710: *rodZ::Himar1* was transduced from CJW2537 into ZG708, selecting for Kan$^R$.

- ZG951: pmbiA-mCherry was integrated into CB15N.

- ZG952: pXGFP4-mreB was integrated into ZG951.

- ZG953: pmbiA-FLAGx2 was integrated into CB15N.

- ZG954: mbiA-FLAGx2 was transduced from ZG953 into ZG956, selecting for Kan$^R$.

- ZG955: pmbiA-FLAGx2 was integrated into ZG697.

- ZG956 and ZG957: pNPTS-mreBG311A and pNPTS-mreBR307C, respectively, were integrated into CB15N chromosome followed by counterselection for sucrose resistance and a screen for the loss of Kan$^R$. The resulting Suc$^R$ Kan$^S$ colonies were subjected to PCR with *mreB*-specific primers, and the PCR products were sequenced to screen for the presence of the desired mutations.

Plasmids

- pBV-mbiA: *mbiA* PCR-amplified from CB15N with NdeI-mbiA F and SacI-mbiA R primers was cloned into pBVMCS-4 via NdeI and SacI sites.
- pBV-mbiA-mCherry: *mbiA-mCherry* PCR-ed from ZG732 with NdeI-mbiA F and SpeI-gfp R primers was ligated into pBVMCS-4 via NdeI and SpeI sites.
- pBV-mbiA-FLAG: *mbiA* PCR-ed from CB15N with NdeI-mbiA F and SacI-FLAG-mbiA R primers was ligated into pBVMCS-4 via NdeI and SacI sites.
- pBV-mbiAΔSE-mCherry and pBV-mbiAY71N-mCherry: the mutant versions of the pBV-mbiA-mCherry plasmid were isolated in the course of the screen for MbiA-resistant mutations.
- pBV-mreB: *mreB* PCR-ed from CB15N with NdeI-mreB F and EcoRI-mreB R primers was ligated into pBVMCS-4 via NdeI and EcoRI sites.
- pEntry-mreBQ26P: *mreBQ26P* PCR-ed from CJW1715 with Gateway *mreB* primers (9) was subjected to a Gateway BP reaction with pDONR221 destination vector.
- pmbiA-FLAG: *mbiA* PCR-amplified from CB15N with NdeI-mbiA F and SacI-mbiA R primers was ligated into pFLGC-1 via NdeI and SacI sites.
- pmbiA-FLAG2: first, mbiA was excised from pmbiA-FLAG and ligated into pFLGC-2 via NdeI and SacI sites. Then, AgeI-FLAG F and AgeI-FLAG R oligonucleotides were annealed to each other by incubating at 85°C for 10min and then gradually reducing the temperature to 25°C with the speed of 1°C per minute. The resulting DNA fragment was ligated into the plasmid from the first step via the AgeI site, and the ligation products were screened for insertion in the proper orientation by sequencing.
- pmbiA-mCherry: *mbiA* was excised from pmbiA-FLAG and ligated into pCHYC-4 via NdeI and SacI sites.
- pNPTS-mbiA: *mbiA* upstream (U) and downstream (D) regions were PCR-ed with mbiA UF and mbiA DUR primers and mbiA UDF and mbiA DR primers, respectively. Sewing PCR was then used to combine *mbiA*U and *mbiA*D fragments into a single *mbiA*UD PCR product via mbiA UF and mbiA DR primers. The resulting DNA segment was cloned into pCR-BLUNT II-TOPO vector and then sub-cloned into pNPTS138 via the EcoRI site.
- pNPTS-mreBG311A and pNPTS-mreBR307C: *mreB* gene along with 322 bp of upstream sequence and 1154 bp of downstream sequence was amplified from the genomic DNA of ZG696 and ZG697, respectively, with EcoRI-PmreB

F and SpeI-mreB DR primers. The resulting PCR product was ligated into pNPTS138 via EcoRI and SpeI sites. Both plasmids were sequenced to insure the absence of mutations other than *mreBG311A* and *mreBR307C*, respectively.

- pTrc-mbiA: *mbiA* PCR-ed from CB15N with EcoRI-SD-mbiA F and SacI-mbiA R primers was ligated into pTrc99a via EcoRI and SacI sites.

- pTrc-mbiA-MRM: *mbiA* was sub-cloned from pTrc-mbiA into pTrc-MRM via EcoRI and SacI sites.

- pTrc-mbiA-MRMG311A: *mreB'-mCherry* was PCR-amplified from pTrc-mbiA-MRM with XmaI-BamHI-SD-mreB F and MreBSW R4 primers. Separately, *'mreB* was amplified from ZG696 genomic DNA with MreBSW F5 and NotI-XbaI-mreB R primers. The two PCR products were combined by sewing PCR with XmaI-BamHI-SD-mreB F and NotI-XbaI-mreB R primers. The final product was cloned into pTrc-mbiA via XmaI and XbaI sites.

- pTrc-mbiAΔSE-MRM and pTrc-mbiAY71N-MRM: *mbiA* PCR-ed from pBV-mbiAΔSE-mCherry and pBV-mbiAY71N-mCherry, respectively, with EcoRI-SD-mbiA F and SacI-mbiA R primers was cloned into pTrc-MRM via EcoRI and SacI sites.

- pTrc-MRM: the 5' part of *mreB* (*mreB'*) was PCR-amplified from CB15N genomic DNA with SpeI-MreBSW F1 and MreBSW R2 primers. Separately, the 3' part of *mreB* (*'mreB*) was PCR-ed from CB15N with MreBSW F5 and MreBSW R6 primers. Also, *mCherry* was PCR-ed from pVRNt with MreBSW F3 and MreBSW R4 primers. Sewing PCR was then used to combine *mreB'* with *mCherry* via SpeI-MreBSW F1 and MreBSW R4 primers. Finally, the resulting *mreB'-mCherry* product was combined by sewing PCR with '*mreB* via SpeI-MreBSW F1 and MreBSW R6 primers. The final product was cloned into pCR-BLUNT II-TOPO vector. Subsequently, *mreB'-mCherry-'mreB* ($mreB^{SW}$) was PCR-ed from the resulting construct with XmaI-BamHI-SD-mreB F and NotI-XbaI-mreB R primers and cloned into pTrc99a via the XmaI and XbaI sites.

- pTrc-MRMG311A: *mreB'-mCherry* was PCR-amplified from pTrc-mbiA-MRM with XmaI-BamHI-SD-mreB F and MreBSW R4 primers. Separately, *'mreB* was amplified from ZG696 genomic DNA with MreBSW F5 and NotI-XbaI-mreB R primers. The two PCR products were combined by sewing PCR with XmaI-BamHI-SD-mreB F and NotI-XbaI-mreB R primers. The final product was cloned into pTrc99a via XmaI and XbaI sites.

- pVGN-mreB: *mreB* was cloned into pVGNt destination vector via Gateway LR reaction with pEntry-mreB.

- pVRN-mbiA: *mbiA* was cloned into pVRNt destination vector via Gateway LR reaction with pEntry-mbiA.

- pXCFP-rodZ: cloned the same way as pXGFPN-2rodZ in (3), but substituting pXCFPN-1 for pXGFPN-2.

- pXGFP4-mreBG311A and R307C: site-directed mutagenesis of pXGFP4-mreB was conducted with mutagenic primer pairs mreBG311A mutF and mreBG311A mutR or mreBR307C mutF and mreBR307C mutR, respectively.

- pXRN-mreBQ26P: *mreBQ26P* was cloned into pXRNk destination vector via Gateway LR reaction with pEntry-mreBQ26P.

- pXyl::mbiA: Gateway LR reaction was conducted between pEntry-mbiA and pMI12.

## Table S1. Strains used in this study

| Strains | Genotype or description | Reference or source |
|---|---|---|
| CB15N | Synchronizable variant of CB15 wild-type *C. crescentus* strain | 1 |
| CJW1715 | *mreBQ26P* | 2 |
| CJW2537 | *rodZ::Himar1* | 3 |
| DH5α | *E. coli* cloning strain: *fhuA2 Δ(argF-lacZ)U169 phoA glnV44 Φ80 Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17* | Bethesda Research Laboratories |
| EG056 | $P_{van}$*::ftsZ-cfp* | 4 |
| JAT880 | $P_{xyl}$*::mCherry-pbp2* $P_{van}$*::yfp-mreB* | Natalie Dye (Unpublished) |
| LS3814 | $P_{xyl}$*::gfp-mreB* | 5 |
| LS4275 | *ΔmreC* pMR10-$P_{xyl}$*::mreC-rfp1* | 6 |
| MT114 | $P_{xyl}$*::ftsZ-yfp* | 7 |
| MT196 | $P_{van}$*::ftsZ-yfp* | 7 |
| YB1585 | *ΔftsZ* $P_{xyl}$*::ftsZ* | 8 |
| ZG472 | $P_{van}$*::gfp-mreB* | This study |
| ZG498 | $P_{van}$*::gfp-mreB ΔmreC* pMR10-$P_{xyl}$*::mreC-rfp1* | This study |
| ZG690 | $P_{van}$*::mCherry-mbiA* | This study |
| ZG691 | $P_{xyl}$*::ftsZ-yfp* $P_{van}$*::mCherry-mbiA* | This study |
| ZG692 | $P_{xyl}$*::gfp-mreB* $P_{van}$*::mCherry-mbiA* | This study |
| ZG693 | *ΔftsZ* $P_{xyl}$*::ftsZ* $P_{van}$*::mCherry-mbiA* | This study |
| ZG694 | $P_{mbiA}$*::mbiA-FLAG* | This study |
| ZG695 | $P_{xyl}$*::mCherry-mreB* $P_{van}$*::ftsZ-cfp* | This study |
| ZG696 | *mreBG311A* transductant | This study |
| ZG697 | *mreBR307C* transductant | This study |
| ZG698 | *mreBG311A* $P_{xyl}$*::gfp-mreBG311A* | This study |
| ZG699 | *mreBR307C* $P_{xyl}$*::gfp-mreBR307C* | This study |
| ZG700 | *mreBG311A* $P_{xyl}$*::gfp-mreBG311A* $P_{van}$*::mCherry-mbiA* | This study |
| ZG701 | *mreBR307C* $P_{xyl}$*::gfp-mreBR307C* $P_{van}$*::mCherry-mbiA* | This study |
| ZG702 | $P_{xyl}$*::cfp-rodZ* | This study |
| ZG703 | $P_{xyl}$*::cfp-rodZ* $P_{van}$*::ftsZ-yfp* | This study |
| ZG704 | *mreBG311A* $P_{xyl}$*::cfp-rodZ* $P_{van}$*::ftsZ-yfp* | This study |
| ZG705 | *mreBR307C* $P_{xyl}$*::cfp-rodZ* $P_{van}$*::ftsZ-yfp* | This study |
| ZG706 | *rodZ::Himar1* $P_{van}$*::GFP-mreB* | This study |
| ZG707 | *mreBQ26P* $P_{xyl}$*::mCherry-mreBQ26P* | This study |
| ZG708 | *ΔmbiA* | This study |
| ZG709 | *ΔmbiA* $P_{xyl}$*::gfp-mreB* | This study |
| ZG710 | *ΔmbiA rodZ::Himar1* | This study |
| ZG732 | $P_{xyl}$*::mbiA-mCherry* | 9 |
| ZG733 | $P_{xyl}$*::mCherry-mreB* | 9 |
| ZG734 | $P_{xyl}$*::mCherry-murG* | 9 |
| ZG735 | $P_{xyl}$*::dipM-mCherry* | 9 |
| ZG951 | $P_{mbiA}$*::mbiA-mCherry* | This study |
| ZG952 | $P_{mbiA}$*::mbiA-mCherry* $P_{xyl}$*::gfp-mreB* | This study |
| ZG953 | $P_{mbiA}$*::mbiA-FLAGx2* | This study |
| ZG954 | *mreBG311A* $P_{mbiA}$*::mbiA-FLAGx2* | This study |
| ZG955 | *mreBR307C* $P_{mbiA}$*::mbiA-FLAGx2* | This study |
| ZG956 | *mreBG311A* allelic replacement | This study |
| ZG957 | *mreBR307C* allelic replacement | This study |

## Table S2. Plasmids used in this study

| Plasmids | Description | Reference or source |
|---|---|---|
| pBV-mbiA | Van-inducible MbiA overexpression | This study |
| pBV-mbiA-FLAG | Van-inducible MbiA-FLAG overexpression | This study |
| pBV-mbiA-mCherry | Van-inducible MbiA-mCherry overexpression | This study |
| pBV-mbiAΔSE-mCherry | Δ(S58-E59) mutant of pBV-mbiA-mCherry | This study |
| pBV-mbiAY71N-mCherry | Y71N mutant of pBV-mbiA-mCherry | This study |
| pBVMCS-4 | Van-inducible high copy vector | 10 |
| pBV-mreB | Van-inducible MreB overexpression | This study |
| pCHYC-4 | Vector for native-site C-terminal mCherry fusions | 10 |
| pCR-BLUNT II-TOPO | Topoisomerase-conjugated cloning vector | Invitrogen |
| pDONR221 | Gateway entry vector | Invitrogen |
| pEntry-mbiA | MbiA Gateway entry vector | 9 |
| pEntry-mreB | MreB Gateway entry vector | 9 |
| pEntry-mreBQ26P | MreBQ26P Gateway entry vector | This study |
| pFLGC-1 | For Spec/Strep$^R$ native-site C-terminal FLAG fusions | 10 |
| pFLGC-2 | For Kan$^R$ native-site C-terminal FLAG fusions | 10 |
| pJS14-Pxyl::rodZ | Xyl-inducible RodZ overexpression | 3 |
| pmbiA-FLAG | For native-site *mbiA-FLAG* fusion | This study |
| pmbiA-FLAGx2 | For native-site *mbiA-FLAGx2* fusion | This study |
| pmbiA-mCherry | For native-site *mbiA-mCherry* fusion | This study |
| pMI12 | Xyl-inducible high copy Gateway destination vector | John Werner |
| pNPTS138 | For constructing deletion plasmids | Alley MRK |
| pNPTS-mbiA | For *mbiA* deletion | This study |
| pNPTS-mreB | For *mreB* deletion | 5 |
| pNPTS-mreBG311A | For *mreBG311A* allelic replacement | This study |
| pNPTS-mreBR307C | For *mreBR307C* allelic replacement | This study |
| pTrc99a | IPTG-inducible *E. coli* cloning vector | 11 |
| pTrc-mbiA | IPTG-inducible MbiA expression | This study |
| pTrc-mbiA-MRM | IPTG-inducible expression of *C.c.* MreB$^{SW}$ and WT MbiA | This study |
| pTrc-mbiA-MRMG311A | IPTG-inducible expression of *C.c.* MreB$^{SW}$G311A and WT MbiA | This study |
| pTrc-mbiAΔSE-MRM | IPTG-inducible expression of *C.c.* MreB$^{SW}$ and Δ(S58-E59) MbiA | This study |
| pTrc-mbiAY71N-MRM | IPTG-inducible expression of *C.c.* MreB$^{SW}$ and Y71N MbiA | This study |
| pTrc-MRM | IPTG-inducible *C.c.* MreB$^{SW}$ expression | This study |
| pTrcMRMG311A | IPTG-inducible *C.c.* MreB$^{SW}$G311A expression | This study |
| pVGNt | TetR Gateway destination vector for van-inducible N-terminal GFP fusions | John Werner |
| pVGN-mreB | Van-inducible GFP-MreB fusion | This study |
| pVRNt | TetR Gateway destination vector for van-inducible N-terminal mCherry fusions | John Werner |
| pVRN-mbiA | Van-inducible mCherry-MbiA fusion | This study |
| pXCFPN-1 | For xyl-inducible N-terminal CFP fusion | 10 |
| pXCFP-rodZ | Xyl-inducible CFP-RodZ | This study |
| pXGFP4-mreB | Xyl-inducible GFP-MreB | 5 |
| pXGFP4-mreBG311A | Xyl-inducible GFP-MreBG311A | This study |
| pXGFP4-mreBR307C | Xyl-inducible GFP-MreBR307C | This study |
| pXRNk | KanR Gateway destination vector for xyl-inducible N-terminal mCherry fusions | John Werner |
| pXRN-mreBQ26P | Xyl-inducible mCherry-MreBQ26P fusion | This study |
| pXyl::mbiA | Xyl-inducible Gateway MbiA overexpression | This study |

## Table S3. Primers used in this study

| Primer Name | Sequence |
|---|---|
| AgeI-FLAG F | 5'-Phosphate - CCGGTCGGACTACAAGGACGATGACGATAAGAA |
| AgeI-FLAG R | 5'-Phosphate - CCGGTTCTTATCGTCATCGTCCTTGTAGTCCGA |
| EcoRI-mreB R | CAGAATTCCTAGGCCAGCGTGGATTCCAG |
| EcoRI-PmreB F | TTGAATTCCATCGCGAAACCCGTGAAGAGC |
| EcoRI-SD-mbiA F | CATGAATTCGCGAGGAAACGCATATGAAGCCTTACATCGAACTCAAG |
| mbiA DR | GCGTGAGCTTGAAGGGCAGG |
| mbiA DUR | GCTCTAGTCGGCCTGGTTCATGATGTAAGGCTTCAAGCTGTCC |
| mbiA UDF | GGACAGCTTGAAGCCTTACATCATGAACCAGGCCGACTAGAGC |
| mbiA UF | GGATCCTCTCCACGATCCTG |
| mreBG311A mutF | GAGATCCGCGATCATACCGCCCTGCCGGTCACGGTCGC |
| mreBG311A mutR | GCGACCGTGACCGGCAGGGCGGTATGATCGCGGATCTC |
| mreBR307C mutF | CGGCCTGGATGCCGAGATCTGCGATCATACCGGCCTGCCG |
| mreBR307C mutR | CGGCAGGCCGGTATGATCGCAGATCTCGGCATCCAGGCCG |
| MreBSW R2 | CTCGCCCTTGCTCACCATCGACGAGCCGCTGCCGTCGGCCGGCGCGCG |
| MreBSW F3 | CGCGCGCCGGCCGACGGCAGCGGCTCGTCGATGGTGAGCAAGGGCGAG |
| MreBSW R4 | GTCGATCGACAGACCTTCCGAGCCGGCCGGGCCCTTGTACAGCTCGTCCATG |
| MreBSW F5 | CATGGACGAGCTGTACAAGGGCCCGGCCGGCTCGGAAGGTCTGTCGATCGAC |
| MreBSW R6 | GGTAGTCAAGCTTGGGATTGG |
| NdeI-mbiA F | TAGACTCATATGAAGCCTTACATCGAACTC |
| NdeI-mreB F | CTAGCATATGTTCTCTTCCCTTTTCGGCGTG |
| NotI-XbaI-mreB R | GCGGCCGCTCTAGATAAGGAATACCGGCCTAGGC |
| SacI-FLAG-mbiA R | GAGCTCTTACTTGTCGTCGTCGTCCTTGTAGTCGCCGTCGGCCTGGTTCATCACGG |
| SacI-mbiA R | CATGAGCTCTAGTCGGCCTGGTTCATCAC |
| SpeI-gfp R | GTCACTAGTTACTTGTACAGCTCGTCCATG |
| SpeI-mreB DR | TTACTAGTGATGCACATCAGCATGGGCAC |
| SpeI-MreBSW F1 | CAGACTAGTGTTCAAGGAACGCCTGACC |
| XmaI-BamHI-SD-mreB F | CCCGGGATCCTCAGTCAGGGCTTTCAATG |

## Supplemental Information References


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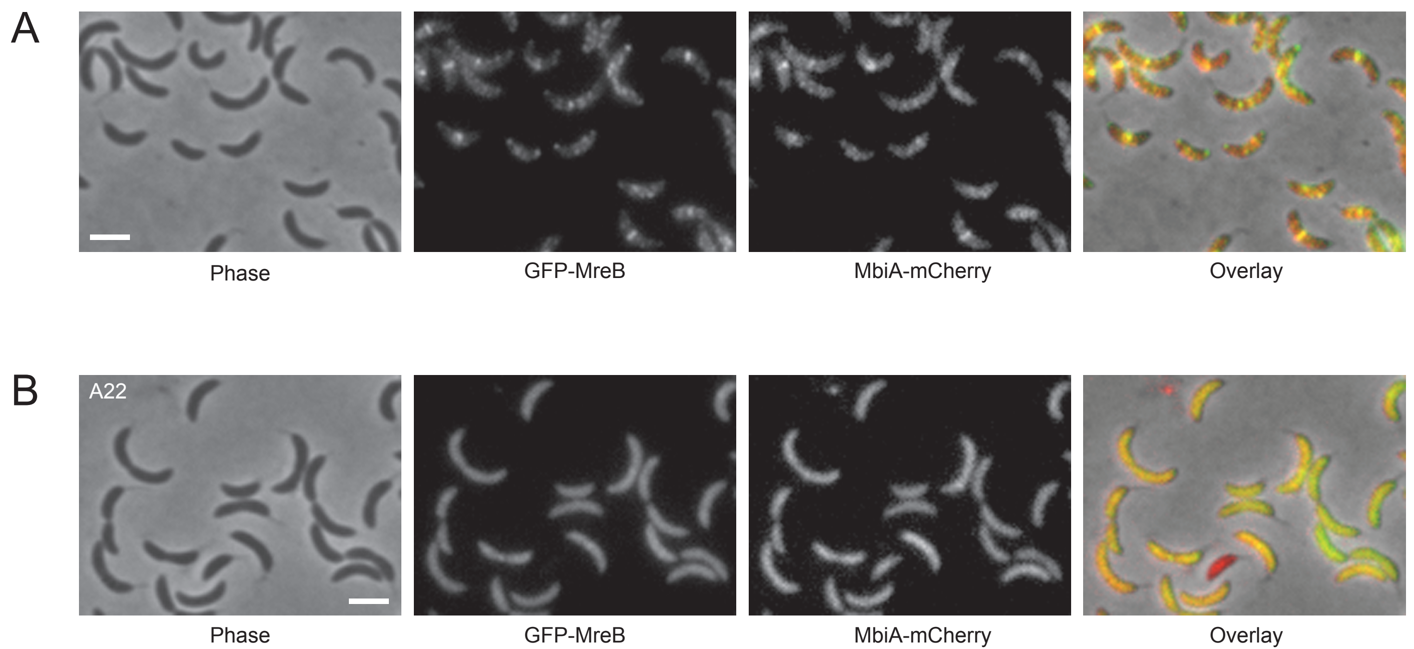


**Figure S1. MbiA endogenous fusion co-localizes with MreB in MreB-dependent fashion**

A. Phase contrast, GFP-MreB fluorescence, MbiA-mCherry fluorescence, and overlay images of ZG952 expressing mbiA-mCherry fusion from the native locus. Cells were grown in PYE with xylose.
B. Phase contrast, GFP-MreB fluorescence, MbiA-mCherry fluorescence, and overlay images of ZG952 grown as in (A) and placed onto agarose pads with 50μg mL-1 of A22. The scale bars are 2μm.

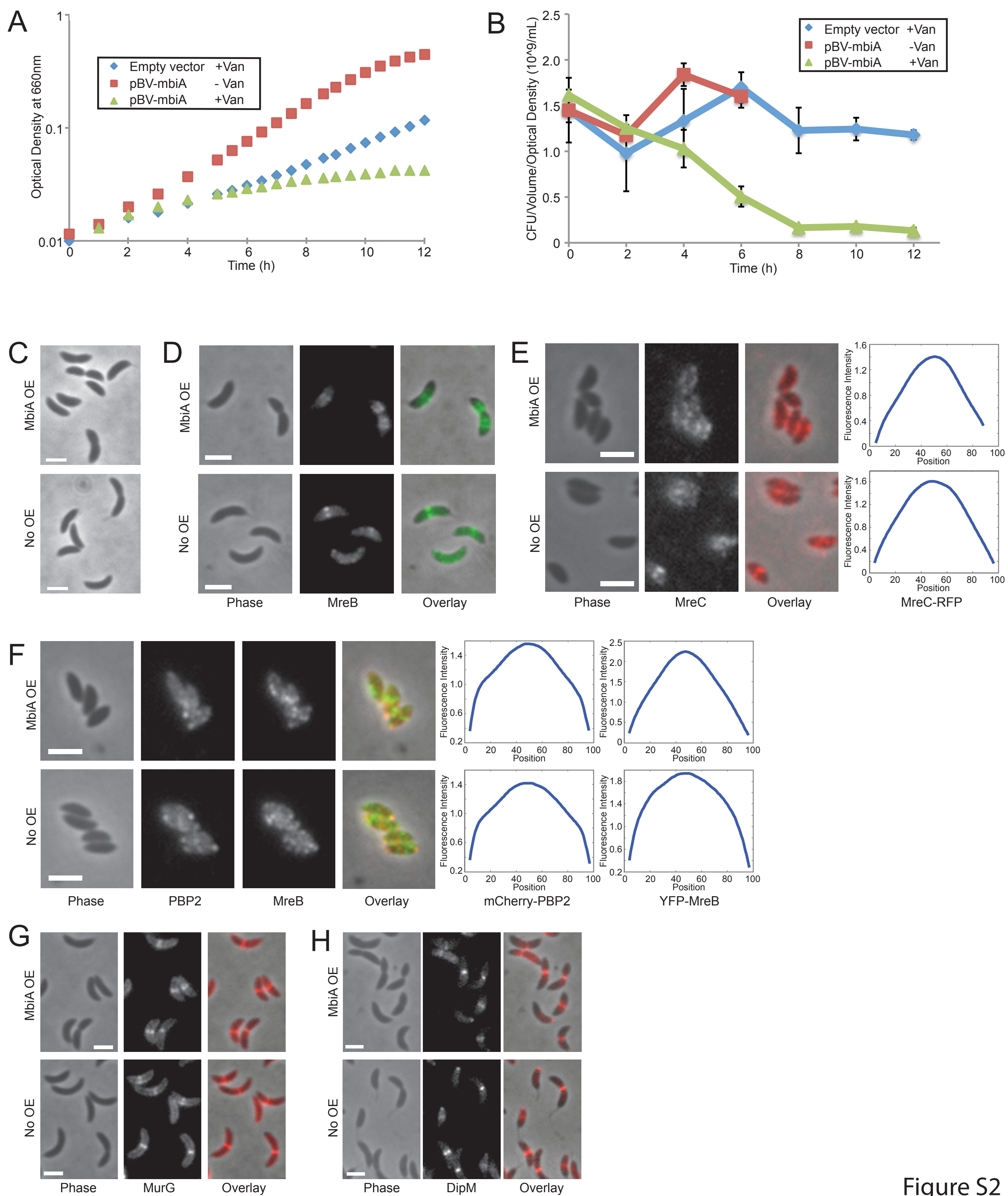


Figure S2

**Figure S2. MbiA overexpression affects cell growth, viability, and morphology, but not the localization of other PG synthesis proteins**

A. Growth curves of CB15N cells carrying either pBV-mbiA or pBVMCS-4 empty vector grown in PYE either with or without vanillate. The results of a single representative experiment are presented.

B. CFU counts for cells from (A) measured at 2h intervals. Latest time points for pBV-mbiA without vanillate yielded too many colonies to count accurately and were omitted from the plot.

C. Phase contrast images of CB15N cells carrying pBV-mbiA grown in PYE with (MbiA OE) or without (No OE) vanillate.

D. Localization of GFP-MreB in unsynchronized LS3814 cells carrying pBV-mbiA grown in PYE with xylose and vanillate (MbiA OE). Cells grown in PYE with xylose only are used as a control (No OE). Phase contrast, GFP-MreB, and overlay images are presented.

E. Localization of MreC-mRFP1 in swarmer cells of LS4275 carrying pBV-mbiA. Cells were grown in PYE with xylose and either with (MbiA OE) or without (No OE) vanillate for 5h prior to synchronization and chemical fixation. Phase contrast, MreC-mRFP1 fluorescence, and overlay images are shown. Plots of average fluorescence intensity with respect to the long axis of the cell are also provided.

F. Co-localization of mCherry-PBP2 and YFP-MreB in swarmer cells of JAT880 carrying either pBV-mbiA (MbiA OE) or pBVMCS-4 (No OE) immediately after synchronization and chemical fixation. Cells were grown in PYE with xylose and vanillate. Phase contrast, mCherry-PBP2 fluorescence, YFP-MreB fluorescence, and overlay images are provided. The plots at the right represent average fluorescence intensity versus position (in % cell length) along the long cell axis for both fusions.

G. Localization of mCherry-MurG in ZG734 cells carrying either pBV-mbiA (MbiA OE) or pBVMCS-4 (No OE) and grown in PYE with xylose and vanillate. Phase contrast, mCherry-MurG fluorescence, and overlay images are presented.

H. Localization of DipM-mCherry in ZG735 cells carrying pBV-mbiA and grown in PYE with xylose and either with (MbiA OE) or without (No OE) vanillate. Phase contrast, DipM-mCherry fluorescence, and overlay images are presented. All scale bars are 2μm.

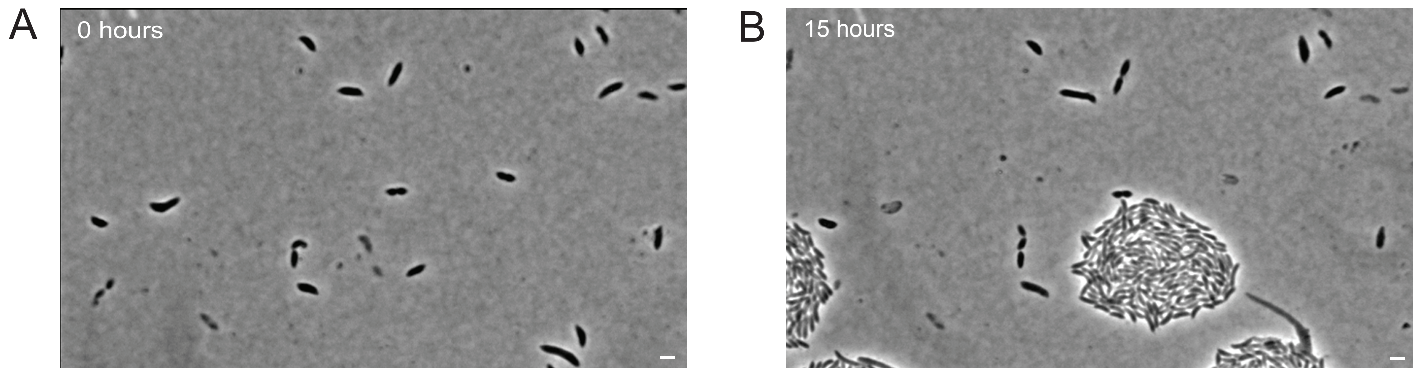


**Figure S3. Few cells recover from MbiA overexpression**

A. Phase contrast image of Caulobacter cells carrying pXyl::mbiA after 8h of growth in PYE with xylose. The image was taken immediately after transfer to a PYE agarose pad with glucose.
B. Same field of cells as in (A) after 15h of growth on the pad with glucose. The scale bars are 2μm.

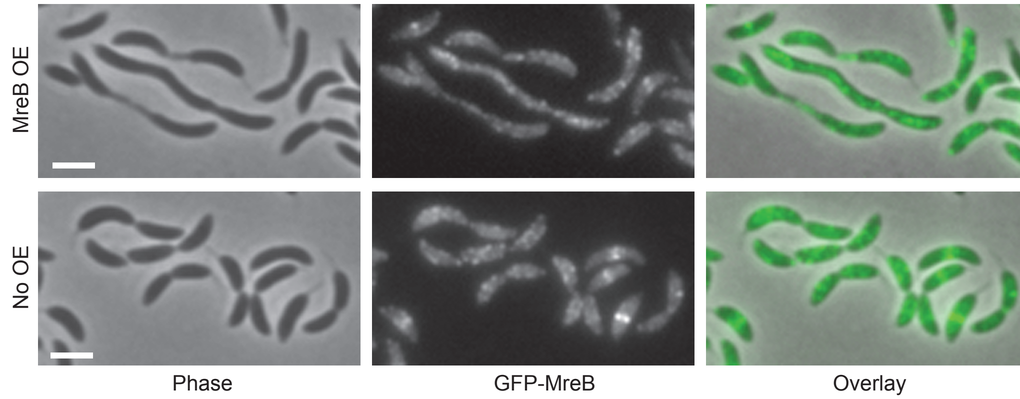


**Figure S4. MreB overexpression does not mimic MbiA overexpression phenotypes**

Localization of GFP-MreB in LS3814 cells overexpressing unfused MreB from pBV-mreB vector. Cells were grown in PYE with xylose and either with (MreB OE) or without (No OE) vanillate. Phase contrast, GFP-MreB fluorescence, and overlay images are shown. The scale bars are 2μm.

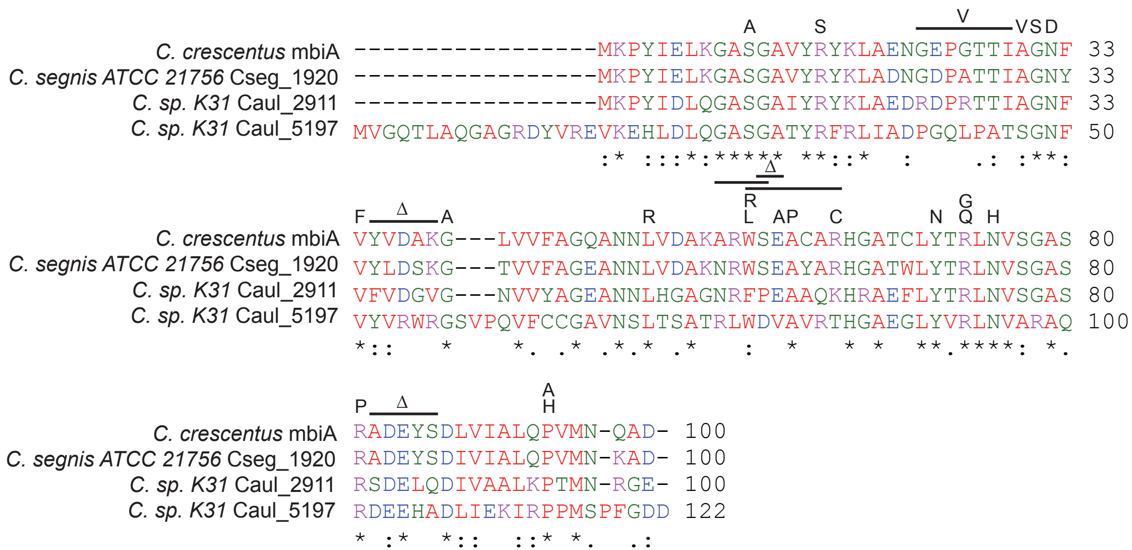


**Figure S5. Non-functional MbiA alleles**

Non-toxic MbiA mutations are presented over an alignment of the three closest MbiA homologs. In-frame deletions are indicated with horizontal lines, with Δ representing deletions that preserve all original codons and V representing the one deletion where merging of two codons created a novel valine residue.

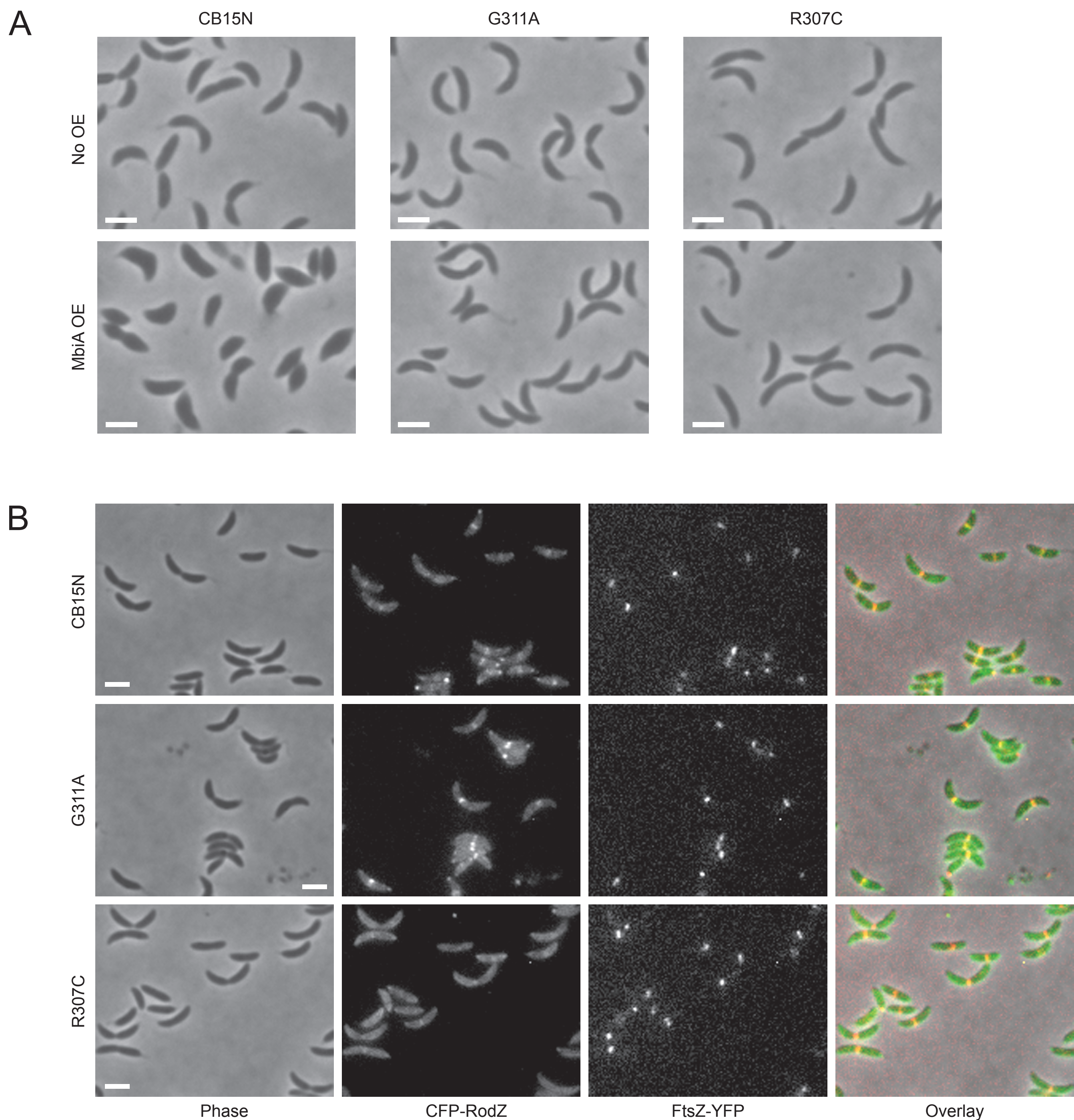


**Figure S6. MreB mutants constructed by allelic replacement are resistant to MbiA overexpression**

A. Phase contrast images of CB15N, G311A (ZG956), and R307C (ZG957) cells carrying pBV-mbiA-mCherry and grown in PYE either with (MbiA OE) or without (No OE) vanillate.

B. Phase contrast, CFP-RodZ fluorescence, FtsZ-YFP fluorescence, and overlay images of WT (ZG703), G311A (ZG704), and R307C (ZG705) cells. The scale bars are 2μm.

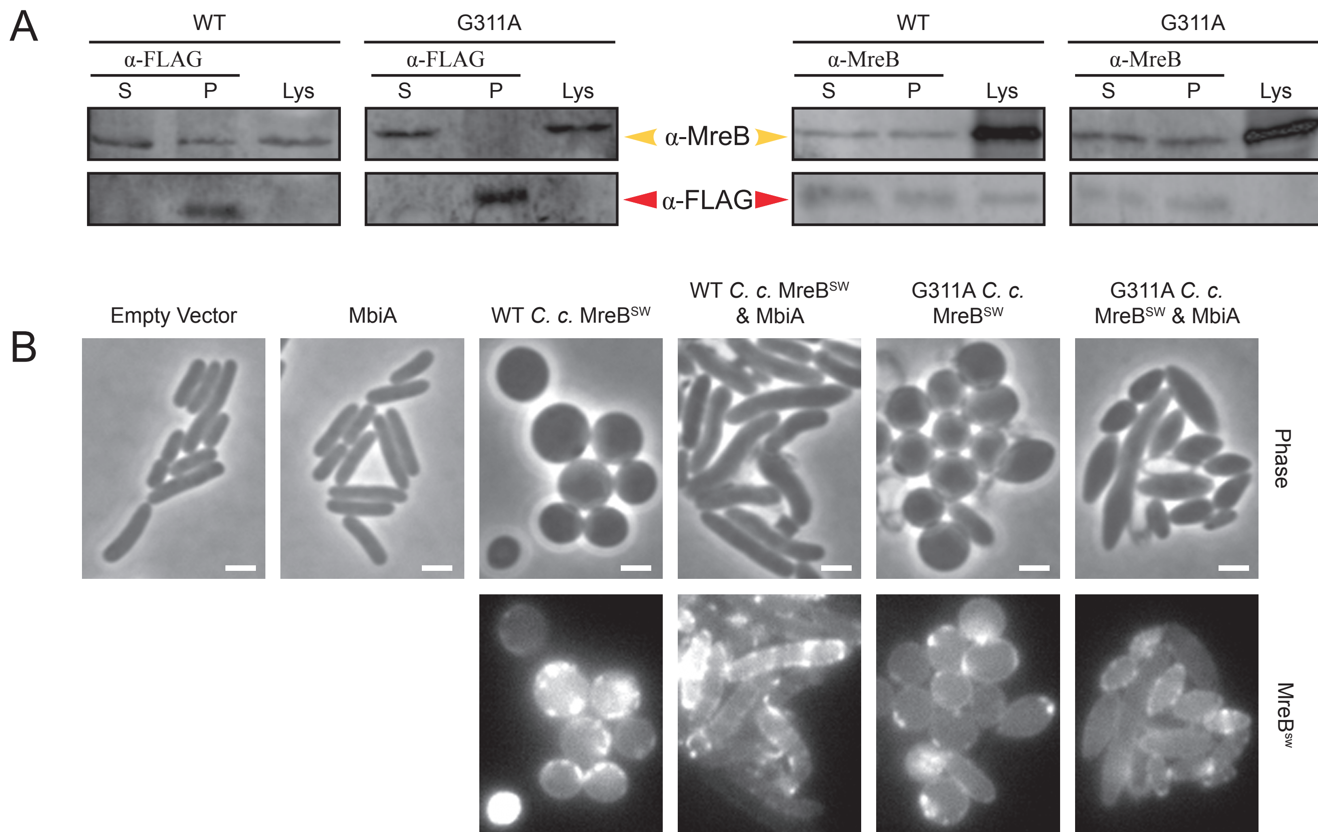


**Figure S7. MbiA interacts with MreB in vivo and in vitro**

A. Immunoblot of co-immunoprecipitation with α-FLAG and α-MreB antibodies (indicated above) in WT (ZG953) or G311A (ZG954) cells encoding an endogenous mbiA-FLAGx2 fusion. The supernatant (S) and the pellet (P) from the co-immunoprecipitation as well as the original lysate (Lys) were loaded on the gel. The immunoblot was performed with the antibodies indicated at the side with arrowheads.

B. Phase contrast images of DH5α E. coli cells carrying pTrc99a empty vector or pTrc-mbiA expression plasmid. Additionally, phase contrast and MreBSW fluorescence images of DH5α expressing WT or G311A Caulobacter MreB sandwich fusion alone (pTrc-MRM and pTrc-MRMG311A, respectively) or with MbiA (pTrc-mbiA-MRM and pTrc-mbiA-MRMG311A, respectively). Cells were grown in LB and induced with 1mM IPTG for 2h. The scale bars are 2µm.

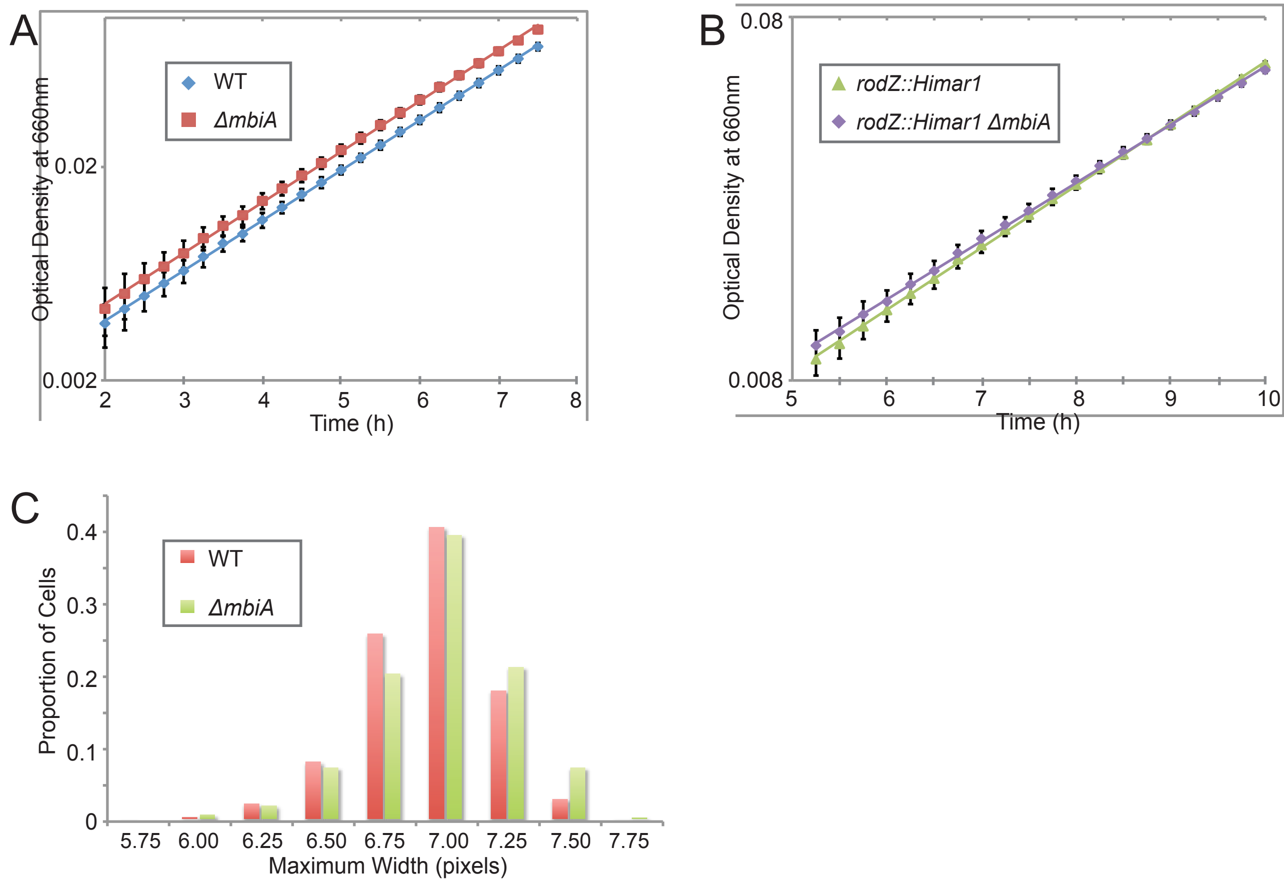


**Figure S8. MbiA deletion has no effect on cell width**

A. Growth curves of CB15N and ΔmbiA (ZG708) cells grown in PYE. The data were taken with the Synergy HT microplate reader, and the average results of 22 replicates of each strain are shown.

B. Growth curves of rodZ::Himar1 (CJW2537) and rodZ::Himar1 ΔmbiA (ZG710) cells grown in PYE with kanamycin. The data were taken with the Synergy HT microplate reader, and the average results of 44 replicates of each strain are shown.

C. Histogram of maximum cell widths in CB15N and ΔmbiA (ZG708) strains. >500 cells of each were analyzed.

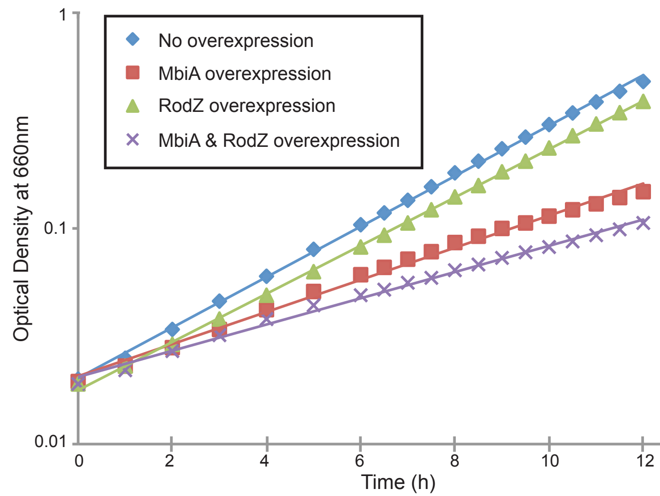


**Figure S9. RodZ overexpression fails to suppress the growth phenotype of MbiA overexpression**

Growth curves of CB15N cells carrying pJS14-Pxyl::rodZ and pBV-mbiA and grown in PYE either with glucose, glucose and vanillate, xylose, or xylose and vanillate. The lines represent best-fit exponential regression curves. A representative result of several experiments is presented.