Supplemental Material

Function and localization dynamics of bifunctional penicillin-binding proteins in *Caulobacter crescentus*

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SUPPLEMENTAL TABLES

Table S1. Strains used in this study.

Strain	Genotype/description	Reference/source
C. crescentus		
CB15N	synchronizable derivative of wild-type strain CB15	Evinger & Agabian (1)
CJW1715	CB15N *mreB*$_{Q26P}$	Aaron et al. (2)
AM52	CB15N Δ*vanA* P*van*::P*van-ftsN* Δ*ftsN*	Möll & Thanbichler (3)
AM160	CB15N *ftsN*::*ecfp-ftsN* P*xyl*::P*xyl-ftsZ-eyfp*	Möll & Thanbichler (3)
AM372	CB15N Δ*pbp1a* Δ*pbpY*	This work
AM373	CB15N Δ*pbp1a* Δ*pbpZ*	This work
AM457	CB15N P*xyl*::P*xyl-venus-pbpY*	This work
AM458	CB15N P*xyl*::P*xyl-venus-pbpZ*	This work
AM472	CB15N Δ*vanA* P*van*::P*van-ftsN* Δ*ftsN* P*xyl*::P*xyl-venus-pbpY*	This work
AM473	CB15N Δ*vanA* P*van*::P*van-ftsN* Δ*ftsN* P*xyl*::P*xyl-venus-pbpX*	This work
DK60	CB15N *ftsZ*::P*iolC-ftsZ*'	This work
JK305	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpY* Δ*pbpZ*	This work
KK1	CB15N Δ*pbpX*	This work
KK12	CB15N Δ*pbp1a* Δ*pbpY* Δ*pbpZ*	This work
KK16	CB15N Δ*pbpY*	This work
KK17	CB15N Δ*pbpZ*	This work
KK18	CB15N Δ*pbp1a*	This work
KK24	CB15N Δ*pbpX* Δ*pbpZ*	This work
KK33	CB15N P*xyl*::P*xyl-venus-pbp1a*	This work
KK37	CB15N Δ*pbpY* Δ*pbpZ*	This work
MT56	CB15N *ftsN*::*ecfp-ftsN* P*xyl*::P*xyl-mreB-eyfp*	This work
MT258	CB15N Δ*dipM*	Möll et al. (4)
MT278	CB15N P*xyl*::P*xyl-venus-pbpX*	This work
MT279	CB15N P*xyl*::P*xyl-venus-pbpC*	Kühn et al. (5)
MT282	CB15N Δ*pbpC*	This work
WS041	CB15N Δ*pbp1a* Δ*pbpX* P*xyl*::P*xyl-pbpX*	This work
WS044	CB15N Δ*pbpX* Δ*pbpY* P*xyl*::P*xyl-pbpX*	This work
WS045	CB15N *venus-pbpY*	This work
WS055	CB15N *venus-pbpX*	This work
WS056	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX*	This work
WS057	CB15N Δ*dipM* P*xyl*::P*xyl-venus-pbpY*	This work
WS058	CB15N Δ*dipM* P*xyl*::P*xyl-venus-pbpX*	This work
WS062	CB15N *ftsZ*::P*iolC-ftsZ* P*xyl*::P*xyl-venus-pbpX*	This work
WS063	CB15N *ftsZ*::P*iolC-ftsZ* P*xyl*::P*xyl-venus-pbpY*	This work
WS064	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX* P*iol*::P*iol-venus-pbpX*	This work
WS065	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX* P*iol*::P*iol-venus-pbpY*	This work
WS066	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX* P*iol*::P*iol-venus-pbp1a*	This work
WS067	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX* P*iol*::P*iol-venus-pbpC*	This work
WS068	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX* P*iol*::P*iol-venus-pbpZ*	This work
WS070	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX* P*iol*::P*iol-pbpX*	This work
WS071	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX* P*iol*::P*iol-pbpY*	This work
WS072	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX* P*iol*::P*iol-pbp1a*	This work
WS073	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX* P*iol*::P*iol-pbpC*	This work
WS074	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX* P*iol*::P*iol-pbpZ*	This work
WS075	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX* P*iol*::P*iol-venus*	This work
WS076	CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX* P*iol*::pAMIOL-4	This work
WS084	CB15N *mreB*$_{Q26P}$ P*xyl*::P*xyl-venus-pbpX*	This work
WS085	CB15N *mreB*$_{Q26P}$ P*xyl*::P*xyl-venus-pbpY*	This work
WS090	CB15N Δ*pbp1a* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P*xyl*::P*xyl-pbpX*	This work
E. coli		
TOP10	cloning strain	Invitrogen
XL1-Blue	cloning strain	Stratagene
BTH101	bacterial two-hybrid reporter strain	Karimova et al. (6)

Table S2. Plasmids used in this study.

Plasmid	Genotype/description	Reference/source
pKT25	Plasmid for constructing C-terminal fusions to T25, Kan^R	Karimova et al. (6)
pUT18C	Plasmid for constructing C-terminal fusions to T18, Amp^R	Euromedex
pVCFPN-4	Integrating plasmid used for creating N-terminal fusions to eCFP under the control of P*van*, Gent^R	Thanbichler et al. (7)
pVVENN-1	Integrating plasmid used for creating N-terminal fusions to Venus under the control of P*van*, Spec^R	Thanbichler et al. (7)
pVVENN-4	Integrating plasmid used for creating N-terminal fusions to Venus under the control of P*van*, Gent^R	Thanbichler et al. (7)
pXCFPN-4	Integrating plasmid used for creating N-terminal fusions to eCFP under the control of P*xyl*, Gent^R	Thanbichler et al. (7)
pXMCS-4	Integrating plasmid for integrating P*xyl* upstream of genes of interest	Thanbichler et al. (7)
pXVENN-1	Integrating plasmid used for creating N-terminal fusions to Venus under the control of P*xyl*, Spec^R	Thanbichler et al. (7)
pXVENN-2	Integrating plasmid used for creating N-terminal fusions to Venus under the control of P*xyl*, Kan^R	Thanbichler et al. (7)
pNPTS138	*sacB*-containing suicide vector for double homologous recombination, Kan^R	M.R.K. Alley, unpublished
pAMIOL-4	Integrating plasmid used for the expression of genes under the control of P*iol*, Gent^R	This work
pAM104	pUT18C carrying *malG* (AA1-77)-'MAS'-*dipM* (AA26-609)	Möll et al. (4)
pAM105	pKT25 carrying *malG* (AA1-77)-'MAS'-*dipM* (AA26-609)	Möll et al. (4)
pAM203	pXVENN-2 carrying *pbpY*	This work
pAM211	pXVENN-2 carrying *pbpZ*	This work
pDK122	pXMCS-4 carrying P*iol*-*ftsZ*', Gent^R	This work
pKK001	pXVENN-2 carrying *pbp1a*	This work
pKK004	pNPTS138-based plasmid for constructing an in-frame deletion in *pbpX*	This work
pKK007	pNPTS138-based plasmid for constructing an in-frame deletion in *pbpY*	This work
pKK009	pNPTS138-based plasmid for constructing an in-frame deletion in *pbpZ*	This work
pKK010	pNPTS138-based plasmid for constructing an in-frame deletion in *pbp1a*	This work
pMT921	pNPTS138-based plasmid for constructing an in-frame deletion in *pbpC*	This work
pMT898	pXVENN-2 carrying *pbpX*	This work
pSS092	pKT25 carrying *ftsL*	This work
pSS094	pUT18C carrying *ftsL*	This work
pSS096	pUT18C carrying *ftsN*	Möll et al. (4)
pSS102	pKT25 carrying *ftsN*	Möll et al. (4)
pWS30	pKT25 carrying *pbpX*	This work
pWS34	pUT18C carrying *pbpX*	This work
pWS39	pXVENN-1 carrying *pbpX* instead of *venus*	This work
pWS40	pVVENN-1 carrying *pbpX*	This work
pWS41	pVVENN-1 carrying *pbpY*	This work
pWS42	pKT25 carrying *pbp1a*	This work
pWS43	pUT18C carrying *pbp1a*	This work
pWS48	pKT25 carrying *pbpZ*	This work
pWS49	pUT18C carrying *pbpZ*	This work
pWS50	pVVENN-4 carrying *pbpX*	This work
pWS52	pAMIOL-4 carrying *venus-pbpX*	This work
pWS58	pNPTS138-based plasmid for replacing the native *pbpY* gene with *venus-pbpY*	This work
pWS59	pNPTS138-based plasmid for replacing the native *pbpX* gene with *venus-pbpX*	This work
pWS60	pAMIOL-4 carrying *venus-pbpY*	This work
pWS61	pAMIOL-4 carrying *venus-pbp1a*	This work
pWS62	pAMIOL-4 carrying *venus-pbpC*	This work
pWS63	pAMIOL-4 carrying *venus-pbpZ*	This work
pWS64	pUT18C carrying *pbpC*	This work
pWS65	pKT25 carrying *pbpC*	This work
pWS66	pUT18C carrying *pbpY*	This work
pWS67	pKT25 carrying *pbpY*	This work
pWS68	pAMIOL-4 carrying *pbpX*	This work
pWS69	pAMIOL-4 carrying *pbpY*	This work
pWS70	pAMIOL-4 carrying *pbp1a*	This work
pWS71	pAMIOL-4 carrying *pbpC*	This work
pWS72	pAMIOL-4 carrying *pbpZ*	This work
pWS73	pAMIOL-4 carrying *venus*	This work

Table S3. Strain and plasmid construction.

Strain/Plasmid	Construction
Strains	
AM372	In-frame deletion of *pbp1a* in KK16 by double homologous recombination using pKK010
AM373	In-frame deletion of *pbp1a* in KK17 by double homologous recombination using pKK010
AM457	Integration of pAM203 in CB15N
AM458	Integration of pAM211 in CB15N
AM472	Integration of pAM203 in AM52
AM473	Transfer of P*xyl*::P*xyl-venus-pbpX* from MT278 into AM52 by phage transduction
DK60	Integration of pDK122 in CB15N
JK305	Deletion of *pbpC* in KK12 by double homologous recombination using pMT921
KK1	Deletion of *pbpX* in CB15N by double homologous recombination using pKK004
KK12	Deletion of *pbp1a* in KK37 by double homologous recombination using pKK010
KK16	Deletion of *pbpY* in CB15N by double homologous recombination using pKK007
KK17	Deletion of *pbpZ* in CB15N by double homologous recombination using pKK009
KK18	Deletion of *pbp1a* in CB15N by double homologous recombination using pKK010
KK24	Deletion of *pbpZ* in KK1 by double homologous recombination using pKK009
KK33	Integration of pKK001 in CB15N
KK37	Deletion of *pbpZ* in KK16 by double homologous recombination using pKK009
MT278	Integration of pMT898 in CB15N
MT279	Integration of pMT906 in CB15N
MT282	Integration of pMT921 in CB15N
WS041	Integration of pWS39 in KK18 and deletion of *pbpX* by double homologous recombination using pKK004
WS044	Integration of pWS39 in KK16 and deletion of *pbpX* by double homologous recombination using pKK004
WS045	Substitution of *pbpY* with *venus-pbpY* in CB15N by double homologous recombination using pWS58
WS055	Substitution of *pbpX* with *venus-pbpX* in CB15N by double homologous recombination using pWS59
WS056	Integration of pWS39 in JK305 and deletion of *pbpX* by double homologous recombination using pKK004
WS057	Integration of pAM203 in MT258
WS058	Integration of pMT898 in MT258
WS062	Integration of pMT898 in DK60
WS063	Integration of pAM203 in DK60
WS064	Integration of pWS52 in WS056
WS065	Integration of pWS60 in WS056
WS066	Integration of pWS61 in WS056
WS067	Integration of pWS62 in WS056
WS068	Integration of pWS63 in WS056
WS070	Integration of pWS68 in WS056
WS071	Integration of pWS69 in WS056
WS072	Integration of pWS70 in WS056
WS073	Integration of pWS71 in WS056
WS074	Integration of pWS72 in WS056
WS075	Integration of pWS73 in WS056
WS076	Integration of pAMIOL-4 in WS056
WS084	Integration of pMT898 in CJW1715
WS085	Integration of pAM203 in CJW1715
WS090	Integration of pWS39 in KK12 and deletion of *pbpX* by double homologous recombination using pKK004
Plasmids	
pAMIOL-4	The upstream region of *cc1298* (*iolC)* was PCR-amplified using primers iolPuni_72f and iolPrev_73r, cut with HindIII and AseI, and ligated into equally treated pXCFPN-4.
pAM203	*pbpY* was PCR-amplified from CB15N chrom. DNA using primers pbpY_323f and oKK19, cut with KpnI and NheI, and ligated into equally treated pXVENN-2.
pAM211	*pbpZ* was PCR-amplified from CB15N chrom. DNA using primers pbpZ_328f and oKK6, cut with KpnI and NheI, and ligated into equally treated pXVENN-2.
pDK122	The *iolC* promoter region was PCR-amplified from CB15N chrom. DNA using primers PiolC3-for2 and PiolC3-rev and cut with HindIII and NdeI. *ftsZ* was PCR-amplified from CB15N chrom. DNA using primers ftsZ-1 and ftsZs-revNheI and cut with NdeI and NheI. The fragments were triple-ligated into HindIII/NheI-cut pXMCS-4.
pKK001	*pbp1a* was PCR-amplified from pMT705 using primers oKK1 and oKK2, cut with KpnI and NheI, and ligated into equally treated pXVENN-2.
pKK004	The upstream and downstream regions of *pbpX* were PCR-amplified from CB15N chrom. DNA using primers oKK7/oKK8 and oKK9/oKK10. The reaction products were treated with HindIII/BamHI and BamHI/EcoRI, respectively, and ligated into HindIII/EcoRI-cut pNPTS138.
pKK007	The upstream and downstream regions of *cc1875* were PCR-amplified from CB15N chrom. DNA using primers oKK18/oKK19 and oKK20/oKK21. The reaction products were treated with HindIII/NheI and NheI/EcoRI, respectively, and ligated into HindIII/EcoRI-cut pNPTS138.
pKK009	The upstream and downstream regions of *cc3570* were PCR-amplified from CB15N chrom. DNA using primers oKK27/oKK28 and oKK29/oKK30. The reaction products were treated with NheI/EcoRI and HindIII/EcoRI, respectively, and ligated into HindIII/NheI-cut pNPTS138.
pKK010	The upstream and downstream regions of *pbp1a* were PCR-amplified from CB15N chrom. DNA using primers oKK23/oKK24 and oKK25/oKK26. The reaction products were treated with HindIII/EcoRI and NheI/EcoRI, respectively, and ligated into HindIII/NheI-cut pNPTS138.
pSS092	*ftsL* was PCR-amplified from CB15N chrom. DNA using primers oSS192 and oSS206, cut with EcoRI and BglII, and ligated into equally treated pKT25.
pSS096	*ftsL* was PCR-amplified from CB15N chrom. DNA using primers oSS192 and oSS206, cut with EcoRI and BglII, and ligated into equally treated pUT18C.

Table S3. continued

Strain/Plasmid	Construction
pMT921	Plasmid pMT906 was restricted with XcmI/BgIII, treated with T4 DNA polymerase and self-ligated. The *pbpC* gene bearing an in-frame deletion was isolated by restriction with KpnI/NheI and subsequent T4 polymerase treatment and ligated into EcoRV-cut pNPTS138. Aberrant XcmI/BgIII junction: CCACCCATC.
pMT906	*pbpC* was PCR-amplified from CB15N chrom. DNA using primers pbpC_for and pbpC_rev2, cut with KpnI and NheI, and ligated into equally treated pXVENN-1.
pMT898	*pbpX* was PCR-amplified from CB15N chrom. DNA using primers pbpX-for and pbpX-rev, cut with BgIII and NheI, and ligated into equally treated pXVENN-1.
pWS30	*pbpX* was PCR-amplified from pMT898 using primers pbpX_7f and pbpX_8r, cut with KpnI and EcoRI, and ligated into equally treated pKT25.
pWS34	*pbpX* was PCR-amplified from pMT898 using primers pbpX_7f and pbpX_8r, cut with KpnI and EcoRI, and ligated into equally treated pUT18C.
pWS39	*pbpX* was isolated of pAM203 using NdeI/SacI and ligated into equally treated pXVENN-1.
pWS40	*pbpX* was PCR-amplified from CB15N chrom. DNA using primers pbpX_16f and pbpX_8r, cut with KpnI and EcoRI and ligated into equally treated pVVENN-1.
pWS41	*pbpY* was PCR-amplified from pAM203 using primers pbpY_323f and pbpY_337r, cut with KpnI and EcoRI, and ligated into equally treated pVVENN-1.
pWS42	*pbp1a* was PCR-amplified from CB15N chrom. DNA using primers pbp1a_17f and pbp1a_18r, cut with KpnI and EcoRI, and ligated into equally treated pKT25.
pWS43	*pbp1a* was PCR-amplified from CB15N chrom. DNA using primers pbp1a_17f and pbp1a_18r, cut with KpnI and EcoRI, and ligated into equally treated pUT18C.
pWS48	*pbpZ* was PCR-amplified from CB15N chrom. DNA using primers pbpZ_22f and cc3570_21r, cut with KpnI and EcoRI, and ligated into equally treated pKT25.
pWS49	*pbpZ* was PCR-amplified from CB15N chrom. DNA using primers pbpZ_22f and cc3570_21r, cut with KpnI and EcoRI, and ligated into equally treated pUT18C.
pWS50	*pbpX* was PCR-amplified from CB15N chrom. DNA using primers pbpX_16f and pbpX_8r, cut with KpnI and EcoRI, and ligated into equally treated pVVENN-4.
pWS52	*venus-pbpX* was isolated from pWS50 by restriction with NdeI/EcoRI and ligated into AseI/EcoRI-treated pAMIOL-4.
pWS58	*venus-pbpY* was isolated from pWS41 by restriction with NdeI/EcoRI. The upstream region of *pbpY* was PCR-amplified from CB15N chrom. DNA using primers pbpY_27f and pbpY_26r and cut with HindIII/NdeI. The two fragments were then triple-ligated into HindIII/EcoRI-cut pNPTS138.
pWS59	*venus-pbpX* was isolated from pWS40 by restriction with NdeI/EcoRI. The upstream region of *pbpX* was PCR-amplified from CB15N chrom. DNA using primers pbpX_25f and pbpX_24r and cut with HindIII/NdeI. The two fragments were then triple-ligated into HindIII/EcoRI-cut pNPTS138.
pWS60	*pbpY* was isolated of pWS41 using KpnI/EcoRI and ligated into equally treated pWS52.
pWS61	*pbp1a* was PCR-amplified from CB15N chrom. DNA using primers pbp1a_29f and pbp1a_18r, cut with KpnI and EcoRI, and ligated into equally treated pWS52.
pWS62	*pbpC* was PCR-amplified from CB15N chrom. DNA using primers pbpC_30f and pbpC_31r, cut with KpnI and EcoRI, and ligated into equally treated pWS52.
pWS63	*pbpZ* was PCR-amplified from CB15N chrom. DNA using primers pbpZ_28f and cc3570_21r, cut with KpnI and EcoRI, and ligated into equally treated pWS52.
pWS64	*pbpC* was PCR-amplified from CB15N chrom. DNA using primers pbpC_32f and pbpC_31r, cut with KpnI and EcoRI, and ligated into equally treated pUT18C.
pWS65	*pbpC* was PCR-amplified from CB15N chrom. DNA using primers pbpC_32f and pbpC_31r, cut with KpnI and EcoRI, and ligated into equally treated pKT25.
pWS66	*pbpY* was PCR-amplified from CB15N chrom. DNA using primers pbpY_35f and pbpY_337r, cut with KpnI and EcoRI, and ligated into equally treated pUT18C.
pWS67	*pbpY* was PCR-amplified from CB15N chrom. DNA using primers pbpY_35f and pbpY_337r, cut with KpnI and EcoRI, and ligated into equally treated pKT25.
pWS68	*pbpX* was PCR-amplified from CB15N chrom. DNA using primers pbpX_36f and pbpX_8r, cut with NdeI and EcoRI, and ligated into AseI/EcoRI-treated pAMIOL-4.
pWS69	*pbpY* was PCR-amplified from CB15N chrom. DNA using primers pbpY_37f and pbpY_6r, cut with NdeI and EcoRI, and ligated into AseI/EcoRI-treated pAMIOL-4.
pWS70	*pbp1a* was PCR-amplified from CB15N chrom. DNA using primers pbp1a_38f and pbp1a_18r, cut with NdeI and EcoRI, and ligated into AseI/EcoRI-treated pAMIOL-4.
pWS71	*pbpC* was PCR-amplified from CB15N chrom. DNA using primers pbpC_39f and pbpC_31r, cut with NdeI and EcoRI ,and ligated into AseI/EcoRI-treated pAMIOL-4.
pWS72	*pbpZ* was PCR-amplified from CB15N chrom. DNA using primers pbpZ_40f and pbpZ_21r, cut with NdeI and EcoRI, and ligated into AseI/EcoRI-treated pAMIOL-4.
pWS73	pWS52 was restricted with KpnI/EcoRI, treated with T4 DNA polymerase, and self-ligated.

Table S4. Oligonucleotides

Restriction sites are indicated by capital letters.

Designation	Sequence (5' - 3')
oKK1	tatGGTACCatgtctgatcctaccgaccctgcagcga
oKK2	tataGCTAGCtcaggggcgcggctccggc
oKK6	tataGCTAGCctattctcccttgatccgcaccttggcg
oKK7	atAAGCCTtggcgctcatcagcacgagatcgacc
oKK8	atGGATTCcggatttccgccgaagggtccgttc
oKK9	atGGATCCgagcctccgcccgcggaccagttgc
oKK10	atGAATTCagcgcgaggccctgacgcttctgac
oKK18	aaatAAGCTTggcgttggcccagcgggtgtacatgc
oKK19	tttaaGCTAGCacacaaaagccggatccgctgagcggg
oKK20	aaattGCTAGCgatcgccgacagcaccgccacgccc
oKK21	atGAATTCaccgggcggtcagccagatcctgct
oKK23	aaatAAGCTTttgagcaatgtccccaagcattcgg
oKK24	aaGAATTCgggaacgtcgtctgcagggtcggta
oKK25	aaGAATTCccgctggcccccccgccgccggagc
oKK26	aatttGCTAGCccgtaggccaggatgcgggcgttcg
oKK27	tttaAAGCTTcgatgcaggagcgctcggtggccct
oKK28	aaGAATTCacgggcttcatctccccaaaactca
oKK29	aaGAATTCgtgaccgccaaggtgcggatcaagg
oKK30	aaattGCTAGCggtcaagcacatcatggtcgtgggc
oSS192	atGAATTCtcatcgcaacgccccctggacttg
oSS206	atatAGATCTCatgacggcggctggcgtcttcaatc
pbpX-for	tatAGATCTatggcgaacggacccttcggcgga
pbpX-rev	tataGCTAGCctagtacggcaactggtccgcgggc
pbpX_7f	atatGGTACCgatggcgaacggacccttcgg
pbpX_8r	atatGAATTCctagtacggcaactggtccgcgg
pbpX_16f	atatGGTACCatggcgaacggacccttcggcgga
pbpX_24r	atatCATATGgatcgtattgttcctaccgcacagac
pbpX_25f	atatAAGCTTgcttccgggttcacctccgg
pbpX_36f	atatCATATGgcgaacggacccttcgg
pbp1a_17f	atatGGTACCgatgtctgatcctaccgaccctgcag
pbp1a_18r	atatGAATTCtcaggggcgcggctccg
pbp1a_29f	atatGGTACCatgtctgatcctaccgaccctgcag
pbp1a_38f	atatCATATGtctgatcctaccgaccctgcagac
pbpY_6r	atatGAATTCtcagtaaagcccgctcagcggatc
pbpY_26r	atatCATATGgcgacctccgtcgaacggg
pbpY_27f	atatAAGCTTcgccgactccggtccggac
pbpY_35f	atatGGTACCgatggatctgaaacccaccgaacgatg
pbpY_37f	atatCATATGgatctgaaacccaccgaacgatg
pbpY_323f	aGGTACCatggatctgaaacccaccgaacgatgg
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pbpC_for	ttGGTACCatgaacgactggacgctgccgcccta
pbpC_rev2	tataGCTAGCctagtagggcaggttgtccgggggcgg
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pbpC_31r	atatGAATTCctagtagggcaggttgtccgggg
pbpC_32f	atatGGTACCgatgaacgactggacgctgccg
pbpC_39f	atatCATATGaacgactggacgctgccgc
pbpZ_21r	atatGAATTCCtattctcccttgatccgcaccttggc
pbpZ_22f	atatGGTACCgatgtcatcccggacgacccgc
pbpZ_28f	atatGGTACCatgtcatcccggacgacccgc
pbpZ_40f	atatCATATGtcatcccggacgacccg
pbpZ_328f	aGGTACCatgtcatcccggacgacccgcagggtc
PiolC3-for2	tatAAGCTTcaatcccactggaccatatgttcc
PiolC3-rev	tatCATATGtccggttccacgtcactctgcg
ftsZ-1	ttttCATATGgctatttctctttccgcgccgc
ftsZs-revNheI	tatGCTAGCtcacttcgaagtggaagggcttgg
cc3570_21r	atatGAATTCctattctcccttgatccgcaccttggc
iolPuni_72f	aaaAAGCTTgggaaacctgtacgcagagagtcgg
iolPrev_73r	tATTAATggttccacgtcactctgcgaccggc

Table S5. Muropeptide analysis

		Muropeptide composition of peptidoglycan isolated from the indicated strains (%)					
	Relative PG content (%)	**Tetra**	**PentaGly**	**Penta**	**Tetra-PentaGly**	**Tetra-Tetra**	**Tetra-Penta**
WT	100	28	12	15	8	13	8
WS070 (PbpX)	111	30	18	13	11	7	8
WS071 (PbpY)	65	33	20	15	9	7	6
WS072 (Pbp1A)	58	26	23	20	8	5	5
WS073 (PbpC)	66	24	21	14	12	9	7
WS074 (PbpZ)	34	17	29	16	14	5	7

SUPPLEMENTAL FIGURES

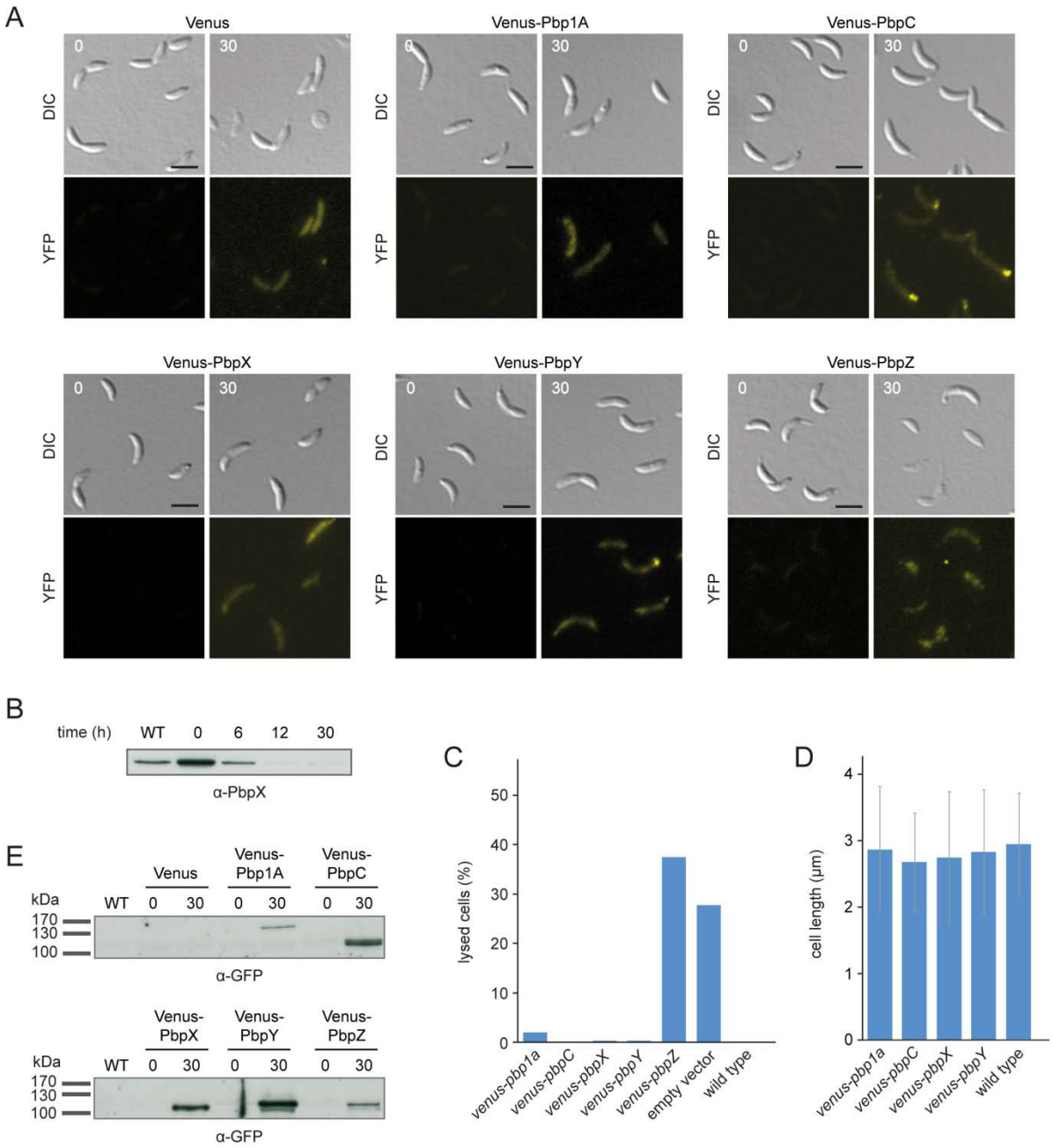

Figure S1. Functionality of fluorescently tagged bPBP derivatives. (A) Microscopic analysis of bPBP-deficient cells expressing single Venus-bPBP fusions. Strains carrying inositol-inducible copies of *venus* (WS075), *venus-pbp1a* (WS066), *venus-pbpC* (WS067), *venus-pbpX* (WS064), *venus-pbpY* (WS065) or *venus-pbpZ* (WS068) were grown in PYE medium containing 0.3 % xylose, washed, and transferred into PYE medium supplemented with 0.3 % myo-inositol (t = 0 h) and cultivated for another 30 h. At the indicated time points, cells were analyzed by DIC and fluorescence microscopy. The cultures were diluted when necessary to ensure exponential growth throughout the course of the experiment. Scale bar: 3 µm. **(B)** Time course of PbpX depletion. Cells of strain WS076 (CB15N Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P_{xyl}::P_{xyl}-*pbpX* P_{iol}::pAMIOL-4) were grown in PYE medium containing 0.3 % xylose, washed, and transferred into PYE medium containing 0.3 % myo-inositol (t = 0 h). At the indicated time points, samples were taken and analyzed by immunoblotting with anti-PbpX antiserum. **(C)** Quantification of cell lysis in the cultures described in (A). At t = 30 h, the fraction of lysed (ghost) cells was determined for each strain (n > 300). **(D)** Average length of the cells in the cultures described in (A). Measurements were performed at t = 30 h (n = 100 per strain; error bars = SD). **(E)** Immunoblot analysis of the strains described in (A) at t = 0 h and t = 30 h.

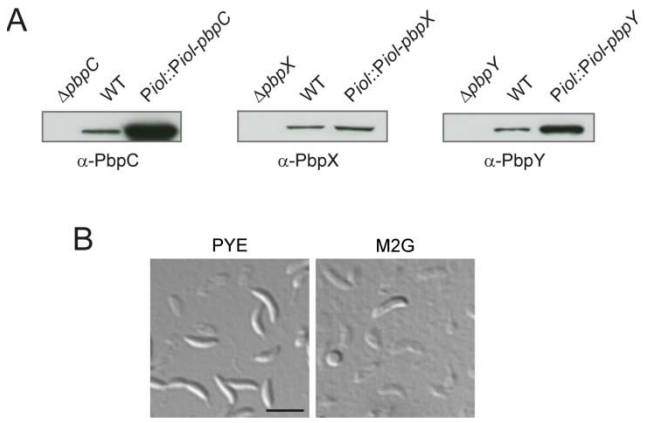

Figure S2. Expression levels of bPBPs and complementation analysis with PbpC. (A) Levels of PbpC, PbpX and PbpY upon expression from the native or inositol-inducible promoters. Strains CB15N (WT), WS073 (Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P_{xyl}::P_{xyl}-*pbpX* P_{iol}::P_{iol}-*pbpC*), WS070 (Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P_{xyl}::P_{xyl}-*pbpX* P_{iol}::P_{iol}-*pbpX*), and WS071 (Δ*pbp1a* Δ*pbpC* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P_{xyl}::P_{xyl}-*pbpX* P_{iol}::P_{iol}-*pbpY*) were grown in PYE medium containing 0.3 % xylose, washed, and transferred into PYE medium containing 0.3 % myo-inositol. After 30 h of cultivation in exponential phase, samples were taken and analyzed by immunoblotting with the indicated antisera. Strains MT282 (Δ*pbpC*), KK1 (Δ*pbpX*) and KK16 (Δ*pbpY*) were used to control for the specificity of the antibodies. **(B)** Complementation analysis with PbpC. Cells of strain WS090 (Δ*pbp1a* Δ*pbpX* Δ*pbpY* Δ*pbpZ* P_{xyl}::P_{xyl}-*pbpX)* were grown in PYE and M2G medium, respectively, containing 0.3 % xylose. Cells were washed, transferred into the same medium lacking inducer, and visualized by DIC microscopy after 45 h of exponential growth. Scale bar: 3 µm.

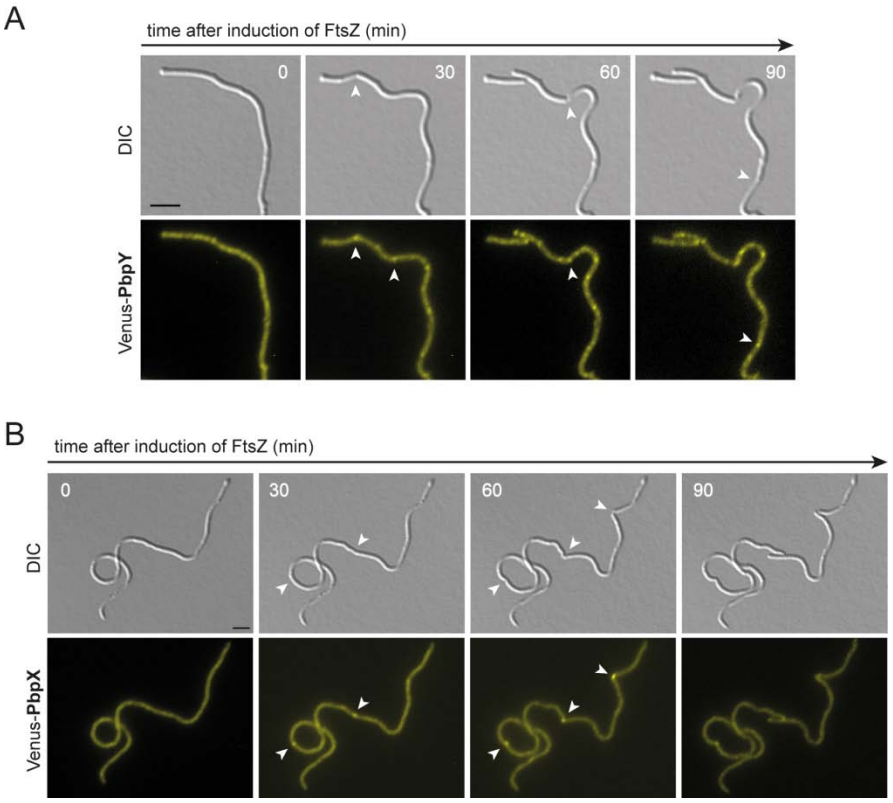

Figure S3. Dependency of PbpX and PbpY midcell localization on the cell division protein FtsZ. Cells of **(A)** strain WS062 *(ftsZ::*P_{iol}*-ftsZ* P_{xyl}*::*P_{xyl}*-venus-pbpX)* and **(B)** WS063 (*ftsZ*::P_{iol}*-ftsZ* P_{xyl}::P_{xyl}*-venus-pbpY*) were grown in M2G medium containing 0.3 % inositol, washed three times with M2 salts, and subsequently grown for 9 h in M2G medium lacking inducer to deplete FtsZ. Three hours prior to analysis, expression of *venus-pbpX* and *venus-pbpY*, respectively, was induced by addition of 0.3 % xylose. Cells were transferred onto M2G-agarose pads supplemented with 0.3 % xylose and 0.3 % inositol and visualized at the indicated time points by DIC and fluorescence microscopy. Scale bar: 3 µm.

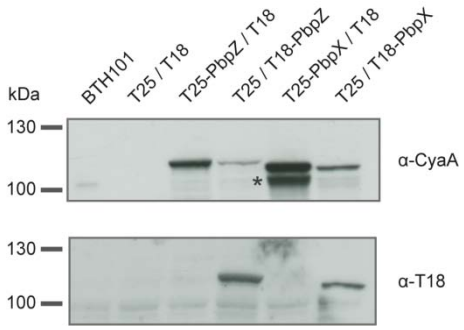

Figure S4. Expression levels of bacterial two-hybrid fusion proteins. Cells of *E. coli* BTH101 harboring pWS48 (pKT25-*pbpZ*), pWS49 (pUT18C-*pbpZ*), pWS30 (pKT25-*pbpX*), pWS34 (pUT18C-*pbpX*) or the empty pKT25 and pUT18C plasmids, respectively, were grown to exponential phase in LB medium supplemented with the respective antibiotics. Expression of the hybrid genes was induced with 0.5 mM IPTG for 3 h. Subsequently, samples were withdrawn and analyzed by immunoblotting with an anti-CyaA antibody (serum L24023 (8)), which recognizes both the T25 and, less efficently, the T18 fragment (α-CyaA), and a monoclonal antibody directed against the C-terminal region of the T18 fragment (α-T18). The asterisk indicates a degradation product of T25-PbpX.

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