Table S1. Strains used in the study

Strains	Description	Source
CB15N	Synchronizable derivative of CB15	1
CJW2141	*pbp3Y270N* (PBP3^{Ts}) *CC2570::Gent*	2
LS117	*Δbla*	Alley, MRK
ZG1020	*ΔpbpX*	This study
ZG1021	*Δpbp1a*	This study
ZG1022	*ΔpbpY*	This study
ZG1023	*ΔpbpC*	This study
ZG1024	*ΔpbpZ*	This study
ZG1025	*ΔmtgA*	This study
ZG1026	*ΔpbpX Δpbp1a*	This study
ZG1027	*ΔpbpX ΔpbpY*	This study
ZG1028	*ΔpbpX ΔpbpC*	This study
ZG1029	*ΔpbpX ΔpbpZ*	This study
ZG1030	*ΔpbpX ΔmtgA*	This study
ZG1031	*Δpbp1a ΔpbpY*	This study
ZG1032	*Δpbp1a ΔpbpC*	This study
ZG1033	*Δpbp1a ΔpbpZ*	This study
ZG1034	*Δpbp1a ΔmtgA*	This study
ZG1035	*ΔpbpY ΔpbpC*	This study
ZG1036	*ΔpbpY ΔpbpZ*	This study
ZG1037	*ΔpbpY ΔmtgA*	This study
ZG1038	*ΔpbpC ΔpbpZ*	This study
ZG1039	*ΔpbpC ΔmtgA*	This study
ZG1040	*ΔpbpC ΔmtgA*	This study
ZG1041	*Δbla ΔpbpX*	This study
ZG1042	*Δbla Δpbp1a*	This study
ZG1043	*Δbla ΔpbpY*	This study
ZG1044	*Δbla ΔpbpC*	This study
ZG1045	*Δbla ΔpbpZ*	This study
ZG1046	*Δbla ΔmtgA*	This study
ZG1047	P_{pbpX}*::msfGFP-pbpX*	This study
ZG1048	P_{pbpY}*::msfGFP-pbpY*	This study
ZG1049	P_{pbpC}*::msfGFP-pbpC*	This study
ZG1050	*Δpbp1a ΔpbpY ΔpbpC ΔpbpZ ΔmtgA*	This study
ZG1051	P_{pbpX}*::pbpX (E101Q E158Q)*	This study
ZG1052	P_{pbpX}*::pbpXS366A*	This study
ZG1065	CB15N *pbp3Y270N CC2570::Gent*	This study
ZG1066	*ΔpbpX pbp3Y270N CC2570::Gent*	This study
ZG1067	*Δpbp1a pbp3Y270N CC2570::Gent*	This study
ZG1068	*ΔpbpY pbp3Y270N CC2570::Gent*	This study
ZG1069	*ΔpbpC pbp3Y270N CC2570::Gent*	This study
ZG1070	*ΔpbpZ pbp3Y270N CC2570::Gent*	This study

ZG1071	*ΔmtgA pbp3Y270N CC2570::Gent*	This study
ZG1072	P_{pbp1a}*::msfGFP-pbp1a*	This study
ZG1073	P_{pbp1a}*::msfGFP-pbp1a ΔpbpX ΔpbpY*	This study
ZG1074	P_{pbpC}*::msfGFP-pbpC ΔpbpX ΔpbpY*	This study
ZG1150	P_{xyl}*::pbpX Δpbp1a ΔpbpY ΔpbpC ΔpbpZ ΔmtgA*	This study

Table S2. Plasmids used in the study

Plasmids	Description	Source
pNPTS138	Vector for making deletion constructs	Alley, MRK
pNPTS-msfGFP-pbp1a	For making native-site msfGFP-PBP1A fusion	This study
pNPTS-msfGFP-pbpC	For making native-site msfGFP-PbpC fusion	This study
pNPTS-msfGFP-pbpX	For making native-site msfGFP-PbpX fusion	This study
pNPTS-msfGFP-pbpY	For making native-site msfGFP-PbpY fusion	This study
pNPTS-mtgA	For deletion of *mtgA*	This study
pNPTS-pbp1a	For deletion of *pbp1a*	This study
pNPTS-pbpC	For deletion of *pbpC*	This study
pNPTS-pbpX	For deletion of *pbpX*	This study
pNPTS-pbpX (E101Q E158Q)	For allelic replacement of *pbpX* with E101Q E158Q double mutant variant	This study
pNPTS-pbpX S366A	For allelic replacement of *pbpX* with S366A mutant variant	This study
pNPTS-pbpY	For deletion of *pbpY*	This study
pNPTS-pbpZ	For deletion of *pbpZ*	This study
pRXMCS-5	Low copy vector for xylose-inducible expression	3
pRX-pbpX (E101Q E158Q)	PbpX (E101Q E158Q) expression vector	This study
pXMCS-5	Integrating vector for constructing xylose-regulated depletions by single recombination	3
pXyl-pbpX'	For depletion of *pbpX*	This study

Table S3. Primers used in the study

Primer	Sequence
0252 DUR	CTCTAGTACGGCAACTGGTCGAAGGGTCCGTTCGCCATGATC
0252E101Q R	CTGGCGGTCTTGGATGGCGATGAACGCCGCCGGGAC
0252E101Q F	CATCGCCATCCAAGACCGCCAGTTCTATCAC
0252E158Q R	CAGGATCAGCTGCTGGGCCTTGCGGCGATAGTTC
0252E158Q F	CAAGGCCCAGCAGCTGATCCTGGCCATCTGG
0252S366A R	CTTGAAGGCCGCTCCGGCCTGACGACGGGCGGTG
0252S366A F	GTCAGGCCGGAGCGGCCTTCAAGCCGTTCGTC
0252 UDF	GATCATGGCGAACGGACCCTTCGACCAGTTGCCGTACTAGAG
0252U-sfgfp F	CAATACGATCATGAGTAAAGGTGAAGAACTGTTC
1516 DUR	GGTTCGTCGGTAGGCGAGGTGTCGGTAGGATCAGACATAGG
1516 UDF	CCTATGTCTGATCCTACCGACACCTCGCCTACCGACGAACC
1516U-sfgfp F	CGCCAAGCCTATGAGTAAAGGTGAAGAACTGTTC
1875 DUR	GGCTTTTGTGTGGGCTTACCGATGGCCATCCATCGTTCGG
1875 UDF	CCGAACGATGGATGGCCATCGGTAAGCCCACACAAAAGCC
1875U-sfgfp F	CGAACGATGGATGAGTAAAGGTGAAGAACTGTTC
3277 DUR	CTCTAGTAGGGCAGGTTGTCCCCGTCGTCGAACTTGTAGG
3277 UDF	CCTACAAGTTCGACGACGGGGACAACCTGCCCTACTAGAG
3277U-sfgfp F	GACGCAAACCGTGAGTAAAGGTGAAGAACTGTTC
3570 DUR	CTCCCTTGATCCGCACCTTGGCGATCAGGACCTTGGTCAGCC
3570 UDF	GGCTGACCAAGGTCCTGATCGCCAAGGTGCGGATCAAGGGAG
AgeI-0252 R	CTACCGGTGGCCTCTCTAGTACGGCAAC
EcoRI-0252 Fint180	TTGAATTCGCCCTCGATCAACTATCTGGAC
EcoRI-0252 UF	TTGAATTCGGATGACGTTGAAGGCGATC
EcoRI-0252 UF550	TTGAATTCGGAAGTGGCTGGAGTTGTCG
EcoRI-1516 UF	TTGAATTCCGACAAGGAGAACATCCTCG
EcoRI-1875 UF	TTGAATTCGAAGGTGGTCGTGATCGACG
EcoRI-3277 UF	TTGAATTCGTCTCGCGCTTCAACGCCAAG
EcoRI-3570 UF	TTGAATTCGGCTGGAGAACTCGGTCAAG
EcoRI-KpnI-0252 F	TTGAATTCTTGGTACCATGGCGAACGGACCCTTCGG
EcoRI-KpnI-1516 F	TTGAATTCTTGGTACCATGTCTGATCCTACCGACCC
EcoRI-KpnI-1875 F	TTGAATTCTTGGTACCATGGCCATCGCGGGCGTGGCGGTG
EcoRI-KpnI-3277 F	TTGAATTCTTGGTACCATGAACGACTGGACGCTGCCG
EcoRI-mtgA UF	CAGAATTCCAGCTTCCACGTGAGCGTGTG
KpnI-sfgfp R	TTGGTACCGCTGCCTTTGTAGAGTTCATCCATGCC
MCSC-EcoRI-0252 UF	GTATCGATAAGCTTGATATCGAATTCCAGGATCTTCTGGGTCTCG
MCSC-EcoRI-1875 UF	GTATCGATAAGCTTGATATCGAATTCGAAGGTGGTCGTGATCGACG
MCSC-SpeI-0252 R	GTGGCGGCCGCTCTAGAACTAGTCCTCTCTAGTACGGCAACTGG
MCSC-SpeI-1875 R	GTGGCGGCCGCTCTAGAACTAGTCCTCGCGCAATGGACGTCAG
mtgA DUR	CTAAGCCACGCAGTCAGCCAGCACGAAACGCCCCACTTGTC
mtgA UDF	GACAAGTGGGGCGTTTCGTGCTGGCTGACTGCGTGGCTTAG

NdeI-0252 F	TTTTCATATGGCGAACGGACCCTTCGG
NheI-0252 Rint	CAGCTAGCTTAGATCGAGGGCTGACGCTTGAC
sfgfp-0252 UR	CTTCACCTTTACTCATGATCGTATTGTTCCTAC
sfgfp-1516 UR	CTTCACCTTTACTCATAGGCTTGGCGCTCGCTTC
sfgfp-1875 UR	CTTCACCTTTACTCATCCATCGTTCGGTGGGTTTC
sfgfp-3277 UR	CTTCACCTTTACTCACGGTTTGCGTCAGGCTCTG
SpeI-0252 DR	TTACTAGTCACGCGATCTAGAGGCGATG
SpeI-0252 Rint1340	TTACTAGTCCAGCTGGATCTTGCTGGTG
SpeI-1516 DR	TTACTAGTGATGGTGCCGACGATGGCGTTG
SpeI-1516 Rint	TTACTAGTCCAGTTGCTGGGTGATGGTCGAG
SpeI-1875 DR	TTACTAGTGGAATGTGCGTGAGACGCAC
SpeI-3277 DR	TTACTAGTGAGCGAACAGGCCTTGATCG
SpeI-3277 R	TTACTAGTCCTAACGCGCTCTAGTAGGG
SpeI-3570 DR	TTACTAGTCGCTTCAATGTCGACCTGTC
SpeI-mtgA DR	TTACTAGTCGGCCATCGCCTTGATCAAG

Strain and Plasmid Construction

Strains

ZG1020-1025: For the single deletions of the GTase-encoding genes, the pNPTS-pbpX, pNPTS-pbp1a, pNPTS-pbpY, pNPTS-pbpC, pNPTS-pbpZ, and pNTPS-mtgA were integrated into CB15N chromosome. Then, the first integrants were submitted to counterselection with sucrose, and Suc^R colonies were screened for Kan^S. Finally, the Kan^S colonies were screened by PCR to identify those that contained the appropriate deletions.

ZG1026-1046: For the double deletions of the GTase genes and for the double mutants in a TG gene and *bla*, pNPTS vectors originally used to construct the GTase deletion strains were re-integrated into the corresponding deletion strains for the use as a selective marker (for example, pNPTS-pbp1a was re-integrated into *Δpbp1a*). *φCr30* phage lysates were subsequently made from these integrants and used to transduce either *Δbla* or another single GTase mutant. The integrated plasmid was then cured by counterselection with sucrose, and Suc^R Kan^S colonies were isolated. In the case of several strains encoding *ΔpbpX* as one of the two mutations, the incidence of suppressor mutants necessitated using double homologous recombination with pNPTS-pbpX vector to introduce *ΔpbpX* into other genetic backgrounds, as described for the single deletion (ZG1020). All double mutants were checked by PCR to ensure the presence of the desired deletions.

ZG1047-1049, ZG1072: For the construction of msfGFP fusions to GTases encoded at the native loci, pNPTS-msfGFP-pbpX, pNPTS-msfGFP-pbp1a, pNPTS-msfGFP-pbpY, or pNPTS-msfGFP-pbpC were integrated into CB15N chromosome, and the integrants were subjected to sucrose counterselection. Suc^R Kan^S colonies were screened by PCR to confirm the presence of msfGFP.

ZG1050: For the construction of the PbpX-Only (*Δpbp1a ΔpbpY ΔpbpC ΔpbpZ ΔmtgA*) strain, the procedure described above for constructing double GTase deletions was repeated a total of four times to introduce additional mutations into the original single deletion background. The presence of all desired deletions in the final strain was confirmed by PCR.

ZG1051-1052: For the allelic substitutions of *pbpX* with mutant variants, pNPTS-pbpX(E101Q E158Q) and pNPTS-pbpXS366A 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 *pbpX*-specific primers, and the PCR products were sequenced to screen for the presence of the desired mutations.

ZG1065-1071: For the construction of strains with $PBP3^{Ts}$ genotype, the *CC2570::Gent* gene insert linked to the *pbp3* gene in CJW2141 was transduced into CB15N and the single GTase deletion mutants, selecting for gentamycin resistance. The resulting $Gent^R$

colonies were patched in duplicate onto PYE + gentamycin plates and incubated at RT and 37°C to screen for temperature sensitivity.

ZG1073-4: For the construction of strains encoding msfGFP fusions to PBP1A or PbpC in *ΔpbpX ΔpbpY* mutant background, ZG1072 and ZG1049, respectively, were successively transduced with lysates produced from ZG1020 with reintegrated pNPTS-pbpX and ZG1022 with reintegrated pNPTS-pbpY. Both plasmids were cured through counterselection for Suc^R and screening for Kan^S colonies.

ZG1150: To construct PbpX depletion strain in the *Δpbp1a ΔpbpY ΔpbpC ΔpbpZ ΔmtgA* mutant background, pXyl-pbpX' plasmid was integrated into the PbpX-Only strain (ZG1050) by single recombination.

Plasmids

pNPTS-msfGFP-pbp1a: *pbp1a* upstream region (U) was PCR-amplified from CB15N with EcoRI-1516 UF and sfgfp-1516 UR primers. Also, *msfGFP* was PCR-amplified with 1516U-sfgfp F and KpnI-sfgfp R primers, and a 5' fragment of *pbp1a* itself was PCR-amplified with EcoRI-KpnI-1516 F and SpeI-1516 Rint primers. The *pbp1a* gene fragment was ligated into pNPTS138 via EcoRI and SpeI sites. Meanwhile, the U PCR product and *msfGFP* were combined by sewing PCR with EcoRI-1516 UF and KpnI-sfgfp R primers, and the resulting product was cloned into pCR-Blunt-II via TOPO Cloning kit (Invitrogen). Finally, the *CC1516U-msfGFP* insert was subcloned into the pNPTS138 derivative containing *pbp1a* 5' fragment via EcoRI and KpnI sites.

pNPTS-msfGFP-pbpC: *pbpC* upstream region was PCR-amplified from CB15N with EcoRI-3277 UF and sfgfp-3277 UR primers. Also, *msfGFP* was PCR-amplified with 3277U-sfgfp F and KpnI-sfgfp R primers, and *pbpC* itself was PCR-amplified with EcoRI-KpnI-3277 F and SpeI-3277 R primers. The *pbpC* gene PCR product was ligated into pNPTS138 via EcoRI and SpeI sites. Meanwhile, the upstream PCR product and *msfGFP* were combined by sewing PCR with EcoRI-3277 UF and KpnI-sfgfp R primers. Finally, the resulting PCR product was ligated into the pNPTS138 derivative containing *pbpC* via EcoRI and KpnI sites.

pNPTS-msfGFP-pbpX: *pbpX* upstream region was PCR-amplified from CB15N with MCSC-EcoRI-0252 UF and sfgfp-0252 UR primers. Also, *msfGFP* was PCR-amplified with 0252U-sfgfp F and KpnI-sfgfp R primers, and *pbpX* itself was PCR-amplified with EcoRI-KpnI-0252 F and MCSC-SpeI-0252 R primers. The *pbpX* gene PCR product was ligated into pNPTS138 via EcoRI and SpeI sites. Meanwhile, the upstream PCR product and *msfGFP* were combined by sewing PCR with MCS-EcoRI-0252 UF and KpnI-sfgfp R primers. Finally, the resulting PCR product was ligated into the pNPTS138 derivative containing *pbpX* via EcoRI and KpnI sites.

pNPTS-msfGFP-pbpY: *pbpY* upstream region was PCR-amplified from CB15N with MCSC-EcoRI-1875 UF and sfgfp-1875 UR primers. Also, *msfGFP* was PCR-amplified

with 1875U-sfgfp F and KpnI-sfgfp R primers, and *pbpY* itself was PCR-amplified with EcoRI-KpnI-1875 F and MCSC-SpeI-1875 R primers. The *pbpY* gene PCR product was ligated into pNPTS138 via EcoRI and SpeI sites. Meanwhile, the upstream PCR product and *msfGFP* were combined by sewing PCR with MCS-EcoRI-1875 UF and KpnI-sfgfp R primers. Finally, the resulting PCR product was ligated into the pNPTS138 derivative containing *pbpY* via EcoRI and KpnI sites.

pNPTS-mtgA: This plasmid is used to construct an in-frame deletion of *mtgA (CC0325)*, leaving intact only the first 5 and the last 6 amino acid-encoding codons. To make the plasmid, the upstream (U) and downstream (D) regions of *mtgA* were PCR-amplified with EcoRI-mtgA UF and mtgA DUR primers and mtgA UDF and SpeI-mtgA DR primers, respectively. The resulting PCR products were then combined by sewing PCR with EcoRI-mtgA UF and SpeI-mtgA DR primers. Finally, the sewing PCR product was ligated into pNPTS138 plasmid via EcoRI and SpeI sites.

pNPTS-pbp1a: This plasmid is used to construct an in-frame deletion of *pbp1a (CC1516)*, leaving intact only the first 6 and the last 26 codons of the coding sequence. To make the plasmid, the upstream (U) and downstream (D) regions of *pbp1a* were PCR-amplified with EcoRI-1516 UF and 1516 DUR primers and 1516 UDF and SpeI-1516 DR primers, respectively. The resulting U and D PCR products were then combined by sewing PCR with EcoRI-1516 UF and SpeI-1516 DR primers. Finally, the sewing PCR product was ligated into pNPTS138 plasmid via EcoRI and SpeI sites.

pNPTS-pbpC: This plasmid is used to construct an in-frame deletion of *pbpC (CC3277)*, leaving intact the first 14 and the last 5 amino acid-encoding codons. To make the plasmid, the upstream (U) and downstream (D) regions of *pbpC* were PCR-amplified with EcoRI-3277 UF and 3277 DUR primers and 3277 UDF and SpeI-3277 DR primers, respectively. The resulting U and D PCR products were then combined by sewing PCR with EcoRI-3277 UF and SpeI-3277 DR primers. Finally, the sewing PCR product was ligated into pNPTS138 plasmid via EcoRI and SpeI sites.

pNPTS-pbpX: This plasmid is used to construct an in-frame deletion of *pbpX (CC0252),* leaving intact the first 6 and the last 5 codons (not counting the stop codon). To make the plasmid, the upstream (U) and downstream (D) regions of *pbpX* were PCR-amplified with EcoRI-0252 UF and 0252 DUR primers and 0252 UDF and SpeI-0252 DR primers, respectively. The resulting U and D PCR products were then combined by sewing PCR with EcoRI-0252 UF and SpeI-0252 DR primers. Finally, the sewing PCR product was ligated into pNPTS138 plasmid via EcoRI and SpeI sites.

pNPTS-pbpX(E101Q E158Q): 550bp of upstream sequence and the 5' part of *pbpX* up to the mutated 101st codon were PCR-amplified with EcoRI-0252 UF550 and 0252E101Q R primers. The part of *pbpX* between the 101st and 158th mutated codons was amplified with 0252E101Q F and 0252E158Q R primers. Also, the part of *pbpX* between the 158th and 447th codons was amplified with 0252E158Q F and SpeI-0252 Rint1340 primers. Then, sewing PCR was used to combine the first two PCR products via EcoRI-0252 UF550 and 0252E158Q R primers. Finally, this sewing PCR product

was combined with the *pbpX*(158-447) PCR product via another round of sewing PCR, this time using EcoRI-0252 UF550 and SpeI-0252 Rint1340. The final PCR product was ligated into pNPTS138 via EcoRI and SpeI sites.

pNPTS-pbpXS366A: the part of the *pbpX* gene between the 60th and the mutant 366th codons was PCR-amplified with EcoRI-0252 Fint180 and 0252S366A R primers. Also, the 3' part of the gene starting from the mutant 366th codon was amplified with 0252S366A F and MCSC-SpeI-0252 R primers. The two PCR products were combined, using sewing PCR with EcoRI-0252 Fint180 and MCSC-SpeI-0252 R primers. Finally, the resulting sewing PCR product was ligated into pNPTS138 via EcoRI and SpeI sites.

pNPTS-pbpY: This plasmid is used to construct an in-frame deletion of *pbpY (CC1875)*, leaving intact the first 3 and the last 14 amino acid-encoding codons. The upstream (U) and downstream (D) regions of *pbpY* were PCR-amplified with EcoRI-1875 UF and 1875 DUR primers and 1875 UDF and SpeI-1875 DR primers, respectively. The resulting U and D PCR products were then combined by sewing PCR with EcoRI-1875 UF and SpeI-1875 DR primers. Finally, the sewing PCR product was ligated into pNPTS138 plasmid via EcoRI and SpeI sites.

pNPTS-pbpZ: This plasmid is used to construct an in-frame deletion of *pbpZ (CC3570)*, leaving intact the first 22 and the last 8 codons of the coding sequence. The upstream (U) and downstream (D) regions of *pbpZ* were PCR-amplified with EcoRI-3570 UF and 3570 DUR primers and 3570 UDF and SpeI-3570 DR primers, respectively. The resulting U and D PCR products were then combined by sewing PCR with EcoRI-3570 UF and SpeI-3570 DR primers. Finally, the sewing PCR product was ligated into pNPTS138 plasmid via EcoRI and SpeI sites.

pRX-pbpX(E101Q E158Q): *pbpX(E101Q E158Q)* was PCR-amplified from ZG1051 genomic DNA with NdeI-0252 F and AgeI-0252 R primers and cloned into pRXMCS-5 via NdeI and AgeI sites.

pXyl-pbpX': The first 189 bp of *pbpX* coding sequence were amplified from CB15N genomic DNA, using NdeI-0252 F and NheI-0252 Rint primers. The reverse primer introduced a TAA stop codon after the 189-bp gene fragment. The PCR fragment was then cloned into pXMCS-5 via NdeI and NheI sites.

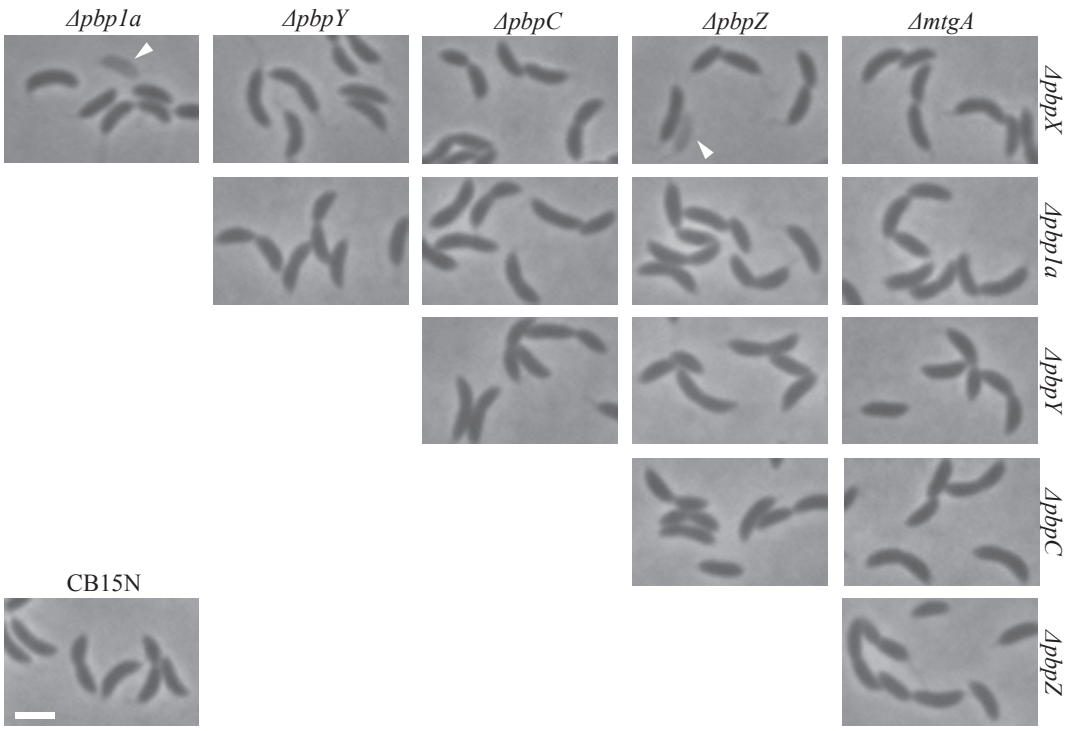
References for Strains and Plasmids

1. Evinger, M., and Agabian, N. Envelope-associated nucleoid from *Caulobacter crescentus* stalked and swarmer cells. *J Bacteriol.* 132(1)**:** 294-301. 1977.

2. Costa, T., Priyadarshini, R., and Jacobs-Wagner, C. Localization of PBP3 in *Caulobacter crescentus* is highly dynamic and largely relies on its functional transpeptidase domain. *Mol Microbiol.* 70(3): 634-51. 2008.

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

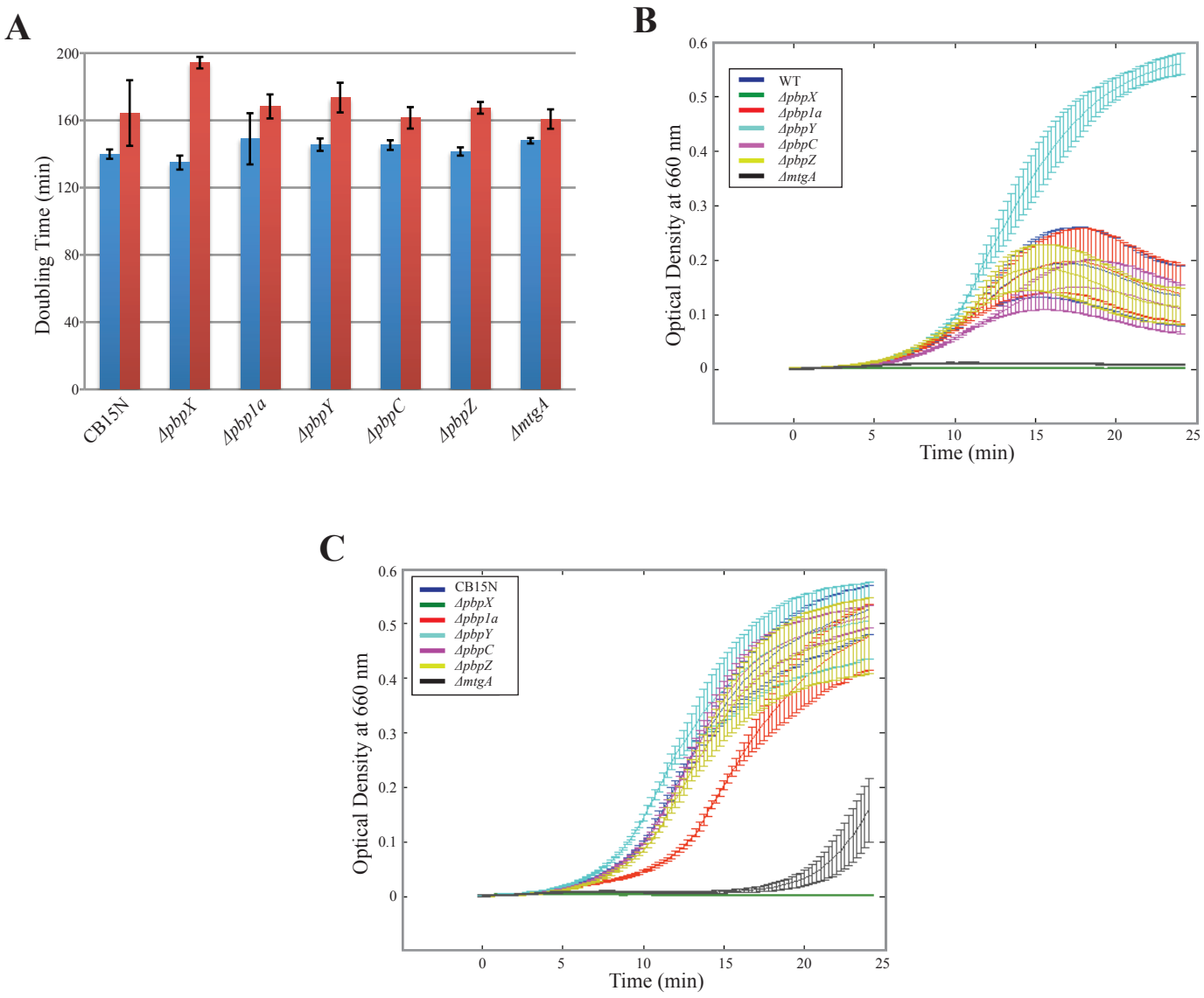
Figure S1



Supplementary Figure 1. Double GTase deletions do not significantly affect cell shape

Phase contrast images of the double deletion mutants arranged as a table. In all cases, arrowheads indicate lysed ghost cells. Wild-type CB15N is provided as a control. The scale bar is 2 μm.

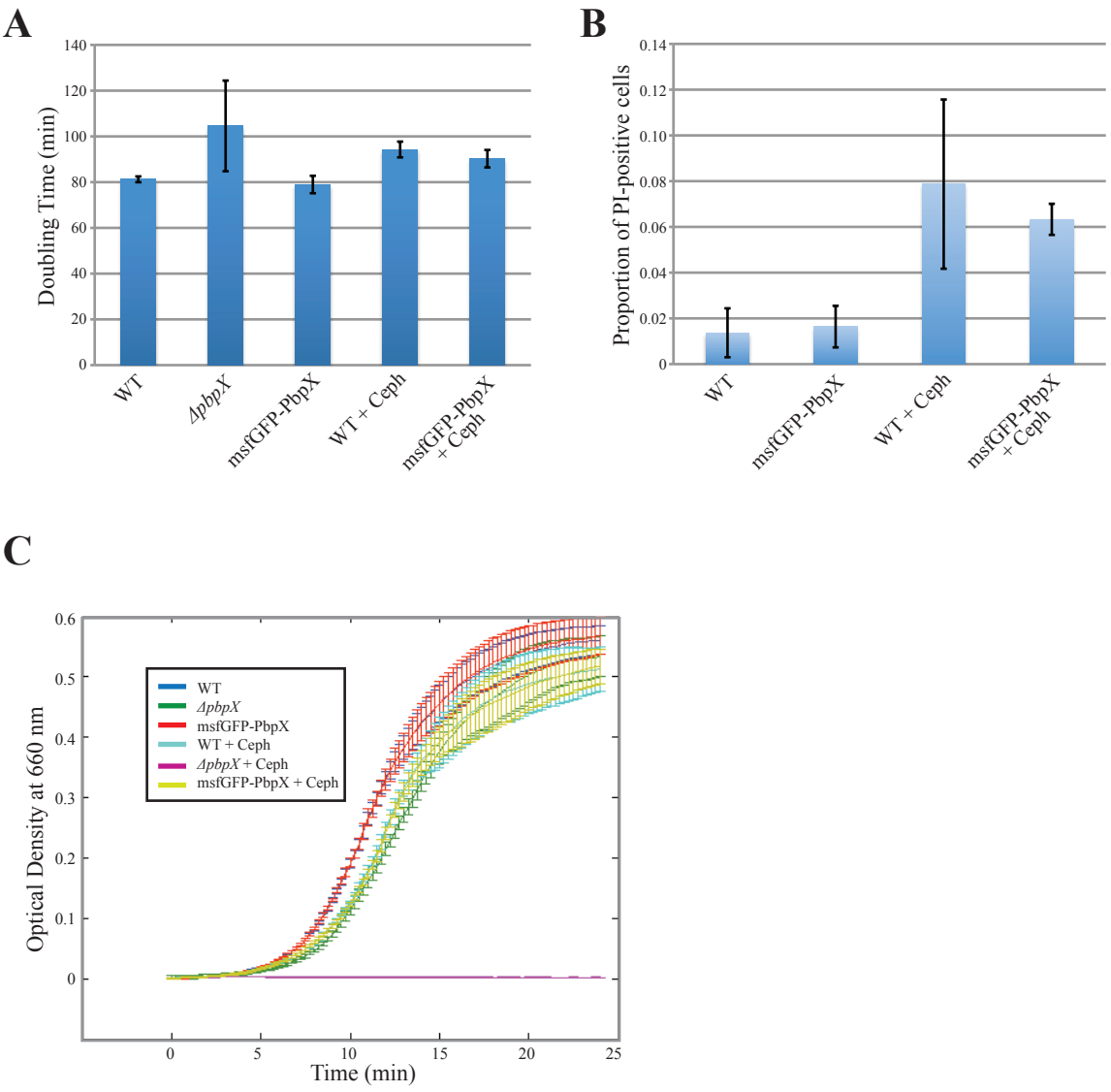
Figure S2



Supplementary Figure 2. *pbpX* deletion sensitizes cells to osmotic shock, mecillinam, and cephalexin

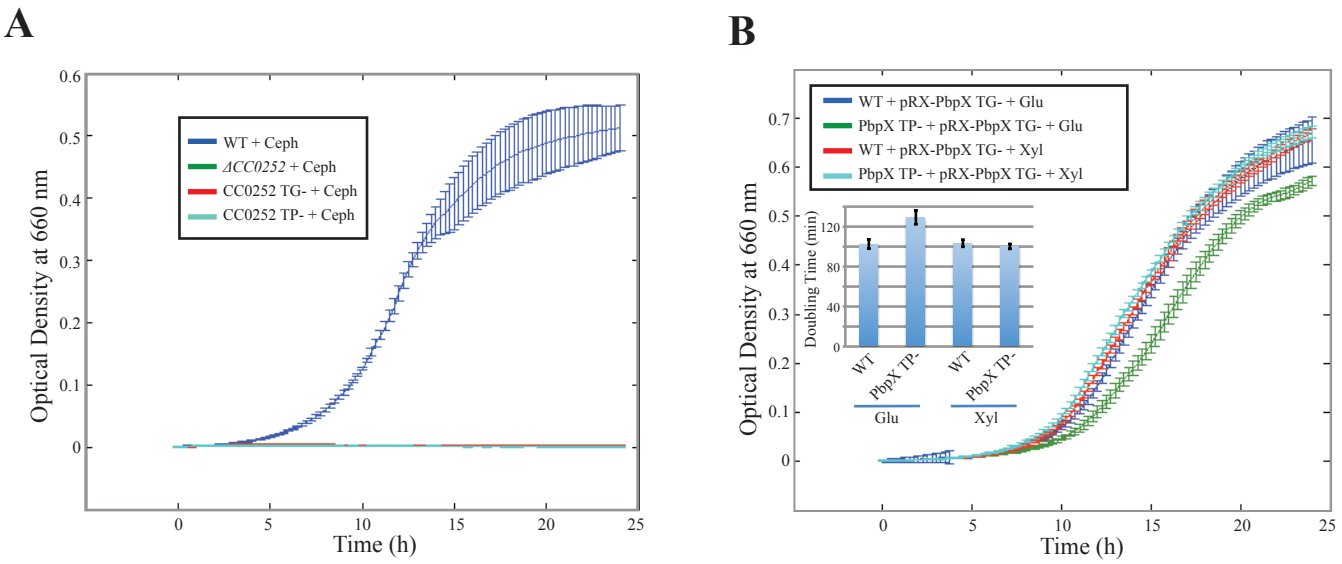
A. Doubling times (in minutes) of the single GTase deletion strains grown in M2G (blue) or M2G with 150 mM sucrose (red). Note that *ΔpbpX* grows at wild-type rate in regular minimal medium, likely due to slower growth lifting some of the strain from the PG remodeling machinery. **B.** Growth curves of wild-type and single TG deletion strains grown with 15 µg/ml mecillinam. To circumvent *Caulobacter*'s natural β-lactam resistance, TG deletions were introduced into *Δbla* genetic background for this experiment. **C.** Growth curves of wild-type (CB15N) and single TG deletion strains grown with 9 µg/ml cephalexin. All values represent the averages of 6 biological replicates.

Figure S3



Supplementary Figure 3. msfGFP-PbpX fusion is fully functional

A. Doubling time of msfGFP-PbpX strain with and without cephalexin. CB15N and *ΔpbpX* growth rates are provided for comparison, but *ΔpbpX* with cephalexin datum was omitted due to no growth. **B.** Proportion of PI-positive msfGFP-PbpX-encoding and wild-type cells with and without cephalexin treatment. **C.** Growth curve of the strain encoding msfGFP-PbpX at the native locus, *ΔpbpX*, and wild-type (CB15N) with and without cephalexin, with each curve showing the average of 14 biological replicates. All error bars represent SD.



Supplementary Figure 4. Transglycosylase and transpeptidase mutants of PbpX are both cephalexin-sensitive and complement each other

A. Growth curve of the TG- and TP- PbpX mutants, *ΔpbpX*, and wild-type (CB15N) grown with cephalexin. **B.** Growth curve of the WT and PbpX TP- strains carrying pRX-PbpX TG- plasmid and grown in the presence of either glucose (no induction) or xylose (induction). The inset shows the doubling times of both strains in both conditions. All error bars represent SD.