SUPPLEMENTARY INFORMATION

FtsW is a peptidoglycan polymerase that is functional only in complex with its cognate penicillin-binding protein

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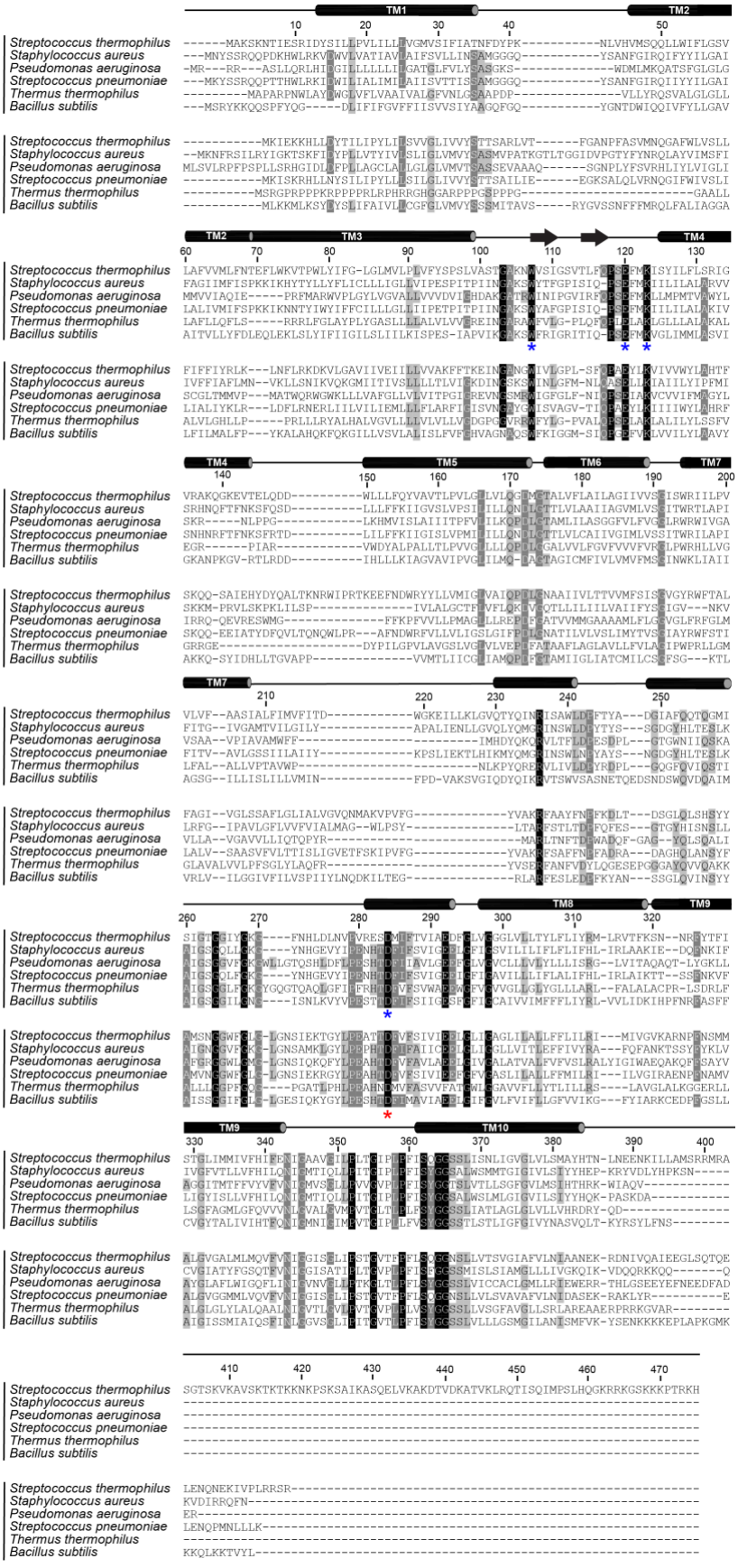
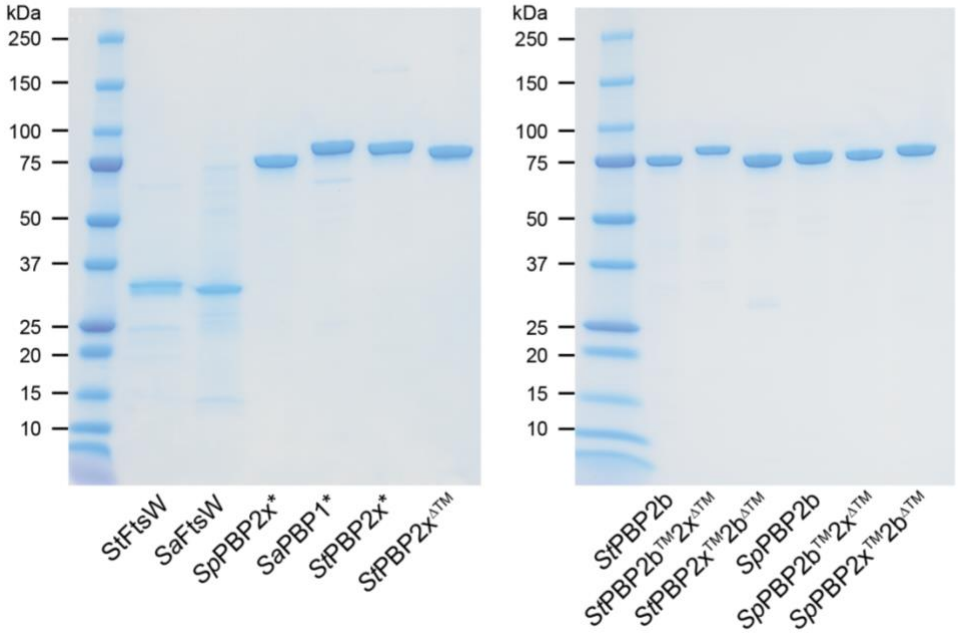

RodA
FtsW
Streptococcus thermophilus
Staphylococcus aureus
Pseudomonas aeruginosa
Streptococcus pneumoniae
Thermus thermophilus
Bacillus subtilis
TM1
TM2
TM3
TM4
TM5
TM6
TM7
TM8
TM9
TM10

Supplementary Figure 1: Sequence alignment of FtsW and RodA. Sequence conservation analysis of 20 RodA sequences and 20 FtsW sequences from diverse taxa was performed as described previously.[1] Representative examples of RodA and FtsW from 6 species are shown. Residues with 98%, 80% and 60% identity in the 40 sequences are colored in black, gray and light gray, respectively. Secondary structure elements are shown above the alignment based on the *T. thermophilus* RodA crystal structure.[1] The red asterisk (*) denotes the conserved aspartate residue mutated in this study. The blue asterisk (*) highlights residues that were previously reported to be essential in *B. subtilis* RodA polymerase activity *in vitro*.[1,2]



Supplementary Figure 2: Coomassie-stained gels of the *Staphylococcus* and *Streptococcus* FtsW and bPBPs with ~2 µg of total protein loaded per lane. Similar results were obtained three times for FtsW and two times for bPBPs.

St = S. thermophilus; *Sp = S. pneumoniae*; *Sa = S. aureus*

*Sp*PBP2x* = *Sp*PBP2x^{S337A}; *Sa*PBP1* = *Sa*PBP1^{S314A}; *St*PBP2x* = *St*PBP2x^{S343A}

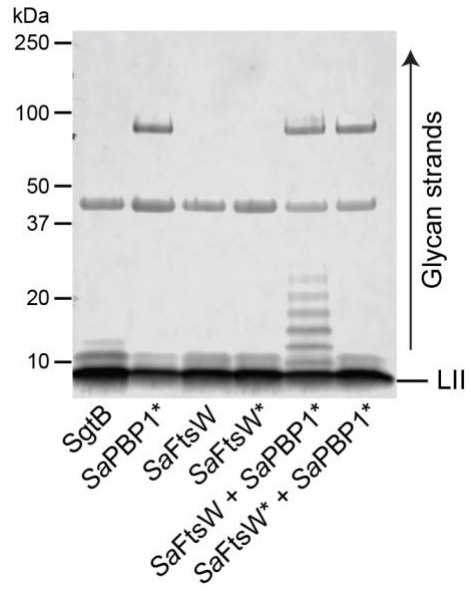
*St*PBP2x$^{\Delta TM}$ = *St*PBP2x$^{G53\text{-}D755}$

*St*PBP2b^{TM}2x$^{\Delta TM}$ = *St*PBP2b$^{M1\text{-}M58}$*St*PBP2x$^{G53\text{-}D755}$

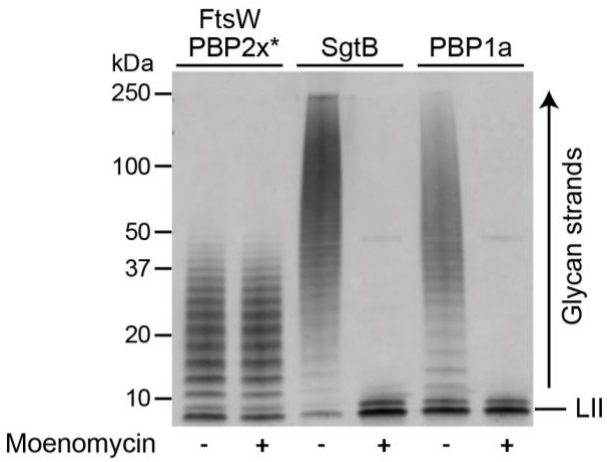
*St*PBP2x^{TM}2b$^{\Delta TM}$ = *St*PBP2x$^{M1\text{-}I52}$*St*PBP2b$^{Q59\text{-}H704}$

*Sp*PBP2b^{TM}2x$^{\Delta TM}$ = *Sp*PBP2b$^{M6\text{-}Y38}$*Sp*PBP2x$^{T50\text{-}D750}$

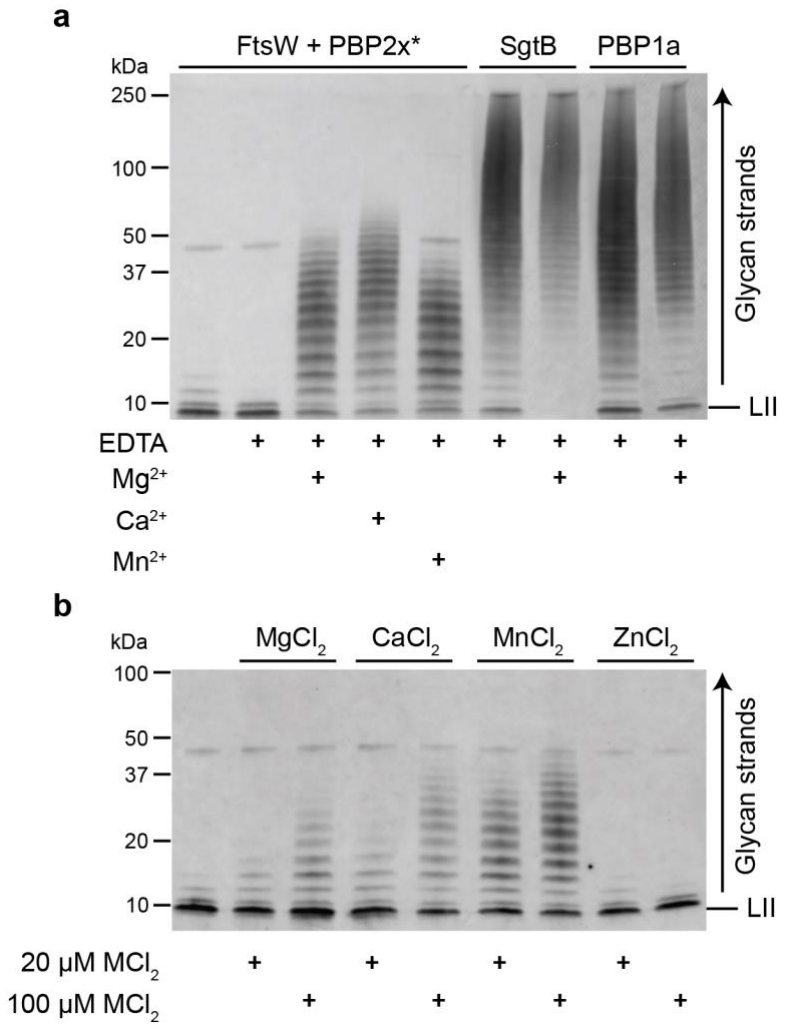
*Sp*PBP2x^{TM}2b$^{\Delta TM}$ = *Sp*PBP2x$^{M1\text{-}G49}$*Sp*PBP2b$^{M39\text{-}N685}$



Supplementary Figure 3: *S. aureus* FtsW polymerizes *S. aureus* Lipid II in the presence of its cognate bPBP. *S. aureus* FtsW (2.5 µM) or SgtB (MGT; 1 µM) was incubated with Lipid II (10 µM) and PBP1* (2.5 µM). Moenomycin (2 µM) was added to all reactions to eliminate background polymerization by aPBPs or MGTs. The resulting polymer was labeled with BDL and detected by western blot as in Fig. 1c. Bands observed around 45 kDa and 85 kDa correspond to *E. faecalis* PBPX and *S. aureus* PBP1*, respectively, which bind BDL. A representative blot of four independent experiments is shown. An asterisk indicates a catalytically inactive variant. FtsW* = *Sa*FtsW^{D287A}; PBP1* = *Sa*PBP1^{S314A}

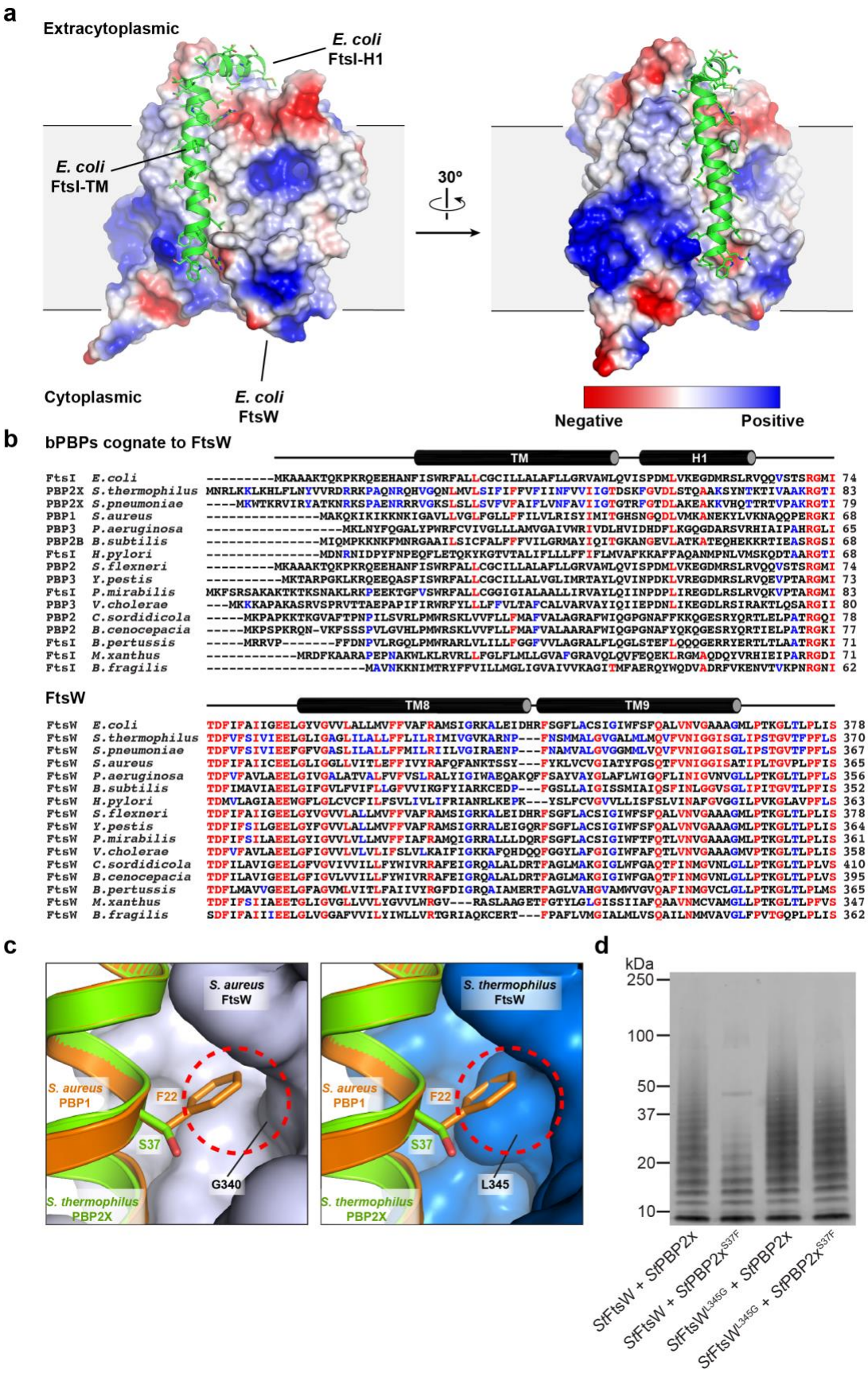


Supplementary Figure 4: *S. thermophilus* FtsW polymerizes *E. faecalis* Lipid II in the presence of moenomycin. *S. thermophilus* FtsW (0.5 µM), *S. aureus* SgtB (MGT; 0.5 µM) and *S. pneumoniae* PBP1a (aPBP; 0.5 µM) were incubated with Lipid II (10 µM). Moenomycin (5 µM) was added to the indicated samples. Polymer was labeled with BDL using *Ef*PBPX and detected by western blot as in Fig 1c. A representative blot of two independent experiments is shown. An asterisk indicates a catalytically inactive variant. PBP2x* = *St*PBP2x^{S343A}

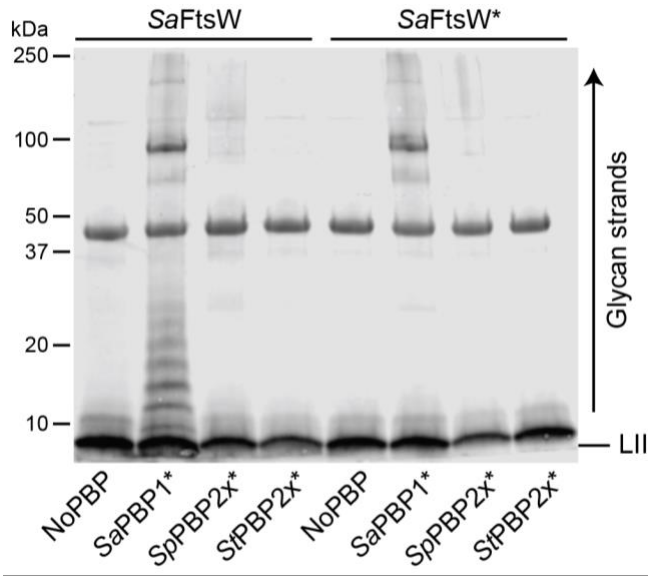


Supplementary Figure 5: *S. thermophilus* FtsW requires divalent cations for PGT activity. (a) *S. thermophilus* FtsW (0.5 µM) and PBP2x* (1 µM) were combined with *E. faecalis* Lipid II (10 µM) in a reaction buffer supplemented with EDTA (10 mM) and the indicated metal chloride (12.5 mM). Analogous reactions were conducted with *S. aureus* SgtB (MGT; 0.5 µM) and *S. pneumoniae* PBP1a (aPBP; 0.5 µM). The polymer products were labeled with BDL using *Ef*PBPX and detected by western blot as in Fig. 1c. A representative blot of three independent experiments is shown. An asterisk indicates a catalytically inactive variant. PBP2x* = *St*PBP2x^{S343A}

(b) *E. faecalis* Lipid II (10 µM) was pre-labeled with BDL (2 mM) using *Ef*PBPX (10 µM) in a reaction buffer supplemented with EDTA (10 µM). After heat-inactivation of *Ef*PBPX, *S. thermophilus* FtsW (0.5 µM) and PBP2x (1 µM) were added to the reaction along with the indicated concentration of metal chloride (MCl_2). The polymerization reaction was quenched after 15 min and the product was detected via western blot. In contrast to other cations tested, Zn^{2+} was unable to rescue polymerase activity. A representative blot of two independent experiments is shown.

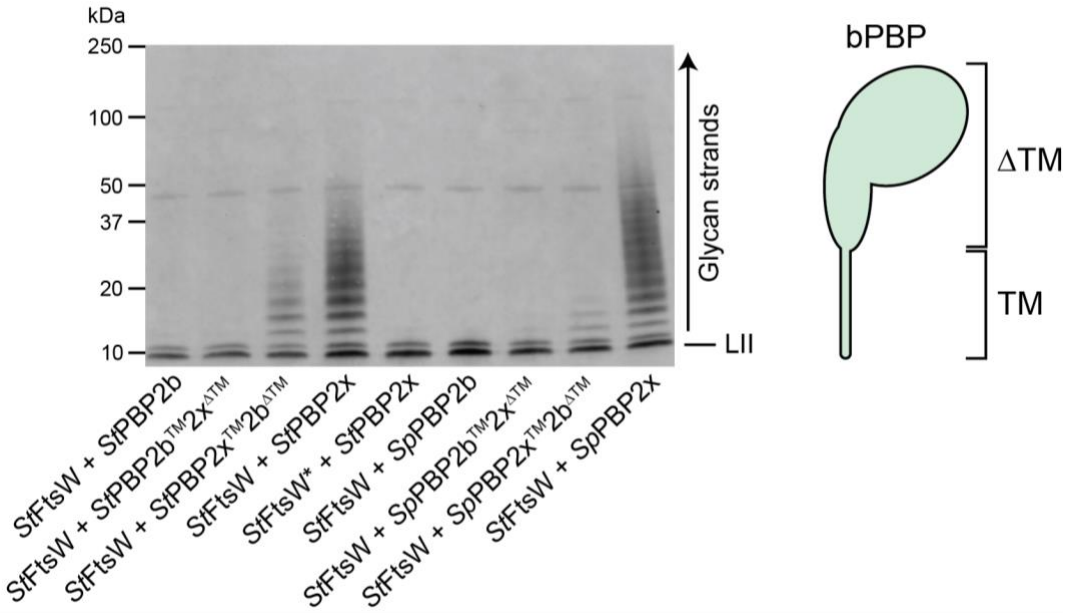

a
Extracytoplasmic
E. coli FtsI-H1
E. coli FtsI-TM
30°
Cytoplasmic
E. coli FtsW
Negative
Positive
b
bPBPs cognate to FtsW
TM
H1
FtsI E.coli ---------MKAAAKTQKPKRQEEHANFISWRFALLCGCILLALAFLLGRVAWLQVISPDMLVKEGDMRSLRVQQVSTSRGMI 74
PBP2X S.thermophilus MNRLKKLKHLFLNYVVRDRRKPAQNRQHVGQNLMVLSIFIFFVFIINFVVIIGTDSKFGVDLSTQAAKSYNTKTIVAAKRGTI 83
PBP2X S.pneumoniae ----MKWTKRVIRYATKNRKSPAENRRRVGKSLSLLSVFVFAIFLVNFAVIIGTGTRFGTDLAKEAKKVHQTTRTVPAKRGTI 79
PBP1 S.aureus ---------------MAKQKIKIKKNKIGAVLLVGLFGLLFFILVLRISYIMITGHSNGQDLVMKANEKYLVKNAQQPERGKI 68
PBP3 P.aeruginosa ------------------MKLNYFQGALYPWRFCVIVGLLLAMVGAIVWRIVDLHVIDHDFLKGQGDARSVRHIAIPAHRGLI 65
PBP2B B.subtilis ---------------MIQMPKKNKFMNRGAAILSICFALFFFVILGRMAYIQITGKANGEVLATKATEQHEKKRTIEASRGSI 68
FtsI H.pylori ---------------MDNRNIDPYFNPEQFLETQKYKGTVTALIFLLLFFIFLMVAFKKAFFAQANMPNLVMSKQDTAARGTI 68
PBP2 S.flexneri ---------MKAAAKTQKPKRQEEHANFISWRFALLCGCILLALAFLLGRVAWLQVISPDMLVKEGDMRSLRVQQVSTSRGMI 74
PBP3 Y.pestis ----------MKTARPGKLKRQEEQASFISWRFALLCGCILLALVGLIMRTAYLQVINPDKLVREGDMRSLRVQEVPTARGMI 73
FtsI P.mirabilis MKFSRSAKAKTKTKSNAKLRKPEEKTGFVSWRFALLCGGIGIALAALLIRVAYLQIINPDPLIREGDLRSLRVQKVPTARGMI 83
PBP3 V.cholerae ---MKKKAPAKASRVSPRVTTAEPAPIFIRWRFYLLLFFVLTAFCALVARVAYIQIIEPDNLIKEGDLRSIRAKTLQSARGII 80
PBP2 C.sordidicola -----MKPAPKKTKGVAFTPNPILSVRLPMWRSKLVVFLLFMAFVALAGRAFWIQGPGNAFFKKQGESRYQRTLELPATRGQI 78
PBP2 B.cenocepacia -----MKPSPKRQN-VKFSSSPVLGVHLPMWRSKLVVFLLFMAFVALAARAFWIQGPGNAFYQKQGESRYQRTIELPATRGKI 77
FtsI B.pertussis -----MRRVP-------FFDNPVLRGQLPMWRARLVLILLFGGFVVLAGRALFLQGLSTEFLQQQGERRYERTLTLAATRGKI 71
FtsI M.xanthus ------------MRDFKAARAPEPNAKWLKLRVRLLFGLFLMLLGVAFGRAVQLQVFEQEKLRGMAQDQYVRHIEIPARRGDI 71
FtsI B.fragilis ----------------------MAVNKKNIMTRYFFVILLMGLIGVAIVVKAGITMFAERQYWQDVADRFVKENVTVKPNRGNI 62
FtsW
TM8
TM9
FtsW E.coli TDFIFAIIGEELGYVGVVLALLMVFFVAFRAMSIGRKALEIDHRFSGFLACSIGIWFSFQALVNVGAAAGMLPTKGLTLPLIS 378
FtsW S.thermophilus TDFVFSIVIEELGLIGAGLILALLFFLILRIMIVGVKARNP---FNSMMALGVGALMLMQVFVNIGGISGLIPSTGVTFPFLS 370
FtsW S.pneumoniae TDFVFSIVIEEFGFVGASLILALLFFMILRIILVGIRAENP---FNAMVALGVGGMMLVQVFVNIGGISGLIPSTGVTFPFLS 367
FtsW S.aureus TDFIFAIICEELGLIGGLLVITLEFFIVYRAFQFANKTSSY---FYKLVCVGIATYFGSQTFVNIGGISATIPLTGVPLPFIS 365
FtsW P.aeruginosa TDFVFAVLAEELGIVGALATVALFVFVSLRALYIGIWAEQAKQFFSAYVAYGLAFLWIGQFLINIGVNVGLLPTKGLTLPFLS 356
FtsW B.subtilis TDFIMAVIAEELGIFGVLFVIFLLGFVVIKGFYIARKCEDP---FGSLLAIGISSMIAIQSFINLGGVSGLIPITGVTLPFIS 352
FtsW H.pylori TDMVLAGIAEEWGFLGLCVCFILFSVLIVLIFRIANRLKEPK---YSLFCVGVVLLISFSLVINAFGVGGILPVKGLAVPFLS 363
FtsW S.flexneri TDFIFAIIGEELGYVGVVLALLMVFFVAFRAMSIGRKALEIDHRFSGFLACSIGIWFSFQALVNVGAAAGMLPTKGLTLPLIS 378
FtsW Y.pestis TDFIFSILGEELGYFGVVLALLMVFFVAFRAMSIGRRALEIGQRFSGFLACSIGIWFSFQALVNVGAAAGMLPTKGLTLPLIS 364
FtsW P.mirabilis TDFIFSILAEELGYIGVVLVLLMVFFIAFRAMQIGRRALLLDQRFSGFLACSIGIWFTFQTLVNVGAAAGMLPTKGLTLPLIS 361
FtsW V.cholerae TDFVFAVLAEELGFIGVVLVLVLIFSLVLKAIFIGKKAFQHDQQFGGYLAFGIGIWFAFQTLVNVGAAAGMVPTKGLTLPLIS 358
FtsW C.sordidicola TDFILAVIGEELGFVGVIVVILLFYWIVRRAFEIGRQALALDRTFAGLMAKGIGLWFGAQTFINMGVNLGLLPTKGLTLPLVS 410
FtsW B.cenocepacia TDFILAVIGEELGFIGVLVVILLFYWIVRRAFEIGRQALALDRTFAGLMAKGIGIWFGAQTFINMGVNLGLLPTKGLTLPLVS 395
FtsW B.pertussis TDFLMAVVGEELGFAGVMLVITLFAIIVYRGFDIGRQAIAMERTFAGLVAHGVAMWVGVQAFINMGVCLGLLPTKGLTLPLMS 365
FtsW M.xanthus TDFIFSIIAEETGLIGVGLLVVLYGVVLWRGV---RASLAAGETFGTYLGLGISSIIAFQAAVNMCVAMGLLPTKGLTLPFVS 347
FtsW B.fragilis SDFIFAIIIEELGLVGGAFVVILYIWLLVRTGRIAQKCERT---FPAFLVMGIALMLVSQAILNMMVAVGLFPVTGQPLPLIS 362
c
S. aureus FtsW
S. aureus PBP1
F22
S37
G340
S. thermophilus PBP2X
S. thermophilus FtsW
S. aureus PBP1
F22
S37
L345
S. thermophilus PBP2X
d
kDa
250
100
50
37
20
10
StFtsW + StPBP2x
StFtsW + StPBP2x S37F
StFtsW L345G + StPBP2x
StFtsW L345G + StPBP2x S37F

Supplementary Figure 6: The predicted complexes between FtsW and its cognate bPBP are largely governed by steric compatibility. (a) Predicted model of *E. coli* FtsW-FtsI (PBP3) interface based on evolutionary covariation. The TM and first alpha helix (H1) of FtsI (green) displays strong evolutionary couplings to TM8 and TM9 of FtsW. FtsW is colored according to electrostatic potential. The model is adapted from Ovchinnikov *et al.*[3] (b) Sequence alignment of FtsW and its cognate bPBP from diverse bacterial taxa. For clarity, only regions with strong inter-protein evolutionary covariation (TM-H1 and TM8-9 for bPBPs and FtsW, respectively) are shown. Red residues are shared by *S. thermophilus, S. pneumoniae* and *S. aureus* FtsW/bPBP. Blue residues are shared by *S. thermophilus* and *S. pneumoniae* FtsW/bPBP. Secondary structure prediction is based on the predicted models of *E. coli* FtsW and FtsI, and multiple sequence alignment was performed by Clustal X.[3,4] (c) Example of a species-specific steric compatibility at the predicted interface between FtsW and its cognate bPBP. (d) A S37F substitution in the *St*PBP2x TM helix predicted to destabilize FtsW-bPBP interaction results in the reduction of polymerase activity. A compensatory L345G substitution in *St*FtsW predicted to relieve the steric hindrance restores polymerase activity. A representative blot of two independent experiments is shown.



Supplementary Figure 7: *S. aureus* FtsW requires its cognate bPBP for PGT activity. *S. aureus* FtsW (2.5 µM) was incubated with *S. aureus* Lipid II (10 µM) and a bPBP from *S. aureus* (*Sa*PBP1, 2.5 µM), *S. pneumoniae* (*Sp*PBP2x, 2.5 µM) or *S. thermophilus* (*St*PBP2x, 2.5 µM) in the presence of moenomycin (2 µM). The polymer products were labeled with BDL using *Ef*PBPX and detected by western blot as in Fig. 1c. Bands observed around 45 kDa and 85 kDa correspond to self-labeling by *E. faecalis* PBPX and *S. aureus* PBP1*, respectively. A representative blot of four independent experiments is shown. An asterisk indicates a catalytically inactive variant.

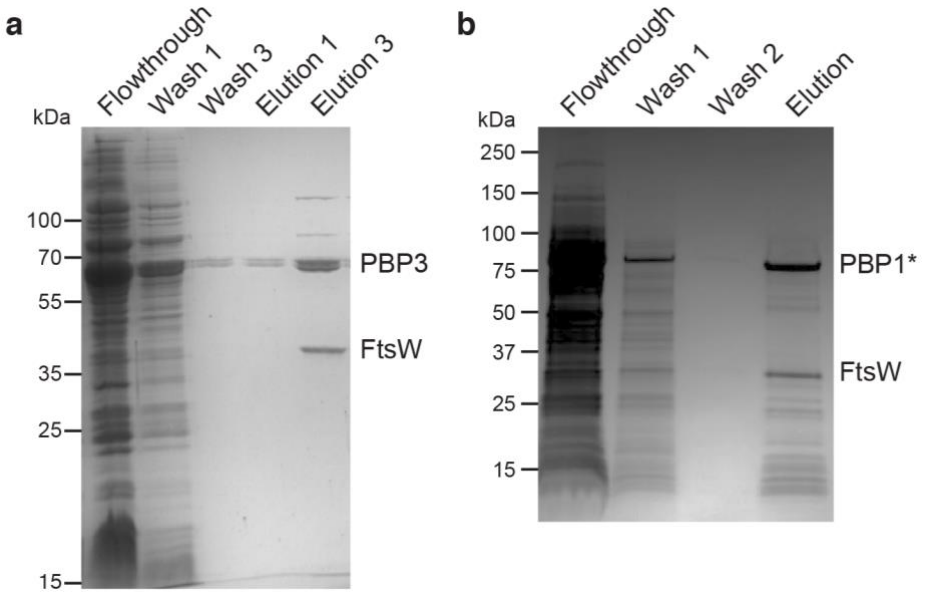
FtsW* = *Sa*FtsW^{D287A}; *Sp*PBP2x* = *Sp*PBP2x^{S337A}; *Sa*PBP1* = *Sa*PBP1^{S314A}; *St*PBP2x* = *St*PBP2x^{S343A}



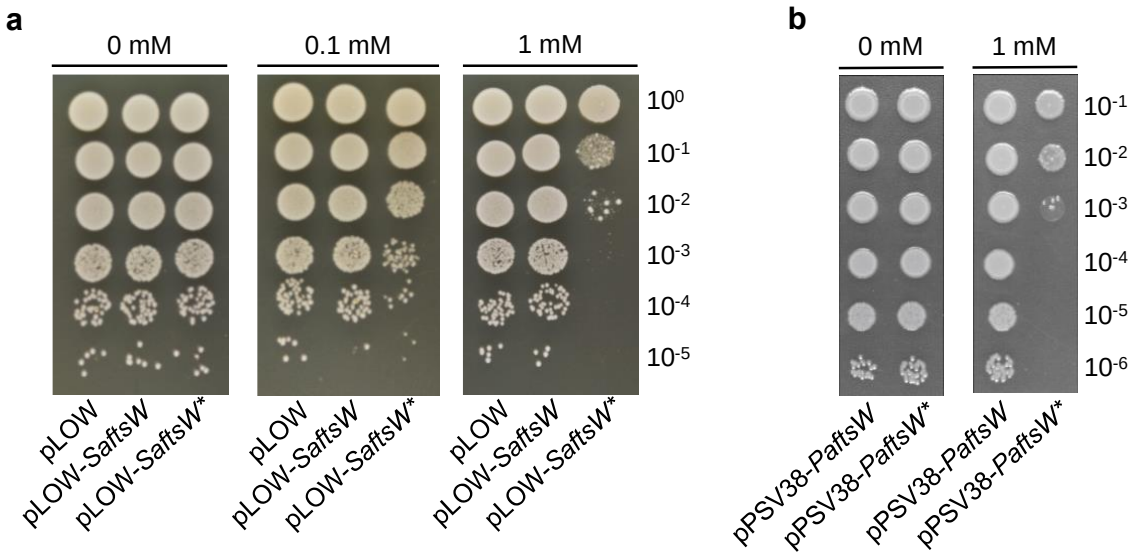
Supplementary Figure 8: *S. thermophilus* FtsW requires the TM helix of its cognate/near-cognate bPBP for PGT activity. *S. thermophilus* FtsW (0.5 µM) and bPBP (1 µM) were combined in a reaction buffer and the polymer products were detected by western blot as in Fig. 1c. A representative blot of three independent experiments is shown. *St*FtsW* = *St*FtsW^{D292A}

2b^{TM}2x$^{\Delta TM}$ = Chimeric PBP containing the TM domain of PBP2b and the extracellular domain of PBP2x

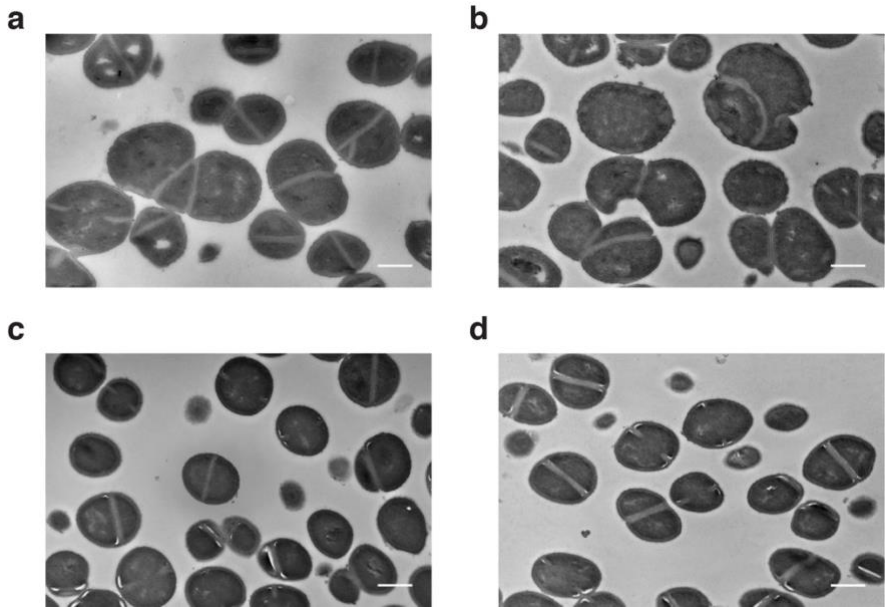
2x^{TM}2b$^{\Delta TM}$ = Chimeric PBP containing the TM domain of PBP2x and the extracellular domain of PBP2b



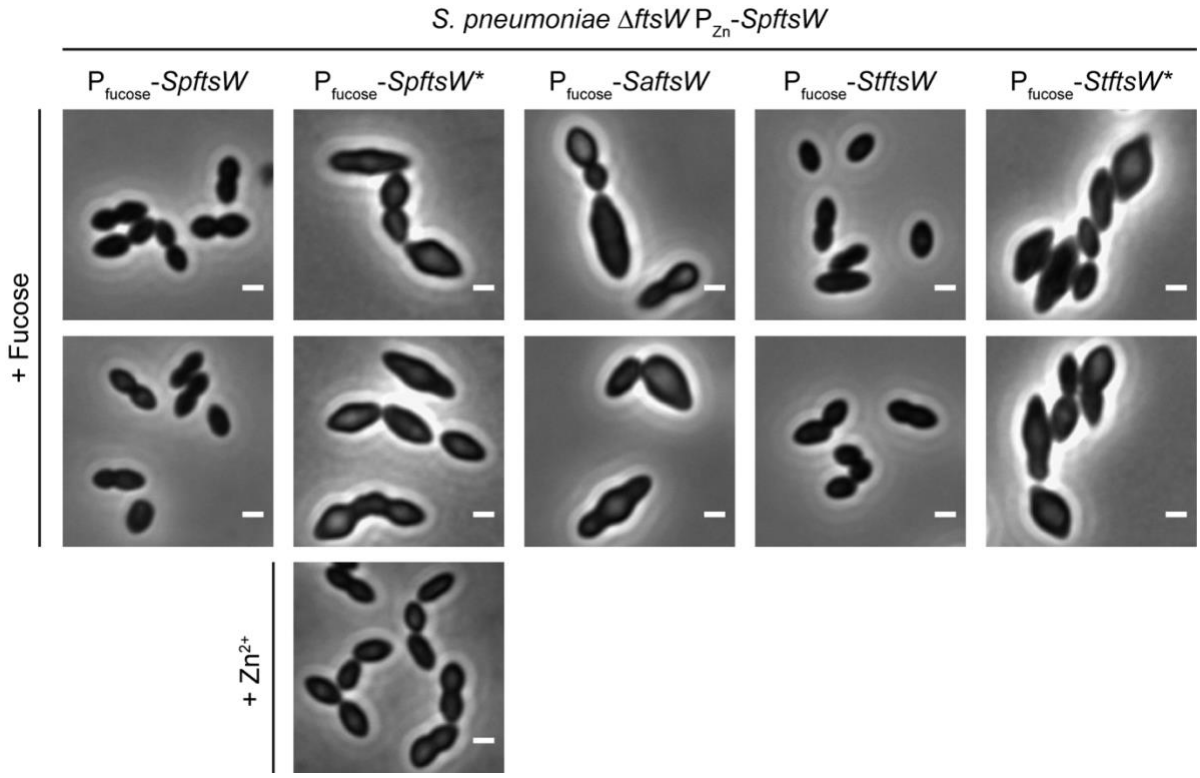
Supplementary Figure 9: Active FtsW-bPBP complexes can be co-purified. Coomassie staining shows the co-expression and purification of (a) *P. aeruginosa* FtsW and PBP3 and (b) *S. aureus* FtsW and PBP1***** (see methods). Representative gels of two independent purifications are shown. Identity of the proteins was assigned by western blot. Note that *Pa*PBP3 purifies as a doublet of bands that both bind Bocillin-FL. The doublet may result from C-terminal processing as has been observed for *E. coli* PBP3.[5]
PBP1***** = *Sa*PBP1^{S314A}



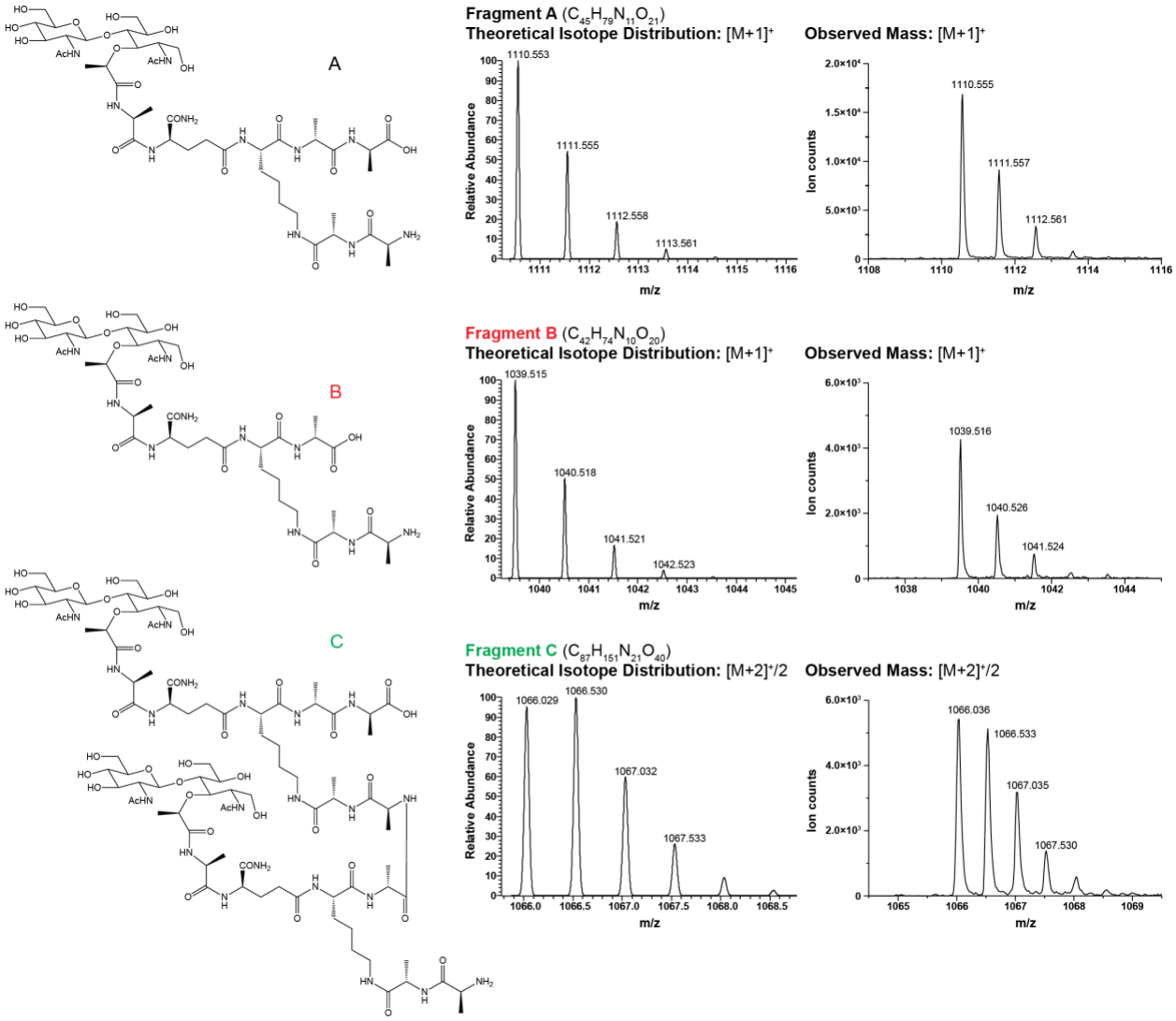
Supplementary Figure 10: Overexpression of FtsW* causes impaired fitness. (a) *S. aureus* cultures in mid-log phase were normalized to OD_{600} = 0.2 and serially diluted before 5 µL of each sample was spotted on TSB plates containing erythromycin supplemented with the indicated concentration of IPTG. (b) *P. aeruginosa* overnight cultures were normalized to OD_{600} = 2.4 and serially diluted before 5 µL of each sample was spotted on LB plates containing gentamycin supplemented with the indicated concentration of IPTG. Representative results of two independent experiments are shown for both *S. aureus* and *P. aeruginosa*. An asterisk indicates a gene encoding a catalytically inactive variant. *SaftsW** = $SaftsW^{D287A}$; *PaftsW** = $PaftsW^{D275A}$



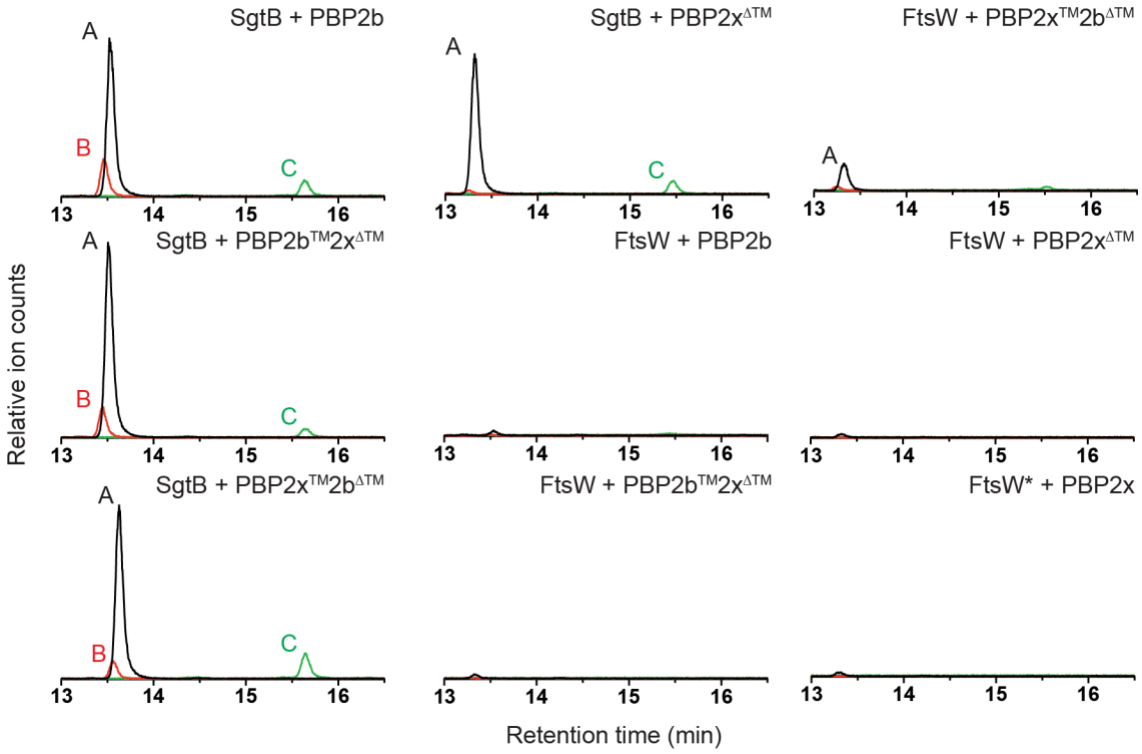
Supplementary Figure 11: Representative electron microscopy images of *S. aureus* strains from two independent experiments. Cells overexpressing FtsW* revealed aberrant morphology (a & b). Images of cells (strain AT196) lacking inducer (c) and cells (strain AT195) overexpressing wild-type FtsW (d) are shown for comparison. Scale bar = 500 nm. See Supplementary Table 3 for strain genotypes.



Supplementary Figure 12: Phase contrast images of *S. pneumoniae* strains in the presence of the indicated inducer. Representative images of two independent experiments are shown. Scale bar = 1 µm.



Supplementary Figure 13: Chemical structures and mass spectra of the muropeptide products of *S. thermophilus* bPBP crosslinking reactions following mutanolysin digestion and reduction. Representative mass spectra of five independent experiments are shown.



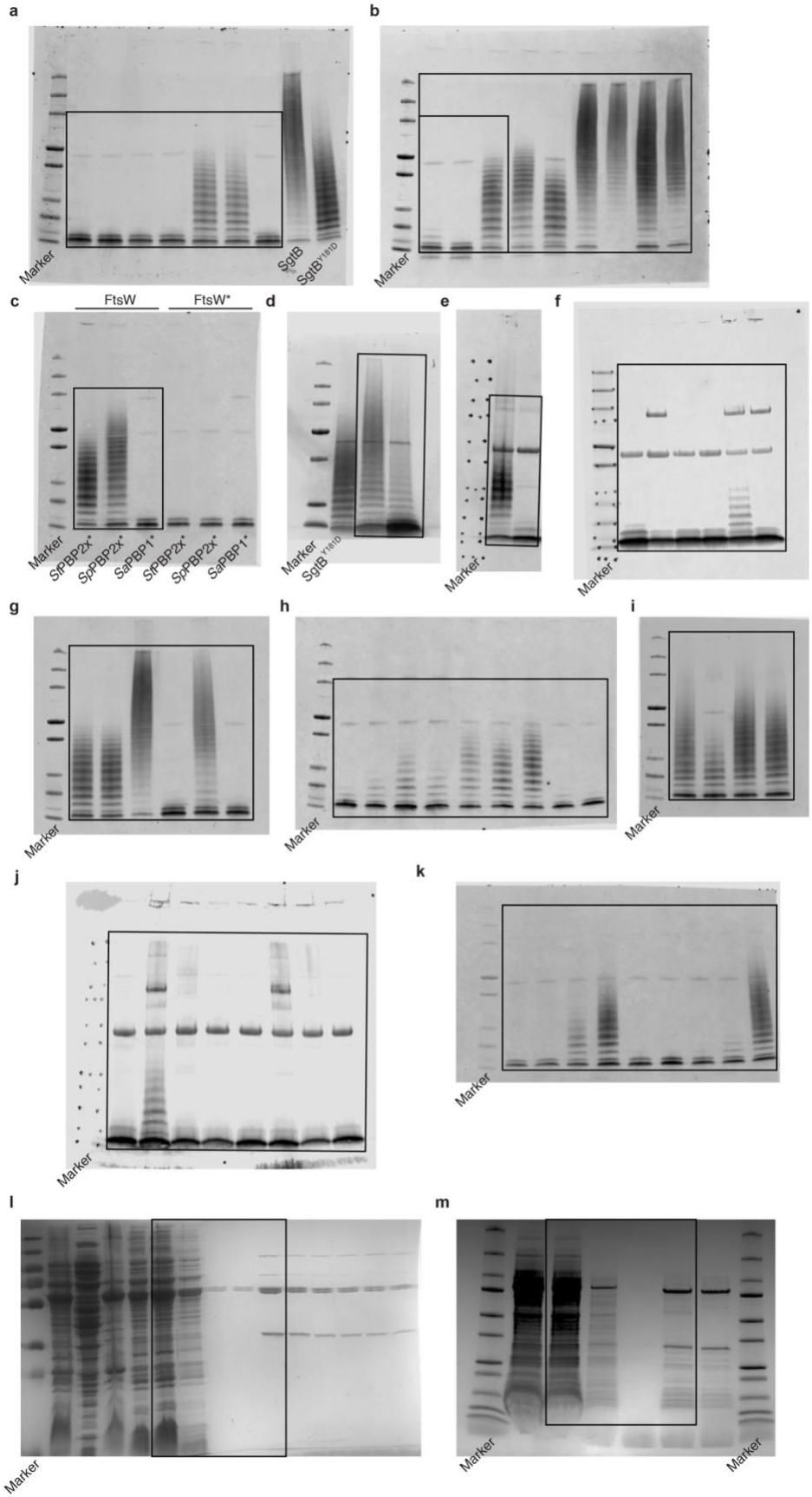
Supplementary Figure 14: *S. thermophilus* bPBPs do not require their native TM domain for crosslinking peptidoglycan. SgtB (1 µM) or *S. thermophilus* FtsW (0.5 µM) was incubated with *S. thermophilus* bPBP (1.25 µM) in a reaction buffer containing *E. faecalis* Lipid II (20 µM). The resulting polymer was digested with mutanolysin and analyzed by LC-MS as in Fig 3a. Crosslinking is detected by the appearance of peak C, a crosslinked muropeptide. Representative chromatograms of two independent experiments are shown.

PBP2b^{TM}2x$^{\Delta TM}$ = *St*PBP2b$^{M1\text{-}M58}$*St*PBP2x$^{G53\text{-}D755}$

PBP2x^{TM}2b$^{\Delta TM}$ = *St*PBP2x$^{M1\text{-}I52}$*St*PBP2b$^{Q59\text{-}H704}$

PBP2x$^{\Delta TM}$ = *St*PBP2x$^{G53\text{-}D755}$

FtsW* = *St*FtsW^{D292A}


a
Marker
SgtB
SgtB^{Y181D}
b
Marker
c
FtsW
FtsW*
Marker
*Sf*PBP2x*
*Sp*PBP2x*
*Sa*PBP1*
*Sf*PBP2x*
*Sp*PBP2x*
*Sa*PBP1*
d
Marker
SgtB^{Y181D}
e
Marker
f
Marker
g
Marker
h
Marker
i
Marker
j
Marker
k
Marker
l
Marker
m
Marker
Marker

Supplementary Figure 15: Full membrane and gel images from Fig. 1d (a), Fig. 1e and Supplementary Fig. 5 (b), Fig. 1f (c), Fig. 2a (d), Fig 2b (e), Supplementary Fig. 3 (f), Supplementary Fig. 4 (g). Supplementary Fig. 5b (h), Supplementary Fig. 6d (i), Supplementary Fig. 7 (j), Supplementary Fig. 8 (k), Supplementary Fig. 9a (l) and Supplementary Fig. 9b (m). Black boxes indicate the cropped region for each figure.

S. aureus	% of cells with septal defects (# of cells)
WT No inducer (245)	0 % (0)
WT + 1 mM IPTG (234)	0 % (0)
D287A No inducer (203)	0 % (0)
D287A + 1 mM IPTG (212)	~19 % (40)

Supplementary Table 1: Only *S. aureus* cells overexpressing FtsW* displayed aberrant septal formation. Every cell in the electron micrograph field was analyzed for septum formation defects. The total number of cells analyzed is shown in parenthesis.

P. aeruginosa	Cell length (μm)	Cell width (μm)
WT No inducer (295)	3.55 ± 0.75	1.02 ± 0.09
WT + 1 mM IPTG (181)	3.65 ± 0.70	1.01 ± 0.05
D275A No inducer (380)	3.65 ± 0.71	1.02 ± 0.04
D275A + 1 mM IPTG (183)	11.54 ± 4.79	0.91 ± 0.11

Supplementary Table 2: Measurement of the cell length and width of *P. aeruginosa* samples (Mean ± SD, Oufti).[6] Cells overexpressing FtsW* displayed a statistically significant difference in cell length compared to other samples ($p < 0.0001$, one-way ANOVA with Bonferroni's post hoc test, GraphPad Prism 7.0). The total number of cells measured is shown in parenthesis.

Supplementary Table 3. Bacterial strains used in this study

Strain	Description*	Reference
E. coli		
XL1-Blue	Host strain for plasmid cloning	Stratagene
DH5α	Host strain for plasmid cloning	Invitrogen
BL21(DE3)	Expression strain for protein production	7
C43(DE3)	BL21(DE3) derivative strain for protein production	8
CAM333	C43(DE3) Δ*ponB*, Δ*pbpC*, Δ*mtgA*	2
S. aureus		
RN4220	Wild-type	9
AT194	RN4220 pLOW; Erm^R	This study
AT195	RN4220 pLOW-*SaftsW*; Erm^R	This study
AT196	RN4220 pLOW-*SaftsW*^{D287A}; Erm^R	This study
P. aeruginosa		
PAO1	Wild-type	C.S. Harwood
LSM7	PAO1 pPSV38-*PaftsW*; $Gent^R$	This study
LSM8	PAO1 pPSV38-*PaftsW*^{D275A}; $Gent^R$	This study
S. pneumoniae		
D39	Δ*cps*	10
AT030	D39 Δ*cps*, Δ*bgaA*::*erm*; Erm^R	11
AT031	D39 Δ*cps*, Δ*bgaA*::*kan*; Kan^R	11
R6	Wild-type	10
AT202	R6 *cpsO*::(P_{zn}-*SpftsW*, *kan*)::*cpsN*; Kan^R	This study
AT203	R6 *cpsO*::(P_{zn}-*SpftsW*, *kan*)::*cpsN*, Δ*ftsW*::*erm*; Erm^R, Kan^R	This study
AT204	R6 *cpsO*::(P_{zn}-*SpftsW*, *kan*)::*cpsN*, Δ*ftsW*::*erm*, Δ*bgaA*::(P_{fucose}-*SpftsW*, *tet*); Erm^R, Kan^R, Tet^R	This study
AT205	R6 *cpsO*::(P_{zn}-*SpftsW*, *kan*)::*cpsN*, Δ*ftsW*::*erm*, Δ*bgaA*::(P_{fucose}-*SpftsW*^{D289A}, *tet*); Erm^R, Kan^R, Tet^R	This study
AT206	R6 *cpsO*::(P_{zn}-*SpftsW*, *kan*)::*cpsN*, Δ*ftsW*::*erm*, Δ*bgaA*::(P_{fucose}-*SaftsW*, *tet*); Erm^R, Kan^R, Tet^R	This study
AT207	R6 *cpsO*::(P_{zn}-*SpftsW*, *kan*)::*cpsN*, Δ*ftsW*::*erm*, Δ*bgaA*::(P_{fucose}-*StftsW*, *tet*); Erm^R, Kan^R, Tet^R	This study
AT208	R6 *cpsO*::(P_{zn}-*SpftsW*, *kan*)::*cpsN*, Δ*ftsW*::*erm*, Δ*bgaA*::(P_{fucose}-*StftsW*^{D292A}, *tet*); Erm^R, Kan^R, Tet^R	This study

* See Supplementary Table 4 for list of abbreviations

Supplementary Table 4. Plasmids used in this study

Plasmid	Description*	Reference
pET28b(+)	IPTG-inducible protein expression vector; Kan^R	Novagen
pATPL107	His_6-*St*FtsW expression vector; Kan^R	This study
pATPL121	His_6-*St*FtsW^{D292A} expression vector; Kan^R	This study
pATPL185	His_6-*St*FtsW^{L345G} expression vector; Kan^R	This study
pMW2179	His_6-*Sa*FtsW expression vector; Kan^R	This study
pMW2193	His_6-*Sa*FtsW^{D287A} expression vector; Kan^R	This study
pMW2194	*Sa*PBP1-His_6 expression vector; Kan^R	This study
pMW2180	*Sa*PBP1^{S314A}-His_6 expression vector; Kan^R	This study
pET22b(+)	IPTG-inducible protein expression vector; $Carb^R$	Novagen
pATPL091	*St*PBP2x-His_6 expression vector; $Carb^R$	This study
pATPL123	*St*PBP2x$^{G53\text{-}D755}$-His_6 expression vector; $Carb^R$	This study
pATPL112	*St*PBP2x^{S343A}-His_6 expression vector; $Carb^R$	This study
pATPL180	*St*PBP2x^{S37F}-His_6 expression vector; $Carb^R$	This study
pATPL169	*St*PBP2b-His_6 expression vector; $Carb^R$	This study
pATPL172	*St*PBP2b$^{M1\text{-}M58}$*St*PBP2x$^{G53\text{-}D755}$-His_6 expression vector; $Carb^R$	This study
pATPL173	*St*PBP2x$^{M1\text{-}I52}$*St*PBP2b$^{Q59\text{-}H704}$-His_6 expression vector; $Carb^R$	This study
pATPL017	*Sp*PBP2x-His_6 expression vector; $Carb^R$	This study
pATPL067	*Sp*PBP2x^{S337A}-His_6 expression vector; $Carb^R$	This study
pATPL084	*Sp*PBP2b-His_6 expression vector; $Carb^R$	This study
pATPL170	*Sp*PBP2b$^{M6\text{-}Y38}$*Sp*PBP2x$^{T50\text{-}D750}$-His_6 expression vector; $Carb^R$	This study
pATPL171	*Sp*PBP2x$^{M1\text{-}G49}$*Sp*PBP2b$^{M39\text{-}N685}$-His_6 expression vector; $Carb^R$	This study
pATPL044	*Sp*PBP1a-His_6 expression vector; $Carb^R$	This study
pATPL051	*Sp*PBP1a^{S370A}-His_6 expression vector; $Carb^R$	This study
pETDuet-1	IPTG-inducible protein expression vector containing two multiple cloning sites; $Carb^R$	Novagen
pETDuet-FLAG	Modified pETDuet-1 containing a N-terminal His_6-SUMO-FLAG tag at the first site and C-terminal His_6 tag at the second site; $Carb^R$	This study
pLSM3	His_6-SUMO-FLAG-*Pa*FtsW & *Pa*PBP3-His_6 expression vector; $Carb^R$	This study
pLSM6	His_6-SUMO-FLAG-*Pa*FtsW^{D275A} & *Pa*PBP3-His_6 expression vector; $Carb^R$	This study
pMW2144	His_6-SUMO-FLAG-*Sa*FtsW & *Sa*PBP1^{S314A}-His_6 expression vector; $Carb^R$	This study
pMW2176	His_6-SUMO-FLAG-*Sa*FtsW^{D287A} & *Sa*PBP1^{S314A}-His_6 expression vector; $Carb^R$	This study
pAM174	Encodes arabinose-inducible Ulp1$^{L403\text{-}K621}$ protease; Cm^R	2
pMW1010	*E. faecalis* His_6-PBPX expression vector; Kan^R	12
pPBP4	*S. aureus* His_6-PBP4 expression vector; Kan^R	13
pMgt1	*S. aureus* SgtB-His_6 expression vector; $Carb^R$	14
pET24bSgtBY181D	*S. aureus* SgtB^{Y181D}-His_6 expression vector; Kan^R	15
pLOW	*S. aureus* expression vector containing IPTG-inducible P_{spac} promoter; Erm^R	16
pLOWftsW	*S. aureus* P_{spac}-*Sa*FtsW expression vector; Erm^R	This study
pLOWftsWD287A	*S. aureus* P_{spac}-*Sa*FtsW^{D287A} expression vector; Erm^R	This study

*Abbreviations: $Carb^R$, ampicillin/carbenicillin resistance; Cm^R, chloramphenicol resistance; Erm^R, erythromycin resistance; $Gent^R$, gentamycin resistance; Kan^R, kanamycin resistance; Tet^R, tetracycline resistance

Supplementary Table 4. Plasmids used in this study (cont.)

Plasmid	Description*	Reference
pPSV38	*P. aeruginosa* expression vector containing IPTG-inducible P_{lacUV5} promoter; $Gent^R$	17
pLSM8	*P. aeruginosa* P_{lacUV5}-*Pa*FtsW expression vector; $Gent^R$	This study
pLSM9	*P. aeruginosa* P_{lacUV5}-*Pa*$FtsW^{D275A}$ expression vector; $Gent^R$	This study
pLEM023	*S. pneumoniae bgaA* integration vector containing zinc-inducible P_{czc} (P_{zn}) promoter; $Carb^R$, Tet^R	11
pATPL139	*S. pneumoniae bgaA*::P_{zn}-*Sp*FtsW integration vector; $Carb^R$, Tet^R	This study
pAKF205	*S. pneumoniae bgaA* integration vector containing fucose-inducible P_{fcsK} (P_{fucose}) promoter; $Carb^R$, Tet^R	11
pATPL137	*S. pneumoniae bgaA*::P_{fucose}-*Sp*FtsW integration vector; $Carb^R$, Tet^R	This study
pATPL138	*S. pneumoniae bgaA*::P_{fucose}-*Sp*$FtsW^{D289A}$ integration vector; $Carb^R$, Tet^R	This study
pATPL141	*S. pneumoniae bgaA*::P_{fucose}-*Sa*FtsW integration vector; $Carb^R$, Tet^R	This study
pATPL142	*S. pneumoniae bgaA*::P_{fucose}-*St*FtsW integration vector; $Carb^R$, Tet^R	This study
pATPL143	*S. pneumoniae bgaA*::P_{fucose}-*St*$FtsW^{D292A}$ integration vector; $Carb^R$, Tet^R	This study

*Abbreviations: $Carb^R$, ampicillin/carbenicillin resistance; Cm^R, chloramphenicol resistance; Erm^R, erythromycin resistance; $Gent^R$, gentamycin resistance; Kan^R, kanamycin resistance; Tet^R, tetracycline resistance

Supplementary Table 5. Oligonucleotide primers used in this study

Primer	Sequence (5'-3')*
oAT01	GTACATATGAAAATTGAAAAGAAACATCTTTTGG
oAT02	ACTAAGCTTCTAACGACTTCTTCTCAAAGGTA
oAT03	CCTTTGTGTTTTCAATTGTTATTGAAGAGTT
oAT04	CTGTTGTTGCTTCTGGGAGATAAC
oAT05	TCTCATGGGGATGCAAGTTTTTGTTAATATCGGTG
oAT06	TGCATCCCCATGAGAGCCCCCACACC
oAT07	GTACATATGAACAGACTTAAAAAACTTAAACAT
oAT08	ACTAAGCTTGTCTCCTAAAGTAATGGTAATCTT
oAT09	GTACATATGGGAACCGATAGTAAGTTTGGTGTTG
oAT10	AGCCAGGGGCAACCATGAAAGTGATGCTCTTGT
oAT11	TGGTTGCCCCTGGCTCATACTGAGCTTCA
oAT12	TATTTTTATCTTTTTTGTTTTTATTATCAAC
oAT13	AAGAGAACCATCAGGTTCTG
oAT14	GTACATATGACATCATTTTGGGAAAAAAATAGTCAAA
oAT15	ACTAAGCTTGTGAGTATTTTGATAAAGTTCCATGATAG
oAT16	CATATAGGTCAAACGCGCAAAA
oAT17	TTTTGCGCGTTTGACCTATATGGGAACCGATAGTAAGTTTGGTGTTG
oAT18	AATAATTACCACAAAGTTGATAATAAAA
oAT19	TTTTATTATCAACTTTGTGGTAATTATTCAGGTTTATAACAAATCTTTTTATACTAA
oAT20	GTAGCTAGCACTGAATATATGAAGAATTTTAGAAGTATTTTACGG
oAT21	ACTGCGGCCGCTTAATTAAATTGTCTTCTTATATCAACTTTTTGTTG
oAT22	ACCACATACAGCATTTATTTTTGCAATTATTTG
oAT23	TCTGGTAAATAGCCCAATTTC
oAT24	AGGAGATATACCATGGGCAAGCAAAAAATTAAAATTAAAAAAA
oAT25	TGCTCGAGTGCGGCCGCGTCCGACTTATCCTTGTC
oAT26	CGAGCCTGGAGCAACATTTAAATC
oAT27	TATGTGTTTTGATAAAGGTCATTTG
oAT28	GTACATATGAAGTGGACAAAAAGAGTAATCCGTT
oAT29	ACTAAGCTTGTCTCCTAAAGTTAATGTAATTTTTTTAATG
oAT30	GCCACTATGAAAGTGATGATGTTG
oAT31	ACCTGGCTCATAGTTACTTTGG
oAT32	GTACATATGAGAAAATTTAACAGCCATTCGATTCC
oAT33	ACTAAGCTTATTCATTGGATGGTATTTTTGATACAGA
oAT34	ATACAACAAACGACCAATAATG
oAT35	CATTATTGGTCGTTTGTTGTATACAGGCACTCGCTTTGGAAC
oAT36	CCCAATAATGACCGCAAAATT
oAT37	AATTTTGCGGTCATTATTGGGATGCAGGTTTTGAACAAGGATT
oAT38	GTACATATGAACAAACCAACGATTCTGC
oAT39	ACTCCTCGAGTGGTTGTGCTGGTTGAGGAT

* Underlined sequences are restriction sites introduced in primers.

Supplementary Table 5. Oligonucleotide primers used in this study (cont.)

Primer	Sequence (5'-3')*
oAT40	GCAACTATGAAACCGATCACAGACTATGCTCC
oAT41	TCCCCAGTCGCGGTTTGTTT
oAT42	TTTCAAGGCCCTGGGGGGTCATCCATGCTGTCGGTGTTGCGCCC
oAT43	TTAAGCATTATGCGGCCGCAAGCTTTTACCTTTCATCGGCAAAGTCCTCTTC
oAT44	TATATTAGTTAAGTATAAGAAGGAGATATACATATATGAAACTGAATTATTTCCAGGG CGCC
oAT45	GGTGCTCGAGTGCGGCCGCAAGCTTGCCACGCCCTCCTTTTGCGG
oAT46	GCGCCCGCAAAAGGAGGGCGTGGCAAGCTTGCGGCCGCACTCGA
oAT47	AAGGGGCGCAACACCGACAGCATGGATGACCCCCCAGGGCC
oAT48	AGTTCAACGAAGAGGACTTTGCCGATGAAAGGTAAAAGCTTGCGGCCGCATAATGCT TAA
oAT49	GGCGCCCTGGAAATAATTCAGTTTCATATATGTATATCTCCTTCTTATACTTAACTAAT ATACTAAGAT
oAT50	CTGCCGGAGGCGCACACCGCCTTCGTCTTCGCCGTGCTT
oAT51	CCTGGGGGGTCATCCACTGAATATATGAAGAATTTTAGA
oAT52	CTCGAATTCGGATCCTTAATTAAATTGTCTTCTTATATCA
oAT53	GGAGATATACATATGGCGAAGCAAAAAATTAAAATTAAAA
oAT54	AGACTCGAGGGTACCTCAGTGGTGGTGGTGGTGGTGCTCGAGTGCGGCCGCAAGCTTG TCCGACTTATCCTTGTCAGT
oAT55	GGATCCGAATTCGAGCTCGGC
oAT56	GGATGACCCCCCAGGGCC
oAT57	GGTACCCTCGAGTCTGGT
oAT58	CATATGTATATCTCCTTCTTATAC
oAT59	ATTGTCGACATTGTAGAATTGGATGACTGAATATATGAAGAAT
oAT60	TAAGGATCCTTACTTGTCGTCATCGTCTTTGTAGTCATTAAATTGTCTTCTTATATCAAC TTTTTG
oAT61	TTACCAGAACCACATACAGCATTTATTTTTGCAATTATTTGCGAAGAA
oAT62	ATTGCAAAAATAAATGCTGTATGTGGTTCTGGTAAATAGCCC
oAT63	TAATGAGCTCACGGGAGGAAAGATGCTGTCGGTGTTGCGCCC
oAT64	TAATTCTAGATTACCTTTCATCGGCAAAGTCCTCTTC
oAT65	ACTCCTCGAGAAAGGAGGTAAGAAAAATGAAGATTAGTAAGAGGCACTT
oAT66	ACTGGATCCCTACTTCAACAGAAGGTTCATTGGT
oAT67	GGTAAACCCATCCTAGCCCA
oAT68	GGAGATCCCCAAGTAATCGTGGGTCTAGAGATGATTTTAATTACAGG
oAT69	GAGAGCACAGATACGGCGTTTCTAATATGTAACTCTTCCCAATATAG
oAT70	TCCATTATGTTTACGAGAACTTTC
oAT71	CACGATTACTTGGGGATCTCC
oAT72	TCCTGCCTTTCCTCCCTCCCATGTATACCACTTGGGCGC
oAT73	GAGGGAGGAAAGGCAGGA
oAT74	CGCCGTATCTGTGCTCTC
oAT75	ACCCGATAAGAGGTTTCATCCG

* Underlined sequences are restriction sites introduced in primers.

Supplementary Table 5. Oligonucleotide primers used in this study (cont.)

Primer	Sequence (5'-3')*
oAT76	GCAGATGTATTGGTAACTCTCTCA
oAT77	TCCTGCCTTTCCTCCCTCCATAGTATCACCACTCTACTAGGATAC
oAT78	GAGAGCACAGATACGGCGCTAAGTTGTACCGAGAATTGGAAA
oAT79	TCTCTGACTTGCTTGCTGAC
oAT80	GAGAGATTTGAGGGAAGATCACG
oAT81	ACTCCTCGAGAAAGGAGGTAAGAAAAATGAAGAATTTTAGAAGTATTTTACGGTAT
oAT82	ACTGGATCCTTAATTAAATTGTCTTCTTATATCAACTTTTT
oAT83	ACTCCTCGAGAAAGGAGGTAAGAAAAATGAAAATTGAAAAGAAACATCTTTTGG
oAT84	ACTGGATCCCTAACGACTTCTTCTCAAAGGTA
oAT85	CCTTTGTCTTTTCTATCGTGATTGAAGA
oAT86	CTGTATGAGCTTCTGGCAAATAACC
oAT87	CCACTATCACAAGGTATTACAAACG
oAT88	GAACATTATTCAAGTAAGCGTTCAC

* Underlined sequences are restriction sites introduced in primers.

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