Supplementary information for: 1 The cell cycle regulator GpsB functions as cytosolic adaptor 2 for multiple cell wall enzymes 3 4 Robert M. Cleverley¹, Zoe J.Rutter¹, Jeanine Rismondo^{2,6}, Federico Corona^{3,7}, Ho-Ching Tiffany 5 Tsui⁴, Fuad A. Alatawi⁵, Richard A. Daniel⁵, Sven Halbedel², Orietta Massidda^{3,8}, Malcolm E. 6 Winkler⁴ and Richard J. Lewis¹ 7 8 ¹Institute for Cell and Molecular Biosciences, University of Newcastle, Newcastle upon Tyne, 9 United Kingdom, NE2 4HH. 10 ²FG11 Division of Enteropathogenic bacteria and Legionella, Robert Koch Institute, Burgstrasse 37, 11 D-38855 Wernigerode, Germany. 12 ³Dipartimento di Scienze Chirurgiche, Università di Cagliari, Cagliari, 09100, Italy. 13 ⁴Department of Biology, Indiana University Bloomington, Bloomington, IN 47405, USA. 14 ⁵Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, University of 15 Newcastle, Newcastle upon Tyne, United Kingdom, NE2 4AX. 16 ⁶Present address: Section of Microbiology and MRC Centre for Molecular Bacteriology and 17 Infection, Imperial College London, London, United Kingdom, SW7 2DD. 18 ⁷Present address: Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, 19 University of Newcastle, Newcastle upon Tyne, United Kingdom, NE2 4AX. 20 ⁸Present address: Department CIBIO, University of Trento, via Sommarive 9, 38123 Povo, Italy. 21 22 Correspondence and requests for materials should be addressed to R.J.L (email: r.lewis@ncl.ac.uk) 23 Keywords: peptidoglycan / cell division / divisome / penicillin binding protein / GpsB 24 25

26 Supplementary Note 1

By circular dichroism measurements of purified recombinant proteins, wild-type BsPBP1₁₋₃₂ has α -27 helical content of 14 %, corresponding to ~5 residues (Supplementary Figure 1B), consistent with 28 the 6 α -helical amino acids in the structure of the *Bs*GpsB₅₋₆₄:*Bs*PBP1₁₋₁₇ complex (Figure 1B, 1C). 29 $BsPBP1_{1-32}^{Ala10Pro}$ is mostly random coil with α -helical content of 2 % (one amino acid) and a >8-30 fold reduction in binding affinity for $BsGpsB_{1-68}$. $BsPBP1_{1-32}$ ^{Ser7Ala} has a slightly higher α -helical 31 content (19%; ~6 amino acids) than the wild-type, presumably because the helix is initiated at Asn6 32 in this peptide. Asparagine also preferentially occupies the N-cap position in α -helices¹ and 33 promotes α -helix formation when introduced at the N-terminus of model peptides². If B_sPBP1₁ 34 $_{32}^{Asn6}$ was the N-cap, its sidechain would hydrogen bond to the amide nitrogen of BsPBP1₁₋₃₂^{Glu9}, 35 negatively impacting on the interaction between its amide and $BsGpsB_{5-64}^{Asp35}$, and explaining 36 the >5-fold reduced binding affinity of this mutant. 37

38

*Lm*PBPA1₁₋₁₅ and *Lm*PBPA1₁₋₁₅^{Gln10Pro} are essentially random coil peptides, consistent with the disorder of the *Lm*PBPA1₁₋₁₅ in the *Bs*GpsB₅₋₆₄^{Lys32Glu}:*Lm*PBPA1₁₋₁₅ structure. The molar ellipticity signal at 222 nm in CD is 40% higher in wild-type *Lm*PBPA1₁₋₁₅ at TFE concentrations of 40, 60 and 80% than in *Lm*PBPA1₁₋₁₅^{Gln10Pro} (**Supplementary Figure 1D**). Q10 in the *Lm*PBPA1 peptide is completely disordered in the structure of *Bs*GpsB₅₋₆₄^{Lys32Glu}:*Lm*PBPA1₁₋₁₅, (**Figure 2A**), and the impact of *Lm*PBPA1₁₋₂₀^{Gln10Pro} likely reflects an effect on the peptide conformation rather than a loss of contacts to GpsB.

47 Supplementary Note 2

Proteins with established roles in growth, division and morphogenesis, including the early cell 48 division proteins FtsZ (lmo2032), FtsA (lmo2033), EzrA (lmo1594), ZapA (lmo1229) and SepF 49 (lmo2030), the late division proteins DivIB (lmo2034), DivIC (lmo0217), FtsL (lmo2040), FtsW 50 (lmo1071) as well as the elangosomal proteins MreB (lmo1548), MreC (lmo1547), MreD 51 (lmo1546), MreBH (lmo1713), Mbl (lmo2525), RodA (lmo2427) and RodZ (lmo1395) were 52 screened for interaction wth LmGpsB by BACTH. The nucleoid occlusion factor Noc (lmo2794), 53 four other high molecular weight penicillin binding proteins (HMW PBPs) PBP A2 (Imo2229), 54 PBPB1 (lmo1438), PBPB2 (lmo2039) and PBPB3 (lmo0441) as well as the PG N-deacetylase PgdA 55 (Imo0415) were also included. In contrast to B. subtilis, which has FtsW, RodA and SpoVE, a 56 sporulation-specific homologue of FtsW/RodA, the L. monocytogenes genome contains four 57 additional FtsW/RodA homologues encoded by lmo0421, lmo2428, lmo2687 and lmo2688. Their 58 function is presently unknown, but they were also included in the screen for GpsB interaction 59 partners. 60

63 Supplementary Table 1: Dissociation constants of GpsB:PBP complexes

GpsB binding partner and sequence	mutation	GpsB protein	$K_{\rm d} \mu { m M}$
TAMRA-BsPBP1 ₁₋₃₂	Wildtype		120 ± 10
<i>GS</i> M ₁ <i>A</i> DQFNSREARRKAN <i>C</i> KSSPSPKKGKKRKKGG ₃₂	Ser7Ala		>700
	Arg8Ala		>1700
	Arg8Lys		>2000
	Ala10Pro	BSGpSB ₁₋₆₈	>1000
	Arg11Ala		>600
	Arg11Lys		390 ± 20
	Arg28Ala		90 ± 10
	Wildtype	BsGpsB ₁₋₆₈ Glu17Ala	>1300
	Wildtype	$BsGpsB_{1-68}$ ^{Tyr25Phe}	>2000
	Wildtype	$BsGpsB_{1-68}^{Asp31Ala}$	>1900
	Wildtype	BsGpsB ₁₋₆₈ ^{Asp35Ala}	>2000
	Wildtype	<i>Bs</i> GpsB	160 ± 10
Fluorescein-BsPBP1 ₁₋₃₂	Wildtype		100 ± 10
<i>GS</i> M ₁ <i>A</i> DQFNSREARRKANSKSSPSPKKGKKRKK <u>C</u> G ₃₂	Arg8Ala	D-CD	>500
	Ala10Pro	BSGPSB ₁₋₆₈	>500
	Arg28Ala		130 ± 20
Fluorescein-LmPBPA11-20	Wildtype		190 ± 40
GSM1ADKPQTRSQYRNKQSGG <u>C</u> K20	Arg8Ala		>3000
	Arg8AlaSer16Arg	B a C ma D Lys32Glu	>2000
	Gln10Pro	BSOPSD ₁₋₆₈	>1500
	Tyr11Ala		430 ± 40
	Arg12Ala		800 ± 40
	Wildtype	<i>Lm</i> GpsB ₁₋₇₃	200 ± 20
TAMRA-SpPBP2a ₂₃₋₄₅	Wildtype		80 ± 20
<i>GSM</i> D ₂₃ SDSTILRRSRSDRKKLAQV <u>C</u> PI ₄₅	Arg31Lys		150 ± 10
	Arg33Lys		360 ± 30
	Arg31LysArg33Lys	SpGpsB ₁₋₆₃	>2000
	Ser32Ala		210 ± 15
	Arg33Ala		530 ± 50
	Arg36Ala		270 ± 20
	Wildtype	$SpGpsB_{1-63}^{Asp33Ala}$	>3000
Fluorescein-SpPBP2b ₁₋₁₇	Wildtype	SpGpsB ₁₋₆₃	>3600
Fluorescein-GM ₁ RLICMRKFNSHSIPIR ₁₇			
Fluorescein-SpPBP2x ₁₋₂₉	Wildtype	SpGpsB ₁₋₆₃	370 ± 30
<i>GSGSG</i> M ₁ <i>E</i> WTKRVIRYATKNRKSPAENRRRVGKSL <u><i>C</i></u> S			
Fluoroescein-BsYpbE ₁₋₂₁	Wildtype	BsGpsB ₁₋₆₈	13 ± 1
Fluoroescein-J ₁ TNJSRVERRKAQNLYEDQNA ₂₁	Wildtype	BsGpsB ₁₋₆₈ ^{Tyr25Phe}	>500
	Wildtype	$BsGpsB_{1-68}^{Asp31Ala}$	>500
Fluoroescein-BsYrrS ₁₋₁₈	Wildtype	BsGpsB ₁₋₆₈	430 ± 20
$GSM_1GNNQSRYENRDKRRKAN_{18}CG$	Wildtype	BsGpsB ₁₋₆₈ ^{Tyr25Phe}	>3000
	Wildtype	$BsGpsB_{1-68}^{Asp31Ala}$	>3000

64

65 Dissociation constants for the interaction of PBP cytoplasmic mini-domain peptides and other

⁶⁶ binding partners with N-terminal domains of GpsB proteins measured by fluorescence polarization.

The subscripts in the peptide sequences represent the residue numbers in the relevant protein; the 67 italics denote non-native residues at the termini resulting from the recombinant method used to 68 produce peptides. All peptides were labelled with a TAMRA or fluorescein fluorophore at the 69 underlined cysteine at the C-terminus in all cases except for BsYpbE₁₋₂₁, SpPBP2b₁₋₁₇ and BsPBP1₁. 70 ₃₂. The $BsYpbE_{1-21}$ and $SpPBP2b_{1-17}$ peptides were labelled at the N-terminus with fluorescein; in 71 the former peptide norleucine (abbreviated with a 'J') replaced the naturally-occurring methionine 72 to avoid sulphoxidation during synthesis caused by proximity of the fluorophore. In B_sPBP1_{1-32} , 73 cysteine replaced PBP1^{Ser16}, which is remote from the protein:peptide interface in the structure of 74 the *Bs*GpsB₅₋₆₄:*Bs*PBP1₁₋₁₇ complex. A *Bs*PBP1₁₋₃₂ peptide labelled at its C-terminus (at residue 31) 75 had the same affinity for $B_s Gp_{1-68}$ in FP experiments³ as the equivalent residue-16 labelled 76 peptide, above. The affinity of 31-labelled peptides also has the same pattern of sensitivity to R8A, 77 A10P and R28A point mutations as 16-labelled peptides. 78

79

The affinities measured herein by FP with soluble protein fragments very likely translate to higher affinities in bacterial cells since the FP measurements do not take into account likely avidity effects that would enhance the affinity of the interaction if full-length GpsB proteins were used in combination with their full-length integral membrane protein interaction partners in the context of a biological membrane. Affinity measurements in solution of components that ordinarily interact only in the context of a membrane have indeed been found to be misleading⁴.

Recipient	<i>pbp2a</i> genotype of	Number of $\Delta pbp1a$::P _c - <i>erm</i> transformants
strain	recipient strain	at 24 h (colony size after streaking; strain) ^a
IU1824	$pbp2a^+$	>500 (medium ^b , IU13444)
IU13256	$\Delta pbp2a$	0
IU13258	$\Delta 2-49^{\rm c}$	0
IU14256	R31A	> 500 (medium, IU14294)
IU14259	R31K R33K	> 500 (medium, IU14296)
IU14263	R33A	> 500 (medium, IU14298)
IU14400	R31A S32A R36A	>500 (medium, IU14416)
IU13180	$\Delta 32-37$ (Δ SRSDRK)	>500 (medium, IU13446)
IU14396	Δ31-36	>500 (medium, IU14414)
IU14394	Δ29-36	>500 (medium, IU14412)
IU13298	Δ27-38	>500 (medium, IU13448)
IU13301	Δ26-45	>500 (small, IU13450)
IU14502	Δ2-22	>500 (small, IU14516)

87 Supplementary Table 2. *Appla*::P_c-*erm* transformation efficiencies and colony sizes

88

^aThe recipient *S. pneumoniae* strains are described in Supplementary Table 3. The transformation
and visualization of colonies was performed as described in the Supplementary Materials and
Methods. The numbers of colonies are normalized to 1 mL of transformation mixture.

⁹² ^bThe streaked colonies of strains with the $\Delta pbp1a$::P_c-*erm* genotype were smaller than isogenic ⁹³ *pbp1a*⁺ parent (IU1824).

^cThe cytoplasmic region of *Sp*PBP2a comprises the first 56 residues.

96 Supplementary Table 3: Strains used in this study

name	relevant characteristics / genotype	Ab ^{R*}	source/ reference				
L. monocyto	L. monocytogenes strains						
EGD-e	wildtype, serovar 1/2a strain	None	lab collection				
LMJR19	$\Delta gpsB$ (lmo1888)	None	3				
LMS57	$\Delta pbpA1$ (lmo1892)	None	5				
LMS64	$\Delta pbpA2$ (lmo2229)	None	5				
LMS211	$pbpAI^{\Delta N}$	None	This work				
LMS215	pbpA1 T7A	None	This work				
LMS216	pbpA1 R8A	None	This work				
LMS217	pbpA1 Y11A	None	This work				
LMS218	pbpA1 R12A	None	This work				
LMS219	$pbpA1 T7A \Delta pbpA2$	None	This work				
LMS220	$pbpA1 R8A \Delta pbpA2$	None	This work				
LMS221	$pbpA1 Y11A \Delta pbpA2$	None	This work				
LMS222	$pbpA1 R12A \Delta pbpA2$	None	This work				
LMS229	pbpA1 R8A R12A	None	This work				
LMS230	pbpA1Q10P	None	This work				
LMS232	$pbpA1 R8A R12A \Delta pbpA2$	None	This work				
LMS233	$pbpA1 Q10P \Delta pbpA2$	None	This work				
S. pneumon	ae strains	_					
IU1824 ^c	D39 $rpsL1 \Delta cps2A'$ - $cps2H'$ = D39 $rpsL1$ $\Box cps$	St	6				
IU1945	$D39 \Delta cps2A'-cps2H'= D39 \Delta cps$	None	6				
E177	D39 $\Delta cps \Delta pbp1a::P_c-erm$	Е	7				
K166	D39 $\Delta cps \Delta pbp2a::P_c-[kan-rpsL^+]$	Κ	7				
IU4888	D39 $\Delta cps \Delta gpsB \ll aad9 // \Delta bgaA::kan-t1t2-P_{fcsK}-gpsB^+$	K Sp	8				
IU4970	D39 $\Delta cps mreC$ -L-FLAG ³ -P _c -erm	Е	9				
IU5458	D39 $\Delta cps gpsB-L-FLAG^3-P_c-erm$	Е	8				
IU5838	D39 $\Delta cps gpsB$ -FLAG-P _c -erm	E	8				
IU6442	D39 $\Delta cps \Delta gpsB \ll aad9 phpP$ (G229D)	Sp	10				
IU6810	D39 $\Delta cps ezrA$ -HA-Pc- kan	Κ	10				
IU6819	D39 $\Delta cps pbp2x$ -FLAG ³ -P _c -erm	Е	11				
IU7434	D39 $\Delta cps \ stkP$ -FLAG ² -P _c -erm	Е	11				
IU7853	D39 $\Delta cps rpsL1 \Delta pbp2a::P_c-[kan-rpsL^+]$ (IU1824 X $\Delta pbp2a::P_c-[kan-rpsL^+]$ from K166)	Κ	This work				
IU8122	D39 $\Delta cps \Delta bgaA::tet-P_{Zn}-RBS^{ftsA}-ftsZ^+$	Т	12				
IU8496	D39 $\Delta cps \Delta divIVA::P_c-erm$ (IU1945 X fusion $\Delta divIVA::P_c-erm$)	Е	This work				
IU11051	D39 $\Delta cps gpsB^+$ -P _c -erm (IU1945 X fusion $gpsB^+$ -P _c -erm)	E	This work				
IU11286	D39 $\Delta cps \Delta bgaA::tet-P_{Zn}-RBS^{ftsA}-gpsB^+$ (IU1945 X fusion $\Delta bgaA::tet-P_{Zn}-gpsB^+$)	Т	This work				
IU11314	D39 $\Delta cps gpsB-L-FLAG^3-P_c-erm pbp2x-HA-P_c-kan$	EK	10				

name	relevant characteristics / genotype	Ab ^{R*}	source/ reference
IU11316	D39 Δcps gpsB-L-FLAG ³ -P _c -erm pbp2b-HA-P _c -kan	ΕK	10
IU11388	D39 $\Delta cps \Delta gpsB \ll aad9//\Delta bgaA::tet-P_{Zn}-RBS^{ftsA}-gpsB^+$ (IU11286 X $\Delta gpsB \ll aad9$ from IU4888)	T Sp	This work
IU11488	D39 $\Delta cps gpsB^+$ -P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11286 X gpsB ⁺ -P _c -erm from IU11051)	ΤE	This work
IU11880	D39 $\Delta cps ezrA$ -HA-P _c -kan-pbp2x-FLAG ³ -P _c -erm (IU6810 X pbp2x-FLAG ³ -P _c -erm from IU6819)	ΕK	This work
IU12077	D39 Δcps ezrA-HA-P _c -kan-stkP-FLAG ² -P _c -erm (IU7434 X ezrA-HA-P _c -kan from IU6810)	ΕK	This work
IU12361	D39 Δcps gpsB D29A-P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11388 X fusion gpsB D29A-P _c -erm)	ΤE	This work
IU12363	D39 Δcps gpsB D33A-P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11388 X fusion gpsB D33A-P _c -erm)	ΤE	This work
IU12440	D39 Δcps gpsB Y23A-P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11388 X fusion gpsB Y23A-P _c -erm)	ΤE	This work
IU12612	D39 Δcps gpsB V28A-P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11388 X fusion gpsB V28A-P _c -erm)	ΤE	This work
IU12615	D39 Δcps gpsB L32A-P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11388 X fusion gpsB L32A-P _c -erm)	ΤE	This work
IU12788	D39 $\Delta cps rpsL1 \Delta bgaA::kan-P_{Zn}-RBS^{fisA}-khpA^+$	K	12
IU13121	D39 $\Delta cps gpsB$ I36A-P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11388 X fusion gpsB I36A-P _c -erm)	ΤE	This work
IU13141	D39 Δcps gpsB D29A-P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11388 X gpsB D29A-P _c -erm from IU12361)	ΤE	This work
IU13180	D39 $\Delta cps rpsL1 pbp2a \Delta 32-37$ (IU7853 X fusion $pbp2a \Delta 32-37$)	St	This work
IU13256	D39 $\Delta cps rpsL1 \Delta pbp2a$ (IU7853 X fusion $\Delta pbp2a$ markerless)	St	This work
IU13258	D39 $\Delta cps rpsL1 pbp2a \Delta 2-49$ (IU7853 X fusion $pbp2a \Delta 2-49$)	St	This work
IU13298	D39 $\Delta cps rpsL1 pbp2a \Delta 27$ -38 (IU7853 X fusion $pbp2a \Delta 27$ -38)	St	This work
IU13301	D39 $\Delta cps rpsL1 pbp2a \Delta 26-45$ (IU7853 X fusion $pbp2a \Delta 26-45$)	St	This work
IU13364	D39 Δcps gpsB Y23A-FLAG-P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11388 X fusion gpsB Y23A-FLAG-P _c -erm)	ΤE	This work
IU13366	D39 Δcps gpsB V28A-FLAG-P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11388 X fusion gpsB V28A-FLAG-P _c -erm)	ΤE	This work
IU13368	D39 \Delta cps gpsB D29A-FLAG-Pc-erm//\Delta bgaA::tet-Pzn-RBS ^{ftsA} -gpsB ⁺ (IU11388 X fusion gpsB D29A-FLAG-Pc-erm)	ΤE	This work
IU13370	D39 Δcps gpsB L32A-FLAG-P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11388 X fusion gpsB L32A-FLAG-P _c -erm)	ΤE	This work
IU13372	D39 Δcps gpsB D33A-FLAG-P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11388 X fusion gpsB D33A-FLAG-P _c -erm)	ΤE	This work
IU13374	D39 Δcps gpsB I36A-FLAG-P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11388 X fusion gpsB I36A-FLAG-P _c -erm)	ΤE	This work
IU13442	D39 Δcps gpsB-FLAG-P _c -erm// $\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺ (IU11388 X gpsB-FLAG-P _c -erm from IU5838)	ΤE	This work
IU13444	D39 $\Delta cps rpsL1 \Delta pbp1a::P_c-erm$ (IU1824 X $\Delta pbp1a::P_c-erm$ from E177)	St E	This work
IU13446	D39 $\Delta cps rpsL1 pbp2a \Delta 32-37 \Delta pbp1a::P_c-erm (IU13180 X \Delta pbp1a::P_c-erm from E177)$	St E	This work
IU13448	D39 $\Delta cps rpsL1 pbp2a \Delta 27$ -38 $\Delta pbp1a$::Pc-erm (IU13298 X $\Delta pbp1a$::Pc-erm from E177)	St E	This work
IU13450	D39 $\Delta cps rpsL1 pbp2a \Delta 26-45 \Delta pbp1a::P_c-erm (IU13301 X \Delta pbp1a::P_c-erm from E177)$	St E	This work
IU14256	D39 $\Delta cps rpsL1 pbp2a$ R31A (IU7853 X fusion $pbp2a$ R31A)	St	This work
IU14259	D39 $\Delta cps rpsL1 pbp2a$ R31K R33K (IU7853 X fusion $pbp2a$ R31K R33K)	St	This work
IU14263	D39 $\Delta cps rpsL1 pbp2a$ R33A (IU7853 X fusion $pbp2a$ R33A)	St	This work
IU14294	D39 $\Delta cps rpsL1 pbp2a R31A \Delta pbp1a::P_c-erm (IU14256 X \Delta pbp1a::P_c-erm from E177)$	St E	This work
IU14296	D39 Δ <i>cps rpsL1 pbp2a</i> R31K R33K Δ <i>pbp1a</i> ::P _c - <i>erm</i> (IU14259 X Δ <i>pbp1a</i> ::P _c - <i>erm</i> from E177)	St E	This work
IU14298	D39 $\Delta cps rpsL1 pbp2a R33A \Delta pbp1a::P_c-erm (IU14263 X \Delta pbp1a::P_c-erm from E177)$	St E	This work
IU14318	D39 $\Delta cps rpsL1 \Delta bgaA::kan-P_{Zn}-RBS^{ftsA}-pbp2a^+$ (IU1824 X fusion $\Delta bgaA::kan-P_{Zn}-RBS^{ftsA}-pbp2a^+$)	St K	This work
IU14365	D39 $\Delta cps rpsL1 \Delta pbp2a$ markerless// $\Delta bgaA$::kan-P _{Zn} -RBS ^{ftsA} -pbp2a ⁺ (IU13256 X $\Delta bgaA$::kan-P _{Zn} -RBS ^{ftsA} -pbp2a ⁺ from IU14318)	St K	This work
IU14381	D39 $\Delta cps rpsL1 \Delta pbp2a$ markerless// $\Delta bgaA$::kan-P _{Zn} -RBS ^{ftsA} -pbp2a ⁺ $\Delta pbp1a$::P _c -erm (IU14365 X $\Delta pbp1a$::P _c -erm from E177)	St K E	This work
IU14383	D39 $\Delta cps \Delta gpsB \ll aad9//\Delta bgaA::tet-P_{Zn}-RBS^{ftsA}-gpsB^+ \Delta pbp1a::P_c-erm (IU11388 X \Delta pbp1a::P_c-erm from E177)$	T Sp E	This work

name	relevant characteristics / genotype	Ab ^{R*}	source/ reference
IU14394	D39 Δ <i>cps rpsL1 pbp2a</i> Δ29-36 (IU7853 X fusion <i>pbp2a</i> Δ29-36)	St	This work
IU14396	D39 Δ <i>cps rpsL1 pbp2a</i> Δ31-36 (IU7853 X fusion <i>pbp2a</i> Δ31-36)	St	This work
IU14400	D39 Δcps rpsL1 pbp2a R31A S32A R36A(IU7853 X fusion pbp2a R31A S32A R36A)	St	This work
IU14412	D39 Δ <i>cps rpsL1 pbp2a</i> Δ29-36 Δ <i>pbp1a</i> ::P _c - <i>erm</i> (IU14394 X Δ <i>pbp1a</i> ::P _c - <i>erm</i> from E177)	St E	This work
IU14414	D39 $\Delta cps rpsL1 pbp2a \Delta 31-36 \Delta pbp1a::P_c-erm (IU14396 X \Delta pbp1a::P_c-erm from E177)$	St E	This work
IU14416	D39 Δ <i>cps rpsL1 pbp2a</i> R31A S32A R36A Δ <i>pbp1a</i> ::P _c - <i>erm</i> (IU14400 X Δ <i>pbp1a</i> ::P _c - <i>erm</i> from E177)	St E	This work
IU14502	D39 $\Delta cps rpsL1 pbp2a \Delta 2-22$ (IU7853 X fusion $pbp2a \Delta 2-22$)	St	This work
IU14516	D39 $\Delta cps rpsL1 pbp2a \Delta 2-22 \Delta pbp1a::P_c-erm (IU14502 X \Delta pbp1a::P_c-erm from E177)$	St E	This work

*Ab^R relates to the antibiotic resistance marker used, St = streptomycin, E = erythromycin, K = kanamycin, T = tetracyclin, Sp = spectinomycin

99 Supplementary Table 4: Plasmids used in this study

name	relevant characteristics	Two-hybrid construct	source/ reference
General	•	· · · ·	
pMAD	bla erm bgaB		13
pKNT25	$kan P_{lac}-cya(T25)$	T25	14
pUT18	$amp P_{lac}-cya(T25)$	T18	14
pKT25	$kan P_{lac}-cya(T25)$	T25	14
pUT18C	$amp P_{lac}-cya(T18)$	T18	14
pKT25 <i>zip</i>	$kan P_{lac}-cya(T25)$ zip	T25-Zip	14
pUT18C <i>zip</i>	$amp P_{lac}-cya(T18) zip$	T18-Zip	14
B. subtilis BACTH			
pFA101	$kan P_{lac}$ - $cya(T25)$ - $ypbE$	T25-BsYpbE	This work
pFA103	$kan P_{lac}$ - $cya(T25)$ - $rodZ$	T25-BsRodZ	This work
pFA104	$kan P_{lac}$ - $cya(T25)$ - $yrrS$	T25-BsYrrS	This work
pFA105	$bla P_{lac}$ - $cya(T18)$ - $ypbE$	T18-BsYpbE	This work
pFA107	$bla P_{lac}$ - $cya(T18)$ - $rodZ$	T18-BsRodZ	This work
pFA108	$bla P_{lac}$ -cya(T18)-yrrS	T18-BsYrrS	This work
pKT25-yrrR	$kan P_{lac}$ - $cya(T25)$ - $yrrR$	T25-BsYrrR	This work
pUT18C-yrrR	$bla P_{lac}$ - $cya(T18)$ - $yrrR$	T18-BsYrrR	This work
pKT25-gpsB	$kan P_{lac}$ - $cya(T25)$ - $gpsB$	T25-BsGpsB	15
pUT18C-gpsB	$bla P_{lac}$ - $cya(T18)$ - $gpsB$	T18-BsGpsB	15
pKT25-gpsB'	$kan P_{lac}$ - $cya(T25)$ - $gpsB'_{1-65}$	T25-BsGpsB ₁₋₆₅	15
pUT18C-gpsB'	$bla P_{lac}$ - $cya(T18)$ - $gpsB'_{1-65}$	T18-BsGpsB ₁₋₆₅	15
pKT25-'gpsB	$kan P_{lac}$ - $cya(T25)$ - $gpsB_{66-98}$	T25-BsGpsB ₆₆₋₉₈	15
pUT18C-'gpsB	$bla P_{lac}$ - $cya(T18)$ - ' $gpsB_{66-98}$	T18-BsGpsB ₆₆₋₉₈	15
pKT25-ponA	$kan P_{lac}$ - $cya(T25)$ - $ponA$	T25-BsPBP1	15
pUT18C-ponA	bla P _{lac} -cya(T18)-ponA	T18-BsPBP1	15
L. monocytogenes BAC	TH		
pJR233	kan P _{lac} -cya(T25)-mreBH(lmo1713)	T25-LmMreBH	This work
pJR236	$kan P_{lac}$ -mreBH-cya(T25)	LmMreBH-T25	This work
pJR242	$kan P_{lac}$ - $cya(T25)$ - $mreC(lmo1547)$	T25-LmMreC	This work
pJR243	$kan P_{lac}$ -mreC-cya(T25)	LmMreC-T25	This work
pJR250	$kan P_{lac}$ - $cya(T25)$ - $pbpA2(lmo2229)$	T25-LmPBPA2	This work
pSH236	$kan P_{lac}$ - $cya(T25)$ - $pbpB1(lmo1438)$	T25-LmPBPB1	This work
pSH235	$kan P_{lac}$ - $cya(T25)$ - $pbpB2(lmo2039)$	T25-LmPBPB2	This work
pSH237	$kan P_{lac}$ - $cya(T25)$ - $pbpB3(lmo0441)$	T25-LmPBPB3	This work
pSH437	$kan P_{lac}$ - $cya(T25)$ - $pbpA1_{\Delta GT-\Delta TP}$	T25- Lm PBPA1 _{ΔGT-ΔTP}	16
pSH484	$bla P_{lac}$ - $gpsB^{\Delta N(202-339)}$ - $cya(T18)$	$LmGpsB_{AN}$ -T18	This work
pSH485	$kan P_{lac}-cya(T25)-pbpA1_{AGT-ATP}^{T7A}$	$T25-LmPBPA1_{AGT-ATP}^{T7A}$	This work
pSH486	$kan P_{lac}-cya(T25)-pbpA1_{AGT-ATP}^{R8A}$	T25-LmPBPA1	This work
pSH487	$kan P_{lac}-cya(T25)-pbpA1_{AGT-ATP}^{YIIA}$	T25-LmPBPA1 _{AGT-ATP} ^{YIIA}	This work
pSH488	$kan P_{lac}-cya(T25)-pbpA1_{AGT-ATP}^{R12A}$	T25-LmPBPA1 _{AGT-ATP} ^{R12A}	This work
pSH489	$kan P_{lac}-cya(T25)-pbpA1_{AGT-ATP}^{KI4A}$	T25-LmPBPA1 _{AGT-ATP} ^{K14A}	This work
pSH490	$kan P_{lac}-cya(T25)-pbpA1_{AGT-ATP}^{K20A}$	T25-LmPBPA1 $_{\Lambda GT-\Lambda TP}$ ^{K20A}	This work
pSH491	$kan P_{lac}-cya(T25)-pbpA1_{AGT-ATP}^{K2IA}$	T25-LmPBPA1 $_{\Lambda GT-\Lambda TP}$	This work
pSH492	$kan P_{lac}-cya(T25)-pbpA1_{AGT-ATP}^{K22A}$	T25-LmPBPA1 $_{\Lambda GT-\Lambda TP}$ K22A	This work
pSH493	$kan P_{lac}-cya(T25)-pbpA1_{AGT-ATP}^{K25A}$	T25-LmPBPA1 _{AGT-ATP} ^{K25A}	This work
pSH494	$kan P_{lac}-cya(T25)-pbpA1_{AGT-ATP}^{R26A}$	T25-LmPBPA1 _{AGT-ATP} ^{R26A}	This work
pSH495	$kan P_{lac}-cya(T25)-pbpA1_{AGT-ATP}^{K28A}$	T25-LmPBPA1 _{AGT-ATP} ^{K28A}	This work
pSH496	$kan P_{lac}$ - $cya(T25)$ - $pbpA1_{AGT-ATP}^{R29A}$	$T25-LmPBPA1_{AGT-ATP}^{R29A}$	This work
S. pneumoniae BACTH			
pKNT25_gpsB	$kan P_{lac}-gpsB-cya(T25)$	SpGpsB-T25	10
pUT18_gpsB	$amp P_{lac}-gpsB-cya(T18)$	SpGpsB-T18	10
pFC101	$kan P_{lac} gps B^{Y23A} - cva(T25)$	SpGpsB ^{Y23A} -T25	This work
pFC102	$amp P_{lac}-gps B^{Y23A}-cya(T18)$	SpGpsB ^{Y23A} -T18	This work
pFC103	$kan P_{lac} gps B^{V28A} - cya(T25)$	SpGpsB ^{V28A} -T25	This work
pFC104	$amp P_{lac}-gps B^{V28A}-cva(T18)$	SpGpsB ^{V28A} -T18	This work
pFC105	$kan P_{lac}-gps B^{D29A}-cva(T25)$	SpGpsB ^{D29A} -T25	This work
pFC106	$amp P_{lac}-gps B^{D29A}-cva(T18)$	SpGpsB ^{D29A} -T18	This work
L.A		· · · ·	

name	relevant characteristics	Two-hybrid construct	source/ reference
pFC107	$kan P_{lac}$ - $gpsB^{L32A}$ - $cya(T25)$	SpGpsB ^{L32A} -T25	This work
pFC108	$amp P_{lac}-gps B^{L32A}-cya(T18)$	SpGpsB ^{L32A} -T18	This work
pFC109	$kan P_{lac}-gps B^{D33A}-cya(T25)$	<i>Sp</i> GpsB ^{D33A} -T25	This work
pFC110	$amp P_{lac}-gps B^{D33A}-cya(T18)$	SpGpsB ^{D33A} -T18	This work
pFC111	$kan P_{lac}$ - $gpsB^{I36A}$ - $cya(T25)$	SpGpsB ^{I36A} -T25	This work
pFC112	$amp P_{lac}-gps B^{I36A}-cya(T18)$	SpGpsB ^{I36A} -T18	This work
pFC113	$kan P_{lac}$ - $cya(T25)$ - $mreC$	T25-SpMreC	This work
pFC114	$amp P_{lac}$ - $cya(T18)$ - $mreC$	T18-SpMreC	This work
pFC115	kan P _{lac} -cya(T25)-pbp2a	T25-SpPBP2a	This work
pFC116	$amp P_{lac}$ - $cya(T18)$ - $pbp2a$	T18-SpPBP2a	This work
pFC117	$kan P_{lac}$ - $cya(T25)$ - $pbp2a_{\Delta 32-37}$	T25-SpPBP2a $_{\Delta 32-37}$	This work
pFC119	$kan P_{lac}$ - $cya(T25)$ - $pbp2a_{\Delta 27-38}$	T25- <i>Sp</i> PBP2a $_{\Delta 27-38}$	This work
pFC121	$kan P_{lac}$ - $cya(T25)$ - $pbp2a_{\Delta 26-45}$	Т25- <i>Sp</i> РВР2а _{Δ26-45}	This work
pFC123	$kan P_{lac}$ - $cya(T25)$ - $pbp1a$	T25-SpPBP1a	This work
pFC124	$amp P_{lac}$ - $cya(T18)$ - $pbp1a$	T18-SpPBP1a	This work
pFC125	$kan P_{lac}$ - $cya(T25)$ - $pbp2b$	T25-SpPBP2b	This work
pFC126	$amp P_{lac}$ - $cya(T18)$ - $pbp2b$	T18-SpPBP2b	This work
pFC127	$kan P_{lac}$ - $cya(T25)$ - $pbp2x$	T25-SpPBP2x	This work
pFC128	$amp P_{lac}-cya(T18)-pbp2x$	T18-SpPBP2x	This work
L. monocytogenes fosfor	nycin sensitivity		
pSH497	bla erm bgaB-recU-pbpA1 (lmo1892)		This work
pSH503	bla erm bgaB- 'recU-pbpA1' $_{\Delta N}$		This work
pSH504	bla erm bgaB-´recU-pbpA1´ ^{T7A}		This work
pSH505	bla erm bgaB-´recU-pbpA1´ ^{R8A}		This work
pSH506	bla erm bgaB-´recU-pbpA1 ^{YIIA}		This work
pSH507	bla erm bgaB-´recU-pbpA1 ^{~RI2A}		This work
pSH508	bla erm bgaB-´recU-pbpA1 ^{R8A R12A}		This work
pSH509	bla erm bgaB-´recU-pbpA1 ^{~Q10P}		This work

Name	Sequence, 5' – 3'	Template
B. subtilis B	ACTH	•
Construction	of T25-fusions to <i>B. subtilis ypbE</i>	
ypbE-F	GGTATCTAGAGACGAACATGTCGAGAGTAGAGAG	1.60
ypbE-R	CTAGGTACCTACCCTATTCATCCATTAAAGG	108
Construction	of T25-fusions to B. subtilis rodZ	
rodZ-F	TGATCTAGAGTCATTGGATGATCTCCAAGCGGC	169
rodZ-R	TGAGGTACCACTTCATAAGTCGTTAACGATCC	108
Construction	n of T25-fusions to <i>B. subtilis yrrS</i>	
yrrS-F	ATGTCTAGAGAGAGCAATTAATCAATCTCGTTATGAAAATCG	169
yrrS-R	ATCGGTACCTGTTTATTTTAGCTTTTCTACTTTTGTCGGCTTCAAG	108
Construction	n of T25-fusions to <i>B. subtilis yrrR</i>	
yrrR-F	GACTCTAGAGAAGATATCGAAACGAATGAAGC	169
yrrR-R	TTAGGTACCTTATTTCTGATTGATTTCAATTTCGTGTAC	108
L. monoctoge	enes BACTH	
Construction	n of T25-fusions to <i>L. monocytogenes sepF</i>	
JR334	GCGCTCTAGAAGGACTATCGAATAAATTTAAGTCATTC	ECD
JR335	GCGCGGTACCGCCATAAAGTTTTGTTCATCGAGCATTTC	EGD-e
Construction	n of T25-fusions to <i>L. monocytogenes zapA</i>	
JR337	GCGCCTGCAGCAAATGAGAGAAATAAAGTAGTGAC	ECD a
JR338	GCGCGGTACCGCATCTCTTCCTTTAATTCGAGCCAG	EGD-e
Construction	n of T25-fusions to <i>L. monocytogenes ezrA</i>	
JR339	GCGCTCTAGAATACTACATGTTAATCGGCTTTATTATC	ECD a
JR340	GCGCGGTACCGCTTCAATATCGTCGACGCTTACTTTTG	EGD-e
Construction	of a T25-fusion to <i>L. monocytogenes divIB</i>	
JR353	GCGCTCTAGAAGCTGAAAATAAACGAGTAATTTCCATTG	ECD a
JR354	GCGCGGTACCGCTTCATTTGTTTCTTTCTTCTTTTAGC	EGD-e
Construction	of a T25-fusion to <i>L. monocytogenes divIC</i>	
JR355	GCGCTCTAGAAAAAAAAGCCAAATCAAAAGTGGCGAG	ECD a
JR356	GCGCGGTACCGCCTCTTTTGTTTCGAATTCTCTTCTG	EOD-e
Construction	n of T25-fusions to L. monocytogenes mreC	
JR364	GCGCCTGCAGCGCCACAATTTTTTCTCAATAAACGTTTG	ECD a
JR365	GCGCGGTACCGCTTGGCCTCCAGTCGTGTCTG	EGD-e
Construction	n of T25-fusions to L. monocytogenes mreBH	
JR368	GCGCTCTAGAATTTGGAACAACAACTATTGGAATTG	EGD a
JR369	GCGCGGTACCGCATTTTTAGCTAATTTATCTGCAAAAACG	
Construction	n of a T25-fusion to L. monocytogenes pbpA2	
SHW153	GACTCTAGAGGACAAATTCAAACAGCAACTTATT	EGD-e

100 Supplementary Table 5: Oligonucleotide primers used in this study for BACTH

SHW154	CTTAGG	TACCTTACCTATCGAATCGATTAAGTTTC	T
Construction	$f_0 T_25_f$	inaccinacciancoancoana anna	
SHW155		AGTGAAACGGCGTATAGGTAACATG	1
SHW155	CGCGGA		- EGD-e
Construction	of a T25-f	insign to L. monocytogenes nbnR1	
SHW157	TGAAAG	TAAATTTTAGAAAAAAGAA	Т
SHW158	CCGGA	ATTCTTAATTTTCGGTTTGTTCTGATTG	- EGD-e
Construction	of a T25-f	Susion to L. monocytogenes nbnB3	
SHW159	AACTGC	CAGTGGCTAGTTATGGTGGGAAAAAG	EGE
SHW160	CGCGGA	ATCCTTATACATACTTTCAATTACAGG	- EGD-e
S. pneumonia	e BACTH		_
Construction	of a T25-f	usion to S. pneumoniae gpsB	
gpsB_PF	AACTGC	CAGGATGGCAAGTATTATTTTTCAGCG	D39 and
gpsB_BR	CGGGAT	TCCTCAAAATCTGAGTTATCTAAAATTTG	mutants
Construction	of a T25-f	fusion to <i>S. pneumoniae mreC</i>	
mreC_XF	GCTCTA	GAGATGAACCGTTTTAAAAAATCAAAAT	D20
mreC_BR	CGGGAT	TCCTTATGAATTCCCCACTAATTCTATC	- D39
Construction	of a T25-f	fusion to S. pneumoniae pbp2a	
pbp2a_XF	CGTCTA	GATATGAAATTAGATAAATTATTTGAGAAATTTCTTTCTCTTTTTAAAAAAGAAACAAG	D39 and
pbp2a_BR	CGGGAT	TCCTTAGCGAAATAGATTGACTATCGAATCCC	mutants
Construction	of a T25-f	fusion to S. pneumoniae pbp1a	
pbp1a_XF	GCTCTA	GAGATGAACAAACCAACGATTCTGCG	D20
pbp1a_BR	CGGGAT	TCCTTATGGTTGTGCTGGTTGAGGAT	D39
Construction	of a T25-f	usion to <i>S. pneumoniae pbp2x</i>	
<i>pbp2x_</i> XF	CGTCTA	GAGATGAAGTGGACAAAAAGAGTAATCC	D20
pbp2x_BR	CGGAAT	TCTTAGTCTCCTAAAGTTAATGTAAT	- D39
Construction	of a T25-f	fusion to S. pneumoniae pbp2b	-
pbp2b XF	GCGGA	TCCCATGAGACTGATTTGTATGAG	
<i>pbp2b</i> BR	CGGAA	TTCCTAATTCATTGGATGGTATTTTTG	- D39
Sequencing a	nd verifica	tion of S. pneumoniae BACTH	
pKT25 579F		GTTCGCCATTATGCCGCATC	
pKT25_802R		GGATGTGCTGCAAGGCGATT	
pUT18C_484	F	GATGTACTGGAAACGGTGC	
pUT18C_660	R	CTTAACTATGCGGCATCAGAGC	
pKNT25/pUT	18_49F	CGCAATTAATGTGAGTTAGC	
pKNT25_328	R	TTGATGCCATCGAGTACG	
pUT18_304R		CGAGCGATTTTCCACAACAA	
<i>pbp2a_</i> 1010F		AGAGCTGGACCAAAACTACC	
<i>pbp2a</i> _1106R	2	CGGTTCGAGAGCTACACTTC	

<i>pbp1a_</i> 980F	GCAAGTCGCTTCTACCATTG
<i>pbp1a_</i> 1126R	CAAGGCAGGAGCATAGTCTG
<i>pbp2x_</i> 1055F	CCTTTCCAGGAGGAGAAGTC
<i>pbp2x_</i> 1177R	CCAACGTTACTTGAGTGTGC
<i>pbp2b_</i> 943F	GGCTTTCCAAGATAGCGTGG
<i>pbp2b_</i> 1047R	AAACCGCACCTGTTTTTGGG

102	Supplementar	v Table 6: Ol	igonucleotide	primers used in	this study	for L. monocvto	genes and S.	pneumoniae s	train construction
		,						p	

L. monoctog	enes strain construction			
Introduction	n of T7A into <i>pbpA1</i>			
SHW744	CCGCAGGCAAGATCTCAGTATCGCAATAAAC	mSII427		
SHW754	AGATCTTGCCTGCGGTTTATCTGCCTCTAG	рэп457, рец407	pbpA1 ^{T7A}	
SHW745	AGATCTTGCCTGCGGTTTATCTGCCAT	рзп497		
Introduction	n of R8A into <i>pbpA1</i>			
SHW746	CAGACAGCATCTCAGTATCGCAATAAACAAAG	pSH437,	$nhn A I^{R8A}$	
SHW747	CTGAGATGCTGTCTGCGGTTTATCTGCC	pSH497	popAI	
Introduction	n of R12A into <i>pbpA1</i>			
SHW748	CAGTATGCAAATAAACAAAGTGGTGGTTCTAAA	pSH437,	$nhn \Lambda I^{RI2A}$	
SHW749	TTTATTTGCATACTGAGATCTTGTCTGCGG	pSH497	popAI	
Introduction	n of R8A R12A into <i>pbpA1</i>			
SHW750	CAGACAGCATCTCAGTATGCAAATAAACAAAGTGGTGGTTCTAAA	nSU407	nhn A 1 ^{R8AR12A}	
SHW751	GTTTATTTGCATACTGAGATGCTGTCTGCGGTTTATCTGCC	p311497	popAl	
Deletion of	SpsB N-terminus			
SHW752	TCGACTCTAGAGCCGCTTAGAACTTCAACACAACC	nSU226	ansB	
SHW753	TAAGCGGCTCTAGAGTCGACCTGCAGG	p311220		
Introduction	n of Y11A into <i>pbpA1</i>			
SHW755	TCTCAGGCTCGCAATAAACAAAGTGGTGG	nSU/37	nhn 1 ^{YIIA}	
SHW756	ATTGCGAGCCTGAGATCTTGTCTGCGG	p311437	рорлі	
Introduction	of K14A into <i>pbpA1</i>			
SHW757	CGCAATGCACAAAGTGGTGGTTCTAAAAAG	nSH/37	$n h n \Lambda 1^{K14A}$	
SHW758	ACTTTGTGCATTGCGATACTGAGATCTTGTC	p511457	рорлі	
Introduction	n of K20A into <i>pbpA1</i>			
SHW759	GGTTCTGCAAAGAAATCCCAAAAACGAGG	nSH/37	$nhnA I^{K20A}$	
SHW760	TTTCTTTGCAGAACCACCACTTTGTTTATTG	p511457	рорлі	
Introduction	of K21A into <i>pbpA1</i>			
SHW761	TCTAAAGCGAAATCCCAAAAACGAGGAAAAC	nSH/37	$nbn4 1^{K21A}$	
SHW762	GGATTTCGCTTTAGAACCACCACTTTGTTTATTG	p511457	рорлі	
Introduction	of K22A into <i>pbpA1</i>			
SHW763	AAAAAGGCATCCCAAAAACGAGGAAAACG	nSH/37	$nbn4 1^{K22A}$	
SHW764	TTGGGATGCCTTTTTAGAACCACCACTTTG	p511+57	popul	
Introduction	n of K25A into <i>pbpA1</i>			
SHW765	TCCCAAGCACGAGGAAAACGAGTAGCAG	nSH/37	$nbn4 1^{K25A}$	
SHW766	TCCTCGTGCTTGGGATTTCTTTTAGAACCAC	P311437	popili	
Introduction	n of R26A into <i>pbpA1</i>			
SHW767	CAAAAAGCAGGAAAACGAGTAGCAGCG	pSH427 phpA1 ^{R26A}		
SHW768	TTTTCCTGCTTTTTGGGATTTCTTTTTAGAACC	P311437	ρυρκι	

Introduction	n of K28A into <i>pbpA1</i>		
SHW769	CGAGGAGCACGAGTAGCAGCGAATATTTTC	pSH437	nbn 4 1 ^{K28A}
SHW770	TACTCGTGCTCCTCGTTTTTGGGGATTTCTTTTAG	pSH457	popAI
Introduction	n of R29A into <i>pbpA1</i>		
SHW771	GGAAAAGCAGTAGCAGCGAATATTTTCAAAAC	pSU/37	nhn 4 1 ^{R29A}
SHW772	TGCTACTGCTTTTCCTCGTTTTTGGGATTTCTT	p311437	рорат
Construction	n of pMAD- <i>pbpA1</i> ´(pSH497)		
SHW773	GCGCGCGGATCCCGAAGGTACGTTCTATTTATGAG	ECD a	nhn 41'
SHW774	GCGCGCCCATGGGTTGGAGCGGTTCCGGATAAG	EOD-e	рорат
Deletion of p	bbpA1 N-terminus		
SHW775	TATTTAGTCGACCATTAAAAATCTCTCTCCTTTAAA	pSU407	nhn 41'
SHW776	TTAATGGTCGACTAAATATACGTATTCTGGTGGAACCCC	pSH497	$popAT \Delta N$
Correction of	of pMAD-pbpA1´(pSH497)		
SHW777	GATATCGGATCCATTGGTTACCCTAACGGCAAGAAG	nSII 407	mbm 4.1.
SHW778	ACCAATGGATCCGATATCGCCCGACGCGAGG	ръп497	popAI
Introduction	n of Q10P into <i>pbpA1</i>		
SHW787	AGATCTCCGTATCGCAATAAACAAAGTGGTGG	nSII 407	mbm 4 1010P
SHW788	GCGATACGGAGATCTTGTCTGCGGTTTATC	ръп497	popA1-
S. pneumoni	ae strain construction		
Construction	n of IU8496 (Δ <i>divIVA</i> ::P _c -erm)		
TT242	GGGAATGGAATGGATAAAGAAGGTAGAAGA	D20	Upstream of <i>divIVA</i> to 8 bp
SC216	CATTATCCATTAAAAAATCAAACGGATCCTTACTTACTTA	D39	before divIVA ORF
SC215	TAACCGTCCAGTTATTATTAAGTAAGTAAGGATCCGTTTGATTTTTAATGGATAATGTG	E177	B array
SC218	GCTGGTGTTGGACCTGTCGGATGCACTGGAGGGGCCCCTTTCCTTATGCTTTTGGAC	E1//	r _c -erm
SC217	GTCCAAAAGCATAAGGAAAGGGGGCCCCTCCAGTGCATCCGACAGGTCCAACACCAGC	D20	Downstream of divIVA
TT238	TTCAGCAAGGGCTGACTCAGATGACCATGA	D39	Downstream of alvivA
Construction	n of IU11051 (gpsB ⁺ -P _c -erm)		
TT196	GCCAAGCCCTGAGACAAATAGTAGTCGTTGGT	D20	Unstream of $an a P + an a P^+$
TT905	ACAAATTTTGGGCCCGGTTAAAAATCTGAGTTATCTAAAATTTGTTTACCAAA	D39	Opstream of gpsb + gpsb
TT906	GTAAACAAATTTTAGATAACTCAGATTTTTAACCGGGCCCAAAATTTGTTTG	1115020	D and downstream of an D
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT	103838	P_c - <i>erm</i> + downstream of <i>gpsb</i>
Construction	n of IU11286 ($\Delta bgaA$::tet-P _{Zn} -RBS ^{ftsA} -gpsB ⁺)		
TT657	CGCCCCAAGTTCATCACCAATGACATCAAC	1110100	5' fragment $\Delta bgaA::tet-P_{zn}$
JC03	CTTTCGCTGAAAAAATAATACTTGCCATTACATCGCTTCCTCTCTATCTTCCTTGTTATA	108122	RBS ^{ftsA}
JC04	GGAAGATAGAGAGGAAGCGATGTAATGGCAAGTATTATTTTTCAGCGAAAG	D20	middle freement $ang D^+$
JC05	GTTTATGAGAAAGTAAGTTCTTTTAAAAATCTGAGTTATCTAAAATTTGTTTACCAAAAA	D39	mode fragment gpsb
JC06	AAACAAATTTTAGATAACTCAGATTTTTAAAAGAACTTACTT		
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC	108122	5 UguA fragment
Constructio	n of IU12361 ($gpsB^{D29A}$ - P_c - erm)		
TT196	GCCAAGCCCTGAGACAAATAGTAGTCGTTGGT	D39	Upstream and 5' gpsB ^{D29A}

JM072	ATGACATCGTCTAAAAACTCGGCAACTTCTACTTTATTATAGCC						
JM073	GGCTATAATAAAGTAGAAGTTGCCGAGTTTTTAGACGATGTCAT	III11051	$3'gpsB^{D29A}$ -P _c -erm +				
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT	1011051	downstream				
Construction	n of IU12363 ($gpsB^{D33A}$ -P _c - erm)	-					
TT196	GCCAAGCCCTGAGACAAATAGTAGTCGTTGGT	D20	Unstream and 5' and D ^{D33A}				
JM074	TCCTTGATGACATCGGCTAAAAACTCGTCAACTTCTA	D39	Opsiteani and S gpsb				
JM075	TAGAAGTTGACGAGTTTTTAGCCGATGTCATCAAGGA	III11051	$3'gpsB^{D33A}-P_c-erm+$				
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT	1011031	downstream				
Construction	n of IU12440 (gpsB ^{Y23A} -P _c -erm)						
TT196	GCCAAGCCCTGAGACAAATAGTAGTCGTTGGT	D20	Lingtroom and 5' and PY23A				
JM070	CAACTTCTACTTTATTAGCGCCACGGACTTCACG	D39	Opstream and 5 gpsb				
JM071	CGTGAAGTCCGTGGCGCTAATAAAGTAGAAGTTG	III11051	$3'gpsB^{Y23A}-P_c-erm+$				
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT	1011031	downstream				
Construction	n of IU12612 ($gpsB^{V28A}$ -P _c - erm)						
TT196	GCCAAGCCCTGAGACAAATAGTAGTCGTTGGT	D20	Unstroom and 5' and PV28A				
JM091	CATCGTCTAAAAACTCGTCAGCTTCTACTTTATTATAGCC	D39	Opsitealli and 5 gpsb				
JM092	GGCTATAATAAAGTAGAAGCTGACGAGTTTTTAGACGATG	III11051	$3'gpsB^{V28A}$ -P _c -erm +				
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT	1011031	downstream				
Construction	n of IU12615 ($gpsB^{L32A}$ -P _c - erm)						
TT196	GCCAAGCCCTGAGACAAATAGTAGTCGTTGGT	D20	Lingtroom and 5' ang PL32A				
JM093	CTTGATGACATCGTCTGCAAACTCGTCAACTTCTAC	D39	Opstream and 5 gpsb				
JM094	GTAGAAGTTGACGAGTTTGCAGACGATGTCATCAAG	III11051	$3'gpsB^{L32A}-P_c-erm+$				
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT	1011031	downstream				
Construction	n of IU13121 ($gpsB^{I36A}$ -P _c - erm)						
TT196	GCCAAGCCCTGAGACAAATAGTAGTCGTTGGT	D20	Unstroom and 5' and P ^{I36A}				
JM076	CATAGGTTTCATAGTCCTTGGCGACATCGTCTAAAAAC	D39	Opsitealli and 5 gpsb				
JM077	GTTTTTAGACGATGTCGCCAAGGACTATGAAACCTATG	III11051	$3'gpsB^{I36A}$ -P _c -erm +				
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT	1011031	downstream				
Construction	n of IU13180 ($pbp2a_{\Delta 32-37}$)						
P226	GGTACGACAACGAAATGTCATACACTGCAC	D30	Unstream and 5' nhn2a				
JM057	TTTTCGAATCGGACCTACTTGGGCTAATTTGCGACGTAAGATAGTAGAATCAGAGTCCTC	D39	Opsitically and 5 $pop_{2u_{\Delta 32-37}}$				
JM058	GAGGACTCTGATTCTACTATCTTACGTCGCAAATTAGCCCAAGTAGGTCCGATTCGAAAA	D30	3' nhn2a i downstroom				
P227	TCTGTTCCCGTGTGATCCGACAAATCCT	D39	$5 pop_2 u_{\Delta 32-37}$ + downstream				
Construction	n of IU13256 (Δ <i>pbp2a</i> markerless, Δ31-712)						
P226	GGTACGACAACGAAATGTCATACACTGCAC	030	Upstream and 5' 90 bp of				
TT1013	TTTGAGCCTTTTCCTTAATCTTCGCACGTAAGATAGTAGAATCAGAGTCCTCTAGTTCAC	D39	pbp2a				
TT1014	AACTAGAGGACTCTGATTCTACTATCTTACGTGCGAAGATTAAGGAAAAGGCTCAAACAA	D39	<i>3</i> ' 60 <i>bp</i> of <i>pbp2a</i> +				
P227	TCTGTTCCCGTGTGATCCGACAAATCCT	D39	downstream				
Construction	n of IU13258 ($pbp2a_{\Delta 2-49}$)						
P226	GGTACGACAACGAAATGTCATACACTGCAC	D39	Upstream and 5' $pbp2a_{\Delta 2-49}$				

TT1015	AGTATAAGGATAATCTTTGTTAGATGATACATGCGTTTATTTTATCATCTTCATCATAGG					
TT1016	AAGATGATAAAATAAACGCATGTATCATCTAACAAAGATTATCCTTATACTAGGTTTGAG	D20	2' nhn2a downstroom			
P227	TCTGTTCCCGTGTGATCCGACAAATCCT	D39	5 $pop2a_{\Delta 2-49}$ + downstream			
Construction	n of IU13298 ($pbp2a_{\Delta 27-38}$)					
P226	GGTACGACAACGAAATGTCATACACTGCAC	D20	Lingtroom and 5' nhm2 a			
TT1020	AATCGGACCTACTTGGGCTAAAGAATCAGAGTCCTCTAGTTCACTTGTTTCTT	D39	Opsite and 3 $pop_{2a_{\Delta 27-38}}$			
TT1021	AACAAGTGAACTAGAGGACTCTGATTCTTTAGCCCAAGTAGGTCCGATTCGA	D20	2'			
P227	TCTGTTCCCGTGTGATCCGACAAATCCT	D39	5 $pop2a_{\Delta 27-38}$ + downstream			
Construction	n of IU13301 ($pbp2a_{\Delta 26-45}$)					
P226	GGTACGACAACGAAATGTCATACACTGCAC	D20				
TT1022	TAACGACGCCAGAATTTTCGATCAGAGTCCTCTAGTTCACTTGTTTCTTTTT	D39	Opstream and 5 $pbp2a_{\Delta 26-45}$			
TT1023	AGAAACAAGTGAACTAGAGGACTCTGATCGAAAATTCTGGCGTCGTTATCAT	D20	2'			
P227	TCTGTTCCCGTGTGATCCGACAAATCCT	D39	$5 pop_{2a_{\Delta 26-45}}$ + downstream			
Construction	n of IU13364 (gpsB ^{Y23A} -FLAG-P _c -erm)					
AL298	GAGGGAAGGCACCAGCCTTGATTTCA	1112440	Lington and an PY23A			
TT262	TTATTTATCATCATCATCTTATAATCAAAATCTGAGTTATCTAAAATTTGTTTACCAAA	1012440	Upstream and gpsB			
TT263	TAACTCAGATTTTGATTATAAAGATGATGATGATAAATAA	1115020				
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT	105858	$\Gamma LAO - \Gamma_c - erm + downsuream$			
Construction	n of IU13366 ($gpsB^{V28A}$ -FLAG-P _c - erm)					
AL298	GAGGGAAGGCACCAGCCTTGATTTCA	H112612	Unstructure and an pV28A			
TT262	TTATTTATCATCATCATCTTATAATCAAAATCTGAGTTATCTAAAATTTGTTTACCAAA	1012012	Upstream and gpsB			
TT263	TAACTCAGATTTTGATTATAAAGATGATGATGATAAATAA	1115020				
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT	103838	$FLAG-P_c$ - <i>erm</i> + downstream			
Construction	n of IU13368 (gpsB ^{D29A} -FLAG-P _c -erm)					
AL298	GAGGGAAGGCACCAGCCTTGATTTCA	П112141	Lingtroom and an pD29A			
TT262	TTATTTATCATCATCATCTTATAATCAAAATCTGAGTTATCTAAAATTTGTTTACCAAA	1013141	Upstream and gpsB			
TT263	TAACTCAGATTTTGATTATAAAGATGATGATGATAAATAA	1115020	ELAC De anna i downstroom			
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT	103838	$FLAG-P_c$ - <i>erm</i> + downstream			
Construction	n of IU13370 ($gpsB^{L32A}$ -FLAG-P _c - erm)					
AL298	GAGGGAAGGCACCAGCCTTGATTTCA	III12615	Unstroom and ang PL32A			
TT262	TTATTTATCATCATCATCTTTATAATCAAAATCTGAGTTATCTAAAATTTGTTTACCAAA	1012013	Opsiteani and gpsb			
TT263	TAACTCAGATTTTGATTATAAAGATGATGATGATAAATAA	1115929	ELAC D and downstroom			
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT	103838	$FLAG-F_c$ - <i>erm</i> + downstream			
Construction	n of IU13372 (gpsB ^{D33A} -FLAG-P _c -erm)					
AL298	GAGGGAAGGCACCAGCCTTGATTTCA	III12262	Unstream and ans P D33A			
TT262	TTATTTATCATCATCATCTTTATAATCAAAATCTGAGTTATCTAAAATTTGTTTACCAAA	1012303	Opsiteani and gpsb			
TT263	TAACTCAGATTTTGATTATAAAGATGATGATGATGATAAATAA	1115838	FLAC P arm + downstream			
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT	103636	$\Gamma LAO - \Gamma_c - erm + uownsureann$			
Construction	n of IU13374 (gpsB ^{136A} -FLAG-P _c -erm)					
AL298	GAGGGAAGGCACCAGCCTTGATTTCA	IU13121	Upstream and gpsB ^{Y23A}			

TT262	TTATTTATCATCATCATCTTATAATCAAAATCTGAGTTATCTAAAATTTGTTTACCAAA				
TT263	TAACTCAGATTTTGATTATAAAGATGATGATGATAAATAA	1115929	ELAC D and downstroom		
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT	103838	$FLAG-F_c$ -erm + downstream		
Construction	n of IU14256 (<i>pbp2a</i> ^{R31A})				
P226	GGTACGACAACGAAATGTCATACACTGCAC	D30	Unstream and 5' nhn2a ^{R31A}		
TT1056	TTTCGATCACTACGAGAGGCACGTAAGATAGTAGAATCAG	D39	Opsiteani and 5 popzu		
TT1057	CTGATTCTACTATCTTACGTGCCTCTCGTAGTGATCGAAA	D30	3' nhn 2 a ^{R31A} + downstroom		
P227	TCTGTTCCCGTGTGATCCGACAAATCCT	D39	5 pop2a + downstream		
Construction	n of IU14259 (<i>pbp2a</i> ^{R31K R33K})				
P226	GGTACGACAACGAAATGTCATACACTGCAC	D20	Lingtroom and 5' nhn 2 R ^{31K} R ^{33K}		
TT1058	CTAATTTTTTCGATCACTCTTAGATTTACGTAAGATAGTAGAATCAG	D39	Opstream and 3 pbp2a		
TT1059	CTGATTCTACTATCTTACGTAAATCTAAGAGTGATCGAAAAAAATTAG	D30	3' nhn 2 a ^{R31K R33K} + downstroom		
P227	TCTGTTCCCGTGTGATCCGACAAATCCT	D39	5 pop2a + downstream		
Construction	n of IU14263 (<i>pbp2a</i> ^{R33A})				
P226	GGTACGACAACGAAATGTCATACACTGCAC	D20	Unstroom and 5' nhn2a ^{R33A}		
JM047	TTTTCGATCACTGGCAGAGCGACGTAAGAT	D39	Opsiteani and 5 popza		
JM048	ATCTTACGTCGCTCTGCCAGTGATCGAAAA	D20	2' nhn 2 a ^{R33A} + downstroom		
P227	TCTGTTCCCGTGTGATCCGACAAATCCT	D39	5 pop2a + downstream		
Construction	n of IU14318 (ΔbgaA::kan-P _{Zn} -RBS ^{ftsA} -pbp2a ⁺)				
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	1112700	5' fragment $\Delta bgaA::kan-P_{zn}$ -		
JC07	ATTTCTCAAATAATTTATCTAATTTCATTACATCGCTTCCTCTCTATCTTCCTTGTTATA	1012/88	RBS ^{ftsA}		
JC08	GAAGATAGAGAGGAAGCGATGTAATGAAATTAGATAAATTATTTGAGAAATTTCTTTC	D20	middle frequent $nhn2a^+$		
JC09	ACTGGTTTATGAGAAAGTAAGTTCTTTTATTAGCGAAATAGATTGACTATCGAATCCC	D39	findule fragment <i>popza</i>		
JC10	ATTCGATAGTCAATCTATTTCGCTAATAAAAGAACTTACTT	D30	3' hagh fragmont		
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC	D39	5 OguA fragment		
Construction	n of IU14394 ($pbp2a_{\Delta 29.36}$)				
P226	GGTACGACAACGAAATGTCATACACTGCAC	D20	Unstream and 5' nhn2a		
TT1091	CGGACCTACTTGGGCTAATTTTTTGATAGTAGAATCAGAGTCCTCTAGTTCACTTGTTTC	D39	Opstream and $5 pop_2 u_{\Delta 29-36}$		
TT1092	AGTGAACTAGAGGACTCTGATTCTACTATCAAAAAATTAGCCCAAGTAGGTCCGATT	D30	3' nhn2a - L downstroom		
P227	TCTGTTCCCGTGTGATCCGACAAATCCT	D39	5 $pop_2a_{\Delta 29-36}$ + downstream		
Construction	n of IU14396 ($pbp2a_{\Delta 31-36}$)				
P226	GGTACGACAACGAAATGTCATACACTGCAC	D20	Unstream and 5' nhn2a		
TT1093	GGACCTACTTGGGCTAATTTTTTACGTAAGATAGTAGAATCAGAGTCCTCTAGTTCAC	D39	Opstream and $3 pop_2a_{\Delta 31-36}$		
TT1094	CTAGAGGACTCTGATTCTACTATCTTACGTAAAAAATTAGCCCAAGTAGGTCCGATT	D20	2' nhn2a - downstroom		
P227	TCTGTTCCCGTGTGATCCGACAAATCCT	D39	5 $pop2a_{\Delta 31-36}$ + downstream		
Construction	n of IU14400 (<i>pbp2a</i> ^{R31A S32A R36A})				
P226	GGTACGACAACGAAATGTCATACACTGCAC	D20	Upstream and 5' <i>pbp2a</i> ^{R31A S32A}		
TT1095	ATTTTTTGCATCACTACGAGCGGCACGTAAGATAGTAGAATCAGAGTCCTCTAGTTCAC	2039	R36A		
TT1096	TCTACTATCTTACGTGCCGCTCGTAGTGATGCAAAAAATTAGCCCAAGTAGGTCCGATT	D20	$3' pbp2a^{R31A S32A R36A} +$		
P227	TCTGTTCCCGTGTGATCCGACAAATCCT	D39	downstream		

Construction	n of IU14502 ($pbp2a_{\Delta 2-22}$)		
P226	GGTACGACAACGAAATGTCATACACTGCAC	D20	Unstream and 5' nhn2a
TT1097	GCGACGTAAGATAGTAGAATCAGAGTCCATGCGTTTATTTA	D39	Opstream and 5 $pop2a_{\Delta 2-22}$
TT1098	GATGAAGATGATAAAATAAACGCATGGACTCTGATTCTACTATCTTACGTCGCTCTC	D20	2' mbm2g / downgtmoorm
P227	TCTGTTCCCGTGTGATCCGACAAATCCT	D39	$5 pop_2a_{\Delta 2-22}$ + downstream

103 Supplementary Table 7: Oligonucleotide primers used in this study for recombinant protein

production

Name	Sequence, 5' – 3'
Construction of plasmid exp	ressing recombinant BsGpsB ₅₋₆₄
BsGpsB5start	GATGCTTCATATGAAAGTAAAGCTTTCTGCGAAAGAAATTTTGG
BsGpsB64stop	CTGTTTCTCGAGGGCTTCTTCAAGCTGTTTTTTCAGCTG
Introduction of E17A to BsC	SpsB ₁₋₆₃
BsGpsBE17A5	CTGCGAAAGAAATTTTGGAAAAAGCATTTAAAACAGGCGTTAGAGGC
BsGpsBE17A3	GCCTCTAACGCCTGTTTTAAATGCTTTTTCCAAAATTTCTTTC
Introduction of Y25F to BsG	bpsB ₁₋₆₃
BsGpsBY25F5	CAGGCGTTAGAGGCTTCAAGCAAGAAGACGTTGAC
BsGpsBY25F5	GTCAACGTCTTCTTGCTTGAAGCCTCTAACGCCTG
Introduction of D31A to Bs(SpsB ₁₋₆₃
BsGpsBD31A5	CGTTAGAGGCTACAAGCAAGAAGACGTTGCCAAATTTTTAGATATGATTATT
	AAGG
BsGpsBD31A3	CCTTAATAATCATATCTAAAAATTTGGCAACGTCTTCTTGCTTG
	ACG
Introduction of D35A to Bs(GpsB ₁₋₆₃
BsGpsBD35A5	GAAGACGTTGACAAATTTTTAGCTATGATTATTAAGGATTATGAAACCTTCC
	ATC
BsGpsBD35A3	GATGGAAGGTTTCATAATCCTTAATAATCATAGCTAAAAATTTGTCAACGTC
_	TTC
Introduction of S16C to BsP	BP1 ₁₋₁₇
BsPBP1S16C5	GCTCGACGAAAAGCGAACTGCAAATCGAGTCCTTCACC
BsPBP1S16C3	GGTGAAGGACTCGATTTGCAGTTCGCTTTTCGTCGAGC
Introduction of S7A to BsPE	3P1 ₁₋₁₇
BsPBP1S7A5	CCATGGCAGATCAATTTAACGCCCGTGAAGCTCGACGAAAAGC
BsPBP1S7A3	GCTTTTCGTCGAGCTTCACGGGCGTTAAATTGATCTGCCATGG
Introduction of R8A to BsPI	3P1 ₁₋₁₇
BsPBP1R8A5	GCAGATCAATTTAACAGCGCTGAAGCTCGACGAAAAGCGAACTG
BsPBP1R8A3	CAGTTCGCTTTTCGTCGAGCTTCAGCGCTGTTAAATTGATCTGC
Introduction of A10P to BsP	BP1 ₁₋₁₇
BsPBP1A10P5	GATCAATTTAACAGCCGTGAACCTCGACGAAAAGCGAACTGC
BsPBP1A10P3	GCAGTTCGCTTTTCGTCGAGGTTCACGGCTGTTAAATTGATC
Introduction of R11A to BsH	BP1 ₁₋₁₇
BsPBP1R11A5	CAATTTAACAGCCGTGAAGCTGCACGAAAAGCGAACTGCAAATCG
BsPBP1R11A3	CGATTTGCAGTTCGCTTTTCGTGCAGCTTCACGGCTGTTAAATTG
Introduction of R28A to BsH	BP1 ₁₋₃₂
BsPBP1R28A5	CACCGAAAAAAGGCAAGAAAGCAAAAAAGGGCGGATAGTTTAAAAAG
BsPBP1R28A3	CTTTTTAAACTATCCGCCCTTTTTTGCTTTCTTGCCTTTTTTCGGTG
Introduction of R8K to BsPl	BP1 ₁₋₁₇
BsPBP1AR8K5	CATGGCAGATCAATTTAACAGCAAAGAAGCTCGACGAAAAGCGAACTGC
BsPBP1AR8K3	GCAGTTCGCTTTTCGTCGAGCTTCTTTGCTGTTAAATTGATCTGCCATG
Introduction of R11K to Bs	BP1 ₁₋₁₇
BsPBP1AR11K5	CAGATCAATTTAACAGCCGTGAAGCTAAACGAAAAGCGAACTGCAAATCG
BsPBP1AR11K3	CGATTTGCAGTTCGCTTTTCGTTTAGCTTCACGGCTGTTAAATTGATCTG
Construction of plasmid exp	ressing recombinant MBP-LmPBPA1 ₁₋₂₀
LmPBPA1ncoI5	GATTTTCCATGGCAGATAAACCGCAGACAAG
LmPBPA1xhoI3	GTAGACCCTCGAGAACGTTTTACTTGGGTTGCATAGTTATAAC
Introduction of S19C, K21S	TOP to MBP-LmPBPA1 _{1,20} to generate MBP-LmPBPA1 _{1,20} ^{S19C}
LmPBPA1S19CK21STOP5	CAGTATCGCAATAAACAAAGTGGTGGTGGTTGTAAATAGAAATCCCAAAAACGA
	GG
LmPBPA1S19CK21STOP3	CCTCGTTTTTGGGATTTCTATTTACAACCACCACTTTGTTTATTGCGATACTG
Introduction of R8A to LmP	BPA1 ₁₋₂₀ S19C
LmPBPA1R8A5	GGCAGATAAACCGCAGACAGCATCTCAGTATCGCAATAAACAAAG
LmPBPA1R8A3	CTTTGTTTATTGCGATACTGAGATGCTGTCTGCGGTTTATCTGCC
Introduction of O10P to Lm	PBPA1 _{1.20} ^{S19C}
LmPBPA1Q10P5	GATAAACCGCAGACAAGATCTCCGTATCGCAATAAACAAAGTGG
LmPBPA1Q10P3	CCACTTTGTTTATTGCGATACGGAGATCTTGTCTGCGGTTTATC

LmPBPA1Y11A5 GGCAGATAAACCGCAGACAAGATCTCAGGCTCGCAATAAACAAAGTGGTGG TrG LmPBPA1Y11A3 CAACCACCACTTGTTTATTGCGAGCCTGAGATCTTGTCGCGGTTTATCTGC C ImPBPA1R12A5 GATAAACCGCCACACTTGTTTATTGCCAAGTCTCAGTATCACAAAGTGGTGGTTGT LmPBPA1R12A5 ImPBPA1R12A5 GATAAACCGCCACTTGTTTATTGGCAATCGAGATCTGAGATCTGCGGTTGTAACAAAGTGGTGGTTGT LmPBPA1S16R5 CTCCAGTATCGCAATAAACAACGTGGTGGTTGTAAATAGAAACCCC LmPBPA1S16R5 CTCAGTATCGCAATAAACAACGTGGTGGTTGTAAATAGAAATCCC LmPBPA1S16R5 CTCAGTATCGGCAATCAACCACCACGTGGTTGTAAATAGAAATCCGC LmPBPA1S16R5 CTCAGTATCGGCAATCGCCAAGGTCGCGACGTCGCAACCCCGGC LmPBPA1S0 GCGGCCCTCGGGCCTCCGGCACGTCGCCAGACCCCAGC SpPBP2Ancol5 CGGCCCCTCGGGCCCTCGGCAGATCGCGCAGC SpPBP2Ancol5 CGGCCCCTCGGGCCTCGCGCGCAGTTGGGACATCTTGGGGCAAATTTTGGGCTC SpPBP2Ancol5 CGAGCCCCCCGGGCCGCGCAGGCGCGCGCGCGCGCGCGC	Introduction of Y11A to Lm	PBPA1 ₁₋₂₀ ^{S19C}								
LmPBPAIVIIA3 CAACCACCACTTTGTTTATTGCGAGGCTGAGATCTTGTCGCGGTTATCTGC C C C Introduction of R12A to ImPIPAI_m3ACCCGCAGACAAGATCTCAGTATGCCCAATAAACAACGTGGTGGTTG LmPBPAIR12A3 CAACCACCACCACTTIGTTATTGGCATACGAGATCTGAGATCTGCGGTTATC Introduction of S16R to ImPBPAIrag CAACCACCACCACTTIGTTTATTGGCATACGAGATCTGAGATCTGCGGTTGTCAAATAGAAACCCC LamPBPAISI6R5 CITCAGTATCGGCATAAAACACGTGGGTTGTAAATAGAAATCCCC LamPBPAISI6R5 CITCAGTATCGGCATACGGCACCTGGCGTTGTTAATGGCGATCGGAGATCGGAGA SpPBP2Ancol5 GGAACAAGTGAACCCATGGACTCTGATTGTACTATCTACGAGC SpPBP2Ancol5 CGGCCCCTCGGGCCCTGGCGCGCAGATCGGCAGC Dirdduction of G43C, R46STOP1 GMPS-SpPBP2ana GGGCCCTCGGCGCCGCGAGATCTGGGACATCTGGGCAAATTTTTGGGCT SpPBP2Ancol5 CGGCCCCTCGGGCCGCAGAATTGGGGCAATTGGAGAAAATTGGGCGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG	LmPBPA1Y11A5	GGCAGATAAACCGCAGACAAGATCTCAGGCTCGCAATAAACAAAGTGGTGG TTG								
Introduction of R12A to <i>Lm</i> ² BPA1L ₂₀ ³⁰⁶ Lm ² BPA1R12A3 GATAAACCGCAGACAAGATCTCAGTAGCCAATAAACAAAGTGGGTGG	LmPBPA1Y11A3	CAACCACCACTTTGTTTATTGCGAGCCTGAGATCTTGTCTGCGGTTTATCTGC								
Introduction of SIG to LPBP PT1_sa GGCTACTTACTGCCACCACCACCACCACCACCACCACCACCACTGCAGTACGCAACAAAGTGCGGGGGGGG	Introduction of D12A to Im	C SIPC								
Jam Drain 12,3. ORL MARCEGE CANTAGE TO CONTROL CONTRUCT CONTROL CONTROL CONTROL CONTROL CONTRUCT CONTROL CONTROL CONTR	Introduction of K12A to Lmi	$CATA \land ACCCC \land C \land C \land AC ATCTC \land CT \land TCCC \land AT \land A \land C \land \land A CTCCTCCTCCTC$								
Dimber AINE 123 CREATE AND ADDRESS Dimber AINE 123 CREATE ADDRESS LimpBPA1516R5 CTCAGTATCGCAATAAACAACGTGGTGGTTGTAAATAGAAATCCC LimpBPA1516R5 CTCAGTATCGCAATAAACAACGTGGTGGTTGTAATAGAAATCCC LimpBPA1516R5 CGGATTCTATTTTACAACCACCACGTGTGTTATTGGGATACTGAGG Construction of plasmid expressing recombinant MBP-SpPBP2a ₃₂₄₆ SpPB2Aabol3 SpPBP2Anb03 CCGGCCCTCGAGCATCCTGCCAGAGTTCCCAGTTGTACTATCTTACG SpPBP2AG43CR46STOP5 GTAAACAAGTAGCACCATGCCAGAGTTCCCAGAGTTGCCGAGT SpPBP2AG43CR46STOP5 GTATAACGACGCCCCAGAATTTCCAAATCGGACATACTTGGGCTAATTTTTTTCG ATCAC GTTACGGCTAATTTTTTTTCGACCACTTTCAGGCGCTCTAAAAGTGGACGAAAAAAATTAGCC CAAG CCAAG CTCTGATTCTACTATCTTACGTCGCCTCTAAAAGTGATCGAAAAAAATTAGCCC SpPBP2AR33K5 CTCTGATTCTACTATCTTACGTCGCGCTCTAAAAGTGAGAAAAAATTAGCCC CAAG SpPBP2AR33K3 CTTGGGCTAATTTTTTTCGATCACTTCTACGTGCGCTCGTAAGATAGTAGAAAAATTAG CCC SpPBP2AS32A3 GGCCTAATTTTTTTCGATCACTACCTACGAGCGCGACGTAAGATAGAAAAATTAG CCC SpPBP2AR33A5 GACTCTGATTCTACTATCTTACGTCGCTCTGCGCTAGTGATCGAAAAAAATTAG CCC SpPBP2AR33A5 GGCTAATTTTTTTCGATCACTACGTACGAGGCGACGTAAGAAGAAGAAGAAGAAGAAGACG GCC SpPBP2AR33A5 GGCTAATTTTTTTCGGATCACTACGAGGCGCGCGCGTAAGGTAGGAAGAAGAAGAAGAGCCC	LIIIF DFAINIZAS									
Introduction of SIGK 10 ZMF 0F AL 1,20 CLATER PAIS 16RS CICAGTATCGCAATAAACAACGTGGTGGTTGTAAATAGAAATCCC Lm/BPAIS 16RS GGGATTTCTATTTACAACCACCACCACCTGTTTATGCGATACTGAG CONSTRUCTION of plasmid expressing recombinant MBP-SpPBP2a ₁₂₋₄₆ SpPBP2Anol3 CCGGCCCCTGGAGCATCCTGCGCGAGGTGGGCAGC CGGCCCCTGGAGCATCCTGGCCGGCAGGCTGGGCAGC SpPBP2AG43CR46STOP 5 GTGATGGAAAAAAATTAGCCCAAGTATGTCGGATGGAAAATTCTGGGCT SpPBP2AG43CR46STOP 5 GTGATGGAAAAAAATTAGCCCAAGTATGTCGGATTGAAAATCTGGGCT SpPBP2AG43CR46STOP 5 GTGATGGAAAAAAATTAGCCCAAGTATGTCGGATTGAAAATTCTGGGCT SpPBP2AG43CR46STOP 5 GTGATGGAAAAAAATTAGCCCAAGTATGTGGGACATACTTGGGCTAATTTTTTCG ATCAC Introduction of 833K to MBP-SpPBP2a ₂₃₋₄₆ fue SpPBP2AR33K3 CTTGGGTAATTTTTTCGATCACTTTAGGTGGCGTCGAAAAAAAA	Introduction of S16D to Lun	CAACCACCACITIOTITATIOOCATACTOAOATCITOTCTOCOOTITATC								
LmcPPA1S1663 CICAGNTICOCATIAACCACCACCACCTIGUTATATACCAATACUCC LmPBPA1S16R3 GGATTICACATITACAACCACCACCACCTIGUTTATTACG SpPBP2.acol5 GAAACAAGTGAACCACCACCACCACGATICTACTACTGAG SpPB2.acol5 GAAACAAGTGAACCACCACCACGATICTACTATCTACG SpPB2.acol5 GAAACAAGTGAACCACCACCACGAGTGGCAGC Introduction of G43C, R45STOP1 to MBP-SpPP2ayagt ⁶⁴⁰⁰ Introduction of G43C, R45STOP1 to MBP-SpPP2ayagt ⁶⁴⁰⁰ SpPBP2AG43CR46STOP3 GTTATC GAAACAAACTATCGGACCAGACATACTGGGCAAAATTTTTTTCGAAAATTCGGGCTAATTTTTTTCGATCC SpPBP2AG43CR46STOP3 GATAACGACGCCCAGAATTTTCAAATCGGACATACTGGGCTAAAAAAAA	Introduction of S16K to LmP									
LmrPrAISING L00GATTICIATITACAACCACCACGTIGITTATITOCGATACTAGAG Construction of plasmid expressing recombinant MBP-SpPBP2a ₂₀ , as SpPBP2Anc015 GAAACAACTGGAACCCATGGACTCTGATTCTACTATCTTACG SpPBP2Anb013 CCGGCCCCTCGACGATCCTGGACTCCTGCAGCAGCC Introduction of G43C, R46STOP to MBP-SpPBP2a ₂₀ , as to generate MBP-SpPBP2a ₂₀ , as SpPBP2AG43CR46STOP3 GTGATCGAAAAAAATTAGCCCAAGTATGTCCGACTATTGGACAAATTTTTCGACGC SpPBP2AG43CR46STOP3 GATAACGACGCCAGAATTTCAAAACGGACATACTTGGGCTAAATTTTTTCGA SpPBP2AG43CR46STOP3 GATAACGACGCCAGAATTTCAAAACGGACATACTTGGGCTAAATTTTTTTCG SpPBP2AG43CR46STOP3 GATCAC SpPBP2AG43CR46STOP3 GATAACGACGCCAGAAATTTCTACTATCTTACGTCGCCTCTAAAAGTGAGATAGTAGAAAAAATTAGCC SpPBP2AR33K5 CTCTGATTCTACTATCTTACGTCGCCCTCAAAAGTGAGATAGTAGAAAAAAAA	LIIPDPAISIORS									
Construction of plasmid expressing recombinant MIP'SPYBP23:46 SpPBP2Action of Construction of	LmPBPAISI6R3									
SpPBP2Aneols GAAACAACIGAACCCATGGACTCTGATCTACTATCTTACGAG Introduction of G43C, R46STOP to MBP-SpPBP2a ₂₁₂₄ ob generate MBP-SpPBP2a ₂₁₂₄ GaC GTGATCGAAAAAAATTAGCCCAAGTATGTCCGACTTGAAAATTCTGGCGTC SpPB2AG43CR46STOP3 GTGATCGAAAAAAATTAGCCCAAGTATGTCCGACTATGGAGCTAATTTTTTCGA ATCAC GTAAACGAACGCCAGAATTTTCAAAATCGGACATACTTGGGGTAATTTTTTCG SpPBP2AG43CR46STOP3 GATAACGACGCCAGAATTTTCAAATCGGACATACTTGGGGTAATTTTTTTCG SpPB2AG43CR46STOP3 GATAACGACGCCAGAATTTTCAAATCGGACATACTTGGGGTAATTTTTTTCG SpPB2AR33K5 CTCTGGATTCTACTATCTTACGTCGCTCTAAAAGTGATCGAAAAAAATTAGCC SpPB2AR33K3 CTCTGGGTAATTTTTTCGATCACTTCTACGTCGGCGCTGTAGTGAAGATAGTAGAAACA SpPB2AR33K3 CTGGGCTAATTTTTTCGATCACTACGTCGGCGCTGTAGTGAACGAAAAAAATTAG SpPB2AS32A3 GGGCTAATTTTTTCGATCACTACGACGCGCACGTAAGATAGTAGAAAAAATTAG SpPB2AR33A3 GGGCTAATTTTTTTGGATCACTACGACGCGCACGTAAGATAGTAGAAACAG SpPB2AR33A3 GGGCTAATTTTTTTTCGATCACTGCGCCTCGCTAGTGATGGAAGATAGAAAAAATTAG SpPB2AR33A3 GGGCTAATTTTTTTTTTTTTTTTTTTTTTTTTGGAGGACTACGAAGGCGACGTAAGATAGAACAG SpPBP2AR33A3 GGGCTAATTTTTTTTTTTTTTTTTTTTTTTTTCGAGGACCTAGGAGCGACGTAAGAAAAAATTAG SpPBP2AR33A3 GGGCTAATTTTTTTTTCGATCACTGCGCCTCGTAGTGAAGAAGAGAGACCAGGAGGACGTAAGAACAG SpPBP2AR33A3 GGGCTAATTTTTTTTCGATCCTGCGCCTCGTAGTGAGAGCAAGAGGAGCAACAGG SpPBP2AR33A3	Construction of plasmid exp	ressing recombinant MBP-SpPBP2a ₂₃₋₄₆								
SpPBP2AAb015 CCGCCCCCCGGAGAACCCCGCCAGAATCCCGCCCC Introduction of G43C, R45STOP to MBP-SpPB2a ₁₂₄ , to generate MBP-SpPB2a_23, to generate MBP-S	SpPBP2Ancol5	GAAACAAGTGAACCCATGGACTCTGATTCTACTATCTTACG								
Introduction of G43.C, R46S IOP to MBP-3p/PB/2a_t_a, to generate MBP-3p/PB/2a_t_a Common Supplementation of Case Supplementatin Supplementation of Case Supplementation of Case Supple	SpPBP2Axhol3									
SpPBP2AG43CR46STOP5 GTATCC SpPBP2AG43CR46STOP3 GATAACGACGCCAGAATTTTCAAATCGGACATACTTGGGCTAATTTTTTTCG ATCAC ATCAC SpPBP2AG43CR46STOP3 GATAACGACGCCAGCAATTTTCAAATCGGACATACTTGGGCTAATTTTTTTCG SpPBP2AR33K5 CTTGGGCTAATTTTTTTCGATCACTTTCAGAGCGACGTAAGATAGTAGAAAAATTAGCC SpPBP2AR33K3 CTTGGGCTAATTTTTTTCGATCACTTTAGAGCGACGTAAGATAGTAGAAAAAATTAGC SpPBP2AR33K3 CTTGGGCTAATTTTTTTCGATCACTTCGGCGCCCGCAGTGAGTG	Introduction of G43C, R46S	TOP to MBP-SpPBP2a ₂₃₋₄₆ to generate MBP-SpPBP2a ₂₃₋₄₅								
SpPBP2AG43CR46STOPS GATAACGACGCCAGAATTTTCAAATCGGACATACTTGGGCTAATTTTTTTCG Introduction of R33K to MBP-SpPBP2a ₂₁₄₅ ^{casc} SpPBP2AR33K5 CTCTGATTCTACTATCTTACGTCGCTCTAAAAGTGATCGAAAAAAATTAGCC CAGAG CAGAG SpPBP2AR33K5 CTTGGGCTAATTTTTTTCGATCACTTTTAGAGCGACGTAAGATAGTAGAAAC AGAG GGC SpPBP2AR33K5 GACTCTGATTCTACTATCTACGTCGCGCGCTGTAGTGATCGAAAAAAATTAG CCC SpPBP2AS32A5 GGGCTAATTTTTTTTCGATCACTACGAGGCGGACGTAAGATAGTAGAAACAG AGTC AGTC SpPBP2AS32A5 GGGCTAATTTTTTTTCGATCACTACGAGGCGCGCGCGACGTAAGATAGTAGAAACAG AGTC AGTC SpPBP2AS33A5 GACTCTGATTCTACTACTACGAGGCGCGCGCGCGAGAAAAAAATTAG CCC SpPBP2AR33A5 GACTCTGATTTTTTTCGATCACTAGCGCGCCTCGGAAGAAAAAATTAG SpPBP2AR33A5 GGACTACTTGGTCCTCGATGTCCTGGAAAAAAATTAGCCAAGATAGTAGAACGA SpPBP2AR33A5 GGACATACTTGGGCTCATGTAGTCCTCGAAAAAAATTAGCCCAAGTAGTGCC SpPBP2AD3SP5 CTATCTACGTCGCTCTCGATGTCCTCGAAAAAAATTAGCCCAAGTATGTCCC SpPBP2AB3SP3 GGACATACTTGGGCTCAAGTTTTTTTTCGAGGACTACGAGGCACGTAAGGACCATGGACCC SpPBP2AR31KR33K5 CAGGCTCCATGGACTCTGATCTCACTACGAGAACAACGCAGGGCCCTGGCCCCTGGAAACCAAGGCCCCGGGCAGGCA	SpPBP2AG43CR46STOP5	GTGATCGAAAAAAATTAGCCCAAGTATGTCCGATTTGAAAAATTCTGGCGTC GTTATC								
Introduction of R33K to MBP-SpPBP2a _{22.46} ^{edac} SpPBP2AR33K5 CTCTGATTCTACTATCTTACGTCGCTCTAAAAGTGATCGAAAAAAATTAGCC AGAG AGAG Introduction of S32A to MBP-SpPBP2a _{22.45} ^{edac} SpPBP2AR33K3 SpPBP2AR33K3 GACTCTGATTCTACTATCTACGTCGCGCCGCTGAGTGATCGAAAAAAATTAGC SpPBP2AR33A5 GACTCTGATTCTACTATCTACGTCGCGCGCGCGCGAGGAAGAAAAAAATTAG CCC SpPBP2AS32A3 GGGCTAATTTTTTTCGATCACTACGAGGCGCGACGTAAGATAGTAGAATCAG AGTC AGTC SpPBP2AR33A5 GACCTCGATTCTACTACTACGTCGCGCTCTGCTAGTGATCGAAAAAAAA	SpPBP2AG43CR46STOP3	GATAACGACGCCAGAATTTTCAAATCGGACATACTTGGGCTAATTTTTTCG ATCAC								
SpPBP2AR33K5 CTCTGATTCTACTATCTTACGTCGCTCTAAAAGTGATCGAAAAAAATTAGCC CAAG SpPBP2AR33K3 CTTGGGCTAATTTTTTCGATCACTTTTAGAGCGACGTAAGATAGTAGAATC AGAG Introduction of S32A to MBP-SpPBP2a ₁₂₄₅ Gac SpPBP2AR33A5 GACTCTGATTCTACTATCTTACGTCGCGCTCTGAGTGATCGAAAAAAATTAG CCC SpPBP2AS32A5 GGCTAATTTTTTTCGATCACTACGAGGCGCGACGTAAGATAGTAGAAACAATTAG CCC SpPBP2AS33A5 GGCCTAATTTTTTCGATCACTACGAGGCGCACGTAAGATAGTAGAAACAATTAG CCC SpPBP2AR33A5 GACCTCTGATTCTACTATCTACGTCGCTCTGCTAGTGATCGAAAAAAATTAG CCC SpPBP2AR33A5 GGCCTAATTTTTTCGATCACTAGCAGAGGGACGAAGATAGTAGAAACAATAG AGTC Introduction of D35P to MBP-SpPBP2a ₂₂₄₅ Gac SpPBP2AD35P5 CTATCTTACGTCGCTCTGTAGTCCTCGAAAAAAATTAGCCCAAGTATGTCC SpPBP2AD35P5 CTATCTTACGTCGCTCTGTAGTCCTCGAAAAAAATTAGCCCAAGTAAGATA G SpPBP2AD35P5 CTATCTTACGTCGCTCTGTAGTCCTCGAAAAAAATTAGCCCAAGTAGATA G SpPBP2AD35P5 CTATCTTACGTCGCTCTGAAGTCTACGAACACGAGGCGCACGTAAGATA G SpPBP2AR31KR33K5 CGAGCGTCCATGGACTCTGATTCTACTACGTCAGGAACCACGAGGGCCACGAAGAACA G SpPBP2AR31KR33K5 CGAGCGTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCCGTAGTGAACCACG G SpPBP2AR31KR33K5 CGAGCACTATTGGACTACTACTACGGCACGCAGTAGAACAAGAGTCCATGGAAC SpPBP2AR31KR33K5 GAGGACATATGGCAAGTATTACTTTTCACGTCGCCACGACGTCCGCAGTGGCCATGGAACCCTG G	Introduction of R33K to MB	P-SpPBP2a ₂₃₋₄₅ G43C								
CAAG SpBP2AR33K3 CTTGGGCTAATTTTTTTCGATCACTTTTAGAGCGACGTAAGATAGTAGAATC AGAG Introduction of S32A to MBP-SpPEP2a ₃₂₄₅ ^{casc} SpPBP2AS32A5 GACTCTGATTCTACTATCTTACGTCGCGCGCGACGTAAGATAGTAGAAAAAATTAG CCC SpPBP2AS32A3 GGGCTAATTTTTTTCGATCACTACGAGCGCGACGTAAGATAGTAGAAAAAATTAG CCC Introduction of R33A to MBP-SpPEP2a ₃₂₄₅ ^{casc} SpPBP2AR33A5 GACTCTGATTCTACTATCTTACGTCGCTCTGCTAGTGATCGAAAAAAATTAG CCC SpPBP2AR33A3 GGGCTAATTTTTTTCGATCACTAGCAGAGCGACGTAAGATAGTAGAATCAG AGTC Introduction of D35P to MBP-SpPEP2a ₃₂₄₅ ^{casc} SpPBP2AD35P5 CTATCTTACGTCGCTCTCGTAGTCCTCGAAAAAAAATTAGCCCAAGGTATGTCC SpPBP2AD35P5 SCTATCTTACGTCGCCTCTCGTAGTCCTCGAAAAAAATTAGCCCAAGGTAAGATAGTAG AGT Introduction of R31K, R33K to MBP-SpPEP2a ₃₂₄₅ ^{casc} SpPBP2AR31KR33K5 CAGGGTTCCATGGACTCTGATTCTACTATCTACGAAGAGCGACGTACGAGAGCCACTG G SpPBP2AR31KR33K3 CGATCACTTTTAGATTACGTAAGATAGTAGAATCAGAGTCCATGGAACCCT G SpPBP2AR31KR33K3 CGATCACTTTTAGATTCACTGCCGCACGTCGCTCTGTAGTGTAGTCG SpPBP2A128AL29A5 SGTCCATGGACTCTGGACTCGTGACGCACGTAGGAATCAGAGTCCATGGAAC Construction of lasmid expressing recombinant SpGpsB ₁₄₃ SpGpsBh13 CCACATCTCGAGGCAAGTATTATTTTTTCAGGC SpGpsB1103del5 CCACATCTGGAGAACTATTAGCTATATAAAAACGGGCCCGATTAAAGAGTATCC SpGpsB11	SpPBP2AR33K5	CTCTGATTCTACTATCTTACGTCGCTCTAAAAGTGATCGAAAAAAATTAGCC								
SpFBP2AR5365 CHOUGETAATTITITICGATCACTITIAGAGCGACGTAAGATAGTAGATAGTAGAATC AGAG Introduction of S32A to MBP-SpPBP2a ₃₂₄₅ ^{cdBC} SpBP2AS32A5 GACTCTGATTCTACTATCTTACGTCGCGCGCGACGTAAGATAGTAGAAAAAATTAG CCC SpPBP2AS32A3 GGGCTAATTTTTTTCGATCACTACGAGGGCGCACGTAAGATAGTAGAAAAAATTAG CCC SpPBP2AR33A5 GACTCTGATTCTACTATCTTACGTCGCTCTGCTAGTGATCGAAAAAAATTAG CCC SpPBP2AR33A5 GACTCTGATTTTTTTCGATCACTAGCAGAGCGACGTAAGATAGTAGAATCAG AGTC Introduction of D35P to MBP-SpPBP2a ₃₂₄₅ ^{cdBC} SpPBP2AB35P5 CTATCTTACGTCGCTCTCGTAGTCCTCGAAAAAAATTAGCCCAAGTAGTAGCC SpPBP2AD35P5 CTATCTTACGTCGCTCTCGTAGTCCTCGAAAAAAATTAGCCCAAGGTAAGATAGTAGAAC SpPBP2AD35P5 CTATCTTACGTCGCCTCTCGATGTCTCACACAGAGAGCGACGTAAGATAGTAGAACG SpPBP2AB35P5 CAGGGTTCCATGGGCCTAATTTTTTTCGAGGACTACCGAGAGCGACGTAAGATAGTAGAAGTCC SpPBP2AR31KR33K5 CAGGGTTCCATGGACTCTGGATTCTACTATCTACGTAAAATCTAAAAGTGATC SpPBP2AR31KR33K3 CGATCACTTTTAGATTACGTAAGATAGTAGAAACAGAGGTCCATGGAACCCT G G Introduction of 128A, L29A to MBP-SpPBP2a ₃₂₄₅ ^{cdBC} SpPBP2A128AL29A5 GTCCATGGACACTACTGGACGCGCCCCTCCGTCTCGTAGGTGCCATGGAAC Costruction of lasmid expressing recombinant SpCpsB1 ₄₄₃ SpGpsBhul3 CCACATCTCGAGACACTATTATTTTTTCAAGGCCCCATAAAGAGTATCC SpGpsBhu3de1 CCACATCTCGAGACACTATGGCAAA		CAAG								
Introduction of S32A to MBP-SpPBP2a ₃₁₋₄₆ ^{GARC} SpPBP2AS32A5 GACTCTGATTCTACTATCTACGTCGCGCTCGTAGTGATCGAAAAAAATTAG CCC SpPBP2AS32A3 GGGCTAATTTTTTTCGATCACTACGAGCGCGCGCGTAAGATAGTAGAATCAG AGTC Introduction of R33A to MBP-SpPBP2a ₃₁₋₄₅ ^{GARC} GACTCTGATTCTACTATCTACGTCGCTCTGCTAGTGATCGAAAAAAATTAG CCC SpPBP2AR33A5 GACTCTGATTCTACTACTACCACGAGCGAGCGTAAGATAGTAGAATCAG AGTC Introduction of D35P to MBP-SpPBP2a ₃₁₋₄₆ ^{GARC} SpPBP2AD35P5 CTATCTTACGTCGCTCTCGTAGTCCTCGAAAAAAATTAGCCCAAGTAGTACC SpPBP2AD35P5 CTATCTTGGGCTAATTTTTTTCGAGGACTACGAGAGCGAAGGCGAACGTAAGATA G GGACATACTTGGGCTAATTTTTTTCGAGGACTACGAGAGCGAACGTAAGATA SpPBP2AD35P5 CTAGCTTTACGTCGCTCTCGTAGTCTCACTACGAGAGCGAACGTAAGATA Introduction of R31K, R33K to MBP-SpPBP2a ₃₂₋₄₆ ^{GARC} GGACTACTTGGACTCTGATTCTACTATCTTACGTAAGAATCAGAGGTCCATGGAACCCT SpPBP2AR31KR33K5 CAGGGCTCCATGGACTCTGATTCTACTATCTACGAGAGTCCATGGAACCCT G SpPBP2AR31KR33K3 CGATCACTTTTAGATTACGTAAGAAAGTAGTAGAAGTCAGAGTCCATGGAACC G SpPBP2AR31KR33K3 CGATCACTTGTGATCTGATCTCATGCCCCCCCCCCCCCGCCCCCCCC	SpPBP2AR33K3	AGAG								
SpPBP2AS32A5 GACTCTGATTCTACTATCTTACGTCGCGCTCGTAGTGATCGAAAAAAATTAG CCC SpPBP2AS32A3 GGGCTAATTTTTTTCGATCACTACGAGCGCGACGTAAGATAGTAGAATCAG AGTC Introduction of R33A to MBP-SpPBP2a ₂₁₋₄ , GBC SpPBP2AR33A5 GACTCTGATTCTACTACTACTACGTCGCTCTGCTAGTGATCGAAAAAAATTAG CCC SpPBP2AR33A3 GGGCTAATTTTTTTCGATCACTAGCAGAGCGACGTAAGATAGTAGAATCAG AGTC Introduction of D35P to MBP-SpPBP2a ₂₁₋₄₆ , GBC SpPBP2AD35P5 CTATCTTACGTCGCTCTCGTAGTCCTCGAAAAAAATTAGCCCAAGTATGTCC SpPBP2AD35P3 GGACATACTTGGCTCTCGTAGTCCTCGAAAAAAATTAGCCCAAGTAAGT	Introduction of S32A to MBI	P-SpPBP2a ₂₃₋₄₅ G43C								
SpPBP2AS32A3 GGGCTAATTTTTTTCGATCACTACGAGCGCGACGTAAGATAGTAGAATCAG AGTC Introduction of R33A to MBP-SpPBP2a ₂₁₄₆ ^{GARC} SpPBP2AR33A5 GACTCTGATTCTACTATCTACGTCGCTCTGCTAGTGATCGAAAAAAATTAG CCC SpPBP2AR33A3 GGGCTAATTTTTTTCGATCACTAGCAGAGCGACGTAAGATAGTAGAAACAG AGTC Introduction of D35P to MBP-SpPBP2a ₂₁₄₆ ^{GARC} SpPBP2AD35P5 CTATCTTACGTCGCTCTCGTAGTCCTCGAAAAAAATTAGCCCAAGTAGTCC SpPBP2AD35P3 GGACATACTTGGGCTAATTTTTTTCGAGGACACACGAGAGCGACGTAAGATA G Introduction of R31K, R33K to MBP-SpPBP2a ₂₁₄₅ ^{GARC} SpPBP2AR31KR33K5 CAGGGTTCCATGGACTCTGATTCTACTACGTAGAAATCTAAAAGTGAAC G Introduction of R128A, L29A to MBP-SpPBP2a ₂₁₄₅ ^{GARC} SpPBP2A128AL29A5 GTTCCATGGACCTTGATTCTACTGCGCACGTCGCTCTCGTAGTGATCG SpPBP2A128AL29A5 SpCasBahol3 GCACATCACTACGAGAGCGACGTAGCAGTAGAAATCAGAGTCATGGAAC SpGpsBhol3 SpGCACATCTCGAGGACATATGGCAAGTATTATTTTTTCAGCG SpGpsBhol3 GCACATCTCGAGAACTACTGCGCACGTCGCAGTACGAGTCATGGAAC SpGpsBh11Neol SGCATATCTGCGGAGTATTGTGGAGCACTATAGTTATC Introduction of Nesite to plasmid expressing recombinant SpGpsB1 ₄₄₃ SpGpsBh11Neol GCATATCCTGGAGTATTATTTTTTTTCAGGGCCCATTATTTTTTCAGGG SpGpsBh11Neol GCATATCCTGGAGTATATGGCAAAAATCGAGATACTCCG SpGpsBh11Neol SGCTATCTGGAGTGGCAAAAAATAATGGGGCCCATTATTTTTTTCAGGG SpGpsBh103del3 CGCTATCCGAGGAGTATACTGGAAAAAGGTAATCCG SpGpsBh103del3 CGCTAACTGG	SpPBP2AS32A5	GACTCTGATTCTACTATCTTACGTCGCGCTCGTAGTGATCGAAAAAAATTAG CCC								
Introduction of R33A to MBP-SpPBP2a2445 Case SpPBP2AR33A5 GACTCTGATTCTACTATCTTACGTCGCTCTGCTAGTGATCGAAAAAAATTAG CCC SpPBP2AR33A3 GGGCTAATTTTTTTCGATCACTAGCAGAGCGACGTAAGATAGTAGAAACAG AGTC Introduction of D35P to MBP-SpPBP2a245 GGACATACTTGGGCTCCTCGTAGTCCTCGAAAAAAATTAGCCCAAGATAGTCC SpPBP2AD35P5 CTATCTTACGTCGCTCTCGTAGTCCTCGAAAAAAAATTAGCCCAAGAATAGT G GGACATACTTGGGCTAATTTTTTTCGAGGACTACGAGAGCGACGTAAGATA G Introduction of R31K, R33K to MBP-SpPBP2a265 CGACTCCATGGACTCTGATTCTACTATCTACGAAAAAATTAAAGTGATC G SpPBP2AR31KR33K5 CAGGGTTCCATGGACTCTGATTCTACTATCATAGAATCAGAGTCCATGGAACCCAT G SpPBP2AR31KR33K5 CAGGGTTCCATGGACTCTGATTCTACGCGCACGTCGCTCCGTAGTGAGACCCATGGAACC SpPBP2A128AL29A5 GTTCCATGGACTCTGATTCTACTACGCGCACGTCGCTCCGTAGTGAGTCG SpPBP2A128AL29A5 CGATCACTACGAGAGCGACGTGCGGCAGCAGGAGACACCCCGCGCGCAGTAGGAACC Construction of plasmid expressing recombinant SpGpsB143 GGACAACATATGGCAAGTATTATTTTTCAGC SpGpsBhol3 GCACATACCATGGCAAGTATTAATTTTTTCAGCG SpGpsBhol3 GCACATACCAGGCAAGTATTAACTACTAAAAATCTGAGTTATC Introduction of Noi site to plasmid expressing recombinant SpGpsB143 GGACATACCATGGACATACTACTTAAAAATCTGAGTTATC SpGpsBh113ko1 CGATACCATGGCCATGGACAAAAAATAAAGGCCCATGGACCATAGGACCATGGACAAAAAGGCCATGGACAAAAAATAAAGGCCCATGGACAAAAAATAAAGG	SpPBP2AS32A3	GGGCTAATTTTTTCGATCACTACGAGCGCGACGTAAGATAGTAGAATCAG AGTC								
SpPBP2AR33A5 GACTCTGATTCTACTACTACTACTACGTCGCCTCGCTAGTGATCGAAAAAAATTAG CCC SpPBP2AR33A3 GGGCTAATTTTTTTCGATCACTAGCAGAGCGACGTAAGATAGTAGAAAAAATTAG CCC Introduction of D35P to MBP-SpPBP2a22.45 GaC SpPBP2AD35P5 CTATCTTACGTCGCTCTCGTAGTCCTCGAAAAAAATTAGCCCAAGTATGTCC SpPBP2AD35P3 GGACATACTTGGGCTAATTTTTTTCGAGGACTACGAGAGCGACGTAAGATA G Introduction of R31K, R33K to MBP-SpPBP2a23.45 GaC SpPBP2AR31KR33K5 CAGGGTTCCATGGACTCTGATTCTACTATCTTACGTAAAATCTAAAAGTGATC G SpPBP2AR31KR33K5 CGATCACTTTTAGATTTACGTAAGATAGTAGAAATCAGAGTCCATGGAACCCT G SpPBP2AR31KR33K3 CGATCACTTTAGATTTACGTAAGATAGTAGAAATCAGAGTCCATGGAACCCT G SpPBP2A128AL29A5 GTTCCATGGACTCTGATTCTACTGCGCGCACGTCGCTCTCGTAGTGATCG SpPBP2A128AL29A5 CGATCACTACGAGAGCGACGTGCGGCACGTAGAATCAGAGTCCATGGAAC Construction of plasmid expressing recombinant SpCpsB1 ₄₆₃ SpGpsBMol5 SpGpsBM015 GAGAGACATATGGCAAGTATTATTTTTTCAGCG SpGpsBM11Ncol GCTATACCTGGGAAACTATTGCTAAAATCTGAGGTTATC Deletion of residues 1-3 to generate plasmid expressing recombinant SpCpsB1 ₄₆₃ SpGpsB103del5 CCACTACTGGGAAATCTTAATTTTTTCAGGGCCACTTATTTTTTCAGCG SpPBP2X5 GCGCGGAAAAAATAATGGCCACCTGAAAAAAGAGTTATCC Deletion of residues 1-3 to generate plasmid expressing recombinant SpCpsB1 ₄₆₃ <t< td=""><td>Introduction of R33A to MB</td><td>P-SpPBP2a2245</td></t<>	Introduction of R33A to MB	P-SpPBP2a2245								
CCC SpPBP2AR33A3 GGGCTAATTTTTTCGATCACTAGCAGAGCGACGTAAGATAGTAGAATCAG AGTC Introduction of D35P to MBP-SpPBP2a3245 GTACTTACGTCGCTCTCGTAGTCCTCGAAAAAAAATTAGCCCAAGTATGTCC SpPBP2AD35P5 CTATCTTACGTCGCTCTCGTAGTCCTCGAAAAAAAATTAGCCCAAGTATGTCC SpPBP2AD35P3 GGACATACTTGGGCTAATTTTTTTCGAGGACTACGAGAGCGACGTAAGATA G Introduction of R31K, R33K to MBP-SpPBP2a3245 GGAC SpPBP2AR31KR33K5 CAGGGTTCCATGGACTCTGATTCTACTATCTTACGTAAATCTAAAAGTGAAC G SpPBP2AR31KR33K5 CAGGGTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTGTAGTGAACCCT G SpPBP2AR31KR33K5 CGATCACTTTAGATTTACGTAAGATAGAAATCAGAGTCCATGGAACCCT G SpPBP2AR31KR33K5 CGATCACTATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCG SpPBP2AR31KR33K5 CGATCACTAGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCG SpPBP2A128AL29A5 GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCG SpGpsBndel5 GACACACTATGGCAAGTATTATTTTTTCAGC SpGpsBndel5 GACACACTCTCGAGTAACTACTTAAAATCTGAGTTATC Introduction of Neol site to plasmid expressing recombinant SpGpsB143 GCACATACTGGCAAGTATTATTTTTCAGGCG SpGpsBM11Ncol CGATACCTCGAGAAATCATCTTAAAAATCTGAGTTATC Deletion of residues 1-3 to generate plasmid expressing recombinant SpGpsB143 CGCACATACTGGAAATCATTAAAAATCTGAGGCCCATAATAAAGGTC SpGpsB1103del5 CCACTACTGGAAAATATTT	SpPBP2AR33A5	GACTCTGATTCTACTATCTTACGTCGCTCTGCTAGTGATCGAAAAAAATTAG								
SpPBP2AR33A3 GGGCTAATTTTTTTCGATCACTAGCAGAGCGACGTAAGATAGTAGAAATCAG AGTC Introduction of D35P to MBP-SpPB2a ₂₃₋₄₅ . GGC SpPBP2AD35P5 CTATCTTACGTCGCTCTCGTAGTCCTCGAAAAAAATTAGCCCAAGTATGTCC SpPBP2AD35P3 GGACATACTTGGGCTAATTTTTTTCGAGGACTACGAGAGCGACGTAAGATA G Introduction of R31K, R33K to MBP-SpPBP2a ₂₃₋₄₅ . GGC SpPBP2AR31KR33K5 CAGGGTTCCATGGACTCTGATTCTACTATCTTACGTAAATCTAAAAGTGATC G SpPBP2AR31KR33K3 CGATCACTTTTAGATTTACGTAAGATAGAAACAGAGGTCCATGGAACCCT G Introduction of 128A, L29A to MBP-SpPBP2a ₂₃₋₄₅ . SpPBP2A128AL29A5 GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCG SpPBP2A128AL29A5 GTTCCATGGACTCTGATTCACTGCCGCAGTAGAATCAGAGTCCATGGAAC Construction of plasmid expressing recombinant SpGpsB ₁₋₆₃ SpGpsBhd15 SpGpsBhd15 GAGAGACATATGGCAAGTATTATTTTTTTCAGC SpGpsBh11Ncol GCTATACCTAGGAAACTACTTAAAAATCTGAGTTATC Introduction of Ncol site to plasmid expressing recombinant SpGpsB ₁₋₆₃ SpGpsBh11Ncol GCATATCTGAGAACTATTTATTTTTTCAGCG SpGpsBh11Ncol GCATATCTGAGAACTATTTATTTTTTCAGGG SpGpsBh103del5 CCACTACTGAGAAATATGGCACCCTGAAATAATCGGGCCATTATTTTTTCAGGG SpGpsBh103del5 CCACTACTGAGAATATATGCCCCCTGAAATAAAGATCTGAGTGG SpGpsBl103del5 CCATACTGAGAAATATGGCGCCCTGAAAAAAAAAAAGG	~F	CCC								
Introduction of D35P to MBP-SpPBP2a ₂₃₄₅ SpPBP2AD35P5 CTATCTTACGTCGCTCTCGTAGTCCTCGAAAAAAATTAGCCCAAGTATGTCC SpPBP2AD35P3 GGACATACTTGGGCTAATTTTTTTCGAGGAACTACGAGAGCGACGTAAGATA G Introduction of R31K, R33K to MBP-SpPBP2a ₂₃₄₅ ^{C43C} SpPBP2AR31KR33K5 CAGGGTTCCATGGACTCTGATTCTACTATCTTACGTAAAATCTAAAAGTGATC G G SpPBP2AR31KR33K3 CGATCACTTTTAGATTTACGTAAGATAGTAGAAATCAGAGTCCATGGAACCCT G G SpPBP2AR31KR33K3 CGATCACTTTTAGATTTACGTAAGATAGTAGAAATCAGAGTCCATGGAACCCT G G SpPBP2AR31KR33K3 CGATCACTTTTAGATTTACGTAAGATAGTAGAAATCAGAGTCCATGGAACCCT G G SpPBP2A128AL29A5 GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCG SpPBP2A128AL29A5 CGATCACTACGAGAAGCGACGTGCGGCAGTAGAATCAGAGTCCATGGAAC Construction of plasmid expressing recombinant SpGpsB _{1.63} SpGpsBndel5 SpGpsBhdel5 GAGAGCATATGGCAAGTATTGCAAGTATTTTTTTCAGC SpGpsBM11Nco1 GCACATCTCGAGTAACTACTTAAAAATCTGAGTTATC Introduction of Ncol site to plasmid expressing recombinant SpGpsB _{1.63} SpGpsB1103del5 SpGpsB1103del5 CCACTACTGAGAACTATTTATTTTTCAGGGGCCCATTATTTTTTCAGCG SpGpsB1103del5 CCACTACT	SpPBP2AR33A3	GGGCTAATTTTTTCGATCACTAGCAGAGCGACGTAAGATAGTAGAATCAG								
Introduction Dots to Dots Control addes SpPBP2AD35P5 CTATCTTACGTCGCTCTCGTAGTCCTCGAAAAAAATTAGCCCAAGTATGTCC SpPBP2AD35P3 GGACATACTTGGGCTAATTTTTTTCGAGGACTACGAGAGCGACGTAAGATA G G Introduction of R31K, R33K to MBP-SpPBP2a ₂₃₋₄₅ casc SpPBP2AR31KR33K3 CGAGGTCCATGGACTCTGATTCTACTATCTTACGTAAAATCTAAAAGTGATC G G SpPBP2AR31KR33K3 CGATCACTTTTAGATTTACGTAAGATAGTAGAAATCAGAGTCCATGGAACCCT G G SpPBP2AR31KR33K3 CGATCACTTTTAGATTTACGTACGCGCACGTCGCTCTCGTAGTGATCG SpPBP2AR31KR33K3 CGATCACTATGGAGCGACGTGCGGCACGTCGGCTCTCGTAGTGATCG SpPBP2A128AL29A5 GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCG SpPBP2A128AL29A5 CGATCACTACGAGGAGCGACGTGCGGCAGTAGAATCAGAGTCCATGGAAC Construction of plasmid expressing recombinant SpGpsB1-63 SpGpsBnde15 SpGpsBhd13 GCACATCTCGAGTAACTACTTAAAAATCTGAGTATC Introduction of Ncol site to plasmid expressing recombinant SpGpsB1-63 SpGpsB103 SpGpsBM11NoI CGATATCTCGAGAACTATTATTTTTCAGGGCGCATTATTTTTCAGCG SpGpsB103del5 CCACTACTGAGAACTATTATTTTCAGGGCCCCTGAAAATAAGATCTGAGTAGG SpGpsB103del5 CCACTACTGAGAACTATATGCGAAAAATAAAGAGTCTGGAAAAAGATCCG SpBPB2x5 GCGGAGTAAGCCATGGAGTGGAAAAAGAACCTTGAGAA	Introduction of D35P to MR	P. SnPBP29 ^{G43C}								
SpPBP2AD3573 CGACATACTGGGCTAATTTTTTCGAGGACTACGAGAGCGACGTAAGATA G GACATACTTGGGCTAATTTTTTCGAGGACTACGAGAGCGACGTAAGATA G Introduction of R31K, R33K to MBP-SpPBP2a2,345 SpPBP2AR31KR33K5 CAGGGTTCCATGGACTCTGATTCTACTATCTTACGTAAATCTAAAAGTGATC G G SpPBP2AR31KR33K3 CGATCACTTTTAGATTTACGTAAGATAGTAGAATCAGAGTCCATGGAACCCT G G Introduction of 128A, L29A to MBP-SpPBP2a2,345 GTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCG SpPBP2A128AL29A5 GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCG SpPBP2A128AL29A5 CGATCACTACGAGAGCGACGTGCGGCAGTAGAATCAGAGTCCATGGAAC Construction of plasmid expressing recombinant SpGpsB1,63 SpGACATATCTGAGTAACTACTTAAAAATCTGAGTTATC SpGpsBnde15 GAGAGAATATGGCAAGTATTATTTTTTCAGCG SpGpsBh11Nco1 GCTATACCATGGCAAGTATTATTTTTTCAGCG SpGpsB111Nco1 CGATATCCGAGGAACTACTTAAAATATCGAGGTTATC Deletion of residues 1-3 to generate plasmid expressing recombinant SpGpsB4,63 SpGpsB1103de15 CCACTACTGAGAATCTTTATTTTTCAGGGCGCCATTATTTTTTCAGCG SpGpsB103de15 CCACTACTGAGAATCTTTAATTTTTTCAGGGGCACATAAGATCTGGG SpGpsB103de15 CCACTACTGAGAATATGGCACCATGAAAATAAGATTCTCAGTAGTGG SpGpsB103de15 CCACTACTGAGAATCTTTATTTTTCAGGGCACAAAAAGATTCTCAGTAGTGG	SpPRP2AD35P5	ΟΤΑΤΟΤΤΑΟΩΤΟΩΟΤΟΤΟΤΟΩΤΑΩΤΟΟΤΟΩΑΑΑΑΑΑΑΤΤΑΩΟΟΟ ΑΔΩΤΑΤΩΤΟΟ								
Spin Di ZADSATS Generate plasmid expressing recombinant SpGpsB163 SpBP2AR31KR33K5 CAGGGTTCCATGGACTCTGATTCTACTATCTTACGTAAATCTAAAAGTGATC G SpPBP2AR31KR33K3 CGATCACTTTTAGATTTACGTAAGATAGAATCAGAGTCCATGGAACCCT G SpPBP2AR31KR33K3 CGATCACTTTTAGATTTACGTAAGATAGTAGAATCAGAGTCCATGGAACCCT G SpPBP2AR31KR33K3 CGATCACTACTGAGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCG SpPBP2AR31KR33K3 GTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCG SpPBP2A128AL29A5 GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCG SpPBP2A128AL29A5 CGATCACTACGAGAGCGACGTGCGCAGTAGAATCAGAGTCCATGGAAC Construction of plasmid expressing recombinant SpGpsB163 GCACATCTCGAGTAACTACTTAAAAATCTGAGTTATC Introduction of Ncol site to plasmid expressing recombinant SpGpsB163 SpGpsBM11Ncol SpGpsBM11Ncol GCATATCTCGAGTAACTACTACTAAAAATCTGAGTTATC Deletion of residues 1-3 to generate plasmid expressing recombinant SpGpsB463 SpGpsB1103del5 SpGpsB1103del5 CCATACTGAGAATCTTATTATTTTTCAGGGCCCATTATTTTTCAGCG SpGpsB1103del5 CCACTACTGAGAATCTTATTATTTTTTCAGGGCCCATTATTTTTTCAGCG SpGpsB1103del5 CCATACTGAGAATCTTATTATTTTTTCAGGGCCCATTATTTTTTCAGCG SpGpsB1103del5 CCATACTGAGATCTTGATGTGCAAAACCTTGAGAAAAAGATCTCG SpPBP2x3st1 CGTTACTTGAGTGTGCAAAACCTTGAGAAAAAGTC SpPB	SpPBP2AD35P3	GGACATACTTGGGCTAATTTTTTTCGAGGACTACGAGAGCGACGTAAGATA								
Introduction of R31K, R33K to MBP-spPBP2a2:45"SpPBP2AR31KR33K5CAGGGTTCCATGGACTCTGATTCTACTATCTTACGTAAATCTAAAAGTGATC GSpPBP2AR31KR33K3CGATCACTTTTAGATTTACGTAAGATAGTAGAATCAGAGTCCATGGAACCCT GIntroduction of I28A, L29A to MBP-SpPBP2a:445GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCCGTAGTGATCGSpPBP2A128AL29A5GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCCGTAGTGATCGSpPBP2A128AL29A5CGATCACTACGAGAGCGACGTGCGGCAGTAGAATCAGAGTCCATGGAACConstruction of plasmid expressing recombinant SpGpsB1.63SGCACATCTCGAGTAACTACTTAAAAATCTGAGTTATCSpGpsBhdel5GAGAGACATATGGCAAGTATTATTTTTCAGCGSpGpsBM11Nco1GCTATACCATGGCAAGTATTATTTTTCAGCGSpGpsBM11Nco1GCATATCTCGAGTAACTACTTAAAAATCTGAGTTATCDeletion of residues 1-3 to generate plasmid expressing recombinant SpGpsB1.63SpGpsB1t03del5CCACTACTGAGAATCTTTATTTTCAGGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGDepsB1t03del5CCACTACTGAGAATCTTTATTTTCAGGGCGCCATTATTTTTCAGCGSpGpsB1t03del5CCACTACTGAGAATCATTAATGGCGCCCTGAAAAATAAAGATTCTCAGTAGTGGDepsB2x5GCGGAGTAAGCCATGGACAAAACGACAAAAGGTCSpPBP2x5CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2x300CL31SL32STGAAACAGACGCCAGGAGTGGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC		G CALLER CARCELLA CALLER CALLE								
SpPBP2AR31KR33KSCAGGGTTCCATGGACTCTGATTCTATCTATCTTACGTAAATCTAAAGTGATC GSpPB2AR31KR33K3CGATCACTTTTAGATTTACGTAAGATAGTAGAATCAGAGTCCATGGAACCCT GIntroduction of I28A, L29A to MBP-SpPBP2a2,45GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCGSpPBP2A128AL29A5GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCCGTAGTGATCGSpPBP2A128AL29A5CGATCACTACGAGAGCGACGTGCGGCAGTAGAATCAGAGTCCATGGAACConstruction of plasmid expressing recombinant SpGpsB1,63SGCACATCTCGAGTAACTACTAAAAATCTGAGTTATCSpGpsBhdel5GAGAGACATATGGCAAGTATTATTTTTCAGCSpGpsBh11NcoIGCTATACCATGGCAAGTATTATTTTTCAGCGSpGpsBM11NcoICGATATCTCGAGTAACTACTTAAAAATCTGAGTTATCDeletion of residues 1-3 to g=reate plasmid expressing recombinant SpGpsB4,63SpGpsB1t03del5CCACTACTGAGAAACTACTTAAAAATCTGAGGTCATCAGGGGGCCATTATTTTTCAGCGSpGpsB1t03del5CCACTACTGAGAAATAATGGCGCCCTGAAAATAAAGATTCTAGTGGGGCConstruction of plasmid expressing recombinant SpGpsB4,63SpGpsB1t03del5CGCTGAAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGDepsP2x351CGTTACTGAGTGTGCAAAACCTTGAGAAAAAGGTAATCCGSpPBP2x351CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2x351CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2x3512CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2x3512GAAAACAGACGACGAGAGTTGGAAAAAGTCSpPBP2x3512GAAAACAGACGACGAGAGTTGGAAAAAGTCGSpPBP2x3512GAAAACAGACGACGAGAGTTGGAAAAAGTCGSpPBP2x3512GAAAACAGACGACGAGAGTTGGAAAAAGTCGGGTTCATAATCTGTCTTTGTTTOP5TTGCC	Introduction of K31K, K33K	to MBP-SpPBP2 a_{23-45}								
SpPBP2AR31KR33K3CGATCACTTTTAGATTTACGTAAGATAGTAGAATCAGAGTCCATGGAACCCT GIntroduction of I28A, L29A to MBP-SpPBP2a2345GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCGSpPBP2A128AL29A5GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCCGTAGTGATCGSpPBP2A128AL29A5CGATCACTACGAGAGCGACGTGCGGCAGTAGAATCAGAGTCCATGGAACCConstruction of plasmid expressing recombinant SpGpsB1.63GAGAGACATATGGCAAGTATTATTTTTCAGCSpGpsBndeI5GAGAGACATATGGCAAGTATTATTTTTTCAGCGSpGpsBM11NcolGCTATACCATGGCAAGTATTATTTTTCAGCGSpGpsBM11NcolGCTATACCATGGCAAGTATTATTTTTCAGCGSpGpsBM11NcolCGATATCTCGAGTAACTACTTAAAAATCTGAGTTATCDeletion of residues 1-3 to g==rate plasmid expressing recombinant SpGpsB4.63SpGpsB1t03del5CCACTACTGAGAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGConstruction of plasmid expressing recombinant SpGpsB4.63SpGpsB1t03del3CGCTGAAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGConstruction of plasmid expressing recombinant SpGpsB4.63SpGpsB1t03del3CGCTGAAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGSpPBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAGAGTAATCCGSpPBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAGAGTAATCCGSpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2x3s0CL31SL32STGAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	SpPBP2AR31KR33K5	G								
Introduction of I28A, L29A to MBP-SpPBP2a23-45SpPBP2A128AL29A5GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCGSpPBP2A128AL29A5CGATCACTACGAGAGCGACGTGCGGCAGTAGAATCAGAGTCCATGGAACConstruction of plasmid expressing recombinant SpGpsB1-63GAGAGACATATGGCAAGTATTATTTTTTCAGCSpGpsBnde15GAGAGACATATGGCAAGTATTACTTAAAAATCTGAGTTATCIntroduction of Ncol site to plasmid expressing recombinant SpGpsB1-63GCACATCTCGAGTAACTACTTAAAAATCTGAGTTATCSpGpsBM11NcolGCTATACCATGGCAAGTATTATTTTTTCAGCGSpGpsBM11NcolGCATATCTCGAGTAACTACTTAAAAATCTGAGTTATCDeletion of residues 1-3 to generate plasmid expressing recombinant SpGpsB4-63SpGpsB1to3del5CCACTACTGAGAATCTTTATTTTTCAGGGCGCCATTATTTTTTCAGCGSpGpsB1to3del5CCACTACTGAGAATCTTTATTTTTCAGGGCGCCATTATTTTTCAGCGSpGpsB1to3del3CGCTGAAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGConstruction of plasmid expressing recombinant SpPBP2x1-29SpPBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAAGAGTCASpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAAACCTCGAGAAAAGTCSpPBP2x3s0CL31SL32STGAAAACAGACGCAGAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	SpPBP2AR31KR33K3	CGATCACTTTTAGATTTACGTAAGATAGTAGAATCAGAGTCCATGGAACCCT G								
SpPBP2AI28AL29A5GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCGSpPBP2AI28AL29A5CGATCACTACGAGAGCGACGTGCGGCAGTAGAATCAGAGTCCATGGAACConstruction of plasmid expressing recombinant SpGpsB1.63GAGAGACATATGGCAAGTATTATTTTTTCAGCSpGpsBnde15GAGAGACATCTCGAGTAACTACTTAAAAATCTGAGTTATCIntroduction of NcoI site to plasmid expressing recombinant SpGpsB1.63GCTATACCATGGCAAGTATTATTTTTTCAGCGSpGpsBM11NcoIGCTATACCATGGCAAGTATTATTTTTTCAGCGSpGpsBM11NcoIGCTATACCATGGCAAGTATCTTAAAAATCTGAGTTATCDeletion of residues 1-3 to generate plasmid expressing recombinant SpGpsB4.63SpGpsB1t03de15CCACTACTGAGAATCTTTATTTTCAGGGCGCCCATTATTTTTCAGCGSpGpsB1t03de15CCACTACTGAGAATCTTTATTTTCAGGGGCGCCATTATTTTTCAGCGSpGpsB1t03de13CGCTGAAAAAATAATGGCGCCCTGAAAAATAAAGAATTCAGTAGTGGConstruction of plasmid expressing recombinant SpBP2x1.29SpPBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAAGAGTAATCCGSpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2x330CL31SL32STGAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	Introduction of I28A, L29A t	to MBP-SpPBP2a ₂₃₋₄₅ ^{G43C}								
SpPBP2AI28AL29A5CGATCACTACGAGAGCGACGTGCGGCAGTAGAATCAGAGTCCATGGAACConstruction of plasmid expressing recombinant SpGpsB1.63GAGAGACATATGGCAAGTATTATTTTTTCAGCSpGpsBxhoI3GCACATCTCGAGTAACTACTTAAAAATCTGAGTTATCIntroduction of Ncol site to plasmid expressing recombinant SpGpsB1.63GCACATCTCGAGTAACTACTTAAAAATCTGAGTTATCSpGpsBM11NcolGCTATACCATGGCAAGTATTATTTTTTCAGCGSpGpsBM11NtolCGATATCTCGAGTAACTACTTAAAAATCTGAGTTATCDeletion of residues 1-3 to g=reate plasmid expressing recombinant SpGpsB4.63SpGpsB1to3del5CCACTACTGAGAATCTTTATTTTCAGGGCGCCATTATTTTTCAGCGSpGpsB1to3del5CCACTACTGAGAATCTTTATTTTCAGGGCGCCATTATTTTTCAGCGSpGpsB1to3del3CGCTGAAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGConstruction of plasmid expressing recombinant SpPB2x1.29SpPBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAGGTAATCCGSpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2x330CL31SL32STGAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	SpPBP2AI28AL29A5	GTTCCATGGACTCTGATTCTACTGCCGCACGTCGCTCTCGTAGTGATCG								
Construction of plasmid expressing recombinant SpGpsB1-63SpGpsBndeI5GAGAGACATATGGCAAGTATTATTTTTTCAGCSpGpsBxhoI3GCACATCTCGAGTAACTACTTAAAAATCTGAGTTATCIntroduction of NcoI site to plasmid expressing recombinant SpGpsB1-63SpGpsBM11NcoIGCTATACCATGGCAAGTATTATTTTTTCAGCGSpGpsBM11XhoICGATATCTCGAGTAACTACTTAAAAATCTGAGTTATCDeletion of residues 1-3 to generate plasmid expressing recombinant SpGpsB4-63SpGpsB1to3del5CCACTACTGAGAATCTTTATTTTCAGGGCGCCCATTATTTTTCAGCGSpGpsB1to3del3CGCTGAAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGConstruction of plasmid expressing recombinant SpBP2x1-29SpBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAGAGTAATCCGSpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAGCCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2x330CL31SL32STGAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	SpPBP2AI28AL29A5	CGATCACTACGAGAGCGACGTGCGGCAGTAGAATCAGAGTCCATGGAAC								
SpGpsBnde15GAGAGACATATGGCAAGTATTATTTTTCAGCSpGpsBxho13GCACATCTCGAGTAACTACTTAAAAATCTGAGTTATCIntroduction of NcoI site to plasmid expressing recombinant SpGpsB1.63SpGpsBM11NcoIGCTATACCATGGCAAGTATTATTTTTTCAGCGSpGpsBM11XhoICGATATCTCGAGTAACTACTTAAAAATCTGAGTTATCDeletion of residues 1-3 to g=n=rate plasmid expressing recombinant SpGpsB4.63SpGpsB1to3del5CCACTACTGAGAATCTTTATTTTCAGGGCGCCCATTATTTTTCAGCGSpGpsB1to3del5CCACTACTGAGAATCTTTATTTTCAGGGCGCCCATTATTTTTCAGCGSpGpsB1to3del3CGCTGAAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGConstruction of plasmid expressing recombinant SpPBP2x1.29SpPBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAAGAGTCASpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAAGTCSpPBP2x330CL31SL32STGAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	Construction of plasmid exp	ressing recombinant SpGpsB1 63								
SpGpsBxhol3GCACATCTCGAGTAACTACTTAAAAATCTGAGTTATCIntroduction of NcoI site to plasmid expressing recombinant SpGpsB1.63SpGpsBM11NcoIGCTATACCATGGCAAGTATTATTTTTCAGCGSpGpsBM11XhoICGATATCTCGAGTAACTACTTAAAAATCTGAGTTATCDeletion of residues 1-3 to generate plasmid expressing recombinant SpGpsB4.63SpGpsB1to3del5CCACTACTGAGAATCTTTATTTTCAGGGCGCCATTATTTTTCAGCGSpGpsB1to3del5CCACTACTGAGAATCTTTATTTTCAGGGCGCCATTATTTTTCAGCGSpGpsB1to3del3CGCTGAAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGConstruction of plasmid expressing recombinant SpPBP2x1.29SpPBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAGAGTAATCCGSpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2xS30CL31SL32STGAAAACAGACGCAGAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	SpGpsBndeI5	GAGAGACATATGGCAAGTATTATTTTTTCAGC								
Introduction of NcoI site to plasmid expressing recombinant SpGpsB1-63SpGpsBM11NcoIGCTATACCATGGCAAGTATTATTTTTTCAGCGSpGpsBM11XhoICGATATCTCGAGTAACTACTTAAAAAATCTGAGTTATCDeletion of residues 1-3 to generate plasmid expressing recombinant SpGpsB4-63SpGpsB1to3del5CCACTACTGAGAATCTTTATTTTCAGGGCGCCATTATTTTTCAGCGSpGpsB1to3del3CGCTGAAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGConstruction of plasmid expressing recombinant SpPBP2x1-29SpPBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAGAGTAATCCGSpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2xS30CL31SL32STGAAAACAGACGCAGAGATTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	SpGpsBxhoI3	GCACATCTCGAGTAACTACTTAAAAATCTGAGTTATC								
SpGpsBM11NcolGCTATACCATGGCAAGTATTATTTTTTCAGCGSpGpsBM11NcolCGATATCTCGAGTAACTACTTAAAAATCTGAGTTATCDeletion of residues 1-3 to generate plasmid expressing recombinant SpGpsB4.63SpGpsB1to3del5CCACTACTGAGAATCTTTATTTTCAGGGCGCCATTATTTTTCAGCGSpGpsB1to3del3CGCTGAAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGConstruction of plasmid expressing recombinant SpBP2x1.29SpBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAGAGTAATCCGSpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2xS30CL31SL32STGAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTGTTTOP5TTGCC	Introduction of Ncol site to n	lasmid expressing recombinant SpGpsB1 (2								
SpGpsBM11XhoICGATATCTCGAGTAACTACTTAAAAAATCTGAGTTATCDeletion of residues 1-3 to generate plasmid expressing recombinant SpGpsB4.63SpGpsB1to3del5CCACTACTGAGAATCTTTATTTTCAGGGCGCCATTATTTTTCAGCGSpGpsB1to3del3CGCTGAAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGConstruction of plasmid expressing recombinant SpBP2x1.29SpBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAAGAGTAATCCGSpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2xS30CL31SL32STGAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	SpGpsBM11NcoI	GCTATACCATGGCAAGTATTATTTTTTCAGCG								
Deletion of residues 1-3 to generate plasmid expressing recombinant SpGpsB4.63SpGpsB1to3del5CCACTACTGAGAATCTTTATTTTCAGGGGCGCCATTATTTTTCAGCGSpGpsB1to3del3CGCTGAAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGConstruction of plasmid expressing recombinant SpPBP2x1.29SpPBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAAGAGTAATCCGSpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAAGTCSpPBP2xS30CL31SL32STGAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	SpGpsBM11XhoI	CGATATCTCGAGTAACTACTTAAAAATCTGAGTTATC								
SpGpsB1to3del5CCACTACTGAGAATCTTTATTTTCAGGGCGCCATTATTTTTCAGCGSpGpsB1to3del3CGCTGAAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGGConstruction of plasmid expressing recombinant SpPBP2x1.29SpPBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAGAGTAATCCGSpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2xS30CL31SL32STGAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	Deletion of residues 1-3 to ge	nerate plasmid expressing recombinant $SnGnsR_{a}$								
SpGps21actionContention and international conduction of the content of	SpGpsB1to3del5	CCACTACTGAGAATCTTTATTTTCAGGGCGCCATTATTTTTCAGCG								
Construction of plasmid expressing recombinant SpPBP2x1-29SpPBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAGAGTAATCCGSpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2xS30CL31SL32STGAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	SpGpsB1to3del3	CGCTGAAAAATAATGGCGCCCTGAAAATAAAGATTCTCAGTAGTGG								
SpPBP2x5GCGGAGTAAGCCATGGAGTGGACAAAAGAGTAATCCGSpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2xS30CL31SL32STGAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	Construction of plasmid even	ressing recombinant SnPBP2x1 20								
SpPBP2x3st1CGTTACTTGAGTGTGCAAAACCTTGAGAAAAAGTCSpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAAGTCSpPBP2xS30CL31SL32STGAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	SpPBP2x5	GCGGAGTAAGCCATGGAGTGGACAAAAAGAGTAATCCG								
SpPBP2x3st2CGTTACTTGAGTGTGCAAAACCTCGAGAAAAGTCSpPBP2xS30CL31SL32STGAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTTOP5TTGCC	SpPBP2x3st1	CGTTACTTGAGTGTGCAAAAACCTTGAGAAAAAGTC								
SpPBP2xS30CL31SL32ST GAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTT OP5 TTGCC	SnPBP2x3st7	CGTTACTTGAGTGTGCAAAAACCTCGAGAAAAAGTC								
OP5 TTGCC	SpPBP2xS30CL31SL32ST	GAAAACAGACGCAGAGTTGGAAAAAGTCTGTGTTCATAATCTGTCTTTGTTT								
	OP5	TTGCC								

SpPBP2xS30CL31SL32ST	GGCAAAAACAAAGACAGATTATGAACACAGACTTTTTCCAACTCTGCGTCT
OP3	GTTTTC
SpPBP2xinsgly5	CCTGTACTTCCAGGGTTCCGGATCTGGAATGGAGTGGACAAAAAGAG
SpPBP2xinsgly3	CTCTTTTTGTCCACTCCATTCCAGATCCGGAACCCTGGAAGTACAGG
Construction of plasmid exp	ressing recombinant <i>Bs</i> YpbE ₈₀₋₂₄₀
YpbEndeI5	CTTATTTCATATGAAGAGCCACCCGGATAATCATG
YpbExhoI3	CGTAAACTCGAGATAATACCCTATTCATCCATTAAAGG
Construction of plasmid exp	ressing recombinant <i>Bs</i> YpbE ₁₃₀₋₂₄₀
YpbEtruncatencoi5	GCTTCTCCATGGAAGATTCCAAGCCAAAAGAGC
YpbEtruncatencoi3	GCTGCTGCCCATGGTATATCTC
Construction of plasmid exp	ressing recombinant BsYrrS
YrrSndei5	GCAGAACATATGAGCAATAATCAATCTCGTTATG
YrrSxhoi3	CGGCTGCTCGAGTTTATTTTAGCTTTTCTACTTTTGTC
Construction of plasmid exp	ressing recombinant <i>Bs</i> YrrSΔ ₁₃₋₁₆
YrrS ₁₃₋₁₆ delete5	CTCGTTATGAAAATCGTGATGCCAATTTAGTGCTTAACATTTTAATCG
YrrS ₁₃₋₁₆ delete3	CGATTAAAATGTTAAGCACTAAATTGGCATCACGATTTTCATAACGAG



107 108

8 Supplementary Figure 1. Biophysical effects of mutations in *Bs*GpsB and *Bs*PBP1.

(A) A sequence alignment of GpsB proteins with the intermolecular interactions from the BsGpsB₅₋ 109 ₆₄:BsPBP1₁₋₁₇ complex highlighted above: black filled circles - residues in hydrogen bonds or salt 110 bridges; yellow circles - residues in van der Waals' interactions; black unfilled circles - interfacial 111 residues utilising main chain atoms; coloured circles below - residues mutated in this study: 112 SpGpsB Y23, V27, L32, D33, I36 (red); LmGpsB Y27, D33 and D37 (cyan); BsGpsB E17, Y25, 113 D31, D33 (yellow). BsGpsB α -helices $\alpha 1$ and $\alpha 2$ are denoted as cyan cylinders. The sequence of 114 $B_{s}PBP1_{1-17}$ peptide (below) is annotated as above and the helical region denoted as a green cylinder. 115 (**B**) The wild-type folding of $BsGpsB_{1-68}$ variants was confirmed by circular dichroism. Protein 116 secondary structure unfolding was monitored with the CD ellipticity signal at 222 nm as a function 117 of temperature. (C) Alanine substitutions of conserved asparate and glutamate residues in BsGpsB₁. 118 $_{68}$ cannot bind to TAMRA-labelled wild-type BsPBP1₁₋₃₂ as measured by fluorescence polarisation. 119 The K_d values are tabulated in **Supplementary Table 1**. (**D**) The helical content of BsPBP1₁₋₃₂ 120 peptides provides a molecular rationale for the reduced affinity of BsPBP11-32 variants for BsGpsB. 121 The absolute molar ellipticity signal at 222 nm, which is linearly proportional to helix content¹⁷, 122 was measured by circular dichroism for BsPBP11-32 peptides. (E) The position of the fluorophore 123 does not affect the interaction of labelled BsGpsB₁₋₃₂ peptides with BsGpsB₁₋₆₈. A peptide labelled 124 at the C-terminus with fluorescein binds $BsGpsB_{1-68}$ with the same affinity as a peptide labelled 125 with TAMRA at Ser16Cys (Figure 1D and Supplementary Table 1). (F) A sequence alignment of 126 the cytoplasmic minidomains of representative Bacillaceae PBP1 orthologues highlights the 127 importance of the invariant Ser7, Arg8 and Arg11 (blue highlight) for GpsB-binding. The helical 128 region of PBP1 is depicted above by a red cylinder. These sequences are representatives from the 129 branches of a *Bacillaceae* family phylogenetic tree¹⁸. Exceptions lacking a PBP1 orthologue with a 130 SRxxR(R/K) motif include Brevibacillus brevis, Paenibacillus sp., Lysinibacillus sphaericus, 131

132 Oceanobacillus iheynensis and Geobacillus sp. strains C56_T3 and Y412MC61.



Supplementary Figure 2: *Lm*PBPA1:*Lm*GpsB interactions depend on a conserved arginine.

(A) The LmPBPA1 TRSQYRN motif is conserved in all publicly available Listeria sequences. 135 Basic amino acids (cyan) are more abundant than negatively-charged residues (red). LmPBPA1^{Thr7} 136 and $LmPBPA1^{Arg8}$ are highlighted in blue. (**B**) $LmPBPA1_{1-20}^{Arg8}$ is the most critical $LmGpsB_{1-73}$ 137 binding determinant. The binding of fluorescein-labelled LmPBPA1₁₋₂₀ variants to LmGpsB₁₋₇₃ and 138 its surrogate, $B_s Gps B_{1-68}^{Lys32Glu}$ was monitored by FP. The cognate $Lm Gps B_{1-73}:Lm PBPA1_{1-20}$ 139 interaction is represented by the solid black curve; all other interactions involve the BsGpsB1 140 $_{68}^{Lys32Glu}$ surrogate. The calculated dissociation constants are listed in **Supplementary Table 1**. (C) 141 In the presence of the helix-stabilizing additive, trifluoroethanol (TFE), wild-type $LmPBPA1_{1-15}$ has a greater helical character than $LmPBPA1_{1-15}^{Gln10Pro}$. (**D**) Mutations in the cytoplasmic minidomain 142 143 of *pbpA1* have little impact on growth of a $\Delta pbpA2$ mutant at 42°C. L. monocytogenes contains two 144 bi-functional PBPs, PBPA1 and PBPA2¹⁹, but at least one is requored for viability⁵. If the 145 *Lm*PBPA1:*Lm*GpsB interaction is essential for PBPA1 function, the Thr7Ala, Arg8Ala, Tyr11Ala 146 and Arg12Ala exchanges in *pbpA1* might not be tolerated with a *pbpA2* deletion. However, strains 147 LMS219 (pbpA1T7A \DeltapbpA2), LMS220 (pbpA1R8A \DeltapbpA2), LMS221 (pbpA1Y11A \DeltapbpA2), 148 LMS222 (pbpA1R12A \DeltapbpA2), LMS232 (pbpA1R8A R12A \DeltapbpA2) and LMS233 (pbpA1O10P 149 $\Delta pbpA2$) are viable and even grow in BHI broth at 42°C. The only significant growth defect 150 (marked with asterisks, P < 0.01, t-test) was observed for strain LMS232 (*pbpA1R8A R12A \DeltapbpA2*) 151 in comparison to the *pbpA2* null mutant. Strains EGD-e (wt), LMJR19 ($\Delta gpsB$), LMS57 ($\Delta pbpA1$) 152 and LMS64 ($\Delta pbpA2$) were included as controls. (E) The free amino acid L-arginine does not 153 displace fluorescein-labelled LmPBPA1₁₋₂₀ peptide from LmGpsB₁₋₇₃ even when present at more 154 than 100-fold excess relative to the LmGpsB₁₋₇₃ protein. In this experiment, the fluorescein-labelled 155 peptide is at 40 nM concentration and the $LmGpsB_{1-73}$ protein at 200 μ M. 156



158

Supplementary Figure 3: Extending the GpsB interactome in B. subtilis and L. monocytogenes 159 (A) The LysM domain of BsYpbE binds peptidoglycan as a dimer. Almost all the monomeric LysM 160 domain of BsYpbE, BsYpbE₁₃₀₋₂₄₀; a disulphide cross-linked dimeric form of BsYpbE, BsYpbE₁₃₀₋ 161 ²⁴⁰^{Ser132Cys}; and the BSA control is found in the supernatant (s) in the absence of PG (left hand side 162 of the SDS-PAGE gel). By contrast, the majority of dimeric BsYpbE₁₃₀₋₂₄₀ Ser132Cys is found in the 163 pellet (p) in the presence of PG whereas monomeric B_{s} YpbE₁₃₀₋₂₄₀ and BSA are still found in the 164 supernatant. The blue smear towards the bottom of the gel is PG that has been pelleted by 165 centrifugation. (B) BsYrrS interacts directly with BsPBP1. SPR sensorgram of serial injections of 166 increasing concentrations (62.5 nM - 1 μ M) of BsYrrS_{A13-16} over a BsPBP1-immobilised chip 167 surface. The black dashed line represents the reference-subtracted sensorgrams and the red-dashed 168 line represents the fit using single-cycle kinetics with a 1:1 binding model yielding a dissociation 169 constant of 20 ± 0.2 nM. BsYrrS_{$\Delta 13-16$} was used to reduce non-specific binding to the chip surface. 170 (C) The interaction of LmGpsB with selected cell division proteins is dependent upon the LmGpsB 171 N-terminal domain. The agar plates in this BACTH of full-length LmGpsB and variants lacking its 172 N- (Δ N) or C-terminal domain (Δ C) against cell division proteins were photographed after 24 and 173 48 hrs at 30°C. pKT25 (T25) without a fusion partner was used as a negative control. (D) Tyr27, 174 Asp33 and Asp37 in LmGpsB are essential for some, but not all, of the interactions against selected 175 cell division proteins. The agar plates in this BACTH were photographed after 48 hrs at 30°C. 176 177



178 Supplementary Figure 4. *Sp*GpsB mutations affect *Sp*PBP2a-binding but not expression.

(A) Sequence alignment of Streptococcaceae PBP2a orthologues reveals that Arg31, Ser32 and 179 Arg36 (blue highlights) and the RSxxxR motif (boxed) are conserved. The conservation of Ser32 180 reflects either its interaction with SpGpsB^{Asp33} and/or its stabilization of the type I β-turn between 181 Arg30 and Arg33 in SpGpsB₁₋₆₃:SpPBP2a₂₃₋₄₅ (molecule 1). The serine/glycine distribution is 182 consistent with preferential occupancies of the *i* and i+2 (serine) and i+3 (glycine) positions in type 183 I β -turns²⁰. A diverse set of sequences were selected based on a phylogenetic tree of *Streptococci*²¹. 184 The RSxxxR motif is conserved in the *mitis*, *salivarius* and *anginosus* subgroups but not in *bovis*, 185 mutans and pyogenes. S. gordonii, in the mitis subgroup, is an exception and encodes SRSGR 186 (yellow highlights, below) that is a near perfect match to the SRxxR(R/K) motif in *Bacillaceae* 187 PBP1 proteins. (B) The reciprocal BACTH analysis of the SpGpsB-T25 data in Figure 5A confirms 188 the interactions of SpGpsB with SpPBP2a and SpMreC. The agar plates were photographed after 40 189 hrs incubation at 30°C. (C) BACTH of the self-interactions of SpGpsB variants reveals that all 190 proteins retain the ability to interact implying that the mutations do not affect protein folding. The 191 agar plates were photographed after 40 hrs incubation at 30°C. (D) SpGpsB-FLAG variants are 192 expressed at wild-type levels. Western analyses of S. pneumoniae strains showing the expression of 193 SpGpsB-FLAG variants, detected with an anti-FLAG antibody as described in the Methods. Anti-194 MreC was performed as a loading control. Strains used are $gpsB^+$ (IU11488, $gpsB^+$ -P_c-erm//P_{Zn}-195 gpsB⁺), gpsB⁺-F (IU13442, gpsB⁺-FLAG-P_c-erm//P_{Zn}-gpsB⁺), Y23A (IU13364, gpsB Y23A-FLAG-196 P_{c} -*erm*// P_{Zn} -*gpsB*⁺), V28A (IU13366, *gpsB* V28A-FLAG- P_{c} -*erm*// P_{Zn} -*gpsB*⁺), D29A (IU13368, 197 gpsB D29A-FLAG-P_c- $erm/P_{Zn}-gpsB^+$), L32A (IU13370, gpsB L32A-FLAG-P_c- $erm/P_{Zn}-gpsB^+$), 198 D33A (IU13372, gpsB D33A-FLAG-P_c-erm//P_{Zn}-gpsB⁺), and I36A (IU13374, gpsB I36A-FLAG-199 P_c -*erm*// P_{Zn} -*gpsB*⁺). The expected molecular mass of GpsB-FLAG is 13.7 kDa. 200



Supplementary Figure 5. Phenotypes of mutant strains lacking the SpPBP2a RSxxxR motif. 202 Truncation of residues 27-38 or 26-45 of SpPBP2a in a $\Delta pbp1a$ genetic background results in 203 longer doubling times and abnormal morphologies. Representative growth curves (A) and phase-204 contrast micrographs (B) of S. pneumoniae strains IU1824 (D39 Acps rpsL1 parent), IU13256 205 (Δ*pbp*2a), IU13444 (Δ*pbp*1a), IU13446 (*pbp*2 $a_{\Delta 32-37}$ Δ*pbp*1a), IU13448 (*pbp*2 $a_{\Delta 27-38}$ Δ*pbp*1a) and 206 IU13450 (*pbp2a* $_{\Delta 26-45} \Delta pbp1a$); doubling times are reported below (**B**). Deleting *Sp*PBP2a residues 207 27-38 or 26-45 in a $\Delta pbp1a$ background resulted in highly variable cell sizes: wide and narrow 208 arrows point to abnormally large and small cells, respectively. All micrographs were taken at mid 209 exponential phase (OD₆₂₀ ≈ 0.15) and are at the same magnification (scale bar = 1 µm). (C) The 210 expression of SpPBP2a truncated variants is like wild-type. Western analyses of S. pneumoniae 211 strains IU1824 ($pbp1a^+$, $pbp2a^+$), IU13256 ($pbp1a^+$, $\Delta pbp2a$), IU13444 ($\Delta pbp1a$, $pbp2a^+$), IU13446 212 $(\Delta pbp1a, pbp2a_{\Delta 32-37})$, IU13448 $(\Delta pbp1a, pbp2a_{\Delta 27-38})$ and IU13450 $(\Delta pbp1a, pbp2a_{\Delta 26-45})$. The 213 expected molecular masses are 80.9 kDa (WT SpPBP2a), 80.2 kDa (SpPBP2a_{A32-37}), 79.4 kDa 214 (SpPBP2a_{$\Delta 27-38$}) and 78.5 kDa (SpPBP2a_{$\Delta 26-45$}). NS indicate non-specific bands that were also 215 present in the $\Delta pbp2a$ strain. (D) Truncation of the RSxxxR GpsB-binding motif in SpPBP2a results 216 in a progessive decrease in interaction with SpGpsB but not with SpMreC. The interactions of the 217 SpPBp2a Δ 32-37, Δ 27-38, Δ 26-45 truncated variants with SpGpsB and SpMreC were analysed by 218 BACTH. Samples were photographed after 36 hrs incubation at 30°C. (E) SpPBp2a interacts 219 directly with SpMreC in BACTH. Samples were photographed after 40 hrs incubation at 30°C. 220



Interaction of SpGpsB with other proteins as revealed by different assays

	PBP2a	MreC	PBP1a	PBP2x	PBP2b	StkP	EzrA
BACTH	+++	+++	+++	++	-	+ ^a	+ ^a
FP	+++	nd	nd	++	-	nd	nd
Co-IP	++ ^a	++a	+a	_a	++ ^a	++ ^a	++ ^a

^{+++:} strong signal, ++: signal present, +: weak signal, -: signal absent, nd: not determined. a: reported in Rued *et al.*, 2017

222 Supplementary Figure 6. *Sp*GpsB interacts with different cell division proteins.

(A) SpGpsB interacts with SpPBP1a, SpPBP2x, SpStkP and SpEzrA, as well as SpMreC and 223 SpPBP2a, but not with SpPBP2b, as detected by BACTH. Tyr23, Asp29, Asp33 and Ile36 are 224 essential for some, but not all, of the interactions against these cell division proteins. The agar plates 225 were photographed after 40 hrs at 30°C. Some of the interactions of SpGpsB and its allelic variants 226 in Figure 5A are reproduced here for the sake of comparison and consistency with Supplementary 227 228 Figure 3D. (B) By FP $SpGpsB_{1-63}$ interacts with TAMRA-labelled $SpPBP2a_{23-45}$ (red) and fluorescein-labelled $SpPBP2x_{1-29}$ (blue), but not fluorescein-labelled $SpPBP2b_{1-17}$ (black). The 229 relevant dissociation constants are listed in Supplementary Table 1. (C) Left panel, pairwise co-IP 230 detection of SpPBP2x with SpStkP-F² and SpMreC-L-F³, but not with SpGpsB-L-F³ using anti-231 PBP2x to detect SpPBP2x (prey) complexed with FLAG-tagged proteins. Strains used were IU1945 232 (WT), IU5458 (gpsB-L-F³), IU7434 (stkP-F²), IU11314 (gpsB-L-F³ pbp2x-HA), IU11316 (gpsB-L-233 F³ pbp2b-HA), and IU4970 (mreC-L-F³). The expected molecular mass of SpPBP2x is 82.4 kDa. 234 Middle panel, pairwise co-IP detection of SpGpsB and SpDivIVA with SpStkP-F², but not with 235 SpPBP2x-F³ using an anti-GpsB serum to detect SpGpsB and SpDivIVA (preys) complexed with 236 FLAG-tagged proteins. Strains used were IU6810 (ezrA-HA, non-FLAG-tagged control), IU12077 237 (*ezrA*-HA *stkP*- F^2), and IU11880 (*ezrA*-HA *pbp2x*- F^3). Right panel, characterisation of anti-*Sp*GpsB 238 serum generated from rabbits immunized with purified SpGpsB₁₋₆₃. Lysates were prepared from 239 WT (IU1945), \(\Delta divIVA\) (IU8496) or \(\Delta gpsB\) (IU6442) strains. The band at \(~13\) kDa is \(SpGpsB\) 240 (expected molecular mass is 12.6 kDa) and that at ~50 kDa is SpDivIVA (expected molecular mass 241 is 30.3 kDa, but typically runs at ~50 kDa on SDS-PAGE). (D) Summary of the interaction of 242 SpGpsB with other proteins as revealed by BACTH (data from A), FP (B) and co-IP (C and 10). 243 244

A Lactococci class A PBPs

						- 7								7	0								2	0						
L. lactis subsp. lactis						M	IS	Е	Ν	Ν	Ν	F	SI	RF	RN	1 K	K	E	S	K	K	NS	S L	. K	ί.	L	Ρ	N	LR	1
L. lactis subsp. tructae						M	I P	Е	Ν	к	Ν	F	SI	RF	R 5	S K	K	Е	т	G	ΚI	KS	5 L	. K	ί.	L	Ρ	κ	I R	1
L. lactis subsp. cremoris						Μ	I P	Е	Ν	κ	Ν	F	SI	RIF	2 8	S K	K	E	т	G	κı	KS	5 L	. K	ί.	Т	Ρ	κ	IR	1
L. plantarum		Μ		Е	N	ΙE	E	κ	κ	S	s	L	SI	R	SE	EK	N	S	D	P	G	LC) F	ł K	11	κ	Α	κı	KR	1
L. raffinolactis		Μ	T	Е	N	E	V	κ	κ	S	s	L	SI	R	SF	RK	N	۷	D	Ρ	E) F	ł K	V	κ	Т	KI	KR	1
L. chungangensis		Μ	T	E	N	ΙE	V	κ	κ	s	S	L	SI	R	5 0) K	N	۷	D	Ρ	E	LC) F	{ K	V I	κ	т	κı	KR	1
L. petauri	•				M	۱S	E	κ	D	Q	Ν	F	SI	R	RA	۱ K	N	κ	κ	S	SI	KK	ί.		1	Е	Ν	N	NE	i
L. garvieae					M	IS	E	κ	D	Q	Ν	F	SI	R F	R A	۱ K	G	κ	κ	S	ТΙ	KK	(N	IΤ	D	κ	Q	Ν	ΙE	
L. fujiensis						Μ	IS	Е	Ν	Q	Ν	F	SI	RF	R 8	S K	S	Q	κ	S	ΚI	ΚE	ΕL	. I		κ				

. .

~~

B Leuconostoc/Weisella class A PBPs

	1 10 20 30	
L. mesenteroides	M A N D K A N Q W S R V N R N H K M Y D Q Y P A Q E P P R P P K P N G P K Q	3 S
L. pseudomesenteroides	MANDNSNQW <mark>SR</mark> VD <mark>R</mark> NQNMYDNQPGQEPPRPRPKGK(зS
L. citreum	MANDNQWSRVNRNQNLYDNHPATEPPTIPPHYG . KO	à G
L. gelidum	MANENQWSRVNRNRNMYDNHPATEPPRIPKSN	
L. carnosum	MANENQWSRVNRNSNTYDSYPATEPPKTPRPPKKNC	GΝ
L. lactis	MANDNQWSRVNBNQNLYDNHPATEPPTIPPHHD. KO	G
L. garlicum	MANDNQWSRVNRNQNLYDNHPATEPPTIPPHHG. KC	GG
W. confusa	MTEEMSRVQRLRSKKKT	
W. viridescens	MSDESSRVSRQDPKKNPNSRSQ	
W. koreensis	MPDQNLSRANRTHPKKKRAN	
W. halotolerans	MTEDDMSRSQRNRSAGATASR.S	
W. paramesenteroides	MTEEMSRVERGRNSTKNNKRPTOPKP	
W. hellenica	MTEKMŠRVSRGQQNTNNNKRPSQPKKTN	
W. kandleri	MSGQKLSRTKRTNKKRVNKS	
W. soli	MAEHLPSRGSRTGSASITKKRNHIPRNPN	
W. cibaria	MTEEMSRVQRNAQTSRK	
W. bombi	MTENMSRVERGORNTNNNORPSKPKKNN	
W. thailandensis	MTEEMSBVERGRNSTKNNKRPSQPNP	
W. ceti	MADEOSBMKBTKKSSAK KOK	
W. minor	MSDETS BT SBONSNSNNSGB	
W. iogaeieotgali	MTEEMSBVERGBNSTKNNKBPSOPNP	
jeguejee.gun		501 I.S.

C Enterococci PBP2a orthologues

	1		10	20	30
E. faecalis	M	ANEQSR	/ SRRNYQ	STKKTP	KKSSPKKAPGKTK
E. faecium	M	IANEQTRS	SSRRQKQ	PTPKKSVKK	(NSGKDSGKSSGTHKK
E. durans	M	I P R K D T R	< K R N Q K K	KQKWFVPK.	
E. canis	M	IANEQSRA	ATRSSRS	QQSNKAPK.	PKGTGKKSVC
E. quebecensis	M T T	DEIGSRA	AARHGHT	PASNN	TENTPSNGGKKPKKK
E. moraviensis	M T T	DEISSR	AARHGHT	PASSN	TGNLSTNGGKKPKKK
E. termitis	M T T	DEISSR	AARHGHT	STSNG	NTNIPSNGGKKPKKK
E. silesiacus	M T T	DEISSR	AARHGHT	TTSNG	TVNMPSNGGKKPKKK
E. caccae	M T T	DEIGSR	AARHGHT	SVTNST	ESTSPSNGGKKPKKK
E. haemoperoxidus	MTT	DEIGSR	AARHGHT	PVSNGT	EN. TPSNGGKKPKKK
E. italicus	M A	N ESR	ΓΝ ΠΗΚΚ.	EPQEKT	
E. sulfureus	M A	N ESR	SRRQTT	KASRPSSRP	ΡSKQTPKKKKPK
E. pallens	M A	K. TSRS	SEKRPKK	ATKQ	KGTKRNGKKV
E. hermanniensis	M S	SN TSRS	SQKNKRT	TPGK	KMKSKNKKGO
E. gilvus	M A	N. PSRS	SQKSKRT	PQGK	KPAQKSKQNF
E. devriesei	M A	K. PSRS	SQKNSRT	TQGK	КТ QPKMKKNF
E. avium	M A	N. PSRS	SQKKKKRT	SQGK	E P K Q K N K K N F
E. malodoratus	M A	N. PSRS	SQKSMRT	TQGK	KPKSKTKKSF
E. raffinosus	M A	N. PSRS	SQKKMRT	AQGK	KPASKKTKNF
E. asini	M T	TDTPSR	AARNQNK	KSGKQPPK.	KNGKNNKPKKKRSVO
E. canintestini	M G	ANDTSSR/	ASRHN	GNNAPN.	KKPKKLKKKTKNSVO
E. dispar	M G	NDTPSR	ASRHN	GNNAPQ.	KNPKKMKKKTKNSAC
E. cecorum	M A	NQTSTR	KAKHHQK	КРМ	NKKSKQNSSC
E. columbae	M A	NNQPSR	(GRHQQS	THRK	
E. phoeniculicola	M T	DHLQSRS	SSRRKET	KSTNNS	NQSKKPKKKRSVO
E. mundtii	M	IPTNQTRS	SSKRSTS	SSSPKK	KRGKQSTKKGTGKDRM
E. thailandicus	M	ANEQTR	SRRKAS	PPQKKGTKS	SSGNG SGKQKNGK
E. ratti	M	ISNEQTR	Г S <mark>R</mark> R K S P	PSK.K.TNG	Q I R K K N V G K D N K K K K S
E. hirae	M	IANEQTR	Γ S <mark>R</mark> R N S P	SSSKK.TSC	Q T R N K T S G K G S G H K K F
E. villorum	M	ANKQTR	FSRRKSP	PSSTK.NKG	TRIKNAGKGPKKHRF
E. saccharolyticus	M A	NQQNSR	/ SRHKTK	ASKP	SKRKTPKQKNKRSIC
E. aquimarinus		MSNNTR	FQRHESP	К	KKTVKNKKKRSTO
E. casseliflavus	MMSMA	NESQSR	r s r h d s k	KGTA K	KASKPPKANGKRSIG
E. gallinarum	MISMA	KESQSR	ASRHDSK	KAAAA Y	KGPKQKKPKGKRTIG

245

246 Supplementary Figure 7. The SRxxR(R/K) motif is conserved in other *Lactobacillales* PBPs.

Sequence alignments are shown for class A PBPs from *Lactococci* (A), *Leuconostoc/Weisella* (B) 247 and Enterococci (C); the L. and W. prefixes in the species names in panel B correspond to 248 Leuconostoc and Weissella respectively. The SRxx(R/K) motif is highlighted in blue. Binding and 249 structural studies reported herein are consistent with GpsB binding tolerating either an Arg or a Lys 250 at the underlined position in the $SRxx\underline{R}(R/K)$ sequence. A phylogenetically diverse set of 251 representative Leuconstoc/Weisella and Enterococci genomes were chosen based on respective 252 phylogenetic trees^{22,23}. The motif is widely conserved in all families, with the exception of 253 Oenococcus oeni, L. fallax and L. ficulneum/L. pseudoficulneum/L. fructosum sub-groups within 254 *Leuconstoc*. This alignment, and all others, was created in $ALINE^{24}$. 255



258 Supplementary Figure 8. Electron density maps for peptides in the GpsB structures.

Stereograms of the final, Refmac-weighted $2mF_{obs}$ -DF_{calc} electron density maps for (**A**) BsGpsB₅₋₆₄ $_{64}:Bs$ PBP1₁₋₁₇, contoured at 0.09 electrons per Å³; (**B**) BsGpsB₅₋₆₄ $_{Lys^{32Glu}}:Lm$ PBPA1₁₋₁₅, (contoured at 0.14 electrons per Å³; (**C**) SpGpsB₄₋₆₃:SpPBP2a₂₇₋₄₀, molecule 1 and (**D**) SpGpsB₄₋₆₃:SpPBP2a₂₇₋₄₀, molecule 2 (both contoured at 0.5 electrons per Å³. The colours, interactions and view is the same as in **Figure 2C**; interfacial residues are shown as sticks, other amino acids as lines.

264 Supplementary References

- 1. Kumar, S. & Bansal, M. Dissecting alpha-helices: position-specific analysis of α -helices in globular proteins. *Proteins* **31**, 460-476 (1998).
- Doig, A.J. & Baldwin, R.L. N- and C-capping preferences for all 20 amino acids in α helical peptides. *Prot. Sci.* 4, 1325-1336 (1995).
- Johnson, C.L. et al The antibacterial toxin colicin N binds to the inner core of
 lipopolysaccharide and close to its translocator protein. *Mol Microbiol.* 92, 440-452 (2014).
- 4. Rismondo, J. et al. Structure of the bacterial cell division determinant GpsB and its interaction with penicillin binding proteins. *Mol. Microbiol.* **99**, 978-998 (2016).
- 273 5. Rismondo, J., Möller, L., Aldridge, C., Gray, J., Vollmer, W. & Halbedel, S. Discrete and
 274 overlapping functions of peptidoglycan synthases in growth, cell division and virulence of
 275 *Listeria monocytogenes. Mol. Microbiol.* 95, 332-351 (2015).
- Lanie, J.A. et al. Genome sequence of Avery's virulent serotype 2 strain D39 of *Streptococcus pneumoniae* and comparison with that of unencapsulated laboratory strain R6. *J. Bacteriol.* 189, 38-51 (2007).
- Tsui, H.C. et al. Suppression of a deletion mutation in the gene encoding essential PBP2b
 reveals a new lytic transglycosylase involved in peripheral peptidoglycan synthesis in
 Streptococcus pneumoniae D39. *Mol. Microbiol.* 100, 1039-1065 (2016).
- Land, A.D. et al. Requirement of essential Pbp2x and GpsB for septal ring closure in
 Streptococcus pneumoniae D39. Mol. Microbiol. 90, 939-955 (2013).
- 284 9. Land, A.D. & Winkler, M.E. The requirement for pneumococcal MreC and MreD is
 285 relieved by inactivation of the gene encoding PBP1a. *J. Bacteriol.* 193, 4166-4179 (2011).
- 28610.Rued, B.E. et al. Suppression and synthetic-lethal genetic relationships of $\Delta gpsB$ mutations287indicate that GpsB mediates protein phosphorylation and penicillin-binding protein288interactions in *Streptococcus pneumoniae* D39. *Mol. Microbiol.* 103, 931-957 (2017).

- Tsui, H.C. et al. Pbp2x localizes separately from Pbp2b and other peptidoglycan synthesis
 proteins during later stages of cell division of *Streptococcus pneumoniae* D39. *Mol. Microbiol.* 94, 21-40 (2014).
- 292 12. Zheng, J.J., Perez, A.J., Tsui, H.T., Massidda, O. & Winkler, M.E. Absence of the KhpA
 293 and KhpB (JAG/EloR) RNA-binding proteins suppresses the requirement for PBP2b by
 294 overproduction of FtsA in *Streptococcus pneumoniae* D39. *Mol. Microbiol.* 106, 793-814
 295 (2017).
- Arnaud, M., Chastanet, A. & Debarbouille, M. New vector for efficient allelic replacement
 in naturally nontransformable, low-GC-content, gram-positive bacteria. *Appl. Environ. Microbiol.* 70, 6887-6891 (2004).
- 14. Karimova, G., Pidoux, J., Ullmann, A. & Ladant, D. A bacterial two-hybrid system based on
 a reconstituted signal transduction pathway. *Proc. Natl. Acad. Sci. USA* 95, 5752-5756
 (1998).
- Solution 15. Claessen, D., Emmins, R., Hamoen, L.W., Daniel, R.A., Errington, J. & Edwards, D.H.
 Control of the cell elongation-division cycle by shuttling of PBP1 protein in *Bacillus subtilis. Mol. Microbiol.* 68, 1029-1046 (2008).
- Rismondo, J., Wamp, S., Aldridge, C., Vollmer, W. & Halbedel, S. Stimulation of PgdA dependent peptidoglycan N-deacetylation by GpsB-PBP A1 in *Listeria monocytogenes*.
 Mol. Microbiol. 107, 472-487 (2018).
- Chen, Y.H., Yang, J.T. & Martinez, H.M. Determination of the secondary structures of
 proteins by circular dichroism and optical rotatory dispersion. *Biochemistry* 22, 4120-4131
 (1972).
- 311 18. Schmidt, T.R., Scott, E.J. 2^{nd} & Dyer, D.W. Whole-genome phylogenies of the family 312 *Bacillaceae* and expansion of the sigma factor gene family in the *Bacillus cereus* species-313 group. *BMC Genomics* **12**, 430 (2011).

- Korsak, D., Markiewicz, Z., Gutkind, G.O. & Ayala, J.A. Identification of the full set of
 Listeria monocytogenes penicillin-binding proteins and characterization of PBPD2
 (Lmo2812). *BMC Microbiol.* 10, 239 (2010).
- 317 20. Hutchinson, E.G. & Thornton, J.M. A revised set of potentials for β -turn formation in 318 proteins. *Prot. Sci.* **3**, 2207-16 (1994).
- Gao, X.Y., Zhi, X.Y., Li, H.W., Klenk, H.P. & Li, W.J. Comparative genomics of the
 bacterial genus *Streptococcus* illuminates evolutionary implications of species groups. *PLoS One* 9, e101229 (2014).
- Chelo, I.M., Zé-Zé, L. & Tenreiro, R. Congruence of evolutionary relationships inside the
 Leuconostoc-Oenococcus-Weissella clade assessed by phylogenetic analysis of the 16S
 rRNA gene, *dnaA*, *gyrB*, *rpoC* and *dnaK*. *Int. J. Syst. Evol. Microbiol.* 57 276-286 (2007).
- 325 23. Zhong, Z. *et al.* Comparative genomic analysis of the genus *Enterococcus*. *Microbiol Res.*326 **196**, 95-105 (2017).
- Bond, C.S. & Schüttelkopf, A.W. ALINE: a WYSIWYG protein-sequence alignment editor
 for publication-quality alignments. *Acta Crystallogr.* D65 510-512 (2009).