Bacillus subtilis YpeB holds SleB inactive preventing cortex peptidoglycan degradation during spore dormancy

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Plasmid constructions

pYG34[*yvbJ::sleB (cat)(amp)*] was constructed in a 2-way ligation with a PCR product containing *sleB* amplified with oCB120 and oYG121 using the gDNA of *B. subtilis 168* as template, and the plasmid pCB06 cut with EcoRI and BamHI. pCB06 is a is double crossover integration vector at the *yvbJ* locus with a *Cm* cassette.

pYG530 [*MCS-proC His-SUMO-FLAG-ypeB (amp)*] was constructed in a 2-way isothermal assembly reaction with a PCR product containing *ypeB* amplified with primers oY992 and oYG993 using gDNA of *B. subtilis* 168 as template, and the plasmid backbone derived from pLA73 (MCS-proC His-SUMO-FLAG-MCS) (1), which was restricted with BamHI and XhoI.

pYG531 [*sleB His-SUMO-FLAG-ypeB (amp)*] was constructed in a 2-way isothermal assembly reaction with a PCR product containing *sleB* amplified with primers oYG994 and oYG995 using gDNA of *B. subtilis* 168 as template, and the plasmid backbone was derived from pYG530 restricted with NcoI and NotI.

pYG532 [*MCS-proC His-SUMO-FLAG-ypeB(Bc) (amp)*] was constructed in a 2-way isothermal assembly reaction with a PCR product containing *ypeB(Bc)* amplified with primers oYG994-F and oYG995-R using gDNA of *B. cereus* as template, and the plasmid backbone was derived from pLA73 (MCS-proC His-SUMO-FLAG-MCS) (1), which was restricted with BamHI and XhoI.

pYG533 [*sleB(Bc) His-SUMO-FLAG-ypeB(Bc) (amp)*] was constructed in a 2-way isothermal assembly reaction with a PCR product containing *sleB(Bc)* amplified with primers oYG996 and oYG997 using gDNA of *B. cereus* as template, and the plasmid backbone was derived from pYG532 restricted with NcoI and NotI.

pYG547 [*SP(dsbA)-sleB(Bc) His-SUMO-FLAG-ypeB(Bc) (amp)*] was constructed in a 2-way isothermal assembly reaction with a PCR product containing *SP(dsbA)-sleB(Bc)*, in which the native signal peptide of *sleB(Bc)* was replaced with the signal peptide of *dsbA* in 3 steps: firstly the *sleB(Bc)*-1 was amplified with primers oYG997and oYG1015 using gDNA of *B. cereus* as template, and *sleB(Bc)-2* was derived from *sleB(Bc)-1* with primers oYG997 and oYG1016, and *SP(dsbA)-sleB(Bc)* was derived from *sleB(Bc)-2* with primers oYG997 and oYG1017. The plasmid backbone was derived from pYG532 with primers oYG190 and oYG1014.

pYG548 [*SP(dsbA)-sleB His-SUMO-FLAG-ypeB (amp)*] was constructed in a 2-way isothermal assembly reaction with a PCR product containing *SP(dsbA)-sleB*, in which the native signal peptide of *sleB* was replaced with the signal peptide of *dsbA* in 3 steps: firstly the *sleB-1* was amplified with primers oYG995 and oYG1018 using gDNA of *B. subtilis 168* as template, and *sleB-2* was derived from *sleB-1* with primers oYG995 and oYG1016, and *SP(dsbA)-sleB* was derived from *sleB-2* with primers oYG995 and oYG1017. The plasmid backbone was derived from pYG530 with primers oYG190 and oYG1014.

pYG549 [*SP(pelB)-sleB(Bc) His-SUMO-FLAG-ypeB(Bc) (amp)*] was constructed in a 2-way isothermal assembly reaction with a PCR product containing *SP(pelB)-sleB(Bc)*, in which the native signal peptide of *sleB(Bc)* was replaced with the signal peptide of *pelB* in 3 steps: firstly the *pelB-sleB(Bc)*-1 was amplified with primers oYG997and oYG1019 using gDNA of *B. cereus* as template, and *pelB-sleB(Bc)-2* was derived from *pelB-sleB(Bc)-1* with primers oYG997 and oYG1020, and *SP(pelB)-sleB(Bc)* was derived from *pelB-sleB(Bc)-2* with primers oYG997 and oYG1021. The plasmid backbone was derived from pYG532 with primers oYG190 and oYG1014.

pYG550 [*SP(pelB)-sleB His-SUMO-FLAG-ypeB (amp)*] was constructed in a 2-way isothermal assembly reaction with a PCR product containing *SP(pelB)-sleB*, in which the native signal peptide of *sleB* was replaced with the signal peptide of *pelB* in 3 steps: firstly the *pelB-sleB-1* was amplified with primers oYG995 and oYG1022 using gDNA of *B. subtilis 168* as template, and *pelB-sleB-2* was derived from *pelB-sleB-1* with primers oYG995 and oYG1020, and *SP(pelB)-sleB* was derived from *pelB-sleB-2* with primers oYG995 and oYG1021. The plasmid backbone was derived from pYG530 with primers oYG190 and oYG1014.

pYG566 [*SP(dsbA)-sleB-proC His-SUMO-FLAG-ypeB (amp)*] was constructed in a 2-way isothermal assembly reaction with a PCR product containing *SP(dsbA)-sleB-proC* amplified with oYG1017 and oYG1051, using pYG548 as template, and the plasmid backbone was derived from pYG530 with primers oYG1050 and oYG190.

pYG681 [*yvbJ::sleB-ypeB(spec)(amp)*] was constructed in a 2-way ligation with a PCR product containing *sleB-ypeB* amplified with oYG105 and oYG106 using the gDNA of *B. subtilis 168* as template, and the plasmid pCB43 cut with EcoRI and BamHI. pCB43 is a is double crossover integration vector at the *yvbJ* locus with a *spec* cassette.

pYG45 [*yvbJ::sleB-ypeB(cat)(amp)*] was constructed in a 2-way ligation with a PCR product containing *sleB-ypeB* amplified with oYG105 and oYG106 using the gDNA of *B. subtilis 168* as template, and the plasmid pCB06 cut with EcoRI and BamHI. pCB06 is a is double crossover integration vector at the *yvbJ* locus with a *Cm* cassette.

pYG638 [*yvbJ::sleB N190A-ypeB(cat)(amp)*] was constructed by site-directed mutagenesis using primers oYG1187 and oYG1188, and pYG45 as template.

pYG636 [*yvbJ::sleB N191A-ypeB(cat)(amp)*] was constructed by site-directed mutagenesis using primers oYG1183 and oYG1184, and pYG45 as template.

pYG689 [*yvbJ::sleB N190A N190A-ypeB(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1189 and oYG1190, and pYG681 as template.

pYG640 [*yvbJ::sleB R194A Q198A-ypeB(cat)(amp)*] was constructed by site-directed mutagenesis using primers oYG1191 and oYG1192, and pYG45 as template.

pYG683 [*yvbJ::sleB F237A-ypeB(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1175 and oYG1176, and pYG681 as template.

pYG684 [*yvbJ::sleB A181L-ypeB(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1195 and oYG1196, and pYG681 as template.

pYG685 [*yvbJ::sleB A233L-ypeB(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1179 and oYG1180, and pYG681 as template.

pYG686 [*yvbJ::sleB A181V-ypeB(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1193 and oYG1194, and pYG681 as template.

pYG687 [*yvbJ::sleB A233V-ypeB(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1181 and oYG1182, and pYG681 as template.

pYG688 [*yvbJ::sleB Y251A-ypeB(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1177 and oYG1178, and pYG681 as template.

pYG703 [*yvbJ::sleB A233L-ypeB E101K(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1268 and oYG1269, and pYG685 as template.

pYG705 [*yvbJ::sleB A181L-ypeB V87I(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1276 and oYG1277, and pYG684 as template.

pYG706 [*yvbJ::sleB A181L-ypeB V185I(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1278 and oYG1279, and pYG684 as template.

pYG707 [*yvbJ::sleB F237A-ypeB E101K(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1268 and oYG1269, and pYG683 as template.

pYG708 [*yvbJ::sleB F237A-ypeB Q143R(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1270 and oYG1271, and pYG683 as template.

pYG709 [*yvbJ::sleB F237A-ypeB V31A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1272 and oYG1273, and pYG683 as template.

pYG710 [*yvbJ::sleB F237A-ypeB H84R(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1274 and oYG1275, and pYG683 as template.

pYG711 [*yvbJ::sleB F237A-ypeB V87I(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1276 and oYG1277, and pYG683 as template.

pYG712 [*yvbJ::sleB F237A-ypeB V185I(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1278 and oYG1279, and pYG683 as template.

pYG535 [*yhdG::sleB-ypeB(spec)(amp)*] was constructed in a 2-way ligation with a PCR product containing *sleB-ypeB* amplified with oYG105 and oYG106 using the gDNA of *B. subtilis 168* as template, and the plasmid pCB33 cut with EcoRI and BamHI. pCB33 is a is double crossover integration vector at the *yhdG* locus with a *spec* cassette.

pYG731 [*yhdG::sleB-ypeB H44A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1319 and oYG1320, and pYG535 as template.

pYG732 [*yhdG::sleB-ypeB E45A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1321 and oYG1322, and pYG535 as template.

pYG553 [*yhdG::sleB-ypeB H44A E45A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1026 and oYG1027, and pYG535 as template.

pYG556 [*yhdG::sleB-ypeB R147E(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1032 and oYG1033, and pYG535 as template.

pYG757 [*yhdG::sleB-ypeB M154A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1360 and oYG1361, and pYG535 as template.

pYG758 [*yhdG::sleB-ypeB N157A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1362 and oYG1363, and pYG535 as template.

pYG557 [*yhdG::sleB-ypeB M154A N157A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1034 and oYG1035, and pYG535 as template.

pYG752 [*yhdG::sleB-ypeB Y280A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1350 and oYG1351, and pYG535 as template.

pYG753 [*yhdG::sleB-ypeB N284A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1352 and oYG1353, and pYG535 as template.

pYG559 [*yhdG::sleB-ypeB Y280A N284A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1038 and oYG1039, and pYG535 as template.

pYG736 [*yhdG::sleB-ypeB Y280A I282A N284A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1329 and oYG1330, and pYG559 as template.

pYG733 [*yhdG::sleB-ypeB I282A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1323 and oYG1324, and pYG535 as template.

pYG734 [*yhdG::sleB-ypeB T249A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1325 and oYG1326, and pYG535 as template.

pYG751 [*yhdG::sleB-ypeB K210A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1335 and oYG1336, and pYG535 as template.

pYG742 [*yhdG::sleB-ypeB K210A T249A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1325 and oYG1326, and pYG751 as template.

pYG735 [*yhdG::sleB-ypeB Y323A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1327 and oYG1328, and pYG535 as template.

pYG739 [*yhdG::sleB-ypeB I446A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1331 and oYG1332, and pYG535 as template.

pYG740 [*yhdG::sleB-ypeB Y323A I446A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1331 and oYG1332, and pYG735 as template.

pYG759 [*yhdG::sleB-ypeB T93A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1364 and oYG1365, and pYG535 as template.

pYG760 [*yhdG::sleB-ypeB L94A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1366 and oYG1367, and pYG535 as template.

pYG741 [*yhdG::sleB-ypeB T93A L94A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1333 and oYG1334, and pYG535 as template.

pYG754 [*yhdG::sleB-ypeB E444A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1354 and oYG1355, and pYG535 as template.

pYG755 [*yhdG::sleB-ypeB E444R(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1356 and oYG1357, and pYG535 as template.

pYG767 [*yhdG::P_{sleB}-(HindIII-XhoI)-ypeB R147E (amp)*] was constructed in a 2-way isothermal assembly reaction with a PCR product containing *P_{sleB}-(HindIII-XhoI)* amplified with oCB63 and oYG999, using pYG556 as template, and the plasmid backbone was also derived from pYG556 with primers oYG998 and oYG1002.

pYG768 [*yhdG::P_{sleB}-(HindIII-XhoI)-ypeB E444R (amp)*] was constructed in a 2-way isothermal assembly reaction with a PCR product containing *P_{sleB}-(HindIII-XhoI)* amplified with oCB63 and oYG999, using pYG755 as template, and the plasmid backbone was also derived from pYG755 with primers oYG998 and oYG1002.

pYG779 [*yhdG::sleB N267I-ypeB R147E(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1407 and oYG1408, and pYG556 as template.

pYG780 [*yhdG::sleB D192V-ypeB E444R(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1409 and oYG1410, and pYG755 as template.

pYG783 [*yhdG::sleB N267I-ypeB E45K(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1407 and oYG1408, and pYG554 as template.

pYG784 [*yhdG::sleB D192V-ypeB E45K(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1409 and oYG1410, and pYG554 as template.

pYG796 [*yhdG::sleB D192V-ypeB M154A N157A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1409 and oYG1410, and pYG557 as template.

pYG799 [*yhdG::sleB N267I-ypeB M154A N157A(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1407 and oYG1408, and pYG557 as template.

pYG802 [*yhdG::sleB D192V-ypeB(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1409 and oYG1410, and pYG535 as template.

pYG803 [*yhdG::sleB N267I-ypeB(spec)(amp)*] was constructed by site-directed mutagenesis using primers oYG1407 and oYG1408, and pYG535 as template.

All plasmids were confirmed by Sanger sequencing.

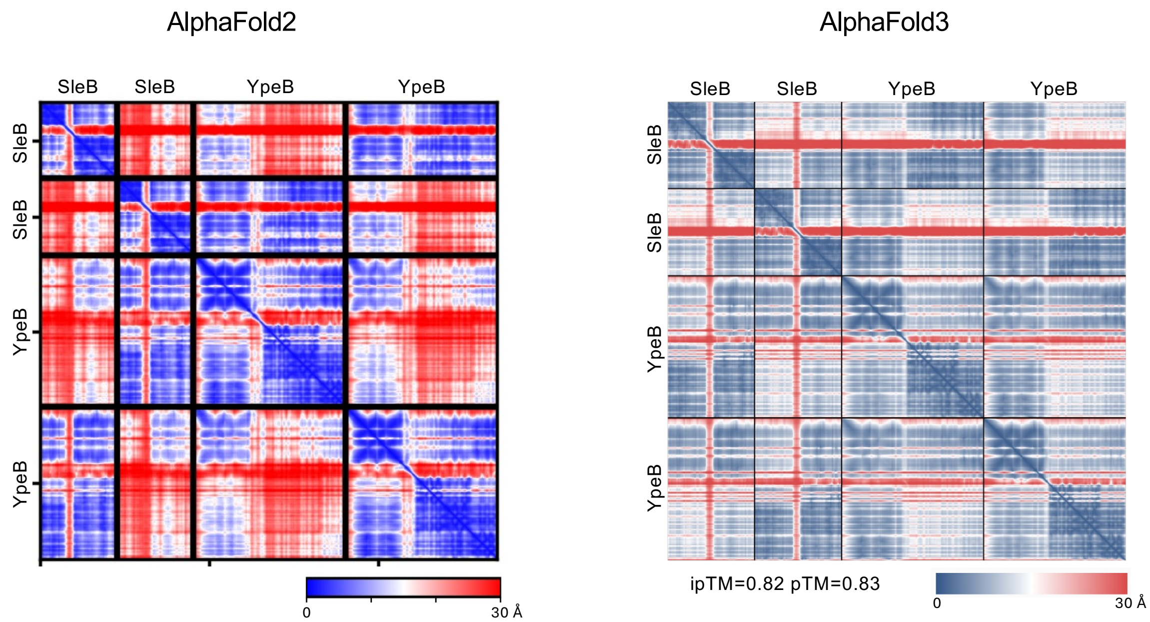

Figure S1. Predicted alignment error (pAE) plots for $SleB_2$-$YpeB_2$ AlphaFold-Multimer predictions. Plots of the predicted alignment error (pAE) in Å of all residues against all residues for the top-ranked AlphaFold2 and AlphaFold3 models for the $SleB_2$-$YpeB_2$ tetramer. Low error (blue) corresponds to well-defined relative domain positions. The interface predicted template modeling score (ipTM) and the template modeling score (pTM) for the AlphaFold3 model are shown below the plot.

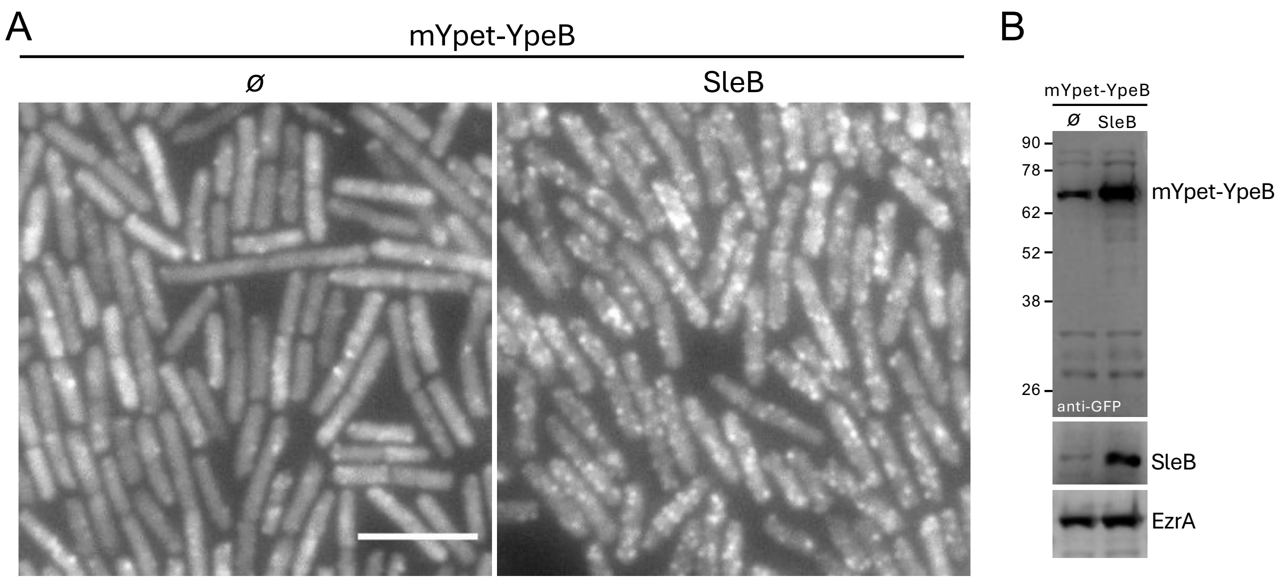

Figure S2. SleB expression stabilizes YpeB and promotes mYpet-YpeB fluorescent foci when expressed during growth. (A) Representative fluorescence images of exponentially growing *B. subtilis* cells expressing mYpet-YpeB in the absence (ø) or presence of SleB. The mYpet-YpeB fusion was expressed under the control of a xylose-regulated promoter (P_{xylA}) with 3.3 mM xylose, and SleB was expressed under the control of an IPTG-regulated promoter ($P_{hyperspank}$) with 1 mM IPTG. Scale bar indicates 5 µm. **(B)** Representative immunoblot of lysates from the strains in (A). mYpet-YpeB and SleB were detected with anti-GFP and anti-SleB antibodies. EzrA controls for loading. Molecular weight standards (in kDa) are shown on the left.

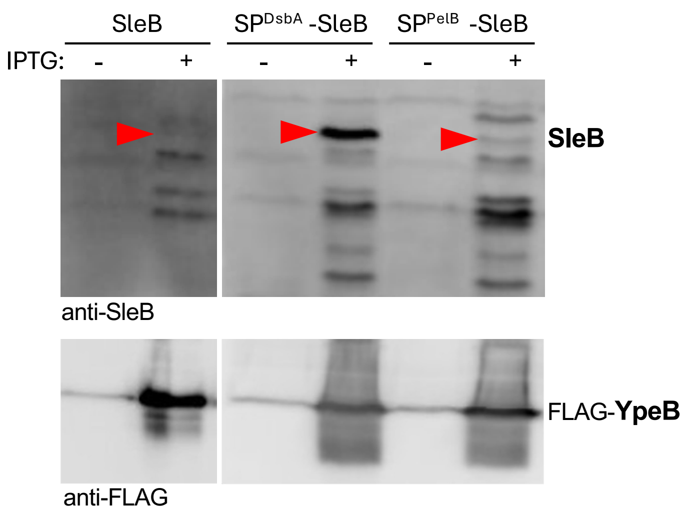

Figure S3. *E. coli* expression of SleB is enhance when its native signal peptide is replaced with *E. coli*'s DsbA signal peptide. Representative immunoblots of SleB and FLAG-YpeB co-expressed in *E. coli* with 0.5 mM IPTG. Cells expressing wild-type *sleB* and *sleB* variants with the signal peptide (SP) from *E. coli* DsbA and from *Erwinia carotovora* PelB are shown. The band corresponding to SleB is indicated (red caret).

Figure S4
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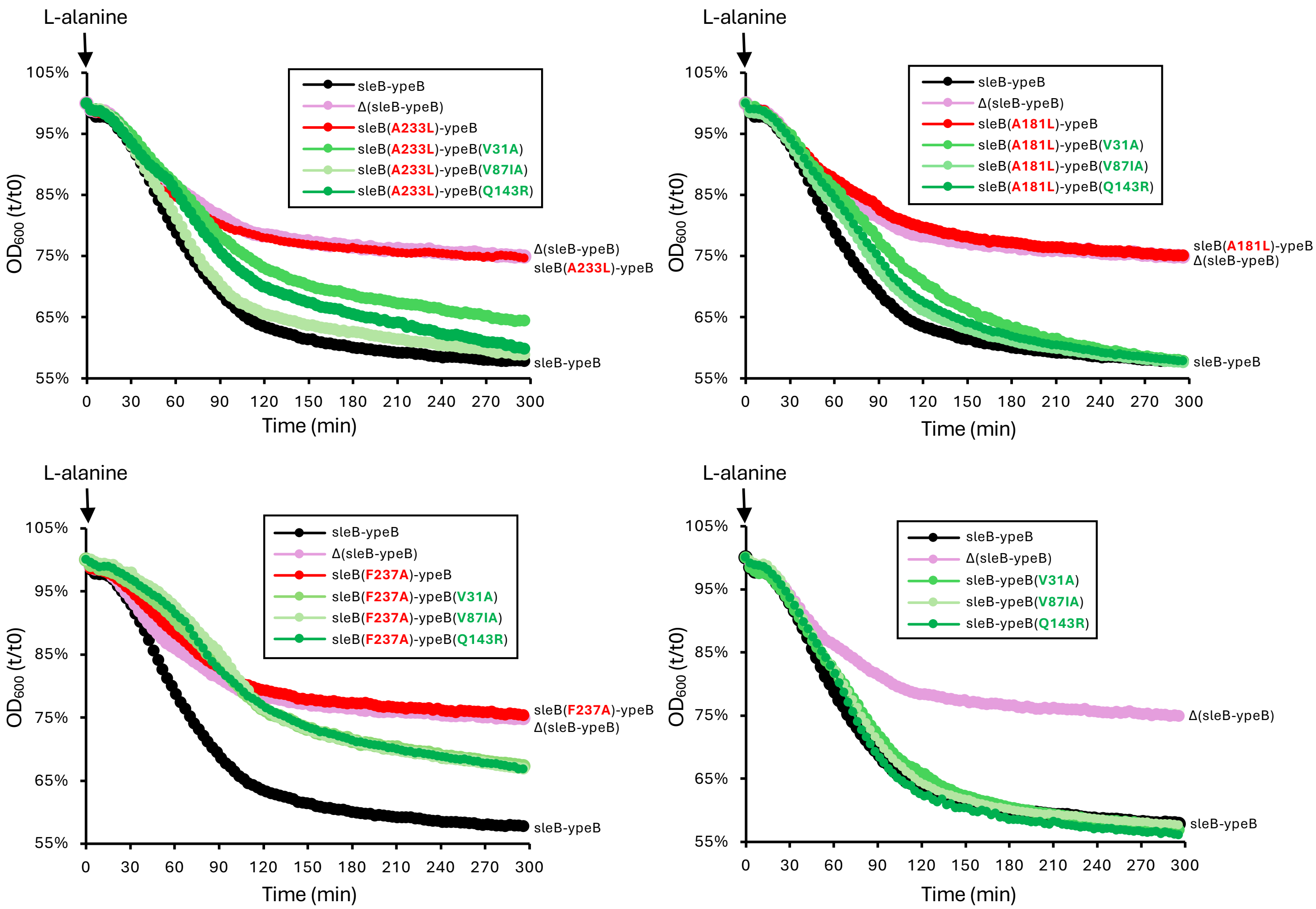

Figure S4. YpeB suppressors mutants are germination-competent. Representative graphs of spore germination in response to L-alanine as assayed by a reduction in optical density (OD_{600}). Histodenz-purified phase-bright spores of the indicated strains lacking *cwlJ*, were induced to germinate with 10 mM L-alanine, and OD_{600} was monitored over time. The data are plotted as the percent reduction in OD_{600} relative to time 0.

Figure S5
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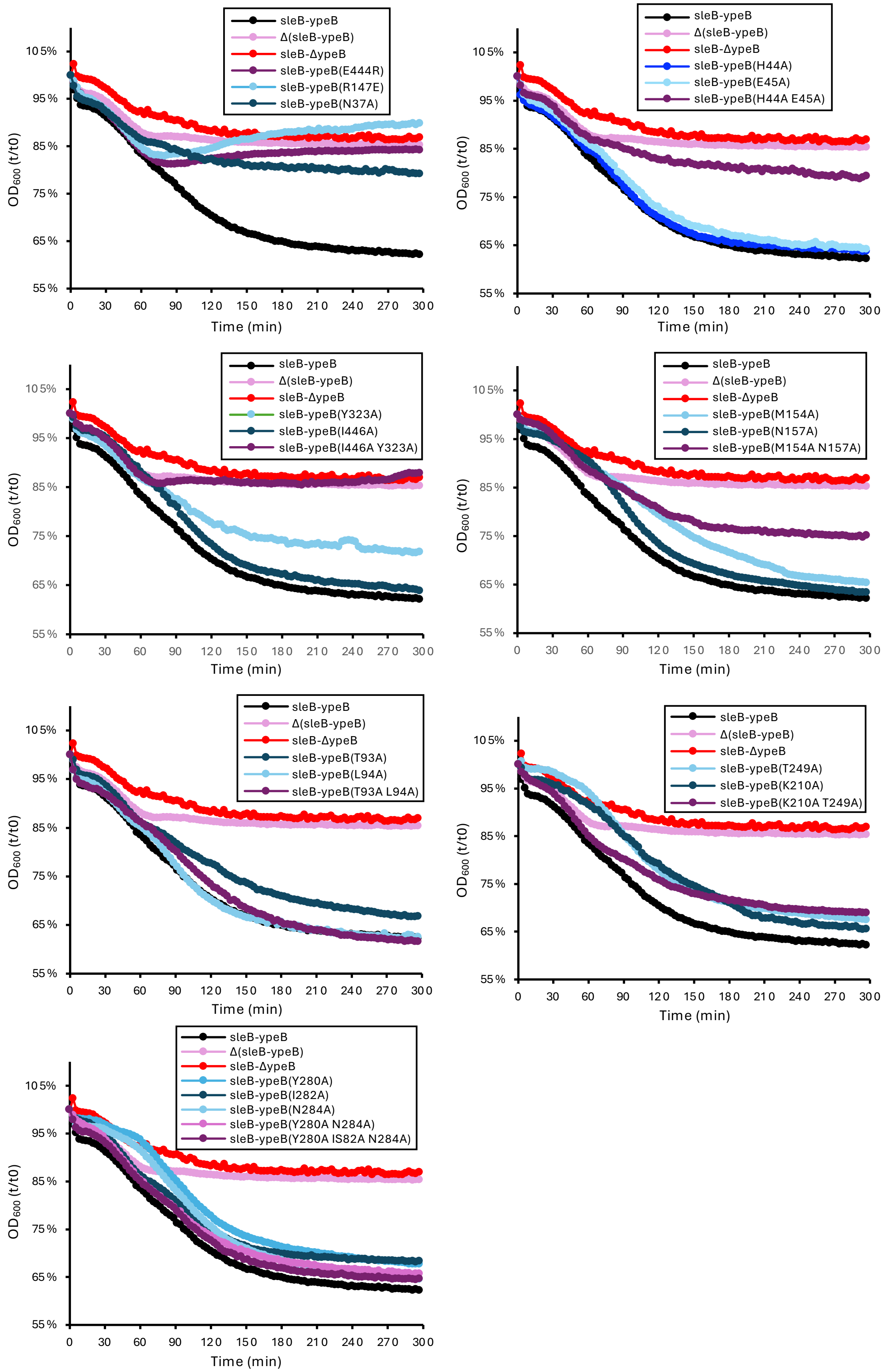
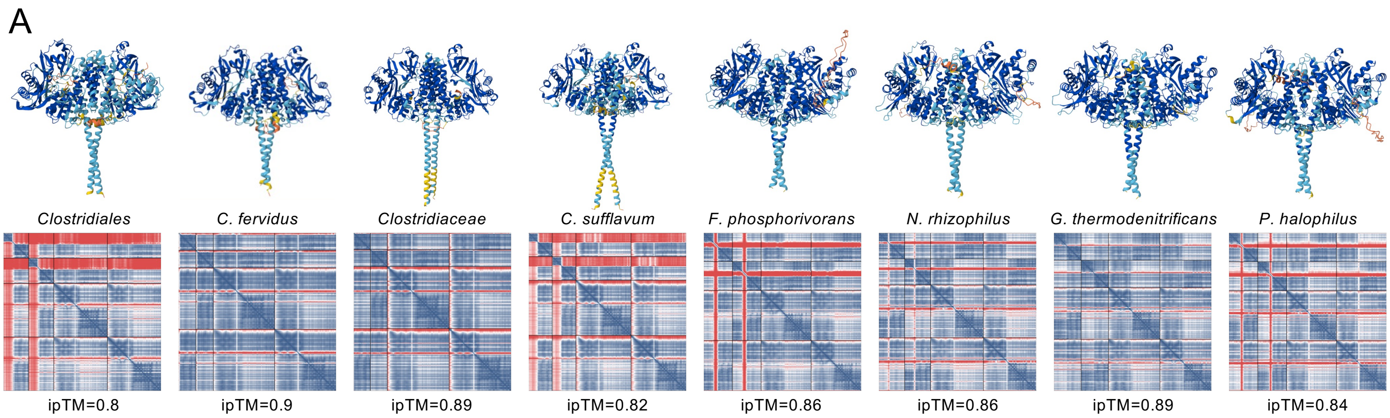


Figure S5. Analysis of spore germination of the YpeB mutants. Representative graphs of spore germination in response to L-alanine as assayed by a reduction in optical density (OD_{600}). Histodenz-purified phase-bright spores of the indicated strains lacking *cwlJ*, were induced to germinate with 10 mM L-alanine, and the OD_{600} was monitored over time. The data are plotted as the percent reduction in OD_{600} relative to time 0. In two cases, *ypeB*(M154A, N157A) and *ypeB*(K210A, T249A), the germination defects were more modest than expected based on the sporulation efficiencies in Figure 3B. Spores used for germination were generated on sporulation agar plates at 30˚C while the sporulation efficiency was determined from cells sporulated in liquid sporulation medium at 37˚C, potentially explaining the discrepancies.



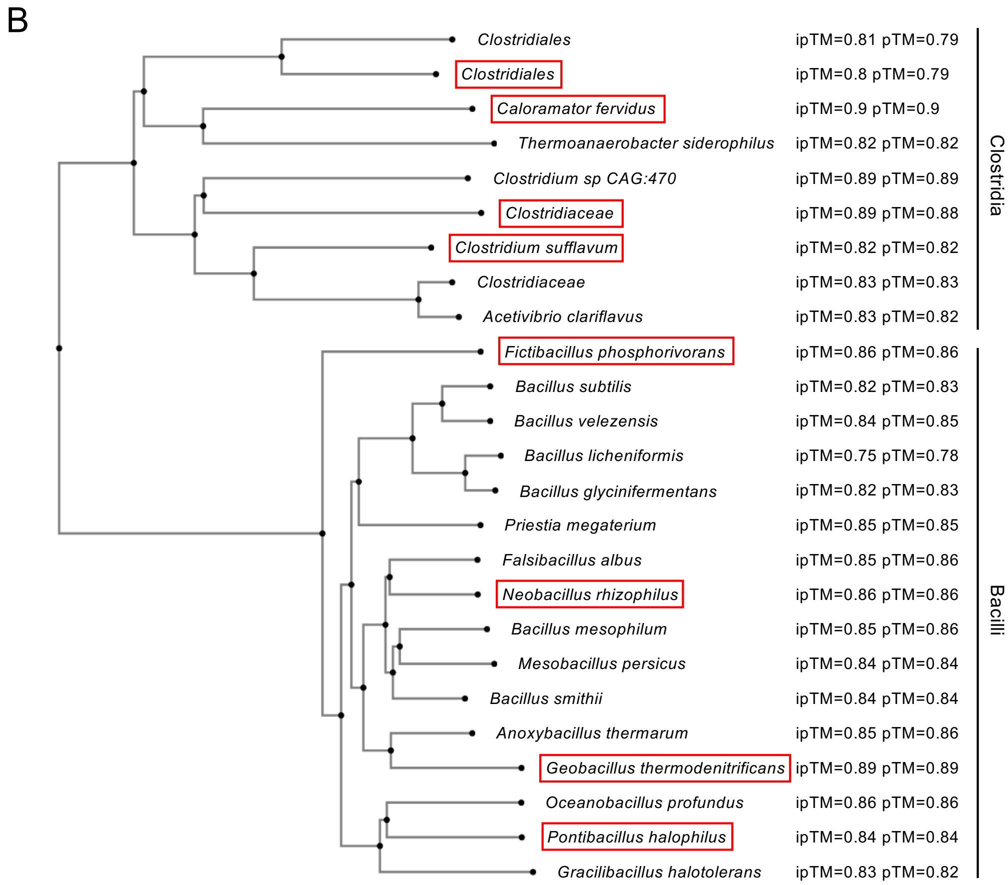


Figure S6. The structure of the *B. subtilis* YpeB$_2$-SleB$_2$ complex is likely conserved across diverse endospore formers in the Bacillota. (A) AlphaFold3-predicted structures of YpeB$_2$-SleB$_2$ tetrameric complexes from 8 endospore forming bacteria. The models are colored based based on the predicted local distance difference test (pLDDT), a per-residue measure of local confidence. The higher confidence scores are dark blue. Plots of the predicted alignment error (pAE) in Å of all residues against all residues for the top-ranked models are shown below. Low error (blue) corresponds to well-defined relative domain positions. The interface predicted template modeling score (ipTM) for each model is shown below the pAE plot. **(B)** Phylogenetic tree of spore-forming Clostridia and Bacilli for which a YpeB$_2$-SleB$_2$ tetrameric complex was predicted by AlphaFold3. The ipTM and template modeling (pTM) score for the top model is indicated to the right of the species. Uniprot IDs for all proteins analyzed can be found in Table S4. The predicted structures and pAE plots of the YpeB$_2$-SleB$_2$ complexes for the species boxed in red are shown in (A).

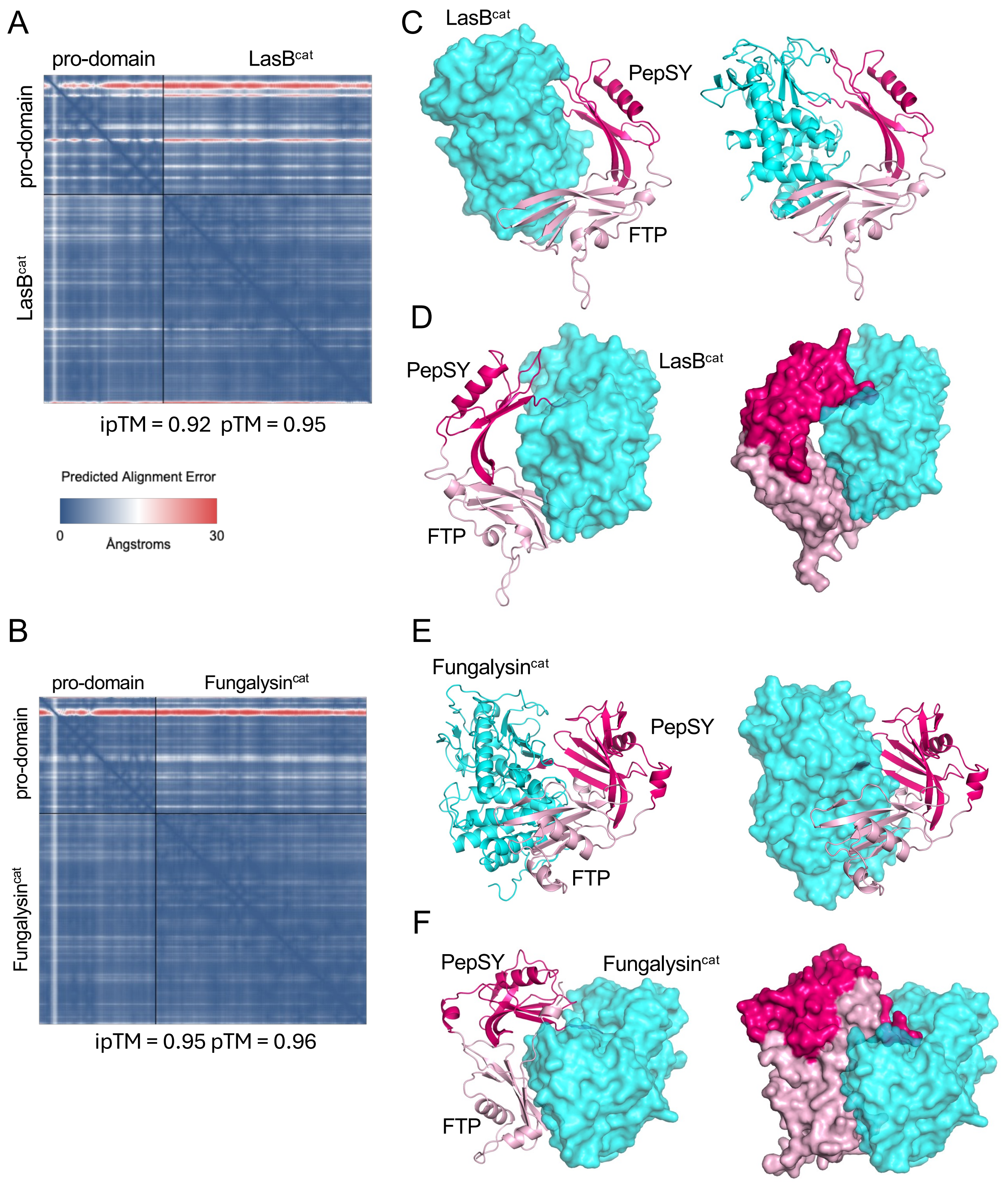


Figure S7. LasB and Fungalysin pro-domains composed of PepSY and FTP domains embrace their catalytic domains. (A-B) Plots of the predicted alignment error (pAE) in Å of the AlphaFold3 model of LasB and Fungalysin and their cleaved pro-domains. **(C)** Predicted structure of LasB's catalytic domain (LasB^{cat}) and its cleaved pro-domain. The PepSY domain (dark pink), FTP domain (light pink), and LasB^{cat} (cyan) are rendered as ribbons and surface. **(D)** A rotated view of the predicted complex highlighting the "embrace" of the catalytic domain by both PepSY and FTP domains. **(E)** Predicted structure of Fugalysin's catalytic domain (Fungalysin^{cat}) and its cleaved pro-domain. The PepSY domain (dark pink), FTP domain (light pink), and Fungalysin^{cat} (cyan) are rendered as ribbons and surface. **(D)** A rotated view of the predicted complex highlighting the "embrace" of the catalytic domain by both PepSY and FTP domains.

Figure S8
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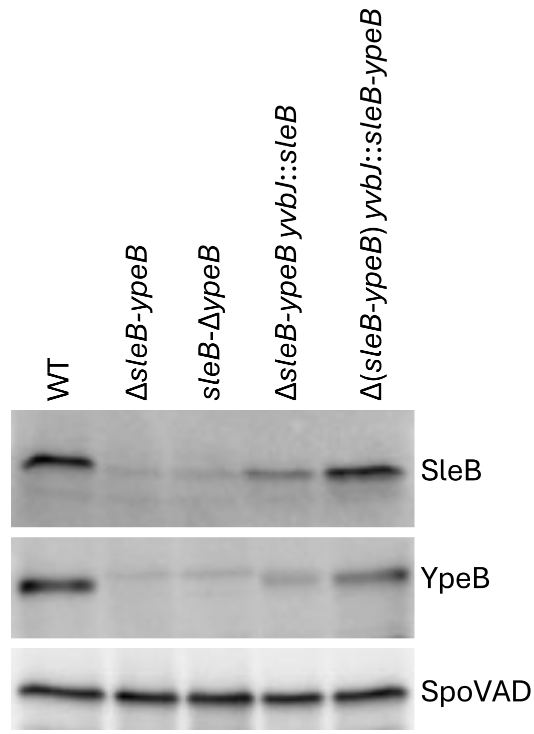

Figure S8. The levels of SleB and YpeB in dormant spores are influenced by the presence of the two genes in a single operon. Representative immunoblots of SleB and YpeB from spore lysates of the indicated strains. SpoVAD controls for loading.

Supplemental Table 1. Strains used in this study

Strains	Genotype	Source	Figures
bDR2414	*Bacillus subtilis 168 (trpC2) "wild-type"*	(2)	1, S1
bdr4178	*ΔypeB::erm*	This work	S8
bYG02	*ΔsleB::lox72*	This work	S8
bYG1073	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A181L)-ypeB(E101K) spec*	This work	2
bYG1074	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A181L)-ypeB(V31A) spec*	This work	2, S4
bYG1075	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A181L)-ypeB(Q143R) spec*	This work	2, S4
bYG1076	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A233L)-ypeB(V31A) spec*	This work	2, S4
bYG1077	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A233L)-ypeB(H84R) spec*	This work	2
bYG1078	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A233L)-ypeB(V87I) spec*	This work	2, S4
bYG1079	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A233L)-ypeB(Q143R) spec*	This work	2, S4
bYG1081	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A233L)-ypeB(V185I) spec*	This work	2
bYG1086	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB-ypeB(Q143R) spec*	This work	S4
bYG1087	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB-ypeB(V31A) spec*	This work	S4
bYG1089	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB-ypeB(V87I) spec*	This work	S4
bYG1091	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(F237A)-ypeB(E101K) spec*	This work	2
bYG1092	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(F237A)-ypeB(Q143R) spec*	This work	2, S4
bYG1093	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(F237A)-ypeB(V31A) spec*	This work	2, S4
bYG1094	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(F237A)-ypeB(H84R) spec*	This work	2
bYG1095	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(F237A)-ypeB(V87I) spec*	This work	2, S4
bYG1109	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(F237A)-ypeB(V185I) spec*	This work	2
bYG1110	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A181L)-ypeB(V87I) spec*	This work	2, S4
bYG1111	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A181L)-ypeB(V185I) spec*	This work	2
bYG1112	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A233L)-ypeB(E101K) spec*	This work	2
bYG153	*ΔsleB::lox72 yvbJ::sleB Cm*	This work	S8
bYG159	*Δ(sleB-ypeB)::lox72 yvbJ::sleB-ypeB cat*	This work	S8
bYG1590	*Δ(sleB-ypeB)::erm yvbJ::Pxyl-mYpet-ypeB cat*	This work	S2
bYG1619	*Δ(sleB-ypeB)::erm yvbJ::Pxyl-mYpet-ypeB cat amyE::Phyperspank-sleB spec*	This work	S2
bYG1700	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::kan*	This work	3, 4, S5
bYG1704	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(H44A E45A) spec*	This work	3, S5
bYG1707	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(R147E) spec*	This work	3, 4, S5
bYG1708	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(M154A N157A) spec*	This work	3, S5
bYG1710	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(Y280A N284A) spec*	This work	3, S5
bYG1714	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB-ypeB spec*	This work	2, 3, 4, S4, S5
bYG1864	*ΔcwlJ::lox72*	This work	2, 4
bYG1977	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(R194A Q198A)-ypeB Cm*	This work	2
bYG1978	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::Kan*	This work	2, S4
bYG2000	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A181V)-ypeB spec*	This work	2
bYG2001	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A233V)-ypeB spec*	This work	2
bYG2002	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(Y251A)-ypeB spec*	This work	2
bYG2003	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(N190A N191A)-ypeB spec*	This work	2
bYG2006	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(N191A)-ypeB Cm*	This work	2
bYG2045	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(F237A)-ypeB spec*	This work	2, S4
bYG2046	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A181L)-ypeB spec*	This work	2, S4
bYG2047	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(A233L)-ypeB spec*	This work	2, S4
bYG2070	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yvbJ::sleB(N190A)-ypeB Cm*	This work	2
bYG2182	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(N37A) spec*	This work	3, S5
bYG2184	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(H44A) spec*	This work	3, S5
bYG2185	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(E45A) spec*	This work	3, S5

bYG2187	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(I282A) spec*	This work	3, S5
bYG2188	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(T93A L94A) spec*	This work	3, S5
bYG2189	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(I446A) spec*	This work	3, S5
bYG2190	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(T249A) spec*	This work	3, S5
bYG2192	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(Y280A I282A N284A) spec*	This work	3, S5
bYG2201	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(Y323A) spec*	This work	3, S5
bYG2207	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(I446A Y323A) spec*	This work	3, S5
bYG2208	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(T249A K210A) spec*	This work	3, S5
bYG2252	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(K210A) spec*	This work	3, S5
bYG2253	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(Y280A) spec*	This work	3, S5
bYG2254	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(N284A) spec*	This work	3, S5
bYG2256	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(E444R) spec*	This work	3, 4, S5
bYG2258	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(M154A) spec*	This work	3, S5
bYG2259	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(N157A) spec*	This work	3, S5
bYG2260	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(T93A) spec*	This work	3, S5
bYG2261	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB-ypeB(L94A) spec*	This work	3, S5
bYG2327	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB(N267I)-ypeB(E444R) spec*	This work	4
bYG2348	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB(D192V)-ypeB(R147E) spec*	This work	4
bYG2377	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB(N267I)-ypeB(E45K) spec*	This work	4
bYG2378	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB(D192V)-ypeB(E45K) spec*	This work	4
bYG2381	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB(N267I)-ypeB(R147E) spec*	This work	4
bYG2386	*ΔcwlJ::lox72 ΔypeB::tet*	This work	3, 4, S5
bYG2405	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB(D192V)-ypeB(M154A N157A) spec*	This work	4,
bYG2411	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB(D192V)-ypeB(E444R) spec*	This work	4
bYG2412	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB(N267I)-ypeB spec*	This work	4
bYG2414	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB(N267I)-ypeB(M154A N157A) spec*	This work	4
bYG2415	*ΔcwlJ::erm Δ(sleB-ypeB)::lox72 yhdG::sleB(D192V)-ypeB spec*	This work	4
bYG64	*ΔcwlJ::lox72 ΔsleB::lox72*	This work	2, 4

Supplemental Table 2. Plasmids used in this study.

Plasmid	Genotype	Source
pLA73	*MCS-proC His-SUMO-FLAG-MCS (amp)*	(1)
pAM174	*arabinose-inducible Ulp1[L403-K621] protease (cat)*	(3)
pCB43	*yvbJ::spec (amp)*	laboratory stock
pCB06	*yvbJ::cat (amp)*	laboratory stock
pCB33	*yhdG::spec (amp)*	laboratory stock
pYG34	*yvbJ::sleB (Cm)(amp)*	This work
pYG530	*MCS-proC His-SUMO-FLAG-ypeB (amp)*	This work
pYG531	*sleB His-SUMO-FLAG-ypeB (amp)*	This work
pYG532	*MCS-proC His-SUMO-FLAG-ypeB(Bc) (amp)*	This work
pYG533	*sleB(Bc) His-SUMO-FLAG-ypeB(Bc) (amp)*	This work
pYG547	*SP(dsbA)-sleB(Bc) His-SUMO-FLAG-ypeB(Bc) (amp)*	This work
pYG548	*SP(dsbA)-sleB(Bc) His-SUMO-FLAG-ypeB(Bc) (amp)*	This work
pYG549	*SP(pelB)-sleB(Bc) His-SUMO-FLAG-ypeB(Bc) (amp)*	This work
pYG550	*SP(pelB)-sleB His-SUMO-FLAG-ypeB (amp)*	This work
pYG566	*SP(dsbA)-sleB-proC His-SUMO-FLAG-ypeB (amp)*	This work
pYG681	*yvbJ::sleB-ypeB(spec)(amp)*	This work
pYG45	*yvbJ::sleB-ypeB(cat)(amp)*	This work
pYG638	*yvbJ::sleB N190A-ypeB(cat)(amp)*	This work
pYG636	*yvbJ::sleB N191A-ypeB(cat)(amp)*	This work
pYG689	*yvbJ::sleB N190A N190A-ypeB(spec)(amp)*	This work
pYG640	*yvbJ::sleB R194A Q198A-ypeB(cat)(amp)*	This work
pYG683	*yvbJ::sleB F237A-ypeB(spec)(amp)*	This work
pYG684	*yvbJ::sleB A181L-ypeB(spec)(amp)*	This work
pYG685	*yvbJ::sleB A233L-ypeB(spec)(amp)*	This work
pYG686	*yvbJ::sleB A181V-ypeB(spec)(amp)*	This work
pYG687	*yvbJ::sleB A233V-ypeB(spec)(amp)*	This work
pYG688	*yvbJ::sleB Y251A-ypeB(spec)(amp)*	This work
pYG703	*yvbJ::sleB A233L-ypeB E101K(spec)(amp)*	This work
pYG705	*yvbJ::sleB A181L-ypeB V87I(spec)(amp)*	This work
pYG706	*yvbJ::sleB A181L-ypeB V185I(spec)(amp)*	This work
pYG707	*yvbJ::sleB F237A-ypeB E101K(spec)(amp)*	This work
pYG708	*yvbJ::sleB F237A-ypeB Q143R(spec)(amp)*	This work
pYG709	*yvbJ::sleB F237A-ypeB V31A(spec)(amp)*	This work
pYG710	*yvbJ::sleB F237A-ypeB H84R(spec)(amp)*	This work
pYG711	*yvbJ::sleB F237A-ypeB V87I(spec)(amp)*	This work
pYG712	*yvbJ::sleB F237A-ypeB V185I(spec)(amp)*	This work
pYG535	*yhdG::sleB-ypeB(spec)(amp)*	This work
pYG731	*yhdG::sleB-ypeB H44A(spec)(amp)*	This work
pYG732	*yhdG::sleB-ypeB E45A(spec)(amp)*	This work
pYG553	*yhdG::sleB-ypeB H44A E45A(spec)(amp)*	This work
pYG556	*yhdG::sleB-ypeB R147E(spec)(amp)*	This work
pYG757	*yhdG::sleB-ypeB M154A(spec)(amp)*	This work
pYG758	*yhdG::sleB-ypeB N157A(spec)(amp)*	This work
pYG557	*yhdG::sleB-ypeB M154A N157A(spec)(amp)*	This work
pYG752	*yhdG::sleB-ypeB Y280A(spec)(amp)*	This work
pYG753	*yhdG::sleB-ypeB N284A(spec)(amp)*	This work
pYG559	*yhdG::sleB-ypeB Y280A N284A(spec)(amp)*	This work
pYG736	*yhdG::sleB-ypeB Y280A I282A N284A(spec)(amp)*	This work

pYG733	*yhdG::sleB-ypeB I282A(spec)(amp)*	This work
pYG734	*yhdG::sleB-ypeB T249A(spec)(amp)*	This work
pYG751	*yhdG::sleB-ypeB K210A(spec)(amp)*	This work
pYG742	*yhdG::sleB-ypeB K210A T249A(spec)(amp)*	This work
pYG735	*yhdG::sleB-ypeB Y323A(spec)(amp)*	This work
pYG739	*yhdG::sleB-ypeB I446A(spec)(amp)*	This work
pYG740	*yhdG::sleB-ypeB Y323A I446A(spec)(amp)*	This work
pYG759	*yhdG::sleB-ypeB T93A(spec)(amp)*	This work
pYG760	*yhdG::sleB-ypeB L94A(spec)(amp)*	This work
pYG741	*yhdG::sleB-ypeB T93A L94A(spec)(amp)*	This work
pYG754	*yhdG::sleB-ypeB E444A(spec)(amp)*	This work
pYG755	*yhdG::sleB-ypeB E444R(spec)(amp)*	This work
pYG767	*yhdG::*P_{sleB}*-(HindIII-XhoI)-ypeB R147E (amp)*	This work
pYG768	*yhdG::PsleB-(HindIII-XhoI)-ypeB E444R (amp)*	This work
pYG779	*yhdG::sleB N267I-ypeB R147E(spec)(amp)*	This work
pYG780	*yhdG::sleB D192V-ypeB E444R(spec)(amp)*	This work
pYG783	*yhdG::sleB N267I-ypeB E45K(spec)(amp)*	This work
pYG784	*yhdG::sleB D192V-ypeB E45K(spec)(amp)*	This work
pYG796	*yhdG::sleB D192V-ypeB M154A N157A(spec)(amp)*	This work
pYG799	*yhdG::sleB N267I-ypeB M154A N157A(spec)(amp)*	This work
pYG802	*yhdG::sleB D192V-ypeB(spec)(amp)*	This work
pYG803	*yhdG::sleB N267I-ypeB(spec)(amp)*	This work

Supplemental Table 3. Oligonucleotides used in this study.

oligo	sequence	use
oCB63	gactggtttttgctcagacaa	pYG767, pYG768
oCB120	cgcGAATTCGGTGGTTTGGTTTGCGTAAAGCG	pYG34
oCB121	cgcGGATCCTCTGATCATTTATCACACCTC	pYG34
oYG1002	ttgtctgagcaaaaaccagtc	pYG767, pYG768
oYG1014	gcggccgcataatgcttaagtcgaacag	pYG547, pYG548, pYG549, pYG550
oYG1015	GCTGGTTTAGTTTTAGCGTTTAGCGCATCGGCGGCAttttctaatcaagtcattcaaag	pYG547
oYG1016	atgAAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAG	pYG547, pYG548
oYG1017	ctttaagaaggagatataccatggatgAAAAAGATTTGGCTGGCGCT	pYG547, pYG548, pYG566
oYG1018	GCTGGTTTAGTTTTAGCGTTTAGCGCATCGGCGGCATTTTCGAATCAGGTCATTCAAAG	pYG548
oYG1019	CTGCTGCTCCTCGCTGCCCAGCCGGCGATGGCCGCAttttctaatcaagtcattcaaag	pYG549
oYG1020	atgAAATACCTGCTGCCGACCGCTGCTGCTGGTCTGCTGCTCCTCGCTGCCCAG	pYG549, pYG550
oYG1021	ctttaagaaggagatataccatggatgAAATACCTGCTGCCGACCGCT	pYG549, pYG550
oYG1022	CTGCTGCTCCTCGCTGCCCAGCCGGCGATGGCCGCATTTTCGAATCAGGTCATTCAAAG	pYG550
oYG1026	GAAAATAACTATCAGCGGGCGTTTGCTGCGCTTACCTATCAGGTGGATCAGCT	pYG553
oYG1027	AGCTGATCCACCTGATAGGTAAGCGCAGCAAACGCCCGCTGATAGTTATTTTC	pYG553
oYG1032	CAGTCCGAAGATATACAAAATGAATTGGAACATGTTCAGCACCTTGTCATGAG	pYG556
oYG1033	CTCATGACAAGGTGCTGAACATGTTCCAATTCATTTTGTATATCTTCGGACTG	pYG556
oYG1034	TGCGTCATGTTCAGCACCTTGTCGCGAGCAAAGCCCTTCGCTGGATGGACGTAGAAATG	pYG557
oYG1035	CATTTCTACGTCCATCCAGCGAAGGGCTTTGCTCGCGACAAGGTGCTGAACATGACGCA	pYG557
oYG1038	AGAAGGGCGGGCATCCGGTAGCCTTGATCCAAGCCAGAGAAGTGAAAGATCAGAAAATC	pYG559
oYG1039	GATTTTCTGATCTTTCACTTCTCTGGCTTGGATCAAGGCTACCGGATGCCCGCCCTTCT	pYG559
oYG105	ctggcGAATTCAAGTGGCTTACTCTTTCTTTAGTTG	pYG681, pYG45, pYG535
oYG1050	ctctggcggaagcggaggatccACTAGTGAAGACCAAGTAGATCCGCGGCTC	pYG566
oYG1051	ggatcctccgcttccgccagagcctccCTCACAGAAAATGTGTTTACCGATTC	pYG566
oYG106	cagcgGGATCCCACGTTTTTATTAAAGCACGTAATG	pYG681, pYG45, pYG535
oYG1175	CAAATTCAGTAGCGGGGGTTATTGCTGAGCCGCTTGCCTTCACAGCA	pYG683
oYG1176	TGCTGTGAAGGCAAGCGGCTCAGCAATAACCCCCGCTACTGAATTTG	pYG683
oYG1177	CAGCAGTAGCCGACGGACAAATTGCCATGCAGCCGAATGAAACGGCAC	pYG688
oYG1178	GTGCCGTTTCATTCGGCTGCATGGCAATTTGTCCGTCGGCTACTGCTG	pYG688
oYG1179	AGCCCGTTATTTCCAAATTCAGTACTGGGGGTTATTTTTGAGCCGCTTG	pYG685
oYG1180	CAAGCGGCTCAAAAATAACCCCCAGTACTGAATTTGGAAATAACGGGCT	pYG685
oYG1181	AGCCCGTTATTTCCAAATTCAGTAGTGGGGGTTATTTTTGAGCCGCTTG	pYG687
oYG1182	CAAGCGGCTCAAAAATAACCCCCACTACTGAATTTGGAAATAACGGGCT	pYG687
oYG1183	GAACATGCCTGGTGGATTTTCCAACGCCGATATCAGGCTGCTTGCTCAAG	pYG636
oYG1184	CTTGAGCAAGCAGCCTGATATCGGCGTTGGAAAATCCACCAGGCATGTTC	pYG636
oYG1187	AGCGAACATGCCTGGTGGATTTTCCGCCAACGATATCAGGCTGCTTGCTC	pYG638

oYG1188	GAGCAAGCAGCCTGATATCGTTGGCGGAAAATCCACCAGGCATGTTCGCT	pYG638
oYG1189	GAACATGCCTGGTGGATTTTCCGCCGCCGATATCAGGCTGCTTGCTCAAG	pYG689
oYG1190	CTTGAGCAAGCAGCCTGATATCGGCGGCGGAAAATCCACCAGGCATGTTC	pYG689
oYG1191	TGGTGGATTTTCCAACAACGATATCGCGCTGCTTGCTGCAGCGGTTTATGGCGAAGCCC	pYG640
oYG1192	GGGCTTCGCCATAAACCGCTGCAGCAAGCAGCGCGATATCGTTGTTGGAAAATCCACCA	pYG640
oYG1193	AGCAAGAAAACAGGATGCGGTAGTAGCGAACATGCCTGGTGGATTTTC	pYG686
oYG1194	GAAAATCCACCAGGCATGTTCGCTACTACCGCATCCTGTTTTCTTGCT	pYG686
oYG1195	AGCAAGAAAACAGGATGCGGTACTAGCGAACATGCCTGGTGGATTTTC	pYG684
oYG1196	GAAAATCCACCAGGCATGTTCGCTAGTACCGCATCCTGTTTTCTTGCT	pYG684
oYG1268	ACATTAATGCCGTTTAATAAAACTAAAGAGCTATTATCAAAGATCGGCGA	pYG703, pYG707
oYG1269	TCGCCGATCTTTGATAATAGCTCTTTAGTTTTATTAAACGGCATTAATGT	pYG703, pYG707
oYG1270	CTATATCAGCAGTCCGAAGATATACGAAATGAATTGCGTCATGTTCAG	pYG708
oYG1271	CTGAACATGACGCAATTCATTTCGTATATCTTCGGACTGCTGATATAG	pYG708
oYG1272	GAACACCAGGAAAAAGACGCAGCTCTTCTTCATGCTGAAAATAACTATC	pYG709
oYG1273	GATAGTTATTTTCAGCATGAAGAAGAGCTGCGTCTTTTTCCTGGTGTTC	pYG709
oYG1274	GTGGAGGATTACATCAGAAGCTCGTAACAGCGTCAGTCAGCTGCCGCT	pYG710
oYG1275	AGCGGCAGCTGACTGACGCTGTTACGAGCTTCTGATGTAATCCTCCAC	pYG710
oYG1276	ACATCAGAAGCTCATAACAGCATCAGTCAGCTGCCGCTTACATTAATG	pYG705, pYG711
oYG1277	CATTAATGTAAGCGGCAGCTGACTGATGCTGTTATGAGCTTCTGATGT	pYG705, pYG711
oYG1278	ACGATTATCAACAGCTTTAAAACAATCGAAAAAAATGTTGGTGCATTC	pYG706, pYG712
oYG1279	GAATGCACCAACATTTTTTTCGATTGTTTTAAAGCTGTTGATAATCGT	pYG706, pYG712
oYG1319	GAAAATAACTATCAGCGGGCGTTTGCTGAGCTTACCTATCAGGTGGA	pYG731
oYG1320	TCCACCTGATAGGTAAGCTCAGCAAACGCCCGCTGATAGTTATTTTC	pYG731
oYG1321	ACTATCAGCGGGCGTTTCATGCGCTTACCTATCAGGTGGATCAGC	pYG732
oYG1322	GCTGATCCACCTGATAGGTAAGCGCATGAAACGCCCGCTGATAGT	pYG732
oYG1323	AGGGCGGGCATCCGGTATACTTGGCCCAAAACAGAGAAGTGAAAGATC	pYG733
oYG1324	GATCTTTCACTTCTCTGTTTTGGGCCAAGTATACCGGATGCCCGCCCT	pYG733
oYG1325	AAGTGGTAAAGAGCGGAAAAAAAGCAAATCGCGATGTATATAGCA	pYG734, pYG742
oYG1326	TGCTATATACATCGCGATTTGCTTTTTTTCCGCTCTTTACCACTT	pYG734, pYG742
oYG1327	GGAAATTGATGAAAGTGCCCAAGCTGATAAAATCGGTGTATTTTCATATG	pYG735
oYG1328	CATATGAAAATACACCGATTTTATCAGCTTGGGCACTTTCATCAATTTCC	pYG735
oYG1329	AGGGCGGGCATCCGGTAGCCTTGGCCCAAGCCAGAGAAGTGAAAGATC	pYG736
oYG1330	GATCTTTCACTTCTCTGGCTTGGGCCAAGGCTACCGGATGCCCGCCCT	pYG736
oYG1331	GAAAAACTAAAAAATGCAGAACCTGCATATAAAGACCTATAAAAACAGGAG	pYG739, pYG740
oYG1332	CTCCTGTTTTTATAGGTCTTTATATGCAGGTTCTGCATTTTTTAGTTTTTC	pYG739, pYG740
oYG1333	CAGCGTCAGTCAGCTGCCGCTTGCAGCAATGCCGTTTAATAAAACTGAAGA	pYG741
oYG1334	TCTTCAGTTTTATTAAACGGCATTGCTGCAAGCGGCAGCTGACTGACGCTG	pYG741
oYG1335	CACCAGTACGAAAAAAGAAGAGGCAGGCTTCAGCCATCTGAAGGGA	pYG751
oYG1336	TCCCTTCAGATGGCTGAAGCCTGCCTCTTCTTTTTTCGTACTGGTG	pYG751

oYG1350	AGAAGGGCGGGCATCCGGTAGCCTTGATCCAAAACAGAGAAGT	pYG752
oYG1351	ACTTCTCTGTTTTGGATCAAGGCTACCGGATGCCCGCCCTTCT	pYG752
oYG1352	CATCCGGTATACTTGATCCAAGCCAGAGAAGTGAAAGATCAGAAAATC	pYG753
oYG1353	GATTTTCTGATCTTTCACTTCTCTGGCTTGGATCAAGTATACCGGATG	pYG753
oYG1356	GTTGAAAAACTAAAAAATGCACGACCTATATATAAAGACCTATAAAAAC	pYG755
oYG1357	GTTTTTATAGGTCTTTATATATAGGTCGTGCATTTTTTAGTTTTTCAAC	pYG755
oYG1360	GTCATGTTCAGCACCTTGTCGCGAGCAAAAACCTTCGCTGGA	pYG757
oYG1361	TCCAGCGAAGGTTTTTGCTCGCGACAAGGTGCTGAACATGAC	pYG757
oYG1362	AGCACCTTGTCATGAGCAAAGCCCTTCGCTGGATGGACGTAGA	pYG758
oYG1363	TCTACGTCCATCCAGCGAAGGGCTTTGCTCATGACAAGGTGCT	pYG758
oYG1364	AGCGTCAGTCAGCTGCCGCTTGCATTAATGCCGTTTAATAAAAC	pYG759
oYG1365	GTTTTATTAAACGGCATTAATGCAAGCGGCAGCTGACTGACGCT	pYG759
oYG1366	GTCAGTCAGCTGCCGCTTACAGCAATGCCGTTTAATAAAACTGA	pYG760
oYG1367	TCAGTTTTATTAAACGGCATTGCTGTAAGCGGCAGCTGACTGAC	pYG760
oYG1407	AGCAGTGCTGGATGCCATCATTGGCTGGGACCCATCAGAGGAAG	pYG779, pYG783, pYG799, pYG803
oYG1408	CTTCCTCTGATGGGTCCCAGCCAATGATGGCATCCAGCACTGCT	pYG779, pYG783, pYG799, pYG803
oYG1409	CTGGTGGATTTTCCAACAACGTTATCAGGCTGCTTGCTCAAGC	pYG780, pYG784, pYG796, pYG802
oYG1410	GCTTGAGCAAGCAGCCTGATAACGTTGTTGGAAAATCCACCAG	pYG780, pYG784, pYG796, pYG802
oYG190	CATCCATGgtatatctccttcttaaagttaaac	pYG547, pYG548, pYG549, pYG550, pYG556
oYG992	GGTGTTGTTTCAAGGCCCTGGGgGATCCATCAGAGGAATTTTAATCGCCGTGCTTG	pYG530
oYG993	gcagcggtttctttaccagacTCGAGTTATAGGTCTTTATATATAGGTTCTGCA	pYG530
oYG994	ttgtttaactttaagaaggagatataccatggATGAAGTCCAAAGGATCGATTATGGCA	pYG531
oYG994-F	GGTGTTGTTTCAAGGCCCTGGGgGATCCttacgaggtattatcattgtattattaac	pYG532
oYG995	ctgttcgacttaagcattatgcggccgcCTACTCACAGAAAATGTGTTTACCGATTC	pYG531, pYG548, pYG550
oYG995-R	gcagcggtttctttaccagacTCGAGttaatcataaattttttcaacagcttgca	pYG532
oYG996	tgtttaactttaagaaggagatataccatggatgcgccaaaaagctatttttaaaatag	pYG533
oYG997	ctgttcgacttaagcattatgcggccgcctatttacagaaaatatgtttaccga	pYG533, pYG547, pYG549
oYG998	AGAAGCTTatACTAGTgtCTCGAGATATGAGAAAGCATAAAAAGAGGTGTGA	pYG767, pYG768
oYG999	CTCGAGacACTAGTatAAGCTTCTTTTCAAGCCTCCTACTGCAAATTTTTAAG	pYG767, pYG768

Table S4
Proteins analyzed in Figure S6

Bacillus licheniformis (strain ATCC 14580)	SleB: Q65I05_BACLD	YpeB: Q65I06_BACLD
Falsibacillus albus	SleB: A0A3L7JW42_9BACI	YpeB: A0A3L7JW36_9BACI
Anoxybacillus thermarum	SleB: A0A0D0S0X8_9BACL	YpeB: A0A0D0RSH9_9BACL
Priestia megaterium (strain ATCC 14581)	SleB: A0A0B6ABB4_PRIM2	YpeB: A0A0B6AYZ4_PRIM2
Geobacillus thermodenitrificans (strain NG80-2)	SleB: A4IQB2_GEOTN	YpeB: A4IQB1_GEOTN
Bacillus glycinifermentans	SleB: A0A0T6BTH1_9BACI	YpeB: A0A0T6BTG2_9BACI
Fictibacillus phosphorivorans	SleB: A0A160IMZ9_9BACL	YpeB: A0A160IPR8_9BACL
Oceanobacillus profundus	SleB: A0A417YFT7_9BACI	YpeB: A0A417YFT1_9BACI
Neobacillus rhizophilus	SleB: A0A942UAT4_9BACI	YpeB: A0A942UBT1_9BACI
Pontibacillus halophilus (JSM 076056)	SleB: A0A0A5GJL4_9BACI	YpeB: A0A0A5GLS1_9BACI
Mesobacillus persicus	SleB: A0A1H8BSK3_9BACI	YpeB: A0A1H8BSK2_9BACI
Bacillus mesophilum	SleB: A0A7V7RMU9_9BACI	YpeB: A0A7V7UYQ1_9BACI
Gracilibacillus halotolerans	SleB: A0A841RGC4_9BACI	YpeB: A0A841RCJ7_9BACI
Bacillus smithii (7_3_47FAA)	SleB: N0AXJ5	YpeB: N0B254
Clostridiales bacterium	SleB: A0A972A6X9	YpeB: A0A971VA40
Clostridium sp. (CAG:470)	SleB: R7FH44	YpeB: R7FHP6
Caloramator fervidus	SleB: A0A1H5RLY6_9CLOT	YpeB: A0A1H5RK67_9CLOT
Thermoanaerobacter siderophilus SR4	SleB: I8QYE3_9THEO	YpeB: I9ADH5_9THEO
Clostridiales bacterium	SleB: A0A971ZAI8	YpeB: A0A971ZAP5
Clostridiaceae bacterium	SleB: A0A970JCK8	YpeB: A0A970JCX8
Clostridium sufflavum (DSM 19573)	SleB: A0A318XME9_9FIRM	YpeB: A0A318XPE2_9FIRM
Clostridiaceae bacterium	SleB: A0A7X8JVJ4	YpeB: A0A7X8JVG8
Acetivibrio clariflavus (strain DSM 19732)	SleB: G8LZE9_ACECE	YpeB: G8LZF0_ACECE
Bacillus velezensis (strain DSM 23117)	SleB: A7Z643_BACVZ	YpeB: A7Z642_BACVZ

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