Supplemental material for

The PASTA domains of *Bacillus subtilis* PBP2B strengthen the interaction of PBP2B with DivIB

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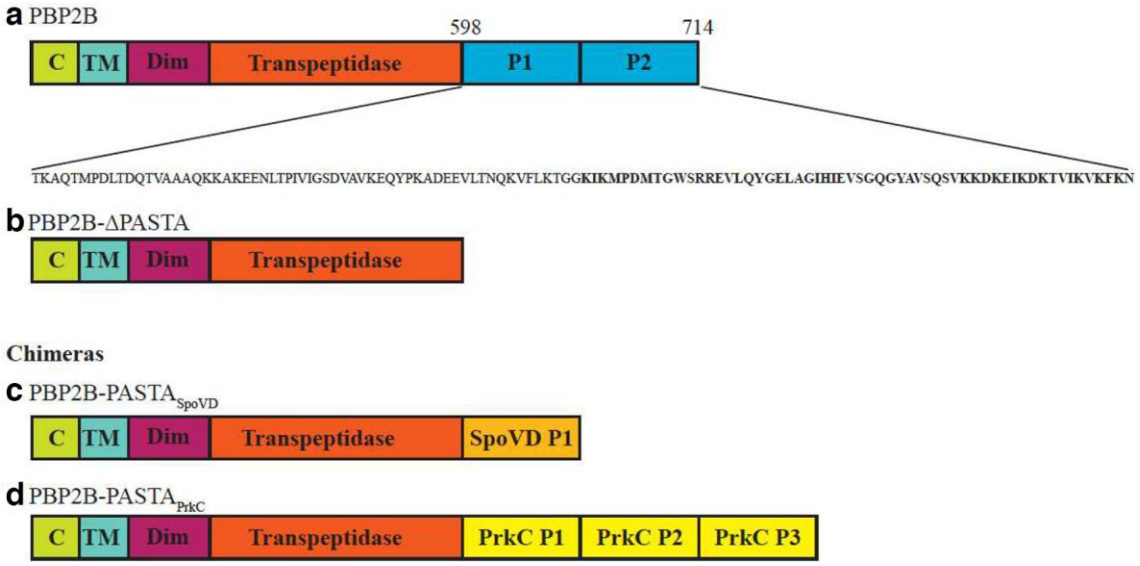

Figure S1. PBP2B variants used in this study. a) PBP2B b) PBP2B-ΔPASTA c) PBP2B-PASTA$_{SpoVD}$ d) PBP2- PASTA$_{PrkC}$. C: cytoplasmic domain, TM: transmembrane, Dim: dimerization domain, P: PASTA domain.

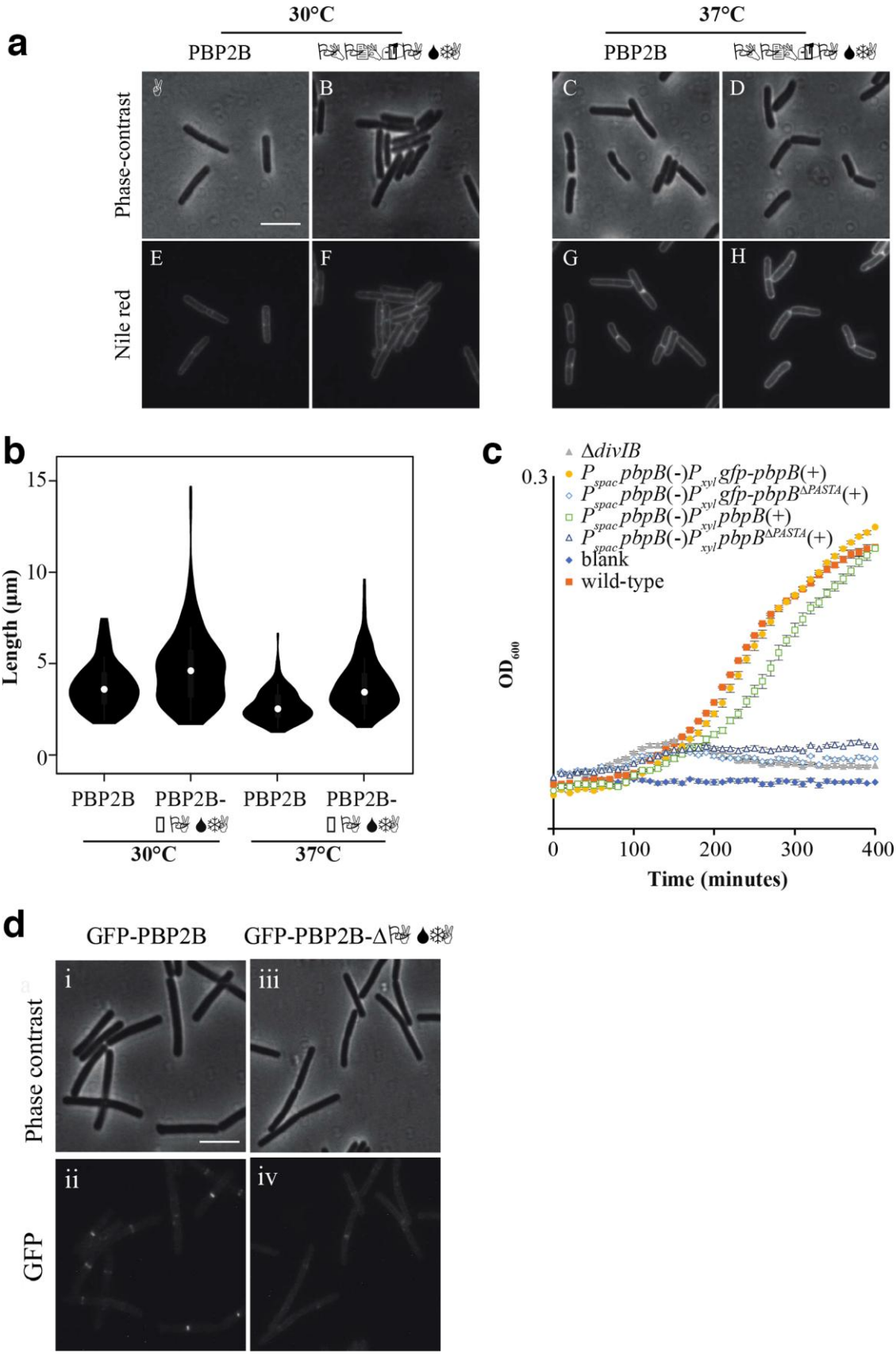

Figure S2. Strains grown in SM medium and localization of GFP-fusions. a) Cells were grown at 30 or 37°C until exponential phase. Membranes were stained using Nile red. Representative results from three independent experiments are shown. Scale bar: 5 µm. b)

Length of PBP2B strains grown on SM medium. Cells were grown in SM medium at 30 or 37°C. As *B. subtilis* forms chains, cells were labelled with Nile red in order to determine the boundaries of single cells. Length of cells was obtained by automated image analysis. The values obtained ($n = 200$ per strain) are shown as box plots. White circles show the medians (P_{xyl} *pbpB* 30°C =3.59 µm, P_{xyl} $pbpB^{\Delta PASTA}$ 30°C=4.61 µm, P_{xyl} *pbpB* 37°C =2.53 µm, P_{xyl} $pbpB^{\Delta PASTA}$ 37°C =3.45 µm); box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles; polygons represent density estimates of data and extend to extreme values. c) Growth curves in SM medium at 48°C, OD_{600} was measured every 10 min. Genotypes are shown and presence (+) or absence (-) of inducer is indicated. (♦) SM medium, blank, (■) wild type (strain 168), (▲) Δ*divIB* (strain Δ*divIB*), (●) P_{spac} *pbpB*(-) P_{xyl} *gfp-pbpB*(+) (strain 4132), (◊) P_{spac} *pbpB*(-) P_{xyl} *gfp-*$pbpB^{\Delta PASTA}$(+) (strain 4133), (□) P_{spac} *pbpB*(-) P_{xyl} *pbpB*(+) (strain 4137) (△) P_{spac} *pbpB*(-) P_{xyl} $pbpB^{\Delta PASTA}$(+) (strain 4138). Representative results from three independent experiments are shown. All experiments were performed in triplicates. The resulting average and standard error are shown for each time point. d) Phase-contrast (i, iii) and fluorescence (ii, iv) microscopy of the strains producing GFP-PBP2B (strain 4132; i, ii) and GFP-PBP2B-ΔPASTA (strain 4133; iii, iv) strains. Cultures were grown in CH-medium at 30°C until exponential phase. Representative results from three independent experiments are shown. Scale bar: 5 µm.

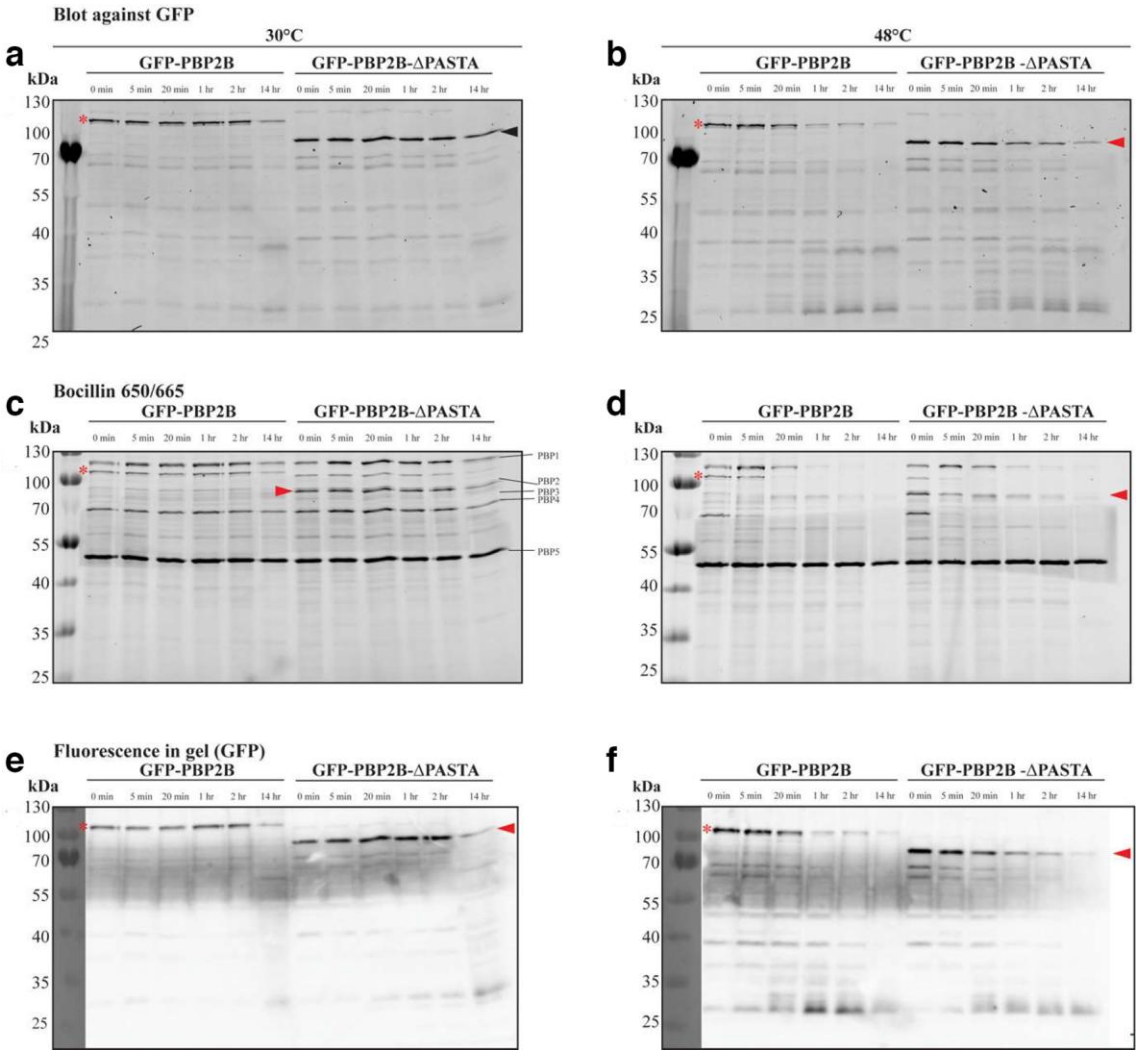

Figure S3. PBP2B and PBP2B-ΔPASTA stability. Membrane proteins of GFP-PBP2B and GFP-PBP2B-ΔPASTA strains were isolated from cells incubated at 30°C. Membranes were incubated at 30°C (a, c, e) or 48°C (b, d, f) at different time points, and then incubated with Bocillin 650/665. GFP was followed by fluorescence in gel (a, b) and western blot using anti-GFP antibodies (e, f). Effects on folding were followed by the ability of Bocillin 650/665 to bind to PBPs (c, d). The star indicates the position of GFP-PBP2B and the triangle the position of GFP-PBP2B-ΔPASTA. Representative results from three independent experiments are shown.

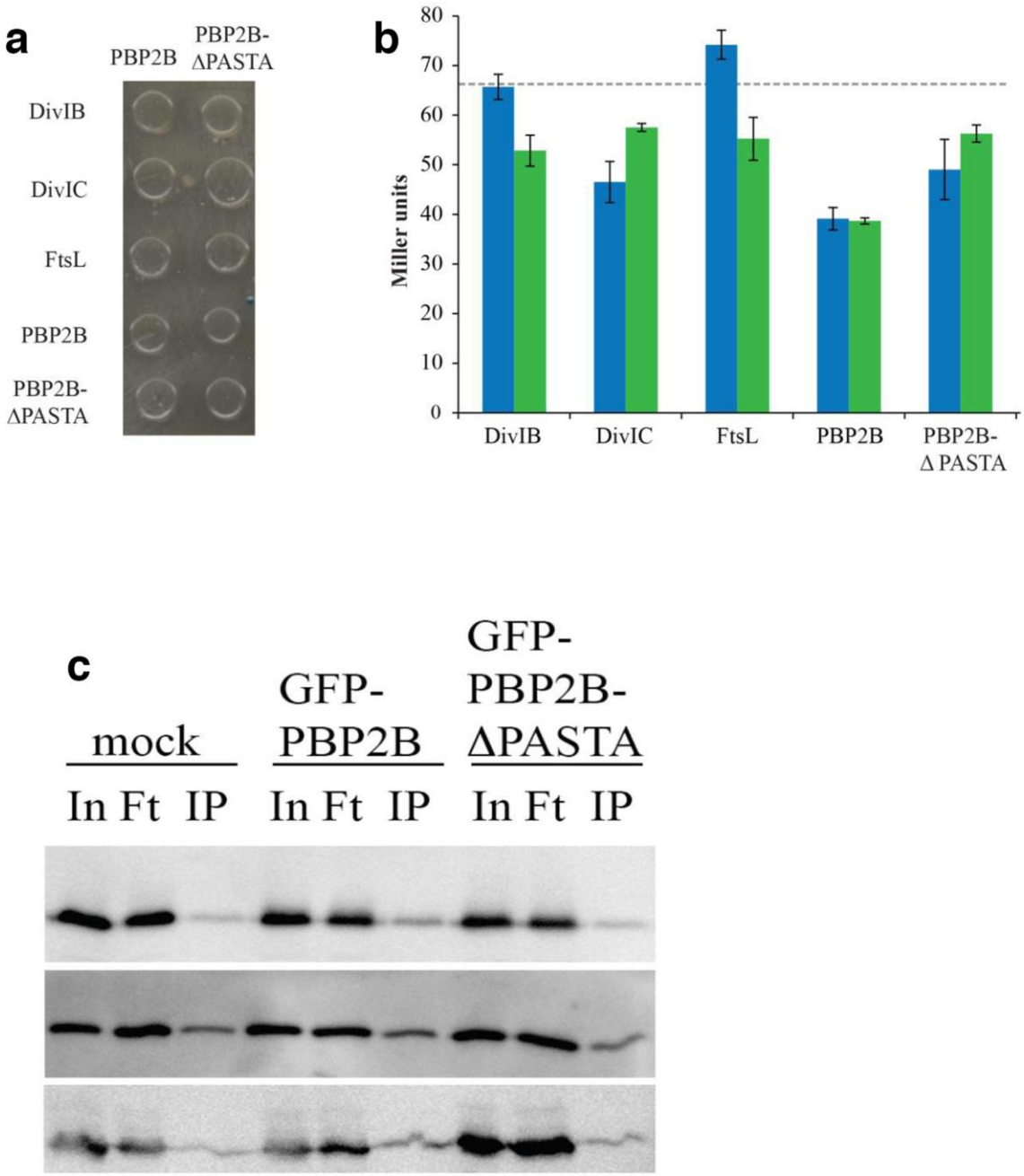

Figure S4. Bacterial two-hybrid and co-Immunoprecipitation controls. a) Bacterial two hybrid interaction assay on plates containing X-Gal. PBP2B, PBP2BΔ-PASTA, DivIB, DivIC and FtsL were cloned into plasmids pKT25 and pUT18C and co-transformed into *E. coli* BTH101. Co-transformants were grown on LB plates containing X-Gal and incubated at 30°C for 36 hrs. Blue colonies are considered indicative of protein-protein interaction. PBP2B and PBP2B-ΔPASTA were used as bait in pUT18C, while prey proteins in pKT25. Representative results from three independent experiments are shown. b) Bacterial two hybrid β-galactosidase assay. Interaction between the fusion of PBP2B and PBP2B-ΔPASTA cloned into pUT18C in combination with the fusion of late division protein cloned into pKT25 Positive control showed an activity of 63278 Miller units and the negative control 66 (shown as dotted line). Representative results from three independent experiments are shown. All experiments were performed in triplicates. The resulting average and standard error are shown for each interaction. c) Western blots of three separate biological replicate co-Immunoprecipitation experiments. Solubilised membranes from strains producing DivIB-

FLAG (mock) or DivIB-FLAG and GFP-PBP2B (GFP-PBP2B) or DivIB-FLAG and GFP-PBP2B-ΔPASTA (GFP-PBP2B-ΔPASTA) were immunoprecipitated using GFP-Trap agarose beads. Input (In), flow-through (Ft) and eluted (IP) material was analysed by SDS-PAGE/Western Blot and the blots were developed using anti-FLAG antibodies. These blots were used to determine the ratio of immunoprecipitated over unbound material in Fig. 4D.

Table S1. List of primers used in this study

Primer	Sequence	Source
PrkC PASTA fw	**ACCAACTGAAAAATCTGACTCAGATAAGGAAGAA**ATG CCTAAG GATGTCAAAATACCT	This work
PrkC PASTA rv	**ATCGATACCGTCGACCCTCGAG**TTAGAGAGAGAATGTC ACTTCA ACT	This work
SpoVD PASTA fw	**ACCAACTGAAAAATCTGACTCAGATAAGGAA**GAAGAC ACAAAA ACAATAGAAGTTCCGA	This work
SpoVD PASTA rv	**ATCGATACCGTCGACCCTCGAG**TTATTCAGTCAAATAC ACGCGT ATC	This work
b2h_divIBfw	GGAGGATCTAGAGATGAACCCGGGTCAAGACC	This work
b2h_divIBrev	GGATTCGGTACCCCCTCAATTTTCATCTTCC	This work
b2h_divICfw	GGAGGATCTAGAGATGAATTTTTCCAGGGAACGA	This work
b2h_divICrev	GGATTCGGTACCGGCTACTTGCTCTTCTTCTCC	This work
b2h_ftsLfw	GGAGGATCTAGAGATGAGCAATTTAGCTTACCAACC	This work
b2h_ftsLrev	GGATTCGGTACCTCATTCCTGTATGTTTTTCAC	This work
b2h_pbpBfw	GGAGGATCTAGAGATGATTCAAATGCCAAAAAAG	This work
b2h_pbpbBrev	GGATTCGGTACCTTAATCAGGATTTTTAAACTTAACCTTG	This work
b2h_pbpbBdPrev	GGATTCGGTACCTTATTCTTCCTTATCTGAGTCAG	This work

* Bold letters correspond to the original plasmid sequence

Table S2. Average of length (µm) of wild-type, PBP2B and PBP2B-ΔPASTA strains under different conditions. Cells were grown in CH or SM medium at 30 or 37°C. As *B. subtilis* forms chains, cells were labelled with Nile red in order to determine the boundaries of single cells. Length of cells was obtained by automated image analysis. The values obtained ($n = 200$ per strain) were averaged. The standard deviation is presented between brackets.

	CH medium		SM medium	
	30°C	37°C	30°C	37°C
wildtype	2.96 (±0.70)	3.14 (±0.84)	1.85 (±0.44)	1.78 (±0.48)
P_{xyl} *pbpB*	3.34 (±0.81)	3.12 (±0.73)	3.78 (±1.28)	2.68 (±0.83)
P_{xyl} *pbpB*$^{\Delta PASTA}$	4.85 (±1.39)	5.20 (±1.81)	4.71 (±1.97)	3.79 (±1.40)
P_{xyl} *pbpB*^{PASTASpoVD}	3.57 (±0.94)	ND	ND	ND
P_{xyl} *pbpB*^{PASTAPrkC}	4.55 (±1.69)	ND	ND	ND

ND- not determined

Table S3. Mann-Whitney U test. Strain lengths were compared to determine if there was statistical difference (n = 200, p < 0.05)

Strains	Conditions	*U*	*p*< 0.05 two-tailed
P_{xyl} *pbpB* vs P_{xyl} $pbpB^{\Delta PASTA}$	CH-medium 30°C	6706.5	0
P_{xyl} *pbpB* vs $pbpB^{\Delta PASTA}$	CH-medium 37°C	4676.5	0
P_{xyl} *pbpB* vs $pbpB^{\Delta PASTA}$	SM-medium 30°C	9321.5	0
P_{xyl} *pbpB* vs $pbpB^{\Delta PASTA}$	SM-medium 37°C	9572.5	0
$pbpB^{\Delta PASTA}$ vs P_{xyl} $pbpB^{PASTASpoVD}$	CH-medium 30°C	8832	0
$pbpB^{\Delta PASTA}$ vs P_{xyl} $pbpB^{PASTAPrkC}$	CH-medium 30°C	16933.5	0.008
P_{xyl} $pbpB^{PASTASpoVD}$ vs P_{xyl} $pbpB^{PASTAPrkC}$	CH-medium 30°C	12156	0