SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. **Cell wall hydrolytic activity of CwlP-SLT, CwlP-M23, CwlP-SLTM23 and full-length CwlP.** SDS gels (lanes M, C, 1, 2 and 3) and zymogram gels (lane 4) containing 14% polyacrylamide. Lane M: protein standard; lane C in panel D: whole cell extract from *Escherichia coli* M15 (no plasmid, control). Lanes 1 and 2: whole cell extracts from *E. coli* JM109 (pQE-SLT) (panel A); *E. coli* JM109 (pQE-M23) (panel B); *E. coli* JM109 (pQE-SLTM23) (panel C); and *E. coli* M15 (pQE-YomI-FL) (panel D), without (lane 1) or with (lane 2) over-expression of the proteins. Lanes 3 and 4: purified protein and zymography, respectively, for CwlP-SLT (panel A), CwlP-M23 (panel B), CwlP-SLTM23 (panel C) and full-length CwlP (panel D). To renature the proteins for the zymography experiments, gels were incubated at 37°C for 3 hours in the renaturation solution (1% Triton X-100, pH 5.0) (panel A), at 40°C for 3 hours in the renaturation solution (1% Triton X-100, pH 7.5) (panel B), or at 37°C for 3 hours (panel C) or overnight (panel D) in the renaturation solution (1% Triton X-100, pH 7.5).

Supplemental Figure S2. **ESI-MS analysis of the peak 2 material shown in Fig. 2A.** Panels A and B show the results for the peak 2 material (a reduced tetrasaccharide) obtained from MS analysis conducted in positive (Panel A) and negative modes (Panel B). The ion peaks for both modes correspond to the $[M+Na]^+$ (*m/z* 999.9) and $[M-H]^-$ (*m/z* 975.7) ions of a reduced tetrasaccharide, GlcNAc-MurNAc-GlcNAc-MurNAcr (M_r, 977).

Supplemental Figure S3. **ESI-MS and -MS/MS analyses of the peak 3 material shown in Fig. 2A.** Panel A shows the result for the peak 3 material (a reduced hexasaccharide) obtained from MS analysis performed in the negative mode. The ion peak corresponds to the $[M-H]^-$ (*m/z* 1,454.9) ion of a reduced hexasaccharide (M_r, 1,456). Panel B shows the result for the peak 3 material obtained from MS/MS analysis performed in the negative mode. The material in peak 3 was identified as GlcNAc-MurNAc-GlcNAc-MurNAc-GlcNAc-MurNAcr. Panel C shows the structure identified and the calculated molecular weight of each fragment in peak 3. Ion series *b* and *y* correspond to the fragment peaks of peak 3 material.

Supplemental Figure S4. **Determination of cleavage sites for CwlP-M23 and LytF by RP-HPLC.** *Bacillus subtilis* peptidoglycan was digested with CwlP-M23 followed by LytF

(D,L-endopeptidase). The free amino acid residues in the sample were labeled with FDNB and then hydrolyzed with HCl, followed by RP-HPLC separation. The sample contained mono-DNP-A_2pm, bis-DNP-A_2pm, and DNP.

Supplemental Figure S5. **Hydrolysis of *Escherichia coli* cells with CwlP-SLT and CwlP-M23 proteins.** *E. coli* JM109 cells (0.3 of OD_{600}) were digested with 0.1 μM of CwlP-SLT at 37°C (pH 5) (closed squares), or with 0.1 μM of CwlP-M23 at 40°C (pH 7.5) (closed triangles). The open circles are *E. coli* JM109 samples without enzymatic digestion (control). The error bars indicate the standard deviations from three independent experiments.