Supporting Information

Ieva and Bernstein 10.1073/pnas.0907912106

SI Materials and Methods

Purification of His-Tagged BamA. Plasmid pET20(b)YaeT, containing the *bamA* gene cloned into pET20(b+) (Novagen), was obtained from Susan Buchanan. BL21 (Stratagene) transformed with pET20(b)YaeT was grown in LB medium, and *bamA* expression was induced during he exponential phase by the addition of 0.5 mM IPTG. After 3 h, the cells were lysed in 8 M urea, and BamA was purified under denaturing conditions on Ni-NTA agarose using the QIAexpressionist protocol (Qiagen). BamA eluted from the resin was further purified by SDS/PAGE on a 4%–12% NuPage minigel (Invitrogen).

1. Renn JP, Clark PL (2007) A conserved stable core structure in the passenger domain β-helix of autotransporter virulence proteins. *Biopolymers* 89:420–427.

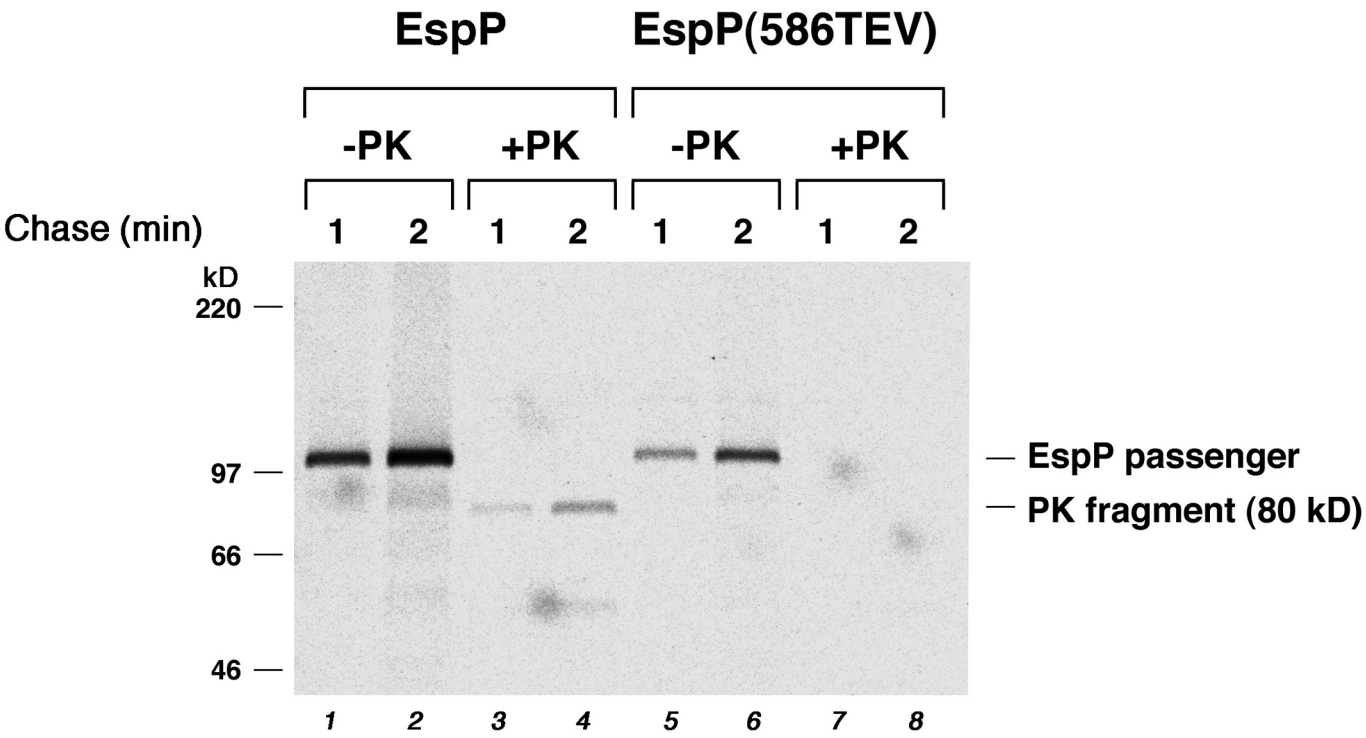

Fig. S1. PK treatment of the WT EspP passenger domain yields an ≈80-kDa fragment. AD202 transformed with pRLS5 or pJH97 were subjected to pulse-chase labeling after the addition of 100 μM IPTG. Culture aliquots were collected at the indicated time points, and cells were removed by centrifugation. Half of the culture medium was then treated with PK, and EspP-containing polypeptides were immunoprecipitated using an N-terminal anti-EspP antiserum.

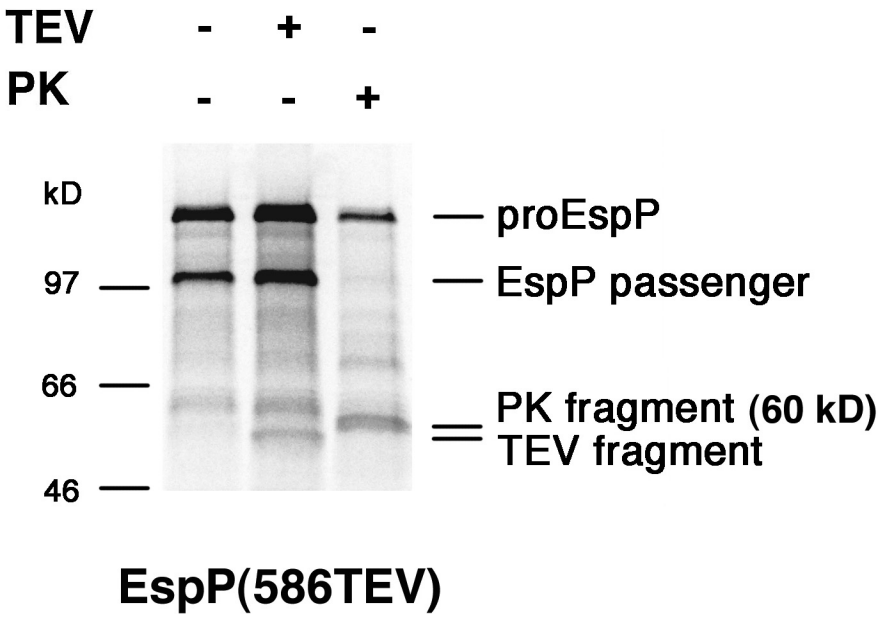

Fig. S2. PK digests the EspP(586TEV) translocation intermediate at a site C-terminal to the TEV insertion. AD202 transformed with pJH97 were pulse-labeled and subjected to a 2 min chase after the addition of 100 μM IPTG. Duplicate culture aliquots were pipetted over ice, and cells were pelleted. One sample was incubated in 33 mM Tris-HCl (pH 8.0), 2 mM EDTA, and 100 μg/mL of lysozyme on ice for 20 min to lyse cells by hypotonic shock. Following the addition of 1 mM DTT, half of the sample was incubated on ice for 2 h with 10 ng/mL of the hyperactive S219V mutant of the TEV protease (a gift from Susan Buchanan). Cells from the second culture sample were treated with PK as described in *Materials and Methods*. EspP-containing polypeptides were then immunoprecipitated using an N-terminal anti-EspP antiserum.

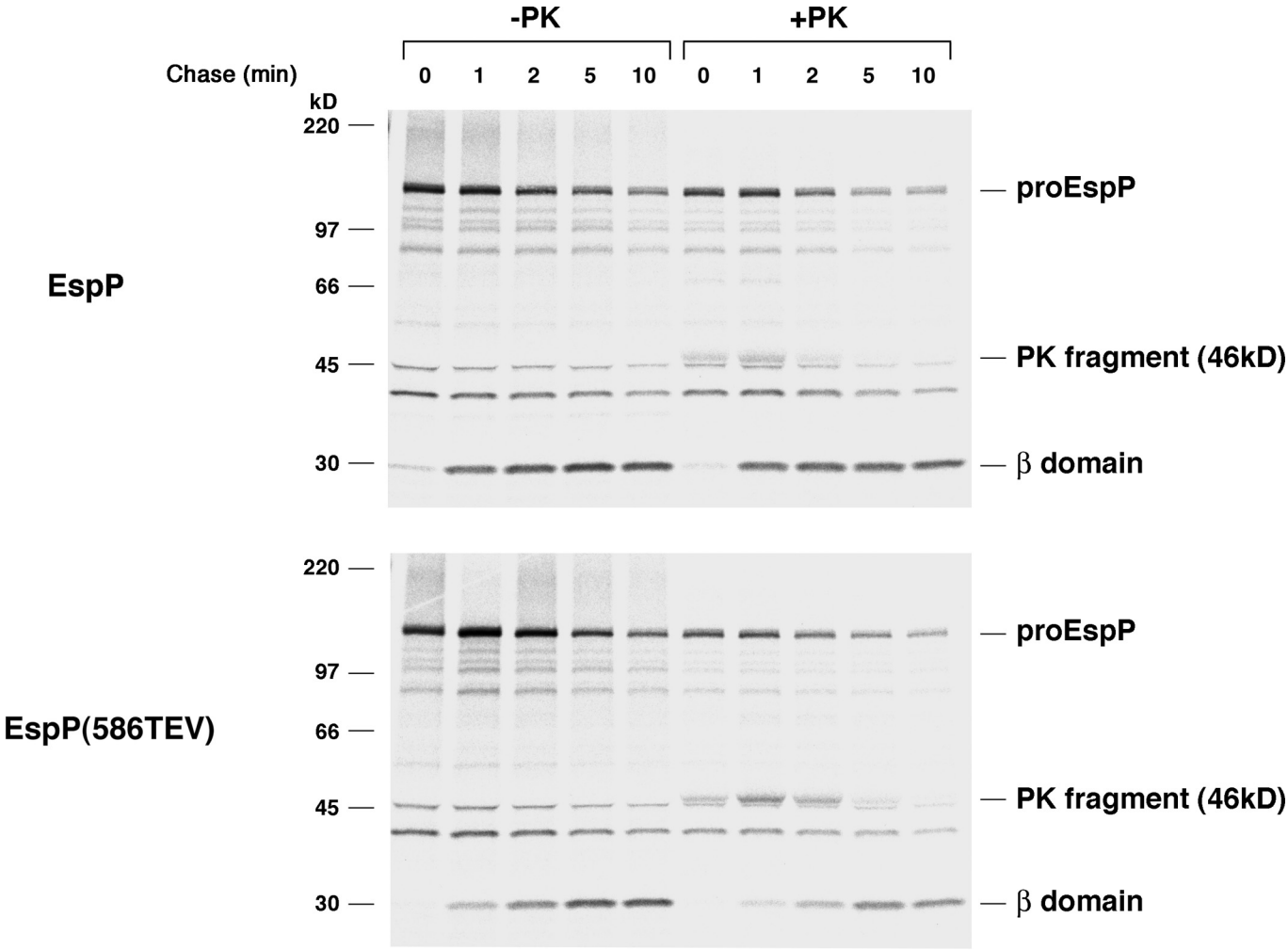

Fig. S3. The C terminus of the EspP(586TEV) passenger domain is rapidly exposed on the cell surface. AD202 transformed with pRLS5 or pJH97 were subjected to pulse-chase labeling after the addition of 100 μM IPTG. Half of the cells were treated with PK, and EspP-containing polypeptides were immunoprecipitated using a C-terminal anti-EspP antiserum. The results show that PK treatment of the pro form of both WT EspP and EspP(586TEV) yields a ≈46-kDa C-terminal fragment consisting of the last ≈140–150 residues of the passenger domain and the entire β domain. The passenger domain moiety presumably corresponds to a C-terminal stable core structure that has been proposed to be a general feature of autotransporters (1). The ≈46-kDa fragment was observed predominantly at 0 min and 1 min time points when WT EspP was examined (*Top Panel*) and was presumably derived from passenger domain molecules that were exposed on the cell surface but not yet cleaved. Interestingly, an elevated level of the fragment was observed at 1 min when EspP(586TEV) was examined, and a significant amount of the fragment also was observed at 2 min (*Bottom Panel*). The elevated levels of the fragment observed at later time points presumably result from the delay in passenger domain cleavage associated with the stalling of passenger domain translocation. These data strongly suggest that before the stalling of translocation, the segment of the EspP(586TEV) passenger domain that is C-terminal to the stall point emerges on the cell surface and folds like the equivalent segment of WT EspP.

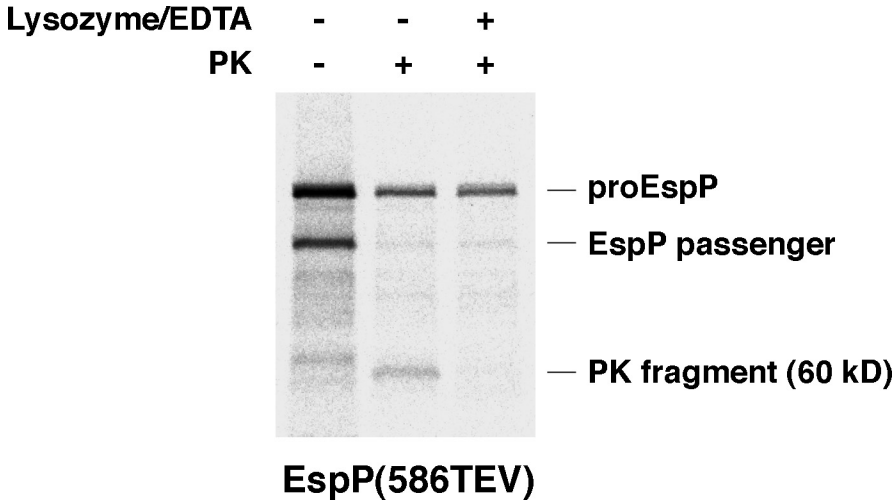

Fig. S4. Permeabilization of the OM exposes a ≈60-kDa N-terminal fragment of EspP(586TEV) to degradation. AD202 transformed with pJH97 were pulse labeled and subjected to a 2 min chase after the addition of 100 μM IPTG. Cells were pipetted over ice, pelleted, and resuspended in spheroplast buffer [33 mM Tris-HCl (pH 8.0) and 40% sucrose]. One-third of the cells were untreated, one-third were treated with PK, and one-third were incubated with 100 μg/mL of lysozyme and 2 mM EDTA on ice for 20 min to permeabilize the OM before PK treatment. EspP-containing polypeptides were then immunoprecipitated using an N-terminal anti-EspP antiserum.

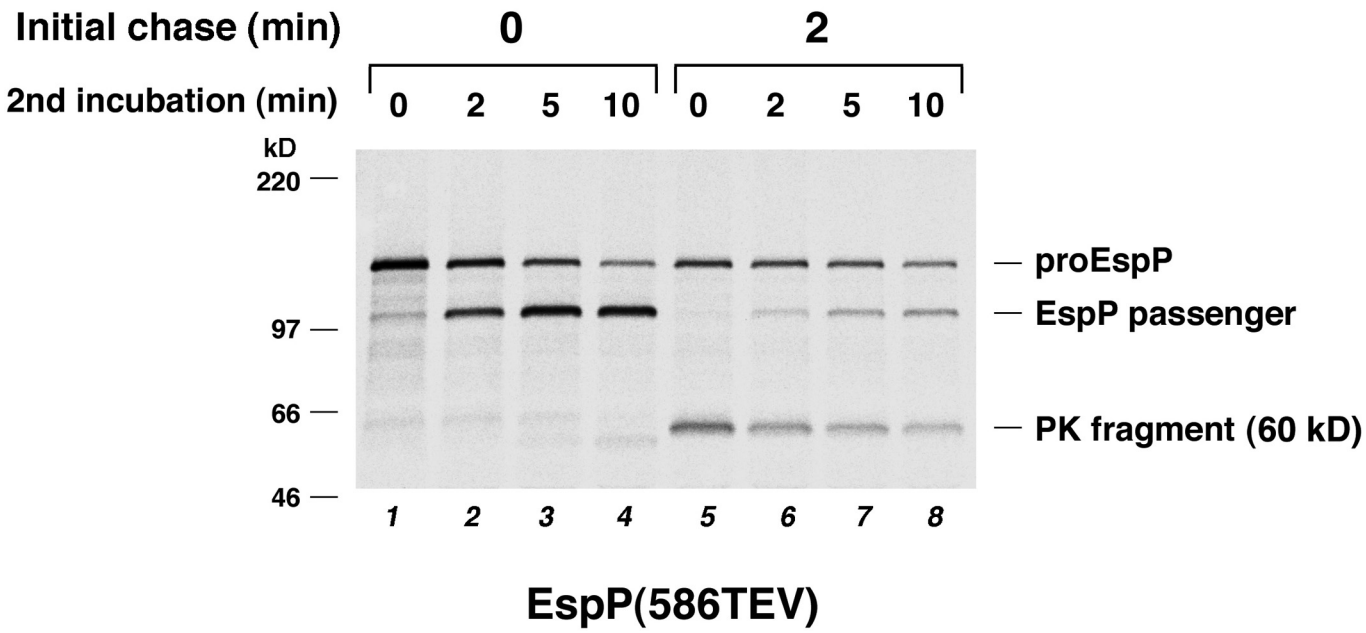

Fig. S5. PK treatment of an EspP(586TEV) translocation intermediate stabilizes a ≈60-kDa N-terminal fragment. AD202 transformed with pJH97 was pulse labeled and subjected to a 0 min or 2 min chase after the addition of 100 μM IPTG. Culture aliquots (4.5 mL) were pipetted over ice, and cells were pelleted (4 °C, 3500 × *g*, 10 min). Then the cells were resuspended in 1 mL of M9 salts, incubated on ice for 15 min with 200 μg/mL of PK, and pelleted in a microfuge (3,000 × *g*, 5 min, 4 °C). The cells were washed in M9 salts twice and then added to 4.5 mL of complete M9 medium supplemented with 50 μg/mL of β-casein. A second incubation at 37 °C was conducted, and 1 mL aliquots were TCA-precipitated at the indicated time points. EspP-containing polypeptides were then immunoprecipitated using an N-terminal anti-EspP antiserum.

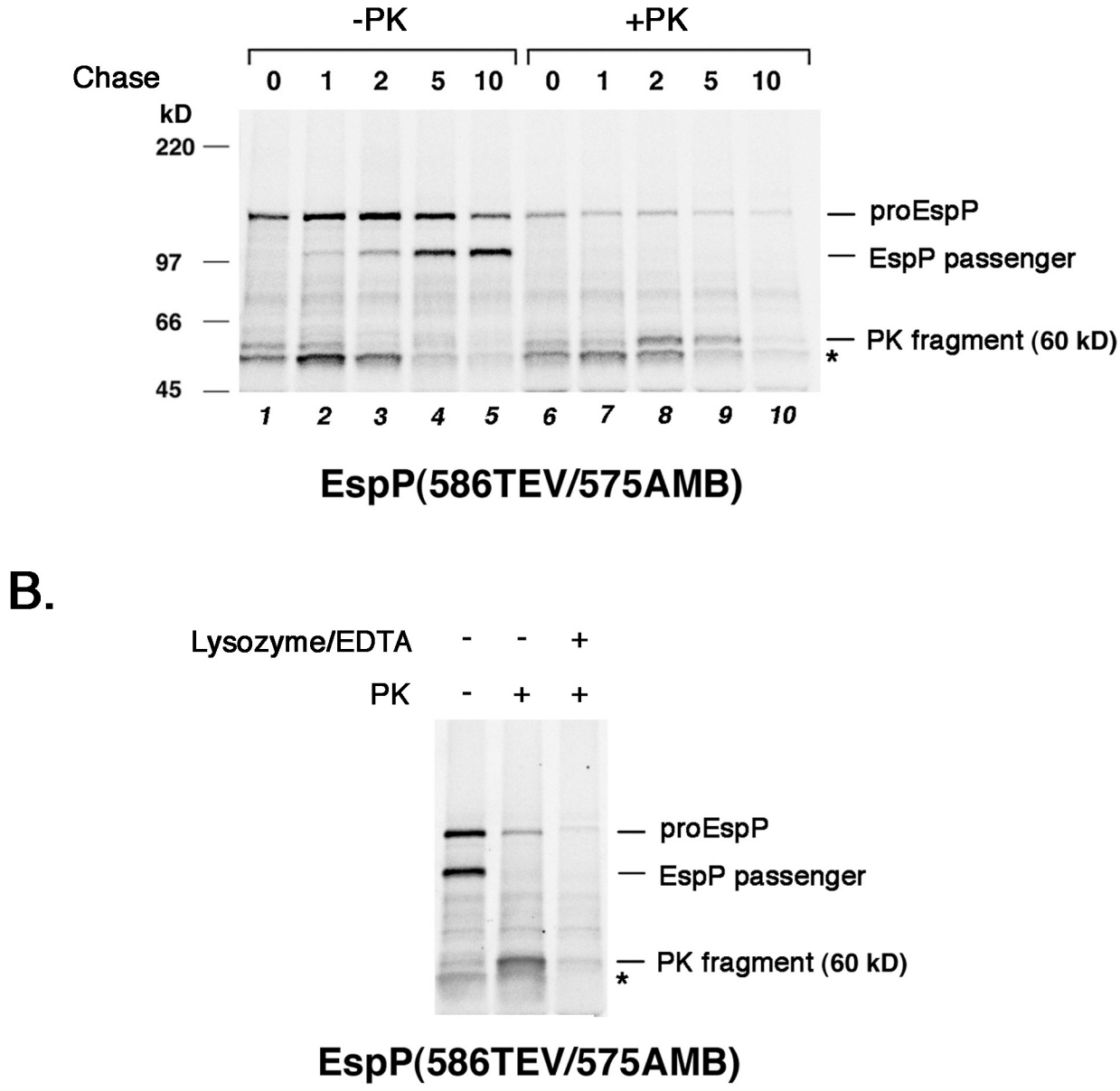

Fig. S6. Translocation of the passenger domain of EspP(586TEV/575AMB) stalls transiently. AD202 was transformed with pDULEBpa and a derivative of pRI23 [P_{lac}-*espP(TEV589)*] harboring an amber codon at residue 575. (*A*) Cells were subjected to pulse-chase labeling after the addition of 200 μM IPTG. Half of the cells were treated with PK, and EspP-containing polypeptides were immunoprecipitated using an N-terminal anti-EspP antiserum. A truncated form of the protein that resulted from translation termination at the amber codon is denoted by an asterisk. (*B*) Cells that were pulse labeled and subjected to a 1 min chase after the addition of 200 μM IPTG were pipetted into a 15 mL tube and chilled on ice. After the cells were pelleted and resuspended in spheroplast buffer, one-third were untreated, one-third were treated with PK, and one-third were incubated with 100 μg/mL of lysozyme and 2 mM EDTA on ice for 20 min before PK treatment. EspP-containing polypeptides were then immunoprecipitated using an N-terminal anti-EspP antiserum.

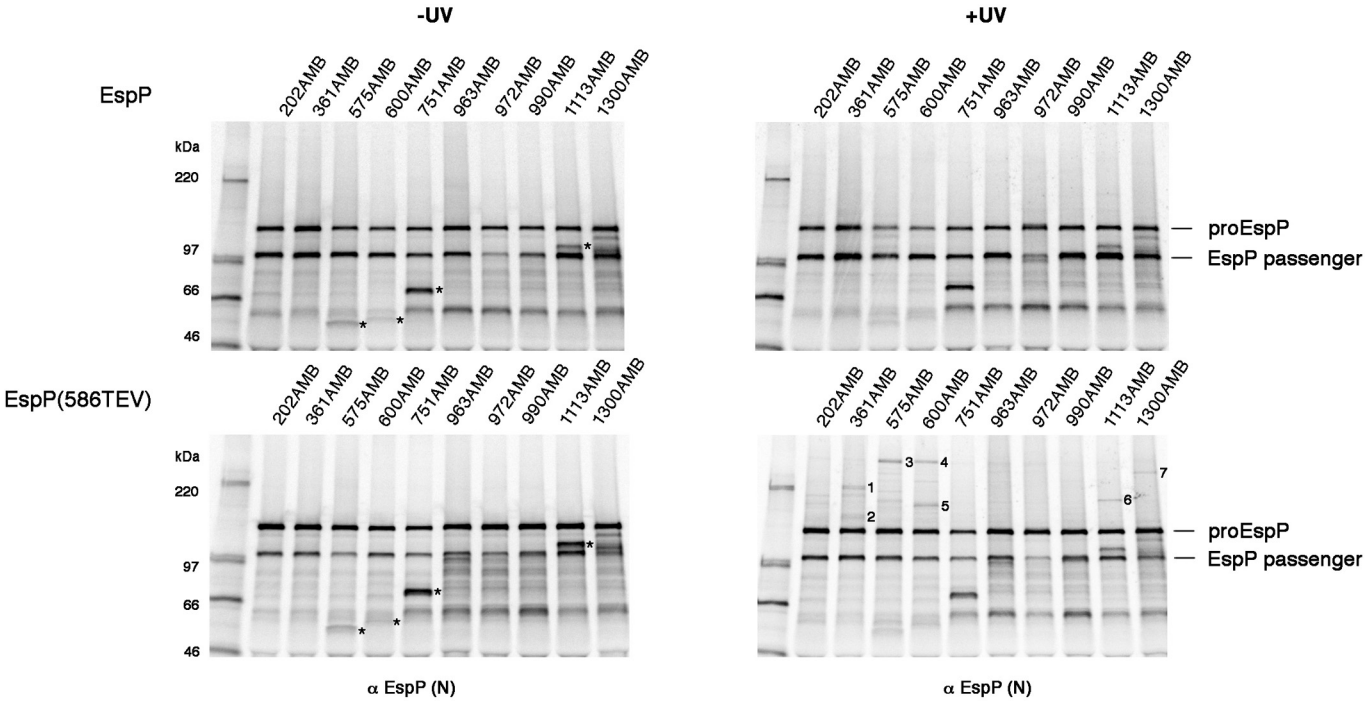

Fig. S7. The site-specific crosslinking of EspP(586TEV) yields high–molecular weight adducts. AD202 was transformed with pDULEBpa and a derivative of pRI22 (P_{lac}-*espP*) or pRI23 [P_{lac}-*espP(586TEV)*] harboring an amber codon at the indicated position. Cells were pulse-labeled and subjected to a 1 min chase after the addition of 200 μM IPTG. Half of each sample was UV irradiated, and EspP-containing polypeptides were immunoprecipitated using an N-terminal anti-EspP antiserum. Crosslinked adducts are numbered 1–7. Truncated forms of the protein that resulted from translation termination at the amber codon are denoted by an asterisk.

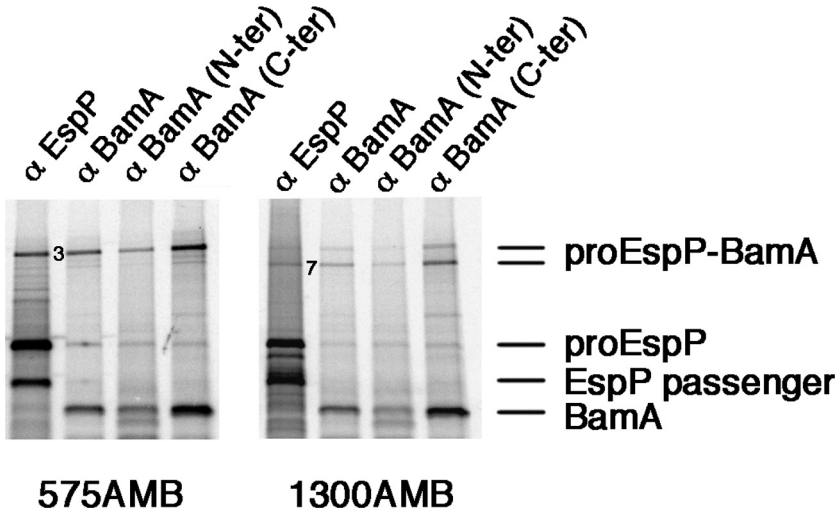

Fig. S8. Crosslinking adduct 7 contains a full-length BamA molecule. AD202 was transformed with pDULEBpa and a derivative of pRI23 [P_{lac}-*espP(586TEV)*] harboring an amber codon at residue 575 or 1300. Cells were pulse-labeled and subjected to a 1-min chase after the addition of 200 μM IPTG. Half of each sample was UV-irradiated, and immunoprecipitations were conducted using polyclonal rabbit antisera raised against an N-terminal EspP peptide, full-length BamA, an N-terminal BamA peptide (SMPVRTGDTVNDEDISNTIRA), and a C-terminal BamA peptide (QPFKKYDGDKAEQFQFNIGKT). Only the UV-irradiated samples are shown.

Table S1. Oligonucleotides used in this study

Pst586(+)	5′-CTCTTGTTCACTACCAGCTCTGCAGCCTATGCGCTTTTTG-3′
PstTev(+)	5′-CGAAAACCTGTACTTCCAGGGTGTGCA-3′
PstTev(−)	5′-CACCCTGGAAGTACAGGTTTTCGTGCA-3′
10H-link	5′-GGCCGCTCCGAAAGATAACCACCATCACCATCACCATCACCATCACCATCT-3′
GC-10H-link	5′-GGCCAGATGGTGATGGTGATGGTGATGGTGATGGTGGTTATCTTTCGGAGC-3′
AMB-F202	5′-GGAGAAAAGAAACTTATCGCATAGAGAGCCGGCTCTGGTGTGGTA-3′
AMB-F361	5′-GATATCCTCCTGAAATCCTCTTAGGATAATGGTGCTGGCGGTCTT-3′
AMB-Y575	5′-CTGAACATGCCATTTAGCGGGATGGAGCTTTCTCTT-3′
AMB-M600	5′-GTGATTATGTTGCAGGATAGCAAAATACAGAAGCTGATGCTG-3′
AMB-F751	5′-GTGACAACTCAAAGTGGTATGTAGTCCACGAGCGATATCAGCATC-3′
AMB-F963	5-CACCAATGAAAATGTCTAGAAAGCCAGTAAACAAAC-3′
AMB-F972	5′-GCCAGTAAACAAACCATTGGTTAGAGTGATGTAACGCCGGTCATT-3′
AMB-W990	5′-ATGACAAAATAACATAGTCACTGACAGGCTA-3′
AMB-F1113	5′-GCTTCCGCCATGTAGGATTCCGGTGCCTA-3′
AMB-F1300	5′-AATTTCCGTTACTCGTAGTGATACTCCGTATTC-3′