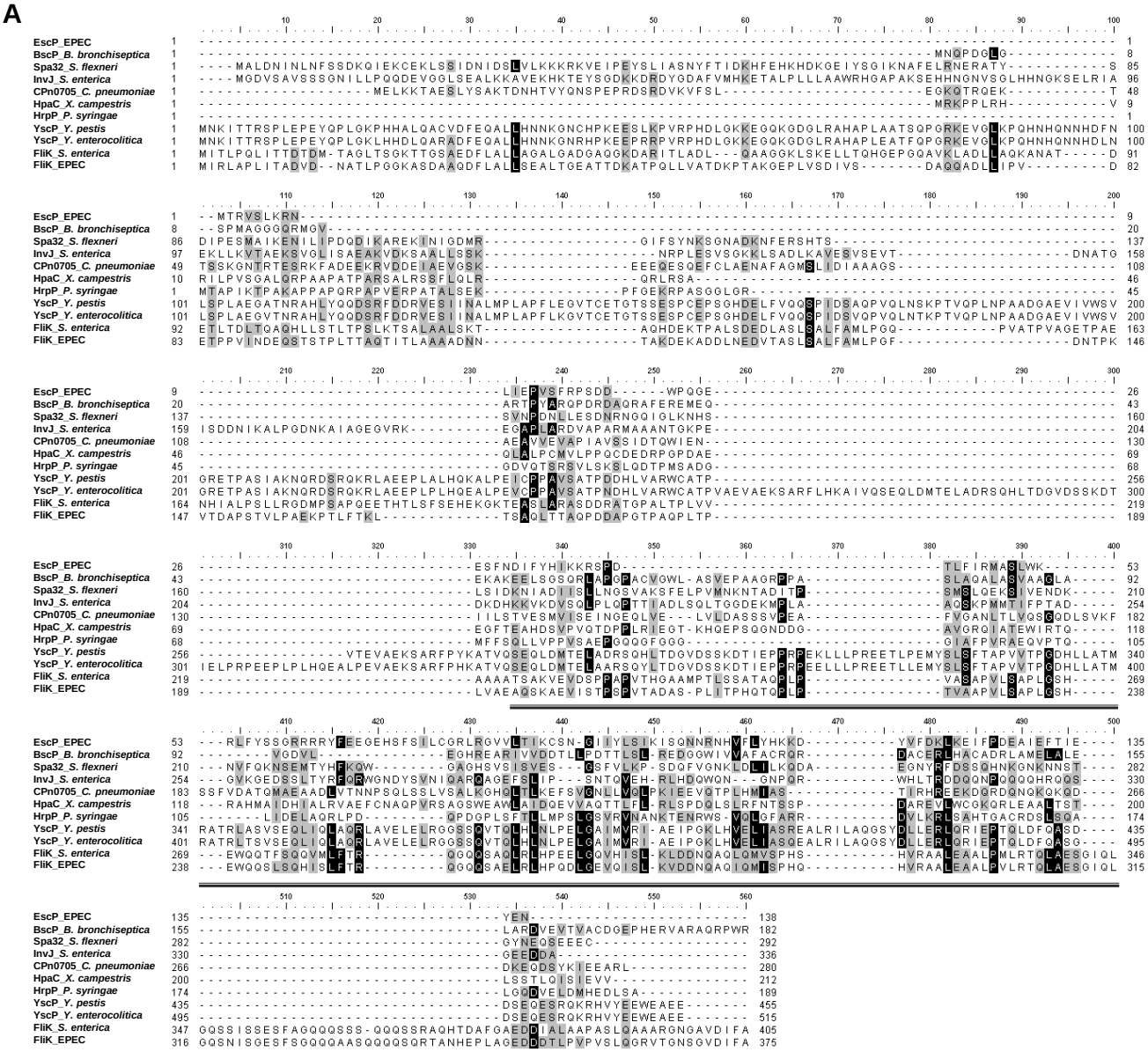
SUPPLEMENTAL MATERIAL

Figure S1

A



B

Protein	Identity/Similarity (%)
BscP_*B. bronchiseptica*	14 / 32
Spa32_*S. flexneri*	9 / 32
InvJ_*S. enterica*	10 / 21
CPn0705_*C. pneumoniae*	12 / 26
HpaC_*X. campestris*	10 / 28

Protein	Identity/Similarity (%)
HrpP_*P. syringae*	12 / 28
YscP_*Y. pestis*	13 / 27
YscP_*Y. enterocolitica*	12 / 25
FliK_*S. enterica*	16 / 35
FliK_EPEC	12 / 30

FIG S1 (A) Multiple sequence alignment of EscP and members of the YscP/FliK family of proteins. Protein sequence alignment was performed using MUSCLE. Residues were shaded using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), when having a consensus greater than 40%; identical residues are shown with a black background and conserved residues with a grey background. The most conserved C-terminal region is underlined. (B) Identity and similarity percentages of paired sequence alignments (Stretcher) between full-length EscP and the conserved C-terminal region (underlined in A) of BscP (NP_888175) from *Bordetella bronchiseptica*, Spa32 (NP_085314) from *Shigella flexneri*, InvJ (NP_461813) from *Salmonella enterica*, CPn0705 (NP_224901) from *Chlamydophila pneumonia*, HpaC (YP_362155) from *Xanthomonas campestris pv. vesicatoria*, HrpP (NP_791225) from *Pseudomonas syringae pv. tomato*, YscP (NP_395176) from *Yersinia pestis*, YscP (AAF70341) from *Yersinia enterocolitica*, FliK (NP_460927) from *Salmonella enterica*, and FliK (YP_002329574) from EPEC.

Figure S2

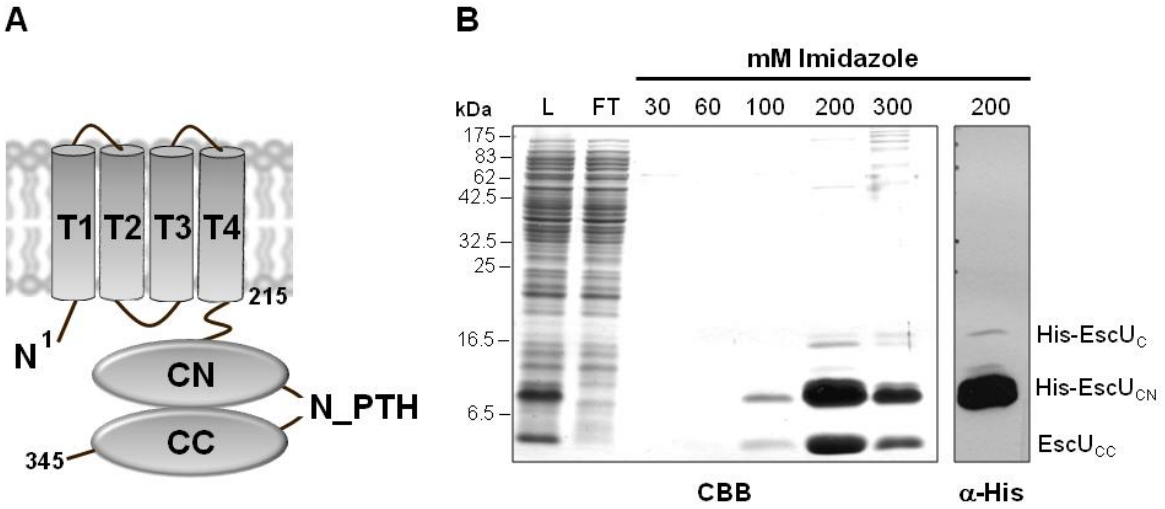


FIG S2 (A) Schematic representation of EscU. EscU has four transmembrane helices (T1 to T4) in its N-terminal domain (residues 1 to 214), followed by a C-terminus cytoplasmic domain (EscU$_C$, residues 215 to 345). The EscU$_C$ domain is auto-cleaved within a conserved NPTH sequence (between N262 and P263). N_PTH, cleaved NPTH domain. (B) Purification and auto-cleavage of His-EscU$_C$ by nickel affinity chromatography. The cleared lysate (L) from cells overproducing His-EscU$_C$ was loaded onto Ni-NTA agarose resin and purification was performed as described in Materials and Methods. Flow-through (FT); washes with 30 and 60 mM imidazole, and elutions with 100, 200 and 300 mM imidazole. All fractions were analyzed by CBB stained SDS-15% PAGE. A small amount of His-EscU$_C$ and increased amounts of its auto-cleavage products, His-EscU$_{CN}$ and EscU$_{CC}$, were detected. The EscU$_{CC}$ sub-domain co-purified with His-EscU$_{CN}$. The 200 mM elution fraction was immunoblotted with anti-His antibody (right panel). Molecular masses of protein standards are indicated on the left of the gel.

Figure S3

A

B

Protein	Identity/Similarity (%)
Orf16 EHEC	97 / 99
Orf16 CR	78 / 89

FIG S3 (A) Multiple sequence alignment of EPEC EscP and homolog proteins from the related A/E pathogens EHEC and CR. Protein sequence alignment was performed using MUSCLE. Residues were shaded using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), when having a consensus greater than 40%; identical residues are shown with a black background and conserved residues with a grey background. The 35 amino acid residues that are missing in the Orf16 protein from *C. rodentium* are underlined. (B) Identity and similarity percentages of paired sequence alignments (Stretcher) between EPEC EscP/Orf16 (AAC38385), Orf16 from EHEC (NP_312593) and Orf16 from CR (AAL06371) completed with the missing amino acid residues.