## SUPPLEMENTAL MATERIAL

## Figure S1

Α

1	10 20 30 40 50 60 70 80 90 100 ••••••••••••••••••••••••••••••••••
1 1 1 1	MNOPOCIGG 8 MALDNINLNFSSDKQIEKCEKLSSIDNIDSI VLKKKRKVEIPEYSLIASNYFTIDKHFEHKHDKGEIYSGIKNAFELRNERATY 85 MODVSAVSSSGNILLPODEVGGLSEALKKAVEKHKTEYSGDKKDROYGDAFVMHKETALPLLLAAWRHGAPAKSEHHNGNVSGLHNNGKSELRIA 96 MELKKTAESLYSAKTDNHTVYONSPEPRDSROVKVFSL 60KOTROEK 748 MELKKTAESLYSAKTDNHTVYONSPEPRDSROVKVFSL 60KOTROEK 748 MEKPLRH 9 1
1 1 1 1	MNKITTRSPLEPEYQPLGKPHHALQACVDFEQALLHNNKGNCHPKEESLKPVRPHDLGKKEGQKGDGLRAHAPLAATSQPORKEVGLKPQHNHQNNHDFN 100 MNKITTRSPLEPEYQPLGKLHHDLQARADFEQALLHNNKGNRHPKEEPRRPVRPHDLGKKEGQKGDGLRAHAPLEATFQPORKEVGLKPQHNHQNNHDLN 100 MITLPQLITTDTDM-TAGLTSGKTTGSAEDFLALLAGALGADGAQGKOARITLADLQAAGGKLSKELLTQHGEPGQAVKLADLLAQKANATD 91 MIRLAPLITADVDNATLPGGKASDAAQDFLALLSEALTGEATTDKATPQLLVATDKPTAKGEPLVSDIVSDAQQADLLIPVD 82
1 8 97 49 10 101 101 92 83	10  120  130  140  150  160  170  180  190  200
9 20 137 159 108 46 45 201 201 164 147	210    220    220    240    250    270    280    290    200
	310    220    330    340    350    360    370    380    390    400      I
	410  400  4
	510  520  540  540  540     I  I  I  I  I  I     I  I  I  I  I  I  I     I  I  I  I  I  I  I  I     I  I  I  I  I  I  I  I     I  I  I  I  I  I  I  I     I  I  I  I  I  I  I  I     I  I  I  I  I  I  I  I  I     I  I  I  I  I  I  I  I  I  I     I  <
	66    67      449    40      49    10      11    111      101    11      102    283      9    20      137    159      146    46      42    2011      101    11      111    111      121    114      141    147      150    222      100    241      121    111      111    111      122    111      132    111      141    111      141    111      111    111      111    111      111    111      111    111      111    111      111    111      111    111      111    111      111    111      111    111      111    111      111    1

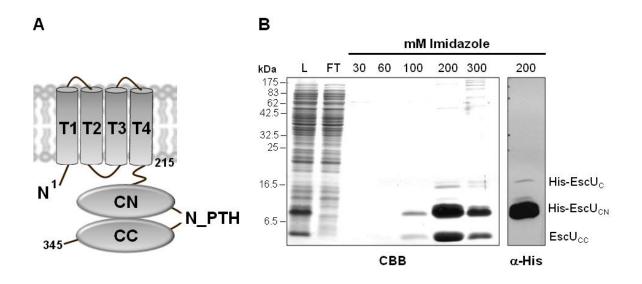
В

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_ <i>P. syringae</i>	12 / 28
YscP_Y. pestis	13 / 27
YscP_Y. enterocolitica	12 / 25
FliK_S. enterica	16 / 35
FliK_EPEC	12 / 30

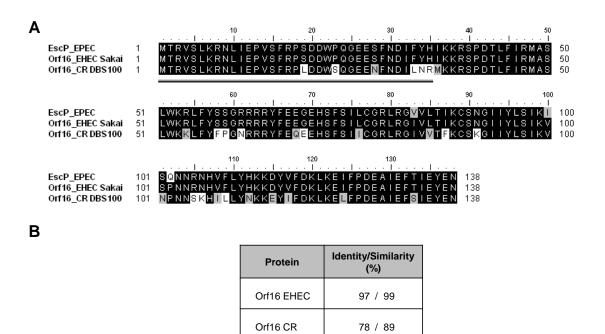
**FIG S1** (A) Multiple sequence alignment of EscP and members of the YscP/FliK family of proteins. Protein sequence alignment was performed using MUSCLE. Residues were shaded using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), when having a consensus greater than 40%; identical residues are 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<sub>C</sub>, residues 215 to 345). The EscU<sub>C</sub> 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<sub>C</sub> by nickel affinity chromatography. The cleared lysate (L) from cells overproducing His-EscU<sub>C</sub> 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<sub>C</sub> and increased amounts of its auto-cleavage products, His-EscU<sub>CN</sub> and EscU<sub>CC</sub>, were detected. The EscU<sub>CC</sub> sub-domain co-purified with His-EscU<sub>CN</sub>. 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



**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.