



Fig. S6. Determination of EspB proteolysis sites.

(A) Schematic of pET21a-EspB-His. (B) Western blot analysis of exogenous rEspB-His using an anti-His antibody that will only detect cleaved EspB if the C-terminal His tag remains intact. (C) Schematic of site directed mutations made to the EspP and *Bt* protease cleavage sites. (D) Western blots of whole cell lysates collected from $\Delta espB$ EHEC complemented with either WT *espB*, *espB* $\Delta Btsite$ or *espB* $\Delta EspPsite$ in pACYC184. EspB appeared to be expressed to similar levels in all 3 strains, RpoA was used as a control for cell numbers. (E) Western blots of supernatants of EHEC $\Delta espB$ complemented with WT EspB or EspB $\Delta Btsite$ cloned into vector pACYC184 in the absence and presence of *Bt* using anti-EspB antisera. There is a decrease in EspB cleavage in the EspB $\Delta Btsite$ strain in the presence of *Bt*. However cleavage is not completely abrogated, this is probably due to the fact that the 36kDa *Bt* cleavage site is intact. We could only map the 34kDa site because we could not completely separate the 37 and 36kDa forms of EspB for cleavage analyses.