Supplemental figure legend

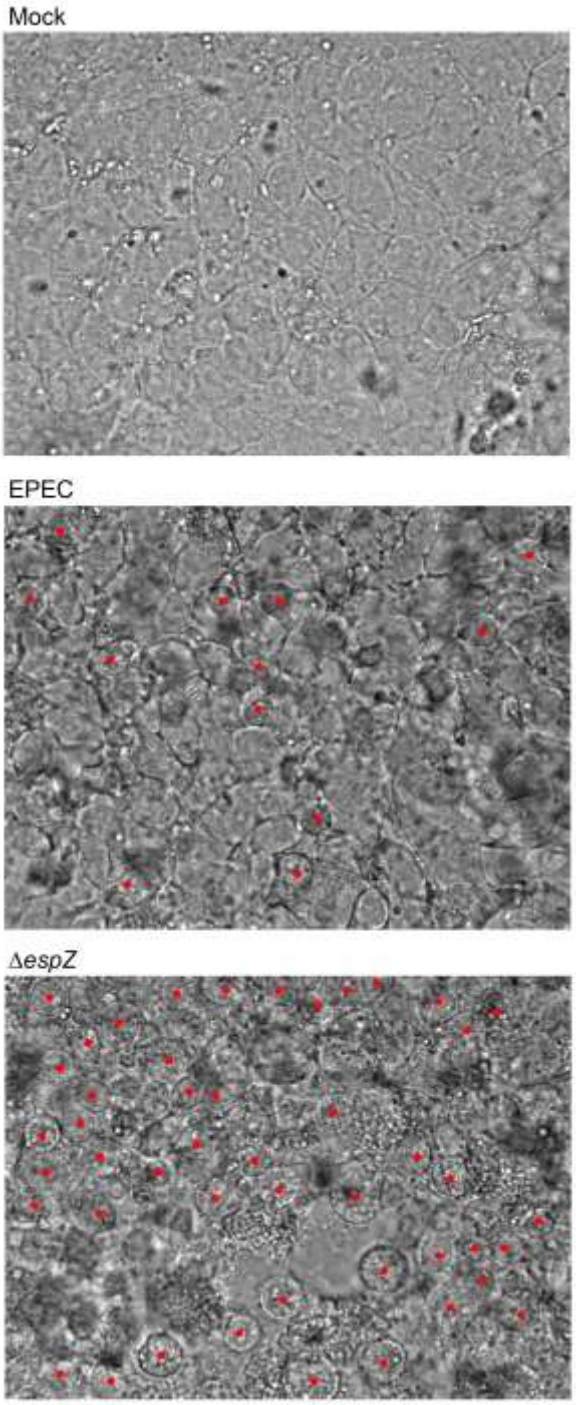


**Figure S1**. Phase contrast images of infected $C2_{BBE}$ cells. Cells were mock-treated or infected with EPEC or Δ*espZ* (MOI=100) for 4 hours. Red dots mark rounded up cells. Images shown are representative of three independent experiments.

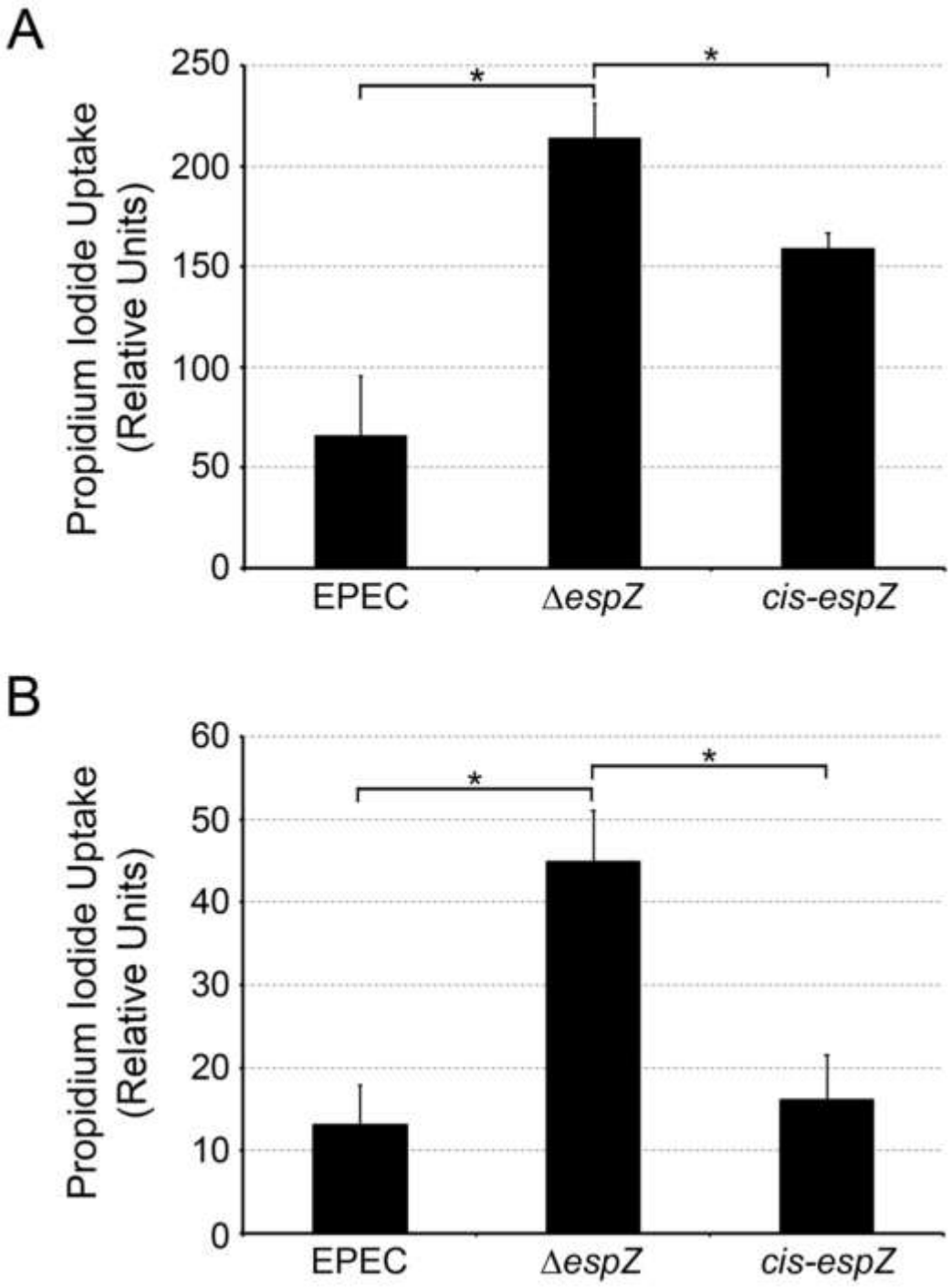


**Figure S2.** Propidium iodide uptake of infected HeLa (A) and $C2_{BBE}$ (B) cells. Cells were infected with EPEC, Δ*espZ* or *cis-espZ* strains at an MOI of 100 for 4 hours. Data shown are the mean increase in PI uptake (n=8) for each treatment relative to uninfected controls and is representative of over three experiments for each cell type. *p<0.05; Student's T-test. Experiments were performed as described in the Methods

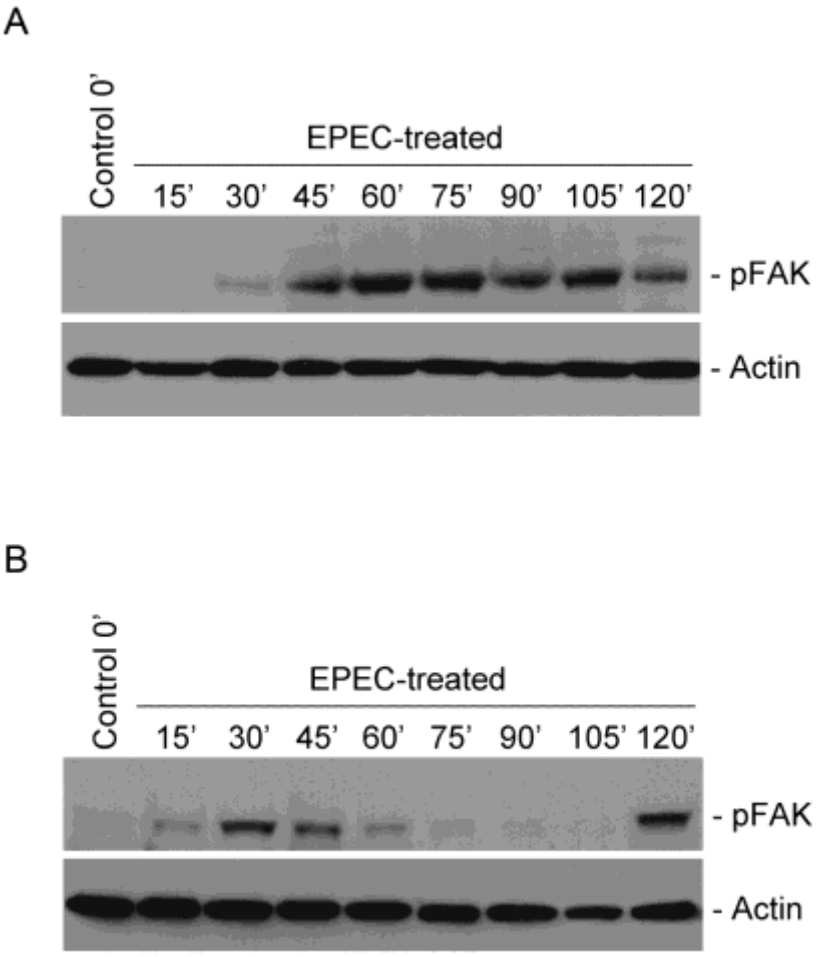


**Figure S3.** EPEC induces FAK phosphorylation. HeLa and $C2_{BBE}$ cells were infected with EPEC. Western blot was performed to monitor phosphorylated FAK (pFAK) in EPEC-infected HeLa (A) and $C2_{BBE}$ (B) cells. Actin levels are shown as loading control.

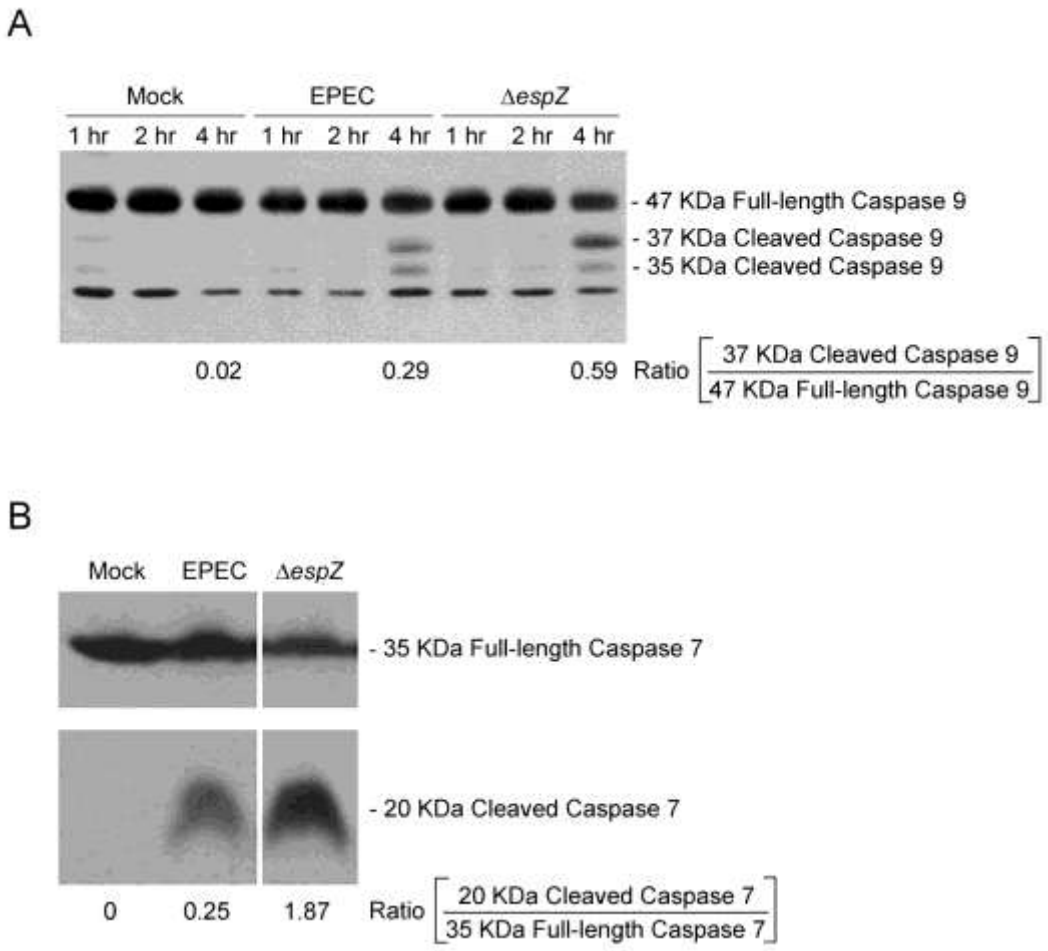


**Figure S4.** EspZ inhibits intrinsic apoptosis. $C2_{BBE}$ cells were infected with EPEC, Δ*espZ*, or plasmid-complemented Δ*espZ* (p*espZ*). Western blot was performed to monitor full-length and cleaved caspase 9 at 1, 2 and 4 hours post-infection (A), as well as full-length and cleaved caspase 7 at 4 hours post-infection (B). Ratios of band intensities of cleaved caspases vs. full-length caspases at 4 hours post-infection are showed below each blot.

## Supplemental Methods

**Cell rounding assay.** $C2_{BBE}$ cells were grown on glass cover slips as described in the methods. When cells reached 100% confluency, growth medium was replaced with serum-free DMEM 14 hours before the start of the infection. Cells were then infected with bacterial strains at an MOI of 100. After 1 hour of infection, medium was changed to remove non-adherent bacteria. At 4 hours post-infection, samples were examined using a Leica DM4000B epifluorescence microscope (Leica Microsystems, Wetzlar, Germany) equipped with a Retiga Exi CCD camera (Qimaging, Surrey, BC, Canada). Images were acquired using SlideBook 4.2 software (Intelligent Imaging Innovations, Denver, CO) and processed using ImageJ 1.44p software (National Institute of Health, USA).

**PI uptake assay**. HeLa or $C2_{BBE}$ cells were seeded in 96 well flat, clear bottom tissue culture dishes. Cells were infected with EPEC, Δ*espZ* or the *cis*-*espZ* strains at an MOI of 100 for 4 hours. Propidium iodide uptake was measured as previously described in the Materials and Methodology section.

**Western blot analysis.** Western blots were performed with polyclonal antibodies to caspase 7 and caspase 9 (Cell Signaling, Danvers, MA), and to phosphorylated FAK and actin (Sigma-Aldrich, St. Louis, MO). Control and infected cells were washed with sterile PBS, scraped, and total protein extracted in cell lysis buffer [20mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerol phosphate, 1mM $Na_3VO_4$, 1 µg/mL leupeptin, and 1 mM PMSF. The extracts were sonicated on ice three times to facilitate cell lysis.

Protein concentrations were determined by the Bradford method. Protein extracts (300 μg) were separated on 12% SDS-PAGE using the Protean II xi apparatus (Bio-Rad, Richmond, CA). Separated proteins were transferred to 0.2 μm nitrocellulose membranes (Transblot Cell Apparatus, Bio-Rad), blocked with 5% nonfat milk in Tris-buffered saline containing Tween 20 (TBST) and then incubated with primary antibodies at appropriate dilutions in membrane blocking solution (Zymed, San Francisco, CA) overnight at $4^{o}$C. Blots were incubated in HRP-conjugated secondary antibody for 1 hour at room temperature. Membranes were washed three times for 5 minutes in TBST between each incubation step. The membranes were then developed with SuperSignal West Femto Chemilluminescent Substrate (Thermo Scientific, Rockford, IL). Densitometry analyses were performed using ImageJ 1.46 (1).

**References**

1. **Schneider, C.A., W.S. Rasband, K.W. Eliceiri.** 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Meth. **9:**671-675.