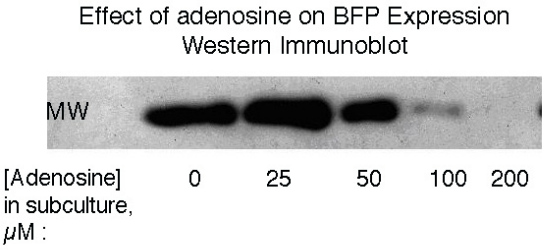
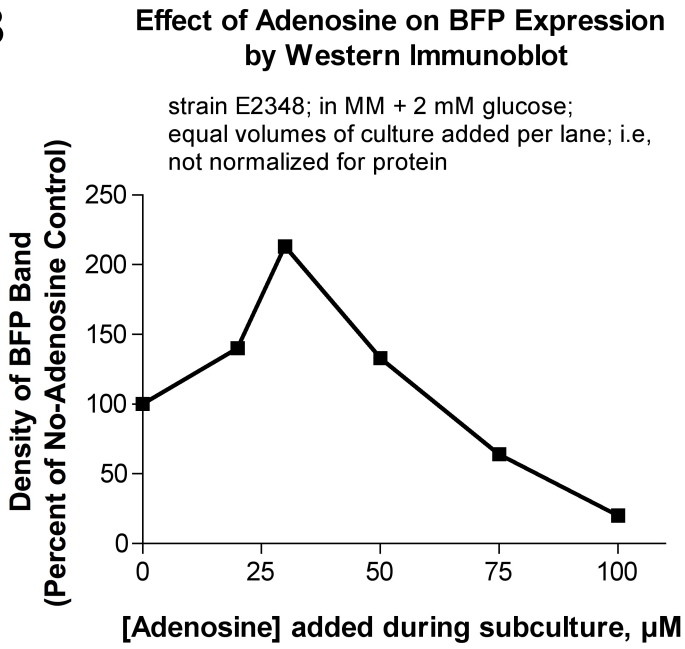
Supporting Figure 1
Effect of Adenosine on Expression of BFP Protein by Western Immunoblot

A

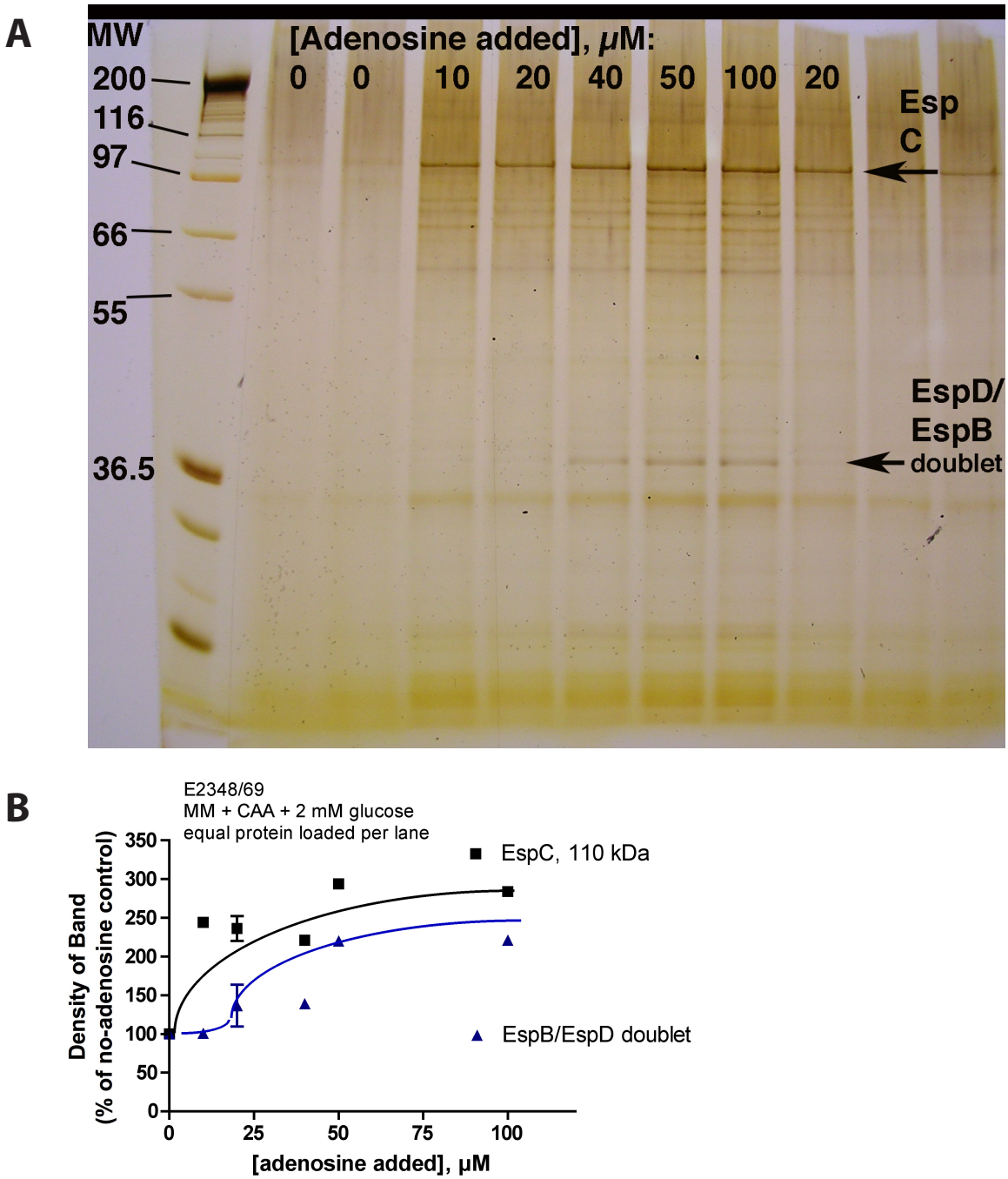


B



Legend: EPEC strain E2348/69 was grown overnight in LB, then subcultured into minimal medium plus 2 mM glucose (alone, or with various concentrations of added adenosine) for 4 h, then samples added to 4X SDS sample buffer with ß-mercaptoethanol.
SDS- Polyacrylamide gel electrophoresis was carried out with equal volumes of sample added per lane, then the proteins were transferred to nitrocellulose. The nitrocellulose was blocked, then Western blotting was carried out using rabbit polyclonal antibody against bundlin, the main structural subunit of BFP, using a dilution of 1: 15,000. The antibody to BFP was a kind gift from Dr. Michael S. Donnenberg, Univ. of Maryland at Baltimore. Western blots were developed by chemiluminescence using the Lumi-Glo kit from Kierkegaard & Perry using a 10 min exposure to X-ray film.
Panel A, Western immunoblot against BFP. Despite a 3-fold increase in bacterial growth, BFP expression dropped to nearly undetectable levels at 100 μM adenosine.
Panel B, densitometry scan of a blot from a different experiment similar to that in Panel A. The increase in BFP at lower adenosine concentrations is due to adenosine stimulation of growth, followed by a fall-off in expression due to inhibition of BFP transcription (see Figs. 2 and 4).

Supporting Fig. 2. Effect of Adenosine on the Abundance of EPEC Secreted Proteins



Legend: EPEC E2348/69 was grown in 10 ml minimal medium plus various concentrations of adenosine for 6 h, then the supernatant medium collected and filtered, then subjected to lyophilization overnight, and resuspended in 1 ml water.

The protein concentration of each sample was determined by Bio-Rad protein assay, and the samples were diluted with water as needed to yield an equal amount of protein per lane. Samples were subjected to SDS-PAGE using Invitrogen Bis-tris minigels, then stained with silver stain (Sterling Silver kit, National Diagnostics, Atlanta, Ga.)

The gel was photographed and densitometry scan was perfomed on a Bio-Rad GS-400 gel scanner using Quantity One image analysis software. Densities of bands were expressed as a percent of the no-adenosine control.

Panel A , photograph of the silver stained gel. MW, molecular weight markers. The positions of the 110 kDa EspC protein band and of the EspD/EspB doublet (39 and 38 kDa, respectively) are shown with arrows.

Panel B, graph of the effect of adenosine on the densities of protein bands, expressed as a percent of the no-adenosine control .

Supporting Figure 3 .

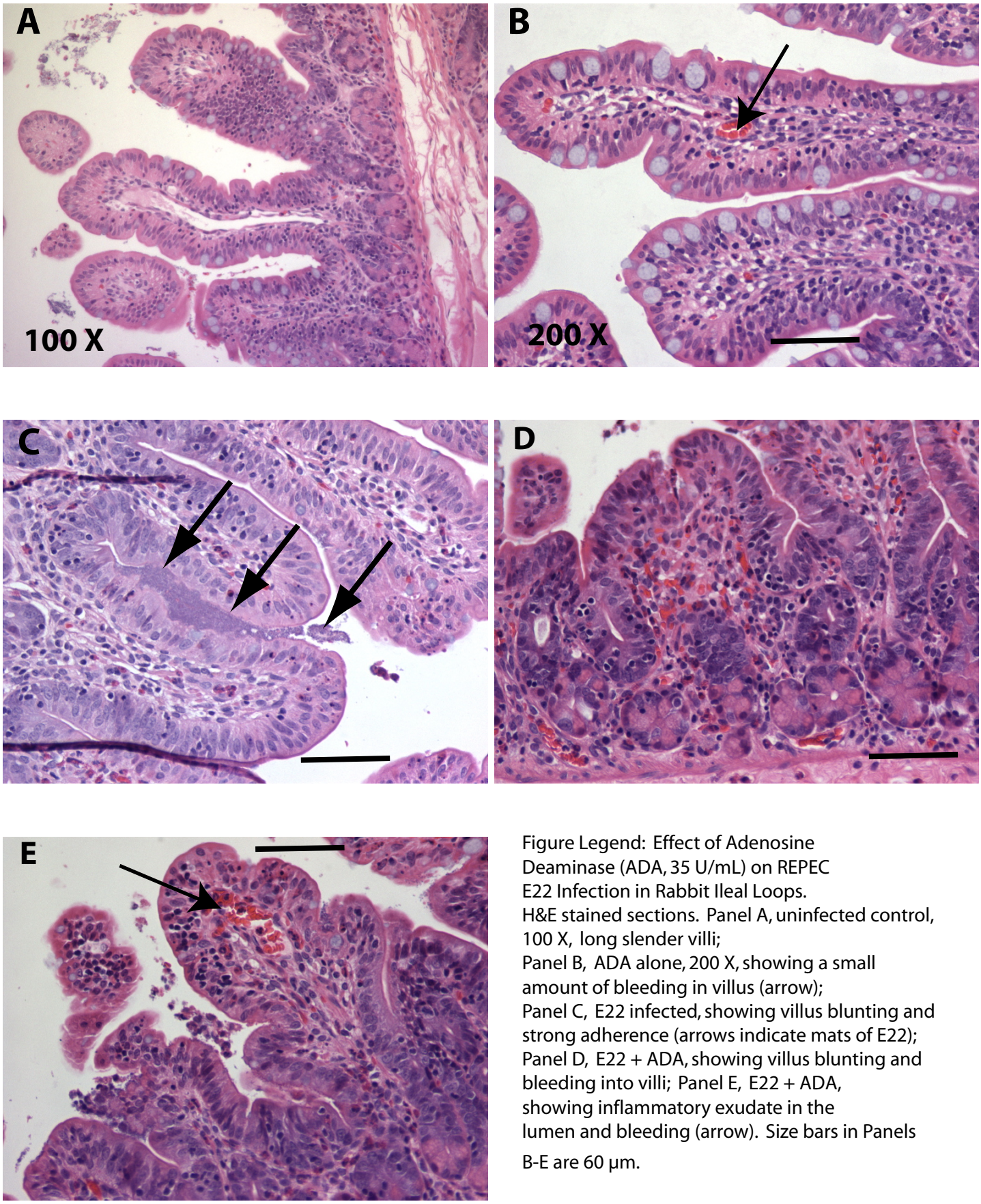


Figure Legend: Effect of Adenosine Deaminase (ADA, 35 U/mL) on REPEC E22 Infection in Rabbit Ileal Loops. H&E stained sections. Panel A, uninfected control, 100 X, long slender villi; Panel B, ADA alone, 200 X, showing a small amount of bleeding in villus (arrow); Panel C, E22 infected, showing villus blunting and strong adherence (arrows indicate mats of E22); Panel D, E22 + ADA, showing villus blunting and bleeding into villi; Panel E, E22 + ADA, showing inflammatory exudate in the lumen and bleeding (arrow). Size bars in Panels B-E are 60 μm.