Supplemental Figure 1.

A and *B* – control immunostaining for testing the specificity of antibodies against Stx1 and Stx2. Representative single 3D projection from a stack of ten 1 μm confocal optical sections obtained by 40X water immersion lens from normal human colonic tissue, *A*: surface region of colon, *B*: crypt region of colon, which were immunostained using antibody against Stx2 (green), Stx1 (red) and nuclear staining (blue by Hoechst). Neither antibody labeled normal human colonic tissue which does not have the Shiga toxins. Bars in (*A*) and (*B*) - 50μm.

C-F – bacteria found associated with epithelial cells in the ileocecal valve sample represent the EHEC *E. coli* O157:H7 strain. Tissue sample was immunostained with (FITC)-labeled anti- *E. coli* O157:H7 (green) antibody and nuclei and bacteria (blue by Hoechst). *C-E*: Representative single 3D projection from a stack of five 0.5 μm confocal optical sections obtained by 100X oil immersion lens, bar in *C* – 20 μm. The associated with epithelium bacteria (arrows in *C*) are positive for *E. coli* O157:H7 antigen (green in *D*). *F*- zoomed region from (*E*) demonstrates the bacteria at the apical surface of epithelial cells are EHEC O157:H7 serotype, bar – 5 μm. Tissue samples in (*E-F*) was also immunostained with Stx1B-Alexa 568 (red) to detect the Gb3 expression and no red fluorescence above the background was detected in epithelial cells.

Supplemental Figure 2. Inhibition of clathrin-dependent endocytosis by CPZ (10 μg/ml) significantly stimulates uptake of HRP at 4 h in T84 cells, but did not affect its

transcytosis at 4 h. Data were obtained from 3 independent experiments. *P<0.05 compared to the control.

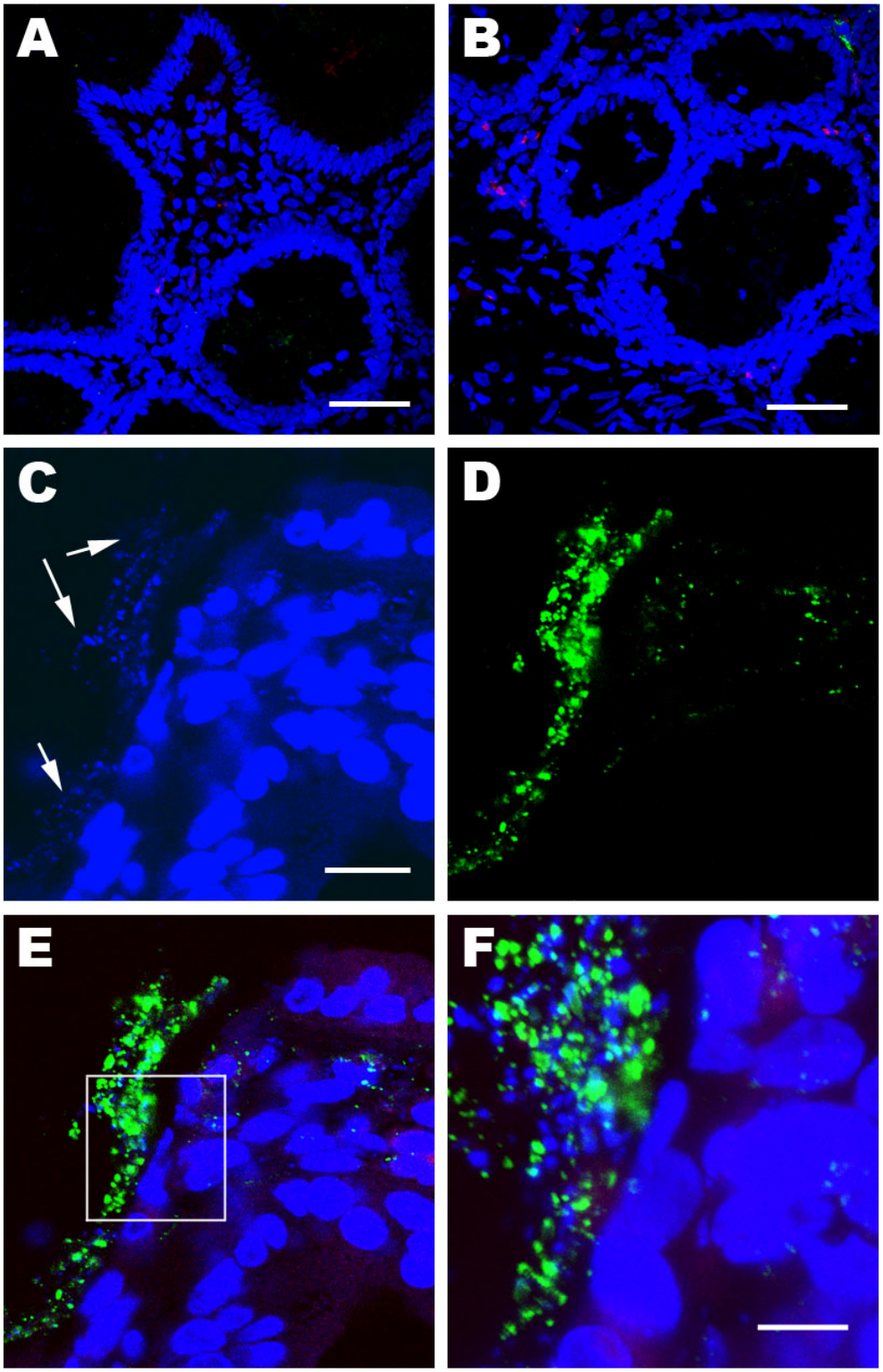
Supplemental Figure 3. TPA significantly stimulates the endocytosis of fluid phase markers in T84 colonic epithelial cells. TPA dose-dependency effect on the uptake of HRP and 3 kDa dextran at 4 h. Low TPA concentration (20 nM) slightly but significantly stimulates HRP endocytosis, but does not increase it further with higher concentrations. The TPA effect at low doses is greater on 3 kDa dextran uptake than on HRP or Stx1B, but also reaches saturation (at 100 nM). Each bar represents the means from 3 independent experiments done in duplicate. *P<0.05 compared to the HRP in control cells; **P<0.05 compared to the 3 kDa dextran in control cells.

Supplemental Figure 4. The fluid phase marker dextran (40 kDa) co-localizes with actin in NEM-induced apical actin ruffles, but not inside the cells. Representative confocal optical sections with a 1.6 μm step (*A, D, G, J*) through the apical and sub-apical regions of T84 cells pretreated for 30 min with 200 μM NEM and co-incubated with dextran – Alexa 488 (green, *C, F, I, L*) for additional 30 min. Cells were fixed and counterstained for F-actin by phalloidin –Alexa 568 (red, *B, E, H, K*). Bar – 10 μm.

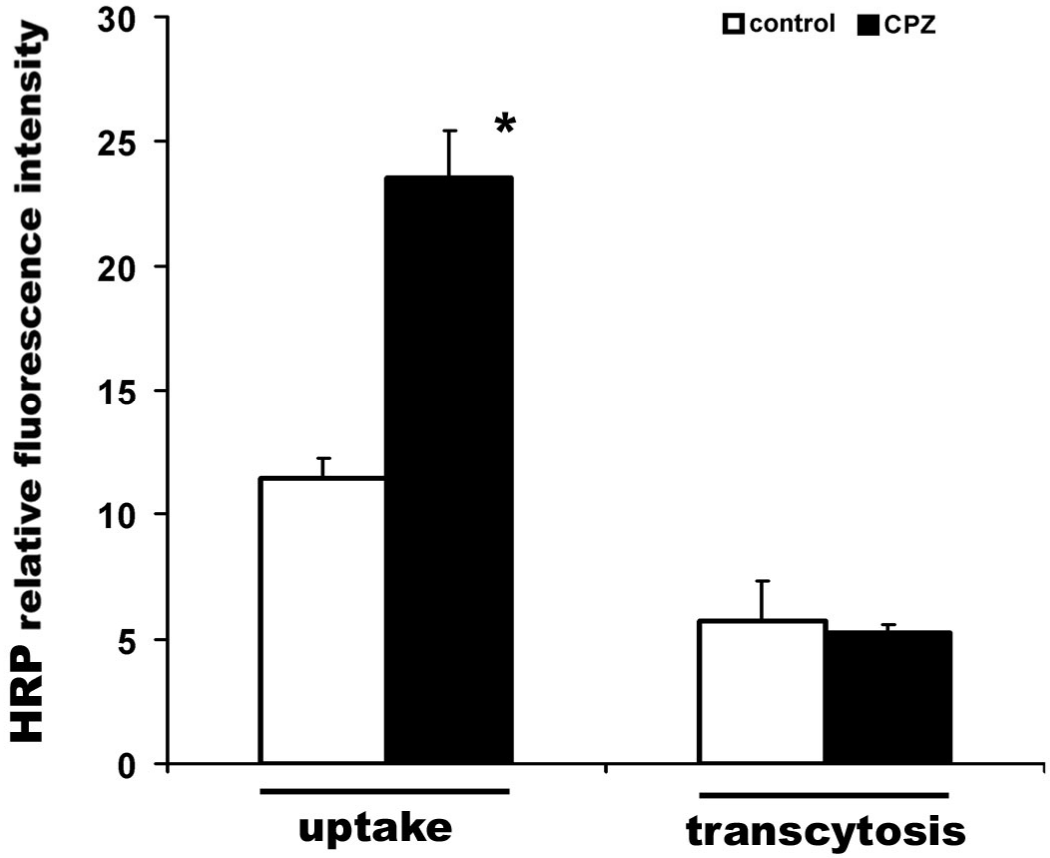
Supplemental Figure 5. Rabbit cecal epithelial cells express the glycosphingolipid Gb3 neither in control conditions nor in the presence of attached RDEC-H19A bacteria – good model to study the mechanism of Stx uptake by receptor-free intestinal epithelial cells.

Representative images of cecal tissue from: (*A*) control rabbit treated with PBS or (*B*) infected for 5 days with RDEC-H19A bacteria that were attached to the epithelial cells (arrows). Staining in (*A*) – nuclei by propidium iodide (red), Gb3 by monoclonal antibody against Gb3 with secondary Alexa 488 Ab (green); staining in (*B*) – nuclei and bacteria by Hoechst (blue), actin by phalloidin-Alexa 488 (green), and Gb3 by Stx1B-Alexa 568 (red). Only some cells in *lamina propria* were positive for Gb3 in both control and experimental rabbits. Similar results were obtained form rabbits infected with RDEC-1 bacteria (data not shown). Bar in (*A*) – 50 μm and in (*B*) - 10 μm.

Supplemental Fig. 1.



Supplemental Fig. 2.



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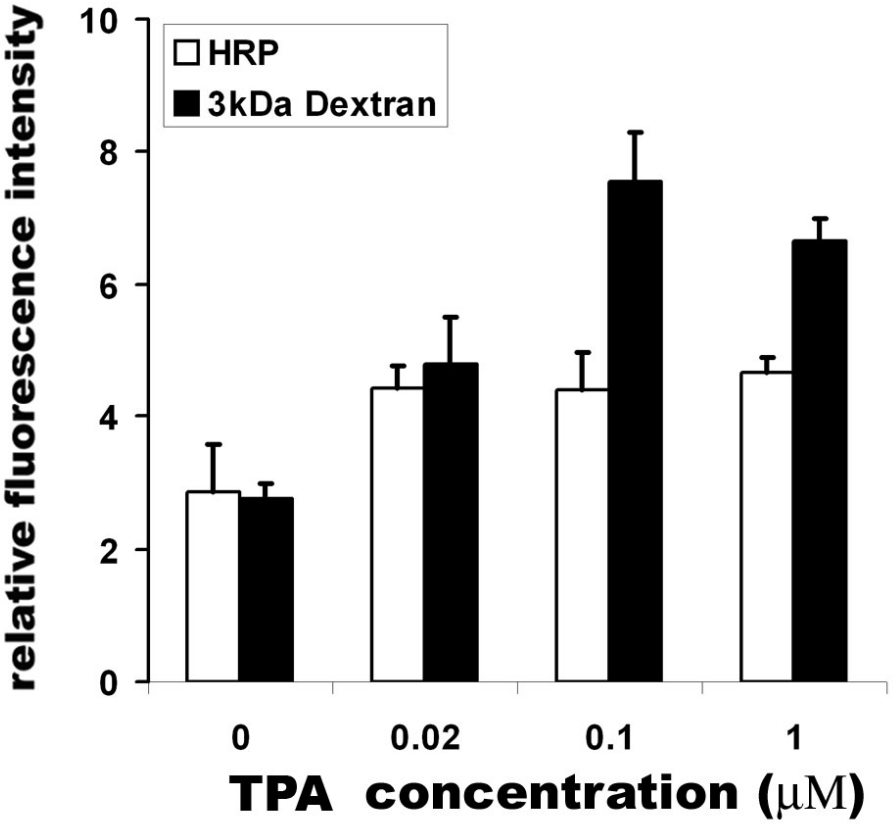
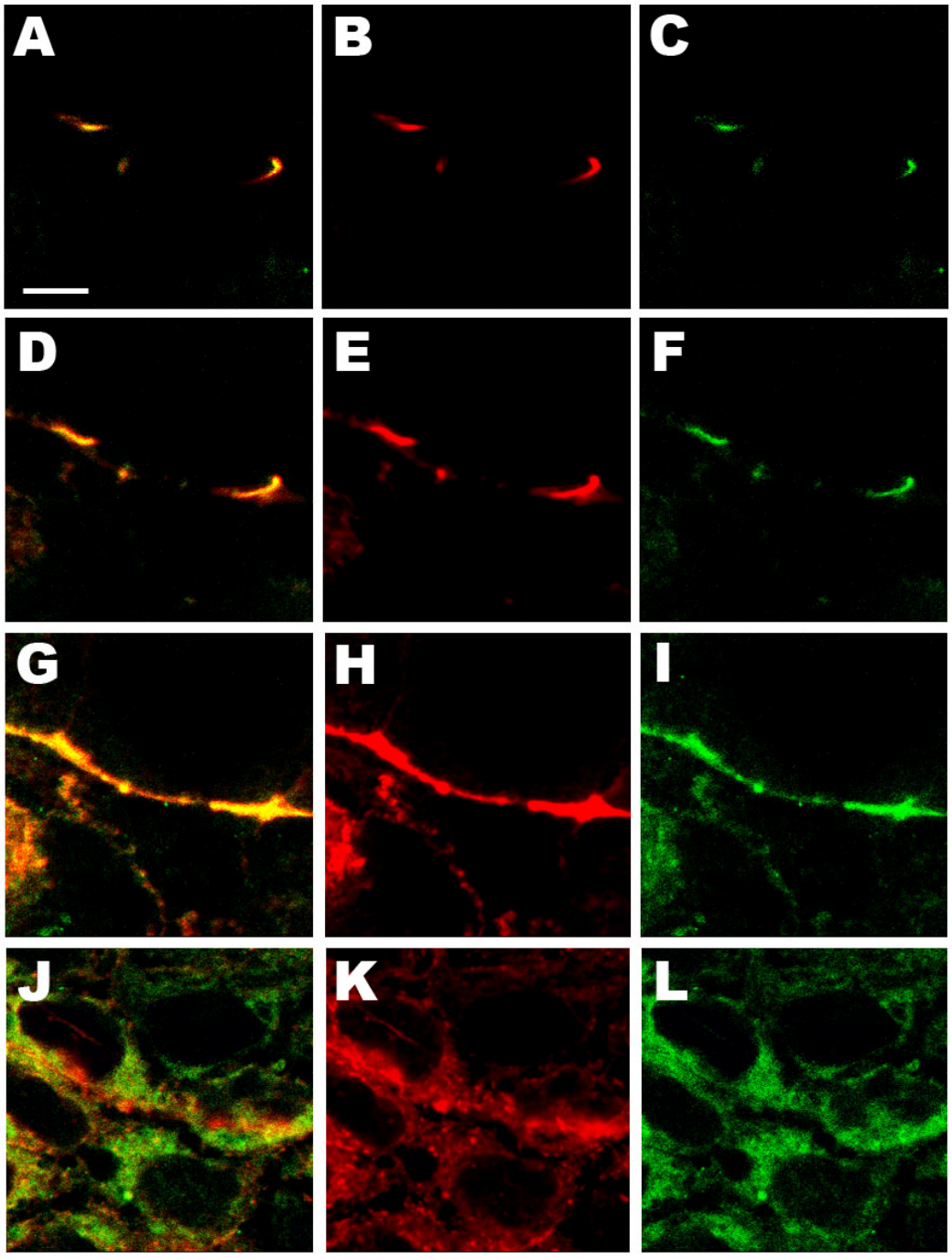


Supplemental Fig. 4.



Supplemental Fig. 5.