Figure S1. EPEC infection induces expression of IFNβ in HT-29 cells. HT-29 cells were grown until confluent then infected with either WT or *ΔescF* EPEC at MOI 50 for 3 hours. RNA was isolated and analyzed by qRT-PCR for IFNβ expression and GAPDH was measured as a control. Data shown is the mean of 3 separate experiments. Error bars indicate standard deviation. p value was calculated using the student t-test. *p<0.05.
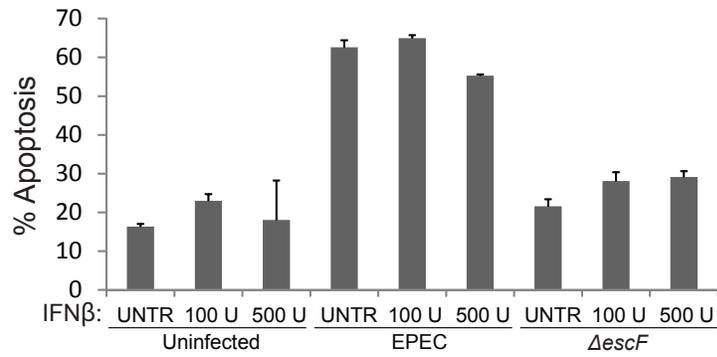
Figure S2. Differentiated Caco-2 cell monolayers were treated with 100 U/ml or 500 U/ml IFNβ for 16 hours. Cells were then infected with either WT or *ΔescF* EPEC at MOI 100 for 3 hours. Apoptotic cells were detected by flow cytometry for cleaved caspase-3 and -7 and the percentage of apoptotic cells is shown. This experiment was performed with 3 replicates for each condition. Error bars indicate standard deviation.
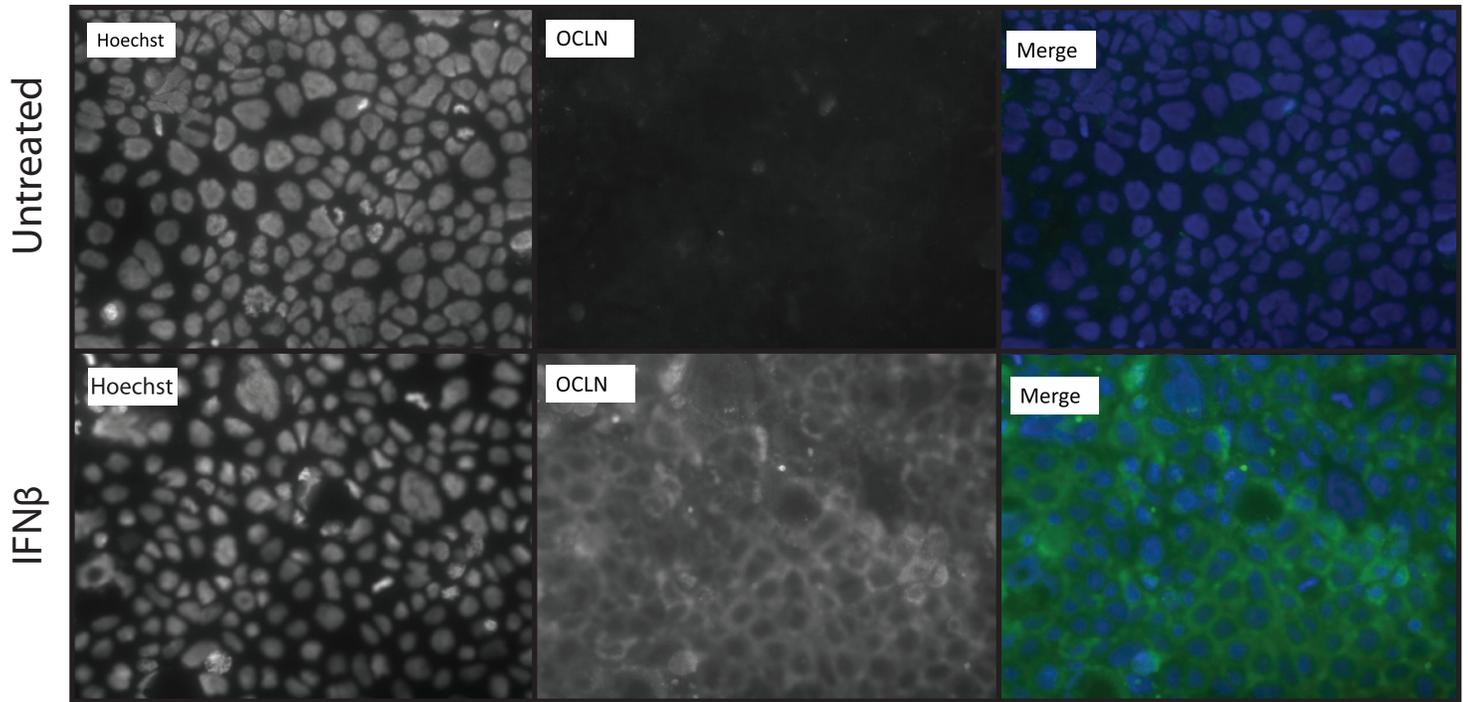
Figure S3. IFN treatment induces occludin expression and localization to paracellular regions. Caco-2 cells were grown on chamber slides for 5 days. Untreated cells and cells treated with 10 U/ml IFNβ were analyzed by immunofluorescense for occludin expression and localization (center panels). Nuclei were stained with Hoechst dye (left panel)s. Representation images of untreated and treated cells are shown.
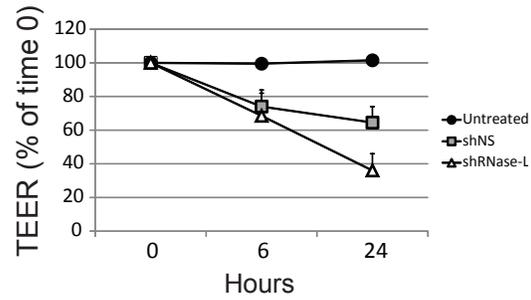
Figure S4. Inflammatory cytokine-induced loss of barrier function is exacerbated in RNase-L deficient cells. Differentiated monolayers of shNS and shRNase-L cells were treated with 10 ng/ml IFNγ for 12 hours followed by 10 ng/ml TNFα treatment for 6 hours and TEER was measured at 6 and 24hours post- TNFα treatment. Datapoints shown are the mean of three separate experiments. Error bars indicate standard deviation.
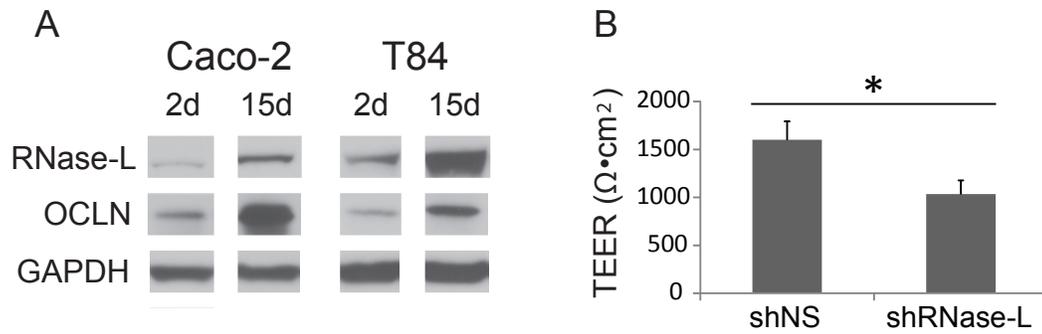
Figure S5. RNase-L expression correlates with differentiation and increased barrier function in IEC monolayers. A) Caco-2 and T84 cells were grown on transwell plates for 15 days and RNase-L and occludin (OCLN) proteins were analyzed by Western Blot. GAPDH was analyzed as a loading control. The image shown is representative of 3 separate experiments. B) TEER was measured in differentiated shNS and shRNase-L monolayers. Datapoints shown are the mean of three separate experiments. Error bars indicate standard deviation. p value was calculated using the student t-test. *p<0.05.