**Supplemental Figure 1.** EspF/SNX9 binding is dispensable in T84 cells for aPKCζ and F-actin pedestal organization, PKC activity, and TJ disruption. T84 monolayers were infected, or not (UI), with wt EPEC, ΔespF, or ΔespF complemented with mutated espF (ΔespF/pestF3) or wt espF (ΔespF/pestF) to assess the localization of aPKCζ and F-actin, PKC kinase activity and TER. (A) aPKCζ aggregates and co-localizes with F-actin under attached bacteria 2 hours post-infection with wt EPEC, ΔespF/pestF3 or ΔespF/pestF. In contrast, reduced co-localization is apparent after infection with ΔespF. Arrowheads indicate regions of co-localization between aPKCζ and F-actin. Scale bars: 10μm (en face); 5μm (z-stack). (B) Schematic representation of aPKCζ (green), F-actin (red), and co-localization (yellow) within pedestals following infection with wt EPEC and EspF mutant strains. (C) Significant increase in PKC activity 2 hours post-infection with wt EPEC and EspF mutant strains compared to UI monolayers. *p<0.05. (D) All EPEC strains significantly reduced TER 3 hours post-infection compared to UI #p<0.001. TER is significantly higher after infection with ΔespF, but not ΔespF/pestF3, compared to wt EPEC infection *p<0.05. TER reported as percent change from baseline.