Supplemental Materials

Molecular Biology of the Cell

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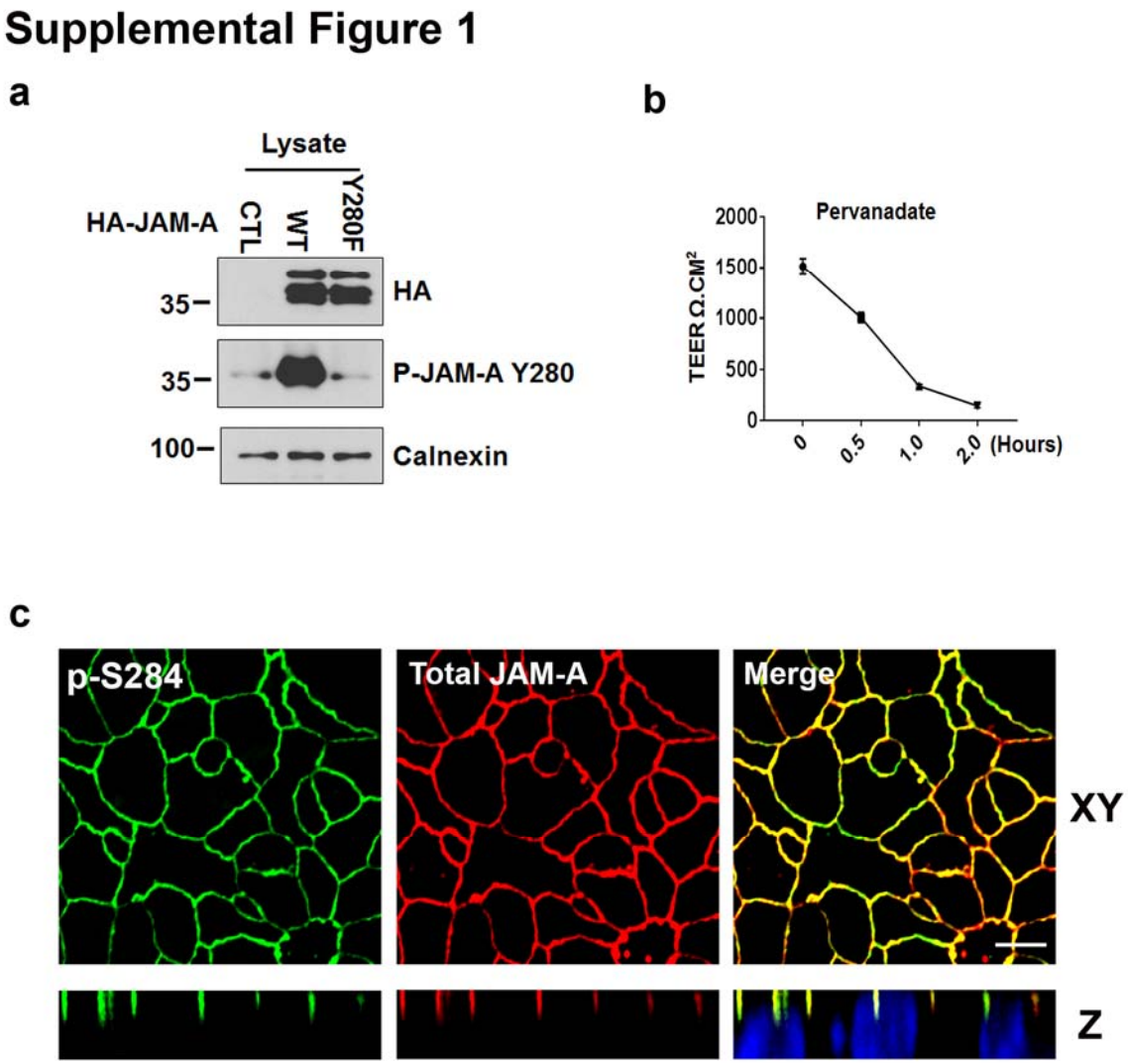
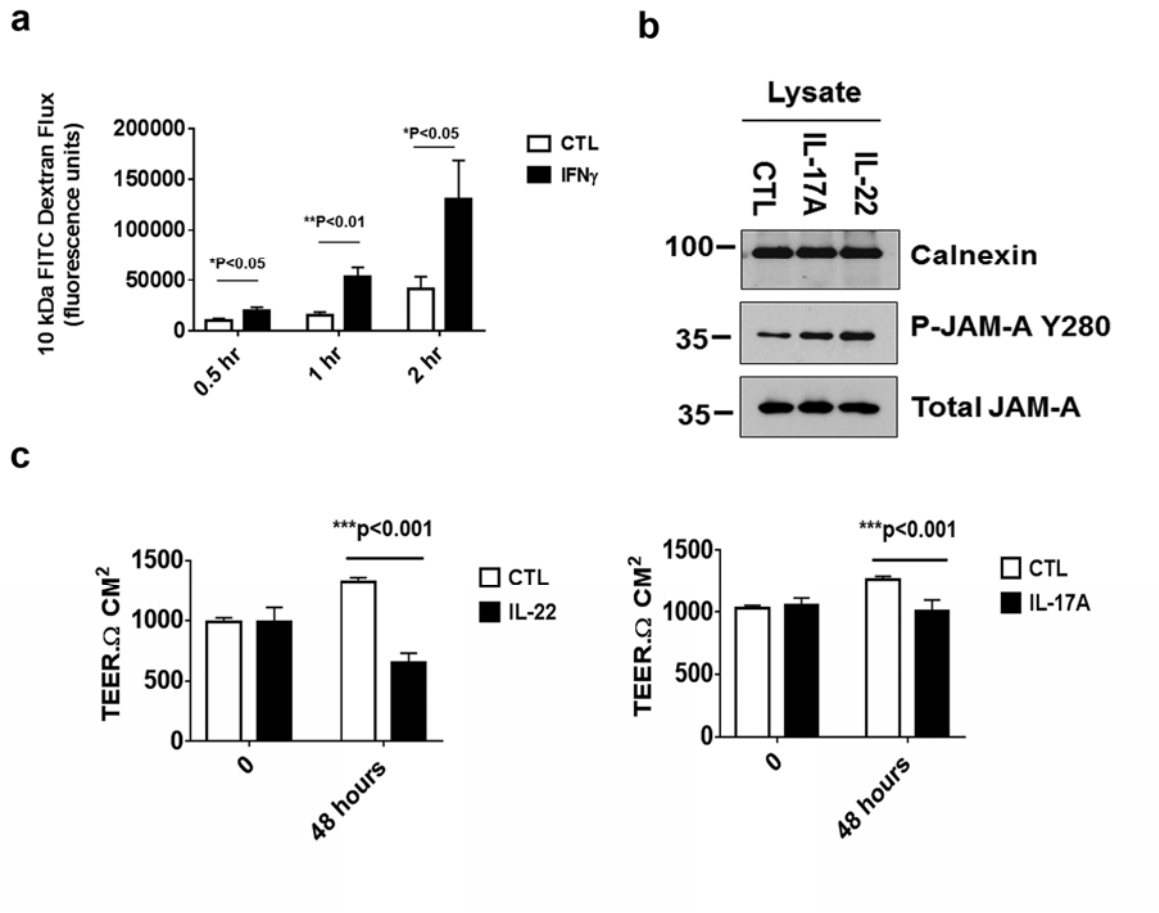
Supplemental materials

Supplemental Figure1. a HA-JAM-A wt, Y280F or empty vector (CTL) were transfected to HEK 293T for 48 hours, then cells were treated with pervanadate for 1 hour before harvesting. Immunoblots were performed as indicated. **b:** Pervanadate treatment results in a dramatic fall in TEER in SK CO-15. **c:** T84 were grown on Transwell filters until fully confluent. Monolayers were fixed, permeabilized with 1% SDS and stained for p-JAM-A S284 and total JAM-A. Confocal Z-stacks indicate that p-JAM-A S284 localizes to TJs. Scale bar:10 μm.



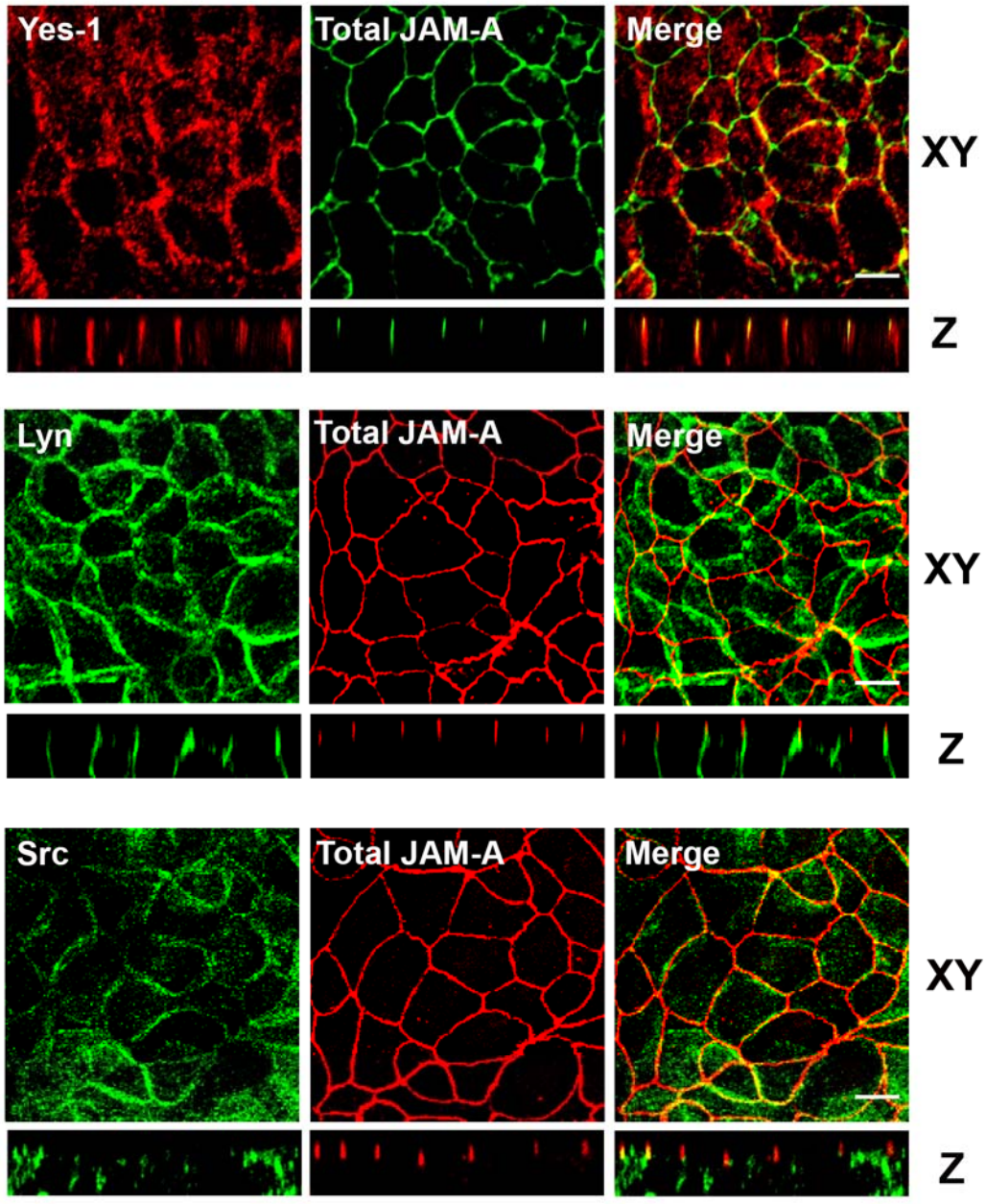
Supplemental Figure 2. Effects of cytokines on barrier and p-JAM-A Y280 in monolayers of SK CO-15 cells and human colonoids. a: Confluent SK CO-15 monolayers were treated with 100 ng/ml of IFNγ for 48 hours and permeability assessed by transepithelial flux of 10 kDa FITC dextran at the time points indicated, Bar graphs represent the mean ± SEM (N=6) of fluorescence intensity measured with a Cytation 5 imaging reader (Bio Tek) between CTL (untreated) and IFNγ treated groups. **b:** Confluent monolayers derived from human colonoids were treated with IL-17A (100 ng/ml) or IL-22 (40 ng/ml) for 48 hours, followed by lysis and immunoblotting for p-JAM-A Y280, total JAM-A and calnexin. **c:** Confluent monolayers of T84 cells were grown on Transwell filters until TEER values reached 1000 $\Omega.CM^2$. Cells were then treated with human recombinant IL-22 (40 ng/ml) or IL-17A (100 ng/ml) for 48 hours followed by assessment of TEER and compared to untreated monolayers (CTL). Bar graphs represent mean ± SEM of three independent experiments. * $p<0.05$, *** $p<0.001$ between two groups as determined by two-tailed student t-test.

Supplemental Figure 2

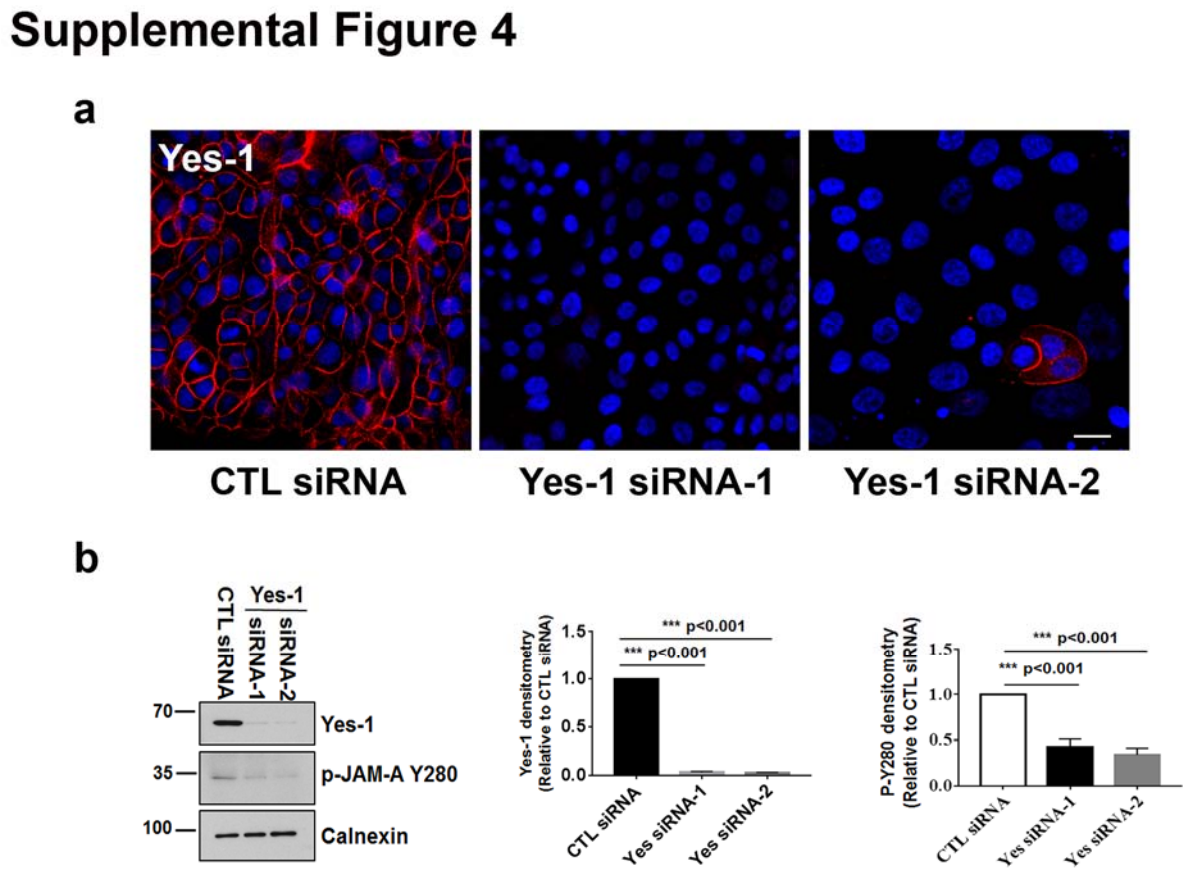


Supplemental Figure 3. Colocalization of JAM-A with Yes-1 in IECs. T84 cells were grown on Transwell filters until confluent. Monolayers were then fixed, permeabilized with 1% SDS and labeled with antibodies against JAM-A and Yes-1, Lyn, or Src. Confocal Z-stacks reveal partial colocalization of Yes-1 with JAM-A in TJs. By contrast there is little co-localization of Lyn/Src with JAM-A. Scale bar 10 μm.

Supplemental Figure 3

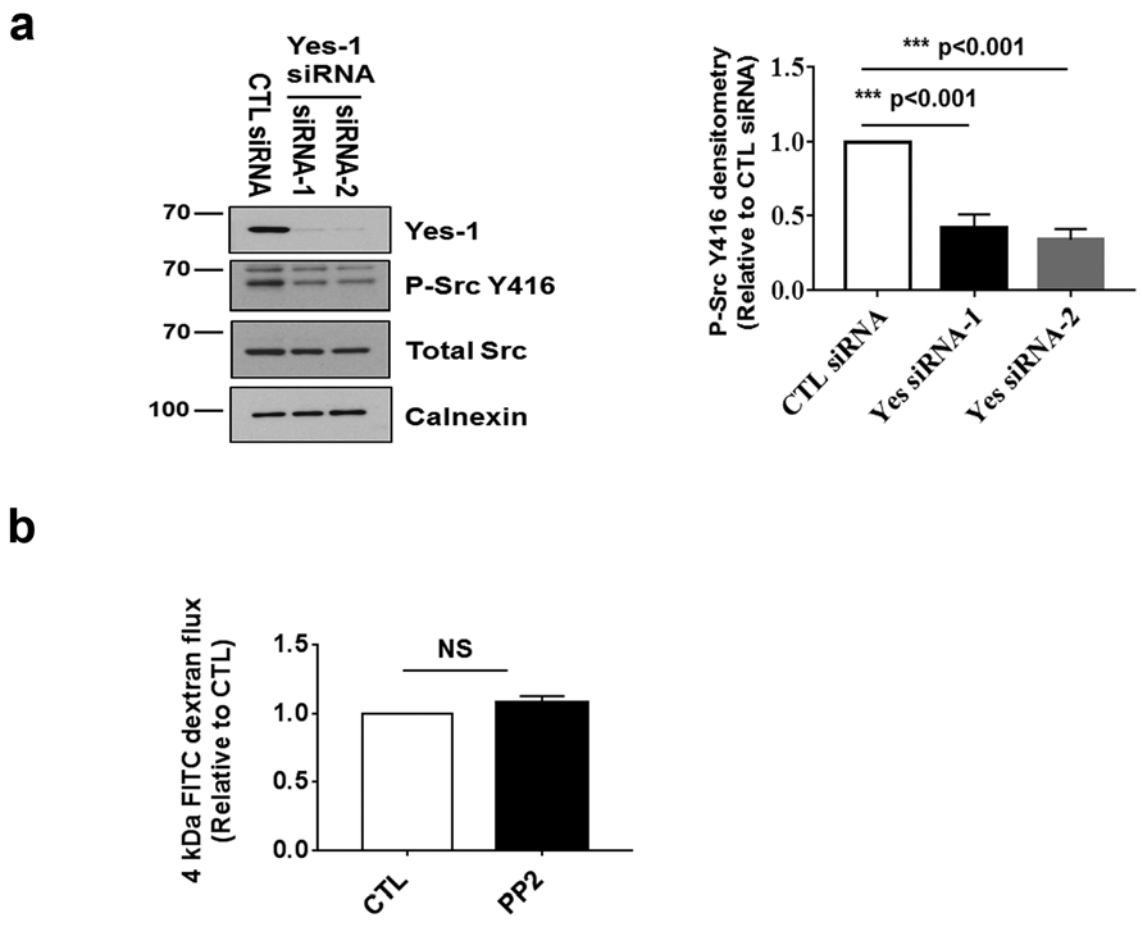


Supplemental Figure 4. Baseline of p-JAM-A Y280 was decreased in Yes-1 knockdown IECs. a. Immunofluorescence of Yes-1 in SK CO-15 cells after 48 hours of transfection with control siRNA or Yes-1 siRNAs. **b.** Cell lysates from control or Yes-1 siRNA were run on SDS-PAGE gels and then immunoblotted with antibodies indicated (left). Quantitation of Yes-1 (middle) and p-JAM-A Y280 (right) immunoblots in Yes-1 siRNA and control siRNA (N=3 individual experiments. Bar graphs represent mean ± SEM). ***p<0.001 between two groups were determined by two-tailed student t-tests. Scale bar: 10 μm.



Supplemental Figure 5. Decreased p-Src Y416 after knockdown of Yes-1 in IECs. **a.** SK-CO15 IECs were treated with Yes-1 siRNAs, lysed, subjected SDS-PAGE and transferred to PDVF membranes. Immunoblots were probed for Yes-1, p-Src Y416, total Src and calnexin. Calnexin served as a loading control (left). Also shown is densitometric quantitation of p-Src Y416 in Yes-1 siRNA and control siRNA (right). **b.** PP2 treatment has no effect on paracellular flux of 4 kDa FITC dextran across epithelial monolayers compared to untreated controls (CTL). Bar graphs represent three independent experiments with mean ± SEM. *** $p<0.001$ between two groups determined by two-tailed student t-test, NS = no significant difference between two groups.

Supplemental Figure 5



Supplemental Figure 6. Increased co-localization of PTPN13 with JAM-A after cytokine exposure. Confluent monolayers of epithelial cells were treated with cytokines IL-17A, IL-22, TNFα, or IFNγ for 48 hours. Immunofluorescence staining with anti-PTPN13 and JAM-A were performed and images obtained with a confocal microscope. Scale bar: 10 μm.

Supplemental Figure 6