Supplemental Materials

Molecular Biology of the Cell

Monteiro et al.

Supplemental Figures

Figure S1: Treatment of confluent T84 monolayers with σ1 for 3 hours led to significant reduction in TER when compared to cells treated with σ1_G381A mutant. Either WT σ1 or σ1_G381A (20 μg/ml) was added to apical and basolateral compartments. TER was evaluated at 1 and 3 hours (A, representative experiment with three independent samples. Bars represent mean +/- SD). SK-CO15 cells stably expressing non-silencing shRNA (NS) or shRNA targeting JAM-A (shJAM-A) were verified by immunoblot. SK-CO15 cells stably downregulated for JAM-A (shJAM-A) do not develop trans-epithelial resistance (TER) after four days of plating when compared to SK-CO15 cells stably expressing non-silencing shRNA (NS) (B, representative experiment with three independent samples. Bars represent mean +/- SD).

Figure S2: JAM-A (JA) co-immunoprecipitates with ZO-2 but not ZO-1 in intestinal epithelial cells. JAM-A immunoprecipitates from cell lysates prepared with a Triton X-100, Sodium deoxycholate and SDS based buffer (RIPA) revealed a 160 kDa ZO-2 immunoreactive band suggesting co-association between JAM-A and ZO-2, but did not reveal a 220 kDa ZO-1 immunoreactive band. Under the same conditions, ZO-2 immunoprecipitates revealed a 220 kDa ZO-1 immunoreactive band suggesting co-association between ZO-2 and ZO-1.

Figure S3: Sample qRT-PCR results verifying knockdown of targets in SK-CO15 cells used for in vitro permeability studies.

Figure S4: Additional PCR demonstrating lack of signal with alternate Rap2a primer pair. A common cDNA template and PCR master mix was prepared and then divided into four tubes containing specific primer pairs (A). Table outlining si/shRNA targets used in this study (B). Table outlining PCR primers used for PCR and qRT-PCR. Beta-actin was used as a housekeeping gene (C).

Figure S1

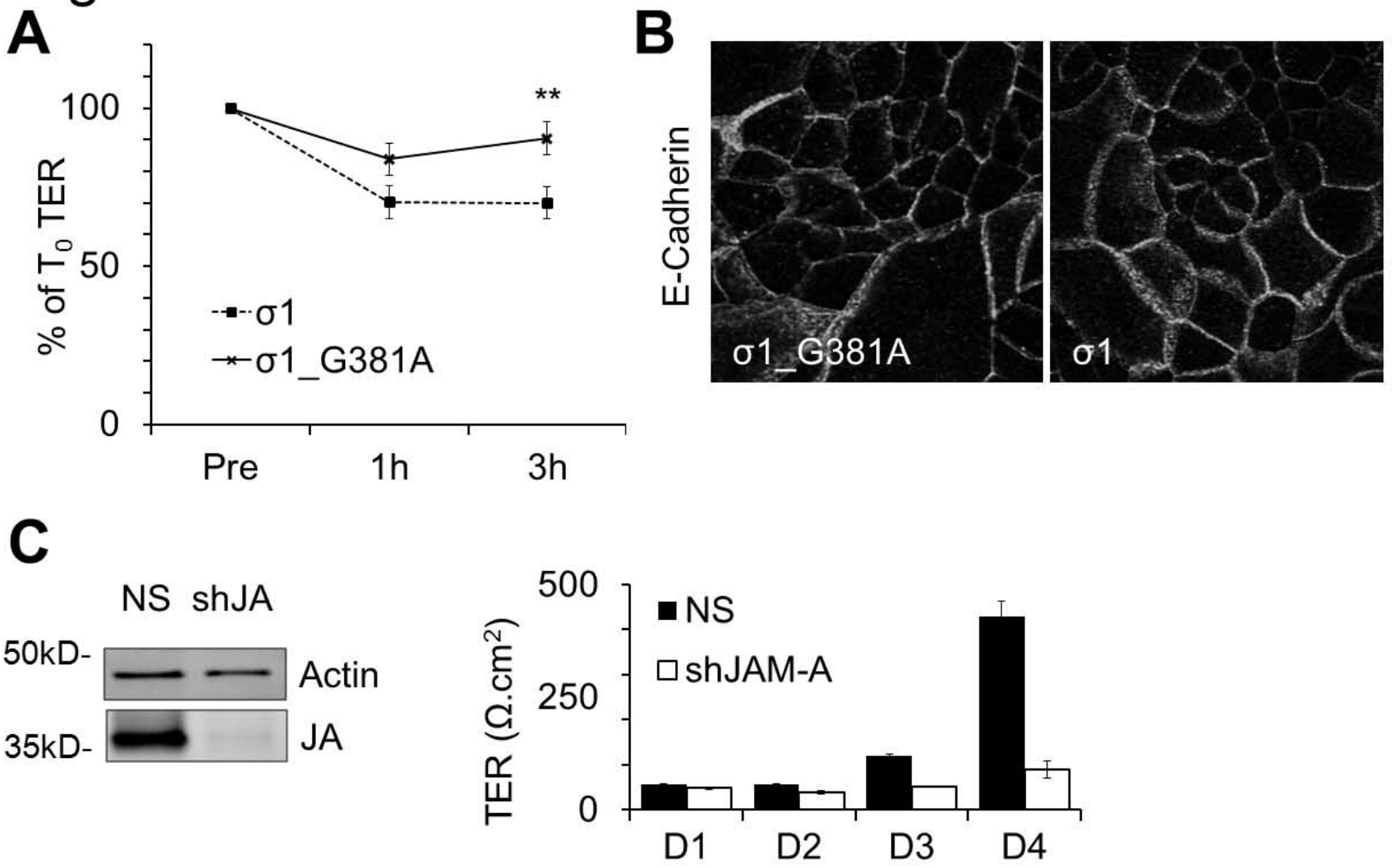

A
100
50
0
% of T0 TER
**
σ1
σ1_G381A
Pre
1h
3h
B
E-Cadherin
σ1_G381A
σ1
C
NS shJA
50kD-
Actin
35kD-
JA
500
250
0
TER (Ω.cm2)
NS
shJAM-A
D1
D2
D3
D4

Figure S2

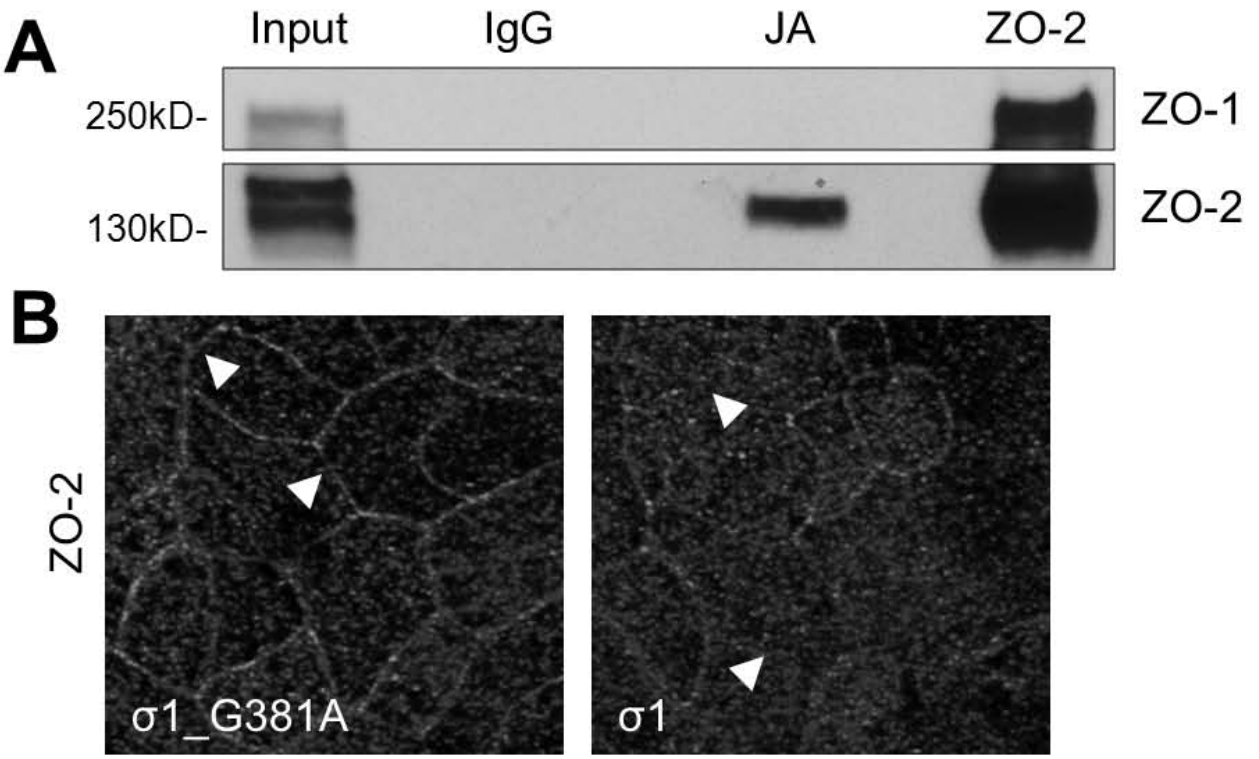
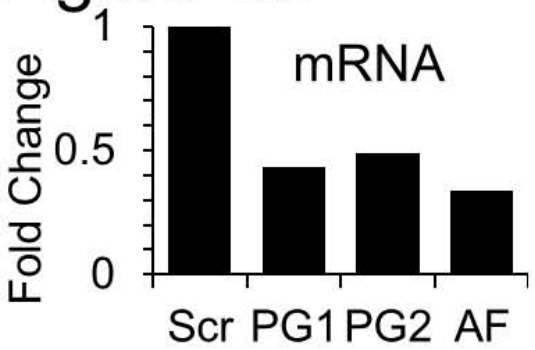


Figure S3


mRNA
Fold Change
1
0.5
0
Scr
PG1
PG2
AF

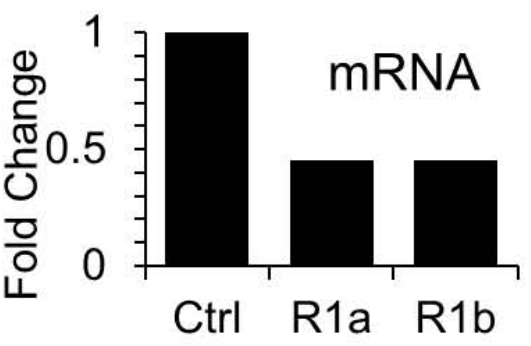
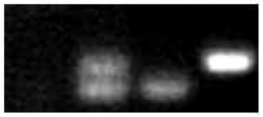

mRNA
Fold Change
1
0.5
0
Ctrl
R1a
R1b

Figure S4

A



B

Target	si/shRNA sequences
Rap1A	5′-GAUCAAUGUUAAUGAGAUATT-3′
Rap1A'	5'-GCGAGUAGUUGGCAAAGAG-3'
Rap1B	5′-CGAGUACUGUGGAUGUGAATT-3′
Rap1B'	5'-GUAUAAUGUCUUAGAUUAATT-3'
Rap2B	5′-GGUGGUCUUAGUAAUAUAATT-3′
Rap2B'	5'-CACUUAAGUUUGAUAUCAATT-3'
Rap2C	5′-GGCUAAUAUACAAGGGUUATT-3′
Rap2C'	5'-UGUUCGUAUUAGAGACAAATT-3'
PDZ-GEF1	5′-GGAAGAAAGUGCCCGUAAATT-3′
PDZ-GEF1'	5′-CCGCUACAUCAUGAUCAGUAA-3′
PDZ-GEF2	5'-CUGCAUGAAUUGUAACUGAA3'
PDZ-GEF2'	CAGGAAGAAGGGACAAACAAA
Afadin	5'-UGAGAAACCUCUAGUUGUA-3'
Afadin'	5'-GUUAAGGGCCCAAGACAUA-3'
JAM-A	5'-GAAGUGAAGGAGAAUUCAATT-3'
JAM-A'	5'-CGGGUGACCUUCUUGCCAATT-3'
Scramble	Proprietary (Qiagen, USA)
shNS	5'-GGAATCTCATTCGATGCATAC-3'
shJAM-A	5'-TGAGAATAATCCTGTGAAGTT-3'
shJAM-A'	5'-ACAACAGGAGAGCTGGTCTTT-3'

C

Target	Forward Primer	Reverse Primer
Rap1A	5′-GGCAAAGAGCAGGGCCAGAATT-3′	5′-CTAGAGCAGCAGACATGATTTC-3′
Rap1B	5′-GGGAAGGAACAAGGTCAAAATC-3′	5′-TTAAAGCAGCTGACATGATG-3′
Rap2A	5′-CATGCTGTTCTGCATGTAAC-3′	5′-CAAGTTCTGCAGTGGAGTAG-3′
Rap2A'	5'-TCCGCGTGAAGCGGTATGAGA-3'	5'-AGGGCTCTGCCTTCGCTGCA-3'
Rap2B	5′-GGCCCTGGCTGAGGAGTGGA-3′	5′-GAGGATCACGCAGGCCGAGC-3′
Rap2C	5'-TGGCCATACCGAGCAGATAAAACTCA-3'	5'-ACAGGTTTACCAAGGCTCAGTTCTGC-3'
PDZ-GEF1	5′-AAATTCGTCACGTTGGCCGAATGG-3′	5′-ACTCCGCCATTTCTTCTTCCGAGT-3′
PDZ-GEF2	5′-TGTTGACTCCATGTCTGCAGCTCT-3′	5′-ACCCAGGGCCATGTTGACTATGAT-3′
Afadin	5'-CCGACATCATCCACCACTGG-3'	5'-CAGCATTCGCATATCAGGTCG-3'
JAM-A	5'-GTTGTCCTGTGCCTACTCGG-3'	5'-CCGTGTCACGGACTTGAAGG-3'
Beta-Actin	5'-TGACCCAGATCATGTTTGAGA-3'	5'-AGTCCATCACGATGCCAGT-3'