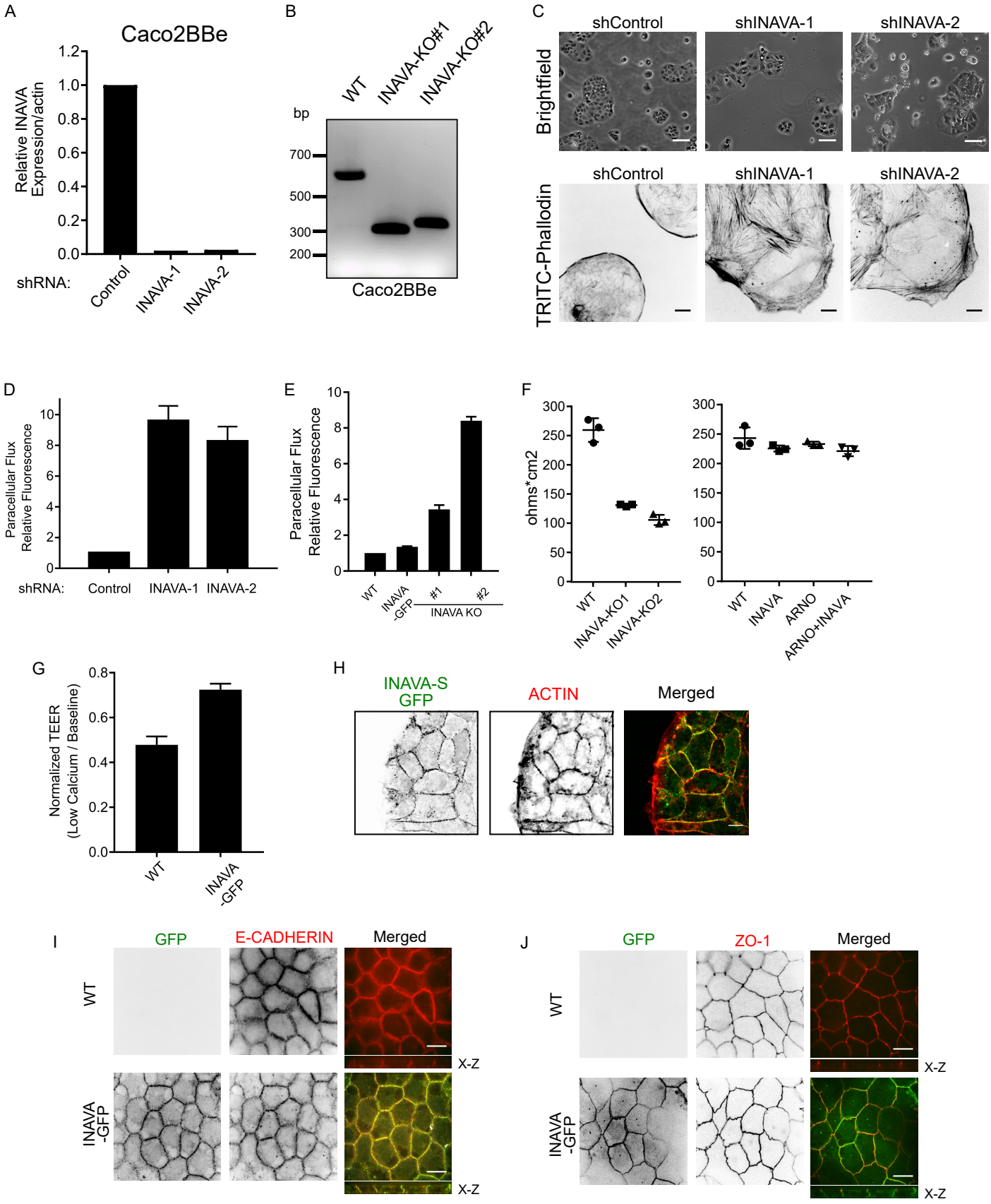
A
Caco2BBe
Relative INAVA Expression/actin
1.2
1.0
0.8
0.6
0.4
0.2
0.0
shRNA:
Control
INAVA-1
INAVA-2
B
WT
INAVA-KO#1
INAVA-KO#2
bp
700
500
300
200
Caco2BBe
C
shControl
shINAVA-1
shINAVA-2
Brightfield
shControl
shINAVA-1
shINAVA-2
TRITC-Phallodin
D
Paracellular Flux
Relative Fluorescence
10
8
6
4
2
0
shRNA:
Control
INAVA-1
INAVA-2
E
Paracellular Flux
Relative Fluorescence
10
8
6
4
2
0
WT
INAVA -GFP
#1
#2
INAVA KO
F
ohms*cm2
300
250
200
150
100
50
0
WT
INAVA-KO1
INAVA-KO2
WT
INAVA
ARNO
ARNO+INAVA
G
Normalized TEER
(Low Calcium / Baseline)
0.8
0.6
0.4
0.2
0.0
WT
INAVA -GFP
H
INAVA-S GFP
ACTIN
Merged
I
GFP
E-CADHERIN
Merged
WT
INAVA -GFP
X-Z
X-Z
J
GFP
ZO-1
Merged
WT
INAVA -GFP
X-Z
X-Z

Figure 1-figure supplement 1. INAVA is important for barrier function in epithelial Caco2BBe cells.
(A) Verification of Caco2BBe INAVA knockdown by qPCR.
(B) Genomic DNA PCR of INAVA locus in Caco2BBe WT and INAVA exon deleted CRISPR knockout lines.
(C) Top, brightfield images of control or INAVA stable knockdown Caco2BBe grown 2 days sparsely on coverslips. Scalebar = 200 μm. Bottom, confocal images of the above cells stained for F-actin (phalloidin-TRITIC). Scalebar = 10 μm.
(D) Permeability assay with 4 kDa FITC-Dextran in control or INAVA knockdown Caco2BBe cells. Cells were cultured on 2-D transwell inserts in triplicate for 3 days and treated apically with 4 kDa FITC-Dextran for 2 hours. Basal chambers were collected and measured for fluorescence. Data representative of two independent experiments, (n=2).
(E) As in (D) but with INAVA CRISPR knockout cells in triplicate. Data representative of two independent experiments.
(F) Transepithelial electrical resistance (TEER) of Caco2BBe WT, INAVA knockout, and INAVA-GFP, ARNO expressing lines grown on 2-D transwell inserts for 3 days. Representative data from two independent experiments are shown.
(G) Relative TEER measurements of Caco2BBe WT and INAVA-GFP stably expressing cells before and after overnight low calcium (50 μM) treatment, Data representative of two independent experiments. Error bars±SEM.
(H) Confocal images of INAVA-S expressing cells grown sparsely on coverslips for 2 days.
(I and J) Confocal images of Caco2BBe cells expressing INAVA-GFP grown on 2-D transwells for 2 weeks. (I) Anti-E-cadherin (adherens junction) and (J) anti-ZO-1 (tight junctions) are cell junction markers. c, TRITC-phalloidin was used to stain F-actin. Scalebar = 10 μm.