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Supplemental Material to:

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Novel mechanism of cytokine-induced disruption of epithelial barriers: Janus kinase and protein kinase D-dependent downregulation of junction protein expression

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Supplemental Figure 1. Chemical activators of PKD induce PKD phosphorylation in model pancreatic epithelium. HPAF-II cells were treated for indicated times with either vehicle, octylindolactam-V (OI-V) or phorbol ester (PMA) (each at 1 μM). Activation of PKD was examined by immunoblotting using antibodies against two different phosphorylated forms of the enzyme. Representative immunoblots show that both chemicals markedly up-regulate expression of phosphorylated PKD species in pancreatic epithelial cells.

Supplemental Figure 2. Inhibition of different PKC isoenzymes does not affect cytokine-induced disruption of the epithelial barrier and junctional disassembly.

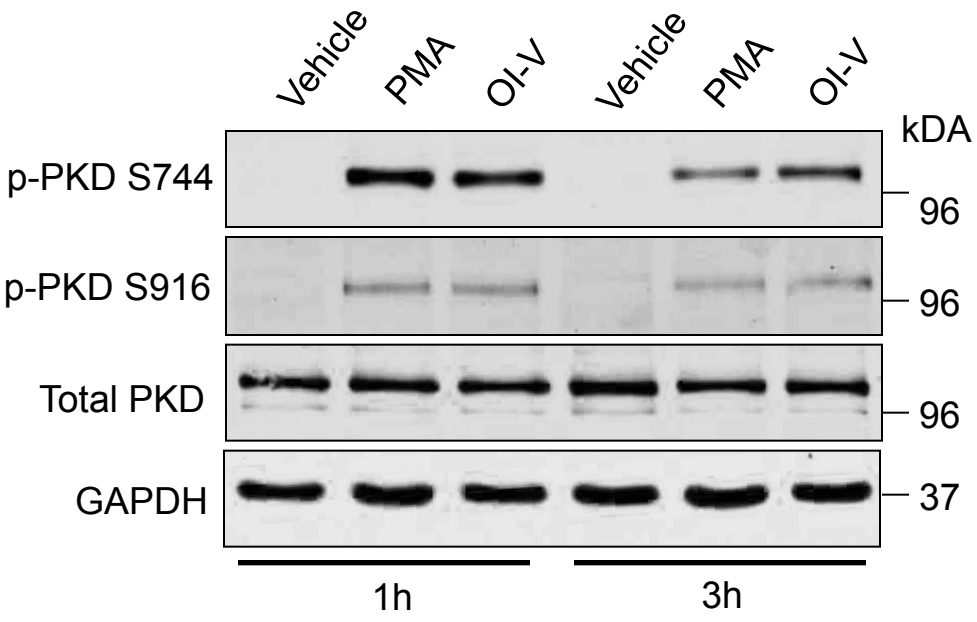
HPAF-II cells were treated for 48 h with IFNγ plus TNFα in the presence of either vehicle or a dual inhibitor of classical and novel PKC, GF-109203X (10 μM), a selective inhibitor of classical PKC, Gö 6983 (10 μM) and an inhibitor of a novel PKC, rottlerin (25 μM). The integrity of the epithelial barrier was examined by TEER measurement, while structure of AJs and TJs was determined by immunofluorescence labeling of E-cadherin and ZO-1 respectively. Neither PKC inhibitor prevents cytokine-driven disruption of the epithelial barrier and AJ/TJ disassembly. Bar, 20 μm

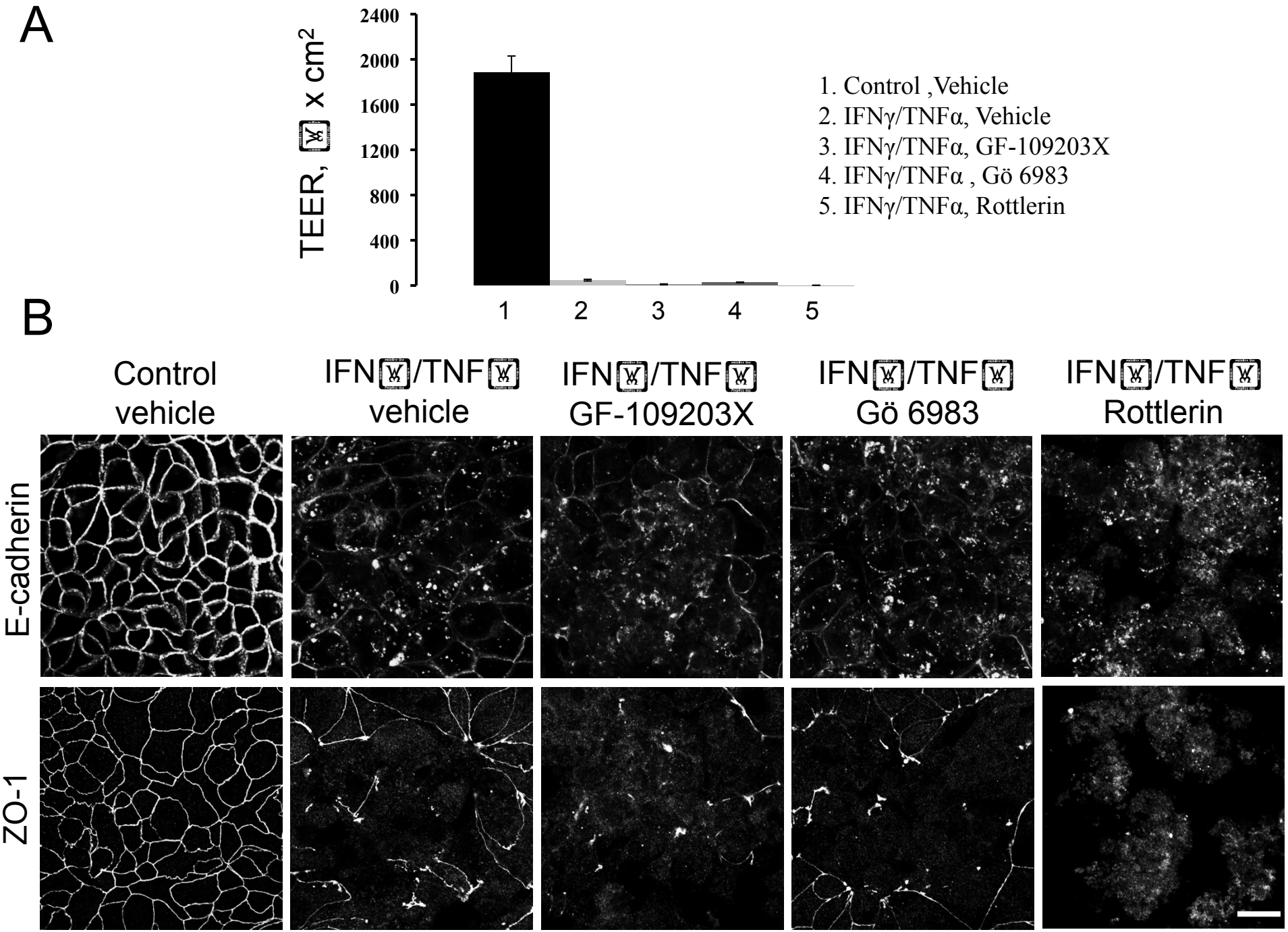
Supplemental Figure 3. Inhibition of PI3 kinase, AMPK, Src and ERK1/2 does not affect cytokine-induced disruption of the epithelial barrier and AJ disassembly.

HPAF-II cells were treated for 48 h with IFNγ plus TNFα in the presence of either vehicle or pharmacological inhibitors of PI3K (LY294002, 20 μM), AMPK (Compound C, 100 μM), Src kinase (PP2, 20 μM), and ERK1/2 (U1026, 10 μM). The integrity of the epithelial barrier was examined by TEER measurements while structure of AJs was determined by immunofluorescence labeling of E-cadherin. Neither inhibitor prevents cytokine-driven disruption of the epithelial barrier and AJ disassembly. Bar, 20 μm

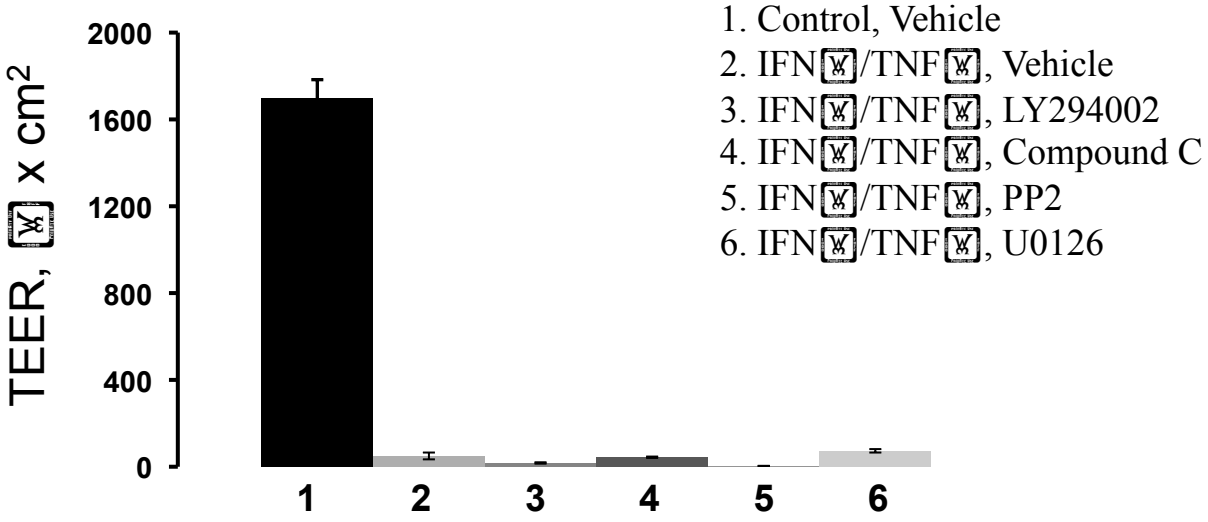
Supplemental Figure 4. siRNA-mediated depletion of p120 catenin and E-cadherin attenuates the development of the pancreatic epithelial barrier.

(A) Immunoblotting analysis shows that E-cadherin-specific siRNA selectively decreases expression of E-cadherin, whereas p120 catenin-specific siRNA downregulates expression of both p120-catenin and E-cadherin proteins in HPAF-II cells. **(B)** TEER measurements demonstrate that E-cadherin and p120 catenin-depleted HPAF-II cell monolayers have lower TEER values compared to control-siRNA-treated cells on day 4 post-transfection. Data is presented as mean ± SE (n = 3); $**p < 0.001$ compared to control siRNA-treated cells.

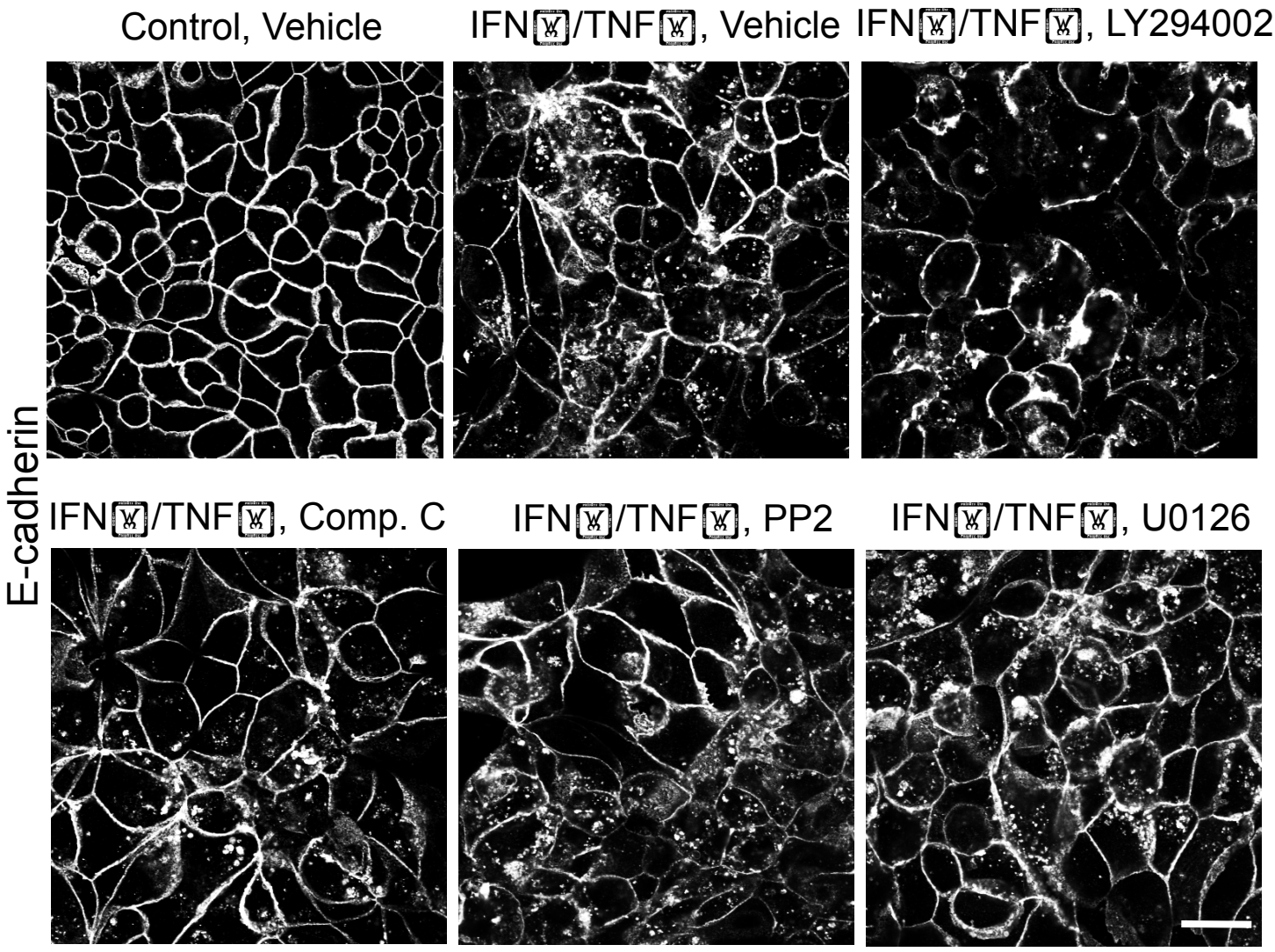

Vehicle
PMA
OI-V
Vehicle
PMA
OI-V
kDA
p-PKD S744
96
p-PKD S916
96
Total PKD
96
GAPDH
37
1h
3h


A
TEER, Ω x cm2
2400
2000
1600
1200
800
400
0
1
2
3
4
5
1. Control ,Vehicle
2. IFNγ/TNFα, Vehicle
3. IFNγ/TNFα, GF-109203X
4. IFNγ/TNFα , Gö 6983
5. IFNγ/TNFα, Rottlerin
B
Control vehicle
IFNγ/TNFα vehicle
IFNγ/TNFα GF-109203X
IFNγ/TNFα Gö 6983
IFNγ/TNFα Rottlerin
E-cadherin
ZO-1

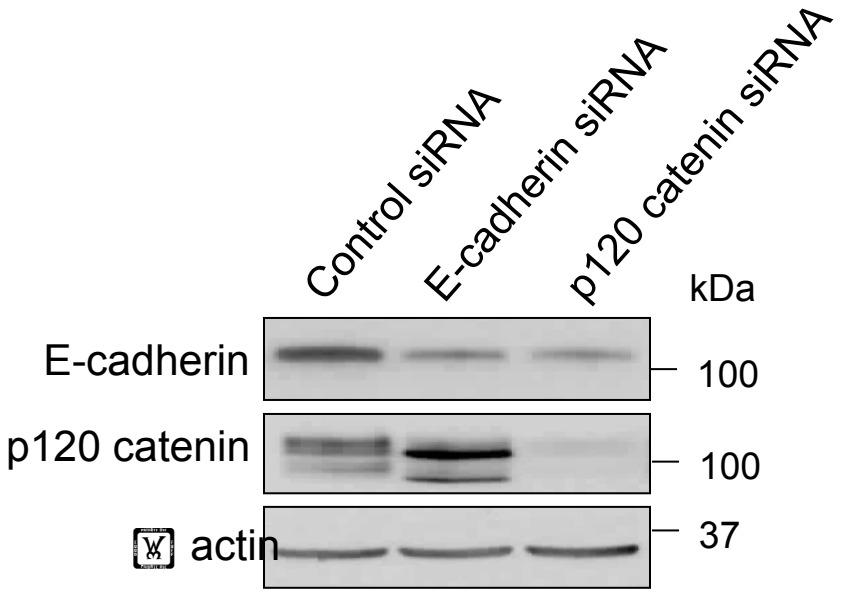
A


TEER, Ω x cm2
2000
1600
1200
800
400
0
1
2
3
4
5
6
1. Control, Vehicle
2. IFNγ/TNFα, Vehicle
3. IFNγ/TNFα, LY294002
4. IFNγ/TNFα, Compound C
5. IFNγ/TNFα, PP2
6. IFNγ/TNFα, U0126

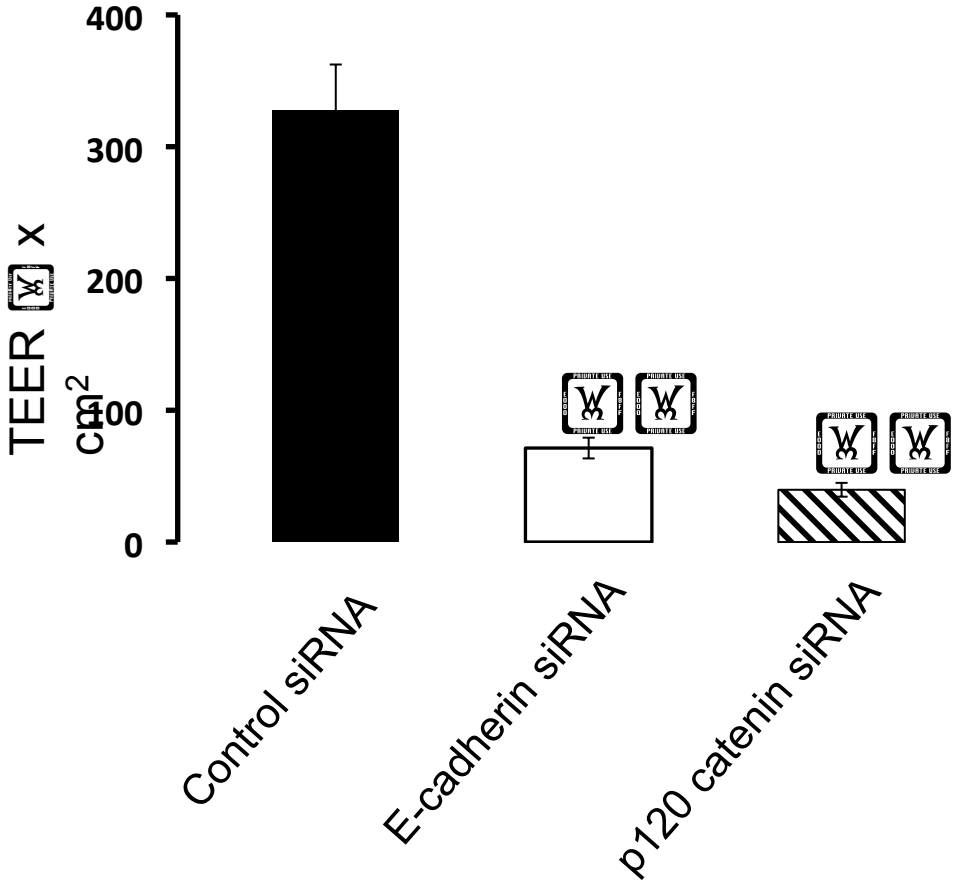
B


Control, Vehicle
IFNγ/TNFα, Vehicle
IFNγ/TNFα, LY294002
E-cadherin
IFNγ/TNFα, Comp. C
IFNγ/TNFα, PP2
IFNγ/TNFα, U0126

A


Control siRNA
E-cadherin siRNA
p120 catenin siRNA
kDa
E-cadherin
100
p120 catenin
100
actin
37

B


400
300
200
100
0
TEER x
cm2
Control siRNA
E-cadherin siRNA
p120 catenin siRNA