Figure S1. Characterization of ATII to ATI cell differnetiation. (A) ATI cells were cultured on a 12-well transwell plate coated with 20 mg/ml fibronectin or PBS as control. TEER was measured every 24 hours after plating (n=4 for each group). **(B)** After day 4, cells were fixed and stained for occludin or zo-1 (red) and counterstained with DAPI (blue). Images were taken at 20x. **(C)** At day 3 after isolation when cells became confluent and displayed an ATI phenotype, 1 μg/ml LPS was added and TEER was measured 24 hours later. **(D)** Following measurement of TEER in C, permeability to 3kD FITC-dextran was assayed. *$p<0.05$; **$p<0.01$. **(E).** Transfection of the β1 subunit also increases other tight junction proteins, including zo-2 and claudin-18. AT1 cells (n=3) were transfected with pCMV-EGFP or pCMV-GFPβ1 plasmids and lused for Western blots 24 hours later.

Figure S2. The β1 subunit mediated upregulation of tight junction proteins is ion-transport independent. (A) ATI cells (day 3 after isolation) were transfected with plasmid expressing the mouse β2 subunit or pCDNA3 empty plasmid as control. Cells were lysed for western blot analysis after 24 hours. **(B)** ATI cells (day 3 after isolation) were transfected with plasmid expressing the mouse β3 subunit containing a DDK tag or pCDNA3 empty plasmid as control. Cells were lysed for western blot analysis after 24 hours.

Figure S3. Dose-dependent induction of β1 subunit gene expression using a Tet-on system. (A) 16HBE14o- cells were cotransfected with pCMV-tet regulator plasmids and pTet3G-human β1 subunit expressing plasmids by electroporation, followed immediately by addition of 0, 1, 10, 100, and 1000 ng/ml doxycycline. Cells were lysed for western blot analysis 24 hours after electroporation. **(B)** 16HBE14o- cells were cotransfected with pCMV-tet plasmids and tet-

lucifersase plasmids by electroporation. After transfection, cells were treated with 1 μg/ml doxycycline or H_2O as control. Wells of cells (n=3) were lysed with reporter lysis buffer every other day and luminescence was measured. RLU: relative luminescence unit. **(C)** qPCR for *SPC* and *CAV1* shows that dox treatment does not affect the differentiation from ATII to ATI.

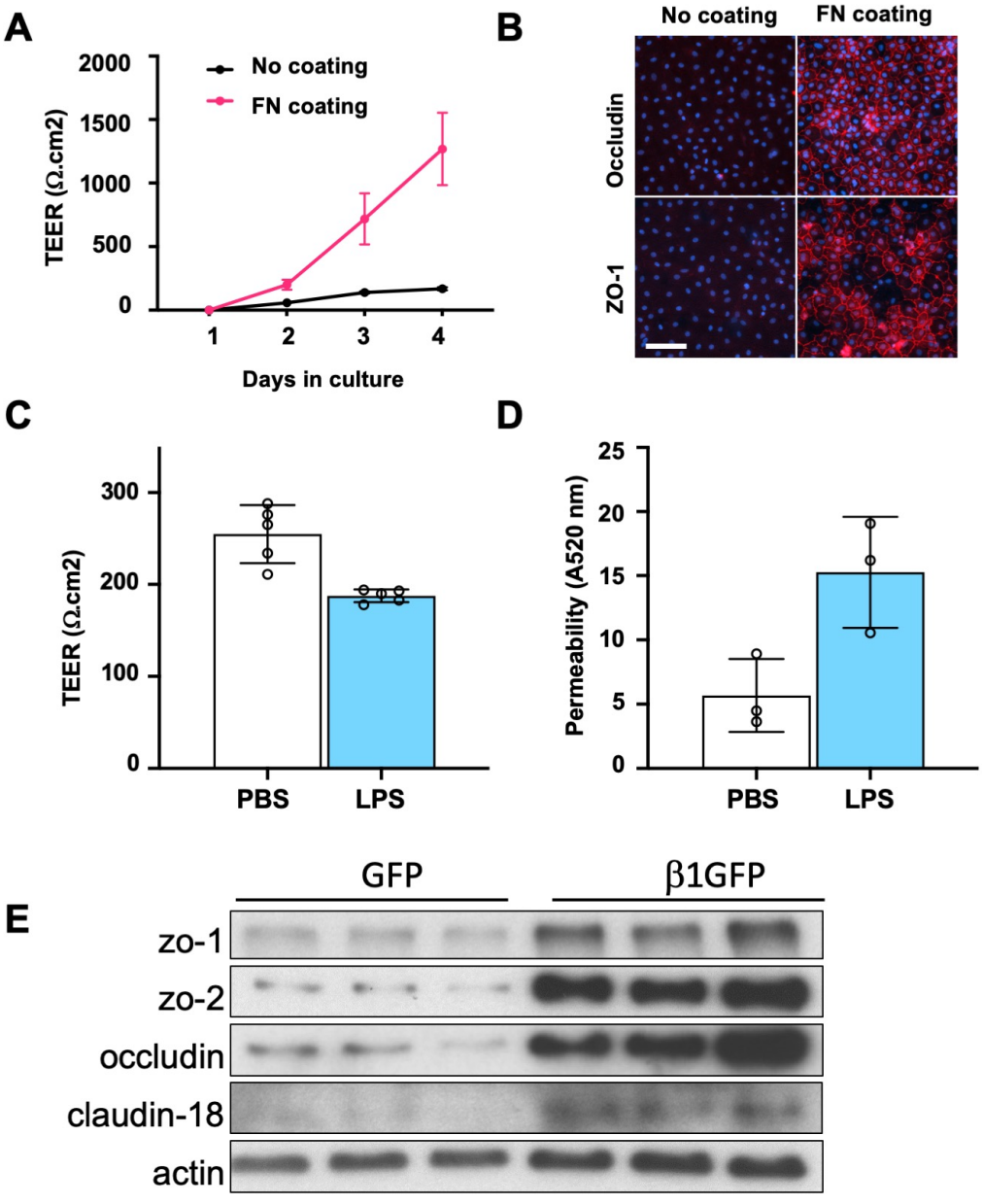
Figure S4. Identifying MRCKα as an interacting protein with the β1 subunit. (A) SDS-PAGE of immunoprecipitation using antibody against the β1 subunit or GFP antibody as control **(B)** Amino acid coverage of MRCKα. Peptides identified by mass spectrometry are marked in red.

Figure S5. Decreased MRCKα level in lung sections from human ARDS patients. (A). Staining of MRCKα from sections of 3 normal control donors and 6 ARDS patients. Three random fields were chosen for each patient for intensity quantitation. Images from ARDS patient #5 were excluded from analysis due to high background signal. **(B)** Representative staining of MRCKα in the airways of control donor and ARDS patient.

Table S1. Number of proteins identified from triplicate MS experiment

Repeat	Control	β1
1	1253	1322
2	1081	1204
3	1325	1581

Figure S1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500
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FN coating
1
2
3
4
Days in culture
B
No coating
FN coating
Occludin
ZO-1
C
300
200
100
0
TEER (Ω.cm2)
PBS
LPS
D
25
20
15
10
5
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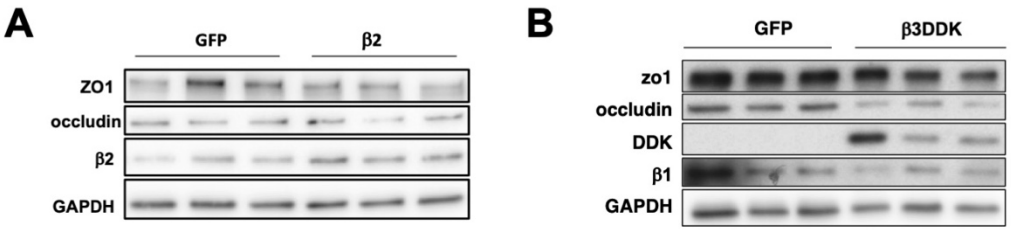
zo-2
occludin
claudin-18
actin

Figure S2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β2
ZO1
occludin
β2
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B
GFP
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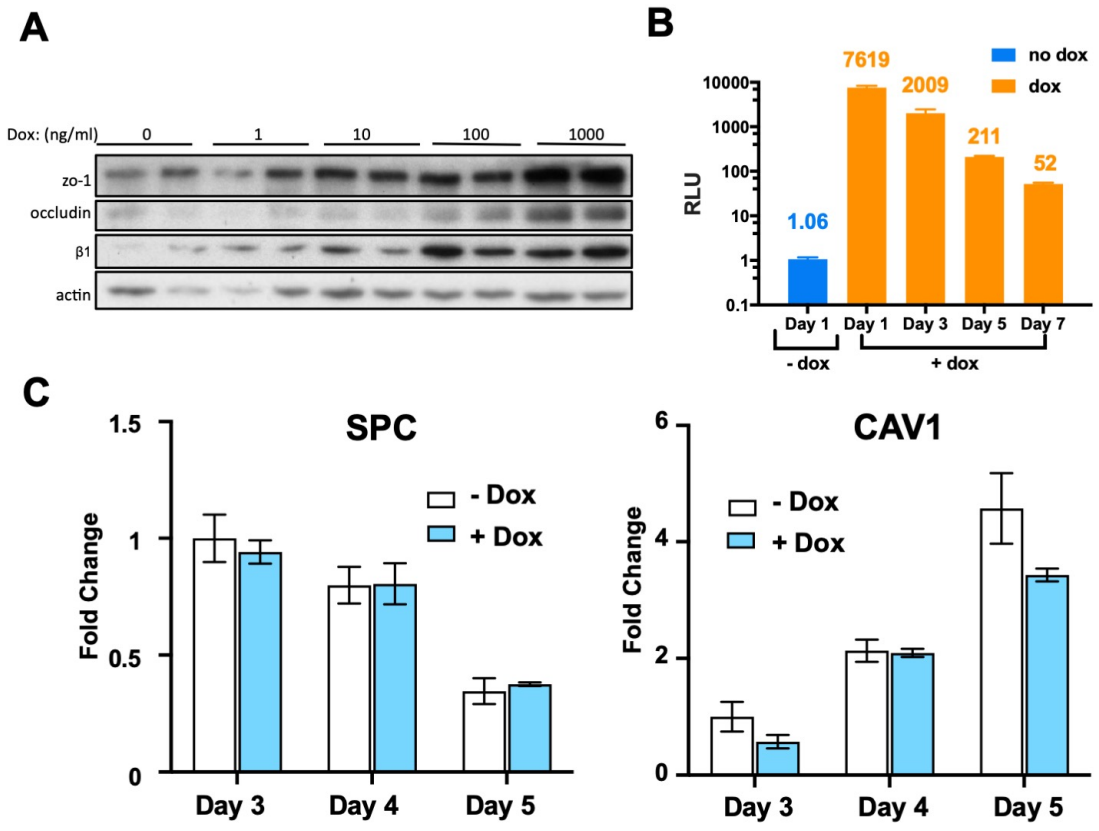
zo1
occludin
DDK
β1
GAPDH

Figure S3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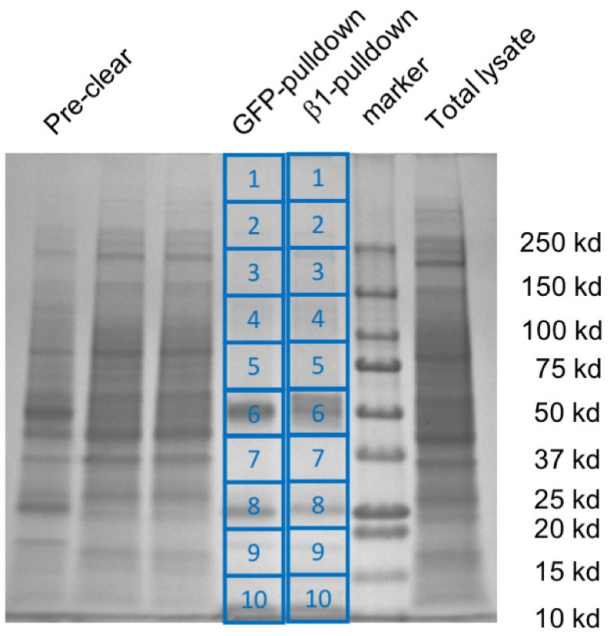
1
10
100
1000
zo-1
occludin
β1
actin
B
no dox
dox
RLU
10000
1000
100
10
1
0.1
7619
2009
211
52
1.06
Day 1
Day 1
Day 3
Day 5
Day 7
- dox
+ dox
C
SPC
- Dox
+ Dox
Fold Change
1.5
1
0.5
0
Day 3
Day 4
Day 5
CAV1
- Dox
+ Dox
Fold Change
6
4
2
0
Day 3
Day 4
Day 5

Figure S4

A



B

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161  DLLTLLSKFE DRLPEDMARF YLAEMVIAID SVHQLHYVHR DIKPDNILMD MNGHIRLADF GSCLKLMEDG TVQSSVAVGT
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1121 GKASQPSVVI SQVIDMRDEE FSVSSVLASD VIHASRKDIP CIFRVTASQL SASNNKCSIL MLADTENEKN KWVGVLSELH
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Figure S5