Supporting Information for Manuscript Entitled:

Lovastatin-induced phosphatidylinositol-4-phosphate 5-kinase diffusion from microvilli stimulates ROMK channels

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FIGURE LEGENDS

Fig. S1. Detection of ROMK1 dimer in mpkCCD$_{c14}$ cells with ROMK1 antibody from Alomone. (*A*) Western blot from control cells or cells transiently transfected with either ROMK1 siRNA or control siRNA. (***B***) Western blot from either control lysate of mpkCCD$_{c14}$ cells or lysate treated with 100 mM DTT.

Fig. S2. Confocal microscopy fluorescent images merged with DIC images show that CTX (green) is mainly located in microvilli (microvilli were visualized through DIC imaging) (*A*) and that ROMK1 (red) is mainly located in planar region (*B*). Images were taken near the apical membrane of mpkCCD$_{c14}$ cells as evidenced by tight junctions (TJ, white arrows). White rectangular boxes indicate zoom-in areas shown by right panels. Blue arrows indicate DIC image of microvilli between ROMK1 channels clustered in planar regions. These images represent data from four separate experiments showing consistent results.

Fig. S3. The whole blots for Fig. 5*D*. Western blot experiments were performed to detect expression levels of either ROMK1 (left) or prominin-1 (right) using proteins either from biotinylation of whole apical membrane and planar regions or from isolated microvilli.

Fig. S4. A double immunoblotting method for Fig. 5*D* to eliminate a possible difference in protein loadings. First, we immunoblotted the membrane with an antibody to ROMK1, then the lower part of the membrane that carries prominin-1 was separated from the top part membrane that carries ROMK1 and was immunoblotted with an antibody to prominin-1. Arrow indicates where the separated membrane was fused back together to show they were from the same gel. In the same lane for microvilli where ROMK1 was undetectable, significant prominin-1 was observed. Conversely, in the same lane for planar regions where prominin-1 was undetectable, significant ROMK1 was observed. These data suggest that neither undetectable ROMK1 in microvilli nor undetectable prominin-1 in planar regions is due to an insufficient protein loading.

Fig. S5. Morphology of Microvilli on the apical membrane of mpkCCD$_{c14}$ cells. Topographic images of the apical membrane of live mpkCCD$_{c14}$ cells were obtained with scanning ion conductance microscopy (SICM), showing that microvilli can be either in ridges or in single forms. Inset panels show zoom-in images. White arrows indicate tight junctions between cells. Cells in both images were from the same culture conditions.

Fig. S6. The whole blot for Fig. 7*B*. Western blot experiments were performed to detect PI(4)P5K expression levels using proteins either from biotinylation of whole apical membrane and planar regions or from isolated microvilli.

Fig. S1

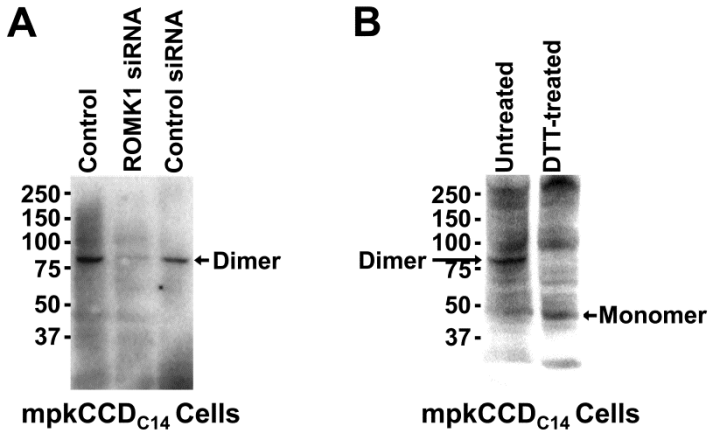

A
Control
ROMK1 siRNA
Control siRNA
250
150
100
75
50
37
Dimer
mpkCCD$_{C14}$ Cells
B
Untreated
DTT-treated
250
150
100
Dimer
75
50
37
Monomer
mpkCCD$_{C14}$ Cells

Fig. S2

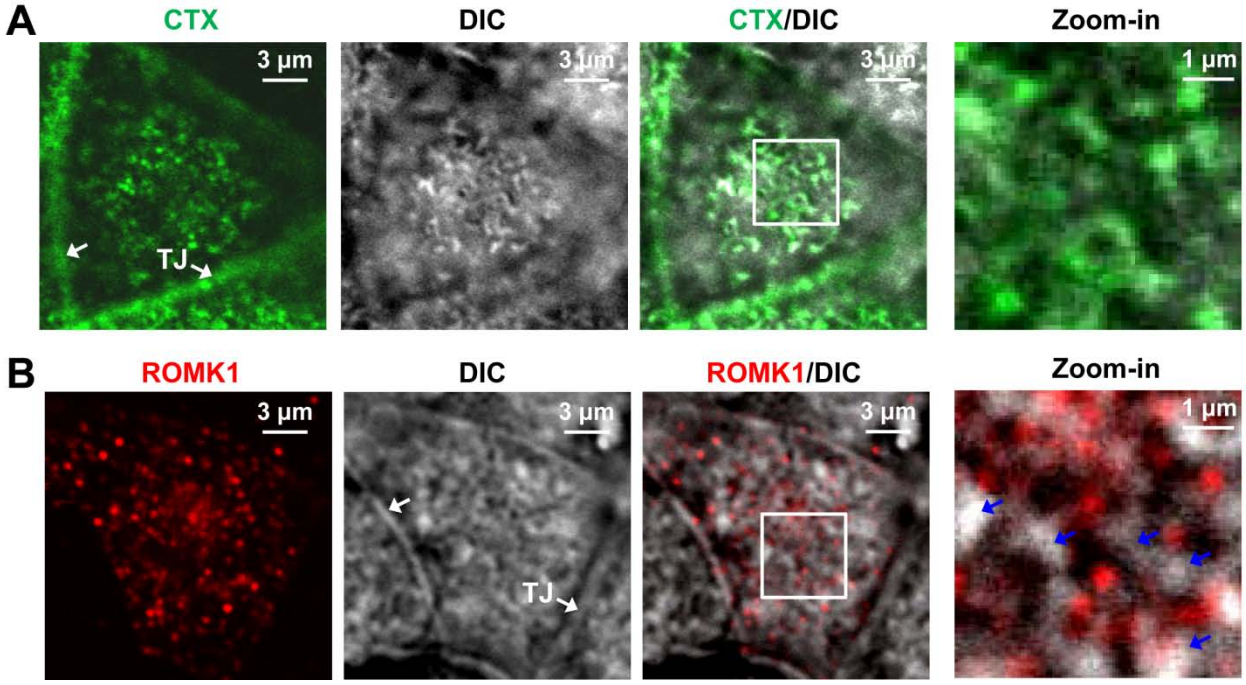

A
CTX
DIC
CTX/DIC
Zoom-in
3 μm
3 μm
3 μm
1 μm
TJ
B
ROMK1
DIC
ROMK1/DIC
Zoom-in
3 μm
3 μm
3 μm
1 μm
TJ

Fig. S3

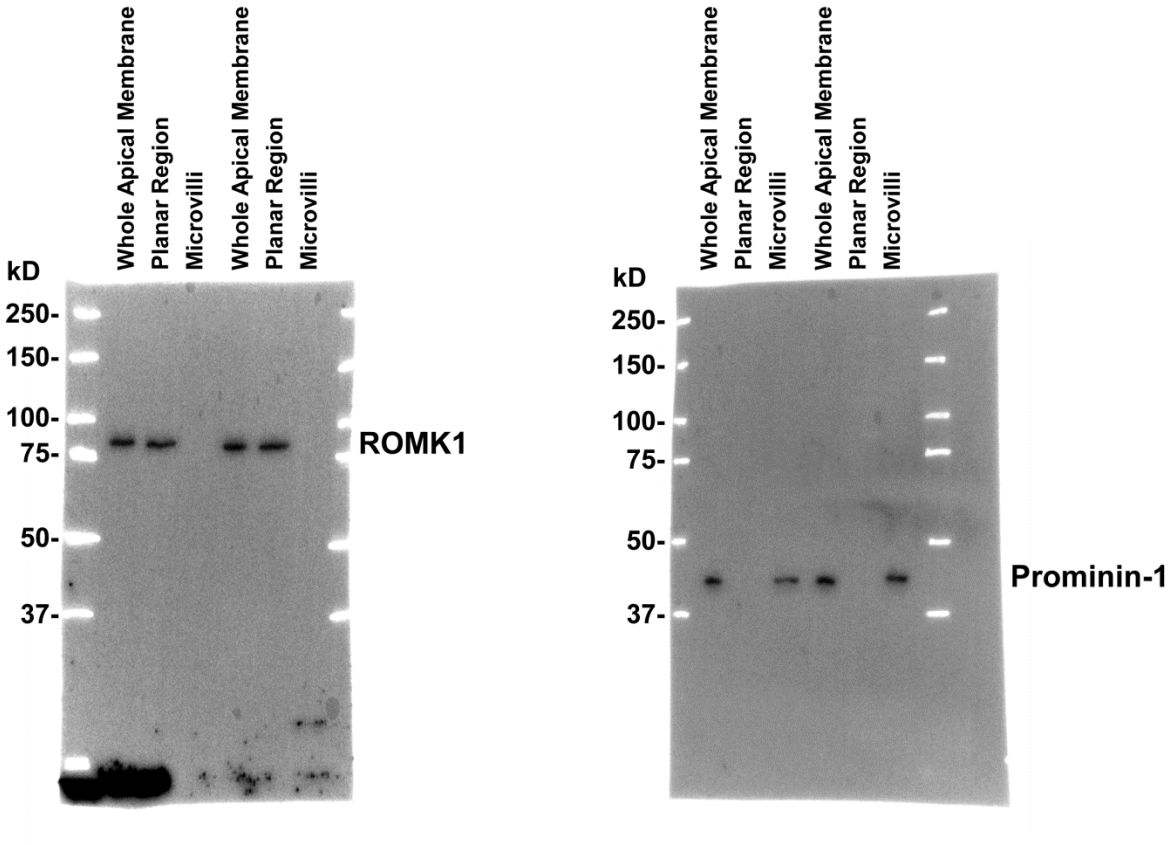

Whole Apical Membrane
Planar Region
Microvilli
Whole Apical Membrane
Planar Region
Microvilli
kD
250-
150-
100-
75-
50-
37-
ROMK1
Whole Apical Membrane
Planar Region
Microvilli
Whole Apical Membrane
Planar Region
Microvilli
kD
250-
150-
100-
75-
50-
37-
Prominin-1

Fig. S4

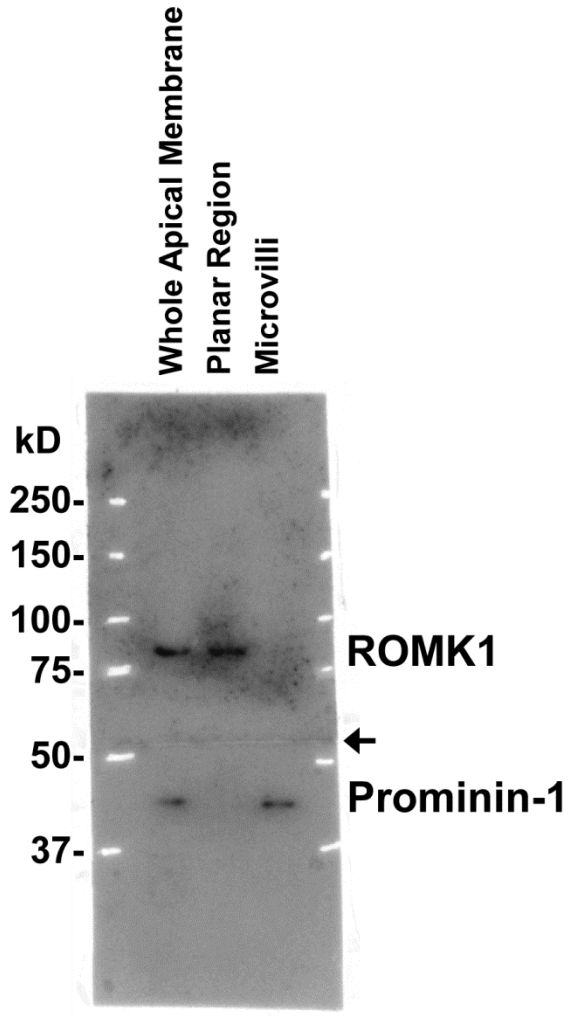

Whole Apical Membrane
Planar Region
Microvilli
kD
250-
150-
100-
75-
50-
37-
ROMK1
Prominin-1

Fig. S5

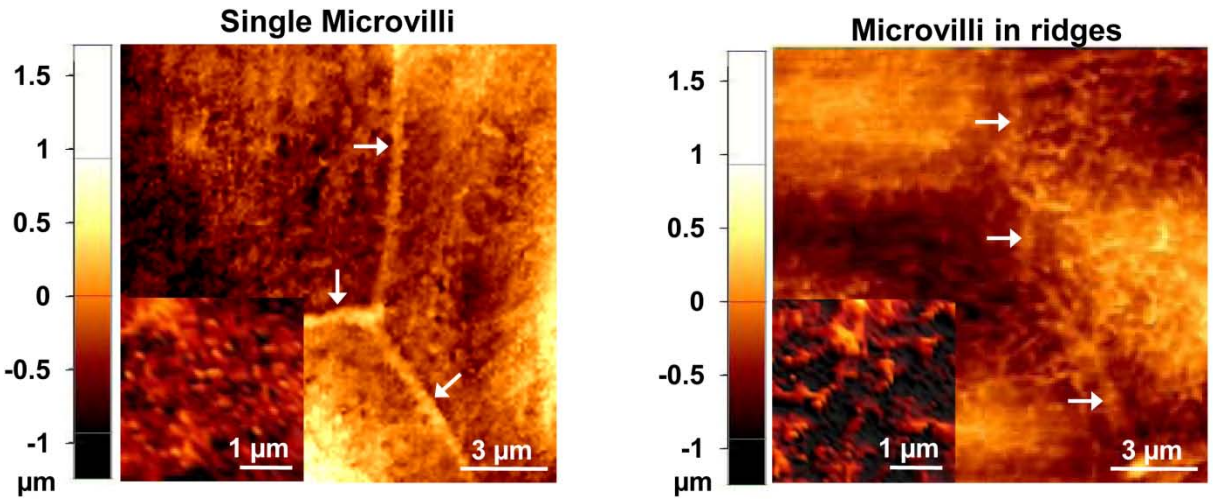
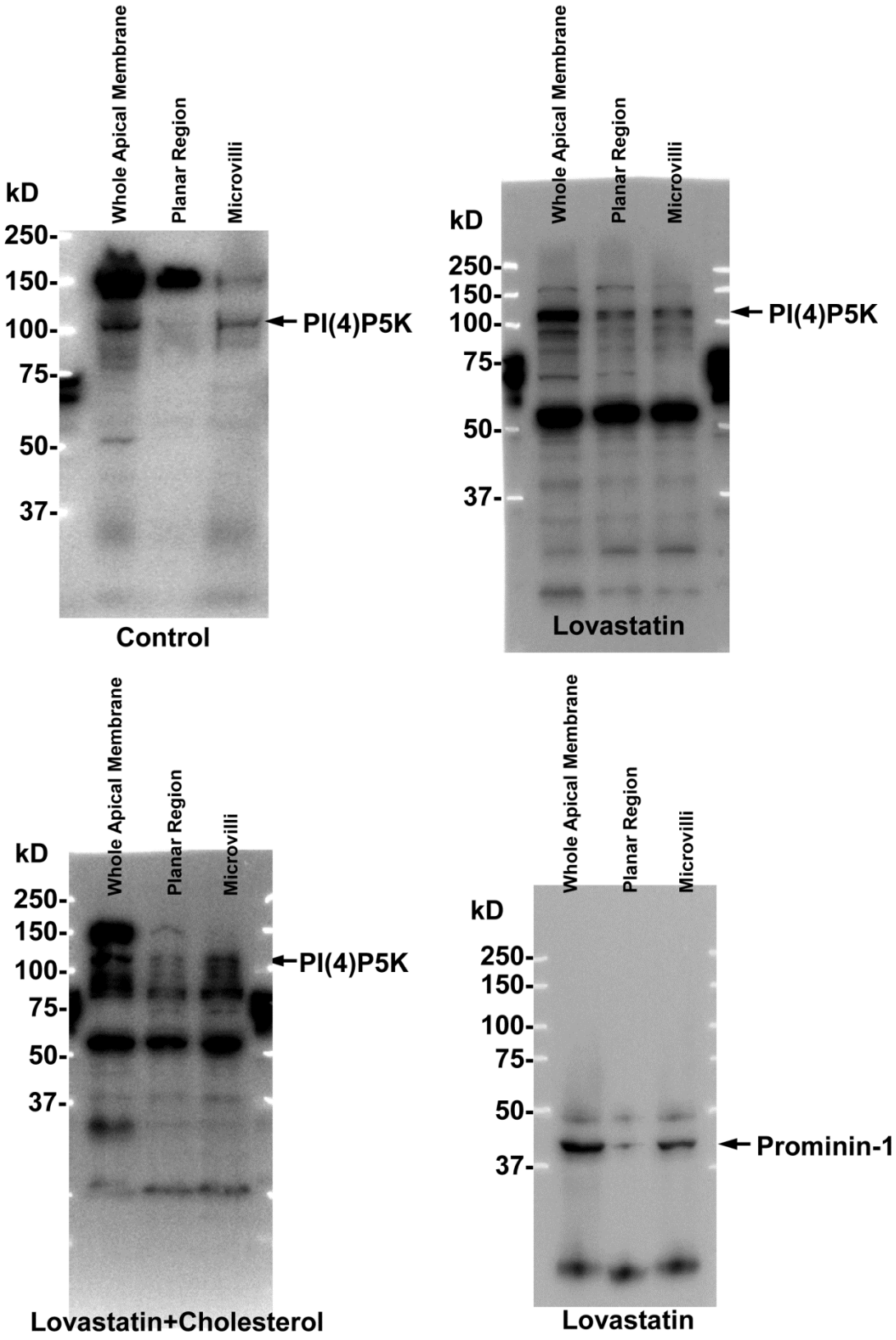

Single Microvilli
1.5
1
0.5
0
-0.5
-1
µm
1 µm
3 µm
Microvilli in ridges
1.5
1
0.5
0
-0.5
-1
µm
1 µm
3 µm

Fig. S6


Whole Apical Membrane
Planar Region
Microvilli
kD
250-
150-
100-
75-
50-
37-
PI(4)P5K
Control
Lovastatin
Lovastatin+Cholesterol
Prominin-1