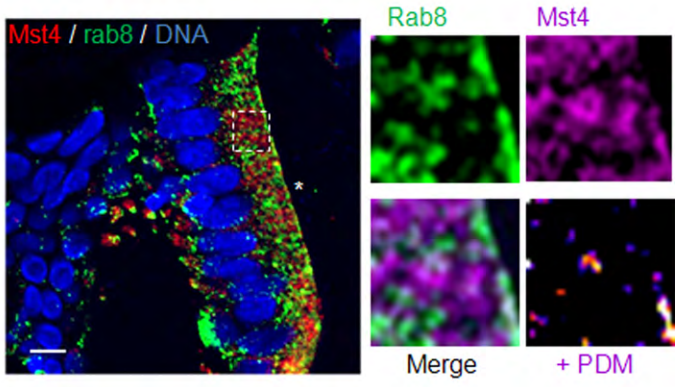

Fig. S1. Analysis of colocalization between Mst4 (red) and rab8 (green) in control patient. Enlargement of the boxed areas are shown on the right. Mst was pseudo-colored magenta. White color in the merged image is indicative for colocalization. Positive (+)PDM image is shown. Asterisk indicate the position of the intestinal lumen. Chromatin (blue) is stained with DRAQ5. Scale bar 10μm

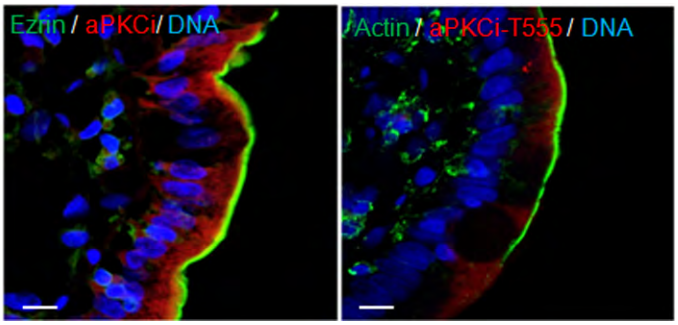

Fig. S2. Analysis of colocalization between aPKCi or aPKC1-T555 (red) with ezrin or actin (green), respectively, in control patient. Asterisk indicates the position of the intestinal lumen. Chromatin (blue) is stained with DRAQ5. Scale bar 10μm

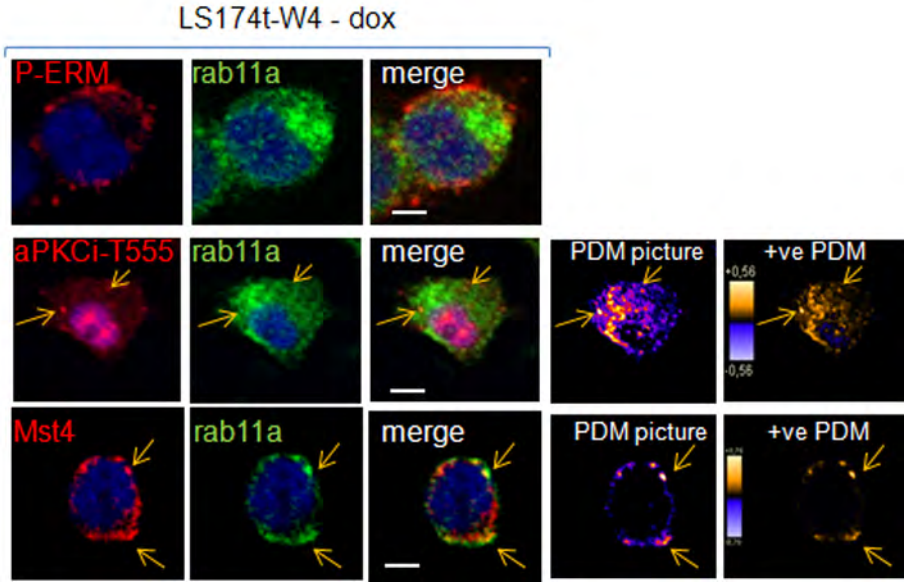

Fig. S3. Codistribution of Mst4, aPKCi and T567-phospho-ERM (all in red) with rab11a (in green) in non-induced (-Dox) LS174T-W4 cells (arrows point to areas of codistribution). For Mst4/rab11a and aPKCi-rab11a, PDM and positive (+)PDM images are presented. Scale bar 5μm

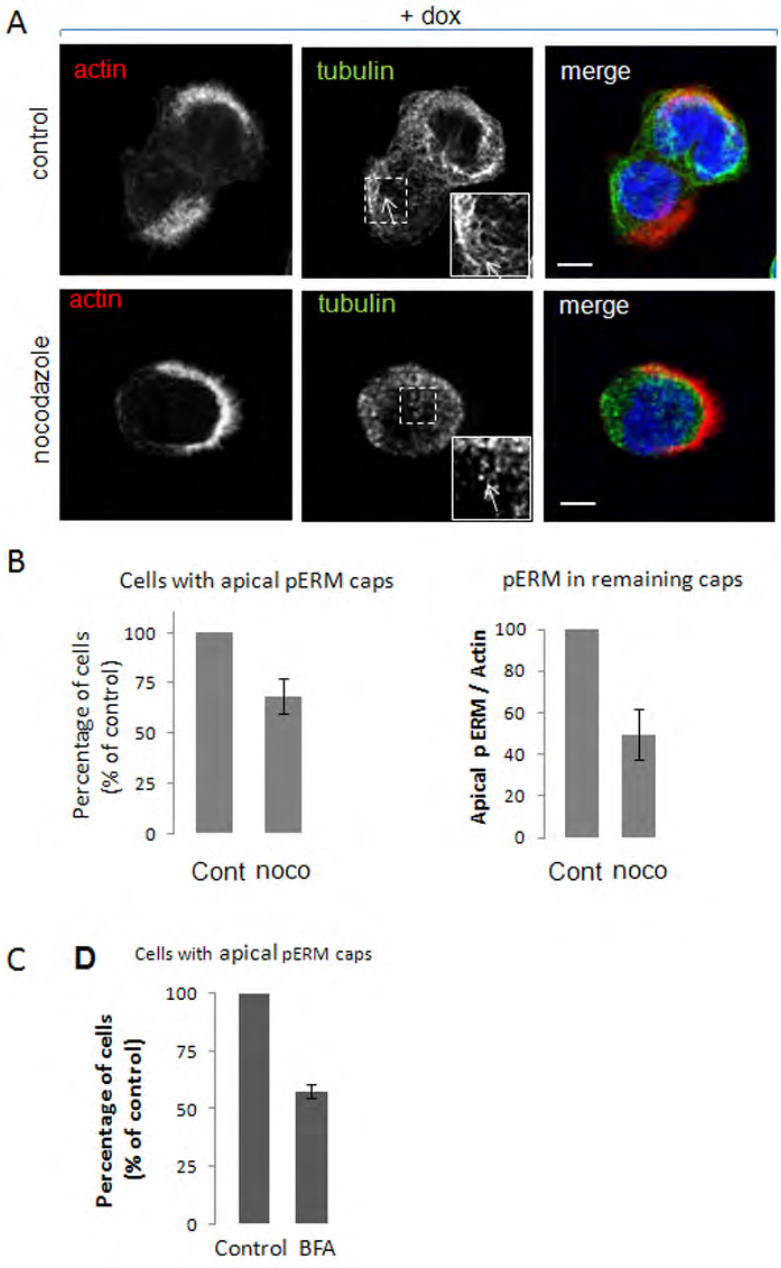

Fig. S4. A) Immunolabeling for actin (red) and tubulin (green) and the merged images in control and nocodazole (noco)-treated docycycline (+dox)-induced LS174T-W4 cells. Insert shows high magnification of the boxed area. Arrows point to tubular tubulin staining (top row) and fragmented tubulin staining (bottom row). Scale bar 5µm. B) quantification of the percentage of cells with a distinguishable p-ERM cap (left) and quantification of the intensity of pERM staining (normalized to actin) (right) in control (cont) and nocodazole (noco)-treated cells. C) quantification of the percentage of cells with a distinguishable p-ERM cap in control and BFA-treated cells.

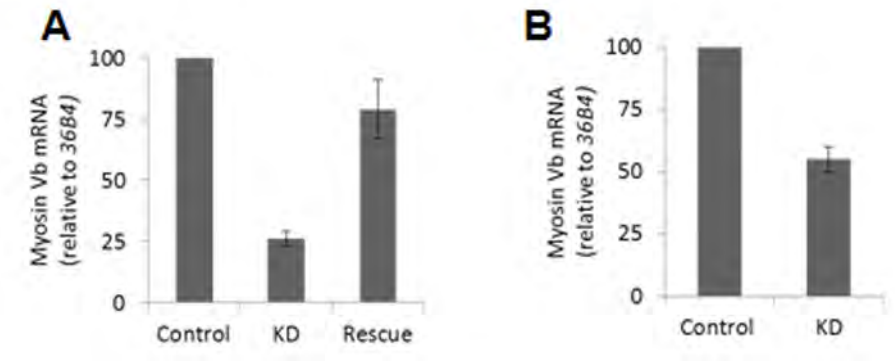

Fig. S5. Myosin Vb mRNA expression level in LS174T-W4 (A) and Caco-2 (B) cells (parental, infected with lentivirus expressing shRNA against myosin Vb, and double infected with lentivirus expressing shRNA against myosin Vb and lentivirus expressing a shRNA-resistant wild type full length *MYO5B*).

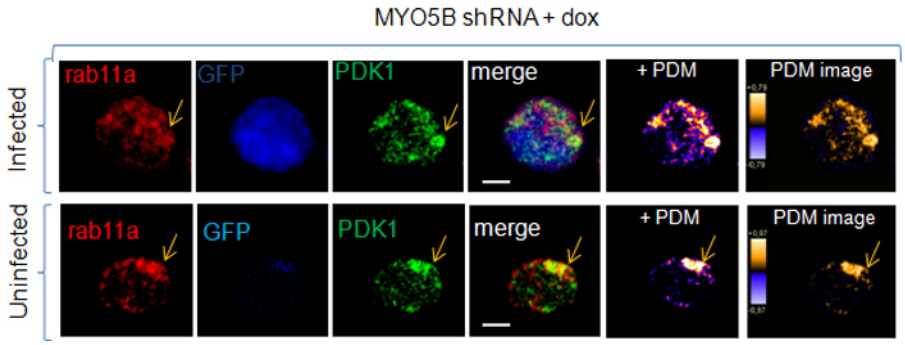

Fig. S6. Analysis of colocalization between PDK1 and rab11a in LS174T-W4 cells. PDK1 (blue) and rab11a (red) in doxycycline-stimulated LS174T-W4 cells transduced with shRNA against myosin Vb. Infected cells (infect) and non-infected cells (uninfect) are GFP-positive (green) and GFP-negative, respectively. Arrow points to the area at the cortex where PDK1 and rab11a colocalize. Also PDM and positive (+)PDM images are shown. Scale bar 5μm.

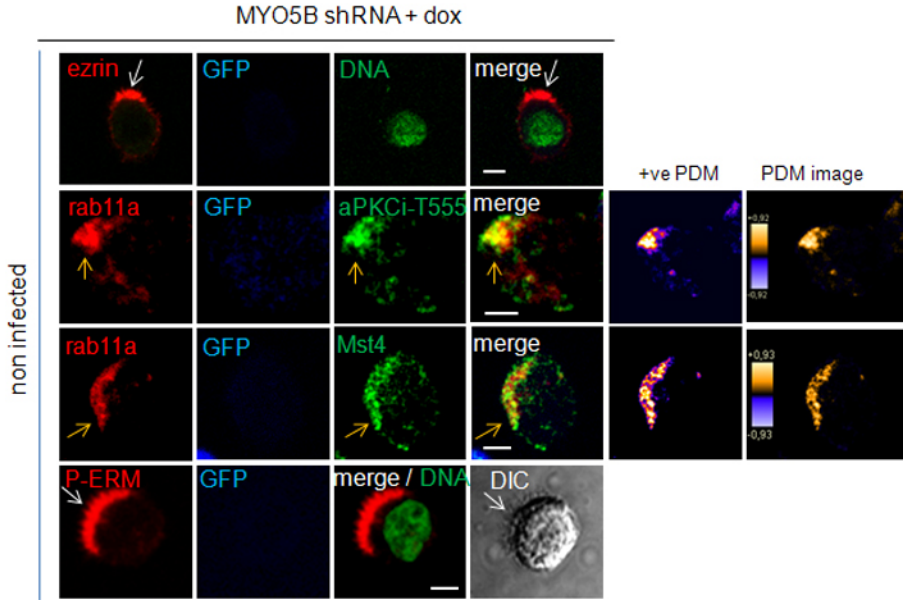

Fig. S7. Shown are the control (GFP-negative) cells of cultures treated with shRNA against myosin Vb. In these cells the polarized distribution of rab11a, Mst4 and aPKCi, and pERM are comparable to non-treated cells. Also PDM and positive (+)PDM images are shown. Scale bar 5µm.

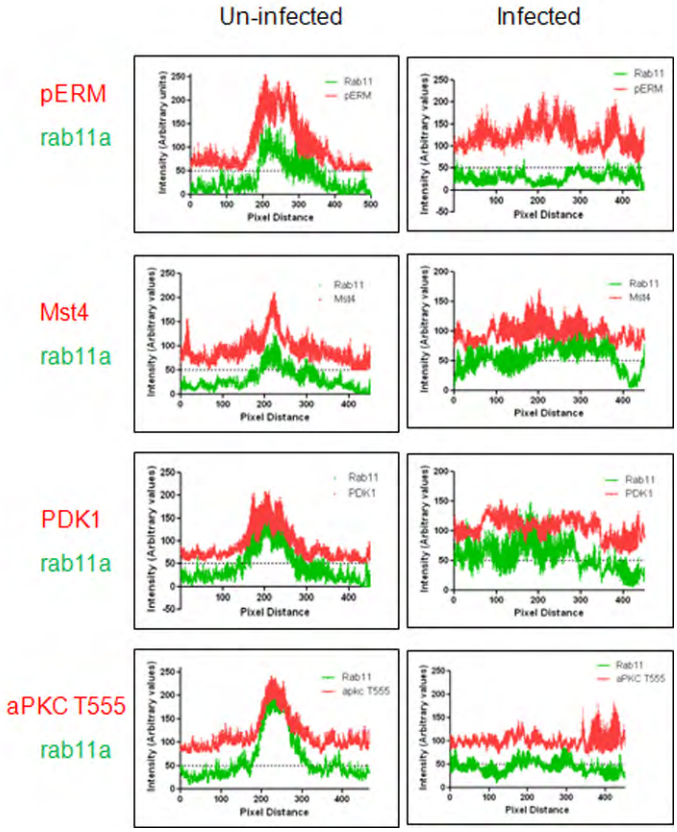

Fig. S8. Fluorescence intensities of rab11a, Mst4, PDK1, aPKCi-T555 together with p-ERM along the cell perimeter of GFP-negative (un-infected) and GFP-positive (infected) LS174T-W4 cells after treatment with lentivirus. Line plots show average of minimal 5 cells per condition. Note the loss of the clear peak of fluorescence intensity in the infected (myosin Vb knockdown) cells as opposed to the un-infected (control) cells, indicating inhibition of the polarized distribution of the proteins at the cell cortex.

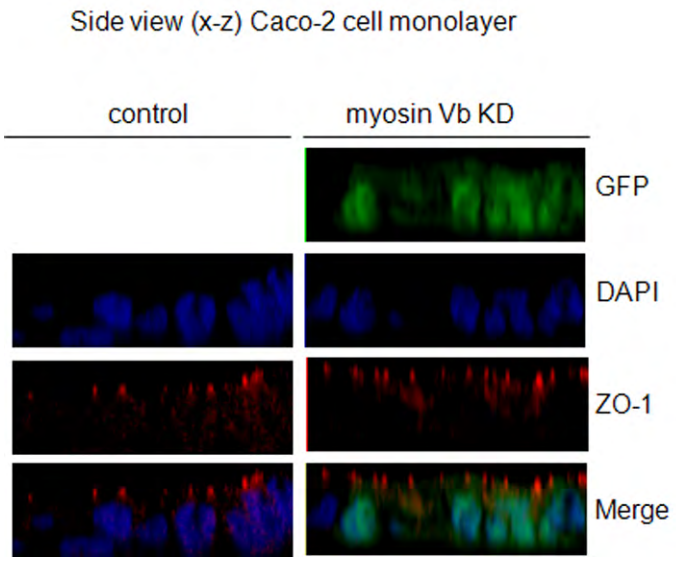

Fig. S9. Immunolabeling of tight junction protein ZO-1 in control Caco-2 cells or Caco-2 cells transduced with shRNA against myosin Vb. Infected cells (infect) and non-infected cells (uninfect) are GFP-positive (green) and GFP-negative, respectively. DNA is stained with DAPI.

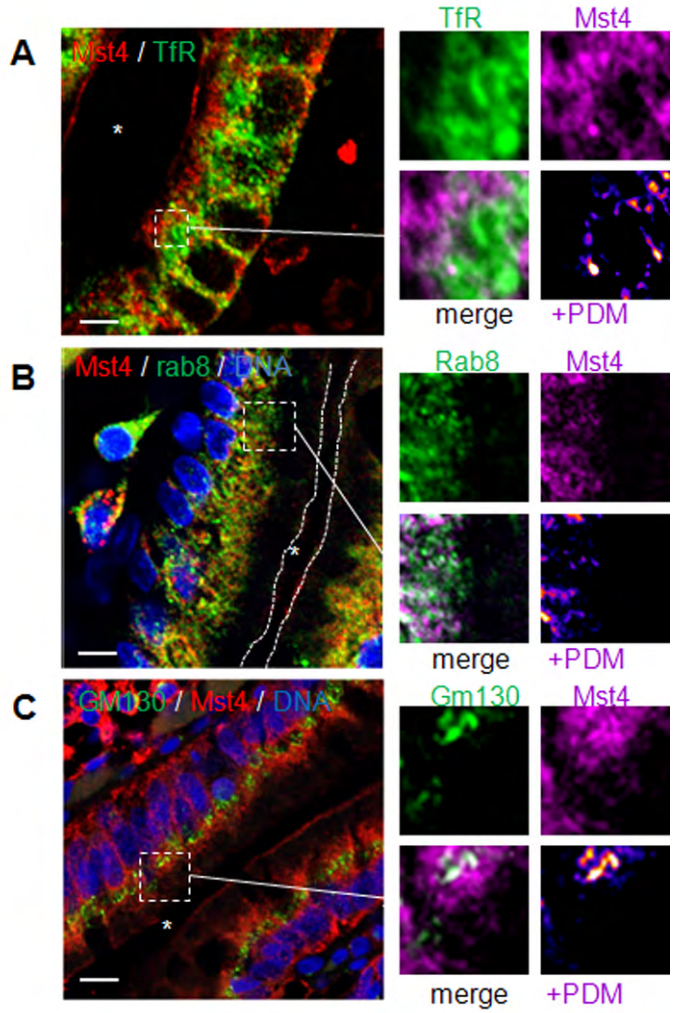

Fig. S10. Analysis of colocalization between Mst4 and transferrin receptor (TfR, A), rab8a (B) or GM130 (C) in MVID enterocytes. A) Mst4 (red) and TfR (green) in MVID patient. Enlargement of the boxed areas are shown on the right where Mst4 is pseudo-colored magenta and also positive (+)PDM images are shown. B) Mst4 (red) and rab8a (green) in MVID patient. Enlargement of the boxed areas are shown on the right where Mst4 is pseudo-colored magenta and also positive (+)PDM images are shown. C) Mst4 (red) and GM130 (green) in MVID patient. Enlargement of the boxed areas are shown on the right where Mst4 is pseudo-colored magenta and also positive (+)PDM images are shown. Asterisks indicate the position of the intestinal lumen. Chromatin (blue) is stained with DRAQ5.

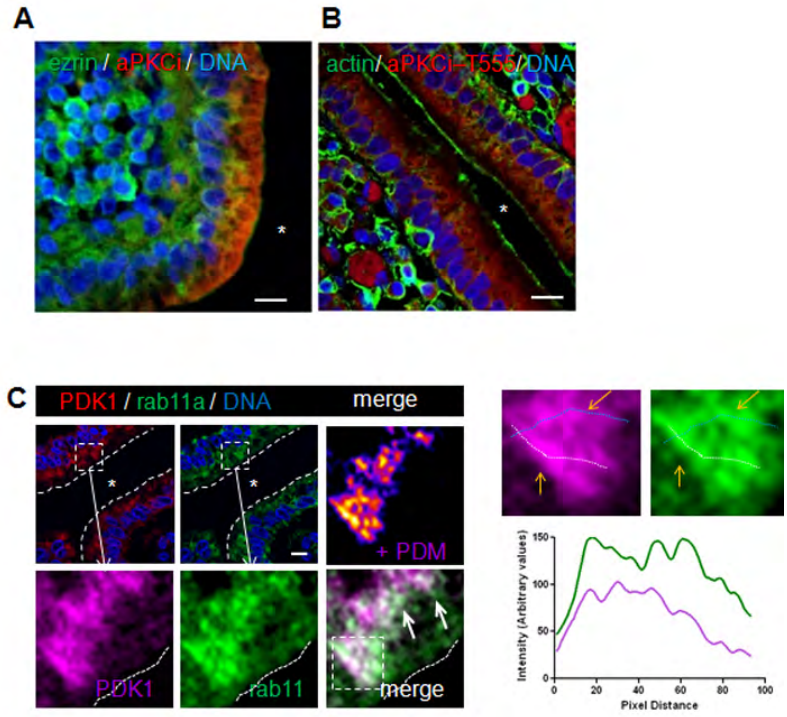

Fig. S11. Distribution of aPKCi (red; A) and aPKCi-T555 (red; B) and ezrin or actin, respectively (green) in MVID patient enterocytes. C) Analysis of colocalization between Rab11a and PDK1 in MVID enterocytes. PDK1 (red, magenta) and Rab11a (green) in MVID patient. Enlargement of the boxed areas are shown on the right where PDK1 is pseudo-colored magenta. Positive (+)PDM images indicate areas of colocalization. Boxed area in the merged panel are enlarged and line plots show fluorescence intensity of the two proteins. Chromatin (blue) is stained with DRAQ5.

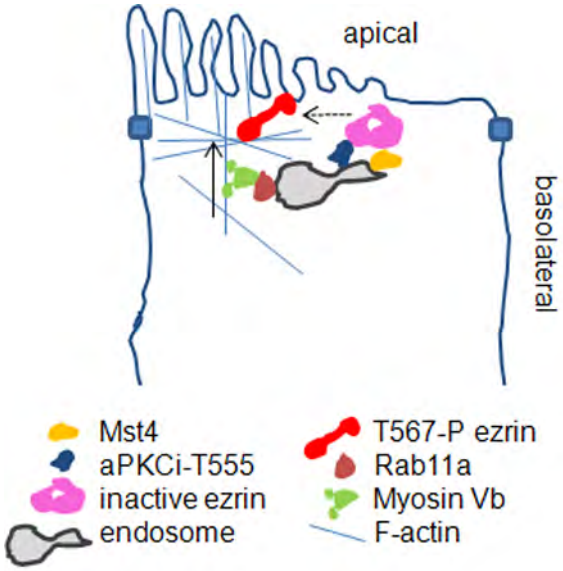

Fig. S12. Cartoon depicting model. See main text for full explanation.

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Primary antibody	**company**	**dilution**
Actin	Sigma	1:1000
Ezrin	TebuBio	1:200
T567-P ERM	Cell Signalling	1:200
Mst4	Epitomics	1:100
Rab11a	BD Bioscience	1:50
Rab8	Abnova	1:20
aPKCi	BD Bioscience	1:100
T555-P PKCi	Invitrogen	1:100
Giantin	Covance	1:200
Transferrin receptor	Invitrogen	1:200
GM130	BD Bioscience	1:200
Alexa Fluor 488- and -543-conjugated secondary antibodies	Invitrogen	1:1000

DRAQ5 was from Cell Signaling (1:5000).