SUPPLEMENTARY MATERIAL

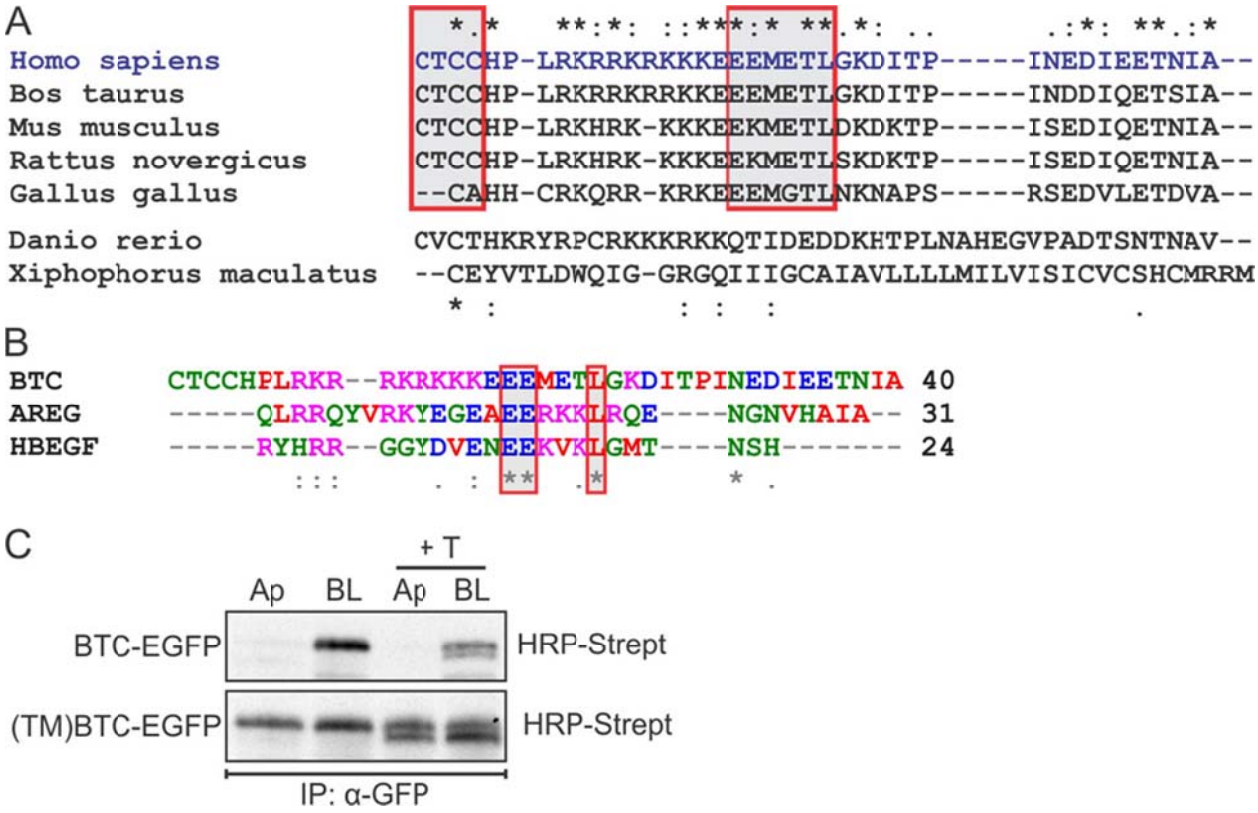


Fig. S1. BTC Cytoplasmic domain alignment and the role of N-glycosylation in BTC trafficking. (A) Cross-species alignment of BTC cytoplasmic domain: the regions showing high sequence homology are shaded in a box with the alignment scores displayed above each aligned aa. * indicates absolutely conserved residues followed by : and . in decreasing order of homology. (B) Conservation of the monoleucine-based basolateral-sorting motif (EEXXXL) within the cytoplasmic domain of AREG, BTC, and HBEGF: the key monoleucine and proximal acidic aa in the basolateral-sorting motif are conserved. (C) BTC N-glycosylation is not required for its apical localization: MDCK cells stably expressing BTC-EGFP and (TM)BTC-EGFP were polarized on Transwell filters and subjected to selective cell-surface biotinylation on either apical (Ap) or basolateral (BL) cell surfaces. A separate pair of filters for each line were preincubated with an N-glycosylation inhibitor, Tunicamycin (2 mg/ml), for 9 hours (+T) and subjected to selective cell-surface biotinylation. Cells were then lysed, immunoprecipitated for GFP, and probed with HRP-streptavidin.

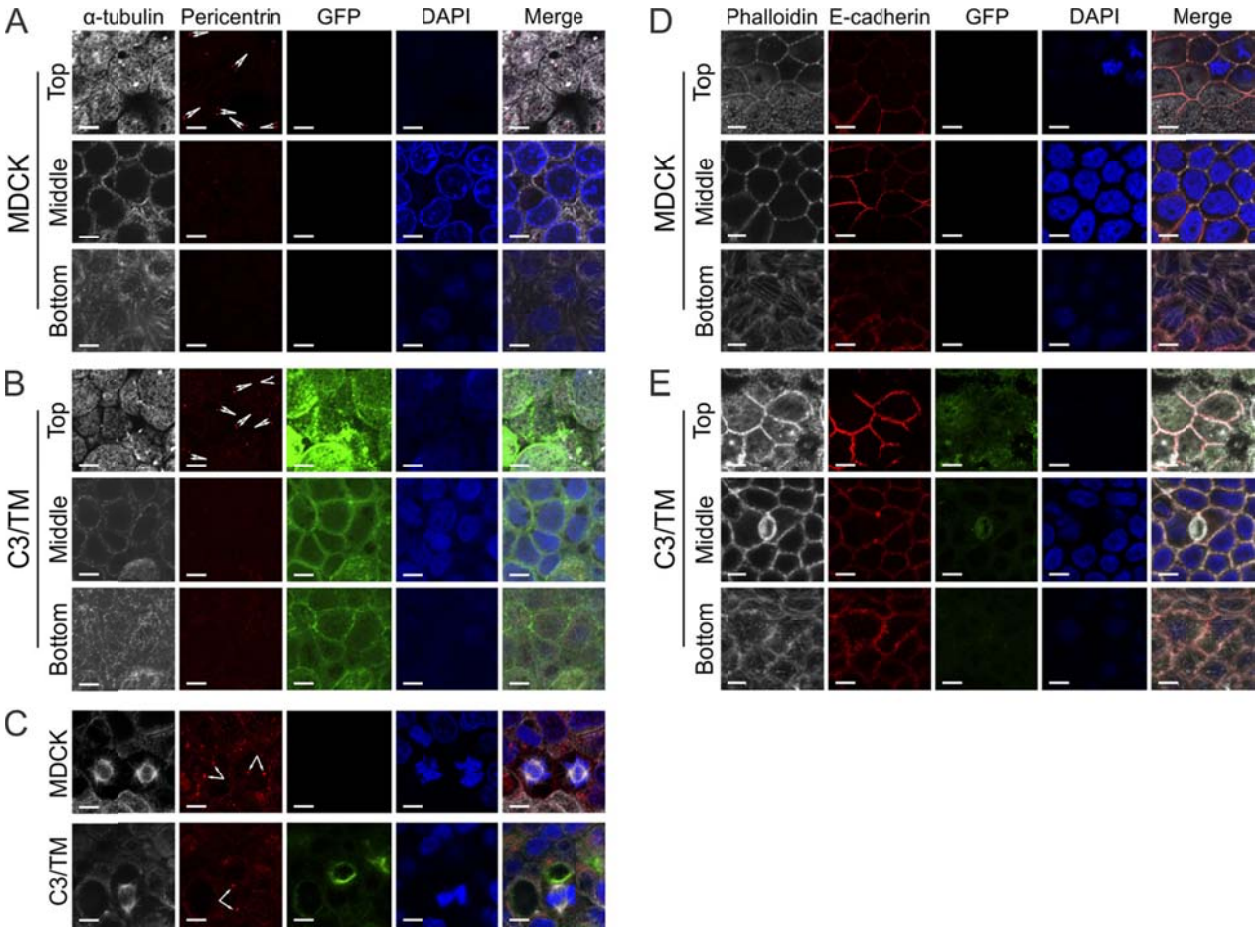


Fig. S2. Cytoskeletal organization of parental and C3/TM-expressing MDCK Transwell cultures. (A) Polarized MDCK were fixed and stained for DAPI (blue), α-tubulin (white), and pericentrin (red). Three xy projections at the top, midde, and bottom position of the Transwell cultures are shown. Pericentrin-stained centrioles in individual cells are indicated with a pair of arrows. (B) Polarized MDCK cells stably expressing (C3/TM)BTC-EGFP were fixed, stained, and displayed as in A. (C) Dividing cells within parental or (C3/TM)BTC-EGFP-expressing MDCK Transwell cultures were fixed and stained as in A. xy projection through the division plane are displayed. (D) Polarized MDCK were fixed and stained for DAPI (blue), phalloidin (white), and E-cadherin (red). Three xy projections at the top, middle, and bottom position of the Transwell cultures are shown. (E) Polarized MDCK cells stably expressing (C3/TM)BTC-EGFP were fixed, stained, and displayed as in D. All scale bars: 10 μm.

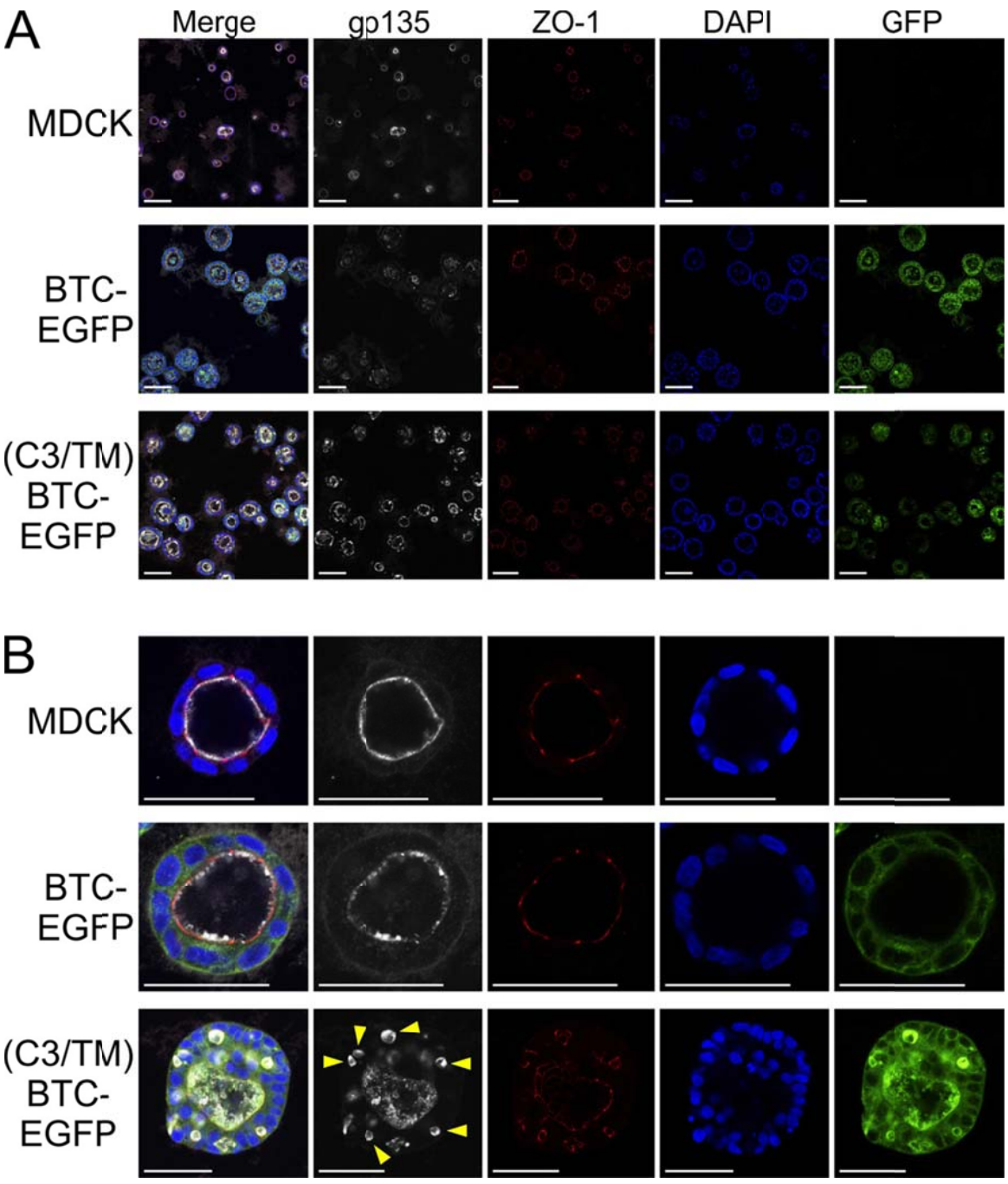


Fig. S3. BTC mistrafficking enhances lateral lumen formation in 3D cultures. (A, B) MDCK cells stably expressing the indicated BTC constructs were cultured in Matrigel for five days and then fixed and stained for ZO-1 (red) and gp135 (white). GFP and DAPI fluorescence are depicted in green and blue, respectively. Both low (A) and high (B) power xy projections show multiple and individual cysts, respectively. Yellow arrowheads in gp135 channel in B indicate lateral lumens. Scale bars: A = 100 μm, B = 50 μm.

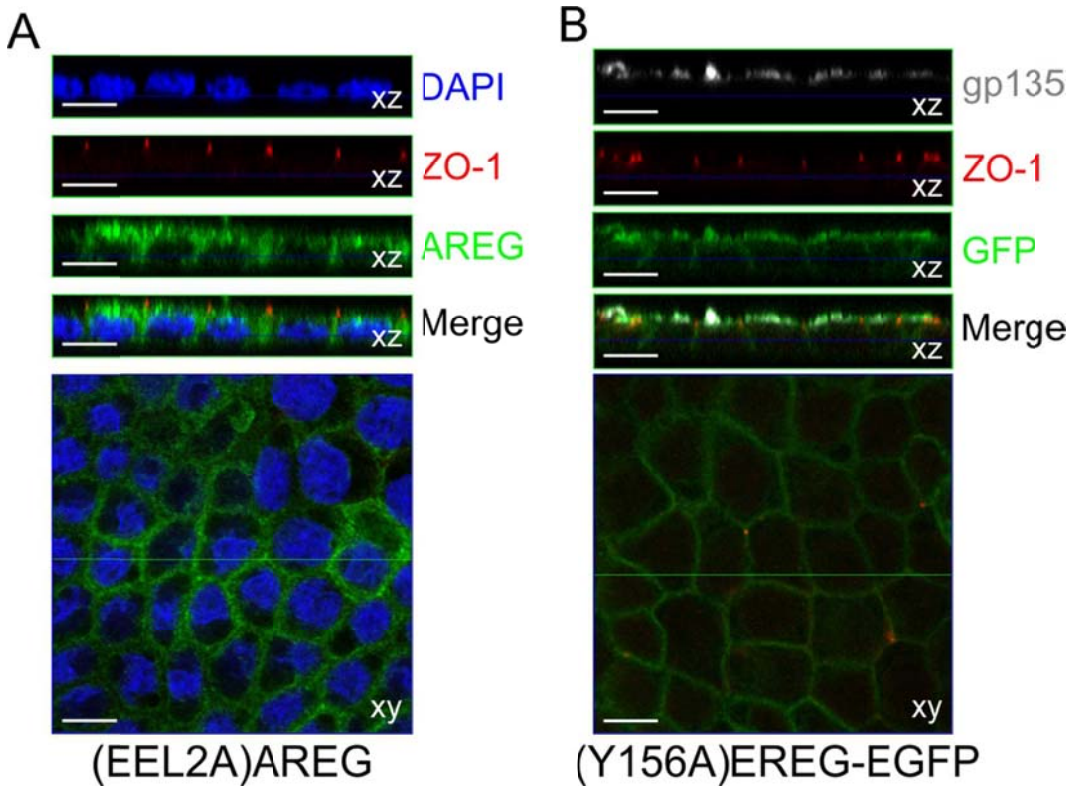
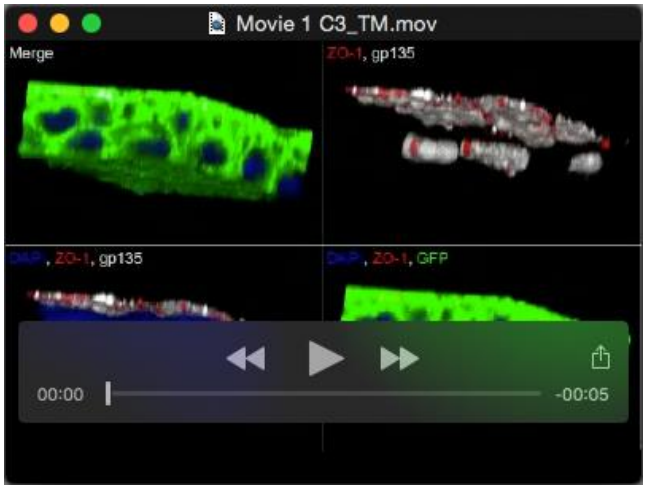
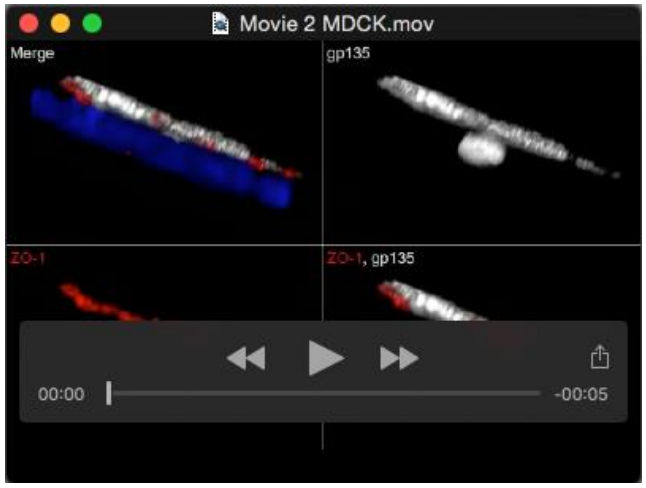


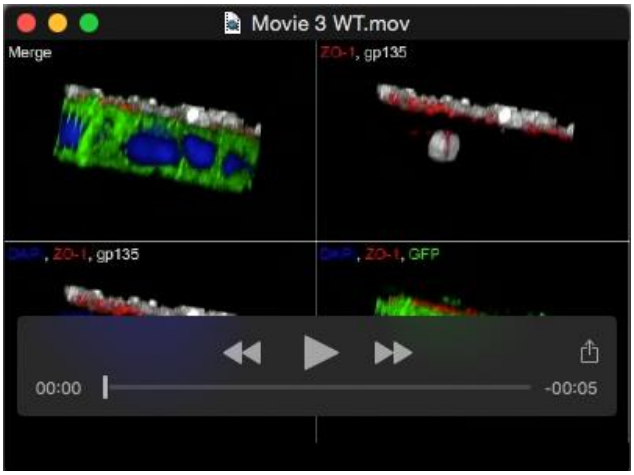
Fig. S4. AREG and EREG mistrafficking does not induce lateral lumens in MDCK cells. (A) Polarized MDCK cells stably expressing the AREG mistrafficking mutant, (EEL2A)AREG, were fixed and immunostained for AREG (green) and ZO-1 (red) and stained for DAPI (blue). (B) Polarized MDCK cells stably expressing the EREG mistrafficking mutant, (Y156A)EREG-EGFP, were fixed and immunostained for gp135 (white), ZO-1 (red), and stained for DAPI (blue); GFP fluorescence is shown in green. Composite xy projections and xz projections in individual channels are displayed. All scale bars: 10 µm.



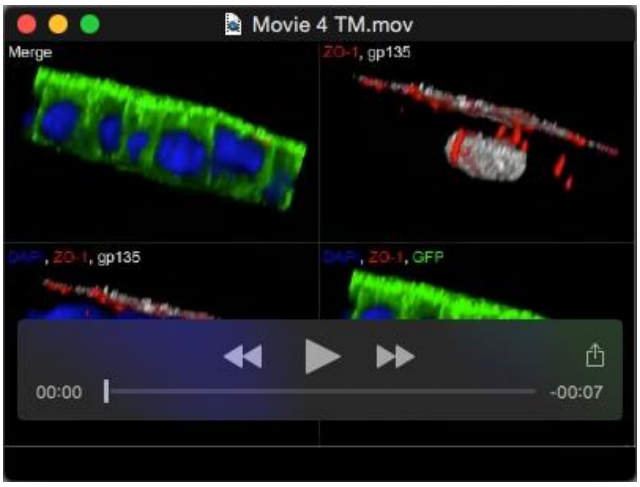
Movie 1. 3D projection of a lateral lumen in (C3/TM)BTC-EGFP-expressing MDCK cells. Polarized MDCK cells stably expressing (C3/TM)BTC-EGFP were fixed and stained for nuclei (DAPI, blue), tight junctions (ZO-1, red), and the apical surface (gp135, white); green color indicates BTC-EGFP fluorescence. A 3D projection of a confocal z-stack containing a lateral lumen is displayed with different combinations of stains.



Movie 2. 3D projection of a lateral lumen in parental MDCK cells. Polarized MDCK cells were fixed and stained for nuclei (DAPI, blue), tight junctions (ZO-1, red), and the apical surface (gp135, white). A 3D projection of a confocal z-stack containing a lateral lumen is displayed with different combinations of stains.



Movie 3. 3D projection of a lateral lumen in BTC-EGFP-expressing MDCK cells. Polarized MDCK cells stably expressing BTC-EGFP were fixed and stained for nuclei (DAPI, blue), tight junctions (ZO-1, red), and the apical surface (gp135, white); green color indicates BTC-EGFP fluorescence. A 3D projection of a confocal z-stack containing a lateral lumen is displayed with different combinations of stains.



Movie 4. 3D projection of a lateral lumen in (TM)BTC-EGFP-expressing MDCK cells. Polarized MDCK cells stably expressing (TM)BTC-EGFP were fixed and stained for nuclei (DAPI, blue), tight junctions (ZO-1, red), and the apical surface (gp135, white); green color indicates BTC-EGFP fluorescence. A 3D projection of a confocal z-stack containing a lateral lumen is displayed with different combinations of stains.