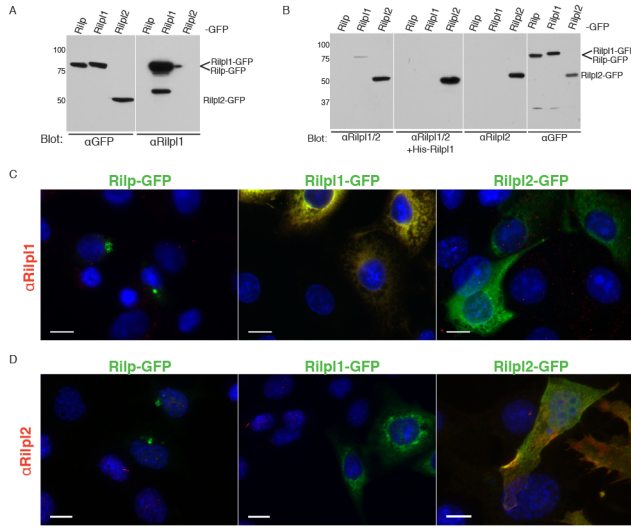## Supplemental Material

Figure S1. Testing of Rilpl1 and Rilpl2 antibodies. (A) Lysates from HEK293T cells transiently transfected with GFP tagged Rilp family proteins were probed for Rilpl1 and GFP. Numbers to the left are molecular weights in kilodaltons. Note that anti-Rilpl1 antibody only recognizes Rilpl1-GFP. (B) Lysates from HEK293T cells transiently transfected with GFP tagged Rilp family proteins were probed for Rilpl2 and GFP. Numbers to the left are molecular weights in kilodaltons. Note that anti-Rilpl1/2 antibody recognizes both Rilpl1 and Rilpl2, where as anti-Rilpl2 only recognizes Rilpl2-GFP. (C) Representative immunofluorescence images of IMCD3 cells transiently transfected with GFP-tagged Rilp family proteins, fixed, and stained for GFP (green), Rilp1 (red), and DNA (DAPI, blue). Bars, 10 μm. Rilpl1 antibody only recognizes Rilpl1-GFP. (D) Representative immunofluorescence images of IMCD3 cells transiently transfected with GFP-tagged Rilp family proteins, serum starved for 24 hrs, fixed, and stained for GFP (green), Rilp2 (red), and DNA (DAPI, blue). Rilpl2 antibody only recognizes Rilpl2-GFP. Bars, 10 μm.
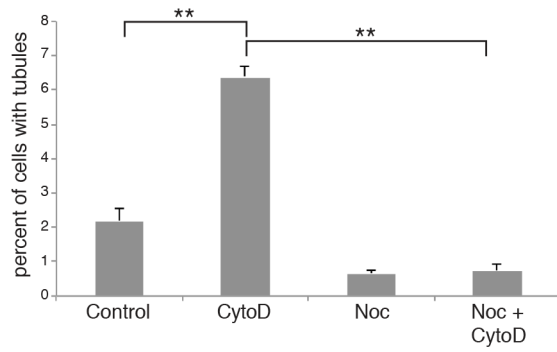
Figure S2. Rilpl2 tubules are dependent on microtubule and actin dynamics. Quantification of the frequency of Rilpl2-LAP tubule formation in serum starved IMCD3 cells treated with vehicle or 1 μM cytochalasin D (CytoD) for 30 min with or without pre-treatment with 5 μg/ml nocodazole (Noc). Results shown are the mean of three independent experiments ± SEM. (500 cells/experiment, **p < 0.01, t-test).
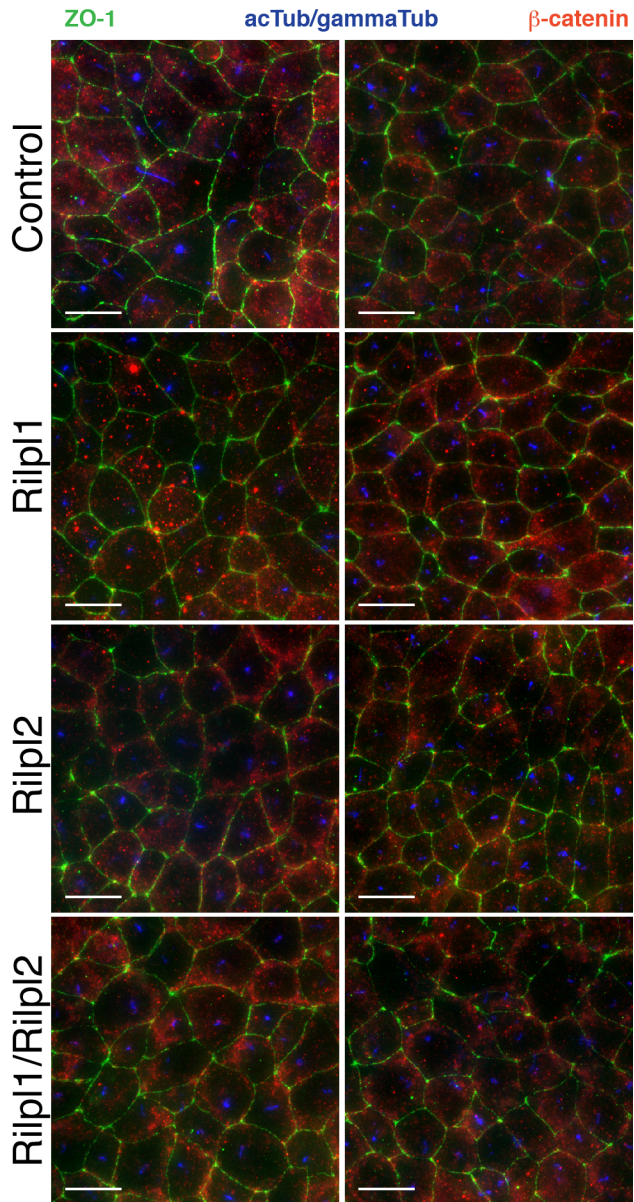
Figure S3. Polarization of Rilp-like protein depleted cells. Representative immunofluorescence images of IMCD3 cells depleted of the indicated protein(s) grown on trans-well filters and stained for ZO-1 (apical junction, green), beta catenin (basolateral surface, red), and gamma and acetylated tubulins (centrosomes and cilia, blue). Images are maximum projections of z-stacks. Bars, 10 μm.

Movie S1. Dynamic localization of Rilpl2-LAP to a primary cilium. Movie shows GFP fluorescence and tdTomato fluorescence in an IMCD3 cell expressing Rilpl2-LAP and tdTomato-inversin (cilium) (Figure 4A). Total time lapsed is 90 min; movie plays at 600x real time.

Movie S2. Rilpl2-LAP forms dynamic tubulovesicular structures. Movie shows GFP fluorescence in an IMCD3 cell expressing Rilpl2-LAP (Figure 4B). Each frame is the maximum projection of a deconvolved z-stack. Total time lapsed is ~ 5 min; movie plays at 68x real time.

Movie S3. Rilpl2-LAP forms dynamic tubulovesicular structures at the base of a primary cilium. Movie shows GFP fluorescence and tdTomato fluorescence in an IMCD3 cell expressing Rilpl2-LAP and tdTomato-inversin (cilium) (Figure 4D). The proximal end of the cilium is on the left. Total time lapsed is 50 min; movie plays at 300x real time.