Hoffmeister et al., http://www.jcb.org/cgi/content/full/jcb.201007050/DC1

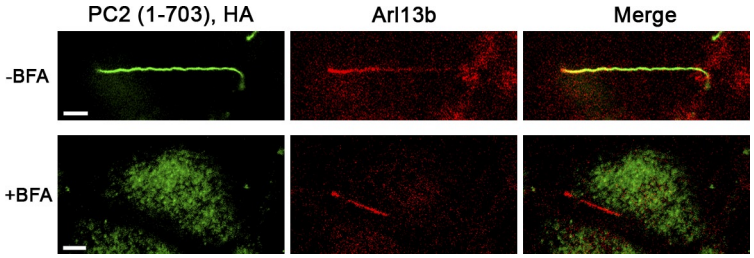


Figure S1. **Trafficking of polycystin-2 to the primary cilium depends on an intact Golgi apparatus.** LtA-2,22 cells were stably transfected with an expression plasmid for HA epitope–tagged polycystin-2 (1–703), and treated with brefeldin A (+BFA) or with the solvent methanol (−BFA). Double staining for the HA epitope and the monomeric G protein Arl13b, another ciliary marker, revealed that in the presence of brefeldin A the truncated polycystin-2 protein no longer reached primary cilia. Bar, 5 µm.

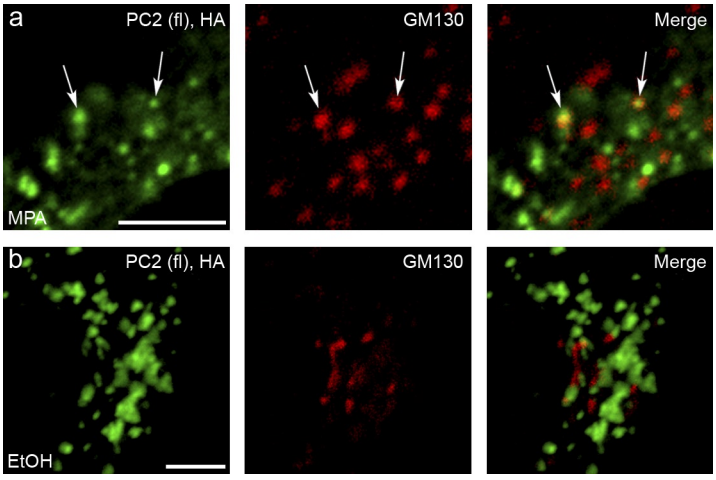


Figure S2. **Colocalization of full-length polycystin-2 and GM130.** Stably transfected LLC-PK_1 cells inducibly producing HA epitope–tagged full-length polycystin-2 were incubated for 6 h with 50 µM mycophenolic acid (MPA). Double staining for the cis-Golgi marker protein GM130 led to the identification of polycystin-2 vesicles colocalizing with GM130 (a, arrows). Cells incubated for 24 h with 0.2% of the solvent ethanol (EtOH) served as a negative control (b). Bar, 5 µm.

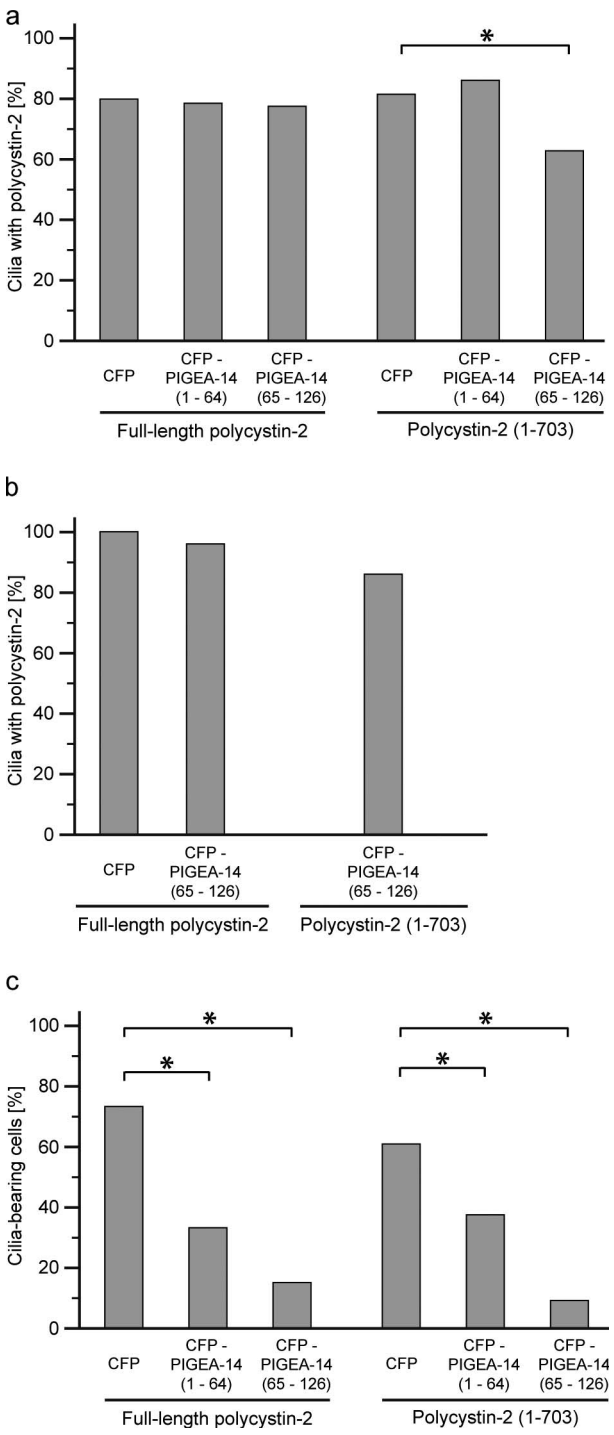


Figure S3. **Ciliary trafficking of polycystin-2 in the presence of PIGEA-14 mutant proteins.** (a) Stably transfected LLC-PK_1 cells inducibly producing HA epitope–tagged full-length polycystin-2 and polycystin-2 (1–703) were transiently transfected with expression constructs encoding ECFP fusion proteins with PIGEA-14 (1–64) and PIGEA-14 (65–126), respectively. Only under one experimental condition was a statistically significant effect observed. (b) Microinjection of the ECFP/PIGEA-14 (65–126) fusion protein had no effect on the trafficking of either polycystin-2 construct. (c) Both PIGEA-14 fusion proteins inhibited the formation of cilia, thus indicating that they are biologically active. In the transient transfection experiments, between 402 and 635 CFP-positive cells were counted, and at least three independent experiments were included. For the microinjection experiments, between 25 and 37 cells were counted. The asterisks indicate statistically significant differences at $P < 0.01$ as determined by a χ^2-test.

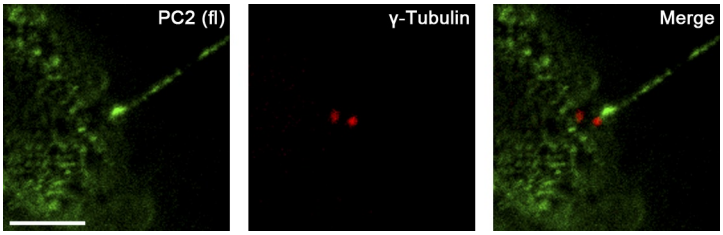
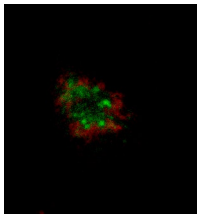


Figure S4. **Double staining for full-length polycystin-2 and γ-tubulin.** LtA-2,22 cells were stably transfected with an expression plasmid for HA epitope–tagged full-length polycystin-2 (PC2 (fl)). Double staining for polycystin-2 and γ-tubulin failed to reveal colocalization of the two proteins. Bar, 5 μm.



Video 1. **Imaging of full-length polycystin-2.** LLC-PK_1 cells stably synthesizing an ECFP fusion protein with *N*-acetylgalactosaminyltransferase-2 (shown in red for better visibility) were transiently transfected with an expression plasmid coding for an EGFP fusion protein with full-length polycystin-2. 3 d later the cells were incubated for 5 h at 15°C before being incubated at 37°C. Pictures were taken every 2 min over a period of 60 min, ~5 min after shifting the cells to 37°C. No overlapping signal can be seen between full-length polycystin-2 and *N*-acetylgalactosaminyltransferase-2.