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Journal Title: Journal of Cell Biology
Volume: Volume 216, Number 7
Publisher: ROCKEFELLER UNIV PRESS | 2017-07-01, Pages 2131-2150
Type of Work: Article
Publisher DOI: 10.1083/jcb.201611138
Permanent URL: https://pid.emory.edu/ark:/25593/s7d42

Final published version: http://dx.doi.org/10.1083/jcb.201611138

Accessed February 10, 2018 11:26 AM EST
BLOC-1 is required for selective membrane protein trafficking from endosomes to primary cilia

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Primary cilia perceive the extracellular environment through receptors localized in the ciliary membrane, but mechanisms directing specific proteins to this domain are poorly understood. To address this question, we knocked down proteins potentially important for ciliary membrane targeting and determined how this affects the ciliary trafficking of fibrocystin, polycystin-2, and smoothened. Our analysis showed that fibrocystin and polycystin-2 are dependent on IFT20, GMAP210, and the exocyst complex, while smoothened delivery is largely independent of these components. In addition, we found that polycystin-2, but not smoothened or fibrocystin, requires the biogenesis of lysosome-related organelles complex-1 (BLOC-1) for ciliary delivery. Consistent with the role of BLOC-1 in sorting from the endosome, we find that disrupting the recycling endosome reduces ciliary polycystin-2 and causes its accumulation in the recycling endosome. This is the first demonstration of a role for BLOC-1 in ciliary assembly and highlights the complexity of pathways taken to the cilium.

Introduction

Many vertebrate cells possess a single nonmotile primary cilium that extends from the cell surface. These cilia perceive the extracellular environment by localizing specific receptors to the ciliary membrane. To date, more than 25 different receptors have been found to be ciliary localized (Hilgendorf et al., 2016). These include the important cystoproteins polycystin-1, polycystin-2, and fibrocystin, which are defective in polycystic kidney disease (PKD) and the hedgehog receptors patched-1 and tissue homeostasis.

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Abbreviations used: BLOC-1, biogenesis of lysosome-related organelles complex-1; IFT, intraflagellar transport; IMCD3, inner medullary collecting duct; MEK, mouse embryonic kidney; NRK, normal rat kidney; PKD, polycystic kidney disease.

The Rockefeller University Press
https://doi.org/10.1083/jcb.201611138

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Interestingly, acute deletion of IFT20 causes opsin accumulation at the Golgi complex, whereas acute deletion of IFT140 causes opsin accumulation in the inner segment plasma membrane (Keady et al., 2011; Crouse et al., 2014). These data are consistent with a model in which IFT20 is important for sorting or trafficking of membrane proteins from the Golgi apparatus to the base of the cilium, where they engage the rest of the IFT system (Follit et al., 2006).

It is not clear that all membrane proteins are trafficked to the cilium by the same route. Early work on opsin transport in frogs and mastigoneme transport in *Ochromonas danica* suggested that these proteins traffic in vesicles directly from the Golgi apparatus to the base of the cilium, where the vesicles dock to the plasma membrane just outside of the cilium before the proteins are transported into the organelle (Bouck, 1971; Papermaster et al., 1985; Deretic et al., 1995). More recent work on smoothened transport suggests that this protein is trafficked to the plasma membrane and laterally moves into the cilium (Milenkovic et al., 2009). Agglutinin transport in *Chlamydomonas reinhardii* uses a similar mechanism (Hunnicutt et al., 1990; Cao et al., 2015). A third pathway whereby proteins are first transported to the plasma membrane followed by endocytosis and delivery to the base of the cilium by the recycling pathway has been proposed, but no proteins are known to take this route (Weisz and Rodriguez-Boulan, 2009; Nachury et al., 2010).

The finding of opsin transport defects when IFT20 is perturbed suggests a role for IFT20-GMAP210 in the direct trafficking pathway from the Golgi complex to the base of the cilium. However, the role of IFT20 in trafficking additional membrane proteins from the Golgi complex and/or through other organelles has not been tested. To assess their importance in trafficking of ciliary membrane proteins, we use a fluorescence-based pulse-chase assay to measure the importance in trafficking of other ciliary membrane proteins, we first block all existing protein with a nonfluorescent derivative and then follow newly synthesized protein with a fluorescent derivative (Sun et al., 2011). The fibrocystin construct consisted of the extracellular domain of CD8 fused to fibrocystin just N-terminal to the transmembrane domain with a SNAP tag placed just before the stop codon. The polycystin-2 construct consisted of the first 703 amino acids fused to SNAP and GFP at the C-terminal end. Similarly, full-length smoothened was tagged with SNAP and GFP at the C-terminal end (Fig. S1, Aa–Ca). The SNAP tag is used to quantify newly synthesized protein, while the CD8 epitope tag (included in the extracellular domain of CD8) and GFP are used to monitor total protein levels at steady state. Each of these chimeric proteins is robustly localized to cilia (Fig. S1, Ab–Cb). Inner medullary collecting duct (IMCD3) Flp-In cell lines that uniformly express proteins are then infected with lentiviral shRNA constructs to knock down the levels of proteins of interest. Reduction of protein was verified by immunoblot, and the amount remaining was typically about ~10%–20% of the controls (Figs. 1, S2, S3, and S4).

The assay starts with blocking all the SNAP-tag binding sites with a cell-permeable nonfluorescent benzylguanine. This substrate is then washed out of the media, and the cells are allowed to synthesize new protein for 1.5 h. The cells are then treated with cycloheximide to inhibit further protein synthesis and shifted to 19°C to prevent the Golgi apparatus from releasing proteins. This causes an accumulation of newly synthesized protein at the Golgi apparatus (Ang et al., 2004). While still keeping the cells in cycloheximide, the cells are shifted back to 37°C, allowing newly synthesized protein to be released from the Golgi complex and trafficked to the cilium. The cells were then fixed at indicated time points after temperature shift back to 37°C, and the amount of newly synthesized and total tagged proteins in the cilium was measured.

For each experiment, we quantified the effectiveness of the shRNA knockdown by charge-coupled device detection of chemiluminescence and displayed results normalized to the un-
Figure 1. IFT20 knockdown affects fibrocystin and polycystin-2 ciliary trafficking but only modestly affects smoothened trafficking to the primary cilium. Quantification of (A) CD8-fibrocystin-CTS-SNAP, (B) polycystin-2(1-703)-GFP-SNAP, and (C) smoothened-SNAP-GFP trafficking from the Golgi apparatus to the primary cilium during lenti-shRNA knockdown of IFT20. (A–C, subpanel a) Selected immunoblot images of IFT20 knockdown and γ tubulin loading control. (A–C, subpanel b) Quantification of knockdown. Mean protein levels plotted from three independent experiments for each condition (control [Con.], NS-shRNA [NS.], shRNA#1, and shRNA#2) and normalized to their corresponding γ tubulin loading control. shRNA-mediated knockdown of IFT20
transfected control sample using γ tubulin or IFT27 as a loading control. For each trafficking experiment, we measured cilia length, ciliary SNAP fluorescence, and other ciliary GFP fluorescence or CD8 immunofluorescence at 0, 1, 2, 4, and 6 h after shift from 19°C to 37°C. The data were then plotted with respect to time, and the trafficking rate was determined from the slope of the curve derived from the SNAP fluorescence. All experiments are plotted and displayed similarly, with the control cell displayed in blue, the nonsilencing shRNA in green, and the two shRNAs targeting the gene of interest in red and black.

Under control conditions, the proteins showed different kinetics of delivery to the cilium (Fig. 1, A–C, subpanel e) and (A–C, subpanel c) mean SNAP ciliary fluorescence plotted from three independent experiments in which 30 cilia were quantified for each condition and (A–C, subpanel a) mean cilia length, (A–C, subpanel b) mean CD8 or GFP ciliary fluorescence, (A–C, subpanel d) mean SNAP fluorescence or CD8 immunofluorescence at 0, 1, 2, 4, and 6 h after shift from 19°C to 37°C. The data were then plotted with respect to time, and the trafficking rate was determined from the slope of the curve derived from the SNAP fluorescence. All experiments are plotted and displayed similarly, with the control cell displayed in blue, the nonsilencing shRNA in green, and the two shRNAs targeting the gene of interest in red and black. Error bars represent SEM.

To gain insight into the mechanism of how IFT20 and GMAP210 interact with the BLOC-1 subunits Pallidin and Sec8 (Fig. 3 A). Flag-GFP and Flag-IFT54 were used as negative and positive controls (Follit et al., 2009). As expected, Flag-GFP did not pull down IFT20, but Flag-IFT54 showed a strong interaction with IFT20 (Fig. 3 Ab). Flag-Palladin brought down endogenous IFT20, while no binding of IFT20 was observed with Flag-KXD1 and Flag-Exo70 (Fig. 3 Ab). Interestingly, Flag-Palladin also precipitated endogenous polycystin-2 (Fig. 3 Ab). Endogenous Exo70 was brought down by Flag-Palladin (Fig. 3 Ab). This is consistent with work showing BLOC-1 interacting with the exocyst components Sec6 and Sec8 (Gokhale et al., 2012). Flag-KXD1 pulled down Exo70 (Fig. 3 Ab), which may be explained by KXD1 interacting with BLOC-1 (Hayes et al., 2011; Yang et al., 2012).

Because no interaction was observed between Flag-Exo70 and endogenous IFT20, we tested to see if Flag-IFT20 could precipitate endogenous Exo70 or Sec8 exocyst subunits (Fig. 3 B). Flag-GFP and Flag-IFT25 were negative controls, and neither interacted with either of the exocyst components. As positive controls, Flag-IFT20 and Flag-IFT25 interacted as expected with IFT88 and Flag-IFT20 with GMAP210 (Fig. 3 B; Follit et al., 2008, 2009; Keady et al., 2011). Importantly, Flag-IFT20 also brought down endogenous Exo70 and Sec8 (Fig. 3 B). The reason for the discrepancy between the two immunoprecipitations is unknown but not uncommon in these types of studies. However, our finding of an interaction between IFT20 and both Exo70 and Sec8 is consistent with prior work showing that the Sec10 subunit of the exocyst could coprecipitate IFT20 and IFT88 (Fogelgren et al., 2011).

Previously we showed that overexpressing the IFT20-binding proteins IFT54 or GMAP210 displaces IFT20 from the Golgi membranes. Importantly, overexpressing other IFT proteins that do not directly bind IFT20 did not have any effect on the distribution of IFT20 in cells (Follit et al., 2009). As a further test for evidence of interaction with IFT20, we overexpressed Flag-KXD1, Flag-Palladin, Flag-Exo70, and, as a positive control, Flag-IFT54 (Fig. 3 C). Flag-IFT54 overexpression completely displaced IFT20 from the Golgi stacks (Fig. 3 Cb). High expression of Flag-KXD1 did not displace IFT20 from the Golgi (Fig. 3 Cc), consistent with a failure to interact in the immunoprecipitation assay. Flag-Palladin overexpression resulted in partial displacement of IFT20 into the cytoplasm and for unknown reasons caused the Golgi apparatus to compact (Fig. 3 Cd). Flag-Exo70 overexpression also caused displacement of IFT20 from the Golgi and induced filopodia formation, similar to what has been described (Fig. 3, Ce and De; Zuo et al., 2006). To ensure that this result was not due to disruption of the Golgi complex by overexpression of our test proteins, we stained with the Golgi marker GMAP210. GMAP210 was not displaced by overexpression of any of the proteins, and Golgi structure was normal except for the compaction observed when

results in >90% reduction of total protein abundance. (A–C, subpanel c) Mean cilia length, (A–C, subpanel d) mean CD8 or GFP ciliary fluorescence, and (A–C, subpanel e) mean SNAP ciliary fluorescence plotted from three independent experiments in which 30 cilia were quantified for each condition and (A–C, subpanel f) linear regression analysis of selected time points of newly synthesized membrane protein delivery to the cilium was completed. Slope values between control and experimental shRNA groups were compared with one another to determine statistical significance. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. Error bars represent SEM.
Flag-Pallidin was expressed (Fig. 3 D). Together the immunoprecipitation and displacement results provide strong evidence that IFT20 interacts with the BLOC-1 subunit pallidin and the exocyst subunit Exo70.

The localization of pallidin at the basal body is partially dependent on IFT20

Because IFT20 binds Exo70 and pallidin, we determined whether their cellular localization is dependent on IFT20. To do this, wild-type and Ift20−/− mouse embryonic kidney (MEK) cells (Jonassen et al., 2008) were transfected with Flag-Pallidin or stained for endogenous Exo70 (Fig. 4). In wild-type cells, Flag-Pallidin partially localizes at the basal body (Fig. 4 Aa). This is consistent with prior work showing other BLOC-1 subunits localizing to the centrosome (Wang et al., 2004). Interestingly, there is a significant decrease in the amount of basal body–localized Flag-Pallidin in the Ift20−/− MEKs, while there is no difference in the total Flag-Pallidin levels in the cell (Fig. 4 A). Endogenous Exo70 partially localizes at the basal body, but there was no difference between control and Ift20−/− cells (Fig. 4 B).

There does not appear to be any effect on IFT20 localization when either pallidin or Exo70 is knocked down (Fig. S6), indicating that IFT20 localization is not dependent on either protein.

The exocyst complex is involved in the ciliary trafficking of fibrocystin and polycystin-2

Previous work had demonstrated a role for exocyst subunit Sec10 in ciliary assembly (Zuo et al., 2009). Because we had found that IFT20 was able to precipitate the exocyst subunits Exo70 and Sec8, we tested the exocyst’s role in trafficking of our model ciliary proteins by knocking down these subunits (Fig. 2, G–L; and Fig. S3). Knockdown of Exo70 to about ∼20% of normal had modest effects on ciliary length
(Fig. S3, Ac–Cc), while knockdown of Sec8 to ∼20% of controls decreased ciliary length to ∼40% of normal (Fig. S3, Dc–Fc). Knockdown of these exocyst subunits reduced the steady-state levels of ciliary fibrocystin and polycystin-2 (Fig. S3, Ad, Bd, Dd, and Ed) and reduced the rate of delivery of these proteins to cilia (Fig. 2, G, H, J, and K; and Fig. S3, Ae, Be, De, and Ee). The reduction of Exo70 or Sec8 did not affect the rate of delivery of newly synthesized smoothened to the cilium (Fig. 2, I and L; and Fig. S3, Ce and Fe), and the steady-state level of ciliary smoothened was only modestly or not affected (Fig. S3, Cd and Fd). This further strengthens the idea that distinct membrane proteins are using different trafficking pathways and specific molecular machineries for ciliary delivery.

Figure 3. IFT20 interacts with the BLOC-1 subunit pallidin and the exocyst subunits Exo70 and Sec8. [A] Flag-GFP (negative IFT20 binding control), Flag-IFT54 (positive IFT20 binding control), Flag-KXD1, Flag-Pallidin, and Flag-Exo70 were expressed in mouse IMCD3 cells. The cells were lysed, immunoprecipitated, with Flag antibody and analyzed by immunoblotting. The left group is the starting material before immunoprecipitation (10% input), and the right group is the precipitated material (IP). Flag-tagged proteins are marked with arrows on the Flag Western blot. Antibodies used for the Western blots are listed on the right side. [B] Flag-GFP (negative binding control), Flag-HC2 (positive binding control), Flag-IFT25 (positive binding control for IFT complex C protein IFT88), and Flag-IFT20 were expressed in mouse IMCD3 cells. The cells were lysed, immunoprecipitated, with Flag antibody and analyzed by immunoblotting. The left group is the starting material before immunoprecipitation (10% input), and the right group is the precipitated material. Proteins are marked with arrows. Antibodies used for the Western blots are listed on the right side. [C] Selected images of IMCD3 cells expressing the Flag-fusion proteins that are listed horizontally at the top of the figure. Flag-fusion proteins were detected with Flag antibody staining (green), endogenous IFT20 antibody staining (red), and nuclei detected with DAPI (blue). (Ca) IFT20 is localized at the Golgi membranes in IMCD3 control cells (arrows). (Cb) Increased pixel intensity of IFT20 in the cytoplasm (arrows) and complete loss of IFT20 from the perinuclear Golgi pool in Flag-IFT54-expressing cells. (Cc) Flag-KXD1-expressing cells show IFT20 localization at the Golgi membranes (arrows) and no IFT20 displacement into the cytoplasm. (Cd) Compact Golgi membranes (asterisks) and increased pixel intensity of IFT20 in the cytoplasm (arrows) and partial displacement from the Golgi membranes in Flag-Pallidin-expressing cells. (Ce) Up-regulated filopodia (asterisks) formation and increased IFT20 pixel intensity in the cytoplasm (arrows) and partial IFT20 displacement from Golgi membranes in cells expressing Flag-Exo70. Bar, 10 µm. [D] Selected images of IMCD3 cells expressing the Flag-fusion proteins that are listed horizontally at the top of the figure. Flag-fusion proteins were detected with Flag antibody staining (green), endogenous GMAP210 antibody staining (red), and nuclei detected with DAPI (blue). (Da) GMAP210 is localized at the Golgi membranes in IMCD3 control cells, (Db) Flag-IFT54-expressing cells, and (Dc) Flag-KXD1-expressing cells (arrows). (Dd) Compact Golgi membranes (asterisks) and GMAP210 are localized at the Golgi membranes (arrows) in Flag-Pallidin-expressing cells. (De) Up-regulated filopodia (asterisks) formation and GMAP210 are localized at the Golgi membranes (arrows) in cells expressing Flag-Exo70. Bar, 10 µm.
BLOC-1 is important for the trafficking of polycystin-2 to primary cilia

BLOC-1 has not been previously implicated in ciliary trafficking, but our finding that pallidin bind IFT20 and polycystin-2 suggests involvement. To test this idea, we assayed ciliary transport after knocking down pallidin and another BLOC-1 subunit dysbindin. Knockdown of pallidin to 10%–15% of normal had no effect on ciliary length (Fig. S4, Ac–Cc). Knockdown of dysbindin to ∼10% of normal affects ciliary assembly (Fig. S4, Dc–Fc). Knockdown of either dysbindin or pallidin had no effect on steady-state levels or rates of delivery of fibrocystin and smoothened to cilia (Fig. 2, M, O, P, and R; and Fig. S4, Ad, Cd, Dd, Fd, Ae, Ce, De, and Fe). In contrast, knockdown of either dysbindin or pallidin reduced the rates of delivery of polycystin-2 to cilia, and dysbindin knockdown decreased steady-state levels of ciliary polycystin-2 (Fig. 2, Q and Fig. S4, Ea, Eb, Ed, and Ec). Our protein interaction data and pulse-chase trafficking data give strong evidence that BLOC-1 is specifically involved in polycystin-2 delivery to cilia. This emphasizes that polycystin-2 is taking a specific pathway to the cilium that is dependent on BLOC-1 machinery.

Perturbation of the exocyst or BLOC-1 complexes decreases endogenous polycystin-2 levels at the primary cilium

To further verify our finding that the exocyst complex and BLOC-1 are involved in trafficking polycystin-2 to the primary cilium, we performed knockdowns in MEK cells and measured endogenous polycystin-2 ciliary levels. MEK cells were chosen because they have more polycystin-2 in their cilia than IMCD3 cells, and because they are mouse cells, the shRNAs developed previously are effective in them.

Similar to what we observed in IMCD3 cells, Exo70 knockdown had little to no effect on ciliary assembly in MEK cells, but Sec8 knockdown decreased ciliary length (Fig. S5, Bd and Cd). Importantly, knockdown of either Exo70 or Sec8...
resulted in decreased steady-state ciliary levels of polycystin-2 (Fig. S5, Ba–Bc), confirming our results with an exogenous chimeric protein. Additionally, knockdown of Sec8 but not Exo70 reduced the ciliary levels of Arl13b at the cilium (Fig. S5, Ca–Cc). Arl13b is a peripheral membrane protein anchored to the cilium by palmitoylated cysteines (Sun et al., 2004; Cevik et al., 2010; Li et al., 2010). It is not clear why the two subunits have different effects on ciliary Arl13b levels, but Sec8 is a core exocyst subunit, while Exo70 is a peripheral membrane-docking subunit (TerBush and Novick, 1995; TerBush et al., 1996; He et al., 2007; Wu et al., 2010). It is possible that core subunits are more important for delivery of Arl13b or that higher levels of Sec8 are needed than Exo70 and the Exo70 levels were not reduced below the critical level.

To confirm the results obtained with the exogenous chimeric protein, we examined the involvement of BLOC-1 in trafficking of endogenous polycystin-2 to primary cilia. Again, MEK cells were used because of their high levels of ciliary polycystin-2. In these cells, knockdown reduced pallidin and dysbindin to 10%–15% of normal (Fig. 5, Ac and Ad). The dysbindin knockdown caused a decrease in cilia length and reduced the steady-state level of ciliary polycystin-2 (Fig. 5 B). Similar to what we saw in IMCD3 cells, pallidin knockdown did not affect cilia length or the levels of ciliary polycystin-2 (Fig. 5 B). This continues to strengthen the evidence that the exocyst complex and BLOC-1 are involved in the trafficking of polycystin-2 to cilia.

**Dtnbp1sdy/sdy and Pldnpa/pa mice possess a cystic kidney phenotype**

The sandy (Dtnbp1<sup>+/−</sup>) and pallid mice (Pldnpa<sup>+/−</sup>) are loss-of-function models of dysbindin and palladin (Huang et al., 1999; Li et al., 2003). Given that our data suggest that BLOC-1 is involved in the trafficking of polycystin-2 to primary cilia, we hypothesized that the Dtnbp1<sup>+/−</sup> and Pldnpa<sup>+/−</sup> mice would have cystic kidneys. Hematoxylin and eosin staining of 5-wk-old Dtnbp1<sup>+/−</sup> and 8-wk-old Pldnpa<sup>+/−</sup> kidneys showed no prominent cyst formation, but the collecting ducts in the papilla were slightly distended in both mouse models (Fig. S7, A, Ba, and Bb). Proximal tubule circumference was also slightly increased in the Dtnbp1<sup>+/−</sup> mice, and both the Dtnbp1<sup>+/−</sup> and Pldnpa<sup>+/−</sup> mice had a slight increase in the mean number of proximal tubule nuclei per cross section (Fig. S7 C). Additionally, the Dtnbp1<sup>+/−</sup> and Pldnpa<sup>+/−</sup> mice had a moderate decrease in ciliary length, and Dtnbp1<sup>+/−</sup> mice showed a modest decrease in the percentage of cilia per basal body (Fig. S7, Bc and Bd).

Given that the first cohort of Dtnbp1<sup>+/−</sup> and Pldnpa<sup>+/−</sup> mice were relatively young, we aged a second cohort for 12 mo to see whether they would present a more severe cystic kidney phenotype. These animals suffer from lung fibrosis, and very few animals survive to 1 yr of age (McGarry et al., 2002). However, we were able to collect three Dtnbp1<sup>+/−</sup> and one Pldnpa<sup>+/−</sup> animal at this age. None of the animals showed prominent cysts, but the collecting ducts were obviously distended (Fig. 6 A). Quantification showed a significant increase in the Dtnbp1<sup>+/−</sup> collecting duct diameter measured in the kidney cortex, at the junction between the outer and inner medulla, and in the inner medulla at the tip of the papilla (Fig. 6 B). The Pldnpa<sup>+/−</sup> also showed increased diameter of collecting ducts, but only one animal was examined. Proximal tubules were also dilated in the two models. In both models, the circumferences were slightly larger, and the number of nuclei per cross section was increased (Fig. 6, D and E). In addition, we noted abnormal position of the proximal tubule nuclei in the two models. Proximal tubule cells typically have well-separated nuclei located near the basal surface. In the mutants, nuclei distribute throughout the cell, including at the apical surface, and often are found next to each other (Fig. 6 D).

There is no difference in cilia assembly and the percentage of cilia per basal body in the Pldnpa<sup>+/−</sup> mice (Fig. 6 C). However, the Dtnbp1<sup>+/−</sup> mice have a decrease in both cilia length and the percentage of cilia per basal body (Fig. 6 C). The Dtnbp1<sup>+/−</sup> and Pldnpa<sup>+/−</sup> mouse phenotype strongly correlates with the biochemical and cell biology data and supports the conclusion that BLOC-1 is involved in trafficking polycystin-2 to primary cilia.

**Polycystin-2 but not fibrocystin or smoothened is retained in the recycling endosome when the C-terminal tail of MyoVb is overexpressed**

The BLOC-1 complex is thought to function at the recycling endosome to extend from sorting endosomes to direct cargos into the recycling endosome and lysosome (Di Pietro et al., 2006; Salazar et al., 2006; Setty et al., 2007; Ryder et al., 2013; Delevoye et al., 2016; Dennis et al., 2016). Because the recycling endosome is localized at the base of the cilium (Westlake et al., 2011), we questioned whether the function of BLOC-1 in trafficking polycystin-2 to cilia might involve its trafficking to or through the recycling endosome. To disrupt trafficking through the recycling endosome, we expressed a fragment of MyoVb that acts as a dominant negative on trafficking through this compartment (Lapierre et al., 2001; Volpicelli et al., 2002). The MyoVb dominant-negative fragment contains the C-terminal Rab11-binding domain but lacks the motor domain, allowing the fragment to bind Rab11-containing vesicles and interfere with their transport. GFP-MyoVb C-terminal tail colocalizes with the recycling endosome marker Rab11 (Fig. S8 A) but not with the Golgi markers IFT20 (Fig. S8 B) and GM120 (Fig. S8 C). Expression of the MyoVb fragment induces compaction of the Golgi complex (Fig. S8, B and C) but had only a small effect on cilia length (Fig. 7, Ad and Bd).

We were unable to package the MyoVb expression vector into lentivirus, as it appears to interfere with some step of the process, and had to rely on electroporation to deliver the construct. MEK cells are difficult to transfet, so we used normal rat kidney (NRK) cells, which also have high levels of ciliary polycystin-2 and are easy to transfect. NRK cells transfected with GFP-MyoVb accumulated polycystin-2 at the recycling endosome (Fig. 7 Aa and Fig. S9, A and B) and had a significant decrease in the steady-state levels of ciliary polycystin-2 (Fig. 7, Ab and Ac). Additionally, we observed a moderate accumulation of endogenous Arl13b at the recycling endosome upon GFP-MyoVb overexpression (Fig. 7 Aa).

To examine the effect of the MyoVb construct on trafficking of smoothened to the cilium, we used Ift27<sup>−/−</sup> NIH 3T3 cells, as the lack of IFT27 causes smoothened to accumulate to high levels in cilia (Eguyether et al., 2014). Expression of GFP-MyoVb C-terminal tail in these cells resulted in a moderate accumulation of smoothened in the recycling endosome (Fig. 7 Ba and Fig. S9, C and D), but there was no difference in ciliary smoothened levels (Fig. 7, Bb and Bc).

We went on to use a Flag-MyoVb construct in our pulse-chase trafficking assays for fibrocystin, polycystin-2, and smoothened. We observed a bolus of polycystin-2 colocalizing...
with the Flag-MyoVb in the recycling endosome after Golgi release (Fig. 8 A). There is a decrease in both the ciliary length (Fig. 8 B) and ciliary trafficking of polycystin-2 (Fig. 8, C and D) in the Flag-MyoVb-overexpressing cells. However, trafficking of fibrocystin and smoothened were not affected by the expression of the MyoVb dominant negative construct (Fig. S11).

To determine whether knockdown of the exocyst or BLOC-1 components results in accumulation of endogenous polycystin-2 in endosome cellular compartments, we stained our shRNA MEK cell lines with the recycling endosome marker Rab11 (Fig. S10). We did not detect accumulation of polycystin-2 in Rab11-positive endosomes in our exocyst or BLOC-1 knockdowns (Fig. S10 B).

Ciliary polycystin-2 is reduced by overexpression of dominant-negative Rab11a

To obtain more evidence that polycystin-2 is using the recycling endosome for trafficking to the primary cilium, we measured ciliary polycystin-2 levels in cells expressing wild-type or mutant

Figure 5.  Dysbindin knockdown decreases endogenous ciliary polycystin-2 levels. (A) Immunoblot and quantification of total protein levels of MEK cells expressing either pallidin or dysbindin lenti-shRNAs. (Aa) Selected immunoblot images showing pallidin knockdown and (Ab) dysbindin knockdown under each condition (control, NS-shRNA, shRNA#1, and shRNA#2). Intensity was normalized to their corresponding γ tubulin or IFT27 loading control. shRNA-mediated knockdown of either pallidin or dysbindin results in >90% reduction of total protein abundance. (B) Immunostaining and quantification of ciliary polycystin-2 and cilia length in MEK cells expressing either pallidin or dysbindin lenti-shRNAs. (Ba) Selected images of polycystin-2 (Pkd2) antibody staining (red), Arl13b antibody staining (green), and nuclei are detected with DAPI (blue). Bars, 10 µm. Insets are 170% enlargements of the cilia. (Bb) Mean steady-state ciliary polycystin-2 is decreased and (Bc) mean ciliary polycystin-2 per micrometer is reduced in cells expressing dysbindin shRNAs. (Bd) Mean cilia length is reduced in cells expressing dysbindin shRNAs. n = 107 cilia per experimental group. Error bars are SEM. Data were analyzed using one-way ANOVA and the Bonferroni multiple-comparisons test. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.
Figure 6. **Dtnbp1**<sup>1dy/sdy</sup> and **Pldnpa**<sup>pa/pa</sup> mice have mildly cystic kidneys. (A) Selected confocal images of 12-mo-old wild-type, **Dtnbp1**<sup>1dy/sdy</sup>, and **Pldnpa**<sup>pa/pa</sup> mouse kidney sections stained with cilia marker Arl13b antibody (red), collecting duct marker AQP2 antibody (green), and basal body marker γ tubulin antibody (blue). Bars, 25 µm. Insets are 285% enlargements. (B) Quantification of mean collecting duct diameters in three regions of the kidney; (Ba) cortex (wild-type and **Dtnbp1**<sup>1dy/sdy</sup> n = 183 collecting ducts per group, **Pldnpa**<sup>pa/pa</sup> n = 61 collecting ducts), (Bb) medulla to papilla transition (wild-type and **Dtnbp1**<sup>1dy/sdy</sup> n = 183 collecting ducts per group, **Pldnpa**<sup>pa/pa</sup> n = 61 collecting ducts), and (Bc) tip of the papilla (wild-type and **Dtnbp1**<sup>1dy/sdy</sup> n = 33 collecting ducts per group, **Pldnpa**<sup>pa/pa</sup> n = 11 collecting ducts). There is an increase in collecting duct diameter in the **Dtnbp1**<sup>1dy/sdy</sup> and **Pldnpa**<sup>pa/pa</sup> cortex and medulla-to-papilla transition. Additionally, the **Dtnbp1**<sup>1dy/sdy</sup> papilla tip collecting duct diameter is wider. (C) Quantification of mean kidney cilia length and the percentage of cilia per basal body. (Ca) Wild-type and **Dtnbp1**<sup>1dy/sdy</sup> n = 300 cilia per group, **Pldnpa**<sup>pa/pa</sup> n = 100 cilia. Cilia in the **Dtnbp1**<sup>1dy/sdy</sup> kidneys are shorter compared with control. (Cb) Wild-type and **Dtnbp1**<sup>1dy/sdy</sup> n = 315 basal bodies per group, **Pldnpa**<sup>pa/pa</sup> n = 105 basal bodies. Percentage cilia per basal body is reduced in the **Dtnbp1**<sup>1dy/sdy</sup> kidneys. (D) Selected confocal images of 12-mo-old wild-type, **Dtnbp1**<sup>1dy/sdy</sup>, and **Pldnpa**<sup>pa/pa</sup> kidney sections stained with proximal tubule marker *Lotus tetragonolobus* agglutinin (LTA; green) and nuclei with Dapi (red). Most tubules are proximal, and some are marked with “P.” The few distal convoluted tubules are marked with “D.” Arrows label proximal tubule nuclei, and asterisks indicate red blood cells. Bars, 25 µm. Insets are 172% enlargements. (E) Quantification of mean kidney proximal tubule nuclei circumference, mean number of proximal tubule nuclei, and nuclei number per proximal tubule circumference. Wild-type and **Dtnbp1**<sup>1dy/sdy</sup> n = 225 proximal tubules per group, **Pldnpa**<sup>pa/pa</sup> n = 73 proximal tubules. (Ca) **Dtnbp1**<sup>1dy/sdy</sup> and **Pldnpa**<sup>pa/pa</sup> proximal tubules have an increased circumference. (Cb) **Dtnbp1**<sup>1dy/sdy</sup> and **Pldnpa**<sup>pa/pa</sup> proximal tubules have an increased mean number of nuclei. (Cc). Increase in nuclei number per proximal tubule circumference. n = 3 mice per wild-type and **Dtnbp1**<sup>1dy/sdy</sup> groups and n = 1 mouse per **Pldnpa**<sup>pa/pa</sup> group. Tissue from the wild-type and **Dtnbp1**<sup>1dy/sdy</sup> mice was harvested at precisely 12 mo of age. Two of the three **Pldnpa**<sup>pa/pa</sup> mice died of a known lung fibrosis phenotype during the aging process. Tissue from the remaining **Pldnpa**<sup>pa/pa</sup> mouse was harvested at 11 mo and 19 d of age. Error bars are SEM. Data were analyzed using one-way ANOVA and the Bonferroni multiple-comparisons test. ***, P < 0.001; ****, P < 0.0001.
forms of Rab11a that are thought to disrupt the recycling endosome (Hehnly and Duxsey, 2014). Overexpressing wild-type Rab11a or Rab11aQ70L, which is thought to block GTP hydrolysis and keep the protein in a constitutively active GTP-bound form, had no effect on ciliary levels of polycystin-2. However, overexpressing Rab11aS25N, which is thought to act as a dominant negative by binding guanine exchange factors, reduced ciliary polycystin-2 levels (Fig. 9, A–C). None of these constructs affected ciliary length (Fig. 9 D). Our finding that ciliary polycystin-2 levels are reduced in cells expressing Rab11aS25N is consistent with dominant-negative forms of Rab11 disrupting the recycling endosome (Westlake et al., 2011; Hehnly and Duxsey, 2014) and further supports our model that polycystin-2 is trafficked to cilia through the recycling endosome.
Cilia monitor the extracellular environment through specific receptors concentrated in their membranes, but we know little about how membrane proteins reach this organelle. Our prior work showed that IFT20 was required for delivery of polycystin-2 to primary cilia and rhodopsin to photoreceptor outer segments. IFT20 localizes to the Golgi complex by interaction with the golgin protein GMAP210 and is part of the IFT complex localized at cilia. This dual localization suggests that IFT20 could sort or traffic vesicles destined for the cilium, but details of how it accomplishes this are lacking. In our present work, we find that IFT20 interacts with the exocyst and the BLOC-1 complexes, and we find that polycystin-2, fibrocystin, and smoothened each has unique requirements for delivery to cilia. Fibrocystin requires IFT20-GMAP210 and the exocyst. Polycystin-2 requires IFT20-GMAP210, the exocyst, and BLOC-1. However, smoothened delivery is largely independent of all of these proteins.

Discussion

Figure 8. Overexpression of MyoVb perturbs polycystin-2 trafficking to the primary cilium. (A) Selected trafficking images of polycystin-2-GFP-SNAP IMCD Flp-In cells overexpressing Flag-MyoVb C-terminal tail. GFP and Arl13b antibody staining (green), SNAP TMR STAR (red), and Flag-MyoVb (blue). Insets depict newly synthesized polycystin-2-GFP-SNAP trafficking to the cilium in control or Flag-MyoVb-overexpressing cells. Insets also show accumulation of newly synthesized polycystin-2-GFP-SNAP in the recycling endosome in the Flag-MyoVb-overexpressing cells. Insets are 200% enlargements of the cilia and 170% enlargements of the recycling endosome. Bars, 10 µm. (B) Mean cilia length of polycystin-2-GFP-SNAP IMCD Flp-In control and Flag-MyoVb-overexpressing cells after the Golgi release. Cilia are shorter in Flag-MyoVb-overexpressing cells. (C) Mean SNAP ciliary fluorescence of Polycystin-2-GFP-SNAP delivery to the cilium after Golgi release. Polycystin-2 ciliary trafficking decreased in Flag-MyoVb-overexpressing cells. (D) Linear regression slope values of newly synthesized polycystin-2-GFP-SNAP in IMCD Flp-In control and Flag-MyoVb-overexpressing cells after the Golgi release using data points taken at 0, 1, 2, 4, and 6 h. Mean SNAP ciliary fluorescence and mean ciliary length were plotted from three independent experiments in which 30 cilia were quantified for each condition (control and Flag-MyoVb) at each time point (n = 90 total cilia per time point). Error bars represent SEM. Data were analyzed using the unpaired Student’s t test. *, P < 0.05; **, P < 0.01; ****, P < 0.0001.
The exocyst was required for the delivery of polycystin-2 and fibrocystin to cilia. This complex consists of eight proteins (Sec3/Exoc1, Sec5/Exoc2, Sec6/Exoc3, Sec8/Exoc4, Sec10/Exoc5, Sec15/Exoc6, EXO70/Exoc7, and EXO84/Exoc8) conserved from yeast to mammals (TerBush et al., 1996; Kee et al., 1997; Heider et al., 2016). The exocyst tethers vesicles at target sites before membrane fusion (Heider and Munson, 2012; Luo et al., 2014). In polarized cells, the exocyst localizes along sites of secretion on the lateral membrane and at the base of the cilium (Grindstaff et al., 1998; Rogers et al., 2004; Mazelova et al., 2009). Knockdown of Sec10 disrupts ciliogenesis in Madin-Darby canine kidney cells, and targeted deletion of Sec10 in the mouse kidney causes ciliary defects and cyst formation (Zuo et al., 2009; Fogelgren et al., 2015; Seixas et al., 2016). We found that IFT20 interacted with the Exo70 and Sec8 subunits. However, it is likely that IFT20 interacts with the entire exocyst complex, as the Sec10 subunit also interacts with IFT20, and a large-scale proteomic study found IFT20 binding to multiple components (Exo5, Exo7, Exo8, and Exo6B; Fogelgren et al., 2011; Huttlin et al., 2015). We found that the localization of the exocyst at the base of the cilium is independent of IFT20, indicating that the exocyst is not delivered by IFT. This suggests the exocyst may capture or anchor vesicles containing ciliary cargos at the base of the cilium before membrane fusion. Because the exocyst can interact with IFT proteins, it may facilitate connections between ciliary membrane proteins and the IFT complex before entry into the cilium. However, the exocyst also interacts with BLOC-1 proteins, so it may have roles in capturing ciliary cargos from the endosome (Fig. 10).

The BLOC-1 complex, which was required for trafficking of polycystin-2 to cilia contains eight proteins (pallidin, dysbindin, muted, snapin, cappucino, BLOS1, BLOS2, and BLOS3; Falcón-Pérez et al., 2002; Moriyama and Bonifacino, 2002; Ciciotte et al., 2003; Li et al., 2003; Starcevic and Dell’Anglica, 2004; Mullin et al., 2011). This complex is best known for trafficking of proteins from the endosome system to the lysosome and lysosome-related organelles such as melanosomes and platelet dense-granules (Di Pietro et al., 2006; Setty et al., 2007, 2008; Sitaram et al., 2012). BLOC-1 defects cause Hermansky-Pudlak syndrome, in which patients exhibit severe hypopigmentation and defects in platelet dense-granules (Di Pietro et al., 2006; Setty et al., 2007, 2008; Sitaram et al., 2012). BLOC-1 defects cause schizophrenia, as a leading susceptibility gene encodes the dysbindin subunit, and brain tissue from schizophrenia patients has reduced dysbindin mRNA and protein levels (Talbot et al., 2004; Weickert et al., 2008; Mullin et al., 2011). The involve-
ment of BLOC-1 in schizophrenia is not clear, but BLOC-1 is involved in dopamine and glutamate release (Numakawa et al., 2004; Kumamoto et al., 2006). Additionally, BLOC-1 regulates membrane protein targeting to synaptic vesicles and delivery of dopamine receptors to the cell surface (Iizuka et al., 2007; Ji et al., 2009; Newell-Litwa et al., 2009; Marley and von Zastrow, 2010; Larimore et al., 2011).

Before our work, the BLOC-1 complex had no ciliary connections. However, we found interactions of BLOC-1 components with IFT20 and polycystin-2 and a requirement for this complex in the transport of polycystin-2 to cilia. Our finding that BLOC-1 is required for ciliary trafficking of polycystin-2 to cilia suggests that defects in BLOC-1-encoding genes should cause cystic kidneys. Previous studies showed that BLOC-1 mouse models have lower rates of lysosomal enzyme secretion into urine, but no other analysis of kidney structure or function has been done (Novak and Swank, 1979). We found that young Dtnbp1sdy/sdy and Pldnpa/pa mice had dilations of the collecting ducts, suggesting that they may become cystic with age. A second cohort of mice was aged 12 months. The animals have lung fibrosis, and most did not survive the year (McGarry et al., 2002). The survivors did not have prominent cystic disease, unlike the phenotype of Pkd2 and Ift20 mutant animals (Wu et al., 1998; Jonassen et al., 2008). However, both Dtnbp1sdy/sdy and Pldnpa/pa animal models showed significant dilation of the collecting ducts and proximal tubules. Additionally, Dtnbp1sdy/sdy mice have reduced ciliation and shortened cilia, which is similar to what we observed in cell culture after knockdown. This suggests that BLOC-1 defects trigger increased proliferation of tubule cells, but compensatory mechanisms keep the tubules from greatly expanding. We also noted increased numbers of abnormally positioned nuclei in the Dtnbp1sdy/sdy and Pldnpa/pa proximal tubules. The disrupted nuclei positioning in the Dtnbp1sdy/sdy and Pldnpa/pa animals may be indicative of a loss of cell polarization within the kidney tubule. Little is known about this phenotype but aberrant nuclei positioning is associated with pathology in muscular dystrophy and hearing loss (Sullivan et al., 1999; Zhang et al., 2007; Lei et al., 2009; Gundersen and Worman, 2013; Horn et al., 2013).
BLOC-1 localizes to recycling endosome tubules that extend from sorting endosomes, where it controls tubule formation and trafficking of membrane proteins to melanosomes (Di Pietro et al., 2006; Salazar et al., 2006; Setty et al., 2007; Ryder et al., 2013; Delevoyle et al., 2016; Dennis et al., 2016). Given that BLOC-1 is involved in polycystin-2 trafficking, we hypothesized that polycystin-2 is traversing the recycling endosome compartment before delivery to the cilium. To perturb trafficking through the recycling endosome, we expressed the C-terminal tail of the myosin motor MyoVb (Lapi erre et al., 2001; Volpicelli et al., 2002). This MyoVb fragment interacts with Rab11 but lacks motor activity (Lapi erre et al., 2001). This construct caused polycystin-2 to accumulate in the recycling endosome and reduced the amount on cilia. In addition, expression of the Rab11aS25N dominant-negative form of Rab11a perturbs ciliary polycystin-2 trafficking. Although this is the first demonstration of a protein traveling through the recycling endosome to reach the cilium, the recycling endosome is involved in the early steps of cilia formation and the recycling endosome remains at the centrosome after ciliary assembly (Westlake et al., 2011; Hennly et al., 2012; Hennly and Doxsey, 2014). In addition to our data, other connections between the endosome system and trafficking of polycystin-2 to cilia are beginning to emerge. The lipid kinase P13K-C2α localizes to the pericentriolar-recycling endosome at the base of the cilium and knockdown of this kinase reduced ciliary polycystin-2 (Franco et al., 2016). Similarly, knockdown of the endosome protein SDCCAG3 reduces ciliary polycystin-2 (Yu et al., 2016).

Summary
Our work uncovered previously unappreciated complexity in membrane protein trafficking pathways to cilia (Fig. 10). These pathways include an IFT20-independent pathway whereby proteins travel to the plasma membrane before lateral diffusion into the cilium, a second IFT20- and exocyst-dependent pathway from the Golgi complex to the ciliary base, and a third endosomal pathway whereby proteins traverse the recycling endosome on the way to the cilium. This complexity suggests that through evolution, the cell customized preexisting cellular trafficking pathways and machineries to generate distinct ciliary membrane protein trafficking routes.

Materials and methods

Cell culture
NRK, IMCD3 (Rauchman et al., 1993) and IMCD3 Flp-In (Mukhopadhyay et al., 2010) cells were grown in 47.5% DMEM (4.5 g/liter glucose) 47.5% F12, 5% FBS, 100 U/ml penicillin, and 100 g/ml streptomycin (all from Gibco) at 37°C in 5% CO₂. MEK cells were grown -TGC TGT TGA CAG TGA GCG CGG TCTAATTTGACTTGTGTATTTGAAAGCGCACTATGTAATCAACAGCTCAATTAGACACATGCCTACTGCCTCGGAG3′; IFT20 shRNA #2: 5′-TGCTTGGTACAGTGAGCCGCGGCGCTGAGAGACTTTGTTGGA AAAAAATTTGGAAAGCGCACTATGTAATCAACAGCTCAATTAGACACATGCCTACTGCCTCGGAG3′; GMAP shRNA #1: 5′-TGC TGTGTGACATGACCGGCGGCGCTGAGAGACTTTGTTGGA AAAAAATTTGGAAAGCGCACTATGTAATCAACAGCTCAATTAGACACATGCCTACTGCCTCGGAG3′; GMAP shRNA #2: 5′-TGC TGTGTGACATGACCGGCGGCGCTGAGAGACTTTGTTGGA AAAAAATTTGGAAAGCGCACTATGTAATCAACAGCTCAATTAGACACATGCCTACTGCCTCGGAG3′; Exo70 shRNA #1: 5′-TGCTTGGTACAGTGAGCCGCGGCGCTGAGAGACTTTGTTGGA AAAAAATTTGGAAAGCGCACTATGTAATCAACAGCTCAATTAGACACATGCCTACTGCCTCGGAG3′; Exo70 shRNA #2: 5′-TGCTTGGTACAGTGAGCCGCGGCGCTGAGAGACTTTGTTGGA AAAAAATTTGGAAAGCGCACTATGTAATCAACAGCTCAATTAGACACATGCCTACTGCCTCGGAG3′.
1.25 × 10^5 cells per well of a six-well cell culture plate. After 48 h, µg/ml Polybrene (Sigma-Aldrich). Virus was added to cells plated at F12, 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, and 5 reagent. After 48 h, the virus was harvested and filtered through a 0.45-

pHAGE lentivirus production

Lentivirus production using the pHAGE system was previously described (Eguyther et al., 2014). In brief, the pHAGE system uses four packaging vectors (Tat, Rev, Gag/Pol, and VSV-G). The virus was packaged by cotransfecting 1× 10^6 HEK293T cells per well of a six-well cell culture plate with the backbone vector and the four packaging vectors in the following proportions: (backbone, 5; Tat, 0.5; Rev, 10; envelope, 5). Cells were harvested and filtered through a 0.45-

Fluorescence pulse-chase trafficking assay

The fluorescence pulse-chase assay was previously described (Follit et al., 2009). Cells were washed once with cold 1× PBS + 1 mM PMSF. They were lysed with CellLytic buffer (Sigma-Aldrich) supplemented with 0.1% NP-40 (Sigma-Aldrich), 0.1% CHAPS (Sigma-Aldrich), and Iglehart, 2013) for 72 h. Cells were washed three times with cold 1× PBS, then scraping the cells into protein-denaturing buffer and passing it through a 22-gauge needle. Samples were run on SDS-PAGE and transblotted to polyvinylidene difluoride membrane at 4°C overnight. Polyvinylidene difluoride membrane was equilibrated with 1× TBST (150 mM NaCl, 1% Tween 20, and 50 mM Tris, pH 7.5) for 10 min, and membranes were blocked with 5% nonfat dry milk in 1× TBST supplemented with 1% fish gelatin (Sigma-Aldrich) for 30 min. Primary antibodies were diluted in the blocking solution as recommended by the manufacturer and incubated with the membrane for 2 h at room temperature. Goat anti-rabbit IgG (H+L) or goat anti-mouse IgG (H+L) were used to detect the primary antibodies. Immunoblots were acquired using either a LAS-3000 imaging system (Fujiﬁlm) or a Molecular Imager Chemi Doc XRS+ imaging system.
Histology
Harvesting and fixing tissues. Wild-type and Dtnbp1sh/sh and Pldnpa/pa mice were sacrificed with CO2 narcosis followed by cervical dislocation. Kidneys were harvested and fixed in 4% paraformaldehyde in 1× PBS overnight at 4°C, followed by paraffin embedding (Jonassen et al., 2008).

Hematoxylin and eosin staining. Paraffin tissue sections were dewaxed using SafeClear (Thermo Fisher Scientific) and rehydrated with graded aqueous solutions of ethanol. The sections were stained in CAT Hematoxylin solution (Biocare Medical) for 8 min followed by differentiation in 2% hydrochloric acid in 70% ethanol for 30 s. The sections were washed in running tap water for 5 min before Bluing in Tacha’s Bluing Solution (Biocare Medical) for 1 min followed by washing in running tap water for 5 min. Next, the samples were dipped ten times in 95% ethanol, counterstained in Edgar Degas Eosin solution (Biocare Medical) for 1 min and washed three times in 100% ethanol for 1 min. Last, the sections were cleared in two changes of xylene for 5 min and mounted with Permount (Thermo Fisher Scientific; San Agustin et al., 2016).

Immunohistochemistry. Paraffin sections were dewaxed and rehydrated as described above. Antigen retrieval was performed in 10 mM sodium citrate, pH 6.0, in an autoclave for 40 min at 121°C. Samples were cooled to ambient temperature and equilibrated in 1× TBS (50 mM Tris, pH 7.4) for 5 min, followed by blocking in 4% nonimmune goat serum, 0.1% cold water fish skin gelatin (Sigma-Aldrich), and 0.1% Triton X-100, in 1× TBST (150 mM NaCl, 1% Tween 20, and 50 mM Tris, pH 7.5) for 30 min. Sections were washed in 1× TBST and incubated with primary antibodies or 488-conjugated Lotus tetragonolobus agglutinin (Vector Laboratories) diluted in 0.1% cold water fish skin gelatin in 1× TBST overnight at 4°C. Next, samples were washed with 1× TBST and incubated with 1:1,000 dilutions of secondary antibodies diluted in 0.1% cold water fish skin gelatin (Sigma-Aldrich), and 0.1% sodium citrate, pH 6.0, in an autoclave for 40 min at 121°C. Samples were subjected to either unpaired Student’s t test or one-way ANOVA and Bonferroni or Tukey multiple comparisons test (*, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001). Data distribution was assumed to be normal but was not formally tested.

Online supplemental material
Fig. S1 depicts schematics of the ciliary membrane protein CD8/GFP and SNAP tag constructs, ciliary membrane protein trafficking rates, and cilia length after temperature shift to 19°C. Fig. S2 depicts ciliary membrane protein trafficking assays during GMAP210 shRNA knockdown. Fig. S3 shows ciliary membrane protein trafficking assays during exocyst shRNA knockdown. Fig. S4 shows ciliary membrane protein trafficking assays during BLOC-1 shRNA knockdown. Fig. S5 depicts steady-state ciliary levels of polycystin-2 and Arl13b during exocyst shRNA knockdown. Fig. S6 depicts steady-state basal body levels of IFT20 during pallidin or Exo70 shRNA knockdown. Fig. S7 shows kidney collecting duct diameter and proximal tubule circumference in young Dtnbp1sh/sh and Pldnpa/pa mice. Fig. S8 shows that dominant-negative MyoVb colocalizes with Rab11 and causes the Golgi complex to compact. Fig. S9 shows that polycystin-2 accumulates in Rab11-positive endosomes when dominant-negative MyoVb is expressed. Fig. S10 illustrates that polycystin-2 is not detected in Rab11-positive endosomes when either the exocyst or BLOC-1 is knocked down. Fig. S11 shows that dominant-negative MyoVb expression does not affect fibrocystin or smoothened trafficking to the primary cilium.

Acknowledgments
We thank Dr. Jovenal San Agustin for assistance with histology and Drs. Maxence Nachury, Peter Jackson, Jagesh Shah, Esteban Dell’Angelico, and Stephen Doxsey for sharing reagents.

This work was supported by funding from the National Institutes of Health grants GM060992 and DK103632 to G.J. Pazour and GM077569 and NS088503 to V. Faundez.

The authors declare no competing financial interests.

Author contributions: W.J. Monis and G.J. Pazour conceived the project and designed experiments. W.J. Monis performed all experimental research and data analysis. V. Faundez contributed advice, mouse tissue, and other reagents. W.J. Monis, V. Faundez, and G.J. Pazour wrote the manuscript.

Submitted: 22 November 2016
Revised: 24 March 2017
Accepted: 3 May 2017

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