Burke et al., http://www.jcb.org/cgi/content/full/jcb.201406140/DC1

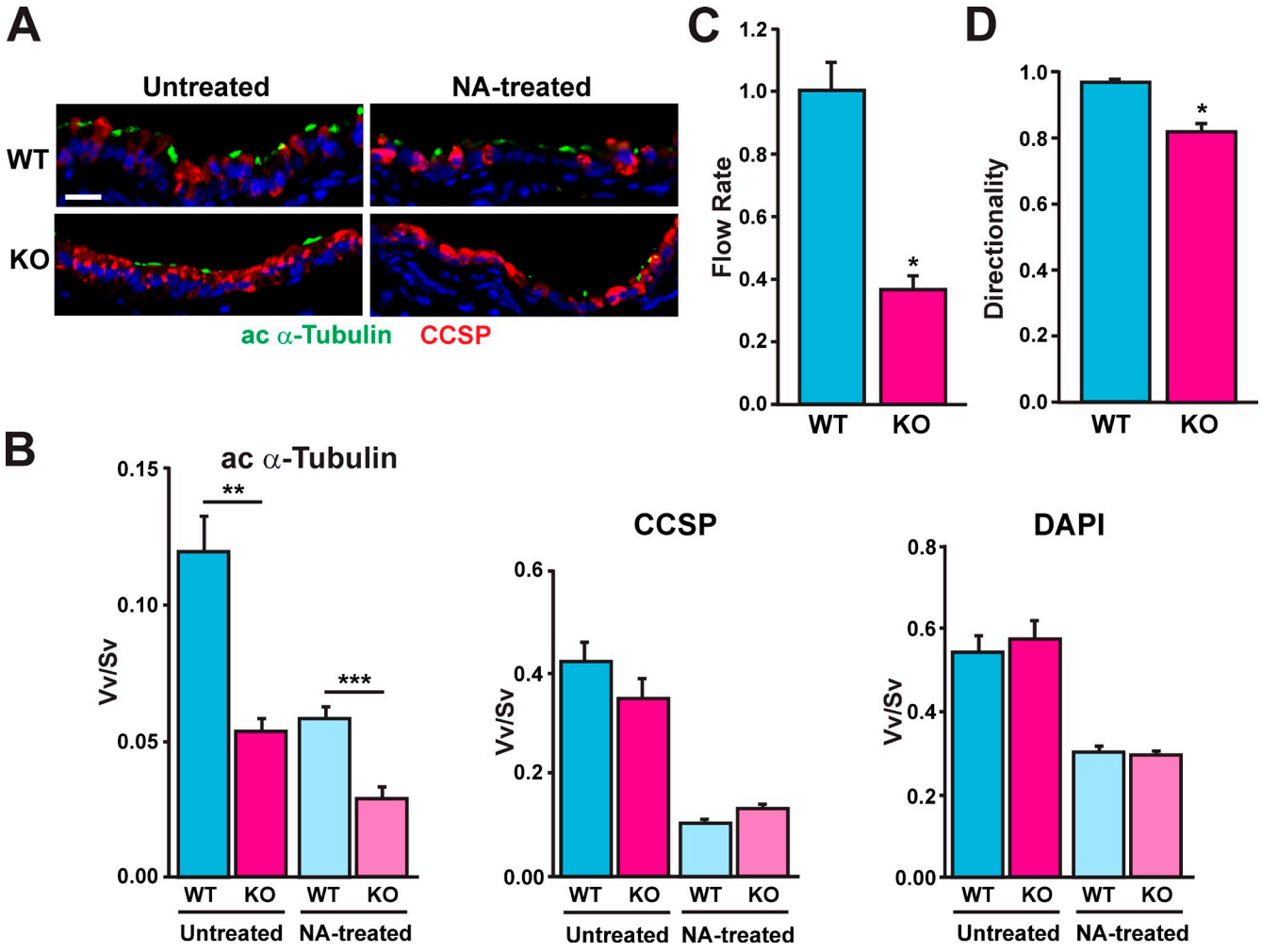


Figure S1. **CbyKO airways display a paucity of cilia and impaired mucociliary transport.** (A) Bronchiolar airway sections from untreated and naphthalene (NA)-treated (13-d recovery) adult WT and CbyKO mice were immunostained for the ciliary axoneme marker acetylated (ac) α-tubulin and for Clara cell marker CCSP. Nuclei were stained with DAPI (blue). Bar, 20 µm. (B) Stereological analysis of A. Bronchiolar regions were imaged at 200× magnification. Three to four images per mouse were used to calculate the volume density of cell markers (V_V) and the surface area of the epithelial basement membrane per reference volume (S_V). Note that there was a significant reduction in airway cilia (acetylated α-tubulin) in CbyKO mice under both untreated or naphthalene-treated conditions compared with WT mice, whereas no major differences were observed in Clara cell (CCSP) or nuclear (DAPI) volume densities. $n = 5$ mice/genotype/condition. Data are presented as means ± SEM. **, $P < 0.01$; ***, $P < 0.001$. (C and D) Measurement of cilia-generated flow (C) and directionality (D) in the tracheal epithelium. Fluorescent microspheres were applied to adult tracheal explants and tracked under epifluorescent illumination. WT values are set as 1. $n = 3$ for each genotype. Data are presented as means ± SEM. *, $P < 0.05$.

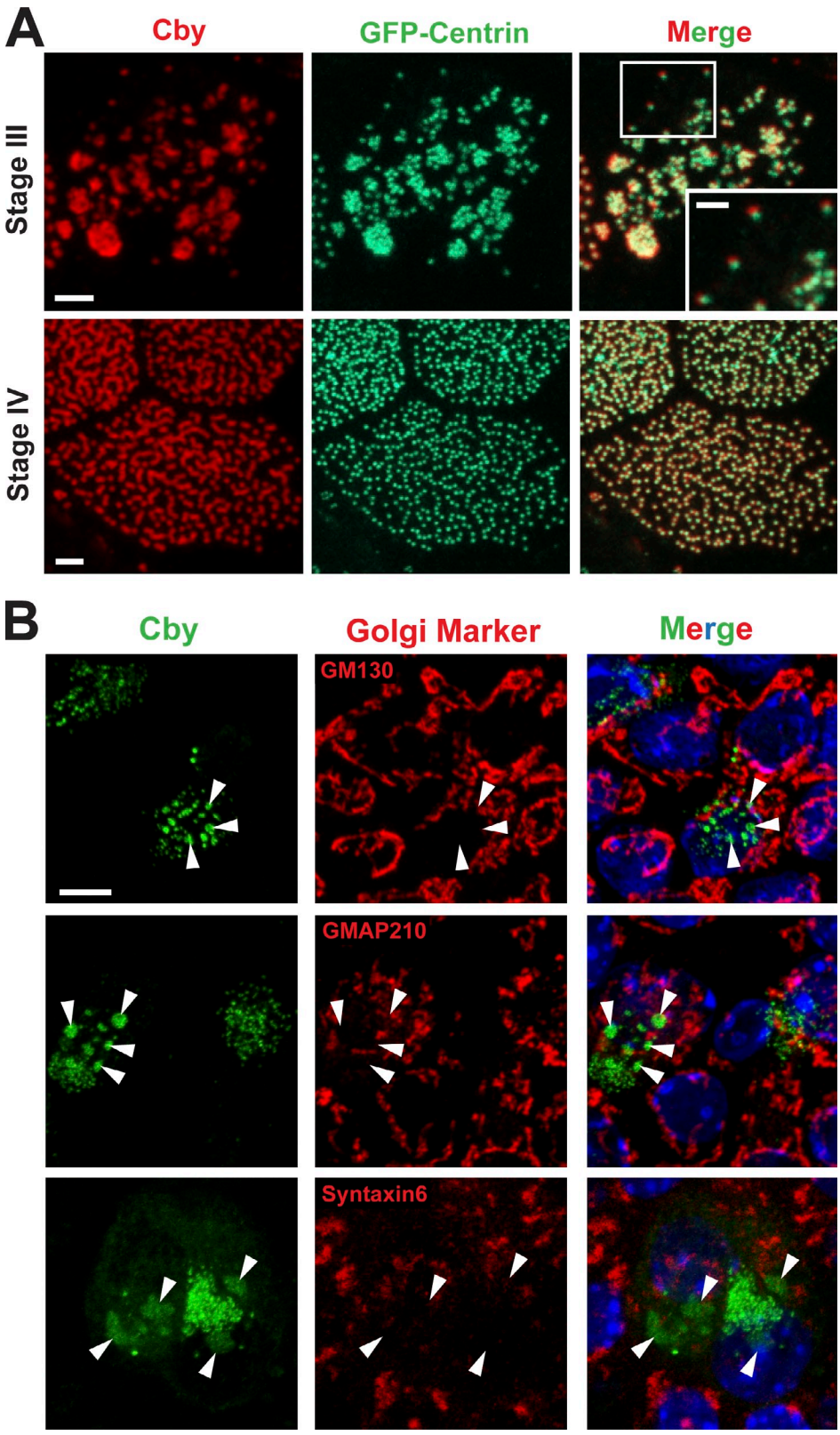


Figure S2. **Cby localization in relation to centrin and Golgi markers in ciliated cells.** (A) Localization of Cby and centrin. MTECs were prepared from GFP–centrin-2 transgenic mice and stained with Cby antibody at the indicated stages. Bars: (main images) 2 µm; (inset) 1 µm. (B) Distribution of diffuse Cby-positive areas and Golgi markers at early stages of ciliated cell differentiation. MTECs at early differentiation stages were colabeled with antibodies for Cby and GM130 (cis-Golgi), GMAP210 (cis-Golgi), or syntaxin 6 (trans-Golgi). Nuclei in the merged image were visualized by DAPI (blue). Arrowheads indicate areas of diffuse Cby staining. Bar, 5 µm.

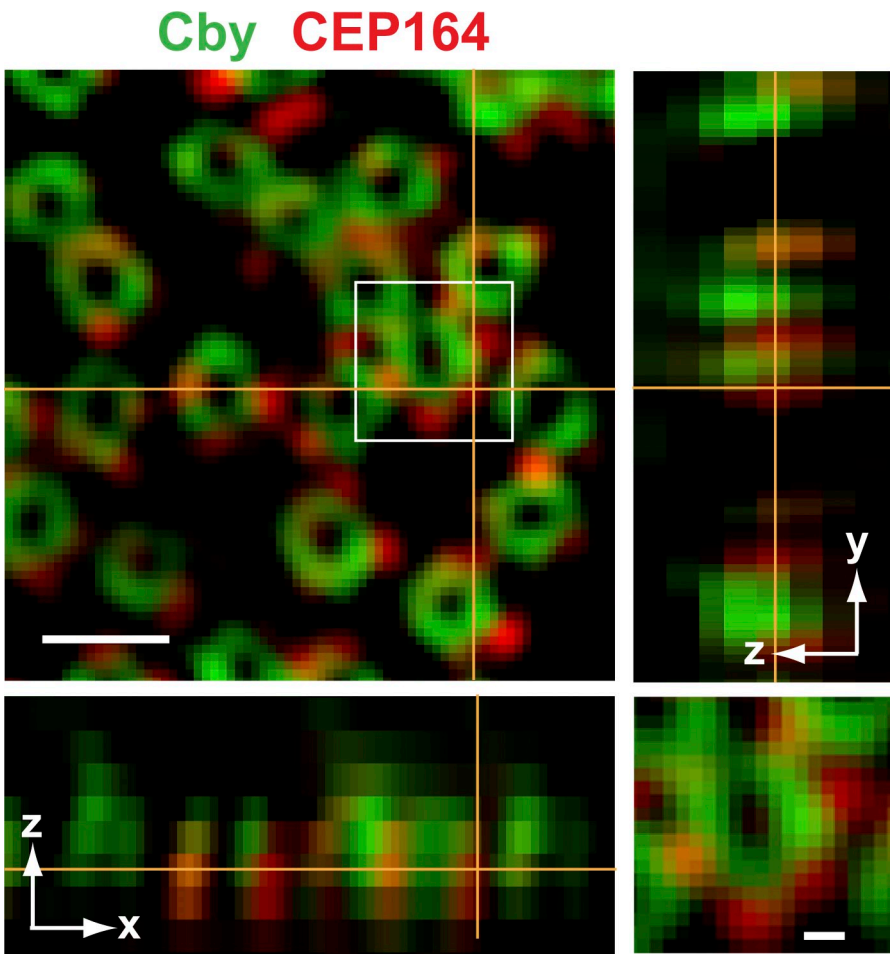


Figure S3. **Cby localizes more apically than CEP164 at the ciliary base.** Fully differentiated MTECs were costained with antibodies against Cby and the transition fiber protein CEP164 and imaged by 3D-SIM. The images are maximum intensity projections of 10 optical sections collected at 125-nm intervals in the z axis. Top-down (x-y) and side (y-z on the right and x-z on the bottom) views are shown. Bar, 0.5 µm. The squared area is shown in higher magnification at the bottom right corner (bar, 0.1 µm).

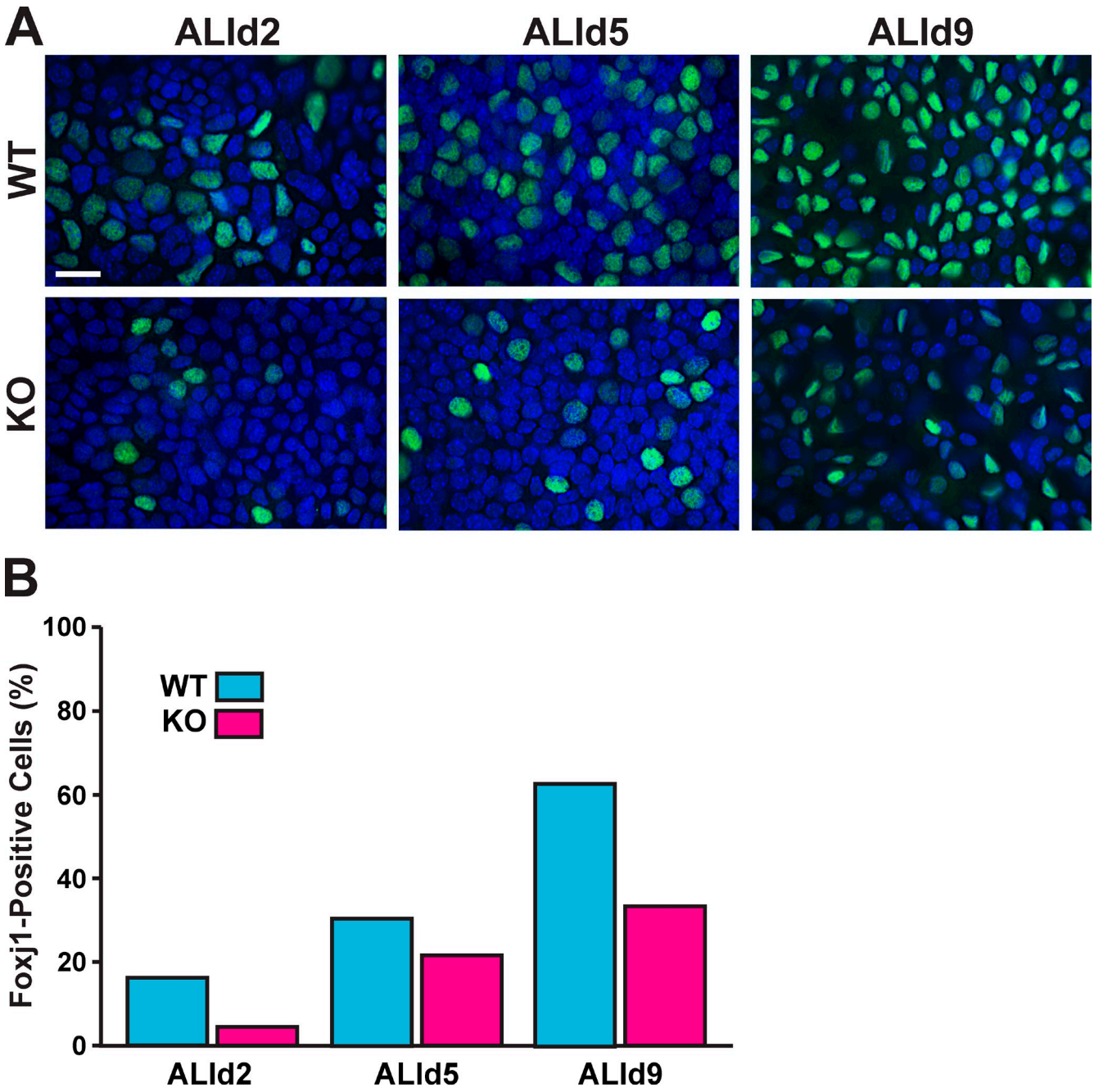


Figure S4. **CbyKO MTECs exhibit a reduction in Foxj1-positive ciliated cell populations.** (A) WT and CbyKO MTECs were fixed at ALId2, d5, and d9 and subjected to immunostaining with Foxj1 antibody (green). Nuclei were visualized by DAPI (blue). Representative images from three independent MTEC preparations are shown. Bar, 5 µm. (B) Quantification of Foxj1-positive cells. At least 200 cells/genotype/ALI day were counted for each MTEC preparation, and results shown are representative of three independent MTEC preparations.

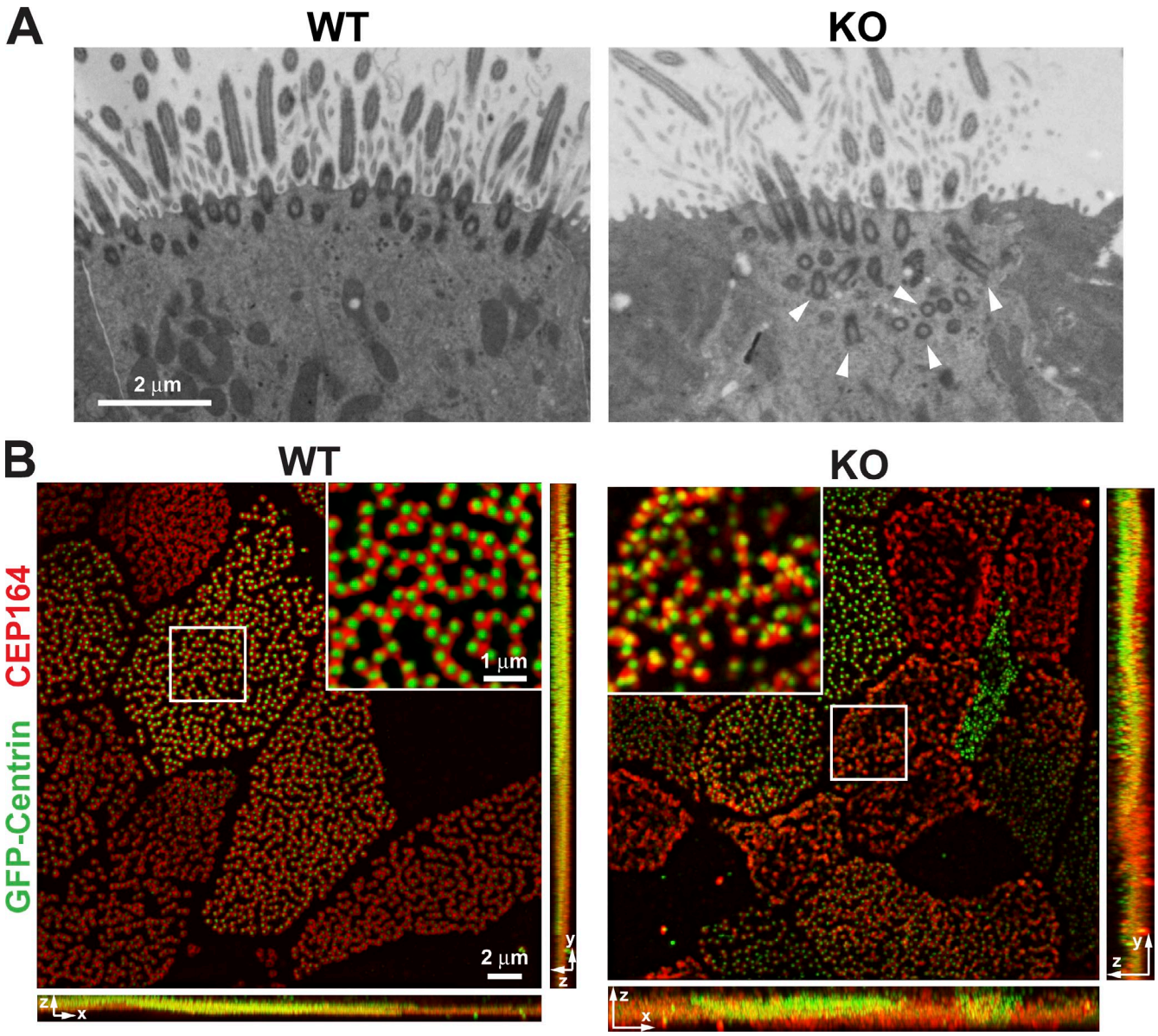
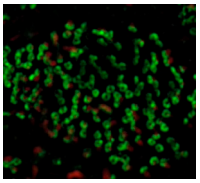


Figure S5. **Mispositioning of basal bodies in CbyKO ciliated cells.** (A) EM images of ciliated cells in adult mouse tracheas. In CbyKO, arrowheads indicate mispositioned basal bodies distantly located from the apical surface. (B) MTECs were prepared from GFP–centrin-2 transgenic mice, fully differentiated, and immunostained with the CEP164 antibody. The images are projections of z sections collected at 125-nm intervals. Top-down views and the entire fields in x-z (bottom) and y-z (right) directions are shown. In WT, along the z axis, basal bodies were properly docked and tightly aligned within ~1 μm at the apical cell surface, whereas in CbyKO, they were scatted over ~3 μm. Note that in CbyKO, many basal bodies appeared poorly polarized, but CEP164 was properly recruited to the basal bodies (insets).

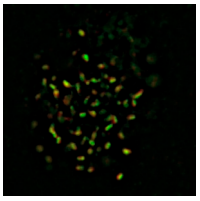
Table S1. **Antibodies used for immunofluorescence microscopy**

Antigen	Species	Source	Fixation
Cby8-2	Mouse IgG2a	Cyge et al., 2011	M/MA/PFA
Cby27-11	Mouse IgG1	Cyge et al., 2011	MA
CEP164	Rabbit	HPA037606; Sigma-Aldrich	MA
CEP164	Rabbit	Graser et al., 2007; E.A. Nigg[a]	M
CEP164	Rabbit	Schmidt et al., 2012; G. Pereira[b]	M
Foxj1	Mouse IgG1	Pan et al., 2007; S.L. Brody[c]	PFA
IFT20	Rabbit	Pazour et al., 2002; G.J. Pazour[d]	MA
GMAP210	Rabbit	Follit et al., 2008; G.J. Pazour	MA
GM130	Mouse IgG1	610823; BD	MA
Rab8a	Rabbit	55296-1-1AP; Proteintech	MA
Syntaxin 6	Mouse IgG1	610635; BD	PFA
Acetylated α-tubulin	Mouse IgG2b	T7451; Sigma-Aldrich	MA/PFA
γ-Tubulin	Mouse IgG1	T6557; Sigma-Aldrich,	MA

M, methanol; MA, 1:1 methanol/acetone.
[a]University of Basel, Basel, Switzerland.
[b]German Cancer Research Center, Heidelberg, Germany.
[c]Washington University, St. Louis, MO.
[d]University of Massachusetts, Worcester, MA.



Video 1. **Z-stack SIM images of Cby and cilia in ciliated cells.** Fully differentiated MTECs were fixed, colabeled with antibodies for Cby (green) and the ciliary axoneme marker acetylated α-tubulin (red), and imaged by superresolution 3D-SIM with a 100× objective (Nikon). A total of 33 images were collected at 125-nm z steps.



Video 2. **Z-stack SIM images of Cby and CEP164 in a stage II ciliated cell.** Stage II MTECs were fixed, colabeled with antibodies for Cby (green) and the distal appendage marker CEP164 (red), and imaged by superresolution 3D-SIM with a 100× objective (Nikon). A total of 33 images were collected at 125-nm z steps.

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