Badgandi et al., https://doi.org/10.1083/jcb.201607095

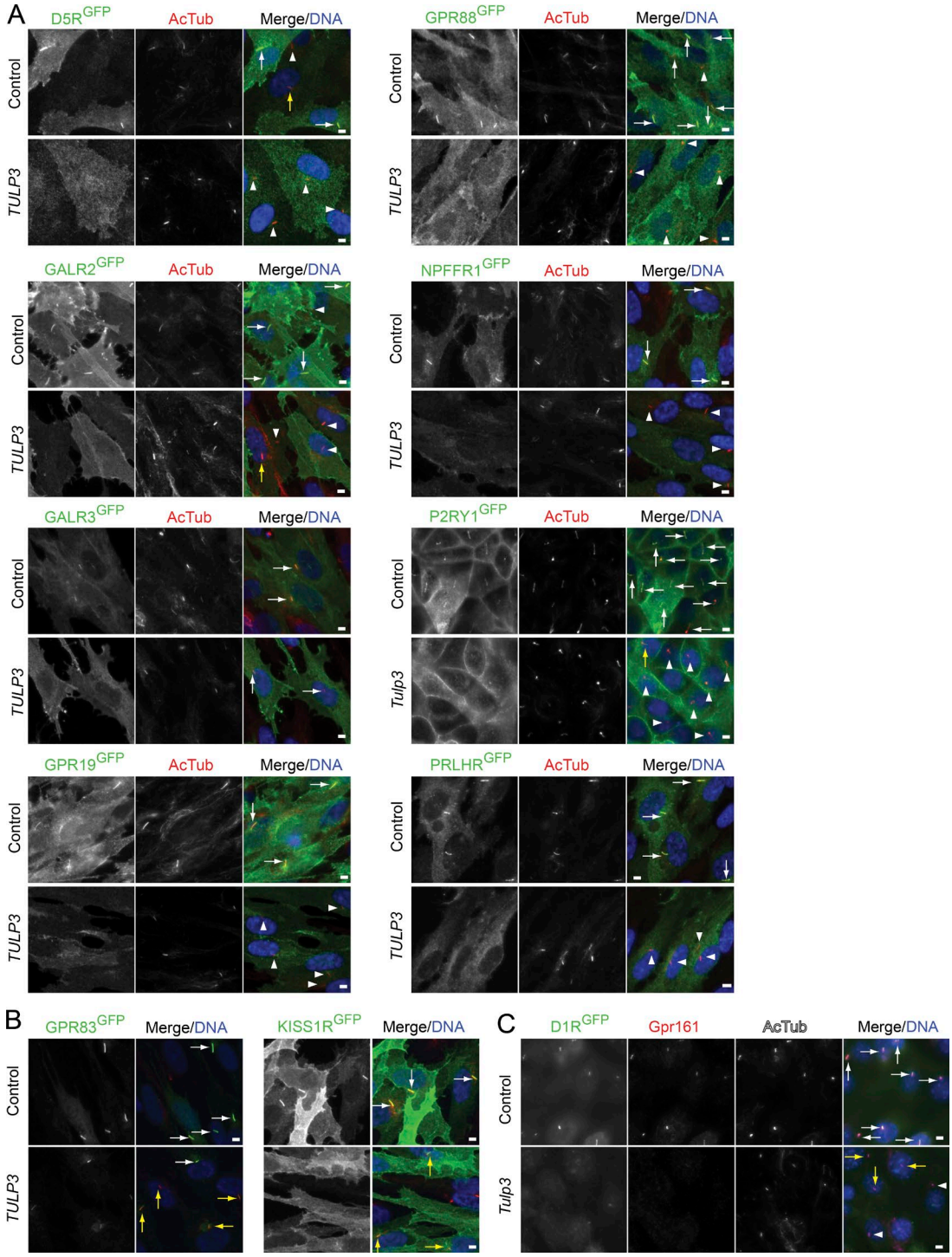

Figure S1. **TULP3 determines localization of multiple rhodopsin family GPCRs to primary cilia.** (A and B) Stable RPE hTERT and IMCD3 Flp-In cell lines expressing the indicated GPCRs as in Fig. 1 (A and B), stained for GFP (green), acetylated tubulin (AcTub, red), and DNA (blue). (C) Stable IMCD3 cell line–expressing D1R as in Fig. 1 C, stained for GFP (green), Gpr161 (red), acetylated tubulin (magenta), and DNA (blue). White arrows and arrowheads indicate cilia that are positive and negative for GFP, respectively. Yellow arrows indicate cilia with reduced GFP intensity. Bars, 5 µm. All images in individual lines treated with different conditions were imported from ImageJ using similar parameters.

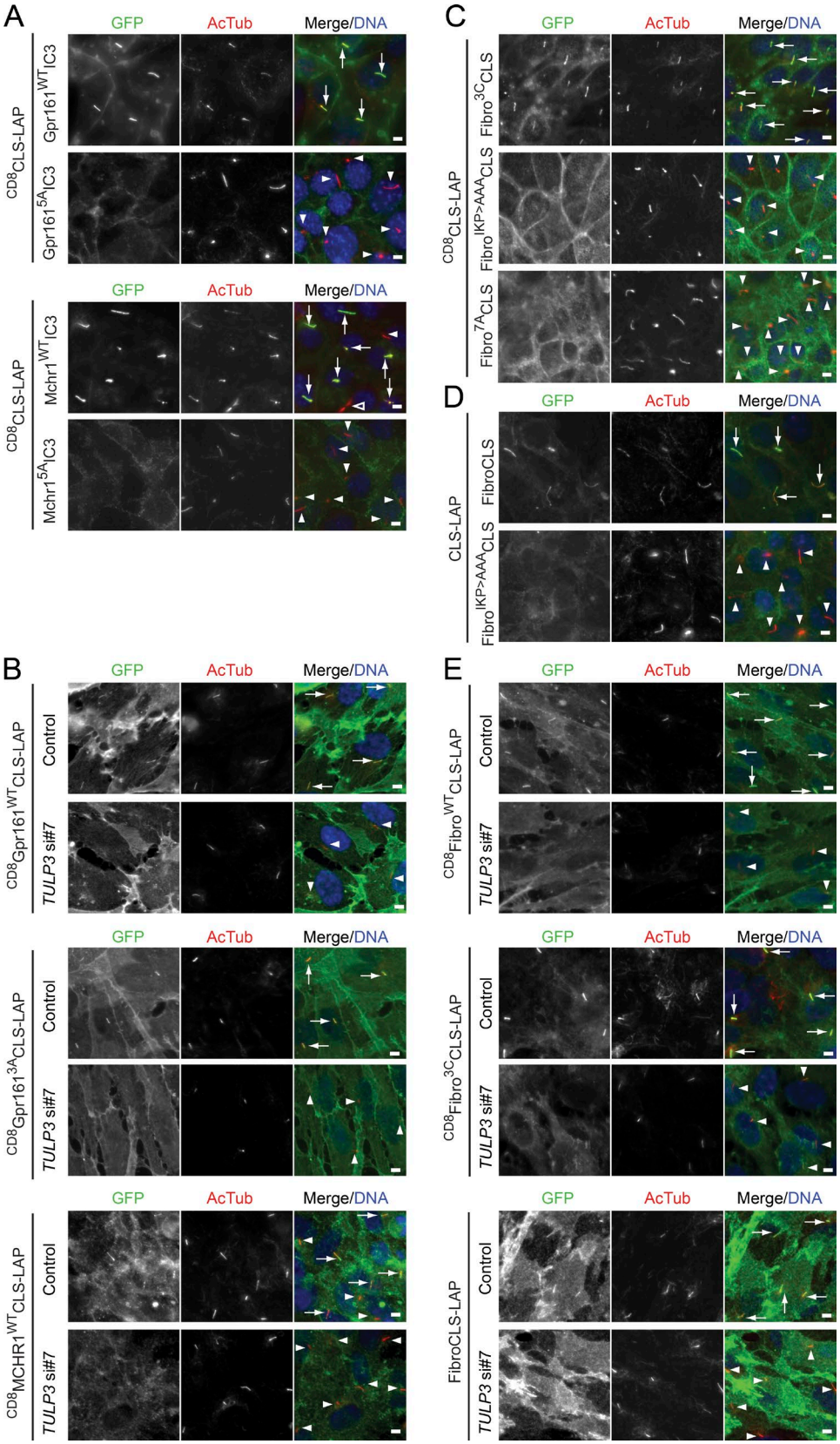

Figure S2. **CLSs from GPCRs and fibrocystin require Tulp3 for trafficking to cilia.** (A, C, and D) Stable IMCD3 cell lines expressing the indicated CLS fusions and mutants as in Fig. 2, stained for GFP (green), acetylated tubulin (AcTub, red) and DNA (blue). (B and E) Stable RPE hTERT cell lines expressing the indicated CLS fusions treated with the indicated siRNAs as in Fig. 2 B, stained for GFP (green), acetylated tubulin (red) and DNA (blue). Bars, 5 µm. White arrows and arrowheads indicate cilia that are GFP positive and negative. The unfilled arrowhead indicates a cytokinetic bridge. All images in individual lines treated with different conditions were imported from ImageJ using similar parameters.

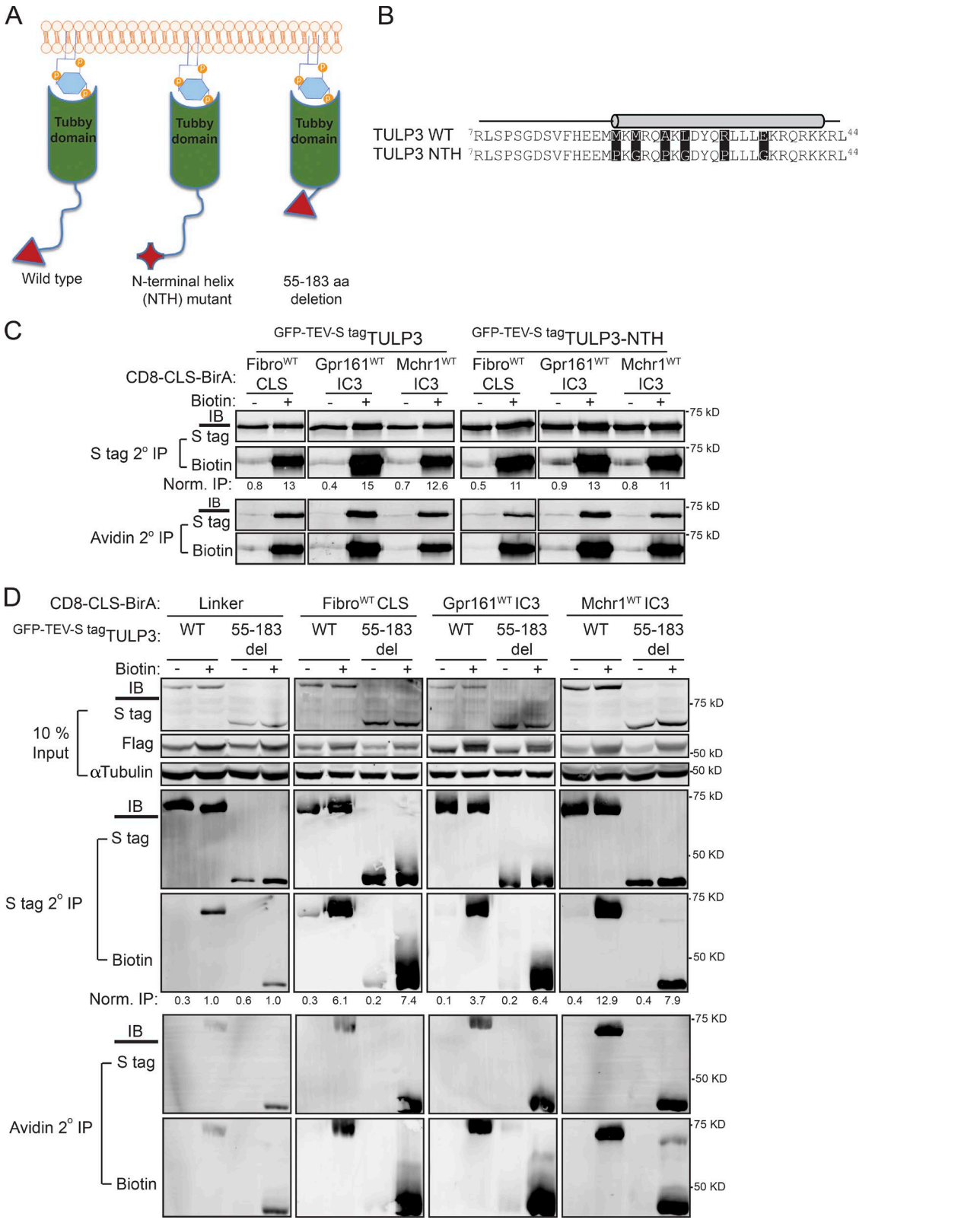

Figure S3. **Proximity biotinylation assays determine CLS-Tulp3 membrane proximity.** (A) Diagram representating TULP3 with the N-terminal IFT nonbinding helix and 55–183 aa–deleted mutants. (B) Mutations used to disrupt the helical nature of the IFT binding region are shown. (C and D) TULP3 mutants tested using the BirA* proximity assay as in Fig. 3 but expressing the respective TULP3 wild-type and mutant constructs are shown. del, deleted; NTH, N-terminal helix.

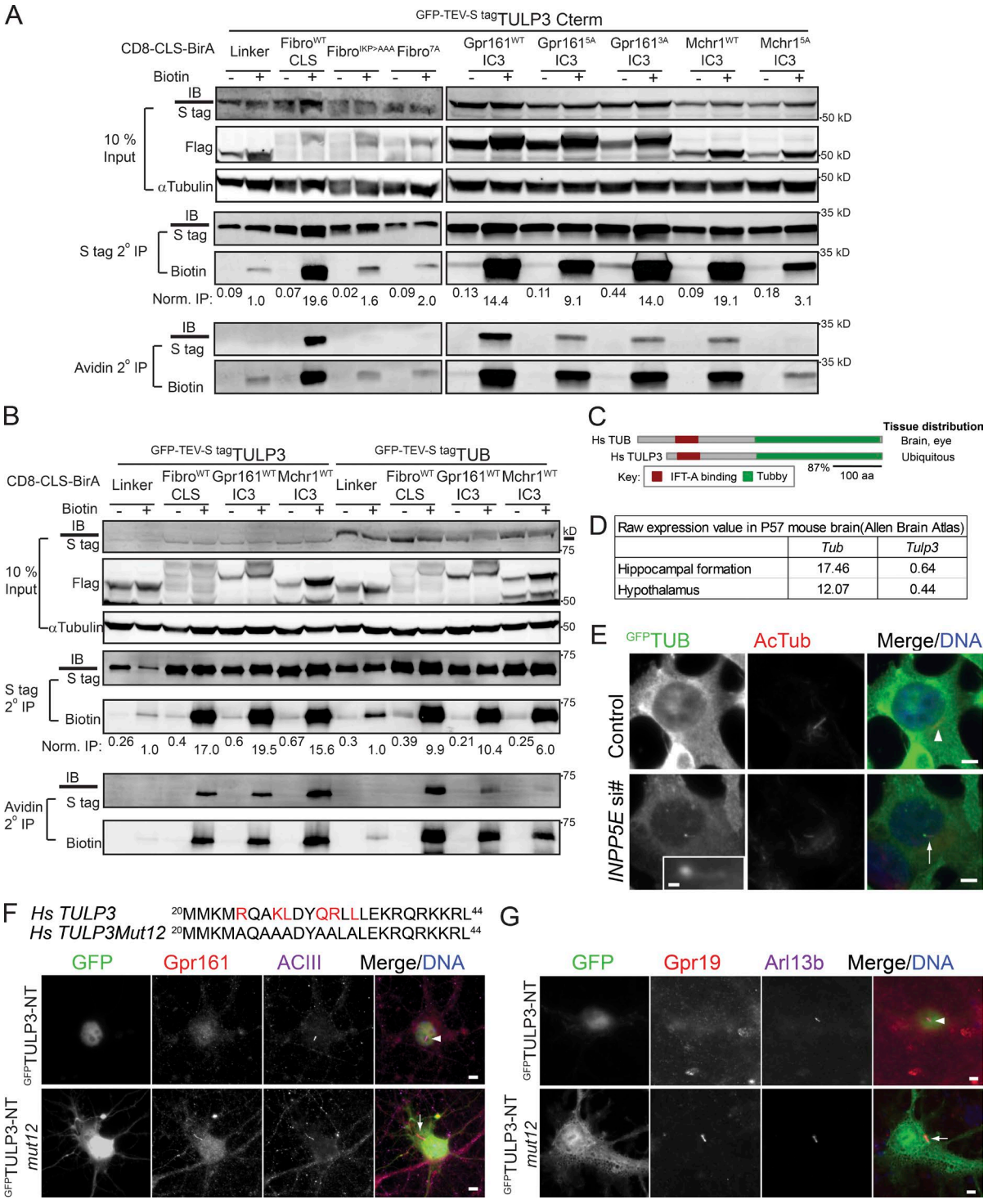

Figure S4. **The tubby domain mediates CLS interactions and differential effects of Tub on ciliary GPCR trafficking.** (A and B) T-Rex-293 cells were cotransfected with CD8-CLS-BirA* and the LAP-tagged tubby domain of TULP3- (A) or LAP-TULP3/TUB isoform b (B)–expressing constructs and processed for the tandem immunopurification (IP) procedure used for the detection and quantification of the extent of biotin labeling on LAP-TULP3/TUB as indicated in Fig. 3 A. (C) Comparison of the domain structures of Tub versus TULP3. The percentage aa similarity of the tubby domain of TULP3 with respect to the canonical TUB tubby domain is shown beneath the scheme. (D) A table depicting relative levels of *Tub/Tulp3* transcripts in adult mouse brains from the Allen Brain Atlas (experiments 71764449 and 76003606 for *Tub* and *Tulp3*, respectively). (E) ARPE cells stably expressing LAP–TUB isoform b were transfected with the indicated 100 nM siRNAs for 72 h and serum starved for the last 24 h before fixation and immunostained for GFP, acetylated tubulin, and DNA. Inset shows a cilium positive for GFPTUB. The white arrow and arrowhead represent GFPTUB-positive and -negative cilia, respectively. (F) Embryonic day 16.5 DIV5 hippocampal neurons from wild-type mice were transfected with the indicated GFP-TULP3 constructs, fixed at DIV8, and immunostained for Gpr161 (red) and DyLight594-labeled ACIII (magenta). The white arrow and arrowhead indicate Gpr161-positive and -negative cilia, respectively. *TULP3Mut12* mutations in the IFT-A binding helix is shown on top (Mukhopadhyay et al., 2010). (G) Embryonic day 18.5 DIV5 glia from wild-type mice were transfected with the indicated constructs, fixed at DIV8, and immunostained for Gpr19 (red) and Arl13b (magenta). The white arrow and arrowhead indicate Gpr19-positive and -negative cilia, respectively. Bars: (main images) 5 µm; (E, inset) 1 µm.

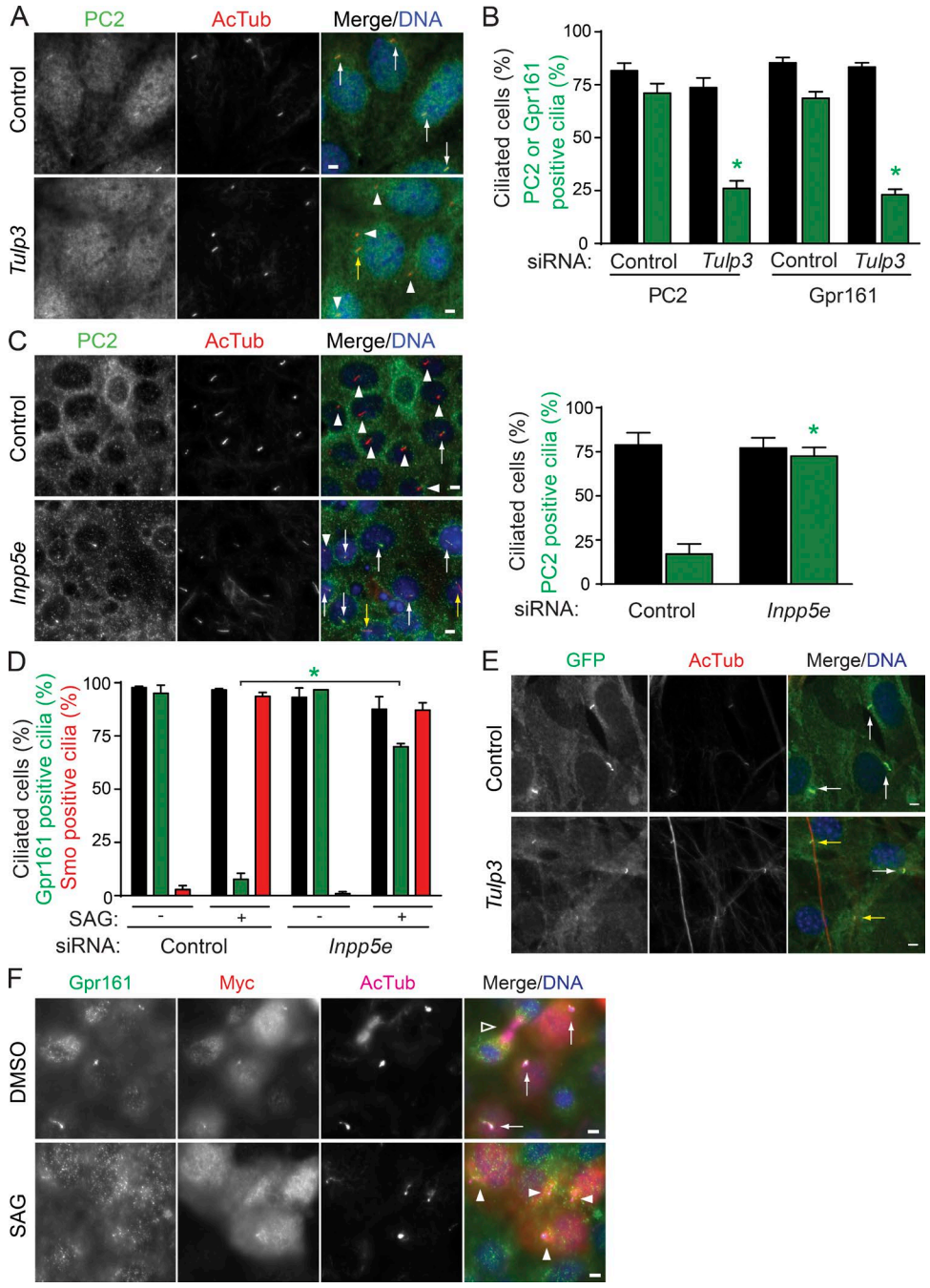

Figure S5. **PC1/2 trafficking to cilia and TULP3/TUB-mediated trafficking to ciliary membrane.** (A and B) mIMCD-K2 cells were sequentially transfected with the indicated 200 nM siRNAs twice for 72 h (see Materials and methods) and serum starved for the last 36 h before fixation, and cells were then immunostained for PC2 using a commercial rabbit polyclonal antibody (H280; Gainullin et al., 2015) or Gpr161, acetylated tubulin, and DNA. PC2- or Gpr161-positive cilia were counted in three experiments and are shown below. PC2-positive and -negative cilia are marked by white arrows and arrowheads, respectively. The yellow arrow marks cilia positive for PC2 but not apparent in the maximal projection image in the respective panels. Total number of cells counted is >530 per condition. (C) IMCD3 cells were sequentially transfected with the indicated 200 nM siRNAs twice for 72 h (see Materials and methods) and serum starved for the last 24 h before fixation and then immunostained for PC2 using anti-mouse PC2 rabbit polyclonal serum, acetylated tubulin, and DNA. PC2-positive and -negative cilia are marked by white arrows and arrowheads, respectively. Yellow arrows mark cilia positive for PC2 but not apparent in the maximal projection image in the respective panels. The total number of cells counted is >700 per condition for PC2. (D) IMCD3 cells were forward transfected with 100 nM siRNAs for 72 h and serum starved ± SAG (500 nM) for the last 24 h before fixation and immunostained for Gpr161 or Smo, acetylated tubulin, and DNA. Gpr161- and Smo-positive cilia were counted in three experiments for Gpr161 and three fields from one experiment for Smo. Total number of cells counted are >600 for Gpr161 and >400 for Smo. (B–D) Data represent means ± SD from three (B) or two (C) experiments; *, $P < 0.001$ with respect to corresponding siRNA controls. (E) NIH 3T3 Flp-In cells stably expressing Gpr161^{V158E}-GFP mutant as in Fig. 7 B were immunostained for GFP (green), acetylated tubulin (red), and DNA (blue). White and yellow arrows indicate cilia that have high and low GFP intensities, respectively. (F) IMCD3 Flp-In cells stably and inducibly expressing ^{Myc}TULP3 N terminus (1–183 aa) were starved in the presence of doxycycline for 20 h (4 µg/ml). After washing, cells were fixed or treated with SAG (500 nM) for 2 h in starvation medium before fixing and immunostaining for Gpr161 (green), Myc (red), acetylated tubulin (magenta), and DNA (blue). White arrows and arrowheads indicate Myc-positive cells that are Gpr161 positive or negative in cilia, respectively. The open white arrowhead marks a cytokinetic ridge. Bars, 5 µm.

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