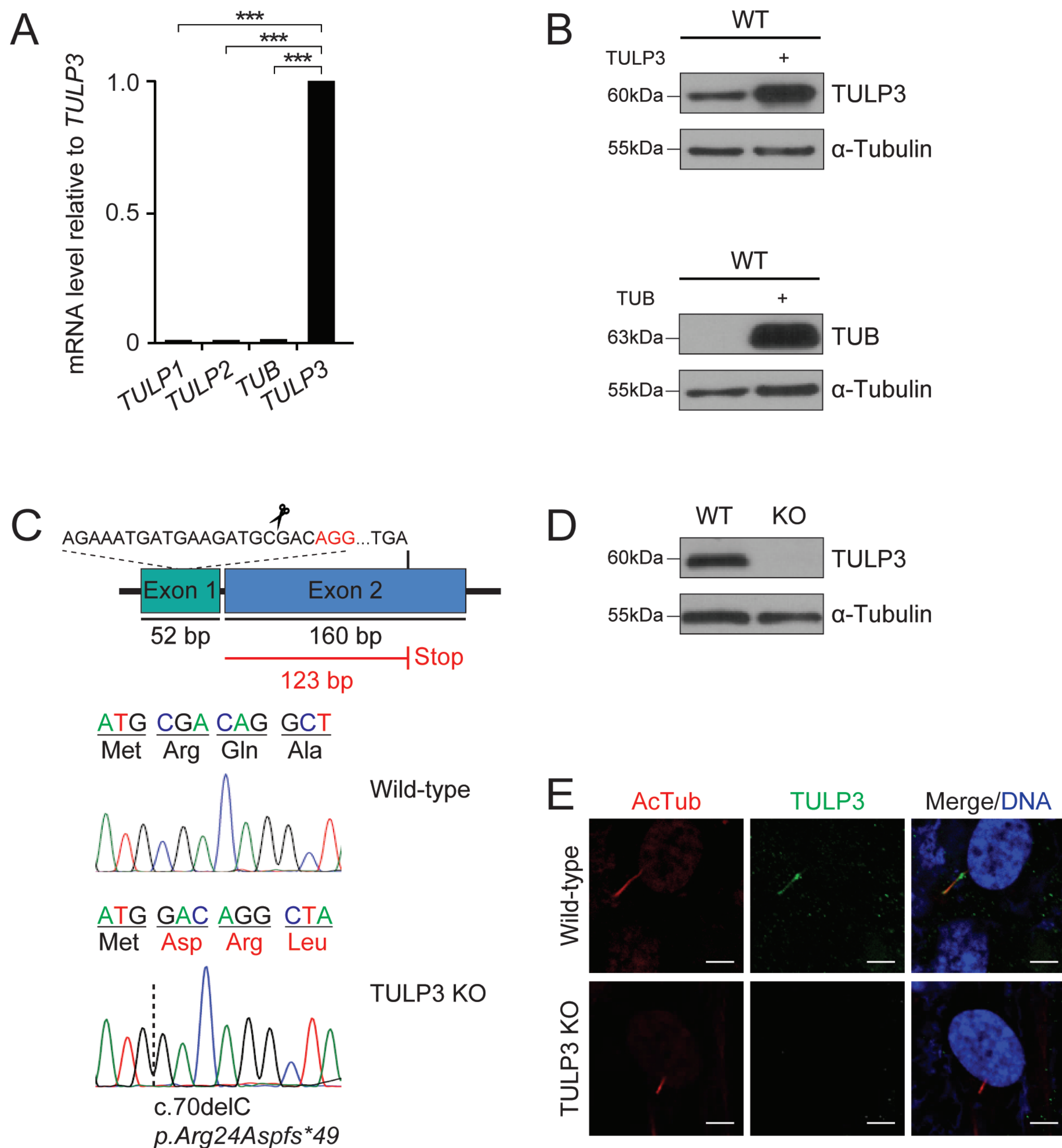
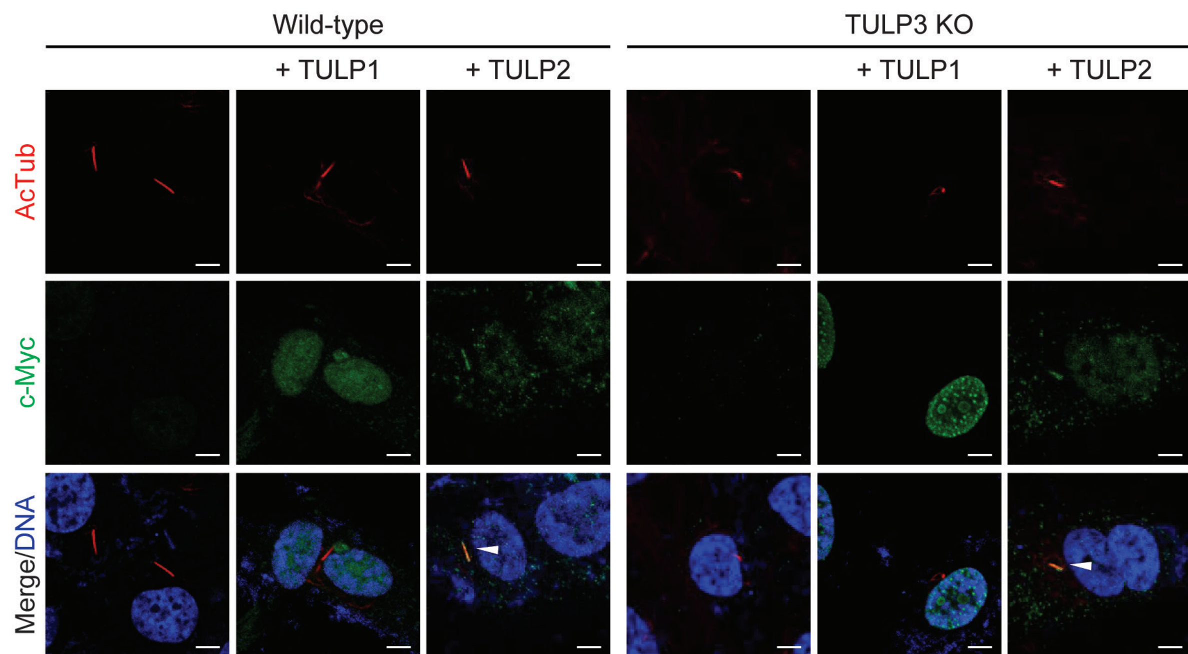
Molecules and Cells

Supplementary Table S1. Sequences of qPCR primers

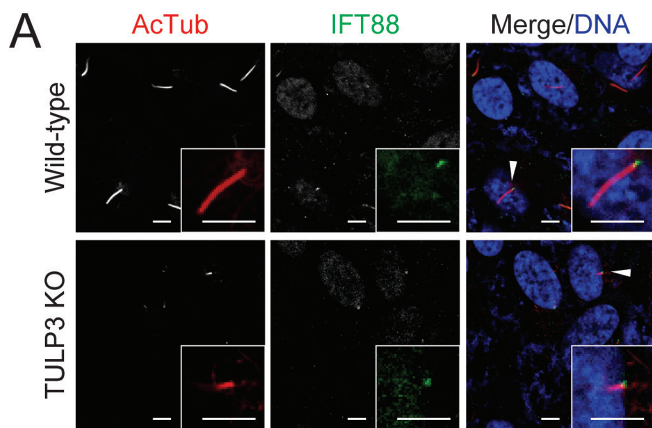
Gene	Forward	Reverse
TULP1	5'-ACCCTACCAATCTGTCCCGA-3'	5'-TTAGTGCTGTACCCACGCTG-3'
TULP2	5'-TCCCTCTGAGCCGTCTTTCT-3'	5'-AGCTTCTGCAGCCTCATAGC-3'
TULP3	5'-AAAAGGGGAATGGATCGGGG-3'	5'-GCTGTTTTGCTCTTTTTCCGC-3'
TUB	5'-TGGGCACCAAGTTCACTGTT-3'	5'-CCTGGGACAATCACGCTCAT-3'
GAPDH	5'-GAGTCAACGGATTTGGTC-3'	5'-TTGATTTTGGAGGGATCT-3'

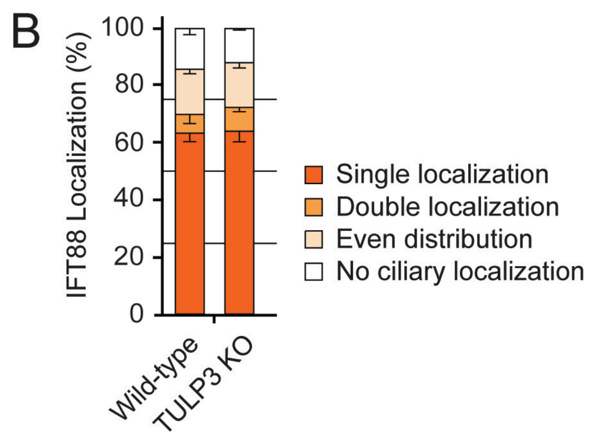


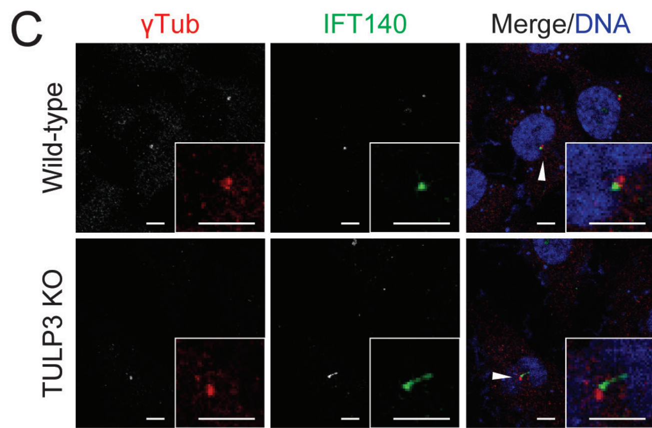
Supplementary Fig. S1. Generation of a TULP-free RPE1 cell line using CRISPR/Cas9. (A) Transcript levels of each *TULP* in RPE1 cells examined by quantitative RT-PCR. Data are presented as means ± SEM from four independent experiments. One-way ANOVAs with Tukey's *post-hoc* tests. $***P < 0.001$. (B) Immunoblot analysis for TULP3 and TUB in RPE1 cells. Ectopic expression of each protein was loaded as a control in the right lane. (C) Generation of a TULP-free RPE1 cell line (*TULP3* KO RPE1) using CRISPR/Cas9. Above, schematic showing the targeted region of the *TULP3* genomic locus, specifically the gRNA targeted sequence in exon 1. Below, alignment comparing control and *TULP3* KO RPE1 cells. It confirms the presence of a frameshift mutation in exon 1 and the resulting premature stop codon at the distal end of exon 2. (D) Immunoblot analysis for TULP3 in wild-type and *TULP3* KO RPE1 cells. (E) Immunocytochemical analysis of TULP3 in wild-type and *TULP3* KO RPE1 cells. Cells were stained for the axonemal marker Ac-tubulin and TULP3. Nuclei were stained with To-Pro3. Scale bars, 5 μm.



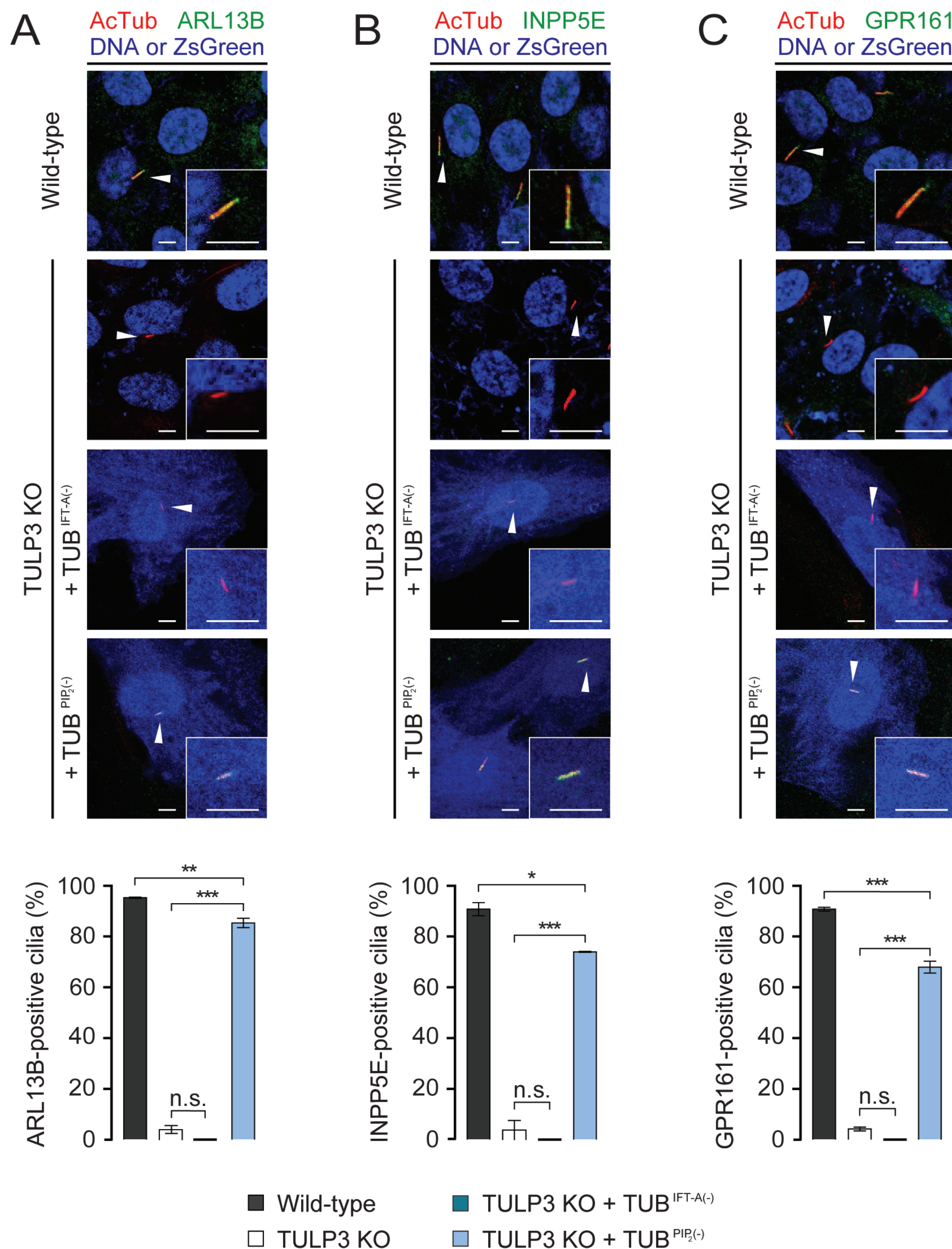
Supplementary Fig. S2. Localization of TULP1 and TULP2 in RPE1 cells. Immunocytochemical analysis of TULP1 and TULP2 in wild-type and *TULP3* KO RPE1 cells. Cells were stained with antibodies specific for the axonemal marker Ac-tubulin, as well as c-Myc for TULP1 and TULP2. Nuclei were stained with To-Pro3. TULP2-positive cilia are marked by arrowheads. Scale bars, 5 μm.



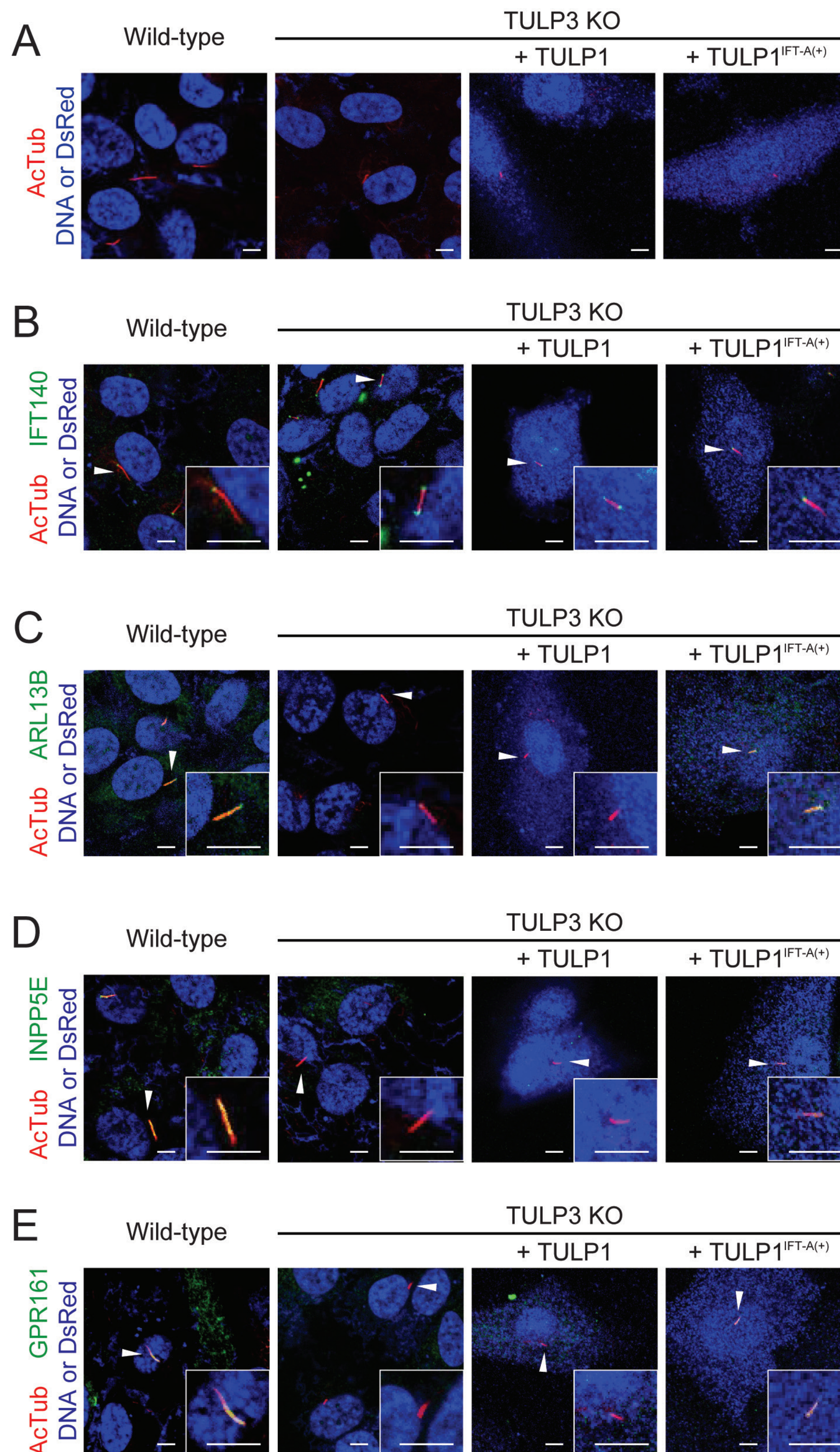




Supplementary Fig. S3. TULP3 functions differently in the ciliary regulation of IFT140 (IFT-A) and IFT88 (IFT-B). (A) Wild-type and *TULP3* KO RPE1 cells, stained with antibodies specific for the axonemal marker Ac-tubulin, IFT88, and To-Pro3. Insets, enlarged images of the cilia marked with arrowheads. Scale bars, 5 μm. (B) Quantification of IFT88 localization. Data are presented as means ± SEM from three independent experiments. 90-120 ciliated cells were counted for each condition. Pearson's χ^2 test. (C) IFT140 localization in wild-type and *TULP3* KO RPE1 cells stained with antibodies specific for the ciliary base marker γ-tubulin, IFT140, and To-Pro3. Insets, enlarged images of the cilia marked with arrowheads. Scale bars, 5 μm.



Supplementary Fig. S4. TUB's IFT-A-binding domain is essential for ciliary membrane protein trafficking. (A-C) Wild-type RPE1 and *TULP3* KO RPE1 cells, transfected with the indicated forms of TUB. Cells were stained with antibodies specific for the axonemal marker Ac-tubulin, as well as for ARL13B (A), INPP5E (B), and GPR161 (C). Transfected cells were identified with ZsGreen. Non-transfected cells were identified with the nuclear marker To-Pro3. Insets, enlarged images of the cilia marked with arrowheads. Scale bars, 5 μm. A quantification of the cilia positive for each protein appears below each set of images. Data are presented as means ± SEM from three independent experiments. More than 100 ciliated cells were observed per condition. One-way ANOVAs with Tukey's *post-hoc* tests. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.



Supplementary Fig. S5. Improving TULP1's IFT-A-binding properties enhances ciliary membrane protein trafficking. (A) Wild-type RPE1 and *TULP3* KO RPE1 cells, transfected with the indicated forms of TULP1. Cells were stained with antibodies specific for the axonemal marker Ac-tubulin. Transfected cells were identified with DsRed staining. Nontransfected cells were identified with the nuclear marker To-Pro3. Scale bars, 5 μm. (B-E) Wild-type and *TULP3* KO RPE1 cells, transfected with the indicated forms of TULP1. Cells were stained with antibodies specific for the axonemal marker Ac-tubulin, as well as for IFT140 (B), ARL13B (C), INPP5E (D), and GPR161 (E). Transfected cells were identified with DsRed staining. Non-transfected cells were identified with the nuclear marker To-Pro3. Insets, enlarged images of cilia marked with arrowheads. Scale bars, 5 μm.