Figure S1. Flow diagram for determining ARL13B and INPP5E protein content in cilia of fibroblasts.
Figure S2. ARL13B cilia staining of fibroblasts. Original deconvoluted images of immunostaining of fibroblasts derived from skin biopsies of JBTS-10, JBTS-153, JBTS-3504, MKS-158, INPP5E-171, ARL13B-277 and controls. ARL13B (green), gamma tubulin (g-tub; white) and cilia (acetylated tubulin, red; scale bar 5 µm).

Figure S3. siRNA knockdown of Mks1 in IMCD3 cells results in decreased MKS1 protein, fewer cilia and decreased ciliary ARL13B and INPP5E levels. (A) RT-QPCR detects lower mRNA levels of Mks1 after siRNA depletion compared to control siRNA transfected IMCD3 cells (p<0.02). Error bars represent SEM (n = 3). (B) Quantification of cilia frequency in IMCD3 cells treated with control siRNA or Mks1 siRNA for 72 hours (p<0.004). Error bars represent SEM (n = 3). (C) Immunoblot of MKS1 of IMCD3 lysates transfected with siControl or siMks1 oligonucleotides for 56 hours. Less MKS1 protein is
detected in siMks1-treated versus siControl-treated IMCD3 cells. β-actin is used as loading control. (D) Immunostaining of IMCD3 cells treated with siControl or siMks1 for 48 hours. Basal body (MKS1, white) and cilia (acetylated tubulin, green) staining shows less MKS1 protein at the base of primary cilia in siMks1-treated versus siControl-treated IMCD3 cells. (E) Immunostaining of IMCD3 cells treated with siControl or siMks1 for 72 hours. ARL13B and INPP5E cilia staining (red) does not colocalize with cilia (acetylated tubulin, green) in siMks1 treated cells. (F) Immunoblot of ARL13B and INPP5E of IMCD3 lysates transfected with siControl or siMks1 oligonucleotides for 56 hours. ARL13B and INPP5E protein levels are unchanged by MKS1 depletion. β-actin is used as loading control. (G) RT-QPCR with primers recognizing mouse Mks1 and human MKS1 detects higher levels of human MKS1 expression in siControl and siMks1 oligonucleotide (56 hours) treated IMCD3 cells transfected (32 hours) with wild-type MKS1 allele (Two-way ANOVA, Bonferroni test *p<0.05, **p<0.01, ***p<0.001). Error bars represent SEM (n = 3).
Figure S4. Reduced ciliary ARL13B and INPP5E in fibroblasts from individuals with ARL13B- and INPP5E-related Joubert syndrome. (A) Immunostaining of fibroblasts derived from skin biopsies of ARL13B-277 and INPP5E-171. ARL13B (green), gamma tubulin (g-tub; white) and cilia (acetylated tubulin, red; scale bar 5 µm). Brightness and contrast were identically adjusted across photos for visualization purposes; original data is in Figure S2. (B) Only ARL13B-277 fibroblasts have less ARL13B in the cilium than control (Tukey whiskers). *** indicates p<0.001 (Kruskal-Wallis test, n > 100 cilia in 2 batches, see Methods and Fig S1 for details). (C) Immunostaining of fibroblasts derived from skin biopsies of ARL13B-277 and INPP5E-171. INPP5E (green), gamma tubulin (white) and cilia (acetylated tubulin, red; scale bar 5 µm). Brightness and contrast were identically adjusted across photos for visualization purposes; original data is in Figure S5. (D) Both mutant fibroblasts have less INPP5E in the cilium than control (Tukey whiskers). *** indicates p<0.001 (Kruskal-Wallis test, n > 100 cilia in 2 batches, see Methods and Fig S1 for details). Ctrl-117 images are included in Figure 2A (Fig S2) for ARL13B and 4A (Fig S5) for INPP5E. (E) Co-staining of INPP5E (white) with the TZ marker RPGRIP1L (green) in 48 hour serum starved RPE cells. No significant overlap of RPGRIP1L and INPP5E is observed (scale bar 10 µm). (F) Immunoblot of MKS1 of lysates of fibroblasts derived from skin biopsies of JBTS-10, JBTS-153, JBTS-3504, MKS-158, INPP5E-171, and controls. β-actin is used as loading control. Densitometry was performed using the Gel Analysis functionality in Fiji (n=2).
Figure S5. INPP5E cilia staining of fibroblasts. Original deconvoluted images of immunostaining of fibroblasts derived from skin biopsies of JBTS-10, JBTS-153, JBTS-3504, MKS-158, INPP5E-171, ARL13B-277 and controls. INPP5E (green), gamma tubulin (g-tub; white) and cilia (acetylated tubulin, red; scale bar 5 µm).