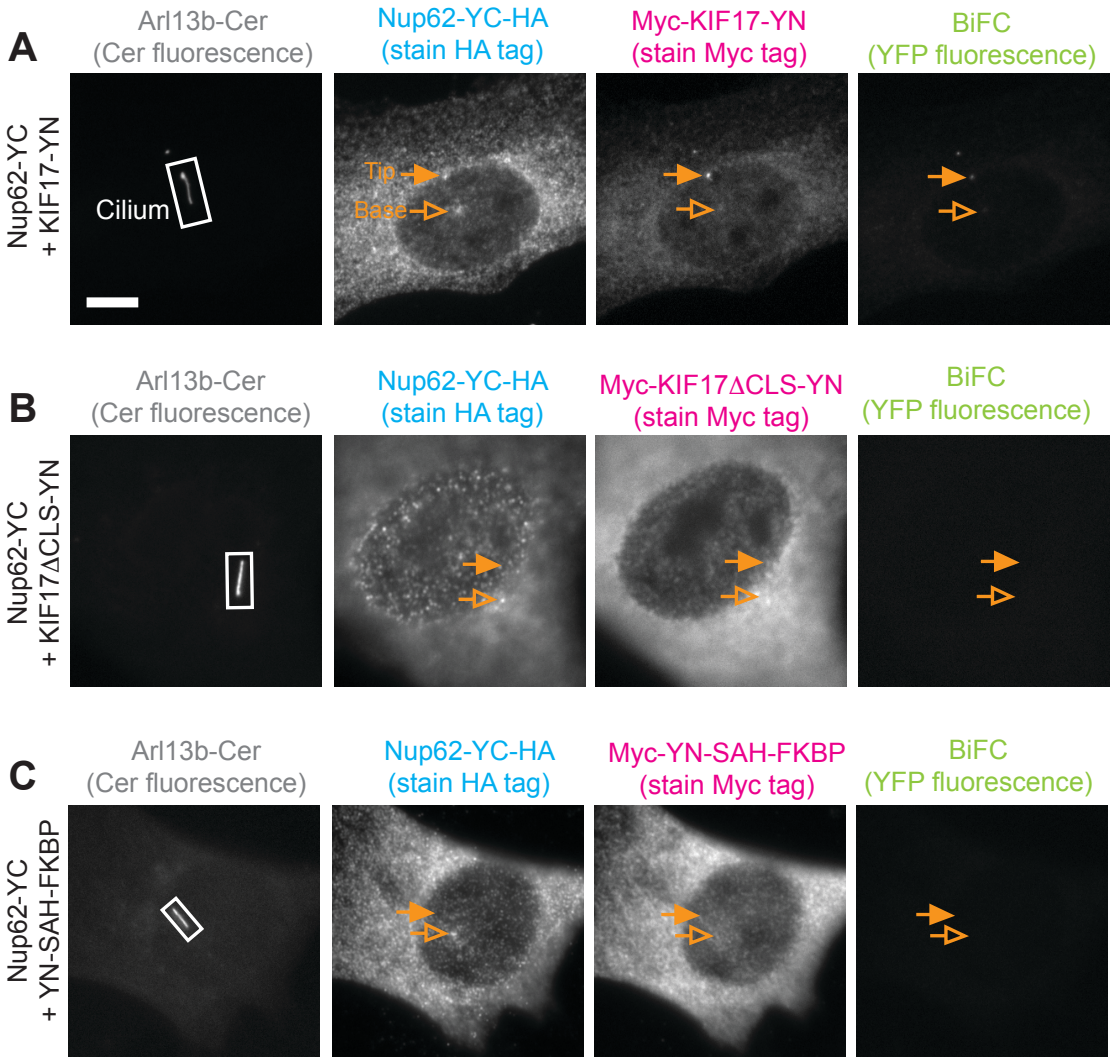

Figure S1. BiFC interactions between NUP62 and transiting KIF17 or control proteins, Related to Figure 1. (A-C) uncropped images of cells shown in Figure 1C-E. Proteins were detected with antibodies to the epitope tags. Scale bar, 5 μm. Filled arrows indicate the tip of the cilium, open arrows indicate the base of the cilium.

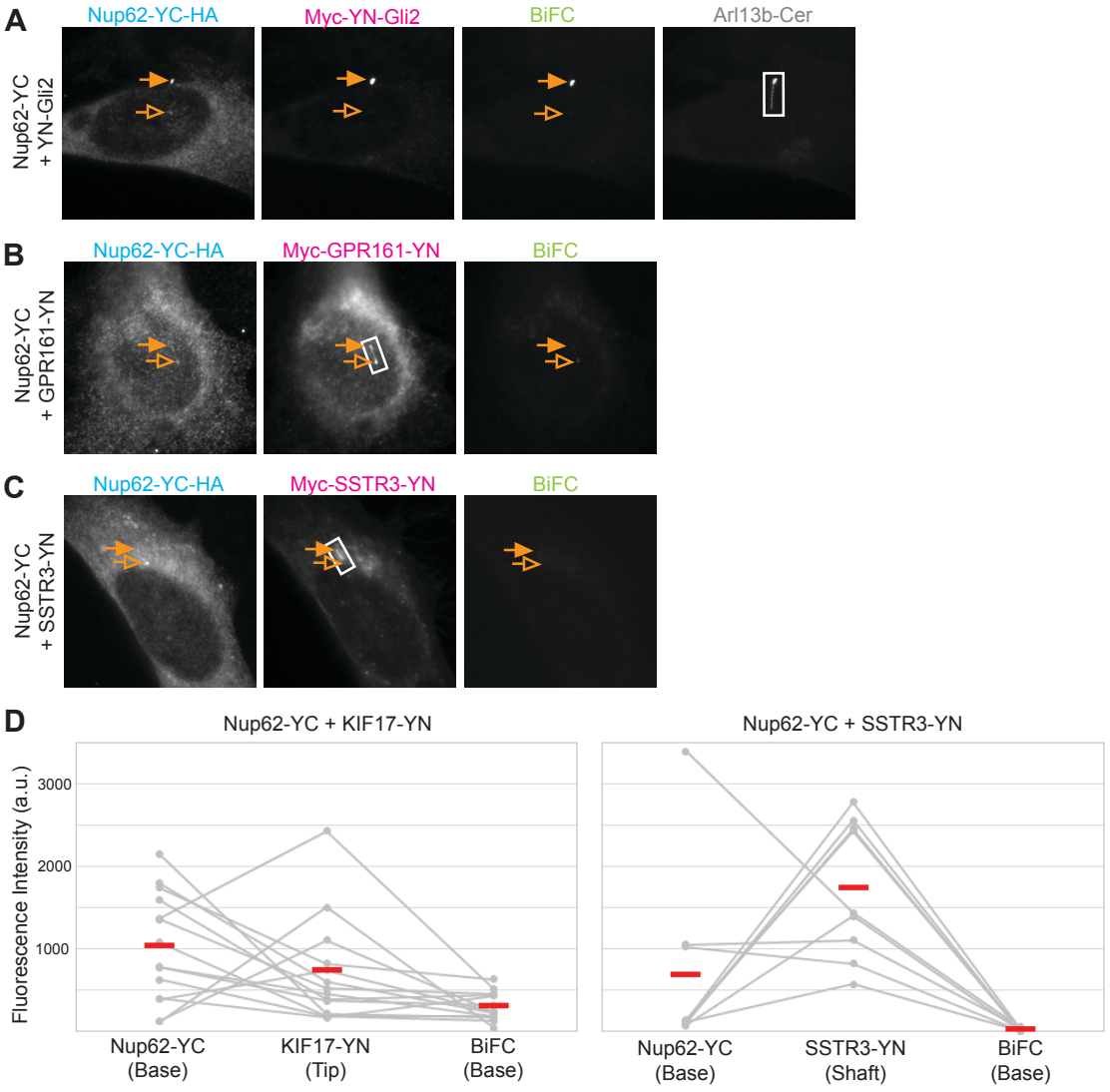

Figure S2. BiFC interactions between Nup62 and transiting cilia proteins, Related to Figure 2. (A-C) uncropped images of cells shown in Figure 2B-D. Proteins were detected with antibodies to the HA or Myc epitope tags. Scale bar, 5 μm. Filled arrows indicate the tip of the cilium, open arrows indicate the base of the cilium. **(D)** BiFC interactions are independent of protein expression level. Quantification of the fluorescence intensities of Nup62-YC and the transiting proteins KIF17-YN (left, scored as 10/11 positive for BiFC at the base in Figure 2E) or SSTR3-YN (right, scored as 0/9 positive for BiFC at the base in Figure 2E). For each measurement (Nup62-YC, KIF17-YN, SSTR3-YN, and BiFC), the fluorescence intensities measured in individual cells are shown as dots and the measurements for each cell are connected by a line. The red bars indicate the mean fluorescence intensity across all cells. a.u. = arbitrary units. By using the same imaging conditions across experiments, the units are comparable between the various panels and figures.

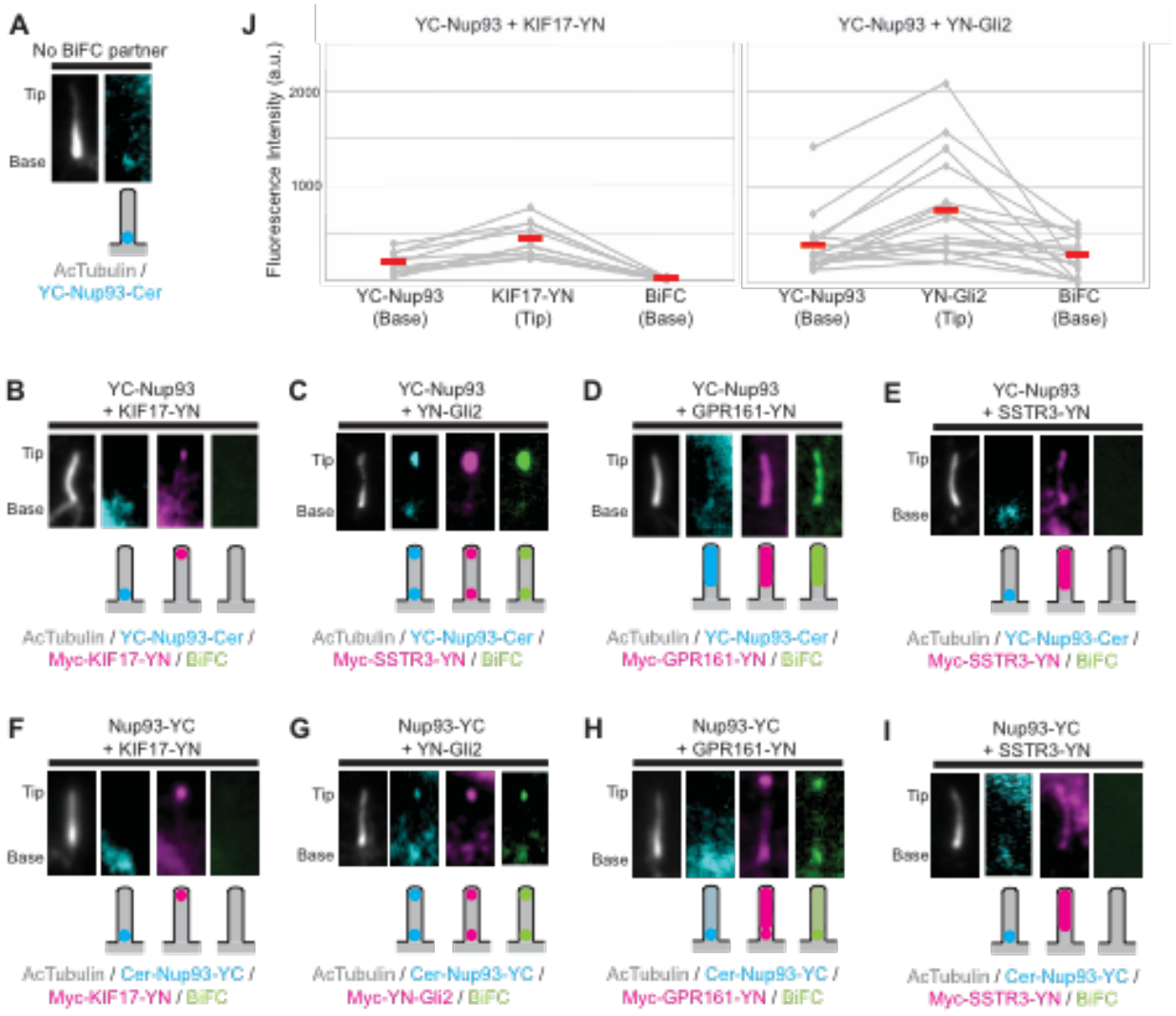

Figure S3. BiFC interactions between Nup93 and transiting cilia proteins, Related to Figure 2. (A) Representative image of the primary cilium and schematic depiction of YC-Nup93 localization in the absence of a BiFC partner. **(B-E)** Representative images and schematic depictions of all BiFC interactions tested for YC-NUP93. **(F-I)** Representative images and schematic depictions of all BiFC interactions tested for NUP93-YC. **(J)** BiFC interactions are independent of protein expression level. Quantification of the fluorescence intensities of YC-Nup93 and the transiting proteins KIF17-YN (left graph, scored as 0/10 positive for BiFC at the base in Figure 2F) or YN-Gli2 (right graph, scored as 11/17 positive for BiFC at the base in Figure 2F). For each measurement (YC-Nup93, KIF17-YN, YN-Gli2, and BiFC), the fluorescence intensities measured in individual cells are shown as dots and the measurements for each cell are connected by a line. The red bars indicate the mean fluorescence intensity across all cells. a.u. = arbitrary units. By using the same imaging conditions across experiments, the units are comparable between the various panels and figures.

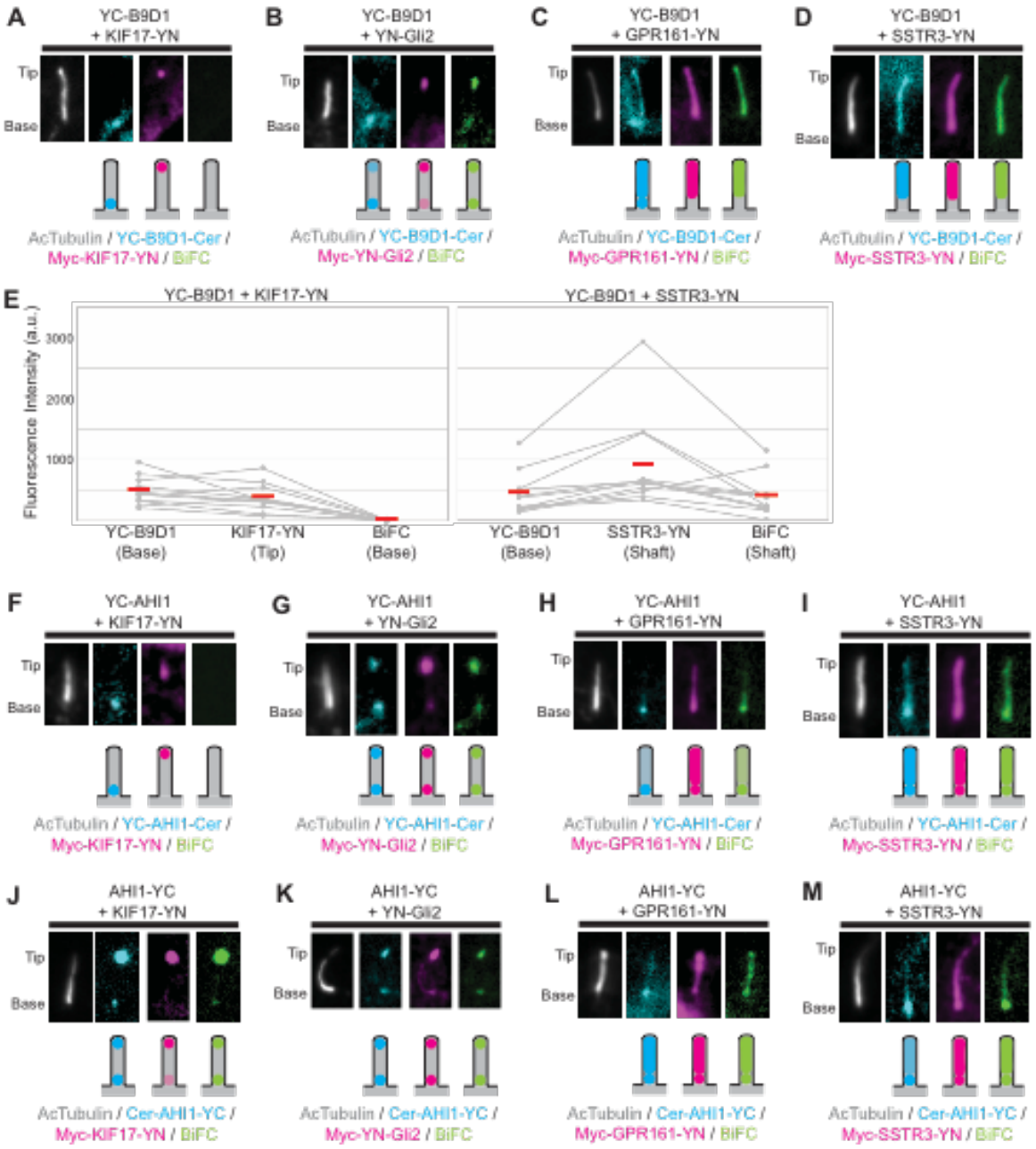

Figure S4. BiFC interactions between MKS components and transiting cilia proteins, Related to Figure 3. (A-D) Shown are representative images of the primary cilium for all BiFC interactions tested for YC-B9D1. **(E)** BiFC interactions are independent of protein expression level. Quantification of the fluorescence intensities of YC-B9D1 and the transiting proteins KIF17-YN (left graph, scored as 0/11 positive for BiFC at the base in Figure 3E) or SSTR3-YN (right graph, scored as 10/11 positive for BiFC in the shaft in Figure 3E). For each measurement (YC-Nup93, KIF17-YN, SSTR3-YN, and BiFC), the fluorescence intensities measured in individual cells are shown as dots and the measurements for each cell are connected by a line. The red bars indicate the mean fluorescence intensity across all cells. a.u. = arbitrary units. By using the same imaging conditions across experiments, the units are comparable between the various panels and figures. **(F-M)** Shown are representative images of the primary cilium for all BiFC interactions tested for (F-I) YC-AHI1 or (J-M) AHI1-YC. Some of these images are also shown in Figure 3A-D.

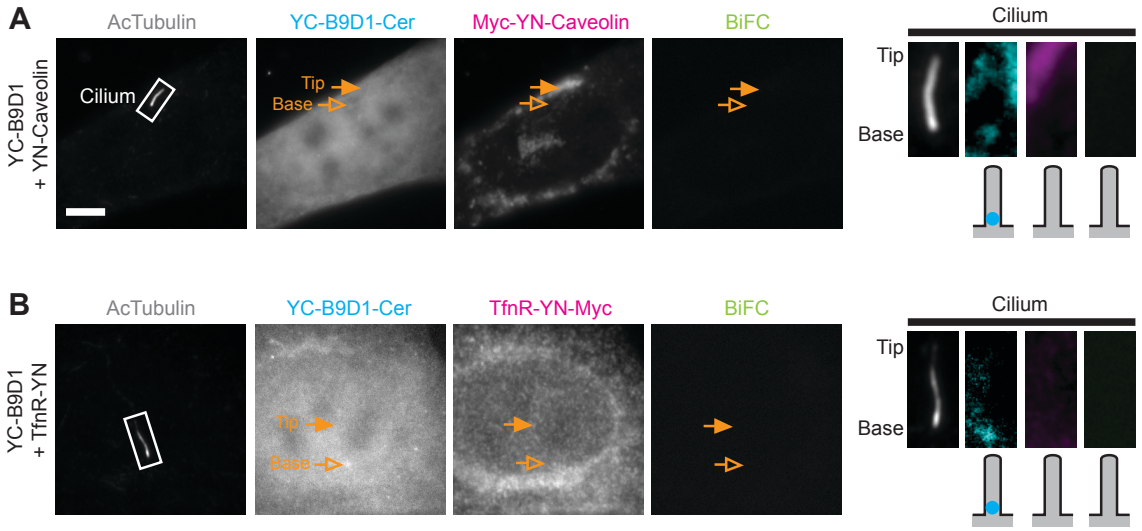

Figure S5. BiFC controls for B9D1 and non-ciliary transmembrane proteins, Related to Figure 3. No BiFC interactions were detected between YC-B9D1 and the non-ciliary proteins (A) YN-Caveolin or (B) TfnR-YN (n = 10 cells each, graphs not shown). Representative images of the entire cells and cropped regions containing the cilium are shown. Scale bar, 5 μm.

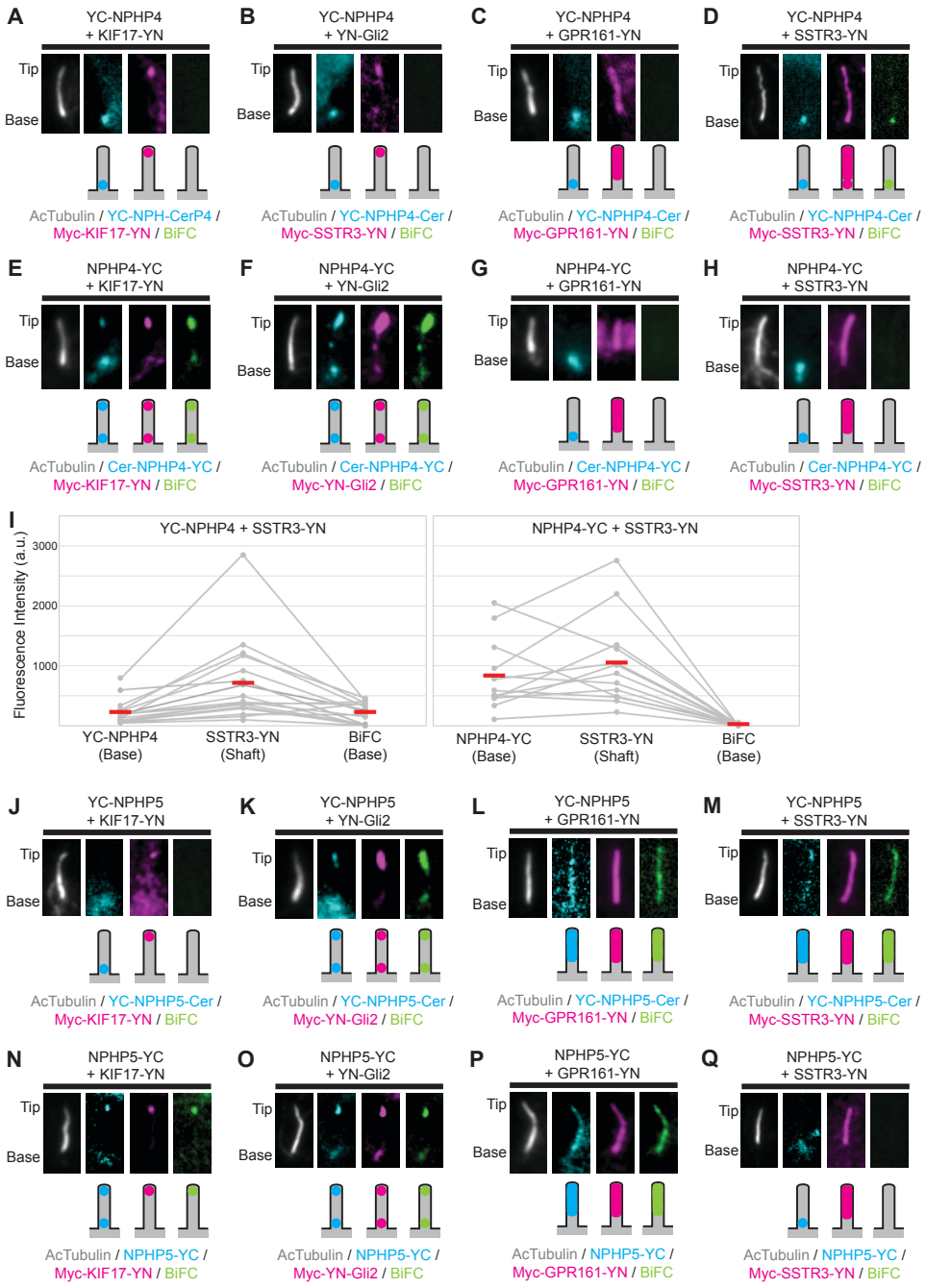

Figure S6. BiFC interactions between NPHP components and transiting cilia proteins, Related to Figure 4. (A-H) Shown are representative images of the primary cilium for all BiFC interactions tested for (A-D) YC-NPHP4 and (E-H) NPHP4-YC. **(I)** BiFC interactions are independent of protein expression level. Quantification of the fluorescence intensities of the transiting protein SSTR3-YN with YC-NPHP4 (left graph, scored as 11/17 positive for BiFC at the base in Figure 4E) or with NPHP4-YC (right graph, scored as 0/13 positive for BiFC at the base in Figure 4E). For each measurement (YC-NPHP4, NPHP4-YC, SSTR3-YN, and BiFC), the fluorescence intensities measured in individual cells are shown as dots and the measurements for each cell are connected by a line. The red bars indicate the mean fluorescence intensity across all cells. a.u. = arbitrary units. By using the same imaging conditions across experiments, the units are comparable between the various panels and figures. **(J-Q)** Shown are representative images of the primary cilium for all BiFC interactions tested for (J-M) YC-NPHP5 or (N-Q) NPHP5-YC.

Table S1, Related to Figures 1-4. List of BiFC constructs

Class	Name in main text	Full name of construct	YFP fragment	Other tag	Relevant Figureures
Soluble	KIF17-YN	Myc-KIF17-YN	mCitrine(1-172)	Myc	Figures 1-4
Soluble	YN-Gli2	Myc-YN-Gli2	mCitrine(1-172)	Myc	Figures 1-4
Soluble	KIF17DCLS-YN	Myc-KIF17ΔCLS-YN	mCitrine(1-172)	Myc	Figure 1D,F
Soluble	YN-SAH-FKBP	Myc-YN-SAH-FKBP	mCitrine(1-172)	Myc	Figure 1E,F
Transmembrane	GPR161-YN	Myc-GPR161-YN	mCitrine(1-172)	Myc	Figures 2-4
Transmembrane	SSTR3-YN	Myc-SSTR3-YN	mCitrine(1-172)	Myc	Figures 2-4
Transmembrane	YN-Caveolin	Myc-YN-Caveolin	mCitrine(1-172)	Myc	Figure S5A
Transmembrane	TfnR-YN	TfnR-YN-Myc	mCitrine(1-172)	Myc	Figure S5B
NUP	Nup62-YC	Nup62-YC-HA	Venus(155-238)	HA	Figures 1, 2
NUP	YC-Nup93	YC-Nup93-Cer	Venus(155-238)	Cerulean	Figure 2
NUP	Nup93-YC	Cer-Nup93-YC	Venus(155-238)	Cerulean	Figure 2
MKS	YC-B9D1	YC-B9D1-Cer	Venus(155-238)	Cerulean	Figure 3
MKS	YC-AHI1	YC-AHI1-Cer	Venus(155-238)	Cerulean	Figure. 3
MKS	AHI1-YC	Cer-AHI1-YC	Venus(155-238)	Cerulean	Figure 3
NPHP	YC-NPHP4	YC-NPHP4-Cer	Venus(155-238)	Cerulean	Figure 4
NPHP	NPHP4-YC	Cer-NPHP4-YC	Venus(155-238)	Cerulean	Figure 4
NPHP	YC-NPHP5	YC-NPHP5-Cer	Venus(155-238)	Cerulean	Figure 4
NPHP	NPHP5-YC	Cer-NPHP5-YC	Venus(155-238)	Cerulean	Figure 4