SUPPLEMENTAL FIGURE 1. Immunopurification of sucrose density gradient-purified IFT A proteins. The 16 S fraction of sucrose density gradient purified IFT A was incubated with an anti-IFT139 (139.1) resin that was subsequently washed with HMDEK-300 buffer and then eluted with acidic glycine (pH 2.0). The protein band corresponding to IFT43 was excised, digested with trypsin and analyzed using MALDI-TOF mass spectrometry.

SUPPLEMENTAL FIGURE 2. The structure of the poorly conserved N-terminal half of IFT43 is predicted to be disordered. *A,* Taxonomic relatedness of IFT43 homologous sequences found in diverse organisms. *B,* Sequence alignment of the IFT43 homologous sequences reveals poor conservation within approximately half of the protein corresponding to the N-terminal 145 amino acids of the *Chlamydomonas* IFT43. *C,* PONDR-FIT analysis of the *Chlamydomonas* IFT43 reveals that the N-terminal half of the protein has a strong propensity for disordered structure.

SUPPLEMENTAL FIGURE 3. Distribution of IFT172 and FLA10 kinesin in the *ift121* mutant is similar to wild-type cells. *A-F,* Epifluorescent and transmitted light images. *A,B,* Using Alexa Fluor 488-labeled secondary antibodies, IFT172 localization (monoclonal 172.1) in the parental strain, CC503, shows strong labeling near the basal body region with punctate staining throughout the flagella. *C,D,* Using Alexa Fluor 488-labeled secondary, FLA10 localization (polyclonal FLA10N) of *ift121* cells shows strong labeling near the basal body region with lesser amounts of FLA10 concentrated near the distal ends of some of the shortened flagella (broad arrows). Flagellar MTs were visualized with Alexa Fluor 596-labeled secondary following incubation with anti-acetylated tubulin. E,F, Localization of IFT172 (Alexa Fluor 488) in *ift121* cells with short flagella revealed strong pooling near the base of the organelle and, although it was not quantified, a significant presence of IFT172 near the distal ends of most flagella (broad arrows). Immunofluorescence of parental and mutant strains was performed using IFT primary antibodies and monoclonal anti-acetylated tubulin (clone 6-11B-1, Sigma) following cold methanol fixation of cells as described previously (20). Scale bars equal 5 μm.