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Supplemental Data

Mutations in *PIH1D3* Cause X-Linked Primary Ciliary Dyskinesia with Outer and Inner Dynein Arm Defects

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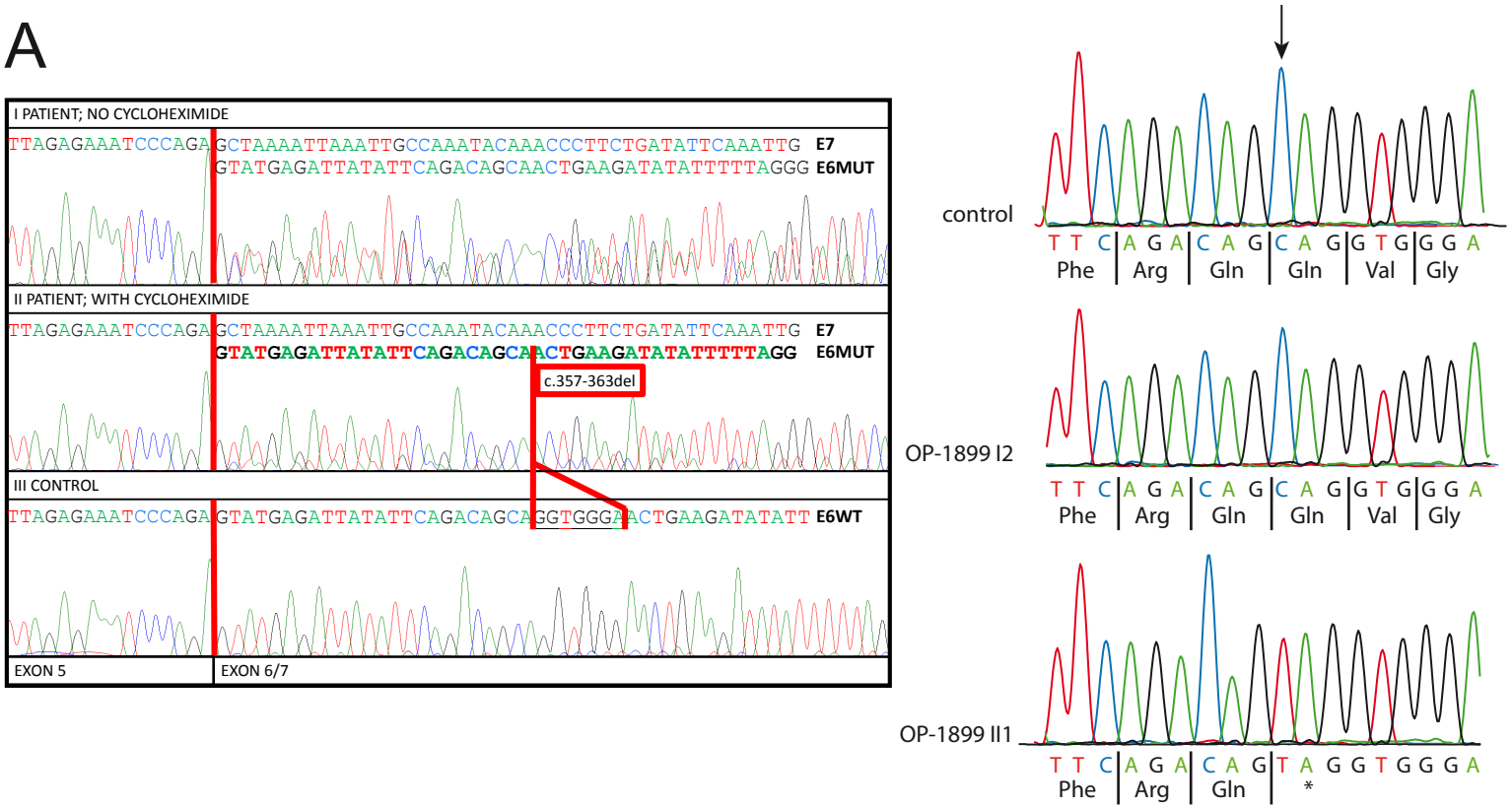

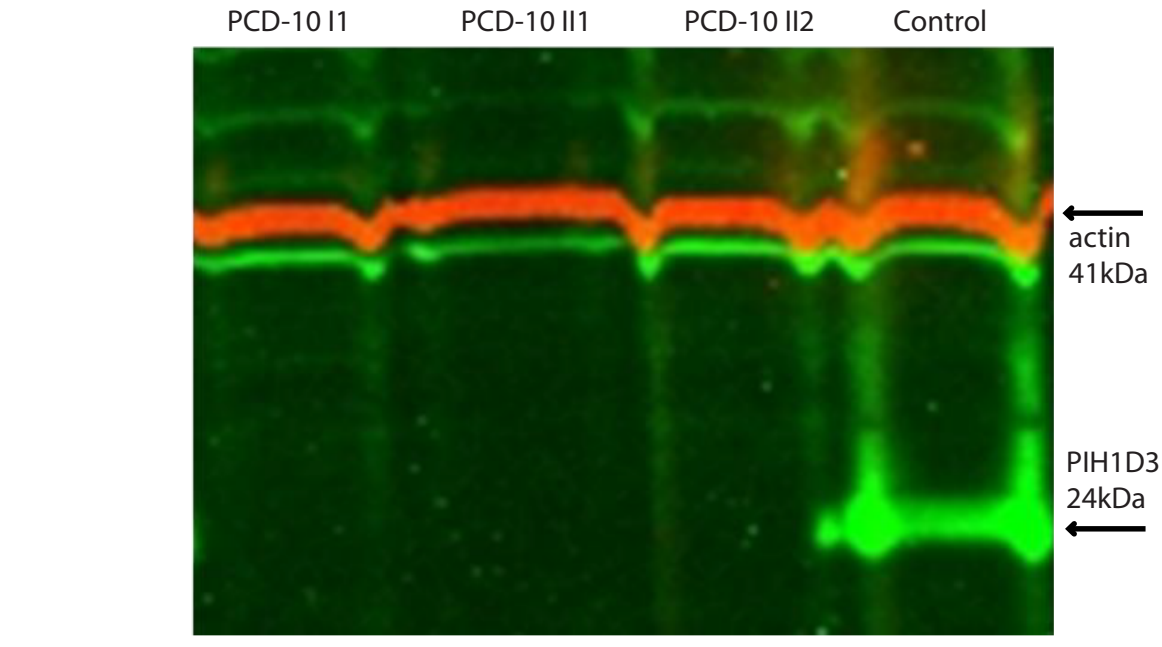

Figure S1. Results of the mutational analyses in *PIH1D3* and expression of PIH1D3 in respiratory cells. (**A**) cDNA sequences of transcripts from cultured respiratory cells of a patient from family PCD-10, with the c.357_363del variant in *PIH1D3* (I), showing two aberrant transcripts: one transcript with the 7 base deletion in exon 6 (E6MUT) and one that shows exon skipping of exon 6 (E7). The cells were cultured with cycloheximide, which blocks the translation step in protein synthesis and consequently inhibits nonsense mediated decay (NMD) (II). This sample shows mainly the E6MUT transcript, indicating that the E6MUT transcript is produced predominantly, but is more subject to NMD in sample A than the exon skipping transcript. In respiratory cells of healthy controls only the wild type (E6WT) transcript is found (III). In family OP-1899 the hemizygous nonsense mutation within exon 6 of *PIH1D3* results in a premature stop codon. (**B**) The expression of PIH1D3 was analyzed by immunoblotting in whole cell lysates of nasal brush biopsies of three patients (PCD-10 I1, PCD-10 II1and PCD-10 II2) with the c.357_363del variant and one healthy control. Actin was used to normalize the amount of protein per well.

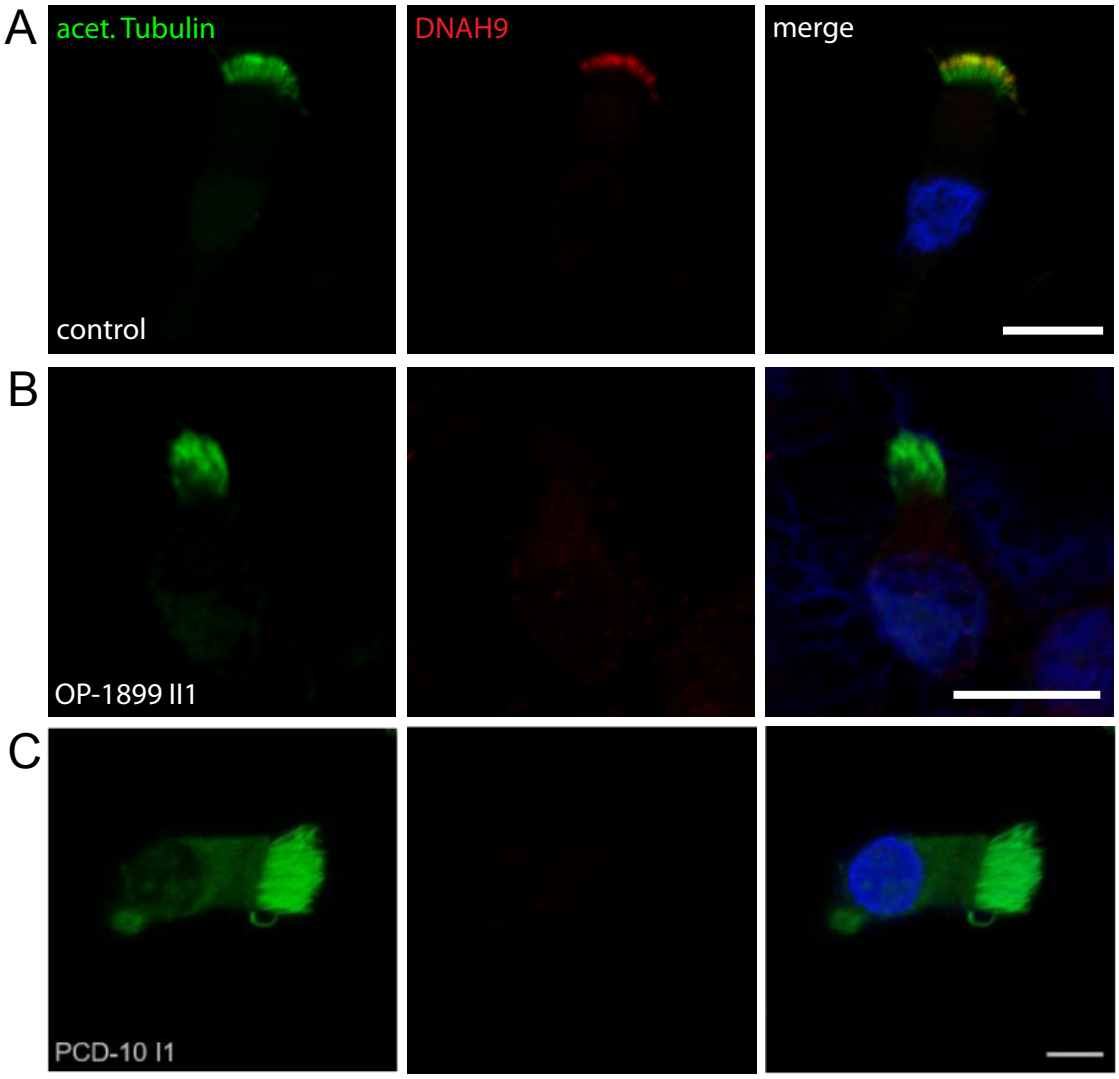

Figure S2. Loss-of-function *PIH1D3*-mutations result in absence of the outer dynein arm heavy chain DNAH9.

(**A**) Respiratory epithelial cells from control and PCD individuals OP-1899 II1 (**B**) and PCD-10 I1 (**C**) were double-labeled with antibodies directed against DNAH9 (red) and acetylated tubulin (green). Acetylated tubulin localizes to the entire length of the cilia, whereas DNAH9 localization is restricted to the distal part of the cilia in control cells (**A**). In contrast, in *PIH1D3*-mutant cells, DNAH9 was absent or severely reduced from ciliary axonemes (**B-C**). Nuclei were stained with Hoechst33342 or DAPI (blue). Scale bars represent 10µm.

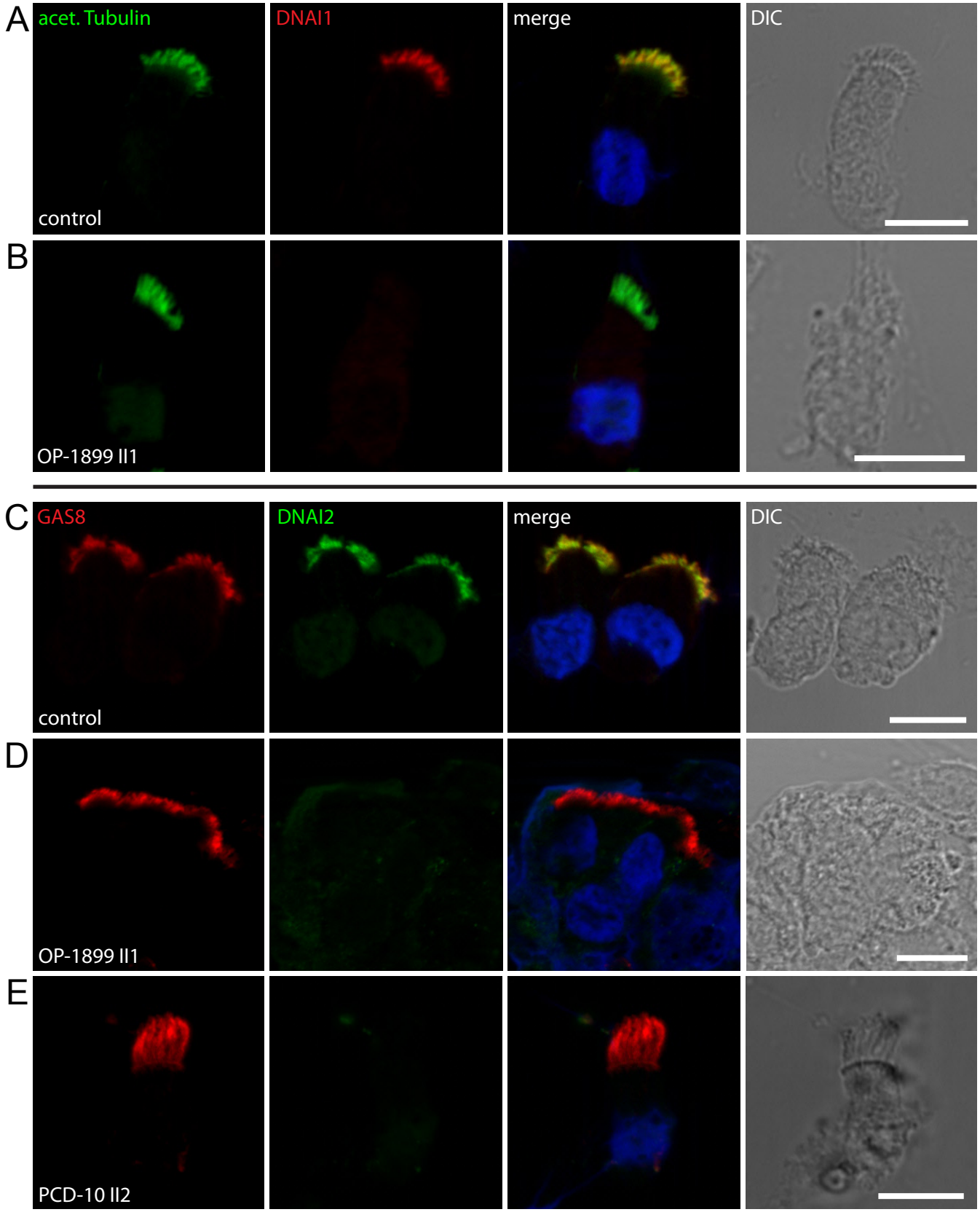

Figure S3. *PIH1D3*-mutant respiratory cilia are deficient for the outer dynein arm intermediate chains DNAI1 and DNAI2.

Cells were double-labeled with antibodies directed against acetylated tubulin (green) and DNAI1 (red). Both proteins colocalize along the cilia in cells from the unaffected control (yellow) (**A**). In contrast, in cells of *PIH1D3*–mutant individuals DNAI1 was absent from or severely reduced in the ciliary axonemes (**B**). (**C-D**) Axonemal localization of the ODA intermediate chain DNAI2. Cells were double-labeled with antibodies directed against DNAI2 (green) and GAS8 (red). Both proteins colocalize along the cilia in cells from the unaffected control (yellow) (**C**). In *PIH1D3*-mutant respiratory cilia, DNAI2 was absent from or severely reduced in the ciliary axonemes, whereas GAS8 showed a normal distribution pattern (**D-E**).
Nuclei were stained with Hoechst33342 (blue). Scale bars represent 10 µm.

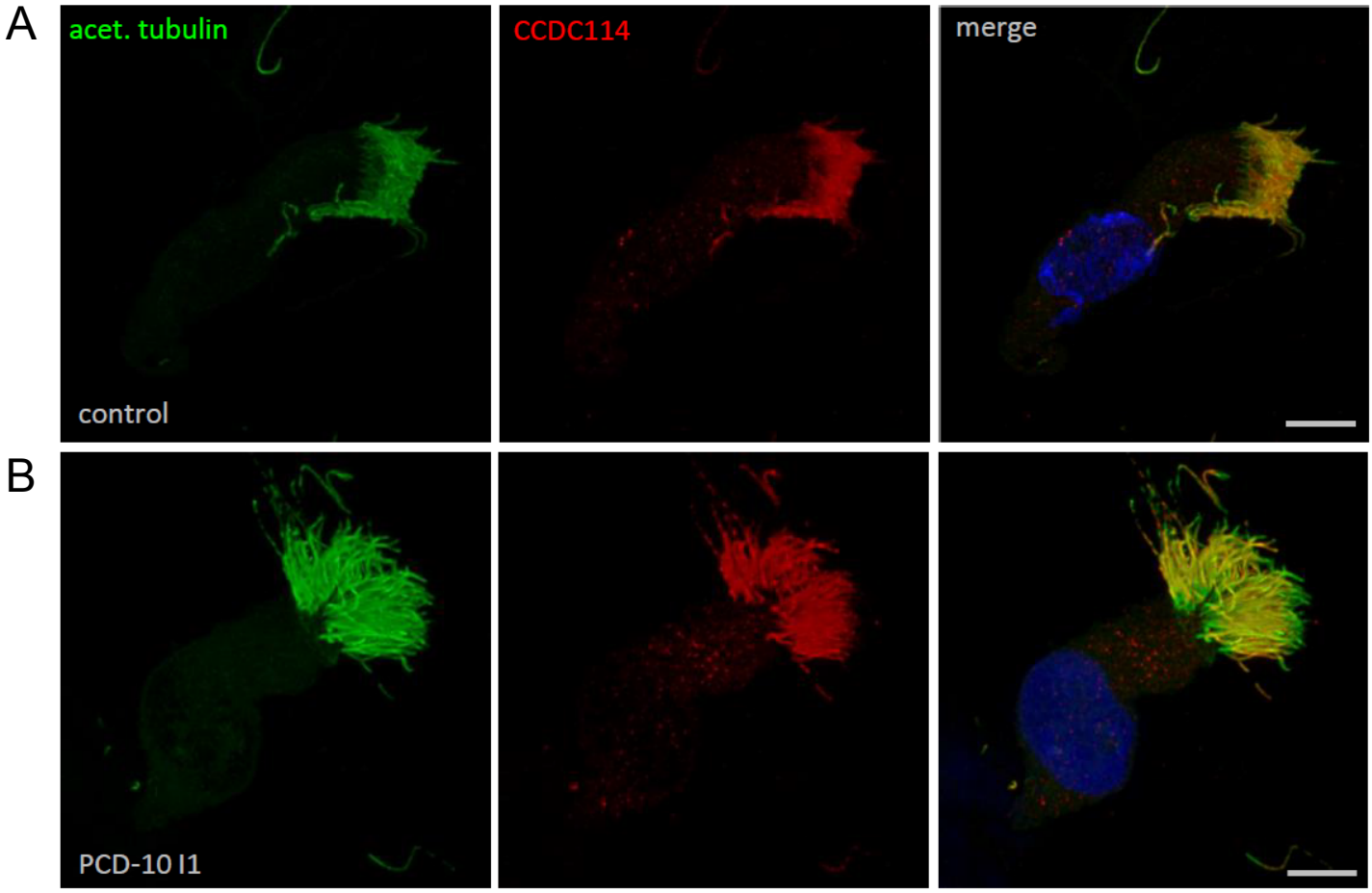

Figure S4. The outer dynein arm docking complex (ODA-DC) is not affected in *PIH1D3*-mutant cells. Respiratory cilia were double-labeled with antibodies directed against acetylated tubulin (green) and CCDC114 (red). CCDC114 colocalize with acetylated tubulin along the cilia from unaffected controls (**A**) and in *PIH1D3*-mutant axonemes (**B**) (yellow), indicating that the ODA-DC is not affected by loss of function of PIH1D3. Nuclei were stained with Hoechst33342 (blue). Scale bars represent 10 µm.

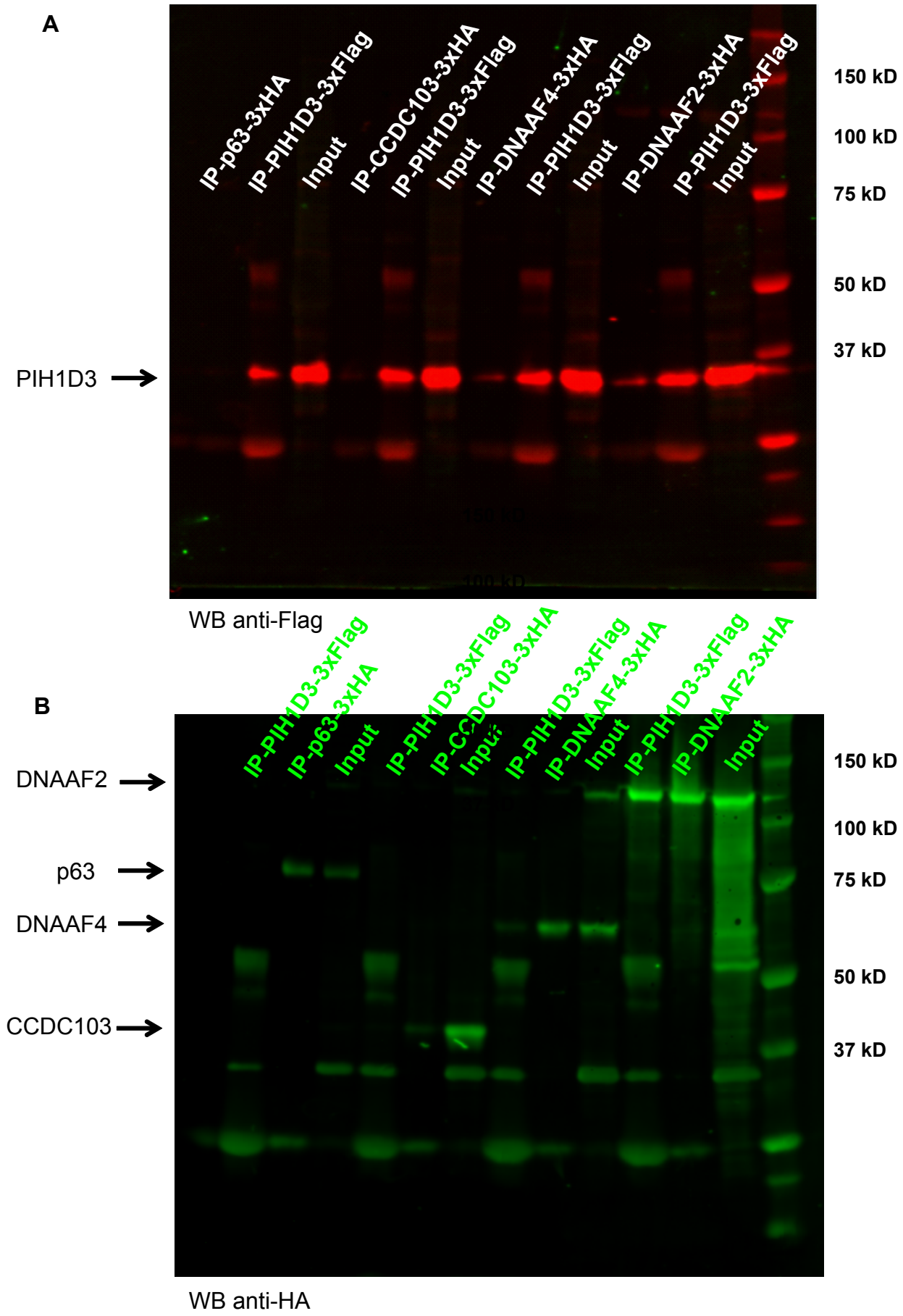

Figure S5. Full gel images of the co-immunoprecipitation results shown in Figure 4. (A) Western-Blot anti-Flag. (B) Western Blot anti HA.

Table S1. Relative mRNA expression of *PIH1D3* compared to household gene VCP in c.357_363del mutant cells.

	Relative mRNA expression of PIH1D3 (SD)	Percentage
With cycloheximide	0.317 (0.026)	100%
Without cycloheximide	0.076 (0.005)	24%

Table S2. Antibodies and dyes

Antibody	**Catologue number**	**Company**
Rabbit polyclonal anti-PIH1D3	ab151121	Abcam
Mouse monoclonal anti-Actin	ab14128	Abcam
IRDye 800 CW goat anti-rabbit IgG	P/N 925-68071	LI-COR Biosciences
IRDye 800 CW goat anti-rabbit IgG	926-32211	LI-COR Biosciences
IRDye 680 CW goat anti-mouse IgG	P/N 925-68070	LI-COR Biosciences
IRDye 680 CW goat anti-mouse IgG	926-32220	LI-COR Biosciences
Mouse monoclonal anti- acetylated tubulin	T7451	Sigma
Rabbit polyclonal anti-CCDC114	HPA042524	Atlas antibodies
Mouse monoclonal to DNAI2	H00064446-M01	Abnova
Rabbit polyclonal anti-DNAI1	HPA021649	Atlas antibodies
Rabbit polyclonal anti-DNAI2	HPA050565	Atlas antibodies
Rabbit polyclonal anti-DNAH9	HPA052641	Atlas Antibodies
Rabbit polyclonal anti-GAS8	HPA041311	Atlas antibodies
Alexa Fluor 546-conjugated goat antibodies to rabbit	A11035	Life technologies
Alexa Fluor 488-conjugated goat antibodies to mouse	A11029	Life technologies
mouse anti-FLAG	clone M2 F9291)	Sigma
mouse anti-HA	H9658	Sigma
Hoechst33342	14533	Sigma
DAPI	D8417	Sigma Aldrich
Mouse monoclonal anti-DNAH5	Omran et al, 2008	
Rabbit polyclonal anti-DNALI1	Rashid et al, 2006	

Table S3. Genes tested for interaction with *PIH1D3.*

Gene tested	**Interaction with *PIH1D3***
CCDC103	-
CCDC114	-
CCDC151	-
C21orf59	-
DNAAF1	-
DNAAF2	**+**
DNAAF3	-
DNAAF4	**+**
DNAH11	-
DNAI1	-
DNALI1	-
HSP90	**+**
IFT46	-
LRRC6	-
TTC25	-
TXNDC3	-
WDR69	-
ZMYND10	-

+: interaction observed, -: no interaction observed

Supplemental References

Omran, H. et al. (2008). Ktu/PF13 is required for cytoplasmic pre-assembly of axonemal dyneins. Nature *456*, 611–616.

Rashid, S. et al. (2006). The murine Dnali1 gene encodes a flagellar protein that interacts with the cytoplasmic dynein heavy chain 1. Mol. Reprod. Dev. *73*, 784–794.