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Supplemental Data

Loss-of-Function Mutations in *RSPH1* Cause Primary Ciliary Dyskinesia with Central-Complex and Radial-Spoke Defects

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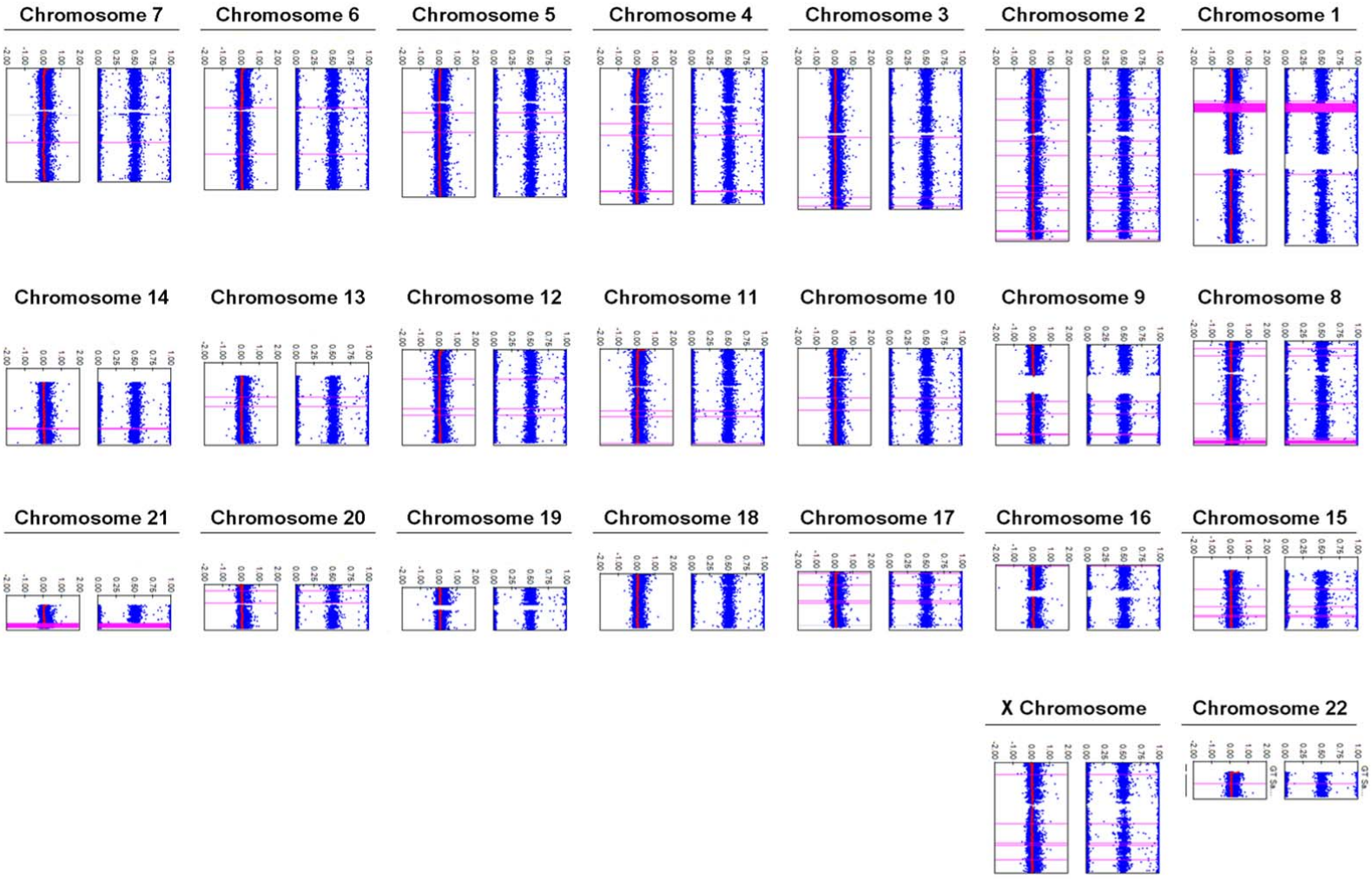
Chromosome 7
Chromosome 6
Chromosome 5
Chromosome 4
Chromosome 3
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Chromosome 1
Chromosome 14
Chromosome 13
Chromosome 12
Chromosome 11
Chromosome 10
Chromosome 9
Chromosome 8
Chromosome 21
Chromosome 20
Chromosome 19
Chromosome 18
Chromosome 17
Chromosome 16
Chromosome 15
X Chromosome
Chromosome 22

Figure S1. Homozygosity Mapping in Individual DCP940

DCP940 was genotyped with the HumanCytoSNP-12 chip from Illumina® and the data were analyzed with the Genome Studio® and CNV partition 3.1.6 softwares (Illumina) as well as with an in-house script designed to identify homozygous regions. Each blue dot represents one individual single-nucleotide polymorphism (SNP). For each chromosome, the upper panel shows the B allele frequency. For each SNP, a low B allele frequency indicates that the individual is homozygous for the A allele; intermediate values mean they are heterozygous and high B allele frequency means that they are homozygous for the B allele. Regions of homozygosity larger than 100 kb are shaded in purple. Three large regions of homozygosity were identified: one on chromosome 1 (between rs638965 and rs1332636 – 13.8 Mb); the second on chromosome 21 (between rs2836415 and rs7278087 – 8.3 Mb), containing *RSPH1*; and the third one on chromosome 8 (between rs11781101 and rs7837240 – 6 Mb). For each chromosome, the lower panel represents the log R ratio, which is the logged ratio of observed probe density to expected probe density. A null log R ratio indicates the absence of loss or gain of material.

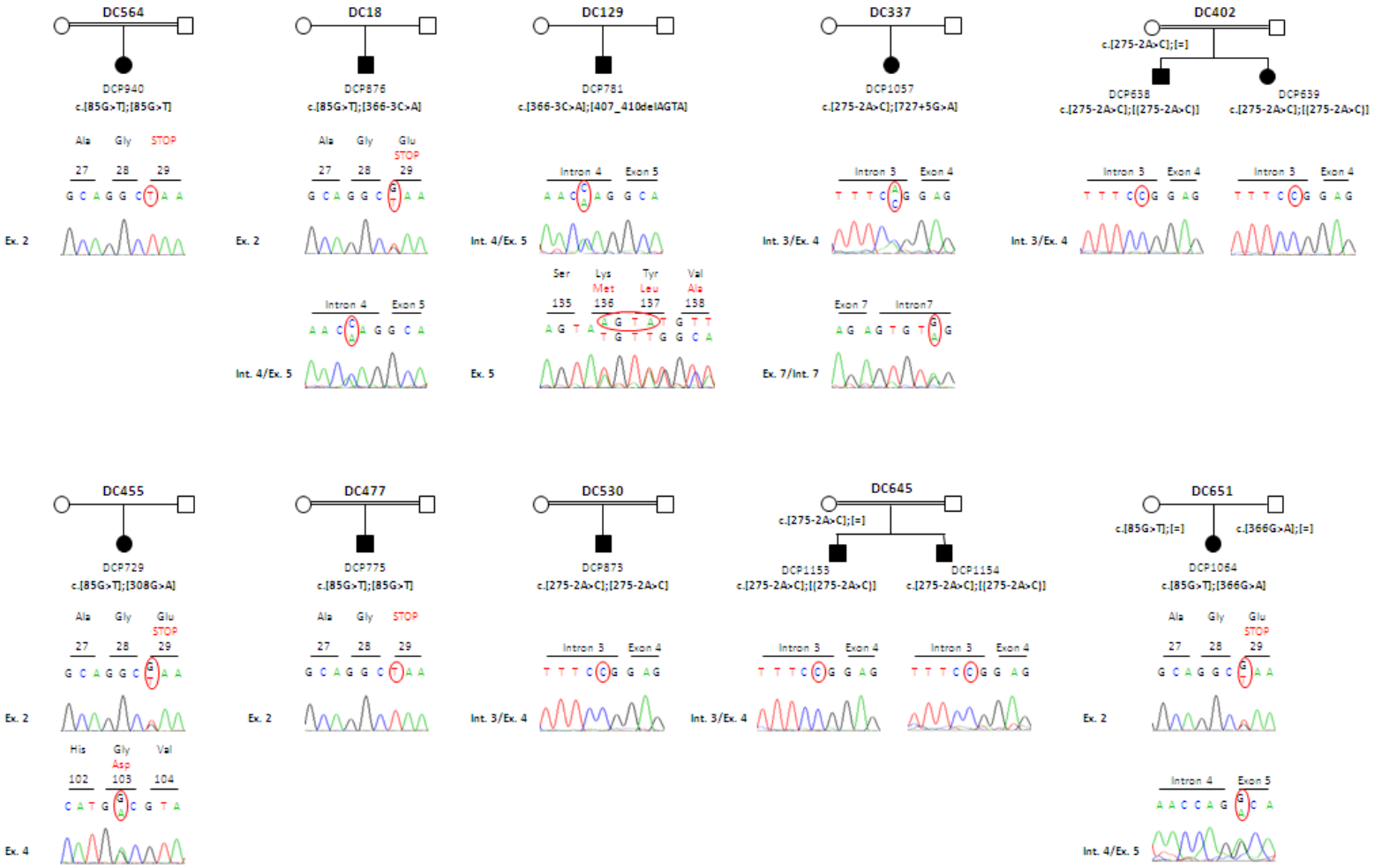

Figure S2. *RSPH1* Mutations Identified in Individuals with PCD

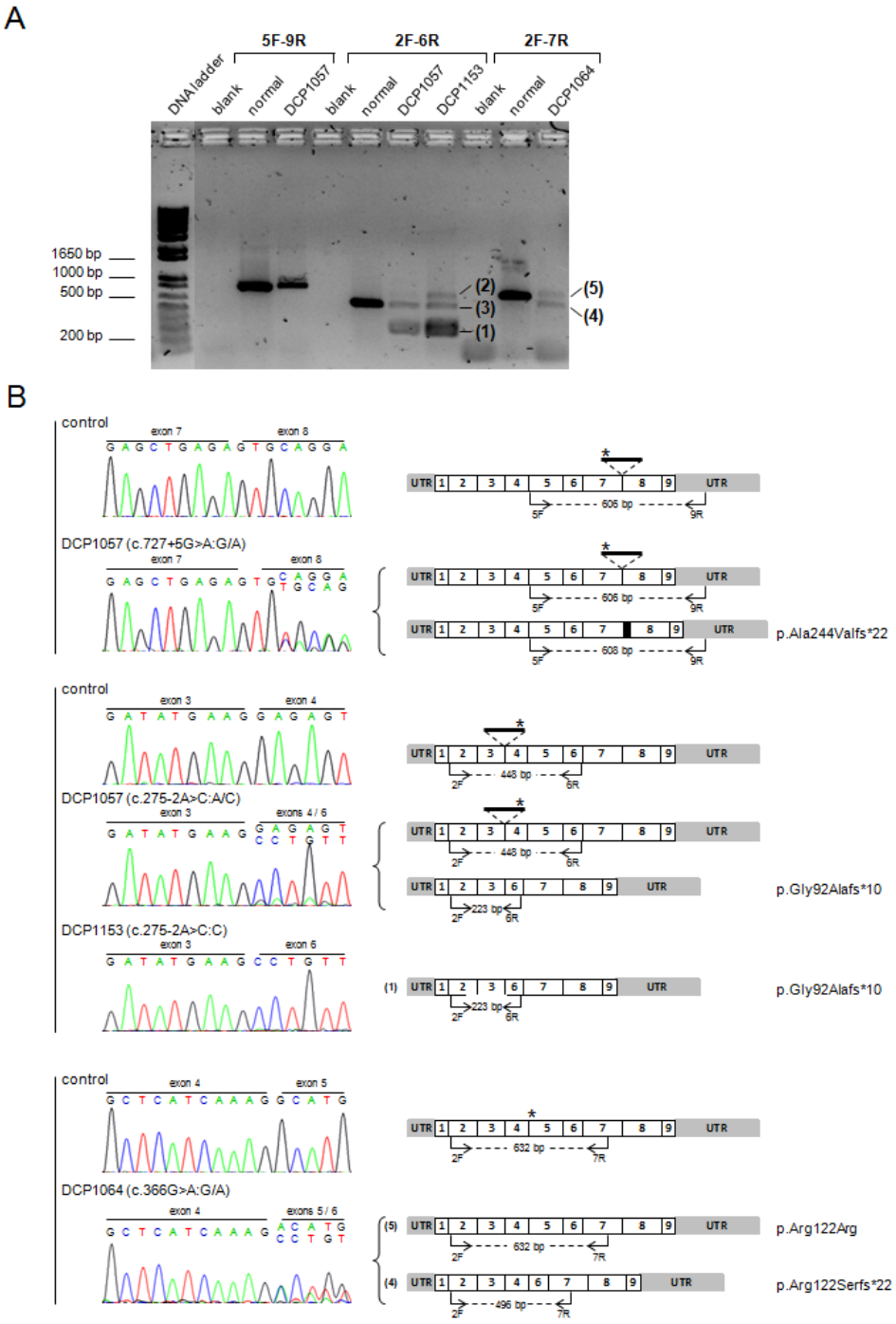

Figure S3. Consequences of the c.727+5G>A, c.275-2A>C, and c.366G>A Mutations on *RSPH1* RNA Splicing, as Assessed by RT-PCR Amplification from Nasal Brushing Samples

(A) RT-PCR amplifications of *RSPH1* transcripts using three exonic primer sets (5F-9R, 2F-6R, and 2F-7R). For each amplification, blank is a negative control (without cDNA), normal

a normal control. The ladder is the 1kb+ DNA ladder from Invitrogen (SKU #10787). As a positive control of reverse transcription and PCR, *DNAI1* transcripts were amplified in each sample (data not shown).

(B) *RSPH1* electrophoregrams corresponding to the direct sequencing of the PCR products shown in (A) for both a control and affected individual(s) (left). The exonic organization of the observed *RSPH1* cDNA is schematized (right). Exons are indicated by empty or grey boxes, depicting translated or untranslated sequences, respectively. The localization of the mutation is indicated on the control cDNA diagram by a star. The expected consequence of the mutation on translation is indicated on the right side of the figure. We have isolated the amplified DNA from the different bands seen on (A) for DCP1153, prior to their direct sequencing. This analysis revealed that band (1) (the major molecular species) indeed corresponds to a transcript lacking exons 4 and 5 (as illustrated in (B)); the fainter bands (2) and (3) (minor molecular species) result from the inclusion of the last 150 bp of intron 3 through the activation of a cryptic splice donor site located in this intron (band (2)), and from a deletion of the first three bases of exon 4 that, if translated, would result in the deletion of a glycine residue (p.Gly92del) that is invariant throughout evolution from *Chlamydomonas reinhardtii* to the human (band (3)) (data not shown). For DCP1064, the electrophoregrams corresponding to the direct sequencing of the RT-PCR product generated with primers 2F-7R is shown in (B). Two molecular species were identified: one transcript lacking exon 4 (corresponding to the major band (4) in (A)), and the normal transcript (corresponding to the minor band (5) in (A)). Primers and RT-PCR conditions are available upon request.

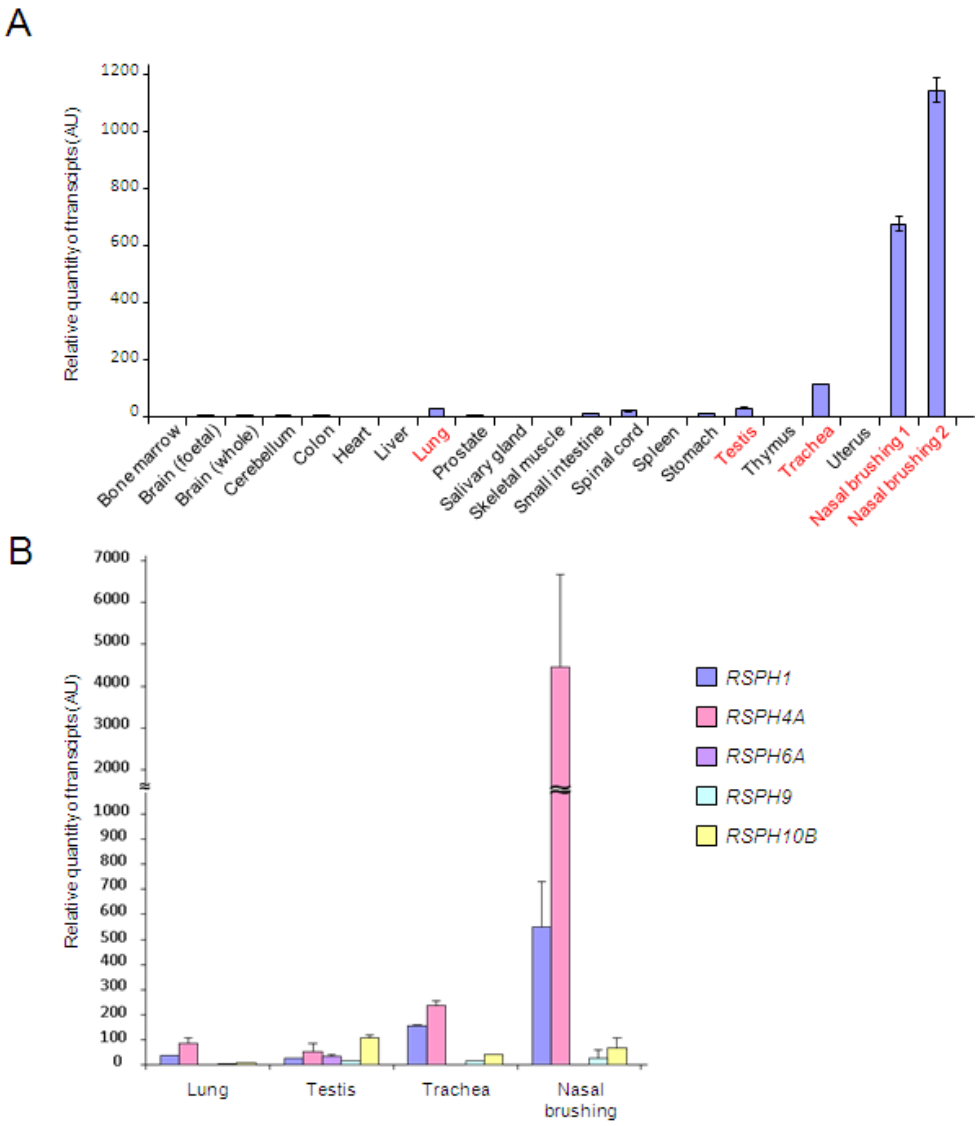

Figure S4. Expression Analysis of Human *RSPH1*, as Assessed by Means of Quantitative RT-PCR Analysis

(A) *RSPH1* transcripts are found at the highest levels in nasal brushing and to a lesser extent in trachea and testis.

(B) Expression of *RSPH1* transcripts in lung, testis, trachea and nasal brushing, as compared with that of four other genes encoding RS head proteins.
Values are the mean±SD of three independent experiments. 500ng of total RNA from different human tissues obtained from Clontech (Takara Bio Europe/Clontech, Saint Germain en Laye, France) or from nasal brushings extracted with the RNeasy Mini Kit (Quiagen) were primed with 2.5 mM of oligodT and then subjected to reverse transcription with the Reverse Transcriptor kit from Roche, following the manufacturer's instructions. cDNAs were amplified in the Light Cycler LC480 (Roche/Boehringer, Mannheim, USA) with the Mesa Blue qPCR MasterMix Plus for SYBR Assay (Eurogentec). The ubiquitously expressed

ERCC3 gene was used as an internal control to normalize the data. The sequences of the primers used in the experiment are the following:

RSPH1:
forward 5’ GAAGAGAGCCGGGAGTATGA 3’
reverse 5’ TTCTTCTTCTTCAGAATTAATGTTTCC 3’

RSPH4A:
forward 5’ GCAGTCCTTCAATCCAACCT 3’
reverse 5’ TGACCCCAGCCTATGTAGAAA 3’

RSPH6A:
forward 5’ TACTCAGTGGCCGTTGTGC 3’
reverse 5’ CCAGCCGATGTAGATGTTCTC 3’

RSPH9:
forward 5’ CCGCTATGATCGGGTTCTC 3’
reverse 5’ TCCACTCTGTGCAGTTCAGG 3’

RSPH10B:
forward 5’ GAAAAGGTTCGTGGGCTGT 3’
reverse 5’ CCATGCATGAGTCCTTCTGA 3’

ERCC3:
forward 5’ ACTGGATGGAGCTGCAGAAT 3’
reverse 5’ GACATAGGGCACCAGACCTC 3’

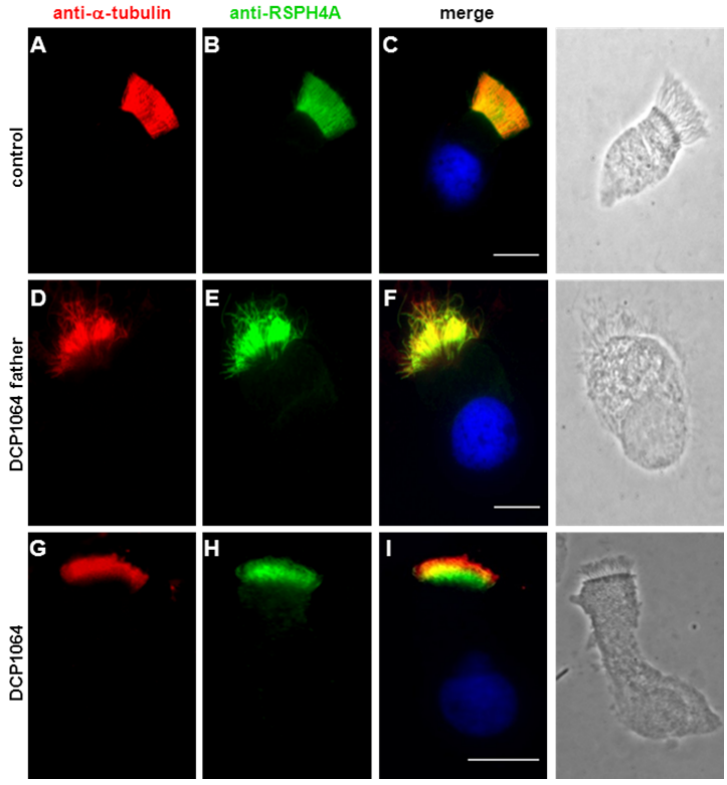

Figure S5. RSPH4A Localizes to Cilia in Airway Epithelial Cells of Healthy Individuals and DCP1064

In airway epithelial cells from a healthy individual (A-C), the healthy father of DCP1064 (D-F), and individual DCP1064 (G-I), RSPH4A (green) localizes within cilia (red) (for details, see the main text). Airway epithelial cells were examined after labeling with a rabbit anti RSPH4A polyclonal antibody (Sigma HPA031196, 1:100, 37°C, 1hr) and a secondary goat anti-rabbit Alexa Fluor-488 (green) antibody (Invitrogen A11034). For controls, we used an antibody directed against acetylated α-tubulin (mouse monoclonal [6-11B-1], abcam ab24610, 1:700) for the visualization of axonemal microtubules, revealed by a secondary goat anti-mouse Alexa Fluor-594 (red) antibody (Invitrogen, A11032). Nuclei were stained with DAPI (Sigma, 32670). White scale bars represent 10 μm.

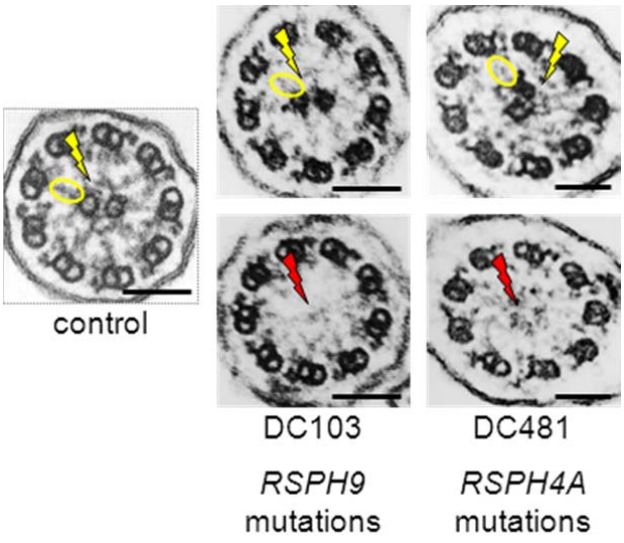

Figure S6. CC and RS Defects in Respiratory Cilia of Individuals with *RSPH4A* or *RSPH9* Mutations

The electron micrographs of cross-sections of cilia from a control, one individual (from family DC103) with mutations in *RSPH9* and one individual (from family DC481) with mutations in *RSPH4A* are shown. For each affected individual, two sections are shown: one with a normal configuration showing the presence of CC and RS (top), and the other with an abnormal axonemal configuration characterized by CC and RS abnormalities (bottom). The yellow flashes show the presence of normal CC in the normal control and in the affected individuals; RS are encircled in yellow in the normal control and in cilia with a CC from affected individuals. The red flashes show CC defects in affected individuals. Black scale bars represent 0.1 μm.

Table S1. Mutations Identified in *RSPH4A* and *RSPH9*

Family	Gene	Allele 1			Allele 2		
		Name	**Frequency (EVS/dbSNP)**	**Predicted effect (PolyPhen)**	**Name**	**Frequency (EVS/dbSNP)**	**Predicted effect (PolyPhen)**
DC5	*RSPH4A*	**c.1916+2T>A**	ND/ND		**c.1916+2T>A**	ND/ND	
DC330	*RSPH4A*	**c.1391G>A** p.Gly464Glu	ND/ND	probably damaging (1.00)	**c.1391G>A** p.Gly464Glu	ND/ND	probably damaging (1.00)
DC397	*RSPH4A*	**c.1916+2T>A**	ND/ND		**c.1916+2T>A**	ND/ND	
DC390	*RSPH4A*	**c.667delA** p.Ser223Alafs*15	ND/ND		**c.1720delA** p.Thr424Glnfs*19	ND/ND	
DC398	*RSPH4A*	**c.1391G>A** p.Gly464Glu	ND/ND	probably damaging (1.00)	**c.1391G>A** p.Gly464Glu	ND/ND	probably damaging (1.00)
DC480	*RSPH4A*	**c.462_469del** p.Gln155Thrfs*12	ND/ND		**c.1351C>T** p.Gln451*	ND/ND	
DC481	*RSPH4A*	**c.430C>T** p.Gln144*	ND/ND		NI	ND/ND	
DC103	*RSPH9*	**c.3G>A** p.Met1?	ND/ND	probably damaging (0.98)	**c.3G>A** p.Met1?	ND/ND	probably damaging (0.98)
DC38	*RSPH9*	c.804_806del p.Lys268del	ND/ND		c.804_806del p.Lys268del	ND/ND	
DC367	*RSPH9*	**c.52C>T** p.Gln18*	ND/ND		**c.52C>T** p.Gln18*	ND/ND	
DC439	*RSPH9*	c.804_806del p.Lys268del	ND/ND		c.804_806del p.Lys268del	ND/ND	

ND: not described; NI: not identified; novel mutations are indicated in bold.

Table S2. Quality criteria (QC) used for the WES analysis performed on the DNA sample from DCP940

Targeted size (bp)	70,091,974
Depth 1x (%)	99.04
Depth 4x (%)	97.39
Depth 10x (%)	94.18
Depth 25x (%)	84.51
Mean depth (x)	72.27

The QC set used was the default settings of the Eris© web-software from IntegraGen (Evry, France).

Table S3. Frequency of identified *RSPH1* mutations in EVS, dbSNP and Ensembl

Mutation	**EVS**	**dbSNP**	**Ensembl**
c.85G>T	0.00038 (all alleles)	0.0005	<0.001
c.275-2A>C	0.001 (European-American alleles)	NA	NA
c.308G>A	ND	ND	ND
c.366-3C>A	ND	ND	ND
c.366G>A	ND	ND	ND
c.407_410delAGTA	ND	ND	ND
c.727+5G>A	ND	ND	ND

NA: not available; ND: not described