SUPPLEMENTARY

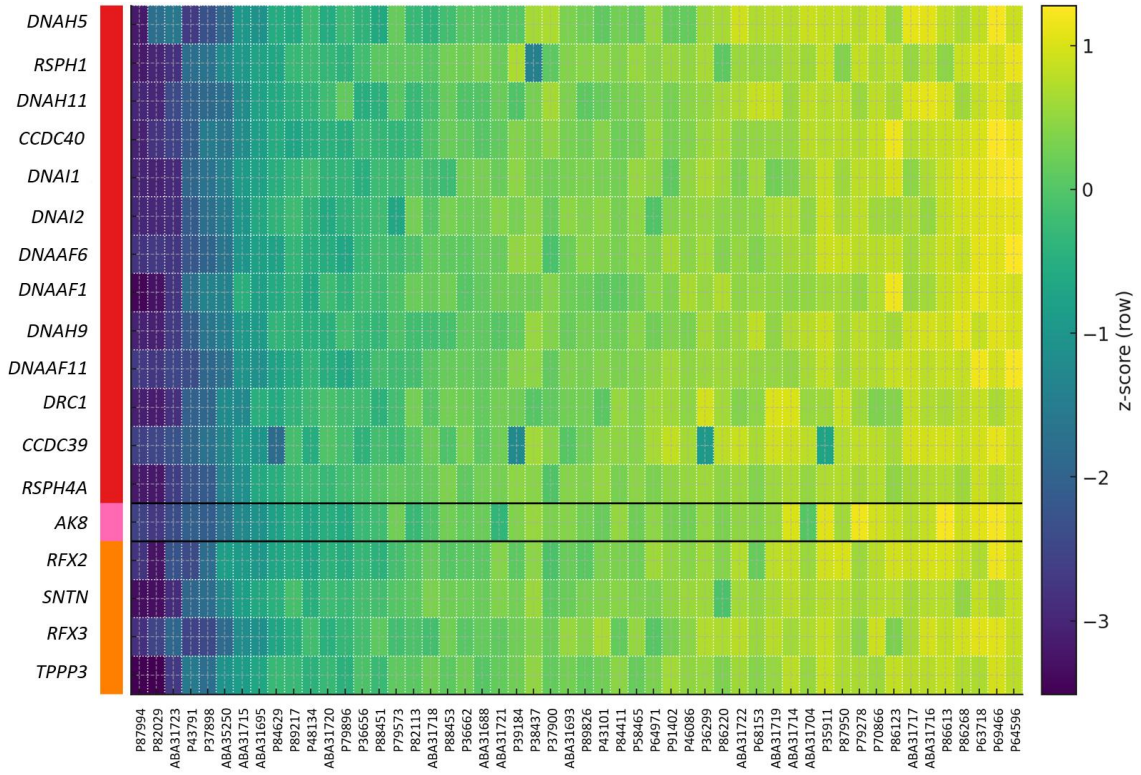


Figure S1. Heatmap showing the normalised gene expression levels (Z-score per row) across all samples. Thirteen genes associated with primary ciliary dyskinesia (PCD) are represented in red, one candidate PCD gene in pink, and four non-PCD ciliary genes in orange. Each column represents an individual, and each row corresponds to a specific gene. The color scale for z-score indicates relative expression levels, with purple representing low expression and yellow representing high expression. Samples are arranged from the lowest to the highest level of ciliary transcriptome capture. Individuals from the supplementary group are codified by a number prefixed with "ABA".

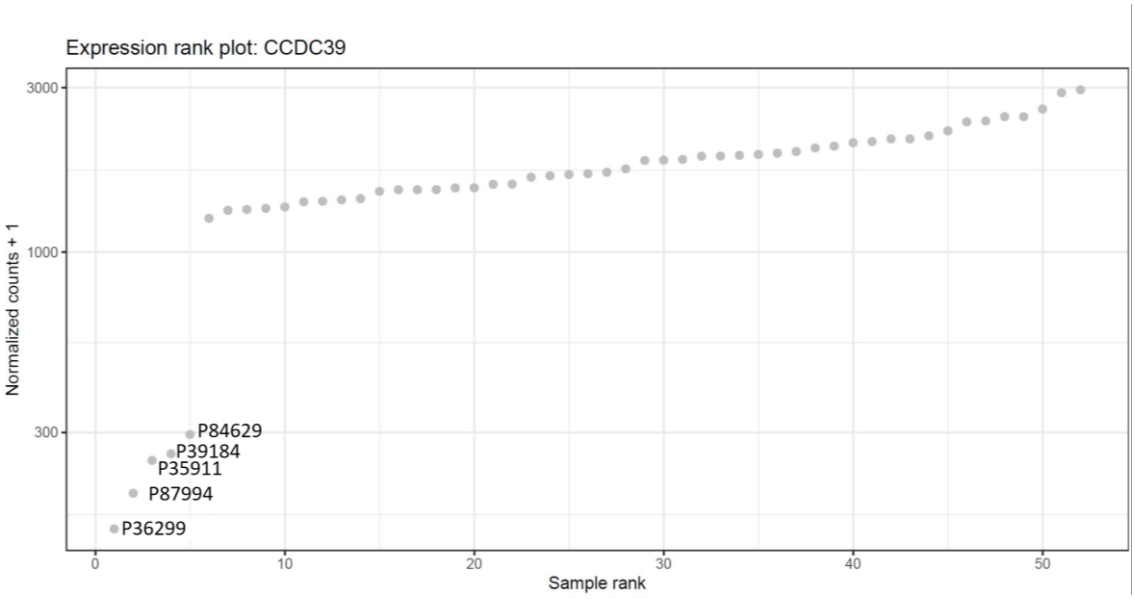


Figure S2. Expression rank plot of *CCDC39* across samples. Each point represents one sample. Samples with low *CCDC39* expression levels are labelled.

Figure S3. RNA-seq results in patient P86268. (A) IGV screenshot showing the deletion c.3810del in heterozygous state in the *DNAH11* gene in the RNA-seq analysis. (B) Sashimi plot of the aberrant splicing event as a consequence of the c.8941-3C>T variant identified in *DNAH11* in patient P86268 (red) in comparison to a control sample (blue). In the patient, 54 splice junction reads supported the exclusion of 22 nucleotides from exon 55.

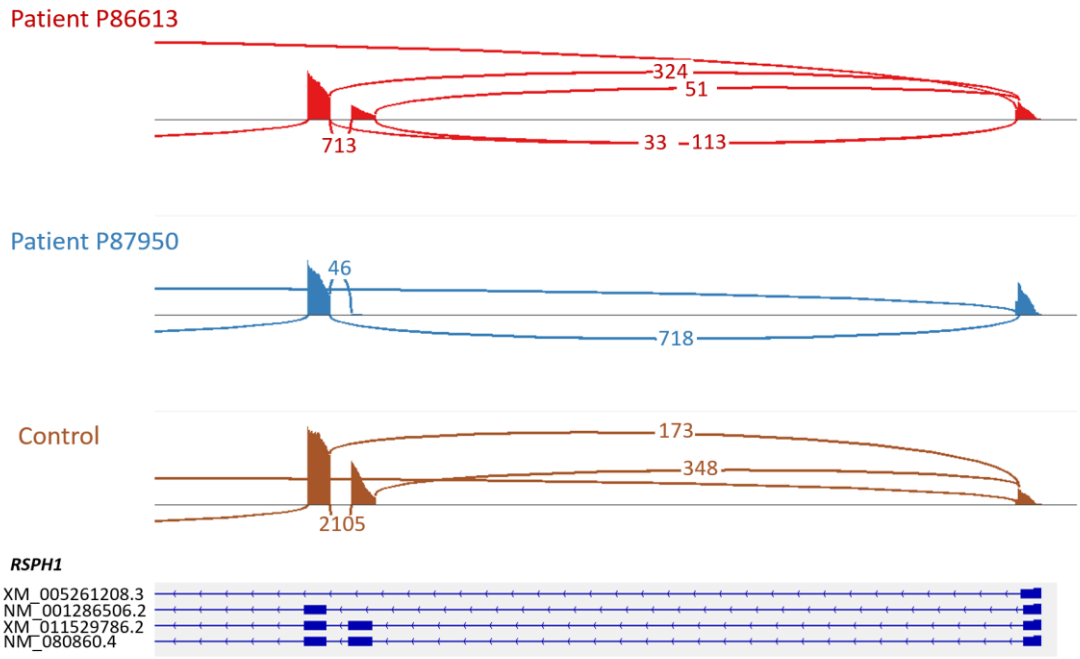


Figure S4. Altered splicing of *RSPH1* exon 2 as a consequence of the pathogenic c.85G>T variant. Patient P86613 (red) carried this variant in heterozygous state, while patient P87950 carried it in homozygosity. Control sample is represented in brown.

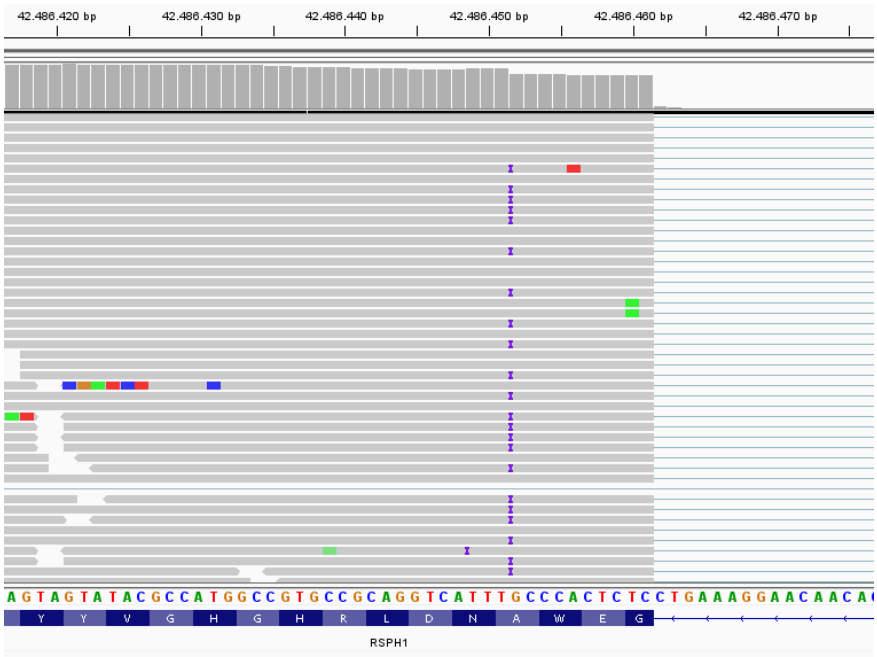


Figure S5. IGV screenshot showing the *RSPH1*:c.287dup in heterozygosity in patient P86613. The duplication is indicated as a purple bar.

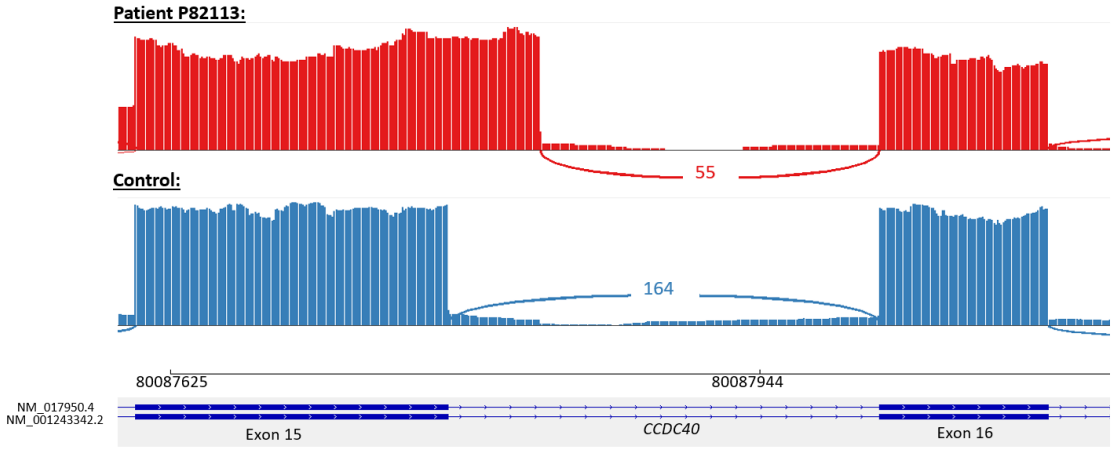


Figure S6. Alteration in *CCDC40* found in patient P82113. Sashimi plot comparing patient P82113 (red) with a representative control (blue). The c.2619+45G>A variant activates a cryptic 5' splice donor, resulting in inclusion of 50 bp of intron 15, thus producing a 50-nucleotides elongation of exon 15.

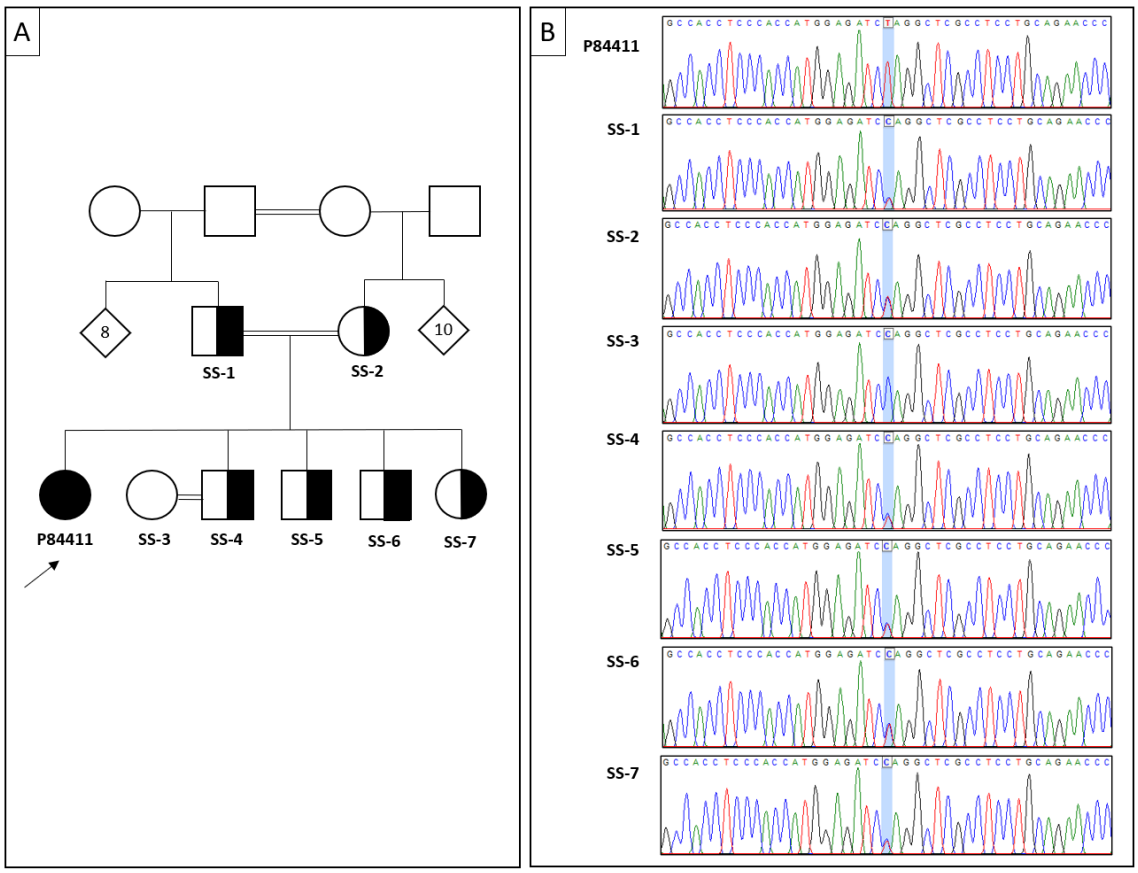


Figure S7. Segregation study of the *AK8*:c.1243C>T variant found in homozygosity in patient P84411. (A) Pedigree tree of family from patient P84411. The presence of the variant was tested in several family members (SS). (B) Electropherograms revealed that only patient P84411 was homozygous for the *AK8*:c.1243C>T variant.

Supplementary table 1.

Patients included in the present study and their previously reported genetic results (Carretero-Vilarroig, 2025)

Group	Patient	Gene	Allele 1	Allele 2
Positive control group	P35911	*CCDC39*	c.2550del;p.(Glu851Argfs*15)	c.2550del;p.(Glu851Argfs*15)
	P36299	*CCDC39*	c.1072del;p.(Thr358Glnfs*3)	c.1072del;p.(Thr358Glnfs*3)
	P39184	*CCDC39*	c.357+1G>C;p.(?)	c.2505_2506del;p.(His835Glnfs*4)
	P84629	*CCDC39*	c.357+1G>C;p.(?)	c.357+1G>C;p.(?)
	P87994	*CCDC39*	c.357+1G>C;p.(?)	c.357+1G>C;p.(?)
	P86268	*DNAH11*	c.3810del;p.(Gly1272Aspfs*21)	c.8941-3C>T;p.(?)
	P86613	*RSPH1*	c.85G>T;p.(Glu29*)	c.287dup;p.(Asn96Lysfs*2)
	P87950	*RSPH1*	c.85G>T;p.(Glu29*)	c.85G>T;p.(Glu29*)
Study group	P70866	*DNAAF11*	c.76G>C;p.(Asp146His)	ND
	P36662	*DNAAF1*	c.1300_1322del;p.(Gly434Profs*4)	ND
	P79896	*RSPH1*	c.85G>T;p.(Glu29*)	ND
	P86220	*HYDIN*	c.1030A>T;p.(Arg344*) (AF=0.52)¥	ND
	P37898	*RSPH1*	c.85G>T;p.(Glu29*)	ND
		HYDIN	c.6889G>T;p.(Glu2297*) (AF=0.52)¥	ND
	P39700	*RSPH1*	c.85G>T;p.(Glu29*)	ND
		HYDIN	c.6889G>T;p.(Glu2297*)¥	ND
	P89217	*HYDIN*	c.6685C>T;p.(Arg2229*) (AF=0.28)¥	c.6889G>T;p.(Glu2297*) (AF=0.27)¥
	P91402	*HYDIN*	c.6685C>T;p.(Arg2229*)¥	c.6889G>T;p.(Glu2297*)¥
	P36656	ND	-	-
	P43101	ND	-	-
	P46086	ND	-	-
	P48134	ND	-	-
	P58465	ND	-	-
	P38437	ND	-	-
	P82029	ND	-	-
	P68153	ND	-	-
	P64596	ND	-	-
	P79278	ND	-	-
	P43791	ND	-	-
	P63718	ND	-	-
	P84411	ND	-	-
	P69466	ND	-	-
	P79573	ND	-	-
	P86123	ND	-	-
	P64971	ND	-	-
	P88453	ND	-	-
	P88451	ND	-	-
	P82113	ND	-	-
	P89826	ND	-	-

AF = allele frequency observed in Exome sequencing; Patients P39700 and P91402 were analysed via PCR according to the results obtained for their siblings (P37898 and P89217, respectively). ¥Data previously unreported. ND = not detected.

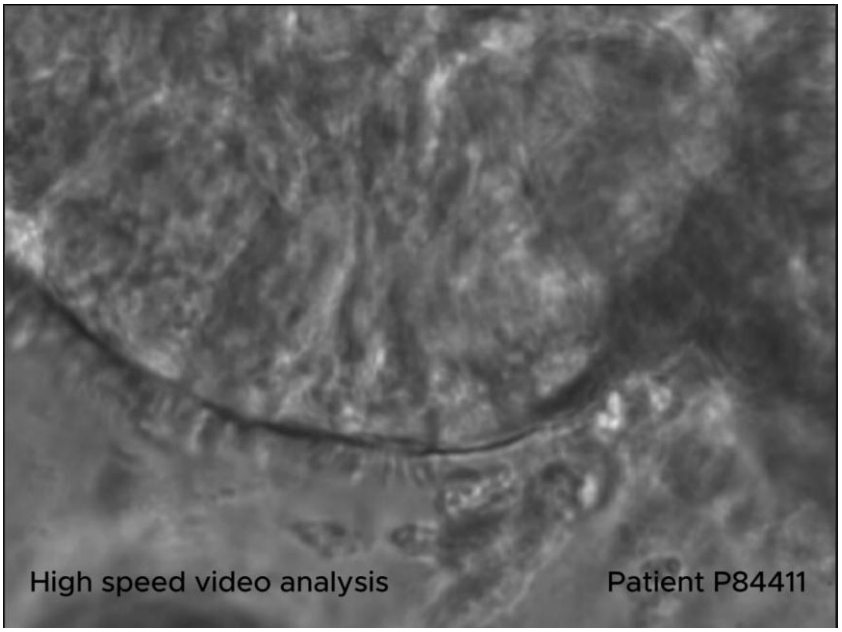
Supplementary table 2. Aberrant expression findings.

Sample ID	geneID	Gene name	pValue	padjust	zScore	l2fc	Raw counts	Mean Raw counts	Norm counts	Mean Corrected	theta	Aberrant By Sample	Aberrant By Gene	padj_rank	Padjust Genes to test on all samples	foldChange
P35911	ENSG00000284862.3	CCDC39	0,003833808	1	-2,88	-2,77	638	2995,15	247,91	1691,39	4,41	0	0	8474	0,968606518	0,15
P36299	ENSG00000284862.3	CCDC39	0,000618824	1	-3,62	-3,44	340	2995,15	156,44	1691,39	4,41	0	0	8474	0,156344947	0,09
P38437	ENSG00000160188.10	RSPH1	1,97E-09	0,000279841	-6,36	-3,43	1169	13845,25	838,5	9044,43	12,7	3	1	1,5	4,97E-07	0,09
P39184	ENSG00000284862.3	CCDC39	0,004628138	1	-2,8	-2,71	306	2995,15	259,21	1691,39	4,41	2	0	8475	0,584646483	0,15
P64971	ENSG00000188596.11	CFAP54	1,71E-09	0,000298303	-6,5	-4,68	92	2066,96	51,99	1349,23	8,41	1	1	1	NA	0,04
P79573	ENSG00000129654.8	FOXJ1	0,000780696	1	3,07	0,67	8636	10663,83	11896,38	7470,8	44,15	4	0	8483,5	0,197241687	1,59
P84629	ENSG00000284862.3	CCDC39	0,007983484	1	-2,59	-2,51	110	2995,15	295,02	1691,39	4,41	0	0	8474	1	0,18
P88453	ENSG00000104450.13	SPAG1	1,17E-09	0,000204638	-6,35	-2,25	1179	6794,08	1271,32	6036,87	24,87	1	1	1	2,96E-07	0,21
P91402	ENSG00000157423.18	HYDIN	0,003048208	1	-3,18	-1,14	4101	11942,71	3253,2	7161,8	19,07	0	0	8474	0,385062888	0,45

Supplementary table 3. Altered splicing events.

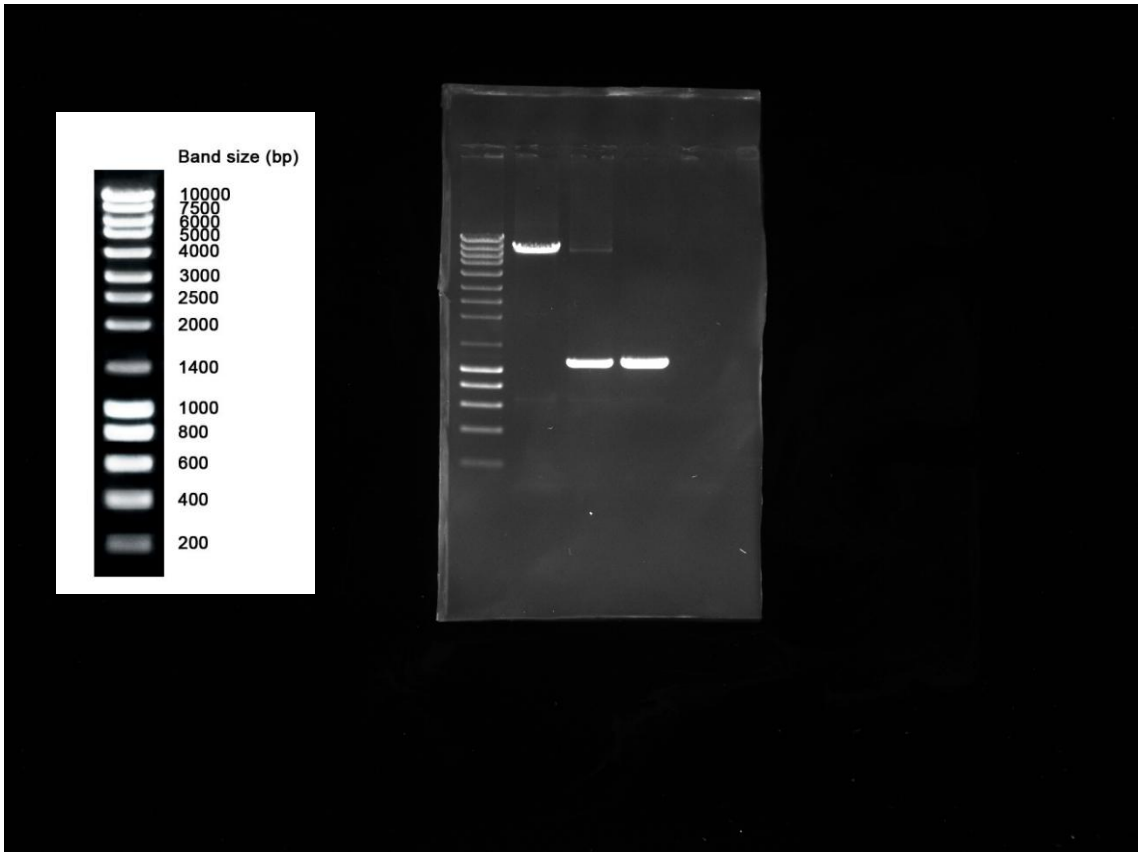
Sample ID	Seq names	start	end	width	strand	hgnc Symbol	pValue	padjust	psiValue	deltaPsi	counts	total Counts	mean Counts	mean Total Counts	non split Counts	non split Proportion	annotated Junction	Potential Impact
P38437	chr21	42482709	42485668	2960	-	RSPH1	4,67E-13	8,53E-07	0,22	-0,73	51	230	2786,17	2939,83	129	0,56	both	(partial) intron Retention
P38437	chr21	42485805	42486370	566	-	RSPH1	2,26E-11	1,55E-05	0,53	-0,45	125	236	2783,98	2869,69	111	0,47	both	(partial) intron Retention
P38437	chr21	42477445	42481929	4485	-	RSPH1	2,54E-11	1,55E-05	0,12	0,11	32	267	32,02	3792,12	74	0,28	start	Exon Elongation
P38437	chr21	42477445	42482636	5192	-	RSPH1	3,62E-11	1,65E-05	0,58	-0,37	158	271	3636,12	3834,44	78	0,29	both	(partial) intron Retention
P38437	chr21	42482709	42484676	1968	-	RSPH1	6,11E-10	0,00018604	0,16	0,15	36	225	24,46	2943,29	128	0,57	start	Exon Elongation
P38437	chr21	42489735	42492757	3023	-	RSPH1	1,52E-09	0,00039725	0,1	0,1	10	97	9,25	2584,71	10	0,1	end	Exon Elongation
P38437	chr21	42476048	42477290	1243	-	RSPH1	1,93E-06	NA	0,83	-0,15	207	249	3953,46	4032,65	34	0,14	both	Annotated Intron reduced Usage
P38437	chr21	42486462	42492757	6296	-	RSPH1	8,60E-06	NA	0,57	-0,32	70	123	2545,06	2828,48	21	0,17	both	(partial) intron Retention
P82113	chr17	80087827	80088010	184	+	CCDC40	3,88E-13	1,77E-07	0,86	0,85	51	59	3,1	163,38	8	0,14	end	Exon Elongation
P82113	chr17	80087777	80088010	234	+	CCDC40	1,31E-08	0,001705	0	-0,65	0	117	138,52	171	66	0,56	both	Annotated Intron reduced Usage
P86268	chr7	21750365	21765449	15085	+	DNAH11	1,53E-07	0,011993	0,35	0,34	66	187	2,21	135,71	57	0,3	start	Exon Truncation
P86268	chr7	21750365	21765427	15063	+	DNAH11	4,32E-06	NA	0,44	-0,45	57	130	64,92	70,13	0	0	both	Annotated Intron reduced Usage
P86613	chr21	42493080	42496119	3040	-	RSPH1	4,52E-07	0,054991	0,23	0,21	141	606	9,75	383,25	24	0,04	none	Exon Elongation
P87950	chr21	42492864	42492965	102	-	RSPH1	1,27E-09	0,00077333	0,06	-0,78	46	813	1299,75	1494,77	25	0,03	both	Annotated Intron reduced Usage
P87950	chr21	42492864	42496132	3269	-	RSPH1	5,09E-07	0,077469	0,52	0,43	733	1412	162,37	1854,48	607	0,43	start	Exon Skipping
P87950	chr21	42486462	42496119	9658	-	RSPH1	3,06E-05	NA	0,22	0,14	637	2870	219,69	2828,88	21	0,01	start	Exon Skipping
P88453	chr8	100225340	100231155	5816	+	SPAG1	4,53E-10	0,00041386	0,27	-0,68	19	71	597,19	640,13	30	0,42	both	(partial) intron Retention
P88453	chr8	100233538	100239239	5702	+	SPAG1	3,57E-07	0,081491	0,65	-0,27	52	80	671,98	718,4	15	0,19	both	(partial) intron Retention
P88453	chr8	100194269	100213089	18821	+	SPAG1	8,88E-06	NA	0,13	-0,58	11	82	87,42	129,96	69	0,84	both	Annotated Intron reduced Usage
P88453	chr8	100213429	100213818	390	+	SPAG1	4,59E-05	NA	0,28	-0,44	8	29	126,88	167,06	11	0,38	both	(partial) intron Retention

Supplementary Movie 1. High speed video analysis of patient P84411.



This video compilation comprises four recordings of patient P84411 obtained from different viewing angles, showing stiff cilia with incomplete bending. The recordings are presented both at normal speed and in slow motion to facilitate detailed assessment of ciliary coordination, and waveform. Multiple viewing angles provide complementary visualization of ciliary motility and allow a more comprehensive evaluation of the ciliary beat pattern.

Supplementary information



Unprocessed agarose gel electrophoresis scan demonstrating the insertion in patient P88453 within the *SPAG1* gene sequence. Lane 1 corresponds to the 10-kb DNA ladder, while lanes 2–5 show the PCR products from patient P88453, the maternal sample, the negative control, and the no-template control, respectively.