The American Journal of Human Genetics, Volume *86*

Supplemental Data

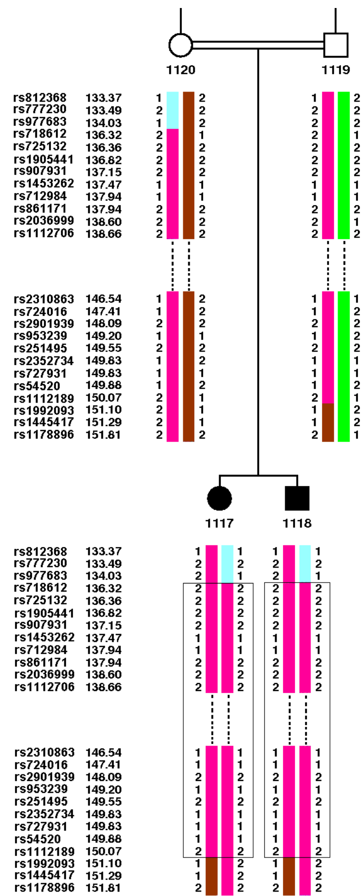
Cranioectodermal Dysplasia, Sensenbrenner Syndrome, Is a Ciliopathy Caused by Mutations in the *IFT122* Gene

Joanna Walczak-Sztulpa, Jonathan Eggenschwiler, Daniel Osborn, Desmond A. Brown, Francesco Emma, Claus Klingenberg, Raoul C. Hennekam, Giuliano Torre, Masoud Garshasbi, Andreas Tzschach, Malgorzata Szczepanska, Marian Krawczynski, Jacek Zachwieja, Danuta Zwolinska, Philip L. Beales, Hans-Hilger Ropers, Anna Latos-Bielenska, and Andreas W. Kuss

Figure S1. Linkage results for family CED-01

(A) Haplotype of the only linkage interval with significant parametric LOD score on Chr3. Markers from both borders of the interval are shown. The most inclusive region of homozygosity is located between rs977683 and rs1992093. **(B)** Genehunter multipoint parametric LOD score plot for the linkage interval on Chr3.

A)



B)

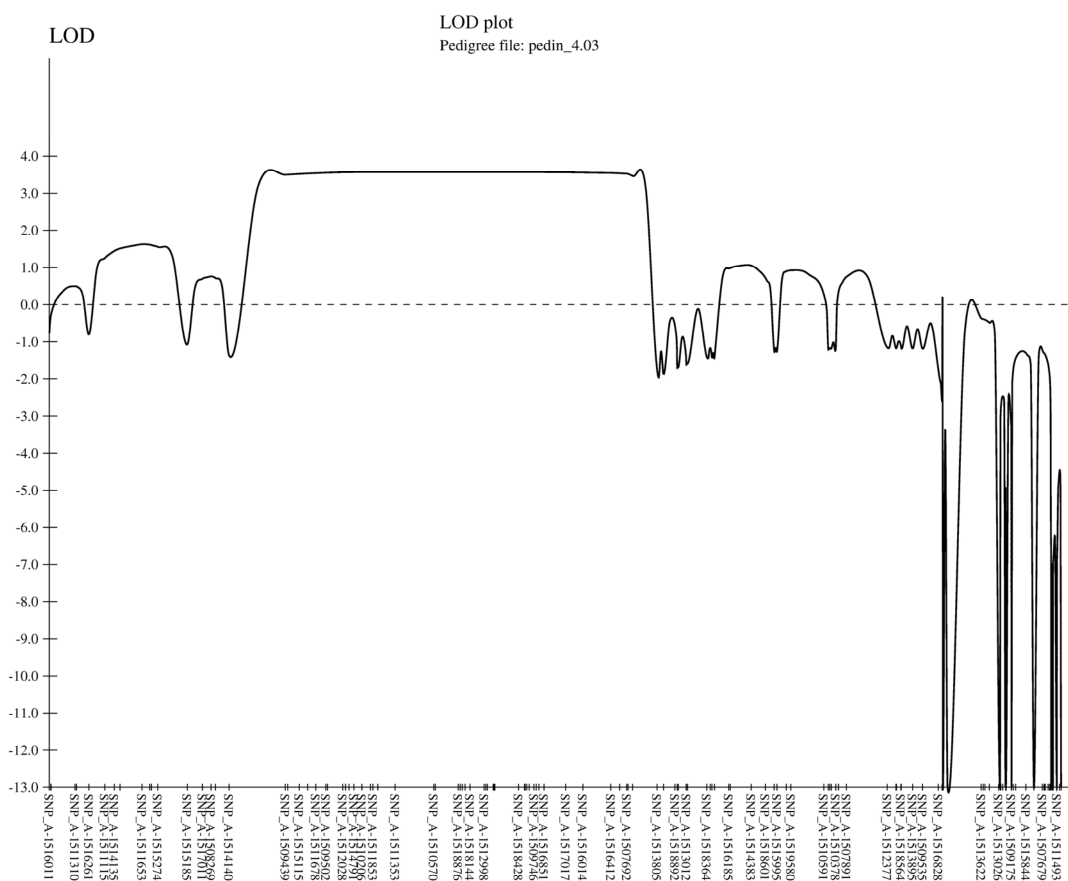
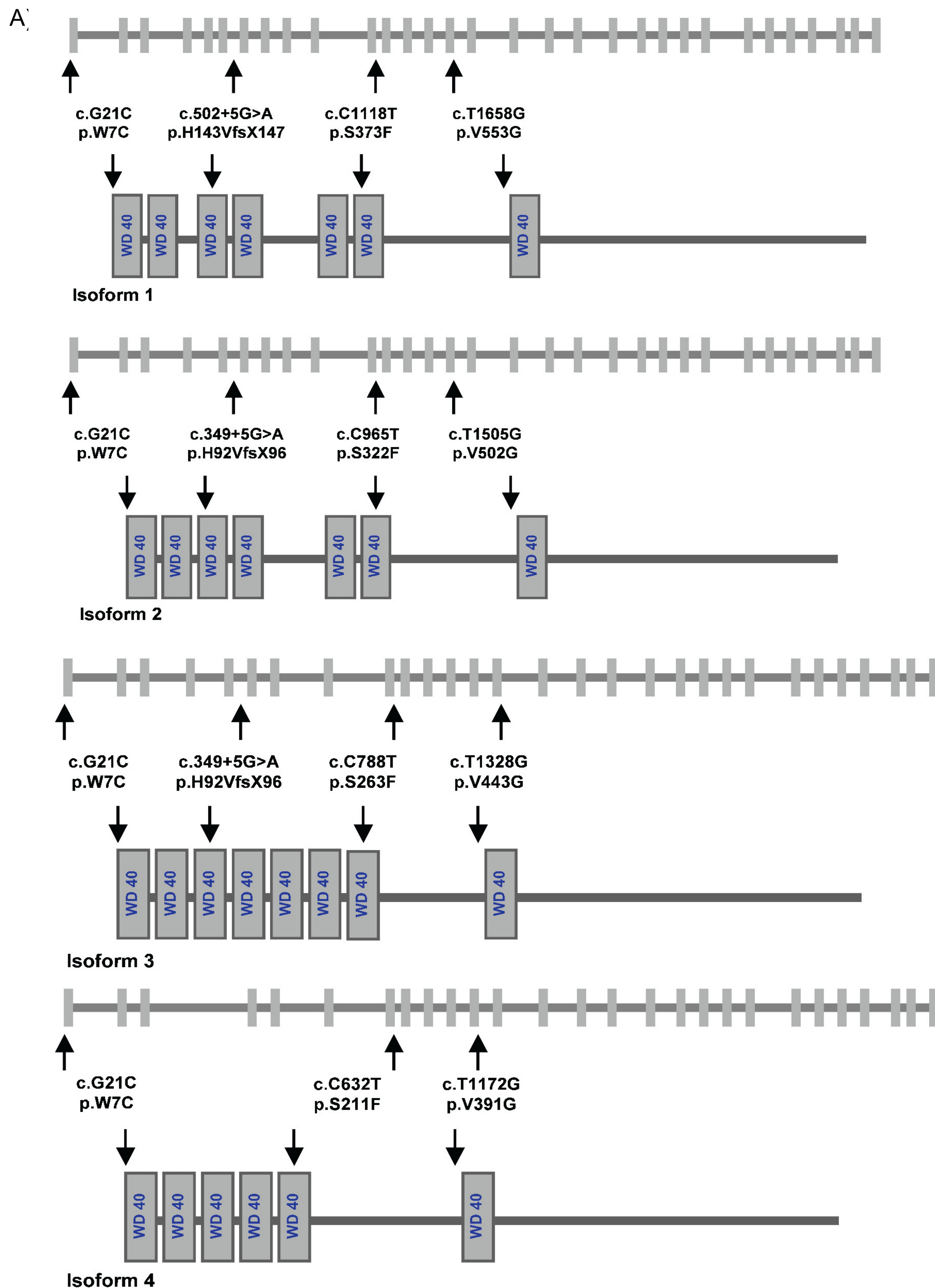


Figure S2. (A) Localization of *IFT122* mutations

Schematic representation of IFT122 isoforms, with light grey blocks representing the exons (upper panel). The lower panel shows a schematic of the gene product. Arrows indicate the positions of the mutations. **(B) Localization of primers for RT-PCR in *IFT122* transcripts.** Numbered boxes represent exons; 1-41bp, 2-67bp, 3-85bp, 4-79bp, 5-153bp, 6-77bp, 7-67bp, 8-147-bp, 9-177bp, 10-76bp, 11-192bp.



B)

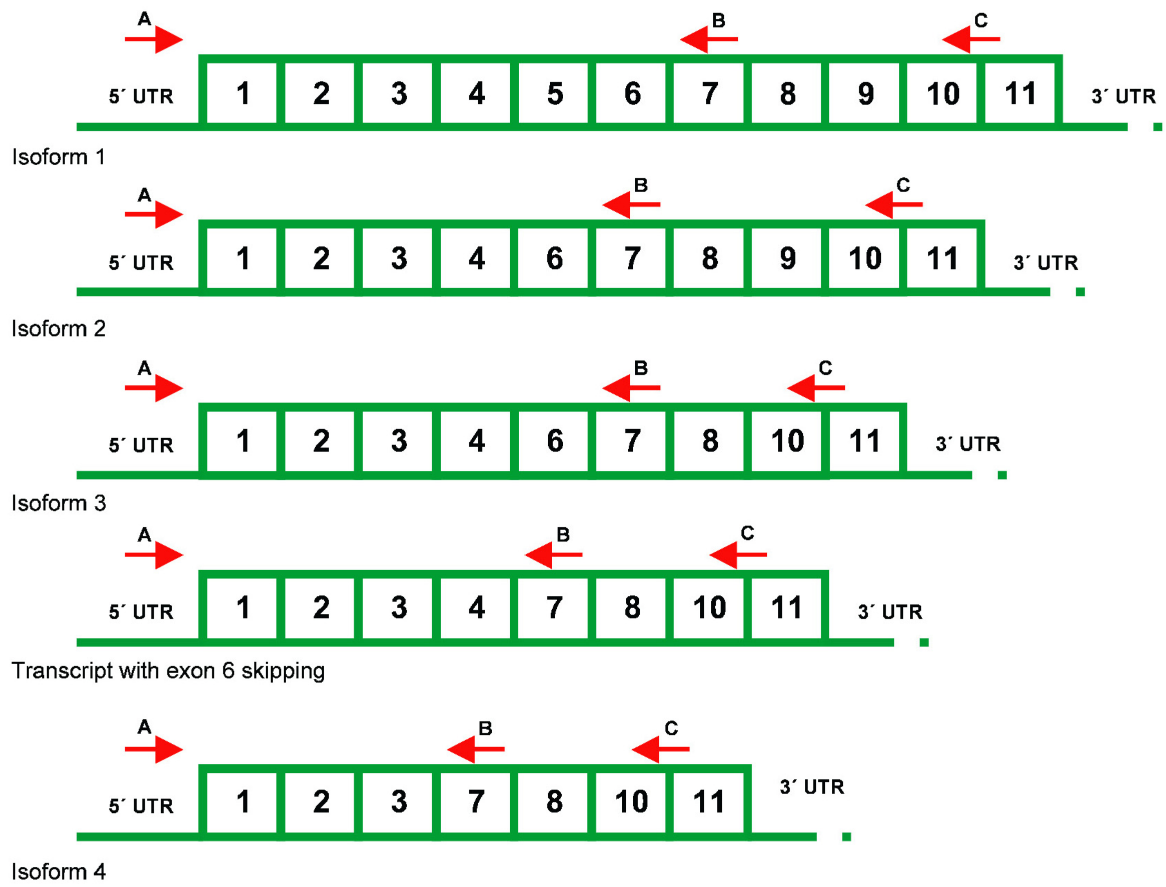


Figure S3. RT-PCR results

Agarose gelelectrophoresis profiles of RT-PCR reactions with different primer combinations (A+B and A+C; see Figure S2 for relative locations) are shown. NUA specific primers were used to control for cDNA synthesis. A Hyper Ladder IV (Bioline) was used as size standard (M).

The corresponding transcripts for each band are indicated below.

Amplicons with primers A+B:

Isoform 1 (predicted product size 668bp): no band/not expressed

1 = isoform 2 and 3 (product size 515bp),

2 = new transcript with exon 6 skipping (product size 438bp),

3 = isoform 4 (product size 359bp).

Amplicons with primers A+C:

Isoform 1 (predicted product size 1084bp): no band/not expressed

Isoform 2 (predicted product size 931bp): no band/not expressed

1 = isoform 3 (product size 754bp),

2 = new transcript with exon 6 skipping (product size 677bp),

3 = isoform 4 (product size 598bp).

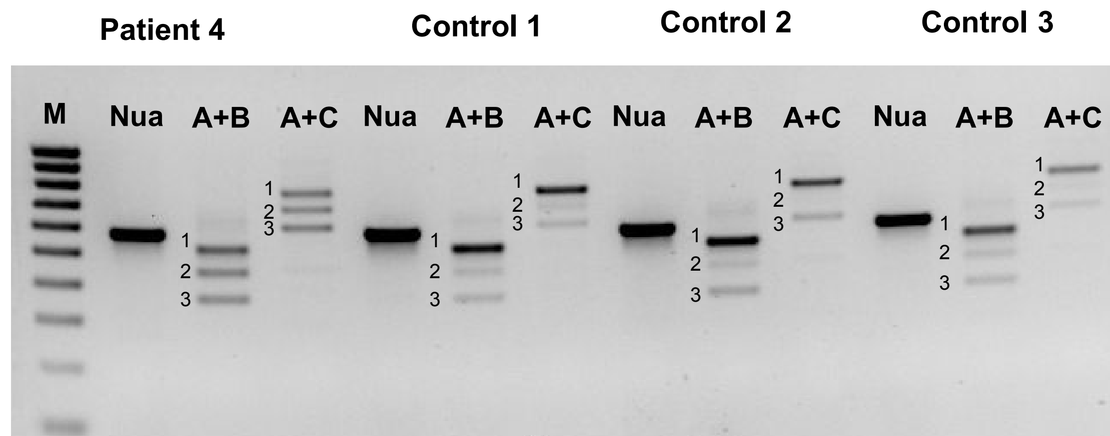


Figure S4. Specific RT-PCR shows that IFT122 splice site morpholino causes loss of Ift122 RNA

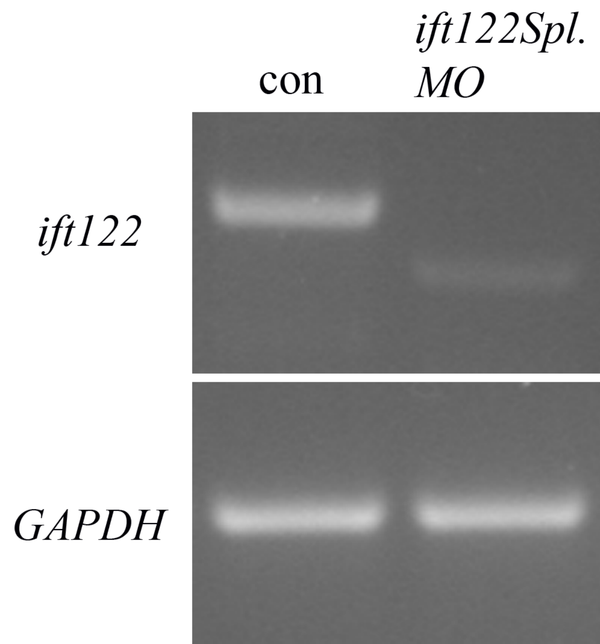


Table S1. Candidate genes selected for sequencing of protein coding regions

1.	*A4GNT*	**21.**	*DZIP1L*	**41.**	*MSL2L1*	**61.**	*RPN1*
2.	*ABTB1*	**22.**	*EEFSEC*	**42.**	*NCK1*	**62.**	*RUVBL1*
3.	*ACPL2*	**23.**	*EPHB1*	**43.**	*NMNAT3*	**63.**	*RYK*
4.	*ACPP*	**24.**	*FAIM*	**44.**	*NPHP3*	**64.**	*SEC61A1*
5.	*AMOTL2*	**25.**	*FAM62C*	**45.**	*NUDT16*	**65.**	*SLC25A36*
6.	*ANAPC13*	**26.**	*FLJ35880*	**46.**	*PAQR9*	**66.**	*SLCO2A1*
7.	*ARMC8*	**27.**	*FOXL2*	**47.**	*PCCB*	**67.**	*SOX14*
8.	*ASTE*	**28.**	*GATA2*	**48.**	*PCOLCE2*	**68.**	*SRPRB*
9.	*ATP1B3*	**29.**	*GK5*	**49.**	*PIK3CB*	**69.**	*STAG1*
10.	*BFSP2*	**30.**	*GRK7*	**50.**	*PLS1*	**70.**	*TF*
11.	*C3orf36*	**31.**	*H1FX*	**51.**	*PODXL2*	**71.**	*TFDP2*
12.	*CDV3*	**32.**	*IFT122*	**52.**	*PPP2R3A*	**72.**	*TMEM108*
13.	*CEP63*	**33.**	*IL20RB*	**53.**	*RAB43*	**73.**	*TMEM22*
14.	*CEP70*	**34.**	*KLHDC6*	**54.**	*RAB6B*	**74.**	*TOPBP1*
15.	*CHST2*	**35.**	*KY*	**55.**	*RAB7A*	**75.**	*TRH*
16.	*CLDN18*	**36.**	*MBD4*	**56.**	*RASA2*	**76.**	*TRIM42*
17.	*CLSTN2*	**37.**	*MGLL*	**57.**	*RBP1*	**77.**	*TRPC1*
18.	*CNBP*	**38.**	*MRAS*	**58.**	*RBP2*	**78.**	*TXNDC6*
19.	*COPB2*	**39.**	*MRPL3*	**59.**	*RHO*	**79.**	*UBE1DC1*
20.	*DBR1*	**40.**	*MRPS22*	**60.**	*RNF7*		

Primer design

All primers were designed using the ExonPrimer and Primer3 softwares.

PCR

PCR reaction	[µl]
DNA (~100ng/µl)	1 µl
H_2O	9,5 µl
BIO-X-ACT (Bioline)	12,5 µl
Primer-F (1:10)	1 µl
Primer-R (1:10)	1 µl
Total	**25 µl**

The following PCR conditions were applied:

1. 96°C – 3 min
2. 94°C – 30 sec
3. 65°C – 30 sec ⇒ 55°C
   (-0.5°C per cycle)
4. 72°C – 1 min

5. go to step 2 – **20x**
6. 94°C – 30 sec
7. 55°C – 30 sec
8. 72°C – 1min
9. go to step 6 - **30x**
10. 72°C - 10 min

Table S2. Primers for PCR-amplification of *IFT122*

IFT122	Forward primer	Revers primer
Exon 1	CACCAATCAAATCCATTCTCG	CCCCTCAAACACGCTGC
Exon 2	CATTAGCAGACTTCATCATTTTGG	AAATAGCCCATTTCCATCCC
Exon 3	AAAATAGCAGTAGCAACCCTGC	GAACTTGCAACTTGTACTCCAGC
Exon 4	TTCTCTCTGGGAAATGCTGG	TTTCTAAGAGGCTTTTGCTGC
Exon 5-6	ACTAAAACGGGTTGAGACGC	CCCTCAGCCATTGAAGGTAG
Exon 7	CTGGAGATCCATCTAAGTTGTTG	CACAAGACCTTCTCTGCTGC
Exon 8	ACCTTCTGTGGGGTGCTTC	TTTGAATGTAGGGAGACCTCAG
Exon 9	CTCCTGATCTCGAGCGATTC	TCTGTCCTGGCAAGTCTGTG
Exon 10	GCCAGGACTTCCTTGTTCC	GCAAATGCTCAATAAGCAGG
Exon 11-12	TTGCAATGGTTATGGATTCG	TTTCAAGTGCCCTGTCACC
Exon 13	TTAATTTCTCTGCCTTGCACC	TGGGCACCCTATCACTGG
Exon 14	GAAAGATCTCCTTGGGGAGG	CTAGACGGGTCACCCACG
Exon 15	AGAGCACATGGGATTCCAAC	AGGGACTGGGAAGCTAGAGG
Exon 16	AAGCCCAGGGTGGTTCTC	CTGGTCTTGTCAGCCTTGC
Exon 17	TTCCATGGCTCTGAAAACAG	CTGCAGTCTATGGCCTCTCC
Exon 18	CTTGCTCCCTTCCTCTCCTC	CTGCCCCTAAAACAGGAACC
Exon 19	CCTTTGTAAAGGCTGCTTCC	GACACCGCACGTGAGAATAC
Exon 20	TGCTGCTAGAAAAGCCTGATG	TGCCTTGTGCAATCTCTGTG
Exon 21	AATGAATGGACATACGGCTTG	CCTGTGTCCCAGGGCTC
Exon 22	GCCAAGTACAGTGTTTTCATGG	TGTCTTGGCAGTAACTTCTCC
Exon 23	CTGCCTCCTGTGTGACTTCC	ATCTTGGTGTCAGAGTGGGG
Exon 24	AGTTGGCAGCCACAGACAC	CTTTAGATATGCCCGGGACC
Exon 25	TTATTGGTGTCTGCCTTTTGG	TCTTGGGGCCATTAGACAAG
Exon 26	ACTCCAAGGACAGGCAGTG	GCAAGAAGGCATGAGATGG
Exon 27	ACCCAAGTGACAGGGCTTG	CTCTCTTTCTGGGGCATTTG
Exon 28	AAGCAACTCTGTGGTCACCC	CCCAGACCCCTCACACAG
Exon 29	TGGAGGCTAGGGTCTGTCTC	GTGCACAGGAGAAGGGATG
Exon 30	ACAGATGTCTCACTGTGGCTG	AGAGGGACATTTGGGCAAG
Exon 31	CAGGCGTAGGGCTGATG	ATTCCGTGGTTACACAAGGC

RT-PCR

RNA extraction and cDNA synthesis

Total RNA was extracted from the blood of patient (CED-03, case 4) and three controls using the PAXgene blood RNA kit (Qiagen). We generated cDNA using the SuperScript III Reverse Transcriptase (Invitrogen) together with random hexamers (Promega). This cDNA was used to perform RT- PCRs and qPCRs.

RT-PCR reaction	[µl]
cDNA	1 µl
H_2O	9,5 µl
BIO-X-ACT (Bioline)	12,5 µl
Primer-F (1:10)	1 µl
Primer-R (1:10)	1 µl
Total	**25 µl**

The following RT-PCR programme was applied:

1. 94°C – 1 min
2. 94°C – 30 sec
3. 55°C – 45 sec
4. 72°C – 45 sec
5. go to step 2 – **35x**
6. 15°C – ∞

Table S3. Primers for RT-PCR

	Primer name	Sequence
A.	IFT122_RT-5´UTR-1-f	GGTAACGCAGGTAGCCAAAG
B.	IFT122_RT-ex7-r	GATCTTGCTGCTTGATTTGTG
C.	IFT122_RT-ex10_11-r	CCAATCTGTTTTCCACTCAGC
D.	IFT122_RT-ex6-r	GCCAGTTGATGAGTAATAGGATTG
E.	IFT122_RT-ex3_7-r	GAGACCACAACCCATCCTTC
F.	IFT122_RT-ex4_7-r	CACAACCGTGTACTTCAGAATG

Combination of primers:

A+D were specific to amplify and sequence new transcript (with exon 6 skipping)
A+E were specific to amplify and sequence isoform 4
A+F were specific to amplify and sequence isoform 3

Quantitative PCR (qPCR)

To quantify the RT-PCR results we performed real-time PCR with primer combinations that were specific for isoform 3, isoform 4 and the transcript N (with exon 6 skipping) of IFT122. The experiments were carried out on an Applied Biosystems 7900 instrument, in 96-Well Optical Reaction Plates (Applied Biosystems, Foster City, CA, United States).
The following programme was applied:

stage 1: 50°C for 2min,
stage 2: 95°C for 10min,
stage 3: 95°C for 15s and 55°C for 1min, for 40 cycles and,
stage 4: 95°C for 15s, 60°C for 15s and 95°C for 15s.

Additional dissociation curves of the products were created. The final reaction volume of 30µl consisted of 15µl of SYBR®Green PCR Master Mix (Applied Biosystems), 2.5µM of each primer (5µl), and 10ul of cDNA (1:10 dilution). Each amplicon was analysed in triplicate. Expression of the *GAPDH* gene was used for normalisation.

Table S4. Primers for qPCR

	IFT122_Isoforms	**Primer name**	**Sequence**	**Product size**
1	**isoform3**	IFT122_ isoform3_ex6_7_f	TCCAGTGACTTTGGGTTGTG	
2		IFT122_ isoform3_ex8_10_r	GATGTCATTACGTTCCTCTCTTG	246bp
3	**ex6_skipping _transcript**	IFT122_ new_transcript_ex4_7_f	GCATTCTGAAGTACACGGTTG	
4		IFT122_ new_transcript_ex8_r	CAGCGCCAGGTACTGACC	114bp
5	**isoform4**	IFT122_ isoform4_ex2_3_f	GCAGATTACTGGTTTATGACACCTC	
6		IFT122_ isoform4_ex3_7_r	GAGACCACAACCCATCCTTC	108bp