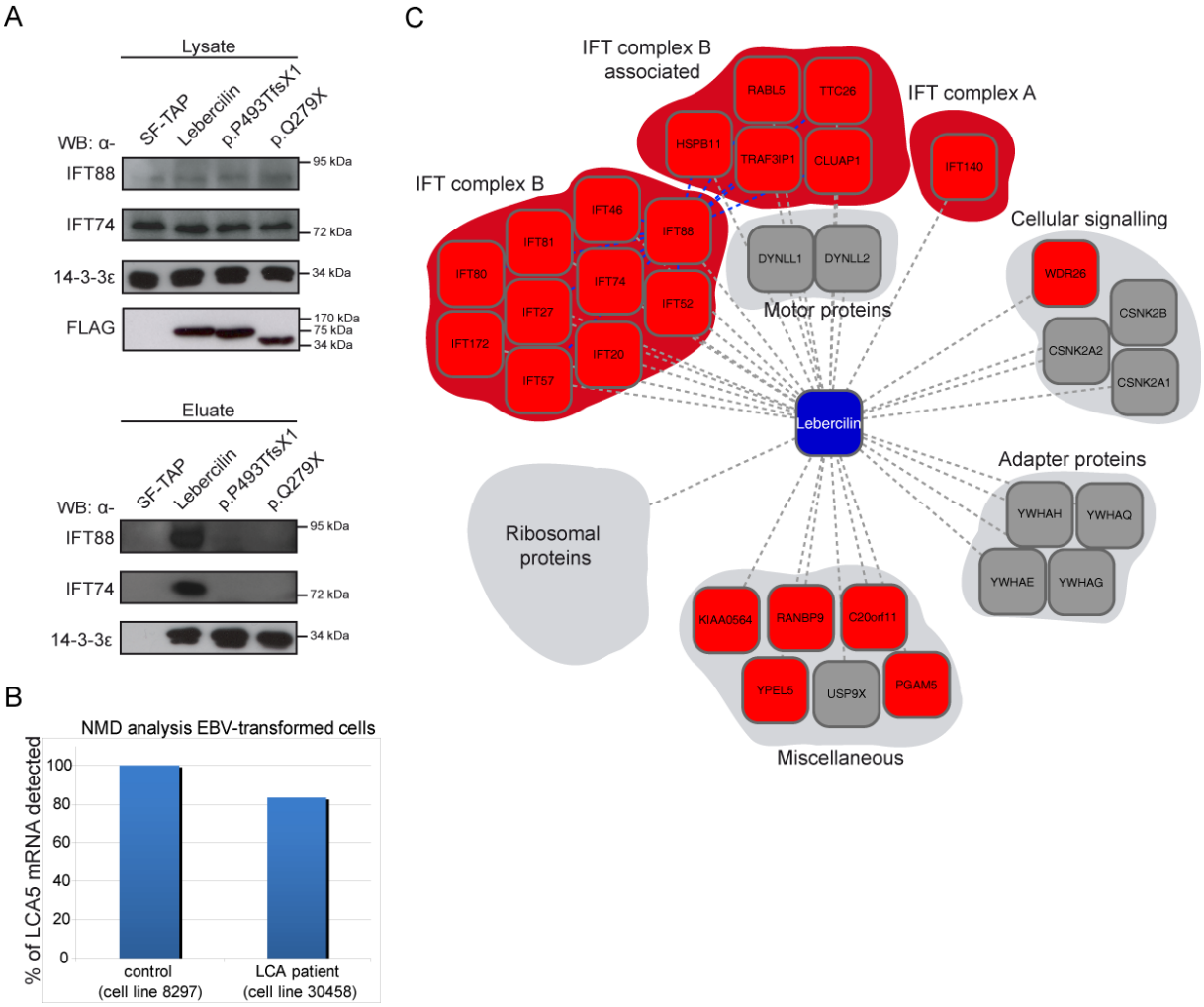
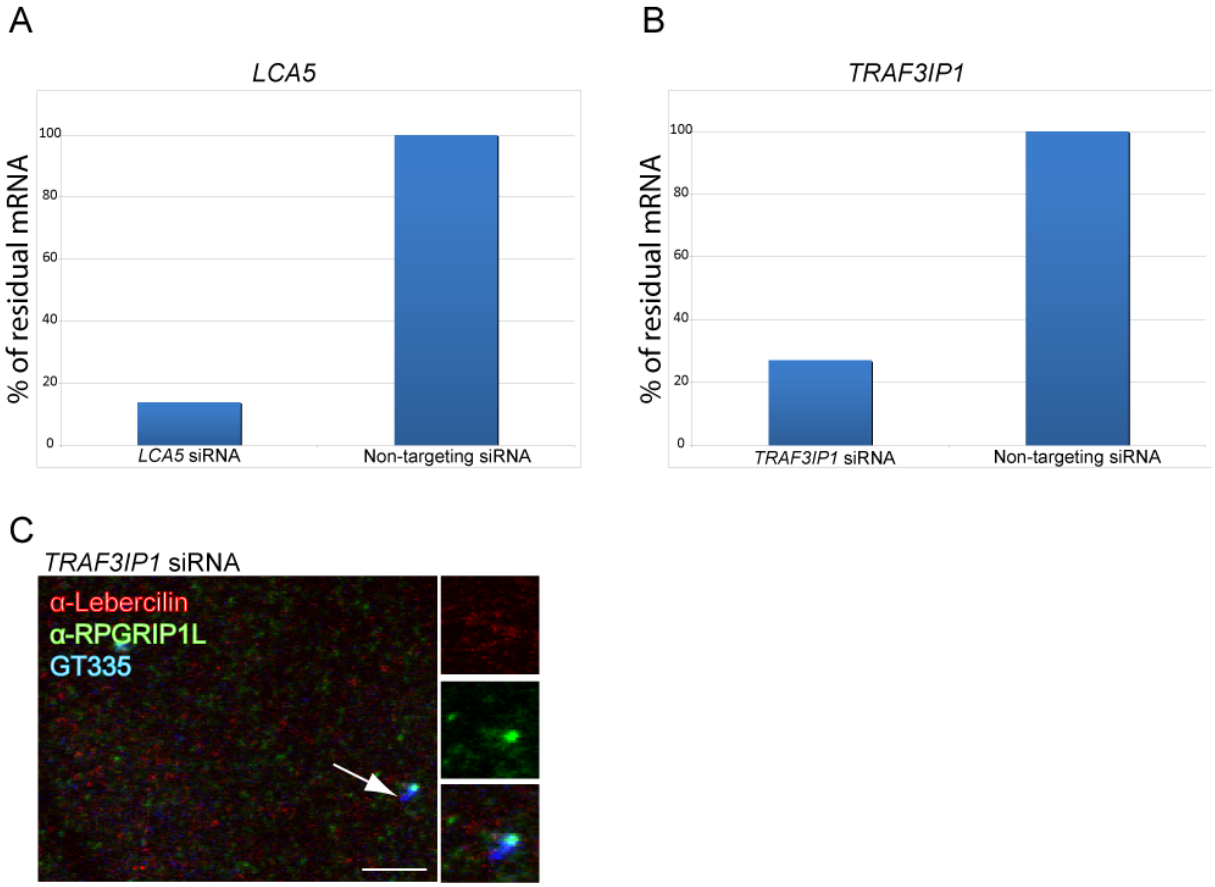
Supplementary Figure 1



Loss of IFT association to the Lebercilin protein complex.

(**A**) Loss of interaction of IFT proteins to both Lebercilin variants (p.P493TfsX1, p.Q279X) was confirmed by western blot. Both IFT74 and IFT88 could easily be detected in the Lebercilin Strep eluates but not in Strep eluates of p.P493TfsX1 and p.Q279X. The presence of the 14-3-3ε protein was not altered in these eluates, supporting mass spectrometric measurements (Supplementary Table 2). The expression levels of IFT88, IFT74 and 14-3-3ε were not altered due to the expression of Lebercilin and its mutants. Lebercilin and both mutant were expressed in HEK293T cells and migrate at the expected molecular weight. (**B**) *LCA5* mRNA expression is not significantly decreased in EBV-transformed lymphocytes derived from an LCA patient, harbouring a homozygous p.P493TfsX1 mutation in *LCA5* (cell line 30458; family 27240 (4)) compared to a control EBV-transformed lymphocytes (cell line 8297), as determined by qPCR. (**C**) Data obtained by quantitative protein complex analysis and comparison are represented. All proteins identified to be part of the Lebercilin protein complex are shown. Proteins, showing loss of binding to both Lebercilin-p.P493TfsX1 and p.Q279X are shown in red. Blue lines show the interconnectivity as determined by the IFT88-SF-TAP analysis (Supplementary Table 3).

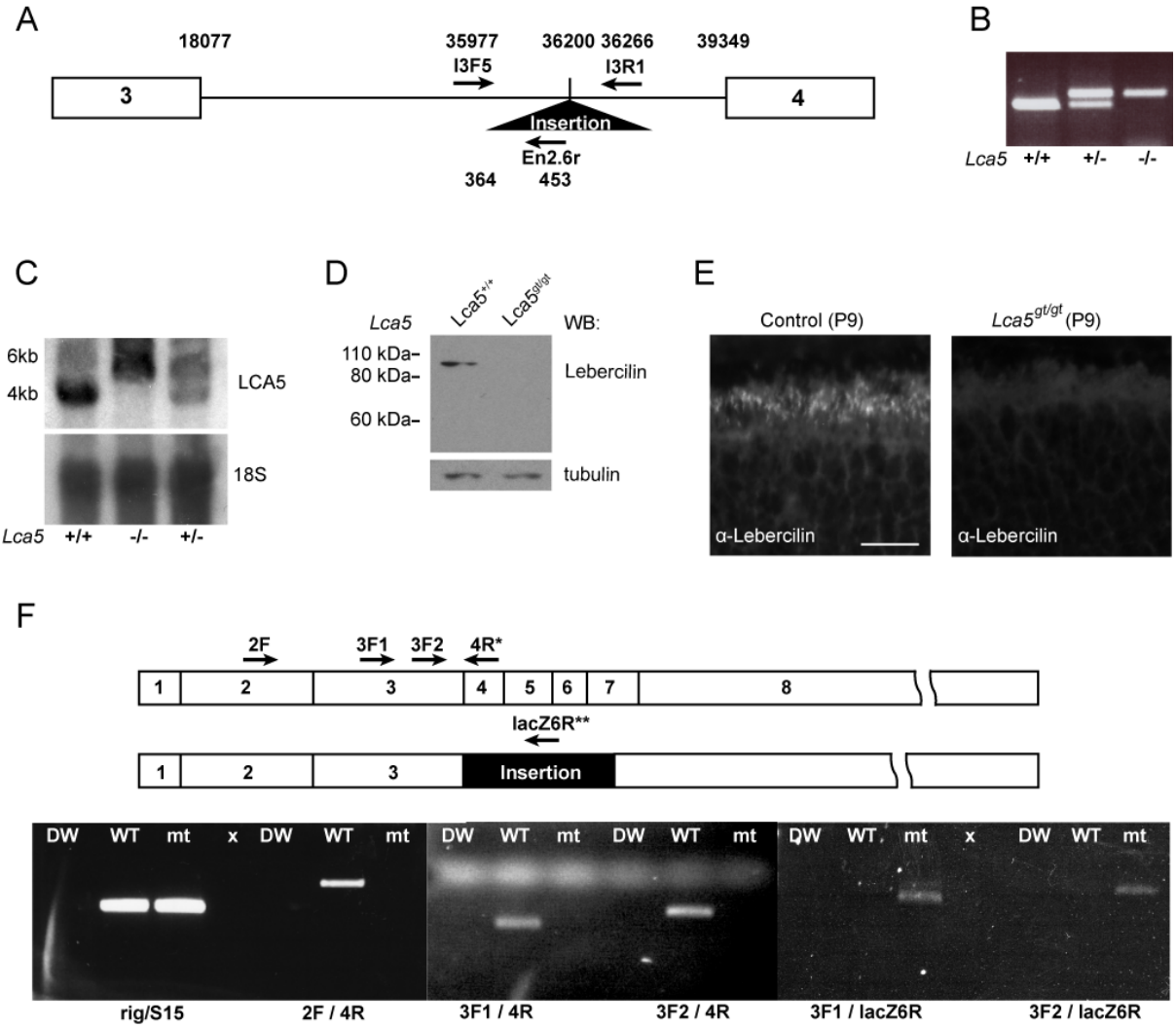
Supplementary Figure 2



Analysis of *LCA5* and *TRAF3IP1* knockdown.

(**A, B**) Knockdown efficiency resulting from a 72 hours transfection of *LCA5* siRNA (15nM) (**A**) and *TRAF3IP1* siRNA (15nM) (**B**) respectively in hTERT-RPE1 cells, measured via qPCR. (**C**) siRNA-mediated knockdown of TRAF3IP1 affects ciliogenesis and subsequent Lebercilin localization (white arrow). Anti-RPGRIP1L and GT335 stain basal body and primary cilium, respectively. Bar= 5 µm.

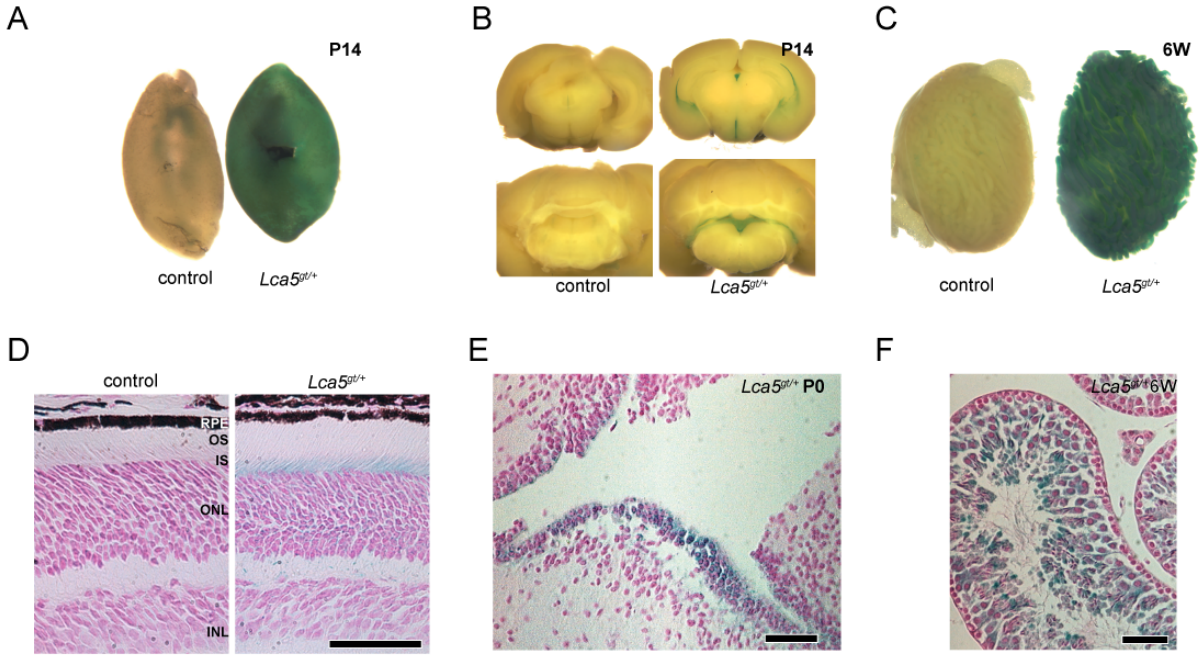
Supplementary Figure 3



Generating *Lca5* gene trap mice.

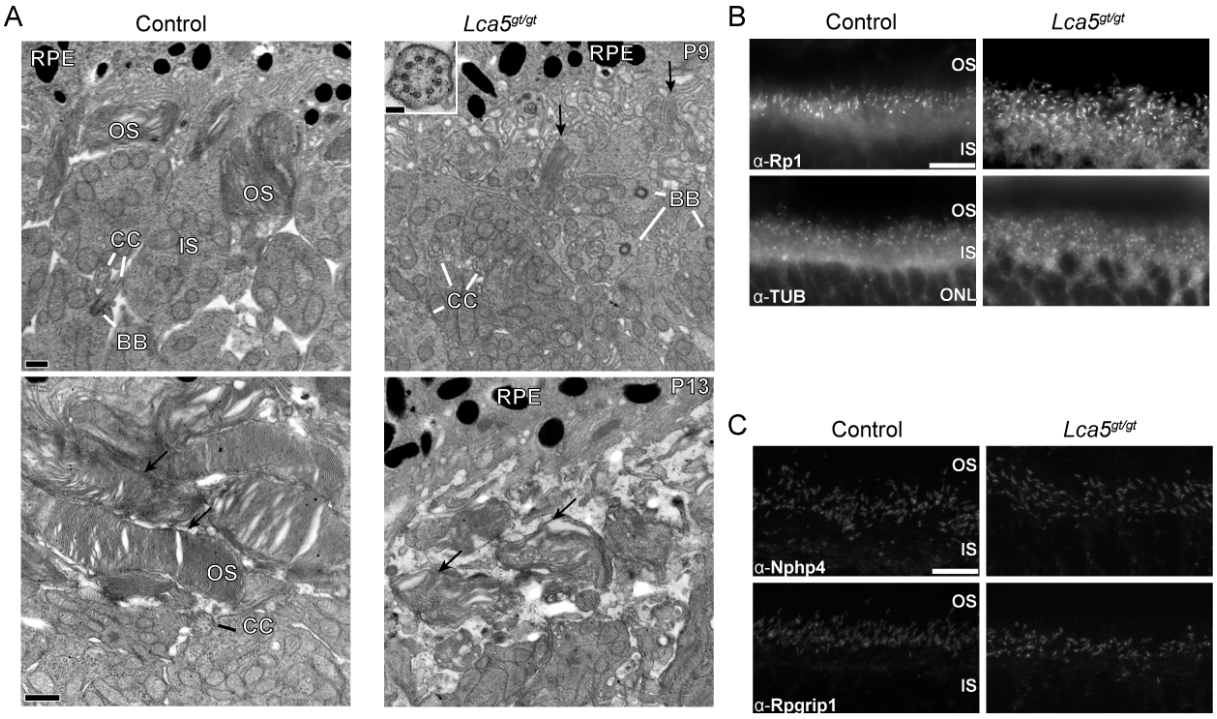
(**A**) Genomic diagram of *Lca5* extending from exon 3 to 4. The gene trap is inserted into intron 3 (triangle) of *Lca5*. Numbers above the diagram indicate the genomic coordinates. Numbers below correspond to the gene trap backbone (pGT01Xr). Genotyping primers are indicated by arrows. (**B**) Genomic PCRs from tail DNA of $Lca5^{+/+}$, $Lca5^{+/gt}$ and $Lca5^{gt/gt}$ mice with indicated primer sets, generate a 290 bp or 402 bp PCR product for wildtype and gene trap alleles respectively. (**C**) Northern blot analysis of total RNA from $Lca5^{+/+}$, $Lca5^{gt/+}$ and $Lca5^{gt/gt}$ retina, probed with a N-terminal 860 bp *Lca5* fragment. A transcript of ~4 kb was detected in wildtype mice, whereas a larger transcript was detected in $Lca5^{gt}$ mice. 18S RNA probe serves as loading control. (**D**) Western blot of retinal lysates of $Lca5^{+/+}$ and $Lca5^{gt/gt}$ mice (P12), stained with anti-Lebercilin 1702#2. Lebercilin is expressed in $Lca5^{+/+}$ retina, but absent in $Lca5^{gt/gt}$ retina. γ-tubulin serves as loading control. (**E**) Retina of $Lca5^{gt/gt}$ mice, stained with anti-Lebercilin (4), show loss of Lebercilin expression in photoreceptors. Bar= 20 µm. (**F**) Top: Diagram of *Lca5* cDNA. The splice acceptor site in the gene trap (black box) induces missplicing of *Lca5*. RT-PCR primers are indicated with arrows and exons are numbered. Primers to detect either wildtype *Lca5* (4R) or *Lca5* gene trap (lacZ6R) are indicated with single and double asterisks respectively. Bottom: RT-PCR results of $Lca5^{+/+}$and $Lca5^{gt/gt}$ mice. Primer set *rig/S15* serves as loading control. DW: distilled water. Mt: mutant.

Supplementary Figure 4



***Lca5* expression analysis in tissues using the β-galactosidase reporter in the gene trap.**
(**A, D**) In the retina, the entire photoreceptor layer and a subset of ganglion cells were *LacZ* positive at P14. (**B, E**) In the brain, reporter gene activity was observed in cells lining the third and the lateral ventricles at P0 and P14. (**C, F**) Strong reporter activity was detected in the testis as well at 6 weeks, with both Sertoli cells and spermatogonia stained positively for *LacZ* expression. RPE: retinal pigment epithelium, OS: outer segment, IS: inner segment, ONL: outer nuclear layer. OPL: outer plexiform layer, INL: inner nuclear layer, IPL: inner plexiform layer, GC: ganglion cell layer. Bar= 50 μm.

Supplementary Figure 5



Clinical and functional assessment of *Lca5*$^{gt/gt}$ mice.

(**A**) OS development is defective in *Lca5*$^{gt/gt}$ retinas. Transmission EM of control and *Lca5*$^{gt/gt}$ retina at P9 and P13. The initiation of OS in *Lca5*$^{gt/gt}$ mice is delayed and OS are shorter than in wildtype controls. OS of *Lca5*$^{gt/gt}$ mice are disorganized and discs appear to be oriented improperly (arrows) when compared to control OS. The structure of the CC in *Lca5*$^{gt/gt}$ mice appears to be normal. Bars= 500 nm, and 100 nm. (**B**) In *Lca5*$^{gt/gt}$retina, the localization of Rp1, an axoneme marker, is comparable to controls. The number of stained basal bodies (anti-γ-tubulin, TUB) in mutant *Lca5* retina is similar to controls but dispersed over the IS. Bar= 10 µm. (**C**) The localization of ciliary disease gene products, Nphp4 and Rpgrip1 is not altered in *Lca5*$^{gt/gt}$ retina. Retinal sections were obtained at P14. OS: outer segment, IS: inner segment, ONL: outer nuclear layer, CC: connecting cilium, BB: basal body. Bar= 10 µm.

Supplementary Figure 6

A

p.P493TfsX1

001 MGERAGSPGT DQERKAGKHH YSYLSDFETP QSSGRSSLVS SSPASVRRKN
050 PKRQTSDGQV HHQAPRKPSP KGLPNRKGVR VGFRSQSLNR EPLRKDTDLV
100 TKRILSARLL KINELQNEVS ELQVKLAELL KENKSLKRLQ YRQEKALNKF
150 EDAENEISQL IFRHNNEITA LKERLRKSQE KERATEKRVK DTESELFRTK
200 FSLQKLKEIS EARHLPERDD LAKKLVSAEL KLDDTERRIK ELSKNLELST
250 NSFQRQLLAE RKRAYEAHDE NKVLQKEVQR LYHKLKEKER ELDIKNIYSN
300 RLPKSSPNKE KELALRKNAA CQSDFADLCT KGVQTMEDFK PEEYPLTPET
350 IMCYENKWEE PGHLTLDLQS QKQDRHGEAG ILNPIMEREE KFVTDEELHV
400 VKQEVEKLED EWEREELDKK QKEKASLLER EEKPEWETGR YQLGMYPIQN
450 MDKLQGEEEE RLKREMLLAK LNEIDRELQD SRNLKYPVLP LLT

58.2% sequence coverage

B

p.Q279X

001 MGERAGSPGT DQERKAGKHH YSYLSDFETP QSSGRSSLVS SSPASVRRKN
050 PKRQTSDGQV HHQAPRKPSP KGLPNRKGVR VGFRSQSLNR EPLRKDTDLV
100 TKRILSARLL KINELQNEVS ELQVKLAELL KENKSLKRLQ YRQEKALNKF
150 EDAENEISQL IFRHNNEITA LKERLRKSQE KERATEKRVK DTESELFRTK
200 FSLQKLKEIS EARHLPERDD LAKKLVSAEL KLDDTERRIK ELSKNLELST
250 NSFQRQLLAE RKRAYEAHDE NKVLQKEVQ

45.5% sequence coverage

Peptide identifications for Lebercilin mutants.
The peptides identified for the p.P493TfsX1 (**A**) and p.Q279X (**B**) Lebercilin mutants are highlighted in green. The peptides were identified from heavy labelled samples with more than 95% identification probability as determined by Mascot search against a human subset of the Swiss Prot database and Scaffold analysis. For the p.P493TfsX1 mutant 58.2% sequence coverage (**A**) and for the p.Q279X mutant 45.5% sequence coverage (**B**) could be observed over virtually the complete theoretical sequence.

Supplementary table 1

			WT/SF-TAP		p.P493TfsX1/WT		p.Q279X/WT	
Gene name	Protein name	Uniprot	Enrichment ratio	Significance (A)	Binding ratio	Significance (A)	Binding ratio	Significance (A)
Lebercilin								
LCA5	Lebercilin	Q86VQ0	11.92	8.947E-12	NA		NA	
Motor proteins								
DYNLL1	Dynein light chain 1, cytoplasmic	P63167	12.87	1.938E-12	0.77	1.228E-01	0.04	1.279E-17
DYNLL2	Dynein light chain 2, cytoplasmic	Q96FJ2	9.64	4.646E-10	0.98	4.218E-01	0.03	1.986E-18
Intraflagellar transport complex B								
IFT20	Intraflagellar transport protein 20 homolog	Q8IY31	8.19	7.643E-09	0.09	2.463E-22	0.04	1.540E-17
IFT27	Intraflagellar transport protein 27 homolog	Q9BW83	3.78	2.708E-04	0.09	6.077E-23	0.07	2.270E-12
IFT46	Intraflagellar transport protein 46 homolog	A8K0F6	6.68	1.891E-07	0.05	7.290E-32	0.04	8.010E-17
IFT52	Intraflagellar transport protein 52 homolog	Q9Y366	7.28	5.112E-08	0.11	1.415E-18	0.04	3.930E-17
IFT57	Intraflagellar transport protein 57 homolog	Q9NWB7	6.56	2.521E-07	0.18	3.288E-12	0.06	5.160E-14
IFT74	Intraflagellar transport protein 74 homolog	Q96LB3	6.38	3.740E-07	0.07	2.208E-25	0.06	6.580E-14
IFT80	Intraflagellar transport protein 80 homolog	Q9P2H3	3.68	3.630E-04	0.09	3.283E-22	0.06	6.820E-13
IFT81	Intraflagellar transport protein 81 homolog	Q8WYA0	5.39	3.924E-06	0.08	1.063E-24	0.05	1.300E-14
IFT88	Intraflagellar transport protein 88 homolog	Q13099	5.10	8.070E-06	0.07	2.302E-26	0.03	3.020E-18
IFT172	Intraflagellar transport protein 172 homolog	Q9UG01	8.64	3.153E-09	0.10	6.741E-21	0.06	3.720E-13
Intraflagellar transport complex B-associated								
CLUAP1	Clusterin-associated protein 1	Q96AJ1	8.38	5.298E-09	0.14	4.309E-15	0.05	1.200E-15
HSPB11	Heat shock protein beta-11	Q9Y547	3.57	4.874E-04	0.12	5.452E-18	0.07	7.680E-12
RABL5	Rab-like protein 5	Q9H7X7	5.00	1.052E-05	0.05	8.277E-34	0.05	1.930E-15
TRAF3IP1	Intraflagellar transport protein 54 homolog	A8MTK4	5.66	2.055E-06	0.07	8.327E-28	0.04	2.430E-16
TTC26	Tetratricopeptide repeat protein 26	A0AVF1	4.46	4.245E-05	0.10	4.634E-21	0.06	1.540E-13
Intraflagellar transport complex A								
IFT140	Intraflagellar transport protein 140 homolog	Q96RY7	3.61	4.387E-04	0.09	1.844E-22	0.06	3.050E-13
Cellular Signalling								
CSNK2A1	Casein kinase 2, alpha 1	B2R6D7	5.97	9.782E-07	0.69	5.754E-02	0.11	3.380E-09
CSNK2A2	Casein kinase 2, alpha 2	P19784	5.80	1.462E-06	0.73	8.186E-02	0.08	2.810E-11
CSNK2B	Casein kinase 2, beta	Q5SRQ6	6.13	6.684E-07	0.50	2.056E-03	0.12	3.570E-08
WDR26	WD repeat-containing protein 26	Q9H7D7	4.48	4.064E-05	0.15	5.276E-15	0.07	1.130E-12
Adapter proteins								
YWHAE	14-3-3 protein epsilon	P62258	3.36	8.818E-04	1.08	4.438E-01	0.97	4.480E-01
YWHAG	14-3-3 protein gamma	P61981	4.89	1.377E-05	1.19	3.193E-01	0.90	3.568E-01
YWHAH	14-3-3 protein eta	Q04917	4.54	3.469E-05	1.21	3.022E-01	0.94	4.049E-01
YWHAQ	14-3-3 protein theta	P27348	4.17	9.234E-05	1.06	4.649E-01	1.03	4.563E-01
Miscellaneous								
KIAA0564	KIAA0564	A3KMH1	6.26	4.998E-07	0.09	1.238E-21	0.09	4.230E-10
PGAM5	Phosphoglycerate mutase family member 5	Q96HS1	6.68	1.894E-07	0.15	7.942E-15	0.05	1.190E-15
C20orf11	Protein C20orf11	Q9NWU2	5.65	2.098E-06	0.07	1.151E-25	0.05	1.010E-14
RANBP9	Ran-binding protein 9	Q96S59	6.92	1.115E-07	0.13	5.373E-17	0.04	4.570E-16
YPEL5	Protein yippee-like 5	P62699	6.37	3.878E-07	0.14	1.066E-15	0.09	1.640E-10
USP9X	Probable ubiquitin carboxyl-terminal hydrolase FAF-X	Q93008	4.57	3.218E-05	1.20	3.093E-01	0.30	7.257E-04
Ribosomal proteins								
MRPS33	28S ribosomal protein S33	Q9Y291	3.42	7.456E-04	1.58	7.675E-02	0.63	9.037E-02
RPLP0	60S acidic ribosomal protein P0	P05388	3.86	2.155E-04	1.86	2.346E-02	0.76	1.980E-01
RPLP1	60S acidic ribosomal protein P1	P05386	3.36	9.007E-04	1.51	9.873E-02	1.00	4.369E-01
RPL10A	60S ribosomal protein L10a	P62906	5.11	7.880E-06	1.57	7.722E-02	1.30	2.613E-01
RPL12	60S ribosomal protein L12	P30050	4.75	1.992E-05	1.32	2.017E-01	1.22	3.331E-01
RPL13	60S ribosomal protein L13	P26373	5.36	4.304E-06	2.49	1.463E-03	0.86	2.977E-01
RPL13A	60S ribosomal protein L13a	P40429	5.06	9.098E-06	1.90	1.991E-02	1.21	3.422E-01
RPL14	60S ribosomal protein L14	P50914	4.20	8.477E-05	1.95	1.561E-02	1.11	4.457E-01
RPL18	60S ribosomal protein L18	Q07020	5.57	2.526E-06	1.82	2.817E-02	1.20	3.545E-01
RPL21	60S ribosomal protein L21	P46778	5.97	9.799E-07	1.43	1.378E-01	1.31	2.534E-01
RPL27	60S ribosomal protein L27	P61353	4.53	3.496E-05	1.97	1.426E-02	1.30	2.620E-01
RPL3	60S ribosomal protein L3	P39023	4.19	8.817E-05	2.59	9.255E-04	0.87	3.014E-01
RPL30	60S ribosomal protein L30	A8MYX5	4.50	3.813E-05	1.81	2.930E-02	1.14	4.121E-01
RPL32	60S ribosomal protein L32	P62910	5.82	1.394E-06	2.28	3.794E-03	0.50	2.921E-02
RPL35A	60S ribosomal protein L35a	P18077	5.24	5.718E-06	1.73	4.118E-02	1.18	3.735E-01
RPL36	60S ribosomal protein L36	Q9Y3U8	4.65	2.613E-05	2.77	4.287E-04	1.62	9.255E-02
RPL4	60S ribosomal protein L4	P36578	5.88	1.194E-06	2.11	7.807E-03	0.93	3.673E-01
RPL6	60S ribosomal protein L6	B2R4K7	5.08	8.553E-06	1.83	2.716E-02	0.73	1.750E-01
RPL7A	60S ribosomal protein L7a	P62424	3.39	8.243E-04	1.85	2.410E-02	0.95	3.854E-01
RPL8	60S ribosomal protein L8	P62917	4.99	1.066E-05	2.07	9.341E-03	1.21	3.437E-01
RPL28	cDNA FLJ57954	B4DEP9	4.76	1.955E-05	1.58	7.533E-02	1.16	3.977E-01
RPL29	cDNA FLJ78093	A8K0H3	4.12	1.069E-04	1.78	3.241E-02	0.66	1.125E-01
RPL15	Ribosomal protein L15	B4DEN1	5.11	7.850E-06	2.20	5.243E-03	1.07	4.920E-01
RPL7P23	RPL7P23	A8MY04	5.24	5.798E-06	1.76	3.556E-02	1.21	3.444E-01

Quantitative Lebercilin protein complex analysis

All proteins detected as specific interactors of wildtype Lebercilin (WT/SF-TAP) are listed, including the enrichment ratio and statistical value (Significance (A)). Additionally, the results are shown for two LCA-causative Lebercilin variants (p.P493TfsX1 and p.Q279X). The ratio of binding for both mutants, compared to wildtype Lebercilin and statistical value for altered binding (Significance (A)) are shown. Proteins, which show loss of binding to both Lebercilin mutants are highlighted in red. Proteins, showing altered binding to one mutant only are highlighted in yellow.

Supplementary Table 2

Chlamydomonas Entrez Gene ID	Chlamydomonas locus tag	Chlamydomonas gene symbol	Chlamydomonas Alias	Note	Human Entrez Gene ID	Human Gene Symbol	Human Entrez Gene aliases	PBLAST Best reciprocal Human Protein	PBLAST Best reciprocal Chlamydomonas Protein
5725752	CHLREDRAFT_139789	CHLREDRAFT_139789	IFT122A	IFT-A	55764	IFT122	SPG|WDR10|WDR10p|WDR140	NP_060732.2	XP_001700201.1
5727567	CHLREDRAFT_195385	FAP118	IFT122B	IFT-A	57539	WDR35	KIAA1336|MGC33196	NP_065830.2	XP_001702021.1
5721885	CHLREDRAFT_126867	FAP60	IFT139	IFT-A	79809	TTC21B	FLJ11457|Nbla10696|THM1	NP_079029.3	XP_001696517.1
5721602	CHLREDRAFT_192205	IFT140		IFT-A	9742	IFT140	DKFZp564L232|FLJ10306|FLJ30571 |KIAA0590|WDTC2|c305C8.4|c380F5.1|gs114	NP_055529.2	XP_001696098.1
5719170	CHLREDRAFT_143468	FAP66	IFT144	IFT-A	57728	WDR19	FLJ23127|KIAA1638|ORF26|PWDMP	NP_079408.3	XP_001693746.1
5722198	CHLREDRAFT_142470	MOT41	IFT43	IFT-A	112752	C14orf179	FLJ32173|MGC16028	NP_001096034.1	XP_001696653.1
5717250	CHLREDRAFT_183240	IFT172		IFT-B	26160	IFT172	DKFZp434A163|KIAA1179|SLB|osm-1|wim	NP_056477.1	XP_001691740.1
5727460	CHLREDRAFT_182072	IFT20		IFT-B	90410	IFT20		AAP50265.1	XP_001701966.1
5715694	CHLREDRAFT_129193	FAP156	IFT27	IFT-B	11020	IFT27	RAYL|RABL4	NP_001171172.1	XP_001689745.1
5716734	CHLREDRAFT_108954	FAP32	IFT46	IFT-B	56912	IFT46	C11orf2|FLJ21827|C11ORF60	NP_001162089.1	XP_001691140.1
5717848	CHLREDRAFT_24116	BLD1	IFT52	IFT-B	51098	IFT52	C20orf9|CGI-53|NGD5|dJ1028D15.1	NP_057088.2	XP_001692161.1
5724201	CHLREDRAFT_98642	IFT57		IFT-B	55081	IFT57	ESRRBL1|FLJ10147|HIPPI|MHS4R2	NP_060480.1	XP_001698648.1
5715315	CHLREDRAFT_136521	IFT74	IFT-71/IFT72	IFT-B	80173	IFT74	CCDC2|CMG-1|CMG1|FLJ22621|MGC111562	NP_001092693.1	XP_001689563.1
5718874	CHLREDRAFT_24171	IFT80		IFT-B	57560	IFT80	ATD2|KIAA1374|MGC126543|WDR56	NP_065851.1	XP_001693341.1
5722735	CHLREDRAFT_138649	IFT81		IFT-B	28981	IFT81	CDV-1|CDV-1R|CDV1|CDV1R|MGC102777 |MGC4027	NP_054774.2	XP_001697224.1
5725696	CHLREDRAFT_24421	IFT88		IFT-B	8100	IFT88	D13S1056E|DAF19|MGC26259 |RP11-172H24.2|TG737|TTC10|hTg737	NP_783195.2	XP_001700100.1
5715530	CHLREDRAFT_195877	FAP9	IFT22	IFT-B associated	64792	RABL5	DKFZp761N0823|FLJ13225|FLJ14117	NP_073614.1	XP_001689669.1
5723882	CHLREDRAFT_98791	FAP232	IFT25	IFT-B associated	51668	HSPB11	C1orf41|HSPCO34|PP25	NP_057210.2	XP_001698507.1
5725022	CHLREDRAFT_185392	FAP116	IFT54	IFT-B associated	26146	TRAF3IP1	DKFZp434F124|MIP-T3|MIPT3	NP_056465.2	XP_001699472.1
5722691	CHLREDRAFT_192420	FAP22		IFT-B associated	23059	CLUAP1	FLJ13297|KIAA0643	NP_055856.1	XP_001697203.1
5724373	CHLREDRAFT_81760	DYF13		IFT-B associated	79989	TTC26	FLJ12571|MGC163211|dyf-13	NP_079202.2	XP_001698769.1
5717991	CHLREDRAFT_128801	FAP259		IFT-B associated	92104	TTC30A	FLJ13946|FLJ77601	NP_689488.3	XP_001692406.1

Orthology mapping of *Chlamydomonas*, *Caenorhabditis, Trypanosoma* and *Danio* IFT (-associated) proteins to humans.

Two IFT protein complexes were identified via biochemical purification in *Chlamydomonas*. IFT complex A (containing IFT43, IFT122A/122B, IFT139, IFT140 and IFT144) and IFT complex B (containing IFT20, IFT27, IFT46, IFT52, IFT57/55, IFT74/72, IFT80, IFT81, IFT88, IFT172) (9, 10, 17). Orthology mapping by the reciprocal best hit approach of PBLAST analyses of *Chlamydomonas*, *Caenorhabditis, Trypanosoma* and *Danio* IFT proteins, suggests that the C14orf179, WDR35, IFT122, TTC21B, WDR19 and IFT140 proteins are part of human IFT complex A, and IFT20 (26), IFT27, IFT46, IFT52 (20, 23-25), IFT57, IFT74, IFT80, IFT81, IFT88 and IFT172 are part of human IFT complex B. Future studies will reveal if the IFT complex B associated proteins: RABL5 (18, 22, 26), TTC30A/B (21, 26), TTC26 (19), CLUAP1 (21, 23, 26), HSPB11 (25) and TRAF3IP1 (28) are part of the IFT machinery.

Supplementary Table 3

Gene name	Protein name	Uniprot	IFT88-SF-TAP #1	IFT88-SF-TAP #2	IFT88-SF-TAP #3
Intraflagellar transport complex B proteins					
IFT27	Intraflagellar transport protein 27 homolog	Q9BW83	100% (2)	100% (3)	100% (2)
IFT46	Intraflagellar transport protein 46 homolog	Q9NQC8	100% (2)	100% (5)	100% (2)
IFT52	Intraflagellar transport protein 52 homolog	Q9Y366	100% (15)	100% (18)	100% (9)
IFT57	Intraflagellar transport protein 57 homolog	Q9NWB7	100% (10)	100% (19)	100% (9)
IFT74	Intraflagellar transport protein 74 homolog	Q96LB3	100% (5)	100% (9)	100% (5)
IFT80	Intraflagellar transport protein 80 homolog	Q9P2H3	100% (4)	100% (14)	100% (3)
IFT81	Intraflagellar transport protein 81 homolog	Q8WYA0	100% (3)	100% (4)	
IFT88	Intraflagellar transport protein 88 homolog	Q13099	100% (34)	100% (41)	100% (38)
IFT172	Intraflagellar transport protein 172 homolog	Q9UG01	100% (34)	100% (41)	100% (38)
Intraflagellar transport complex B-associated proteins					
CLUAP1	Clusterin-associated protein 1	Q96AJ1	100% (3)	100% (2)	94% (1)
HSPB11	Heat shock protein beta-11	Q9Y547		100% (3)	100% (2)
RABL5	Rab-like protein 5	Q9H7X7	100% (4)	100% (5)	100% (3)
TTC26	Tetratricopeptide repeat protein 26	A0AVF1	100% (8)	100% (12)	100% (10)
TTC30A	Tetratricopeptide repeat protein 30A	Q86WT1	100% (2)	100% (9)	100% (3)
TTC30B	Tetratricopeptide repeat protein 30B	Q84P2	89% (1)	100% (3)	
TRAF3IP1	TRAF3-interacting protein 1	A8MTK4		100% (2)	100% (2)
Varia					
TMEM43	Transmembrane protein 43	Q9BTV4	100% (8)	100% (6)	100% (7)
ATP1A1	Sodium/potassium-transporting ATPase	P05023	100% (8)	100% (5)	100% (6)
SLC25A4	ADP/ATP translocase 1	P12235	89% (1)	100% (2)	100% (2)
SLC25A5	ADP/ATP translocase 2	P05141	100% (2)	100% (2)	
TUBB6	Tubulin beta-6 chain	Q9BUF5		100% (2)	100% (2)

IFT88 SF-TAP analysis in HEK293T cells.

Shown are the protein names, the Uniprot (http://www.uniprot.org/) accession numbers, identification probabilities and the number of unique peptides detected for each protein in three independent experiments, as determined by Scaffold analysis (Proteome Software). Proteins detected in the SF-TAP control were excluded, as well as known contaminants like keratins. All known IFT complex B proteins, except IFT20, and all proteins described to associate with IFT complex B were identified in the IFT88-SF-TAP analysis, demonstrating that these complexes are formed in HEK293T cells.

Supplementary Table 4

Gene Symbol	RefSeq Accession Number	Gene ID	siRNA ID	Sense siRNA Sequence	Antisense siRNA Sequence
SiSel_NC1	NA	NA	s813	UAACGACGCGACGACGUAAtt	UUACGUCGUCGCGUCGUUAtt
LCA5	NM_181714	167691	s46679	CAACUAGGAAUGUAUCCAAtt	UUGGAUACAUUCCUAGUUGgt
LCA5	NM_181714	167691	s46677	GCCCUAAGGGUCUACCAAAtt	UUUGGUAGACCCUUAGGGCtt
LCA5	NM_181714	167691	s46678	GAAUUAAGGAGCUAUCGAAtt	UUCGAUAGCUCCUUAAUUCtt
TRAF3IP1	NM_015650	26146	s25157	GAAGCUAAUAUUAACUCAAtt	UUGAGUUAAUAUUAGCUUCtt
TRAF3IP1	NM_015650	26146	s25159	GGAUGAUAAUUCAGCUAGUtt	ACUAGCUGAAUUAUCAUCCga
TRAF3IP1	NM_015650	26146	s25158	CAAGCUCUCUAGUGACGAUtt	AUCGUCACUAGAGAGCUUGtt

Overview of the target sequences.
Three siRNAs (Ambion, Silencer Select) targeting a single gene were combined and used in a final concentration of 15 nM. Non-targeting siRNA (SiSel_NC1) served as negative control (15 nM).

Supplementary Table 5

qPCR primers used

Gene	Gene-ID	Forward (5'>3') primer	Reverse (3'>5') primer
hsGUSB	2990	AGAGTGGTGCTGAGGATTGG	CCCTCATGCTCTAGCGTGTC
hsLCA5	167691	CATCGCTGGTCAGTTCTTC	GGTAGACCCTTAGGGCTTGG
hsLCA5	167691	AATCGTCTGCCAAAGTCCTC	AGGTCTGCAAAATCACTCTGG
hsTRAF3IP1	26146	GAAGGGCCTCTACACAGACG	ACCACGTCTATGGCCTTTTG

RT-PCR primers used

Primername	Sequence
E3F1	AGAAACTGGTTTCGGCGAG
en2R1	ATGCATCTGCGTTCTTCTT
E2F1	ATTGCCAGGACACCAAGACT
E4R1	CTGGAAACTGTTCGTGCTCA
E3F2	GGGCGACAGAGAAAAGAGTG
LacZ6R	TGGCGAAAGGGGGATGTG
rig/S15F	TTCCGCAAGTTCACCTACC
rig/S15R	CGGGGCCGGCCATGCT TTACG

Genotyping primers used

Primername	Sequence
I3F5	AGAGAGCCATTTGCTTTGAGA
I3R1	CTACCATGCAGACCCAAGGT
En2.6R	CGGTACCAGACTCTCCCATC

Overview of primers used in this study.
Primers used for quantitative PCR (qPCR), RT-PCR and genotyping.