Supplemental Materials

Molecular Biology of the Cell

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RABL2 interacts with the IFT-B complex and CEP19, and participates in ciliary assembly

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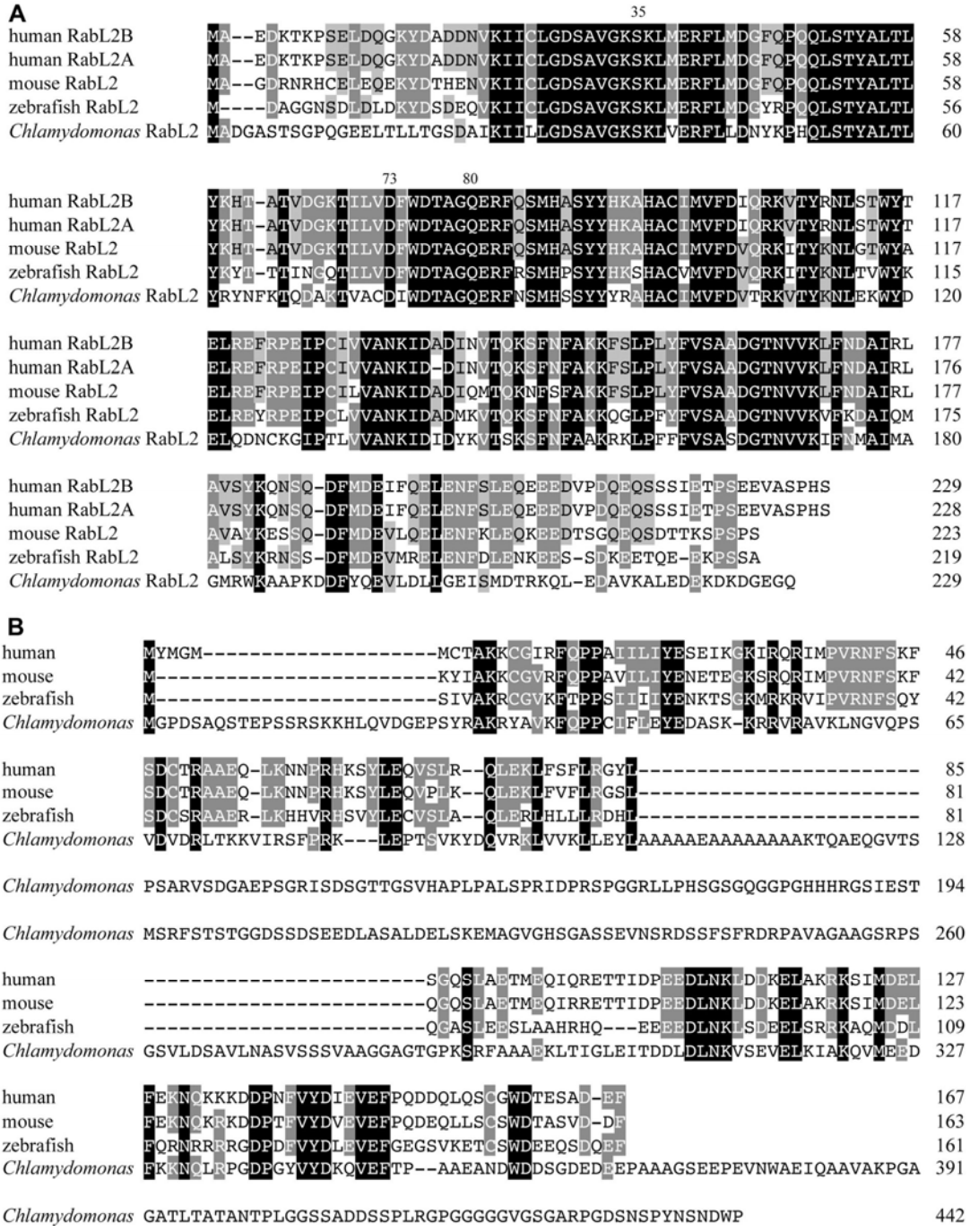

Fig. S1. Alignments of RABL2 and CEP19 sequences

(A) Human RABL2A (NP_009013) and RABL2B (NP_001003789), mouse RABL2 (NP_081093), zebrafish RABL2 (NP_001038428), and *Chlamydomonas reinhardtii* RABL2 (XP_001697212) sequences are aligned. Residues conserved in all members are shown in black boxes, and those conserved in four members are shown in grey boxes. (B) Human (NP_116287), mouse (NP_080168), zebrafish (NP_001028906), and *Chlamydomonas reinhardtii* CEP19 sequences are aligned. The *Chlamydomonas* CEP19 sequence was translated from the clonedcDNA sequence (see Results), which was deposited to DDBJ with accession number LC257670. Residues conserved in all members are shown in black boxes, and those conserved in three members are shown in grey boxes.

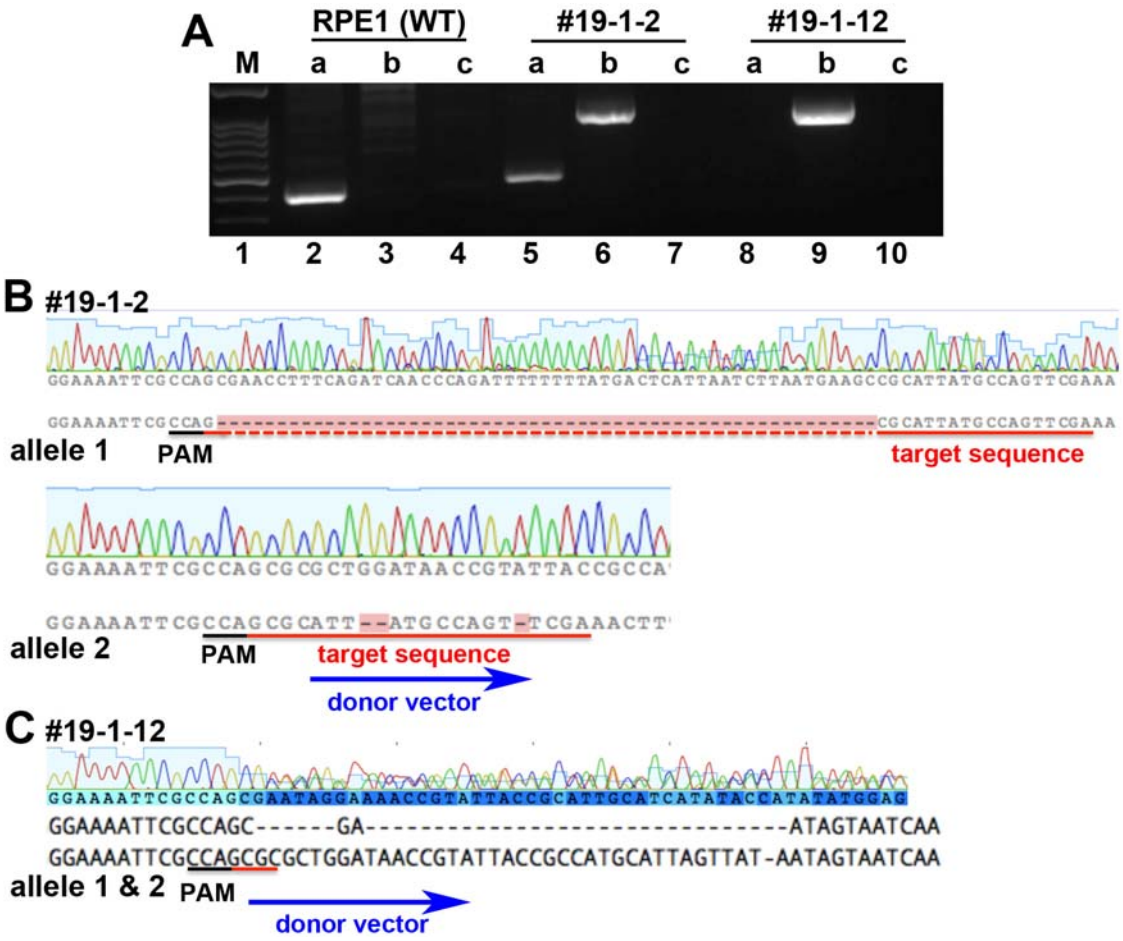

Fig. S2. Genomic PCR and sequencing to confirm donor vector integration or adeletion in the selected *CEP19*-KO cell lines

(A) Genomic DNA was extracted from control RPE1 cells (lanes 2–4), and from *CEP19*-KO cell lines (#19-1-2, lanes 5–7; and #19-1-12, lanes 8–10) established using a donor knock-in vector containing a target sequence (see Table S2), which targets the coding region within exon 2 of the human *CEP19* gene. The DNA was subjected to PCR using primer pair a (primers 1 + 2; lanes 2, 5, and 8), pair b (primers 1 + 3; lanes 3, 6, and 9), or pair c (primers 2 + 3; lanes 4, 7, and 10) (see Table S2) to detect alleles with a small insertion, deletion, or no repair, with donor vector forward integration, or with reverse integration, respectively. Lane 1, a 100-bp ladder marker in which the most intense band is 500 bp. (B and C) Alignments of allele sequences of cell lines #19-1-2 (B) and #19-1-12 (C) that weredetermined by direct sequencing of the genomic PCR products with the reference sequence encompassing the coding sequence of exon 2. The double peaks in the chromatogram of #19-1-12 were separated computationally. Red and black lines indicate the target sequences and PAM sequence, respectively. Blue arrows indicate the direction of vector integration.

Movie S1. Video microscopy of a control *Chlamydomonas*strain, a *rabl2*strain, and its transformant with the RABL2 expression vector

Note that the threemovies are played at the same frame rate, but the *rabl2*mutant shows virtually no motion owing to the lack of flagella.

Table S1. Plasmid vectors used in this study

No.	Vector	Insert
1	pcDNA3-HAC	Human RABL2B
2	pcDNA3-HAC	Human RABL2B(S35N)
3	pcDNA3-HAC	Human RABL2B(D73G)
4	pcDNA3-HAC	Human RABL2B(Q80L)
5	pEGFP-N1	Human RABL2B
6	pEGFP-N1	Human RABL2B(S35N)
7	pEGFP-N1	Human RABL2B(D73G)
8	pEGFP-N1	Human RABL2B(Q80L)
9	pmCherry-N1	Human RABL2B
10	pmCherry-N1	Human RABL2B(S35N)
11	pmCherry-N1	Human RABL2B(D73G)
12	pmCherry-N1	Human RABL2B(Q80L)
13	pEGFP-C1	Human CEP19
14	pEGFP-C1	Human CEP19(16-167)
15	pCAG-EGFP-C	Human CEP19(31-167)
16	pEGFP-C1	Human CEP19(1-150)
17	pEGFP-C1	Human CEP19(1-120)
18	pmCherry-C1	Human CEP19
19	pTagRFP-T-C1	Human CEP19
20	pRRLsinPPT-EGFP-C-IRES-Zeo	Human CEP19
21	pRRLsinPPT-EGFP-C-IRES-Zeo	Human CEP19(1-120)
22	pRRLsinPPT-EGFP-C-IRES-Zeo	Human CEP19(91-167)
23	pEGFP-C1	Human FGFR1OP
24	pEGFP-C1	Human FGFR1OP(1-179)
25	pEGFP-C1	Human FGFR1OP(1-352)
26	pEGFP-C1	Human FGFR1OP(180-379)
27	pEGFP-C1	Human FGFR1OP(353-379)
28	pEGFP-C1	Human FOR20
29	pmCherry-C1	Human CEP350(3071-3117)
30	pIC2L-BCCPC-3×HA	*Chlamydomonas reinhardtii* RABL2

Table S2. Antibodies used in this study

Antibody	Manufacturer	Clone or catalog number	Dilution (purpose)
Monoclonal mouse anti-Ac-α-tubulin	Sigma-Aldrich	6-11B-1	1:500 (immunofluorescence)
Monoclonal mouse anti-γ-tubulin	Sigma-Aldrich	GTU88	1:1,000 (immunofluorescence)
Monoclonal mouse anti-RABL2	OriGene	OTI4A8	1:200 (immunofluorescence) 1:1,000 (immunoblotting)
Polyclonal rabbit anti-CEP19	OriGene	AP09929PU-N	1:300 (immunofluorescence)
Monoclonal mouse anti-FGFR1OP	Abnova	2B1	1:10,000 (immunofluorescence)
Monoclonal mouse anti-ODF2	Abnova	1A1	1:200 (immunofluorescence)
Polyclonal rabbit anti-IFT88	Proteintech	13967-1-AP	1:200 (immunofluorescence) 1:1,000 (immunoblotting)
Monoclonal mouse anti-actin	EMD Millipore	C4	1:2,000 (immunoblotting)
Monoclonal rat anti-HA	Roche Applied Science	3F10	1:1,000 (immunoblotting)
Monoclonal mouse anti-GFP	BD Biosciences	JL-8	1:1,000 (immunoblotting)
Polyclonal rabbit anti-mRFP	MBL Life Science	PM005	1:1,000 (immunoblotting)
Polyclonal rabbit anti-tRFP	Evrogen	AB233	1:1,000 (immunoblotting)
AlexaFluor-conjugated secondary	Molecular Probes	A21240, A11034, A21127	1:1,000 (immunofluorescence)
DyLight 649-conjugated secondary	Jackson ImmunoResearch	115-495-209	1:1,000 (immunofluorescence)
Peroxidase-conjugated secondary	Jackson ImmunoResearch	115-035-166, 111-035-144	1:3,000 (immunoblotting)

Table S3. Oligo DNAs used in this study

No.	Name	Sequence
1	hCep19-G2-FW	5'-CGGACTAGAACCAGATCTTCTG-3'
2	hCep19-G2-RV	5'-CTGAAGCTCACTGAGAGTAAG-3'
3	pTagBFP-N-RV	5'-GTTGTCCACGGTGCCCTCCATGTAC-3'
4	hCep19-gRNA2-S	5'-CACCGTTCGAACTGGCATAATGCGC-3'
5	hCep19-gRNA2-AS	5'-AAACGCGCATTATGCCAGTTCGAAC-3'
6	hCep19-gRNA2-donor-AS	5'-TCCAGCGCATTATGCCAGTTCGAAC-3'
7	CrRabL2-G Fw	5'-ACGTCATAGACCAACCCTCG-3'
8	CrRabL2-G Rv	5'-ACCGTCCAAACTCACAGTCC-3'
9	5end-cassette-Rv	5'-GCACCAATCATGTCAAGCCT-3'
10	3end-cassette-Fw	5'-GACGTTACAGCACACCCTTG-3'
11	CrRabL2-G-Rv2	5'-CTCCTGGTAGAAGTCGTCC-3'
12	CrRabL2-NS	5'-CGGGATCCATGGCTGATGGGGCGAGCACG-3'
13	CrRabL2-CAS	5'-CGGAATTCATTGCCCCTCGCCGTCCTTGTCC-3'
14	CrCep19-NS-BamH1	5'-CGGGATCCACCATGGGGCCGGACTCAGCACAG-3'
15	CrCep19-CAS-EcoR1	5'-CGGAATTCCTAAGGCCAGTCGTTGCTGTTG-3'