

Fig. S1. Genomic PCR and sequencing to confirm donor vector integration in selected *IFT22*-KO cell lines

(A) Genomic DNA was extracted from control RPE1 cells (lanes 2–4), and from the *IFT22*-KO cell lines (#22-2-3, lanes 5–7; and #22-2-4, lanes 8–10) established using a donor knock-in vector. The DNA was subjected to PCR using primer pair a (primers G + H; lanes 2, 5, and 8), pair b (primers C + G; lanes 3, 6, and 9), and pair c (primers C + H; lanes 4, 7, and 10) (see Table S3). (B and C) Alignments of allele sequences of cell lines #22-2-3 (B) and #22-2-4 (C) determined by direct sequencing of the genomic PCR products with the reference sequence encompassing the *IFT22* coding sequence. Red and black lines indicate the target sequences and protospacer adjacent motif (PAM) sequence, respectively. Blue arrows indicate the direction of vector integration. The #22-2-3 cell line has a one-nucleotide insertion in one *IFT22* allele and a reverse integration of the donor knock-in vector in the other allele (B); and the #22-2-4 cell line has a five-nucleotide deletion in one *IFT22* allele and a reverse integration of the donor knock-in vector in the other allele (C).

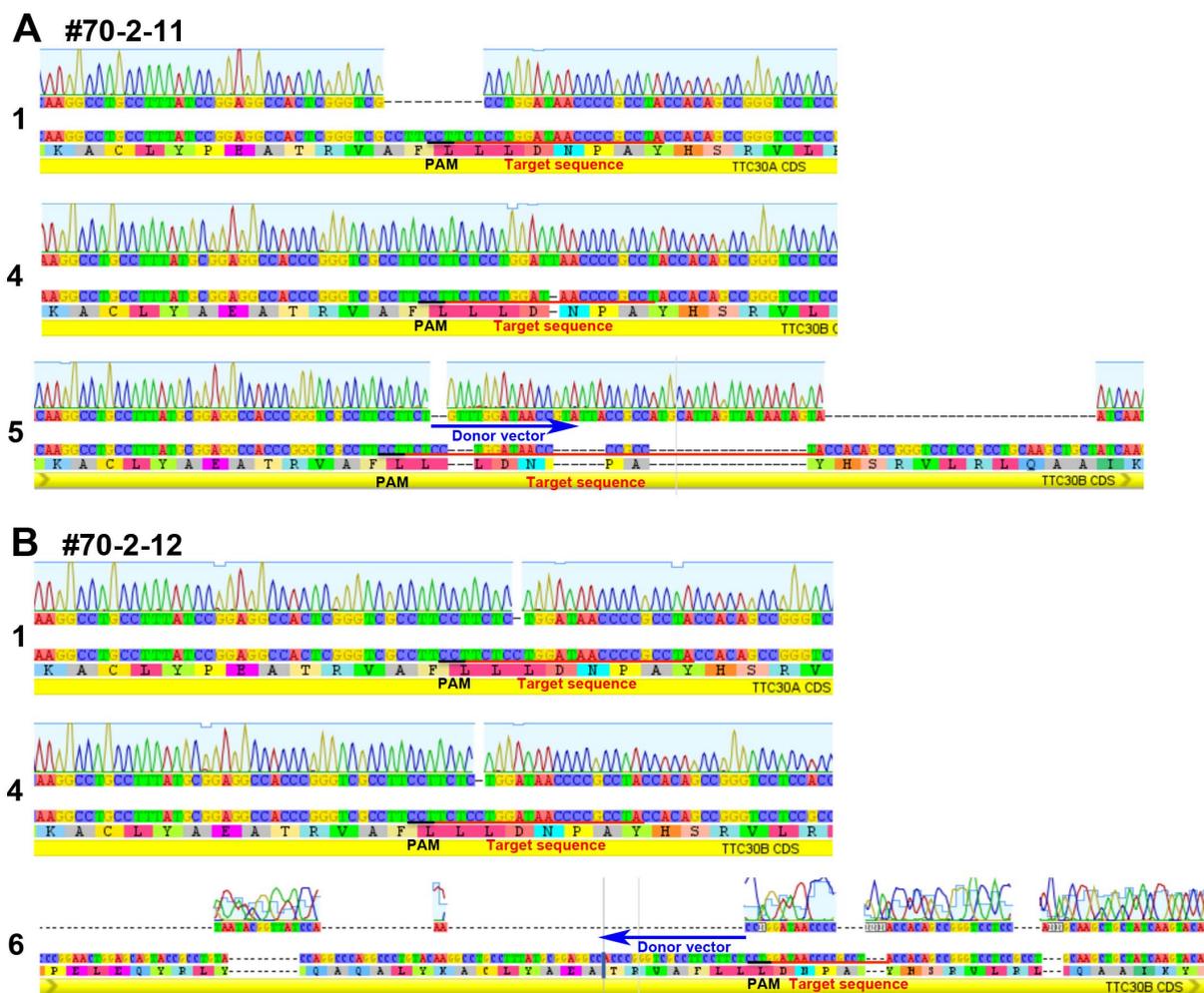


Fig. S2. Alignments of allele sequences of the *IFT70*-KO cell lines

(A) The PCR products of the #70-2-11 genomic DNA shown in Fig. 2B, middle panel, lanes 1, 4, and 5, and those of the #70-2-12 genomic DNA shown in Fig. 2B, bottom panel, lanes 1, 4, and 6, were subjected to direct sequence analysis with the reference sequence encompassing the *IFT70* coding sequence. Red and black lines indicate the target sequence and PAM sequence, respectively. Blue arrows indicate the direction of vector integration. The #70-2-11 cell line has a 10-nucleotide deletion in one of the *IFT70A* alleles, a one-nucleotide insertion in one of the *IFT70B* alleles, and a forward integration of the donor vector in the other *IFT70B* allele; and the #70-2-12 cell line has a one-nucleotide deletion in one of the *IFT70A* alleles, a one-nucleotide deletion in one of the *IFT70B* alleles, and a reverse integration of the vector in the other *IFT70B* allele.

Table S1. Plasmid vectors used in this study

No	Vector	Insert	Reference
1	pCAG2-EGFP-C	Human IFT70A	This study
2	pCAG2-mCherry-C	Human IFT70A	This study
3	pCAG2-mCherry-C	Human IFT70A(Δ N1:48-665)	This study
4	pCAG2-mCherry-C	Human IFT70A(Δ N2:85-665)	This study
5	pCAG2-mCherry-C	Human IFT70A(Δ C1:1-626)	This study
6	pCAG2-mCherry-C	Human IFT70A(Δ C2:1-581)	This study
7	pCAG2-EGFP-C	Human IFT70B	Katoh et al. (2016)
8	pRRLsinPPT-EGFP-C	Human IFT70A	This study
9	pRRLsinPPT-EGFP-C	Human IFT70B	This study
10	pRRLsinPPT-tRFP-T-C	Human IFT70A	This study
11	pRRLsinPPT-tRFP-T-C	Human IFT70A(Δ N1:48-665)	This study
12	pRRLsinPPT-tRFP-T-C	Human IFT70A(Δ C1:1-626)	This study
13	pCAG-EGFP-C	Human IFT52	Katoh et al. (2016)
14	pCAG-EGFP-C	Human IFT88	Katoh et al. (2016)
15	pCAG-mCherry-C	Human IFT52	Katoh et al. (2016)
16	pCAG-mCherry-C	Human IFT88	Katoh et al. (2016)
17	pmCherry-C	Human IFT22	This study

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 (IF)
Monoclonal mouse anti- γ -tubulin	Sigma-Aldrich	GTU88	1:1,000 (IF)
Polyclonal rabbit anti-IFT139	Sigma-Aldrich	HPA035495	1:1000 (IB)
Polyclonal rabbit anti-ARL13B	Proteintech	17711-1-AP	1:500 (IF)
Polyclonal rabbit anti-IFT88	Proteintech	13967-1-AP	1:200 (IF); 1:1,000 (IB)
Polyclonal rabbit anti-IFT140	Proteintech	17460-1-AP	1:100 (IF)
Polyclonal rabbit anti-IFT70	Proteintech	25352-1-AP	1:100 (IF); 1:500 (IB)
Polyclonal rabbit anti-IFT52	Proteintech	17534-1-AP	1:1,000 (IB)
Polyclonal rabbit anti-ARL13B	Proteintech	17711-1-AP	1:1,000 (IF)
Polyclonal rabbit anti-GPR161	Proteintech	13398-1-AP	1:200 (IF)
Monoclonal mouse anti-GFP	BD Biosciences	JL-8	1:1,000 (IB)
Polyclonal rabbit anti-RFP	MBL Life Science	PM005	1:1,000 (IB)
Polyclonal rabbit anti-tRFP	Evrogen	AB233	1:1,000 (IB)
Monoclonal mouse anti- β -tubulin	EMD Millipore	KMX-1	1:2,000 (IB)
Monoclonal mouse anti-actin	EMD Millipore	C4	1:2,000 (IB)
AlexaFluor-conjugated secondary	Molecular Probes	A11034, A21240, A21147	1:1,000 (IF)
Peroxidase-conjugated secondary	Jackson ImmunoResearch	115-035-166, 111-035-144	1:3,000 (IB)

IF, immunofluorescence; IB, immunoblotting.

Table S3. Oligo DNAs used in this study

No.	Name	Sequence
1	pTagBFP-N-RV2 (primer C)	5'-CGTAGAGGAAGCTAGTAGCCAGG-3'
2	IFT70A-genome-FW (primer A)	5'- CGTGGCAAAGTAACCCGTCG -3'
3	IFT70A-genome-RV (primer B)	5'- CTTGGTGAGTCTCCGAAGCTG -3'
4	IFT70B-genome-FW (primer D)	5'- TGCGACAAGGAAACCGGCAG -3'
5	IFT70B-genome-RV (primer E)	5'- TATGGTAAGTTTCCGGAGGAC -3'
6	Donor-vector-Seq-FW (primer F)	5'- CACCTCTGACTTGAGCGTCG-3'
7	IFT70-gRNA#2-S	5'- CACCGAGGCGGGGTTATCCAGGAGA-3'
8	IFT70-gRNA#2-AS	5'- AAACTCTCCTGGATAACCCCGCCTC-3'
9	IFT22-genome-FW (primer G)	5'- ATAGGTGCCCAACCACAC-3'
10	IFT22-genome-RV (primer H)	5'- GCCTAGGGTACAGTAGGTGC-3'
11	IFT22-gRNA#2-S	5'- CACCGATGAAGGATGCTCATGGAG-3'
12	IFT22-gRNA#2-AS	5'- AAACCTCCATGAGCATCCTTCATC-3'