

Supplemental Materials

Molecular Biology of the Cell

Hirano et al.

Supporting information

Intraflagellar transport-A complex mediates ciliary entry as well as retrograde trafficking of ciliary G protein-coupled receptors

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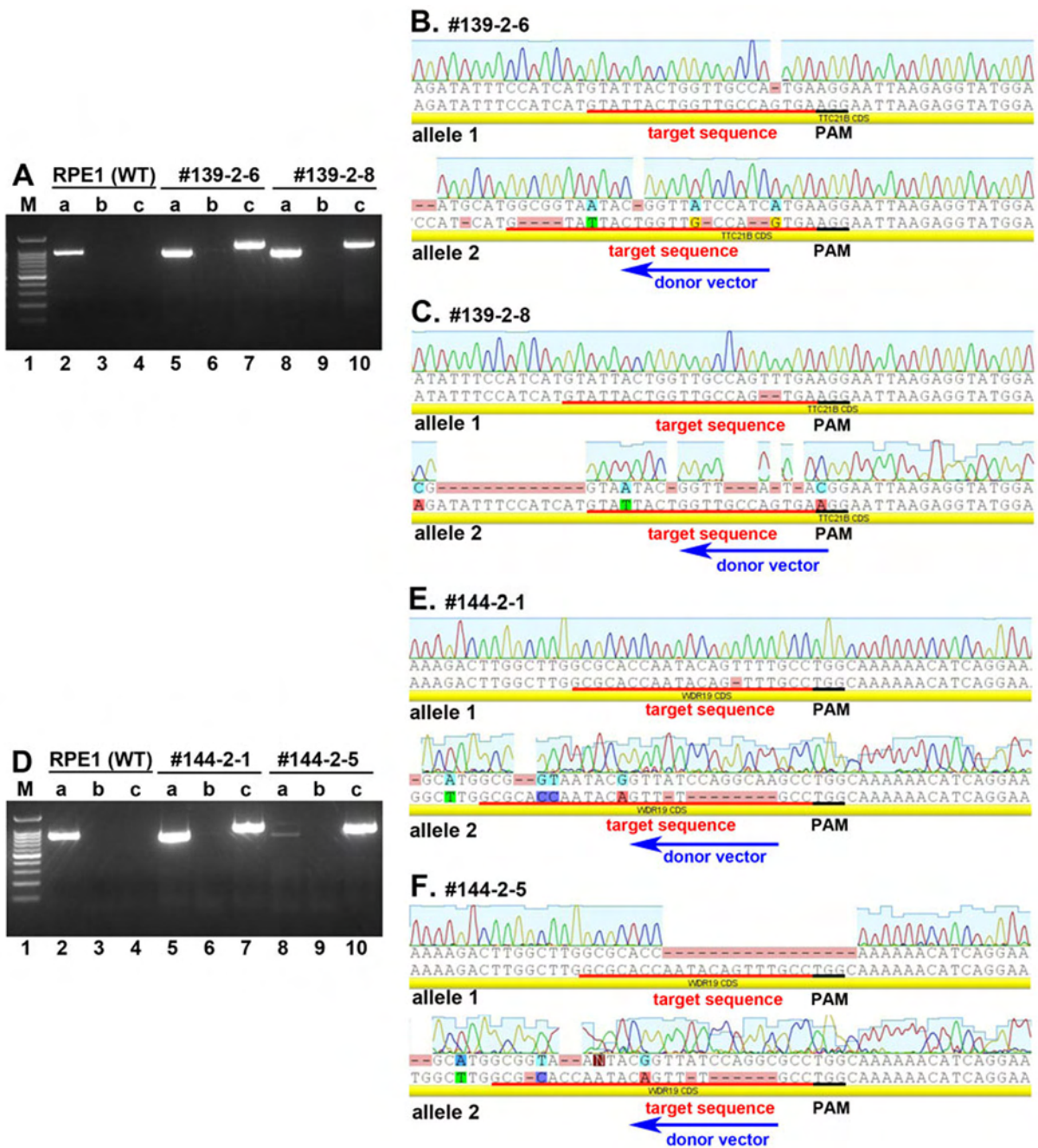


Fig. S1. Genomic PCR and sequencing to confirm donor vector integration or small deletions or insertions in the selected *IFT139*-KO and *IFT144*-KO cell lines

(A) Genomic DNA was extracted from control hTERT-RPE1 cells (lanes 2–4), and from *IFT139*-KO cell lines (#139-2-6, lanes 5–7; and #139-2-8, 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 *IFT139* 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 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 #139-2-6 and #139-2-8 determined by direct sequencing of the genomic PCR products with the reference sequence encompassing the coding sequence of exon 2. Red and black lines indicate the target sequences and PAM sequence, respectively. Blue arrows indicate the direction of vector integration. (D) Genomic DNA was extracted from control hTERT-RPE1 cells (lanes 2–4), and from *IFT144*-KO cell lines (#144-2-1, lanes 5–7; and #144-2-5, 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 *IFT144* gene. The DNA was subjected to PCR in a similar manner to that described in (A). (E) and (F), alignments of allele sequences of cell lines #144-2-1 and #144-2-5 determined by direct sequencing of the genomic PCR products with the reference sequence encompassing the coding sequence of exon 2.

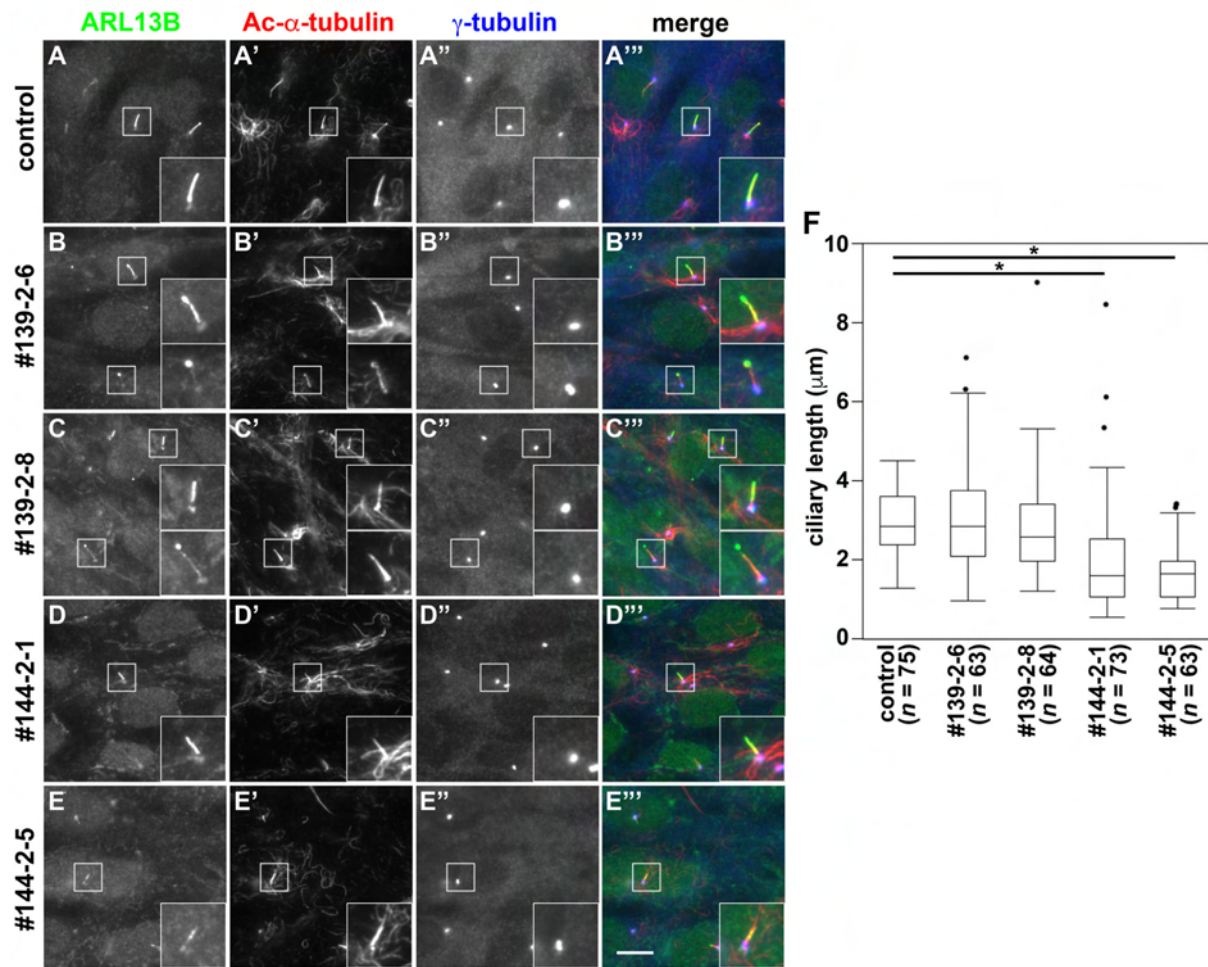


Fig. S2. Localization of ARL13B in *IFT139*-KO and *IFT144*-KO cells

Control RPE1 cells (A), the *IFT139*-KO cell lines #139-2-6 (B) or #139-2-8 (C), or the *IFT144*-KO cell lines #144-2-1 (D) or #144-2-5 (E) were serum-starved for 24 h and triple immunostained for ARL13B (A–E), Ac- α -tubulin (A'–E'), and γ -tubulin (A''–E''). Merged images are shown in A'''–E'''. Insets indicate enlarged images of the boxed regions. Scale bar, 10 μ m. (F) Ciliary lengths (lengths of Ac- α -tubulin staining) of individual control cells, and *IFT139*-KO and *IFT144*-KO cells were measured and expressed as box and whisker plots with the median values. *, $p < 0.0001$ (Student *t*-test).

Table S1: Sources of cDNAs used in this study

cDNA	Alternative names	Ref. sequences	CDS (bp)	Protein (a.a.)	Species	Sources
IFT43		NM_001102564	627	208	human	Cloned from a cDNA library by PCR
IFT121	WDR35/IFTA-1	NM_001006657	3546	1181	human	Kazusa DNA Res. Inst. Clone ID: pF1KA1336
IFT122	WDR10/DAF-10	NM_018262	3549	1182	human	Obtained by modification of NM_052990 (Riken BRC clone ID: IRAL009C12)
IFT139	THM1/TTC21B	NM_024753	3951	1316	human	Cloned from a cDNA library by PCR
IFT140	CHE-11	NM_014714	4389	1462	human	Riken BRC clone ID: IRAK069L05
IFT144	WDR19/DYF-2	NM_025132	4029	1342	human	DNAFORM clone ID: 100068972
TULP3		NM_003324	1329	442	human	Riken BRC clone ID: IRAK069G14

Table S2: Plasmids used in this study

Vectors	Inserts
pEGFP-C1	IFT43
pCAG2-EGFP-C	IFT121
pCAG2-EGFP-C	IFT122
pCAG2-EGFP-C	IFT139
pCAG2-EGFP-C	IFT140
pCAG2-EGFP-C	IFT144
pEGFP-C1	TULP3
pmCherry-C1	IFT43
pCAG2-mCherry-C	IFT121
pCAG2-mCherry-C	IFT122
pCAG2-mCherry-C	IFT139
pCAG2-mCherry-C	IFT140
pCAG2-mCherry-C	IFT144
pmCherry-C1	TULP3
pCAG2-HA-C	IFT122
pRRL.sinPPT-EGFP-C	IFT43
pRRL.sinPPT-EGFP-C	TULP3
pRRL.sinPPT-TagRFP-T-C	IFT139
pRRL.sinPPT-TagRFP-T-C	IFT144
pRRL.sinPPT-EGFP-N	SSTR3
pRRL.sinPPT-EGFP-N	MCHR1
pGEX-6P1	GFP-nanobody

Table S3: Oligo DNAs used in this study

Names	Sequences
pTagBFP-N-RV (primer 3)	5'-GTTGTCCACGGTGCCCTCCATGTAC-3'
IFT139-genome-FW (primer 1)	5'-GGTCTGGCAATGGAAGCCTCTTG-3'
IFT139-genome-RV (primer 2)	5'-TACTAATGAAACCTCTGCCAGAGG-3'
IFT139-gRNA#2-S	5'-CACCGTATTACTGGTTGCCAGTGA-3'
IFT139-gRNA#2-AS	5'-AAACTCACTGGCAACCAGTAATAC-3'
IFT139-gRNA#2-donor-AS	5'-TCCATCACTGGCAACCAGTAATAC-3'
IFT144-genome-FW (primer 1)	5'-TCCGTGCCACAGATAATTAAGTG-3'
IFT144-genome-RV (primer 2)	5'-CACTGCACCTGGCCTAAATGTTTC-3'
IFT144-gRNA#2-S	5'-CACCGCGCACCAATACAGTTTGCC-3'
IFT144-gRNA#2-AS	5'-AAACGGCAAACCTGTATTGGTGCGC-3'
IFT144-gRNA#2-donor-AS	5'-TCCAGGCAAACCTGTATTGGTGCGC-3'

Table S4: Antibodies used in this study

Antibodies	Manufacturers	Clone or catalog numbers	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)
Polyclonal rabbit anti-IFT139	Sigma-Aldrich	HPA035495	1:1,000 (immunoblotting)
Polyclonal rabbit anti-ARL13B	Proteintech	17711-1-AP	1:1,000 (immunofluorescence)
Polyclonal rabbit anti-IFT88	Proteintech	13967-1-AP	1:200 (immunofluorescence)
Polyclonal rabbit anti-IFT140	Proteintech	17460-1-AP	1:100 (immunofluorescence)
Polyclonal rabbit anti-GPR161	Proteintech	13398-1-AP	1:200 (immunofluorescence)
Polyclonal rabbit anti-SMO	Abcam	ab38686	1:100 (immunofluorescence)
Monoclonal mouse anti-FOP	Abnova	2B1	1:10,000 (immunofluorescence)
Monoclonal mouse anti-GFP	BD Biosciences	JL-8	1:1,000 (immunoblotting)
Polyclonal rabbit anti-RFP	MBL Life Science	PM005	1:1,000 (immunoblotting)
Monoclonal rat anti-HA	Roche Applied Science	3F10	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)