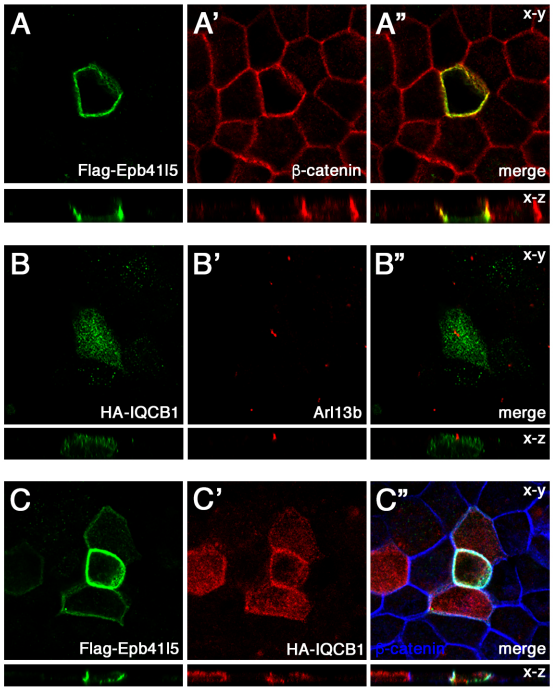


Fig. S1. Subcellular localization of Epb41l5 and IQCB1 in MDCK cells

(A-A") Epb41l5 was localized at the basolateral membrane of MDCK cells. MDCK cells expressing exogenous Flag-Epb41l5 were immunostained by anti-Flag and Adherens Junction (AJ) protein, β-catenin. (B-B") IQCB1 was localized at the apical cytoplasm. MDCK cells expressing exogenous HA-IQCB1 were immiunostained by anti-HA and ciliary protein Arl13b. (C-C") Co-expression of Epb41l5 and IQCB1 in MDCK cells. IQCB1 co-expression did not alter Epb41l5 localization at the basolateral membrane. Epb41l5 co-expression may decrease IQCB1 accumulation in the apical cytoplasm.

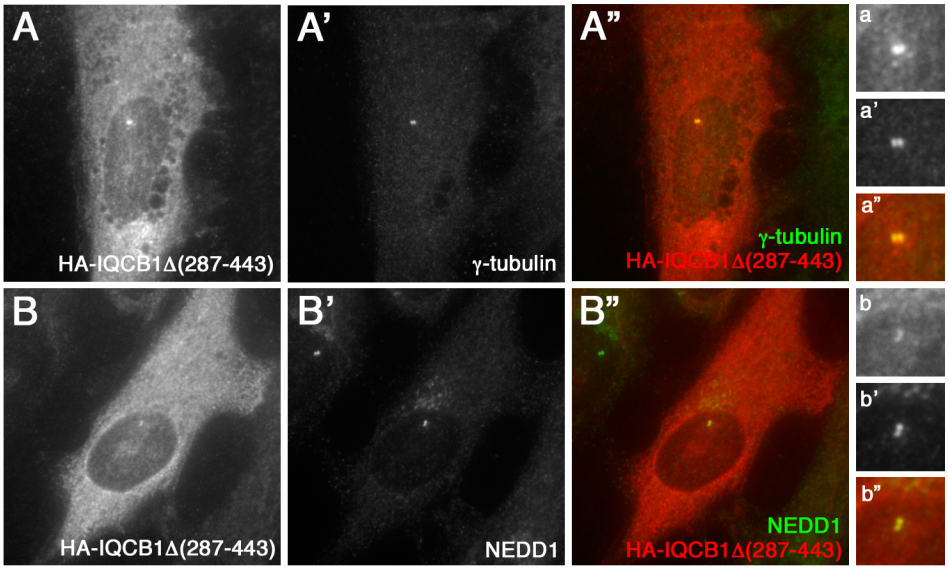


Fig. S2. Subcellular localization of IQCB1Δ(287-443) in in hTERT-RPE1 cells

(A-A") IQCB1Δ(287-443) was co-localized with endogenous γ-tubulin, a centrosome protein. (a-a") Enlarged images of a portion of Fig.S2A. (B-B") IQCB1Δ(287-443) was co-localized with endogenous NEDD1, a centrosome protein. (b-b") Enlarged images of a portion of Fig.S2B.

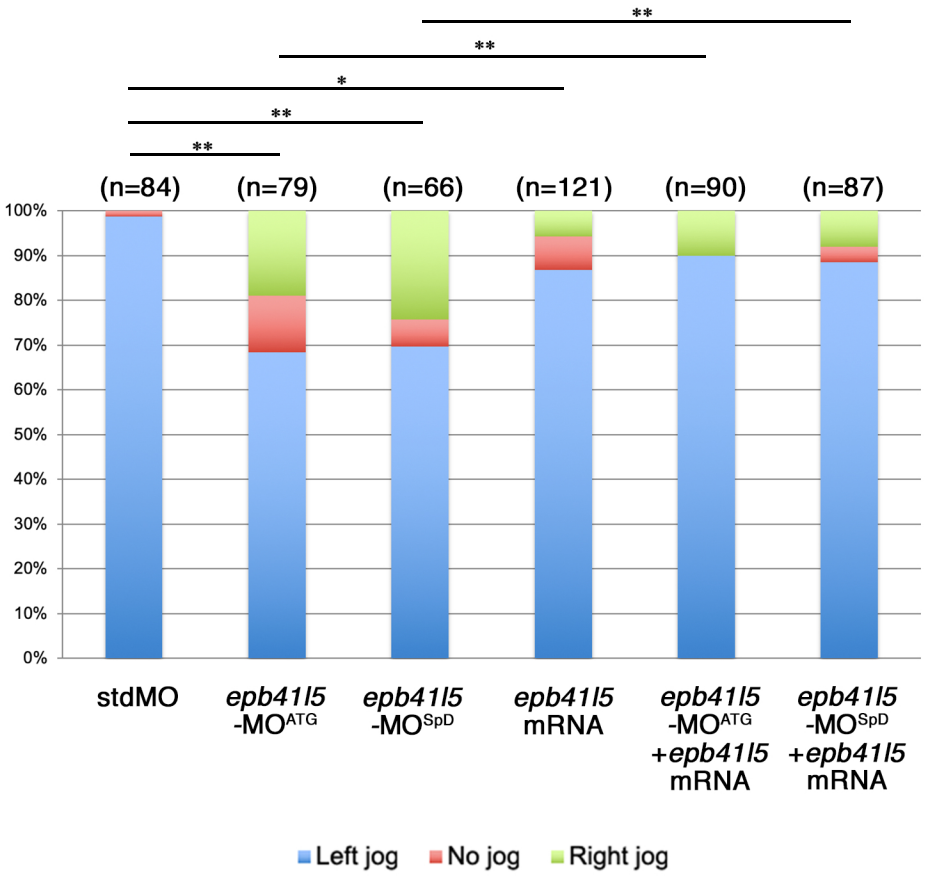
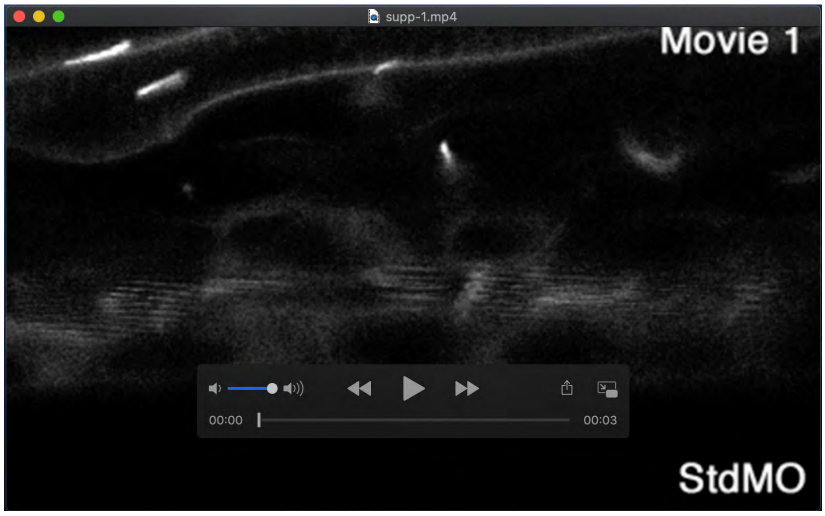


Fig. S3. LR patterning defects in *epb41l5*-MO^{ATG} morphants or *epb41l5*-MO^{SpD} morphants were rescued by co-injection of *epb41l5* mRNA

epb41l5-MO^{ATG} or *epb41l5*-MO^{SpD} morpholinos were injected with or without mRNA encoding full-length Epb41l5 at 1-2 cell stage. The direction of heart jogging at 24 hpf was scored as left jog, no jog or right jog. ** $p<0.01$, * $p<0.05$, n.s. not significant.



Movie 1. Time-lapse imaging of pronephric cilia in wild-type embryos

Pronephric cilia motility at 28-30 hpf. Images were captured at 5 second intervals for 3 min. Pronephric cilia in control wild-type embryos were difficult to image since them moved much faster than the scanning speed of a conventional confocal microscope. n=5

Movie 2. Time-lapse imaging of pronephric cilia in *epb41l5*-MO^{ATG} morphants. Pronephric cilia motility at 28-30 hpf. Images were captured at 5 second intervals for 3 min. Pronephric cilia in *epb41l5*-MO^{ATG} morphants were more clearly captured compared to wild-type embryos (Movie 1), suggesting that these cilia had reduced motility. n=5

Movie 3. Time-lapse imaging of pronephric cilia in *epb41l5*-MO^{SpD} morphants. Pronephric cilia motility at 28-30 hpf. Images were captured at 5 second intervals for 3 min. Pronephric cilia in *epb41l5*-MO^{SpD} morphants were more clearly captured compared to wild-type embryos (Movie 1), suggesting that these cilia had reduced motility. n=5

Table S1

Name	Sequence (5'-3')	Purposes
hIQCB1-F-BamHI	GGATCCTGAAGCCAACAGGTACAGA	hIQCB1 expression vector
hIQCB1-F1-BamHI-157	GGATCCCTGATTCTCTCTTCTGGC	hIQCB1 expression vector
hIQCB1-R1-XhoI-stop157	CTCGAGTTAAACATGGCCTCCCA	hIQCB1 expression vector
hIQCB1-R2-XhoI-stop290	CTCGAGCTATTCTACTTCCTGATAGAC	hIQCB1 expression vector
hIQCB1-F2-BamHI-287	GGATCCATCAGGAAGTAGAAGAGC	hIQCB1 expression vector
hIQCB1-F3-BamHI-439	GGATCCAGAAACTATTTGCTCCTTG	hIQCB1 expression vector
hIQCB1-R3-XhoI-stop443	CTCGAGTCAAGGAGCAAATAGTTTCT	hIQCB1 expression vector
hIQCB1-R-XhoI-598	CTCGAGCTAAGGTGGTTTGGTTC	hIQCB1 expression vector
hIQCB1Δ(287-443)-F	TCCGACCATTGGGCTTAAAAG	hIQCB1 expression vector
hIQCB1Δ(287-443)-R	GGAGGACTCCAAGAACTC	hIQCB1 expression vector
hEpb41l5-shRNA#1	GAGATGGAACTGGCTATTTTT	hEpb41l5 knockdown in human cell lines
hEpb41l5-shRNA#2	GTTCAGATTCGTGCCTATTCAG	hEpb41l5 knockdown in human cell lines
zEpb41l5 splice donor gRNA 1	GCGTAATACGACTCACTATAGGGGAGAGCAGAAAAGGGTAGCAGTTTTAGAGCTAGAAATAGC	Template oligo for gRNA1 (target sequence is underlined)
zEpb41l5 splice donor gRNA 2	GCGTAATACGACTCACTATAGGGGCAGGGATGAAACAAACCTGGGTTTTAGAGCTAGAAATAGC	Template oligo for gRNA2 (target sequence is underlined)
zEpb41l5 splice donor gRNA 3	GCGTAATACGACTCACTATAGGGGCTCACAATGTCATTGCCACCGTTTTAGAGCTAGAAATAGC	Template oligo for gRNA3 (target sequence is underlined)
zEpb41l5-SpD-F	GCTCTGTTTCCCCTCTTCCT	PCR primer to test gene editing
zEpb41l5-SpD-R	AGGTTTTATTGACCACCAAGC	PCR primer to test gene editing
zEpb41l5-RT-PCR-F	GCTCTGTTTCCCCTCTTCCT	RT-PCR primer
zEpb41l5-RT-PCR-R	GCGTAATACGACTCACTATAGGGGCTCACAATGTCATTGCCACCGTTTTAGAGCTAGAAATAGC	RT-PCR primer
epb41l5-MO-ATG	AGTTTATTCAACTCACCGGCAGGTC	Translation blocking morpholino for zebrafish *epb41l5*
epb41l5-MO-SpD	TAGCAGGGATGAAACAAACCTGGT	Splicing blocking morpholino for zebrafish *epb41l5*
iqcb1-MO	TCAAATCTGAATACCTGAGGAGGTC	Splicing blocking morpholino for zebrafish *iqcb1*
tp53-MO	GCGCCATTGCTTTGCAAGAATTG	morpholino for *p53*

Table S2

Name	Manufacture	Catalog number	Dilution
Primary antibodies			
rat anti-HA (clone 3F10)	Roche	11867423001	1/100 (IF)
mouse anti-HA (clone 16B12)	Covance	MMS-101P	1/300 (IF) 1/2000 (WB)
mouse anti-Flag (clone 2H8)	Cosmo Bio	KAL-KO602	1/500 (IF) 1/2000 (WB)
mouse anti-AcTub (clone 6-11B-1)	Sigma Aldrich	T7451	1/300 (IF)
rabbit anti-myc	Sigma Aldrich	C3956	1/500 (IF) 1/2000 (WB)
rabbit anti-Arl13b	Proteintech	17711-1-AP	1/500 (IF) 1/2000 (WB)
rabbit anti-γ-Tubulin	Abcam	ab11321	1/300 (IF)
mouse anti-NEDD1	Abnova	H00121441-MO5	1/300 (IF)
rabbit anti-β-catenin	Sigma Aldrich	PLA0230	1/500 (IF)
Secondary antibodies			
HRP anti-mouse IgG	Cell Signaling	7074	1/3,000 (WB)
HRP anti-rabbit IgG	Cell Signaling	7076	1/3,000 (WB)
Alexa Fluor 488 anti-rabbit IgG	Invitrogen	A-11034	1/200 (IF)
Alexa Fluor 488 anti-mouse IgG	Invitrogen	A-11029	1/200 (IF)
Alexa Fluor 647 anti-rabbit IgG	Invitrogen	A-21245	1/200 (IF)
Cy3 anti-mouse IgG	Jackson ImmunoResearch	715-165-151	1/500 (IF)
Cy3 anti-rabbit IgG	Jackson ImmunoResearch	711-165-152	1/500 (IF)
Cy3 anti-rat IgG	Jackson ImmunoResearch	712-165-153	1/500 (IF)