Supplemental Materials

Molecular Biology of the Cell

Hamada et al.

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Interaction of WDR60 intermediate chain with TCTEX1D2 light chain of the dynein-2 complex is crucial for ciliary protein trafficking

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Video S1. TIRF microscopy of RPE1 cells expressing EGFP-DYNC2LI1

Scale bar: 5 μm

Video S2. TIRF microscopy of the #1D2-2-9 cells expressing EGFP-DYNC2LI1

Scale bar: 5 μm

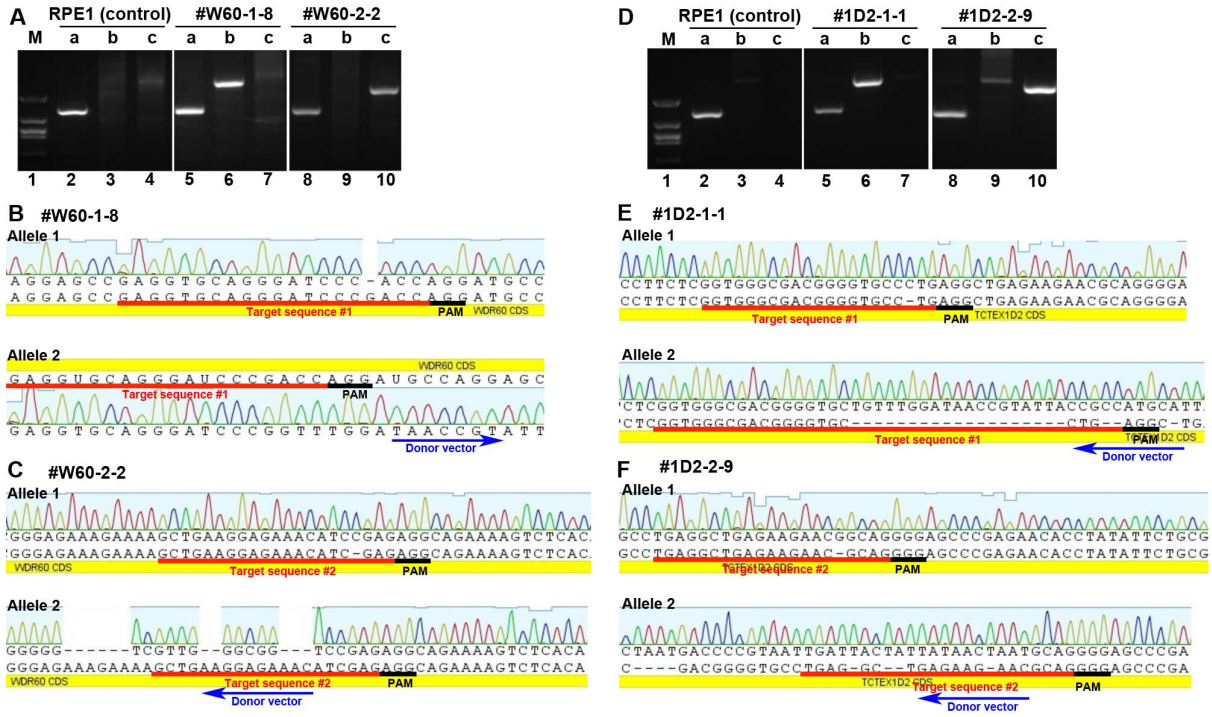

Fig. S1. Genomic PCR and sequencing to confirm donor vector integration or small deletions or insertions in selected *WDR60*-KO and *TCTEX1D2*-KO cell lines

(A) Genomic DNA was extracted from control hTERT-RPE1 cells (lanes 2–4), and from *WDR60*-KO cell lines (#W60-1-8, lanes 5–7; and #W60-2-2, lanes 8–10) established using the all-in-one vector containing a target sequence (see Table S3) and the donor knock-in vector. 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 S3) to detect alleles with a small indel, or with a forward or reverse integration of the donor vector, respectively. Lane 1, fragments of the *DdeI*-digested pSP64 plasmid. (B) and (C), alignments of allele sequences of cell lines #W60-1-8 and #W60-2-2 determined by direct sequencing of the genomic PCR products with the reference sequence. Red and black lines indicate the target sequences and PAM sequence, respectively. Blue arrows indicate the direction of donor vector integration. (D) Genomic DNA was extracted from control hTERT-RPE1 cells (lanes 2–4), and from *TCTEX1D2*-KO cell lines (#1D2-1-1, lanes 5–7; and #1D2-2-9, lanes 8–10) established using the all-in-one vector containing a target sequence (see Table S3) and the donor knock-in vector. 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 #1D2-1-1 and #1D2-2-9 determined by direct sequencing of the genomic PCR products with the reference sequence.

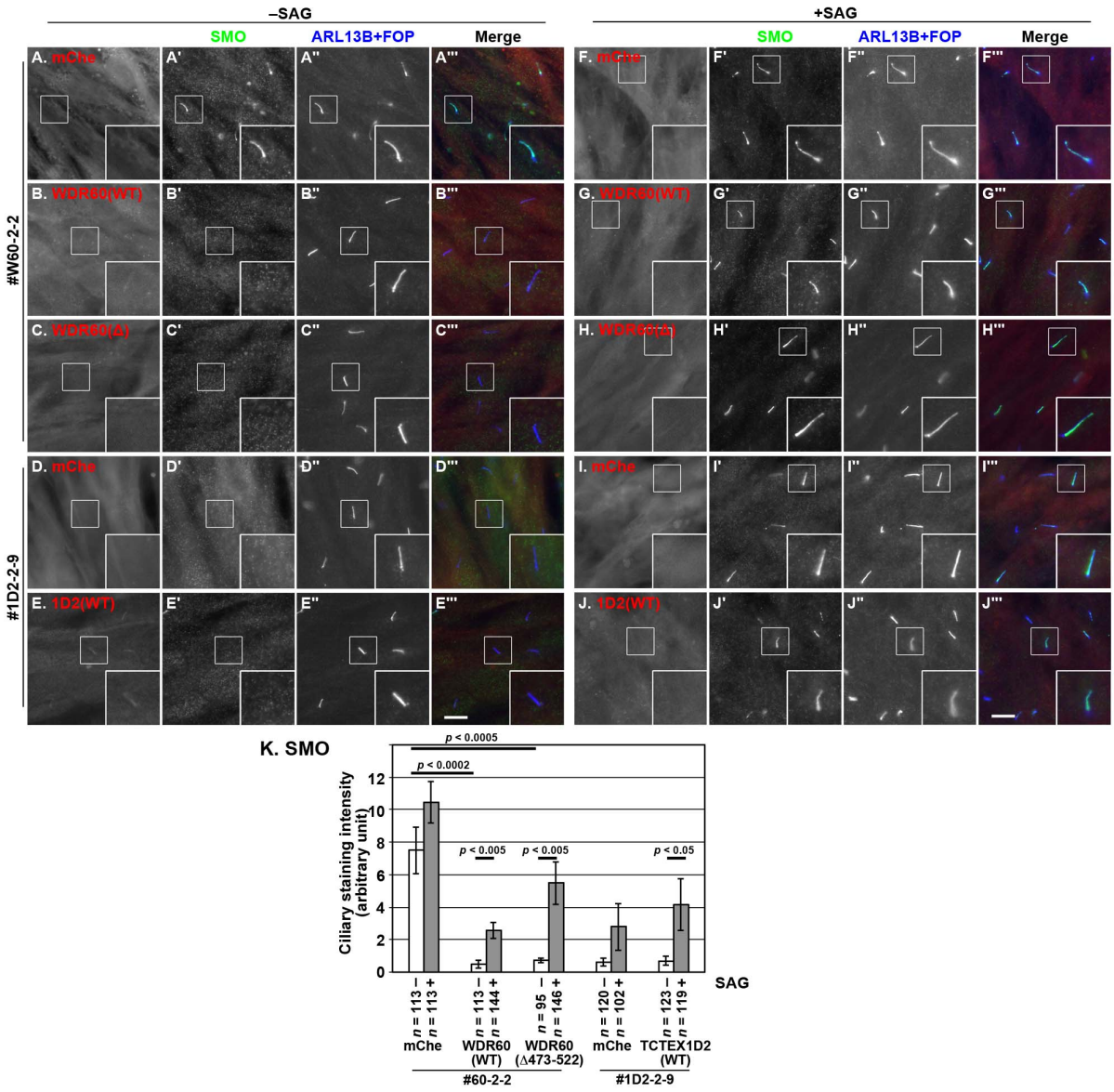

Fig. S2. Rescue of SMO localization in *WDR60*-KO and *TCTEX1D2*-KO cells upon the respective expression of a WDR60 or TCTEX1D2 construct

The #W60-2-2 cell line stably expressing mChe (A, F), mChe-fused WDR60(WT) (B, G), or WDR60(Δ473–522) (C, H), or the #1D2-2-9 cell line stably expressing mChe (D, I) or mChe-TCTEX1D2(WT) (E, J) were cultured and treated with SAG as described in the legend for Fig. 4, and quadruple immunostained for either RFP (A–J), SMO (A′–J′), and ARL13B+FOP (A″–J″). (K) Relative staining intensities for SMO were estimated and expressed as described in the legend for Fig. 4. Values are means ± S.D. of three independent experiments. In each set of experiments, 31–50 ciliated cells were analyzed, and the total numbers of ciliated cells analyzed (*n*) are shown. *p*, one-way ANOVA followed by Tukey post-hoc analysis for comparison among the cell lines, and the Student *t*-test for comparison between the cells with and without SAG treatment.

Table S1. Sources of cDNAs used in this study

cDNA	Ref. sequence	CDS (bp)	Protein (a.a.)	Species	Source
DYNC2H1	NM_001377	12,924	4,307	Human	Kazusa DNA Res. Inst. clone ID: bj00195y2
DYNC2LI1	NM_001193464	1,059	352	Human	Riken BRC clone ID: IRAL016M21
WDR34	NM_052844	1,611	536	Human	Riken BRC clone ID: IRAL028A16
WDR60	NM_018051	3,201	1,066	Human	Riken BRC clone ID: IRAL034P23
DYNLL1	NM_001037494	270	89	Human	Synthesized by Geneart (Regensburg, Germany)
DYNLL2	NM_080677	270	89	Human	Synthesized by Geneart (Regensburg, Germany)
DYNLRB1	NM_014183	291	96	Human	Synthesized by Geneart (Regensburg, Germany)
DYNLRB2	NM_130897	291	96	Human	Synthesized by Geneart (Regensburg, Germany)
DYNLT1	NM_006519	342	113	Human	Synthesized by Geneart (Regensburg, Germany)
DYNLT3	NM_006520	351	116	Human	Synthesized by Geneart (Regensburg, Germany)
TCTEX1D2	NM_152773	429	142	Human	Synthesized by Geneart (Regensburg, Germany)

Table S2. Plasmids used in this study

Vector	Insert	Reference
pCAG2-EGFP-N	DYNC2H1(tail; 1–1,650)	This study
pCAG2-EGFP-C	DYNC2LI1	This study
pCAG2-EGFP-C	WDR34	This study
pCAG2-EGFP-C	WDR60	This study
pCAG2-EGFP-C	DYNLL1	This study
pCAG2-EGFP-C	DYNLL2	This study
pCAG2-EGFP-C	DYNLRB1	This study
pCAG2-EGFP-C	DYNLRB2	This study
pCAG2-EGFP-C	DYNLT1	This study
pCAG2-EGFP-C	DYNLT3	This study
pCAG2-EGFP-C	TCTEX1D2	This study
pCAG2-mCherry-N	DYNC2H1(tail; 1–1,650)	This study
pCAG2-mCherry-C	DYNC2LI1	This study
pCAG2-mCherry-C	WDR34	This study
pCAG2-mCherry-C	WDR60	This study
pCAG2-mCherry-C	DYNLL1	This study
pmCherry-C1	DYNLL2	This study
pCAG2-mCherry-C	DYNLRB1	This study
pCAG2-mCherry-C	DYNLRB2	This study
pCAG2-mCherry-C	DYNLT1	This study
pCAG2-mCherry-C	DYNLT3	This study
pCAG2-mCherry-C	TCTEX1D2	This study
pCAG2-EGFP-C	WDR60(375–1066)	This study
pCAG2-EGFP-C	WDR60(473–1,066)	This study
pCAG2-EGFP-C	WDR60(523–1066)	This study
pCAG2-EGFP-C	WDR60(Δ473–522)	This study
pRRLsinPPT-mCherry-C	WDR60	This study
pRRLsinPPT-mCherry-C	WDR60(Δ473–522)	This study
pRRLsinPPT-mCherry-C	TCTEX1D2	This study
pRRLsinPPT-EGFP-C	DYNC2LI1	This study
pDonor-tBFP-NLS-Neo (Universal)	–	Katoh et al. (2017)
peSpCas9 (1.1)-2×gRNA	–	Katoh et al. (2017)
pGEX-6P1	GFP-nanobody	Katoh et al. (2015)

Table S3: Antibodies used in this study

Antibody	Manufacturer	Clone or catalog number	Dilution (purpose)
Polyclonal rabbit anti-IFT88	Proteintech	13967-1-AP	1:500 (IF)
Polyclonal rabbit anti-GPR161	Proteintech	13398-1-AP	1:200 (IF)
Polyclonal rabbit anti-IFT140	Proteintech	17460-1-AP	1:100 (IF)
Polyclonal rabbit anti-SMO	Abcam	ab38686	1:100 (IF)
Monoclonal rabbit anti-ARL13B	Abcam	N29566	1:500 (IF)
Monoclonal mouse anti-FOP	Abnova	2B1	1:10,000 (IF)
Monoclonal mouse anti-GFP	BD Biosciences	JL-8	1:1,000 (IB)
Monoclonal mouse anti-RFP	MBL	3G5	1:5,000 (IF)
Polyclonal rabbit anti-RFP	MBL	PM005	1:1,000 (IB)
AlexaFluor-conjugated secondary	Molecular Probes	A11034, A21127, A21137, A21241, A21242	1:1,000 (IF)
Peroxidase-conjugated secondary	Jackson ImmunoResearch	115-035-166, 111-035-144	1:3,000 (IB)

IF, immunofluorescence; IB, immunoblotting

Table S4: Oligo DNAs used in this study

Name	Sequence
pTagBFP-N-RV2 (primer 3)	5'-CGTAGAGGAAGCTAGTAGCCAGG-3'
WDR60-genome-FW (primer 1)	5'-GAAACTGGGATTGGAAAGCAG-3'
WDR60-genome-RV (primer 2)	5'-GCAGCGAGCTATCAACCTGTC-3'
WDR60-exon 3-gRNA#1-S	5'-CACCGAGGTGCAGGGATCCCGACC-3'
WDR60-exon 3-gRNA#1-AS	5'-AAACGGTCGGGATCCCTGCACCTC-3'
WDR60-exon 3-gRNA#2-S	5'-CACCGCTGAAGGAGAAACATCGAG-3'
WDR60-exon 3-gRNA#2-AS	5'-AAACCTCGATGTTTCTCCTTCAGC-3'
TCTEX1D2-genome-FW (primer 1)	5'-CCTCAAGTCCAGGAGCCTCAG-3'
TCTEX1D2-genome-RV (primer 2)	5'-CGCCCAAACACTGAGGAAG-3'
TCTEX1D2-exon 1-gRNA#1-S	5'-CACCGGTGGGCGACGGGGTGCCTG-3'
TCTEX1D2-exon 1-gRNA#1-AS	5'-AAACCAGGCACCCCGTCGCCCACC-3'
TCTEX1D2-exon 1-gRNA#2-S	5'-CACCGTGAGGCTGAGAAGAACGCAG-3'
TCTEX1D2-exon 1-gRNA#2-AS	5'-AAACCTGCGTTCTTCTCAGCCTCAC-3'