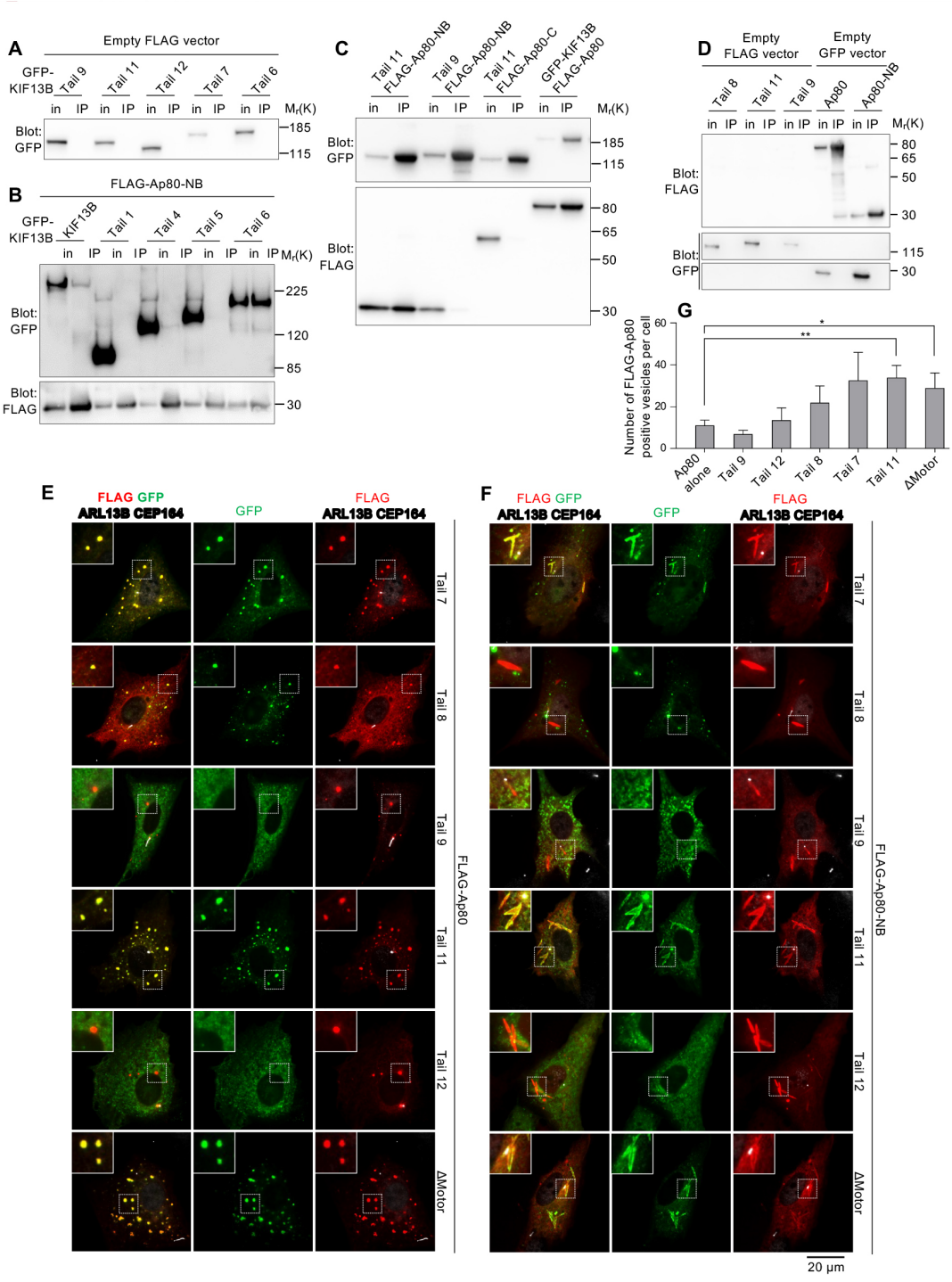


Fig. S1. Control experiments for Figure 2. (A) FLAG IP analysis of HEK293T cells co-expressing empty FLAG vector and the indicated GFP-KIF13B tail fusions. Input (in) and IP pellets (IP) were subjected to SDS-PAGE and immunoblot analysis using GFP antibody. **(B)**

Journal of Cell Science • Supplementary information

FLAG IP analysis as in (B) using HEK293T cells co-expressing FLAG-Ap80-NB and either full length GFP-KIF13B (KIF13B) or the indicated GFP-KIF13B tail fusions. **(C)** GFP IP analysis of HEK293T cells co-expressing different combinations of FLAG-Ap80 fusions and GFP-KIF13B tail fusions as indicated. GFP fusions were IPed using anti-GFP conjugated beads and input (in) and IP pellets (IP) subjected to immunoblot analysis using indicated antibody. Note that the two last lanes are the same as those depicted in Figure 1B. **(D)** FLAG IP analysis of HEK293T cells co-expressing empty FLAG vector with indicated GFP-KIF13B tail fusions and cells co-expressing GFP alone with either FLAG-Ap80 or FLAG-Ap80-NB. FLAG fusions were IPed using anti-FLAG conjugated beads and input (in) and pelleted proteins (IP) subjected to immunoblot analysis using indicated antibody. **(E, F)** IFM analysis of RPE1 cells co-expressing FLAG-Ap80 **(E)** or FLAG-Ap80-NB **(F)** and different GFP-KIF13B tail fusions as indicated. Antibodies against ARL13B and CEP164 (pseudocolored white) mark the cilium-centrosome axis. Insets show enlargements of regions with vesicles or tubular structures induced by FLAG-Ap80/Ap80-NB. **(G)** Quantification of the number of FLAG-Ap80 positive vesicles per cell expressing FLAG-Ap80 alone or together with different GFP-KIF13B tail fusions (21-50 cells analyzed per condition, n=3, except for Δmotor where n=2). Bars represent mean ±SD. * and **; $P \leq 0.05$ and 0.01, respectively (one-way ANOVA).

Journal of Cell Science • Supplementary information

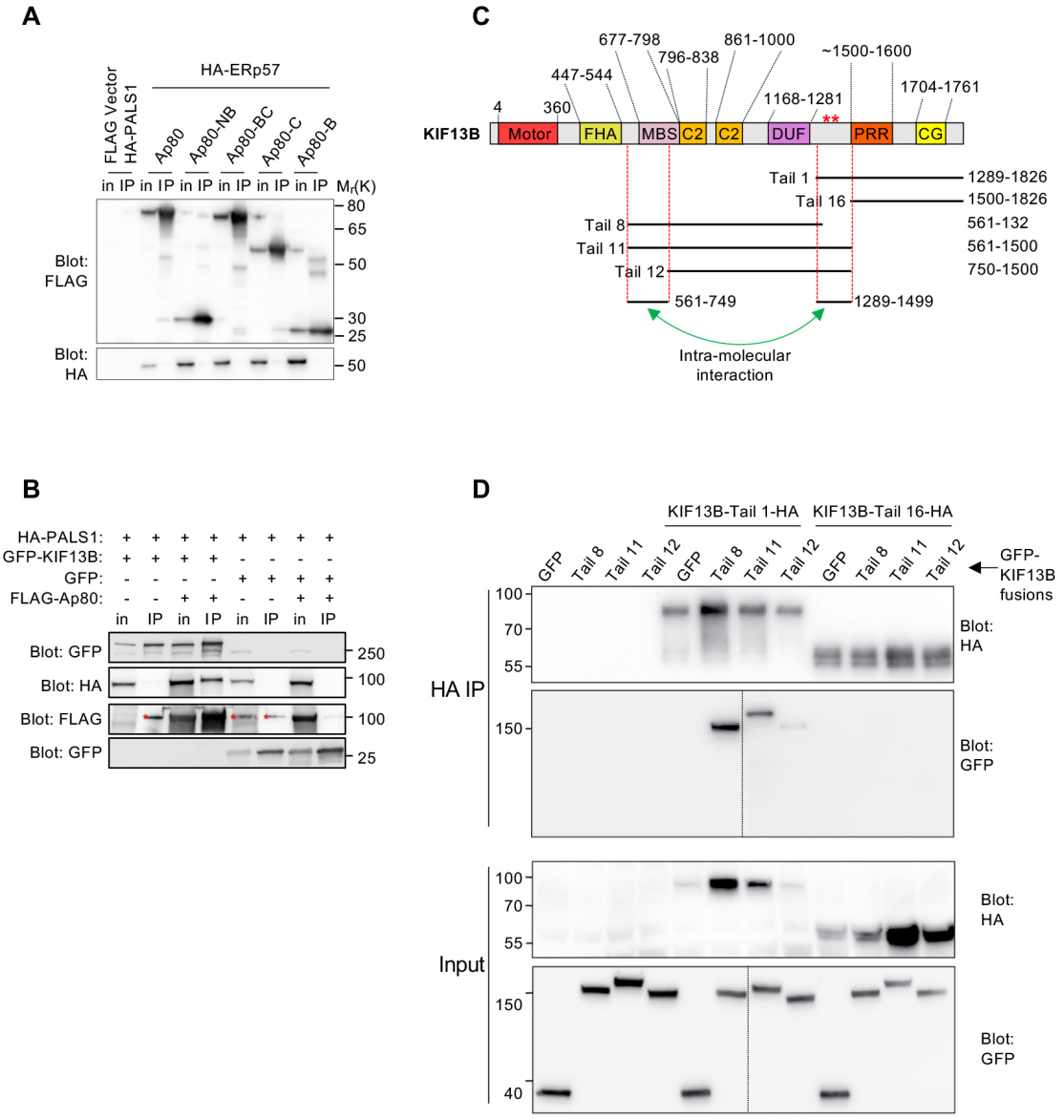
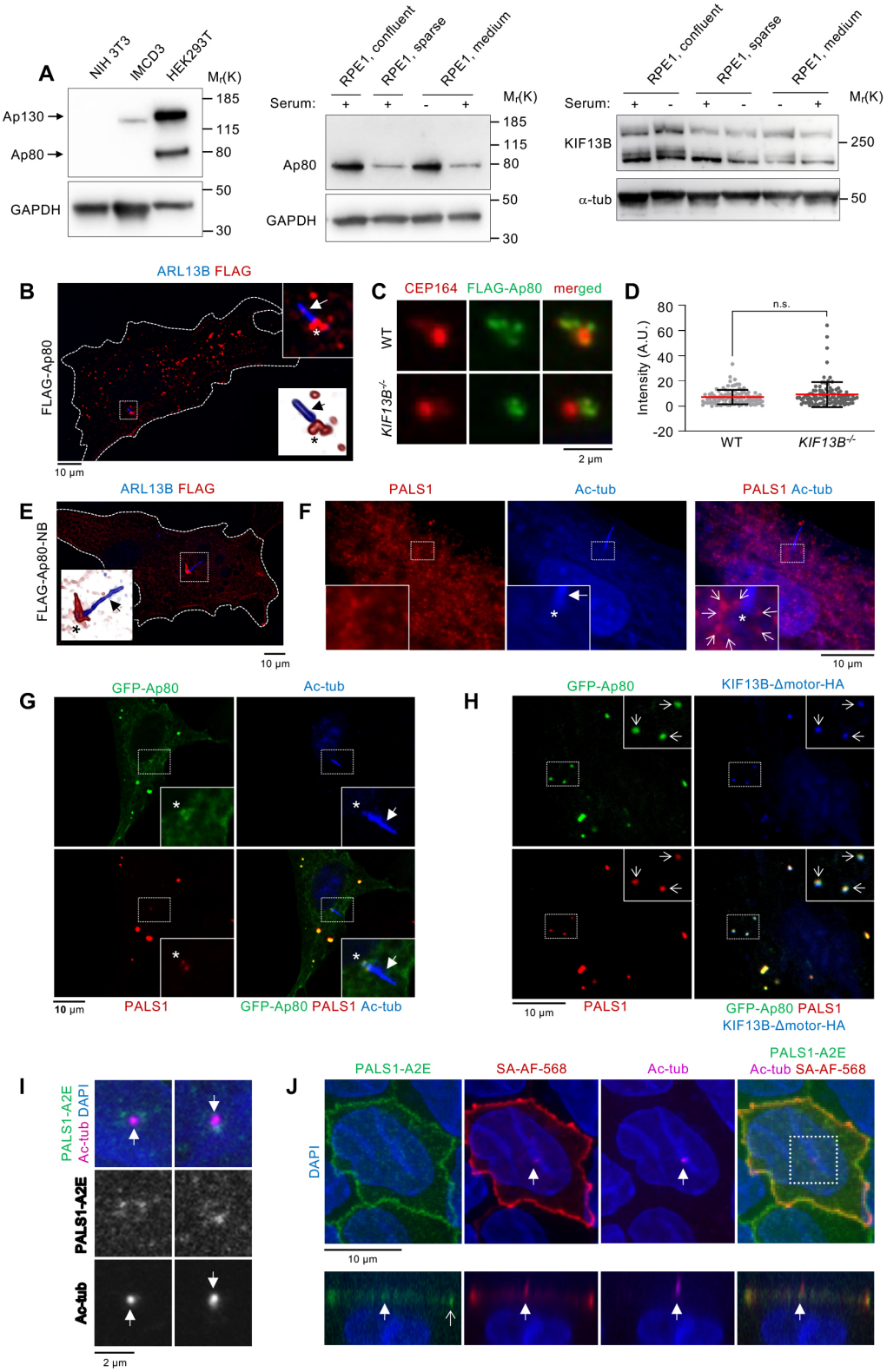


Fig. S2. Control IP experiments for Figure 2. (A) Control experiment for Figure 2E. FLAG IP analysis of HEK293T cells co-expressing HA-ERp57 and the indicated FLAG-Ap80 constructs and cells co-expressing empty FLAG vector and HA-PALS1. **(B)** GFP IP analysis of HEK293T cells expressing the indicated fusion proteins. Input (in) and IP pellet (IP) fractions were analyzed by immunoblotting using indicated antibodies. **(C, D)** Schematics of GFP- or HA-tagged KIF13B tail fragments **(B)** used for HA IP analysis in HEK293T cells, followed by immunoblotting with GFP or HA antibodies as indicated **(D)**. Note that KIF13B-Tail 1-HA (residues 1289-1826) binds to GFP-Tail 8 and 11 that both contain the MBS domain whereas KIF13B-Tail 16-HA (residues 1500-1826) does not. The GFP blots in panel D were assembled from two gels run in parallel, with the assembly sited indicated with dotted lines.

Journal of Cell Science • Supplementary information



Journal of Cell Science • Supplementary information

Fig. S3. Ap80, KIF13B and PALS1 localization in ciliated RPE1 cells. Related to Figure 3. **(A)** Immunoblot analysis of cell lysates from NIH3T3, IMCD3, HEK293T (left panel), and RPE1 (middle and right panel) cells using antibodies as indicated. RPE1 cells were grown with (+) or without (-) serum and to indicated confluency. KIF13B migrates as a double band in these cells as shown before (Schou et al., 2017). **(B, C)** IFM of FLAG-Ap80 in RPE1 cells using indicated antibodies. In (B) ARL13B marks the cilium (arrows), and asterisks indicate the ciliary base. In (C), transfected WT and *KIF13B*$^{-/-}$ cells (Schou et al., 2017) were analyzed in parallel and CEP164 antibody was used to mark the centrosome/mother centriole. **(D)** Quantification of relative centrosomal levels of FLAG-Ap80 in WT and *KIF13B*$^{-/-}$ cells, based on images as in (C). Between 29 and 39 cells were analyzed per condition (n=3). Bars represent mean ± SD. P value results from unpaired two-tailed t-test (n.s., $P \geq 0.05$, not significant). **(E)** IFM of FLAG-Ap80-NB in RPE1 cells using indicated antibodies; ARL13B marks the cilium (arrows). Asterisks indicate the ciliary base. Inset in (C) shows 3D isosurface rendering on captured z stacks of the boxed area. **(F)** IFM of untransfected cells using antibodies against acetylated α-tubulin (Ac-tub) and PALS1. Closed arrow and asterisk mark the cilium and centrosome, respectively, while open arrows indicate periciliary staining of PALS1 in the enlarged insert. The image shown is representative of 39 ciliated cells examined. **(G, H)** IFM of cells expressing GFP-Ap80 without **(G)** and with co-expression of KIF13B-ΔMotor-HA **(H)** using antibodies as indicated. Closed arrows point to cilia; asterisks mark the ciliary base; open arrows point to cellular puncta where GFP-Ap80, KIF13B-ΔMotor-HA and endogenous PALS1 co-localize. In (G, H), 74% of the GFP-Ap80 puncta (n=80) were positive for endogenous PALS1; in (H) 53% of GFP-Ap80 puncta were positive for both PALS1 and KIF13B-ΔMotor-HA, and 21% were positive for KIF13B-ΔMotor-HA but not PALS1 (n=57). We did not observe co-localization of KIF13B-ΔMotor-HA and PALS1 in the absence of GFP-Ap80. **(I)** Confocal images of filter-grown PALS1-A2E MDCK-II cells (Tan et al., 2020) stained with acetylated tubulin antibody (magenta) to mark the cilium (closed arrow). DNA is stained with DAPI. **(J)** Confocal images of filter-grown PALS1-A2E MDCK-II cells subjected to APEX2-mediated proximity biotinylation for 1 min, followed by staining with fluorescently labeled streptavidin (SA-AF-568; red) and acetylated tubulin antibody (magenta) to mark the cilium (closed arrow). Upper and lower panels show the same cell viewed from the top and side, respectively. Open arrow shows PALS1 staining at the cell-cell junction.

Journal of Cell Science • Supplementary information

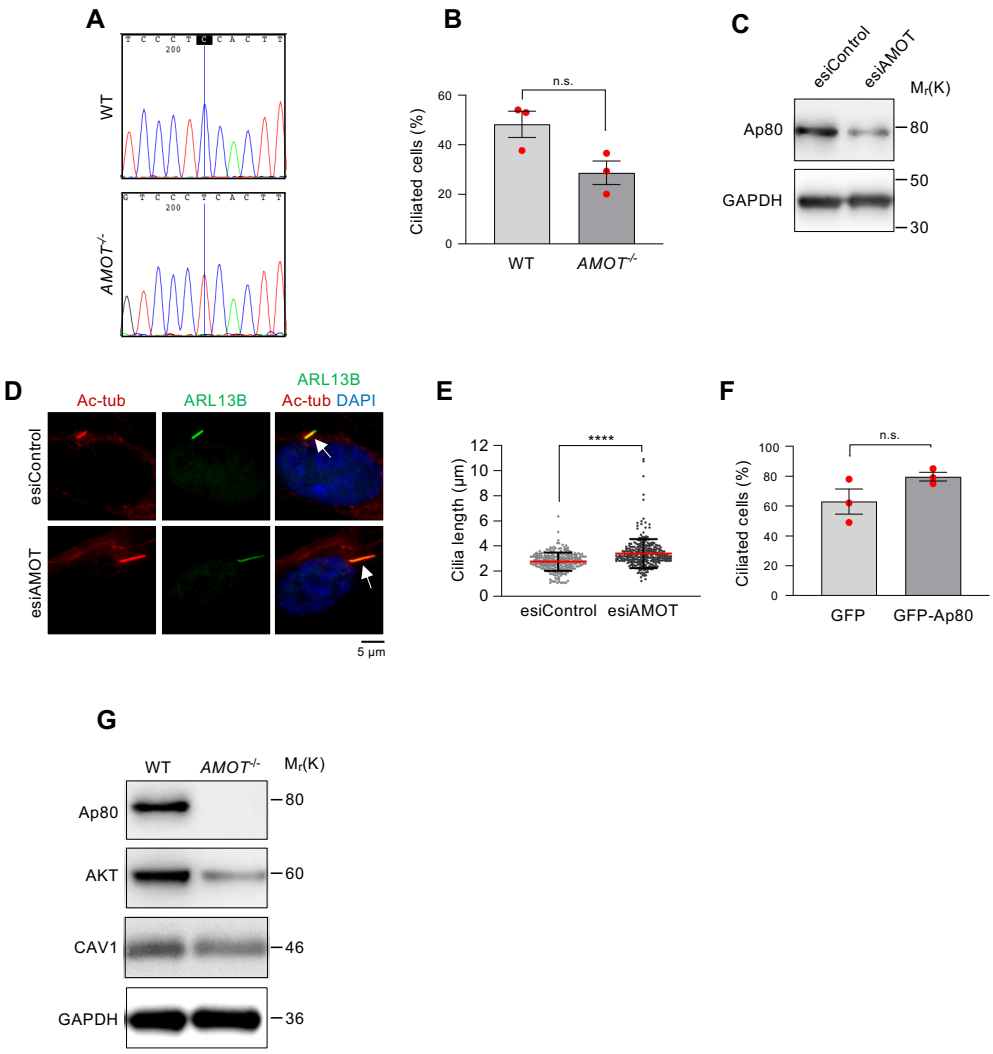


Fig. S4. Characterization of AMOT-depleted cells. Related to Figure 3. (A) Chromatogram illustrating the genomic sequence of WT control RPE1 cells (upper panel) and the *AMOT*$^{-/-}$ clone (lower panel) in the region targeted by CRISPR/Cas9. The marked cytosine is deleted in the *AMOT*$^{-/-}$ clone, causing a premature stop codon. **(B)** Quantification of ciliation frequency of WT (parental) and *AMOT*-/- RPE1 cells. At least 100 cells were analyzed per condition per experiment (n=3). Data are represented as mean ± SD and significance was determined using an unpaired, two-tailed Student's t test (n.s., P ≥ 0.05, not significant). **(C)** Immunoblot analysis of WT RPE1 cells treated with esiAMOT or control esiRNA. Blots were probed with indicated antibodies. Quantification of band intensities showed an average Ap80 protein level of 49.1% ±11.6% in esiAMOT treated cells relative to esiControl treated cells (n=3). **(D)** IFM analysis of serum-deprived WT cells treated with esiAMOT or control esiRNA. Cells were stained with antibodies against acetylated a-tubulin (AcTub, red) and ARL13B (green) to mark the cilium (arrows). DNA was stained with DAPI (blue). **(E)** Quantification of cilia length based on data as

Journal of Cell Science • Supplementary information

shown in (D), with at least 69 cilia measured in total per condition (n=3). Data are represented as mean ± SD and significance was determined using an unpaired, two-tailed Student's *t* test: ****, P≤0.0001. (**F**) Quantification of ciliation frequencies in RPE1 cells expressing GFP or GFP-Ap80. Between 32-102 transfected cells were analyzed per condition per experiment (n=3). Data are represented as mean ± SD and significance was determined using an unpaired, two-tailed Student's *t* test (n.s., P ≥ 0.05, not significant). (**G**) Immunoblot analysis of WT and *AMOT*$^{-/-}$ RPE1 cells using the indicated antibodies. Note the reduced level of AKT in the *AMOT*$^{-/-}$ cells. CAV1 and GAPDH were used as loading control.

Journal of Cell Science • Supplementary information

Table S1. Primers used in this study.

Primer name	Sequence 5'-3' *	Used for
Ap80F	CCGAATTCTATGCCTCGGGCTCAGCCA	FLAG-Ap80-NB GFP-Ap80
Ap80F_HindIII	CCAAGCTTATGCCTCGGGCTCAGCCA	Ap80-GFP
Ap80(735)R	CAGGATCCTTATTCTGAAACGTTGGTGGGCTG	FLAG-Ap80-NB FLAG-Ap80-B
Ap80(106)F	CCGAATTCTAACCGGAACTTGAGGCAAGA	FLAG-Ap80-BC FLAG-Ap80-B
Ap80FL_R	CAGGATCCTTAGATGAGATATTCCACCATCTC	FLAG-Ap80-BC FLAG-Ap80-C GFP-Ap80
Ap80_R_BamH 1	AAGGATCCGCGATGAGATATTCCACCATCTCTGC A	Ap80-GFP
Ap80(670)F	ACGAATTCTAGGGAACTGGAATCCCTGAG	FLAG-AP80-C
13B_561F	CCGGTACCTCCATGAAGAACGAGAATAGTTC	GFP-Tail 7, 8, 11
KIF13B_1702R (13B_5106R)	AAGGATCCTCATCGGAGCCACTCCGG	GFP-Tail 7, 9
13B_3981R	CAGGATCCTCAATTGGCTGCCATTCTTGCTA	GFP-Tail 8
KIF13B_750F	AAAAAGGTACCATTTGGTCTTTGGAAAAACTGG AC	GFP-Tail 9, 12
KIF13B_1500R	AAGGATCCTCAGATGTCCGGGCTGGCTG	GFP-Tail 11, 12
ZAN_030	AAAAGGTACCATGTCTCATCGAAGTTCTATTCCT	Tail 1-HA
ZAN_102	AAAAGGTACCATGATCAGGGTGACCAGGATGG	Tail 16-HA
ZAN_031	AAAATCTAGATTATCAAGCGTAATCTGGAACAT CGTATGGGTAGCTGGCCCAGGATTTC	Tail 1-HA, Tail 16-HA
Ap130 1r	CAGTGCTAGGGCCTGGAATC	Sequencing of *AMOT*$^{-/-}$ RPE1 cells
Ap130 2f	GACTGGATTTCCTGCCCCTG	Sequencing of *AMOT*$^{-/-}$ RPE1 cells

* Relevant restriction endonuclease sites are underlined.

Journal of Cell Science • Supplementary information

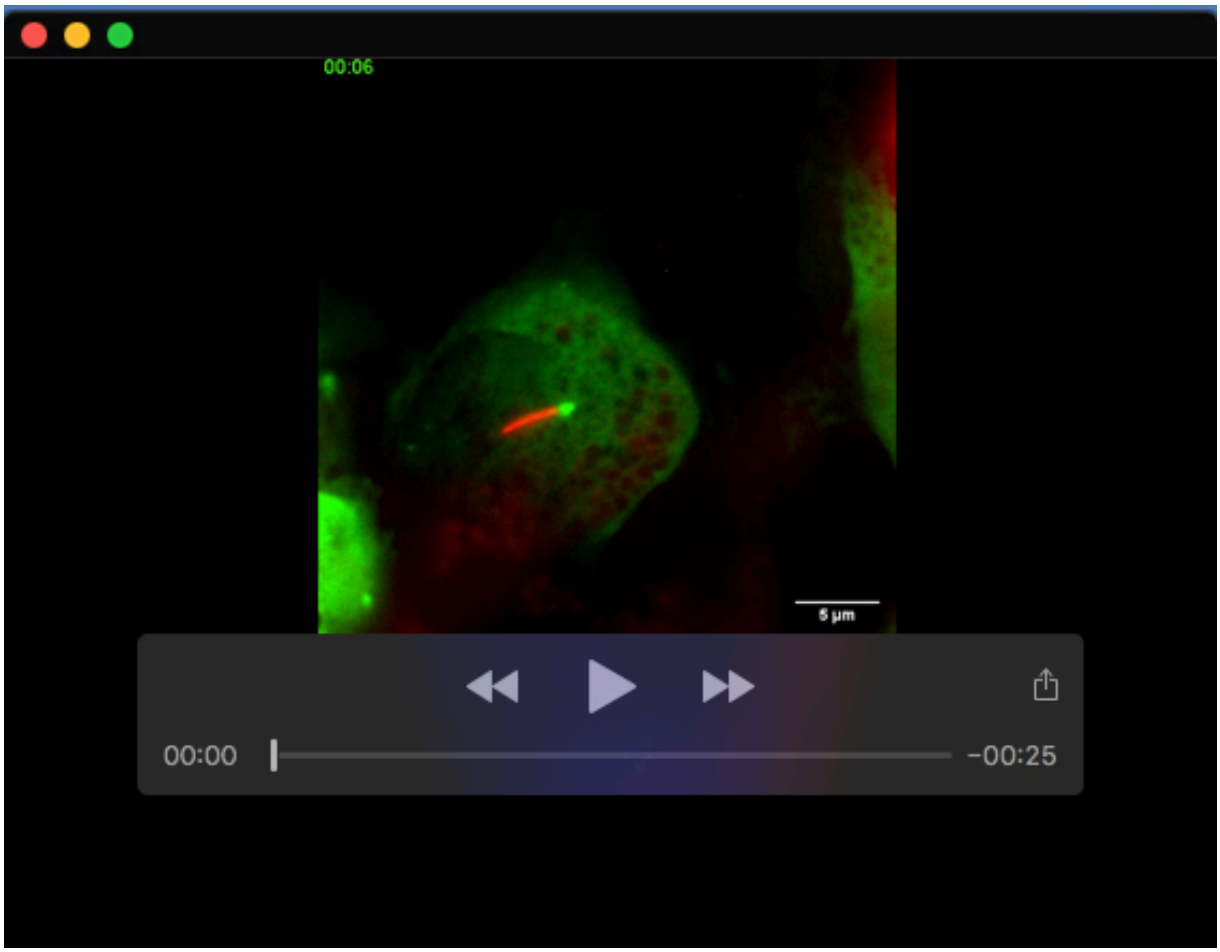
Table S2. Plasmids used in this study.

Vector	Protein product encoded by insert*	Reference/source
pEGFP-C1	none	Clontech
pEGFP-N1	none	Clontech
pFLAG-CMV2	none	Sigma
pCMV-FLAG	Ap130	(Yi et al., 2013)
pCMV-FLAG	Ap80	(Yi et al., 2013)
pFLAG-CMV2	Ap80 aa 1-245 (NB)	This study
pFLAG-CMV2	Ap80 aa 35-675 (BC)	This study
pFLAG-CMV2	Ap80 aa 222-675 (C)	This study
pFLAG-CMV2	Ap80 aa 35-245 (B)	This study
pEGFP-C1	Ap80	This study
pEGFP-N1	Ap80	This study
pEGFP	DLG1 (rodent)	Gift from Jeffrey Miner
pcDNA3	HA-ERp57	(Appenzeller-Herzog et al., 2008)
pEGFP-C1	KIF13B	(Asaba et al., 2003)
Modified pEGFP-C1 vector with BirA insertion	KIF13B aa 607-1826 (Tail 2; designated C2 in (Serra-Marques et al., 2020))	(Lansbergen et al., 2006, Serra-Marques et al., 2020)
Modified pEGFP-C1 vector with BirA insertion	KIF13B aa 752-1826 (Tail 3; designated C3 in (Serra-Marques et al., 2020))	(Lansbergen et al., 2006, Serra-Marques et al., 2020)
pEGFP-C1	KIF13B aa 1289-1826 (Tail 1)	(Schou et al., 2017)
pEGFP-C1	KIF13B aa 1000-1826 (Tail 4)	(Schou et al., 2017)
pEGFP-C1	KIF13B aa 840-1826 (Tail 5)	(Schou et al., 2017)
pEGFP-C1	KIF13B aa 561-1826 (Tail 6)	(Schou et al., 2017)
pEGFP-C1	KIF13B aa 561-1702 (Tail 7)	This study
pEGFP-C1	KIF13B aa 561-1327 (Tail 8)	This study
pEGFP-C1	KIF13B aa 750-1702 (Tail 9)	This study
pEGFP-C1	KIF13B aa 561-1500 (Tail 11)	This study

pEGFP-C1	KIF13B aa 750-1500 (Tail 12)	This study
pcDNA3	KIF13B-Δmotor-eGFP	(Lamason et al., 2010)
pcDNA3	KIF13B-HA	(Lamason et al., 2010)
pcDNA3	KIF13B-Δmotor-HA	(Lamason et al., 2010)
pcDNA3.1	KIF13B aa 1289-1826 (Tail 1-HA)	This study
pcDNA3.1	KIF13B aa 1500-1826 (Tail 16-HA)	This study
pBa-eGFP	MARK2	Addgene #66706
Gateway plasmid	3xHA-PALS1	(Gosens et al., 2007)
pEGFP-N1	PALS1	(Tan et al., 2020)
pSpCas9(BB)-2A-Puro (PX459)	25 nt oligo targeting exon 1 in AMOT: 5'-AATACCGTGGTCCCTCCACTTGG -3'	This study; (Ran et al., 2013)

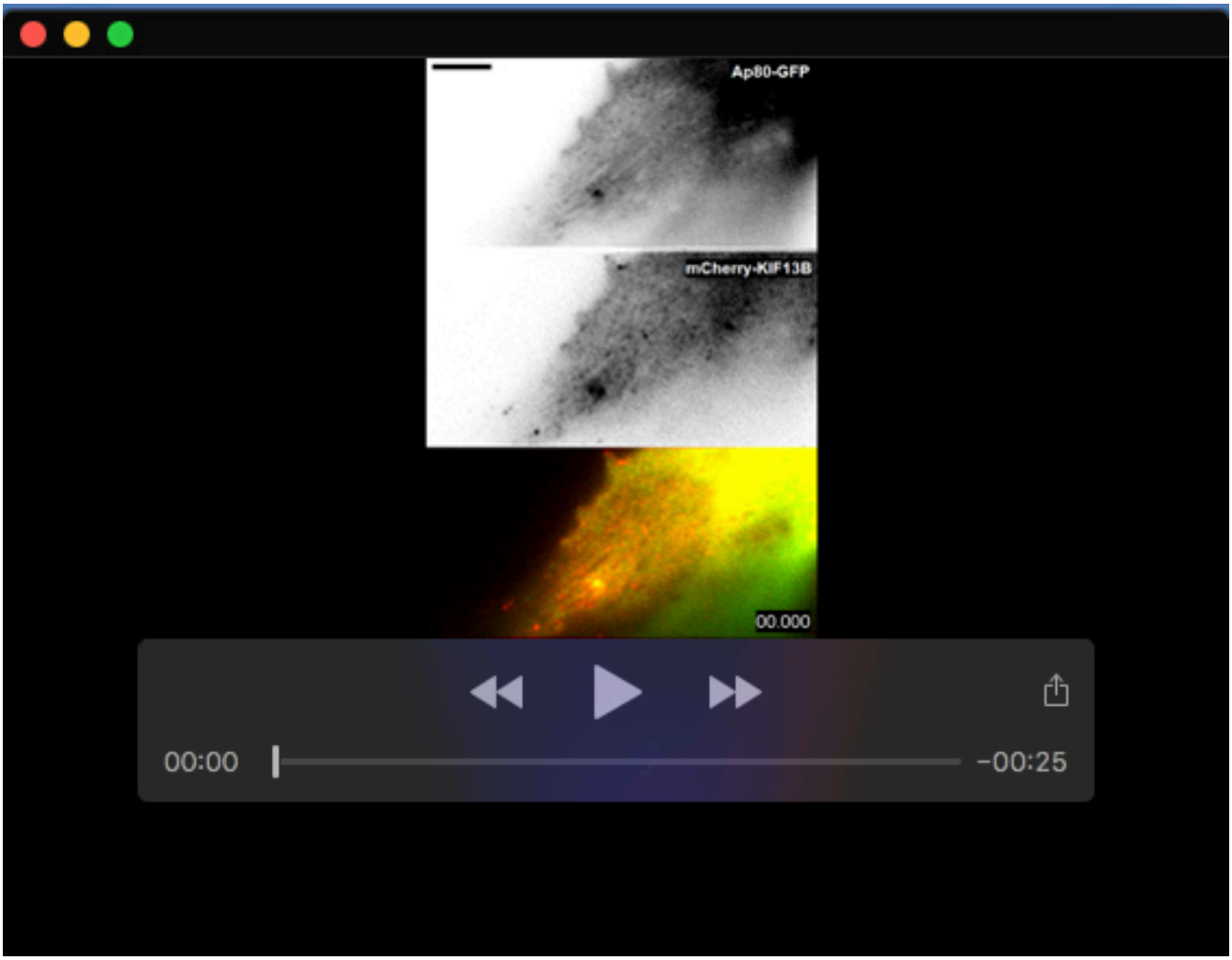
*Unless otherwise indicated, names refer to human proteins.

Journal of Cell Science • Supplementary information



Movie 1. Example of live imaging of Ap80-GFP in RPE1 cells stably expressing SMO-tRFP. Time-lapse movie of a cell co-expressing SMO-tRFP and Ap80-GFP, recorded on a spinning disk confocal microscope, with time indicated in seconds.

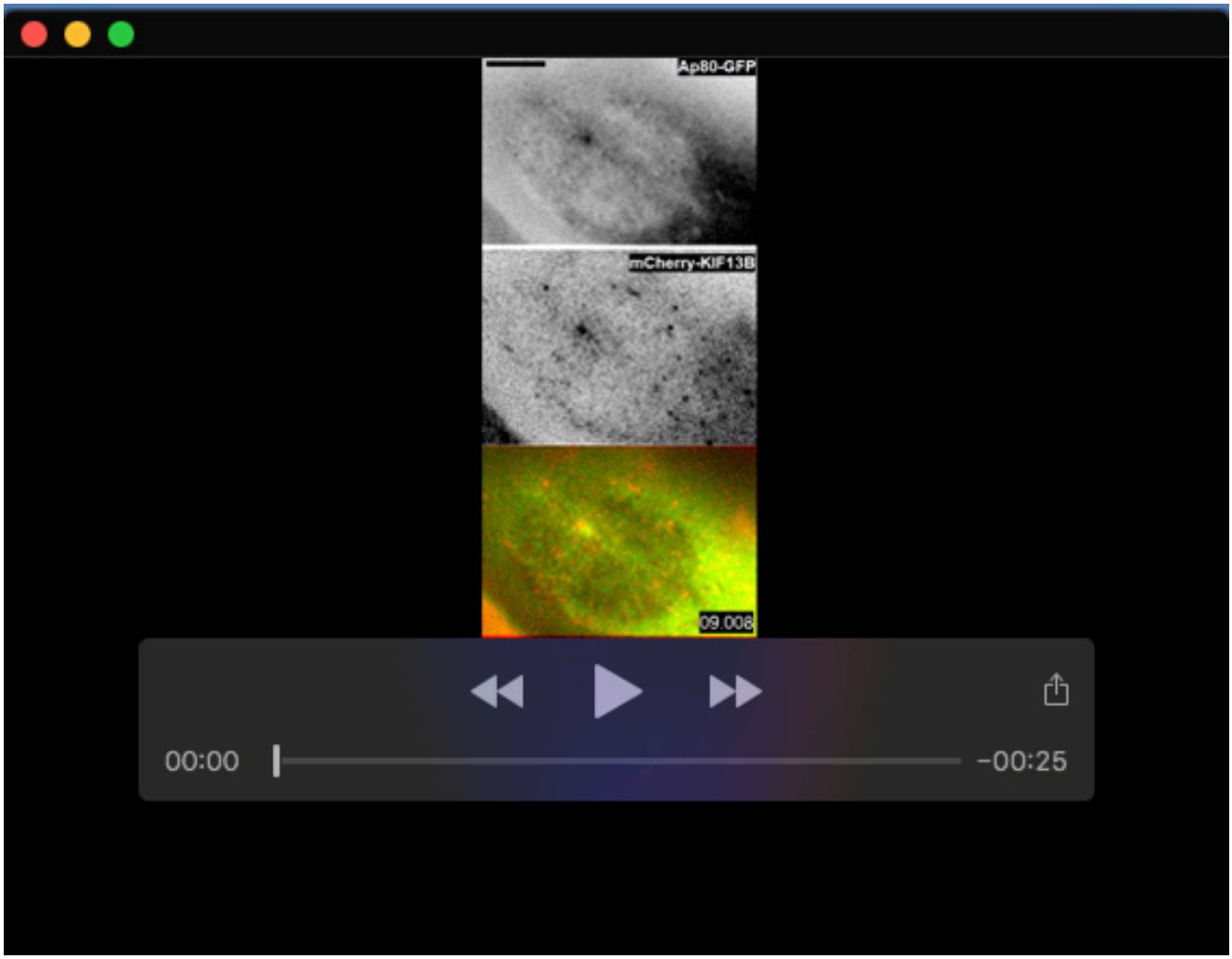
Journal of Cell Science • Supplementary information



Movie 2. Live-cell imaging of mCherry-KIF13B and Ap80-GFP.

RPE1 cells co-expressing Ap80-GFP and mCherry-KIF13B were imaged using TIRF microscopy. No colocalization between Ap80-GFP and mCherry-KIF13B was detected except at the centrosome region. Scale bar 5 µm. 5 frames per second stream imaging with 20 ms exposure.

Journal of Cell Science • Supplementary information



Movie 3. Another example of live-cell imaging of mCherry-KIF13B and Ap80-GFP. RPE1 cells co-expressing Ap80-GFP and mCherry-KIF13B were imaged using TIRF microscopy. No colocalization between Ap80-GFP and mCherry-KIF13B was detected except at the centrosome region. Scale bar 5 µm. 5 frames per second stream imaging with 20 ms exposure.

Journal of Cell Science • Supplementary information

Figure S5

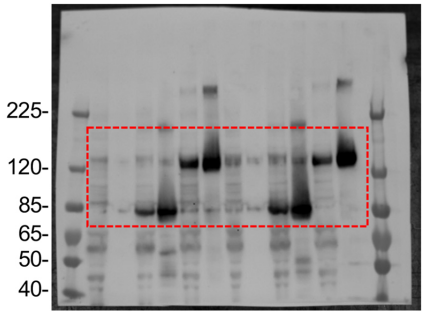


Figure 1A, FLAG blot

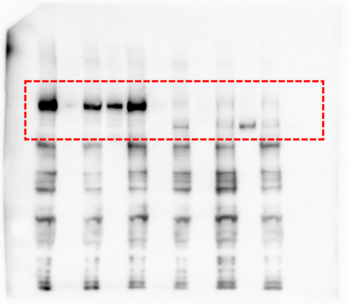


Figure 1A, HA blot

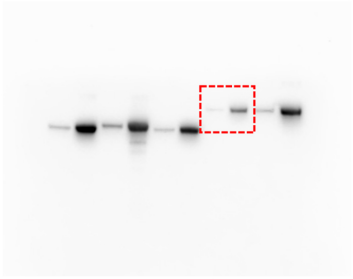


Figure 1B, GFP blot

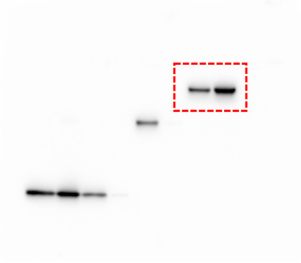


Figure 1B, FLAG blot

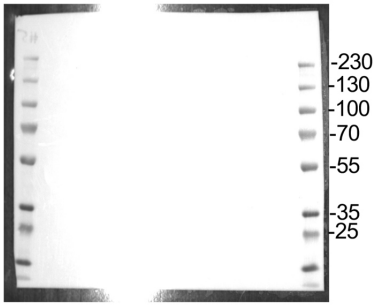


Figure 1B, membrane w.
MW markers for FLAG blot

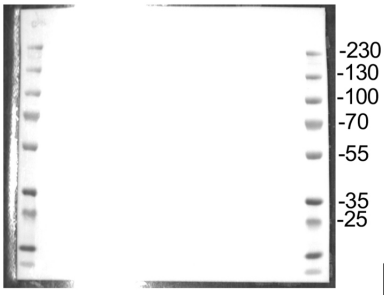


Figure 1B, membrane w.
MW markers for GFP blot

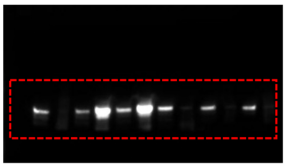


Figure 1D, HA blot

Figure 1D, membrane w.
MW markers for HA blot

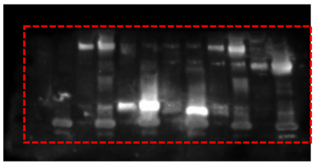


Figure 1D, FLAG blot

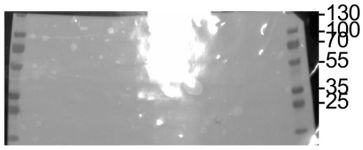


Figure 1D, membrane w.
MW markers for FLAG blot

Journal of Cell Science • Supplementary information

Figure S5 (continued)

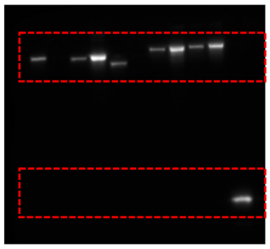


Figure 2B, GFP blot

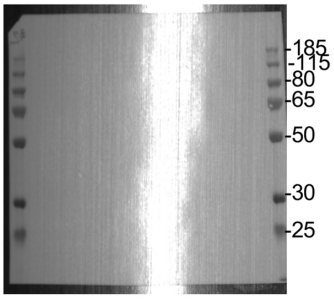


Figure 2B, MW markers for GFP blot

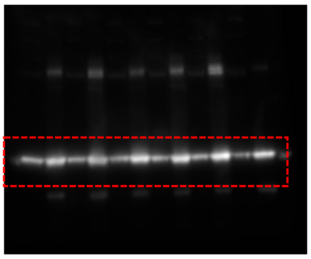


Figure 2B, FLAG blot

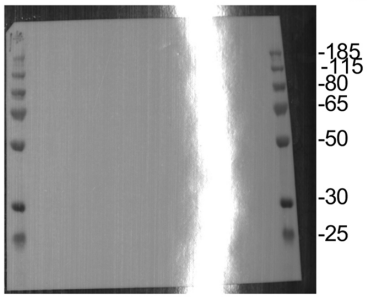


Figure 2B, membrane w. MW markers for FLAG blot

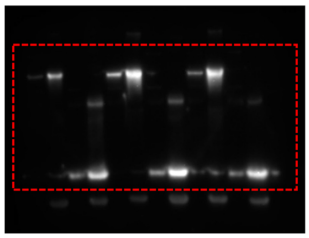


Figure 2C, FLAG blot

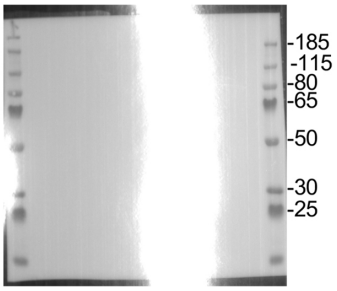


Figure 2C, membrane w. MW markers for FLAG blot

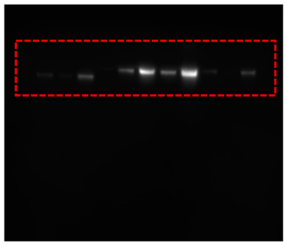


Figure 2C, GFP blot

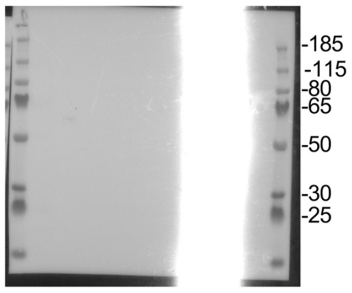


Figure 2C, membrane w. MW markers for GFP blot

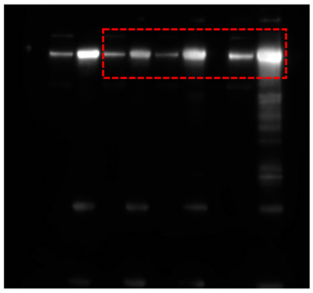


Figure 2D, FLAG blot

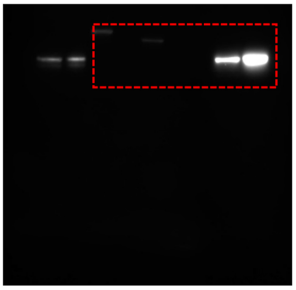


Figure 2D, GFP/HA blot

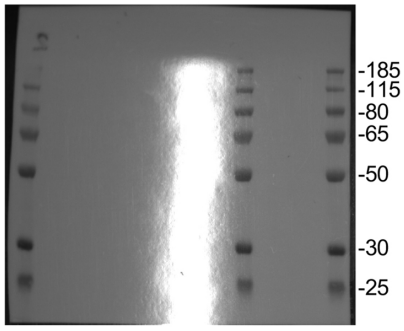


Figure 2D, membrane w. MW markers for both blots

Journal of Cell Science • Supplementary information

Figure S5 (continued)

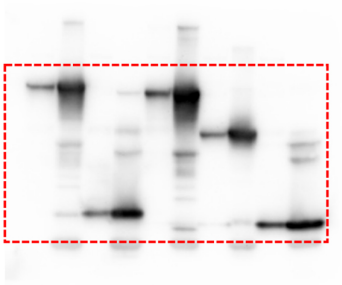


Figure 2E, FLAG blot

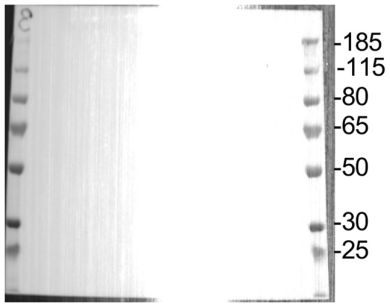


Figure 2E, membrane w. MW marker for FLAG blot

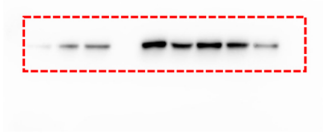


Figure 2E, HA blot

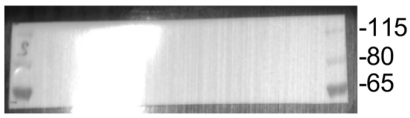


Figure 2E, membrane w. MW marker for HA blot

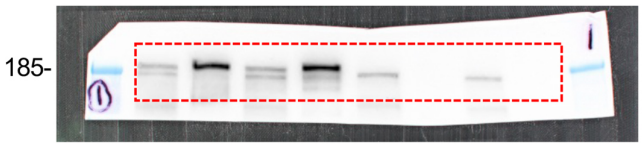


Figure 2F, GFP blot (upper)

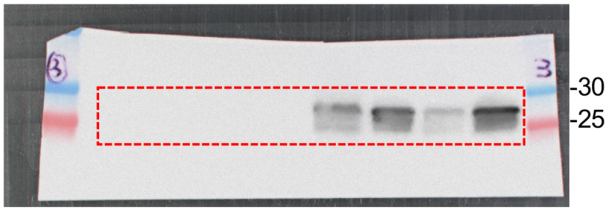


Figure 2F, GFP blot (lower)

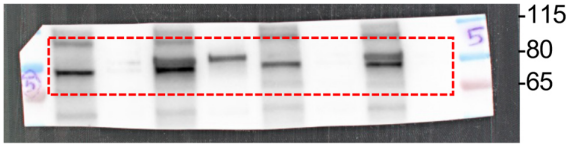


Figure 2F, HA blot

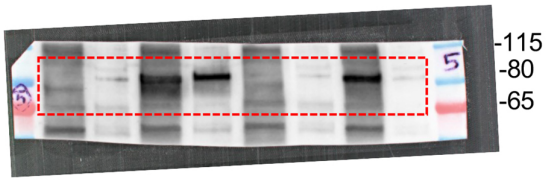


Figure 2F, FLAG blot

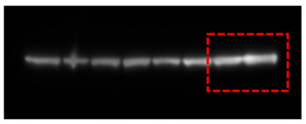


Figure 3D, GAPDH blot

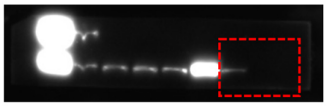


Figure 3D, Ap80 blot

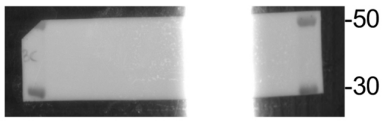


Figure 3D, membrane w. MW markers for GAPDH blot

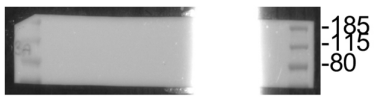


Figure 3D, membrane w. MW markers for Ap80 blot

Journal of Cell Science • Supplementary information

Figure S5 (continued)

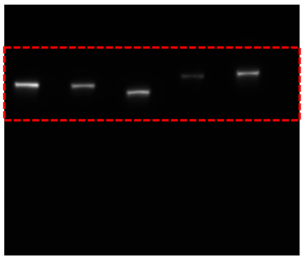


Figure S1A, GFP blot

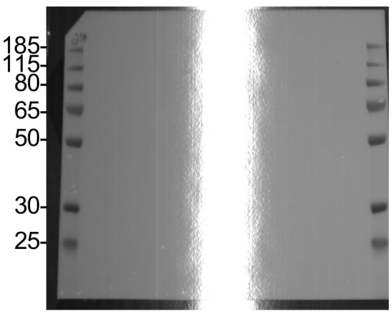


Figure S1A, membrane w. MW marker for GFP blot

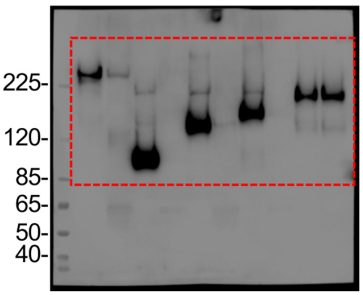


Figure S1B, GFP blot

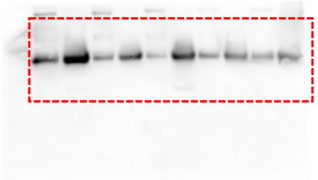


Figure S1B, FLAG blot

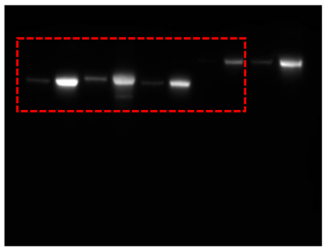


Figure S1C, GFP blot

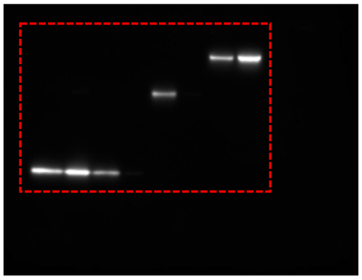


Figure S1C, FLAG blot

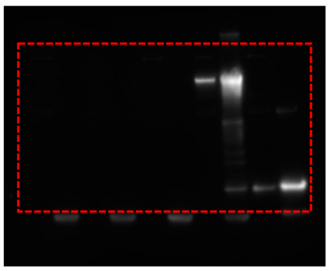


Figure S1D, FLAG blot

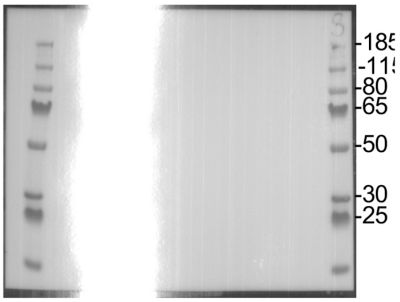


Figure S1D, membrane w. MW markers FLAG blot

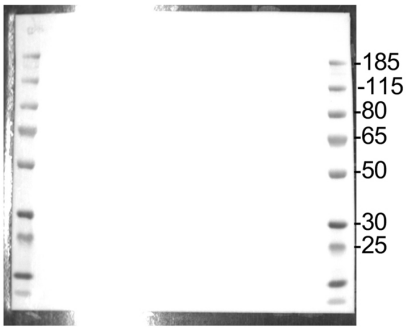


Figure S1C, membrane w. MW marker for both blots

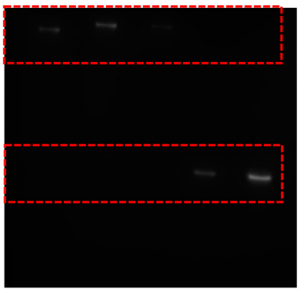


Figure S1D, GFP blot

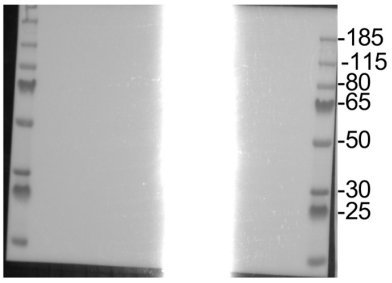


Figure S1D, GFP blot membrane w. MW markers

Journal of Cell Science • Supplementary information

Figure S5 (continued)

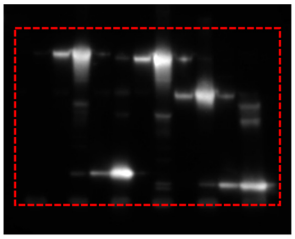


Figure S2A, FLAG blot

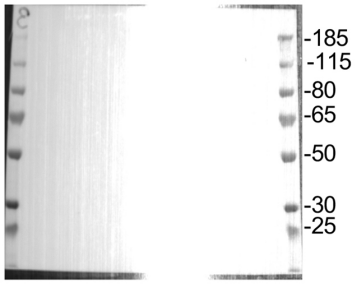


Figure S2A, FLAG blot membrane w. MW markers

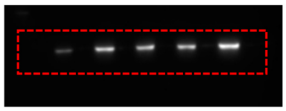


Figure S2A, HA blot

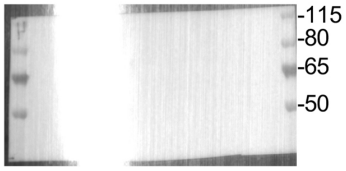


Figure S2A, HA blot membrane w. MW markers

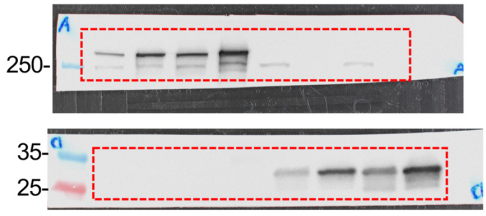


Figure S2B, GFP blots

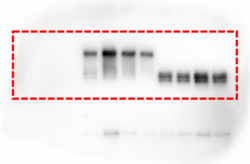


Figure S2D, IP HA blot

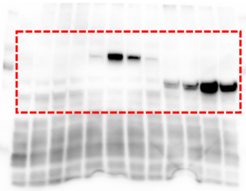


Figure S2D, input HA blot

Figure S2B, HA blot

Figure S2D, input GFP blot

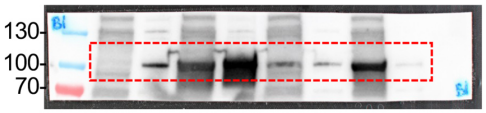


Figure S2B, FLAG blot

Figure S2D, IP GFP blot

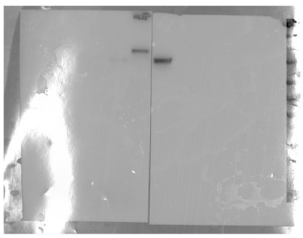


Figure S2D, IP GFP blot (white light image)

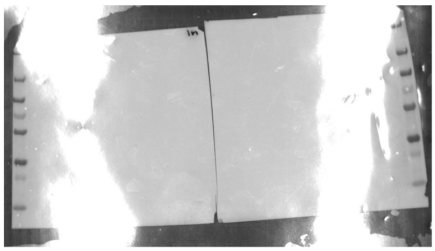


Figure S2D, input GFP blot (white light image)

Journal of Cell Science • Supplementary information

Figure S5 (continued)

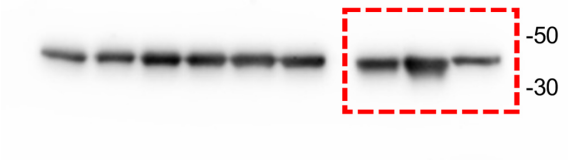


Figure S3A, left panel, GAPDH blot

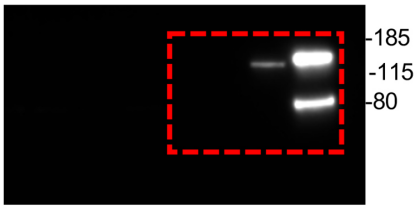


Figure S3A, left panel, AMOT blot

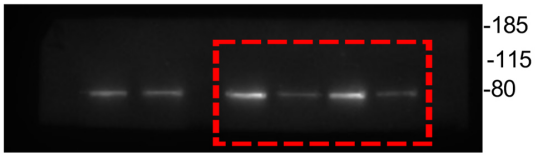


Figure S3A, middle panel, AMOT blot

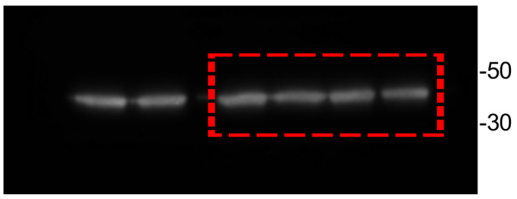


Figure S3A, middle panel, GAPDH blot

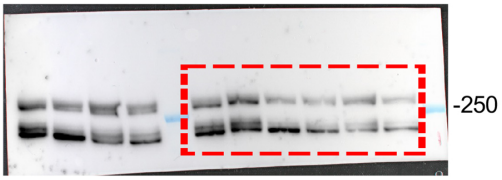


Figure S3A, right panel, KIF13B blot

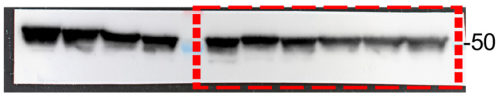


Figure S3A, right panel, α–tubulin blot

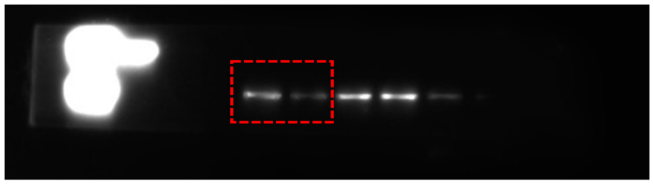


Figure S4C, AMOT blot

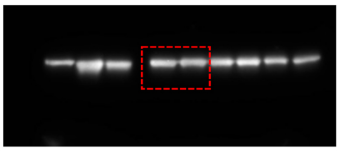


Figure S4C,GAPDH blot

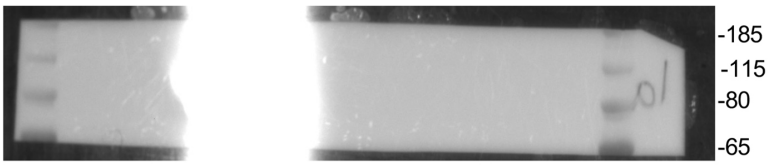


Figure S4C, membrane w. MW marker for AMOT blot

Figure S4C, membrane w. MW marker for GAPDH blot

Journal of Cell Science • Supplementary information

Figure S5 (continued)

Figure S4G, AMOT blot

Figure S4G, AKT blot

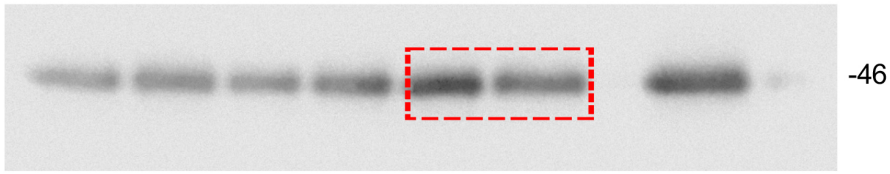


Figure S4G, CAV1 blot

Figure S4G, GAPDH blot

Fig. S5. Original, uncropped immunoblots. Blots are organized in order of appearance with corresponding figure panels indicated below each blot. Boxed areas indicate reagions of the blots used in the respective figure panels. Molecular mass markers are indicated in kDa.

Journal of Cell Science • Supplementary information