Supplementary Figure S1

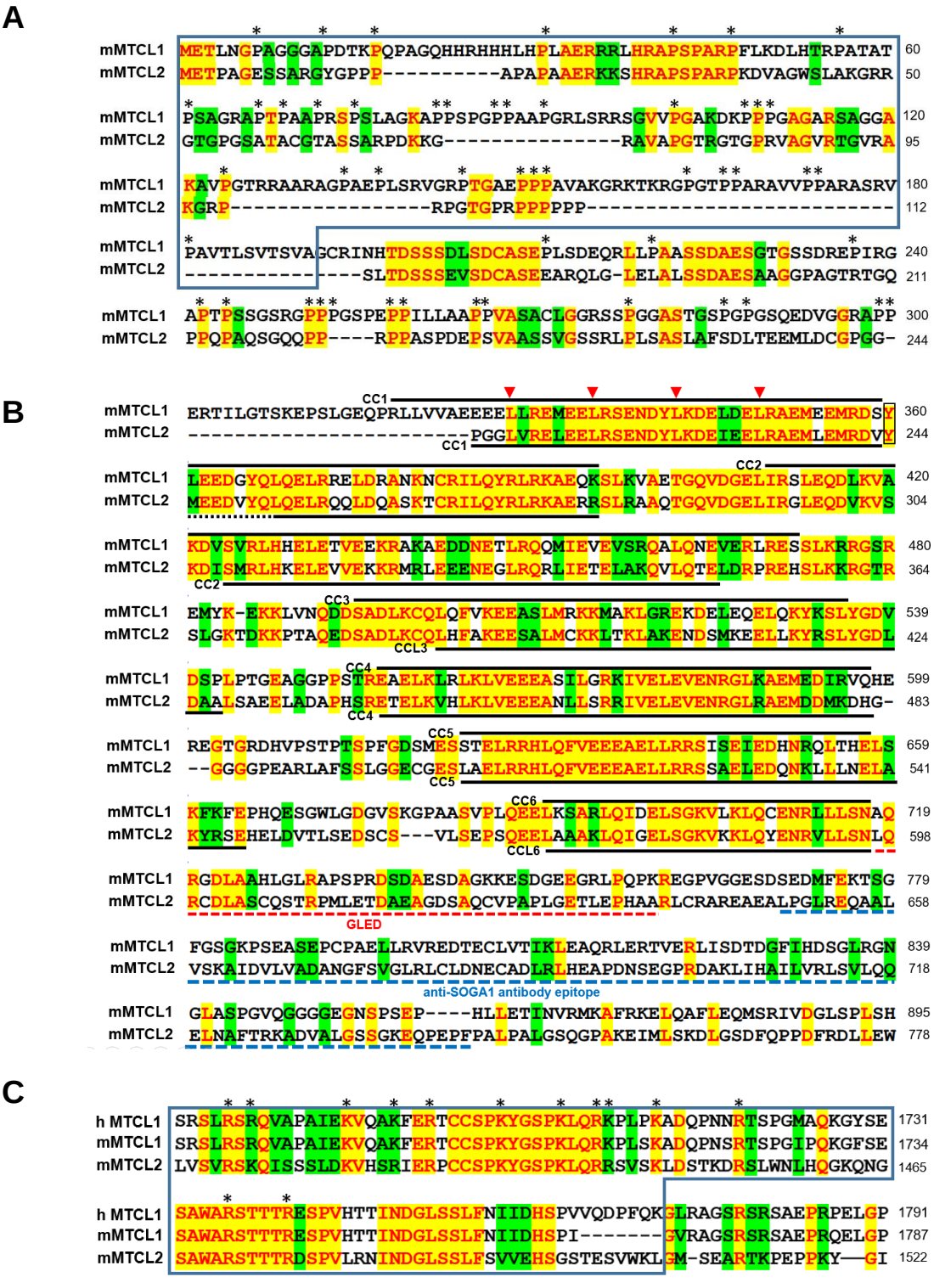


Fig. S1. Sequence alignment of amino acid sequences of mouse MTCL1 and 2.

(A) The N-terminal sequences. Boxed region corresponds to N-MTBD of MTCL1. Asterisks indicate the positions of proline highly condensed in this region. (B) The N-terminal coiled-coil region. The positions of each coiled-coil motif (CC) or coiled-coil-like motif (CCL) of MTCL1 or 2 are indicated by bold lines on the top or bottom of each sequence, respectively. GLED sequence of MTCL2 is underlined by a red dashed line. Four leucine residues mutated in 4LA or 4LP mutants are indicated red arrowheads. A tyrosine residue that disrupts the periodicity of CC1 is boxed. Blue dotted lines indicate the region corresponding to the epitope for anti-SOGA1 antibody. (C) The sequences of the C-terminal MT-binding regions. Because MTCL1 C-MTBD (boxed) was defined for human protein (Sato et al., 2013), the human sequence of MTCL1 is also included in this alignment. The region of mouse MTCL2 corresponding to MTCL1 C-MTBD is designated the “KR-rich region” since the conserved basic residues (asterisks) are condensed.

Supplementary Figure S2

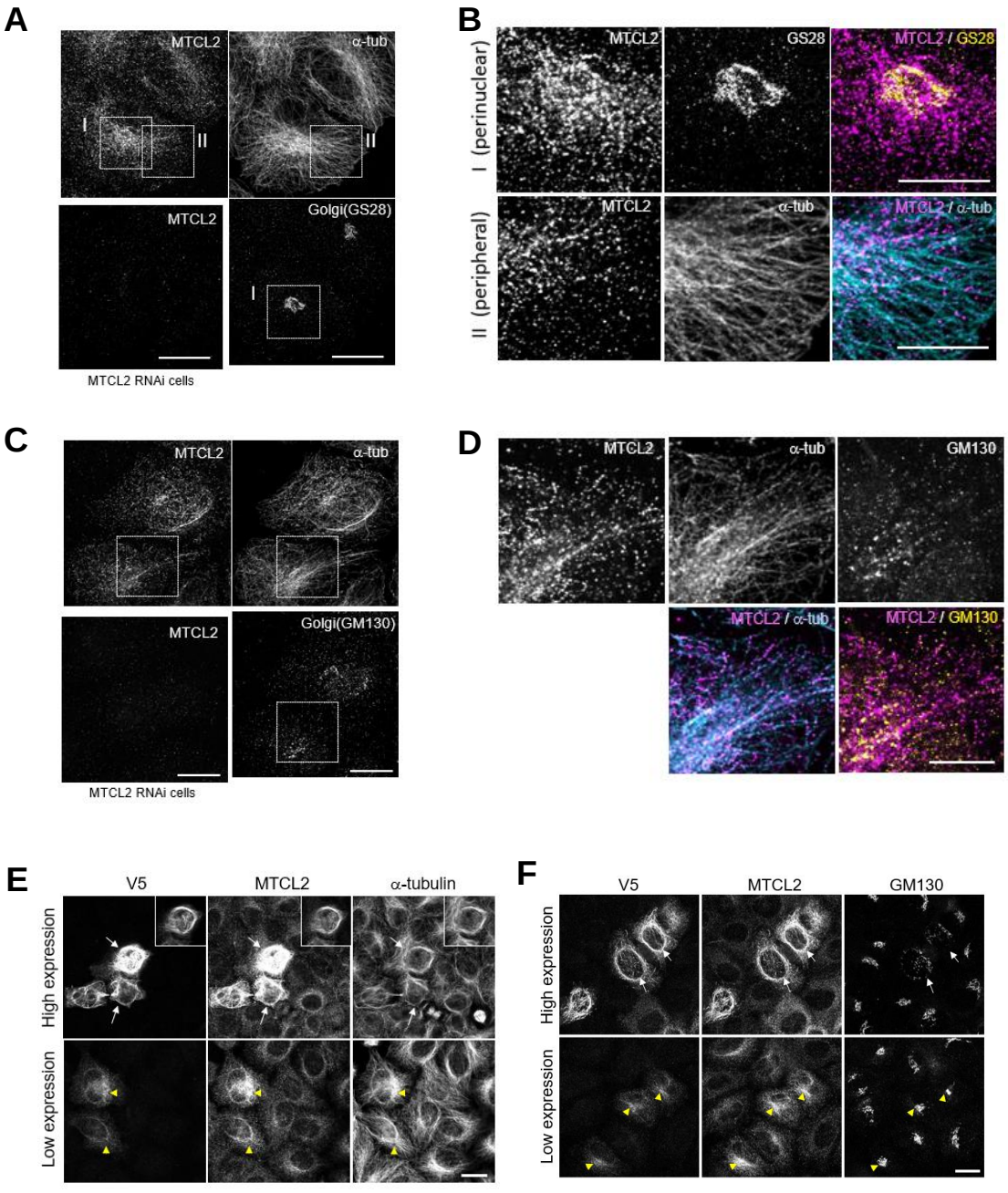


Fig. S2. Confirmations of subcellular localization of MTCL2.

(A) HeLa-K cells fixed with 4% paraformaldehyde were stained with anti-SOGA1 (MTCL2) together with anti-α-tubulin and anti-GS28 antibody. The specificity of anti-SOGA1 signals is indicated by their disappearance in MTCL*2*-knockdown cells subjected to the same procedures (see a lower left panel). Scale bar: 20 μm. (B) Boxed regions in (A) are enlarged to examine the colocalization of MTCL2 on the Golgi and MTs more closely. Scale bar: 10 μm. (C) HeLa-K cells were fixed with 4% paraformaldehyde after brief treatment of an extraction buffer containing 0.5% TX-100 and 4 mM EGTA. The specificity of anti-SOGA1 staining signals is indicated by their disappearance in MTCL*2*-knockdown cells subjected to the same procedures (see a lower left panel). Scale bar: 20 μm. (D). The boxed region in (C) is enlarged to examine the colocalization of MTCL2 on the Golgi and MTs more closely. Scale bar: 10 μm. (E and F) Localization of exogenously expressed MTCL2 mimics that of endogenous proteins at low expression levels. HeLa-K cells stably harboring 6xV5-tagged mouse MTCL2 expression vector (pOSTet15.1) were cultured in the presence of 100 ng/mL doxycycline and stained with the indicated antibodies. Scale bar: 20 μm. Arrows indicate cells highly expressing exogenous MTCL2, whereas yellow arrowheads indicate cells expressing exogenous MTCL2 at a level comparable to endogenous MTCL2. The insets in (E) show alternative images of a cell located at the center of the panel, in which contrasts of the individual staining signals are adjusted separately to provide unsaturated images.

Supplementary Figure S3

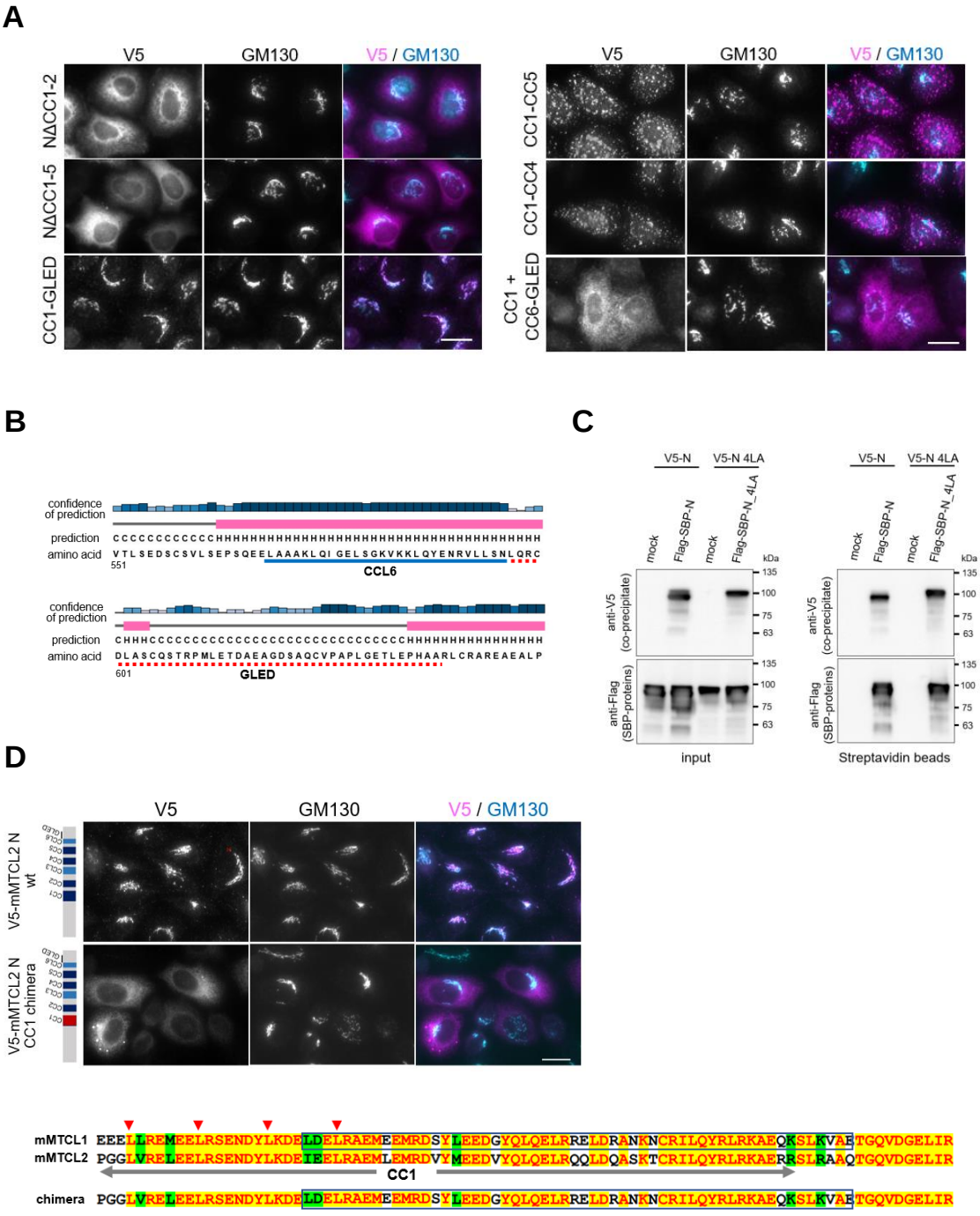


Fig. S3. The essential sequence required for the Golgi association of the MTCL2 N fragment.

(A) Subcellular localization of the indicated mutants expressed in HeLa-K cells (see Fig. 5A). Scale bar: 20 μm. (B) The amino acid sequence of GLED and its secondary structure redicted using PSIPED (http://bioinf.cs.ucl.ac.uk/psipred/). (C) A streptavidin pull-down experiment was performed for soluble extracts (input) of HEK293 cells expressing V5-N with Flag-SBP-N or V5-N 4LA with Flag-SBP-N 4LA, as indicated. In mock samples, empty backbone vectors for Flag-SBP constructs were transfected with each V5 construct. (D) Subcellular localization of the CC1 chimera of the N fragment, in which the highly conserved CC1 sequence of MTCL2 was seamlessly exchanged with that of MTCL1. Scale bar, 20 μm. The amino acid sequence of CC1 in the chimera mutant is shown below.

Supplementary Figure S4

A

wt

MT distribution (skewness)

	n			p-value	
	NS	KD	res	NS/KD	KD/rescue
1st	56	56	23	1.50E-04	2.03E-03
2nd	99	79	58	3.96E-07	8.40E-03
3rd	62	88	55	3.27E-04	8.44E-05
sum	217	223	136	5.77E-10	1.71E-06

4LA

	n			p-value	
	NS	KD	res	NS/KD	KD/rescue
1st	81	94	51	5.64E-08	0.600
2nd	123	93	71	1.86E-03	0.270
3rd	77	125	93	0.050	0.890
sum	281	312	215	7.46E-11	0.984

ΔKR

	n			p-value	
	NS	KD	res	NS/KD	KD/rescue
1st	65	56	45	2.01E-02	0.490
2nd	112	106	58	5.88E-05	0.534
3rd	73	106	47	7.37E-03	0.072
sum	250	268	150	1.07E-07	0.1056

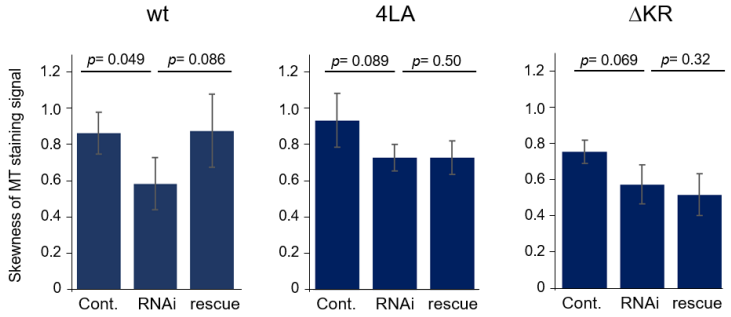
Golgi ribbon expansion angle

	n			p-value	
	NS	KD	res	NS/KD	KD/rescue
1st	196	149	196	2.77E-13	2.20E-16#
2nd	358	339	177	2.20E-16#	2.20E-16#
3rd	114	114	73	1.01E-05	6.85E-05
sum	668	602	446	2.20E-16#	2.20E-16#

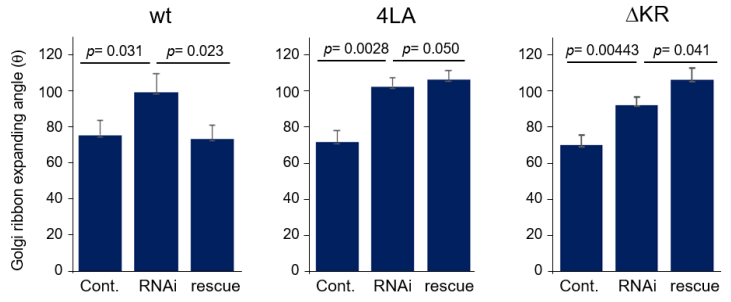
	n			p-value	
	NS	KD	res	NS/KD	KD/rescue
1st	85	65	47	6.85E-12	0.579
2nd	134	79	68	4.00E-11	0.766
3rd	120	126	50	8.88E-05	0.028*
sum	339	270	165	2.20E-16	0.265

	n			p-value	
	NS	KD	res	NS/KD	KD/rescue
1st	95	81	56	1.60E-09	0.698
2nd	83	113	88	4.31E-03	1.95E-03*
3rd	102	110	41	6.25E-04	0.169
sum	280	304	185	1.32E-12	3.64E-03*

B



C



D

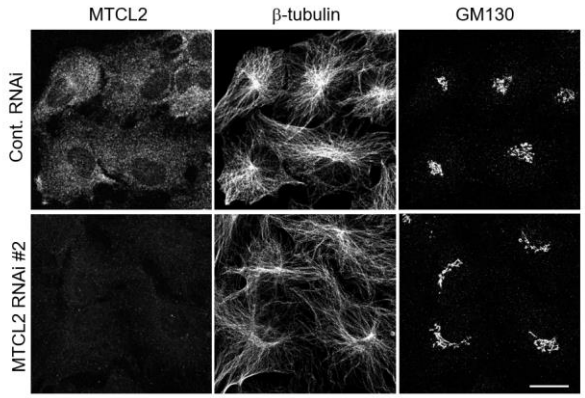


Fig. S4. Statistical data for technical replicates of the rescue experiments.

(A) Numbers of biological replicates (n) and *p* values estimated by the Wilcoxon test are listed for each rescue experiment replicated three times. Top, experiments to examine rescue activity for MT distribution. Bottom, Golgi ribbon compactness. The *p* values indicated by # mean less than 2.20e-16. Expression of MTCL2 mutants (4LA, ΔKR) tended to worsen the knockdown phenotypes of MTCL2, sometimes resulting in low *p* values in KD/rescue comparison, as indicated by asterisks. Note that essential trends of each MTCL2 mutant shown in Fig. 6 and Supplementary Figure S5 are highly reproduced except in an experiment (yellow cell) in which the MTCL2-knockdown effect was rather low. (B and C) Mean of biological replicates in each experiment listed in (A) was averaged in three technical replicates and compared between each condition. Data represent the mean ± S.D. of three independent experiments for MT distribution (B) and Golgi ribbon compactness (C). The *p* value was estimated using Student's t-test assuming a one-tailed distribution and two-sample unequal variance. (D) RPE1 cells transfected with control or MTCL2 siRNAs were subjected to immunofluorescence analysis using the indicated antibodies. Note that reduced accumulation of MTs around the Golgi and lateral expansion of the Golgi ribbon were observed in this cell line. Scale bar: 20 μm.

Supplementary Figure S5

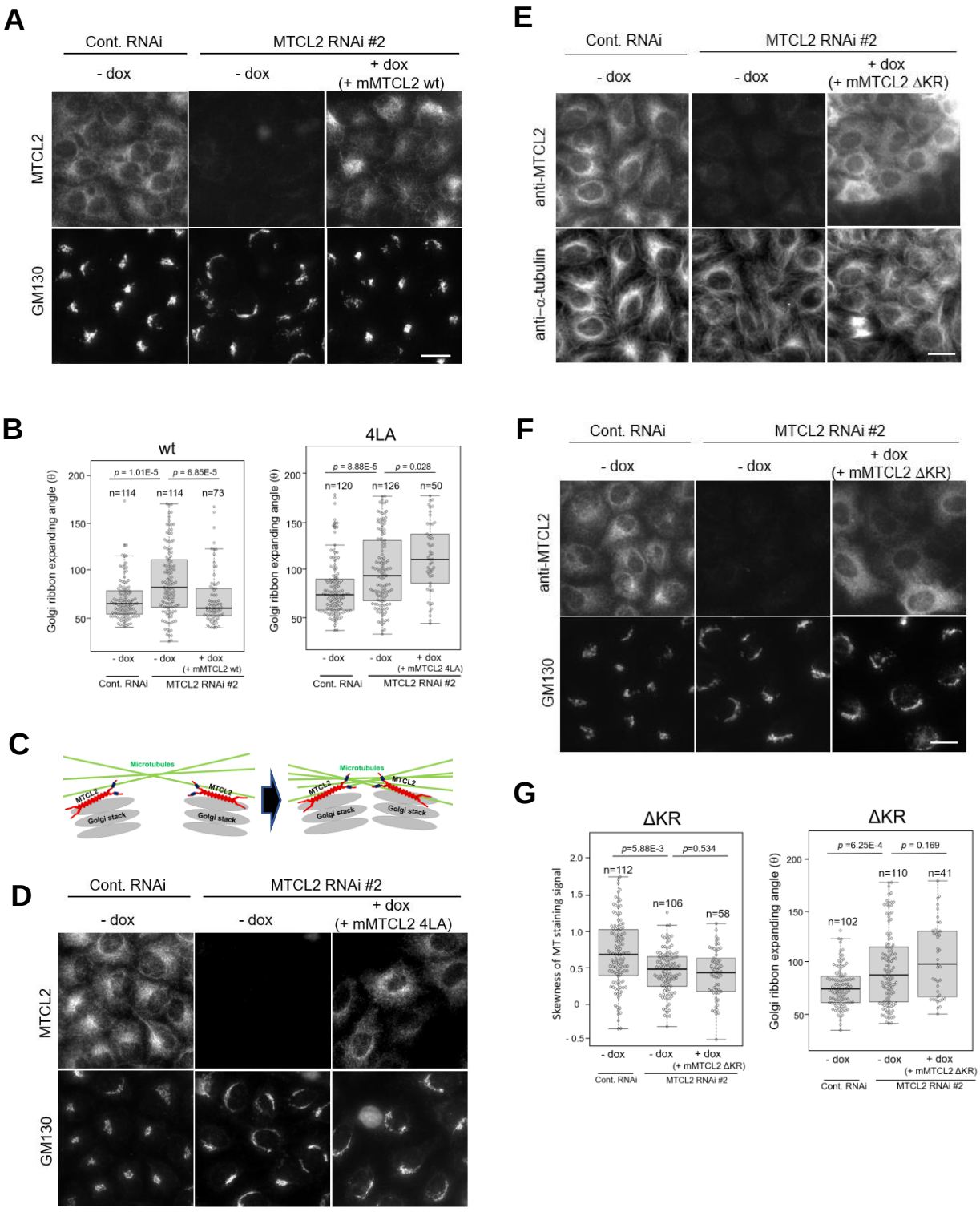


Fig. S5. MTCL2 promotes clustering of the Golgi stacks in a Golgi-association-dependent manner.

(A) HeLa-K cells stably harboring pOSTet15.1 expression vector for mouse MTCL2 were transfected with siRNAs for control or MTCL2 knockdown (#2) in the presence or absence of 100 nM doxycycline and doubly stained with anti-SOGA1 (MTCL2) and anti-GM130 antibodies, as indicated on the left. Note that cells subjected to control RNAi show compact Golgi ribbon structures at one side of the perinuclear region. Such Golgi ribbon structures become laterally expanded around the nucleus in MTCL2-knockdown cells (-dox), whereas exogenous expression of RNAi-resistant MTCL2 (+dox) strongly restores their compactness. Scale bar: 20 μm. (B) Box plots of the angle distribution in each condition (left, data for wt rescue; right, data for 4LA mutant rescue shown in (D)). The lines within each box represent medians. Data represent the results of the indicated number (n) of cells from a typical experiment (biological replicates). The *p* values were estimated using the Wilcoxon test. Statistical data of technical replicates (three independent experiments) are demonstrated in Supplementary Fig. S4.
(C) A model explaining how MT accumulation secondarily increases clustering of individual Golgi stacks.
(D) HeLa-K cells stably harboring pOSTet15.1 expression vector for mouse MTCL2 4LA were subjected to the same experimental procedure as in (A). Note that compactness of Golgi ribbon was not restored by expression of mouse MTCL2 4LA. Scale bar: 20 μm. (E-G) HeLa-K cells stably harboring pOSTet15.1 expression vector for mouse MTCL2 ΔKR were transfected with siRNAs for control or MTCL2 knockdown (#2) in the presence or absence of 100 nM doxycycline (dox). Perinuclear accumulation of MTs (E and left panel in G) and expansion of the Golgi ribbon around the nucleus (F and right panel in G) were analyzed in the same manner as described in (A) and (B). Scale bar: 20 μm.

Supplementary Figure S6

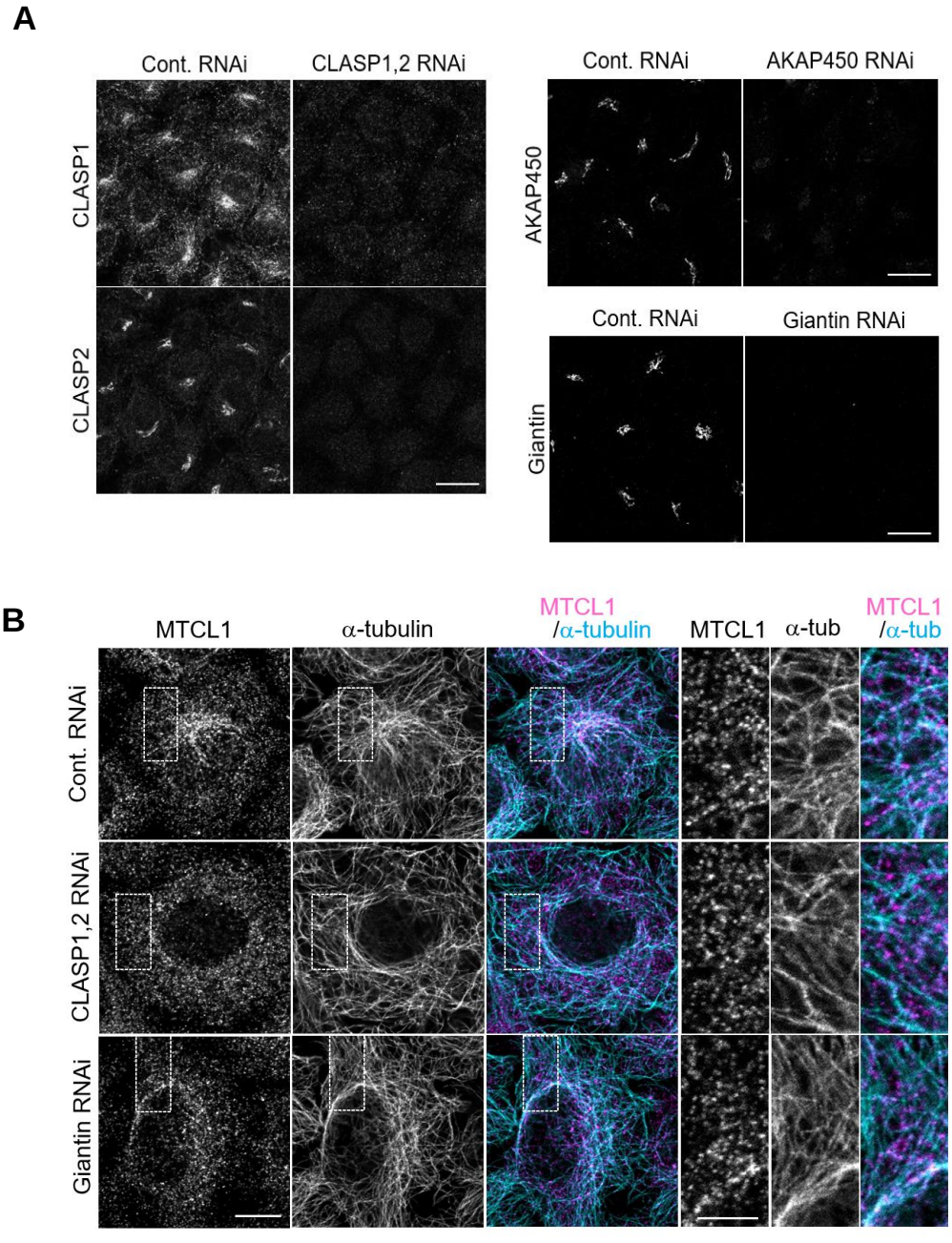


Fig. S6. Knockdown effects of CLASP, AKAP450, and giantin.

(A) Reduced expression of target proteins of the indicated siRNAs is shown. Scale bar: 20 μm. (B) Colocalization of endogenous MTCL2 with MTs in the indicated knockdown cells was examined in HeLa-K cells. Scale bar: 10 μm. Boxed regions are enlarged in the right panels. Scale bar: 5 μm.

Supplementary Figure S7

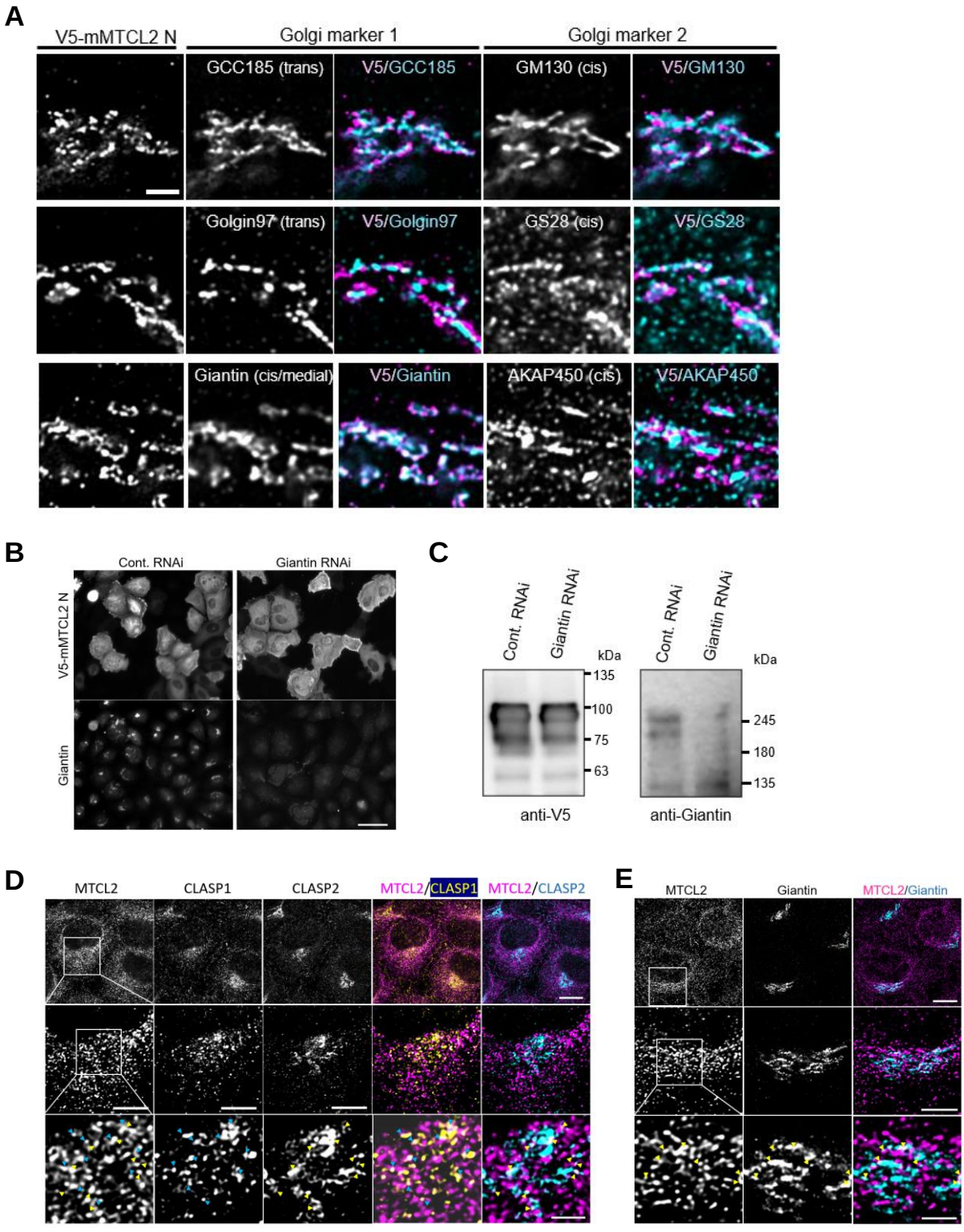


Fig. S7. Giantin is involved in the Golgi association of the MTCL2 N fragment.

(A) Subcellular localization of V5-mMTCL2 N fragment in HeLa-K cells was compared with that of Golgi-resident proteins using super-resolution microscopy. Scale bar: 2μm. Note that the N-terminal fragment of MTCL2 shows colocalization with the cis/medial Golgi protein giantin/GOLGB1 most clearly. The fragment showed distinct localization from cis Golgi marker proteins, suggesting that it is mainly associated with the medial Golgi cisternae. (B) Levels of V5-mMTCL2 N fragment in control and giantin-knockdown cells were compared through immunostaining analysis using the indicated antibodies after paraformaldehyde fixation, which prevented leakage of cytosolic protein during fixation. Scale bar: 50 μm. (C) Levels of V5-mMTCL2 N fragment in control and giantin-knockdown cells were compared through western blotting analysis using total cell extracts. (D and E) Subcellular localization of endogenous MTCL2 in HeLa-K cells was compared with that of CLASPs (D) and giantin (E) using super-resolution microscopy. Boxed regions are serially enlarged in the middle and bottom panels. Arrowheads indicate the regions where each protein shows colocalization with MTCL2. Scale bars: 10 μm (top), 5 μm (middle), and 2 μm (bottom).

Supplementary Figure S8 "Blot Transparency"

Fig.1B

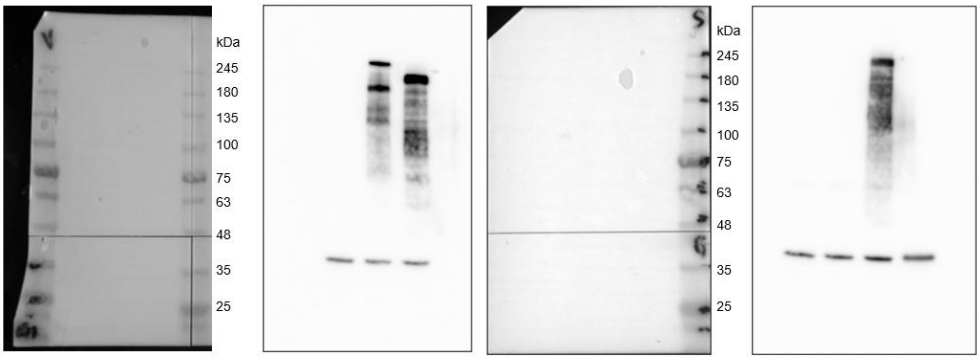


Fig.1C

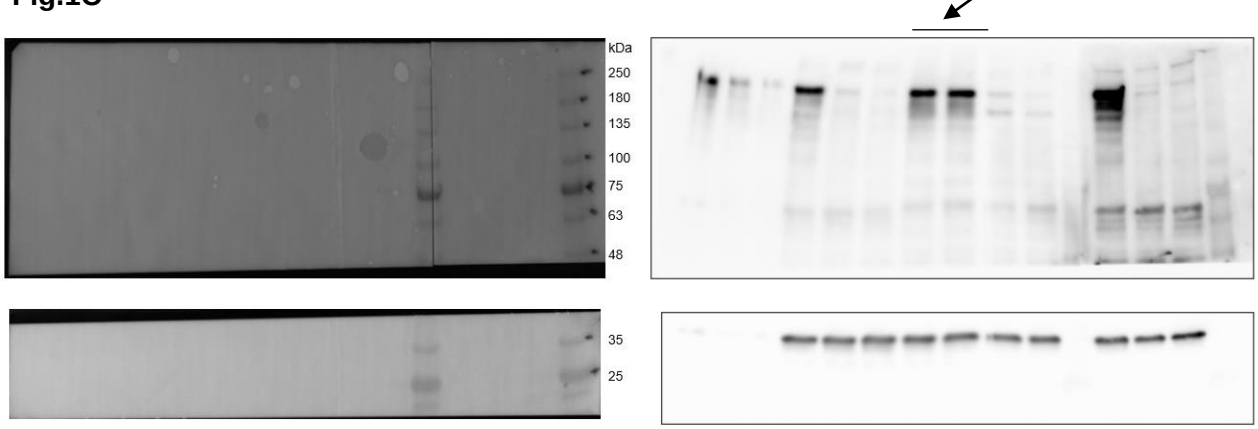


Fig.1D

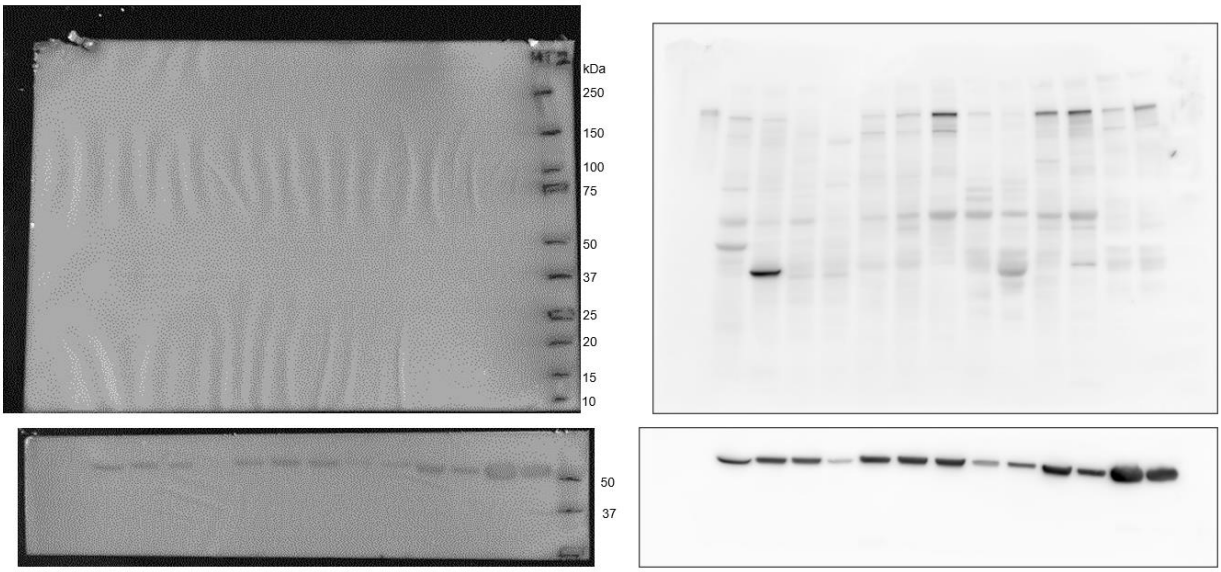


Fig.3C

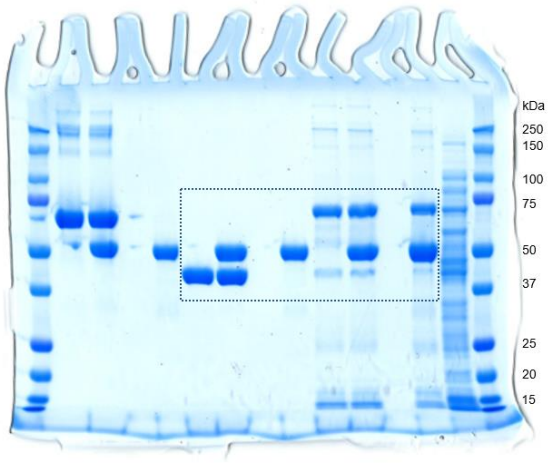


Fig.3E

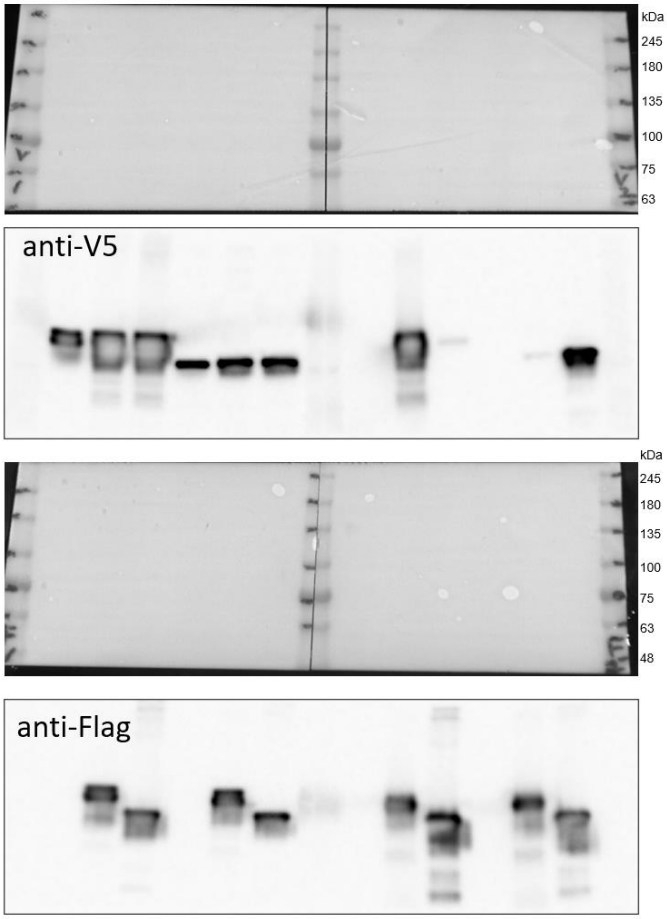
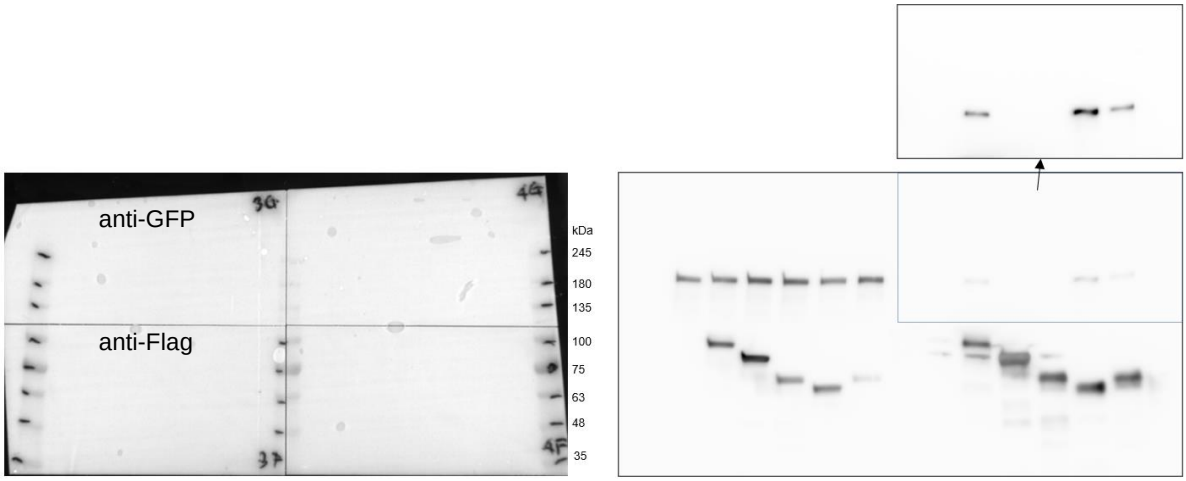
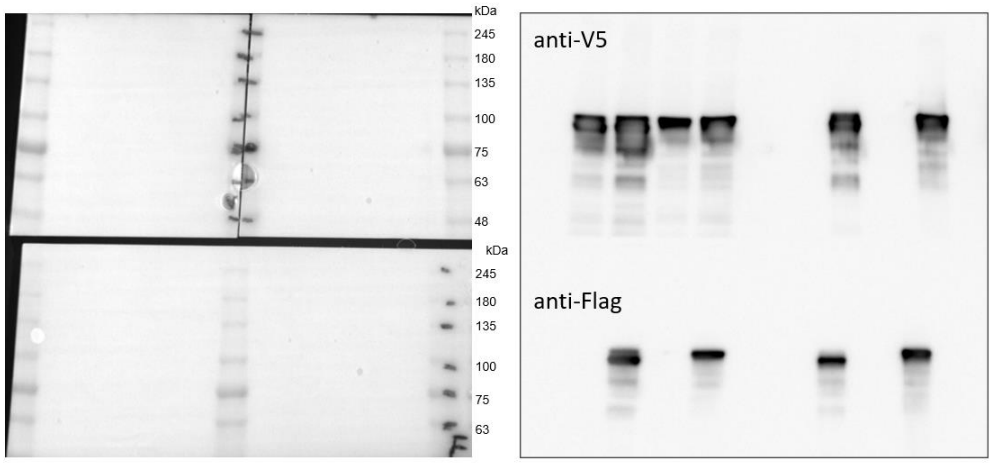


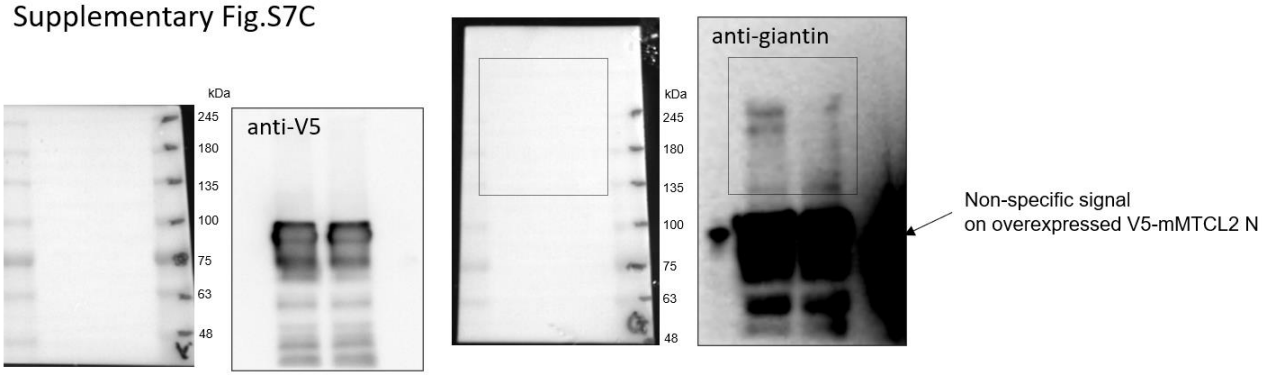
Fig.8B

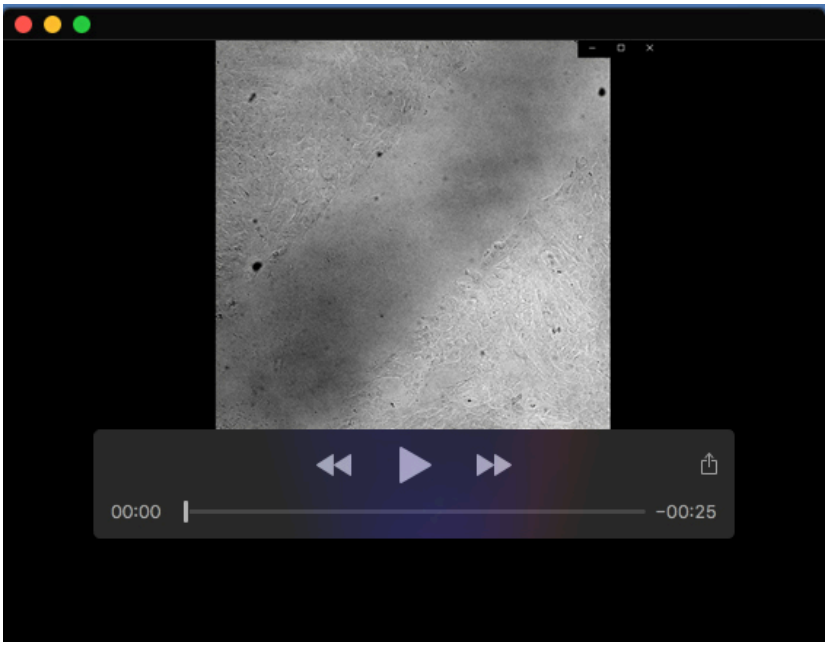


Supplementary Fig.S3C

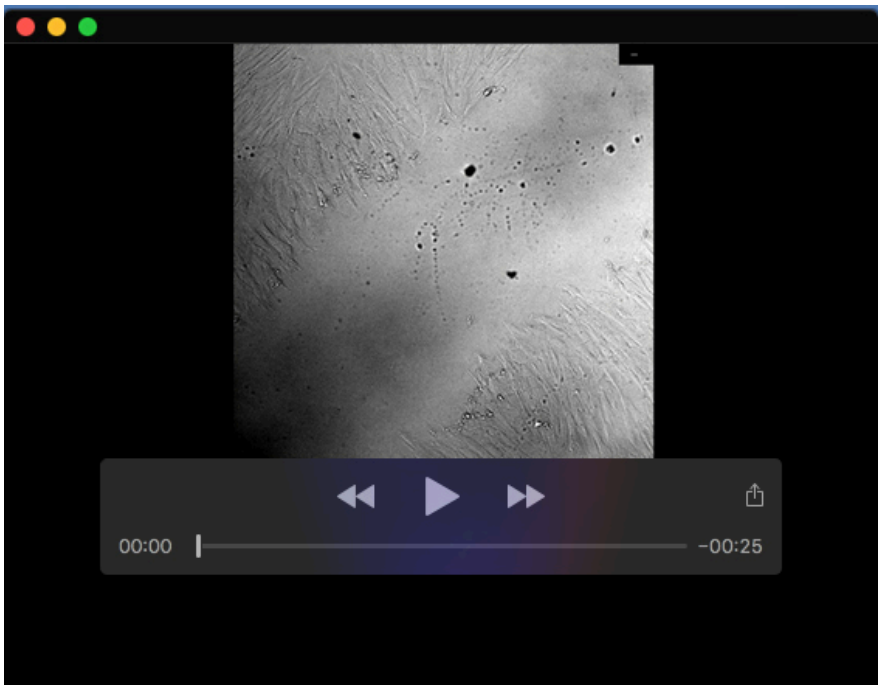


Supplementary Fig.S7C





Movie 1. Wound healing of RPE1 cells subjected to control knockdown. Differential interference contrast images of cells were taken every 10 min for 440 min. The video speed is 6 fps. Representative frames of this movie are shown in Fig. 7B.



Movie 2. Wound healing of RPE1 cells subjected to MTCL2 knockdown. Data were collected as described in the supplementary material Movie 1 legend. Representative frames of this movie are shown in Fig. 7B.