Supplemental material

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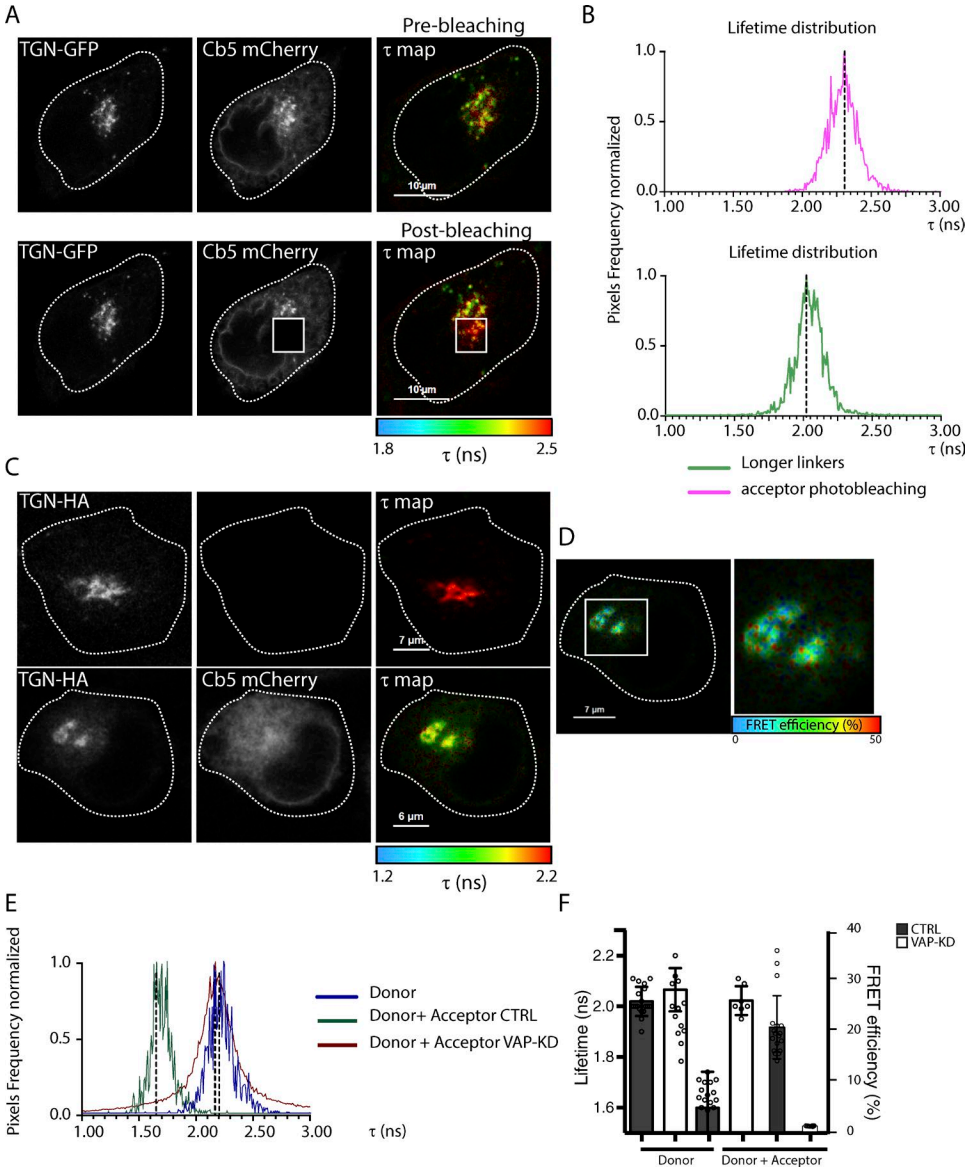


Figure S1. **Validation of the FRET–FLIM approach to visualize ERTGoCS. (A)** Immunofluorescence (IF) images (gray) and FLIM (in color) showing donor (TGN46-GFP) lifetime in the presence of the acceptor (mCherry-Cb5) before and after photobleaching of the acceptor in the squared region. The FLIM images show the spatial variation of the mean fluorescence lifetime (τ) of TGN46-GFP (donor): τ values are represented by a pseudocolor scale ranging from 1.8 to 2.5 ns. After acceptor photobleaching, the TGN46-GFP lifetime becomes longer, almost identical to lifetime measurements of donor alone (Fig. 2 D). **(B)** The τ value distribution curves of the donor when using reporter proteins after acceptor photobleaching (top) and with the longer form of the linkers (bottom). **(C)** FLIM (in color) and (gray) images of HeLa cells expressing the TGN46-HA (donor) alone (top) or TGN46-HA and mCherry-Cb5 (donor+acceptor; bottom). HA was detected using the Alexa Fluor 430–conjugated anti-HA antibody. The FLIM images show the spatial variation of the mean fluorescence lifetime (τ) of Alexa Fluor 430: τ values are represented by a pseudocolor scale ranging from 1.2 to 2.2 ns. **(D)** The spatial distribution of the FRET efficiency (FRET [%] map) is depicted (color scale ranges from 0 to 50%). **(E)** Mean τ value distribution curves of Alexa Fluor 430 (donor) conjugated with anti-HA antibody in cells expressing TGN46-HA alone (blue line) or TGN46-HA and mCherry-Cb5 (green line) in control cells and TGN46-HA and mCherry-Cb5 in VAP-KD cells (red line). **(F)** Quantification of average donor lifetime and FRET efficiency in donor alone or donor+acceptor in control and VAP-KD HeLa cells. *n* = 20. Data are means ± SD; Student's *t* test.

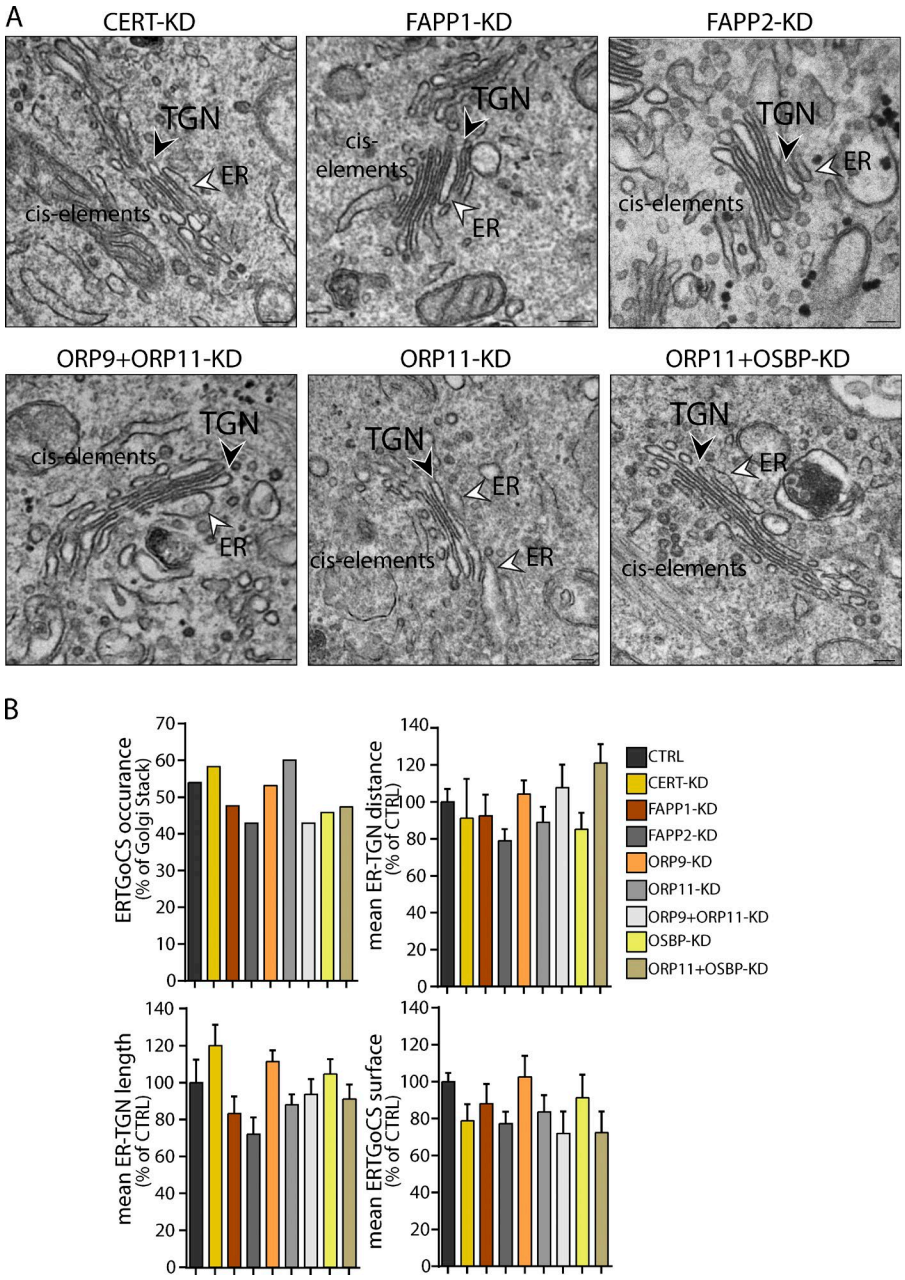


Figure S2. **Morphometric analysis of ERTGoCS in cells depleted of lipid transfer proteins found to be dispensable for ERTGoCS integrity by FLIM-FRET. (A)** Electron micrographs of CERT-KD, FAPP1-KD, FAPP2-KD, ORP9+ORP11-KD, ORP11-KD, and ORP11+OSBP-KD cells. Scale bar, 200 nm. **(B)** Quantification of ERTGoCS occurrence, mean ERTGoCS distance, length, and surface corresponding to the knockdown conditions analyzed in A. Quantifications were performed as in Fig. 1, B and C, and as described in Materials and methods. Data are mean ± SEM; Student's *t* test. *n* = 20–30 Golgi stacks. No condition was significantly different compared with the control.

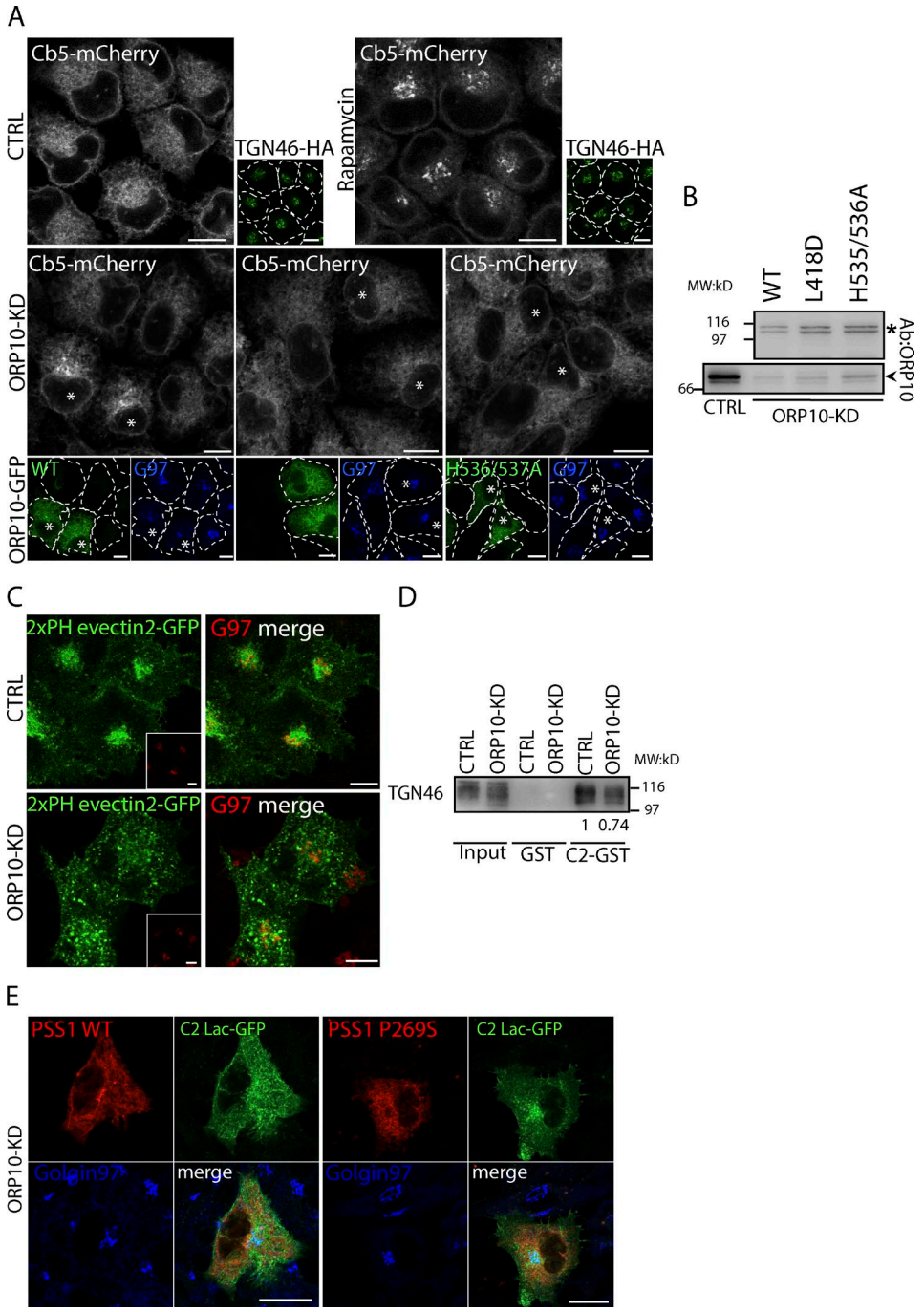


Figure S3. **ORP10 controls PS levels at the TGN. (A)** Immunofluorescence images related to Fig. 5 D. In the top row, control (CTRL) HeLa cells stably expressing the TGN46-FRB-HA and mCherry-T2A-FKBP-Cb5 reporter proteins were left untreated (left) or were treated with 200 nM rapamycin for 2 min (right). After treatment with rapamycin, the two probes heterodimerize stabilizing mCherry-Cb5 at the TGN46-HA-positive area (insets). In the bottom two rows, ORP10-KD cells stably expressing the TGN46-FRB-HA-mCherry-FKBP-Cb5 construct were transfected with GFP-tagged WT or mutant forms (L418D and H535/536A) of ORP10 and were treated with 200 nM rapamycin for 2 min. Asterisks mark overexpressing cells. Quantification was based on mCherry-Cb5 colocalization with TGN46 (see Materials and methods). Golgin-97 staining (blue). Scale bar, 10 µm. **(B)** Expression levels of the WT or mutant forms (L418D and H535/536A) of ORP10 in ORP10-KD cells evaluated by Western blot with anti-ORP10 antibody. The arrowhead and the asterisk indicate endogenous and GFP-tagged ORP10, respectively. **(C)** Mock (CTRL) and ORP10-KD cells were probed for PS localization using the GFP-tagged 2xPH domain of evectin2 (Uchida et al., 2011). Cells were colabeled with the Golgi marker Golgin-97 (insets) and show similar results obtained in Fig. 5 E. Scale bar, 10 µm. **(D)** PS-affinity isolation of TGN membranes. Postnuclear supernatants (see Materials and methods) from control (CTRL) and ORP10-KD cells were preincubated with the LacC2 domain fused to GST (C2-GST) or with GST and then incubated with glutathione magnetic beads. Subcellular fractions retained on the beads were eluted with sample buffer and analyzed by SDS-PAGE and Western blot with anti-TGN46 antibody. Numbers indicate the ratio between TGN46 retained on the beads and TGN present in the input and are expressed as the percentage of control. **(E)** PS distribution in ORP10-KD cells expressing mCherry-tagged WT (WT mCherry) or constitutively active (P269SmCherry) PS synthase 1, using the C2 domain of lactadherin (C2-Lact-GFP; top row). The bottom row shows Golgin-97 staining. Scale bar, 10 µm.

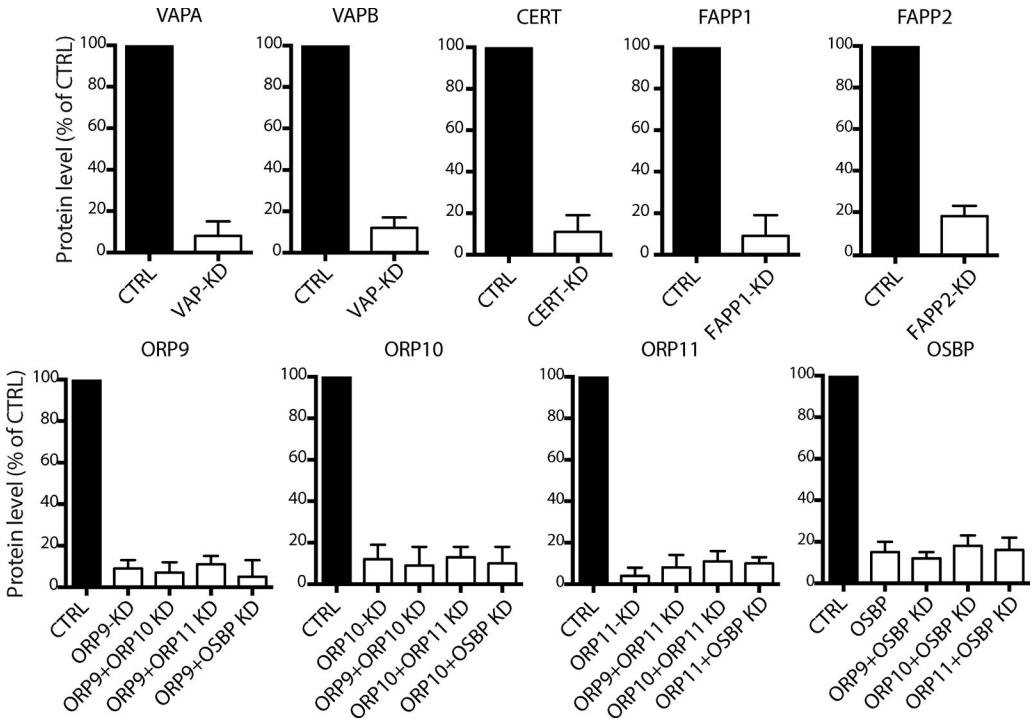
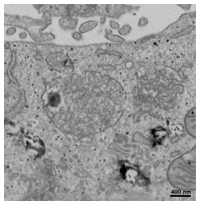


Figure S4. **Effect of the siRNAs used in the study on target protein levels.** Quantification by Western blot of the indicated proteins targeted by the indicated siRNAs. Means ± SD of three independent experiments.



Video 1. **FIB-SEM analysis of ERTGoCS.** 3D organization of ERTGoCS in HepG2 cells analyzed by FIB-SEM. The ER is highlighted in red and the TGN is highlighted in green.

Table S1. **List of primers and cloning strategies used in this study**

Plasmid	Template for PCR	Primer set	Vector backbone	Enzyme sites
TGN46-FRB-HA-GFP		Subcloned from TGN46-FRB-HA-CFP	pEGFP-N1	Nhe I/Age I
mCherry-FKBP	FKBP cDNA	5′-GAAGGATCCAGGGGGAATGGGAGTGCAGGTGGA AAC-3′/5′-CTAAGATCTCCGCCTCCCTCCAGTTTTAGA AGCTCCACA-3′	pmCherryC3	BglII
mCherry-FKBP-Cb5	Venus-C17	5′-GCCGAATTCTGGGTGGAGGAGGTTCAGGAG-3′/ 5′-GAGGGTACCGCTCATTAATCTTCAGCCATGTACA-3′	mCherry-FKBP	EcoR I/Kpn I
*m*Cherry-FKBP-C17-IRES-neo2	mCherry-FKBP-C17	5′-AATACCGGTACCATGGTGAGCAAGGGCG-3′/ 5′-CTTGCGGCCGCTCATTAATCTTCAGCCATG-3′	pIRES-neo2	AgeI/NotI
T2A-mCherry-FKBP-C17-IRES-neo2	Primer annealing	5′-TTAAGACCGGTGAGGGCAGGGGCAGCCTGCTGA CCTGCGGCGACGTGGAGGAAAACCCTGGCCCCT-3′/ 5′-CCGGAGGGGCCAGGGTTTTCCTCCACGTCGCCG CAGGTCAGCAGGCTGCCCCTGCCCTCACCGGTC-3′	mCherry-FKBP-C17-IRES-neo2	AflII/AgeI
TGN46-FRB-HA-GFP-T2A-mCherry-FKBP-C17-pIRES-neo2	TGN46-FRB-HA-GFP	5′-TATGCTAGCACCACCATGCGGTTCGTAGT-3′/5′-TAT ACCGGTTCCCTTGTACAGCTCGTCCATGC-3′	mCherry-FKBP-C17-T2A-pIRES-neo2	AgeI/NotI
TGN46-FRB-HA-T2A-mCherry-FKBP-C17-pIRES-neo2	TGN46-FRB-HA-GFP	5′-TATGCTAGCACCACCATGCGGTTCGTAGT-3′/ 5′-GTCACCGGTCGCGTAGTCTGGTACGTCG-3′	T2A-mCherry-FKBP-C17-pIRES-neo2	NheI/AgeI
pmCherry-N1-FKBP	FKBP cDNA	5′-GAAGGATCCAGGGGGAATGGGAGTGCAGGTGGA AAC-3′/5′-CTAAGATCTCCGCCTCCCTCCAGTTTTAGA AGCTCCACAT-3′	pmCherry-N1	BamHI
PH-FFAT-FLAG	PH-FFAT-CHERRY	5′-GAGAGATCTACTGGGGGCTCGGGCGG-3′/ 5′-TATGTCGACTCTGGTTCTCTTTTCCTTTTTG-3′	p3XFLAG (CMV-14)	BglII/SalI
pmCHERRY-FKBP-C17-longer linker (Várnai et al., 2007)	Primer assembling for longer linker (Várnai et al., 2007)	First forward 5′-GGATCTGGTGCTGGTGCAGGCGCT GGCGCCAT-3′ Second reverse 5′-TCCCTAGCTGCTGCCTCTCTTGCT GCAGCTTCTCTGGCAGCGGCCTCTCTAGCGGCGGCT TCAGAATTCAGAATGGCGCCAGCGCCTG-3′ Third forward 5′-GGCAGCAGCTAGGGAAGCAGCTGC ACGTGAAGCCGCAGCCAGAGAGGCTGCTGCTCGCGA AGCTGCCGCACGGGAAGCTGCTGCTAGAAA-3′ Fourth reverse 5′-CACTCTTGAATTTCTAGCAGCAGC TTCCCGT-3′	pmCHE RRY-FKBP-C17	PIPE vector primers: 5′-GAA GCTGCTGCTAGAAATTCAAGA GTGTCCGAAACTCTGATCACT ACCGTTG-3′/5′-GCCTGCACC AGCACCAGATCCTCCCTCCAG TTTTAGAAGCTCCAC-3′
TGN46-FRB-HA-GFP-longer linker (Várnai et al., 2007)	pmCHERRY-FKBP-C17-longer linker (Várnai et al., 2007)	5′-GGATCTGGTGCTGGTGCAGGCGCTGGCGCCAT-3′/ 5′-CACTCTTGAATTTCTAGCAGCAGCTTCCCGT-3′	TGN46-FRB-HA-GFP	PIPE vector primers: 5′-GAA GCTGCTGCTAGAAATTCAAGA GTGCTATCTAGAATCCTCTGG CATGAG-3′ and 5′-GCCTGC ACCAGCACCAGATCCGGGATC CGACTTCTGGTCCA-3′
GFP-2XPH EVECTIN	cDNA EVECTIN	First PH: 5′-AGATCTCGAGCTCAAGCTTCGAATTCT ATGGCGTTTGTGAAGAGTGGC-3′/5′-CGCCATGGTACC GTCGACGTTTGTCCTAGAATCTTGGAGT-3′ Second PH: 5′-GGACAAACGTCGACGGTACCATGGCGT TTGTGAAGAGTGG-3′/5′-TCAGTTATCTAGATCCGG TGGATCCTCAGTTTGTCCTAGAATCTTGGAGT-3′	pEGFP-C1	PIPE vector primers: 5′-GGA TCCACCGGATCTAGATAA CTGA-3′/5′-GAATTCGAAGCT TGAGCTCGAGATCT-3′
GST-TEV- C2-Lact	C2-Lact-GFP	5′-GGTGGGGGTGGATCAGGAAGTGAAGAAAACCTG TACTTCCAGAGTGGGTCAGGTTCTGGAGGATCCCAG GAATTCTGCACTGAACCCCTAGGC-3′/5′-CACGATGCG GCCGCTCGAGCTAACAGCCCAGCAGCTCC-3′	pGEX	PIPE vector primers: 5′-GAG CGGCCGCATCGTG-3′/5′-CTT CACTTCCTGATCCACCCCCAC CTTTTGGAGGATGGTCGCC-3′
GFP-ORP10	pEGFP-C1	Subcloned from pEGFP-C1	CHERRY-ORP10 siRNA resistant	AgeI/HindIII

Table S1. **List of primers and cloning strategies used in this study *(Continued)***

Plasmid	Template for PCR	Primer set	Vector backbone	Enzyme sites
CHERRY-ORP10 siRNA resistant	CHERRY-ORP10	First pair: 5′-ACTCAGCTTCGAGCTTGTGCCAAGTAT CATATGGAGATGAATTCTAAGAGTGCTC-3′/5′-GAG CACTCTTAGAATTCATCTCCATATGATACTTGGCAC AAGCTCGAAGCTGAGT-3′	Site-directed mutagenesis	
		Second pair: 5′-TAAGGAAGAGACGGAATTGGGCGT CATGGAGGACCAAAGATCAATAATTCTTCATCTCAT TTCACAACTCAAACTT-3′/5′-AAGTTTGAGTTGTGA AATGAGATGAAGAATTATTGATCTTTGGTCCTCCAT GACGCCCAATTCCGTCTCTTCCTTA-3′		
		Third pair: 5′-CACAGGGTTACCGCAGAAGTGAAGCAT AATCCCACAAATACAATTGTTTGTAAAGCCCATGGG GAATG-3′/5′-CATTCCCCATGGGCTTTACAAACAATT GTATTTGTGGGATTATGCTTCACTTCTGCGGTAACC CTGTG-3′		
ORP10-L418D		5′-GATTTGACCAAGGTGGTGGATCCTACCTTTATCCTG GAGAAGC-3′/5′-GCTTCTCCAGGATAAAGGTAGGAT CCACCACTTGGTCAAATC-3′	Site-directed mutagenesis	
ORP10-H535/536A		5′-GTTTGTGGCTGAGCAAGTGTCCGCTGCCCCACCCAT CTCCTGCT-3′/5′-AGCAGGAGATGGGTGGGGCAGCGG ACACTTGCTCAGCCACAAAC-3′	Site-directed mutagenesis	

Table S2. **siRNA sequences used in this study**

Gene name	Duplex number	Sequence (sense strand)
VAPA	#1	5′-CCACAGACCUCAAAUUCAA-3′
	#2	5′-GGCAAAACCUGAUGAAUUA-3′
	#3	5′-CCUGAGAGAUGAAGGUUUA-3′
	#4	5′-CAAGGAAACUAAUGGAAGA-3′
VAPB	#1	5′-GUAAGAGGCUGCAAGGUGA-3′
	#2	5′-CCACGUAGGUACUGUGUGA-3′
	#3	5′-UGUUACAGCCUUUCGAUUA-3′
	#4	5′-GUAAUUAUUGGGAAGAUUG-3′
FAPP1	#1	5′-CGAAGAACCUACUCAGAUA-3′
	#2	5′-GCAUAAAGAUGGCAGUUUG-3′
	#3	5′-UCACAACGCUUGAGGAAUG-3′
	#4	5′-GAACCAGUAUCUACACUUC-3′
FAPP2	#1	5′-GAGAUAGACUGCAGCAUAU-3′
	#2	5′-GAAUUGAUGUGGGAACUUU-3′
	#3	5′-GAAAUCAACCUGUAAUACU-3′
	#4	5′-CCUAAGAAAUCCAACAGAA-3′
ORP9	#1	5′-GAGGAGCACAAGAGCGUUA-3′
	#2	5′-ACCAAGAAGUUGCCUAUAA-3′
	#3	5′-GCUCAUAUCUGGACCAAAU-3′
	#4	5′-CCAAAGCGCUUAAUAGAUU-3′
ORP10	#1	5′-CAACCCAACCAACACCAUU-3′
	#2	5′-GGAUCAGCGUAGUAUAAUU-3′
	#3	5′-AAUACCACAUGGAAAUGAA-3′
ORP11	#1	5′-GAGACUUAAUUAGACGAAU-3′
	#2	5′-GAAUCUGGACUAUUAGCGA-3′
	#3	5′-GCGUGACAAUGGUUGGAGA-3′
OSBP1	#1	5′-CACAACUGUACACAACAUU-3′
	#2	5′-GAUAGAUCAGUCUGGCGAA-3′
CERT	#1	5′-GAAGAUGACUUUCCUACAA-3′
	#2	5′-GAAGUUGGCUGAAAUGGAA-3′
	#3	5′-GCGAGAGUAUCCUAAAUUU-3′
	#4	5′-UCAAAGGGAUAAAGUGGUA-3′

References

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