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Supplementary Materials for

The structure and global distribution of the endoplasmic reticulum network are actively regulated by lysosomes

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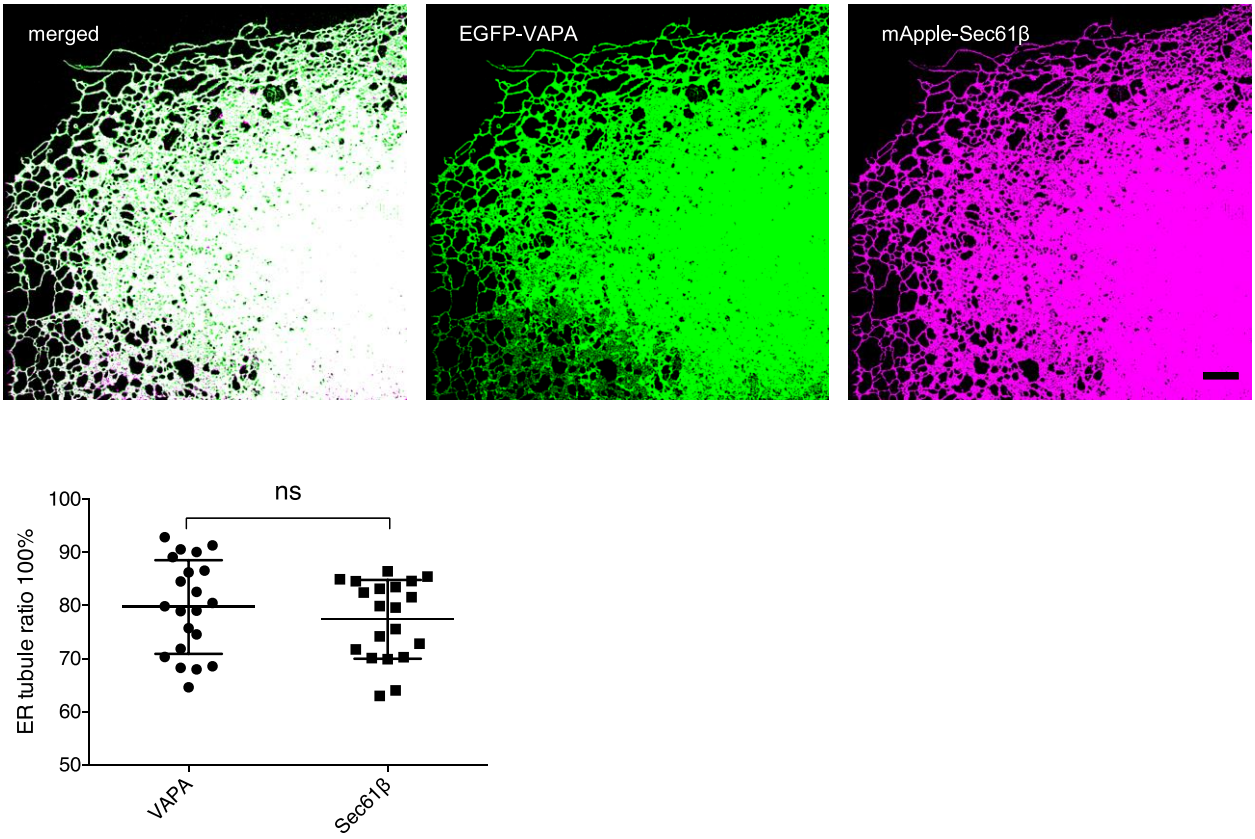


Fig. S1. ER morphology assay in VAPA- or Sec61β - expressing cells.

Top panel: a representative COS-cell expressing EGFP-VAPA and mApple-Sec61β shows full colocalization of the two ER-specific proteins.

Bottom panel: quantification of ER tubule ratio in COS-7 cells expressing EGFP-VAPA or mApple-Sec61β, showing overexpression of VAPA does not alter the balance between tubule and sheet domain. ns: no significance (student's t test). See table S9.

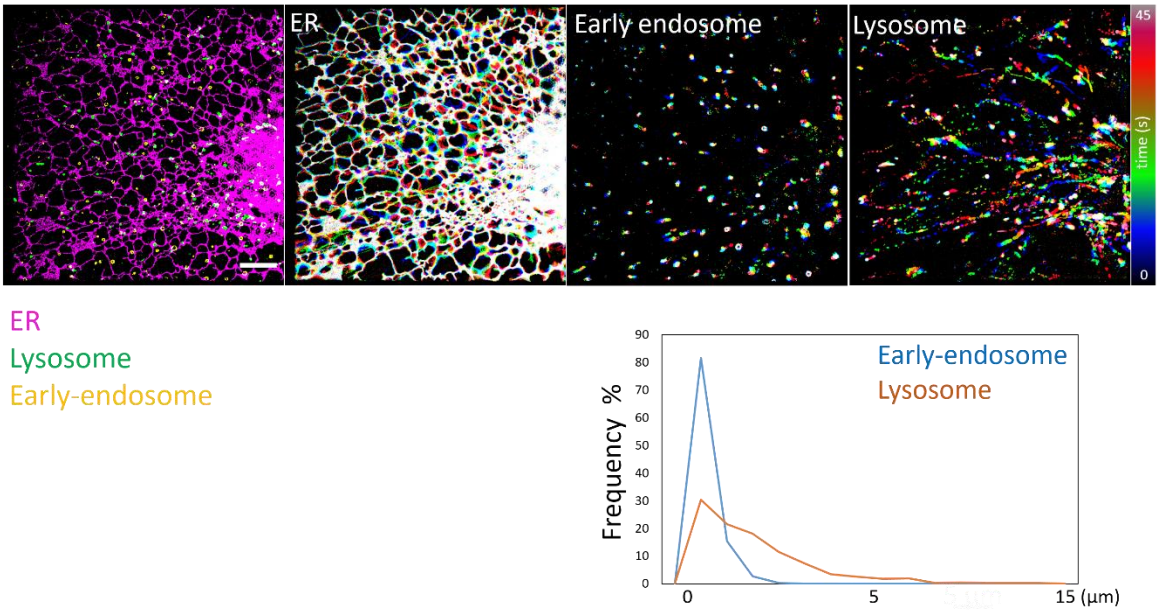


Fig. S2. Comparison of organelle dynamics between early endosome and lysosome.
Top panel: first left, SIM image from a time-lapse recording of a COS-7 cell expressing Rab5-EGFP (yellow) and mApple-Sec61β (magenta), stained with SiR-lysosome (green). Second left to right: images of ER, early endosome, and lysosome, all color coded to represent time. Bottom pabel: Histograms of net displacement of early endosomes and lysosomes during recording. 3025 early endosomes and 1000 lysosomes were analyzed from three different experiments. Scale bar: 5 µm. (see Movie S2 and table S10).

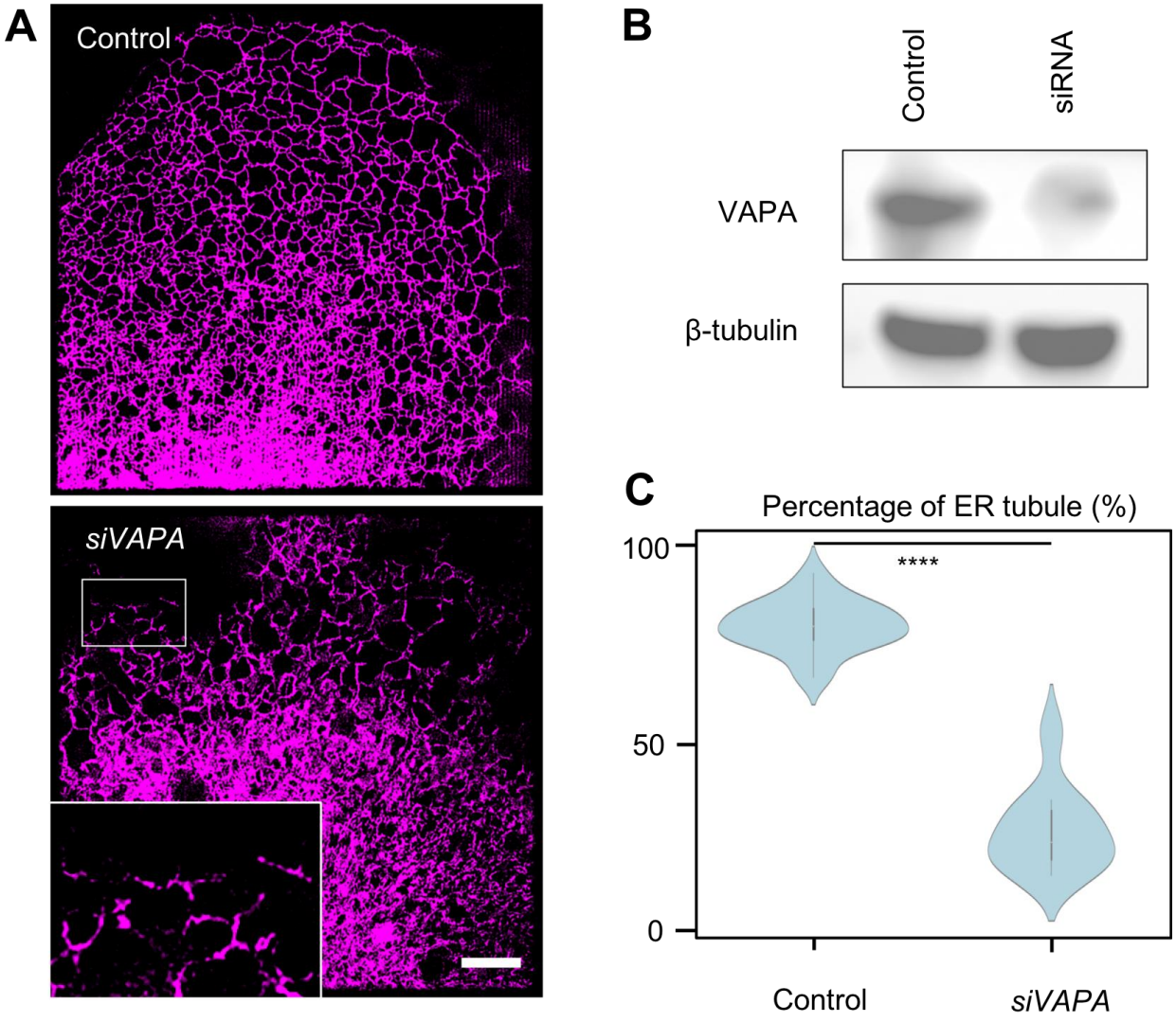


Fig. S3. VAPA knockdown leads to reduced ER tubules.

(A) A representative COS-7 cell expressing Sec61β-EGFP (magenta) with siRNA treatment of VAPA. An enlarged region demonstrates that discontinued tubules appeared in the cell treated with *siVAPA*. (B) Western blot validation of VAPA depletion.

(C) Percentage of the ER comprising tubules upon knockdown of VAPA. Data are shown as ± S.E.M. **** = $P<0.0001$ (Student's t test). Data from 20 images from 3 independent experiments were analyzed for each condition. See table S11.

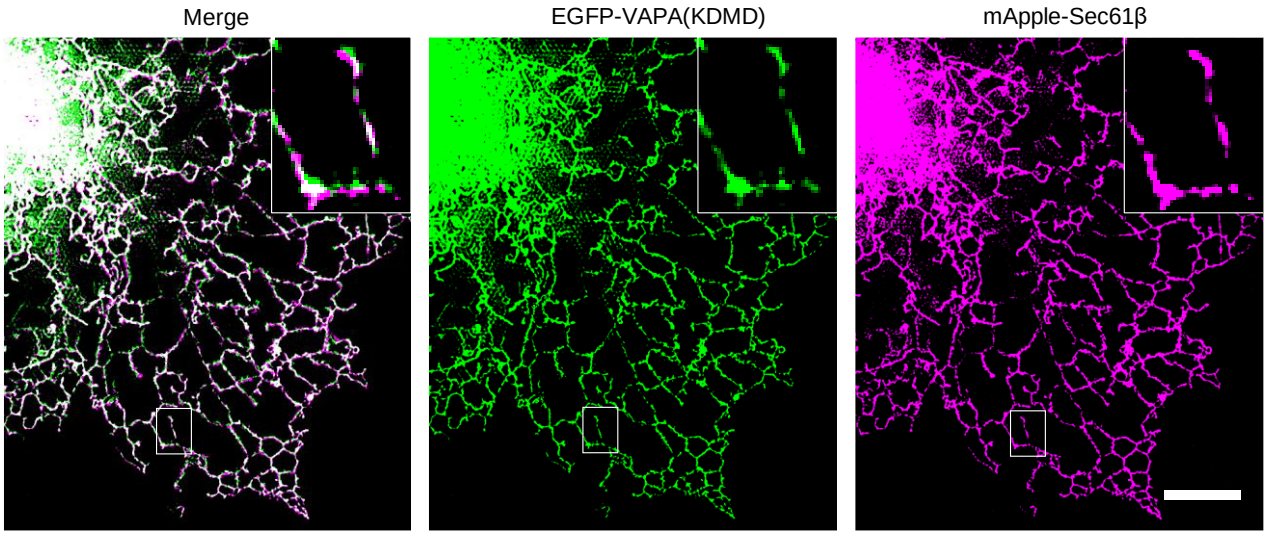


Fig. S4. The distribution of mutant VAPA in the cell is not affected by the KD/MD mutation. Representative COS-7 cell expressing EGFP-VAPA (KD/MD) (green), and mApple-Sec61β (magenta) shows full colocalization of the two ER-specific proteins. An enlarged region demonstrates that both VAPA (KD/MD) and Sec61β positive ER structures feature the same discontinuities in ER tubules.

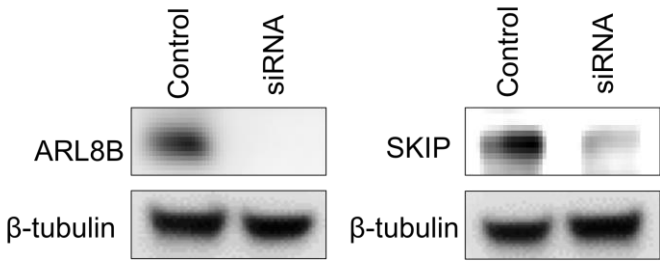


Fig. S5. Western blots to confirm depletion of Arl8b and SKIP.

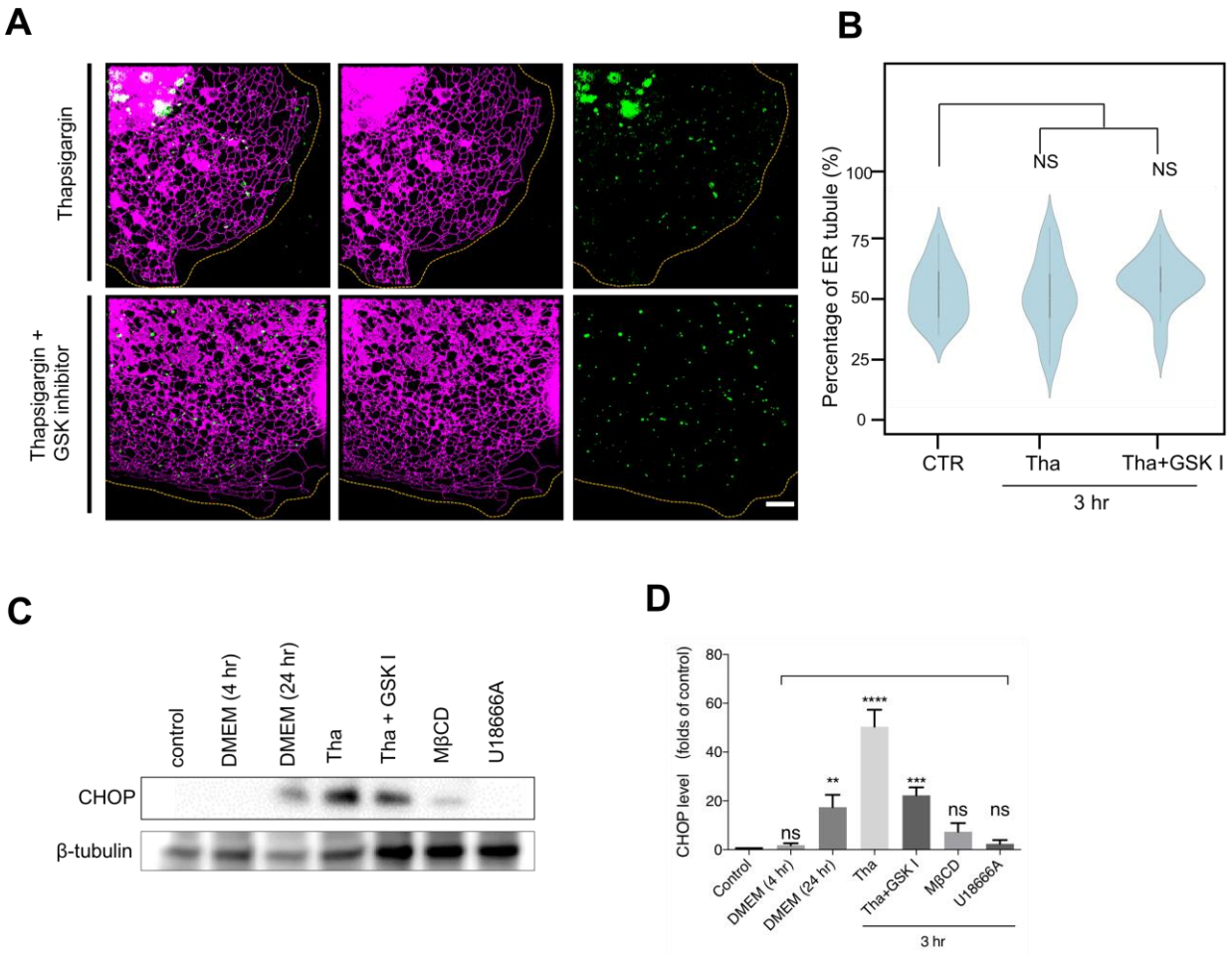


Fig. S6. ER stress examination.

(**A**) Representative COS-7 cells expressing mEmerald-Sec61β (magenta) and stained with Sir-Lysosome (green), after 3 h of Thapsigargin treatment (500 nM) or Thapsigargin (500 nM) plus GSK inhibitor (5 μM). (**B**) Quantification of ER tubule percentage under the two treatment. (**C**) Immunoblot analysis with antibodies against CHOP revealed elevation of ER stress in Thapsigargin treated COS-7 cells. (**D**) Quantification of the CHOP levels in immunoblots (*N*=3). See table S12.

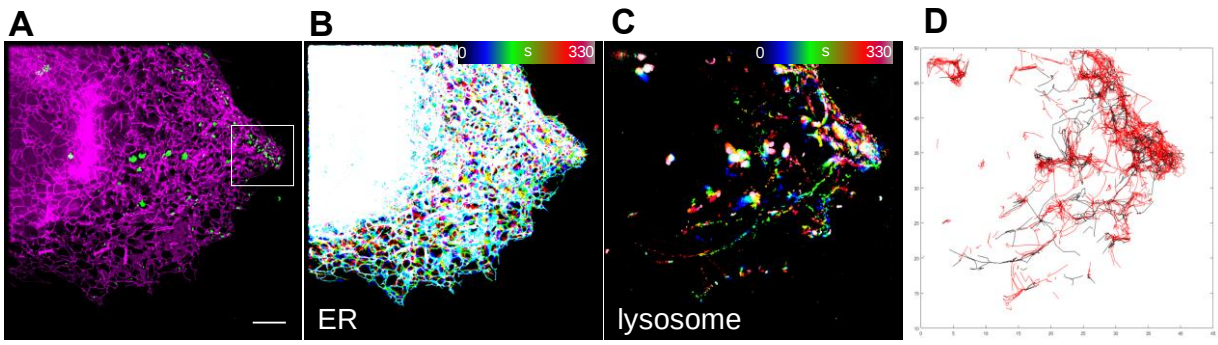


Fig. S7. Motion tracking of lysosomes in prolonged serum starvation.
(**A**) Representative COS-7 cells expressing mEmerald-Sec61β (magenta) and stained with SiR-Lysosome (green), after 24 h of DMEM treatment. Images are color-coded to represent time, and show, (**B**), ER and, (**C**), lysosome motion. (**D**) Lysosome tracks, color coded according to direction of motion. Red: lysosome move towards the protrusion highlighted by the white box. Black: lysosome tracks that move away from the protrusion. Units of x- and y-axes: μm. Scale bar: 5 μm. (See Movie S7)

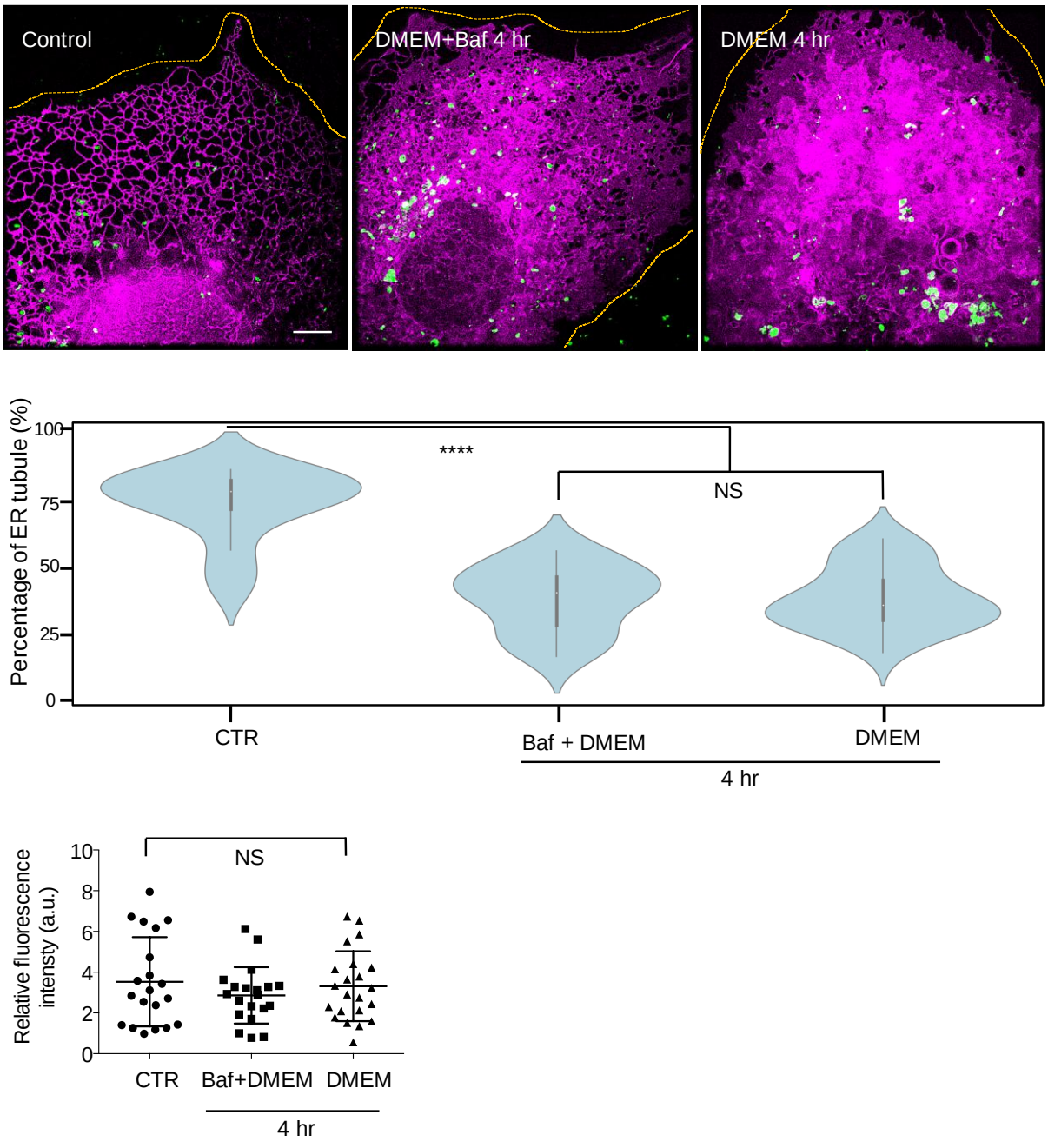


Fig. S8. A reduction in ER tubular network content upon nutrient starvation is not caused by ER-phagy.

Top row: Representative SIM images of the distribution of lysosomes (green) and ER (magenta) upon nutrient starvation. From left to right: no treatment control; 4 hr serum starvation and Bafilomycin A1; 4 hr serum starvation. The ER is visualized via Sec61β-EGFP, shown in magenta, and lysosomes (green color) are labeled by SiR-Lysosome. Middle row: Quantification of ER tubule percentage under the above treatments. See table S13. Bottom row: quantification of lysosome intensity of the cells in the above treatments. This shows that the administration of Baf did not lead to a measurable decrease of the lysosome intensity.

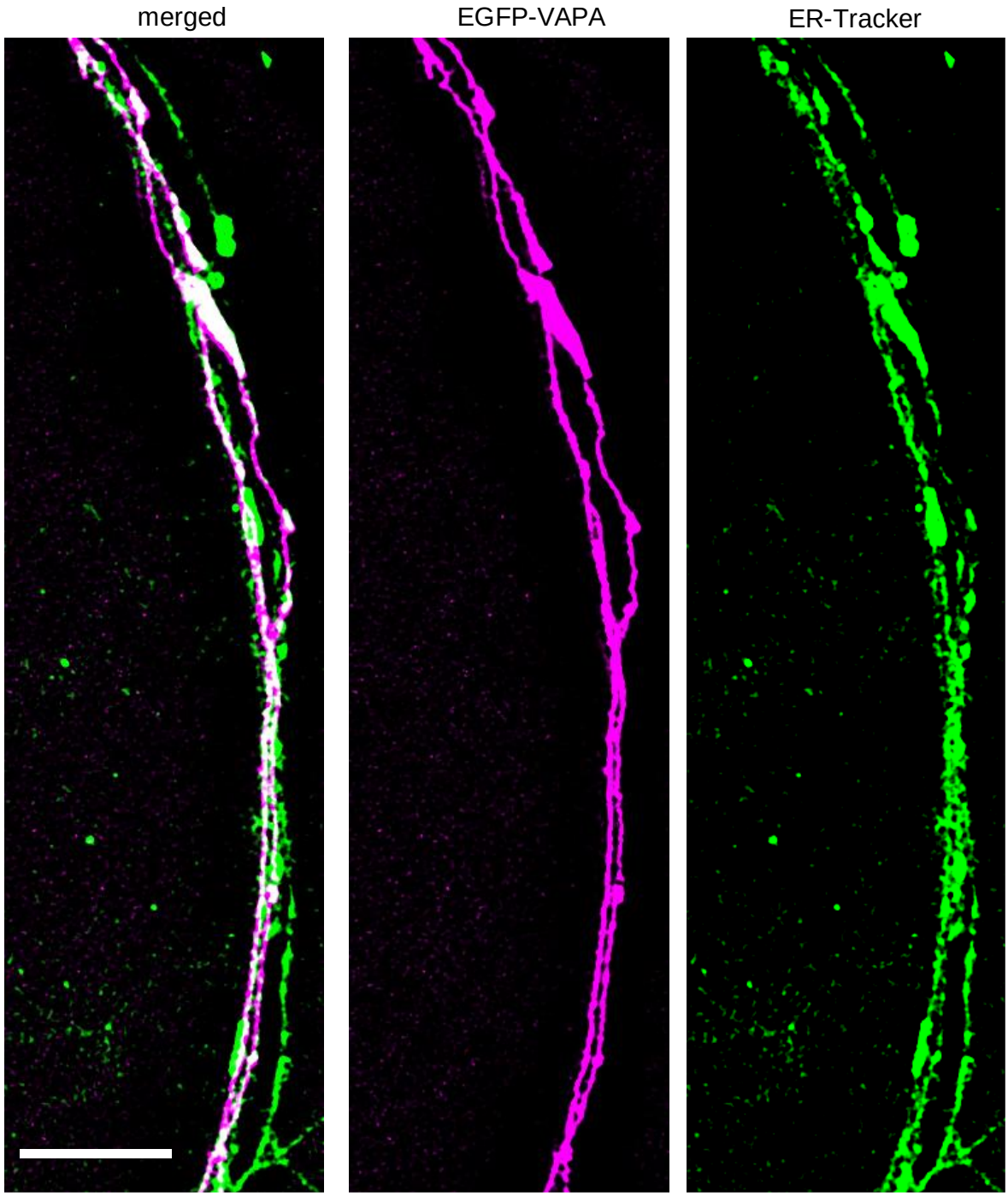


Fig. S9. Colocalisation of VAPA and ER-Tracker-Red in a *Xenopus laevis* axons.
The fragmented structure visualized by ER-Tracker was likely due to due to heterogeneous staining or phytotoxicity, which was largely limited using the fluorescent protein-coupled ER marker VAPA.

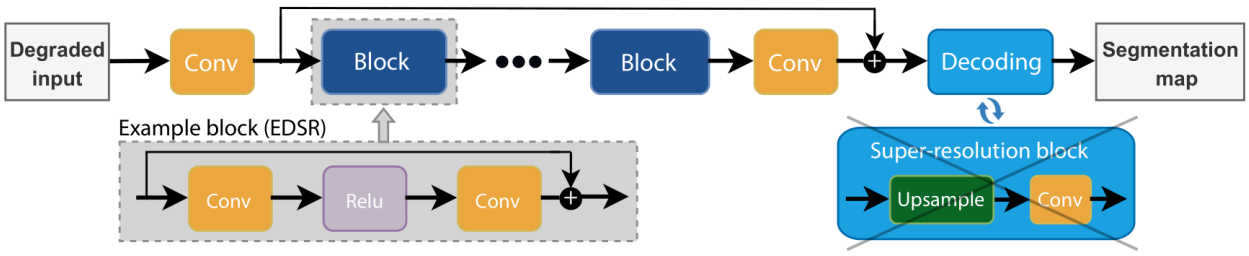


Fig. S10. Architecture of the residual CNN used for segmentation. The overall structure follows that of EDSR and RCAN, except for the replacement of the super-resolution block with a decoder module that reduces the number of feature channels to the number of unique classes in the segmentation map using a convolutional layer with a corresponding number of output channels and a kernel size of 1x1. This operation is sometimes referred to as feature pooling.

Table S1.

Quantification of ER elongation events in Fig. 1d and e. Three independent experiments were performed to measure the ER tubule elongation length, duration and efficiency.

Fig. 1D

Condition	***N* (cells)**	***N* (events)**	**Elongation length ± SD (µm)**
ER	39	305	2.23±0.084
ER+LY	39	175	6.72±0.269

Fig. 1E

Condition	***N* (cells)**	***N* (events)**	**Elongation duration ± SD (s)**
ER	39	305	3.54
ER+LY	39	175	8.92

Fig. 1F

Condition	***N* (cells)**	***N* (events)**	**Successful elongation length (µm)**	**Unsuccessful elongation length (µm)**
ER	39	305	442.92	237.18
ER+LY	39	175	1169.73	1.77
Total	39		1612.65	238.95

Experiment 1		**ER**	**ER+LY**
N (cells)		23	23
N (events)		165	105
Successful elongation length (µm)	A	246.87	719.62
Unsuccessful elongation length (µm)	B	130.32	1.76
Elongation efficiency (%)	A/(A+B)	65.44	99.75
Elongation duration (s)		530	931

Experiment 2		**ER**	**ER+LY**
N (cells)		6	6
N (events)		59	27
Successful elongation length (µm)	A	80.705	167.195
Unsuccessful elongation length (µm)	B	41.127	0
Elongation efficiency (%)	A/(A+B)	66.24	100
Elongation duration (s)		83.5	166

Experiment 3		**ER**	**ER+LY**
N (cells)		10	10
N (events)		81	43
Successful elongation length (µm)	A	115.341	282.911
Unsuccessful elongation length (µm)	B	65.721	0
Elongation efficiency (%)	A/(A+B)	63.70	100
Elongation duration (s)		259.5	445.5

Table S2.

Quantification of ER-LY coupled motion in either EGFP-VAPA(KD/MD) or EGFP-VAPA expressing COS-7 cells. Expression of KD/MD mutant of VAPA resulted a significant proportion of lysosomes detached from ER during motions (66%). As noted in the following table, the proportion of lysosomes contacted with ER also decreased from 97.7% (VAPA) to 75.5% (KD/MD).

	VAPA	**KD/MD**
Total lysosome No.	710	634
Lysosomes connected with ER	694	479
Lysosomes coupled with ER in motions	70	56
Detached lysosomes from coupled motions	0	37
N (cells)	11	10
N (repeats)	2	2

Table S3.

Quantification of lysosome velocity in the events of ER-LY detachment. Three independent experiments were performed to measure the velocities of lysosomes at five continuous time points.

Time point (s)	**-1.5**	**0**	**1.5**	**3**	**4.5**
average velocity (μm/s)	0.39	0.48	1.047	0.65	0.46
N (events)	22	22	22	22	22
N (cells)	15	15	15	15	15
N (repeats)	3	3	3	3	3

Table S4.

Quantification of tubular domain among the whole ER in two independent experiments of different gene knock-downs by siRNA.

Treatment	**CTR (control)**	**siArl8b**	**siSKIP**
Percentage of ER tubules (%)	80.496315	24.32851	25.12999
N (cells)	40	20	20
N (repeats)	2	2	2

Table S5.
Quantification of tubular domain among the whole ER in two independent chemogenetic experiments.

Treatment	Vehicle	Inducer
Percentage of ER tubules (%)	80.51	25.86
N (cells)	20	20
N (repeats)	2	2

Table S6.
Quantification of tubular domain among the whole ER in in two independent experiments of different metabolic treatments.

Treatment	**CONTROL**	**DMEM 4hr**	**DMEM 24 hr**	**sArl8b DMEM 24 hr**	**siSKIP DMEM 24 hr**	**U18666A**	**MβCD**	**siArl8b MβCD**	**siSKIP MβCD**
Percentage of ER tubules (%)	61.83	38.31	54.45	24.93	25.91	9.27	78.15	23.96	31.70
N (cells)	41	29	27	15	15	47	34	15	16
N (repeats)	2	2	2	2	2	2	2	2	2

Table S7.
Quantification of ER breakages and connections in either EGFP-VAPA(KD/MD) or EGFP-VAPA expressing RGC axons. Four independent experiments were performed to measure the number of ER tubule breakages and connections, either via ER only or lysosome-driven elongation.

Fig.5D

KD/MD

Experiment	***N* (axons)**	**ER breakage gaps**	**Gap connection**	**Lysosome-driven connection**
1	92	51	2	0
2	34	8	0	0
3	89	44	7	2
4	6	0	0	0
Total	221	103	9	2

VAPA

Experiment	*N* (axons)	ER breakage gaps	Gap connection	Lysosome-driven connection
1	40	3	3	2
2	17	0	0	0
3	18	4	4	3
4	10	3	3	1
Total	85	10	10	6

Fig. 5E and F
Quantification of average axon length and the average ER fragment length in EGFP-VAPA or EGFP-VAPA(KD/MD) expressing axons.

	KDMD	VAPA
Axon length (μm)	47.86	66.281
ER length (μm)	28.47	57.70
N (axons)	152	118

Table S8.
Quantification of length of either EGFP-VAPA(KD/MD) or EGFP-VAPA expressing RGC axons after overnight culture. Three independent experiments were performed for quaitification.

	KD/MD with discontinued ER	with intact ER	**VAPA** with discontinued ER	with intact ER
axon length ± SD (μm	39.56	61.54	46.84	72.4
N (axons)	98	56	42	84

Table S9.
Quantification of tubular domain among the whole ER in either EGFP-VAPA or mApple-Sec61β expressing cells.

	VAPA	Sec61β
Percentage of ER tubules (%)	79.67	77.38
N (cells)	20	20
N (repeats)	3	3

Table S10.
Comparison of the displacements between early endosomes and lysosomes. Three independent experiments of time-lapse Fast-SIM imaging were performed to measure the displacements of each motion tracks via TrackMate, Fiji.

	Early endosome		**Lysosome (Late endosome)**	
Experiment	N(tracks)	Displacement ± SD (μm)	N (tracks)	Displacement ± SD (μm)
1	1039	0.57	328	2.46
2	1036	0.43	273	3.02
3	947	0.50	399	2.28

Table S11.
Quantification of tubular domain among the whole ER in in three independent experiments of VAPA gene depletion.

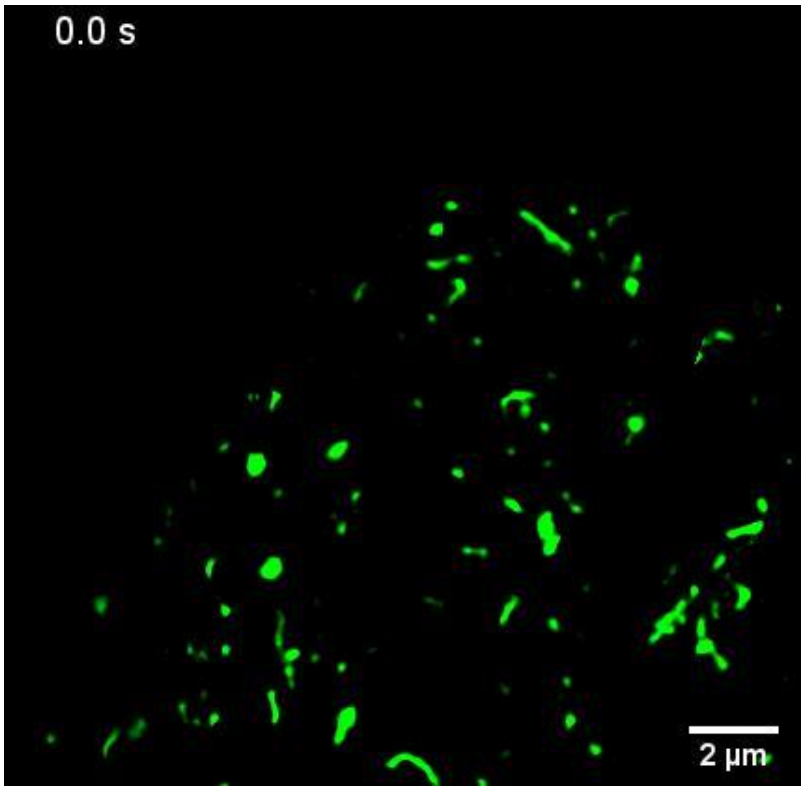
	Control	**siVAPA**
Percentage of ER tubules (%)	80.51	20.45
N (cells)	20	20
N (repeats)	3	3

Table S12.
Quantification of tubular domain among the whole ER in three independent experiments of different ER stress treatments.

Treatment	**Control**	**Thapsigargin (3hr)**	**Tha+GSK Inhibitor (3hr)**
Percentage of ER tubules (%)	61.83	59.82	66.93
N (cells)	42	58	38
N (repeats)	3	3	3

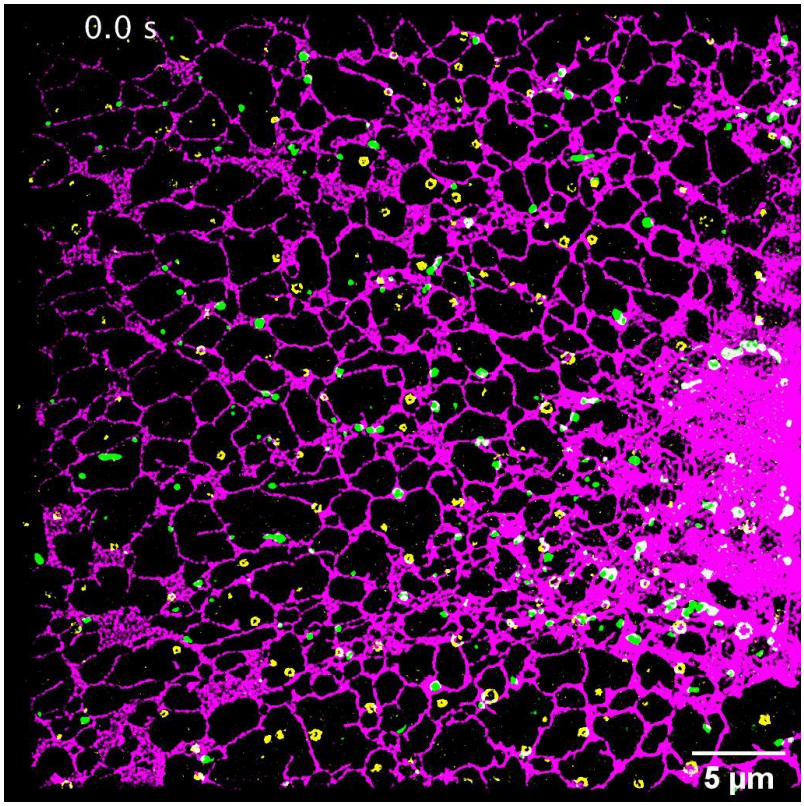
Table S13.
Quantification of tubular domain among the whole ER in two independent experiments of different treatments.

	Control	**Baf + DMEM (4 hr)**	**DMEM (4hr)**
Percentage of ER tubules (%)	78.65	36.54	36.51
N (cells)	23	20	28
N (repeats)	2	2	2

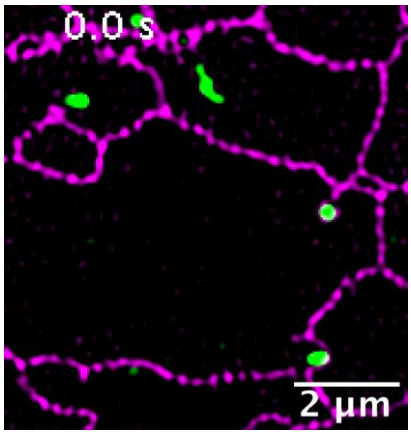


Movie S1.

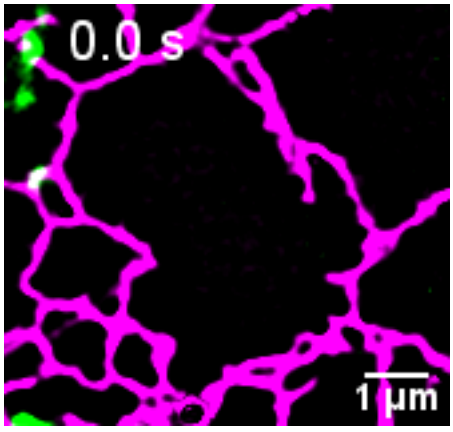
Live cell imaging by SIM demonstrates the dynamic motions of lysosomes. A representative 100 frames of whole recording. COS-7 cells were stained by SiR-Lysosome and a region was imaged over 12.5 mins at 1.5 s/frame, from which Trackmate was used to extract lysosome positions at each time point for further analysis.



Movie S2.
Live cell imaging by SIM demonstrates the dynamic motions of early endosomes and lysosomes. A COS-7 cell expressing Rab5-EGFP (yellow) and mApple-Sec61β-C1 (magenta), stained with SiR-lysosome (green) was imaged over 58.5 s at 1.5 s/frame. Data were extracted by TrackMate to compare the organelle dynamics between early endosomes and lysosomes.



Movie S3.
Live cell imaging by SIM demonstrates the coupled motions of the growing tip of a newly formed ER tubule (magenta) and associated lysosome (green).
A region of a COS-7 cells expressing EGFP-VAPA (magenta) and lysosomes labeled with SiR-lysosome (green) was imaged at 1.5 s/frame. The tip of ER forms three-way junctions.

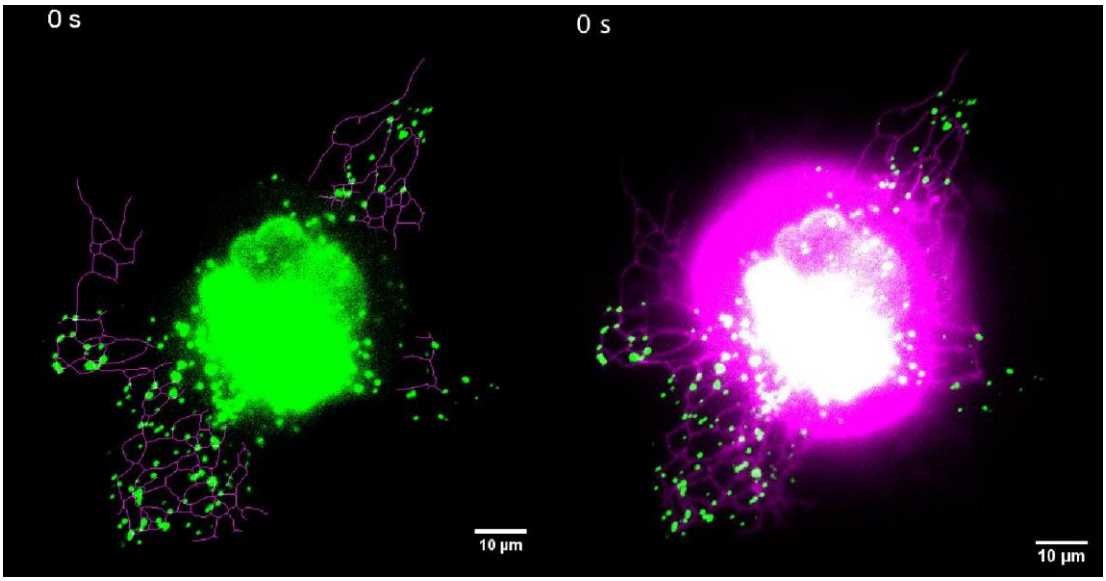


Movie S4.
Live cell imaging by SIM demonstrates retraction of ER tubules when not associated lysosomes.
A region of a COS-7 cells expressing EGFP-VAPA (magenta) and lysosomes labeled with SiR-lysosome (green) was imaged at 1.5 s/frame. The tubules are unstable and retracts after a period of elongation.

Movie S5.

Live cell imaging by SIM demonstrates breakage of the connection between the growing tip of a newly-formed ER tubule and a lysosome.

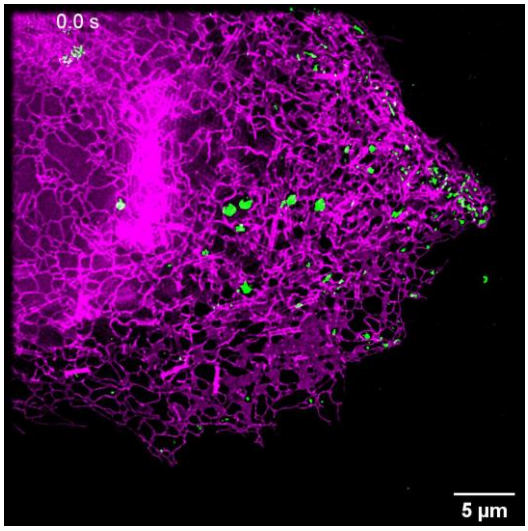
A region of a COS-7 cells expressing EGFP-VAPA (KD/MD) (magenta) and lysosomes labeled with SiR dye (green) was imaged at 1.5 s/frame. The tubule retracted after its detachment from a moving lysosome that was observed to gain an increase in velocity simultaneously. The lysosome positions at each time point were extracted by Trackmate to analyze the lysosome velocity changes.



Movie S6.

Live cell imaging of optogenetically controlled lysosome motions and their guided ER reshaping.

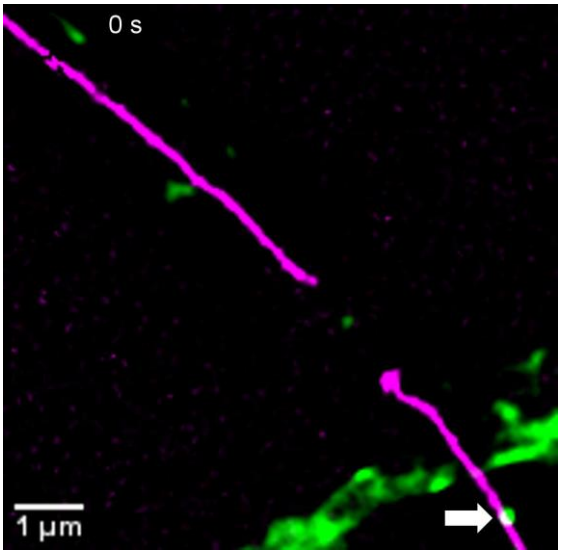
A COS-7 cell expressing LAMP1-mCherry-iLID (green), YFP-ER (magenta) and KIF1-VVDfast were imaged by widefield microscope over 4 mins at 2 s/frame. Left: ER channel of the widefield images was reconstructed to extract the skeletons by a trained artificial neural network for further quantification (see methods). Right: original widefield imaging.



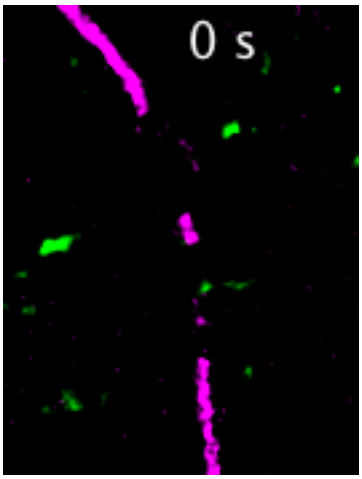
Movie S7.

Live cell imaging by SIM demonstrates anterograde motions of lysosomes and ER network protrution under prolonged starvation.

COS-7 cells expressing EGFP-VAPA (magenta) and lysosomes labeled with SiR dye (green) were treated with serum-free DMEM for 24 hr then imaged at 1.5 s/frame. The lysosome positions at each time point were extracted by Trackmate to analyze the movement direction.



Movie S8.
Live cell imaging by SIM demonstrates the reconnection of ER tubules by a moving lysosome in an VAPA expressing RGC axon.
RGC axons expressing EGFP-VAPA (magenta) and stained with SiR-Lysosome (green) were imaged at 5 s/frame.



Movie S9.
Live cell imaging by SIM demonstrates the persistent breakage of ER tubules in an VAPA(KD/MD) expressing RGC axon.
RGC axons expressing EGFP-VAPA(KD/MD) (magenta) and stained with SiR-Lysosome (green) were imaged at 5 s/frame.