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Supplementary Information for

ER-phagy requires Lnp1, a protein that stabilizes rearrangements of the ER network

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This PDF file includes:

Figs. S1 to S6
Table S1

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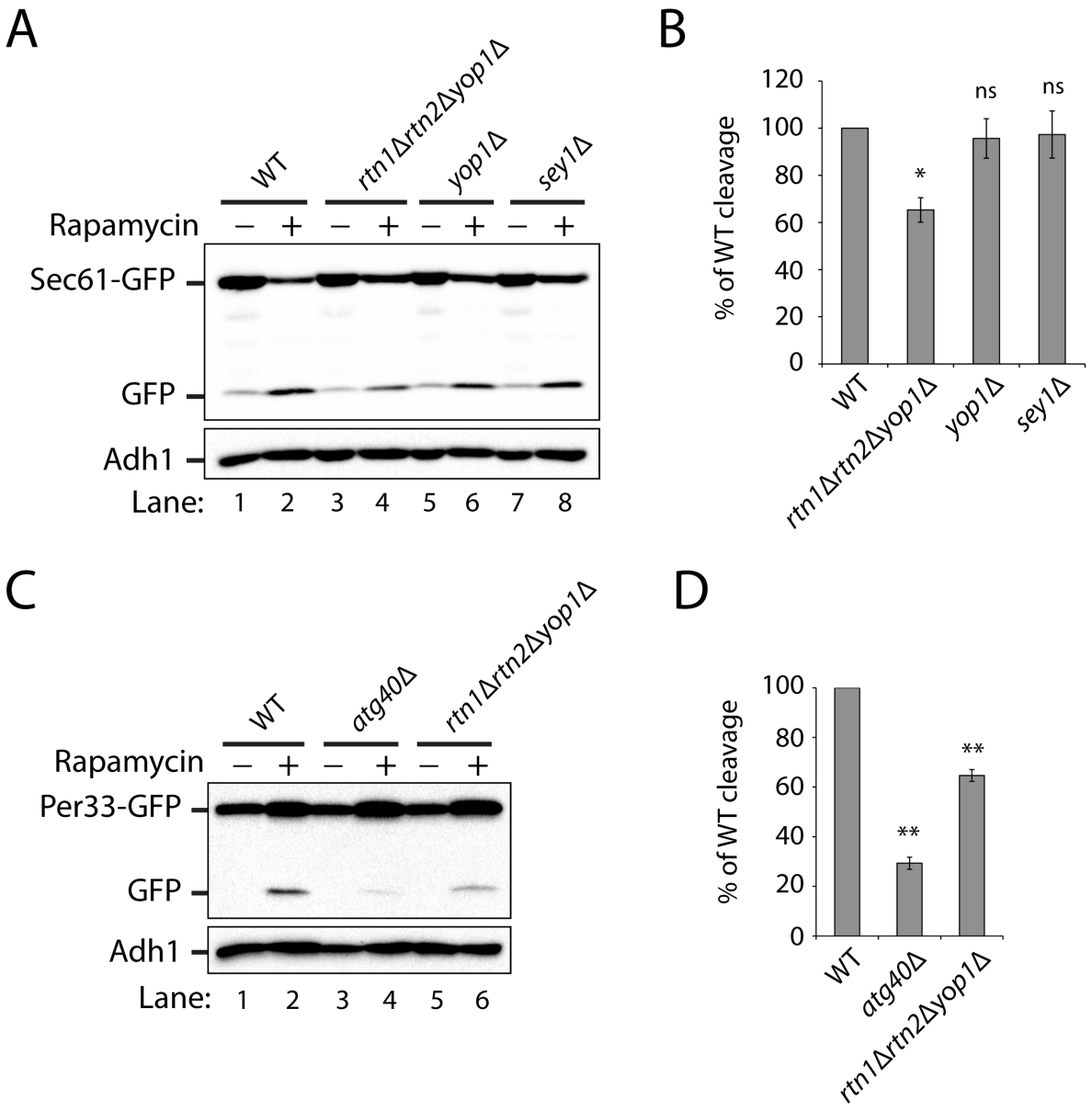

Figure S1. The *rtn1Δ rtn2Δ yop1Δ* triple mutant exhibits a modest ER-phagy defect.

(A) Western blot analysis showing the cleavage of Sec61-GFP in WT, *rtn1Δ rtn2Δ yop1Δ*, *yop1Δ* and *sey1Δ* mutant cells after 0 or 24 hr of rapamycin treatment at 30°C. (B) Quantitation of the percent of cleaved GFP divided by total Sec61-GFP for WT and mutant cells treated with rapamycin for 24 hr. The data was normalized to the WT control. Error bars represent S. E. M.

from three separate experiments. *P < 0.05 Student's t-test, ns: non-significant. (C) Western blot analysis showing the cleavage of Per33-GFP in WT, *atg40Δ* and *rtn1Δ rtn2Δ yop1Δ* mutant cells after 0 or 12 hr of rapamycin treatment at 30^{o}C. (D) Quantitation of the percent of cleaved GFP divided by total Per33-GFP for WT and mutant cells treated with rapamycin for 12 hr. The data was normalized to the WT control. Error bars represent S. E. M. from three separate experiments. **P < 0.01 Student's t-test.

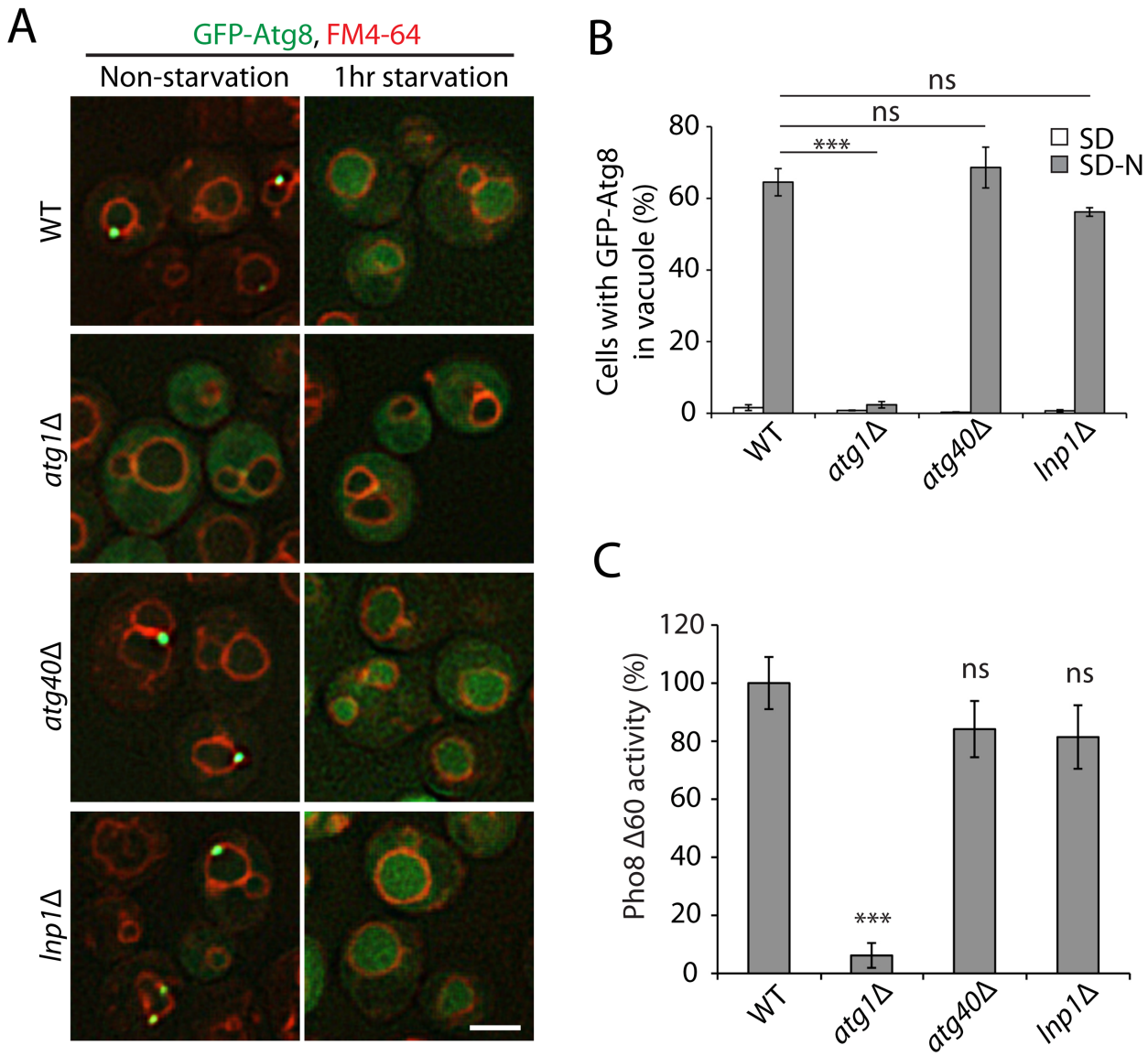

Figure S2. Macroautophagy is not impaired in the *lnp1*Δ mutant.

(A) Cells expressing GFP-Atg8 were grown in SD (SC with 2% glucose) or SD-N (starvation) media for 1 hr at 30°C before the vacuole was stained with FM4-64. Subsequently, the cells were harvested and directly examined by fluorescence microscopy. (B) The percent of cells with GFP in the vacuole in part (A) was quantified. Error bars represent S. E. M. for three separate experiments. ***P < 0.001 Student's t-test, ns: non-significant. (C) Cells expressing the

cytoplasmic form of vacuolar alkaline phosphatase (Pho8Δ60) were grown for 2 hr at 30°C in SD-N media. Alkaline phosphatase assays were performed as described in the Materials and Methods, and the Pho8Δ60 activity was normalized to WT. Error bars represent S. E. M. for three separate experiments. ***P < 0.001 Student's t-test, ns: non-significant. The scale bar in (A) is 2 μm.

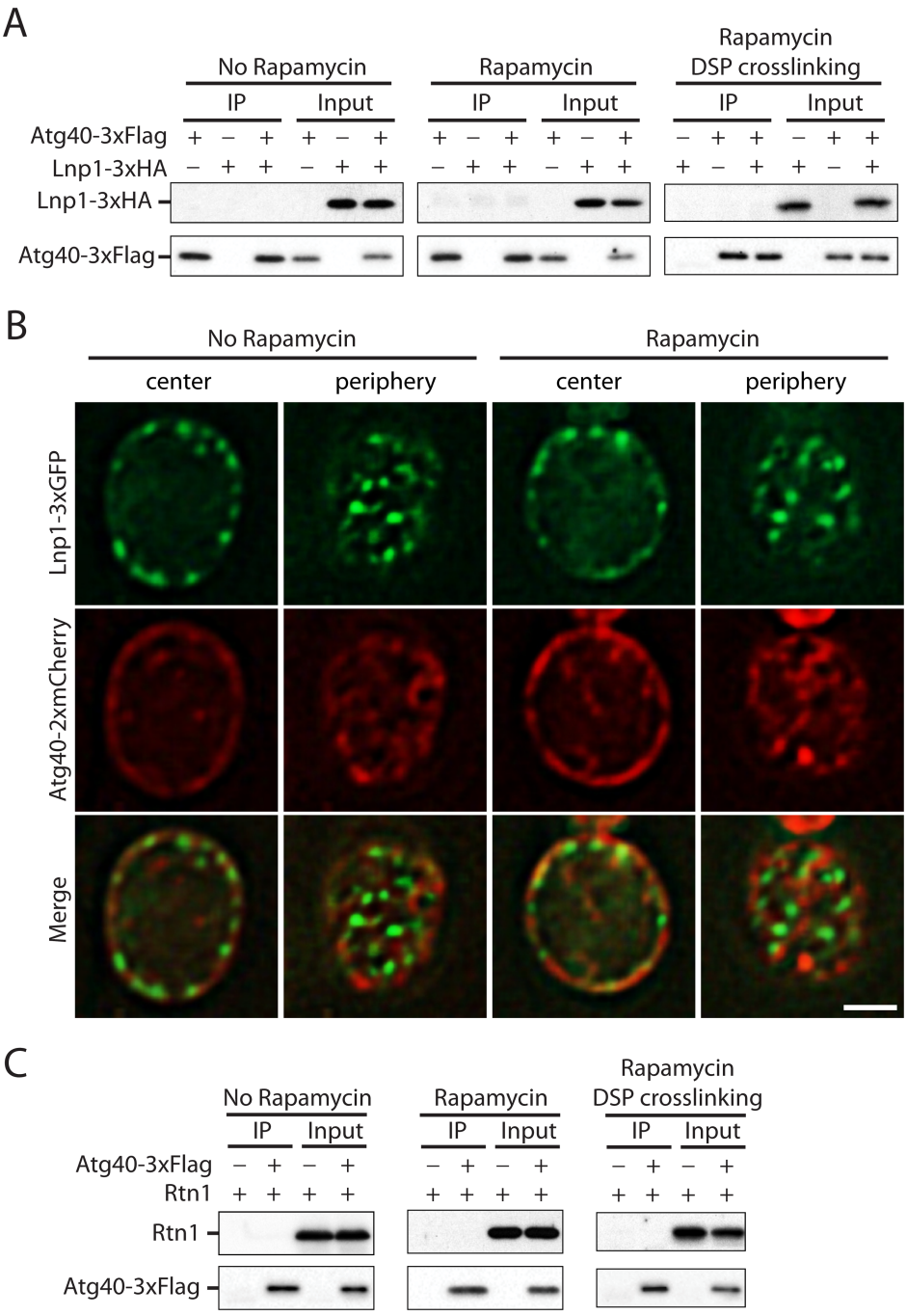

Figure S3. Atg40 does not interact with Lnp1 or Rtn1.

(A) Atg40-3xFLAG was precipitated from lysates, with or without crosslinking, as described in the Materials and Methods and the precipitates were blotted for the presence of Lnp1-3xHA. (B)

Cells expressing Lnp1-3xGFP and Atg40-2xmCherry were grown at 30°C in SC media without or with rapamycin (200 ng/ml) for 3 hr before they were harvested and directly examined by fluorescence microscopy. (C) Atg40-3xFLAG was precipitated from lysates, with or without crosslinking, as described in the Materials and Methods and the precipitates were analyzed for the presence of Rtn1 by western blot analysis. The scale bar in (B) is 1 μm.

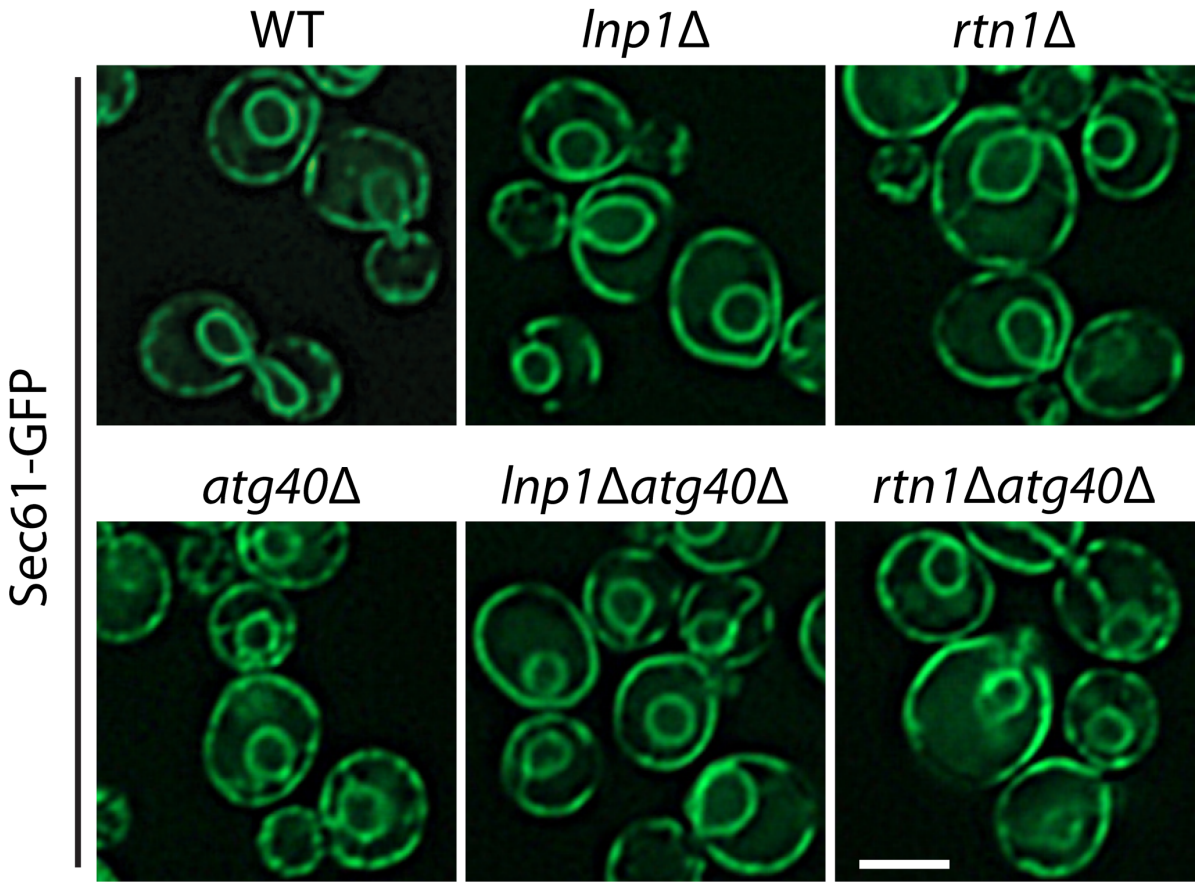

Figure S4. The loss of Atg40 does not disrupt ER structure or accentuate the ER structure defects of *lnp1*Δ or *rtn1*Δ mutants.

WT and mutant cells expressing Sec61-GFP were grown in SC media to early log phase, harvested and directly examined by fluorescence microscopy. The scale bar is 2 μm.

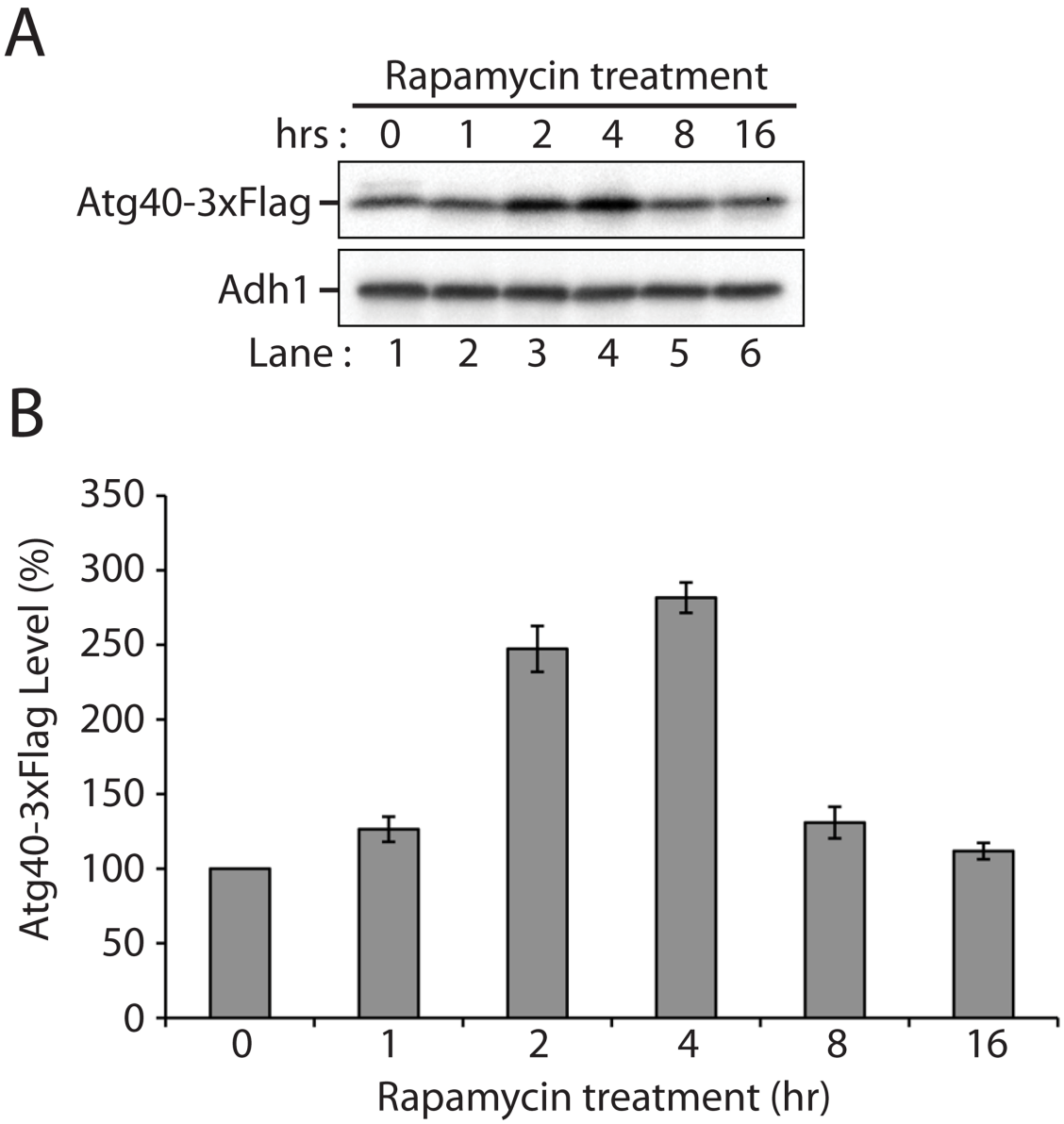

Figure S5. The level of Atg40 increases transiently during rapamycin treatment.

(A) Cells expressing Atg40-3xFLAG were grown in SC media containing 200 ng/ml of rapamycin at 30°C. Samples were harvested at the indicated time points, and lysates were prepared as described in the Materials and Methods. The levels of Atg40-3xFLAG were determined by western blot analysis. Alcohol dehydrogenase (Adh1) was used as a loading

control. (B) Atg40-3xFLAG was normalized to the 0 time point and the amount of Adh1 in each sample. Error bars represent S. E. M. for three separate experiments.

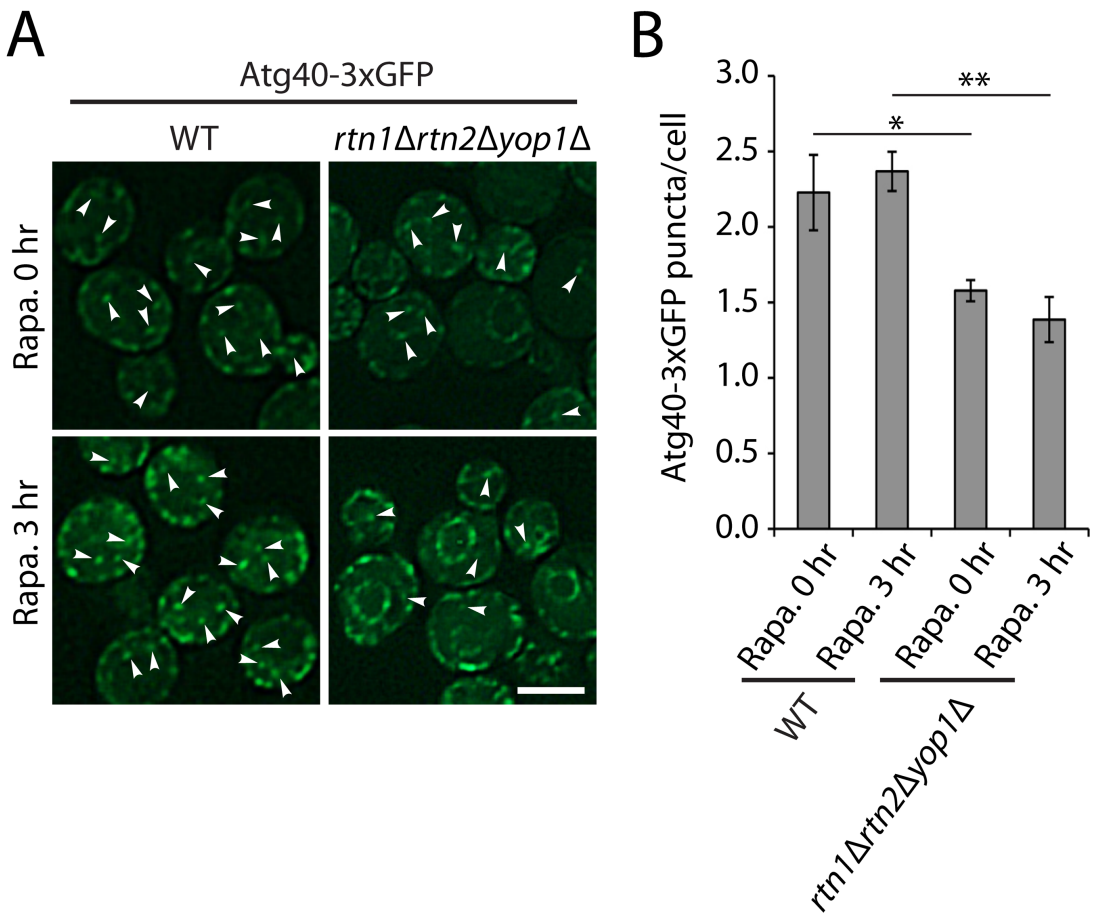

Figure S6. Atg40 redistributes to the nuclear envelope in *rtn1*Δ *rtn2*Δ *yop1*Δ cells.

(A) WT and *rtn1Δ rtn2Δ yop1Δ* cells expressing Atg40-3xGFP were treated for 3 hr with rapamycin (200 ng/ml) in SC media at 30°C. Subsequently, cells were harvested and directly examined by fluorescence microscopy. Arrowheads mark Atg40 puncta located in the cell interior. (B) Quantitation of Atg40-3xGFP puncta in the cell interior. Puncta were quantitated from the following number of cells: WT at 0hr, N = 358; WT at 3hr, N = 403; *rtn1*Δ *rtn2*Δ *yop1*Δ at 0hr, N =322; *rtn1*Δ *rtn2*Δ *yop1*Δ at 3hr, N =375. Error bars represent S. E. M. from three separate experiments. *P < 0.05, **P < 0.01 Student's t-test. The scale bar in (A) is 2 μm.

Table S1. Yeast strains used in this study.

Strain #	Genotype
SFNY 2094	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, SEC61-GFP::LEU2*
SFNY 2095	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, rtn1Δ::KanMX6, SEC61-GFP::LEU2*
SFNY 2096	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, sey1Δ::KanMX6, SEC61-GFP::LEU2*
SFNY 2269	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, lnp1Δ::His3MX6, SEC61-GFP::LEU2*
SFNY 2270	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, lnp1Δ::His3MX6, sey1Δ::URA3, SEC61-GFP::LEU2*
SFNY 3138	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, rtn2Δ::KanMX6, SEC61-GFP::LEU2*
SFNY 2837	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, atg40Δ::KanMX6, SEC61-GFP::LEU2*
SFNY 2271	*MAT alpha, Gal+, ura3-52, leu2-3,112, his3Δ200, rtn1Δ::KanMX6, rtn2Δ::URA3, yop1Δ::URA3, SEC61-GFP::LEU2*
SFNY 3123	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, ypt7Δ::His3MX6, SEC61-2xRFP::LEU2, pRS416-GFP-Atg8 (URA3, CEN)*
SFNY 3124	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, ypt7Δ::His3MX6, atg40Δ::KanMX6, SEC61-2xRFP::LEU2, pRS416-GFP-Atg8 (URA3, CEN)*
SFNY 3125	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, ypt7Δ::His3MX6, lnp1Δ::KanMX6, SEC61-2xRFP::LEU2, pRS416-GFP-Atg8 (URA3, CEN)*
SFNY 2566	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, pho8Δ60::URA3*
SFNY 2978	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, atg40Δ::KanMX6, pho8Δ60::URA3*
SFNY 2979	*MAT alpha, Gal+, ura3-52, leu2-3,112, his3Δ200, atg1Δ::His3MX6, pho8Δ60::URA3*
SFNY 2980	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, lnp1Δ::His3MX6, pho8Δ60::URA3*
SFNY 2834	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, pRS416-GFP-Atg8 (URA3, CEN)*
SFNY 2857	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, atg40Δ::KanMX6, pRS416-GFP-Atg8 (URA3, CEN)*
SFNY 2835	*MAT alpha, Gal+, ura3-52, leu2-3,112, his3Δ200, atg1Δ::His3MX6, pRS416-GFP-Atg8 (URA3, CEN)*
SFNY 2558	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, lnp1Δ::His3MX6, pRS416-GFP-Atg8 (URA3, CEN)*
SFNY 3013	*MAT alpha, Gal+, ura3-52, leu2-3,112, his3Δ200, atg1Δ::His3MX6, SEC61-GFP::URA3*
SFNY 3438	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, ATG40-3xGFP::URA3, Sec61-2xRFP::LEU2*
SFNY 3439	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, lnp1Δ::His3MX6, ATG40-3xGFP::URA3, Sec61-2xRFP::LEU2*
SFNY 3440	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, ATG40-3xGFP::URA3*
SFNY 3441	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, lnp1Δ::His3MX6, ATG40-3xGFP::URA3*
SFNY 3442	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, sey1Δ::His3MX6, ATG40-3xGFP::URA3*
SFNY 3443	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, lnp1Δ::His3MX6, sey1Δ::KanMX6, ATG40-3xGFP::URA3*
SFNY 3444	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, rtn1Δ::His3MX6, rtn2Δ::KanMX6, yop1Δ::KanMX6, ATG40-3xGFP::URA3*
SFNY 3113	*MAT a, Gal+, Gal+, ura3-52, leu2-3,112, his3Δ200, PER33-GFP::LEU2*
SFNY 3114	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, lnp1Δ::His3MX6, PER33-GFP::LEU2*

SFNY 3115	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, atg40Δ::KanMX6, PER33-GFP::LEU2*
SFNY 3116	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, rtn1Δ::His3MX6, PER33-GFP::LEU2*
SFNY 3117	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, rtn2Δ::KanMX6, PER33-GFP::LEU2*
SFNY 3118	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, yop1Δ::KanMX6, PER33-GFP::LEU2*
SFNY 3119	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, sey1Δ::His3MX6, PER33-GFP::LEU2*
SFNY 3120	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, lnp1Δ::His3MX6, sey1Δ::KanMX6, PER33-GFP::LEU2*
SFNY 3121	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, rtn1Δ::His3MX6, rtn2Δ::KanMX6, yop1Δ::KanMX6, PER33-GFP::LEU2*
SFNY 2955	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, ATG40-3xFLAG::KanMX6*
SFNY 2962	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, ATG40-3xGFP::URA3, ATG11-2xmCherry::LEU2*
SFNY 2963	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, lnp1Δ::His3MX6, ATG40-3xGFP::URA3, ATG11-2xmCherry::LEU2*
SFNY 2961	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, LNP1-3xGFP::His3MX6, ATG40-2xmCherry::URA3*
SFNY 2957	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, lnp1Δ::His3MX6, atg40Δ::KanMX6, SEC61-GFP::LEU2*
SFNY 2958	*MAT a, Gal+, ura3-52, leu2-3,112, his3Δ200, rtn1Δ::URA3, atg40Δ::KanMX6, SEC61-GFP::LEU2*