Supplemental material

Shima et al., https://doi.org/10.1083/jcb.201809032

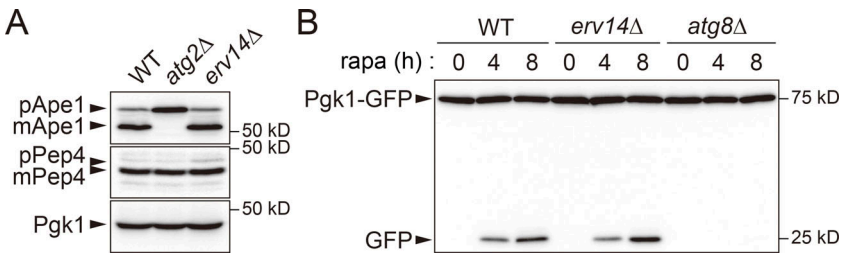

Figure S1. **Erv14 is not important for autophagy. (A)** Cells were grown to mid-log phase, treated with rapamycin for 2 h, and examined by immunoblotting using antibodies against Ape1, Pep4, and Pgk1. mApe1, mature Ape1; pApe1, Ape1 proform; mPep4, mature Pep4; pPep4, Pep4 proform. **(B)** Cells expressing Pgk1-GFP were examined by immunoblotting using anti-GFP antibodies as described in A.

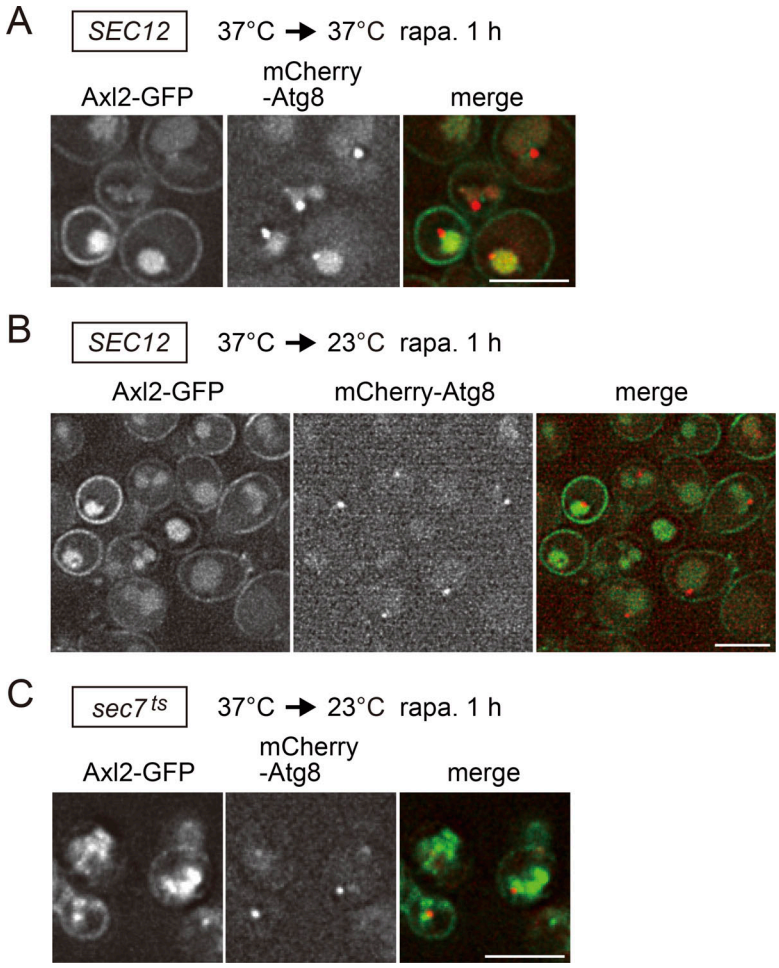

Figure S2. **Axl2-GFP is transferred from the ER to autophagy-related membranes, not via the plasma membrane or Golgi apparatus. (A and B)** *SEC12 SSA1pro-AXL2-GFP mCherry-ATG8* cells were incubated at 37°C for 1 h to allow expression of Axl2-GFP, treated with rapamycin for 1 h at 37°C (A) or 23°C (B), and then observed by fluorescence microscopy. **(C)** *sec7*^{ts} *SSA1pro-AXL2-GFP mCherry-ATG8* cells were incubated at 37°C for 1 h, treated with rapamycin at 23°C for 1 h, and then observed by fluorescence microscopy. Scale bars, 5 µm.

Table S1. **Yeast strains used in this study**

Name	Genotype	Figure/Reference/Source
X2180-1A	*MATa SUC2 mal mel gal2 CUP1*	Yeast Genetic Stock Center
HMSF162	X2180-1A *sec12-4*	Novick et al., 1980
YSMT309	YNH137 *trp1Δ::nat*NT2 *TRP1proΔ::pRS304-p2HSE-AXL2-GFP*	None
YSMT698	YNH137 *trp1Δ::nat*NT2 *TRP1proΔ::pRS304-p2HSE-AXL2-GFP leu2::ATG8pro-2xmCherry-ATG8-hph*NT1	Figs. 1 C and 2 A
YSMT710	YNH137 *trp1Δ::nat*NT2 *TRP1proΔ::pRS304-p2HSE-AXL2-GFP SEC63-mCherry-hph*NT1	Fig. 1 B
YSMT711	YSMT710 *erv14Δ::kan*MX4	Fig. 1 B
YSMT700	YSMT698 *erv14Δ::kan*MX4	Fig. 1 C
YSMT699	YSMT698 *atg2Δ::kan*MX4	Fig. 2 A
YSMT708	YSMT698 *ura3Δ::zeo*NT3 *ura3::GPDpro-APE1-pRS306*	Fig. 2 B
YSMT739	YSMT698 *erv14Δ::kan*MX4 *ura3Δ::zeo*NT3 *ura3::GPDpro-APE1-pRS306*	Fig. 2 B
YSMT700	YSMT698 *ypt7Δ::kan*MX4	Fig. 2 (C–E)
YSMT699	YSMT698 *erv14Δ::kan*MX4 *ypt7Δ::zeo*NT3	Fig. 2 (C–E)
YSMT314	YSMT309 *ATG8pro-2xmCherry-ATG8-hph*NT1 *ypt7Δ::kan*MX4	Fig. 3 (A, B, and E–J)
YSMT483	YSMT309 *ATG8pro-2xmCherry-ATG8-hph*NT1 *erv14Δ::zeo*NT3 *ypt7Δ::kan*MX4	Fig. 3 (C and B)
W303-1A	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*	Thomas and Rothstein, 1989
AIDEG1	W303-1A *ADE2 ura3-1::pNHK53* (*ADH1pro-OsTIR1-9Myc*)	Fig. S1 A
AIDEG22	AIDEG1 *atg2Δ::kan*MX4	Fig. S1 A
YSMT469	AIDEG1 *erv14Δ::kan*MX4	Fig. S1 A
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Brachmann et al., 1998
YNH1344	BY4741 *PGK1-EGFP-his*MX4	Fig. S1 B
YNH1345	BY4741 *PGK1-EGFP-his*MX4 *atg8Δ::zeo*NT3	Fig. S1 B
YSMT482	BY4741 *PGK1-EGFP-his*MX4 *erv14Δ::zeo*NT3	Fig. S1 B
YSMT612	X2180-1 *trp1Δ::nat*NT2 *TRP1proΔ::pRS304-p2HSE-AXL2-GFP leu2::ATG8pro-2xmCherry-ATG8-hph*NT1	Fig. S2 (A and B)
YSMT607	X2180-1 *sec7-1 trp1Δ::nat*NT2 *TRP1proΔ::pRS304-p2HSE-AXL2-GFP leu2::ATG8pro-2xmCherry-ATG8-hph*NT1	Fig. S2 C

References

Brachmann, C.B., A. Davies, G.J. Cost, E. Caputo, J. Li, P. Hieter, and J.D. Boeke. 1998. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast.* 14:115–132. https://doi.org/10.1002/(SICI)1097-0061(19980130)14:2<115::AID-YEA204>3.0.CO;2-2

Novick, P., C. Field, and R. Schekman. 1980. Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. *Cell.* 21:205–215. https://doi.org/10.1016/0092-8674(80)90128-2

Thomas, B.J., and R. Rothstein. 1989. The genetic control of direct-repeat recombination in *Saccharomyces*: the effect of *rad52* and *rad1* on mitotic recombination at *GAL10*, a transcriptionally regulated gene. *Genetics.* 123:725–738.