Supplementary Information

Trs130 participates in autophagy through GTPases Ypt31/32 in *Saccharomyces cerevisiae*

Zou *et al.*,

Table S1. Yeast strains and plasmids used in this study

A. Strains

Strain	Alias	Genotype	Source
NSY991	VSY459, TRS130	*MATa leu2-3,112 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9 ura3-52 TRS130-HA::HIS3MX6*	(1)
NSY992	*trs130ts*	*MATa leu2-3,112 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9 ura3-52 trs130-(33 aa truncation)-HA^{ts}::HIS3MX6*	(1)
YLY2360	GFP-Atg8 TRS130	NSY991, *GFP-ATG8::URA3*	This study
YLY2361	GFP-Atg8 *trs130ts*	NSY992, *GFP- ATG8::URA3*	This study
YLY2741	GFP-Atg8 RFP-Ape1 TRS130	NSY991, *GFP-Atg8::URA3,RFP-APE1::LEU2*	This study
YLY2742	GFP-Atg8 RFP-Ape1 *trs130ts*	NSY992, *GFP-ATG8::URA3,RFP-APE1::LEU2*	This study
YLY4081	TRS130 *trs85Δ*	YLY2741, *trs85Δ::Kan*	This study
YLY4083	*trs130ts trs85Δ*	YLY2742, *trs85Δ::Kan*	This study
YLY3661	TRS130 *atg1Δ*	YLY2741, *atg1Δ::Kan*	This study
YLY3662	*trs130ts atg1Δ*	YLY2742, *atg1Δ::Kan*	This study
NSY1176	Trs130-HA Trs120-myc	*MATα leu2-3,112 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9 ura3–52 TRS130-HA::HIS3MX6 TRS120-myc::TRP1*	(2)
NSY1177	*trs65ts*	NSY1176*, trs65Δ::Kan*	(2)
YLY2483	GFP-Atg8 TRS65	NSY1176, *GFP-ATG8::URA3*	This study

YLY2484	GFP-Atg8 *trs65ts*	NSY1177, *GFP-ATG8::URA3*	This study
YLY2851	GFP-Atg8 RFP-Ape1 TRS65	NSY1176, *GFP-ATG8::URA3,RFP-APE1::LEU2*	This study
YLY2852	GFP-Atg8 RFP-Ape1 *trs65ts*	NSY1177, *GFP-ATG8::URA3,RFP-APE1::LEU2*	This study
YLY4504	GFP-Atg8 RFP-Ape1 *tca17ts*	YLY2851*, tca17Δ::Kan*	This study
YLY3362	TRS130 *atg11Δ*	YLY2741, *atg11Δ::Hyg*	This study
YLY3365	*trs130ts atg11Δ*	YLY2742, *atg11Δ::Hyg*	This study
YLY3331	TRS130 *atg17Δ*	YLY2741, *atg17Δ::Kan*	This study
YLY3334	*trs130ts atg17Δ*	YLY2742, *atg17Δ::Kan*	This study
YLY3126	TRS130 *atg5Δ*	YLY2741, *atg5Δ::Hyg*	This study
YLY3127	*trs130ts atg5Δ*	YLY2742, *atg5Δ::Hyg*	This study
YLY4205	TRS130 *Atg13Δ*	YLY2741, *atg13Δ::Hyg*	This study
YLY4208	*trs130ts atg13Δ*	YLY2742, *atg13Δ::Hyg*	This study
YLY4068	TRS130 *Atg9Δ*	YLY2741, *atg9Δ::Hyg*	This study
YLY4071	*trs130ts atg9Δ*	YLY2742, *atg9Δ::Hyg*	This study
YLY3212	TRS130 *atg14Δ*	YLY2741, *atg14Δ::Hyg*	This study
YLY3213	*trs130ts atg14Δ*	YLY2742, *atg14Δ::Hyg*	This study
YLY4462	TRS33	*MAT a ura3-52 leu2-3,112 trp1-Δ901 lys2-801 his3-Δ200, TRS130-HA::HIS3MX6, GFP-Atg8::URA3,RFP-APE1::LEU2*	This study
YLY4464	*trs33Δ*	YLY4462, *trs33Δ::Hyg*	This study
YLY4431	TRS120	SEY6210, *TRS130-myc::TRP1* + pRS425-YPT31, *GFP-Atg8::URA3,RFP-APE1::LEU2*	This study

YLY4021	*trs120Δ*	SEY6210, *trs120::HIS3MX6 TRS130-myc::TRP1* + pRS425-YPT31, *GFP-Atg8::URA3,RFP-APE1::LEU2*	This study
YLY2705	Atg9-GFP RFP-Ape1 TRS130	NSY991, *ATG9-3XGFP::URA3,RFP-APE1::LEU2*	This study
YLY2704	Atg9-GFP RFP-Ape1 *trs130ts*	NSY992, *ATG9-3XGFP::URA3,RFP-APE1::LEU2*	This study
YLY2978	Atg9-GFP RFP-Ape1 TRS130 *atg1Δ*	YLY2705, *atg1Δ::Kan*	This study
YLY2979	Atg9-GFP RFP-Ape1 *trs130ts atg1Δ*	YLY2704, *atg1Δ::Kan*	This study
YLY2710	Atg9-GFP RFP-Ape1 TRS65	NSY1176, *ATG9-3XGFP::URA3,RFP-APE1::LEU2*	This study
YLY2870	Atg9-GFP RFP-Ape1 *trs65ts*	YLY2710, *trs65Δ::Hyg*	This study
YLY2746	Atg9-GFP RFP-Ape1 TRS65 *atg1Δ*	YLY2710, *atg1Δ::Kan*	This study
YLY2872	Atg9-GFP RFP-Ape1 *trs65ts atg1Δ*	YLY2746, *trs65Δ::Hyg*	This study
YLY2540	TRS130 Sec7-DsRed	NSY991, *SEC7-DsRed*	This study
YLY2541	*trs130ts* Sec7-DsRed	NSY992, *SEC7-DsRed*	This study
YLY2817	Sec7-DsRed Atg9-3XGFP T RS130	YLY2540, *ATG9-3XGFP::URA3*	This study
YLY2818	Sec7-DsRed Atg9-3XGFP *trs130ts*	YLY2541, *ATG9-3XGFP::URA3*	This study

YLY3091	Sec7-DsRed Atg9-3XGFP TRS130 *atg1Δ*	YLY2817, *atg1Δ::Kan*	This study
YLY3092	Sec7-DsRed Atg9-3XGFP *trs130ts* *atg1Δ*	YLY2818, *atg1Δ::Kan*	This study
YLY2914	Sec7-DsRed GFP-Atg8 TRS130	YLY2540, *GFP-ATG8::URA3*	This study
YLY2917	Sec7-DsRed GFP-Atg8 *trs130ts*	YLY2541, *GFP-ATG8::URA3*	This study
YLY3337	Sec7-DsRed GFP-Atg8 TRS130 *atg1Δ*	YLY2914, *atg1Δ::Kan*	This study
YLY3339	Sec7-DsRed GFP-Atg8 *trs130ts* *atg1Δ*	YLY2917, *atg1Δ::Kan*	This study
YLY3359	TN124	*MATα leu2-3,112 trp1 ura3-52 pho8::pho8Δ60 pho13::LEU2*	(3)
YLY3799	TRS130 (TN124)	TN124, *HIS3Δ::Hyg*, *TRS130-HA::HIS3MX6*	This study
YLY3800	*trs130ts* (TN124)	TN124, *HIS3Δ::Hyg*, *trs130-(33 aa truncation)-HA^{ts}::HIS3MX6*	This study
YLY3956	*atg1Δ* (TN124)	YLY3799, *atg1Δ::Kan*	This study
YLY4060	TRS65 (TN124)	TN124, *HIS3Δ::Hyg*, *TRS130-HA::HIS3MX6 TRS120-myc::TRP1*	This study
YLY4140	*trs65ts* (TN124)	YLY4060*, trs65Δ::Kan*	This study
NSY1081	YPT1	*MAT a ura3-52 Leu2 his3*	(2)
NSY1082	*ypt1ts*	*MAT a ura3-52 Leu2 his3 ypt1A136D*	(2)
YLY2366	GFP-Atg8 YPT1	NSY1081, *GFP-ATG8::URA3*	This study
YLY2367	GFP-Atg8 *ypt1ts*	NSY1082, *GFP-ATG8::URA3*	This study

YLY2417	GFP-Snc1 Sec7-DsRed	*MAT a leu2-3,112 lys2-801 TRS130-HA::HIS3MX6, GFP-Snc1::URA3, SEC7-DsRed*	This study
YLY2418	GFP-Snc1 Sec7-DsRed *trs130ts*	*MAT a leu2-3,112 lys2-801 trs130-(33 aa truncation)-HA^{ts}::HIS3MX6, GFP-Snc1::URA3, SEC7-DsRed*	This study
RSY367	SEC7	*MATa ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-Δ1 can1-100*	R. Scheckman lab
RSY301	*sec7-4*	*MATa ura3-1 his3-11,15 Leu2-3,112 trp1-Δ1*	R. Scheckman lab
YLY4478	SEC7 GFP-Atg8	RSY367, *TRS130-HA::HIS3MX6*, *GFP-ATG8::URA3*	This study
YLY4479	*trs130ts* GFP-Atg8	RSY367, *trs130-(33 aa truncation)-HA^{ts}::HIS3MX6*, *GFP-ATG8::URA3*	This study
YLY4480	*sec7-4* GFP-Atg8	RSY301, *TRS130-HA::HIS3MX6*, *GFP-ATG8::URA3*	This study
YLY4481	*sec7-4* *trs130ts* GFP-Atg8	RSY301, *trs130-(33 aa truncation)-HA^{ts}::HIS3MX6*, *GFP-ATG8::URA3*	This study
YLY40	NSY128	*MATα ade2 his3-200 leu2-3,112 lys2-801 ura3-52*	(4)
YLY44	NSY340 *ypt31Δ* *ypt32ts*	*MATα leu2-3,112 lys2-801 ura3-52 ypt31Δ::HIS3 ypt32A141D*	(4)

B. Plasmids

Plasmid	Alias	Genotype	Source
p1K-*GFP-ATG8*-406	pYL233		(5)
RFP-APE1-305	pYL238		(6)
ATG9-3XGFP-PG5	pYL242		(7)
Cu-Cherry-*ATG8*-415	pYL254		(8)
pNS180	pRS425	2μ, *LEU3,* Amp^{r}	(9)
pNS781	Ypt31	pRS425-*YPT31*	(1)
pYL316	Ypt31S	pRS425-*YPT31* S27N	This study
pYL313	Ypt31Q	pRS425-*YPT31* Q72L	This study
pYL317	Ypt1	pRS425-*YPT1*	This study
pYL120	pRS426	2μ, *URA3,* Amp^{r}	(9)

pYL335		pRS426-Ypt31	This study
pYL338		pRS426-*YPT31* S27N	This study
pYL336		pRS426-*YPT31* Q72L	This study
pYL339		pRS426-*YPT1*	This study
pYL151	YEp351	2μ, *LEU2*	(10)
pYL152		YEp351-*TRS130*	This study
pYL283		YEp351-*YPT32*	This study
pYL225		YEp351-*TRS65*	This study
pYL342		YEp351-*TCA17*	This study

Supplementary Materials and Methods

Growth assay of genetic interaction

For genetic interaction assays, cultures were grown overnight at 26°C in minimal medium (SD-Leu), normalized to the same density and spotted onto SD-Leu plates in serial dilutions of 1:10. Plates were incubated at various temperatures for different numbers of days and photographed.

References

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Figure S1

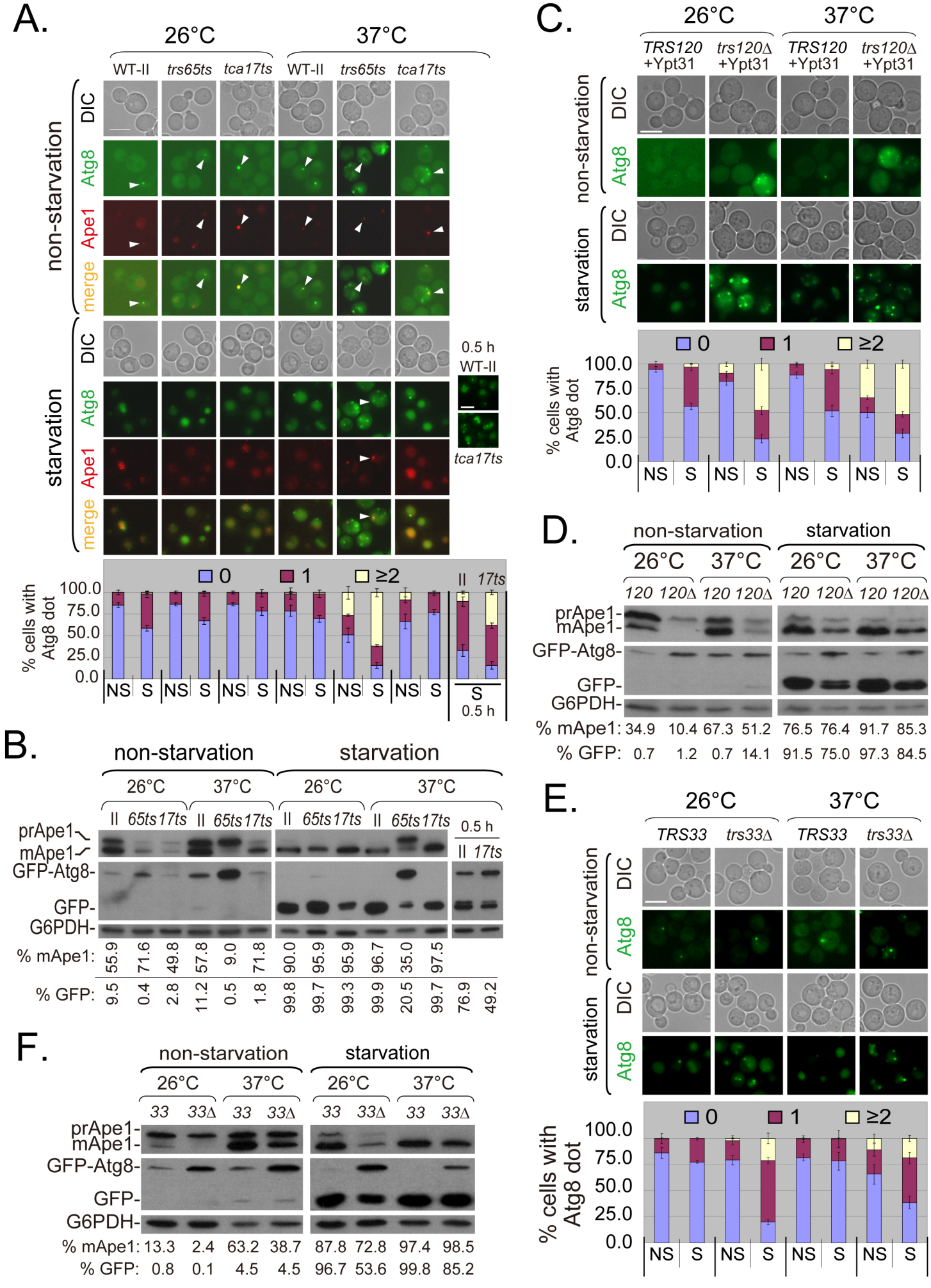
A.
26°C
37°C
WT-II
trs65ts
tca17ts
non-starvation
starvation
DIC
Atg8
Ape1
merge
0.5 h
% cells with Atg8 dot
0
1
≥2
NS
S
B.
non-starvation
starvation
prApe1
mApe1
GFP-Atg8
GFP-
G6PDH
% mApe1:
% GFP:
C.
TRS120 +Ypt31
trs120Δ +Ypt31
D.
120
120Δ
prApe1-
mApe1-
GFP-Atg8-
GFP-
G6PDH-
% mApe1: 34.9 10.4 67.3 51.2 76.5 76.4 91.7 85.3
% GFP: 0.7 1.2 0.7 14.1 91.5 75.0 97.3 84.5
E.
TRS33
trs33Δ
F.
33
33Δ
% mApe1: 13.3 2.4 63.2 38.7 87.8 72.8 97.4 98.5
% GFP: 0.8 0.1 4.5 4.5 96.7 53.6 99.8 85.2

Figure S1. Cvt pathway and starvation-induced autophagy are impaired in other TRAPP II-specific subunit mutants at the restrictive temperature. (A) Atg8 and Ape1 localization in *trs65ts* and *tca17ts* mutant cells. WT-II (for indicating TRAPP II-specific subunits Trs130 and Trs120 are tagged as Trs130-HA Trs120-myc) and mutant cells were tagged using GFP-Atg8 and RFP-Ape1 integration plasmids. Cells were grown and treated as in Figure 1A. GFP-Atg8 morphology of WT-II and *tca17ts* cells starved for 0.5 hour at 37°C is at right. Arrowheads indicate colocalization of Atg8 and Ape1. Bar, 5 μm. The percentage of cells with Atg8 dots was quantitated and presented as in Figure 1A. **(B)** Ape1 maturation was blocked under non-starvation conditions and GFP-Atg8 degradation was reduced under starvation conditions in *trs65ts* mutant cells at 37°C. Cells grown as in (A) but in YPD were subjected to immunoblot assay, quantitated and presented as in Figure 1B. II for WT-II as in (A). **(C)-(F)**: GFP-Atg8 localization in the *trs120*Δ mutant (*trs120*Δ+Ypt31) **(C)** and the *trs33*Δ mutant (*trs33*Δ in Trs130-HA background) (Trs33 was a candidate for TRAPP II-specific subunit in this study) **(E)**; immunoblot assay for autophagy defects in *trs120*Δ mutant **(D)** and *trs33*Δ mutant **(F)**. Experiments were done as in (A) and (B).

Figure S2

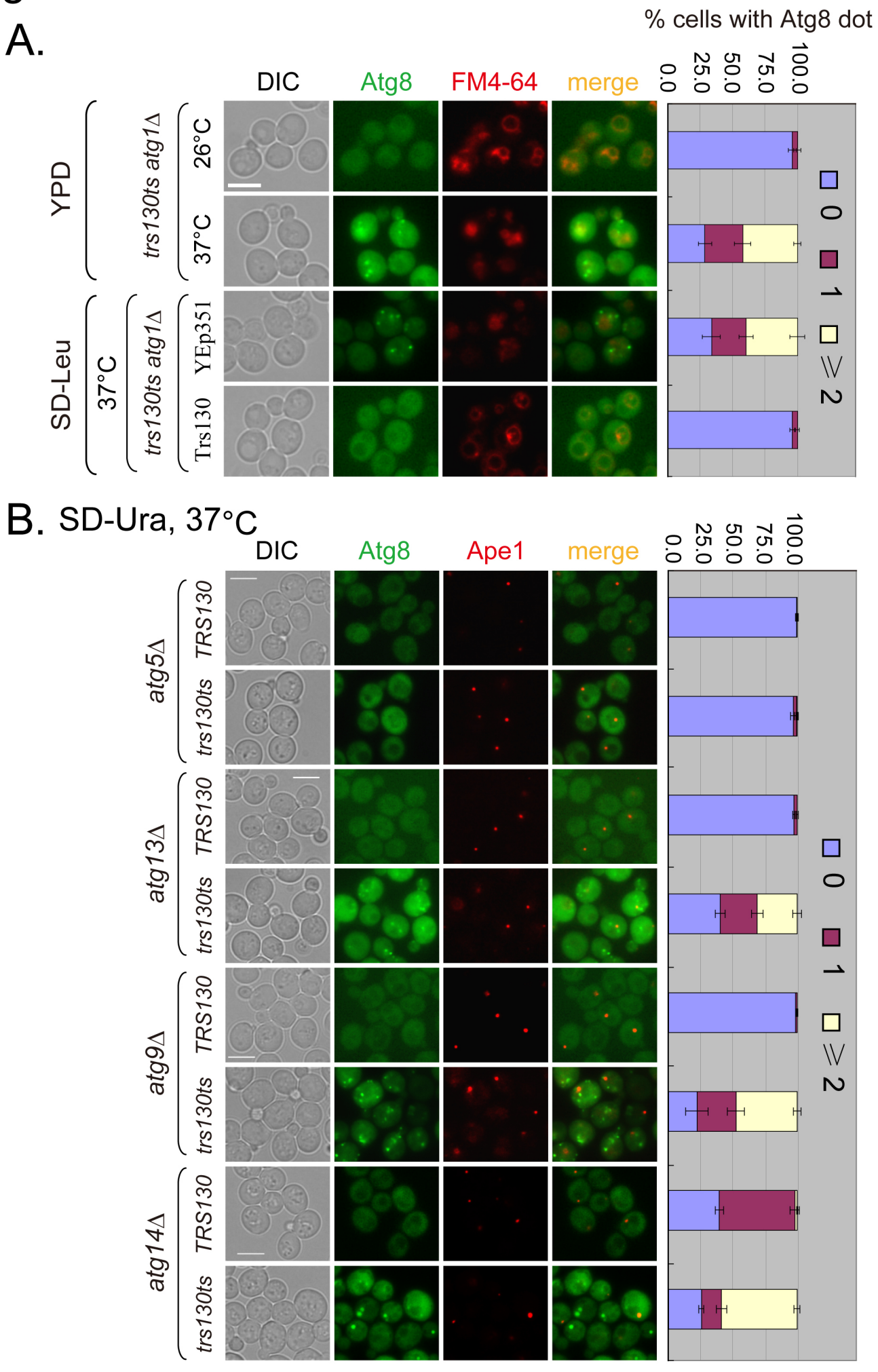
A.
DIC
Atg8
FM4-64
merge
% cells with Atg8 dot
0.0
25.0
50.0
75.0
100.0
YPD
SD-Leu
37°C
trs130ts atg1Δ
26°C
37°C
YEp351
Trs130
0
1
⩾2
B. SD-Ura, 37°C
DIC
Atg8
Ape1
merge
atg5Δ
atg13Δ
atg9Δ
atg14Δ
TRS130
trs130ts

Figure S2. Localization of Atg8 in *trs130ts* mutants with deletion of *ATG* genes without starvation. (A) Multiple GFP-Atg8 dots were formed in *trs130ts atg1*Δ without starvation at the restrictive temperature; this was rescued by Trs130. Cells grown in rich medium (YPD or SD-Leu) to mid-log phase at 26°C were treated as in Figure 1A for non-starvation and stained with FM4-64 during the last hour to detect the vacuole. Bar, 5 μm. **(B)** Yeast strains were grown and treated as described in Figure 3B except that incubation was in SD-Ura for 1.5 hours at 37°C without starvation. Cells were examined by live microscopy imaging for changes in GFP-Atg8 and RFP-Ape1. Bar, 5 μm. The percentage of cells with Atg8 dots was quantitated and presented as in Figure 1A.

Figure S3

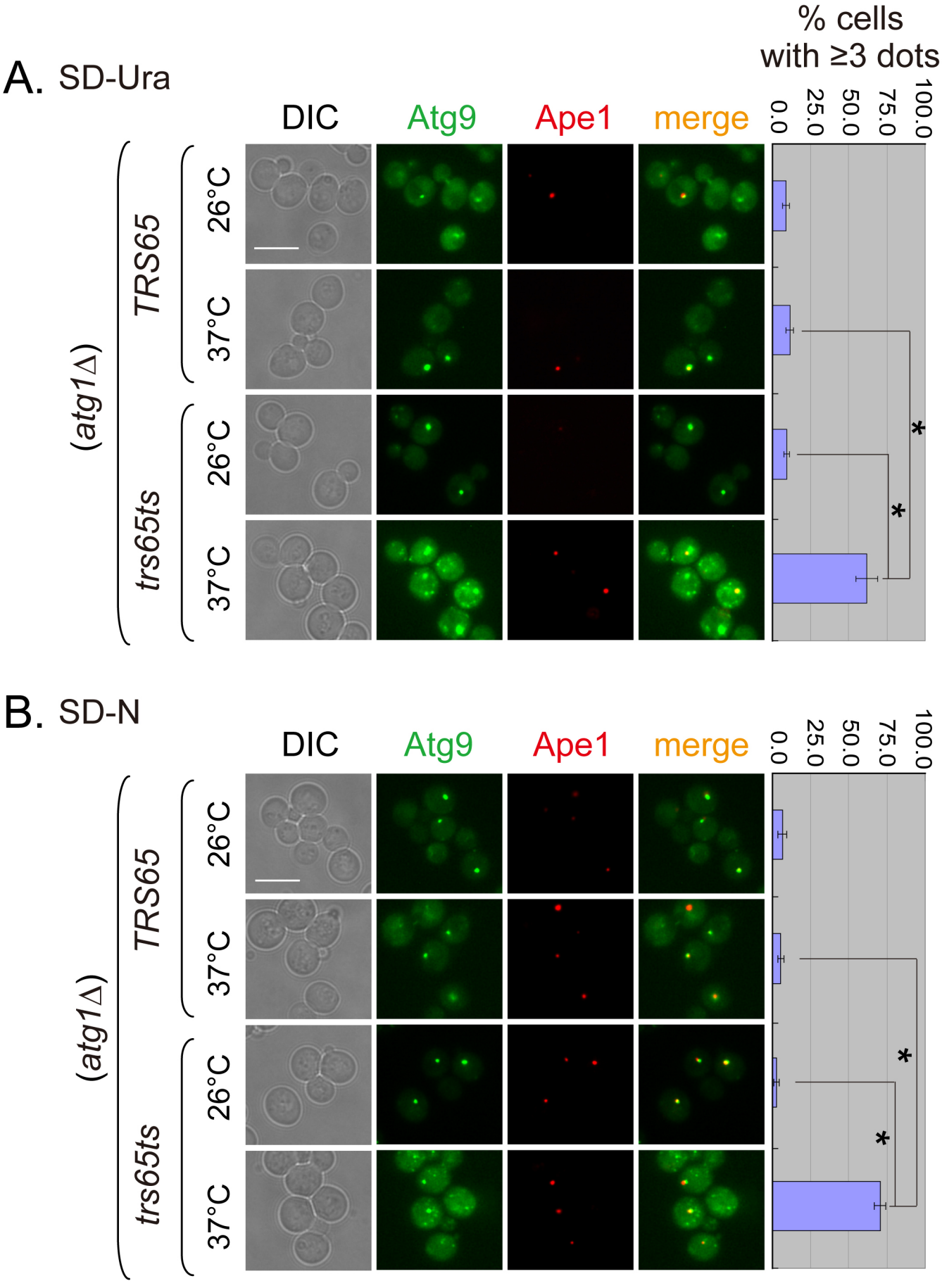
A. SD-Ura
B. SD-N
DIC
Atg9
Ape1
merge
% cells with ≥3 dots
0.0
25.0
50.0
75.0
100.0
(atg1Δ)
TRS65
trs65ts
26°C
37°C
*
*
*
*

Figure S3. Anterograde transport of Atg9 to the PAS is altered in *trs65ts* mutant cells at the restrictive temperature. WT and *trs65ts* mutant cells tagged with Atg9-3XGFP and RFP-Ape1 in an *atg1*Δ background were incubated as described in Figure 4 and examined for fluorescence. **(A)** Non-starvation. **(B)** Starvation. Bar, 7 μm. Data are presented as in Figure 4. Asterisks indicate $P < 0.001$ as highly significant.

Figure S4

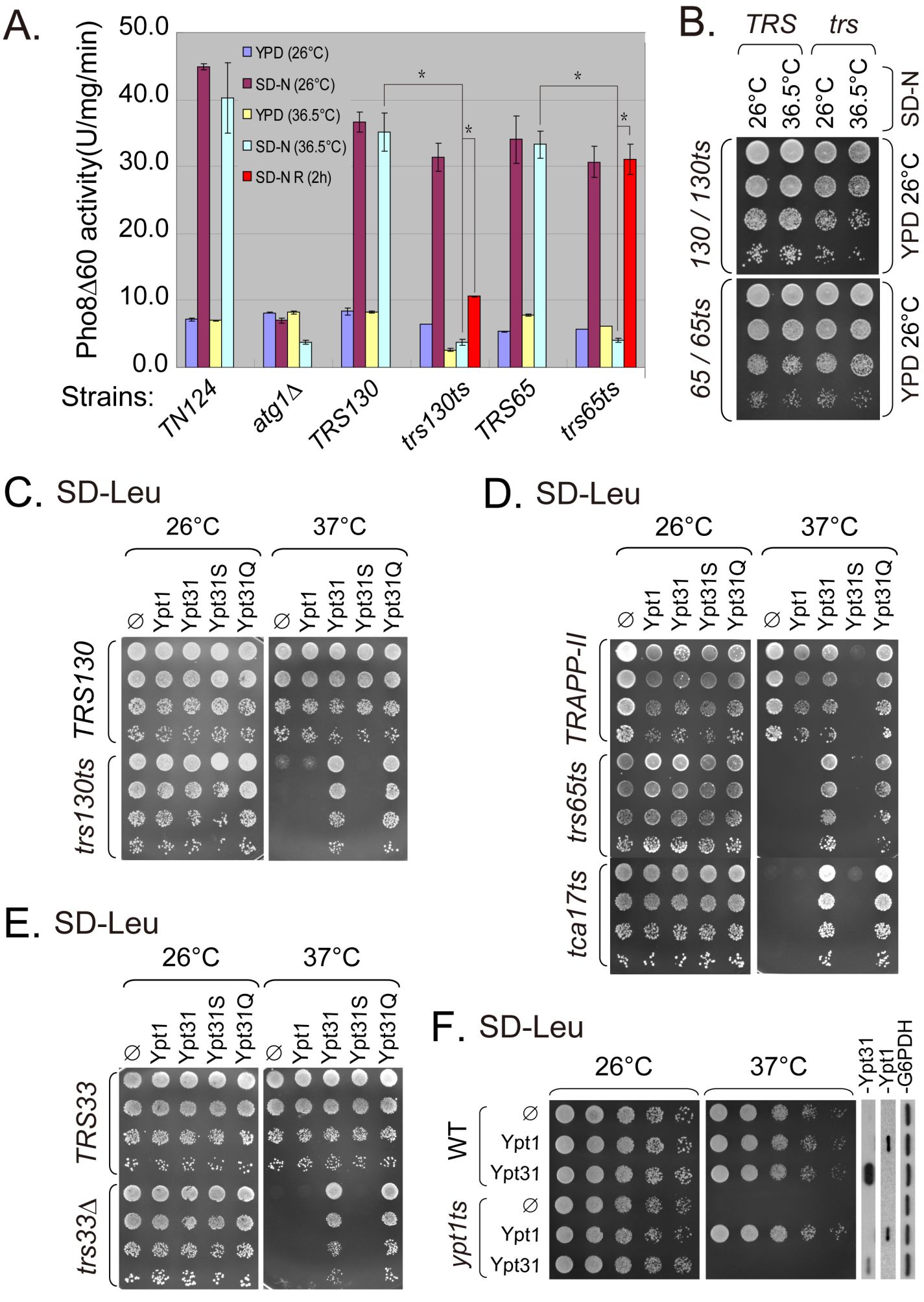
A.
Pho8Δ60 activity(U/mg/min)
50.0
40.0
30.0
20.0
10.0
0.0
YPD (26°C)
SD-N (26°C)
YPD (36.5°C)
SD-N (36.5°C)
SD-N R (2h)
*
*
*
*
Strains:
TN124
atg1Δ
TRS130
trs130ts
TRS65
trs65ts
B.
TRS
trs
26°C
36.5°C
26°C
36.5°C
SD-N
130 / 130ts
YPD 26°C
65 / 65ts
YPD 26°C
C. SD-Leu
26°C
37°C
∅
Ypt1
Ypt31
Ypt31S
Ypt31Q
TRS130
trs130ts
D. SD-Leu
26°C
37°C
∅
Ypt1
Ypt31
Ypt31S
Ypt31Q
TRAPP-II
trs65ts
tca17ts
E. SD-Leu
26°C
37°C
∅
Ypt1
Ypt31
Ypt31S
Ypt31Q
TRS33
trs33Δ
F. SD-Leu
26°C
37°C
-Ypt31
-Ypt1
-G6PDH
WT
∅
Ypt1
Ypt31
ypt1ts
∅
Ypt1
Ypt31

Figure S4. Changes in autophagic flux in some TRAPP II-specific subunit mutants and suppression of growth defects in mutants by Ypt31. (A) Pho8Δ60 activity in *trs130ts* and *trs65ts* mutants. WT (TN124) and the *atg1*Δ (in *TRS130* background) strains were used as positive and negative controls. All cells were treated as in Figures 1B except that 36.5°C was used to guarantee that the *trs130ts* cells would be viable after treatment since the mutants in the TN124 background were more temperature sensitive than the strains in Figures 1A and S1A. The *trs130ts* and *trs65ts* mutants were transferred from 36.5°C in SD-N to 26°C in SD-N for 2 hours recovery and designated SD-N R (2h). Pho8Δ60 assay data are presented as in Figure 7B except with absolute values because of slightly lower Pho8Δ60 activity in *TRS130* than in TN124. Asterisks indicate $P < 0.001$ as highly significant. **(B)** The *trs130ts* and *trs65ts* mutants were viable after treatment at 36.5°C in SD-N for 2 hours. WT and the mutants incubated in SD-N at 26°C and 36.5°C for 2 hours were plated on YPD plates with tenfold serial dilution from top to bottom and grown at 26°C for 2 days. **(C)-(E)** Cells as in Figures 7 and S1 but without RFP-Ape1 tagging were transformed with the indicated plasmids to examine growth. Cells were spotted onto SD-Leu medium with tenfold serial dilution from top to bottom and grown at various temperatures. Ypt31 (WT and GTP-bound form) but not Ypt1 suppressed the growth defect of *trs130ts* mutant cells **(C)**, *trs65ts* and *tca17ts* mutant cells **(D)**, and *trs33*Δ mutant cells **(E)** at 37°C. **(F)** The Ypt1 plasmid in (C)-(E) was used to overexpress Ypt1 in *ypt1ts* to confirm expression and function. Ypt31 served as a negative control. Growth was checked as in (C) and protein expression was examined as in Figure 7C.

Figure S5

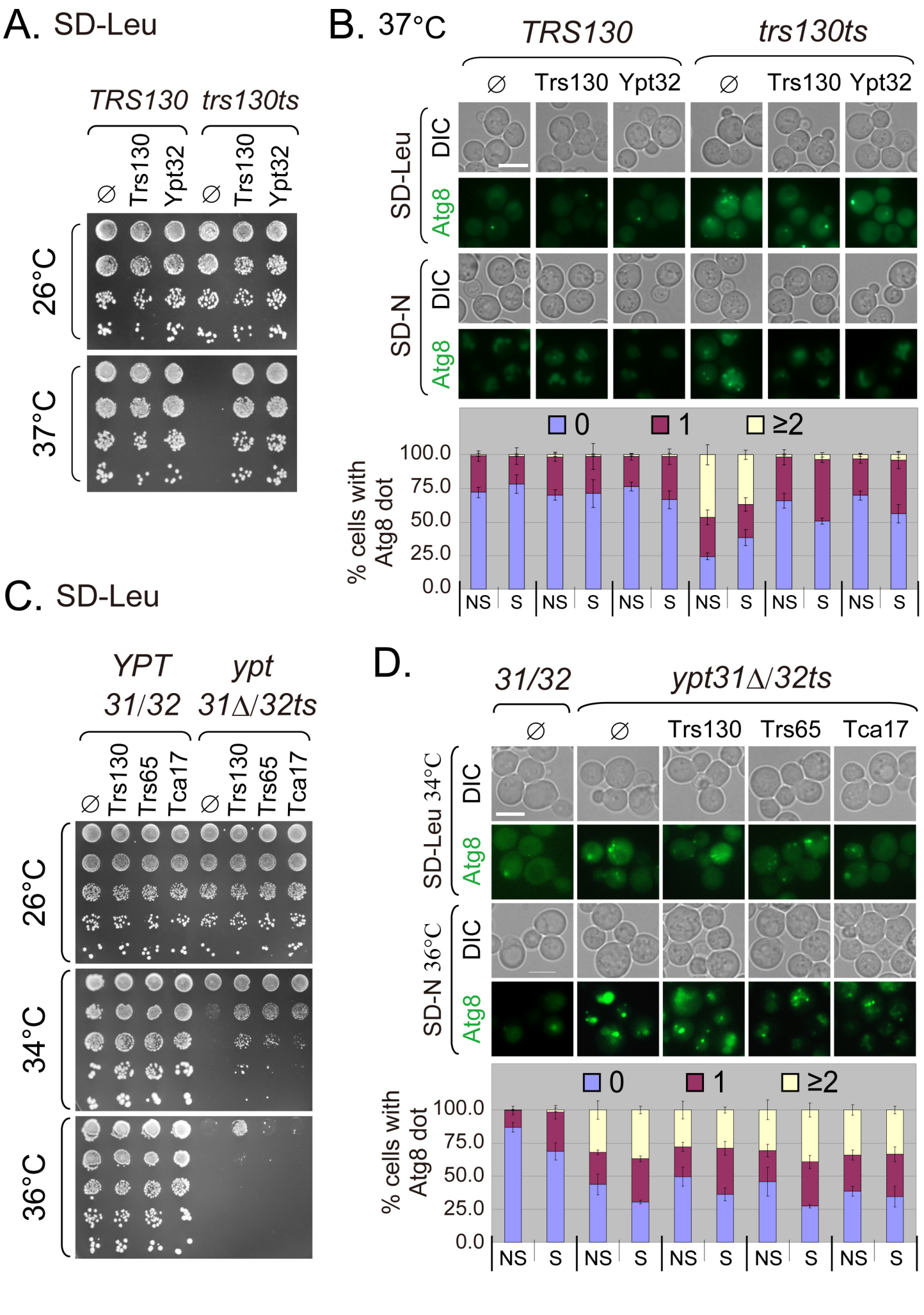
A. SD-Leu
TRS130
trs130ts
∅
Trs130
Ypt32
26°C
37°C
B. 37°C
SD-Leu
SD-N
DIC
Atg8
0
1
≥2
% cells with Atg8 dot
100.0
75.0
50.0
25.0
0.0
NS
S
C. SD-Leu
YPT 31/32
ypt 31Δ/32ts
Trs65
Tca17
34°C
36°C
D.
31/32
ypt31Δ/32ts
SD-Leu 34°C
SD-N 36°C

Figure S5. Ypt32 fully suppresses growth and autophagy defects in *trs130ts* mutant cells, but TRAPP II subunits suppress *ypt31Δ/32ts* growth defects weakly. (A) Trs130 and Ypt32 suppress the growth defect in *trs130ts* mutant cells at the restrictive temperature. Trs130 and Ypt32 in YEp351 were transformed into WT and *trs130ts* mutant cells tagged with GFP-Atg8 and grown as in Figure S4C. **(B)** Trs130 and Ypt32 suppressed the GFP-Atg8 transport defect in *trs130ts* cells. Yeast transformants as in (A) were investigated to determine whether Trs130 and Ypt32 suppressed the GFP-Atg8 transport defect in *trs130ts* cells in synthetic minimal medium (SD-Leu) or starvation medium (SD-N) at the restrictive temperature. Bar, 5 μm. The percentage of cells with Atg8 dots was quantitated and presented as in Figure 1A. **(C)** TRAPP II-specific subunits (Trs130, Trs65 and Tca17) weakly suppressed the growth defect in *ypt31Δ/32ts* mutant cells at the non-permissive temperature (34°C). Trs130, Trs65 and Tca17 in YEp351 (all are functional) were transformed and grown as in Figure S4C. **(D)** TRAPP II-specific subunits (Trs130, Trs65 and Tca17) did not obviously suppress the GFP-Atg8 transport defect in *ypt31Δ/32ts* cells. Yeast transformants as in (C) were investigated to determine whether TRAPP II-specific subunits suppressed the GFP-Atg8 transport defect in *ypt31Δ/32ts* cells in synthetic minimal medium (SD-Leu) at a growth suppression temperature (34°C) or in starvation medium (SD-N) at a temperature at which GFP-Atg8 transport starts to show defects (36°C). Bar, 5 μm. The percentage of cells with Atg8 dots was quantitated and presented as in Figure 1A.