Supplemental Materials

Molecular Biology of the Cell

Chen et al.

Action of Arl1 GTPase and golgin Imh1 in Ypt6-independent retrograde transport from endosomes to the trans-Golgi network

Supplementary Information

Supplementary Figure legends

Fig. S1

Ypt6 is required for the proper localization of Snc1 and Tlg1. Wild-type and *ypt6*Δ cells were transformed with GFP-Snc1, mCherry-Tlg1, Sec7 (late-Golgi marker), Gos1 (mid-Golgi marker), Sed5 (cis-Golgi marker), FYVE (endosome marker), Vps74 (PtdIns4P marker), or GOLPH3 (PtdIns4P marker). Yeast cells transformed with the indicated markers were observed by fluorescence microscopy (Scale bar, 5 μm).

Fig. S2

Ypt6 functions in the retrograde transport of Snc1 from endosomes to the late Golgi. (*A*) Ypt6, Arl1, and Imh1 do not directly participate in the exocytic pathway of Snc1 to the plasma membrane. GFP-Snc1 or GFP-Snc1-PM (the endocytosis-defective mutant) was expressed in the cells as indicated. Live cells were observed in the mid-log phase using fluorescence microscopy. (Scale bar, 5 μm) (*B*) Snc1-GFP puncta in *ypt6*Δ cells do not co-localize with the late Golgi marker Sec7-mRFP. GFP-Snc1 was co-expressed with Sec7-mRFP (late Golgi marker) in cells as indicated. Live cells were observed in the mid-log phase using fluorescence microscopy. The arrows highlight GFP-Snc1 co-localized with Sec7-mRFP. (Scale bar, 5 μm)

Fig. S3

Deletion of Arl1 or Imh1 does not impair proper localization of Snc1 and Tlg1 or cell growth at high temperature. (*A*) The deletion of *ARL1* or *IMH1* does not affect Snc1 and Tlg1 localization. Cells transformed with the indicated organelle markers were observed by fluorescence microscopy (Markers represented as in Fig. S1; Scale bar, 5 μm). (*B*) Deletion of *ARL3*, *SYT1*, *ARL1*, or *IMH1* does not exhibit a temperature-sensitive growth defect. Yeast strains were serially diluted tenfold as indicated, spotted on plates, and incubated at 30°C or 37°C.

Fig. S4

Both Arl1 and Imh1 are required for compensating the Tlg1 transport defects in *ypt6*Δ cells. (*A*) The overexpression of Arl1 or Imh1 restores the late Golgi distribution of Tlg1 in ypt6-null cells. *YPT6* knockout cells with excess Arl1 or Imh1 were co-expressed with mCherry-Tlg1 and Sec7-GFP (late Golgi marker) or GFP-Sed5 (early Golgi marker). Cells were observed in mid-log phase using fluorescence microscopy (Scale bar, 5 μm). At least 100 cells were included in experiments from three independent biological repeats. The ratio of the colocalization between mCherry-Tlg1 and the

Golgi indicators were represented as the Pearson Correlation Coefficient (PCC) using ImageJ plugin Just Another Colocalization Plugin with Costes Automatic Thresholding as described in methods. The data are presented as the mean ± SD and the P values were determined applying one-way ANOVA (***P < 0.001; N.S. Not significant). (*B*) Arl1 and Imh1 act interdependently to suppress defects in Tlg1 retrograde transport in *ypt6*Δ cells. mCherry-Tlg1 was co-expressed with an empty vector, Arl1, or Imh1 in wild-type, *ypt6*Δ, *ypt6arl1*Δ, and *ypt6imh1*Δ cells. Cells were observed in mid-log phase using fluorescence microscopy (Scale bar, 5 μm).

Fig. S5

Overexpression of Arl1 or Imh1 suppresses temperature-sensitive growth defects in *ypt6syt1*Δ, but not *ypt6arl3*Δ cells. Yeast *ypt6syt1*Δ or *ypt6arl3*Δ cells transformed with Arl1 or Imh1, respectively, were serially diluted tenfold as indicated, spotted on plates, and incubated at 30°C or 37°C.

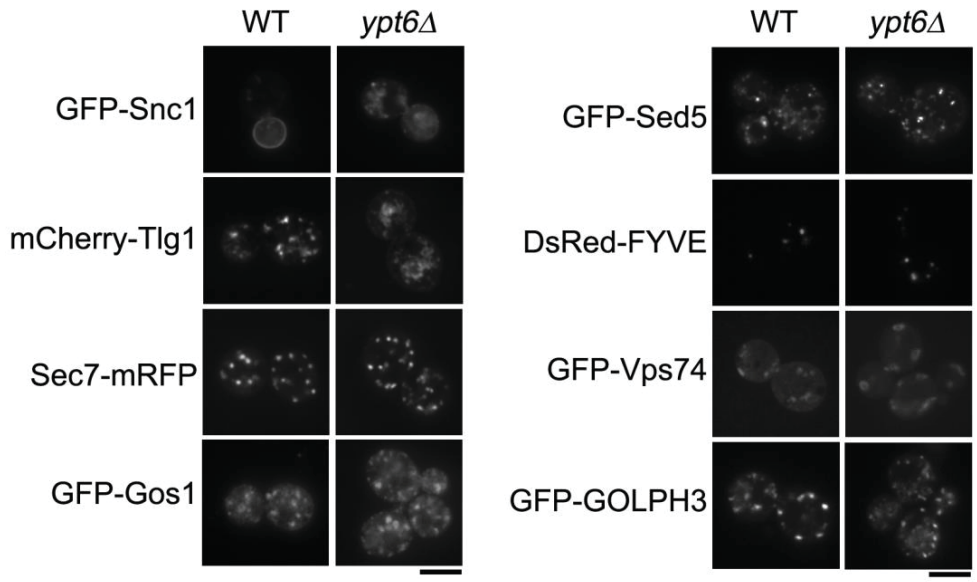
Fig. S6

The Arl1-Drs2-Gea2 ternary complex is not required for suppressing defects in Snc1 transport in *ypt6*Δ cells. (*A*) Overexpression of Arl1 or Imh1 failed to suppress Snc1 transport defects in *ypt6drs2*Δ cells. Arl1 or Imh1 was co-expressed with GFP-Snc1 in wild-type, *ypt6*Δ, and *ypt6drs2*Δ strains to examine the plasma membrane localization of Snc1 (Scale bar, 5 μm). (B) *GEA2* is not involved in the suppression effect of Arl1 and Imh1 in *ypt6*Δ cells. GFP-tagged Snc1 or Tlg1 was co-expressed with Arl1, Imh1, or HA-Ypt6 in *ypt6gea2*Δ cells. Live cells were observed in mid-log phase by fluorescence microscopy (Scale bar, 5 μm).

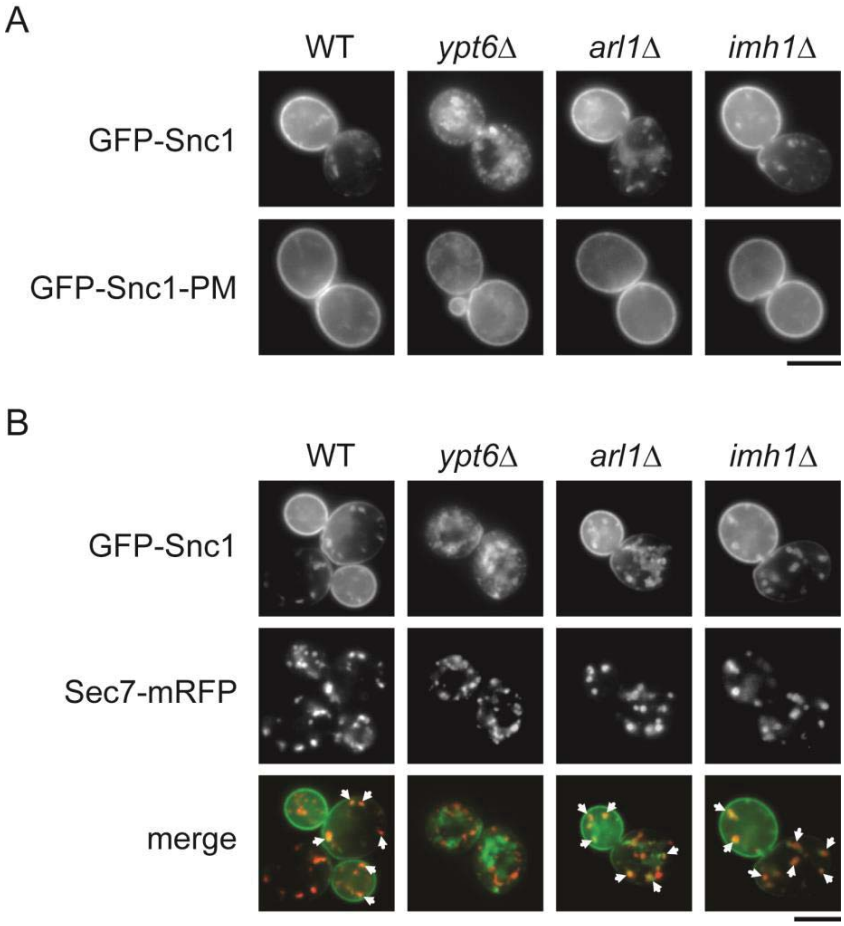
Fig. S7

Deletion of GARP complex subunits results in endosome-to-TGN transport defects. The localization of Snc1 and Tlg1 is affected in *vps52*Δ and *vps53*Δ cells. GARP complex subunit-deletion strains were transformed with GFP-Snc1, mCherry-Tlg1, Sec7 (late-Golgi marker), Gos1 (mid-Golgi marker), Sed5 (cis-Golgi marker), FYVE (endosome marker), Vps74 (PtdIns4P marker), or GOLPH3 (PtdIns4P marker). Yeast cells transformed with the indicated markers were observed by fluorescence microscopy (Scale bar, 5 μm).

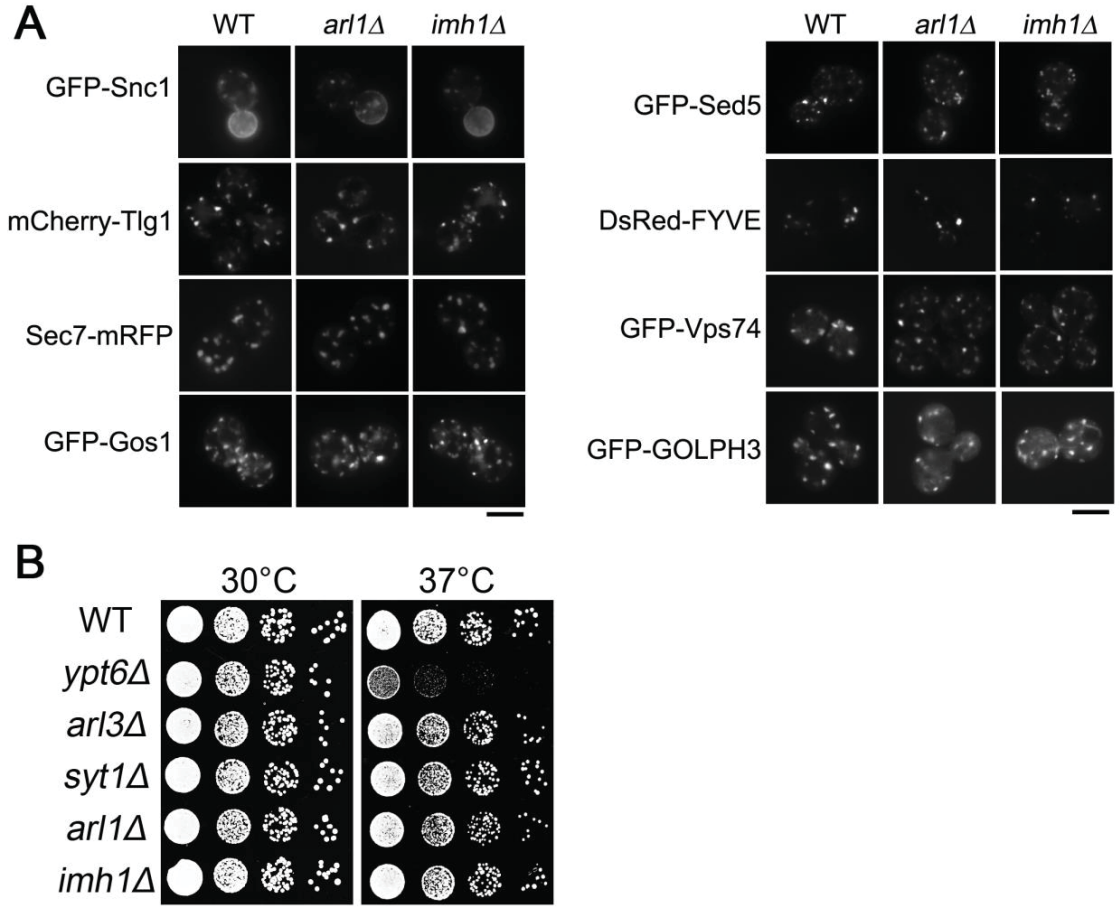
Supplementary Figure 1



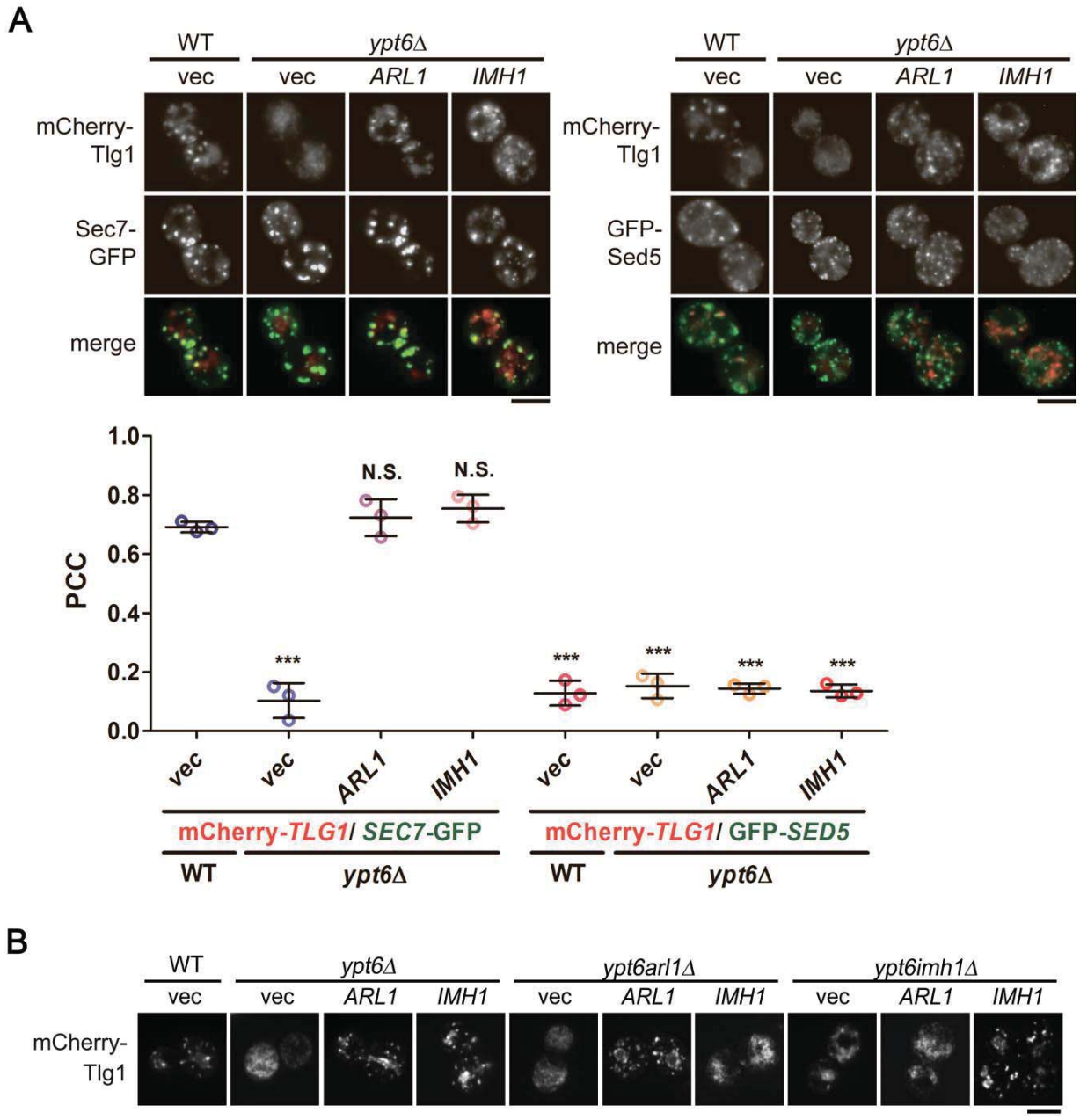
Supplementary Figure 2



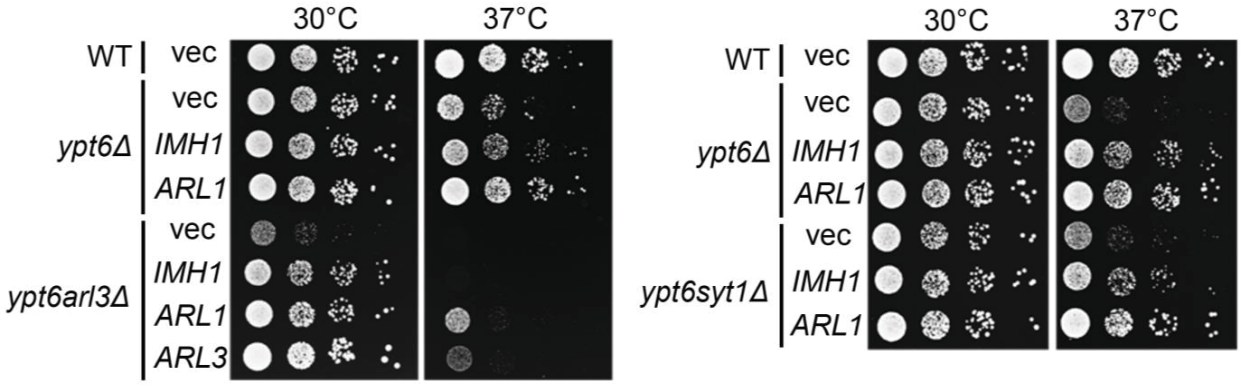
Supplementary Figure 3



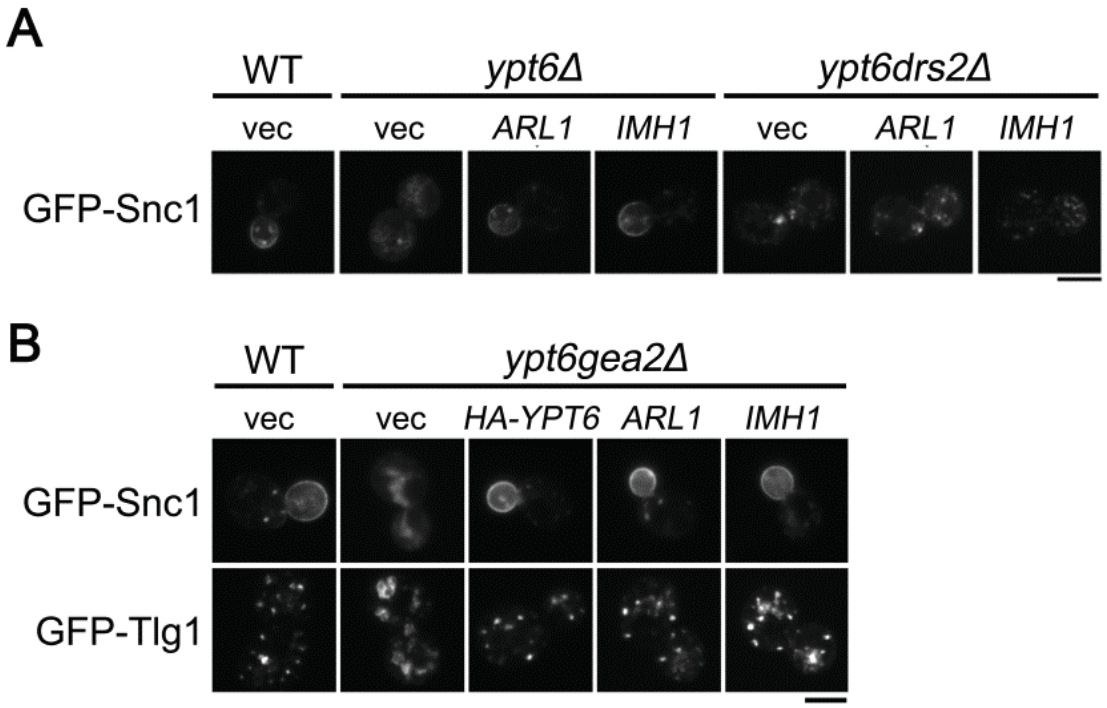
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7

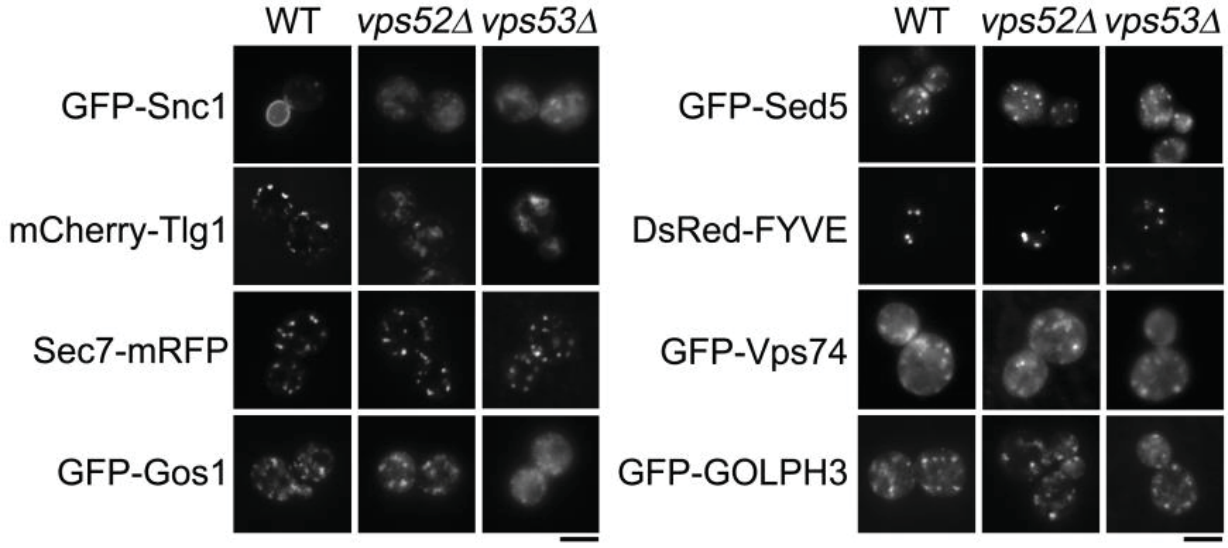


Table S1. Yeast strains used in this study

Strain	Genotype	Reference
BY4741	*MATa his3, leu2, met15, ura3*	Invitrogen
BY4741 *ypt6*Δ	*MATa his3, leu2, met15, ura3 ypt6::KanMX6*	Invitrogen
BY4741 *arl3*Δ	*MATa his3, leu2, met15, ura3 arl3::KanMX6*	Invitrogen
BY4741 *syt1*Δ	*MATa his3, leu2, met15, ura3 syt1::hphMX3*	Invitrogen
BY4741 *arl1*Δ	*MATa his3, leu2, met15, ura3 arl1::KanMX6*	Invitrogen
BY4741 *imh1*Δ	*MATa his3, leu2, met15, ura3 imh1::KanMX6*	Invitrogen
BY4741 *drs2*Δ	*MATa his3, leu2, met15, ura3 drs2::KanMX6*	Invitrogen
BY4741 *gea2*Δ	*MATa his3, leu2, met15, ura3 gea2::KanMX6*	Invitrogen
BY4741 *vps52*Δ	*MATa his3, leu2, met15, ura3 vps52::KanMX6*	Invitrogen
BY4741 *vps53*Δ	*MATa his3, leu2, met15, ura3 vps53::His3MX6*	This study
BY4741 *ypt6arl1*Δ	*MATa his3, leu2, met15, ura3 ypt6::KanMX6,arl1::His3MX6::PGAL1-ARL1*	This study
BY4741 *ypt6imh1*Δ	*MATa his3, leu2, met15, ura3 ypt6::KanMX6, imh1::His3MX6*	This study
BY4741 *ypt6arl3*Δ	*MATa his3, leu2, met15, ura3 ypt6::KanMX6, arl3::His3MX6*	This study
BY4741 *ypt6syt1*Δ	*MATa his3, leu2, met15, ura3 ypt6::KanMX6, syt1::His3MX6*	This study
BY4741 *ypt6vps53*Δ	*MATa his3, leu2, met15, ura3 ypt6::KanMX6, vps53::His3MX6*	This study
BY4741 *ypt6drs2*Δ	*MATa his3, leu2, met15, ura3 ypt6::KanMX6, drs2::His3MX6*	This study
BY4741 *ypt6gea2*Δ	*MATa his3, leu2, met15, ura3 ypt6::KanMX6, gea2::His3MX6*	This study
BY4741 *arl3arl1*Δ	*MATa his3, leu2, met15, ura3 arl1::KanMX6, arl3*	(Chen et al., 2012)

Table S2. Plasmids used in this study

Plasmids	Description
pVT101U	*2-μm, URA3, ADH1p*
pVT101U-HA-*YPT6*	*2-μm, URA3, ADH1p-HA-YPT6*
pVT101U-*ARL3*	*2-μm, URA3, ADH1p-ARL3*
pVT101U-*ARL3*^{Q78L}	*2-μm, URA3, ADH1p-ARL3*^{Q78L}
pVT101U-*ARL3*^{T31N}	*2-μm, URA3, ADH1p-ARL3*^{T31N}
pVT101U-*SYT1*	*2-μm, URA3, ADH1p-SYT1*
pVT101U-*ARL1*	*2-μm, URA3, ADH1p-ARL1*
pVT101U-*ARL1*^{Q72L}	*2-μm, URA3, ADH1p-ARL1*^{Q72L}
pVT101U-*ARL1*^{T32N}	*2-μm, URA3, ADH1p-ARL1*^{T32N}
pVT101U-*ARL1*-HA	*2-μm, URA3, ADH1p-ARL1-HA*
pVT101U-*IMH1*	*2-μm, URA3, ADH1p-IMH1*
pVT101U-*IMH1*^{dN100}	*2-μm, URA3, ADH1p-IMH1*^{dN100}
pVT101U-mCherry-*IMH1*	*2-μm, URA3, ADH1p-mCherry-IMH1*
pVT101U-HA-*IMH1*	*2-μm, URA3, ADH1p-HA-IMH1*
pVT101U-HA-*IMH1*^{dN100}	*2-μm, URA3, ADH1p-HA-IMH1*^{dN100}
pVT101U-HA-GFP-*VPS74*	*2-μm, URA3, ADH1p-HA-GFP-VPS74*
pVT101U-*DRS2*	*2-μm, URA3, ADH1p-DRS2*
pVT101U-*DRS2*^{D560N}	*2-μm, URA3, ADH1p-DRS2*^{D560N}
pVT101U-*DRS2*^{dN160}	*2-μm, URA3, ADH1p-DRS2*^{dN160}
YEplac181	*2-μm, LEU2*
YEplac181-*ARL1*	*2-μm, LEU2, ADH1p-ARL1*
YEplac181-*IMH1*	*2-μm, LEU2, ADH1p-IMH1*
YEplac181-*VPS53*-GFP	*2-μm, LEU2, ADH1p-VPS53-GFP*
YCplac111-*VPS52*-GFP	*CEN, LEU2, ADH1p-VPS52-GFP*
pHS12-*ARL1*-GFP	*CEN, LEU2, ADH1p-ARL1-GFP*
pHS12-*ARL1*-mRFP	*CEN, LEU2, ADH1p-ARL1-mRFP*
pRS313-*VPS52*-GFP	*CEN, HIS3, ADH1p-VPS52-GFP*
pRS313-*VPS53*-GFP	*CEN, HIS3, ADH1p-VPS53-GFP*
pRS315-GFP-*SNC1*	*CEN, LEU2, ADH1p-GFP-SNC1*
pRS315-GFP-*SFT2*	*CEN, LEU2, ADH1p-GFP-SFT2*
pRS315-mCherry-*TLG1*	*CEN, LEU2, ADH1p-mCherry-TLG1*
pRS315-GFP-*TLG1*	*CEN, LEU2, ADH1p-GFP-TLG1*

pRS315-*SEC7*-mRFP	*CEN, LEU2, ADH1p-SEC7-mRFP*
pRS316-*SEC63*-GFP	*CEN, URA3, ADH1p-SEC63-GFP*
pRS416-GFP-*SNC1*	*CEN, URA3, MET3p-GFP-SNC1*
pRS416-GFP-*SNC1*-PM	*CEN, URA3, MET3p-GFP-SNC1*-PM
pRS416-GFP-*GOLPH3*	*CEN, URA3, ADH1p-GFP-GOLPH3*
pRS426-GFP-*SED5*	*2-μm, URA3, ADH1p-GFP-SED5*
SEC7-GFP	*2-μm, URA3, ADH1p-SEC7-GFP*
SEC7-mRFP	*2-μm, URA3, ADH1p-SEC7-mRFP*
GFP-*GOS1*	*2-μm, URA3, ADH1p-GFP-GOS1*