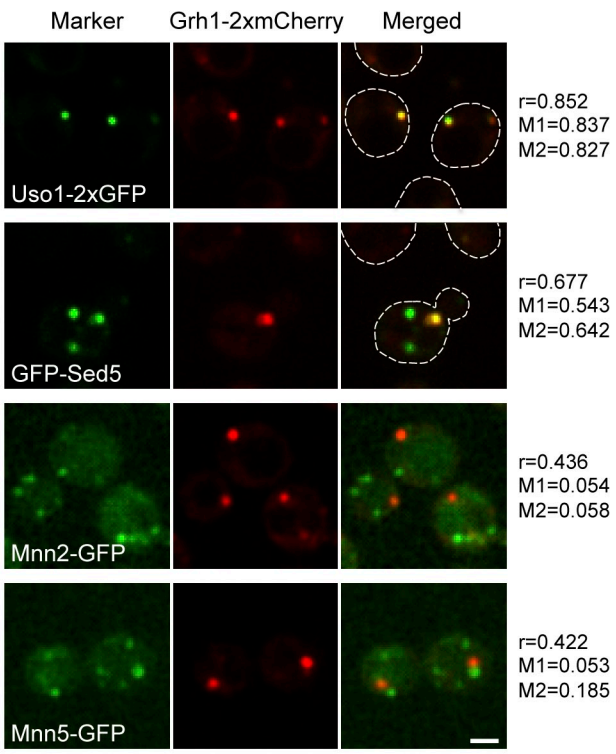
Cruz-Garcia et al., http://www.jcb.org/cgi/content/full/jcb.201407119/DC1

A



B

	Pearson	M1	M2	N (cells)
Uso1 / Grh1	0.834±0.005	0.771±0.016	0.807±0.017	73
Sed5 / Grh1	0.685±0.012	0.420±0.024	0.668±0.028	54
Mnn2 / Grh1	0.405±0.009	0.059±0.011	0.146±0.025	57
Mnn5 / Grh1	0.434±0.011	0.056±0.014	0.108±0.023	54

Figure S1. **Quantitative colocalization analysis of Grh1 and Golgi proteins.** (A) Cells coexpressing Grh1-2×mCherry and Uso1-2×GFP, GFP-Sed5, Mnn2-GFP, or Mnn5-GFP were grown in complete medium, then washed and incubated in 2% potassium acetate. After 2 h of starvation, z stacks were acquired by spinning-disk confocal fluorescence microscopy. The panels show representative single confocal sections of the GFP and mCherry signals and the corresponding merged images. The numbers next to the merged images indicate the values of the Pearson's (r) and Manders' (M1 and M2) coefficients of the corresponding cropped stack of confocal sections. M1 refers to the proportion of the GFP signal overlapping with a signal in the mCherry channel over its total intensity. M2 refers to the proportion of the mCherry signal overlapping with a signal in the GFP channel over its total intensity. Broken lines indicate cell boundaries. Bar, 2 µm. (B) Quantitation of the colocalization of the different proteins in A, as measured by the Pearson's and Manders' coefficients between the GFP and mCherry signals. Values represent the mean ± SEM of the indicated number of cells (N).

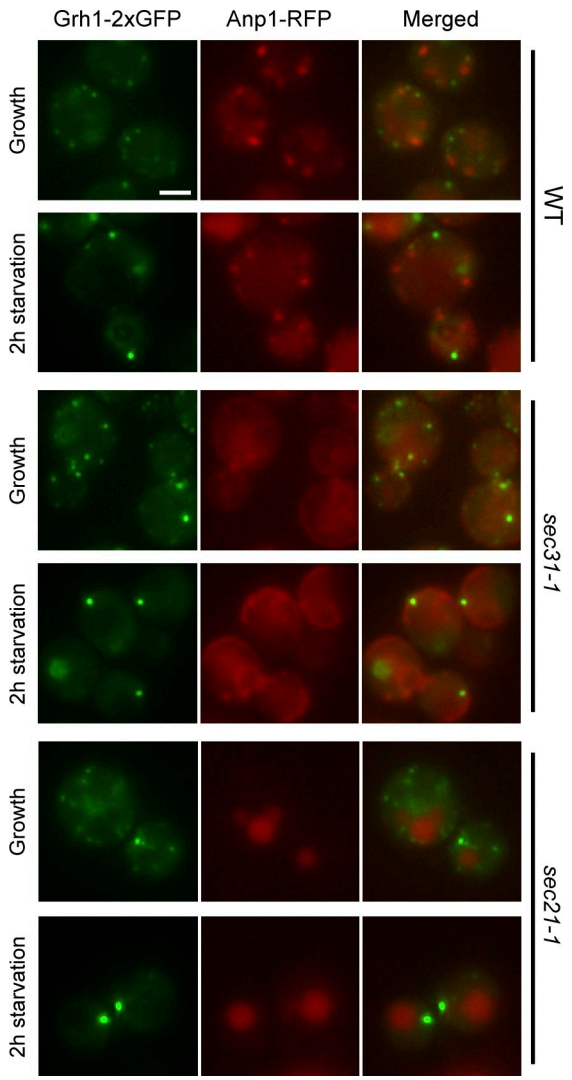


Figure S2. **CUPS formed in COPII or COPI mutant cells do not contain the early Golgi marker Anp1.** Wild-type, *sec31-1*, and *sec21-1* cells coexpressing Grh1-2×GFP and Anp1-RFP were grown to mid-log phase at 25°C, then preincubated at 37°C for 45 min followed by starvation in 2% potassium acetate at 37°C for 2 h. Cells were imaged by fluorescence microscopy just before starvation (Growth) and within 2 h of starvation. Bar, 2 µm.

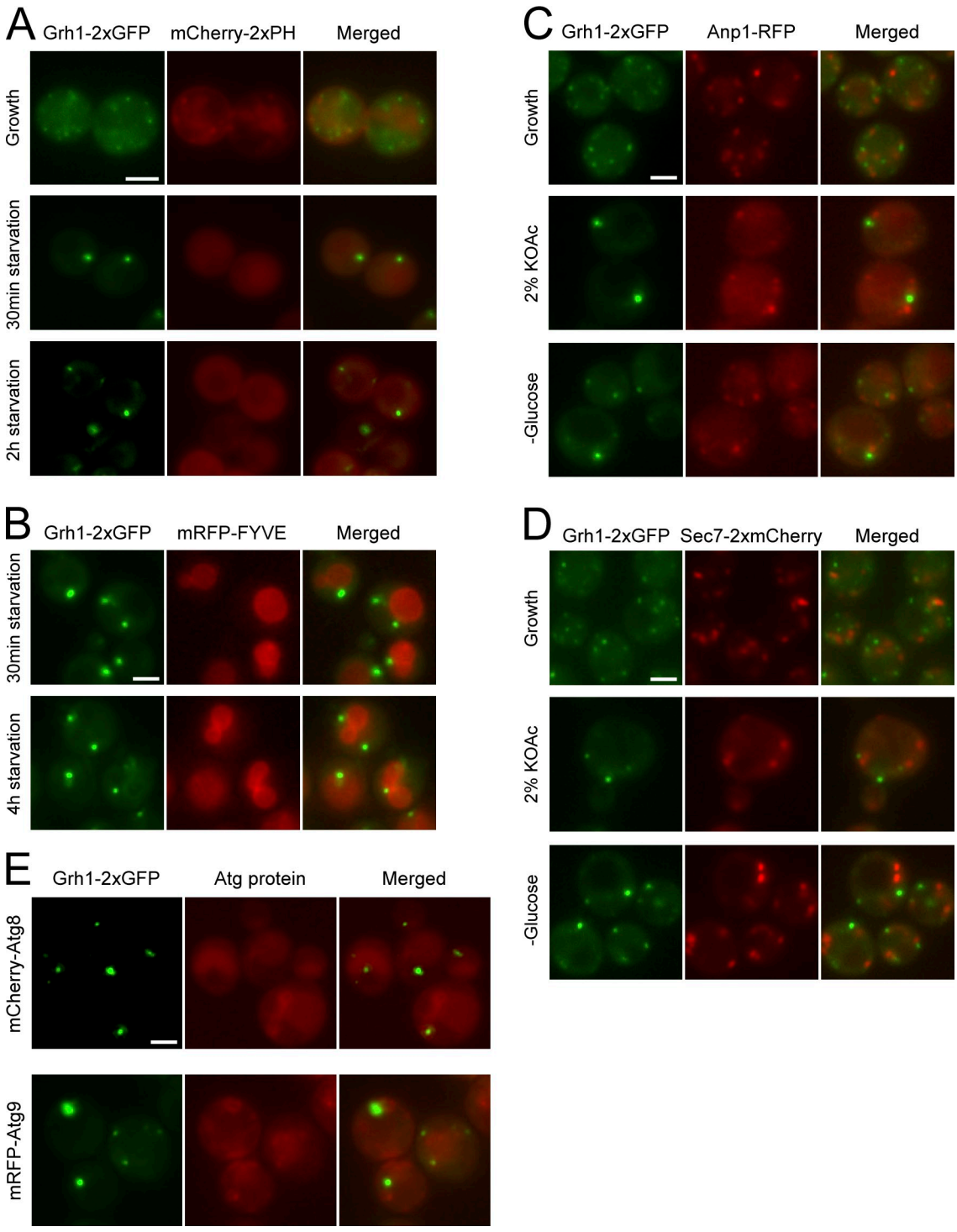


Figure S3. **Colocalization analysis of Golgi-, endosome-, and autophagosome-specific proteins and lipids in CUPS.** (A and B) Cells coexpressing Grh1-2×GFP and mCherry-Osh2-2×PH (A) or mRFP-EEA1-FYVE (B) were grown in complete medium, then washed and incubated in 2% potassium acetate for 2 h. Cells were observed by fluorescence microscopy just before starvation (Growth) and at the indicated time points after starvation. (C and D) Cells coexpressing Grh1-2×GFP and Anp1-RFP (C) or Sec7-2×mCherry (D) were grown in rich medium, washed and cultured in 2% potassium acetate or SC-D (−glucose) medium for 2 h, and visualized by fluorescence microscopy. (E) Grh1-2×GFP–expressing cells transformed with plasmids coding for mCherry-Atg8 or mRFP-Atg9 were grown in complete medium, starved for 4 h in 2% potassium acetate, and imaged by fluorescence microscopy. Bar, 2 µm.

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

Name	Genotype	Source
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	EUROSCARF
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	EUROSCARF
DCY001	BY4741 *GRH1-2×GFP-HIS3MX6*	This study
DCY002	BY4741 *GRH1-2×mCherry-CaURA3*	This study
DCY003	BY4741 *GRH1-2×mCherry-CaURA3 USO1-2xGFP-HIS3MX6*	This study
Uso1-GFP	BY4741 *USO1-GFP-HIS3MX6*	Invitrogen
DCY004	BY4741 *USO1-GFP-HIS3MX6 grh1Δ::KanMX4*	This study
DCY005	BY4741 *GRH1-2×GFP-HIS3 vps34Δ::KanMX4*	This study
uso1-1[a]	BY4741 *uso1-1-KanMX*	C. Boone
sec13-1[b]	BY4741 *sec13-1-KanMX*	C. Boone
sec31-1[b]	BY4741 *sec31-1-KanMX*	C. Boone
sec21-1[c]	BY4741 *sec21-1-KanMX*	C. Boone
sec7-1[b]	BY4741 *sec7-1-KanMX*	C. Boone
pik1-104[b]	BY4741 *pik1-104-KanMX*	C. Boone
sec20-1[b]	BY4741 *sec20-1-KanMX*	C. Boone
sly1-ts[b]	BY4741 *sly1-ts-KanMX*	C. Boone
DCY006	BY4741 *GRH1-2×GFP-HIS3MX6 uso1-1-KanMX*	This study
DCY007	BY4741 *GRH1-2×GFP-HIS3MX6 sec13-1-KanMX*	This study
DCY008	BY4741 *GRH1-2×GFP-HIS3MX6 sec31-1-KanMX*	This study
DCY009	BY4741 *GRH1-2×GFP-HIS3MX6 sec21-1-KanMX*	This study
DCY010	BY4741 *GRH1-2×GFP-HIS3MX6 sec7-1-KanMX*	This study
ACY040	BY4741 *GRH1-2×GFP-HIS3MX6 pik1-104-KanM*	This study
vam7Δ	BY4741 *vam7Δ::KanMX4*	EUROSCARF
DCY011	BY4741 *GRH1-2×GFP-HIS3MX6 vam7Δ::KanMX4*	This study
DCY012	BY4741 *GRH1-2×GFP-HIS3MX6 sec20-1-KanMX*	This study
DCY013	BY4741 *GRH1-2×GFP-HIS3MX6 sly1-ts-KanMX*	This study
ACY041	BY4742 *GRH1-2×mCherry-CaURA3*	This study
Mnn2-GFP	BY4741 *MNN2-GFP-HIS3MX6*	Invitrogen
Mnn5-GFP	BY4741 *MNN5-GFP-HIS3MX6*	Invitrogen
DCY014	*MATa GRH1-2×mCherry-CaURA3 MNN2-GFP-HIS3MX6*	This study
DCY015	*MATa GRH1-2×mCherry-CaURA3 MNN5-GFP-HIS3MX6*	This study
Anp1-RFP	BY4742 *ANP1-RFP-KanMX*	E. O'Shea[d]
DCY016	BY4742 *ANP1-RFP-NatMX*	This study
DCY017	*MATa GRH1-2×GFP-HIS3MX6 ANP1-RFP-NatMX*	This study
DCY018	*MATa GRH1-2×GFP-HIS3MX6 ANP1-RFP-NatMX sec31-1-KanMX*	This study
DCY019	*MATa GRH1-2×GFP-HIS3MX6 ANP1-RFP-NatMX sec21-1-KanMX*	This study
DCY020	BY4741 *GRH1-2×GFP-HIS3MX6 SEC7-2×mCherry-CaURA3*	This study

[a]The mutation in the *uso1-1* allele is a truncation of most of its C-terminal tail.
[b]The mutation in this temperature-sensitive allele is unknown.
[c]The mutation in the *sec21-1* allele is G482D.
[d]Harvard University, Cambridge, MA.