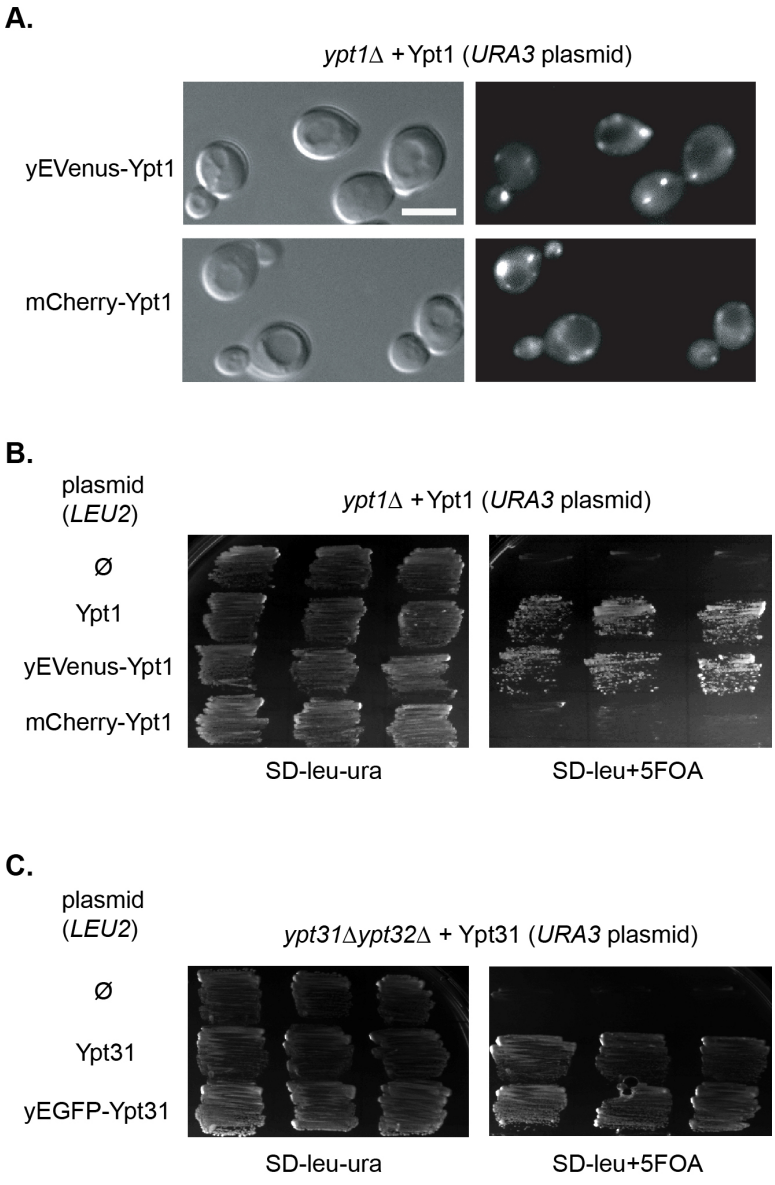
Supplemental Figures and Legends

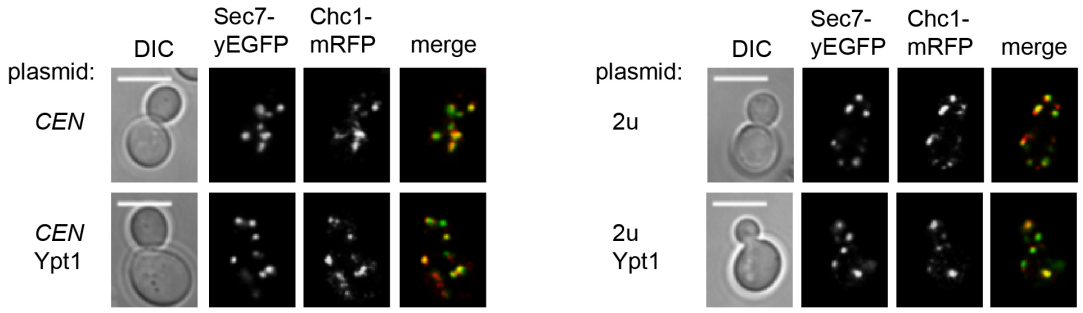


D.

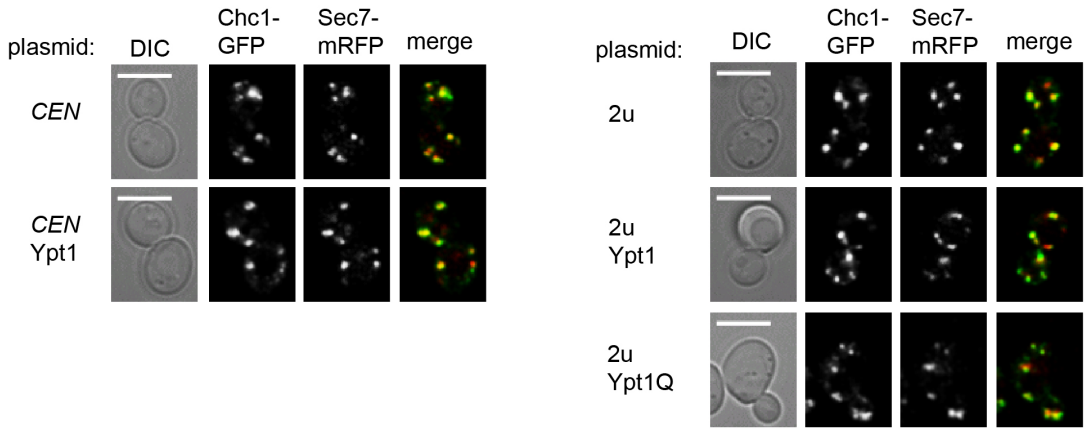
Puncta per cell	# of cells (n)	**# of puncta average**	std dev
yEVenus-Ypt1	10	**10.3**	2
yEGFP-YPT31 /ypt32Δ	10	**11.5**	2

Figure S1. Functionality of fluorescently tagged Ypt1 and Ypt31 (Related to Figures 2-4). A. Both yEVenus-Ypt1 and mCherry-Ypt1 show punctate fluorescence signal. Cells expressing the fluorescently tagged Ypt1 proteins from a *CEN* plasmid were visualized by live-cell microscopy. Shown are: DIC (left) and yEVenus-Ypt1 or mCherry-Ypt1 (right). Bar 5 mm. **B.** yEVenus-Ypt1, but not mCherry-Ypt1, is functional as a sole copy. Cells carrying *ypt1Δ* on the chromosome and expressing *YPT1* from a *CEN URA3* plasmid were transformed with a *CEN LEU2* plasmid expressing Ypt1 from it own promoter and terminator (from top to bottom): Ø (empty vector control), Ypt1, yEVenus-Ypt1 (Ypt1 tagged at its N-terminus with yeast-codon-optimized enhanced Venus), or mCherry-Ypt1. Growth of transformants is shown on SD-Ura-Leu (left), and the ability of cells to lose the *URA3* plasmid is shown on SD-Leu+5FOA (right). Whereas Ypt1 and yEVenus-Ypt1 are functional, mCherry-Ypt1 is not. **C.** yEGFP-Ypt31 is functional as a sole copy. Cells carrying *ypt31Δ ypt32Δ* on the chromosome and expressing *YPT31* from a *CEN URA3* plasmid were transformed with a *CEN LEU2* plasmid expressing Ypt31 from its own promoter and terminator (from top to bottom): Ø (empty vector control), Ypt31, yEGFP-Ypt31 (Ypt31 tagged at its N-terminus with yeast-codon-optimized enhanced GFP). Growth of transformants is shown on SD-Ura-Leu (left), and the ability to lose the *URA3* plasmid is shown on SD-Leu+5FOA (right). Both Ypt31 and yEGFP-Ypt31 can support cell growth. Results shown in this figure represent at least two independent experiments. **D.** Table shows quantifications from two independent expreiments of total number of puncta of Ypt1 and Ypt31 per cell by live-cell microscopy.

A. Sec7 and Chc1 (yEGFP/mRFP)



B. Sec7 and Chc1 (mRFP/GFP)

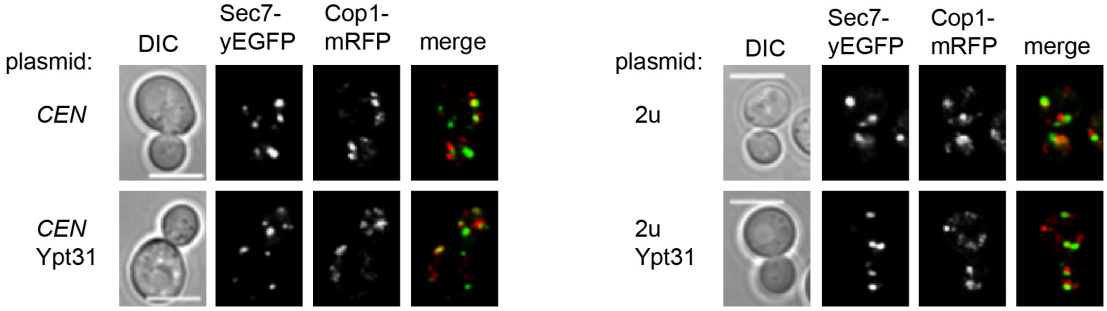


C.

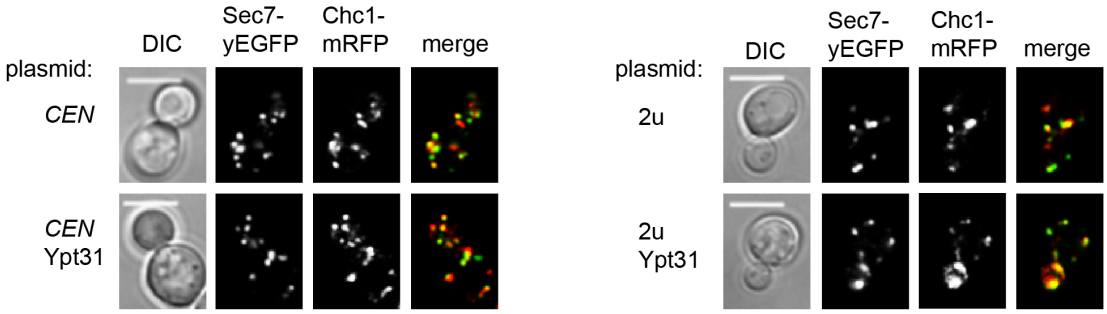
Strain	Plasmid	# of cells (n)	co-localization (%) of Green with Red	std dev	# Green puncta/slice	std dev	# co-localized puncta/slice	# not co-localized puncta/slice	std dev
Cop1-mRFP Sec7-yEGFP	Ø *CEN*	20	18.5	1	4.3	0.1	0.9		
	Ypt1 *CEN*	20	31.2	1	3.8	0.6	1.1		
	Ø 2µ	20	18.7	1	4.3	0.2	0.9		
	Ypt1 2µ URA3	20	42.6	1	3.6	0.2	1.4		
	Ypt1 2µ LEU2	30	40	0	4	0.2	1.5		
	Ypt1-GTP 2µ	30	55	6	4.7	0.2	2.5		
Sec7-mRFP Chc1-GFP	Ø *CEN*	28	78.8	3	5.2		4	1.2	0.4
	Ypt1 *CEN*	28	75.1	4	5.6		4.1	1.5	0.3
	Ø 2µ	28	64.5	3	5.6		3.6	2.1	0.2
	Ypt1 2µ	28	78.8	3	5.4		4.1	1.3	0.1
	Ypt1-GTP 2µ	28	83.5	3	5.4		4.4	1	0.2
Sec7-yEGFP Chc1-mRFP	Ø *CEN*	20	88.5	3	4.2		3.6	0.6	
	Ypt1 *CEN*	20	91	4	3.7		3.3	0.4	
	Ø 2µ	20	88.9	0	3.6		3.1	0.5	
	Ypt1 2µ	20	89.5	5	3.9		3.5	0.4	

Figure S2. Ypt1 overexpression does not affect Sec7 and Chc1 co-localization (Related to Figures 6-7). A. Cells expressing Sec7-yEGFP and Chc1-mRFP from their endogenous loci were transformed with *CEN* (left) and 2m (right) plasmids, empty (top) and for overexpression of Ypt1 (bottom). Information from this experiment was used in Figure 7E. **B.** Cells expressing Sec7-mRFP and Chc1-yEGFP from their endogenous loci were transformed with *CEN* (left) and 2m (right) plasmids, empty (top) and for overexpression of Ypt1 (bottom). **C.** Table shows quantifications from two independent experiments for panels A and B of this figure and Figures 6 and 7. The co-localization level of the two markers was determined using live cell microscopy. Bar, 5µm.

A. Cop1 and Sec7



B. Sec7 and Chc1



C.

Strain	Plasmid	# of cells (n)	co-localization (%) of Green with Red	std dev	# Green puncta/slice	std dev	# co-localized puncta/slice	# not co-localized puncta/slice	std dev
Cop1-mRFP Sec7-yEGFP	Ø *CEN*	20	14.3	5	4.6	0.2	0.7		
	Ypt31 *CEN*	20	20.1	3	4.5	0.6	0.9		
	Ø 2μ	20	18.7	1	4.3	0.2	0.9		
	Ypt31 2μ	20	19.5	3	4.2	0.1	0.8		
Sec7-mRFP Chc1-GFP	Ø *CEN*	20	81.1	1	5.2		4.1	1.1	0
	Ypt31 *CEN*	20	70.5	3	6.3		4.5	1.9	0
	Ø 2μ	28	64.5	3	5.6		3.6	2.1	0.2
	Ypt31 2μ	28	64.4	4	6.3		4	2.3	0.3
	Ypt31-GTP 2μ	28	45.1	3	8.4		3.6	4.9	0.4
Sec7-yEGFP Chc1-mRFP	Ø *CEN*	20	90.9	1	4		3.6	0.4	
	Ypt31 *CEN*	20	90.3	3	4.5		4.1	0.4	
	Ø 2μ	20	88.9	0	3.6		3.1	0.5	
	Ypt31 2μ	20	88	4	4.4		3.9	0.5	

Figure S3. Effects of Ypt31 overexpression on co-localization of Golgi markers. (Related to Figures 6-7). **A.** Overexpression of Ypt31 does not affect the level of co-localization of Cop1 and Sec7. Cells expressing Sec7-yEGFP and Cop1-mRFP from their endogenous loci were transformed with *CEN* (left) and 2m (right) plasmids, empty (top) and for overexpression of Ypt31 (bottom). Information from this experiment was used in Figure 6E-F. **B.** Overexpression of Ypt31 does not affect the level of co-localization of Sec7 with Chc1. Cells expressing Sec7-yEGFP and Chc1-mRFP from their endogenous loci were transformed with *CEN* (left) and 2m (right) plasmids, empty (top) and for overexpression of Ypt31 (bottom). **C.** Table shows quantifications from two independent experiments for panels A and B of this figure and Figures 6 and 7. The co-localization level of the two markers was determined using live cell microscopy. Bar, 5μm.

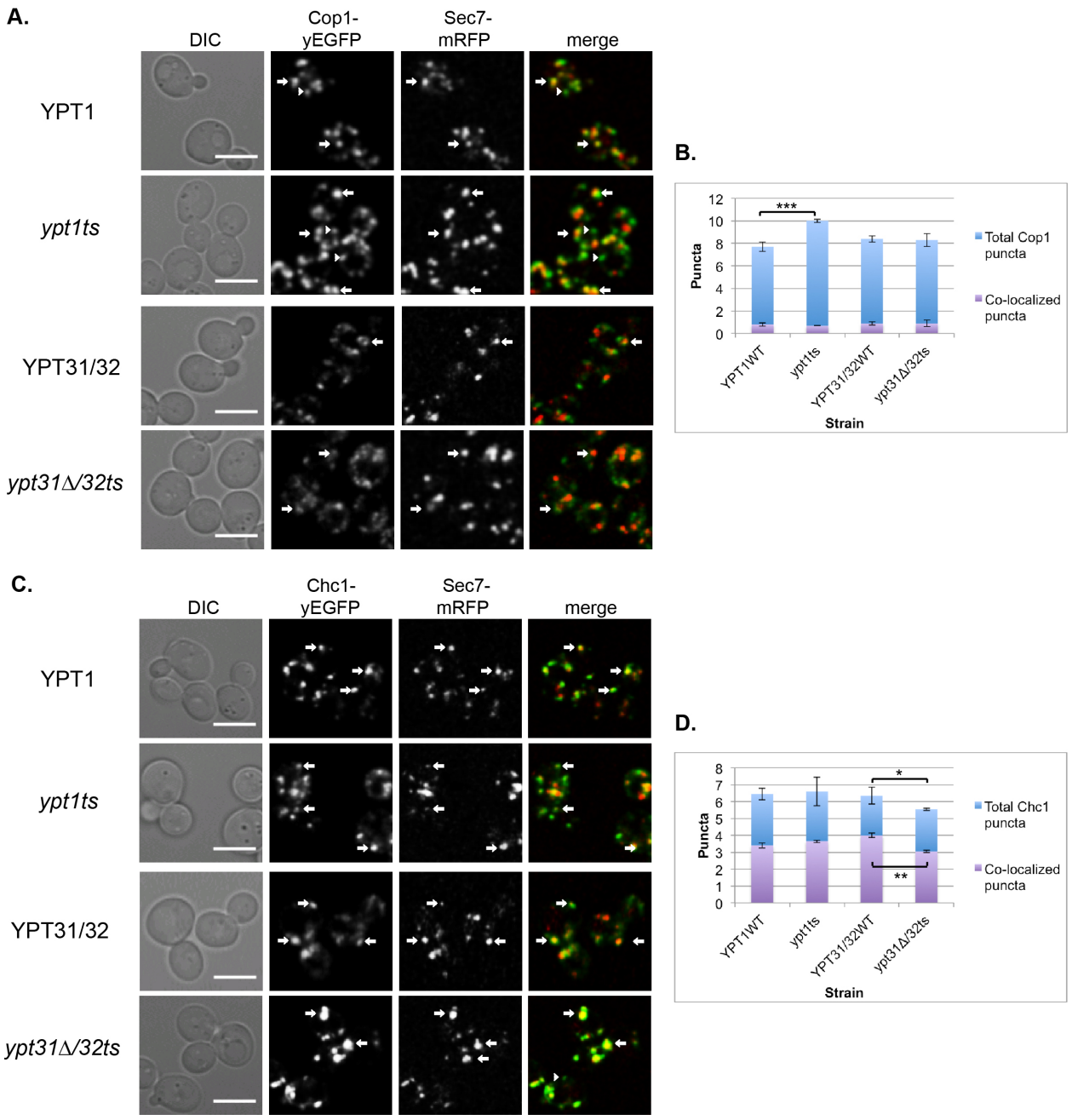


Figure S4. The effect of *ypt1* and *ypt31/32* loss-of-function mutations on the co-localization of Golgi markers. (Related to Figures 6-7). The effect of *ypt1ts* and *ypt31Δ/32ts* mutations on the co-localization of Golgi markers was determined at the permissive temperature using live-cell fluorescence microscopy: **A-B.** Cop1-Sec7. **C-D.** Sec7-Chc1. Wild type and mutant cells expressing tagged Golgi markers from their endogenous promoters were analyzed as described for Figures 6-7. Panels A and C: Shown from top to bottom: WT (*YPT1*), *ypt1ts*, WT (*YPT31/YPT32*), *ypt31Δ/32ts*. Arrows show co-localization; arrowheads show green-only puncta. Bar, 5μm. Bar graphs showing the number of Cop1 (B) or Chc1 (D) puncta: Total puncta (blue+purple bars), and puncta that co-localize with Sec7 (purple bars). Error bars and +/- represent STDEV; brackets with stars represent p-value from significant (*) to highly significant (***); p-values of all other pairs were not significant. Quantifications from two independent experiments are detailed in Table S2.

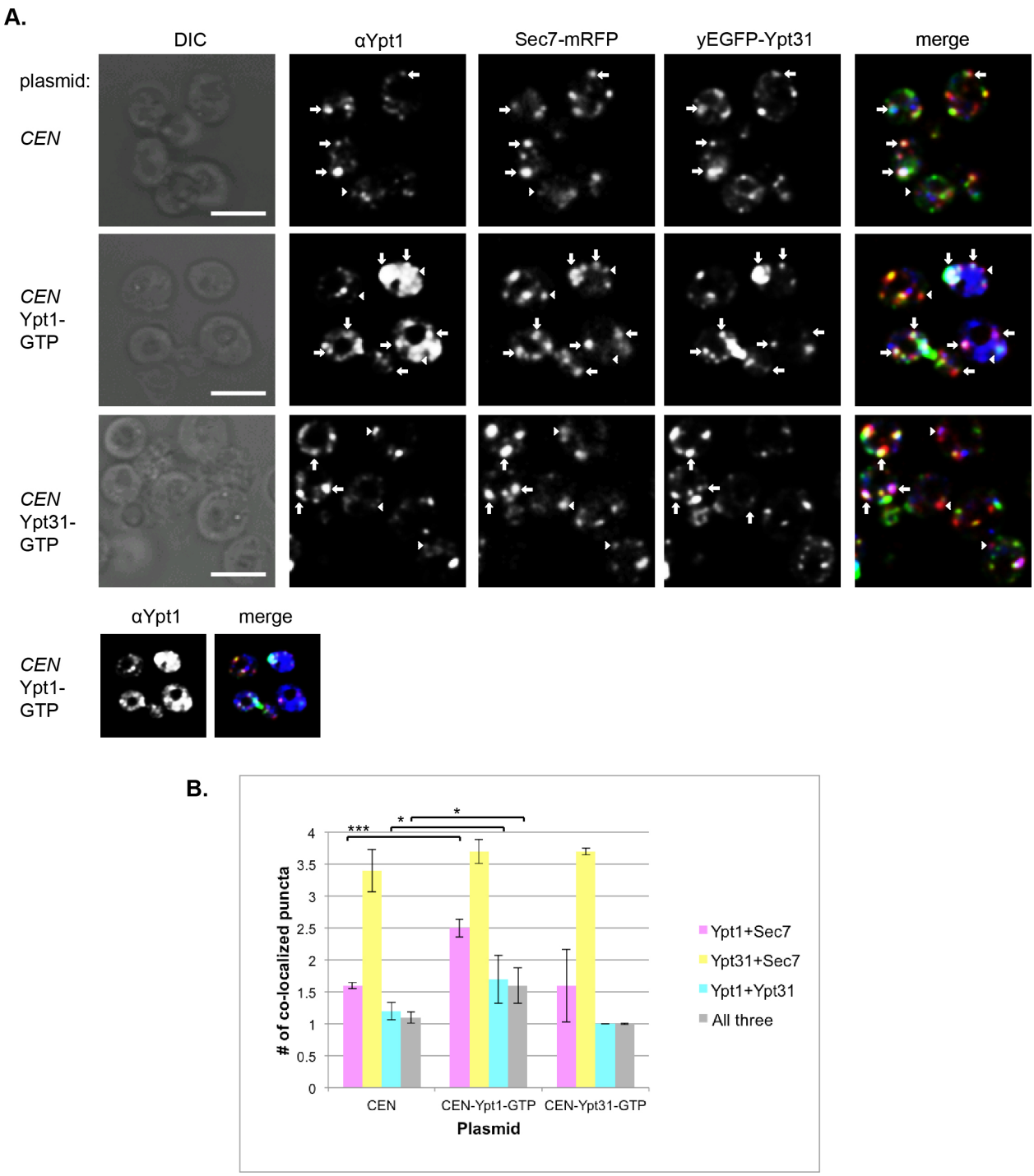


Figure S5. The effect of Ypt1 or Ypt31 activation on their co-localization at the Sec7-marked Golgi compartment (Related to Figure 6-7). A. Cells were transformed with a CEN plasmid: empty (top), expressing Ypt1-GTP (middle), or Ypt31-GTP (bottom). Three-color IF microscopy was done as described in the legend for Figure 5C. The brightness of the Ypt1 staining in cells overexpressing Ypt1-GTP was reduced to enable the co-localization analysis (two bottom panels show the cells before the adjustment). Bar, 5μm. **B.** Bar graph showing the number of puncta in transformants of the three plasmids (from left-to-right): empty (CEN), Ypt1-GTP and Ypt31-GTP. The effect of each plasmid on co-localization of two or three proteins is color-coded (see key on the right): Ypt1+Sec7 (pink), Ypt31+Sec7 (yellow), Ypt1+Ypt31 (blue), and all three proteins (grey). Error bars and +/- represent STDEV; brackets with stars represent p-value; p-values for all other pairs were not significant. Quantifications from two independent experiments are detailed in Table S3.

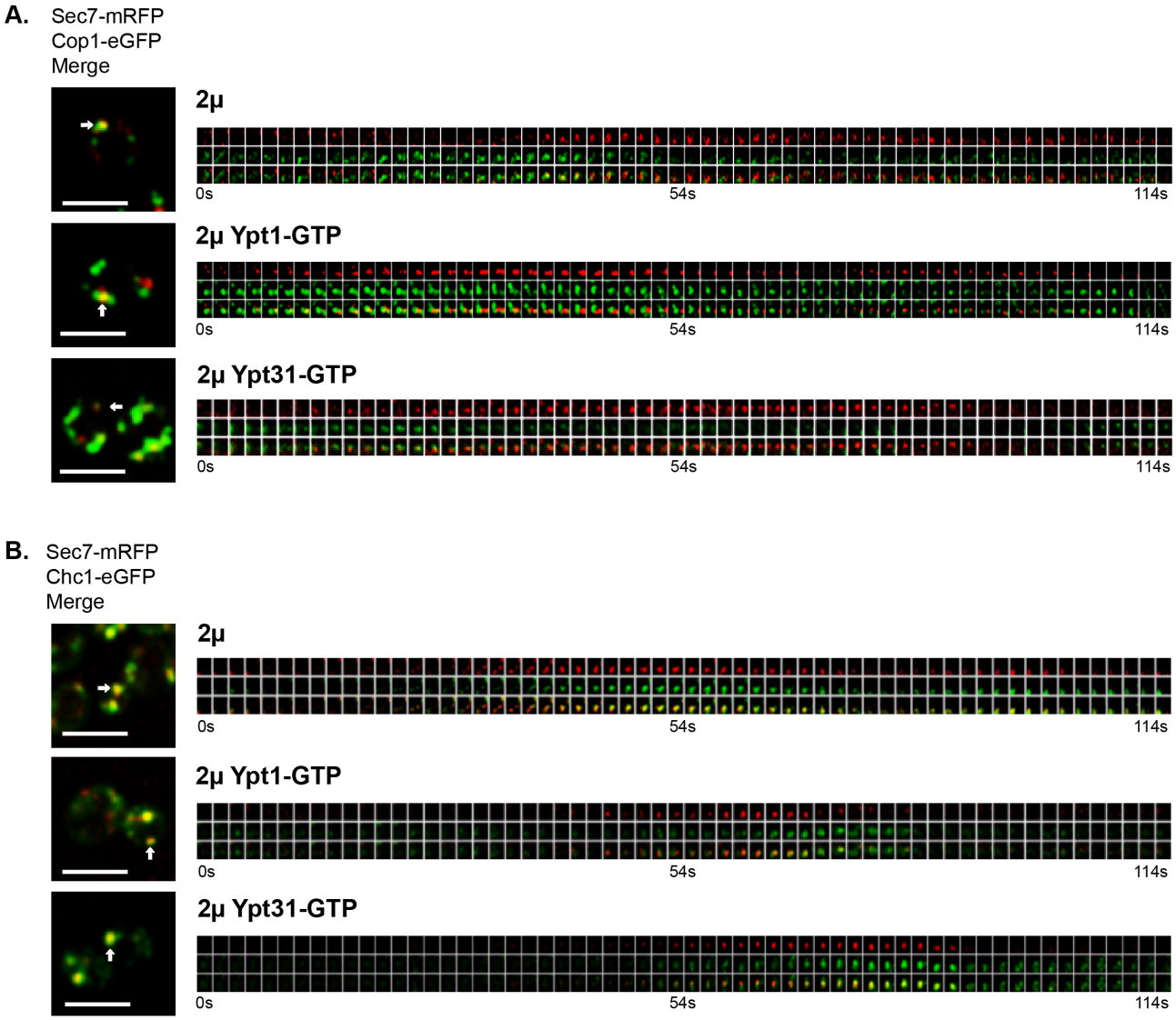


Figure S6. The effect of overexpression of activated Ypt1 and Ypt31 on Golgi cisternal progression. (Related to Figure 8) Three-channel kymographs of puncta used for graphs shown in Figure 8A-F. **A-C**: Cop1-GFP and Sec7-mRFP. **D-F**: Sec7-mRFP and Chc1-GFP. Cells expressing a Golgi marker pair were transformed with one of the indicated 2m plasmids: empty, YPT1-GTP or Ypt31-GTP. For each transformant, left panel shows a merged live-cell image with a white arrow pointing to the punctum selected for the kymograph, which is shown to its right. For each kymograph, shown from top-to-bottom: mRFP, GFP, merge, time (secs). Bar, 5μm.

Supplemental Tables

Table S1. Distribution of Ypt1 on the Golgi using three-color IF microscopy (Related to Figure 3).

Cop1-mRFP Sec7-yEGFP α-Ypt1	# of cells (n)	co-localization (%) of Sec7 with Ypt1	std dev	# Sec7 puncta/slice	# co-localized puncta/slice	co-localization (%) of Ypt1 with Sec7	std dev	# Ypt1 puncta/slice
	24	33.1	3	5	1.5	23.4	2	6.6
		co-localization (%) of Sec7 with Cop1	std dev	# co-localized puncta/slice		co-localization (%) of Ypt1 with Cop1	std dev	# co-localized puncta/slice
		14.7	0	0.7		54.2	6	3.4
co-localization of three markers	# of cells (n)	co-localization (%) of Ypt1+Sec7 with Cop1	std dev		# of cells (n)	co-localization (%) of Cop1+Sec7 with Ypt1	std dev	
	20	46.7	7		14	93.8	9	
Chc1-mRFP Sec7-yEGFP α-Ypt1	# of cells (n)	co-localization (%) of Sec7 with Ypt1	std dev	# Sec7 puncta/slice	# co-localized puncta/slice	co-localization (%) of Ypt1 with Sec7	std dev	# Ypt1 puncta/slice
	24	32.3	1	4.5	1.4	23	2	6.3
		co-localization (%) of Sec7 with Chc1	std dev	# co-localized puncta/slice		co-localization (%) of Ypt1 with Chc1	std dev	# co-localized puncta/slice
		58.2	3	2.6		11.9	4	0.66
co-localization of three markers	# of cells (n)	co-localization (%) of Ypt1+Chc1 with Sec7	std dev		# of cells (n)	co-localization (%) of Ypt1+Sec7 with Chc1	std dev	
	12	100	0		19	60.3	3	

Table S2. The effect of *ypt1* and *ypt31/32* loss-of-function mutations on the co-localization of Golgi markers (Related to Figure S4).

	Strain	# of cells (n)	co-localization (%) of green with Sec7	std dev	**# green puncta/slice**	**std dev**	**p-value**	**# co-localized puncta/slice**	**std dev**	**p-value**
Sec7-mRFP Chc1-GFP	YPT1	20	11.1	2	**7.7**	**0.4**	**n/a**	**0.8**	**0.1**	
	ypt1ts	20	7.8	1	**10.0**	**0.1**	**0.0007*** **	**0.7**	**0**	
	YPT31/32	20	11.9	1	**8.4**	**0.3**	**n/a**	**0.9**	**0.1**	
	ypt31Δ/32ts	20	11.0	4	**8.3**	**0.6**	**0.8297**	**0.9**	**0.3**	
Sec7-mRFP Cop1-GFP	YPT1	20	54.9	1	**6.5**	**0.4**	**n/a**	**3.4**	**0.1**	
	ypt1ts	20	56.8	1	**6.6**	**0.8**	**0.7318**	**3.7**	**0.1**	**0.0374***
	YPT31/32	20	63.6	7	**6.4**	**0.5**	**n/a**	**4.0**	**0.1**	
	ypt31Δ/32ts	20	56.6	3	**5.6**	**0.1**	**0.0395***	**3.1**	**0.1**	**0.0042****

Bolded numbers were used for graphs in Figure S4B,D.

Table S3. The effect of Ypt1 or Ypt31 activation on their co-localization at the Sec7-marked Golgi compartment (Related to Figure S5)

***yEGFP-YPT31/ypt32Δ* Sec7-mRFP α-Ypt1**		**# of Ypt1+Ypt31 puncta**	% coloc Ypt1 with Ypt31	% coloc Ypt31 with Ypt1	**# of Ypt1+Sec7 puncta**	% coloc Ypt1 with Sec7	% coloc Sec7 with Ypt1	**# of Ypt31+Sec7 puncta**	% coloc Ypt31 with Sec7	% coloc Sec7 with Ypt31
CEN	**ave**	**1.17**	0.20	0.20	**1.57**	0.28	0.35	**3.43**	0.57	0.77
n=30	**±**	**0.14**	0.02	0.04	**0.05**	0.02	0.00	**0.33**	0.05	0.09
CEN-Ypt1-GTP	**ave**	**1.73**	0.29	0.27	**2.50**	0.43	0.54	**3.73**	0.60	0.81
n=30	**±**	**0.38**	0.04	0.07	**0.14**	0.02	0.04	**0.19**	0.02	0.02
		p=0.0240			**p=0.0006**			**p=0.3474**		
CEN-Ypt31-GTP	**ave**	**1.00**	0.16	0.16	**1.60**	0.28	0.40	**3.70**	0.61	0.83
n=30	**±**	**0.00**	0.01	0.01	**0.57**	0.11	0.17	**0.05**	0.06	0.02
		p=0.4038			**p=0.8926**			**p=0.3934**		

***yEGFP-YPT31/ypt32Δ* Sec7-mRFP α-Ypt1**		# of red Sec7	# of green Ypt31	# of blue Ypt1	**# of puncta co-loc of all three**	% of Ypt1+Ypt31 with Sec7
CEN	**ave**	4.43	6.03	5.67	**1.07**	0.92
n=30	**±**	0.05	0.24	0.38	**0.09**	0.03
CEN-Ypt1-GTP	**ave**	4.63	6.33	5.97	**1.60**	0.93
n=30	**±**	0.05	0.00	0.52	**0.28**	0.04
					p=0.0350	
CEN-Ypt31-GTP	**ave**	4.43	6.27	5.90	**0.93**	0.93
n=30	**±**	0.05	0.47	0.33	**0.00**	0.00
					p=0.5132	

Bolded numbers were used for graphs in Figure S5B.

Supplemental Experimental Procedures

Yeast Strains

NSY number	Alias	Genotype	Reference
NSY862	Cop1-mRFP	*mat alpha his3 delta1 leu2 delta 0 lys2 delta 0 ura3 delta 0 COP1::mRFP-kanMX6*	1
NSY1733	Cop1-mRFP Vrg4-yEGFP	*NSY862 VRG4::yEGFP-nat*	this study
NSY1734	Cop1-mRFP Sec7-yEGFP	*NSY862 SEC7::yEGFP-nat*	this study
NSY1735	Cop1-mRFP Chc1-GFP	*NSY862 CHC1::GFP-hyg*	this study
NSY863	Chc1-mRFP	*mat alpha his3 delta1 leu2 delta 0 lys2 delta 0 ura3 delta 0 CHC1::mRFP-kanMX6*	1
NSY1736	Chc1-mRFP Vrg4-yEGFP	*NSY863 VRG4::yEGFP-nat*	this study
NSY1737	Chc1-mRFP Cop1-GFP	*NSY863 COP1::GFP-hyg*	this study
NSY1738	Chc1-mRFP Sec7-yEGFP	*NSY863 SEC7::yEGFP-nat*	this study
NSY825	WT- BY4741	*mat a leu2 delta 0 ura3 delta 0 his3 delta 1 met15 delta 0*	2
NSY1739	Sec7-mCherry	BY4741 SEC7::mCherry-NatR	this study
NSY1740	Vrg4-yEGFP	BY4741 VRG4::yEGFP-NatR	this study
NSY1221	yEGFP-Ypt31/ypt31Δ	*mat alpha ade2 ura3-52 leu2-3,112 his3 delta 200 lys2 ypt32::kan yEGFP-Ypt31(LYS2) in Ypt31 locus*	this study
NSY1741	yEGFP-Ypt31/ypt31Δ Sec7-mRFP	*mat alpha ade2 ura3-52 leu2-3,112 his3 delta 200 lys2 ypt32::kan yEGFP-Ypt31(LYS2) in Ypt31 locus SEC7::mRFP-HygR*	this study
NSY1742	Chc1-GFP	BY4741 CHC1::GFP-HygR	this study
NSY1743	Chc1-GFP Sec7-mRFP	BY4741 CHC1::GFP-HygR SEC7::mRFP-G418R	this study
NSY1744	Sec7-mRFP	BY4741 SEC7::mRFP-G418R	this study
NSY1745	Cop1-GFP Sec7-mRFP	BY4741 SEC7::mRFP-G418R COP1::GFP-HygR	this study
NSY128	WT- DBY4985	*mat alpha ade2 his3 delta 200 leu2-3,112 lys2-801 ura3-52*	David Botstein
NSY1746	Chc1-GFP	NSY128 CHC1::GFP-HygR	this study

NSY1747	Chc1-GFP Sec7-mRFP	NSY128 CHC1::GFP-HygR SEC7::mRFP-G418R	this study
NSY1748	Cop1-GFP	NSY128 COP1::GFP-HygR	this study
NSY1749	Cop1-GFP Sec7-mRFP	NSY128 COP1::GFP-HygR SEC7::mRFP-G418R	this study
NSY1541	*ypt1Δ* + pRS316-Ypt1	NSY128 pRS316-YPT1 yptt1 delta::HygR	3
NSY302	*ypt31Δ/ypt32Δ* + pRS316-Ypt31	*mat alpha ade2 his3 delta 200 leu2-3 lys2 ura3-52 deltaYPT31::HIS2 pRS316-Ypt31 deltaYPT32::KanR pRS316-Ypt31*	4
NSY220	WT- YPT1	*mat alpha ura3-52 lys2 his4*	5
NSY222	*ypt1ts*	*mat alpha ura3-52 his4 ypt1A136D*	5
NSY125	WT- YPT31/32	*mat a ura3-52 lys2 his4*	6
NSY348	*ypt31Δ/ypt32ts*	*mat a ura3-52 lys2 his4-539 deltaYPT31::HIS3 ypt32-A141D*	6
NSY1750	YPT1 Sec7-mRFP	NSY1081 SEC7::mRFP-G418R	this study
NSY1754	YPT1 Sec7-mRFP Cop1-GFP	NSY1081 SEC7::mRFP-G418R COP1::GFP-HygR	this study
NSY1756	YPT1 Sec7-mRFP Chc1-GFP	NSY1081 SEC7::mRFP-G418R CHC1::GFP-HygR	this study
NSY1751	*ypt1ts* Sec7-mRFP	NSY1082 SEC7::mRFP-G418R	this study
NSY1758	*ypt1ts* Sec7-mRFP Cop1-GFP	NSY1082 SEC7::mRFP-G418R COP1::GFP-HygR	this study
NSY1760	*ypt1ts* Sec7-mRFP Chc1-GFP	NSY1082 SEC7::mRFP-G418R CHC1::GFP-HygR	this study
NSY1752	YPT31/32 Sec7-mRFP	NSY125 SEC7::mRFP-G418R	this study
NSY1762	YPT31/32 Sec7-mRFP Cop1-GFP	NSY125 SEC7::mRFP-G418R COP1::GFP-HygR	this study
NSY1764	YPT31/32 Sec7-mRFP Chc1-GFP	NSY125 SEC7::mRFP-G418R CHC1::GFP-HygR	this study
NSY1753	*ypt31Δ/ypt32ts* Sec7-mRFP	NSY348 SEC7::mRFP-G418R	this study
NSY1766	*ypt31Δ/ypt32ts* Sec7-mRFP Cop1-GFP	NSY349 SEC7::mRFP-G418R COP1::GFP-HygR	this study
NSY1768	*ypt31Δ/ypt32ts* Sec7-mRFP Chc1-GFP	NSY350 SEC7::mRFP-G418R CHC1::GFP-HygR	this study

Plasmids

PNS number	Alias	Genotype	Reference
pNS245	empty *CEN*	pRS315 *CEN*, *LEU2*, Amp^r	7
pNS1430	yEVenus-Ypt1	pRS315-yEVenus-*YPT1*	8
pNS1431	mCherry-Ypt1	pRS315-mCherry-*YPT1*	This study
pNS1364	*CEN* Ypt1	pRS315-*YPT1*	9
pNS661	GFP-Ypt31	pRS315-GFP-*YPT31*	Ruth Collins
pNS221	*CEN* Ypt31	pRS315-*YPT31*	4
pNS246	empty *CEN*	pRS316 *CEN*, *URA3*, Amp^r	7
pNS636	*CEN* Ypt1	pRS316-*YPT1*	Ruth Collins
pNS220	*CEN* Ypt31	pRS316-*YPT31*	4
pNS719	empty *CEN*	pRS317 *CEN*, *LYS2*, *Amp^r*	10
pNS939	*CEN* Ypt31	pRS317-*YPT31*	This study
pNS994	yEGFP-Ypt31	pRS317-yEGFP-*YPT31*	This study
pNS180	empty 2µ	pRS425 2µ, *LEU2*, Amp^r	7
pNS993	2µ Ypt1	YEp423-*YPT1 LEU2*	5
pNS1556	2µ Ypt1-GTP	YEp423-*YPT1Q67L*	This study
pNS781	2µ Ypt31	pRS425-*Ypt31*	Scott Emr
pNS782	2µ Ypt31-GTP	pRS425-*YPT31Q72L*	Scott Emr
pNS274	empty 2µ	YEp24 2µ, *URA3*, Amp^r	New England Biolabs, MA
pNS489	2µ Ypt1	YEp24-*YPT1*	11
pNS229	2µ Ypt31	YEp24-*YPT31*	6

pNS1527	tagging plasmid	pFA6a-mRFP-G418^r	1
pNS1557	tagging plasmid	pFA6a-GFP-Hyg^r	This study
pNS1506	tagging plasmid	pKT127-yEGFP replaced with mCherry-G418r replaced with Natr	12
pNS1533	tagging plasmid	pKT127-yEGFP-G418r replaced with Nat^r	This study
pNS1558	*CEN* Ypt1-GTP	pRS315-YPT1Q67L	This study
pNS1559	*CEN* Ypt31-GTP	pRS315-YPT31Q72L	This study

Plasmid construction

Plasmid for expression of mCherry-Ypt1 was constructed by sub-cloning mCherry to replace yEVenus in pNS1430. pNS220 (pRS316-Ypt31) was described in (Jedd et al., 1997). pNS939 (pRS317-Ypt31) was made by sub-cloning the Ypt31 containing ClaI-XbaI fragment from pRS316-Ypt31 into pRS317. pNS939 (pRS317-yEGFP-Ypt31) was made as follows: first, PciI site was removed from the vector backbone and introduced upstream of Ypt31 by site-directed mutagenesis, then yEGFP was cloned in frame with Ypt31 using PciI. pNS993 was previously described(Liang et al., 2007). pNS1556 (Ypt1Q67L in a 2μ *LEU2*) was made by site-directed mutagenesis of pNS993. pNS1557 (pFA6a-GFP-HygR) was made by replacing G418R cassette with HygR cassette using BglII and SacI sites. pNS1533 (pKT-yEGFP-NatR) was made by replacing G418R cassette with NatR cassette using BglII and SacI sites. The *CEN* plasmids pNS1558 and pNS1559 were constructed by sub-cloning Ypt1Q67L and Ypt31Q72L from the 2μ plasmids pNS1556 and pNS782, respectively, using the Sal1 and Xba1 sites of pNS245 (*CEN Leu2*).

Yeast strain construction and growth

Golgi proteins were tagged on the chromosome at their C-termini with mRFP, GFP, mCherry, or yEGFP using a standard technique (Wach et al., 1997) using appropriate plasmids listed in the plasmid table. All GFP-tagged strains were checked to ensure that the tagged product is stable by using immuno-blot analysis and mouse monoclonal anti-GFP antibodies (Roche Diagnostics).

Media preparation and yeast culture growth were done as previously described (Segev and Botstein, 1987).

Supplemental References

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