Supplemental figures legends

Figure S1. The p24 complex is required for efficient ER exit of GPI-anchored proteins. Pulse-chase analysis of the ER-to-Golgi transport in wild-type and *emp24*Δ strains. Cells were radiolabeled for 5 min, chased for 15 min at 24°C, and lysed. GPI-anchored proteins, CPY and Pre-pro-α-factor were immunoprecipitated, resolved by SDS-PAGE, and analyzed by PhosphoImager. GPI-anchored proteins: p, ER-precursor form; m, Golgi form. CPY: p1, ER-precursor form; p2, Golgi precursor form; m, mature form. Pre-pro-α-factor: p, ER-precursor form. GPI-APs: GPI-anchored proteins.

Figure S2. ERES formation is not influenced by *erv14*Δ and *emp24*Δ mutations. Fluorescent micrographs of live wild-type, *erv14*Δ, *emp24*Δ, *sec31-1, sec31-1 erv14*Δ and *sec31-1 emp24*Δ cells expressing Sec13-GFP at 37°C. Raw images. Scale bar: 5 µm.

Figure S3. The p24 complex is not required for protein sorting upon ER exit. (A) Fluorescent micrographs of live *sec31-1* and *sec31-1 emp24*Δ cells expressing Hxt1-CFP (cyan) and Cwp2-Venus (yellow) at 37°C. (B) Quantification of several micrographs described in (A). The graph displays the means of the percentage of co-localization per cell between Cwp2-Venus dots and Hxt1-CFP dots in *sec31-1* (n=47), and in *sec31-1 emp24*Δ (n=58). Images deconvoluted by 10 iterations. Open arrow heads: non co-localizing dots. Scale bar: 5µm.

Figure S4. Emp24p is present in GPI-anchored protein containing ERES. (A) Live images of *sec31-1* ERV14-mCi (yellow) cells expressing CFP-Hxt1p (blue) at 37°C. (B) Quantification of several micrographs described in (A). (B) In the left panel, the number of dots of Erv14-mCi and CFP-Hxt1p per cell was averaged. In the right panel, the graph plots the quantification of the co-localization between Erv14-mCi and CFP-Hxt1p dots. (n=52). (C) Live images of *sec31-1* cells expressing Emp24-CFP (blue) and Ccw14-Venus (yellow) at 37°C. (D) Quantification of several micrographs described in (C). (D) In the left panel, the number of dots of Emp24-CFP and Ccw14-Venus per cell was averaged. In the right panel, the graph plots the quantification of the co-localization between Emp24-CFP and Ccw14-Venus dots. (n=72). (A,C) Scale bar: 5 µm. White arrow heads: co-localizing dots. The images were deconvoluted by 10 iterations.

Figure S5. Native co-immunoprecipitation assay between Emp24p and Gas1p in *per1*Δ and *gup1*Δ remodeling mutant strains. Wild type, *per1*Δ, *gup1*Δ and *emp24*Δ mutant cells expressing Gas1-HA were solubilized in 1% digitonin and analyzed by native immunoprecipitation (IP) with anti-Emp24p antibody followed by immunoblotting with anti-HA peroxidase antibody. Totals (T) represent a fraction of the solubilized input material.

Figure S6. Functional fluorescent fusion of Erv14p and Emp24p. (A) Fluorescent micrographs of live wild-type, *erv14*Δ and ERV14-mCi cells expressing CFP-Hxt1p at 30°C. (B) Fluorescent micrographs of live wild-type cells, *emp24*Δ cells transformed with YCplac111 and *emp24*Δ cells transformed with YCplac111-Emp24-CFP expressing Cwp2-Venus at 30°C. (A,B) Raw images. The white arrow points out ER localization of cargoes. Scale bar: 5 µm.

Figure S7. The p24 complex is stable in remodeling mutants. (A) Co-immunoprecipitation of Emp24-CFP with Erp2p in wild-type, *erv25*Δ and *bst1*Δ cells. Lysates (L) were incubated with the monoclonal anti-GFP antibody. (B) Stability of Emp24p after cycloheximide treatment in wild-type, *erv25*Δ, *bst1*Δ and *per1*Δ cells at 30°C. (C) Protein levels of Emp24p and Erv25p in the remodeling mutants at 37°C.

Figure S8. Co-localization of a GPI-anchored protein with a vacuolar marker in wild type and mutant strains. Live images of wild-type, *ret1-1*, *bst1*Δ and *ret1-1 bst1*Δ expressing Ccw14-Venus at 24°C. The dye CellTracker blue-CMAC was added to cultures to allow visualization of the vacuole. Raw images. Scale bar: 5 µm.

Fig. S1

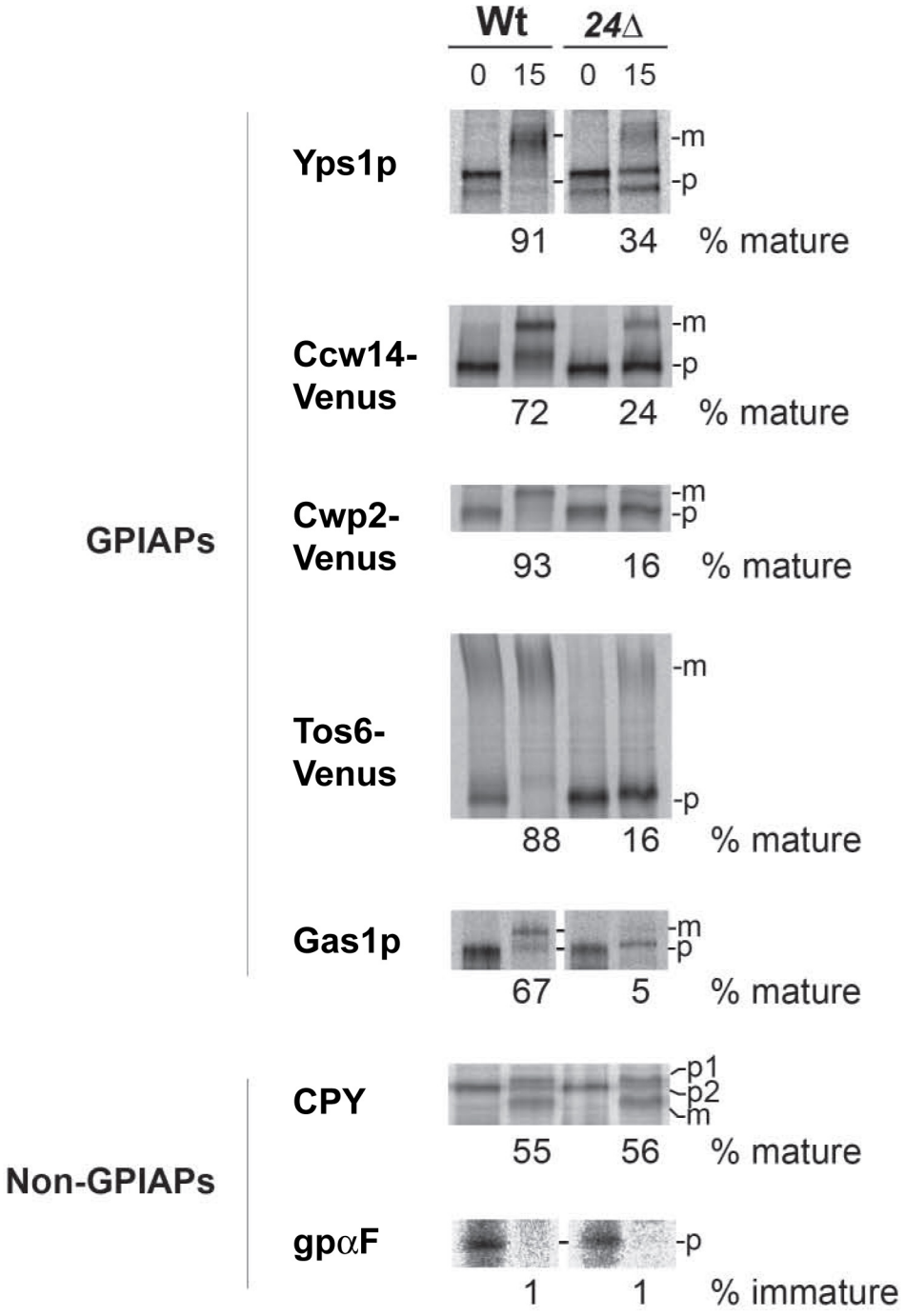

Wt
24Δ
0 15 0 15
GPIAPs
Yps1p
-m
-p
91 34 % mature
Ccw14-Venus
-m
-p
72 24 % mature
Cwp2-Venus
-m
-p
93 16 % mature
Tos6-Venus
-m
-p
88 16 % mature
Gas1p
-m
-p
67 5 % mature
Non-GPIAPs
CPY
-p1
-p2
-m
55 56 % mature
gpαF
-p
1 1 % immature

Fig. S2

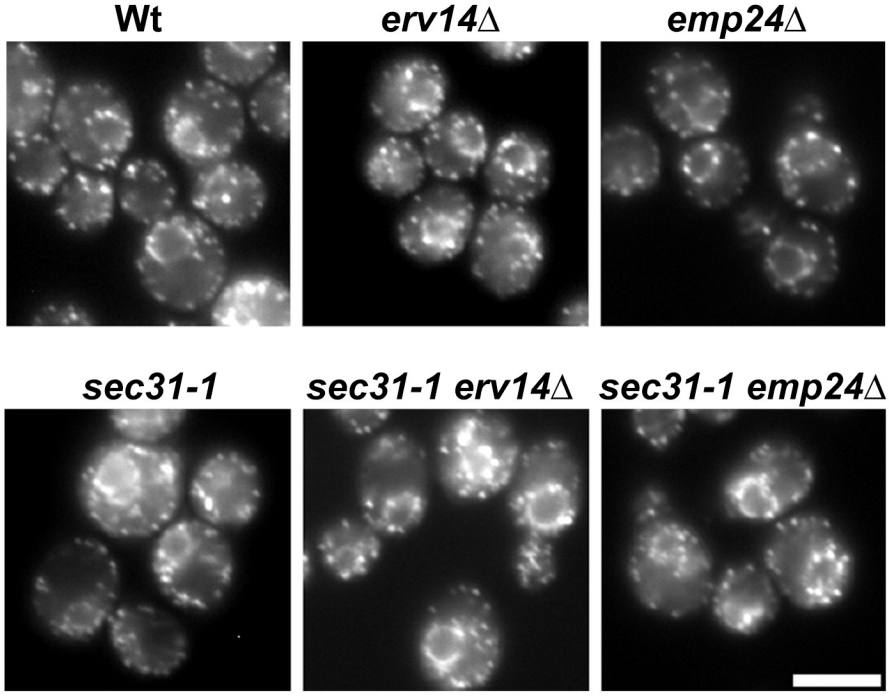
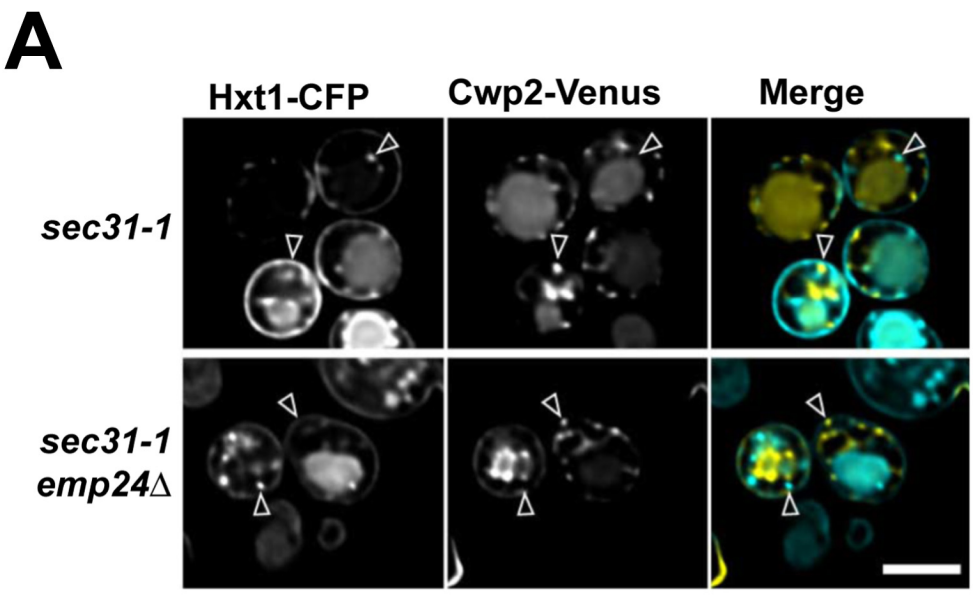


Fig. S3


A
Hxt1-CFP
Cwp2-Venus
Merge
sec31-1
sec31-1
emp24Δ

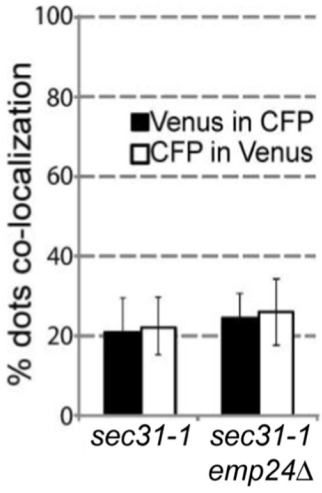

B
% dots co-localization
100
80
60
40
20
0
Venus in CFP
CFP in Venus
sec31-1
sec31-1
emp24Δ

Fig. S4

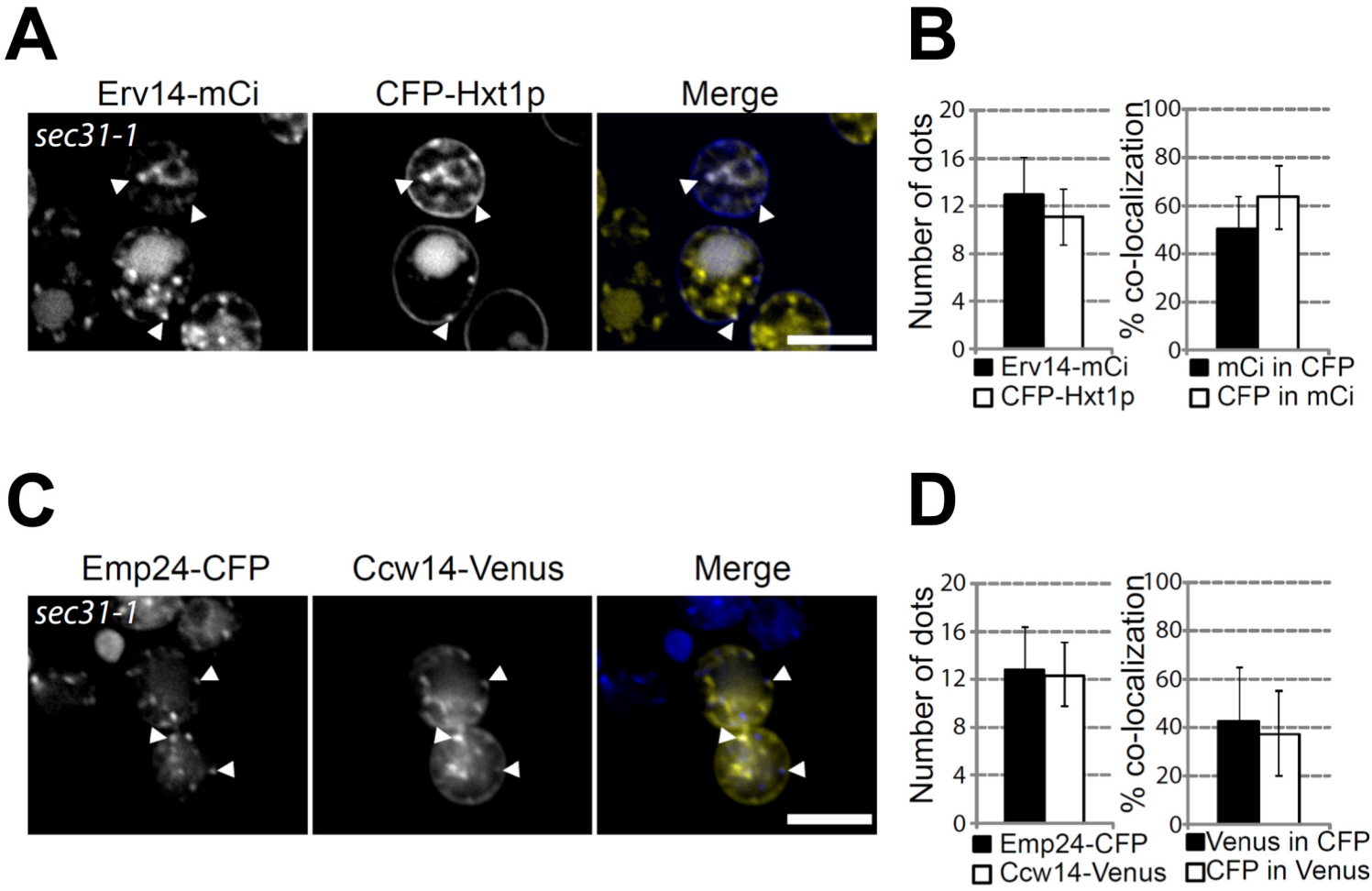

A
Erv14-mCi
CFP-Hxt1p
Merge
sec31-1
B
Number of dots
% co-localization
Erv14-mCi
CFP-Hxt1p
mCi in CFP
CFP in mCi
C
Emp24-CFP
Ccw14-Venus
Merge
sec31-1
D
Number of dots
% co-localization
Emp24-CFP
Ccw14-Venus
Venus in CFP
CFP in Venus

Fig. S5

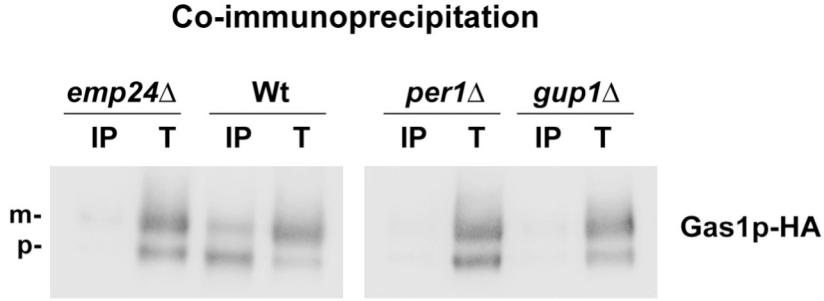

Co-immunoprecipitation
emp24Δ
Wt
per1Δ
gup1Δ
IP
T
IP
T
IP
T
IP
T
m-
p-
Gas1p-HA

Fig. S6

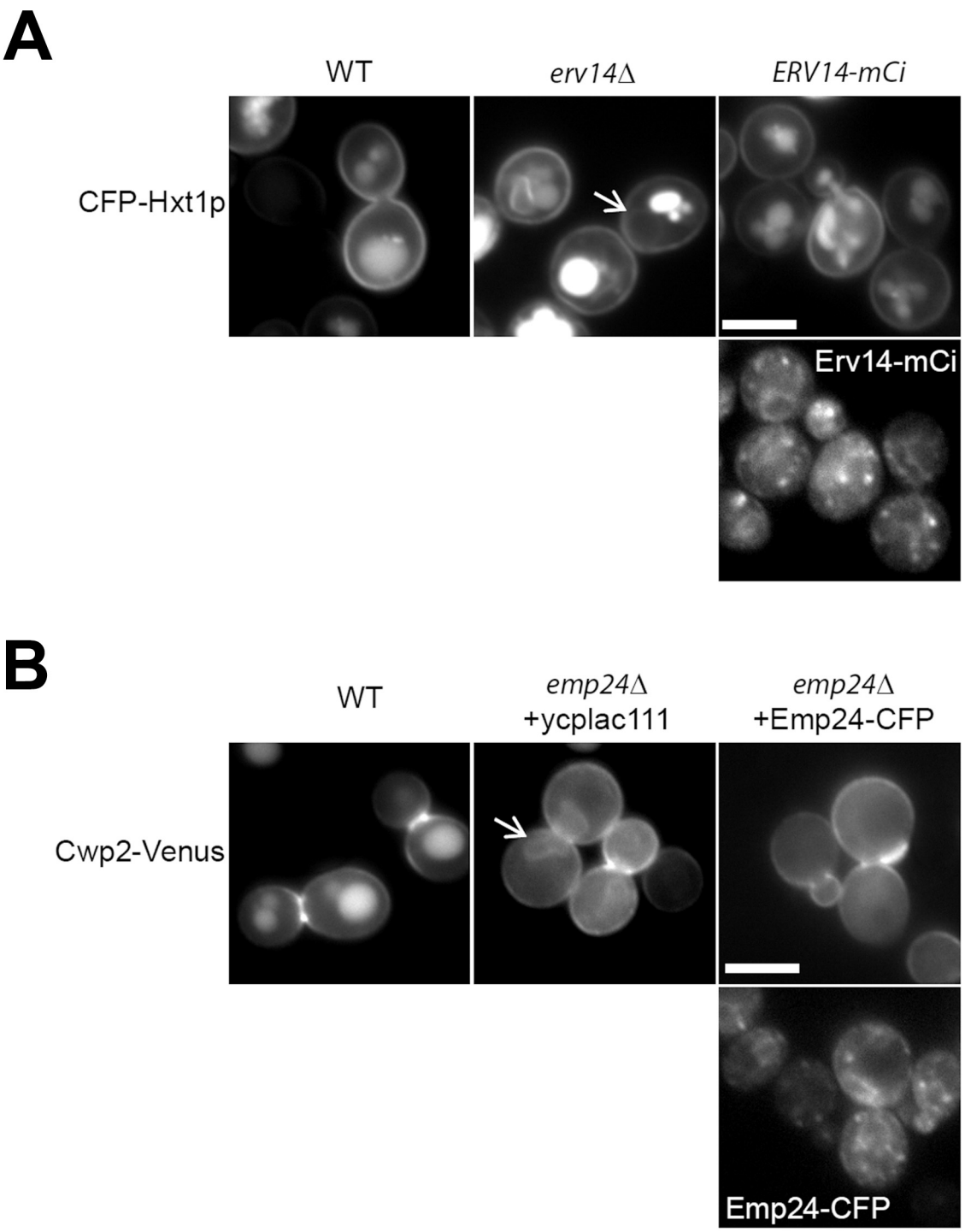

A
WT
erv14Δ
ERV14-mCi
CFP-Hxt1p
Erv14-mCi
B
WT
emp24Δ
+ycplac111
emp24Δ
+Emp24-CFP
Cwp2-Venus
Emp24-CFP

Fig. S7

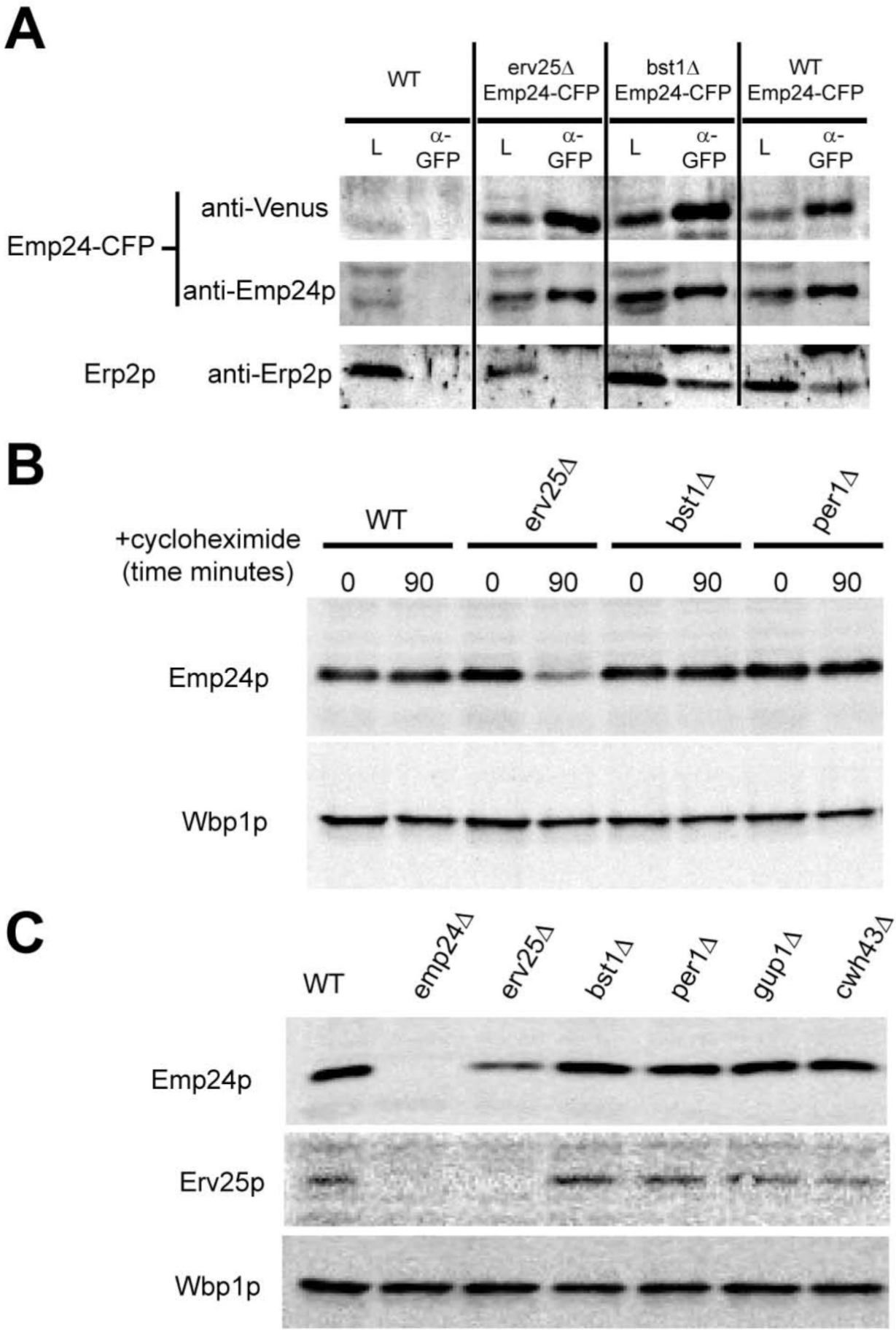

A
WT
erv25Δ Emp24-CFP
bst1Δ Emp24-CFP
WT Emp24-CFP
L
α-GFP
Emp24-CFP
anti-Venus
anti-Emp24p
Erp2p
anti-Erp2p
B
+cycloheximide (time minutes)
WT
erv25Δ
bst1Δ
per1Δ
0
90
Emp24p
Wbp1p
C
WT
emp24Δ
erv25Δ
bst1Δ
per1Δ
gup1Δ
cwh43Δ
Emp24p
Erv25p
Wbp1p

Fig. S8

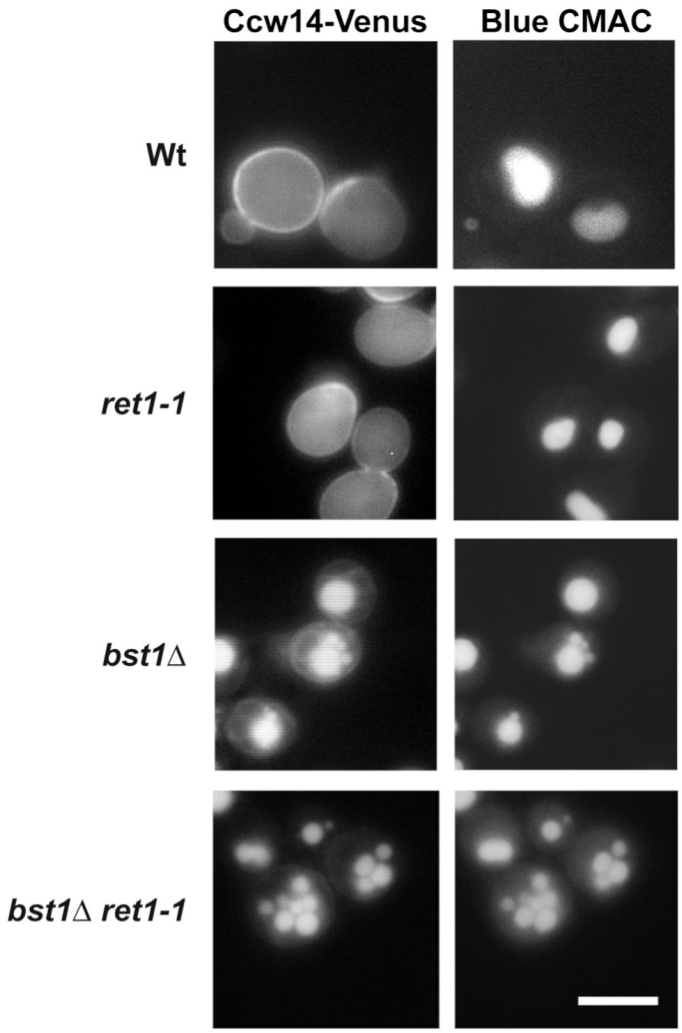


Table S1. List of the strains used in this study.

Number	***Name***	***Genotype***
RH2874	*WT*	*Mat α leu2 lys2 trp1 ura3 bar1*
RH6153	*erv29Δ*	*Mat α erv29Δ::KanMX ura3 leu2 lys2 trp1 bar1*
RH5698	*gpi1Δ*	*Mat α gpi1::TRP1 ade2 ade3 his3 leu2 trp1 ura3*
RH5877	*sec31-1*	*Mat a sec31-1 leu2 ura3 lys2 trp1 his3*
RH6910	*emp24Δ*	*Mat a emp24Δ::KanMX leu2 ura3 lys2 trp1 his3*
RH6912	*sec31-1 emp24Δ*	*Mat a sec31-1 emp24Δ::KanMX leu2 ura3 lys2 trp1*
RH6878	*erv14Δ*	*Mat a erv14Δ::KanMX leu2 ura3 lys2 trp1*
RH6881	*sec31-1 erv14Δ*	*Mat a sec31-1 erv14Δ::KanMX leu2 ura3 lys2 trp1*
RH7016	*erv14-mCi*	*Mat α erv14-mCi-SpHIS5 leu2 ura3 lys2 trp1 his3*
RH7017	*sec31-1 erv14-mCi*	*Mat α sec31-1 erv14-mCi-SpHIS5 leu2 ura3 lys2 trp1 his3*
RH7021	*WT*	*Mat a leu2 ura3 met15 his3*
RH7022	*emp24Δ*	*Mat a emp24Δ::KanMX leu2 ura3 met15 his3*
RH7023	*erv25Δ*	*Mat a erv25Δ::KanMX leu2 ura3 met15 his3*
RH7025	*bst1Δ*	*Mat a bst1Δ::KanMX leu2 ura3 met15 his3*
RH7026	*per1Δ*	*Mat a per1Δ::KanMX leu2 ura3 met15 his3*
RH7085	*bst1Δ emp24Δ*	*Mat a bst1Δ::KanMX emp24Δ::KanMX ura3 leu2 lys2 trp1 his3*
RH7096	*bst1Δ*	*Mat a bst1Δ::KanMX ura3 leu2 lys2 trp1 his3*
RH7097	*sec31-1 bst1Δ*	*Mat a sec31-1 bst1Δ::KanMX ura3 leu2*
MMY596	*per1Δ*	*Mat a per1Δ::KanMX ura3 leu2 his4 trp1 ade2*
MMY756	*emp24 3xHA*	*Mat a emp24 3xHA::KanMX ura3 leu2 his3*
MMY631	*emp24 3xHA*	*Mat α emp24 3xHA::KanMX ura3 leu2 his3 lys2*
BY4742	*gas1Δ*	*Mat α gas1Δ::KanMX ura3 leu2 his3 lys2*
MMY664	*emp24 3xHA gas1Δ*	*Mat a emp24-3xHA::KanMX gas1Δ::KanMX ura3 leu2 his3 lys2*
RH2863	*ret1-1*	*Mat α ret1-1 ura3 leu2 his4 lys2 trp1*
MMY946	*ret1-1 bst1Δ*	*Mat α ret1-1 bst1Δ ura3 leu2 his3 lys2 ade2 trp1*
RSY1800*	*lst1Δ*	*Mat a lst1::HIS3 ura3 leu2 his3 lys2 ade2 trp1*
MMY982	*gas1 3xHA emp24Δ*	*Mat a gas1 3xHA::URA3 emp24Δ::KanMX leu2 his3 trp1 ade2*
VGY1202‡	*gas1 3xHA*	*Mat a gas1 3xHA::URA3 leu2 his3 trp1 ade2 can1*
MMY848	*gas1 3xHA bst1Δ*	*Mat a gas1 3xHA::URA3 bst1Δ::KanMX leu2 his3 trp1*
MMY850	*gas1 3xHA per1Δ*	*Mat a gas1 3xHA::URA3 per1Δ::KanMX leu2 his3 trp1 ade2*
MMY852	*gas1 3xHA gup1Δ*	*Mat α gas1 3xHA::URA3 gup1Δ::KanMX leu2 his3*

*Randy Schekman yeast collection, ‡Veit Goder yeast collection