Supplemental Materials

Molecular Biology of the Cell

Dalton et al.

Figure S1: Sorting of the Snc1 chimera and Neo1 is not affected in Vps13 point mutantsA) Invertase activity overlay assay shows that plasmids expressing wild-type and mutant forms of Vps13 are able to restore GSS localization in a *vps13*Δ mutant (upper panel). Cell surface chimera levels were quantified by densitometry (lower panel). a.u. (arbitrary units), n=3, One-Way ANOVA with a Dunnett-corrected post-hoc test: ****p<0.0001.B) The Neo1 accumulation in large puncta seen in *vps13*Δ mutants was complemented by wild-type and mutant pVps13 plasmids. Top: live-cell fluorescence microscopy of *vps13*Δ strains co-expressing pADHpr-GFP-Neo1 and Vps13 wild-type or mutant plasmids. Scale bar, 2μm. Bottom: quantification of the number of large Neo1 puncta/cell. Error bars report standard error of the mean, >300 cells/trial, n=4.One-Way ANOVA with a Dunnett-corrected post-hoc test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure S2: Identification of mutants that cause Golgi retention of Neo1 A) Plot of Neo1/Sec7 colocalization versus the average number of Neo1 puncta/cell. Indicated mutants were subject to further analysis. B) Quantitation after small-scale re-imaging identifies a subset of mutants with increased Neo1/Sec7 colocalization. Error bars report standard error of the mean, >300 cells/trial, n=3. One-Way ANOVA with a Dunnett-corrected post hoc test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure S3: The Snx3 sorting signal is present in the first 195 amino acids of Neo1. A) Representative live-cell fluorescence images of strains expressing GFP-Neo1-ALP truncation mutants and stained with the vacuolar dye FM4-64. (B) Live-cell fluorescence microscopy shows a second Snx3 sorting signal downstream of amino acid 140 may be active in the GFP-Neo1-ALP chimera. Left: representative images. Scale bar, 2μm. Right: percentage of cells showing GFP-Neo1-ALP at vacuolar rim. Error bars report standard error of the mean, >100 cells/trial, n=3. One-Way ANOVA with a Dunnett-corrected post hoc test comparisons to wild-type (1-195): *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. C) Quantitation of vacuolar localization. Left: Schematic representation of truncation mutants. Right: Percentage of cells showing GFP-Neo1-ALP at the vacuolar rim, >100 cells, n=1.

Figure S4: T-Coffee alignment of the Neo1 N-terminal tail in *Saccharomyces cerevisiae* and other closely related yeasts. Colors reflect the JalviewClustalX color scheme.

Figure S5: TheN-terminal tail of Neo1 is not essential for viability. 10x serial dilutions of strains expressing the wild-type or mutant forms of pGFP-Neo1 under the native promoter, or an empty vector control, were grown on selective medium containing galactose (SG-URA) or dextrose (SD-URA) to repress *GAL1pr-NEO1* expression. Plates were incubated at 30°C or 37°C for 36 hours. All mutant forms of Neo1 supported viability even at high temperatures.

Figure S6: Trifluoperazine sensitivity may reflect changes in lipid asymmetry. 10x serial dilutions of strains expressing the indicated wild-type and mutant forms of pGFP-Neo1 under the native promoter were grown on glucose-containing media (YPD +/- trifluoperazine) to repress expression of *GALpr-NEO1*.

Figure S7: The Neo1 FEM>AAA mutation does not cause missorting of the CPY receptor Vps10. Serial dilutions (1:2) of strains were grown on synthetic medium containing dextrose to repress *GALpr-NEO1* expression in applicable strains. Plates were incubated at 30°C for 16 hours with a nitrocellulose disc overlay, which was probed for the presence of CPY. The numbers above the blot indicate the concentration of yeast in OD_{600}/mL

Table S1
Genome-wide invertase z scores

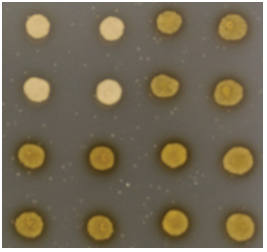
Table S2
Genes identified in the CLIK group for mutants with reduced cell surface GSS levels.

Table S3
High Content Imaging T-statistic raw values.

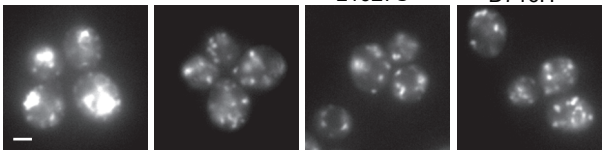
Table S4
Yeast Strains used in this study

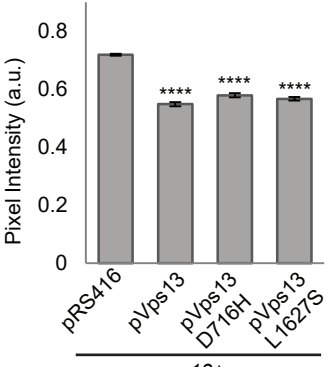
Table S5
Plasmids used in this study.

A


pRS416
pVps13 L1627S
pVps13
pVps13 D716H

B


GFP-Neo1
vps13Δ
pRS416
pVps13
pVps13 L1627S
pVps13 D716H


Pixel Intensity (a.u.)
0.8
0.6
0.4
0.2
0
****
****
****
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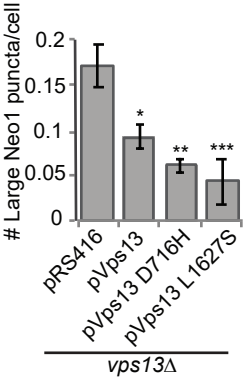
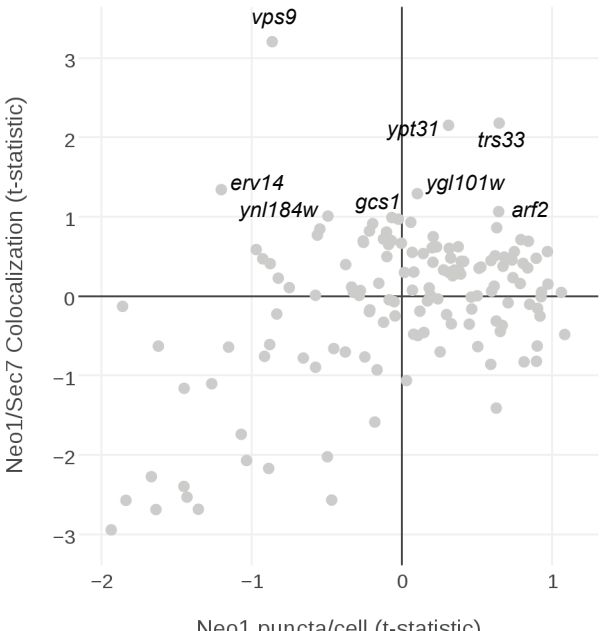
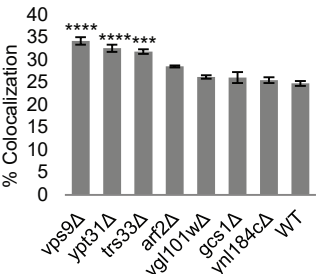
pVps13
pVps13 D716H
pVps13 L1627S
vps13Δ

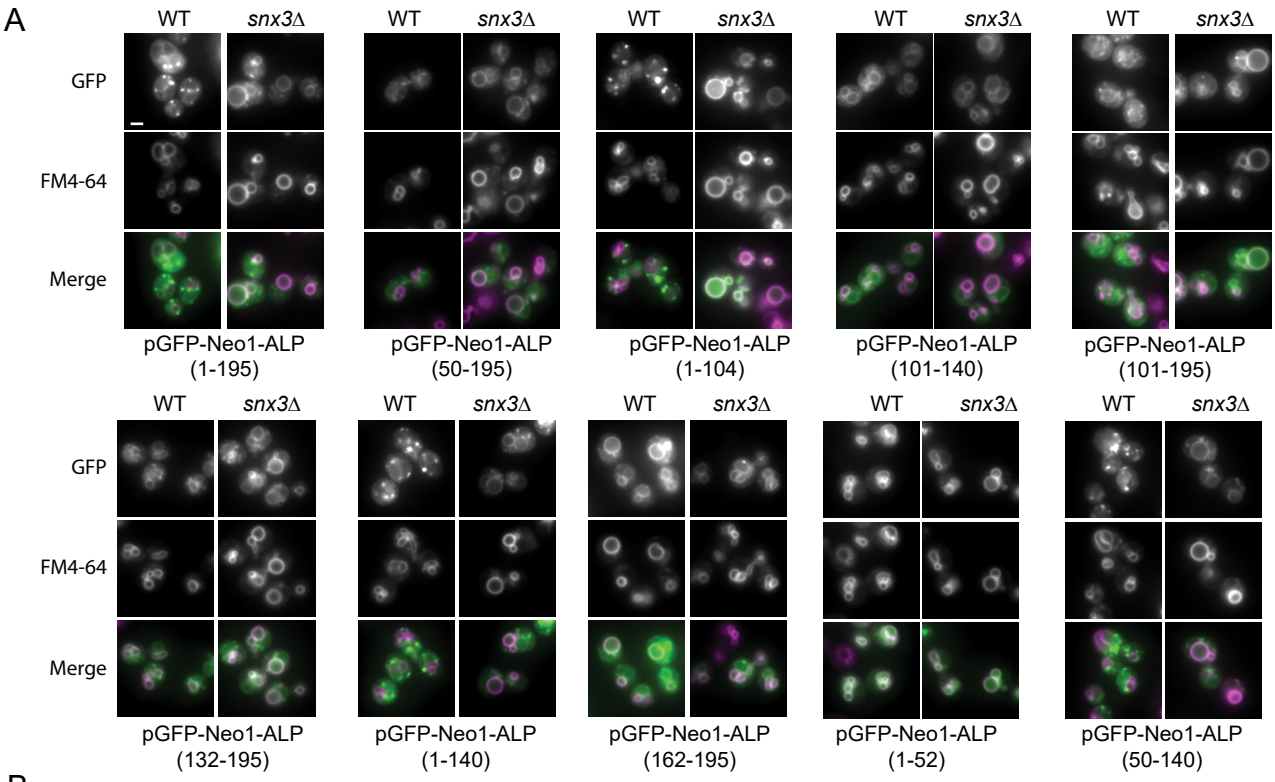

Large Neo1 puncta/cell
0.2
0.15
0.1
0.05
0
*
**
***
pRS416
pVps13
pVps13 D716H
pVps13 L1627S
vps13Δ

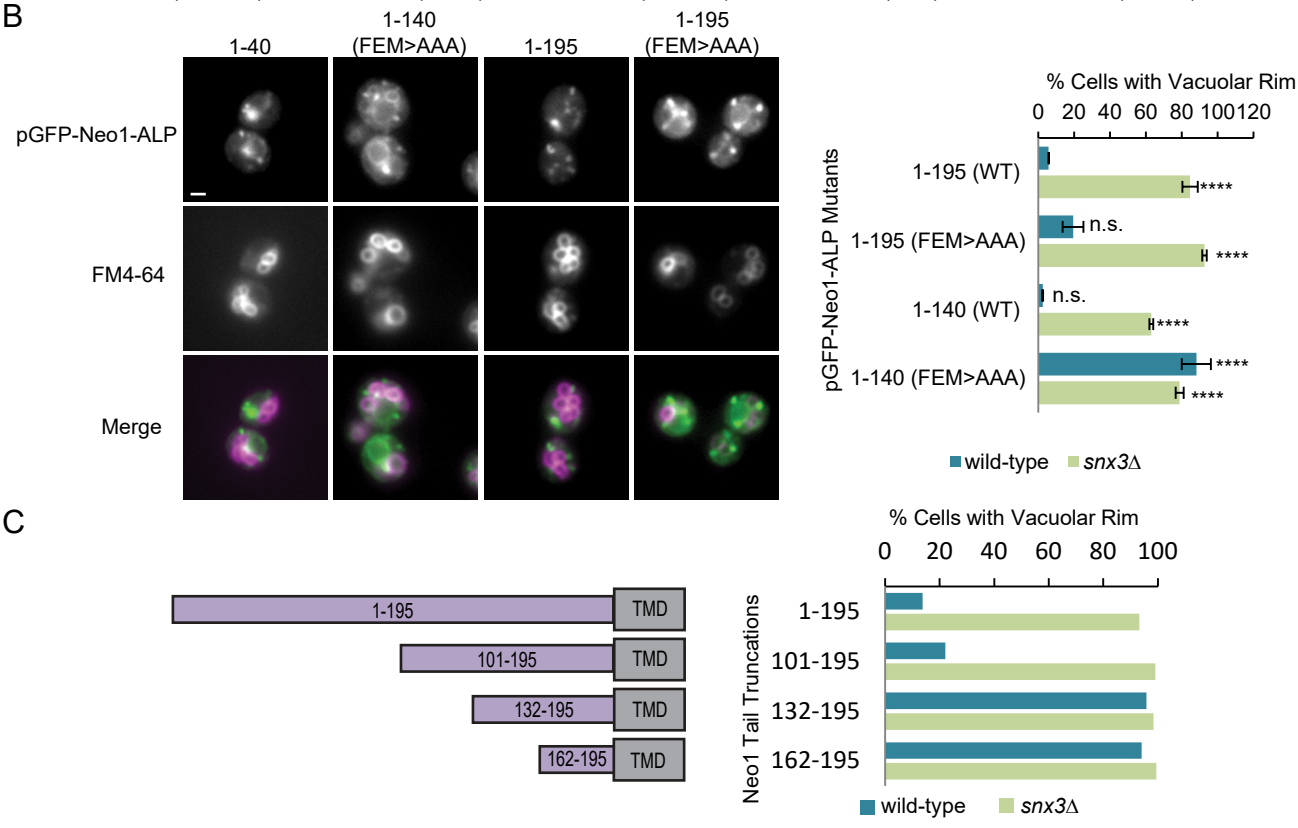
A


vps9
ypt31
trs33
erv14
ygl101w
ynl184w
gcs1
arf2
Neo1/Sec7 Colocalization (t-statistic)
Neo1 puncta/cell (t-statistic)
3
2
1
0
−1
−2
−3
−2
−1
0
1

B


% Colocalization
40
35
30
25
20
15
10
5
0
****
****
***
vps9Δ
ypt31Δ
trs33Δ
arf2Δ
ygl101wΔ
gcs1Δ
ynl184cΔ
WT


A
WT
snx3Δ
GFP
FM4-64
Merge
pGFP-Neo1-ALP (1-195)
pGFP-Neo1-ALP (50-195)
pGFP-Neo1-ALP (1-104)
pGFP-Neo1-ALP (101-140)
pGFP-Neo1-ALP (101-195)
pGFP-Neo1-ALP (132-195)
pGFP-Neo1-ALP (1-140)
pGFP-Neo1-ALP (162-195)
pGFP-Neo1-ALP (1-52)
pGFP-Neo1-ALP (50-140)


B
1-40
1-140 (FEM>AAA)
1-195
1-195 (FEM>AAA)
pGFP-Neo1-ALP
FM4-64
Merge
% Cells with Vacuolar Rim
0 20 40 60 80 100120
pGFP-Neo1-ALP Mutants
1-195 (WT)
1-195 (FEM>AAA)
1-140 (WT)
1-140 (FEM>AAA)
n.s.
****
wild-type
snx3Δ

C
1-195
101-195
132-195
162-195
TMD
% Cells with Vacuolar Rim
0 20 40 60 80 100
Neo1 Tail Truncations
wild-type
snx3Δ

Figure S4

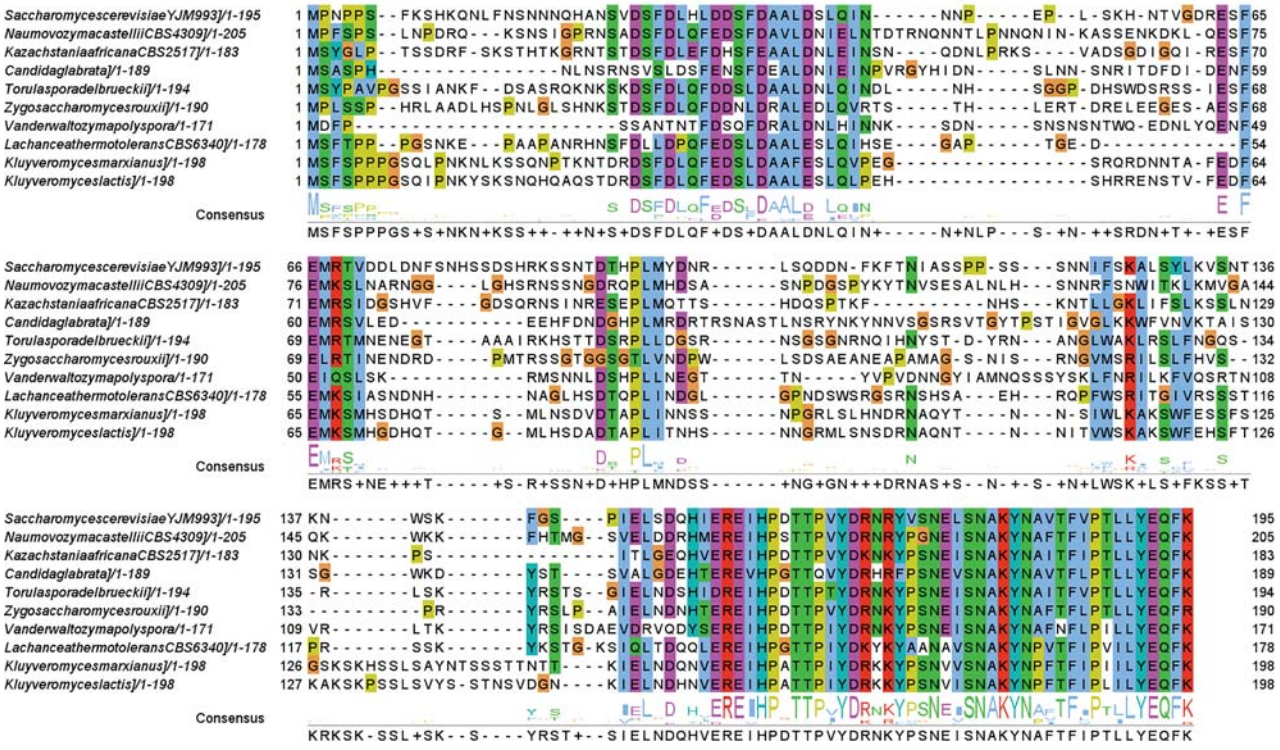

SaccharomycescerevisiaeYJM993]/1-195
NaumovozymacastelliiCBS4309]/1-205
KazachstaniaafricanaCBS2517]/1-183
Candidaglabrata]/1-189
Torulasporadelbrueckii]/1-194
Zygosaccharomycesrouxii]/1-190
Vanderwaltozymapolyspora/1-171
LachanceathermotoleransCBS6340]/1-178
Kluyveromycesmarxianus]/1-198
Kluyveromyceslactis]/1-198
Consensus

Figure S5

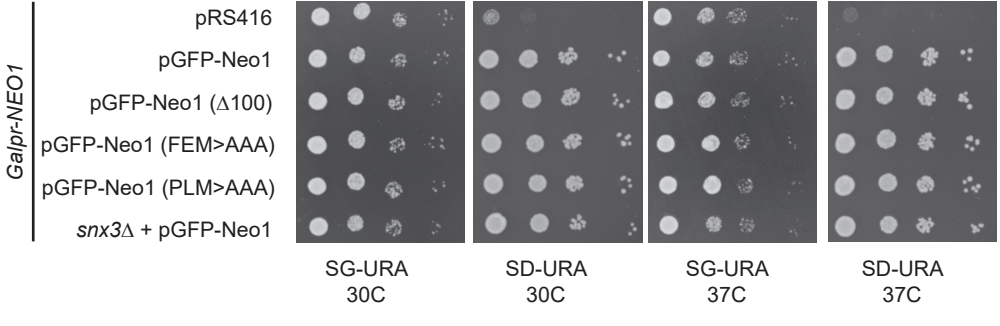

Gal pr-NEO1
pRS416
pGFP-Neo1
pGFP-Neo1 (Δ100)
pGFP-Neo1 (FEM>AAA)
pGFP-Neo1 (PLM>AAA)
snx3Δ + pGFP-Neo1
SG-URA 30C
SD-URA 30C
SG-URA 37C
SD-URA 37C

Figure S6

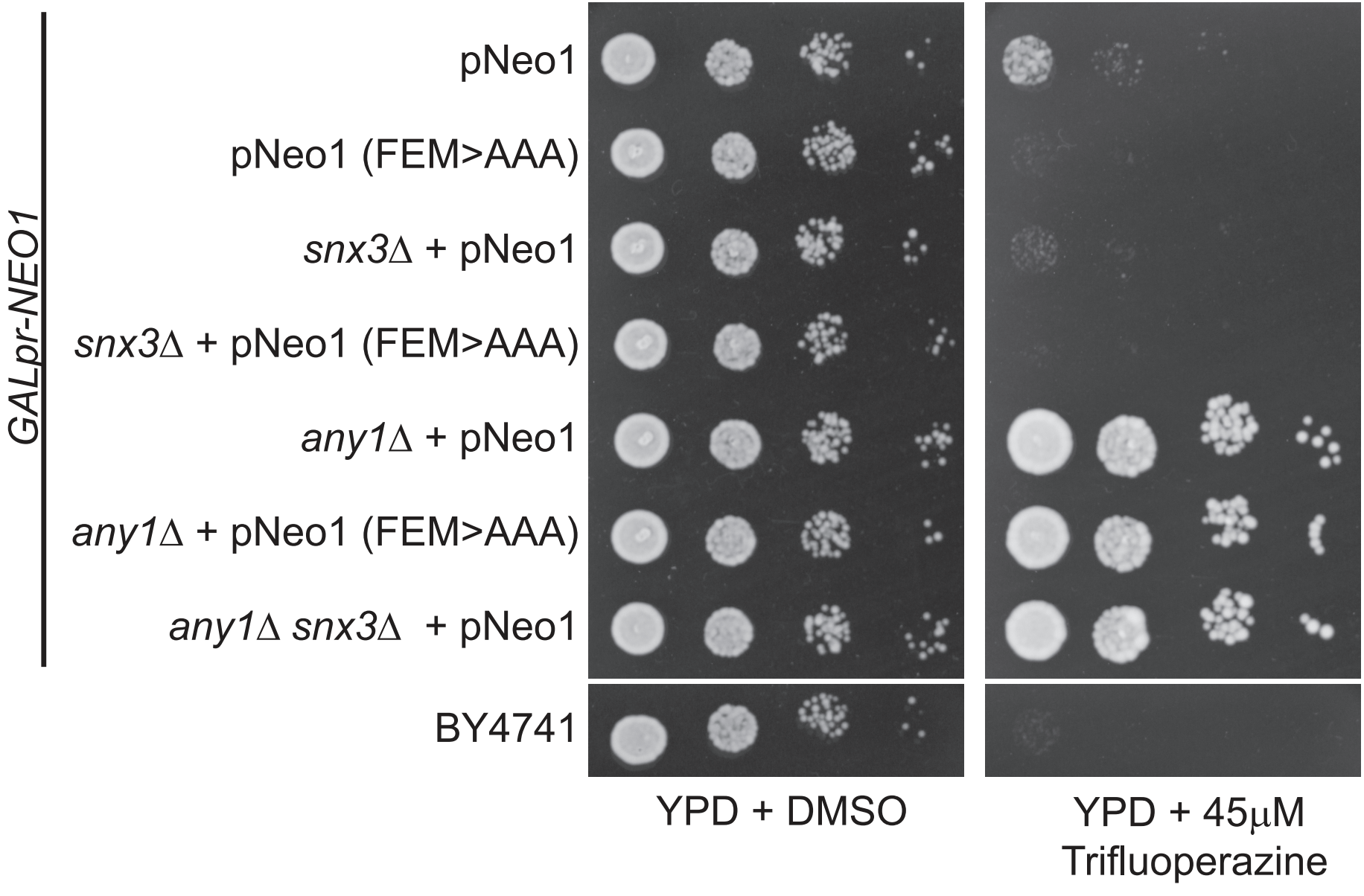
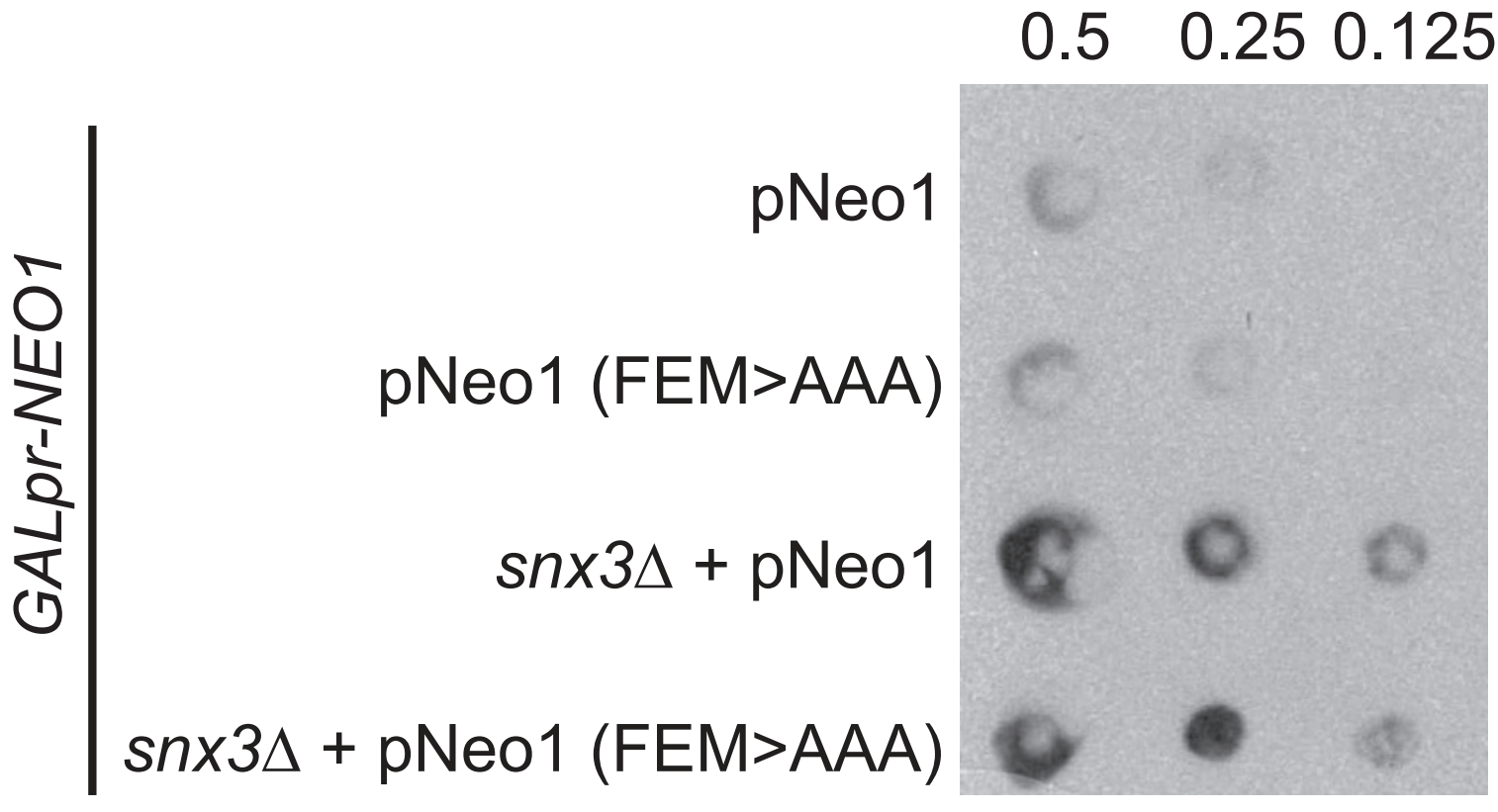

GALpr-NEO1
pNeo1
pNeo1 (FEM>AAA)
snx3Δ + pNeo1
snx3Δ + pNeo1 (FEM>AAA)
any1Δ + pNeo1
any1Δ + pNeo1 (FEM>AAA)
any1Δ snx3Δ + pNeo1
BY4741
YPD + DMSO
YPD + 45μM Trifluoperazine

Figure S7


0.5
0.25
0.125
GALpr-NEO1
pNeo1
pNeo1 (FEM>AAA)
snx3Δ + pNeo1
snx3Δ + pNeo1 (FEM>AAA)