Figure S1.

A. Cells were grown to logarithmic phase at 25 or 30ºC. Cells grown at 25ºC were also shifted to 37ºC for 2 hours. Equal cell numbers were treated to generate spheroplasts, and the cell wall was analyzed by western blot to determine the amount of extracellular Bgl2. Total Bgl2 was determined by solubilising equal number of non-spheroplasted cells in SDS sample buffer.

B. The indicated strains were grown to logarithmic phase at 25 or 30ºC. Cells grown at 25ºC were also shifted to 37ºC for 2 hours. Equal cell number were treated to generate spheroplasts, washed to remove the cell wall and analyzed by western blot to determine the amount of intracellular Bgl2.

Figure S2.

Wild type and *cof1-8* cells grown at 30ºC were fractionated on a continuous 30-60% sucrose gradient. Bgl2 location with respect to ER and Golgi membrane was determined by western blot. ER and Golgi membrane containing fractions were detected by western blot of Kar2 and Mnn9, respectively. Quantitation of protein present in each fraction and relative percentages were determined for three independent experiments. These were analyzed and plotted using GraphPad Prism software. Error bars represent SEM.

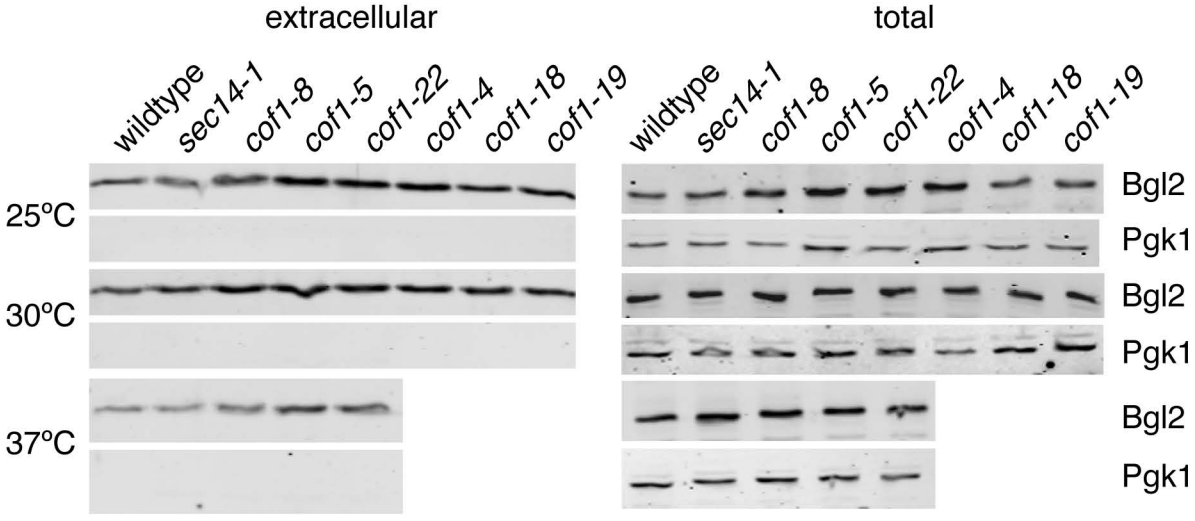
Figure S3.

A. Invertase secretion was determined in the indicated strains by measuring enzymatic activity in external versus total fractions of cells. Percentage of extra-cellular versus total was determined for 8 independent experiments. Error bars represent SEM.

B. Pma1-GFP was expressed exogenously in wild type and *cof1-8* cells. Localization was determined in live cells grown to logarithmic phase at 30ºC. Scale bar = 5µM

Figure S1

A


extracellular
total
wildtype
sec14-1
cof1-8
cof1-5
cof1-22
cof1-4
cof1-18
cof1-19
25°C
30°C
37°C
Bgl2
Pgk1
Bgl2
Pgk1
Bgl2
Pgk1

B

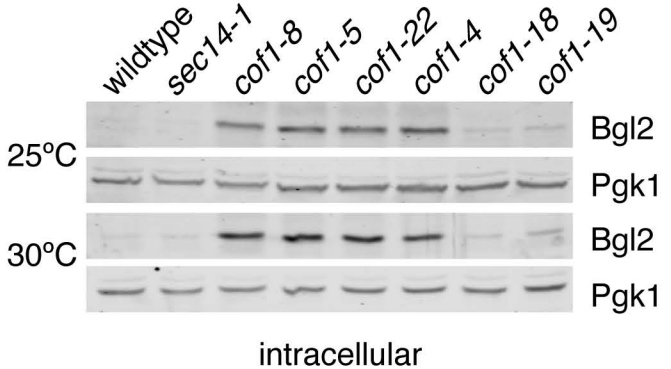
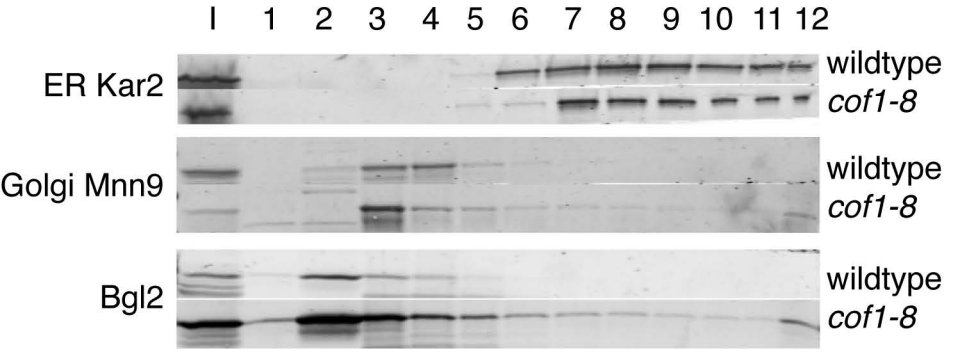

wildtype
sec14-1
cof1-8
cof1-5
cof1-22
cof1-4
cof1-18
cof1-19
25°C
30°C
Bgl2
Pgk1
Bgl2
Pgk1
intracellular

Figure S2


I 1 2 3 4 5 6 7 8 9 10 11 12
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wildtype
cof1-8
Golgi Mnn9
wildtype
cof1-8
Bgl2
wildtype
cof1-8

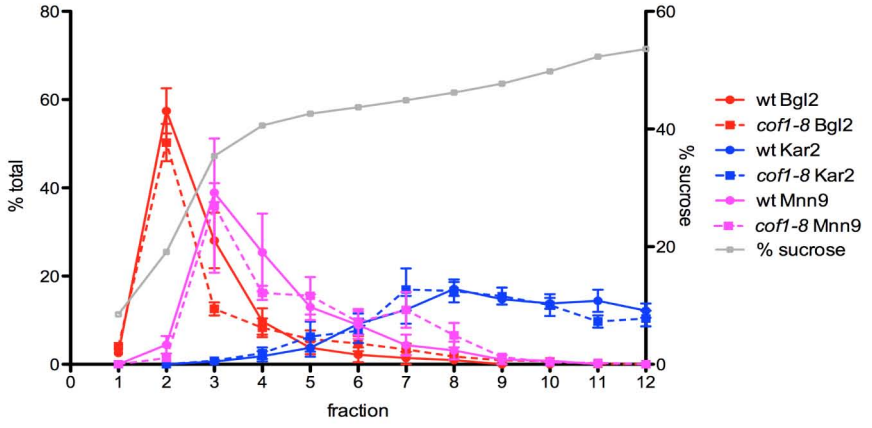
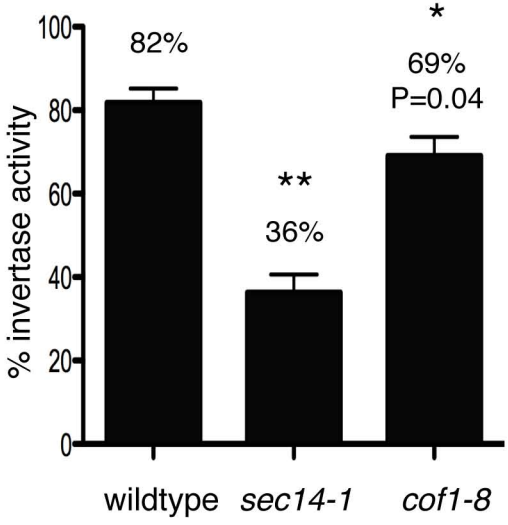
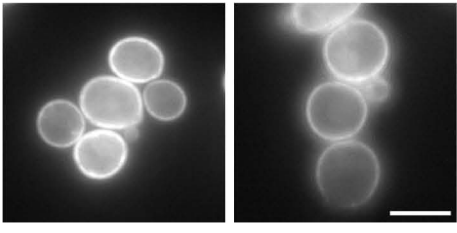

% total
% sucrose
fraction
0
20
40
60
80
0
1
2
3
4
5
6
7
8
9
10
11
12
wt Bgl2
cof1-8 Bgl2
wt Kar2
cof1-8 Kar2
wt Mnn9
cof1-8 Mnn9
% sucrose

Figure S3

A



B



Pma1-GFP