

## 1 Supplemental material for

### 2 Characterization of yeast mutants lacking alkaline ceramidases *YPCI* and *YDCI*

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### 6 Supplemental Materials and Methods

7 **Materials.** FM4-64 was from Molecular Probes, 5'-Fluoroorotic Acid (FOA) from Toronto Research  
8 Chemicals, cycloheximide, phytosphingosine, 2,4-dinitrophenylhydrazine and Lucifer Yellow from  
9 Sigma. [<sup>14</sup>C]serine was from ARC, St. Louis, MO; monomethylamine (33% in ethanol) was from  
10 Fluka AG, Buchs, Switzerland. Anti-DNP antibodies were from Dako. Protease inhibitors were from  
11 Roche Diagnostics GmbH, Mannheim, Germany. 3,3'-dihexyloxycarbocyanine iodide was from  
12 AnaSpec.

13 **Synthetic Genetic Array.** SGA analysis was performed as described previously (Collins et al., 2010).  
14 Briefly, the query strains (Y7092, FBY5162, FBY5173) were robotically crossed against an array of  
15 4978 individual *MATa* knockouts of nonessential genes to generate double or triple mutant arrays. The  
16 resulting double and triple mutants were then screened for genetic interactions affecting cell growth.  
17 When selecting for triple mutants, the plates were further replicated in parallel on plates containing  
18 also Aureobasidin A at a concentration, which did not give visible growth inhibition of WT cells (0.03  
19 µg/ml). They also were replicated onto plates containing 25 µM PHS, or 100 mM Ca<sup>2+</sup>, or onto  
20 inositol free medium at 37°C and at 37°C in normal medium. The measurement of growth and  
21 following analysis and visualization of the high-throughput screen data were conducted with the help  
22 of the ScreenMill software (Dittmar et al., 2010). Additionally, the interactions pointed by the screen  
23 were verified by independent crosses, tetrad dissection or random sporulation and by serial dilution  
24 plating to assess colony sizes, cloning efficiency and growth rates.

25 **Metabolic labeling of cells with [<sup>14</sup>C]serine, lipid extraction, mild base treatment and thin-layer**  
26 **chromatography.** Cells were grown in synthetic minimal medium. 3.0 OD<sub>600</sub> units of exponentially  
27 growing cells (i.e. 3 ml of a culture having an OD<sub>600</sub> of 1.0) were harvested, resuspended in 250 µl of  
28 the same medium supplemented with 10 µg/ml of cycloheximide (CHX). After 10 min of  
29 preincubation, 4 µCi of [<sup>14</sup>C]serine were added and cells were incubated for 40 min at 30°C. Then the  
30 samples were diluted with 750 µl of fresh minimal medium supplemented with CHX and labeling was  
31 continued for a further 120 min. Labeling was terminated by adding NaN<sub>3</sub> and NaF (10 mM final  
32 concentrations) and chilling cells on ice. Cells were resuspended in chloroform:methanol (2:1) and  
33 broken with glass beads in the cold. The extract was kept apart and the pellet was re-extracted  
34 sequentially with chloroform:methanol (1:1) and EtOH:H<sub>2</sub>O:Et<sub>2</sub>O:Pyridin: 25% NH<sub>4</sub>OH  
35 (15:15:5:1:0.018), which achieves quantitative extraction of all complex sphingolipids (Hanson and

36 Lester, 1980). Extracts were combined and solvent was evaporated under vacuum in a rotary  
37 evaporator. Incorporation into lipids usually amounted to 5 % of added radioactivity. Where indicated,  
38 lipids were subjected to mild base hydrolysis with mono-methylamine (MMA). Lipids were  
39 resuspended in 400  $\mu$ l of MMA (33% in ethanol) or, as a negative control, in methanol, and incubated  
40 at 53°C for 1 hour. Then, solvents were evaporated under vacuum. All lipids were resolved by  
41 ascending TLC on silica gel plates after having been desalted by Folch partitioning as described (Folch  
42 et al., 1957). Extracts from metabolically labeled cells were resolved with chloroform/methanol/glacial  
43 AcOH (90:1:9) or CHCl<sub>3</sub>:MeOH:KCl (55:45:5) solvent systems. When the untreated and deacylated  
44 lipid extract was run side by side, material from an equivalent number of cells was spotted.  
45 Radioactivity was detected and quantified by one- and two-dimensional radioscanning using a  
46 Berthold radioscanner and visualized by fluorography or radioimaging using the Bio-Rad Molecular  
47 Imager FX.

48 **Isolation of detergent resistant membranes and Triton X-100 solubilization assay.** For the  
49 isolation of detergent resistant membranes, published protocols were used (Bagnat et al., 2000;  
50 Malinska et al., 2004). Crude membranes corresponding to 200  $\mu$ g protein were incubated in 300  $\mu$ l  
51 cold TNE buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA) containing protease  
52 inhibitors (Roche Diagnostics GmbH, Mannheim, Germany) and 1% Triton X-100 for 30 min on ice.  
53 Subsequently, the samples were overlaid with an Optiprep (Nycomed) step gradient and centrifuged  
54 for 3 h at 208,000  $\times$  g in a Beckman SW60 rotor at 4°C. After centrifugation, six equal fractions were  
55 collected, and the proteins were immunodetected on Western blots.

56 **FM4-64 staining to monitor endocytosis.** FM4-64 was used to stain vacuoles and endosomes as  
57 described previously (Baggett et al., 2003). Yeast cells were cultured at 24°C. Five separate aliquots of  
58 1 ml each were centrifuged and cooled on ice. Each pellet was resuspended in 50  $\mu$ l of ice cold FM4-  
59 64 (20  $\mu$ g/ml). Tubes were incubated for 20 min in an ice-water bath to allow the dye to label the  
60 plasma membrane. For the time point zero, 1 ml ice-cold rich medium without any carbon source was  
61 added, cells were centrifuged 3 min at 300  $\times$  g, 4°C and washing was repeated. The pellet was  
62 resuspended in 50  $\mu$ l of rich medium with no carbon source and kept on ice from this point onward.  
63 Remaining tubes were washed two times with cold rich medium containing a carbon source and finally  
64 resuspended in 1 ml of the same. These tubes were placed into a water bath at 26°C with shaking.  
65 Tubes were removed after 5, 10, 20, and 45 min. When tubes were removed, the cells were washed  
66 twice with ice-cold rich medium without a carbon source and resuspended in 50  $\mu$ l of rich medium  
67 with no carbon source and kept on ice. For fluorescent visualization, cells were mounted onto  
68 Concanavalin A (ConA)-coated cover slips and observed with a rhodamine/TRITC filter.

69 **Lucifer yellow (LY) accumulation in the vacuole.** Fluid-phase endocytosis was assayed using the  
70 dye LY as described previously (Baggett et al., 2003). Yeast cells were cultured in YPD medium  
71 overnight to an OD<sub>600</sub> of  $\sim$  0.1 at 30°C. One ml aliquots of cell suspension were sedimented by  
72 centrifuging 2 min at 800  $\times$  g at room temperature. The cell pellet was resuspended in 90  $\mu$ l of YPD

73 and then 10  $\mu$ l of 40 mg/ml of LY was added. Holes were pierced through the top of the tubes to allow  
74 aeration of the cells during the LY uptake step. Tubes were incubated at 24°C for 1.5 hrs in the dark.  
75 Next, 1 ml of ice-cold phosphate buffer with 10 mM of NaN<sub>3</sub> and NaF was added and the tubes were  
76 centrifuged. Washing was repeated three times, resuspending the pellet between washes. Cells were  
77 mounted on ConA-coated cover slips and viewed by fluorescence microscopy using a FITC filter.

78 **CPY secretion assay.** Cells were grown overnight to OD<sub>600</sub> 1-2. 10 OD<sub>600</sub> units were collected,  
79 washed and resuspended in 1 ml of water. Tenfold dilutions of the various strains were deposited onto  
80 YPD plates, incubated for 3 days at 30°C and the next day overlaid with nitrocellulose. After 12 h of  
81 incubation at 30°C, the nitrocellulose filter was washed with water and processed for Western blotting  
82 using anti-CPY antibodies.

83 **Protein carbonylation assay.** The level of protein carbonylation was assessed as described before  
84 (Dirmeier et al., 2002).

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## 86 **Supplemental Figure legends**

87 **Fig. S1. The localization of mtGFP, Vph1p, Sec63p, Sec7p and Sed5p is normal in yy $\Delta$  cells.**

88 WT and yy $\Delta$  cells expressing either mtGFP, *VPH1*-GFP, *SEC63*-GFP, *SEC7*-DsRed or GFP-*SED5*  
89 from single copy vectors were grown to exponential phase at 30°C, using galactose as a carbon source  
90 for the mtGFP and Vph1p expression. mtGFP contains GFP fused to the first 69 amino acids of the  
91 subunit 9 of the F<sub>0</sub> ATPase from *Neurospora crassa*, under control of the *GALI* promoter  
92 (Westermann and Neupert, 2000).

93 **Fig. S2. yy $\Delta$  cells show normal kinetics of endocytosis. a,** WT and yy $\Delta$  cells were grown to early  
94 log phase on YPD medium at 24°C. Cells were incubated with FM4-64 (20  $\mu$ g/ml final concentration)  
95 in an ice bath for 20 min, washed and then further incubated at 26°C. After 0, 5, 10, 20 and 45 min  
96 cells were visualized under the fluorescence microscope. **b,** exponentially growing cells were  
97 incubated for 1.5 h at 24°C in rich YPD medium containing lucifer yellow (LY, 4 mg/ml), washed and  
98 viewed under the fluorescent microscope.

99 **Fig. S3. CPY and Gas1p are targeted normally in yy $\Delta$ .** **a,** tenfold dilutions of the various strains  
100 were deposited onto YPD plates, incubated for 3 days at 30°C and overlaid with nitrocellulose. The  
101 nitrocellulose filter was processed for Western blotting using anti-CPY antibodies. *vps4 $\Delta$*  cells are  
102 deficient in vacuolar targeting and serve as positive control. **b,** cell membranes of WT and yy $\Delta$  cells  
103 containing either *CANI*-GFP, *GAS1*-GFP, or *SEC63*-GFP were incubated with 1% of Triton X-100 on  
104 ice for 30 min and then loaded at the bottom of a step-density Optiprep gradient (Bagnat et al., 2000).  
105 After centrifugation, six fractions were collected and analyzed in a Western blot for the presence of the  
106 GFP-marked proteins, the *SEC63*-GFP serving as a detergent sensitive control. Fractions 1-2 contain  
107 the detergent resistant membranes floating on top of the gradient, fractions 4 – 6 the soluble proteins

108 not associated with detergent resistant membrane domains.

109 **Fig. S4. Serine incorporation into lipids in *yyΔΔ* cells is qualitatively normal.** **a**, WT and *yyΔΔ*  
110 cells were cultured with or without 3 μg/ml of AbA for 1 h. Then the cells were labeled with  
111 [<sup>14</sup>C]serine for 160 min at 30°C in the same medium as used for preincubation. The extracted lipids  
112 were deacylated or not with MMA, therewith leaving sphingolipids intact but hydrolyzing labeled  
113 glycerophospholipids. Lipids were resolved by TLC in chloroform:methanol:glacial AcOH (90:1:9). **b**,  
114 the same as in A but the lipids were resolved in CHCl<sub>3</sub>:MeOH:0.25% KCl in H<sub>2</sub>O (55:45:5), with IPC-  
115 C, IPC-D and MIPC highlighted with a red asterisk. Cers = ceramides.

116 **Fig. S5. a, hydroxylation or desaturation of fatty acids decreases their affinity for Ypc1p.**  
117 Reverse ceramidase activity of microsomal detergent extracts of 1Δ.YPC1 cells were assayed in  
118 presence of various concentrations of unlabeled fatty acids (0 – 300 nmol) as described in Fig. 5C. **b**,  
119 **long chain base specificity of Ypc1p-dependent microsomal reverse ceramidase activity.** The  
120 Ypc1p-dependent ceramide synthase activity was assayed under standard conditions but replacing  
121 PHS by LCBs that are not normally present in yeast cells. The amounts of ceramide-[<sup>3</sup>H]C16 are  
122 indicated as a percentage of the amounts obtained in the standard assay (5 nmol PHS). Result of a test  
123 done in duplicate is indicated.

124 **Fig. S6. Growth of *ypc1Δ*, *ycd1Δ* and *yyΔΔ* cells on non-fermentable carbon sources.** *Ypc1Δ*,  
125 *ycd1Δ*, *yyΔΔ* cells and their isogenic WT were grown to exponential phase, collected and resuspended  
126 at OD<sub>600</sub> of 1.0 in media with different carbon sources, such as dextrose (2%, YPD), ethanol (3%,  
127 YPEthanol), glycerol (2%, YPGlycerol) and lactate (2%, YPLactate). Cell density was measured at  
128 indicated times by measuring OD<sub>600</sub>.

129 **Fig. S7. Protein carbonylation in the presence of H<sub>2</sub>O<sub>2</sub>.** *yyΔΔ* and WT strains were grown to  
130 exponential phase in YPD medium, cultures were supplemented with 1 mM H<sub>2</sub>O<sub>2</sub> and further grown  
131 for 24 hours at 30°C. After culturing, cells had reached densities of OD<sub>600</sub> ≈ 10 and were harvested,  
132 spheroplasts were prepared and lysed in hypotonic medium. ER-derived microsomes and  
133 mitochondrial membranes were isolated by sedimentation at 12,100 × g, remaining cellular  
134 membranes by subsequent centrifugation at 100'000 × g yielding also the cytosolic supernatant  
135 fraction. 5 μg of the proteins from each fraction were derivatized with 2,4-dinitrophenylhydrazine. The  
136 derivatized proteins were separated by SDS-PAGE and probed with anti-DNP antibodies on a Western  
137 blot.

138 **Fig. S8. Chronological life span of *ypc1Δ* and *ycd1Δ* cells.** *Ypc1Δ::kanMX* and *ycd1Δ::kanMX*  
139 deletions in the BY background (EUROSCARF collection) were grown to stationary phase (OD<sub>600</sub> ≈  
140 15) in YPD and then transferred to sterile water (day 0). Cells were kept at 25°C without shaking and  
141 CFUs were determined by plating cells at the indicated days onto YPD onto 4 plates at different  
142 dilutions. Viability is given as percent of colonies counted at day 0 (=100%), which was > 400 CFUs  
143 for all strains. (Due to caloric restriction and to the possibility to feed on dying cells, the CFUs drop

144 relatively slowly.)

145 **Fig. S9. Localization of Ypc1p-GFP in exponentially growing and stationary cells.**

146 FY1679.YPC1-GFP cells were viewed after having been grown at 30°C in complete synthetic medium  
147 to late log phase viewed ( $OD_{600} = 0.9$ ) and stationary phase ( $OD_{600}$  of 10). Cells were analyzed using a  
148 Delta vision Deconvolution microscope (Applied Precision, Issaquah, WA) with 100x oil objective  
149 and individual Z stacks are shown. (This microscope is different from the one used for Fig. 6B).  
150 Diffuse cytosolic fluorescence comes from other Z stacks and is due to amplification. Comparison  
151 with Nomarski pictures shows that vacuoles are spared. The white bar represents 6.4  $\mu\text{m}$ . Two  
152 consecutive Z stacks of the same cells are shown in the red box.

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Strains	Genotype	Reference
WT (BY4742)	<i>MAT<math>\alpha</math> his3<math>\Delta</math>1 leu2<math>\Delta</math>0 lys2<math>\Delta</math>0 ura3<math>\Delta</math>0</i>	EUROSCARF, (Frankfurt, GE)
yy $\Delta$ (FBY2182)	BY4742, but <i>ypc1<math>\Delta</math>::kanMX ydc1<math>\Delta</math>::kanMX<math>\Delta</math>::natMX4</i>	This study
ypc1 $\Delta$	BY4742, but <i>ypc1<math>\Delta</math>::kanMX</i>	EUROSCARF
ydc1 $\Delta$	BY4742, but <i>ydc1<math>\Delta</math>::kanMX</i>	EUROSCARF
Y7092	<i>MAT<math>\alpha</math> can1<math>\Delta</math>::STE2pr-Sp_his5 lyp1<math>\Delta</math> his3<math>\Delta</math>1 leu2<math>\Delta</math>0 ura3<math>\Delta</math>0 met15<math>\Delta</math>0</i>	C. Boone
ypc1 $\Delta$ (FBY5162)	Y7092, but <i>ypc1<math>\Delta</math>::LEU2</i>	This study
yy $\Delta$ (FBY5173)	Y7092, but <i>ypc1<math>\Delta</math>::LEU2 ydc1<math>\Delta</math>::natMX4</i>	This study
WT.URA3 (FBY5319)	<i>MAT<math>\alpha</math> can1<math>\Delta</math>::STE2pr-Sp_his5 lyp1<math>\Delta</math> his3<math>\Delta</math>1 leu2<math>\Delta</math>0::loxP-LEU2-loxP ura3<math>\Delta</math>0 met15<math>\Delta</math>0</i> containing pNP302	This study
WT.YPC1 (FBY5320)	<i>MAT<math>\alpha</math> can1<math>\Delta</math>::STE2pr-Sp_his5 lyp1<math>\Delta</math> his3<math>\Delta</math>1 leu2<math>\Delta</math>0::loxP-LEU2-loxP ura3<math>\Delta</math>0 met15<math>\Delta</math>0</i>	This study
yy $\Delta$ .GAL-YPC1 (FBY5179)	As FBY2182, but containing pBF842	This study
yy $\Delta$ .GAL-HIS3 (FBY5181)	As FBY2182, but containing pBF841	This study
yy $\Delta$ .YPC1 (FBY5182)	As FBY2182, but containing pYPC1-URA3	This study
yy $\Delta$ .URA3 (FBY5180)	As FBY2182, but containing pRS316	This study
WT.Fus-Mid (FBY5280)	As BY4742, but containing pTPQ55	This study
yy $\Delta$ .Fus-Mid (FBY5290)	As FBY2182, but containing pTPQ55	This study
FY1679.YPC1-GFP	<i>MAT<math>\alpha</math>/<math>\alpha</math> ura3-52/ura3-52 trp1<math>\Delta</math>63/TRP1 leu2<math>\Delta</math>1/LEU2 his3<math>\Delta</math>200/HIS3</i> containing pYPC1-GFP	(Natter et al., 2005)
yy $\Delta$ .CAN1-GFP (FBY5171)	As FBY2182, but containing pCAN1-GFP	This study
WT.CAN1-GFP (FBY5172)	As BY4742, but containing pCAN1-GFP	This study
yy $\Delta$ .GAS1-GFP (FBY5187)	As FBY2182, but containing Yep24-GAS1.GFP	This study
WT.GAS1-GFP (FBY5186)	As BY4742, but containing Yep24-GAS1.GFP	This study
yy $\Delta$ .FUR4-GFP (FBY5197)	As FBY2182, but containing YCplac33-FUR4.GFP	This study
WT.FUR4-GFP (FBY5204)	As BY4742, but containing YCplac33-FUR4.GFP	This study
yy $\Delta$ .PMA1-GFP (FBY5274)	As FBY2182, but containing pPMA1-GFP	This study
WT.PMA1-GFP (FBY5273)	As BY4742, but containing pPMA1-GFP	This study
yy $\Delta$ .SEC63-GFP (FBY5165)	As FBY2182, but containing pSEC63-GFP	This study
WT.SEC63-GFP (FBY5166)	As BY4742, but containing pSEC63-GFP	This study
ypc1 $\Delta$ vps4 $\Delta$ (FBY5293)	<i>MAT<math>\alpha</math> can1<math>\Delta</math>::STE2pr-Sp_his5 lyp1<math>\Delta</math> his3<math>\Delta</math>1 leu2<math>\Delta</math>0 ura3<math>\Delta</math>0 met15<math>\Delta</math>0 ypc1<math>\Delta</math>::LEU2 vps4<math>\Delta</math>::kanMX</i>	This study
vps4 $\Delta$	<i>MAT<math>\alpha</math> his3<math>\Delta</math>1, leu2<math>\Delta</math>0, met15<math>\Delta</math>0, ura3<math>\Delta</math>0 vps4<math>\Delta</math>::kanMX</i>	EUROSCARF
yy $\Delta$ .PHM5-GFP	As FBY2182, but containing pPHM5416	This study

WT.PHM5-GFP	As BY4742, but containing pPHM5416	This study
yyΔΔ.SNC1-GFP	As FBY2182, but containing pGS416-SNC1	This study
WT.SNC1-GFP	As BY4742, but containing pGS416-SNC1-GFP	This study
yyΔΔ.SSO1-GFP	As FBY2182, but containing pSSO1416-GFP	This study
WT.SSO1-GFP	As BY4742, but containing pSSO1416-GFP	This study
yyΔΔ.STE2-GFP	As FBY2182, but containing pSTE2416	This study
WT.STE2-GFP	As BY4742, but containing pSTE2416	This study
yyΔΔ.mtGFP (FBY5184)	As FBY2182, but containing pYES-mtGFP	This study
WT.mtGFP (FBY5183)	As BY4742, but containing pYES-mtGFP	This study
yyΔΔ.VPH1-GFP (FBY5169)	As FBY2182, but containing pVPH1-GFP	This study
WT.VPH1-GFP (FBY5170)	As BY4742, but containing pVPH1-GFP	This study
yyΔΔ.SEC7-RFP (FBY5193)	As FBY2182, but containing pTQ128	This study
WT.SEC7-RFP (FBY5195)	As BY4742, but containing pTQ128	This study
yyΔΔ.SED5-GFP (FBY5201)	As FBY2182, but containing pSED5-GFP	This study
WT.SED5-GFP (FBY5202)	As BY4742, but containing pSED5-GFP	This study
YPK9	<i>MATa ade2-101<sup>ochre</sup> his3-Δ200 leu2-Δ1 lys2-801<sup>amber</sup> trp1-Δ63 ura3-52</i>	(Jiang et al., 1998)
1Δ.YPC1	YPK9 <i>lag1Δ::TRP1</i> containing pPK183	This study
2Δ.YPC1	YPK9 <i>lag1Δ::TRP1 lac1Δ::URA3</i> containing pPK183	(Jiang et al., 2004)
FBY7478	<i>MAT a/α his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, lys2Δ0/LYS2, ura3Δ0/ura3Δ0, met15Δ0/met15Δ0, LYP1/lyp1Δ, CAN1/can1Δ::STE2pr-Sp_his5</i>	
FBY7479	<i>MAT a/α his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, lys2Δ0/LYS2, ura3Δ0/ura3Δ0, met15Δ0/met15Δ0, LYP1/lyp1Δ, CAN1/can1Δ::STE2pr-Sp_his5 ypc1::kanMX4/YPC1 ydc1::kanMX4::natMX/YDC1</i>	This study
FBY7480	<i>MAT a/α his3Δ1/his3Δ1, leu2Δ0/LEU2, lys2Δ0/LYS2, ura3Δ0/ura3Δ0, met15Δ0/met15Δ0, LYP1/lyp1Δ, CAN1/can1Δ::STE2pr-Sp_his5, ypc1::kanMX4/ypc1::kanMX::ura3::LEU2, ydc1::kanMX4::natMX/ydc1::natMX</i>	This study

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161 **Table S2. Plasmids.**

pNP302	CEN ARS <i>URA3</i> , <i>ADH1</i> promoter	C. De Virgilio
pYPC1-URA3	<i>YPC1</i> in pNP302	This study
pBF841	2 $\mu$ <i>HIS3</i> , <i>GAL</i> promoter	N. Ramachandra
pBF842	<i>YPC1</i> in pBF841	N. Ramachandra
pSTE2416	<i>STE2</i> -GFP in pRS416 CEN <i>URA3</i> , <i>TPII</i> promoter	F. Reggiori
pPHM5416	GFP- <i>PHM5</i> in pRS416 CEN <i>URA3</i> , <i>TPII</i> promoter	(Reggiori and Pelham 2001)
pSSO1416	GFP- <i>SSO1</i> in pRS416 CEN <i>URA3</i> , <i>TPII</i> promoter	F. Reggiori
pGS416-SNC1	GFP- <i>SNC1</i> in pRS416 CEN <i>URA3</i> , <i>TPII</i> promoter	(Lewis et al., 2000) H. Pelham
pSED5-GFP	GFP- <i>SED5</i> CEN <i>URA3</i> <a href="http://www2.brc.riken.jp/cache/dna/8658">http://www2.brc.riken.jp/cache/dna/8658</a>	A. Nakano
pTQ128	<i>SEC7</i> -DsRed in CEN <i>LEU2</i> , <i>ADH1</i> promoter	K. Simons
pYES-mtGFP	mtGFP in 2 $\mu$ <i>URA3</i> , <i>GAL</i> promoter	(Westermann and Neupert, 2000)
pVPH1-GFP	<i>VPH1</i> -GFP CEN <i>URA3</i>	R. Schneiter
pSEC63-GFP	<i>SEC63</i> -GFP in 2 $\mu$ <i>URA3</i>	R. Schneiter
pCAN1-GFP	<i>CAN1</i> -GFP in 2 $\mu$ <i>URA3</i> , <i>ADH1</i> promoter	W. Tanner
pFUR4-GFP	YCplac33- <i>FUR4</i> -GFP CEN <i>URA3</i> , endogenous promoter	W. Tanner
pPMA1-GFP	<i>PMA1</i> -GFP CEN <i>URA3</i> , endogenous promoter	R. Schneiter
YE <sub>p</sub> 24-GAS1.GFP	<i>GAS1</i> -GFP in 2 $\mu$ <i>URA3</i> , endogenous promoter	L. Popolo
pTPQ55	Fus-Mid-GFP in CEN <i>URA3</i> , <i>GAL</i> promoter	K. Simons
pYPC1-GFP	<i>YPC1</i> -GFP in pRS416 <i>URA3</i> <i>TEF1</i> promoter	(Natter et al., 2005)
pPK183	<i>YPC1</i> in 2 $\mu$ with endogenous promoter <i>LEU2</i>	(Jiang et al., 2004)

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