Supplemental Material

Supplemental Tables

Table S1: Primers used in this study

Primer	Sequence
PCT1-LEU2-1	5'GCATACGCCAGTCACTTTTCTTTATTGTATTGTTTAACTTCGTAAGATGCAAGAGTTCGA3' [1]
PCT1-LEU2-2	5'GGTTGGTTGAAGGGAGAGAGAAAGAGACGCACAGAGATTCCCTCCTCCTTGTCAATATTA3' [1]
SCT1-HH-1	5'GAAGAAGAGGAAGAAGAAGAAGAAGGGAAAGAAGGAGATGCGTCCCACCACCATCATCATCAC3' [1]
SCT1-HH -2	5'GATTTAGTTTCTAGTATCTTCACCGTCAGTAAATGGCAT ACTATAGGGAGACCGGCAGATC3' [1]
pYES-SCT1-1	5'GCGTCTCGGATCCATGCCTGCACCAAAACTCACGG3' [2]
pYES-SCT1-2	5'GCGTCTCCTCGAGTCGACTCATTAAGCGTAGTCTG3' [2]
pESC-OLE1-1	5'GGGCCCATGCCAACTTCTGGAAC3' [2]
pESC-OLE1-2	5'GGGCCCACAAAGAACTTACCAGTTTCG3' [2]
SCT1-G253L-1	5'GGGATCTTTCCTGAA***CT***TGG***A***TCCCACGACAGAAC3' [3]
SCT1-G253L-2	5'GTTCTGTCGTGGGA***T***CCA***AG***TTCAGGAAAGATCCC3' [3]

(1) Underlined sequences correspond to nucleotides upstream and downstream (reverse complementary) of the *PCT1* and *SCT1* gene.

(2) Underlined sequences correspond to beginning and ending (reverse complementary) sequences of the *SCT1*-*HISHA* and the *OLE1* gene.

(3) Bases in bold italics correspond to changes in the sequence to introduce a point mutation and a BamHI site.

Supplemental Figure Legends

Figure S1. PE molecular species profile of *pct1* and *sct1pct1* cells. The PE species profiles of *pct1* and *sct1pct1* cells grown to mid-log phase in SSL, were analyzed by ESI-MS/MS in neutral loss scans for 141 atomic mass units in the positive ion mode. The relative signal intensity is shown for species that contribute at least 1% of total PE and 36:1 PE (to facilitate comparison with the PC species profiles in Figure 1). Error bars represent the SD (*pct1*: n=10; *sct1pct1*: n=3).

Figure S2. The effect of Sct1p overexpression on phospholipid and neutral lipid composition (A, B), and the effect of deleting *SCT1* on the synthesis of neutral lipids (C). *sct1pct1* pYES2 and pYES2-*SCT1* cells were grown to mid-log phase in SGR. Lipids were extracted and analyzed by TLC. (A) The relative abundance of glycerophospholipids as % of total phospholipid phosphorus was determined by phosphorus determination; the error bars represent the variation (n=2). (B) TLC plate showing the neutral lipid composition of *sct1pct1* cells overexpressing Sct1p *vs.* the empty vector control; Ori, origin; PL, phospholipid; ST, sterol; DAG, diacylglycerol; FA, fatty acid; STE, steryl ester. (C) *pct1* and *sct1pct1* cells were grown to midlog phase in SL, incubated with [^{14}C]-acetate for 1 h and subjected to lipid extraction. Lipid extracts were analyzed by TLC, and lipids quantified by phosphor imaging as detailed in Materials and methods. The incorporation of [^{14}C]-acetate in neutral lipids is shown as percentage of the total incorporation in the respective pools.

Figure S3. The effect of Sct1p overexpression on the molecular species profiles of the four major glycerophospholipids. Total lipid extracts of *sct1pct1* pYES2 and pYES2-*SCT1* cells grown to mid-log phase in SGR, were analyzed by ESI-MS/MS for PC (parent ion scans for *m/z* 184 in the positive ion mode), PS (neutral loss scans for 87 atomic mass units in the negative ion mode), PE (neutral loss scans for 141 atomic mass units), and PI (parent ion scans for *m/z* 241 in the negative ion mode). (A) ESI-

MS/MS spectra of PC with the highest peak set at 100%. (B) Molecular species composition of the phospholipid classes indicated, expressed as relative signal intensity (percentage of the total signal intensity), which corresponds to the relative abundance of the molecular species for PC. (C) Saturated acyl chain content of the phospholipid classes indicated, calculated from the molecular species composition depicted in panel B. Error bars represent the SD for PC and PE (n=4) and the variation for PI and PS (n=2).

Figure S4. Fatty acid composition of cells co-overexpressing Ole1p with non-tagged and with tagged Sct1p. *sct1pct1* cells transformed with the indicated plasmids were cultured to mid-log phase in SGR and analyzed for their fatty acid contents by GC. The relative abundance (mol%) of the 6 major fatty acids is shown with the error bars representing the variation (n=2).

Figure S5. The expression and phosphorylation level of Sct1p are similar in wild type cells cultured on SD and SL at 30 and 37^{o}C. Wild type (*BY4741 SCT1HH*) cells were grown to mid-log phase in the indicated media at the indicated temperatures, and immunoblotting was performed as described.

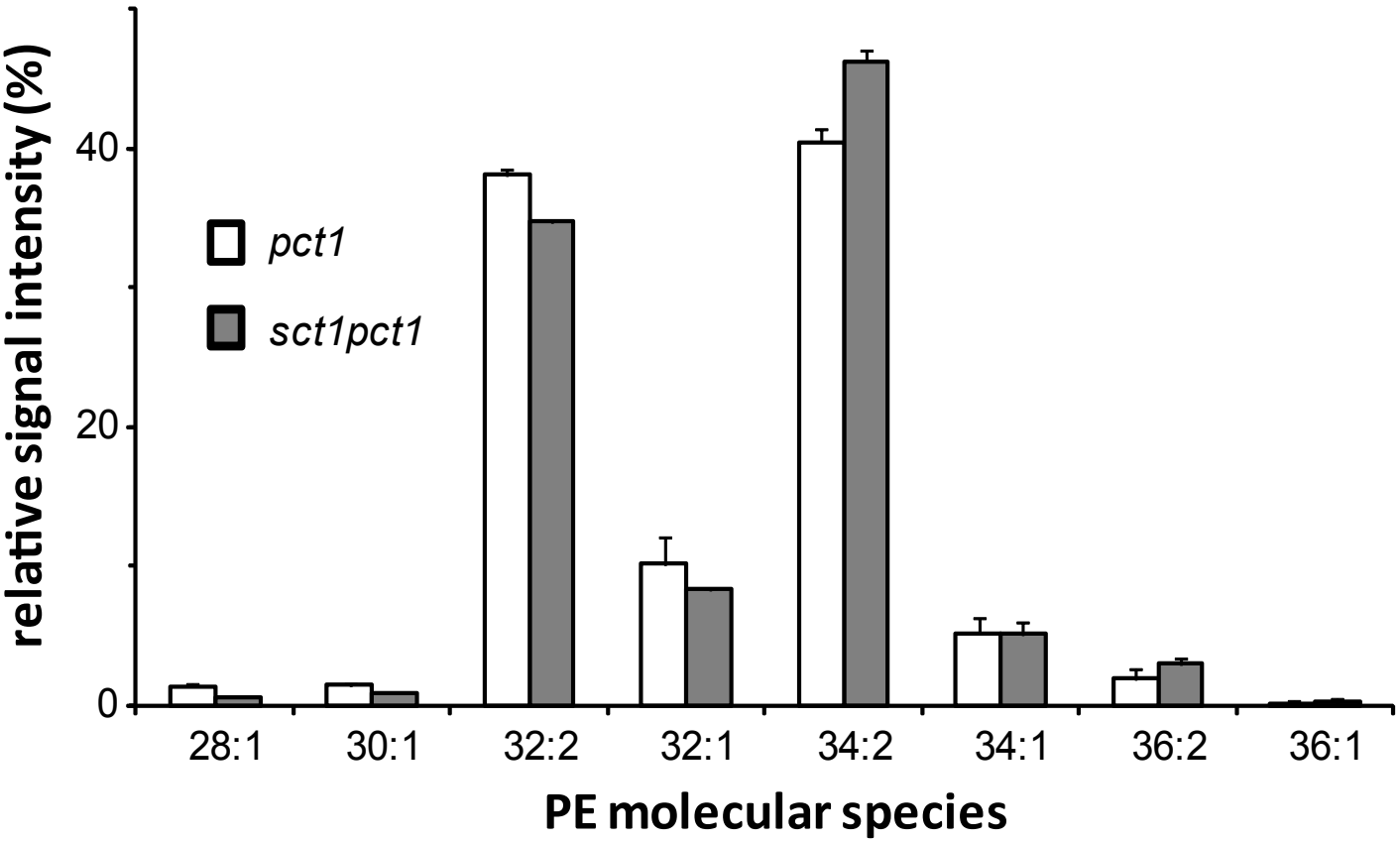
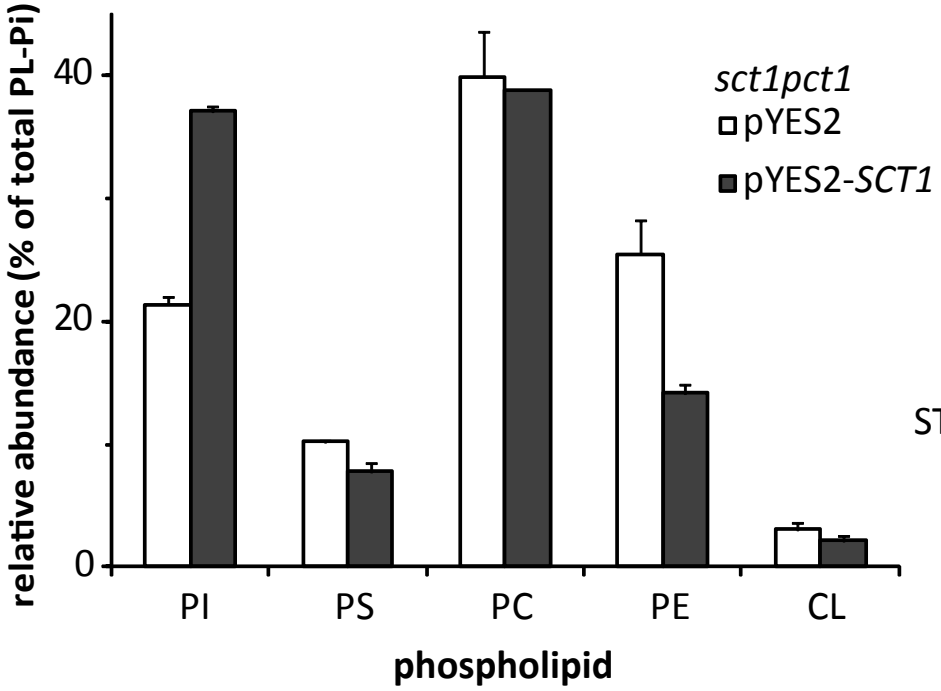
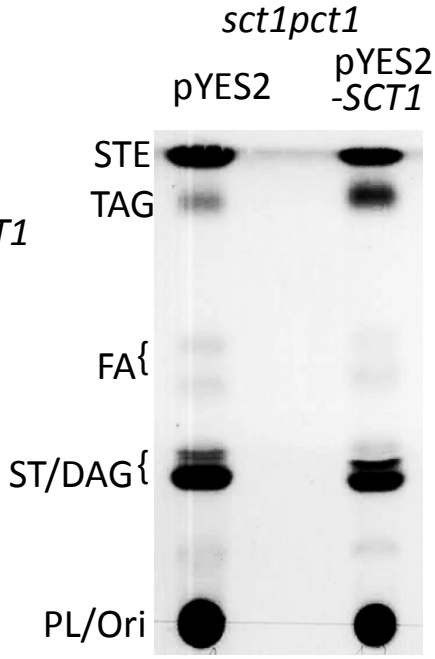

relative signal intensity (%)
40
20
0
pct1
sct1pct1
28:1
30:1
32:2
32:1
34:2
34:1
36:2
36:1
PE molecular species

Figure S1

A



B



C

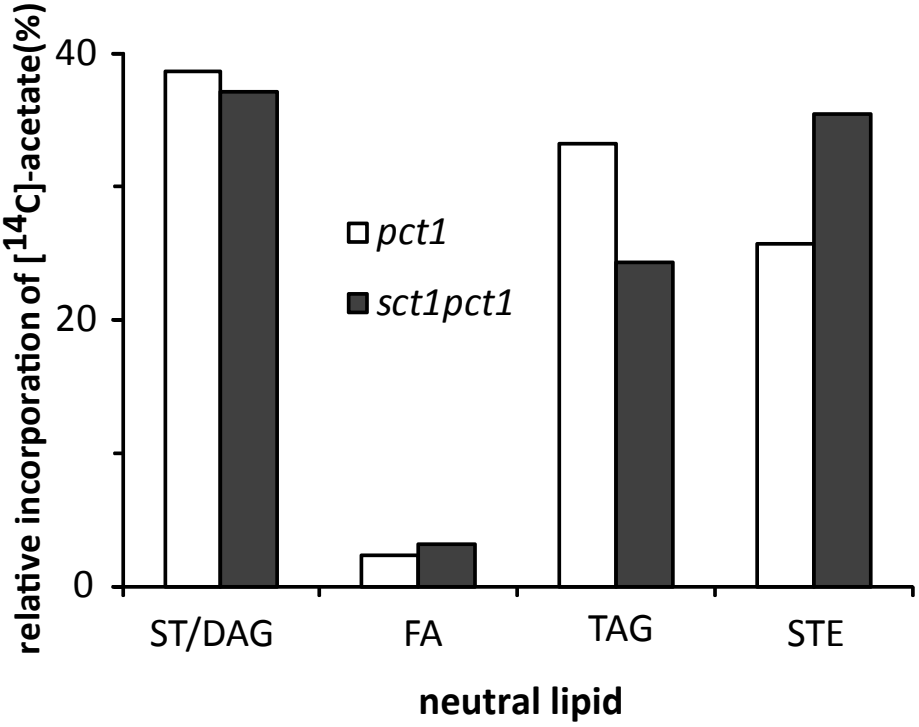
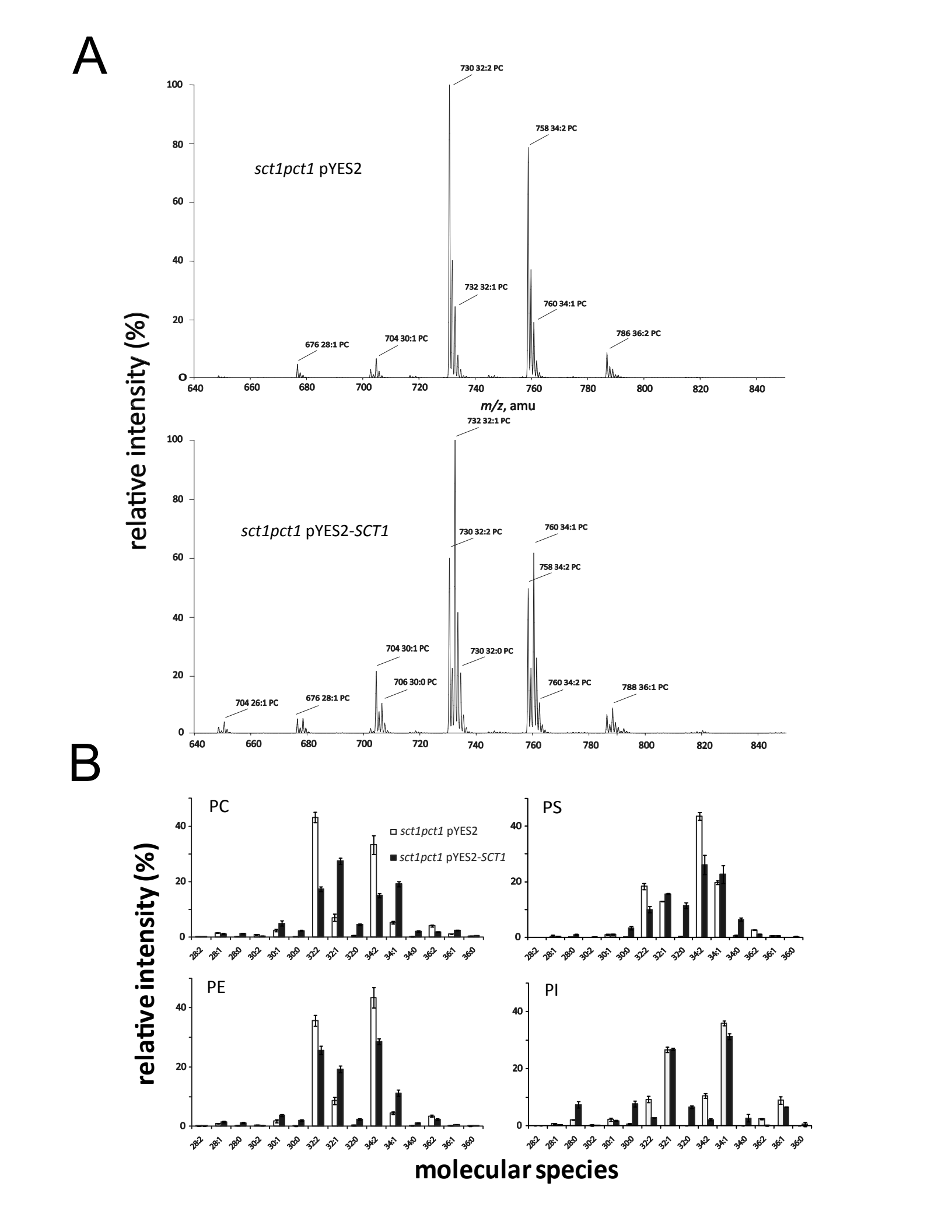


Figure S2


A
730 32:2 PC
758 34:2 PC
sct1pct1 pYES2
732 32:1 PC
760 34:1 PC
676 28:1 PC
704 30:1 PC
786 36:2 PC
m/z, amu
732 32:1 PC
sct1pct1 pYES2-SCT1
730 32:2 PC
760 34:1 PC
758 34:2 PC
704 30:1 PC
730 32:0 PC
706 30:0 PC
760 34:2 PC
788 36:1 PC
704 26:1 PC
676 28:1 PC
relative intensity (%)
B
PC
sct1pct1 pYES2
sct1pct1 pYES2-SCT1
PS
PE
PI
molecular species

C

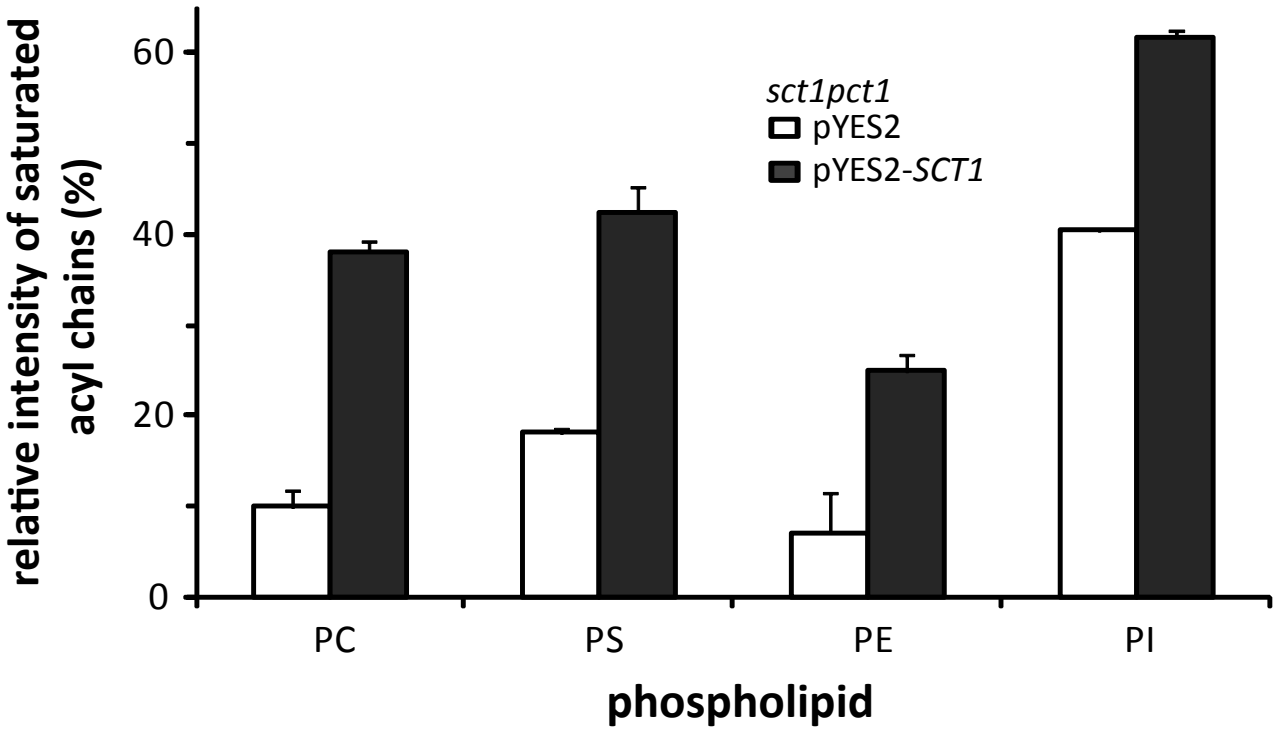

relative intensity of saturated acyl chains (%)
60
40
20
0
sct1pct1
pYES2
pYES2-SCT1
PC
PS
PE
PI
phospholipid

Figure S3

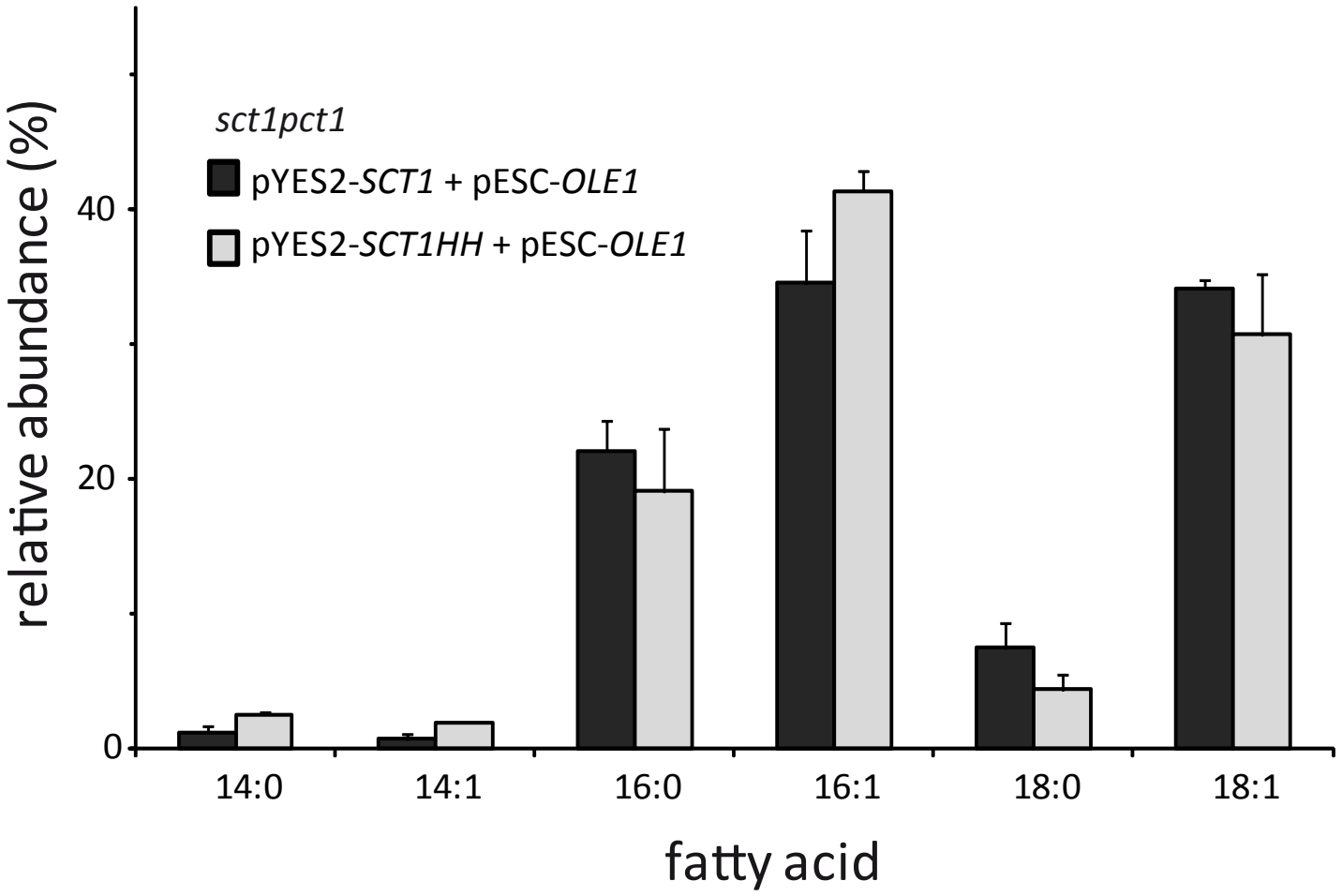

sct1pct1
pYES2-SCT1 + pESC-OLE1
pYES2-SCT1HH + pESC-OLE1
relative abundance (%)
0
20
40
14:0
14:1
16:0
16:1
18:0
18:1
fatty acid

Figure S4

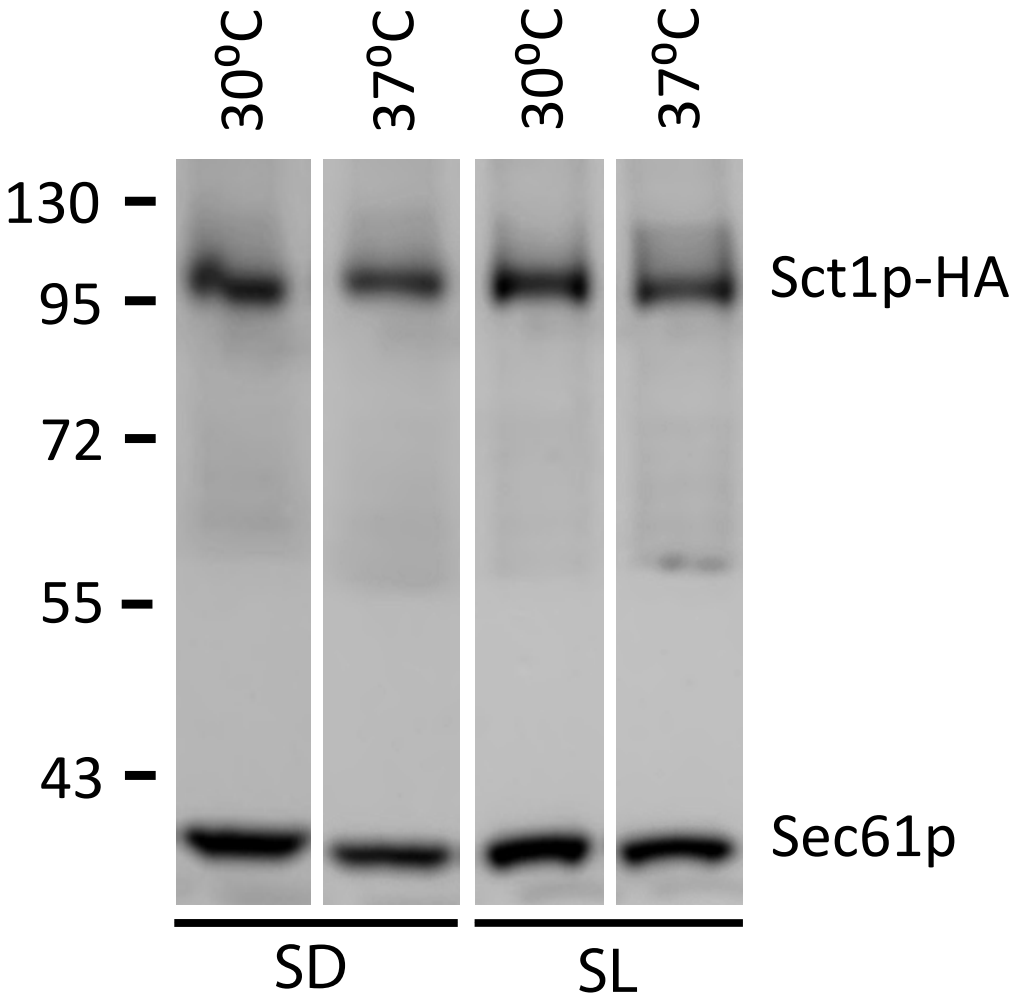


Figure S5