Supplemental information to

YEAST CELLS LACKING ALL KNOWN CERAMIDE SYNTHASES CONTINUE TO MAKE COMPLEX SPHINGOLIPIDS AND TO INCORPORATE CERAMIDES INTO GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) ANCHORS

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Supplemental experimental procedures

Origin of materials. DNA sequencing services were provided by MICROSYNTH, Balgach, Switzerland. Myriocin was from SIGMA (St.Louis, MO), Aureobasidin A from Takara Shuzo Co. (Shiga, Japan). PHS and DHS were from Avanti Polar (Alabaster, Alabama). PHS and DHS (stocks = 2.5mM in methanol), myriocin (stock = 1mg/ml in methanol) and aureobasidin A (stock = 0.5mg/ml in ethanol) were added to molten agar before pouring plates.

Cell labeling, lipid extraction, mild base treatment and thin-layer chromatography. Unless stated, metabolic labeling, cell disruption, lipid extraction and analysis were carried out as previously described (38). Where indicated, lipids were subjected to mild base treatment using NaOH as described (Becker and Lester, 1980, J Bacteriol, 142, 747-54), but incubating 1h at 37°C. Solvent systems 55:45:5 or 55:45:10 [chloroform:methanol:0.25%KCl] were used in ascending TLC on silica gel plates. Radioactivity was detected and quantified by one- and two-dimensional radioscanning (Berthold) and visualized by fluorography or radioimaging using the Bio-Rad Molecular Imager FX.

GPI remodeling. Preparation and analysis of [^{3}H]*myo*-inositol labeled GPI anchor lipids were done as described (37) with minor modifications. After delipidation proteins were incubated for 15 min at 60°C instead of 37°C. For octyl-Sepharose chromatography the pH was adjusted to 5.5 instead of 4.5. Lipids were mixed with "cold" lipids extracted from 1 A_{600} unit of cells and analyzed by TLC in solvent 55:45:5.

Mass spectrometry analysis of lipid extracts. Mass spectrometry of lipids was done essentially as described (13) with modifications. Exponentially growing cells (A_{600} of 1-3) were extracted as described (41), using procedure IIIB. Aliquots in $CHCl_3$-CH_3OH were heated and stirred at 65°C for 1 min to solubilize $M(IP)_2C$. For detection of lyso-IPCs and ceramides in 4Δ strains, lipid extracts were deacylated using methanolic NaOH and desalted. The indicated amounts were immediately injected into a normal phase PVA SIL HPLC column (YMC Europe GmbH, D-46514 Scherbeck, Germany, 1 x 150 mm) and eluted at 45 µl/min. Solvents were the following: A, hexane-isopropanol (98:2); B, $CHCl_3$-isopropanol (65:35); C, CH_3OH. The program used to change solvent composition over time to give ratios A:B:C was as follows: 0 min 12:88:0; 3 min 10:74:16; 4 min 8:61:31; 5 through 16 min 0:0:100; 18 min 0:100:0; 22 through 29 min 12:88:0. Ions in the effluent were ionized by electrospray ionisation with an electrode potential of 3.5 kV and the masses of negative ions were detected by a

Bruker Esquire-LC quadrupole ion trap mass spectrometer. Alternatively, we used a Thermo Scientific LTQ Orbitrap XL mass spectrometer after flow split (1:100) giving a flow rate of 450 nl/min. Total ion counts represent the integration of the particular ion throughout the elution region. Alternatively, lipid extracts were analyzed by direct infusion mass analysis using a LTQ Orbitrap XL equipped with Triversa NanoMate (Advion Biosciences, Ithaca) as previously described (39).

Supplemental calculation

The deletion of the 4 ceramide synthases affects ceramide incorporation into GPI anchors less severely than IPC biosynthesis.

We estimate that the amount of base resistant GPI anchors made is reduced only by about 2 fold in 4Δ cells whereas the amounts of IPCs and MIPCs is reduced 20 fold. The argument goes as follows: In Fig. 3A we see that of the 80 μCi [^{3}H]inositol added to cells, W4Δ.SLC1-1 incorporated 45 μCi, W4Δ.LAC1 cells 33 μCi into free lipids. It should be noted that the W4Δ.LAC1 cells carry *LAC1* and *LAG1* on a single plasmid so that with regard to acyl-CoA dependent ceramide synthases they can be considered to be normal and serve as a control. We calculate from cpm found in GPI anchors (Fig. 3) that GPI-associated inositol represents 0.34% of total inositol-containing free lipids in W4Δ.SLC1-1, 0.76% in W4Δ.LAC1. The fraction of ceramide containing GPIs is 41 and 45%, respectively, so that the ceramide-containing GPIs represent 0.168% (0.41 x 0.34%) and 0.342% (0.45 x 0.76%) of inositolphospholipids in W4Δ.SLC1-1 and W4Δ.LAC1, respectively. We conclude that the synthesis of ceramide containing GPI anchors in W4Δ.SLC1-1 is 49.1% of the one in W4Δ.LAC1. Quantitation of free [^{3}H]IPCs plus [^{3}H]MIPCs in the same type of lipid extracts of [^{3}H]inositol-labeled cells shows that W4Δ.SLC1-1 cells contain 0.91 – 1.1% of incorporated radioactivity in these free sphingolipids, in comparison to 13.5 or 26.9 % in corresponding wt cells (supplemental Table SV). Thus, by metabolic labeling, loss of ceramide synthases brings IPCs down 15 to 25 fold whereas ceramide containing GPI anchors are down 2 fold. Also the direct comparison shows that in wt cells, IPC in GPI anchors account for 1.25% of total IPCs (0.342%/[26.91% + 0.342%]), whereas in the 4Δ mutant the percentage is 13.2% (0.168%/[1.1 + 0.168%]).

Supplemental Figure legends

Fig. S1. **Exogenous water-soluble ceramides cannot rescue growth of 4Δ.SLC1-1 on complete synthetic media.** Ten fold dilutions of W4Δ.LAC1 and Y4Δ.LAG1 cells were plated onto SDaaUAI with or without 0.5 mM hexanoyl-DHS either in the presence or absence of FOA. Plates were incubated at 30 °C.

Fig. S2. Media requirements of 4Δ cells. Cells were plated either on LM or on synthetic complete medium at 30°C.

Fig. S3. Systematic analysis of gene deletions in 4Δ.SLC1-1 strains. Gene deletions were verified by PCR in 4Δ and control strains. Arrows in upper parts of each panel indicate the position and direction of the primers. "F" is used for sense-strand primers, "R" for anti-sense primers. Primer sequences are available in Table SIII. For each gene deletion 3 or 4 PCR reactions were performed using different combinations of primers. Products were migrated on 1%-agarose gel and revealed using ethidium bromide. **A**, checking the replacement of *LAG1* by *TRP1*. **B**, checking the replacement of *LAC1* by *LEU2*. **C**, checking the replacement of *YPC1* by KanMX. **D**, checking the replacement of *YDC1* by NatMX. **E**, checking for the presence of pBM150-LAG1 or pBM150-LAC1-LAG1. **F**, checking for the presence of the conserved TLC motif found in all acyl-CoA dependent ceramide synthases (8). Results of PCRs are shown under the corresponding schemes. The PCR using primers F14/R15 in panel F amplified an unexpected 800 base pair fragment. The fragment was sequenced and corresponds to a fragment of YNL152w.

Fig. S4. No anomalous ceramides are present in GPI anchors of 4Δ cells. Panels show experiments of the same kind as shown in Fig. 3.

A, when preparing anchor lipids, the bulk of GPI anchor peptides requires 50% propanol to elute from the octyl-sepharose column used during purification. Here the more polar anchor peptides already eluting at 25% propanol from octyl-sepharose were analyzed in the same experiment as shown in Fig. 3. Samples in lanes 2 and 9 were already run in Fig. 3, lanes 2 and 8. Note that, due to the incomplete removal of detergent, the mobility of all anchor lipids obtained from 25% propanol fraction is lower than the mobility of the same lipids obtained from the 50% propanol fraction. An aliquot of the free lipids is shown in lane 1.

Result: 4Δ.SLC1-1 cells do not make any abnormal, more polar GPI anchor lipids. Note that small amounts of lyso-PI containing anchors (marked with asterisk) are not abnormal, since lyso-GPI anchors are normal intermediates during anchor lipid remodeling (Fig. 1C) and are also seen in wt cells (lane 8). (Some polar lipids are seen in lcb1Δ SLC1-1 cells (lane 7), but it was not tested if they are anchor lipids and if they are mild base resistant.)

B, cells were precultured in LM and labeled in SDaaUA with 100 μCi of [^{3}H]inositol for 120 min at 30 °C. The released anchor lipids (A) and a comparable amount of free lipids (F) were divided into two aliquots, one of which was deacylated using monomethylamine (MMA). The total amount or radioactivity in the free lipid extracts is indicated at the bottom (μCi F). Lane 10 contains anchor peptides not treated with nitrous acid.

C, anchor lipids of 4Δ.SLC1-1 cells are compared to the normal profiles found in YPK9 wt cells and *plc1*Δ and *pgc1*Δ cells. Lane 1 contains an aliquot of the lipid extract of [^{3}H]inositol-labeled wt cells, lane 8 contains anchor peptides not treated with nitrous acid.

Result: GPI anchors with apparently normal TLC mobility are also made in Y4Δ.SLC1-1 cells, as in W4Δ.SLC1-1 cells, and also in this strain the amount of ceramide-containing anchors was comparable to the one in wt cells.
YPK9.4Δ cells can be maintained alive by Lass5, a mammalian *LAG1* homologue and ceramide synthase, which mainly uses C16:0 and C18:0 fatty acids. As reported before, in such 4Δ.Lass5 cells, the free IPCs mainly contain C16 and C18 fatty acids and migrate aberrantly on TLC, while GPI anchors contain IPC-3 and IPC-4 having the same mobility as in wt cells, and can be assumed to contain C26:0 and C24:0 (29). This is reproduced here in lane 3. In this particular instance it is conceivable that the ceramides used for GPI anchoring are also made by the unknown ceramide synthesis pathway but it cannot be excluded that they are made by Lass5, which may not be entirely specific for C16:0 and C18:0 fatty acids. In contrast, the origin of ceramides in GPI anchors of 4Δ.SLC1-1 cells cannot be traced to any known ceramide synthase.

Fig. S5. Synthesis of mild base resistant inositides in 4Δ cells is reduced by myriocin and AbA. In parallel with the experiment shown in Fig. 4B, we also tested the ability of drugs to inhibit biosynthesis of anomalous inositolphosphorylsphingolipids in Y4Δ.SLC1-1 (FBY2219) and, as a control, W303.SLC1-1 (FBY2192), i.e. wt cells containing *SLC1-1*. Cells were grown in LM, washed 2x with SDaaUA and resuspended in the same. After preincubation for 10 min with inhibitors (40µg/ml of myriocin or 2µg/ml of AbA) cells were labeled by addition of 10 µCi of [^{3}H]inositol in presence of the same drugs and incubation at 30°C for 2 hours. Aliquots containing the indicated amounts of radioactivity (µCi) were either deacylated by NaOH treatment (+) or left untreated (-), desalted and analyzed by TLC in solvent 55:45:10 and fluorography. Residual IPCs and MIPCs in 4Δ cells are marked by crosses (+), lipid a is indicated with an asterisk (*). Residual PI indicates that mild base hydrolysis was not complete in some lanes.

Fig. S6. Lipid profiling of 4Δ cells. A, four different 4Δ cells were grown in LM at 30°C. Lipids were extracted, deacylated and aliquots of 0.1 A_{600} units were analyzed by LC-ESI-MS on an ion trap mass spectrometer. Ion counts corresponding to lyso-IPCs are plotted. While there was no internal standard to allow direct comparison of the quantities of lyso-IPCs in these four strains, it is apparent that they all display a high ratio of PHS20-containing over PHS18-containing species. **B,** W303.4Δ (FBY958) cells were grown to exponential phase in LM, the various wt strains and an elo3Δ strain were grown in ordinary complete synthetic media. Lipids were extracted using $ClCH_3$-CH_3OH (2:1) and were analyzed by ESI-MS in positive and negative ion mode on a QSTAR spectrometer as described (39). For each strain, the sum of all positive ions corresponding to known lipid species (9 different lipid classes) was set to 100% and the intensities of all ions corresponding to LCBs were expressed as a percentage thereof. Similarly, the sum of all negative ions corresponding to known lipid species (17 different lipid classes) were set to 100% and the intensities for all known species of phosphatidylserine (PS), lyso-IPCs and LCB-phosphates (LCB-P) were expressed as a percentage

thereof. The plot only shows those lipid species, which were significantly under- or overrepresented in W303.4Δ cells. It appears that 4Δ cells have very high amounts not only of lyso-IPCs, but also of LCBs and LCB-phosphates.

Fig. S7. Lcb1Δ SLC1-1 cells do not make mild base resistant inositides. A, aliquots (3µCi) of the free lipid extracts of W4Δ.SLC1-1 and lcb1Δ SLC1-1 cells generated during the analysis of GPI anchor lipids in Fig. 3, lanes 2 and 7, respectively, were deacylated by NaOH treatment (+) or mock incubated (-). Samples were analyzed by TLC using the solvent 55:45:10. The TLC plate was then sprayed with EN^{3}HANCER, placed on a film and exposed for 4 weeks. While several mild base resistant lipids are present in W4Δ.SLC1-1, no such lipids are visible in lcb1Δ SLC1-1 cells. The latter only contains a trace of residual PI and some barely migrating glycerol-phospho-[^{3}H]inositol, which has not been completely removed during the desalting procedure after mild base hydrolysis. **B** shows the result of an identical experiment except that lcb1Δ SLC1-1 cells were grown without (lanes 3, 6) or with (lanes 2, 5) PHS, and that only 0.2 µCi of untreated lipid extract were spotted (lanes 1 - 3), whereas 3 µCi of lipid extract was deacylated to obtain the resistant lipids shown in lanes 4 - 6.

Fig. S8. 4Δ cells do not contain DNA other than from *S.cerevisiae*. DNA from W303.4Δ and W4Δ.SLC1-1 cells was prepared and used as template for low stringency PCR at low temperature with primers YPC1-VF1 and YPC1-VR1 (lane 2) or LAG1.VF1 and LAG1.VR1 (lane 3), corresponding to the coding sequences of *YPC1* and *LAG1,* respectively. As these genes are deleted in both of the above strains, annealing temperatures were 12 or 20 °C lower than required for specific amplification of *YPC1* and *LAG1*. The products were separated on agarose and boxed bands were excised and used as templates for a further round of PCR amplification. The products of this second PCR were sequenced, but only 5 of the 9 bands yielded DNA sequence. Nucleotide blasting with these sequences showed that they all originate from *S.cerevisiae.*

Supplemental Tables

Table SI. Yeast *Saccharomyces cerevisiae* strains

Strains	Genotype	Reference
YPK9	*MAT***a** *ade2-101*^{ochre} *his3-Δ200 leu2-Δ1 lys2-801*^{amber} *trp1-Δ63 ura3-52*	(26)
Y2D.LAC1 (913)	Same as YPK9, but *lac1::LEU2 lag1::TRP1* containing pBM150-LAC1	(26)
Y4Δ.LAG1 (FBY2171)	As YPK9 but *lac1::LEU2 lag1::TRP1 ydc1::natMX ypc1::kanMX4* containing pBM150-LAG1	(29)
Y4Δ.mSLC1-1/LAG1 (FBY2174)	As Y4Δ.LAG1but also containing pBF27	This study
Y4Δ.mSLC1/LAG1 (FBY2176)	As Y4Δ.LAG1 but also containing pBF26	This study
Y4Δ.mSLC1-1 FBY2187	As YPK9 but *lag1::TRP1, lac1::LEU2 ydc1::natMX ypc1::kanMX4* containing pBF27	This study
Y4Δ.SLC1-1 (FBY2219)	As YPK9 but *lag1::TRP1, lac1::LEU2 ydc1::natMX ypc1::kanMX4* containing pBF212	This study
Y4Δ.SLC1-1a (FBY945)	As Y4Δ.SLC1-1, but *ypc1::kanMX4::ADE2*	This study
Y4Δ.LAG1a (FBY1183)	As Y4Δ.LAG1 but *lac1::ADE2*	This study
Y4Δ.LAG1/AUR1 (FBY988)	As Y4Δ.LAG1a but also harboring pYC272-AUR1-LEU2	This study
Y4Δ.AUR1 (FBY992)	As YPK9 but *lac1::ADE2 lag1::TRP1 ydc1::natMX ypc1::kanMX4* harboring pYC272-AUR1-LEU2	This study
Y4Δ.Lass5a = 4Δ.Lass5-ADE2 (FBY947)	As YPK9 but *lag1::TRP1 lac1::LEU2 ydc1::natMX ypc1::KanMX4::ADE2* containing p413MET-Lass5	(29)
W303-1A	*MAT***a** *can1-100 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1*	(26)
W303-1B	*MAT***a** *can1-100 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1*	(26)
W4Δ.LAC1 (FBY2169)	As W303-1A but *lac1::LEU2 lag1::TRP1 ydc1::natMX ypc1::kanMX4* containing pBM150-LAC1-LAG1	This study
W4Δ.mSLC1-1/LAG1 (FBY2178)	As W4Δ.LAC1 but also containing pBF27	This study
W4Δ.mSLC1/LAG1 (FBY2180)	As W4Δ.LAC1 but also containing pBF26	This study
W4Δ.mSLC1-1 FBY2189	As W303-1A but *lac1::LEU2 lag1::TRP1 ydc1::natMX ypc1::kanMX4* containing pBF27	This study
W4Δ.SLC1-1 (FBY2220)	As W3031A but *lac1::LEU2 lag1::TRP1 ydc1::natMX ypc1::kanMX4* containing pBF212	This study
W4Δ.SLC1-1a (FBY943)	As W4Δ.SLC1-1 but *ypc1::kanMX::ADE2*	This study
W4Δ.SLC1-1/UBI4 (FBY1201)	As W4Δ.SLC1-1 but containing also YEp352-UBI4	This study
W303.4Δ (FBY958)	As W303-1A but *lac1::LEU2 lag1::TRP1 ydc1::natMX ypc1::kanMX4*	(13)
W303.4Δ (FBY958-Lnew)	As W303-1A but *lac1::LEU2 lag1::TRP1 ydc1::natMX ypc1::kanMX4* (grown for several weeks in continuous liquid culture)	This study
W303.mSLC1	As W303-1B but containing pBF26	This study

(FBY2185)		
W303.SLC1-1 (FBY2192)	As W303-1B but containing pBF212	This study
SJ21R	*ura3-52 leu2-3,112 ade1*	K. Athenstaedt
lcb1Δ SLC1-1 (4R3-17C)	As SJ21R but *lcb1::URA3 SLC1-1 MEL1*	R. Dickson
pmi40 (C4)	*MAT***a** *leu2-3,112 ura3-52 pmi 40^{ts}*	Sipos et al.**
pmi40 lcb1Δ SLC1-1 (FBY952)	*lcb1Δ SLC1-1 pmi40^{ts}*	This study
lcb1-100	*MAT***a** *end8-1 leu2 ura3 his4 bar1*	R. Schneiter
(FBY999)*	*csg1::kanMX4 csh1::kanMX4 his3Δ1 leu2Δ0 ura3Δ0* containing pBF26	This study
(FBY9100)*	*CSG1 csh1::kanMX4 his3Δ1 leu2Δ0 ura3Δ0* containing pBF26	This study
(FBY9101)*	*csg1::kanMX4 CSH1 his3Δ1 leu2Δ0 ura3Δ0* containing pBF26	This study
(FBY9102)*	*CSG1 CSH1 his3Δ1 leu2Δ0 ura3Δ0* containing pBF26	This study
(FBY9103)*	*CSG1 CSH1 his3Δ1 leu2Δ0 ura3Δ0* containing pBF27	This study
(FBY9104)*	*csg1::kanMX4 CSH1 his3Δ1 leu2Δ0 ura3Δ0* containing pBF27	This study
(FBY9105)*	*csg1::kanMX4 csh1::kanMX4 his3Δ1 leu2Δ0 ura3Δ0* containing pBF27	This study
(FBY9106)*	*CSG1 csh1::kanMX4 his3Δ1 leu2Δ0 ura3Δ0* containing pBF27	This study
(FBY9107)*	*CSG1 csh1::kanMX4 his3Δ1 leu2Δ0 ura3Δ0* containing pBF212	This study
(FBY9108)*	*csg1::kanMX4 CSH1 his3Δ1 leu2Δ0 ura3Δ0* containing pBF212	This study
(FBY9109)*	*csg1::kanMX4 csh1::kanMX4 his3Δ1 leu2Δ0 ura3Δ0* containing pBF212	This study
(FBY9110)*	*CSG1 CSH1 his3Δ1 leu2Δ0 ura3Δ0* containing pBF212	This study
BY4742	*MAT***a** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	EUROSCARF, (Frankfurt, GE)
plc1Δ	BY4742, but *plc1::kanMX*	EUROSCARF
pgc1Δ	BY4742, but *pgc1::kanMX*	EUROSCARF
elo3Δ	BY4742, but *elo3::kanMX*	EUROSCARF
ypc1Δ ydc1Δ (FBY2182)	Same as BY4742, but *ypc1::kanMX4 ydc1::kanMX4::natMX*	This study

*FBY999 through 9102 are the four meiotic products of a cross of a BY4742 *csg1::kanMX* with a BY4741 *csh1::kanMX*, both strains containing pBF26, FBY9103 through 9106 are the four meiotic products of a cross of a BY4742 *csg1::kanMX* with a BY4741 *csh1::kanMX*, both strains containing pBF27, and FBY9107 through 9110 are the four meiotic products of a cross of a BY4742 *csg1::kanMX* with a BY4741 *csh1::kanMX*, both strains containing pBF212.

**Sipos, G., Puoti, A. and Conzelmann, A. (1994) EMBO J 13, 2789-2796

Table SII. Plasmids

pBF26	*SLC1* in p423Met25 (2µ *HIS3 MET25* promoter)	(28)
pBF27	*SLC1-1* in p423Met25 (2µ *HIS3 MET25* promoter)	(28)
pBF212	*SLC1-1* in p413Met25 (CEN ARS *HIS3 MET25* promoter)	(28)
pBM150-LAC1-LAG1	CEN ARS *URA3*, contains both, *LAC1* and *LAG1* under *GAL1,10* promoter	M. Jazwinsky
pBM150-LAG1	CEN ARS *URA3,* pBM150 having *LAG1* behind *GAL1,10* promoter	M. Jazwinsky
pBM150-LAC1	CEN ARS *URA3,* pBM150 having *LAC1* behind *GAL1,10* promoter	M. Jazwinsky
pYC272-AUR1-LEU2	*LEU2* in YC272 (2µ *AUR1-C*)	This study
YEp352-UBI4	*UBI4* in Yep352 (2µ *URA3)*	(53)

Table SIII. Oligonucleotide primers

Name of primer	Short name	Oligonucleotide sequence 5'-> 3'
TSC13XhoI.R1		ttccgctcgagcggccTCAAAATACAAATGGAATCAAGAA
TSC13HindIII.F1		ccttcccaagcttcatacaATGCCTATCACCATAAAAAGCC
LEU2Not1.F1		ccatgcggccgcAGGCCGTTTCTGACAGAGTAA
LEU2Not1.R1		atgggcggccgcTCGAGGAGAACTTCTAGTATATC
LAG1.VF2	F1	TCCGTCAAGACTAATATCGATA
TRP1.VR2	R1	TGAGTAGTATGTTGCAGTCTTT
LAG1.VF3	F2	TTAGCGGGAAAAGCAAATTGCA
LAG1.VR3	R2	CGAGAGCTGGCCAAAGAGG
LAG1.VF1	F3	GAGTTTCAGGGCCCTTTG
LAG1.VR1	R3	GCTCTCATCAGAATCACTGTCAC
LAC1-VF1	F4	CCTCCTTGGCTTGTTGCATCC
LEU2R	R4	ATGGTCTTAAGTTGGCGTACA
LAC1.VF2	F5	TTGTATGTACCATTCGGATTTATGG
LAC1-VR1	R5	GATATACTAAAGCAACGCTCAT
KanMX4-F1	F6	GGATCTTGCCATCCTATGGAACTGC
LAC1.VR2	R6	GGAGTACTGTCAGTTGGAGTAGTGG
YPC1-VF2	F7	CCCACTAGTGCGGACGGATT
YPC1-R5	R7	ACGTTATTTTGCCGCCGACG
YPC1-VF1	F8	GAGAGTTCTGTCCCCGGCG
YPC1-VR2	R8	GGTACATGTCCCGAATTAGCTA
YDC1-VF1	F9	CTGATCGTAGCCATTCGGCTCA
YPC1-VR1	R9	GATTGATCTTCTACGTATGGGC
YDC1-KO.F1	F10	GTCCGATAGCGTACGCCAACC
NatMX4-VR1	R10	GTAAGCCGTGTCGTCAAGAGTGGTAC
YDC1-VF2	F11	CCAGAAGCCCCGATTGAAGG
YDC1-KO.R1	R11	GATGGGAGTAACTGCTGCATGTG
114.F1	F12	TCAGAGCTCATTGAAGTACGGATTAGAAGCCGCCG
YDC1-VR2	R12	TTACAATTGGCCCCAGATACTC
LGA1.R1	R13	TCTTGAACAACCACAAATCA
SLC1.F1		CCAACTAGTCTAGAATACAATGAGTGTGATAGGTAGGTTCTTGTATTACTTGAGG
SLC1.R1		CCACTCGAGAATTCTTAATGCATCTTTTTTACAGATGAACCTTCGTTATG
LAG1cm.F1	F13	GCCAAGAAAGGATTACAAG
LAG1cm.R1	R14	CAGATAGTTTAATGTCTTAGAC
LAC1cm.F1	F14	AGATCATAACGAGTTGACTTT
LAC1cm.R1	R15	AATAATTTAGAGTTTTGGAGAA

Table SV. Small amounts of mild base resistant lipids are present in 4Δ.SLC1-1 cells

Experiment	Strain	% IPC-MIPC*	% abnormal*°	%mild base resistant*
1	YPK9	13.50	1.36	14.87
2	Y4Δ.SLC1-1	0.83	1.51	2.33
1	Y4Δ.mSLC1-1	0.99	1.31	2.30
1	W303	26.91	2.40	29.31
2	W4Δ.SLC1-1	0.86	1.27	2.12
4	W4Δ.SLC1-1	0.75	1.05	1.80
3	W4Δ.mSLC1-1	1.46	1.77	3.22
1	W4Δ.mSLC1-1	1.33	2.23	3.56
	Mean of 4Δ strains	1.04	1.52	2.55
	Standard deviation	+/- 0.29	+/- 0.42	+/- 0.68

The [^{3}H]inositol-labeled lipids were quantified in 4 independent experiments by Berthold radioscanning of TLC plates, experiment 4 corresponding to the lipid extract of the experiment shown in Fig. 3, lane 2. Cells were grown to exponential phase and metabolically labeled with [^{3}H]inositol during 2 h; between 40 and 70% of added radioactivity was incorporated into lipids. Equal aliquots of lipid extracts were treated with mild base or left untreated and run on TLC.

* The mild base resistant lipids of indicated types detectable after treatment with NaOH are expressed as a percentage of labeled lipids present in the extract before mild base treatment.

° The major abnormal mild base resistant lipid was lyso-IPC.

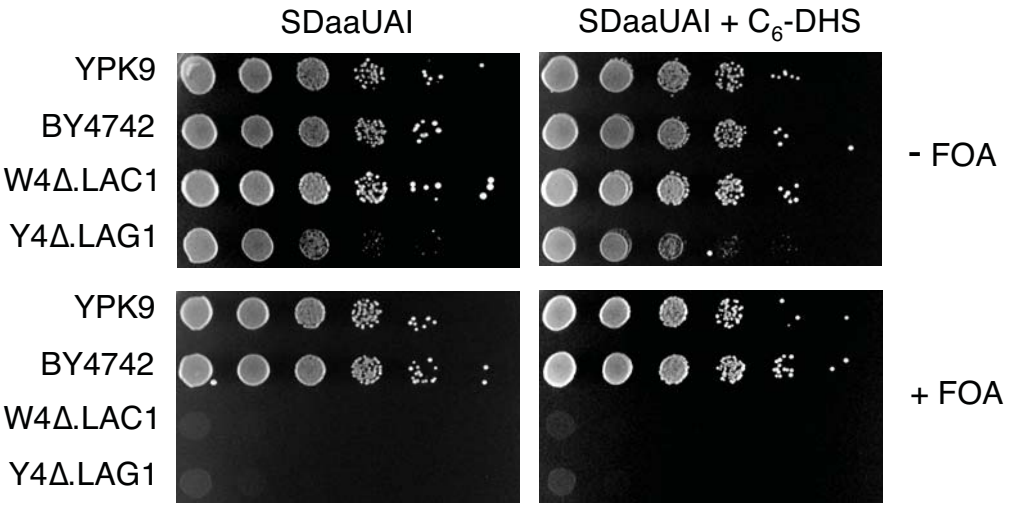
SDaaUAI
SDaaUAI + C_6-DHS
YPK9
BY4742
W4Δ.LAC1
Y4Δ.LAG1
- FOA
YPK9
BY4742
W4Δ.LAC1
Y4Δ.LAG1
+ FOA

Fig. S1

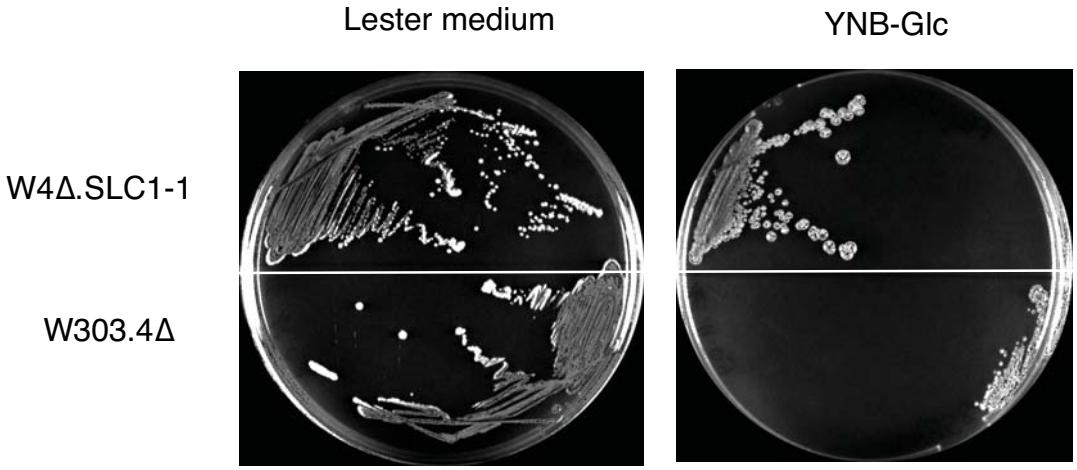

Fig. S2

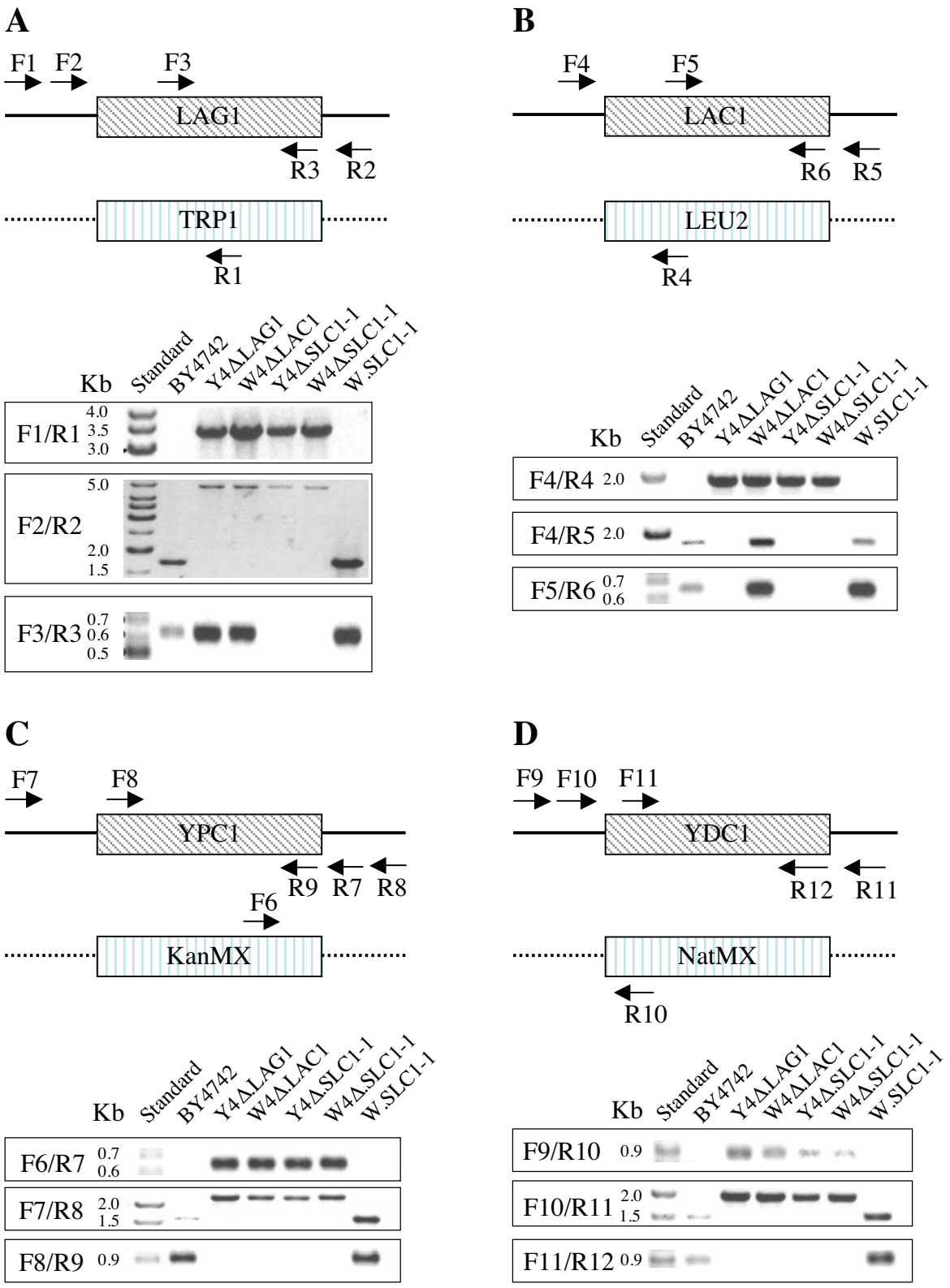
A
F1
F2
F3
LAG1
R3
R2
TRP1
R1
Kb
Standard
BY4742
Y4ΔLAG1
W4ΔLAC1
Y4ΔSLC1-1
W4ΔSLC1-1
W.SLC1-1
F1/R1
4.0
3.5
3.0
F2/R2
5.0
2.0
1.5
F3/R3
0.7
0.6
0.5
B
F4
F5
LAC1
R6
R5
LEU2
R4
F4/R4
2.0
F4/R5
2.0
F5/R6
0.7
0.6
C
F7
F8
YPC1
R9
R7
R8
F6
KanMX
F6/R7
0.7
0.6
F7/R8
2.0
1.5
F8/R9
0.9
D
F9
F10
F11
YDC1
R12
R11
NatMX
R10
F9/R10
0.9
F10/R11
2.0
1.5
F11/R12
0.9

Fig. S3A-D

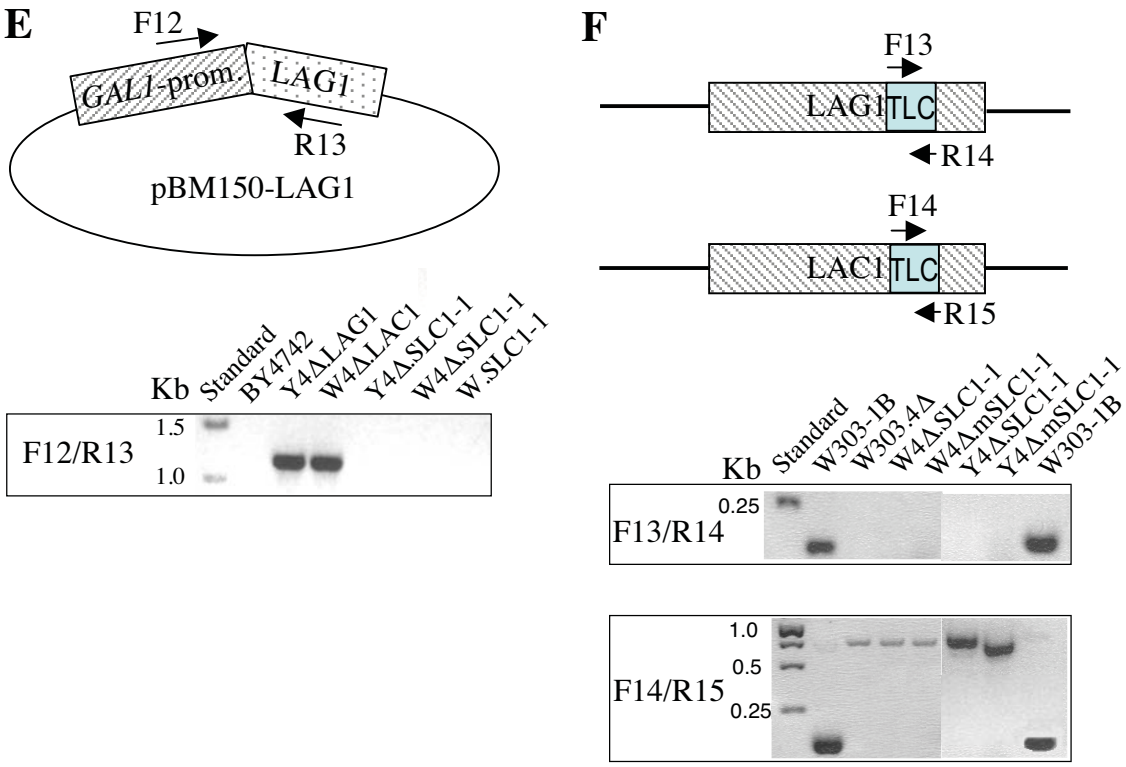
E
F12
GAL1-prom.
LAG1
R13
pBM150-LAG1
Kb
Standard
BY4742
Y4ΔLAG1
W4ΔLAC1
Y4ΔSLC1-1
W4ΔSLC1-1
W.SLC1-1
F12/R13
1.5
1.0
F
F13
LAG1
TLC
R14
F14
LAC1
TLC
R15
Kb
Standard
W303-1B
W303.4Δ
W4ΔSLC1-1
W4ΔmSLC1-1
Y4ΔSLC1-1
Y4ΔmSLC1-1
W303-1B
F13/R14
0.25
F14/R15
1.0
0.5
0.25

Fig. S3 E-F

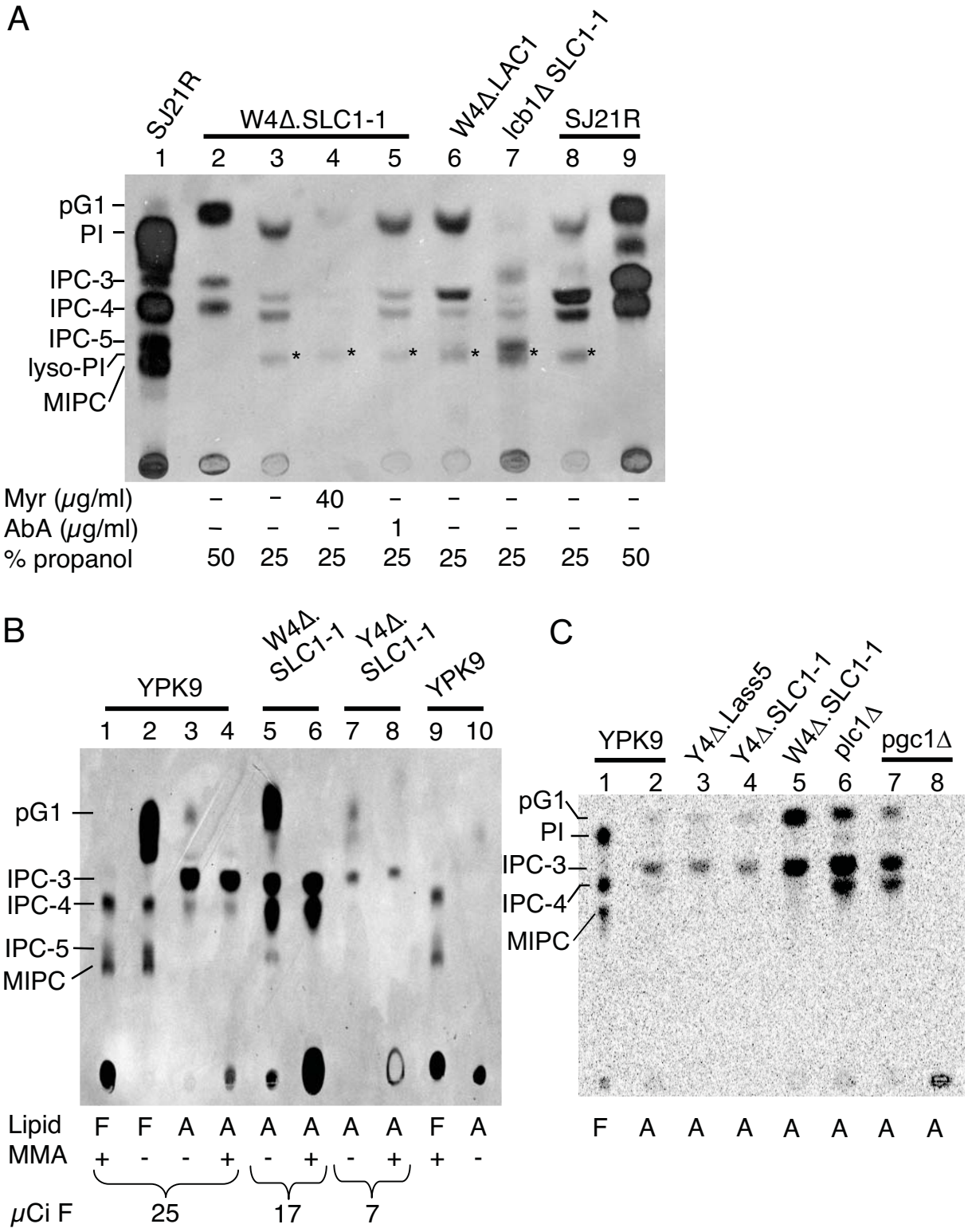
A
SJ21R
W4Δ.SLC1-1
W4Δ.LAC1
lcb1Δ SLC1-1
SJ21R
1 2 3 4 5 6 7 8 9
pG1
PI
IPC-3
IPC-4
IPC-5
lyso-PI
MIPC
Myr (μg/ml) – – 40 – – – – –
AbA (μg/ml) – – – 1 – – – –
% propanol 50 25 25 25 25 25 25 50
B
YPK9
W4Δ. SLC1-1
Y4Δ. SLC1-1
YPK9
1 2 3 4 5 6 7 8 9 10
pG1
IPC-3
IPC-4
IPC-5
MIPC
Lipid F F A A A A A A F A
MMA + - - + - + - + + -
μCi F 25 17 7
C
YPK9
Y4Δ.Lass5
Y4Δ.SLC1-1
W4Δ.SLC1-1
plc1Δ
pgc1Δ
1 2 3 4 5 6 7 8
pG1
PI
IPC-3
IPC-4
MIPC
F A A A A A A A

Fig. S4

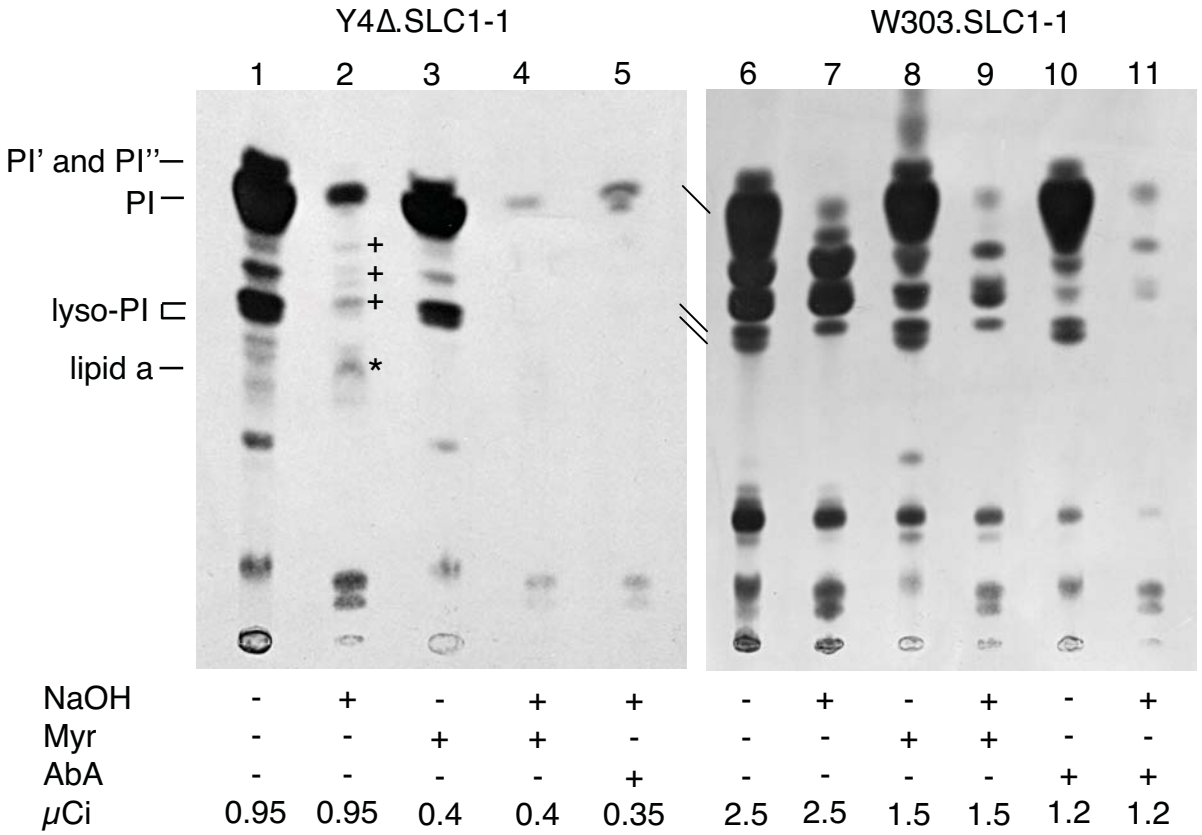
Y4Δ.SLC1-1
W303.SLC1-1
1 2 3 4 5 6 7 8 9 10 11
PI' and PI''
PI
lyso-PI
lipid a
+
+
+
*
NaOH - + - + + - + - + - +
Myr - - + + - - - + + - -
AbA - - - - + - - - - + +
μCi 0.95 0.95 0.4 0.4 0.35 2.5 2.5 1.5 1.5 1.2 1.2

Fig. S5

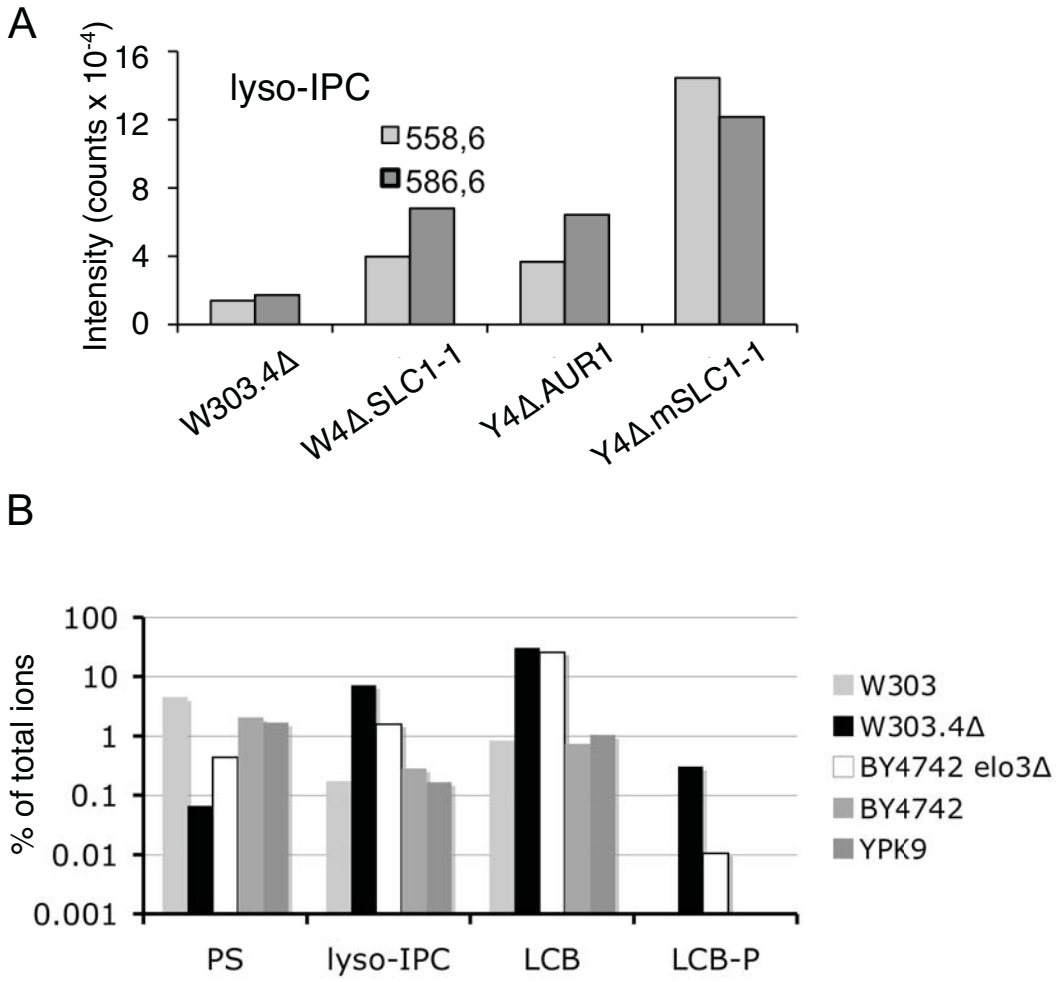
A
lyso-IPC
558,6
586,6
Intensity (counts x 10-4)
16
12
8
4
0
W303.4Δ
W4Δ.SLC1-1
Y4Δ.AUR1
Y4Δ.mSLC1-1
B
% of total ions
100
10
1
0.1
0.01
0.001
PS
lyso-IPC
LCB
LCB-P
W303
W303.4Δ
BY4742 elo3Δ
BY4742
YPK9

Fig. S6

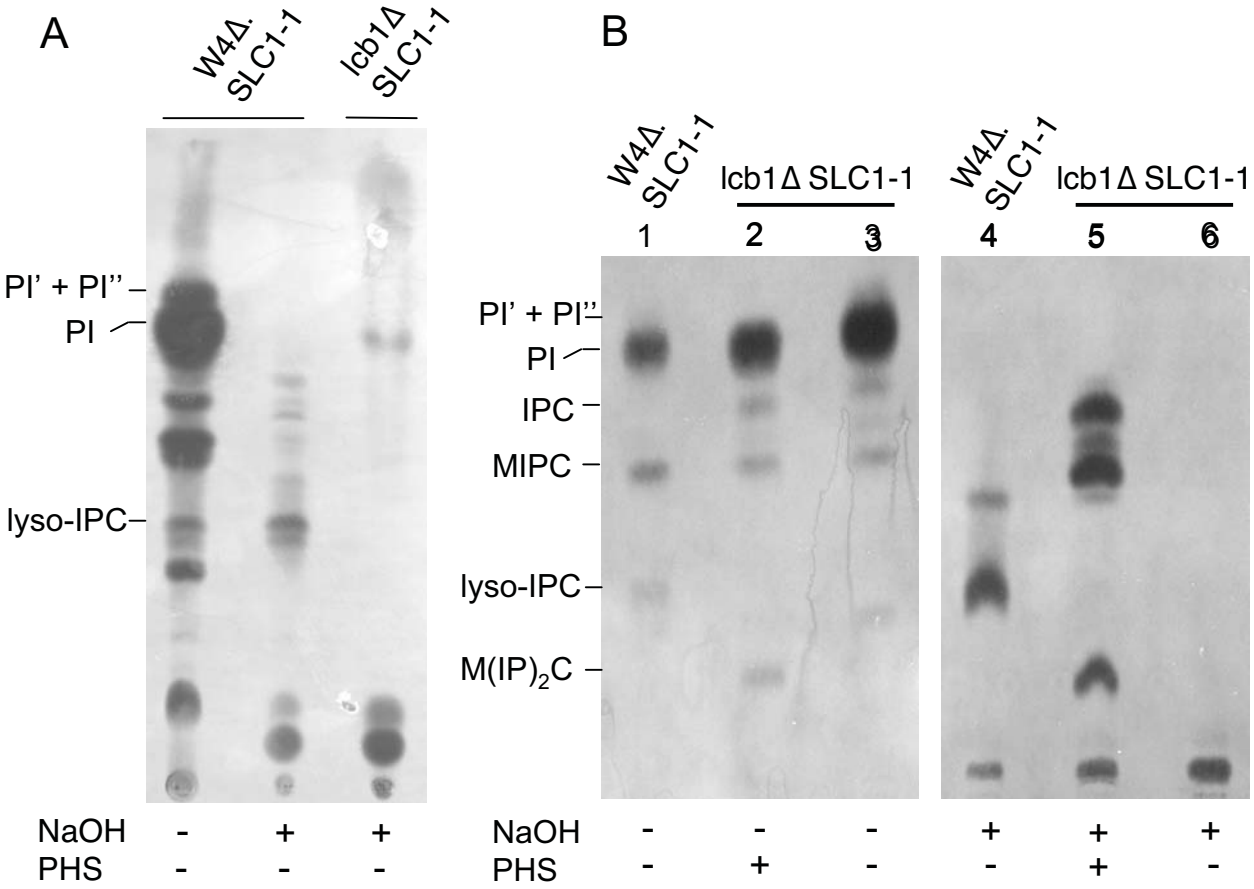
A
W4Δ. SLC1-1
lcb1Δ SLC1-1
PI' + PI''
PI
lyso-IPC
NaOH - + +
PHS - - -
B
W4Δ. SLC1-1
lcb1Δ SLC1-1
W4Δ. SLC1-1
lcb1Δ SLC1-1
1 2 3 4 5 6
PI' + PI''
PI
IPC
MIPC
lyso-IPC
M(IP)2C
NaOH - - - + + +
PHS - + - - + -

Fig. S7

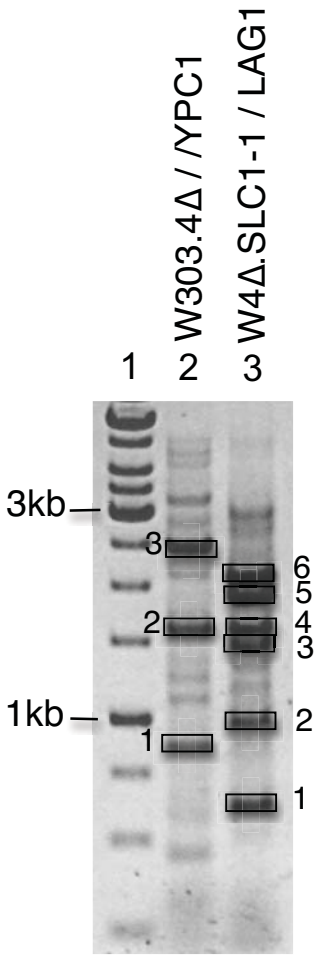

Fig. S8