Supporting Information

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SI Materials and Methods

Chemicals and Lipid Standards. Chemicals and solvents were purchased from Sigma–Aldrich and Merck. Synthetic lipid standards were from Avanti Polar Lipids, Larodan Fine Chemicals, and Steraloids. PI 17:0–17:0 was provided by Christoph Thiele (MPI-CBG, Dresden). IPC, MIPC, $M(IP)_2C$ and stigmasta-5,7,22-trienol were purified as described below.

Purification of Lipid Standards. Sphingolipid standards IPC, MIPC and $M(IP)_2C$ were isolated from crude sphingolipid extracts of *sur2*Δ*scs7*Δ [provided by Teresa Dunn (Uniformed Services University of the Health Sciences)] as previously described (1). All lipid standards were subjected to quality control by mass spectrometry and phosphate analysis. Stigmasta-5,7,22-trienol was isolated by preparative chromatography after alkaline hydrolysis of stigmasta-5,7,22-trienol acetate (Sigma–Aldrich). Purified stigmasta-5,7,22-trienol was subjected to quality control by quantitative mass spectrometry using synthetic ergosterol and 5,7-cholestadien-3β-ol (Steraloids Inc.).

Spike Amounts for Quantitative Lipidomics. Before lipid extraction the yeast cell lysates were mixed with 20 μl internal lipid standard mixture providing a spike of 23 pmol of PA 17:0–14:1, 47 pmol of PE 17:0–14:1, 12 pmol of PG 17:0–14:1, 39 pmol of PS 17:0–14:1, 47 pmol of PC 17:0–14:1, 50 pmol of PI 17:0–17:0, 12 pmol of DAG 17:0–17:0, 12 pmol of Cer 18:0;3/18:0;0, 15 pmol of TAG 17:1–17:1–17:1, 50 pmol of LCB 17:0;2, 50 pmol of LCBP 17:0;2, 10 pmol of LPI 17:0, 10 pmol of LPC 17:1, 20 pmol of LPE 17:1, 15 pmol of LPS 17:1, 15 pmol of LPA 17:1, 15 pmol of CL 15:0–15:0:15:0–16:1, 40 pmol of IPC 18:0;2/26:0;0, 40 pmol of MIPC 18:0;2/26:0;0, 20 pmol of $M(IP)_2C$ 18:0;2/26:0;0 and 140 pmol of stigmasta-5,7,22-trienol.

Estimating Lipid Extraction Recovery. Isolated 17:1 and 2:1 phase lipid extracts were spiked with a second mixture of internal standards (Table S1). The recovery of IPC, MIPC and $M(IP)_2C$ species was estimated using *elo3*Δ cell lysates. The recovery of ergosterol was estimated using cell lysates from *erg6*Δ. Lipid recovery of the two-step lipid extraction procedure was compared to the Bligh and Dyer lipid extraction protocol (2) performed with chloroform/methanol/H_2O (1:1:0.9, V/V/V) at room temperature. Lipid extracts were vacuum evaporated, dissolved in 100 μl chloroform/methanol (1:2) and analyzed by quantitative mass spectrometry as described below.

Lipid Analysis by Quadrupole Time-of-flight Mass Spectrometry (QSTAR Pulsar-*i*). Glycerophospholipid and DAG species were detected by MPIS analysis (3–5): 10 μl lipid extract was loaded on top of 10 μl 0.4 mM methylamine in methanol in a polypropylene 96-well plate (Eppendorf). The plate was covered with aluminum foil. Samples were infused in negative ion mode using a back pressure of 0.5 psi and ionization voltage of −0.9 kV. PC, Cer and TAG species recovered in 17:1 phase lipid extracts were monitored by consecutive positive ion mode PIS *m/z* 184.1 (3, 5) and MRM analysis, respectively: 10 μl lipid extract was loaded on top of 12.9 μl 13.3 mM ammonium acetate in 2-propanol in a 96-well plate, and infused using a back pressure of 1.25 psi and ionization voltage of 0.95 kV.

For the quantification of ergosterol (6) and intermediate sterol lipids 40 μl aliquots of 17:1 phase lipid extracts were loaded in a 96-well plate, vacuum evaporated and acetylated using 110 μl of acetic anhydride/chloroform (1/12, V/V). Samples were then dissolved in 20 μl 7.5 mM ammonium acetate in chloroform/methanol/2-propanol (1:2:4, V/V/V) and analyzed by MRM in positive ion mode using the transitions [ergosterol acetate+NH_4]$^+$ *m/z* 456.4 → 379.34, [stigmasta-5,7,22-trienol acetate+NH_4]$^+$ *m/z* 470.4 → 393.35, [ergostatetraenol acetate+NH_4]$^+$ *m/z* 454.4 → 377.32, [ergostadienol acetate+NH_4]$^+$ *m/z* 458.4 → 381.35, [cholestatrienol acetate+NH_4]$^+$ *m/z* 442.4 → 365.32 and [cholestadienol acetate+NH_4]$^+$ *m/z* 444.4 → 367.34. Table S2 (below) provides a summary of the applied mass spectrometric methods. Acquired spectra were processed by Lipid Profiler software (MDS Sciex) for isotope correction, identification and quantification of detected lipid species as previously described (3).

Lipid Analysis by Linear Ion Trap-orbitrap Mass Spectrometry (LTQ Orbitrap). IPC, MIPC, $M(IP)_2C$, LCBP and anionic lysoglycerophospholipid species recovered in the 2:1 phase lipid extracts were detected by negative ion mode FT MS analysis (1) applying low *m/z* range scans (*m/z* 200–605) and high *m/z* range scans (*m/z* 505-1400) with target mass resolution of 100000 (fwhm). The 2:1 phase lipid extracts were infused using methylamine as outlined above. TAG, LPC, LPE and LCB species recovered in the 17:1 phase lipid extracts were monitored by positive ion mode FT MS analysis (1, 7) applying looped low *m/z* range scans (*m/z* 260–530) and high *m/z* range scans (*m/z* 500-1050) with target mass resolution of 100000 (fwhm). The 17:1 phase lipid extracts were infused using ammonium acetate as outlined above. The structures of detected lipid precursors were verified by MS^n analysis (1, 7).

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2. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37:911–917.
3. Ejsing CS, *et al.* (2006) Automated identification and quantification of glycerophospholipid molecular species by multiple precursor ion scanning. *Anal Chem* 78:6202–6214.
4. Ekroos K, Chernushevich IV, Simons K, Shevchenko A (2002) Quantitative profiling of phospholipids by multiple precursor ion scanning on a hybrid quadrupole time-of-flight mass spectrometer. *Anal Chem* 74:941–949.
5. Ekroos K, *et al.* (2003) Charting molecular composition of phosphatidylcholines by fatty acid scanning and ion trap MS3 fragmentation. *J Lipid Res* 44:2181–2192.
6. Liebisch G, *et al.* (2006) High throughput quantification of cholesterol and cholesteryl ester by electrospray ionization tandem mass spectrometry (ESI-MS/MS). *Biochim Biophys Acta* 1761:121–128.
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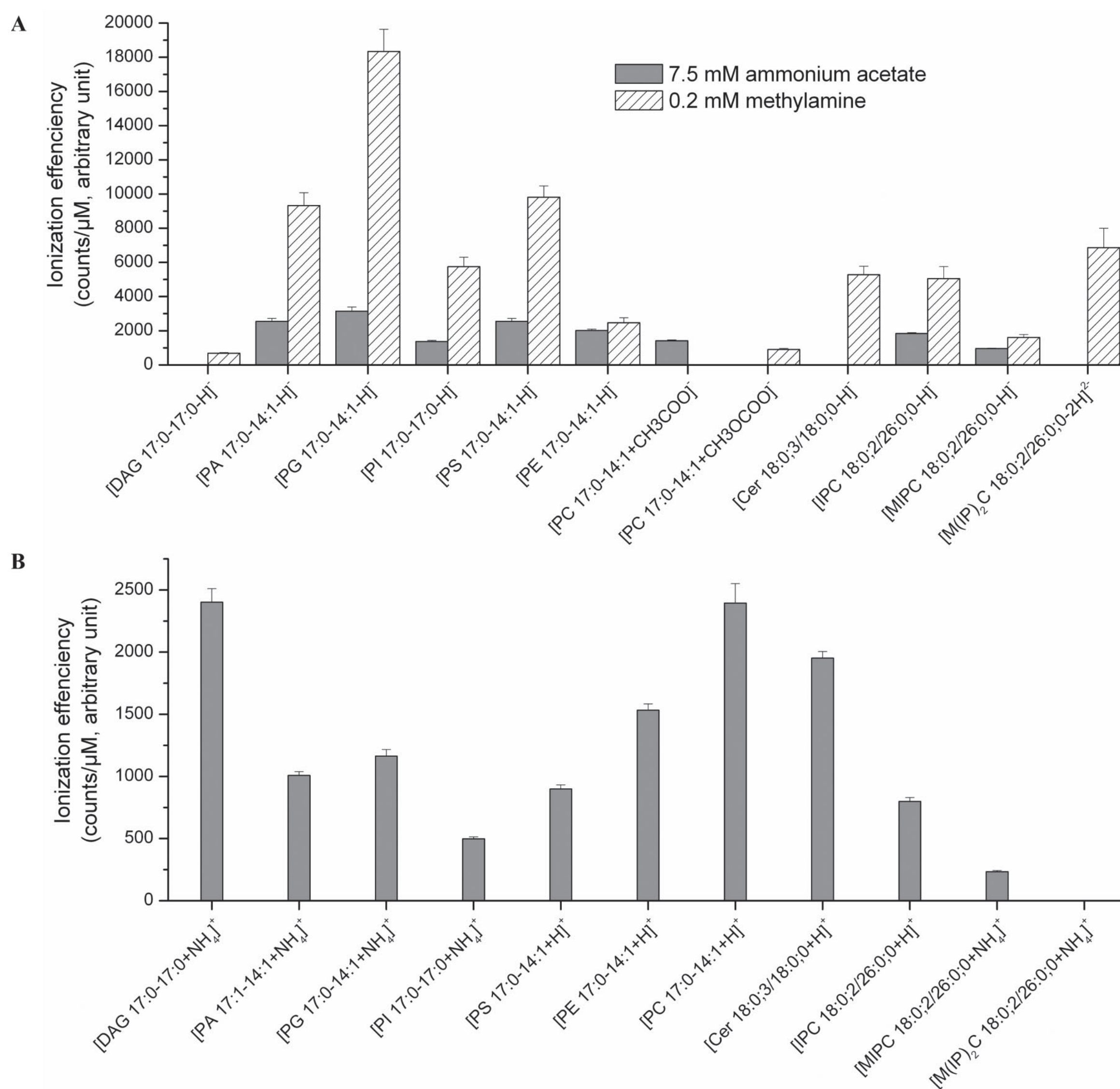

Fig. S1. Selective ionization enables sensitive detection of anionic glycerophospholipid and sphingolipid species. (*A*) Negative ionization efficiency. A mixture of the specified lipid standards was analyzed by time-of-flight mass spectrometry in negative ion mode. The lipid mixture was infused using 7.5 mM ammonium acetate in chloroform/methanol/2-propanol (1:2:4) or 0.2 mM methylamine in chloroform/methanol (1:5). Ionization efficiency was calculated as the lipid precursor intensity divided by its concentration. Note that using methylamine renders PC methyl carbonate adduct ions (CH_3OCOO^-). (*B*) Positive ionization efficiency. The mixture of the lipid standards was analyzed by in positive ion mode. The lipid mixture was infused using 7.5 mM ammonium acetate in chloroform/methanol/2-propanol (1:2:4). Ionization efficiency was calculated as the lipid precursor intensity divided by its concentration. Error bars indicate ± SD. (n = 3).

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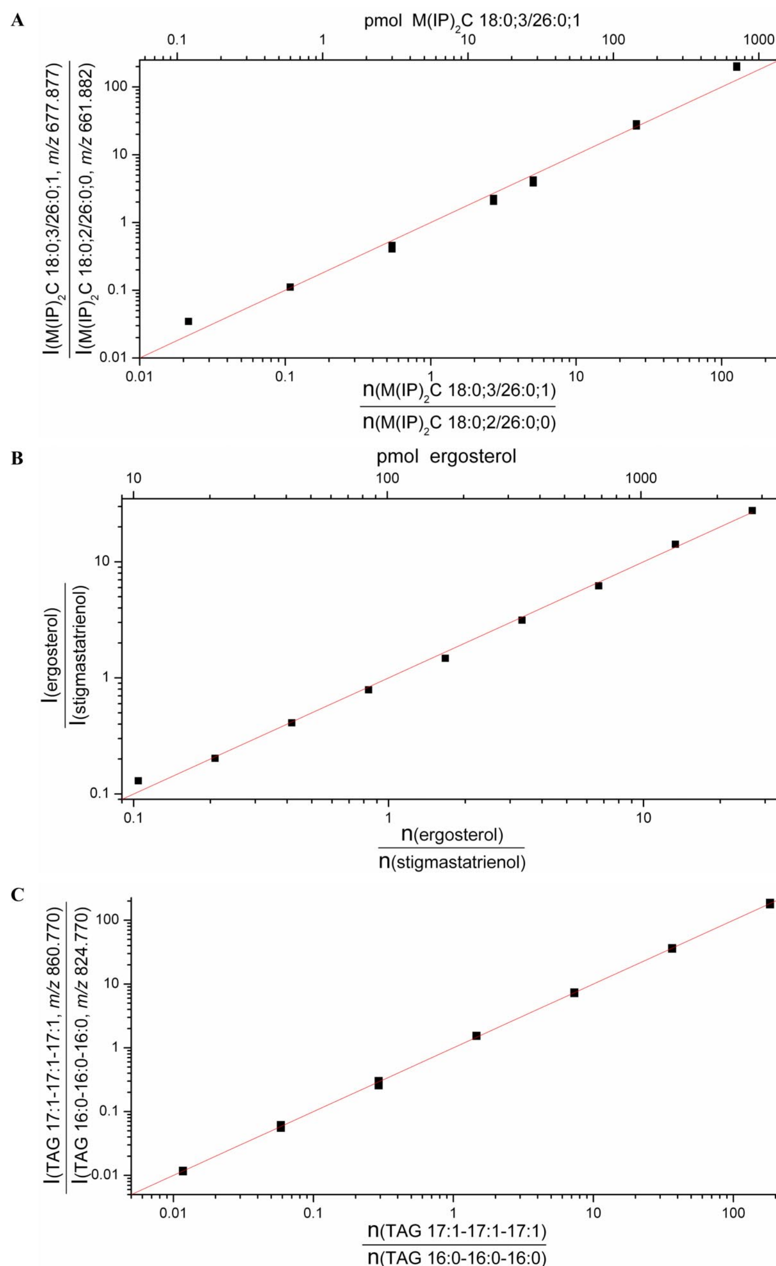

Fig. S2. Dynamic quantification of the shotgun lipidomics platform. (*A*) Dynamic quantification range of $M(IP)_2C$ analysis. Purified wild-type $M(IP)_2C$ 18:0;/26:0;1 was titrated relative to a constant amount of internal standard $M(IP)_2C$ 18:0;2/26:0;0, and spiked into *elo3*Δ cell lysates followed by two-step lipid extraction. The isolated 2:1 phase lipid extracts were analyzed in negative ion mode using 0.2 mM methylamine and high-mass resolution FT MS. The upper *x* axis shows the absolute spike amount of $M(IP)_2C$ 18:0;3/26:0;1 (0.2–700 pmol). The lower *x* axis shows the relative spike amount of $M(IP)_2C$ 18:0;3/26:0;1 compared to $M(IP)_2C$ 18:0;2/26:0;0. The *y* axis shows the peak intensity ratio of *m/z* 677.877 ($M(IP)_2C$ 18:0;3/26:0;1) relative to *m/z* 661.882 ($M(IP)_2C$ 18:0;2/26:0;0). Values derived from FT MS analysis of two independent titration experiments. The line indicates the linear function with slope 1. (*B*) Dynamic quantification range of ergosterol analysis. Ergosterol standard was titrated relative to a constant amount of internal standard stigmastatrienol, and spiked into *erg6*Δ cell lysates followed by lipid extraction using chloroform/methanol (17:1). The 17:1 phase lipid extracts were vacuum evaporated, and mixed with acetic anhydride to produce sterol acetate. Derivatized samples were analyzed in positive ion mode using ammonium acetate and MRM analysis with the transitions *m/z* 456.4 → 379.34 ([ergosterol acetate+NH_4]$^+$) and *m/z* 470.4 → 393.35 ([stigmastatrienol acetate+NH_4]$^+$). The upper *x* axis shows the absolute spike amount of ergosterol (10–2700 pmol). The lower *x* axis shows the spike amount of ergosterol relative to the internal standard stigmastatrienol. The *y* axis shows the peak intensity ratio *m/z* 379.34 in the MS/MS spectrum of *m/z* 456.4 (I(ergosterol)) relative to *m/z* 393.35 in the MS/MS spectrum of *m/z* 470.4 (I(stigmastatrienol)). The line indicates the linear function with slope 1. (*C*) Dynamic quantification range of TAG analysis. TAG 17:1–17:1–17:1 was titrated relative to a constant amount of TAG 16:0–16:0–16:0, and spiked into wild-type cell lysates followed by lipid extraction using chloroform/methanol (17:1). The 17:1 phase lipid extracts were vacuum evaporated. The lipid extracts were analyzed in positive ion mode using 7.5 mM ammonium acetate and high-mass resolution FT MS. The lower *x* axis shows the spike amount of TAG 17:1–17:1–17:1 relative to the standard TAG 16:0–16:0–16:0. The *y* axis shows the peak intensity ratio of *m/z* 860.770 (TAG 17:1–17:1–17:1) relative to *m/z* 824.770 (TAG 16:0–16:0–16:0). Values derived from FT MS analysis of two independent titration experiments. The line indicates the linear function with slope 1.

Fig. S3. Comparative lipidomics of wild-type BY4741 cultured at 24 °C and 37 °C. (*A*) Molecular lipid composition of wild-type BY4741 cultured at 37 °C (quantification of 131 molecular lipid species). (*B*) Lipid class composition. (*C*) Fatty acid profile of all detected glycerophospholipid and glycerolipid species. Error bars indicate ± SD. (n = 4).

A mol% *elo1*Δ pmol

B mol% *elo2*Δ pmol

C mol% *elo3*Δ pmol

Fig. S4. Molecular lipid composition of (*A*) *elo1*Δ (quantification of 159 molecular lipid species), (*B*) *elo2*Δ (quantification of 149 molecular lipid species) and (*C*) *elo3*Δ (quantification of 162 molecular lipid species). Error bars indicate ± SD. ($n = 4$).

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Fig. S5. Molecular composition of (*A*) PI and LPI, (*B*) PC and LPC, (*C*) PE and LPE, (*D*) PS and LPS, (*E*) PA and LPA, (*F*) DAG species and (*G*) TAG species. Error bars indicate ± SD. ($n = 4$).

E

mol% PA and LPA species

BY4741
*elo1*Δ
*elo2*Δ
*elo3*Δ

LPA 14:0, LPA 16:1, LPA 16:0, LPA 18:1, PA 12:0-16:1, PA 12:0-16:0, PA 14:1-16:0, PA 14:0-16:1, PA 16:1-16:1, PA 16:0-16:1, PA 16:0-16:0, PA 16:1-18:1, PA 16:0-18:1, PA 18:0-16:1, PA 18:1-18:1, PA 18:0-18:1

F

mol% DAG species

BY4741
*elo1*Δ
*elo2*Δ
*elo3*Δ

DAG 12:0-16:1, DAG 12:0-16:0, DAG 14:1-16:1, DAG 14:0-16:1, DAG 14:0-16:0, DAG 16:1-16:1, DAG 16:0-16:1, DAG 16:0-16:0, DAG 16:1-18:1, DAG 16:0-18:1, DAG 18:1-18:1, DAG 18:0-18:1

G

mol% TAG species

BY4741
*elo1*Δ
*elo2*Δ
*elo3*Δ

Fig. S5. Continued.

Table S1. Lipid recovery of the 2-step lipid extraction procedure

Lipid class	17:1 phase lipid extract	2:1 phase lipid extract	Bligh and Dyer	Lipid standard 1	Lipid standard 2
DAG	91%	6%	85%	DAG 17:0–17:0	DAG 16:0–16:0
TAG	99%	1%	ND	TAG 17:1–17:1–17:1	TAG 16:0–16:0–16:0
PE	81%	4%	99%	PE 17:0–14:1	PE 17:0–17:0
LPE	40%	33%	ND	LPE 17:1	LPE 14:0
PC	95%	4%	80%	PC 17:0–14:1	PC 17:0–17:0
LPC	72%	11%	ND	LPC 17:1	LPC 15:0
PG	83%	12%	85%	PG 17:0–14:1	PG 17:0–17:0
LCB*	81%	18%	ND	LCB 17:0;2	LCB 18:0;3
Cer	95%	4%	100%	Cer 18:0;3/18:0;0	Cer 18:0;2/24:0;0
Ergosterol†	92%	8%	85%	Ergosterol	Stigmasta-5,7,22-trienol
PA	6%	94%	86%	PA 17:0–14:1	PA 17:0–17:0
LPA	3%	57%	ND	LPA 17:1	LPA 14:0
PS	10%	90%	100%	PS 17:0–14:1	PS 17:0–17:0
LPS	2%	65%	ND	LPS 17:1‡	none
PI	4%	95%	80%	PI 17:0–17:0	PI 17:0–14:1
LPI	1%	70%	ND	LPI 17:0§	none
CL	20%	72%	84%	CL 15:0–15:0–15:0–16:1	CL 14:0–14:0–14:0–14:0
LCBP*	0%	61%	ND	LCBP 17:0;2	LCBP 18:0;3
IPC	2%	95%	95%	IPC 18:0;2/26:0;0	IPC 18:0;3/26:0;1
MIPC	2%	84%	87%	MIPC 18:0;2/26:0;0	MIPC 18:0;3/26:0;1
$M(IP)_2C$	1%	74%	19%	$M(IP)_2C$ 18:0;2/26:0;0	$M(IP)_2C$ 18:0;3/26:0;1

Coefficient of variation for estimated recoveries was between 1 and 10%. Values were estimated from 4 independent experiments. Lipid standards were spiked into 0.2 OD_{600} units of *elo3*Δ cell lysates unless another yeast strain is specified. The Blight and Dyer protocol was executed using chloroform/methanol/H_2O (1:1:0.9, V/V/V). ND, not determined.

*Performed using wild-type BY4741 cell lysate.

†Performed using *erg6*Δ cell lysate.

‡Extraction recovery was estimated by comparison to PS 17:0–17:0.

§Extraction recovery was estimated by comparison to PI 17:0–14:1.

Table S2. Lipid standards and mass spectrometric methods used for quantitative lipid analysis

Lipid class	Lipid extract	Internal lipid standard	MS methods
DAG	17:1 phase lipid extract	DAG 17:0–17:0	−MPIS
PE		PE 17:0–14:1	−MPIS
PG		PG 17:0–14:1	−MPIS
PC		PC 17:0–14:1	+PIS *m/z* 184.1/ -MPIS
TAG		TAG 17:1–17:1–17:1	+FT MS/ +MRM
LPE		LPE 17:1	+FT MS
LPC		LPC 17:1	+FT MS
LCB		LCB 17:0;2	+FT MS
Cer		Cer 18:0;3/18:0;0	+MRM
Sterols		Stigmastatrienol*	+MRM (after acetylation)
PA	2:1 phase lipid extract	PA 17:0–14:1	−MPIS
PS		PS 17:0–14:1	−MPIS
PI		PI 17:0–17:0	−MPIS
LPA		LPA 17:1	−FT MS
LPS		LPS 17:1	−FT MS
LPI		LPI 17:0	−FT MS
CL		CL 15:0–15:0–15:0–16:1	−FT MS
LCBP		LCBP 17:0;2	−FT MS
IPC		IPC 18:0;2/26:0;0†	−FT MS
MIPC		MIPC 18:0;2/26:0;0†	−FT MS
$M(IP)_2C$		$M(IP)_2C$ 18:0;2/26:0;0†	−FT MS

+ and − indicate positive and negative ion mode analysis, respectively. FT MS analysis was performed using looped low *m/z* range and high *m/z* range scans (see *SI Material and Methods* for details).
*Synthesized from stigmasta-5,7,22-trienol acetate (see *SI Material and Methods* for details).
†Isolated from the mutant strain *sur2*Δ*scs7*Δ (see *SI Material and Methods* for details).

Other Supporting Information Files

Dataset S1