Supplementary material

Prieto et al. (2019)

Contents:

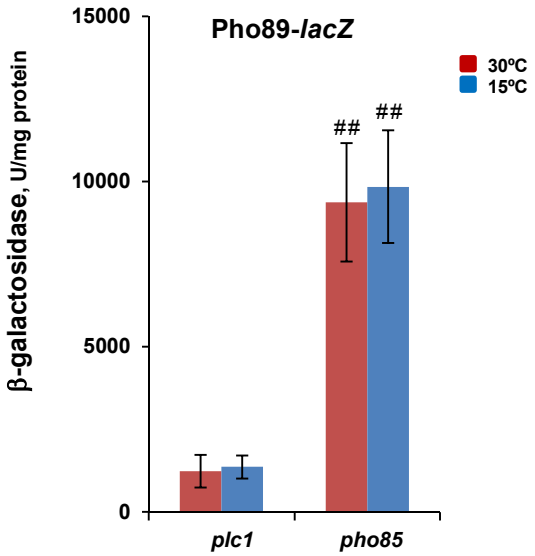

Fig. S1. The expression of *PHO89* does not vary in cold-shocked cells of the *plc1* and *pho85* mutants. PHO89*::lacZ* transformants of the CEN.PK2-1C mutants *plc1* and *pho85* were grown at 30ºC in SCD, transferred to 15ºC for 3 h, and the β-galactosidase activity of cellular extracts was analyzed as indicated in the Materials and methods section. Data represent the mean value (± SD) of three independent experiments. Activity differences between *plc1* and *pho85* samples were statistically significant at both 30 and 15ºC (##; $p < 0.01$).

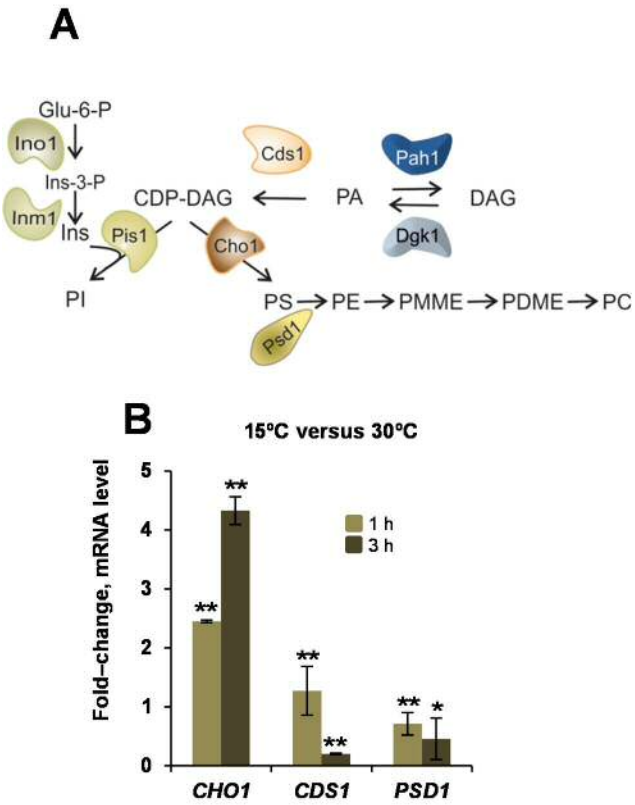

Fig. S2. Cold stimulates the expression of UAS_{INO} sequences containing genes. A) Schematic representation of pathways in which the phospholipid biosynthetic genes *CHO1*, which encodes the phosphatidylserine synthase, *CDS1*, the yeast CDP-diacylglycerol synthase and *PSD1*, encoding the phosphatidylserine decarboxylase, are involved. Details are given in the text and reviews [79]. B) Cells of the CEN.PK2-1C wild-type (wt) strain were grown to the mid-logarithmic phase in SCD medium at 30ºC. An aliquot was withdrawn for the analysis and the rest of the culture was shifted to 15ºC for 1 h or 3 h. Samples were analyzed for total mRNA levels of the mentioned genes by qPCR as indicated in the Materials and methods section. Expression differences between control (30ºC) and cold-treated (15ºC) samples are represented as the fold-change (*; $p<0.05$) (**; $p<0.01$). Data represent the mean (± SD) of at least three independent experiments.

Table S1. The *Saccharomyces cerevisiae* strains used in this study

Strain	Genotype	Reference or source
CEN.PK2-1C	*MATa ura3-52 his3-Δ1 leu-2-3,112 trp1-289*	M. Rose
CEN.PK2-1C *inp51*	CEN.PK2-1C *inp51::natMX4*	[4]
CEN.PK2-1C *plc1*	CEN.PK2-1C *plc1::kanMX4*	This study
CEN.PK2-1C *vip1*	CEN.PK2-1C *vip1::hygMX4*	[4]
CEN.PK2-1C *pho85*	CEN.PK2-1C *pho85*::*hygMX4*	[4]
CEN.PK2-1C Pah1-Myc	CEN.PK2-1C *PAH1*-13myc::*His3MX6*	[4]
CEN.PK2-1C Ypk1-HA	CEN.PK2-1C *YPK1*-3HA::*His3MX6*	This study
CEN.PK2-1C Orm2-HA	CEN.PK2-1C *ORM2*-3HA::*His3MX6*	This study
CEN.PK2-1C *pho85* Pah1-Myc	CEN.PK2-1C *pho85*::*hygMX4 PAH1*-13myc::*His3MX6*	This study
CEN.PK2-1C *pho85* Ypk1-HA	CEN.PK2-1C *pho85*::*hygMX4 YPK1*-3HA::*His3MX6*	This study
CEN.PK2-1C *pho85* Orm2-HA	CEN.PK2-1C *pho85*::*hygMX4 ORM2*-3HA::*His3MX6*	This study
KKT268 Ypk1-HA	*MATa LYS2 ura3Δ0 his3Δ1 leu2Δ0 MET15 fpk1Δ::HphMX4 fpk2Δ::KanMX6 YPK1*-3HA::*His3MX6*	[98]

[98] Nakano K, Yamamoto T, Kishimoto T, Noji T, Tanaka K (2008) Protein kinases Fpk1p and Fpk2p are novel regulators of phospholipid asymmetry. *Mol Biol Cell* 19: 1783-1797.

Table S2. The oligonucleotides used in this study

Name	Sequence	Used for
KAN-S2	GTCAAGGAGGGTATTCTGG	Verification integration
YPK1-F2	ACAGCTAGGTAGCTCAATGGTGCAAGGTAGAAGCATTAGA CGGATCCCCGGGTTAATTAA	Genetic fusion of *HA* to *YPK1*
YPK1-R1	AAATTGCGCCATTGGTACAGTTGCTTCATCTTGAACACAG GAATTCGAGCTCGTTTAAAC	Genetic fusion of *HA* to *YPK1*
YPK1-V	ATTTGGTGGCTGGACATACG	Verification fusion of *HA* to *YPK1*
ORM2-F2	GAATATCCATCCCTGGTATTACGGGCCGTGCTCAAATTAGT CGGATCCCCGGGTTAATTAA	Genetic fusion of *HA* to *ORM2*
ORM2-R1	ACATATATATATATATATATATACATATATGCGTATAGGCA GAGCCAAGAATTCGAGCTCGTTTAAAC	Genetic fusion of *HA* to *ORM2*
ORM2-V	CTGGGAATTACGCATAGA	Verification fusion of *HA* to *ORM2*
PAH1-F2	AATTCGATGACGATGAATTCGACGAAGATGAATTCGAAGATC GGATCCCCGGGTTAATTAA	Genetic fusion of *MYC* to *PAH1*
PAH1-R1	AGTATGGATCGTTATAAATAATATTCGGCTACAAGAATCGAA TTCGAGCTCGTTTAAAC	Genetic fusion of *MYC* to *PAH1*
PAH1-V	CACGAAGGGAGCAAAGTG	Verification fusion of *MYC* to *PAH1*
PLC1-F1	TAAACGTACAACGGTAAGGTCATTCACGCAGTGTATATGCGTA CGCTGCAGGTCGAC	*PLC1* disruption
PLC1-R1	TGTATTGTTCCCCCTCCATGTTAAACAACGGAATGTGACGATC GATGAATTCGAGCTC	*PLC1* disruption
PLC1-V1	ACAGTTACTTTCACCAAGAG	Verification *PLC1* disruption
ACT1-F	GGATCTTCTACTACATCAGC	Quantification by qRT-PCR of *ACT1* mRNA
ACT1-R	CACATACCAGAACCGTTATC	Quantification by qRT-PCR of *ACT1* mRNA
INO1-F	ATTGCTCCAATCACCTCCG	Quantification by qRT-PCR of *INO1* mRNA
INO1-R	CCGAAGTAGTTTGGTTGC	Quantification by qRT-PCR of *INO1* mRNA
ORM2-F	TGAAGAGTCTCCGCTTACC	Quantification by qRT-PCR of *ORM2* mRNA
ORM2-R	TCCATTTGGGCGTCGACC	Quantification by qRT-PCR of *ORM2* mRNA
LCB3-F	AGCATTGGTGGTTTCCTTTG	Quantification by qRT-PCR of *LCB3* mRNA
LCB3-R	CCAGGGTGACTCCAAACACT	Quantification by qRT-PCR of *LCB3* mRNA
LCB4-F	TCGTCAAATATGCTGCCAAA	Quantification by qRT-PCR of *LCB4* mRNA
LCB4-R	AGGTACTGGTTCCGTCATCG	Quantification by qRT-PCR of *LCB4* mRNA
LCB5-F	GCCACTGGACAAACAATCCT	Quantification by qRT-PCR of *LCB5* mRNA
LCB5-R	ACCCAATTCAAACCTTGCAG	Quantification by qRT-PCR of *LCB5* mRNA
YSR3-F	ACTGGTATGGCCAACAAAGC	Quantification by qRT-PCR of *YSR3* mRNA
YSR3-R	AAACAAGCCCCATGCTACAC	Quantification by qRT-PCR of *YSR3* mRNA
DPL1-F	TAGTCGGTGCAGCAATGAAG	Quantification by qRT-PCR of *DPL1* mRNA
DPL1-R	GGCTTTTGTAGGGCATTGAA	Quantification by qRT-PCR of *DPL1* mRNA

Table S3. The plasmids used in this study

Plasmid	Description	Source or reference
pFA6a-kanMX4	pFA-yeast plasmid containing the *kan*^{r} gene, which provide resistance to the drug geneticine. kanMX4 cassette template	[99]
pFA6a-3HA-His3MX6	pFA6a-His3MX6-derived plasmid containing sequences encoding 3 tandem repeats of the influenza virus hemagglutinin epitope	[49]
pPHO89::*lacZ*	Plasmid that contains the *E. coli lacZ* gene under the control of the *PHO89* gene promoter.	[93]
pRS414-7x2-PHO5-GFP-hPLC	Plasmid that contains two repeats of Phospholipase C δ1 PH-domain fused to GFP under the control of the *PHO5* gene promoter	Tim Levine

[99] Wach A, Brachat A, Pöhlmann R, Philippsen P (1994) New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast* 10: 1793-1808.

TABLE S4. Composition, total carbon length and total double bond of TAG molecular species found in *S. cerevisiae* wild-type cells grown at 30ºC and cold-shocked at 15ºC for 3 h.

	mol% ± SD[a]	
TAG species	30ºC	15ºC
C38:0	0.12 ± 0.07	nd
C38:1	0.15 ± 0.01	0.06 ± 0.03
C40:0	0.23 ± 0.06	0.11 ± 0.02*
C40:1	0.56 ± 0.05	0.31 ± 0.01**
C42:0	0.36 ± 0.09	0.28 ± 0.03
C42:1	1.77 ± 0.07	1.37 ± 0.06*
C42:2	1.87 ± 0.05	0.99 ± 0.01**
C44:0	0.41 ± 0.18	0.31 ± 0.18
C44:1	2.41 ± 0.02	2.44 ± 0.06
C44:2	3.88 ± 0.04	2.61 ± 0.06**
C44:3	0.14 ± 0.01	nd
C46:1	1.95 ± 0.08	2.07 ± 0.02*
C46:2	5.47 ± 0.09	4.35 ± 0.05**
C46:3	1.87 ± 0.02	0.97 ± 0.02**
C48:1	1.76 ± 0.15	1.93 ± 0.03
C48:2	9.91 ± 0.16	9.75 ± 0.09
C48:3	13.63 ± 0.14	12.02 ± 0.10**
C50:2	9.88 ± 0.10	11.35 ± 0.05**
C50:3	22.57 ± 0.23	23.37 ± 0.39
C52:2	5.29 ± 0.09	7.05 ± 0.63*
C52:3	10.87 ± 0.17	13.45 ± 0.19**
C54:2	1.63 ± 0.03	2.19 ± 0.08**
C54:3	1.32 ± 0.07	1.68 ± 0.03**
C56:1	0.21 ± 0.12	0.20 ± 0.02
C56:2	0.34 ± 0.02	0.32 ± 0.01
C56:3	0.17 ± 0.04	0.15 ± 0.09
C58:2	0.58 ± 0.01	0.49 ± 0.03*
C60:2	0.73 ± 0.05	0.57 ± 0.33*
Total carbon length		
C38	0.27 ± 0.07	0.06 ± 0.03
C40	0.78 ± 0.10	0.42 ± 0.01*
C42	4.00 ± 0.19	2.64 ± 0.03**
C44	6.84 ± 0.20	5.25 ± 0.12**
C46	9.29 ± 0.01	7.38 ± 0.09**
C48	25.30 ± 0.23	23.71 ± 0.21**
C50	32.45 ± 0.29	34.72 ± 0.43*
C52	16.16 ± 0.20	20.51 ± 0.45**
C54	2.95 ± 0.03	3.87 ± 0.06**
C56	0.65 ± 0.11	0.62 ± 0.10
C58	0.58 ± 0.01	0.49 ± 0.03*
C60	0.01 ± 0.00	nd
Total double bond		
C:0	1.12 ± 0.39	0.59 ± 0.16
C:1	8.73 ± 0.23	8.34 ± 0.16*
C:2	39.58 ± 0.32	39.48 ± 0.75
C:3	50.57 ± 0.31	51.59 ± 0.74

[a]Values are mean ± SD of 3 independent replicates. Significance level: * $p < 0.05$; ** $p < 0.01$. nd, non detected.

TABLE S5. Composition, total carbon length and total double bond of SE molecular species found in *S. cerevisiae* wild-type cells grown at 30ºC and cold-shocked at 15ºC for 3 h.

	mol% ± SD[a]	
SE species	30ºC	15ºC
C10:0	0.24 ± 0.01	0.4 ± 0.03*
C12:0	0.71 ± 0.06	1.02 ± 0.13*
C14:0	0.99 ± 0.05	1.50 ± 0.10**
C14:1	0.82 ± 0.04	1.35 ± 0.05**
C16:0	12.70 ± 0.18	16.18 ± 0.02*
C16:1	43.19 ± 0.43	52.83 ± 6.57
C18:0	8.36 ± 0.11	7.4 ± 1.08
C18:1	32.04 ± 0.33	24.31 ± 2.15*
C20:0	0.95 ± 0.07	0.81 ± 0.11*
Total carbon length		
C10	0.24 ± 0.01	0.40 ± 0.03*
C12	0.71 ± 0.06	1.02 ± 0.13*
C14	1.80 ± 0.05	2.85 ± 0.13**
C16	55.89 ± 0.40	63.62 ± 2.77*
C18	40.40 ± 0.40	31.71 ± 3.22*
C20	0.95 ± 0.07	0.81 ± 0.11
Total double bond		
C:0	23.95 ± 0.30	26.57 ± 0.27**
C:1	76.05 ± 0.30	73.43 ± 0.27**

[a]Values are mean ± SD of 3 independent replicates. Significance level: * $p < 0.05$; ** $p < 0.01$. nd, non detected.

TABLE S6. Composition, total carbon length and total double bond of DAG molecular species found in *S. cerevisiae* wild-type cells grown at 30ºC and cold-shocked at 15ºC for 3 h.

	mol% ± SD[a]	
DAG species	30ºC	15ºC
C30:0	0.94 ± 0.04	1.27 ± 0.68
C30:1	2.35 ± 0.09	4.47 ± 0.06**
C30:2	0.51 ± 0.04	1.76 ± 0.12**
C32:1	13.07 ± 0.09	13.95 ± 0.17*
C32:2	24.12 ± 0.38	22.69 ± 0.25*
C34:1	18.77 ± 0.08	15.33 ± 0.10**
C34:2	30.21 ± 0.30	31.11 ± 1.19
C36:0	6.26 ± 0.01	6.01 ± 0.80
C36:1	3.08 ± 0.05	3.02 ± 0.04
C42:1	1.02 ± 0.59	1.19 ± 0.69
Total carbon length		
C30	3.80 ± 0.10	7.50 ± 0.79*
C32	37.19 ± 0.44	36.65 ± 0.16
C34	48.98 ± 0.27	46.43 ± 1.26*
C36	9.34 ± 0.06	9.03 ± 0.77
C42	0.68 ± 0.59	0.40 ± 0.69
Total double bond		
C:0	0.94 ± 0.04	1.27 ± 0.68
C:1	41.13 ± 0.51	40.15 ± 0.89*
C:2	57.94 ± 0.55	58.58 ± 1.36

[a]Values are mean ± SD of 3 independent replicates. Significance level: * $p < 0.05$; ** $p < 0.01$. nd, non detected.

TABLE S7. Composition, total carbon length and total double bond of PA molecular species found in *S. cerevisiae* wild-type cells grown at 30ºC and cold-shocked at 15ºC for 3 h.

	mol% ± SD[a]	
PA species	30ºC	15ºC
C28:1	1.45 ± 0.22	2.03 ± 0.23
C30:1	2.34 ± 0.20	3.03 ± 0.27**
C30:2	0.86 ± 0.50	1.68 ± 0.46
C32:1	11.54 ± 0.41	12.67 ± 0.35
C32:2	23.41 ± 0.63	24.21 ± 1.87
C34:1	19.34 ± 0.90	15.94 ± 1.24*
C34:2	39.89 ± 0.46	39.07 ± 1.18
C36:2	1.46 ± 0.10	1.39 ± 0.22
Total carbon length		
C28	1.12 ± 0.25	1.92 ± 0.76
C30	2.92 ± 0.51	4.7 ± 0.71*
C32	34.94 ± 0.57	36.87 ± 1.52
C34	59.23 ± 1.06	55.01 ± 2.38
C36	1.46 ± 0.10	1.39 ± 0.22
Total double bond		
C:0	nd	nd
C:1	34.67 ± 1.08	33.66 ± 1.15
C:2	65.33 ± 1.08	66.34 ± 1.15

[a]Values are mean ± SD of 3 independent replicates. Significance level: * $p < 0.05$; ** $p < 0.01$. nd, non detected.

TABLE S8. Composition, total carbon length and total double bond of PC molecular species found in *S. cerevisiae* wild-type cells grown at 30ºC and cold-shocked at 15ºC for 3 h.

	mol% ± SD[a]	
PC species	30ºC	15ºC
C28:0	0.27 ± 0.01	nd
C28:1	3.26 ± 0.15	2.83 ± 0.13*
C28:2	0.06 ± 0.03	0.07 ± 0.04
C30:0	0.11 ± 0.02	nd
C30:1	2.66 ± 0.04	1.54 ± 0.04**
C30:2	1.65 ± 0.04	2.19 ± 0.03**
C32:1	6.22 ± 0.07	1.69 ± 0.15**
C32:2	39.66 ± 0.07	45.66 ± 0.42**
C34:1	3.6 ± 0.08	0.54 ± 0.13**
C34:2	38.99 ± 0.21	42.87 ± 0.23**
C36:1	0.61 ± 0.01	0.07 ± 0.04**
C36:2	2.90 ± 0.04	2.60 ± 0.03**
C38:2	0.06 ± 0.03	nd
Total carbon length		
C28	3.56 ± 0.16	2.88 ± 0.16*
C30	4.42 ± 0.02	3.72 ± 0.05**
C32	45.88 ± 0.04	47.34 ± 0.31**
C34	42.59 ± 0.18	43.41 ± 0.14**
C36	3.51 ± 0.05	2.65 ± 0.07**
C38	0.04 ± 0.03	nd
Total double bond		
C:0	0.38 ± 0.03	nd
C:1	16.35 ± 0.29	6.65 ± 0.47**
C:2	83.27 ± 0.28	93.35 ± 0.47**

[a]Values are mean ± SD of 3 independent replicates. Significance level: * $p < 0.05$; ** $p < 0.01$. nd, non detected.

TABLE S9. Composition, total carbon length and total double bond of PE molecular species found in *S. cerevisiae* wild-type cells grown at 30ºC and cold-shocked at 15ºC for 3 h.

	mol% ± SD[a]	
PE species	30ºC	15ºC
C26:0	0.11 ± 0.01	0.08 ± 0.01
C26:1	0.13 ± 0.01	0.17 ± 0.02*
C28:0	0.25 ± 0.02	0.17 ± 0.00**
C28:1	0.82 ± 0.04	0.91 ± 0.07
C30:1	1.51 ± 0.00	1.49 ± 0.01*
C30:2	0.37 ± 0.02	0.72 ± 0.02**
C32:1	9.52 ± 0.17	8.96 ± 0.05*
C32:2	23.83 ± 0.12	22.13 ± 0.26**
C34:1	10.75 ± 0.02	10.26 ± 0.09**
C34:2	48.45 ± 0.36	50.92 ± 0.36*
C36:2	4.12 ± 0.12	4.14 ± 0.03
C38:2	0.13 ± 0.01	0.09 ± 0.05
Total carbon length		
C26	0.24 ± 0.01	0.24 ± 0.02
C28	1.07 ± 0.05	1.08 ± 0.07
C30	1.88 ± 0.02	2.21 ± 0.01**
C32	33.35 ± 0.19	31.09 ± 0.31**
C34	59.21 ± 0.34	61.18 ± 0.42*
C36	4.12 ± 0.12	4.14 ± 0.03
C38	0.13 ± 0.01	0.06 ± 0.05
Total double bond		
C:0	0.36 ± 0.02	0.25 ± 0.01
C:1	22.74 ± 0.23	21.79 ± 0.08*
C:2	76.90 ± 0.23	77.96 ± 0.08*

[a]Values are mean ± SD of 3 independent replicates. Significance level: * $p < 0.05$; ** $p < 0.01$. nd, non detected.

TABLE S10. Composition, total carbon length and total double bond of PG molecular species found in *S. cerevisiae* wild-type cells grown at 30ºC and cold-shocked at 15ºC for 3 h.

	mol% ± SD[a]	
PG species	30ºC	15ºC
C32:1	26.94 ± 3.13	27.77 ± 0.47
C32:2	6.82 ± 3.94	14.02 ± 0.53
C34:1	55.38 ± 1.26	34.79 ± 0.13**
C34:2	15.41 ± 1.81	23.41 ± 0.92**
Total carbon length		
C32	29.21 ± 1.41	41.80 ± 0.94**
C34	70.79 ± 1.41	58.20 ± 0.94**
Total double bond		
C:1	82.32 ± 2.13	62.57 ± 0.41**
C:2	17.68 ± 2.13	37.43 ± 0.41**

[a]Values are mean ± SD of 3 independent replicates. Significance level: * $p < 0.05$; ** $p < 0.01$. nd, non detected.

TABLE S11. Composition, total carbon length and total double bond of PI molecular species found in *S. cerevisiae* wild-type cells grown at 30ºC and cold-shocked at 15ºC for 3 h.

	mol% ± SD[a]	
PI species	30ºC	15ºC
C24:0	0.13 ± 0.07	0.33 ± 0.02
C26:0	2.74 ± 0.14	3.83 ± 0.09**
C26:1	0.37 ± 0.02	1.13 ± 0.02**
C28:0	4.68 ± 0.13	4.75 ± 0.14*
C28:1	0.86 ± 0.05	2.62 ± 0.07**
C30:0	1.74 ± 0.01	0.92 ± 0.01**
C30:1	2.84 ± 0.08	5.42 ± 0.09**
C30:2	0.26 ± 0.00	1.27 ± 0.02**
C32:1	22.37 ± 0.15	23.85 ± 0.08**
C32:2	4.76 ± 0.13	7.10 ± 0.02**
C34:1	40.72 ± 0.46	31.34 ± 0.25**
C34:2	8.31 ± 0.09	9.57 ± 0.08**
C36:1	9.35 ± 0.32	6.86 ± 0.10**
C36:2	0.95 ± 0.01	1.01 ± 0.02*
Total carbon length		
C24	0.13 ± 0.07	0.33 ± 0.02
C26	3.11 ± 0.16	4.97 ± 0.10**
C28	5.54 ± 0.17	7.37 ± 0.20**
C30	4.84 ± 0.08	7.6 ± 0.12**
C32	27.13 ± 0.21	30.95 ± 0.08**
C34	49.03 ± 0.39	40.91 ± 0.28**
C36	10.30 ± 0.32	7.87 ± 0.09**
Total double bond		
C:0	9.21 ± 0.33	9.84 ± 0.24**
C:1	76.51 ± 0.50	71.22 ± 0.24**
C:2	14.29 ± 0.21	18.94 ± 0.03**

[a]Values are mean ± SD of 3 independent replicates. Significance level: * $p < 0.05$; ** $p < 0.01$. nd, non detected.

TABLE S12. Composition, total carbon length and total double bond of PS molecular species found in *S. cerevisiae* wild-type cells grown at 30ºC and cold-shocked at 15ºC for 3 h.

	mol% ± SD[a]	
PS species	30ºC	15ºC
C26:0	nd	0.53 ± 0.03
C28:0	0.44 ± 0.07	0.57 ± 0.02
C28:1	0.53 ± 0.04	1.21 ± 0.06**
C30:1	1.18 ± 0.09	2.40 ± 0.06**
C32:1	13.64 ± 0.29	17.97 ± 0.32**
C32:2	6.83 ± 0.09	8.93 ± 0.30**
C34:1	33.89 ± 0.62	29.59 ± 0.79*
C34:2	41.64 ± 0.79	35.37 ± 0.82*
C36:1	0.58 ± 0.05	1.10 ± 0.21*
C36:2	1.46 ± 0.12	2.53 ± 0.34*
Total carbon length		
C26	nd	0.53 ± 0.03
C28	0.93 ± 0.10	1.81 ± 0.01
C30	1.18 ± 0.09	2.40 ± 0.06**
C32	20.46 ± 0.36	26.90 ± 0.07**
C34	75.53 ± 0.18	64.96 ± 0.62**
C36	2.04 ± 0.12	3.62 ± 0.46*
Total double bond		
C:0	0.44 ± 0.07	0.91 ± 0.32*
C:1	49.64 ± 0.65	52.26 ± 1.27
C:2	49.93 ± 0.70	46.82 ± 0.97*

[a]Values are mean ± SD of 3 independent replicates. Significance level: * $p < 0.05$; ** $p < 0.01$. nd, non detected.