Supplementary Material

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

Name	Relevant Genotype	Source
BY4742	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	EUROSCARF
elo3Δ	BY4742; MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 elo3::kanMX4*	EUROSCARF
scs7Δ	BY4742; MATα *his3 Δ 1 leu2 Δ 0 lys2 Δ 0 ura3Δ0 scs7::kanMX4*^{r}	EUROSCARF
W303-1A	MATa ade2-1; *ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*	Cerantola et al, 2009
lac1Δ lag1Δ	W303-1A; *MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 lac1::LEU2 lag1::TRP1*	Cerantola et al, 2009
yor1Δ	BY4742; *MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 yor1::kanMX4*	EUROSCARF
orm1Δ orm2Δ	BY4742; *MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 orm1::clonNAT orm2::kanMX4*	Lab collection

Supplementary Figure Legends

Figure S1. Fragmentation of C17-containing ceramide-C.

Cells were incubated with 25 μM C17-PHS for 60 min, lipids were extracted and analyzed by mass spectrometry. C17-ceramide C at *m/z* = 696.5 was fragmented and product ions were analyzed in negative (panel A) and positive mode (panel B).

Figure S2. Complex sphingolipids are stable.

Cells were pulsed with C17-PHS (50 μM) for 90 min, washed, and then allowed to grow for the indicated period of time. Lipids were extracted and both C17- and C18-PHS containing complex sphingolipids were quantified. Values represent mean ±SD of three independent determinations.

Figure S3. Elevated concentrations of C17-PHS inhibits ceramide and IPC synthesis in the Orm mutant.

Wild-type and *orm1*Δ *orm2*Δ mutant cells were incubated with different concentrations of C17-PHS for 45 min and newly synthesized C17 Cer-C (panel A) and C17 IPC-C (panel B) were quantified. Values represent mean ±SD of three independent determinations. Asterisks denote statistical significance (*P<0.05; **P<0.001; ***P<0.0001; n.s. (non significant)).

Figure S4. The media composition affects steady-state levels of ceramide but not the rate of ceramide and IPC synthesis.

Comparison of steady-state levels of ceramide (panel A) and IPC-C (panel B) of wild-type and Orm mutant cells cultivated either in rich (YPD) or synthetic (SD) media. Comparison of the rate of ceramide (panel C) and IPC-C (panel D) synthesis of wild-type and Orm mutant cells cultivated either in rich (YPD) or synthetic (SD) media. Cells were incubated with C17-PHS (10 μM) for 45 min. Values represent mean ±SD

of three independent determinations. Asterisks denote statistical significance (*P<0.05; ***P<0.0001; n.s. (non significant)).

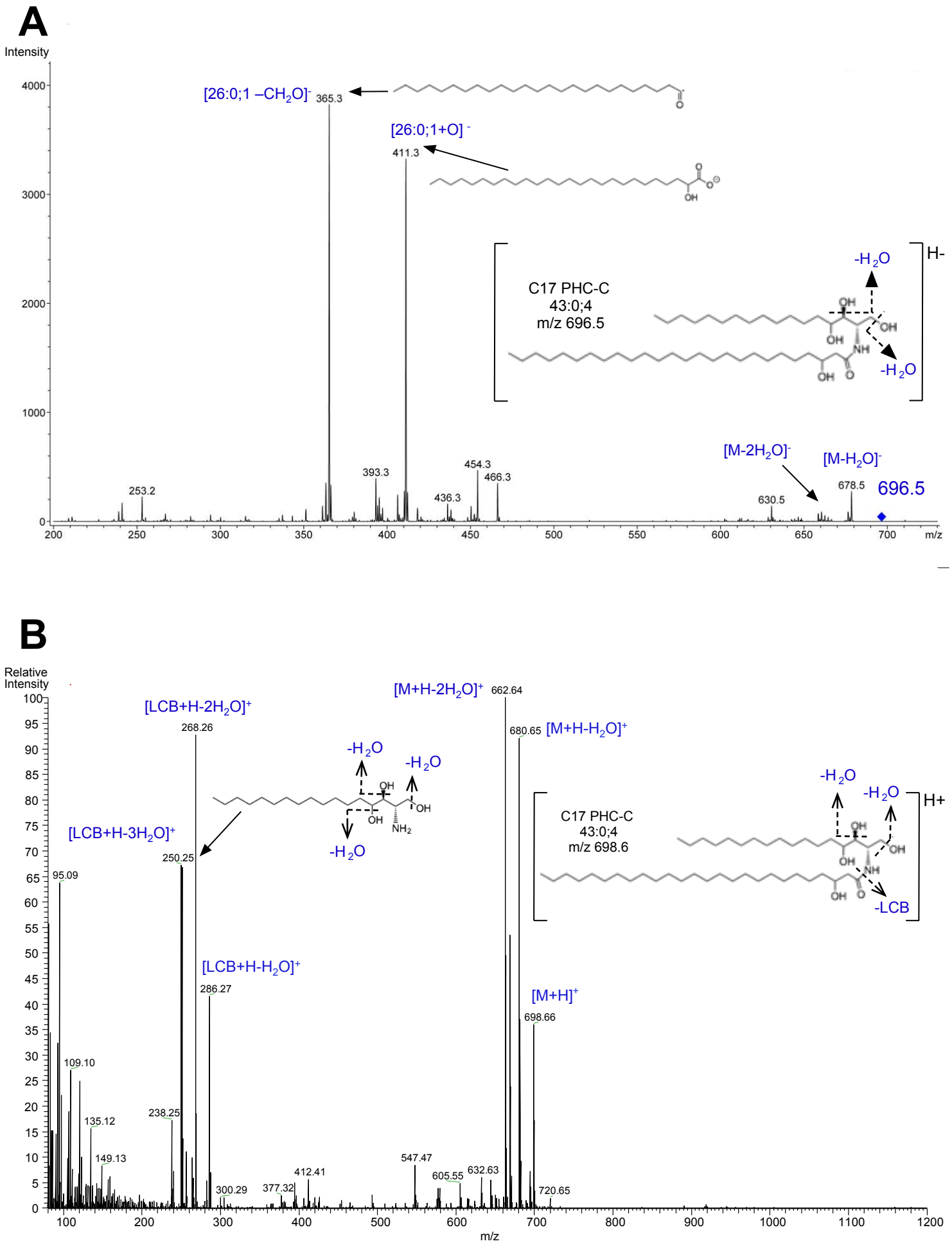
A
Intensity
[26:0;1 –CH2O]- 365.3
[26:0;1+O] -
411.3
C17 PHC-C
43:0;4
m/z 696.5
-H2O
-H2O
H-
[M-2H2O]-
[M-H2O]-
696.5
253.2
393.3
436.3
454.3
466.3
630.5
678.5
m/z
B
Relative
Intensity
[LCB+H-2H2O]+
268.26
[M+H-2H2O]+ 662.64
680.65 [M+H-H2O]+
-H2O
-H2O
-H2O
[LCB+H-3H2O]+
250.25
95.09
C17 PHC-C
43:0;4
m/z 698.6
-H2O
-H2O
H+
-LCB
[LCB+H-H2O]+
286.27
[M+H]+
698.66
109.10
135.12
149.13
238.25
300.29
377.32
412.41
547.47
605.55
632.63
720.65
m/z

Figure S1

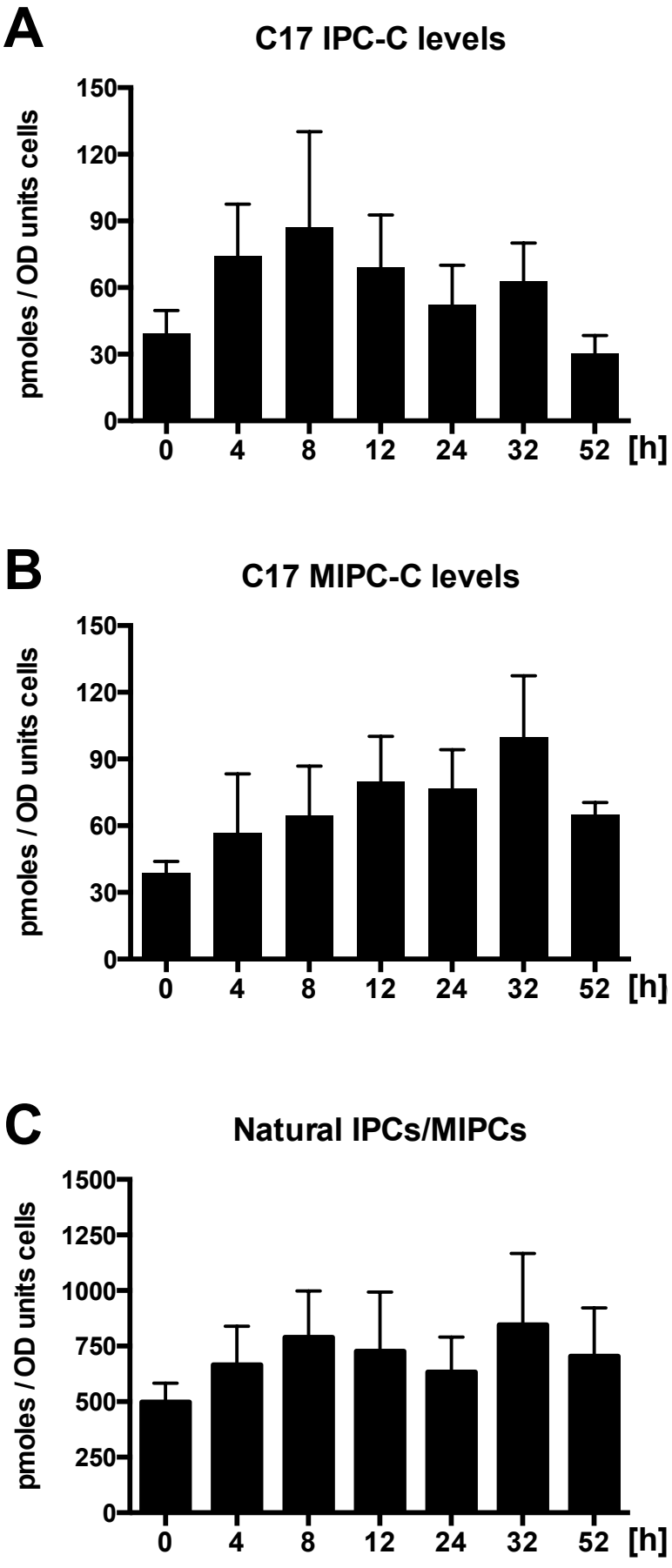
A
C17 IPC-C levels
pmoles / OD units cells
150
120
90
60
30
0
0
4
8
12
24
32
52
[h]
B
C17 MIPC-C levels
pmoles / OD units cells
150
120
90
60
30
0
0
4
8
12
24
32
52
[h]
C
Natural IPCs/MIPCs
pmoles /OD units cells
1500
1250
1000
750
500
250
0
0
4
8
12
24
32
52
[h]

Figure S2

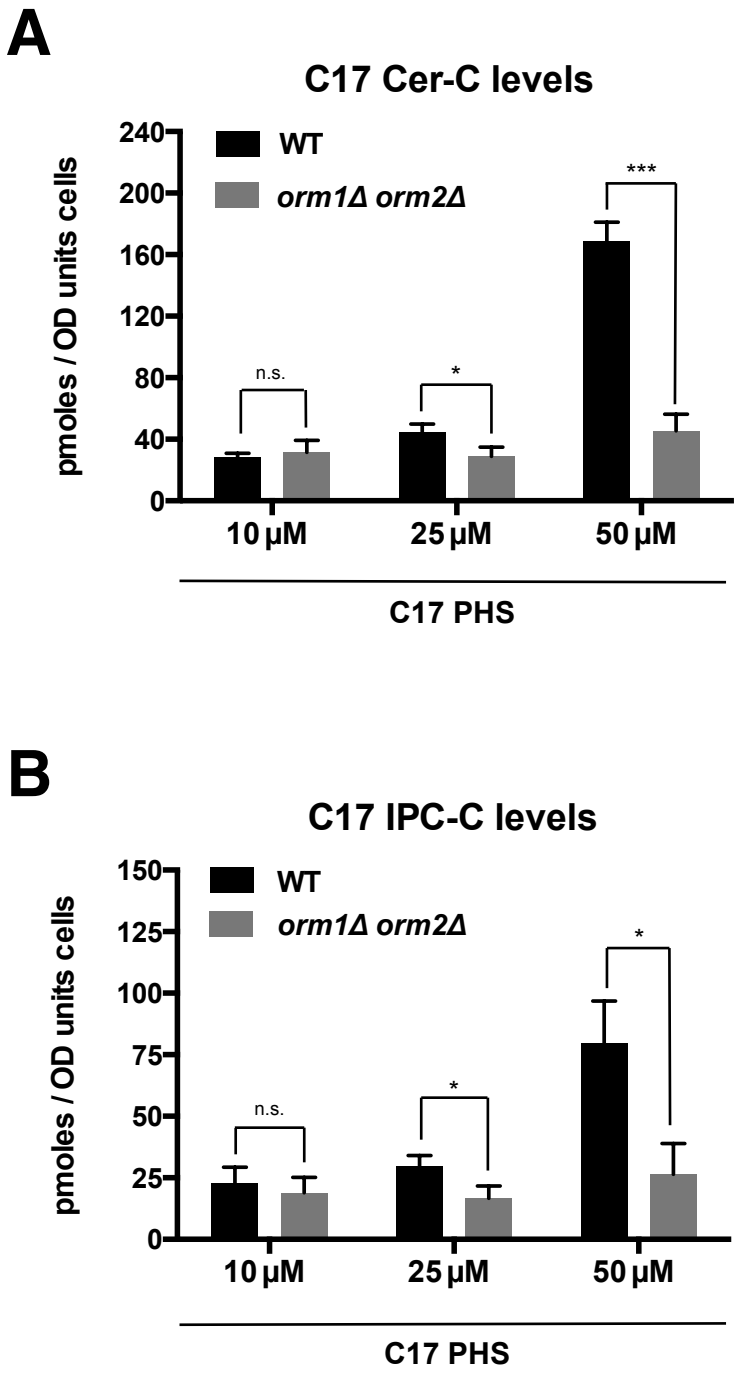
A
C17 Cer-C levels
pmoles / OD units cells
240
200
160
120
80
40
0
WT
orm1Δ orm2Δ
n.s.
*
***
10 µM
25 µM
50 µM
C17 PHS
B
C17 IPC-C levels
pmoles / OD units cells
150
125
100
75
50
25
0
WT
orm1Δ orm2Δ
n.s.
*
*
10 µM
25 µM
50 µM
C17 PHS

Figure S3

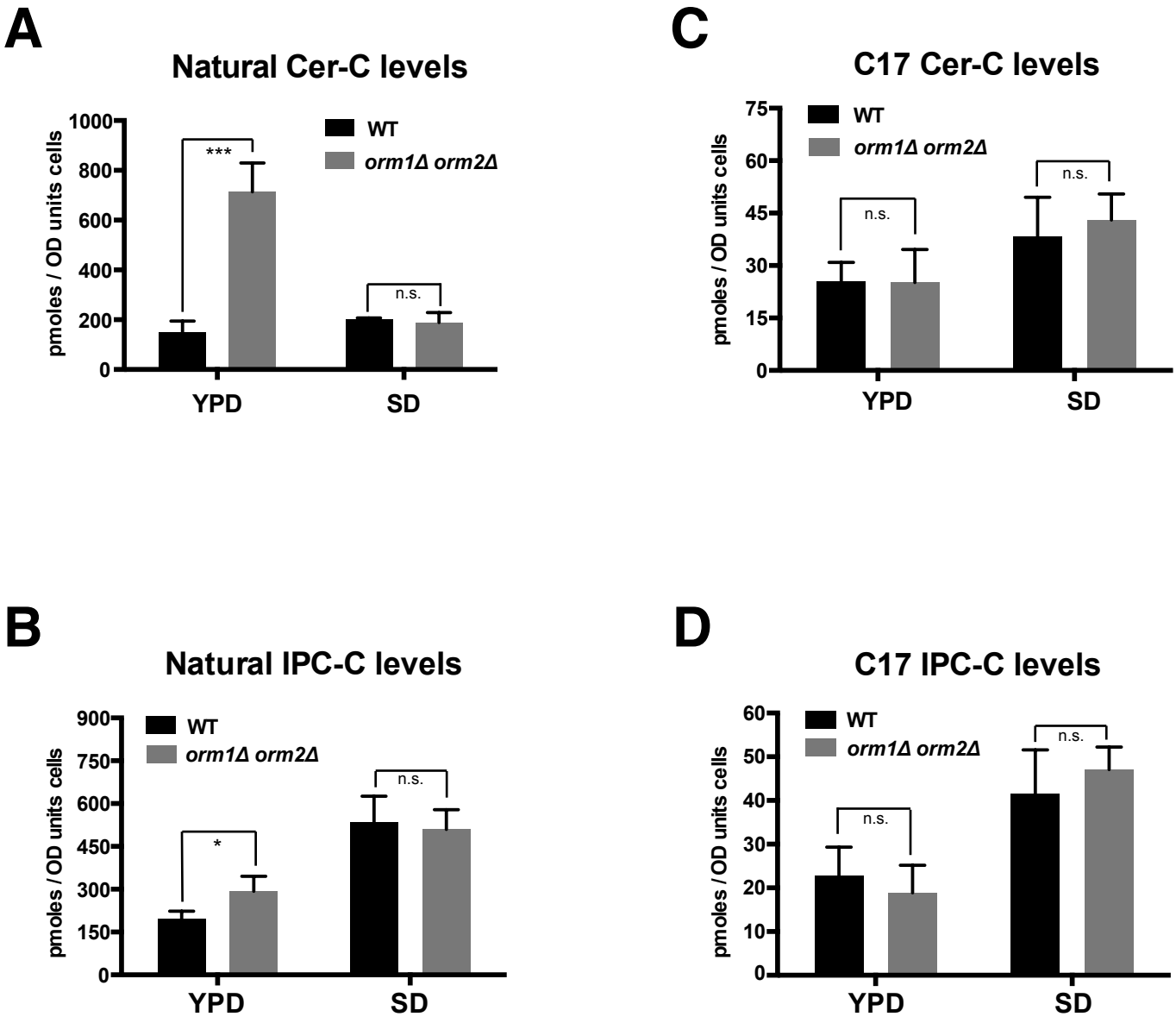
A
Natural Cer-C levels
pmoles / OD units cells
1000
800
600
400
200
0
WT
orm1Δ orm2Δ
***
n.s.
YPD
SD
C
C17 Cer-C levels
pmoles / OD units cells
75
60
45
30
15
0
WT
orm1Δ orm2Δ
n.s.
n.s.
YPD
SD
B
Natural IPC-C levels
pmoles / OD units cells
900
750
600
450
300
150
0
WT
orm1Δ orm2Δ
*
n.s.
YPD
SD
D
C17 IPC-C levels
pmoles / OD units cells
60
50
40
30
20
10
0
WT
orm1Δ orm2Δ
n.s.
n.s.
YPD
SD

Figure S4