

Supplementary Material

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

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

Supplementary Figure Legends

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

Cells were incubated with 25 μ M 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 μ M) 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 \pm 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 \pm 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 μ M) for 45 min. Values represent mean \pm SD

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

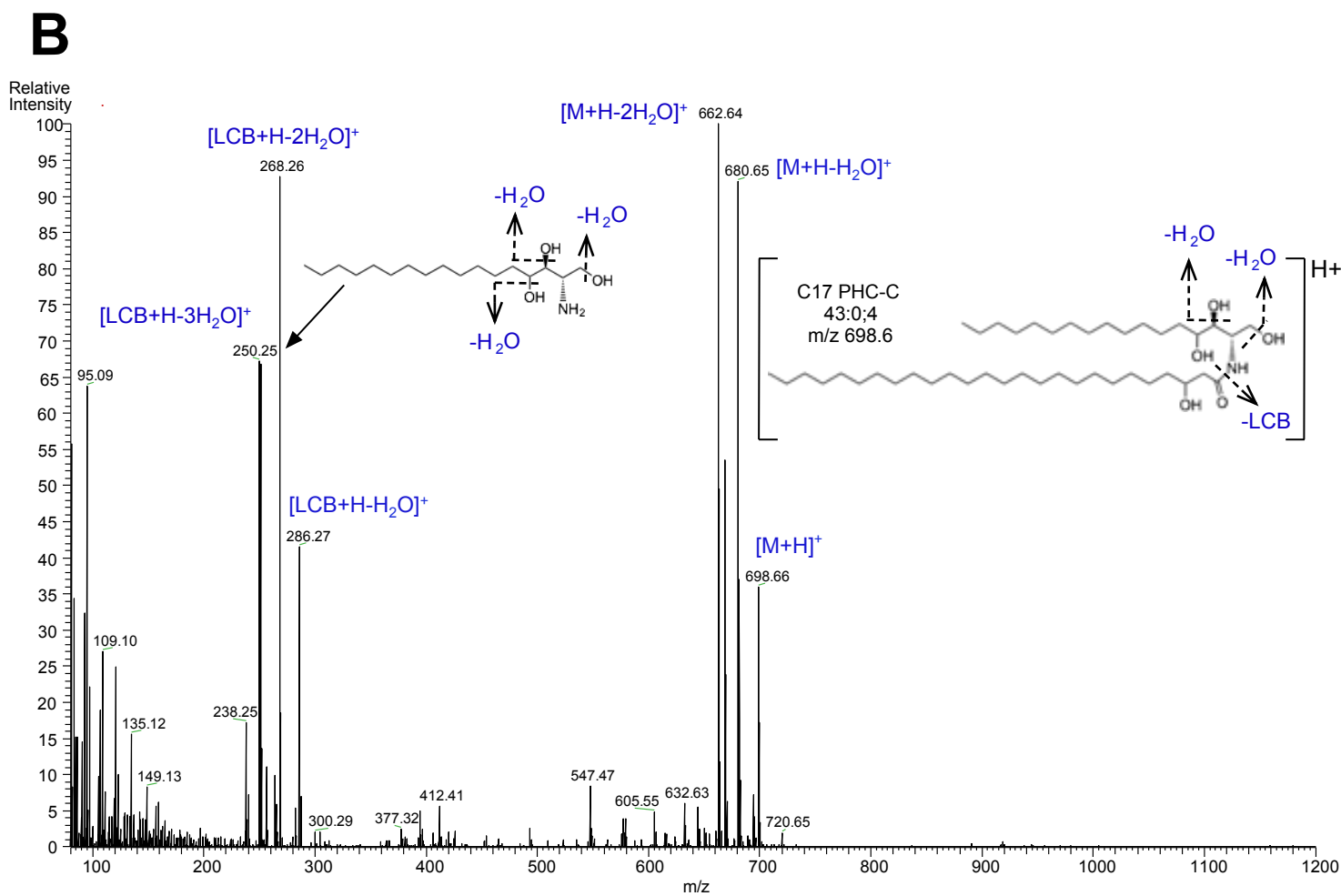
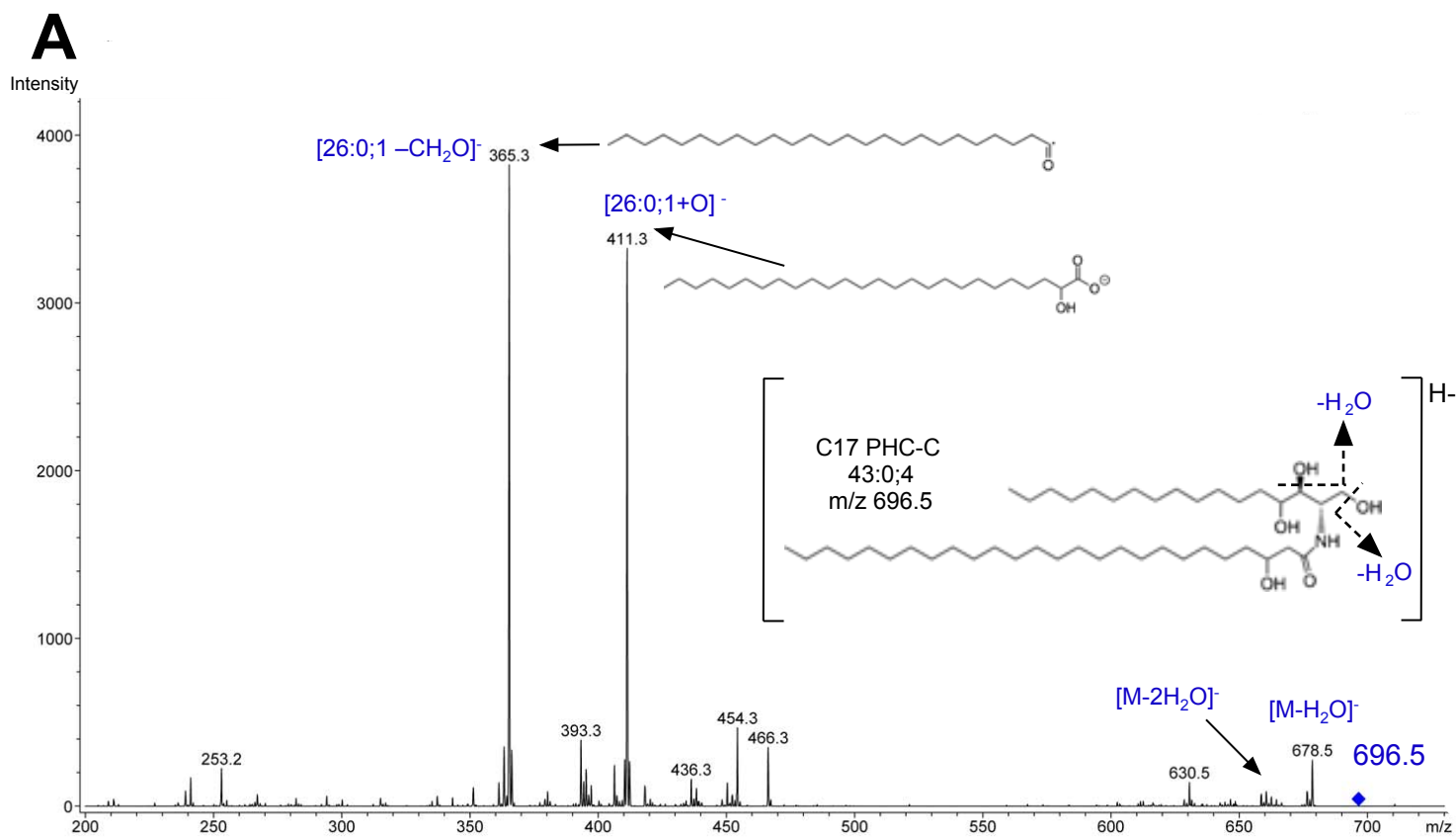
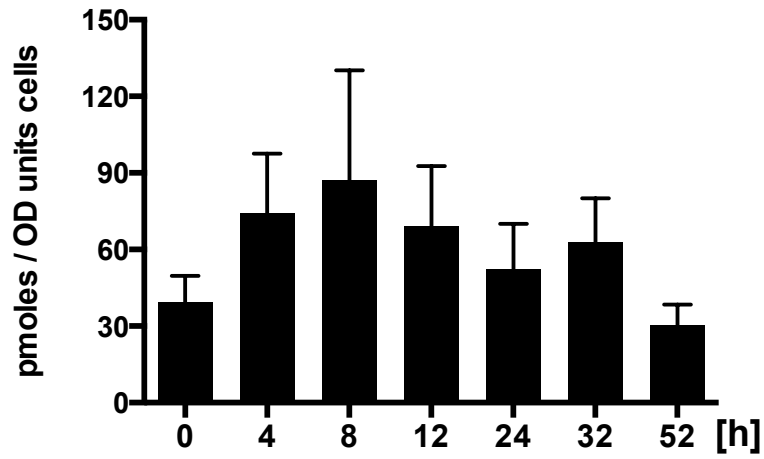
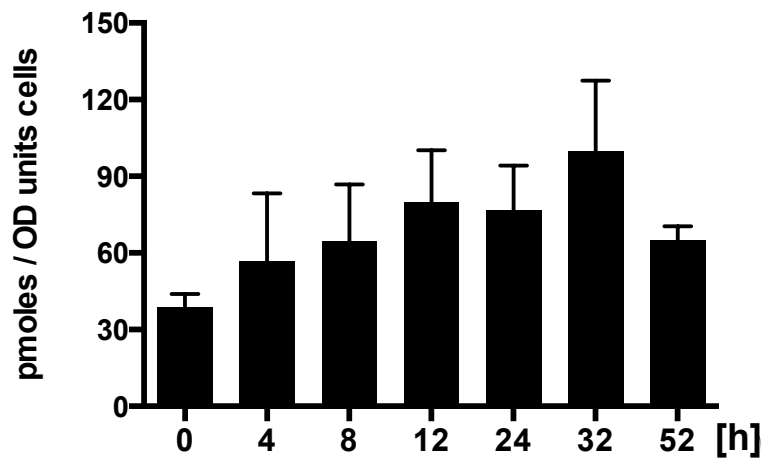
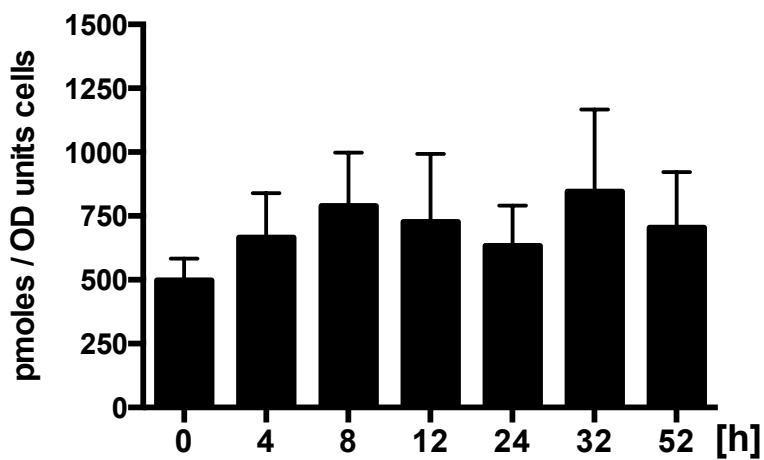
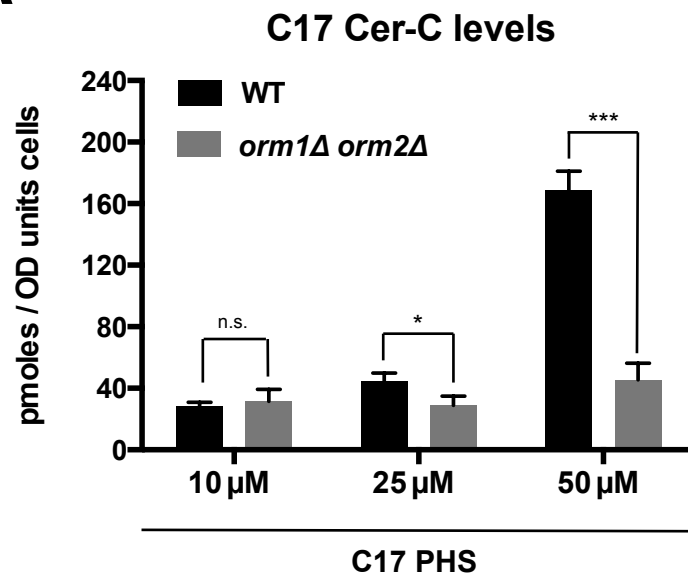
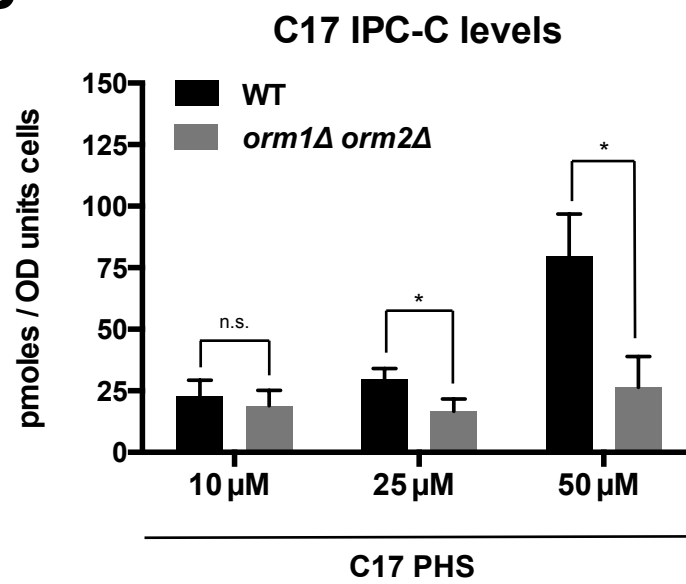


Figure S1

A**C17 IPC-C levels****B****C17 MIPC-C levels****C****Natural IPCs/MIPCs****Figure S2**

A**B****Figure S3**

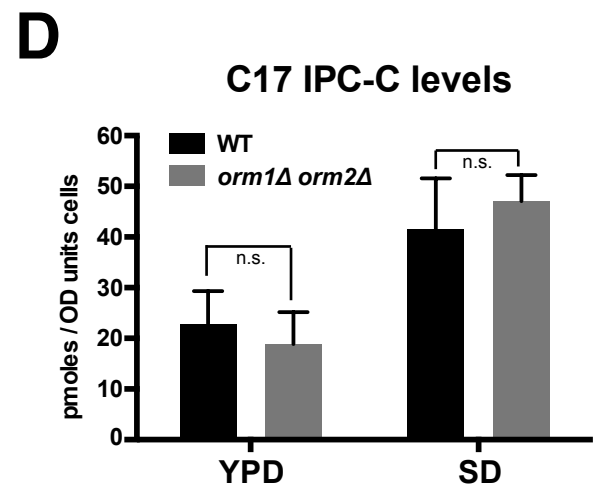
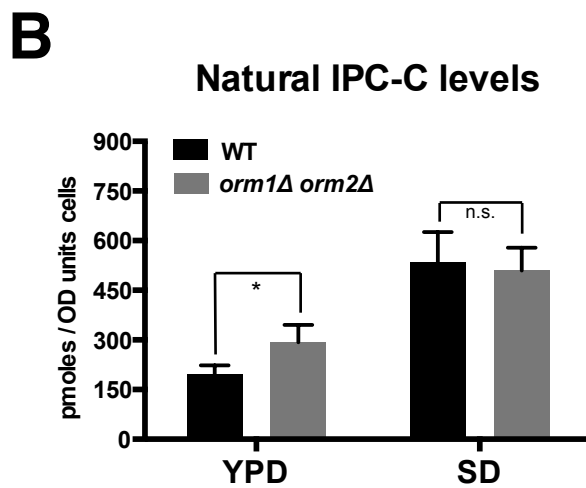
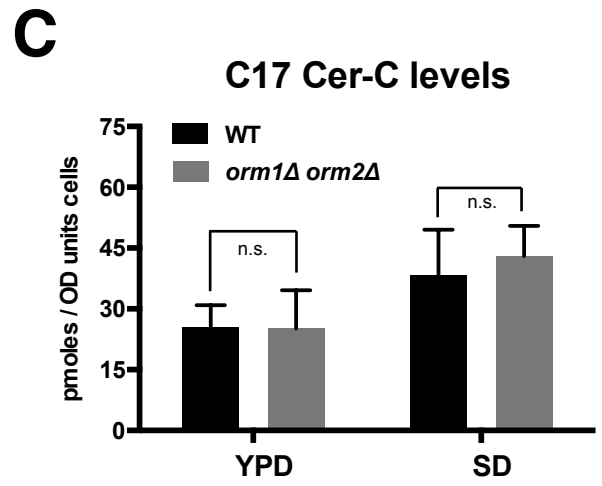
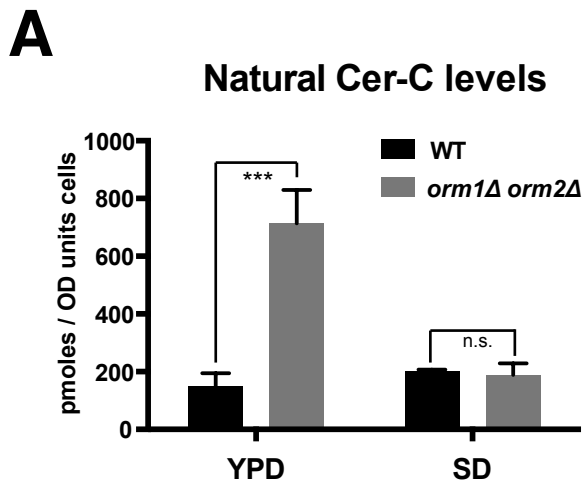


Figure S4