Published in "Proceedings of the National Academy of Sciences - PNAS, 2010
doi: 10.1073/pnas.0911617107" which should be cited to refer to this work.

Supporting Information

Han et al. 10.1073/pnas.0911617107

SI Methods

Strains. Strains used in this study were predominantly in the BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) or BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) background. *orm2Δ::kan*^{r}, *lcb3Δ::kan*^{r}, *sur2Δ::kan*^{r}, and *rsb1Δ::kan*^{r} cells are from the systematic deletion project in the BY4741 background. A strain with *ORM2* tagged at the C terminus with a TAP tag in the BY4741 background was purchased from Open Biosystems; TAP-tagged Orm2 is functional because the cells are not sensitive to tunicamycin. SHY54 is the *ORM2-TAP* strain with *TRP1* replaced with *URA3* by marker swap (1), and *lcb2*Δ, marked with *TRP1*, introduced by transformation with PCR products amplified using pML2 as template with primers 683 and 684 (2). Primer sequences available by request. SHY53 is a strain with *ORM1* tagged at the C terminus with a TAP tag in the BY4741 strain background, generated by marker swap of *HIS3* for *TRP1* first, followed by transformation with PCR products amplified using pBS1479 as template with primers 666 and 667 (3). HXY1 is *orm1*Δ in the BY4742 background; the knockout, marked by resistance to clonNAT (Werner BioAgents), was generated by transformation with PCR products amplified using pAG25 as template with primers 538 and 530 (4). HXX1 is a cross between *orm1Δ::clonNAT*^{r} (HXY1) and *orm2Δ::kan*^{r}. ACX144 is a cross between *lcb3Δ::kan*^{r} and HXX1-7D (*MATα orm2Δ::kan*^{r} *orm1Δ::clonNAT*^{r}); a tetrad in which resistance to geneticin segregated 2:2 was selected so that resistant ascospores ACX144-1B and ACX144-1D are *orm1*Δ *orm2*Δ *lcb3*Δ and *orm2*Δ *lcb3*Δ mutants, respectively. ACX 154 was made similarly by crossing *sur2Δ::kan*^{r} and HXX1-7D; ACX154-5B is an *orm1*Δ *orm2*Δ *sur2*Δ mutant. ACX164 is a cross between HXY1 and SHY20, which is *MATa orm2Δ::HIS3* generated by transformation of HXX1-2A with PCR products amplified using pFA6a-HIS3MX6 as the template with primers 551 and 598 (2). ACX164-1C is a *MATα orm1Δ::clonNAT*^{r} *orm2Δ::HIS3* mutant. ACX165 is a cross between ACX164-1C and *lac1Δ::kan*^{r}; ACX165-7C is an *orm1*Δ *orm2*Δ *lac1*Δ triple mutant. ACX 167 is a cross between ACX164-1C and *lag1Δ::kan*^{r}; ACX167-7A is a *lag1*Δ *orm1*Δ *orm2*Δ triple mutant. ACX176 is a cross between *opi1Δ::kan*^{r} (ACX173-12B) and *orm1Δ::clonNAT*^{r} *orm2Δ::HIS3* (ACX164-1C). ACX161 is a cross between *rsb1Δ::kan*^{r} and HXX1-2D; PCR was used to identify double and triple mutants because *rsb1*Δ and *orm2*Δ are both marked with *kan*^{r} in this cross. Because *ORM2* is 109 bp away from the stop codon of the neighboring gene (*NIT3*), a strain was constructed bearing a *HIS3*-marked insertion into the coding sequence of *ORM2*. SHY21 is *MATa orm2::HIS3* generated by transformation of HXX1-2A with PCR products amplified using pFA6a-HIS3MX6 as the template with primers 612 an 613. The *orm2::HIS3* insertion mutant displays tunicamycin sensitivity like the knockout.

Plasmids. pSH14 and pSH16 carry *ORM1* in a *HIS3*-marked centromeric plasmid and a *LEU2*-marked 2-μ plasmid, respectively; they were constructed by placing the 2.0-kb SpeI-XhoI fragment from p2DL07 (5) (gift from Greg Prelich) in pRS313 and pRS425 (6). pSH15 and pSH17 are *LEU2*-marked centromeric and 2-μ plasmids, respectively, bearing *ORM2*; they were constructed by placing the 2.5-kb PstI-XhoI fragment from p3d04 (5) in pRS315 and pRS425.

pES67 is a *LEU2*-marked centromeric plasmid bearing HA-CPY* under the control of a *GAL1* promoter (7) (gift from D. Ng, National University of Singapore). pYEP96 is a *TRP1*-marked 2-μ plasmid bearing *hsf1-R206S*, a constitutively active mutant of *HSF1* (8) (gift from D. Winge, University of Utah Health Sciences Center). pJC104, a *URA3*-marked 2-μ plasmid bearing four tandem repeats of *UPRE* fused to *lacZ*, is a gift from P. Walter (University of California, San Francisco) (9). pRS316-RSB1-3xHA and pRS426-RSB1-3xHA are *URA3*-marked centromeric and 2-μ plasmids, respectively, described previously (10), and are gifts from S. Mowe-Rowley (University of Iowa). An *INO1-lacZ* reporter, pJH359, is a gift from Susan Henry (Cornell University). pRC12 is a high-copy *LEU2*-marked plasmid (YEplac181) bearing *SCS2*, described previously (11) (gift from Tom Petes, Duke University). pRS315-*LCB1*-HA and pRS315-*LCB2*-HA are gifts from Teresa Dunn (Uniformed Services University of the Health Sciences) (12). pRH7 is myc-tagged *ERG 11* on a pRS316 backbone (13), a gift from Rolf Craven (University of Kentucky).

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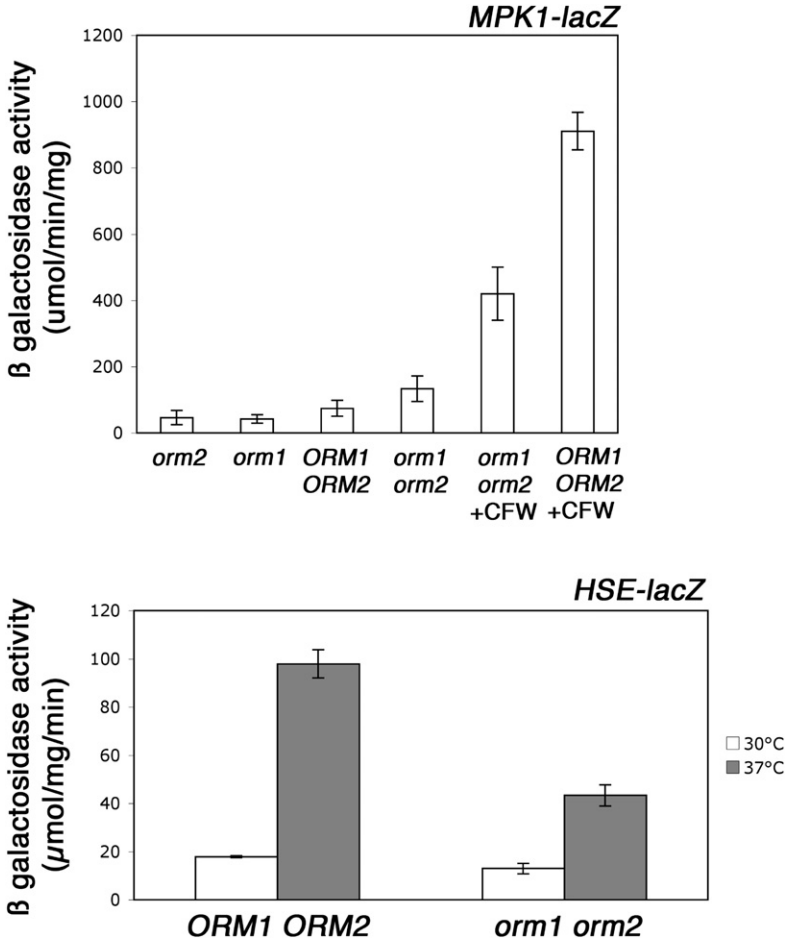

Fig. S1. Cell wall stress response and heat shock response. Cells were transformed with a cell wall stress reporter (*MPK1-lacZ*), a gift from D. Levin (Johns Hopkins University) (1). Cells were shifted to yeast extract/peptone/dextrose medium for 6 h at 25 °C and then incubated for an additional 1 h in the absence or presence of calcofluor white (40 μg/mL). Heat shock response was measured in cells bearing pCM63-SSA3-lacZ, a *URA3*-marked 2-μ plasmid with the *SSA3 HSE* fused to *lacZ* (2), a gift from D. Thiele (Duke University). Cells were grown to midlog at 30 °C in synthetic complete medium and then incubated for an additional 1 h at 30 °C or 37 °C before cells were harvested. Assays were performed in duplicate on at least three independent colonies.

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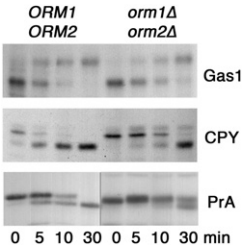

Fig. S2. ER-to-Golgi transport in *orm1*Δ *orm2*Δ cells. Wild-type and *orm1*Δ *orm2*Δ cells were grown overnight at room temperature to midlog phase in minimal medium. Cells were then pulse-labeled with Expre^{35}S^{35}S for 5 min and chased for various times. Cells were lysed, and immunoprecipitations with anti–carboxypeptidase Y, anti-Gas1, and anti-PrA were normalized to acid-precipitable cpm. Immunoprecipitations were analyzed by SDS/PAGE and fluorography.

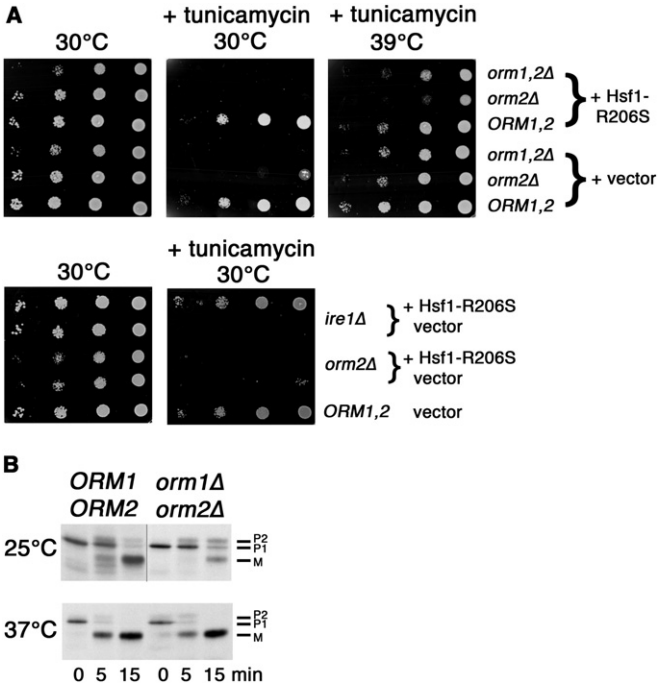

Fig. S3. Suppression of *orm1*Δ *orm2*Δ at high temperature is not mediated by heat shock response. (*A*) Suppression of impaired growth on tunicamycin by incubation at 39 °C; suppression is not mimicked by constitutively active Hsf1. Wild-type, *orm2*Δ, *orm1*Δ *orm2*Δ, or *ire1*Δ cells bearing vector or *hsf1-R206S* were serially diluted and spotted onto plates with synthetic complete medium with or without tunicamycin (1 μg/mL). Plates were incubated at 30 °C or 39 °C. (*B*) Pulse-chase analysis of ER-Golgi transport. Wild-type and *orm1*Δ *orm2*Δ cells were shifted to 37 °C for 15 min before pulse-labeling for 5 min with Expre^{35}S^{35}S and chase for various times. Carboxypeptidase Y (CPY) was immunoprecipated from lysate and analyzed by SDS/PAGE and fluorography. ER-localized P1, Golgi-modified P2, and proteolytically processed M forms of CPY are indicated.

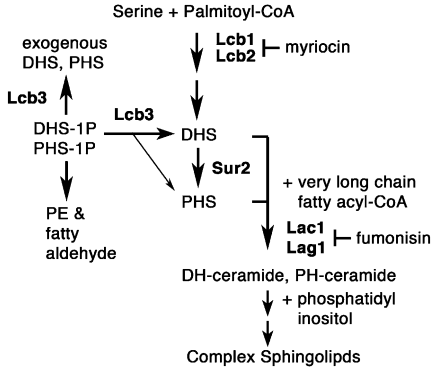

Fig. S4. Schematic diagram of the sphingolipid biosynthesis pathway.

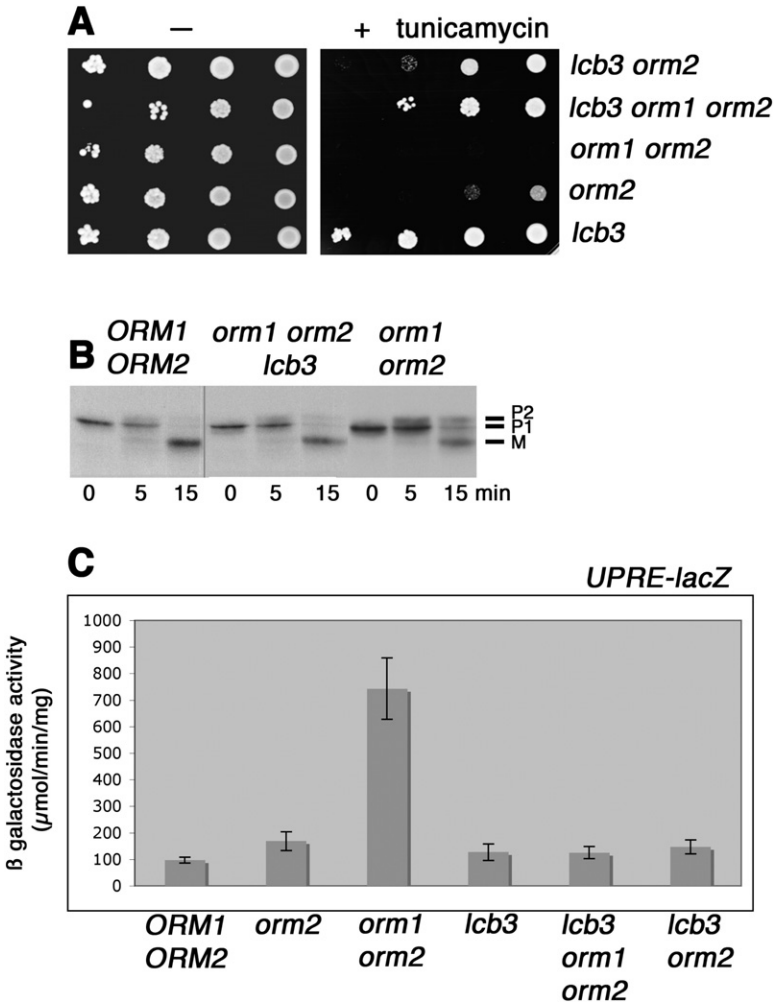

Fig. S5. An *lcb3*Δ mutant, defective in sphingolipid synthesis, is a suppressor of *orm1*Δ *orm2*Δ cells. (*A*) Suppression of tunicamycin sensitivity of *orm1*Δ *orm2*Δ mutants. Cells (ACX144 ascospores) were serially diluted and spotted onto plates with synthetic complete medium with or without tunicamycin (1 μg/mL) at 30 °C. (*B*) Slow ER-to-Golgi transport is suppressed by *lcb3* mutation. Wild-type (HXX1-2A), *orm1*Δ *orm2*Δ (HXX1-2D), and *lcb3*Δ *orm*Δ*1 orm2*Δ (ACX144-1B) triple mutants were pulse-labeled as described in Fig. S2 legend. (*C*) Unfolded protein response is suppressed in *lcb3*Δ *orm1*Δ *orm2*Δ cells. Exponentially growing cells bearing a *UPRE-lacZ* reporter were lysed for β-galactosidase activity measurements. Assays were performed in duplicate at least three independent times.

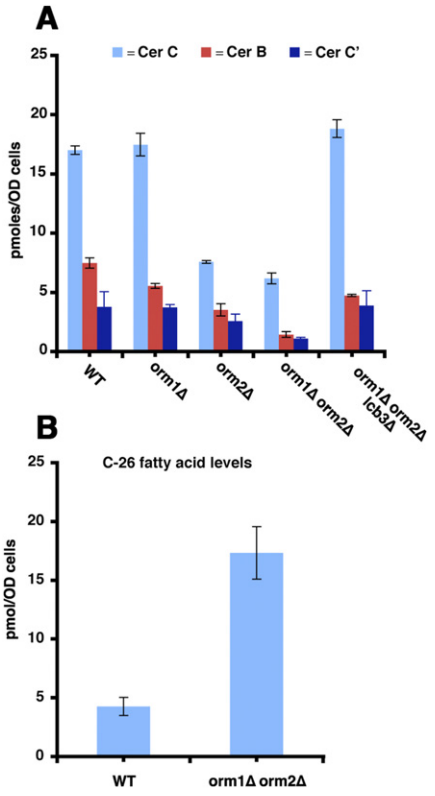

Fig. S6. Ceramide (*A*) and C26 fatty acid (*B*) levels in *orm1*Δ *orm2*Δ cells. Ceramide and long chain fatty acid levels were measured in wild-type and *orm1*Δ *orm2*Δ cells by mass spectrometry as described in the main text (*Methods*).

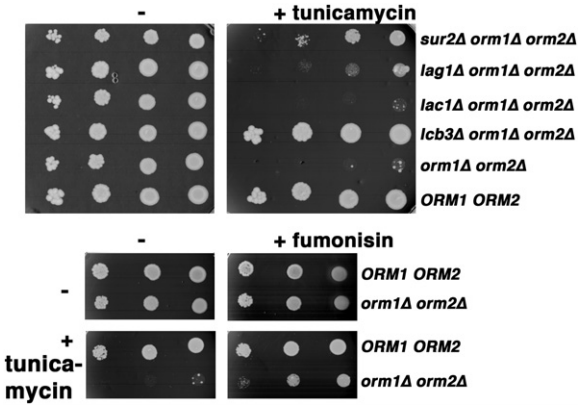

Fig. S7. Weak suppression of *orm1*Δ *orm2*Δ cells upon inhibition of later steps in the sphingolipid pathway. Cells were serially diluted and spotted on plates with synthetic complete medium with 2% glucose. *Top:* With or without tunicamycin (0.5 μg/mL). *Bottom:* With or without tunicamycin (0.5 μg/mL) with or without 10 μM fumonisin. Strains are as follows: wild-type (HXX1-2A), *orm1*Δ *orm2*Δ (HXX1-7D or ACX164-1C), *lcb3*Δ *orm1*Δ *orm2*Δ (ACX144-1B), *lag1*Δ *orm1*Δ *orm2*Δ (167-7A), and *lac1*Δ *orm1*Δ *orm2*Δ (ACX165-7C).