Table 1. The yeast strains used in this study.

Strain	**Genotype**
DDY1102	*MATa/MATα his3-Δ200/his3-Δ200 ura3-52/ura3-52 leu2-3,112/leu2-3,112 lys2-801/LYS2 ade2-1/ADE2*
DDY904	*MATα his3-Δ200 leu2-3, 112, ura3-52*
DDY5101	*MATa his3-Δ200 leu2-3, 112 ura3-52 3XFLAG-ORM1::NAT*
DDY5102	*MATα his3-Δ200 leu2-3, 112 ura3-52 3XFLAG-ORM2::NAT*
DDY5103	*MATa his3-Δ200 leu2-3, 112 ura3-52 orm2Δ::CgURA3 3XFLAG-ORM1::NAT*
DDY5104	*MATα his3-Δ200 leu2-3, 112 ura3-52 orm1Δ::CgLEU2 3XFLAG-ORM2::NAT*
DDY5105	*MATα his3-Δ200 leu2-3, 112 ura3-52 pph21Δ::CgHIS3*
DDY5106	*MATα his3-Δ200 leu2-3, 112 ura3-52 pph22Δ::CgLEU2*
DDY5107	*MATα his3-Δ200 leu2-3, 112 ura3-52 pph22Δ::CgLEU2 pph21Δ::CgHIS3*
DDY5108	*MATa his3-Δ200 leu2-3, 112 ura3-52 cdc55Δ::CgHIS3*
DDY5109	*MATα his3-Δ200 leu2-3, 112 ura3-52 rts1Δ::CgURA3*
DDY5110	*MATα his3-Δ200 leu2-3, 112 ura3-52 tpd3Δ::CgLEU2*
DDY5111	*MATa his3-Δ200 leu2-3, 112 ura3-52 pph21Δ::CgHIS3 3XFLAG-ORM2::NAT*
DDY5112	*MATa his3-Δ200 leu2-3, 112 ura3-52 pph22Δ::CgLEU2 3XFLAG-ORM2::NAT*
DDY5113	*MATα his3-Δ200 leu2-3, 112 ura3-52 pph22Δ::CgLEU2 pph21Δ::CgHIS3 3XFLAG-ORM2::NAT*
DDY5114	*MATa his3-Δ200 leu2-3, 112 ura3-52 cdc55Δ::CgHIS3 3XFLAG-ORM2::NAT*
DDY5115	*MATα his3-Δ200 leu2-3, 112 ura3-52 rts1Δ::CgURA3 3XFLAG-ORM2::NAT*
DDY5116	*MATα his3-Δ200 leu2-3, 112 ura3-52 tpd3Δ::CgLEU2 3XFLAG-ORM2::NAT*
DDY5117	*MATa his3-Δ200 leu2-3, 112 ura3-52 orm1Δ::CgLEU2 orm2Δ::CgURA3*
DDY5118	*MATa his3-Δ200 leu2-3, 112 ura3-52 orm1Δ::CgLEU2 3XFLAG-orm2 (S46A, S47A, S48A)::NAT*
DDY5119	*MATa his3-Δ200 leu2-3, 112 ura3-52 orm1Δ::CgLEU2 3XFLAG-orm2 (S46D, S47D, S48D)::NAT*
DDY5120	*MATa his3-Δ200 leu2-3, 112 ura3-52 orm1Δ::CgLEU2 3XFLAG-orm2 (S46A, S47A, S48A)::NAT*
DDY5121	*MATa his3-Δ200 leu2-3, 112 ura3-52 orm1Δ::CgLEU2 3XFLAG-orm2 (S46D, S47D, S48D)::NAT*
DDY5122	*MATa his3-Δ200 leu2-3, 112 ura3-52 cdc55Δ::CgHIS3 orm1Δ::CgLEU2*
DDY5123	*MATa his3-Δ200 leu2-3, 112 ura3-52 cdc55Δ::CgHIS3 orm1Δ::CgLEU2 orm2Δ::CgURA3*
DDY5124	*MATa his3-Δ200 leu2-3, 112 ura3-52 cdc55Δ::CgHIS3 orm1Δ::CgLEU2 3XFLAG-ORM2::NAT*
DDY5125	*MATa his3-Δ200 leu2-3, 112 ura3-52 cdc55Δ::CgHIS3 orm1Δ::CgLEU2 3XFLAG-orm2 (S46A, S47A, S48A)::NAT*
DDY5126	*MATa his3-Δ200 leu2-3, 112 ura3-52 cdc55Δ::CgHIS3 orm1Δ::CgLEU2 3XFLAG-orm2 (S46D, S47D, S48D)::NAT*
DDY5127	*MATa his3-Δ200 leu2-3, 112 ura3-52 pph21Δ::CgHIS3 orm1Δ::CgLEU2*

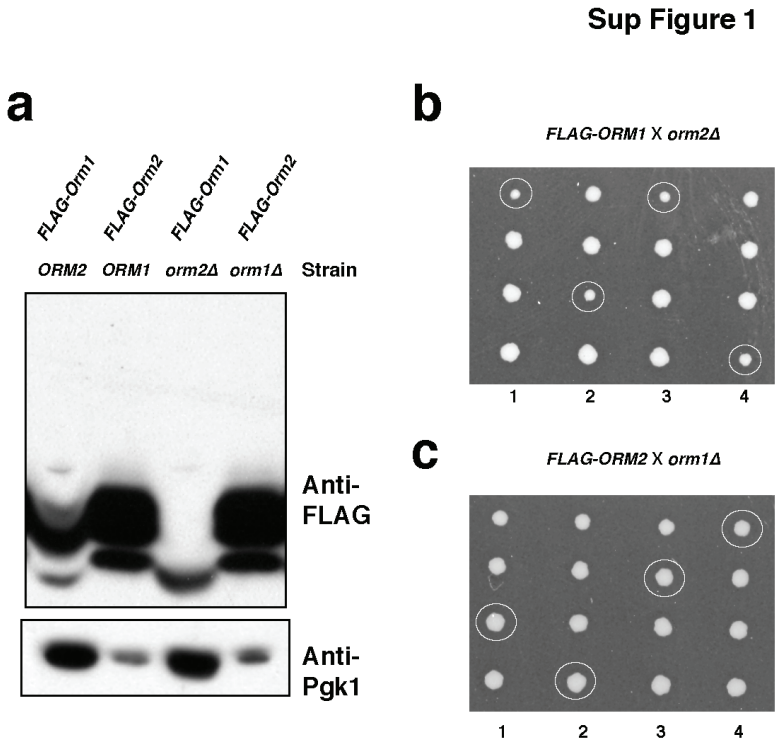
DDY5128	*MATa his3-Δ200 leu2-3, 112 ura3-52 pph21Δ::CgHIS3 orm1Δ::CgLEU2 orm2Δ::CgURA3*
DDY5129	*MATa his3-Δ200 leu2-3, 112 ura3-52 pph21Δ::CgHIS3 orm1Δ::CgLEU2 3XFLAG-ORM2::NAT*
DDY5130	*MATa his3-Δ200 leu2-3, 112 ura3-52 pph21Δ::CgHIS3 orm1Δ::CgLEU2 3XFLAG-orm2 (S46A, S47A, S48A)::NAT*
DDY5131	*MATa his3-Δ200 leu2-3, 112 ura3-52 pph21Δ::CgHIS3 orm1Δ::CgLEU2 3XFLAG-orm2 (S46D, S47D, S48D)::NAT*
DDY5132	*MATa his3-Δ200 leu2-3, 112 ura3-52 rts1Δ::CgURA3 orm1Δ::CgLEU2*
DDY5133	*MATa his3-Δ200 leu2-3, 112 ura3-52 rts1Δ::CgURA3? orm1Δ::CgLEU2 orm2Δ::CgURA3*
DDY5134	*MATa his3-Δ200 leu2-3, 112 ura3-52 rts1Δ::CgURA3 orm1Δ::CgLEU2 3XFLAG-ORM2::NAT*
DDY5135	*MATa his3-Δ200 leu2-3, 112 ura3-52 rts1Δ::CgURA3 orm1Δ::CgLEU2 3XFLAG-orm2 (S46A, S47A, S48A)::NAT*
DDY5136	*MATa his3-Δ200 leu2-3, 112 ura3-52 rts1Δ::CgURA3 orm1Δ::CgLEU2 3XFLAG-orm2 (S46D, S47D, S48D)::NAT*
DDY5137	*MATα his3-Δ200 leu2-3, 112 ura3-52 pph22Δ::CgLEU2 pph21Δ::CgHIS3 ura3-52::pph21 (E102K)::URA3 3XFLAG-ORM2::NAT*
DDY5138	*MATa his3-Δ200 leu2-3, 112 ura3-52 lcb5Δ::CgURA3 lcb4Δ::CgLEU2 3XFLAG-ORM2::NAT*
DDY5139	*MATα his3-Δ200 leu2-3, 112 ura3-52 pkh1Δ::CgLEU2 pkh2Δ::CgHIS3 ura3-52::pkh1-as::URA3 3XFLAG-ORM2::NAT*
DDY5140	*MATα his3-Δ200 leu2-3, 112, ura3-52 cdc55Δ::CgHIS3 lcb1-100*
DDY5141	*MATα his3-Δ200 leu2-3, 112, ura3-52 lcb1-100*
DDY5142	*MATα his3-Δ200 leu2-3, 112, ura3-52 ypk1Δ::CgLEU2, ypk2Δ:: CgHIS3, ura3-52::*ypk1-as::URA3, *Flag-orm2:: NAT*
DDY5143	*MATα his3-Δ200 leu2-3, 112, ura3-52 lcb3Δ::CgHIS3, 3XFLAG-ORM2::NAT*

Table 2, The primers used in this study.

Name	Primer sequence
HindIII-ORM2-F	GGGAAGCTTATTAAATTTAGGGTCCCCGGCA
Flag-ORM2-inter-R	TTGTAATCGATGTCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTCCATGATGAAACTGTTTCTTATGCGTTTTTA
Flag-ORM2-inter-F	ATGGACACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGATTGACCGCACTAAAAACGAATCT
XhoI-ORM2-R	GGGCTCGAGGGCGTAGGTATATGCTATGACATGA
NatR-ORM2-F	CCCAAGCTTACATGGAGGCCCAGAATACCCTCCTTGACA
NatR-ORM2-R	CCCACTAGTACTCCAACAACGACAGCGGATGCGCTGTTCTGCGGATGCGTGACCGTATCCGCAAAGAACGAAGCAGTATAGCGACCAGCATTCACATA
HindIII-ORM1-F	GGGAAGCTTAGCGCCACCTGTCTTCAG
Flag-ORM1-inter-R	TCCTTGTAATCGATGTCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTCCATTAACTAAATGGATAAAAATGCAAT
Flag-ORM1-inter-F	ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGACCGAATTAGATTATCAAGGAACT
XhoI-ORM1-R	GGGCTCGAGCTTCAGCATCTTTTTCCCCA
NatR-ORM1-F	CCCCTCGAGACATGGAGGCCCAGAATACCCTCCTTGACA
NatR-ORM1-R	CCCGGGCCCATATTATAGCCTTTATCAACAATGGAATCCCAACAATTATCTAATTACCCACATATATCTCACCAGTATAGCGACCAGCATTCACATA
ORM2-3A-F	CCATAGGAGAAGACGGGCAGCCGCCGTAATATCACATGTGGAACAGG
ORM2-3A-R	CCTGTTCCACATGTGATATTACGGCGGCTGCCCGTCTTCTCCTATGG
ORM2-3D-F	CCAGTGACCGACCATAGGAGAAGACGGGATGACGACGTAATATCACATGTGGAACAGG
ORM2-3D-R	CCTGTTCCACATGTGATATTACGTCGTCATCCCGTCTTCTCCTATGGTCGGTCACTGG
PKH1-XhoI-F	CCGCTCGAGCGGCAACTGATGATACTGATATGCC
PKH1-SacII-R	TCCCCGCGGGGAGCTACATACGATGTAGGCTA
PKH1-F187V/L203A-F	GCTGAATGGCACCAAGGGCATAGTTAAGCTTTTCTTCACTTTCCAGGACGAGGCAAGCTTGTATTTCCT-TGCTGAATATGCCCCCCACGGTGATTTC
PKH1-F187V/L203A-R	GAAATCACCGTGGGGGGCATATTCAGCAAGGAAATACAAGCTTGCCTCGTCCTGGAAAGTGAAGAAAAGCTTAACTATGCCCTTGGTGCCATTCAGC
PPH21-BamHI-F	GGGGGATCCTTCTGTCGTGCTTGAAAGATTT
PPH21-NotI-R	GGGGCGGCCGCTCCAATGGCAAGACGTCTGAACT

PPH21-E102K-F	GGCGGTGGACGTGTTGCAGTTCAAGGAGAATGTTAAACCAATTAACGTGCC
PPH21-E102K-R	GGCACGTTAATTGGTTTAACATTCTCCTTGAACTGCAACACGTCCACCGCC
OYS227	GGAGAACTATGGCCAAACAGC
OYS228	CCTGCTATACATGGCCGCTG
OYS267	TCCAATCACCGGAAAAATTATACTTTGTTGCAGCGTTTATCAATGGTGGTGAGTTG
OYS268	CAACTCACCACCATTGATAAACGCTGCAACAAAGTATAATTTTTCCGGTGATTGGA

Supplemental Figure Legends

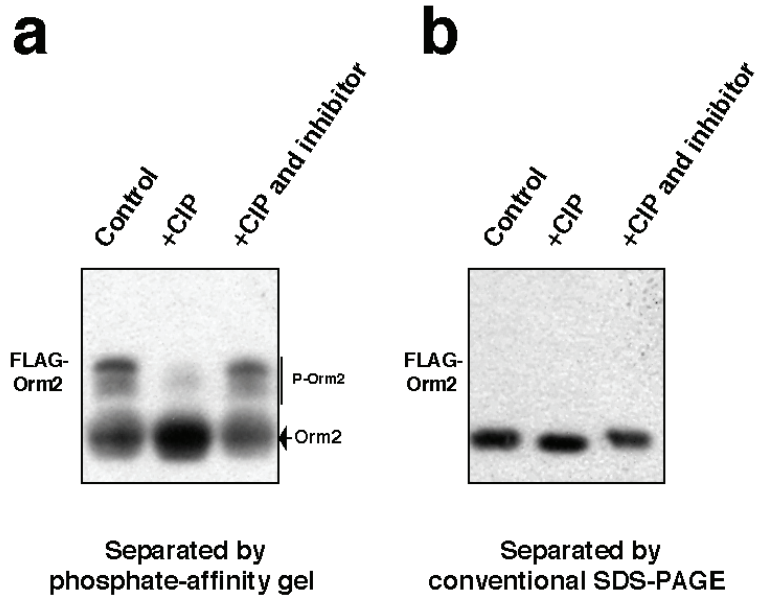


Supplemental Figure 1,

Functionality of FLAG tagged Orm proteins.

(a), Phosphorylation status of FLAG-Orm1 and FLAG-Orm2 in various strain backgrounds. Western blots reveal Orm1 or Orm2 phosphorylation patterns after separation on phosphate affinity gels. The 3- phosphoglycerate kinase 1(Pgk1) was used as a loading control. Note that ten times as much FLAG-Orm1-containing cell extracts were loaded compared to the FLAG-Orm2 extracts. (b) and (c), Tetrad analysis of heterozygous diploid strains obtained by crossing 3XFLAG tagged Orm1 and *orm2*Δ (b) or crossing 3XFLAG tagged Orm2 and *orm1*Δ (c). Circled colonies in (b) represent *FLAG-ORM1 orm2*Δ mutants and circled colonies in (c) represent *FLAG-ORM2 orm1*Δ mutants. Note that 3XFLAG tagged Orm1 in *orm2*Δ strain, but not 3XFLAG tagged Orm2 in *orm1*Δ strain, exhibits slow cell growth.

Sup Figure 2

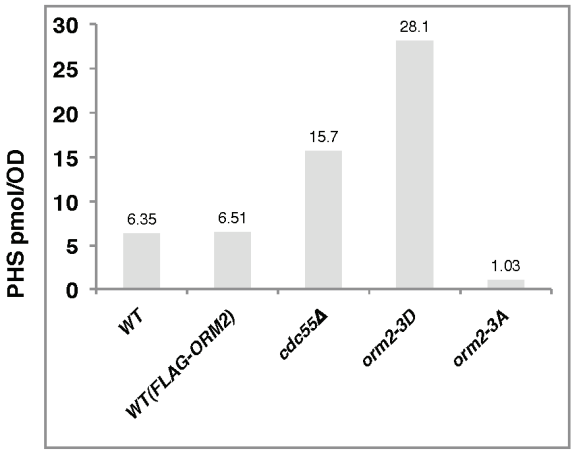


Supplemental Figure 2,

Calf intestine phosphatase (CIP) treatment of Orm2 protein.

Immunoprecipitations (IP) were performed with cell lysates using anti-FLAG resin (Sigma), as previously described by Breslow *et al* [1]. The IP samples were treated with CIP in the presence and absence of phosphatase inhibitor (sodium orthovanadate). Western blots show the phosphorylation patterns after separation on a phosphate affinity gel (a) or on a conventional SDS-PAGE gel (b). Note that the phosphate affinity gel greatly improves the separation of phosphorylated Orm2 from unphosphorylated Orm2.

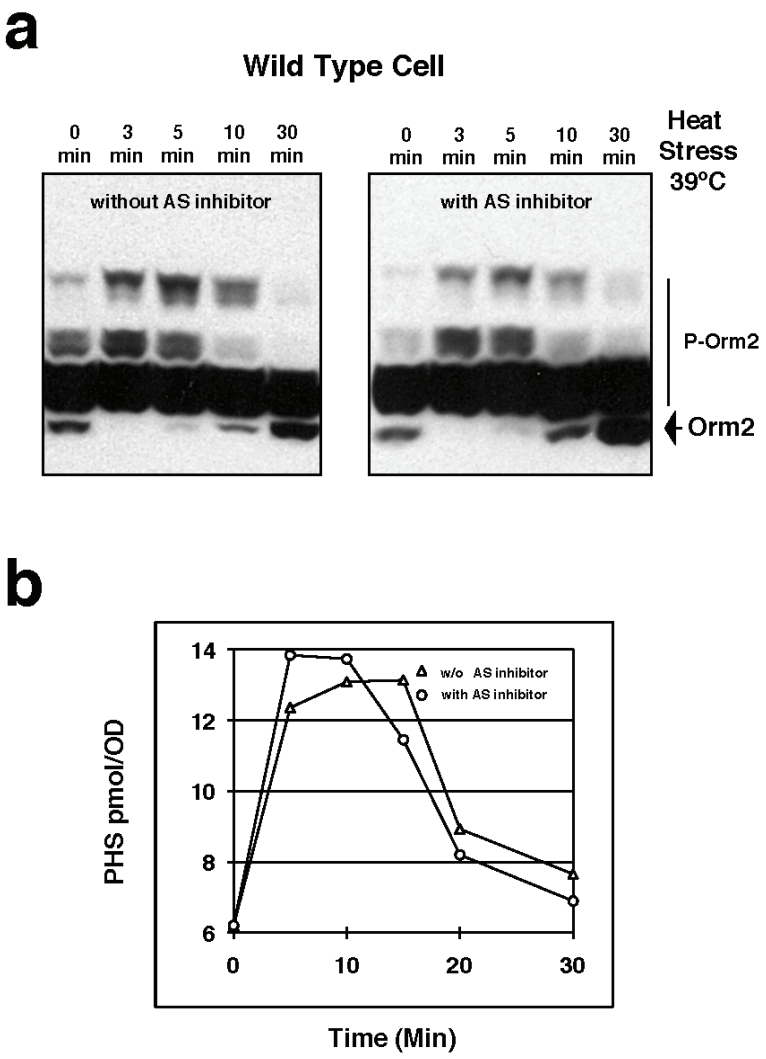
Sup Figure 3



Supplemental Figure 3,

Sphingoid base concentrations in various mutants. The C18-PHS concentration was determined in indicated mutants by HPLC.

Sup Figure 4



Supplemental Figure 4,

3-MOB-PP1 does not affect heat stress induced Orm phosphorylation and sphingoid base production in wild-type cells. (a), Orm2 phosphorylation upon heat stress in the absence and the presence of 3-MOB-PP1 in wild-type cells. (b), Concentration of C18-PHS in the wild type cells cultured in the absence (line with triangle) and the presence (line with circle) of 50μM 3-MOB-PP1 under heat stress for indicated times.

References

1, Breslow, D. K. *et al.* Orm family proteins mediate sphingolipid homeostasis. *Nature* **463**, 1048-1053, doi:10.1038/nature08787 (2010).