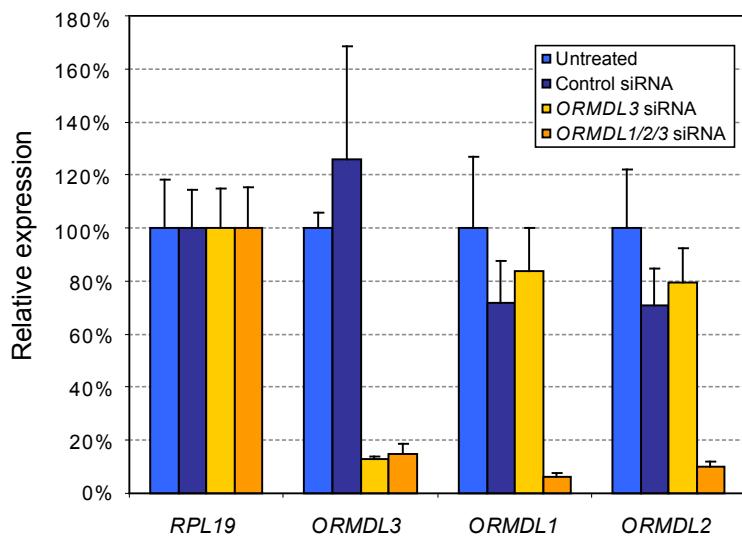


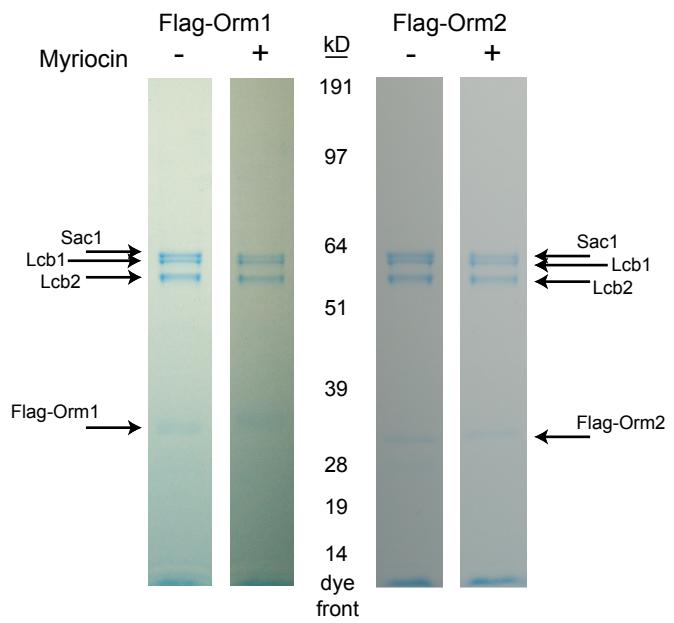
Supplementary Figure 1. A functional role for Sac1 in sphingolipid metabolism.

a, Lipidomic analysis of LCB levels in the indicated strains are shown (average \pm s.d., n = 3). **b**, Serial ten-fold dilutions of the indicated strains were spotted on plates with 0 or 400 ng/ml myriocin and imaged after 24-48 hr of growth. The *sac1-8* allele of *SAC1* is a catalytically inactive *SAC1* mutant described previously¹. **c**, *ORM1/ORM2* and *SAC1* deletion mutants exhibit synthetic lethality. Representative tetrads from a *orm1Δ/orm2Δ* x *sac1Δ* cross are shown, with circles indicating spores with the *orm1Δ/orm2Δ/sac1Δ* genotype that fail to grow.

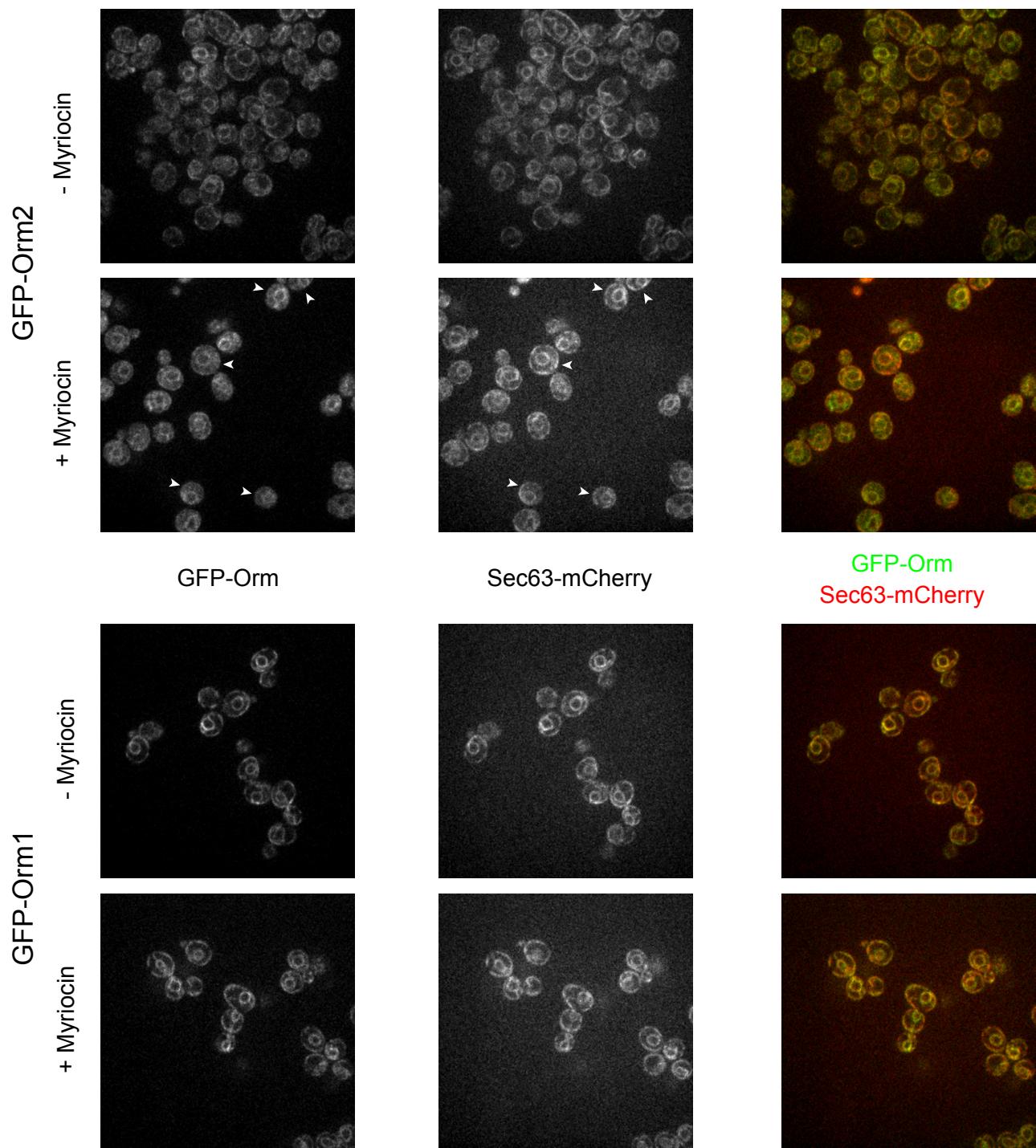


Supplementary Figure 2. ORMDL gene expression after RNAi treatment

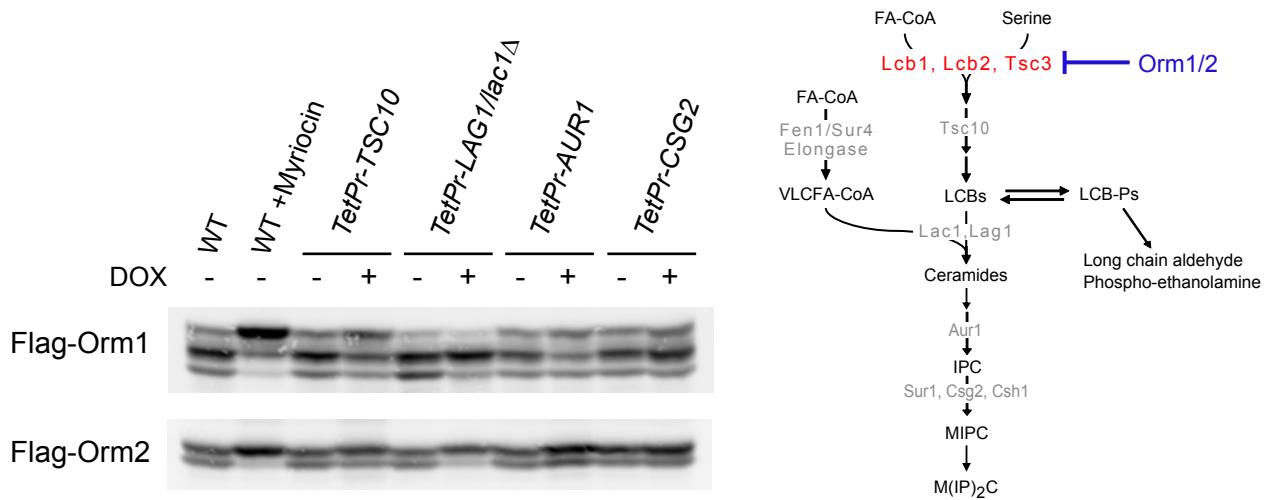
Gene expression was quantified for the indicated genes after transfection of Hela cells with siRNAs against the indicated genes. RT-PCR was performed with primers for the indicated genes using *RPL19* as a reference. Data are normalized to expression levels in untreated cells (average \pm s.d., n = 3).



Supplementary Figure 3. Myriocin treatment does not prevent formation of the Orm1/2-Lcb1/2-Sac1 complex. Colloidal-stained SDS/PAGE gels are shown for native affinity purifications from strains expressing 3xFlag-Orm1 or 3xFlag-Orm2. Prior to harvest, the indicated strains were grown for 12-16 hr in standard rich media or media supplemented with 150 ng/ml myriocin.

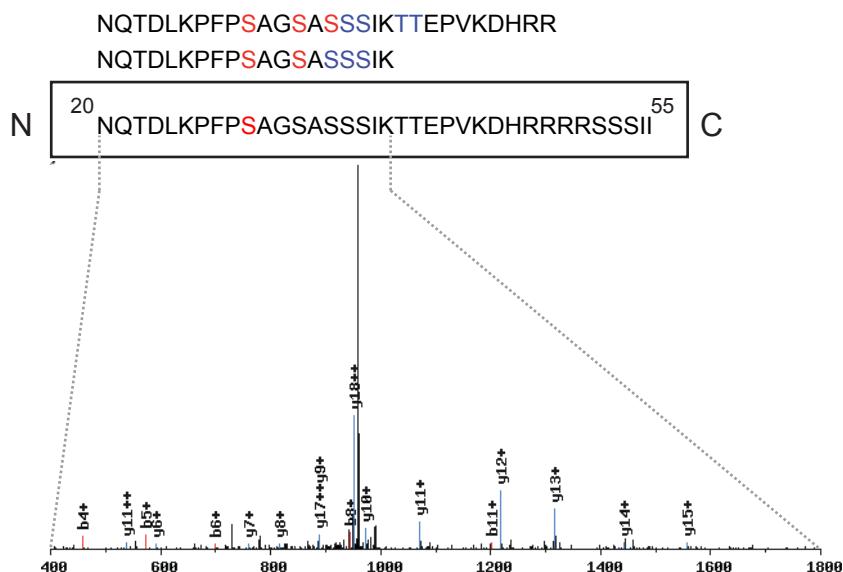


Supplementary Figure 4. Orm2 localization changes in response to myriocin treatment.
 GFP-Orm2 and GFP-Orm1 were visualized in strains grown in rich media with or without myriocin. Sec63-mCherry was used as a marker for ER localization. Arrowheads in images of GFP-Orm2 indicate a reduction in cortical ER localization in response to myriocin treatment. Median filtering was applied to reduce image noise.

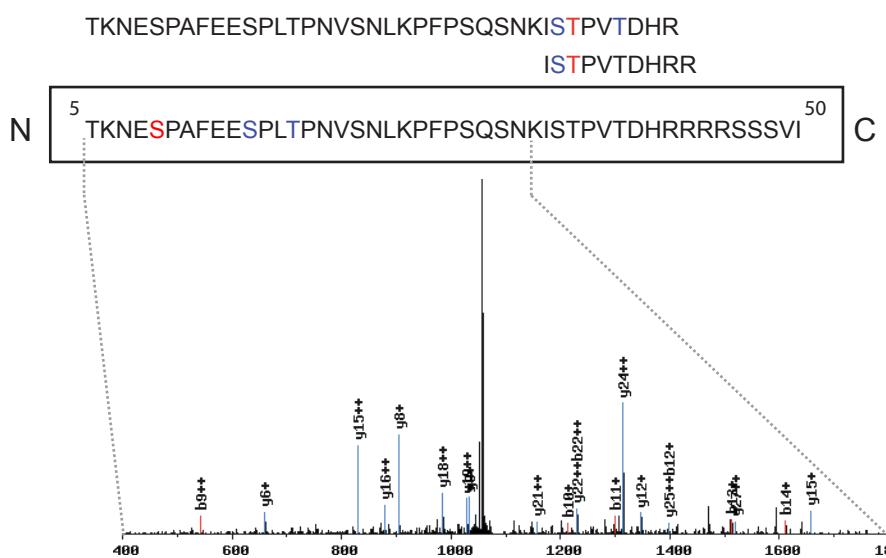


Supplementary Figure 5. Disruptions to sphingolipid synthesis that act downstream of Lcb1/2 but upstream of Aur1 induce Orm1/2 phosphorylation. Tetracycline-repressible promoters were inserted in front of the indicated genes in strains expressing 3xFlag-Orm1 or 3xFlag-Orm2 (note: *pTet-LAG1* was combined with deletion of *LAC1*). Lysates were prepared before and after gene expression shut-off (treatment with 5 µg/ml doxycycline for 14-16 hr) and analyzed by Western blot against the Flag epitope after separation on phosphate-affinity SDS/PAGE gels. Note: gel-to-gel differences in banding pattern are due to variability in phosphate-affinity gel resolution.

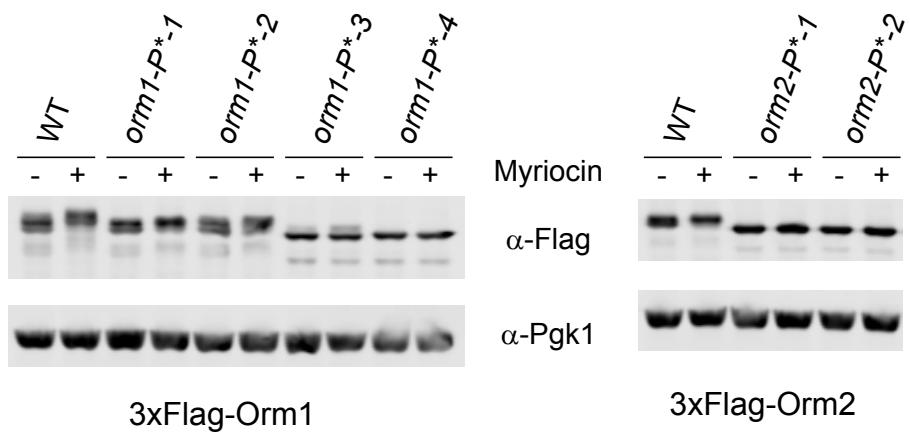
Orm1



Orm2



Supplementary Figure 6. Mass spectrometry identifies phosphorylated residues on Orm1 and Orm2. Phospho-peptides from immuno-precipitated 3xFlag-Orm1 and 3xFlag-Orm2 proteins were analyzed using an Orbitrap mass spectrometer. For both Orm1 and Orm2, three phospho-peptides were identified. All Orm2 phospho-peptides were singly phosphorylated, whereas singly, doubly and triply phosphorylated peptides were identified for Orm1. The most probable phosphorylation site assignments are highlighted in red, and alternative assignments (with a lower probability) are highlighted in blue. For the boxed peptides NQTDLKPFP*AGSASSSIK (Orm1) and TKNES*PAFEESPLTPNVSNLKPFPSQSNK (Orm2), annotated tandem mass spectra are shown, where S* indicates the site of phosphorylation.



Supplementary Figure 7. Wild type and phospho-mutant forms of Orm1 and Orm2 show similar expression levels. Lysates from strains expressing 3xFlag-tagged wildtype (WT) or phospho-mutant alleles of *ORM1* and *ORM2* were prepared after growth for 12-16 hr in 0 or 150 ng/ml myriocin. Western blots of these lysates were probed against the Flag epitope and against Pgk1 as a loading control.

Supplementary Methods

Plasmid sequences below are derived in part from published constructs described in Gari et al.² and Shaner et al.³

pFA6a-NATMX4_pTEF2_eGFP_Adh1-tm-AG

Primer annealing sequences used for PCR are underlined. TEF2 promoter sequence is in bold font.

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pFA6a-NATMX4_pTDH3_eGFP_Adh1-tm-AG

Primer annealing sequences used for PCR are underlined. TDH3 promoter sequence is in bold font.

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ACTATA

pFA6a-NAT-MX4-Tet-Act-pTet

A fusion PCR product of the pFA6a-NATMX4 marker, Tet-Activator, and 4XTet-Operator promoter was generated and cloned into the pCR2.1-TOPO vector

(Invitrogen). The sequence of the cloned PCR product is given below (NAT marker is underlined, TetO sites are in bold font):

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pNTI8_mCherry_URA

Primer annealing sequences used for PCR are underlined. mCherry sequence used for C-terminal tagging is in bold font.

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TTAAGTGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGTACTGCTGTCGATTGATACTA
ACGCCGCCATCCAGTGTGAAACGAGCTCGAATTCATCGATG

Protein ID	<u>IP 3xFlag-Orm1</u>		<u>IP 3xFlag-Orm2</u>	
	# of peptides	% Sequence Coverage	# of peptides	% Sequence Coverage
Sac1	42	74.6	33	62.1
Lcb1	23	40.9	26	43.5
Lcb2	23	41.0	22	37.1
Orm1	10	49.1	4	19.4
Orm2	6	25.0	8	31.9
Tsc3	3	30.0	1	17.5

Supplementary Table 1. Orm-associated proteins identified by mass spectrometry.
 Proteins found in immunoprecipitations of 3xFlag-Orm1 and 3xFlag-Orm2 were analyzed by mass spectrometry. Peptide coverage information for identified proteins corresponding to the bands indicated in **Fig. 2a** are shown above.

Supplementary Notes

References for Supplementary Information

1. Kearns, B. G. et al. Essential role for diacylglycerol in protein transport from the yeast Golgi complex. *Nature* 387, 101-5 (1997).
2. Gari, E., Piedrafita, L., Aldea, M. & Herrero, E. A set of vectors with a tetracycline-regulatable promoter system for modulated gene expression in *Saccharomyces cerevisiae*. *Yeast* 13, 837-48 (1997).
3. Shaner, N. C. et al. Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. *Nat Biotechnol* 22, 1567-72 (2004).