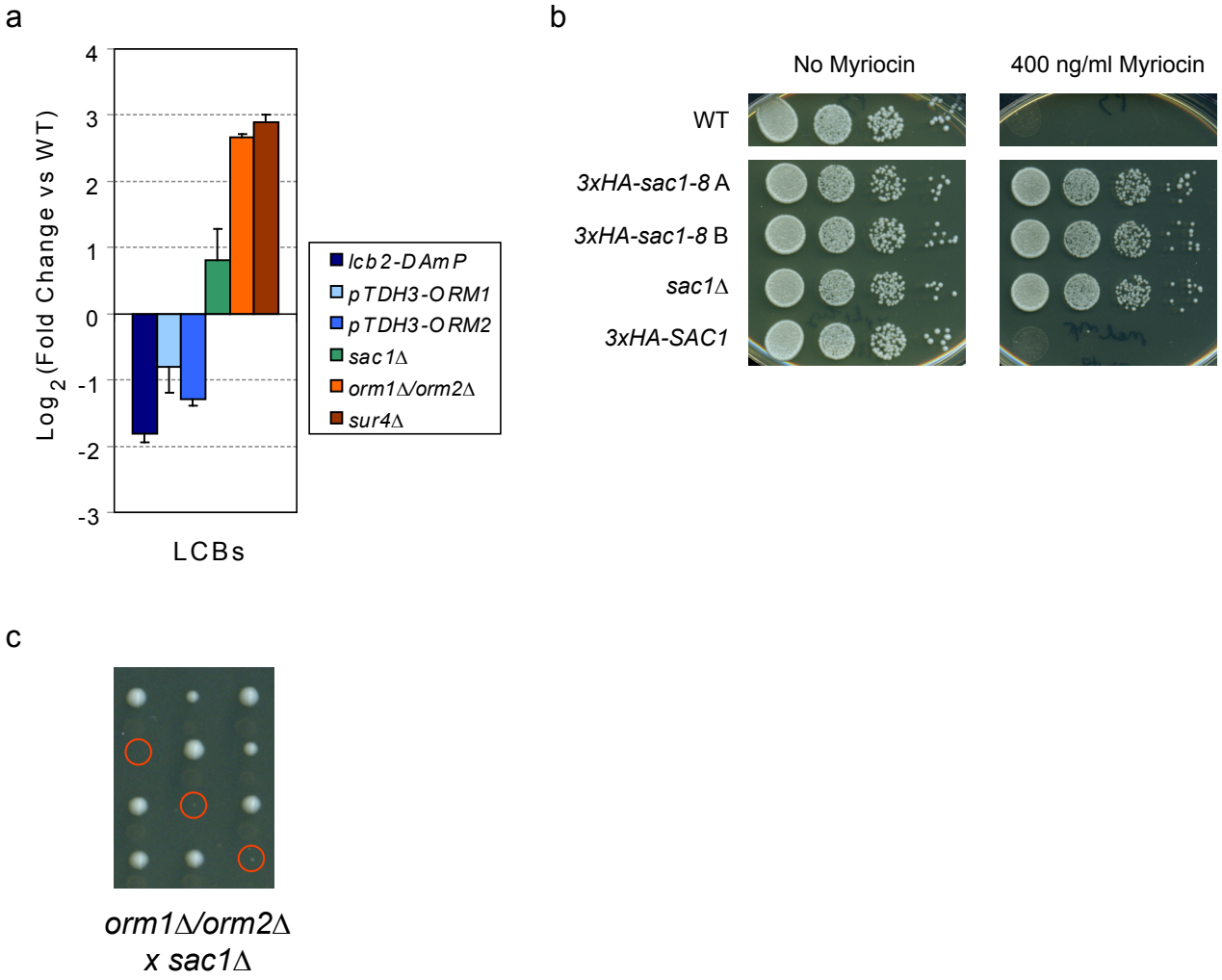
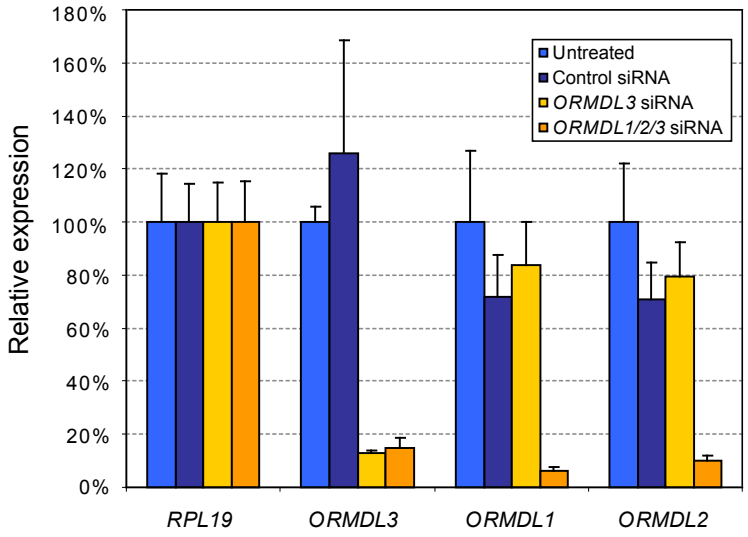
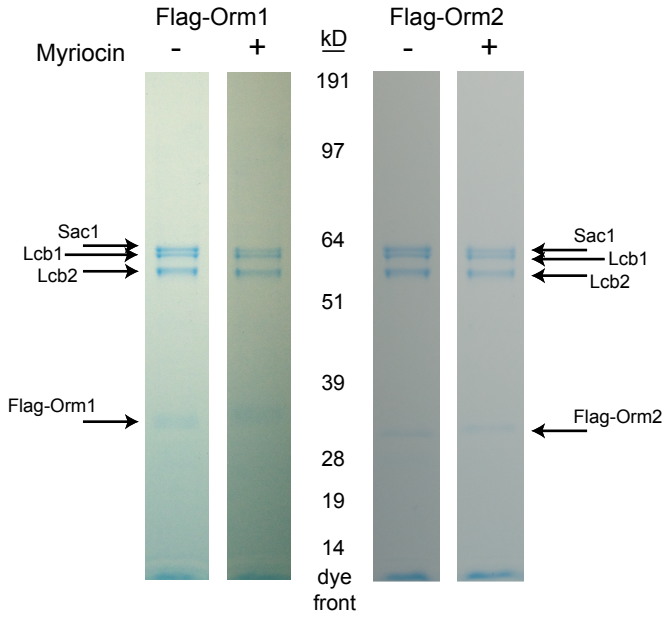


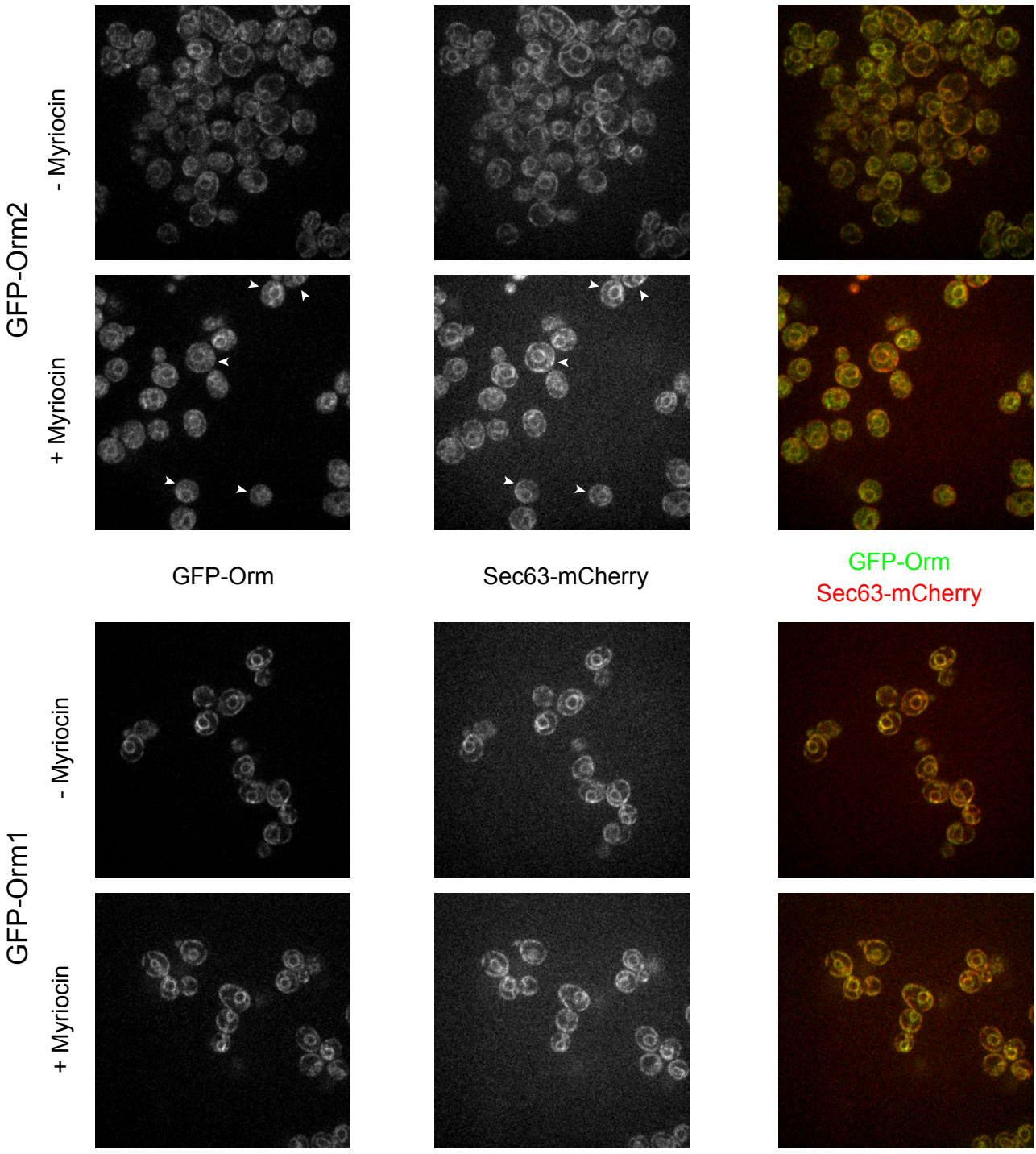

**Supplementary Figure 1. A functional role for Sac1 in sphingolipid metabolism.**
**a**, Lipidomic analysis of LCB levels in the indicated strains are shown (average ± s.d., n = 3). **b**, Serial ten-fold dilutions of the indicated strains were spotted on plates with 0 or 400 ng/ml myriocin and imaged after 24-48 hr of growth. The *sac1-8* allele of *SAC1* is a catalytically inactive *SAC1* mutant described previously[1]. **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.



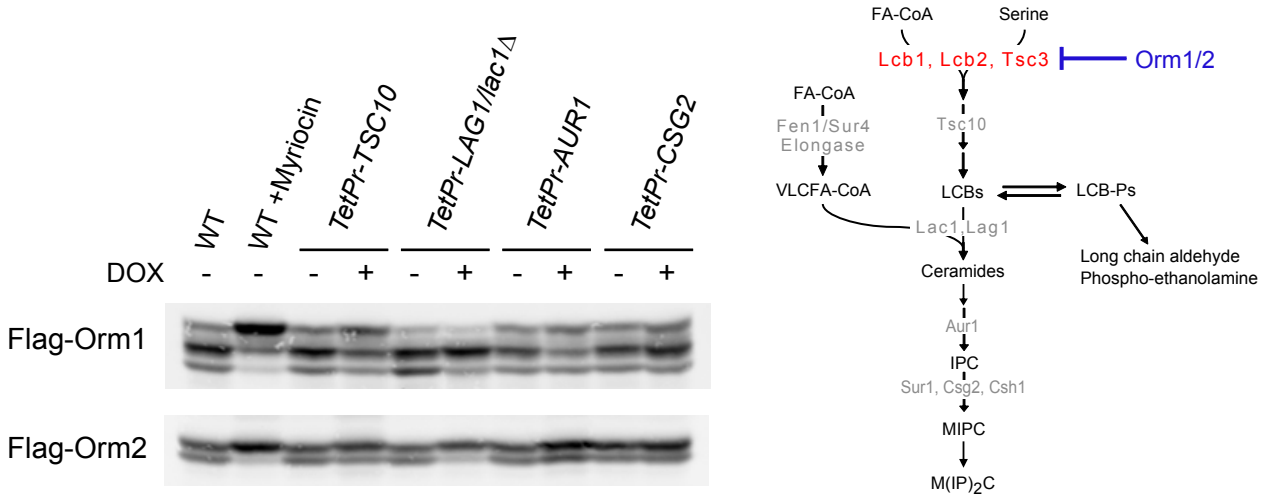

**Supplementary Figure 2. *ORMDL* gene expression after RNAi treatment**
Gene expression was quantified for the indicated genes after transfection of Hela cells with siRNAs against the indicated genes. RT-PCR was performed with primers for the indicated genes using *RPL19* as a reference. Data are normalized to expression levels in untreated cells (average ± 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.

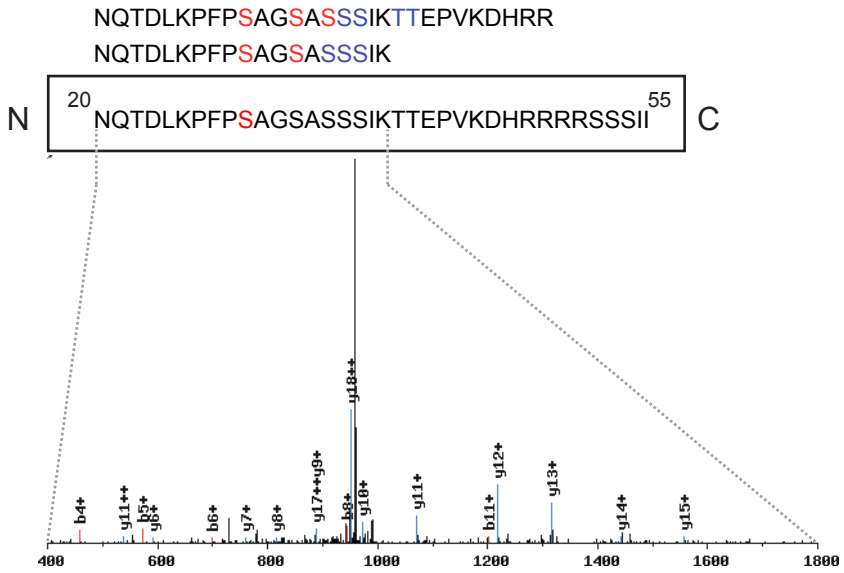



**Supplementary Figure 4. Orm2 localization changes in response to myriocin treatment.** GFP-Orm2 and GFP-Orm1 were visualized in strains grown in rich media with or without myriocin. Sec63-mCherry was used as a marker for ER localization. Arrowheads in images of GFP-Orm2 indicate a reduction in cortical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

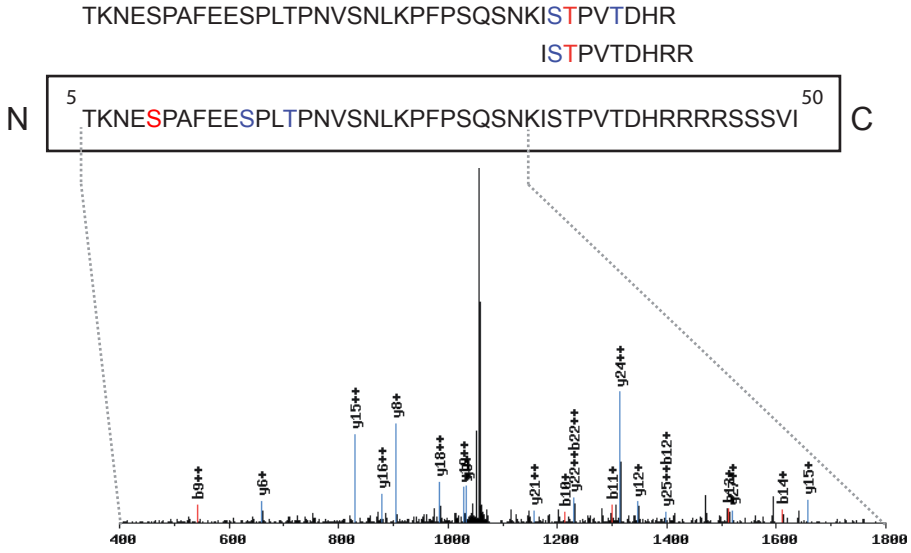



**Supplementary Figure 5. Disruptions to sphingolipid synthesis that act downstream of Lcb1/2 but upstream of Aur1 induce Orm1/2 phosphorylation.** Tetracycline-repressible promoters were inserted in front of the indicated genes in strains expressing 3xFlag-Orm1 or 3xFlag-Orm2 (note: *pTet-LAG1* was 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.

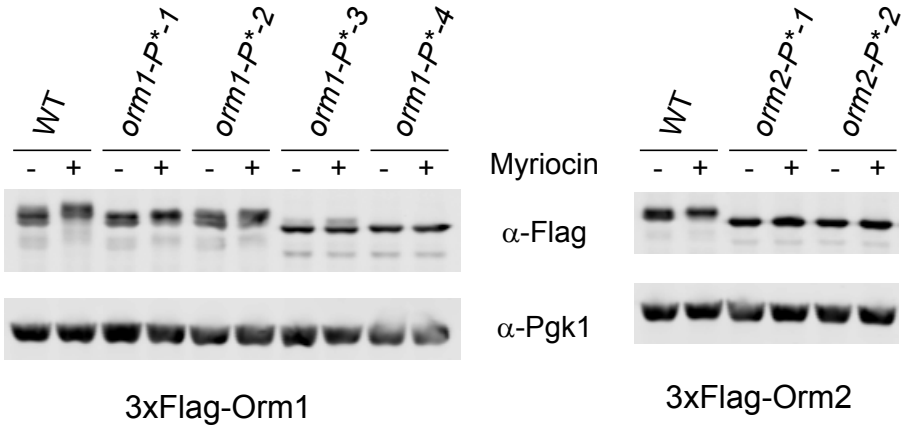
Orm1




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 NQTDLKPFPS*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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ccgcatatgatcaattcaaggccgaataagaaggctggctctgcaccttggtgatcaaataattcgatagcttgtcg
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**aagtgaaagtcgagtttaccactccctatcagtgatagagaaaagtgaaagtcgagtttaccactccctatcagtga**
**tagagaaaagtgaaagtcgag**ctcggtaccctatggcatgcatgtgctctgtatgtatataaaactcttgttttctt
cttttctctaaatattctttccttatacattaggtcctttgtagcataaattactatacttctatagacacgcaaac
acaaatacacacactaaattaccggatcaattcgggATG

**pNTI8_mCherry_URA**

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

ATATCAGATCCACTAGTGGCCTATGCGGCCGCGGATCTGCCGGTCTCCCTATAGTGAGTCGTATTAATTTCGATAAG
CCAGGTTAACCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCT
CGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTA
TCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGC
CGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGC
GAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTG
CCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCT
CAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTAT
CCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATT
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ATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCA
CCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTG
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GATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTG
ACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTC
CCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTAT
CCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTT
GTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATC
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TGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCA
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GTACTGAGAGTGCACCATATGGACATATTGTCGTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACA
CATACGATTTAGGTGACACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGCAGGTCGACGGATCGGTGACG
GTGCTGGTTTAATTAACATG**GTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGAGATTCAAG**
**GTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCAGACCCTACGAGGGCAC**
**CCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGT**
**ACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAG**
**TGGGAGAGAGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTT**
**CATCTACAAGGTGAAGTTGAGAGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGG**
**AGGCCTCCTCCGAGAGAATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGAC**
**GGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGT**
**CAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATTGTGGAACAATATGAAAGAGCTGAAGGTAGAC**
**ATTCTACTGGTGGTATGGATGAATTGTACAAA**TAAGGCGCGCCACTTCTAAATAAGCGAATTTCTTATGATTTATGA
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CACCTCTACCGGCAGATCCGCTAGGGATAACAGGGTAATATAGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCAC
CCGGCCAGCGACATGGAGGCCCAGAATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTG
TCGCCCGTACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCACGGCGCGAA
GCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCCCTCACAGACGCGTTGAATTGTCCCCAC
GCCGCGCCCCTGTAGAGAAATATAAAAGGTTAGGATTTGCCACTGAGGTTCTTCTTTCATATACTTCCTTTTAAAAT
CTTGCTAGGATACAGTTCTCACATCACATCCGAACATAAACAACCATGACAGTCAACACTAAGACCTATAGTGAGAG
AGCAGAAACTCATGCCTCACCAGTAGCACAGCGATTATTTCGATTAATGGAACTGAAGAAAACCAATTTATGTGCAT
CAATTGACGTTGATACCACTAAGGAATTCCTTGAATTAATTGATAAATTAGGTCCTTATGTATGCTTAATCAAGACT
CATATTGATATAATCAATGATTTTTCCTATGAATCCACTATTGAACCATTATTAGAACTTTCACGTAAACATCAATT

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TTAGGACAACAATATAGAACTGTTGATGAAGTTGTTAGCACTGGAACTGATATTATCATTGTTGGTAGAGGATTGTT
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TTAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGATACTA
ACGCCGCCATCCAGTGTCGAAAACGAGCTCGAATTCATCGATG

| | IP 3xFlag-Orm1 | | IP 3xFlag-Orm2 | |
|---|---|---|---|---|
| Protein ID | # 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).