

Adeyo et al., <http://www.jcb.org/cgi/content/full/jcb.201010111/DC1>

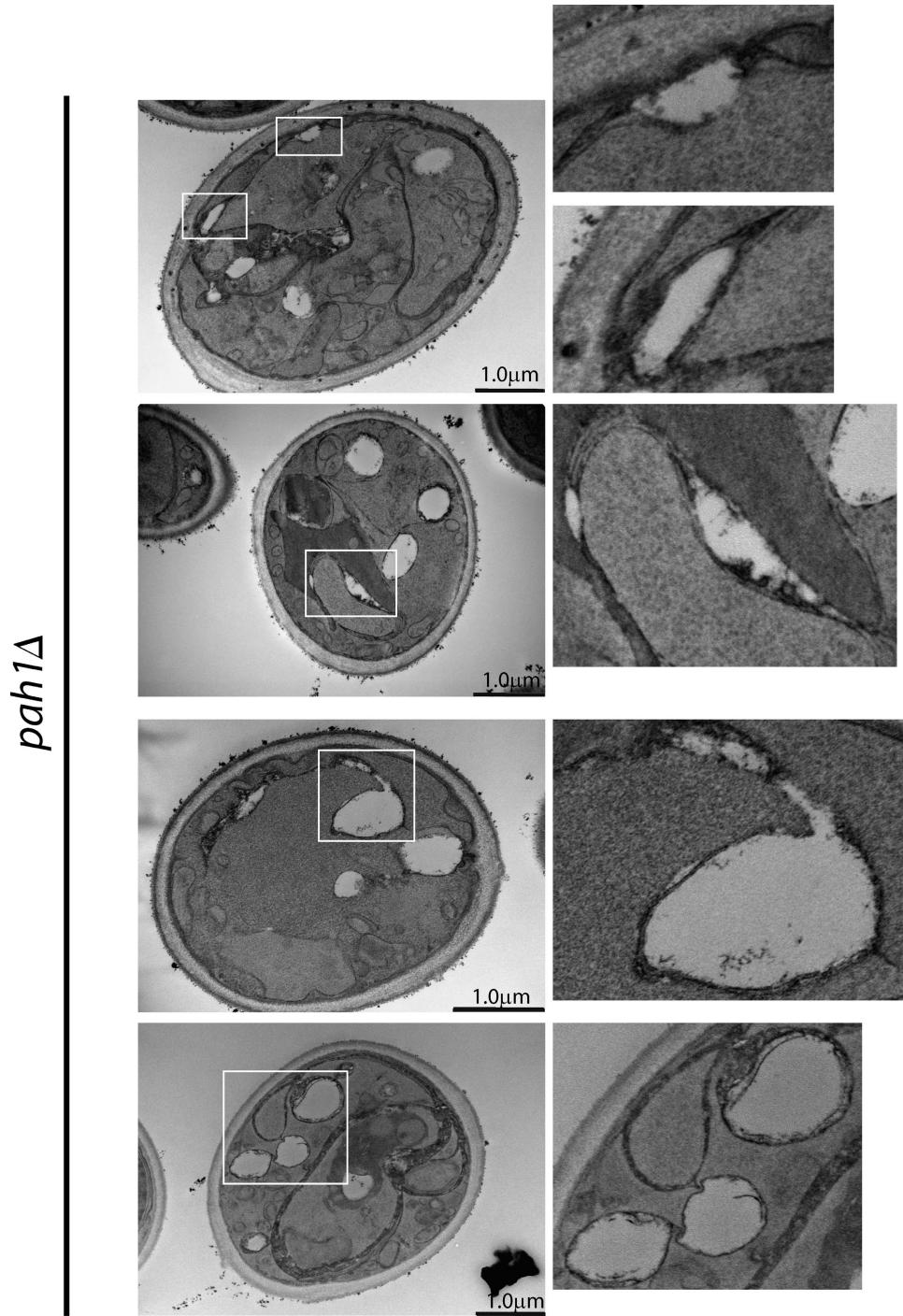


Figure S1. Electron micrographs of *pah1Δ* cells demonstrating large neutral lipid inclusions in membranes. Cells were cultured overnight in oleic acid medium. The images in the right column are enlarged sections of the images in the left column.

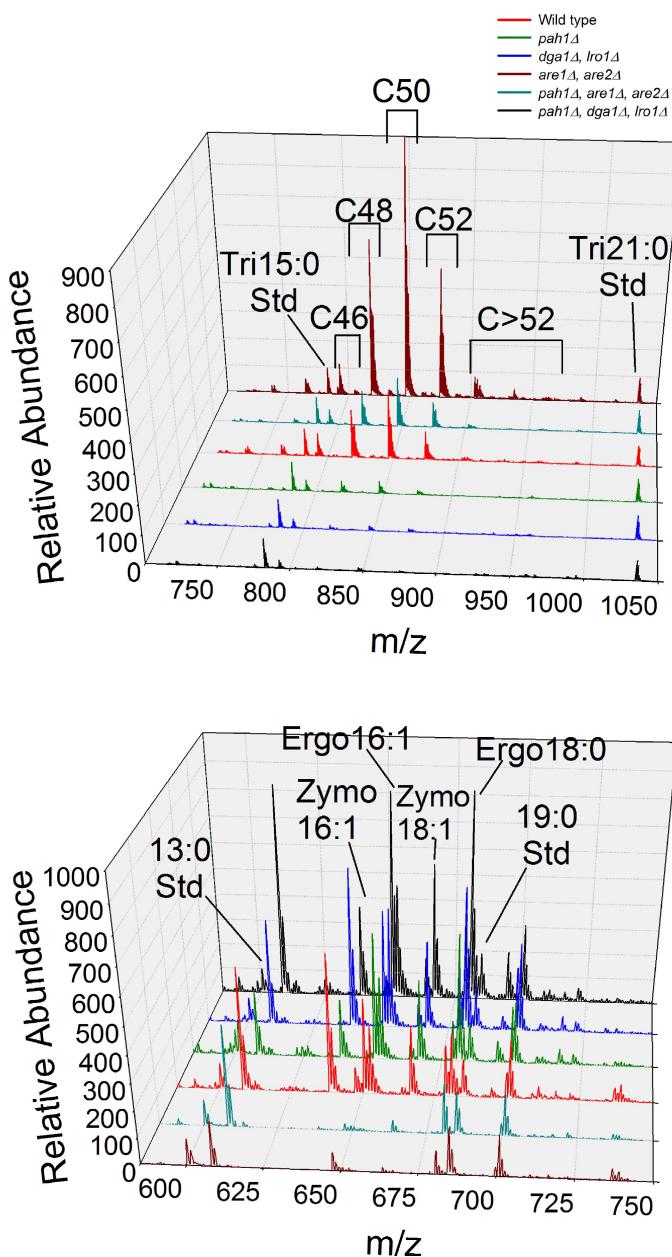


Figure S2. Mass spectroscopic analysis of TAGs and STEs from the indicated strains, all cultured in SD. The amount of specific species are listed in Tables S2 and S3. Ergo, ergosterol; Std, lipid standard; Zymo, zymosterol; m/z, mass to charge ratio.

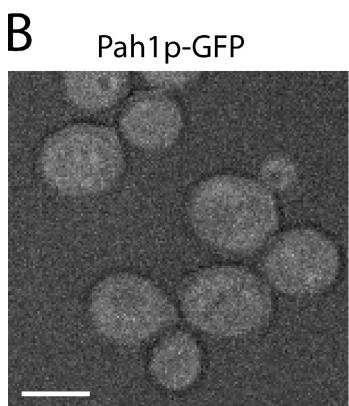
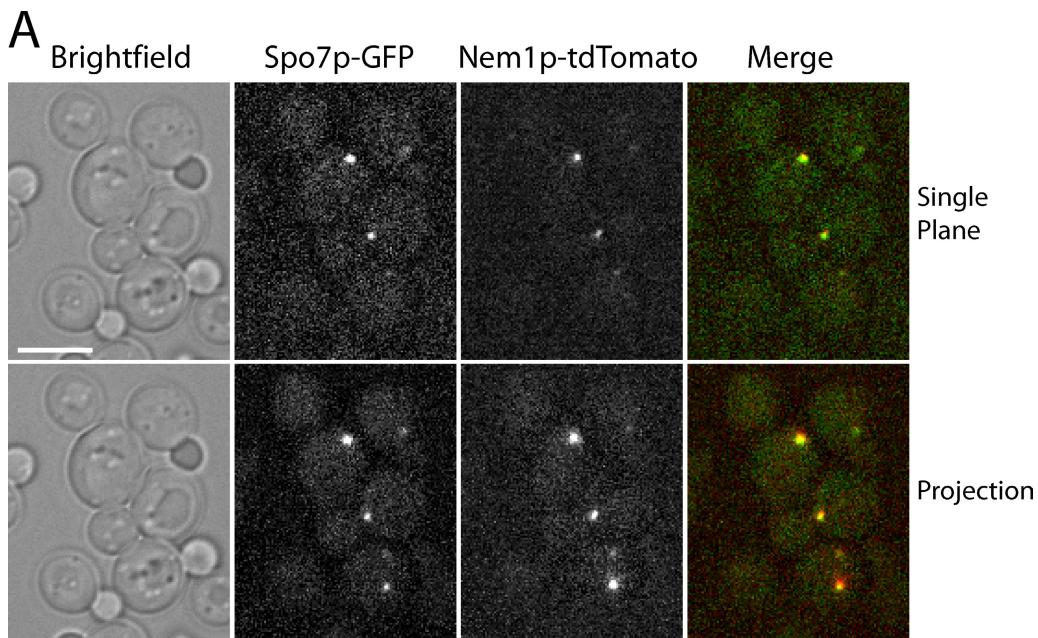
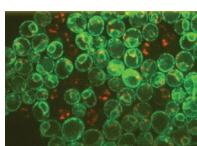


Figure S3. **Localization of Pah1p, Nem1p, and Spo7p (chromosomally tagged) cultured in SD medium.** (A) Colocalization of Spo7-GFP with Nem1p-tdTomato. (top row) Fluorescence images from a midplane section; (bottom row) fluorescence images from a projection of the corresponding z stack. (B) Cytoplasmic localization of Pah1p-GFP. Bars, 5 μ m.



Video 1. **Three-dimensional reconstruction of the field of cells shown in Fig. 6 A.** Lipid droplets in cells expressing Nem1p-mCherry were stained with BODIPY to generate the three-dimensional projection shown. Green, droplets; pink, Nem1p within 0.65 μ m of a droplet, the limit of resolution; blue, Nem1p-mCherry beyond 0.65 μ m of a droplet.



Video 2. **Lipid droplets move laterally on the ER surface but do not dissociate.** Living cells expressing mCherry-tagged Erg6p in the genome to label lipid droplets and GFP-HDEL expressed on a plasmid to label the ER (Szymanski et al., 2007) were subjected to time-lapse fluorescence microscopy for 2 min at 5-s intervals. Lateral movement of droplets was common, but dissociation from the ER was not observed.

Table S1. Plasmids and strains used in this study

| Plasmid | Relevant characteristics | Source or reference |
|---------------------------------|--|-----------------------------------|
| pRS313 | <i>E. coli</i> /yeast vector with <i>HIS3</i> | Sikorski and Hieter, 1989 |
| pOA101 | pRS313 containing <i>PAH1</i> 5' untranslated region (0.7 kb), <i>PAH1</i> coding sequence (2.6 kb), and 3' untranslated region (0.5 kb) inserted into <i>Xba</i> I– <i>Sac</i> II sites | This study |
| pOA102 | pOA101 containing G80R mutation in <i>PAH1</i> coding sequence | This study |
| pOA103 | pOA101 containing D396E mutation in <i>PAH1</i> coding sequence | This study |
| pOA104 | pOA101 containing D400E mutation in <i>PAH1</i> coding sequence | This study |
| pRS315 | <i>E. coli</i> /yeast vector with <i>LEU2</i> | Sikorski and Hieter, 1989 |
| pRS315-PGK | pRS315 containing <i>PGK1</i> promoter and terminator | Binns et al., 2006 |
| pRS315-PGK-CFP-HDEL | pRS315-PGK containing CFP-HDEL inserted into <i>Xba</i> I– <i>Sac</i> II sites | Szymanski et al., 2007 |
| pRS316-PGK-CFP-HDEL | pRS316-PGK containing CFP-HDEL inserted into <i>Xba</i> I– <i>Sac</i> II sites | Szymanski et al., 2007 |
| Strain | Genotype (all <i>S. cerevisiae</i>) | |
| BY4742 | Mata his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 | Thermo Fisher Scientific |
| BY4742-16601 | BY4742 (<i>nem1</i> Δ::Kan') | Thermo Fisher Scientific |
| BY4742-10399 | BY4739 (<i>spo7</i> Δ::Kan') | Thermo Fisher Scientific |
| BY4742-SL001 | BY4742 strain with mCherry at the C terminus of the <i>NEM1</i> ORF | This study |
| BY4742-DB001 | BY4742 strain with mCherry at the C terminus of the <i>ERG6</i> ORF | Szymanski et al., 2007 |
| BY4742-KS001 | BY4742 (<i>pah1</i> Δ::URA3) | This study |
| SCY328 | MATα ade2-1 his3-11,15 leu2-3,112, trp1-1ura3-1 can1 | Valachovic et al., 2006 |
| SCY1998 | <i>dga1</i> Δ::URA3, <i>lro1</i> Δ::LEU2 | Gift from S. Sturley ^a |
| SCY1703 | <i>are1</i> Δ::HIS3, <i>are2</i> Δ::LEU2 | Gift from S. Sturley ^a |
| SCY2021 | <i>dga1</i> Δ::URA3, <i>lro1</i> Δ::URA3, <i>are1</i> Δ::LEU2, <i>are2</i> Δ::HIS3 | Gift from S. Sturley ^a |
| OAS001 | SCY328 (<i>pah1</i> Δ::TRP1) | This study |
| OAS002 | <i>dga1</i> Δ::URA3, <i>lro1</i> Δ::LEU2, <i>pah1</i> Δ::TRP1 | This study |
| OAS003 | <i>are1</i> Δ::HIS3, <i>are2</i> Δ::LEU2, <i>pah1</i> Δ::TRP1 | This study |
| OAS004 | <i>dga1</i> Δ::URA3, <i>lro1</i> Δ::URA3, <i>are1</i> Δ::LEU2, <i>are2</i> Δ::HIS3, <i>pah1</i> Δ::TRP1 | This study |
| OAS005 | SCY328 (<i>dkg1</i> Δ::HIS3) | This study |
| OAS006 | SCY328 (<i>dkg1</i> Δ::HIS3, <i>pah1</i> Δ::TRP1) | This study |
| OAS007 | SCY1998 (<i>dkg1</i> Δ::HIS3) | This study |
| BY4741-Spo7p-GFP | BY4741 with GFP at the C terminus of the <i>SPO7</i> ORF | Invitrogen |
| BY4741-Spo7p-GFP-Nem1p-tdTomato | BY4741-Spo7p-GFP with tdTomato at the C terminus of the <i>NEM1</i> ORF | This study |
| BY4741-Pah1p-GFP | BY4741 with GFP at the C terminus of the <i>PAH1</i> ORF | Invitrogen |

^aColumbia University, New York, NY.

Table S2. TAG composition; mean mole percent values from two independent experiments

| m/z | TAG (total acyl chain length/ number of double bonds) | Wild type | <i>pah1Δ</i> | <i>are1Δ</i> <i>are2Δ</i> | <i>dga1Δ</i> <i>lro1Δ</i> | <i>pah1Δ</i> <i>are1Δ</i> <i>are2Δ</i> | <i>pah1Δ</i> <i>dga1Δ</i> <i>lro1Δ</i> |
|-------|--|-----------|--------------|------------------------------|------------------------------|--|--|
| 736.6 | 42:2 | 1.8 | 2.3 | 0.6 | 2.0 | 1.0 | 2.6 |
| 738.7 | 42:1 | 1.6 | 1.6 | 0.4 | 1.0 | 0.5 | 1.3 |
| 740.7 | 42:0 | 0.3 | 0.3 | 0.0 | 0.8 | 0.1 | 1.1 |
| 764.7 | 44:2 | 3.1 | 3.3 | 1.5 | 1.7 | 1.8 | 2.2 |
| 766.7 | 44:1 | 2.0 | 2.8 | 0.8 | 1.3 | 1.1 | 1.7 |
| 768.7 | 44:0 | 0.1 | 0.4 | 0.0 | 0.6 | 0.2 | 0.9 |
| 790.7 | 46:3 | 1.5 | 2.0 | 0.9 | 1.3 | 1.5 | 2.5 |
| 792.7 | 46:2 | 3.9 | 3.7 | 1.9 | 0.3 | 4.2 | 0.0 |
| 794.7 | 46:1 | 1.5 | 2.4 | 0.6 | 1.9 | 0.9 | 2.7 |
| 796.7 | 46:0 | 0.2 | 0.4 | 0.1 | 1.7 | 0.2 | 1.1 |
| 818.7 | 48:3 | 17.9 | 16.4 | 16.5 | 10.1 | 18.0 | 6.7 |
| 820.7 | 48:2 | 8.2 | 8.5 | 5.9 | 4.0 | 5.3 | 3.5 |
| 822.8 | 48:1 | 1.2 | 1.2 | 0.6 | 1.2 | 0.4 | 1.7 |
| 846.8 | 50:3 | 25.9 | 20.4 | 30.6 | 21.0 | 27.2 | 13.1 |
| 848.8 | 50:2 | 7.7 | 8.4 | 6.7 | 8.1 | 7.2 | 5.7 |
| 850.8 | 50:1 | 0.7 | 0.9 | 0.7 | 1.0 | 0.5 | 1.7 |
| 852.8 | 50:0 | 0.5 | 0.6 | 0.5 | 1.0 | 0.6 | 1.6 |
| 874.8 | 52:3 | 10.1 | 7.8 | 18.1 | 12.0 | 14.5 | 6.1 |
| 876.8 | 52:2 | 3.7 | 4.3 | 5.4 | 7.5 | 6.4 | 3.1 |
| 878.8 | 52:1 | 1.2 | 2.1 | 1.1 | 1.9 | 0.7 | 2.6 |
| 880.8 | 52:0 | 0.6 | 0.8 | 0.5 | 0.8 | 0.6 | 1.7 |
| 904.8 | 54:2 | 1.4 | 1.2 | 2.6 | 3.3 | 2.0 | 3.4 |
| 906.8 | 54:1 | 0.9 | 0.9 | 1.1 | 0.9 | 0.6 | 1.3 |
| 908.9 | 54:0 | 0.5 | 0.8 | 0.4 | 1.2 | 0.4 | 1.7 |
| 932.9 | 56:2 | 0.5 | 0.8 | 0.4 | 2.5 | 0.5 | 6.1 |
| 934.9 | 56:1 | 0.8 | 1.2 | 0.9 | 2.9 | 0.7 | 5.1 |
| 960.9 | 58:2 | 0.6 | 1.0 | 0.3 | 3.2 | 1.0 | 7.7 |
| 962.9 | 58:1 | 0.4 | 0.8 | 0.3 | 0.9 | 0.4 | 1.9 |
| 988.9 | 60:2 | 0.9 | 1.5 | 0.6 | 2.3 | 1.1 | 6.1 |
| 990.9 | 60:1 | 0.3 | 1.1 | 0.2 | 1.6 | 0.5 | 2.8 |

The data are graphed in Fig. S2.

Table S3. StE composition; mean mole percent values from two independent experiments

| m/z | Sterol group (acyl chain) | Wild type | pah1Δ | dga1Δ lro1Δ | pah1Δ dga1Δ lro1Δ |
|-------|--|-----------|-------|----------------|-------------------------|
| 612.6 | Zymosterol (14:0) | 1.1 | 1.3 | 0.4 | 0.8 |
| 624.6 | Ergosterol (14:0) | 1.0 | 1.6 | 0.5 | 1.1 |
| 626.6 | Fecosterol/Episterol (14:0) | 0.9 | 1.5 | 0.5 | 0.9 |
| 638.6 | Zymosterol (16:1) | 23.9 | 9.9 | 19.1 | 10.2 |
| 640.6 | Zymosterol (16:0) | 2.3 | 1.6 | 1.2 | 1.6 |
| 650.6 | Ergosterol (16:1) | 17.1 | 20.9 | 15.5 | 21.8 |
| 652.6 | Ergosterol (16:0) ^a | 10.0 | 11.7 | 14.1 | 10.3 |
| 654.6 | Fecosterol/Episterol (16:0) | 2.1 | 2.1 | 2.0 | 1.8 |
| 666.6 | Zymosterol (18:1) | 12.7 | 14.0 | 11.2 | 14.0 |
| 668.6 | Zymosterol (18:0) | 1.4 | 2.0 | 0.9 | 2.2 |
| 678.6 | Ergosterol (18:1) | 9.5 | 7.6 | 8.6 | 10.4 |
| 680.6 | Ergosterol (18:0) ^b | 12.0 | 20.4 | 22.0 | 20.2 |
| 682.7 | Fecosterol/Episterol (18:0) ^c | 2.1 | 2.6 | 1.8 | 2.3 |
| 708.7 | Lanosterol (18:1) | 1.5 | 1.4 | 1.3 | 1.2 |
| 710.7 | Lanosterol (18:0) | 2.5 | 1.6 | 0.9 | 1.2 |

The data are graphed in Fig. S2. *are1Δare2Δ* and *pah1Δare1Δare2Δ* were not determined because of small amounts or no lipid present.

^aSmaller amounts of Fecosterol/Episterol (16:1) were also present at this m/z.

^bSmaller amounts of Fecosterol/Episterol (18:1) and Lanosterol (16:1) were also present at this m/z.

^cSmaller amounts of Lanosterol (16:0) were also present at this m/z.

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