

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

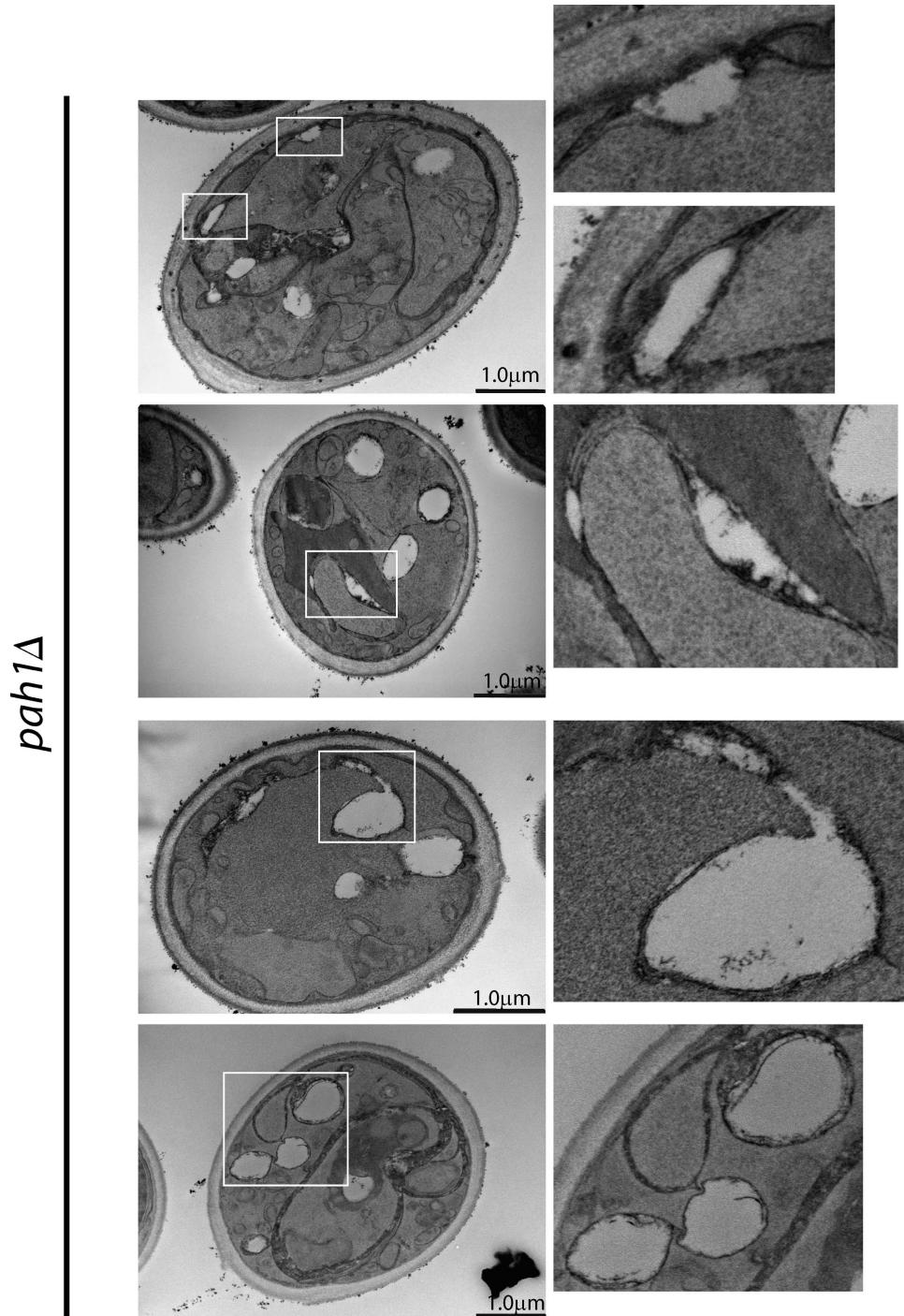


Figure S1. Electron micrographs of *pah1Δ* cells demonstrating large neutral lipid inclusions in membranes. Cells were cultured overnight in oleic acid medium. The images in the right column are enlarged sections of the images in the left column.

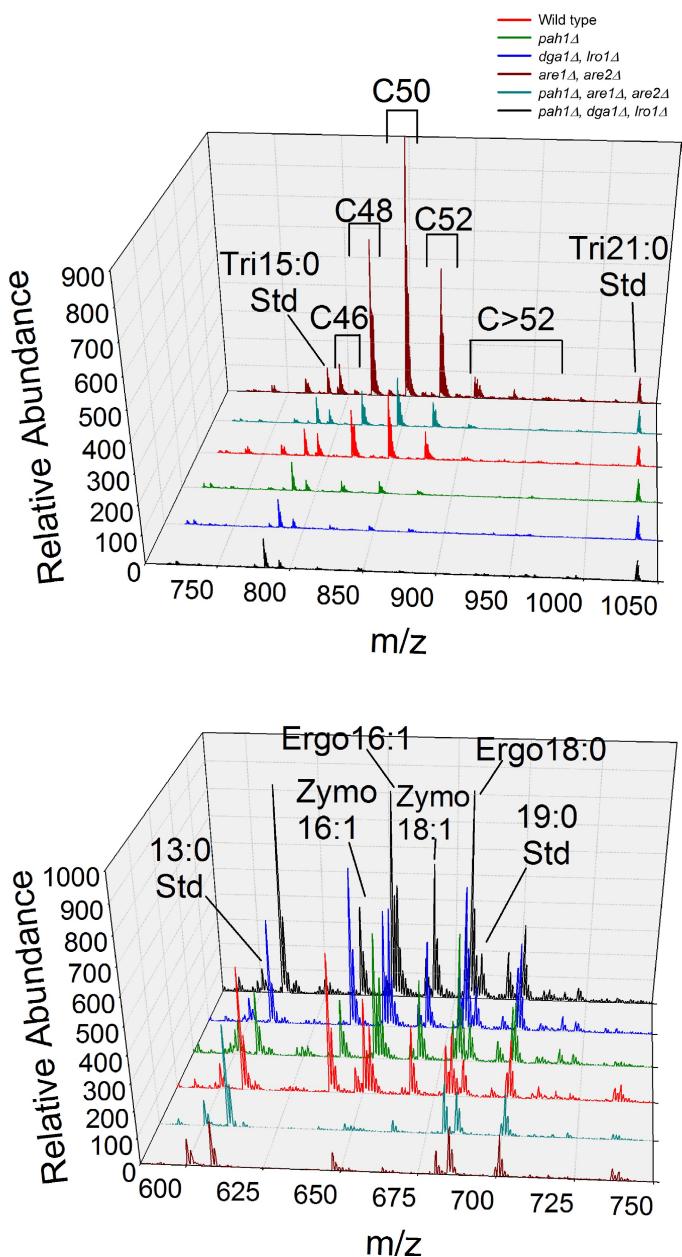


Figure S2. Mass spectroscopic analysis of TAGs and STEs from the indicated strains, all cultured in SD. The amount of specific species are listed in Tables S2 and S3. Ergo, ergosterol; Std, lipid standard; Zymo, zymosterol; m/z, mass to charge ratio.

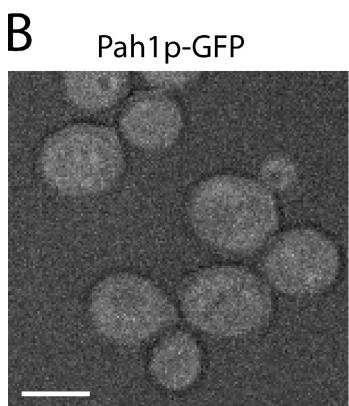
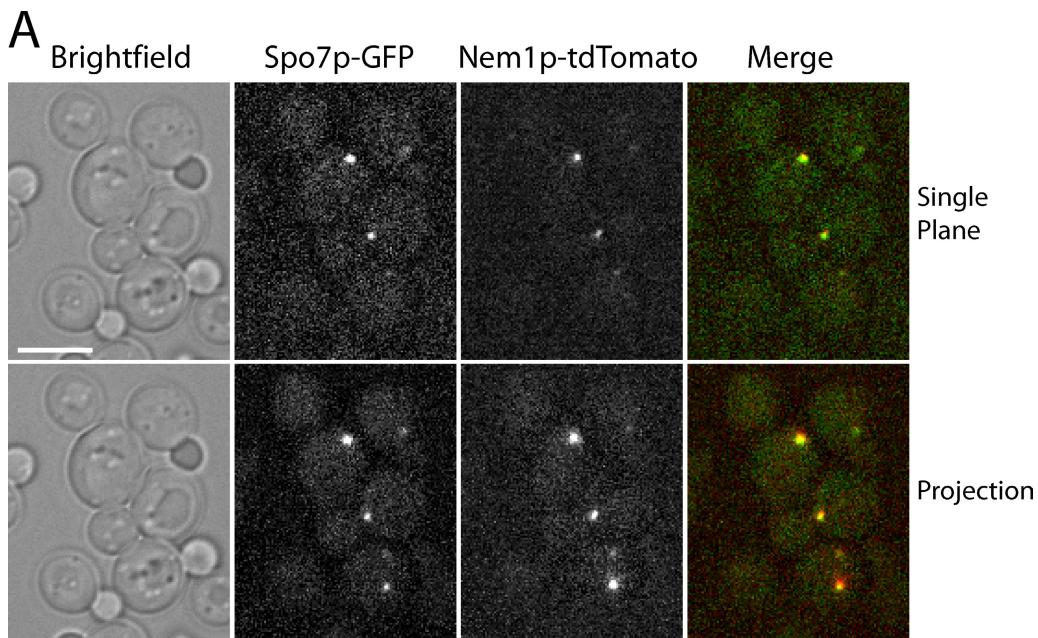
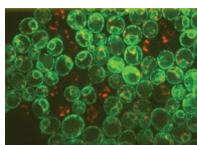


Figure S3. **Localization of Pah1p, Nem1p, and Spo7p (chromosomally tagged) cultured in SD medium.** (A) Colocalization of Spo7-GFP with Nem1p-tdTomato. (top row) Fluorescence images from a midplane section; (bottom row) fluorescence images from a projection of the corresponding z stack. (B) Cytoplasmic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