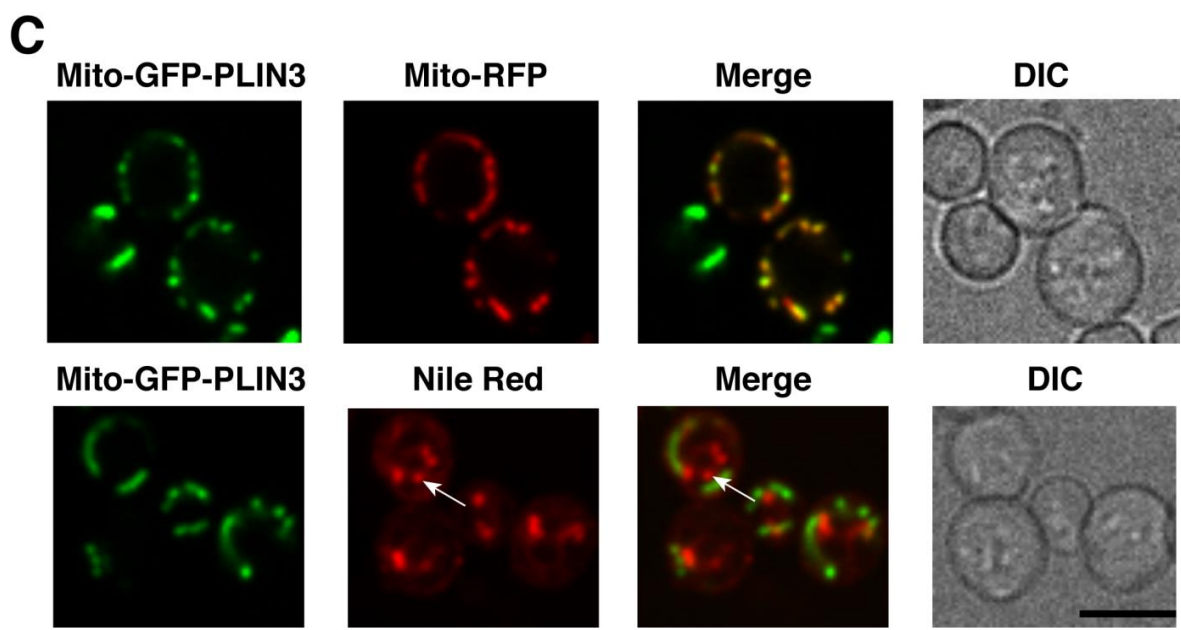
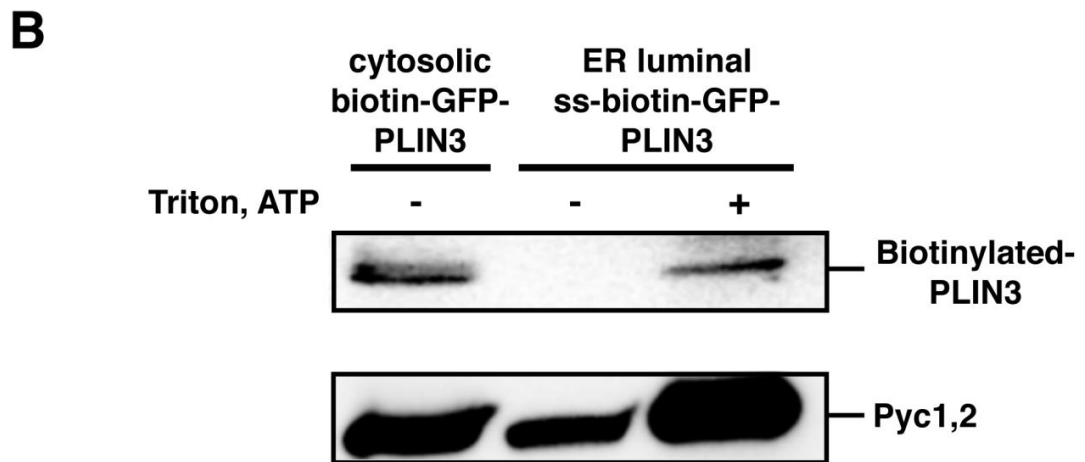
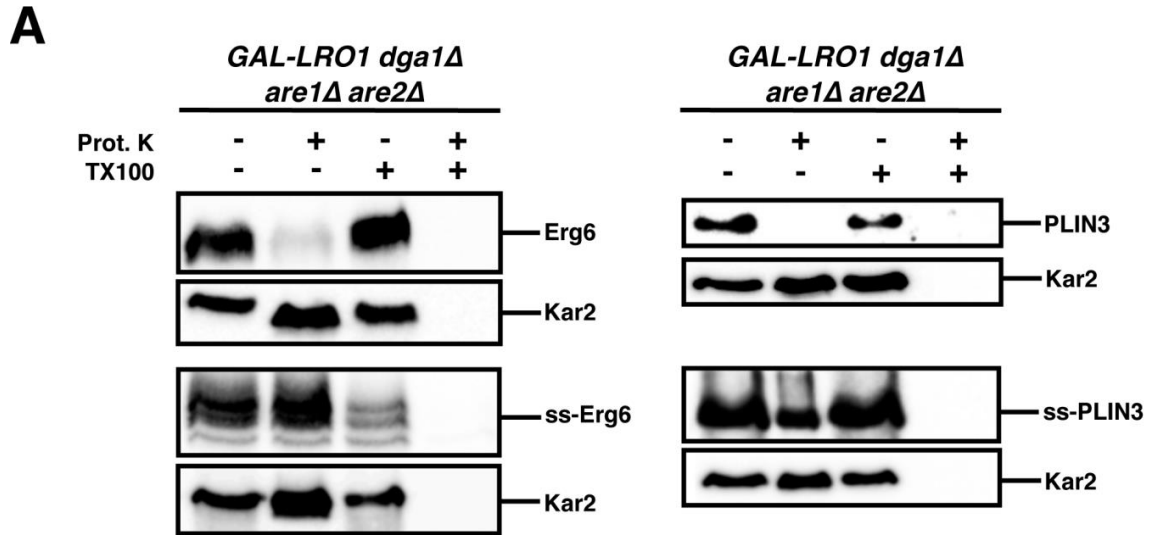


Supplementary Material

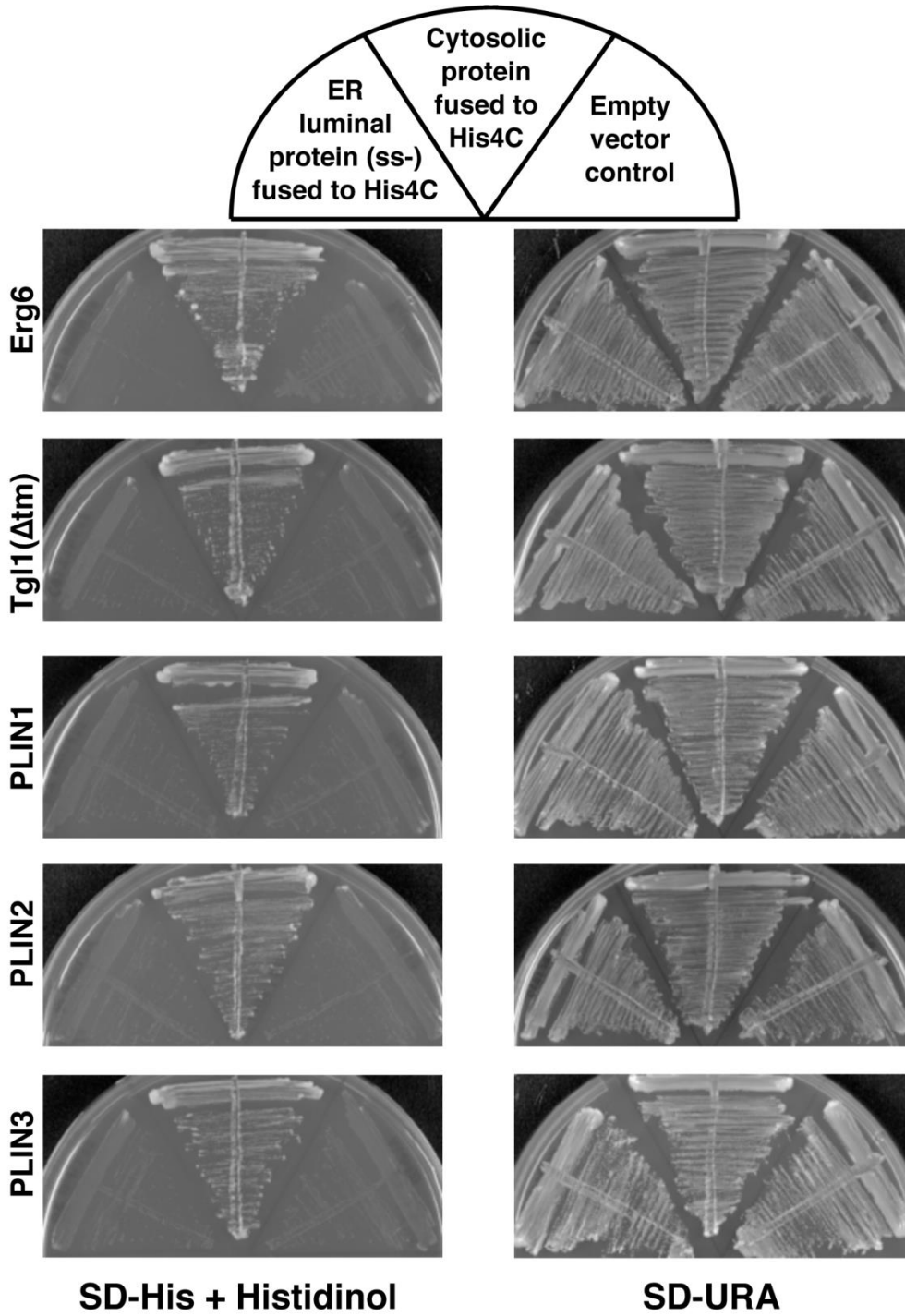
Supplementary Figures



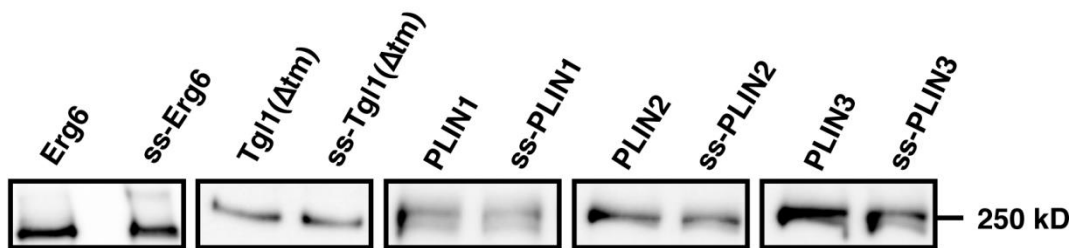
**Figure S1.** ss-containing marker proteins are protease protected in cells lacking LDs, protected from biotinylation, and PLIN3 can efficiently be targeted to mitochondria.

A) ss-Erg6 and ss-PLIN3 are protease protected when expressed in cells lacking LDs. Cells of the indicated genotype were cultivated in glucose containing medium, microsomes were isolated and subjected to digestion by proteinase K (Prot. K) in the presence and absence of Triton X-100 (TX100). Proteins were separated by SDS-PAGE and Western blots were probed with antibodies against GFP and Kar2. B) A signal sequence containing biotinylatable PLIN3 is protected from biotinylation by the cytosolic biotin-CoA ligase *in vivo*. Cell extracts were prepared from cells expressing a cytosolic or signal sequence containing biotinylatable version of PLIN3. Where indicated, the extract was incubated for 2 h at 30°C with 1% Triton X-100, with free biotin, CoA, and an ATP regenerating system. Proteins were then separated by SDS-PAGE and biotinylated proteins were detected with avidin conjugated to horseradish peroxidase. The native, biotinylated protein pyruvate carboxylase Pyc1,2 (130 kD) serves as a loading control. C) PLIN3 containing a mitochondrial targeting signal colocalizes with the mitochondrial marker mito-RFP but not with LDs. Cells expressing GFP-PLIN3, containing an N-terminal mitochondrial targeting signal, were analyzed by confocal microscopy. Colocalization was observed between the fusion protein and the mitochondria marker mito-RFP, but not between mito-GFP-PLIN3 and Nile red-stained LDs. Bar, 5 µm.

**A**

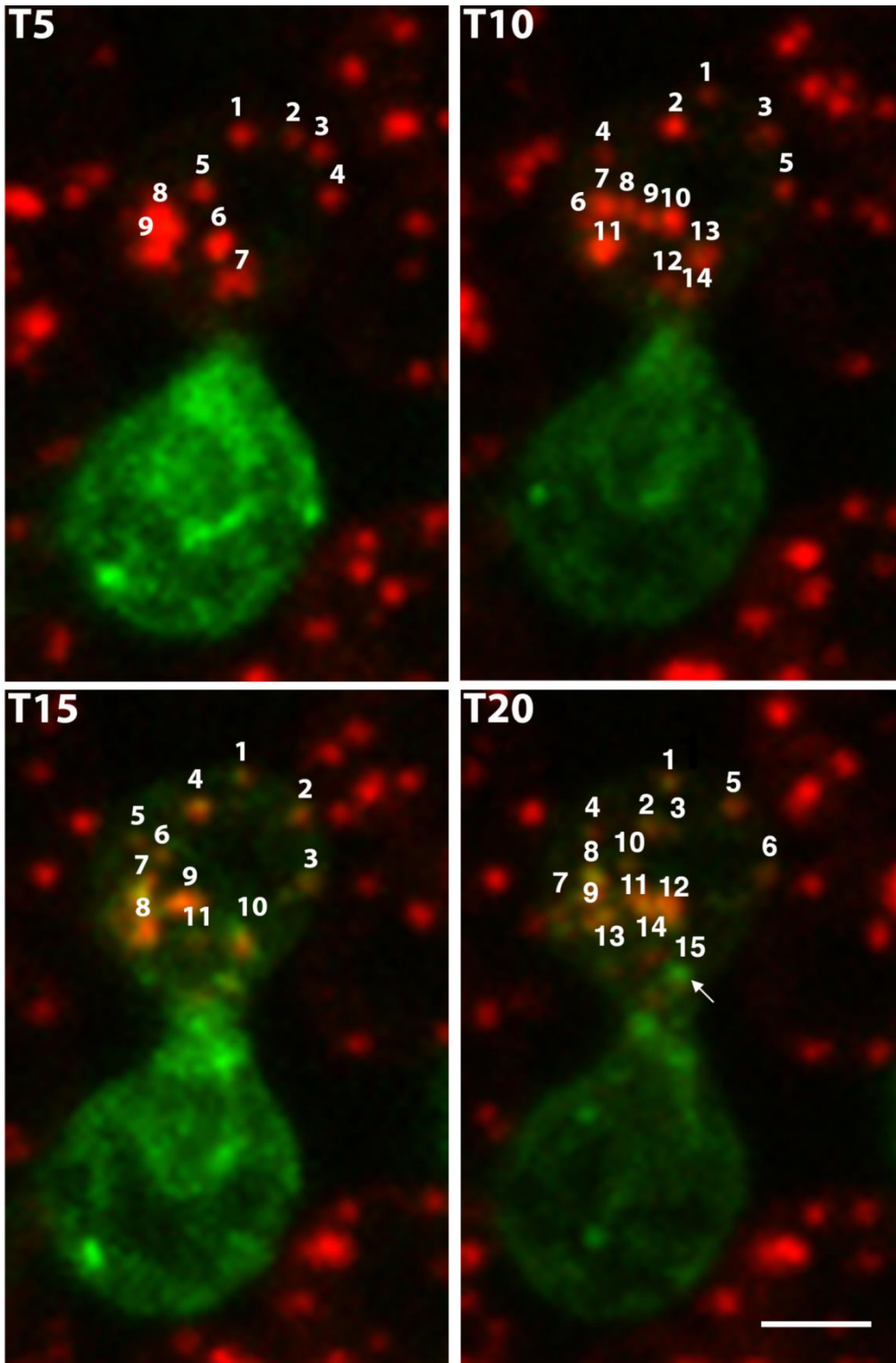


**B**



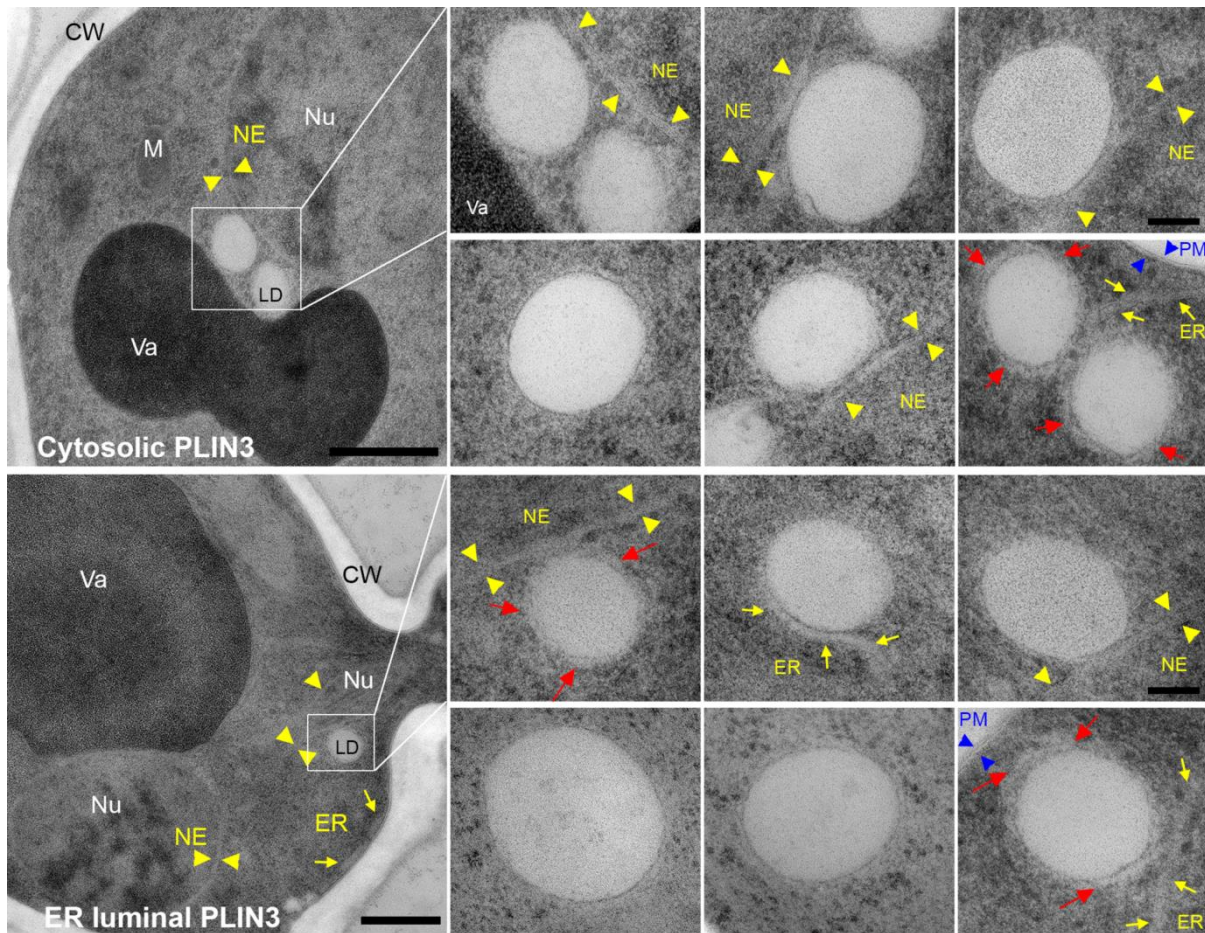
**Figure S2.** Signal sequence containing LD marker proteins are stringently translocated.

A) Histidine auxotrophic cells expressing the indicated LD marker proteins fused to the invertase-His4C dual topology reporter were streaked on plates lacking histidine but containing histidinol and on plates lacking uracil to control for the presence of the plasmid. Absence of growth is consistent with an ER luminal localization of the respective fusion protein. B) The dual topology reporters are expressed. Western blot using an antibody against GFP showing expression of the cytosolic and ss-containing variants of the indicated LD marker proteins.



**Figure S3.** Quantification of the rate of ss-GFP-PLIN3 onto LDs marked by Erg6-mCherry upon cell fusion.

MAT $\alpha$  4 $\Delta$  cells lacking LDs (*lro1 $\Delta$  dga1 $\Delta$  are1 $\Delta$  are2 $\Delta$* ) expressing ss-GFP-PLIN3 were mated with wild-type MAT $\alpha$  cells expressing the LD marker Erg6-mCherry, and mating progression was analyzed by time-lapse spinning disk confocal microscopy with 7 confocal sections, separated by 0.6  $\mu$ m, taken every 1 min. Maximal intensity projections from the 5, 10, 15, and 20 min time points are displayed. GFP and mCherry intensities of every LD were normalized to the total GFP and mCherry intensities present in the respective mating partner. Individual LDs are numbered to measure the ratio of GFP/mCherry fluorescence. An outlier with a high GFP/mCherry ratio at the T20 time point is indicated by the arrow. Bar, 2  $\mu$ m.



**Figure S4.** Expression of ER luminal PLIN3 does not affect the morphology and ER association of LDs.

Cells expressing either cytosolic PLIN3 or ss-PLIN3 were cryofixed and processed for EM. The nuclear envelope (NE) is indicated by yellow arrowheads, the ER is indicated by yellow arrows, the plasma membrane (PM) is indicated by blue arrowheads. Ribosome free zones covering LDs are indicated by red arrows. CW, cell wall; M, mitochondria; Nu, nucleus; Va, vacuole. Bar in whole cell images, 500 nm; in LD blow ups, 100 nm.

**Table S1: Yeast strains used in this study**

<b>Name</b>	<b>Relevant Genotype</b>	<b>Source</b>
RSY1533	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Euroscarf
RSY5226	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i> <i>pGREG506-ADH1-ERG6-GFP-URA3</i>	This study
RSY5361	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pRS416-</i> <i>ADH1-TGL1<sup>30-548</sup>-GFP-URA3</i>	This study
RSY5305	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i> <i>pGREG506-ADH1-SS<sup>PRY1</sup>-ERG6-GFP-URA3</i>	This study
RSY5360	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pRS416-</i> <i>ADH1-SS<sup>PRY1</sup>-TGL1<sup>30-548</sup>-GFP-URA3</i>	This study
RSY4640	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i> <i>pGREG576-ADH1-GFP-PLIN1-URA3</i>	Jacquier <i>et al.</i> , 2013
RSY4639	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i> <i>pGREG576-ADH1-GFP-PLIN2-URA3</i>	Jacquier <i>et al.</i> , 2013
RSY4647	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i> <i>pGREG576-ADH1-GFP-PLIN3-URA3</i>	Jacquier <i>et al.</i> , 2013
RSY5470	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i> <i>pGREG506-ADH1-SS<sup>PRY1</sup>-GFP-PLIN1-URA3</i>	This study
RSY5525	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pRS416-</i> <i>ADH1-SS<sup>PRY1</sup>-GFP-PLIN2-URA3</i>	This study
RSY5472	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i> <i>pGREG506-ADH1-SS<sup>PRY1</sup>-GFP-PLIN3-URA3</i>	This study
RSY3021	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0</i> <i>GAL-LRO1::TRP1 dga1::loxP are1::KanMX</i> <i>are2::KanMX</i>	Lab collection
RSY4645	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0</i> <i>GAL-LRO1::TRP1 dga1::loxP are1::KanMX</i> <i>are2::KanMX pGREG576-ADH1-GFP-PLIN2-</i> <i>URA3</i>	Jacquier <i>et al.</i> , 2013
RSY5729	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0</i> <i>GAL-LRO1::TRP1 dga1::loxP are1::KanMX</i> <i>are2::KanMX pRS416-ADH1-SS<sup>PRY1</sup>-GFP-</i>	This study



<i>PLIN2-URA3</i>		
RSY4641	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dga1::loxP are1::KanMX are2::KanMX pGREG576-ADH1-GFP-PLIN3-URA3</i>	Jacquier <i>et al.</i> , 2013
RSY5306	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dga1::loxP are1::KanMX are2::KanMX pGREG506-ADH1-SS<sup>PRY1</sup>-ERG6-GFP-URA3</i>	This study
RSY5308	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dga1::loxP are1::KanMX are2::KanMX pGREG506-ADH1-SS<sup>PRY1</sup>-GFP-PLIN3-URA3</i>	This study
RSY5718	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dga1::loxP are1::KanMX are2::KanMX pGREG506-ADH1-ERG6-GFP-URA3</i>	This study
RSY5715	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dga1::loxP are1::KanMX are2::KanMX HRD1::HIS3</i>	This study
RSY5717	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pGREG506-ADH1-SS<sup>PRY1</sup>-ERG6-GFP-URA3 pGREG503-ADH1-ERG6-mCherry-HIS3</i>	This study
RSY5719	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dga1::loxP are1::KanMX are2::KanMX pGREG506-ADH1-ERG6-GFP-URA3 pADH-mCherryHDEL-LEU2::CEN</i>	This study
RSY5720	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dga1::loxP are1::KanMX are2::KanMX pGREG506-ADH1-SS<sup>PRY1</sup>-ERG6-GFP-URA3 pADH-mCherryHDEL-LEU2::CEN</i>	This study
RSY5721	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dga1::loxP are1::KanMX are2::KanMX pRS416-ADH1-TGL1<sup>30-548</sup>-GFP-URA3 pADH-mCherryHDEL-LEU2::CEN</i>	This study

RSY5722	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dgal::loxP are1::KanMX are2::KanMX pRS416-ADH1- SS<sup>PRY1</sup>-TGL1<sup>30-548</sup>-GFP-URA3 pADH-mCherryHDEL-LEU2::CEN</i>	This study
RSY5723	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dgal::loxP are1::KanMX are2::KanMX pGREG576-ADH1-GFP-PLIN1-URA3 pADH-mCherryHDEL-LEU2::CEN</i>	This study
RSY5724	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dgal::loxP are1::KanMX are2::KanMX pGREG576-ADH1- SS<sup>PRY1</sup>-GFP-PLIN1-URA3 pADH-mCherryHDEL-LEU2::CEN</i>	This study
RSY5725	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dgal::loxP are1::KanMX are2::KanMX pGREG576-ADH1-GFP-PLIN3-URA3 pADH-mCherryHDEL-LEU2::CEN</i>	This study
RSY5726	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dgal::loxP are1::KanMX are2::KanMX pGREG576-ADH1- SS<sup>PRY1</sup>-GFP-PLIN3-URA3 pADH-mCherryHDEL-LEU2::CEN</i>	This study
RSY5727	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dgal::loxP are1::KanMX are2::KanMX hrd1::HIS3 pRS416-ADH1-GFP-PLIN2-URA3 pADH-mCherryHDEL-LEU2::CEN</i>	This study
RSY5728	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1Δ0 GAL-LRO1::TRP1 dgal::loxP are1::KanMX are2::KanMX hrd1::HIS3 pRS416-ADH1- SS<sup>PRY1</sup>-GFP-PLIN2-URA3 pADH-mCherryHDEL-LEU2::CEN</i>	This study
RSY5531	<i>MATα his4-401 leu2-3 trp1-1 ura3-52 HOL1-1 SUC2::LEU2</i>	A. Conzelmann
RSY5521	<i>MATα his4-401 leu2-3 trp1-1 ura3-52 HOL1-1 SUC2::LEU2 pJK90-TPI-ERG6-3(HA)-SUC2-HIS4C-URA3</i>	This study
RSY5522	<i>MATα his4-401 leu2-3 trp1-1 ura3-52 HOL1-1 SUC2::LEU2 pJK90-TPI-TGL1(30-548)-3(HA)-SUC2-HIS4C-URA3</i>	This study
RSY5520	<i>MATα his4-401 leu2-3 trp1-1 ura3-52 HOL1-1 SUC2::LEU2 pJK90-TPI-GFP-PLIN1-3(HA)-</i>	This study

<i>SUC2-HIS4C-URA3</i>		
RSY5518	<i>MATα his4-401 leu2-3 trp1-1 ura3-52 HOL1-1 SUC2::LEU2 pJK90-TPI-GFP-PLIN2-3(HA)-SUC2-HIS4C-URA3</i>	This study
RSY5519	<i>MATα his4-401 leu2-3 trp1-1 ura3-52 HOL1-1 SUC2::LEU2 pJK90-TPI-GFP-PLIN3-3(HA)-SUC2-HIS4C-URA3</i>	This study
RSY5597	<i>MATα his4-401 leu2-3 trp1-1 ura3-52 HOL1-1 SUC2::LEU2 pJK90-TPI-SS<sup>PRY1</sup>-GFP-PLIN1-3(HA)-SUC2-HIS4C-URA3</i>	This study
RSY5598	<i>MATα his4-401 leu2-3 trp1-1 ura3-52 HOL1-1 SUC2::LEU2 pJK90-TPI-SS<sup>PRY1</sup>-GFP-PLIN2-3(HA)-SUC2-HIS4C-URA3</i>	This study
RSY5599	<i>MATα his4-401 leu2-3 trp1-1 ura3-52 HOL1-1 SUC2::LEU2 pJK90-TPI-SS<sup>PRY1</sup>-GFP-PLIN3-3(HA)-SUC2-HIS4C-URA3</i>	This study
RSY5464	<i>MATα his4-401 leu2-3 trp1-1 ura3-52 HOL1-1 SUC2::LEU2 pJK90-TPI-SS<sup>PRY1</sup>-ERG6-3(HA)-SUC2-HIS4C-URA3</i>	This study
RSY5736	<i>MATα his4-401 leu2-3 trp1-1 ura3-52 HOL1-1 SUC2::LEU2 pJK90-TPI-SS<sup>PRY1</sup>-TGL1(30-548)-3(HA)-SUC2-HIS4C-URA3</i>	This study
RSY5809	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pGREG576-ADH1-Biotin-GFP-PLIN3-URA3</i>	This study
RSY5810	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pRS416-ADH1-SS<sup>PRY1</sup>-Biotin-GFP-PLIN3-URA3</i>	This study
RSY5741	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pVT100U-mito-GFP-PLIN3-URA3</i>	This study
RSY5807	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pVT100U-mito-GFP-PLIN3-URA3 pMito-RFP-LEU</i>	This study
RSY5811	<i>MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ p503-ADH1-Erg6-mCherry-HIS3</i>	This study
RSY5812	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ are1::KanMX are2::KanMX dgal::KanMX lro1::KanMX pGREG506-ADH1-SS<sup>PRY1</sup>-PLIN3-URA3</i>	This study