Supplemental Information

Extended Experimental Procedures

Yeast Strains and Plasmid Construction

Gene deletion and tagging was done by homologous recombination of PCR fragments. Ice2p, Erg6p and Tgl3p were genomically tagged at the N-terminus using a GFP-HIS3 cassette amplified from pFA6a-GFP-HIS3 (Longtine et al., 1998). N-terminal genomic tagging of Erg6p with RFP was done by using a RFP-KanMX cassette amplified from pFA6a-mRFP-KanMX6 (Huh et al., 2003). A deletion of *ice2* was generated using a PCR fragment containing the URA3 marker and flanking regions of the *ice2* gene, amplified from plasmid pRS316. To generate plasmids carrying C-terminal GFP tagged versions of Ice2p, Ice2p amino acids 241-357 (Ice2(cyt)), and Dga1p, GFP was amplified from pFA6a-GFP-KanMX, digested with XhoI/HindIII and ligated into XhoI/HindIII sites of p415-ADHpr (Nguyen et al., 2012). Ice2p and Ice2p aa241-357 (Ice2(cyt)) were amplified from genomic DNA and, after digestion with XbaI / SmaI, they were ligated into the XbaI/SmaI sites of p415-ADHpr-GFP. Dga1p was amplified from genomic DNA and ligated into XbaI/HindII sites of p415-ADHpr-GFP after digestion. To generate a plasmid carrying C-terminal mRFP-tagged Dga1p, the plasmid encoding Dga1-GFP was digested with HindIII / XhoI, to replace GFP with PCR-amplified and HindIII / XhoI-digested mRFP. To generate a plasmid encoding a C-terminal GFP-tagged version of Ice2p, lacking the cytoplasmic loop (aa241-357, ∆cyt), Ice2∆cyt was generated by fusion of two PCR products, Ice2 1-241 and Ice2 357-492. The resulting PCR product was digested with SmaI / XbaI and ligated into SmaI / XbaI sites of p415-ADHpr-GFP. For expression of an RFP tagged version of Ice2p aa241-357 (Ice2(cyt)) in COS7 cells, Ice2 aa241-357 was amplified from genomic DNA and, after digestion with SalI /SacII, ligated into the SalI/SacII site of pAcRFP-C1.

In vitro phosphatidylserine conversion assay

To measure the conversion of PS to PE in vitro (Figure S2B), yeast cells were grown at 30°C to early-logarithmic phase in SC medium containing 2% Glucose. Crude mitochondria (containing ER-mitochondrial contact sites) were purified as previously described (Daum et al., 1982). One hundred µL crude mitochondria (100 µg protein) in 0.6 m mannitol, 20 mm Tris pH 7.4 and 0.6 mm $MnCl_2$ were incubated with 0.1 µCi L-[^{14}C(U)]-serine at 30°C. After 20 min, 40 mM unlabeled serine was added, and PS synthesis was arrested by addition of 5 mM EDTA. The conversion of PS to PE was then followed during a 45 min incubation period at 30°C. The reaction was stopped by adding 1 mL chloroform:methanol 2:1 (v/v). After shaking for 1 h, lipids were extracted, the organic phase was washed with 100 µL 0.9% NaCl (w/v), and dried at 65°C. Lipids were resuspended in 15 µL chloroform, separated on thin-layer chromatography plates (Silica 60) in chloroform/methanol/25% ammonium hydroxide (50/25/6 v/v/v). They were visualized using a phosphoimager (BioRad, PMI). Radioactive lipids were quantified using ImageJ software. Data reported are the mean +/- SD for three experiments.

Table S1: Yeast strains used in this study

Strain ID	Genotype	Source
BY4741	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	(Brachmann et al., 1998)
YDM104	*MAT**a*** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ICE2::KanMX	Open Biosystems
YDM194	*MAT**a*** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ICE2::GFP-HIS3	This study
YDM218	*MAT**a*** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TGL3::GFP-HIS3	This study
YDM222	*MAT**a*** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ICE2::KanMX TGL3::GFP-HIS3	This study
YDM260	*MAT**a*** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ERG6::GFP-HIS3	This study
YDM261	*MAT**a*** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ICE2::KanMX ERG6::GFP-HIS3	This study
YDM316	*MAT**a*** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ERG6::RFP-KanMX	This study
YDM320	*MAT**a*** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ERG6::RFP-KanMX ICE2::GFP-HIS3	This study
YDM383	*MAT**a*** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TGL3::KanMX	Open Biosystems
YDM388	*MAT**a*** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 DGA1::KanMX	Open Biosystems
YDM397	*MAT**a*** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TGL3::KanMX ICE2::GFP-HIS3	This study
YDM415	*MAT**a*** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 DGA1::KanMX ICE2::URA3	This study

Table S2: Plasmids used in this study

ID	Name	Reference
158	p415-ADHpr-Ice2-GFP	This study
185	p415-ADHpr-Dga1-GFP	This study
193	p415-ADHpr-Ice2 (aa241-357)-GFP	This study
189	pAcRFP-C1-Ice2 aa241-357	This study
210	p415-ADHpr-Dga1-mRFP	This study
224	p415-ADHpr-Ice2Δcyt-GFP	This study

Table S3: Genetic interactions of Ice2; selected, from DRYGIN Database

Negative genetic interactions	Positive genetic interactions
Dgk1, Scs2, Ino2, Scs3, Psd2, Ino4, Cho2, Opi3, Psd1, Are2, Pct1, Erg12, Sct1 (Gat2), Ect1, CKI1	Fld1, Scs7, Pah1, Erg6, Erg3, Erg25

References

Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P., and Boeke, J.D. (1998). Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14, 115-132.

Daum, G., Gasser, S.M., and Schatz, G. (1982). Import of proteins into mitochondria. Energy-dependent, two-step processing of the intermembrane space enzyme cytochrome b2 by isolated yeast mitochondria. The Journal of biological chemistry 257, 13075-13080.

Huh, W.K., Falvo, J.V., Gerke, L.C., Carroll, A.S., Howson, R.W., Weissman, J.S., and O'Shea, E.K. (2003). Global analysis of protein localization in budding yeast. Nature 425, 686-691.

Koh, J.L., Ding, H., Costanzo, M., Baryshnikova, A., Toufighi, K., Bader, G.D., Myers, C.L., Andrews, B.J., and Boone, C. (2010). DRYGIN: a database of quantitative genetic interaction networks in yeast. Nucleic acids research 38, D502-507.

Longtine, M.S., McKenzie, A., 3rd, Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J.R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast 14, 953-961.

Nguyen, T.T., Lewandowska, A., Choi, J.Y., Markgraf, D.F., Junker, M., Bilgin, M., Ejsing, C.S., Voelker, D.R., Rapoport, T.A., and Shaw, J.M. (2012). Gem1 and ERMES do not directly affect phosphatidylserine transport from ER to mitochondria or mitochondrial inheritance. Traffic 13, 880-890.

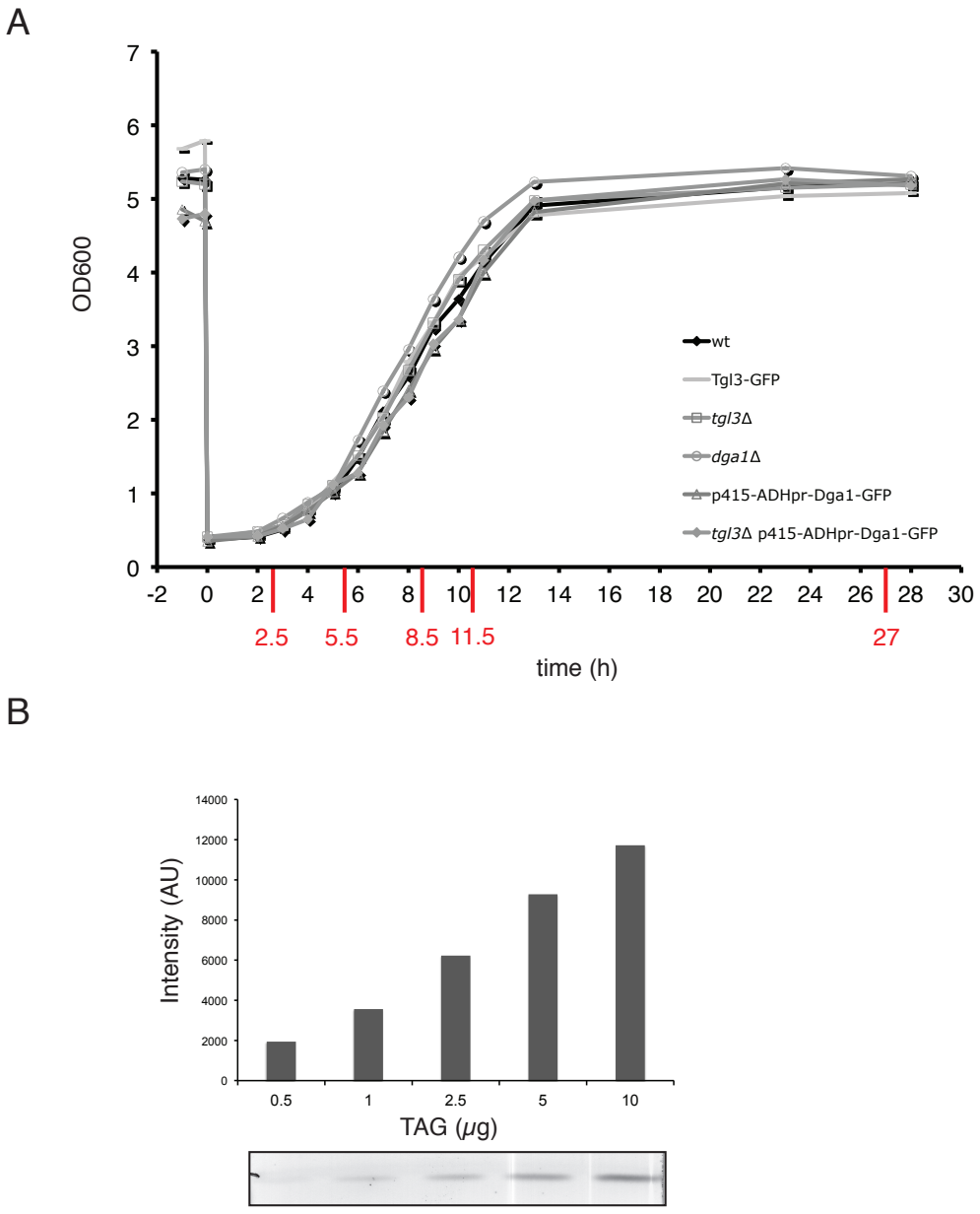


Figure S1. Time-points analyzed during the growth of different yeast strains and validation of TAG analysis by iodine staining, related to Figure 1.

(A) Wild type (WT) and the indicated mutant cells were diluted from stationary phase into fresh medium and their growth was followed by measuring the OD at 600nm. Samples, analyzed in Figure 1, were taken at the time points labeled in red. (B) The linearity of the TAG assay was verified by separating the indicated amounts of TAG by TLC, followed by iodine staining. TLC plates were scanned and quantified using ImageJ.

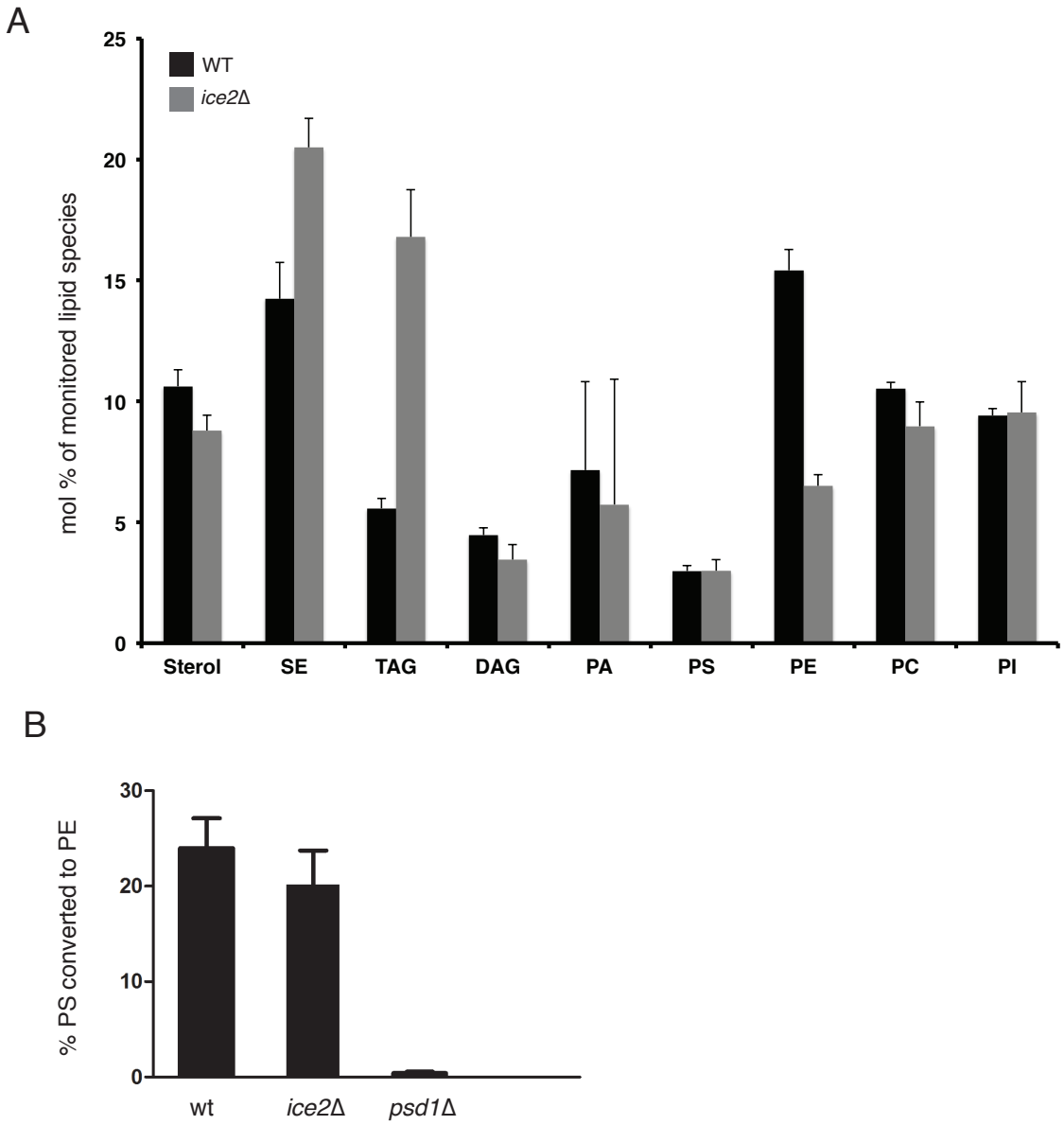


Figure S2. Ice2p affects phospholipid levels independently of mitochondrial decarboxylation of PE, related to Figure 3.

(A) Lipid composition of WT and *ice2*Δ cells, harvested at 0.6 A_{600nm}-unit/ml, were determined after lipid extraction and analysis by quantitative mass spectrometry. Data from three independent experiments are shown as mean +/- SD. (B) The conversion of PS to PE was determined *in vitro* using crude mitochondria incubated with radioactive serine. The percentage of radiolabeled PS converted to PE in WT and mutant mitochondria is shown. Bars and error bars represent the mean +/- SD from three independent experiments.

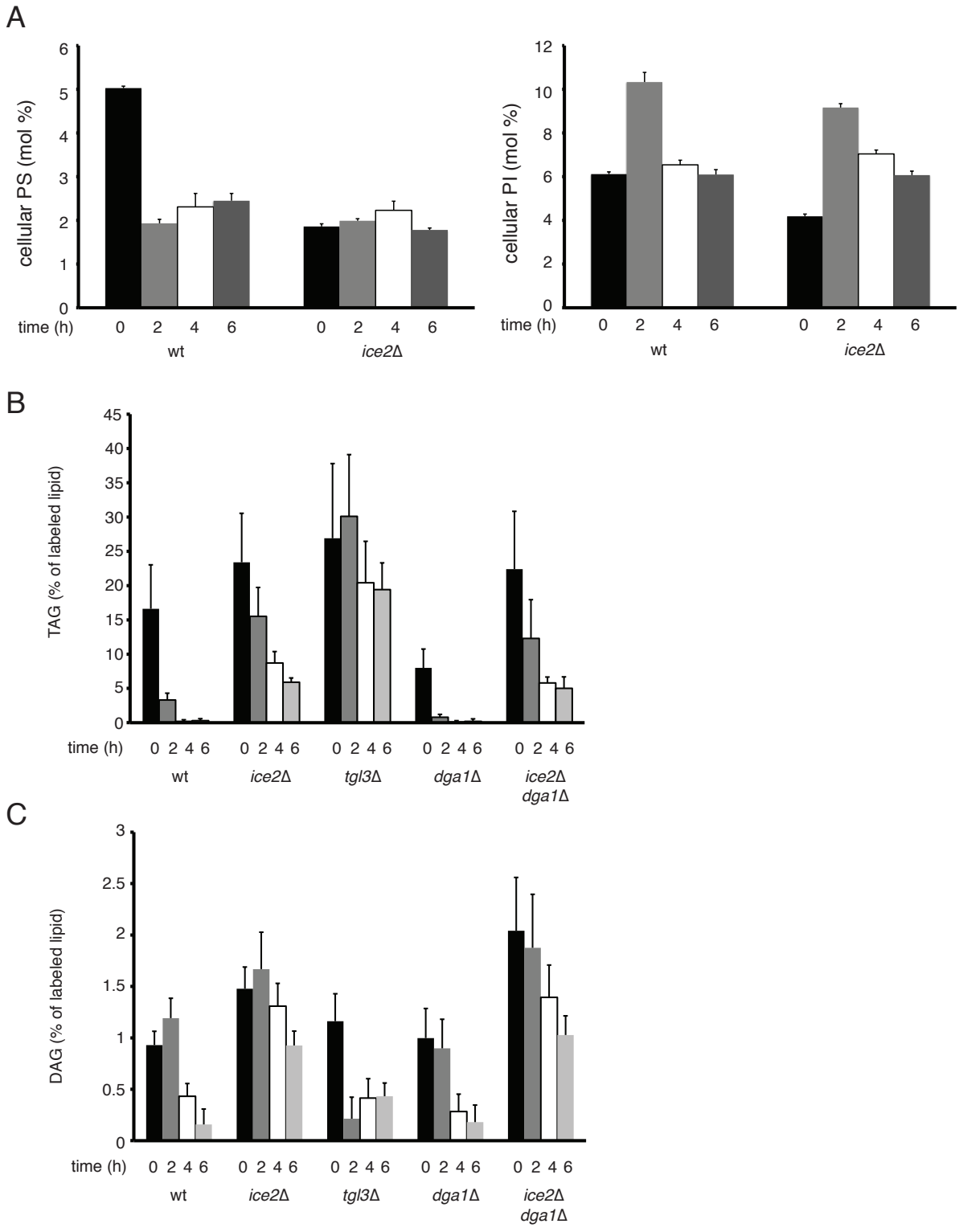
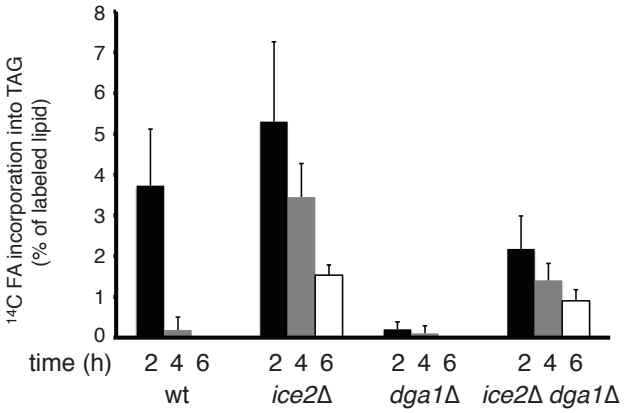

A
cellular PS (mol %)
cellular PI (mol %)
time (h)
0 2 4 6
wt
ice2Δ
B
TAG (% of labeled lipid)
wt
ice2Δ
tgl3Δ
dga1Δ
ice2Δ dga1Δ
C
DAG (% of labeled lipid)

Figure S3. Lipid composition and DAG accumulation during growth resumption in cells lacking Ice2p, related to Figure 4.

(A) The lipid composition of wild type (WT) and *ice2*Δ cells was analyzed after dilution of cells from stationary phase into fresh medium containing 10 µg/ml cerulenin. Samples were taken at different time points after dilution and PS and PI were analyzed by quantitative mass spectrometry. Data from two independent experiments, analyzed in duplicates, are shown as mean +/- SD. (B) Wild type (WT) cells and the indicated mutants were grown to stationary phase in the presence of ^{14}C-acetic acid and diluted into fresh medium containing 10 µg/ml cerulenin. The levels of labeled TAG were determined at different time points by TLC, followed by phosphorimaging and analysis by ImageJ. Labeled TAG was normalized to the total radioactivity in the chloroform-extracted fraction. Data from three independent experiments are shown as mean +/- SD. (C) As in (B), but labeled DAG was analyzed.

A



B

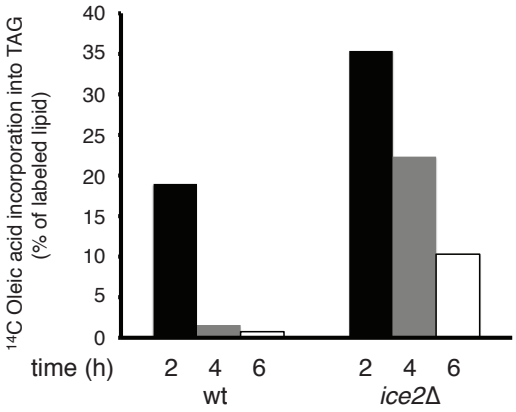


Figure S4. The re-esterification of DAG to TAG is dependent on Dga1p, related to Figure 5.

(A) The incorporation of ^{14}C-palmitic acid into TAG was determined for wild type (WT), and the indicated mutant cells at different time points after dilution from stationary phase into fresh medium containing 10µg/ml cerulenin and ^{14}C-palmitic acid. The lipids were separated by TLC and quantitated by phosphorimaging and analysis by ImageJ. TAG levels were normalized to the total radioactivity in the chloroform-extracted fraction. and are presented as mean +/- SD of four independent expriments. (B) As in (A), but with ^{14}C-oleic acid.

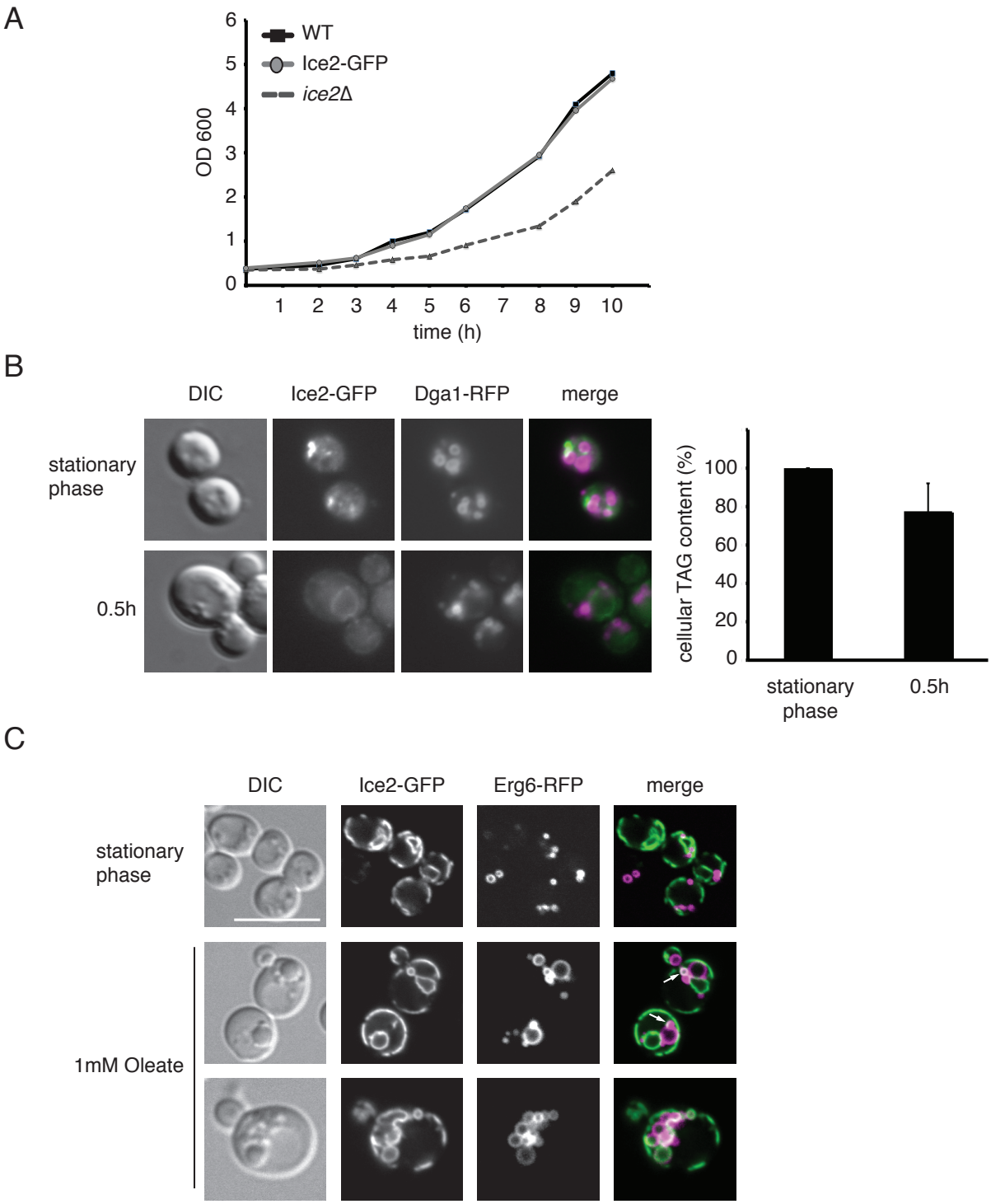

A
WT
Ice2-GFP
ice2Δ
OD 600
time (h)
B
DIC
Ice2-GFP
Dga1-RFP
merge
stationary phase
0.5h
cellular TAG content (%)
stationary phase
0.5h
C
DIC
Ice2-GFP
Erg6-RFP
merge
stationary phase
1mM Oleate

Figure S5. Re-localization of Ice2p-GFP, related to Figure 6.

(A) Wild type (WT) cells, *ice2*Δ cells, or cells in which the endogenous ICE2 gene was replaced by a GFP-tagged version, were diluted from stationary phase into fresh medium and their growth was followed by measuring the OD at 600nm. (B) The localization of Ice2-GFP was analyzed by fluorescence microscopy at stationary phase and 0.5h after dilution into fresh medium. The cells also expressed Dga1-mRFP from a plasmid as a marker for LDs. The graph shows TAG levels at stationary phase and 30min after dilution into fresh medium. Lipids were extracted at the indicated time points and analyzed by TLC followed by iodine staining and quantification using ImageJ. (C) The localization of Ice2p-GFP, overexpressed from a plasmid, was analyzed by fluorescence microscopy at stationary phase and 6h after dilution into fresh medium containing 1 mM oleate. The cells also expressed Erg6p-RFP as a marker for LDs. Arrows indicate Ice2-GFP positive structures in contact with Erg6-RFP labeled LDs. Scale bar: 10 μm.

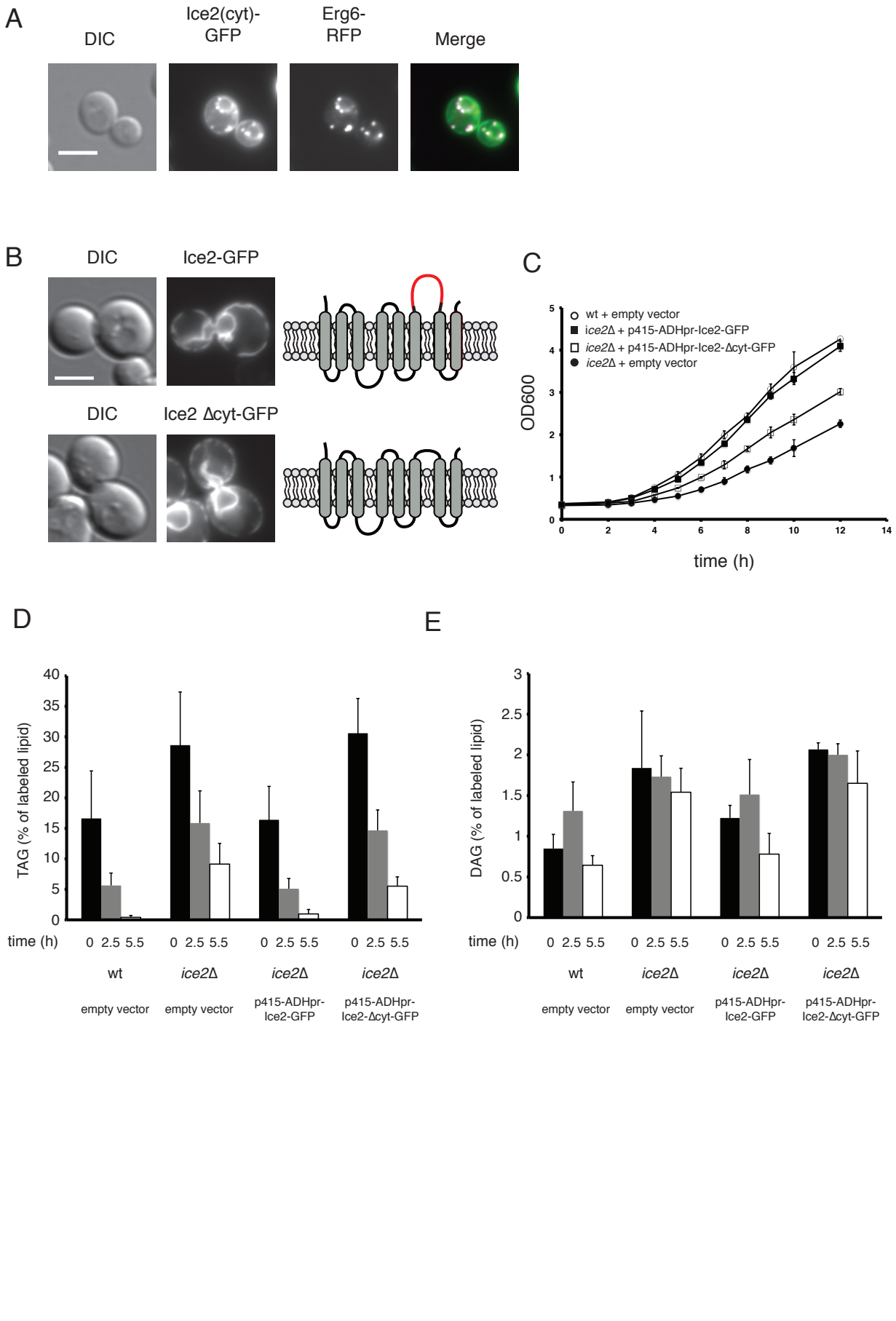

A
DIC
Ice2(cyt)-GFP
Erg6-RFP
Merge
B
DIC
Ice2-GFP
DIC
Ice2 Δcyt-GFP
C
wt + empty vector
ice2Δ + p415-ADHpr-Ice2-GFP
ice2Δ + p415-ADHpr-Ice2-Δcyt-GFP
ice2Δ + empty vector
OD600
time (h)
D
TAG (% of labeled lipid)
time (h)
0 2.5 5.5
wt
empty vector
ice2Δ
empty vector
ice2Δ
p415-ADHpr-Ice2-GFP
ice2Δ
p415-ADHpr-Ice2-Δcyt-GFP
E
DAG (% of labeled lipid)
time (h)
0 2.5 5.5
wt
empty vector
ice2Δ
empty vector
ice2Δ
p415-ADHpr-Ice2-GFP
ice2Δ
p415-ADHpr-Ice2-Δcyt-GFP

Figure S6 (related to Figure 7). A cytoplasmic domain of Ice2p interacts with LDs and is required for Ice2p function.

(A) The localization of the cytoplasmic loop of Ice2p (Ice2(cyt)-GFP),expressed from a plasmid, was analyzed by fluorescence microscopy in exponentially growing cells. The cells also expressed Erg6-RFP as a marker for LDs. Scale bar: 5 µm. (B) *ice2*Δ mutant cells overexpressing Ice2-GFP or Ice2 Δcyt-GFP from a plasmid were analyzed by fluorescence microscopy in exponentially growing cells. (C) Growth of wild type cells or *ice2*Δ cells overexpressing Ice2p-GFP or Ice2 Δcyt-GFP from a plasmid under the ADH promoter. (D) Wild type (WT) and *ice2*Δ mutant cells carrying the indicated plasmids were grown to stationary phase in the presence of ^{14}C-acetic acid and diluted into fresh medium containing 10 µg/ml cerulenin. The levels of labeled TAG were determined at different time points by TLC, followed by phosphorimaging and analysis by ImageJ. Labeled TAG was normalized to the total radioactivity in the chloroform-extracted fraction. Data from three independent experiments are shown as mean +/- SD. (E) As in (D), but labeled DAG was analyzed.