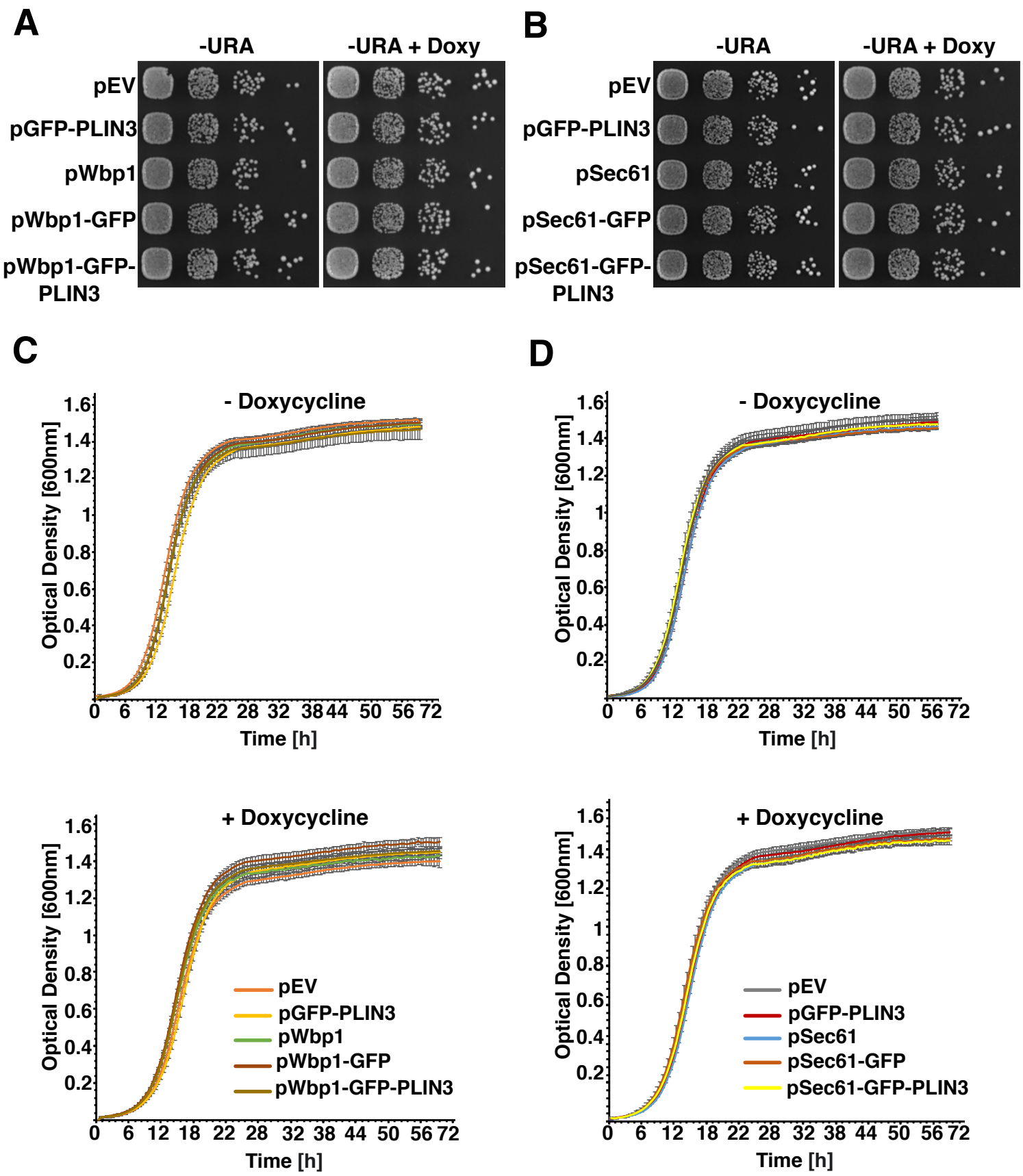

Figure S1

Figure S1. Expression of membrane-anchored PLIN3 does not impact cell growth. A, B) Cells expressing the membrane anchored LD reporters grow similarly to wild-type cells on solid media. Wild-type cells transformed with an empty vector (pEV), pGFP-PLIN3, pWbp1, pWbp1-GFP or pWbp1-GFP-PLIN3 (panel A) were grown to exponential phase, 10-fold serially diluted and stamped on solid minimal media lacking or containing doxycycline (10 µg/ml), to repress expression of the respective proteins. Plates were incubated for 3 days at 30°C. Cells transformed with the indicated Sec61-based plasmids (panel B) were treated and stamped as described in A. C, D) Cells expressing the membrane anchored LD reporters grow similarly to wild-type cells in liquid media. Strains shown in panels A and B were inoculated in liquid minimal media lacking (top panels) or containing doxycycline (10 µg/ml), lower panel, and growth at 30°C was monitored on a Bioscreen C reader at 600nm. Growth curves represent means ± SD of three independent measurements.

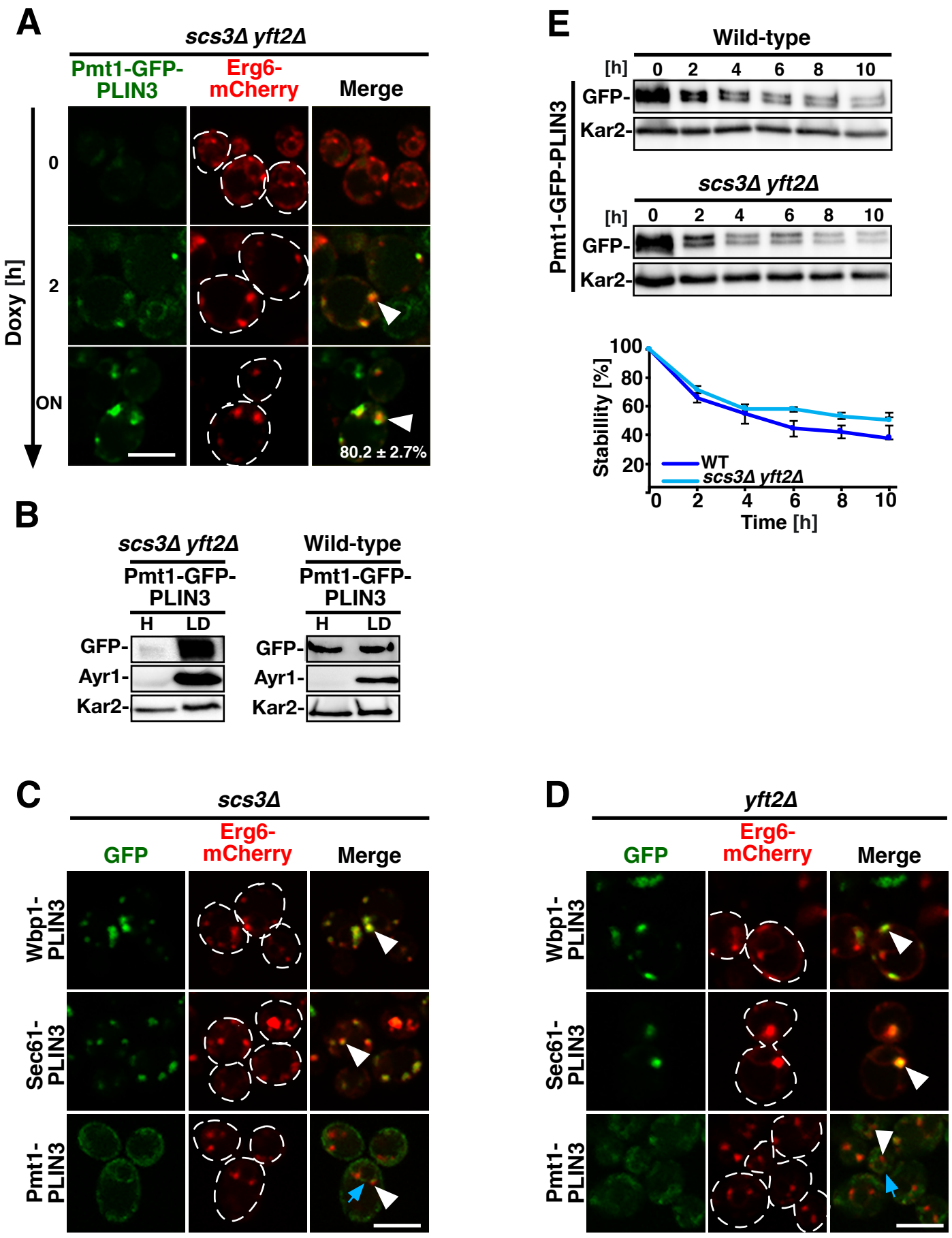
A
scs3Δ yft2Δ
Pmt1-GFP-PLIN3
Erg6-mCherry
Merge
Doxy [h]
0
2
ON
80.2 ± 2.7%
B
scs3Δ yft2Δ
Wild-type
Pmt1-GFP-PLIN3
H
LD
GFP-
Ayr1-
Kar2-
C
scs3Δ
GFP
Erg6-mCherry
Merge
Wbp1-PLIN3
Sec61-PLIN3
Pmt1-PLIN3
D
yft2Δ
E
Wild-type
[h]
0 2 4 6 8 10
Pmt1-GFP-PLIN3
GFP-
Kar2-
scs3Δ yft2Δ
Stability [%]
100
80
60
40
20
WT
scs3Δ yft2Δ
Time [h]

Figure S2

Figure S2. Scs3 and Yft2 share a redundant function in restricting access of Pmt1-GFP-PLIN3 to LDs.

A) Localization of Pmt1-GFP-PLIN3 to pre-existing LDs. FIT double mutant (scs3Δ yft2Δ) cells expressing Pmt1-GFP-PLIN3 and Erg6-mCherry cultivated in minimal medium. Expression of the membrane proximal PLIN3 reporter was induced at time 0 by removing doxycycline (-Doxy) and the localization of the marker protein was analyzed by fluorescence microscopy. White arrowheads depict the presence of the reporters at LDs. Pmt1-GFP-PLIN3 colocalizes to 80% with Erg6-mCherry in the scs3Δ yft2Δ double mutant. N>100 LDs. Scale bar, 5 µm.

B) Pmt1-GFP-PLIN3 is enriched on isolated LDs in FIT double mutant cells but not in wild-type. LDs were isolated by flotation on step density gradients and an equal amount of protein (10 µg) from the homogenate (H) and the isolated LD fraction were probed by Western blotting with antibodies against GFP, the LD-localized protein Ayr1, and the luminal ER protein Kar2.

C, D) One of the FIT proteins is sufficient to prevent localization of the ER luminal reporter to LDs. Cells lacking one of the FIT proteins, either Scs3 (C) or Yft2 (D) but expressing the indicated membrane-anchored LD reporters together with Erg6-mCherry were cultivated in media containing oleic acid and the localization of the reporters was analyzed by confocal microscopy. White arrowheads indicate punctuate LD localization, blue arrows indicate localization in the ER. Scale bar, 5 µm.

E) Stability of Pmt1-GFP-PLIN3 is not affected in wild-type compared to scs3Δ yft2Δ double mutant cells. Cells were cultivated in minimal medium and poisoned by the addition of cycloheximide (10 µg/ml). Aliquots were removed at the indicated time points and analyzed by Western blotting. The chimeric protein was detected with an antibody against GFP, and detection of Kar2 serves as a loading control. Relative protein stability over time is plotted in the graph.

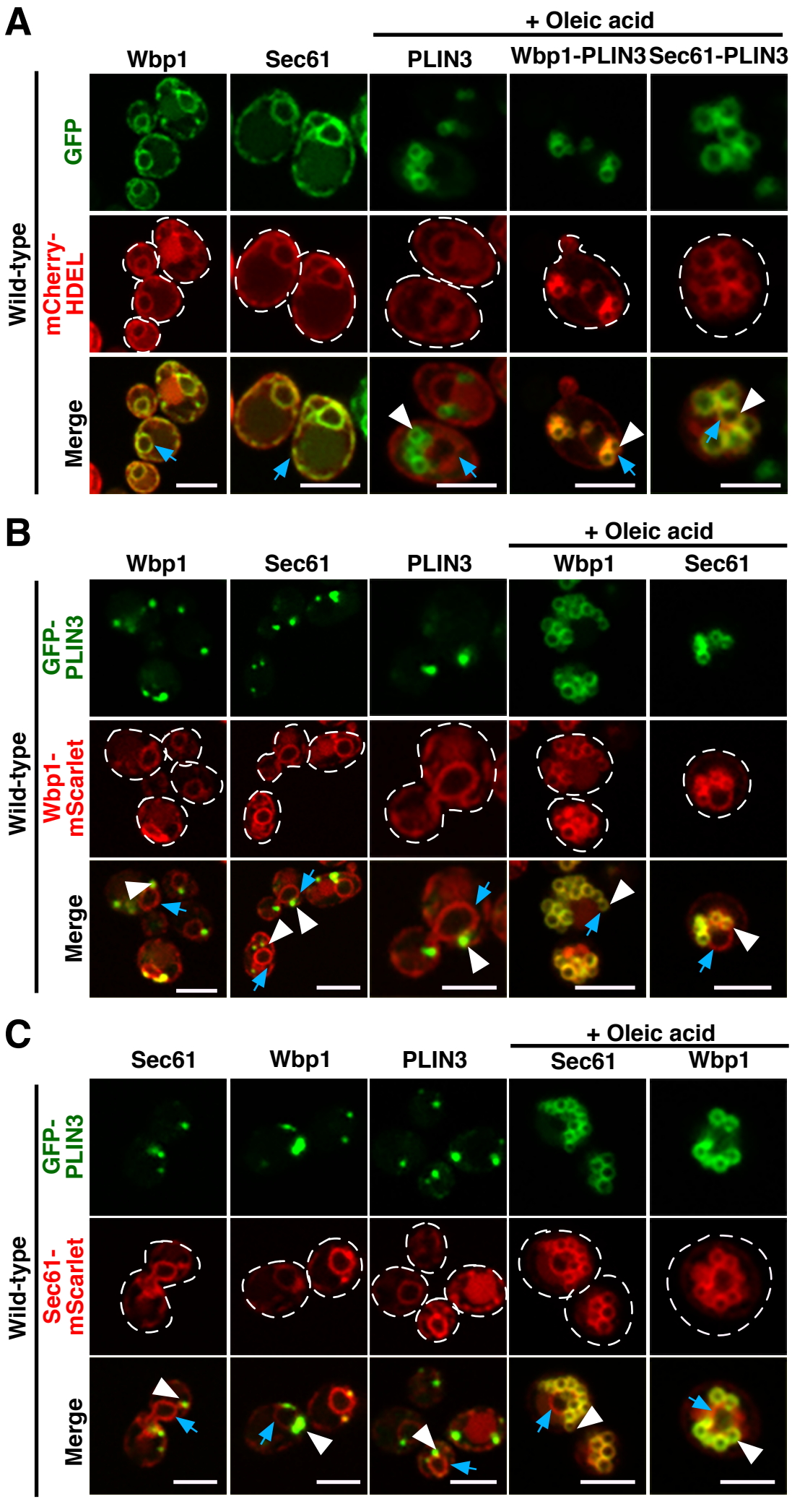

Figure S3

Figure S3. Wbp1-GFP and Sec61-GFP are homogenously distributed within the ER membrane and LD peripheral Wbp1-GFP-PLIN3 and Sec61-GFP-PLIN3 colocalize with the ER.

A) Wbp1-GFP or Sec61-GFP uniformly stain the ER membrane and colocalize with the ER luminal marker mCherry-HDEL. Wild-type cells expressing the indicated fluorescently-tagged proteins were grown in media lacking or containing oleic acid. Unlike the soluble PLIN3-GFP, the membrane-anchored PLIN3 (Wbp1-GFP-PLIN3 and Sec61-GFP-PLIN3) colocalize with the ER marker mCherry-HDEL when cells were cultivated in oleic acid containing media. Scale bar, 5 µm,

B, C) Membrane-anchored PLIN3 does not impair the overall morphology of the ER membrane in cells grown without oleic acid. Wild-type cells expressing the ER markers Wbp1-mScarlet (panel B) or Sec61-mScarlet (panel C) together with the soluble GFPPLIN3 or the GFP-tagged membrane-anchored PLIN3 (Wbp1-GFP-PLIN3 and Sec61-GFP-PLIN3) were grown either with or without oleic acid. Blue arrows designate the ER and white arrowheads indicate LDs. Scale bar, 5 µm.

Table S1. *Saccharomyces cerevisiae* strains used in this study

Name	*Relevant Genotype*	Source
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Lab collection
RSY 5870	*MATa his3Δ1 leu2Δ0 ura3Δ0 scs3::KanMX yft2::KanMX*	Lab collection
RSY 5428	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ERG6-VC::HIS3*	Lab collection
RSY 6664	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 scs3::KanMX*	Euroscarf
RSY 6665	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 yft2::KanMX*	Euroscarf
RSY 6245	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ERG6-mCherry::HIS3*	W. Prinz

Table S2. Plasmids used in this study

Plasmids	Source
pCM189-*tetO$_7$-GFP-PLIN3/URA3*	This Study
pCM189-*tetO$_7$-WBP1-GFP-PLIN3/URA3*	This Study
pCM189-*tetO$_7$-SEC61-GFP-PLIN3/URA3*	This Study
pCM189-*tetO$_7$-WBP1-mScarlet-PLIN3/URA3*	This Study
pCM189-*tetO$_7$-SEC61-mScarlet-PLIN3/URA3*	This Study
pCM189-*tetO$_7$-PMT1-GFP-PLIN3/URA3*	This Study
pCM189-*tetO$_7$-PMT1-mScarlet-PLIN3/URA3*	This Study
pCM189-*tetO$_7$-WBP1-mScarlet/URA3*	This Study
pCM189-*tetO$_7$-SEC61-mScarlet/URA3*	This Study
pCM189-*tetO$_7$-WBP1-GFP-PLIN3/HIS3*	This Study
pCM189-*tetO$_7$-SEC61-GFP-PLIN3/HIS3*	This Study
pCM189-*tetO$_7$-GFP-PLIN3/HIS3*	This Study
pCM189-*tetO$_7$-WBP1-GFP/URA3*	This Study
pCM189-*tetO$_7$-SEC61-GFP/URA3*	This Study
pCM189-*tetO$_7$-WBP1/URA3*	This Study
pCM189-*tetO$_7$-SEC61/URA3*	This Study
pCM189-*tetO$_7$-WBP1-VN/LEU2*	This Study
pCM189-*tetO$_7$-SEC61-VN/LEU2*	This Study
pCM189-*tetO$_7$-ERG6-VC/URA3*	This Study
pCM189-*tetO$_7$-OM14-GFP/URA3*	This Study
pCM189-*tetO$_7$-OM14-GFP-PLIN3/URA3*	This Study
pMito-*RFP/LEU2*	Nunnari lab
pGREG505-*ADH1-ERG6-mCherry/LEU2*	Lab Collection
pRS415-*ADH1-mCherry-HDEL/LEU2*	Lab Collection
pCMV-*mCherry-PLIN2*	Lab Collection
pELF1-*OST48-GFP-PLIN3*	This Study
pCMV-*POMT1-GFP-PLIN3*	This Study
pCMV-*SEC61A1-GFP-PLIN3*	This Study