Choudhary et al., http://www.jcb.org/cgi/content/full/jcb.201505067/DC1

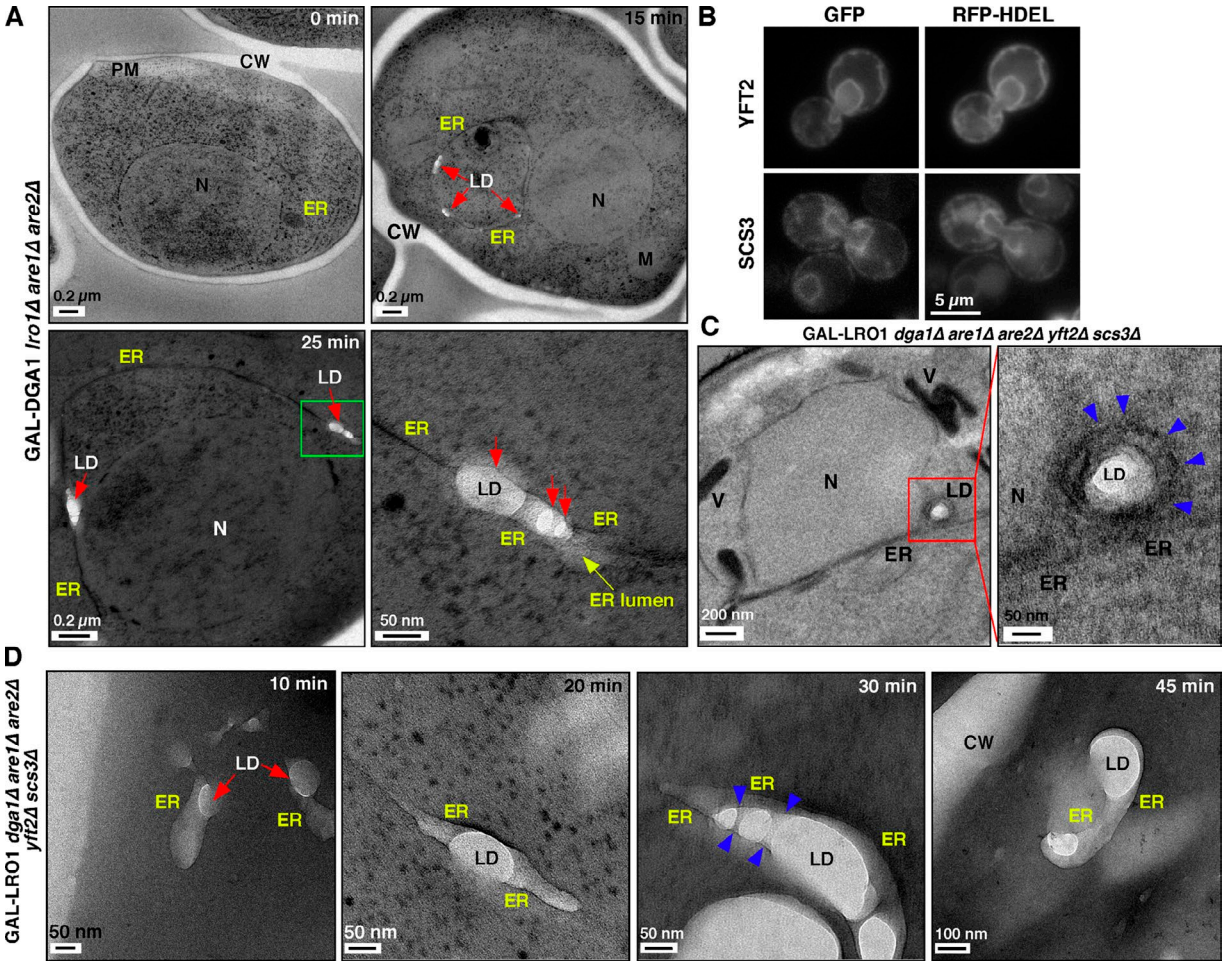

Figure S1. **Nascent LDs in cells lacking FIT proteins are membrane wrapped.** (A) *GAL-DGA1 lro1Δ are1Δ are2Δ* cells were gown to mid-logarithmic growth phase in a medium containing raffinose, galactose was added to induce the Dga1p expression, and the cells were visualized by EM either 15 or 25 min later. Cells lack any detectable LDs when TAG synthesis is not induced (0 min). The boxed region is shown at higher magnification. Red arrows indicate nascent LDs in the ER membrane. Yellow arrow denotes the ER lumen. CW, cell wall; M, mitochondria; N, nucleus; PM, plasma membrane. (B) Cells expressing Scs3-GFP or Yft2-GFP from plasmids were grown to mid-logarithmic growth phase and imaged live. The cells also expressed the ER marker ss-RFP-HDEL. (C) *GAL-LRO1 dga1Δ are1Δ are2Δ scs3Δ yft2Δ* cells were grown to mid-logarithmic growth phase in a medium with raffinose. Galactose was added to the medium to induce TAG formation, and cells were grown for 45 min, chemically fixed, and imaged by EM. The boxed region is shown in higher magnification. (D) *GAL-LRO1 dga1Δ are1Δ are2Δ scs3Δ yft2Δ* cells were grown to mid-logarithmic growth phase in a medium with raffinose. Galactose was added to the medium to induce TAG formation, and cells were cryofixed after 10, 20, 30, or 45 min and imaged by EM. Arrows denote nascent LDs, and arrowheads indicate the bifurcated ER membrane.

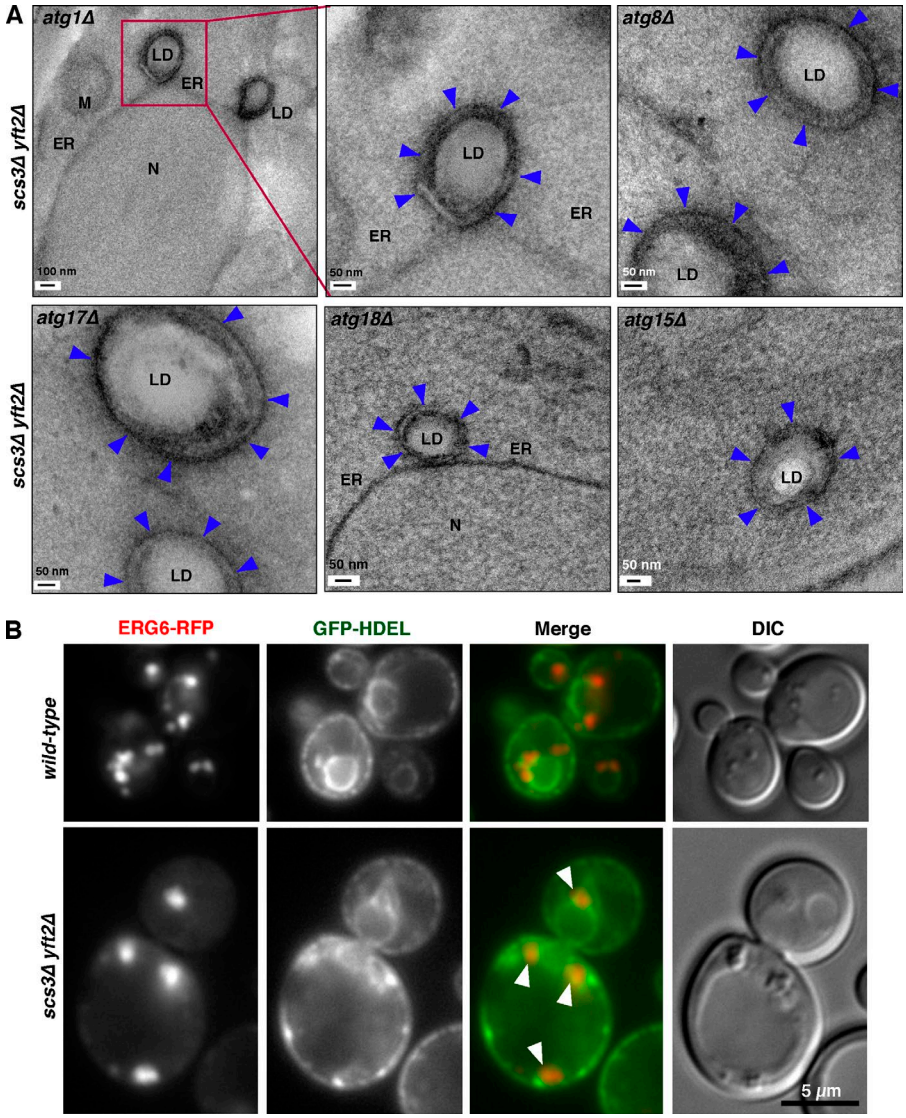

Figure S2. **FIT mutants lacking autophagy proteins have membrane-wrapped LDs.** (A) LDs in FIT mutant cells missing autophagy proteins. Strains lacking the FIT genes and the indicated autophagy (*atg*) genes were grown in YPD, chemically fixed, and visualized by EM. Most LDs in these strains were membrane wrapped as in cells lacking only the FIT proteins. The boxed region in triple mutant (*yft2Δ scs3Δ atg1Δ*) is shown in higher magnification. Arrowheads indicate the membrane-wrapping LDs. M, mitochondria; N, nucleus. (B) LDs do not undergo autophagy in FIT mutants. Wild-type and *scs3Δ yft2Δ* cells expressing the LD marker Erg6-RFP and the ER marker GFP-HDEL were visualized by fluorescence microscopy. LDs are not inside the vacuole, and the vacuole does not show red fluorescence that would result from degradation of Erg6-RFP inside the vacuole. DIC, differential interference contrast.

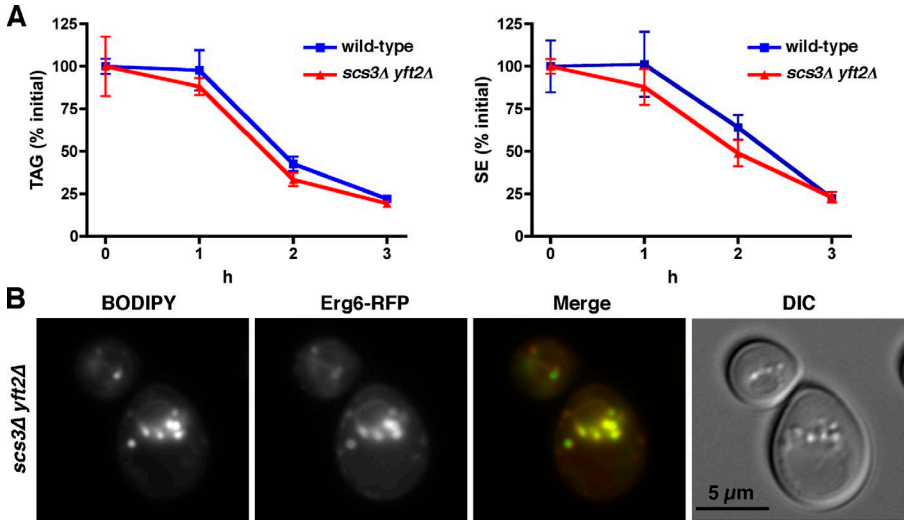

Figure S3. **Characterization of yeast cells lacking Scs3p and Yft2p.** (A) Neutral lipids are mobilized normally in cells lacking FIT proteins. Cells were labeled to saturation in YPD containing [^{3}H]palmitate. The cells were then diluted into fresh YPD media containing cerulenin and terbinafine. Samples were taken at the indicated times and the percent of TAG and SE determined (mean ± SD, n = 3 independent experiments). (B) Erg6-RFP is present on all LDs. Cell lacking the FIT proteins and expressing the LD marker Erg6-RFP were stained with BODIPY to visualize LDs. Note that all LDs are bound by the cytosolic peripheral membrane protein Erg6-RFP, indicating that LDs in the ER membrane remain exposed to the cytosol. DIC, differential interference contrast.

Table S1. ***S. cerevisiae* strains and plasmids used in this study**

Strain/plasmid	Genotype/description
VCY04[a]	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 met15 are1::KanMX, are2::KanMX trp1::URA3 GAL-LRO1:TRP1 dga1::lox-HIS3-lox ERG6-GFP :HIS3*
VCY07[a]	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 met15 are1::KanMX, are2::KanMX trp1::URA3 lro1::lox-HIS-lox GAL-GFP-DGA1::HIS*
VCY15	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 met15* + pERG6-RFP-URA + pKar2-GFP-HDEL-LEU
VCY18	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 met15 scs3::KanMX yft2::HIS3* + pERG6-RFP-URA3 + pKar2-GFP-HDEL-LEU2
VCY30	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 met15are1::KanMX are2::KanMX trp1::URA3 GAL-LRO1:TRP1 dga1::loxP scs3::loxP yft2::lox-HIS3-lox*
VCY42	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 met15scs3::HIS3 yft2::KanMXpERG6-RFP-URA*
VCY52	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 met15pRFP-HDEL-URA*
VCY55	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 met15scs3::HIS3 yft2::KanMX* + pRFP-HDEL-URA
VCY71	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 yft2::KanMx GAL-SCS3::HIS3*
NOY49	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 yft2::KanMx*
NOY50	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 scs3::HIS3*
NOY53	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 scs3::HIS3 yft2::KanMX*
NOY456	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 scs3::HIS3 yft2::NatMX atg1::HYG*
NOY457	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 scs3::HIS3 yft2::NatMX atg8::KanMX*
NOY460	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 scs3::HIS3 yft2::NatMX atg17::KanMX*
NOY465	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 scs3::lox-HIS3-lox yft2::NatMX atg15::KanMX*
NOY640	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 scs3::lox-HIS3-lox yft2::NatMX atg18::KanMX*
NOY624	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* + pNO24 + pLK205
NOY625	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* + pNO17 + ss-RFP-HDEL[NatMX]
pNO17	Encodes Scs3-GFP under MET25 promoter/URA3/CEN
pNO24	Encodes YFT2-sfGFP under GDP promoter/LEU2/CEN
pLK205	Encodes ss-dsRED-HDEL under TPI1 promoter/URA3/CEN

sfGFP, superfold GFP.
[a]Obtained from R. Schneiter (University of Fribourg, Fribourg, Switzerland).

Table S2. **Primers used for siRNA knockdown of *fit2* in 3T3 L1 fibroblasts and for CRISPR/Cas9 *fitm*-2 deletion in *C. elegans***

Primer	Sequence
FIT2 siRNA-1 (sense)	5′-CAACGUGUAUUUUGUUAAATT-3′
FIT2 siRNA-1 (antisense)	5′-UUUAACAAAAUACACGUUGAG-3′
FIT2 siRNA-2 (sense)	5′-UCAUUGCCCUUACCAACUATT-3′
FIT2 siRNA-2 (antisense)	5′-UAGUUGGUAAGGGCAAUGAAA-3′
FIT2 qPCR F	5′-CGCAACGTCCTCAACGTGTAT-3′
FIT2 qPCR R	5′-ATGCTCCTGTCTGATGCCCT-3′
FITM-2 gRNA-1	5′-GCGTGCTATTTGGGGAAGCGTTTTAGAGCTAGAAATAGCAAGT-3′
FITM-2 gRNA-2	5′-CAGCATTCGGCGTGCTATTGTTTTAGAGCTAGAAATAGCAAGT-3′
FITM-2 repair oligo	5′-ATATACTTACAGCAACGAAAATCCCGAGTGCAGCATTCGGCGTGCTATTTTAGGAAGCTTATCGCTTTGTTGTGGAGTCTCTGAAAAA TCAACTCATTTTGTCAAAAACAA-3′
FITM-2 PCR F	5′-TCGGGTTTTCCGATTAATTCG-3′
FITM-2 PCR R	5′-CTAGATTCTGATTAACTTCTGC-3′
FITM-2 seq	5′-ATGTCAACAAGGCGTAGTTC-3′