Velázquez et al., http://www.jcb.org/cgi/content/full/jcb.201508102/DC1

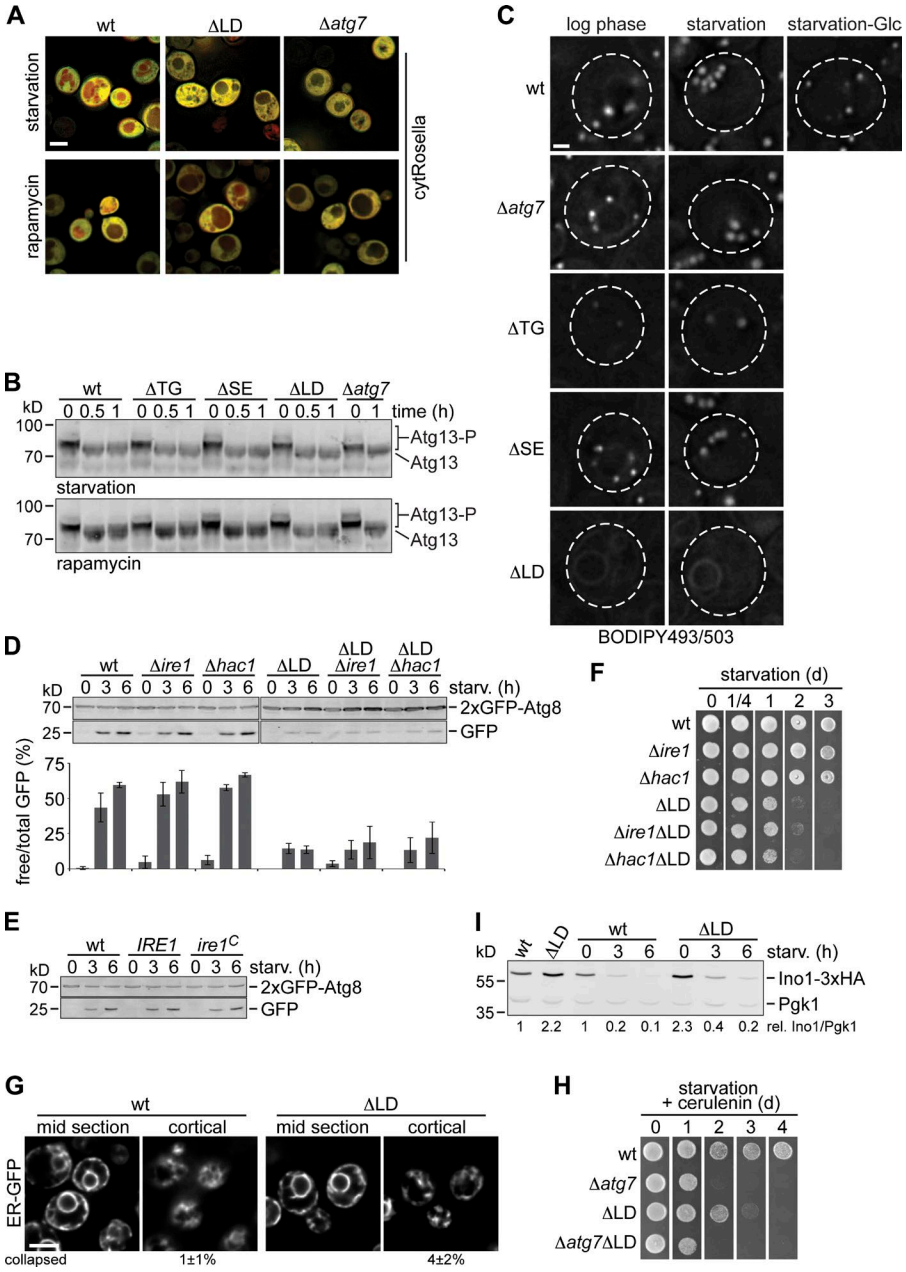

Figure S1. **Additional characterization of cells deficient in LD biogenesis.** (A) wt, ΔLD, and Δ*atg7* cells expressing a plasmid-encoded cytRosella (pHluorin-mCherry) were grown to log phase, starved or treated with rapamycin, and imaged by fluorescence microscopy after 6 h. Red fluorescent signal in vacuoles indicates autophagy flux. Bar, 5 µm. (B) Atg13 phosphorylation during starvation or rapamycin treatment by whole-cell extraction and Western blot analysis using an α-Atg13 antibody. (C) Fluorescence microscopy images of the data shown in Fig. 2 A. Dashed lines indicate cell boundaries. Bar, 1 µm. (D) Autophagy flux in wt, Δ*ire1*, Δ*hac1*, ΔLD, ΔLDΔ*ire1*, and ΔLDΔ*hac1* cells expressing *2xGFP-ATG8* during starvation (starv.). Data are mean ± SD (n = 3). (E) Autophagy flux in wt or Δ*ire1* cells coexpressing *2xGFP-ATG8* and a plasmid encoded *IRE1* or constitutively active *IRE1* allele (*ire1*^{C}), respectively, during starvation (starv.). (F) Survival of indicated strains during starvation. (G) wt and ΔLD cells expressing GFP-HDEL (ER-targeted GFP) were imaged after 1 h of rapamycin treatment. Images show single cortical and mid sections of the same cells. Quantifications of cells with a collapsed ER network are means ± SDs (≥150 cells; n = 3). (H) Survival of indicated strains during starvation + cerulenin (10 µg/ml). (I) wt and ΔLD expressing genomically 3xHA-tagged *INO1* were grown to log phase and shifted to starvation (starv.). Cells were analyzed at indicated time points by whole-cell extraction and Western blot analysis using α-HA and α-Pgk1 antibodies.

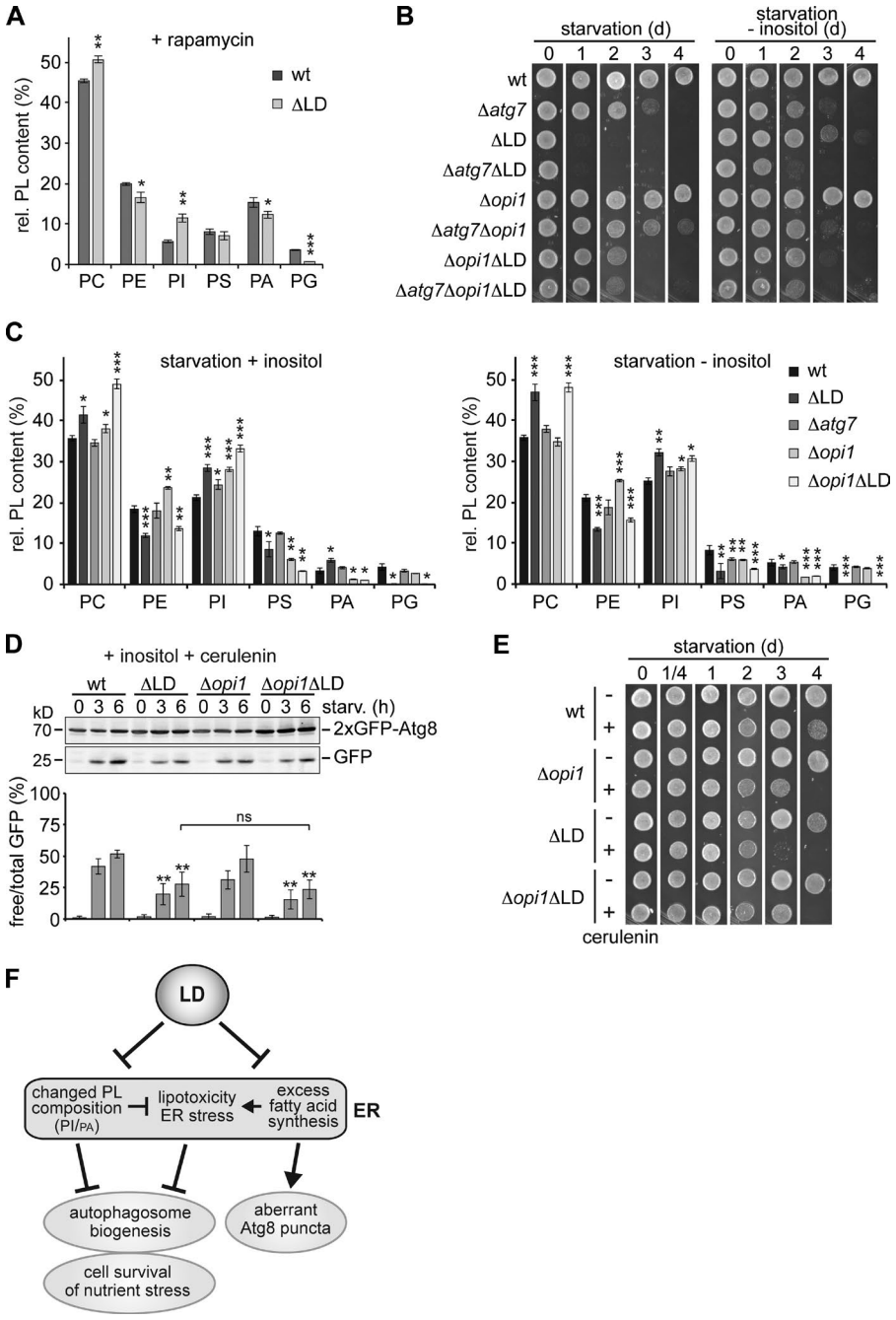

Figure S2. **Additional characterization of PL composition, cell survival, autophagy flux of LD-deficient cells in dependence of OPI1, and FA synthesis.** (A) wt and ΔLD cells were treated with rapamycin for 6 h, and PLs were analyzed by mass spectrometry as described in the Materials and methods. Relative (rel.) distribution of PC, PE, PI, PS, PA, and PG is shown as means ± SD (n = 3). (B) Survival of indicated strains during starvation ± inositol (2 mg/l). (C) wt, ΔLD, Δ*atg7*, Δ*opi1*, and Δ*opi1*ΔLD cells were starved ± inositol (2 mg/l) for 6 h, and PLs were analyzed as in A. (D) Autophagy flux in wt, ΔLD, Δ*opi1*, and Δ*opi1*ΔLD cells starved (starv.) in the presence of cerulenin (10 µg/ml). Data are means ± SDs (n = 4). (E) Survival of indicated strains during starvation ± cerulenin (10 µg/ml) in inositol-free media. (F) Model for LD-mediated ER homeostasis required for autophagy and cell survival during nutrient stress. *t* test in A, C, and D: *, P < 0.05; **, P < 0.01; ***, P < 0.001.

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

Name	Genotype
YMG1	w303 *ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100*
YMG2	w303 Δ*atg7::kanMX6*
YMG3	w303 Δ*dga1::TRP1* Δ*lro1::HIS3*
YMG4	w303 Δ*are1::TRP1* Δ*are2::HIS3*
YMG5	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3*
YMG6	w303 Δ*opi1::natMX6*
YMG7	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3* Δ*opi1::natMX6*
YMG8	w303 pRS306-pr^{ATG8}-*2xyEGFP-ATG8*
YMG9	w303 Δ*atg7*::kanMX4 pRS306-pr^{ATG8}-*2xyEGFP-ATG8*
YMG10	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* pRS306-pr^{ATG8}-*2xyEGFP-ATG8*
YMG11	w303 Δ*are1::TRP1* Δ*are2::HIS3* pRS306-pr^{ATG8}-*2xyEGFP-ATG8*
YMG12	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3* pRS306-pr^{ATG8}-*2xyEGFP-ATG8*
YMG13	w303 Δ*opi1::natMX6 pRS306-prATG8-2xyEGFP-ATG8*
YMG14	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3* Δ*opi1::natMX6 pRS306-prATG8-2xyEGFP-ATG8*
YMG15	w303 Δ*ura3::4xUPRE-GFP-URA3*
YMG16	w303 Δ*atg7::kanMX6* Δ*ura3::4xUPRE-GFP-URA3*
YMG17	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*ura3::4xUPRE-GFP-URA3*
YMG18	w303 Δ*are1::TRP1* Δ*are2::HIS3* Δ*ura3::4xUPRE-GFP-URA3*
YMG19	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3* Δ*ura3::4xUPRE-GFP-URA3*
YMG20	w303 Δ*opi1::natMX6* Δ*ura3::4xUPRE-GFP-URA3*
YMG21	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3* Δ*opi1::natMX6* Δ*ura3::4xUPRE-GFP-URA3*
YMG22	w303 Δ*ire1::natMX6 pRS306-prATG8-2xyEGFP-ATG8*
YMG23	w303 Δ*hac1::natMX6 pRS306-prATG8-2xyEGFP-ATG8*
YMG24	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3* Δ*ire1::natMX6 pRS306-prATG8-2xyEGFP-ATG8*
YMG25	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3* Δ*hac1::natMX6 pRS306-prATG8-2xyEGFP-ATG8*
YMG26	w303 Δ*ire1::natMX6 pRS306-prATG8-2xyEGFP-ATG8 pRS315-prIRE1-IRE1*
YMG27	w303 Δ*ire1::natMX6 pRS306-prATG8-2xyEGFP-ATG8 pRS315-prIRE1-ire1C*
YMG28	w303 *INO1-3xHA-kanMX6*
YMG29	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3 INO1-3xHA-kanMX6*
YMG30	w303 *SEC13-mCherry-kanMX6* pRS306-pr^{ATG8}-*2xyEGFP-ATG8*
YMG31	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3 SEC13-mCherry-kanMX6* pRS306-pr^{ATG8}-*2xyEGFP-ATG8*
YMG32	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3* Δ*atg7::kanMX4*
YMG33	w303 pRS305-*yEGFP-HDEL*
YMG34	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3* pRS305-*yEGFP-HDEL*
YMG35	w303 Δ*opi1::natMX6* pRS305-*yEGFP-HDEL*
YMG36	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3* Δ*opi1::natMX6* pRS305-*yEGFP-HDEL*
YMG37	w303 pRS315-pr^{ADH1}-*pHluorin-mCherry*
YMG38	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3* pRS315-pr^{ADH1}-*pHluorin-mCherry*
YMG39	w303 Δ*atg7::kanMX6* pRS315-pr^{ADH1}-*pHluorin-mCherry*
YMG40	w303 Δ*opi1::natMX6* Δ*atg7::kanMX4*
YMG41	w303 Δ*dga1::TRP1* Δ*lro1::HIS3* Δ*are1::TRP1* Δ*are2::HIS3* Δ*opi1::natMX6* Δ*atg7::kanMX4*