## Supplemental Information

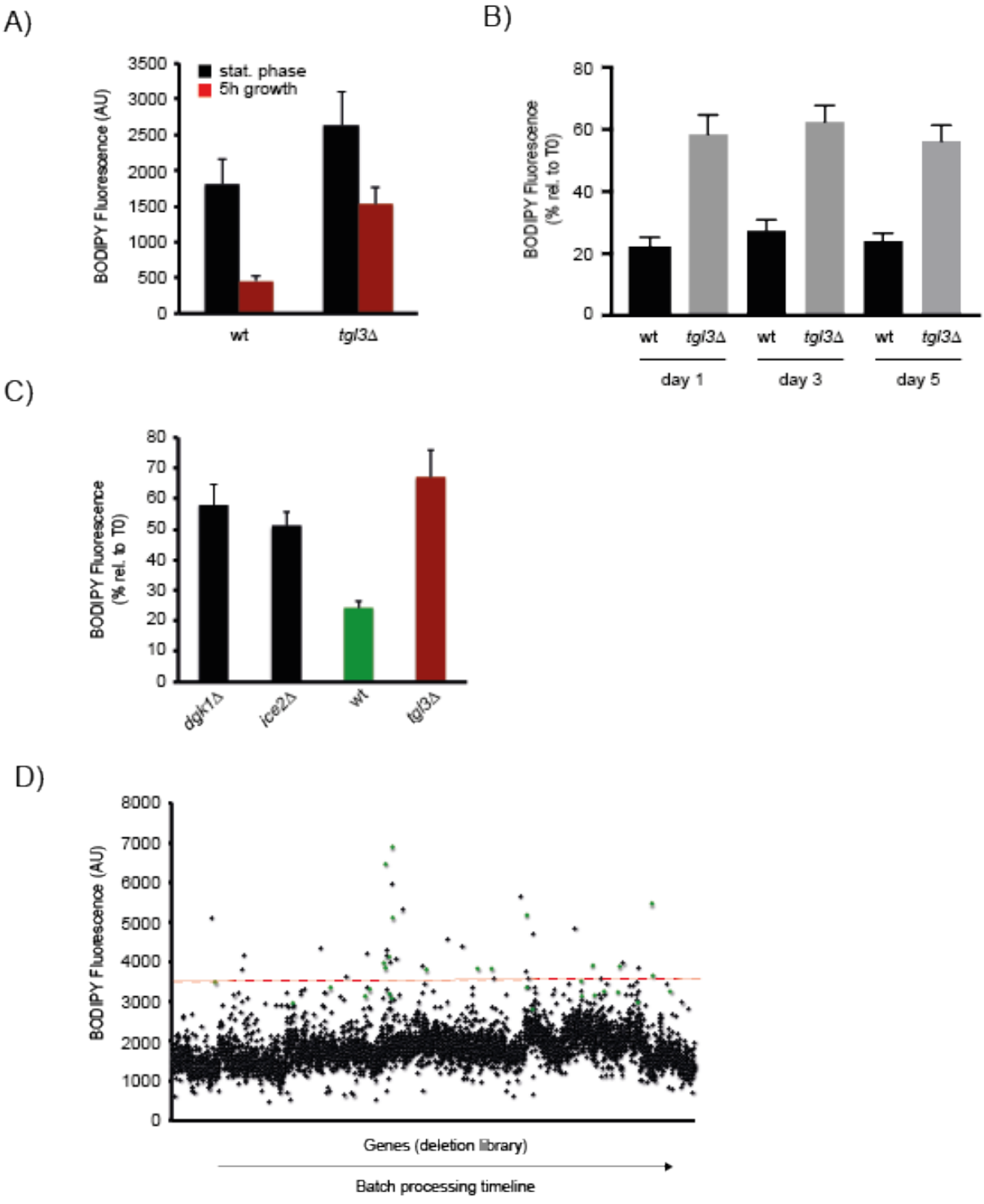


Figure S1: **Flow cytometry-based quantification of BODIPY493/503 labeled lipid droplets**. (A) The lipid droplet BODIPY493/503-fluorescence at stationary phase (T0) and after 5h growth in fresh medium containing cerulenin (T5) was measured in WT and *tgl3*Δ mutant cells. Values present fluorescence raw data from controls of the genome-wide screen shown in Figure 1B (n=55). (B) Stability of flow-cytometry based measurements of LD consumption. The indicated strains were processed and analyzed as in (A). Samples were measured at day1, re-measured at day3, day5 and stored at 4°C in the dark between measurements. LD consumption is presented as

Journal of Cell Science • Supplementary information

BODIPY493-503 fluorescence at T0 relative to T5, in percent. (n=12). (C) Flow-cytometry-based measurement of LD consumption in mutants known to affect LD dynamics. The indicated strains were analyzed as in (B) (n= 4). (D) Lipid droplet BODIPY493/503-fluorescence of deletion library mutant strains analyzed in the genome-wide screen at stationary phase. The dashed red line indicates an BODIPY493/503 fluorescence intensity ≥2xSD of *tgl3*Δ mutant control cells (n=55). Green dots indicate mutant strains that were previously described to alter LD dynamics (see Figure S3).

Journal of Cell Science • Supplementary information

Figure S2

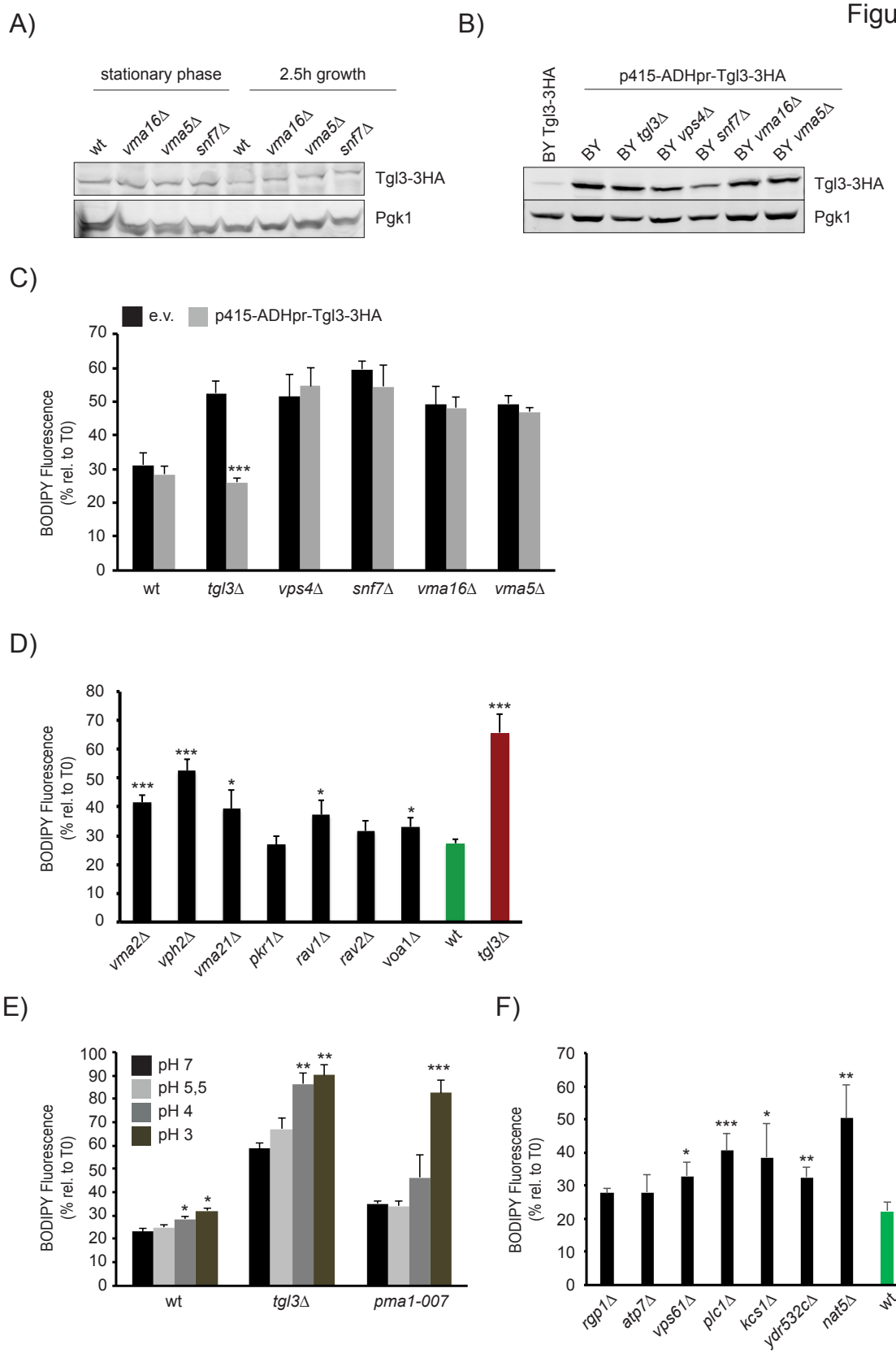


Fig. S2: **Tgl3p expression levels in ESCRT and *vma* mutant strains and the effect of Tgl3p overexpression, V-ATPase assembly mutants and pH on LD consumption** (A) Expression levels of Tgl3p. The indicated strains expressing genomically 3HA-tagged Tgl3p were grown to stationary phase and diluted into fresh

Journal of Cell Science • Supplementary information

medium containing cerulenin for 2.5h. Protein extracts were analyzed by SDS-PAGE followed by Western blotting. (B) as in (A) but the indicated strains expressing genomically 3HA-tagged Tgl3p or overexpressing 3HA-tagged Tgl3p from a plasmid were grown to early exponential phase and analyzed. (C) Flow-cytometry-based measurement of LD consumption. The indicated strains carrying empty vector or plasmid overexpressing Tgl3-HA were analyzed as in Fig. S1B (n=6). (D) as (C), but the indicated mutants, implicated in the assembly of the V-ATPase were analyzed (n=4). (E) as in (C) but the indicated strains, grown to stationary phase in medium pH 5.5 and diluted into fresh medium of the indicated pH containing cerulenin were analyzed (n=3). (F) as in (C), but the indicated strains were analyzed (n=4). Data are presented as mean ± SD; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, versus e.v. (C), WT (D,F) or pH 5.5 control (E).

Figure S3

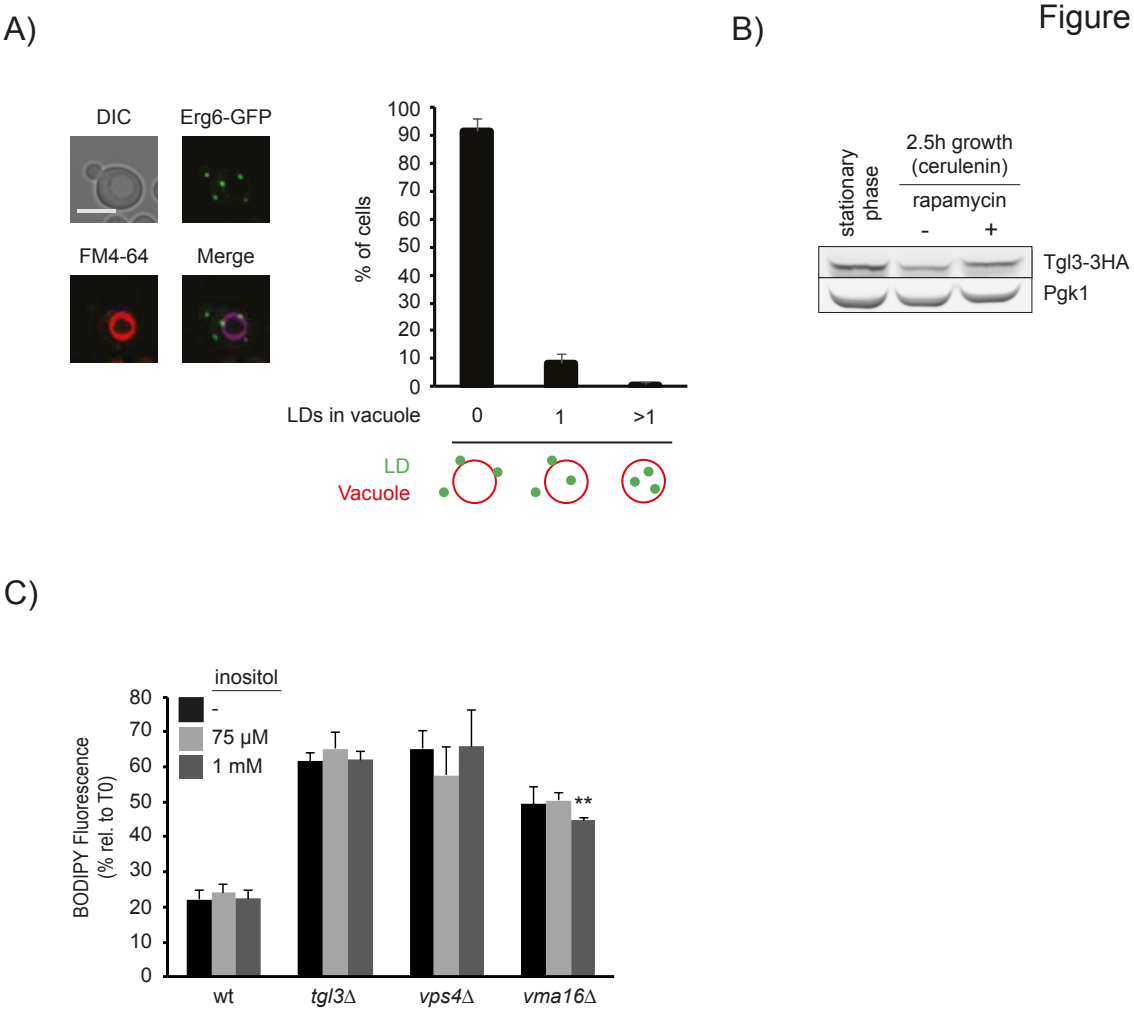


Figure S3: **Fluocerscence microscopy analysis of lipophagy, Tgl3p expression during rapamycin treatment and the effect of inositol supplementation on LD consumption**. (A) Cells expressing Erg6p-GFP were diluted from stationary phase for 1 h into fresh medium containing cerulenin and FM4-64 to stain vacuoles. Cells were chased for 1.5 h in fresh medium containing cerulenin without FM6-64 and analyzed by fluorescence microscopy. For the quantification of LD autophagy, eight microscopy images were analyzed (n= 428 cells). Scale bar: 5 µm. (B) Expression levels of Tgl3p. Cells expressing genomically 3HA-tagged Tgl3p were grown to stationary phase and diluted into fresh medium containing cerulenin in the presence or absence of rapamycin. Protein extracts were analyzed by SDS-PAGE followed by

Journal of Cell Science • Supplementary information

Western blotting. (C) Flow-cytometry-based measurement of LD consumption as in S1B. The indicated strains grown to stationary phase and diluted into fresh medium containing cerulenin and the indicated concentration of inositol were analyzed (n=4). Data are presented as mean ± SD; ** $P < 0.01$, versus WT.

Table S1: Yeast strains used in this study. Strains used in Figure 1C,F,G; 2A,B,D; 4A,C,D; 5 A,B; 6C were from the deletion library (GE Dharmacon).

| Strain ID | Genotype | Source |
|---|---|---|
| BY4741 | *MAT***a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 | (Brachmann et al., 1998) |
| YDM104 | *MAT***a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ICE2::KanMX | (Markgraf et al., 2014) |
| YDM288 | *MAT***a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TGL3::3HA-KanMX | This study |
| YDM372 | *MAT***a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 pRS415-ADH | This study |
| YDM383 | *MAT***a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TGL3::KanMX | (Markgraf et al., 2014) |
| YDM433 | *MAT***a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 DGK1::KanMX | Open Biosystems |
| YDM480 | *MAT***a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SNF7::KanMX | Open Biosystems |
| YDM481 | *MAT***a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 VPS4::KanMX | Open Biosystems |
| YDM483 | *MAT***a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 VPS4::KanMX TGL3::URA3 | This study |
| YDM644 | *MAT***a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 VMA16::KanMX | Open Biosystems |
| YDM654 | *MAT***a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 VMA5::KanMX | Open Biosystems |
| YDM655 | YDM644; TGL3::3HA-HIS3 | This study |
| YDM656 | YDM654; TGL3::3HA-HIS3 | This study |
| YDM657 | YDM480; TGL3::3HA-HIS3 | This study |
| YDM666 | *MAT***a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 YGL007W::KanMX | Open Biosystems |

Table S2: Plasmids used in this study

| ID | Name | Source |
|---|---|---|
| 77 | *pRS415-ADHpr* | (Nguyen et al., 2012) |
| 190 | *pRS415-ADHpr-Erg6-mRFP* | (Markgraf et al., 2014) |
| 256 (pMB65) | *pRS415-Vps4-ts2-29* | (Dimaano et al., 2008) |
| 263 | *p416-GPDpr-C1δ-GFP* | (Ganesan et al., 2015) |
| 277 | *p415-ADHpr-Tgl3-3HA* | This study |

Table S3: Genes with strong phenotype on LD dynamics in stationary phase (BODIPY493/503-fluorescence ≥2x SD BODIPY493/503-fluorescence of *tgl3*Δ cells). The effect of individual mutants was confirmed in separate flow cytometry analyses.

| Gene | Reference |
|---|---|
| *ANP*1, CHC1, *ERD*1, *MDM*20, | This study; (Fei et al., 2008) *mld*; (Szymanski et al., 2007) |
| *MNN*10, *MNN*11, *RPB*4, *GON*7, *KRE*6,*YLR244C*, *SNF*2, *RTT*109, *SWI*3, *ELM*1, *SNF*6, | This study; (Fei et al., 2008) *mld* |
| *CAX*4 | This study; (Fei et al., 2008) *fld* |
| *INO*2, *INO*4, *OPI*3, *CKB*1 | This study; (Fei et al., 2011) |
| *SWI6*, *SWI*4, CLC1, *RML*2, *NPL*3, *HFI*1, *HOF*1, *GAS*1, *VPS*3, *CTK*2, *CTS*1, *GLO*3, *CDC*26, *CCR*4, *FYV*4, *SPT*3, *HTL*1, *IRC*14, *MRP*21 | This study |

*mld,* many lipid droplet; *fld,* few lipid droplet

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Journal of Cell Science • Supplementary information