Gao et al., https://doi.org/10.1083/jcb.201610013

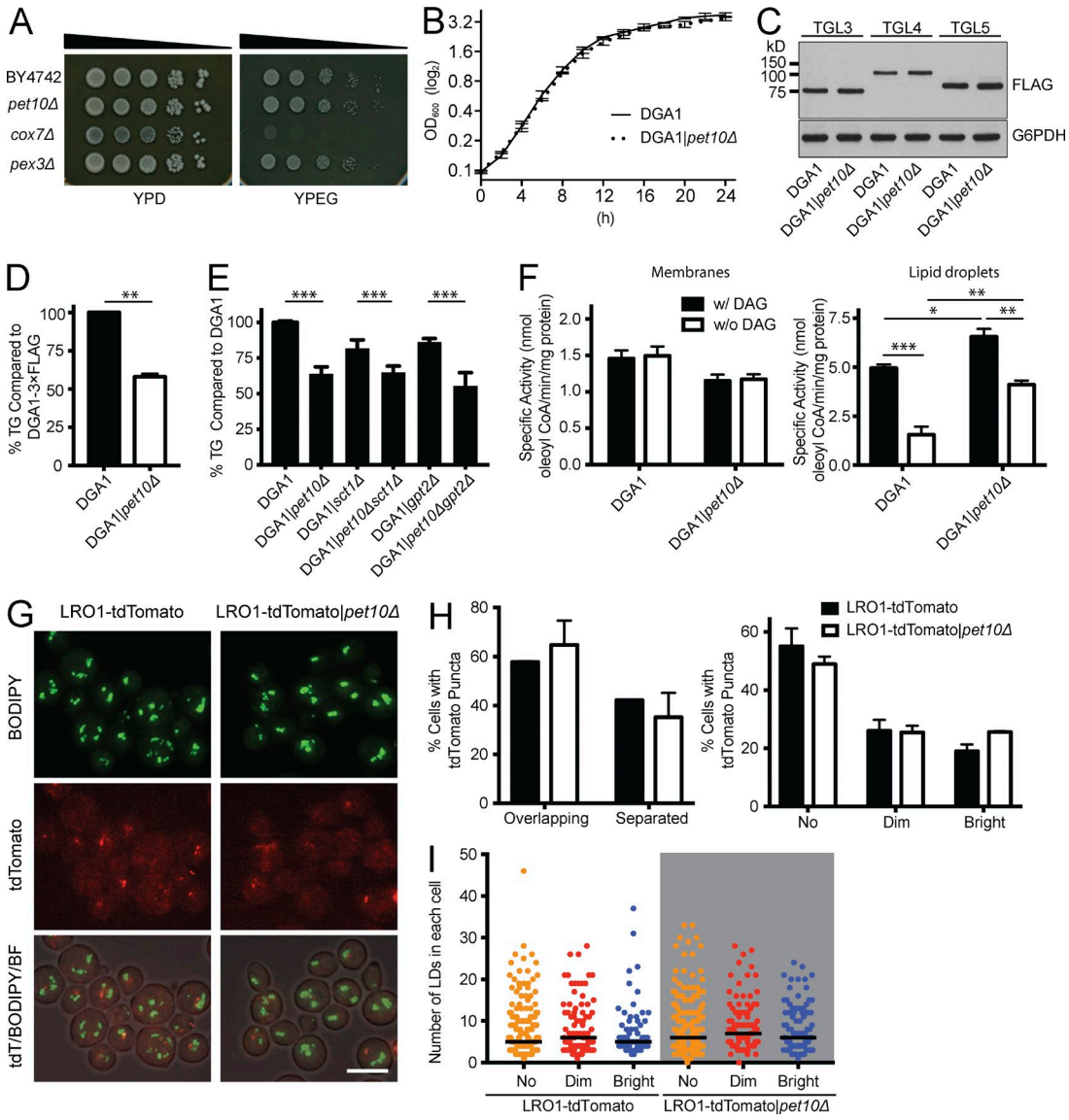

Figure S1. **Behaviors of Dga1p and Lro1p.** (A) Serial 10-fold dilutions of BY4742 (wild type), *pet10Δ*, *cox7Δ*, or *pex3Δ* (all in BY4742 background) were grown on YPD (dextrose) or YPEG (ethanol glycerol, requiring mitochondrial respiration) plates for 2 d. (B) Growth curve of DGA1 and DGA1|*pet10Δ* in SD medium. Error bars represent range of duplicate cultures from a representative experiment. (C) Immunoblots of 3×FLAG-labeled Tgl3p, Tgl4p, or Tgl5p in DGA1 or DGA1|*pet10Δ* strains with anti-G6PDH as loading control. (D) TG accumulation in DGA1-3×FLAG and DGA1-3×FLAG|*pet10Δ*. (E) TG accumulation in the indicated strains. (F) Specific DGAT activity, with or without exogenous DAG, from crude membranes (left) or droplets (right). (G) Lro1-tdTomato puncta in the indicated strains. Bar, 5 µm. (H, left) Distribution of cells containing Lro1-tdTomato spots that were overlapping or separated from BODIPY-stained droplets in the indicated strains. (H, right) Distribution of cells in which Lro1-tdTomato puncta were absent, dim, or bright in the indicated strains. (I) Number of lipid droplets (LDs) per cell in cells with absent, dim, or bright Lro1-tdTomato spots in the indicated strains. Black bars represent median values. For D–F and H, error bars represent SEM over all replicate experiments. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

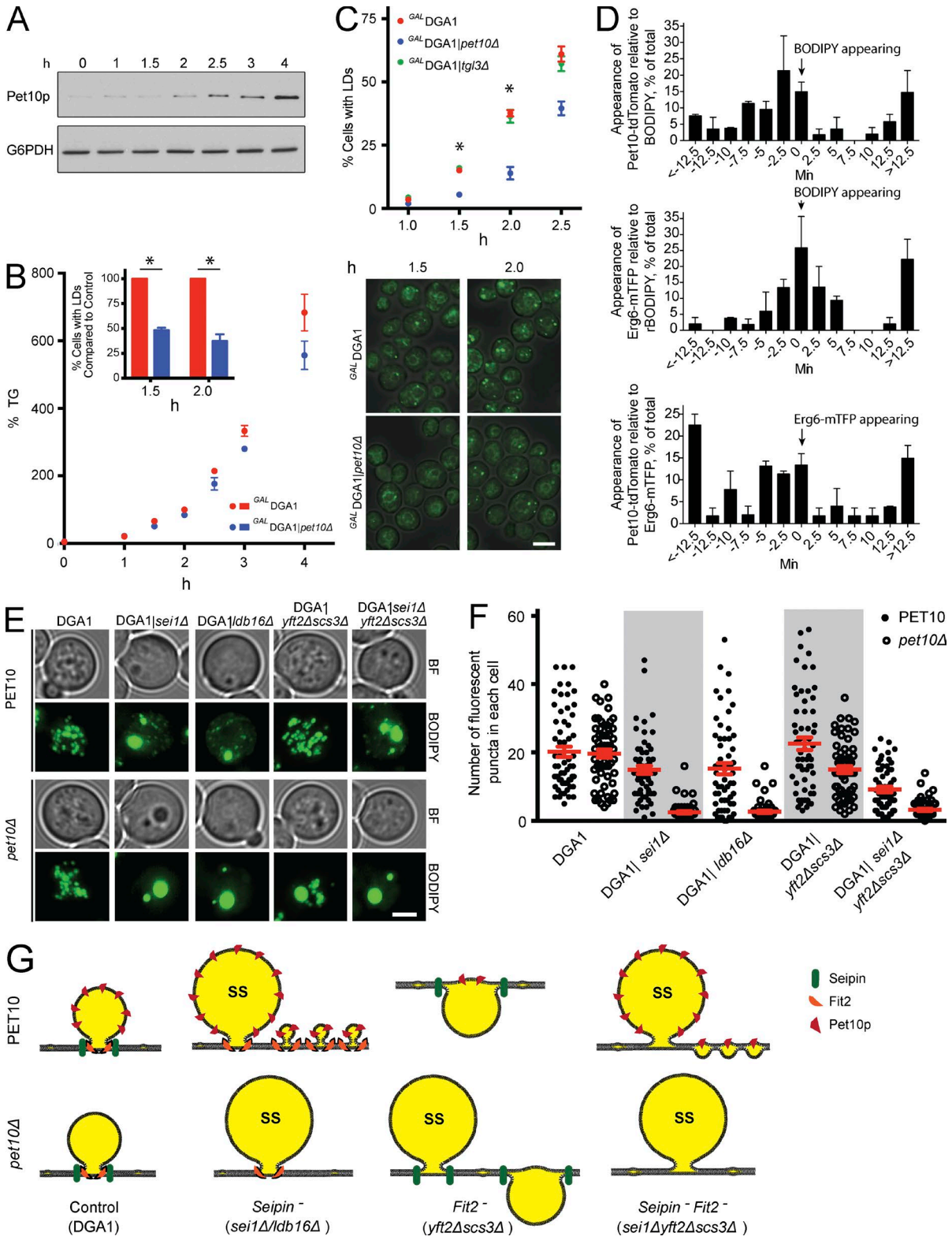

Figure S2. **Pet10p and droplet biogenesis.** (A) Immunoblot of endogenous Pet10p during induction of *DGA1* and droplets. (B, left) TG accumulation after *DGA1* induction in the indicated strains; the 2-h time point in the ^{GAL}DGA1 strain is set at 100%. (B, inset) Percentage of cells with droplets, determined with BODIPY, in ^{GAL}DGA1|*pet10Δ* (compared with ^{GAL}DGA1) from the same cultures at early time points. (B, right) Representative micrographs, BODIPY staining. (C) Lipid droplet (LD) appearance is compared in ^{GAL}DGA1|*pet10Δ* versus ^{GAL}DGA1|*tgl3Δ*. (D) Droplets from cells coexpressing Pet10-tdTomato and Erg6-mTFP were induced identically to those in Fig. 5 G. 58 BODIPY puncta that appeared during the observation period were counted. Data in A–D are each from two independent experiments. Bar, 5 µm. (E) Representative single cells from the experiment from Fig. 6 (A and D) are shown at higher magnification. Bar, 2 µm. (F) The number of droplets in each cell from the experiment illustrated in E are plotted, regardless of the presence of supersized (SS) droplets. Droplets in 60 cells from each strain were scored. (G) A diagram of hypothetical representative droplets from the experiment in Fig. 6 is shown. Bilayers are oriented with cytosol above and ER lumen below. For B–D and F, error bars represent SEM over all replicate experiments. *, $P < 0.05$.

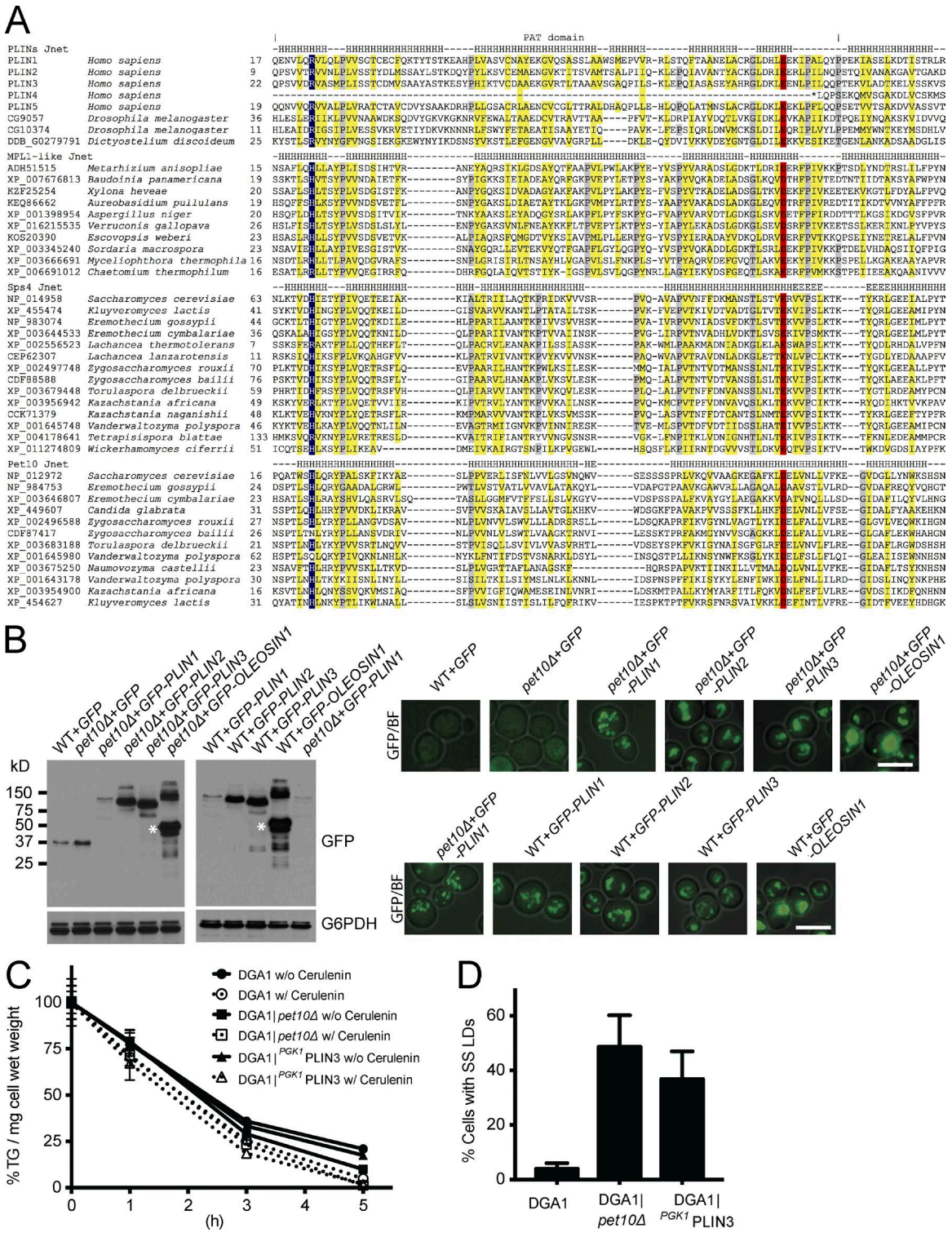

Figure S3. **Pet10p family sequence conservations and relationships.** (A) A multiple-sequence alignment illustrates the relationship between select metazoan perilipin PAT domains (including an additional helical segment) and those from representative Mpl1p, Sps4p, and Pet10p family sequences. Sequence positions are colored according to conserved amino acid properties: mainly hydrophobic (yellow), mainly small (gray), mainly basic (blue), and mainly acidic (red). Predicted Jnet (Cuff and Barton, 2000) secondary structures (H for helix and E for extended strand) for the top representative family sequence are indicated above each group. The sequences are labeled to the left with either the gene name or the NCBI accession number, followed by the scientific name and the residue number of the first position in the alignment. (B, left) Anti-GFP immunoblot of the indicated strains in which exogenous GFP-tagged proteins were expressed in W303-1A or the corresponding *pet10Δ* strain as indicated; anti-G6PDH blot as loading control is below. Asterisks indicate the predicted molecular mass of GFP-Oleosin1. (B, right) Images of cells from the indicated strains. Bars, 5 µm. (C) The change of TG levels in the indicated strains with or without cerulenin or DMSO vehicle added at t_0. (D) The percentage of cells cultured in OA with supersized (SS) droplets (>1.0 µm in diameter) is shown for the indicated strains. For C and D, error bars represent SEM over all replicate experiments.

Provided online are two Excel tables. Table S1 shows proteomics analysis of isolated lipid droplets from indicated strains and Table S2 shows strains used in this study.

Reference

Cuff, J.A., and G.J. Barton. 2000. Application of multiple sequence alignment profiles to improve protein secondary structure prediction. *Proteins.* 40:502–511. http://dx.doi.org/10.1002/1097-0134(20000815)40:3<502::AID-PROT170>3.0.CO;2-Q