Xu et al., http://www.jcb.org/cgi/content/full/jcb.201201139/DC1

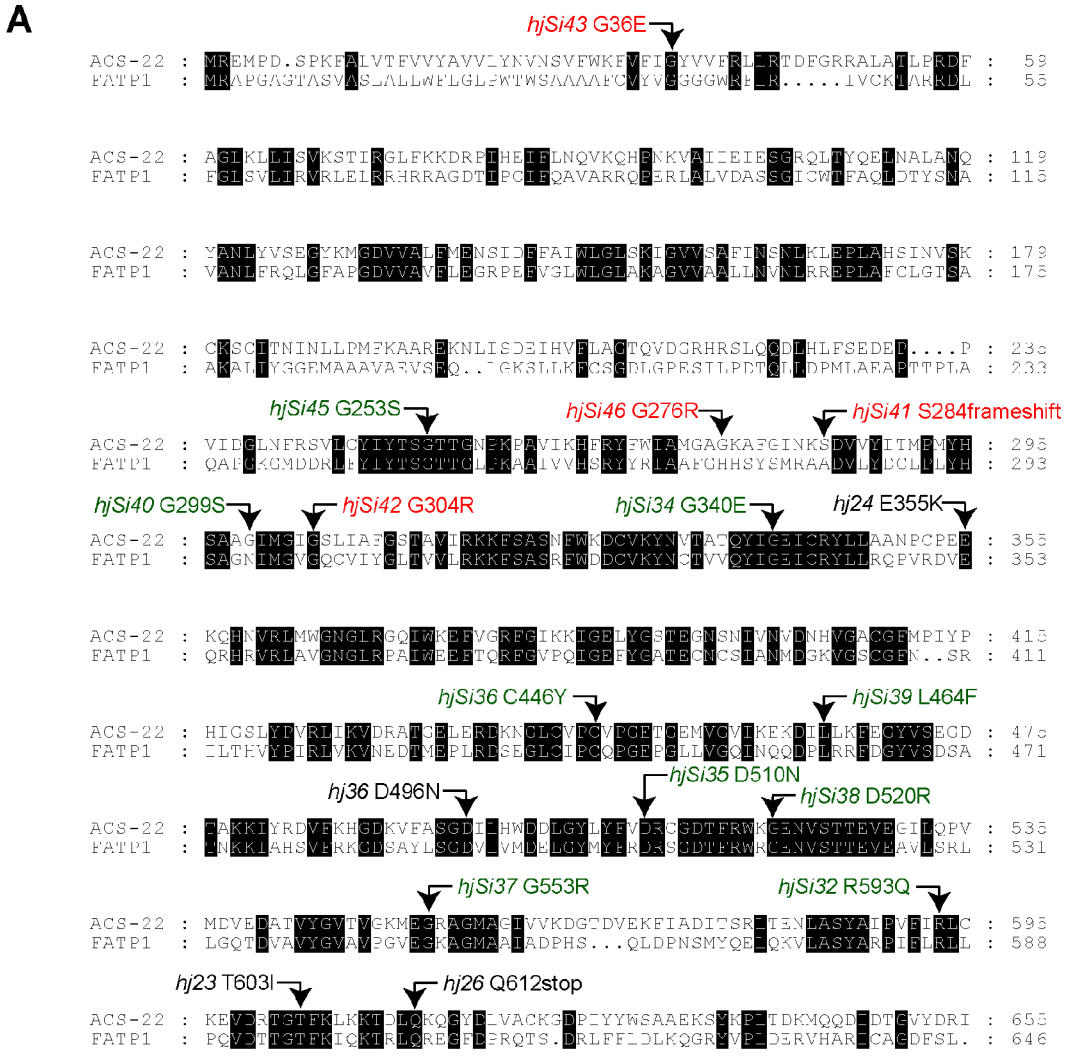

Figure S1. **Molecular lesions in the endogenous *acs-22* gene and single-copy *vha-6p::acs-22::gfp-3×FLAG* transgenes.** (A) Sequence alignment of ACS-22 and mouse FATP1. Mutant alleles of *acs-22* are in black. Molecular lesions that affect expression of ACS-22::GFP-3×FLAG fusion protein are in red, whereas those that do not affect or abolish its expression are in green. (B) Western blotting using an anti–ACS-22 antibody indicated that endogenous ACS-22 protein was absent in animals carrying *hj24*, *hj25*, or *hj26* alleles, whereas ACS-22 expression was unaffected in animals carrying *hj23* and *hj36* alleles or in *daf-22(ok693)* mutant animals. A nonspecific band is marked with an asterisk. (C) Western blotting using an anti-FLAG antibody detected ACS-22::GFP-3×FLAG fusion proteins expressed from single-copy transgenes in *daf-22(ok693); acs-22(hj26)* mutant animals that lacked endogenous ACS-22. The α-tubulin blots served as loading controls for B and C. WT, wild type.

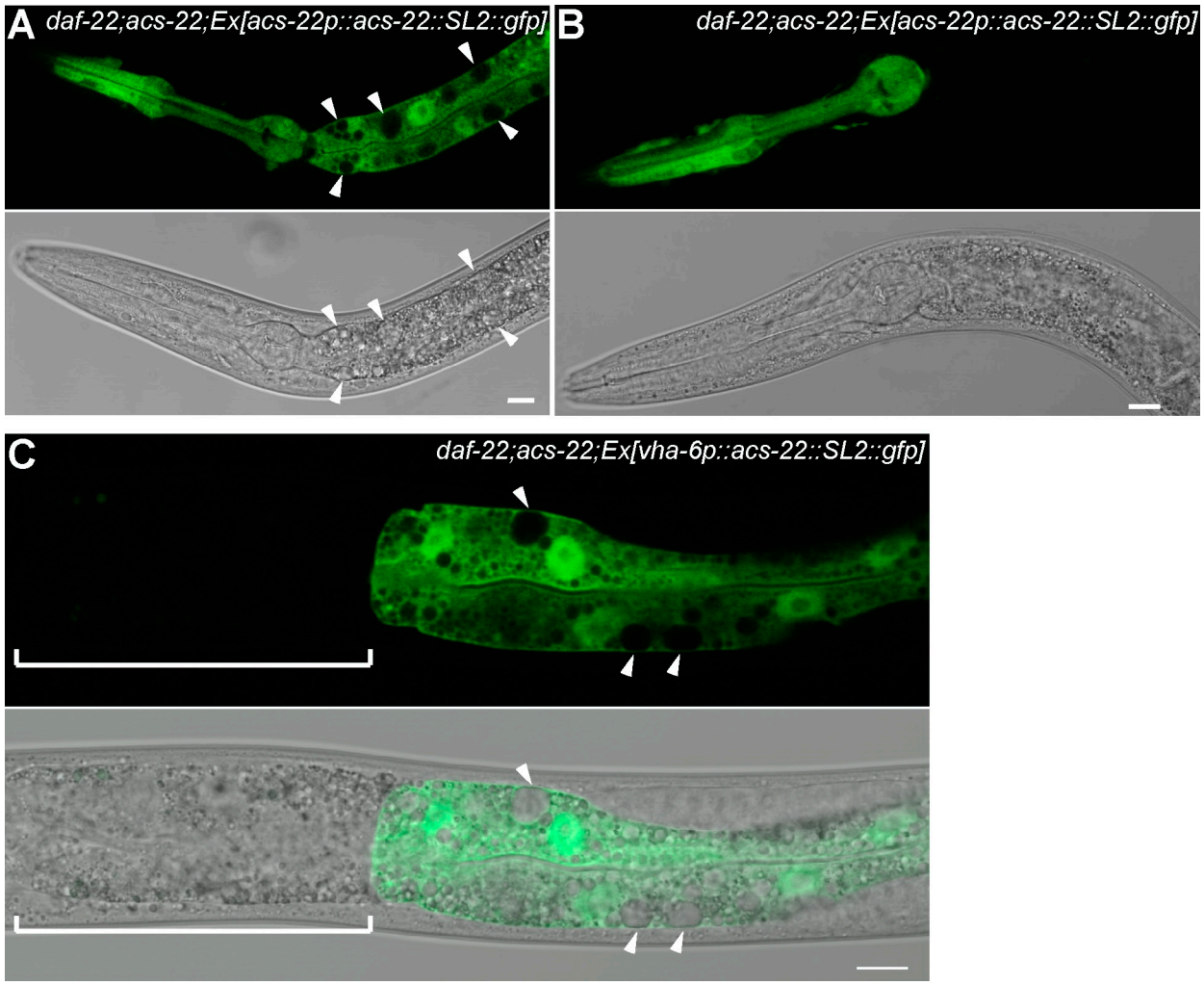

Figure S2. **ACS-22 acts cell autonomously to promote LD expansion.** (A) Expression of an *acs-22p::acs-22::SL2-gfp* transgene in the intestine and the pharynx restored LD expansion in a *daf-22(ok693); acs-22(hj26)* L4 animal. Expanded LDs were marked by white arrowheads. (B) Expression of an *acs-22p::acs-22::SL2-gfp* transgene in the pharynx alone did not restore LD expansion in a *daf-22(ok693); acs-22(hj26)* L4 animal. (C) Mosaic expression of a *vha-6p::acs-22::SL2-gfp* transgene in a subset of intestinal cells restored LD expansion in the same cells of a *daf-22(ok693); acs-22(hj26)* L4 animal. Expanded LDs were marked by white arrowheads. No expanded LDs were detected in intestinal cells (marked with white brackets) that did not express the transgene (GFP negative) in the same animal. Bars, 10 μm.

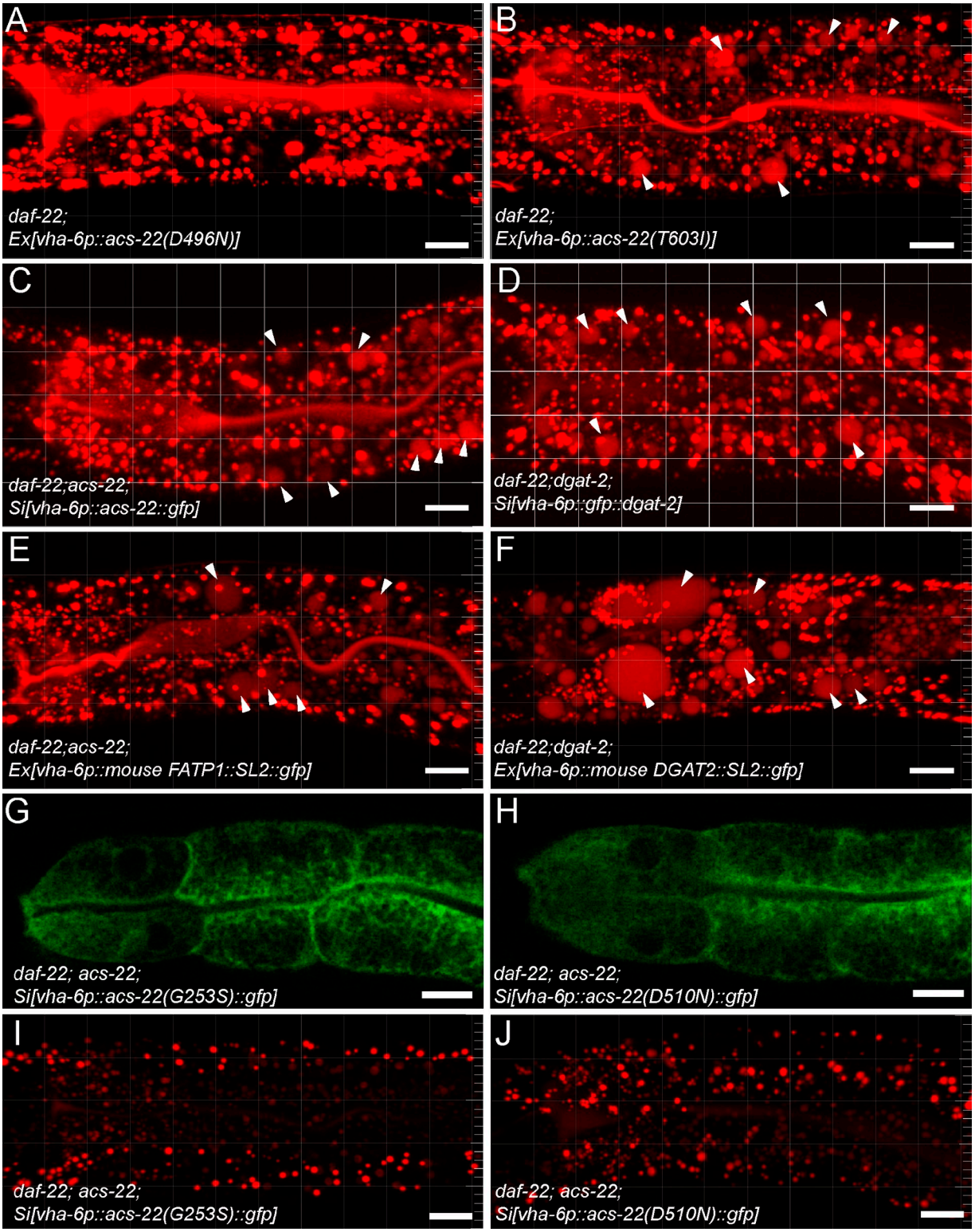

Figure S3. **LD expansion in mutant animals carrying *acs-22* or *dgat-2* transgenes.** Representative images of larval stage L4 animals stained with red BODIPY-C12 (except G and H in which GFP fluorescence is shown). Expanded LDs >3 μm in diameter were stained and indicated by white arrowheads. (A) Overexpression of ACS-22 (D496N) suppressed LD expansion in *daf-22(ok693)* animals. (B) Overexpression of ACS-22 (T603I) did not affect LD expansion in *daf-22(ok693)* animals. (C) *hjSi29[vha-6p::acs-22::gfp]* expressed the ACS-22::GFP fusion protein and restored LD expansion in *daf-22(ok693); acs-22(hj26)* mutant animals. (D) *hjSi56[vha-6p::gfp::dgat-2]* expressed the GFP::DGAT-2 fusion protein and restored LD expansion in *daf-22(ok693); dgat-2(hj44)* mutant animals. (E) Overexpression of mouse FATP1 compensated for a loss of *acs-22* function and restored LD expansion in *daf 22(ok693); acs-22(hj26)* animals. (F) Overexpression of mouse DGAT2 compensated for a loss of *dgat-2* function and restored LD expansion in *daf-22(ok693); dgat-2(hj44)* animals. (G and H) Mutant ACS-22::GFP fusion proteins retained their localization to the ER. (I and J) Mutant ACS-22::GFP fusion proteins failed to support LD expansion in *daf-22(ok693); acs-22(hj26)* mutant animals. No expanded LDs >3 μm in diameter were observed. Bars, 10 μm.

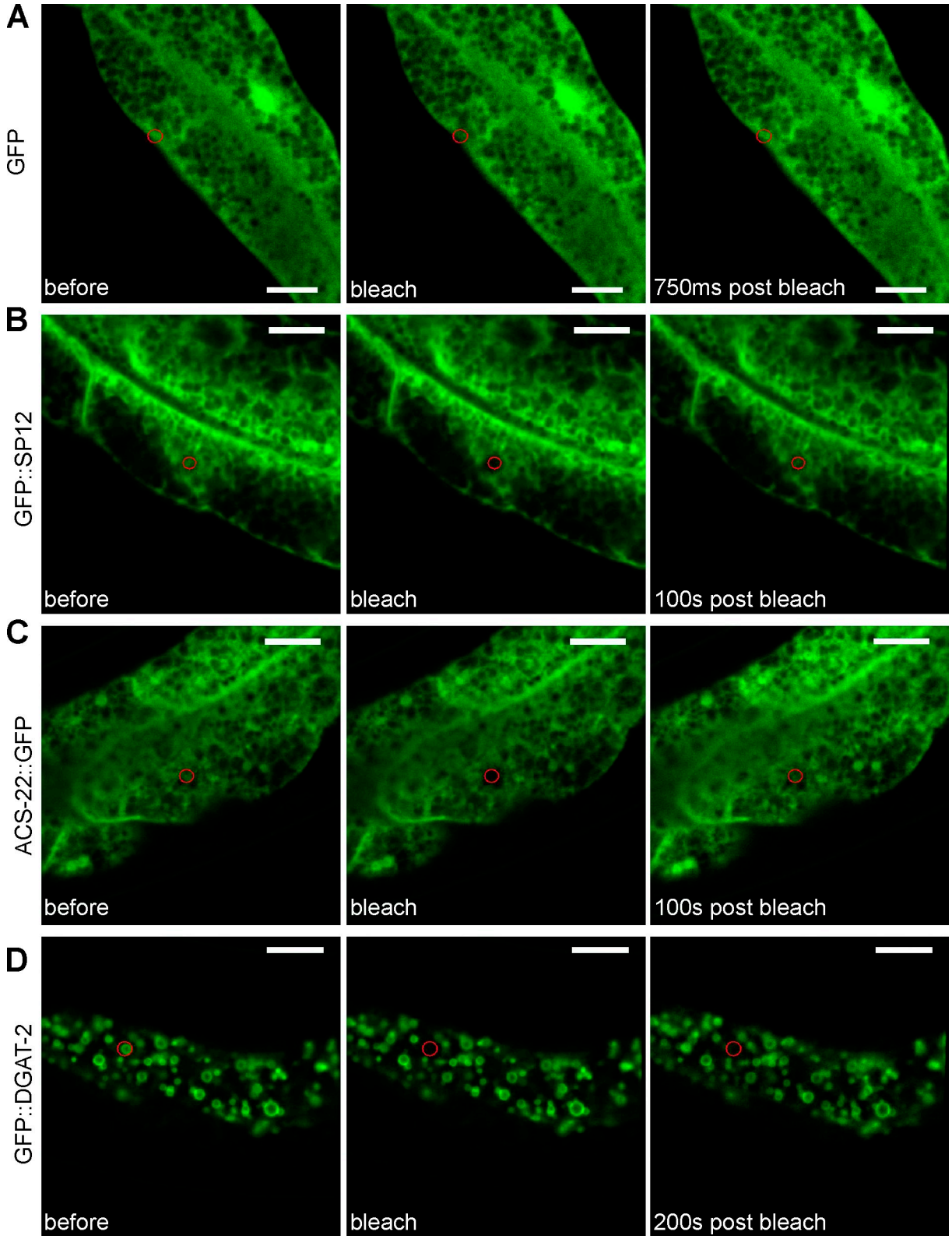

Figure S4. **ACS-22 and DGAT-2 are ER and LD resident proteins, respectively.** Animals at larval stage L4 were subjected to photobleaching (2 µm diameter area marked by red circles). Representative images before, during, and after photobleaching of each strain are shown. (A) *hjSi22[vha-6p::gfp]*; (B) *hjIs14 [vha-6p::gfp::C34B2.10(SP12)]*; (C) *acs-22(hj26); hjSi29[vha-6p::acs-22::gfp]*; (D) *dgat-2(hj44); hjSi56[vha-6p::gfp::dgat-2]*. Bars, 10 µm.

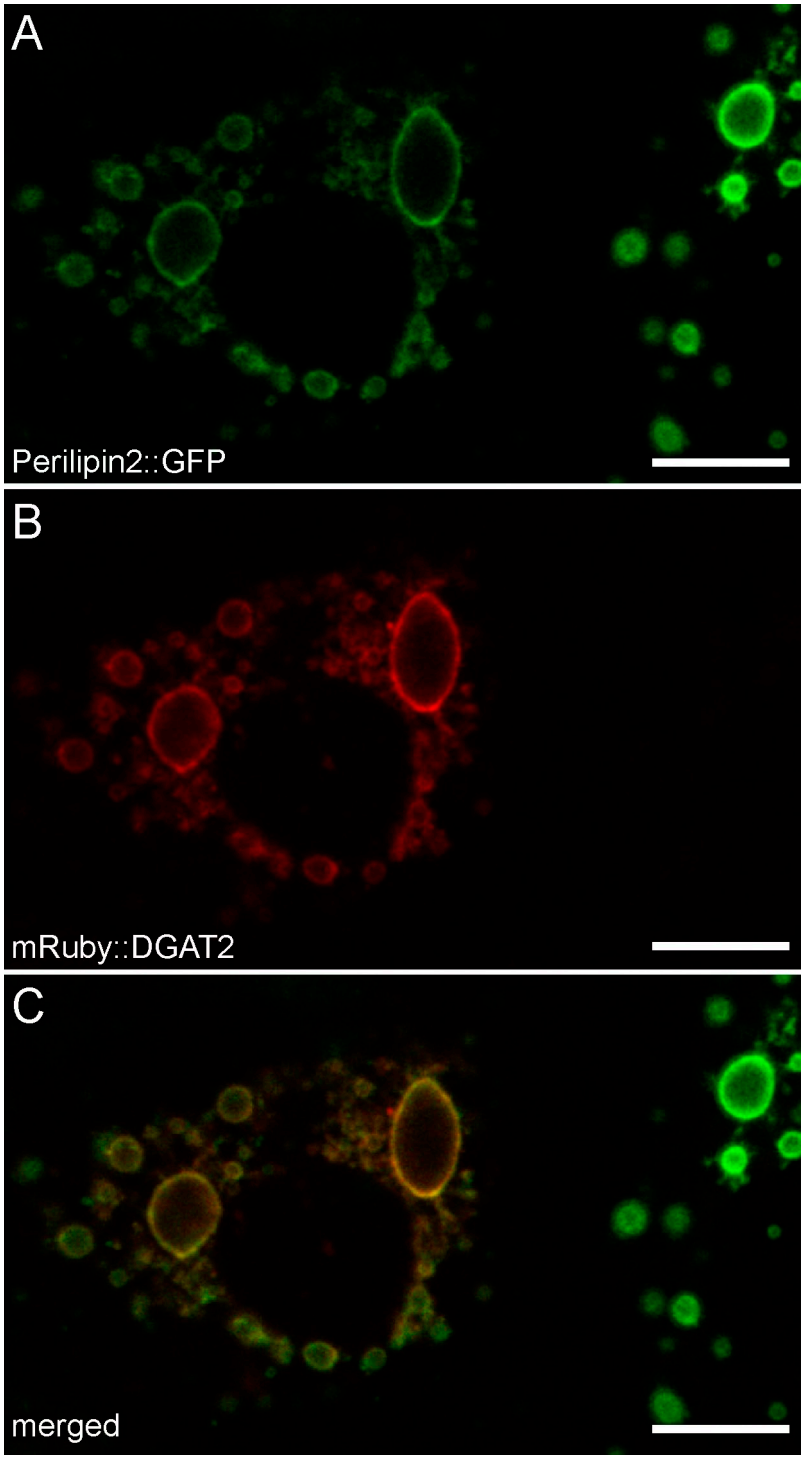

Figure S5. **Colocalization of DGAT2 and Perilipin2.** Representative images of oleic acid–loaded COS7 cells that expressed Perilipin2::GFP and mRuby::DGAT2. The fusion proteins colocalized on the LD surface. The cell on the right only expressed Perilipin2::GFP and served as a control for spectral separation of green and red fluorescence signals. Bars, 10 µm.

Table S1. **Knockdown of acyl-CoA synthetase function by RNAi**

Gene name	Suppression of LD expansion in *daf-22* animals?
acs-1[a]	No
acs-2[b]	No
acs-3[b]	No
acs-4[a]	No
acs-5[a]	No
acs-6[b]	No
acs-7[a]	No
acs-9[a]	No
acs-10[b]	No
acs-11[a]	No
acs-12[a]	No
acs-13[a]	No
acs-14[b]	No
acs-15[a]	No
acs-16[b]	No
acs-17[a]	No
acs-18[a]	No
acs-19[a]	No
acs-20[a]	No
acs-21[a]	No
acs-22[a]	Yes

LD expansion in *daf-22(ok693)* mutant animals was scored at larval stage L4. Expanded LDs were visualized by C1-BODIPY-C12 staining. RNAi experiments were performed in 6-well plates at 25°C with each RNAi clone tested in duplicate wells in independent plates. Each well was scored by two independent researchers.
[a]RNAi clones obtained from the Ahringer library.
[b]RNAi clones made in this study.