Supporting Information

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SI Materials and Methods

Lipid Droplet Isolation. Lipid droplets were recovered by flotation centrifugation following the procedure of Liu et al. (1), and suspended in equal volumes of medium for staining, microscopic examination, and MS. On this basis, lipid droplets isolated from mutant plants were more abundant than from wild-type. Routinely, equal fresh weights of plant tissues were homogenized and suspended in 20 mM Tricine-HCl (pH 7.8) and 250 mM sucrose. The suspension was then overlaid with 0.5 volume of 20 mM Pipes (pH 7.4), 100 mM KCl, and 2 mM $MgCl_2$ before centrifugation (60,000 × *g*, 90 min; Beckman 100Ti rotor, Beckman TL-100 centrifuge). The fat pad on the surface and residue around the wall of the tubes were collected and analyzed.

Direct Organelle MS Conditions. Approximately one-dozen lipid droplets, isolated from either mutant or wild-type leaves, were collected in a glass nanospray tip (1-μm pore size; New Objective), microextracted into $CHCl_3$:MeOH in 10 mM ammonium acetate, and analyzed immediately by nanospray ionization MS using an LCQ Deca XP Plus quadrupole ion trap. Triacylglycerol (TAG) molecular species were identified as their ammonium adducts $[M+NH_4]^+$. Typical scanning conditions were carried out in positive ion mode with a 0.8 to 1.2 kV spray voltage, scan range of 500 to 1,000 *m/z*, 200 °C capillary temperature, three microscans per full scan with a maximum injection time of 200 ms.

Direct Infusion Electrospray Ionization-MS (TAG Quantification). TAG and steryl ester molecular species from whole tissue extracts were identified by neutral loss fragmentation spectra in tandem (2) and quantified against tri-15:0 and cholestryl ester (13:0), respectively. Typical scanning conditions for a direct infusion rate of 12.5 μL/min were carried out in positive ion mode with a 3- to 3.5-kV spray voltage, 40-V cone voltage, and a scan range of 200 *m/z* to 1,050 *m/z*. The desolvation and source temperatures were maintained at 200 and 80 °C, respectively, and the desolvation and cone gas flows were set at 300 and 80 L/h, respectively. Tandem scans (MS/MS), whether detecting the precursor ions that lost a particular acyl chain in neutral-loss mode or single precursor-product species, were performed with collision energy of 30 V, with a scan range from 400 *m/z* to 1,050 *m/z*. Tripentadecanoyl glycerol (tri-15:0) was added at the time of extraction and used as a quantitative standard for TAG content (steryl esters were quantified against a cholesteryl decanoate standard and normalized to the tri-15:0 TAG standard).

1. Liu P, et al. (2004) Chinese hamster ovary K2 cell lipid droplets appear to be metabolic organelles involved in membrane traffic. *J Biol Chem* 279:3787–3792.
2. Bartz R, et al. (2007) Lipidomics reveals that adiposomes store ether lipids and mediate phospholipid traffic. *J Lipid Res* 48:837–847.

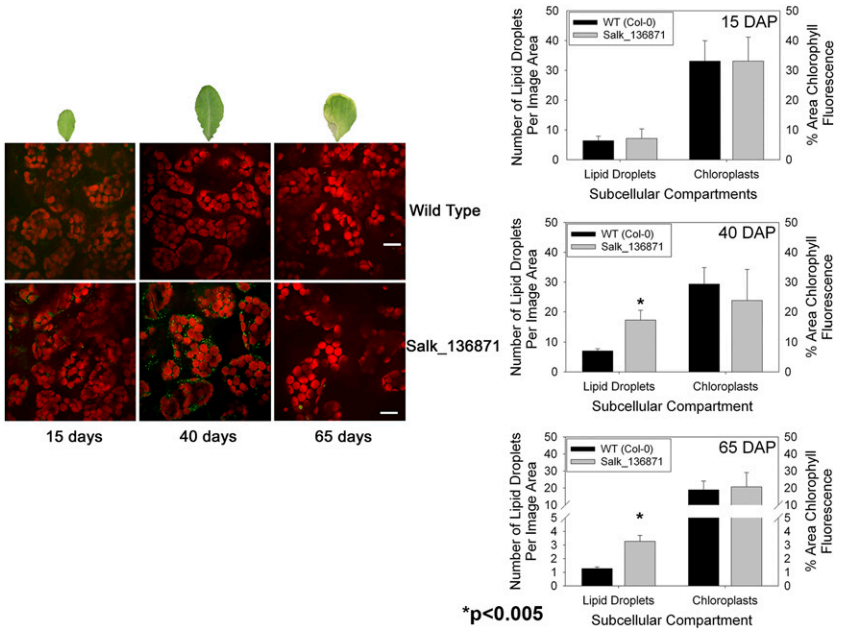

Fig. S1. Temporal differences in lipid droplet accumulation in wild-type and *cgi-58*-disrupted *Arabidopsis* plant cells. Representative confocal fluorescence micrographs of *Arabidopsis* leaves emerged at 15, 40, or 65 d (wild-type and T-DNA mutant, Salk_136871). Lipid droplets are green and chloroplasts are red; Imaging acquisition conditions were the same as for Fig. 4. The numbers of lipid droplets and the percent area of chloroplasts in mesophyll cells were quantified by morphometric analysis. Averages and SDs are plotted from 10 digital images of 25,000 μm^2, each over several leaves at each stage. There were significant differences (*$P < 0.005$) between the numbers of lipid droplets in mutants and wild-type leaves at 40 and at 65 d, but not at 15 d. Abundant droplets were most evident in mature fully expanded leaves of mutants. No statistical differences were observed in the relative percentage of chloroplasts between mutant and wild-type leaves. (Scale bars, 20 μm.)

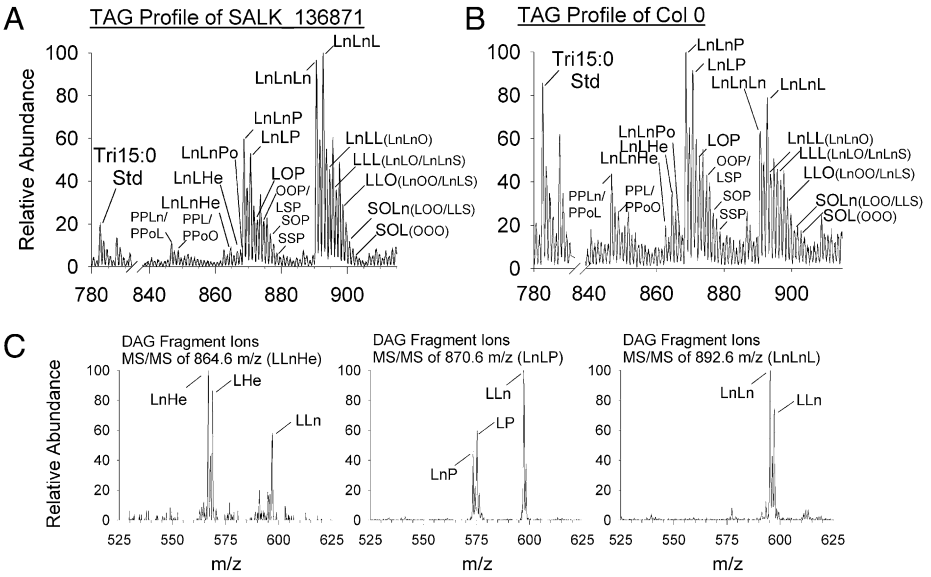

Fig. S2. Electrospray ionization MS of TAG molecular species in wild-type and *cgi-58* T-DNA knockout plants. Representative positive ion MS analysis of neutral lipids extracted from wild-type (*A*) and *cgi-58* (*B*) plants. TAG species are identified as ammonium adducts $[M + NH_4]^+$ and quantified against an added TAG standard (tri-15:0). Peaks are labeled according to the three fatty acids present in each TAG molecular species, and low abundance TAG species with the same molecular mass are indicated in parentheses. Positions of fatty acids on the glycerol backbone were not determined. Fatty acid abbreviations are: He, 16:3-hexadecatrienoic acid; L, 18:2-linoleic acid; Ln, α-18:3-linolenic acid; P, 16:0-palmitic acid; Po, 16:1-palmitoleic acid; S, 18:0-stearic acid. (*C*) Regions of several relevant, representative MS/MS scans showing the diagnostic diacylglycerol fragmentation ions minus a free fatty acid compared with known masses (1). Molecular ions were in some cases a combination of isomers as revealed in MS/MS (e.g., first two panels, each with three combinations of diacylglycerols (DAG) species derived from same parent ion, but with a different fatty acid loss).

1. Byrdwell WC (2005) The bottom-up solution to the triacylglycerol lipidome using atmospheric pressure chemical ionization mass spectrometry. *Lipids* 40:383–417.

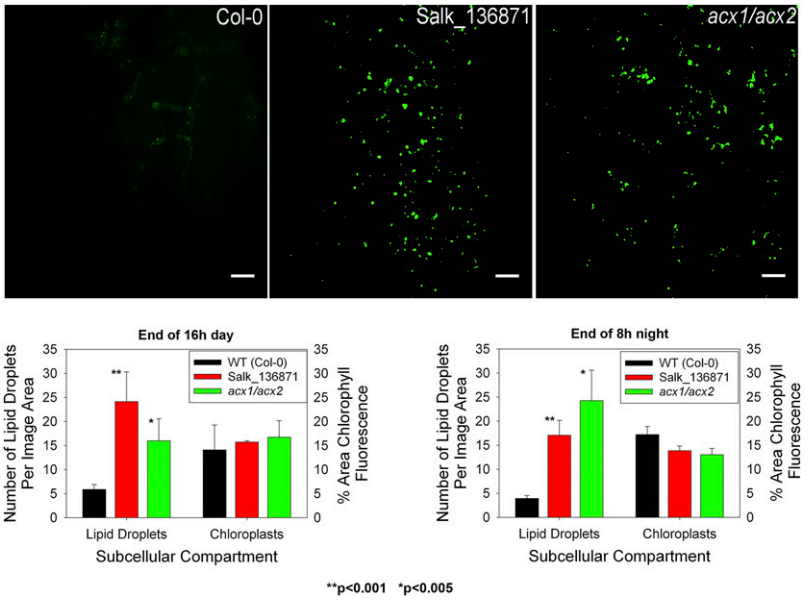

Fig. S3. Comparison of lipid droplet accumulation in *cgi-58* disrupted plants with acyl CoA oxidase double mutants (*acx1/acx2*), previously shown by Slocombe et al. (1) to accumulate lipid droplets in leaves. Representative confocal fluorescence micrographs of mature leaves of *Arabidopsis* WT (Col-0), the Salk_136871 mutant, and the *acx1/acx2* double knockout. Lipid droplets are green and imaging acquisition conditions were the same as for Fig. 4. Numbers of lipid droplets and the percent area of chloroplasts in mesophyll cells were quantified by morphometric analysis (*Lower*). Averages and SDs are plotted for 10 digital images of 25,000 μm^2, each taken from several leaves. There were significant differences between the amounts of lipid droplets in both mutants compared with wild-type leaves ($P < 0.005$) imaged at the end of a 16-h day and at the end of an 8-h night. No statistical differences were observed in the relative percentage of chloroplasts between mutants and wild-type. (Scale bar, 20 μm.)

1. Slocombe SP, et al. (2009) Oil accumulation in leaves directed by modification of fatty acid breakdown and lipid synthesis pathways. *Plant Biotechnol J* 7:694–703.

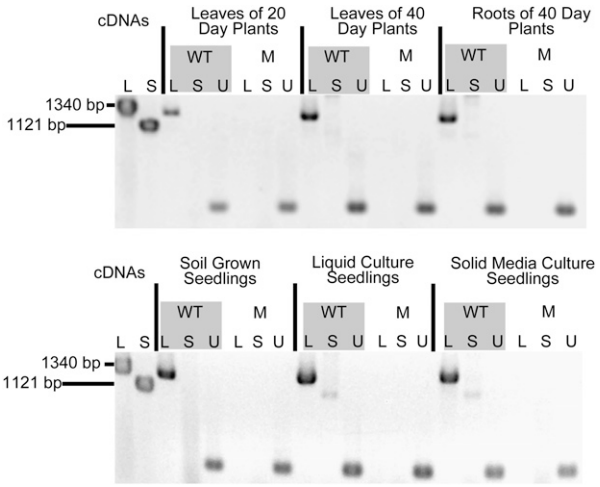

Fig. S4. Expression of At4g24160 (*CGI-58*) isoforms in different tissues and different developmental stages. Complementary DNAs corresponding to the two alternatively spliced At4g24160 (At4g24160.1 and At4g24160.2) transcripts (Fig. 1*B*) were amplified from seedling mRNA and used to verify sequences predicted in Fig. 1. Analysis of mRNA abundance using semiquantitative RT-PCR revealed that the longer splice variant (1,340 bp; lanes labeled "L" in each sample) was expressed in both leaves and roots of wild-type plants cultivated under a variety of conditions. The shorter splice variant (1,121 bp; lanes labeled "S"), however, was only detected at very low levels in leaves of 40-d-old plants, and seedlings grown on liquid or solid media. Notably, no transcripts for either the longer or shorter form of At4g24160 were detected in the T-DNA mutant plants (M). The level of ubiquitin transcripts expressed in the same wild-type and mutant plants (lanes labeled "U") are shown as a control.

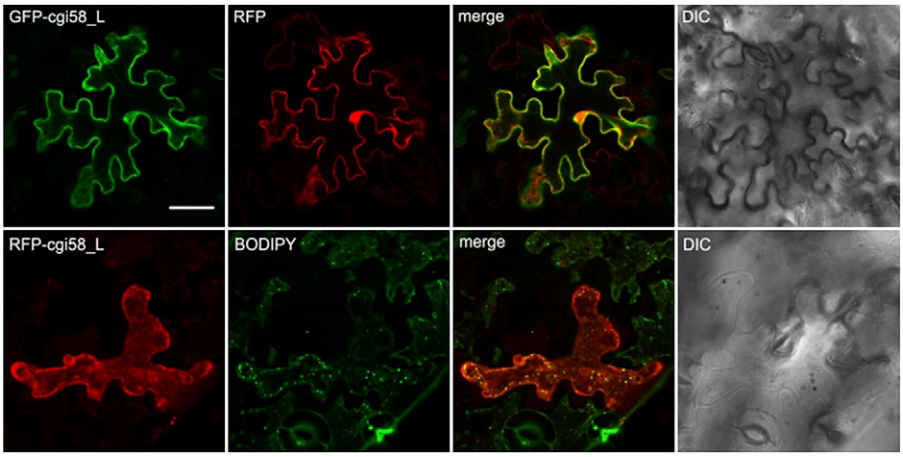

Fig. S5. CGI-58 localizes to the cytosol in *Arabidopsis* leaves. In transient expression studies, a GFP-CGI-58 fusion protein (consisting of GFP fused to the N terminus of the longer isoform of CGI-58; GFP-cgi58_L) was transiently coexpressed (via biolistic bombardment) with the red fluorescent protein (RFP) in epidermal cells of 25 d-old *Arabidopsis* leaves (*Upper*). The merged image of the two fluorophores indicates colocalization in the cytosol. The image to the right is of the same leaf cells acquired by differential interference contrast (DIC) microscopy. In a similar experiment, transiently expressed RFP-cgi-58_L localizes to the cytosol, and not to endogenous lipid droplets (stained with BODIPY). (Scale bar, 20 μm.)