Supplementary File	Title
Supplementary Figure 1	Co-localization between BODIPY and SRS signals in lipid droplets in mammalian 293 cells.
Supplementary Figure 2	Image artifact of CARS due to lipid unrelated structure.
Supplementary Figure 3	Quantification of fat storage in the *daf-2(e1370)* mutants based on CARS microscopy.
Supplementary Figure 4	New genetic regulators of fat storage identified using label-free SRS microscopy through RNAi screening.
Supplementary Table 1	Summary of newly identified genes responsible for fat storage.

Online Methods:

The apparatus of SRS microscopy setup is similar to what was described in Reference 7.

Laser arrangement: A Nd:YVO_4 Pump laser (picoTRAIN, High-Q Laser Production GmbH) provided an output pulse train at 1064 nm with 7 ps pulse width and 76 MHz repetition rate. This served as the Stokes beam. The frequency doubled output at 532 nm was used to synchronously seed an optical parametric oscillator (OPO) (Levante Emerald, APE GmbH) to produce a mode-locked pulse train (the idler beam of the OPO is blocked with an interferometric filter) from 700 to 900 nm. This served as the Pump beam. The wavelength tuning of the OPO involved a coarse temperature tuning of a non-critically phase matched LBO crystal and a fine angle tuning of a stacked Lyot filter. The intensity of the 1064 nm Stokes beam was modulated by an acousto-optical modulator (AOM) (Model 3080-122, Crystal technology) at 10 MHz driven by a square-wave function generator. Modulation depth was more than 80% when the Stokes beam was gently focused inside the AOM crystal. The Pump beam was spatially overlapped with the Stokes beam with a dichroic mirror (1064 DCRB, Chroma Technology). The temporal overlap between Pump and Stokes pulse trains was ensured with a mechanical delay stage and measured with an auto-correlator (PulseCheck, APE GmbH).

Microscopy arrangement: Pump and Stokes beams wer coupled into a modified laser scanning upright microscope (BX61WI/FV300, Olympus) optimized for near IR throughput. The beam size was matched to fill the back-aperture of objectives. For fast whole worm lipid screening, a 20 × air objective (UPlanAPO, 0.75 N.A., Olympus) was used as an excitation lens. For high-resolution imaging, a 60 × water objective (UPlanAPO/IR, 1.2 N.A, Olympus) was then used. The forward going Pump and Stokes beams after passing through the sample was collected in transmission with a high N.A. condenser lens (oil immersion, 1.4 N.A, Nikon) which was aligned following Kohler illumination. A telescope was then used to image the scanning mirrors onto a large area (10 by 10 mm) Si photodiode (FDS1010, Thorlabs) to avoid (e.g. descan) beam motion during laser scanning. The photodiode was reversed bias by 60V from a DC power supply to increase the saturation threshold. A high O.D. bandpass filter (890/220 CARS, Chroma Technology) was used to block the Stokes beam completely and transmit the Pump beam only.

SRS imaging: The output current of the photodiode was electronically pre-filtered by a band-pass filter (BBP-10.7, Mini Circuits) to suppress both the 76 MHz laser pulsing and the low-frequency contribution due to laser scanning cross the scattering sample. It was then fed into a radio frequency lock-in amplifier (SR844, Stanford Research Systems) terminated with 50 Ω to demodulate the stimulated Raman loss signal of the Pump beam. The x-output (in-phase component) of the lock-in amplifier was fed back into the A/D converter of the microscope input. The time constant was set for 10 μs (with no additional filter applied in lock-in amplifier). For imaging, 512 by 512 pixels were acquired for one frame with a 100 μs of pixel dwell time.

CARS imaging: CARS signal was collected by the same excitation objective in the backward direction and directed onto a red-sensitive photomultipier tube (PMT) (R3896, Hamamatsu) mounted at a non-descanned epi- position. In front of the CARS PMT, a combination of a short pass filter and a bandpass filter (ET750sp-2p8, H660/20, Chroma Technology) were used to block the Pump and Stokes beams and any induced two-photon fluorescence.

Fluorescence imaging: Two-photon excited fluorescence images of Nile red or BODIPY stained *C. elegans*, and PAT protein transfected or BODIPY stained 293 cells were collected in the same setup used for the SRS microscopy measurements by using only the 1064 nm Stokes beam. The two-photon fluorescence emission was detected in the backward direction by a PMT (R3896, Hamamatsu) equipped with two filters (ET750sp-2p8, H580/30 (Nile Red), 550/30 (YFP) or 510/20 (BODIPY), Chroma Technology). The fluorescence stack was first recorded (with the 817 nm beam was blocked), followed by the corresponding SRS stack of the same specimen by unblocking the 817 nm beam.

Strains: N2 Bristol was used as the wild-type strain. We have also used the following *C. elegans* strains and mutant alleles: *daf-2(e1370)III*, *nre-1(hd20)lin-15b(hd126)X*, and *mgIs[ges-1p::K04A8.5::gfp]*.

RNAi feeding and screening: RNAi bacteria were cultured 12 -14 h in LB with 50 μg/mL Carbenicillin and seeded onto 12-well agar plates containing 5 mM isopropylthiogalactoside (IPTG). The plates were allowed to dry in a laminar flow hood and incubated at room temperature overnight to induce dsRNA expression. Approximately 20 - 30 synchronized worms at the first larval stage were placed into each well of the RNAi bacteria containing plates. The worms were kept at 20°C for 4 days until day-2 adulthood. Adult worms were washed off plates using M9 buffer with 0.1% sodium azide and transferred into 8-well chambered microscope cover glasses. Fat storage phenotype was imaged and screened by the SRS microscopy system.

SRS and CARS quantification: For each genotype, approximately 15 anesthetized animals were mounted on a microscope slide with a centered agar pad. Five images were randomly captured as described in the section "SRS imaging" and "CARS imaging". 20 × air objective has larger depth of view, and adult worms are ~ 90μm in diameter and further flattened when mounted onto slides. Thus projection images were captured instead of taking 3-D sections, which greatly accelerated the analysis process. Using Image J, mean intensity of SRS or CARS signals in the animal anterior was calculated and subtracted from the mean intensity in the signal free area. The average of five images was presented.

TLC-GC quantification: ~ 5×10^3 adult worms were washed off large regular worm plates using M9 buffer, filtered through 35 μm nylon mesh to remove eggs and L1 larvae, and

placed onto empty plates for 20 min at room temperature to clean up undigested bacteria in the gut. The animals were collected into 400 μL of PBS and homogenized through grinding followed by sonication. 50 μL of homogenate was utilized to measure protein concentration, and the other 250 μL of samples were used for lipid extraction with 5 mL of Chloroform: Methanol (1:1) overnight at 4°C. The extract was washed with 1 mL of 0.15 M KCl and lipids were recovered in the chloroform phase and dried under nitrogen. Add 1 μg of tritridecanoin (C13:0 triglyceride, Nu-Chek Prep) as internal standard. Different lipid classes were separated by thin layer chromatography on Silica gel plates, and the triglyceride fraction was recovered for fatty acid transesterization. The resulted fatty acid methyl esters were analyzed by gas chromatography and their levels were normalized to internal standard (C13:0). The triglyceride concentration was calculated based on fatty acid methyl ester levels and further normalized to the protein quantity.

Cell culture: HEK293 cells were grown in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 2 mmol/L L-glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin at 37°C with 5% CO_2 and 95% humidity. The constructs peri-YFP, LSDP5-YFP, and ADFP-YFP were transfected into the 293 cells using FuGene 6 transfection reagent (Roche) according to the manufacturer's instructions. 6 hr after transfection, the cells were incubated with growth medium supplemented with 400 μM oleic acid complexed with bovine serum albumin (BSA). The cell images were captured in DMEM without phenol red using a 60 × water objective, after 12 hr fatty acid supplement. For BODIPY staining, the cells were incubated with growth medium containing 2 μg/mL BODIPY for 60 min and imaged in DMEM without phenol red using a 60 × water objective.