Supplementary information for

Label-free Quantitative Analysis of Lipid Metabolism in Living *Caenorhabditis elegans*

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Identification and analysis of autofluorescent lipid species. We observed both CARS and TPEF signal for each autofluorescent lipid species. However, TPEF signals were significantly higher than CARS signal. Therefore, all autofluorescent lipid species are dominated by TPEF signal which we assigned a blue color. Close examination of each autofluorescent lipid species also reveals an area with CARS signal which we assigned a red color. It is important to note that the ratio between CARS and TPEF signal varies among autofluorescent lipid species. Thus, it might appear from first glance that all autofluorescent lipid species has dominant TPEF (blue) signal, but only some of them have weak CARS (red) signal. Careful observation revealed both CARS and TPEF signal for ALL autofluorescent lipid species. Note the weak contribution from lipids (~2840 cm^{-1}) to the Raman spectra of a typical autofluorescent lipid droplet (**Fig. 1d**, blue curve). It should also be noted that CARS signal is quadratically dependent on the number of CH_2 molecular vibration. Therefore, an autofluorescent lipid species with two-fold fewer lipids would yield four-fold less CARS signal. The nonlinear dependence of CARS signal on molecular concentration could explain the large variation of CARS signal (red) among autofluorescent lipid species.

CARS is a chemically-selective imaging technique. At $\omega_p - \omega_S = 2840\ cm^{-1}$, CARS signal (red) arises from CH_2 molecular vibration. Thus, CARS signal is a reliable measure of CH_2 or neutral lipid concentration. TPEF signal arises only from autofluorescent lipid species. Thus, TPEF signal (blue) is a reliable measure of autofluorescent lipid species concentration. Therefore, we used CARS signal (red) and TPEF signal (blue) to measure the levels of neutral lipids and autofluorescent lipid species, respectively.

We observed the following properties in regard to autofluorescent particles in *C. elegans*: 1) The autofluorescent particles contain lipids identifiable with CARS imaging. 2) Genetic deletion of sterol regulatory element binding protein, a transcription factor controlling lipid synthesis, suppresses expression of the autofluorescent particles. And 3) Genetic deletion of superoxide dismutase, an anti-oxidant enzyme, leads to an increase in expression of the autofluorescent particles. Our observations are consistent with several previous studies where autofluorescent particles were identified and associated with lipids, oxidative stress, and lifespan of C. elegans (Clokey et al. & Hosokawa et al.).

References

Clokey, G.V. & Jacobson, L.A. The autofluorescent lipofuscin granules in the intestinal-cells of Caenorhabditis elegans are secondary lysosomes. *Mech. Ageing Dev.* **35**, 79-94 (1986).

Hosokawa, H., Ishii, N., Ishida, H., Ichimori, K., Nakazawa, H. & Suzuki, K. Rapid accumulation of fluorescent material with aging in an oxygen-sensitive mutant Mev-1 of Caenorhabditis elegans. *Mech. Ageing Dev*. **74**, 161-170 (1994).

Supplementary movie S1. 3-D distribution of neutral (red/white) and oxidative lipid species (blue) in a living larval L2 *C. elegans*. Movie is a composite of 25 frames at 1 μm interval along vertical axis taken with simultaneous CARS and TPEF imaging.

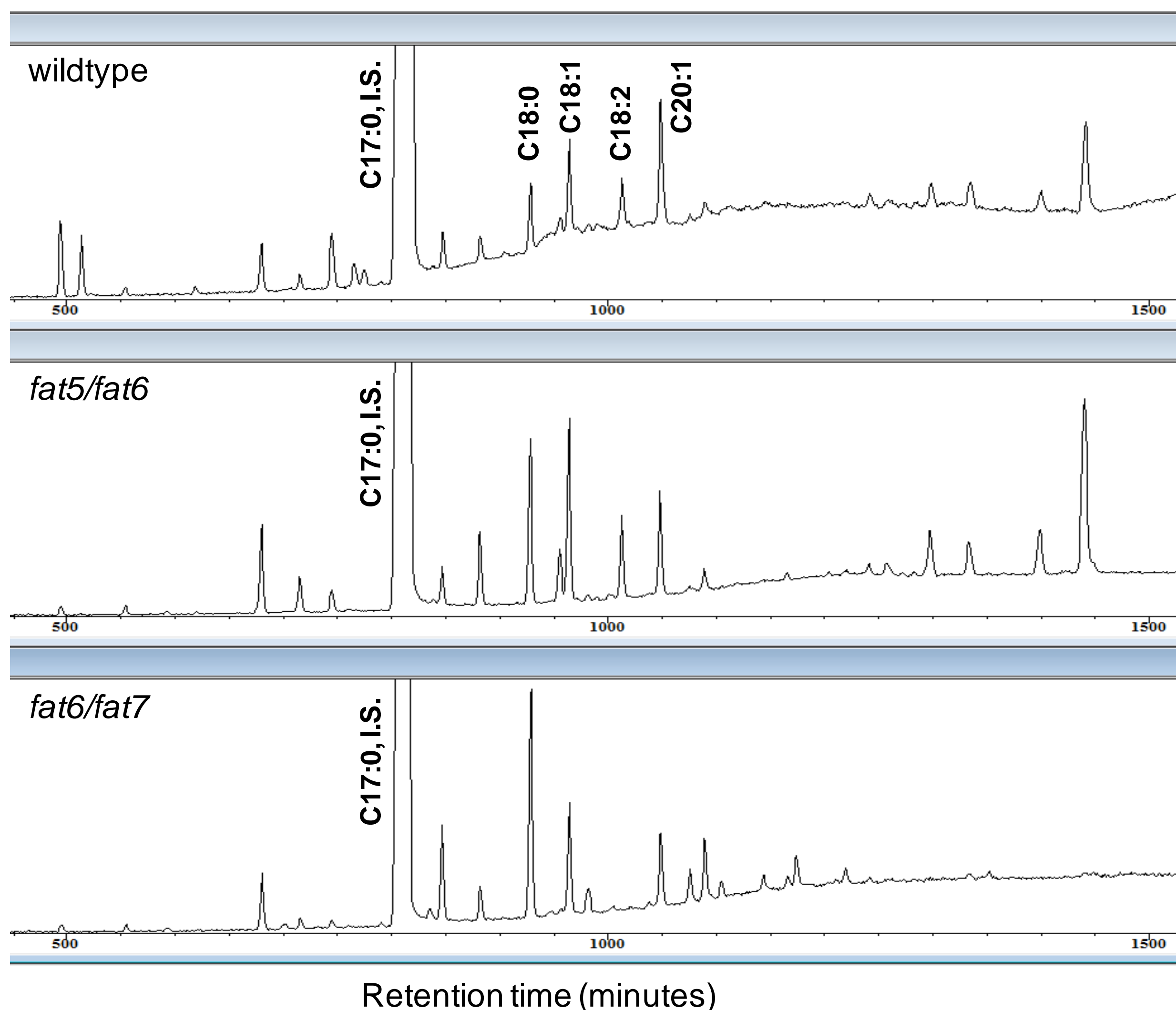

Figure S1. GC-MS analyses of fatty acid methyl esters in wildtype and mutant *C. elegans*. Total lipids are extracted using previously described protocols. Lipids extracts were spiked with heptadecanoic acid C17:0 for internal standard (I.S., Cat. No. 51633, Sigma-Aldrich, St Louis, MO). GC-MS was performed using a ThermoQuestGCQ mass spectrometer (ThermoFinnigan, San Jose, CA). Fatty acid methyl esters were separated using a capillary column DBWAX (J&W Scientific, Folsom, CA). Gas chromatography was programmed for an initial temperature of 120°C for 1 minute followed by an increase of 10°C per minute to 190°C followed by an increase of 2°C per minute to 200°C. Mass spectrometry was performed using electron energy of 70eV, ion source temperature of 200°C, isobutane as chemical ionization gas, and injector temperature of 250°C. Fatty acid methyl esters extracted from *C. elegans* are identified by cross-referencing with the retention times and molecular weights of known fatty acid methyl ester mixture (Supelco, Bellefonte, PA, Cat. No. 18919-1AMP) analyzed with the same GC-MS instruments and experimental conditions (data not shown).

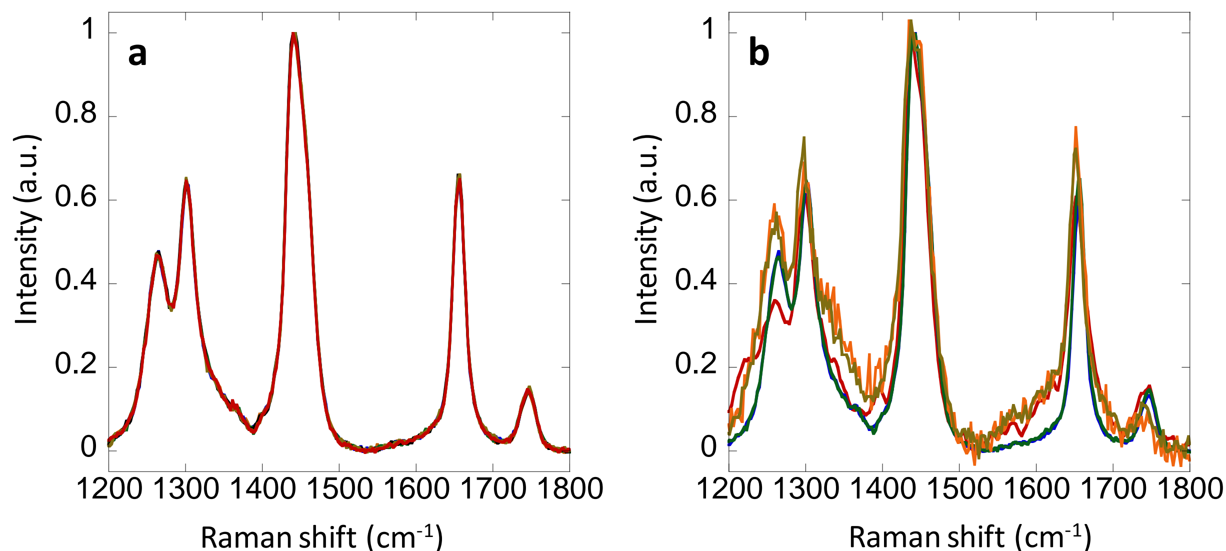

Figure S2. Variability in Raman spectra of neutral lipid droplets in wildtype *C. elegans*. (**a**) Overlay of 6 Raman spectra from 6 different neutral lipid droplets within a single wildtype worm. No difference in Raman spectra is observed between neutral lipid droplets within a living worm. (**b**) Overlay of 6 Raman spectra of neutral lipid droplets from 6 different wildtype worms. Slight variability is observed in the peak intensity ratio I_{1660}/I_{1445}, which measures lipid-chain unsaturation, among lipid droplets from different worms.

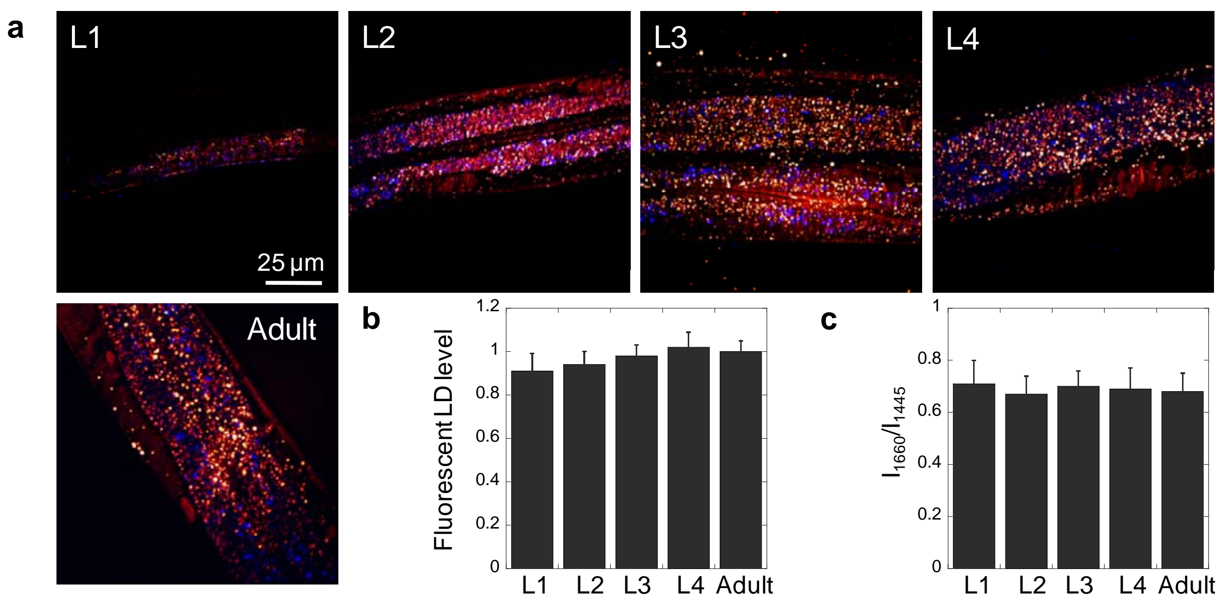

Figure S3. Expression level of autofluorescent lipid species and lipid-chain unsaturation as functions of developmental stages. (**a**) CARS (red) and TPEF (blue) imaging of wildtype *C. elegans* at larval L1, L2, L3, L4, and adult stages. L2 and L3 images show 2 side-by-side worms. All images are presented as stacks of ~25 frames taken at 1 um interval along vertical axis. (**b**) Expression level of autofluorescent lipid species as a function of developmental stages. Expression levels are normalized to 1 for adult and comparatively larval *C. elegans*. Error bars represent distribution across 6 worms analyzed. (**c**) Lipid-chain unsaturation as a function of developmental stages. Error bars represent distribution across 9 lipid droplets measured in 3 worms at each developmental stage.