Supporting Information

In Vivo Metabolic Fingerprinting of Neutral Lipids with Hyperspectral Stimulated Raman Scattering Microscopy

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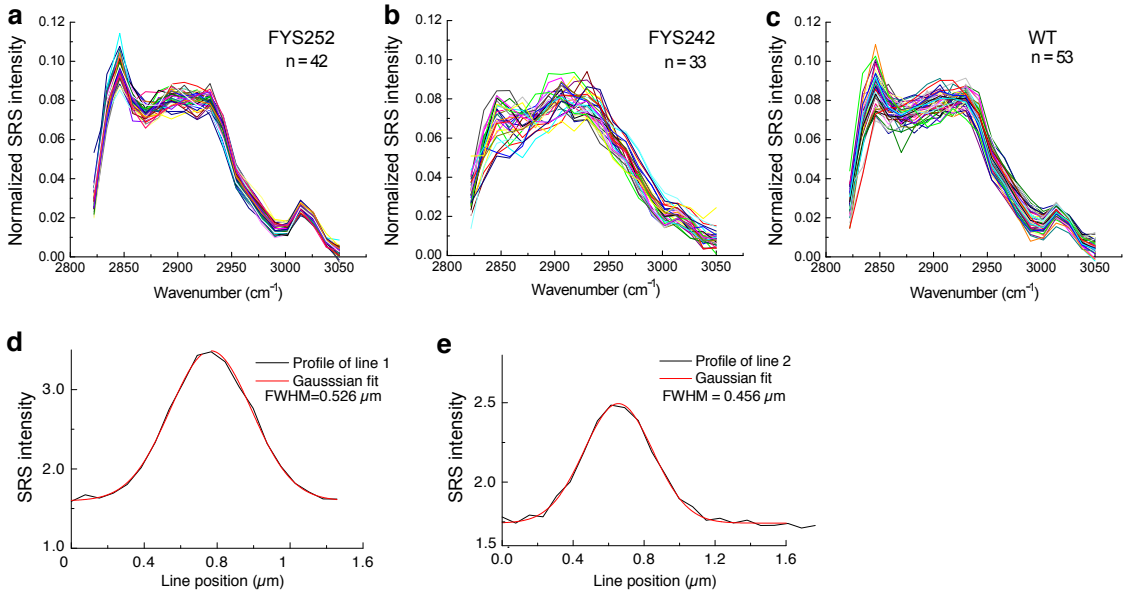

Figure S1. Spectra of individual yeast LDs by hsSRS.
(a-c) Spectra of individual LDs in FYS252 (a), FYS242 (b) and wild-type (c) yeast strains. (d and e) SRS intensity line profiles of two representative droplets and their Gaussian fitting. Assuming a cubic relationship between the SRS intensity and the size of the droplets, we estimated the system lateral resolution to be around 380 nm. Most of the LDs analyzed in yeast cells fall in the 200 ~ 400 nm size range

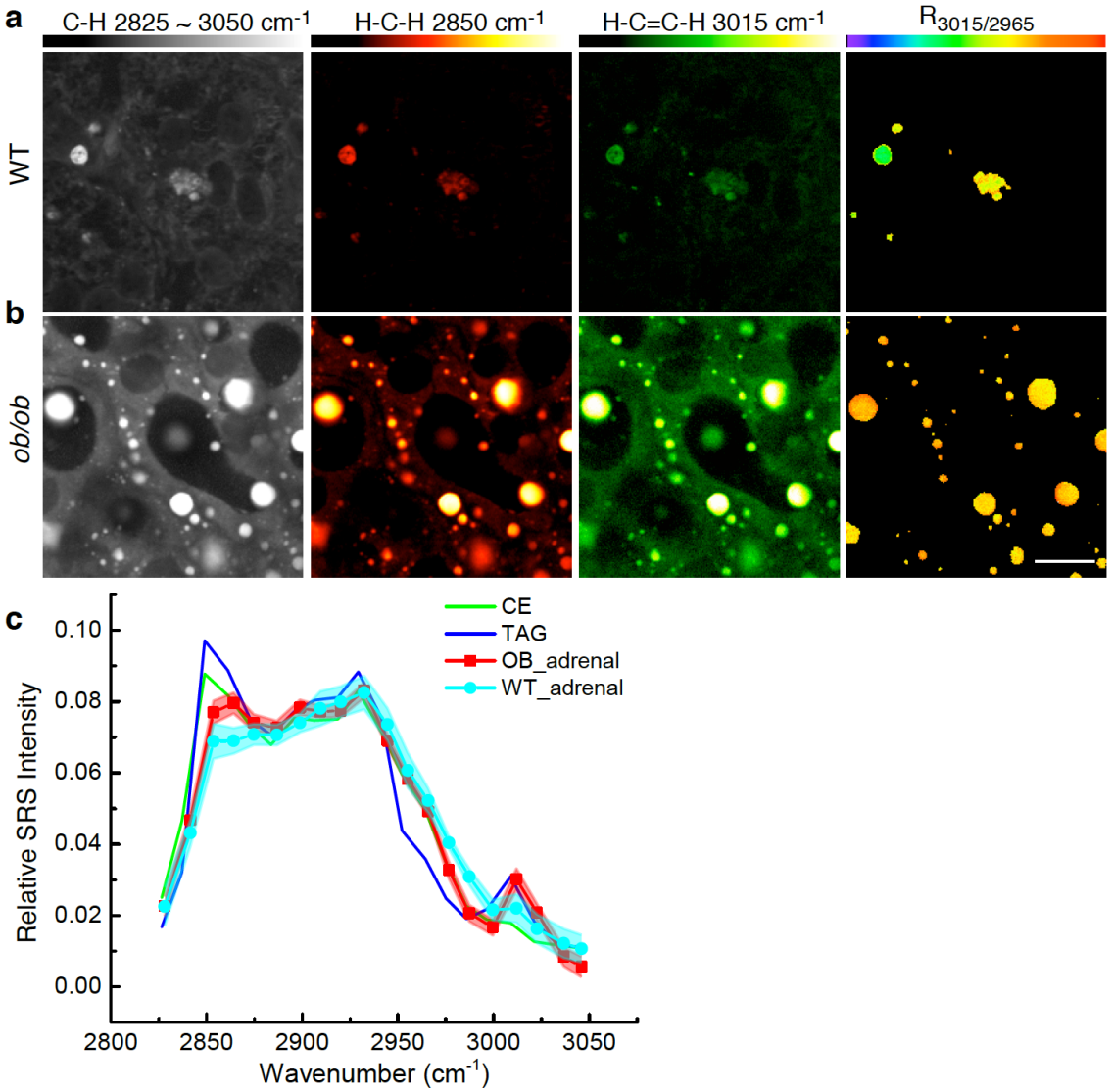

Figure S2. Visualization of lipid compositional changes associated with obesity in the adrenal gland.

(a-b) Different lipid phenotypes of the adrenal gland in wild-type (WT) (a) and *ob*/*ob* (b) mice were visualized with hsSRS. The maximum intensity projection of 20 slices from 2825 cm^{-1} to ~ 3050 cm^{-1} show the overall tissue morphology. The H-C-H bonds in all fatty acyl chains and the C=C-H bonds in unsaturated fatty acyl chains were visualized at 2850 cm^{-1} and 3015 cm^{-1}, respectively. The $R_{3015/2965}$ images reveal predominant distribution of CE in the adrenal gland of WT mouse and the $R_{3015/2965}$ levels are increased in the ob/ob mouse. Scale bar =10 μm. (c) The average spectra of LDs in the adrenal gland of WT and ob/ob mice show lipid compositional changes associated with obesity. The spectra of WT and the ob/ob mouse are both similar to that of pure CE. However the signal at 3015 cm^{-1} is dramatically increased in the ob/ob mouse. Shading along the line represents the standard deviation.

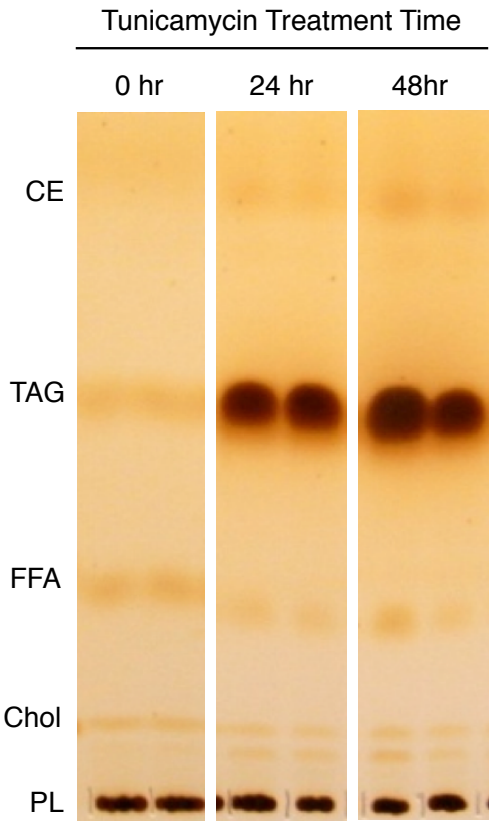

Figure S3. TLC results confirm increased levels of TAG upon ER stress.

Wild-type mice were injected with tunicamycin to induce ER stress in the liver. Total lipids were extracted from the liver at 0 hour, 24 hours and 48 hours of the treatment, and analyzed using TLC. TAG levels are dramatically increased after the treatment, while CE levels remain largely unchanged. CE: cholesteryl esters; TAG: triacylglycerols; FFA: free fatty acids; Chol: cholesterol; PL: phospholipids.

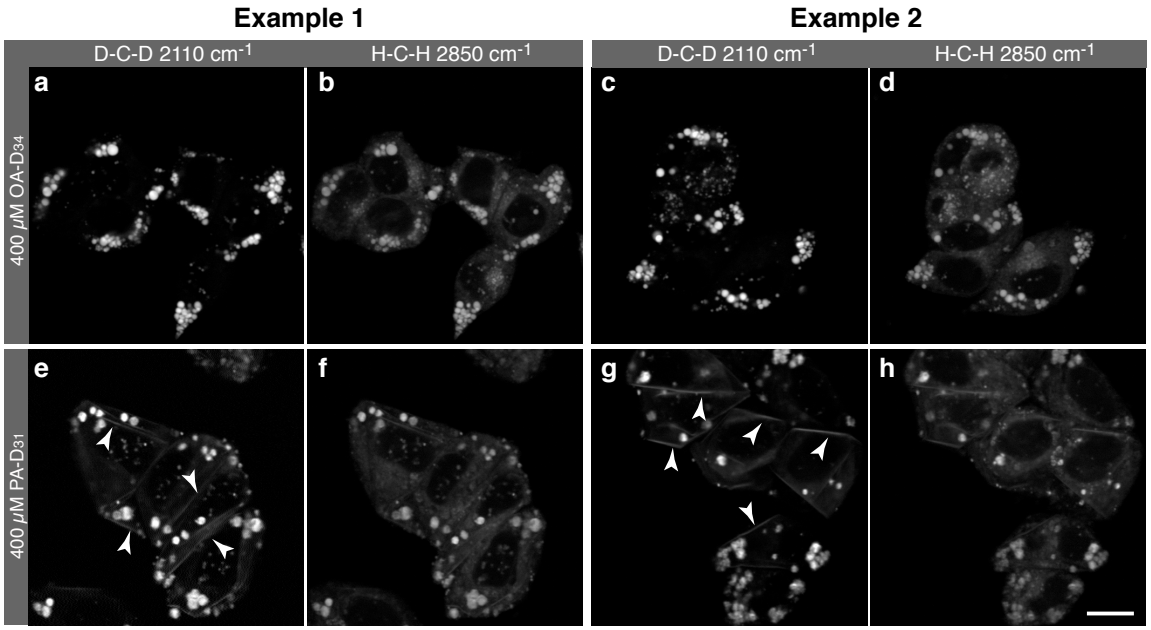

Figure S4. Abnormal membrane-like structures caused by saturated fatty acid supplementation.

McA-RH7777 hepatic cells were fed with either 400 μM of oleic acid-D_{34} (OA-D_{34}, a-d) or palmitic acid-D_{31} (PA-D31, e-h) for 7 hours and then imaged with hsSRS in the C-D region for deuterated fatty acids (a, c, e, g) and in the C-H region for total lipids (b, d, f, h). Arrowheads indicate abnormal membrane-like structures, which are only present in PA-D_{31} supplemented cells. For each condition, two examples are shown. Scale bar = 10 μm.

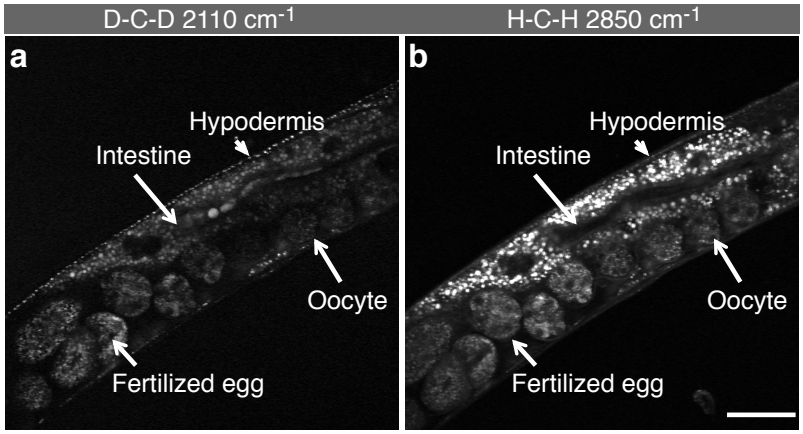

Figure S5. Transportation and incorporation dynamics of fatty acids in *C. elegans*.

Day-1-old wild-type *C.elegans* were supplemented with OA-D_{34} for 5 hours, and imaged by hsSRS. (a) C-D signals at 2110 cm^{-1} reveal OA-D_{34} distribution in different tissues. (b) C-H signals at 2850 cm^{-1} show total lipid distribution. Scale bar = 20 μm.

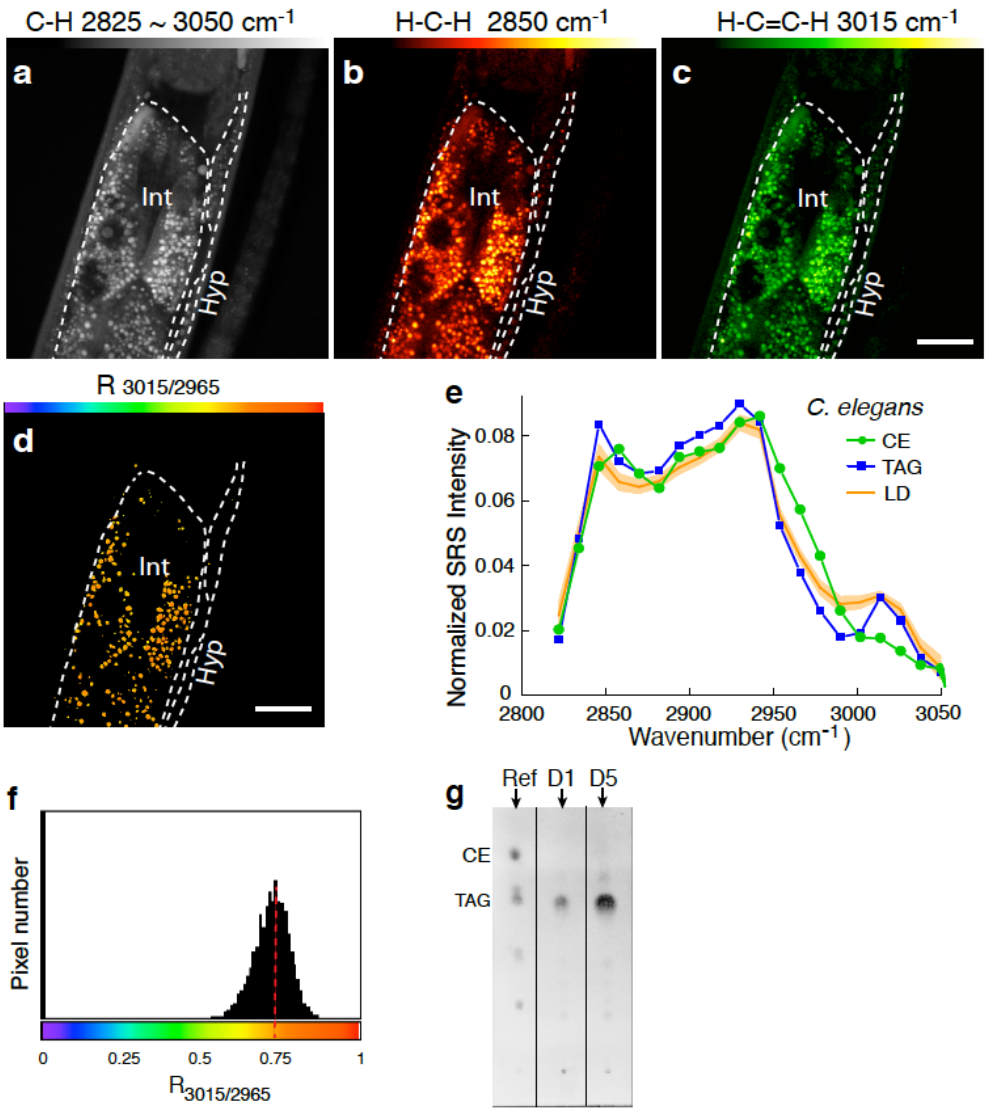

Figure S6. Visualization of predominat TAG distribution in *C. elegans* LDs by hsSRS.

(a-c) Different lipid phenotypes were acquired in live wild-type *C.elegans* adults by hsSRS. Maximum intensity projection of 20 slices from 2825~3050 cm^{-1} shows the overall morphology (a). H-C-H bonds in all fatty acyl chains (b) and H-C=C-H bonds (c) in unsaturated fatty acyl chains were visualized at 2850 cm^{-1} and 3015 cm^{-1}, respectively. LDs were detected in both intestinal (Int) and hypodermal (Hyp) cells. (d) The $R_{3015/2965}$ image reveals that *C. elegans* LDs contain predominantly TAG. (e) The average spectrum of LDs in *C. elegans* is close to that of pure TAG. (f) $R_{3015/2965}$ signals show a distribution with a peak at 0.75 that is the same as pure TAG. (g) TLC results verify predominant TAG distribution in *C. elegans*. Reference standard (Ref), total lipid extracts of day-1 and day-5 wild-type adults (D1 and D5) are shown in the TLC image. Scale bar = 20 μm.