**Supplementary Materials and Methods**

**Nonlinear optical microscopy equipment.**

Nd:Vanadate laser (picoTrain, High-Q), optical parametric oscillator (OPO; Levante, Emerald OPO, Applied Physics &amp; Electronics Inc.), acousto-optic modulator (12465, Crystal Technology, Inc.), 60X water objective (1.2NA, UPlanSApo, Olympus), microscope (IX71, Olympus). The CARS and SRS signals were separated with a dichroic mirror (Di02-R785-25x36, Semrock Inc.). The CARS signal was detected by a Hamamatsu photomultiplier tube (R2658, Hamamatsu Photonics, Inc.) with a 650 +/-50nm bandpass filter. The Raman loss in the pump beam was detected by a photodiode (FDS1010, Thorlabs, Inc.) and a high O.D. bandpass filter (Semrock, Inc.) was placed in front of the photodiode to block the Stokes beam. The modulated signal from the photodiode was filtered with an electronic bandpass filter (BBP-10.7+, Mini-Circuits, Inc.) to suppress the 76-MHz signal from laser pulsing and low frequency signals from scanning. The filtered voltage signal was demodulated by a homemade lock-in amplifier and sent to the computer for display.

For all imaging, 512 by 512 pixels were acquired for one frame with 10 μs pixel dwell time. The average laser power on the sample was kept constant at 15mW for the Stokes beam. For the pump beam, we used 15mW for imaging normal lipid distribution (817nm) and 20mW for imaging deuterated signals (864nm) to minimize sample photodamage. We also took an off-resonant image (pump 890nm, 35mW) for each individual measurement.

The cellular redox states and SHG signals were measured on a commercial Zeiss laser scanning microscope (LSM510) equipped with a femtosecond laser source (Chameleon, Coherent Inc.). The laser beam was focused onto the cells through a 40X water objective (0.8NA, LUMPlanFl, Olympus). Two photon excited fluorescence of NADH was excited at 740nm and collected with 480nm±50nm bandpass filters in front of the photomultiplier tube (PMT). TPEF of FAD+ was excited at 900nm and collected with 530nm±50nm bandpass filters in front of the PMT. SHG signals of collagen fibers were imaged simultaneously using a 450nm±10nm bandpass filter in front of a second PMT. All images were acquired using 512 by 512 pixels with 6 μs pixel dwell time. We used 10mW at the sample for NADH imaging and 15mW for FAD+ imaging. To calibrate for system performance variations on different dates/times, we acquired images of a freshly-prepared fluorescein solution (0.02 μM at pH7) prior to and following each measurement session as a reference.

Because CARS and ORR measurements were performed on two different microscopes, multi-modal images from identical acini were obtained by using localizing grids at the bottom of each imaging dish. Small differences in CARS and ORR image-pair morphological appearance were observed likely due to differences in imaging dish orientation, image plane depth, and biochemical origins of the optical contrast.

**Cell culture.** PME (PCS-600-010), T47D (HTB-133), and MDA-MB-231 (HTB-26) were obtained from American Type Culture Collection (ATCC). MDA-MB-231 is a triple negative breast cancer cell line and does not express ERα; T47D is an ER positive breast cancer cell line and the ERα is overexpressed both in nuclear and cytoplasmic membranes; and PME cells express normal levels of ERα in the membrane. PME cells were cultured with Mammary Epithelial Cell Basal Medium added with growth kit. Both MDA-MB-231 and T47D breast cancer cells obtained from ATCC were cultured at 37 °C and 5% CO2 in advanced DMEM/F-12 culture medium. The culture medium was regularly changed every two to three days to remove waste and provide the cells with fresh nutrients to grow. The cells were monitored on a daily basis and washed and passaged at 80% confluency. For all the experiments, cells were washed three times with Hanks buffer and detached from flasks using TrypLE. We collected all the cells, centrifuged and re-suspended them in regular culture medium, and counted with a hemocytometer. For lipid dynamic studies, the advanced DMEM culture medium was changed to serum free medium for cell cycle synchronization for 12 hours. Although synchronization was not independently confirmed, serum starvation is a standard protocol that has been shown to induce quiescence and block the cells in phase G0/G1 [1].

**Metabolic measurements.** The power of the laser and the gain on the PMTs were kept constant for all the measurements during the experiments. Before the metabolic measurements, standard fluorescein and olive oil solutions were imaged as reference samples for TPEF and CARS respectively. Glucose and lipid metabolic changes were measured every other day for 12 days. We first excited NADH at 740nm, then excited NAD+ at 900nm to measure the concentration of both components in the same subcellular location. The lipid content was then measured with CARS. We returned to the same acinus structure for each measurement using coordinates etched at the bottom of the gridded imaging dish.

**Hyperspectral SRS Imaging.** The region of interest was identified with regular SRS imaging. The pump laser wavelength was tuned from 803nm to 820nm (0.3nm stepwise) by computer control of the crystal temperature, Lyot filter and cavity length of the OPO. The laser power was monitored by a photodiode detecting the reflection from a cover glass before the scanner box. The power was kept constant for all the wavelengths throughout the experiments. After the scanning process, we obtained an imaging stack that contains the Raman spectral information for each pixel from 2791cm-1 to 3055cm-1.

For hyperspectral SRS stacks, we used vertex component analysis (VCA) to retrieve the three most prominent spectral features from the images. The VCA algorithm was written in Matlab and discussed in detail in [2]. The three main spectral components were identified as end members and were assigned different colors (red, green and blue in this case). Each hyperspectral cube of each pixel was fitted with a linear combination of the three components and given a corresponding RGB color.

**Spontaneous Raman spectroscopy.** Spontaneous Raman spectra were acquired with a commercial Raman microscope (InVia Confocal, Renishaw) at room temperature. A 532nm diode laser was focused onto the sample through the 60X water objective (1.2NA, UPlanSApo, Olympus). The laser power was kept at 12mW and the exposure time was 40s in order to acquire spectra from 500cm-1 to 3200cm-1. All the spontaneous Raman spectra were smoothed with a Savitzgy-Golay algorithm and the backgrounds were subtracted with polynomial fitting. The data processing was done in Matlab.

**TPEF image processing.** The fluorescent intensities of NADH and FAD+ were calibrated with the fluorescein reference sample as,

in which, is the calibrated signal, is the direct measured signal intensity, is the minimum intensity in the image, is the mean intensity of the fluorescein sample measured before the experiment and is the mean intensity of the reference sample measured at day 1.

 The ORR optical redox ratio (FAD+/(NADH+FAD+)) was calculated in the cytoplasm regions pixel by pixel. For 3D experiments, the redox ratios of all cells in the entire 3D volume were averaged and stored as a representative redox ratio for the whole acinus. For 2D experiments, the ORRs of all cells in the field of view were averaged.

**SRS imaging pre-processing.** Fast Fourier transforms (FFT) were performed on SRS images (Matlab) to remove the interference from power supply. Each column of the images was treated with 1D FFT and the 60Hz frequency band was eliminated. Then, we removed nonspecific signals by subtracting off-resonant SRS images from SRS images of normal lipid distribution and deuterated signals. The processed images were exported and saved as TIFF files. The background-subtracted normal lipid SRS images and the matching deuterated lipid SRS images were analyzed in ImageJ.

**Collagen Fiber Orientation Quantification.** We implemented the fiber orientation algorithm as described in [3]. In brief, the fiber orientation at each pixel is defined as the weighted circular mean at all possible angles:

where A is the orientation angle of the fiber at the pixel, is the possible angle and is the weight for the vectors at each angle. at each possible angle was determined as the product of weighted intensity variation and weighted vector length:

 and

in which is the weighted intensity variation along the possible angles, is the weighted vector length at the possible angles, L is the vector length and is the standard deviation of a Gaussian function. For all our experiment, = 1.

Next, the boundary of the cells was identified by Otsu thresholding and boundary detection functions provided by Matlab. For each pixel on the boundary, the normal vector was calculated as,

where ( , ) is the coordinate of pixels on the cell cluster boundary. The normal vectors of each pixel on the boundary were compared to the collagen fiber orientation at the same location.

The regions of malignant transformation in the broken acini were manually selected and cropped in ImageJ. The stacks of malignant regions in broken acini, growth arrested regions in broken acini and normal acini structures were analyzed by customized software independently. For each analysis, the orientation differences between the normal vector of the cell boundary and corresponding collagen orientations were stored in a 1D matrix which was used to make the rose plot. Stacks of 5 different acini structures were analyzed and compared.

**Reference**

1. Rosner, M., K. Schipany, and M. Hengstschlager, *Merging high-quality biochemical fractionation with a refined flow cytometry approach to monitor nucleocytoplasmic protein expression throughout the unperturbed mammalian cell cycle.* Nat Protoc, 2013. **8**(3): p. 602-26.

2. Tabarangao, J.T. and A.D. Slepkov, *Mimicking Multimodal Contrast with Vertex Component Analysis of Hyperspectral CARS Images.* Journal of Spectroscopy, 2015. **2015**: p. 8.

3. Quinn, K.P. and I. Georgakoudi, *Rapid quantification of pixel-wise fiber orientation data in micrographs.* J Biomed Opt, 2013. **18**(4): p. 046003.