SUPPLEMENTARY MATERIALS AND METHODS

Nonlinear Microscopy

Freshly isolated adipose tissue was washed with PBS, stained with 50 μM Rhodamine 123 (Life Technologies, Carlsbad, CA) in PBS for 15 minutes at 37°C, washed twice with PBS, and then submerged in PBS on a glass bottom dish (#1.5 glass, uncoated; In Vitro Scientific, Mountain View, CA). A custom built coherent anti-Stokes Raman scattering (CARS) and two-photon excited fluorescence (TPEF) microscope was used to visualize lipids and active mitochondria within the adipose tissue. A pulsed laser (Nd:Vanadate, 2 ps, 76 MHz; PicoTrain High Q Lasers, Rankweil, Austria) generated a fundamental 1064 nm beam and a 532 nm beam. The latter was coupled into an optic parametric oscillator (Levante Emerald OPO; APE, Berlin, Germany) to generate a 817 nm beam that was then overlapped in time and space with the fundamental beam in the focus of an inverted microscope (Eclipse TE2000-E with C2 confocal scanning head; Nikon, Tokyo, Japan). Tight focus was ensured with an oil immersion objective (40x, NA 1.30; Nikon). The samples were kept at 37°C in the focus plane using an on-stage-incubator (Okolab, Ottaviano, Italy). Lipids were visualized via the 2845 cm\(^{-1}\) symmetric CH\(_2\) stretching vibration and the CARS signal was collected above the sample. The TPEF signals of Rhodamine 123 (ex.: 817 nm, em.: 495-530 nm) were collected in epi direction. All signals were recorded using single photon counting detectors (Becker&Hickl GmbH, Berlin, Germany). For each sample, multiple 3D z-stacks were collected to ensure that each cell was analyzed at its center plane. The analysis of lipid droplets from CARS images has been described in detail previously (Brannmark, et al. 2014). In brief, single cells were selected at their center plane, their area was measured, and the lipid/mitochondria signals were split, thresholded, and the covering area in relation to the full cell area was determined. In addition to
the individual quantification of lipid content and mitochondrial activity, a metabolic activity index was determined from the ratio of the mitochondrial activity to the lipid content as a reflection of the metabolic activity of the tissue.

**Oil Red O Staining and Quantification of Lipid Accumulation in HIB-1B Cells**

HIB-1B cells cultured in 24-well plates (Grainer Bio-One, Frickenhausen, Germany) were fixed for 20 minutes in 4% v/v phosphate-buffered formaldehyde and incubated with 0.5% w/v Oil Red O (Sigma-Aldrich, St. Louis, MO) in 60% v/v isopropanol for 30 minutes at room temperature. The Oil Red O-stained area was assessed in 2-3 randomly selected 200x fields per well in 5-10 wells per test condition using the ImageJ software (1.47v; Wayne Rasband, National Institutes of Health, Bethesda, MD).

**Mitochondrial Staining in HIB-1B Cells**

HIB-1B cells cultured in 4-well chamber slides (Sarstedt, Numbrecht, Germany) were stained with 50 nM MitoTracker Red (Invitrogen, Carlsbad, CA) for 20 minutes at 37°C. The cover slip was mounted using Prolong Gold Antifade Mountant (Invitrogen). Mitochondrial area was assessed in 3-4 randomly selected 200x fields per well in 8 wells per test condition using the ImageJ software.

**β-Oxidation in HIB-1B Cells**

β-oxidation was measured as previously described (Nerstedt, et al. 2012). In brief, radiolabeled palmitate ([9,10-3H(N)]-palmitic acid; PerkinElmer, Waltham, MA) was evaporated and then dissolved in palmitate (Sigma-Aldrich) and DMEM (Lonza, Basel, Switzerland) containing 1
g/l (5.5 mM) glucose and 1% v/v fatty acid-free bovine serum albumin (BSA; Sigma-Aldrich) to give a final concentration of 0.5 µCi/ml [9,10-3H(N)]-palmitic acid and 110 µM palmitate. HIB-1B cells cultured in 24-well plates were incubated with the palmitate solution for 2 hours at 37°C and medium was collected. The remaining labeled palmitate in 0.5 ml medium was precipitated by adding 50 µl 20% w/v BSA and 27 µl 70% v/v perchloric acid followed by vortexing and centrifugation. 50 µl 20% w/v BSA was added to the supernatant, followed by vortexing and centrifugation, and this step was repeated once. The radioactive water formation as the product of free fatty acid oxidation was quantified in a liquid scintillation counter (Tri-Carb 2800TR; PerkinElmer).

**NEFA Uptake in HIB-1B Cells**

HIB-1B cells were cultured in black 96-well plates (Grainer Bio-One) and fatty acid uptake was measured using the QBT Fatty Acid Uptake Assay Kit (Molecular Devices, Sunnyvale, CA) following the manufacturer’s instructions.

**Incorporation of [14C]-Oleate into triacylglycerol (TAG) in HIB-1B Cells**

HIB-1B cells cultured in 24-well plates were incubated with culture media containing 0.5 µCi/ml [14C]-oleic acid (PerkinElmer), 360 µM oleic acid, and 1% v/v fatty acid-free BSA for 1.5 hours. Cells were collected for lipid extraction, followed by lipid separation by thin-layer chromatography on silica gel plates. Radiolabeled TAGs were detected by iodine vapor and quantified by a scintillation counter.
**Lipolysis in HIB-1B Cells**

HIB-1B cells were cultured in 24-well plates and stimulated with 10 nM isoproterenol for 2 hours. The concentration of glycerol in culture medium was measured using Glycerol Assay Kit (Sigma-Aldrich).

**References**
