Cells were then lysed and analyzed by immunoprecipitation for the crosslinked proteins as described above.

Statistical analysis

Sample size, mean, and significance p values are indicated in the text and figure legends. Error bars in the experiments represent standard deviation (SD) from either independent experiments or independent samples. Statistical analyses were performed using GraphPad Prism 7, or reported by the relevant computational tools.

Table S1: Differential gene scores for genetic screens, Related to Figures 1, 2 and 6.

Table S2: Proteomics data for CHP1 and GPAT4 co-immunoprecipitation, Related to Figure 4.

Table S3: Oligonucleotides used in this study, Related to STAR Methods.

Supplemental Methods
Real time PCR assays

For HeLa cells, RNA was isolated using the RNeasy Kit (Qiagen) according to the manufacturer's protocol. For 3T3-F442A cells, cells were lysed in-well with 0.5 mL of Trizol (Life Technologies) and RNA was isolated using manufacturer's protocol. RNA was spectrophotometrically quantified and equal amounts were used for cDNA synthesis with the Superscript III RT Kit (Invitrogen). qPCR analysis was performed on a ABI Real Time PCR System (Applied Biosystems) with the SYBR green Master Mix (Applied Biosystems). *Rpl32* was used as control for HeLa while *Tbp* was used as control for 3T3-F442A. Primer sequences are indicated in **Table S3**.

Polar metabolite profiling for palmitoyl-CoA quantification

Jurkat cells (3 x 10^6 cells per replicate) were cultured as triplicates in 6-well plates and treated for 24 hrs with the indicated conditions prior to collection of cells and washing with 1 mL of cold 0.9% NaCl. Polar metabolites were extracted in 0.5 mL of cold 80% methanol containing internal standards (Cambridge Isotope Laboratories). After extraction, samples were nitrogen-dried and stored at -80C until analysis by LC-MS. Analysis was conducted on a QExactive benchtop orbitrap mass spectrometer equipped with an Ion Max source and a HESI II probe, which was coupled to a Dionex UltiMate 3000 UPLC system (Thermo Fisher Scientific). External mass calibration was performed using the standard calibration mixture every 7 days.

Dried polar samples were resuspended in 100 µL water and 2 µL were injected into a ZIC-pHILIC 150 x 2.1 mm (5 µm particle size) column (EMD Millipore). Chromatographic separation was achieved using the following conditions: Buffer A was 20 mM ammonium carbonate, 0.1% ammonium hydroxide; buffer B was acetonitrile. The column oven and autosampler tray were held at 25℃ and 4℃, respectively. The chromatographic gradient was run at a flow rate of 0.150 mL/min as follows: 0–20 min.: linear gradient from 80% to 20% B; 20–20.5 min.: linear gradient from 20% to 80% B; 20.5–28 min.: hold at 80% B. The mass spectrometer was operated in full-scan, polarity switching mode with the spray voltage set to 3.0 kV, the heated capillary held at 275℃, and the HESI probe held at 350℃. The sheath gas flow was set to 40 units, the auxiliary gas flow was set to 15 units, and the sweep gas flow was set to 1 unit. The MS data acquisition was performed in a range of 70–1000 m/z, with the resolution set at 70,000, the AGC target at 10e6, and the

maximum injection time at 20 msec. Relative quantitation of polar metabolites was performed with XCalibur QuanBrowser 2.2 (Thermo Fisher Scientific) using a 5 ppm mass tolerance and referencing an in-house library of chemical standards. Metabolite levels were normalized to the total protein amount for each condition.

Seahorse fatty acid oxidation assay

HeLa cells were seeded into Seahorse XF 96-well plates at 30,000 cells/well. Seahorse fatty acid oxidation assay was carried out following manufacturer's protocol using the Seahorse XF Palmitate-BSA fatty acid oxidation (FAO) Substrate (Agilent) on a Seahorse XFe96 Analyzer (Agilent). Maximal respiration due to exogenous fatty acids was calculated as the maximal Palm:BSA-Eto rate minus maximal BSA-Eto rate minus oxygen consumption rate due to uncoupling by fatty acids.

Immunofluorescence

For immunofluorescence assays in 6-well plates, 150,000 cells were seeded on coverslips previously coated with fibronectin. 12 hours later, cells were treated with 1 mM oleate or control BSA. Subsequently, cells were stained with 1 μM ER-Tracker Blue-White DPX in HBSS for 30 min at 37℃ before fixing for 15 min with 4% paraformal dehyde in PBS at room temperature. After three washes with PBS, cells on the coverslips were permeabilized by incubation with 0.2% Triton X-100 in PBS for 10 min prior to another three PBS washes. After incubation with blocking solution (5% normal donkey serum in PBS) for 1 hour, coverslips were incubated with the indicated primary antibodies (1:500 in blocking solution) for an additional hour, and washed 3 times with PBS. Secondary Alexa Fluor 488 donkey anti-rabbit antibody was diluted in blocking solution (1:250) and added to the coverslips for 45 min at room temperature, prior to three washes with PBS. Coverslips were finally mounted onto slides with Prolong Gold antifade mounting media (Invitrogen). Images were taken on an inverted LSM 780 laser scanning confocal microscope (Zeiss).

Click chemistry

Immunoprecipitation of the FLAG-CHP1 constructs were carried out as described above. Before the elution step, beads were incubated with 22 μL lysis buffer with 3 μL freshly prepared click chemistry reaction cocktail (azido-rhodamine (0.55 μL, 10 mM stock solution in DMSO), tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (0.55 μL, 50 mM freshly prepared stock solution in deionized water), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (1.36 μL, 10 mM stock solution in DMSO/t-butanol) and $CuSO_4 \cdot 5H_2O$ (0.55 μL, 50 mM freshly prepared stock solution in deionized water)) at room temperature for 1 h. Beads were then eluted and analyzed by SDS-PAGE as described above. Gel was scanned on a GE Healthcare Typhoon 9400 variable mode imager with excitation and emission at 532 nm and 580 nm, respectively. The same gel was then transferred onto a PVDF membrane for downstream Western blotting analysis.