Supplementary Methods

Treatment

Mice bearing established tumors were randomly allocated to the treatment groups. The anti-VEGF monoclonal antibody B20.4-1.1 (Genentech) was administered by i.p. injection twice per week at 5 mg/kg, unless otherwise noted. Chemotherapy with 5-fluorouracil (5-FU, Selleck) was administered via i.p. injection twice per week at a dose of 50 mg/kg. β-aminopropionitrile (BAPN, Sigma-Aldrich) was administered by oral gavage twice per week at 100 mg/kg. Etomoxir (Selleck) was administered via i.p. injection twice per week at a dose of 15 mg/kg. PF-573228 (Selleck) was administered by oral gavage twice per week at a dose of 5 mg/kg. Verteporfin (Selleck) was administered via i.p. injection twice per week at a dose of 10 mg/kg.

Haematoxylin and Eosin (HE), Masson's trichrome staining, and Picrosirius Red staining

Tumor tissues were 10% formalin-fixed (24h), paraffin-coated, sliced into 4 μm segments, with consequent staining through Hematoxylin & Eosin, Masson's trichrome and Picrosirius Red (all Solarbio) in line with individual kit protocols. Stained slices were captured through a slide-scanning imaging platform (Shengqiang). Morphometric analysis of hepatic fibrosis was conducted blindly. Through 5-randomized-field-determinations per slide, the fibrillar collagen-stained area per total area was measured using ImageJ software.

Immunohistochemical (IHC) analyses

Tissue samples were 4% formaldehyde-fixed (over-night), with consequent paraffin. Serial sections (4 μm) were incubated with horseradish peroxidase (HRP)–labelled polymer anti-rabbit or anti-mouse antibody, combined with 3,30-diaminobenzidine (DAKO). Images were obtained using a slide scanning imaging system (Shengqiang). For CPT1A immuohistochemical staining, the staining intensity of each section was scoredas 0 (negative), 1 (weak), 2 (moderate), or 3 (strong), and the staining extent depended on the positive percentage of the stained area was scored as 0 (0%), 1 (1%-25%), 2 (26%-50%), 3 (51%-75%), or 4 (76%-100%). The product of the above two values was the final staining score (0-12) of each slice for CPT1A expression.

Immunofluorescence (IF) on tissue samples Tumor tissues were 10% formalin-fixed (24h), paraffin-coated, sliced into 4 μm segments, deparaffinized with xylene, rehydrated with ethanol. 0.3% hydrogen peroxide in methanol was used to inhibit endogenous peroxidase. After being permeabilized with 0.5% Triton X-100 for 30 min

and three washing steps, samples were blocked in 10% bovine serum albumin (BSA) for 1 h and incubated overnight at 4 °C with primary antibody CD31 (Servicebio), followed by incubation with Cy3-labeled goat anti-rabbit secondary antibodiy for 90 min at room temperature with protection from light. DAPI (Beyotime) was used to stain cell nuclei. Images were obtained using a confocal microscope (Nikon). The density of vessels was calculated with ImageJ software.

Bioluminescent in vivo imaging

The tumors were assessed using bioluminescent in vivo imaging on day 28 after the construction of the liver metastasis model. Briefly, the tumor-bearing mice were i.p. injected with Beetle Luciferin Potassium Salt (Promega) in vivo grade solution (150 mg/kg). Within 10–15 min after injection, bioluminescent signals were captured using an in vivo imaging system (Thermo Fisher Scientific).

Co-culture

HSCs and colon cancer cells were co-cultured in two ways. The first method was by mixing CFSE-labelled HSCs with colon cancer cells and seeding them on polyacrylamide gels in 6-well cell culture plates. Specifically, HSCs were suspended and washed by PBS twice to remove excess FBS, then labelled with 1μmol/L CFSE (Selleck) in PBS at 37 °C in the dark for 10 min. After washing with 2% FBS medium, the CFSE-labelled HSCs were mixed with an equal number of colon cancer cells and co-cultured for 48 h. The cells were then isolated by fluorescence activated cell sorting (FACS) and processed for further analysis. The second method was using a Transwell system (Corning). To test whether lipid transfer required direct cell-cell contact, HSCs on polyacrylamide gels in the lower well were co-cultured with colon cancer cells in the upper well of the Transwell system for 48 h.

Conditioned medium

HSCs were cultured until confluency and then for 48 h in antibiotic-free, 1% FBS media. The supernatant was collected and filtered using a 0.22 μm filter. The colon cancer cells were cultured in 1% FBS medium for 24 hours before adding the HSC-filtered supernatant.

Lipid transfer experiments

HSCs were seeded onto polyacrylamide gels in 6-well cell culture plates. HSCs were treated with either DMSO or the indicated inhibitors for 24 h before switching back to basal medium. Lipids in HSCs were labelled with 5 μM BODIPY C-16 (Invitrogen) in Stellate Cell Medium for 4 h in the dark. Extracellular BODIPY was eliminated from the cells by washing thrice with 1x Hank’s balanced salt solution (HBSS) containing 0.2% fatty-acid-free BSA. After washing, the HSCs on the bottom

of the well the polyacrylamide gels were co-cultured with colon cancer cells on the top, separated by a Transwell (Corning) insert, for 24 h. The colon cancer cells were transferred to confocal dishes and incubated for 6 h. The cells were fixed and imaged using a confocal microscope (Nikon). BODIPY in colon cancer cells was quantified using ImageJ software by calculating total fluorescence intensity.

Ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS)

The freshly collected conditioned medium, which was derived from LX-2 and JS-1 cells treated as indicated, was immediately stored at −80 °C until use. Targeted metabolomics was performed using Metabo-Profile. The samples were thawed in an ice -bath to diminish sample degradation. 20μL of the medium sample was added to a 96-well plate and transferred to an Eppendorf EpMotion Workstation (Eppendorf Inc., Hamburg, Germany). Ice-cold methanol (180 μL) with partial internal standards was automatically added to each sample and vortexed vigorously for 5 min. The plate was centrifuged at 4000×g for 30 min (Allegra X-15R; Beckman Coulter, Inc., Indianapolis, IN, USA) and returned to the workstation. Forty microlitres of the supernatant was transferred to a clean 96-well plate, and 40μL of freshly prepared derivative reagents was added to each well. The plate was sealed and the derivatization was carried out at 30 °C for 60 min. After derivatization, 420 μL ice-cold acetonitrile was added to dilute the sample. Then the plate was stored at -20 °C for 20 min and followed by 4000×g centrifugation at 4 °C for 30 min. The supernatant (135 μL) was then transferred to a new 96-well plate. Serial dilutions of the derivatised stock standards were added to the left wells. Finally, the plates were sealed for LC-MS analysis. An ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) system (ACQUITY UPLC-Xevo TQ-S, Waters Corp., Milford, MA, USA) was used to quantitate all targeted FFAs. The raw data files generated by UPLC-MS/MS were processed using the MassLynx software (v4.1, Waters, Milford, MA, USA) to perform peak integration, calibration, and quantitation of each metabolite. The self-developed iMAP platform (v1.0, Metabo-Profile, Shanghai, China) was used for statistical analyses, including PCA, OPLS-DA, univariate analysis and pathway analysis. A total of 35 types of medium-and long-chain fatty acids were detected.

Lipid droplet staining

To measure cellular lipid content, HSCs were labelled with 5 μM BODIPY 493/503 (Invitrogen) for 30 min at 37 °C in the dark. After 30 min, extracellular BODIPY was eliminated from the cells by washing thrice with 1x HBSS containing 0.2% fatty-acid-free BSA. After washing, the cells were fixed with 4% paraformaldehyde at room temperature for 30 min and then incubated with DAPI (Beyotime) for 10 min. Finally,

the cells were washed with PBS, observed, imaged using a confocal microscope (Nikon), and signals were quantified using ImageJ software.

Fatty acid oxidation rate assay

Cell mitochondrial proteins (Cpr) were obtained through the Cell Mitochondria Isolation Kit (Beyotime) , with quantification through the BCA assay kit (Fudebio). Fatty-acid oxidation (FAO) rates were determined through Carnitine Palmitoyltransferase 1 (CPT1) Assay Kit (Comin). Briefly, the FAO rate was evaluated spectrophotometrically via interactions across DNTB and CoA-SH, discharged through palmitoyl-CoA. Mitochondrion-derived proteomic content (40 μL) was introduced into 880 μL carnitine + 40 μL palmitoyl CoA within a fresh cuvette, whereby such absorbance was determined (412 nm) through spectrophotometric methods (OD1). Consequently, such solutions were placed into incubation (37 °C and 120s), with a subsequent second absorbance determination under identical conditions (412nm; OD2). Variations across OD read-outs served for quantifying CoA-SH discharge level. CPT1 function was determined through:

$$\text{nmol/min/mg} = 880 \times (OD2 - OD1)/Cpr.$$

The entirety of experimental runs was conducted across three separate occasions.

Cellular ROS analysis

Cultures were harvested and trypsinised for obtaining individual cellular suspensions, followed by 1 μM DCF-DA labelling (Beyotime). Post-incubating (37 °C and 30 min), cellular suspensions were exposed to two PBS-rinse steps, with consequent flow-cytometric analyses.

ATP quantification

Post-cellular lysis contents (20mL) underwent ATP assay kit protocol (Beyotime). These were introduced to ATP-determining diluted solution (10mL), with luminescence registered through SpectraMax M5 luminescence determining platform. Proteomic content intensities were determined through the BCA assay kit (Fudebio). Cellular relative ATP levels were calculated as the ratio of ATP to protein values.

Cellular oxygen consumption rates (OCR) analysis

Cellular OCR assessed FAO actual propensity through the Seahorse XF96 extracellular-flux analyser. DLD1 (30,000 cells per well) or MC38 (20,000 cells per well) cells were plated within a 96-well XF Cell Culture Microplate (Agilent Seahorse). Post-overnight incubation to allow attachment, all media were exchanged with DMEM augmented by glucose (0.5mM), 0.5mM carnitine (0.5mM), glutamine (1mM) together with 1% FBS for 4 h at 37 °C. Forty-five min before the assay, the FAO assay KHB

buffer (sodium chloride (111mM) + potassium chloride (4.7mM) + calcium chloride (1.25mM) + magnesium sulphate (2mM) + sodium hypophosphate(1.2mM)) - augmented through glucose (2.5mM), carnitine (0.5mM), in conjunction with HEPES (5mM)- were refreshed, with pH optimized for 7.4. Baseline OCRs were determined for such an environment, combined to oligomycin (3 μM), carbonyl cyanide-4-trifluoro methoxy phenyl hydrazone (FCCP) (0.6 μM), and rotenone/antimycin A (2 μM) being separately introduced within all wells. Oxygen consumption rates were normalized to protein values, measured after assay completion.

Western blotting

Overall proteomic content was collected from colon-derived tissue samples and cellular suspensions through RIPA lysis buffer, augmented through protease and phosphatase inhibitors (Fudebio). All samples underwent 8–10% SDS-PAGE gel-chromatography and were consequently transported over PVDF membranes (Millipore). Membrane- blocking employed 5% BSA (minimum of 60 minutes at room temperature with consequent incubation with primary antibodies (over-night, 4 °C). Post-TBST-rinse-step (30 min), membranes were incubated with an HRP-conjugated secondary antibody (60 minutes, room temperature. Post-rinsing, the proteins were visualized through X-ray films. The primary antibodies used are listed in Supplementary Table S3.

RNA isolation and quantitative polymerase chain reaction (qPCR) analysis

All transcriptomic contents were collected from cellular samples through FastPure Kit (Vazyme), in line with kit protocol. Reverse transcriptions were conducted through HiScript 1st-Strand cDNA Synthesis Kit (Vazyme). qPCR was conducted through the QuantStudio 6 Flex platform (Thermo Fisher Scientific). Targeted-gene expression profiling was normalized through subtraction of corresponding tuberculosis threshold cycle (Ct) read-out. Primer sequences are listed in Supplementary Table S4.

Cell IF staining Cells on hydrogels were fixed with 4% paraformaldehyde at 4 °C for 30 min. and permeabilized with 0.2% Triton X-100 (Solarbio) for 5 min before IF. After permeabilized with 0.2% Triton X-100 (Solarbio) and blocked with 10% BSA, cells were incubated with primary antibody α-SMA (abcam) at 4 °C overnight. Then, cells were incubated with green fluorescence-conjugated goat anti-rabbit antibody Alexa Fluor 488 (Beyotime) at room temperature for 1 h and then incubated with DAPI (Beyotime) for 10 min. Finally, cells were washed with PBS, observed and captured under a confocal microscope (Nikon), and signals were quantified using the ImageJ software.

Non-Esterified Fatty Acid (NEFA) assay

The supernatant from HSCs was collected and filtered using a 0.22-mm filter. A Non-Esterified Free fatty acids assay kit (Jiancheng, Nanjing) was used to measure the medium NEFA content according to the manufacturer's instructions. 4 μL of double-distilled water, 1.00 mmol/L standards, medium samples were added to a 96-well plate respectively. 200 μL of reagent 1 was added to each well. After incubation at 37 °C for 5 min, the absorbance of the mixtures was measured at 546 nm by spectrophotometry (A1). 50 μL of reagent 2 was added to each well. After incubation at 37 °C for 5 min, the absorbance of the mixtures was measured at 546 nm by spectrophotometry (A2). NEFA content was calculated according to the following formula: △A=A2-A1, NEFA content =(△Asample-△Ablank)/(△Astandards-△Ablank)×1.00 mmol/L.

EdU assay

A Cell-Light EdU Apollo 567 In Vitro Imaging Kit (Ribobio) was used to analyze cell proliferation. All procedures were based on the manufacturer's instructions.

HUVEC tubule formation assay

CMs from HSCs were used to treat colon cancer cells with or without 50μM Etomoxir (Selleck) for 24 h. After washing with PBS twice, colon cancer cells were cultured back in basal medium for another 24 h. The supernatant of colon cancer cells was collected and filtered using a 0.22-mm filter. Tubule formation assay for HUVEC was performed as previously described. Briefly, HUVECs were seeded at a density of 1×10^5 cells/well in 24-well plates. Thereafter, the different CMs from cancer cells were added, and the cells were cultured for 48 h. A μ-Slide Angiogenesis 12-well plate (ibidi) was polymerized by Matrigel (Corning) for 30 min at 37 °C. HUVECs (1×10^4) were incubated in 50μl corresponding CMs with either IgG or 9% Bevacizumab for 12 h before image taking. The capillary tubes were photographed by a 100X bright-field microscope (Nikon) and quantified by measuring the number of nudes. Three independent experiments were required for each treatment.

Chicken chorioallantoic membrane (CAM) assay

CMs from HSCs were used to treat colon cancer cells with or without 50μM Etomoxir (Selleck) for 24 h. After washing with PBS twice, colon cancer cells were cultured back in basal medium for another 24 h. The supernatant of colon cancer cells was collected and filtered using a 0.22-mm filter. Day-6 fertilized chicken eggs (Xinxingdahuanong Breeding) were chosen to perform the CAM assay. To expose the CAM, a window about 1.0 cm in diameter was opened in the eggshell. A sterile rubber ring in 0.5 cm diameter was placed on the CAM before 100 μl CMs from colon cancer cells with indicated treatment was added. The window was closed using a piece of

steriled adhesive tape, and eggs were placed in a 37 °C incubator with 80–90% relative humidity for 3 d. CAMs were fixed by stationary solution (methanol: acetone=1:1) for 15 min before it was cut and harvested. Photos were taken by a digital camera (Cannon) and the effects of CM on angiogenesis were assessed through assessing the density of vessels.