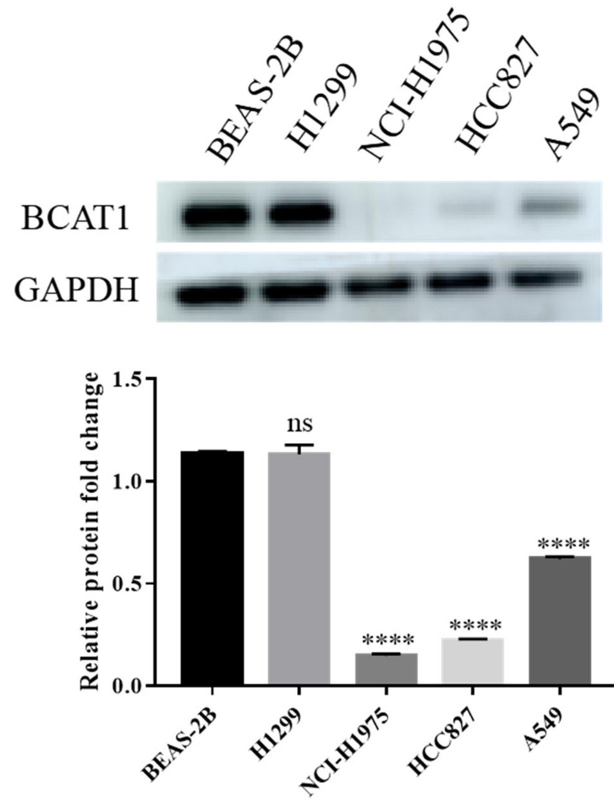

Fig. S1 Western blot analysis of branched-chain aminotransferase 1 (BCAT1) in immortalized normal lung cell line (BEAS-2B) and lung adenocarcinoma (LUAD) cell lines. Data are expressed as mean±SD, *n*=3. ** *P*<0.0001; ^{ns} not significant.**

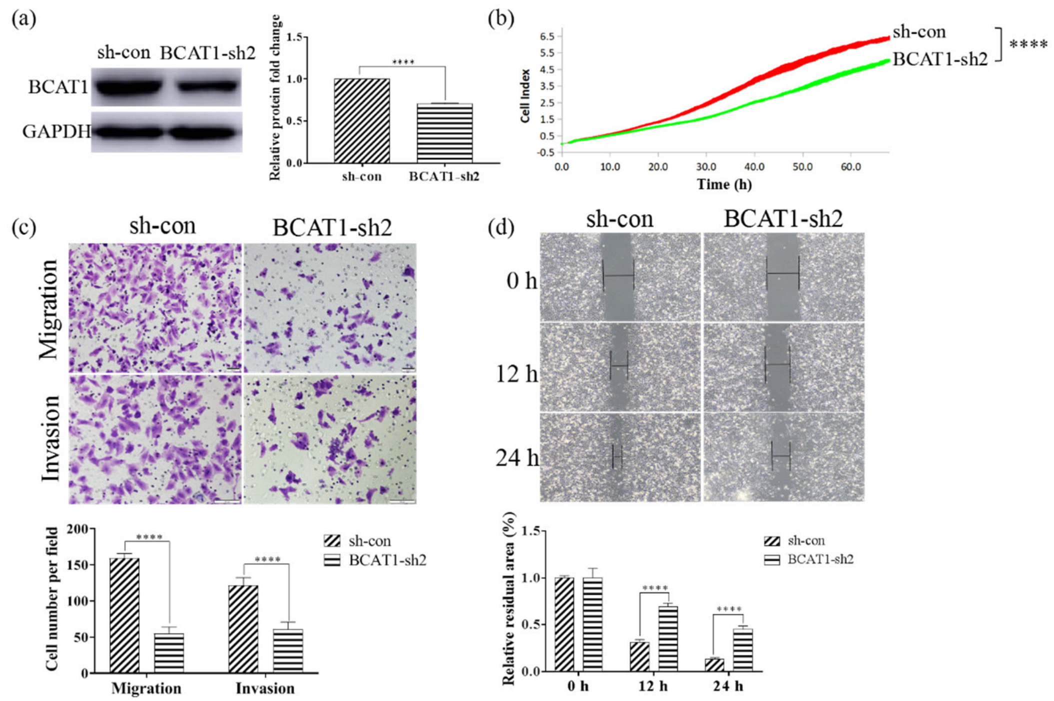

Fig. S2 Branched-chain aminotransferase 1 (BCAT1) knockdown inhibited cell proliferation, migration and invasion. (a) Western blot, (b) Cell proliferation rate detection, and (c-d) Migration, invasion, and wound healing assays of BCAT1 knockdown and control lung adenocarcinoma (LUAD) cells. BCAT1-sh2 and sh-con: BCAT1 knockdown and control LUAD cell lines. Data are expressed as mean±SD, *n*=3. * *P*<0.05; ** *P*<0.01.; * *P*<0.001.; **** *P*<0.0001.**

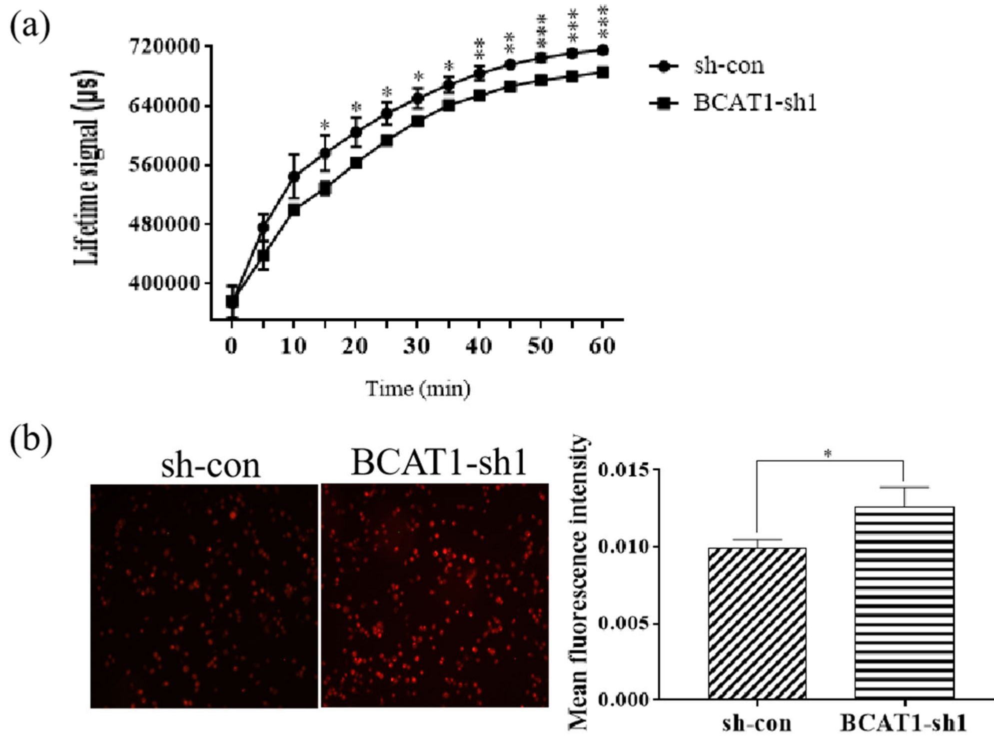

Fig. S3 Branched-chain aminotransferase 1 (BCAT1) knockdown decreased mitochondrial aerobic respiration and increased oxygen species (ROS) content. (a) Oxygen consumption rate (OCR) and (b) Cellular ROS level measurement in BCAT1 knockdown and control lung adenocarcinoma (LUAD) cells. BCAT1-sh1 and sh-con: BCAT1 knockdown and control LUAD cell lines. Data are expressed as mean±SD, *n*=3. * *P*<0.05; ** *P*<0.01.; * *P*<0.001.; **** *P*<0.0001.**

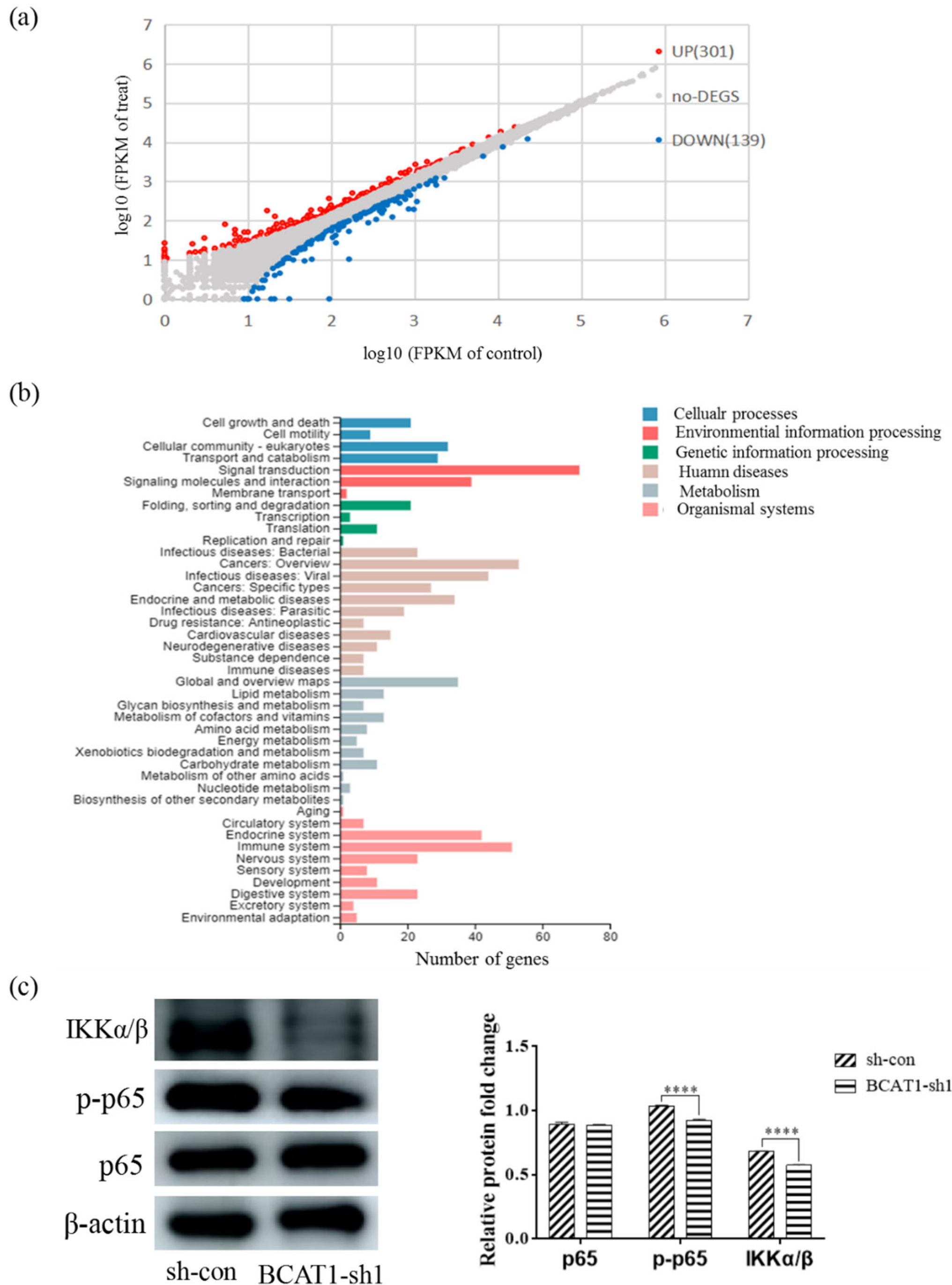

Fig. S4 **(a) Scatter plot of global gene expression pattern in branched-chain aminotransferase 1 (BCAT1) overexpressing and control cells. (b) Kyoto-Encyclopedia of Genes and Genomes (KEGG) pathway classification analysis of differentially expressed genes (DEGs). (c) Western blot analysis of p65, p-p65 and IKKα/β in BCAT1 knockdown and control LUAD cells. p-p65: phosphorylated p65; IKKα/β: inhibitor of kappa B kinase α/β; BCAT1-sh1 and sh-con: BCAT1 knockdown and control LUAD cell lines. Data are expressed as mean±SD, *n*=3. **** *P*<0.0001.**

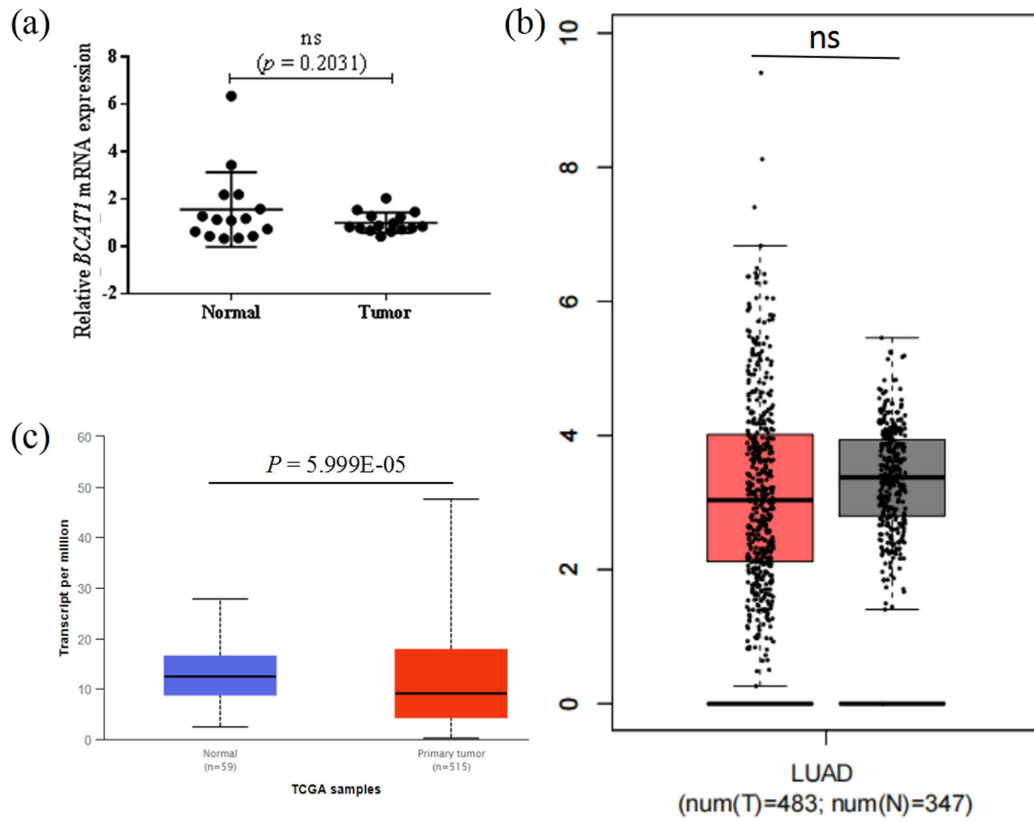

Fig. S5 (a) Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis of branched-chain aminotransferase 1 (*BCAT1*) in fifteen pairs of tissue samples from in lung adenocarcinoma (LUAD) patients. (b) and (c) Transcription level of *BCAT1* in tissues of LUAD patients from Gene Expression Profiling Interactive Analysis (GEPIA) and Cancer Genome Atlas (TCGA) databases. ^{ns} not significant.

Material and methods

Cell culture

The immortalized normal lung cell line (BEAS-2B) and LUAD cell lines including NCI-H1975, H1299, A549 and HCC827 were cultured in RPMI-1640 or DMEM with 10% FBS and incubated at 37 °C in a humidified incubator with 5% CO_2. BEAS-2B was purchased from the Chinese Academy of Sciences Typical Culture Preservation Committee Cell Bank. A549 was purchased from Genetic Testing Biotechnology Corporation. HCC827 was gifted by Proteomics and Signal Transduction Center, Zhengzhou University. H1299 and NCI-H1975 were gifted by Henan Institute of Medical and Pharmaceutical Sciences, Zhengzhou University.

Reagents

Antibodies used included BCAT1 (Sangon Biotech, China), phospho-NF-κB p65 (Ser536) (CST, USA), IKKα/β (Wanleibio, China), IKBα (Wanleibio, China), p65 (Wanleibio, China), GAPDH (Sangon Biotech, China), β-actin (proteintech, China), E-cadherin (proteintech, China), Vimentin (proteintech, China), Antibody cocktail to aerobic respiration (CST, USA). PDTC, L-Valine, L-Isoleucine, and L-Leucine (MCE, USA).

IHC and Evaluation

The tissue microarrays consisted of 86 pairs of tumor and surrounding normal lung tissue samples that were collected from 47 male and 39 female patients, aged between 30 and 84. These patients were diagnosed with primary LUAD without distal metastasis. This study was conducted with the informed consent obtained from all subjects. All experiments were performed in accordance with relevant guidelines and regulations of Declaration of Helsinki and has been approved by the Research Ethics Committee of Zhengzhou University. Anti-BCAT1 antibody was used for IHC detection of BCAT1 expression according to the manufacturer's suggested protocols. In brief, paraffin sections were dewaxed by heat treatment in EDTA buffer, pH=9. After blocking for 30 min with 3% bovine serum albumin (BSA), tissue sections were incubated with anti-BCAT1 antibody diluted 1:400 overnight at 4 °C. Then they were incubated with the secondary antibody (HRP labeled) at room temperature for 50 min. The staining

intensity of tissues was scored as 0 (negative), 1 (1+), 2 (2+), 3 (3+). For statistical evaluation, tissues were scored as 0 (non-staining); 1, 1–25%; 2, 26–50%; 3, 51–75%; 4, 76–100% (positive staining). BCAT1 protein expression was evaluated as negative (0), weak positive (1, 2), positive (3, 4) according to the staining positive rate score. The total histological score was defined as the product of "staining intensity score" and "staining positive rate score", and 0-5 indicated BCAT1 low expression while 6-12 indicated BCAT1 high expression.

Lentivirus transduction and Stable cell line construction

BCAT1 overexpression, shRNA and control lentiviral particles were purchased from Genechem and Hanbio Biotechnology (China). The shRNA sequences targeting BCAT1 were as follows: BCAT1-sh1: 5'-GGATCAAGAATGGGTCCCATATTCA-3', BCAT1-sh2: 5'-GTCCCAAGTATGTAAGAGCCTGGAAA-3'. The shRNA control sequence was: 5'-TTCTCCGAACGTGTCACGTAA-3'. A549 cells were seeded in 6-well plates and infected with BCAT1 overexpression, shRNA or control lentivirus respectively, followed by incubation overnight with 5 μg/mL polybrene (Sigma-Aldrich, USA). Stable BCAT1 knockdown and overexpression cells were selected with 2 μg/mL puromycin.

Protein extraction and Western blot

Cells were lysed in RIPA buffer (Solarbio, China) for 30 min at 4 °C and then centrifuged at 12000 rpm for 15 min. The supernatant was collected and total protein was quantified using the BCA kit (Solarbio, China). Total protein was boiled and separated by 10% SDS–PAGE gel, and then transferred to PVDF membrane (Merck Millipore, USA). Membranes were blocked in 5% skim milk powder in TBS-T (TBS plus 0.5% Tween-20) at room temperature for 2 hours, and then blots were incubated with primary antibody at 4 °C overnight. The membrane was probed with secondary antibody, and signals were detected with ECL Substrate (Beyotime Biotech, China) and Amersham Imager 600 System (General Electric Company, USA). Protein expression was quantified using Image J software, with GAPDH or β-actin as an internal control.

RNA Extraction and qRT-PCR

The cDNA microarray was purchased from Shanghai Outdo Biotech, China. Total

RNA was extracted from cells with TRIzol Reagent (Invitrogen, USA) according to the manufacturer's instruction and cDNA was synthesized using the PrimeScript RT Reagent Kit (TaKaRa Clontech, China). qRT-PCR was performed using the ChamQ™ Universal SYBR® qPCR Master Mix (Vazyme Biotech, China) and QuantStudio 5 Real-Time PCR System (Thermo Scientific, USA). The target mRNA expression level was quantified using the $2^{-\Delta\Delta Ct}$ method with normalized to the endogenous gene ACTB. The following primer sequences were used for qRT-PCR: *BCAT1* forward, 5'-TGGAGAATGGTCCTAAGCTG-3', reverse, 5'-GCACAATTGTCCAGTCGCTC-3'; *NFKBIB* forward, 5'-GCCCTGACCGTACTCCCGAC-3', reverse, 5'-GGCCTCC ACTGCCAAATGAA-3'; *BCKDK* forward, 5'-CTTCTGAAAAGTGCTCGGTAC-3', reverse, 5'-CTTCTGGAAGGCACGGATATAT-3'; *BCKDHA* forward, 5'-GAGGCAGG TGTGCTGATGTATCG-3', reverse, 5'-AGTGGAGAGGAGATAGTGACGAAGTG-3'; *BCKDHB* forward, 5'-GGTGGCTCATTTTACTTTCCAG-3', reverse, 5'-AGGATCT TTGGCCAATGAGTTA-3'; *ECHS1* forward, 5'-GTGATATCATCTATGCCGGTGA-3', reverse, 5'-GTCTCAACAGGACAAATCTTGC-3'; *SOD1* forward, 5'-GATGACTTGG GCAAAGGTGGAAATG-3', reverse, 5'-CCAATTACACCACAAGCCAAACGAC-3'; *SOD2* forward, 5'-CGCCCTGGAACCTCACATCAAC-3', reverse, 5'-AACGCCTCCT GGTACTTCTCCTC-3'; *CAT* forward, 5'-CTCAGGTGCGGGCATTCTATGTG-3', reverse, 5'-GGTGGACCTCAGTGAAGTTCTTGAC-3'; *GPX1* forward, 5'-GCAACC AGTTTGGGCATCAGGAG-3', reverse, 5'-CACCGTTCACCTCGCACTTCTC-3'; *PGC1α* forward, 5'-ACCAAACCCACAGAGAACAG-3', reverse, 5'-GGGTCAGAGG AAGAATAAAGTTG-3'; *NRF-1* forward, 5'-AGTGCTTAGCCCTTGATGAAGA-3', reverse, 5'-GCTCTGAAGTGACCTCTGGTAT-3'; *TFAM* forward, 5'-ATGGCGTTTC TCCGAAGCAT-3', reverse, 5'-TCCGCCCTATAAGCATCTTGA-3'; *GAPDH* forward, 5'-CTCAAGGG CATCCTGGGCTA-3', reverse, 5'-CGTCAAAGGTGGAGGAGTGG -3'; *ACTB* forward, 5'-GGCATCCACGAAACTA CCTT-3', reverse, 5'-CTCCTGCTT GCTGATCCACA-3'. Primers used for qRT-PCR were synthesized by Shenggong, China.

RNA sequencing and Informatics analysis

RNA-seq was performed with BCAT1 overexpression and control A549 cells

(each with three replicates) using BGISEQ-500 sequencing system. In brief, RNA sample was extracted and the quality was assessed, followed by generation of cDNA libraries. Gene expression levels for each sample were quantified using the FPKM method (fragments per kilobase of transcript per million mapped reads) and processed for analysis. DEGs were screened by fold change≥1.5 and the adjusted *P* value≤0.01. Gene Ontology (GO) annotation proteome was performed from the UniProt-GOA database (http://www.ebi.ac.uk/GOA). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was based on database (http://www.genome.jp/kegg/).

Cell proliferation assay

The cell proliferation ability was measured by using the xCELLigence Real-Time Cell Analyzer (RTCA)-MP system (Acea Biosciences, USA), which provided a continuous and quantitative measurement of the cell index to monitor cellular growth status. First, 50 μL of culture medium was added in each well of E-Plate 12 (Roche Applied Science, USA) to detect the baseline. Then cells were suspended in 100 μL medium and seeded in E-Plate 12 with a density of 5×10^4 cells/mL. After 30 min, the E-Plate 12 was inserted into RTCA-MP device and incubated at 37 °C with 5% CO_2. Cell index was measured every 5 min to reflect the cellular proliferation ability.

CCK-8 assay

BCAAs (leucine, isoleucine, and valine) and PDTC were dissolved in ddH_2O, after sterilization with 0.22 μm filter, they were added into culture medium. Then cells were collected and suspended in the culture medium and seeded in a 96-well plate (3000 cells/well). For BCAAs treatment, cells were incubated for 30 h. For PDTC treatment, cells were incubated for 48 h. Then 10 μL CCK-8 reagent was added into each well, and cells were incubated for 1-4 h at 37 °C in the dark. The absorbance (A) value at 450 nm wavelength was detected with a microplate reader (Thermo, USA).

Colony formation assay

Cells were seeded into 6-well plates (1000 cells/well). Then cells were cultured at 37 °C with 5% CO_2 for approximately one week. After that, clones were washed with PBS and then fixed with 4% paraformaldehyde, followed by staining with 1% crystal violet for 30 min. The colonies were examined and counted with a microscope.

Wound-healing assay

Cells were seeded with a density of 10^6 cells/well in 6-well plates, when the cell density reached 95%, monolayers were scratched with a 10 μL pipette tip to create scratch wounds. The suspended cells were washed away with PBS, and then cultured in RPMI-1640 medium with 2% FBS. The distance between scratches was measured at 0 h, 12 h, 24 h, and 48 h, and the relative residual area (%) was calculated using the following equation: (current gap distance/original gap distance)×100%.

Cell migration and invasion assays

For migration assay, 5×10^4 cells were suspended in serum-free medium and seeded in the top chambers, then the lower chambers were filled with 600 μL medium containing 10% FBS. After incubation for 8–12 h, the migratory cells were fixed with methanol and stained with a 0.1% crystal violet solution. Then cells were counted with a microscope (magnification, ×200; Olympus BX53, Japan). For invasion assay, the difference was that Matrigel (BD Biosciences, USA) was diluted in serum-free medium (1:6) and added to the upper chamber before cells were seeded.

For PDTC treatment, PDTC was dissolved in ddH_2O, after sterilization with 0.22 μm filter, it was added into culture medium. Then cells were collected and suspended in the culture medium and seeded in the top chambers (5×10^4 cells/chamber). The lower chambers were filled with 600 μL medium containing 10% FBS. After incubation for 24 h, cells were fixed, stained, and counted as above.

Xenograft mice model

Animal study was performed in accordance with the guidelines of the National Act on the Use of Laboratory Animals (P. R. China), approved by the Animal Ethics Committee of Zhengzhou University. This study was reported in accordance with ARRIVE guidelines. BALB/c nude mice (female, aged 4 weeks) were upraised in SPF conditions in Henan Key Laboratory for Pharmacology of liver diseases, with a 12 h light/dark cycle and free access to food and water. BCAT1 overexpression and control A549 cells ($5x10^6$ in 100 μL PBS) were subcutaneously injected into the flanks of mice, respectively. Five weeks after injection, mice were sacrificed by cervical dislocation and the tumors were collected for analysis. Tumor volume was evaluated with the

formula: tumor volume=(width^2×length)/2.

OCR detection

Briefly, cells (8 x 10^4) were seeded into a 96-well plate and then cultured at 37 °C with 5% CO_2 for overnight. Next, the BBoxiProbeTM R01 (BestBio, China) and oxygen blocking fluid were added into the 96-well plate chambers in sequence at 37 °C, and the fluorescence intensity at the excitation wavelength of 468 nm was detected by the CLARIOstar Plus (BMG, Germany).

Intracellular ROS content determination

About 1×10^5 cells/well were seeded in a 6-well plate. When the cell density reached 80%, dihydroethidium (DHE, 10 μmol/L) was added into chambers, and cells were incubated at 37 °C for 20 min in the darkness. Later, cells were washed twice with PBS, the fluorescence intensity which represented ROS content was detected at 535 nm with Eclipse TS100 microscope (Nikon, Japan). About 250 cells were selected randomly for fluorescence intensity calculation with ImageJ software.

Statistical analysis

All statistical analyses were performed using SPSS 21.0 and visualized with GraphPad Prism 8.0. Data are shown as mean±standard deviation (SD). A P value≤0.05 was considered as statistically significant.