

Data S1. Supplementary materials and methods

MTT assay. U87, U251, T98G and LN229 cells were seeded in 96-well plates at a density of 2×10^3 cells/well and incubated overnight at 37°C in a 5% CO₂ incubator. After 0, 24, 48 and 72 h, 10 μ l MTT solution (5 mg/ml; cat. no. ST316; Beyotime Institute of Biotechnology) were added to each well, and the plates were incubated at 37°C for 4 h. Formazan crystals were dissolved in 100 μ l DMSO (cat. no. D8371; Beijing Solarbio Science & Technology Co., Ltd.), and absorbance was measured at 490 nm using the Multiskan™ Spectrum microplate reader (Thermo Fisher Scientific, Inc.).

Cell Counting Kit-8 (CCK-8) assay. U251 cells were seeded in 96-well plates at a density of 2.5×10^3 cells/well. Cells were treated with 0, 2.5, 5, 10 and 15 mM N-acetyl-cysteine, 0, 2, 4, 6 and 8 μ M azacitidine or 0, 5, 10, 15 and 20 μ M C646 for 48 h at 37°C. A total of 10 μ l CCK-8 reagent (cat. no. MC0301; Henan Jackson Biotechnology Co., Ltd.) was added to each well, and the plates were incubated for 2 h. Absorbance was measured at 450 nm using the Multiskan Spectrum microplate reader (Thermo Fisher Scientific, Inc.).

Colony formation assay. U251 cells were seeded in 6-well plates at a density of 1×10^3 cells/well and incubated for 14 days at 37°C. Colonies (defined as visible cell aggregates containing ≥ 50 cells) were fixed with 4% paraformaldehyde for 10 min at room temperature, stained with crystal violet for 5 min at room temperature, and counted. The colony formation rate was calculated using the following formula: (Number of colonies/1,000) $\times 100$.

Real-time cell analysis (RTCA). U251 cell proliferation was monitored using the xCELLigence RTCA S16 instrument (Agilent Technologies, Inc.). A total of 4×10^4 cells/well were seeded in E-S16 plates containing 150 μ l fresh medium. Background measurements were taken using wells containing medium only. Cell proliferation was monitored in real time.

3D tumor sphere experiment. U251 cells were seeded into a 96-well Round Bottom Ultra-Low Attachment Microplate (cat. no. 7007; Corning, Inc.) at a density of 1×10^3 cells/well. They were then centrifuged at 100 \times g for 5 min at room

temperature to facilitate aggregation and sphere formation. After 48 h of incubation at 37°C, dense cell spheroids were observed under an inverted optical microscope (Nikon Eclipse Ts2; Nikon Corporation). To simulate a 3D growth environment, spheroids were embedded in Matrigel (cat. no. 354234; Corning, Inc.); Pre-cooled 24-well glass-bottom plates (cat. no. 801006; Wuxi NEST Biotechnology Co., Ltd.) and pipette tips were used to aspirate 250 μ l Matrigel (8 mg/ml), which was then dispensed into the plates. Spheroids were carefully transferred to the center of Matrigel-coated wells and incubated at 37°C for 30 min to allow solidification. Complete medium (500 μ l) was subsequently added slowly along the well walls. Spheroid growth was monitored at 0, 24, 48 and 72 h using Z-stack scanning with an AX confocal microscope (Nikon Corporation). The 'Freehand Selection' tool in ImageJ software v1.8.0.345 (National Institutes of Health) was employed to calculate spheroid areas, with final diffusion areas determined by subtracting the baseline (0 h) measurements from subsequent time points.

ACC1 overexpression plasmid transfection. Vector and acetyl-CoA carboxylase 1 (ACC1) plasmids (backbone: pcDNA3.1, GeneCopoeia, Inc.) were extracted using the EndoFree Mini Plasmid Kit II (cat. no. DP118; Tiangen Biotech Co., Ltd.), and their concentration and purity were measured using a microvolume spectrophotometer (NanoPhotometer® NP80; Implen, Inc.). For plasmid transfection, U251 cells were seeded in a 35 mm diameter cell culture dish at a density of 3×10^5 cells and cultured overnight. The next day, cells were transfected at 37°C for 48 h with 2.5 μ g vector plasmid or ACC1 plasmid using 5 μ l Lipofectamine™ 2000 Transfection Reagent (Thermo Fisher Scientific, Inc.). Cells were then collected 24 h after transfection for follow-up experiments.

Free fatty acid (FFA) measurement. Cellular FFA levels were measured using the FFA Content Assay Kit (cat. no. BC0595; Beijing Solarbio Science & Technology Co., Ltd.), according to the manufacturer's instructions. A total of 8×10^6 U251 cells were used per assay. Samples were normalized to protein content determined using a BCA assay. Absorbance was measured at 550 nm using the Multiskan Spectrum microplate reader (Thermo Fisher Scientific, Inc.).

Figure S1. KD of ACC1 promotes U251 cell proliferation, migration and invasion. (A) WB of ACC1 in four glioma cell lines. MTT analysis of (B) U87-OE, (C) U251-KD, (D) T98G-KD and (E) LN229-KD cell proliferation. (F) Phase-contrast images of shNC and shACC1 U251 cells. (G) Quantification of the cell yield from equal cell inoculation and identical culture time. (H) Real-time cell analysis of proliferation of shNC and shACC1 U251 cells. (I) Colony formation images of shNC and shACC1 U251 cells. (J) Quantification of colony formation efficiency in shNC and shACC1 U251 cells. (K) Flow cytometric analysis of the cell cycle in shNC and shACC1 U251 cells. (L) Quantification of cell cycle distribution in shNC and shACC1 U251 cells. (M) WB of ACC1, cyclin B1, cyclin D1 and p21 in shNC and shACC1 U251 cells. (N) Semi-quantification of ACC1, cyclin B1, cyclin D1 and p21 protein levels in shNC and shACC1 U251 cells. (O) Confocal images of shNC and shACC1 U251 spheroids at indicated time points. (P) Quantification of spheroid diffusion area. Scale bars, 100 μ m. Error bars represent the mean \pm standard deviation from three independent experiments. * P <0.05; ** P <0.01; *** P <0.001. ACC1, acetyl-CoA carboxylase 1; sh, short hairpin; NC, negative control; WB, western blotting; KD, knockdown; OD, optical density; OE, overexpression.

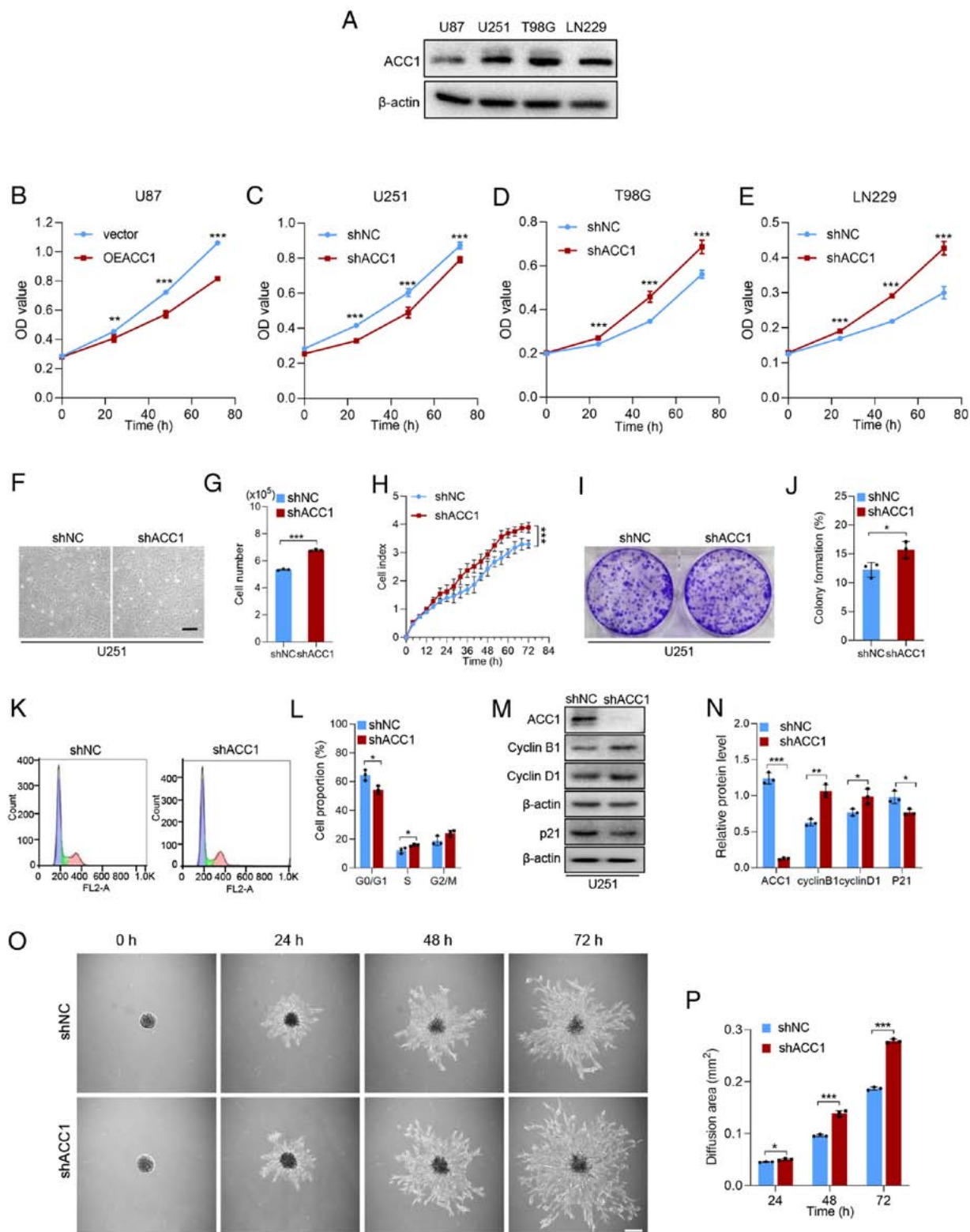


Figure S2. Knockdown of ACC1 reduces the expression level of SDH in U251 cells. Reverse transcription-quantitative PCR of SDHA/B/C/D mRNA in (A) U87, (B) U251, (C) T98G and (D) LN229 cells. Western blotting of SDHA/B in (E) U87, (F) U251, (G) T98G and (H) LN229 cells. Semi-quantification of SDHA/B protein levels in (I) U87, (J) U251, (K) T98G and (L) LN229 cells. Error bars represent the mean \pm standard deviation from three independent experiments. ** $P < 0.01$ and *** $P < 0.001$. ACC1, acetyl-CoA carboxylase 1; SDH, succinate dehydrogenase; OE, overexpression; sh, short hairpin; NC, negative control.

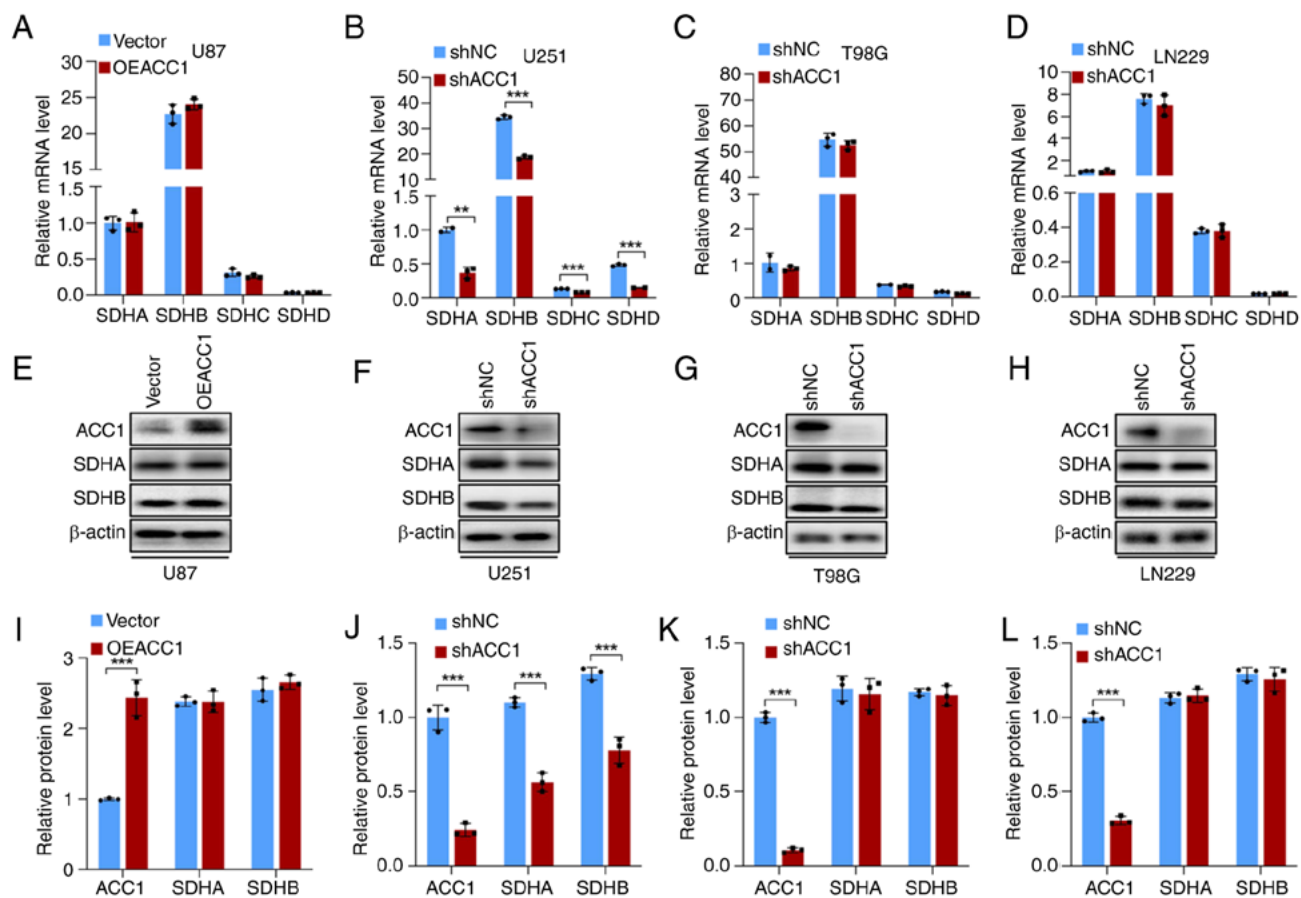


Figure S3. Drug dose effects on U251 proliferation (Cell Counting Kit-8 assay). (A) NAC at 5 mM, (B) Aza at 4 μ M and (C) C646 at 5 μ M demonstrated no inhibitory effects on proliferation and were selected for subsequent experiments. Error bars represent the mean \pm standard deviation from three independent experiments. **P<0.01; ***P<0.001. NAC, N-acetyl-cysteine; Aza, azacitidine.

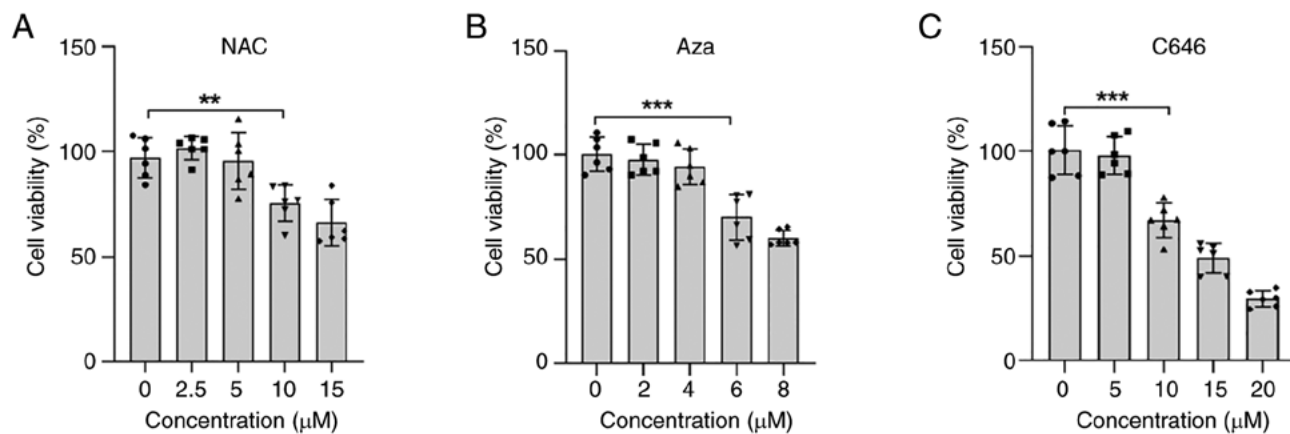


Figure S4. ACC1 OE reverses ACC1-knockdown phenotypes. (A) Images of Transwell migration and invasion assays after ACC1 OE. (B) Quantification of Transwell migration and invasion assays after ACC1 OE. (C) Wound-healing images after ACC1 OE. (D) Quantification of wound-healing assay after ACC1 OE. (E) Flow cytometric analysis of ROS after ACC1 OE. (F) Quantification of ROS levels after ACC1 OE. (G) Western blotting of ACC1, SDHA, SDHB, H3K9ac, DNMT1, vimentin, H3, fibronectin and PAI-1 after ACC1 OE. (H) Semi-quantification of ACC1, SDHA, SDHB, H3K9ac/H3, DNMT1, vimentin, fibronectin and PAI-1 protein levels after ACC1 OE. Scale bars, 100 μ m. Error bars represent the mean \pm standard deviation from three independent experiments. * P <0.05; ** P <0.01; *** P <0.001. # P <0.05; ### P <0.001. ACC1, acetyl-CoA carboxylase 1; DNMT, DNA methyltransferase; H3K9ac, histone H3 acetylation at lysine 9; SDH, succinate dehydrogenase; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; sh, short hairpin; NC, negative control; OE, overexpression.

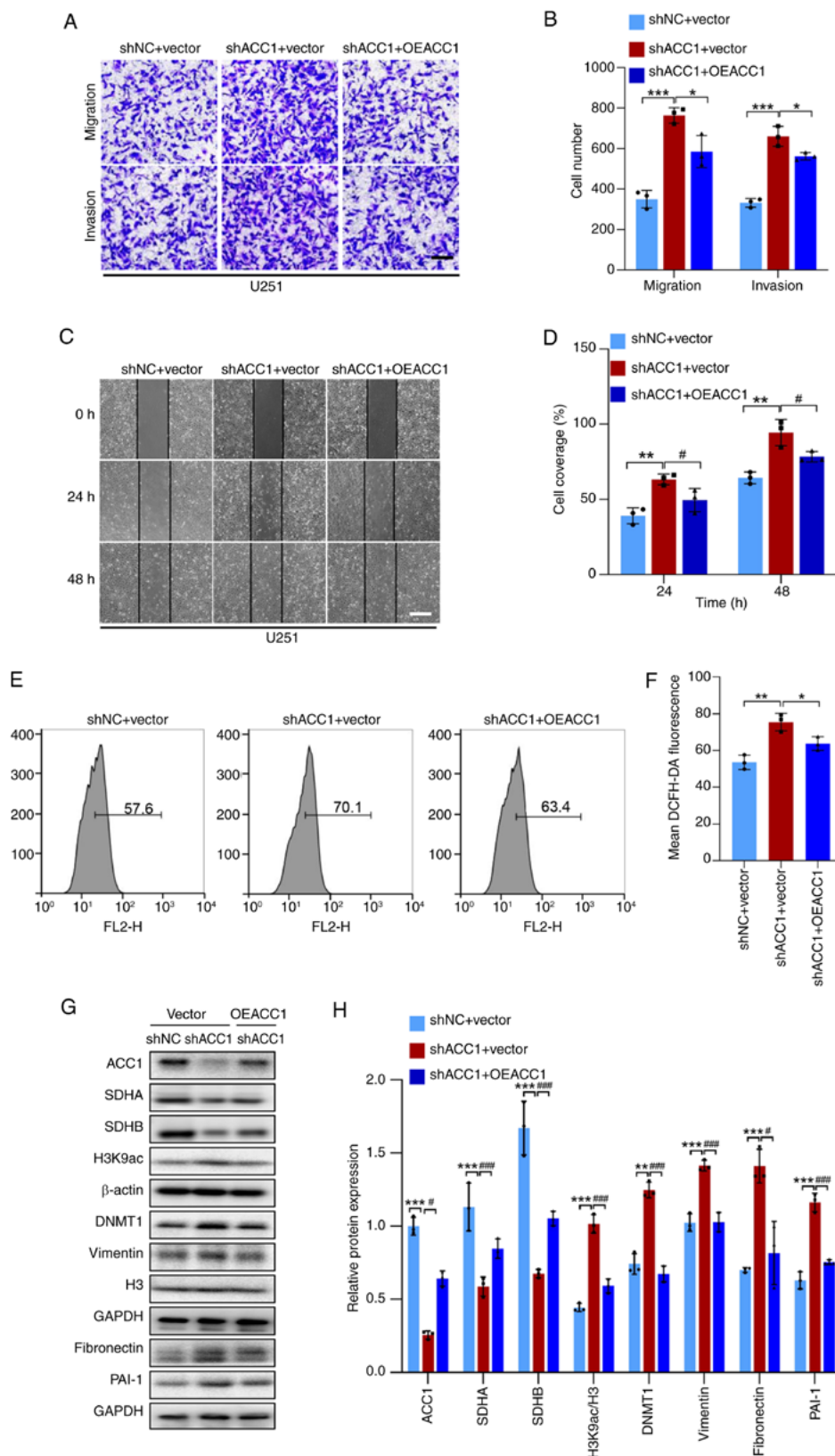


Figure S5. Upregulation of DNMTs and downregulation of SDH subunits in glioma (TCGA/CPTAC). (A) DNMT1, (B) DNMT3A and (C) DNMT3B mRNA levels in glioma (TCGA). (D) DNMT1, (E) DNMT3A and (F) SDHA protein levels in glioma (CPTAC). (G) SDHB, (H) SDHC and (I) SDHD protein levels in glioma (CPTAC). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. TCGA, The Cancer Genome Atlas; CPTAC, Clinical Proteomic Tumor Analysis Consortium; DNMT, DNA methyltransferase; GBM, glioblastoma multiforme.

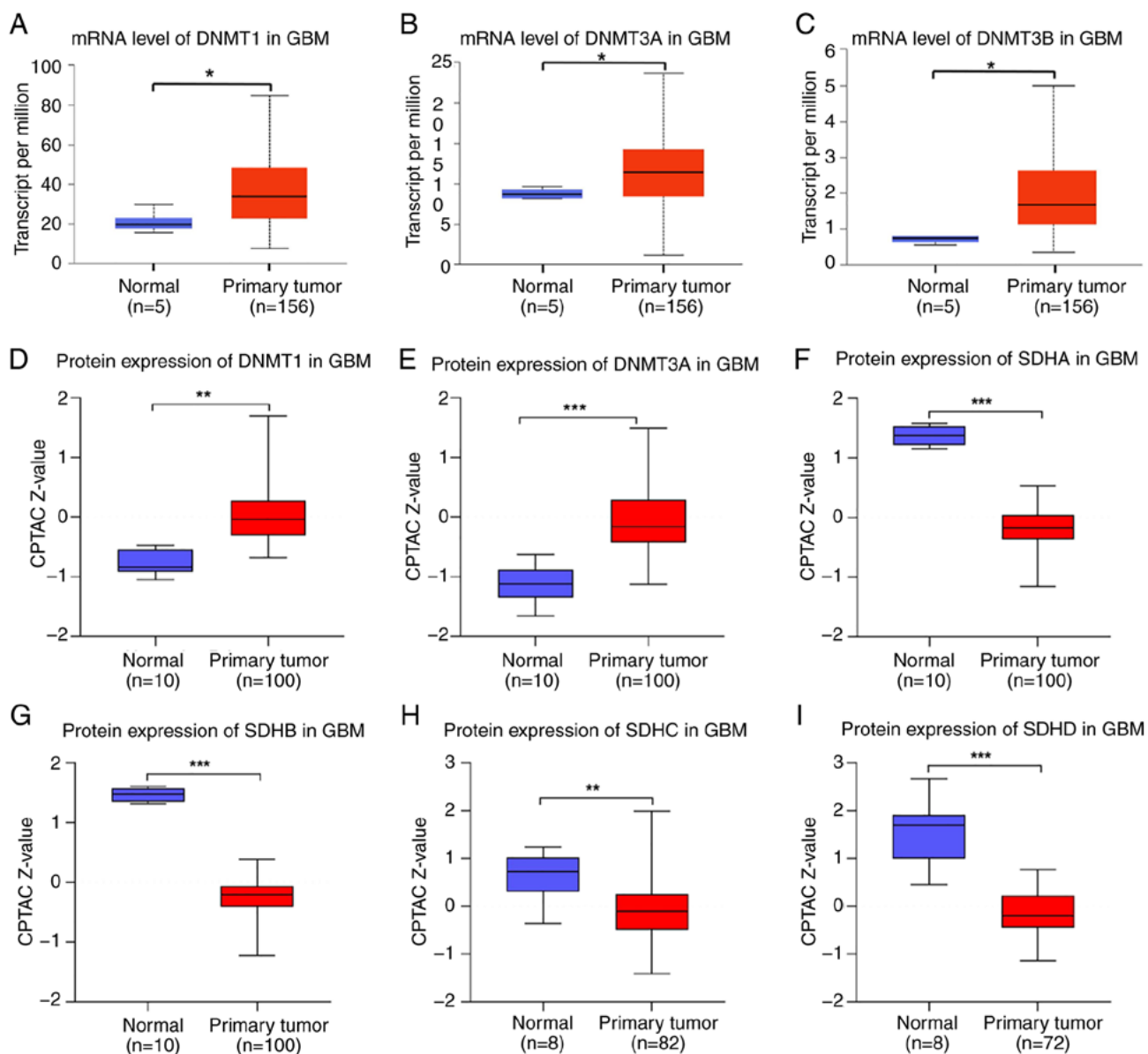


Figure S6. Knockdown of ACC1 does not affect fatty acid synthesis in U251 cells. (A) Intracellular FFA content was measured by fluorometric assay. (B) Volcano plots indicating upregulated and downregulated molecules. Reverse transcription-quantitative PCR of (C) SREBF1 and (D) FASN mRNA levels in shNC and shACC1 U251 cells. (E) Western blotting of ACC1, SREBP1 and FASN in shNC and shACC1 U251 cells. (F) Semi-quantification of ACC1, SREBP1 and FASN protein levels. Error bars represent the mean \pm standard from three independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. ACC1, acetyl-CoA carboxylase 1; FFA free fatty acid; SREBF1, sterol regulatory element-binding transcription factor 1; FASN, fatty acid synthase; ns, not significant; sh, short hairpin; NC, negative control.

