

Original Article

Quercetin targets BCAT1 to potentiate CD8⁺ T cell immunity by rewiring branched-chain amino acid metabolism against colorectal cancer metastasis

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ABSTRACT

Colorectal cancer (CRC) metastasis is a major cause of cancer-related death, highlighting the need for therapies that target immune-metabolic pathways. Here, we show that quercetin inhibits CRC progression via dual actions: directly suppressing tumor cell growth and migration, while systemically reinvigorating CD8⁺ T cell mediated immunity. *In vitro*, quercetin potently inhibited CRC cell proliferation, migration, and survival. In an experimental lung metastasis mouse model, quercetin enhanced the effector function of CD8⁺ T cells, as evidenced by increased production of Granzyme B, Perforin, and IFN- γ in metastases, blood, and tumor-draining lymph nodes. Metabolomic profiling uncovered a pronounced remodeling of branched-chain amino acid (BCAA) metabolism following quercetin intervention. Screening via the HERB database identified BCAA transaminase 1 (BCAT1) as a candidate target, confirmed by molecular docking and surface plasmon resonance (SPR). Importantly, the combination of oral leucine and quercetin produced a synergistic effect, significantly boosting CD8⁺ T cell cytotoxicity and dramatically reducing the lung metastatic burden. Taken together, these data identify BCAT1 as a direct target of quercetin and elucidate a resultant immunometabolic circuit that bolsters CD8⁺ T cell function, providing a rationale for targeting this pathway in advanced CRC.

Introduction

Colorectal cancer (CRC) remains one of the most daunting challenges in oncology, contributing substantially to global cancer-related mortality (Sekiguchi et al., 2025; Siegel et al., 2022; Tamura et al., 2026). This high fatality is overwhelmingly driven by metastatic dissemination, wherein the disease progresses to incurable stages. While targeted therapies and immune checkpoint inhibitors have revolutionized modern oncology, a large subset of patients with metastatic CRC derives only minimal or transient benefit from these interventions (Al-Hujaily et al., 2022; Biller and Schrag, 2021; H. Huang et al., 2025). Primary and acquired resistance mechanisms, often orchestrated by the immunosuppressive tumor microenvironment (TME), remain a principal obstacle (Arora et al., 2026). Within this milieu, dysregulated metabolic

pathways create a state of nutrient deprivation and generate toxic byproducts that actively suppress immune effector cells. Notably, cytotoxic CD8⁺ T cells, which are indispensable for eliminating malignant cells, display severe functional exhaustion in the TME, partly due to fierce competition for critical metabolites such as glucose and amino acids (Pelka et al., 2021; Puzanov et al., 2026; Yao et al., 2023). These insights have refocused therapeutic development on immunometabolic pathways as actionable targets (Leone and Powell, 2020; Wang et al., 2025; Zhao et al., 2026b). Consequently, identifying pharmacological agents that remodel the TME's metabolic landscape to restore CD8⁺ T cell efficacy represents a rational strategy. This approach may offer translational potential for overcoming current therapeutic limitations in advanced CRC.

Branched-chain amino acids (BCAAs), including leucine, isoleucine,

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and valine are essential nutrients that serve as both metabolic building blocks and signaling molecules (Hu et al., 2025; Yoneshiro et al., 2019; Zheng et al., 2024). Their catabolism begins with the branched-chain aminotransferases BCAT1 and BCAT2 (L. Huang et al., 2025; Liu et al., 2025), which are frequently overexpressed in CRC and linked to tumor growth and chemoresistance (Han et al., 2023; Nong et al., 2022; Xu et al., 2021; Young et al., 2021). Paradoxically, while cancer cells exploit BCAAs for anabolic processes, CD8⁺ T cells in the TME suffer from BCAA deprivation, leading to impaired mitochondrial fitness and reduced cytotoxicity (Tosello et al., 2024; Wetzel et al., 2023). Recent studies suggest that BCAA supplementation can enhance T cell function (Hu et al., 2025; Xu et al., 2025), but systemic delivery risks fueling tumor progression (Corsetti et al., 2025; Wei et al., 2020; Xiong et al., 2025). Thus, strategies to selectively modulate BCAA metabolism in immune cells rather than tumor cells are urgently needed to break this therapeutic conundrum.

Quercetin, a dietary flavonoid abundant in apples, onions, and berries (Batiha et al., 2020), has garnered attention for its pleiotropic anti-cancer effects, including pro-apoptotic, anti-angiogenic, and cell-cycle inhibitory properties (H. Huang et al., 2025; Seo et al., 2019). Preclinical studies demonstrate its efficacy against CRC by suppressing Wnt/ β -catenin and PI3K/AKT pathways (Duan et al., 2025; Mehrabadi, 2025; Sun et al., 2024; Yang et al., 2019). Intriguingly, quercetin also exhibits immunomodulatory potential, such as reducing regulatory T cell infiltration and promoting dendritic cell maturation (Cui et al., 2025; Manni et al., 2023; Verna et al., 2021). However, its direct molecular targets and mechanisms within the immunometabolic axis particularly in CD8⁺ T cells remain enigmatic. Given the dual role of BCAAs in tumor and immune cell biology, we posited that quercetin might reconcile this metabolic conflict by selectively rewiring BCAA utilization in CD8⁺ T cells.

We hypothesized that quercetin suppresses CRC not only by directly targeting tumor cells but also by augmenting CD8⁺ T cell function via a specific metabolic pathway. Using an integrated strategy, including *in vitro* assays, murine metastasis models, metabolomics, and bioinformatic analysis, we identified BCAT1 as a direct target of quercetin. Our results reveal a novel mechanism: quercetin binds to BCAT1, reprograms BCAA metabolism in CD8⁺ T cells, and enhances their cytotoxicity, ultimately inhibiting CRC metastasis.

Here, we employed an integrative approach combining *in vitro* functional assays, metabolomics, *in vivo* metastasis models, and computational target prediction to dissect quercetin's immunometabolic actions. We identified BCAT1 as a direct physical target of quercetin and delineated its role in reprogramming BCAA metabolism to reinvigorate CD8⁺ T cell function. Our findings unveil a novel mechanism whereby quercetin-BCAT1 binding augments CD8⁺ T cell cytotoxicity and suppresses metastatic outgrowth, offering a rationale for targeting BCAA metabolism in advanced CRC therapy.

Materials and methods

Clinical samples

A total of 9 human CRC tissue samples were obtained from patients who underwent surgical resection at the Affiliated Haian Hospital of Nantong University. The inclusion criteria were as follows: (1) pathologically confirmed primary CRC; (2) availability of well-preserved formalin-fixed paraffin-embedded (FFPE) tissue blocks; (3) complete demographic and clinicopathological data; and (4) no prior history of neoadjuvant chemotherapy, radiotherapy, or immunotherapy before surgery. The exclusion criteria included: (1) presence of other concomitant malignancies; (2) history of autoimmune diseases or active chronic infections; and (3) severe tissue necrosis insufficient for multiplex immunofluorescence (mIHC) evaluation. The patient cohort included 7 males and 2 females, with an age range of 48–87 years. The disease stages included Stage II–IV (Table S1). All participants provided

written informed consent prior to inclusion. The study protocol was reviewed and approved by the Ethics Committee of Haian People's Hospital (Approval No 2025-L025).

Cells

SW480 and MC38 cells were obtained from the Cell Bank of the Chinese Academy of Sciences. Cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and cultured at 37 °C under 5% CO₂. Furthermore, all cell lines were routinely authenticated by STR profiling and tested negative for mycoplasma contamination before experimentation.

Cell viability assay (CCK-8)

Cells were seeded in 96-well plates at densities of 1×10^4 , 5×10^3 , and 2.5×10^3 cells per well for 24-, 48-, and 72-hour time points, respectively, and incubated overnight. The cells were then treated with quercetin (MCE) at concentrations of 0, 12.5, 25, 50, 100, and 150 μ M. At each indicated time point, 10 μ l of CCK-8 reagent (APExBIO) was added to each well, followed by incubation for 40 min. The absorbance at 450 nm was measured using a microplate reader, and the background absorbance from blank wells was subtracted from each sample value before calculating the cell viability.

Wound healing assay

For the wound healing assay, SW480 and MC38 cells were seeded in 6-well plates at a density of 1×10^6 cells per well and cultured until reaching approximately 95% confluence. A straight scratch wound was then created using a sterile 200 μ l pipette tip held perpendicular to the bottom of the well. After gentle washing twice with PBS to remove detached cells, 2 ml of serum-free medium was added to each well. Images of the scratch area were captured at 0 h post-scratch using an inverted optical microscope to establish the baseline. Following image acquisition, the serum-free medium was discarded and replaced with DMEM medium containing different concentrations of quercetin supplemented with 1% serum to minimize interference from cell proliferation during migration analysis. Images were taken at 24 h and 48 h after scratching. All images were analyzed using ImageJ software to measure and quantify the wound area. Wound closure rate was calculated according to the following formula: Wound closure (%) = [(Area at 0 h – Area at the indicated time point) / Area at 0 h] $\times$ 100%.

Apoptosis assay

Cells were seeded in 6-well plates at a density of 5×10^4 cells per well and incubated overnight. The cells were treated with quercetin at concentrations of 0, 25, 50, 100, and 150 μ M for 48 h. After treatment, the supernatant was removed, and the cells were washed once with PBS. Cells were detached using 500 μ l of EDTA-free trypsin (KeyGEN), neutralized, and collected by centrifugation at 1000 rpm for 5 min. The cell pellet was washed twice with PBS and resuspended in 500 μ l of binding buffer. Annexin V-FITC (2.5 μ l) and PI (2.5 μ l) (KeyGEN) were added to each sample, followed by incubation in the dark for 15 min. Samples were gently mixed and analyzed by flow cytometry. Data acquisition was performed using a EasyCell 206A1 (Wellgrow, Shenzhen China) flow cytometer equipped with multiple excitation lasers, specifically including a blue/yellow-green laser and a red laser. For each sample, at least 10,000 events were acquired.

Colony formation assay

Cells were seeded in 6-well plates at a density of 600 cells per well and incubated for 3 days. The cells were then treated with quercetin at concentrations of 0, 25, 50, and 100 μ M. The cells were then cultured in

complete medium containing indicated concentrations of quercetin. To ensure continuous drug exposure, the quercetin-containing medium was refreshed every 3 days throughout the 14-day incubation period. After incubation, cells were washed once with PBS and fixed with 1 ml of 4% paraformaldehyde (BOSTER) for 15 min. Following fixation, cells were washed three times with PBS and air-dried. Colonies were stained with 1 ml of crystal violet (Beyotime) in the dark for 20 min, rinsed, air-dried, and photographed. Colonies containing >50 cells were counted manually using ImageJ.

Animal tumor models and in vivo treatment

Specific pathogen-free (SPF) 6–8-week-old male C57BL/6 mice (weighing 18–22 g) were obtained from Laboratory Animal Center of Nantong University (Nantong, China). All mice were maintained in the SPF facility of Laboratory Animal Center of Nantong University. For the *in vivo* metastasis models, the mice were randomly assigned to different treatment groups ($n = 3$ –5 mice per group). To minimize subjective bias, the investigators were blinded to the group allocation during the assessment of lung metastatic nodules and subsequent histological analyses. All mice were housed under standard conditions with free access to food and water. All experimental protocols were approved by the Animal Ethics Committee of the Laboratory Animal Center of Nantong University (Approval No S20251015–001).

To model colorectal cancer lung metastasis in mice, MC38 cells (2.5×10^5) were administered via tail vein injection. The mice were monitored twice daily, in the morning and evening. Approximately 18 days post-injection, all animals were humanely euthanized. To evaluate the effects of quercetin and leucine, mice with established colorectal cancer lung metastases were divided into three groups. The quercetin group received intraperitoneal injections of quercetin (10 mg/kg; MCE) every other day. The leucine group was administered leucine (10 mg/kg) orally on the same schedule. The combination group received both agents concurrently, following the identical dosing regimen.

For CD8⁺ T cell depletion experiments, animals were administered 100 μ g of depleting or corresponding isotype control monoclonal antibodies via intraperitoneal injection. The anti-CD8 α antibody (clone 53–6.7, BE0004–1, BioXCell) was used to deplete CD8⁺ T cells, with a rat IgG2a isotype control (clone 2A3, BE0089, BioXCell) as control. Antibody administration commenced prior to tumor inoculation, with a dose given 1 day before tumor challenge, followed by once-weekly injections.

Isolation of lymphocytes from mouse tissues

Peripheral blood (100 μ l per mouse) was diluted 1:9 with PBS and centrifuged at $450 \times g$ for 15 min. The pellet was treated with ACK lysis buffer 5 min to lyse red blood cells, washed in PBS, and resuspended in PBS containing 1% FBS. Inguinal lymph nodes (ILN) were excised and rinsed in PBS. They were then mechanically dissociated by pressing through a syringe plunger onto a 100 μ m cell strainer. The resulting homogenate was filtered through the strainer, centrifuged at $300 \times g$ for 7 min, and treated with ACK lysis buffer for 5 min to remove red blood cells. After washing in PBS, cells were resuspended in PBS supplemented with 1% FBS. Lung metastases were dissected into 2–3 mm fragments and subjected to enzymatic digestion at 37 °C for 30 min in RPMI-1640 medium containing 10% heat-inactivated FBS (ABW), 1% penicillin-streptomycin (Beyotime), and 1% Collagenase III (Worthington Biochemical). The digested tissue was passed through a 100- μ m cell strainer and centrifuged at $300 \times g$ for 7 min. The pellet was resuspended in 40% Percoll. A 40%–70% Percoll density gradient was used for lymphocyte isolation by centrifugation at $800 \times g$ for 15 min. The interface containing enriched lymphocytes was collected, treated with ACK lysis buffer for 5 min to remove red blood cells, washed with PBS, and finally resuspended in PBS supplemented with 1% FBS. These procedures were performed as previously described.^[56,57]

Flow cytometry and T cell profiling

For surface staining, cells were seeded at 1×10^6 per well in a 96-well plate and incubated with fluorophore-conjugated antibodies against CD45, CD3, B220, CD8, and CD4 (diluted 1:200 in PBS containing 2% FBS) for 30 min at 4 °C. To assess intracellular production of GZMB, perforin, and IFN- γ , cells were stimulated for 6 h with Cell Activation Cocktail (containing Brefeldin A, BioLegend) prior to fixation and permeabilization. For intracellular staining of p-S6, cells were fixed and permeabilized using the True-Phos Perm Buffer (BioLegend) and fixation Buffer (BioLegend). Subsequently, cells were stained with Phospho-S6 (Ser235, Ser236) Monoclonal Antibody (APC, Thermo Fisher).

Negative selection and in vitro culture of CD8 T cells

Under sterile conditions, lymphocytes were isolated from lung metastatic lesions and spleen using the tissue processing method described previously. CD8⁺ T cells were then enriched by negative selection using a mouse CD8⁺ T Cell Isolation Kit (BEVAER). The purified cells were cultured on plates pre-coated with anti-CD3 (3 μ g/ml) and anti-CD28 (1 μ g/ml) antibodies. After 48 h, quercetin was added to the culture medium at a final concentration of 10 μ M.

Mouse T cell BCAT1 knockdown assay

Following 48 h activation with anti-CD3/CD28, primary mouse CD8⁺ T cells were subjected to BCAT1 knockdown using the ProteanFect Max Primary T Cell Transfection Kit (PT03, Nanoportal Biotech), according to the manufacturer's protocol.

Histopathological analysis

The extent of lung metastasis was initially evaluated based on gross morphological observation and the lung weight-to-body weight ratio. Lung tissues containing metastatic lesions were fixed in formalin and embedded in paraffin. Sequential sections were prepared from paraffin-embedded lung blocks using a Leica microtome and subsequently stained with hematoxylin and eosin (H&E). Whole slide imaging of the sections was performed using a KFBIO digital pathology slide scanner, with the maximal cross-sectional area of the lung captured for further analysis.

Multiplex immunofluorescence staining (mIHC)

Formalin-fixed, paraffin-embedded (FFPE) tissue sections (4 μ m thick) from 9 colorectal cancer patients, with or without metastasis, were deparaffinized and rehydrated. Heat-induced epitope retrieval was performed by incubating sections in EDTA buffer (pH 9.0) at 99 °C for 30 min. After blocking, sections were incubated with primary antibodies at 37 °C for 60 min: anti-CD8 (Zsbio), anti-CD4 (Zsbio), anti-BCAT1 (Abcam), anti-BCAT2 (Abcam), anti-PD-1 (Abcam), and anti-TCF-1 (CST). Subsequently, sections were incubated with HRP-conjugated secondary antibodies at 37 °C for 20 min. Tyramide signal amplification (TSA) was performed using fluorophore-conjugated tyramides as follows: CF430 (Biotium, 96,053) for CD8, AF488 (AAT, 11,070) for CD4, AF594 (AAT, 11,082) for PD-1, Cy3 (AAT, 11,065) for TCF-1, Cy5 (AAT, 11,066) for BCAT2, and XTSA690 (AKF-, AXT6710000) for BCAT1, each incubated at room temperature for 10 min. Between each TSA cycle, antibodies were stripped by incubating sections in EDTA buffer (pH 9.0) at 99 °C for 8 min. Finally, nuclei were counterstained with DAPI.

Formalin-fixed, paraffin-embedded (FFPE) tissue sections (4 μ m thick) of lung metastases from mice treated with four different drug regimens were deparaffinized and rehydrated. Heat-induced epitope retrieval was performed by incubating the sections in EDTA buffer (pH

9.0) at 99 °C for 30 min. After blocking, sections were incubated with primary antibodies at 37 °C for 60 min: anti-CD8 (Proteintech), anti-EPCAM (Abcam), and anti-p-S6 (Proteintech). Subsequently, sections were incubated with HRP-conjugated secondary antibodies at 37 °C for 20 min. Tyramide signal amplification (TSA) was carried out using fluorophore-conjugated tyramides as follows: AF594 (ATT, 11,082) for CD8, AF488 (AAT, 11,070) for EPCAM, and XTSA690 (alphaxbio, AXT6710000) for p-S6, each incubated at room temperature for 10 min. Between TSA cycles, antibodies were stripped by incubating the sections in EDTA buffer (pH 9.0) at 99 °C for 8 min. Finally, nuclei were counterstained with DAPI.

Measurement of intracellular branched-chain amino acids (BCAAs)

To quantify the intracellular BCAA levels, primary CD8⁺ T cells were harvested after the indicated *in vitro* treatments and washed thoroughly with cold PBS to remove any residual BCAA from the culture medium. The cells were then lysed, and the intracellular BCAA concentrations were determined using a Branched-Chain Amino Acid (BCAA) Assay Kit (Cat. No S0535S; Beyotime Biotechnology, Shanghai, China) according to the manufacturer's instructions. The absorbance was measured at 450 nm using a microplate reader, and the absolute BCAA concentrations were calculated based on the standard curve and normalized to the total cell number.

Western blot

Total cellular protein was extracted using a lysis buffer suitable for both Western blotting and immunoprecipitation. For each sample, 20 µg of protein was separated by SDS-PAGE and transferred onto a PVDF membrane. The membrane was then blocked with 5% skimmed milk in PBS for 1 h at room temperature. Following blocking, the membrane was incubated overnight at 4 °C with primary antibodies targeting specific proteins, including anti-BCAT1, anti-BCAT2, anti-BCKDH- α , anti-BCKDH- β , anti-DBT, anti-p-S6, and anti- β -actin. After primary antibody incubation, the membrane was probed with an HRP-conjugated secondary antibody for 1 h at room temperature. Protein detection was performed using an enhanced chemiluminescence (ECL) substrate, and signals were visualized by chemiluminescent imaging.

Quantitative reverse transcription PCR

Reverse transcription quantitative PCR (RT-qPCR) was performed as follows. Total RNA was extracted from tumor cells or cultured cells using Trizol reagent (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized from the extracted RNA using a High Capacity RNA-to-cDNA Kit (Applied Biosystems, Vazyme) following the manufacturer's protocol. Real-time PCR was carried out using SYBR Green Master Mix (Vazyme). Gene expression levels were determined based on threshold cycle (Ct) values measured during the linear amplification phase. All data were normalized to GAPDH expression and analyzed using the $\Delta\Delta C_t$ method for relative quantification.

Molecular docking

The crystal structure of BCAT1 (PDB ID: 7NWA) was preprocessed in PyMOL 3.0, including the removal of heteroatoms (*e.g.*, solvent molecules, ions, and small molecules) and structural repairs. Both the protein receptor and the small-molecule ligand (quercetin) were then prepared in AutoDockTools 4 and saved in .pdbqt format. Global docking of the processed receptor and ligand was conducted using AutoDock Vina 1.2.5. Default docking parameters were applied: grid spacing = 0.375 Å and exhaustiveness = 8. The prepared .pdbqt files were used as input, and the docking simulation was run under the specified settings. The resulting poses were ranked by docking score, and the top-scoring conformation was selected for further analysis. Protein-ligand

interactions were analyzed in Discovery Studio Visualizer 2024, and structural visualizations were generated with PyMOL 3.0 (Ali et al., 2026; Eberhardt et al., 2021; Morris et al., 2009).

Molecular dynamics simulation

Molecular dynamics simulations were performed using the GRO-MACS software package (Van Der Spoel et al., 2005). The protein receptor was described using the AMBER99SB-ILDN all-atom force field, while the ligand parameters and topology were generated using the General AMBER Force Field to ensure compatibility (Lindorff-Larsen et al., 2010; Wang et al., 2004). The protein-ligand complex was immersed in a cubic simulation box 4 with a minimum distance of 1.0 nm between the solute and the box edges. The system was solvated using the SPC216 water model, and Na⁺ or Cl⁻ ions were added to neutralize the total net charge. Energy minimization was initially conducted using the steepest descent algorithm to remove any steric clashes. Subsequently, the system underwent equilibration in two successive stages: a constant volume ensemble and a constant pressure ensemble. Following equilibration, a 100 ns production MD simulation was executed with GPU acceleration enabled via the CUDA toolkit. Structural stability and flexibility were assessed by calculating the Root Mean Square Deviation, Root Mean Square Fluctuation, and Radius of Gyration. Intermolecular interactions were evaluated through hydrogen bond profiling and center-of-mass distance analysis. Finally, the Free Energy Landscape was constructed based on the relative Gibbs free energy, which was calculated from the probability distribution of the first two principal components (PC1 and PC2).

Surface plasmon resonance (SPR) analysis

Surface plasmon resonance analysis was performed using a Biacore system. The running buffer for all steps was 1 × PBS-pH (pH 7.4). For ligand immobilization, a CM5 sensor chip was installed according to the manufacturer's instructions. Flow channel 2 was activated with a 1:1 mixture of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) at a flow rate of 10 µl/min. Recombinant human BCAT1 protein, diluted to 50 µg/ml in sodium acetate buffer (pH 5.0), was then immobilized onto the activated channel at the same flow rate, yielding a final immobilization level of approximately 9655 response units (RU). Remaining active groups were blocked with ethanolamine-HCl (pH 8.5). Channel 1 underwent the same activation and blocking procedure but was injected with sodium acetate buffer without protein to serve as a reference surface for background subtraction. Solvent correction was performed prior to analyte binding assays using a calibration series with DMSO concentrations ranging from 4.5% to 5.8% to account for buffer effects. For the affinity measurement (kinetic analysis), quercetin was diluted in the running buffer containing 5% DMSO to generate a series of concentrations (0.3125, 0.625, 1.25, 2.5, 5, and 10 µM) using a two-fold serial dilution. The analyte was then injected over the BCAT1-immobilized chip and reference surfaces from the lowest to the highest concentration at 30 µl/min for 150 s (association phase), followed by buffer flow for dissociation. The sensor surface was regenerated with 10 mM glycine-HCl (pH 2.0) between cycles. Binding kinetics were analyzed using the Biacore Insight Evaluation Software. Sensorgrams were double-referenced and globally fitted to a 1:1 Langmuir binding model to determine the association rate (K_a), dissociation rate constant (K_d), and dissociation constant (K_D).

Statistical analysis

Flow cytometry data were analyzed using FlowJo v10.8.1 (BD Biosciences). Statistical analyses were performed in GraphPad Prism 10 and R v4.1.2 within RStudio. Comparisons between two independent groups were analyzed using the unpaired Student's *t*-test. Comparisons among three or more groups with a single independent variable were conducted

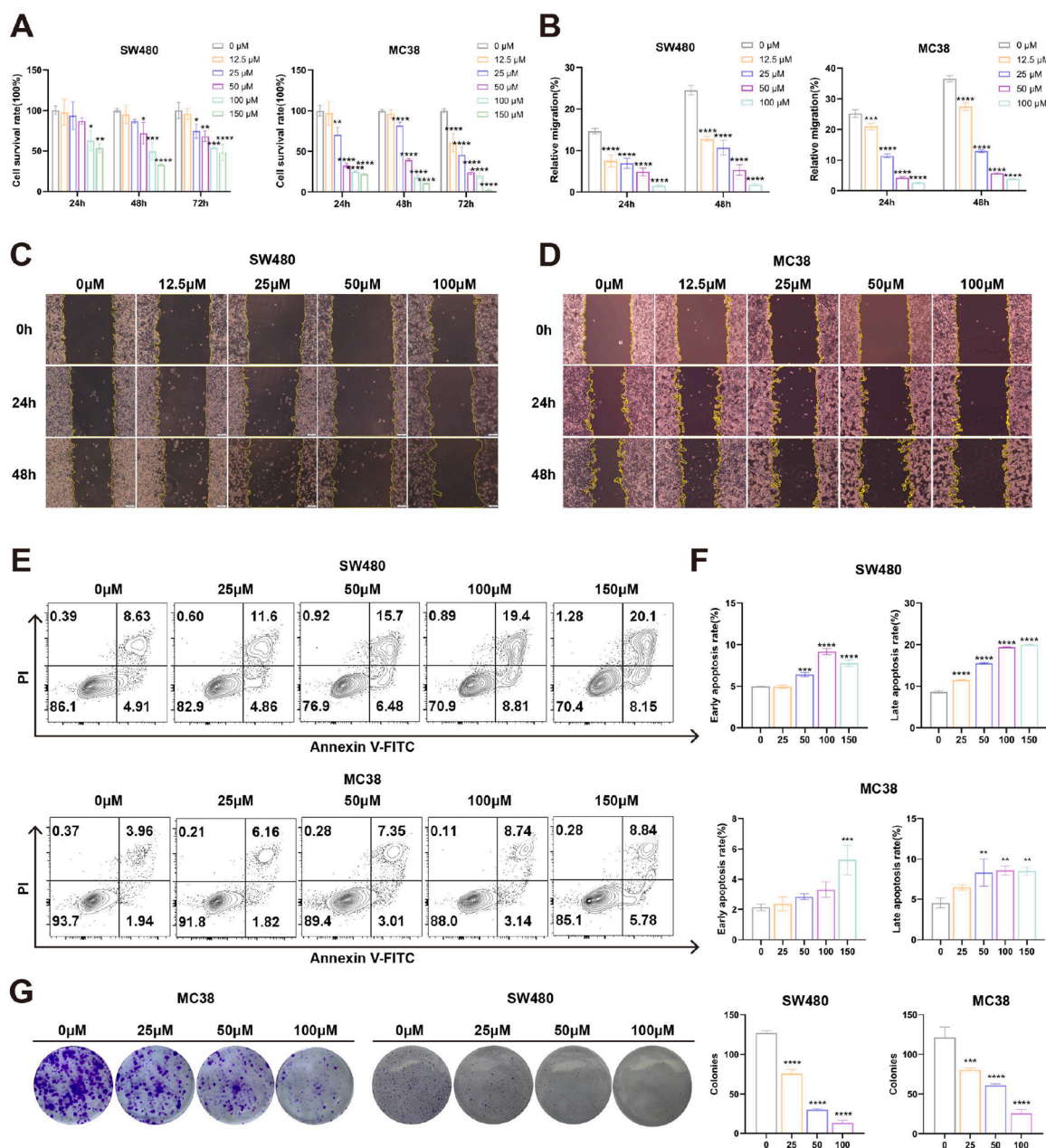


Fig. 1. *In vitro* inhibition of colorectal cancer cell growth and metastasis by quercetin. (A) Viability of human SW480 and mouse MC38 cells treated with quercetin at indicated doses, as assessed by CCK-8 assay ($n = 3$). (B) Quantification of wound healing/migration capacity of SW480 and MC38 cells after quercetin treatment ($n = 3$). (C) Representative images of wound healing assays in SW480 cells. (D) Representative images of wound healing assays in MC38 cells. (E) Flow cytometry plots showing apoptosis (Annexin V-FITC/PI staining) in SW480 and MC38 cells after treatment with quercetin. (F) Quantitative analysis of early and late apoptosis from (E) ($n = 3$). (G) Colony formation assays and corresponding quantification in SW480 and MC38 cells treated with quercetin ($n = 3$).

using one-way analysis of variance (ANOVA), whereas data involving two independent variables were evaluated using two-way ANOVA, followed by Tukey's or Bonferroni's post-hoc tests as appropriate. All experiments were performed in at least three independent biological replicates. All data are expressed as mean $\pm$ standard error of the mean (SEM) from at least three independent experiments. The exact sample sizes (n) are specified in the respective figure legends. A P -value < 0.05 was considered statistically significant. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

Results

In vitro inhibition of colorectal cancer cell growth and metastasis by quercetin

To evaluate the direct anti-tumor effects of quercetin, we first examined its impact on the viability, migration, apoptosis, and clonogenicity of CRC cells *in vitro*. Using CCK-8 and wound healing assays, we found that quercetin dose- and time-dependently suppressed the proliferation of both human SW480 and mouse MC38 cells and significantly inhibited their migratory capacity (Fig. 1A–D). Further analysis by Annexin V-FITC/PI staining demonstrated that quercetin promoted early and late apoptosis in a dose-dependent manner in both cell lines

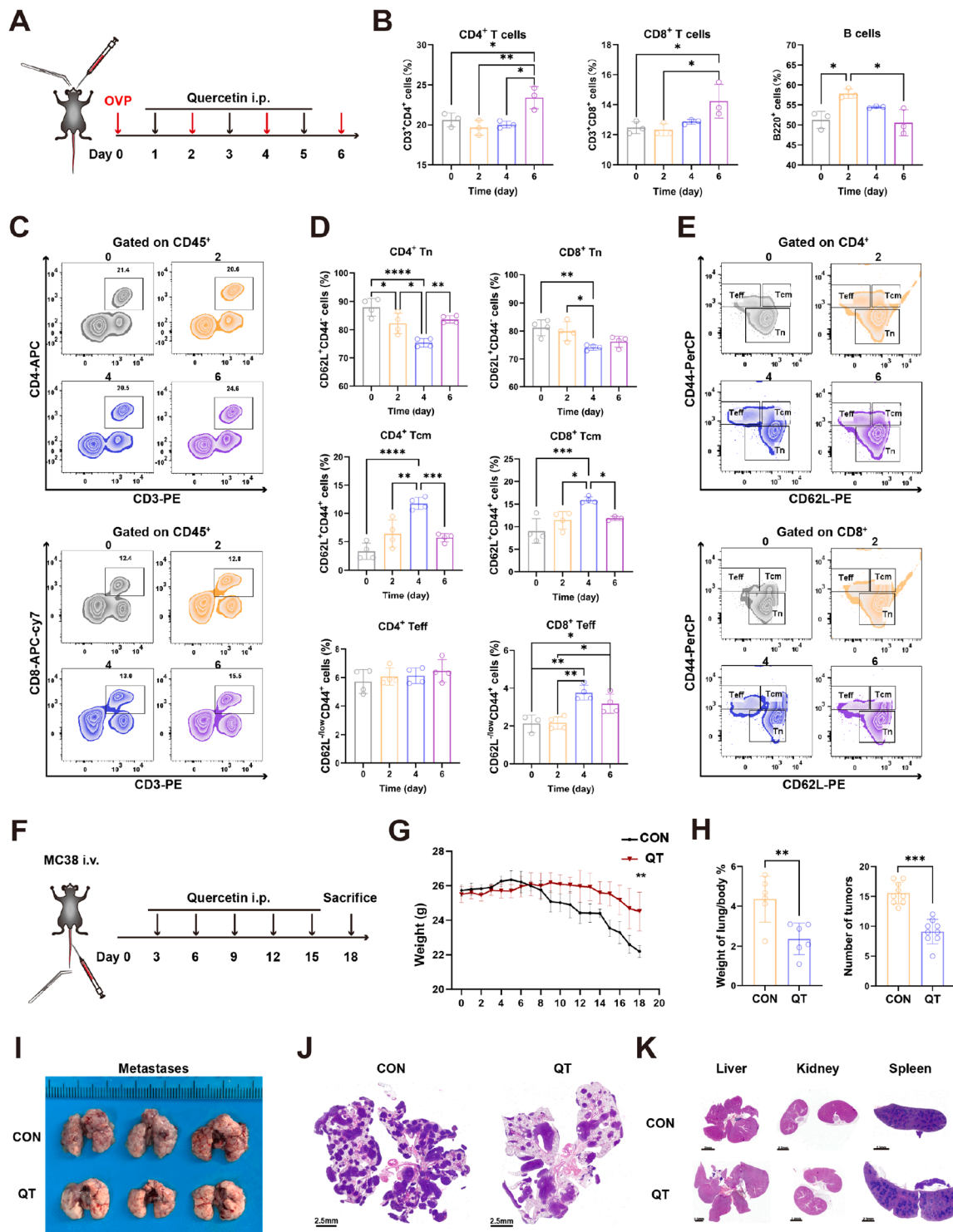


Fig. 2. Quercetin promotes effector differentiation of CD8⁺ T cells in peripheral blood and suppresses experimental colorectal cancer lung metastasis *in vivo*. (A) Schematic of the administration and sampling timeline. Healthy mice received intraperitoneal injections of quercetin on days 1, 3, and 5, with orbital blood collection on days 0, 2, 4, and 6. (B) Quantification of the proportions of CD4⁺ T cells, CD8⁺ T cells, and B cells in peripheral blood over time, as assessed by flow cytometry ($n = 3$). (C) Representative flow cytometry plots showing CD4⁺ and CD8⁺ T cell populations in peripheral blood on indicated days. (D) Quantification of Tn, Tcm, and Teff subsets within CD4⁺ and CD8⁺ T cell compartments over time ($n = 4$). (E) Representative flow cytometry plots depicting CD4⁺ (Tn, Tcm, Teff) and CD8⁺ (Tn, Tcm, Teff) subsets on indicated days. (F) Schematic of the experimental lung metastasis model and treatment schedule. MC38 cells were injected intravenously to establish lung metastases, followed by quercetin or vehicle treatment. (G) Body weight change of mice in control and quercetin-treated groups following tumor inoculation. (H) Left: Lung-to-body weight ratio of mice in control and quercetin-treated groups. Right: Quantification of visible metastatic nodules on the lung surface ($n = 6$). (I) Representative gross images of lungs from control and quercetin-treated mice. (J) Representative H&E-stained sections of lung tissues showing metastatic foci. (K) Representative H&E-stained sections of liver, kidney, and spleen from control and quercetin-treated mice.

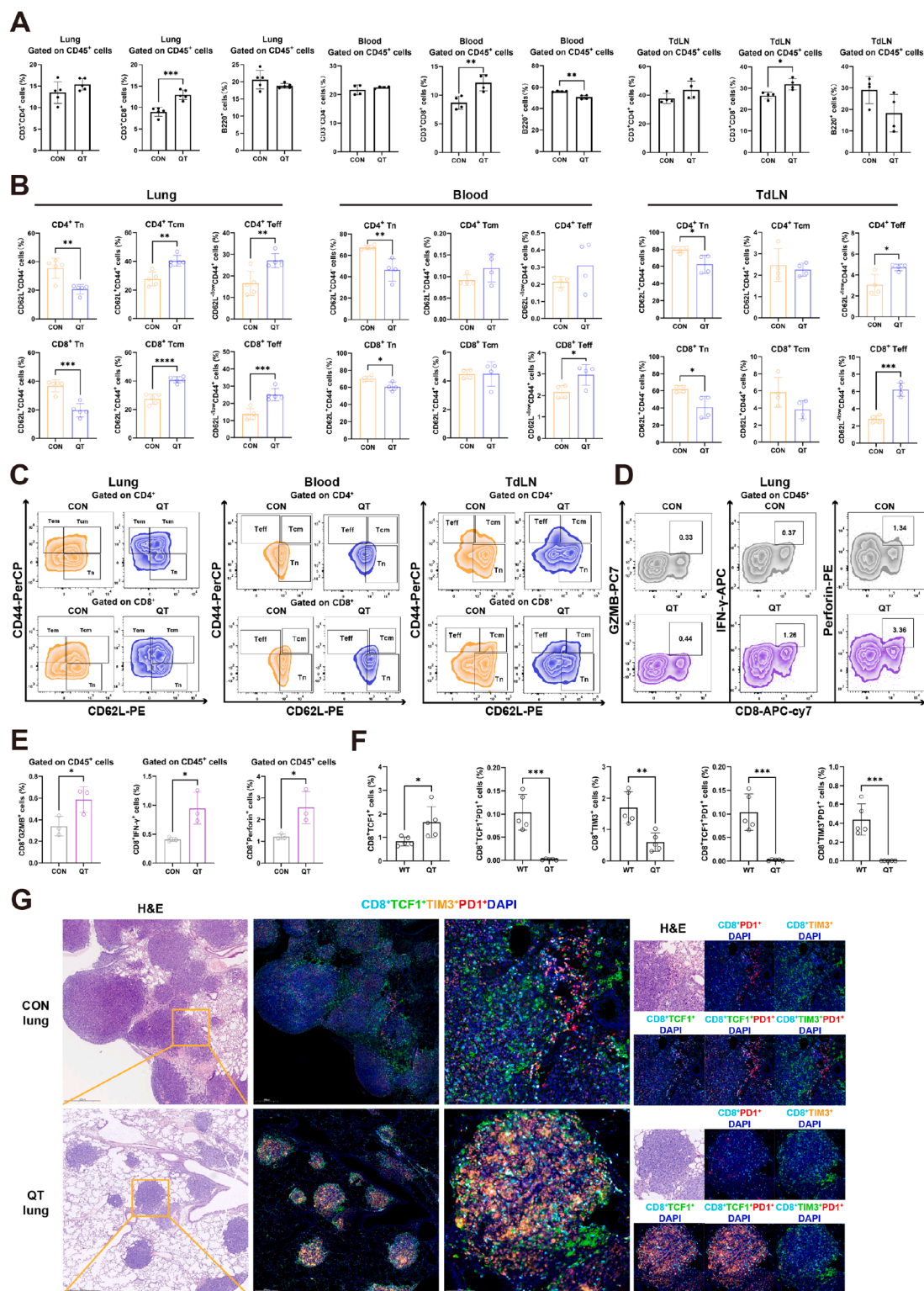


Fig. 3. Quercetin reinvigorates effector function and alleviates exhaustion of CD8⁺ T cells in the tumor microenvironment. (A) Proportions of CD4⁺ T cells, CD8⁺ T cells, and B220⁺ B cells in peripheral blood ($n = 4$), TdLNs ($n = 4$), and lung metastatic foci ($n = 5$) from control and quercetin-treated mice. (B) Frequencies of Tn, Tcm, and Teff subpopulations among CD4⁺ and CD8⁺ T cells in lung metastatic foci ($n = 5$), peripheral blood ($n = 4$), and TdLNs ($n = 4$). (C) Representative flow cytometry plots illustrating CD4⁺ (Tn, Tcm, Teff) and CD8⁺ (Tn, Tcm, Teff) T cell subsets in lung metastatic foci, peripheral blood, and TdLNs. (D) Representative flow cytometry plots of CD8⁺ T cells from lung metastatic foci expressing IFN- γ , GZMB, and perforin. (E) Quantification of IFN- γ ⁺, GZMB⁺, and perforin⁺ CD8⁺ T cells within lung metastatic foci. (F) Quantification of CD8⁺TCF-1⁺, CD8⁺PD-1⁺ and CD8⁺TIM-3⁺ T cells within lung metastatic foci by mIHC. (G) Representative images of H&E staining and multiplex immunofluorescence in lung metastatic sections from control and quercetin-treated mice.

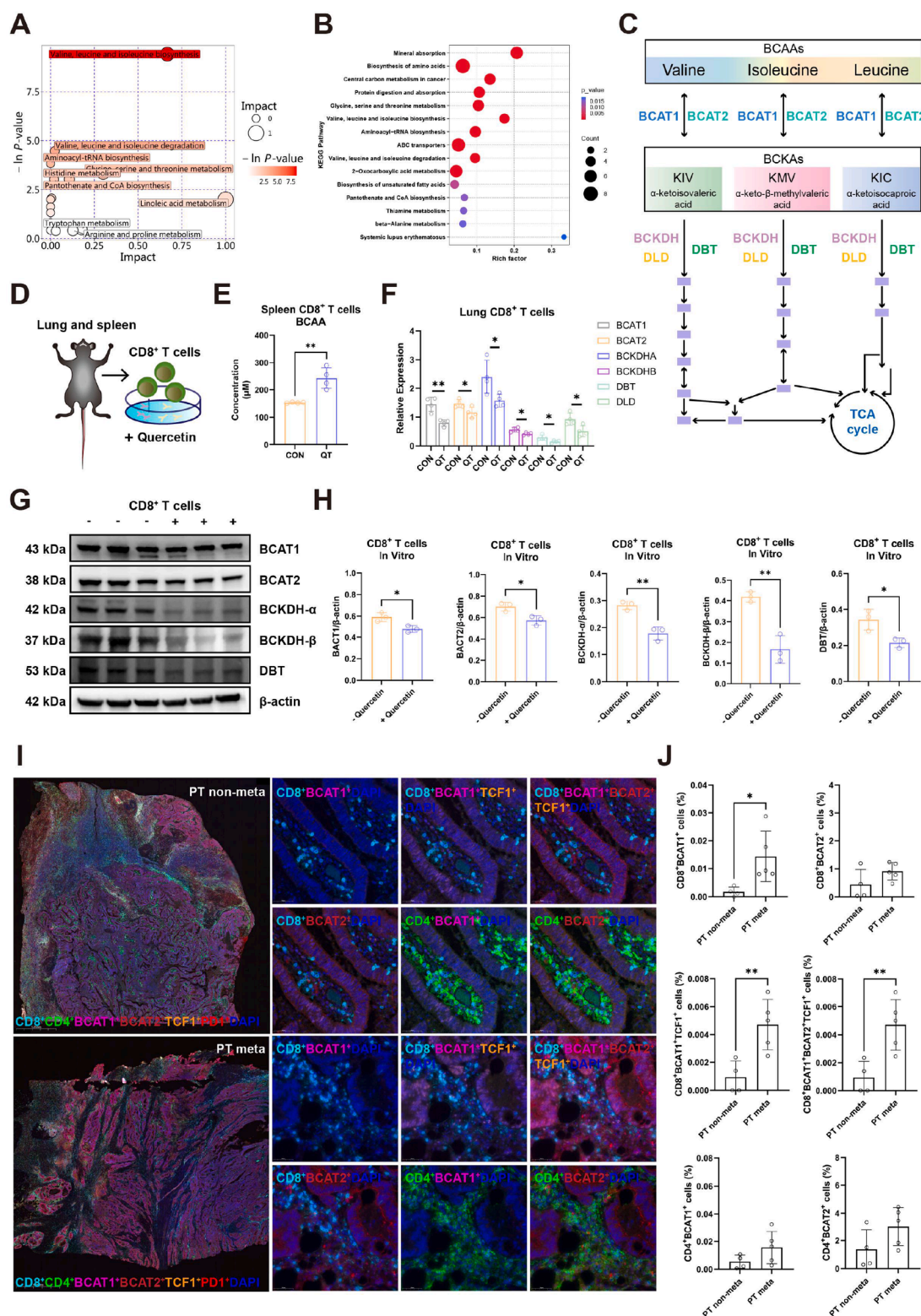


Fig. 4. Quercetin reprograms branched-chain amino acid metabolism in CD8⁺ T cells. (A) Bubble plot depicting untargeted metabolomics analysis of lung metastatic foci from control and quercetin-treated tumor-bearing mice. (B) KEGG pathway enrichment analysis showing significantly altered metabolic pathways in lung metastases following quercetin treatment. (C) Schematic overview of the BCAA catabolic pathway. (D) Schematic of the experimental workflow: CD8⁺ T cells were isolated from lung metastatic foci of tumor-bearing mice and activated *in vitro* with anti-CD3/CD28 antibodies in the presence or absence of quercetin. (E) Intracellular BCAA concentrations in primary CD8⁺ T cells. CD8⁺ T cells were isolated from spleen, activated *in vitro*, and treated with or without quercetin. (F) mRNA expression levels of key BCAA-catabolizing enzymes (BCAT1, BCAT2, etc.) in activated CD8⁺ T cells treated with quercetin *in vitro*, measured by RT-qPCR (n = 4). (G) Representative Western blot images showing protein expression of BCAA pathway enzymes in activated CD8⁺ T cells after quercetin treatment *in vitro*. (H) Quantification of Western blot results from panel G, normalized to β-actin. (I) Representative mIHC images of primary colorectal cancer tissues from patients with or without distant metastasis. (J) Quantification of mIHC data from panel I, comparing the frequencies of CD8⁺BCAT1⁺ T cells and CD8⁺BCAT1⁺TCF1⁺ T cells, etc. in primary tumors of patients with or without metastasis.

(Fig. 1E-F). Notably, quercetin induced a pronounced increase in late apoptosis in SW480 cells, detectable even at 25 μ M. Moreover, colony formation assays confirmed its potent inhibitory effect on long-term clonogenic survival (Fig. 1G). Collectively, these results indicate that quercetin exerts direct growth-suppressive and anti-metastatic actions on CRC cells *in vitro*.

Quercetin promotes the effector differentiation of CD8⁺ T cells in peripheral blood

Prompted by its direct anti-tumor activity *in vitro*, we next evaluated the immunomodulatory potential of quercetin *in vivo* (Fig. 2A). Healthy mice received intraperitoneal quercetin on days 1, 3, and 5 (Fig. S1A). Serial orbital blood samples were collected on days 0, 2, 4, and 6 for comprehensive immunophenotyping by flow cytometry. Analysis revealed dynamic and time-dependent alterations in major lymphocyte subsets. By day 6, the proportions of both CD4⁺ and CD8⁺ T cells were significantly elevated. In contrast, B cell frequency showed a transient increase on day 2 before declining on days 4 and 6 (Fig. 2B-C and Fig. S2). Examination of T cell differentiation states showed a progressive decline in naive CD4⁺ T cells (Tn, CD62L⁺CD44⁻), which reached its nadir on day 4 before a slight rebound on day 6. Conversely, the proportion of CD4⁺ central memory T cells (Tcm, CD62L⁺CD44⁺) showed a significant increase on day 4, while effector CD4⁺ T cells (Teff, CD62L^{low}CD44⁺) did not exhibit a marked change. A similar trend was observed in CD8⁺ T cells: the naive subset declined, whereas CD8⁺ Tcm increased significantly on day 4. Notably, the proportion of CD8⁺ Teff rose markedly by day 4 and, despite a slight decrease by day 6, remained significantly higher than baseline levels. Collectively, these data indicate that quercetin treatment *in vivo* induces systemic immunostimulation, characterized by the expansion of total T cells and the promotion of effector differentiation, particularly within the CD8⁺ T cell compartment (Fig. 2D-E and Fig. S2).

In vivo suppression of experimental colorectal cancer lung metastasis by quercetin

Based on these immunostimulatory effects observed in healthy mice, we proceeded to establish an experimental model of colorectal cancer lung metastasis to evaluate the therapeutic potential of quercetin against tumor dissemination (Fig. 2F). MC38 cells were intravenously injected to initiate lung metastasis, followed by quercetin or vehicle treatment. Quercetin-treated mice exhibited a significantly attenuated loss of body weight compared to the control group (Fig. 2G). Consistent with reduced metastatic burden, the lung-to-body weight ratio was markedly decreased, and the number of visible metastatic nodules on the lung surface was significantly reduced in the quercetin group (Fig. 2H). Gross anatomical observation and subsequent H&E staining of lung tissues confirmed a substantial decrease in metastatic foci (Fig. 2I-J). Histological examination of other major organs revealed no overt signs of quercetin-induced toxicity, although a notable increase in spleen size was observed in the treatment group, suggesting a possible systemic immune response (Fig. 2J-K).

Quercetin reinvigorates effector function and alleviates exhaustion of CD8⁺ T cells

To investigate whether the reduction in metastasis was linked to enhanced anti-tumor immunity, we analyzed immune cell phenotypes in the peripheral blood, lung metastatic foci, and tumor-draining lymph nodes (TdLNs) by flow cytometry. Quercetin treatment profoundly altered systemic and local T-cell landscapes. Systemically, the proportion of CD8⁺ T cells in the peripheral blood was significantly increased (Fig. 3A and Fig. S3). Within the tumor microenvironment and draining sites, a consistent shift from naive to effector phenotypes was observed. In both TdLNs and lung metastatic foci, quercetin treatment led to a

significant reduction in the frequency of naive CD8⁺ T cells, accompanied by a concomitant and significant increase in CD8⁺ Teff. Although the total proportion of CD4⁺ T cells in TdLNs and metastatic foci did not change significantly, their composition was notably altered: naive CD4⁺ T cells decreased, whereas CD4⁺ Teff cells increased. Quercetin treatment also significantly enhanced the effector function of CD8⁺ T cells within lung metastatic foci (Fig. 3B-D and Fig. S4). Functional analysis revealed a substantial increase in the frequency of CD8⁺ T cells producing IFN- γ , as well as those expressing the key cytotoxic mediators Granzyme B (GZMB) and Perforin, collectively indicating a potent boost in their cytotoxic activity (Fig. 3E).

We further assessed T cell exhaustion by mIHC staining. Quercetin treatment significantly reduced the proportion of exhausted CD8⁺ T cells in lung metastases, as showed by decreased frequencies of PD-1⁺CD8⁺ and TIM-3⁺CD8⁺ cell populations (Fig. 3F-G). This reduction in canonical exhaustion markers suggests that quercetin not only enhances effector function but also alleviates the exhausted state of CD8⁺ T cells within the tumor microenvironment.

Quercetin exerts anti-metastatic efficacy in a CD8⁺ T cell-dependent manner

To definitively ascertain whether CD8⁺ T cells are indispensable for the anti-metastatic effects of quercetin *in vivo*, we performed an antibody-mediated CD8⁺ T cell depletion in the MC38 lung metastasis model (Fig. S5A). To verify the efficacy of the CD8⁺ T cell depletion, we performed flow cytometric analysis of the peripheral blood. The results confirmed that the anti-CD8 neutralizing antibody successfully eliminated the CD8⁺ T cell population, achieving a depletion efficiency of >98%, ensuring the reliability of our subsequent *in vivo* observations (Fig. S5B). Macroscopic observation and H&E staining of the lungs revealed that while quercetin administration dramatically suppressed the formation of metastatic nodules in immunocompetent mice, this profound therapeutic efficacy was virtually abolished upon CD8⁺ T cell depletion (Fig. S5C-D). Quantitative analysis further demonstrated that in the absence of CD8⁺ T cells, quercetin failed to significantly reduce the lung-to-body weight ratio or the total number of lung metastatic tumors (Fig. S5E). These compelling *in vivo* data strongly indicate that the protective role of quercetin against colorectal cancer metastasis is critically dependent on the presence of functional CD8⁺ T cells.

Quercetin reprograms branched-chain amino acid metabolism in CD8⁺ T cells

Given the observed functional reinvigoration of CD8⁺ T cells, we hypothesized that quercetin might exert its effects by remodeling their metabolic state, a key determinant of T cell effector potential. Untargeted metabolomic analysis of lung metastatic foci from tumor-bearing mice revealed profound alterations in branched-chain amino acid (BCAA) metabolism following quercetin treatment (Fig. 4A-C).

To directly examine whether quercetin modulates BCAA-related metabolic pathways in CD8⁺ T cells within the complex tumor microenvironment of lung metastases, CD8⁺ T cells were first isolated from lung metastatic foci of untreated tumor-bearing mice, activated *in vitro*, and treated with quercetin for transcriptional analysis (Fig. 4D). We first quantified the intracellular BCAA levels using a biochemical assay. The results demonstrated that quercetin treatment induced a significant intracellular accumulation of BCAAs within these primary CD8⁺ T cells (Fig. 4E). Transcriptional analysis showed that quercetin significantly downregulated mRNA expression of key BCAA-catabolizing enzymes, notably BCAT1 and BCAT2, as well as several other enzymes involved in this pathway (Fig. 4F). To confirm these findings *in vitro*, CD8⁺ T cells were then magnetically purified from spleens of quercetin-treated mice for protein-level validation by Western blotting (Fig. 4G). Consistent with the transcriptional data, protein levels of BCAT1 and BCAT2, and other relevant metabolic enzymes were also markedly reduced. These

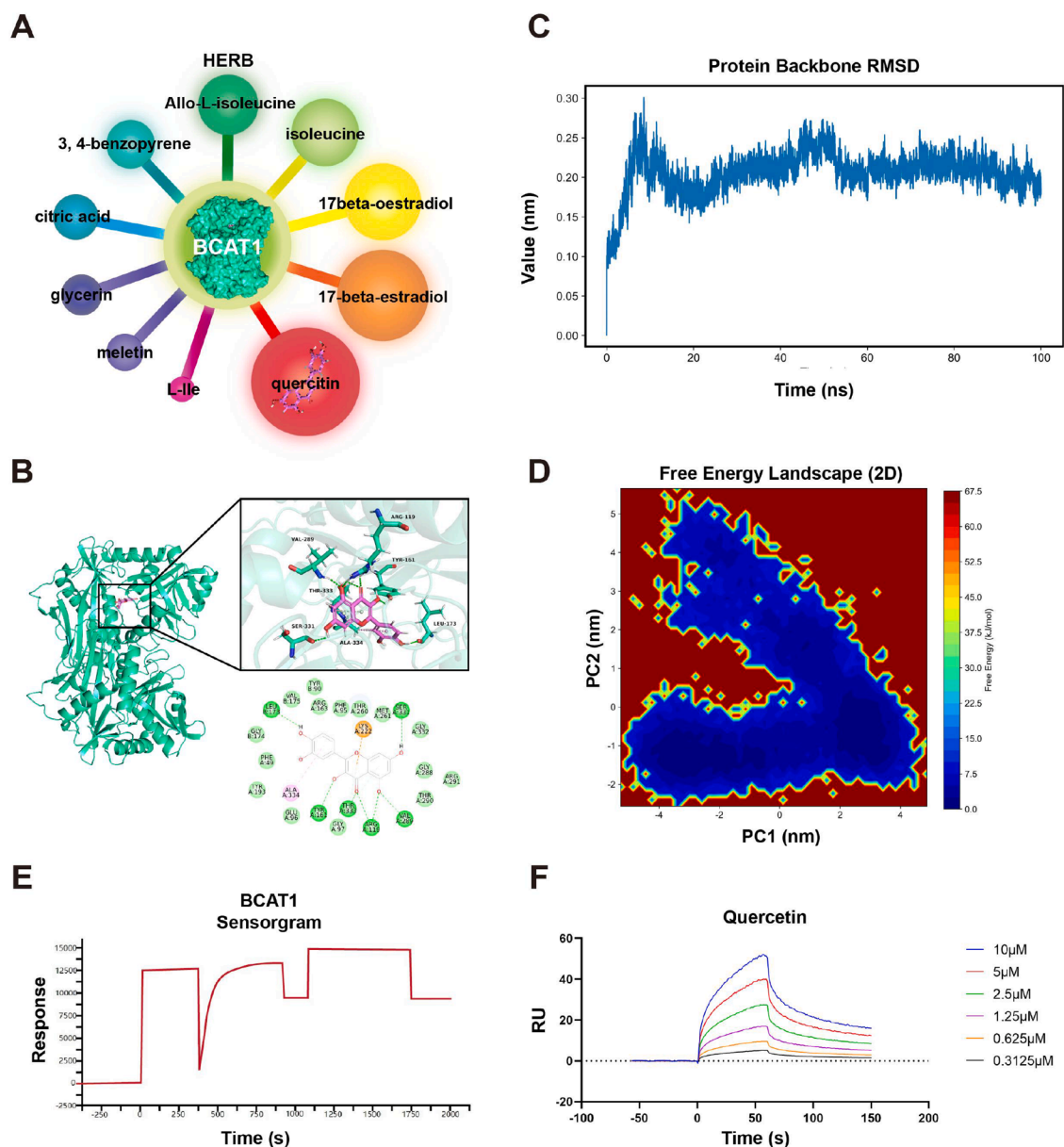


Fig. 5. Quercetin directly binds to and inhibits BCAT1. (A) Screening of the HERB database identified quercetin as a candidate ligand for BCAT1. (B) Molecular docking simulation predicted a high binding affinity between quercetin and BCAT1. (C–D) Detailed view of the predicted binding interface between quercetin and BCAT1. (E) Representative sensorgram showing the immobilization of human BCAT1 protein onto the SPR chip. A total of 9655 response units (RU) of protein were conjugated. (F) SPR binding sensorgrams and derived kinetic parameters (association rate constant K_a 1.70×10^3 (1/Ms), dissociation rate constant K_d 6.98×10^{-3} (1/s), and dissociation constant K_D 4.11×10^{-6} (M)) confirm a strong and specific interaction between quercetin and BCAT1.

results demonstrate that quercetin broadly suppresses BCAA metabolism in $CD8^+$ T cells by downregulating multiple key enzymes (Fig. 4H).

Having established that quercetin suppresses BCAA catabolism in $CD8^+$ T cells, we next investigated its concurrent impact on tumor cells. Interestingly, quercetin treatment also downregulated BCAT1 expression in MC38 cells in a dose-dependent manner (Fig. S6A–B). To rigorously assess the mechanistic dependence on BCAT1, we performed an *in vitro* targeted knockdown in MC38 cells. While either quercetin treatment or BCAT1 silencing (siBcat1) independently suppressed downstream mTORC1 signaling, evidenced by reduced p-S6 levels, the combination of quercetin and siBcat1 induced a profound, additive suppression of p-S6 expression (Fig. S6C–D). These results suggest that in tumor cells, quercetin exerts a multi-targeted dual effect, impairing metabolic flexibility through BCAT1 suppression while concurrently inhibiting upstream survival signals.

To visualize this divergent metabolic rewiring *in situ*, we evaluated

mTORC1 activation within the lung metastatic microenvironment using mIHC. Strikingly, while quercetin suppressed overall p-S6 levels in the tumor bulk, it specifically enhanced p-S6 signaling within infiltrating $CD8^+$ T cells (Fig. S6E–F). This indicates a favorable metabolic competition, wherein nutrients in the microenvironment are preserved from tumor utilization and preferentially sequestered by T cells to fuel their activation.

To explicitly confirm that this quercetin-induced metabolic shift translates to enhanced immune-mediated cytotoxicity, we performed an *ex vivo* co-culture assay with MC38 cells and primary $CD8^+$ T cells. Although quercetin alone induced moderate tumor cell apoptosis, the addition of $CD8^+$ T cells synergistically amplified MC38 cell death, significantly increasing both early and late apoptosis rates (Fig. S6G–H). Crucially, flow cytometric analysis of the co-cultured $CD8^+$ T cells revealed that quercetin treatment significantly upregulated their intracellular p-S6 levels, which was accompanied by a robust increase in the

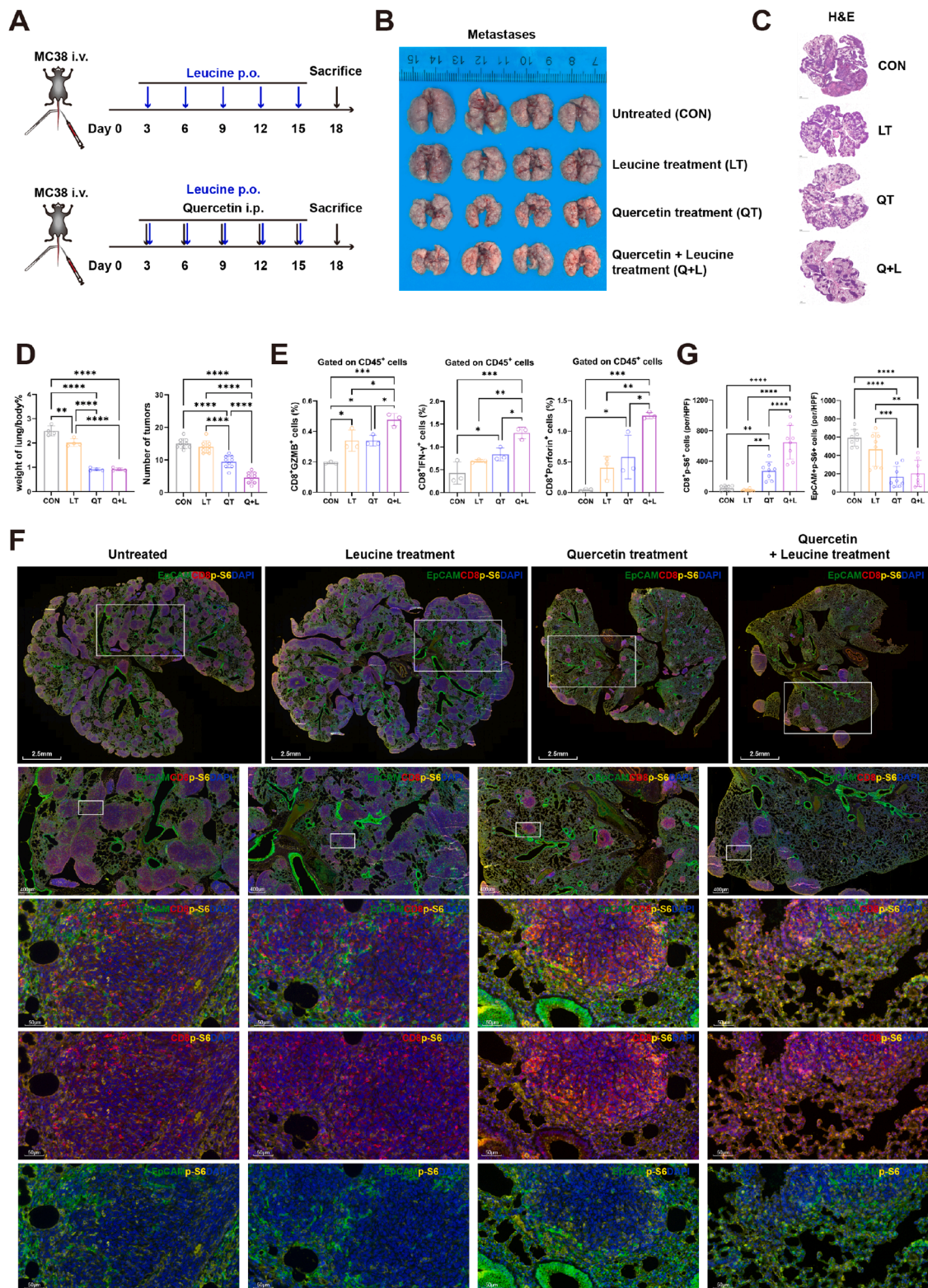


Fig. 6. Synergistic enhancement of anti-tumor immunity and lung metastasis suppression by quercetin and leucine. (A) Schematic of the *in vivo* treatment strategy. Mice with established MC38 lung metastases were treated with quercetin, leucine, or their combination. (B) Representative gross images of lungs from untreated control and treatment groups. (C) Representative H&E-stained sections of lung tissues showing metastatic foci. (D) Lung-to-body weight ratios of mice across different treatment groups ($n = 4$) and quantification of visible metastatic nodules on lung surfaces. (E) Flow cytometric analysis of cytotoxic CD8⁺ T cells from lung metastatic foci. (F) Representative mIHC images of lung metastatic sections stained for EpCAM (green), CD8 (red), p-S6 (yellow), and DAPI (blue). (G) Quantitative analysis of CD8⁺p-S6⁺ cells and EpCAM⁺p-S6⁺ cells per high-power field (HPF) across indicated groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

production of effector molecules, including GZMB, IFN- γ , and Perforin (Fig. S6I-J). Taken together, these data demonstrate that quercetin unleashes a synergistic anti-tumor attack by directly crippling tumor cell metabolism while concomitantly potentiating CD8⁺ T cell effector function through mTORC1 activation.

Having established that quercetin rewires BCAA metabolism and activates the mTORC1/S6 signaling pathway, we next sought to confirm whether BCAT1 acts as the direct functional target for quercetin-mediated CD8⁺ T cell potentiation. We silenced Bcat1 in primary murine CD8⁺ T cells using specific siRNA, and the knockdown efficiency was robustly validated by RT-qPCR (Fig. S7A-B). In the *in vitro* co-culture system, quercetin treatment significantly enhanced the capacity of siNC-transfected CD8⁺ T cells to induce apoptosis in MC38 tumor cells (Fig. S7C-D). Furthermore, quercetin promoted the effector T cell polarization (Fig. S7E-F) and upregulated the secretion of cytotoxic molecules, including Perforin, IFN- γ , and GZMB (Fig. S7G-H). Critically, following Bcat1 knockdown, quercetin treatment no longer exerted a significant effect on these responses. Specifically, quercetin-induced activation of p-S6 was entirely dependent on BCAT1 expression, as treatment with quercetin in the siBcat1 group did not lead to a further significant increase in the proportion of p-S6 positive cells compared with the siBcat1 group without quercetin (Fig. S7I-J).

To investigate the clinical relevance of BCAA metabolism in T cells, we analyzed primary tumor tissues from 9 colorectal cancer patients using mIHC staining (Fig. 4I). Our results revealed that patients with distant metastases exhibited a significantly higher proportion of CD8⁺BCAT1⁺ T cells within the primary tumor, as well as a notable increase in CD8⁺BCAT1⁺TCF1⁺ T cells. In contrast, frequencies of CD8⁺BCAT2⁺ or CD4⁺BCAT1⁺ T cells showed no significant correlation with metastatic status (Fig. 4J). These findings suggest that elevated BCAT1 expression in CD8⁺ T cells, particularly within a TCF1⁺ subset, may be associated with tumor metastasis in colorectal cancer.

Quercetin directly binds to and inhibits BCAT1

To elucidate the molecular mechanism underlying quercetin-mediated BCAT1 suppression, we first performed an *in silico* screening of potential BCAT1 binders using the HERB database. This analysis identified quercetin as a candidate ligand (Fig. 5A). Subsequent molecular docking simulations predicted a high binding affinity between quercetin and BCAT1 (Fig. 5B). Detailed analysis of the binding interface revealed a network of interactions stabilizing the complex, including hydrogen bonds, Pi-hydrogen bonds, electrostatic (Pi-cation), hydrophobic (Pi-alkyl), and van der Waals forces. Specifically, hydrogen bonds were formed with BCAT1 residues Ser331, Tyr161, Val289, Arg119, and Thr333 on chain A, as well as Leu173 on chain B. Additional interactions included a Pi-hydrogen bond with Thr333 (chain A), a Pi-cation interaction with Lys222 (chain A), and a Pi-alkyl interaction with Ala334 (chain A). These computational data strongly support quercetin as a direct BCAT1 binder, providing a structural rationale for its inhibitory effect (Fig. 5C-D).

To biophysically validate this prediction, we then employed surface plasmon resonance (SPR) analysis to assess the direct interaction between recombinant Human BCAT1 protein and quercetin (Fig. 5E-F). The SPR binding kinetics confirmed a strong and specific interaction. Quercetin exhibited rapid association and slow dissociation with Human BCAT1 protein, indicating high binding affinity and stable complex formation, which aligns with the molecular docking results and provides direct biophysical evidence for quercetin's role as a BCAT1 binder.

Synergistic enhancement of anti-tumor immunity and lung metastasis suppression by quercetin and leucine

To investigate the functional interaction between quercetin and BCAAs metabolism *in vivo*, we established four treatment cohorts in the MC38 lung metastasis model: untreated control (CON), leucine

monotherapy (LT), quercetin monotherapy (QT), and a combination of quercetin plus leucine (Q+L) (Fig. 6A). Macroscopic and histological evaluation of lung metastases revealed a pronounced reduction in metastatic burden following quercetin and combination treatments (Fig. 6B-C). Consistent with this observation, both the lung-to-body weight ratio and the number of visible tumor nodules were significantly decreased, with the Q+L combination therapy exerting the most profound metastatic suppressive effect (Fig. 6D).

To determine whether this enhanced efficacy was driven by local immune activation, we evaluated the functional state of CD8⁺ T cells within the metastatic foci using flow cytometry. While monotherapies showed trends of immune activation, the Q+L combination synergistically upregulated the frequencies of GZMB⁺, IFN- γ ⁺, and Perforin⁺ CD8⁺ T cells, indicating a massive boost in local anti-tumor cytotoxicity (Fig. 6E and Fig. S8).

To address the metabolic fate of the supplemented leucine, we evaluated *in situ* mTORC1 activation indicated by p-S6 using mIHC. We co-stained for EpCAM to identify tumor cells and CD8 for T cells within the metastatic microenvironment (Fig. 6F). In the leucine monotherapy (LT) group, p-S6 signaling was predominantly enriched within EpCAM⁺ tumor cells, confirming that tumors avidly utilize exogenous leucine. However, in the combination (Q+L) group, this metabolic landscape was completely inverted. Quercetin treatment profoundly suppressed p-S6 in EpCAM⁺ tumor cells, while the p-S6 signal became strikingly concentrated and co-localized with infiltrating CD8⁺ T cells. Quantification confirmed a significant decrease in EpCAM⁺p-S6⁺ cells alongside a dramatic surge in CD8⁺p-S6⁺ cells specifically in the Q+L cohort (Fig. 6G). These *in situ* data provide compelling visual and quantitative proof that quercetin prevents tumor cells from exploiting extracellular leucine, allowing reinvigorated T cells to metabolically hijack the nutrient to fuel their own cytotoxic response.

Together, these results indicate that quercetin not only exerts standalone anti-metastatic activity but also synergizes with leucine to further suppress lung metastasis, supporting the therapeutic potential of co-targeting BCAA metabolism to boost anti-tumor immunity and control metastatic spread.

Discussion

The natural flavonoid quercetin has attracted considerable attention for its broad anticancer properties, traditionally ascribed to direct pro-apoptotic and anti-proliferative effects on tumor cells (Lotfi et al., 2023; Maleki Dana et al., 2021; Maugeri et al., 2023; Xie and Wei, 2025). However, growing evidence underscores the decisive role of the tumor microenvironment and host immunity in metastatic progression (De Visser and Joyce, 2023; Grant and Ferrer, 2025; Reck et al., 2025; Zhao et al., 2026a). Within this framework, the immunomodulatory potential of dietary compounds such as quercetin remains relatively unexplored (Han et al., 2025; Kurek-Górecka et al., 2025; Yu et al., 2026). Our work critically redefines quercetin's function as a central orchestrator of the immunometabolic microenvironment. We demonstrate that quercetin suppresses colorectal cancer metastasis not merely through direct cytotoxicity, but primarily by profoundly remodeling anti-tumor immunity via the targeted inhibition of BCAT1-mediated branched-chain amino acid catabolism.

A central mechanistic advance of this study lies in elucidating the dual-mechanism by which quercetin differentially regulates mTORC1 signaling in tumor cells *versus* CD8⁺ T cells. As demonstrated by our analysis using siBcat1 in MC38 cells, quercetin effectively phenocopies the metabolic stress induced by BCAT1 ablation. Furthermore, the additive collapse of p-S6 observed with the combination of siBcat1 and quercetin aligns with the well-documented polypharmacology of this natural product, which may concurrently antagonize upstream survival kinases in cancer cells. Conversely, in CD8⁺ T cells, quercetin-mediated BCAT1 inhibition prevents the degradation of leucine, allowing its intracellular accumulation to act as a potent signaling bolus that triggers

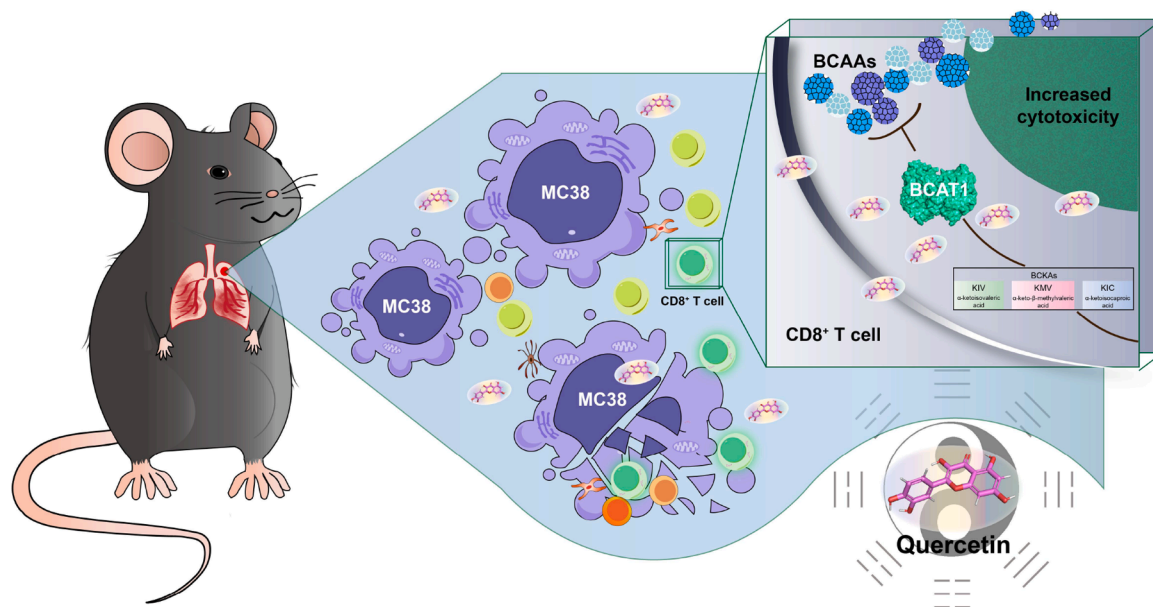


Fig. 7. Mechanism diagram of quercetin targets BCAT1 to potentiate CD8⁺ T cell immunity.

mTORC1 activation. This metabolic rewiring successfully reverses T cell exhaustion, downregulating PD-1 and TIM-3 while significantly boosting the production of GZMB, IFN- γ , and Perforin.

This divergent metabolic rewiring resolves the apparent leucine paradox and provides a rationale for our observed synergy between quercetin and leucine supplementation. By specifically evaluating *in situ* p-S6 via mIHC, we provided direct evidence for a metabolic competition model within the tumor microenvironment. In the presence of quercetin, reinvigorated T cells preferentially sequestered the supplemented leucine to fuel their cytotoxic response, leaving the BCAT1-impaired tumor cells metabolically starved. Importantly, this synergistic effect is likely highly dose-dependent.

The low dose of leucine (10 mg/kg) utilized in our study acts specifically as a signaling trigger for T cells; however, excessive dietary leucine supplementation could paradoxically risk fueling tumor anabolism. In a clinical setting, indiscriminate leucine supplementation could theoretically pose a risk of promoting tumor growth. However, it is crucial to note that in our study, leucine was administered in combination with quercetin, which exerts direct anti-neoplastic effects and inhibits BCAT1-mediated metabolic reprogramming. We hypothesize that this combination therapy may 'tilt the scales' in favor of T cell reinvigoration over tumor fuel, but the safety and precise dosage of such a strategy require rigorous evaluation in future clinical trials.

Clinically, our multiplex analysis of primary colorectal cancer tissues identified a significant enrichment of BCAT1⁺CD8⁺TCF1⁺ T cells in patients with distant metastases. We speculate that this specific subpopulation may represent a distinct progenitor exhausted state characterized by unique and elevated metabolic demands. If validated, this population could potentially serve as a predictive cellular indicator or immunological correlate to identify patients most likely to respond to BCAA-targeted immunotherapies. However, we must acknowledge the limitation of our small clinical sample size ($n = 9$), which necessitates rigorous validation in larger, independent patient cohorts.

Several other limitations of our study warrant consideration and provide avenues for future research. First, although our *ex vivo* assays using siRNA-mediated Bcat1 knockdown in CD8⁺ T cells clearly demonstrate the cell-intrinsic role of the BCAT1 pathway, the lack of *in vivo* T cell-specific Bcat1 genetic knockout models still limits our ability to definitively isolate the systemic contribution of T cell-intrinsic BCAT1 inhibition from the direct anti-tumor effects of quercetin in a complex tumor microenvironment. Second, a methodological limitation of our

study is the lack of direct metabolic flux assays. While our data clearly indicate the modulation of the BCAT1-BCAA-mTORC1 signaling axis based on steady-state measurements, we did not employ stable isotope tracing to dynamically quantify the intracellular metabolic rewiring in CD8⁺ T cells. Future investigations utilizing advanced mass spectrometry-based fluxomics will be essential to map the precise dynamic flow of BCAA catabolism following quercetin treatment. Third, the human clinical cohort analyzed via multiplex immunofluorescence in our study was limited in sample size ($n = 9$) and lacked a formal statistical power analysis. These clinical data serve primarily as an exploratory, proof-of-concept observation to support our preclinical findings. Future studies involving larger, statistically powered, and independent multicenter clinical cohorts are essential to definitively validate the clinical and translational significance of the BCAT1 pathway. Finally, a critical next step will be to evaluate this metabolic strategy in combination with established immune checkpoint blockade therapies to determine if co-targeting BCAA metabolism can overcome primary or acquired resistance in advanced CRC.

Importantly, we fully acknowledge that quercetin is a highly pleiotropic molecule with well-documented polypharmacology. Its profound *in vivo* anti-metastatic efficacy observed in our study is likely a synergistic consequence of its direct cytotoxicity against tumor cells combined with its newly identified immunometabolic modulation via the BCAT1 axis. Therefore, while our data establish BCAT1 as a direct and critical target for quercetin-mediated CD8⁺ T cell reinvigoration, the overall therapeutic outcome should be cautiously interpreted as a multi-targeted network effect rather than being exclusively dependent on a singular BCAT1-centric mechanism.

In summary, this study establishes quercetin as a targeted modulator of immunometabolism. By directly inhibiting BCAT1, quercetin orchestrates a favorable metabolic competition that cripples tumor cell metabolism while simultaneously reinvigorating CD8⁺ T cell effector function. The observed synergy with leucine supplementation suggests a potential strategy to therapeutically exploit nutrient metabolism, laying a solid conceptual foundation for BCAA-targeted interventions in metastatic colorectal cancer. (Fig. 7).

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Chuyi Wang: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Project administration. **Yunxing Xu:** Writing – review & editing, Methodology, Resources, Data curation, Funding acquisition. **Pengcheng Ding:** Formal analysis, Data curation. **Yunhai Ding:** Visualization, Formal analysis, Data curation. **Liangfeng Zheng:** Validation, Data curation. **Tianyi Li:** Validation, Data curation. **Shouliang Cheng:** Validation, Data curation. **Chen Zhang:** Validation. **Feiyue Ji:** Supervision, Resources, Methodology. **Xiaojiang Liu:** Supervision, Resources. **Kai Chen:** Writing – review & editing, Resources, Methodology, Conceptualization. **Guiyuan Gu:** Supervision, Validation, Resources, Conceptualization. **Xiaomin Lu:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.phymed.2026.158382](https://doi.org/10.1016/j.phymed.2026.158382).

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