Research article

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PLOD1 promotes the malignancy of hepatocellular carcinoma by facilitating the NF-κB/IL-6/STAT3-dependent TCA cycle

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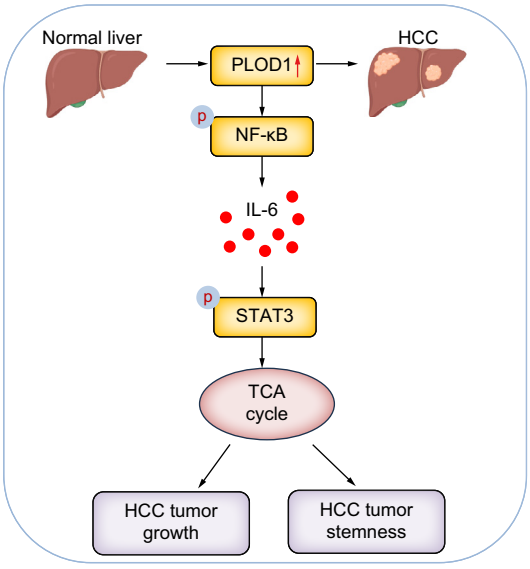
Graphical abstract



Highlights:

- Elevated expression of PLOD1 predicts poor prognosis in patients with HCC.
- PLOD1 promotes HCC proliferation and stemness.
- PLOD1 promotes HCC proliferation and stemness by facilitating the activation of the NF-κB/IL-6/STAT3 signaling pathway.
- PLOD1 accelerates HCC TCA cycle metabolic reprogramming.

Impact and implications:

The roles and underlying mechanisms of PLOD1 in the progression of HCC remain unclear. In this study, we report that PLOD1 is highly expressed in patients with HCC and promotes the proliferation and stemness of HCC cells by activating the NF-κB/IL-6/STAT3-dependent TCA cycle. Knocking down hepatic PLOD1 using adeno-associated virus results in reduced progression of HCC in mice, suggesting that PLOD1 may serve as a potential therapeutic target for HCC.

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PLOD1 promotes the malignancy of hepatocellular carcinoma by facilitating the NF-κB/IL-6/STAT3-dependent TCA cycle

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Background & Aims: Procollagen lysyl hydroxylase 1 (PLOD1) is crucial in regulating collagen synthesis and cross-linking. However, its roles and underlying mechanisms in the progression of hepatocellular carcinoma (HCC) remain unclear. Herein, we aimed to investigate the underlying biological functions and mechanisms of PLOD1 in HCC.

Methods: The expression levels of PLOD1 in HCC were measured by qPCR, Western blot, and immunohistochemistry. Cell proliferation, apoptosis, and stemness were examined by CCK8, flow cytometry, sphere formation, and aldehyde dehydrogenase activity assays. The subcutaneous tumorigenicity model, orthotopic tumorigenicity model, and hepatotoxin-induced HCC model were used for *in vivo* experiments. RNA-sequence and untargeted metabolomics analysis were performed to identify underlying mechanisms.

Results: PLOD1 is found to be highly expressed in both human (p <0.0001) and mouse HCC (p <0.01) and is associated with a poor prognosis (p = 0.047). *In vitro* and *in vivo* experiments reveal that overexpression of PLOD1 promotes the proliferation and stemness of HCC cells. Meanwhile, the depletion of PLOD1 attenuates the occurrence and growth of HCC, leading to cell cycle arrest (p <0.01) and apoptosis (p <0.001) in HCC. Mechanistically, PLOD1 positively regulates the NF-κB/IL-6/STAT3 signaling pathway and accelerates TCA cycle metabolic reprogramming. Blocking the NF-κB/IL-6/STAT3 signaling pathway and TCA cycle can effectively mitigate PLOD1-induced proliferation and stemness of HCC cells.

Conclusions: Our study uncovers the PLOD1/NF-κB/IL-6/STAT3 axis as a therapeutic target for inhibiting the progression and stemness of HCC.

Introduction

Hepatocellular carcinoma (HCC) accounts for approximately 90% of primary liver cancer cases and remains a significant threat to human life and health. It is the third most common leading cause of cancer-related deaths globally.[1] The World Health Organization (WHO) estimated that HCC will contribute to more than one million human deaths in 2030.[2] Multiple factors contribute to the progression of HCC, such as infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), heavy alcohol consumption, aflatoxin B1 contaminated food, and non-alcoholic fatty liver disease (NAFLD).[3,4] Currently, the main therapeutic approaches for patients with HCC include surgery, chemotherapy, molecular-targeted therapy, and immunotherapy. Because of the late presentation of symptoms, most patients with HCC are diagnosed at advanced stages and are therefore not suitable for surgical resection.[5] Additionally, only a limited number of patients with HCC are responsive to related molecular-targeted therapy and immunotherapy.[6] Therefore, it is important to further investigate the molecular mechanisms underlying HCC and identify new potential targets for the treatment of HCC.

Metabolic reprogramming is one of the most common features of tumor cells, including HCC, which facilitates tumor cell proliferation and survival.[7,8] Glycolysis and the tricarboxylic acid (TCA) cycle are the two main metabolic pathways that provide energy to the cell. Although numerous studies have shown that tumor cells are more likely to obtain energy through glycolysis, recent research has indicated that the TCA cycle is abnormally elevated in various tumors, such as melanoma, breast cancer, and HCC.[9–11] Targeting the TCA cycle presents a novel strategy for the treatment of HCC.[12,13] However, the regulatory mechanisms of the TCA cycle in HCC require further investigation.

The signal transducer and activator of transcription 3 (STAT3) is a critical transcription factor engaged in the development of cancers and inflammatory diseases. In HCC, STAT3 is usually aberrant activated. STAT3 can be activated by various cytokines or growth factors such as interleukin-6 (IL-6), leukemia inhibitory factor (LIF), and connective tissue growth

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factor (CTGF).[14,15] Activated STAT3 promotes tumorigenesis, metastasis, and immune evasion by facilitating the expression of downstream oncogenic target genes.[16,17] As an activator of STAT3, the expression of IL-6 is tightly controlled by NF-κB. Thus, the crosstalk between NF-κB and STAT3 plays a pivotal role in the progression of cancers and inflammatory diseases.[18,19] Several studies have reported that the crosstalk between NF-κB and STAT3 facilitated the proliferation and metastasis of HCC.[20–22] However, the specific role of the NF-κB/STAT3 axis in the metabolic reprogramming of HCC has not been extensively studied.

The procollagen lysyl hydroxylase (PLOD) family includes PLOD1, PLOD2, and PLOD3. PLODs are required for the modulation of collagen synthesis and cross-linking, which promote the maturation of the extracellular matrix. PLOD1 has been linked to diabetic wound healing, Epstein–Barr virus replication, and tumor occurrence.[23–25] Though increased PLOD1 expression has been associated with several types of cancer, such as glioblastoma, gastric cancer, and HCC,[25–27] its specific roles and underlying mechanisms in the progression of HCC remain unclear. In this study, we report that PLOD1 is highly expressed in both human and mouse HCC, correlating with poor prognosis. Overexpression of PLOD1 promotes the occurrence, development, and stemness of HCC. RNA-sequence and untargeted metabolomics analysis indicate that PLOD1 facilitates the malignancy of HCC by strengthening the NF-κB/IL-6/STAT3 signaling pathway and the TCA cycle. Our findings suggest that PLOD1 could serve as a potential therapeutic target for HCC.

Materials and methods

Lentivirus preparation and stable cell lines construction

HEK293T cells were seeded into six-well plates (6.5×10^5 cells per well) overnight for lentivirus packaging. A sample of 2.5 μg of the lentiviral vector (pLKO.1-shScramble, pLKO.1-shPLOD1, pLenti-CMV-GFP/puro or pLenti-CMV-PLOD1), 1 μg of pMDLg/pRRE, 0.4 μg of pRSV-Rev, and 0.6 μg of pMD2.G were co-transfected into HEK293T cells using polyethylenimine. After 48 h, the viruses were harvested and filtered through a 0.45-μm strainer. HCC cells were seeded into 12-well plates (1×10^5 cells per well) overnight and infected with lentivirus for 48 h to generate stable cell lines. The cells were then selected with 2 μg/ml puromycin.[28]

Cell viability assay

HCC cells were seeded into 96-well plates (2×10^3 cells per well). After 3 or 5 days, 10 μl CCK8 was added to each well and incubated at 37 °C for 1.5 h. The absorbance value of each well was measured at 450 nm.

Colony formation assay

HCC cells were seeded into six-well plates (2×10^3 cells per well) and cultured for 2 weeks. Then, the cells were fixed with 4% paraformaldehyde in PBS for 20 min and stained with 0.1% crystal violet for 10 min.

Sphere formation assay

HCC cells were seeded into a 24-well ultra-low attachment plate (Corning, New York, USA; 1×10^3 cells per well) and cultured in serum-free DMEM-F12 (Servicebio, Wuhan, China) supplemented with 20 ng/ml epidermal growth factor (Sino Biological, Beijing, China), 20 ng/ml bFGF (ABclonal, Wuhan, China), 1 × B27 (Thermo, Waltham, USA), and 4 μg/ml insulin (Shanghai Zhongqiaoxinzhou Biotech, Shanghai, China). The cells were cultured for 7 days, spheres were photographed and counted under a light microscope.

ALDH activity assay

A total of 5×10^6 HCC cells were collected and the activity of aldehyde dehydrogenase (ALDH) was detected by an ALDH Activity Assay Kit (BC0750, Solarbio, Beijing, China) according to the manufacturer's instructions.

PDH activity assay

A total of 5×10^6 HCC cells were collected and the activity of pyruvate dehydrogenase (PDH) was detected by a PDH Activity Assay Kit (YFX0134, YFXBIO, Nanjing, China) according to the manufacturer's instructions.

EdU cell proliferation assay

HCC cells were seeded into co-focal dishes (2×10^5 cells per well) and cultured for 24 h. After being stained with 5-ethynyl-2′-deoxyuridine (EdU) (Beyotime, Shanghai, China) and DAPI, EdU^+ cells were observed under a fluorescence microscope.

Mouse tumor model

For the subcutaneous tumorigenicity model, 2×10^6 HCCLM3 cells were injected subcutaneously into 6-week-old BALB/c male nude mice. Tumor size was measured weekly and tumor volume was calculated using the formula: length × width × width/2. Mice were euthanized after 5 weeks and xenografted tumor tissues were dissected for immunohistochemical (IHC) analysis.

For the orthotopic tumorigenicity model, 1×10^6 HCCLM3 cells were injected into the liver of 6-week-old BALB/c male nude mice. Four weeks later, the mice were euthanized and the tumor volume was measured.

For the hepatotoxin-induced HCC model, 2-week-old male C57BL/6 mice were administered intraperitoneally with diethylnitrosamine (DEN) (25 mg/kg). Four weeks after the DEN injection, the mice received intraperitoneal injections of 10% CCl_4 (10 ml/kg, dissolved in corn oil) once a week for 12 weeks. At 22 weeks postpartum, the mice were administered with AAV2/8-shNC or AAV2/8-shPlod1 (2×10^{11} vg/mouse) virus via tail vein injection. At 29 weeks postpartum, mice were euthanized, tumor numbers were recorded and the volume of the largest tumor was measured.

ELISA

Supernatants from HCC cells were collected. Levels of human IL-6 (BioLegend, California, USA) were measured using ELISA according to the manufacturer's instructions.

Flow cytometry analysis for liver cancer stem cells

HCC cells were collected and resuspended in 100 μl staining buffer (2% FBS in PBS). The cells were stained with phycoerythrin (PE) anti-human CD24 and allophycocyanin (APC) anti-

human CD133 antibodies on ice for 30 min in the dark. After washing twice and resuspending in 500 μl staining buffer, the cells were analyzed using flow cytometry (CytoFLEX) and data were analyzed with CytExpert software.

RNA-sequence

shNC and shPLOD1-HCCLM3 cells (1 × 10^6 cells per sample) were lysed in Trizol. The gene expression profiles were examined by RNA sequencing in BioMarker (Beijing, China). The differentially up- and downregulated genes were screened with a criterion of absolute fold change >2 and *p* value <0.05. KEGG pathway enrichment analyses were carried out with Enrichr tools to focus on the function of differential expression genes.

Untargeted metabolomics analysis

shNC and shPLOD1-HCCLM3 cells (5 × 10^6 cells per sample) were collected and stored at -80 °C. The cells were then sent to Cosmos Wisdom (Hangzhou, China) for LC-MS analysis.

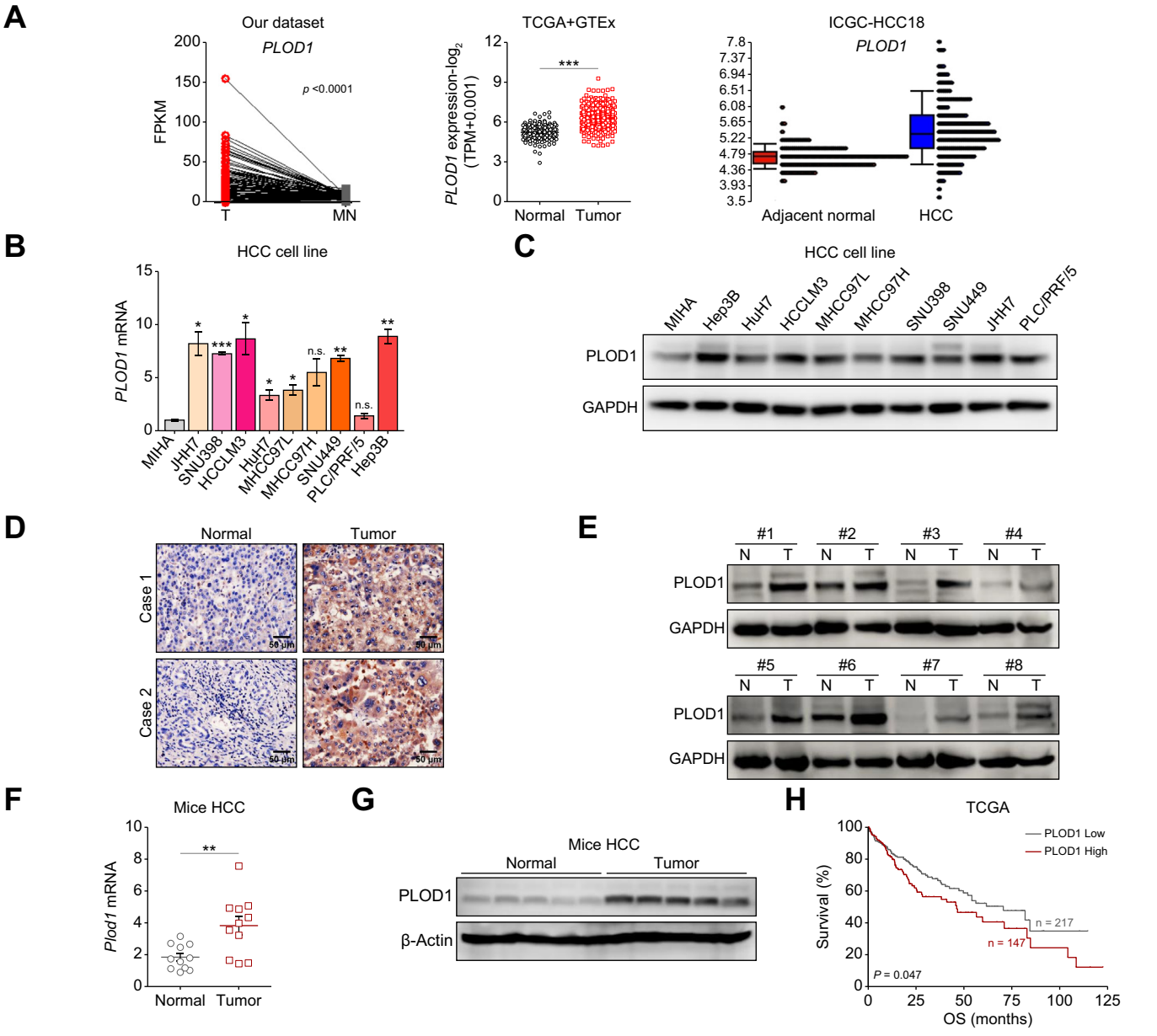

Fig. 1. PLOD1 is upregulated in HCC and correlated with poor outcomes. (A) PLOD1 is upregulated in our in-house HCC dataset and validated in The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC) datasets. (B) *PLOD1* mRNA expression levels in human normal liver cells (MIHA) and HCC cell lines were measured by qPCR. (C) PLOD1 protein expression levels in human normal liver cells (MIHA) and HCC cell lines were assessed by Western blot. (D) Representative image of IHC staining of PLOD1 in human HCC tissues and paired normal liver tissues. (E) Protein levels of PLOD1 in human HCC tissues and paired normal liver tissues were assessed by Western blot. (F) Plod1 mRNA expression levels in mice HCC tissues (hepatotoxin-induced mice HCC model) and normal liver tissues were measured by qPCR. (G) Protein levels of PLOD1 in mice HCC tissues (hepatotoxin-induced mice HCC model) and normal liver tissues were assessed by Western blot. (H) The expression level of PLOD1 was associated with the survival of patients with HCC using the Kaplan–Meier plotter. Data are shown as mean ± SEM and were analyzed using Student's two-tailed *t* test. **p* <0.05; ***p* <0.01; ****p* <0.001; ns, not significant. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HCC, hepatocellular carcinoma; ICGC, International Cancer Genome Consortium; IHC, immunohistochemical; PLOD1, procollagen lysyl hydroxylase 1; qPCR, quantitative PCR; TCGA, The Cancer Genome Atlas.

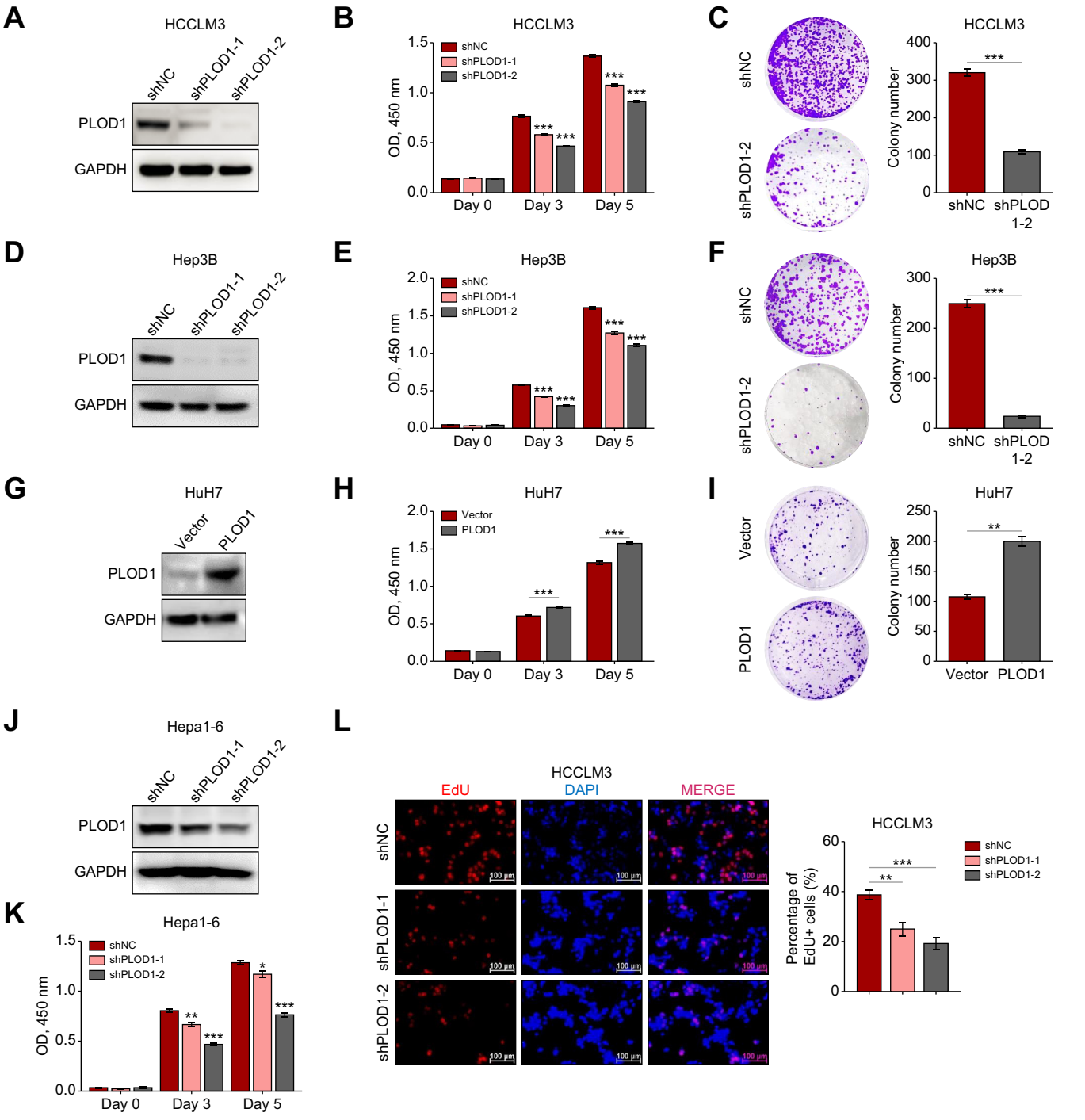

Fig. 2. PLOD1 promotes the proliferation of HCC cells. (A–C) Stable knockdown of PLOD1 in HCCLM3 cells; the expression of PLOD1 was assessed by Western blot. The growth or the viability of HCCLM3 cells was measured by CCK8 or colony formation assay. (D–F) Stable knockdown of PLOD1 in Hep3B cells; the protein level of PLOD1 in Hep3B cells was assessed by Western blot. The growth or the viability of Hep3B cells was measured by CCK8 or colony formation assay. (G–I) Overexpression of PLOD1 in HuH7 cells; the protein level of PLOD1 in HuH7 cells was assessed by Western blot. The growth or the viability of HuH7 cells was measured by CCK8 or colony formation assay. (J,K) Stable knockdown of Plod1 in Hepa1-6 cells; the protein level of Plod1 in Hepa1-6 cells was assessed by Western blot. The viability of Hepa1-6 cells was measured by CCK8. (L) Stable knockdown of PLOD1 in HCCLM3 cells, the proliferation of HCC cells was evaluated using an EdU staining assay. Data are shown as mean ± SEM and were analyzed with Student's two-tailed *t* test. **p* <0.05; ***p* <0.01; ****p* <0.001. EdU, 5-ethynyl-2′-deoxyuridine; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HCC, hepatocellular carcinoma; PLOD 1, procollagen lysyl hydroxylase 1.

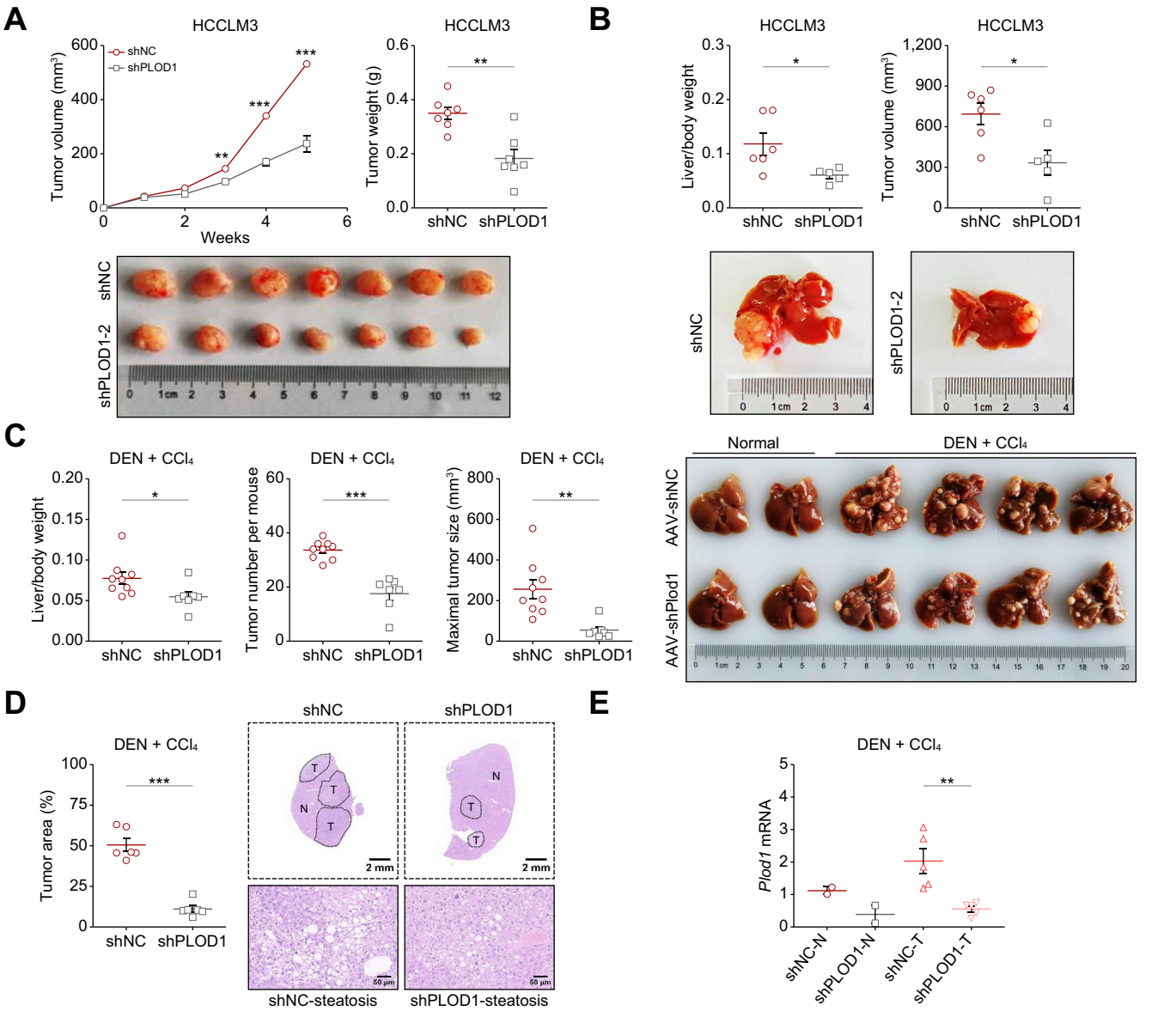

Fig. 3. PLOD1 promotes the progression of HCC *in vivo*. (A) shNC or shPLOD1-HCCLM3 cells were injected subcutaneously into BALB/c nude mice, the tumor volumes were measured weekly and the tumor weights were measured after 5 weeks. (B) shNC or shPLOD1-HCCLM3 cells were injected into the liver of BALB/c nude mice, and the tumor volumes were measured after 4 weeks. (C) Hepatotoxin-induced HCC model mice were administered with AAV2/8-shNC or AAV2/8-shPlod1 (2×10^{11} vg/mouse) via tail vein injection for 7 weeks. Tumor numbers were recorded and the volume of the largest tumor was measured. (D) Livers from the mice described in C were stained with H&E, and tumor areas were measured. (E) The expression levels of *Plod1* in mice normal liver tissues or mice HCC tissues were measured by qPCR. Data are shown as mean ± SEM and were analyzed with Student's two-tailed *t* test. **p* <0.05; ***p* <0.01; ****p* <0.001. CCl_4, carbon tetrachloride; DEN, diethylnitrosamine; HCC, hepatocellular carcinoma; PLOD 1, procollagen lysyl hydroxylase 1; qPCR, quantitative PCR.

Metabolite detection

HCC cells were collected and stored at -80 °C. The levels of citric acid were measured using the Citric Acid Content Assay Kit (AKAC003M, Boxbio, Beijing, China), and the levels of malic acid were detected by the Malic Acid Content Assay kit (BC5490, Solarbio, Beijing, China), according to the manufacturer's instructions.

Statistical analysis

All data analysis was performed using GraphPad Prism software (GraphPad Software, San Diego, CA, USA) and presented as the mean ± SEM. Student's *t* test was used to compare the two groups. A *p* value <0.05 was considered to indicate significance.

Results

PLOD1 is associated with HCC malignancy

To investigate the potential role of PLOD1 in HCC, we first analyzed the differential expression levels of PLOD1 in adjacent-normal liver tissues and HCC tumor tissues from the public dataset. We found that the expression of PLOD1 was

upregulated in HCC tumor tissues (Fig. 1A Fig. S1A). We further examined the expression levels of PLOD1 in normal hepatocytes (MIHA) and various HCC cell lines. The results indicated that PLOD1 expression was notably higher in HCC cells than in normal hepatocytes (Fig. 1B and C). Additionally, IHC staining and Western blot assays confirmed that PLOD1 expression was elevated in HCC tumor tissues compared with adjacent-normal liver tissues (Fig. 1D and E). To validate these findings in human HCC, we also assessed PLOD1 expression in a mouse model of HCC. Quantitative PCR (qPCR) and Western blot analyses showed that PLOD1 was also overexpressed in HCC tumor tissues from the mice (Fig. 1F and G). Furthermore, we assessed the correlation between PLOD1 expression levels and the prognosis of patients with HCC. As

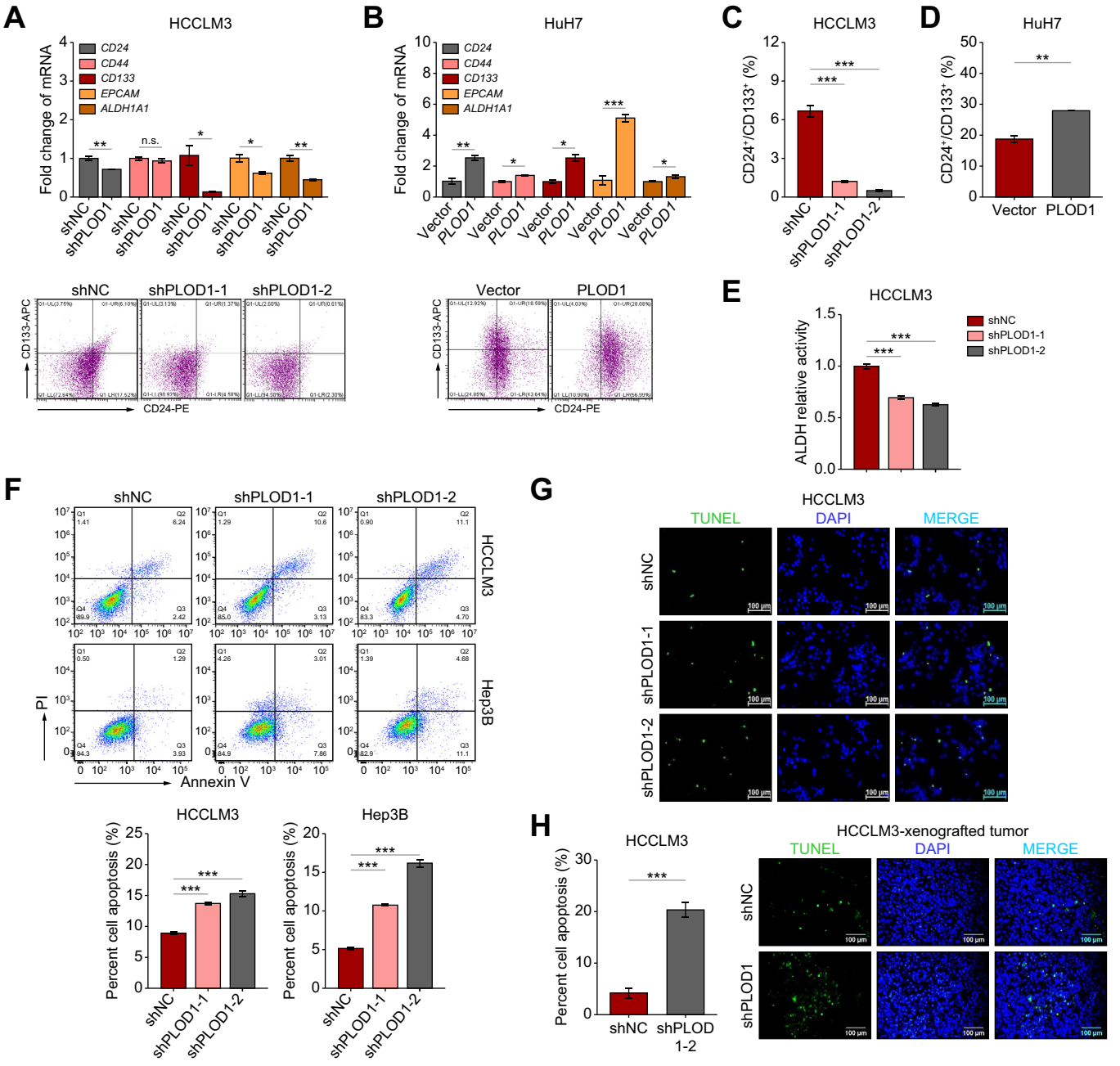

Fig. 4. PLOD1 promotes stemness and apoptosis resistance in HCC. (A) Stable knockdown of PLOD1 in HCCLM3 cells by shRNA, the expression levels of *CD24, CD44, CD133, EPCAM*, and *ALDH1A1* were detected by qPCR. (B) Overexpression of PLOD1 in HuH7 cells, the expression levels of *CD24, CD44, CD133, EPCAM*, and *ALDH1A1* were detected by qPCR. (C) Stable knockdown of PLOD1 in HCCLM3 cells by shRNA, $CD24^+/CD133^+$ cells were analyzed using FACS. (D) Overexpression of PLOD1 in HuH7 cells, $CD24^+/CD133^+$ cells were analyzed using FACS. (E) Stable knockdown of PLOD1 in HCCLM3 cells was performed using shRNA, and the activity of ALDH was measured. (F) Stable knockdown of PLOD1 in HCC cells, apoptotic cells were analyzed using FACS. (G) Stable knockdown of PLOD1 in HCCLM3 cells with shRNA; apoptotic cells were examined using the TUNEL assay. (H) Apoptotic cells in HCCLM3 xenografted tumor tissues were examined using the TUNEL assay. Data are shown as mean ± SEM and were analyzed with Student's two-tailed *t* test. **p* <0.05; ***p* <0.01; ****p* <0.001; ns, not significant. HCC, hepatocellular carcinoma; PLOD 1, procollagen lysyl hydroxylase 1; qPCR, quantitative PCR.

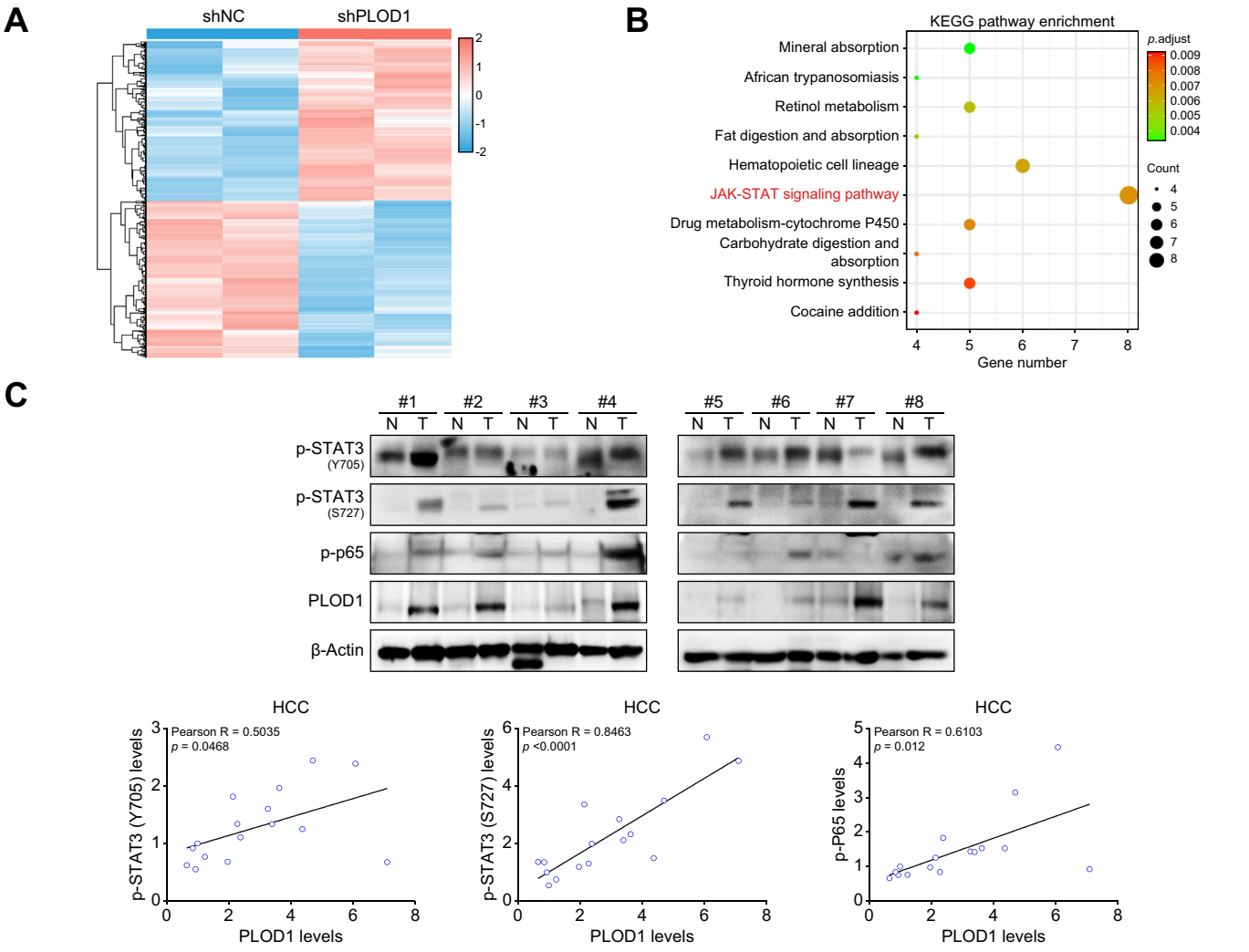

Fig. 5. PLOD1 promotes the activation of NF-κB/IL-6/STAT3 pathway. (A) Stable knockdown of PLOD1 in HCCLM3 cells by shRNA, the differentially expressed genes were analyzed by RNA-sequence. (B) KEGG pathway enrichment analysis from the differentially expressed genes in A. (C) Levels of PLOD1, p-p65, and p-STAT3 in human HCC tissues and paired normal liver tissues were assessed by Western blot. (D) Knockdown or overexpression of PLOD1 in HCC cells, the levels of p-STAT3, STAT3, p-p65, p65, and PLOD1 were analyzed by Western blot. (E) The levels of p-STAT3, p-p65, and PLOD1 in mice HCC tissues were analyzed by Western blot. (F) The levels of p-STAT3, p-p65, and PLOD1 in HCCLM3 xenografted tumor tissues were analyzed by IHC. (G) Knockdown or overexpression of PLOD1 in HCC cells, the levels of IL-6 mRNA were measured by qPCR. (H) Knockdown or overexpression of PLOD1 in HCC cells, the levels of IL-6 secreted by HCC cells were detected by ELISA. Data are shown as mean ± SEM and were analyzed with Student's two-tailed *t* test. ***p* <0.01; ****p* <0.001. HCC, hepatocellular carcinoma; PLOD 1, procollagen lysyl hydroxylase 1; qPCR, quantitative PCR; STAT3, signal transducer and activator of transcription 3.

shown in Fig. 1H and Fig. S1B, elevated expression of PLOD1 was negatively correlated with the overall survival of patients with HCC. These findings demonstrate that PLOD1 is significantly overexpressed in HCC and is associated with the malignancy of HCC.

PLOD1 promotes the proliferation of HCC cells *in vitro*

To clarify the biological functions of PLOD1 in the growth of HCC, we initially knocked down PLOD1 in human HCC cells using shRNA, and the proliferation of HCC cells was evaluated by CCK8 and colony formation assays. We found that knocking down PLOD1 dramatically attenuated the growth of HCCLM3 and Hep3B cells (Fig. 2A–F). Next, we overexpressed PLOD1 in HuH7 cells. The CCK8 and colony formation assays indicated that the overexpression of PLOD1 significantly expedited the proliferation of HuH7 cells (Fig. 2G–I). Additionally, knocking down Plod1 in mouse HCC cells also suppressed their growth (Fig. 2J and K). Consistent with these findings, the EdU staining assay demonstrated that the deficiency of PLOD1 impaired the proliferation of HCC cells (Fig. 2L). Collectively, our results indicate that PLOD1 promotes the proliferation of HCC cells *in vitro*.

Given that metastasis is a significant barrier to improving the prognosis of HCC, we also investigated the impact of PLOD1 on the migration of HCC cells. Transwell and wound-healing assays showed that the knockdown of PLOD1 had minimal effect on the motility of the HCC cells (Fig. S2A and B). Similarly, the overexpression of PLOD1 did not significantly influence the migration and invasion of HCC cells (Fig. S2C). These results suggest that PLOD1 does not affect the metastatic process of HCC.

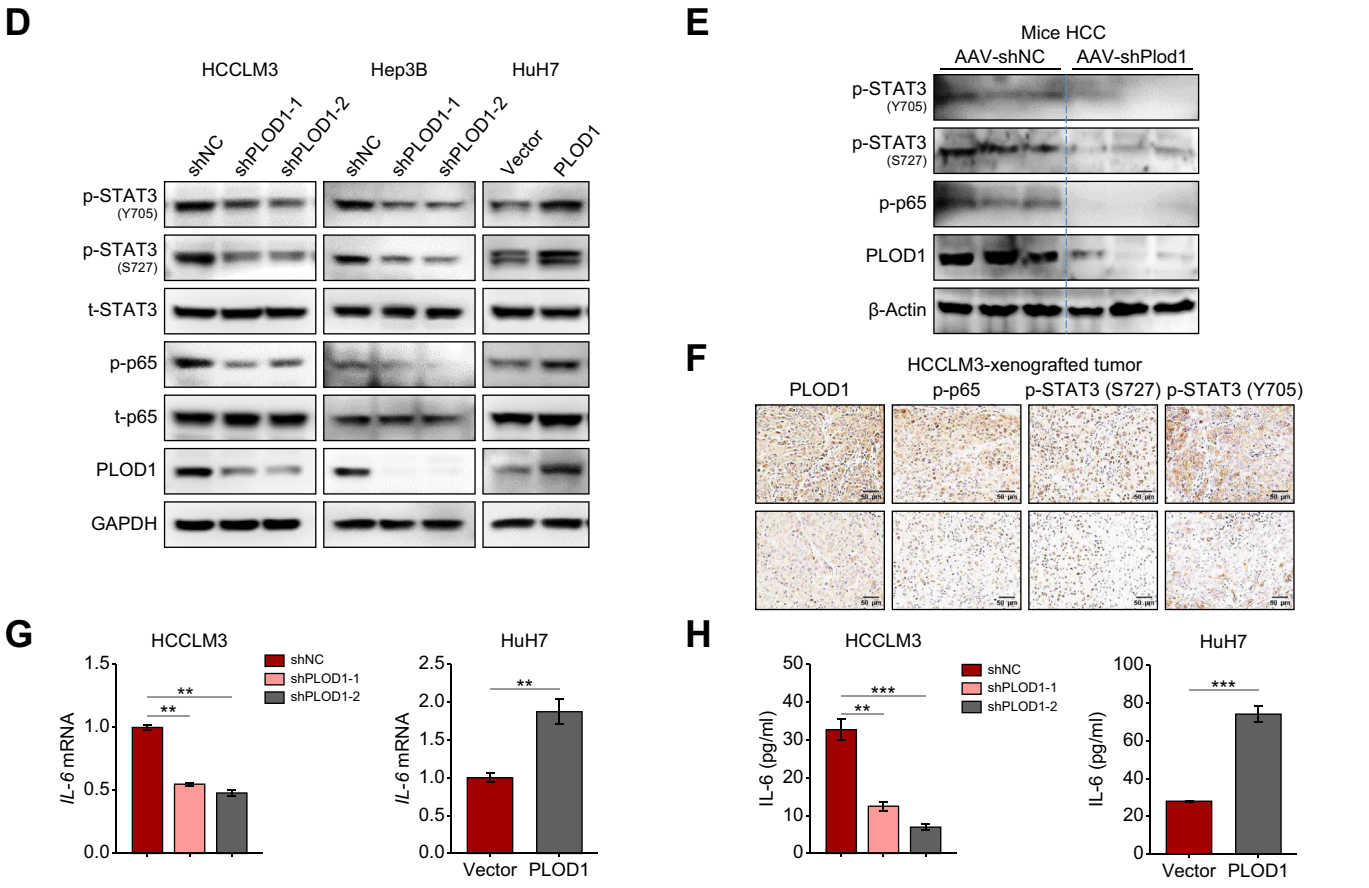

Fig. 5. ***(continued)***

PLOD1 facilitates HCC growth *in vivo*

To further confirm the role of PLOD1 in regulating HCC, a subcutaneous xenograft mouse model was established to evaluate the influence of PLOD1 on HCC growth *in vivo*. PLOD1-knockdown and control HCCLM3 cells were subcutaneously injected into nude mice. As shown in Fig. 3A, the PLOD1 knockdown group exhibited reduced tumor volumes and weights compared with the control group. IHC staining for KI67 showed that PLOD1 knockdown diminished the proliferative capability of HCC cells (Fig. S3A). This biological function of PLOD1 was further investigated in an orthotopic tumorigenicity model. We observed a significantly lower liver/body weight ratio and decreased orthotopic tumor volumes in the PLOD1 knockdown group (Fig. 3B). These results suggest that PLOD1 facilitates HCC growth *in vivo*. To better evaluate the function of PLOD1 in the development of HCC, a hepatotoxin-induced HCC model was established. After treatment with DEN and repeated carbon tetrachloride, mice received tail vein injections of AAV2/8-shNC or AAV2/8-shPlod1 (2×10^{11} vg/mouse). As shown in Fig. 3C, the liver/body weight ratio, tumor numbers, and maximum tumor volumes were all significantly reduced in the AAV2/8-shPlod1 group. H&E staining assay also revealed that liver tumors and liver steatosis were markedly decreased in the Plod1 knockdown group (Fig. 3D). qPCR results confirmed the successful knockdown of Plod1 in the livers of mice (Fig. 3E). Overall, these data suggest that PLOD1 facilitates the progression of HCC *in vivo*.

PLOD1 deficiency inhibits stemness, enhances apoptosis, and induces S-phase cell cycle arrest in HCC

Stemness, apoptosis resistance, and rapid cell division are key factors contributing to the malignancy of tumor cells. To investigate how PLOD1 facilitates HCC progression, we evaluated whether PLOD1 affects cell stemness, cell apoptosis, and the cell cycle of HCC. CD24, CD44, CD133, EPCAM, and ALDH1A1 are generally acknowledged as cell surface markers of liver cancer stem cells (LCSCs).[29,30] We found that most of these markers were downregulated after PLOD1 knockdown. In comparison, overexpression of PLOD1 promoted the expression of these markers (Fig. 4A and B). Consistent with these, flow cytometry analysis unveiled that knockdown of PLOD1 decreased the population of CD24+/CD133+ LCSCs, whereas overexpression of PLOD1 increased this process (Fig. 4C and D). In addition, the knockdown of PLOD1 reduced the activity of the ALDH enzyme and sphere formation, whereas overexpression of PLOD1 enhanced these processes (Fig. 4E, Fig. S4A–C). These data suggest that PLOD1 promotes the stemness of HCC. Subsequently, annexin v/propidium iodide (PI) staining analysis indicated that silencing PLOD1 increased the percentage of apoptotic cells in HCCLM3 and Hep3B cells

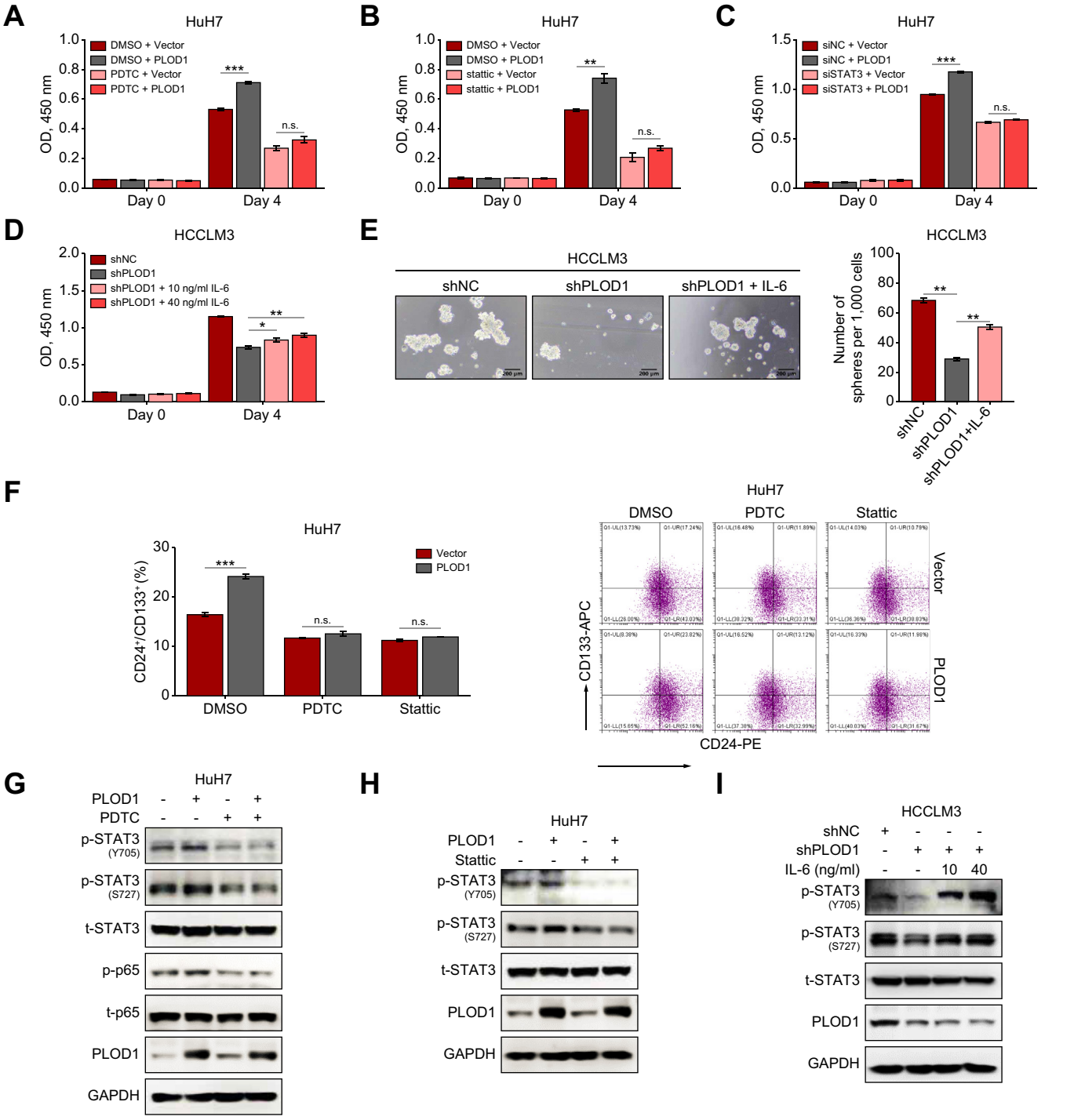

Fig. 6. PLOD1 promotes the progression of HCC dependent on NF-κB/IL-6/STAT3 pathway. (A) Overexpression of PLOD1 in HuH7 cells, the cells were treated with or without PDTC for 4 days, and the viability of cells was measured by CCK8. (B) Overexpression of PLOD1 in HuH7 cells, the cells were treated with or without stattic for 4 days, and the viability of cells was measured by CCK8. (C) Overexpression of PLOD1 in HuH7 cells, the cells were transfected with or without STAT3 siRNA. The viability of cells was measured by CCK8. (D) Stable knockdown of PLOD1 in HCCLM3 cells by shRNA, the cells were treated with indicated concentrations of IL-6 for 4 days, and the viability of cells was measured by CCK8. (E) Stable knockdown of PLOD1 in HCCLM3 cells by shRNA, the cells were treated with indicated concentrations of IL-6 for 4 days, sphere formation ability was measured using a sphere formation assay. (F) Overexpression of PLOD1 in HuH7 cells, the cells were treated with or without PDTC and stattic for 24 h, $CD24^+/CD133^+$ cells were analyzed using FACS. (G) Overexpression of PLOD1 in HuH7 cells, the cells were treated with or without PDTC for 2 h, levels of p-STAT3, STAT3, p-p65, p65, and PLOD1 were analyzed using Western blot. (H) Overexpression of PLOD1 in HuH7 cells, the cells were treated with or without stattic for 2 h, levels of p-STAT3, STAT3, and PLOD1 were analyzed using Western blot. (I) Stable knockdown of PLOD1 in HCCLM3 cells by shRNA, the cells were treated with indicated concentrations of IL-6 for 1 h, levels of p-STAT3, STAT3, and PLOD1 were analyzed by Western blot. Data are shown as mean ±SEM and analyzed with Student's two-tailed *t* test. **p* <0.05; ***p* <0.01; ****p* <0.001; ns, not significant. HCC, hepatocellular carcinoma; PDTC, pyrrolidinedithiocarbamate; PLOD 1, procollagen lysyl hydroxylase 1; qPCR, quantitative PCR; STAT3, signal transducer and activator of transcription 3.

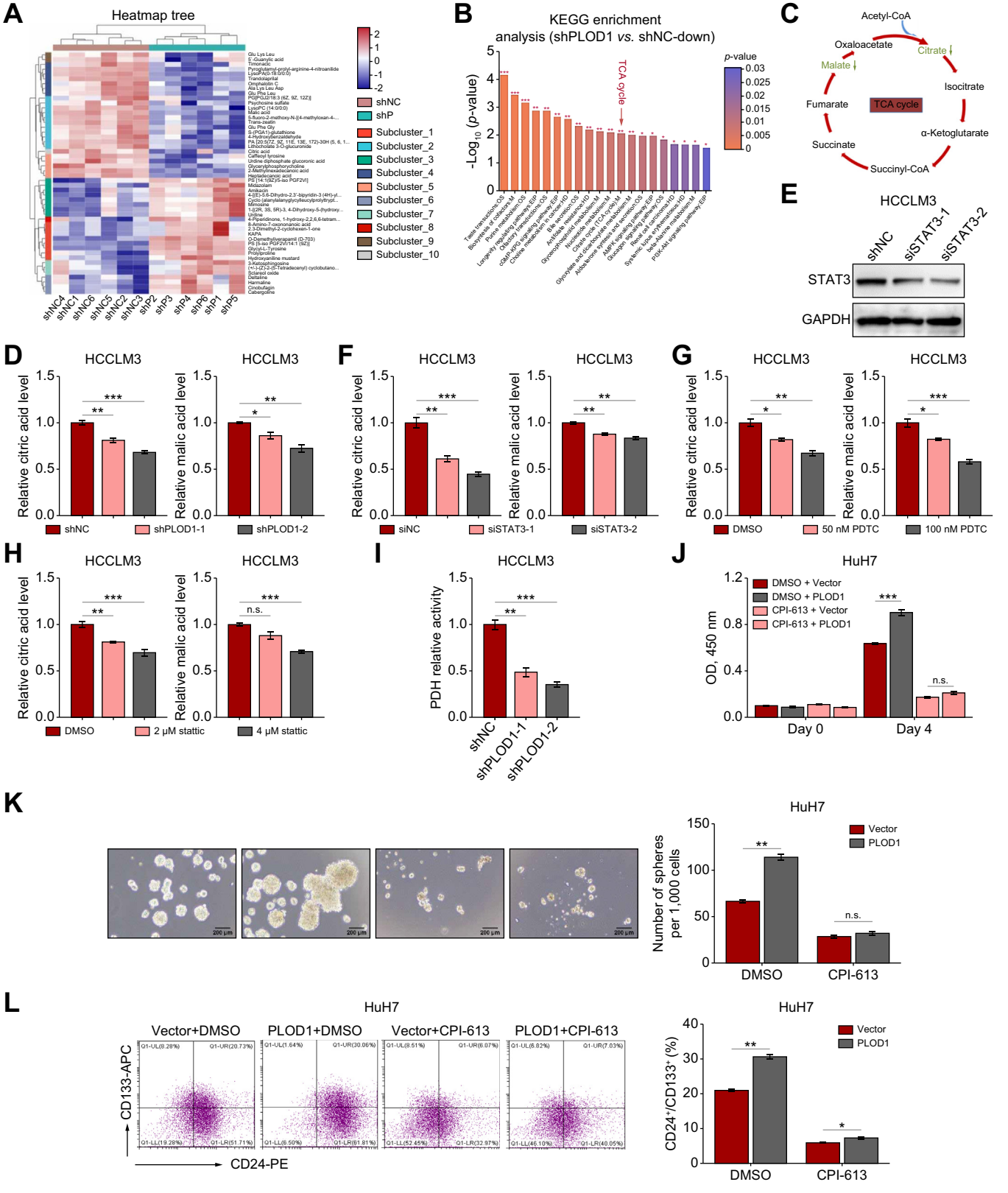

Fig. 7. PLOD1 promotes TCA cycle. (A) Stable knockdown of PLOD1 in HCCLM3 cells by shRNA, untargeted metabolomics analysis were performed. (B) KEGG enrichment analysis for downregulated metabolites. (C) Schematic diagram of the TCA cycle. (D) Stable knockdown of PLOD1 in HCCLM3 cells by shRNA, the levels of citric acid and malic acid were measured. (E) HCCLM3 were transfected with STAT3 siRNA for 72 h, and the level of STAT3 was analyzed using Western blot. (F) HCCLM3 were transfected with STAT3 siRNA for 72 h, the levels of citric acid and malic acid were measured. (G) HCCLM3 cells were treated with PDTC for 24 h, and the levels of citric acid and malic acid were measured. (H) HCCLM3 cells were treated with stattic for 24 h, and the levels of citric acid and malic acid were measured. (I)

(Fig. 4F). TUNEL staining revealed that knockdown of PLOD1 also increased the percentage of apoptotic cells in HCCLM3 xenografted tumors and PLOD1-deficiency HCCLM3 cells (Fig. 4G and H, Fig. S4D). To investigate the impact of PLOD1 on the HCC cell cycle, shNC and PLOD1-knockdown HCCLM3 cells were stained with PI and analyzed by flow cytometry. The results showed that knocking down PLOD1 increased the percentage of cells at the S phase (Fig. S4E). Thus, these results demonstrate that PLOD1 deficiency inhibits stemness, enhances apoptosis, and induces S-phase cell cycle arrest in HCC.

PLOD1 promotes the activation of NF-κB/IL-6/STAT3 axis

To unravel the underlying molecular mechanisms by which PLOD1 contributes to the malignancy of HCC, we conducted RNA-sequence analysis on control HCCLM3 cells and PLOD1-knockdown HCCLM3 cells. The downstream genes of PLOD1 were selected for pathway enrichment analysis. Fig. 5A and B shows that the JAK-STAT signaling pathway is highly associated with downstream PLOD1. The constitutive activation of STAT3 is known to contribute to malignancy in various cancers, including HCC.[31] We speculated that the activity of STAT3 might play a role in PLOD1's promotion of HCC malignancy. Furthermore, a previous study reported that PLOD1 can bind to IκBα, thus promoting the activation of the NF-κB signaling pathway in glioblastoma.[25] More importantly, IL-6, a downstream inflammatory factor of NF-κB, is a crucial cytokine that activates STAT3, facilitating malignancy in cancers.[32] Therefore, we investigated whether NF-κB/IL-6/STAT3 axis is modulated by PLOD1 in HCC. We first assessed the correlation between PLOD1 and the status of NF-κB/STAT3 in human HCC samples. As shown in Fig. 5C, the expression level of PLOD1 was positively correlated with the phosphorylation levels of NF-κB and STAT3. Moreover, we observed that knocking down PLOD1 in HCC cells dramatically reduced the phosphorylation of NF-κB and STAT3 (Fig. 5D). In comparison, overexpressing PLOD1 heightened the phosphorylation of these proteins (Fig. 5D). This trend was also consistent in hepatotoxin-induced HCC models and xenograft mouse models, where the phosphorylation levels of NF-κB and STAT3 were all downregulated in the shPLOD1 group (Fig. 5E and F). Moreover, qPCR and ELISA assays corroborated that the expression of inflammatory factor IL-6 was controlled by PLOD1 in HCC cells (Fig. 5G, H). In addition to these, we found that PLOD1 interacts with IκBα and enhances its phosphorylation in HCC cells (Fig. S5A and B), which was consistent with previous research.[25] Taken together, these findings unveil that PLOD1 promotes the activation of the NF-κB/IL-6/STAT3 axis in HCC.

PLOD1 promotes HCC progression dependent on the NF-κB/IL-6/STAT3 pathway

We then further investigated whether PLOD1 promotes the progression of HCC by activating the NF-κB/IL-6/STAT3 pathway. Initially, we confirmed that blocking the activity of NF-κB and STAT3 strikingly attenuated the growth of HCC cells (Fig. S6A and B). Next, we treated both control HuH7 cells and PLOD1-overexpression HuH7 cells with NF-κB and STAT3 inhibitors to evaluate cell viability. As shown in Fig. 6A and B, the overexpression of PLOD1 in HuH7 cells significantly encouraged the growth of the cells. However, this PLOD1-raised cell growth was effectively eliminated by the NF-κB and STAT3 inhibitors. Furthermore, knockdown of STAT3 using siRNA also suppressed PLOD1-raised cell growth (Fig. 6C). Supporting these findings, we found that the inflammatory factor IL-6 partially restored the inhibitory effects of PLOD1-shRNA on HCC cell growth and sphere formation (Fig. 6D and E). In addition, the increase in the population of CD24+/CD133+ LCSCs induced by PLOD1 was diminished by NF-κB and STAT3 inhibitors (Fig. 6F). These data suggest that PLOD1 promotes the progression and stemness of HCC dependent on NF-κB/IL-6/STAT3 pathway. To further substantiate these results, we examined the phosphorylation levels of NF-κB and STAT3 in these HCC cells. The results also showed that overexpression of PLOD1 in HuH7 cells led to increased phosphorylation of both NF-κB and STAT3, whereas application of NF-κB and STAT3 inhibitors diminished this process (Fig. 6G and H). The decreased phosphorylation levels of STAT3 mediated by PLOD1-shRNA were drastically restored by the inflammatory factor IL-6 (Fig. 6I). Thus, our results indicate that PLOD1 promotes the malignancy of HCC by activating the NF-κB/IL-6/STAT3 signaling pathway.

PLOD1 strengthens the TCA cycle in HCC

Metabolic reprogramming is a crucial feature of tumor progression. According to the pathway enrichment analysis of RNA-sequence, we identified not only the JAK-STAT signaling pathway, but also notable alterations in metabolic pathways such as fat digestion and absorption, and carbohydrate digestion and absorption. These findings reveal that PLOD1 may contribute to metabolism alterations in HCC. To further determine the specific metabolites regulated by PLOD1 in HCC, control HCCLM3 cells and PLOD1-knockdown HCCLM3 cells were used for untargeted metabolomics analysis. The results showed that metabolites involved in the TCA cycle (citric acid and malic acid) were decreased after PLOD1 knockdown (Fig. 7A–C). The TCA cycle is a vital metabolic pathway that underpins tumor progression, and previous studies have shown that it is controlled by STAT3 signaling. Importantly, acetyl-CoA, which is generated through fat and carbohydrate metabolism, is an essential source of the TCA cycle.[33,34] Given these insights, the TCA cycle may be the critical reason for PLOD1 promotes HCC malignancy and we then further verify whether PLOD1, NF-κB, and STAT3 modulate the TCA cycle. We observed that knockdown of PLOD1 and STAT3 significantly reduced citric acid and malic acid levels in HCC cells (Fig. 7D–F). Furthermore, the use of pharmacological inhibitors targeting NF-κB and STAT3 also led to

Stable knockdown of PLOD1 in HCCLM3 cells by shRNA and the activity of PDH was measured. (J) Overexpression of PLOD1 in HuH7 cells, the cells were treated with or without CPI-613 for 4 days, and the viability of cells was measured with CCK8. (K) Overexpression of PLOD1 in HuH7 cells, the cells were treated with or without CPI-613, and sphere formation ability was measured by sphere formation assay. (L) Overexpression of PLOD1 in HuH7 cells, the cells were treated with or without CPI-613 for 24 h, and $CD24^{+}/CD133^{+}$ cells were analyzed using FACS. Data are shown as mean ± SEM and analyzed with Student's two-tailed *t* test. **p* <0.05; ***p* <0.01; ****p* <0.001; ns, not significant. PLOD 1, procollagen lysyl hydroxylase 1; STAT3, signal transducer and activator of transcription 3; TCA cycle, tricarboxylic acid cycle.

the downregulation of these metabolites in HCC cells (Fig. 7G and H). A previous study has reported that STAT3 associates with the pyruvate dehydrogenase complex E1 (PDC-E1), facilitating the conversion of pyruvate to acetyl-CoA, thereby promoting the TCA cycle.[35] To verify whether PLOD1 promotes the TCA cycle depending on PDH activity, we further explored the influence of PLOD1 on the enzyme activity of PDH. We found that the knockdown of PLOD1 resulted in reduced PDH enzyme activity (Fig. 7I). Most notably, the use of a PDH inhibitor CPI-613 (also known to inhibit the TCA cycle) significantly abolished the growth, sphere formation, and population of CD24+/CD133+ LCSCs of HCC cells induced by PLOD1 overexpression (Fig. 7J–L, Fig. S6C).[13] Together, these observations imply the critical role of PLOD1 in facilitating the TCA cycle in HCC.

Discussion

Surgical resection is an effective treatment option for patients with early-stage HCC. Unfortunately, a large number of patients are diagnosed at an advanced stage and are no longer suitable for surgical intervention because of the heterogeneity and complexity of HCC. Consequently, the availability of treatment strategies for patients with advanced-stage HCC remains limited.[36,37] It is crucial to fully understand the detailed molecular mechanisms underlying HCC progression and to develop new therapeutic strategies. PLOD1, a lysyl hydroxylase, plays a vital role in collagen cross-linking and deposition.[38] A previous study showed that PLOD1 was highly upregulated in HCC and correlated with poor overall survival in patients with HCC.[27] However, the roles and underlying mechanisms of PLOD1 in the progression of HCC are still unknown. In our study, we found that PLOD1 is highly expressed in both human and mouse HCC. We further demonstrated that knockdown of PLOD1 attenuated the occurrence and growth of HCC both *in vitro* and *in vivo*. RNA-sequence and untargeted metabolomics analyses revealed that PLOD1 promoted the malignancy of HCC by strengthening the NF-κB/IL-6/STAT3 signaling pathway and TCA cycle. These findings indicate that PLOD1 has an oncogenic role in HCC and may serve as a potential target for HCC treatment.

A previous study demonstrated that PLOD1 is highly expressed in glioblastoma multiforme (GBM) and is associated with poor prognosis in patients. PLOD1 facilitates tumor viability, proliferation, migration, and malignant mesenchymal subtype transition of GBM.[25] In our current study, we observed that PLOD1 promoted the proliferation of HCC both *in vitro* and *in vivo*, although it had little influence on the migration of HCC cells. Stemness, apoptosis resistance, and rapid cell division are common features of tumor progression.[39–41] To determine how PLOD1 enhances the growth of HCC, the stemness, apoptosis resistance, and cell cycle of HCC cells were analyzed. We found that knockdown of PLOD1 inhibited HCC cell stemness, promoted HCC cell apoptosis, and induced S-phase cell cycle arrest in HCC. β-Catenin, STAT3, transforming growth factor beta, NOTCH, and Epithelial-mesenchymal transition (EMT) pathways were crucial for promoting cancer cell stemness.[42–46] In this study, we demonstrated that the effect of PLOD1 on the stemness of HCC cells is dependent on STAT3 signaling. Cancer stem cells play an important role in tumor initiation, growth, metastasis, and drug resistance.[47–49] However, in this study, we have not elucidated the role of PLOD1 in drug resistance in HCC, which could be an important scientific question for future research.

Metabolic reprogramming is one of the most common features of tumor cells. This study utilized RNA sequencing to investigate the underlying molecular mechanism by which PLOD1 contributes to HCC malignancy. We found that knocking down PLOD1 significantly inhibited the JAK-STAT signaling pathway. Additionally, we also observed a correlation between PLOD1 expression and processes related to fat and carbohydrate metabolism. Thus, to further uncover the potential role of PLOD1 in the metabolic reprogramming of HCC, control HCCLM3 cells and PLOD1-knockdown HCCLM3 cells were used for untargeted metabolomics analysis. The results showed that the knockdown of PLOD1 decreased the metabolites involved in the TCA cycle (citric acid and malic acid). Considering that the TCA cycle is the hub of the three major nutrients and also contributes to the malignancy of tumors, we hypothesized that the TCA cycle may be critical for PLOD1 in promoting HCC progression.[10] Our results also confirmed that pharmacological inhibition of the TCA cycle abolished PLOD1-induced HCC progression and stemness. Previous studies have noted TCA cycle metabolites are pivotal for modulating signaling pathways in immune cells.[50,51] However, in this study, we have not explored whether PLOD1 mediated-TCA cycle is involved in the immune regulation of HCC. Therefore, the detailed potential functions of PLOD1 in HCC still need further study. Our untargeted metabolomics analysis also revealed alterations in other implicated pathways, such as purine metabolism and choline metabolism, following PLOD1 knockdown. The role of these pathways in the promotion of HCC progression by PLOD1 also requires additional exploration.

In summary, this study reveals that PLOD1 is overexpressed in HCC and has an oncogenic role in HCC. Our results demonstrate that PLOD1 promotes HCC progression by facilitating the NF-κB/IL-6/STAT3-dependent TCA cycle. The PLOD1/NF-κB/IL-6/STAT3 axis may offer a promising therapeutic target for inhibiting HCC malignancy and stemness.

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Abbreviations

ALDH, aldehyde dehydrogenase; CCL_4, carbon tetrachloride; CTGF, connective tissue growth factor; DEN, diethylnitrosamine; EdU, 5-ethynyl-2′-deoxyuridine; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GBM, glioblastoma multiforme; HCC, hepatocellular carcinoma; ICGC, International Cancer Genome Consortium; IHC, immunohistochemical; LCSCs; liver cancer stem cells; LIF,

leukemia inhibitory factor; NAFLD, non-alcoholic fatty liver disease; PDH, pyruvate dehydrogenase; PI, propidium iodide; PLOD 1, procollagen lysyl hydroxylase 1; qPCR, quantitative PCR; STAT3, signal transducer and activator of transcription 3; TCA, tricarboxylic acid; WHO, World Health Organization.

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Conflicts of interest

The authors have declared that no competing interests exist.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Supervised the project and funding support: HX, FZ.Conceived and designed the experiments: HX, CZ, YZ. Performed the experiments: CZ, YZ, YP, ZM. Analyzed the data: CZ, YP. Wrote the original manuscript: CZ. Reviewed the manuscript: all authors.

Data availability statement

The data supporting this study's findings are available from the corresponding authors, upon reasonable request.

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Supplementary data

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References

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[1] Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–249.

[2] Villanueva A. Hepatocellular carcinoma. New Engl J Med 2019;380:1450–1462.

[3] **Yang C**, **Zhang H**, Zhang L, et al. Evolving therapeutic landscape of advanced hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2023;20:203–222.

[4] Akinyemiju T, Abera S, Ahmed M, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the Global Burden of Disease Study 2015. JAMA Oncol 2017;3:1683–1691.

[5] Xing X, Hu E, Ouyang J, et al. Integrated omics landscape of hepatocellular carcinoma suggests proteomic subtypes for precision therapy. Cell Rep Med 2023;4:101315.

[6] Zheng Y, Wang Y, Lu Z, et al. PGAM1 inhibition promotes HCC Ferroptosis and synergizes with Anti-PD-1 immunotherapy. Adv Sci (Weinh) 2023;10:e2301928.

[7] **Pavlova NN**, **Zhu J**, Thompson CB. The hallmarks of cancer metabolism: still emerging. Cell Metabol 2022;34:355–377.

[8] **Chen T**, **Xie S**, Cheng J, et al. AKT1 phosphorylation of cytoplasmic ME2 induces a metabolic switch to glycolysis for tumorigenesis. Nat Commun 2024;15:686.

[9] Liu N, Yan M, Tao Q, et al. Inhibition of TCA cycle improves the anti-PD-1 immunotherapy efficacy in melanoma cells via ATF3-mediated PD-L1 expression and glycolysis. J Immunother Cancer 2023;11:e007146.

[10] Du D, Liu C, Qin M, et al. Metabolic dysregulation and emerging therapeutical targets for hepatocellular carcinoma. Acta Pharm Sin B 2022;12:558–580.

[11] Lulli M, Del Coco L, Mello T, et al. DNA damage response protein CHK2 regulates metabolism in liver cancer. Cancer Res 2021;81:2861–2873.

[12] Xing T, Li L, Chen Y, et al. Targeting the TCA cycle through cuproptosis confers synthetic lethality on ARID1A-deficient hepatocellular carcinoma. Cel Rep Med 2023;4:101264.

[13] Yang C, Lee D, Zhang MS, et al. Genome-wide CRISPR/Cas9 library screening revealed dietary restriction of glutamine in combination with inhibition of pyruvate metabolism as effective liver cancer treatment. Adv Sci (Weinh) 2022;9:e2202104.

[14] Johnson RW, Finger EC, Olcina MM, et al. Induction of LIFR confers a dormancy phenotype in breast cancer cells disseminated to the bone marrow. Nat Cel Biol 2016;18:1078–1089.

[15] Makino Y, Hikita H, Kato S, et al. STAT3 is activated by CTGF-mediated tumor-stroma cross talk to promote HCC progression. Cell Mol Gastroenterol Hepatol 2023;15:99–119.

[16] **Kim H**, **Jang B**, Zhang C, et al. Targeting stem cells and dysplastic features with dual MEK/ERK and STAT3 suppression in gastric carcinogenesis. Gastroenterology 2024;166:117–131.

[17] **Haq ATA**, **Yang PP**, Jin C, et al. Immunotherapeutic IL-6R and targeting the MCT-1/IL-6/CXCL7/PD-L1 circuit prevent relapse and metastasis of triple-negative breast cancer. Theranostics 2024;14:2167–2189.

[18] Ishteyaque S, Singh G, Yadav KS, et al. Cooperative STAT3-NFkB signaling modulates mitochondrial dysfunction and metabolic profiling in hepatocellular carcinoma. Metab Clin Exp 2024;152:155771.

[19] Saha K, Subramenium Ganapathy A, Wang A, et al. Alpha-tocopherylquinone-mediated activation of the aryl hydrocarbon receptor regulates the production of inflammation-inducing cytokines and ameliorates intestinal inflammation. Mucosal Immunol 2023;16:826–842.

[20] **Zhou Q**, **Tian W**, **Jiang Z**, et al. A positive feedback loop of AKR1C3-mediated activation of NF-κB and STAT3 facilitates proliferation and metastasis in hepatocellular carcinoma. Cancer Res 2021;81:1361–1374.

[21] **Wang X**, **Sun W**, **Shen W**, et al. Long non-coding RNA DILC regulates liver cancer stem cells via IL-6/STAT3 axis. J Hepatol 2016;64:1283–1294.

[22] **Meng F**, **Zhang S**, **Song R**, et al. NCAPG2 overexpression promotes hepatocellular carcinoma proliferation and metastasis through activating the STAT3 and NF-κB/miR-188-3p pathways. EBioMedicine 2019;44:237–249.

[23] Hu M, Wu Y, Yang C, et al. Novel long noncoding RNA lnc-URIDS delays diabetic wound healing by targeting Plod1. Diabetes 2020;69:2144–2156.

[24] Dheekollu J, Wiedmer A, Soldan SS, et al. Regulation of EBNA1 protein stability and DNA replication activity by PLOD1 lysine hydroxylase. PLoS Pathog 2023;19:e1010478.

[25] **Wang Z**, **Shi Y**, Ying C, et al. Hypoxia-induced PLOD1 overexpression contributes to the malignant phenotype of glioblastoma via NF-κB signaling. Oncogene 2021;40:1458–1475.

[26] Wang D, Zhang S, Chen F. High expression of PLOD1 drives tumorigenesis and affects clinical outcome in gastrointestinal carcinoma. Genet Test Mol Biomarkers 2018;22:366–373.

[27] **Yang B**, **Zhao Y**, Wang L, et al. Identification of PLOD family genes as novel prognostic biomarkers for hepatocellular carcinoma. Front Oncol 2020;10:1695.

[28] **Zhang C**, **Wang X**, **Fang D**, et al. STK39 is a novel kinase contributing to the progression of hepatocellular carcinoma by the PLK1/ERK signaling pathway. Theranostics 2021;11:2108–2122.

[29] Chen J, Xu Z, Huang H, et al. SETD1A drives stemness by reprogramming the epigenetic landscape in hepatocellular carcinoma stem cells. JCI Insight 2023;8:e168375.

[30] **Yu J**, **Li W**, **Hou GJ**, et al. Circular RNA cFAM210A, degradable by HBx, inhibits HCC tumorigenesis by suppressing YBX1 transactivation. Exp Mol Med 2023;55:2390–2401.

[31] **Jiang Y**, **Xu Y**, **Zhu C**, et al. STAT3 palmitoylation initiates a positive feedback loop that promotes the malignancy of hepatocellular carcinoma cells in mice. Sci Signa 2023;16:eadd2282.

[32] Song Q, Zhang X, Liu W, et al. Bifidobacterium pseudolongum-generated acetate suppresses non-alcoholic fatty liver disease-associated hepatocellular carcinoma. J Hepatol 2023;79:1352–1365.

[33] Amaya ML, Inguva A, Pei S, et al. The STAT3-MYC axis promotes survival of leukemia stem cells by regulating SLC1A5 and oxidative phosphorylation. Blood 2022;139:584–596.

[34] Assante G, Chandrasekaran S, Ng S, et al. Acetyl-CoA metabolism drives epigenome change and contributes to carcinogenesis risk in fatty liver disease. Genome Med 2022;14:67.

[35] Xu YS, Liang JJ, Wang Y, et al. STAT3 undergoes acetylation-dependent mitochondrial translocation to regulate pyruvate metabolism. Sci Rep 2016;6:39517.
[36] Chen J, Feng W, Sun M, et al. TGF-β1-induced SOX18 elevation promotes hepatocellular carcinoma progression and metastasis through transcriptionally upregulating PD-L1 and CXCL12. Gastroenterology 2024;167:264–280.
[37] **Benichou E**, **Seffou B**, **Topçu S**, et al. The transcription factor ChREBP Orchestrates liver carcinogenesis by coordinating the PI3K/AKT signaling and cancer metabolism. Nat Commun 2024;15:1879.
[38] Li L, Hua R, Hu K, et al. SIRT6 deficiency causes ovarian hypoplasia by affecting Plod1-related collagen formation. Aging Cell 2024;23:e14031.
[39] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–674.
[40] **Zhang Q**, **Tsui YM**, **Zhang VX**, et al. Reciprocal interactions between malignant cells and macrophages enhance cancer stemness and M2 polarization in HBV-associated hepatocellular carcinoma. Theranostics 2024;14:892–910.
[41] Lee TK, Guan XY, Ma S. Cancer stem cells in hepatocellular carcinoma - from origin to clinical implications. Nat Rev Gastroenterol Hepatol 2022;19:26–44.
[42] Fan L, Tian C, Yang W, et al. HKDC1 promotes liver cancer stemness under hypoxia through stabilizing β-catenin. Hepatology 2024. https://doi.org/10.1097/HEP.0000000000001085.
[43] Liu Y, Xu Q, Deng F, et al. HERC2 promotes inflammation-driven cancer stemness and immune evasion in hepatocellular carcinoma by activating STAT3 pathway. J Exp Clin Cancer Res 2023;42:38.
[44] Liu K, Tian F, Chen X, et al. Stabilization of TGF-β receptor 1 by a receptor-associated adaptor dictates feedback activation of the TGF-β signaling pathway to maintain liver cancer stemness and drug resistance. Adv Sci (Weinh) 2024;11:e2402327.
[45] Fang S, Liu M, Li L, et al. Lymphoid enhancer-binding factor-1 promotes stemness and poor differentiation of hepatocellular carcinoma by directly activating the NOTCH pathway. Oncogene 2019;38:4061–4074.
[46] **Huang Y**, **Hong W**, Wei X. The molecular mechanisms and therapeutic strategies of EMT in tumor progression and metastasis. J Hematol Oncol 2022;15:129.
[47] **Huang Z**, **Zhang K**, Jiang Y, et al. Molecular glue triggers degradation of PHGDH by enhancing the interaction between DDB1 and PHGDH. Acta Pharm Sin B 2024;14:4001–4013.
[48] **Park M**, **Jung E**, Park JM, et al. The HSP90 inhibitor HVH-2930 exhibits potent efficacy against trastuzumab-resistant HER2-positive breast cancer. Theranostics 2024;14:2442–2463.
[49] **Liu HY**, **Sun XJ**, Xiu SY, et al. Frizzled receptors (FZDs) in Wnt signaling: potential therapeutic targets for human cancers. Acta Pharm Sin 2024;45:1556–1570.
[50] Bei J, Chen Y, Zhang Q, et al. HBV suppresses macrophage immune responses by impairing the TCA cycle through the induction of CS/PDHC hyperacetylation. Hepatol Commun 2023;7:e0294.
[51] Peace CG, O'Carroll SM, O'Neill LAJ. Fumarate hydratase as a metabolic regulator of immunity. Trends Cel Biol 2024;34:442–450.

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