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Original Article

PDK3 drives colorectal carcinogenesis and immune evasion and is a therapeutic target for boosting immunotherapy

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Abstract: Pyruvate Dehydrogenase Kinase 3 (PDK3) has emerged as a significant player in various cancer types, yet its specific impact on cancers including colon cancer remains ambiguous. Through pan-cancer analysis using TCGA data, we found that the expression of *PDK3* and the composition of the immune microenvironment for different tumors were highly heterogeneous across tumors. *PDK3* is highly expressed in colorectal cancer and may promote tumor proliferation by activating PI3K-AKT signaling. In addition, we found that *PDK3* was able to inhibit tumor antigen presentation signals to suppress immune killing. High *PDK3* expression predicts less $CD8^+$ T cell infiltration and effector function. Moreover, inhibition of *PDK3* expression bolstered $CD8^+$ T cell-mediated cytotoxicity $CD8^+$ T cell infiltration and activation in vivo. Notably, *PDK3* was found to facilitate STAT1 activation and elevate programmed death-ligand 1 (PD-L1) expression in colon cancer cells. Importantly, *PDK3* inhibition combination with PD-1 blockade significantly activates the infiltrated $CD8^+$ T cells to suppress tumor growth and improves the survival benefit in several murine tumor models. In summary, these findings underscore PDK3's role in fueling colon cancer growth by orchestrating PI3K-AKT signaling and PD-L1 expression and dampening $CD8^+$ T cell function.

Keywords: Colorectal cancer, Pyruvate Dehydrogenase Kinase 3, immune infiltration

Introduction

Protein kinase D3 (PDK3), a member of the protein kinase D (PKD) family, has recently emerged as a pivotal regulator in the intricate landscape of tumorigenesis [1]. With cancer being one of the leading causes of mortality worldwide, understanding the molecular mechanisms underlying its development and progression is of paramount importance. PDK3, implicated in various cellular processes ranging from proliferation to apoptosis, has garnered significant attention for its potential role in promoting or suppressing tumor growth [2]. Tumorigenesis is a multifaceted process characterized by the aberrant regulation of signaling pathways governing cell proliferation, differentiation, and survival. Dysregulation of these pathways can lead to uncontrolled cell growth and the formation of malignant tumors. In this context, the involvement of PDK3 in modulating key signaling cascades implicated in tumorigenesis has sparked considerable interest among researchers.

Cancer immunotherapy, which harnesses the power of the immune system to target and eradicate tumors, has revolutionized cancer treatment [3]. However, tumor immune evasion remains a significant challenge, limiting the effectiveness of current immunotherapeutic approaches. Tumor immune evasion encom-

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passes a variety of mechanisms through which cancer cells evade detection and destruction by the immune system. These mechanisms include the downregulation of major histocompatibility complex (MHC) molecules, which are crucial for presenting tumor antigens to T cells [4], as well as the upregulation of immune checkpoint molecules, such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which inhibit T cell activation and function [5]. Additionally, tumors can recruit immunosuppressive cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), to create an immunosuppressive microenvironment that facilitates immune evasion and tumor progression [6]. Furthermore, PDK3-mediated alterations in metabolic pathways may contribute to immune evasion by creating a metabolically hostile microenvironment that impairs the function of immune cells. For example, increased glycolytic metabolism in tumor cells can lead to the accumulation of lactate and acidification of the tumor microenvironment, which suppresses the function of cytotoxic T cells and promotes immune evasion.

Understanding the intricate relationship between PDK3 and tumor immune evasion holds promise for the development of novel immunotherapeutic strategies to overcome this critical barrier to effective cancer treatment. Targeting PDK3 and its downstream signaling pathways may enhance the antitumor immune response, restore immune cell function, and improve the efficacy of existing immunotherapies. Moreover, PDK3 expression and activity may serve as potential biomarkers for predicting response to immunotherapy and guiding treatment decisions in cancer patients.

Methods

Data extraction and data processing

From the TCGA database (https://portal.gdc.cancer.gov/), genetic mutation data, transcriptome data, and clinical data of 33 different human cancer types samples were gathered.

Cell culture

CT26 and MC38 mouse colon cancer cells, as well as human colon cancer cells HCT116 and RKO, were procured from the National Infrastructure of Cell Line Resource and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS. Cells were incubated with 5% CO_2 at 37°C.

shRNA and vector construction

An shRNA fragment targeting the CDS of human *PDK3* was generated using a pair of primers (forward primer: 5'-C C G G A G A G T T G G T A T A T G C A G A G T T C T C G A G T T A T C G G T T G G A A C C T T C A A T T T T T T G-3'); of mouse *Pdk3* was generated using a pair of primers (forward primer: 5'-C C G G C C C A C C T G T A A T G T A G C T G A T C T C G A G T T A T C G G T T G G A A C C T T C A A T T T T T T G-3') and cloned into the plasmid pLKO.1-TRC (Addgene 10878) as described in the TRC protocols http://www.broadinstitute.org.

Lentiviral transduction of tumor cells

Lentivirus-induced *PDK3*-knockdown was conducted in HCT116, SW480, MC38, and CT26 cells. 4 μg PLKO.1 plasmid of shCtrl or sh PDK3, 3 μg psPAX2 packaging vector and 1 μg VSVG enveloped vector were co-transfected into HEK-293T cells in 10 cm cell-culture dishes. After 48 and 72 hr, virus-containing supernatant was collected and filtered through a 0.45 mm PES Syringe Filter (Thermo Fisher), and then were used to infect tumor cells in the presence of 8 mg/ml Polybrene. Finally, 2 μg/ml puromycin (Solarbio) for indicated cells was added to select the positive infected cells for 5 days and stable cell lines were further maintained under puromycin.

Western blot

1 milliliter of cell lysis buffer (50 milliliter Tris base (pH 7.4), 150 milliliter Sodium chloride, 1% Nonidet P-40, 0.5% Sodium deoxycholate, 0.1% Sodium dodecyl sulfate, 5 milliliter Ethylene Diamine Tetraacetic Acid, and 1 milliliter Benzylsulfonyl fluoride) was used to lyse transfected cells. As stated, immunoblot analyses were carried out.

Flow cytometry

Tumor tissues from shCtrl or sh*Pdk3* MC38 and CT26 were digested at 37°C for 30 minutes using 1 mg/ml Collagenase D and 0.1 mg/ml DNase I (Roche). The digestion was halted with EDTA, and cells were filtered through 70

μm cell strainers, then washed twice with PBS containing 1 mM EDTA and 2% FBS (staining buffer). Cells were resuspended in the staining buffer and stained with the following antibodies on ice for 30 minutes: anti-CD45, anti-CD8, anti-IFN-γ, and anti-Granzyme B (all from BioLegend).

Cell proliferation assay

Cells were seeded in triplicate at a density of 1,000 cells per well in 96-well plates for MTT assays and incubated for 5 days. The MTT uptake was assessed daily by measuring the absorbance at 450 nm, following the manufacturer's guidelines.

Cell apoptosis assay

Before beginning the assays, 1×10^5 cancer cells were seeded in a 24-well plate. After digestion with EDTA-free trypsin, cells were collected by centrifugation at 300 g for 5 min at 4°C. To prevent false positives, trypsin digestion was carefully controlled to avoid excessive duration. Subsequently, the cells were washed twice with pre-cooled PBS, each time at 300 g, and centrifuged at 4°C for 5 mins. Following the removal of PBS, 100 μL of 1 × Binding Buffer was added to resuspend the cells. Annexin V-FITC (5 μL) was then added and gently mixed. The mixture was protected from light and allowed to react at room temperature for 10-15 mins. Next, 400 μL of 1 × Binding Buffer was added, mixed, and placed on ice. Finally, samples were detected by flow cytometry or fluorescence microscopy within 1 hour.

PDK3-related immune infiltration analysis

The TIMER website (https://cistrome.shinyapps.io/timer/) is a web server designed for in-depth analysis of tumor-infiltrating immune cells. Utilizing this database, researchers investigated the correlation between *PDK3* expression and various immune cell populations, including $CD8^+$ T cells, neutrophils, and macrophages et al.

Mouse model and tumor studies

Eight-week-old male C57BL/6 or naked mice were randomly assigned to groups of five for the in vivo experiments. The mice weighed around 21 g on average when they were first born. PBS was used to prepare 1×10^6 cells per 100 μL for the preparation of both control and stable PDK3 knockdown cells via digestion and resuspension. The right flank of each mouse was then subcutaneously injected with a 100 μL cell solution. Tumor size was measured every three days while the mice were kept in the same housing circumstances for the duration of the study. The formula used to compute the tumor volume was Volume = length × width^2 × 0.5. Mice were humanely put to death on day sixteen by intraperitoneal injection of 150 mg/kg pentobarbital.

Tumor-infiltrating $CD8^+$ T-cell responses

The tumor tissue was divided into small pieces and subjected to an enzymatic digestion process for 30 minutes at 37°C with 200 μg/ml collagenase and 20 μg/ml DNase. After digestion, a 0.45 μm filter was used to filter the tumor tissue suspension, and the single-cell suspension that was left behind was collected. As directed by the manufacturer, Golgiplug Protein Transport Inhibitor was applied to the gathered cells. The cells were then stained for 30 minutes at 4°C using CD8 antibody, intracellular IFN-γ, and GzmB antibody. The BD X20 cytometer was then used to conduct a flow cytometry analysis.

Statistical analysis

Data are presented as the mean ± SD. A Student's t-test was used for comparisons between the two groups. A one-way ANOVA was used for comparison between multiple groups. $P<0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed using GraphPad Prism version 9.5.

Results

PDK3 is upregulated in multiple cancer types

The expression profiles of *PDK3* in several cancers based on data from TCGA were analyzed using GEPIA (http://gepia.cancer-pku.cn/), and the results indicated that *PDK3* mRNA was upregulated in numerous cancers, including breast invasive carcinoma (BRCA), clear cell carcinoma of the kidney (KIRC), acute myeloid leukemia (LAML), and colorectal cancer (COAD and READ) (**Figure 1A**, **1B**). In particular, the

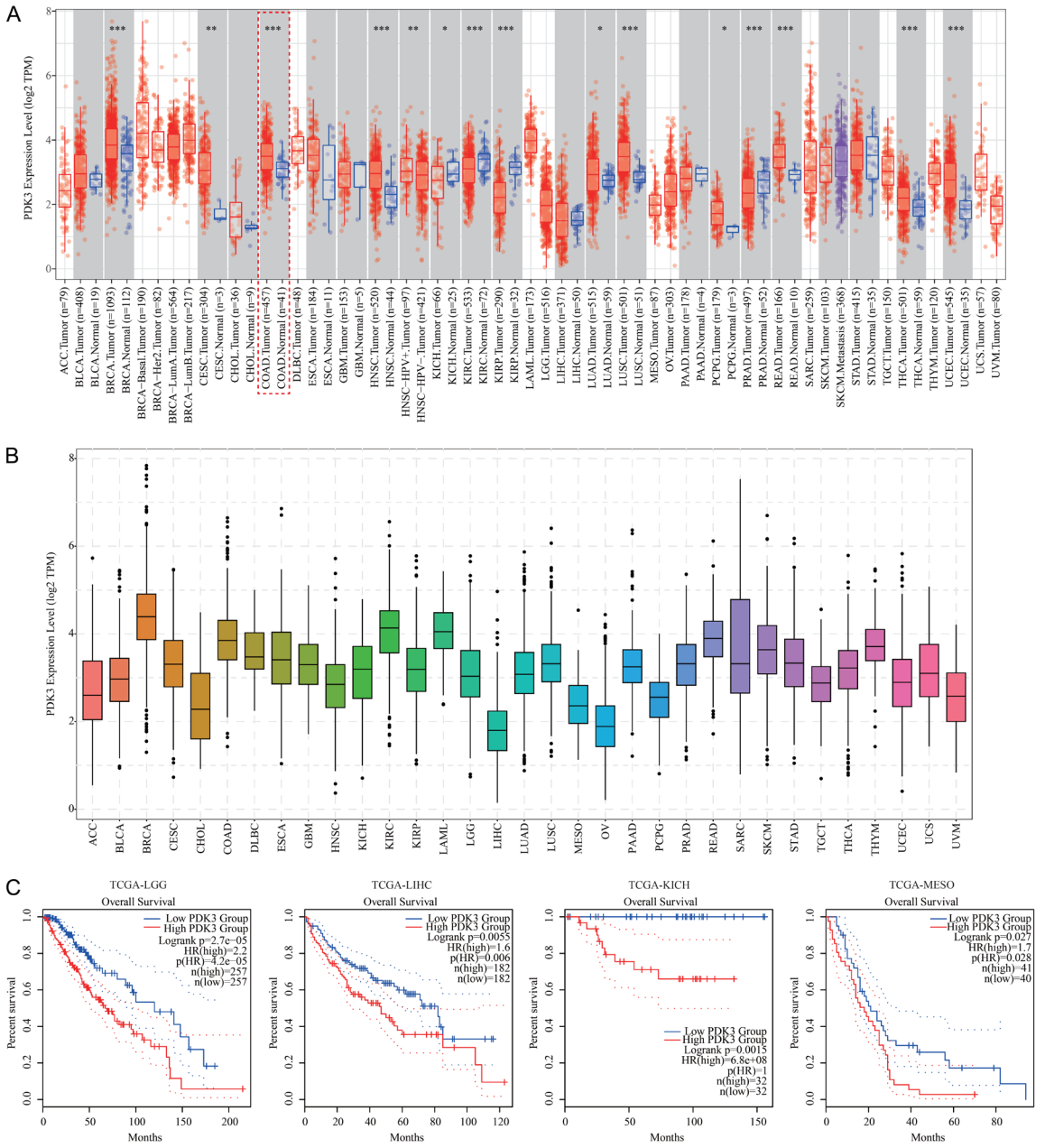


Figure 1. PDK3 expression in several types of cancer and its prognostic effect. A. Differences in the gene expression of PK3 between tumor and normal tissues across 33 different types of solid cancers. B. PDK3 expression in 33 different types of solid cancers. C. Kaplan-Meier curves of overall survival for 4 cancer patients with low or high PDK3 expression.

expression of PDK3 in colon cancer was higher than that in normal tissues (**Figure 1A**). In addition, data on four cancer types from TCGA were also analyzed, and it was found that patients with higher PDK3 expression had markedly lower survival times (**Figure 1C**). Together, these data indicate that PDK3 is upregulated in multiple cancer types and correlated with poor prognostic outcomes.

PDK3 expression correlates with tumor immune infiltration and immune-related molecules in pan-cancers

The association between *PDK3* expression and immune cell infiltration was analyzed to determine its pan-cancer immunological role. The results revealed that *PDK3* is negatively correlated with the immune score and infiltration of

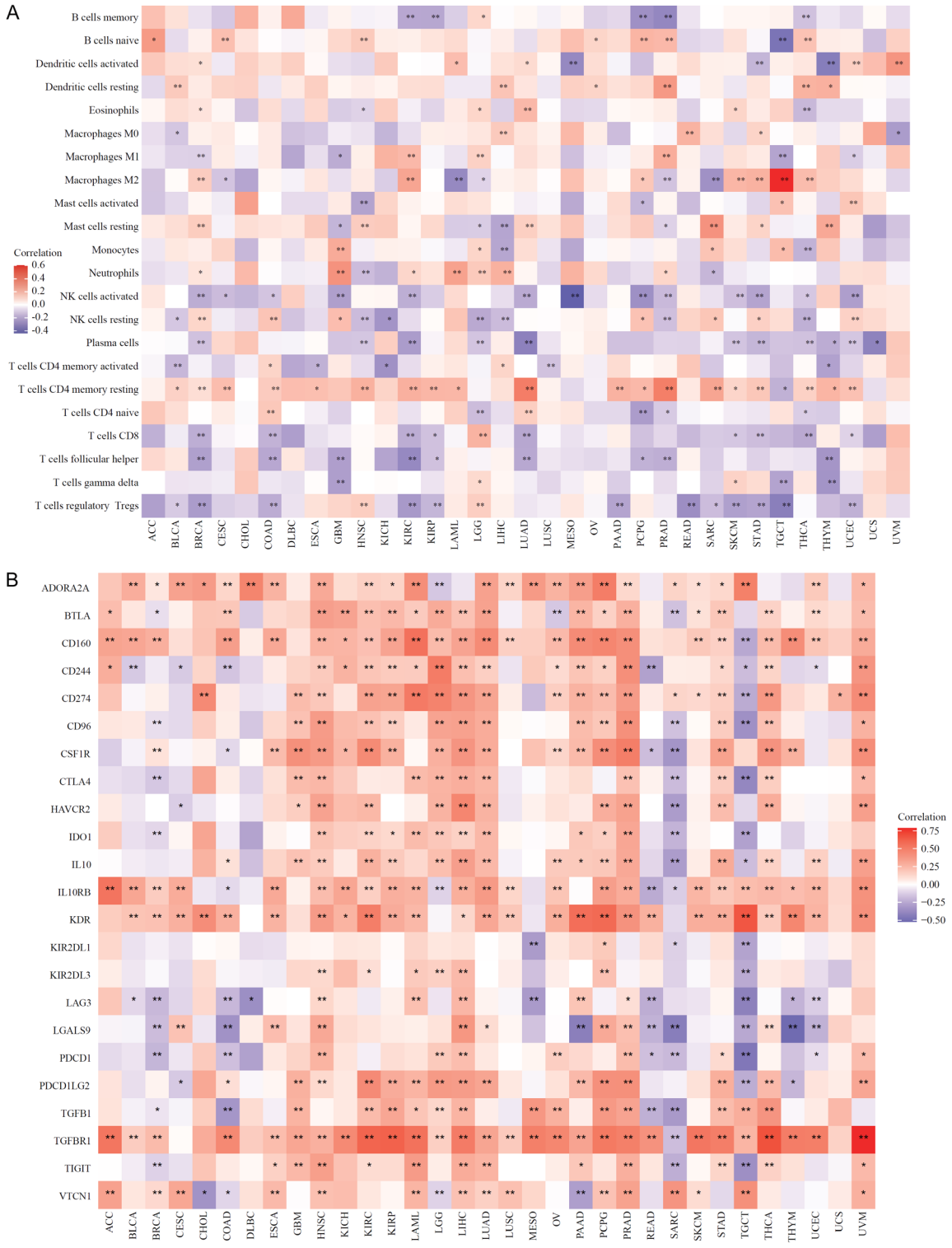


Figure 2. Bioinformatics analysis of the relationship between PDK3 expression and immune-related molecules. A. Correlation between PDK3 gene expression and immune infiltration. B. Correlation between PDK3 gene expression and immune inhibitory molecules.

numerous types of immune cells in multiple cancer types based on the ssGSEA algorithm (**Figures 2A** and S1A). The correlation of *PDK3* expression and several immune-related inhibi-

tion molecules were also performed by Pearson analysis. The result indicated that *PDK3* was significantly correlated with the expression of several immune-related inhibition molecules (**Figure 2B**), including CD160, CTLA-4, TGFBR1, TIGIT and KDR, in multiple cancer types. *PDK3* was also found to be significantly positively correlated with the expression of chemokines and chemokine receptors (Figure S2A, S2B). Meanwhile, *PDK3* was also positively correlated with the expression of some immune checkpoint and other immunomodulatory molecules (Figure S1B, S1C).

PDK3 promotes colon cancer growth through activating PI3K-AKT pathway

PDK3 expression was higher in colon cancer compared to the normal samples in several GEO datasets (**Figure 3A**, **3B**). PDK3 expression in tissues of different TNM stages was analyzed, and the results showed that the expression of *PDK3* in stages, histological subtypes, nodal metastasis status, and TP53 mutation status of colon cancer were significantly higher than that in the normal tissues (**Figure 3C-F**). To determine whether *PDK3* affected colon cancer growth, shRNA-mediated *PDK3* knockdown was performed in HCT116 and RKO human colon cancer cells. As shown in **Figures 3G** and S3A, the mRNA expression and protein levels of *PDK3* in HCT116 and RKO cells were markedly lower following knock down. A cell proliferation assay was performed to observe the effect of *PDK3* expression on the *in vitro* proliferation of HCT116 and RKO cells. As shown in **Figure 3H**, knockdown of *PDK3* expression decreased the proliferation of HCT116 and RKO cells. However, Restoration of *PDK3* expression reverted the cell proliferation inhibition mediated by *PDK3* knockdown (Figure S3B, S3C). Cell proliferation assay was also performed to investigate the effect *PDK3* overexpression. In contrast to *PDK3* knockdown, overexpression of *PDK3* significantly promoted the proliferation of colorectal cancer cells, a result that suggests that *PDK3* is a potential pro-oncogene (Figure S3E). Apoptosis assays also verified that *PDK3* knockdown promotes apoptosis in HCT116 tumor cells (**Figure 3I**). Subcutaneous graft tumor model was then established in BALB/c nude mice by subcutaneously injecting *PDK3*-knockdown or control HCT116 and RKO cells. The mice were sacrificed 16 days after injection. As shown in **Figure 3J** and **3K**, the growth of *PDK3*-knockdown HCT116 and RKO tumors was significantly decreased compared with that of mice injected with control cells. These results suggest that *PDK3* plays an important role in the occurrence and development of colon cancer. In order to investigate the potential mechanisms by which *PDK3* promotes proliferation in colorectal cancer, we used TCGA-COADA data to classify high and low *PDK3* expression groups based on median *PDK3* expression, followed by differential analysis and KEGG signaling pathway analysis. We found that the *PDK3* high-expression group was significantly enriched in the PI3K-AKT signaling pathway (**Figure 3L**), and GSEA analysis demonstrated that *PDK3* expression was significantly and positively correlated with the PI3K-AKT signaling pathway (**Figure 3M**). To verify this finding, we knocked down *PDK3* in HCT116 cells and found that inhibition of *PDK3* significantly reduced the activity of PI3K and AKT as well as the protein content of the downstream target gene c-Myc (**Figure 3N**). We also rescue the AKT protein in the sh*PDK3* HCT116 cells for cell proliferation assay, and result showed that rescuing WT but not the enzyme inactivated T308A AKT expression rescued the cell growth disadvantage caused by *PDK3* knockdown, which indicated that *PDK3* may promote the proliferation of colorectal cancer through the activation of PI3K-AKT signaling (Figure S3D). These results suggested that *PDK3* may promote the proliferation of colorectal cancer through the activation of PI3K-AKT signaling.

PDK3 inhibits tumor antigen presentation and the T-cell killing signals

Given that the prerequisite for tumor killing by $CD8^+$ T cells requires their specific recognition of the tumor's own antigens, which in turn allows them to kill tumor cells, this phenomenon is known as MHC molecular restriction. Therefore, we first analyzed the correlation of tumor mutational load (TMB) and microsatellite instability (MSI) with *PDK3* expression, which, at least in colorectal cancer, showed a negative correlation with TMB and MSI (**Figure 4A**). Subsequently, we analyzed the correlation between *PDK3* and molecules related to MHC-I antigen presentation, and found that *PDK3* was significantly negatively correlated with the

Roles of PDK3 on CRC growth and immune evasion

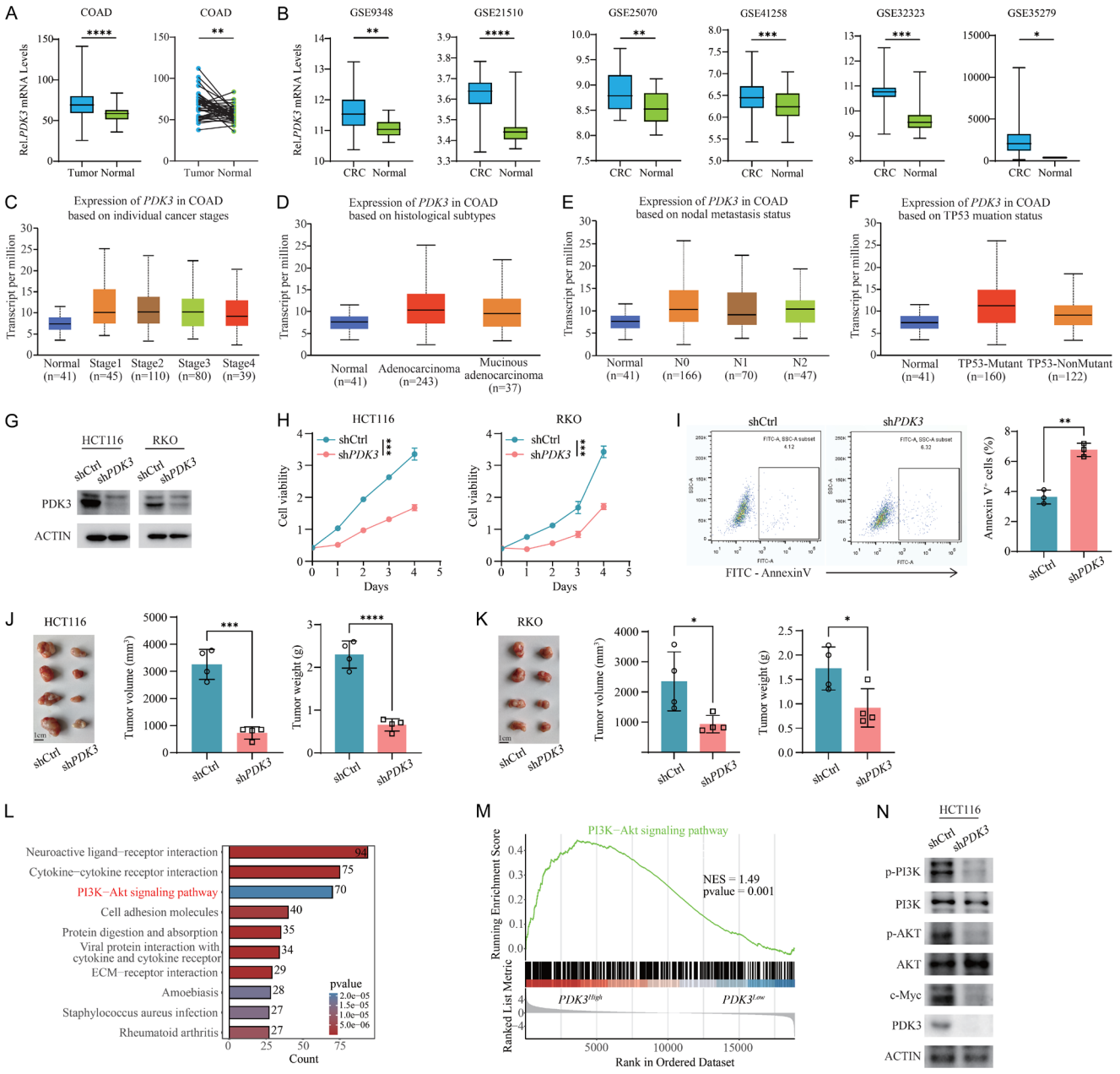
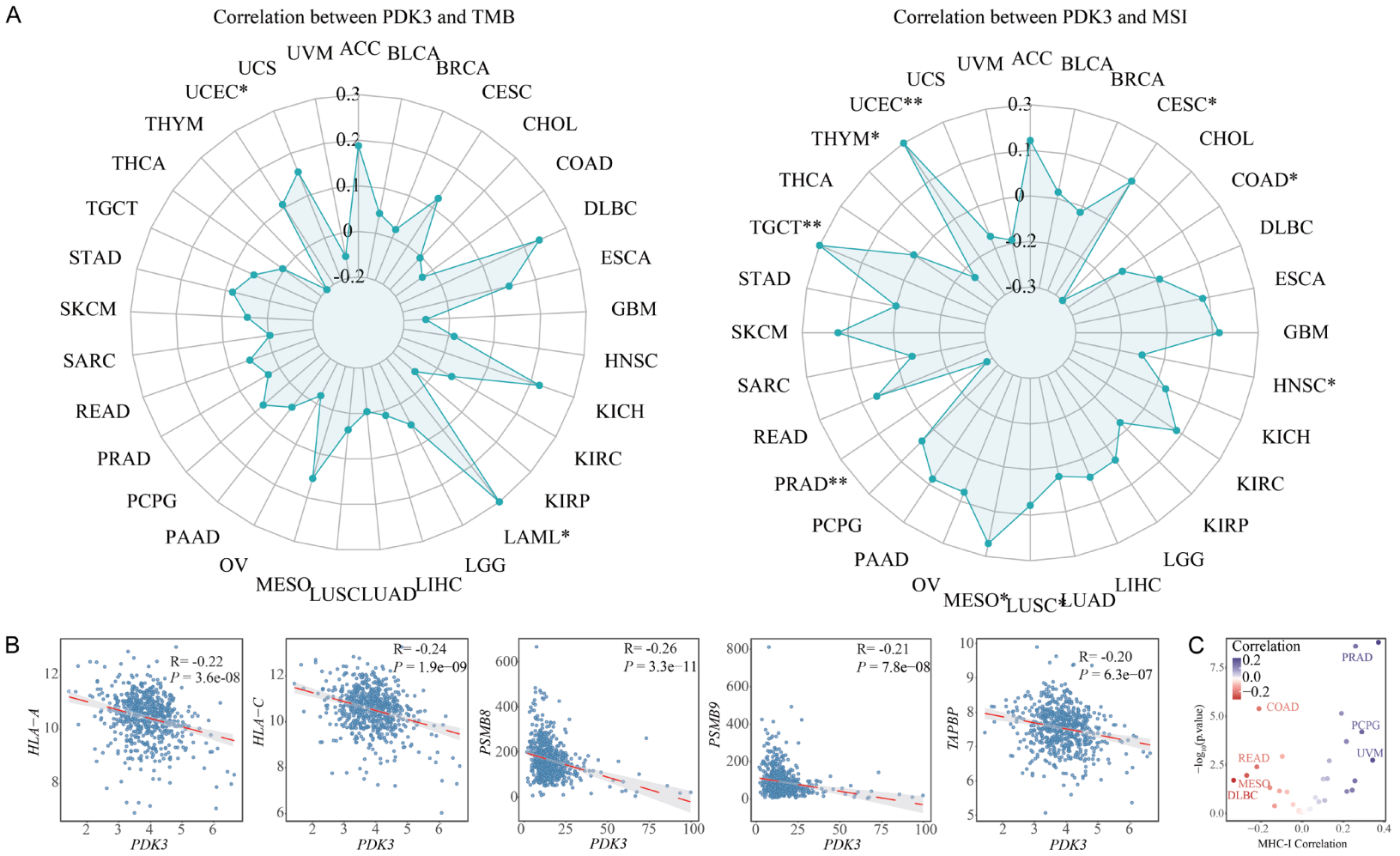


Figure 3. PDK3 promotes CRC growth by activating PI3K-AKT signaling. A, B. PDK3 gene expression level was up-regulated in multiple datasets. C-F. PDK3 expression in colon cancer based on clinical stage, histological subtypes, nodal metastasis, and TP53 mutation status. G. The protein expression levels of PDK3 were analyzed by western blotting. H. Effect of PDK3 on the proliferation of HCT116 and RKO cells was assessed using a CCK-8 assay. I. Cell apoptosis was detected after PDK3 knockdown. J, K. Detection of tumor volume and weight in mice. Subcutaneous tumorigenesis in 8-week-old mice: 1 × 10^6 PDK3-knockdown or control HCT116 and RKO cells were implanted into the mice. L. KEGG analysis based on PDK3-high versus PDK3-low patients. M. GSEA analysis shows that the PI3K-AKT signaling was positive with high PDK expression. N. The protein expression levels of PI3K-AKT signaling were analyzed by western blotting.



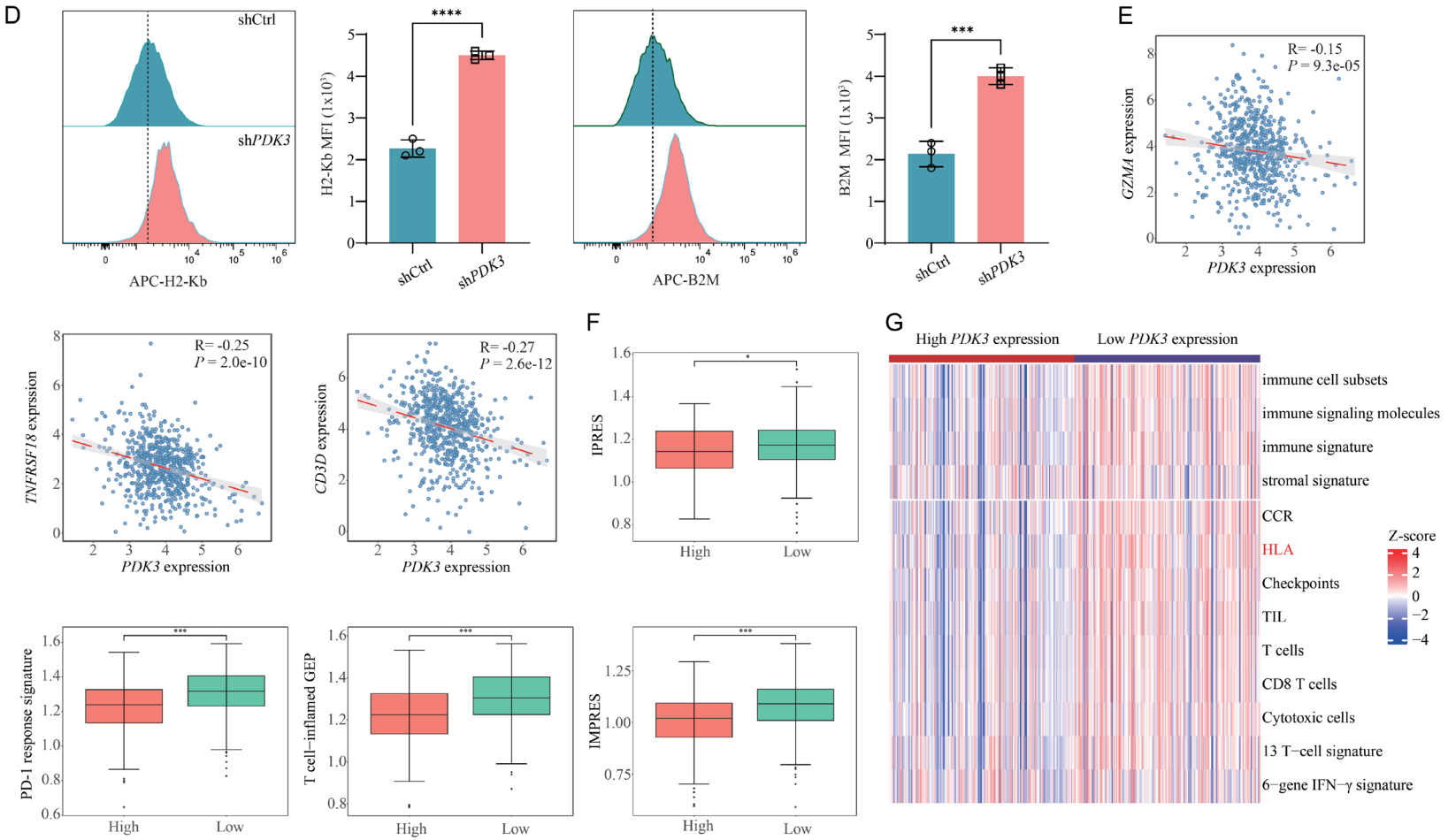
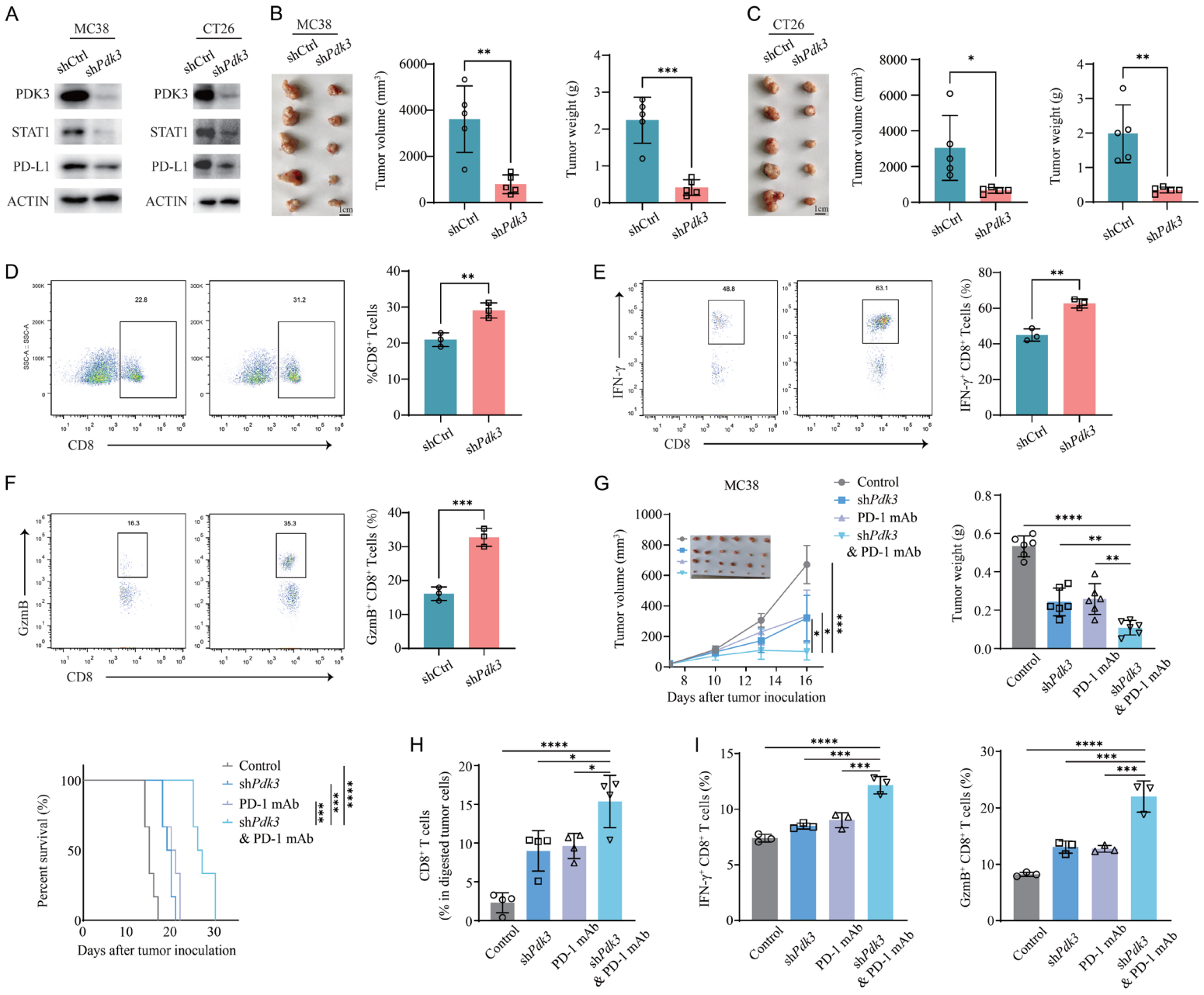


Figure 4. *Pdk3* inhibit antigen presentation and CD8⁺ T cell effect signal. A. Correlation between PDK3 gene expression and MSI and TMB. B. Correlation between PDK3 gene expression and several MHC-I molecules. C. Correlation between PDK3 gene expression and MHC-I based pan-cancer analysis. D. *Pdk3* knockdown increased tumor antigen presentation. E. Correlation between PDK3 gene expression and several CD8⁺ T cell effect molecules. F. Several immune responsive signaling based PDK3 gene expression. G. Several immune-related signaling based PDK3 gene expression.

expression of these antigen presentation-related molecules (**Figure 4B**), as shown by pan-cancer analysis in tumors such as DLBC, including CRC (COAD and READ) (**Figure 4C**). To further validate this biological phenomenon, we knocked down the expression of *PDK3* in MC38 tumor cell lines and further went on to examine the expression of MHC-I molecules. The results revealed that knockdown of PDK3 significantly upregulated the expression of H2-Kb and B2M molecules (**Figure 4D**). It indicates that *PDK3* inhibition significantly enhances antigen presentation in tumors. Our further analysis revealed that the expression of *PDK3* was significantly negatively correlated with the expression of several effector molecules of CD8 T cells (**Figure 4E**), suggesting that we *PDK3* may be able to inhibit the effector function of CD8 T cells in tumors. Furthermore, compared with *PDK3*-low samples, higher enrichment scores of T cell inflamed gene expression profile (GEP), innate anti-PD-1 resistance (IPRES), PD-1 response signature, and immuno-predictive score (IMPRES) signatures were observed in *PDK3*-high samples (**Figure 4F**). *PDK3*-low patients exhibited significant enrichment of signatures identifying immune cells or immune response (**Figure 4G**). Taken together, these data indicate that *PDK3* could inhibit MHC-I presentation and $CD8^+$ T cell effector function signal.

The combination of PDK3 inhibition with PD-1 blockade significantly suppresses tumor growth and enhances the survival rate in mouse tumor models

The above experimental results showed that PDK3 inhibited the activation of $CD8^+$ T c ells in the tumor microenvironment in colon cancer, thus promoting tumor growth in vivo. The mechanism through which PDK3 in tumor cells promoted T cells to induce immune tolerance was next assessed. Tumor cells often expressed higher PD-L1 to promote their immune evasion. Therefore, The expression levels of PD-L1 protein were detected in *Pdk3*-knockdown MC38 and CT26 cells. The results showed that the expression levels of PD-L1 in colon cancer cells with *Pdk3* deletion were lower than that in control cells (**Figure 5A**). To explore the mechanism by which *Pdk3* upregulated PD-L1 expression, the effect of *Pdk3*on the expression of PD-L1 and activation of the JAK/STAT1 signaling pathway was measured. As *Pdk3* increased PD-L1 expression, and as PD-L1 gene expression is regulated by the transcription factor STAT1, whether *Pdk3* affected the activation of STAT1 was assessed. As anticipated, *Pdk3* knockdown markedly decreased the protein levels of STAT1 and in MC38 and CT26 cells (**Figure 5A**). A transplantation tumor model was also then established in C57BL/6 mice by subcutaneously injecting *Pdk3*-knockdown or control MC38 and CT26 cells. The mice were sacrificed 16 days after injection. As shown in **Figure 5B** and **5C**, the growth of *Pdk3*-knockdown MC38 and CT26 cells *in vivo* was significantly decreased compared with that of mice injected with control cells. FACS analysis was then used to examine the changes that occurred in intratumoral immune cell infiltration. The distribution of different immune cell subpopulations was evaluated in the tumor microenvironment. In tumors harvested from mice injected with *Pdk3*-knockdown cells, the percentage of $CD8^+$ cytotoxic T cells was significantly higher than that in the control group (**Figure 5D**). Flow cytometry was further used to analyze the effect of PDK3 on the ability of $CD8^+$ T cells to secrete cytokines. It was found that *Pdk3* knockdown in MC38 cells markedly decreased the secretion of IFN-γ and Granzyme B by $CD8^+$ T cells (**Figure 5E**, **5F**). Based on the molecular mechanism study, we hypothesize that inhibition of *Pdk3* might sensitize tumors to the anti-PD-1 immunotherapy in vivo. To test this hypothesis, we utilized the syngeneic mouse MC38 tumor model to examine how the combination of *Pdk3* inhibition with anti-PD-1 antibody affected tumor growth and mice survival. Strikingly, our results showed that combinational treatment of the *Pdk3* inhibition plus anti-PD-1 antibody significantly suppressed tumor growth and improved the overall survival rates of MC38 tumor-bearing immunocompetent C57BL/ 6 mice compared to either single-agent or control-treated group (**Figure 5G**). Analysis of infiltrated immune cells demonstrated that the *Pdk3* inhibition combined with anti-PD- treatment could significantly increase the percentage of $CD8^+$ T cells. To further address whether the *Pdk3* inhibition affects the activation of tumor-infiltrating $CD8^+$ T cells (**Figure 5H**), we also detected the T-cell activation maker, Granzyme B (GzmB) and IFN-γ, on infiltrated $CD8^+$ T cells in syngeneic MC38 mice tumor model. Our results showed that the *Pdk3*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Control
shPdk3
PD-1 mAb
shPdk3 & PD-1 mAb
Tumor volume (mm³)
Days after tumor inoculation
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Percent survival (%)
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IFN-γ⁺ CD8⁺ T cells (%)
GzmB⁺ CD8⁺ T cells (%)

Figure 5. The combination of PDK3 inhibition with PD-1 blockade significantly suppresses tumor growth and enhances the survival rate in mouse tumor models. A. The protein expression levels of STAT1 and PD-L1 were analyzed by western blotting. B, C. Detection of tumor volume and weight in mice. Subcutaneous tumorigenesis in 8-week-old mice: 1×10^6 *Pdk3*-knockdown or control MC38 and CT26 cells were implanted into the mice. D. Detection of $CD8^+$ T cell infiltration in tumor-infiltrating lymphocytes using flow cytometry. E, F. Detection of IFN-γ^+ and $GzmB^+$ CD8 T cell infiltration in tumor-infiltrating lymphocytes using flow cytometry. G. Tumor growth and weight or Kaplan-Meier survival curves for C57BL/6 mice bearing MC38 tumors with indicated treatments. H. Detection of $CD8^+$ T cell infiltration in tumor-infiltrating lymphocytes using flow cytometry. I. Detection of IFN-γ^+ and $GzmB^+$ CD8 T cell infiltration in tumor-infiltrating lymphocytes using flow cytometry.

inhibition combined with anti-PD-1 treatment significantly elevated the expression of GzmB and IFN-γ on infiltrated $CD8^+$ T cells (**Figure 5I**). Taken together, these results reveal a molecular mechanism and potential strategy of combination therapy of PDK3 inhibition plus anti-PD-1 antibody to enhance the efficacy of antitumor therapy largely through reprograming an inflamed TME.

Discussion

The PDK3 gene encodes a protein kinase called enolase. The function of this enzyme in cells is to regulate the glycolytic pathways involved in metabolic processes, particularly those related to energy production in mitochondria [7]. Specifically, enolase prevents mitochondria from utilizing glucose for energy production by inhibiting one step in the glucose catabolic pathway. This regulation helps cells adapt to energy demands under low oxygen conditions, such as during exercise or hypoxic environments. In some cases, excessive activity of PDK3 may lead to metabolic disorders that are associated with the development of diseases such as diabetes and tumors. Therefore, studying the role of the PDK3 gene and the proteins it encodes in these diseases is important for understanding disease mechanisms and developing therapeutic approaches.

The emerging role of PDK3 in tumorigenesis, emphasizing its involvement in cell cycle regulation, signaling pathways, metabolic reprogramming, and its association with cancer progression. The dysregulation of PDK3 expression has been implicated in the pathogenesis of various cancer types, including breast, prostate, and CRC cancer. High levels of PDK3 expression have been associated with poor prognosis and increased tumor aggressiveness in some malignancies, highlighting its potential as a prognostic biomarker and therapeutic target. Understanding the intricate interplay between PDK3 and tumorigenesis holds promise for the development of novel therapeutic strategies aimed at combating this devastating disease. Our research indicated that PDK3 mRNA expression level was up-regulated in multiple cancer types, including CRCs. And we found that PDK3 inhibition decreased the CRC cells proliferation and promoted apoptosis. We further found that PDK3 could promote CRC growth may through activating PI3K-AKT signaling. However, emerging evidence suggests that PDK3 may play a role in regulating cellular metabolism, another hallmark of cancer. Metabolic reprogramming is a common feature of cancer cells, enabling them to sustain rapid proliferation and adapt to the tumor microenvironment. PDK3-mediated alterations in metabolic pathways, such as glycolysis and lipid metabolism, may contribute to the metabolic shift observed in cancer cells, fueling their growth and survival.

We also found that the emerging role of PDK3 in mediating tumor immune evasion, highlighting its involvement in immune checkpoint regulation, immune cell recruitment, and metabolic reprogramming within the tumor microenvironment. The relationship between PDK3 expression and the activation of the immune system in colon cancer tissues was analyzed, and it was found that PDK3 expression in colon cancer was negatively correlated with tumor antigen presentation and $CD8^+$ T cell infiltration and the expression of T cell effector molecules, such as IFN-γ and GzmB. We also validated these interesting findings through flow cytometry. Next, the effect of PDK3 on tumor growth was assessed in vivo using a subcutaneous transplantation model of colon cancer in mice, and it was found that PDK3 promoted tumor growth in mice and significantly decreased the percentage of IFN-γ^+/$CD8^+$ T cells in the tumor microenvironment. The experimental results are consistent with the results of TCGA correlation analysis, suggesting that PDK3 promotes

the growth of colon cancer by inhibiting the T cell-mediated tumor immune response. The expression of PD-L1 in colon cancer cells was also detected, and it was found that PDK3 suppressed the protein expression levels of PD-L1. Further experiments showed that PDK3 inhibited the activation of the JAK1/STAT1 pathway, which has been demonstrated to decrease PD-L1 expression. It was therefore concluded that PDK3 may increase PD-L1 expression and affect the function of $CD8^+$ T cells.

In the present study, it was determined that PDK3 can play a role in tumor proliferation and immunity and inhibit the killing effect of $CD8^+$ T cells to promote tumor growth in colon cancer. In conclusion, the results of the present study suggested that PDK3 can promote CRC proliferation by activating PI3K-AKT signaling and inhibit $CD8^+$ T cells to promote colon cancer immune evasion may by suppress tumor antigen presentation and increase PD-L1 expression through the JAK1/STAT1 pathway. Hence, our study not only provides a molecular insight but also reveals a potential therapeutic strategy that targeting the PDK3 might enhance the efficacy of anti-PD-1 in treating human cancers.

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Disclosure of conflict of interest

None.

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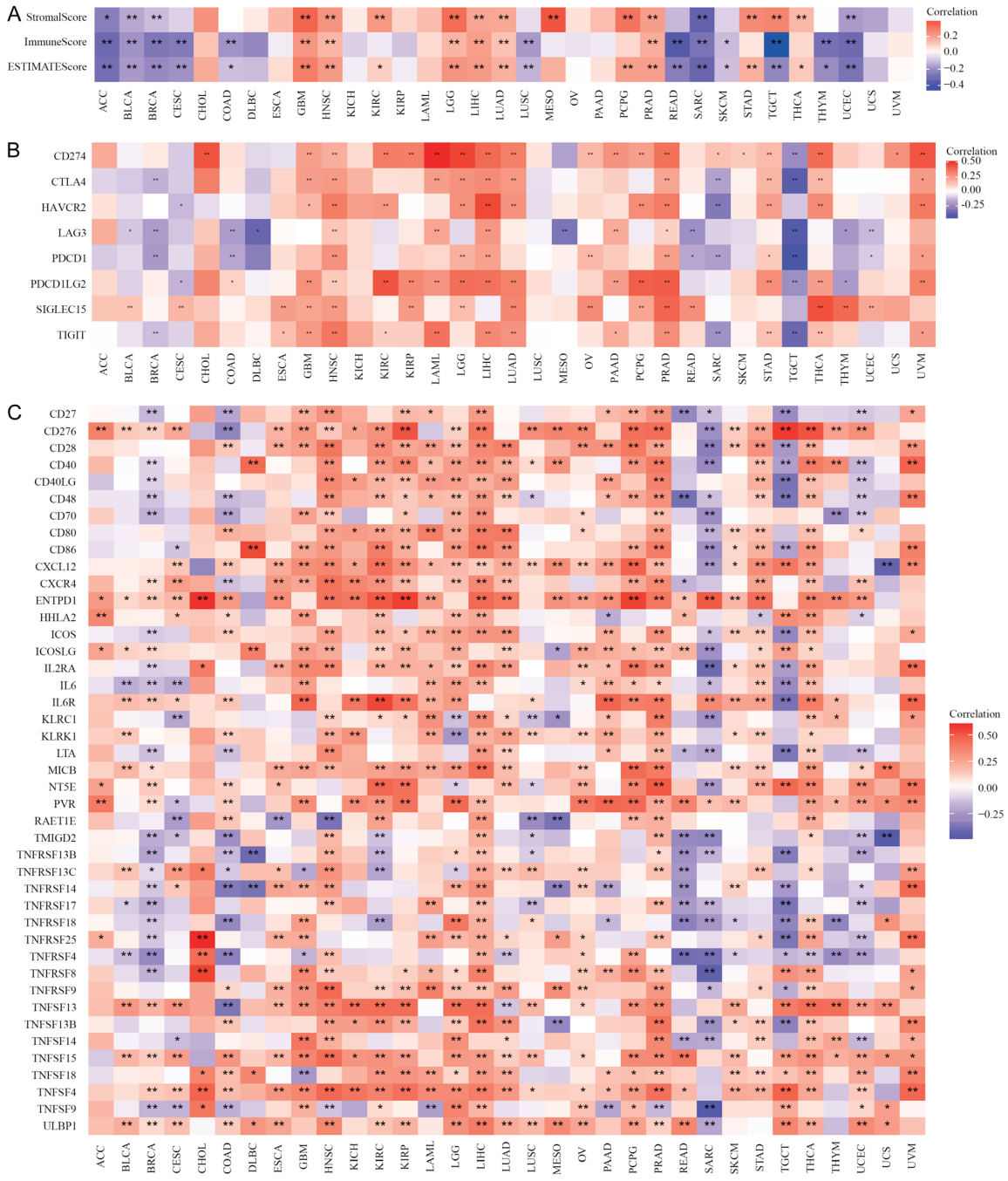

Figure S1. Bioinformatics analysis of the relationship between PDK3 expression and chemokines and chemokine receptors. A. Correlation between PDK3 gene expression and immune score. B. Correlation between PDK3 gene expression and several immune checkpoints. C. Correlation between PDK3 gene expression and several immune stimulators.

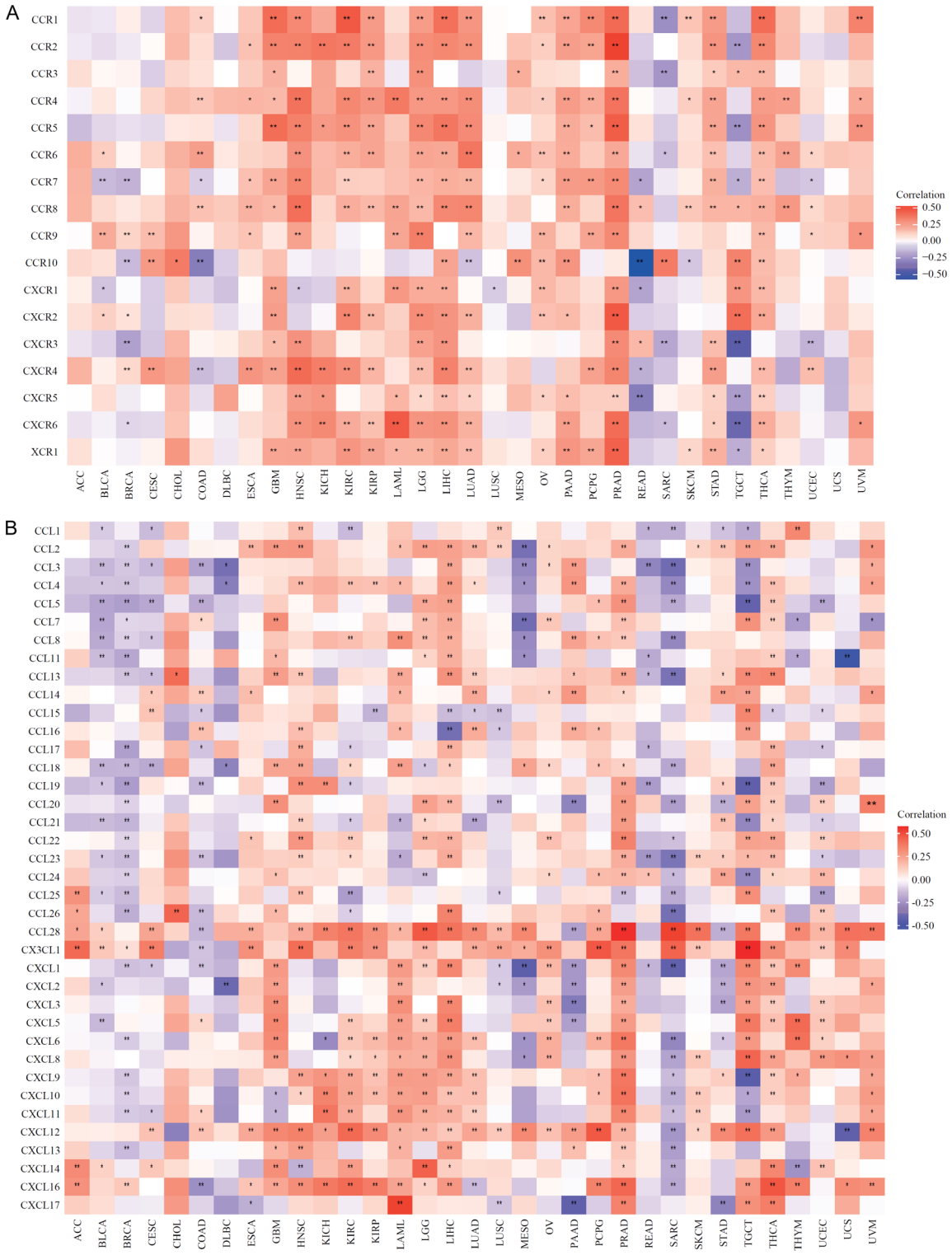

Figure S2. Bioinformatics analysis of the relationship between PDK3 expression and chemokines and chemokine receptors. A. Correlation between PDK3 gene expression and chemokine receptors. B. Correlation between PDK3 gene expression and chemokines.

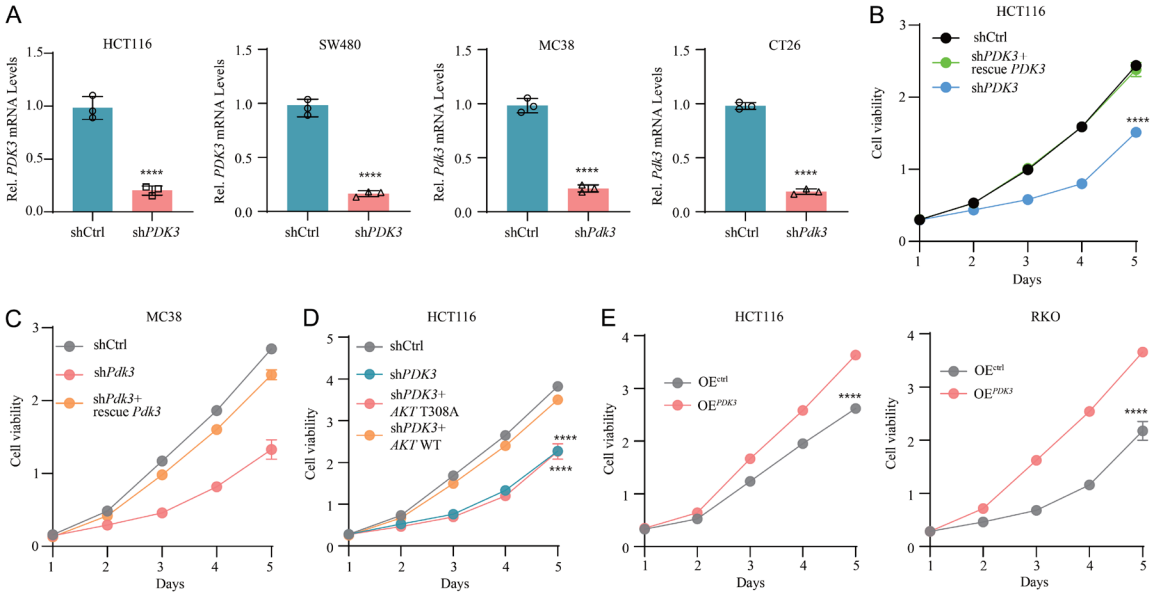

Figure S3. Validation of PDK knockdown. A. qRT-PCR shows PDK3 was successfully knocked down in the indicated cell lines. B-E. Cell proliferation assays of indicated treatment.