

KIR2DL4 promotes the proliferation of renal cell carcinoma cells by PI3K/AKT signaling pathway activation

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Keywords: renal cell carcinoma, killer cell immunoglobulin-like receptor 2DL4, phosphatidylinositol-3-kinase, protein kinase B, natural killer cells

Posted Date: May 7th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-420757/v1>

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Abstract

Background: Killer cell immunoglobulin-like receptor 2DL4 (KIR2DL4) is a transmembrane glycoprotein that is expressed by natural killer (NK) cells and certain subsets of T cells. It has been reported to serve an important role in the immune response. However, its expression profiles and function in solid tumor progression remain poorly defined.

Methods: In the present study, using bioinformatics analysis, immunohistochemistry, immunoblotting, MTT assay, soft agar colony formation assay and a renal cell carcinoma (RCC) cell xenograft model in nude mice, we examined whether KIR2DL4 is expressed by RCC and its possible roles in RCC progression.

Results: We confirmed that KIR2DL4 is overexpressed by RCC cells. MTT and soft agar cloning assays showed that KIR2DL4 knockdown delayed cell proliferation in RCC cell lines, Caki-1 and 769-P, *in vitro*. By contrast, KIR2DL4 overexpression promoted Caki-1 cell proliferation both *in vitro* and *in vivo*, which was observed in a BALB/c-nu/nu xenograft mouse model. Moreover, RNA sequencing data demonstrated that the differentially expressed genes between vector controlled and KIR2DL4-overexpressed Caki-1 cells were highly associated with cancer development, of which those related to the phosphatidylinositol-3-kinase (PI3K)/ protein kinase B (AKT) signaling pathway were particularly enriched. Immunoblotting data showed that the level of AKT phosphorylation was higher in KIR2DL4-overexpressing Caki-1 cells compared with that in the parallel-controlled cells.

Conclusions: Our results indicate that KIR2DL4 is also expressed by RCC cells, which promotes RCC progression through the PI3K/AKT signaling pathway.

Background

Renal cell carcinoma (RCC) is a malignancy that accounts for ~2% of the total global cancer burden [1]. Due to the asymptomatic nature of early RCC, patients with RCC frequently present with distant metastasis at the time of diagnosis [2]. The rates of morbidity and mortality associated with RCC remain on the increase, despite many novel pharmacological agents being previously approved for advanced RCC therapy [3]. Development of high-throughput analysis of the global gene expression profile is valuable for enhancing the understanding of RCC biology and for identifying novel treatment targets [4]. We previously performed an integrated bioinformatics analysis in The Cancer Genome Atlas (TCGA) database and found differential mRNA expression and copy number variation (CNV) in the killer cell immunoglobulin-like receptor (KIR) 2DL4 (KIR2DL4) gene in RCC (<https://figshare.com/s/2b5cd71ddf0b59c371c6>).

KIRs are a family of receptors, which are expressed on the surfaces of natural killer (NK) cells, that play an important role in NK cell functional regulation [5]. KIR2DL4 is a unique member of the KIR family according to its structure, expression, localization and signaling function [6]. KIR2DL4 is referred to as the framework KIR gene since its corresponding mRNA is constitutively expressed in all NK cells [7, 8]. Furthermore, KIR2DL4 is not only expressed in NK and certain subsets of T cells, but it can also be found

in human mast cells and Langerhans cells. The only so far reported ligand of KIR2DL4 is the non-classical HLA class 1 gene HLA-G, leading to the inhibition of the cytolytic NK cell function [9].

Recently, KIR2DL4 expression levels were found to be selectively higher in non-small cell lung cancer and NK/T cell lymphoma, positive KIR 2DL4 expression on tumor cells was correlated with poor prognosis [10, 11].

In the present study, we showed for the first time, to the best of our knowledge, that KIR2DL4 is overexpressed in human RCC cells, where it could promote RCC cell proliferation and growth.

Materials And Methods

Ethics statement

All experimental procedures involving animals were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and Guide for the Care and Use of Laboratory Animals in China. The present study complied with the ethical standards and was approved by the Medical Ethics Committee of Taizhou University Medical School (approval no. 2019-215; Taizhou, China).

TCGA data analysis

RCC tissue samples and their corresponding clinicopathological parameters were downloaded from TCGA database. We selected 525 cases of RCC and 72 cases of solid normal who had RCC. For TCGA data, the edgeR package was used for screening differentially expressed genes (DEGs), using the criteria of adjusted P-value <0.01 and $\log_2|\text{fold change}| > 2$ as the cut-off. To analyze the potential prognostic value of KIR2DL4, the cut-off point was set using the median value of KIR2DL4 expression in TCGA cohort to categorize into high and low expression subgroups.

Cell culture and reagents

Human RCC cell lines, Caki-1 and 769-P, and the 293T cell line were obtained from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences and maintained in the appropriate medium as suggested by the supplier. All cells were cultured at 37°C in a humidified, mycoplasma-free atmosphere with 5% CO₂. Polybrene and puromycin were purchased from MedChemExpress (Shanghai, China).

The pLenti-EF1a-EGFP-P2A-Puro-CMV-KIR2DL4-3Flag plasmid was obtained from OBiO Technology (Shanghai) Corp., Ltd. The insert sequence of KIR2DL4 (accession no. NM_002255) was subcloned into the lentiviral vector.

For the creation of the short hairpin RNA (shRNA) plasmid, the 5'-GGTCTATATGAGAAACCTT-3', 5'-CCCAGCATCAATGGAACAT-3' or 5'-CCTTCTAGTAGTTGGCCTT-3' sequences were subcloned into the pLKD-CMV-G&PR-U6 vector separately to establish a KIR2DL4-shRNA vector. The negative control sequence used was TTCTCCGAACGTGTCACGT.

pMD2.G was a gift from Didier Trono (Addgene plasmid no. 12259; Addgene, Inc.) whereas psPAX2 was a gift from Didier Trono (Addgene plasmid no. 12260; Addgene, Inc.). Both of these aforementioned plasmids were used for lentivirus assembly.

Immunohistochemistry

RCC samples were obtained from Taizhou University Hospital. The patients provided written informed consent prior to participation in the study, which was ethically approved by the Medical Ethics Committee of Taizhou University Hospital (approval no. TZZXYY2019-027; Taizhou, China). The tissues were formalin-fixed and paraffin-embedded, and then cut into 4- μ m sections. The slices were then processed for immunohistochemistry by following the standard methods as previously described[12]. Antibodies against KIR2DL4 were purchased from Abcam.

Immunoblotting

Immunoblotting assay were conducted by following standard procedures [12] using antibodies against KIR2DL4 (Abcam), AKT, phosphorylated AKT (pAKT; Cell Signaling Technology, Inc.) and GAPDH (Santa Cruz Biotechnology, Inc.).

Reverse transcription-quantitative PCR

Total RNA was extracted with TRIzol[®] (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturers' protocol and was reverse transcribed using the PrimeScript[™] RT reagent kit (Takara Biotechnology Co., Ltd.). The resultant cDNA was amplified by qPCR using a KIR2DL4-specific primer pair. The primer sequences for KIR2DL4 were as follows: 5'-GTTGGATCACTGCATTTTCACAC-3' (forward) and 5'-GGCTCAGCATTTGGAAGTT CTATAC-3' (reverse). 5'-GCACCGTCAAGGCTGAGAAC-3' (forward) and 5'-GCCTTCTC CATGGTGGTGAA-3' (reverse) were the primer pairs used for GAPDH. Thermocycling was programmed according to instructions as follows: Initial denaturation at 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec, 60°C for 30 sec and then 95°C for 15 sec, 60°C for 60 sec and 95°C for 15 sec. Gene expression was assessed using the $2^{-\Delta\Delta Cq}$ method and mRNA levels of KIR2DL4 were normalized to those of GAPDH in the identical sample.

Cell viability

Cell viability was assessed using a MTT assay. Briefly, cells (1×10^3 cells per well) were placed in a 96-well plate in complete culture medium. After 4, 24, 48, 72 and 96 h, the media was carefully aspirated before 50 μ l serum-free media and 50 μ l MTT solution was added into each well. The plates were then incubated at 37°C for another 3 h. After incubation, 150 μ l MTT solvent was added into each well before the plates were wrapped in foil and shaken on an orbital shaker for 15 min, then absorbance at 490 nm was read in each well.

Flow cytometry analysis of DNA content

DNA content was measured using flow cytometry (CytoFLEX S; Beckman Coulter, Inc.) after fixation with 70% ethanol and propidium iodide staining. Kaluza Analysis software (Beckman Coulter, Inc.) was used for subsequent analysis.

Colony formation assay

Initially, a 4% low melting-point agarose stock was made with ddH₂O. The 24-well plates were then coated with 0.8% low melting-point agarose in RPMI-1640 medium supplemented with 10% FBS, which was used as the base agarose. Cells were suspended in RPMI-1640 media containing 0.4% agarose and seeded at a density of 1,000 cells per well in a 24-well plate as the top layer and supplemented with 100 µl 10% FBS medium every 3 days. After incubating for 14 days at 37°C under 5% CO₂, cell colony-forming units were counted.

In vivo xenograft model and tumorigenicity

Male BALB/C-nu/nu mice aged 4-5 weeks were obtained from Shanghai SIPPR-Bk Lab Animal Co., Ltd. (Shanghai, China). The mice were housed in sterile cages under laminar airflow hoods at 20°C, in a specific pathogen-free environment under a 12-h light/dark cycle and provided with autoclaved chow and water *ad libitum*. The present study was ethically approved by the Medical Ethics Committee of Taizhou College of Medicine (approval, no. TZYXY2019-215; Taizhou, China). In total, 1x10⁷ parallel-controlled or KIR2DL4-overexpressed Caki-1 cells were transplanted subcutaneously into the flank of six mice 6 mice in each group. Tumor volumes were measured with calipers twice per week and calculated using the formula volume = (width)² x length/2. After 12 weeks, animals were sacrificed by cervical dislocation before the solid tumors were removed and weighed. The largest diameter of tumors isolated from all mice examined was 1.106 cm, with no multiple tumors observed.

mRNA sequencing

Total RNA was extracted using the miRNeasy Mini kit (Cat. no. 217004; Qiagen GmbH) and inspected for quality by electrophoresis using an Agilent Bioanalyzer 2100 (Agilent technologies, Inc.). mRNA was concentrated by Oligo (dT) magnetic beads with 3-5 µg total RNA used.

Next, mRNA was fragmented in a Mg²⁺ solution, which was then reverse transcribed using random primers to synthesize the first strand and the second strand cDNA, which became a double-stranded cDNA. After the 3' ends were adenylated and the 5' ends were repaired, "Y" adapters were ligated to both ends of the double-stranded cDNA. After fragment selection, PCR amplification and purification, the product was inspected for quality by electrophoresis with an Agilent Bioanalyzer 2100 (Agilent technologies, Inc.). In this manner, a standard sequencing library was completely constructed, which was sequenced on a HiSeq sequencer.

Statistical analysis

Statistical analysis was performed with GraphPad Prism version 8 (GraphPad Software, Inc.). Data are presented as the Mean \pm SD. Statistical differences were determined using a Student's t-test or ANOVA. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

KIR2DL4 is overexpressed in RCC

In a previous study, we performed an integrated analysis of TCGA data and found increased KIR2DL4 mRNA expression and copy number variation in the KIR2DL4 gene in RCC (supplementary.xlsx files). In the present study, the KIR2DL4 mRNA expression levels were analyzed further in different grades of RCC using the GEPIA online analysis tool (<http://gepia.cancer-pku.cn/>) and we found that the mRNA expression of KIR2DL4 was increased with increasing RCC grade (Figure 1A), the levels of which (cut-off point was determined using the median value deduced from TCGA cohort for categorization into high and low subgroups) were negatively associated with the survival time of patients (Figure 1B) [13].

It has been previously reported that KIR2DL4 is expressed by NK cells. To test whether it is also expressed by RCC cells, we further performed immunoblot assays to examine the KIR2DL4 expression profiles of 293T cells and RCC cell lines Caki-1 and 769-P. As shown in Figure 2A, 293T cells expressed KIR2DL4 at lower levels, but KIR2DL4 was found to be overexpressed by Caki-1 and 769-P cells. We also conducted immunohistochemical assays on four tissue samples collected from RCC patients who underwent radical nephrectomy. We found that KIR2DL4 is expressed in RCC tissues, with the expression levels being higher compared with that in adjacent normal tissues (Figure 2B).

Knocking down KIR2DL4 expression reduces cell viability and colony formation in Caki-1 and 769-P cells

KIR2DL4 was considered to be a candidate oncogene in RCC according to analysis of the data in TCGA database. To test this hypothesis, we knocked down KIR2DL4 expression in Caki-1 (Figure 3A and B) and 769-P cells (Figure 4A and B) by transfection with KIR2DL4-shRNA and examined its impact on their proliferative phenotypes (Figures 3C-E, 4C and D). The data showed that KIR2DL4 knockdown could markedly reduce RCC cell viability whilst inhibiting colony formation compared with controlled cells.

KIR2DL4 overexpression promotes Caki-1 cell proliferation

Our primary results showed that knocking down KIR2DL4 expression can inhibit cell viability and colony formation. Therefore, we next overexpressed KIR2DL4 in Caki-1 cells by transfecting KIR2DL4 cDNA (that was inserted into a lentiviral vector) and determining its impact on cell viability, colony formation *in vitro* and xenograft growth in nude mice.

As shown in Figure 5A and B, we successfully overexpressed KIR2DL4 in Caki-1 cells. Subsequently, KIR2DL4 overexpression could also significantly increase cell viability (Figure 5C) and promote colony formation (Figure 5D) in Caki-1 cells.

Moreover, to further determine the role of KIR2DL4 in RCC progression, we performed a mouse xenograft assay to test the effects of KIR2DL4 overexpression on RCC cell growth *in vivo*. As shown in Figure 6A and 6C, in BALB/C-nu/nu mice, tumor xenografts formed by injection with Caki-1 cells overexpressing KIR2DL4 emerged earlier and grew faster compared with those formed by Caki-1 cells transfected with the vector control. We also monitored the body weight changes, as shown in the Figure 6B and 6C, there is no significant difference between two groups in the mice body weight.

KIR2DL4 can affect tumor related pathways and the phosphatidylinositol-3-kinase (PI3K)/AKT signaling pathway.

To elucidate the mechanism underlying the KIR2DL4-mediated promotion of RCC progression, we performed RNA-sequencing to identify the profile of DEGs between parallel-controlled and Caki-1 cells overexpressing KIR2DL4 (<https://figshare.com/s/13768e97493047251553> for sequencing data). As shown in Figure 6A, the expression of 265 genes were found to be significantly upregulated, including KIR2DL4, whilst 251 genes were downregulated, following KIR2DL4 overexpression. Furthermore, these differentially expressed genes were analyzed further using Kyoto Encyclopedia of Genes and Genomes (<https://www.kegg.jp/> or <https://www.genome.jp/kegg>), which is a knowledge base for the biological interpretation and analysis of genome sequences and high-throughput sequencing data. The analysis data showed that most of the DEGs were associated with cancer and particularly enriched in the PI3K/AKT signaling pathway (Figure 6B). Immunoblotting assay revealed that pAKT levels were upregulated in KIR2DL4-overexpressing Caki-1 and 769-P cells (Figure 6C). Therefore, these observations indicated that KIR2DL4 may promote RCC progression via the PI3K/AKT pathway, though the underlying mechanism require further examination.

Discussion

In the present study, we showed that KIR2DL4 was overexpressed by RCC cells, whereas the knockdown or overexpression of KIR2DL4 could inhibit or promote RCC cell proliferation, respectively. In addition, most of the DEGs between parallel-controlled and KIR2DL4-overexpressing Caki-1 cells were particularly enriched in the PI3K/AKT signaling pathway, where the levels of pAKT were higher in Caki-1 and 769-P cells overexpressing KIR2DL4, which suggest that KIR2DL4 may promote RCC progression via the PI3K/AKT signaling pathway. However, the underlying mechanism needs to be further investigated.

We analyzed TCGA database and showed that KIR2DL4 mRNA expression was upregulated in RCC with concomitant increases in its DNA copy number. TCGA is a landmark cancer genome project that significantly improves our ability to diagnose, treat and prevent cancer. Therefore, the present study hypothesized that KIR2DL4 is an oncogenic gene of RCC. We next confirmed the expression profile of KIR2DL4 in RCC using immunoblotting and immunohistochemistry assays.

KIR2DL4 was considered to be expressed in all NK cells and some subsets of T cells, which can recognize its homologous ligand HLA-G [14]. Upregulation of HLA-G expression has been found to be related to the immunosuppression and promotion of tumor cell immune evasion [15]. In the present study, we found

that KIR2DL4 was also expressed in RCC tumor tissues. Moreover, its expression level was higher in tumor tissues compared with that in corresponding adjacent normal tissues. We also showed that overexpression of KIR2DL4 promoted the proliferation of Caki-1 and 769-p cells, whereas downregulating KIR2DL4 expression conferred opposite results. Therefore, it was hypothesized that KIR2DL4 is not only expressed on NK cells, but is also expressed in RCC, where it directly promotes RCC progression. This also suggests KIR2DL4 to be a potential target for RCC therapy.

Immunotherapy has been undergoing a resurgence with the FDA approval of nivolumab for patients with advanced RCC in 2015 [16-18]. The HLA-G/KIR2DL4 axis is a particularly interesting pathway in cancer biology. Monoclonal antibodies targeting HLA-G or KIR2DL4 were considered anticancer drug candidates by blocking the interaction between HLA-G and KIR2DL4 [9]. Based on our primary data, we hypothesized that KIR2DL4 mAb would inhibit RCC directly, since KIR2DL4 is expressed in RCC with the overexpression of which promoted RCC progression.

To unravel the underlying mechanism, we performed RNA-sequencing to detect the DEGs between parallel-controlled and KIR2DL4-overexpressing Caki-1 cells. The results demonstrated that the DEGs were highly associated with cancer development and were mainly enriched in the PI3K/AKT signaling pathway. We also revealed higher levels of pAKT in KIR2DL4-overexpressing cells compared with those in parallel-controlled cells. However, we did not find notable expression changes in genes associated with cell cycle progression, although we did observe G₁ phase cell cycle arrest in KIR2DL2-knockdown cells. Therefore, the underlying mechanism should be investigated in future studies. However, it was hypothesized that KIR2DL4 knockdown-induced cell cycle arrest was not a result of changes in any one cell cycle-related gene specifically.

Over the past decade, a number of targeted pharmacological agents have been approved by the Food and Drug Administration (FDA) for RCC therapy, including sorafenib, sunitinib, temsirolimus and everolimus [19-22]. The PI3K/AKT/mTOR pathway is pivotal for RCC therapy, since temsirolimus and everolimus were approved by FDA for RCC therapy 10 years ago [22]. We therefore propose that the expression levels of KIR2DL4 may be a candidate predictive biomarker for temsirolimus and everolimus efficacy in personalized therapy for RCC, similar to mutations in the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α gene and loss of *PTEN*.

Conclusion

The present data showed that KIR2DL4 expression level was higher in RCC compared with that in adjacent normal tissue. Knockdown or overexpression of KIR2DL4 could inhibit or promote RCC cell proliferation, respectively. KIR2DL4 overexpression also promotes PI3K/AKT/mTOR signaling activation, although the underlying mechanism require further study.

Abbreviations

KIR2DL4, Killer cell immunoglobulin-like receptor 2DL4; RCC, renal cell carcinoma; NK, natural killer cell; PI3K, phosphatidylinositol-3-kinase; AKT, protein kinase B; MTT, ; FDA, Food and Drug Administration; TCGA, The Cancer Genome Atlas.

Declarations

Ethics approval and consent to participate

RCC samples were obtained from Taizhou University Hospital. The patients provided written informed consent prior to participation in the study, which was ethically approved by the Medical Ethics Committee of Taizhou University Hospital (approval no. TZZXY2019-027; Taizhou, China). Animal study was ethically approved by the Medical Ethics Committee of Taizhou College of Medicine (approval, no. TZYXY2019-215; Taizhou, China)

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and material

Research data will be made available on request.

Competing interests

The authors declare no conflict of interests with respect to this study.

Funding

This research was supported by Zhejiang Provincial Natural Science Foundation of China (grant nos. LY20H310003 and LGF19H050004), National Natural Science Foundation of China (grant nos. 81802657 and 81201530) and Technology Research Projects of the Science Technology Department of Taizhou (grant nos. 20ywb97 and 1901ky77).

Authors' contributions

DX carried out the cellular studies, participated in the animal studies and drafted the manuscript. MH and CJ carried out the immunoassays. WY participated in the sequence alignment. LY participated in the design of the study and performed the statistical analysis. CG, DX and LX conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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Figures

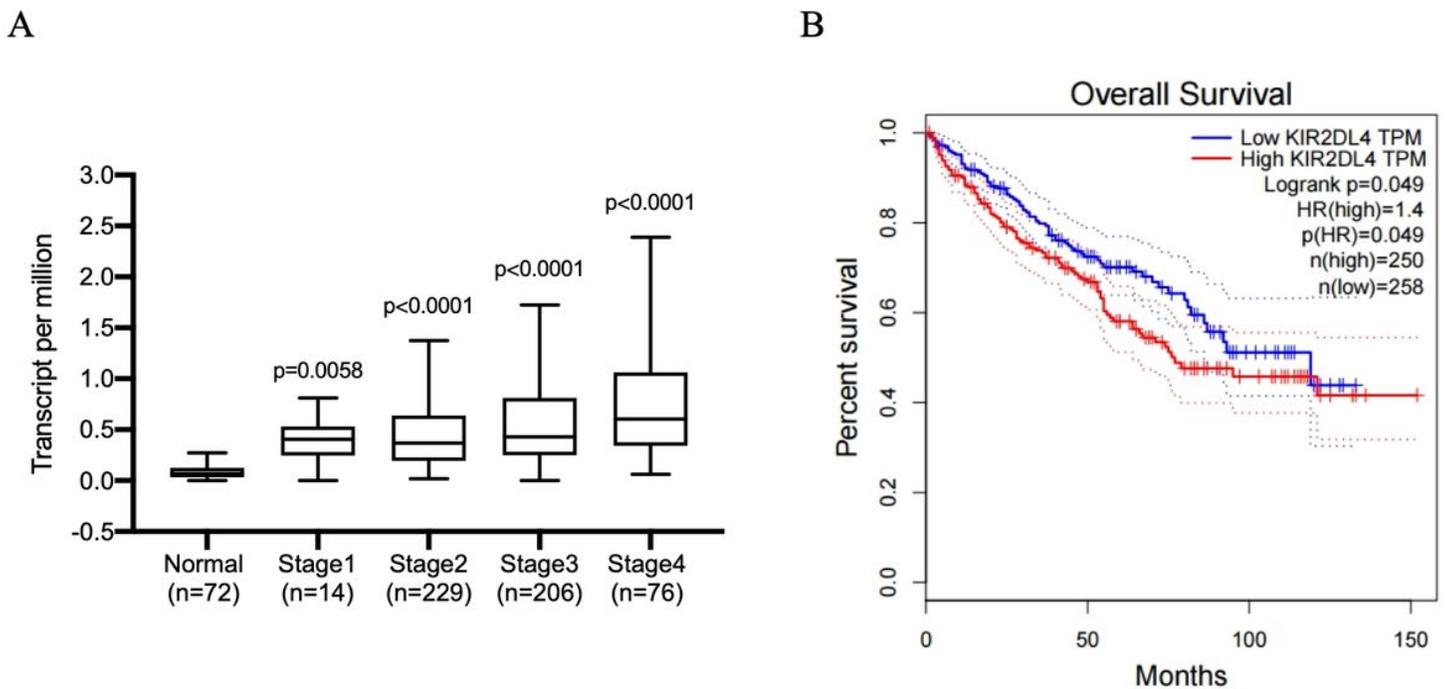


Figure 1

Analysis of the possible association between KIR2DL4 expression and RCC grade and survival using data from The Cancer Genome Atlas. (A) Expression of KIR2DL4 was significantly increased in RCC tissues compared with that in adjacent normal kidney tissues, and positively associated with RCC grade. (B) Patients with higher KIR2DL4 expression exhibited poorer survival rates compared with patients with lower KIR2DL4 expression. KIR2DL4, killer cell immunoglobulin-like receptor 2DL4; RCC, renal cell carcinoma.

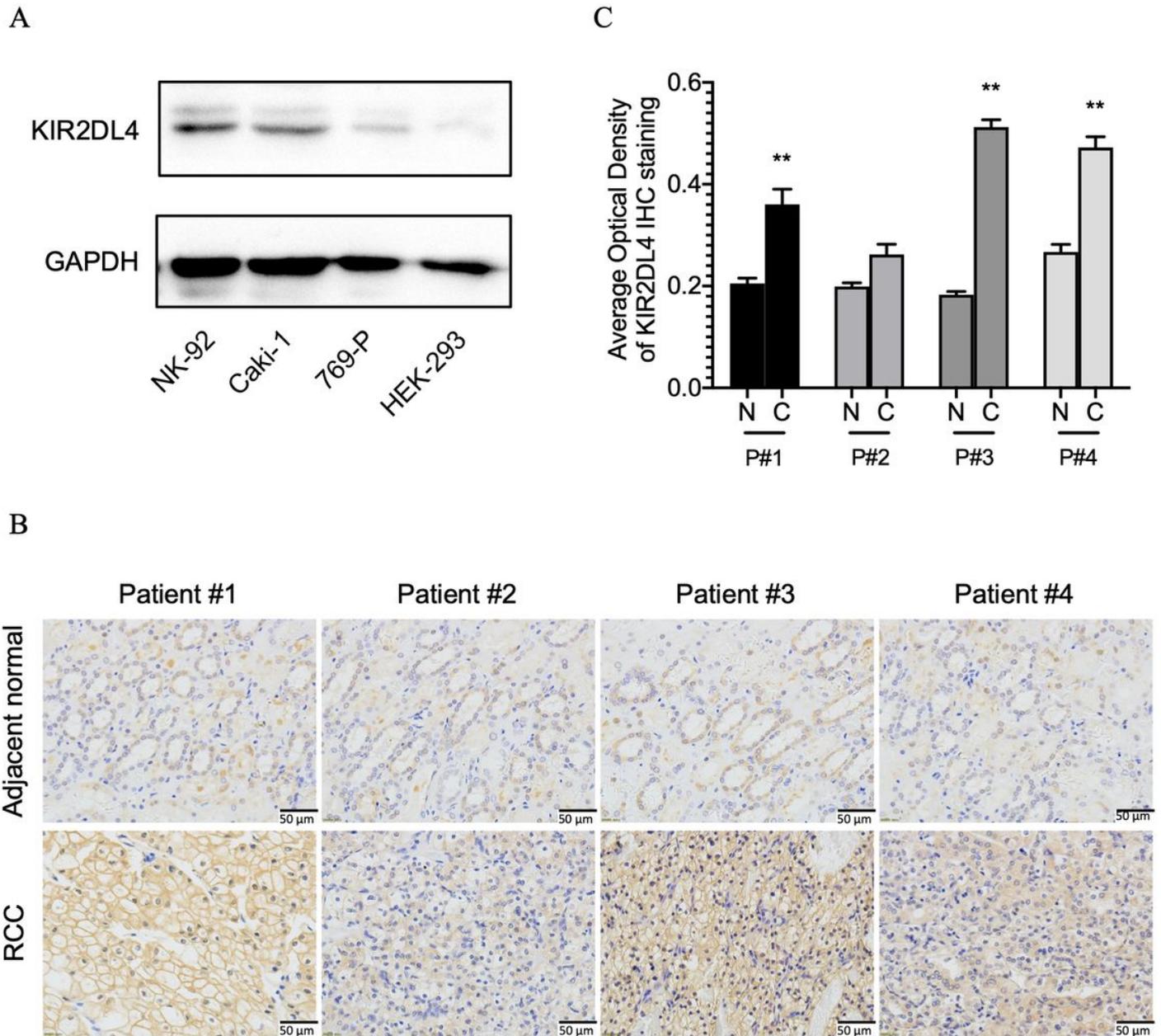


Figure 2

KIR2DL4 is expressed at higher levels in RCC cells compared with that in normal kidney cells. (A) KIR2DL4 protein expression profiles in RCC cell lines. GAPDH was used as the loading control. (B) The expression of KIR2DL4 in RCC and adjacent normal tissues were detected using IHC. (C) KIR2DL4 IHC staining was quantified by measuring the average optical density using ImageJ d1.47. software. ** $P < 0.01$ vs. adjacent normal tissue. KIR2DL4, killer cell immunoglobulin-like receptor 2DL4; RCC, renal cell carcinoma; IHC, immunohistochemistry.

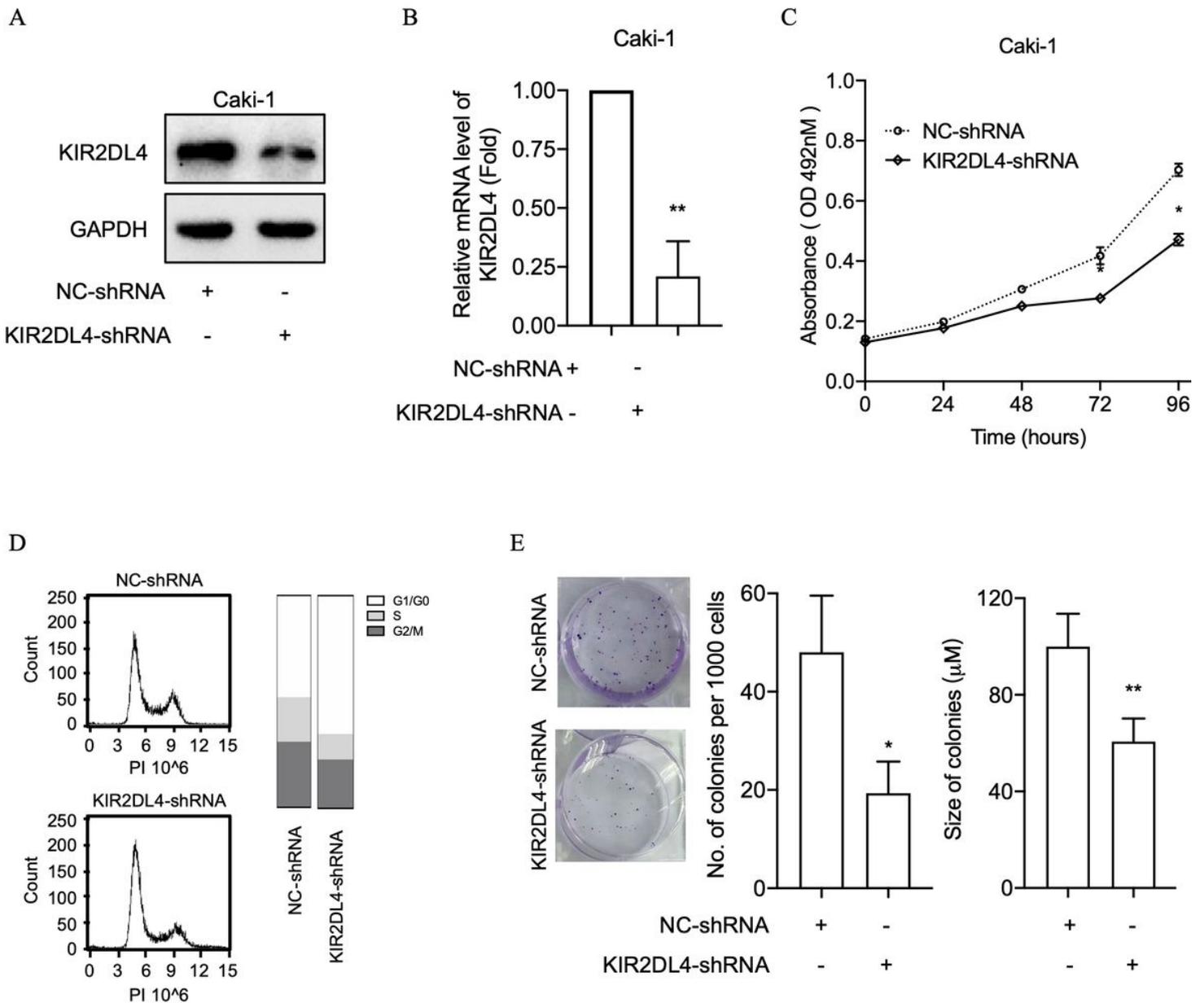


Figure 3

KIR2DL4 knockdown impairs Caki-1 cell viability and colony formation. (A) KIR2DL4 protein expression in Caki-1 cells was significantly reduced after being transfected with KIR2DL4-shRNA. GAPDH was used as a loading control, whereas NC-shRNA was used as the NC. (B) KIR2DL4 knockdown efficiency was evaluated by reverse transcription-quantitative PCR, with all expression levels normalized to those of GAPDH. Data are presented as fold changes relative to the KIR2DL4 levels in control cells and as the mean \pm SD. ** $P < 0.01$. (C) KIR2DL4-knockdown can reduce Caki-1 cell proliferation, mean \pm SD. * $P < 0.05$. (D) The effect of KIR2DL4-knockdown on cell-cycle distribution. DNA content was assessed using flow cytometry. Data shown are representative and presented as the mean \pm SD from at least three independent experiments. (E) KIR2DL4 knockdown can inhibit Caki-1 cell colony formation in soft agar, mean \pm SD. ** $P < 0.01$. KIR2DL4, killer cell immunoglobulin-like receptor 2DL4; shRNA, short hairpin RNA; NC, negative control.

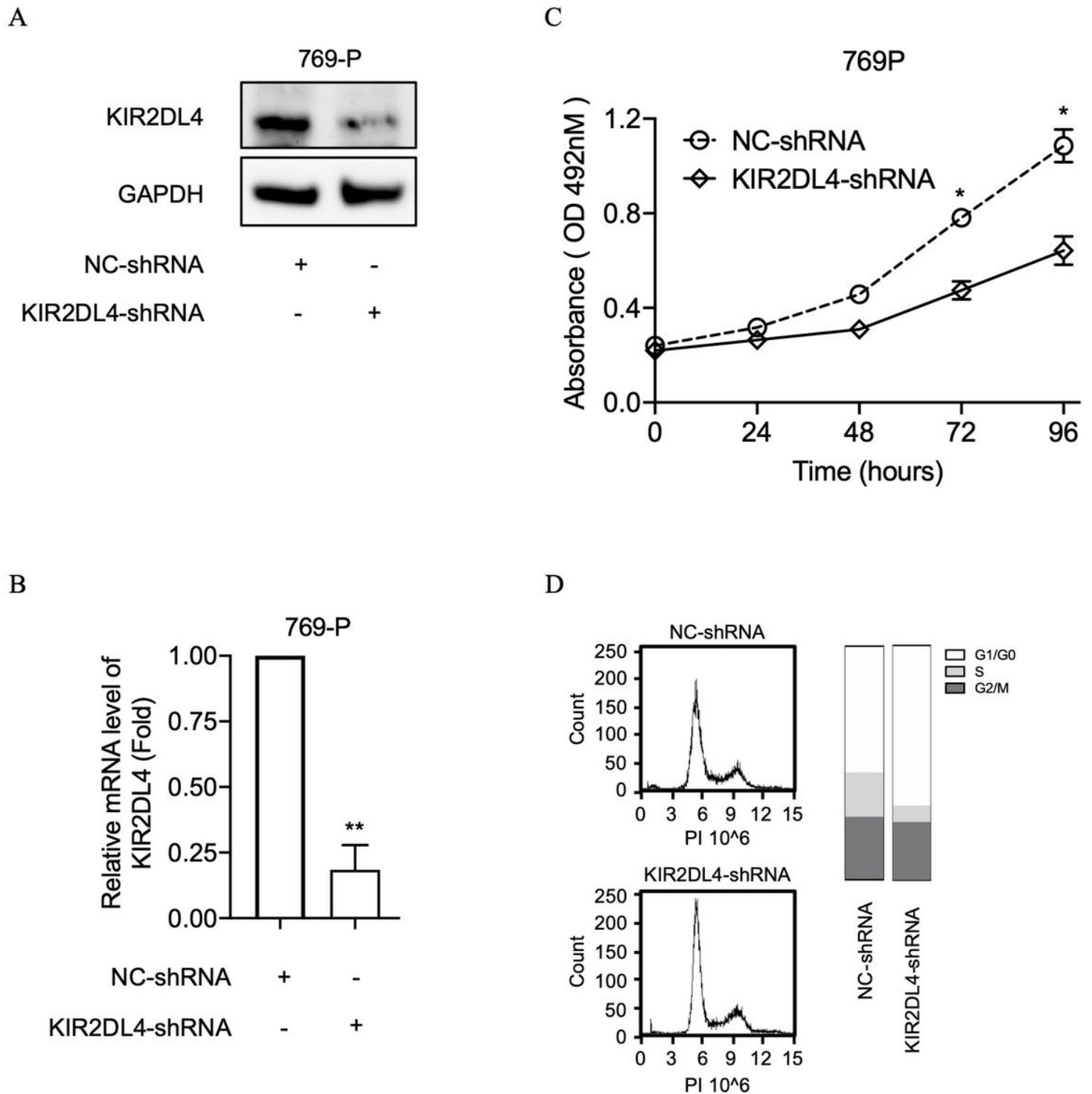


Figure 4

KIR2DL4 knockdown impairs 796-P cell proliferation. (A) KIR2DL4 protein expression in 796-P cells was reduced significantly after being transfected with KIR2DL4-shRNA, GAPDH was used as a loading control, whereas NC-shRNA was used as the NC. (B) KIR2DL4 knockdown efficiency in 796-P cells was evaluated by reverse transcription-quantitative PCR, which was normalized to that of GAPDH. Data are presented as the fold change relative to the KIR2DL4 expression levels in control cells and as the mean \pm SD. **P<0.01.

(C) KIR2DL4 knockdown can suppress 796-P cell proliferation, mean \pm SD. * P <0.05. KIR2DL4, killer cell immunoglobulin-like receptor 2DL4; shRNA, short hairpin RNA; NC, negative control.

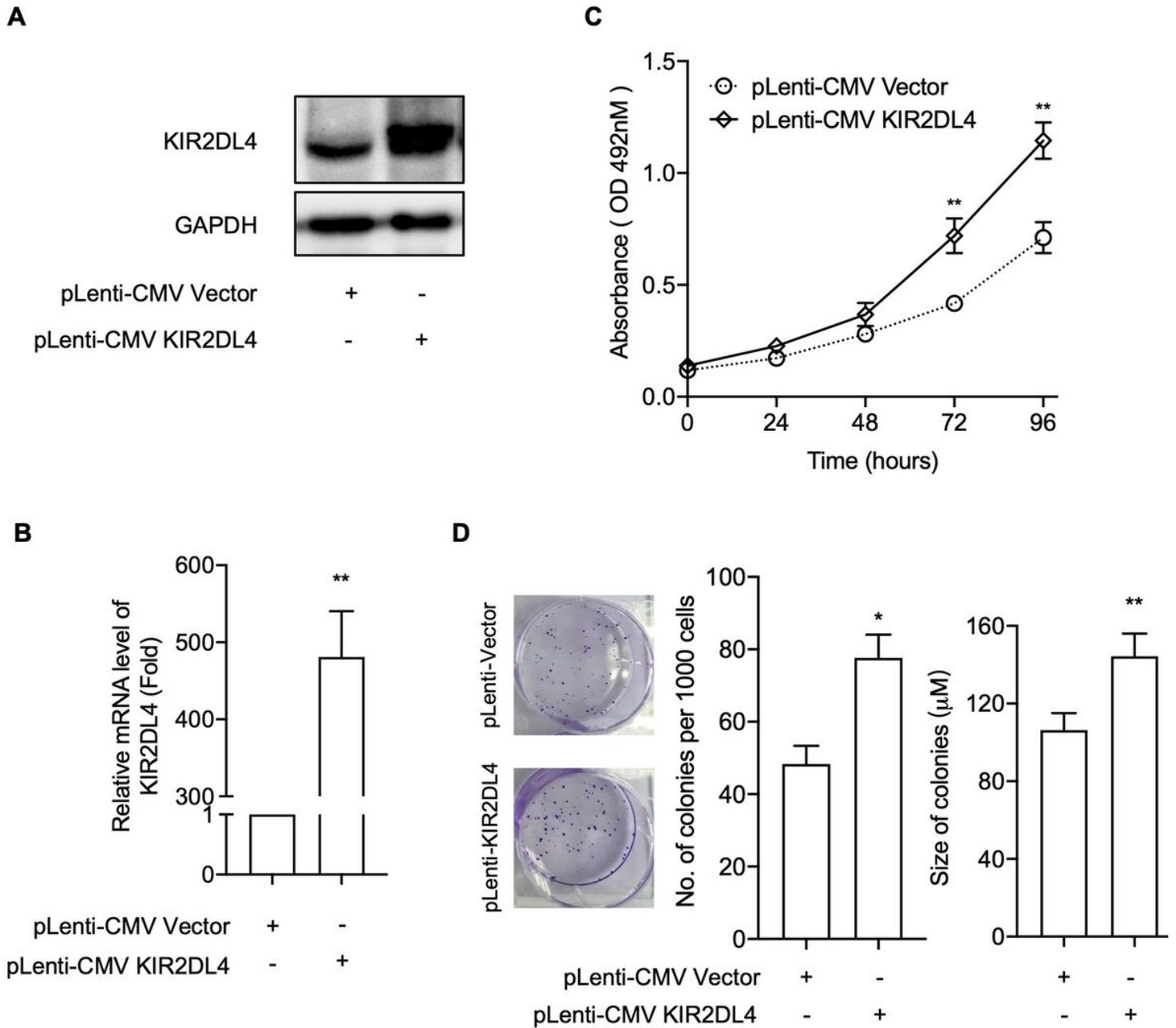


Figure 5

Overexpressing KIR2DL4 promotes Caki-1 cell proliferation in vitro. (A) KIR2DL4 protein expression in Caki-1 cells was significantly increased after being transfected with the KIR2DL4 cDNA lentiviral plasmid, with GAPDH being used as the loading control. (B) KIR2DL4 overexpression efficiency was evaluated by reverse transcription-quantitative PCR, which was normalized to that of GAPDH. Data shown as the fold change relative to KIR2DL4 levels in control cells and were presented as the mean \pm SD. ** P <0.01. (C) KIR2DL4 overexpression can promote Caki-1 cell proliferation, mean \pm SD. * P <0.05. (D) KIR2DL4 overexpression can increase Caki-1 cell colony formation in soft agar, mean \pm SD. * P <0.05 and ** P <0.01. (E and F) Overexpression of KIR2DL4 promoted Caki-1 cell xenograft growth. Tumors formed by KIR2DL4-

overexpressing Caki-1 cells emerged earlier and grew faster compared with those in parallel-controlled cells, mean \pm SD. * $P < 0.05$.

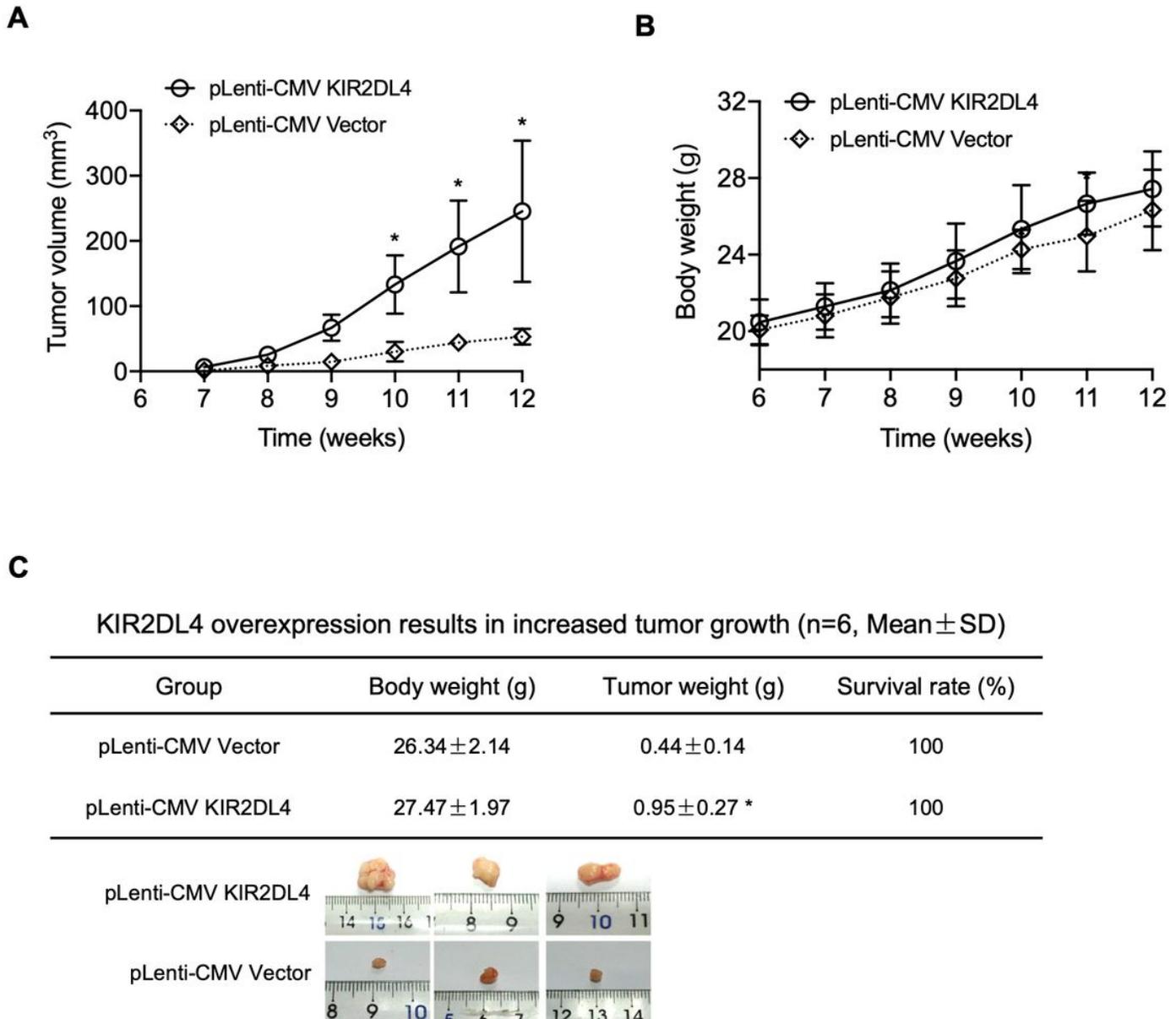


Figure 6

Overexpressing KIR2DL4 promotes Caki-1 cell growth in vivo. (A, B) The tumor volume and body weight curve was shown as the Mean \pm SD. * $P < 0.05$. (C) Table of tumor weight and representative tumor images, Mean \pm SD. * $P < 0.05$. KIR2DL4, killer cell immunoglobulin-like receptor 2DL4.

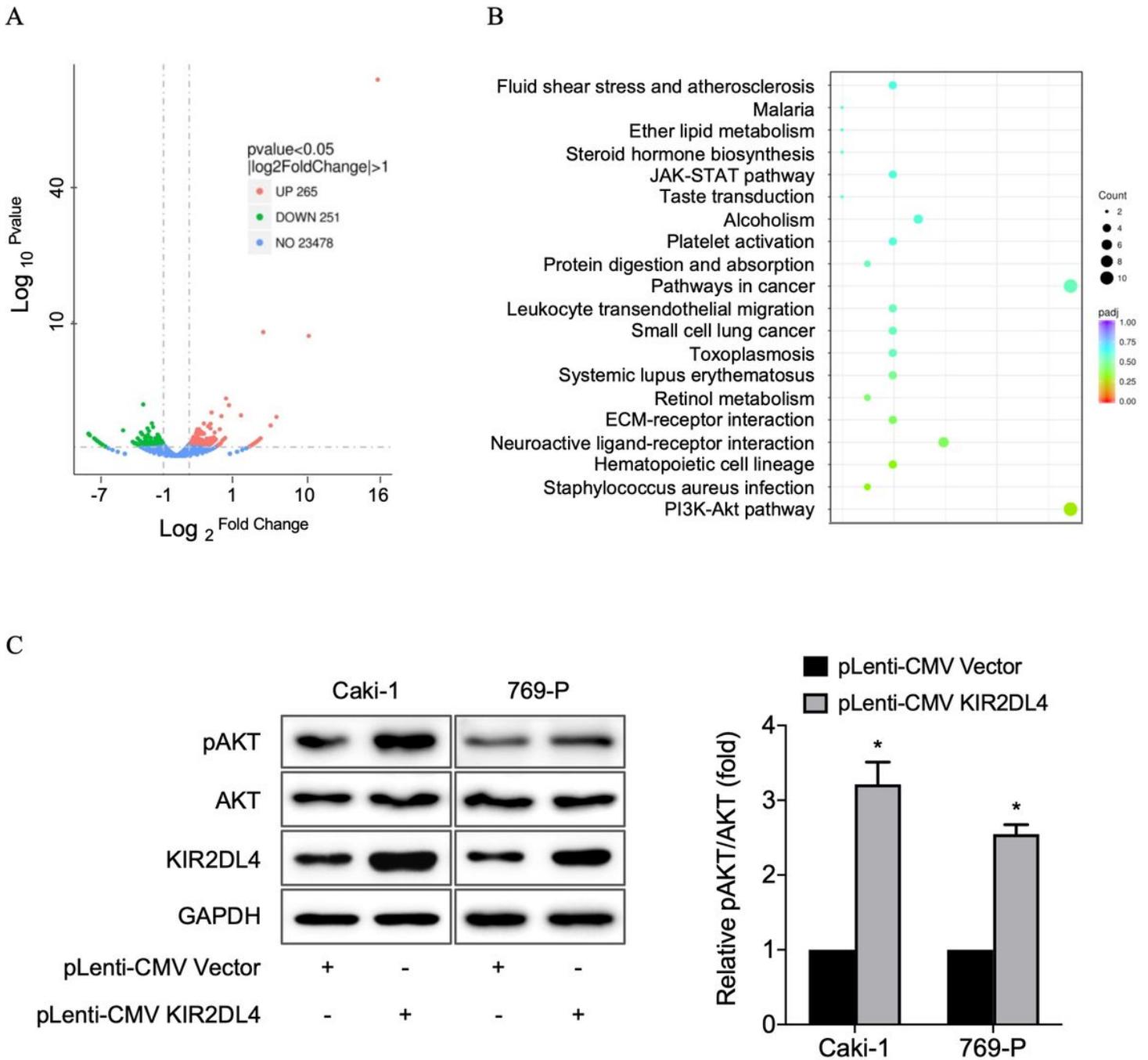


Figure 7

PI3K/AKT signaling pathway is upregulated in Caki-1 cells overexpressing KIR2DL4. (A) Changes in the expression profiles of genes that accompanied KIR2DL4 overexpression. (B) Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis results, showing representative enriched pathways involving the differentially expressed genes found in Caki-1 cells with stable KIR2DL4 overexpression, including the PI3K/AKT pathway- and cancer-associated genes. (C) Phosphorylated AKT levels were analyzed by immunoblotting, with GAPDH used as the loading control. KIR2DL4, killer cell immunoglobulin-like receptor 2DL4; PI3K, phosphatidylinositol-3-kinase; AKT, protein kinase B.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [DEGsbetweenKIR2DL4overexpressedandControlcells.xls](#)