

MiR-489-3p reduced pancreatic cancer proliferation and metastasis by targeting PKM2 and LDHA involving glycolysis

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Abstract

Background: Malignant proliferation and chemotherapy resistance are some of the causes of high mortality in pancreatic cancer. MicroRNAs have for a long period been a hot spot in cancer research and are involved in tumor formation and metabolic stress responses. miR-489-3p is involved in the inhibition of the growth of many tumors. However, its relationship with the growth and metabolism of pancreatic cancer is not clear.

Methods: We used RNA in situ hybridization to analyze the differential expression of miR-489-3p in pancreatic cancer tissues and adjacent tissues. The qRT-PCR experiment detected the content of miR-489-3p in pancreatic cancer cell lines and ordinary pancreatic ductal epithelial cells. Then we did experiments in vivo (subcutaneous tumor formation in nude mice) and in vitro (plate cloning, transwell, glycolysis related experiments) experiments to verify that miR-489-3p can continue the invasion and metastasis of pancreatic cancer and glucose metabolism. Furthermore, we confirmed that LDHA and PKM2 are the two targets of miR-489-3p through dual luciferase reporter gene experiments. Finally, several reply experiments were done to verify the regulation mechanism of miR-489-3p.

Results: We determined that miR-489-3p was under-expressed in pancreatic cancer tissues by RNA in situ hybridization. The function acquisition and deletion and glycolysis experiments confirmed that miR-489-3p could inhibit the proliferation and invasion of Glycolysis. On the analysis of the website, we found that miR-489-3p could target LDHA and PKM, a finding that we verified through the luciferase report experiment. Therefore, we proceeded with recovery experiments on LDHA and PKM2 and concluded that miR-489-3p performed its function by targeting LDHA and PKM2. Finally, in vivo experiments confirmed that highly expressed miR-489-3p inhibited the growth of pancreatic cancer.

Conclusion: In short, this study has identified miR-489-3p as a novel chemotherapy target for pancreatic cancer, but its diagnostic value deserves further study.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a deadly cancer with a poor treatment methods available. Early metastasis and invasion are the main factors leading to its poor prognosis[1, 2]. Only sufficient energy and biosynthetic precursors can sustain this aggressive biology. However, one of the characteristics of the pancreatic cancer microenvironment is dense desmoplasia[3, 4], which results in the creation of enormous solid stress and fluid pressure in the tumors, and compression of the vasculature. Therefore, its hypovascularization decreases the supply of materials for biosynthesis into cancer cells and generates an energy shortage. Nevertheless, tumor cells can adapt to this change by metabolic reprogramming; the most typical example of this is enhanced glycolysis, which was initially named as the "Warburg Effect"[5]. This reprogramming provides energy, macromolecular precursors, and reducing equivalents, which are crucial to the abnormal growth and survival of cancer cells [6]. Although aerobic

glycolysis has been verified in PDAC, its driving mechanism remains hardly known. Hence, the elucidation of this mechanism is essential for the research and treatment of pancreatic cancer.

MicroRNAs, a group of non-coding RNAs of 18–23 nucleotide, are high-profile molecular family that participates in mediating metabolic stress response in cancer [7-9]. For instance, they are widely involved in the regulation of signal pathways such as p53, LKB1/AMPK, c-Myc, and other pathways that regulate metabolic response [10-12]. It has been reported that micro RNAs can, directly and indirectly, promote glycolysis of a variety of tumors, such as pancreatic cancer[13], gastric cancer[14], bladder cancer[15]. Furthermore, literature has found multiple micro RNAs including miR-135[13] and miR-124 [16] to be implicated in the metabolic reprogramming of pancreatic cancer. Since miR-489-3p has been reported to inhibit tumor progression [17, 18], we hypothesized that it can also inhibit the progression of pancreatic cancer and that it functions against glycolysis that is known to promote tumor progression.

In this study, we found that the MiR-489-3p were involved in glucose metabolism reprogramming and malignancy in pancreatic cancer (PC). We found MiR-489-3p to be negative associated with the expression of tumor tissue in PC patients and PC cell lines. To further explore the function of the MiR-489-3p, we conducted loss- and gain-of-function assays to observe proliferation and metastasis in vivo and in vitro, as well as metabolism experiments in cells. The results proved that MiR-489-3p inhibits malignancy and glucose metabolism in PC. We further demonstrated that miR-489-3p targets the critical enzymes for glycolytic flux, LDHA, and PKM2, thus controlling glycolysis and PC progression. This study provides important evidence that miRNA is actively involved in the adaptation of pancreatic cancer cells to the nutrient-poor microenvironment.

Materials And Methods

human pancreatic tumor samples.

A total of 90 cases of pancreatic cancer tissues and adjacent tissues from the pancreatic surgery department of Wuhan University People's Hospital from 2009 to 2019 were collected for RNA in situ hybridization. All patients were diagnosed with pancreatic cancer according to the World Health Organization's diagnostic criteria. All samples were approved by the ethics review committee and the patient's informed consent was obtained.

Cell culture

Two human pancreatic cancer cell lines PANC-1 and MIA-PACA2 were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and both were grown in high-glucose DMEM (Gibco, NY, USA). Both cell lines were cultured at 37 °C in a humidified 5% CO₂ incubator according to ATCC protocols.

RNA in situ hybridization

The miR-489-3p was detected through an indirect labeling method using digoxin. The RNA probe labeling method was used to construct plasmids. and then synthesized by transfection. Sample processing, probe preparation, in situ hybridization, washing, blocking, and finally enzyme reaction detection. The probe was visualized under the optical microscope and images taken.

Cell viability analysis

Cell Counting Kit-8 (Beyotime, China) was used to detect the proliferation of PC cells. Following the instructions of the manufacturer, 100ul of 2×10^3 cells were seeded into a 96-well plate, and 10ul of cck8 reagent was added to each well. Absorbance values were measured every 0, 24, 48, 72, and 96 hours and recorded for statistical analysis.

Colony formation assay

Two types of pancreatic cancer cells were inoculated into six-well plates at a concentration of 500 cells per well and cultured at 37°C and 5% CO₂ for two weeks. The medium was then discarded. The cultures were fixed with 4% paraformaldehyde for 20 minutes and followed by staining with 0.2% crystal violet for 30 minutes. The number of clones was observed under an optical microscope and analyzed with statistical software.

Transwell assays

Panc-1 and mia-paca2 cells of different treatment groups were seeded into the upper chamber of Transwell in a concentration of 1×10^5 per well. 200 ul of a medium containing 10% serum was added to the upper chamber, and 700 ul of serum-free medium added to the lower chamber. The transwell was then incubated for 24 hours at 37°C after which the culture medium was discarded. The transwell was washed with PBS (Hyclone, USA), and fixed with 4% paraformaldehyde(Biosharp, China) for 30 minutes. It was then dyed using 0.5% crystal violet (Solarbio, Beijing, China) solution for 30 minutes. Finally, the transwell was rinsed with pure water, observed under an optical microscope, and images taken. Finally, the software counts and analyzes.

Quantitative real-time PCR

The cells of each group were seeded in a six-well plate, and the total RNA of each group was extracted using Trizol reagent (Invitrogen, CA, USA) when the cells achieved 80% - 90% confluence. The cDNA was then synthesized using a PrimeScript RT reagent kit (Takara) according to the manufacturer's instructions. Real-time quantitative PCR was performed by Powerup SYBR Green PCR Master Mix (Life Technologies).

Luciferase reporter assay

The cells in a good state were seeded into a six-well plate. When cells in the six-well plate reached 50% confluence, they were transfected using lipofectamine reagent (Invitrogen) with miR-489-3p or TALD

promoter-containing luciferin Enzyme reporter genes for 4-6 hours. The medium was replaced with High glucose DMEM and then cultured in an incubator at 37°C for 24 hours. Then disposed according to the product instructions for the double luciferase reporter gene detection kit (Beyotime Biotechnology, China). Briefly, 500 microliters of reporter gene cell lysate were discarded after discarding the medium, and the supernatant was taken for determination after sufficient lysis.

Western blot

The cells of each group were seeded in a six-well plate, and when the band fusion reached 90%, the protein was obtained by lysis with RIPA lysate (beytime, China). BCA reagent (beytime, China) was used to quantify the protein, and then the protein was diluted to the same concentration, and then boiled in a refrigerator at -20 ° C until use. Electrophoresis was performed using Solarbio reagents and 10% separation gel and 5% concentrated gel were prepared according to the manufacturer's instructions. After electrophoresis and transfer, exposure is performed.

Glucose uptake and lactate production measurements

Glucose uptake was determined using the D-2-deoxyglucose method following the manufacturer's instructions. D-2-deoxyglucose used was purchased from Beyotime, China. Lactic acid production was assayed using a Lactic Acid detection kit (Leagene Biological, Beijing) as per the recommendations of the manufacturer.

Cellular ATP level

Experiments were performed using an ATP detection kit (Beyotime Biotechnology, China). Add 200 µl of lysate to each well of the six-well plate according to the product instructions, and centrifuge the supernatant after full lysis. Prepare ATP working solution and add it to the detection tube to measure the luminescence value.

Extracellular acidification rate (ECAR)

Hippocampal experiments were performed using Agilent equipment: Hippocampus XFe24 Micro Edition and XFe24 cartridge. Cells of each group were incubated at 500 ° C for 7.5×10^4 cell seeds at 37 ° C for 1 hour at the hippocampal preparation station.

56 µl glucose (100 mm) (G8270σ), 62 µl oligomycin (10 µM) (σ) 75351 and 69 µl 2 dg (1 meter) (D6134, Sigma) were added to the cartridge wells. The ECAR values were then read.

In vivo assay

A total of 107 panc-1 cells (miR-NC group and miR-489-3p up-regulation group) were injected subcutaneously into 4 weeks old female NCr nude mice (Hua Fukang Biotechnology, Beijing) for tumor growth. The tumor size was measured with calipers every 7 days for a period of 30 days. At the end of the experiment, the mice were euthanized, and subcutaneous tumors were collected for further analyses.

IHC analysis

Immunohistochemical staining was used to detect the expression of proliferation and metabolic indicators. Briefly, the subcutaneous tumor tissue was cut into 3µm sections and then dewaxed. The sections were then incubated with rabbit anti-monoclonal at 4 ° C overnight. After washing three times with PBS, each piece was incubated with goat anti-rabbit IgG for 30 minutes and then stained with 3,3'-diaminobenzidine (DAB). All antibodies used were purchased from Proteintech, USA.

Statistical analyses

The results were analyzed and presented as the mean \pm standard deviation. GraphPad Prism 7.0 (San Diego, California, USA) was used for mapping and statistical analysis. Chi-square test was used to analyze the relationship between miR-489-3p expression level and clinicopathological characteristics in PC. The Kaplan-Meier curve method was used to analyze the overall survival rate. The student's t-test was used for statistical comparison between the two groups. Significance level was set at $P < 0.05$ (*) and $P < 0.05$ (* *).

Results

The expression of miR-489-3p and its relationship with clinical prognosis of patients.

RNA in situ hybridization (ISH) experiments were performed on the tumor tissues of 90 patients with pancreatic cancer to verify the expression of miR-489-3p. We found that miR-489-3p expression in tumor tissues was much lower than in the adjacent tissues (Fig. 1a-b). This finding was confirmed by the quantitative RT-PCR (qRT-PCR) analysis (Fig. 1c). Similarly, miR-489-3p showed lower expression in PC cell lines (Fig. 1d). Based on the pathological characteristics of miR-489-3p expression and clinical relevance of pancreatic cancer (Table 1), we found that miR-489-3p expression was significantly related to tumor size ($P < 0.01$) and distant metastatic ability ($P < 0.05$). When we performed survival analysis on patients who provided tissue samples, it was found that patients with low expression of miR-489-3p had a worse prognosis ($P=0.0323$, $HR=1.843$; Fig1e).

MiR-489-3p inhibits proliferation and invasion of pancreatic cancer.

Based on our finding that miR-489-3p is lowly expressed in PC, we speculated that miR-489-3p could inhibit the progression of pancreatic cancer. Therefore, we transfected panc-1 cells with miR-NC and miR-489-3p mimics, and mia-paca-2 with anti-miR-NC and anti-miR-489-3p inhibitors (Fig. 2a). Several proliferation experiments showed that overexpression of miR-489-3p inhibited PC cell proliferation while the inhibition of miR-489-3p increased the PC cell proliferation (Fig. 2b-e). The invasion experiment revealed that overexpression of miR-489-3p could inhibit the invasion ability of PC cells (Fig. 2f).

MiR-489-3p targets LDHA and PKM2.

Furthermore, we explored the mechanisms by which miR-489-3p regulate PC growth. We found that LDHA and PKM2 were RNA binding protein (RBP) of miR-489-3p by STARBASE3.0 (<http://starbase.sysu.edu.cn/>).^{S1a-b} Therefore, we supposed that miR-489-3p could be performing its biological function by targeting them. To verify this, we overexpressed and inhibited miR-489-3p in PC cells and found that LDHA and PKM2 were decreased and increased, respectively (Fig. 3a-c). And the tissue co-expression correlation map exposed that miR-489-3p was negatively correlated with LDHA and PKM2 (Fig. 3d-e). The binding sequences and mutation sites of LDHA and PKM2 on miR-489-3p are shown in Figure 3f. Overexpression of miR-489-3p significantly reduced the luciferase activity of wild types (WT) of LDHA and PKM2 but not their mutants (Mutt). (Fig. 3g). In contrast, down-regulating miR-489-3p significantly increased the luciferase activity of WT of LDHA and PKM2 but did not have any effect on their mutants. (Fig. 3h). These results indicate that miR-489-3p could target LDHA and PKM2.

MiR-489-3p regulates PC glycolysis.

Since miR-489-3p can target LDHA and PKM2 which were glycolytic-related enzymes, we hypothesized that miR-489-3p could regulate glycolysis. We, therefore, performed glycolysis-related experiments to observe the metabolic parameters of PC tumor cells after overexpression or inhibition of miR-489-3p. We noticed a reduction in the production of lactate, glucose, and ATP by the pancreatic cancer cells after miR-489-3p was overexpressed. Conversely, after the downregulation of miR-489-3p, the production of all these compounds increased (Fig. 4a-c). Seahorse assays showed that reducing the expression of miR-489-3p inhibited the extracellular acidification rate (ECAR) of PC cells (Fig. 4d-e). In order to verify the role of miR-489-3p in pancreatic cancer viability, the CCK8 assays indicated that the proliferation make no difference after overexpressing and inhibiting miR-489-3p under low glucose conditions (Fig.4f-g).

LDHA and PKM2 restore miRNA-mediated proliferation and invasion

Our previous results demonstrated that miR-489-3p could regulate the progression of pancreatic cancer and that miR-489-3p could target LDHA and PKM2. Therefore, we hypothesized that miR-489-3p might be carrying out its function by targeting LDHA and PKM2. The QRT-PCR and western blot (WB) assays showed that overexpression of miR-489-3p could down-regulate LDHA and PKM2, and overexpression of LDHA and PKM2 could restore this change (Fig. 5a-b). Similarly, inhibiting miR-489-3p up-regulated LDHA and PKM2, and down-regulating LDHA and PKM2 restored this phenomenon (Fig. 5c-d). We then conducted several response assays of the functional experiments. Proliferation assays showed that miR-489-3p could target LDHA and PKM2 to regulate the proliferation of pancreatic cancer cells (Fig. 5e-h). Invasion assays showed that miR-489-3p could regulate the invasion ability of PC cells through LDHA and PKM2 (Fig. 5i).

MiR-489-3p supresses glycolysis through LDHA and PKM2.

To confirm whether miR-489-3p also inhibited glycolysis of PC cells by targeting LDHA and PKM2, we overexpressed and knocked down of LDHA and PKM2. Up-regulation of miR-489-3p significantly reduced glucose consumption, lactic acid production, and ATP production, whereas increasing LDHA or PKM2

increased their production. Likewise, down-regulating miR-489-3p markedly increased glucose consumption, lactic acid production, and ATP production levels, whereas lowering LDHA or PKM2 reduced their production (Fig. 6a-f). In addition, the hippocampal XF extracellular flux analyzer showed that LDHA and PKM2 could restore the extracellular acidification rate (ECAR) caused by miR-89-3p (Fig. 6g-h).

Effects of overexpression of miR-489-3P on PC proliferation and metabolism in vivo.

To investigate the effect of miR-489-3p on tumor progression in vivo, we used a xenograft tumor model. The image (Fig. 7a) was taken after 30 days of subcutaneous tumor formation in nude mice. Changes in tumor weight and volume indicated that overexpression of mir-489-3p could inhibit tumor growth (Fig. 7b-c). The expression of miR-489-3p was significantly lower in the control group than in the samples transfected with miR-489-3p mimics (Fig7d). The IHC-stained images of cell proliferation factors showed that overexpression of miR-489-3p could inhibit tumor proliferation (Fig. 7e). Finally, to investigate whether miR-489-3p regulates glycolysis, we used IHC to stain glycolysis-related indicators. Apparently, the staining results showed that after miR-489-3p was overexpressed, tumor glycolysis was also inhibited (Fig. 7f).

Discussion

Pancreatic cancer is a highly malignant tumor of the digestive tract, which is extremely difficult to diagnose and treat. It accounts for 8% of cancer-related mortality worldwide.[19]. The highly invasive nature is one of the causes of the lethality of pancreatic cancer. The occurrence and development of tumors are related to the abnormal expression of specific genes[20]. Therefore, identifying genes related to the growth of pancreatic cancer is very important in the research and treatment of this disease. It is well established that pancreatic cancer is highly resistant to radiotherapy and chemotherapy[21-23]. Scientists have long found that glycolysis intensity is positively correlated with tumor invasion and metastasis [24]. For example, lactic acid, the product of glycolysis, forms an acidic microenvironment that is essential for the transformation of lung metastases to malignant metastases [24]. The inhibition of glycolysis to limit the energy supply to the cancer cells has become an emerging chemotherapy approach .

The role of MiRNAs in tumors is extensive. The miR-489-3p is known to inhibit the growth of a variety of tumors, such as bladder cancer [25], renal cell carcinoma[26], and osteosarcoma[17]. However, its relationship with pancreatic cancer has not been well established. Using microarray analysis, the present study found that the expression of miR-489-3p in pancreatic cancer was lower than that in adjacent tissues, and it was closely related to the prognosis of clinical patients. Our functional experiments also demonstrated that miR-489-3p could inhibit the invasion and metastasis of pancreatic cancer cells. However, the mechanism by which overexpression of miR-489-3p inhibits pancreatic cancer growth metabolism is not yet clear.

Furthermore, a luciferase reporter experiment showed that miR-489-3p could target the expression of LDHA and PKM2. LDHA catalyzes the conversion of pyruvate to lactic acid during glycolysis whereas PKM2 is a rate-limiting enzyme for glycolysis. Previous studies have shown that LDHA is inseparable from the aerobic glycolysis of tumors[27]. There are two isomers of M-type pyruvate kinase: PKM1 and PKM2 and PKM2 is the only form of pyruvate kinase found in cancerous tissues [28, 29]. A transcription factor Hypoxia-Inducible Factor (HIF) is highly expressed in tumors, and one of its subtypes, HIF-1, promotes the expression of the promoter region of the MYC gene. MYC is a transcription factor with a wide range of biological functions, including cellular energy metabolism[30]. It can stimulate the expression of many genes, including LDHA and PKM2[31, 32]. This showed the relevance of LDHA and PKM2 in the study of pancreatic cancer. Since LDHA and PKM2, the targets of miR-489-3p, are glycolysis-related enzymes, we confirmed that miR-489-3p could inhibit glycolysis of pancreatic cancer cells by metabolism experiments .

We then performed functional and glycolysis-related experiments by overexpression or knockdown of LDHA or PKM2. Apparently, the overexpression of LDHA or PKM2 restored the inhibition of pancreatic cancer cell growth and glycolysis caused by overexpression of miR-489-3p. In contrast, knocking down LDHA or PKM2 alleviated the fast growth and hypermetabolism of pancreatic cancer cells caused by inhibition of miR-489-3p. On performing the in vivo experiments, we found that the xenograft tumors in the overexpressing miR-489-3p group were significantly smaller than the NC group. Besides, the immunohistochemistry of the xenograft tumors showed that the proliferative and metabolic markers of the overexpressing miR-489-3p group were lower than the NC group.

The first-line treatment options for pancreatic cancer (FOLFIRINOX and gemcitabine/Nab-paclitaxel) have limited effects and are toxic[33, 34]. In recent years, studies have found that the analysis of circulating miRNAs may improve the choice of the best treatment plan [35]. And metabolic flexibility is necessary for successful metastasis and is essential for effective colonization of distant sites. Though it has been demonstrated that micro RNAs can control tumor growth by regulating glycolysis-related genes[36, 37]. The number of related studies is still rare. Our research demonstrated that aberrant expression of miR-489-3p can modulate the progression of pancreatic cancer via targeting LDHA and PKM2. It may expand the biomarker library for pancreatic cancer.

Conclusion

In summary, our in vivo and in vitro experiments have demonstrated that miR-489-3p inhibits the growth and glycolysis of pancreatic cancer cells. This study has provided new ideas for molecular targeted therapy of pancreatic cancer.

Declarations

Ethics approval and consent to participate

Human samples were obtained from Renmin Hospital of Wuhan University. All patients included in the study provided written informed consent, and the study was approved by the Institutional Ethics Committee of Renmin Hospital of Wuhan University. Patients did not receive financial compensation. All methods were performed in accordance with relevant guidelines and local regulations.

Consent for Publication

All authors have agreed to publish this manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors have no conflicts of interest to disclose

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Authors' contributions

JX Jiang and ZW He contributed to the experiment design, and data analysis. D Zhang, YY Shen and J Wang contributed to the experiment implementation, Tao Liu and JX Jiang contributed to manuscript draft and data analysis. All authors read and approved the final manuscript.

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Table

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

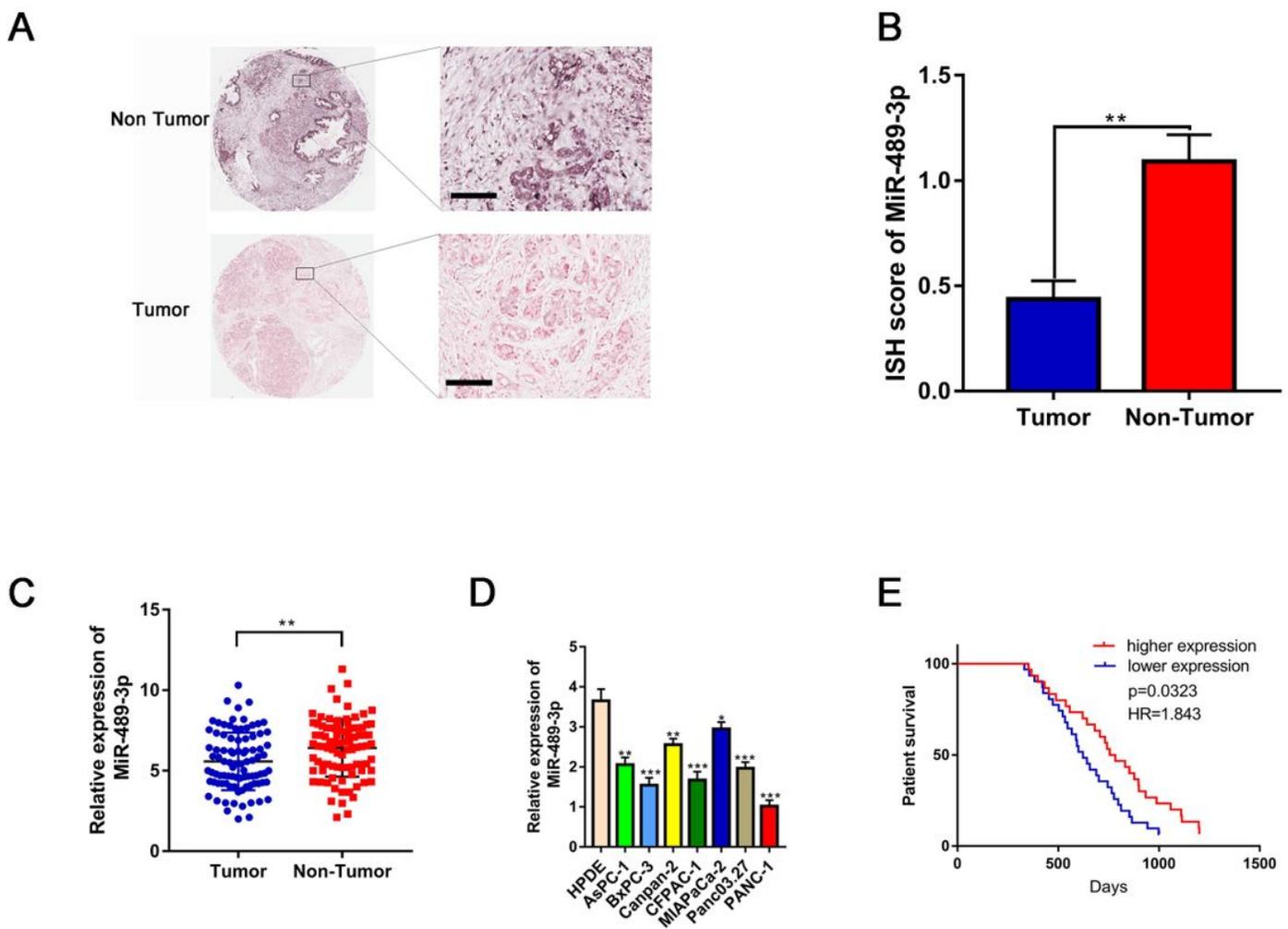


Figure 1

The expression of miR-489-3p and its relationship with clinical prognosis of patients. (a-b) RNA in situ hybridization experiments showed the expression of miR-489-3p in paracancerous and cancerous tissues. The bar stands for 50 microns (c) qRT-PCR analysis of the relative expression of miR-489-3p in adjacent tissues and PC tissues. (d) qRT-PCR showed the relative expression of miR-489-3p in PC cell lines and pancreatic normal duct epithelial cells (HPDE). (e) Kaplan-Meier curve was divided into survival periods by miR-489-3p expression. Among them, patients were divided into high expression group (red) and low expression group (blue) by median expression of miR-489-3p. (*P < 0.05, ** P < 0.01, *** P < 0.001)

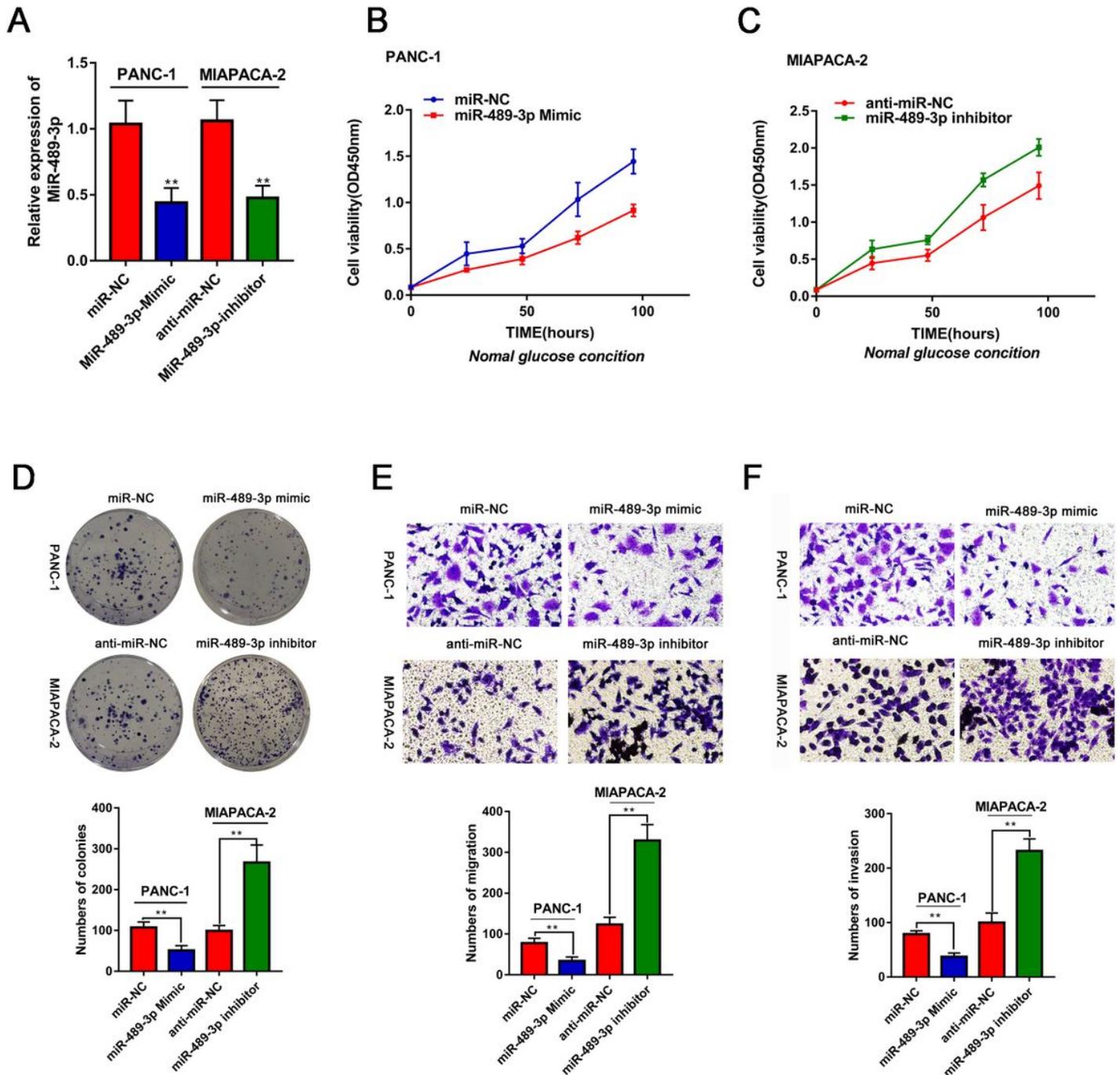


Figure 2

MiR-489-3p inhibits proliferation and invasion of pancreatic cancer. (a-b) qRT-PCR showed the relative expression of miR-489-3p after transfection of miR-489-3p mimic in panc-1 cells and addition of miR-489-3p inhibitor in mia-paca2 cells. (b-e) CCK8 and plate cloning and transwell proliferation assays showed the cell proliferation ability of panc-1 cells transfected with miR-489-3p mimics and mia-paca2 cells with miR-489-3p inhibitors. (f) Transwell invasion assays showed the cell invasion ability of panc-1 cells transfected with miR-489-3p mimics and mia-paca2 cells with miR-489-3p inhibitors. (** P < 0.01)

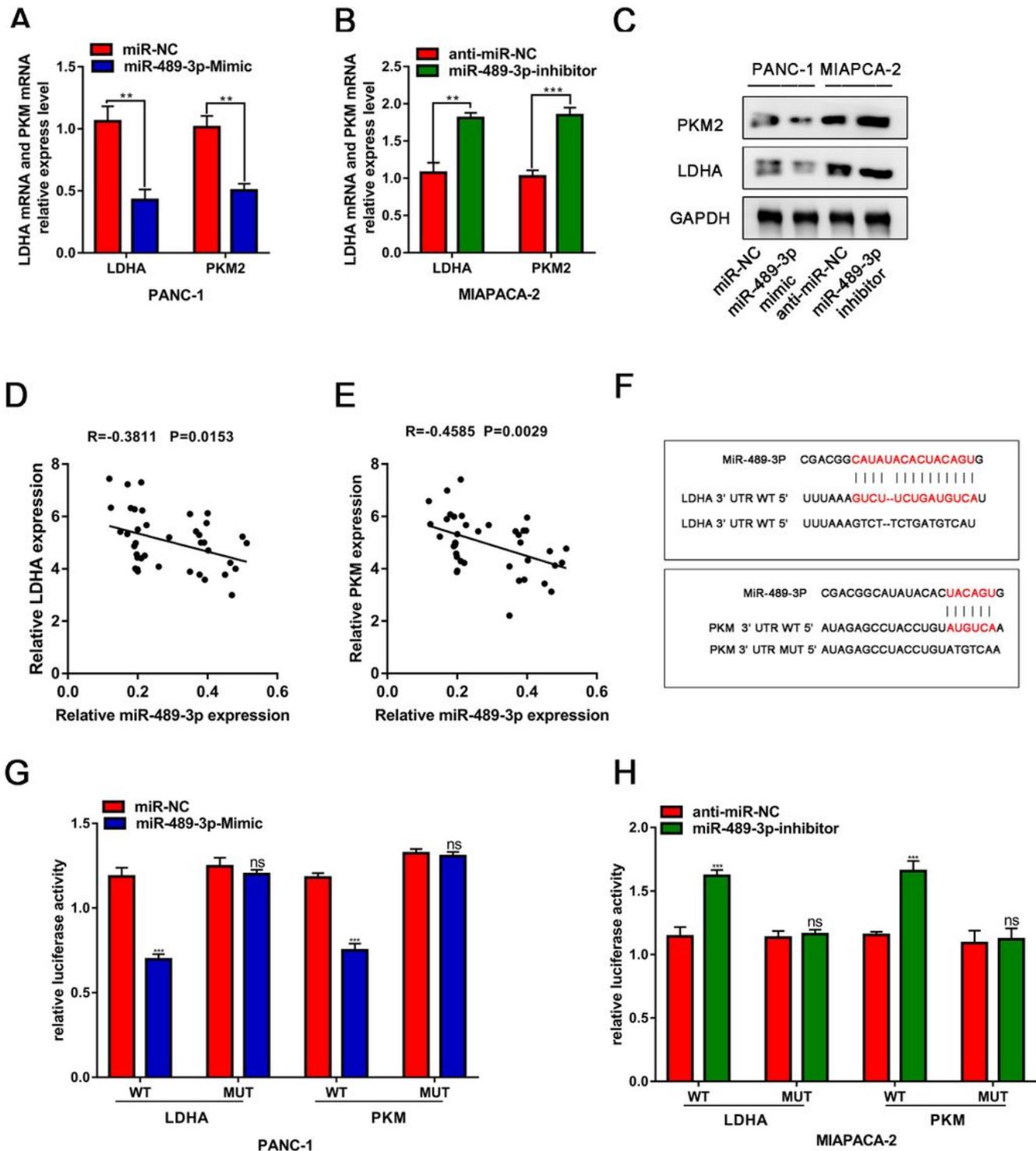


Figure 3

MiR-489-3p targets LDHA and PKM2. (a,c) qRT-PCR and western blot assays showed relative expression of LDHA and PKM2 after panc-1 cells were transfected with miR-489-3p mimics. (b,c) qRT-PCR and western blot assays showed relative expression of LDHA and PKM2 after miR-489-3p inhibitor was added to mia-paca2. (d-e) Spearman rank correlation analysis showed a statistical relationship between miR-489-3p and LDHA and PKM. (f) The predicted binding site of miR-489-3p in human LDHA and PKM gene 3' UTR, and the corresponding sequence in the mutated version. (g-h) Analysis of the luciferase reporter gene indicated a statistical relationship between miR-489-3p and LDHA and PKM. (** P < 0.01, *** P < 0.001)

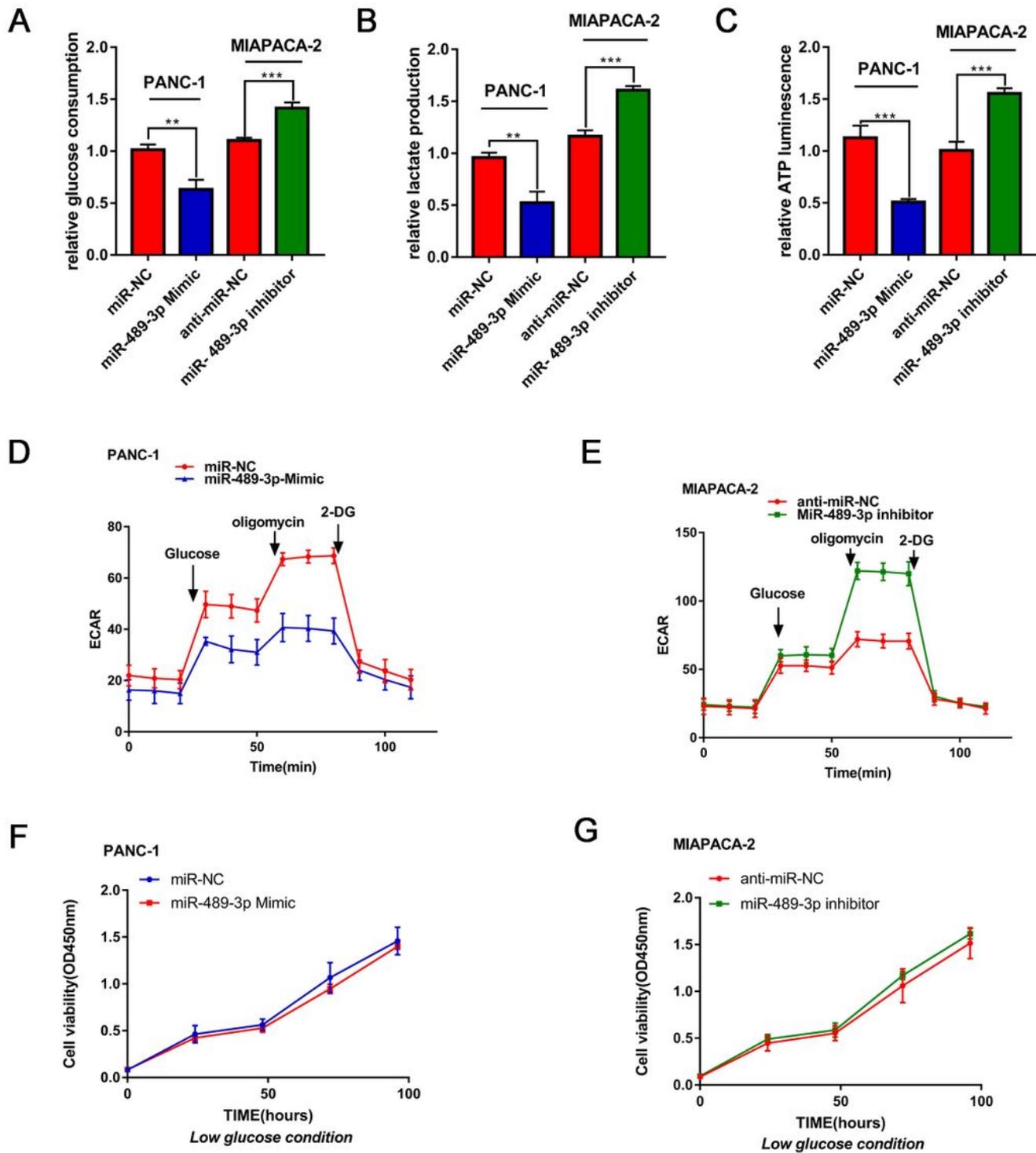


Figure 4

MiR-489-3p regulates PC glycolysis. (a-c) Cell metabolism experiments showed glucose uptake, lactic acid production, and ATP production of miR-489-3p mimics in panc-1 cells and miR-489-3p inhibitors in mia-paca2 cells. (d-e) The hippocampal XF extracellular flux analyzer showed that ECAR was added to miR-489-3p mimics in panc-1 cells and miR-489-3p inhibitors in mia-paca2 cells. (f-g) CCK8 assays showed the survival status of pancreatic cancer cells in low glucose condition. (** P < 0.01, *** P < 0.001)

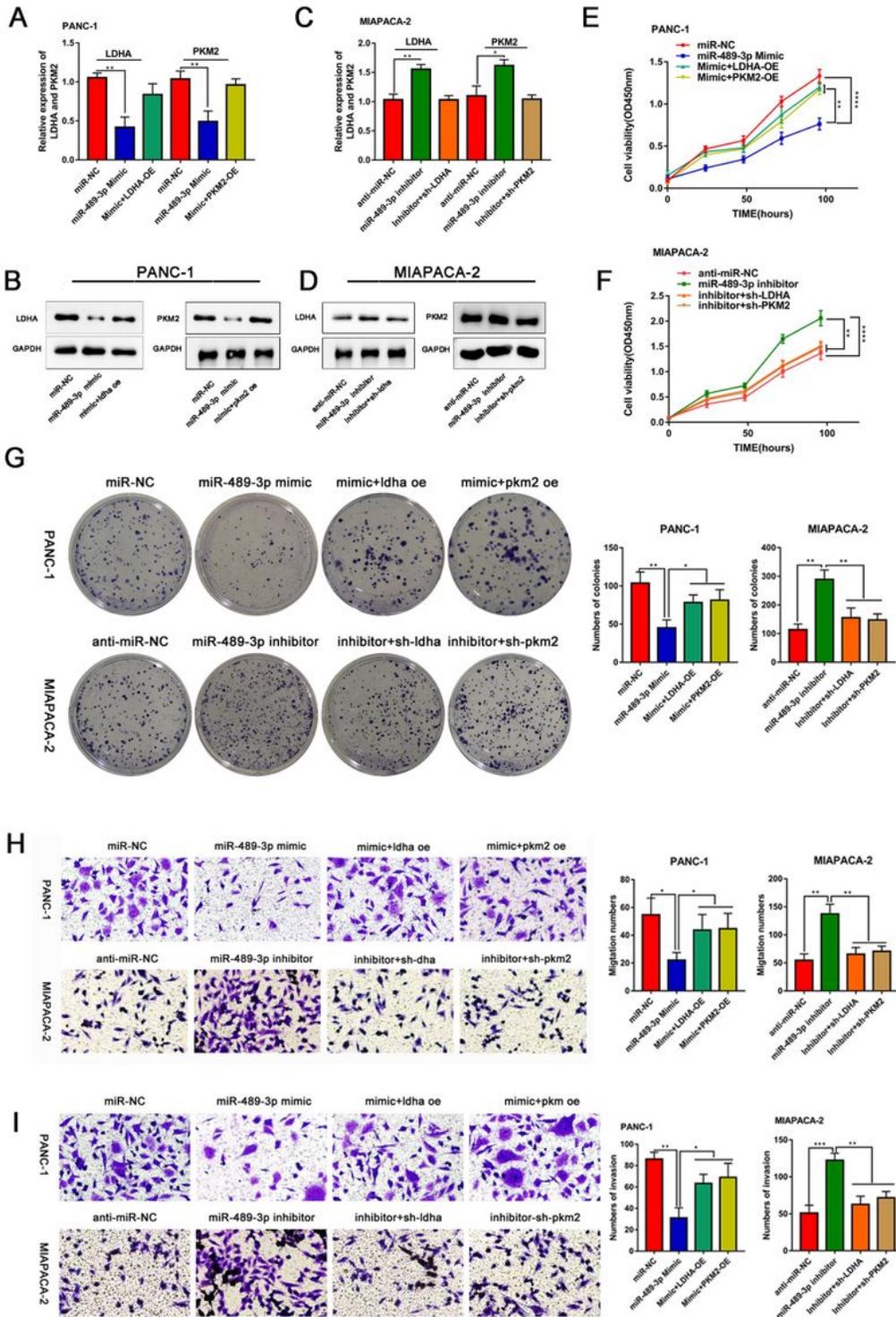


Figure 5

LDHA and PKM2 restore miRNA-mediated proliferation and invasion (a-b) qRT-PCR and western blot experiments showed that the relative expression of LDHA and PKM2 after up-regulating miR-489-3p and LDHA (PKM2). (c-d) qRT-PCR and western blot experiments showed that the relative expression of LDHA and PKM2 after down-regulating miR-489-3p and LDHA (PKM2) simultaneously. (e-h) CCK8 and plate cloning and transwell migration assays showed that the proliferation ability of PC cells after up-

regulating (down-regulating) miR-489-3p and LDHA (PKM2). (i) Transwell invasion assays show that the invasion ability of PC cells after up-regulating (down-regulating) miR-489-3p and LDHA (PKM2) simultaneously. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** < 0.0001)

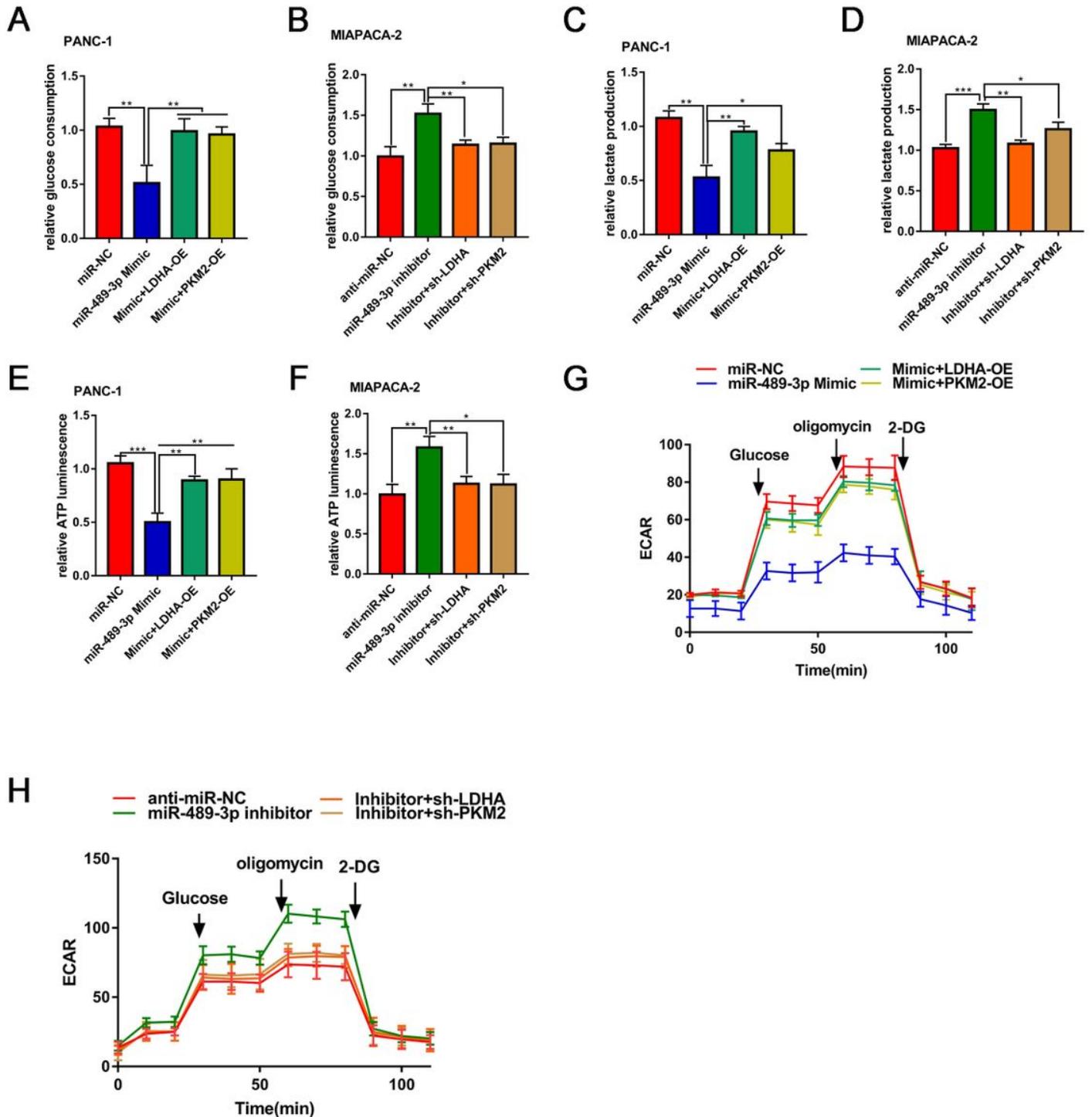


Figure 6

MiR-489-3p suppresses glycolysis through LDHA and PKM2. (a,c,e) Cell metabolism experiments showed that glucose uptake, lactic acid production, and ATP production of PC cells after upregulating miR-489-3p and LDHA (PKM2) simultaneously. (b,d,f) Cell metabolism experiments showed glucose uptake, lactic

acid production, and ATP production of PC cells after miR-489-3p and LDHA (PKM2) were down-regulated simultaneously. (g) The hippocampal XF extracellular flux analyzer showed ECAR after simultaneously up-regulating miR-489-3p and LDHA (PKM2). (h) The hippocampal XF extracellular flux analyzer showed ECAR after simultaneously down-regulating miR-489-3p and LDHA (PKM2).

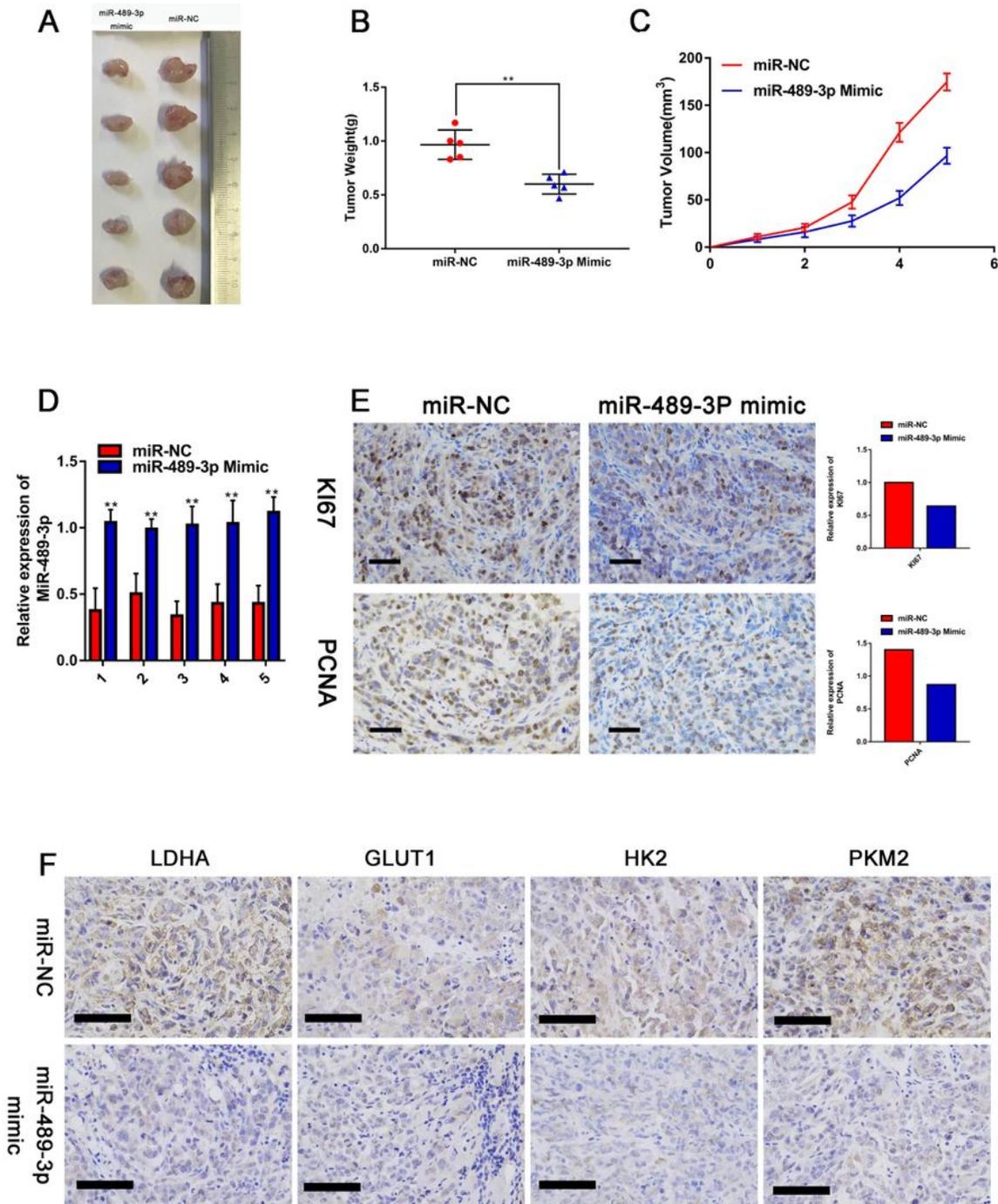


Figure 7

Effects of overexpression of miR-489-3P on PC proliferation and metabolism in vivo. (a) Typical images of nude mice tumors (n = 5), (b) subcutaneous tumor weight, (c) subcutaneous tumor volume, (d) miR-489-3p expression in xenografts by qRT-PCR. (e) Typical IHC staining images of xenografts show Ki-67 and PCNA expression. The bar stands for 50 microns. (f) Typical IHC staining images of xenografts show the expression of metabolic indicators (LDHA, GLUT1, HK2, PKM2). The bar stands for 100 microns.

Supplementary Files

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