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ORIGINAL ARTICLE

Basic Study

FAM53B promotes pancreatic ductal adenocarcinoma metastasis by regulating macrophage M2 polarization

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Abstract

BACKGROUND

Our study investigated the role of FAM53B in regulating macrophage M2 polarization and its potential mechanisms in promoting pancreatic ductal adenocarcinoma (PDAC) metastasis.

AIM

To further investigate the role of FAM53B in regulating macrophage M2 polarization and its potential mechanism in promoting PDAC metastasis. Our goal is to determine how FAM53B affects macrophage M2 polarization and to define its underlying mechanism in PDAC metastasis.

METHODS

Cell culture and various experiments, including protein analysis, immunohistochemistry, and animal model experiments, were conducted. We compared FAM53B expression between PDAC tissues and healthy tissues and assessed the correlation of FAM53B expression with clinical features. Our study analyzed the role of FAM53B in macrophage M2 polarization *in vitro* by examining the expression of relevant markers. Finally, we used a murine model to study the role of FAM53B in PDAC metastasis and analyzed the potential underlying mechanisms.

RESULTS

Our research showed that there was a significant increase in FAM53B levels in PDAC tissues, which was linked to adverse tumor features. Experimental findings indicated that FAM53B can enhance macrophage M2 polarization, leading to increased anti-inflammatory factor release. The results from the mouse model further supported the role of FAM53B in PDAC metastasis, as blocking FAM53B

prevented tumor cell invasion and metastasis.

CONCLUSION
FAM53B promotes PDAC metastasis by regulating macrophage M2 polarization. This discovery could lead to the development of new strategies for treating PDAC. For example, interfering with the FAM53B signaling pathway may prevent cancer spread. Our research findings also provide important information for expanding our understanding of PDAC pathogenesis.

Key Words: FAM53B; Pancreatic ductal adenocarcinoma; Tumor metastasis; Macrophage polarization

Core Tip: Our study investigates FAM53B in regulating macrophage M2 polarization and its potential mechanisms in promoting pancreatic ductal adenocarcinoma (PDAC) metastasis. Our research revealed a significant upregulation of FAM53B in PDAC tissues, which was associated with the malignant features of the tumors. Experimental findings indicated that FAM53B can enhance macrophage M2 polarization, leading to increased release of anti-inflammatory factors. Murine model results further confirmed the role of FAM53B in PDAC metastasis, as inhibiting FAM53B suppressed tumor invasion and metastasis. Therefore, FAM53B promotes PDAC metastasis by regulating macrophage M2 polarization.

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INTRODUCTION

In 2023, it is estimated that the number of new cases and deaths of pancreatic ductal adenocarcinoma (PDAC) in China will reach 134374 and 131203 cases, respectively, so PDAC has become a serious public health problem in China[1-3]. Despite significant advances in cancer research, PDAC remains a deadly disease[4-10]. FAM53B is an important member of the tumor necrosis factor superfamily[11-14]. This cytokine is particularly important for reactivating non-reactive T lymphocytes to achieve an optimal CD8 response in CD8+ T cells[15]. Its receptor, a member of the co-stimulatory receptor and tumor necrosis factor receptor superfamily, was initially found on activated T cells and is transiently expressed on activated T cells that encounter homologous antigens[16-20]. The traditional view is that the FAM53B ligand binds to receptors and transmits signals to cells expressing them, that is, positive signal transduction[21]. The mutual recognition of T cells and FAM53B on antigen-presenting cells can lead to receptor aggregation, activation of downstream TRAFS-mediated cascade signaling, and ultimately T cell activation, proliferation, and survival[22-24]. Moreover, FAM53B induces a strong co-stimulatory signal in T cells, which works in tandem with T cell receptor signals to produce interleukin (IL)-2 and interferon-gamma (IFN-γ)[25]. In addition to inducing effector cytokine production, FAM53B co-stimulated T cell memory and effector differentiation and protected T cells from apoptosis[26-28].

PDAC is a highly aggressive tumor with a worrying morbidity-to-mortality ratio[29]. According to the World Health Organization, PDAC is the seventh leading cause of cancer death worldwide. Despite significant advances in cancer research, survival rates for PDAC are relatively low, in part due to its late diagnosis and rapid metastasis. Therefore, an in-depth study of the metastasis mechanism of PDAC is of vital significance for improving patient prognosis. FAM53B is a protein-coding gene whose function has attracted much attention. In recent years, research has shown that FAM53B plays a key role in a variety of cancers, including PDAC. However, the specific role of FAM53B in PDAC and its effect on the regulation of macrophage M2 polarization on PDAC metastasis remain unclear[30]. The PDAC microenvironment has fewer CD8+ T cells than other parts of the body. This means that the positive co-stimulatory signal of FAM53B may not work as well in treating PDAC. Conversely, the reverse signaling of FAM53B can exert anti-tumor effects by reshaping the tumor microenvironment (TME).

In this study, we examined the role of FAM53B in the development of PDAC, focusing on its possible control of macrophage polarization. This study is expected to open up new frontiers in the research and treatment of PDAC and other cancer types.

MATERIALS AND METHODS

Cell culture and resuscitation

ASPC-1 and U937 cells (CL-0018; Pricella, Wuhan, China) to be resuscitated were removed from the liquid nitrogen tank, and the cells were quickly shaken and thawed in a pre-heated 37 °C constant temperature water bath. ASPC-1 and U937

cells were cultured with Gibco 1640 medium containing 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, United States). Gibco DMEM high-glucose medium with 10% fetal bovine serum was used to grow COLO357, BXPC-3, and PANC-1 cells. The consumables required for the experiment, including the pasteurized straws, were placed on the ultra-clean operating table and sterilized with ultraviolet light for 30 min. After completion, high glucose DMEM complete culture medium and RPMI 1640 complete culture medium were prepared on the operating table with the ratio of basic culture medium: Serum: Penicillomycin double antibody = 9:1:0.1. The cells to be resuscitated were removed from the liquid nitrogen tank and thawed rapidly in a pre-heated 37 °C constant temperature water bath. The thawed cell suspension was then transferred to a sterile centrifuge tube, and 10 mL of complete medium was added and mixed well. The cells were centrifuged at 1000 rpm for 5 min, the supernatant was discarded, and an appropriate amount of complete medium was added to resuspend the cells. The cell suspension was transferred to a culture bottle and placed in an incubator, cultured at 5% carbon dioxide at 37 °C and observed the next day.

Preparation of conditioned medium

A total of 1 × 10^5 BXPC-3 and PANC-1 cells (CL-0019; Pricella) were inoculated in per well of a 6-well plate. After 24 h of culture, the cells were washed twice with sterile phosphate-buffered saline (PBS) solution and serum-free medium (10 mL, A2656101; Thermo Fisher Scientific) was added for 48 h.

U937 cell differentiation

When U937 cells (CL-0022; Pricella) reached the logarithmic growth phase, the cell suspension was transferred to a centrifuge tube and spun at 800 rpm for 5 min. The pellet was resuspended in complete media and cells were counted under the microscope. A total of 1 × 10^6 cells in suspension was transferred into a 6 cm petri dish. phorbol 12-myristate 13-acetate (PMA) was added at a concentration of 100 ng/mL. The cells were incubated for 48 h to induce differentiation into macrophage-like U937 cells.

Generation of FAM53B knockout stable cell lines

CRISPR/Cas9-based lentiviral infection was used to generate stable FAM53B knockdown pancreatic cancer cell lines. Virus for stable FAM53B knockdown was purchased from Shanghai Jikai Company (Shanghai, China). The lentiviral vector was GV493, which contained GFP and a puromycin resistance cassette. The virus titer was higher than 1E+8TU. The two shRNA sequences were: sh1: *5′-CTACTATGTCTTCTTTCAACT-3′*, sh2: *5′-TGGAATACGCCTCTGACGCTT-3*'.

Macrophage induction and polarization

The macrophage model was constructed using U937 cells, and macrophage-like U937 cells were transferred to 6-well plates and transfected when the cell density reached 20-40%. Then, under PMA conditions, IL-13 and IL-4 (20 ng/mL each) were added to M0 macrophage culture medium, and the culture was stimulated for 48 h. M0 macrophages were induced to differentiate into M2 macrophages, thus obtaining THP-1 cell-derived M2 macrophages.

Western blotting and exosome uptake

Exosomal vesicles derived from U937 cells and macrophages were lysed in RIPA lysis buffer. Antibodies against FAM53B (1:1000, A19236; Abclonal, Wuhan, China), GAPDH (1:2000, AC001; Abclonal) and Tubulin were used. Protein samples were adjusted to 0.375 mg/mL, 0.75 mg/mL, 1.5 mg/mL, 3 mg/mL, and Graphpad software (San Diego, CA, United States) was used to generate a standard protein curve. After washing, the membranes were incubated with peroxidase-labeled goat anti-rabbit secondary antibody (1:2000, AS014; Abclonal) at 37 °C for 1 h. The PKH67 Fluorescent Cell Linker Kit was used to identify and stain exosome suspensions. Diluent C (250 μL) was added to 50 μL of exosomes, followed by 1.5 μL diluent C (1.5 μL). The labeled and diluted exosomes were completely suspended with DMEM, and the exosomes were then added to the U937 cell (DAPI staining) culture supernatant with a pipette. After 24 h of culture, fixation, membrane rupture, and nucleation, laser scanning confocal fluorescence microscopy was used to observe whether U937 cells could take up macrophage-derived exosomes.

The gray value was detected by Image J software (version 1.8.0; National Institutes of Health, Bethesda, MA, United States), and the results were calculated by Graphpad Prism (version 9.0; GraphPad Software, San Diego, CA, United States).

Construction of a nude mouse transfer model

Four-week-old female BALB/c nude mice were fed under standard pathogen-free conditions and divided into two groups of seven mice per group. After 2 wk of feeding, the animals were used for downstream experiments. Firstly, transfected PANC-1 cells were digested, centrifuged, and re-suspended to prepare a 5 × 10/100 μL cell suspension. Next, nude mice were anesthetized by intraperitoneal injection of 1% pentobarbital sodium (50 mg/kg), placed in the right lying position, and disinfected with 75% alcohol. A longitudinal 1.0 cm-long incision was made on the left side of the abdomen. The spleen was exposed and gently removed from the abdominal cavity. A 1 mL syringe was inserted into the lower pole of the spleen about 1.0 cm and the tumor cell suspension was slowly injected. After injection, the syringe was removed, and the eye of the needle was pressed with a cotton ball of iodophenol for 2 min. The abdomen was closed, and the mice received routine postoperative feeding. After 4 wk of culture, the nude mice were sacrificed by cervical dislocation, and splenic tumor formation, liver metastasis and other metastases were observed anatomically. Splenic graft tumors and liver metastases were collected, and the results were observed by hematoxylin-eosin staining. The experiment was divided into two groups: sh-NC PANC-1 and sh-FAM53B PANC-1.

International council for harmonization and hepatic encephalopathy staining

International council for harmonization: The slices were washed in PBS solution three times for 5 min each. Slices were placed in a dark box, and the excess PBS around the tissue was absorbed by filter paper. An appropriate amount of anti-rabbit and anti-mouse fluorescent secondary antibody was added, and incubated in a 37 °C incubator for 30 min (the subsequent steps were all performed in a dark room). The slices were washed three times in PBS solution for 5 min each. An appropriate amount of DAPI dye was added and stained for 10 min. After the slices were rinsed in PBS solution three times, they were placed in the dark box and 10 μL anti-fluorescence quench agent was added. The number of positively stained cells under 200 times of visual field was counted by fluorescence microscopy, and five visual fields were randomly counted. These data represent the average of the results obtained by two scorers.

Hepatic encephalopathy staining: The slide was soaked in hematoxylin solution for 5 min, washed with running water, then soaked in 1% hydrochloric alcohol for 3 s, and washed again with running water. The slide was dyed in eosin dye for 1 minute and washed with running water. The slide holder was placed in 50% ethanol for 5 min, 75% ethanol for 5 min, 85% ethanol for 5 min, 95% ethanol for 5 min, 100% ethanol for 5 min, and finally placed in two cylinders of xylene solution for 15 min each.

Colony formation and flow cytometry analysis

Colony formation assay: PDAC cells were separately plated into culture dishes containing medium. The FAM53B overexpression vector was transfected into one group and the other group was used as a blank control. After culture for a period of time, the cells were fixed with the AGAR flower tumor medium, and the number and size of the colonies were recorded.

Flow cytometry analysis: Macrophages were treated to differentiate into M2 type and divided into different groups (FAM53B overexpression group and control group). Fluorescent markers were used to label surface markers or cytokines, and the proportions of different cell subpopulations and the expression levels of specific markers were detected and analyzed by flow cytometry.

Statistical methods

Statistical analysis for this project was conducted using SPSS (version 25.00; IBM Corp., Armonk, NY, United States), including descriptive statistics, *t*-tests, correlation analysis, multiple regression, and survival analysis.

RESULTS

FAM53B expression is high in PDAC

We collected thirty pancreatic cancer tissue samples for this investigation. Additional immunohistochemical staining analyses confirmed that FAM53B expression was substantially higher in pancreatic cancer compared to adjacent non-tumorous tissues ($P < 0.001$) (Figure 1A-C). We tested FAM53B expression in normal pancreatic cells (HPDE6-C7) and pancreatic cancer cells (ASPC-1, PANC-1, BXPC-3, and COLO357) using quantitative reverse transcriptase PCR (qRT-PCR) and protein immunoblotting. FAM53B protein expression was considerably greater in ASPC-1, PANC-1, and BXPC-3 cells compared to HPDE6-C7 cells ($P < 0.05$) (Figure 1D). Significant differences in FAM53B expression were observed at the mRNA level between HPDE6-C7 and ASPC-1, PANC-1, and BXPC-3 ($P < 0.05$); however, FAM53B expression did not differ between COLO357 and HPDE6-C7 cells (Figure 1E and F).

FAM53B knockout inhibited PDAC cells

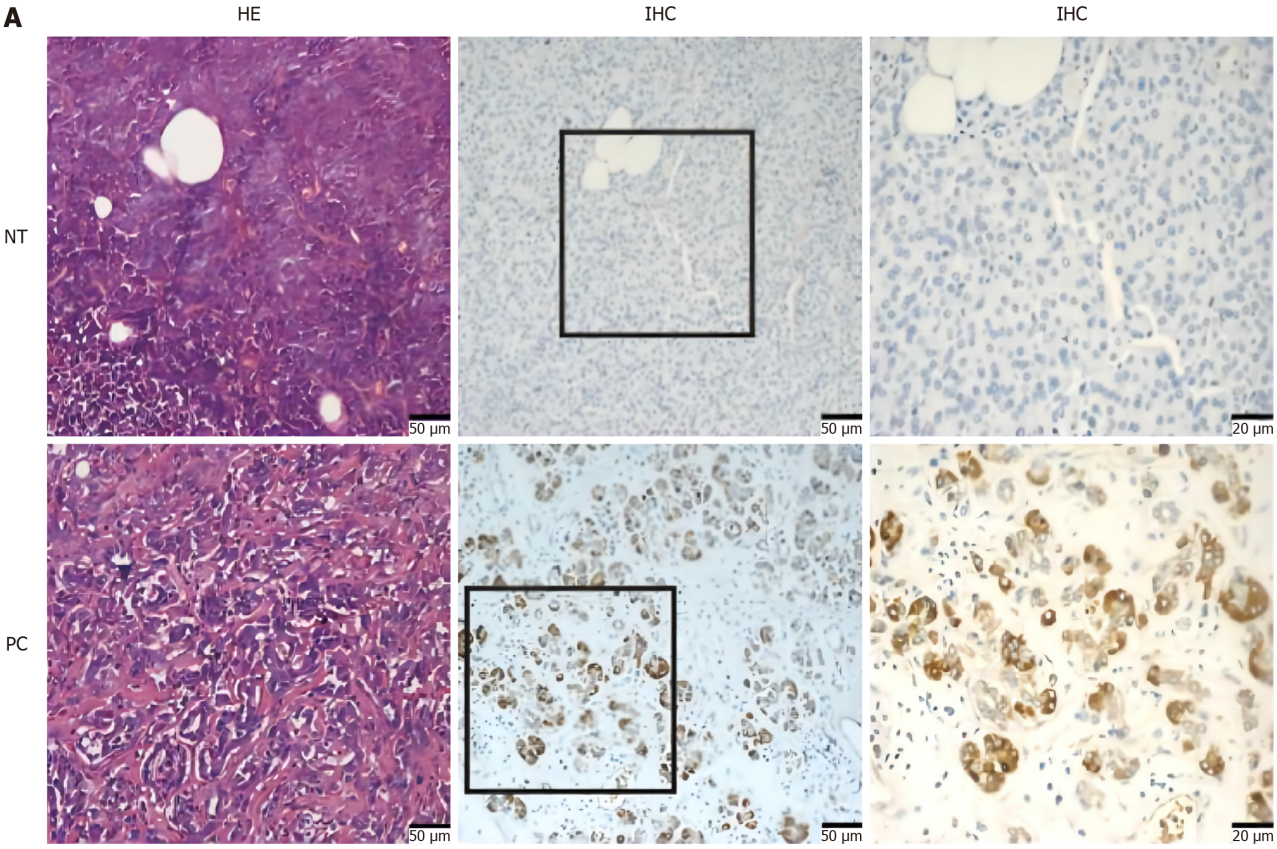
We used cell counting kit-8 (CCK-8) and single-cell cloning to investigate the impact of sh-NC, sh-FAM53B-1, and sh-FAM53B-2 tumor-associated macrophages (TAMs) on pancreatic cancer cell growth. The proliferation of BXPC-3 and PANC-1 cells was unaffected by sh-FAM53B-1 and sh-FAM53B-2 TAMs when co-cultured with sh-NC TAMs (Figure 2A-F). Additionally, FAM53B knockdown macrophages had a higher level of apoptosis in BXPC-3 and PANC-1 compared to those with sh-NCTAMs ($P < 0.05$) (Figure 2G-J).

FAM53B expression associated with M2 polarization

We performed immunofluorescence to examine the relative abundance of M0 macrophages (CD68), M1-type macrophages (CD86), and M2-type macrophages (CD206) in pancreatic cancer tissues. Our findings revealed a statistically significant increase in the number of M2-type macrophages compared to M1-type macrophages ($P < 0.01$) (Figure 3A and B). Furthermore, fluorescence colocalization analysis revealed that immune cells were the primary source of TNFSF9 expression ($P < 0.001$) (Figure 3C). Additional analysis revealed a stronger correlation ($r = 0.722$) between the expression of TNFSF9 and M2 macrophages as opposed to M1 macrophages ($r = 0.599$) (Figure 3D).

FAM53B induces M2 polarization

We compared markers of M1 (CD86) and M2 (CD206) macrophages between knockdown FAM53B TAMs (sh-FAM53B-1 TAMs and sh-FAM53B-2 TAMs) and transfected empty vector TAMs (sh-NC TAMs) using immunofluorescence. In comparison to the sh-NC group, both BXPC-3 and PANC-1 cell supernatants produced TAMs. The expression of the M2-type macrophage marker in sh-FAM53B-1 and sh-FAM53B-2 TAMs was considerably decreased ($P < 0.05$), but the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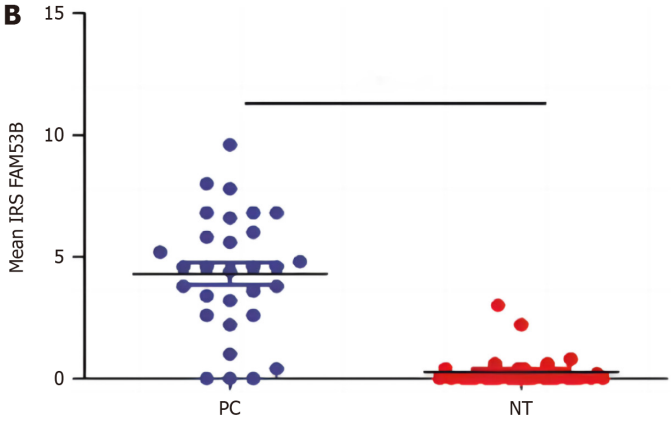
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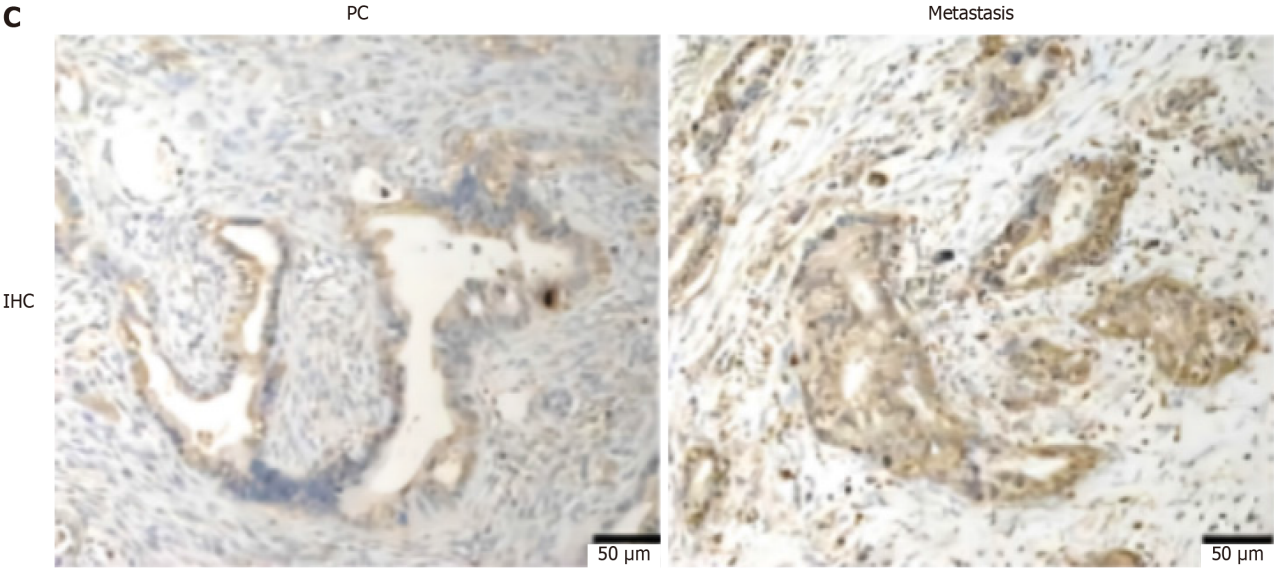
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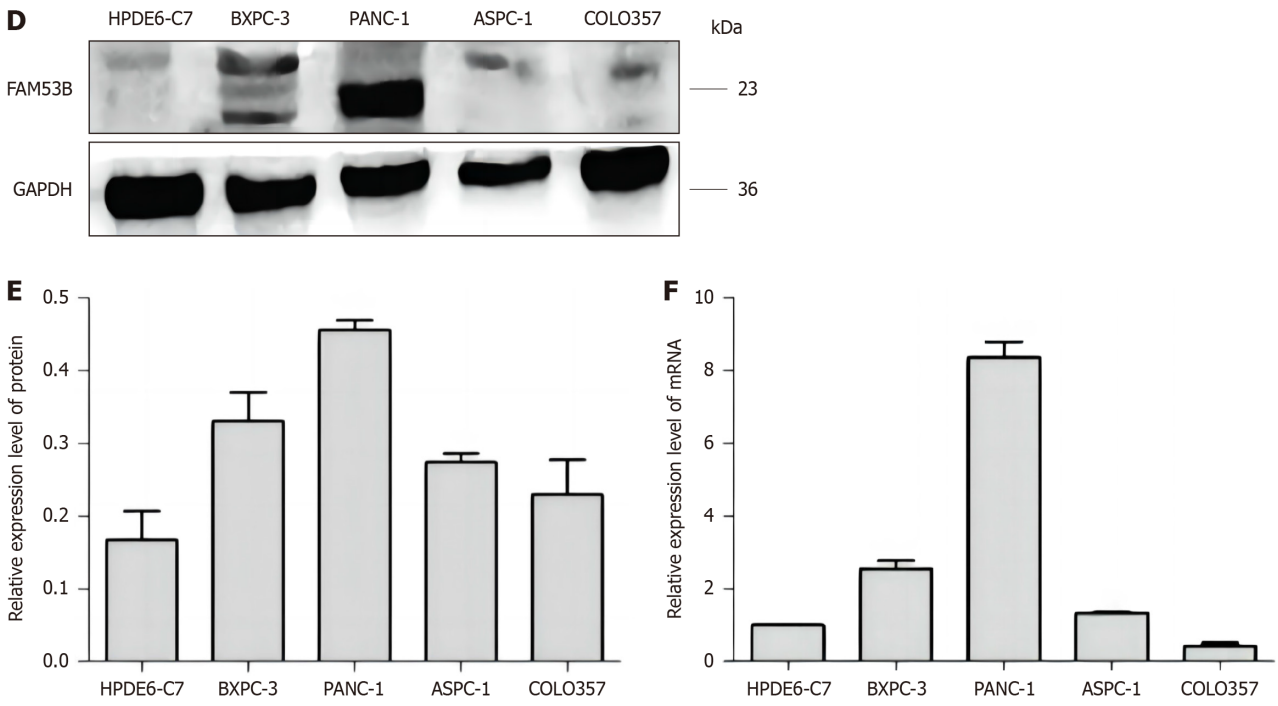


Figure 1 FAM53B expression in pancreatic ductal adenocarcinoma tissue. A: Hepatic encephalopathy staining of pancreatic ductal adenocarcinoma and para-carcinoma tissue (n = 30); B and C: Immunohistochemical staining for immune response score (NT for para-cancer non-tumor tissue, PC for pancreatic ductal adenocarcinoma tissue); D and E: Western blot analysis; F: Quantitative real-time polymerase chain reaction analysis. HE: Hepatic encephalopathy.

expression of the M1-type macrophage marker was not statistically different from the sh-NC group (Figure 4A-E). We then performed qRT-PCR to determine mRNA expression of M1 markers (IL-8 and tumor necrosis factor-alpha) and M2 markers (IL-10 and transforming growth factor beta) in sh-FAM53B-1TAM, sh-FAM53B-2 TAMs, and sh-NC TAMs. The expression of M1 markers was significantly higher in sh-FAM53B-1 TAMs and sh-FAM53B-2 TAMs in comparison to sh-NC TAMs (Figure 4F-G). Conversely, the expression of M2 markers was found to be significantly lower in both TAMs (all P values < 0.05).

M2 polarized macrophages have no effect on proliferation

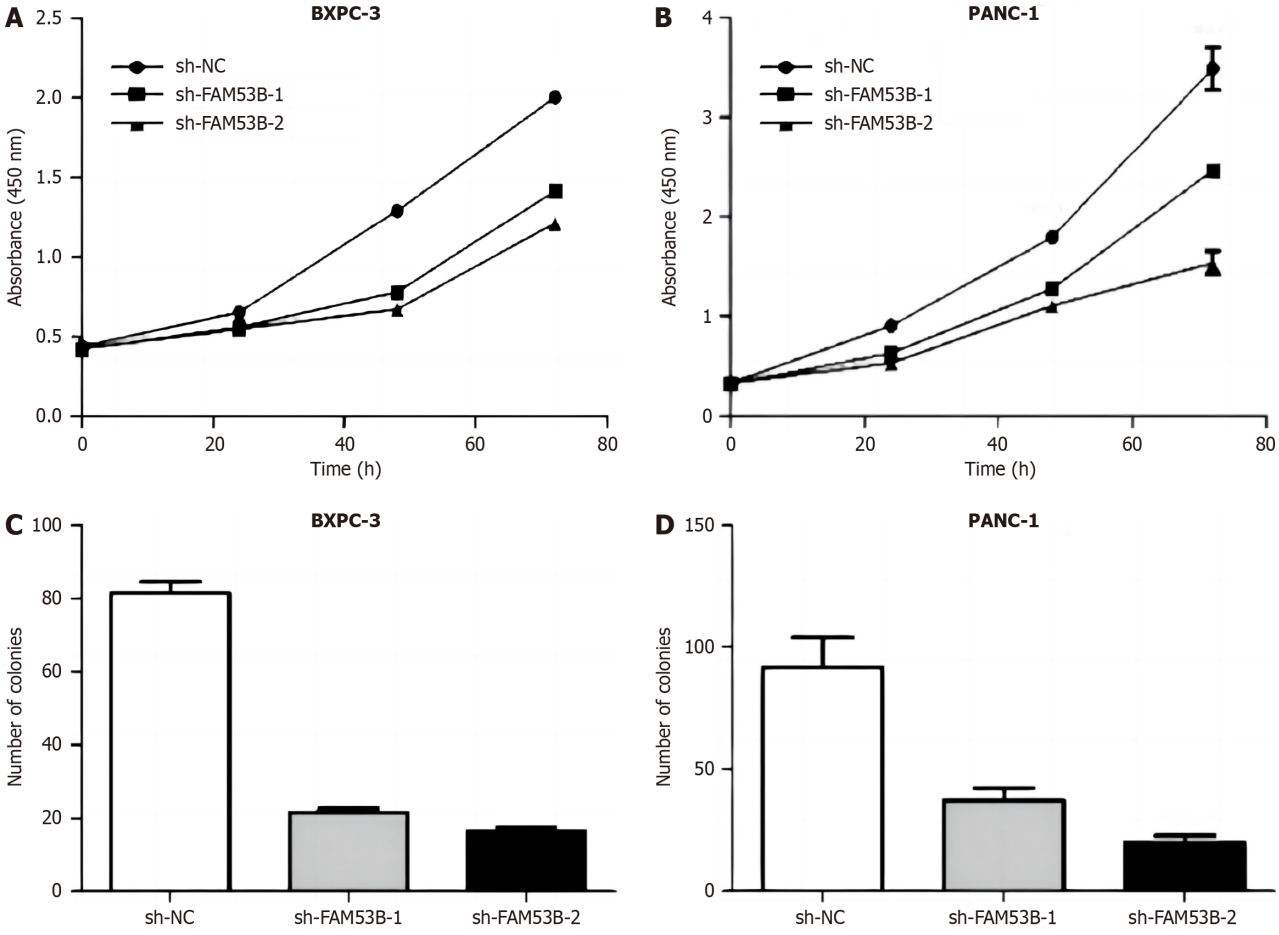
By using CCK-8 and single-cell cloning procedures, researchers were able to investigate the effects of sh-NC, sh-FAM53B-1 TAMs, and sh-FAM53B-2 TAMs on pancreatic cancer cell proliferation. There was no significant alteration in the growth of BXPC-3 and PANC-1 cells when co-cultured with sh-NC, sh-FAM53B-1 or sh-FAM53B-2 TAMs (Figure 5A-F). In comparison to the sh-NC-TAMs group, FAM53B knockdown macrophages induced apoptosis of BXPC-3 and PANC-1 cells (P < 0.05) (Figure 5G-I).

M2 polarized macrophages promote PDAC cell development and invasion

Transwell and matrix gel assays were performed to investigate how down-regulated FAM53B macrophages affect pancreatic cancer cell migration and invasion. A significant reduction in BXPC-3 and PANC-1 cell migration and invasion capacity was observed when treated with sh-FAM53B-1 TAMs and sh-FAM53B-2 TAMs compared to sh-NC TAMs (P < 0.001), as demonstrated in Figure 6A-D. In addition, we performed western blotting to examine the expression of epithelial-mesenchymal-transition-associated proteins (N-cadherin, vimentin, and E-cadherin) in pancreatic cancer cells following Fam53B-knocked macrophage intervention. There was a significant decrease in N-cadherin and vimentin expression in BXPC-3 and PANC-1 cells compared to the sh-NC TAMs group. N-cadherin and vimentin expression was down-regulated by both sh-FAM53B-1 TAMs and sh-FAM53B-2 TAMs. There was a considerable increase in E-cadherin expression (P < 0.05), as shown in Figure 6E-H.

FAM53B induces M2 polarization by activating the Src/FAK/p-Akt/IL-1β signaling pathway

We next tested the function of the Src/FAK/p-Akt/IL-1β signaling pathway in macrophages lacking FAM53B. Compared to sh-NC TAMs, Src, FAK, p-AKT, and IL-1β expression was reduced in sh-FAM53B-1TAMs and sh-FAM53B-2 TAMs in BXPC-3-induced TAMs. AKT expression was significantly elevated (P < 0.05), while P-ERK expression did not exhibit any significant changes (Figure 7A and B). Similarly, after knocking down FAM53B in PANC-1-induced TAMs, the expressions of Src, FAK, P-Akt, and IL-1β in sh-FAM53B-1 TAMs and sh-FAM53B-2TAMS were considerably lower than in the sh-NC TAMs group (P < 0.05). There was a decrease in the expression of P-ERK, while there was a rise in the expression of AKT (P < 0.05) (Figure 7C and D).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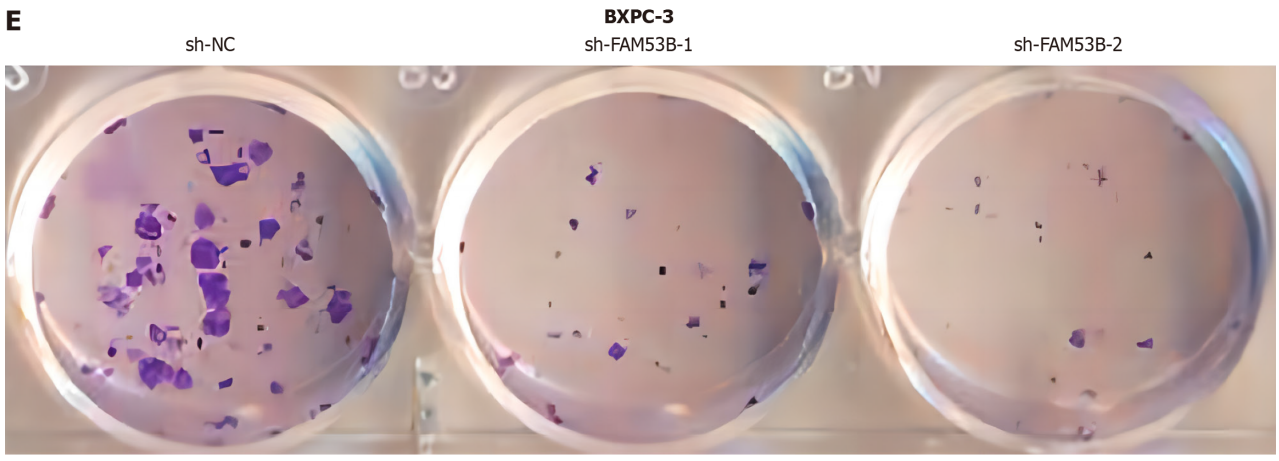
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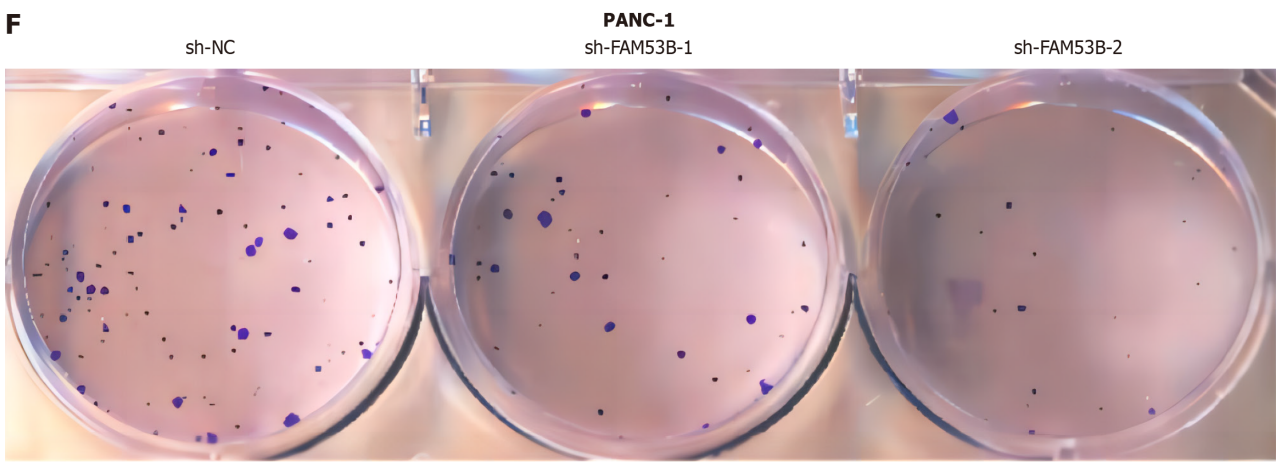
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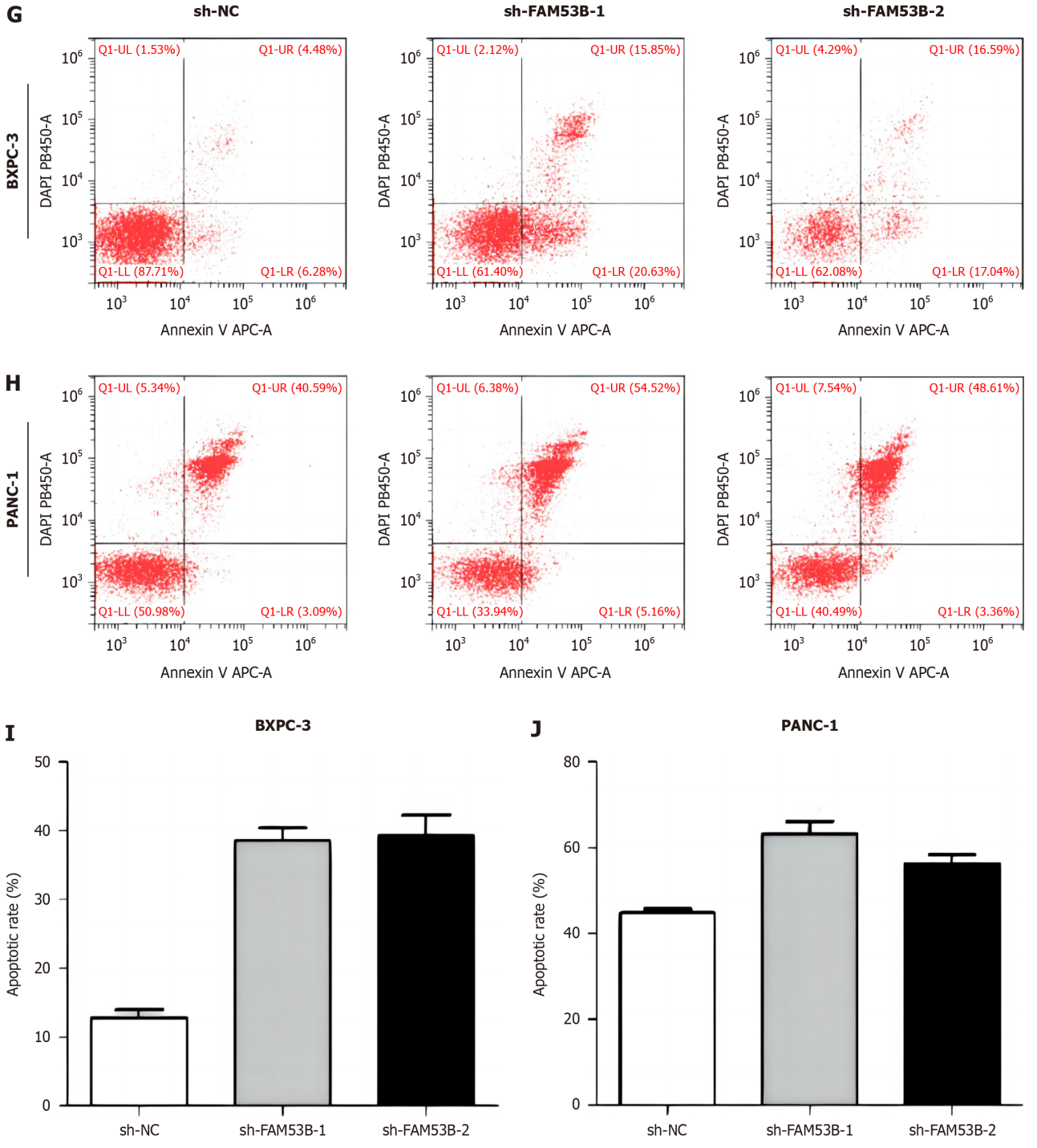


Figure 2 FAM53B knockout inhibited pancreatic ductal adenocarcinoma cell proliferation. A and B: Cell counting kit-8; C-F: Colony formation; G-J: Flow cytometry.

FAM53B induces PDAC metastasis

To develop a model of metastasis, we injected FAM53B knockdown PANC-1 cells into the spleens of nude mice. Within the sh-NC group, every mouse showed clear liver metastasis, which indicates that the metastasis rate was one hundred percent. This was manifested on the liver surface as grayish-yellow spots of varying diameters that were dispersed and partially fused into chunks. We determined that all of the tissue seen in the spleen was tumor tissue, as shown in Figure 8A. A considerably reduced metastatic rate of 28.6% was seen in the sh-FAM53B gene deletion group compared to the sh-NC group, and liver metastasis occurred in 2 out of 7 nude mice. There were still obvious signs of normal spleen tissue. To verify the findings, we performed hepatic encephalopathy staining on tissue sections taken from the liver and the spleen (Figure 8B).

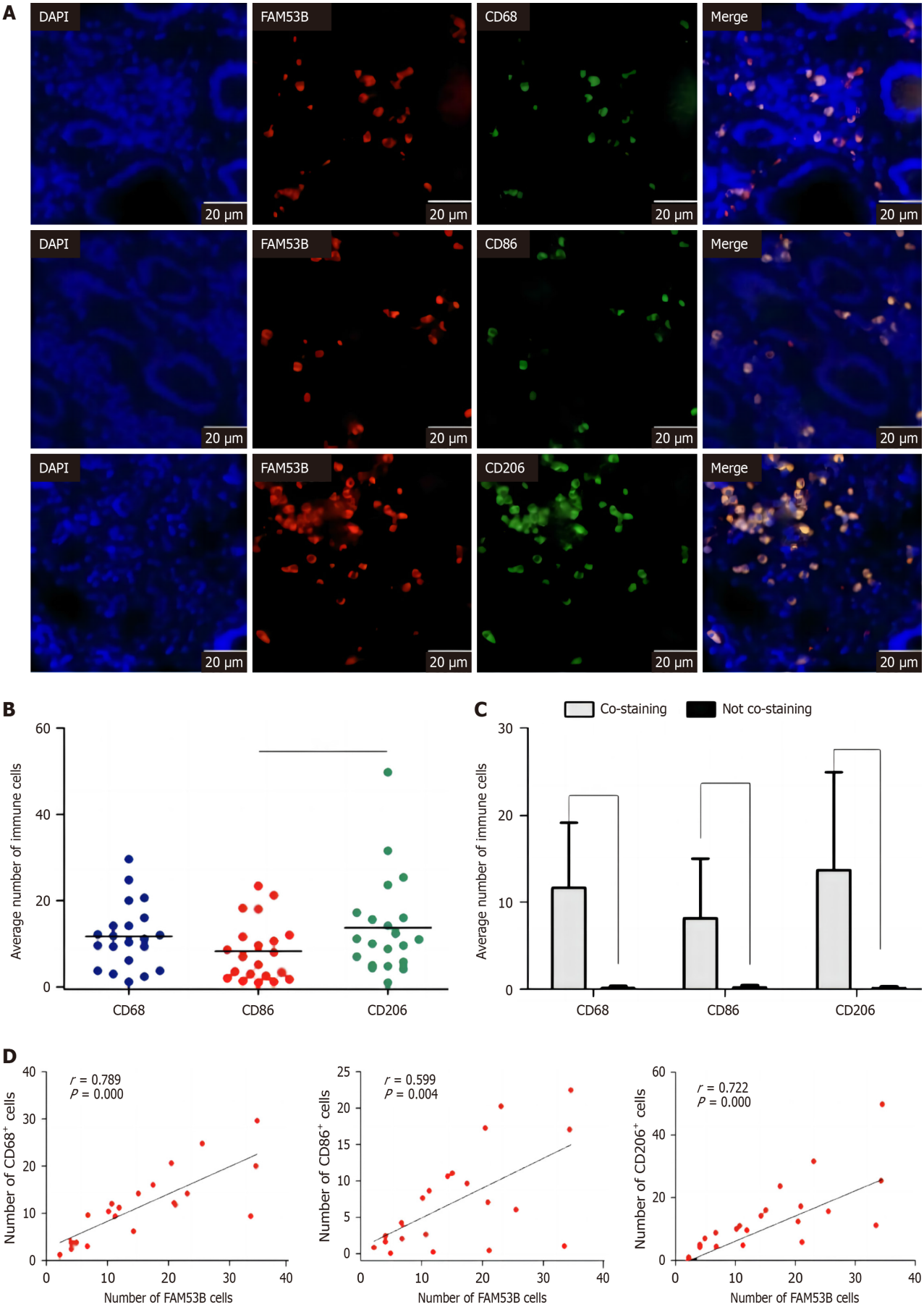
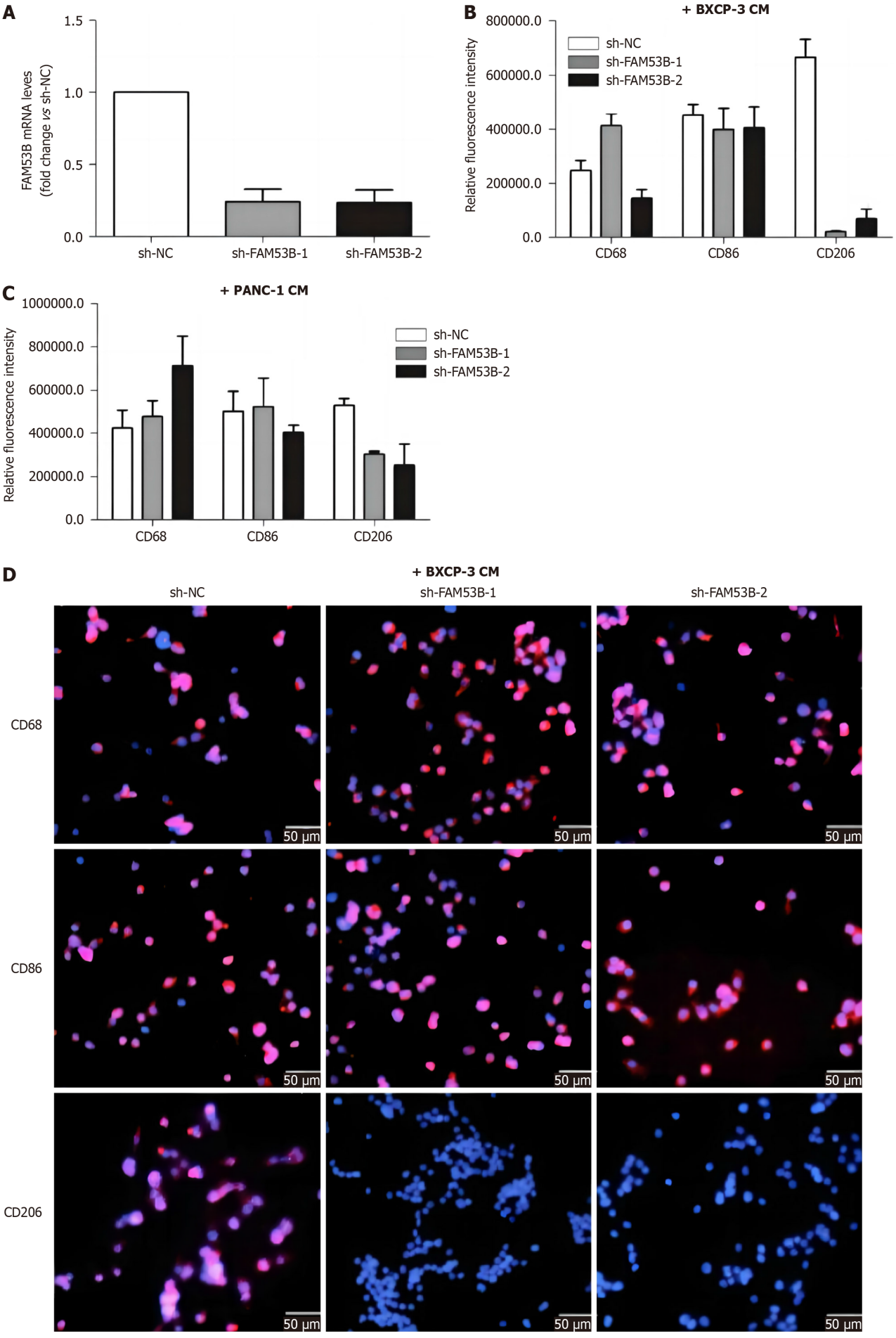


Figure 3 FAM53B expression associated with M2 polarization. A-C: Tissue immunohistochemical test; D: FAM53B (+) associated with M2.


A
FAM53B mRNA leves (fold change vs sh-NC)
1.5
1.0
0.5
0.0
sh-NC
sh-FAM53B-1
sh-FAM53B-2
B
+ BXCP-3 CM
Relative fluorescence intensity
800000.0
600000.0
400000.0
200000.0
0.0
sh-NC
sh-FAM53B-1
sh-FAM53B-2
CD68
CD86
CD206
C
+ PANC-1 CM
Relative fluorescence intensity
1000000.0
800000.0
600000.0
400000.0
200000.0
0.0
sh-NC
sh-FAM53B-1
sh-FAM53B-2
CD68
CD86
CD206
D
+ BXCP-3 CM
sh-NC
sh-FAM53B-1
sh-FAM53B-2
CD68
CD86
CD206
50 μm

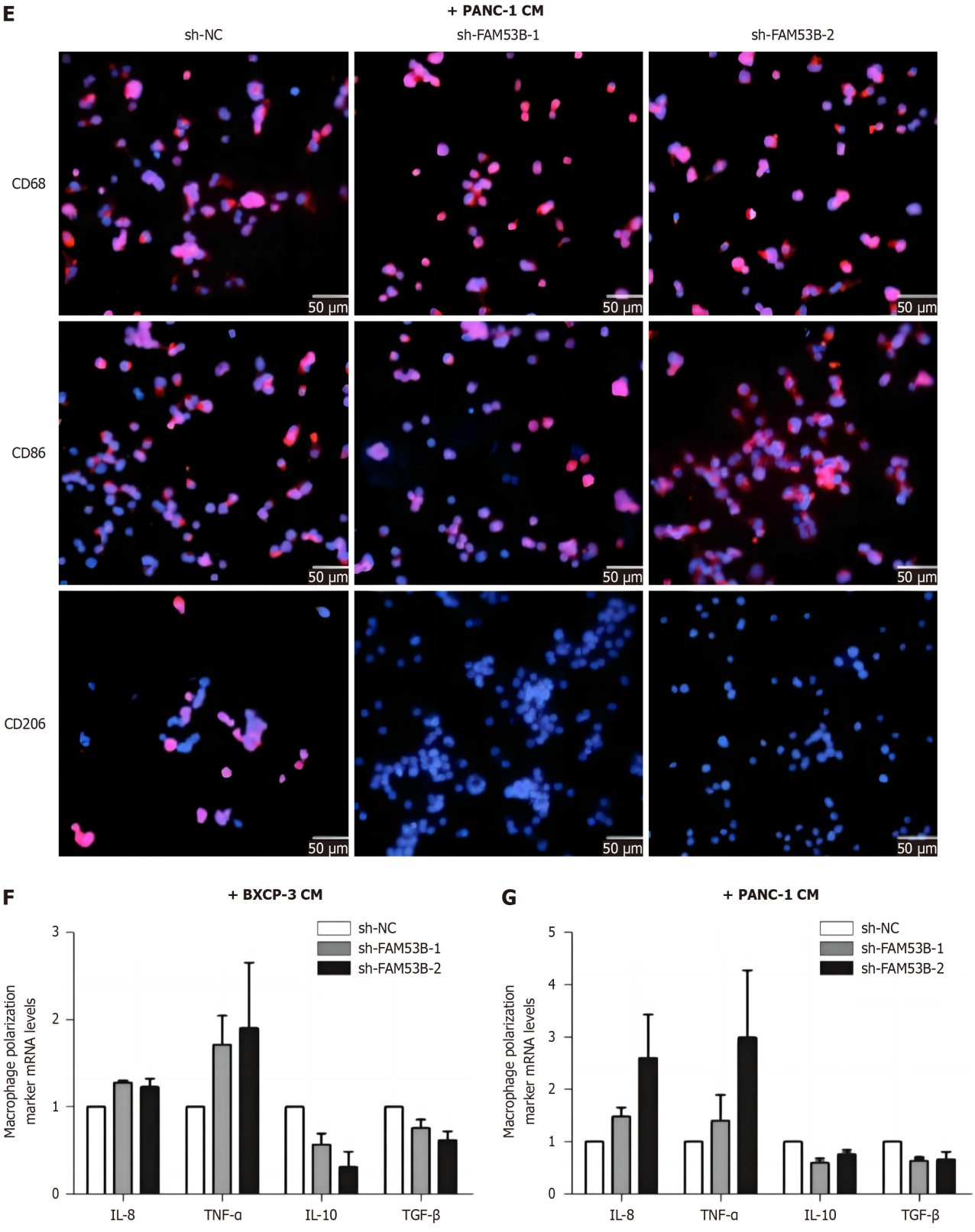
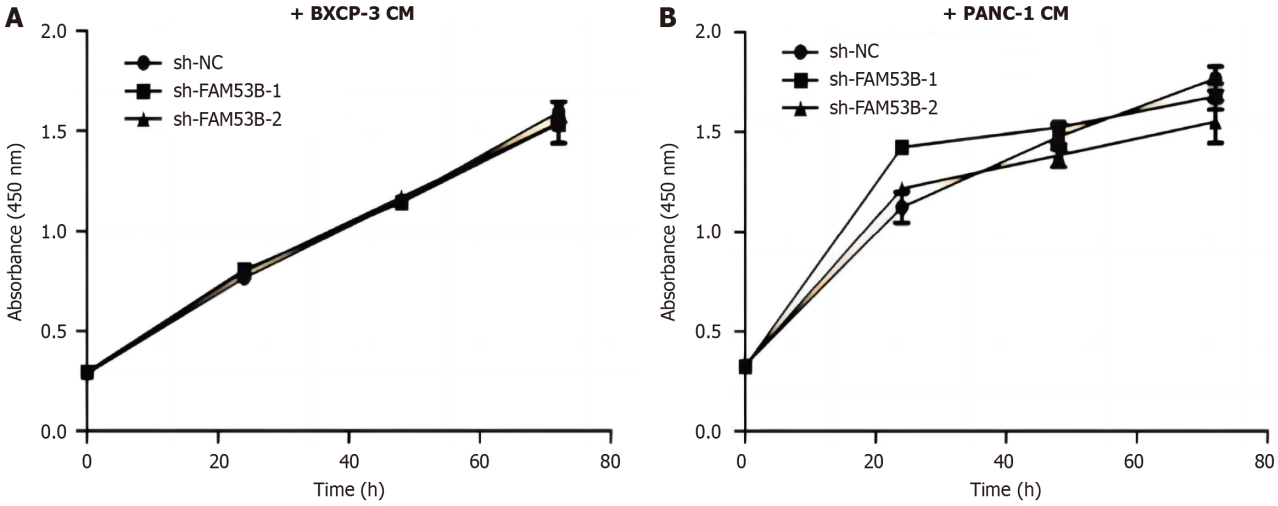
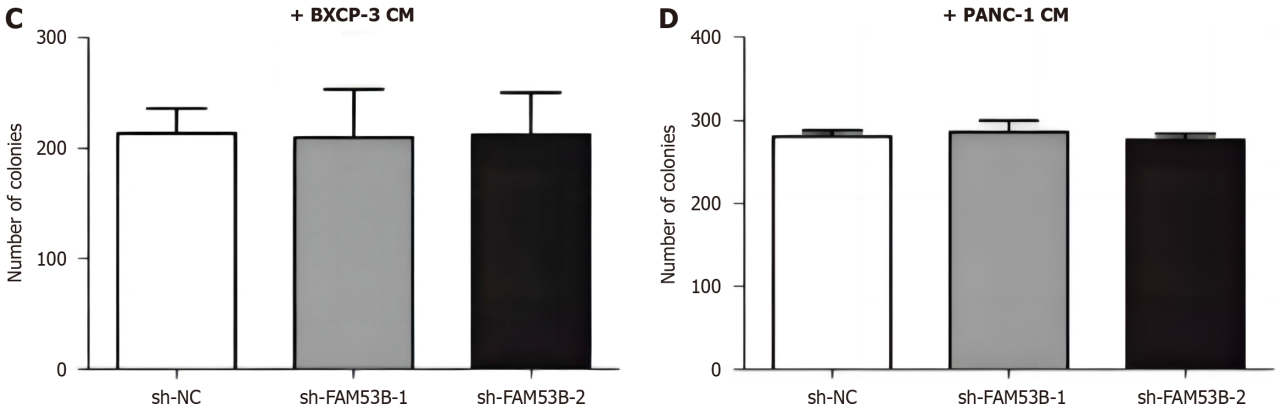


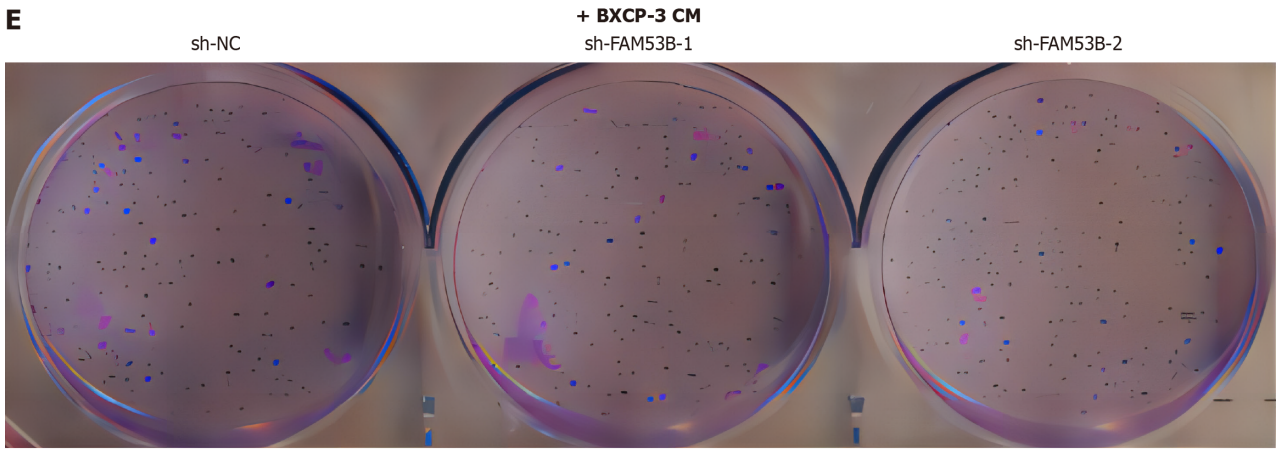
Figure 4 FAM53B induces the polarization of M2. A-E: Tumor-associated macrophages induced; F and G: Expression of M1 macrophage markers.

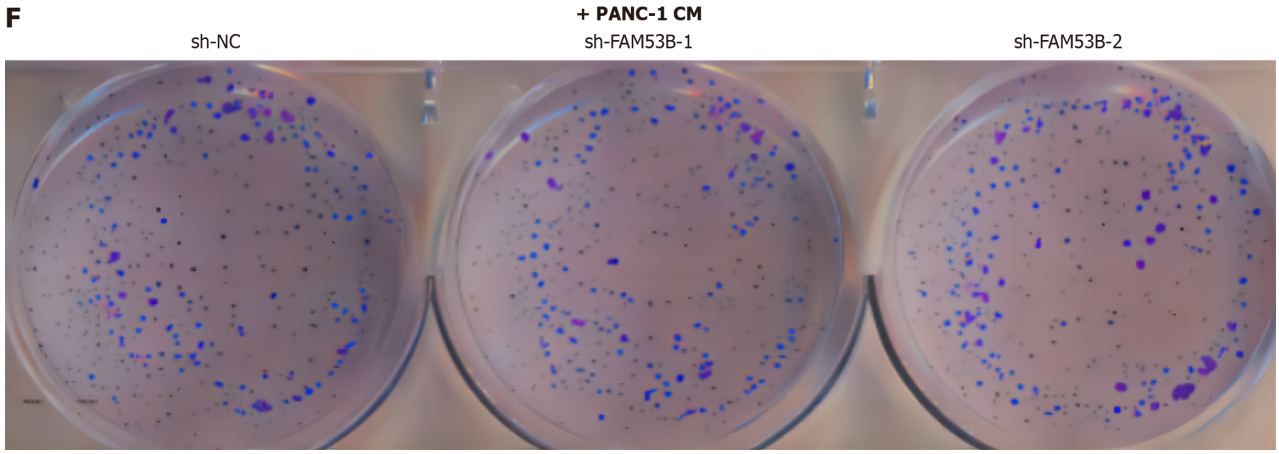
DISCUSSION

As researchers continue to understand the various biological behaviors of cancer and various cancer treatment strategies [31], the survival rate for many cancer types and quality of life of patients have been significantly improved[31-34]. However, the prognosis of PDAC is not optimistic due to its early systemic spread, high vascular invasiveness, difficulty in early diagnosis, and resistance to chemotherapy[35-37]. We focused on exploring whether FAM53B knockdown can change the secretion of tumor cytokines, whether the secreted cytokines can affect macrophage polarization, and whether the macrophages with altered polarization status can affect PDAC cell migration[38-40]. We also found that the activation


A
+ BXCP-3 CM
sh-NC
sh-FAM53B-1
sh-FAM53B-2
Absorbance (450 nm)
Time (h)
B
+ PANC-1 CM
sh-NC
sh-FAM53B-1
sh-FAM53B-2
Absorbance (450 nm)
Time (h)


C
+ BXCP-3 CM
Number of colonies
sh-NC
sh-FAM53B-1
sh-FAM53B-2
D
+ PANC-1 CM
Number of colonies
sh-NC
sh-FAM53B-1
sh-FAM53B-2


E
+ BXCP-3 CM
sh-NC
sh-FAM53B-1
sh-FAM53B-2


F
+ PANC-1 CM
sh-NC
sh-FAM53B-1
sh-FAM53B-2

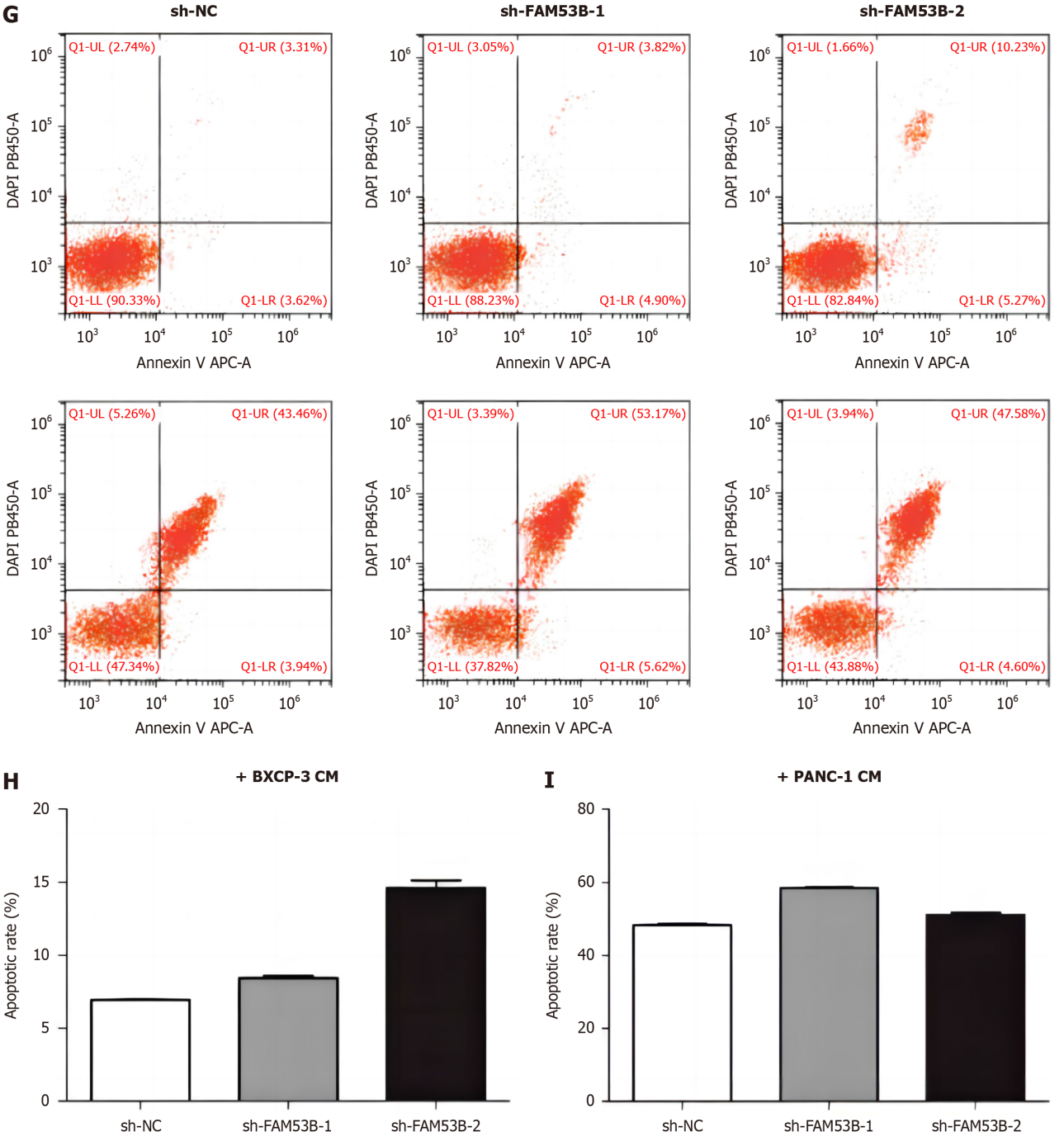
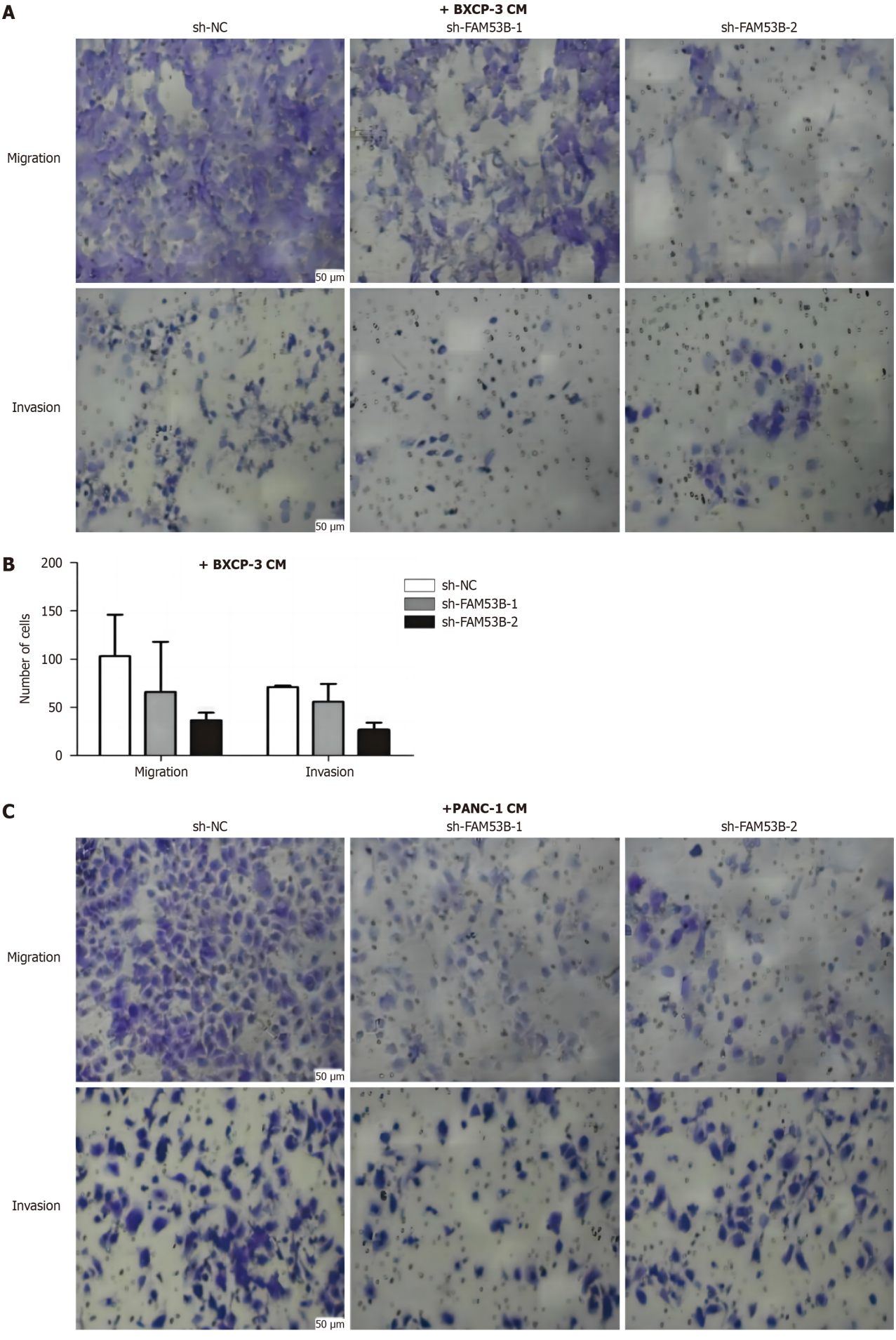


Figure 5 M2 polarized macrophages have no effect on proliferation. A and B: Cell counting kit-8 experiment; C-F: Single cell cloning experiment; G-I: Flow cytometry experiment.

of the macrophage M2 phenotype can induce stronger migration and invasion in the PDAC metastatic AsPC-1 cell line [41]. TME exosomes transmit messages from one cell to another and reprogram recipient cells, and they are the most important communication medium between various cell types. Tumor cell-derived exosomes can regulate the PTEN/PI3K/AKT pathway, SOCS1/STAT6 pathway and integrin signal STAT1 through the miRNA they carry and further regulate the polarization of macrophages toward M2, creating a microenvironment conducive to tumor survival[42].

Our study looked into the part FAM53B plays in PDAC, specifically how it might affect the metastasis of macrophage M2 polarization. Researchers[43] found that FAM53B was significantly increased in PDAC. They also found that blocking this gene could stop cancer from spreading. We did not find that FAM53B-mediated M2 macrophage polarization directly affected cancer cell growth or death, but that it plays a key role in the metastasis process. This result suggests that FAM53B may be involved in metastasis through other pathways without directly affecting proliferation. Our results support the idea that FAM53B is linked to M2 macrophage polarization, which suggests that it plays a major role in controlling the environment around tumors. However, our study requires more in-depth mechanistic studies to elucidate the exact mode of action of FAM53B during the transfer process. We speculate that FAM53B may play a role by influencing macrophage activity, cell migration, and the TME, but the specific mechanism needs to be further explored.


A
+ BXCP-3 CM
sh-NC
sh-FAM53B-1
sh-FAM53B-2
Migration
Invasion
50 μm
50 μm
B
+ BXCP-3 CM
Number of cells
200
150
100
50
0
sh-NC
sh-FAM53B-1
sh-FAM53B-2
Migration
Invasion
C
+PANC-1 CM
sh-NC
sh-FAM53B-1
sh-FAM53B-2
Migration
Invasion
50 μm
50 μm

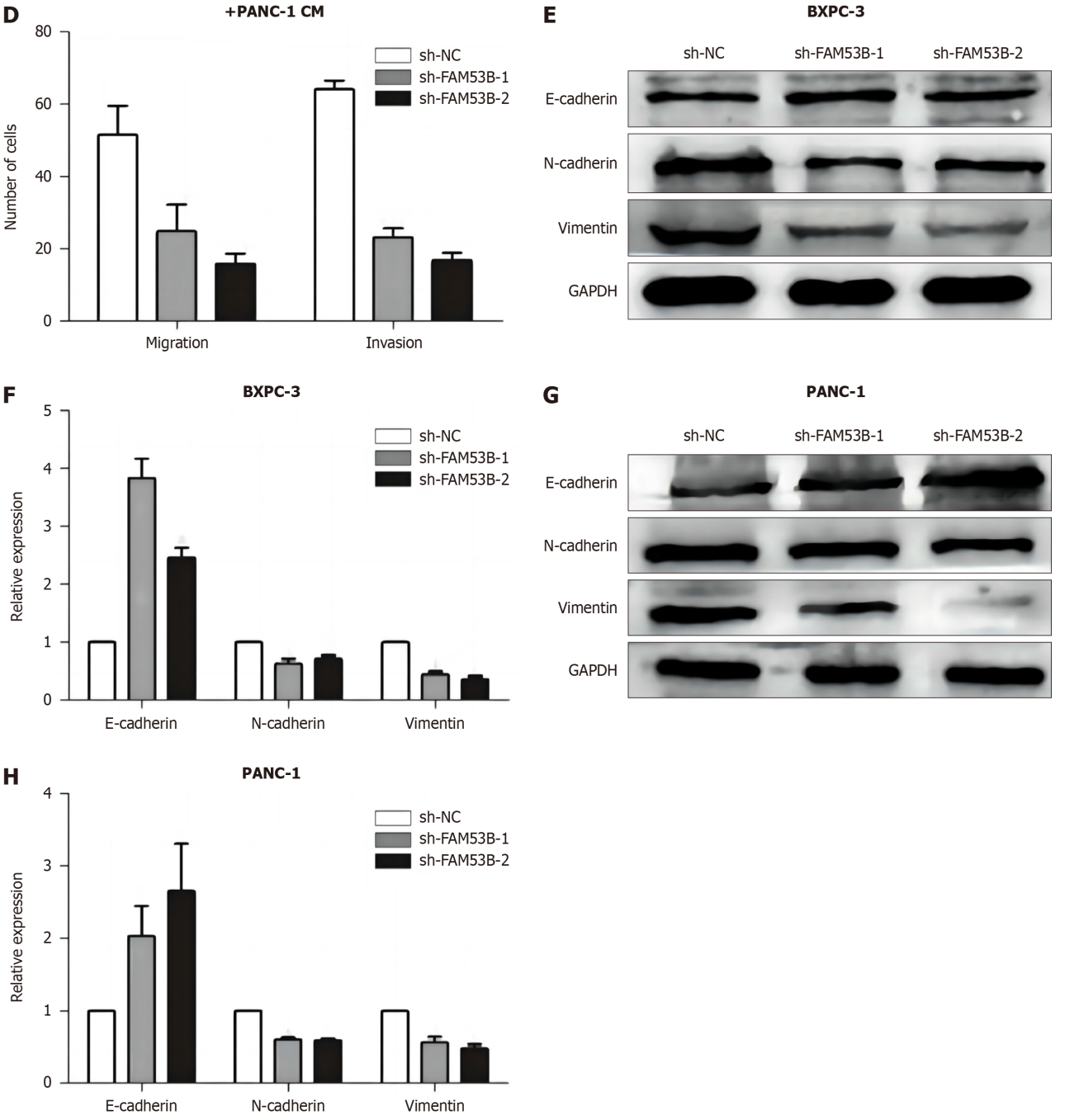


Figure 6 M2 polarized macrophages promote pancreatic ductal adenocarcinoma cell development and invasion. A-D: Transwell experiment; E-H: Western blotting experiment.

Our study shows that although FAM53B-mediated polarization of M2 macrophages does not have a direct effect on PDAC cell proliferation and apoptosis, this does not exclude the possibility of its involvement in the metastasis process. FAM53B may primarily affect macrophage activity and polarization, thereby regulating their function in the TME, but not directly affecting the proliferation and apoptosis of pancreatic cancer cells. In addition, this difference may be related to the specificity of cell signaling pathways. As a regulatory factor, FAM53B may be more likely to take part in certain signaling pathways or control certain cell functions without having an effect on cell proliferation and apoptosis. However, it is very important in controlling the metastasis process.

At the same time, exosomes from M2 macrophages can contain certain miRNAs. This specific cargo can make PDAC cells less sensitive to gemcitabine once it gets to the tumor cells, which helps them become resistant to treatment. The study[44] showed that M2 macrophage-derived exosomes re-educate tumor cells by secreting miR-21, greatly reducing the drug sensitivity of tumor cells to cisplatin. In ovarian cancer, M2-type macrophage-derived exosomes modify the TME by regulating the Treg/Th17 cell ratio, resulting in an ovarian cancer TME immune imbalance, thereby promoting tumor cell immune escape. In glioblastoma, M2-type macrophage-derived exosomes can inhibit the proliferation of CD8+ T cells, reduce cytotoxic activity and IFN-γ levels, and promote immune escape of glioma cells by inhibiting PEG3 expression.

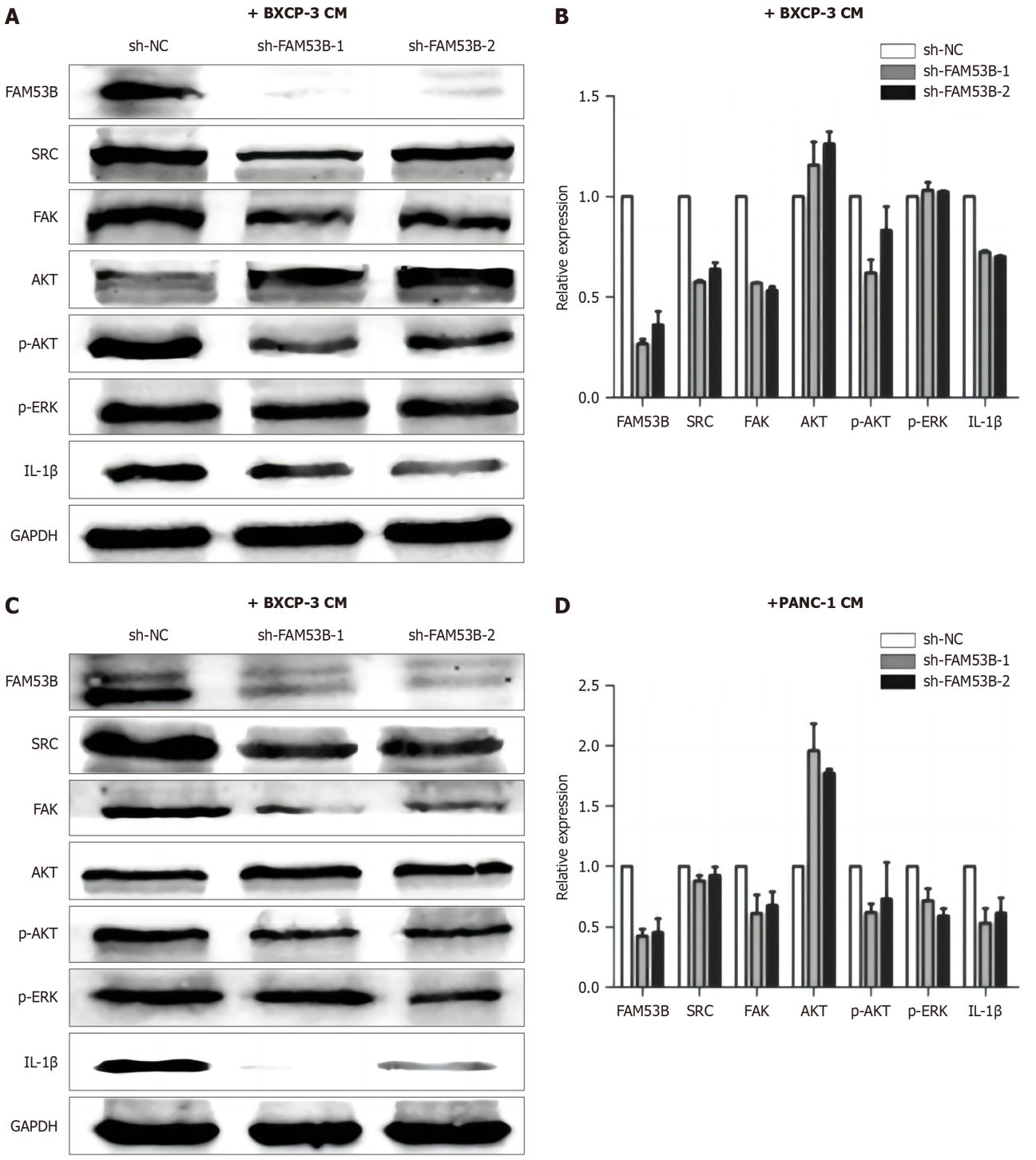


Figure 7 FAM53B induces M2 polarization by activating the Src/FAK/p-Akt/IL-1β signaling pathway. A and B: FAM53B knockout in induced pancreatic ductal adenocarcinoma tumor-associated macrophages (TAMs); C and D: FAM53B knockout in PANC-1-induced pancreatic ductal adenocarcinoma TAMs.

In this study, we found that FAM53B expression was elevated in PDAC cells. This result is contrary to previous studies that found that FAM53B inhibited liver cancer, colorectal cancer, and small-cell lung cancer. FAM53B expression is only associated with the prognosis of PDAC among common tumors of the digestive system.

CONCLUSION

This study revealed the important role of FAM53B in regulating macrophage M2 polarization and promoting the metastasis of PDAC. The experimental results showed that FAM53B promoted the transformation of macrophages into M2 types by influencing specific signaling pathways and played a key regulatory role in PDAC metastasis. Further

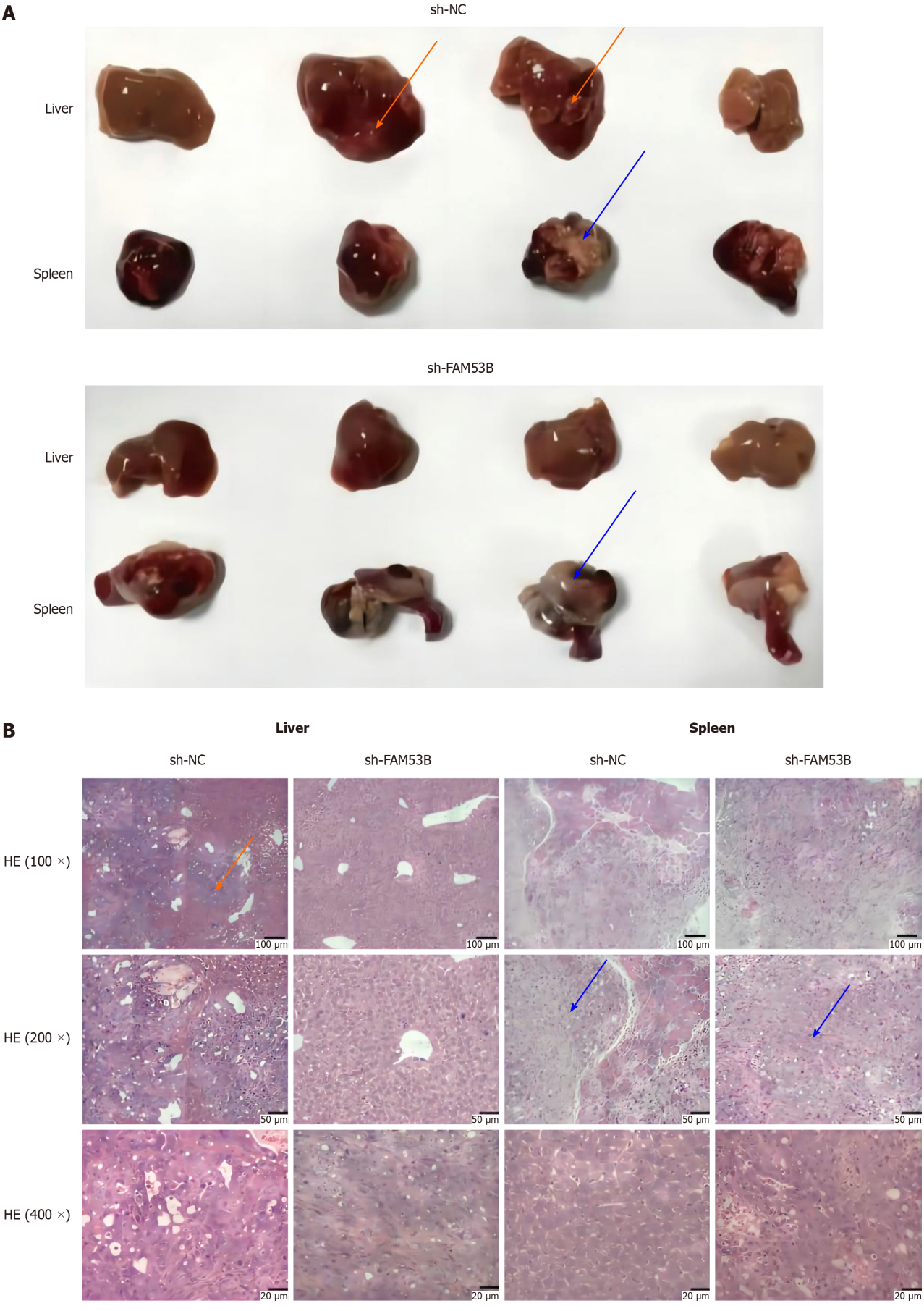


Figure 8 FAM53B induces pancreatic ductal adenocarcinoma metastasis. A: Metastasis model was established in the spleen of nude mice; B: Hepatic encephalopathy staining. HE: Hepatic encephalopathy.

molecular mechanism studies showed that FAM53B may accelerate the PDAC cell metastasis and invasion by regulating several key genes and cytokines. This finding not only deepens the understanding of PDAC development and metastasis but also provides a potential target for future research and treatment.

ARTICLE HIGHLIGHTS

Research background

Our study investigates the role of FAM53B in regulating macrophage M2 polarization and its potential mechanisms in promoting pancreatic ductal adenocarcinoma (PDAC) metastasis.

Research motivation

The motivation for this study stems from a deep interest in the mechanisms of PDAC development and the critical role of macrophages in the tumor microenvironment. Given the highly aggressive nature of PDAC and its propensity to metastasize, we focused on exploring the underlying molecular mechanisms, with a particular focus on FAM53B's role in regulating macrophage M2 polarization. By further investigating the function of FAM53B, we expect to reveal its specific regulatory mechanisms during PDAC metastasis, providing a new perspective for an in-depth understanding of the development of this cancer.

Research objectives

To explore the role of FAM53B in the regulation of macrophage M2 polarization, further study the molecular mechanism that may be involved in promoting PDAC metastasis, and reveal the influence of FAM53B on the M2 polarization of macrophages, as well as the specific regulatory mechanism in PDAC metastasis.

Research methods

Various methods were used to investigate the role of FAM53B in regulating macrophage M2 polarization and promoting PDAC metastasis. A macrophage model regulated by FAM53B expression level was constructed by cell culture and gene knockout techniques. Subsequently, immunocytochemistry and coimmunoprecipitation techniques were used to detect M2 macrophage marker expression and the interaction between FAM53B and related proteins. In animal models, the effect of FAM53B on PDAC metastasis was evaluated by transplanting PDAC cell lines and observing tumor growth and metastasis. At the molecular level, transcriptomic and proteomic methods were used to analyze the changes in the FAM53B-regulated signaling pathway and related gene expression.

Research results

Our research showed that there was a significant increase in FAM53B levels in PDAC tissues, which was linked to the tumors' cancerous features. Experimental findings indicated that FAM53B can enhance macrophage M2 polarization, leading to an increased release of anti-inflammatory factors. The results from the mouse model further supported the role of FAM53B in PDAC metastasis, as blocking FAM53B stopped the tumor from spreading and invading other tissues.

Research conclusions

FAM53B promotes PDAC metastasis by regulating macrophage M2 polarization. This discovery could lead to new ways to treat PDAC.

Research perspectives

The results of this study are expected to provide a new molecular mechanism for in-depth understanding of the development and metastasis of PDAC and provide innovative ideas for the development of relevant therapeutic strategies. By revealing the key role of FAM53B in the regulation of macrophage M2 polarization, it can provide a basis for the design of targeted interventions. Further research could focus on developing FAM53B inhibitors or related therapeutic strategies to reduce the aggressiveness of PDAC and improve therapeutic efficacy. In addition, the interrelationships between FAM53B and other signaling pathways will be explored in depth to provide a more comprehensive understanding of the complex regulatory networks in the tumor microenvironment. This will provide important guidance for future clinical transformation and is expected to bring new breakthroughs in the treatment of PDAC patients.

FOOTNOTES

Author contributions: Pei XZ wrote the manuscript; Cai M, Jiang DW, Chen SH, Wang QQ and Lu HM collected the data; Lu YF conceived and guided the study. All authors reviewed, edited, and approved the final manuscript and critically revised it for important intellectual content, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the West China Hospital of Sichuan University.

Conflict-of-interest statement: The authors declare having no conflicts of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at [email: luyifan927@163.com]. Participants gave informed consent for data sharing.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

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