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MiR-455-3p reduces apoptosis and alleviates degeneration of chondrocyte through regulating PI3K/AKT pathway

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Abstract

Aims: This study aimed to explore the functions of miR-455-3p, PTEN, and PI3K/AKT pathway in osteoarthritis.

Materials and Methods: We used the human bone marrow stem cell (BMSC), healthy chondrocytes, osteoarthritis chondrocytes (OA), and the IL-1β/TNF-α-treated chondrocyte model to explore the relationship between miR-455-3p and PTEN. Mimic or inhibitor was used to transfect chondrocytes to determine whether miR-455-3p can regulate PTEN and influence COL2A1 and MMP13. Apoptosis was detected by flow cytometry. A luciferase report was applied to verify the targeted binding. KO mice was applied to investigate PTEN and pAKT expression and the effect on chondrocytes *in vivo*.

Key findings: MiR-455-3p and PTEN were reverse in chondrogenesis and healthy cartilage versus OA cartilage. Similar trends were noted in IL-1β model. PTEN and MMP13 decreased and COL2A1 increased after overexpressing miR-455-3p, whereas the inhibition showed opposite results. Flow cytometry showed that miR-455-3p could reduce the apoptosis of chondrocytes. The results of luciferase revealed that miR-455-3p could affect fluorescence activity of PTEN by targeting its 3'-UTR. Finally, we found a marked increased in the expression of PTEN in KO mice relative to WT mice, while pAKT levels decreased.

Significance: It can be supported that miR-455-3p can reduce the apoptosis of chondrocytes and alleviate OA through regulating PI3K/AKT pathway, which may be expected to be a target for the treatment of osteoarthritis.

Key Words: osteoarthritis; miR-455-3p; PTEN; PI3K/AKT

1. Introduction：

Osteoarthritis (OA) is one of the most common age-related, degenerative total joint diseases, characterized by cartilage degeneration, synovitis, osteophyte formation, and subchondral bone remodeling, which seriously affects quality of life in the elderly [1, 2]. However, the exact molecular mechanism of osteoarthritis remains unknown. It is widely accepted that osteoarthritis is related to age, obesity, genetic predisposition, long-term mechanical load, trauma, and other factors [3-6]. Growing evidence suggests that osteoarthritis is associated with low-grade inflammation and cholesterol metabolism [1, 7]. Until now, osteoarthritis has been a complicated, multifactorial disease with limited treatment options.

The PI3K/AKT pathway has been found to be vital in promoting tumor cell survival and growth [8, 9]. This pathway also plays a role in regulating key aspects of cellular metabolism, proliferation, apoptosis, and other physiological processes [8-11]. AKT phosphorylation marks the activation of the PI3K/AKT pathway, while PTEN, which facilitates phosphatidylinositol (PI) (3,4,5) P3 dephosphorylation, is an inhibitor of this pathway [10, 12, 13]. Although the role of this pathway in osteoarthritis is not fully understood, Yu X et al. found that activation of PI3K/AKT

resulted in angiogenesis *in vitro*, which may promote the development of OA [14]. Lin CX et al. reported that blocking PI3K/AKT pathway can suppress subchondral osteosclerosis as well as reduce post-traumatic osteoarthritis [15]. However, Song Y et al. discovered FAM3A protects chondrocytes through activating the PI3K/AKT pathway [16]. Thus far, there has been extensive debate about the impact of the PI3K/AKT signaling pathway in osteoarthritis.

MicroRNAs (miRNAs) are a class of non-coding, single-stranded RNAs encoded by endogenous genes that are approximately 22 nucleotides long. They regulate gene expression by promoting degradation of the corresponding mRNAs, or by inhibiting translation by targeting the 3'-UTR of mRNA [17]. In recent years, an increasing number of studies have shown that miRNAs are important regulators of cartilage development and OA development. Our previous work has shown that miR-455-3p was upregulated at an average of 2.97 fold in human adipose stem cell chondrogenesis [18]. In addition, we found that PI3K/AKT pathway was activated in BMSCs after transfecting miR-455-3p agomir [19]. On the basis of these findings, we predicted miR-455-3p could specifically target PTEN using the online Target Scan software. Therefore, we were determined to explore whether miR-455-3p could regulate PTEN thereby playing a protective role in chondrogenic differentiation and chondrocyte degeneration.

2. Materials and methods

The ethics committee of the first affiliated hospital of Sun Yat-Sen University

approved this project (IRB: 2011011) , and all volunteers signed informed consent.

2.1 BMSCs isolation, culture, and chondrogenic induction

BMSCs were provided by four volunteers (4 males, mean±SD age: 20.1±3.3 years) who were undergoing fracture reduction surgery because of femoral fractures due to trauma, and who did not have malignant tumors or metabolic disease. The bone marrow aspirate was added to Ficoll-Paque™ PLUS (GE Healthcare,Uppsala, Sweden), and centrifuged for 18 min at 1000 g. The interfacial mononuclear cells were collected and were cultured in α-MEM basic (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco, Australia) and 1% penicillin and streptomycin (PS, Gibco Life Technology). The medium was changed every 3 days[20].

BMSCs were collected and resuspended in incomplete chondrogenic medium at $2x10^7$ cells/ml. Droplets (12.5 ml) were placed in each well of a 24-well plate. BMSCs droplets were concentrated at 37℃ for 90min, followed by the addition of 500 ml complete chondrogenic medium (Cyagen, USA). After 24 h, the cell droplets coalesced and became spherical. Complete medium was changed every 2 days. Samples of 7, 21, and 35 days were collected for immunohistochemistry and alcian blue staining. And samples of 0, 7, 14, 21, and 35 days were collected for RNA extraction and qPCR verification[19].

2.2 Chondrocyte cell isolation and culture

Healthy chondrocyte cell samples were provided by five volunteers (3 males, 2 females, 34.1±2.3 years) who were undergoing total hip arthroplasty (THA) because

of femoral fractures due to trauma, and who did not have OA or rheumatoid arthritis. OA samples were provided by five volunteers (4 females,1 male, 62.6±5.5 years) who were undergoing total hip arthroplasty (THA) because of osteoarthritis. The cartilages were cut to a size of 1 mm^3 and then digested by 4 mg/mL protease for 1.5 hours and 0.25 mg/mL collagenase P for 6-8 hours. Chondrocytes were cultured in MEM/F-12 (Gibco) and supplemented with 5% FBS (Gibco, Brazil) and 1% PS (Gibco Life Technology) [19].

2.3 Inflammatory models of IL-1β/TNF-α and transfection

Chondrocytes were maintained in 6-well plates and reached 60% to 70% density following stimulating with 5ng/mL IL-1β (Peprotech, USA) or 20ng/mL TNF-α (Peprotech, USA). After 48 hours in culture, samples were collected for real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR), or western blotting after 72 hours.

Mimic (50nmol/L) or inhibitor (100 nmol/L) (RiboBio, Guangzhou, China) was used in transfection. At the same time, we used mimic-NC, inhibitor-NC as controls. After 48 hours in culture, samples were collected for real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR), or western blotting after 72 hours.

2.4 qRT-PCR, western blot, and immunohistochemistry

Total RNA was extracted by miRNeasy Mini Kit (Qiagen, Venlo, Netherlands) , and cDNA was synthesized using PrimeScript™ RT Master Mix (Takara, Shiga,

Japan). qRT-PCR was performed on a CFX96 system (Bio-Rad).

Proteins were extracted using RIPA Lysis Buffer (CWBIO, China), and western blotting was performed as previously described [20]. The primary antibodies used (1:1000 dilution) are as follows [antibody, manufacturer, serial no.]: anti-PTEN, Affinity Biosciences, AF6351; anti-AKT, Proteintech Group, 10176-2-AP; anti-pAKT, Proteintech Group, 66444-1-Ig; anti-MMP13, Proteintech Group, 18165-1-AP; anti-COL2A1, Abcam, EPR12268; anti-GAPDH, Cell Signaling Technology, 14C10.

Immunohistochemical analysis was described previously [19]. For antigen retrieval, sections in 0.1% EDTA were incubated at moderate heat in a microwave for 10 minutes. Section staining was treated with 3% goat serum for 1 hour and then incubated with antibodies including anti-PTEN, anti-pAKT， anti-MMP13, and anti-COL2A1.

2.5 Flow cytometry analysis

Chondrocytes were incubated with Annexin V (FITC) and Propidium Iodide (FL3) (Meilunbio, China) for 15 min. CytoFLEX S (Beckman Coulter, USA) was used for flow cytometry analysis. The results were analyzed using the FlowJo_V10 software.

2.6 Animals and in situ hybridization

The mmu-miR-455-3p global knockout mice were generated by a transcription activator-like effector nuclease (TALEN) system–refer to previous study for detailed

protocol[19]. DNA sequencing analysis was performed after PCR. The wild-type (miR-455-3p+/+), heterozygous (miR-455-3p+/−), and homozygous (miR-455-3p−/−) mice were obtained through the mating of heterozygous male and female mice. In this study, we compared the wild-type mice and homozygous mice. The knee joint of miR-455-3p deletion mice and wildtype mice were harvested at 6 months and fixed in paraformaldehyde(Sigma-Aldrich,St.Louis,MO,United States) at 4 ℃ overnight. Tissues were subsequently dehydrated with a graded series of ethanol, embedded in paraffin, and cut into 5-μm-thick sections. Sections were subjected to in situ hybridization analysis using the miR-455-3p-specific probe (Servicebio, Wuhan, China), or treated with PBS as negative control.

2.7 Luciferase reporter assay

The PTEN 3'-UTR was amplified by PCR using the following primers: forward 5'-GATCGCTCGAGTTTCAATCATAATACCTGC-3' and reverse 5'-GCGGCCAGCGGCCGCT TCTGCCAAATACTACAGTTA-3'. The seed sequences were mutated using the following primers: forward 5'-ACTGTGTTTGTGAGCCCCTCCTTCCCACCGGAAGTCCAGCTTCA-3' and reverse 5'-TGAAGCTGGACTTCCGGTGGGAAGGAGGGGCTCACAAACACAGT -3'. The psi-CHECK2 Vector was embedded into the amplified DNA sequence to obtain PTEN 3'-UTR or mutated PTEN 3'-UTR luciferase vectors. Around $2x10^4$ cells (HEK293T) were transfected with hsa-miR-455-3p mimic/mimic NC (50 nmol/L) , or inhibitor/inhibitor NC (100 nmol/L) . Luciferase activity was determined by the

dual-luciferase Reporter Assay System (E1910) (Promega) 48 h after co-transfection. Luciferase assays were performed in three independent experiments.

2.8 Statistical analysis

The results are displayed as mean±SD. The student's *t* tests were applied to identify differences between the two groups. It was considered statistically significant when $P < 0.05$. IBM SPSS Statistics 25 software (IBM Corporation, Armonk, NY) was used for all statistical analyses.

3. Results

3.1 Variation tendency of PTEN and miR-455-3p in chondrogenic differentiation model

To explore the variation tendency of PTEN and miR-455-3p during chondrogenesis, we used a model of BMSCs induced to a state of chondrogenic differentiation. At days 7, 21, and 35, Alcian staining results showed that BMSCs differentiated into chondrocytes successfully (Figure 1A). miR-455-3p continued to increase in the first 21 days during chondrogenesis, peaked at day 21, and then declined (Figure 1B). PTEN levels were lower in the first 28 days of chondrogenesis and then increased (Figure 1C). Immunohistochemistry result of PTEN (Figure 1A) showed a similar trend to RT-PCR. While, the opposite trend was evident in pAKT immunohistochemistry result (Figure 1A). The expression of COL2A1 and SOX9 was

the same as that of miR-455-3p, which was consistent with the progress of chondrogenic differentiation (Figure 1A, D-E).

3.2 Expression of miR-455-3p and PTEN in OA versus healthy cartilage

We performed qRT-PCR and immunohistochemistry to explore difference in expression of miR-455-3p and PTEN between OA and healthy cartilage. The results of the qRT-PCR analysis indicated that miR-455-3p was lower in OA cartilage than that in healthy cartilage significantly (Figure 2A), while PTEN showed the opposite trend (Figure 2B). This suggested that there may be a regulatory relationship between them. COL2A1 and MMP13 were consistent with the typical phenotypes seen in OA and healthy cartilage (Figure 2C-2D). The immunohistochemistry results were the same as those from the qRT-PCR analysis (Figure 2E-2F). We also found that PTEN levels increased and pAKT levels decreased in OA cartilage, suggesting that the activity of PI3K/AKT pathway was inhibited in osteoarthritis (Figure 2F).

3.3 Expression pattern of miR-455-3p and PTEN in IL-1β/TNF-α model

To explore the influence of IL-1β/TNF-α on chondrocytes, we treated healthy chondrocytes with IL-1β (5 ng/mL)/TNF-α (20 ng/mL) for 48 hours, and then performed qRT-PCR or western blot. We found that miR-455-3p was declined significantly, while PTEN expression increased (Figure 3A). In addition, COL2A1 and ACAN was significantly reduced and MMP13 increased, which confirmed our predictions (Figure 3A). Furthermore, the results of the western blot were similar to those from the qRT-PCR analysis (Figure 3B).

3.4 MiR-455-3p regulates PTEN and cell apoptosis *in vitro*

To determine whether miR-455-3p can regulate PTEN, we transfected IL-1β-treated chondrocytes with miR-455-3p mimic and inhibitor. The results showed that overexpressing miR-455-3p led to the downregulation of PTEN, MMP13, and ADAMTS4, and the upregulation of COL2A1 and ACAN. As expected, the opposite effect was observed when we added the inhibitor (Figure 3C-3D). The western blot showed the same trend (Figure 3E). Additionally, flow cytometry showed that IL-1β could increase the apoptosis rate of chondrocytes. After transfection with mimic/mimic NC, IL-1β could significantly increase the apoptosis rate, which might be related to the cytotoxicity of transfection. However, the apoptosis rate of mimic group was significantly decreased as compared with NC group, suggesting that overexpression of miR-455-3p might reduce the apoptosis of chondrocyte (Figure 4A).

3.5 Comparing expression levels of PTEN and pAKT *in vivo*

To further investigate the expression of miR-455-3p, PTEN, and pAKT *in vivo*, we used miR-455-3p deletion mice that had been previously described. In situ hybridization was used to confirm the successful establishment of miR-455-3p KO mice (Figure 4B).We discovered that KO mice showed signs of osteoarthritis at 6 months when comparing to wild-type (WT) mice according to safranine O staining (Figure 4B). In addition, on analyzing the immunohistochemistry results, we found a marked augment in the expression of PTEN in KO mice relative to WT mice, while

pAKT levels decreased (Figure 4C).

3.6 Effects of PTEN inhibitor on chondrocytes

To confirm whether miR-455-3p functions through PTEN, we used the VO-Ohpic trihydrate (Selleck Chemicals, US) to inhibit the effect of PTEN. According to our preliminary experiments and literature search [21], we set up four different concentration gradients: 0 μM, 0.25 μM, 0.5 μM, and 1 μM for qRT-PCR analysis after 48 h, or western blot analysis after 72 h. We discovered that there was no significant change in PTEN mRNA levels (Figure 5A). While pAKT activity increased as the concentration of VO-Ohpic increases (Figure 5F). Interestingly, we found that at 0.25 μM, the expression of COL2A1 and ACAN were at maximum and that MMP13 and ADAMTS4 was at a minimum. Consequently, as the concentration increased, the expression of COL2A1 declined and that of MMP13 increased, suggesting that PTEN activity may have a positive effect on chondrocyte formation, while excessive inhibition of PTEN activity may have a negative effect (Figure 5B-5E, 5F).

3.7 MiR-455-3p targets with the PTEN 3′-UTR

We discovered that miR-455-3p has a latent targeted binding site within PTEN through an online prediction software (Figure 6A). Thus, we testify to it by a luciferase reporter assay. We constructed wild-type or mutant PTEN 3’-UTRs in the presence or absence of miR-455-3p mimic or inhibitor. In the transfected clones with the PTEN 3’-UTR, the mimic group was significantly different when compared with

NC group ($P<0.01$), as was the inhibitor group versus inhibitor NC group ($P<0.05$). This suggested that hsa-miR-455-3p could affect the fluorescence activity of PTEN gene by binding to its 3'-UTR. However, no difference was observed between the mimic group and NC group in the transfected clones with mut PTEN 3'-UTR plasmids. The same pattern was seen with the inhibitor group and inhibitor NC group ($P>0.05$). This indicated that hsa-miR-455-3p could not affect the fluorescence activity of the mut PTEN gene by binding to its 3'-UTR, and that the binding site mutation was complete (Figure 6B).

4. Discussion

MiR-455-3p has been shown to play a regulatory role in many diseases, such as tumors, pulmonary fibrosis, and osteoarthritis[19,22-23]. In our previous study, we have found that miR-455-3p was upregulated in human adipose stem cell chondrogenesis and knockout of miR-455-3p mice showed signs of osteoarthritis, which suggested that miR-455-3p plays an important role in chondrogenic development and degeneration. In the present study, we observed that miR-455-3p increased in the first 21 days during chondrogenesis and then declined in the late stage of chondrogenic differentiation, while PTEN showed the opposite trend. This indicates that miR-455-3p and PTEN may be associated with the chondrogenesis and cartilage degeneration. And then we found low expression of miR-455-3p but high expression of PTEN in OA cartilage. After overexpressing miR-455-3p, the expression of cartilage-specific genes was increased, while PTEN decreased. There was also a decrease in chondrocyte apoptosis. We also proved that miR-455-3p may

regulate PTEN expression through a luciferase reporter assay. Therefore, we speculated that miR-455-3p functions by regulating PTEN. Finally, we verified this hypothesis in miR-455-3p KO mice.

PTEN, an inhibitor of the PI3K/AKT pathway, is often found to be mutated in tumor cells, which causes uncontrolled proliferation in tumors [24]. Changes of PTEN expression can result in a series of cell activity disorders, such as apoptosis, migration, cell cycle, and so on[25]. However, there are few researches on PTEN and osteoarthritis. And there are different conclusions regarding the expression of PTEN in healthy cartilage relative to OA cartilage. Kenjiro Iwasa et al. and Huang ZH et al. found that PTEN was significantly higher in OA cartilage [26, 27], while Wu XF et al. found that it decreased in OA [28].These inconsistent results may be related to sample collection. In our study, PTEN expression in OA cartilage was also significantly increased. Therefore, we speculated that PI3K/AKT pathway was inhibited in OA and appropriate activation of this pathway may alleviate OA.

Additionally, there is controversy about the role of the PI3K/AKT pathway in OA. Some scholars believe that excessive activated PI3K/AKT pathway can lead to OA, and repression of this pathway will delay the progress of OA by promoting autophagy, attenuating inflammatory response, inhibiting bone sclerosis in subchondral bone, and other factors [29, 30]. However, others believe that the PI3K/AKT pathway activation is a protective factor for OA progression because it promotes chondrocyte proliferation, inhibits apoptosis, and induces cell autophagy [26, 27, 31]. In this study, we used VO-Ohpic at different concentration gradients to

explore this problem preliminarily. We found that moderate concentrations of VO-Ohpic can have a positive effect on chondrocytes, while high concentrations can have a negative effect, which suggested that the PI3K/AKT pathway may play a bidirectional regulatory role, and that moderate activity of PTEN is necessary for chondrocytes.

Recently, a growing number of studies have shown that miRNAs participate in multiple cell signaling pathways such as TGF-β/Smad, NF-κB, Wnt, and PI3K/AKT, in turn regulate cell growth, metabolism, and other aspects and play an indispensable role in osteoarthritis[32-36]. In this study, we proved that miR-455-3p can target PTEN directly to activate the PI3K/AKT pathway involved in cartilage differentiation and degeneration. It has been reported that viscoelastic hydrogels can be prepared in the form of microspheres that can be used as delivery vehicles to control the release of various small molecules, so as to repair osteochondral tissue more safely and effectively[37]. Therefore, the use of these miRNAs for intracellular regulation to delay or even reverse the OA process holds promise. However, we need to further clarify the intracellular regulatory network of miR-455-3p to provide complete theoretical support for clinical application.

This study has some limitations. First, the age of healthy group was younger than that of OA group. OA samples are always older than healthy ones due to sampling limitations. It may be better to compare the OA samples at different stages according to the OARSI and ICRS standards. Second, the studies on the mechanism of miR-455-3p targeting PTEN to regulate the PI3K/AKT signaling pathway are limited.

The deeper underlying mechanisms need further clarification.

5. Conclusion

To summarize, in this study, we can infer that increasing PTEN may increase the expression of ADAMTS4 and MMP13 and decrease COL2A1 and ACAN by inhibiting PI3K/AKT pathway. However, excessive inhibition of PTEN may also accelerate the progress of OA. MiR-455-3p regulated PTEN by directly targeting the PTEN 3'-UTR, and played a protective role in reducing the apoptosis of chondrocytes and alleviating OA.

Abbreviations

PTEN	gene of phosphate and tension homology deleted on chromsome ten
BMSC	bone marrow stem cell
OA	osteoarthritis
FBS	Foetal bovine serum
PS	penicillin and streptomycin
IL-1β	Interleukin -1β
TNF-α	Tumour necrosis factor -α
MMP13	Matrix metalloproteinase -13
COL2A1	collagen type II alpha 1

ADAMTS4	A disintegrin and metalloproteinase with thrombospondin motifs 4
ACAN	aggrecan
SOX9	SRY-Box 9
DNMT3A	DNA methyltransferase -3A
GAPDH	glyceraldehyde‐3‐phosphate dehydrogenase
U6	U6 small nuclear ribonucleic acid
3'-UTR	3'-untranslated region
miR	MicroRNA

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Xingzhao Wen and Hongyi Li designed, performed experiments and wrote manuscript. Hao Sun conceived and collected samples. Anyu Zeng and Ruifu Lin Collated and analyzed data. Jing Zhao and Zhiqi Zhang conceived and supervised the study and wrote the manuscript.

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Availability of data and materials

The data involved in this study are available from the corresponding author on reasonable request.

FIGURE LEGEND

Figure 1: Different expression during chondrogenesis. A: Immunohistochemical results at 7/21/35 days of chondrogenic differentiation of Alcian, COL2A1, PTEN, and pAKT. (Scale:400 μm) B-E: miR-455-3p, PTEN, SOX9, and COL2A1 expression during chondrogenesis by qRT-PCR. * $P<0.05$，** $P<0.01$, *** $P<0.001$ compared to TGFβ3- group respectively. GAPDH for mRNA or U6 for miR-455-3p was used as the internal control.

Figure 2: Different gene expression profile between healthy cartilage and OA cartilage. A-D: miR-455-3p, PTEN, COL2A1, and MMP13 expression in healthy and OA cartilage by qRT-PCR, * $P<0.05$，** $P<0.01$, *** $P<0.001$. E-F: Immunohistochemical results of Alcian, COL2A1, MMP13, PTEN, and pAKT in healthy and OA cartilage. (Scale:400 μm)

Figure 3: IL-1β/TNF-α down-regulates miR-455-3p and miR-455-3p inhibites the expression of PTEN. A: qPCR analyzing chondrocytes treated with IL-1β/TNF-α : IL-1β/TNF-α results in down-regulation of miR-455-3p and up-regulation of PTEN. B: The western blotting results of healthy cartilage and IL-1β treated cartilage. C-D: qRT-PCR analyzing overexpressing or inhibiting miR-455-3p: miR-455-3p inhibits PTEN and regulates related mRNA. E: western blotting analyzing the changes of related protein after transfecting mimic/inhibitor.* $P<0.05$，** $P<0.01$, *** $P<0.001$.

Figure 4: MiR-455-3p reduces the apoptosis rate of chondrocytes and regulates

PI3K/AKT pathway *in vivo*. A: Flow cytometry analyzing chondrocytes treated by IL-1β and mimic: IL-1β could increase apoptosis of chondrocytes slightly while miR-455-3p decreased compared to control group. B: In situ hybridization confirmed the successful establishment of mir-455-3p KO mice and Safranine O staining showed miR-455-3p KO mice developing signs of osteoarthritis. C: PTEN and pAKT expression in miR-455-3p KO mice and WT mice.* $P<0.05$，**$P<0.01$, ***$P<0.001$.(Scale:200 μm)

Figure 5: The effect of different concentration of VO-Ohpic on chondrocytes. A-E: The expression of PTEN, MMP13, COL2A1, ACAN, and ADAMTS4 under different concentration of VO-Ohpic (0 μM, 0.25 μM, 0.5 μM, and 1 μM) by qRT-PCR. F:Western blotting results were consistent with qRT-PCR.* $P<0.05$，**$P<0.01$, ***$P<0.001$.

Figure 6: miR-455-3p could target PTEN directly. A: predicted miR-455-3p binding sequence and PTEN 3'-UTR through target scan website. B: Luciferase assay confirmed that miR-455-3p could bind to PTEN 3'-UTR.* $P<0.05$，**$P<0.01$, ***$P<0.001$.

Table 1: Primers for quantitative real-time polymerase chain reaction (qRT-PCR)

Gene		Primer sequence (5'-3')
hsa-PTEN	F	TGGATTCGACTTAGACTTGACCT
hsa-PTEN	R	GGTGGGTTATGGTCTTCAAAAGG
hsa-COL2A1	F	TGGACGATCAGGCGAAACC
hsa-COL2A1	R	GCTGCGGATGCTCTCAATCT
hsa-ACAN	F	GTGCCTATCAGGACAAGGTCT
hsa-ACAN	R	GATGCCTTTCACCACGACTTC
hsa-MMP13	F	CCAGACTTCACGATGGCATTG
hsa-MMP13	R	GGCATCTCCTCCATAATTTGGC
hsa-ADAMTS4	F	GGTCAAGGTCCCATGTGCAAC
hsa-ADAMTS4	R	GAATGCGGCCATCTTGTCATC
hsa-SOX9	F	AGCGAACGCACATCAAGAC
hsa-SOX9	R	CTGTAGGCGATCTGTTGGGG
hsa-GAPDH	F	GGAGCGAGATCCCTCCAAAAT
hsa-GAPDH	R	GGCTGTTGTCATACTTCTCATGG
hsa-miR-455-3p	F	GCAGTCCATGGGCATATACAC
hsa-U6	F	CAGTGCAGGGTCCGAGGTAT
hsa-U6	R	CAAATTCGTGAAGCGTTCCAT

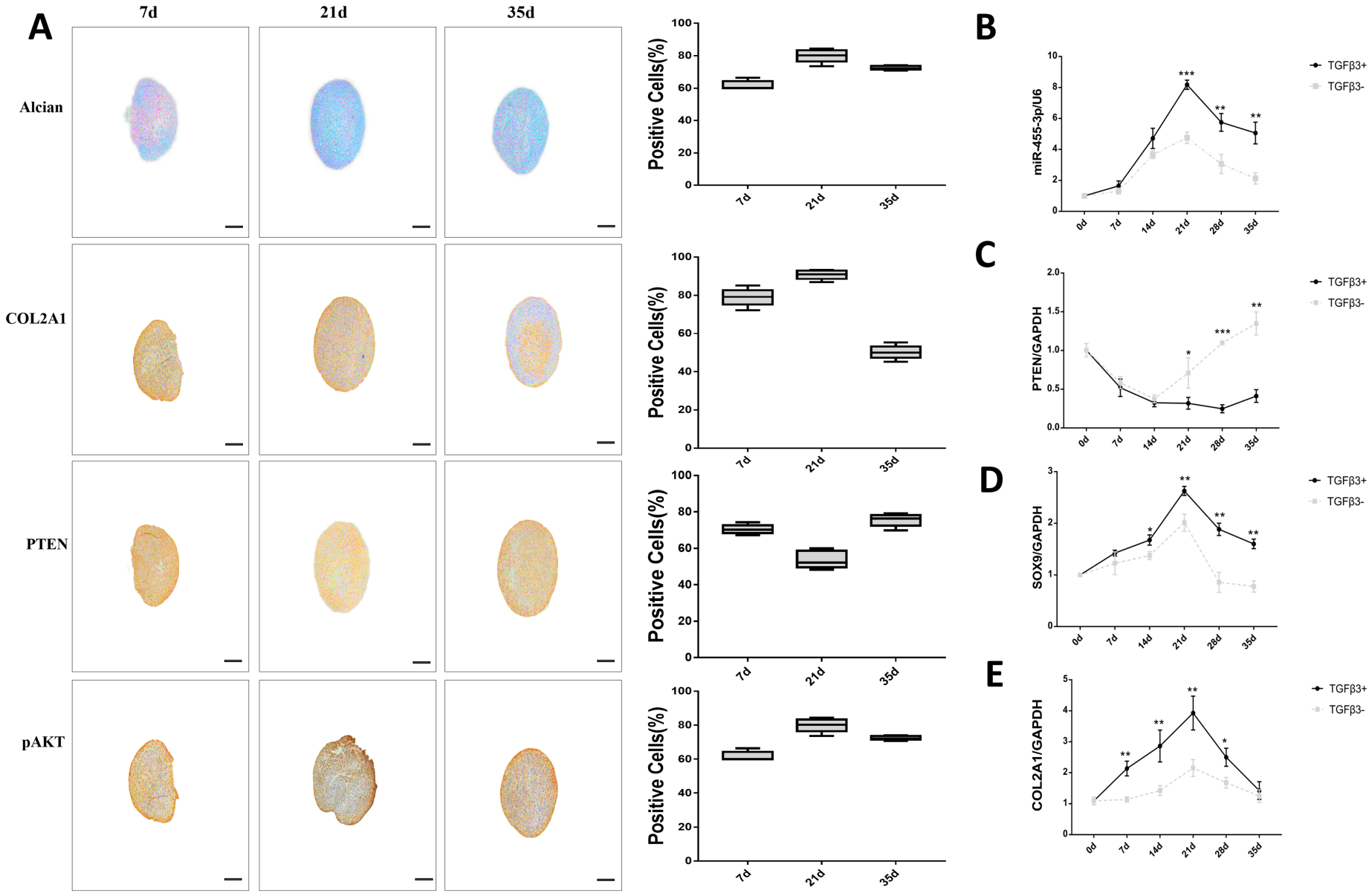
A
7d
21d
35d
Alcian
COL2A1
PTEN
pAKT
Positive Cells(%)
B
miR-455-3p/U6
C
PTEN/GAPDH
D
SOX9/GAPDH
E
COL2A1/GAPDH
TGFβ3+
TGFβ3-
0d
7d
14d
21d
28d
35d

Figure 1

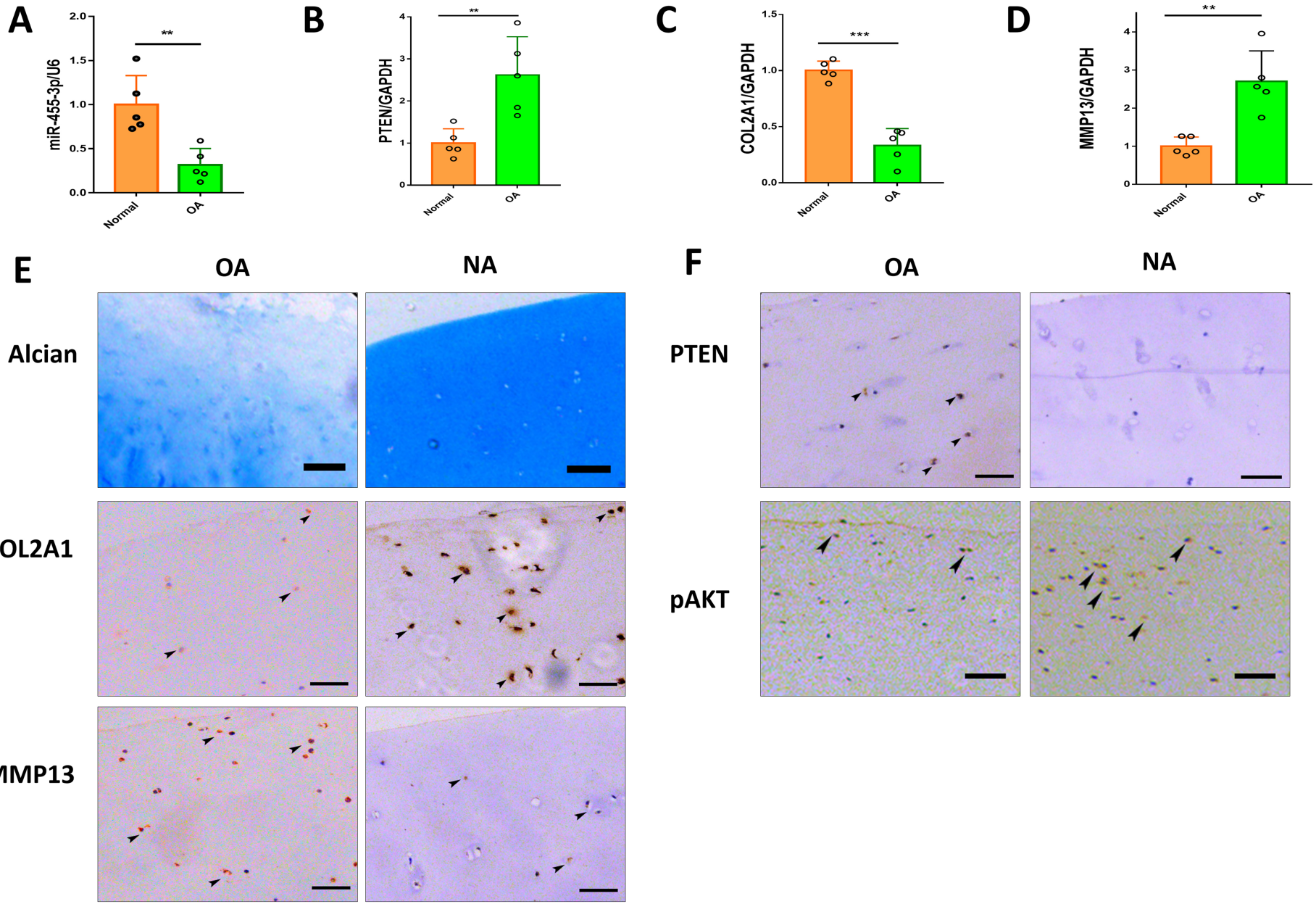
A
miR-455-3p/U6
2.0
1.5
1.0
0.5
0.0
**
Normal
OA
B
PTEN/GAPDH
4
3
2
1
0
**
Normal
OA
C
COL2A1/GAPDH
1.5
1.0
0.5
0.0
***
Normal
OA
D
MMP13/GAPDH
4
3
2
1
0
**
Normal
OA
E
OA
NA
Alcian
COL2A1
MMP13
F
OA
NA
PTEN
pAKT

Figure 2

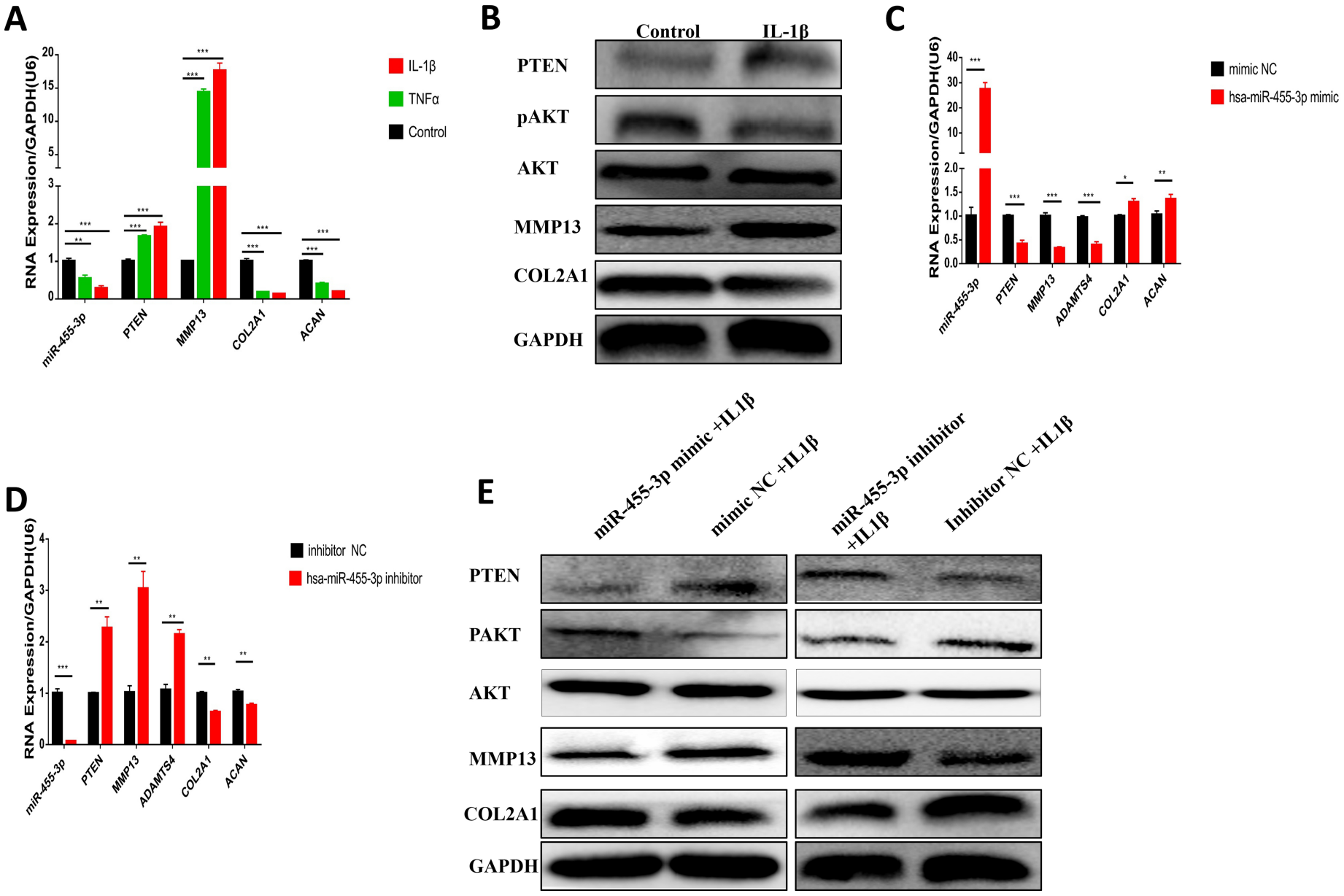
A
RNA Expression/GAPDH(U6)
IL-1β
TNFα
Control
miR-455-3p
PTEN
MMP13
COL2A1
ACAN
B
Control
IL-1β
PTEN
pAKT
AKT
MMP13
COL2A1
GAPDH
C
RNA Expression/GAPDH(U6)
mimic NC
hsa-miR-455-3p mimic
miR-455-3p
PTEN
MMP13
ADAMTS4
COL2A1
ACAN
D
RNA Expression/GAPDH(U6)
inhibitor NC
hsa-miR-455-3p inhibitor
miR-455-3p
PTEN
MMP13
ADAMTS4
COL2A1
ACAN
E
miR-455-3p mimic +IL1β
mimic NC +IL1β
miR-455-3p inhibitor +IL1β
Inhibitor NC +IL1β
PTEN
PAKT
AKT
MMP13
COL2A1
GAPDH

Figure 3

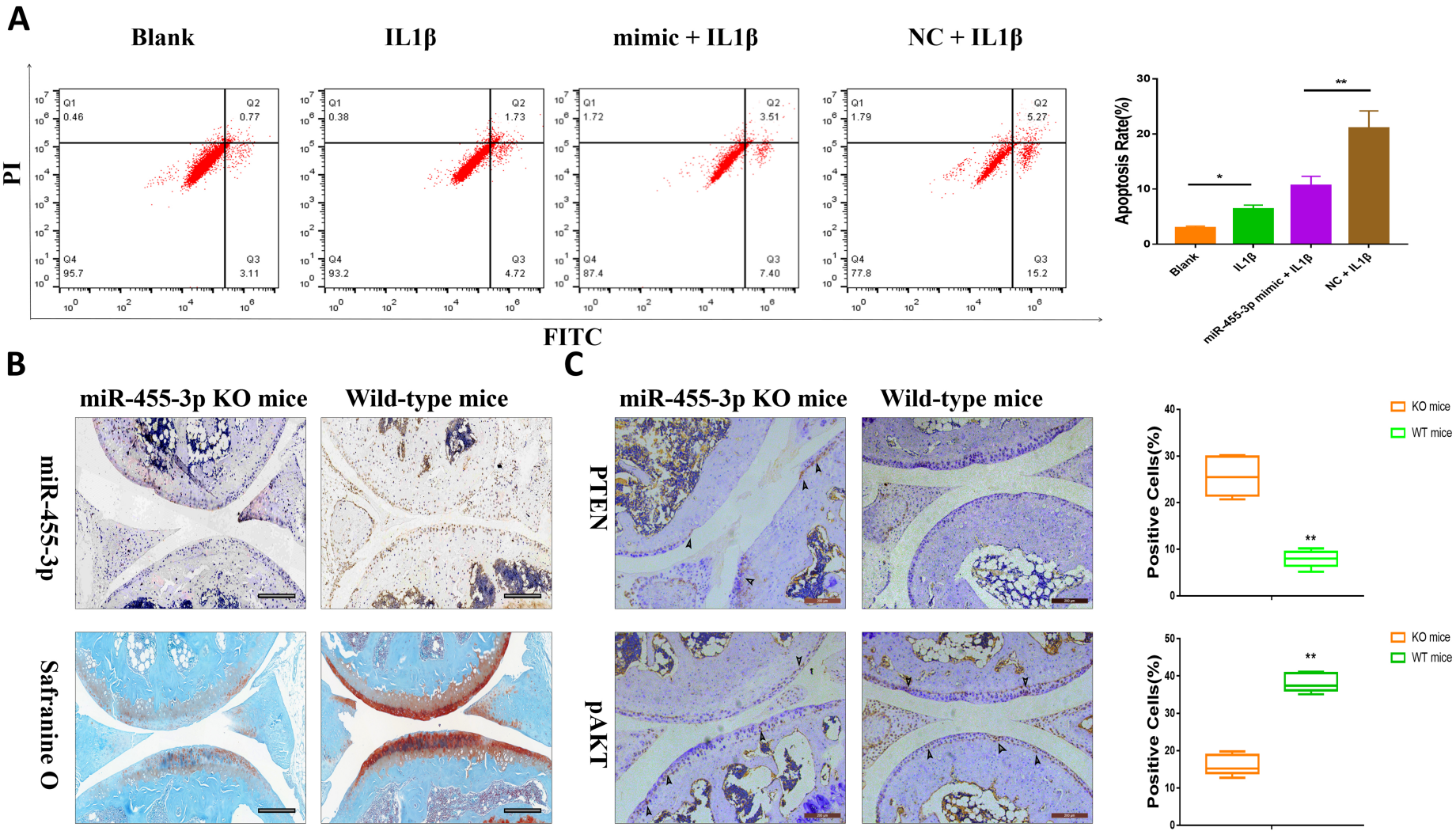
A
Blank
IL1β
mimic + IL1β
NC + IL1β
PI
FITC
Q1 0.46
Q2 0.77
Q4 95.7
Q3 3.11
Q1 0.38
Q2 1.73
Q4 93.2
Q3 4.72
Q1 1.72
Q2 3.51
Q4 87.4
Q3 7.40
Q1 1.79
Q2 5.27
Q4 77.8
Q3 15.2
Apoptosis Rate(%)
Blank
IL1β
miR-455-3p mimic + IL1β
NC + IL1β
B
miR-455-3p KO mice
Wild-type mice
miR-455-3p
Safranine O
C
miR-455-3p KO mice
Wild-type mice
PTEN
pAKT
Positive Cells(%)
KO mice
WT mice

Figure 4

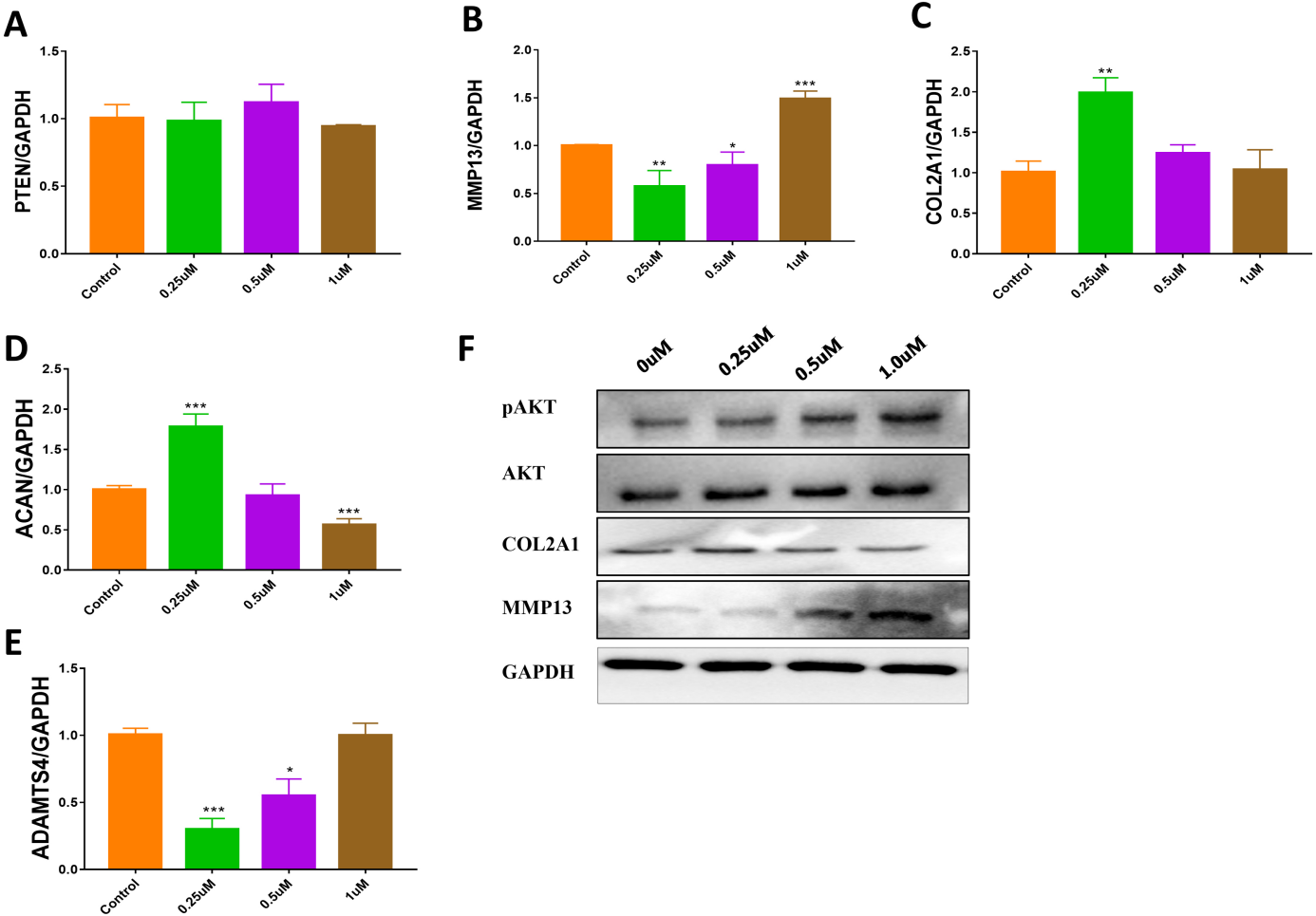
A
PTEN/GAPDH
1.5
1.0
0.5
0.0
Control
0.25uM
0.5uM
1uM
B
MMP13/GAPDH
2.0
1.5
1.0
0.5
0.0
**
*
***
Control
0.25uM
0.5uM
1uM
C
COL2A1/GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
**
Control
0.25uM
0.5uM
1uM
D
ACAN/GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
***
***
Control
0.25uM
0.5uM
1uM
E
ADAMTS4/GAPDH
1.5
1.0
0.5
0.0
***
*
Control
0.25uM
0.5uM
1uM
F
0uM
0.25uM
0.5uM
1.0uM
pAKT
AKT
COL2A1
MMP13
GAPDH

Figure 5

A

Position 1563-1569 of PTEN 3' UTR

```
5'  ...UAUUUGAUAUGCCCA--GACUGCAU...
                ||||||||||  |||||||
3'        CACAUAUACGGGUACCUGACGUA
```

has-miR-455-3p

B

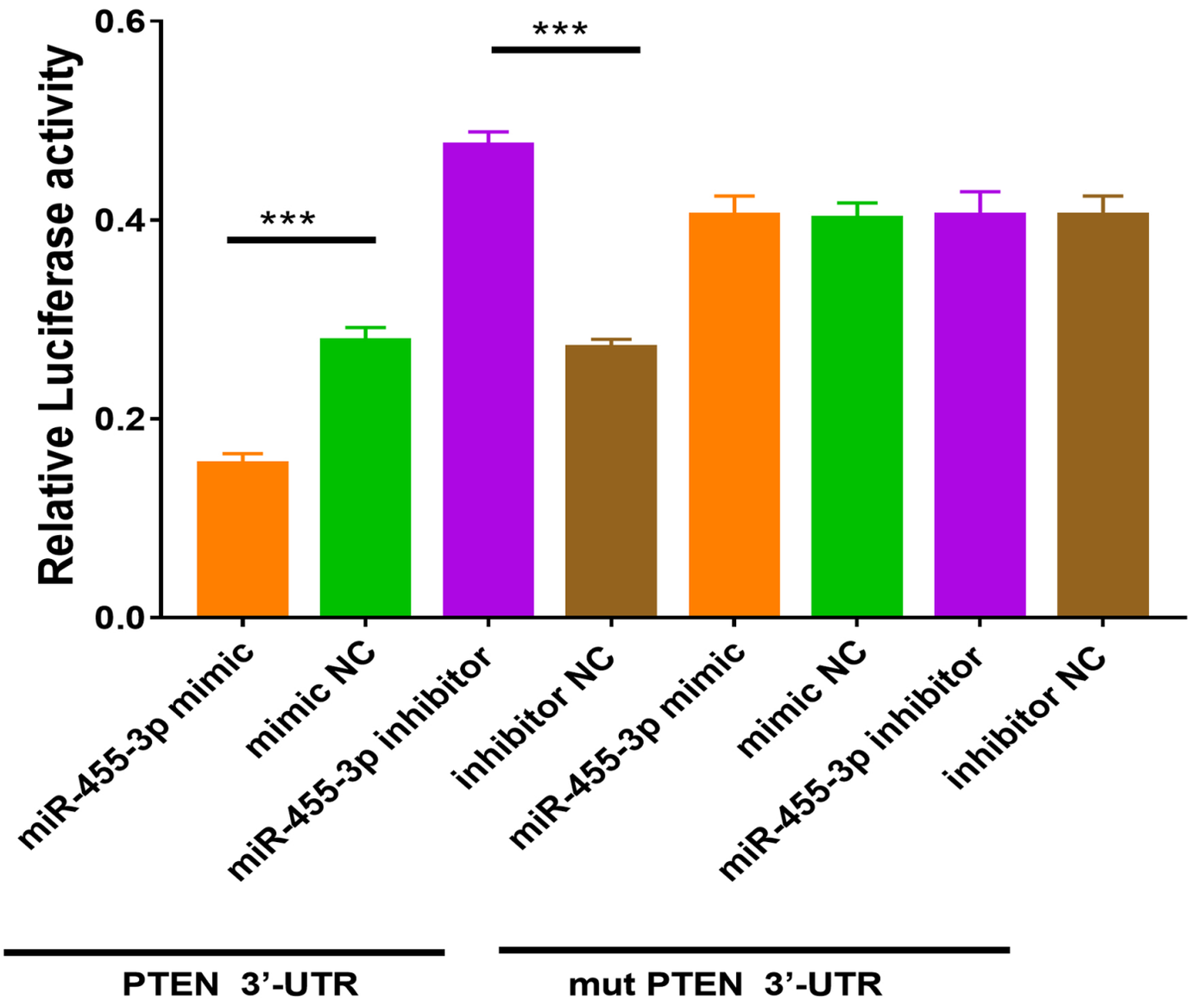

Figure 6