

# Knockout of *miR-17-92* Cluster Protect Against Intervertebral Disc Degeneration by Inhibiting Apoptosis of Nucleus Pulposus Cells in Mice via the Activation of PI3K/Akt Pathway

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## Research Article

**Keywords:** Intervertebral disc degeneration, mouse model, natural degeneration, histological assessment, behavioral parameter

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# Abstract

**Background:** microRNA(*miR*)-17-92 cluster is involved in a variety of physiological and pathological processes, and the purpose of this study is to preliminarily explore the role of *miR*-17-92 cluster in disc degeneration and the corresponding mechanisms.

**Methods:** Hematoxylin and Eosin (HE) and Safranin O Staining were used to evaluate the degeneration of intervertebral disc. qRT-PCR was applied to evaluate the mRNA level of *miR*-17-92 cluster and functional genes of nucleus pulposus (NP) tissues, whose protein level was evaluated with Western-blot. Terminal-Deoxynucleotidyl Transferase Mediated Nick End Labeling (TUNEL) was used to evaluate the apoptotic level of nucleus pulposus cell (NPC).

**Results:** The expression levels of members of the *miR*-17-92 cluster were significantly increased in the NP tissues from patients with intervertebral disc degeneration (IVDD). Furthermore, in the 3-months and 24-months *miR*-17-92-ckKO mice, the degree of IVDD was significantly lower than that of the control group. At the same time, we also detected the expression levels of related functional genes in the NP tissues of mice in two groups. The results showed that the mRNA and protein levels of Bax and Caspase-3 in the knockout group were significantly lower than those in the control group, and the mRNA and protein levels of Bcl-2 were significantly higher. The TUNEL results showed that the apoptosis level of NPCs in the 3-month knockout mice was significantly lower than that in the control group. Finally, the assessment of pathway-related protein levels showed that p-Ser473-Akt expression ratio in the nucleus pulposus of mice in the knockout group were significantly increased, suggesting that the PI3K/Akt pathway was activated after *miR*-17-92 cluster knockout.

**Conclusion:** To sum up, *miR*-17-92 cluster does play an important regulating role in IVDD, and the results showed that *miR*-17-92 cluster could inhibiting NPCs apoptosis by activating PI3K/Akt pathway, eventually producing protective effect against IVDD.

## Introduction

Intervertebral disc degenerative (IVDD) is a common disease in spinal surgery. The clinical symptoms caused by IVDD, including neck and shoulder pain, limb weakness, and paresthesia, will seriously affect the quality of life of patients. At present, the clinical treatment of IVDD is mainly achieved through decompression surgery, which adds a heavy economic burden to the patient's family and even the whole society[1, 2]. Therefore, the development of a non-surgical treatment is imperative and urgent. Generally, studies on IVDD are divided into three levels: (1) studies on the mechanism of IVDD (2) Studies on the non-surgical treatment of IVDD; (3) Studies on drug delivery methods of intervertebral disc (IVD), while the most important part is to clarify the pathological basis of IVDD. In this regard, a large number of articles have discussed and analyzed the relevant mechanisms, and the conclusions obtained have gradually improved the construction of "IVDD signal network". MicroRNA (miR) mechanisms account for a large proportion in the whole network.

miRs are a class of non-coding single-stranded RNA molecules encoded by endogenous genes between 19–25 nucleotides, which inhibit expression by complementing and binding to the 3'UTR region of the target gene. They are involved in various physiological and pathological processes, and play a key regulatory role in many cases. MIR17HG gene is a nucleotide sequence with a length of about 7000bp located on human chromosome 13. After further transcription and shear processing, 7 mature miRs will eventually be formed, including *miR-17-3p*, *miR-17-5p*, *miR-18a-5p*, *miR-19a-3p*, *miR-19b-3p*, *miR-20a-5p* and *miR-92a-3p*, collectively known as the *miR-17-92* family cluster. At present, a large number of studies have confirmed that *miR-17-92* clusters are involved in the development and progression of many diseases[3–5].

There have been abundant studies on IVDD and miRs. For example, miR-21-5p can regulate the process of IVDD by promoting the proliferation of nucleus pulposus cell (NPC) and the degradation of extracellular matrix (ECM)[6–8]. miR-140-5p can regulate IDD by inhibiting inflammatory response and protecting NPCs[9, 10]. In our previous work, we found that *miR-17-92* clusters are involved in cartilage development and also play an important regulatory role in bone healing. Considering that the nucleus pulposus (NP), annulus fibrosus (AF), and endplate of the IVDs are all cartilaginous tissues, we considered whether *miR-17-92* could also regulate the degeneration process of the IVDs. Therefore, the purpose of this study is to verify the above hypothesis[11].

## Materials And Methods

### 1. Patient Materials

Non-degenerated IVD samples and degenerative IVD samples were obtained from idiopathic scoliosis patients and patients with IVDD undergoing disectomy, respectively. All patients performed routine magnetic resonance imaging (MRI) of spine before surgery. This study had been approved by the Ethics Committee of West China Hospital, Sichuan University and the Ethics Approvals have been provided. Informed consent was obtained from all subjects and all methods were carried out in accordance with relevant guidelines and regulations.

### 2. Clinical Evaluation of Disc Degeneration with Magnetic Resonance Imaging (MRI)

The disc degeneration degree was analyzed according to T2-weighted images by using the modified Pfirrmann classification[12].

### 3. Animals and Experimental Groups

The *miR-17-92<sup>flox/flox</sup>* mouse strain used in this study was supported by The Jackson Laboratory and the collagen II (Col2a1)-Cre mouse strain was supported by The Maine Medical Research Center in The United States. By hybridization of *miR-17-92<sup>flox/flox</sup>* with Col2a1-Cre mouse strain, *miR-17-92*-ccKO mice were obtained with specific knockout of *miR-17-92* in chondrocytes, including NPCs. The mice of the control group in this study were C57BL/6 mice (WT), purchased from Beijing HFK Bioscience CO., LTD. All

experimental protocols of the animal part of this study had been approved by the Animal Ethics Committee of West China Hospital, Sichuan University and the Ethics Approvals have been provided. All methods were carried out in accordance with relevant guidelines and regulations.

#### **4. Histopathologic Analysis**

Parts of mouse IVD tissues (Co6-Co8) were extracted for the histopathologic analysis. The target IVDs were excised, then fixed with 4% paraformaldehyde and decalcified with Ethylene Diamine Tetraacetic Acid (EDTA) solution, embedded with paraffin, and cut into 5 $\mu$ m-thick section. The disc specimens were stained with hematoxylin-eosin (HE) and Safranin O staining.

#### **5. Histological Evaluation of Intervertebral Disc Degeneration**

Histological assessment of the degree of IVDD was based on Tam's report[13]. The whole scoring system is divided into three parts to evaluate the histological appearance of NP, AF and NP/AF. The specific scoring system can be referred to Table-1.

#### **6. Terminal-Deoxynucleotidyl Transferase Mediated Nick End Labeling (TUNEL)**

Apoptosis levels of NP tissues in each group were measured utilizing a TUNEL kit (Takara Bio Tech CO., LTD, Beijing, China) following the manufacturer's instructions. Green fluorescence (FITC) indicated DNA fragments and the blue fluorescence (DAPI) indicated cell nucleus. The percentage cells undergoing apoptosis to the total number of NP cells was calculated and considered the apoptotic index.

#### **7. Real-Time Fluorogenic Polymerase Chain Reaction (qRT-PCR)**

Total RNA was extracted from nucleus pulposus tissues using Animal Total RNA Isolation Kit (Foregene CO., LTD, Chengdu, China). The ratio of A260/A280 and concentration of RNA were determined using a Nanodrop ultraviolet spectrophotometer (Thermo CO., LTD, Waltham, MA, USA), with RNA stored at -80°C. For the routine mRNA qRT-PCR, the total RNA (50ng) was reversely transcribed into cDNA with the PrimeScript™ RT Reagent Kit (TaKaRa CO., LTD, Beijing, China).  $\beta$ -actin was used as an internal reference standard. After incubating at 95°C for 30s, the qRT-PCR reaction conditions were set to 40 cycles of "95°C for 10s + 60°C for 30s", and the incubation time at 95°C for 5s. Primers of functional genes used in the study were listed in Table-2. For the miR qRT-PCR, the total RNA (50ng) was reversely transcribed into cDNA with the mix of 8 RT primers, including U6, miR-17-3p, miR-17-5p, miR-18a-5p, miR-19a-3p, miR-19b-3p, miR-20a-5p and miR-92a-3p (RiboBio CO., LTD, Guangzhou, China) and the PrimeScript™ RT Reagent Kit (TaKaRa CO., LTD, Beijing, China). U6 was used as an internal reference standard. After incubating at 95°C for 10min, the qRT-PCR reaction conditions were set to 40 cycles of "95°C for 5s + 60°C for 20s + 70°C for 10s", and the incubation time at 95°C for 5s. qRT-PCR analyses were performed using TB Green Premix Kit (TaKaRa CO., LTD, Beijing, China) on the QuanStudio™ 12K Flex Real-Time PCR System (Thermo CO., LTD, Waltham, MA, USA). The  $2^{-\Delta\Delta C_t}$  method was applied to quantify the relative expression levels of targets genes.

## 8. Western Blot Analysis

Total protein of each group of NP tissue was extracted with RIPA buffer, and the BCA kit (EpiZyme, Shanghai, China.) was used to detect the protein concentration. Protein samples were separated by electrophoresis using a 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA). After processing in a 5% defatted milk in TBST, the blots were incubated with anti- $\beta$ -actin, anti-GAPDH, anti-Akt, anti-p-Ser473-Akt, anti-Bcl-2, anti-Bax, anti-Caspase-3, anti-Cleaved-Caspase-3 (ProteinTech, Wuhan, China.), diluted from 1:500 to 1:1000, at 4°C, for over 12 hours.  $\beta$ -actin and GAPDH served as the internal reference. After washing with TBS containing Tween 20, the appropriate secondary antibody was incubated with the blots at 25°C for 60min, treated with Chemiluminescence Kit (EpiZyme, Shanghai, China.) for 30s, and placed in a dark room for observation. Gray values were analyzed with Image J software v1.46.

## 9. Statistical Analysis

All statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, Illinois). The results are presented as mean  $\pm$  standard deviation. The differences between various groups were analyzed using Student T test.  $P < 0.05$  was considered to indicate statistical significance.

# Results

### 1. Differential Expression of *miR-17-92* Cluster in Patients' NP with Intervertebral Disc Degeneration

The expression of *miR-17-92* cluster was evaluated by qRT-PCR in patients with or without disc degeneration, and the results were shown in Figure-1. As we can see, the members of *miR-17-92* cluster differentially expressed in two groups, and the expression levels were relatively lower in patients without disc degeneration, which at least suggests that *miR-17-92* cluster is correlated with disc degeneration.

### 2. Knockout of *miR-17-92* Cluster Relieve the Degree of Intervertebral Disc Degeneration in Mice

To further prove the correlation between *miR-17-92* cluster and disc degeneration, we constructed genetically-engineered mice with the knockout of *miR-17-92* cluster, and the knockout verification of *miR-17-92* cluster has been shown in Figure-2. Next, we histologically evaluated the caudal IVDs of 3- and 24 month-mice, respectively. With HE and Safranin O Staining (Figure-3A to Figure-3E), we found that, in whatever age group, the disc morphology of the *miR-17-92*-ccKO group was significantly better than that of the control group, which was also confirmed by the histological scores (Figure-3F to Figure-3M).

### 3. Knockout of *miR-17-92* Cluster Protects Intervertebral Disc from Degeneration by Inhibiting Apoptosis of NPCs

We do not yet know with which mechanism *miR-17-92* cluster affects intervertebral disc degeneration, so we examined the expression levels of a range of functional genes associated with nucleus pulposus cells. As shown in Figure-4A to Figure-4D, there were no significant differences in mRNA expression levels

between two groups in Col2a1, Aggrecan, Proliferating Cell Nuclear Antigen (PCNA), Ki67, reminding us that the knockout of *miR-17-92* cluster would not affect the matrix metabolism and proliferation of NPCs. As shown in Figure-4E to Figure-4H, the mRNA expression levels of Bax and Caspase-3 were significantly lower than that of the control group, while the mRNA expression level of Bcl-2 and the ratio of Bax/Bcl-2 in *miR-17-92*-ccKO group was significantly higher. Further, the Western Blot also showed the same results, as shown in Figure-4I to Figure-4M. To obtain a more intuitive result, we performed a direct histological evaluation of apoptosis in the caudal intervertebral discs with TUNEL (Figure-4N to Figure-4T). As shown in Figure-4U, there were relieved apoptosis in *miR-17-92*-ccKO group of 3 months.

#### 4. Knockout of *miR-17-92* Inhibits NPCs apoptosis by Activating PI3K/Akt Pathway

According to existing literature, we examined the classical pathways involved in the apoptosis of NPCs, and finally found the activation of PI3K/Akt in the nucleus pulposus cells of the *miR-17-92*-ccKO group of mice. As shown in Figure-5A, the mRNA level of Akt showed no difference between two groups. Similarly, Akt protein level did not differ significantly between the two groups (Figure-5B and Figure-5C), while the p-Ser473-Akt protein level was significantly up-regulated in *miR-17-92*-ccKO group (Figure-5B and Figure-5D), reminding us that the PI3K/Akt pathway was activated.

## Discussion

The basic research of IVDD has become the clinical hotspot, and as mentioned above, the ultimate goal of the related research is to find the non-surgical methods that can effectively relieve IVDD. Although not every study can be translated into clinical practice, the more complete the mechanism network of IVDD, the more abundant the treatment that can eventually be transformed. Therefore, studies on the mechanism of IVDD provide more possibilities[14].

The construction of non-coding RNA system has provided new ideas for the pathogenesis research of many diseases, including IVDD. Up to now, there have been more than hundreds of studies focusing on the correlation between non-coding RNA and IVDD, among which miR has been well studied. The results of this research provide new materials and possibilities for the construction and improvement of this system.

In previous work, we found that *miR-17-92* cluster was involved in cartilage development and also play an important regulatory role in bone healing. Accordingly, we attempted to verify the effect of *miR-17-92* cluster on IVDD. As described above, we firstly determined that the expression levels of *miR-17-92* cluster in the NP of patients with IVDD were significantly higher than those in the control group. Next, we build the *miR-17-92*-ccKO mice, and evaluate the degenerative levels of IVDs. We found that in 3 months and 24 months of mice, the degenerative levels of IVDs in *miR-17-92*-ccKO mice were much lower than that of the control group, further confirming the regulatory role of *miR-17-92* cluster in IVDD. In subsequent studies, we attempt to preliminary explore the regulating mechanism of *miR-17-92* cluster in the IVDD. There're no significant difference in the expression levels of Col2a1, Aggrecan, PCNA and Ki67 mRNA in the NP tissues of the two groups of mice, suggesting that *miR-17-92* cluster knockout did not affect the

ECM synthesis and proliferation functions of NPCs. The mRNA levels of Bax and Caspase-3 in the nucleus pulposus of the knockout group were significantly lower than those of the control group, while the mRNA levels of Bcl-2 and the ratio of Bcl-2/Bax were significantly higher than those of the control group, and the protein results were also consistent. Therefore, we believe that *miR-17-92* cluster knockout protect IVDs from inhibiting the apoptosis of NPCs. Furthermore, we demonstrate the above conclusions at the histological level with the TUNEL test. Finally, we screened the signaling pathways related to nucleus pulposus apoptosis with the help of existing literature reports, and the results indicated that the activity of PI3K/Akt pathway was significantly increased after *miR-17-92* cluster deletion. Therefore, we believe that *miR-17-92* cluster knockout can inhibit the apoptosis of nucleus pulposus cells by activating the PI3K/Akt pathway, and finally produce a protective effect on the IVDs against degeneration.

The main shortcomings of this paper which need further study in the future are as follows: (1) The elaboration of the relevant mechanism is not intact, which needs to be further analyzed with the mRNA high-throughput sequencing technology; (2) This study did not explore the possibility of clinical transformation.

## Conclusion

To sum up, *miR-17-92* cluster in IVDD does play an important regulating role, and preliminary results showed that *miR-17-92* cluster could activate the PI3K/Akt pathway, which in turn, inhibit the apoptosis of NPCs, eventually producing protective effect on IVDs against degeneration. In the future to further explore the specific mechanism, we will try to turn this conclusion for clinical transformation and application.

## Abbreviations

AF: annulus fibrosus

Col2a1: collagen II

ECM: extracellular matrix

EDTA: Ethylene Diamine Tetraacetic Acid

IVD: intervertebral disc

IVDD: intervertebral disc degeneration

miRNA: microRNA

MRI: magnetic resonance imaging

NP: nucleus pulposus

NPC: nucleus pulposus cell

qRT-PCR: Real-Time Fluorogenic Polymerase Chain Reaction

TUNEL: Terminal-deoxynucleotidyl Transferase Mediated Nick End Labeling

## **Declarations**

### **Ethics approval and consent to participate:**

The contents of the clinical specimen part of this study had been approved by the Ethics Committee of West China Hospital, Sichuan University and the Ethics Approvals have been provided. Informed consent was obtained from all subjects and all methods were carried out in accordance with relevant guidelines and regulations.

### **Animal Ethics approval:**

All experimental protocols of the animal part of this study had been approved by the Animal Ethics Committee of West China Hospital, Sichuan University and the Ethics Approvals have been provided. All methods were carried out in accordance with relevant guidelines and regulations.

### **Consent for publication:**

Not applicable.

### **ARRIVE guidelines:**

The study was carried out in compliance with ARRIVE guidelines.

### **Availability of data and material:**

Data will not be shared. These are the preliminary results of a long-term study. Many of the image content is only a selective display. We don't want to damage the originality of this study by sharing the results too much. But, the data is available from the corresponding author on reasonable request.

### **Competing interests:**

The authors declare that they have no conflict of interest.

### **Funding:**

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### **Authors' contributions:**

Yingjun Guo was responsible for the qRT-PCR, Western-blot, TUNEL and main part of the article writing.

Yang Meng was responsible for the construction of miR-17-92-ccKO mice.

Hao Liu was responsible for the design of concept and modeling device.

Xiaofei Wang was responsible for the histological evaluation of IVDD.

Ying Hong was responsible for the collection of clinical information.

Beiyu Wang was responsible for the figures making and language polishing.

Chen Ding was responsible for the data analysis.

Xijie Yu was responsible for the research and technology instruction.

All authors have reviewed the manuscript.

### **Acknowledgement:**

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## **References**

1. Zhao K, Zhang Y, Kang L, Song Y, Wang K, Li S, Wu X, Hua W, Shao Z, Yang S *et al*: **Epigenetic silencing of miRNA-143 regulates apoptosis by targeting BCL2 in human intervertebral disc degeneration.** *Gene* 2017, **628**:259-266.
2. Wang J, Liu X, Sun B, Du W, Zheng Y, Sun Y: **Upregulated miR-154 promotes ECM degradation in intervertebral disc degeneration.** *J Cell Biochem* 2019.
3. Yang C, Jia X, Zhou J, Sun Q, Ma Z: **The MiR-17-92 Gene Cluster is a Blood-Based Marker for Cancer Detection in Non-Small-Cell Lung Cancer.** *Am J Med Sci* 2020, **360**(3):248-260.
4. Plum PS, Warnecke-Eberz U, Drebber U, Chon SH, Alakus H, Holscher AH, Quaas A, Bruns CJ, Gockel I, Lorenz D *et al*: **Upregulation of miR-17-92 cluster is associated with progression and lymph node metastasis in oesophageal adenocarcinoma.** *Sci Rep* 2019, **9**(1):12113.
5. Liu H, Wu Z, Zhou H, Cai W, Li X, Hu J, Gao L, Feng T, Wang L, Peng X *et al*: **The SOX4/miR-17-92/RB1 Axis Promotes Prostate Cancer Progression.** *Neoplasia* 2019, **21**(8):765-776.
6. Wang WJ, Yang W, Ouyang ZH, Xue JB, Li XL, Zhang J, He WS, Chen WK, Yan YG, Wang C: **MiR-21 promotes ECM degradation through inhibiting autophagy via the PTEN/akt/mTOR signaling pathway in human degenerated NP cells.** *Biomed Pharmacother* 2018, **99**:725-734.
7. Lin H, Zhang W, Zhou T, Li W, Chen Z, Ji C, Zhang C, He F: **Mechanism of microRNA-21 regulating IL-6 inflammatory response and cell autophagy in intervertebral disc degeneration.** *Exp Ther Med* 2017, **14**(2):1441-1444.

8. Chen B, Huang SG, Ju L, Li M, Nie FF, Zhang Y, Zhang YH, Chen X, Gao F: **Effect of microRNA-21 on the proliferation of human degenerated nucleus pulposus by targeting programmed cell death 4.** *Braz J Med Biol Res* 2016, **49**(6).
9. Yang S, Li L, Zhu L, Zhang C, Li Z, Guo Y, Nie Y, Luo Z: **Aucubin inhibits IL-1beta- or TNF-alpha-induced extracellular matrix degradation in nucleus pulposus cell through blocking the miR-140-5p/CREB1 axis.** *J Cell Physiol* 2019, **234**(8):13639-13648.
10. Zhang Q, Weng Y, Jiang Y, Zhao S, Zhou D, Xu N: **Overexpression of miR-140-5p inhibits lipopolysaccharide-induced human intervertebral disc inflammation and degeneration by downregulating toll-like receptor 4.** *Oncol Rep* 2018, **40**(2):793-802.
11. Penolazzi L, Lambertini E, Bergamin LS, Roncada T, De Bonis P, Cavallo M, Piva R: **MicroRNA-221 silencing attenuates the degenerated phenotype of intervertebral disc cells.** *Aging (Albany NY)* 2018, **10**(8):2001-2015.
12. Griffith JF, Wang YX, Antonio GE, Choi KC, Yu A, Ahuja AT, Leung PC: **Modified Pfirrmann grading system for lumbar intervertebral disc degeneration.** *Spine (Phila Pa 1976)* 2007, **32**(24):E708-712.
13. Tam V, Chan WCW, Leung VYL, Cheah KSE, Cheung KMC, Sakai D, McCann MR, Bedore J, Seguin CA, Chan D: **Histological and reference system for the analysis of mouse intervertebral disc.** *J Orthop Res* 2018, **36**(1):233-243.
14. Shao Z, Ni L, Hu S, Xu T, Meftah Z, Yu Z, Tian N, Wu Y, Sun L, Wu A *et al*: **RNA-binding protein HuR suppresses senescence through Atg7 mediated autophagy activation in diabetic intervertebral disc degeneration.** *Cell Prolif* 2021, **54**(2):e12975.

## Tables

**Table-1.** Histological Scores of IVDD

NP Structure		
No.	Description	Scores
<input type="checkbox"/>	Single NP cell mass with no or very little segregation of cells	0
<input type="checkbox"/>	Honey comb or cell cluster formation with <50% cell loss	1
<input type="checkbox"/>	Honey comb or cell cluster formation with >50% cell loss	2
<input type="checkbox"/>	Matrix-rich NP compartment with little or no cells	3
<input type="checkbox"/>	Mineralised matrix in NP/loss of NP compartment	4
NP Clefts/Fissures		
No.	Description	Scores
<input type="checkbox"/>	No clefts	0
<input type="checkbox"/>	Mild clefts/fissures and the length of the longest cleft <50% of NP compartment	1
<input type="checkbox"/>	Severe clefts/fissures and the length of the longest cleft >50% of NP compartment	2
AF Structure		
No.	Description	Scores
<input type="checkbox"/>	Concentric lamellar structure	0
<input type="checkbox"/>	Serpentine AF/Widened AF lamellae/Rounded lamellae cells	1
<input type="checkbox"/>	Reversal of lamellae	2
<input type="checkbox"/>	Lamellae structure not defined and may penetrate into NP compartment	3
<input type="checkbox"/>	Mineralised matrix in AF/loss of AF compartment	4
AF Clefts/Fissures		
No.	Description	Scores
<input type="checkbox"/>	No clefts	0
<input type="checkbox"/>	Mild clefts/fissures and the length of the longest cleft <50% of AF compartment	1
<input type="checkbox"/>	Severe clefts/fissures and the length of the longest cleft >50% of AF compartment	2
AF/NP Boundary		
No.	Description	Scores
<input type="checkbox"/>	Clear cut boundary between the AF and NP	0
<input type="checkbox"/>	Round chondrocyte-like cells at the boundary/discontinuity of the AF/NP boundary	1
<input type="checkbox"/>	Loss of AF/NP boundary	2

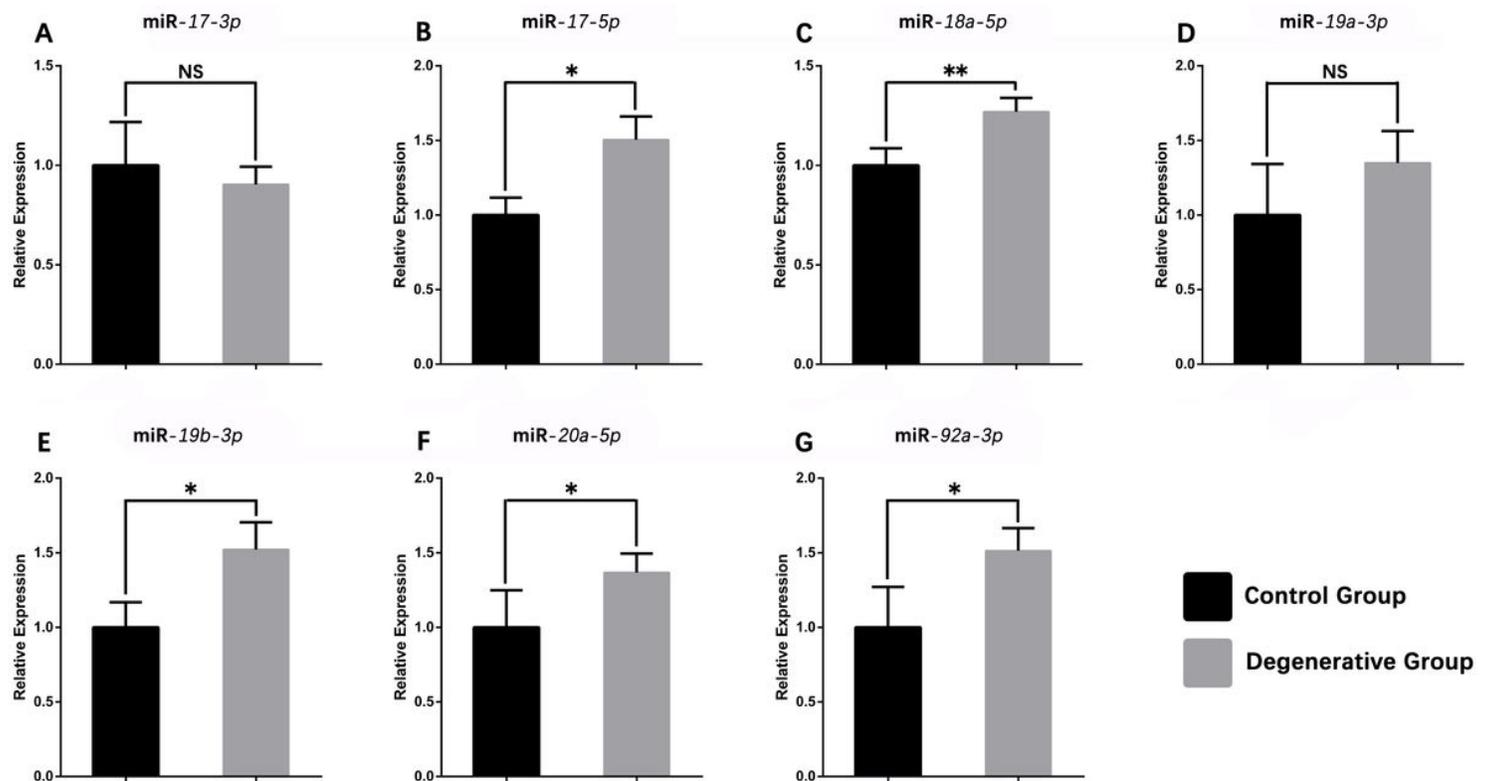
**Note.** NP, nucleus pulposus; AF, annulus fibrosus.

**Table-2.** Primer Sequence Information

Gene Names	Forward Primer	Reverse Primer
<i>β-actin</i>	CTACCTCATGAAGATCCTGACC	CACAGCTTCTCTTTGATGTCAC
<i>Col2a1</i>	TACTGGAGTGACTGGTCCTAAG	AACACCTTTGGGACCATCTTTT
<i>Aggrecan</i>	GGAGACCCAGACAGCAGAAACAAC	GCAGGTGGCTCCATTTCAGACAAG
<i>PCNA</i>	GAAGTTTTCTGCAAGTGGAGAG	CAGGCTCATTTCATCTCTATGGT
<i>Ki67</i>	CACAGAGAACAAAGGTGTGAAG	GGAGACTGCAGAGCTATTTTTTG
<i>Bcl-2</i>	GATGACTTCTCTCGTCGCTAC	GAACTCAAAGAAGGCCACAATC
<i>Bax</i>	TTGCCCTCTTCTACTTTGCTAG	CCATGATGGTTCTGATCAGCTC
<i>Caspase-3</i>	GAAACTCTTCATCATTTCAGGCC	GCGAGTGAGAATGTGCATAAAT
<i>Akt</i>	TGCACAAACGAGGGGAATATAT	CGTTCCTTGTAGCCAATAAAGG

**Note.** Col2a1, collagen II; PCNA, Proliferating Cell Nuclear Antigen.

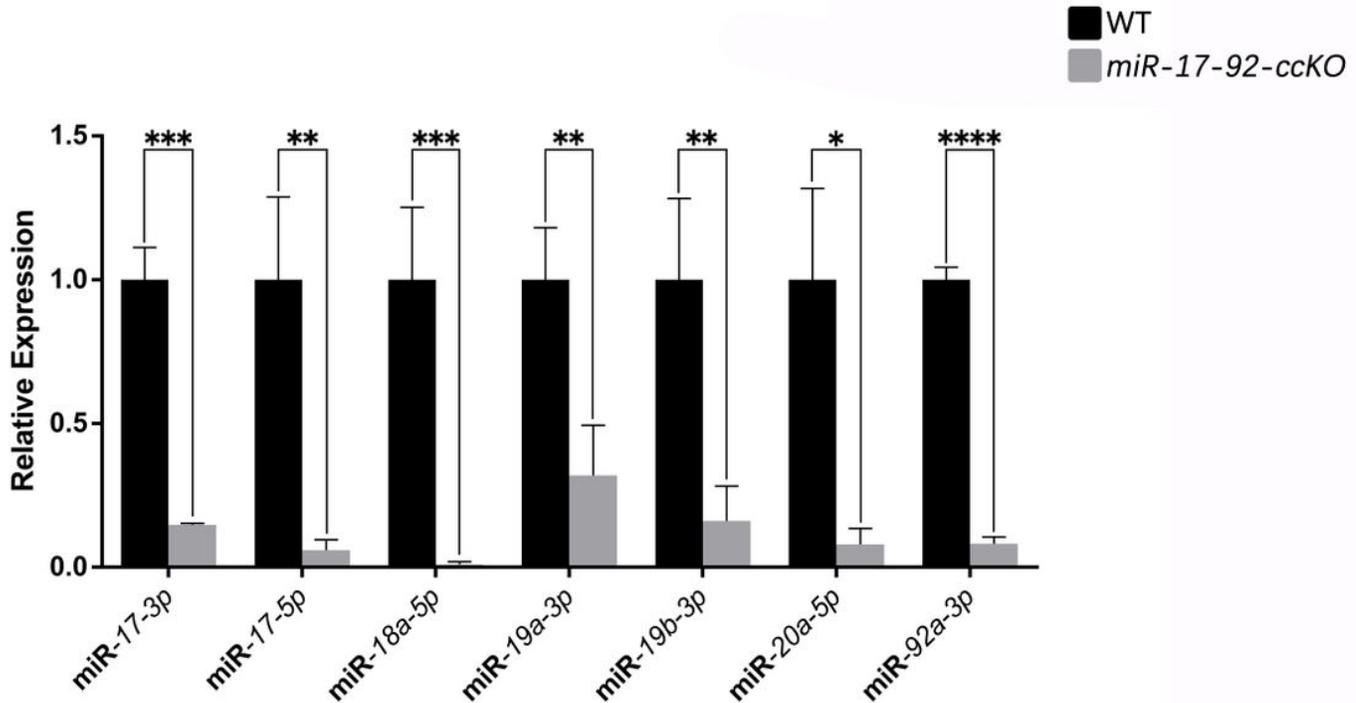
## Figures



**Figure 1**

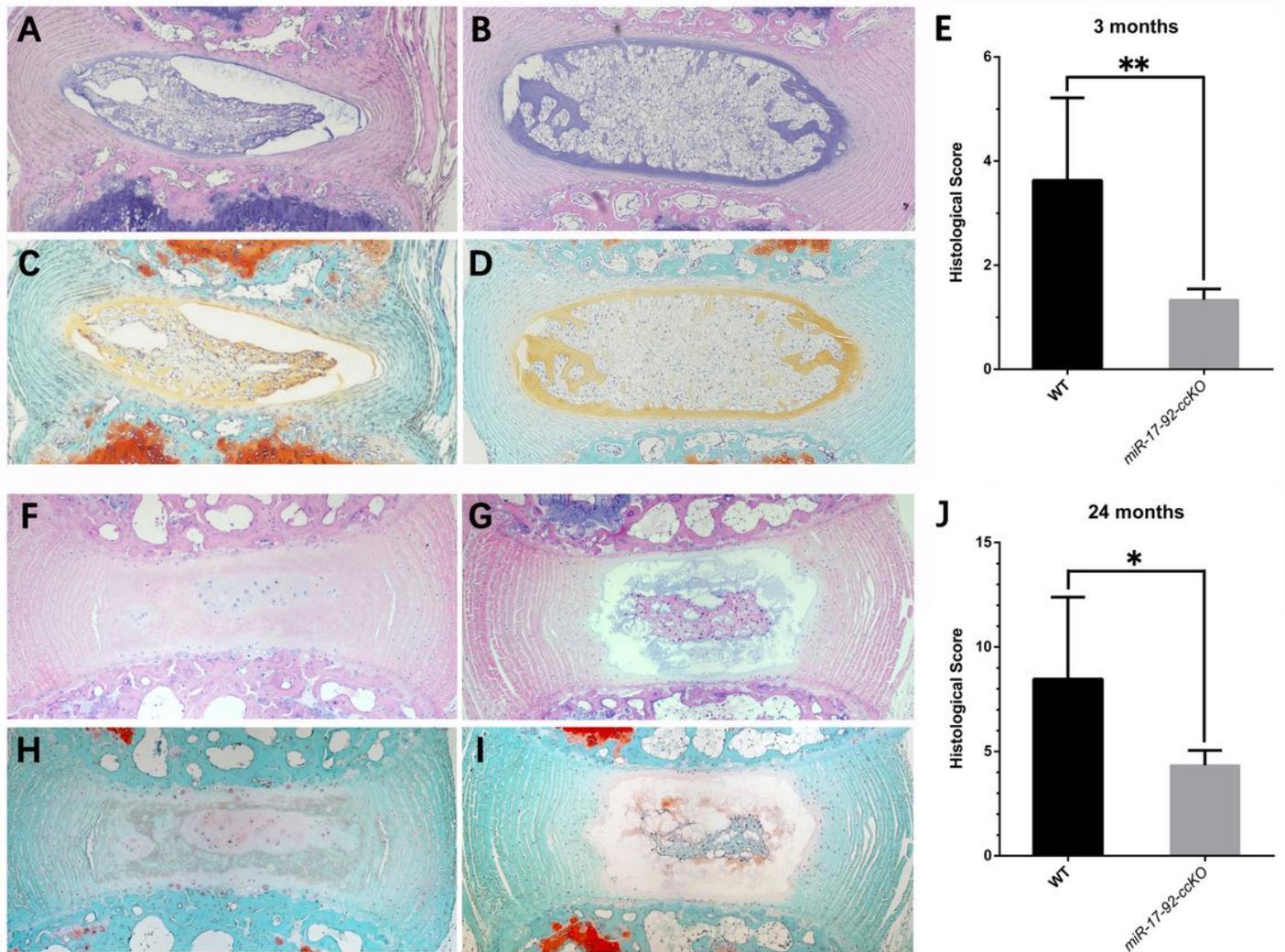
Differential Expression of miR-17-92 Cluster in NP in Control and Degenerative Group of Patients Figure-1A, no significant higher expression level of miR-17-3p was found in degenerative group (P=0.4616).

Figure-1B, there's significant higher expression level of miR-17-5p in degenerative group (P=0.0113).  
 Figure-1C, there's significant higher expression level of miR-18-5p in degenerative group (P=0.0061).  
 Figure-1D, there's significant higher expression level of miR-19a-3p in degenerative group (P=0.2014).  
 Figure-1E, there's significant higher expression level of miR-19b-3p in degenerative group (P=0.0231).  
 Figure-1F, there's significant higher expression level of miR-19b-3p in degenerative group (P=0.0470).  
 Figure-1G, there's significant higher expression level of miR-19b-3p in degenerative group (P=0.0212).



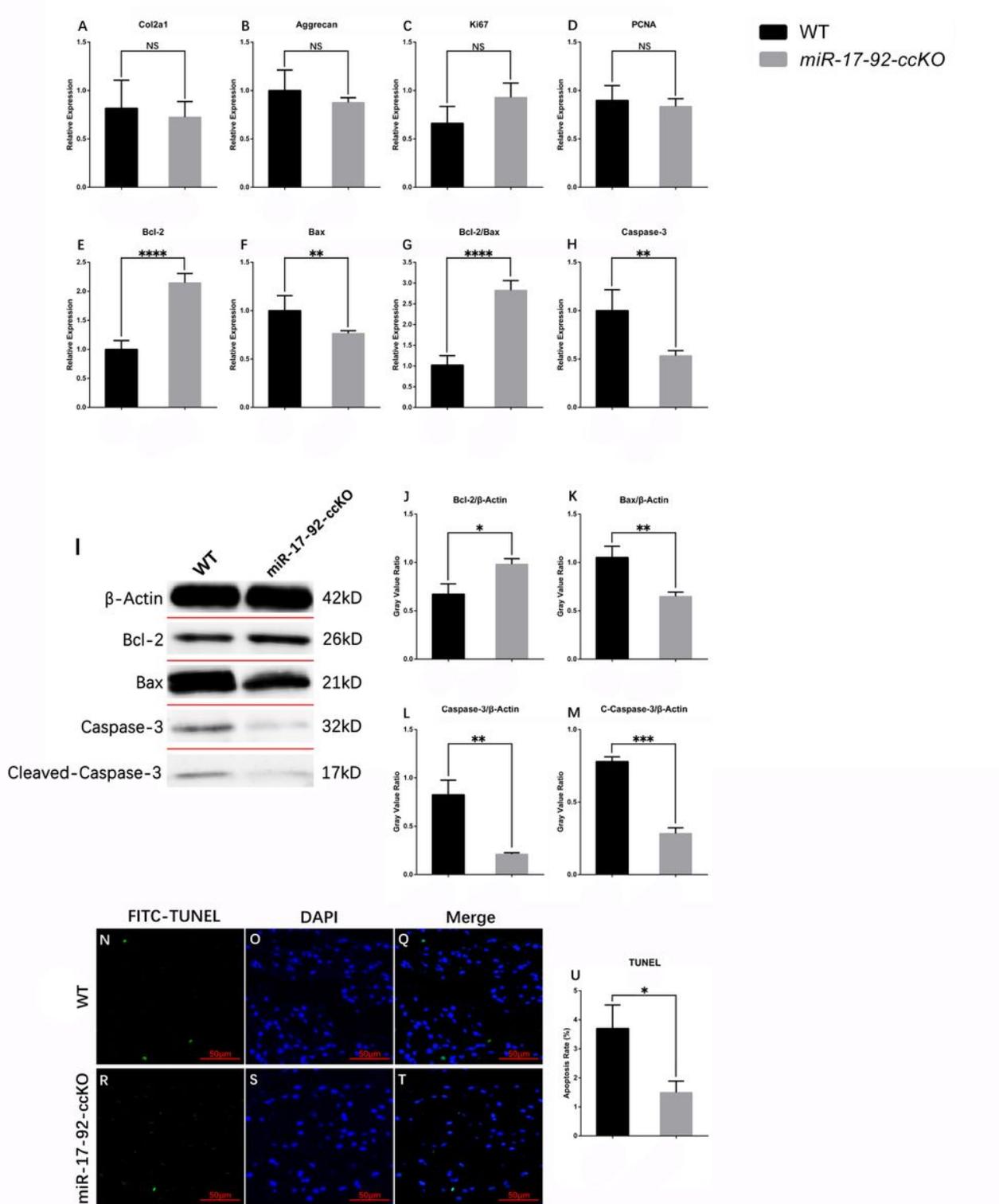
**Figure 2**

Knockout Verification of miR-17-92 Cluster in NP of miR-17-92-ccKO Mice The expression level of members of the miR-17-92 cluster in the NP tissues of miR-17-92-ccKO mice was significantly lower than that of WT mice, indicating that the effect of endochondral knockout was verified in the NP tissues.



**Figure 3**

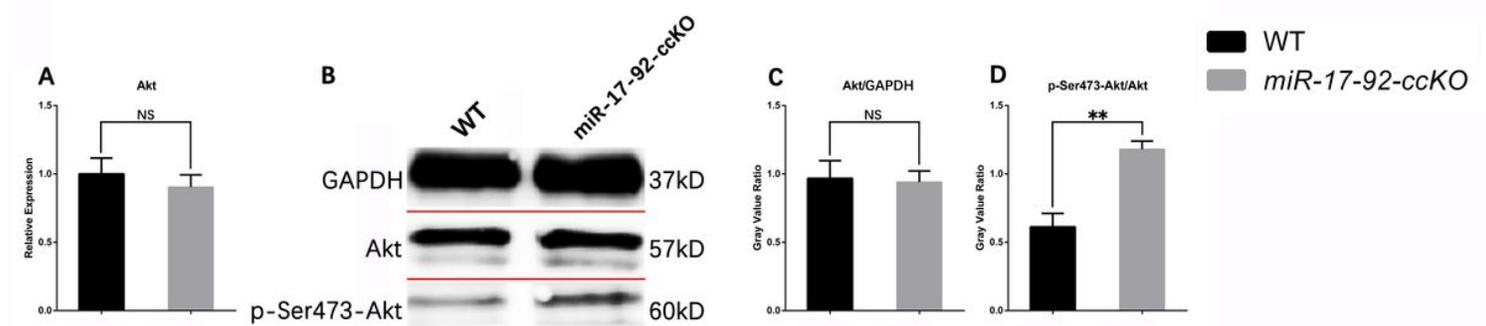
Histological Comparison of IVDs between WT and Degenerative Group of Mice Figure-3A, HE staining of 3-month caudal IVD in WT group. Figure-3B, HE staining of 3-month caudal IVD in miR-17-92-ccKO group. Figure-3C, Safranin O Staining of 3-month caudal IVD in WT group. Figure-3D, Safranin O Staining of 3-month caudal IVD in miR-17-92-ccKO group. Figure-3E, comparison of histological scores of 3-month caudal IVD between WT and miR-17-92-ccKO group (P=0.0071). Figure-3F, HE staining of 24-month caudal IVD in WT group. Figure-3G, HE staining of 24-month caudal IVD in miR-17-92-ccKO group. Figure-3H, Safranin O Staining of 24-month caudal IVD in WT group. Figure-3I, Safranin O Staining of 24-month caudal IVD in miR-17-92-ccKO group. Figure-3J, comparison of histological scores of 24-month caudal IVD between WT and miR-17-92-ccKO group (P=0.0403).



**Figure 4**

Knockout of miR-17-92 Inhibit Apoptosis in NPCs of Mice Figure-4A, there's no significant difference in the mRNA expression level of Col2a1 in NP tissues between WT and miR-17-92-ccKO group of mice (P=0.6569). Figure-4B, there's no significant difference in the mRNA expression level of Aggrecan in NP tissues between WT and miR-17-92-ccKO group of mice (P=0.2478). Figure-4C, there's no significant difference in the mRNA expression level of Ki67 in NP tissues between WT and miR-17-92-ccKO group of

mice ( $P=0.1692$ ). Figure-4D, there's no significant difference in the mRNA expression level of PCNA in NP tissues between WT and miR-17-92-ccKO group of mice ( $P=0.5570$ ). Figure-4E, the mRNA expression level of Bcl-2 in NP tissues is significantly higher in miR-17-92-ccKO than that of WT group of mice ( $P\leq 0.0001$ ). Figure-4F, the mRNA expression level of Bax in NP tissues is significantly higher in WT than that of miR-17-92-ccKO group of mice ( $P=0.0065$ ). Figure-4G, the mRNA expression level ratio of Bcl-2/Bax in NP tissues is significantly higher in miR-17-92-ccKO than that of WT group of mice ( $P\leq 0.0001$ ). Figure-4H, the mRNA expression level of Caspase-3 in NP tissues is significantly higher in WT than that of miR-17-92-ccKO group of mice ( $P=0.0011$ ). Figure-4I, Western-blot results of apoptosis-related genes in NP tissues of WT and miR-17-92-ccKO group of mice (This grouping of blots cropped from different gels). Figure-4J, the protein expression level ratio of Bcl-2/ $\beta$ -Actin in NP tissues is significantly higher in miR-17-92-ccKO than that of WT group of mice ( $P=0.0214$ ). Figure-4K, the protein expression level ratio of Bax/ $\beta$ -Actin in NP tissues is significantly higher in miR-17-92-ccKO than that of WT group of mice ( $P=0.0074$ ). Figure-4L, the protein expression level ratio of Caspase-3/ $\beta$ -Actin in NP tissues is significantly higher in WT than that of miR-17-92-ccKO group of mice ( $P=0.0023$ ). Figure-4M, the protein expression level ratio of C-Caspase-3/ $\beta$ -Actin in NP tissues is significantly higher in WT than that of miR-17-92-ccKO group of mice ( $P=0.0003$ ). Figure-4N, apoptosis-positive cells with FITC-TUNEL label in caudal IVD of 3-month WT group of mice. Figure-4O, cell nucleus with DAPI label in caudal IVD of 3-month WT group of mice. Figure-4Q, merge result of FITC-TUNEL and DAPI in caudal IVD of 3-month WT group of mice. Figure-4R, apoptosis-positive cells with FITC-TUNEL label in caudal IVD of 3-month miR-17-92-ccKO mice of mice. Figure-4S, cell nucleus with DAPI label in caudal IVD of 3-month miR-17-92-ccKO group of mice. Figure-4T, merge result of FITC-TUNEL and DAPI in caudal IVD of 3-month miR-17-92-ccKO mice of mice. Figure-4U, there's a significantly higher apoptotic rate of NPCs in WT than that of miR-17-92-ccKO group of mice ( $P=0.0226$ ).



**Figure 5**

Knockout of miR-17-92 Activates PI3K/Akt Pathway Figure-5A, there's no significant difference in the mRNA expression level of Akt in NP tissues between WT and miR-17-92-ccKO group of mice ( $P=0.8216$ ). Figure-5B, Western-blot results of Akt and p-Ser473-Akt in NP tissues of WT and miR-17-92-ccKO group of mice (This grouping of blots cropped from different gels). Figure-5C, there's no significant difference in the protein expression level ratio of Akt/GAPDH in NP tissues between WT and miR-17-92-ccKO group of

mice (P=0.3620). Figure-5D, the protein expression level ratio of p-Ser473-Akt/Akt in NP tissues is significantly higher in miR-17-92-ccKO than that of WT group of mice (P=0.0074).