

Original Research

# FOSL2 Promotes Chondrogenic Differentiation of Bone Marrow Mesenchymal Stem Cells via Upregulation of CAMK2D in Osteoarthritis

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## Abstract

**Background:** FOSL2 has been linked to inflammation and tissue remodeling, but its role in osteoarthritis (OA) remains unclear. OA is characterized by progressive cartilage loss and limited regenerative capacity. **Methods:** FOSL2 expression was first measured in cartilage samples from OA patients undergoing total knee arthroplasty. Bone marrow mesenchymal stem cells (BMSCs) were then induced to undergo chondrogenic differentiation *in vitro*. FOSL2 was knocked down or overexpressed, and downstream effects on chondrogenic and catabolic markers were assessed. CAMK2D was identified as a potential downstream target by RNA sequencing. Finally, FOSL2-knockdown BMSCs were injected intra-articularly into Sprague-Dawley rats with ACLT-induced OA. **Results:** FOSL2 was lower in cartilage-defective areas than in relatively normal cartilage. During BMSC chondrogenic differentiation, FOSL2 was upregulated along with COLII and SOX9, whereas ADAMTS5, MMP13, and COL10 were downregulated. FOSL2 knockdown impaired chondrogenic potential. FOSL2 regulated CAMK2D expression. *In vivo*, FOSL2-knockdown BMSCs exhibited reduced cartilage repair capacity in rat OA joints. **Conclusions:** FOSL2 promotes BMSC chondrogenic differentiation through CAMK2D and improves cartilage repair in OA. FOSL2 represents a potential therapeutic target for OA.

**Keywords:** osteoarthritis; bone marrow mesenchymal stem cells; cartilage; FOSL2; CAMK2D

## 1. Introduction

Articular chondrocytes (AC) form an extremely tough tissue that covers the surfaces of bones and plays a critical role in joint protection [1]. From a biomechanical perspective, it serves two essential functions: providing a smooth, lubricated surface for near-frictionless joint movement, and acting as a shock absorber to distribute and transmit mechanical loads to the underlying subchondral bone. This tissue is a dense connective structure primarily composed of collagen and glycosaminoglycans [2]. Collagen fibers are oriented perpendicular to the weight-bearing surface and are integrated with a 3D network of highly hydrated glycosaminoglycans [3]. These components form a compressive elastic system with excellent energy dissipation capability [4]. Chondrocyte damage compromises the structural integrity of healthy articular cartilage. Collagen undergoes abnormal remodeling, and the dynamic balance of glycosaminoglycans is disrupted. These molecular and structural alterations result in the collapse of the biomechanical properties of cartilage.

Clinically, osteoarthritis (OA) manifests as joint pain, stiffness, swelling, and dysfunction [5]. As one of the most common diseases in orthopedics, OA has a high disability

rate. Currently, the incidence and prevalence of OA are increasing yearly, especially among the elderly [6,7]. The development of OA is driven by multiple risk factors, including advanced age, obesity, genetic predisposition, previous joint trauma, and abnormal mechanical loading. Most patients experience OA in the knee and hip joints, leading to progressive disability and eventually requiring joint replacement [8]. Notably, knee OA is now recognized as a whole joint disease, with pathological changes extending beyond articular cartilage to include subchondral bone remodeling, synovial inflammation, meniscal degeneration, and biomechanical alterations of the infrapatellar fat pad. The progressive destruction of articular cartilage represents the central pathological event in OA progression, characterized by decreased synthesis of cartilage extracellular matrix, upregulated activity of catabolic enzymes, and chondrocyte hypertrophy, ultimately leading to the loss of cartilage structural integrity [9,10]. At present, there is no cure for OA. Patients rely on lifestyle modifications and pharmacological treatment [11,12,13]. Some medications, such as glucosamine, chondroitin, acetaminophen, and celecoxib, can help relieve OA-related pain [14]. However, these options control symptoms without altering dis-



ease progression. End-stage OA patients require joint replacement surgery [15]. No disease-modifying therapy is currently available, and strategies to prevent and slow OA progression are urgently needed.

Mesenchymal stem cells (MSCs), particularly those derived from bone marrow mesenchymal stem cells (BMSCs), possess the potential to differentiate into chondrocytes and exhibit anti-inflammatory and immunomodulatory properties [16,17]. BMSCs are considered an ideal MSC source and a promising therapeutic strategy for repairing various damaged tissues, including injured cartilage [18,19]. Thus, the chondrogenic differentiation of BMSCs plays an indispensable role in cartilage repair [20,21]. Furthermore, BMSC regeneration has been reported to have great therapeutic potential in knee OA treatment [22,23,24]. The chondrogenic differentiation of MSCs is crucial for successful cartilage regeneration, as differentiated chondrocytes can mediate stable cartilage formation [25]. However, this differentiation process rarely occurs spontaneously. Uncovering the underlying mechanisms driving BMSCs chondrogenesis is therefore essential for effective OA cartilage repair. Therefore, elucidating the key molecular mechanisms driving BMSC chondrogenic differentiation is of great significance for optimizing BMSC-based therapeutic strategies and achieving effective cartilage repair.

Activator protein 1 (AP-1) is a transcription factor complex primarily composed of proteins encoded by early response genes. This complex responds rapidly to internal and external stimuli to regulate intracellular transcription, thereby coordinating cellular behaviors such as proliferation and differentiation [26]. Notably, AP-1 has been reported to drive the production of pro-inflammatory cytokines, including IL-1 $\beta$ , which promotes the progression of OA [27].

Based on amino acid sequence similarity, these transcription factors can be divided into two subfamilies: FOS and JUN [28]. The *FOSL2* gene encodes Fos-related antigen 2 (Fra-2), which is a crucial member of the FOS family [29]. The Fra-2 protein is a 46 kDa protein homologous to FosB and Fra-1 [30,31]. Due to its homology and detection via anti-Fos serum, it was named Fos-related antigen 2 (FOSL2, Fra-2) [30]. FOSL2 plays vital roles in various diseases, such as colorectal cancer, myocardial fibrosis, and rheumatoid arthritis [32,33,34]. Clinical evidence has shown that FOSL2 expression is significantly down-regulated in the synovial tissue of OA patients compared with healthy controls, suggesting its involvement in OA pathogenesis [35]. In the pathogenesis and progression of OA, FOSL2 may play a significant role by modulating joint cartilage inflammation and bone remodeling. The study has shown that FOSL2 expression is indirectly regulated via mmu\_circRNA\_003795 sponging miR-504-3p, and its upregulation enhances BMSC proliferation [36]. BMSCs can differentiate into chondrocytes and fill cartilage defects, thereby slowing OA progression. In skeletal development,

reduced FOSL2 expression results in abnormally large osteoclast formation [37]. Although direct evidence in OA is still limited, these findings suggest that FOSL2 may contribute to OA pathophysiology by influencing both cartilage repair potential and subchondral bone dynamics. This highlights FOSL2 as a promising candidate for further investigation as a therapeutic target.

Based on these unresolved questions, the present study was designed to determine the expression pattern of FOSL2 in OA cartilage and to evaluate its functional involvement in BMSC chondrogenic differentiation *in vitro* and *in vivo*. FOSL2 expression was first compared between damaged and relatively normal cartilage from OA patients. The effects of FOSL2 knockdown and overexpression on BMSC chondrogenic differentiation were then assessed *in vitro*. CAMK2D was further identified as a downstream transcriptional target of FOSL2 through RNA sequencing. Finally, the *in vivo* reparative capacity of FOSL2-knockdown BMSCs was examined in rat models of OA.

## 2. Materials and Methods

### 2.1 Cell Source

Primary rat bone marrow mesenchymal stem cells (Sprague-Dawley rat MSCs) were purchased from Xinrun Biotechnology Co., Ltd. (PR2075, China). According to the supplier's certificate/product information, the cells were originally obtained from the Cell Bank/Stem Cell Bank, SIBCB, Chinese Academy of Sciences. All cells were validated by the supplier using immunophenotypic characterization of mesenchymal stem cell surface markers, showing positivity for CD29, CD44, and CD90 and negativity for CD45 and CD117. The cells were supplied at passage 2 and were confirmed to be negative for mycoplasma and bacterial contamination before use.

These cells were cultured in complete Alpha Minimum Essential Medium ( $\alpha$ -MEM; Gibco, Thermo Fisher Scientific, USA; Cat. No. 12561-056) supplemented with 10% MSC-qualified fetal bovine serum (FBS; Gibco; Cat. No. 12662-029) and 1% penicillin-streptomycin (Gibco; Cat. No. 15140-122). The cells were maintained in a humidified incubator at 37 °C with 5% CO<sub>2</sub>. The culture medium was replaced every 2 to 3 days to remove non-adherent cells and cellular debris. Upon reaching 80–90% confluence, the cells were passaged using 0.25% Trypsin-EDTA (Gibco; Cat. No. 25200-056). Cells from passages 3 to 5 were utilized for all subsequent experiments.

### 2.2 Cartilage Sample Collection From the Knee Joint

This study was approved by the Ethics Committee of the Sixth Affiliated Hospital of Nantong University (Approval No. Lunshen-2022-102) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participating patients prior to their inclusion in the study. Articular cartilage was obtained from 24 patients (11 males and 13 females, aged 55–

85 years) undergoing total knee arthroplasty due to knee osteoarthritis (OA, Kellgren-Lawrence grade III or IV). The inclusion criteria were patients diagnosed with primary knee OA who required total knee arthroplasty. The exclusion criteria included: (1) secondary OA (e.g., post-traumatic or rheumatoid arthritis); (2) history of joint infection, tumors, or severe systemic immune diseases; and (3) intra-articular injections (e.g., corticosteroids or hyaluronic acid) within six months prior to surgery. Typically, the medial cartilage of the knee is more severely damaged than the lateral side. Based on the severity of cartilage erosion, samples were classified as OA (severely damaged) and relatively normal (RN, less damaged) sides. All cartilage samples were harvested from the femoral condyle and stored at  $-80^{\circ}\text{C}$  until use.

### 2.3 Animal Models

Male SD rats (6–8 weeks old, 300–350 g) were purchased from SPF Biotechnology Co., Ltd. (Suzhou, Jiangsu, China). The animals were housed in a Specific Pathogen-Free (SPF) facility under standard laboratory conditions (temperature  $22 \pm 2^{\circ}\text{C}$ , relative humidity  $50 \pm 10\%$ , and a 12 h light/dark cycle) with ad libitum access to standard rodent chow and water. After one week of acclimatization and training, the rats were randomly assigned into four groups ( $n = 10$  per group): sham, OA, BMSC, and BMSC+si-FOSL2. Except for the sham group, which underwent skin incision and suture only, all other groups received anterior cruciate ligament transection and destabilization of the medial meniscus surgery [38]. Briefly, the ACL, medial meniscus, and medial meniscotibial ligament were transected after removing the fat pad. All rats were anesthetized with isoflurane (induction at 3–5%, maintenance at 1.5–2.5%) with oxygen flow at 1–2 L/min. Once anesthetized, the right knee was exposed through a medial parapatellar incision. The patella was dislocated laterally, and the ACL was fully transected with micro-scissors. Complete transection was confirmed by a positive anterior drawer test. If the drawer test result is negative, the model animals will be excluded and not included in any of the experimental groups. Partial meniscectomy was performed to induce joint instability while preserving joint structure.

On day 28 post-surgery, intra-articular injections were performed weekly for four weeks. The injected cells were primary BMSCs derived from SD rats. The treatments were administered as follows: the sham and OA groups received 0.3 mL PBS; the BMSC group received 0.3 mL PBS containing  $1 \times 10^6$  control BMSCs. BMSC+si-FOSL2 group received 0.3 mL of FOSL2-knockdown BMSCs at the same concentration. The selected cell number and injection volume were determined based on established protocols from previous literature investigating BMSC-based therapies for rat OA models, alongside our preliminary optimizations to ensure therapeutic efficacy. At the end of the study period, all rats were euthanized by an intraperitoneal injection of

an overdose of sodium pentobarbital (150 mg/kg, concentration: 3%). The study period was from June 1, 2024 to February 5, 2025.

All animal procedures complied with national animal welfare standards, and the experimental protocol was approved by the Ethics Committee of Jiangsu Vocational College of Medicine (Approval No. SYLL-2025-700) and were conducted in accordance with the Standard Operating Procedures for the Laboratory Animal Center of Jiangsu Medical Vocational College and the relevant institutional animal welfare and ethical requirements. The animal study was conducted and reported in compliance with the ARRIVE 2.0 guidelines [39].

Cartilage damage was evaluated using the Mankin scoring system (range 0–14), which assesses four parameters: (1) structure (0–6 points), (2) cellularity (0–3 points), (3) matrix staining (0–4 points), and (4) tidemark integrity (0–1 point). Higher scores indicate more severe cartilage damage. All sections were scored by two independent observers who were blinded to the group allocation. The mean score of the two observers was used for statistical analysis.

### 2.4 Alcian Blue Staining

BMSCs were induced to undergo chondrogenic differentiation using a chondrogenic differentiation kit (Beoyotime, Nanjing, Jiangsu, China). Briefly, third-passage BMSCs were seeded into 24-well plates at a density of  $1 \times 10^5$  cells/well and incubated with chondrogenic induction medium. After incubation for 0, 3, 7, and 14 days, the cells were fixed with 4% paraformaldehyde for 15 minutes, washed with PBS, and then incubated with 0.1% Alcian Blue 8GX (Solarbio, Beijing, China) for 40 minutes.

### 2.5 Cell Transfection

Lentiviral and siRNA transfection experiments were performed when the cells were actively proliferating and had reached a confluence of 40%–70%. Prior to lentiviral transfection, a gradient of MOI was tested in a 96-well plate to determine the optimal MOI (with a fluorescence expression rate of 80%–90%). Cells were then seeded in 6-well plates at a density of  $5 \times 10^4$  cells/mL and cultured for 24 hours before adding the virus according to the selected MOI of 30 (i.e., 30 transducing units per cell). Recombinant lentiviruses expressing the rat FOSL2 gene (Lv-FOSL2) or the empty vector control (Lv-NC), both carried an EGFP reporter and titrated at  $\geq 1 \times 10^8$  TU/mL. After 8–12 hours of infection, the medium was replaced with complete medium, and after 48–72 hours, cells were selected using complete medium containing 2  $\mu\text{g/mL}$  puromycin. Successfully transfected cells were labeled as Lv-NC and Lv-FOSL2. When the cell confluence exceeded 90%, total protein and RNA were extracted.

For siRNA transfection, cells were seeded in 6-well plates at a density of  $2 \times 10^5$  cells/well one day in advance. When the cell density reached approximately 70% the fol-

**Table 1. Primer sequences.**

| Gene                    | Sequence (5' to 3')                                              | Species |
|-------------------------|------------------------------------------------------------------|---------|
| GAPDH                   | F:5'-AGGTCGGTGTGAACGGATT-3'<br>R:5'-AACTTGCCGTGGGTAGAGTC-3'      | Rat     |
| FOSL2                   | F:5'-GCCAGCAGCTGTCTCC-3'<br>R:5'-ACTGATTTGCACACAGGGC-3'          | Rat     |
| COL II                  | F:5'-CTGCAGAATGGGCAGAGGTAT-3'<br>R:5'-TGGCCCTAATTTTCCACTGGC-3'   | Rat     |
| ADAMTS5                 | F:5'-GCCCACCTAACGGCAAATCT-3'<br>R:5'-ACCACGTAATAGCCAGTGCC-3'     | Rat     |
| MMP13                   | F:5'-GGAGCCCTGATGTTTCCCAT-3'<br>R:5'-GCAGACGCCAGAAGATCTGT-3'     | Rat     |
| SOX9                    | F:5'-AGTACCCGCATCTGCACAAC-3'<br>R:5'-ACGAAGGGTCTCTTCTCGCT-3'     | Rat     |
| CAMK2D                  | F:5'-AGGGACCTGAAGCCTGAGAA-3'<br>R:5'-CTTCATCCCAGAAGGGTGGG-3'     | Rat     |
| COL10                   | F:5'-ACGCCATAAAGAGTAAAGGGAT-3'<br>R:5'-GCTTCCCCGTGGCTGATATT-3'   | Rat     |
| GAPDH                   | F:5'-TGTGGGCATCAATGGATTGG-3'<br>R:5'-ACACCATGTATTCCGGGTCAAT-3'   | Human   |
| FOSL2                   | F:5'-CAGAAATTCCGGGTAGATATGCC-3'<br>R:5'-GGTATGGGTTGGACATGGAGG-3' | Human   |
| si-FOSL2#1 (474-496)    | Target sequence: 5'-GCACTTCAAACCTTGTCTTTCA-3'                    | Rat     |
| si-FOSL2#2 (1988-2010)  | Target sequence: 5'-TGGGACTTAGCAGTGAGTATTGG-3'                   | Rat     |
| si-FOSL2#3 (3551-3573)  | Target sequence: 5'-TCCTCGTTTGAAGATAATTAAG-3'                    | Rat     |
| si-CaMK2D#1 (1812-1834) | Target sequence: 5'-CACCTCTTGTGGCAAAACATCT-3'                    | Rat     |
| si-CAMK2D#2 (1941-1963) | Target sequence: 5'-ATGTTTCTGAGTGCATGAAGTTG-3'                   | Rat     |
| si-CAMK2D#3 (533-555)   | Target sequence: 5'-AAGACATAGTGGCAAGAGAATAT-3'                   | Rat     |
| si-CAMK2D#4 (1102-1124) | Target sequence: 5'-TGCTTGAAGAAATTAATGCTAG-3'                    | Rat     |

lowing day, Lipofectamine 3000 and siRNA (final concentration 50 nM) were diluted separately in Opti-MEM, gently mixed, incubated for a specified period (e.g., 15 minutes), and then added to the cells. The transfection mixture was incubated with the cells for 6 hours. After 48 hours, total protein and RNA were extracted. The siRNA targeting rat FOSL2 (si-FOSL2, three independent target sequences), targeting rat CAMK2D (si-CAMK2D, 4 independent target sequences) and a non-targeting scrambled negative control (si-NC) were designed, synthesized, and HPLC-purified by GenScript (Nanjing, Jiangsu, China). All target sequences are listed in Table 1.

## 2.6 qRT-PCR

Total RNA was extracted from BMSCs and cartilage tissue using the TRIzol reagent kit (Invitrogen, USA). Reverse transcription of total RNA was performed using the HiScript® II Q RT SuperMix for qPCR kit (Vazyme, China). Quantitative PCR was carried out on an Applied Biosystems QuantStudio 5 real-time PCR system (Thermo Fisher Scientific, USA) using the ChamQ SYBR qPCR Master Mix (Vazyme, China). The amplification protocol was as follows: initial denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Melt-

ing curve analysis (65–95 °C, 0.5 °C/5 s) was performed at the end of each run to confirm amplification specificity. Primer sequences used for rat BMSC experiments (FOSL2, Col2a1, Adamts5, Mmp13, Sox9, CAMK2D, Col10a1) and for human cartilage tissue (FOSL2) are listed separately in Table 1. GAPDH was used as the endogenous reference gene for both species. Relative gene expression was calculated using the  $2^{-\Delta\Delta C_t}$  method. All reactions were performed in technical triplicate with at least three independent biological replicates.

## 2.7 Transcriptome Sequencing and Bioinformatics Analysis

Total RNA was extracted from BMSCs in the si-NC and si-FOSL2 groups. Library preparation and sequencing were performed by Azenta Life Sciences/GENEWIZ (Suzhou, Jiangsu, China). Briefly, RNA-seq library construction was performed using a poly(A)-enrichment, strand-specific protocol. After library quality control, the libraries were sequenced on an Illumina sequencing platform to generate 150-bp paired-end reads. Raw sequencing data were converted to FASTQ files using Bcl2fastq (v2.20.0.422). Sequencing quality was assessed using FastQC (v0.10.1). Adapter sequences and low-quality

bases were removed using Cutadapt (v1.9.1). Bases at the 5' or 3' ends with Phred quality scores <20 or ambiguous nucleotides (N) were trimmed, and reads shorter than 75 bp after trimming were discarded. Clean reads were aligned to the rat reference genome (*Rattus norvegicus*) using HISAT2 (v2.2.1) with default parameters. Transcripts were assembled using StringTie (v1.3.3b), and gene expression levels were estimated using HTSeq (v0.6.1) and normalized as FPKM values. Differentially expressed genes between the si-NC/control and si-FOSL22/treatment groups at each time point were identified using DESeq2 (v1.26.0) based on read count data, with thresholds of  $|\log_2 \text{fold change}| > 1$  and  $q\text{-value/FDR-adjusted } p\text{-value} \leq 0.05$ . Volcano plots, Venn diagrams, heatmaps, and other downstream visualizations were generated in R. Across the 24 samples, the average Q30 value of the clean reads was 91.95%, the average total mapping rate was 95.29%, and the average uniquely mapped rate was 87.99%, indicating that the sequencing data were suitable for downstream analyses. The PPI network of DEG-encoded proteins was constructed using the STRING database and visualized in Cytoscape. The transcriptome sequencing data have been deposited in the OMIX database under accession number OMIX019222.

## 2.8 Western Blotting

Total protein was extracted from BMSCs and cartilage tissue using RIPA lysis buffer (Beyotime, Nanjing, Jiangsu, China) supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 × protease inhibitor cocktail. Total protein concentration was determined using a Bicinchoninic Acid (BCA) Protein Assay Kit (Beyotime) before electrophoresis to ensure equal loading. Protein samples (20 µg) were separated by 10% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (Invitrogen). After blocking with 5% non-fat milk, the membranes were incubated overnight at 4 °C with the following primary antibodies: FOSL2 (15832-1-AP, 1:1000, Proteintech), COL II (ab188570, 1:5000, Abcam), SOX9 (67439-1-AP, 1:5000, Proteintech), Col10 (26984-1-AP, 1:500, Proteintech), ADAMTS5 (ab41037, 1:250, Abcam), MMP13 (18165-1-AP, 1:2000, Proteintech), CAMK2D (20667-1-AP, 1:2000, Proteintech), and GAPDH (ab181602, 1:10,000, Abcam). The membranes were then incubated with the corresponding horseradish peroxidase-conjugated secondary antibodies (Beyotime) at room temperature for 2 hours. Protein signals were visualized using the ECL chemiluminescence detection kit (Beyotime) with a chemiluminescence imaging system (BIO-RAD, USA). After image acquisition, protein bands were quantified by densitometric analysis using ImageJ software (v1.52, NIH, USA), and target protein expression was normalized to the corresponding GAPDH loading control.

## 2.9 Hematoxylin-eosin (H&E) Staining

Rat cartilage sections were fixed with 4% PFA and embedded in paraffin, and sectioned at 5 µm thickness. After deparaffinization and rehydration, the sections were stained with H&E. Morphological changes in the cartilage tissue were observed using a microscope (Nikon).

## 2.10 Safranin O-Fast Green Staining

After dewaxing and hydration as in H&E staining, tissue sections were immersed in Fast Green solution for 3 minutes and rinsed with PBS. Sections were then dipped in acetic acid solution for 3 seconds, rinsed with PBS, and stained with Safranin O for 10 seconds, followed by another PBS rinse. After air-drying, sections were mounted with neutral resin and coverslipped.

## 2.11 Immunofluorescence staining

BMSCs were fixed with 4% paraformaldehyde (Beyotime, Nanjing, Jiangsu, China), followed by incubation with the primary antibody MMP13 (18165-1-AP, Proteintech, 1:200) overnight at 4 °C. After three washes with PBS, the cells were incubated with a Cy3-conjugated goat anti-rabbit IgG secondary antibody (Beyotime, Nanjing, Jiangsu, China) for 1 h at room temperature in the dark. Nuclei were counterstained with DAPI (1 µg/mL, Beyotime, Nanjing, Jiangsu, China) for 5 min at room temperature. After three final PBS washes, the cells were mounted with anti-fade mounting medium and observed under an Olympus IX73 fluorescence microscope (Olympus, Japan) equipped with a digital camera. Fluorescence images were captured at ×200 magnification.

## 2.12 Immunohistochemistry (IHC)

Paraffin-embedded sections were dewaxed with xylene and boiled for antigen retrieval. The sections were then incubated with hydrogen peroxide blocking solution for 10–15 minutes to reduce nonspecific background staining caused by endogenous peroxidase activity. The tissues were covered with primary antibody against COL II (Proteintech, 1:100) in working solution and incubated overnight at 4 °C. On the following day, the sections were incubated with anti-rabbit IHC secondary antibody (Abcam, USA) at room temperature for 30 minutes. Subsequently, the tissues were stained with hematoxylin and DAB. Finally, the sections were mounted with coverslips and observed and photographed under a microscope.

## 2.13 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). All data were tested for normality and homogeneity of variance. For paired samples (e.g., comparison of RN vs. OA sides from the same patient in Fig. 1), a paired Student's *t*-test was used. For unpaired two-group comparisons, an independent-samples Student's *t*-test was used. For com-

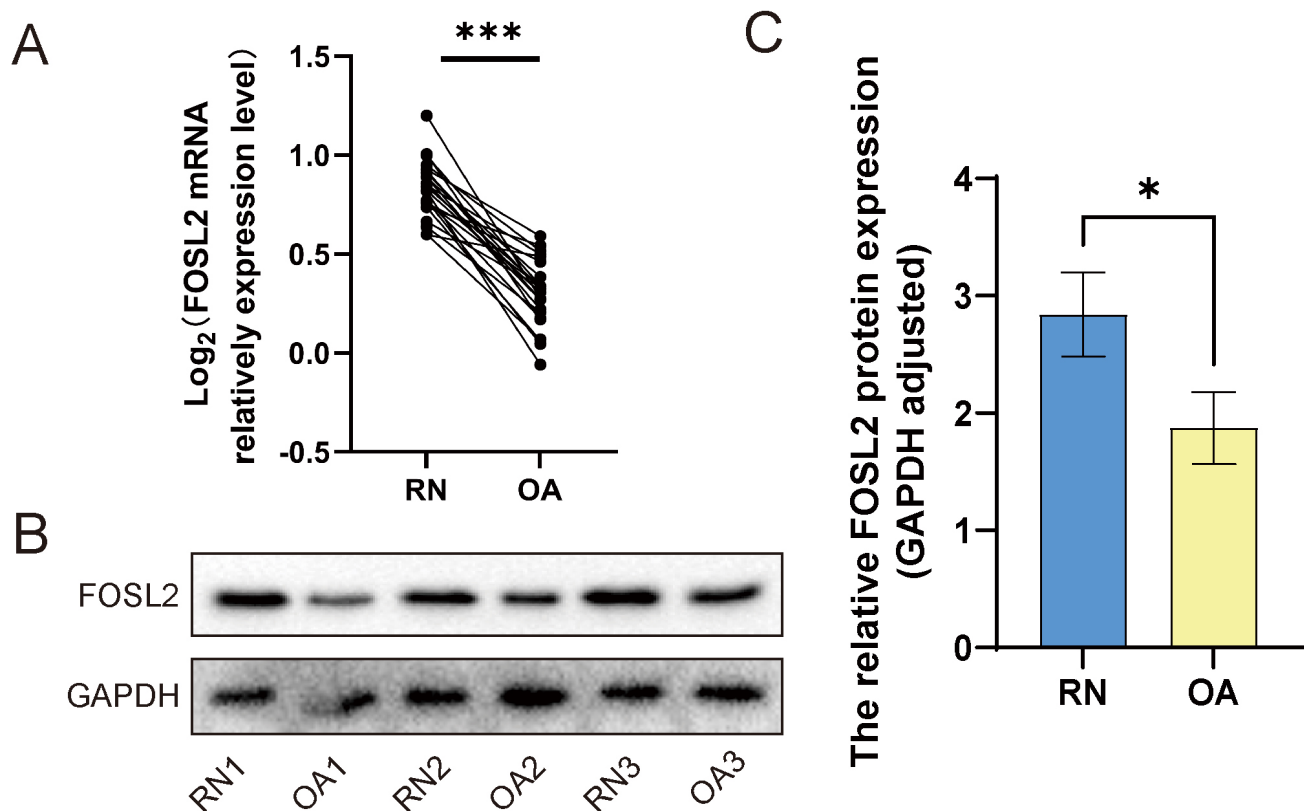


**Fig. 1.** Expression of FOSL2 in the RN and OA sides of osteoarthritic cartilage samples ( $n = 24$ ). (A) Differences in FOSL2 mRNA expression between the damaged side and the relatively normal side of osteoarthritic cartilage; (B,C) Differences in FOSL2 protein expression. Statistical significance was determined by paired Student's *t*-test (RN vs. OA, paired samples from the same patient). RN, relatively normal cartilage; OA, osteoarthritis. \* $p < 0.05$ , \*\*\* $p < 0.001$ .

parisons among three or more groups, one-way ANOVA was applied, followed by Tukey's post hoc test for multiple comparisons.  $p$ -value  $< 0.05$  was considered statistically significant (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

### 3. Results

#### 3.1 FOSL2 is Lowly Expressed in Osteoarthritic Knee Cartilage

To investigate the role of FOSL2 in the progression of OA, we analyzed the expression levels of FOSL2 in clinical samples. Cartilage samples were collected from relatively normal cartilage (RN) and the damaged cartilage (OA) knees of 24 OA patients. According to qRT-PCR results, the mRNA expression level of FOSL2 in OA was significantly lower than that in RN (Fig. 1A). Similarly, western blotting results demonstrated that the protein expression level of FOSL2 in OA was significantly lower than that in RN (Fig. 1B,C).

#### 3.2 FOSL2 Expression Increases During Chondrogenic Differentiation of BMSCs

To explore the role of FOSL2 in the chondrogenic differentiation of BMSCs, the chondrogenic induction medium was used to induce the differentiation of BMSCs.

As shown by Alcian blue staining in Fig. 2A, staining intensity increased with prolonged induction time. Both western blotting and qRT-PCR results indicated that during chondrogenic differentiation, the expression levels of FOSL2 and cartilage-specific markers (COL II, SOX9) gradually increased (Fig. 2B–F,M,N). Conversely, the expression levels of catabolic markers (ADAMTS5, MMP13) and hypertrophic chondrocyte markers (COL10) decreased (Fig. 2G–L). Consistent with the western blotting and qRT-PCR results, representative immunofluorescence staining showed an overall reduction in MMP13-positive signal during chondrogenic differentiation (Fig. 2O).

#### 3.3 Knockdown of FOSL2 Impairs Chondrogenic Differentiation Ability

To further investigate the biological function of FOSL2 in BMSCs, we used siRNA to specifically knock down FOSL2 expression. Western blotting and qRT-PCR results showed that si-2 achieved the most significant knockdown effect (Fig. 3A–C). Compared with the blank control and NC groups, Alcian blue staining was noticeably weaker in the FOSL2 knockdown group (Fig. 3D). Western blotting and qRT-PCR analysis revealed that the expression of chondrogenic markers was reduced follow-

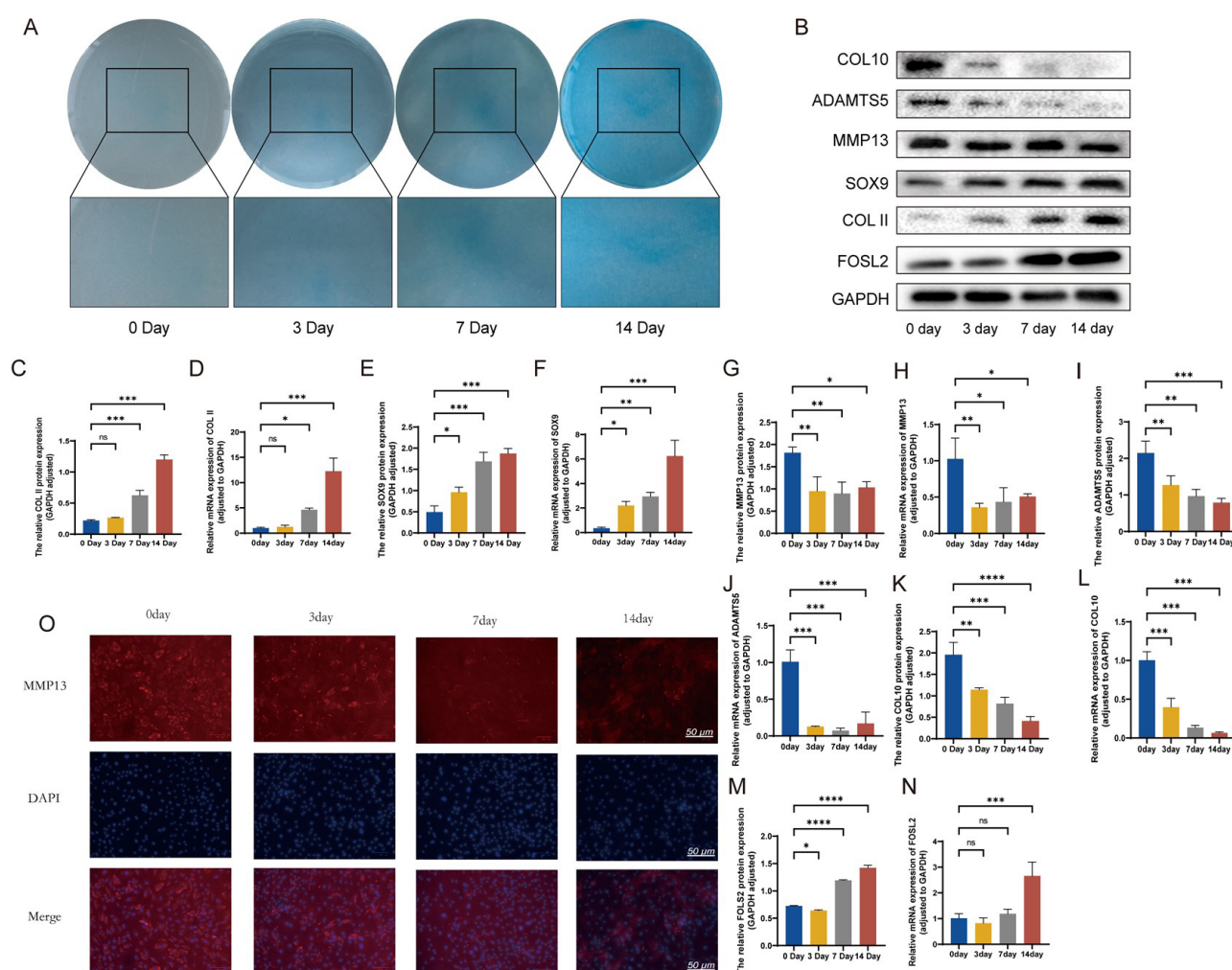


**Fig. 2. BMSC chondrogenic differentiation.** (A) Alcian blue staining of BMSCs after chondrogenic differentiation; (B–N) Expression levels of FOSL2, chondrogenic markers (COL II, SOX9), catabolic markers (MMP13, ADAMTS5), and the hypertrophic chondrocyte marker (COL10) at days 0, 3, 7, and 14 of BMSC chondrogenic differentiation; (O) Representative immunofluorescence images showing MMP13-positive signal at days 0, 3, 7, and 14 of BMSC chondrogenic differentiation. All immunofluorescence images within the same experiment were acquired and displayed using consistent parameters. Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons (4 time points: 0, 3, 7, 14 d). BMSC, bone marrow mesenchymal stem cell. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ , ns:  $p > 0.05$ . Scale bar = 50  $\mu$ m.

ing FOSL2 knockdown (Fig. 3E–J). Additionally, we examined the expression of catabolic markers and the hypertrophic chondrocyte marker in BMSCs. Results showed that the expression of these markers significantly increased upon FOSL2 silencing (Fig. 3K–R). Immunofluorescence staining of MMP13 also showed enhanced signal intensity after FOSL2 knockdown, further confirming the upregulation of MMP13 expression (Fig. 3S).

### 3.4 Overexpression of FOSL2 Enhances Chondrogenic Differentiation

To further investigate the effect of FOSL2 on chondrogenic differentiation of BMSCs, we overexpressed FOSL2 in primary BMSCs. Following transfection with recombinant lentivirus expressing GFP, green fluorescence was ob-

served in both NC and lv-FOSL2 groups (Fig. 4A). Western blotting and qRT-PCR confirmed significant upregulation of FOSL2 expression at both the protein and mRNA levels in the lv-FOSL2 group (Fig. 4B–D). After 14 days of chondrogenic induction, Alcian blue staining revealed markedly increased staining intensity in the FOSL2-overexpressing BMSCs compared to both the blank control and NC groups (Fig. 4E). Western blotting and qRT-PCR further showed that the expression of cartilage-specific markers was significantly higher in the lv-FOSL2 group compared to the NC and normal groups (Fig. 4F–J). We also examined the expression levels of catabolic markers and the hypertrophic chondrocyte marker in BMSCs. The results demonstrated that upon FOSL2 overexpression, the expression of these markers was significantly reduced (Fig. 4F,K–P). Im-

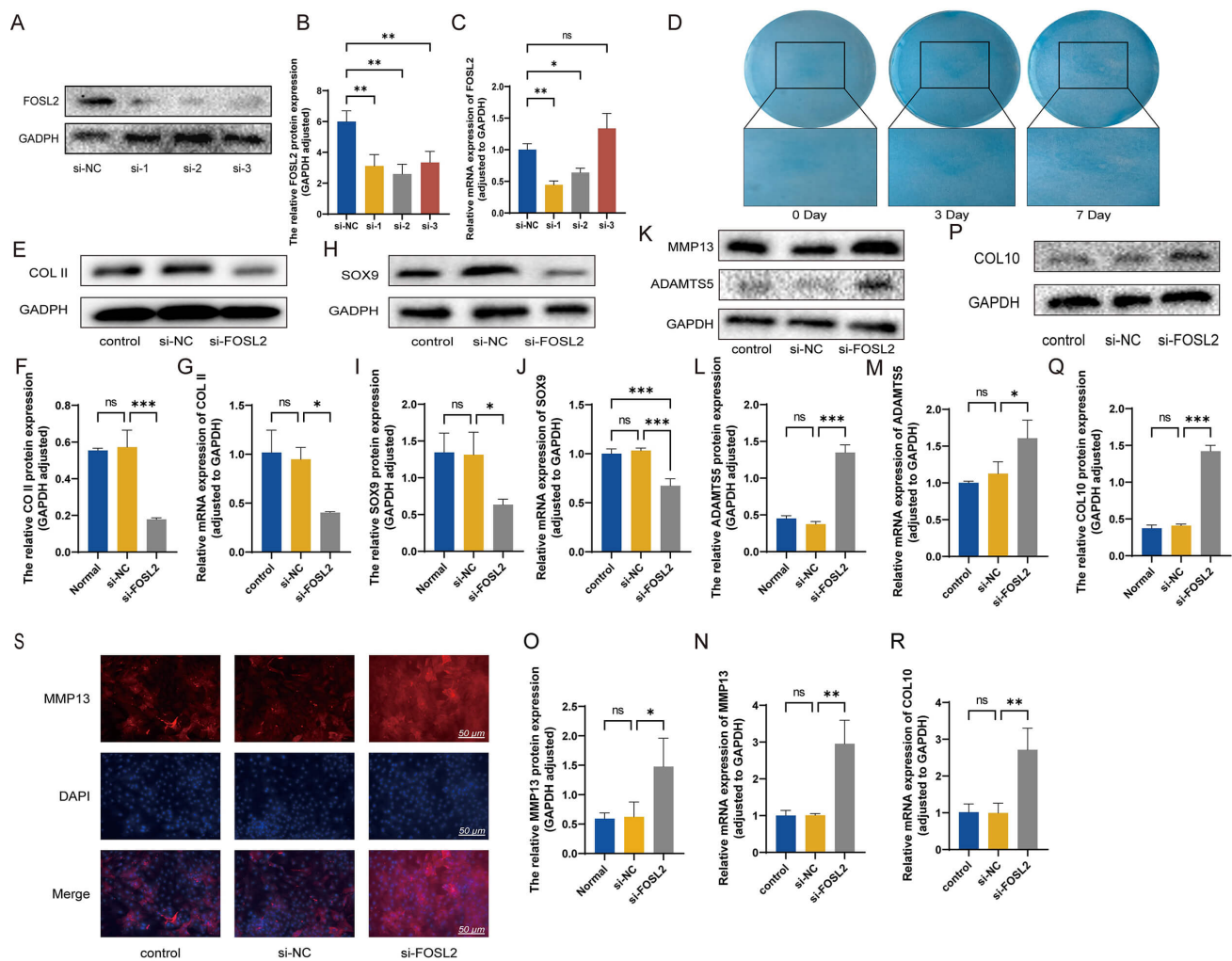


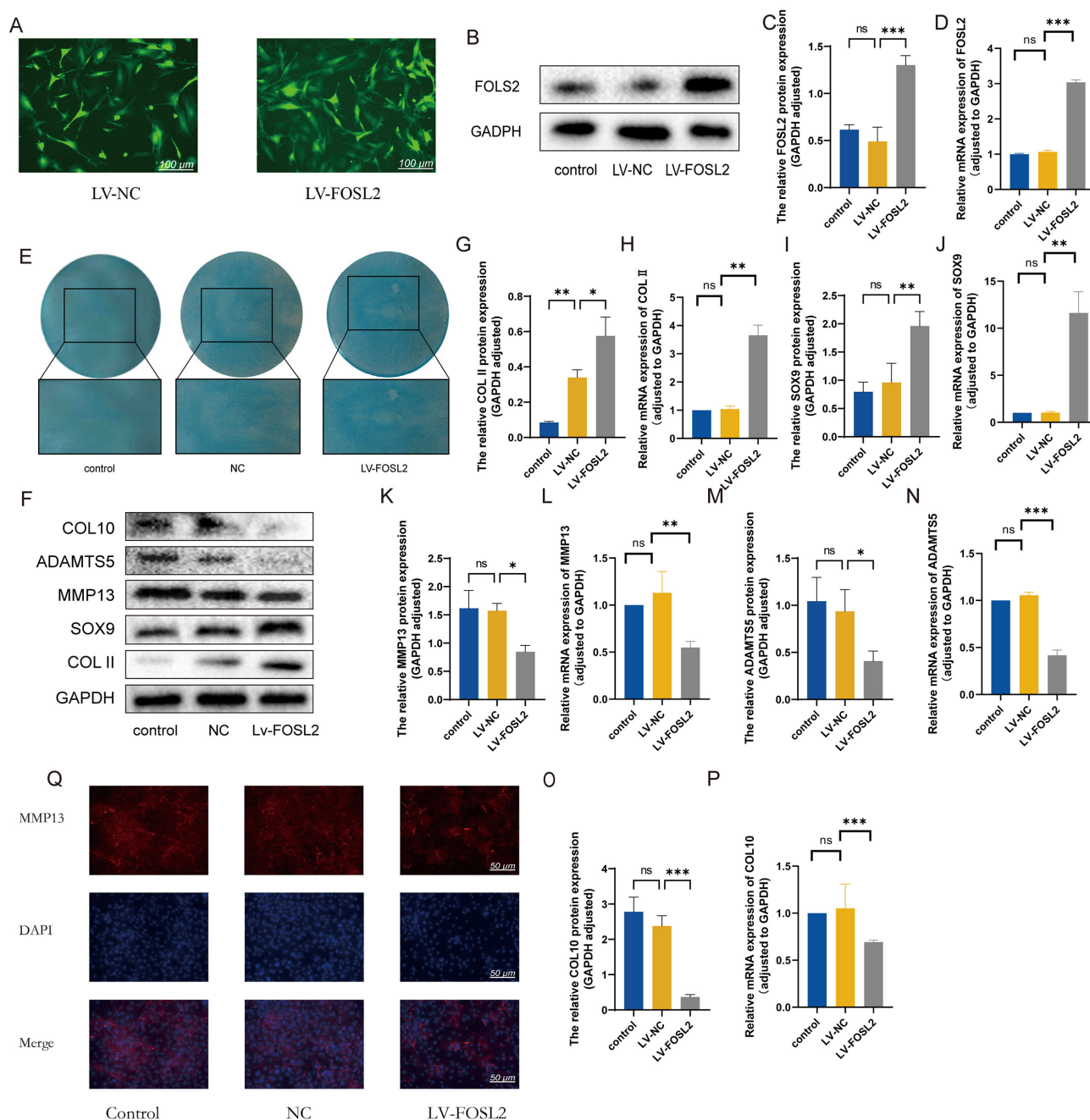
**Fig. 3. Results of BMSC chondrogenic differentiation at Day 14.** (A–C) Selection of siRNA for FOSL2 knockdown; (D) Alcian blue staining comparison between FOSL2 knockdown and control groups; (E–J) Expression levels of chondrogenic markers (COL II, SOX9) at Day 14 of BMSC chondrogenic differentiation; (K–O) Expression levels of catabolic markers (MMP13, ADAMTS5); (P–R) Expression levels of the hypertrophic chondrocyte marker (COL10); (S) Representative immunofluorescence images showing MMP13-positive signal in FOSL2 knockdown and control groups. All immunofluorescence images within the same experiment were acquired and displayed using consistent parameters. Statistical significance was determined by independent-samples Student's *t*-test for the quantitative panels (si-NC vs. si-FOSL2, two-group comparison). \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001. ns: *p* > 0.05. Scale bar = 50  $\mu$ m.

munofluorescence staining of MMP13 showed decreased fluorescence intensity in the FOSL2-overexpression group compared with the control group, further supporting the downregulation of MMP13 (Fig. 4Q).

### 3.5 Transcriptome Analysis Results

To explore the mechanism by which FOSL2 participates in the chondrogenic differentiation of BMSCs, RNA was extracted from BMSCs in the si-NC and si-FOSL2 groups at day 0 and day 14 of chondrogenic induction and subjected to transcriptome sequencing. Differential expression analysis was performed by comparing day 0 and day 14 within each group. Specifically, Fig. 5A shows the day 0 versus day 14 comparison in the si-NC group, whereas

Fig. 5B shows the corresponding comparison in the si-FOSL2 group. No direct comparison between the si-NC and si-FOSL2 groups was performed in the RNA-seq analysis. Upregulated and downregulated DEGs were identified based on the criteria described in the Methods section and are shown in Fig. 5C. These DEGs were input into the STRING database analysis platform. Genes with a combined score  $\geq 0.9$  were retained, and disconnected nodes were removed to identify hub genes. The selected genes were then imported into Cytoscape software for visualization and ranked by degree value (Fig. 5D). The top seven hub genes identified were Actg1, Vcl, Actb, Flna, CAMK2D, Tln1, and Myl6 [40]. In the transcriptome analysis, CaMK2D was identified as a candidate gene showing differential expression in the day 0 versus day 14 compar-



isons(Fig. 5E). Western blotting and qRT-PCR confirmed that CAMK2D expression was increased after FOSL2 overexpression (Fig. 5F–H), and decreased after FOSL2 knockdown (Fig. 5I–K), consistent with the transcriptome results.

### 3.6 Knockdown of CAMK2D Suppresses the Pro-Chondrogenic Effect of FOSL2 Overexpression

To verify whether CAMK2D, as a functional downstream effector of FOSL2, participates in the chondrogenic differentiation process, we performed a rescue experiment by knocking down CAMK2D in BMSCs overexpress-

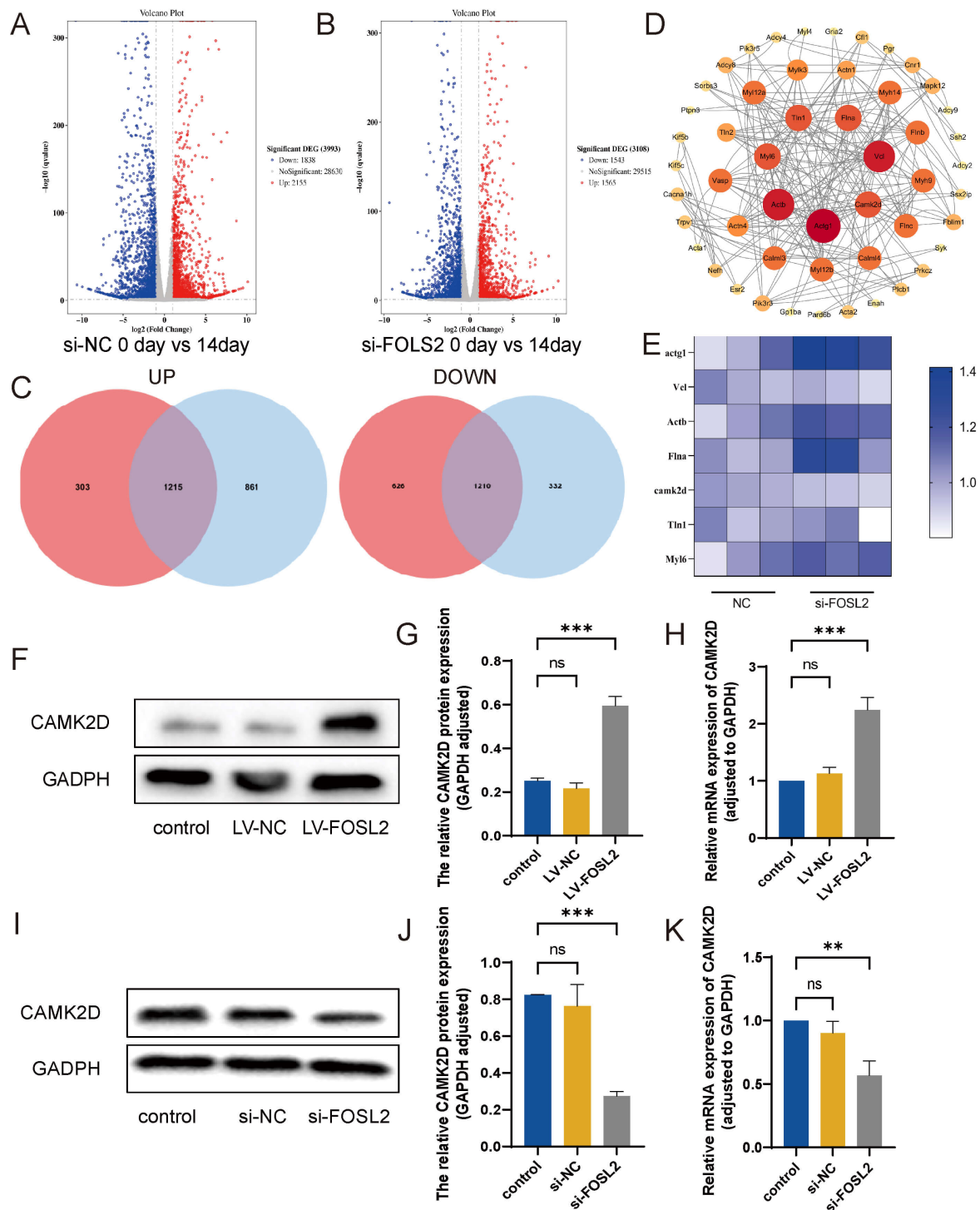


**Fig. 5. Transcriptome results and validation.** (A) Volcano plot of BMSCs at day 0 and day 14 of chondrogenic induction in the si-NC group; (B) Volcano plot of BMSCs at day 0 and day 14 of chondrogenic induction after si-FOSL2 knockdown; (C) Differentially expressed genes; (D) Protein-protein interaction network of DEGs; (E) Heatmap of the top 7 hub genes; (F–H) Validation of CAMK2D expression after FOSL2 overexpression; (I–K) Validation of CAMK2D expression after FOSL2 knockdown. Statistical significance was determined by independent-samples Student's *t*-test (two-group comparisons) and one-way ANOVA followed by Tukey's post hoc test (multi-group comparisons in the validation experiments). \*\**p* < 0.01, \*\*\**p* < 0.001. ns: *p* > 0.05.

ing FOSL2. Four siRNA sequences targeting CaMK2D were designed, and western blotting and qRT-PCR results showed that si-4 had the most significant knockdown ef-

fect (Fig. 6A–C). A BMSCs model overexpressing FOSL2 was established using lentiviral transfection. After 14 days of chondrogenic induction, western blotting and qRT-PCR

results showed that the expression of chondrogenic markers was reduced after CAMK2D knockdown, indicating that the downregulation of CAMK2D reversed the FOSL2-induced upregulation of chondrogenic genes (Fig. 6D–H). The expression levels of catabolic markers and the hypertrophic chondrocyte marker in BMSCs were also measured. The results showed that CAMK2D knockdown reversed the inhibitory effect of FOSL2 on catabolic and hypertrophic markers (Fig. 6I–N). These findings indicate that CAMK2D downregulation can suppress the promotive effect of FOSL2 on BMSCs chondrogenic differentiation.

### 3.7 Injection of FOSL2-Knockdown BMSCs Impaired Cartilage Repair in OA

To investigate the *in vivo* role of FOSL2, a knee OA model was established in SD rats. BMSCs with FOSL2 knockdown were injected into the knee joints of the rats. After 4 weeks, the rats were euthanized, and knee cartilage tissues were collected. HE staining and Safranin O-Fast Green staining revealed that compared to the normal cartilage morphology observed in the Sham group, the OA group exhibited a rough cartilage surface and disordered chondrocyte arrangement. Compared to the rats in the BMSCs group, the rats in the si-FOSL2 + BMSCs group showed a reduced number of chondrocytes and more disordered cell arrangement (Fig. 7A,B), with an increased Mankin score. Immunohistochemical staining further demonstrated a significantly reduced expression level of COL II in the si-FOSL2 + BMSCs group (Fig. 7A,C).

## 4. Discussion

This study investigated the role of FOSL2 in the chondrogenic differentiation of BMSCs. The results indicated that FOSL2 promoted the chondrogenic differentiation of BMSCs. Overexpression of FOSL2 enhanced the chondrogenic process, whereas knockdown of FOSL2 inhibited cartilage formation. Furthermore, *in vivo*, injection of si-FOSL2-transduced BMSCs into the OA joints exhibited attenuated restorative effects on knee joint cartilage in a rat OA model. Transcriptomic analysis further revealed that FOSL2 mediated the chondrogenic differentiation of BMSCs via CAMK2D pathway.

BMSCs have shown great promise in OA treatment, supported by a recent meta-analysis of 13 randomized controlled trials (n = 1150 patients) demonstrating significant pain and function improvements at 6–12 months after BMSC injection [41]. Yet the molecular determinants governing BMSC chondrogenic capacity remain incompletely understood, the efficacy and predictability of BMSC-based therapies are limited. In the present study, we examined FOSL2 as a candidate regulator of BMSC chondrogenic differentiation. FOSL2 expression rose during chondrogenic induction, and manipulating FOSL2 levels in BMSCs altered both cartilage marker expression and catabolic gene profiles. Our findings are consistent with a prior transcrip-

tomic study of *in vitro* BMSC chondrogenesis [42], supporting the validity of our induction system. Notably, our rescue experiments suggest that FOSL2 acts in part through CAMK2D, a calcium/calmodulin-dependent kinase previously implicated in cartilage homeostasis, identifying a FOSL2-CAMK2D axis that may inform future strategies to enhance BMSC-based OA treatment.

FOSL2 is a core AP-1 transcription factor. Previous studies have implicated AP-1/Fra-2 in cartilage development, bone biology, and inflammatory regulation [43,44,45], but its function at the earlier stage of BMSC chondrogenesis is unclear. The only BMSC-related study of FOSL2 to date has focused on FOSL2 regulation by the CGRP/mmu\_circRNA\_003795/miR-504-3p axis in BMSC proliferation, not differentiation [36], leaving the role of FOSL2 in BMSC chondrogenic commitment entirely unexplored. The present study addresses this gap by demonstrating that FOSL2 expression rises during BMSC chondrogenic differentiation and that manipulating FOSL2 levels bidirectionally regulates chondrogenic outcomes. Consistent with its known role in cartilage homeostasis, FOSL2 modulation in BMSCs produced opposing but coordinated effects on cartilage anabolism and catabolism: knockdown enhanced MMP13/ADAMTS5/COL10 expression while reducing COL II/SOX9, whereas overexpression produced the inverse pattern. This dual regulation, in a single cell type, is uncommon and suggests FOSL2 acts as a master switch rather than a simple activator or repressor. Notably, while previous studies on FOSL2 in cartilage biology focused on embryonic skeletal development (Fra-2 knockout studies) [37,44] or adult chondrocyte homeostasis, our findings extend the FOSL2 functional repertoire to adult BMSC-based cartilage regeneration, a clinically relevant setting in which BMSC transplantation is being actively explored for OA therapy. The *in vivo* finding that FOSL2-knockdown BMSCs produced weaker cartilage repair than control BMSCs further distinguishes our work from prior studies by providing direct evidence that BMSC-intrinsic FOSL2 levels govern therapeutic efficacy in an OA model. We further investigated the mechanism through which FOSL2 regulates BMSC chondrogenic differentiation. Based on transcriptomic, qRT-PCR, and western blot results, we identified CAMK2D as a potential functional downstream effector of FOSL2. Although our rescue experiments confirmed the functional hierarchy between FOSL2 and CAMK2D, the precise molecular mechanism underlying this regulation warrants further discussion. As a core member of the AP-1 transcription factor family, FOSL2 typically drives gene expression by physically binding to specific DNA motifs [46,47]. It is highly plausible that FOSL2 directly binds to the AP-1 consensus sequence (TGACTCA) within the CAMK2D promoter. Alternatively, this regulatory relationship might be indirect. Since previous studies indicate that FOSL2 participates in complex competing endoge-

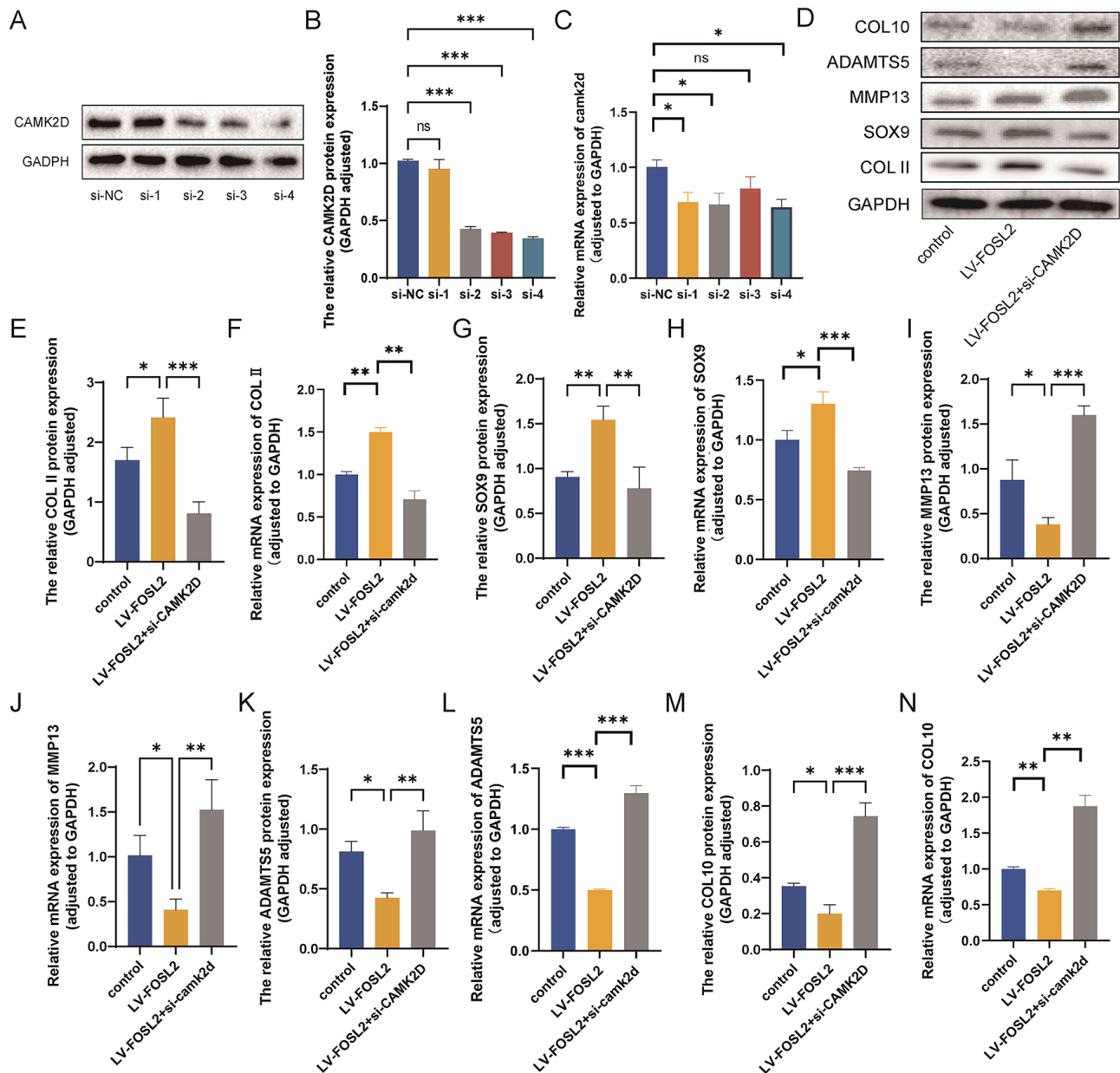


**Fig. 6. Knockdown of CAMK2D suppresses the pro-chondrogenic effect of FOSL2 overexpression.** (A–C) Selection of siRNA for CAMK2D knockdown; (D–H) Expression levels of chondrogenic genes (COLII, SOX9) on day 14 of BMSCs chondrogenic differentiation; (I–L) Expression levels of catabolic markers (MMP13, ADAMTS5); (M,N) Expression levels of the hypertrophic chondrocyte marker (COL10). Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons (3 groups: control, LV-FOSL2, LV-FOSL2+si-CAMK2D). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . ns:  $p > 0.05$ .

nous RNA networks in BMSCs, FOSL2 might influence CAMK2D post-transcriptionally via specific microRNAs or long non-coding RNAs [36]. Moreover, FOSL2 could indirectly modulate CAMK2D levels by regulating intermediate cytokines critical for the chondrogenic microenvironment. CAMK2D is a serine/threonine kinase. It is considered a key component of calcium signaling pathways. CAMK2D contains a calmodulin-binding domain. It is activated in response to increased intracellular calcium levels [48]. While extensively studied in the nervous and car-

diovascular systems, the role of CAMK2D in OA has recently garnered attention. As a crucial regulator of the cell cycle, CaMKII signaling plays a key role in G2/M transition and metaphase-to-anaphase progression [49]. Its influence on chondrocytes is also notable, potentially affecting their proliferation and apoptosis. Moreover, it may have implications for extracellular matrix degradation. Existing studies indicate that CAMK2D is essential for maintaining cartilage homeostasis [50]. It exhibits high expression levels in normal cartilage, particularly in hyaline cartilage and

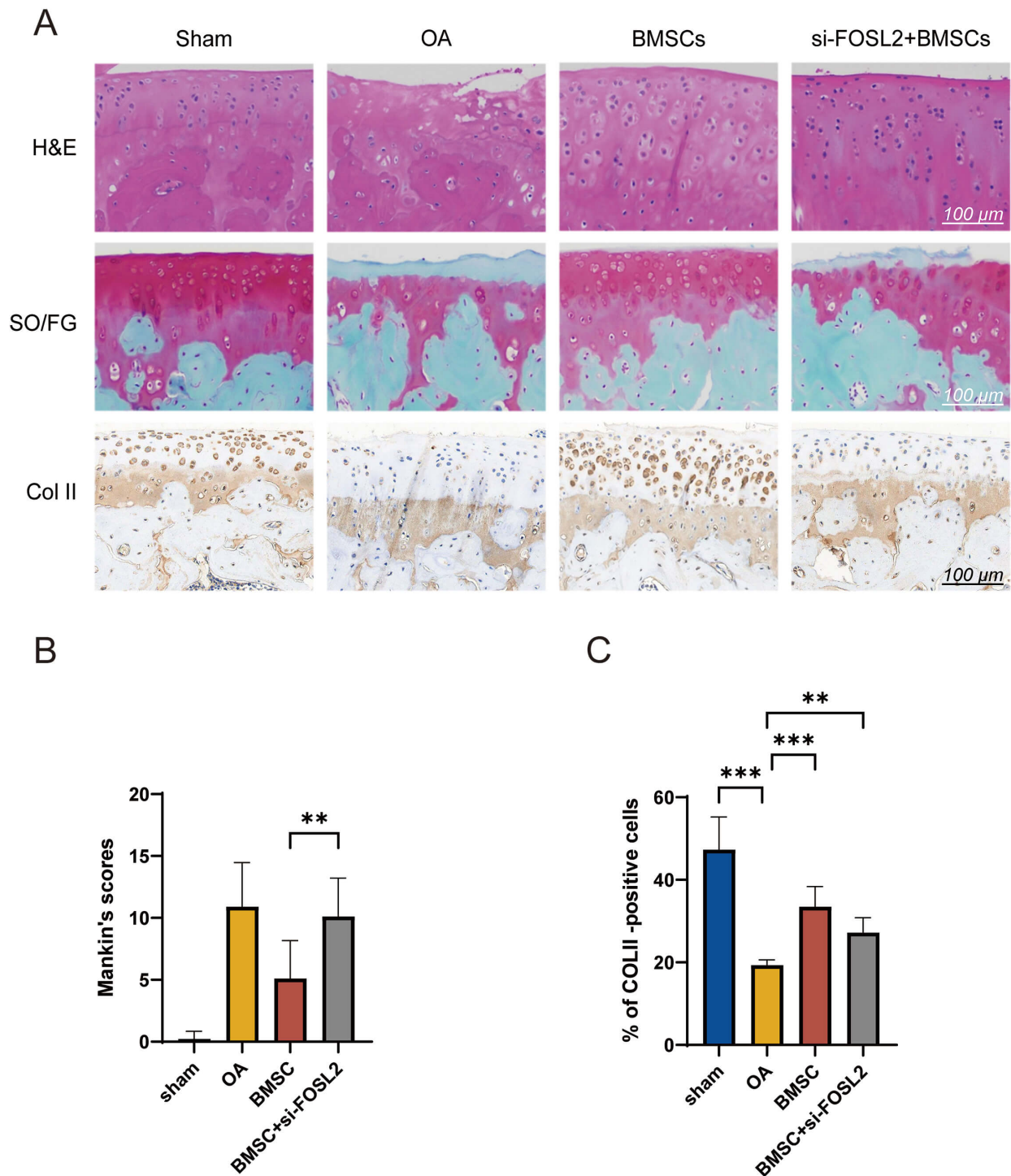


**Fig. 7. Injection of FOSL2-knockdown BMSCs impaired OA cartilage repair.** (A) HE staining, Safranin O-Fast Green staining, and immunohistochemical staining for COL II in the knee joint cartilage; (B) Mankin scores of knee joint cartilage; (C) Semi-quantitative analysis of immunohistochemical staining. Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons (4 groups: sham, OA, BMSC, BMSC+si-FOSL2). \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Scale bar = 100  $\mu$ m.

growth plates. However, its expression is significantly reduced in OA cartilage, highlighting its protective role in cartilage repair and maintenance [51].

Our results showed that FOSL2 overexpression elevated cartilage gene expression and reduced catabolic and

hypertrophic markers on day 14. However, knockdown of CAMK2D in this context reversed these effects. This diminished the chondrogenic promotion and weakened the suppression of catabolic and hypertrophic differentiation, ultimately impairing chondrocyte function. CAMK2D has

also been shown to inhibit M1 macrophage polarization, thereby alleviating OA progression [52]. Both *in vitro* and *in vivo* studies confirm that CAMK2D inhibition exacerbates chondrocyte damage and OA severity. Similar results have demonstrated that inhibiting CAMK2D reduces cartilage gene expression and impairs chondrocyte development in animal OA models. Previous study has also reported that inhibition of CaMKII in pluripotent stem cells suppresses cartilage differentiation [53]. Therefore, we hypothesized that CAMK2D inhibited the differentiation of stem cells into chondrocytes, which was confirmed by experiments. However, this study still has some limitations. It should be noted that our *in vivo* experiments were limited to FOSL2-knockdown BMSCs. The *in vivo* effect of FOSL2-overexpressing BMSCs was not evaluated in the present study. Our *in vitro* data show that FOSL2 overexpression enhances BMSC chondrogenic differentiation. Future studies are warranted to verify whether FOSL2-overexpressing BMSCs exhibit enhanced cartilage repair *in vivo*. Additionally, due to the lack of direct binding evidence, we rigorously defined CAMK2D as a *downstream effector* rather than a *direct target*. Future studies employing Chromatin Immunoprecipitation and dual-luciferase reporter assays are essential to elucidate the exact transcriptional regulatory mechanism between FOSL2 and CAMK2D. Such validation would more directly support a therapeutic role of FOSL2 in BMSC-based OA treatment.

## 5. Limitations

This study has several limitations. First, the *in vivo* experiments only evaluated the cartilage repair capacity of FOSL2-knockdown BMSCs. An FOSL2-overexpressing BMSC group was not included, which would have provided more direct *in vivo* evidence. Second, the mechanistic evidence linking FOSL2 to CAMK2D regulation is based on transcriptomic analysis, expression validation, and rescue experiments. Direct evidence such as chromatin immunoprecipitation and dual-luciferase reporter assays remains to be established. Third, the clinical sample size was relatively modest. All patients had end-stage OA (Kellgren-Lawrence grade III/IV). FOSL2 expression in early-stage OA patients requires further validation. Fourth, only primary rat BMSCs were used in the present study. Extending these findings to established chondrocyte cell lines such as ATDC5 or C28/I2 would further strengthen the conclusions.

## 6. Conclusion

In this study, we found that FOSL2 regulates BMSC chondrogenic differentiation. FOSL2 promoted cartilage anabolism while suppressing catabolic gene expression in BMSCs, and FOSL2-knockdown BMSCs showed reduced cartilage repair in a rat OA model. Rescue experiments suggest that the FOSL2-CAMK2D axis mediates these effects, though direct transcriptional regulation between FOSL2 and CAMK2D awaits further validation. Together, these

findings suggest FOSL2 as a candidate target for enhancing BMSC-based cartilage repair in OA.

## Availability of Data and Materials

The available processed transcriptomic data generated in this study have been deposited in the Open Archive for Miscellaneous Data (OMIX) of the National Genomics Data Center and are publicly available under accession number OMIX019222: <https://ngdc.cnecb.ac.cn/omix/release/OMIX019222>. The datasets and raw data used and analyzed during the current study are available from the corresponding authors upon reasonable request.

## Author Contributions

CL, XO contributed to conceptualization, methodology, investigation, and writing of the original draft. GL, JZ, DB, DX contributed to investigation and data curation. YS contributed to supervision, the acquisition, analysis, or interpretation of data for the work, writing, review, and editing. PC contributed to conceptualization, project administration, supervision, and final manuscript revision. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Affiliated Hospital 6 of Nantong University (Approval No. Lunshen-2022-102) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The animal study was approved by the Ethics Committee of Jiangsu Vocational College of Medicine (Approval No. SYLL-2025-700) and was conducted in accordance with the Standard Operating Procedures for the Laboratory Animal Center of Jiangsu Medical Vocational College and the Regulations for the Administration of Affairs Concerning Experimental Animals of China (GB 14925-2023 Laboratory Animal — Environment and Housing Facilities). The animal study was reported in accordance with the ARRIVE 2.0 guidelines.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/FBL53458>.

## References

- [1] Joukar A, Creecy A, Karnik S, Noori-Dokht H, Trippel SB, Wallace JM, et al. Correlation analysis of cartilage wear with biochemical composition, viscoelastic properties and friction. *Journal of the Mechanical Behavior of Biomedical Materials*. 2023; 142: 105827. <https://doi.org/10.1016/j.jmbbm.2023.105827>
- [2] Eschweiler J, Horn N, Rath B, Betsch M, Baroncini A, Tingart M, et al. The Biomechanics of Cartilage-An Overview. *Life* (Basel, Switzerland). 2021; 11: 302. <https://doi.org/10.3390/life11040302>
- [3] Jin P, Liu L, Chen X, Cheng L, Zhang W, Zhong G. Applications and prospects of different functional hydrogels in meniscus repair. *Frontiers in Bioengineering and Biotechnology*. 2022; 10: 1082499. <https://doi.org/10.3389/fbioe.2022.1082499>
- [4] Chawla D, Kazyak E, Eriten M, Henak CR. Influence of applied stress on energy dissipation and crack growth in articular cartilage. *Journal of the Mechanical Behavior of Biomedical Materials*. 2025; 168: 106997. <https://doi.org/10.1016/j.jmbbm.2025.106997>
- [5] Thysen S, Luyten FP, Lories RJU. Targets, models and challenges in osteoarthritis research. *Disease Models & Mechanisms*. 2015; 8: 17–30. <https://doi.org/10.1242/dmm.016881>
- [6] Hunter DJ, Schofield D, Callander E. The individual and socio-economic impact of osteoarthritis. *Nature Reviews. Rheumatology*. 2014; 10: 437–441. <https://doi.org/10.1038/nrrheum.2014.44>
- [7] Palazzo C, Nguyen C, Lefevre-Colau MM, Rannou F, Poiraudou S. Risk factors and burden of osteoarthritis. *Annals of Physical and Rehabilitation Medicine*. 2016; 59: 134–138. <https://doi.org/10.1016/j.rehab.2016.01.006>
- [8] Veronese N, Harvey NC, Rizzoli R, Fuggle NR, Reginster JY, ESCEO Working Group on the Management of Musculoskeletal Health in the Oldest Old Individuals. Executive Summary: Treatment of Osteoporosis and Osteoarthritis in the Oldest Old. *Drugs & Aging*. 2025; 42: 179–181. <https://doi.org/10.1007/s40266-025-01184-7>
- [9] Pettenuzzo S, Berardo A, Belluzzi E, Pozzuoli A, Ruggieri P, Carniel EL, et al. Mechanical insights into fat pads: a comparative study of infrapatellar and suprapatellar fat pads in osteoarthritis. *Connective Tissue Research*. 2025; 66: 272–283. <https://doi.org/10.1080/03008207.2025.2502591>
- [10] Poole AR. Osteoarthritis as a whole joint disease. *HSS Journal : the Musculoskeletal Journal of Hospital for Special Surgery*. 2012; 8: 4–6. <https://doi.org/10.1007/s11420-011-9248-6>
- [11] Abramoff B, Caldera FE. Osteoarthritis: Pathology, Diagnosis, and Treatment Options. *The Medical Clinics of North America*. 2020; 104: 293–311. <https://doi.org/10.1016/j.mcna.2019.10.007>
- [12] Messier SP, Legault C, Loeser RF, Van Arsdale SJ, Davis C, Ettinger WH, et al. Does high weight loss in older adults with knee osteoarthritis affect bone-on-bone joint loads and muscle forces during walking? *Osteoarthritis and Cartilage*. 2011; 19: 272–280. <https://doi.org/10.1016/j.joca.2010.11.010>
- [13] Deyle GD, Allison SC, Matekel RL, Ryder MG, Stang JM, Gohdes DD, et al. Physical therapy treatment effectiveness for osteoarthritis of the knee: a randomized comparison of supervised clinical exercise and manual therapy procedures versus a home exercise program. *Physical Therapy*. 2005; 85: 1301–1317.
- [14] Zhu X, Wu D, Sang L, Wang Y, Shen Y, Zhuang X, et al. Comparative effectiveness of glucosamine, chondroitin, acetaminophen or celecoxib for the treatment of knee and/or hip osteoarthritis: a network meta-analysis. *Clin Exp Rheumatol*. 2018; 36: 595–602.
- [15] Im GI. Considerations in modern regenerative medicine for osteoarthritis. *EFORT Open Reviews*. 2025; 10: 336–344. <https://doi.org/10.1530/EOR-2025-0050>
- [16] Ham O, Lee CY, Kim R, Lee J, Oh S, Lee MY, et al. Therapeutic Potential of Differentiated Mesenchymal Stem Cells for Treatment of Osteoarthritis. *International Journal of Molecular Sciences*. 2015; 16: 14961–14978. <https://doi.org/10.3390/ijms160714961>
- [17] Koga H, Engebretsen L, Brinckmann JE, Muneta T, Sekiya I. Mesenchymal stem cell-based therapy for cartilage repair: a review. *Knee Surgery, Sports Traumatology, Arthroscopy : Official Journal of the ESSKA*. 2009; 17: 1289–1297. <https://doi.org/10.1007/s00167-009-0782-4>
- [18] Kirsch T, Zhang F, Braender-Carr O, Cowman MK. Protective Effects of a Hyaluronan-Binding Peptide (P15-1) on Mesenchymal Stem Cells in an Inflammatory Environment. *International Journal of Molecular Sciences*. 2021; 22: 7058. <https://doi.org/10.3390/ijms22137058>
- [19] Xu S, Wu X. miR-134 inhibits chondrogenic differentiation of bone marrow mesenchymal stem cells by targeting SMAD6. *Bioscience Reports*. 2019; 39: BSR20180921. <https://doi.org/10.1042/BSR20180921>
- [20] Liu Y, Peng L, Li L, Huang C, Shi K, Meng X, et al. 3D-bioprinted BMSC-laden biomimetic multiphasic scaffolds for efficient repair of osteochondral defects in an osteoarthritic rat model. *Biomaterials*. 2021; 279: 121216. <https://doi.org/10.1016/j.biomaterials.2021.121216>
- [21] He L, He T, Xing J, Zhou Q, Fan L, Liu C, et al. Bone marrow mesenchymal stem cell-derived exosomes protect cartilage damage and relieve knee osteoarthritis pain in a rat model of osteoarthritis. *Stem Cell Research & Therapy*. 2020; 11: 276. <https://doi.org/10.1186/s13287-020-01781-w>
- [22] Cao M, Ou Z, Sheng R, Wang Q, Chen X, Zhang C, et al. Efficacy and safety of mesenchymal stem cells in knee osteoarthritis: a systematic review and meta-analysis of randomized controlled trials. *Stem Cell Research & Therapy*. 2025; 16: 122. <https://doi.org/10.1186/s13287-025-04252-2>
- [23] Sadeghirad B, Rehman Y, Khosravirad A, Sofi-Mahmudi A, Zandieh S, Jomy J, et al. Mesenchymal stem cells for chronic knee pain secondary to osteoarthritis: A systematic review and meta-analysis of randomized trials. *Osteoarthritis and Cartilage*. 2024; 32: 1207–1219. <https://doi.org/10.1016/j.joca.2024.04.021>
- [24] Han X, Yang B, Zou F, Sun J. Clinical therapeutic efficacy of mesenchymal stem cells derived from adipose or bone marrow for knee osteoarthritis: a meta-analysis of randomized controlled trials. *Journal of Comparative Effectiveness Research*. 2020; 9: 361–374. <https://doi.org/10.2217/cer-2019-0187>
- [25] Hollander AP, Dickinson SC, Kafienah W. Stem cells and cartilage development: complexities of a simple tissue. *Stem Cells* (Dayton, Ohio). 2010; 28: 1992–1996. <https://doi.org/10.1002/stem.534>
- [26] Bahrami S, Drablos F. Gene regulation in the immediate-early response process. *Advances in Biological Regulation*. 2016; 62: 37–49. <https://doi.org/10.1016/j.jbior.2016.05.001>
- [27] Yao Q, Wu X, Tao C, Gong W, Chen M, Qu M, et al. Osteoarthritis: pathogenic signaling pathways and therapeutic targets. *Signal Transduction and Targeted Therapy*. 2023; 8: 56. <https://doi.org/10.1038/s41392-023-01330-w>
- [28] Angel P, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochimica et Biophysica Acta*. 1991; 1072: 129–157. [https://doi.org/10.1016/0304-419x\(91\)90011-9](https://doi.org/10.1016/0304-419x(91)90011-9)

- [29] Shaulian E, Karin M. AP-1 in cell proliferation and survival. *Oncogene*. 2001; 20: 2390–2400. <https://doi.org/10.1038/sj.onc.1204383>
- [30] Nishina H, Sato H, Suzuki T, Sato M, Iba H. Isolation and characterization of fra-2, an additional member of the fos gene family. *Proceedings of the National Academy of Sciences of the United States of America*. 1990; 87: 3619–3623. <https://doi.org/10.1073/pnas.87.9.3619>
- [31] Foletta VC, Sonobe MH, Suzuki T, Endo T, Iba H, Cohen DR. Cloning and characterisation of the mouse fra-2 gene. *Oncogene*. 1994; 9: 3305–3311.
- [32] Bian Z, Zhou M, Cui K, Yang F, Cao Y, Sun S, et al. SNHG17 promotes colorectal tumorigenesis and metastasis via regulating Trim23-PES1 axis and miR-339-5p-FOSL2-SNHG17 positive feedback loop. *Journal of Experimental & Clinical Cancer Research* : CR. 2021; 40: 360. <https://doi.org/10.1186/s13046-021-02162-8>
- [33] Seidenberg J, Stellato M, Hukara A, Ludewig B, Klingel K, Distler O, et al. The AP-1 Transcription Factor FosL2 Regulates Autophagy in Cardiac Fibroblasts during Myocardial Fibrogenesis. *International Journal of Molecular Sciences*. 2021; 22: 1861. <https://doi.org/10.3390/ijms22041861>
- [34] Gao P, Yuan S, Wang Y, Wang Y, Li X, Liu T, et al. *Corydalis decumbens* and tetrahydropalmatrubin inhibit macrophages inflammation to relieve rheumatoid arthritis by targeting FosL2. *Journal of Ethnopharmacology*. 2025; 341: 119348. <https://doi.org/10.1016/j.jep.2025.119348>
- [35] Xu W, Wang X, Liu D, Lin X, Wang B, Xi C, et al. Identification and validation of hub genes and potential drugs involved in osteoarthritis through bioinformatics analysis. *Frontiers in Genetics*. 2023; 14: 1117713. <https://doi.org/10.3389/fgene.2023.1117713>
- [36] Ren W, Yang L, Deng T, Wu C, Li Y, Wu J, et al. Calcitonin gene-related peptide regulates FOSL2 expression and cell proliferation of BMSCs via mmu\_circRNA\_003795. *Molecular Medicine Reports*. 2019; 19: 3732–3742. <https://doi.org/10.3892/mmr.2019.10038>
- [37] Bozec A, Bakiri L, Hoeberitz A, Eferl R, Schilling AF, Komnenovic V, et al. Osteoclast size is controlled by Fra-2 through LIF/LIF-receptor signalling and hypoxia. *Nature*. 2008; 454: 221–225. <https://doi.org/10.1038/nature07019>
- [38] Kamekura S, Hoshi K, Shimoaka T, Chung U, Chikuda H, Yamada T, et al. Osteoarthritis development in novel experimental mouse models induced by knee joint instability. *Osteoarthritis and Cartilage*. 2005; 13: 632–641. <https://doi.org/10.1016/j.joca.2005.03.004>
- [39] Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biology*. 2020; 18: e3000410. <https://doi.org/10.1371/journal.pbio.3000410>
- [40] Wang Y, Wagner ES, Yu D, Chen KJ, Keel TJ, Pownder SL, et al. Assessment of osteoarthritis functional outcomes and intra-articular injection volume in the rat anterior cruciate ligament transection model. *Journal of Orthopaedic Research* : Official Publication of the Orthopaedic Research Society. 2022; 40: 2004–2014. <https://doi.org/10.1002/jor.25245>
- [41] Lohrasbi E, Babaie S, Hamedfar H, Pourzeinali S, Toopchizadeh V, Farshbaf-Khalili A. Efficacy based on dose-response and safety of bone marrow-derived mesenchymal stem cells for the treatment of osteoarthritis: a systematic review and meta-analysis. *Stem Cell Research & Therapy*. 2025; 16: 674. <https://doi.org/10.1186/s13287-025-04799-0>
- [42] Schwarzl T, Keogh A, Shaw G, Krstic A, Clayton E, Higgins DG, et al. Transcriptional profiling of early differentiation of primary human mesenchymal stem cells into chondrocytes. *Scientific Data*. 2023; 10: 758. <https://doi.org/10.1038/s41597-023-02686-y>
- [43] Ye N, Ding Y, Wild C, Shen Q, Zhou J. Small molecule inhibitors targeting activator protein 1 (AP-1). *Journal of Medicinal Chemistry*. 2014; 57: 6930–6948. <https://doi.org/10.1021/jm5004733>
- [44] Karreth F, Hoeberitz A, Scheuch H, Eferl R, Wagner EF. The AP1 transcription factor Fra2 is required for efficient cartilage development. *Development* (Cambridge, England). 2004; 131: 5717–5725. <https://doi.org/10.1242/dev.01414>
- [45] Zenz R, Eferl R, Scheinecker C, Redlich K, Smolen J, Schonthaler HB, et al. Activator protein 1 (Fos/Jun) functions in inflammatory bone and skin disease. *Arthritis Research & Therapy*. 2008; 10: 201. <https://doi.org/10.1186/ar2338>
- [46] Eferl R, Wagner EF. AP-1: a double-edged sword in tumorigenesis. *Nature Reviews. Cancer*. 2003; 3: 859–868. <https://doi.org/10.1038/nrc1209>
- [47] Birnhuber A, Biasin V, Schnoegl D, Marsh LM, Kwapiszewska G. Transcription factor Fra-2 and its emerging role in matrix deposition, proliferation and inflammation in chronic lung diseases. *Cellular Signalling*. 2019; 64: 109408. <https://doi.org/10.1016/j.cellsig.2019.109408>
- [48] Leser JM, Torre OM, Gould NR, Guo Q, Buck HV, Kodama J, et al. Osteoblast-lineage calcium/calmodulin-dependent kinase 2 delta and gamma regulates bone mass and quality. *Proceedings of the National Academy of Sciences of the United States of America*. 2023; 120: e2304492120. <https://doi.org/10.1073/pnas.2304492120>
- [49] Skelding KA, Rostas JAP, Verrills NM. Controlling the cell cycle: the role of calcium/calmodulin-stimulated protein kinases I and II. *Cell Cycle* (Georgetown, Tex.). 2011; 10: 631–639. <https://doi.org/10.4161/cc.10.4.14798>
- [50] Nalesso G, Thorup AS, Eldridge SE, De Palma A, Kaur A, Peddireddi K, et al. Calcium calmodulin kinase II activity is required for cartilage homeostasis in osteoarthritis. *Scientific Reports*. 2021; 11: 5682. <https://doi.org/10.1038/s41598-021-82067-w>
- [51] Zhang X, Wang C, Zhao J, Xu J, Geng Y, Dai L, et al. miR-146a facilitates osteoarthritis by regulating cartilage homeostasis via targeting Camk2d and Ppp3r2. *Cell Death & Disease*. 2017; 8: e2734. <https://doi.org/10.1038/cddis.2017.146>
- [52] Lv Z, Xu X, Sun Z, Yang YX, Guo H, Li J, et al. TRPV1 alleviates osteoarthritis by inhibiting M1 macrophage polarization via Ca<sup>2+</sup>/CaMKII/Nrf2 signaling pathway. *Cell Death & Disease*. 2021; 12: 504. <https://doi.org/10.1038/s41419-021-03792-8>
- [53] Saitta B, Elphinstone J, Limfat S, Shkhyan R, Evseenko D. CaMKII inhibition in human primary and pluripotent stem cell-derived chondrocytes modulates effects of TGFβ and BMP through SMAD signaling. *Osteoarthritis and Cartilage*. 2019; 27: 158–171. <https://doi.org/10.1016/j.joca.2018.08.017>