Supplementary Materials

Chemical reagents and antibodies

The chemical reagents used were purchased from Medchemexpress, including 3-MA (HY-19312), rapamycin (HY-10219) and bafilomycin A1 (HY-100558). The specific information of antibodies used in this study was provided in Table S1.

Animal experiments

Mice were housed in a specific-pathogen-free animal facility at Nanjing University. The animals were kept under constant temperature and humidity and controlled 12 h light-dark cycles. Mice were fed a standard laboratory chow and water ad libitum. Surgically induced OA by DMM was performed using 10-week-old male C57BL/6 at the right knee joint followed the procedure published previously. Briefly, mice were anaesthetized with isoflurane supplied in a mouse anaesthesia apparatus, followed with joint surgery on the right joint by sectioning the medial meniscotibial ligament. The sham operation with the control mice of the same age was performed with a similar incision at the right joint capsule without menisci ligament section. For a local delivery, 10 μl of rAAV9 carrying siRNA of METTL3 or negative control labeled with EGFP (1×10^{11} GC) was weekly injected into knee joints of mice 14 days after DMM surgery. Mice were sacrificed at 8 weeks after DMM.

For intra-articular delivery of rAAV9 and rAAV9.HAP-1, 10 μl of rAAV9 or rAAV9.HAP-1 labeled with EGFP was intra-articularly injected into knee joint of mice. 3 weeks after injection, EGFP expression in organs were imaged with IVIS Spectrum imaging system. The sections of the knee joint were examined by laser scanning confocal microscope.

Generation of synovium-targeting rAAV9 vector

The DNA sequence encoding the synovium-targeting peptide motif HAP-1 was codon-optimized. To generate the HAP-1 capsid, a pair of plasmids were used in this study. First, the start codon of VP2 in pAAV9 was mutated to generate a plasmid only

express VP1 and VP3. In another plasmid, the HPA-1 sequence was fused to the N-terminus of AAV9-VP2 ORF. A Kozak sequence and ATG start codon were placed immediately upstream of the HAP-1 sequence allowing for optima expression driven by the CMV promoter. The two plasmids were used in rAAV production (5×10^{12} GC/ml).

Histology

Mouse Knee joint were fixed in 4% paraformaldehyde overnight, dehydrated in ethanol and embedded in paraffin after decalcification with ethylene diamine tetraacetic acid (EDTA). Samples were cut into 5 μm slices and stained with Fast Green and Safranin O. Joint pathology was further quantified by using the OARSI scoring system [1].

Immunohistochemisry

Human OA synovium or normal synovium were fixed with 4% paraformaldehyde overnight and embedded in paraffin before sectioning into 5 μm-thick slices. Then, the sections were deparaffinized with xylene before being rehydrated in water using an ethanol gradient. After washing with water, antigen retrieval was performed and the samples were further incubated with 3% H_2O_2 and blocked with goat serum. The sections were subsequently incubated with rabbit anti-p21 overnight at 4°C. Finally, the sections were washed and incubated with appropriate secondary antibody for 1 h. Antigen-positive cells were visualized using the DAB Substrate kit (ZSGB-BIO, Beijing, China).

Immunofluorescent staining

Cells were washed with PBS and fixed with 4% paraformaldehyde for 10 min. Tissue sections were deparaffinized in xylene, and hydrated with decreasing concentrations of ethanol. Next, cells or tissue section were permeabilized with 0.3% Triton X-100 (Sigma) for 5 min and blocked with bovine serum albumin (Sigma) for 1 h at room temperature, followed with the incubation of the primary antibodies overnight at 4°C.

Samples were washed and further incubated with Alexa Flour 488 or 594 secondary antibodies for 1 h at room temperature. Nuclei were stained with DAPI (Beyotime Biotechnology). Images were taken by a confocal laser microscope (Olympus).
For the quantification of LC3B positive puncta, samples were captured at 5 random vision fields and the mean numbers of LC3B positive puncta were calculated by using imageJ software.

Isolation of FLS and cell culture

Human OA synovium specimens were sourced from OA patients (n=10, age from 50-65) undergoing total knee replacement at Drum Tower Hospital of Nanjing. The normal synovium were obtained from 10 patients who underwent arthroscopic meniscus or anterior cruciate ligament repair surgery at the age from 50-65, which displayed no synovitis and no cartilage injury as confirmed by arthroscopic examination. Detail information of patients were shown in Table S2-4. FLS were isolated from synovial tissues through enzymatic digestion using 2 mg/ml Collagenase A and 0.1 mg/ml DNase I (Roche). The dissociated cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) complemented with 10% fetal bovine serum (FBS). Non-adherent cells were removed after 1 day incubation. The cells were detached with Trypsin-EDTA (Gibco) and split in new medium when reaching approximately 90% confluence.

The immortalized human chondrocyte cell line C28/I2 were established by the laboratory of Professor Mary B Goldring (Hospital for Special Surgery/Weill Medical College of Cornell University, New York, NY), which were isolated from the costal cartilage of a 15 year old female and immortalized using SV-40 large T-antigen [2]. C28/I2 cells were cultured in DMEM/F12 medium supplemented with 10% FBS. The cells were detached with Trypsin-EDTA (Gibco) and split in new medium when reaching approximately 90% confluence.

Plasmid and siRNA transfection

Gene sequences coding for GATA4, ATG7 and METTL3 were cloned into the pcDNA3.1 vector (Invitrogen). The siRNA sequence used in this study were designed and chemically synthesized by RiboBio as: human GATA4, 5′-CGAUAUGUUUGACGACUUC-3′; human ATG7, 5′-CAACAUCCCUGGUUACAAG-3′; human METTL3, 5′-GCACTTGGATCTACGGAAT-3′; human YTHDF1, 5′-GATACAGTTCATGACAATGA-3′; human YTHDF2, 5′-CTGCCATGTCAGATTCCTA-3′; mouse METTL3, 5′-CGGCUAAGAAGUCAAGGAA-3′. Transfection was performed using Lipofectamine3000 (Invitrogen) according to the manufacturer's instruction.

Western blot analysis

Cells or tissues were lysed on ice by RIPA buffer containing with protease and phosphatase inhibitor cocktail (Beyotime Biotechnology) for 30 min. The protein concentration was determined by the BCA protein assay kit (Beyotime Biotechnology). Total protein was further separated by SDA-PAGE before transferring to polyvinylidene difluoride (Millipore) membranes. Then, the membranes were blocked with 5% non-fat milk at room temperature for 1 h, followed with the incubation of corresponding primary antibodies overnight at 4°C. After 5 times washing, the membranes were incubated with HRP-conjucted secondary antibodies at room temperature for 1 h. Protein bands were visualized by using ECL solution (Millipore) and detected with an automatic chemiluminescence imaging analysis system (Tanon, Shanghai, China). The intensity of each band was quantified using NIH ImageJ software.

Quantitative real-time PCR

Total RNA from cells or tissues was extracted by using the TRIzol reagent (Invitrogen) following the manufacturer's instructions. The cDNA was generated by HiScript Q RT SuperMix (Vazyme, Nanjing, China). qPCR was performed by using ChamQ SYBR qPCR Master Mix (Vazyme) with the ABI Step-One Plus™ Real-Time

PCR System (Applied Biosystems). The data were analyzed following the $2^{-\Delta\Delta Ct}$ method and calculated using Gapdh as internal control for normalization. All primers were listed in Table S5.

SA-β-Gal staining

SA-β-Gal staining was performed by using Cell senescence β-galactosidase staining kit (Beyotime Biotechnology) according to the manufacturer's protocol. Briefly, cells were washed with PBS and fixed with 2% PFA and 0.2% glutaraldehyde for 5 min. Then, the cells were washed and incubated with SA-β-Gal staining solution at 37°C for 16 h. After incubation, the cells were washed and imaged by using Eclipse Ni-U microscope (Nikon). Total cells and SA-β-Gal–positive cells were calculated in three random fields per culture dish via imageJ.

ELISA

Cells were washed with PBS and incubated in serum-free DMEM containing antibiotics for 24 h. The conditioned medium were filtered and stored at -80°C. Cell number was determined in each experiment. ELISA was performed using kit purchased from R&D (IL-1β, #DLB50). The data were normalized to cell numbers.

RNA-immunoprecipitation (RIP)-qPCR analysis

RIP analysis was performed by using EZ-Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore Sigma) according to the instruction of the manufacturer. Briefly, cells were lysed with RIP lysis buffer containing with protease and RNase inhibitor. Then, the cell lysates were centrifuged and the supernatant was collected, which was further incubated with magnetic beds conjugated with YTHDF1 or YTHDF2 antibodies overnight at 4°C. After being washed with IP wash buffer for three times, the beads were treated with proteinase K (Millipore Sigma) at 65°C for 0.5 h with occasional shaking. At last, the input and immunoprecipitated RNAs were isolated by TRIzol reagent, and the fold enrichment was detected by qPCR

Methylated RNA immunoprecipitation-PCR (m^6A RIP-qPCR)

The m^6A modification levels in the mRNA of ATG7 was measured by using the magna MeRIP^{TM} m^6A kit according to the manufacturer's instructions (Millipore Sigma). Briefly, the isolated RNA was fragmented by using RNA fragmentation buffer. One tenth of total RNA was saved to be used as the input RNA. Then, the remaining RNAs were immunoprecipitated with magnetic beads A/G coated with m^6A antibody (Millipore Sigma) overnight at 4°C. The m^6A-modified RNAs were eluted with elution buffer after three time washing with immunoprecipitation wash buffer. Finally, eluted RNAs were recovered by ethanol precipitation and then subjected to qPCR assay to determine the m^6A enrichment. The sequences of primers used are presented in Table S6. In addition, total cellular m^6A levels were further detected by EpiQuik m^6A RNA Methylation Quantification Kit (Epigentek). The measurement was performed in triplicate according to the manufacturer's instructions.

Statistical analysis

Results are presented as the mean ± SD. Statistical significances were calculated with Student's t-test for comparisons between 2 groups and ANOVA for multiple group comparisons as showed in figure legends. P values were considered significant at *P < 0.05, **P < 0.01.

1. Pritzker KP, Gay S, Jimenez SA, et al. Osteoarthritis cartilage histopathology: grading and staging. *Osteoarthritis Cartilage* 2006; 14: 13-29.
2. Goldring MB, Birkhead JR, Suen LF, et al. Interleukin-1 beta-modulated gene expression in immortalized human chondrocytes. *J Clin Invest* 1994; 94: 2307-16.