Bone & Joint Research

Supplementary Material

10.1302/2046-3758.103.BJR-2020-0206.R2

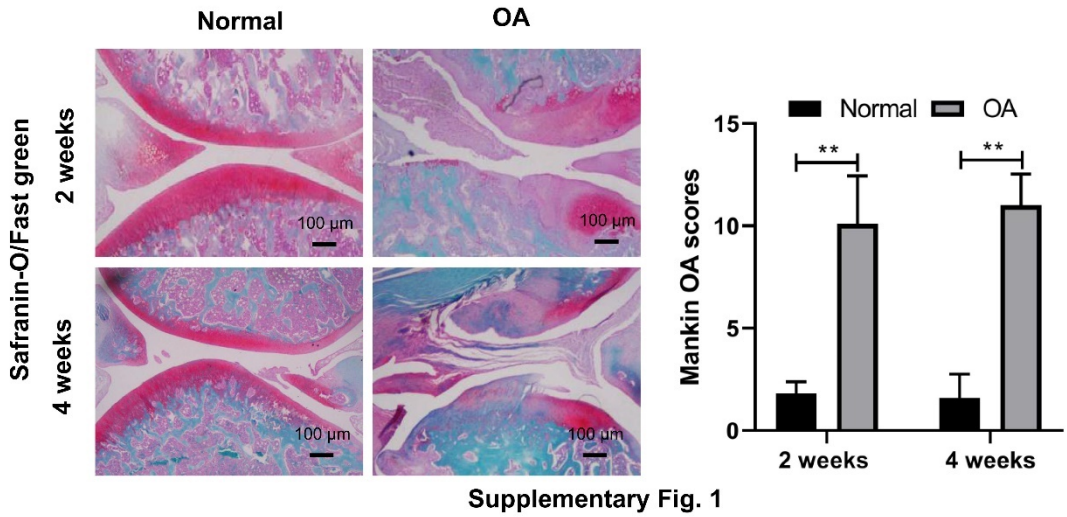
Table i. Primers used for quantitative real-time polymerase chain reaction.

Gene	Abbreviation	Primer	Primer sequence (5'→3')
glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	*GAPDH*-F	TGCACCACCAACTGCTTAGC
		GAPDH-R	GGCATGGACTGTGGTCATGAG
bone gamma-carboxyglutamate protein	*BGLAP*	*BGLAP*-F	ATGAGAGCCCTCACACTCCTC
		BGLAP-R	GCAGCCTTTGTGTCCAAGCAG
Sp7 transcription factor	*SP7*	*SP7*-F	ATGGCGTCCTCCCTGCTTG

		SP7-R	GTTGTTGAGTCCCGCAGAGG
RUNX family transcription factor 2	*RUNX2*	*RUNX2*-F	GGTACCAGATGGGACTGTG
		RUNX2-R	CATAACAGCAGAGGCATTCC
secreted phosphoprotein 1	*OPN*	*OPN*-F	TTCTGGGAGGGCTTGGTTGTC
		OPN-R	TTGGTCGGCGTTTGGCTGAG
alkaline phosphatase	*ALP*	*ALP*-F	ATGGACCGCTTCCCATATGTG
		ALP-R	TCTGGAAGTTGCCCTTGACC
fatty acid binding protein 4	*FABP4*	*FABP4*-F	TGGGCCAGGAATTTGACGAAG
		FABP4-R	CTGCACATGTACCAGGACACC
peroxisome proliferator activated receptor gamma	*PPARγ*	*PPARγ*-F	GGAGACACTGTGTATGGCTG
		PPARγ-R	TGCACTGGCAGCAGTGAAAG
CCAAT enhancer	*C/EBP-α*	*C/EBP-α*-F	CCGCCTTCAACGACGAGTTC

binding protein alpha			
		C/EBP-α-R	TAGTCAAAGTCGCCGCC
preadipocyte factor 1	*Pref-1*	*Pref-1*-F	AGAGATGACCGCGACCGAAG
		Pref-1-R	AGCATTCAGCCCCATAGGTG
lipoprotein lipase	*LPL*	*LPL*-F	TCCAGCTGGACCTAACTTTG
		LPL-R	AGGGGACCCTCTGGTGAATG
aggrecan	*ACAN*	*ACAN*-F	TGCATTCCACGAAGCTAACCTT
		ACAN-R	GACGCCTCGCCTTCTTGAA
melanoma inhibitory activity	*MIA*	*MIA*-F	CCCAGTAGCATTGTCCGAGA
		MIA-R	GGCAGTAGAAATCCCATTTGTCT
collagen type I alpha 2 chain	*COL1A2*	*COL1A2*-F	GCTGGCAGCCAGTTTGAATATAAT
		COL1A2-R	CAGGCGCATGAAGGCAAGT
collagen type II alpha 1 chain	*COL2A1*	*COL2A1*-F	AGAGGTATAATGATAAGGATGTGTGGAAG
		COL2A1-R	GTCGTCGCAGAGGACAGTCC

collagen type X alpha 1 chain	*COL10A1*	*COL10A1*-F	CACGCAGAATCCATCTGAGAAT
		COL10A1-R	CGTTCAGCGTAAAACACTCCAT
glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	*GAPDH*-F	GCAAGTTCAACGGCACAG
		GAPDH-R	GCCAGTAGACTCCACGACA
interleukin 1 beta	*Il-1b*	*Il-1b*-F	TGCAGGCTTCGAGATGAAC
		Il-1b-R	GGGATTTTGTCGTTGCTTGTC
tumour necrosis factor	*TNF-α*	*TNF-α*-F	ATCCCCGAATGTCGATGCCT
		TNF-α-R	TGGGAAGCTCTGAGGGAGGG
interleukin 1 receptor antagonist	*Il-1ra*	*Il-1rn*-F	TCATTGCTGGGTACTTACAAGG
		Il-1rn-R	CTGGAGCTTGGTGTCATCTC
TNF alpha induced protein 6	*TSG-6*	*TSG-6*-F	TCTTTAAATCCCCAGGCTTCC
		TSG-6-R	ACATAGTCAGCCAAACAGCC



Supplementary Fig. 1

Fig. a. Histological evaluation of the articular cartilage after the injection of 1.0 mg monosodium iodoacetate (MIA) at two and four weeks. Safranin-O/Fast green staining of representative specimens. The pictures shown are representative of each group (scale bars = 100 µm). The severity of osteoarthritis (OA) was assessed using the Mankin score system by three independent investigators (including WR and YQZ) in blind fashion. Data shown are mean and standard deviation (SD) from 3 rats/group. **$p < 0.01$ versus normal group.

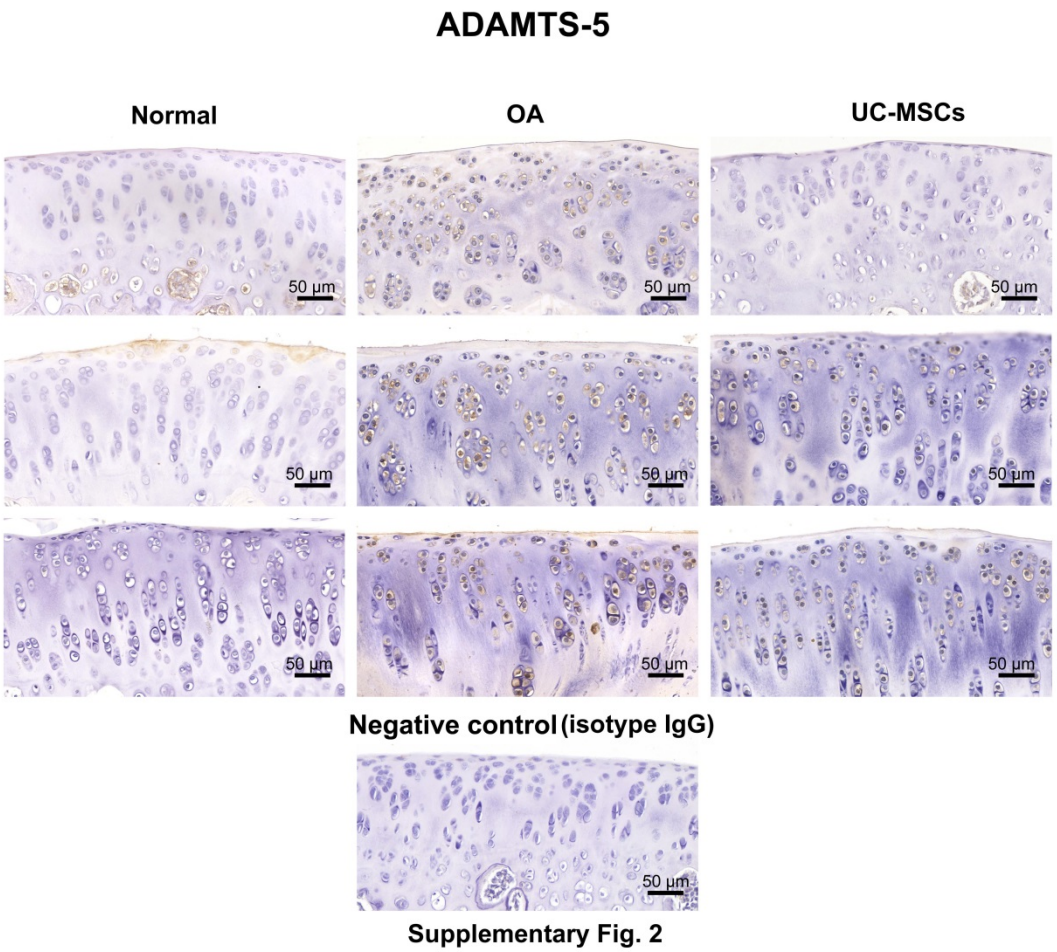


Fig. b. Immunohistochemistry of a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) in the articular cartilage. The expression of ADAMTS-5 in the articular cartilage was determined by immunohistochemistry staining. Three sections were selected from each group. Immunoglobin G (IgG) was used as negative (isotype) control (Scale bar = 50 μm). OA, osteoarthritis; UC-MSCs, umbilical cord mesenchymal stem cells.

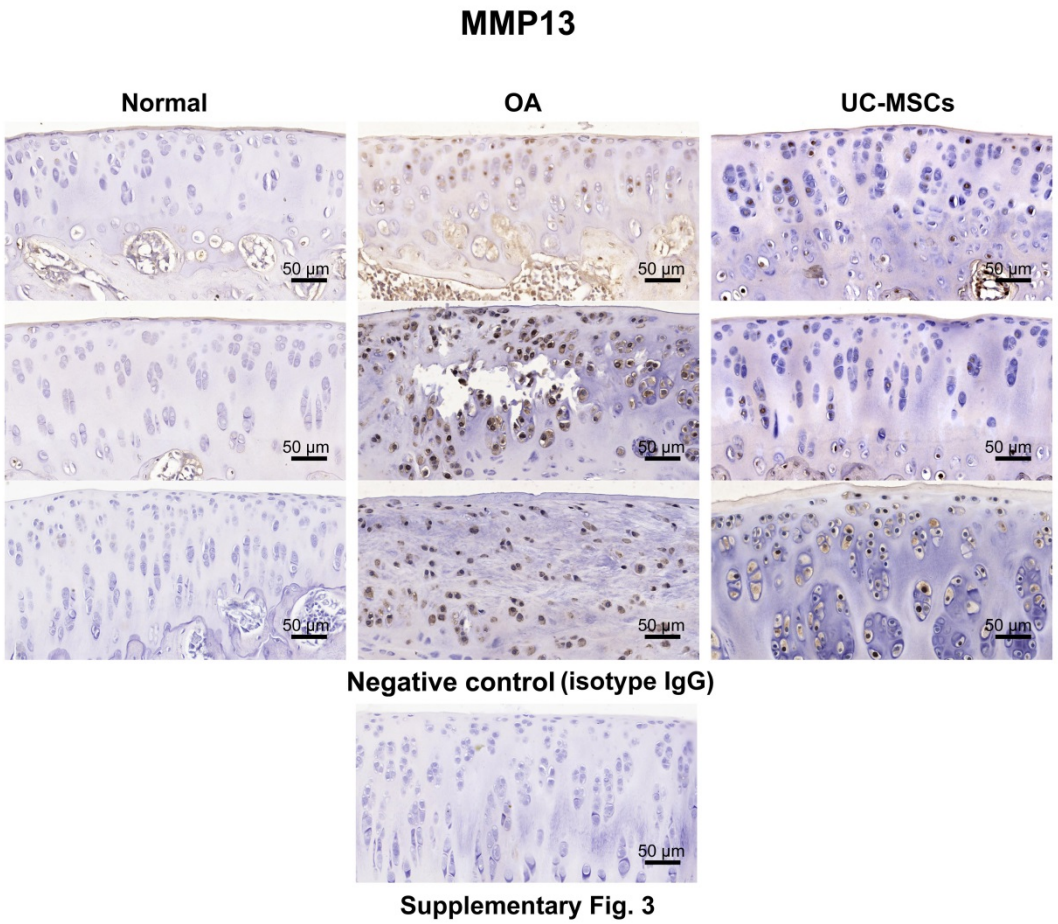


Fig. c. Immunohistochemistry of matrix metalloproteinase-13 (MMP13) in the articular cartilage. The expression of MMP13 in the articular cartilage was determined by immunohistochemistry staining. Three sections were selected from each group. Immunoglobin G (IgG) was used as negative (isotype) control (Scale bar = 50 μm). OA, osteoarthritis; UC-MSCs, umbilical cord mesenchymal stem cells.

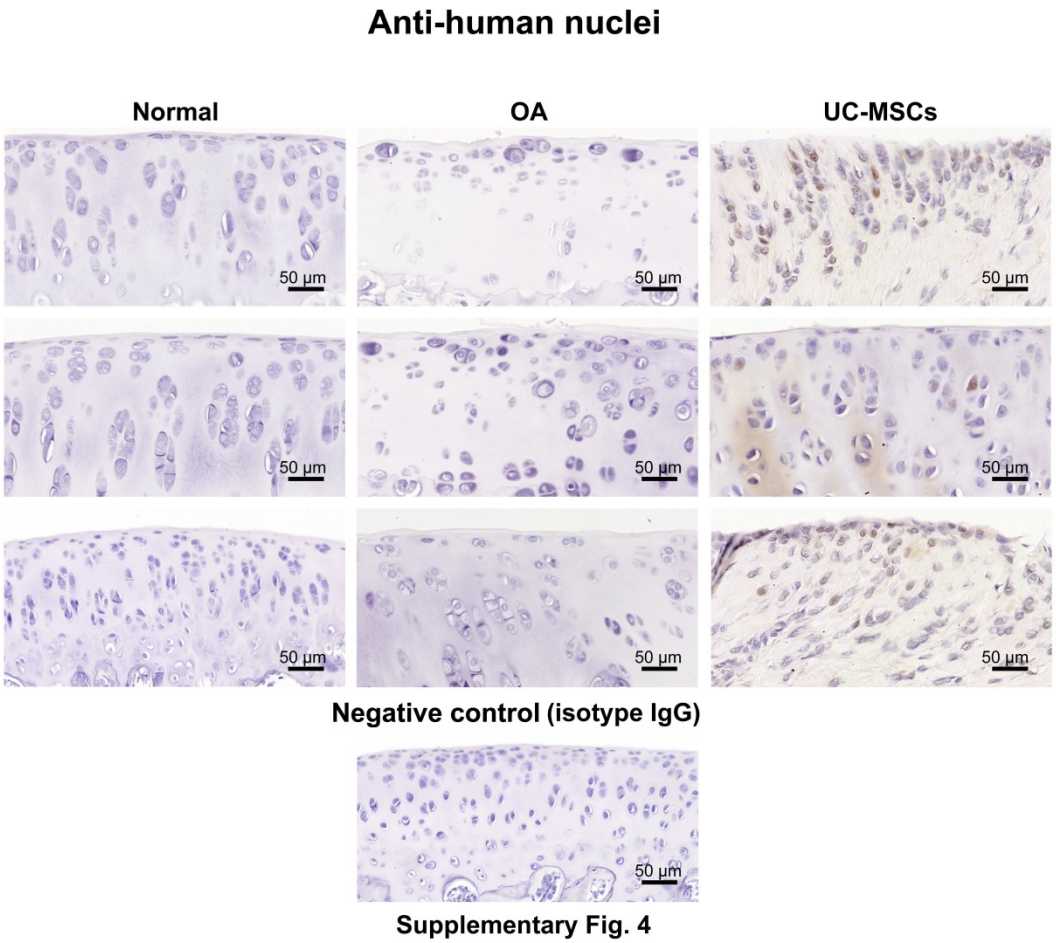


Fig. d. Immunohistochemistry of anti-human nucleic antibody MAB1281 in the articular cartilage. The expression of anti-human nucleic antibody MAB1281 in the articular cartilage was determined by immunohistochemistry staining. Three sections were selected from each group. Immunoglobin G (IgG) was used as negative (isotype) control (Scale bar = 50 μm). OA, osteoarthritis; UC-MSCs, umbilical cord mesenchymal stem cells.

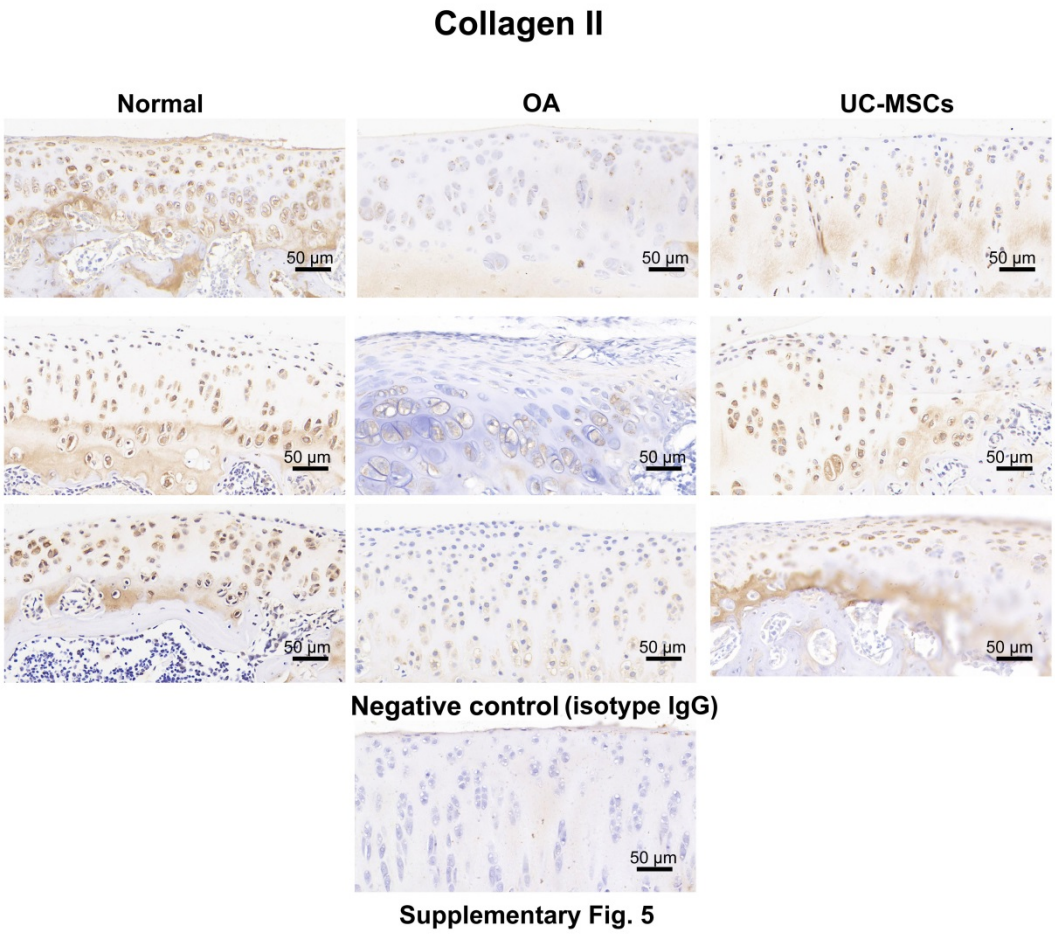


Fig. e. Immunohistochemistry of type II collagen in the articular cartilage. The expression of type II collagen in the articular cartilage was determined by immunohistochemistry staining. Three sections were selected from each group. Immunoglobin G (IgG) was used as negative (isotype) control (Scale bar = 50 μm). OA, osteoarthritis; UC-MSCs, umbilical cord mesenchymal stem cells.

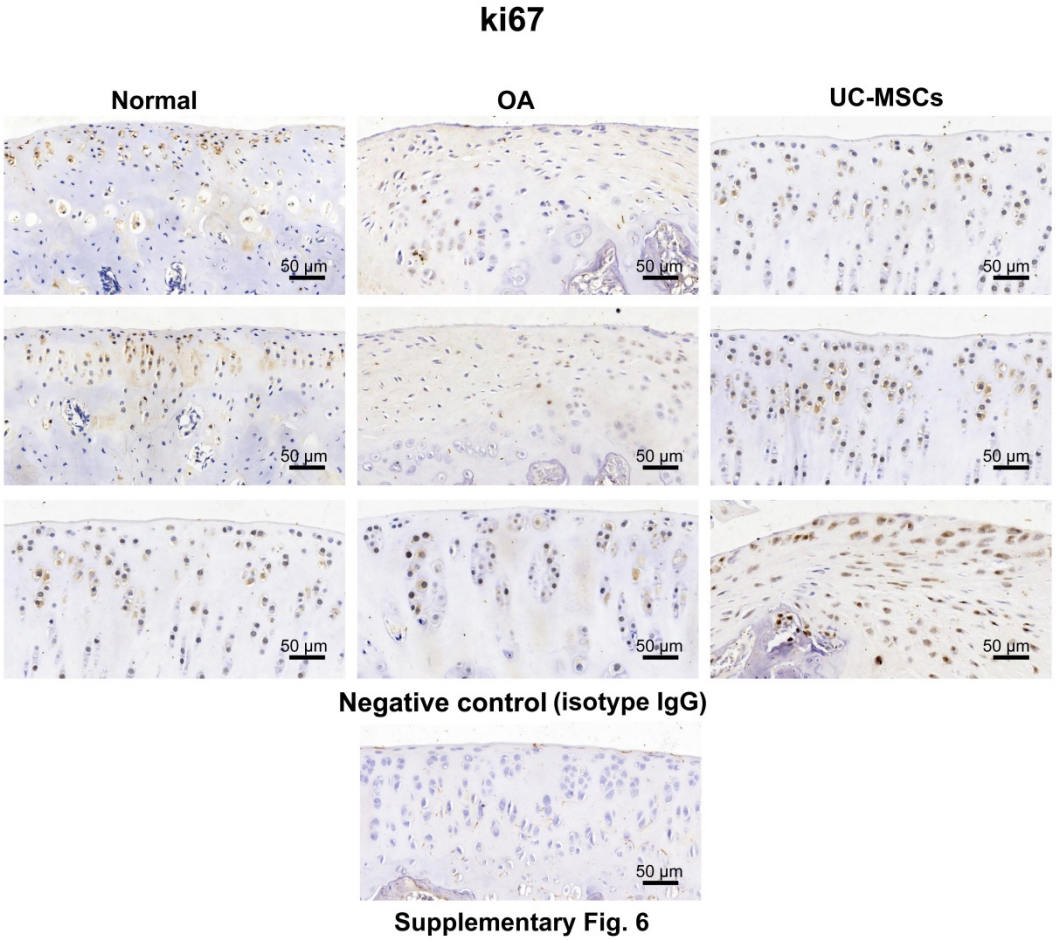


Fig. f. Immunohistochemistry of ki67 in the articular cartilage. The expression of ki67 in the articular cartilage was determined by immunohistochemistry staining. Three sections were selected from each group. Immunoglobin G (IgG) was used as negative (isotype) control (Scale bar = 50 μm). OA, osteoarthritis; UC-MSCs, umbilical cord mesenchymal stem cells.

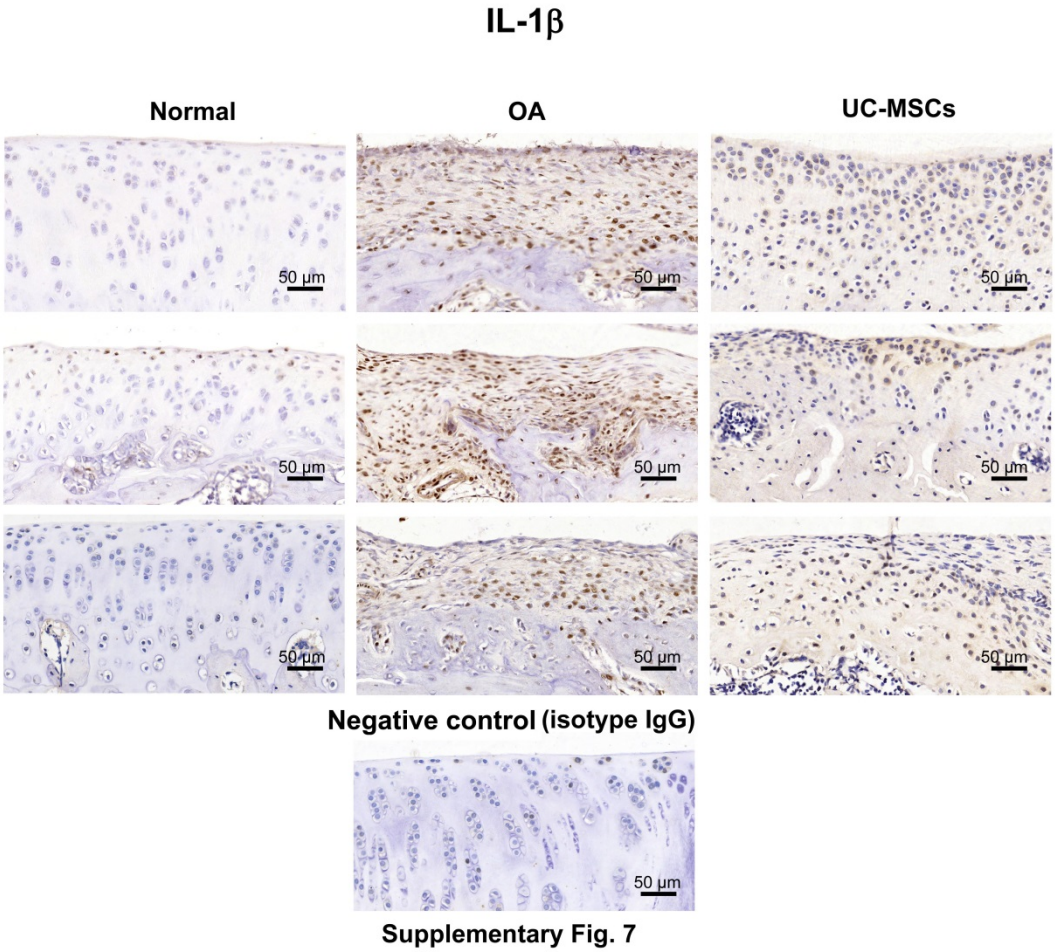


Fig. g. Immunohistochemistry of interleukin-1ß (IL-1ß) in the articular cartilage. The expression of IL-1ß in the articular cartilage was determined by immunohistochemistry staining. Three sections were selected from each group. Immunoglobin G (IgG) was used as negative (isotype) control (Scale bar = 50 μm). OA, osteoarthritis; UC-MSCs, umbilical cord mesenchymal stem cells.

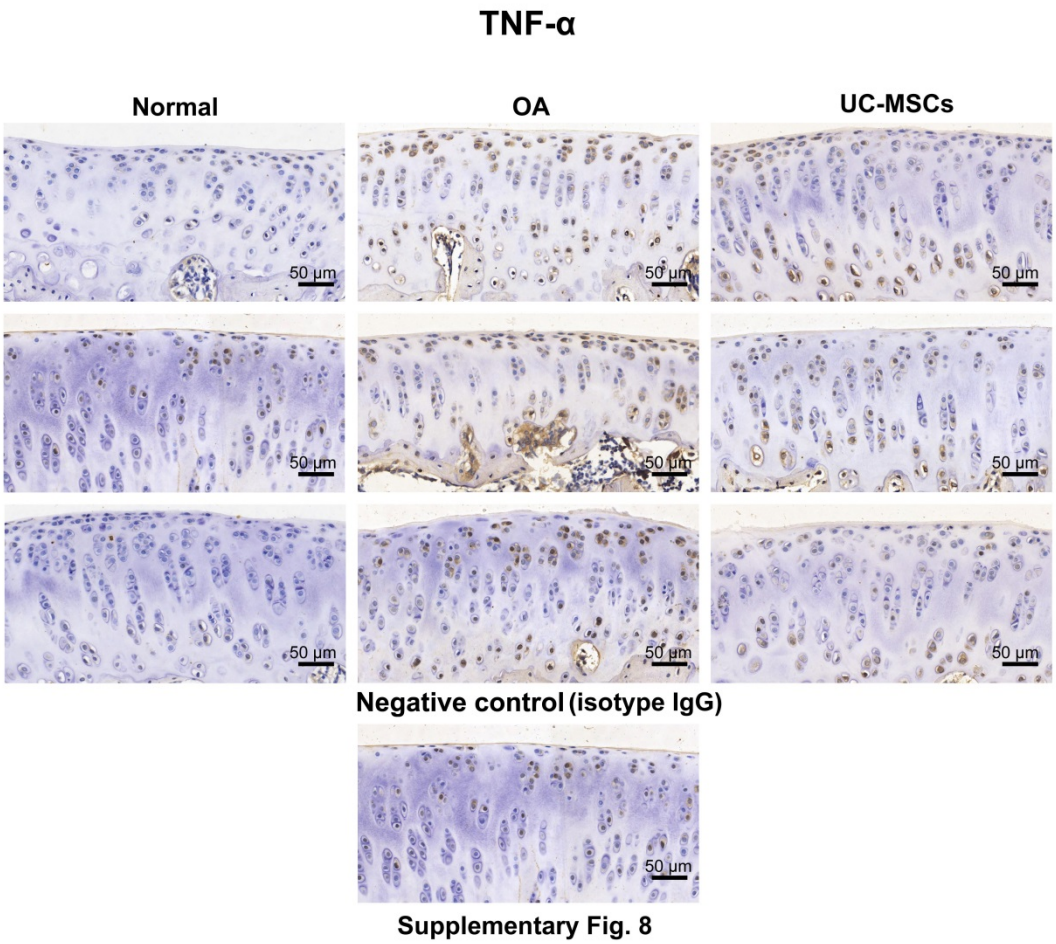


Fig. h. Immunohistochemistry of tumour necrosis factor-α (TNF-α) in the articular cartilage. The expression of TNF-α in the articular cartilage was determined by immunohistochemistry staining. Three sections were selected from each group. Immunoglobin G (IgG) was used as negative (isotype) control (Scale bar = 50 μm). OA, osteoarthritis; UC-MSCs, umbilical cord mesenchymal stem cells.

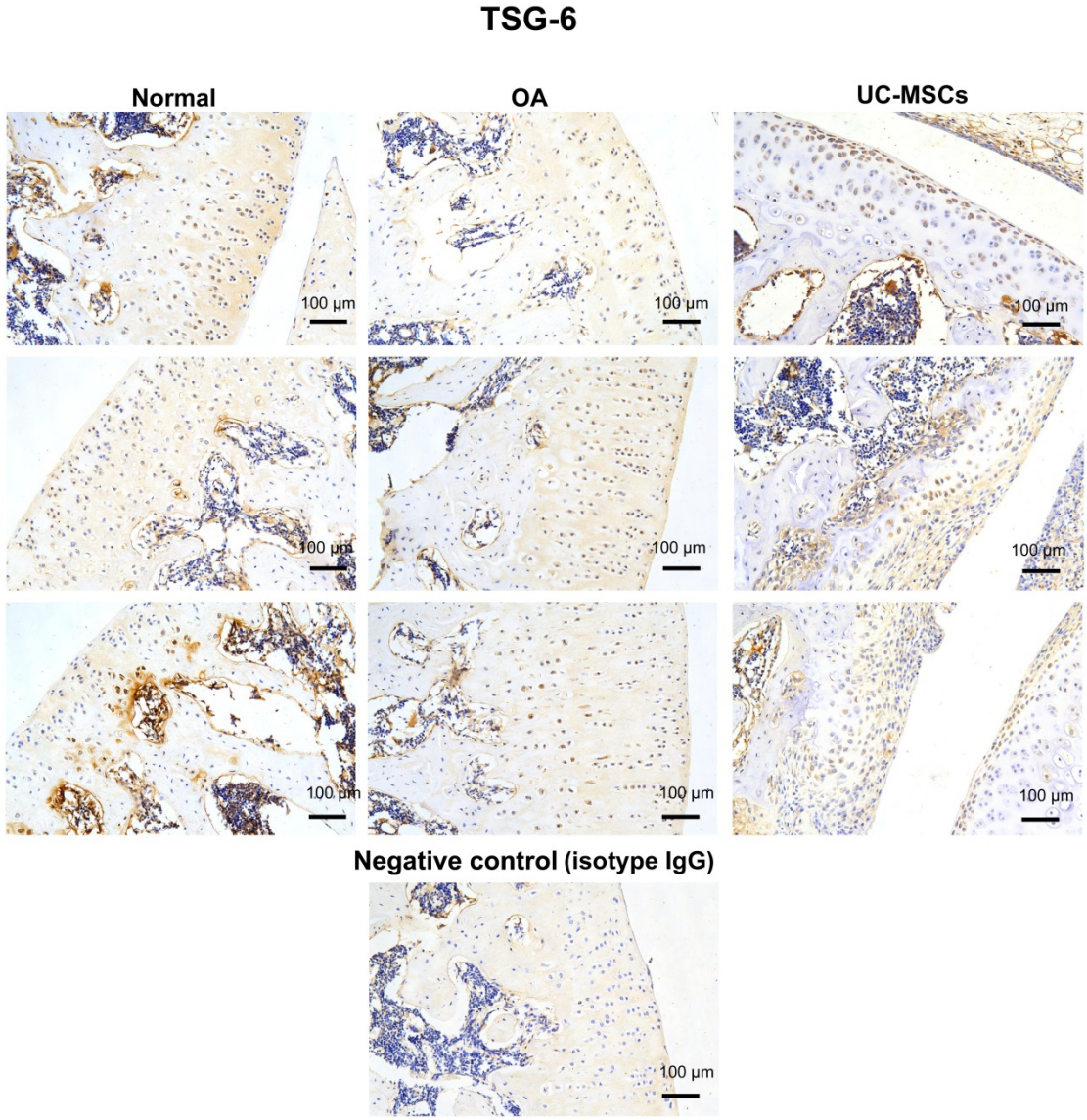


Fig. i. Immunohistochemistry of tumour necrosis factor-α-induced protein 6 (TSG-6) in the articular cartilage. The expression of TSG-6 in the articular cartilage was determined by immunohistochemistry staining. Three sections were selected from each group. Immunoglobin G (IgG) was used as negative (isotype) control (Scale bar = 50 μm). OA, osteoarthritis; UC-MSCs, umbilical cord mesenchymal stem cells.

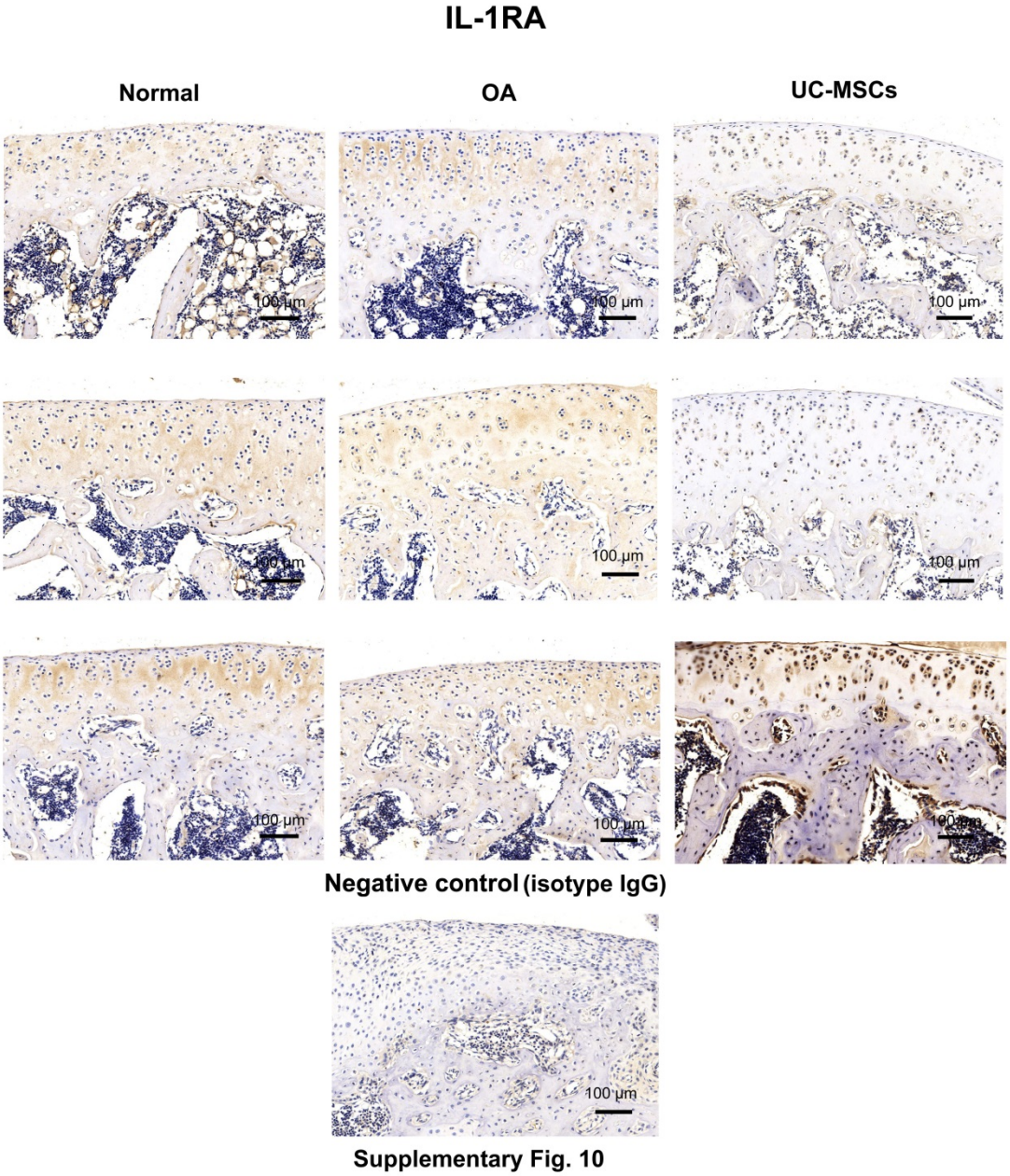
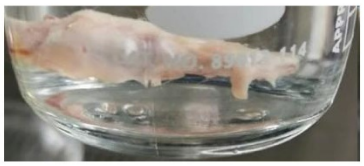
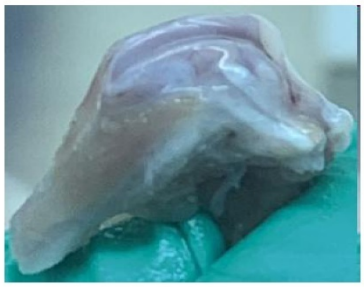


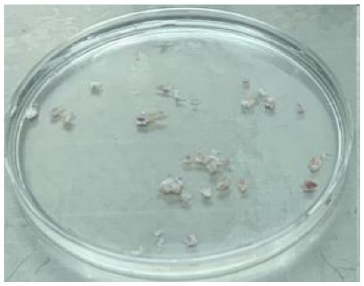
Fig. j. Immunohistochemistry of interleukin-1 receptor antagonist (IL-1RA) in the articular cartilage. The expression of IL-1RA in the articular cartilage was determined by immunohistochemistry staining. Three sections were selected from each group. Immunoglobin G (IgG) was used as negative (isotype) control (Scale bar = 50 μm). OA, osteoarthritis; UC-MSCs, umbilical cord mesenchymal stem cells.



Supplementary Fig. 11a



Supplementary Fig. 11b



Supplementary Fig. 11c

Supplementary Fig. 11d

Fig. k. Isolation of chondrocytes from the articular cartilage of Wistar rats. a) Skin and soft tissues were removed from the right legs of rats. b) Isolate femoral heads, femoral condyles, and tibial plateau using scalpel. c) The cartilage was cut into small pieces, freshly isolated chondrocytes were obtained. d) Cartilage pieces were collected through the centrifuge (n = 3 to 4 per group).

The ARRIVE Essential 10: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/paragraph
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Methods (Page4 Monosodium iodoacetate-induced osteoarthritis model). Methods (Page2-4)
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Methods (Page4 Monosodium iodoacetate-induced osteoarthritis model) Methods (Page4)
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	Methods (Page4) There were no data not included in the analysis Methods(Page4 Monosodium iodoacetate-induced osteoarthritis model)
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Methods(Page4) the animal were randomized into each group all the animals were kept in the same environment, and minimized the potential confounder
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Page 3(Figure 3-captions)

Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Abstract(Page1)and Methods(Page 2-4) There is no hypothesis-testing
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Statistical analysis (Page 6) Statistical analysis (Page 6)
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Methods (Page 2, 180g-200g male Wistar rats were used in this study) Methods (Page 2, health Wistar rats)
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Methods(Page 4) Methods(Page 4) Methods(Page 4) Methods(Page 4)
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Statistical analysis (Page 6) There is no need for The confidence interval.