

Supplementary Materials

Mesenchymal stem cell-derived extracellular vesicles promote macrophage efferocytosis and inflammation resolution in osteoarthritis through the miR-21a-5p/YAP1 axis

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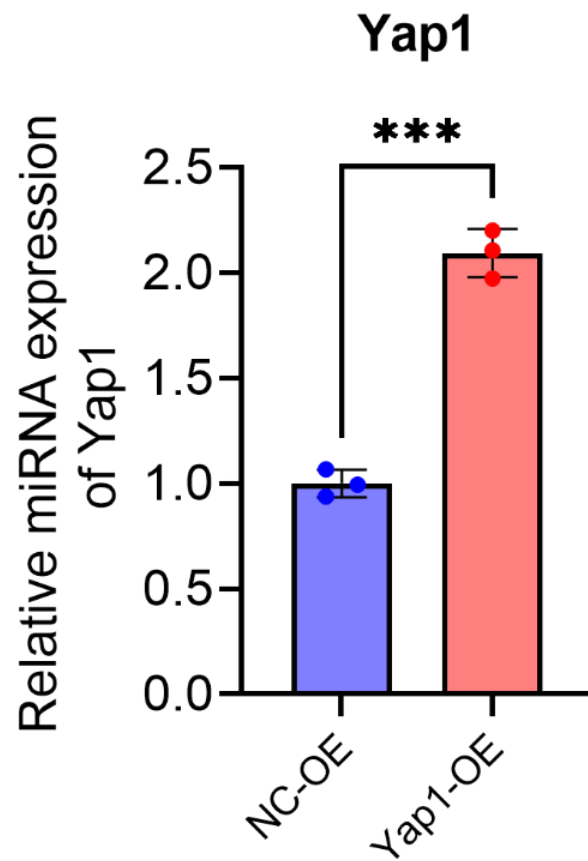
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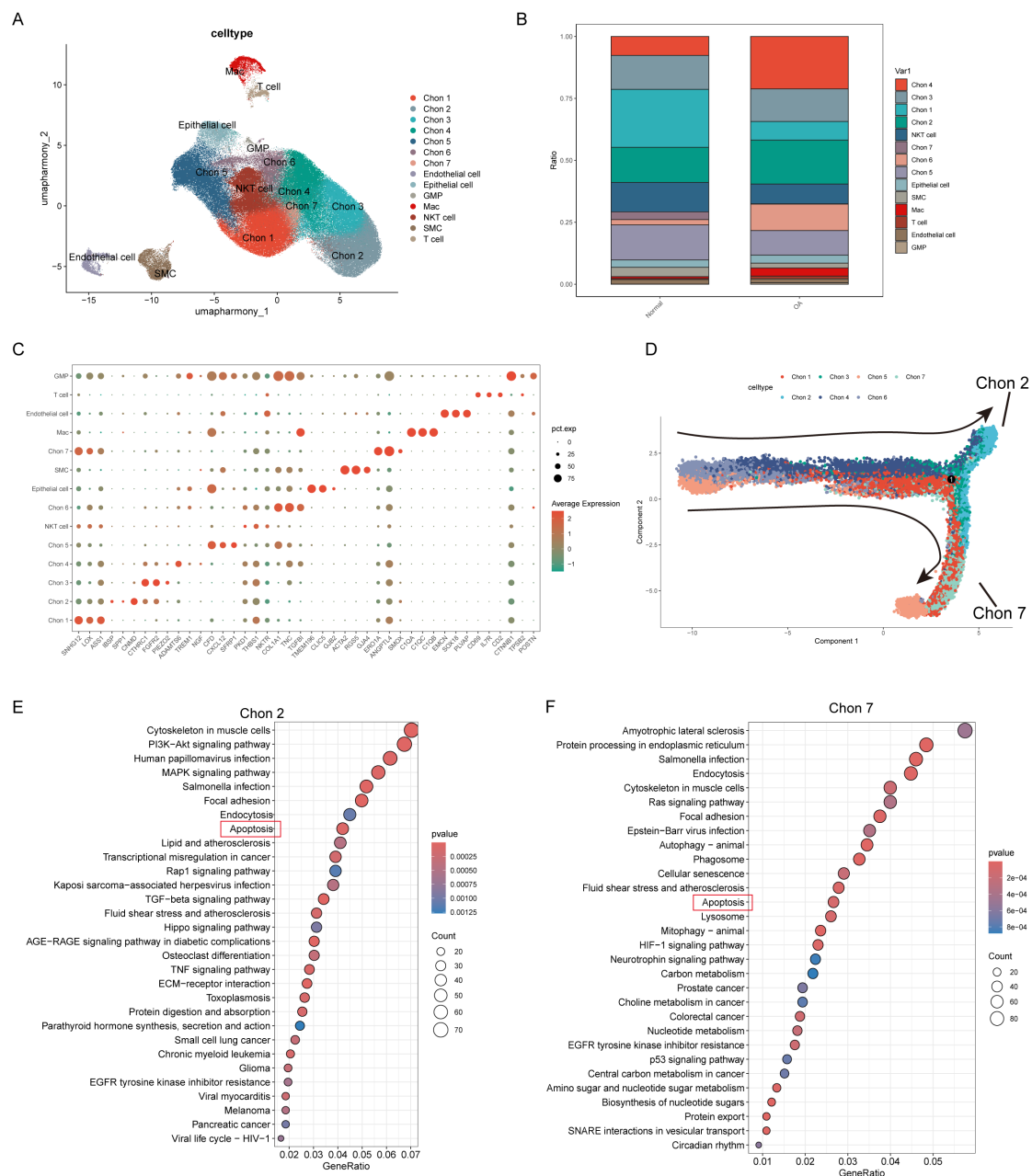
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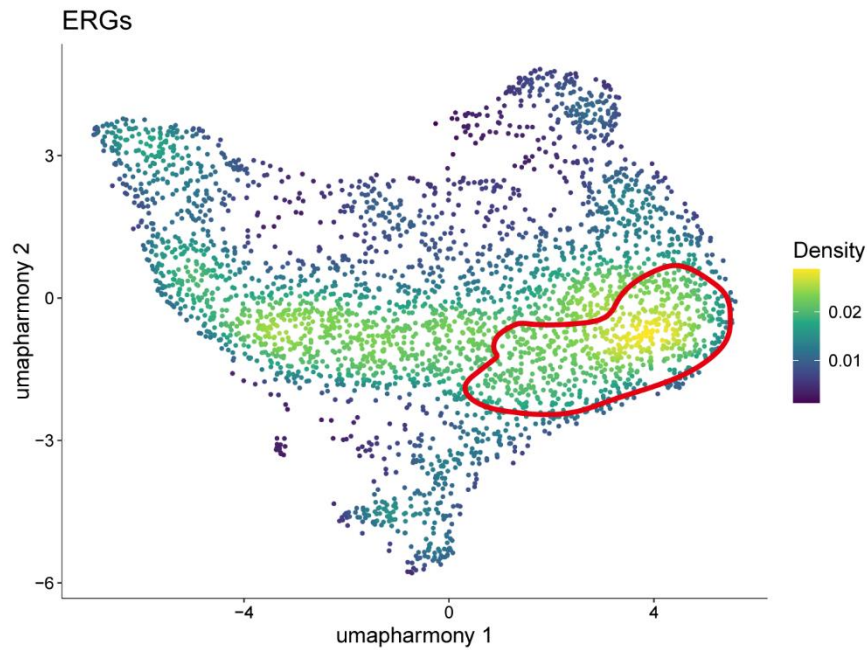
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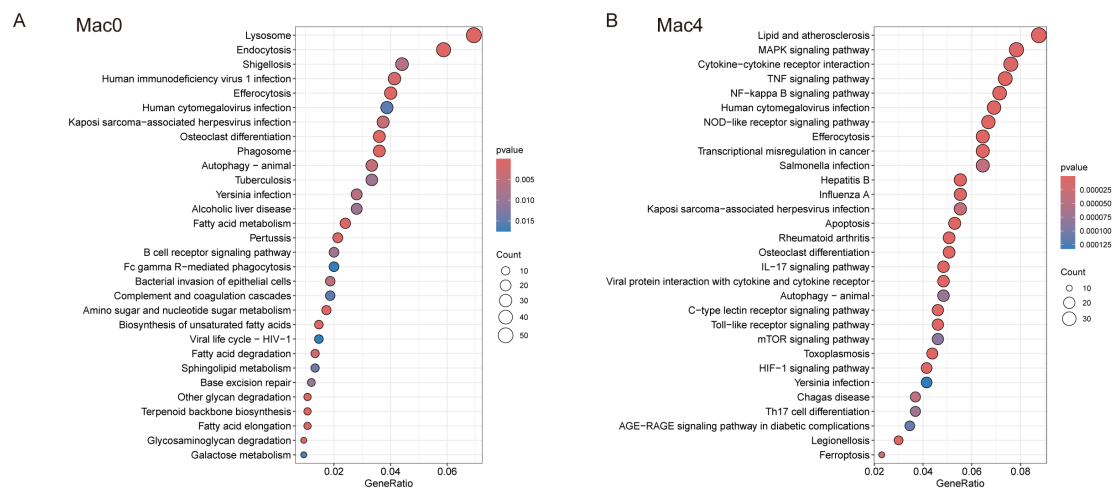
Supplementary Figure 16. qRT-PCR analysis of the Yap1 expression in negative and overexpression groups ($n = 3$ independent biological experiments). NC indicated Vector-NC. Student's t test was used. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.



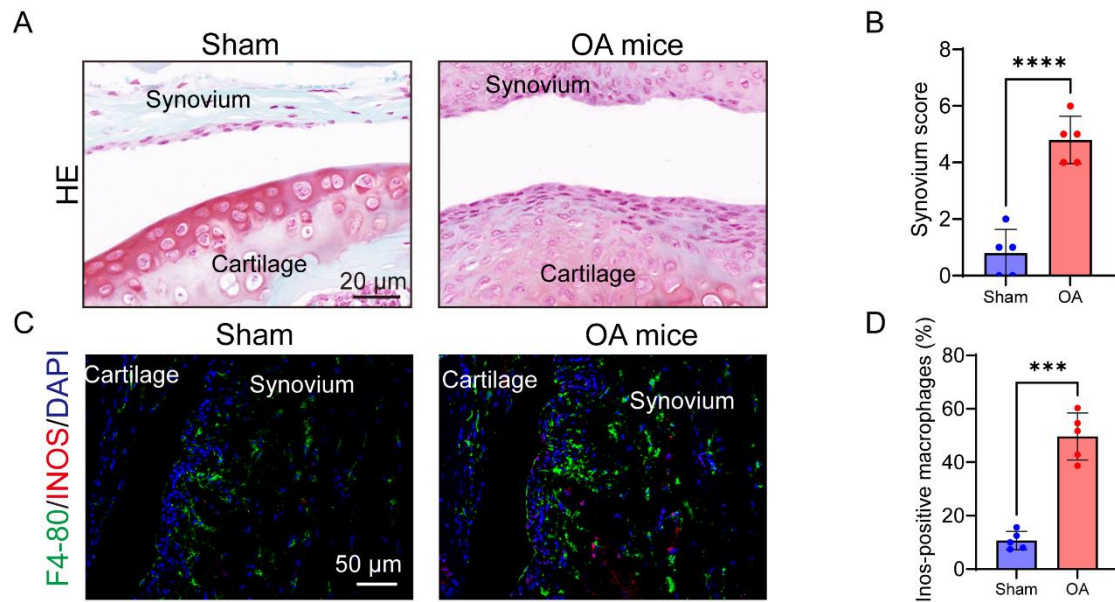
Supplementary Figure 2. Single cell sequencing analysis of human knee tissue. (A) UMAP visualizing human knee joint cells as 14 different subclusters after unsupervised clustering. Each point indicates a single cell, colored according to different cell subclusters; (B) Relative proportion of each cell cluster between the normal group and OA group; (C) Representative molecular signatures for each human cell clusters. The area of the bubbles indicates the proportion of cells expressing the gene, and the color intensity reflects the expression intensity. The area of the bubbles indicates the proportion of cells expressing the gene, and the color intensity reflects the expression intensity; (D) Trajectory differentiation diagram colored by cell types and differentiated states. Cells within terminal state were Chon 2 and 7; (E and F) KEGG analysis of the differential pathways in Chon 2 and 7.



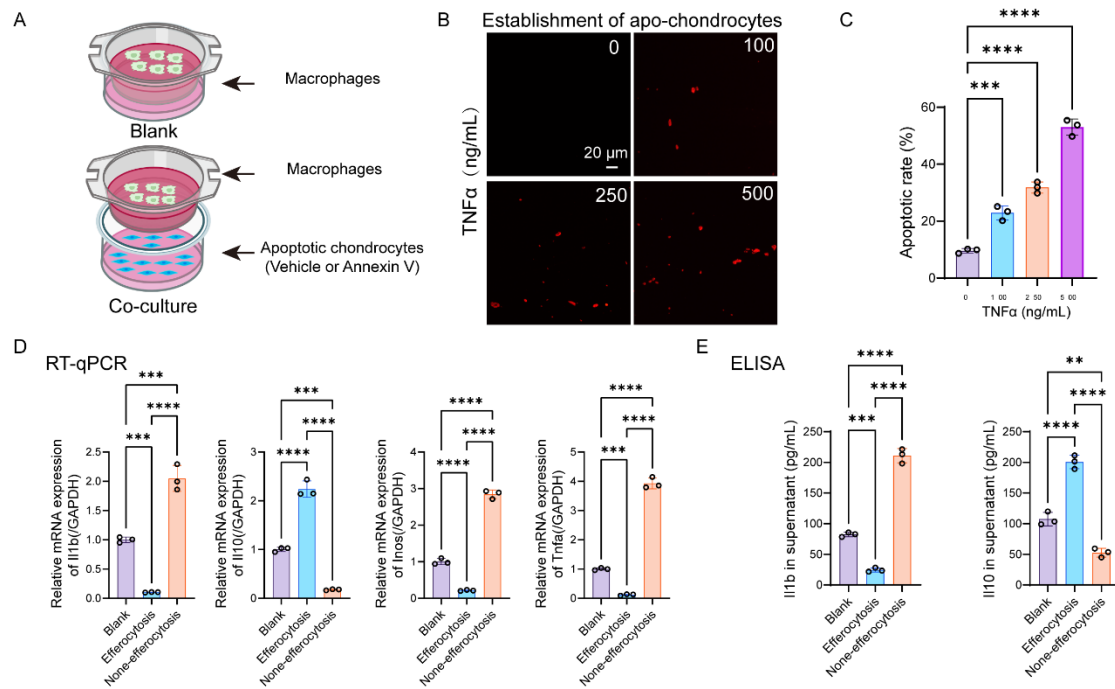
Supplementary Figure 3. UMAP showing the efferocytosis score of macrophage subclusters based on single cell sequencing.



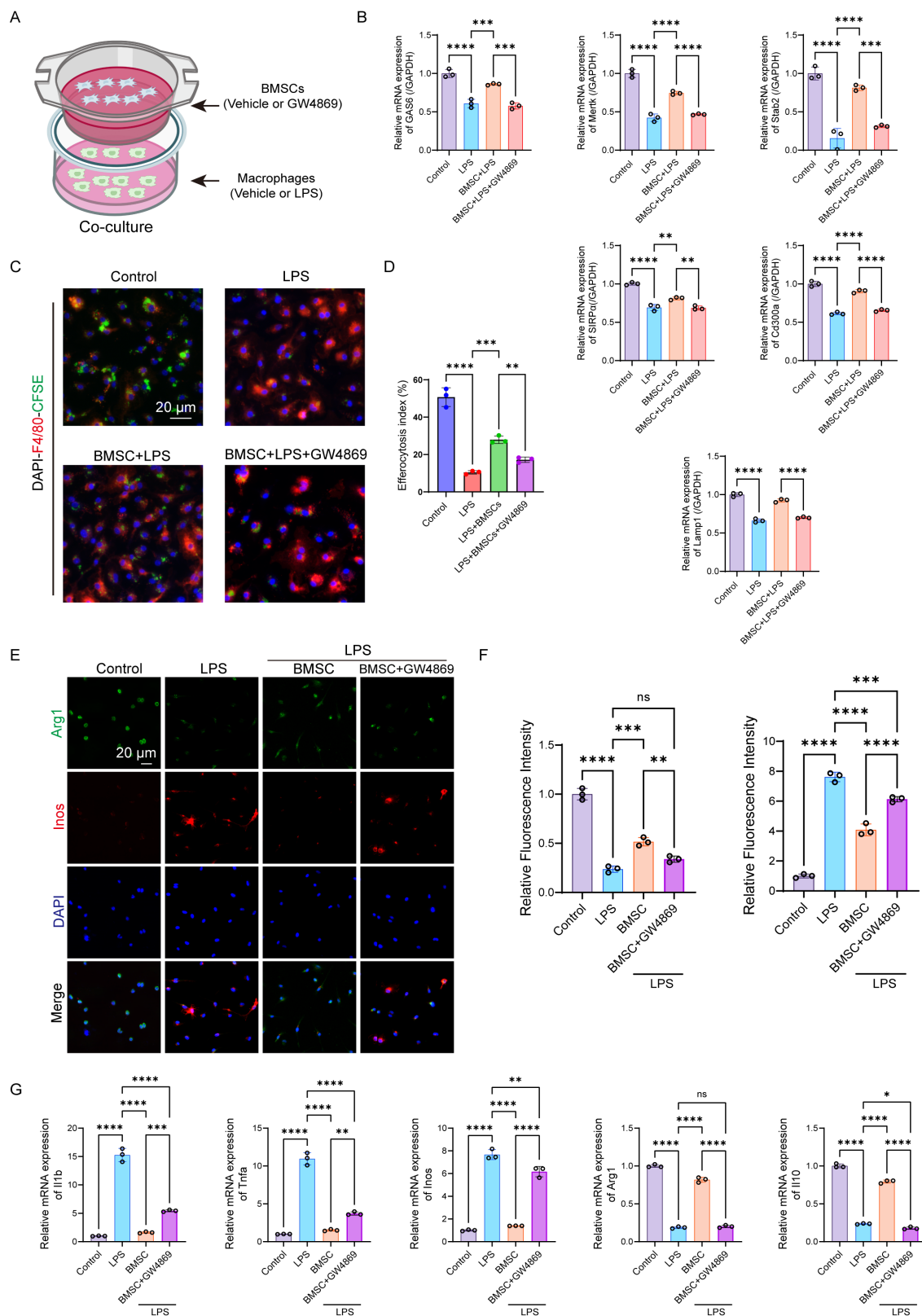
Supplementary Figure 4. KEGG showing the differential pathways of macrophage 0 and 4 clusters based on single cell sequencing.



Supplementary Figure 5. Macrophage polarization in synovial tissue from OA mice. (A) Representative H&E-stained images of knee synovial tissues from sham-operated and DMM-induced OA mice at 28 days after surgery. OA mice exhibited marked synovial thickening and increased cellularity compared with sham controls. Scale bar, 20 μ m; (B) Quantification of synovitis scores based on H&E staining, showing significantly increased synovial inflammation in OA mice compared with sham-operated controls ($n = 5$ mice per group); (C) Representative immunofluorescence images of F4/80 (green), iNOS (red), and DAPI (blue) in knee synovial tissues from sham-operated and DMM-induced OA mice at 28 days after surgery. Increased macrophage accumulation and iNOS expression were observed in the OA synovium. Scale bar, 50 μ m; (D) Quantification of iNOS-positive macrophages, showing a significantly higher proportion of pro-inflammatory macrophages in OA mice than in sham-operated controls ($n = 5$ mice per group). Mann-Whitney U was used in B, and Student's t test was used in D. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

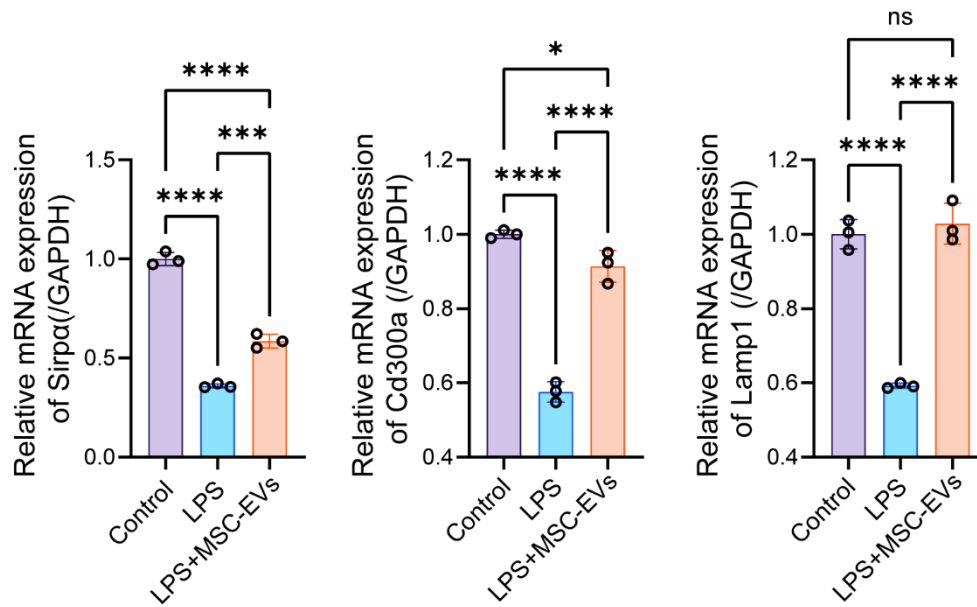


Supplementary Figure 6. Macrophages phenotype underwnt changes in response to chondrocyte apoptosis. (A) Mode pattern of co-incubation of macrophages and apoptotic chondrocytes; (B) Establishment of apoptotic chondrocytes treated by $\text{TNF}\alpha$; (C) Quantitation results of TUNEL staining ($n = 3$ independent biological experiments); (D) qRT-PCR analysis of inflammation-related genes in macrophages co-cultured with apoptotic chondrocytes ($n = 3$ independent biological experiments); (E) ELISA assay detecting the secretion of IL1b and IL10 in macrophages co-cultured with apoptotic chondrocytes ($n = 3$ independent biological experiments). One-way ANOVA followed by Tukey's multiple-comparison test were used for comparison in multiple groups. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

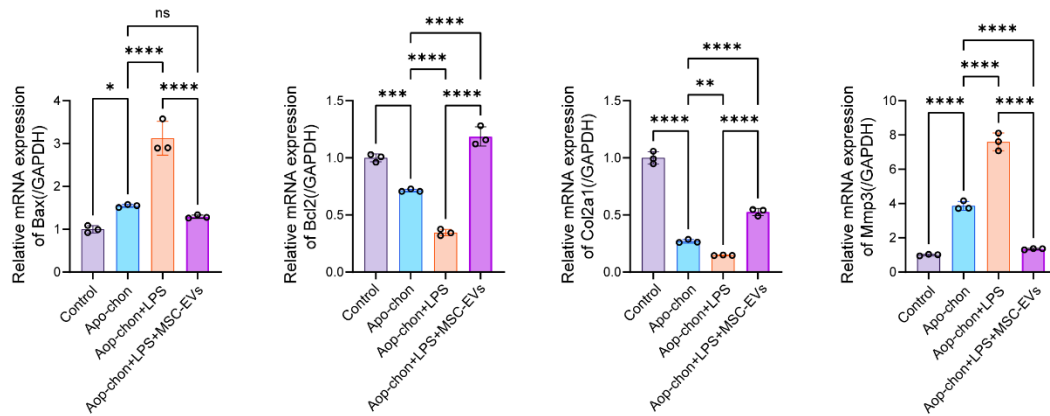


Supplementary Figure 7. Bone marrow-derived MSCs induced enhancement of macrophage efferocytosis and inflammation resolution. (A) Mode pattern of co-incubation of macrophages and BMSCs; (B) qRT-PCR analysis of inflammation- and efferocytosis-related genes in macrophages co-cultured with BMSCs ($n = 3$

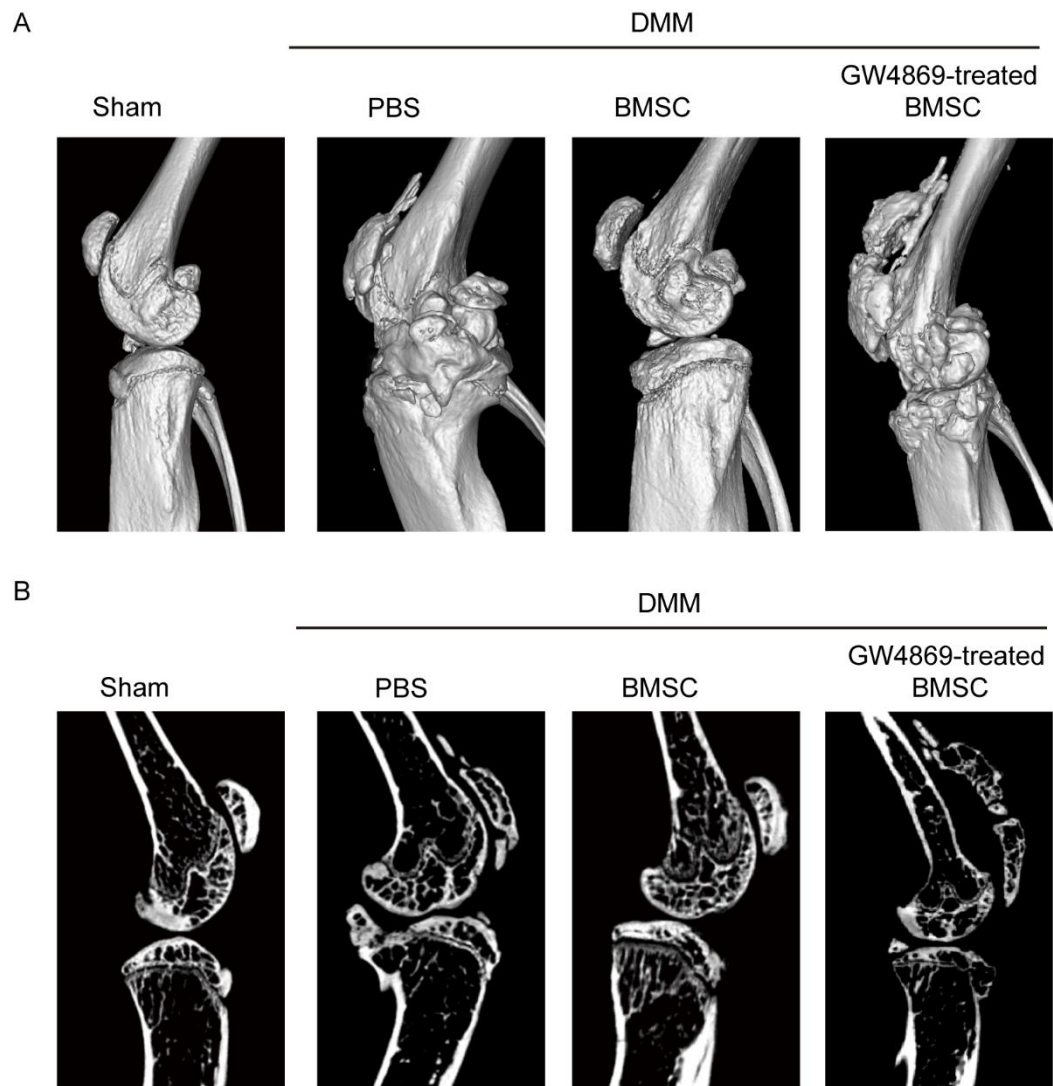
independent biological experiments); (C) Immunofluorescence staining for F4/80 (red) in macrophages and CFSE (green) in apoptotic chondrocytes after phagocytosis for 2 hours. Scale bar: 20 μ m; (D) Quantification of positive macrophages engulfing apoptotic chondrocytes as a proportion of total F4/80-positive cells ($n = 3$ independent biological experiments); (E) Representative images of immunofluorescence staining for Arg1 and Inos in macrophages co-cultured with BMSCs for 24 hours ($n = 3$ independent biological experiments). Scale bar: 20 nm; (F) Quantified results of immunofluorescence for Arg1 and Inos ($n = 3$ independent biological experiments); (G) q-RT-PCR analysis of the effects of BMSCs on inflammation-related genes in macrophages co-cultured with BMSCs for 24 hours ($n = 3$ independent biological experiments). One-way ANOVA followed by Tukey's multiple-comparison test were used for comparison in multiple groups. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.



Supplementary Figure 8. q-RT-PCR analysis of the effects of BMSC-exosomes on efferocytosis-related genes in macrophages co-cultured with BMSCs for 24 hours ($n = 3$ independent biological experiments). One-way ANOVA followed by Tukey's multiple-comparison test were used for comparison in multiple groups. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

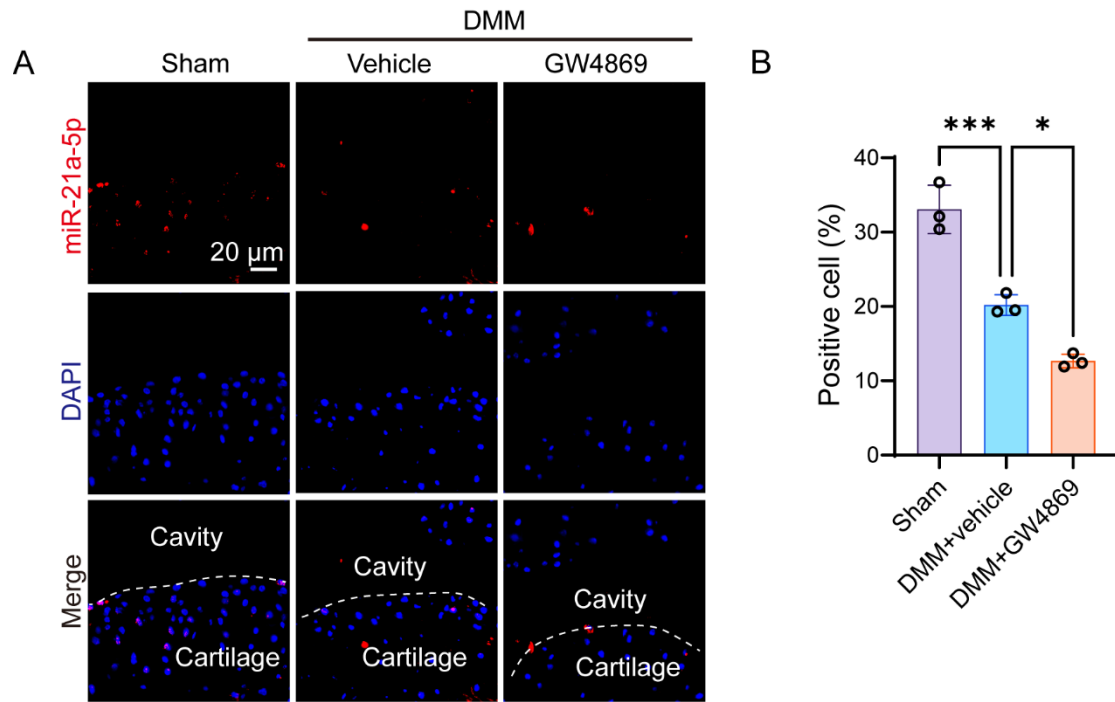


Supplementary Figure 9. qRT-PCR analysis showed the relative gene expression of Bax, Bcl2, Col2a1, and Mmp3 in chondrocytes treated by LPS-induced macrophage (100 ng/mL) or BMSC-exosomes ($n = 3$ independent biological experiments). One-way ANOVA followed by Tukey's multiple-comparison test were used for comparison in multiple groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

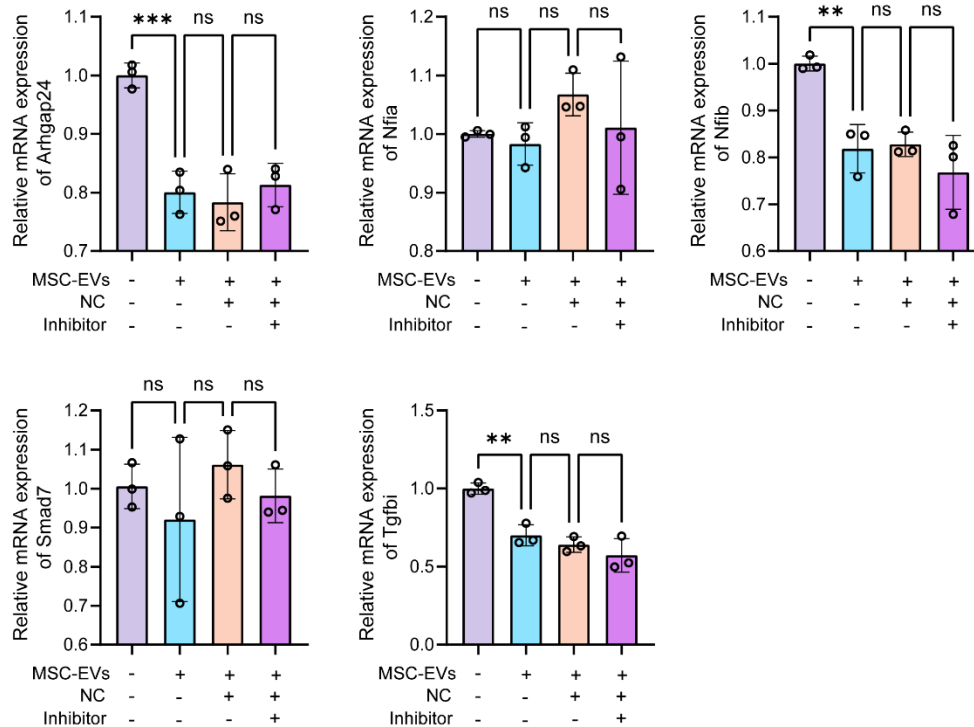


Supplementary Figure 10. Representative micro-CT images of knee joints following

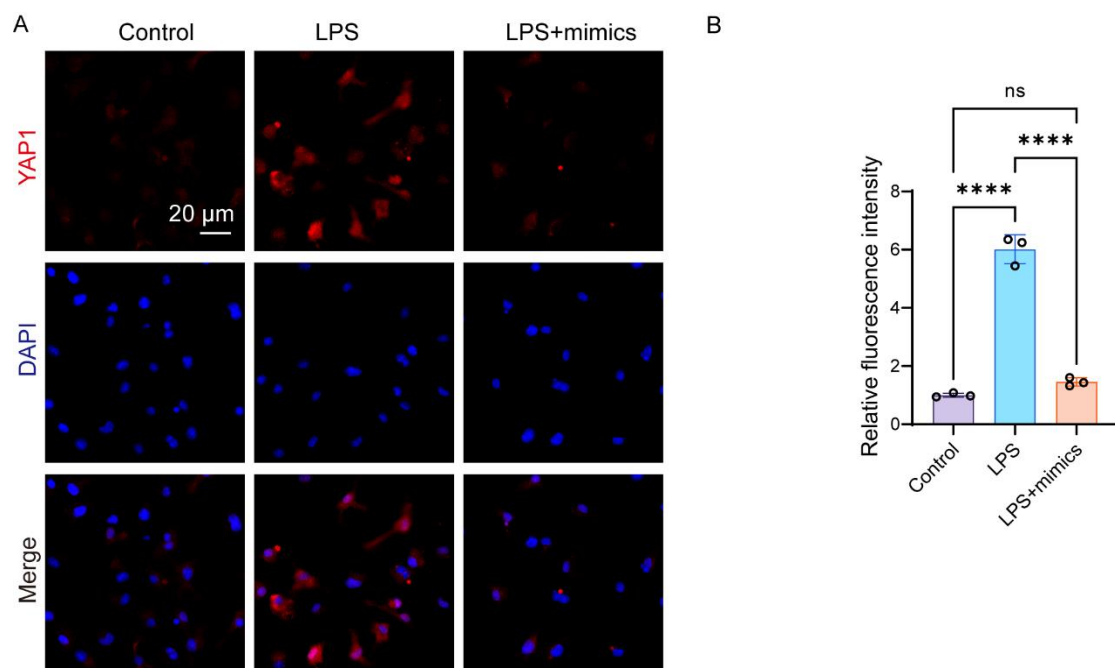
DMM-induced osteoarthritis and different treatments. (A) The representative three-dimensional (3D) reconstructed micro-CT images of the knee joints; (B) The representative two-dimensional sagittal micro-CT sections of the corresponding knee joints. From left to right: Sham, DMM + PBS, DMM + BMSC, and DMM + GW4869-treated BMSC groups. DMM, destabilization of the medial meniscus; BMSC, bone marrow-derived mesenchymal stem cell.



Supplementary Figure 11. *In situ* hybridization analysis revealed the level of miR-21a-5p in the cartilage tissue sham, DMM, or DMM+GW4869 mouse models. (A and B) Representative images and quantitation results of Cy3 for miR-21a-5p in mouse knee cartilage tissue ($n = 3$ independent biological experiments). One-way ANOVA followed by Tukey's multiple-comparison test were used for comparison in multiple groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

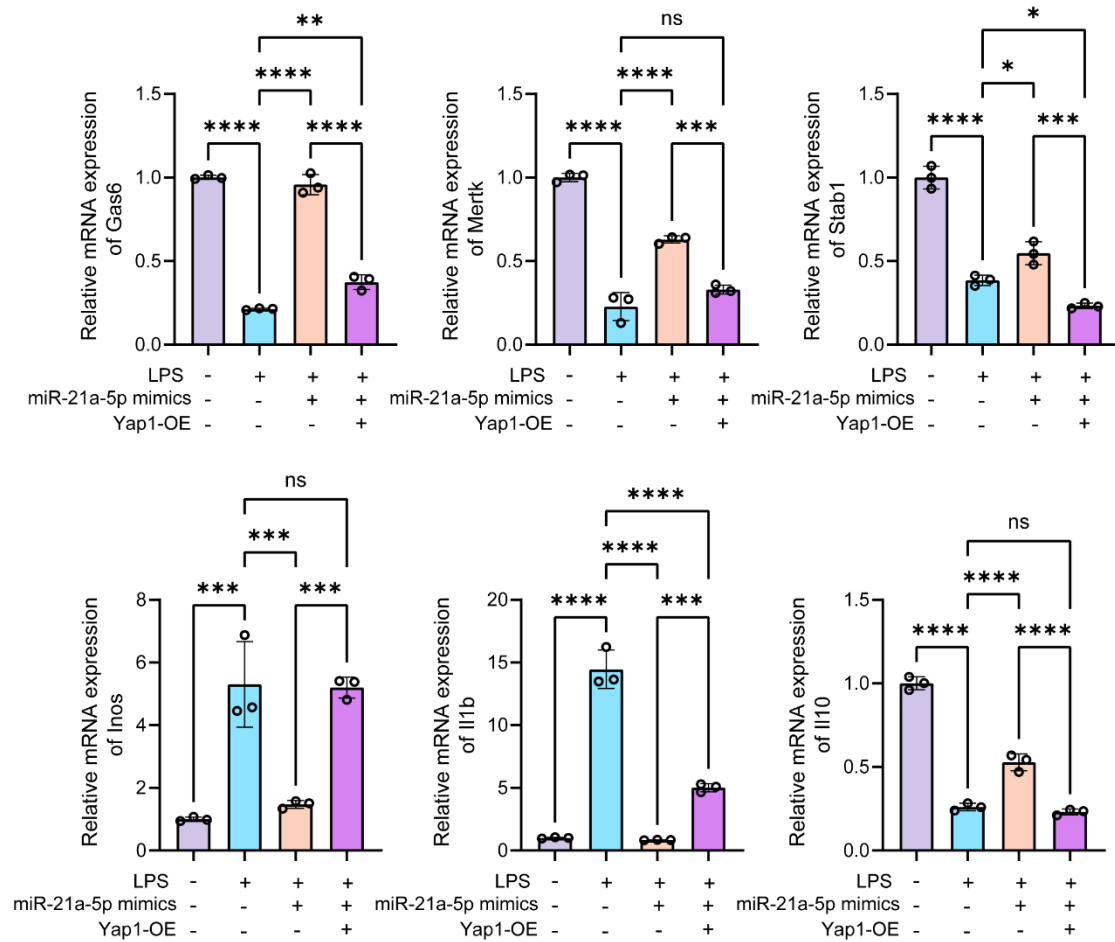


Supplementary Figure 12. Effects of MSC-EVs treatment and inhibitor intervention on the expression of identified genes. qRT-PCR analysis of the expression of the potential target genes in macrophages treated by LPS, BMSC-exos, or miR-21a-5p inhibitor ($n = 3$ independent biological experiments). One-way ANOVA followed by Tukey's multiple-comparison test were used for comparison in multiple groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

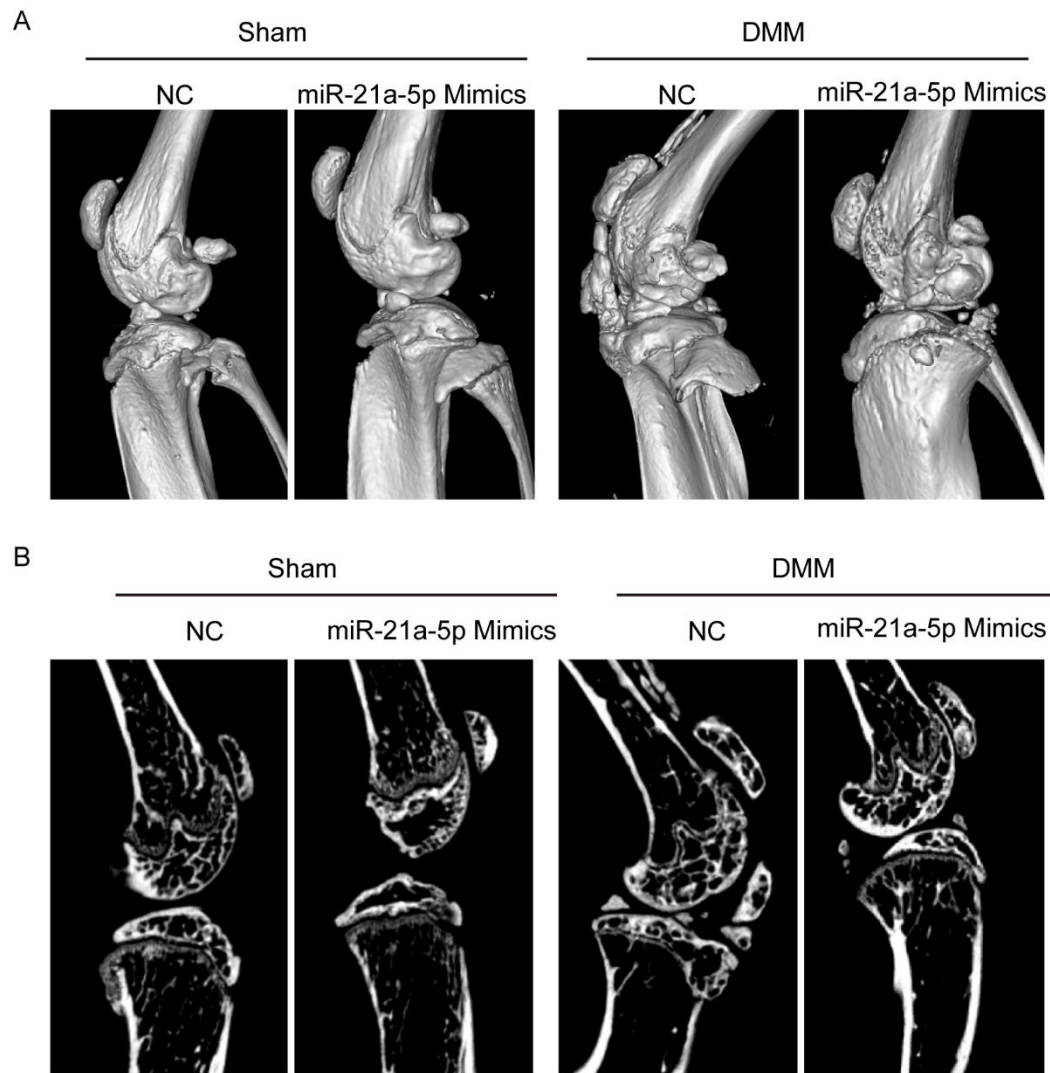


Supplementary Figure 13. miR-21a-5p mimic treatment attenuates LPS-induced

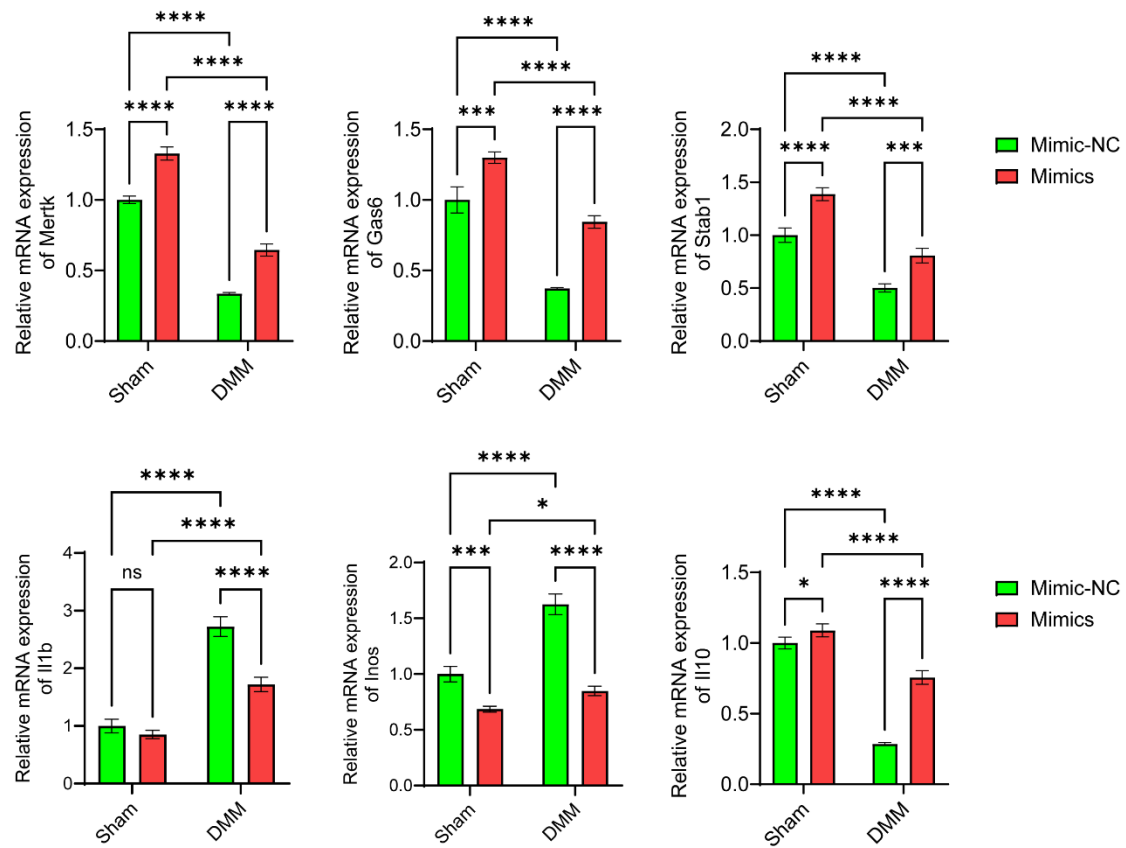
YAP1 expression. Representative images (A) and quantitation results (B) of immunofluorescence staining for Yap1 in macrophages *in vitro* treated by LPS or miR-21a-5p mimics ($n = 3$ independent biological experiments). One-way ANOVA followed by Tukey's multiple-comparison test were used for comparison in multiple groups. Scale bar=20 μm . * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.



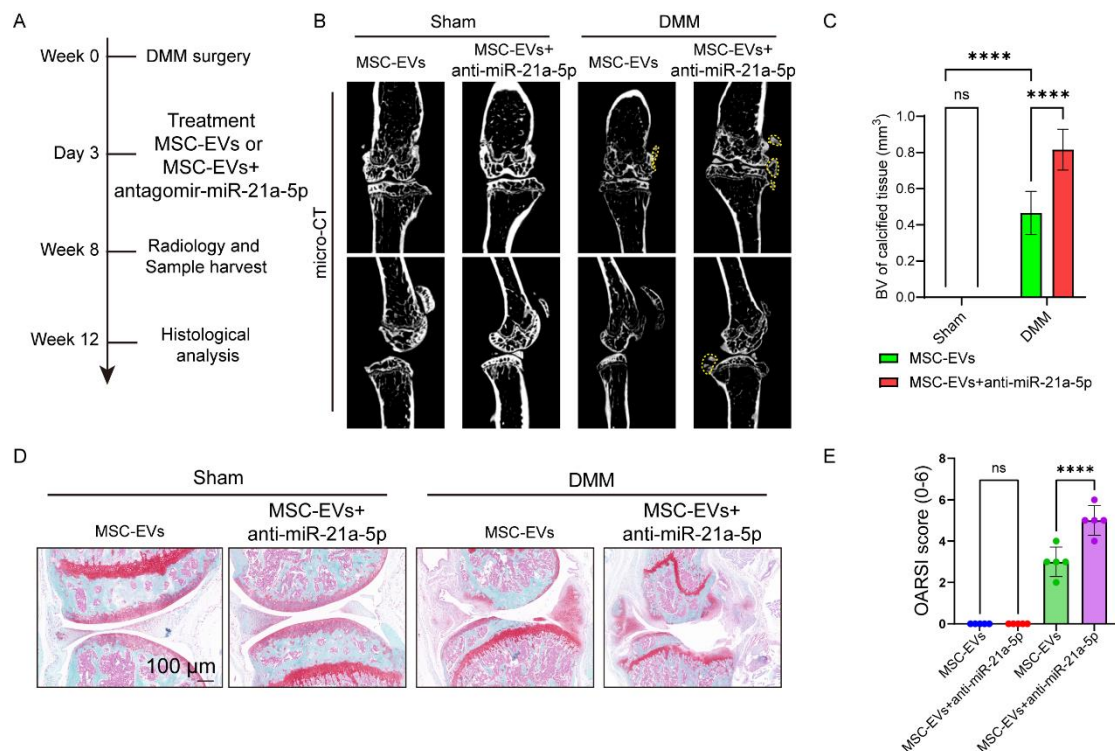
Supplementary Figure 14. qRT-PCR analysis showed the expression of inflammation- and efferocytosis-related genes and efferocytotic index in macrophages with varying treatments. ($n = 3$ independent biological experiments). One-way ANOVA followed by Tukey's multiple-comparison test were used for comparison in multiple groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.



Supplementary Figure 15. Representative micro-CT images of knee joints following miR-21a-5p mimic treatment in Sham and DMM mice. (A) Representative three-dimensional (3D) reconstructed micro-CT images of knee joints from Sham + NC, Sham + miR-21a-5p Mimics, DMM + NC, and DMM + miR-21a-5p Mimics groups; (B) Representative sagittal micro-CT sections of the corresponding knee joints in each group. DMM, destabilization of the medial meniscus; NC, negative control.



Supplementary Figure 16. qRT-PCR of the expression of efferocytosis- and inflammation-related genes in mouse knee joint synovium tissue ($n = 3$ indicates 3 mice per group). Two-way ANOVA followed by Šidák's multiple-comparison test was used for comparison in multiple groups. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.



Supplementary Figure 17. Local inhibition of miR-21a-5p attenuated the protective effects associated with MSC-EV treatment *in vivo*. (A) Schematic illustration of the animal experiment evaluating the functional contribution of miR-21a-5p to the protective effects associated with MSC-EV administration in experimental OA. Purified MSC-EVs were administered intra-articularly alone or together with antagomir-miR-21a-5p; (B and C) Representative micro-CT images of the knee joints and quantitative analysis of osteophyte formation. Osteophytes are indicated in yellow ($n = 5$ mice per group); (D) Representative Safranin O/Fast Green (SOFG) staining of knee joint sections from mice receiving MSC-EVs alone or MSC-EVs together with antagomir-miR-21a-5p. Scale bar: 100 μ m; (E) Quantitative analysis of OARSI scores based on SOFG staining ($n = 5$ mice per group). Mann-Whitney U test for OARSI. Two-way ANOVA followed by Šidák's multiple-comparison test was used for comparison in multiple groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.