

***Supplementary Material for***  
***The therapeutic potential of exosomes vs. matrix-bound***  
***nanovesicles from human umbilical cord mesenchymal***  
***stromal cells in osteoarthritis treatment***

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Figure S1. Characterization of UC-MSC Exosomes, and UC-MSC MBV. (A) Flow cytometry results show the presence of phenotypic markers for UC-MSC (CD105, CD73, CD90, CD29, CD44) and the absence of hematopoietic markers (CD19, CD11beta, CD45, CD34).

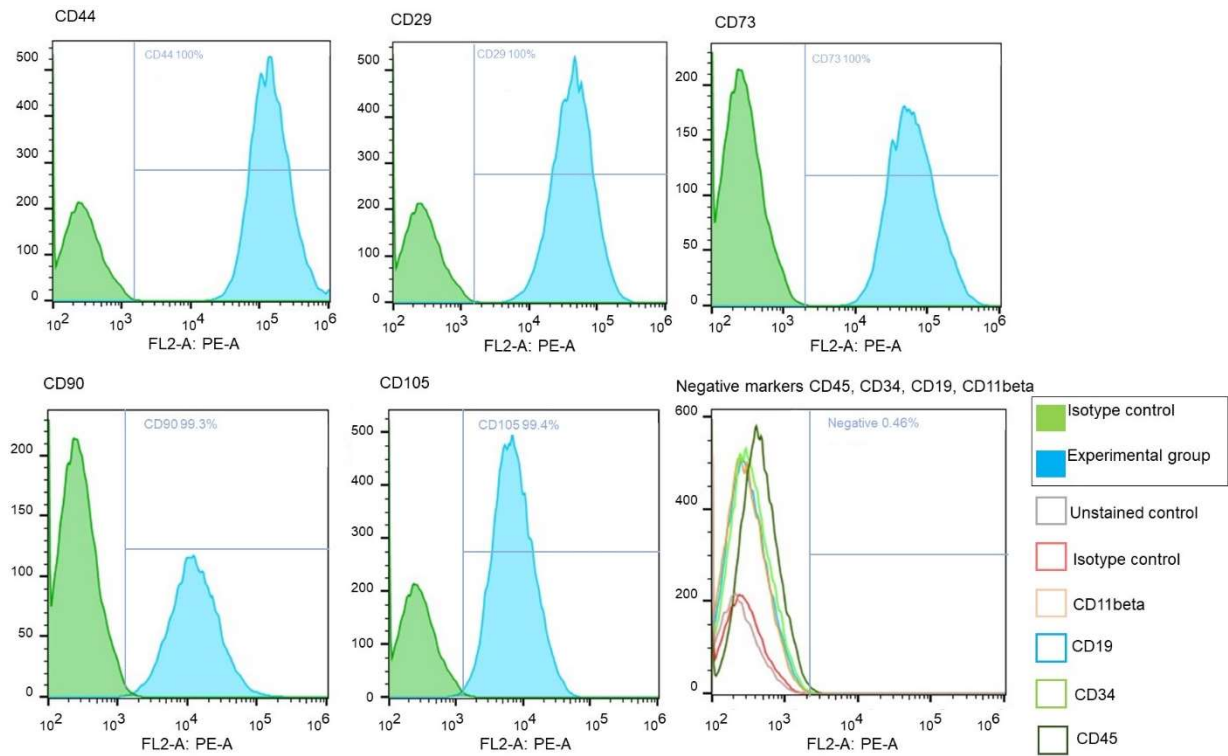
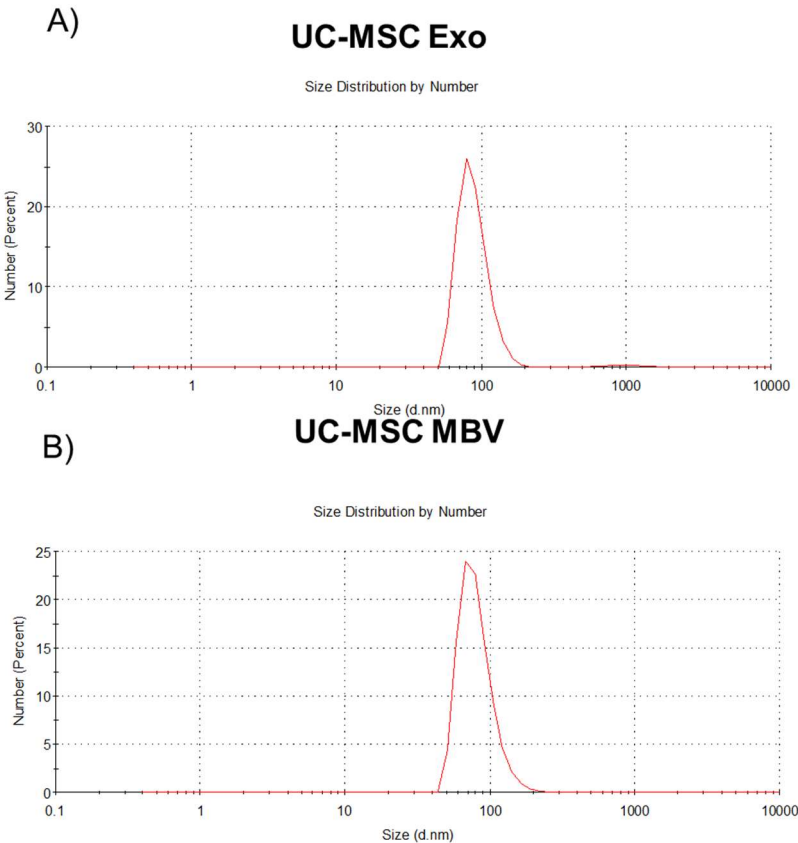


Figure S2. Particle size distribution obtained by nanoparticle dynamic light scattering.



The histogram shows the peaks of particle size distribution for A) UC-MSC Exosomes, B) UC-MSC MBV. The Y-axis shows the distribution of number Particle size distribution (PSD) is the means (percentage), the X-axis shows the distribution of particle Size (d. nm)

Figure S3Visualization of UC-MSC Exo and UC-MSC MBV via transmission electron microscopy

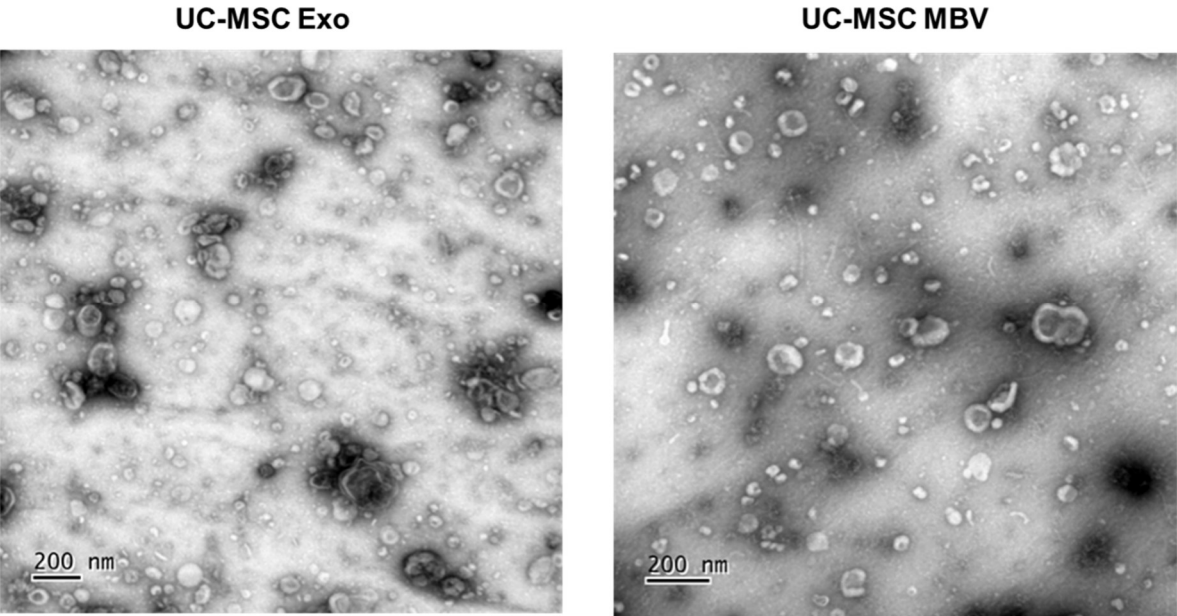


Figure S4. Visualization of UC-MSC Exo and UC-MSC MBV via confocal microscopy with fluorescent staining of extracellular vesicles (UC-MSC Exosomes and UC-MSC MBV) using PKH26 membrane dye.

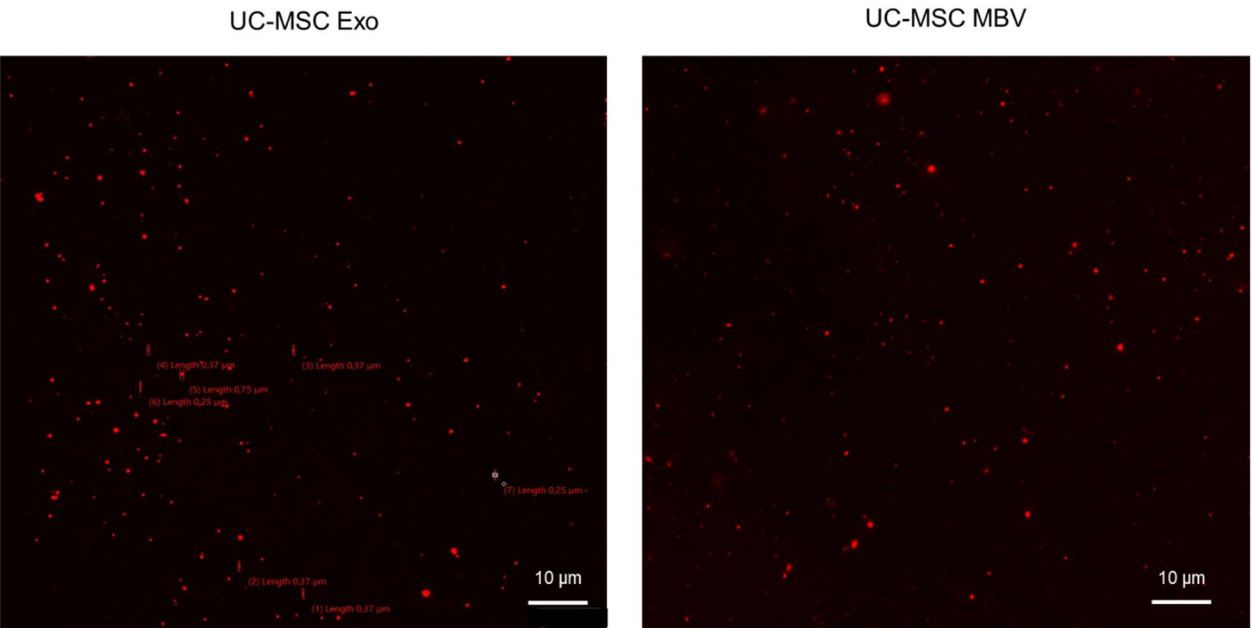


Figure S5. Phagocytosis of UC-MSC Exo and UC-MSC MBV labeled with PKH26 by M1 (IFN- $\gamma$ +LPS) macrophages.

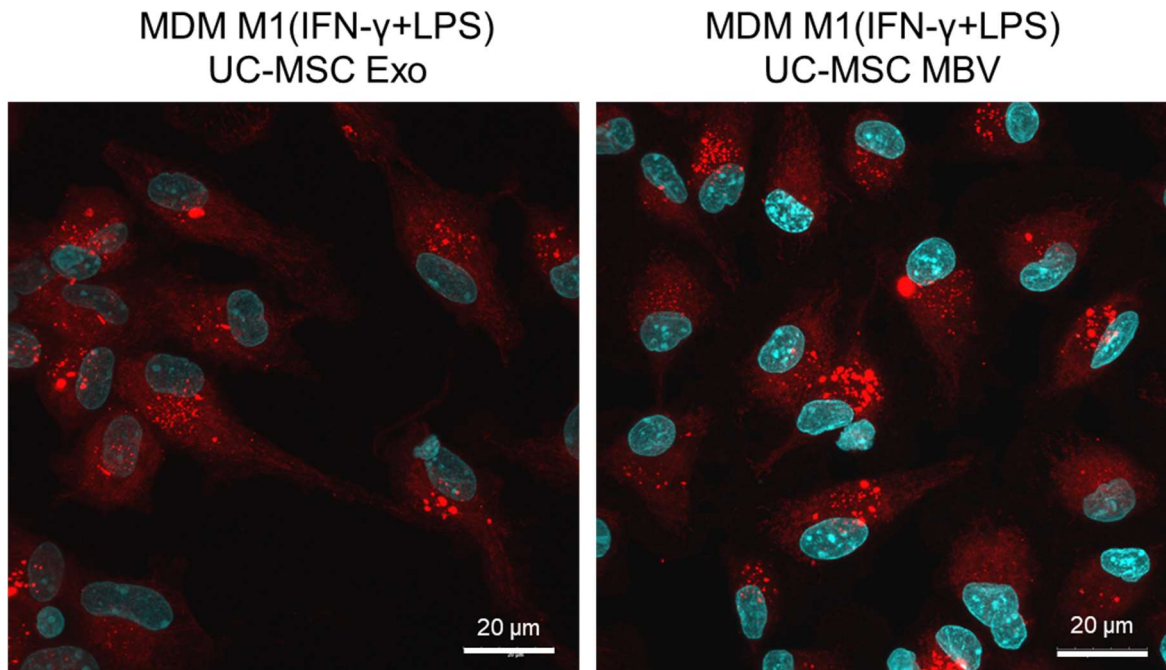
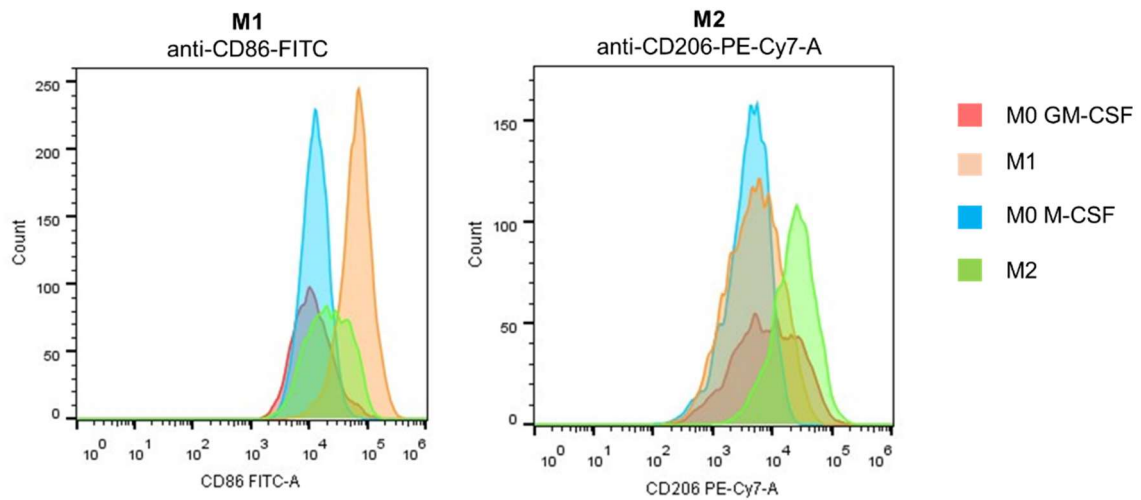


Figure S6. Flow cytometry assessment of MDM polarization markers.



A. The histogram shows the peaks of fluorescence intensity for 4 MDM phenotypes used in the study (M0\_GM, M1, M0\_M, M2). Cells M0\_GM, M1 were cultured with GM-SCF (50 ng/ml) for 6 days, on day 6 M1 were treated with LPS (10 ng/ml) and IFN- $\gamma$  (50 ng/ml). Macrophages of phenotypes M0\_M, M2 were cultured with M-CSF (50 ng/ml) for 6 days, on the 6th day M2 were treated with IL-4 (10 ng/ml). After two days of culture with polarization inducers, macrophages were detached from the plastic surface using accutase after pretreatment with a Versene solution, after which the cells were stained with anti-CD86-FITC and/or CD206-PE-Cy7 antibodies.

**Table S1.** Semi-quantitative scoring of cartilage tissue changes using the Mankin method.

Features		Points
SurfaceIntegrity	Normal	0
	Minor surface changes (thickness variation)	1
	Moderate surface changes (thickness variation)	2
	Pronounced surface changes (thickness variation)	3
	Cracks in the superficial zone	4
	Cracks in the transitional zone	5
	Cracks extending to the calcified zone	6
	Fibrillation and/or absence of the superficial zone	7
	Fibrillation and/or absence of the transitional zone	8
	Fibrillation and/or absence of the calcified zone	9
	Fibrillation and/or absence of subchondral bone	10
CellularDystrophy	Normal	0
	Mild dystrophy	1
	Moderate dystrophy	2
	Severe dystrophy	3
	No cells	4
Cell Cloning	Normal	0
	Individual doublets	1
	Multiple doublets	2
	Doublets and triplets	3
	Multiple "nests" of cells	4

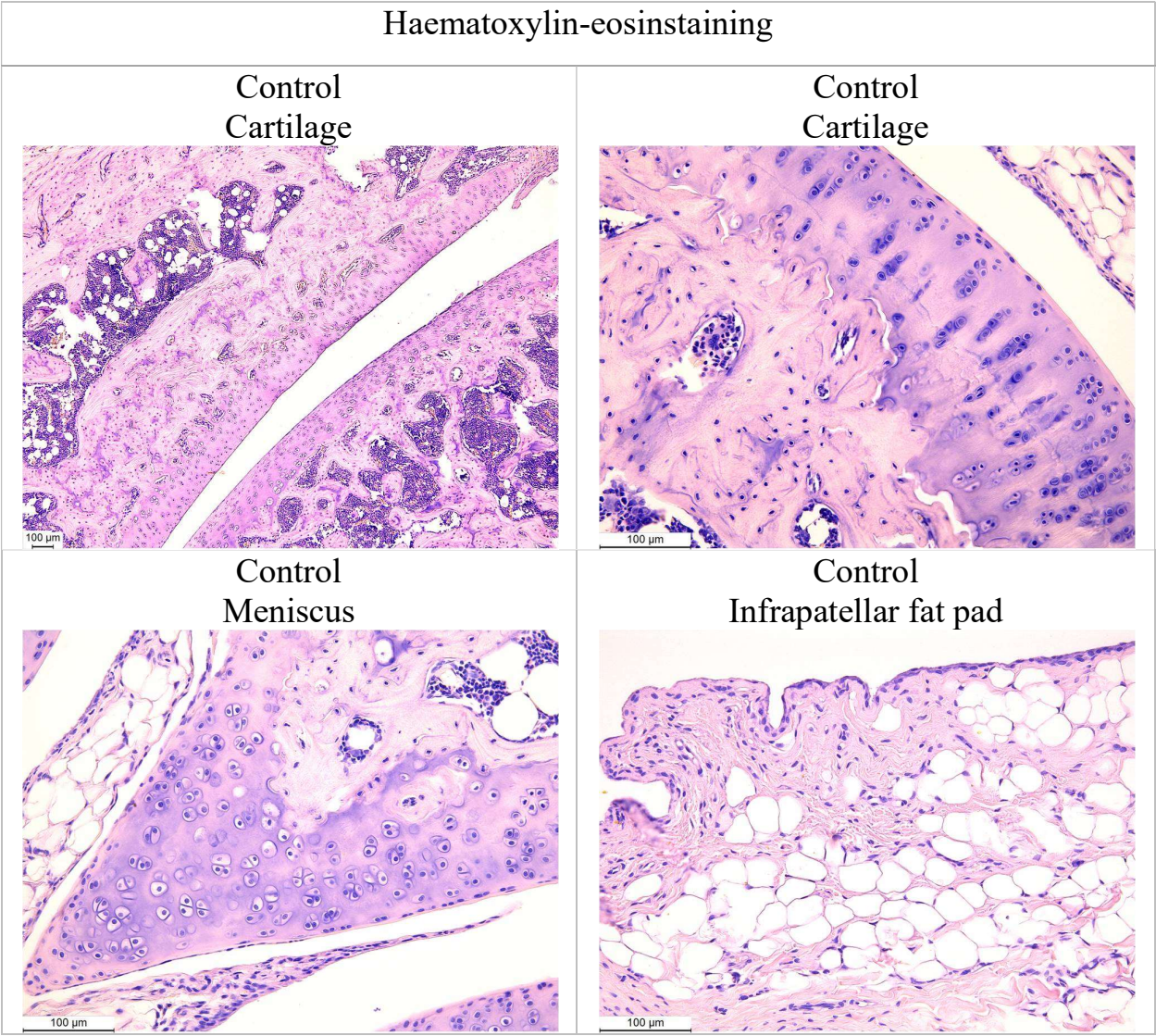


Maximum totalscore: 18 points
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**Table S2.** *Semi-quantitative scoring scale for immunohistochemical analysis data*

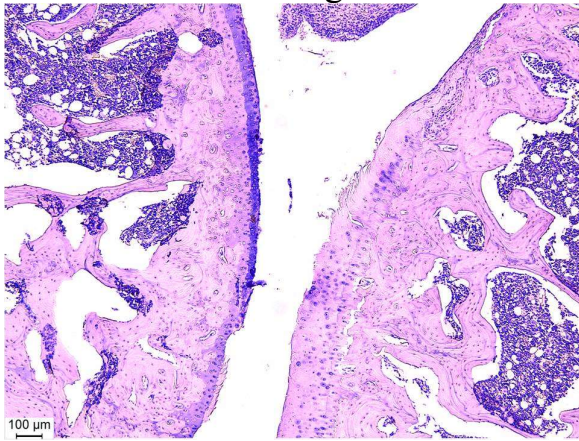
PrevalenceofStaining	StainingIntensity
0 – lessthan 25% ofcells	0 – notdetected
1 – 25-49% ofcells	1 – singlepositivelystainedgranules
2 – 50-74% ofcells	2 – moderate content of positively stained granules
3 – 75% ofcellsormore	3 – highcontentofgranules

**Figure S7.**

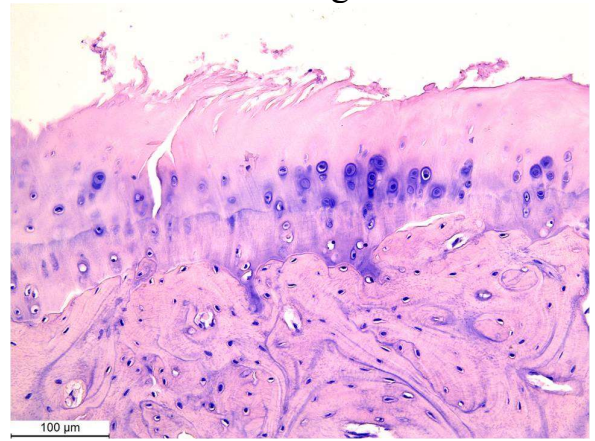




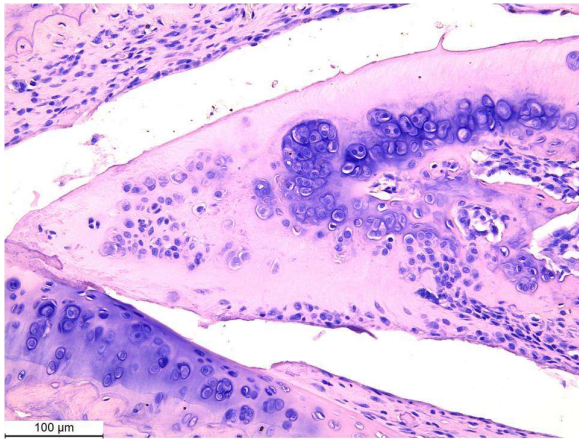
OA  
Cartilage



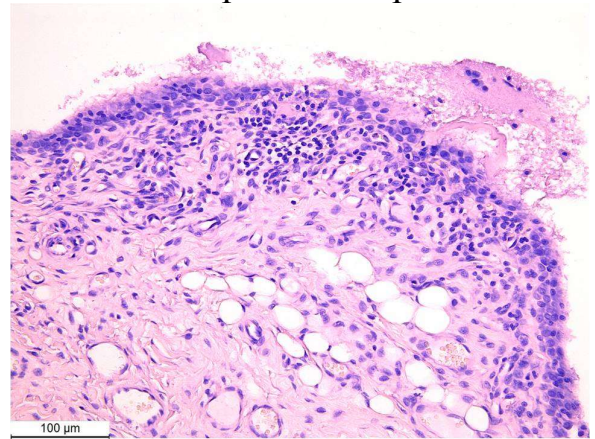
OA  
Cartilage



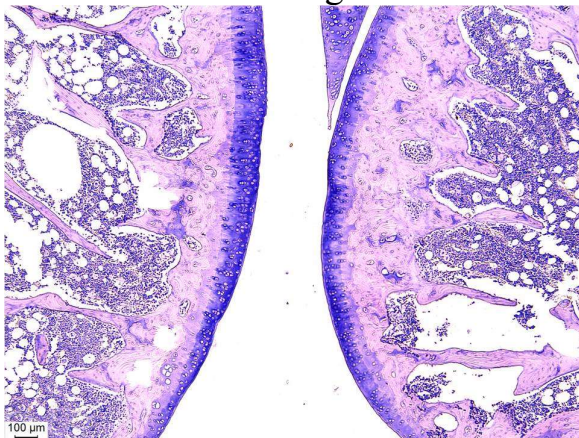
OA  
Meniscus



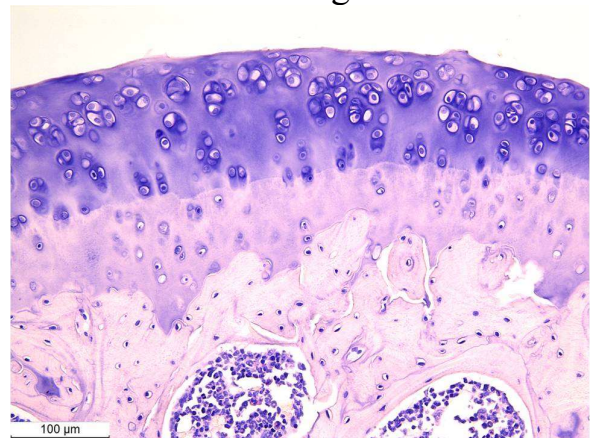
OA  
Infrapatellar fat pad



OA+Exosomes  
Cartilage



OA+Exosomes  
Cartilage



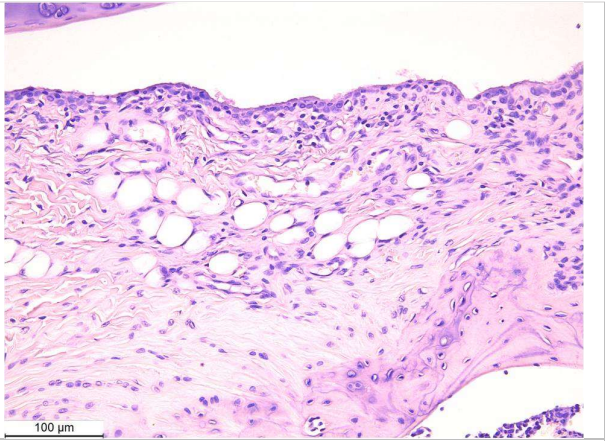
OA+Exosomes  
Meniscus

OA+Exosomes  
Infrapatellar fat pad

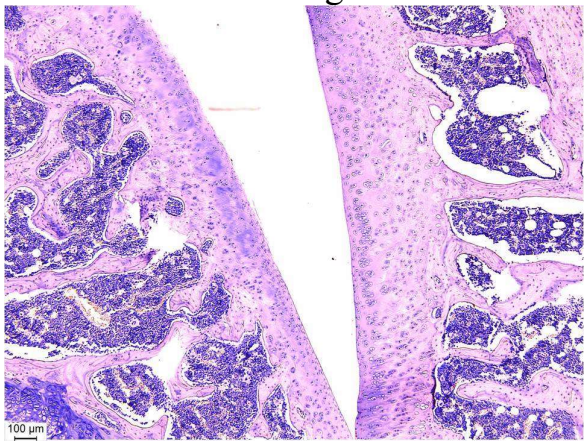




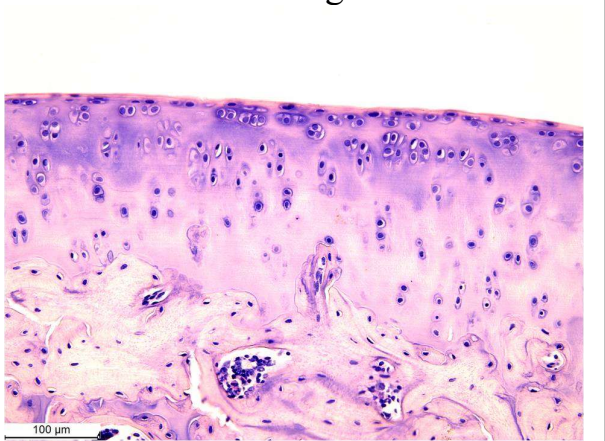
OA+MBV  
Cartilage



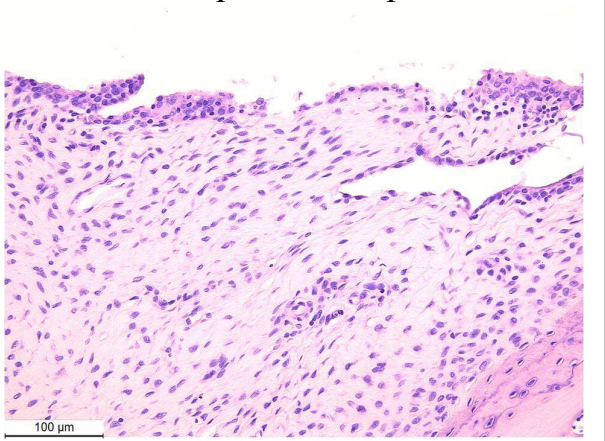
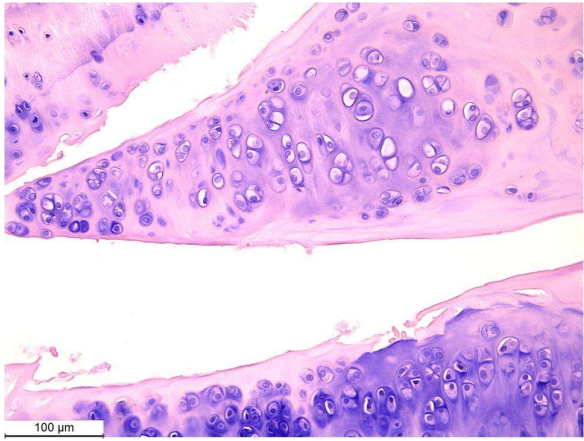
OA+MBV  
Cartilage



OA+MBV  
Meniscus



OA+MBV  
Infrapatellar fat pad

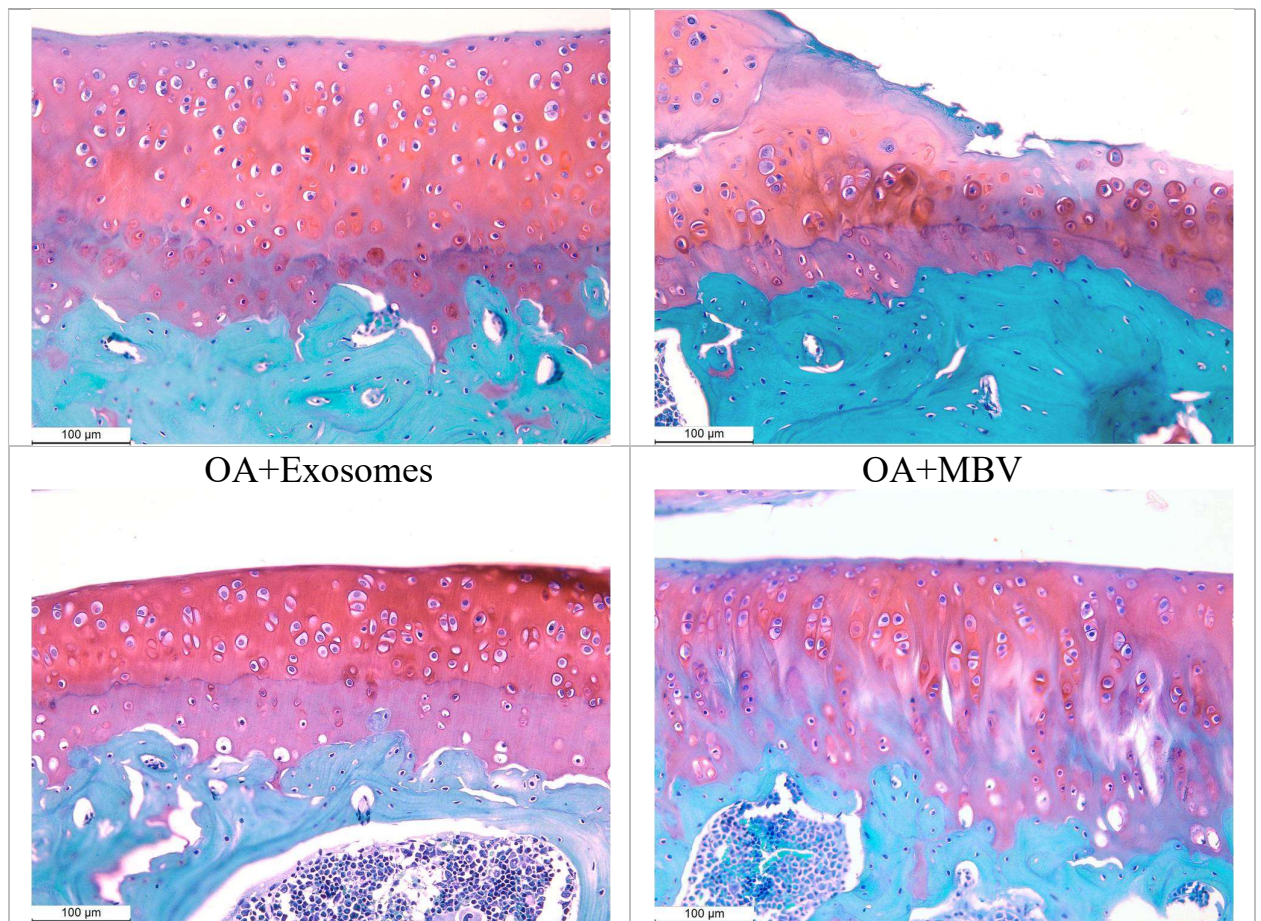


Safranin O/Fast Green staining

Control

OA





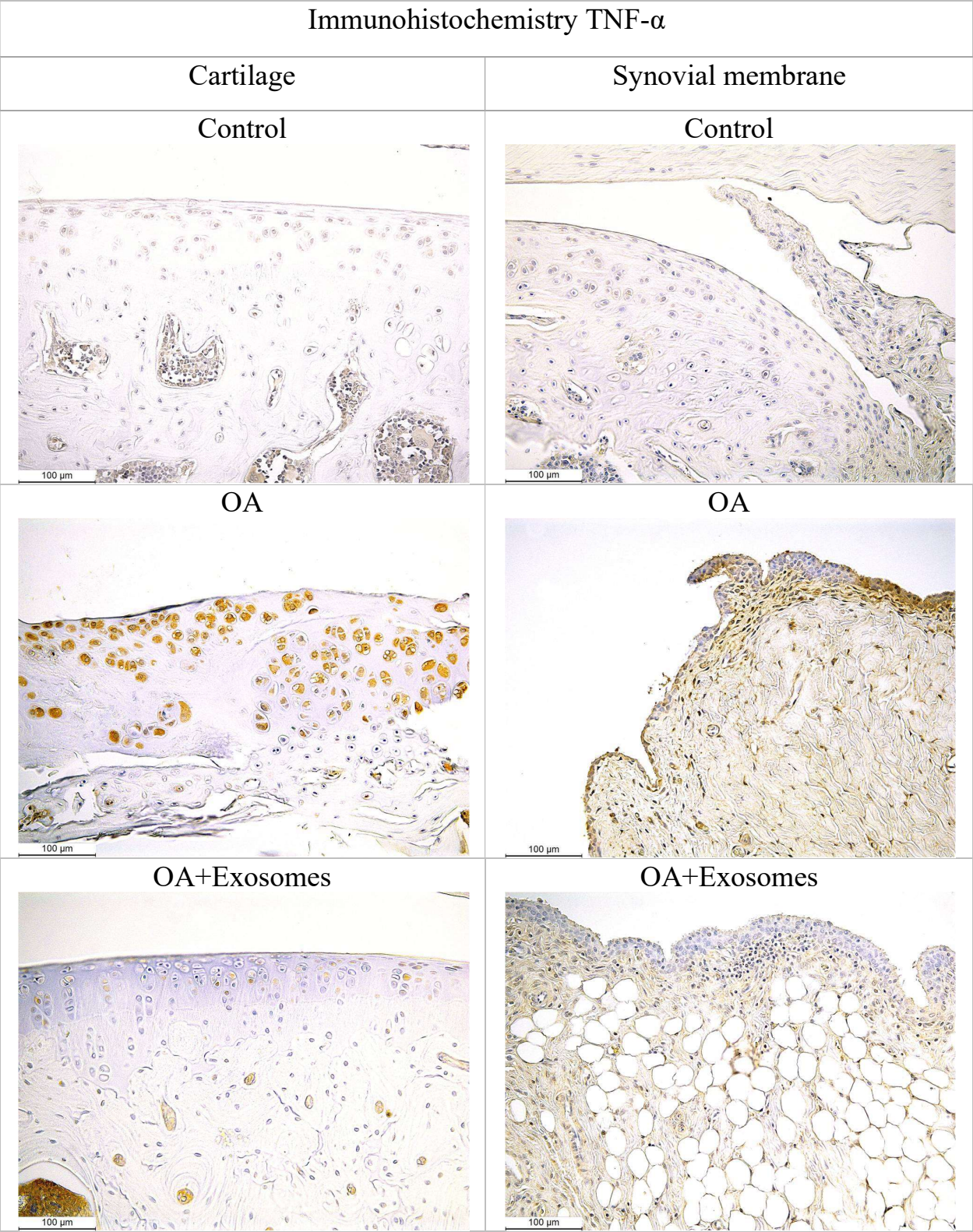
**Figure S7. Histochemical analysis of the impact of EVs (exosomes and MBV) from UC-MSC on the progression of pathological changes in cartilage in rats with surgically induced OA.** A. Histological staining with H&E, Safranin O/Fast Green. The images depict histological sections of animals 3 weeks post OA therapy. Ctrl - intact control without surgery, OA - OA group injected intra-articularly with PBS, OA+Exo - group with intra-articular injection of EVs from UC-MSC secretome, OA+MBV - group with intra-articular injection of MBV from UC-MSC. Scale bar: 100  $\mu$ m, magnification 200x

**Figure S8. Immunohistochemical analysis of the impact of UC-MSC-derived EVs (exosomes and MBV) on the development of inflammation in cartilage of rats with surgically induced OA.**

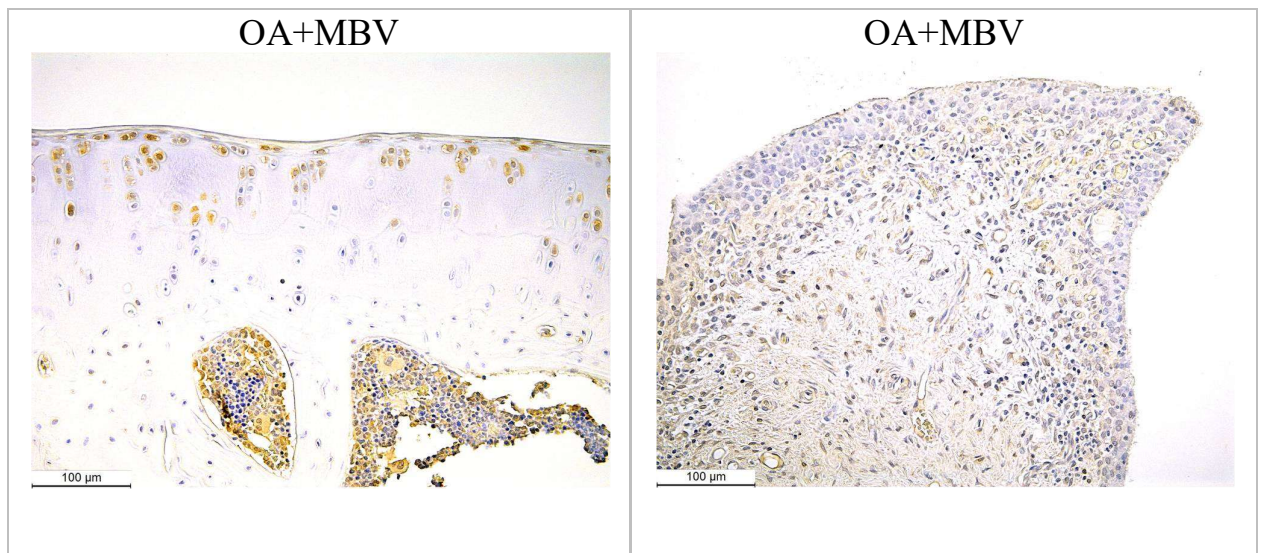
**Figure S8.1., S8.2., S8.3.** IHC analysis of inflammatory markers TNF- $\alpha$ , iNOS, Arg-1— markers of enhanced anti-inflammatory microenvironment. Histological tissue images were obtained 3 weeks post-OA therapy. **Figure 8.4.** In situ PCR analysis of NLRP3 inflammasome in joint tissue samples of experimental groups. Ctrl - intact control without surgery, OA - group with surgically induced OA injected with PBS, OA+Exo - group with intra-articular injection of UC-MSC exosomes, OA+MBV - group with intra-articular injection of UC-MSC MBV. Scale bar: 100  $\mu$ m, magnification 200x



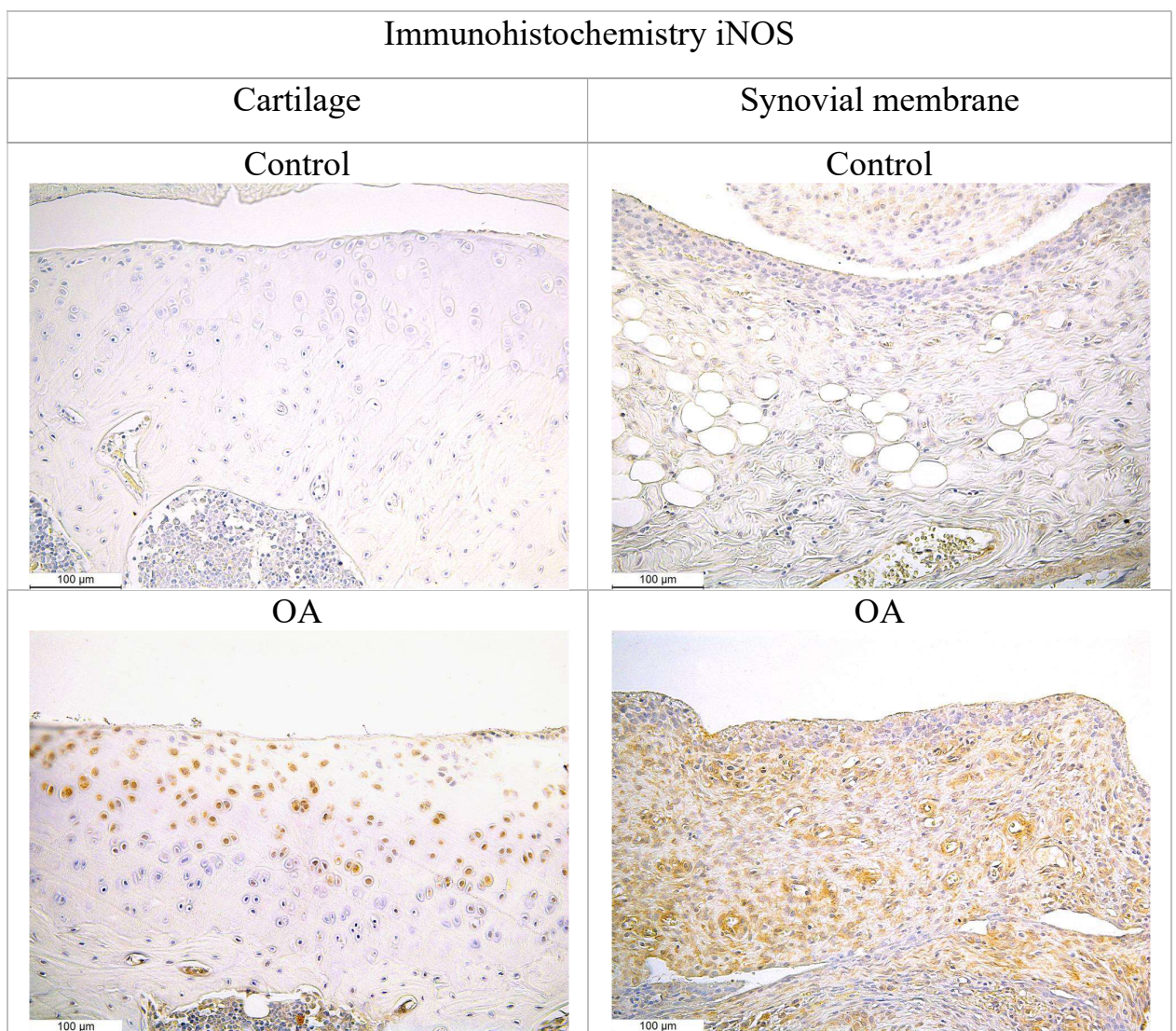
**Figure S8.1. Immunohistochemistry TNF- $\alpha$**



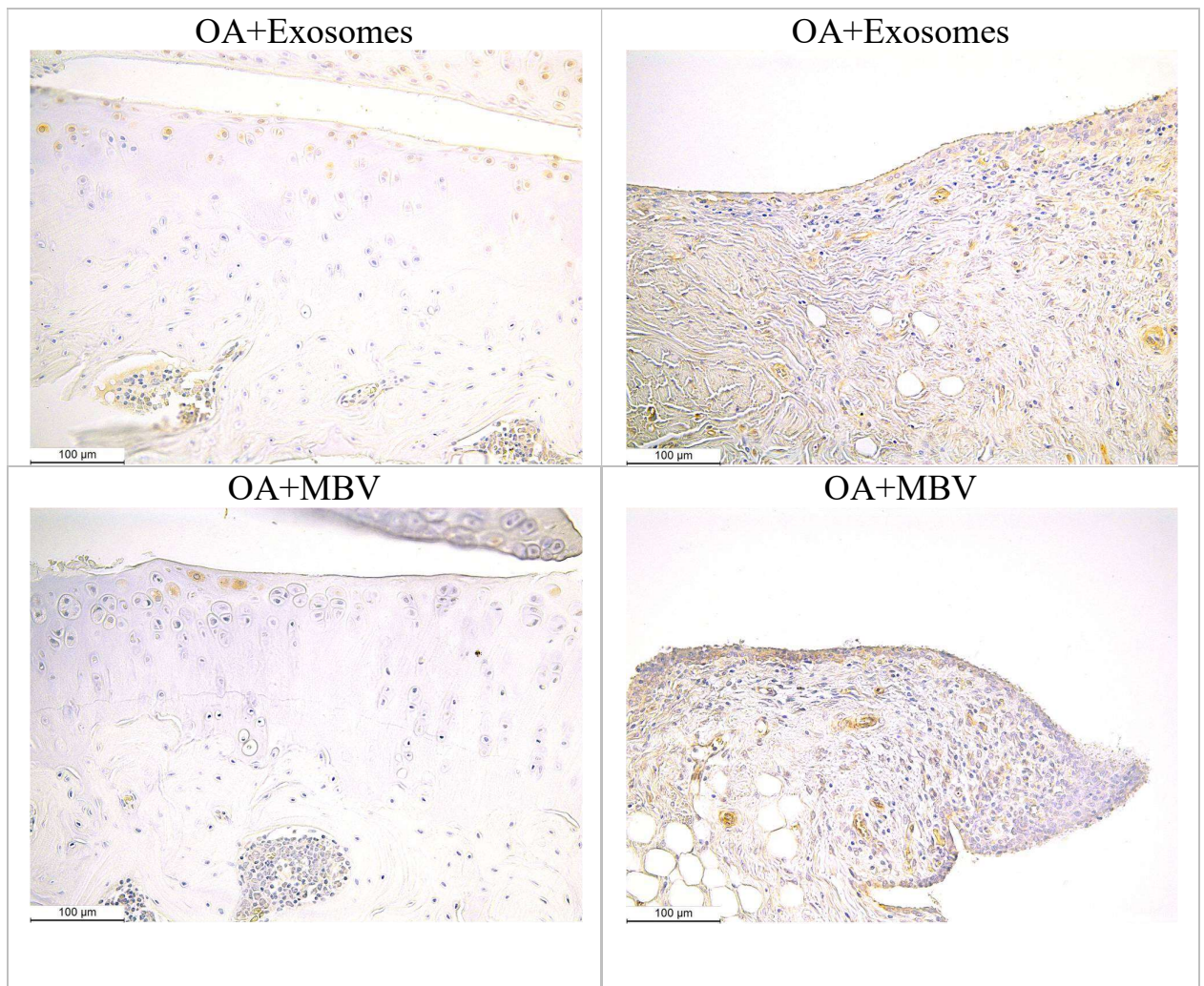




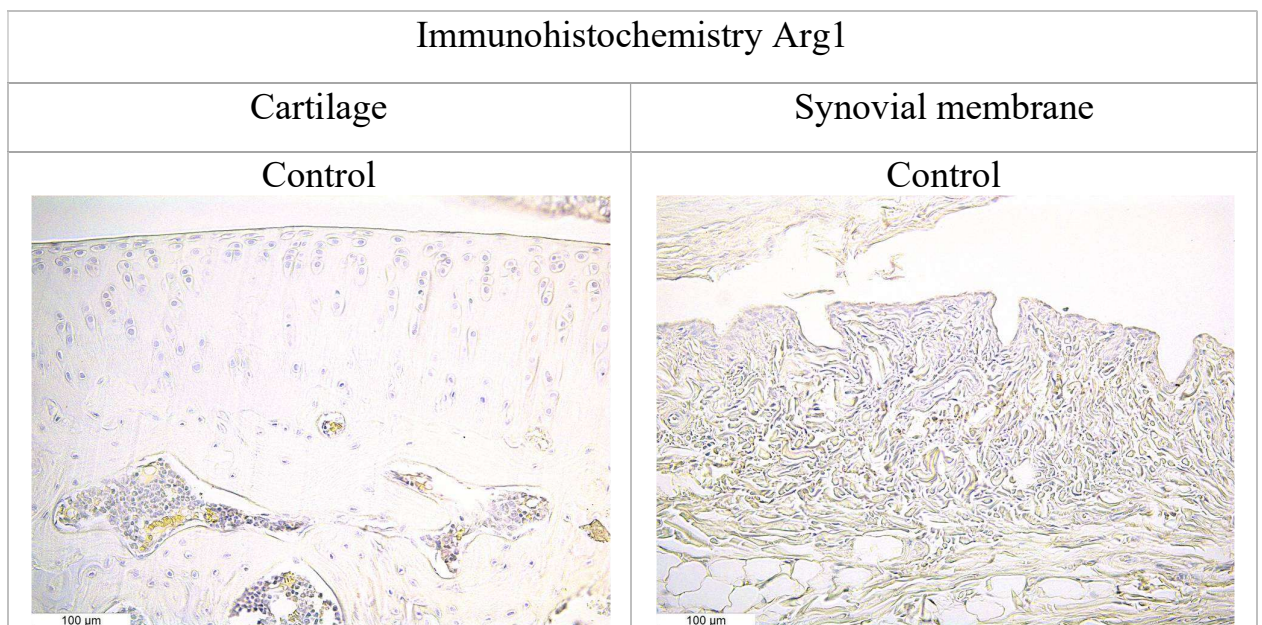
**Figure S8.2. Immunohistochemistry iNOS**



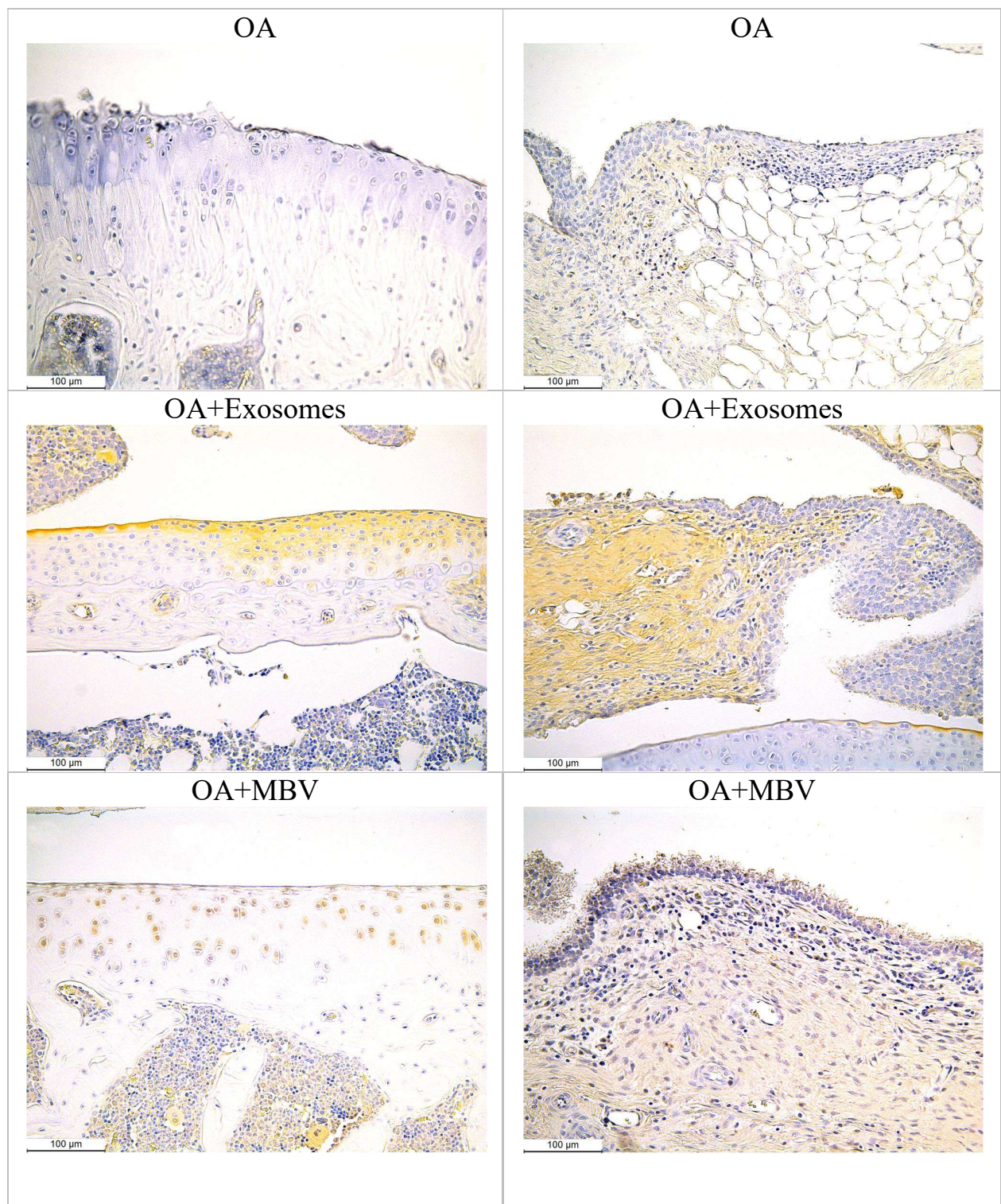




**Figure S8.3. Immunohistochemistry Arg1**



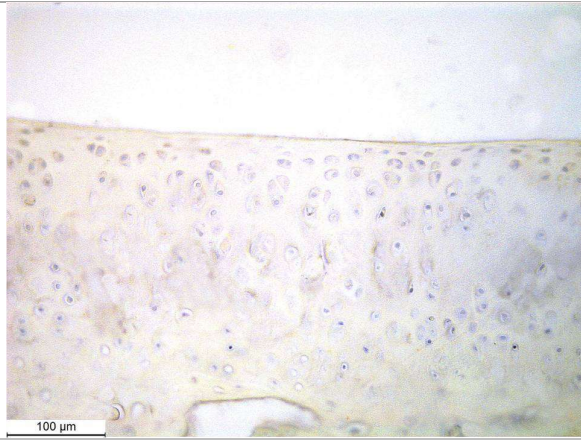




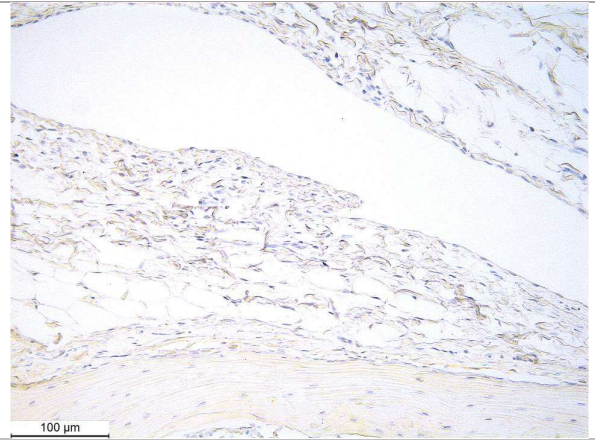
**Figure S8.4. PCR in situ NLRP3**

PCR in situ NLRP3	
Cartilage	Synovial membrane
Control	Control





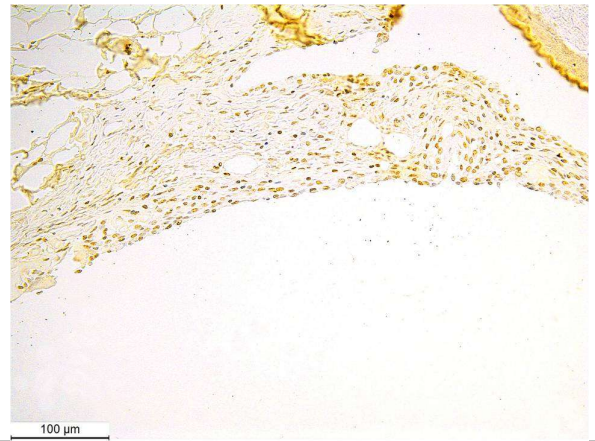
OA



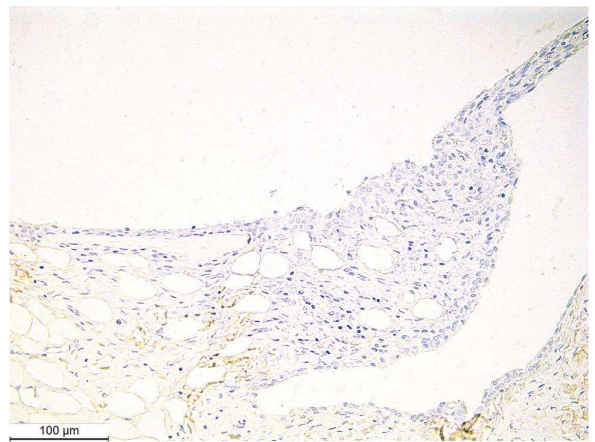
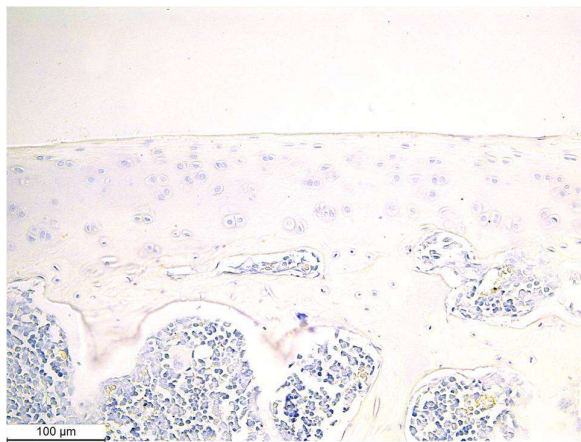
OA



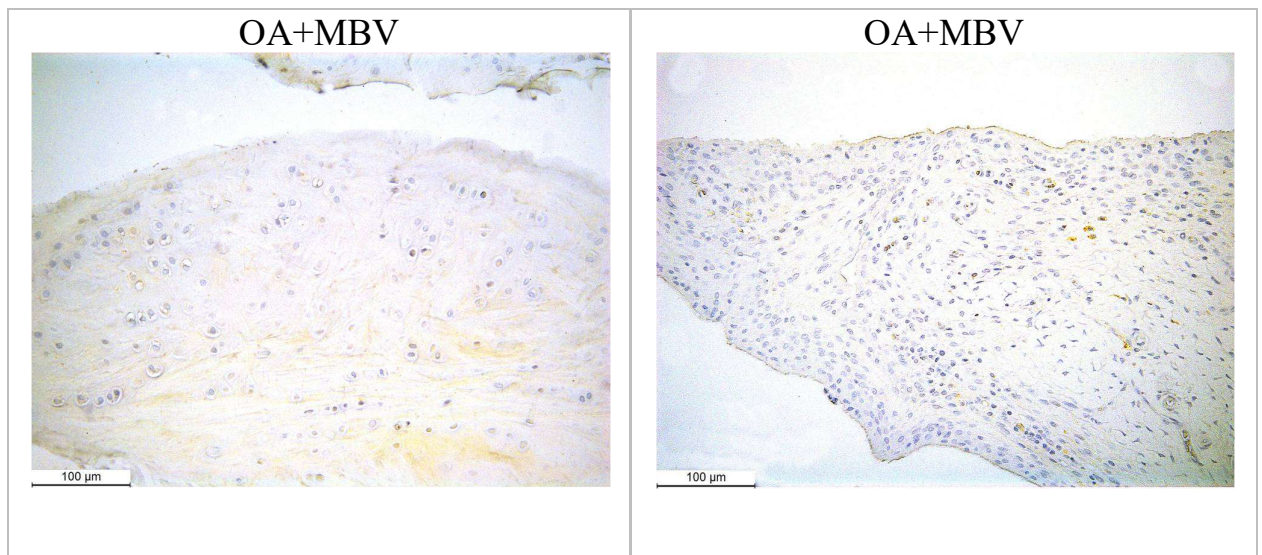
OA+Exosomes



OA+Exosomes







**Figure S9. ICH assay of cartilage and infrapatellar fat pad**

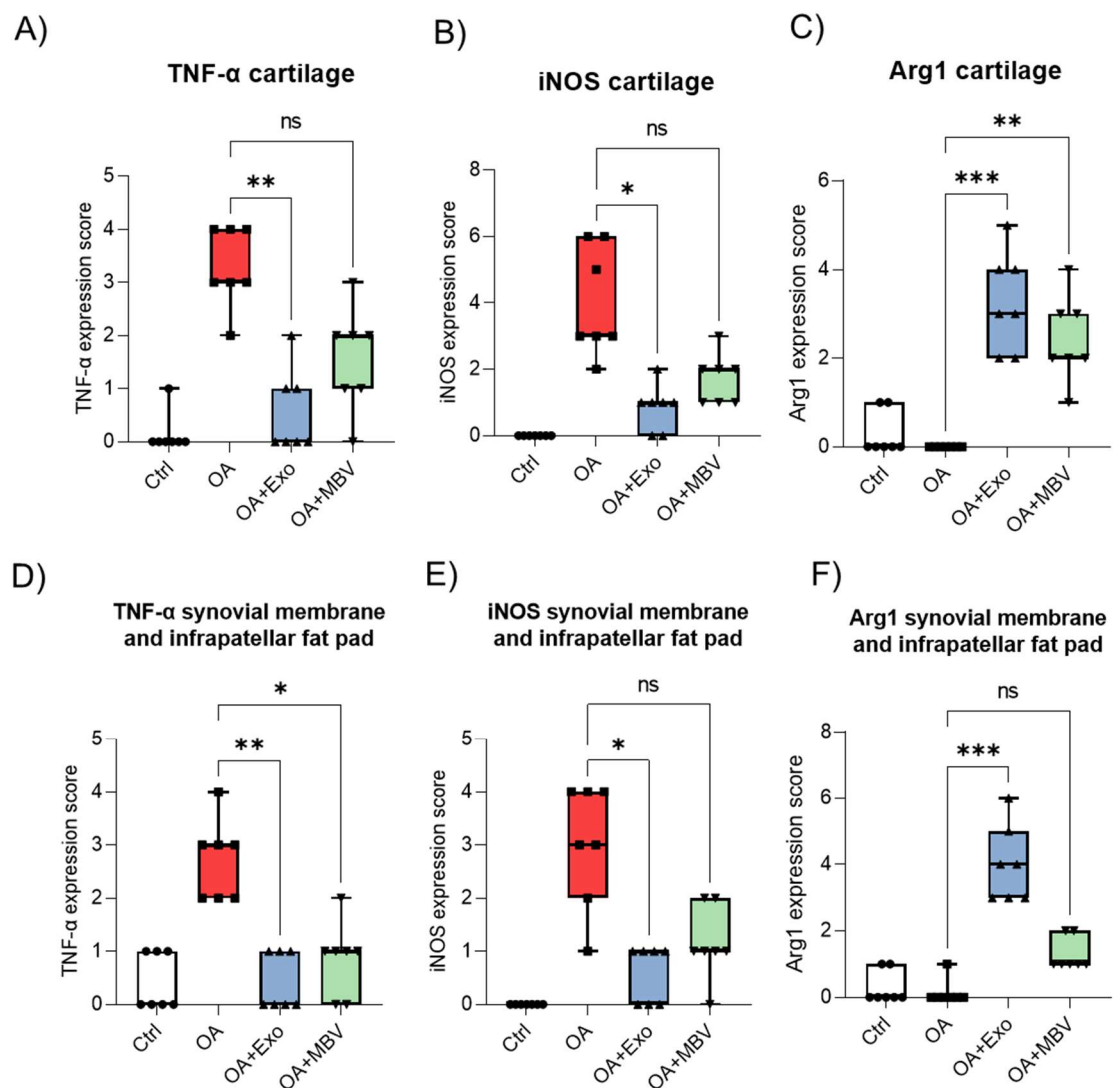


Figure S9. Semi-quantitative scoring of IHC marker expression in joint tissues (cartilage, synovial membrane, infrapatellar fat pad) across different groups.

A. TNF- $\alpha$  cartilage, B. iNOS cartilage, C. Arg1 cartilage, D. TNF- $\alpha$  synovial membrane and infrapatellar fat pad, E. iNOS synovial membrane and infrapatellar fat pad, F. Arg1 synovial membrane and infrapatellar fat pad, \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001. The figures (circle, square, triangle) on the graphs indicate the level of expression of IHC marker expression in joint tissues across different groups: ● (control group); ■ (OA group); ▲ (experimental group + exosomes); ▼ (experimental group + MBV).