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## Supplemental Information

# Synovial Mesenchymal Stem Cell-Derived EV-Packaged miR-31 Downregulates Histone Demethylase KDM2A to Prevent Knee Osteoarthritis

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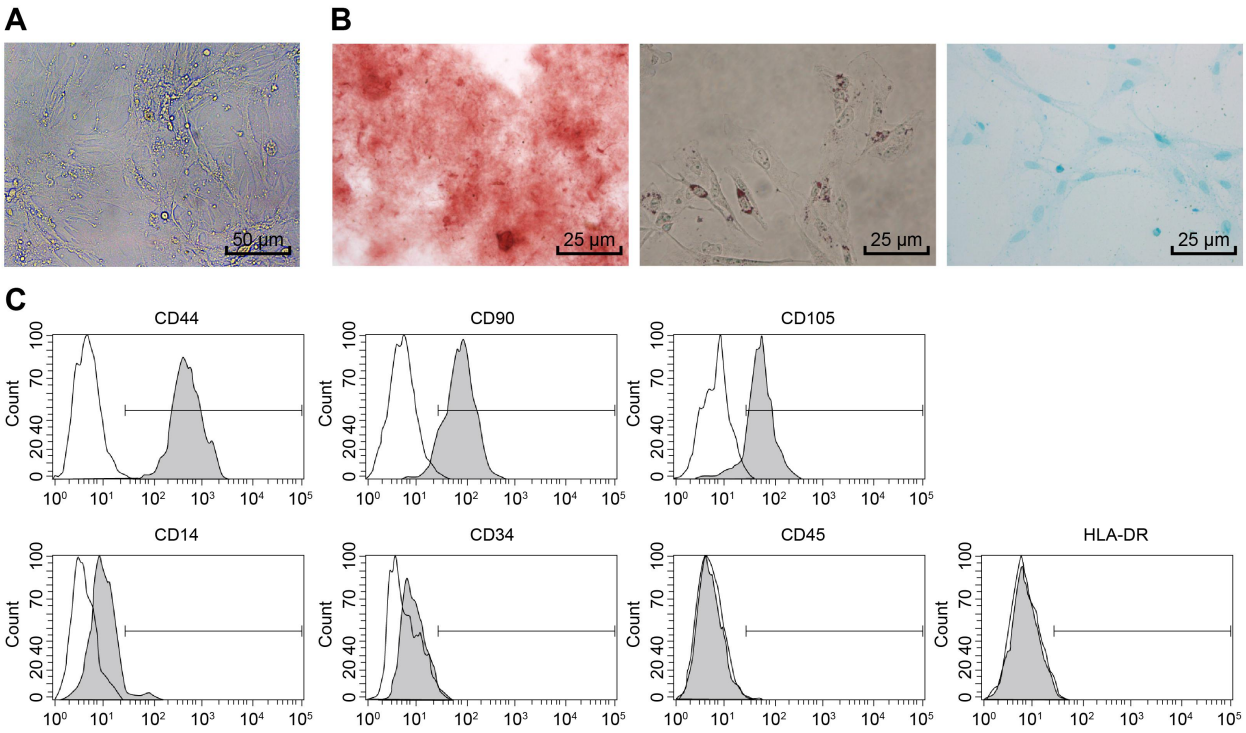
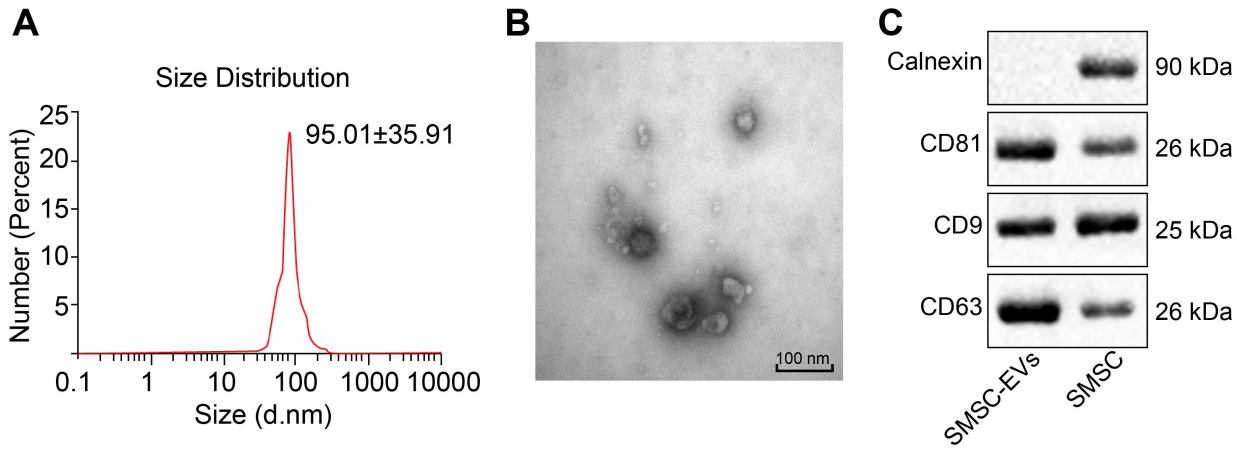


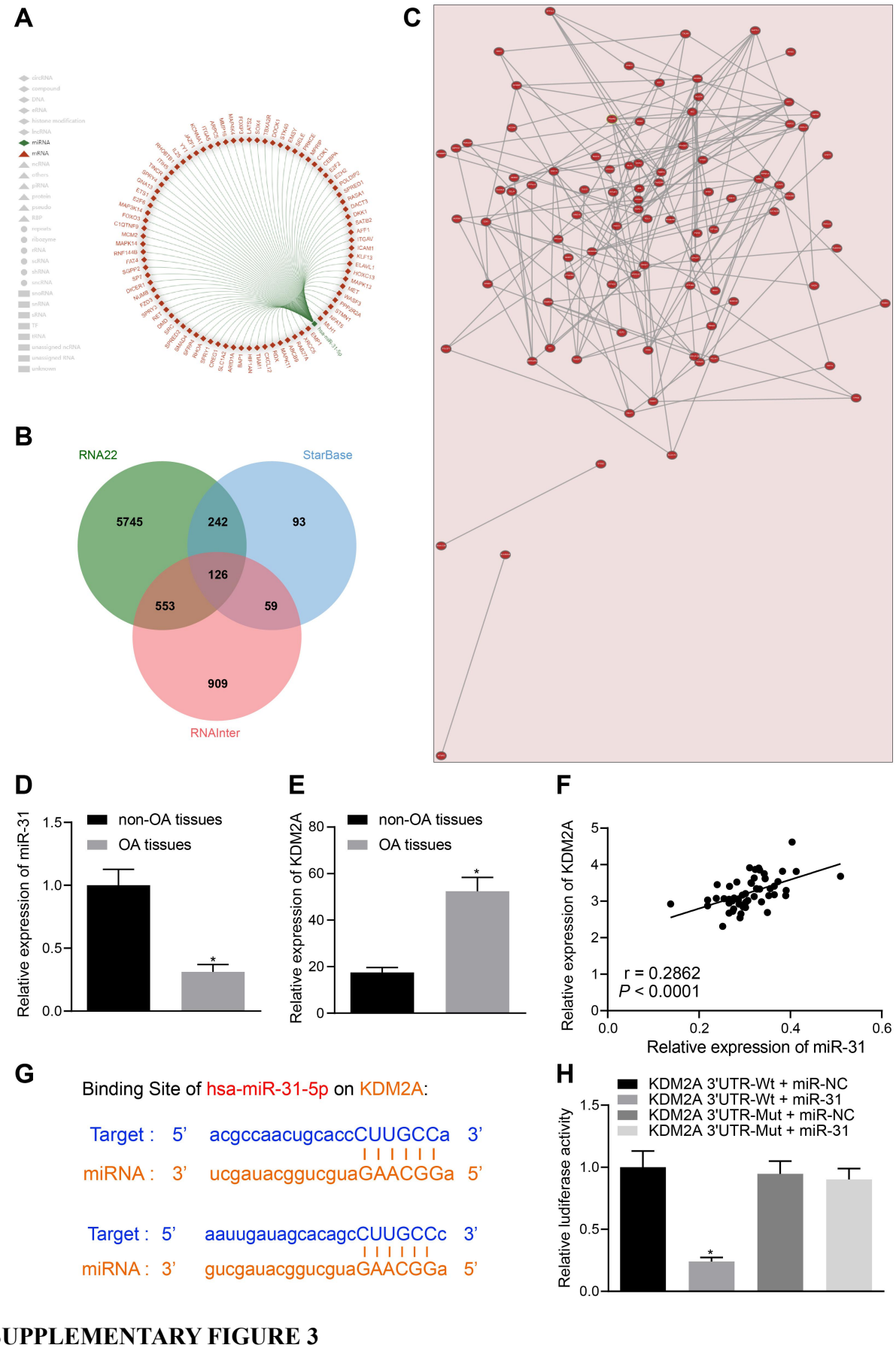

**SUPPLEMENTARY FIGURE 1**

Immunophenotype identification of SMSCs. A, SMSCs showed a typical spindle-like morphology (× 200). B, SMSCs showed pluripotent differentiation ability for osteogenesis (alizarin red staining, × 400), adipogenesis (oil red O staining, × 400) and cartilage formation (× 400). C, Flow cytometric analysis of characteristic cell surface markers of SMSCs. The unfilled curve represents the isotype control, while the solid gray curve represents the measured surface markers (CD44, CD90, CD105, CD14, CD34, CD45, and HLA-DR).




**SUPPLEMENTARY FIGURE 2**

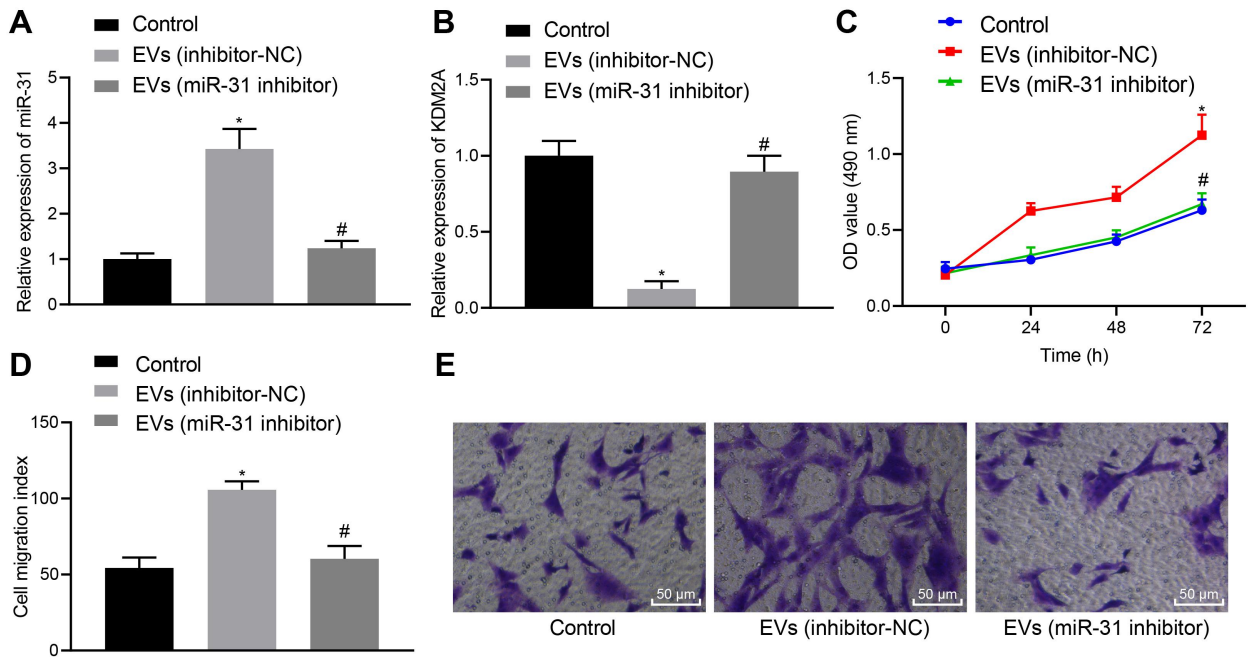
Characterization of the isolated EVs. A, Particle size distribution of EVs measured by DLS. Representative results were obtained from three independently repeated experiments. B, Morphology of EVs observed under a TEM (scale bar = 100 nm). C, EV surface markers (CD81, CD9, CD63 and Calnexin) measured using western blot analysis.




**SUPPLEMENTARY FIGURE 3**

KDM2A is a target gene of miR-31. A, A map of the interaction network between miR-31 and

mRNAs was obtained from the RNAInter website. B,. Venn diagram of the downstream target genes of miR-31 predicted by bioinformatics websites RNA22, StarBase and RNAInter. C, Co-expression network map between 126 genes analyzed by the Coexpedia website. D, miR-31 expression determined by RT-qPCR in articular cartilage tissues of OA (N = 54) and non-OA (N = 36) subjects, * $p < 0.05$ compared with articular cartilage tissues of non-OA subjects. E, Immunohistochemical staining of KDM2A protein in articular cartilage tissues of OA (N = 54) and non-OA (N = 36) subjects, * $p < 0.05$ compared with articular cartilage tissues of non-OA subjects. F, Pearson correlation analysis of miR-31 expression with KDM2A expression in clinical tissue samples. G, Starbase database prediction for the putative miR-31 binding sites in the 3'UTR of KDM2A mRNA. The targeting effect was present in both human and mice. H, Binding of miR-31 to KDM2A confirmed by dual luciferase reporter assay in HEK-293T cells. * $p < 0.05$ compared with HEK-293T cells co-transfected with KDM2A 3'UTR-WT and miR-NC. Data are shown as mean ± standard deviation of three technical replicates. Unpaired t-test was applied for comparison between two groups. Data comparison between multiple groups was performed using one-way ANOVA with Tukey's *post hoc* test. Data comparison between groups at different time points was performed using repeated measures ANOVA with Bonferroni correction.




**SUPPLEMENTARY FIGURE 4**

Role of miR-31 in the chondrocytes isolated from OA. A, The expression of miR-31 determined by RT-qPCR in miR-31 mimic-transfected chondrocytes or chondrocytes treated with EVs from miR-31 inhibitor-transfected SMSCs. B, The expression of KDM2A determined by RT-qPCR in in miR-31 mimic-transfected chondrocytes or chondrocytes treated with EVs from miR-31 inhibitor-transfected SMSCs. C, Chondrocyte proliferation measured by CCK-8 assay following miR-31 mimic transfection or treatment with EVs from miR-31 inhibitor-transfected SMSCs. D, The number of migrated chondrocytes measured by Transwell assay following miR-31 mimic transfection or treatment with EVs from miR-31 inhibitor-transfected SMSCs. E, Representative images of migrating chondrocytes following miR-31 mimic transfection or treatment with EVs from miR-31 inhibitor-transfected SMSCs (× 200). * $p < 0.05$ compared with chondrocytes without any treatment. # $p < 0.05$ compared with chondrocytes treated with EVs from inhibitor-NC-transfected SMSCs. Data are shown as mean ± standard deviation of three technical replicates. Unpaired t-test was applied for comparison between two groups. Data comparison between multiple groups was performed using one-way ANOVA with Tukey's *post hoc* test. Data comparison between groups at

different time points was performed using repeated measures ANOVA with Bonferroni correction.

**Supplementary Table 1.** Primer sequences for RT-qPCR

| RNA/miRNA | Sequence |
|---|---|
| sh-PTTG1-1 | 5’-GGGAAUCCAAUCUGUUGCATT-3’ |
| sh-PTTG1-2 | 5’-GGGAGATCTCAAGTTTCAACA-3’ |
| sh-NC | 5’-UUCUCCGAACGUGUCACGUTT-3’ |
| miR-31 | Forward: 5’-AGGCAAGAUGCUGGCAUAGCU-3’ |
| KDM2A | Forward: 5’-AACCCCAGCTCAAACTTTGAGA-3’<br>Reverse: 5’-GAACCCCAGCTCAAACTTTGAG-3’ |
| E2F1 | Forward: 5′-AGCGGCGCATCTATGACATC-3′<br>Reverse: 5′-GTCAACCCCTCAAGCCGTC-3′ |
| E2F1 | Forward: 5′-AGCGCCTGGCCTATGTGACCTG-3′<br>Reverse: 5′-TCGATGGGGCCTTGTTTGCTCTTA-3′ |
| PTTG1 | Forward: 5’-ACCCGTGTGGTTGCTAAGG-3'<br>Reverse: 5’-ACGTGGTGTTGAAACTTGAGAT-3’ |
| U6 | Forward: 5’- GCAAGGATGACACGCAAATTC-3’ |
| β-actin | Forward: 5’-GTCTTCCCCTCCATCGTG-3’<br>Reverse: 5’-AGGGTGAGGATGCCTCTCTT-3’ |
| β-actin | Forward: 5’- CCTAAGGCCAACCGTGAAAAGATG-3’<br>Reverse: 5’-GGTCCCGGCCAGCCAGGTCCAG-3’ |