Supplementary Materials

Macrophage-derived ectosomal miR-350-3p promotes osteoarthritis progression through downregulating chondrocyte H3K36 methyltransferase NSD1

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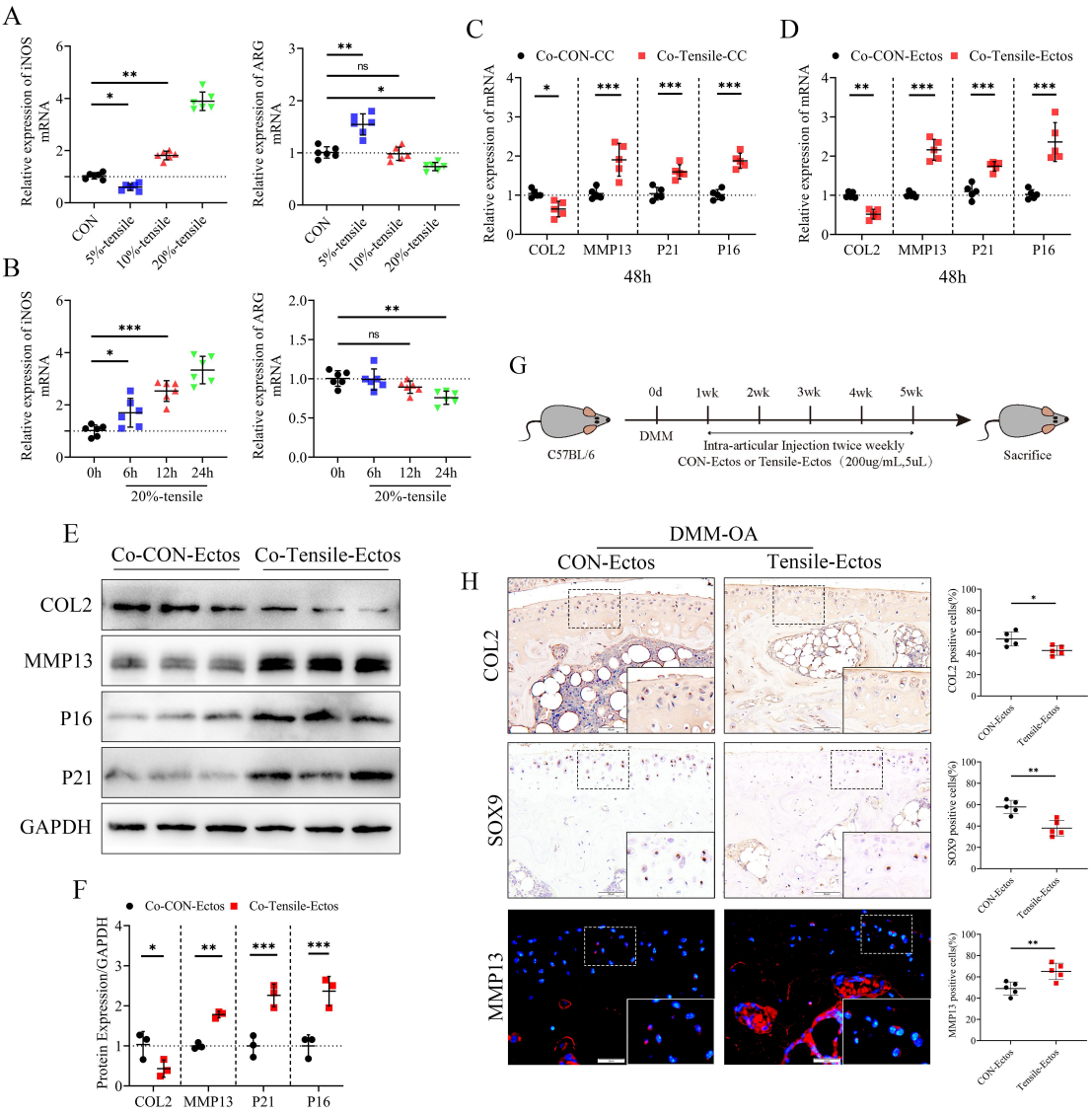
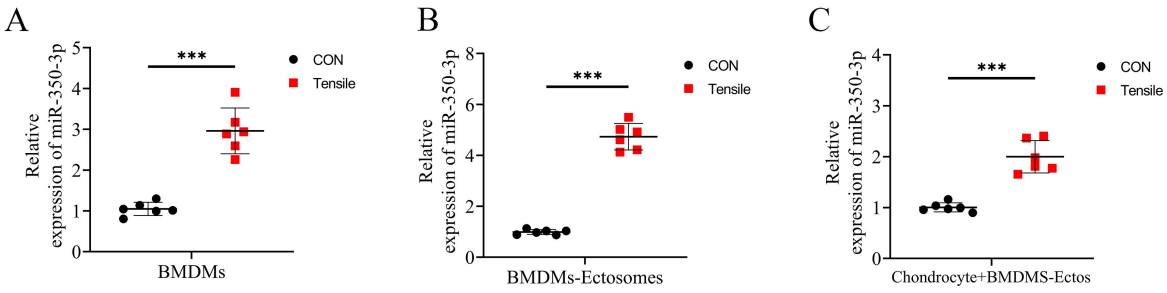
Supplementary Figure 1 to 4



Supplementary figure 1

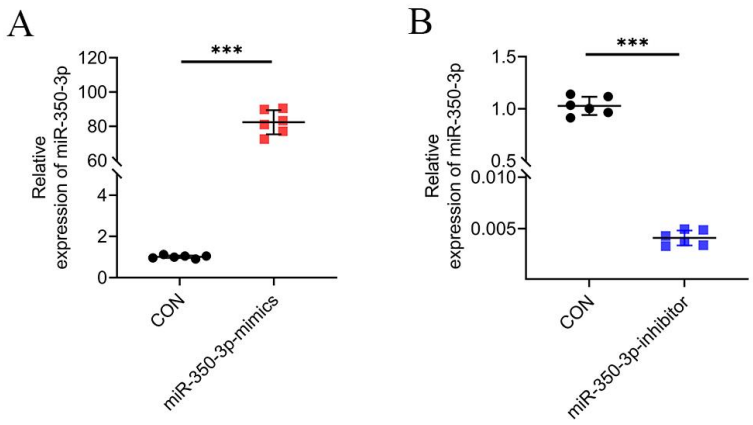
Quantitative PCR analysis of iNOS and ARG in BMDMs treated with 5%, 10% and 20% elongation strain loading, n=6 per point. (B) Quantitative PCR analysis of iNOS and ARG in BMDMs treated with 0.5Hz, 20% elongation strain loading for 0, 6, 12 and 24 hours, n=6 per point. (C) Quantitative PCR analysis of MMP13, COL2, P21 and P16 in chondrocytes co-cultured with supernatants of overloaded-BMDMs for 48h, n=5 per group. (D-F) Quantitative PCR and western blot of MMP13, COL2, P21 and P16 in chondrocytes treated with ectosomes from mechanically overloaded primary macrophages (BMDMs). (G) Scheme of intra-articular injection of CON-Ecots and Tensile-Ecots in DMM-OA mice. (H) IHC/IF staining and

quantification of COL2, SOX9, MMP13 in cartilage from DMM-OA mice treated with CON-Ectos or Tensile-Ectos. Statistical analyses were conducted using unpaired t-test (H), one-way analysis of variance followed by Dunnett's multiple comparison test (A, B) or two-way analysis of variance followed by Sidak's multiple comparison test (C, D, F). *P<0.05, **P<0.01, ***P<0.001, ns not significant.



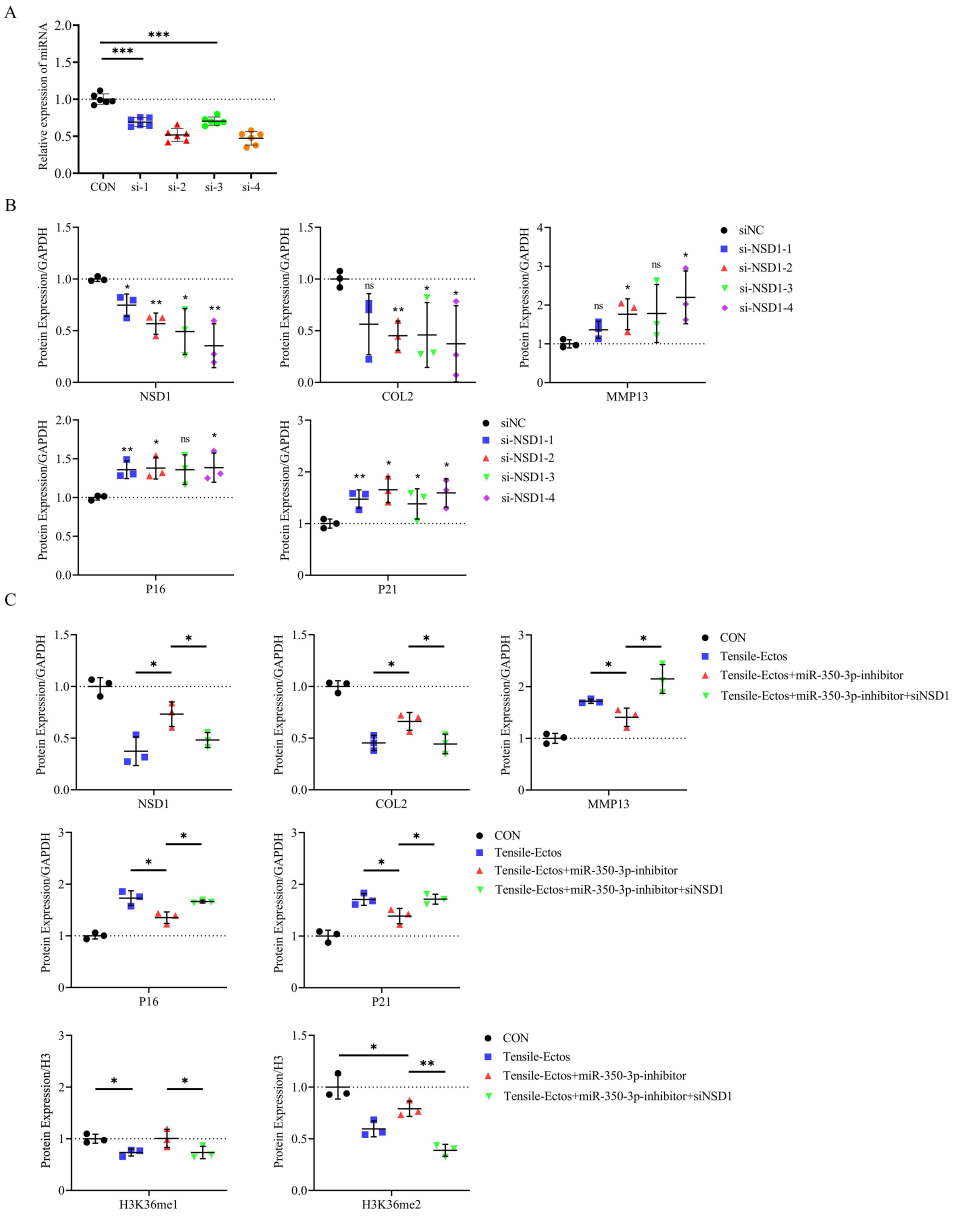
Supplementary figure 2

(A) Quantitative PCR analysis of miR-350-3p in BMDMs treated with 0.5Hz, 20% elongation strain loading for 24h. (B) Quantitative PCR analysis of miR-350-3p in ectosomes from mechanically overloaded primary macrophages (BMDMs). (C) Quantitative PCR analysis of miR-350-3p in chondrocytes treated with CON-Ectos or Tensile-Ectos from BMDMs. Statistical analyses were conducted using Student's t-test. ***P<0.001.



Supplementary figure 3

(A) Relative miRNA expression levels of miR-350-3p in chondrocytes treated with miR-350-3p mimics or mimics-NC (CON). (B) Relative miRNA expression levels of miR-350-3p in chondrocytes treated with miR-350-3p inhibitor or inhibitor-NC (CON). Statistical analyses were conducted using Student's t-test. ***P<0.001.



Supplementary figure 4

(A) Relative mRNA expression levels of NSD1 in chondrocytes treated with miR-350-3p mimics or mimics-NC(CON) and miR-350-3p inhibitor or inhibitor-NC(CON). (B) Relative protein quantification of NSD1, COL2, MMP13, P16 and P21 in chondrocytes treated with si-NSD1 or si-NC. (C) Relative protein quantification of NSD1, COL2, MMP13, P16, P21, H3K36me1 and H3K36me2 in chondrocytes treated with Tensile-Ectos, si-NSD1 and miR-350-3p inhibitor. Statistical analyses were conducted using one-way analysis of variance followed by Tukey's multiple comparison test (A-C). *P<0.05, **P<0.01, ***P<0.001, ns not significant.