

Supplementary Materials

Quantitative polymerase real-time polymerase chain reaction

The sequences of miRNA are shown below:

RNU6B-FOR: 5'-CTCGCTTCGGCAGCACA -3'

RNU6B REV: 5'- AACGCTTCACGAATTTGCGT -3'

miR-193b-3p: 5'- TGGCCCTCAAAGTCCCGCTAA -3'

The primer sequences of mRNA are shown below:

COL2A1-FOR: 5'- GCACCTGCAGAGACCTGAAAC -3'

COL2A1-REV: 5'- GCAAGTCTCGCCAGTCTCCA -3'

SOX9-FOR: 5'- GGAGATGAAATCTGTTCTGGGAATG -3'

SOX9-REV: 5'- TTGAAGGTTAACTGCTGGTGTCTG -3'

AGGRECAN-FOR: 5'- GATGTTCCCTGCAATTACCACCTC -3'

AGGRECAN-REV: 5'- TGATCTCATACCGGTCCTTCTTCTG -3'

COMP-FOR: 5'- ACTCAGTTTCCCCACCATGTC -3'

COMP-REV: 5'- CAACGTCCAGCCTCAAGGTAA -3'

HDAC3-FOR: 5'- ACCAATATGCAAGGCTTCACCAA -3'

HDAC3-REV: 5'- GCCTGTGTAACGCGAGCAGA -3'

RUNX2-FOR: 5'- CACTGGCGCTGCAACAAGA -3'

RUNX2-REV: 5'- CATTCCGGAGCTCAGCAGAATAA -3'

COL10A1-FOR: 5'- CATAAAAGGCCCACTACCCAAC -3'

COL10A1-REV: 5'- ACCTTGCTCTCCTTACTGC -3'

MMP13-FOR: 5'- TCCTGATGTGGGTGAATACAATG -3'

MMP13-REV: 5'- GCCATCGTGAAGTCTGGTAAAAT -3'

Primer sequences for the amplification of the *SOX9* promoter, *COL2A1* promoter, *AGGRECAN* promoter, and *COMP* promoter

COL2A1 Promoter-FOR: 5'- ATTAGTCCACAGGGGCTTTCC -3'

COL2A1 Promoter-REV: 5'- TGAGCTTGGAAGATGCTTGGG -3'

COMP Promoter-FOR: 5'- ACTCAGTTTCCCCACCATGTC -3'

COMP Promoter-REV: 5'- ACCACAGCCCTGCCCAACGT -3'

AGGRECAN Promoter-FOR: 5'- GCCGACCGCAATGCAGATG -3'

AGGRECAN Promoter-REV: 5'- ATGGCGCGCTGTGACACAT -3'

SOX9 Promoter-FOR: 5'- ACGATGCTGTTCTTACACTTTC -3'

SOX9 Promoter-REV: 5'-TGTA ACTTCTTCAAATGGGATG -3'

The target sequences (5' to 3') of the siRNA molecules

siHDAC3-1: 5'-CAGCCGGTTATCAACCAGGTA -3'

siHDAC3-2: 5'-CAGGTAGTGGACTTCTACCAA -3'

Isolation and identification of exosomes

Isolation of exosomes

Exosomes were isolated from 4 mL of cell free plasma. The Plasma were centrifuged

at $500 \times g$ for 10 minutes, then the supernatant was centrifuged at $20,000 \times g$ for 20 min. Exosomes were then harvested by centrifugation at $100,000 \times g$ for 70 min. The exosome pellet was resuspended in PBS and collected by ultracentrifugation at $100,000 \times g$ for 70 min (Sorvall S100AT5 rotor) [1].

Nanoparticle tracking analysis

The size distribution of the exosomes was measured using Nanosizer™ technology (Malvern Instruments, Malvern, UK), and was analysed using Zetasizer software (Malvern).

Transmission electron microscopy (TEM) analysis of exosomes

The morphology of the exosomes was observed by transmission electron microscopy (TEM). The exosomes were loaded onto copper grids coated with Formvar (Structure Probe, Inc., PA, USA). The grids were contrasted using 2% uranyl acetate, dried and then observed using a Philips Morgagni 268D microscope (Philips, Amsterdam, Netherlands).

Isolation of RNA and protein from exosomes

Protein were extracted from exosomes using a Total Exosome Protein Isolation Kit (Invitrogen, Carlsbad, CA, USA) following the instructions supplied. The obtained protein was subsequently used in western blotting.

References:

1. Peinado H, Alečković M, Lavotshkin S, et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. NATURE MEDICINE. 2012; 18: 883–91.

Figure S1

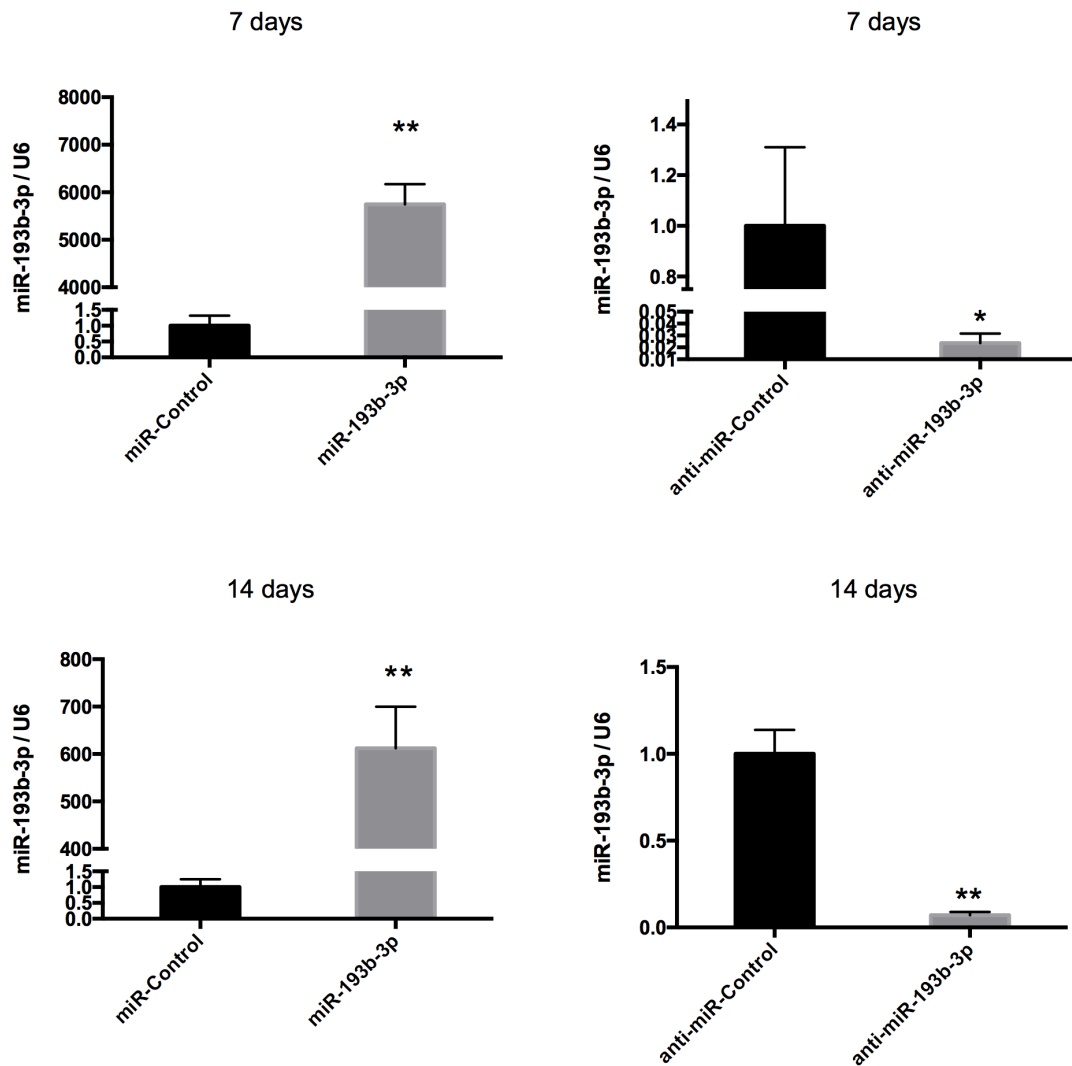


Figure S1. The relative expression levels of miR-193b on day 7, 14 of chondrogenesis of hMSCs. hMSCs were transfected with anti-miR-193b-3p, anti-nonspecific control miRNA (anti-miR-Control), and miR-193b-3p or miR-Control, and then induced to differentiate into chondrocytes for 7 and 14 days. RNU6B was detected as endogenous controls. The data shown represent the mean \pm standard error (SE) of three experiments. *P < 0.05; ** P < 0.001.

Figure S2

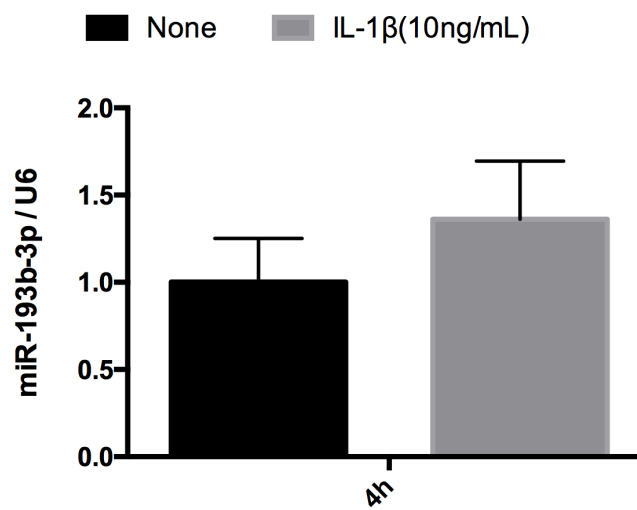


Figure S2. The relative expression levels of miR-193b after 4 h of IL-1 β treatment in PHCs. The relative expression levels of miR-193b-3p at 4 h post-treatment with IL-1 β (10 ng/ mL) or in untreated PHCs. RNU6B were detected as endogenous controls. The data shown represent the mean \pm standard error (SE) of three experiments. *P < 0.05; ** P < 0.001.

Figure S3

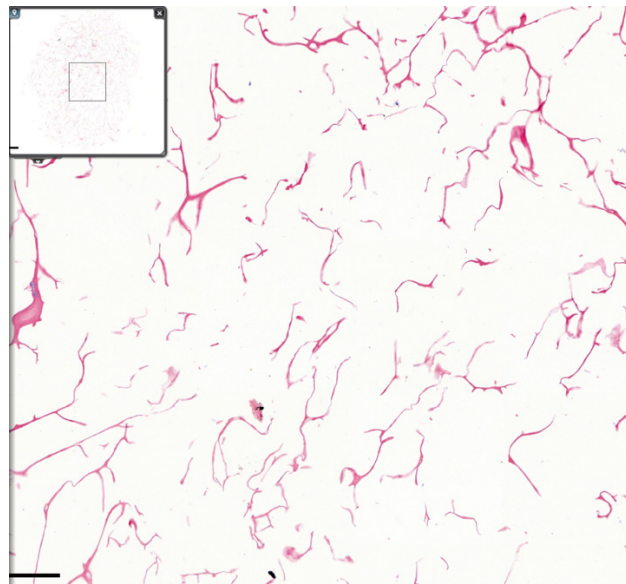


Figure S3. Histological characteristics of the empty scaffold were examined by H&E staining 8 weeks after implantation in nude mice. Top left panel: gross view of the sample. Lower panel: expanded view. Scale bar = 100 μ m.