Supporting Information

Chondrocyte-Targeted MicroRNA Delivery by Engineered Exosomes, towards a Cell-Free Osteoarthritis Therapy

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Table S1. List of primers used for cloning, reverse transcription-polymerase chain reaction (RT-PCR) and real-time quantitative RT-PCR in this work.

Usage	Gene name	Forward primer（5‘-3‘）	Reverse primer（5‘-3‘）
CAP – Lamp2b cloning	CAP primers	tcgaGGCAACAGCACCATGGACTGGAGGGTGATCATCCCCCCCAGGCCCAGCGCCGGCGGCAGCGGCGGCT	ccggaGCCGCCGCTGCCGCCGGCGCTGGGCCTGGGGGGGATGATCACCCTCCAGTCCATGGTGCTGTTGCC
CAP-GFP-Lamp2b cloning	GFP primers	GTAGGCATCCGGAGGAGGCTCTGTGAGC	GTAGGCATCCGGACTTGTACAGCTCGTCCA
miRNA mimic	miR-140-5p	CAGUGGUUUUACCCUAUGGUAG	
Stem-loop RT primer	Rat-U6 RT primer	AACGCTTCACGAATTTGCGTG	
	miR-140-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTACCAT	
	Human-U6 RT Primer	CACGGAAGCCCTCACACCGTGTCGTTC	
Real-time PCR	U6	GCTCGCTTCGGCAGCACA	GAGGTATTCGCACCAGAGGA
	miR-140-5p	CGCGCCAGTGGTTTTACCCT	GTGCAGGGTCCGAGGT
	Rat MMP-13	CTGACCTGGGATTTCCAAAA	ACACGTGGTTCCCTGAGAAG
	Rat GAPDH	GACTTCAACAGCAACTC	TGTAGCCATATTCATTGTCA
	Human GAPDH	GATCATCAGCAATGCCTCCT	TGTGGTCATGAGTCCTTCCA
	Human MMP-13	TGATGACATCAAGAAGGTGGTGAAG	TCCTTGGAGGCCATGTGGGCCAT
	Human ADAMTS5	GCTAGGCGACAAGGACAAGAG	CGCAGGCAGCTTCTTGGTCAG

Table S2. List of antibodies used in this work.

Antibody name	**Catalog number**	**Source**
Anti-MMP13 antibody	ab39012	Abcam
Anti-ADAMTS5 antibody	ab41037	Abcam
Goat Anti-Rabbit IgG H&L (HRP)	ab205718	Abcam
Anti-LAMP2 antibody	L0668-200UL	Sigma Aldrich
Goat anti-Rabbit IgG (H+L), Alexa Fluor 594	A-11037	Thermo Fisher Scientific

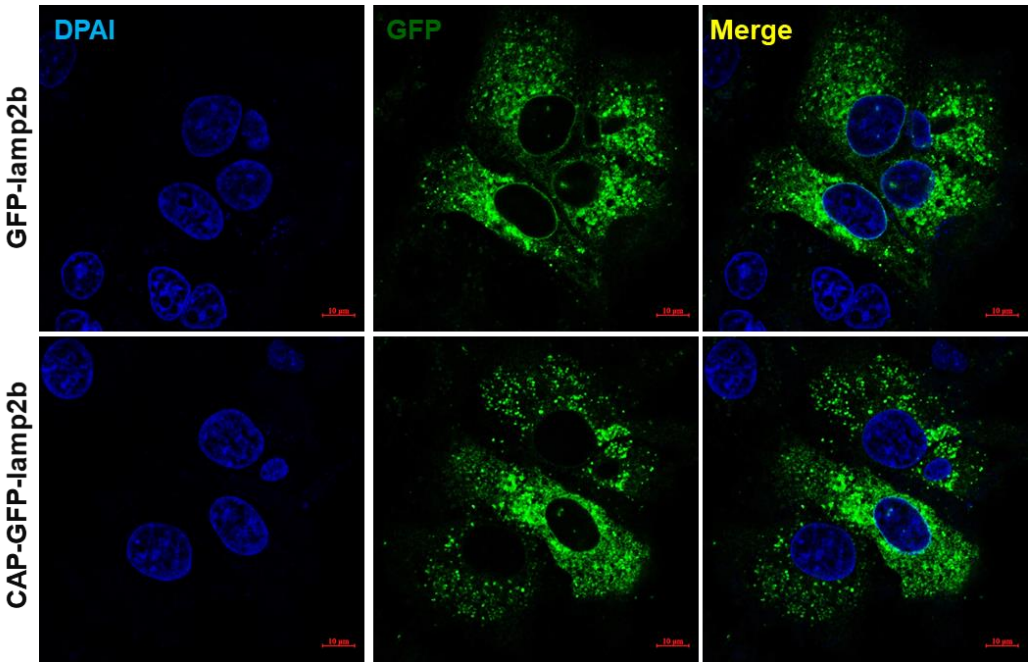

Figure S1. Overexpression of GFP-lamp2b or CAP-GFP-lamp2b in dendritic cells showing green puncta. Dendritic cells were seeded (1×10^6 cells/plate) onto 35-mm confocal dishes. After 24 hours, lipofection transfection was performed by adding 4 μg of GFP-LAMP2b or CAP-GFP--LAMP2b. After 48hr expression, the cells were fixed with 4% paraformaldehyde washed and imaging using a LSM800 confocal microscope after DAPI staining. Scale bar = 10 μm.

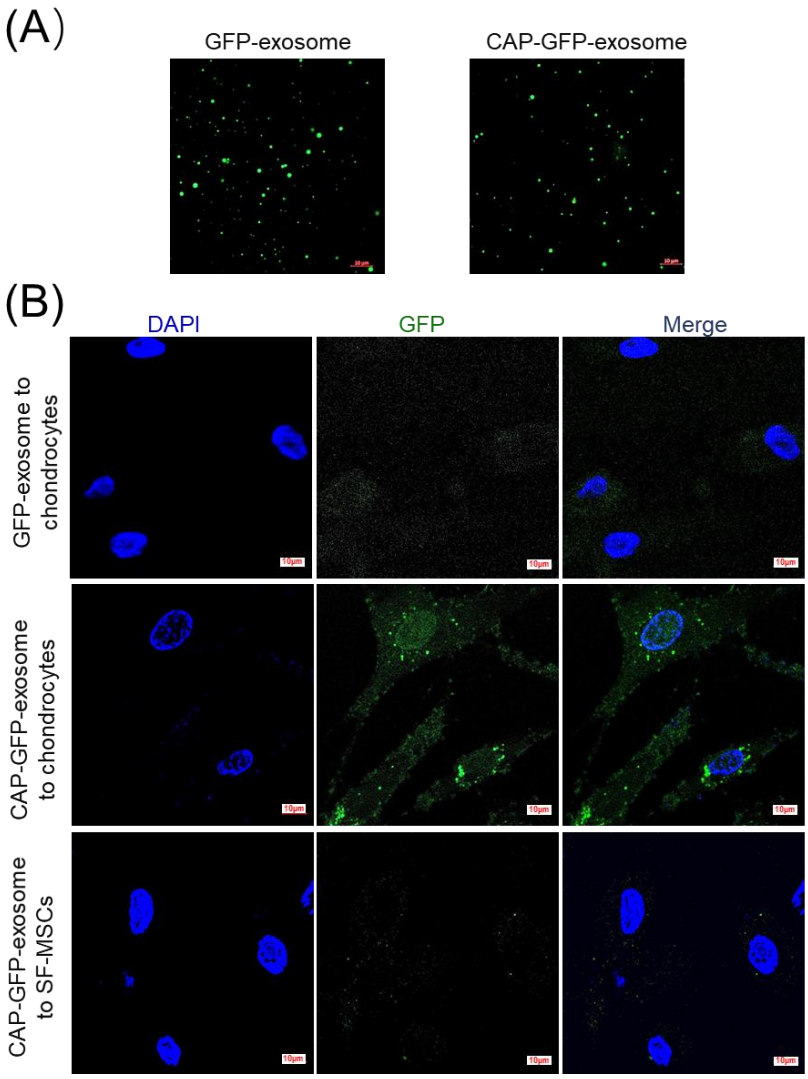

Figure S2. Selective entrance of CAP-GFP-exosomes to chondrocytes but not SF-MSCs. (A) Confocal microscope images of purified CAP-GFP exosomes and GFP-exosomes. (B) Target-specific intracellular delivery of GFP labelled exosomes to different cells. Cells were incubated with GFP-exosome or CAP-GFP-exosome for 2 h. After washing three times with DPBS and a counterstaining of the nuclei with DAPI. Confocal images were detected using an LSM800 confocal microscope (ZEISS, German) at 60×magnification. Scale bars, 10 μm.

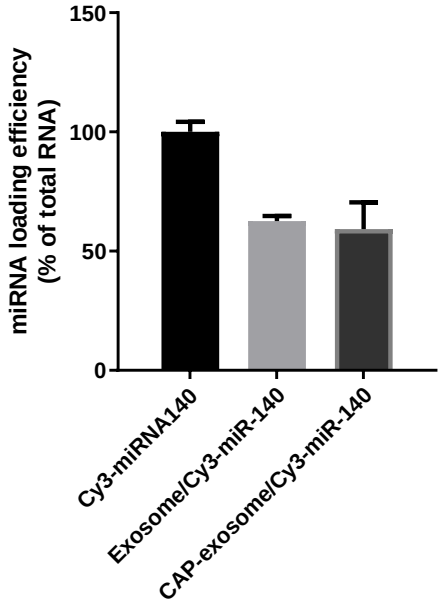

Figure S3. Quantification of the loading efficiency of different exosome preparations. Briefly, 0.5 μ mol of cy3-miR-140 mimic was mixed with 10 μg total exosome in electroporation buffer. After electroporation at 350 V and 150 μF in 4 mm electroporation cuvette using a Gene Pulser II system (Bio-Rad Laboratories, CA), the mixture was incubated at 37℃ for 30 min to allow the membrane of the exosomes to fully recovered. Excess free cy3-miR-140 were separated from loaded exosomes by ultracentrifugation for 1 hour at 120,000 g and 4 ℃. The final pellet (exosomes) was resuspended in PBS and stored at −80 ℃. Both the fluorescence intensities of initially added (total) miRNA and the free unloaded miRNA in the supernatant of the centrifuged were obtained by a fluorospectrophotometer at an excitation wavelength of 532 nm and an emission wavelength of 580 nm. The loading efficiency was calculated by the formula: (total miRNA – free unloaded miRNA) / total miRNA ×100%.

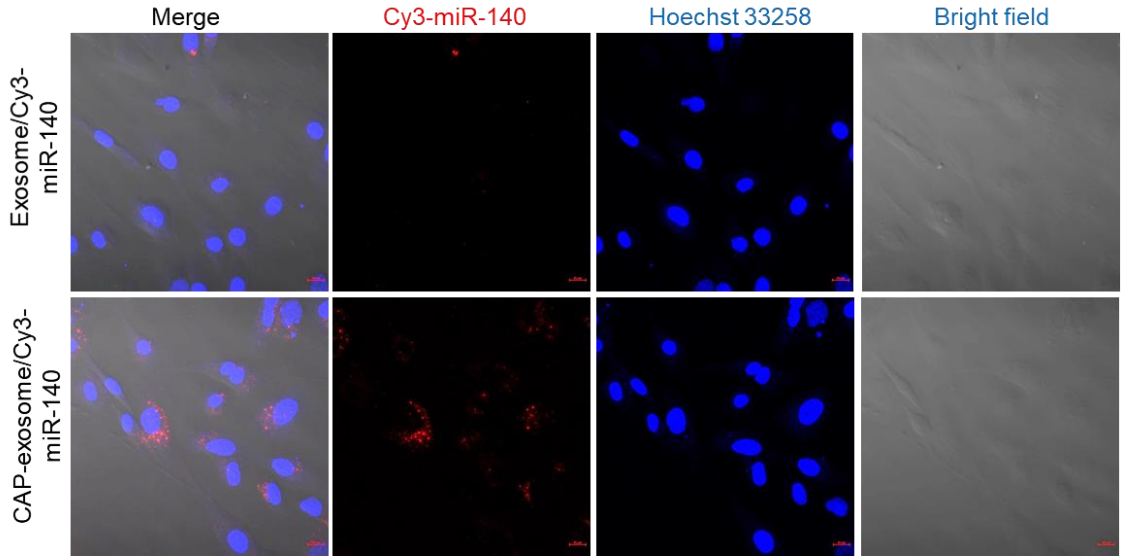

Figure S4. CAP-exosomes showed increased delivery of miRNA-140 to chondrocytes than exosomes without CAP signal. Briefly, chondrocytes were seeded into 24-well plates at a density of 8×10^4 cells per well. The cells were incubated and maintained for 24 h at 37 ℃ in an incubator with 5% CO_2. After incubation with exosome/cy3-miR140 or CAP-exosome/cy3-miR140 for 2 h, cells were rinsed with PBS, fixed and stained by followed by DAPI. Fluorescent images of the intracellular uptake were obtained by laser-scanning microscope at 40× magnification. The scale bar indicates 20 μm.

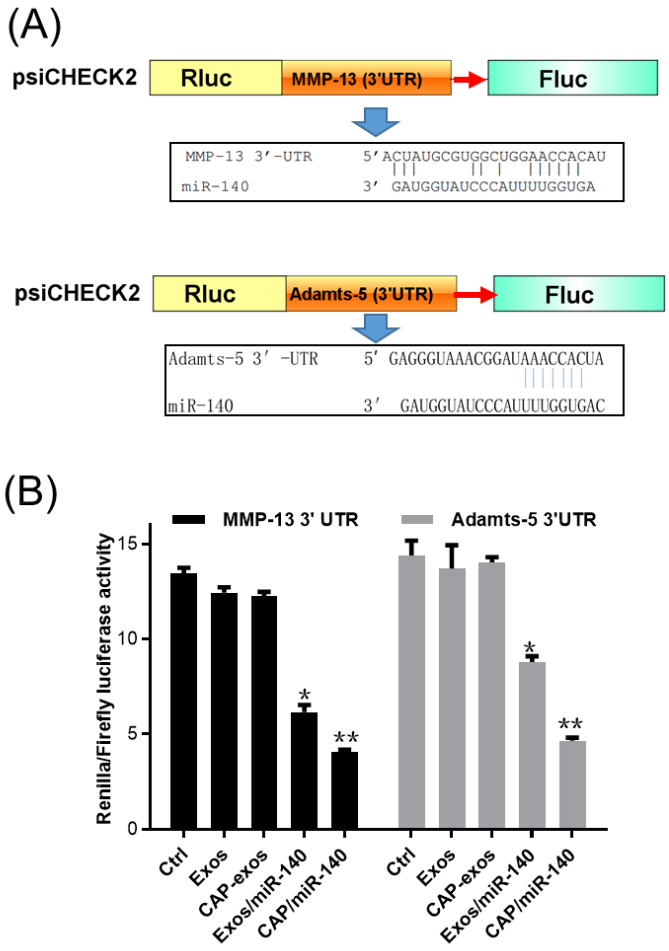

Figure S5. Analysis of the reporter gene activity in chondrocytes confirms that CAP-exosome/miR-140 directly targets the predicted binding sites in the MMP-13 and Adamts-5 3'UTR. (A) Schematic illustration of the complementarity binding site between miR-140 and the UTR site. (B) Luciferase activity measured upon the treatment by different exosome preparations. Briefly, the MMP-13 3′-UTR or Adamts-5 3′-UTR was inserted downstream of Renilla luciferase gene in the psiCHECK -2 vector. 12 h later, the cells were transfected with psiCHECK-2-MMP-13 3′-UTR and psiCHECK-2- Adamts-5 3′-UTR, respectively. 10 μg of exosomes, CAP-exosome or exosomes/miR-140, CAP-exosome/miR-140 were added to cells. After incubation for another 12 h, the luciferase activity was measured according to the dual luciferase reporting system (Promega, Madison, WI, USA). Relative protein levels were expressed as Renilla luciferase normalized against firefly luciferase signals. This assay was done in triplicate under the same conditions. Results represent the mean and s.d. of three independent experiments. *, $p < 0.05$. **, $p < 0.01$.

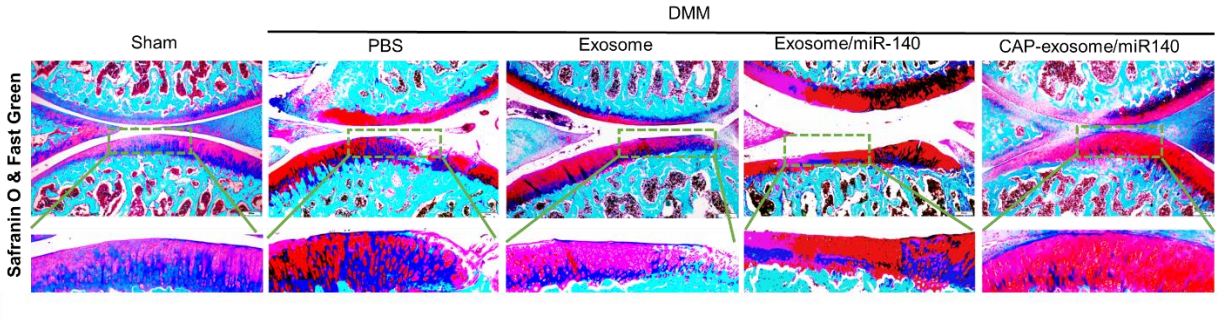

Figure S6. Images of Safranin O and Fast Green staining of cartilages under different treatments. Briefly, sections were stained with solution of 0.l % safranin-O and 0.2% Fast Green. The intensity of Safranin O staining is proportional to the proteoglycan content in the cartilage tissue. Fast Green counterstains the non-collagen sites and provides a clear contrast to the Safranin O staining.

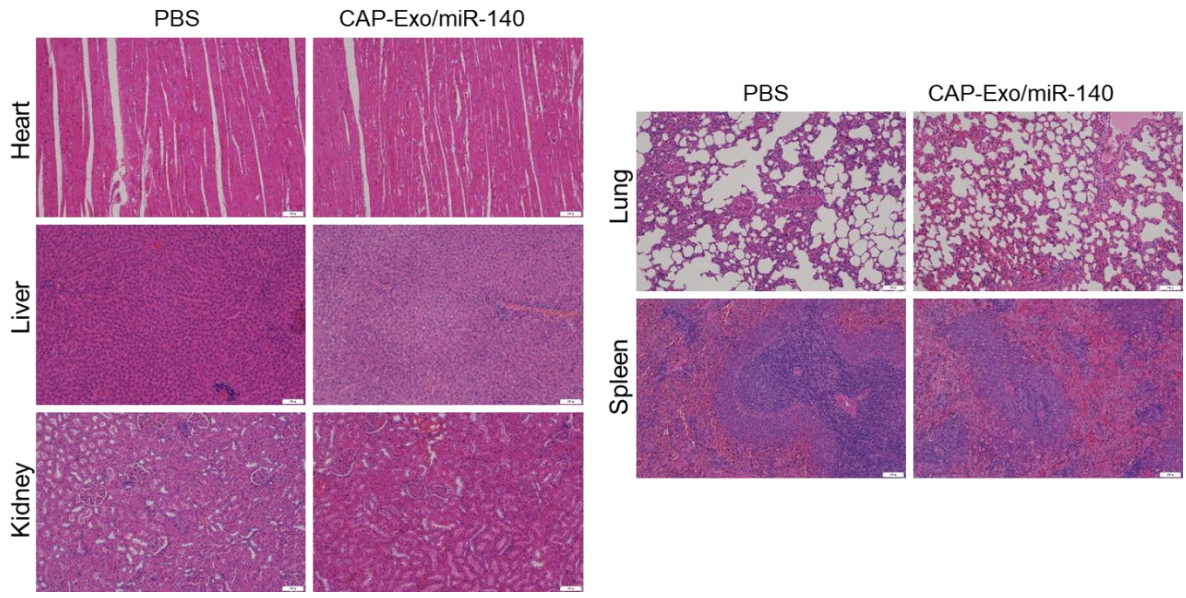

Figure S7. Toxicity of the exosome treatment to major organs. Briefly, the control group was injected with 0.1 mL of PBS into the knee joint cavity, and the experimental group was injected with 0.1 mL of CAP-Exo/miR-140, once a week for 4 weeks. The rats were then sacrificed. The major organs were dissected, and tissue sections were stained by H&E staining. Scale bar, 100 μm.