Polyethylene Glycol-Modified Single-Walled Carbon Nanotubes for Intra-Articular Delivery to Chondrocytes

Supporting Information

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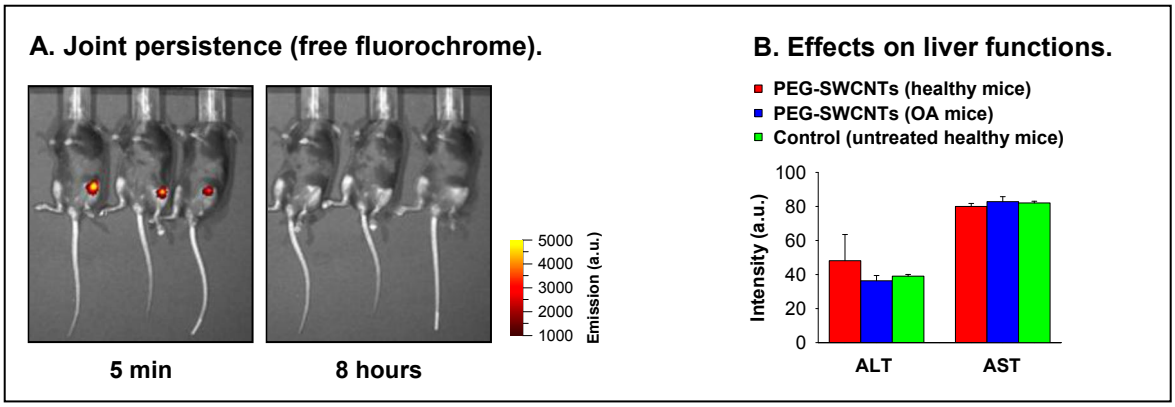

Figure S1. A. Joint persistence of IA-injected free fluorochromes (Seta750). A group (N=5) of healthy 3 month-old female C57BL/6J (B6) mice was unilaterally IA-injected with 0.4 µmoles of free Seta750 in the knee. NIR fluorescence images of animals were taken through an IVIS Spectrum for live imaging during the following 12 hours. NIR images showed that treated knees lost the signal in less than 8 hours. **B. Effects of IA-injected PEG-SWCNTs on liver functions.** Two groups (N=5) of healthy 3 month-old female B6 mice were unilaterally IA-injected in the knee with 5 µg of PEG-SWCNT-750 in 10 µL of PBS or equal volume of vehicle (PBS, control), respectively. PEG-SWCNT-750 were also IA-injected to a group (N=5) of 3 month-old female B6 mice with mild/moderate OA (1 month post-DMM surgery). After 14 days the mice were sacrificed, the blood collected and the levels of AST and ALT measured. The levels of AST and ALT of PEG-SWCNT-750-treated mice did not statistically differ from those of control mice, thus suggesting that the IA-injection of particles did not affect liver functions.

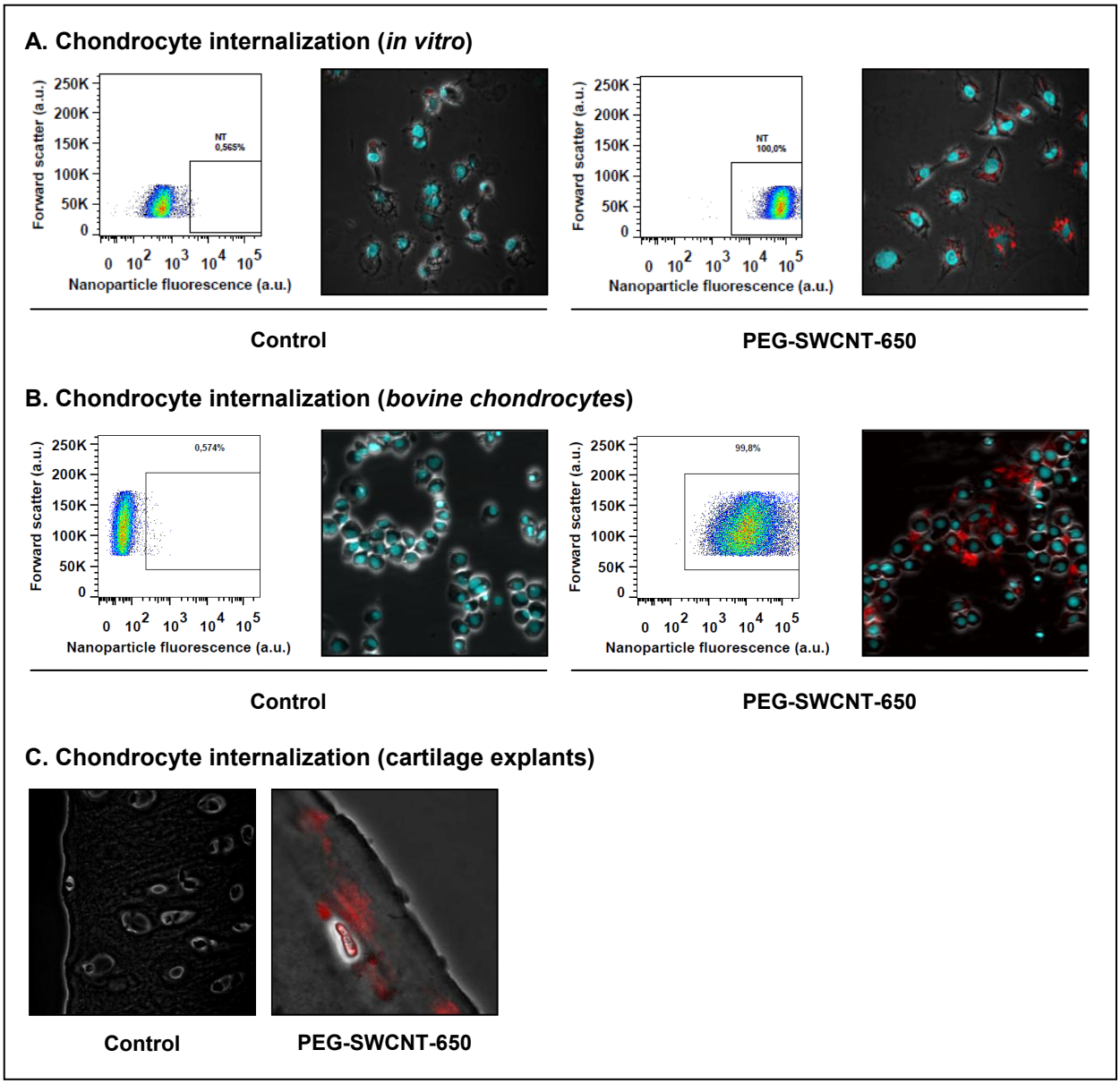

Figure S2. Internalization of PEG-SWCNTs into chondrocytes. A. and **B.** Cultured human TC-28 cells (**A.**) or extracted bovine chondrocytes (**B.**) were incubated with 10 nM of PEG-SWCNT-650 or equal volume of vehicle (PBS, control) for 24 hours. FACS analysis (left panels) showed that PEG-SWCNT-650 were able to accumulate into chondrocytes with an efficiency of 100%. Confocal images (right panels) showed NIR features in PEG-SWCNT-650-treated cells, whereas no NIR fluorescence was detected from control cells. **C.** Bovine cartilage explants were incubated with 10 nM of PEG-SWCNT-650 or equal volume of vehicle (PBS, control) for 7 days, then fixed, embedded in OCT compound and sectioned by a microtome-cryostat. Confocal

images of cartilage cryosections showed that PEG-SWCNT-650 localized in the cytoplasm and nucleus of chondrocytes.

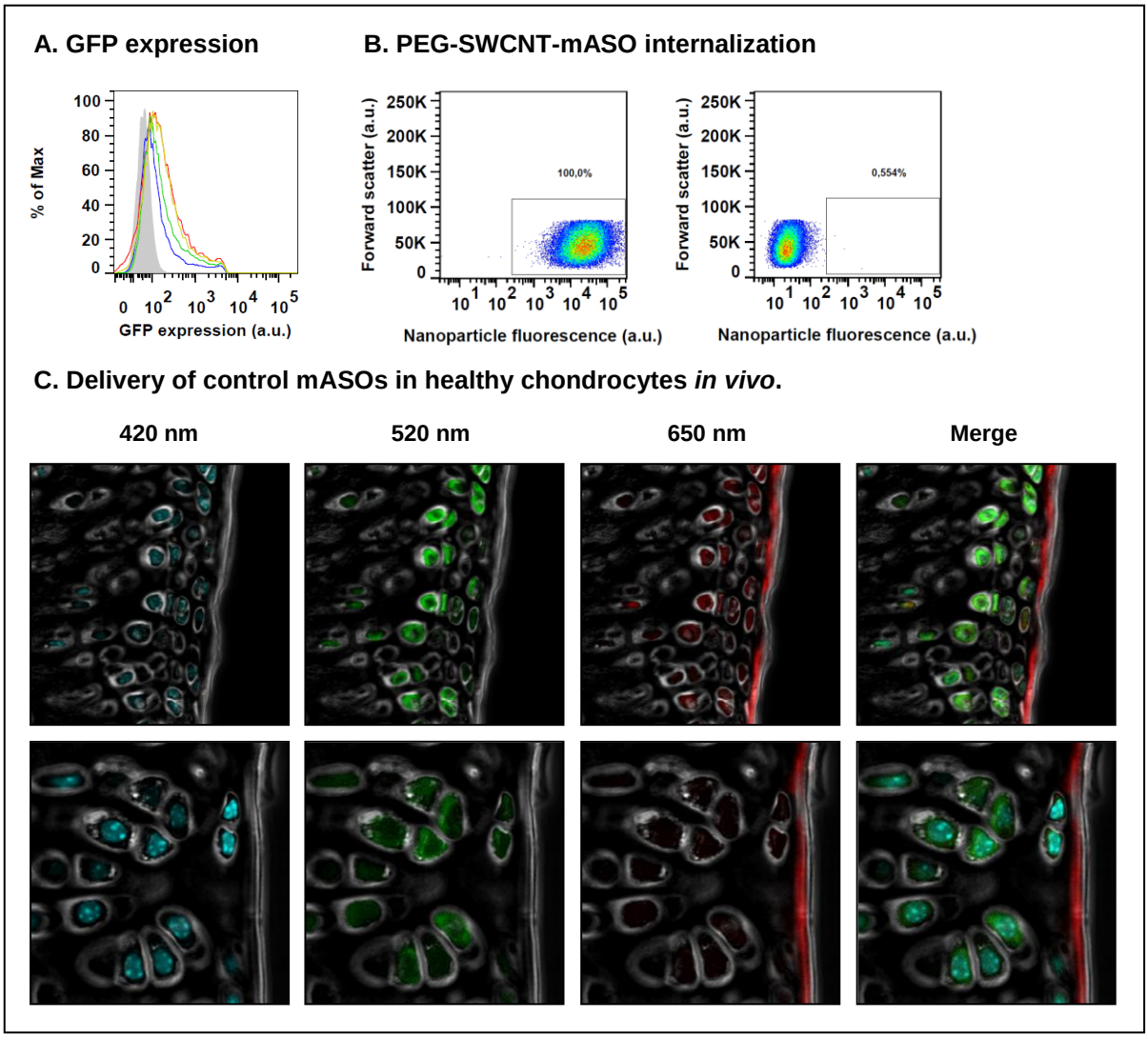

Figure S3. A. and **B. Delivery of anti-GFP mASOs by PEG-SWCNTs *in vitro*. A.** FACS analysis of HEK 293T cells lipofected with pEGFP-N1 plasmid and incubated 48 hours with 10 nM PEG-SWCNT-Ctr (red line), 10 nM PEG-SWCNT-mASOs (green line), an equivalent amount (~500 nM) of free GFP-mASOs (yellow line), co-lipofected with pEGFP-N1 plasmid and ~500 nM of free GFP-mASOs (blue line), or lipofected with PBS (grey line). **B.** Internalization of PEG-SWCNT-mASO in HEK 293T cells after 48 hours (left), gated versus the same cells without nanoparticles (right). PEG-SWCNT-mASOs were able to accumulate into chondrocytes with an efficiency of 100%. **C. Delivery of control mASOs by IA-injected PEG-SWCNTs in healthy chondrocytes *in vivo*.** Healthy (N=3) female C57BL/6-Tg(UBC-GFP)30Scha/J mice were unilaterally IA-treated in the knee with 5 µg of PEG-SWCNT-mASOs in 10 µL of PBS. Contralateral knees were treated with PEG-SWCNT-Ctr. After 3 days mice

were sacrificed and cartilage cryosections prepared. Confocal images showed that both nanotube-positive (upper panels) and -negative (lower panels) cells of IA-PEG-SWCNT-Ctr-treated mice had bright GFP signal.

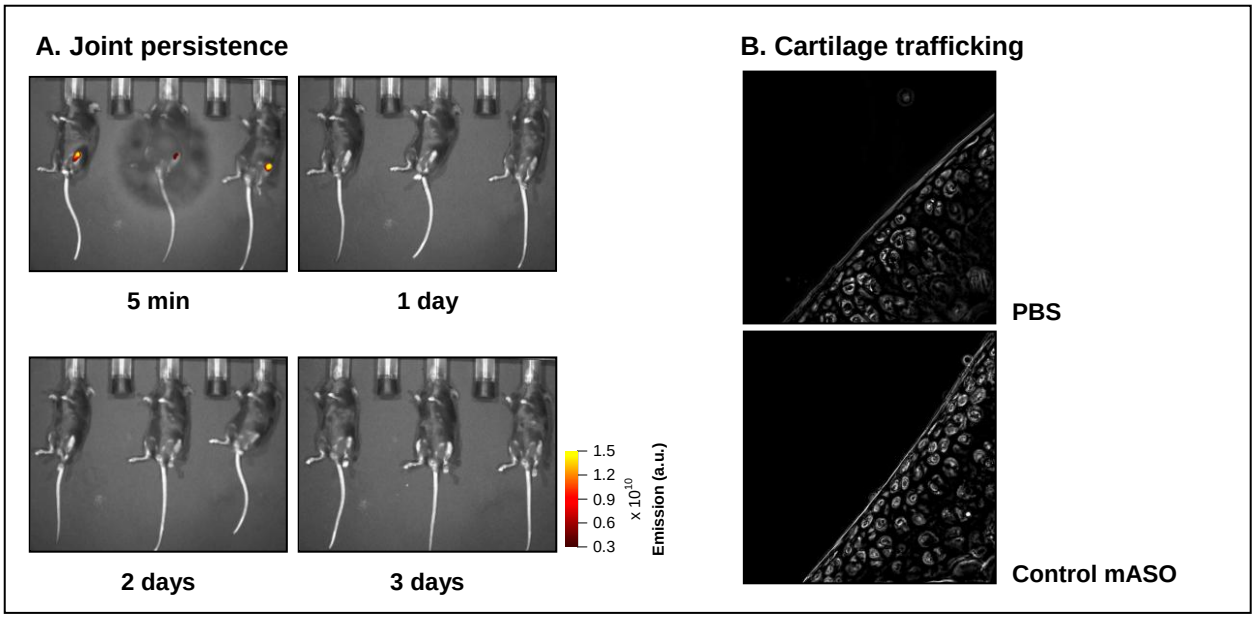

Figure S4. Joint persistence and cartilage trafficking of free control mASOs. A group (N=3) of healthy 3 month-old female C57BL/6J (B6) mice was unilaterally IA-injected with 1 nmole of fluorescein-labeled control mASO in the knee. Contralateral knees were injected with the same volume of PBS. Images of animals were recorded by means of an IVIS Spectrum for live imaging after 5 minutes, 24 hours, 48 hours and 72 hours. Images showed that treated knees lost the signal in less than 24 hours (**A.**). After 3 days mice were sacrificed and cartilage cryosections prepared. Confocal images showed free mASOs did not accumulate in the cartilage ECM or chondrocytes (**B.**).