Supplemental Material

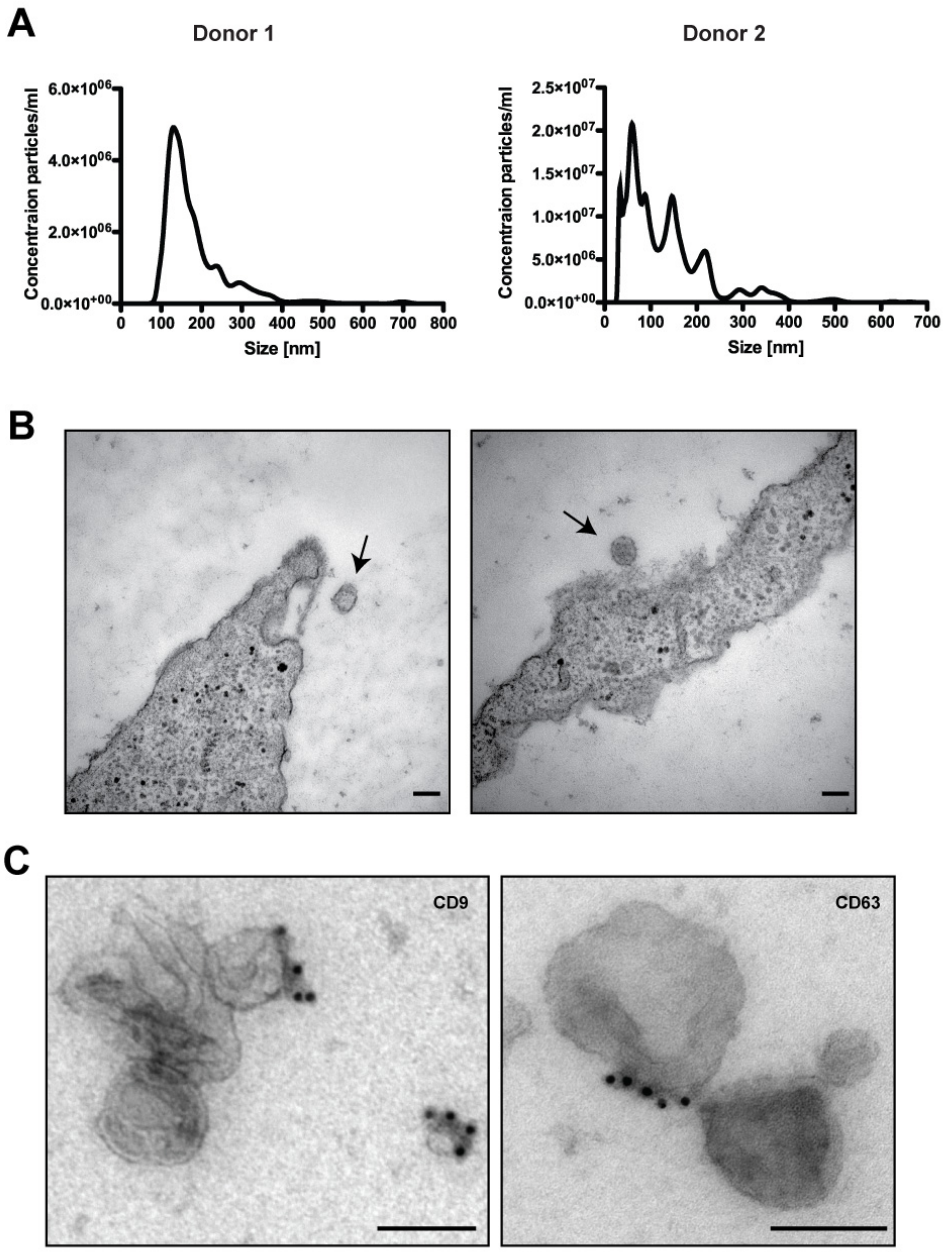

Figure S1. BMMSC-EV characterization. **(A)** NTA profiles of BMMSC-EVs isolated from conditioned medium of two BMMSC donors (100 000g pellet). **(B)** Release of BMMSC EVs captured by Transmission Electron Microscopy of EPON embedded MSCs. Representative micrographs are shown. Arrows indicate secreted vesicles. Scale bar is 100 nm **(C)** Not all MSC-EVs are positive for exosomal markers CD63 and CD9. EVs were isolated from conditioned medium derived from primary bone marrow MSC and subjected to sucrose density gradient centrifugation. The EVs residing in the fractions corresponding to densities 1.1082 to 1.972 g/ml were pooled and analyzed by immuno-electron microscopy. Representative micrographs of 3 independent experiments are shown. Scale bar is 100 nm.

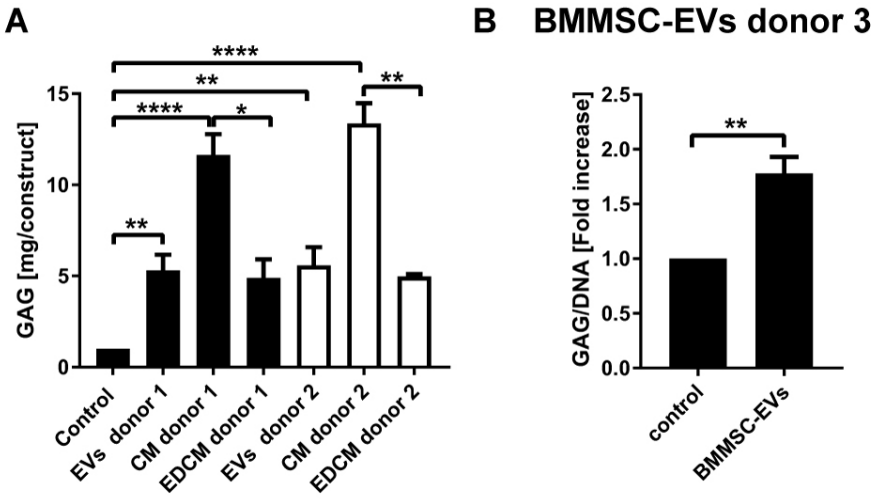

Figure S2. BMMSC-EVs promote proteoglycan production by chondrocytes derived from osteoarthritic patients. Chondrocytes from OA patients were cultured for 28 days in fibrin glue. The BMMSC-EVs, BMMSC conditioned medium (BMMSC-CM), BMMSC conditioned medium depleted from EVs (BMMSC-EDCM) – all equivalent of $500x10^3$ cells from healthy allogeneic BMMSC donors were added every 5 days. For BMMSC-EVs equivalent of $500x10^3$ cells equals: +/- $1,7x10^8$ particles for BMMSC donor 1, +/- $1.8x10^9$ particles for BMMSC donor 2 and +/- $2.43x10^8$ particles for BMMSC donor 3. **(A)** 28 day chondrocytes cultures from OA patients (same as in figure 5 B) were digested and analyzed for proteoglycan content. Glycosaminoglycan content not normalized for DNA is shown. Data of 3 independent experiments are presented as mean +/- SEM. ****p<0.0001**p< 0.002, *p< 0.02. The data are presented as fold increases relative to untreated control. **(B)** Effect of BMMSC-EVs isolated from conditioned medium derived from BMMSC donor 3 on proteoglycan production in two osteoarthritic patients is shown. 28 day chondrocytes cultures from OA patients were digested and analyzed for proteoglycan content. Glycosaminoglycan content normalized for DNA is shown. Data of 2 independent experiments performed in triplicates are presented as mean +/- SEM. **p< 0.005. The data are presented as fold increases relative to untreated control.

Supplemental Material and Methods

Transmission Electron Microscopy of EPON embedded MSCs

Cells were cultured 3 days, 4 days or 7 days and fixed with Karnovsky fixative (2.5% glutaraldehyde and 2% formaldehyde (Electron Microscopy Sciences) in 0.2M Cacodylate buffer, pH 7.4) in equal volume to culture at room temperature medium for 15 min. This was replaced and incubated with fresh Karnovsky fixative for 2 hours at room temperature. Cells were then post-fixed with 1% OsO4 / 1.5% K3Fe(III)(CN)6 in 0.065 M phosphate buffer, for 2 hours at 4°C and finally 1 hour with 0.5% uranyl acetate. Cells were then dehydrated in degraded EtOH series, and embedded in Epon resin (Polysciences). Ultrathin sections of 70 nm were cut and contrasted with uranyl acetate and lead citrate using the AC20 (Leica). The sections were inspected with a Jeol 1010 TEM (Jeol , Europe).

Table: primer sequences for quantitative real time PCR	
ACAN	FOR : 5'- CAACTACCCGGCCATCC -3' REV : 5'- GATGGCTCTGTAATGGAACAC -3'
ALP	FOR : 5'- GGACGGACCCTCGCCAGTGCT -3' REV : 5'- AGAGGGCCACGAAGGGGAACT -3'
COL2A1	FOR : 5'- AGGGCCAGGATGTCCGGCA -3' REV : 5'- GGGTCCCAGGTTCTCCATCT -3'
COL10A1	FOR : 5'- CACTACCCAACACCAAGACA -3' REV : 5'- CTGGTTTCCCTACAGCTGAT -3'
COX2	FOR: 5'- GCCCGACTCCCTTGGGTGTC -3' REV: 5'- TTGGTGAAAGCTGGCCCTCGC -3'
IL1A	FOR : 5'- ATCAGTACCTCACGGCTGCT -3' REV : 5'-CTTCATCTTGGGCAGTCACA -3'
IL1B	FOR : 5'- GCTGAGGAAGATGCTGGTTC -3' REV : 5'- TCCATATCCTGTCCCTGGAG -3'
IL6	FOR : 5'- CCTTCCAAAGATGGCTGAAA -3' REV : 5'- CAGGGGTGGTTATTGCATC -3'
IL8	FOR : 5'- CAGTTTTGCCAAGGAGTGCT -3' REV : 5'- AGTTTTCCTTGGGGTCCAGA -3'
IL17	FOR : 5'- CCGTGGGCTGCACCTGTGTC-3' REV : 5'- GGGAGTGTGGGCTCCCCAGA-3'
RUNX2	FOR: 5'- ATGCTTCATTCGCCTCAC -3' REV: 5'- ACTGCTTGCAGCCTTAAAT-3'
18S	FOR : 5'- GTAACCCGTTGAACCCCATT-3' REV : 5'- CCATCCAATCGGTAGTAGCG -3'
SOX9	FOR : 5'- CCCAACGCCATCTTCAAGG -3' REV : 5'- CTGCTCAGCTCGCCGATGT -3'
WNT7A	FOR : 5'- TGCCCGGACTCTCATGAAC -3' REV : 5'- GTGTGGTCCAGCACGTCTTG -3'