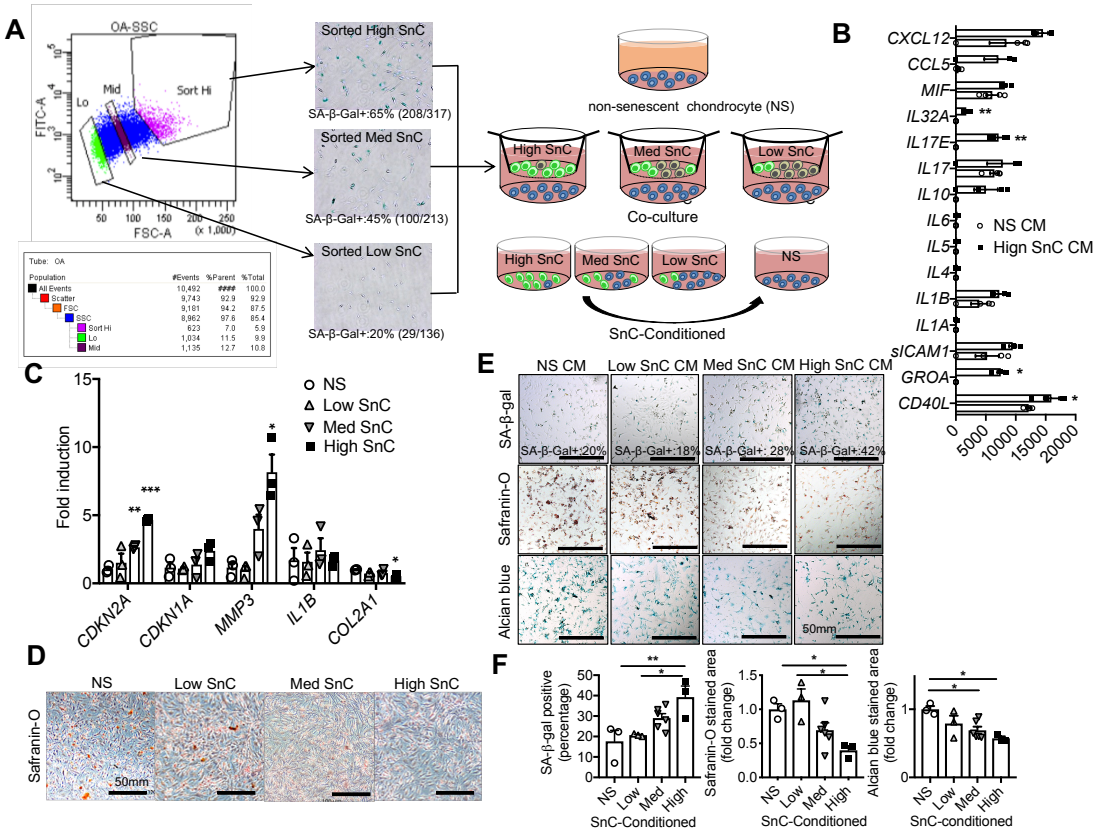
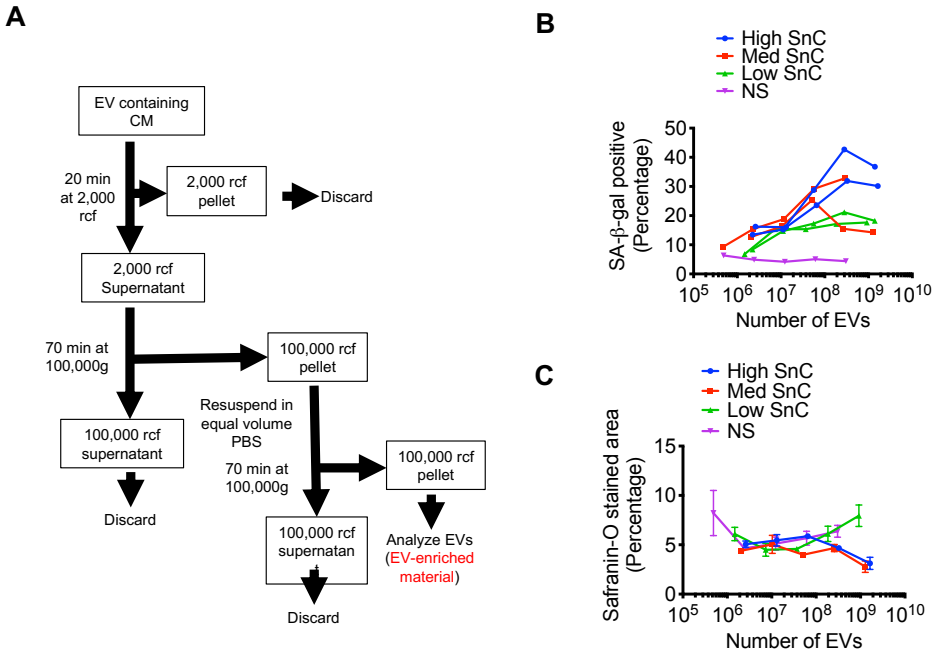
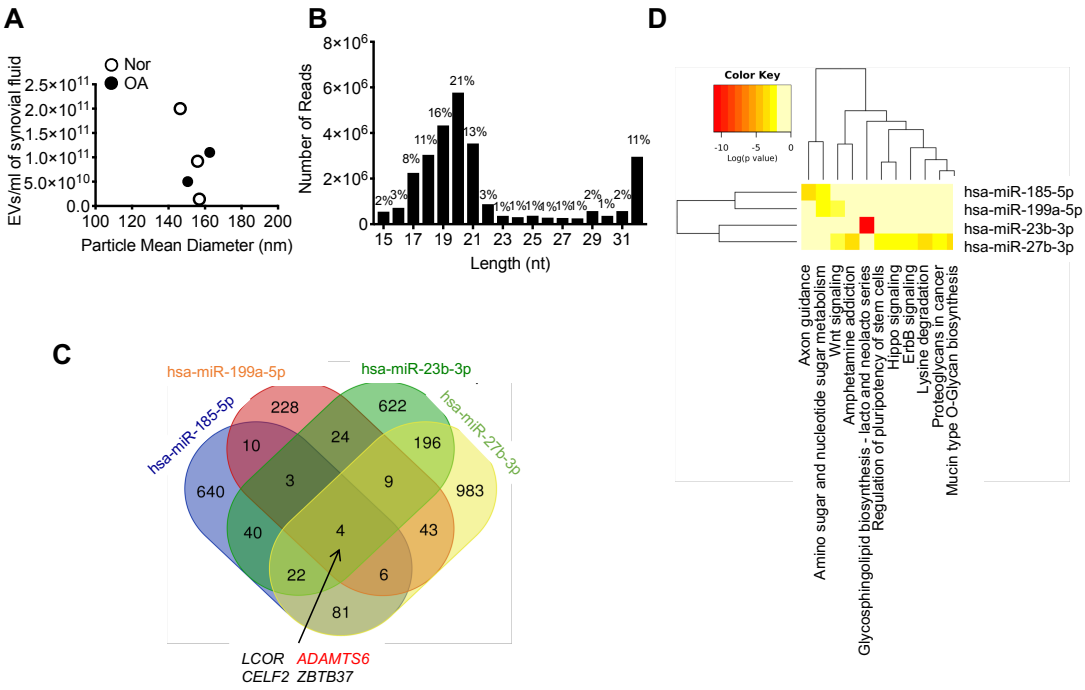
Supplemental figures and legends



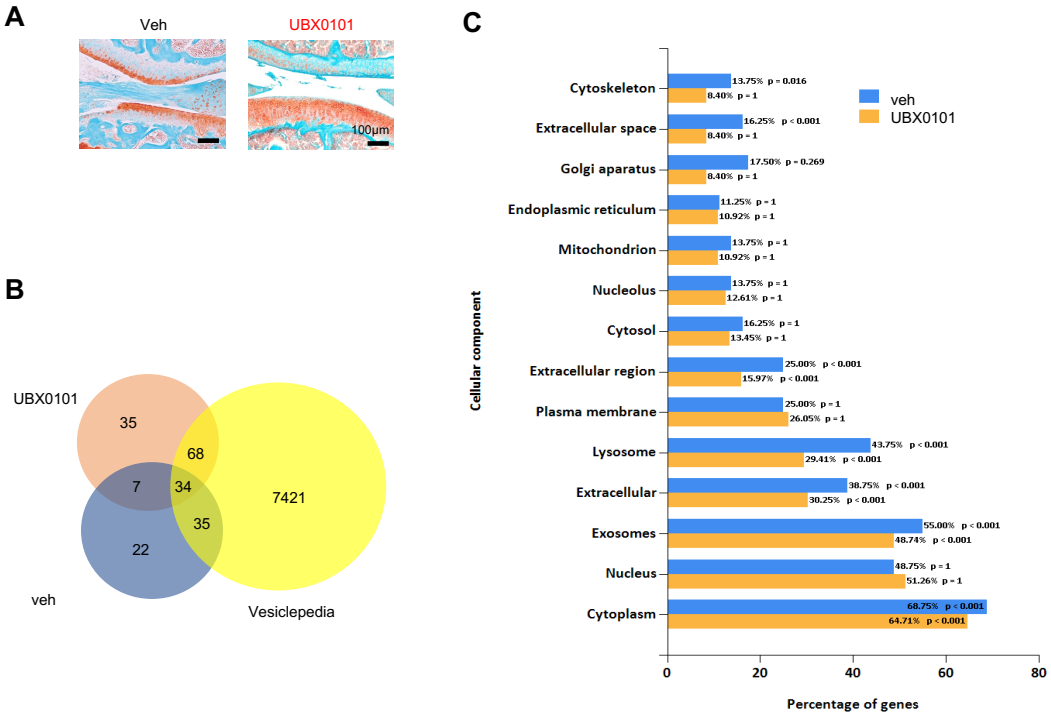
Supplemental Figure 1. SnCs impair cartilage ECM production of neighboring human chondrocytes through secreted factors. (A) Gating strategy to sort senescent human OA chondrocytes from mixed senescent and non-senescent cultures based on size and auto-fluorescence by flow cytometry. Middle panel: representative images of SA-β-Gal activity in cultures with high, medium (med) or low SnCs. Right panel: Experimental design for **C-D** describing co-culture of non-senescent (NS) and senescent chondrocytes or conditioned medium from high, med, and low SnC co-cultures. Control cultures contained only non-senescent chondrocytes. (B) Conditioned media (CM) from NS or high SnCs were analyzed by human cytokine antibody arrays (n = 4 per group). (C) Quantification of mRNA levels for *CDKN2A*, *CDKN1A*, *MMP3*, *IL1B* and *COL2A* normalized to *ACTB* expression (n = 3 per group). In B and C, data are means ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to NS or NS CM as determined by a two-tailed t-test (unpaired). (D) Safranin-O staining of the non-senescent chondrocytes after 7 days of co-culture. (E) Representative images of SA-β-Gal, Safranin-O, and Alcian blue staining of NS 7 days after incubation with CM derived from high, med, and low SnCs. (F) Quantification of SA-β-Gal positive SnCs of high, med and low sized population from which CM was collected. The Safranin-O and Alcian blue stained areas in NS 7 days after an incubation with the CM and were quantified using Image J software (NS, low and high, $n = 3$; med, $n = 6$). per group). Data are means ± SEM. *$P < 0.05$, **$P < 0.01$, as determined by 1-way ANOVA and Tukey's multiple-comparisons test.



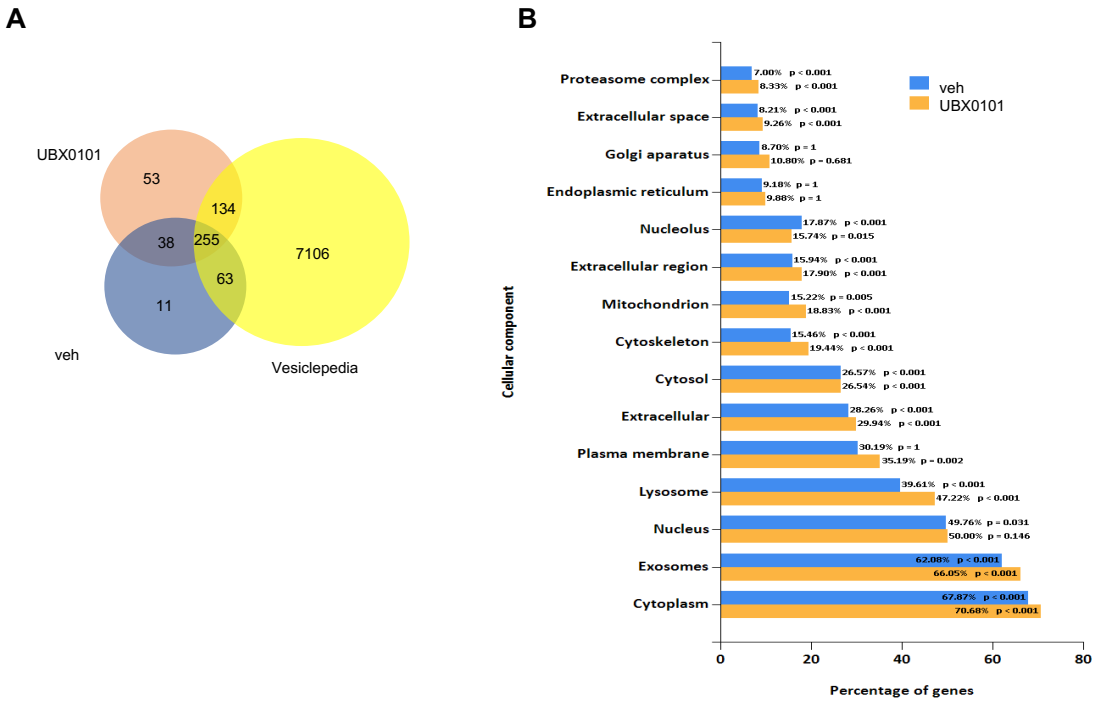
Supplemental Figure 2. Transmission of senescence to non-senescent chondrocytes by EVs from populations containing high, medium (med) and low levels of senescent chondrocytes. (A) Schematic showing the isolation protocol to obtain EVs from CM. (B) SA-β-Gal positive cells and (C) Safranin-O stained areas were quantified in non-senescent chondrocytes (NS) 6 days after incubation with different number of EVs derived from non-senescent chondrocytes or populations containing high, med or low SnCs. In B, data of each replicate is shown as colored dots. In C, data are means ± SEM (n = 3 ~ 6 per each EV number).



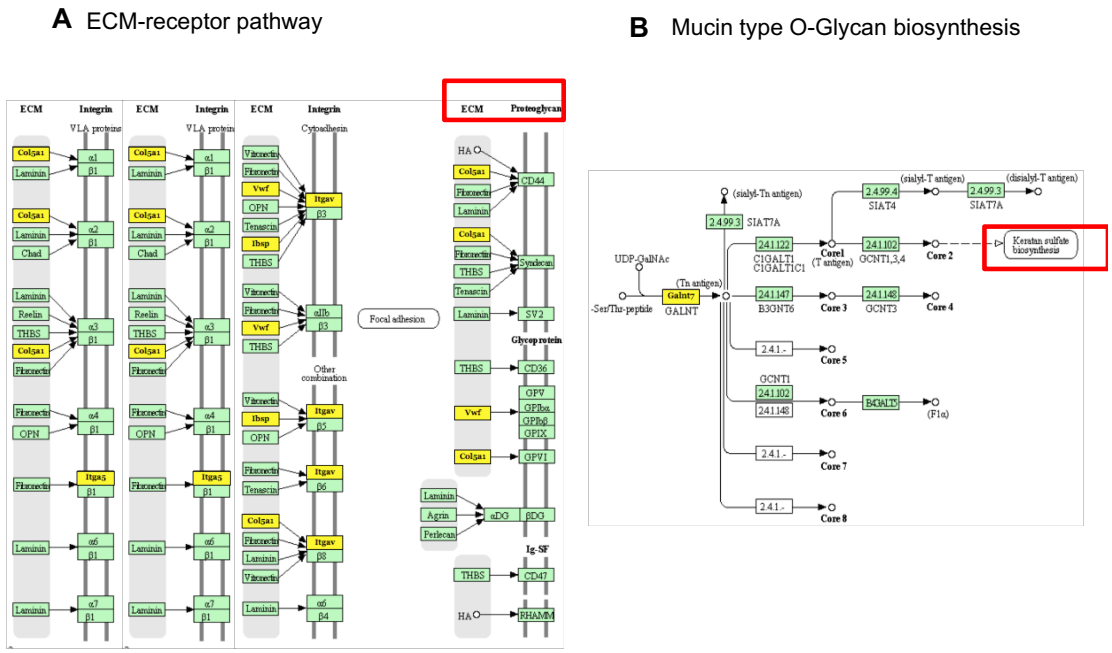
Supplemental Figure 3. EV-derived miRNAs differentially present in human synovial fluid from OA patients. (A) Mean size of EVs (x axis) versus concentration of EVs in synovial fluid (y axis), measured by NTA. There was no significant difference in either measurement between EVs from OA donors ($n = 2$) and EVs from normal donors ($n = 3$). (B) The length distribution of mappable reads, centered at 20 nt, thus illustrating that the miRNA sequencing is reliable (total number of mappable reads: 27,419,206). (C) Venn diagram of the target genes of hsa-miR-199a-5p, -185-5p, -23b-3p and -27b-3p, predicted by DIANA-microT-CDS (v5.0). (D) Heatmap showing statistically significant correlations among the 4 miRNAs differentially expressed between human healthy and OA synovial fluid derived-EVs and the pathways they are thought or known to mediate. The heat map illustrates p-values (log scaled) determined by DIANA-mirPath (v3.0).



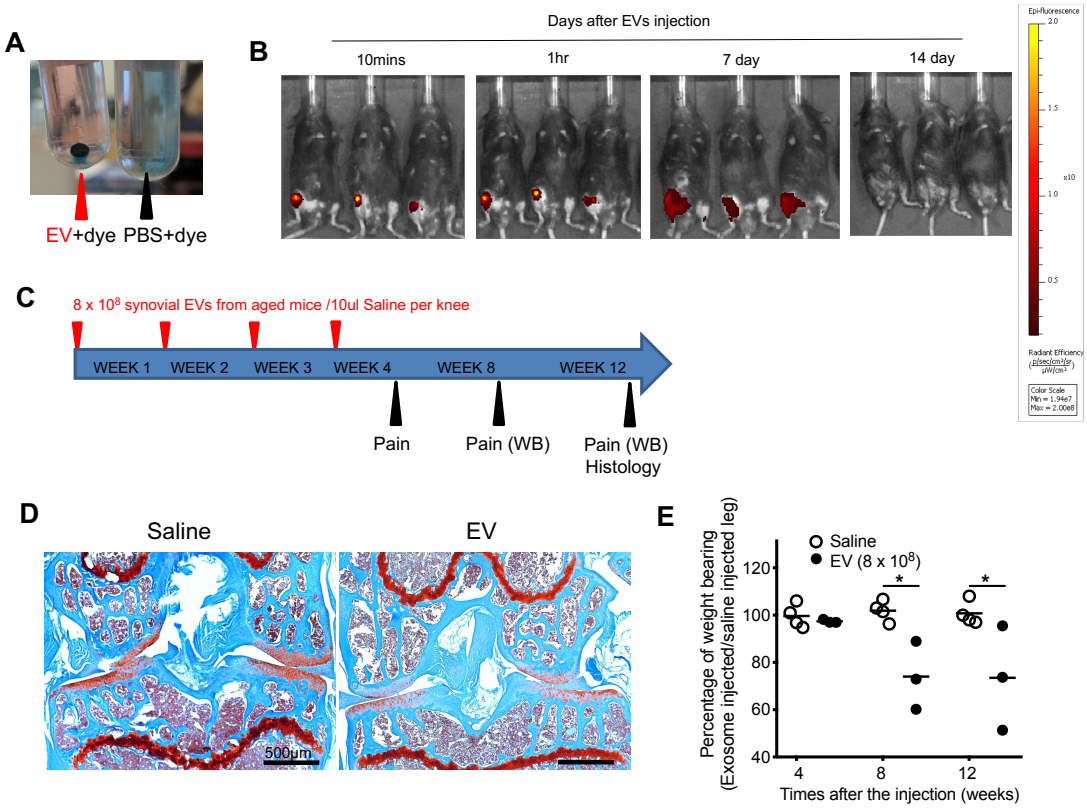
Supplemental Figure 4. The proteomics analysis of synovial EVs derived from young OA mice joints. (A) Representative images of Safranin-O and methyl green staining in articular cartilage of young PTOA mice-treated with vehicle (veh) or the senolytic UBX0101. (B) Venn diagram comparing proteins identified by proteomics of EVs enriched in synovial fluid of young OA mice treated with Veh or UBX0101 and datasets previously reported by vesiclepedia. (C) Gene ontology (GO) analysis of proteins extracted from synovial EVs derived from Veh or UBX0101-treated young OA mice joints compared with Vesiclepedia data to show the cellular origin of proteins by using FunRich software, an open access standalone tool that uses to analyze EV data set of proteins (http://funrich.org/faq).



Supplemental Figure 5. Proteomics characterization of synovial EVs derived from aged OA mice joints. (A) Venn diagram of synovial EV-associated proteins matched to records in the EV databases vesiclepedia. (B) Percentage of proteins in synovial EV enrichments using FunRich. EV enriched proteins represent the 62% or 66.05% of total identified proteins in synovial EVs collected from aged OA mice joints treated with Veh or UBX0101.



Supplemental Figure 6. Mapping of genes targeted within cartilage ECM formation-related pathway by the differentially expressed EV-derived miRNAs after SnCs removal. The map of (A) "ECM-receptor interaction" and (B) "Mucin type O-glycan biosynthesis" pathway mediated by mmu-miR-24, miR-30c, miR-150, miR-92a and miR-125a. In this map, yellow target gene could be regulated by 5 miRNAs to devote to new cartilage ECM formation (proteoglycan and keratin sulfate as a sulfated glycosaminoglycans; highlighted in red box) the joints after the SnCs removal (adapted from the diagram generated by DIANA mirPath v.3 web tool).



Supplemental Figure 7. Administration of EVs derived from synovial fluid of aged (20 months old) mice induce OA-like pathology in young mice (3 months old). (A) The EV pellets were labeled using a lipophilic near-infrared dye. (B) To determine how long injected EVs from aged mouse synovial fluid persisted in the articular joint space, fluorescently labeled EVs were injected IA and tracked by xenolight DIR/IVIS imaging. (C) Schematic of the time course for the experiments in **D-E**. To investigate whether aged mouse synovial fluid-derived EVs induce OA pathology in young mice, $8x10^8$ EVs in 10 µl saline were injected once per week for 4 weeks. (WB; Weight Bearing test) (D) Representative images of joints stained with Safranin-O and methyl green in articular cartilage from saline and EV injected young mice on day 84 after injection; saline, $n = 3$; EV, $n = 3$. Scale bars, 500 µm. (E) The percentage of weight placed on the ACL transected limb versus the contralateral control limb was evaluated as indicated. All data are expressed as means, and each data point represents an individual mouse. One-way ANOVA with Tukey's multiple-comparisons test was used for statistical analysis (saline, $n = 4$; EV, $n = 3$). $^*P < 0.05$.