Supplemental figures and figure legends

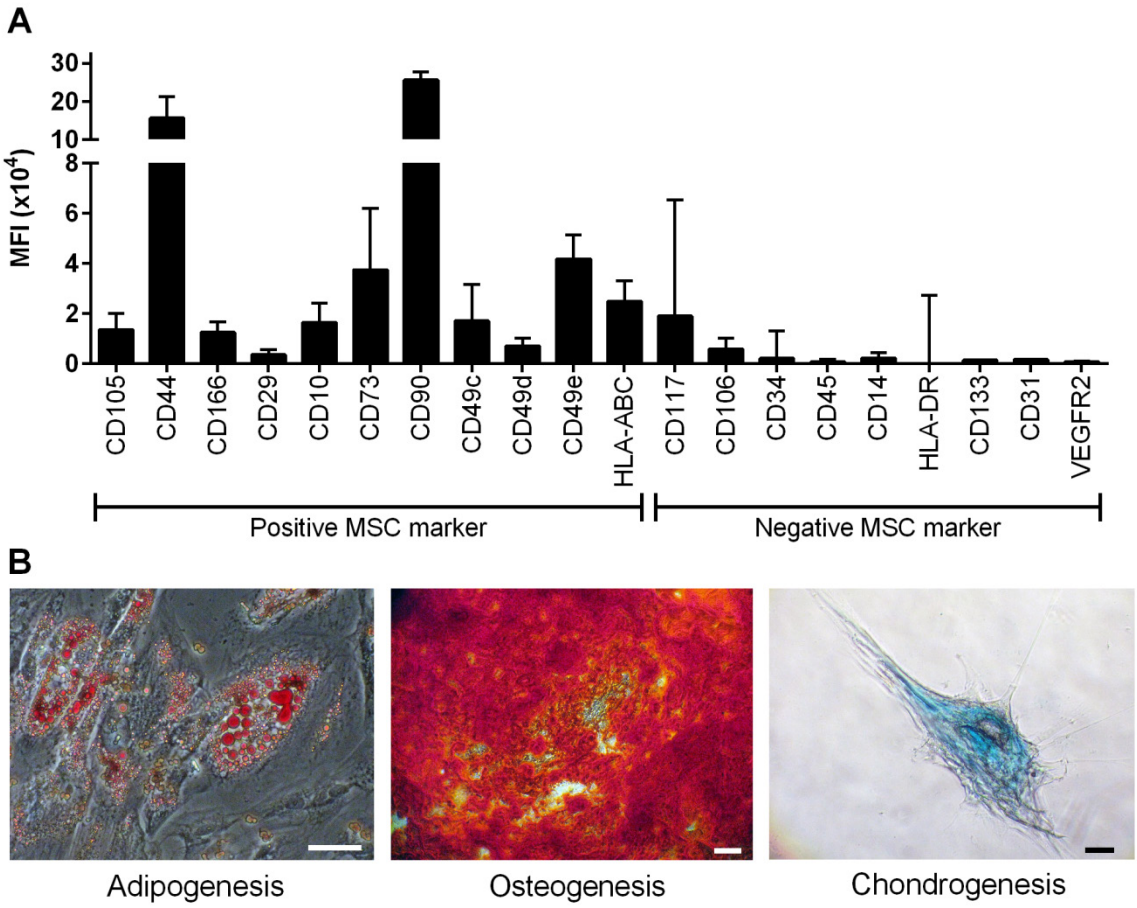
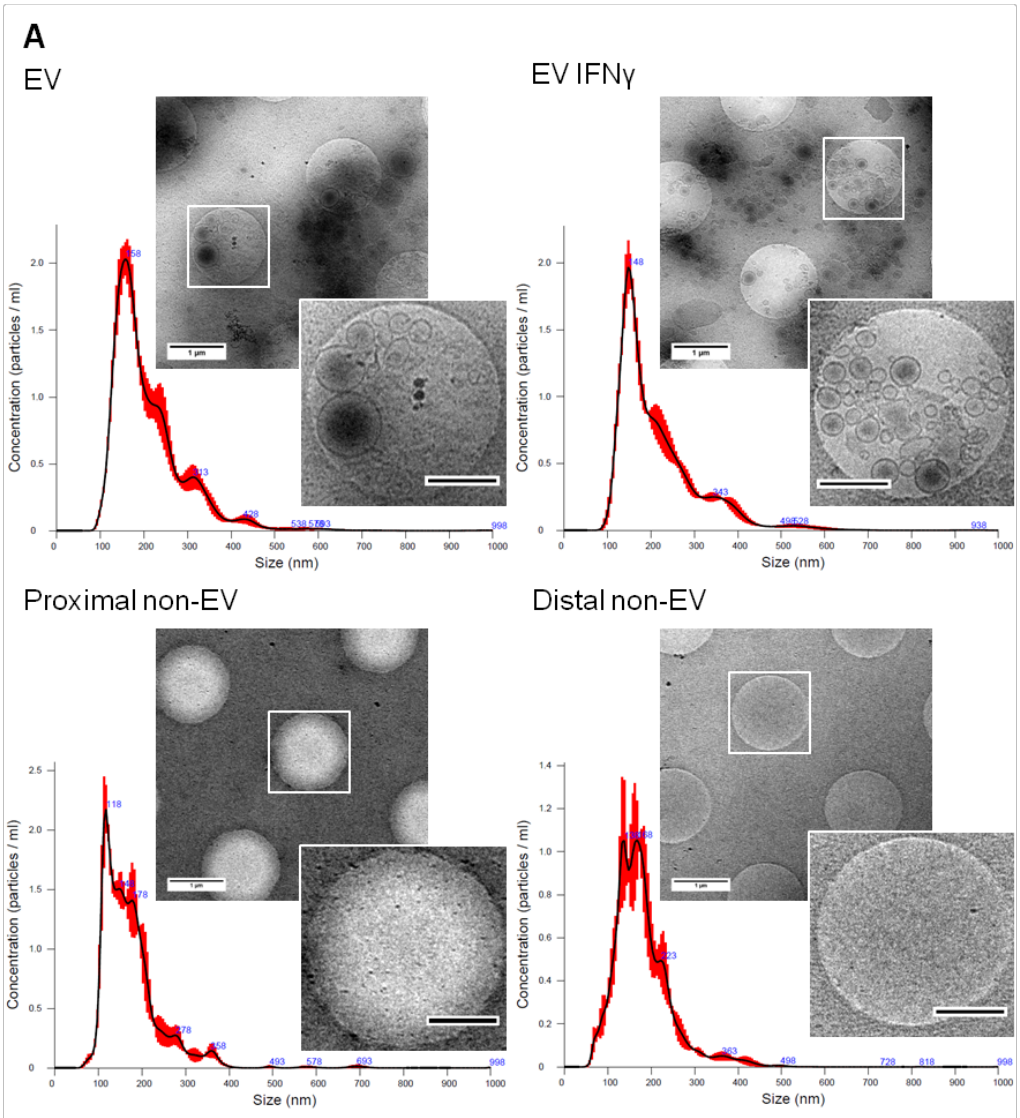


Figure S1. Characterization of UCMSCs by flow cytometry and differentiation assays. (A) UCMSCs were consistently positive for the common MSC markers CD105, CD44, CD166, CD10, CD73, CD90, CD49c, CD49d, CD49e and negative for CD117, CD106, CD34, CD45, CD14, CD29, CD133, CD31 and VEGFR2. Bars represent the mean ± SD of the total cell population's MFI minus the isotype-matched MFI, of seven different UCMSC donors. **(B):** UCMSCs were successfully differentiated towards an adipogenic, osteogenic and chondrogenic lineages. Images show cell cultures after staining, from left to right, lipid droplets with Oil red O, calcium with Alizarin red S, and proteoglycans with Alcian blue. Scale bars = 100 μm.

Related to Figure 1.



B

Sample	Diameter (nm)		Concentration
	Mean ± SD	Mode ± SD	particles/ml ± SD
EV	228.2 ± 25.6	169.6 ± 19.6	$5.51 \cdot 10^{10} \pm 1.14 \cdot 10^{10}$
EV IFNγ	238.7 ± 21.2	157.2 ± 12.5	$3.46 \cdot 10^{10} \pm 7.04 \cdot 10^{9}$
Proximal non-EV	168.4 ± 20.7	124.3 ± 24.3	$3.60 \cdot 10^{10} \pm 2.50 \cdot 10^{10}$
Distal non-EV	179.8 ± 20.6	155.1 ± 10.5	$2.54 \cdot 10^{10} \pm 3.82 \cdot 10^{8}$

Figure S2. UCMSC-EVs presence was confirmed by NTA and Cryo-EM in the EV fraction and absence in proximal and distal non-EV fractions obtained in SEC. (A): NTA profiles (size [nm] versus concentration [particles/ml]) and their corresponding Cryo-EM images of the pooled SEC fractions. Numerous EVs were observed in the EV-fractions from both untreated and IFNγ-primed UCMSCs (left and right, upper images), while proximal and distal non-EV fractions were found completely free of particles (left and right, bottom images). The grid's holes, measuring 1.5µm in diameter, can be clearly distinguished as brighter circles, and are shown in close-up images of the indicated areas. Scale bars = 1µm and 500nm in open field and inserts, respectively. **(B)**: Modal, mean size and mean quantification of particles found in pooled SEC fractions according to NTA analysis. Data of three independent experiments.
Related to Figure 2.

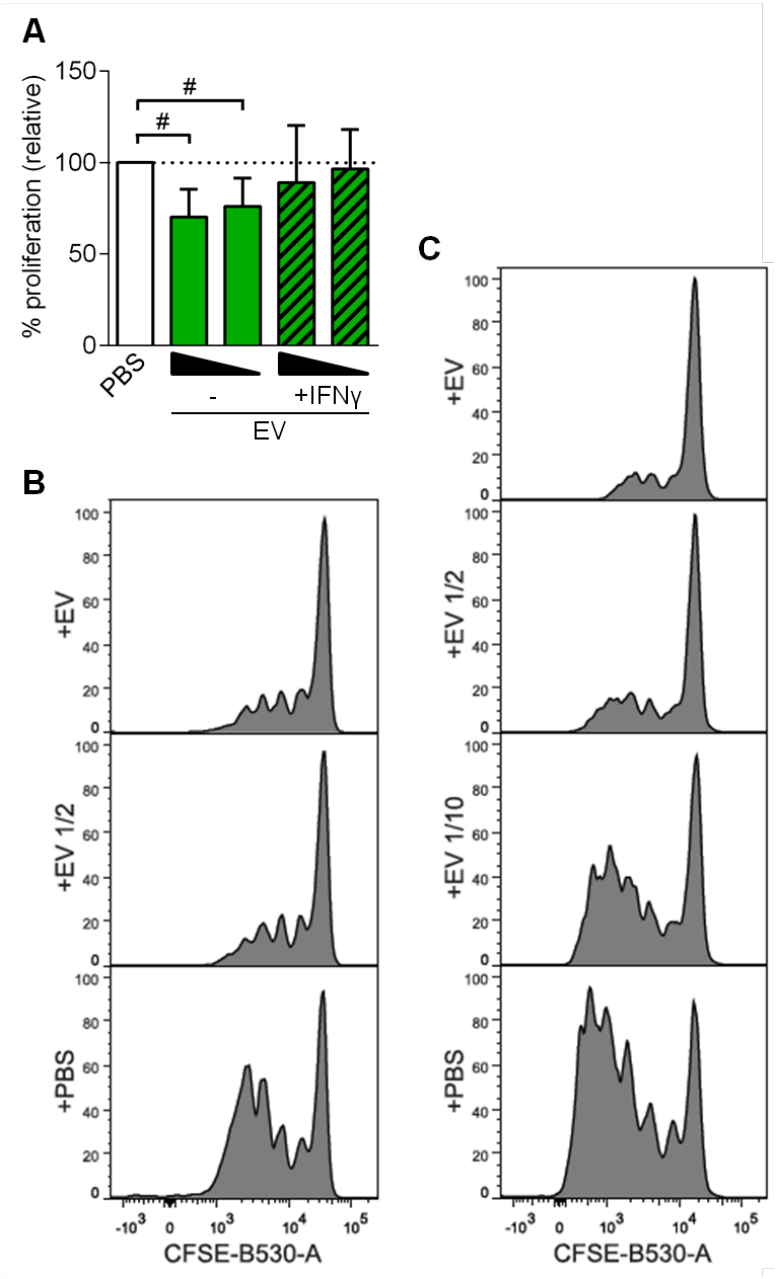


Figure S3. Inhibition of T cell proliferation by EVs is dose-dependent. T cells were stimulated with anti-CD2CD3/CD28 coated microbeads (10:1 ratio) and cultured in the presence of UCMSC-EVs corresponding to $2.5x10^5$, $1.25x10^5$ (1/2 dilution in PBS) or $2.5x10^4$ (1/10 dilution in PBS) initial UCMSCs, without (**A-B**) or after vacuum concentration (**C**). **(A):** Data represents mean ± SD of proliferation relative to the PBS control of seven and three independent experiments of unconditioned and IFNγ-conditioned UCMSC-EVs, respectively. Statistical differences are #$p<0.05$ by Wilcoxon Signed Rank test. **(B, C):** Representative histograms of proliferation analysis by CFSE loss. Generations can be clearly distinguished as CFSE-intensity peaks.

Related to Figures 3 and 5.

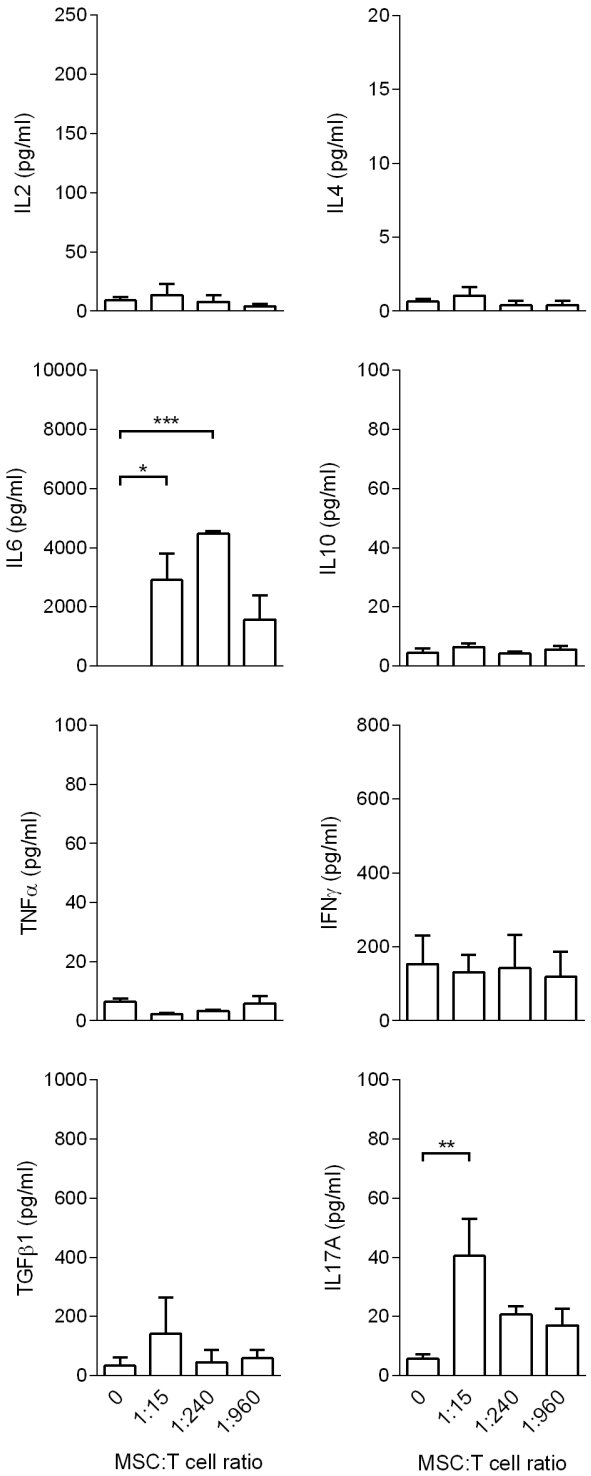


Figure S4. Cytokines found in the supernatants of T cell proliferation assays corresponding to Figure 1D were analyzed by CBA (IL-2, IL-4, IL-6, IL-10, TNF-α, IFNγ and IL-17A) and TGF-β1 ELISA. Bars represent means ± SD of cytokines produced by 50,000 cells, from three independent experiments. Statistical differences are indicated for groups with *p<0.05, **p<0.01 and ***p<0.001 by One-way ANOVA with Tukey's post hoc analysis.

Related to Figure 1 and 4.

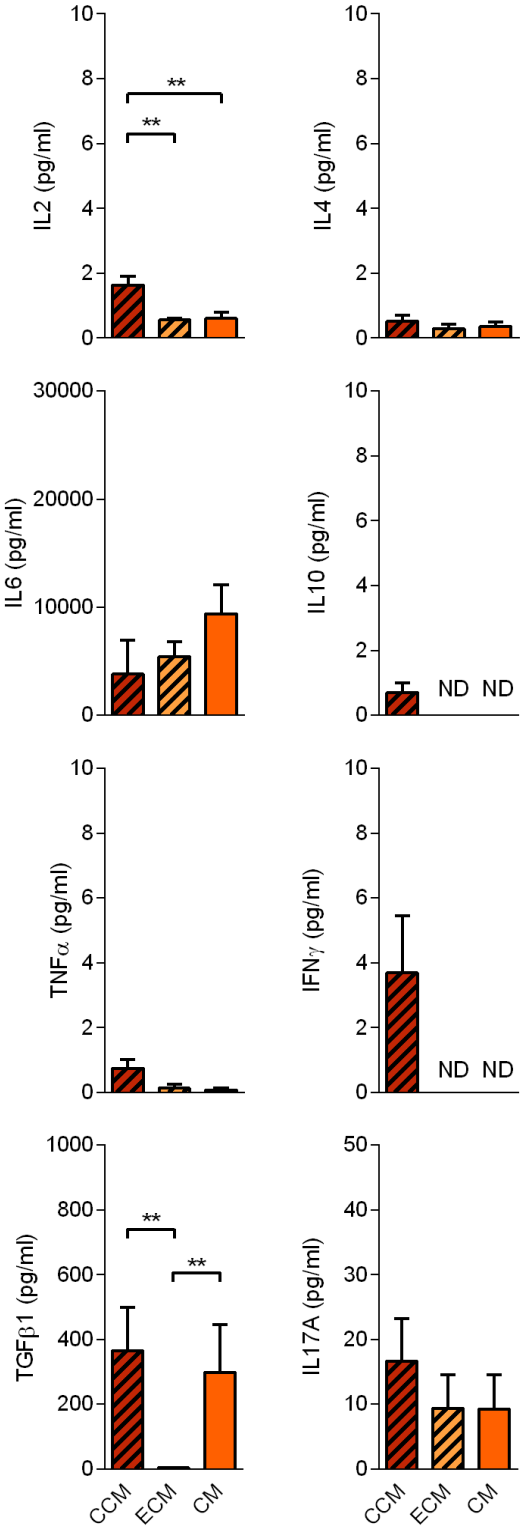


Figure S5. Cytokines found in the CCM, ECM and CM were analyzed by CBA (IL-2, IL-4, IL-6, IL-10, TNF-α, IFNγ and IL-17A) and TGF-β1 ELISA. Bars represent means ± SD of five independent experiments. Statistical differences are indicated for groups with $p<0.05$ by Kruskall-Wallis; *$p<0.05$ and **$p<0.01$ by Mann-Whitney test. ND= not detected.

Related to Figure 4.

Table S1. Primers used for real time PCR:

		Sequence (5'-3')	Tm [ºC]	%GC	Amplicon size [bp]
18S	Forward	TCTTTCTCGATTCCGTGGGT	58.74	50	145
	Reverse	TCTAAGAAGTTGGGGGACGC	59.39	55	
CD80	Forward	CTGCCTGACCTACTGCTTTG	58	55	77
	Reverse	GGCGTACACTTTCCCTTCTC	58	55	
CD163	Forward	CACCAGTTCTCTTGGAGGAACA	59	50	82
	Reverse	TTTCACTTCCACTCTCCCGC	59	55	
CD206	Forward	ACACAAACTGGGGGAAAGGTT	59.99	47.62	174
	Reverse	TCAAGGAAGGGTCGGATCG	58.8	57.89	