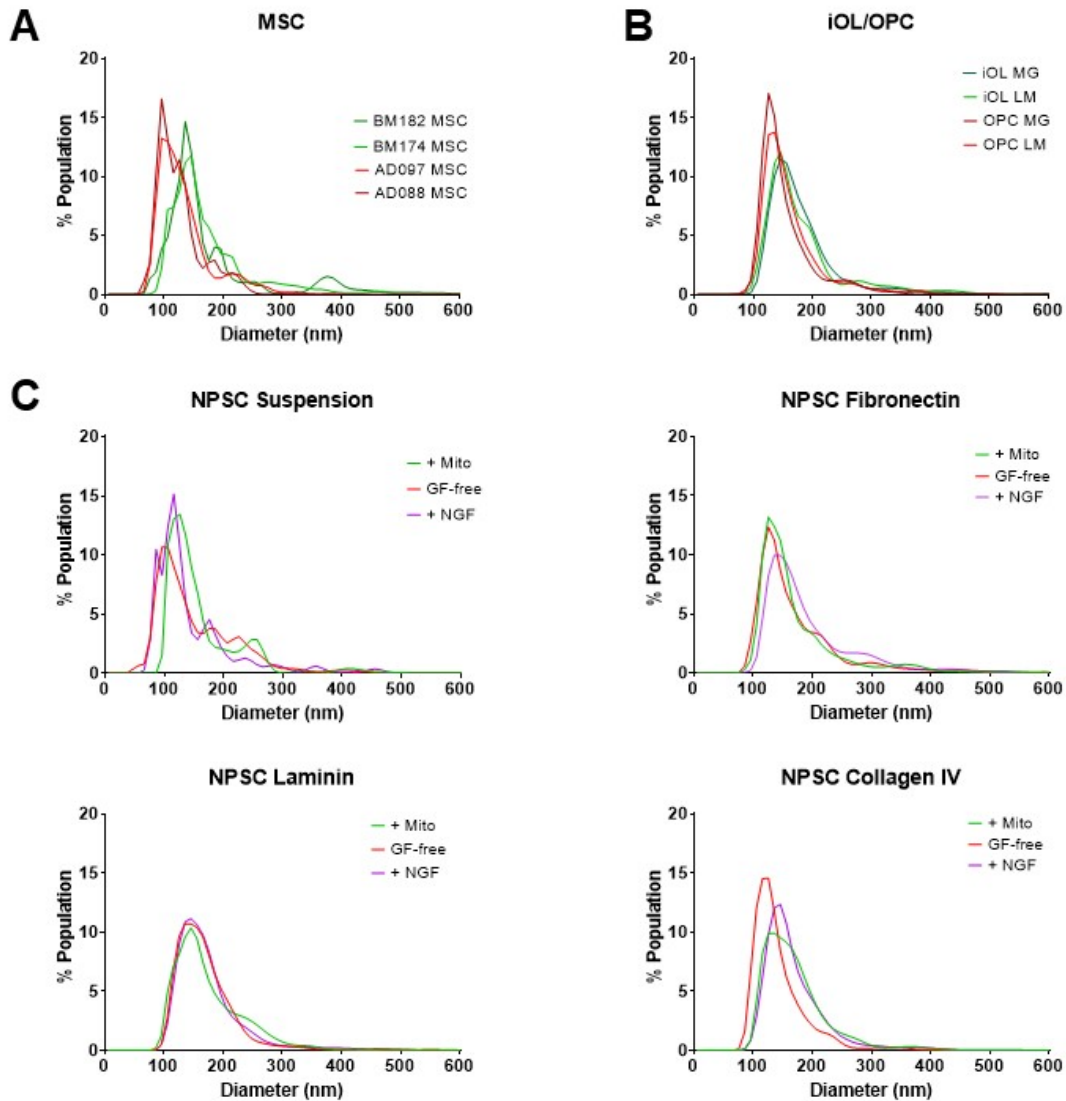
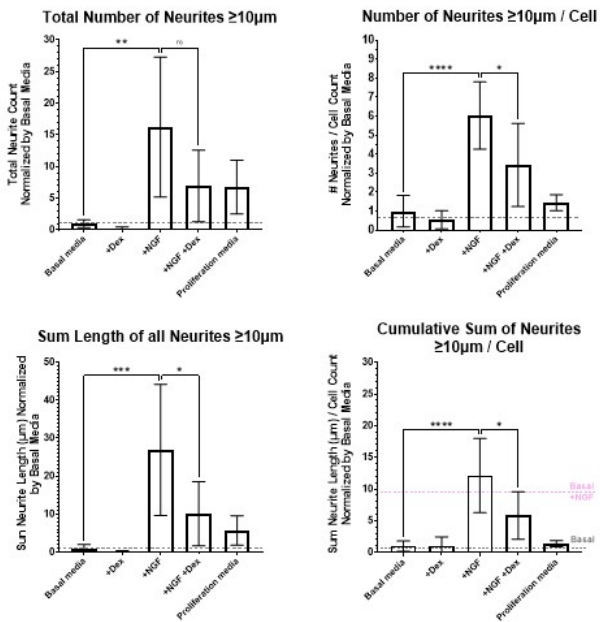
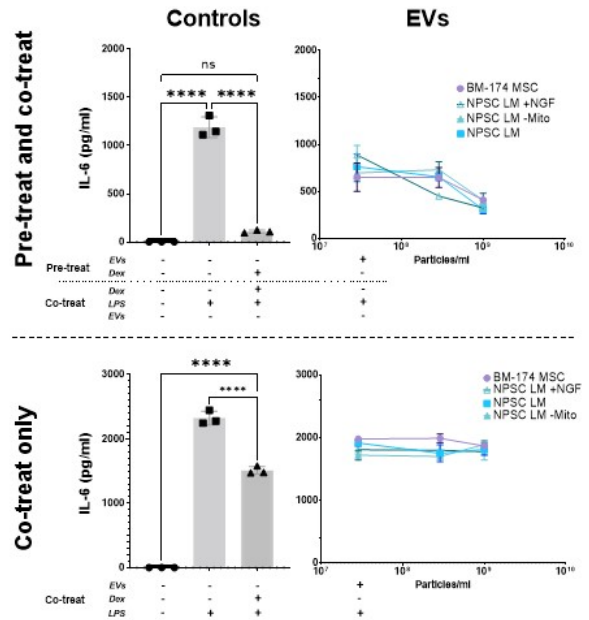


Culture Conditions				Average NTA concentration in EV isolate (particles/ml)	Mean Diameter ± error (nm)	Avg Particle count/Parent Cell	Total EV Isolate Protein Content (mg)/Cell		
Cell Type	Culture	Integrin	Growth Factor						
NPSC	Suspension		EGF/bFGF	2.66E+10	153.2 ± 6.4	1.31E+02	1.28E-08		
					9.42E+10	151.9 ± 8.0	8.01E+01	1.27E-08	
					2.10E+11	147.8 ± 5.2	1.60E+02	1.54E-08	
	Adherent	LM		EGF/bFGF	2.77E+10	144.5 ± 5.1	3.90E+02	6.53E-08	
						2.44E+11	171.2 ± 1.8	3.46E+03	7.25E-08
						2.37E+11	175.6 ± 1.3	1.47E+03	4.27E-08
		MG		EGF/bFGF	2.10E+10	152.9 ± 2.8	4.54E+02	6.80E-08	
						2.91E+11	165.7 ± 2.7	7.29E+03	1.64E-07
						3.20E+10	177.3 ± 1.7	7.18E+02	1.94E-07
		ColIV		EGF/bFGF	6.89E+10	171.4 ± 1.1	1.30E+04	1.15E-07	
						3.06E+11	144.1 ± 3.3	1.04E+04	3.17E-07
						2.70E+11	171.4 ± 1.1	5.50E+03	1.32E-07
FBN		EGF/bFGF	2.39E+10	171.7 ± 2.1	4.65E+02	9.55E-08			
				2.00E+11	176.3 ± 0.8	4.84E+03	1.57E-07		
				3.64E+10	195.7 ± 5.8	4.85E+02	7.88E-08		
OPC	Adherent	LM	OMM	3.41E+10	164.5 ± 2.0	1.67E+03	1.19E-07		
		MG		1.97E+10	157.2 ± 2.0	8.41E+02	8.82E-08		
iOL	Adherent	LM	ODM	5.96E+10	184.7 ± 1.6	1.53E+03	1.20E-07		
		MG		1.06E+11	182.5 ± 2.7	2.30E+03	1.58E-07		
MSC AD061	Adherent			1.59E+10	128.4 ± 1.1	5.08E+02	4.22E-08		
MSC AD088				5.47E+10	127.6 ± 5.2	7.69E+02	5.00E-08		
MSC AD097				6.71E+10	134.6 ± 2.2	7.97E+02	5.37E-08		
MSC BM174				3.50E+10	173.8 ± 5.5	5.11E+02	1.12E-07		
MSC BM180				6.56E+10	167.8 ± 3.1	7.90E+02	7.38E-08		
MSC BM182				4.93E+10	185.7 ± 12.9	6.15E+02	3.42E-08		

Supplemental Table 1. EV characterization and yields. Nanoparticle tracking analysis and bicinchoninic acid assay were used to determine per cell EV yields (particle count and protein content) and EV sample concentrations and mean size across all culture conditions tested.

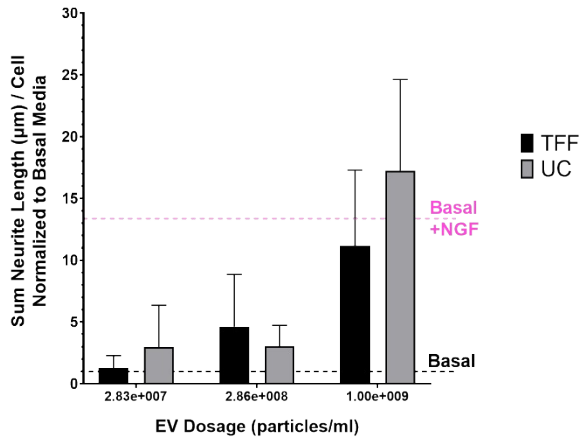


Supplemental Figure 1. EV characterization. Nanoparticle tracking analysis of EVs from **A)** MSCs from different patient donors and tissue sources (bone marrow or adipose), **B)** iOLs and OPCs grown on Matrigel or laminin, and **C)** NPSCs grown in suspension or on fibronectin, laminin, or collagen IV and in the presence or absence of growth factors (EGF/bFGF) or NGF.

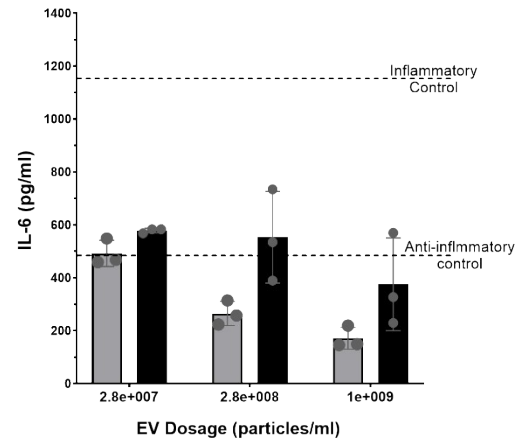
A**B**

Supplemental Figure 2. PC-12 neurite outgrowth assay and RAW264.7 immunoassay development. **A)** Several methods for data analysis were compared and cumulative sum of neurites $\geq 10\mu\text{m}$ normalized by cell count was selected for its ability to maximize dynamic range while controlling for cell count. **B)** Pre- and co-treat vs co-treat only regimens were compared and only pre- and co-treat (upper panel) revealed anti-inflammatory effects of EVs.

UC vs. TFF for Fibronectin+NGF NPSC EVs



EV Isolation Method Does Not Significantly Alter Immunosuppressive Bioactivity of NPSC EVs



Supplemental Figure 3. Bioactivity of NPSC EVs is similar between isolation with ultracentrifugation (UC) and tangential flow filtration with 100kDa membrane (TFF). EVs from NPSCs cultured on fibronectin with NGF were evaluated in A) PC-12 neurite outgrowth assay and B) RAW 264.7 mouse macrophage immunoassay.