Supplementary Material

Raman spectroscopy as a quick tool to assess purity of extracellular vesicle preparations and predict their functionality

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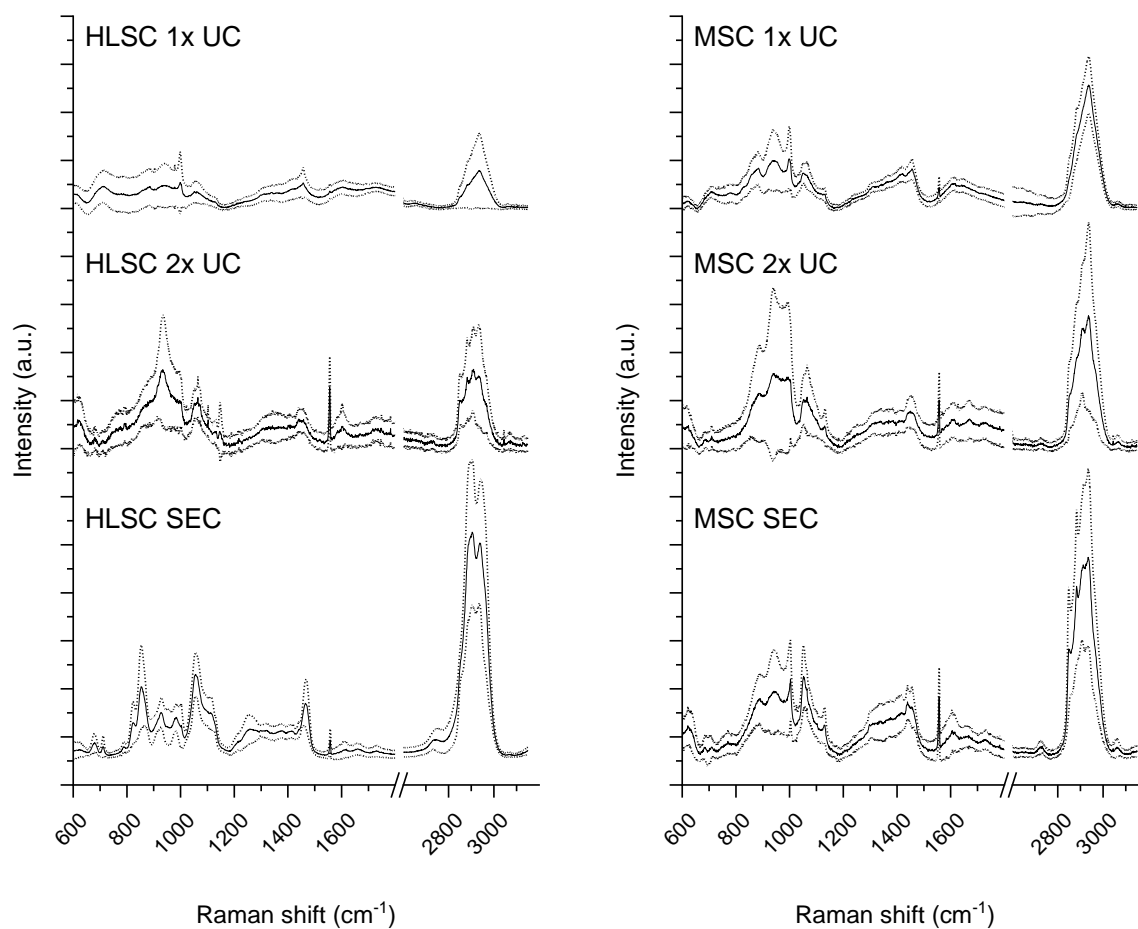
Supplementary Figure 1. Raman spectra of EV samples. Mean Raman spectra obtained on air dried drop of EV samples isolated by 1x UC, 2x UC and SEC protocols from HLSC and MSC supernatants. All spectra were baseline corrected, aligned and normalized before averaging. Dotted lines indicate standard deviation of the signal intensities (at least 60 spectra per sample).
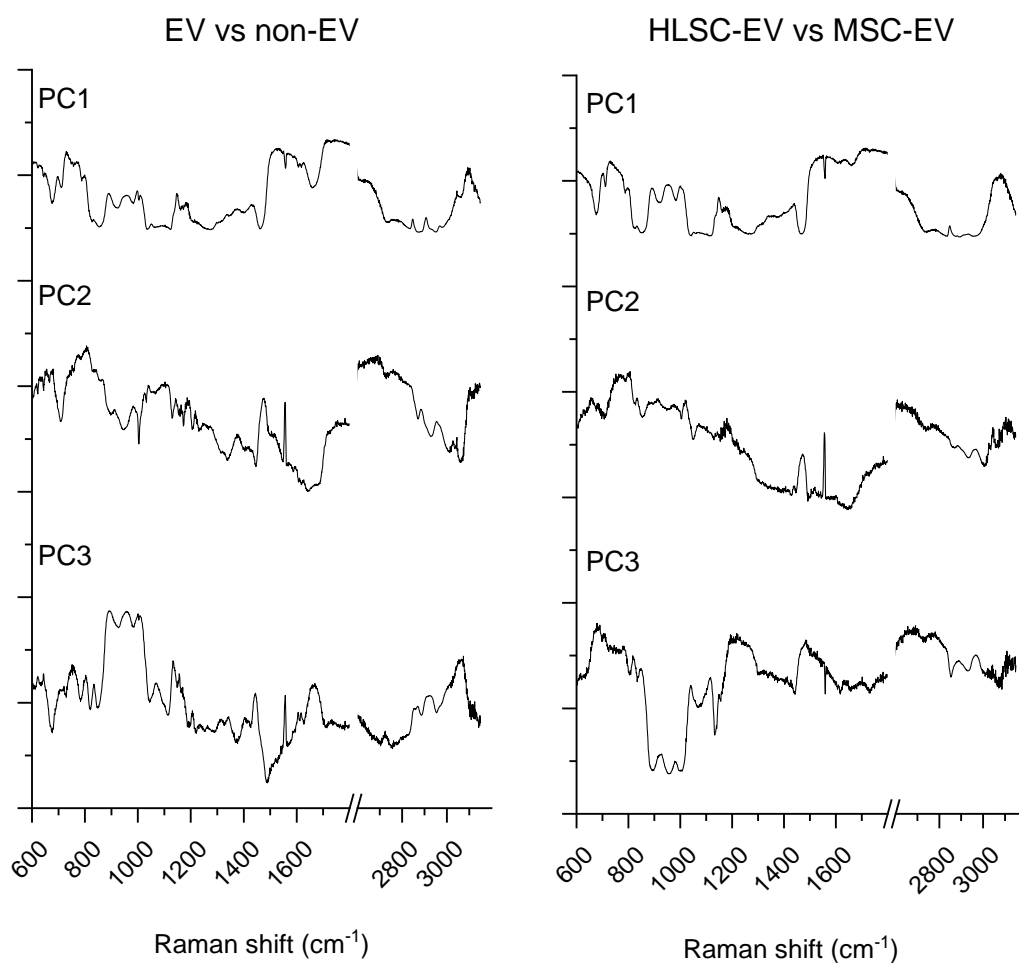
Supplementary Figure 2. Principal Component Analysis (PCA). Loadings of the Principal Component (PC) obtained after multivariate PCA. Left panel: PC1, PC2 and PC3 obtained after PCA analysis of the EV and non-EV Raman spectra non considering the cell source for the analysis, describing the 34.8%, 13.97% and 9.6% of total variance. Right panel: PC1, PC2 and PC3 obtained after PCA analysis of the EV Raman spectra from HLSC and MSC by 1x UC, 2x UC and SEC protocols, describing the 37.1%, 13.4% and 9% of total variance.

cm^{-1}	HLSC-EV			MSC-EV			Origin
	SEC	2x UC	1x UC	SEC	2x UC	1x UC	
679	x	x		x	x	x	*Nucleic acids*
710-713	x	x	x	x	x	x	*Phospholipids*
788	x	x		x			*O-P-O stretching in nucleic acids*
825				x			*Phosphodiester*
855				x			*Pro, Tyr*
928-940	x	x		x	x	x	*Proteins (Pro; Hydroxy-Pro)*
1003	x	x	x	x	x	x	*Phe*
1053-1055	x	x	x	x	x	x	*C-O and C-N stretching in proteins and lipids*
1065	x	x		x	x		*Chain C-C stretching in lipids*
1127-1130	x	x		x	x	x	*C-N stretching of proteins and phospholipids*
1200-1300	x	x	x	x	x	x	*Amide III*
1450	x	x	x	x	x	x	*CH$_2$ and CH$_3$ deformations in proteins and lipids*
1455-1457	x	x	x	x	x	x	*Nucleic acids*
1465				x			*Lipids*
1556.6	x	x	x	x	x	x	*Background spectrum: substrate impurity*
1606	x	x	x	x	x	x	*Phe, Tyr ring vibrations*
1615	x			x	x	x	*Tyrosine, tryptophan, C=C (protein)*
1600-1690	x	x	x	x	x	x	*Amide I*
1728	x	x	x	x	x		*Ester C=O stretches (lipids)*
2883	x	x	x	x	x	x	*CH$_2$ asymmetric stretch in lipids and proteins*
2908	x	x		x	x		*CH$_3$ stretching vibrations*
2937-2940	x	x	x	x	x	x	*C-H vibrations in lipids and proteins*

Supplementary Table 1. Main peak assignments. The assignments of the main peaks that can be identified in the Raman spectra from EV samples are reported (Czamara K. et al., Journal of Raman Spectroscopy (2015); Krafft C. et al., Spectrochimica Acta Part (2005); Movasaghi Z. et al., Applied Spectroscopy Reviews (2007))