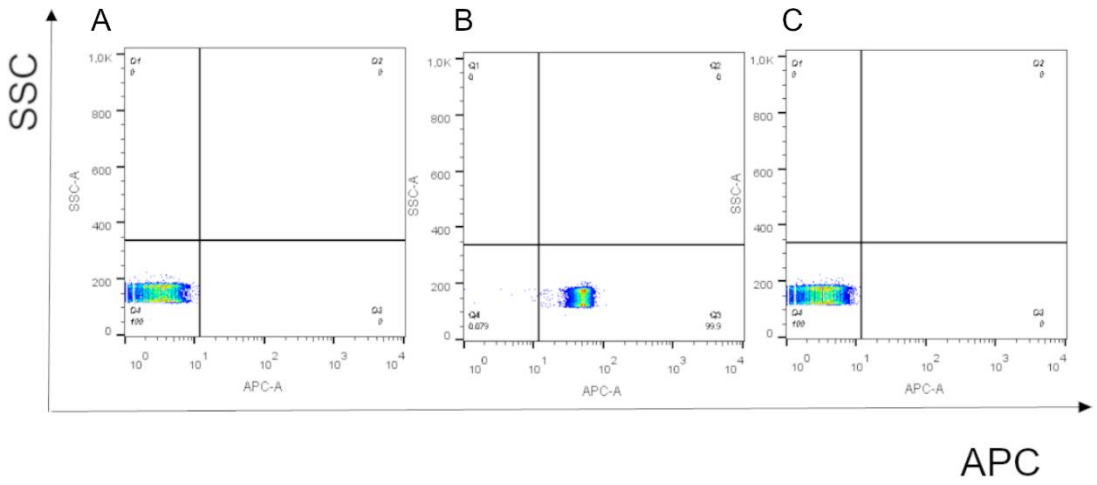
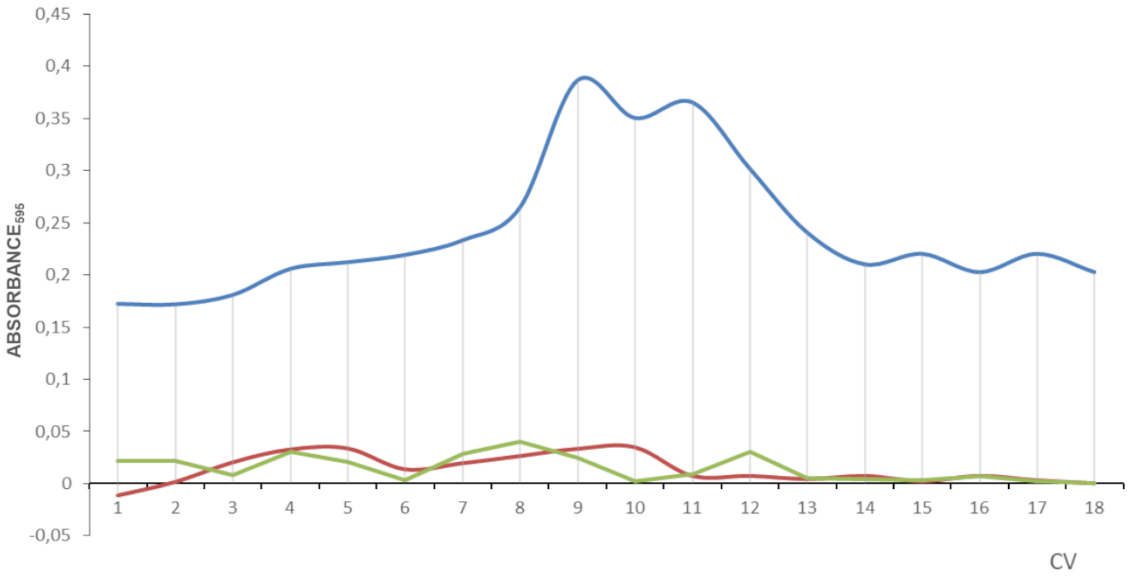
Supplementary material for:

Filipović, L.; Spasojević, M.; Prodanović, R.; Korać, A.; Matijaševic, S.; Brajušković, G.; de Marco, A.; Popović, M. Affinity-Based Isolation of Extracellular Vesicles by Means of Single-Domain Antibodies Bound to Macroporous Methacrylate-Based Copolymer. New Biotechnology 2022, 69, 36–48. https://doi.org/10.1016/j.nbt.2022.03.001.



Supplementary figure 1. Detection of CD9 in EV isolated from conditioned cell culture medium and milk samples: Latex beads coated with (B) EVs and (C) milk used in chromatography were analyzed for the presence of CD9. Gates were set according to the values of autofluorescence of naked beads (A)



Supplementary figure 2. Chromatographic separation of EXO-containing fractions present in the human plasma and control samples. EVs present in plasma pool (blue line) were separated using an immunoaffinity resin composed of five different VHH constructs immobilized on methacrylate-based copolymer as described in the Material and Methods. Controls were set-up in as follows. Chromatography was performed in the absence of plasma sample, running only blocking milk buffer (red line). VHHs were conjugated to the matrix, which was blocked with

glycine and milk (Material and Methods). Following the blocking step, the column was washed with PBS until no protein was detectable by Bradford assay (data not shown) after which the column was treated with glycine and the absorbance of the eluted fractions was recorded. Control of the matrix was performed to test whether methacrylate-based copolymer could directly absorb EVs from plasma sample, without the affinity mediation of VHHs (green line). The co-polymer was blocked with glycine and milk as described in the Material and Method section, the plasma sample was loaded, unbound proteins were washed with PBS until no protein was detectable (data not shown) and the residual protein content was eluted by glycine addition. Absorbance of the eluted fractions was recorded at 595 nm.

Supplementary Table 1. Matrix porosimetry data after blocking and VHH immobilization

The results of two independent experiments are shown.

Measured parameter	**Run1**	**Run2**
Total cumulative volume (cm^3/g)	**0.712**	**0.564**
Bulk density (g/cm^3)	**0.928**	**1.077**
Apparent density (g/cm^3)	**2.740**	**2.740**
Total Hg porosity (%)	**66.13**	**60.71**
Total specific surface area (m^2/g)	**43.4**	**35.5**
Average pore diameter 4V/S (nm)	**65.7**	**63.6**
Median pore diameter za V50% (nm) V50 at (cm^3/g)	**81** **0.356**	**84** **0.282**
Modal pore diameter (nm) at dV/dlogD (cm^3/g, D)	**80** **2.415**	**92** **1.223**