Membrane-binding Peptides for Extracellular Vesicles on-chip Analysis

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Supplementary Information

Characterization of HEK cell line UC isolated sample

Particle counting and sizing
Quantification and sizing of particles isolated from the HEK cell line by UC was performed by Nanoparticle Analysis System (NTA).
Figure S1 shows results of the analysis of the UC sample. NTA provided a mean particle size of 203 ± 3 nm and a concentration of 3.94×10^9 particles/mL.

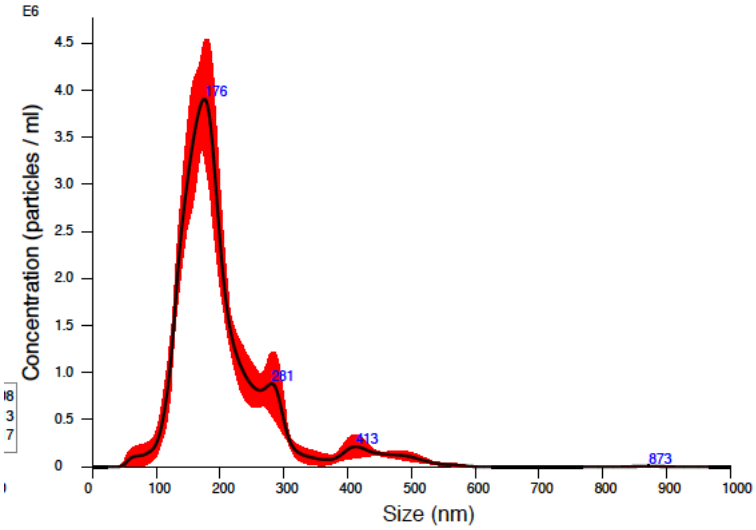

Figure S1: *NTA analysis of the HEK UC isolated sample*

Imaging by TEM
The bulk UC isolated sample was imaged by TEM after staining with uranyl acetate.
Figure S2 shows two different magnification of the UC sample. Lipidic bilayer membrane is clearly visible

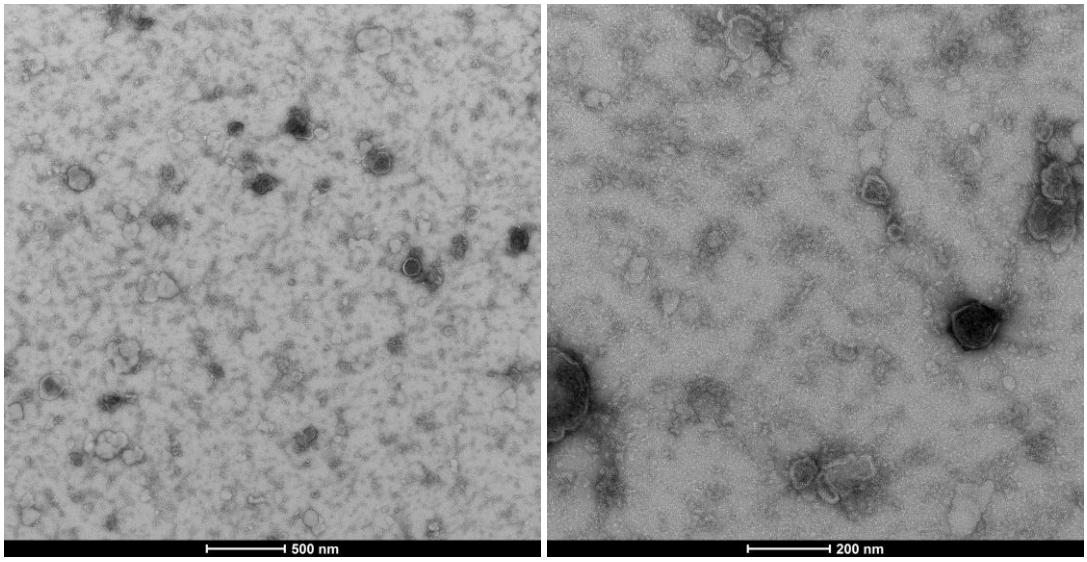

Figure S2: TEM imaging of the UC isolated sample at two different magnifications

Protein analysis by Western Blotting
According to MISEV 2018, to demonstrate the presence of EVs in the UC isolated sample, the presence of transmembrane protein CD63 and CD9 and luminal proteins ALIX and TSG101 was assessed by Western Blotting. The UC preparation resulted positive to all the four proteins (Figure S3)

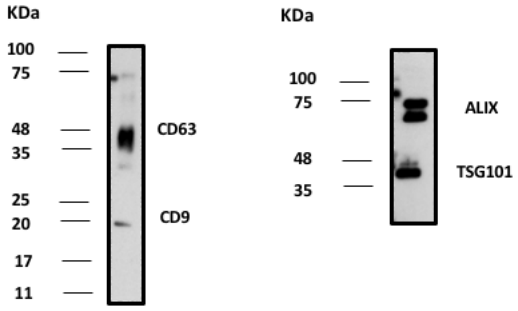

Figure S3: Western Blot analysis of the HEK UC isolated sample. The presence of the transmembrane proteins CD9 and CD63 and the luminal proteins ALIX and TSG101 is verified.

Representative spot images for the entire set of peptides
Representative peptide spot images are provided for BP, BPb, BPt and BPn incubated with $1x10^9$ particles/mL HEK UC sample. Blu dots indicate detected particles. A slight increase in particle binding is visible on the multivalent peptides compared to the linear BP one. The number of particles detected on the negative control BPn is negligible.

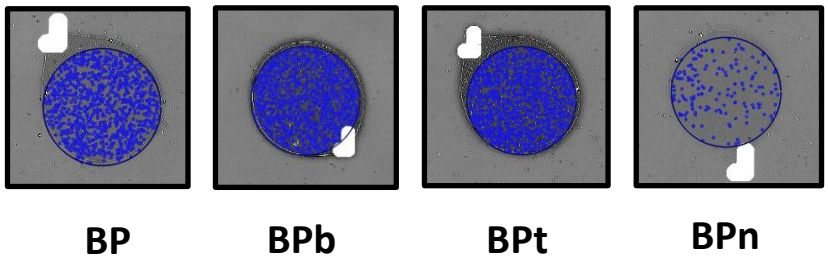

Figure S4: representative spot images for the entire set of peptides. Blue dots indicated detected particles.

Characterization of EVs from human serum isolated by UC and SEC

Particle counting and sizing
Quantification and sizing of particles isolated from the pool of serum sample by UC and SEC were performed by Nanoparticle Analysis System (NTA).
Figure S6 shows results of the analysis of the UC sample. NTA provided a mean particle size of 210 ± 2.5 nm and a concentration of $1.23x10^9$ particles/mL.

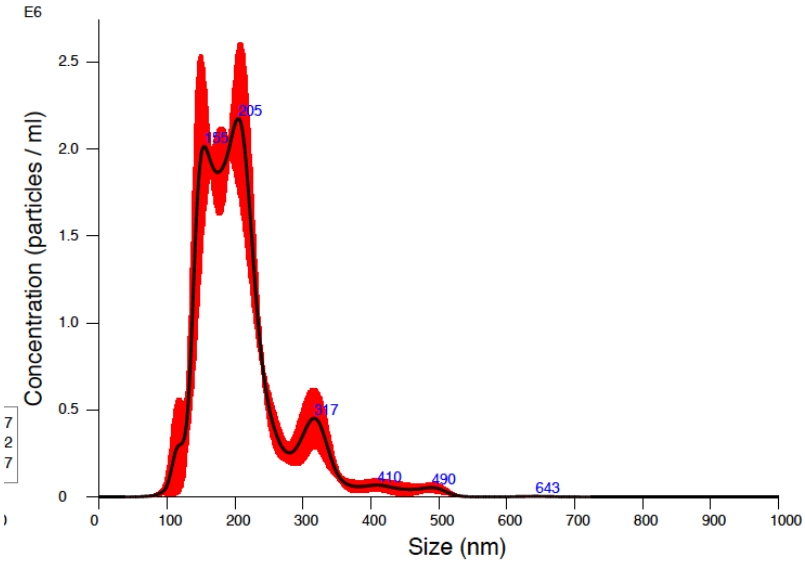

Figure S5: *NTA analysis of the UC isolated sample form human serum*

Figure S6 shows results of the analysis of the SEC isolated sample. NTA provided a mean particle size of 208 ± 2.3 nm and a concentration of $1.27x10^9$ particles/mL.

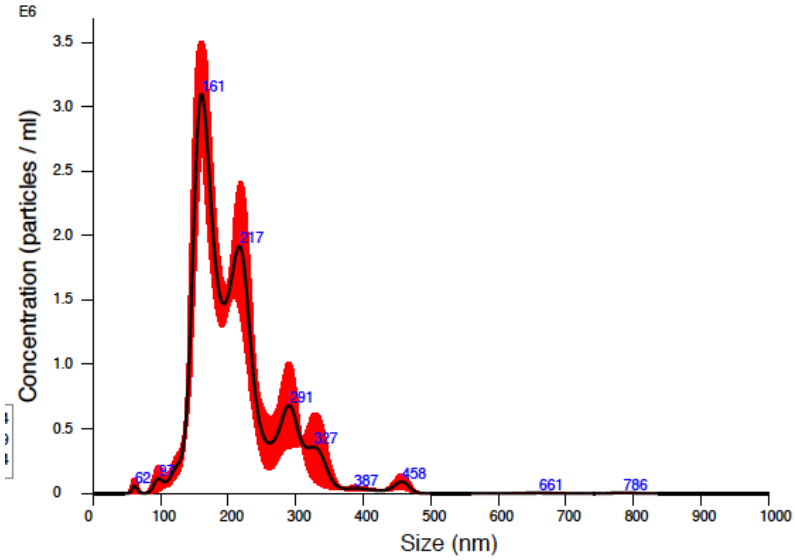

Figure S6: *NTA analysis of the SEC isolated sample from human serum*

Imaging by TEM

The bulk UC and SEC isolated samples from human serum where imaged by TEM after staining with uranyl acetate.

Figure S7 shows two different magnification of the UC sample from human serum

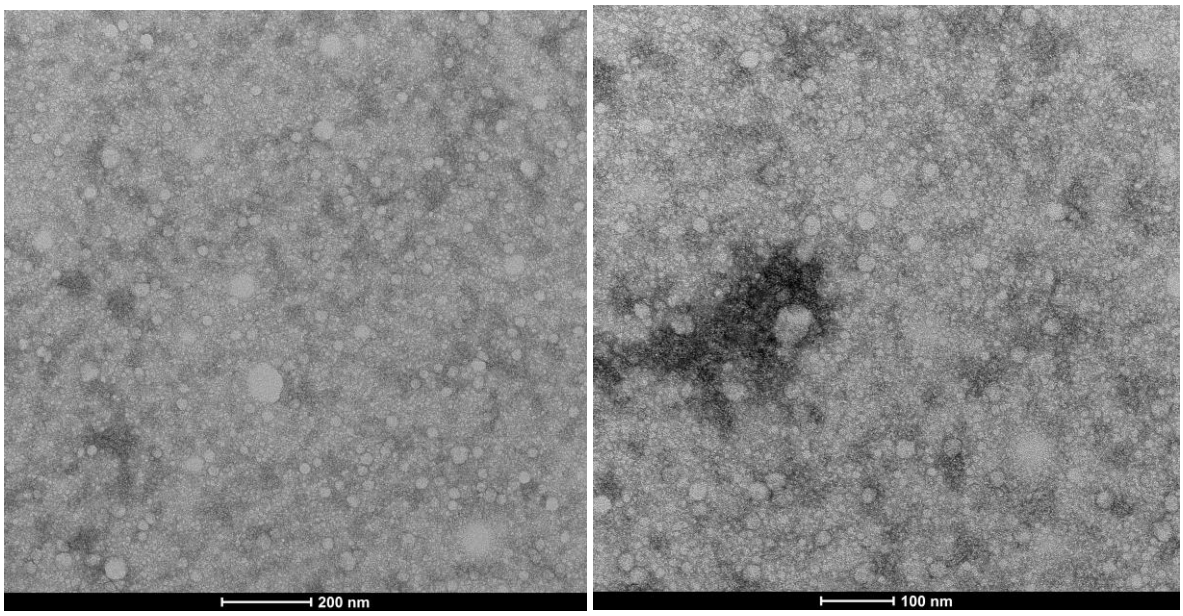

Figure S7: TEM imaging of the UC isolated sample.

Figure S8 shows two different magnification of the SEC sample from human serum

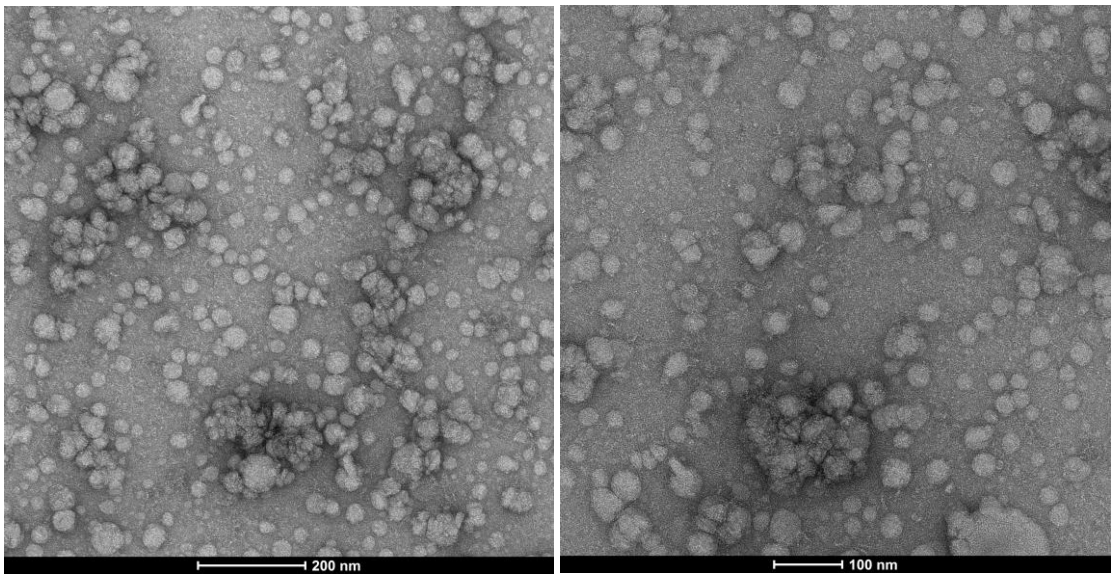

Figure S8: TEM imaging of the SEC isolated sample.

Protein analysis by Western Blotting

According to MISEV 2018, to demonstrate the presence of EVs in UC (A) and SEC (B) isolated samples, the presence of transmembrane protein CD63 and CD9 and cytosolic proteins ALIX and TSG101 was assessed by Western Blotting. The UC preparation resulted positive to all the four proteins whereas the SEC fraction positive to CD63 and TSG101 **(Figure S9)**

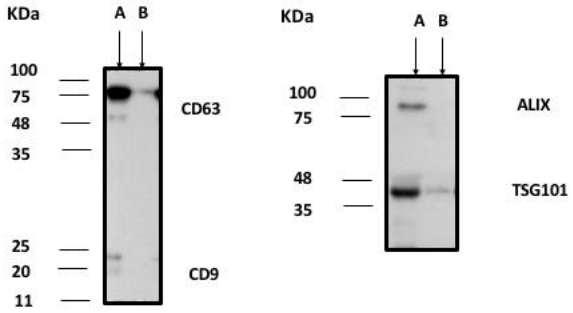

Figure S9: Western Blot analysis of the UC (A) and SEC (B) isolated samples from human serum. The presence of the transmembrane proteins CD9 and CD63 and the luminal proteins ALIX and TSG101 is checked. The UC (A) sample is positive for CD63, CD9; ALIX and TSG101. The SEC (B) sample is positive to CD63 and TSG101.

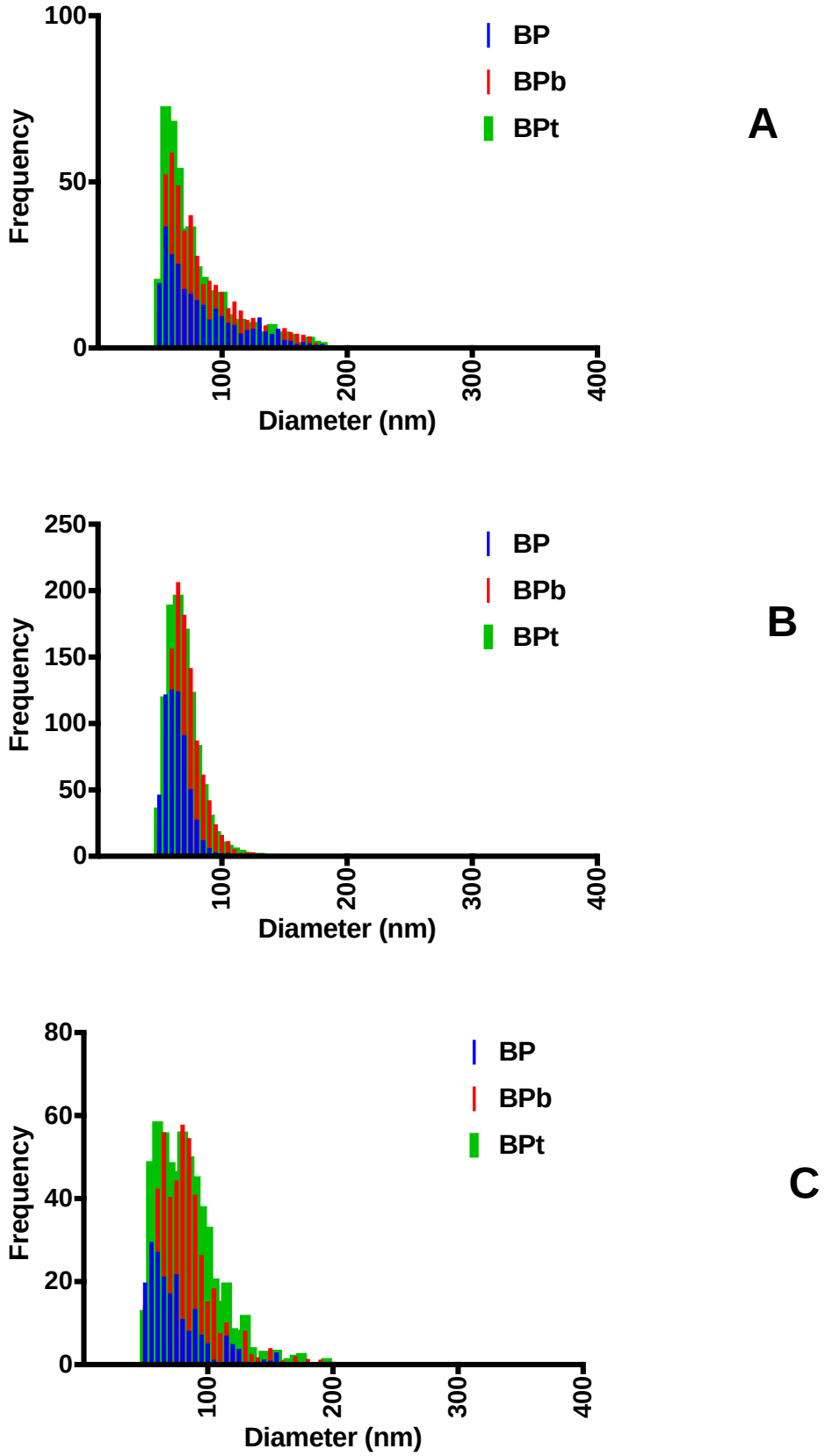

Figure S10: Observed size distribution of captured particles reported as the number of counts detected in each 5nm bin . EVs from pooled human sera captured on BP peptides for A: EVs isolated by ultracentrifugation; B: EVs isolated by SEC; C: un-treated serum

SPRi assay

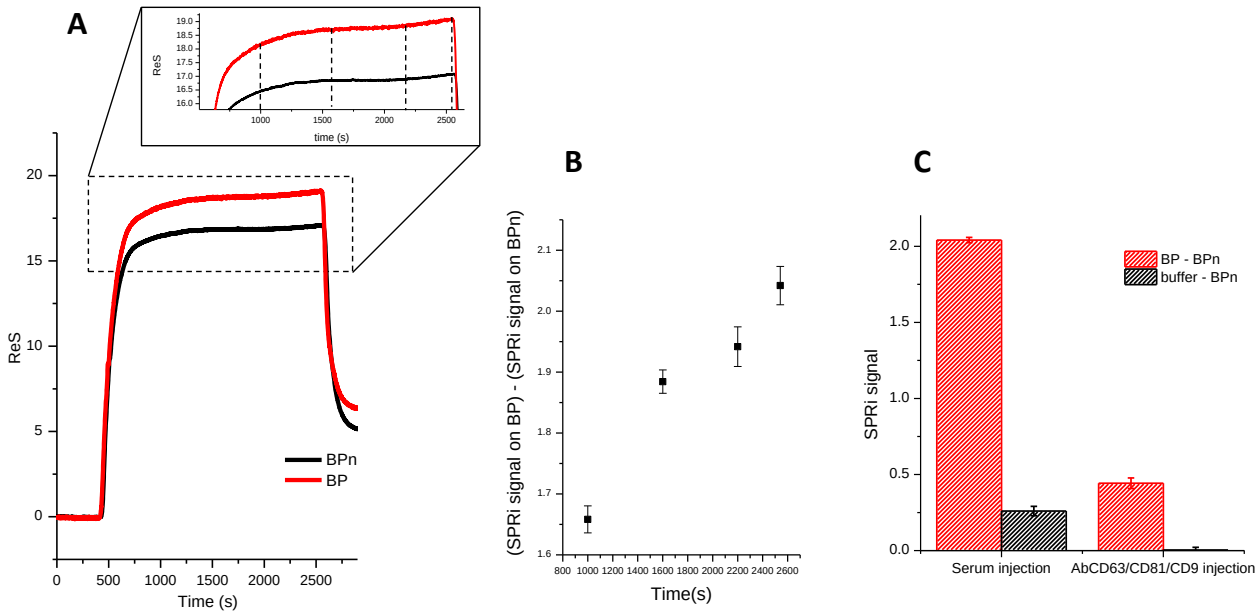

Figure S11
A) SPRi sensorgram related to the injection of serum diluted 1:10 on BP peptide (red line) and BPn (black line); B) Differences in SPRi intensities on BP peptide and BPn during the serum injection, at four different time points (showed in the inset of the figure A, indicating the binding between EVs and the BP peptide spotted on the SPRi biochip; C) SPRi intensities related to the serum injection and the subsequent injection of a mixture of anti-CD63/CD81/CD9 antibodies, collected on BP peptide and on a buffer solution (as negative sample), spotted on the SPRi biochip. The signals confirmed the specific immobilization on BP peptide of EVs carrying CD63, CD81 and CD9.

Oriented immobilization via streptavidin

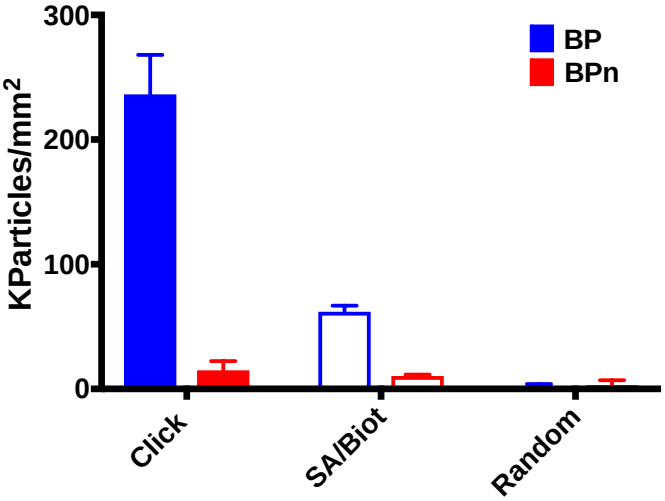

Figure S12: comparison of binding capacity of the SEC isolated EVs from human serum ($1x10^9$ particles/mL) incubated on BP and BPn immobilized by click chemistry, via streptavidin/biotin and by random immobilization

NTA characterization of SEC samples before and after trypsin treatment

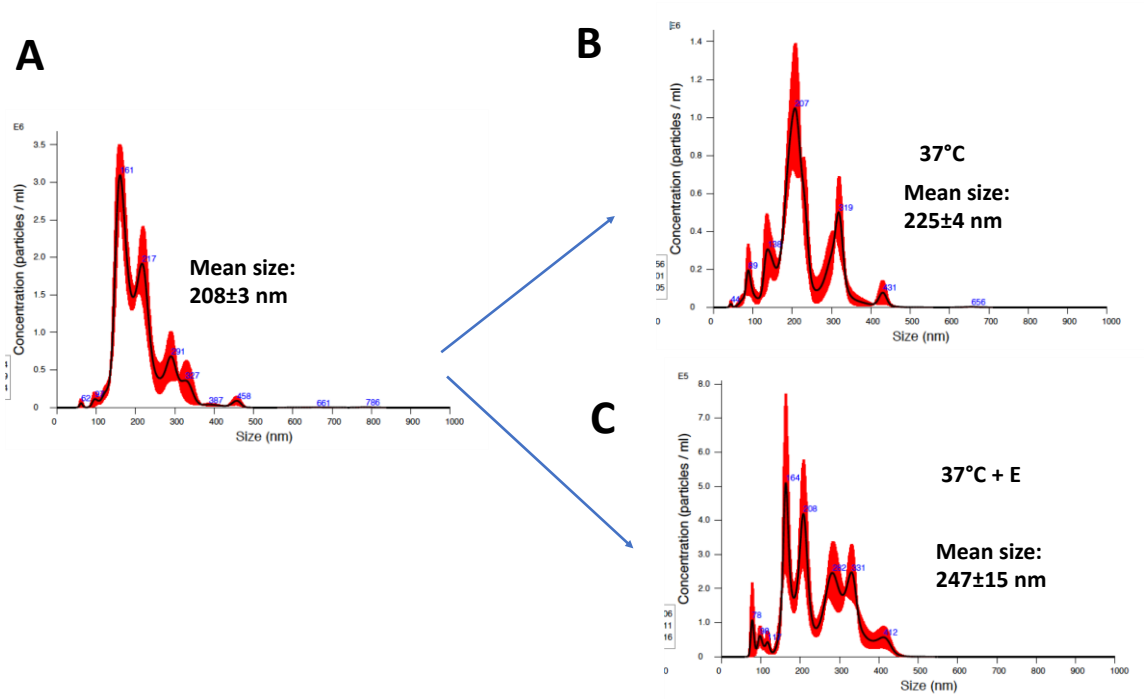

Figure S13: A: SEC isolated EVs from human serum characterized before treatment; B: after 6 hours incubation at 37°C without enzyme.; C: after 6 hours incubation at 37°C in presence of trypsin.