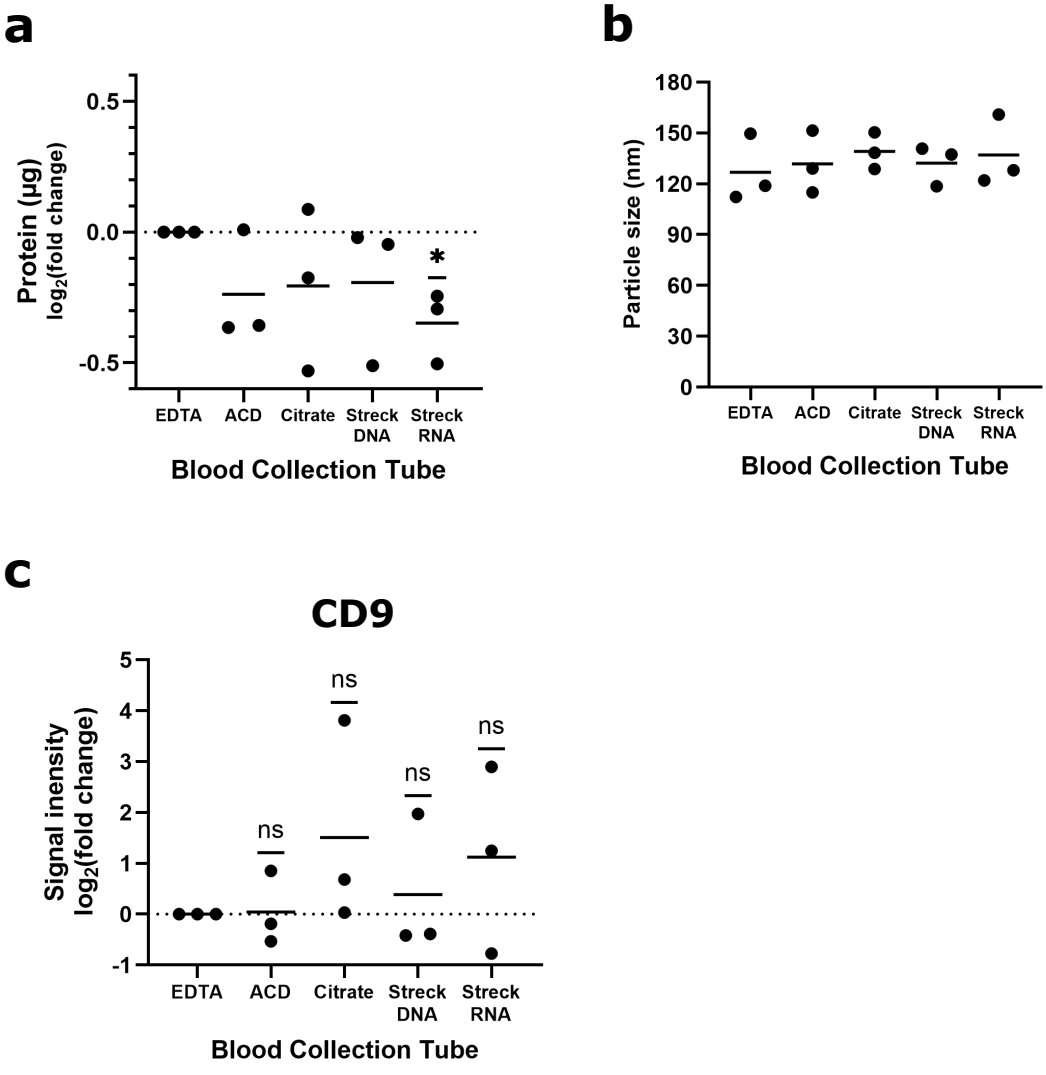


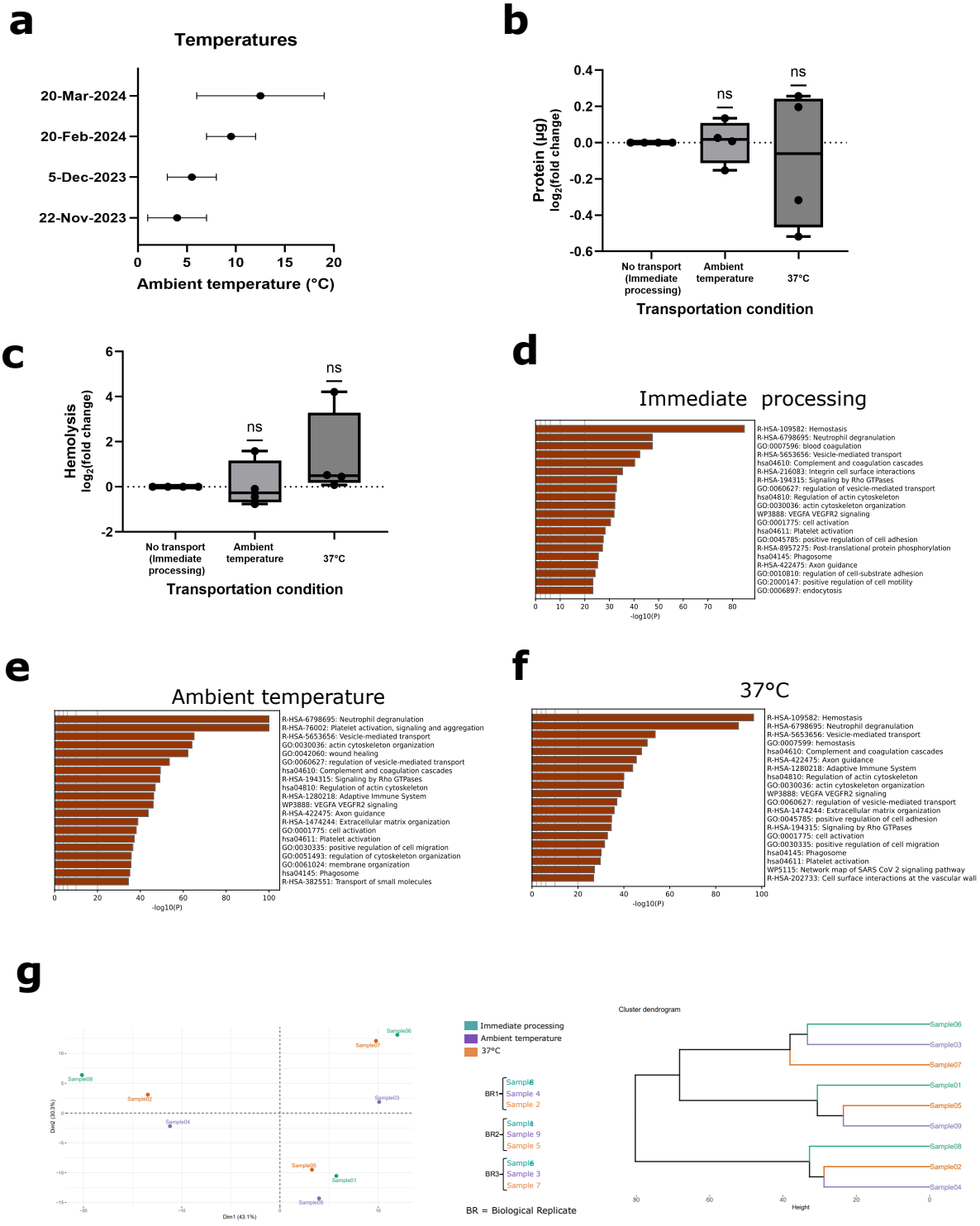
Supplementary Figure S1

Supplementary Figure S1: Blood collection tubes (BCTs) differentially affect isolated EVs

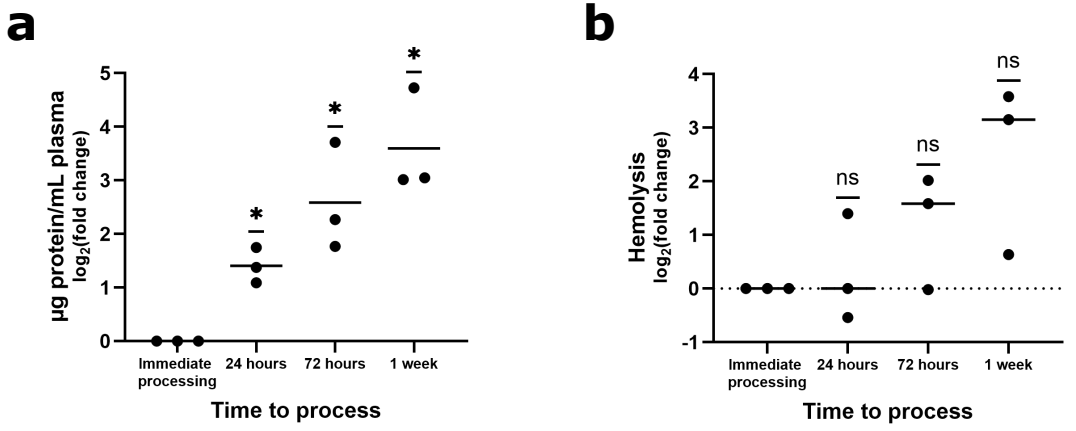
a. Total protein obtained in EV-enriched fractions from each BCT after lysing EV enriched fractions, measured by BCA. Data normalized to the reference BCT (EDTA).

b. Size of the particles recovered in EV-enriched fractions from the different BCTs, measured by NTA.

c. Immunoblotting quantification of CD9. Data in A.-C. is derived from n=3 biological replicates.



Supplementary Figure S2



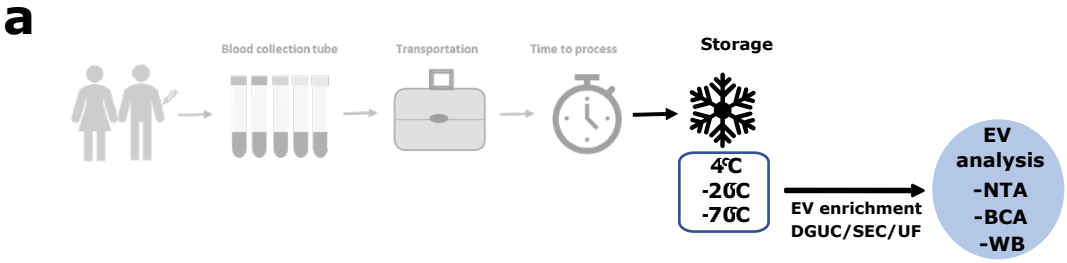
Supplementary Figure S3

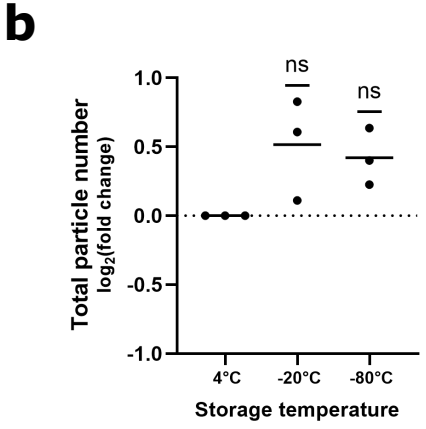
Supplementary Figure S3: Increasing needle-to-processing time induces extraphysiological EV changes

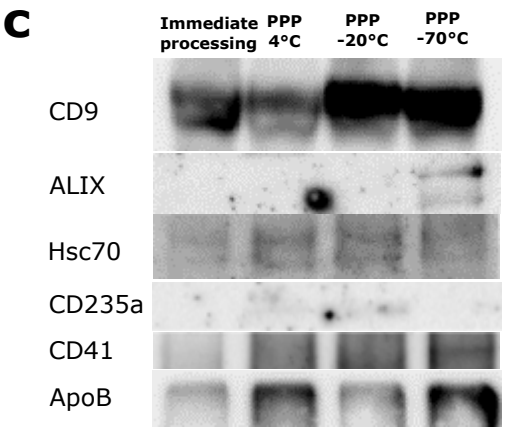
a. EV-derived protein amount per mL plasma at different processing timepoints, measured by BCA (n=3 independent biological replicates).
b. Hemolysis measurement of platelet-poor plasma at different processing timepoints, measured by absorbance at 414nm. Data in A. and B. is normalized to the immediate processing condition (n=3 independent biological replicates).

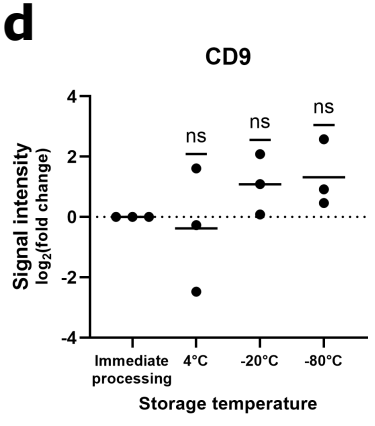
Supplementary Figure S2: Sample transportation temperature influences spectrum of EV-derived proteome

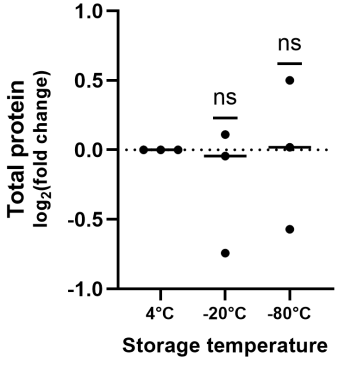
a. Record of the ambient temperatures on the dates of each biological replicate of the transportation experiment.
b. Total protein obtained from each transportation condition after lysing EV-enriched fractions, measured by BCA.
c. Hemolysis of the platelet-poor plasma obtained from the different conditions, measured by absorbance at 414nm.
d-f. Top 20 functional categories identified in the mass spectrometry protein sets of the three transportation conditions according to Metascape enrichment analysis.
g. Mass spectrometry analysis of EV-derived proteins after transportation at different temperatures: principal component analysis (PCA) and hierarchical clustering.

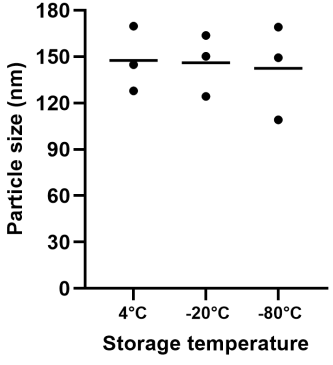












Supplementary Figure S4

Supplementary Figure S4: Storage temperature of platelet-poor plasma does not affect quality of isolated EVs

a. A set of three peripheral blood samples were collected from each healthy volunteer in a Streck Cell-Free DNA BCT. Platelet poor plasma (PPP) was stored for two weeks at 4°C, -20°C or -70°C. EV-enrichment was performed by DGUC, followed by SEC and then UF. EV-enriched fractions were analyzed by nanoparticle tracking analysis (NTA), bicinchoninic acid assay (BCA) and western blot (WB)

b. Particle numbers measured by NTA. Data normalized to storage at 4°C.

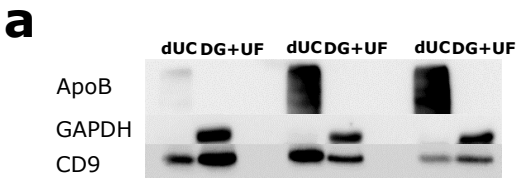
c. Representative immunoblotting from particles purified after the different storage conditions tested.

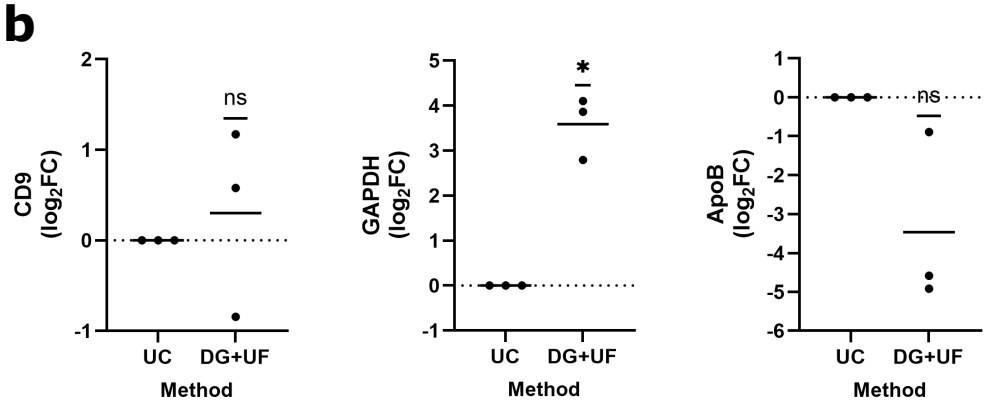
d. Quantification of the immunoblot signal for CD9 of n=3 independent experiments. Data normalized to immediate processing (no storage).

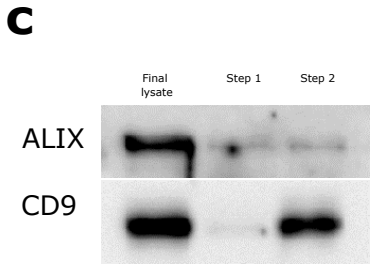
e. Total protein obtained from each storage condition after lysing EV-enriched fractions, measured by BCA.

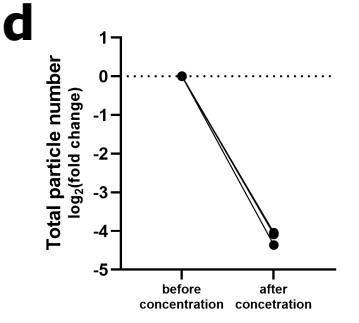
f. Particle size obtained after thawing the PP samples and EV-enrichment.

Data in D.-F. is derived from n=3 biological replicates,









Supplementary Figure S5

Supplementary Figure S5: Choice of the isolation methods severely impacts yield and purity of isolated plasma-derived EVs

a. Immunoblot and b. quantification of EV-derived protein markers purified in n=3 biological replicates either by differential ultracentrifugation or density gradient ultracentrifugation. Data normalized to dUC condition. dUC = differential ultracentrifugation + Size exclusion chromatography, DG+UF = density gradient ultracentrifugation + size exclusion chromatography + ultrafiltration (n=3 biological replicates).

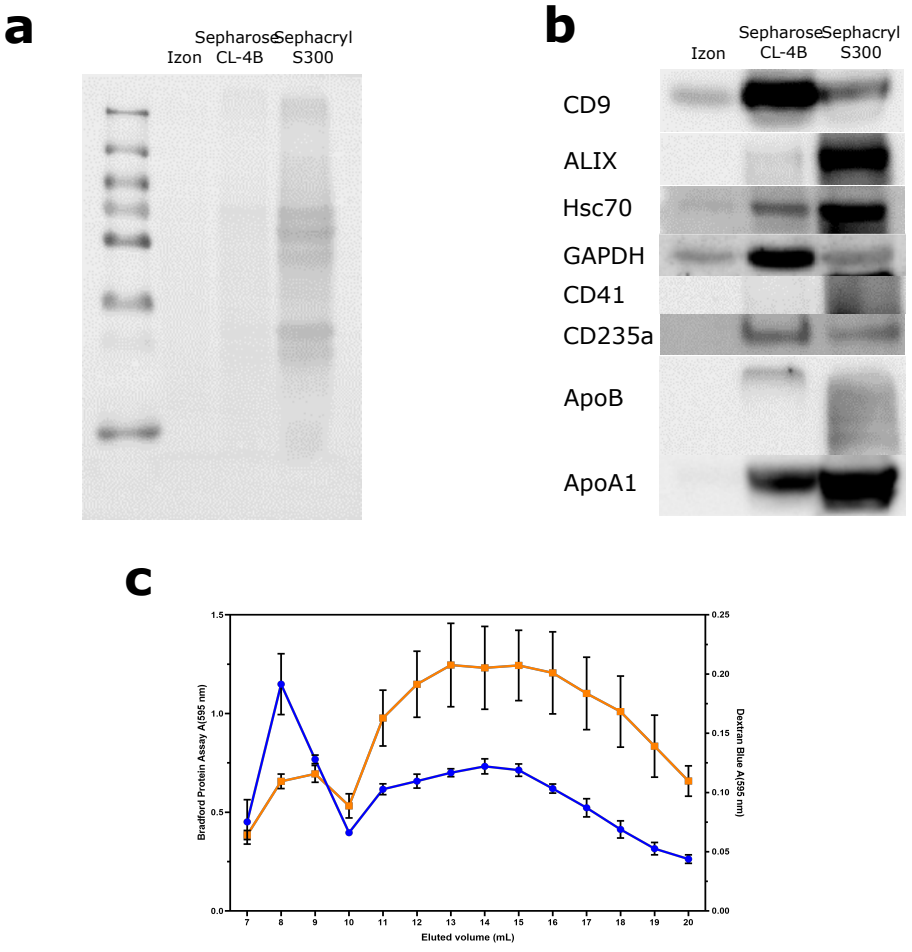
c. Assessment of EV loss throughout the process of isolation by density gradient and size exclusion chromatography.

Final lysate = lysed EVs after removal from Amicon filter following DG and SEC.

Step 1 = EV-enriched volume from the density gradient was collected in an 1.5 mL eppi tube. After removal of this volume, lysis buffer was added to the tube to assess remaining particles.

Step 2 = EV-enriched fractions from SEC were collected directly in an Amicon ultrafiltration unit. After concentrating the sample, the concentrate was removed and lysis buffer was added to the filter to assess remaining particles.

d. Calculated total particle number based on NTA measurement before and after ultrafiltration of EV-enriched fractions (n=3 biological replicates).



Supplementary Figure S6

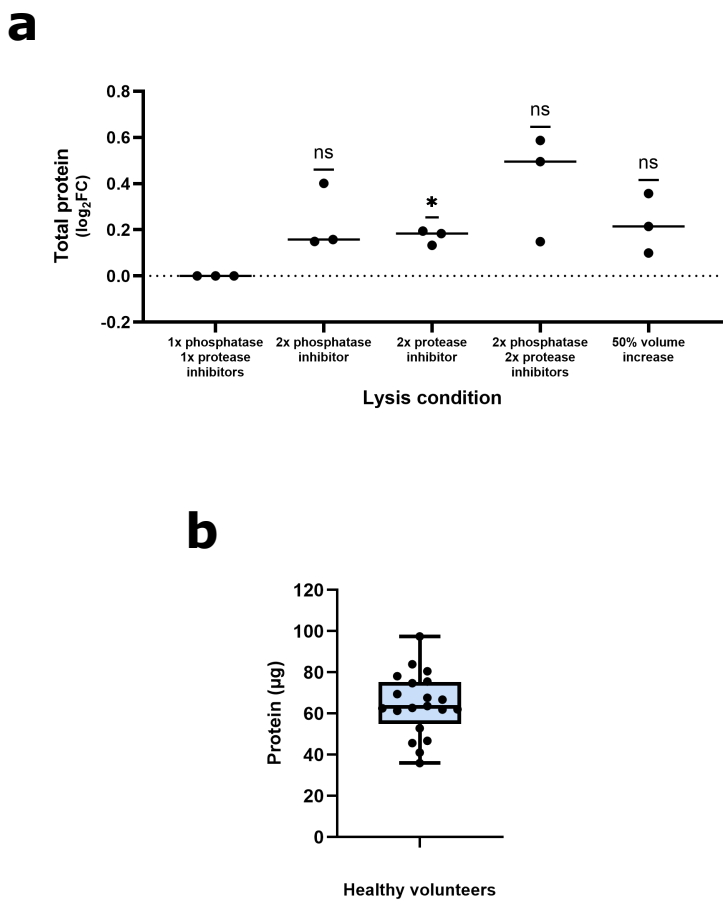
Supplementary Figure S6: Home-made Sepharose CL-4B size exclusion chromatography columns reliably separate EVs from free plasma proteins

a. Representative Ponceau colorimetric and b. immunoblotting image of the pooled EV-enriched fractions obtained after size exclusion chromatography performed with the different resins.

c. Assessment of Sepharose CL-4B elution profiles using as representative standards Dextran Blue (2g/L)(right y axis) and protein mix of Thyroglobulin (2g/L) and bovine serum albumin (BSA, 4g/L) (left y axis) as quality control to evaluate reproducibility (n=6 biological replicates).

d. Elution profiles of Dextran Blue (2g/L) obtained from the same Sepharose CL-4B column freshly prepared, after 7 times or after 11 times usage, respectively.

e. Elution profiles of protein mix (Thyroglobulin (2g/L) + BSA (4g/L)) obtained from the same Sepharose CL-4B as in D., freshly prepared or after 11 times usage.



Supplementary Figure S7

Supplementary Figure S7: EV-derived protein yield depends on lysis buffer composition

a. EV-enriched SEC fractions were lysed with RIPA buffer and the impact of different concentrations of phosphatase and protease inhibitors on protein recovery was measured by BCA (n=3 biological replicates).

b. Protein yields obtained from blood donor healthy volunteers after EV enrichment by DGUC, SEC and UF (n=20).