

Supplementary Figures

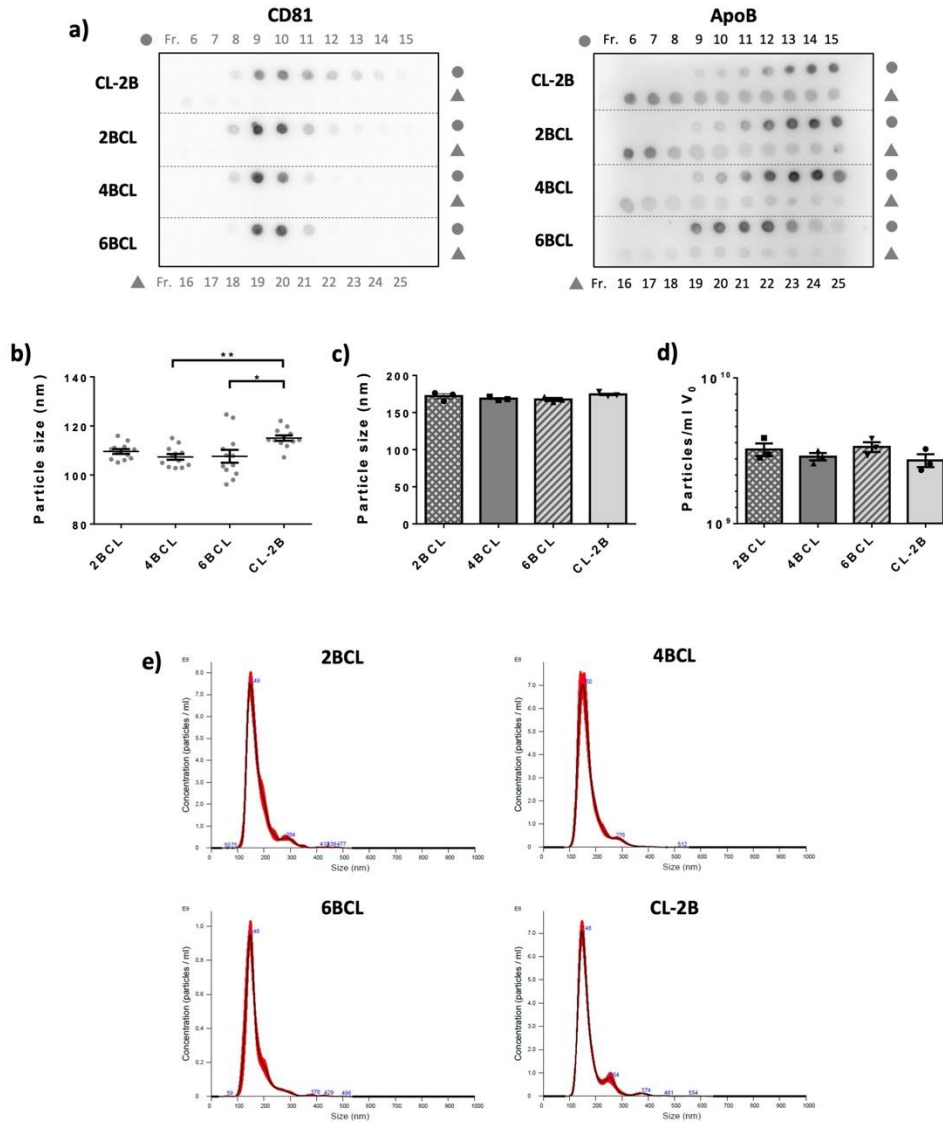


Figure S1. Characterization of EV-enriched fractions obtained by SEC with standard agarose resins. a) Representative dot blots from the analyses of EVs (CD81) and LPP (ApoB) markers in SEC fractions. **b)** Mean particle size of EVs, as derived from TEM images using TEM Exosome Analyzer. The mean \pm SEM from the analyses of 12 images from 2 independent experiments are shown. Significant differences in the One-way Anova statistical test are indicated with * ($P \leq 0.05$) or ** ($P \leq 0.01$). **c)** Mean particle size of particles analyzed by NTA. The mean \pm SEM from 3 independent experiments is shown. **d)** Concentration of particles in EV-enriched fraction pools analyzed by NTA, represented as particles per ml of initial volume of conditioned media (V_0). The mean \pm SEM of 3 independent experiments is shown. **e)** Size profile of particles in pools of EV-enriched fractions analyzed by NTA.

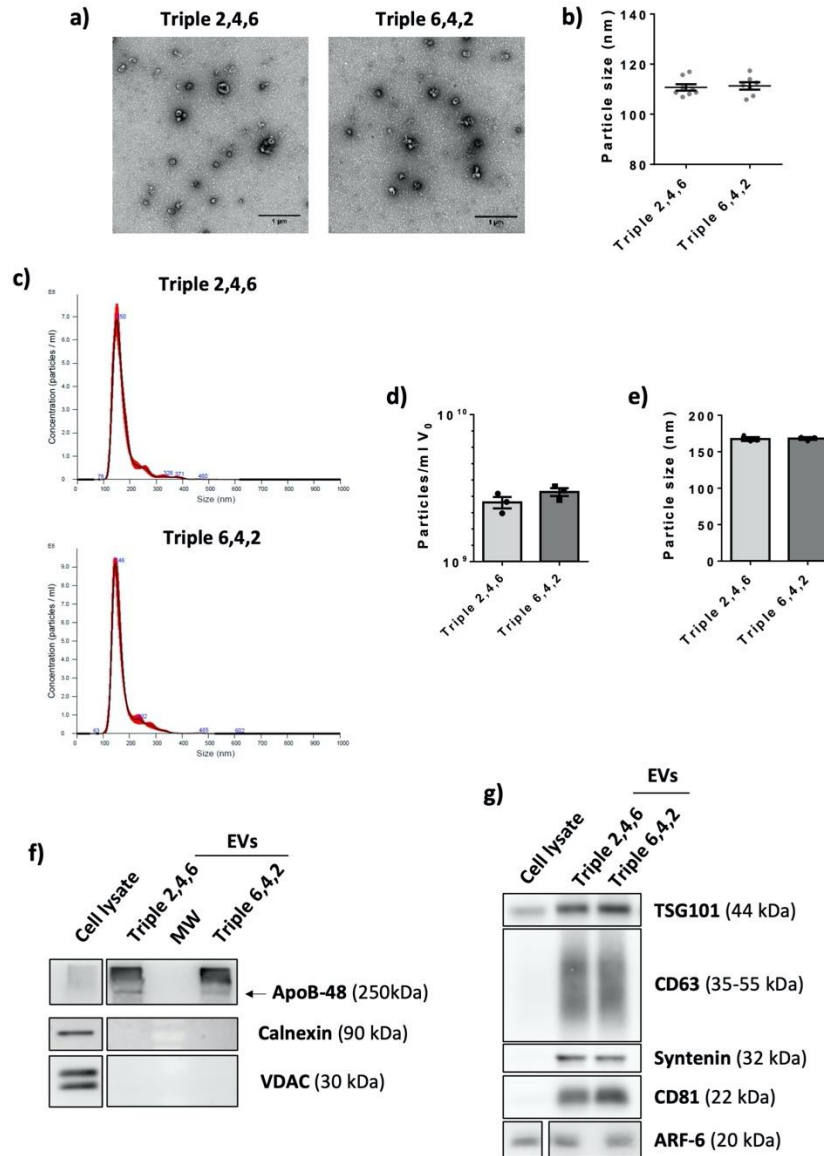


Figure S2. Characterization of EV-enriched fractions obtained by SEC with columns that combined agarose resins of different percentages. **a)** Representative TEM images of negatively stained samples from the EV-enriched fraction pools isolated with the different columns. Bars=1 μ m. **b)** Mean EV diameter of EVs derived from TEM Exosome Analyser on TEM images. 8 images from 2 independent experiments were analyzed and the data was shown as means \pm SEM. **c)** Size profile, **d)** concentration (represented as particles per ml of initial volume of conditioned media (V_0)) and **e)** mean particle size of particles in EV-enriched fraction pools analyzed by NTA. Means \pm SEM of 3 independent experiments are shown. **f)** Western blot analyses of non-EV markers (ApoB, Calnexin or VDAC) or **g)** EV markers (TSG101, CD63, Syntenin-1, CD81 and ARF6) in pools of EV-enriched fractions. Protein content was measured by BCA and a total of 10 μ g was loaded in each lane. The same amount of total cell lysate was included.

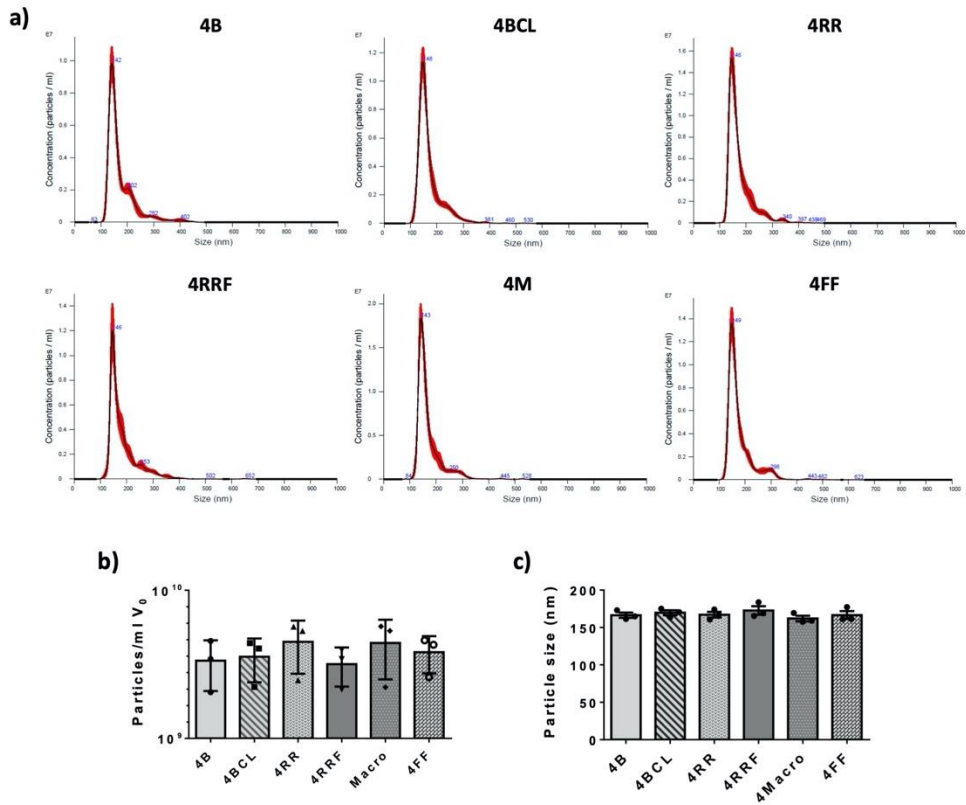


Figure S3. NTA characterization of EV-enriched fractions obtained by SEC with 4% agarose resins of different crosslinking or bead size. a) Size profile, b) Concentration (represented as particles per ml of initial volume of conditioned media (V_0)) and c) Mean particle size of particles in EV-enriched fraction pools analyzed by NTA. Means \pm SEM from 3 independent experiments are shown.

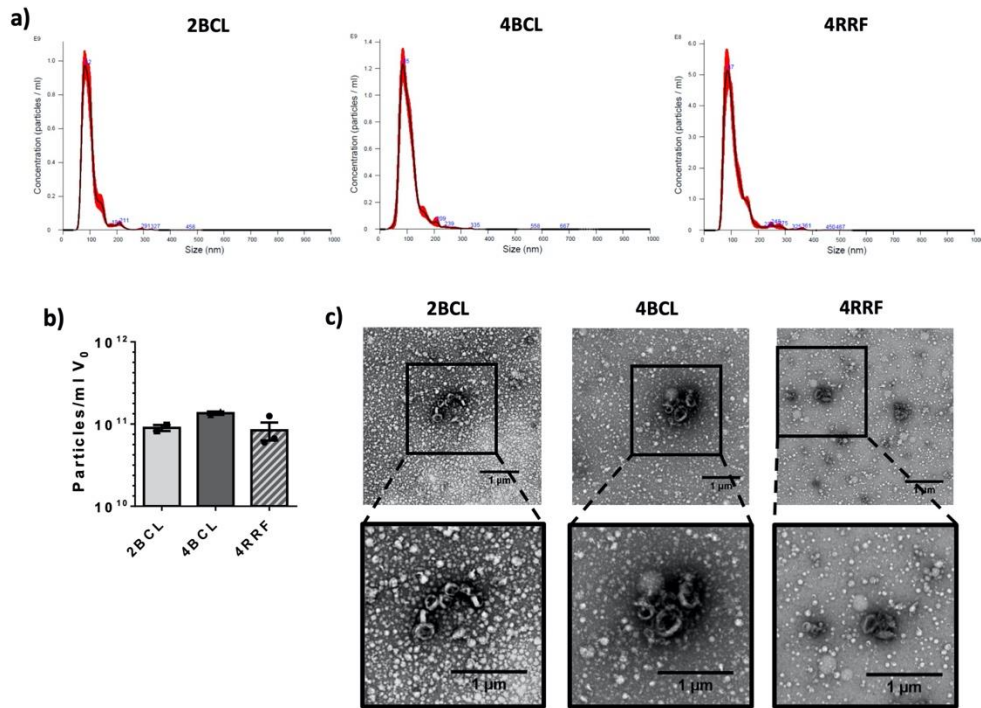


Figure S4. NTA and TEM characterization of EV-enriched fractions obtained by SEC of plasma samples. a) Size profile and **b)** Concentration of particles in EV-enriched fractions pools analyzed by NTA, represented as particles per ml of initial volume of plasma (V_0). Means \pm SEM from 3 independent experiments are shown. **c)** Representative TEM images of negatively stained samples from EV-enriched fractions isolated with the different resins. Bars=1 μ m.