SUPPORTING INFORMATION

Integrated dual-mode chromatography to enrich extracellular vesicles from plasma

Jan Van Deun[1,2], Ala Jo[1,2†], Huiyan Li[1,2†], Hsing-Ying Lin[1,2†], Ralph Weissleder[1,2,3], Hyungsoon Im[1,2], Hakho Lee[1,2,4,5]

1. Center for Systems Biology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114
2. Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114
3. Department of Systems Biology, Harvard Medical School, Boston, MA 02114
4. Center for NanoMedicine, Institute for Basic Science (IBS), Seoul 03722, Republic of Korea
5. Yonsei-IBS Institute, Yonsei University, Seoul, Korea

†These authors contributed equally.

*Corresponding author:
H. Lee, PhD

Center for Systems Biology
Massachusetts General Hospital
185 Cambridge St, CPZN 5206
Boston, MA, 02114
617-726-6487

hlee@mgh.harvard.edu

SUPPORTING FIGURES

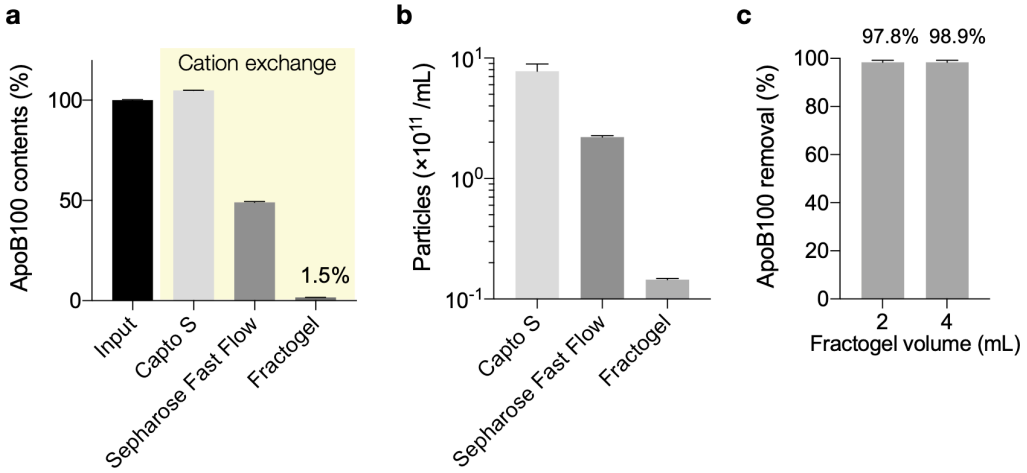

Figure S1. Optimization of cation exchange columns. (a). Cation exchange columns, made of various resins (2 mL for each type), were compared for their capacity for (V)LDL capture. The input standard was prepared by filtering human plasma via SEC. Each exchange column was loaded with 2 mL of the standard sample and the amount of ApoB100 was quantified through ELISA before and after filtration. Fractogel-based filter had the best ApoB100 removal rate (~98%). **(b)** Particle numbers after filtration, measured via nanoparticle tracking analysis, showed a similar trend as in ELISA. **(c)** Two columns packed with different volumes of Fractogel were tested. The overall improvement in the removal yield was similar. All data are from technical duplicates and displayed as mean ± s.d.

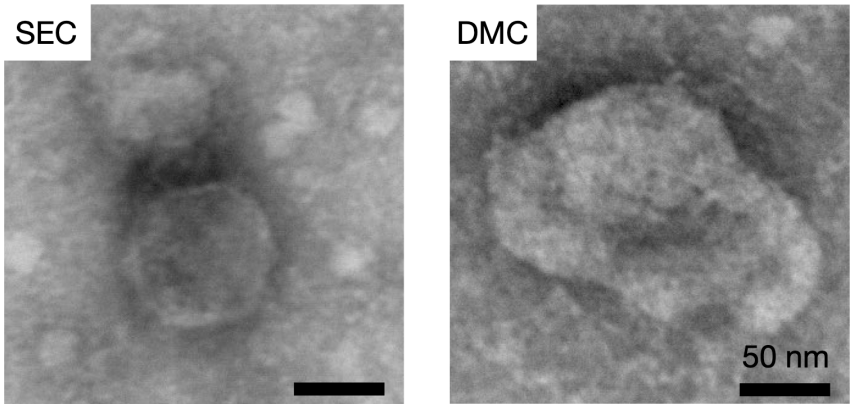

Figure S2. Close-up transmission electron micrographs of SEC- and DMC-prepared human plasma. EVs, negatively stained, appeared dark; LPPs appeared white.

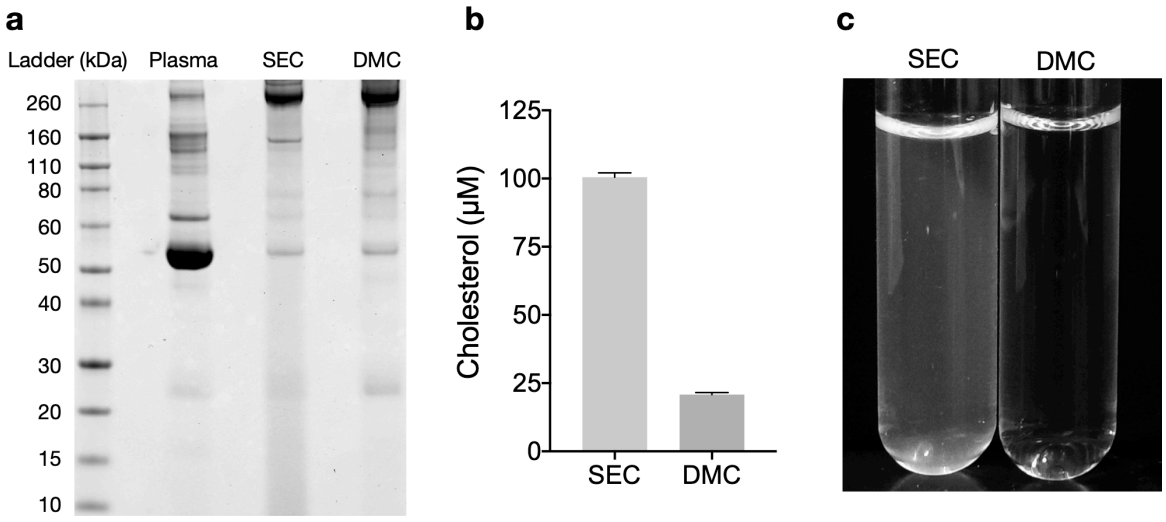

Figure S3. Comparison of protein signature, cholesterol content and appearance of SEC and DMC samples. (a) Equal protein amounts (18 μg) were loaded per sample. Both SEC and DMC depleted soluble proteins from the initial plasma, as evidenced by the decrease of the serum albumin and IgG heavy chain bands (66 kDa and 55 kDa, respectively). DMC preparation resulted in a more specifically enriched sample as evidenced by a distinct pattern of discrete bands compared to SEC. **(b)** Cholesterol concentrations were lower in DMC-prepared EV samples. **(c)** Side-by-side comparison of the appearance of an SEC- and DMC-prepared EV sample.

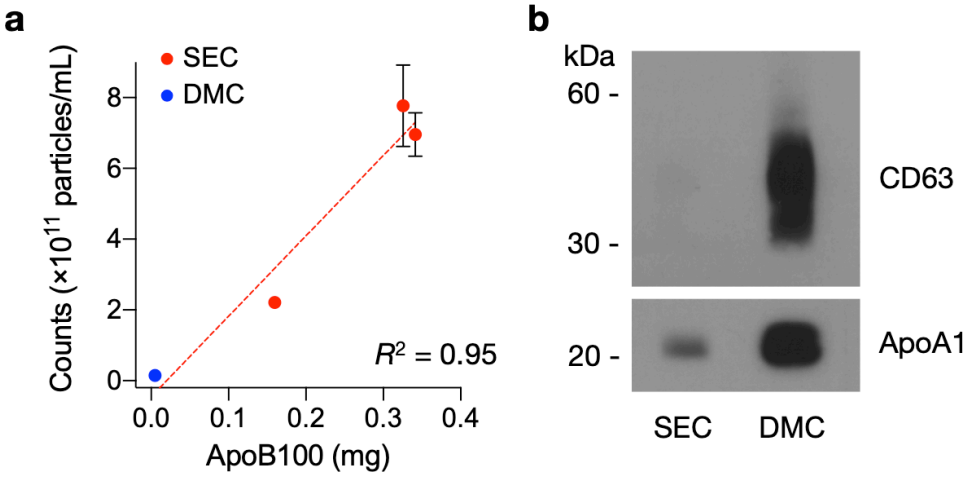

Figure S4. Particle counts in plasma EV fractions. (a) SEC and DMC EV fractions of plasma samples were analyzed for total particle counts (NTA) and ApoB100 amounts (ELISA). Note the good linear correlation (R^2 = 0.95) between these two variables. The slope of the line is non-zero with statistical significance (p = 0.02; two-sided t-test). The data is displayed as mean ± s.d. from technical duplicates. **(b)** SEC or DMC-processed EV-spiked plasma samples were analyzed. Equal number of particles (by NTA) were loaded for Western blotting. CD63 staining was stronger in the DMC sample than in the SEC sample.

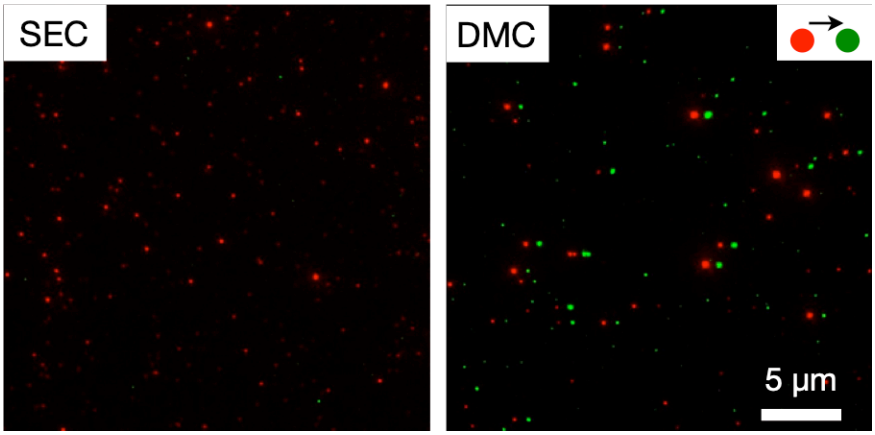

Figure S5. Overlay images of lipid and CD63 channels. Two images in different channels were pixel-shifted for better visualization.

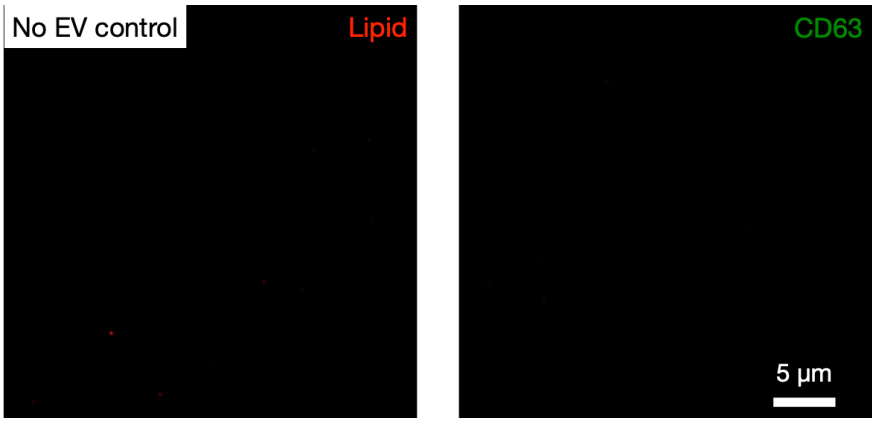

Figure S6. Fluorescent images of a control sample. EV-free samples were subjected to the labeling processes for lipophilic dyes and fluorophore-conjugated anti-CD63 antibodies. No measurable signal was measured in both fluorescent detection channels after the labeling.

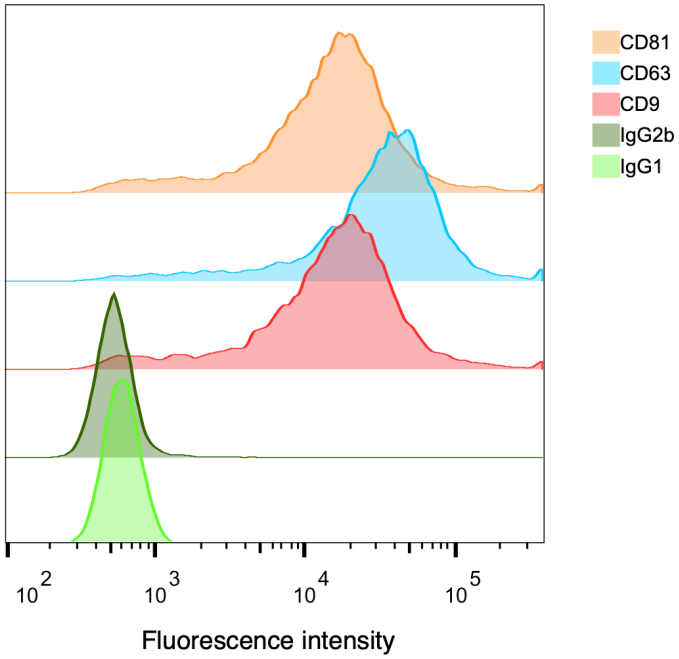

Figure S7. Analysis of tetraspanins in collected EVs. Representative vesicular markers (CD63, CD81, CD9) were measured by bead-based flow cytometry. EVs derived from derived from ES2 cell culture were used. Filtered EVs were captured on aldehyde/sulphate latex beads (4% w/v, 4 μm, Thermo Fisher) and labeled with primary antibodies: anti-CD63 (1:100, clone H5C6, BD Biosciences), CD9 (1:20, clone MM2/57, Millipore Sigma), CD81 (1:40, clone 1.3.3.22, Thermo Fisher) or isotope-matched IgG. For fluorescent labeling, we used AF488-labeled secondary antibodies. All three EV markers were detected in the prepared EV samples. Similar results were obtained for Gli36 EGFRvIII EVs.