Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Label-free ferrohydrodynamic separation of exosome-like nanoparticles

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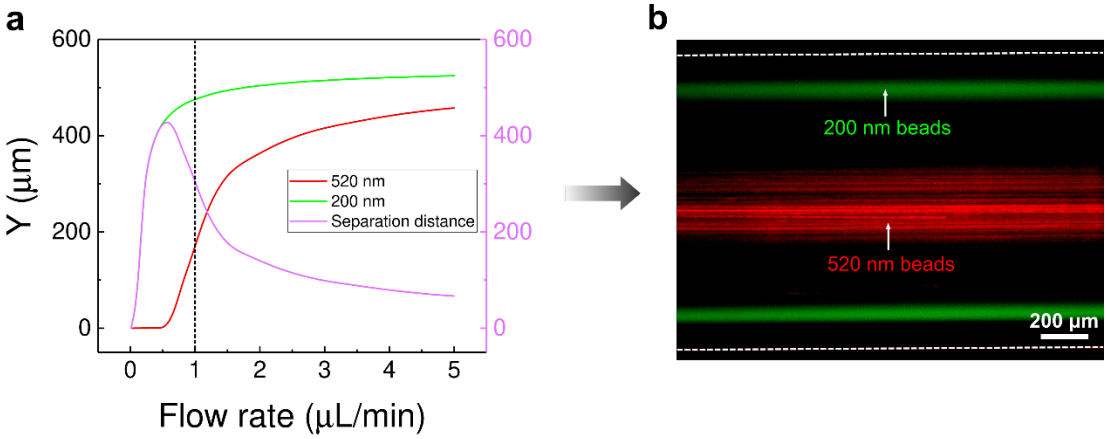

Figure S1. a Simulation results of the separation of 200 nm and 520 nm polystyrene beads at different sample flow rates (0 – 5 µL min^{-1}) in a 0.3% (v/v) ferrofluid in the FerroChip. The ratio of particle flow and sheath flow was 1:5 (sample – 1 µL min^{-1}; sheath – 5 µL min^{-1}). **b** Experimental results of beads separation (green: 200 nm beads, red: 520 nm beads) at the end of the separation region in the FerroChip when the sample flow rate was 1 µL/min and sheath flow rate was 5 µL/min.

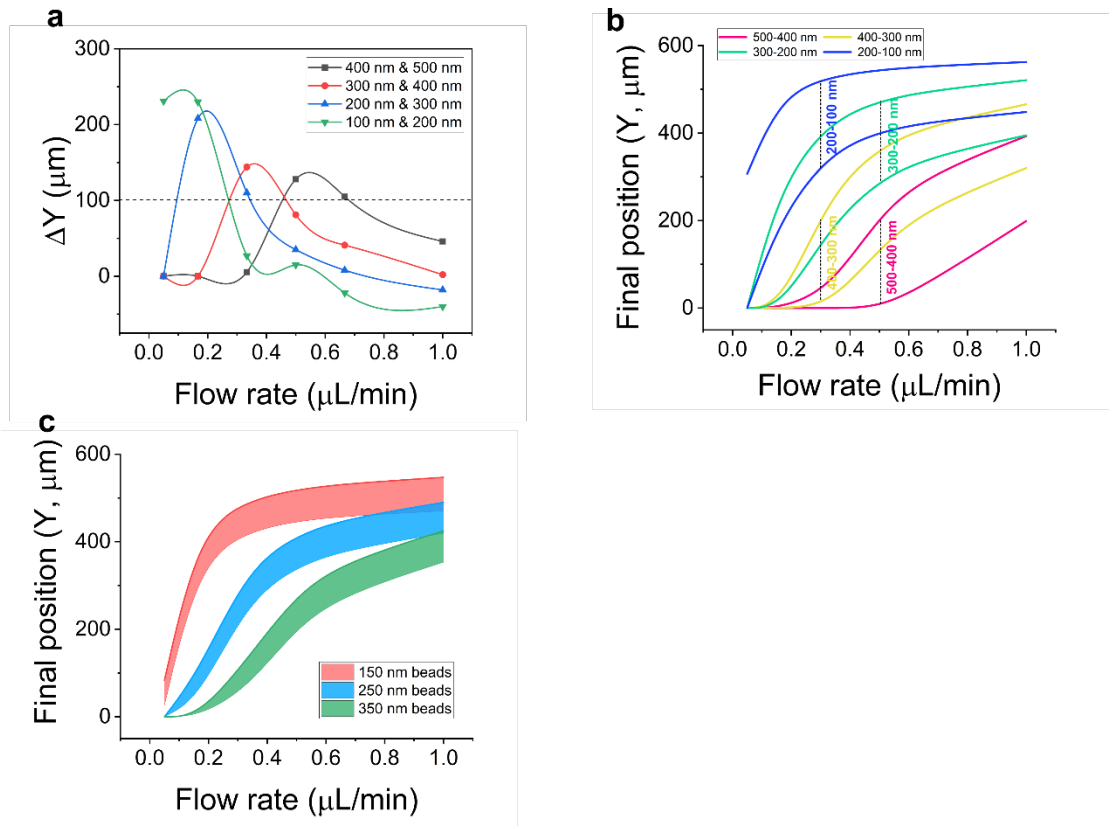

Figure S2. Theoretical size resolution of the FerroChip and its potential in fractionation of a heterogeneous extracellular vesicles (EV) sample. The simulation uses the same device geometry, ferrofluid concentration/composition and magnetic fields of the FerroChip reported in this study. Variable parameters in this simulation include the sample flow rate and particle sizes. **a** Simulation results of binary particles separation with a diameter difference of 100 nm at variable sample flow rates. Y-axis is the separation distance between the two particle streams. The dashed line of 100 μm on the Y-axis is the minimal separation distance with which binary particles are considered to be separated. **b** Simulation results of fractionation of a heterogeneous EV sample in the FerroChip at variable flow rates. Y-axis is the end of separation region in the FerroChip. Bands of EVs with 100 nm diameter range are labeled in different colors. **c** Simulation results of fractionation of a sample with three different sized beads in the FerroChip at variable flow rates. Y-axis is the end of separation region in the FerroChip.

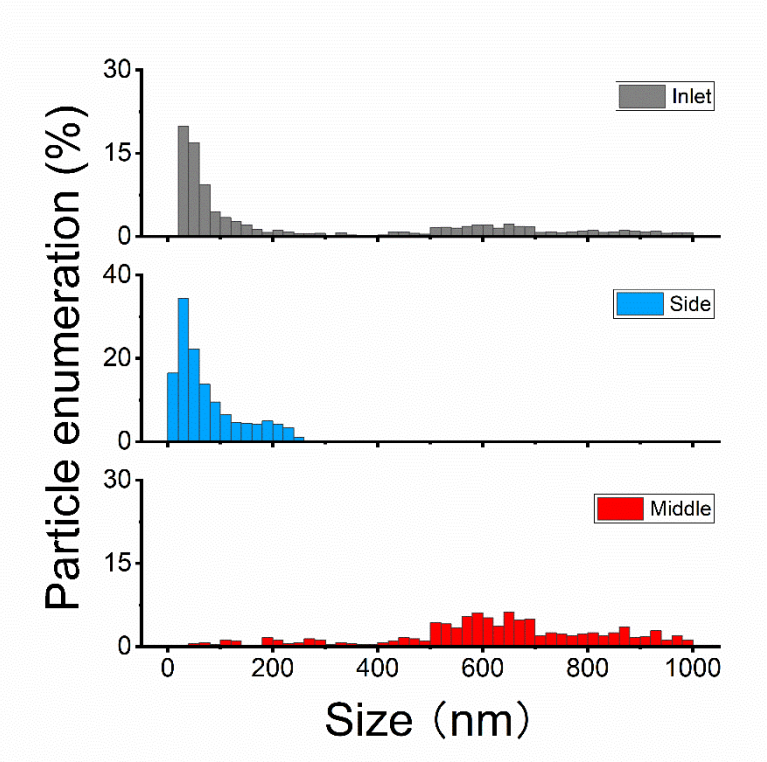

Figure S3. Size distribution of extracellular vesicles (EVs) from MDA-MB-231 breast cancer cell culture media before and after the FerroChip processing. Y-axis is the EV enumeration and X-axis is the diameter of the EVs. Diameters of EVs were determined from atomic force microscopy. Before the FerroChip processing, 1650 EVs were counted from the inlet of the FerroChip, which showed a first peak between 0-100 nm and a second peak at ~600 nm. After processing, 1059 EVs were counted from the side outlets of the FerroChip, which showed a peak between 0-100 nm; 532 EVs were counted from the middle outlet, which showed a peak at ~600 nm.

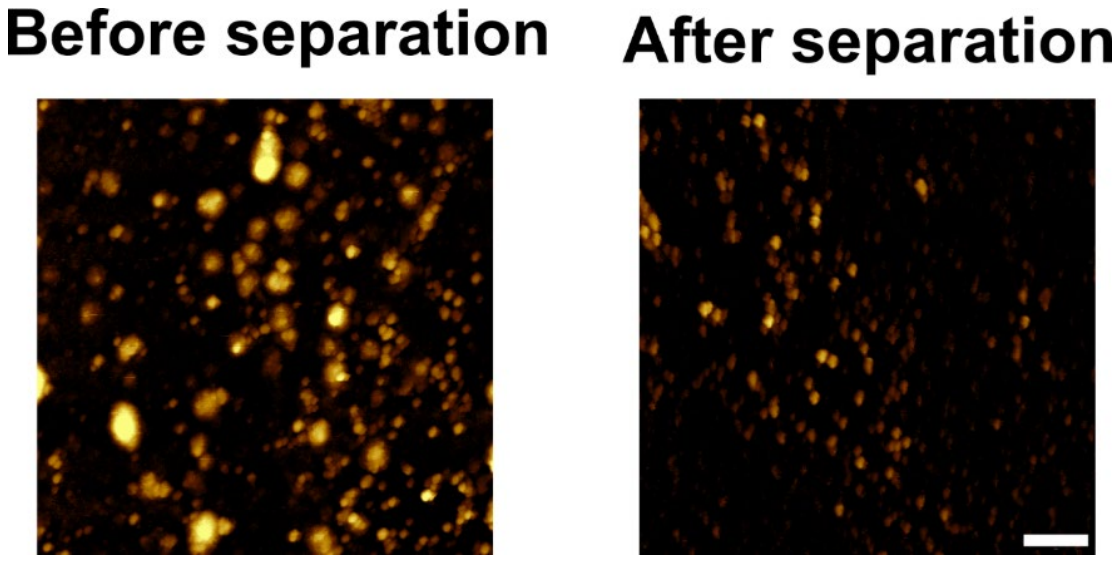

Figure S4. AFM images of extracellular vesicles (EVs) in the FerroChip separation experiment using MDA-MB-231 breast cancer cell culture media from Figure 5a-e in the main text. AFM images of the sample before separation (left, mixture of exosomes and large EVs) and sample after separation (right, EVs collected from side outlet) showned a clear size difference. Sample flow rate is 1 μL min^{-1}, and sheath flow rate is 5 μL min^{-1}. Scale bar: 1 μm.

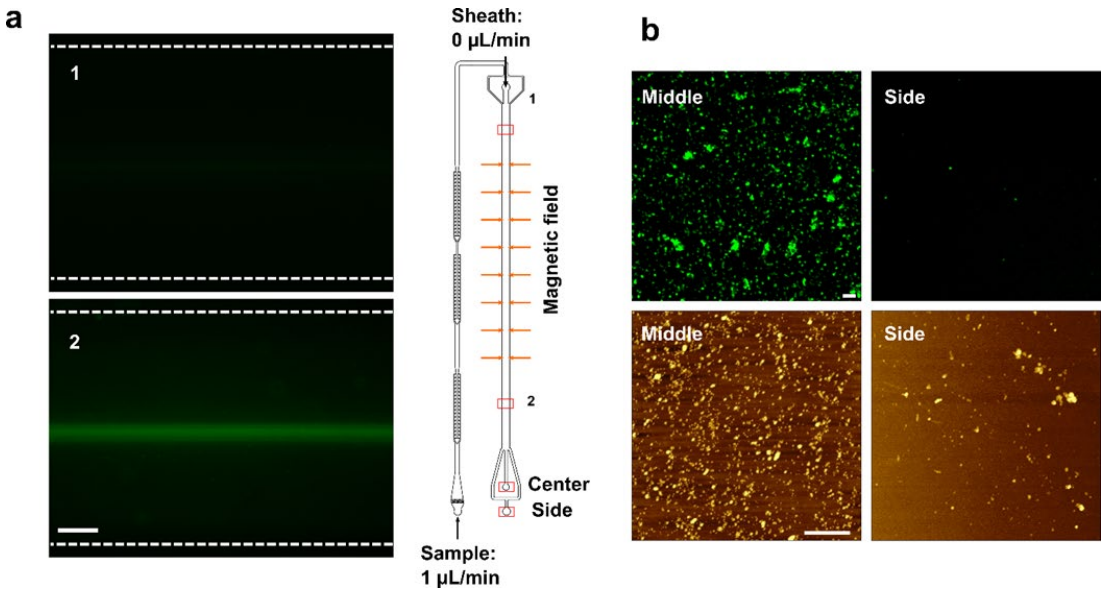

Figure S5. a Extracellular vesicles (from MDA-MB-231 culture medium, green) focusing when the sample flow rate is 1 µL/min and the ferrofluid concentration is 0.3%. The focused EVs were collected in the middle channel and analyzed by both super-resolution fluorescence imaging and atomic force microscopy (AFM). Scale bar: 200 µm. **b** Super-resolution fluorescence images (top) and AFM (bottom) images were used to analyze the vesicles collected from middle and side outlets. These two characterization methods showed a clear enrichment of extracellular vesicles in the middle channel. Scale bars: 1 µm.

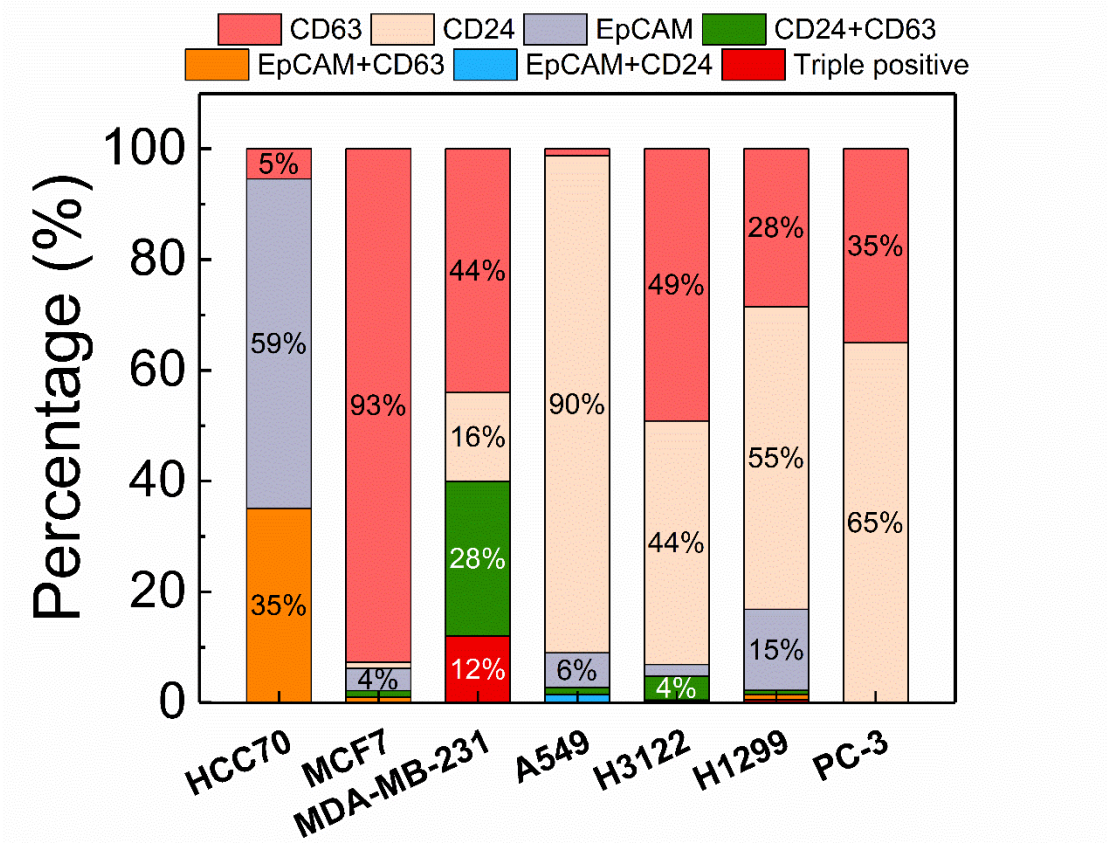

Figure S6. Percentage of antibody presence on the surfaces of separated exosomes (n=1000) from the culture media of 7 cancer cell lines (breast cancer: HCC70, MCF7, MDA-MB-231; lung cancer: A549, H3122, H1299; prostate cancer: PC-3).