Supplementary Information

Quantitative Single-particle Profiling of Extracellular Vesicles via Fluorescent Nanoparticle Tracking Analysis

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Table S1: Antibodies information used in this study. All antibodies' clonality is monoclonal.

Target	Vendor	Cat No.	Clone	Host/Isotype	Lot No.
CD63	Thermo Fisher Scientific	MA5-18149	MEM-259	Mouse/ IgG1	YH4015363
CD63	Abcam	ab309976	EPR5702	Rabbit/ IgG	1054788-1
ApoB	Santa Cruz	sc-393636	A-6	Mouse/ IgG1	K1618
EpCAM	Abcam	ab237395	EPR20532-225	Rabbit/ IgG	1085103-6

Table S2: List of reagents used in this study.

Reagent	Company	Cat No.	Purpose
Non-PEGylated biotinylated liposomes	CD Bioparticles	CDEIMS-1523	Liposomes binding validation
DiO-labelled liposomes	CD Bioparticles	CDL6001F-DO	Fluorescence standards
DiD-labelled liposomes	CD Bioparticles	CDL6001F-DD	Fluorescence standards

Lyophilised exosomes from HT29	HansaBiomed	HBM-HT29-100/2	Cell line extracellular vesicles
Lyophilised exosomes from HEK293	HansaBiomed	HBM-HEK293-100/2	Cell line extracellular vesicles
Very low-density lipoprotein	Merck Life Science	437647-5MG	Lipoprotein binding validation
Phosphate-buffered saline	Sigma-Aldrich	P4417	Buffer
Poly-L-lysine solution	Sigma-Aldrich	P8920	Coverslip coating
Polystyrene 100 nm beads	Particle Metrix	—	Calibration

Cryogenic Electron Microscopy (Cryo-EM)

Cryo-EM was used to determine the morphologies of EV samples. 4 µL EV isolates were applied to glow-discharged copper grids (Quantifoil R2/2, Quantifoil Micro Tools). The grids were blotted for 4.5 s at 4 °C with 100% humidity and plunged into liquid ethane using a Vitrobot Mark IV device (Thermo Fisher Scientific, Waltham, MA, USA). The vitrified samples were stored in liquid nitrogen (-196 °C) prior to cryo-TEM analysis. Cryo-EM data were collected on a Thermo Fisher Talos Arctica transmission electron microscope operated at 200 kV acceleration voltage. Images were recorded on a Falcon III detector (Thermo Fisher Scientific, Waltham, MA, USA).

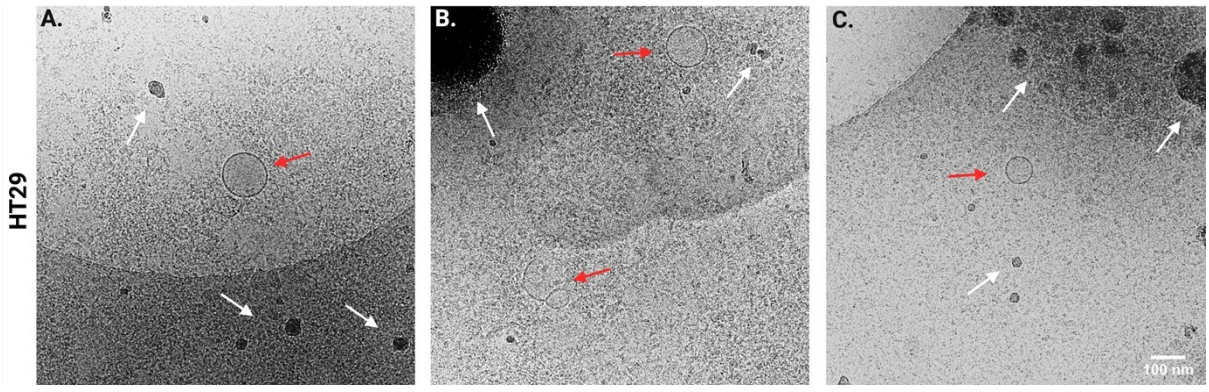

Figure S1. Representative Cryo-EM images of the commercially pursued HT29 EVs, showing the presence of EVs (red arrow) and other impurities (white arrow). Scale bar: 100 nm.

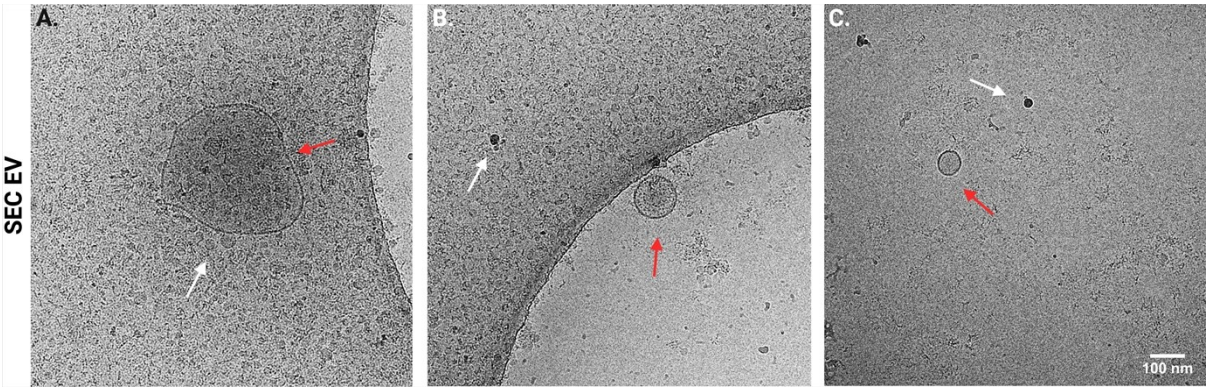

Figure S2. Representative Cryo-EM images of the EVs isolated from human plasma by SEC, showing the presence of EVs (red arrow) and other impurities (white arrow). Scale bar: 100 nm.