Electronic Supplementary Information (ESI)

The convenient exosome separation by phosphatidylserine targeting polymer brush materials

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1. Experimental section

1.1 Reagents and apparatus

DABA was purchased from TCI (Shanghai, China), BTCO was purchased from J&K Scientific Ltd. (Beijing, China), and acetone, acetic acid, dimethylformamide, tetrahydrofuran, and ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 1-Ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide sodium salt (sulfo-NHS) were purchased from McLean (Shanghai, China) . 10 mM PBS (pH 7.4) was purchased from HyClone Company. The ultrapure water was secondary deionized water (18.2 MΩ cm) produced by the Mill-Q ultrapure water synthesis system of Milli-pore Company in the United States. BCA protein concentration assay kit (enhanced) was purchased from Biyuntian (Shanghai, China). Exosome Isolation and Purification Kit (from plasma or serum) was purchased from UmiBio (Shanghai, China).

Field emission-transmission electron microscope (FE-TEM) was Tecnai G2 F20 (FEI Ltd., Czech Republic), microplate reader EMax Plus (MOLECULAR DEVICES, USA), ultracentrifuge Optima-XPN-100 (Beckman Coulter Corporation, U.S). Fourier transform infrared spectrometer from Perkin Elmer, USA. The physical adsorption apparatus was a McMeretic ASAP 2400 from China.

1.2 Experimental

Fabrication of the PBs. The brush-like polymers were fabricated as follows: 3,5-diaminobenzoic acid (DABA, 0.0375 mmol) and 1,3,5-benzeetricarboxaldehyde (BTCO, 0.0565 mmol) were dissolved in 2.5 mL acetone in the centrifuge tubes, respectively. After complete dissolution by sonication, 0.4 mL acetic acid was added to the prepared solutions with a quick mixture for 10 s on a vortex mixer. Then the centrifuge tubes were stood at 25℃ for 2 days. The products of PBs were obtained by washing with organic solvents 3 times to remove the monomers and then dispersed in water to dry at 25℃.

Synthesis of ExoPB-PS. 2 mg PBs was dispersed in MES (15 mM) buffer solution at pH 6.0 and then 40 mg/mL1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 40 mg/mL N-Hydroxysulfosuccinimide sodium salt (Sulfo-NHS) were added to the PBs dispersion for activating the carboxyl groups on the surface of PBs for 2h. The activated materials were washed 3 times and then 0.5 mg/mL PSpep (dissolved in pH 7.2, 10 mM PBS buffer solution) solution or FITC-PSpep solution was added immediately at room temperature overnight. Centrifuge to remove unreacted peptides in the solution, and dry at room temperature for later use.

Preparation of standard exosome solution. Model cells Hep G2 were recovered and seeded in T25 flasks and cultured in a 37°C 5% CO_2 incubator. The cell culture medium was composed of DMEM medium with 10% (V/V) fetal bovine serum (Gibco), and 1% penicillin: streptomycin (Gibco)). MCF-7 and MDA-MB-231 cells were cultured in the same condition as Hep G2. And the cell culture mediums were collected and stored at a low temperature.

Standard exosome solutions were isolated by UC from the cell culture medium of model cells Hep G2. All the centrifuge procedures were conducted at 4°C. The cell debris were firstly removed from the stored cell mediums by centrifugation at 2500 rpm for 15 min. The supernatant was collected and centrifuged at 3000 rpm for 15 minutes, 10,000 rpm for 30 minutes, and 100,000 rpm for 2 hours. Finally, the supernatant was carefully discarded, and the obtained exosome samples were resuspended in phosphate-buffered saline and stored at a low temperature for later use.

The capture of exosomes in the standard exosome solutions. 2 mg ExoPB-PS was placed in 100 μL diluted exosome samples (HEPES buffer, concentration of 2.6×10^{10} Particles/mL), and $CaCl_2$ with final concentrations of 7.5mM was added for adsorption experiments for 60 min. 10000 × g was used as the suitable centrifuge speed to obtain the ExoPB-PS with exosomes. After twice washes with HEPES buffer (pH 7.4) and desorption by EDTA (20 mM) for 30 min, the resulting centrifugation product was diluted 1:1000 for NTA quantification.

The direct exosome isolation from the cell medium. 2 mg ExoPB-PS was directly dispersed in an untreated 2 mL cell culture medium (including cell Hep G2, MCF-7, MDA-MB-231), and the optimized experimental conditions were applied to capture exosomes. TEM and NTA analysis were used to characterize the morphology and quantity of captured exosomes. A human apolipoprotein E ELISA kit was applied to verify the purity. the resulting centrifugation product was diluted at 1:1000 for NTA quantification.

The direct exosome isolation from the plasma sample. The fresh plasma sample was pre-centrifugated with 3000 rpm. 2 mg ExoPB-PS was directly dispersed in plasma and the optimized experimental conditions were applied to capture exosomes. TEM and NTA analysis were used to characterize the morphology and quantity of captured exosomes. Western blot and Human apolipoprotein E ELISA kit were applied to verify the purity and contamination. The resulting centrifugation product was diluted to a suitable concentration for NTA quantification.

The detection of protein content in the samples. The protein concentration in the exosome sample was applied to bicinchoninic acid (BCA) methods. The enhanced BCA Protein Assay Kit (Beyotime) was used to detect the protein concentration in the plasma-related samples. The experimental procedures were carried out according to the instructions of the enhanced BCA Protein Assay Kit. Each sample was tested three times.

Western Blotting (WB). The EV samples were lysed with 20 μL loading buffer (1×, with DTT) and subsequently boiled at 95 °C for 10 min. Then the prepared samples were separated by 10% SDS-PAGE (80 V, 30 min; 120 V, 60 min) followed by transferring onto PVDF membranes (300 mA, 120 min). Later, the PVDF membranes were blocked with blocking solution in TBST buffer for 1 h and then washed three times with TBST buffer for 10 min. The membranes were incubated with the primary antibodies at 4 °C overnight and further washed three times with TBST buffer. After incubation with peroxidase conjugated secondary antibodies for 1 h at RT, the samples were washed three times with TBST buffer for 10 min. The signals were visualized by

a ChemiDoc MP Imaging System (Bio-Rad).

Plasma related exosomes obtained by UC. All the centrifuge procedures were conducted under at 4°C. The cell debris were firstly removed from the fresh plasma sample by the centrifugation at 2500 rpm for 15 min. The supernatant was collected and centrifuged at 3000 rpm for 15 minutes, 10,000 rpm for 30 minutes, and 100,000 rpm for 2 hours. Finally, the supernatant was carefully discarded and the obtained exosome samples were resuspended in phosphate buffered saline and stored at low temperature for later use.

Plasma related exosomes obtained by UF. Plasma samples were first filtered through a 0.22 μm filter, and serum samples were diluted 10-fold with PBS. The plasma sample was added to a 100 kDa ultrafiltration tube, and centrifuged at 4°C, 4000 × g for 30 min. After the centrifugation, an appropriate amount of PBS was added to the 100 kDa ultrafiltration tube and centrifuged again under the same conditions, repeated three times, and then the ultrafiltration was collected. The liquid in the upper layer of the tube is dispensed for use.

Plasma related exosomes obtained by Kit. All operations are carried out according to the instructions given in the manual.

Ethics statement. Normal human serum was obtained from Hospital of Shaanxi Normal University. This study complied with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of People's Republic of China (1988) and the Guidelines on the Humane Treatment of Laboratory Animals (2006). All experimental procedures with serum were approved by the Human and Animal Research Ethics Board of Shaanxi Normal University (NO. 20150323). All participants provided written informed consent.

2. Construction and characterization of ExoPB-PS

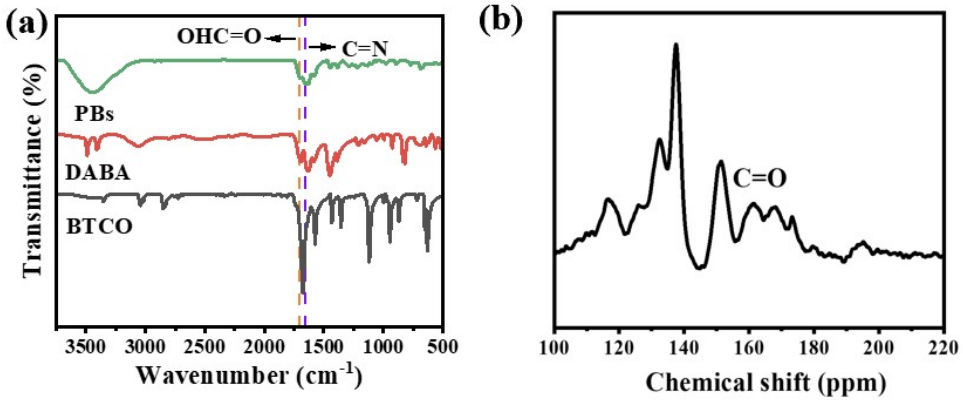

Fig. S1 The FT-IR and 13C spectra of PBs (a, b).

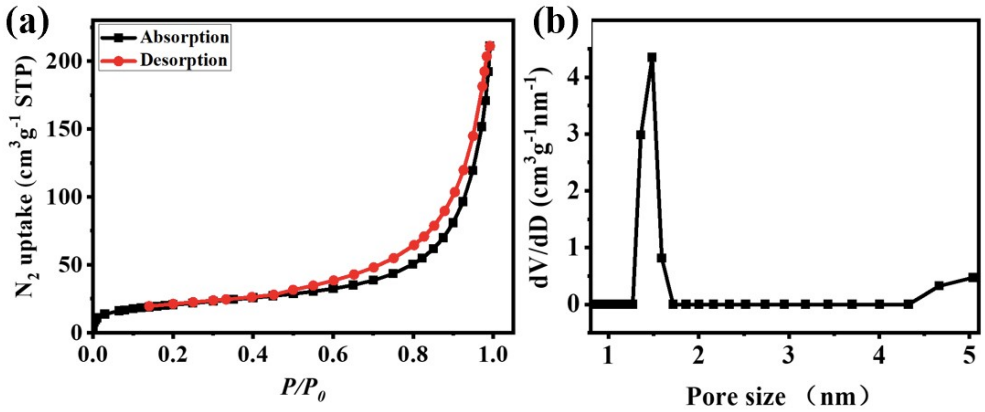

Fig. S2 N_2 adsorption−desorption isotherms and pore-size distribution of PBs.

Fig. S3 Photograph of PBs.

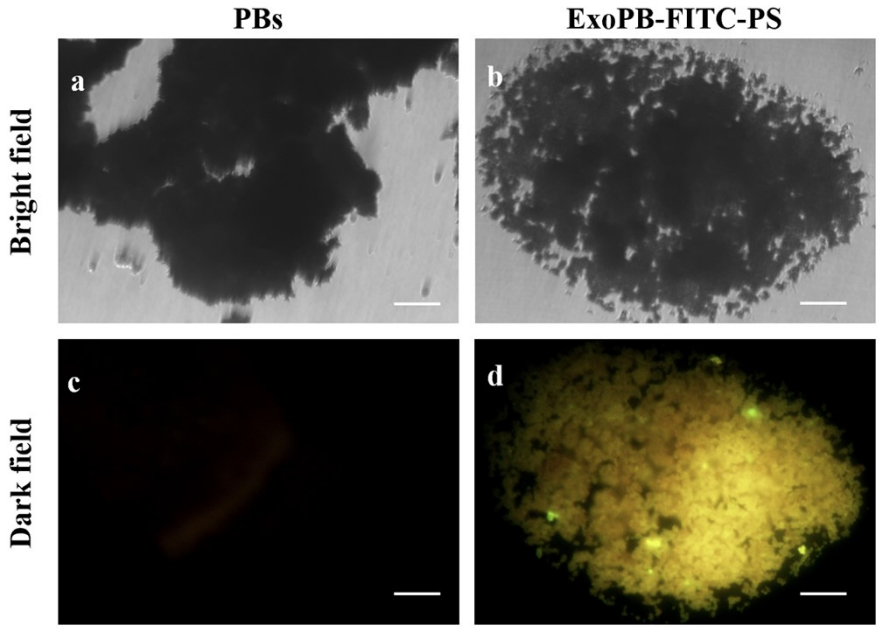

Fig. S4 Fluorescent images of PBs and ExoPB-FITC-PS (excitation wavelength: 484 nm, scale bars: 100 μm).

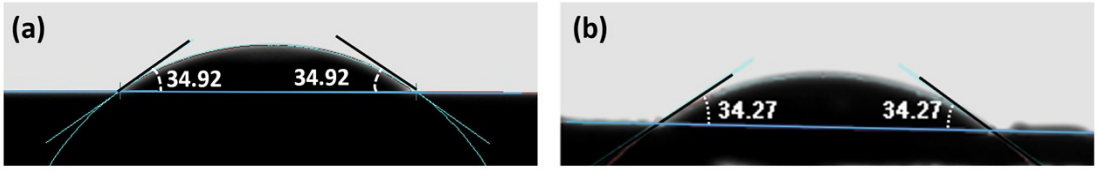

Fig. S5 Contact angle of (a) PBs and (b) ExoPB-PS.

3. Capture verification of ExoPB-PS for exosomes

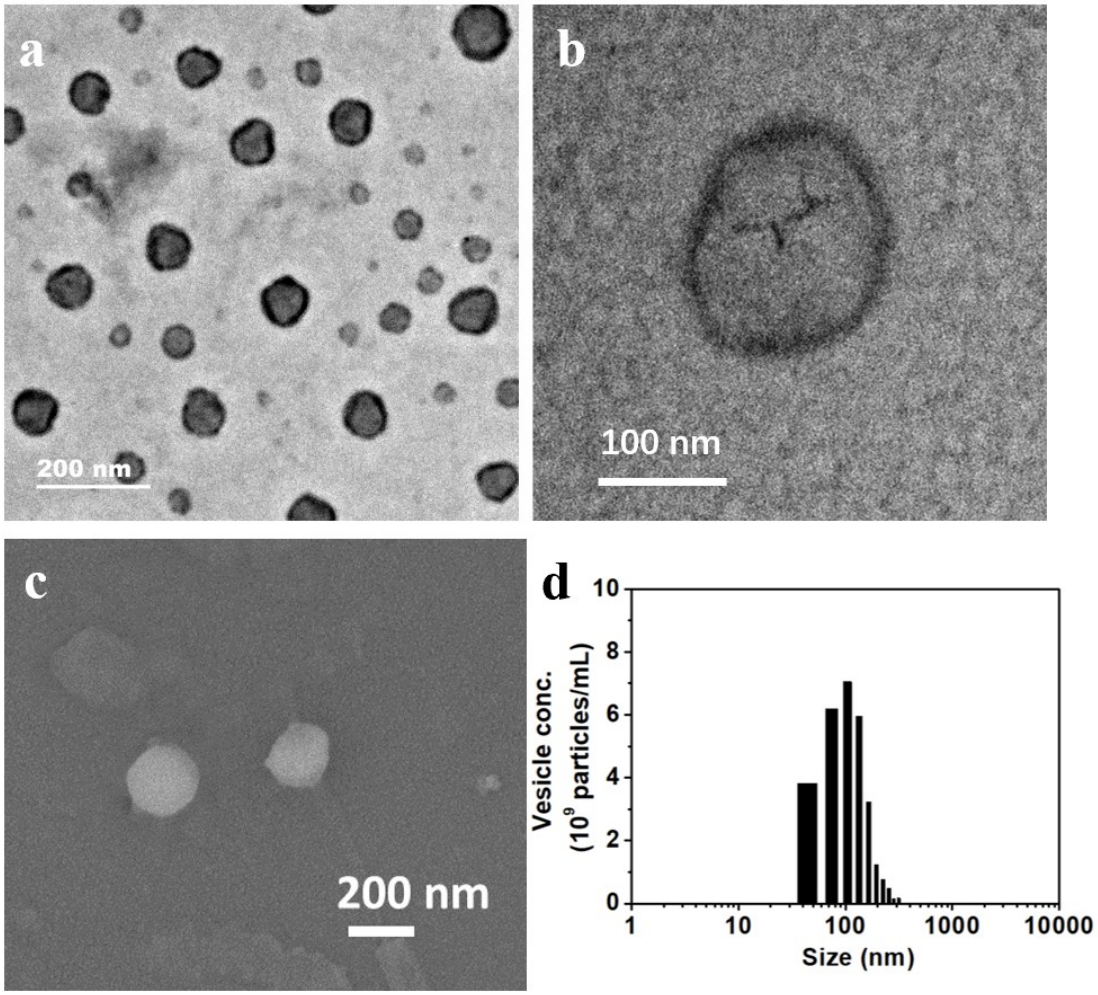

Fig. S6 The (a and b) TEM images, (c) SEM images and (d)NTA data of standard EVs sample obtained by UC.

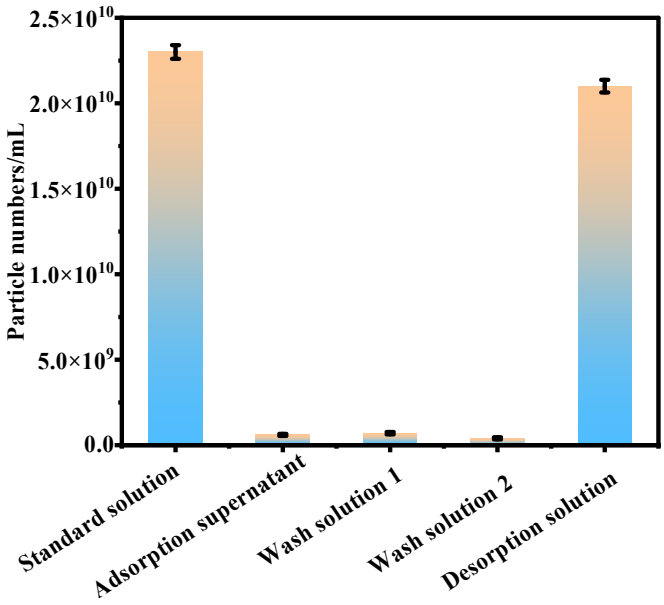

Fig. S7 The particle number of different solution in the all procedure of the ExoPB-PS capture process.

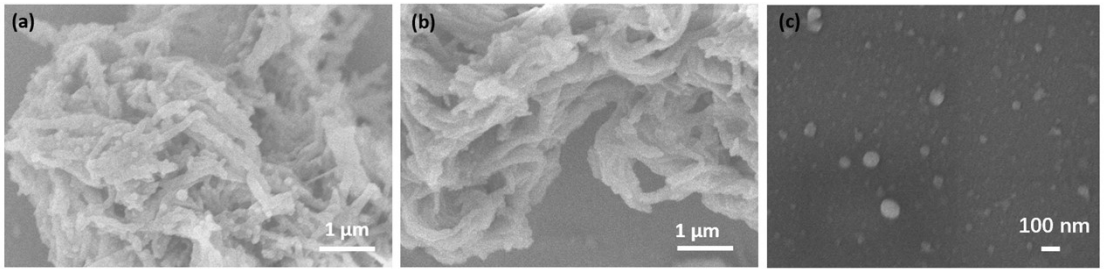

Fig. S8 The SEM images of (a) ExoPB-PS adsorbed with exosomes in the Ca^{2+} solution, (b) the ExoPB-PS after elution, and (c) desorbed exosomes by the EDTA solution.

4. Optimization of the ExoPB-PS capture condition

To maximize the capture efficiency of EVs by ExoPB-PS, a series of experimental conditions for adsorption and desorption were sequentially optimized. Adsorption conditions were firstly optimized. As shown in Figure S9, the optimization of the adsorption conditions was carried out by the concentration of Ca^{2+}, the amount of ExoPB-PS, and the adsorption time. Since the binding of PSpep on the surface of ExoPB-PS to PS on the out layer of exosomes is carried out in a Ca^{2+}-dependent

manner, the concentration of Ca^{2+} in solution plays a key role in the adsorption capacity. Therefore, Fig. S9a is the adsorbed exosome number results of ExoPB-PS under different Ca^{2+} concentrations. When the Ca^{2+} concentration reached 7.5 mM, 86% of exosomes in the standard exosome solution were successfully captured, so 7.5 mM was chosen as the optimal Ca^{2+} concentration in the following experiments. Furthermore, the dosage of the ExoPB-PS and the capture time were also optimized (Fig. S9b, c). In conclusion, the adsorption conditions for exosome capture using ExoPB-PS were determined as follows: 2 mg ExoPB-PS was adsorbed at 7.5 mM Ca^{2+} for 60 min.

The release of EVs was achieved by using an appropriate concentration of EDTA as the eluent. To determine the optimal EDTA concentration for release from EVs after Ca-chelation, 4 different concentrations of EDTA were applied to the elution process after capture (5-40 mM). The NTA analysis results for particle number and size distribution were conducted for the exosome-releasing samples. Particle number increased at EDTA concentration from 5 to 20 mM but decreased at 40 mM (Fig. S10a). Table S1 lists the size distributions of eluted EVs samples under 20 mM and 40 mM EDTA, respectively, showing that aggregated particles (exceeds 200 nm) were appeared at high EDTA concentrations. The particle size is higher than the EVs content of model exosomes in the 200 nm particle size range. Some researchers have mentioned that the EDTA with high concentration would decrease the stability of the membrane, which would lead the vesicle fusion or exosomal aggregation. Therefore, choosing 20 mM as the optimal concentration of EDTA, and 20 min as the elution time for twice times were used as the most suitable exosome elution and release conditions (Fig. S10 b, c).

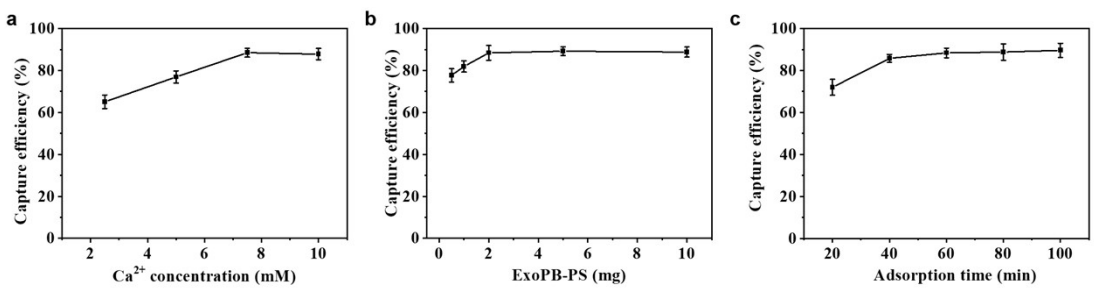

Fig. S9 Capture optimization using model EVs samples. (a) Concentration of Ca^{2+}, (b) dosage of ExoPB-PS and (c) adsorption time.

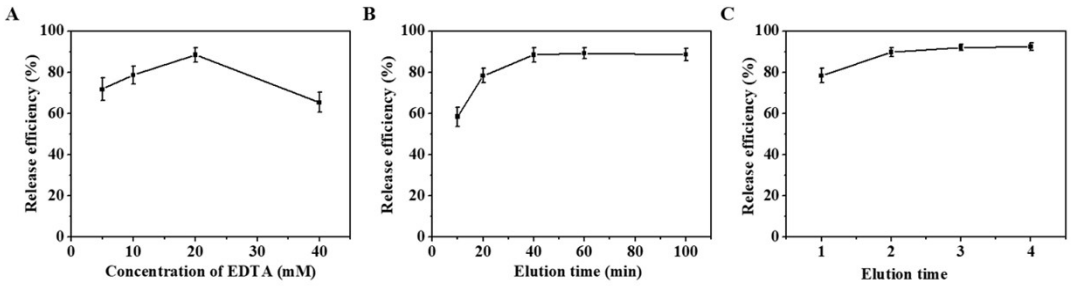

Fig. S10 Released optimization using model EVs samples. (a) Concentration of EDTA, (b)elution time and (c) times.

Table S1 The size distribution percentage of model EVs, EVs released with 20 mM and 40mM EDTA.

Size range	Model EVs	EVs released with 20mM EDTA	EVs released with 40mM EDTA
30-500 nm	7.4%	10.3%	11.6%
50-200 nm	86.7%	83.3%	63.4%
>200 nm	5.9%	6.4%	`25.0%

5. Direct isolation of exosomes from cell medium and plasma sample

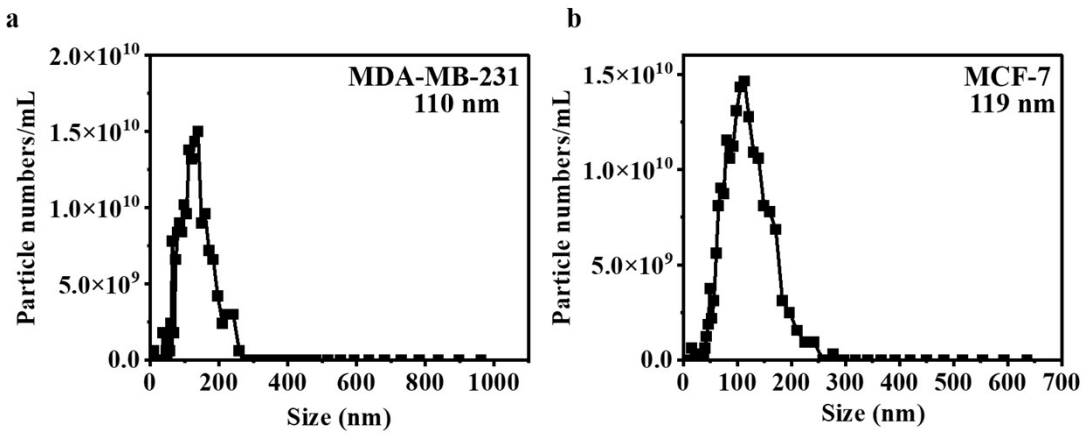

Fig. S10 NTA results obtained by releasing from ExoPB-PS in MDA-MB-231 (a) and MCF-7 cell culture mediums (b).

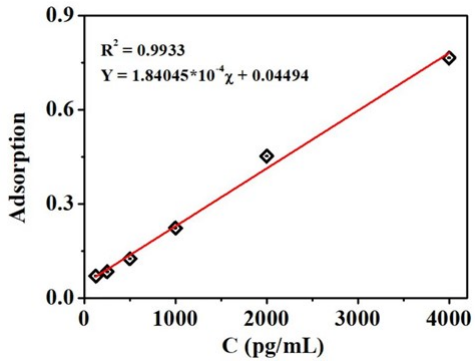

Fig. S11 Standard Curve of ELISA-ApoE kit.

Table S2 The mean size and the particle number of the obtained exosomes by UC and ExoPB-PS in 3 cell mediums.

	UC			ExoPB-PS		
Cell Type	Particle numbers/mL	Mean size (nm)	ApoE Content (pg/mL)	Particle numbers/mL	Mean size (nm)	ApoE Content (pg/mL)
He PG2	1.39×10^{11}	99.6 ± 4.3	873.2	1.88×10^{11}	97.4 ± 3.1	426.3
MDA-MB-231	0.98×10^{11}	109.6 ± 3.6	753.6	1.21×10^{11}	110.3 ± 5.2	405.9
MCF-7	7.54×10^{10}	115.6 ± 3.7	1104.4	8.32×10^{10}	119.5 ± 4.6	662.8

Table S3 The comparison of UC, UF, Kit and ExoPB-PS for capture exosomes in the plasma sample.

	UC	UF	Kit	ExoPB-PS
Particle numbers/mL	5.72×10^{11}	7.2×10^{11}	4.06×10^{11}	6.83×10^{11}
Mean size (nm)	99.3 ± 7.2	109.6 ± 9.8	108.4± 4.1	107.1 ± 3.9
Apo E（pg/mL）	26640	28312	13576	26640