Supporting Information

Integrated Separation and Detection of Exosomes via a Label-Free Magnetic SERS Platform

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EXPERIMENTAL SECTION

Materials. Chloroauric acid hydrated ($HAuCl_4 \cdot 4H_2O$), trisodium citrate, ascorbic acid (AA) and ferric chloride hexahydrate was purchased from Sinopharm Chemical Regent (Shanghai, China), Sodium hydroxide. Mercapto polyethylene glycol amino (SH-PEG-COOH, MW = 2000) was purchased from Ponsure Biological (Shanghai, China). Diethylene glycol (DEG), Formaldehyde was purchased from Macklin (Shanghai, China). High sugar Du's modified medium (DMEM), Fetal bovine serum protein (FBS) was purchased from Gibco. Penicillin streptomycin solution, Cell membrane green fluorescent probe (Dio) were purchased from keygenbio (Jiangsu, China). All reagents were used as per specifications and were not further purified. During the whole working process, ultrapure water was used (≥18 MΩ cm).

Synthesis of Fe_3O_4 Magnetic Nanoparticles. The method reported in the reference was used to synthesize Fe_3O_4 magnetic nanoparticles after slightly fine-tuning[1]. First, 50 mmol sodium hydroxide was dissolved in DEG (20 mL), heated to 120 ℃ under nitrogen protection, and stirred for 1 h. After cooling, the solution was stored at 70 ℃ to obtain NaOH/DEG stock solution. Then, 100 mg of ferric chloride was weighed and dissolved in 17 mL of DEG, and 20 mg of SH-PEG COOH was added after full mixing. After uniform mixing, the reaction solution was heated to 220 ℃ under the protection of nitrogen. After 30 min of reaction, 1.6 mL of NaOH/DEG stock solution prepared in the first step was added to the above solution, vigorously stirred and heated at 220 ℃ for 1 h. After the reaction, a black and turbid solution was

obtained. The mixture solution was cooled to room temperature, centrifugated and washed three times to obtain magnetic nanoparticles with a size of about 12 nm. The magnetic Fe_3O_4 particles obtained were dispersed in 5 mL of deionized water for standby.

Synthesis of Gold Nanoparticles (AuNPs). AuNPs were synthesized by sodium borohydride reduction. Add 0.6 mL $HAuCl_4$ with concentration of 1% into 40 mL ultrapure water, mix well and store at 4 ℃. Then add 0.2 mL potassium carbonate solution with concentration of 0.2 mol. Quickly add 0.4 mL of freshly prepared sodium borohydride aqueous solution (0.5 mg/mL) under vigorous stirring, and repeat for 3-5 times until the solution changes from blue purple to orange red. After continuous stirring for 15 min, gold nanoparticles with a diameter of 2-5 nm were obtained.

Synthesis of Fe_3O_4/Au NPs. Fe_3O_4/Au nanoparticles were synthesized in two steps[2]. First, add 5 mL of Fe_3O_4 magnetic nanoparticles solution uniformly dispersed in the aqueous solution into the colloidal AuNPs solution synthesized in the above step, and use the surface -SH bond of the Fe_3O_4 magnetic nanoparticles to combine with small Au nanoparticles as the seed for the growth of Au shell. Then, add the prepared gold layer growth solution into the above system, use formaldehyde as the reducing agent, and react for several hours without stirring. After the solution turns blue purple, it was centrifugated and the supernatant was discarded. Finally, the precipitation was washed for many times and dispersed in deionized water.

Characterization of Fe_3O_4/Au NPs. The Fe_3O_4/Au nanoparticles were characterized through dynamic light scattering (DLS, Zetasizer Nano ZS90, Malven, also for zeta potential measurement), UV spectrum (UV-3600 Plus, Shimadzu), Fourier Transform Infrared Spectroscopy (FTIR, Bruker Vertex 70), XRD (D8 Advance, Bruker), Raman spectrum (RTS-2, Andor), Scanning electron microscopy (SEM, Regulus-8100, Hitachi) and Transmission Electron Microscope (TEM, with EDX mapping analysis collected by field emission transmission electron microscope TF-G20 of Thermo Fisher Scientific Company), Vibrating Sample Magnetometer (VSM, LakeShore-7404, USA).

Cell Culture and Collection of Exosomes. MCF-7 (breast cancer cells), MDA-MB-231 (breast cancer cells), MCF-10A cells ((human normal breast epithelial cells) were cultured in DMEM (high sugar) medium containing 10% FBS and 1% penicillin streptomycin solution at 37 ℃ and 5% CO_2. HepG2, Hep1-6 and L02 cells (liver cancer cells) were cultured in 1640 (high sugar) medium containing 10% FBS and 1% penicillin streptomycin solution at 37 ℃ and 5% CO_2. All cell lines were obtained from the cell bank of the Chinese Academy of Sciences (Shanghai, China). The cell culture medium was collected after the cells are filled with culture dishes and centrifugated at 3000 rpm to obtain the supernatant for subsequent experiments (n = 10). Then, take 1 mL of cell culture medium and add serum free medium containing Fe_3O_4/Au nanoparticles (0.5 nM) for co-incubation. After 5 h, magnetic separation was carried

out and the sediment was collected.

For comparison with the exosome extraction method of ultracentrifugation, the cell culture supernatant was centrifuged at 120 000g for 2 h using a CS100FNX instrument with an S50ST swing rotor. Then, the pellets of exosomes were gathered and then resuspended in 1× PBS. The mixture was then centrifuged at 100 000g for 70 min. Next, the exosome pellets were resuspended in 1× PBS. The collected exosome pellets were dispersed in PBS solution (500 μL) as the model samples and stored at −20 °C for further use.

Fluorescence Dyeing Experiment. In this work, Dio was used as a cell membrane dye to label exosomes. First, collect the precipitates containing exosomes according to the above steps and disperse them in PBS. Dio was configured as anhydrous DMSO storage solution with a concentration of 5 mM. Then dilute it to 30 μM with PBS for subsequent staining. 30 μM Dio was added into 1 mL PBS dispersion containing exosomes, mixed evenly and incubated at 37 ℃ in dark for 8 min. After incubation, the supernatant was discarded by magnetic separation, and PBS was used to suspend the exosomes again. After repeated three times, the exosomes were observed under confocal fluorescence microscope (LSM 880, Nikon).

SERS Detection. The exosomes precipitate that took in Fe_3O_4/Au nanoparticles was transferred to the capillary tube and placed under the confocal optical microscope (Andor Raman spectrometer, Zolix, China) for further observation and experiment. In

all experiments, a 40x objective lens with a long working distance (Olympus, Japan) and 638 nm laser for excitation were used. The laser power was fixed at 10 mW, and the exposure time was 10 s. When measuring the spectrum, the focus is fixed. Each spectrum was the average value after two collections. Each sample collects at least three signals.

Animal models and serum acquisition. All experiments were approved in compliance with the policy on animal use and ethics established by the Animal Experimentation Ethics Committee of China Pharmaceutical University (Approval Number: SYXK 2021–0011). Six to eight weeks old female BALB/c mice were purchased from the Institute of Comparative Medicine Yangzhou University. 1×10^6 4T1 breast cells were injected into the fourth breast fat pad of the mice for two weeks to establish the tumor-bearing model. Then, serum samples were collected from healthy mice and mice with breast cancer (n = 10). Take 0.1 mL of serum samples and add Fe_3O_4/Au nanoparticles (0.5 nM) for co-incubation. After 5 h, magnetic separation was carried out and the sediment was collected.

Principal Component Analysis (PCA). PCA was used to reduce the dimensions of a dataset while maintaining the characteristics of the dataset that contributed the most to the square difference. Through processing, the most important aspects of the data could be retained. For the spectra collected in each experiment, several variables were selected from the Raman shifts from 500 to 3000 cm^{-1}. Then use the built-in function

in the Origin 2019 data processing software to perform principal component analysis on these spectral data. One point in the obtained analysis data image represented a spectrum, and the elliptical range represented the 95% confidence interval boundary of different data.

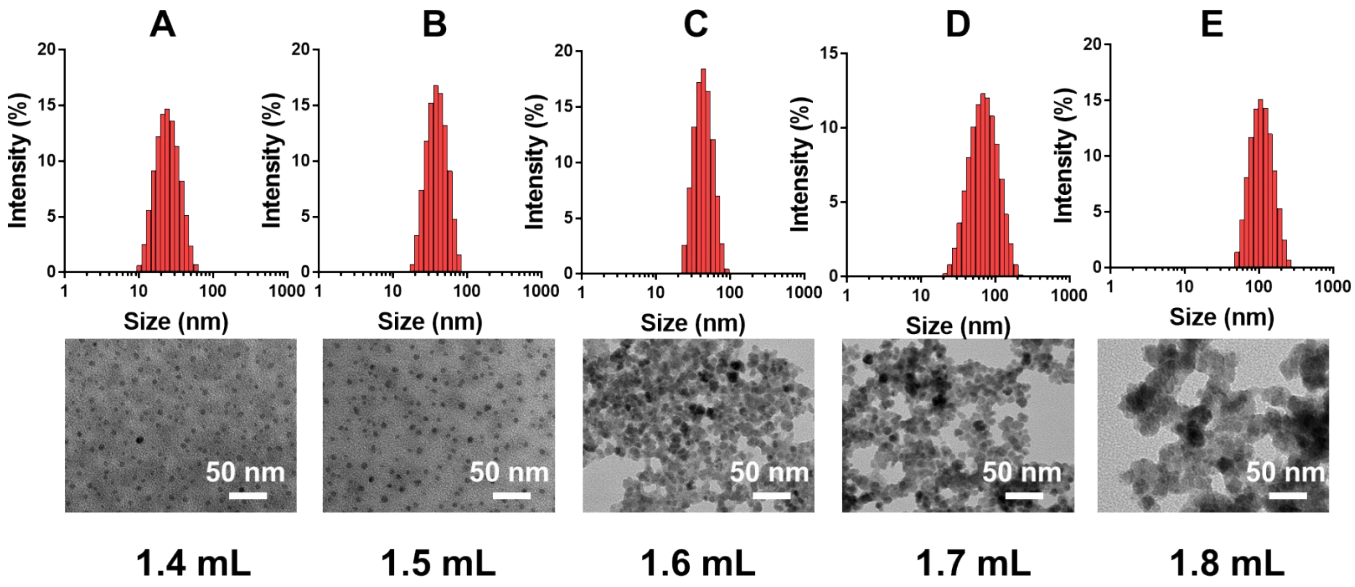
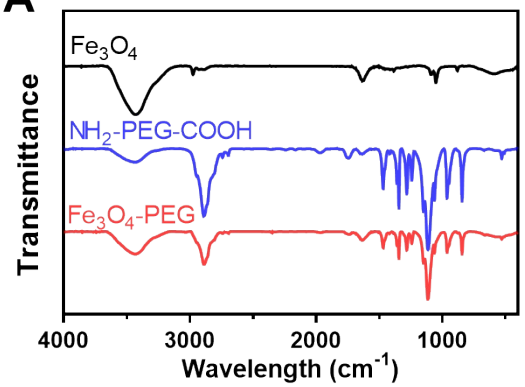


Fig. S1 Size distribution curves and TEM images of Fe_3O_4 NPs prepared using different amount of NaOH/DEG solution (A-E: From 1.4 mL to 1.8 mL). The size of Fe_3O_4 NPs increased from 5 nm to 40 nm (The hydration particle size determined by DLS was larger than TEM), indicating that NaOH/DEG solution could promote the formation and growth of Fe_3O_4 NPs.

A



B

Wavelength (cm^{-1})	Assignment
2700-2900; 1200-1400	C-H
1600-1700	C=O
650-900	N-H
3400-3600	-OH
570	Fe-O

Fig. S2 (A) FTIR analysis. (B) Peak Attributions. The Fe_3O_4-PEG spectrum showed both the characteristic peaks of Fe_3O_4 and NH_2-PEG-COOH, suggesting the successful modification of -COOH onto the surface of Fe_3O_4 NPs.

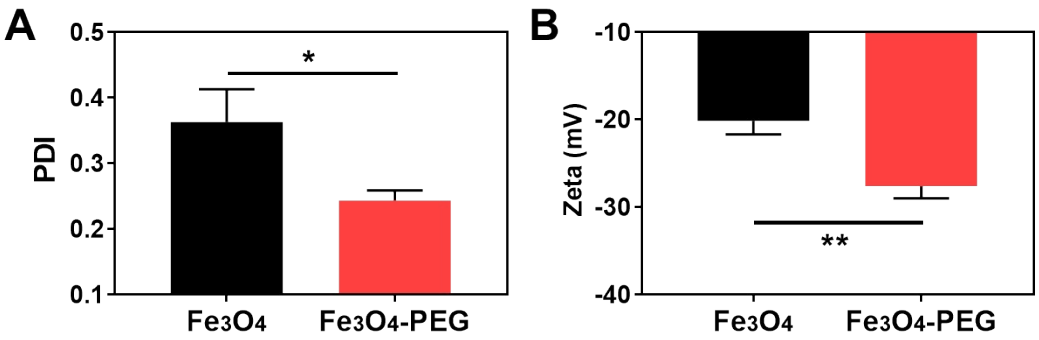


Fig. S3 The polydispersity index (PDI) and Zeta potential determination of Fe_3O_4 and Fe_3O_4-PEG. *$p < 0.05$, **$p < 0.01$.

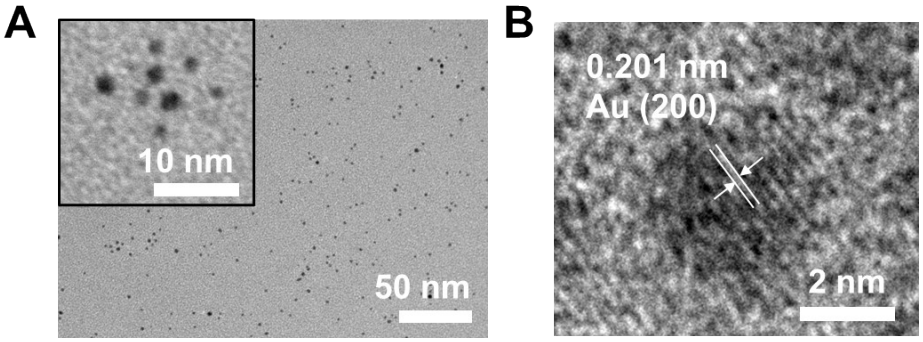


Fig. S4 (A) TEM images of Au NPs prepared using sodium borohydride reduction method. (B) HRTEM images of Au NPs.

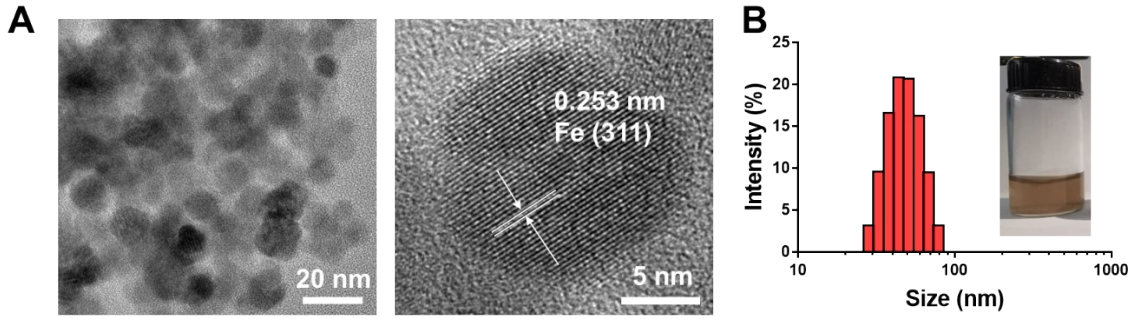


Fig. S5 (A) The TEM image of Fe_3O_4 NPs. (B) The size distribution curve of Fe_3O_4 NPs. The inset showed the graph of the Fe_3O_4 NPs solution.

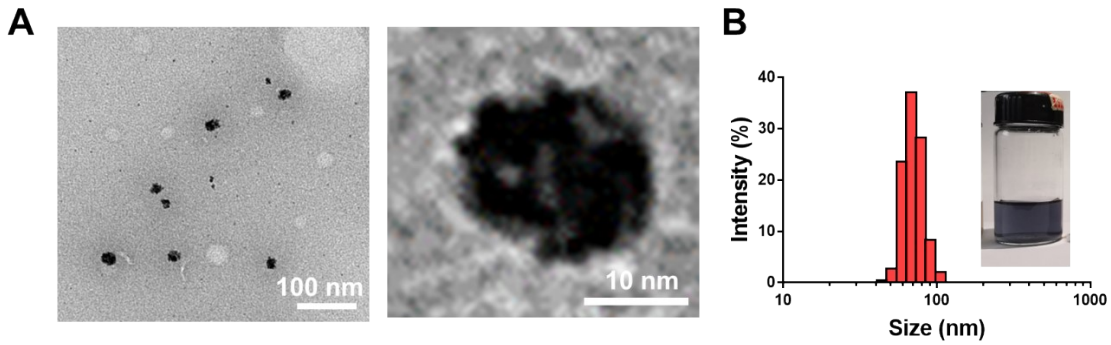


Fig. S6 (A) The TEM image of Fe_3O_4/Au NPs. (B) The size distribution curve of Fe_3O_4/Au NPs. The inset showed the graph of the Fe_3O_4/Au NPs solution.

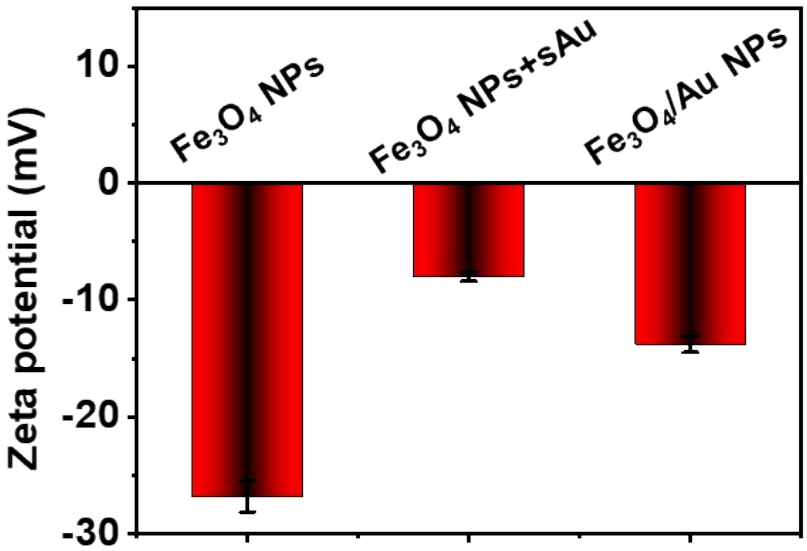


Fig. S7 Zeta potential of Fe_3O_4 NPs, Fe_3O_4+sAu, Fe_3O_4/Au NPs.

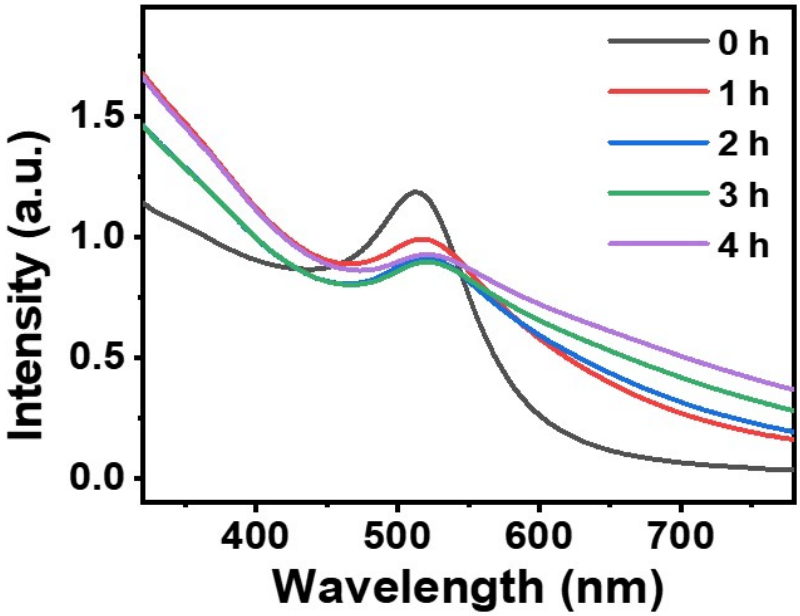


Fig. S8 UV-Visible absorption spectra of Fe_3O_4 NPs incubated with Au NPs for different time.

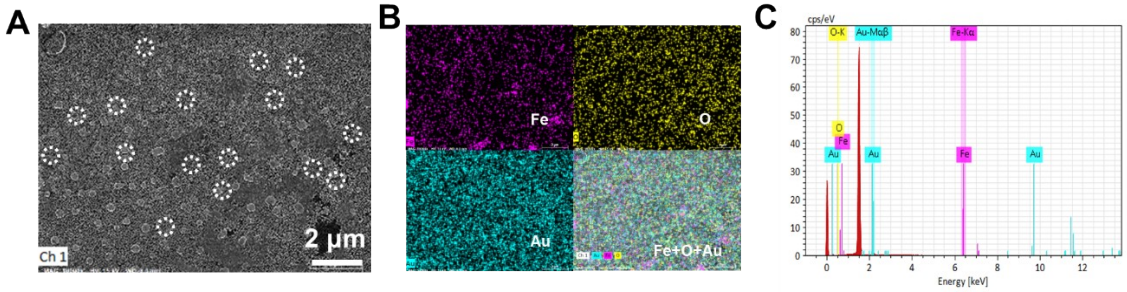


Fig. S9 (A-B) SEM images of Fe_3O_4/Au NPs and the distribution mapping of Fe, O, Au elements. (C) Distribution situation of the Fe, O and Au elements.

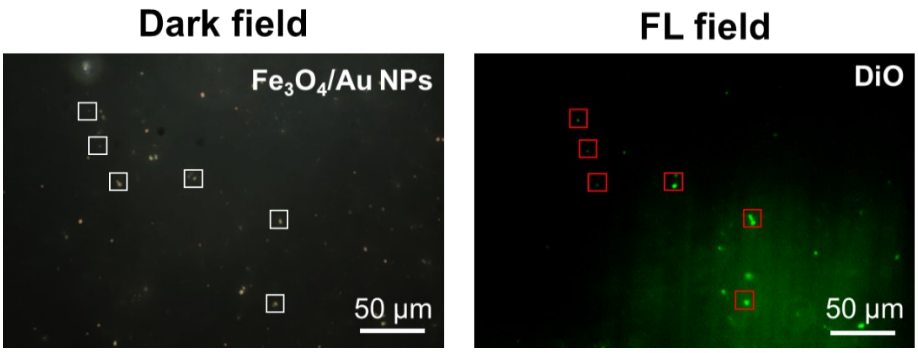


Fig. S10 Colocalization of Fe_3O_4/Au NPs and exosomes derived from HepG2 cell lines. The membrane of exosomes was labeled with DiO (green fluorescence).

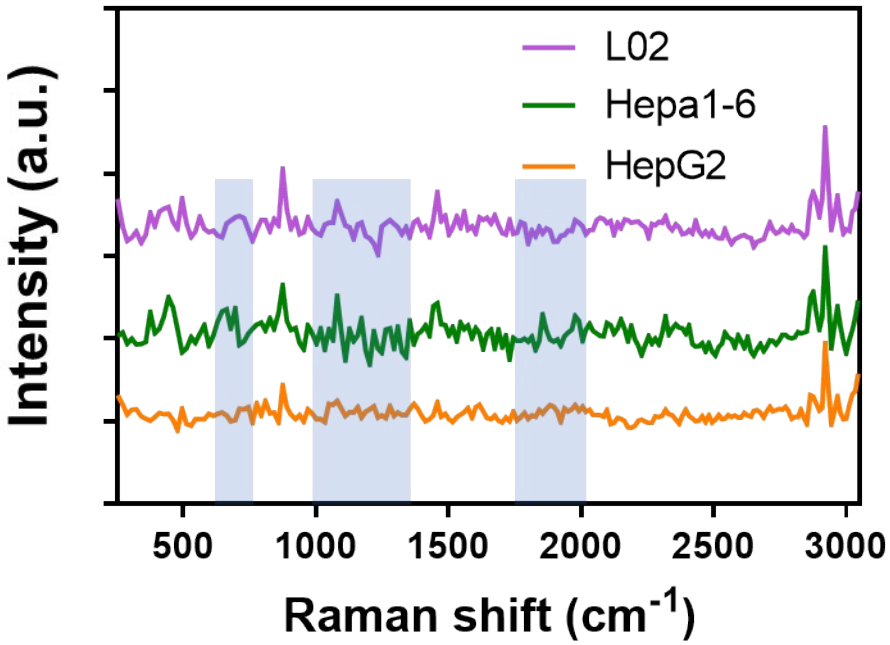


Fig. S11 SERS spectra of exosomes secreted by different liver cancer cell, including HepG2 (liver cancer cells), Hep1-6 (liver cancer cells) and L02 (normal liver cells).

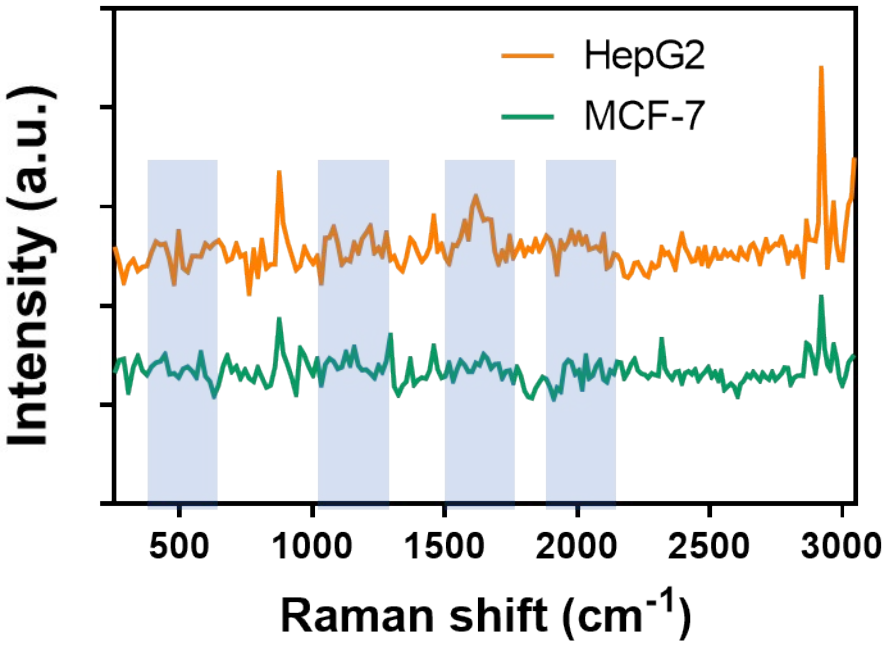


Fig. S12 SERS spectra of exosomes secreted by different cancer cell lines, including HepG2 (liver cancer cells) and MCF-7 (breast cancer cells).

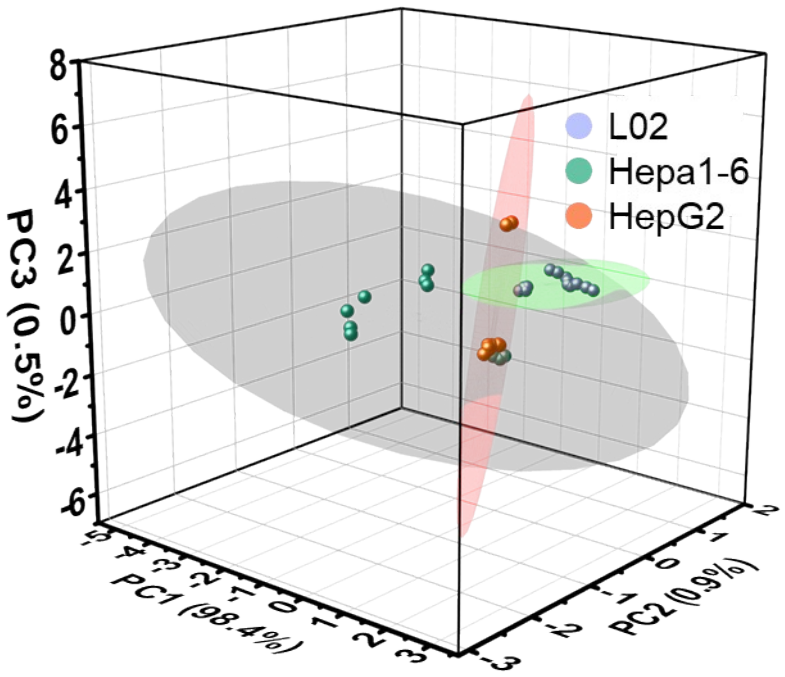


Fig. S13 PCA of SERS data sets. Data could be classified using 95% confidence zone ellipses. When the analyzed sample appeared in an ellipse, it meant that the sample belonged to this group at 95% confidence. The difference displayed in the data was analyzed by PC1, PC2 and PC3 (accounting for 98.4%, 0.9%, 0.5% respectively), accounting for 99.8% of the total difference.

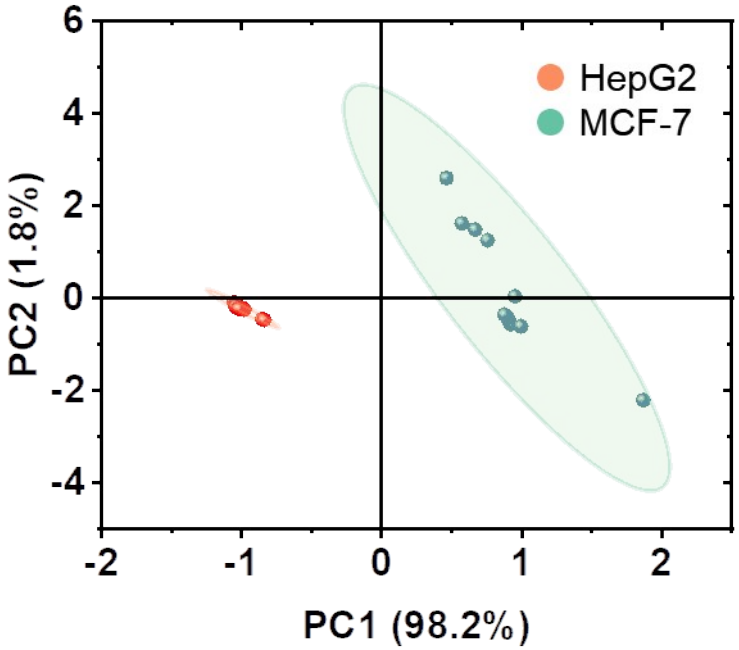
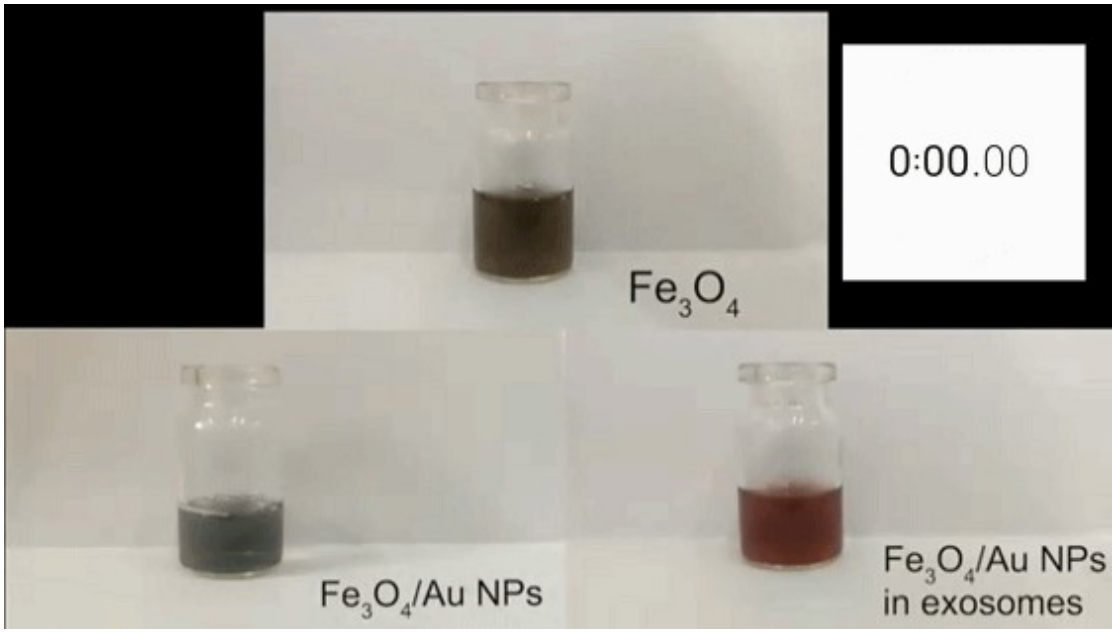


Fig. S14 PCA of SERS data sets. The difference displayed in the data was analyzed by PC1 and PC2 (accounting for 98.2%, 1.8% respectively), accounting for 100.0% of the total difference. The results showed 100% sensitivity and specificity, and could successfully distinguish the exosomes secreted by different cancer cell lines through SERS.



Video S1 The magnetic separation process of both Fe_3O_4 and Fe_3O_4/Au NPs within 1 min.

Table S1 Peak attributions of XRD patterns.

2θ (degree)	**Attributions**	**2θ (degree)**	**Assignment**
30.1	Fe_3O_4 (220)	38.3	Au (111)
35.5	Fe_3O_4 (311)	44.5	Au (200)
43.1	Fe_3O_4 (400)	64.2	Au (220)
53.4	Fe_3O_4 (422)	77.8	Au (311)
57.0	Fe_3O_4 (511)		
62.6	Fe_3O_4 (440)		

Table S2 Peak attributions of Raman spectra from exosomes.

Raman shift (cm^{-1})	**Biomolecule**	**Raman shift (cm^{-1})**	**Biomolecule**
782-792	Nucleic acids (Pyrimidine C, T, U ring)	1174-1177	Proteins (Tyr)
852-870	Proteins (Tyr)	1570-1580	Nucleic acids (Purine A, G ring)
930-960	Proteins (α-Helix backbone)	1670-1690	Nucleic acids (ν(C=O) in pyrimid
1004	Proteins (Phe)	2950-3100	Lipid

Table S3 Peak comparisons of Raman spectra between exosomes from different breast cell lines.

Raman shift (cm^{-1})	Biomolecules	Attributions
588	Proteins	Phe, Tyr
880	Proteins	Trp, Tyr
1229	Nucleic acids	U, C ring; Sugar puckering
1648	Lipids	*ν*(C=C) in acyl chain

Table S4 Peak comparisons of Raman spectra between exosomes from different liver cell lines.

Raman shift (cm^{-1})	Biomolecules	Attributions
713	Proteins	Met
1095	Nucleic acids	Phosphodioxy $\nu_s(PO_2^-)$
1304	Proteins	Backbone $\delta(C_\alpha H)$, $\nu(C_\alpha\text{-}C)$
1855	Lipids	ν(C=C) in acyl chain

Table S5 Peak comparisons of Raman spectra between exosomes from HepG2 and MCF-7 cell lines.

Raman shift (cm^{-1})	Biomolecules	Attributions
611	Proteins	Phe, Tyr
1222	Nucleic acids	U, C ring; Sugar puckering
1616	Proteins	Tyr
1944	Lipids	*v*(C=C) in acyl chain

Table S6 Peak comparisons of Raman spectra between exosomes from healthy and breast-cancer-suffered mice.

Raman shift (cm^{-1})	Biomolecules	Attributions
600	Proteins	Phe, Tyr
1068	Nucleic acids	Phosphodioxy $\nu_s(PO_2^-)$
1642	Lipids	ν(C=C) in acyl chain

Table S7 Comparisons of the existed methods for isolation and detection of exosomes from biological samples.

Isolation methods	Detection methods	Materials	Target markers	Analysis time	Costs	Ref
ExoQuick kit; miRNeasy Micro Kit	Electrochemical	TiO_2@MoS_2 quantum dots	RNA	~3 h	Need primers design; expensive isolation kits	3
Ultracentrifugation	ICP-MS	Au NPs@ up-conversion NPs	Surface markers	~5 h	Need aptamers modification	4
Ultracentrifugation	Fluorescence	Nano-engineered ExoProfile Chip	Surface markers	~18 h	Microfluidic chips are complex and expensive	5
Ultracentrifugation	Colorimetry	Aptamer/AuNPs	Surface markers	~5 h	Need aptamers modification	6
Ultracentrifugation	Electrogenerated Chemiluminescence	g-C3N4@ Galinstan-PDA nanoprobes	Surface markers	~8 h	Need antibodies modification	7
Total Exosome Isolation™ kit	Fluorescence	MLCHA-based photonic crystals	miRNAs	~5 h	Need aptamers modification; expensive isolation kits	8
Ultracentrifugation	SPR	Aptamer/AuNPs	Surface markers	~10 h	Need aptamers modification	9
Magnetic separation	SERS	Fe_3O_4/Au NPs	Exosome content	~5 h	Without capture units modification	This work

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