Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Phenotypic profiling of pancreatic ductal adenocarcinoma plasma-derived small extracellular vesicles for cancer diagnosis and cancer stage prediction: a proof-of-concept study

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Table S1 Particle size and concentration of sEVs measured by NTA

Sample No.	Particle size (nm)	Concentration of sEVs in plasma (particles/mL)
H1 (Healthy sample)	82.1 ± 4.1	(3.62 ± 0.27) ×10^8
H2	94.1 ± 3.5	(2.75 ± 0.15) ×10^8
H3	77.1 ± 9.5	(7.12 ± 0.07) ×10^8
H4	94.1 ± 3.5	(4.47 ± 0.13) ×10^8
H5	66.4 ± 1.6	(6.65 ± 0.67) ×10^8
H6	88.8 ± 5.7	(3.10 ± 0.70) ×10^8
P1 (patient sample)	95.1 ± 1.5	(2.35 ± 0.07) ×10^9
P2	52.1 ± 27.8	(6.75 ± 0.46) ×10^9
P3	103.0 ± 7.9	(2.59 ± 0.14) ×10^9
P4	57.7 ± 33.4	(1.49 ± 0.09) ×10^9
P5	75.5 ± 2.7	(1.60 ± 0.03) ×10^9
P6	88.8 ± 5.7	(2.77 ± 0.78) ×10^9
P7	83.0 ± 3.7	(7.90 ± 0.48) ×10^8
P8	97.3 ± 2.4	(2.52 ± 0.16) ×10^9
P9	76.2 ± 3.2	(1.15 ± 0.12) ×10^9

Table S2 Total sEV protein concentration measured by Micro BCA

Sample No.	Protein concentration in plasma (μg/mL)			Ave (μg/mL)
H1	41.2	35.7	32.6	36.5 ± 4.4
H2	35.8	33.3	47.2	38.8 ± 7.4
H3	39.2	32.8	43.4	38.5 ± 5.3
H4	28.5	47.7	38.3	38.2 ± 9.6
H5	41.2	37.3	47.5	42.0 ± 5.1
H6	36.5	40.8	44.3	40.5 ± 3 .9
P1	77.6	65.7	81.4	74.9 ± 8.2
P2	68.5	70.3	65.9	68.2 ± 2.2
P3	60.4	58.8	70.2	63.1 ± 6.2
P4	63.6	48.4	53.6	55.2 ± 7.7
P5	50.8	44.7	40.5	45.3 ± 5.2
P6	44.1	38.9	45.8	42.9 ± 3.6
P7	42.4	40.2	46.1	42.9 ± 3.0
P8	55.3	45.4	51.2	50.6 ± 5.0
P9	48.4	42.5	38.9	43.3 ± 4.8

Table S3 The normalized SERS signal of individual biomarkers and the average sum of these biomarkers (phenotypic signature) (deviation of signature values were calculated based on $\sqrt{E_1^2 + E_2^2 + E_3^2}$, E1, E2 and E3 were deviations of normalized SERS signal of Glypican 1, EpCAM and CD44V6, respectively)

Sample No.	Glypican 1	EpCAM	CD44V6	Sum
H1	0.07 ± 0.00	0.22 ± 0.06	0.10 ± 0.01	0.39 ± 0.06
H2	0.02 ± 0.02	0.08 ± 0.06	0.04 ± 0.05	0.14 ± 0.08
H3	0.01 ± 0.01	0.07 ± 0.03	0.03 ± 0.02	0.11 ± 0.04
H4	0.00 ± 0.00	0.08 ± 0.03	0.00 ± 0.00	0.08 ± 0.03
H5	0.02 ± 0.01	0.11 ± 0.02	0.04 ± 0.01	0.17 ± 0.02
H6	0.02 ± 0.02	0.11 ± 0.03	0.03 ± 0.02	0.16 ± 0.04
H7	0.04 ± 0.00	0.12 ± 0.01	0.05 ± 0.00	0.21 ± 0.01
H8	0.04 ± 0.01	0.07 ± 0.02	0.07 ± 0.02	0.18 ± 0.03
H9	0.06 ± 0.02	0.21 ± 0.04	0.07 ± 0.03	0.34 ± 0.05
H10	0.08 ± 0.04	0.11 ± 0.03	0.08 ± 0.03	0.27 ± 0.06
H11	0.03 ± 0.00	0.11 ± 0.02	0.07 ± 0.03	0.21 ± 0.04
H12	0.06 ± 0.06	0.10 ± 0.06	0.07 ± 0.06	0.23 ± 0.10
H13	0.06 ± 0.02	0.21 ± 0.04	0.10 ± 0.04	0.37 ± 0.06
P1	0.89 ± 0.54	1.00 ± 0.66	0.68 ± 0.48	2.57 ± 0.98
P2	0.64 ± 0.10	0.74 ± 0.11	0.49 ± 0.13	1.87 ± 0.20
P3	0.29 ± 0.25	0.54 ± 0.44	0.39 ± 0.27	1.22 ± 0.57
P4	0.29 ± 0.06	0.50 ± 0.10	0.34 ± 0.09	1.13 ± 0.15
P5	0.16 ± 0.01	0.31 ± 0.01	0.09 ± 0.09	0.56 ± 0.09
P6	0.12 ± 0.01	0.29 ± 0.04	0.08 ± 0.01	0.49 ± 0.04
P7	0.13 ± 0.03	0.18 ± 0.04	0.10 ± 0.00	0.41 ± 0.05
P8	0.05 ± 0.01	0.46 ± 0.12	0.11 ± 0.02	0.62 ± 0.12
P9	0.06 ± 0.01	0.31 ± 0.17	0.12 ± 0.00	0.49 ± 0.17

Table S4 The definitions of 8th edition of TNM staging system of PDAC by AJCC

	Definition	T stage	N stage	M stage	Overall cancer stage
T1	Maximum tumor diameter ≤2 cm	T1	N0	M0	IA
T2	2 cm<Maximum tumor diameter≤4 cm	T2	N0	M0	IB
T3	Maximum tumor diameter>4 cm	T3	N0	M0	IIA
T4	Tumor involves the celiac axis, common hepatic artery or the superior mesenteric artery	T1-T3	N1	M0	IIB
N0	No regional lymph node metastasis	T4(any T)	Any N (N2)	M0	III
N1	Metastasis in 1-3 regional lymph nodes	Any T	Any N	M1	IV
N2	Metastasis in ≥ 4 regional lymph nodes				
M0	No distant metastasis				
M1	Distant metastasis				

Figure S1. Portable Raman reader (EVA3000PLUS, Shanghai Oceanhood Opto-electronics Tech, China).

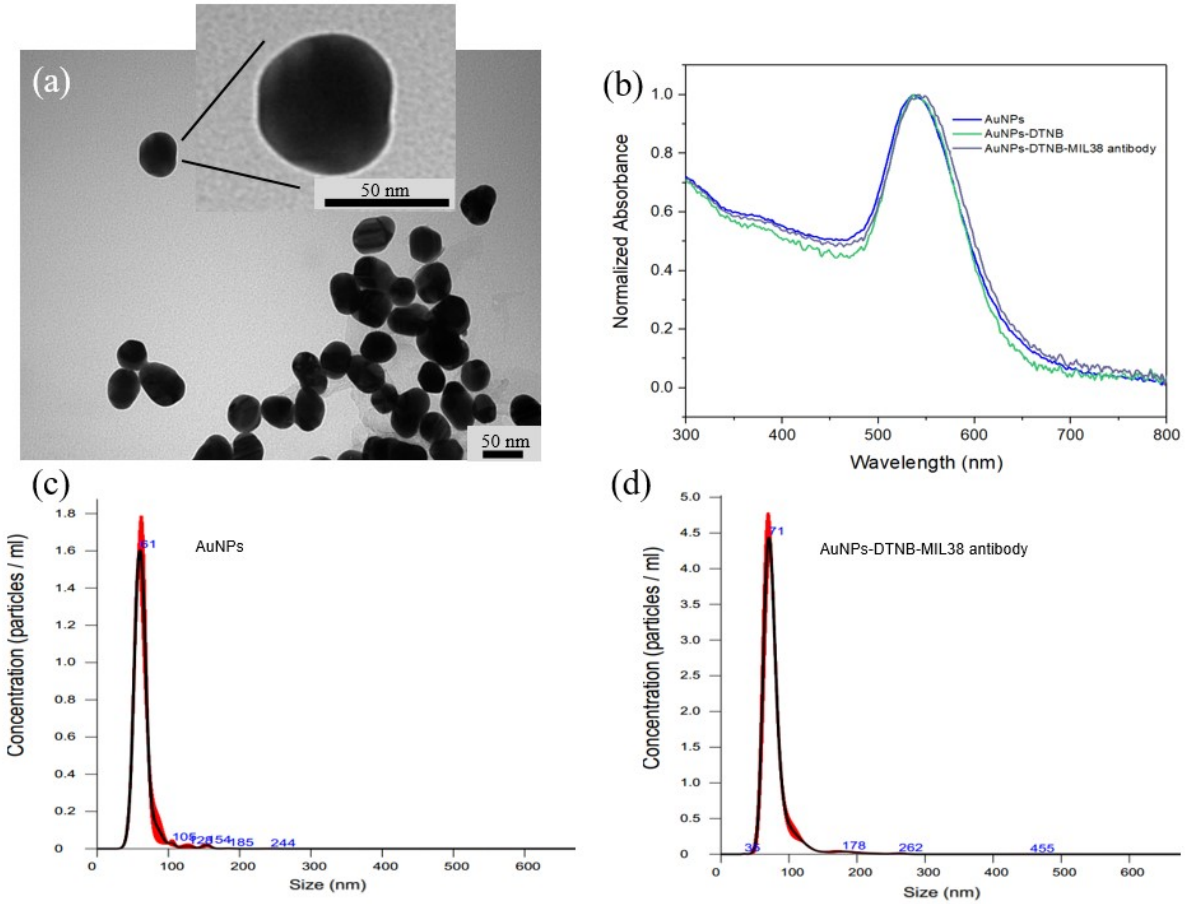

Figure S2. Characterization of SERS nanotags conjugated with MIL38 antibody. (a): TEM images of SERS nanotags with MIL38 antibody, showing round or oval-shaped morphology with a particle size around 65 nm. (b) UV-vis absorption spectra of AuNPs, AuNPs-DTNB and AuNPs-DTNB-MIL-38 antibody (SERS nanotags). The red shift of the UV-Vis absorption spectrum of AuNPs from 537 nm to 539 nm and 543 was seen due to a change of the surrounding refractive index of AuNPs. This confirmed the successful binding of Raman reporter molecules and MIL-38 antibodies onto the AuNPs. (c and d) Particle size distribution of AuNPs and AuNPs-DTNB-MIL-38 antibody measured by NTA. The average size of AuNPs-DTNB-MIL-38 antibody increased to 71 nm from 61 nm (AuNPs), indicating successful conjugation of antibody onto AuNPs.

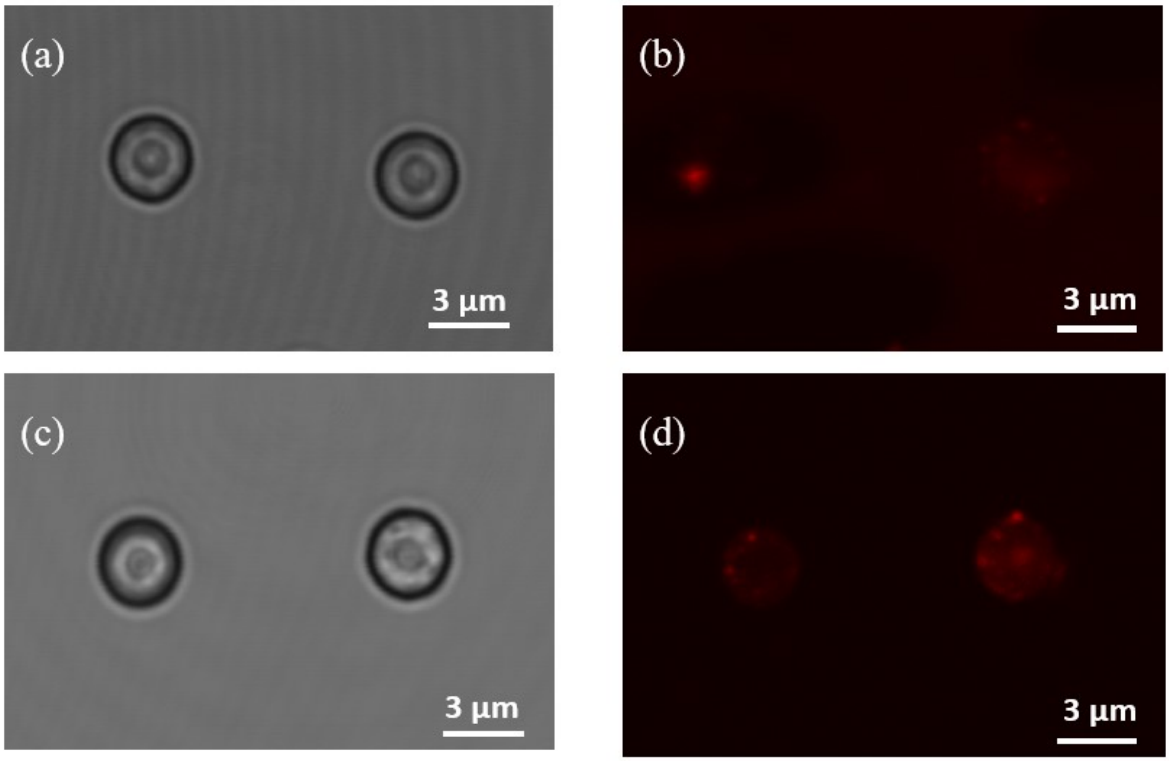

Figure S3. Bright field and confocal fluorescent images of plasma-derived sEVs (stained by DiI) captured by functionalized MBs. (a and b): Control, DiI incubated with functionalized MBs and showed low fluorescence around MBs, indicating low nonspecific binding between DiI and MBs; (c and d): The sEVs captured by functionalized MBs were stained with DiI have strong fluorescence around MBs, indicating the presence of sEVs on MBs.

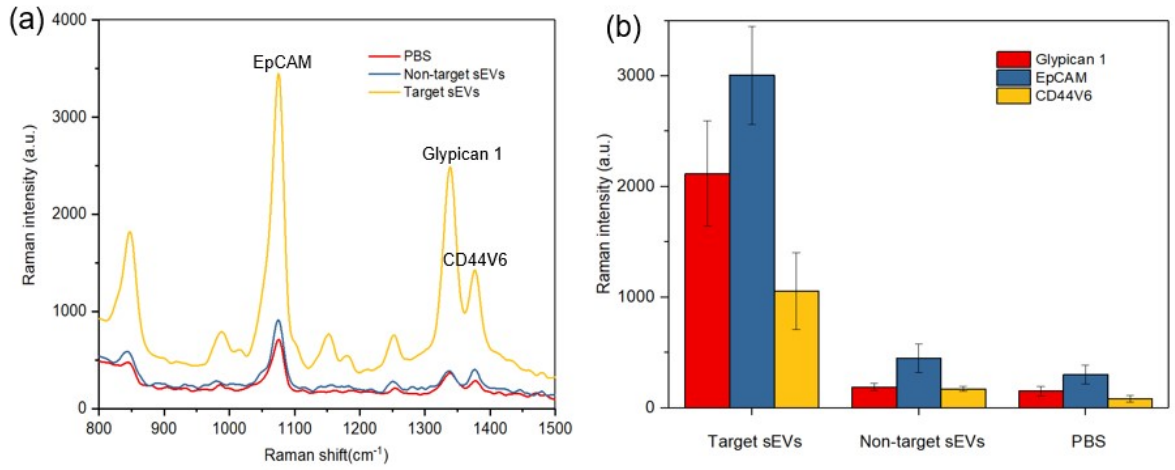

Figure S4. Specificity study of the SERS assay. (a): Raman spectra of PBS, non-target sEVs and target sEVs. (b) Histogram of Raman intensity corresponding to PBS, non-target sEVs and target sEVs. The low SERS signal from PBS and non-target sEVs demonstrated the high specificity of the proposed SERS assay. Target sEVs: patient plasma containing CD63 positive sEVs; non-target sEVs: patient plasma without CD63 positive sEVs (CD63 positive sEVs removed by magnetic beads conjugated with CD63 antibody).

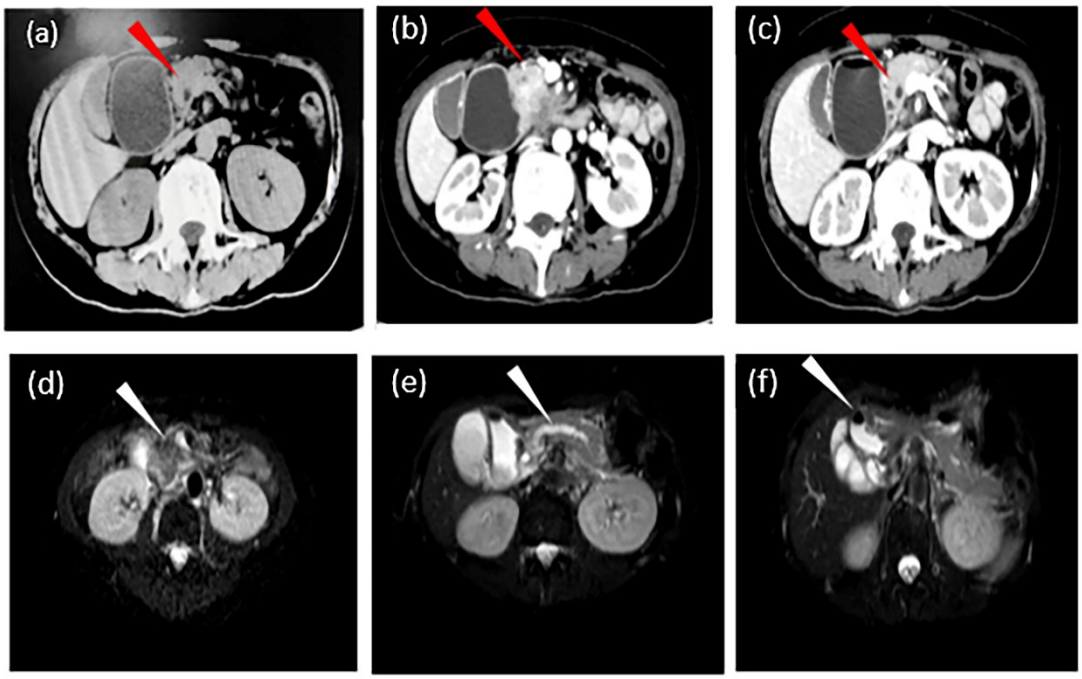

Figure S5. CT and MRI images of P3 (T2N1M0). (a) CT image of pancreas showed the maximum slice size around 31mm*29 mm; (b and c) Enhanced CT images showed dilated descending duodenum, pancreatic duct and bulbous lumen and no abnormality in the size and shape in the neck, body and tail of the pancreas; (d to f) MRI T2 weighted images of pancreas showed dilated distal pancreatic duct and irregular shape of pancreas head.