Electronic Supplementary Information (ESI):

Improving SERS immunoassay for the analysis of ovarian cancer-derived small extracellular vesicles

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Table S1. Summarise of capturing agents in different sEV studies using SERS.

Sources of cancer-derived sEVs	Target capture protein	Ref
SKBR3, T84, and LNCaP	CD63	[1]
SKBR3 and MRC5	CD63	[2]
Panc-1, SW480, and C3	CD63	[3]
HepG2	CD9	[4]
Breast cancer plasma	CD81	[5]

Table S2. LOD of other studies using SERS assay for analysing sEVs

Cancer types	Target molecules	Capturing molecules	LOD	Ref
Prostate cancer	EpCAM	CD63	1.6×10^{-1} particles/µL	[6]
Breast cancer and normal lung cells	HER2	CD63	1.2×10^{3} particles/µL	[2]
Breast cancer, lung cancer, and prostate cancer	H2, CEA, PSMA	CD63	- Breast cancer: 38 particles/µL - Lung cancer: 73 particles/µL - Prostate cancer: 308 particles/µL	[1]
Pancreatic cancer, colorectal cancer, bladder cancer	Glypican-1, EpCAM, CD44v6	CD63	2.3×10^{3} particles/µL	[3]
This work: Ovarian cancer	CA125, EpCAM, CD24	CD9	1.5×10^{5} particles/µL	

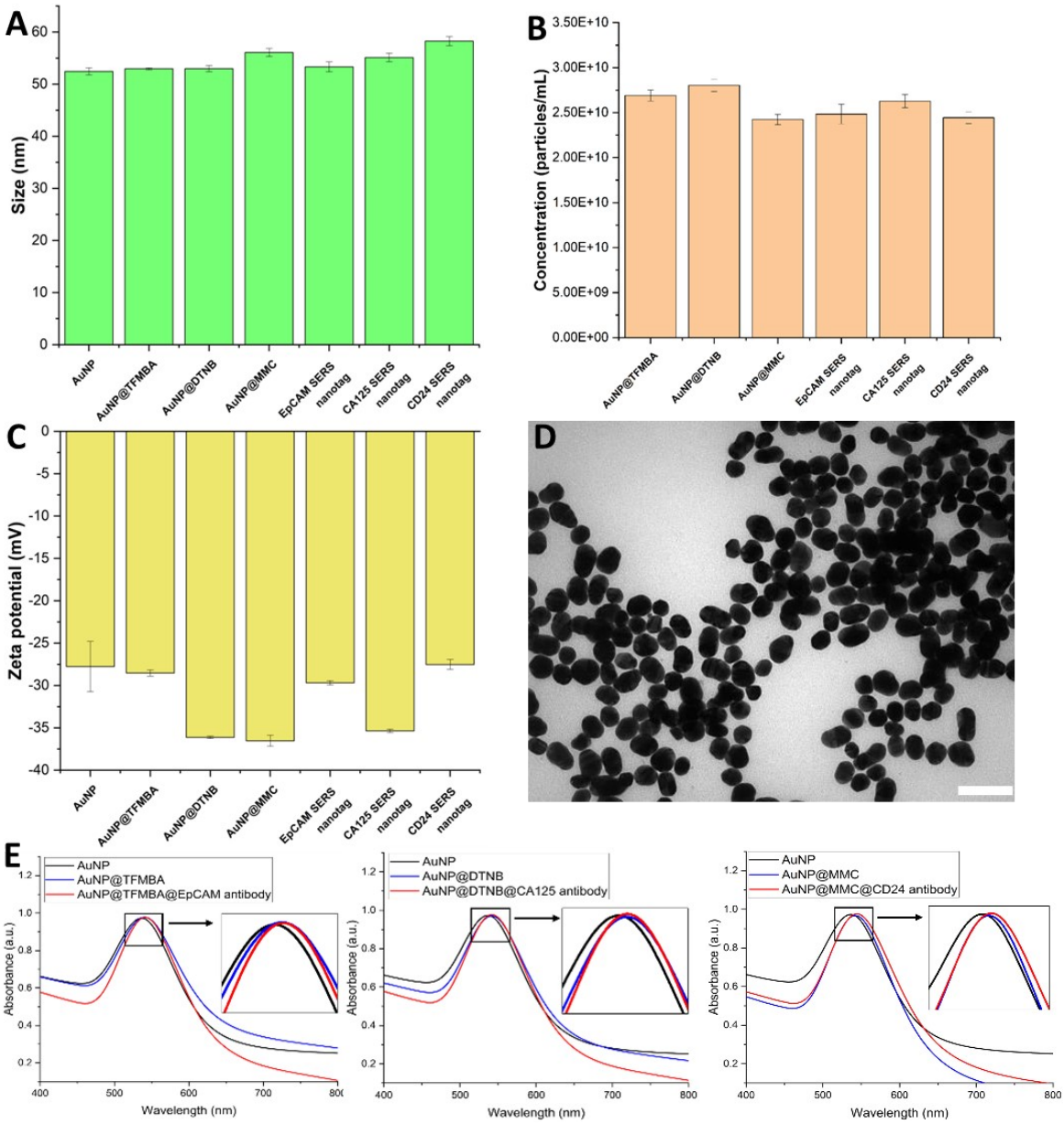

Figure S1. Characterisation of AuNPs, AuNPs@Raman molecules and SERS nanotags by A) size (by NTA); B) concentration (NTA); C) zeta-potential (zetasizer); D) TEM image of AuNP (Scale bar 100 nm); E) UV-vis spectra of AuNP, AuNP@Ra, and SERS nanotags.

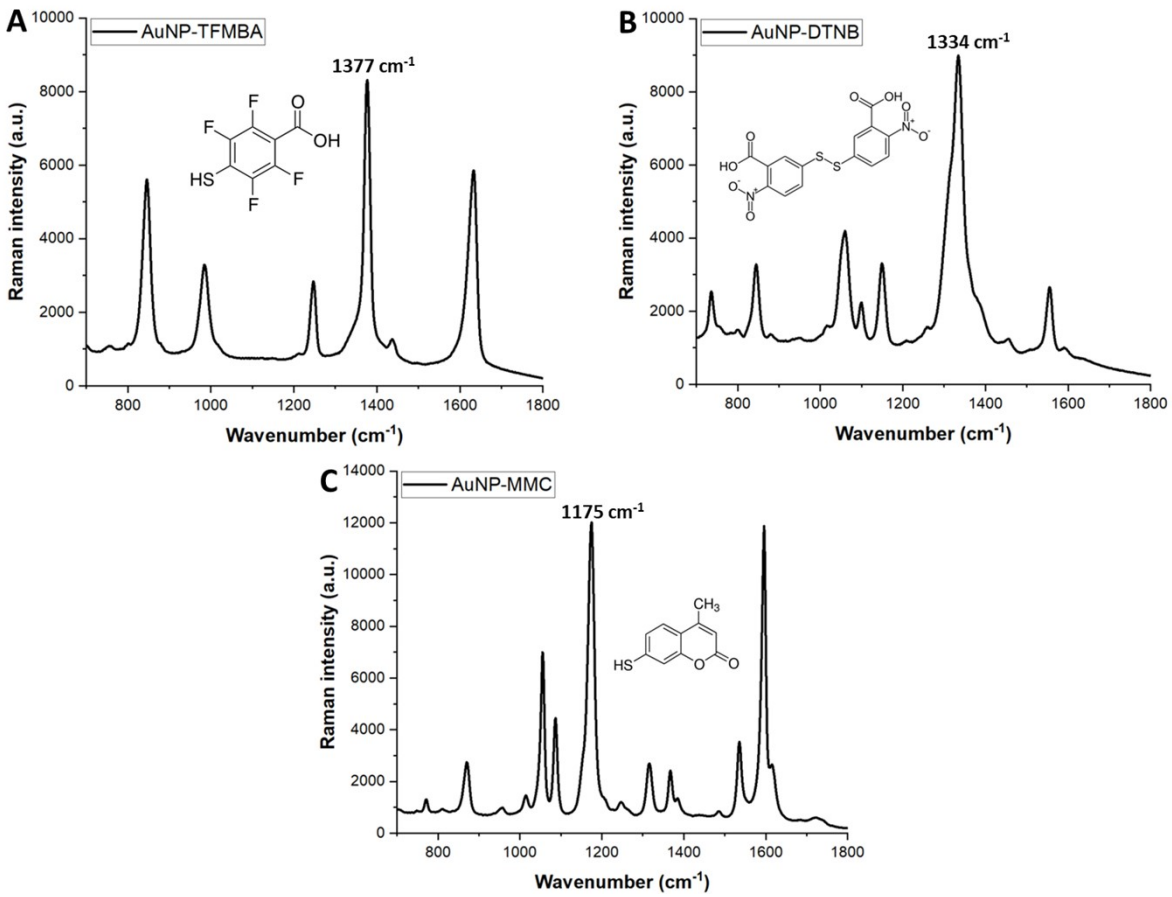

Figure S2. Raman spectra and intensity of gold nanoparticle (AuNP) coating with A) TFMBA Raman molecule, B) DTNB Raman molecules, C) MMC Raman molecules.

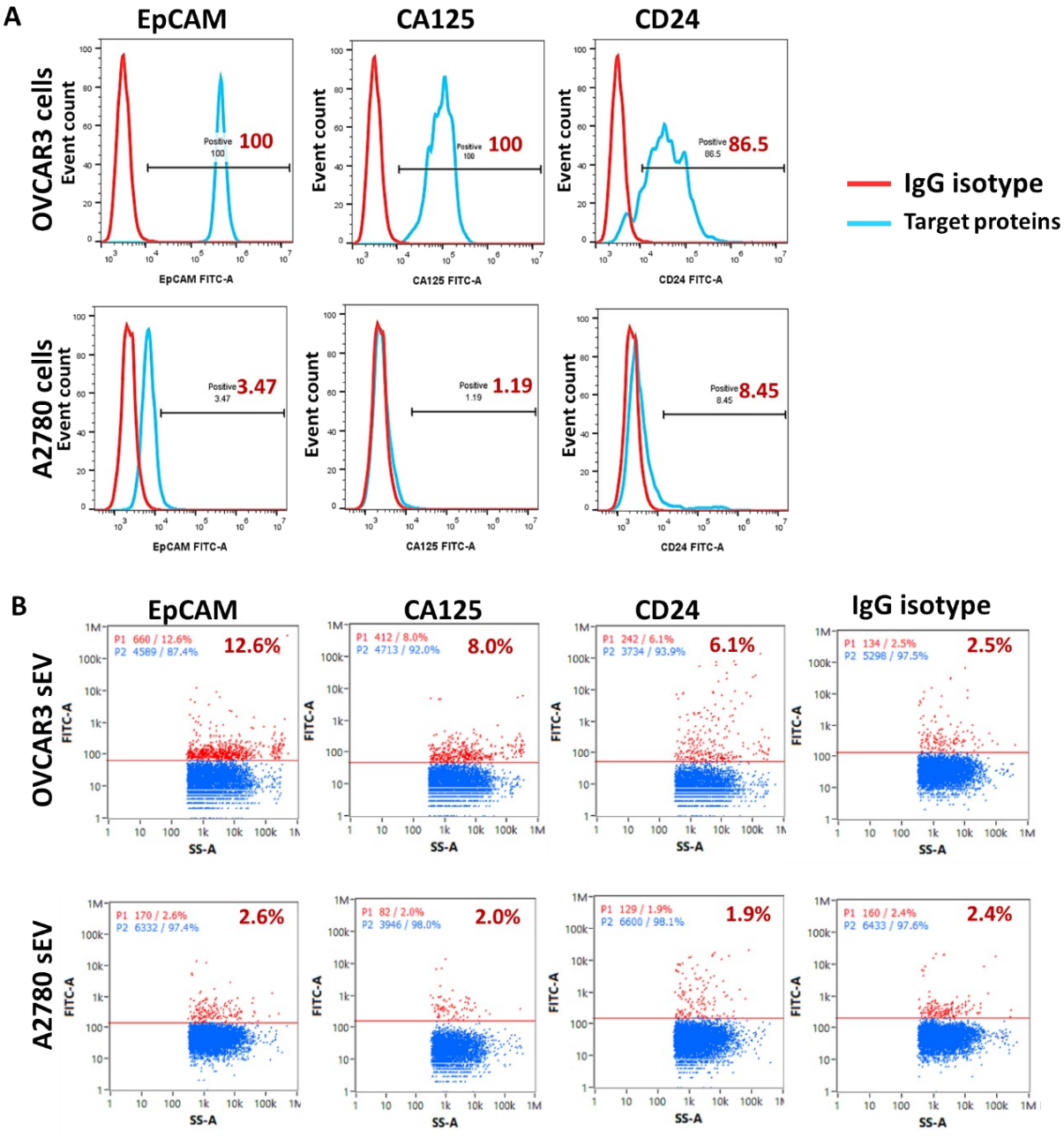

Figure S3. The protein expression level of EpCAM, CA125 and CD24 (A) by flow cytometry in OVCAR3 and A2780 cells, and (B) by NanoFCM in sEVs of OVCAR3 and A2780.

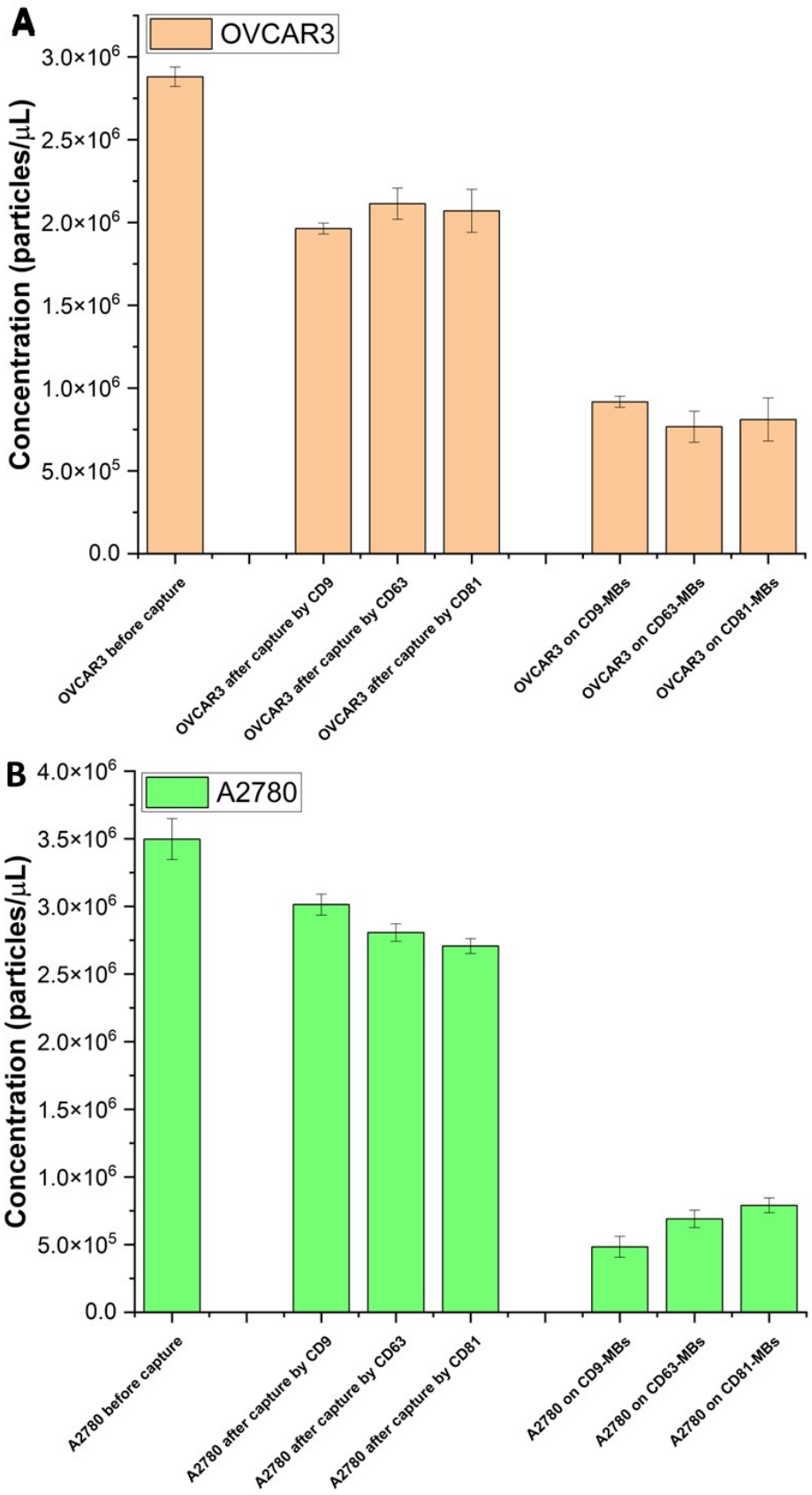

Figure S4. sEVs concentration before and after capturing with different MBs. A) OVCAR3-derived sEVs; B) A2780-derived sEVs.

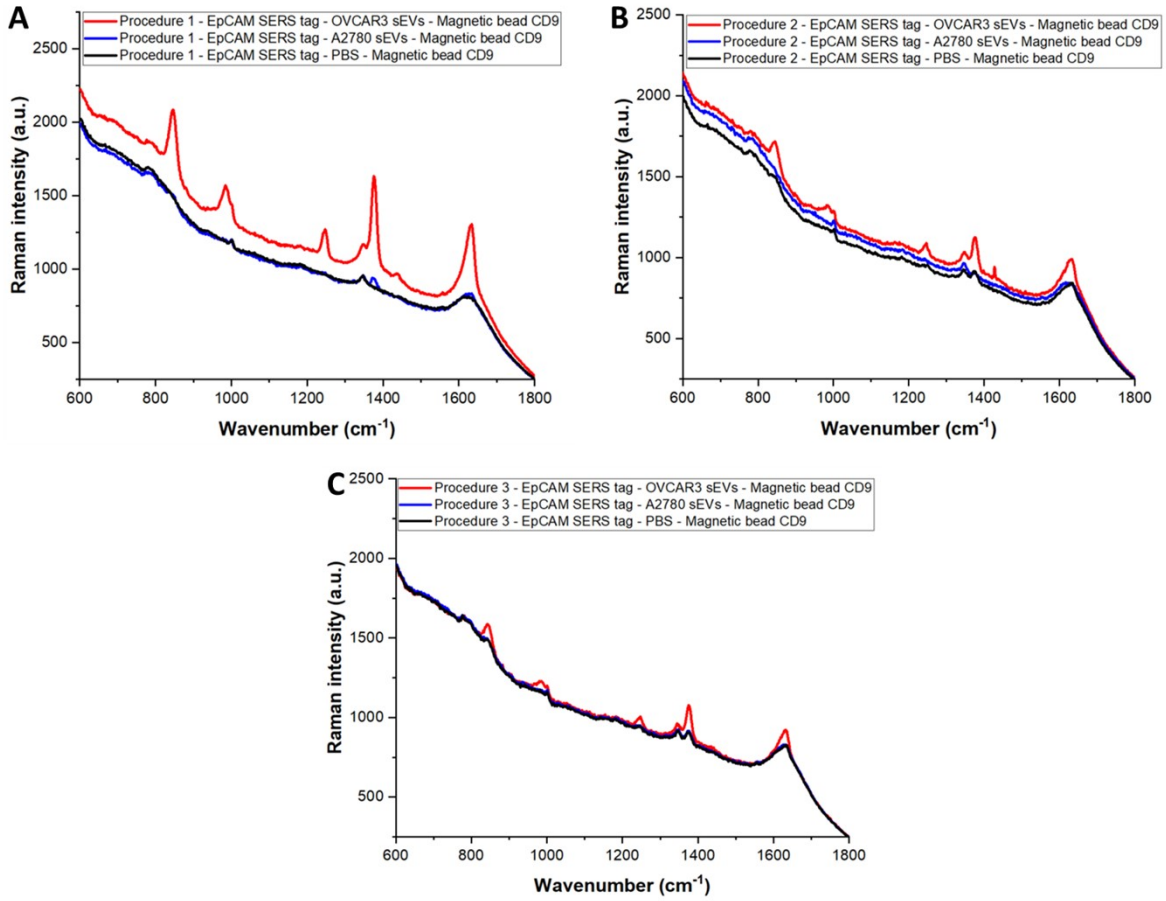

Figure S5. Raman spectrum of different immunocomplex forming procedure A) Procedure 1, B) Procedure 2, C) Procedure 3.

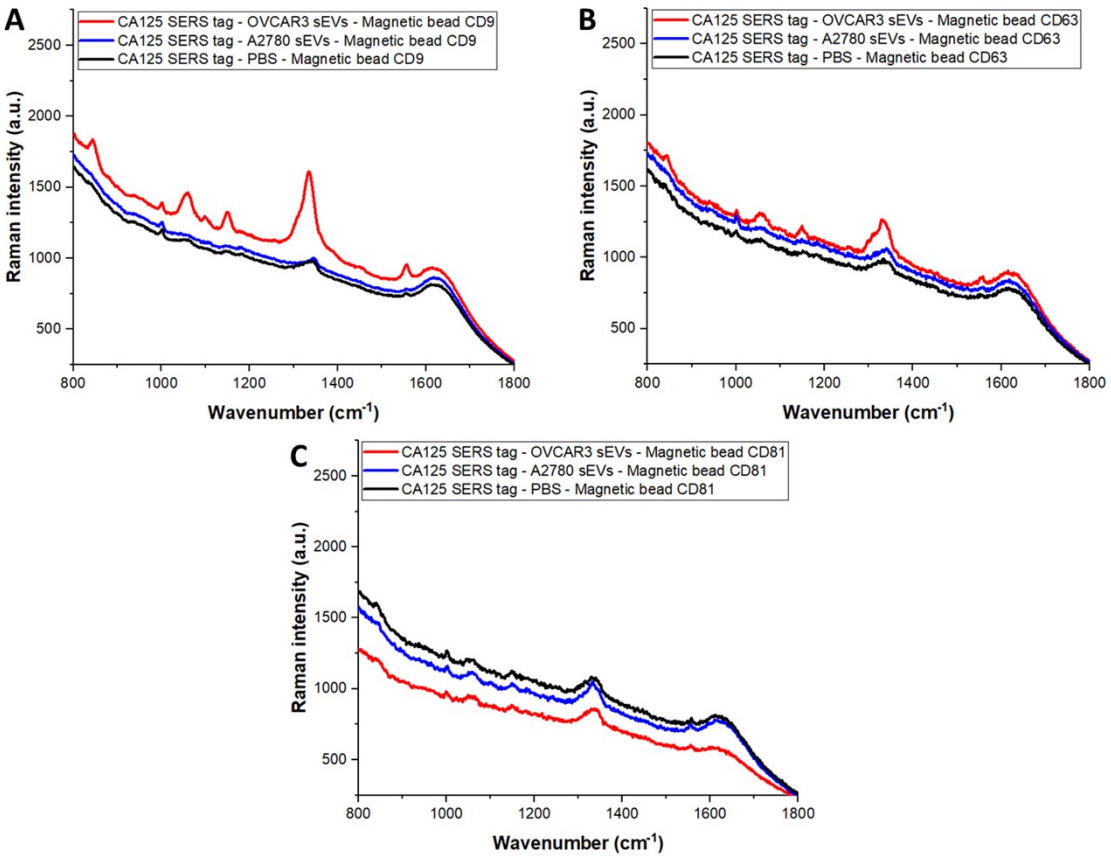

Figure S6. Raman spectrum of anti-tetraspanin antibodies efficiency on capturing cancer-derived sEVs using CA125 SERS nanotags. A) Capturing by magnetic beads conjugated with CD9, B) Capturing by magnetic beads conjugated with CD81, C) Capturing by magnetic beads conjugated with CD63.

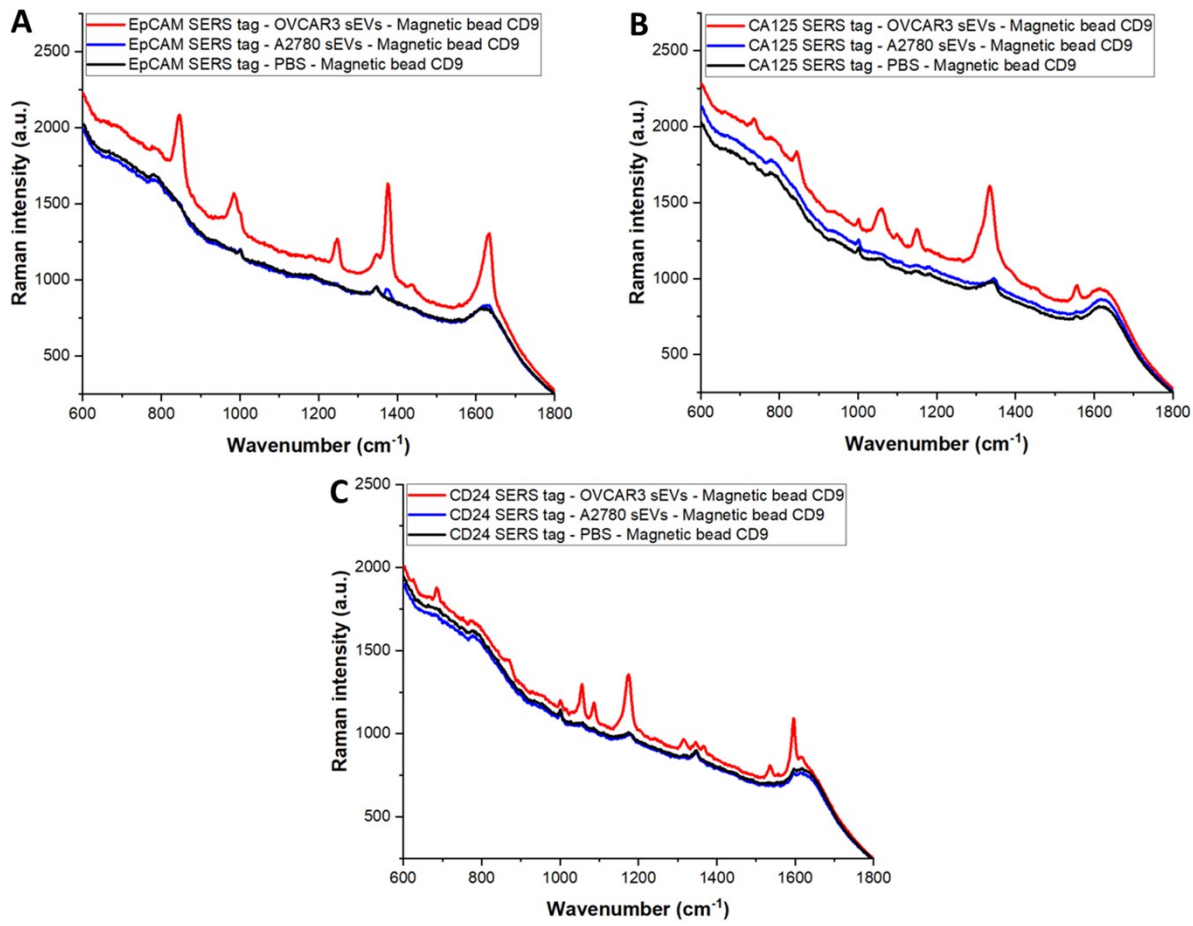

Figure S7. Specificity study of three SERS nanotags on profiling three ovarian cancer biomarkers. A) EpCAM, B) CA125, C) CD24.

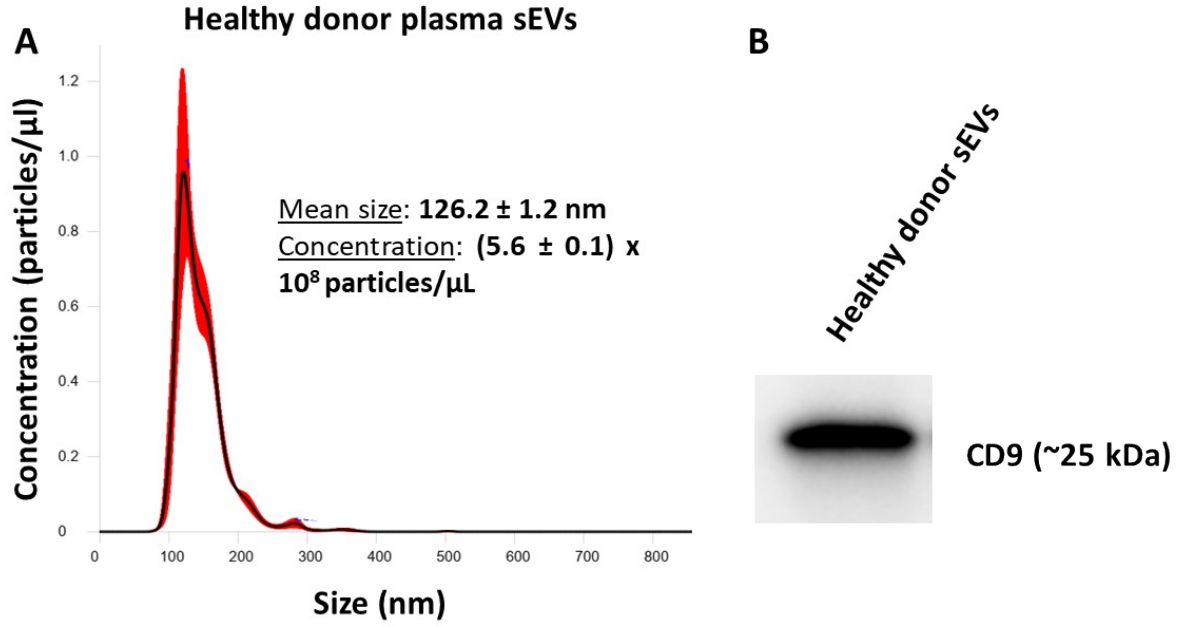

Figure S8. Characterisation of healthy donor plasma sEVs. (A) NTA result on the mean size and concentration of plasma's sEVs; (B) Immunoblotting of healthy donor sEVs with CD9 expression.

References

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