Electronic Supplementary Information

Colorimetric analysis of extracellular vesicles surface proteins based on controlled growth of Au aptasensors

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Results

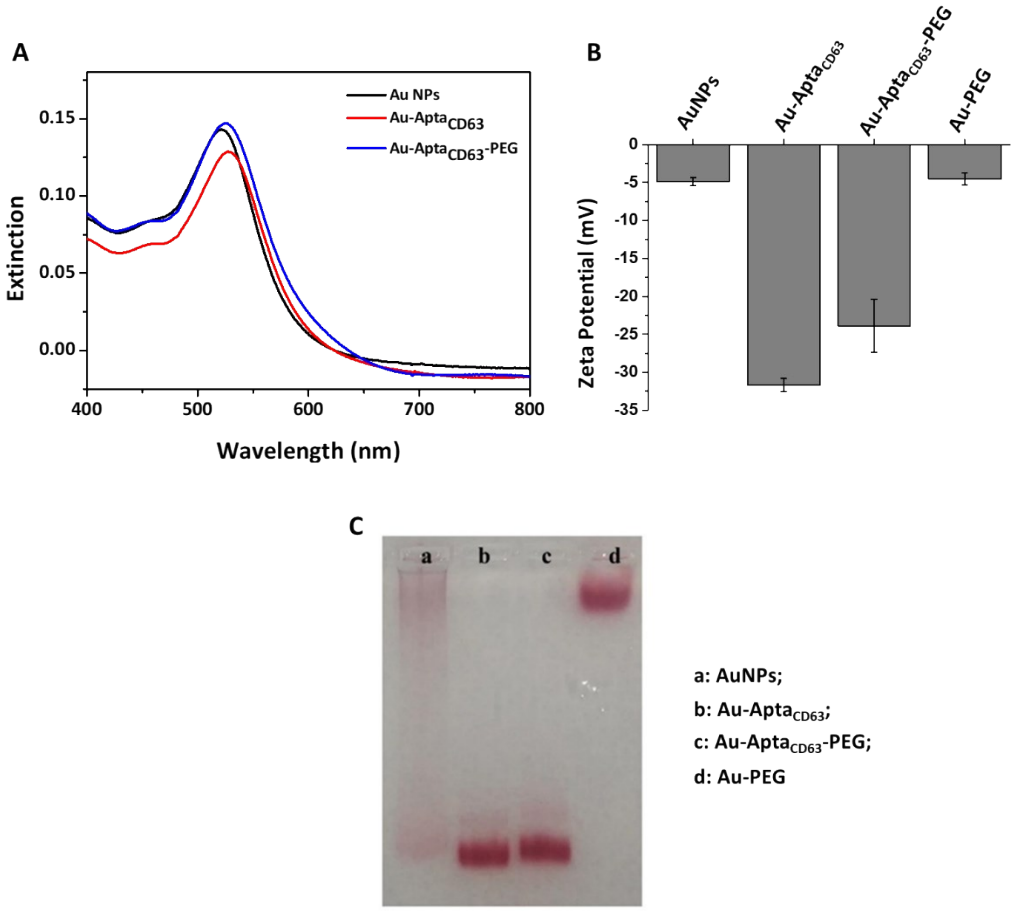

Figure S1 (A) UV-Vis extinction spectra of AuNPs with different surface modification. (B) Zeta potential of AuNPs with different surface modification. (C) Gel electrophoresis measurement of (a) AuNPs; (b) Au-Apta$_{CD63}$; (c) Au-Apta$_{CD63}$-PEG; (d) Au-PEG.

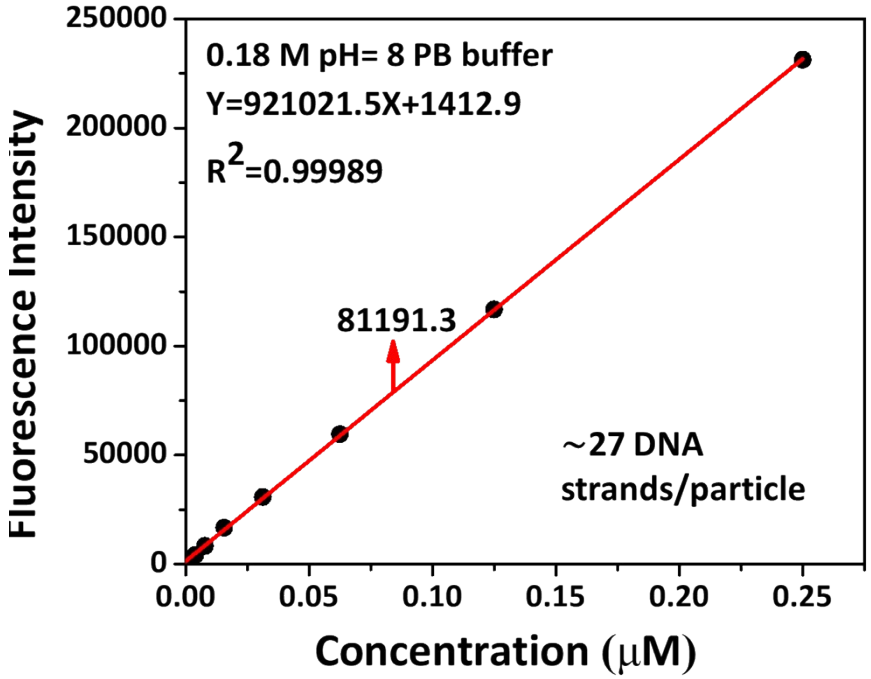

Figure S2 Linear plot of fluorescence intensity in response to various concentrations of 6-FAM labeled aptamers. The red arrow represents fluorescence intensity of aptamers dissociated from the surface of AuNPs.

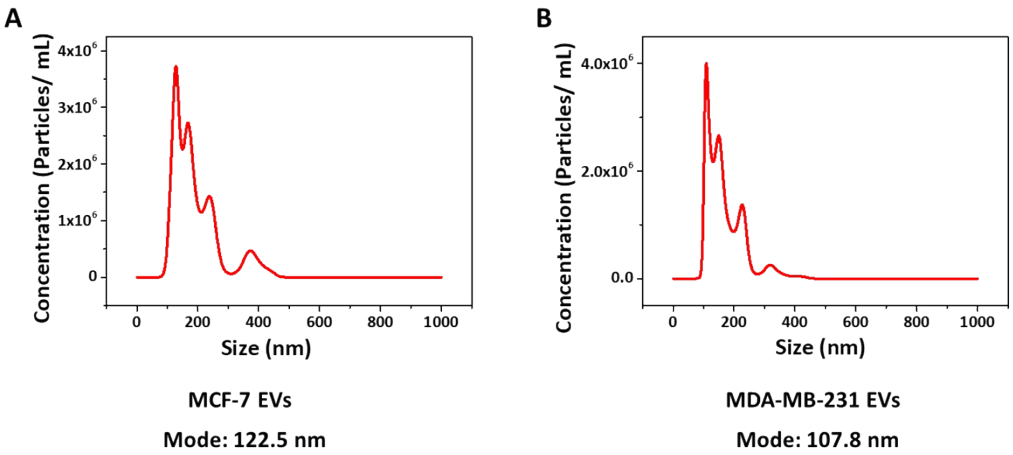

Figure S3 NTA detection of EVs derived from MCF-7 cells and MDA-MB-231 cells.

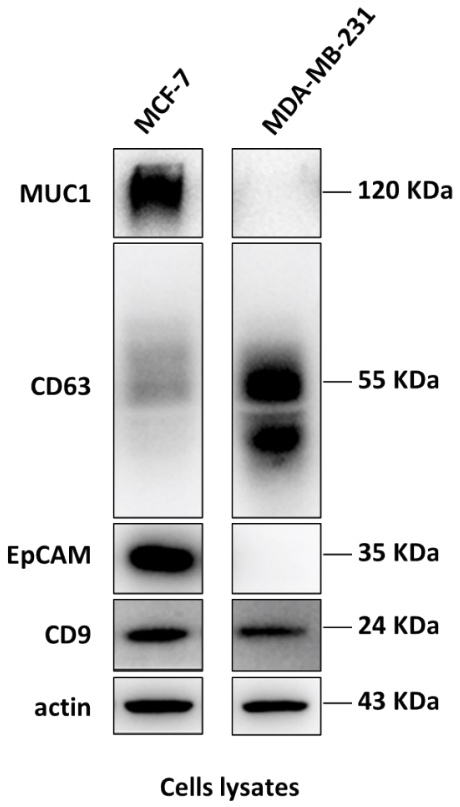

Figure S4 Western blot images of the whole cell lysates of MCF-7 cells and MDA-MB-231 cells.

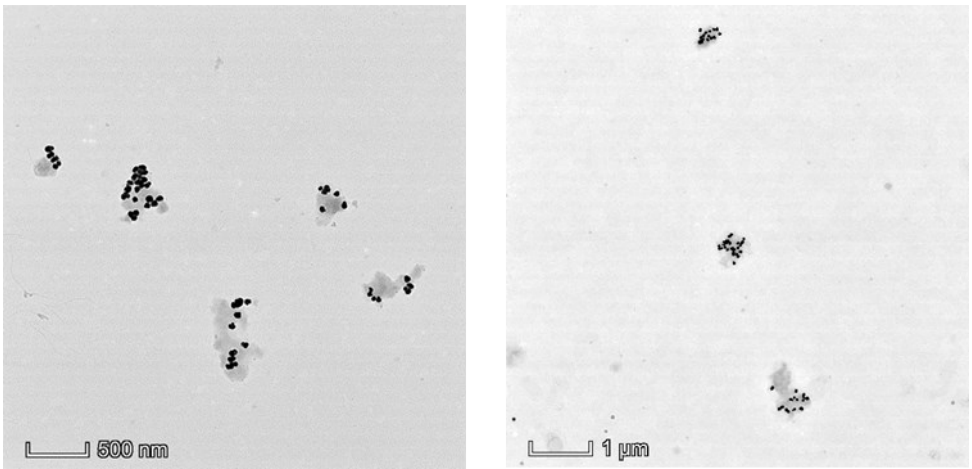

Figure S5 TEM images showing more EVs attached with Au aptasensors.

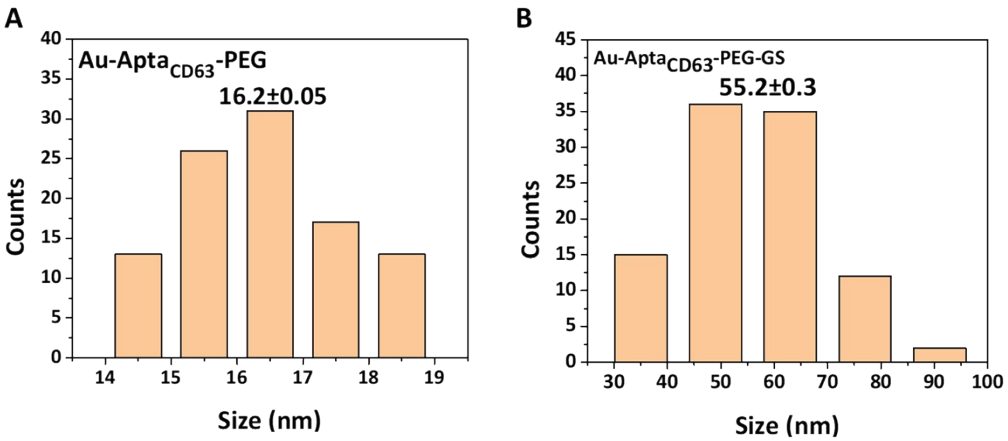

Figure S6 Size distribution of Au-Apta$_{CD63}$-PEG aptasensors (A) before and (B) after growth.

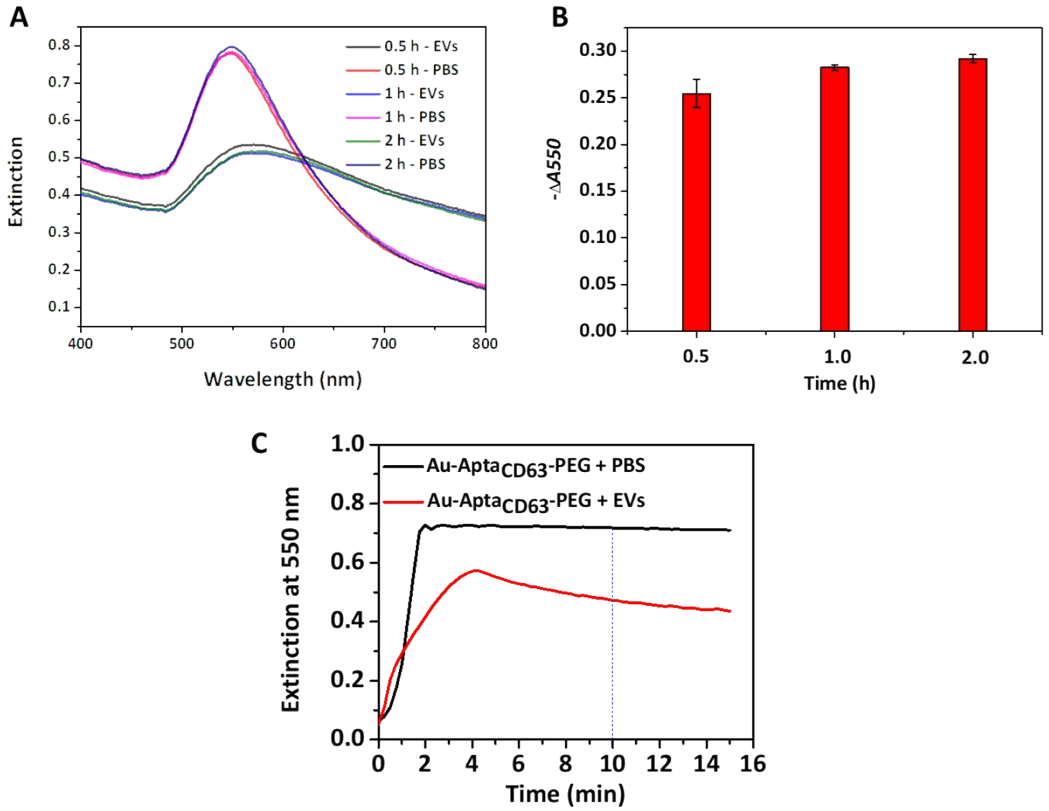

Figure S7 (A) UV-Vis extinction spectra of Au-Apta$_{CD63}$-PEG + MCF-7 EVs incubated for different time durations and after adding the Au growth reagent. (B) Histogram of $-\Delta A_{550}$ versus different incubation times of Au-Apta$_{CD63}$-PEG and 7.5 ng/μL MCF-7 EVs. The error bars represent the standard deviations of three independent measurements. (C) The reaction kinetics of the mixture of Au-Apta$_{CD63}$-PEG and MCF-7 EVs after adding the Au growth reagent.

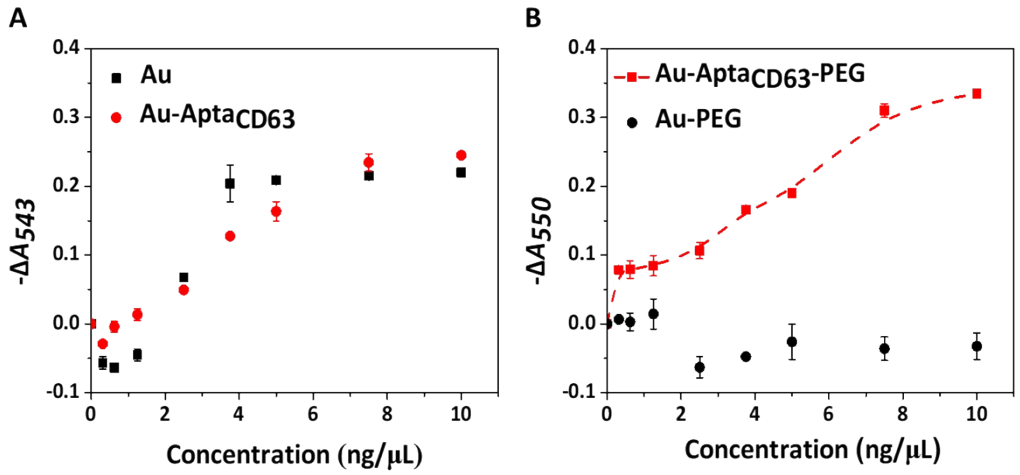

Figure S8 Elimination of nonspecific adsorption by PEG modification. (A) The scatter diagram of $-\Delta A_{543}$ versus various concentrations of MCF-7 EVs. $-\Delta A_{543} = A_{543} - A_{543C}$, where A_{543} is the extinction peak intensity of Au-Apta$_{CD63}$ at 543 nm after incubation with MCF-7 EVs and adding the Au growth reagent, and A_{543C} is that without incubation with EVs. (B) The scatter diagram of $-\Delta A_{550}$ versus various concentrations of MCF-7 EVs. The maximum extinction peak after Au-Apta$_{CD63}$ and Au-Apta$_{CD63}$-PEG growth is at 543 nm and 550 nm, respectively.

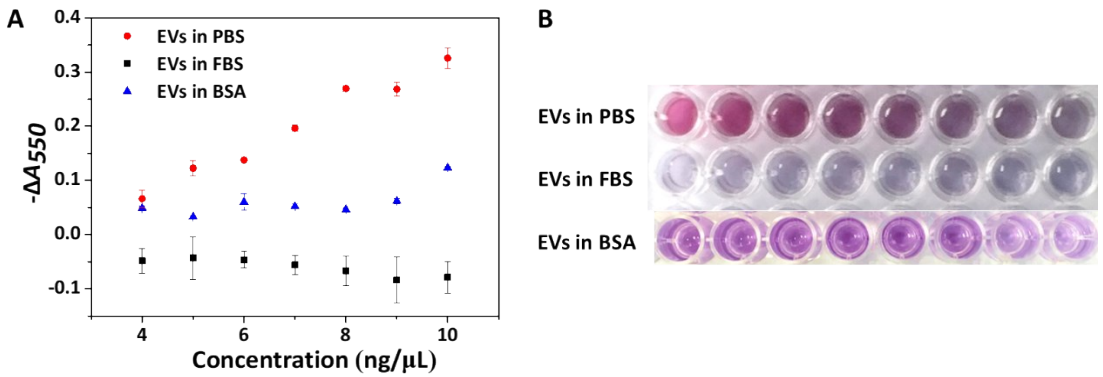

Figure S9 Interference of the detection assay by FBS and BSA. A) The scatter diagram of $-\Delta A_{550}$ versus various concentrations of MCF-7 EVs dispersed in PBS, FBS or BSA/PBS solution (3 ng/μL). B) The corresponding photographs showing the colorimetric detection of EVs with the naked eye.

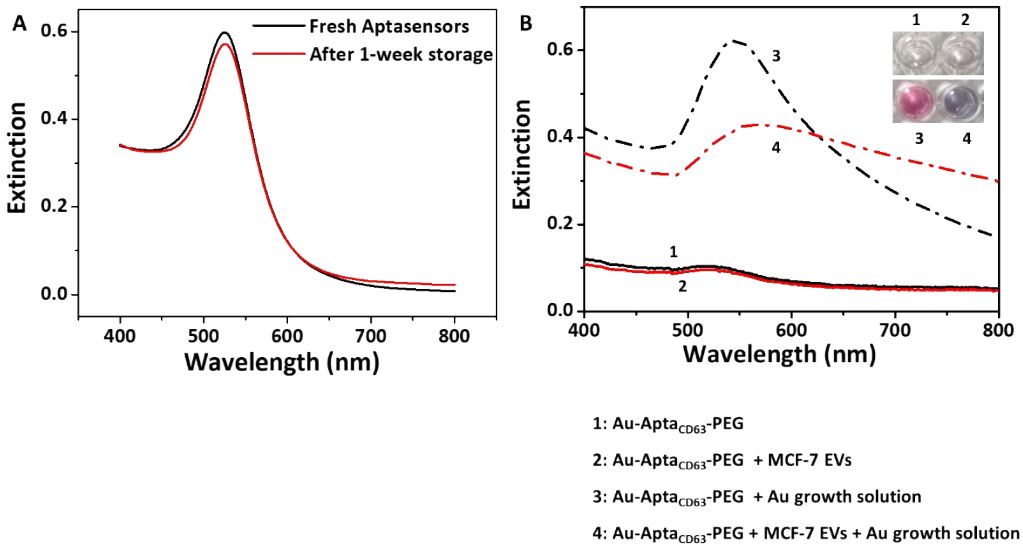

Figure S10 (A) The extinction spectra of freshly prepared aptasensors and the ones after 1-week storage. (B) UV-Vis extinction spectra and corresponding photographs of Au-Apta$_{CD63}$-PEG aptasensors after 1-week storage, incubated without (1,3) or with (2,4) EVs, before (1,2) and after (3,4) growth.

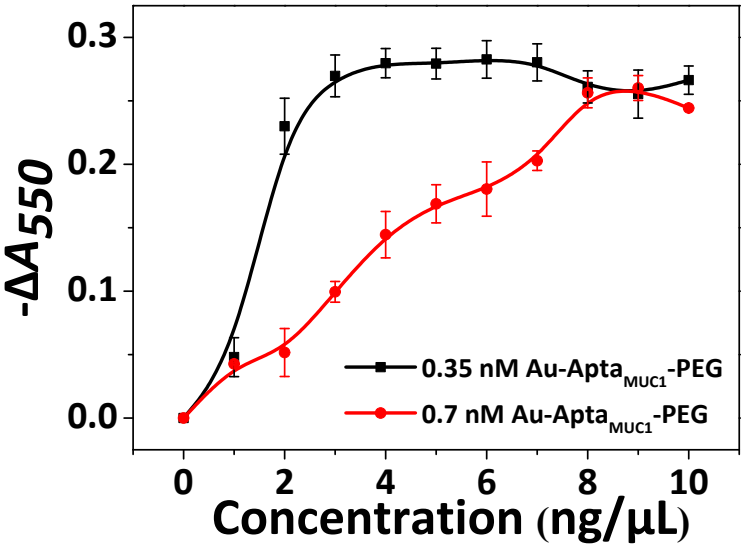

Figure S11 Plots of *–ΔA550* vs. concentration of EVs when incubated with Au-Apta$_{MUC1}$-PEG of different concentrations.

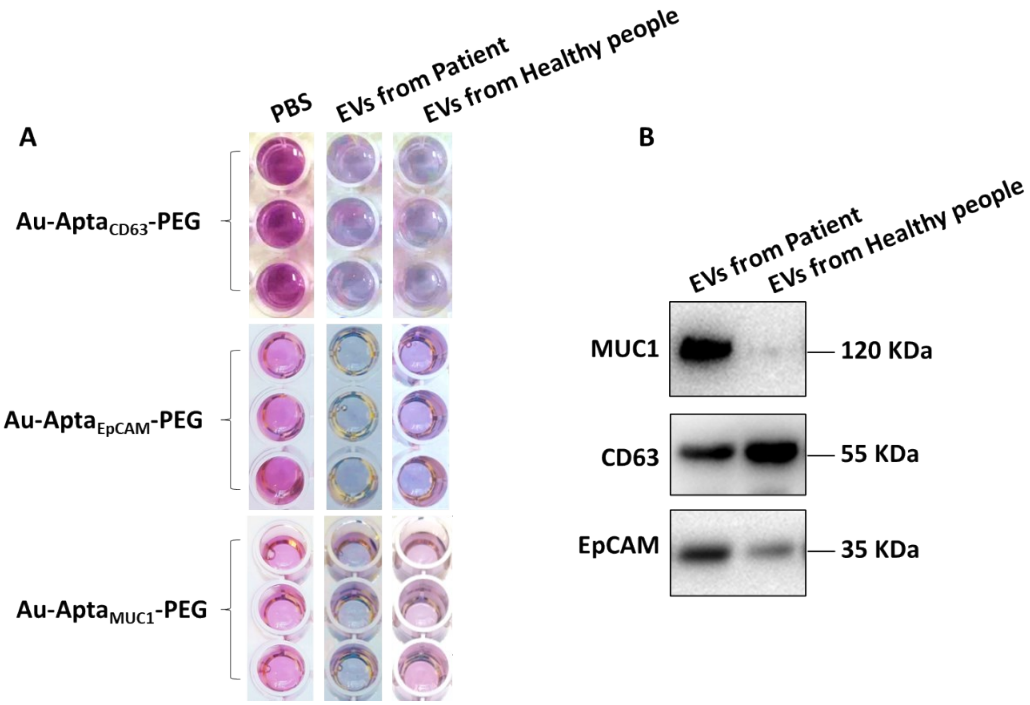

Figure S12 (A) Photographs of Au-Apta$_{CD63}$-PEG, Au-Apta$_{EpCAM}$-PEG, and Au-Apta$_{MUC1}$-PEG aptasensors, incubated with PBS without EVs or with EVs from a patient or a healthy people. (B) Western blot images of EVs from plasma of a breast cancer patient and a healthy people.

Table S1. Comparison of the sensitivity, detection range and time of currently available methods for the detection of EVs

Principle	Targets	Detection range	LOD	Detection time	Refs
Fluorescence	CD63 and EpCAM	0-6×10^5 EVs/μL	2.1×10^4 EVs/μL by targeting EpCAM in EVs from SW480 cells	~40 min	[29]
Fluorescence	CD63, AFP, CEA, EpCAM, PTK-7, PSMA, and PDGF	1.6×10^5-1.6×10^8 EVs/mL	1.6 × 10^5 EVs/mL by targeting CD63 in EVs from HepG2 cells	~30 min	[14]
Electrochemical	CD63 and EpCAM	5×10^2-5×10^6 EVs/μL	1.25×10^2 EVs/μLby targeting CD63 and EpCAM in EVs from MCF-7 cells	NA	[19]
Electrochemical	CD63, EpCAM, CD24, CA125	NA	3×10^4 EVs by targeting CD63 and other protein in EVs from OV90 cells	10 μL/1h	[11]
Electrochemical	EpCAM and PSMA	NA	50 EVs by detecting EpCAM and PMSA in EVs from VCaP cells	~130 min	[30]
SPR	EpCAM, CD24, CA-125, MUC18, EGFR, and HER2	NA	3×10^3 EVs by targeting CD63 in EVs from CaOV3 cells	~60 min	[10]
SERS	EpCAM, CD44, HER2, EGFR, IGFR, CD81, CD63, and CD9	50-5×10^5 EVs/μL	2×10^3 EVs/μLby targeting CD63 in EVs from MDA-MB-231 cells	~2 h	[31]
Lateral flow immunoassay	CD9, CD81 and CD63	NA	8.54×10^5 EVs/μL by targeting CD9, CD81 and CD63 in EVs from Ma-Mel-86c cells	~15 min	[32]
Colorimetric	CD63	1.84×10^6-2.21×10^7 EVs/μL	5.2×10^5 EVs/μLby targeting CD63 in EVs from MCF-7 cells	~40 min	[33]
Colorimetric	CD63, EpCAM, PDGF, PSMA, and PTK7	0-12.8 μg/mL	NA	~20 min	[21]
Colorimetric	CD63and CD9	2.2×10^5-2.4×10^7 EVs/μL	2.2×10^4 EVs/μL by targeting CD63 in EVs from MCF-7 cells	NA	[34]
Colorimetric	CD63, EpCAM, and MUC1	CD63: 6.9×10^4-4.9×10^5 EVs/μL; EpCAM: 2.1×10^5-6.9×10^5 EVs/μL; MUC1: 0-6.2×10^5 EVs/μL	6.2×10^4 EVs/μL by targeting CD63 in EVs from MCF-7 cells; 4.8×10^4 EVs/μL by targeting EpCAM in EVs from MCF-7 cells; 5.4×10^4 EVs/μL by targeting MUC1 in EVs from MCF-7 cells	~70 min	This work