Supporting information

Table S1. Sequences of oligonucleotides used in present work.

Title	Sequences (5' to 3')	labeling
Recognizing probe	CAC TAC AGA GGT TGC GTC TGT CCC ACG TTG TCA TGG GGG GTT GGC CTG TTT TTT GGG AAA GGA TCA GAA CTC CGA GCC GGT CGA AAT AGT GCG ACC TCT GTA	EpCAM aptamer
Cholesterol-substrate	TTT CGA CCG CCA TCC ACG CAC TAT /rA/GG TTC TGA TCT CTT CTC CGA GCC GGT CGA AAT AGT GGG T	cholesterol

Experimental section

sEVs isolation and characterization

A549cells were cultured in 5% CO_2 at 37 °C in DMEM. Following an additional 48 h of cell growth in FBS-free medium, approximately 80 % of the bottle's bottom was covered with A549 cells. Afterwards, the larger micro vesicles and cellular detritus were eliminated from the FBS-free culture media through 300g centrifugation for 10 min at 4 °C. To remove protein aggregates and sizable vesicles, the filtrate was centrifuged at 4 °C for 30 min with 16500 g centrifugation. The sEVs-containing supernatant was filtered through a 0.22 μm pore filter. Following 30 min of centrifugation at 5000 g at 4 °C with a 100 kDa ultrafiltration tube, the filtrate was subjected to precipitation by reintroducing a fresh PBS buffer for centrifugation. This process was repeated three times. Ultimately, the sEVs particle was dissolved in sterile PBS and stored at −80 °C until further use.

Immobilization of CD63 antibody on 96-well plate

A 200 μL of glutaraldehyde (5 mM) was firstly added to a 96-well plate and incubated at 37 °C for 4 h. Then the plate was washed three times with PBS buffer (pH 5.0), followed by washing twice with deionized water. Subsequently, 200 μL of CD63 antibody dispersed in TE buffer (pH 7.4) was added and incubated at 37 °C for another 4 h, followed by washing three times with deionized water to remove the unbound aptamers.

Optimization of experimental parameters

First, 2 μL of sEVs (10^4 particles/μL) and different concentration of the cholesterol-substrate (2 μL) were added to the anti-CD63-coated plate (anti-CD63@plate), and the mixture was incubated at room temperature for 30 min. After removing the unbound cholesterol-substrate, 2 μL of the recognition probe and 2 μL of HP@AuNPs were added to the plate. Subsequently, 1 μL of $MgCl_2$ (250 mM) was added to initiate the movement of the immobilized DNAzyme walker, and the reaction was allowed to proceed at room temperature for 60 min. Emission spectra ranging from 510 to 620 nm were recorded for each sample using an excitation wavelength of 488 nm. The fluorescence intensities at the emission peak of 520 nm were compared. The

optimization of other parameters follows the same procedures.

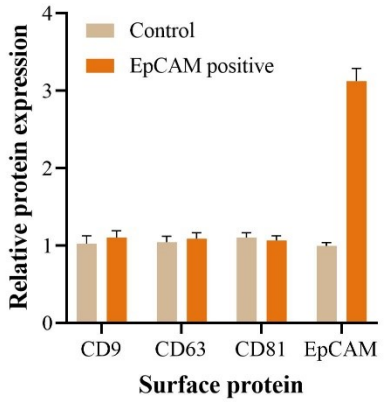

Figure S1. Relative protein expression levels of the control (normal sEVs) and the EpCAM positive sEVs calculated by ELISA method.

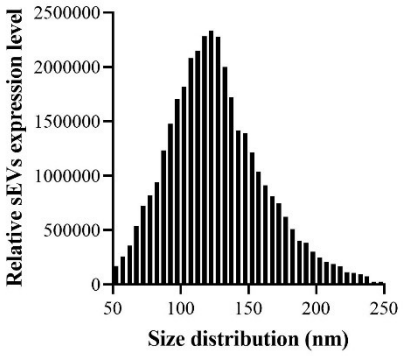

Figure S2. NTA results of the extracted sEVs.

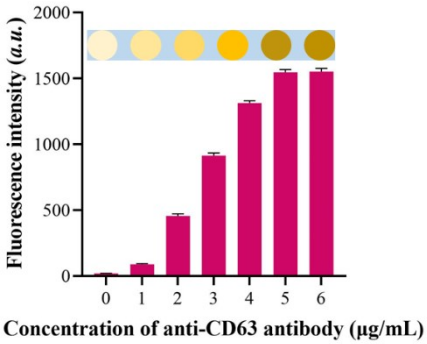

Figure S3. Fluorescence intensity of the anti-CD63@plate when different concentrations of anti-CD63 antibody were mixed with plate.

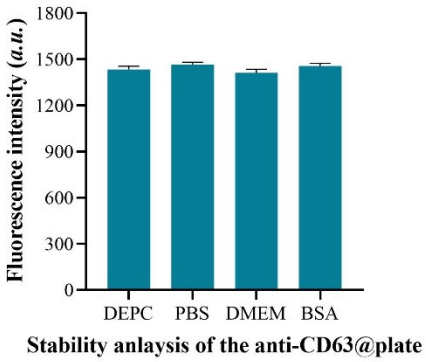

Figure S4. Fluorescence intensity of the anti-CD63@plate when incubated with different buffer solution.

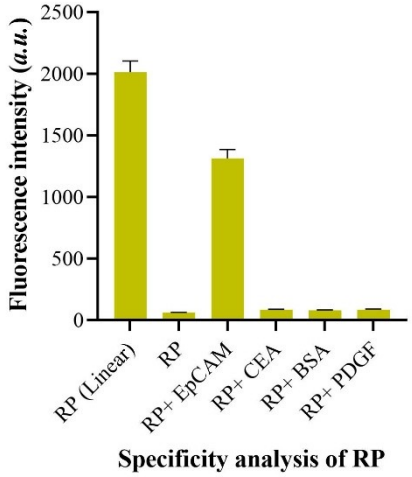

Figure S5. Fluorescence intensity of RP when mixed with EpCAM and interfering molecules.

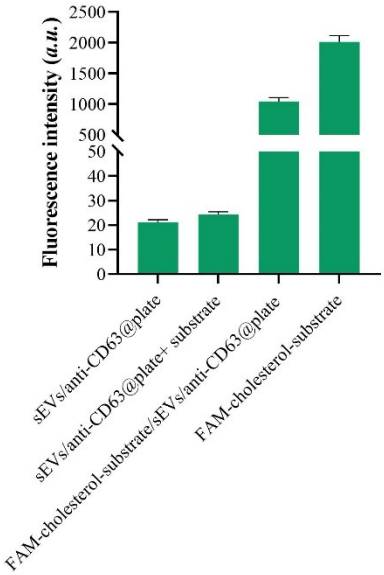

Figure S6. Fluorescence intensity of the FAM-cholesterol-substrate/sEVs/anti-

CD63@plate.

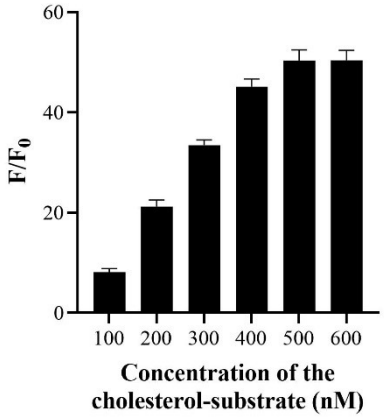

Figure S7. The F/F_0 value (F and F_0 represent the fluorescence intensity with and without sEVs, respectively) of the method when detecting EpCAM positive sEVs with different concentrations of the cholesterol-substrate.

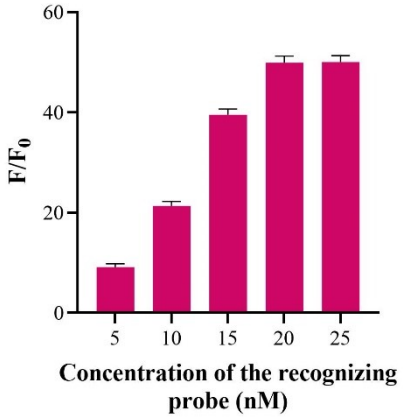

Figure S8. The F/F_0 value (F and F_0 represent the fluorescence intensity with and without sEVs, respectively) of the method when detecting EpCAM positive sEVs with different concentrations of the recognizing probe.