Supporting Information

Supplementary experimental section:

Cell culture and sEVs extraction:

A549 cells were cultured in RPMI-1640 medium with 10% fetal bovine serum (FBS) and maintained in a humidified atmosphere of 5% CO_2 at 37°C. For sEVs isolation, we washed the cells three times with phosphate-buffered saline (PBS) when cells reached 70% confluence and maintained them for an additional 12 hours in medium without FBS; the culture medium was then collected for sEVs isolation by ultracentrifugation according to standard differential centrifugation separation protocols. Finally, transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA) were performed to characterize these isolated EVs: the concentration and size distribution of extracted exosomes were analyzed by NTA, which was performed using the Nanosight NS300 followed by protocol. TEM imaging was carried out using a JEM-1400Plus.

Optimizations of experimental condition:

T4 DNA ligase, phi29 enzyme and dNTPs: Different concentrations of T4 DNA ligase (1 U to 10 U) were utilized for the rolling circle amplification (RCA). The fluorescence signal of SYBR was detected to reflect the formation of RCA products; Different concentrations of T4 DNA ligase (0.5 U to 3 U) were utilized for the rolling circle amplification (RCA). The fluorescence signal of SYBR was detected to reflect the formation of RCA products; Different concentrations of dNTPs (200 nM to 1000 nM) were utilized for the rolling circle amplification (RCA). The fluorescence signal of SYBR was detected to reflect the formation of RCA products;

Incubation time: The mixture containing 10 μL sEVs (10^3 particles/L) and 12 μL detection scaffold solution in 18 μL Tris-HCl buffer was incubated at 37 °C with different time. Then, 50 μL H_2O and 100 nM MB were added into the above reaction mixture for 30 min at 30 °C. The fluorescence signal of FAM moiety was detected by Hitachi F-4700 fluorospectro photometer (Beijing, China).

Statistical Analysis: Each test was repeated at least three independent replicates, which were displayed as the mean ± standard deviation (SD). Data were visualized using software GraphPad Prism 8.0 (CA, USA). The Student's t test was used to analyze comparisons between two groups. Multiple groups were compared with one-way ANOVA and Least-Significant Difference method (LDS). Differences were considered significant at values of $P < 0.05$.

Table S1 Oligonucleotides used in the experiments

| Title	Sequence (5'-3')
Dumbbell structure probe	P-TGA CAT TGT ACC TCA TCC AGG TGG GGT GTA ATA TAC AAT GTC ACT TAG GCA GAC CAA CTA TTC GAC CGG CTC GGA GAA GAG ATG CAG CTT GAA TCC GTG
Trigger	TAC AAT GTC ACA CGG ATT CA
Molecular beacons	(FAM)-CCA CCA TCA CCA ACT AT/rA/GGA AGA GAT GTT TGG TGG-(BHQ1)
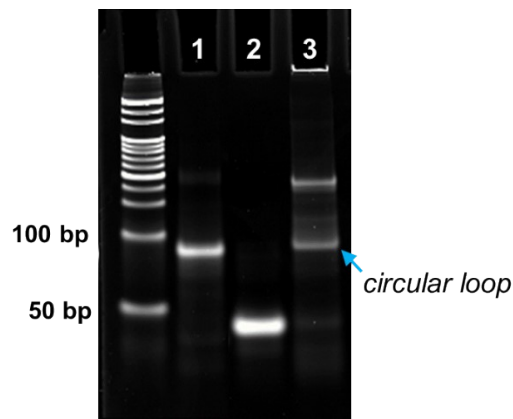
Fig S1. PAGE result of the RCA process. Lane 1: dumbbell probe; Lane 2: trigger; Lane 3: RCA products.
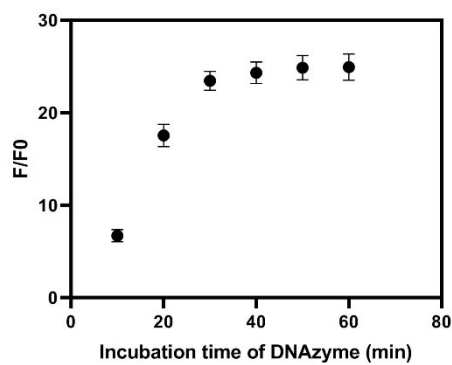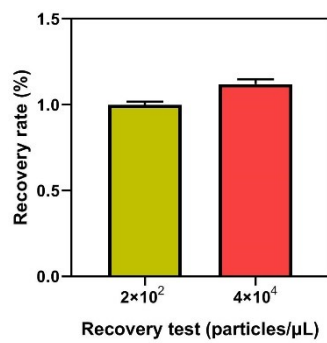

Fig S2. F/F0 result of the method with different DNAzyme incubation time.
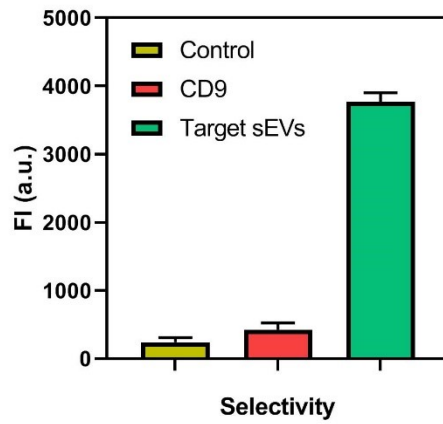
Fig S3. Fluorescence result of the method when detecting target sEVs and CD9 protein.
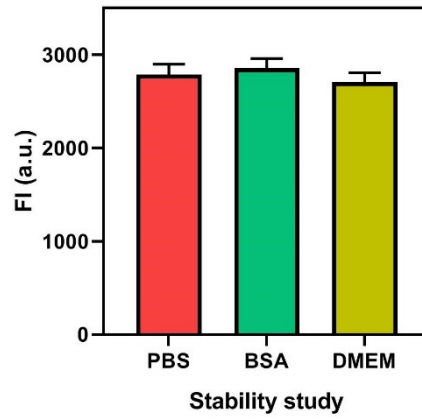
Fig S4. Fluorescence result of the method when detecting target sEVs in PBS buffer, BSA and DEME solutions.

Fig S5. Recovery rate of the method.