Self-Protected Circular DNAzyme for Integrated Enrichment and Quantification of Small Extracellular Vesicles

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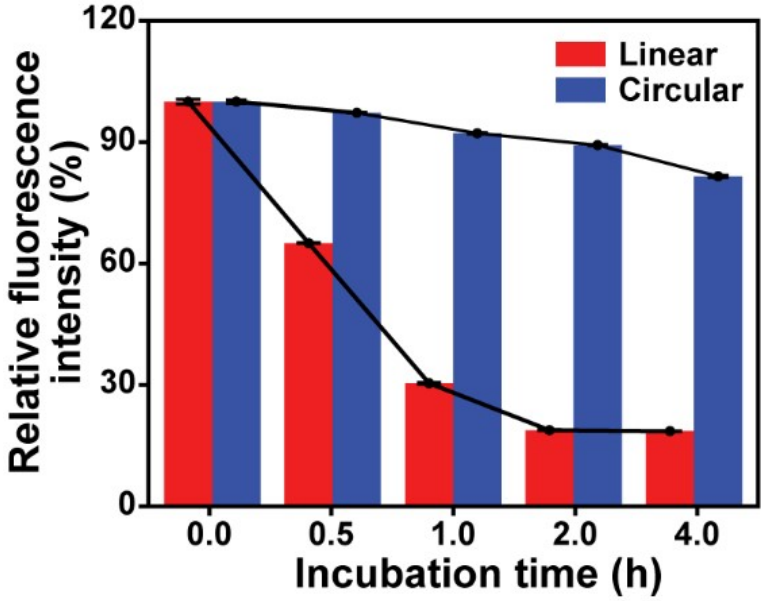

Fig. S1 Stability evaluation of the DNAzyme. Time-dependent fluorescence intensity changes of the Circular DNAzyme and Linear DNAzyme incubated in 10% FBS (n = 3).

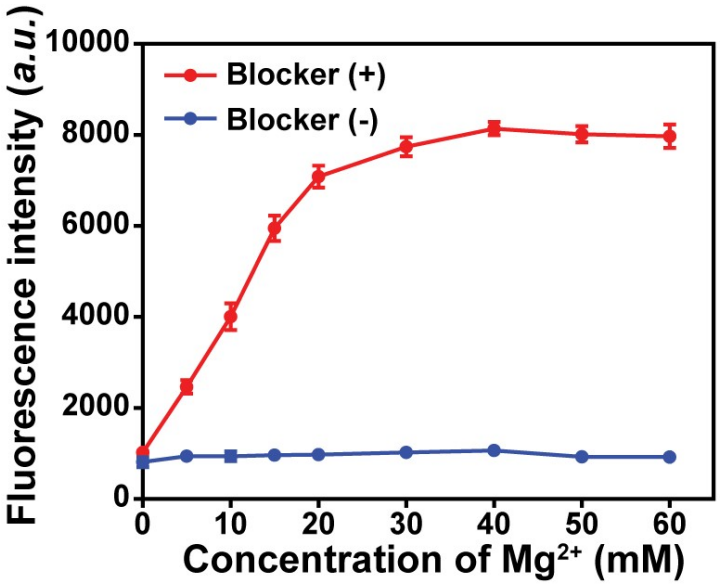

Fig. S2 Optimization of Mg^{2+} concentrations for DNAzyme catalytic system. Mg^{2+} concentrations were from 0 mM to 60 mM (0 mM, 5 mM, 10 mM, 15 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60mM). Error bars represent standard deviation (n = 3).

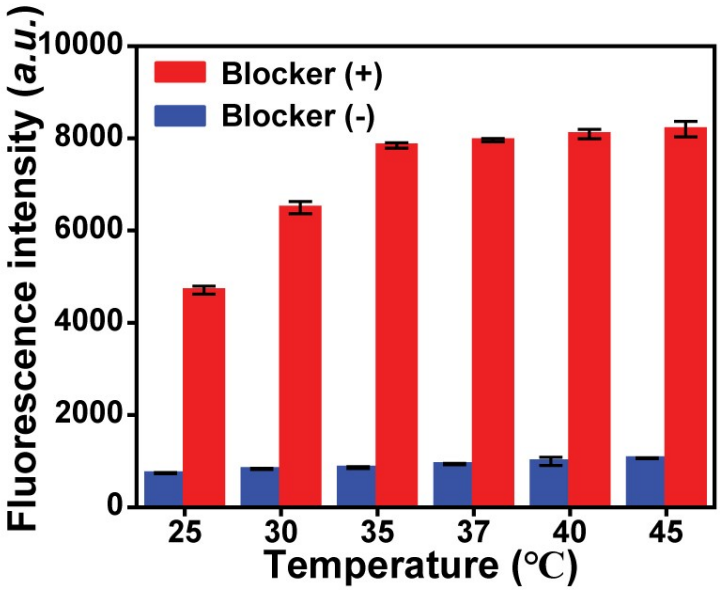

Fig. S3 Optimization of the incubation temperature for DNAzyme catalytic reaction. The reaction temperatures were from 25 ℃ to 45 ℃ (25 ℃, 30 ℃, 35 ℃, 37 ℃, 40 ℃, 45 ℃). Error bars represent standard deviation (n = 3).

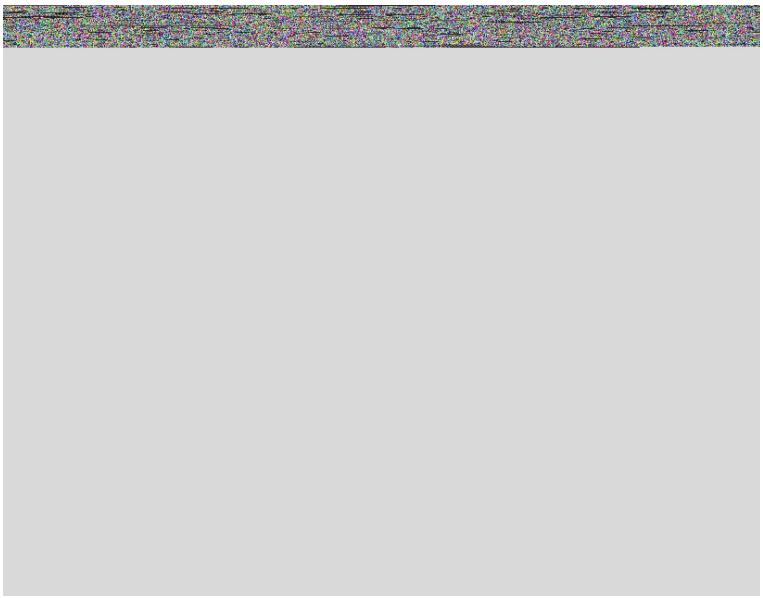

Fig. S4 Effect of incubation time on fluorescence intensity for DNAzyme catalytic reaction. The observation times were from 0 h to 3.5 h (0 h, 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h). Error bars represent standard deviation (n = 3).

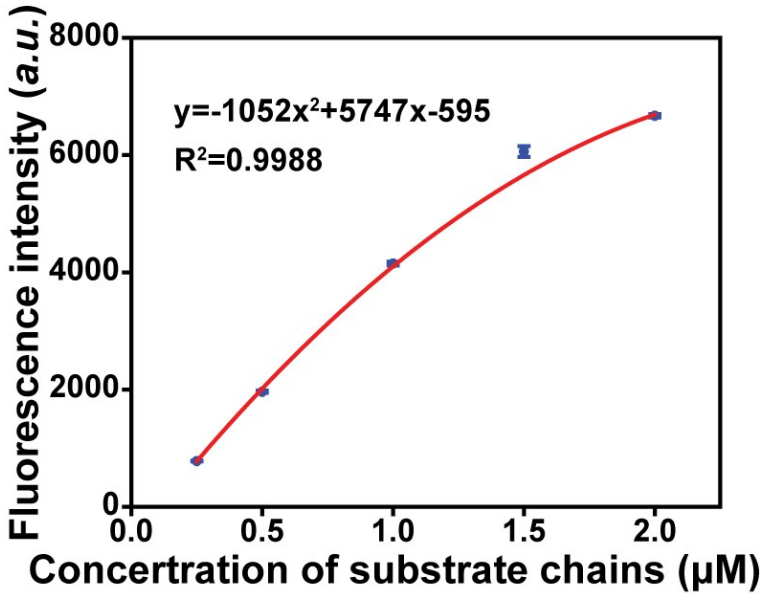

Fig. S5 Calibration curve of different concentrations of substrate strands (n = 3).

Fig. S6 Quantification of the binding efficiency between substrate chains and magnetic beads (n = 3).

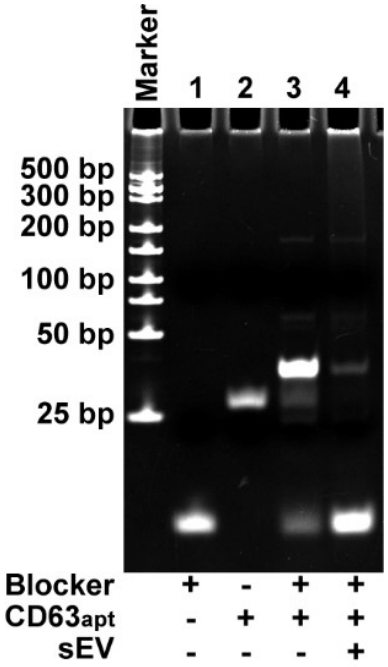

Fig. S7 Native PAGE analysis verifying the assembly of AptCD63/Blocker complex and the specific release of Blocker by sEVs. (marker: 25-500 bp; lane 1: Blocker; line 2: CD63apt; line 3: Blocker + CD63apt; line 4: Blocker + CD63apt + sEV.)

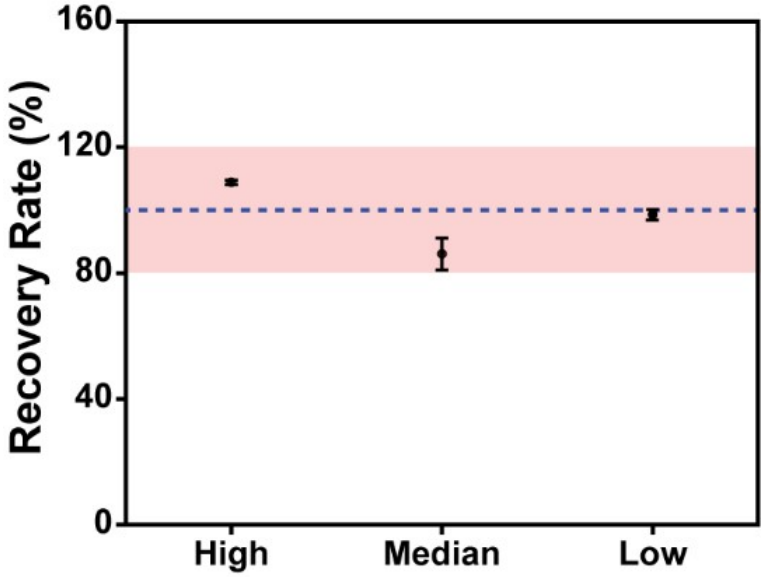

Fig. S8 Spike-and-recovery analysis of sEVs in serum. The plot displays the recovery rates at three different concentration levels (n = 3).

Fig. S9 Quantification of the wound healing test (n = 3).

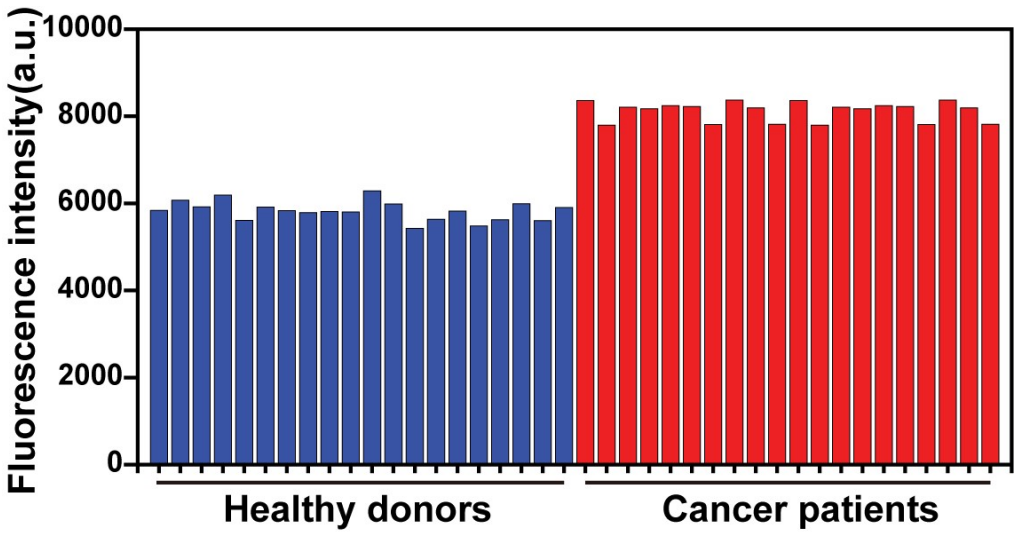

Fig. S10 Fluorescence intensity of clinical 20 healthy individuals and 20 cancer patients analyzed by the proposed method.

Table S1. Sequences of oligonucleotides used in this work

Name	Sequence (5'→3')
DNAzyme	CTACGTGTCTCTTCTCCGAGCcgtagctagccAAAtacagaggttgCGGTCGAAATAGTGCAT
Linear DNAzyme	CTACGTGTCTCTTCTCCGAGCCGGTCGAAATAGTGCAT
blocker	AGGTGGGGTGTATAACAACG
substrate	ATGCACTAT/rA/GGAAGAGACACGTAG
Substrate1	ATGCAC/iBHQ2dT/AT/rA/GGAA/iCy3/GAGACACGTAG
Substrate2	NH_2-C6-TTTTTTTTATGCACTAT/rA/GGAAGAGACACGTAGTTT-Cholesteryl
Substrate2	NH_2-C6-TTTTTTTTATGCAC/iBHQ2dT/AT/rA/GGAA/iCy3/GAGACACGTAGTTT-Cholesteryl
CD63 aptamer	TATACACCCCACCTCGCTCCCGTGACACTAATGCTA

Table S2. The clinical sample information in this study (healthy donors, HD; breast cancer patients, BC)

Sample	Age	Gender	Tumor staging
HD 1	50	Female	-
HD 2	61	Female	-
HD 3	55	Female	-
HD 4	59	Female	-
HD 5	54	Female	-
HD 6	48	Female	-
HD 7	45	Female	-
HD 8	48	Female	-
HD 9	58	Female	-
HD 10	55	Female	-
HD 11	26	Female	-
HD 12	38	Female	-
HD 13	36	Female	-
HD 14	25	Female	-
HD 15	39	Female	-
HD 16	30	Female	-
HD 17	30	Female	-
HD 18	29	Female	-

HD 19	39	Female	-
HD 20	49	Female	-
BC 1	46	Female	I
BC 2	47	Female	I
BC 3	56	Female	I
BC 4	57	Female	I
BC 5	36	Female	I
BC 6	51	Female	I
BC 7	55	Female	I
BC 8	57	Female	I
BC 9	57	Female	I
BC 10	43	Female	I
BC 11	57	Female	I
BC 12	56	Female	I
BC 13	50	Female	I
BC 14	41	Female	I
BC 15	49	Female	I
BC 16	66	Female	I
BC 17	58	Female	I
BC 18	48	Female	I
BC 19	49	Female	I
BC 20	64	Female	I