Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Spherical Nucleic Acid Reporters-based Cascade CRISPR/Cas12a Amplifier for Stable and Sensitive Biosensing of Circulating Tumor DNA

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Experimental Section

Materials and Apparatus. All oligonucleotides sequences (Table S1 in Supporting information), were synthesized and HPLC-purified by Sangon Biotech Co.Ltd (Shanghai, China) and HIPPOBIO Bio-Technology Co.Ltd (Beijing, China). All RNA sequences were purchased from Shanghai GenePharma (Shanghai, China). LbCas12a nuclease and 10×NEB buffer 2.1 were obtained by NEB (New England Biolabs, Ipswich, MA, U.S.A.). T4 DNA Ligase (1000 U μL^{-1}), phi29 DNA Polymerase (10 U μL^{-1}), 10×phi29 DNA Polymerase reaction buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, 100 mM $(NH_4)_2SO_4$, 40 mM DTT), and dNTP (2.5 mM) were obtained from Beyotime Biotechnology Co.Ltd (Shanghai, China). BSA (20 mg mL^{-1}) were provided by NEB (New England Biolabs, Ipswich, MA, U.S.A.). Ultrapure water was obtained from Milli-Q system (Billerica, MA, USA). All other reagents were analytically pure and can be used without further purification. NanoDrop 2000c Spectrophotometer (Waltham, MA, USA) was used to quantify the concentration of all oligonucleotides. The morphology of nanomaterials was characterized by TEM (Hitachi JEM-2100, Japan). The UV-vis spectrum was tested on UV-2450 (Shimadzu, Japan). Both DLS characterization and Zeta potential characterization were obtained by Malvern Zetasizer Nano ZS90 (Worcestershire, UK). The fluorescence spectra were measured with the Edinburgh FS5 spectrofluorometer (Livingston, UK).

Preparation of AuNPs. The AuNPs were prepared using sodium citrate reduction method. The 0.01 wt% $HAuCl_4$ was added to a clean round-bottomed flask, boiling for 5 min, followed by the addition of 1 mL 3 wt% sodium citrate, and boiling

for 30 min. After that, the solution was naturally cooled to room temperature and stored at 4 ℃ to obtain AuNPs.

Preparation of SNA reporters. The thiolated DNA strands (as shown in Table S1) were activated by incubating with TCEP at 37 ℃ for 1.5 h. After that, 3 nM AuNPs were incubated with activated DNA strands, 0.01% Tween 20, and 10 μL Citrate-HCl (0.5 M pH 7.5). 3 M NaCl was dropwise added to the above solution every 1 h, and the final concentration of NaCl was 1 M. Finally, the solution was incubated overnight at 37 ℃ and washed with enzyme-free water for 3 times (16200 g, 20 min, 4 ℃).

The DNA density on the surface of AuNPs was then measured. In brief, the SNA reporters were treated with 20 mM β-mercapto-ethanol (2-mercapto-Ethanol, ME) at room temperature to cleave FAM-labeled thiolated DNA from gold nanoparticles. The gold aggregation was separated by centrifugation, and the fluorescence of the supernatant was measured on a fluorescence spectrometer. The molar concentration of the DNA is converted by the interpolated fluorescence signal of the standard linear curve, which is prepared by measuring the fluorescence intensity of same FAM-labeled thiolated DNA at known concentration. The concentration of AuNPs is measured at UV-Vis spectrophotometer.

RCA reaction. The RCA reaction includes two parts: ligation reaction and amplification reaction. First at all, 10 nM padlock probe, target ctDNA (PIK3CA E542KM) of different concentrations, and 15 U μL^{-1} T4 ligase, were mixed in 1×T4 ligase reaction buffer. The ligation reaction was incubated at 20 ℃ for 20 min, and

then was heat-treated at 65 ℃ for 5 min to inactivate the T4 ligase. In the amplification reaction, 0.5 mM dNTP, 0.4 mg mL^{-1} BSA, 0.1 U μL^{-1} phi29 enzyme, and 4 μL phi29 buffer was added to the above reaction solution, incubated at 30 ℃ for 30 min, and heated at 65 ℃ to obtain long ssDNA product.

ctDNA detection in buffer. 1 nM LbCas12a protein and 10 nM crRNA were incubated in 1×NEB buffer 2.1 at 37 ℃ for 30 min to form a stable binary complex. Then, the LbCas12a/crRNA complex was mixed with the RCA product and incubated at 37 ℃ for 60 min. In the sensitivity experiment, different concentrations of target were incubated with the LbCas12a/crRNA complex. Next, 10 μL SNA reporters were added to the mixture at 37 ℃. Finally, the fluorescence spectra were recorded by using the Edinburgh FS5 spectrofluorometer with the excitation wavelength of 488 nm.

ctDNA detection in serum samples. A standard addition method was adopted to detect the recovery rate of different concentrations of ctDNA target in 10% serum samples. In general, a 10 μL serum sample was added into 90 μL TAE buffer, then the solution was heated to 95 ℃ for 15 min, quickly cooled to 4 ℃, and centrifuged (16000 g, 20 min, 4 ℃) to obtain the supernatant. Then, according to the standard addition method, the samples with different concentrations of PIK3CA E542KM were quantitative detected. For the double-blind experiment, all samples were randomly prepared by one researcher, and another researcher who did not know the details performed the detection experiment.

Table S1. Sequences of oligonucleotides used in this work.

Name	Sequences(5'-3')
PIK3CA E542KM	CTCAGTGATTTTAGAGAGAGGAT
Padlock	AAATCACTGAGTTTATCATGTATTATAATTTCGTATGTAAGCTA CCTGAGATCTTCTGTACAATTGATCCTCTCTCTA
TS	ATCTCAGGTAGCTTACATACGAAATTA
15nt FQ	FAM-TTTTTTTTTTTTTTT-BHQ1
10nt SNA reporter	SH-TTTTTTTTTT-FAM
15nt SNA reporter	SH-TTTTTTTTTTTTTTT-FAM
25nt SNA reporter	SH-TTTTTTTTTTTTTTTTTTTTTTTTT-FAM
35nt SNA reporter	SH-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM
PIK3CA E542KN	CTCAGTGATTTCAGAGAGAGGAT
KRAS G12DM	GCCTACGCCATCAGCTCCAACT
mismatch-PIK3CA E542KM	CTCAGAGATTTAAGAGTGAGGAT
Lb-crRNA	UAAUUUCUA CUAAGUGUAGAUGUAUGUAAGCUACCU GAG

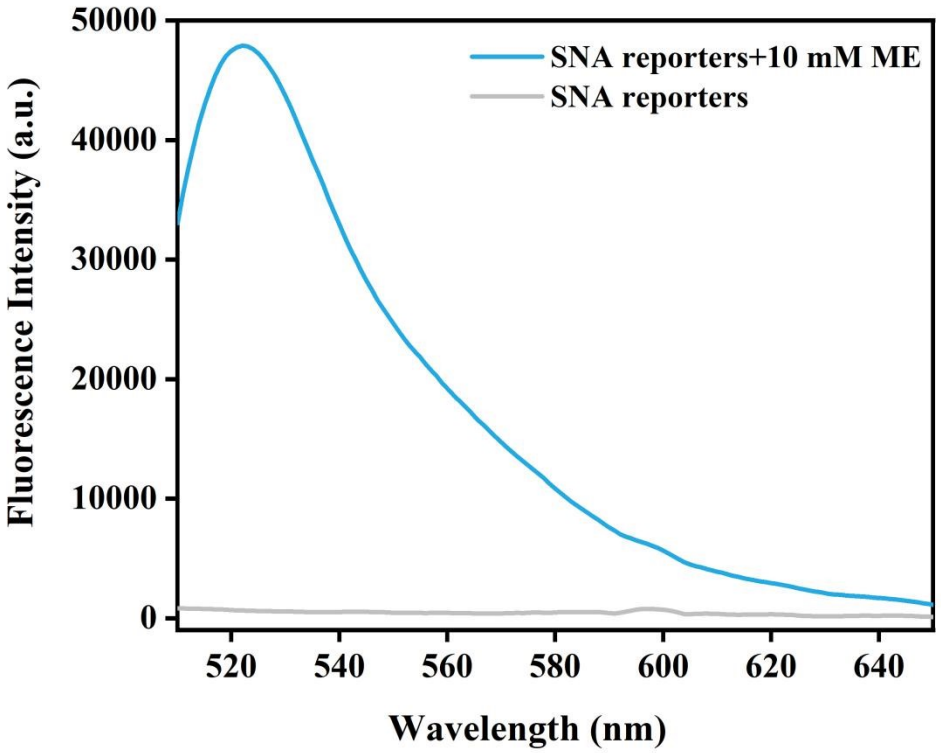

Figure S1. Fluorescence spectra of SNA reporters cleaved by 10 mM 2-Hydroxy-1-ethanethiol (ME).

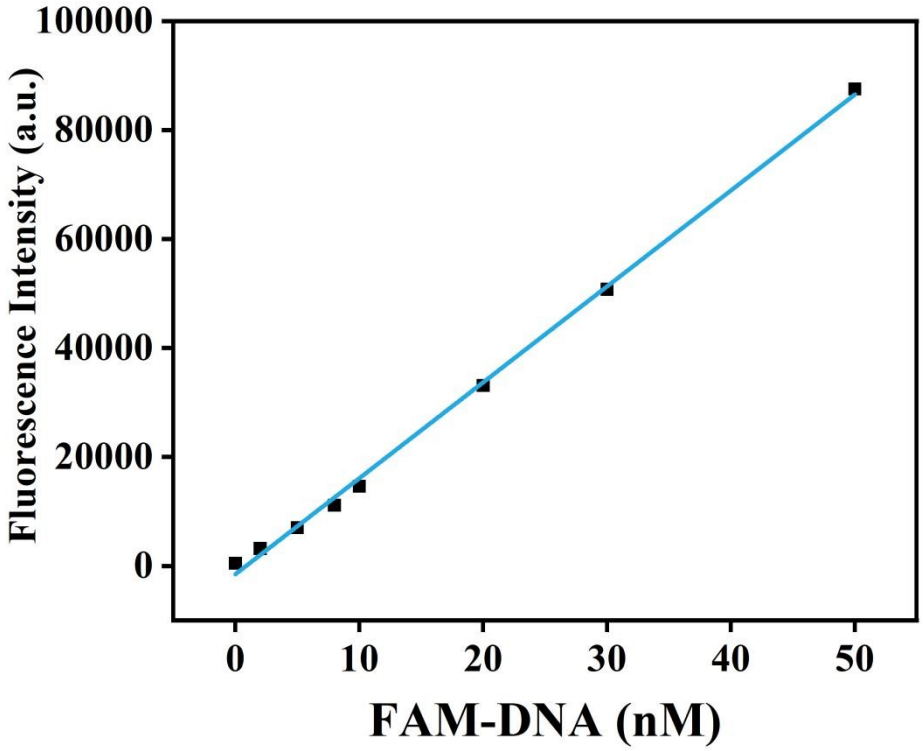

Figure S2. Fluorescence intensity of FAM-labeled thiolated DNA at known concentration.

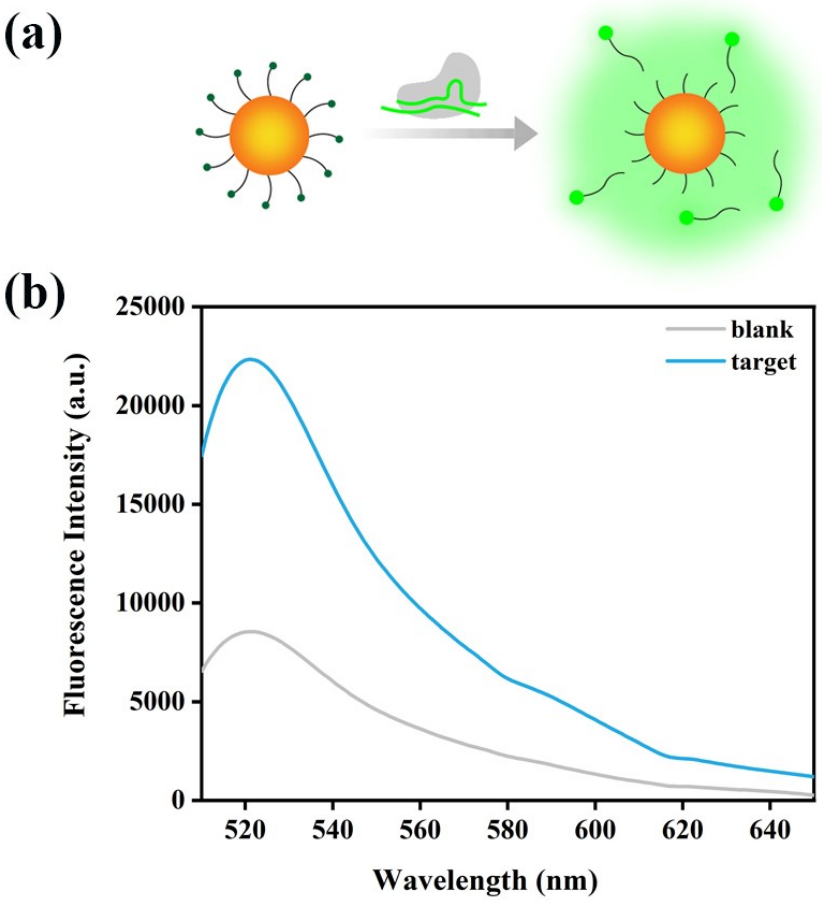

Figure S3. Schematics (a) and fluorescence spectra (b) of SNA reporters cleaved by activated CRISPR/Cas12a.

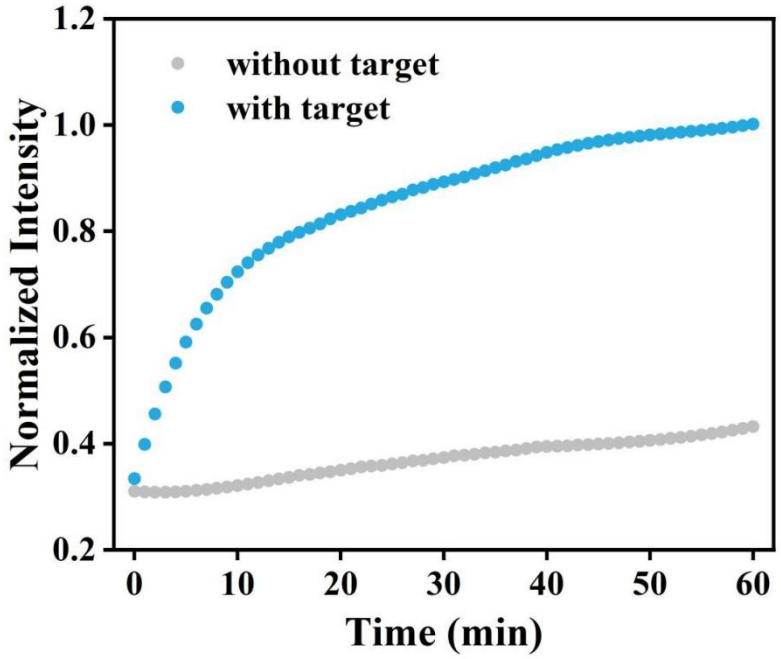

Figure S4. Time course of Cas12a reaction with or without target.

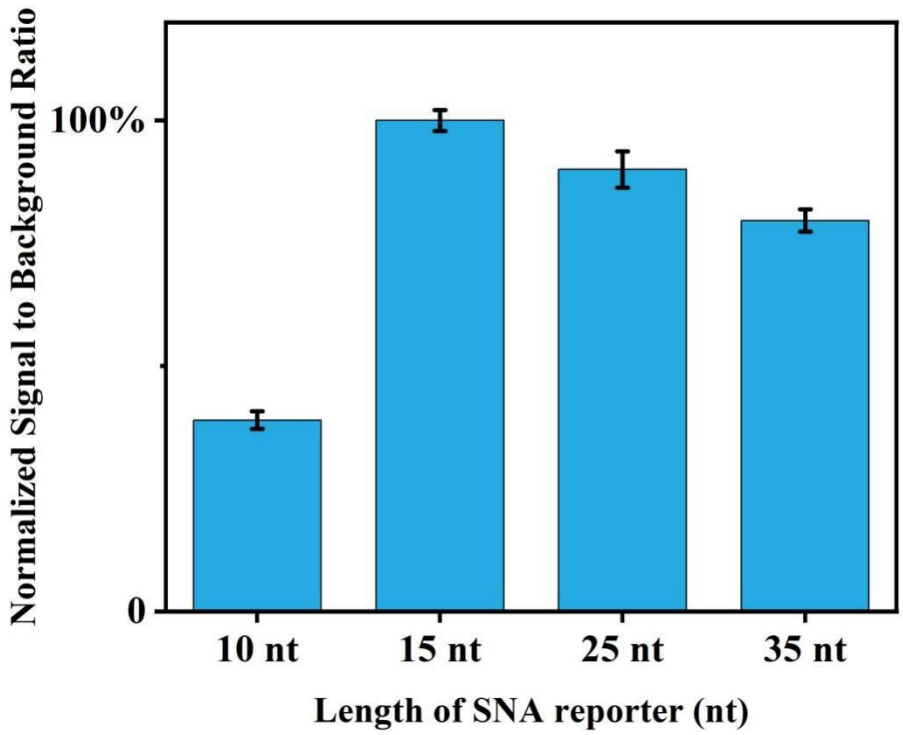

Figure S5. Optimization of DNA length for SNA reporters. For each length, fluorescence intensity of the probe with target is divided by that of probe without target to obtain the signal-to-background ratio.

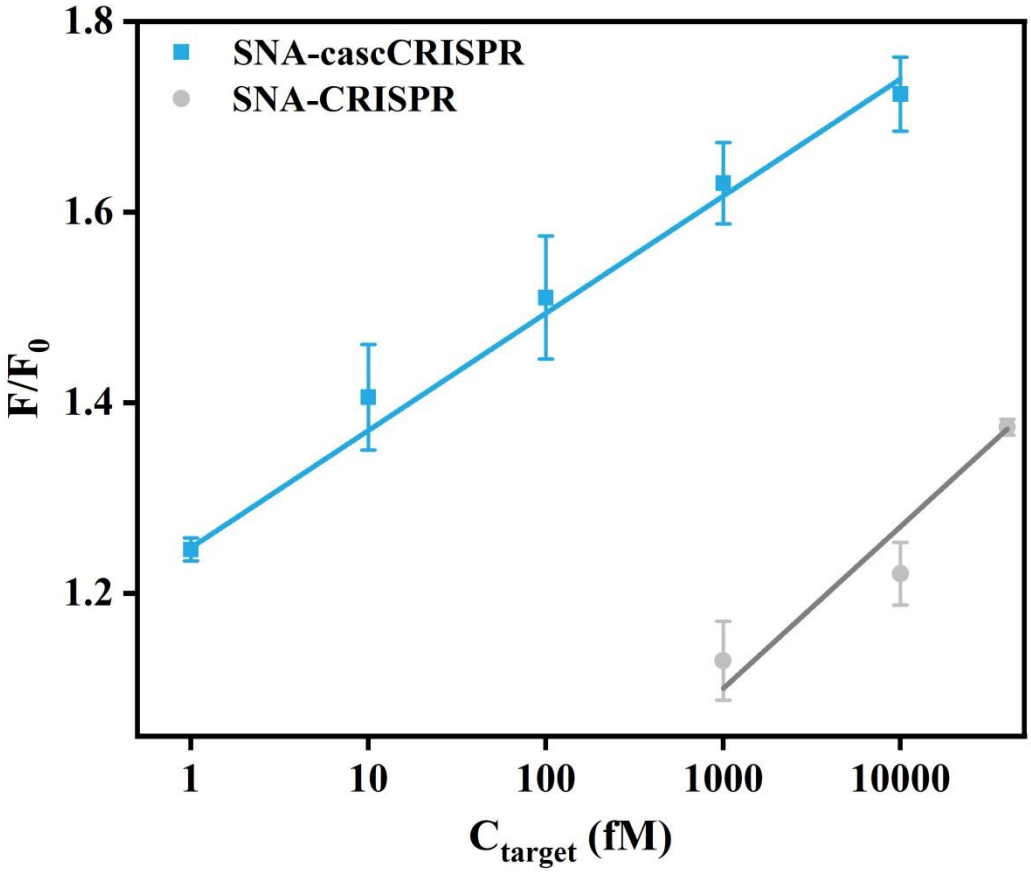

Figure S6. Standard curve of fluorescence response of SNA-cascCRISPR (blue) and SNA-CRISPR (grey) to different concentrations of ctDNA.

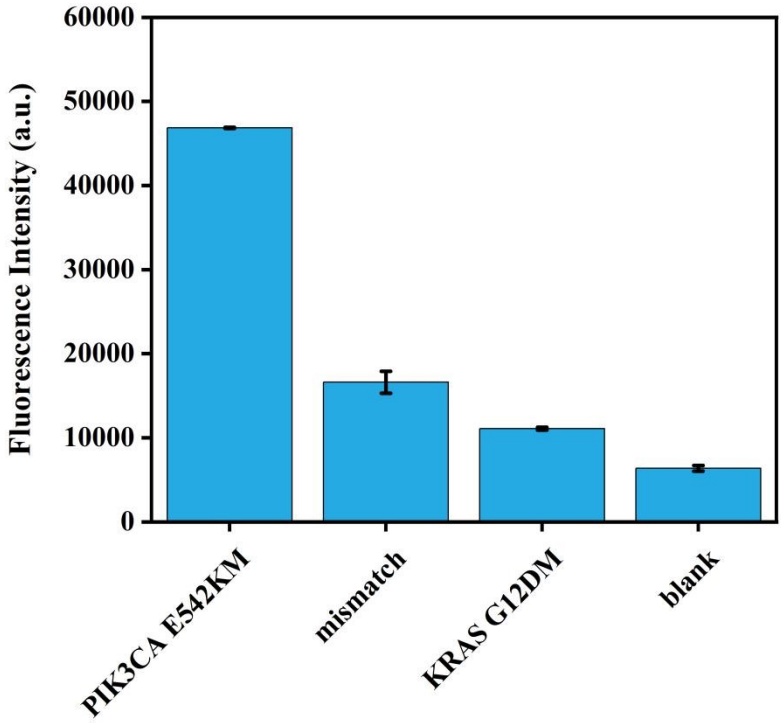

Figure S7. Fluorescence intensity of SNA-cascCRISPR in the presence of target and other nucleic acids.

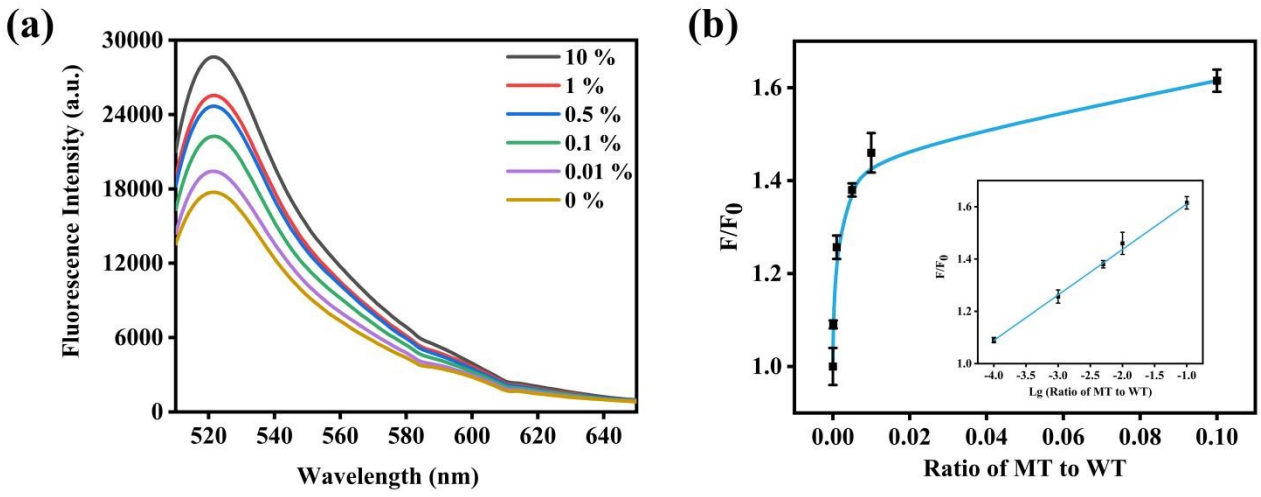

Figure S8. (a) Fluorescence response of mutant and wild-type targets (total concentration of 10 nM) mixed in different proportions of mutant-type target. (b) The signal-to-back ratio response curve of wild-type and mutant-type mixed in different proportions (linear intervals in the inset). MT: wild-type, WT: mutant-type.

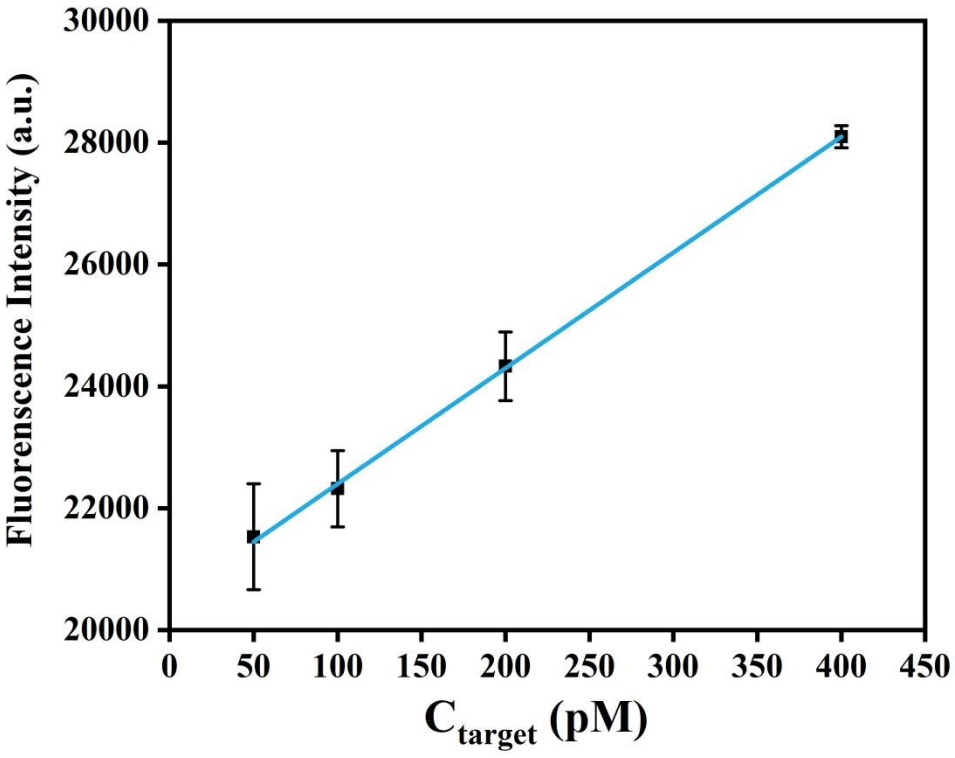

Figure S9. Standard curve of fluorescence response in 10% serum.

Ethics Statement

Serum samples of healthy people were collected from the Xiangya Hospital Central South University (Hunan, Changsha). All experiments were performed in accordance with the Guidelines of Clinical Sample Management Rules of Hunan Cancer Hospital and Xiangya Hospital of Central South University, which were reviewed and approved by the Ethics Committee at Hunan Cancer Hospital and Xiangya Hospital of Central South University. Informed consents were received from the blood donors of this project.