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Supporting Information

A programmed terminal extension strategy to light up multiple beacons for DNA and cellular telomerase detection

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Experimental section

Reagents and materials.

Nanopure water (18.2 MΩ; Millpore Co., USA) was used in all experiments. Hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 3H_2O$), 99.99%, sodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), 99%, and tris(2-carboxyethyl)phosphine (TCEP) were purchased from Alfa Aesar and used without further purification. Terminal deoxynucleoside transferase (TdT) was purchased from Beyotime Biotechnology Co. Ltd. (Nantong, China). RIPA lysis buffer was obtained from New Cell & Molecular Biotech (Suzhou, China). APure telomerase was obtained from commercial ELISA kit (Yibang Biotechnology; Suzhou, China). Other chemicals were purchased from Sigma-Aldrich and used as received without any further purification. All oligonucleotides were synthesized and HPLC purified by Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences were as follows: (1) 5′-GAGCATACATAGGGTTTCTC-3′, (1’) 5′-GAGCATACAT*G*GGGTTTCTC-3′ (one-base mismatch), (1’’) 5′-GAGCATAC*GC*AGGGTTTCTC-3′ (two-base mismatch), (1’’’) CATTCGTCTTAGAGAGCTAC (random sequence), (2) 5′-GAGAAACCCTATGTATGCTCTTTT-$(CH_2)_6$-SH-3′, (3) 5′-TAMRA-GCTAAAAAAAAAAAATTAGC-$(CH_2)_6$-SH-3’, (4) 5’-$(CH_2)_6$-SH-AATCCGTCGAGCAGAGTT-3’, (5) 5’-TAMRA-CGTTACCCTAACCCTAACGTTTT-$(CH_2)_6$-SH-3′, (6) 5’-

AAAAAAAAAAAAAAAAA-$(CH_2)_6$-SH-3'.

Preparation of AuNPs.

Citrate-capped AuNPs with an average diameter of 13 nm were synthesized by the citrate reduction method. Briefly, sodium citrate (5 mL, 38.8 mM) was added rapidly to boiling solution of $HAuCl_4$ (50 mL, 1 mM) with vigorously stirring, and then the color of the solution changed quickly from pale yellow to deep red. Before slowly cooling to room temperature, the solution was boiled while stirring for additional 15 min to ensure complete reaction. The concentration of AuNPs solutions was determined to be 11 nM using the absorbance values at 520 nm with extinction coefficient of 2.7×10^8 M^{-1} cm^{-1}.

Preparation of DNA-modified AuNPs.

The (2)/(3)-modified AuNPs were synthesized by incubating the prepared AuNPs colloid with TCEP activated strands (2) and (3) (the final concentration 3 μM, molar ratio = 1:2) at room temperature for 8 h. Repeat was conducted for a total of six salting steps with at least 30 min intervals between the steps to reach a final salt concentration of 0.2 M NaCl. To remove the excess DNA, the solution was centrifuged for 30 min at 13000 rpm after the salt aging process. The red precipitate was washed and re-centrifuged, then dispersed in 1 mL solution (20 mM Tris–HCl buffer with 100 mM NaCl at pH 7.2). The solution was stored at 4 °C for use. The (4)(5)-DNA-modified AuNPs were synthesized using the same method.

Cell culture and cellular protein extraction.

All of cell lines were obtained from Cell Bank at the Chinese Academy of Sciences (Shanghai, China). They were all seeded in DMEM medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY), penicillin (100 mg mL^{-1}), and streptomycin (100 mg mL^{-1}) at 37 °C in a 5% CO_2 atmosphere. Cells were collected in the exponential phase of growth, and 10 million cells were dispensed in a 1.5 mL EP tube, washed twice with ice-cold PBS (pH 7.4) solution and then resuspended in 100 mL of ice-cold CHAPS lysis buffer (10 mM Tris–HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 5 mM mercaptoethanol, 0.5% (w/v) CHAPS, 10% (v/v) glycerol). The solution was incubated on ice for 30 min and centrifuged for 20 min at 12000 rpm, 4 °C. Without disturbing the pellet, the cleared lysate was carefully transferred to a fresh RNase-free EP tube, immediately use for telomerase assay or frozen at -80°C.

DNA detection.

The analyses were performed in a reaction volume of 200 μL that included phosphate buffer solution, 10 mM, pH = 7.4, and 200 mM NaCl. The sensing modules: (2)/(3)-AuNPs (50 pM) and different concentrations of the target (4) were reacted for 30 min and then subjected to centrifugation. Then, the precipitate was dispersed in 50 μL TdT-containing buffer (1mM dTTP, 200 mM potassium cacodylate, 5 U of TdT, 0.05% (v/v) Triton X-100, and 125 mM Tris, pH 7.2) and incubated at 37 °C for 1 h to achieve signal production and amplification.

Telomerase detection.

The analyses were performed in an extension reaction volume of 200 μL that included 20 mM Tris–HCl, 1.5 mM $MgCl_2$, 100 mM KCl, 0.005% Tween 20, 1 mM EGTA, 200 μM dNTP, BSA 0.1 mg mL^{-1}, pH 8.0. The sensing modules: (4)/(5)-AuNPs (50 pM) and different concentrations of telomerase were reacted at 37 °C for 40 min, which allows telomerase extension and strand hybridization. Telomerase extracts were diluted in lysis buffer with respective number of cells. The extracts with different cell counts (5 μL, corresponding to 0-2 $\times 10^6$ cells) were added to 185 μL of extension reaction solution, then add 10 μL AuNPs probe. Then the solution was incubated at 37°C for 40 min. For negative control experiments, telomerase extracts were heated at 95 °C for 10 min.

Fluorescence measurements.

All the fluorescence measurements were carried out on a F-7000 fluorometer with excitation at 547 nm and emission at 575 nm for the hairpin fluorescence probes labeled by TAMRA as a fluorophore. When the samples were excited at 547 nm, a slit width of 5 nm, the emission was scanned from 560 to 620 nm, the scanning speed was 1200 nm/min for all other experiments.

In Situ Imaging of Intracellular Telomerase Activity.

A volume of 100 μL of 1×10^6 mL^{-1} cells (MCF-7, Hela, A549, MDA-MB-231 or HL-7702) were seeded in each confocal dish for 24 h. After incubation with 20 μL probe at 37 °C for 2h, the cells were and sent for fluorescent confocal imaging or flow cytometric detection.

Results and discussion

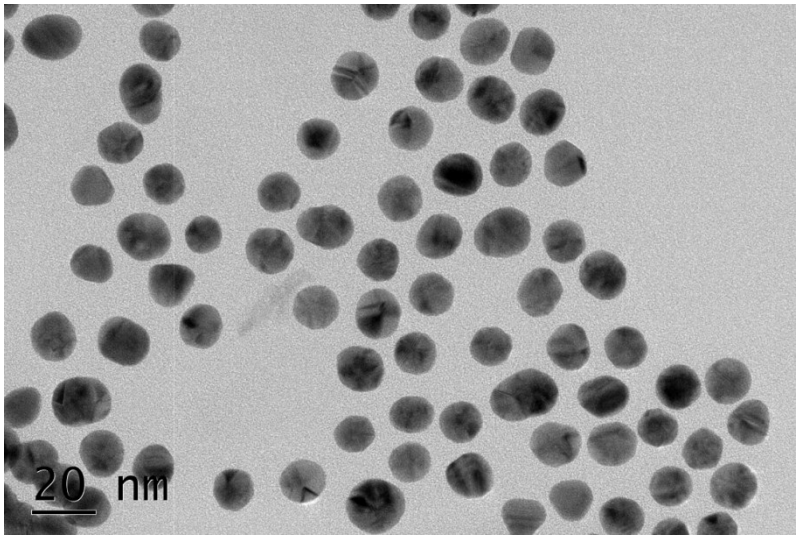

Fig. S1. The synthesized DNA-modified gold nanoparticle.

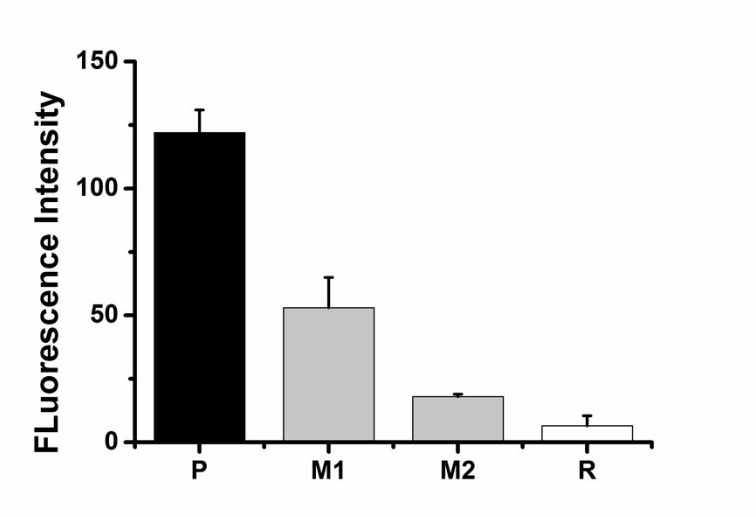

Fig. S2. Response of the proposed assay to its perfectly complementary target and mismatched targets (P: perfectly complementary target, M1: one-base mutant, M2: two-base mutant, R: random DNA strand).

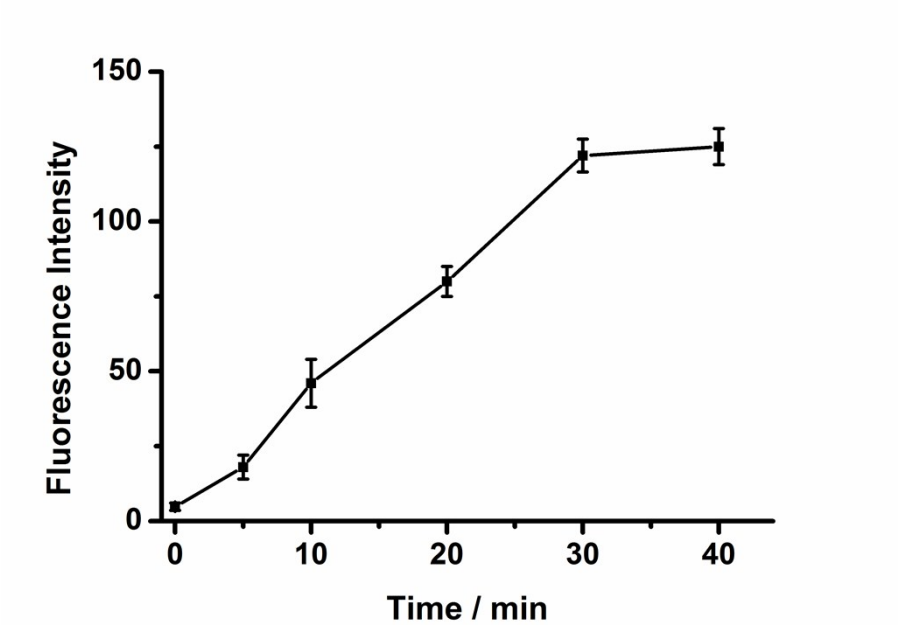

Fig. S3. Optimization hybridization time of target (1) onto the (2)/(3) modified AuNPs.

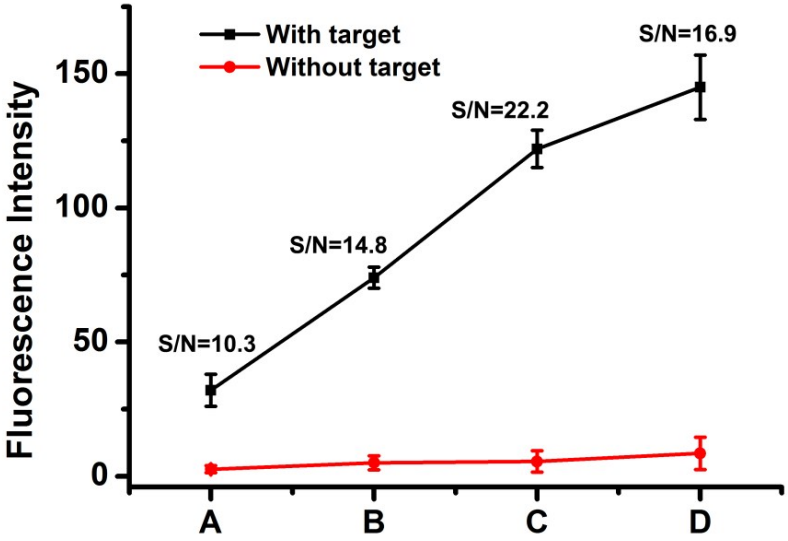

Fig. S4. Optimization of the molar ratio of strand (2) and (3) modified onto the AuNPs (A: 1:0.5, B: 1:1, C: 1:2, and D: 1:3).

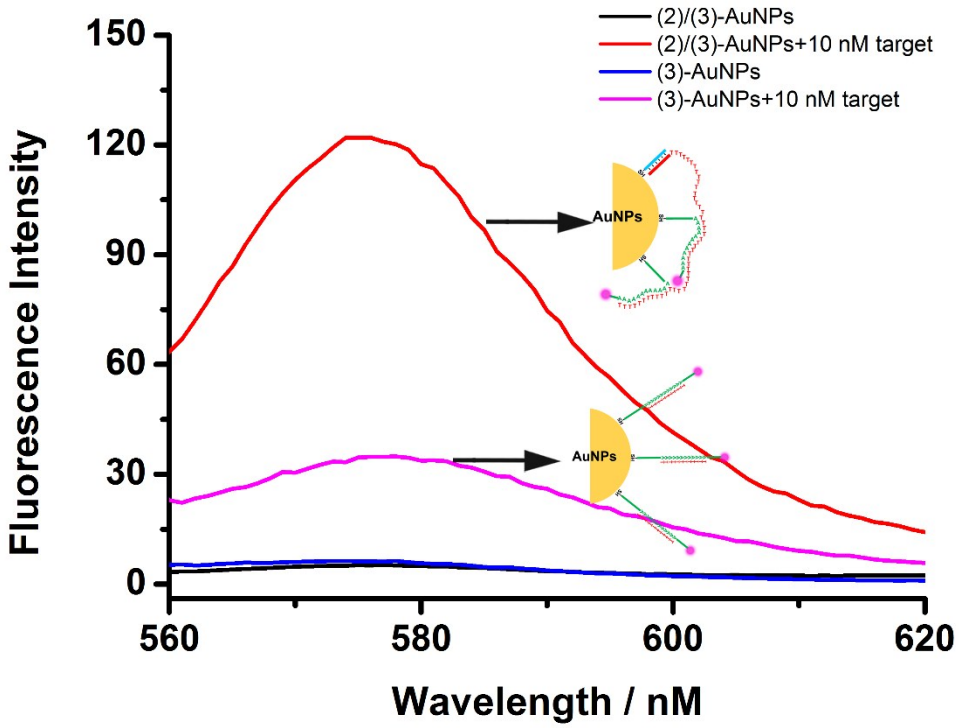

Fig. S5. Comparison of the proposed nanoprobe and strand (3) modified AuNPs which both nanoparticles are responded to 10 nM target.

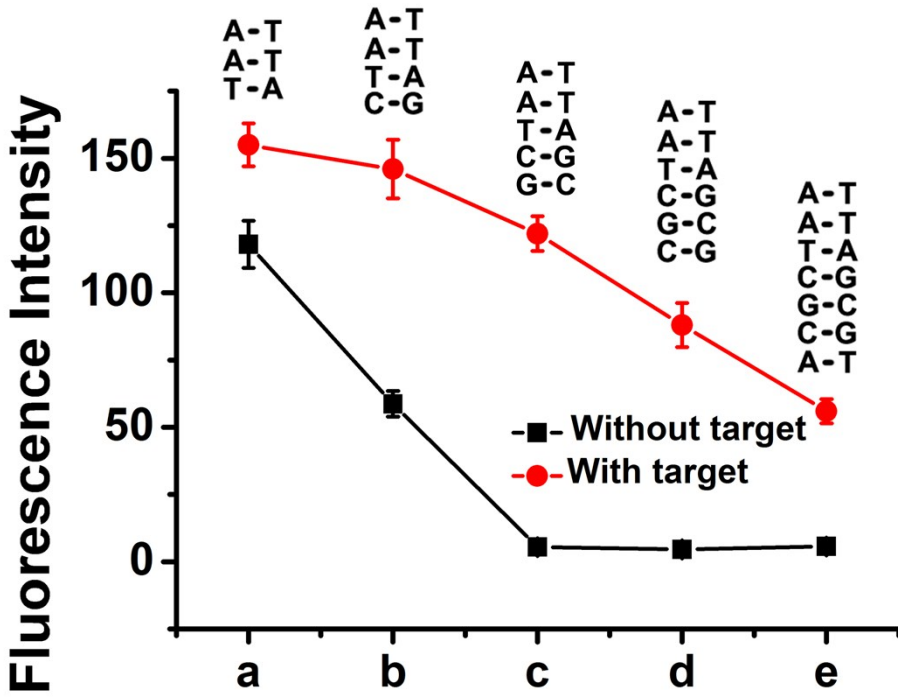

Fig. S6. Optimization of the proposed assay through adjusting the stem sequence of probe (3).

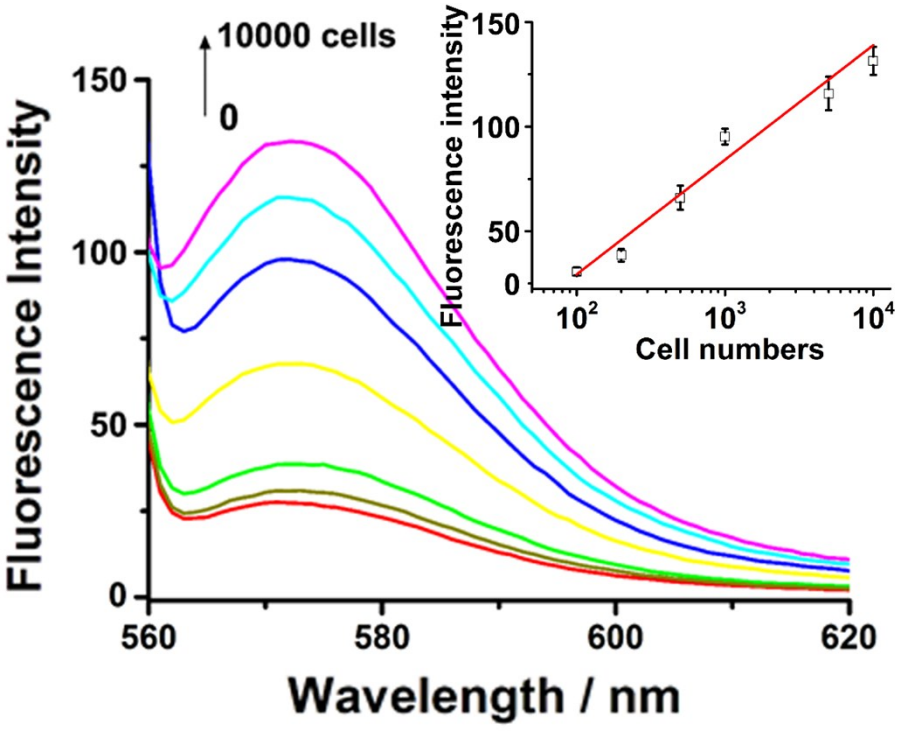

Fig. S7. Detection of telomerase extracted from cancer cell using the proposed (4)/(5)-AuNPs (from bottom to top: 0, 100, 200, 500, 1000, 5000, 10000 cells).

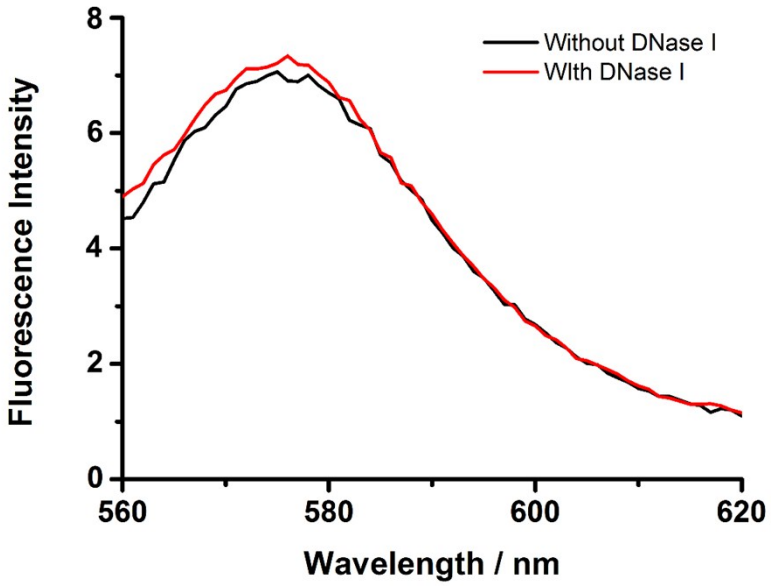

Fig. S8. The stability of the proposed nanoprobe after nuclease treatment for 2 h.

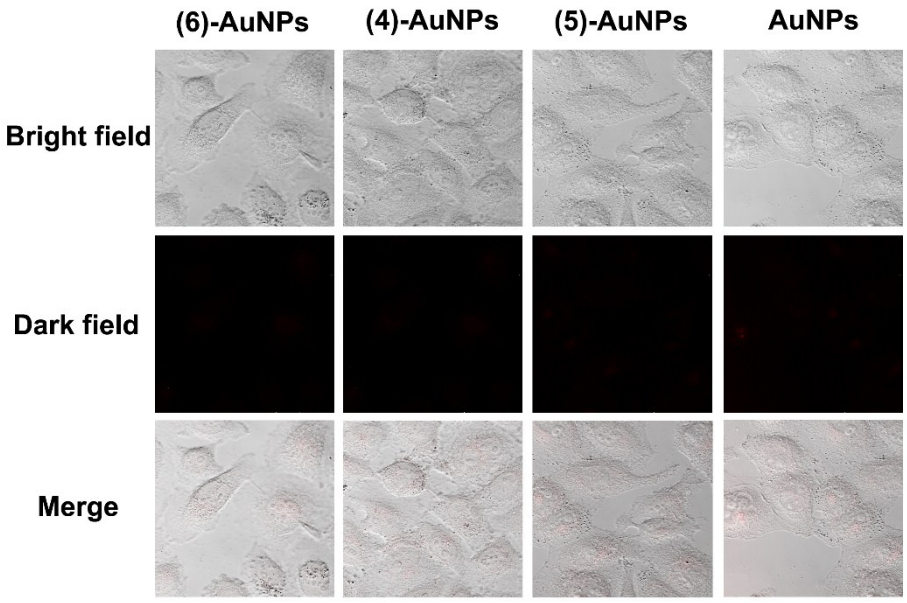

Fig. S9. Control experiments of the proposed nanoprobe for cellular telomerase

detection.

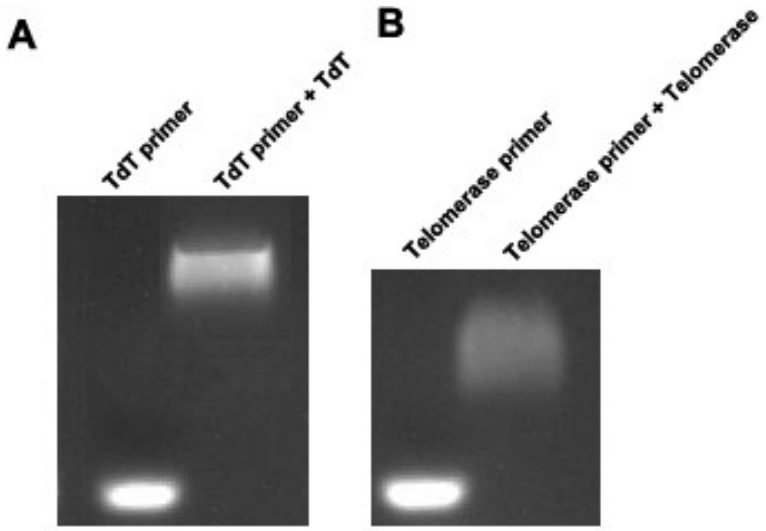

Fig. S10. Gel electrophoresis results of the primer extended by TdT and telomerase.

Table S1. Performance comparisons between the proposed assay and previous literatures for telomerase detection.

Assay	LOD	Reaction step	Detection range	Cellular detection	Ref.
Electrochemistry	10^3 cells	One step	10^3-10^5 cells	No	1
Surface enhanced Raman scattering	1 cell/mL	Two steps	1-10^4 cells/mL	No	2
Gel electrophoresis	5 cells	Four steps	10-100 cells	No	3
Fluorescence	5 cells	Three steps	0-1000 cells	No	4
Fluorescence	Single cell analysis	Two steps	-	Yes (Lipofectamine required)	5
Fluorescence	Single cell analysis	One step	10^2-10^4 cells	Yes (No lipofectamine required)	This work

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