Electronic Supplementary Information

A triple-amplification strategy for sensitive detection of telomerase at single-cell level

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EXPERIMENTAL SECTION

Chemicals and Materials. All oligonucleotides (Table S1) were synthesized and HPLC purified by Sangon Biotech Co., Ltd (Shanghai, China). Human apurinic/apyrimidinic (AP) endonuclease (APE 1), 10× NEBuffer 4 (500 mM potassium acetate, 200 mM tris-acetate, 100 mM magnesium acetate, 10 mM DTT, pH 7.9), Bst. DNA polymerase (large fragment) and 10× ThermoPol reaction buffer (200 mM Tris-HCl, 100 mM ammonium sulfate $(NH_4)_2SO_4$), 100 mM potassium chloride (KCl), 20 mM magnesium sulfate ($MgSO_4$), 1% Triton X-100, pH 8.8) were purchased from New England Biolabs (Beijing, China). The deoxynucleotide triphosphates (including dATPs, dTTPs, dCTPs, and dGTPs) were obtained from TaKaRa Bio. Inc. (Dalian, China). SYBR Gold was obtained from Life Technologies (Carlsbad, CA, USA). Bovine serum albumin (BSA)

was purchased from Sigma Aldrich Company (St. Louis, MO, USA). TRAPeze 1× CHAPS lysis buffer was purchased from Millipore (Bedford, MA, USA). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). Human cervical carcinoma cell line (HeLa cells), human embryonic kidney cell line (HEK-293T cells) and human embryonic lung fibroblast cell line (MRC-5 cells) were purchased from Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Ultrapure water was prepared by a Millipore filtration system (Millipore, Milford, MA, USA) and used in all experiments.

Table S1. Sequences of the Oligonucleotides[α]

note	sequence (5′-3′)
telomerase substrate primer	5'-AAT CCG TCG AGC AGA GTT-3'
synthetic telomerase product (TPC 7)	5'-AAT CCG TCG AGC AGA G***TT AGG GTT AGG GTT AGG GTT AGG GTT AGG GTT AGG G***-3'
assistant probe	5'-GAA GGC GGG CGA CAG CCC TAA XCC TAA CCC T-NH_2-3'
circular template	5'-CGC CCG CCT TCA CGT TGC TTC GAT TCG CAT CTA AGG CAC TCT TTA GGG CTG T-3'
signal probe	5'-FAM-TCG CAT CXA AGG CAC-BHQ-3'

[α]The italic bold letters in the TPC 7 indicate the telomeric repeats. The letter X in the assistant probe and signal probe indicates the abasic site.

Cell Culture and Preparation of Telomerase Extracts. HeLa cells, HEK-293T cells and

MRC-5 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were cultured at 37 ºC in a humidified atmosphere containing 5% CO_2. In the exponential phase of growth, cells were collected with trypsinization, washed twice with ice-cold phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer, pH 7.4), and pelleted at 2,000 rpm for 10 min at 4 ℃. About 1×10^6 million cells were resuspended in 200 μL of ice-cold 1× CHAPS lysis buffer (0.5 % CHAPS, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 5 mM mercaptoethanol, 10% glycerol, 10 mM Tris-HCl, pH 7.5), incubated on ice for 30 min, and then centrifuged at 12,000× g for 20 min at 4 ℃. After centrifugation, the supernatant was carefully transferred into a fresh tube and stored at -80 ℃ until use. For the control experiments, the telomerase extracts were pre-treated at 85 ℃ for 10 min prior to the measurement.

Telomerase Assay. First, the primer extension reaction was carried out in 10 μL of solution containing 2 μL of telomerase extracts, 100 nM telomerase substrate primer, 30 mM Tris-HCl (pH 8.3), 3 mM $MgCl_2$, 70 mM KCl, 1 mM EGTA, 0.05% (v/v) Tween 20, dNTPs (250 μM each). After 20-min telomerase extension at 37 ℃, 400 nM assistant probe, 6 U of APE 1 and 1× NEBuffer 4 (50 mM potassium acetate, 20 mM tris-acetate, 10 mM magnesium acetate, 1 mM DTT, pH 7.9) were added to the mixture and incubated at 37 ℃ for 30 min, followed by heating at 65 ℃ for 20 min to inactive APE 1. Second, 2 μL of cleavage products were incubated with a solution containing 1× ThermoPol reaction buffer (20 mM Tris-HCl, 10 mM ammonium sulfate ($(NH_4)_2SO_4$), 10 mM potassium chloride (KCl), 2 mM magnesium sulfate ($MgSO_4$), 1% Triton X-100, pH 8.8), 10 U of Bst. DNA polymerase, 500 μM dNTPs (dATP, dUTP, dGTP, dCTP each),

and 50 nM circular template in a final volume of 20 μL at 65 ℃ for 2 h. Third, 20 μL of RCA products was mixed with 1× NEBuffer 4 (50 mM potassium acetate, 20 mM tris-acetate, 10 mM magnesium acetate, 1 mM DTT, pH 7.9), 6 U of APE 1 and 800 nM signal probes in 40 μL of solution, and incubated at 37 ℃ for 40 min.

The fluorescence spectra were measured at room temperature using a F-7000 fluorescence spectrophotometer (Hitachi, Japan) equipped with a xenon lamp as the excitation source. The spectra were recorded in the range from 500 to 650 nm at an excitation wavelength of 490 nm. The excitation and emission slits were set for 5.0 and 5.0 nm, respectively. The fluorescence intensity at 520 nm was used for data analysis.

Gel Electrophoresis. The 2% agarose gel electrophoresis analysis of RCA products was carried out in 1× TAE (40 mM Tris-ethylic acid, 2 mM EDTA) at a 110 V constant voltage for 50 min. The gels were stained by SYBR gold and analyzed by ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA, USA).

To verify the stability of single-stranded assistant probes and signal probes in the presence of APE 1, we performed nondenaturating polyacrylamide gel electrophoresis (PAGE). The cleavage reaction was performed in 10 μL of reaction solution containing 2 μM assistant probes (or signal probes), 6 U of APE 1 and 1× NEBuffer 4 (50 mM potassium acetate, 20 mM tris-acetate, 10 mM magnesium acetate, 1 mM DTT, pH 7.9) at 37 ℃ for 40 min, followed by heating at 65 °C for 20 min to inactive APE 1. The cleavage products stained with SYBR Gold were analyzed by 14% polyacrylamide gel electrophoresis (PAGE) in 1× TBE buffer (9 mM boric acid, 9 mM Tris-HCl, 0.2 mM EDTA, pH 7.9) at 110 V constant voltage for 60 min at room temperature. The gel was

analyzed by ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA, USA).

Inhibition Assay. The Hela cells were cultured in serum-containing Dulbecco's modified Eagle's medium and incubated for 6 h prior to the addition of inhibitor MST-312 [N, N'-bis (2,3-dihydroxybenzoyl)-1, 2-phenylenediamine]. After incubation with various-concentration MST-312 for 72 h, the cells were collected for telomerase extraction according to the procedures described above, and the telomerase activity of 100000 Hela cells was measured. The fluorescence signals in response to different-concentration MST-312 were obtained by the F-7000 spectrometer, and the relative activity (*RA*) of telomerase was calculated according to equation 1:

$$RA\ (\%) = \frac{F_i - F_0}{F_t - F_0} \times 100\% \qquad (1)$$

where F_0 is the fluorescence intensity in the absence of telomerase extracts, F_t is the fluorescence intensity in the presence of telomerase extracts, and F_i is the fluorescence intensity in the presence of both telomerase extracts and MST-312.

SUPPLEMENTARY RESULTS

Verification of the stability of single-stranded assistant probes and signal probes in the presence of APE 1. To verify the stability of single-stranded assistant probes and signal probes in the presence of APE 1, we performed nondenaturating polyacrylamide gel electrophoresis (PAGE). A distinct band of 30 nt in the presence of APE 1 (Fig. S1, lane 2) equivalent to the complete assistant probe in the absence of APE 1 (Fig. S1, lane 1) and a distinct band of 14 nt in the presence of APE 1 (Fig. S1, lane 4) equivalent to the complete signal probe in the absence of APE 1 (Fig. S1, lane 3) are observed, respectively. These results suggest that the intact AP site in either

single-stranded assistant probe or single-stranded signal probe cannot be cleaved by APE 1.

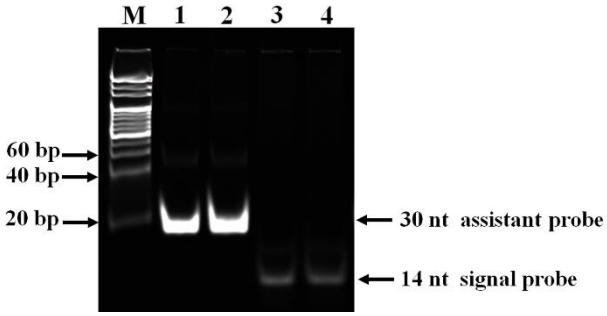

Fig. S1. PAGE analysis of the stability of single-stranded assistant probes and single-stranded signal probes in the presence of APE 1. Lane M, DNA marker; lane 1, 2 μM assistant probes; lane 2, 2 μM assistant probes + 6 U of APE 1; lane 3, 2 μM signal probe; lane 4, 2 μM signal probe + 6 U of APE 1.

Optimization of Experimental Conditions. To obtain the best assay performance, we optimized the experimental conditions including the concentrations of assistant probes and signal probes, the amount of Bst DNA polymerase, and the reaction time of Bst DNA polymerase, respectively. We employed the synthetic telomerase product TPC 7, which corresponds to TS primer extended with seven telomeric repeats $(TTAGGG)_7$, as a model to optimize the experimental condition. As shown in Fig. S2A, the F/F_0 value enhances with the increasing concentration of assistant probe from 100 to 400 nM, followed by the decrease beyond the concentration of 400 nM (F and F_0 are the fluorescence intensity in the presence and absence of TPC 7, respectively). Thus, 400 nM assistant probe is used in the subsequent experiments.

We further optimized the amount of Bst DNA polymerase and the reaction time of Bst DNA polymerase. As shown in Fig. S2B, the F/F_0 value improves with the increasing amount of Bst DNA polymerase from 2 to 10 U, followed by the decrease beyond the amount of 10 U (F and F_0

are the fluorescence intensity in the presence and absence of TPC 7, respectively). Thus, 10 U of Bst DNA polymerase is used in the subsequent research. As shown in Fig. S2C, the F/F_0 value enhances with the Bst DNA polymerase reaction time from 0 to 120 min and reaches the highest value at 120 min (F and F_0 are the fluorescence intensity in the presence and absence of TPC 7, respectively). Thus, 120 min of Bst DNA polymerase reaction time is used in the subsequent research.

We further optimized the concentration of signal probe. The high-concentration signal probes will result in the high hybridization efficiency and consequently the high cleavage efficiency and the high fluorescence signal, but the high-concentration signal probes might cause high background signal. As shown in Fig. S2D, the F/F_0 value improves with the increasing concentration of signal probe from 0.2 to 0.8 μM and reaches the highest value at 0.8 μM (F and F_0 are the fluorescence intensity in the presence and absence of TPC 7, respectively). Thus, 0.8 μM signal probe is used in the subsequent research.

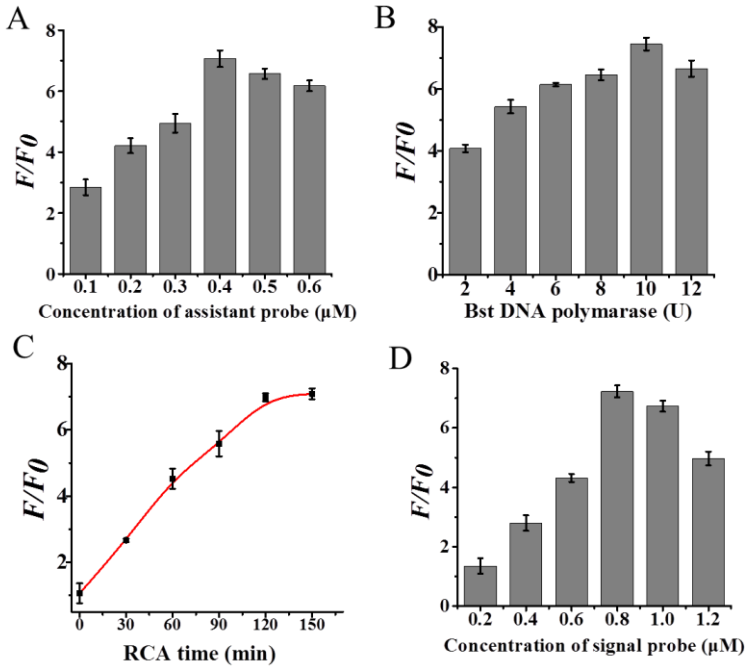

Fig. S2 (A) Variance of the F/F_0 value with the concentration of assistant probe. (B) Variance of

the F/F_0 value with the amount of Bst DNA polymerase. (C) Variance of the F/F_0 value with the reaction time of Bst DNA polymerase. (D) Variance of the F/F_0 value with the concentration of assistant probe. F and F_0 are the fluorescence intensities in the presence and absence of TPC 7, respectively. The TPC 7 concentration is 100 pM. Error bars show the standard deviations of three experiments.

Fluorescence detection of synthetic telomerase product TPC7.

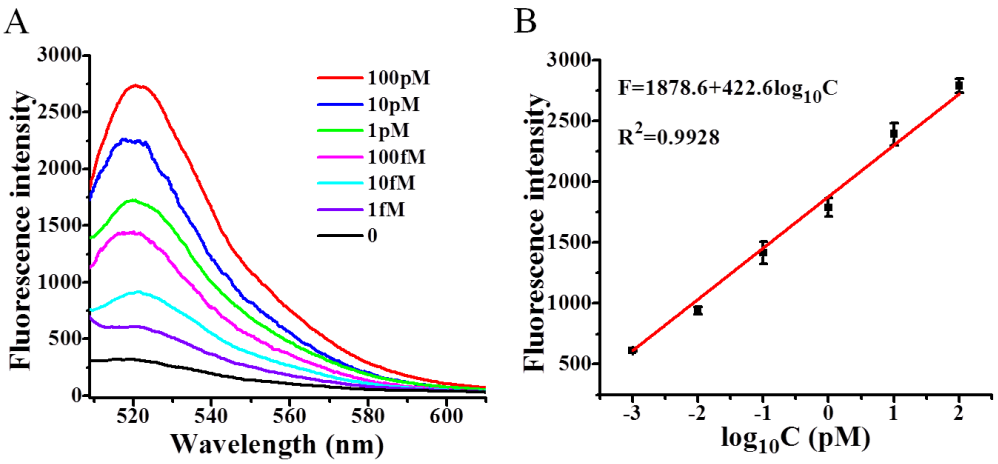

Fig. S3 (A) Measurement of fluorescence emission spectra in response to different-concentration TPC 7 in the range from 0 to 100 pM. (B) The log-linear correlation between the fluorescence intensity at 520 nm and the TPC 7 concentration. Error bars show the standard deviations of three experiments.