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Electronic Supplementary Information (ESI)

for

A Sensitive Fluorescence Strategy for Telomerase Detection in Cancer Cells Based on T7 Exonuclease-Assisted Target Recycling Amplification

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1. Experimental Section

Materials and Instrumentation

T7 Exonuclease was obtained from New England Biolabs (Ipswich, MA). Tween-20, β-mercaptoethanol, ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), ethylene diamine tetraacetic acid (EDTA), sodium deoxycholate, 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) and 3'-azido-3'-deoxythymidine (AZT) were purchased from Sigma-Aldrich. Trisodium citrate was from Amresco (Solon, OH). All other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and used without further purification. All the water used in the work was RNase-free. All oligonucleotides used in this work were synthesized from Takara Biotechnology Co. Ltd. (Dalian, China). The sequences of the oligonucleotides were given in Table S1. All fluorescence measurements were performed using a low scatter micro cell (parts No. 650-0171 from Hitachi) on a Hitachi F-7000 fluorescence spectrofluorometer (Kyoto, Japan) equipped with an R928F photomultiplier tube. The excitation

wavelength was set to 480 nm with a 5 nm slit, and the emission spectrum was collected in the range from 500 to 620 nm with a 5 nm interval and a 5 nm slit. The fluorescence readings were automatically processed using the FL Solutions Software (Ver. 2.1). The pH was measured by a model 868 pH meter (Orion). High performance liquid chromatography analysis was performed on a LC-20A chromatography (Shimadzu, Japan) equipped with fluorescence detector (RF-10AXL) at an excitation wavelength of 480 nm and an emission wavelength of 520 nm with both excitation and emission sides of 15 nm spectral bandwidth.

Table S1. Sequences of oligonucleotides used in this work

DNA	Sequences (5' to 3')
TS primer	5′-AATCCGTCGAGCAGAGTT-3′
Taqman probe	5'-*FAM*-CCC T(*TAMRA*)AA CCC TAA-3'
synthetic sequence with four TTAGGG repeats	5′-AATCCGTCGAGCAGAGTT AGGG TTAGGG TTAGGGTTAGGG-3′

Cell Culture

HeLa cells were gained from cell bank of Xiangya Central Experiment Laboratory of Central South University of China. The cells were cultured in RPMI 1640 (GIBICO) medium supplemented with 15% fetal calf serum (Invitrogen), 100 U/ml penicillin and 100 U/ml gentamicin, and the cells were maintained at 37 ℃ in humidified atmosphere (95% air and 5% CO_2).

Preparation of Telomerase Extract from HeLa Cells

The telomerase extracts were prepared according to the previously reported protocol.[S1] First, HeLa cells were collected in the exponential phase of growth and counted. Then 1×10^6 cells were transferred into an RNase-free 1.5 ml EP tube and washed twice with ice-cold PBS by centrifugation at 1,800 rpm for 5 min. After discarding the supernatant carefully, the cells were resuspended in 200 μL of ice-cold

NP-40 lysis buffer (10 mM Tris-HCl, pH 8.0, 1 mM $MgCl_2$, 1 mM EDTA, 1% NP-40, 0.25 mM sodium deoxycholate, 10% glycerol, 150 mM NaCl, 5 mM β-mercaptoethanol, 0.1 mM AEBSF), and incubated for 30 min on ice, and then centrifuged at 12 000 rpm for 20 min at 4 ℃. Finally, the supernatant was collected carefully and transferred to microcentrifuge tube and stored at -80 ℃ until ready for analysis. The telomerase extracts from other cancer cells were prepared in the similar protocol.

Telomerase Extension Reaction

Telomerase extracts were first diluted with lysis buffer to 5 μL of a series of different concentrations and then added into 20 μL of the telomerase extension reaction buffer (20 mM Tris-HC1, pH 8.3, 1.5 mM $MgC1_2$, 1 mM EGTA, 63 mM KCl, 0.05% Tween 20, 0.2 mM dATP, 0.2 mM dGTP, 0.2 mM dTTP, and 5 nM TS primer). The mixtures were then incubated at 30 ℃ for 2 h. For the control experiments, telomerase extracts were heat-treated at 95 ℃ for 10 min or pre-incubated with 100U RNase ONE at 37 ℃ for 30 min. In the inhibition experiments, different volumes of 3'-azido-3'-deoxythymidine (AZT, 50 mM) solution were also added into the telomerase extension reaction buffer to achieve the desired final concentrations.

Detection of Telomerase Activity

After the telomerase extension reaction, the reaction products were directly added to 50 μL of T7 Exonuclease reaction system containing 20 mM Tris-Ac (pH 7.9), 50 mM KAc, 10 mM $Mg(Ac)_2$, 1 mM DTT, 50 nM Taqman probe and 10U T7 Exonuclease. The mixtures were incubated at 25 ℃ for 60 min and diluted to 100 μL with H_2O, and then the fluorescence intensities were recorded immediately at room temperature in a quartz cuvette (with path length of 10 mm and inside width of 1 mm) on an F-7000 spectrofluorometer (Hitachi, Japan) with the detector of photomultiplier tube. The excitation wavelength was 480 nm and the emission wavelengths were in

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the range from 500 to 620 nm with the slit widths of both excitation and emission of 5 nm.

2. References

S1 B. S. Herbert, A. E. Hochreiter, W. E. Wright and J. W. Shay, *Nat Protoc*., 2006, **1**, 1583-1590.

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3. Supplementary Figures

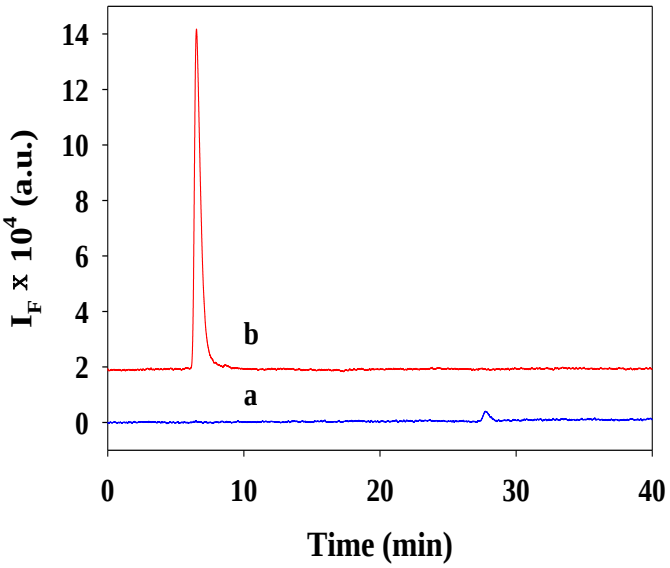

Fig. S1 High performance liquid chromatography analysis of the Taqman probe after incubated with (a) lysis buffer and (b) telomerase extracts from 1000 HeLa cells.

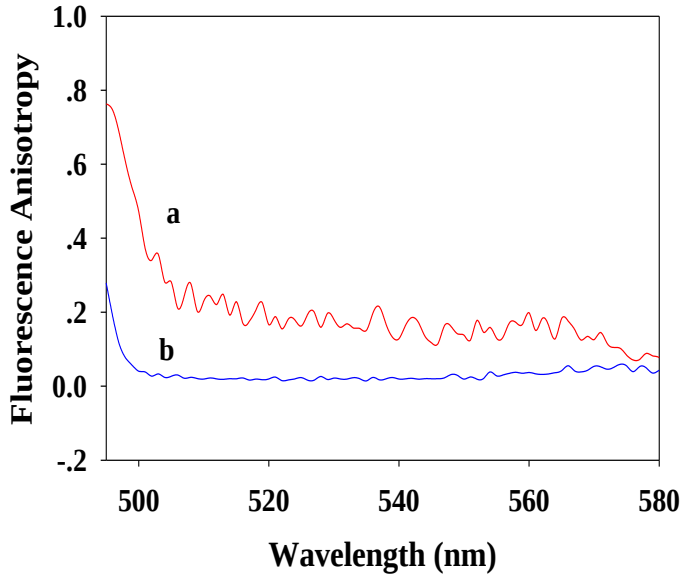

Fig. S2 Fluorecence anisotropy analysis of the Taqman probe after incubated with (a) lysis buffer and (b) telomerase extracts from 1000 HeLa cells.

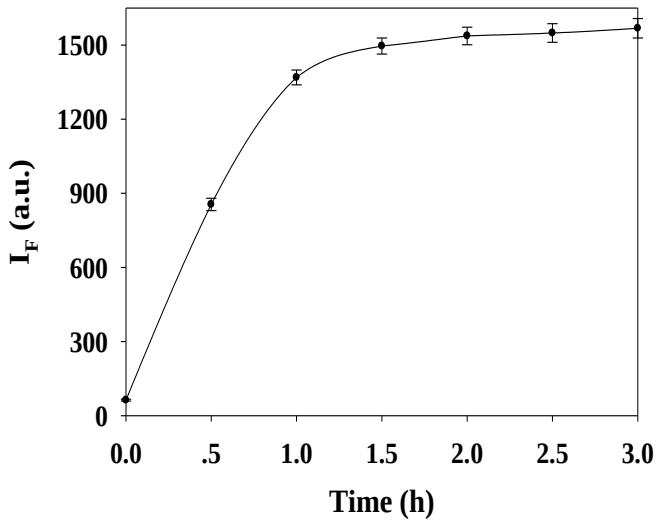

Fig. S3 Effect of the telomerase extension reaction time on the fluorescence intensity. The reaction system contained 5 nM TS primer, 20 mM Tris-HC1, pH 8.3, 1.5 mM $MgC1_2$, 1 mM EGTA, 63 mM KCl, 0.05% Tween 20, 0.2 mM dATP, 0.2 mM dGTP, 0.2 mM dTTP.

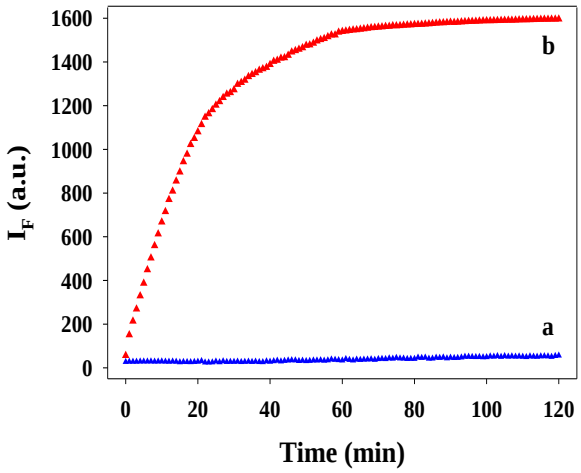

Fig. S4 The effect of the T7 Exonuclease reaction time on the fluorescence intensity with (a) lysis buffer and (b) telomerase extracts from 1000 HeLa cells. The reaction was performed at 25 ℃ with 10 U T7 Exonuclease. Telomerase extension reaction was carried out at 30 ℃ for 2 h.

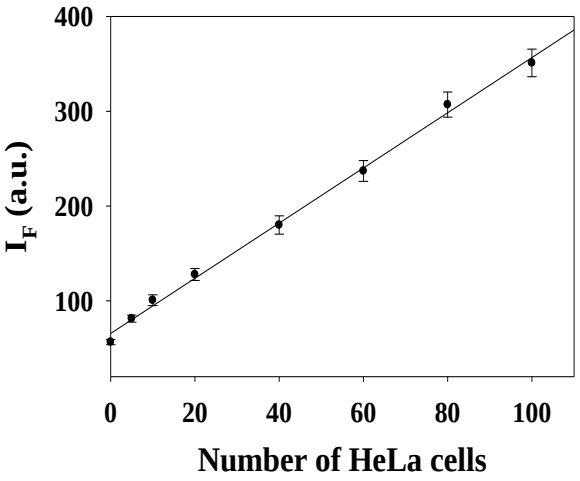

Fig. S5 The relationship between the fluorescence intensity and different numbers of and HeLa cells. Error bars are standard deviation for five repetitive assays.

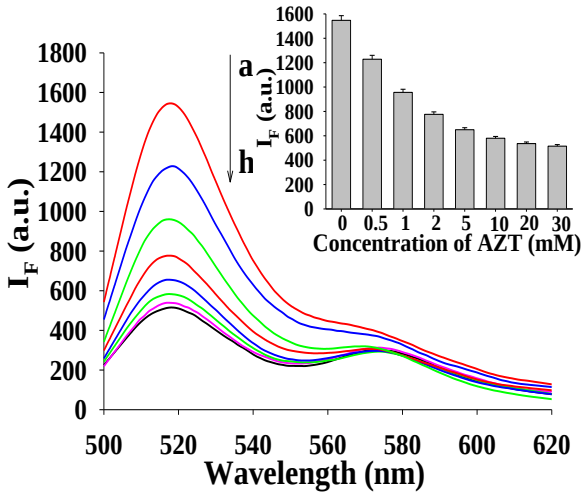

Fig. S6 Fluorescent spectra and bar graph (inset) in response to different concentratins of inhibitor (AZT), the curves from a to h represent 0, 0.5, 1, 2, 5, 10, 20 and 30 mM AZT, respectively.

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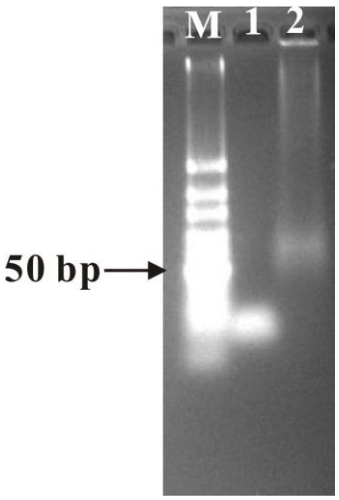

Fig. S7 Agarose gel (4%) electrophoresis experiments. Lane M: DNA size marker, lane 1: TS primer incubated with lysis buffer, lane 2: TS primer incubated with telomerase extracts. Because of the varying molecular weight for the product from telomerase-catalyzed elongation, the product is shown as a weakly fluorescent band spanning a large molecular weight range in the image.