Supporting Information

Highly sensitive detection of telomerase based on a DNAzyme strategy: method development and its application for in situ detection

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Materials and methods

General information: The oligonucleotides and dNTPs were purchased from Invitrogen Technology(Shanghai, China). RiboLock™ RNase Inhibitor and dNTPs were purchased from Fermentas Inc. Tris base, $MgCl_2$, KCl, EGTA, glycerol, β-mercaptoethanol, CHAPS, Tween 20, BSA and PMSF were purchased from Sigma Inc. HS578BST and HeLa cell lines were obtained from the Committee on Type Culture Collection of Chinese Academy of Sciences. Fluorescence was measured by LS55 Perkin Elmer. All measurements were performed at room temperature.

Cell culture: HeLa cells and HS578BST cells were cultured in Dulbecco's modified Eagles medium (DMEM). All cells were supplemented with 10% fetal bovine serum and 100 U/ml 1% antibiotics penicillin/streptomycin and maintained at 37 °C in a 100% humidified atmosphere containing 5% CO_2 at 37 °C.

Preparation of cellular crude of telomerase: Trypsinized cells ranging from 200 to 10^5 were washed with PBS twice. Resuspend the cell pellet in 200 μL of 1 × CHAPS Lysis Buffer/10^6 cells for 30 mins on ice. The recipe of 1 × CHAPS lysis buffer was 10 mM Tris–HCl at pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 10% glycerol, 10 U/mL RiboLock™ RNase Inhibitor, 5 mM β-mercaptoethanol, 0.5% CHAPS and 0.1 mM PMSF. After lysis, the solution was centrifuged at 15000 g for 30 mins. The telomerase was contained in the supernatant, which was used to do the telomere extension. The whole process should prevent RNA degradation.

Telomere extension reaction: Extension reaction was performed in a buffer containing 20 mM Tris-HCl(pH 8.3), 1.5 mM $MgCl_2$, 63 mM KCl, 0.05% Tween 20, 1 mM EGTA, 100 μM deoxynucleoside triphosphates, BSA(0.1 mg/mL), 100 nM of Telo probe and different amounts of telomerase crudes.

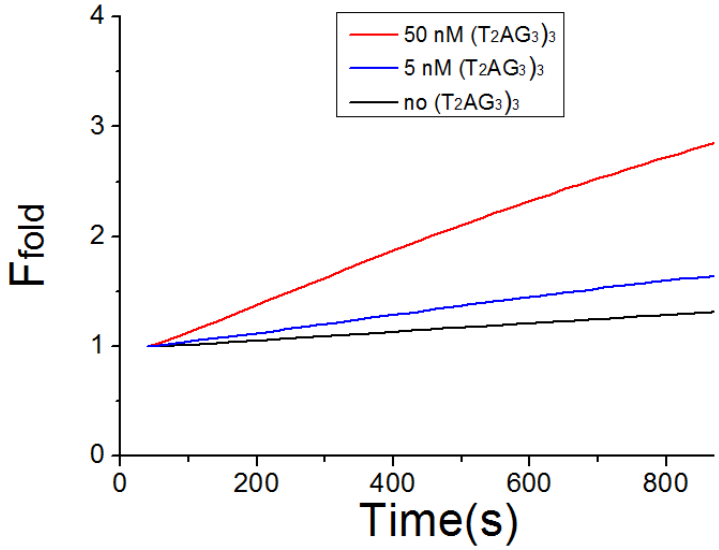
Cleavage reaction and fluorescence measuring: The cleavage reaction was incubated in a buffer containing 66 mM tris-HCl(pH 7.6) and 6.6 mM Mg^{2+}; and then MB substrate was added, making the final concentration to be 0.1 μM. The detection was conducted in the Kinetics mode of a LS55 Perkin Elmer. The excitation and emission wavelengths were set at 494 and 518 nm, respectively.

Confocal fluorescence imaging for in situ detection of telomerase: All cells were plated on chamber slides for 24 h. Then slides with cells were washed with cold phosphate buffer saline(PBS) twice. Two hundred μL of extension buffer contained 20 mM Tris-HCl(pH 8.3), 1.5 mM $MgCl_2$, 63 mM KCl, 0.05% Tween 20, 1 mM EGTA, 100 μM deoxynucleoside triphosphates, BSA(0.1 mg/mL) and 100 nM of Telo probe. The slides were incubated at 22 °C for 30 mins in a dark box. After extension ,the slides were washed with cold PBS twice, followed by incubation in a buffer containing 66 mM tris-HCl(pH 7.6), 6.6 mM Mg^{2+} and 100 nM MB substrate for 45 mins. After the cleavage reaction, the slides were washed with cold PBS twice. Cells were examined using a fluorescence microscope(Nikon C1-Si, Tokyo, Japan).

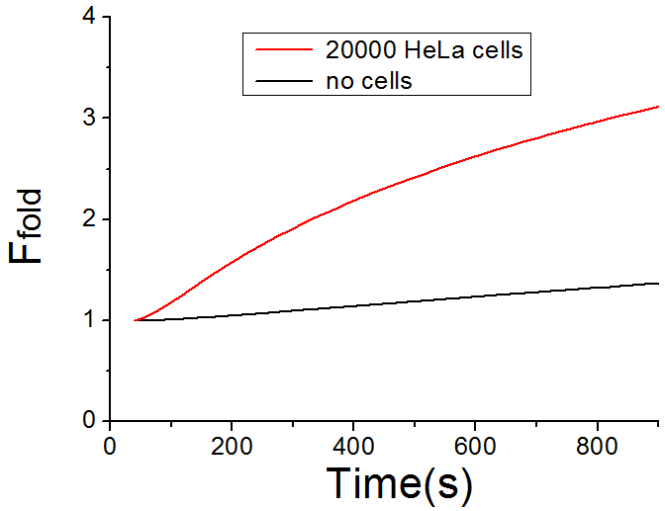
Flow cytometry analysis for telomerase detection: About 5×10^5 cells were seeded in a 6-well plate and incubated at 37 ^{o}C for 16 h. Then the cells in positive sample were incubated in 200 μL of extension buffer containing 20 mM Tris-HCl(pH 8.3), 1.5 mM $MgCl_2$, 63 mM KCl, 0.05% Tween 20, 1 mM EGTA, 100 μM deoxynucleoside triphosphates, BSA(0.1 mg/mL) and 100 nM of Telo 2 probe. The cells in control sample were incubated in the extention buffer without telo 2 probe added. The extension was performed at 22 °C for 30 mins in a dark box. After extension ,the cells were washed with cold PBS twice, followed by incubation in a buffer containing 66 mM tris-HCl(pH 7.6), 6.6 mM Mg^{2+} and 100 nM MB substrate for 45 mins. After the cleavage reaction, the cells were washed with cold PBS twice. Cells were harvested and examined using flow cytometry on a Beckmen Coulter Eltra flow cytometer. Statistical analysis was performed using FlowJo Version 7 Analysis Software.

Table S1 Sequences of oligomers used for detection of telomerase.

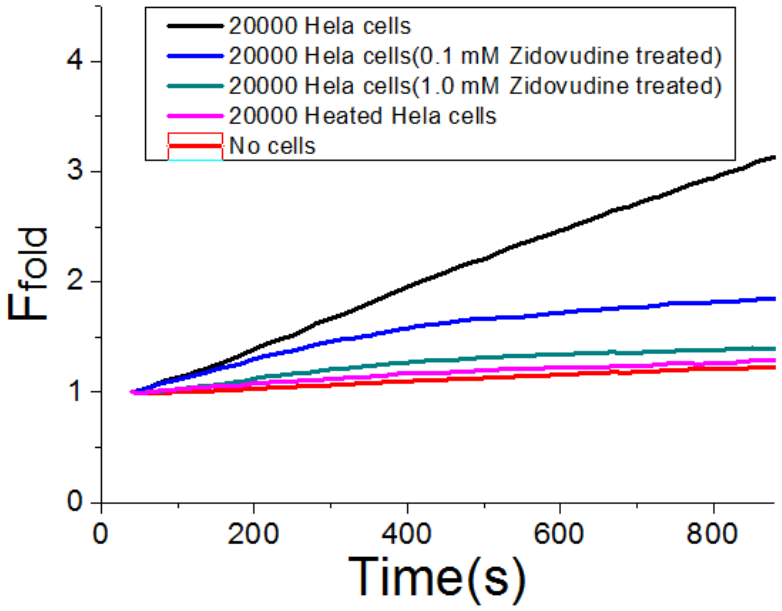
Oligomer	Sequence(from 5'to 3')
Telo	GGGTTAGGGTTA CATCTCTTCTCCGAGCCGGTCGAAATAGTGGGTG AAA$(TAACCC)_5$AACAATCCGTCGAGCAGAGTT
Telo1	GGGTTAGGGT CATCTCTTCTCCGAGCCGGTCGAAATAGTGGGTG AAA$(TAACCC)_5$AACAATCCGTCGAGCAGAGTT
Telo2	GGGTTAGGGTT CATCTCTTCTCCGAGCCGGTCGAAATAGTGGGTG AAA$(TAACCC)_5$AACAATCCGTCGAGCAGAGTT
Telo3	GGGTTAGGGTTAG CATCTCTTCTCCGAGCCGGTCGAAATAGTGGGTG AAA$(TAACCC)_5$AACAATCCGTCGAGCAGAGTT
Telo4	GGGTTAGGGTTAGG CATCTCTTCTCCGAGCCGGTCGAAATAGTGGGTG AAA$(TAACCC)_5$AACAATCCGTCGAGCAGAGTT
MB substrate for 817 DNAzyme	/FAM/-CCACCACATTCAAATTCACCAACTATrAGGAAG-AGATGTTACGAGGCGGTGGTGG-/BHQ/



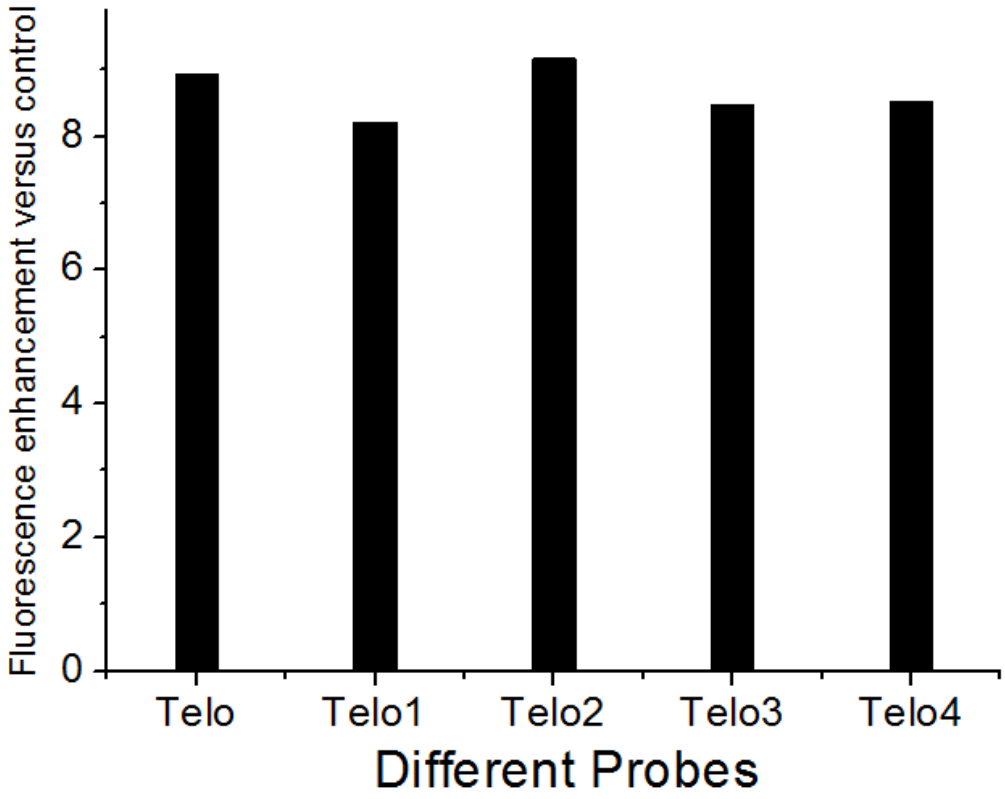
Supporting Information Figure S1: Fluorescence absorbance change upon analysis of different concentration of added $d(T_2AG_3)_4$



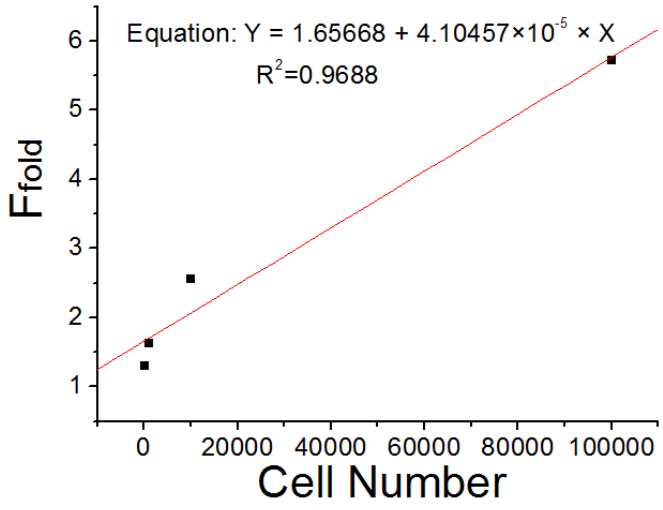
Supporting Information Figure S2: Fluorescence changes upon analyzing telomerase originating from 2×10^4 HeLa cells.



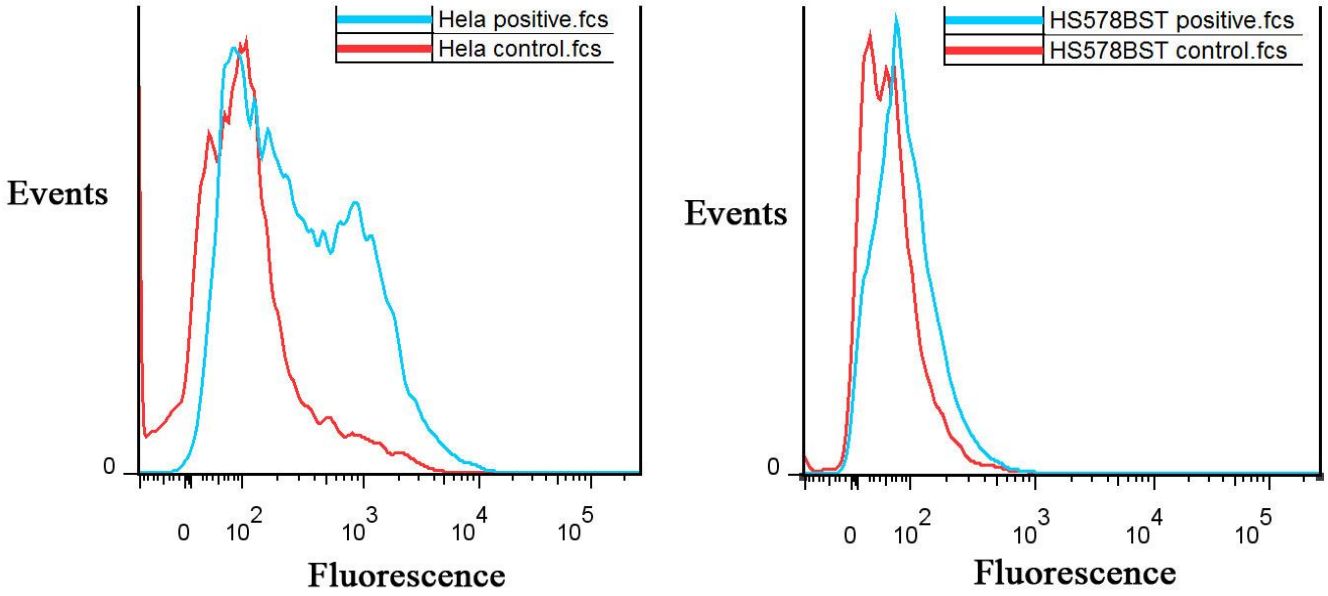
Supporting Information Figure S3: Fluorescence changes upon analyzing telomerase activity originating from HeLa cells. Zidovudine was chosen as the inhibitor of telomerase. HeLa cells were randomly divided into drug and no drug groups. Cells in the drug group were incubated in culture medium supplemented with Zidovudine at the indicated concentrations for 24h. The same culture medium without Zidovudine was used for the cells in the no drug group. The fluorescence in Zidovudine-treated HeLa cells was evidently lower than the one in no drug cells. For a negative control, the cells were heated at 70 ^{o}C for 15 mins. The concentrations for Telo and MB substrate are 50 nM and 100 nM, respectively.



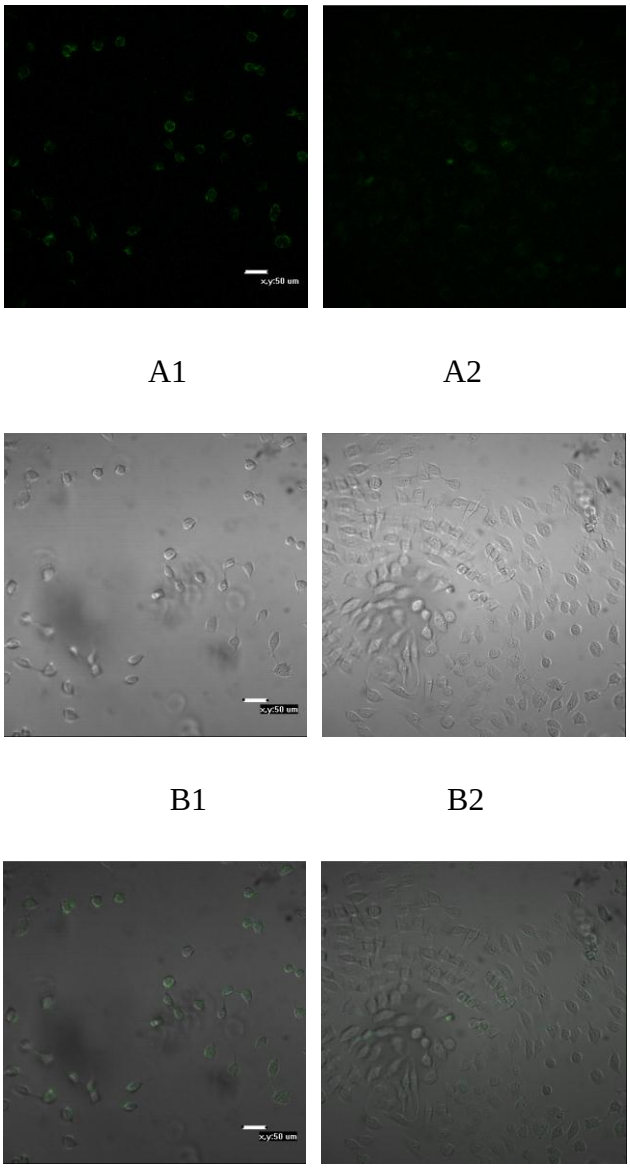
Supporting Information Figure S4: Bars represent the fluorescence ratio values of (F/F_0-1) upon analyzing 2×10^4 HeLa cells over control of the different probes, where F_0 and F are the fluorescence signals at the beginning and after a time interval of 15 mins, respectively. Telo2 possess a best signal over background.



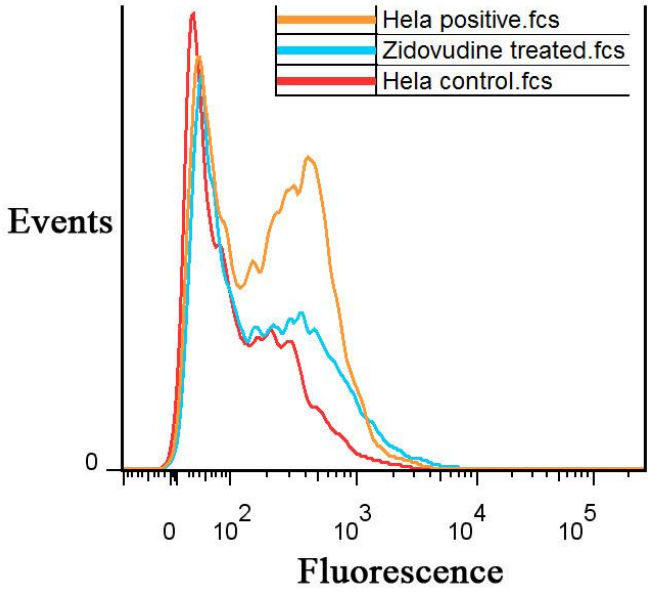
Supporting Information Figure S5: The fluorescence enhancement was determined after a time interval of 15 mins. Fitting curve of the fluorescence enhancement for different amounts of HeLa cells. The curve was plotted with the fluorescence enhancement over background vs cell number.



Supporting Information Figure S6: Flow Cytometry analysis of HeLa cells and HS578BST cells based on the 8-17 DNAzyme strategy. In the positive samples, the cells were incubated in the extension buffer in the presence of Telo 2 probe, while there was no Telo 2 probe in the control cells. The harvested cells were analyzed as described under "Materials and methods." There was an evident fluorescence difference between the positive sample and the control one of HeLa cells, while a much decreased fluorescence difference could be observed in HS578BST cells.



Supporting Information Figure S7: Fluorescence confocal microscopy analysis of HeLa cells based on the 8-17 DNAzyme strategy. Zidovudine was chosen as the inhibitor of telomerase. HeLa cells were randomly divided into positive and the control groups. Cells in the positive group were incubated in culture medium supplemented with Zidovudine at the concentration of 1.0 mM for 24h. The same culture medium without Zidovudine was used for the cells in the control group. The fluorescence in Zidovudine-treated HeLa cells was evidently lower than the one in control cells. A, The green passage (excitation 488 nm, emission 530 nm). B, the phase-contrast passage. C, the colocalization passage. Sample1: Control HeLa cells; Sample2: 1 mM Zidovudine treated HeLa cells.



Supporting Information Figure S8: Flow Cytometry analysis of HeLa cells based on the 8-17 DNAzyme strategy. Cells in the experimental group were treated with Zidovudine at the concentration of 1.0 mM for 24h. The fluorescence in Zidovudine-treated HeLa cells was evidently lower than the one in the positive sample. The harvested cells were analyzed as described under "Materials and methods."