Electronic Supplementary Information

Visualizing Telomerase Activity for Identification of Tumours by Hybridization-Triggering Ratiometric Fluorescences

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EXPERIMENTAL SECTION

Animal use protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Institute of Hefei Physical Science (Chinese Academy of Sciences).

Materials. All DNA strands used in this work were obtained from Sangon Biotech (Shanghai, China). The PBS buffer (136.89 mM NaCl, 2.67 mM KCl, 8.24 mM Na_2HPO_4, and 1.76 mM KH_2PO_4, pH 7.4), CHAPS lysis buffer, DNA marker, Diethyl pyrocarbonate (DEPC) and tris-acetic-EDTA (TAE) buffer were all purchased from Sangon Biological Engineering Technology & Co., Ltd. (Shanghai, China). The standard telomerase product was purchased from Chemicon International, Inc. (Billerica, MA).

Cell lines. HeLa (cervical tumour cell), 293T (embryonic kidney tumour cell), A549 (lung tumour cell), N87 (stomach tumour cell) and MRC-5 (normal lung cell), were purchased from Shanghai Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, China). MCF-10A (normal breast cell) and QSG (normal liver cell) were obtained from Shanghai Bioleaf biotech Co. Ltd. Hep G2 (liver tumour cell) was supplied from Center of Medical Physics and Technology, Hefei Institutes of Physical Science, Chinese Academy of Sciences.

Cell Cultures. HeLa, MRC-5, A549, N87 and 293T cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. QSG, Hep G2, and MCF-10A cells were cultured in 1640 medium with 10% FBS and 1% penicillin-streptomycin. The cells were maintained in a humidified atmosphere (95% air and 5% CO_2) at 37 °C. The densities of cells were measured by cell-counting board prior to the use of cells.

Cell Lysates Preparation. Cells were lysed by using the NP-40 method according to the documented protocol (R. Qian, L. Ding and H. Ju, *J. Am. Chem. Soc.*, 2013, **135**, 13282-13285). Firstly, cells were exfoliated from culture dish by use of trypsin, washed three times with PBS and centrifugated at 1200 rpm for 5 min at 4 °C. The cell number was determined with a Hausser Petroff-Hausser cell counter (USA). Then, the cells were resuspended in cooled lysis buffer at a concentration of 1.0×10^7 cells/mL and kept at 4 °C for 30 min under shaking. Finally, the mixture were centrifuged at 11,000 rpm for 20 min and flash frozen in liquid nitrogen. The as-prepared lysate was stored at -80 °C for further use.

Telomerase Activity Detection. Different volumes of pure telomerase or cellular lysates containing specific number of cells (0, 100, 500, 1000, 2000, 5000, 10 000, 100 000 or 200 000) were incubated with mixtures consisting of 10 μL TP (10 μM), 10 μL MB (10 μM), 4 μL dNTPs (10 mM) and 20 μL 10× TRAP buffer at 37 °C for 1.5 h (in all experiments, the total volume was adjusted to 200 μL with DEPC-treated water). Then, the fluorescent spectra of product were measured with an excitation of 485 nm. In control experiments, the telomerase in the HeLa cell lysates was first heated at 90 °C for 30 min, and then the identical operation and measurement were performed.

PCR amplification of TP strand. The reaction for the extension of TP strand was performed using cell lysates or commercial telomerase. 2 μL of cell lysates or telomerase reagent was mixed with 3 μL TP strand, 16 μL water, 2.5 μL 10× TRAP buffer, 0.5 μL dNTPs (10 mM), 0.5 μL TRAP primer mix and 0.5 μL Taq polymerase (5 units/μL) in a centrifuge tube. Then, the mixture was incubated at 37 °C for 30 min to elongate TP primer. The elongated TP strands were amplified with the PCR instrument for 33 circular steps, and each step includes the incubation at 94 °C for 30 s, 60 °C for 30 s and 70 °C for 1 min.

Gel electrophoresis. The working principle of this strategy was verified by using the technique of gel electrophoresis. 10 μL samples were mixed with 2 μL of 6× loading buffer, and then injected into polyacrylamide hydrogel. Electrophoresis was taken out in tris-acetic-EDTA (TAE) at 120 V for 55 min. Then the gel was stained with 4S red plus for 5 min and imaged with a gel imaging system.

Establishment of tumour models. Athymic nude mice (four week old) were obtained from Nanjing Biomedical Research Institute (Nanjing University, China). All mice were maintained in a

specific pathogen-free room and used according to the animal care regulations of Hefei Institutes of Physical Science, Chinese Academy of Sciences, and all efforts were made to minimize animal suffering. To obtain the human cervix tumour in mice, one million HeLa cells were suspended in 100 μL DMEM, and mixed with 100 μL matrigel (BD Biosciences). The mixture was injected into the subcutaneous space under the armpit of mice. The HeLa-implanted mice were maintained until the size of tumours reached ~400 mm^3. Tumour volumes were calculated as follows: tumour volume (mm^3) = [(W^2 × L)/2] in which width (W) is defined as the smaller of the two measurements and length (L) is defined as the larger one. Other tumour models were also established according to the similar method.

Telomerase from Tissue Samples. The mice were euthanized and the tumour or normal tissus were taken out. The tissues were washed, weighted and pulverized in liquid nitrogen before lysed using CHAPS lysis buffer. The mixture was centrifuged at 11000 rpm for 20 min after incubated at 4 °C for 30 min, and then the supernatant was collected. The as-prepared tissue lysates were stored at -80 °C for further experiments.

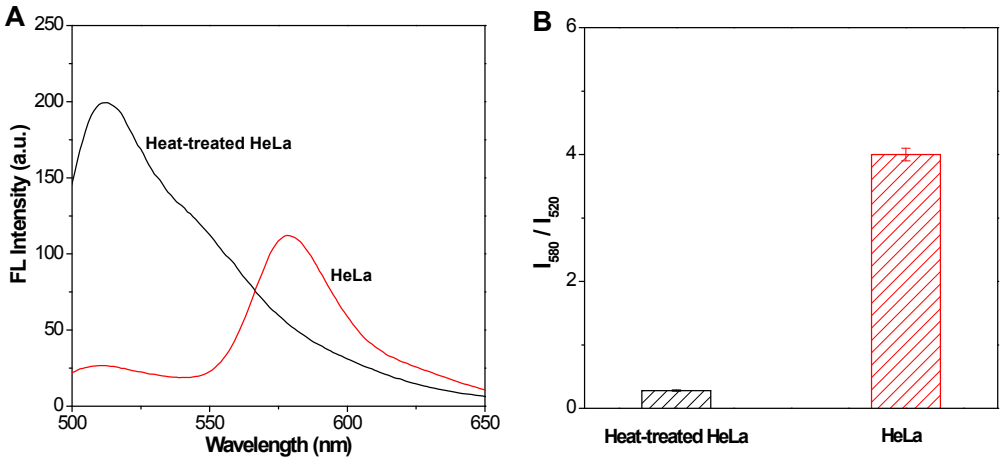

Figure S1. Control experiments for telomerase activity in HeLa cell lysates. (A) Control experiment by telomerase-inactivated HeLa cell lysates. In the control experiment, the cell lysates were heated to inactivate telomerase there. A mixing solution containing 10 μL TP (10 μM), 10 μL MB (10 μM), 4 μL 10 mM dNTPs, 20 μL 10× TRAP buffer and 146 μL DEPC water was co-incubated with 10 μL of HeLa lysate or heat-treated HeLa lysate at 37 °C for 1.5 h. Fluorescent spectra were then measured. (B) Fluorescence intensity ratio (I_{580}/I_{520}) histogram of HeLa lysates and heat-inactive HeLa lysates. The error bars represent standard deviation (± SD) from three measurements.

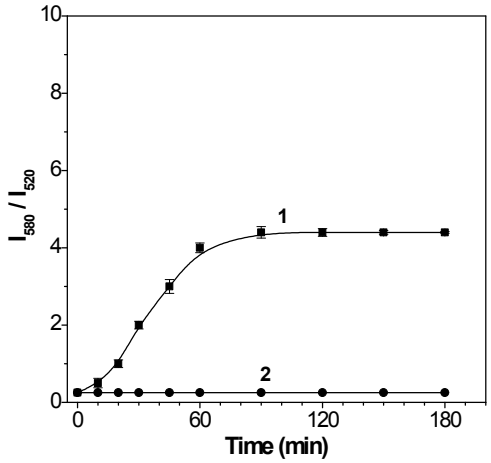

Figure S2. Reaction dynamics of the strategy with telomerase in HeLa lysate. 10 μL TP (10 μM) and 10 μL MB (10 μM) were mixed with 4 μL dNTPs (10 mM), 20 μL 10× TRAP buffer, and 146 μL DEPC water. The mixture was co-incubated with 10 μL of HeLa cell lysate (100,000 cells) or DEPC water. The time-dependent fluorescent enhancements were measured: (1) HeLa cell lysate; (2) blank DEPC water. The error bars represent standard deviation (± SD) from three measurements.

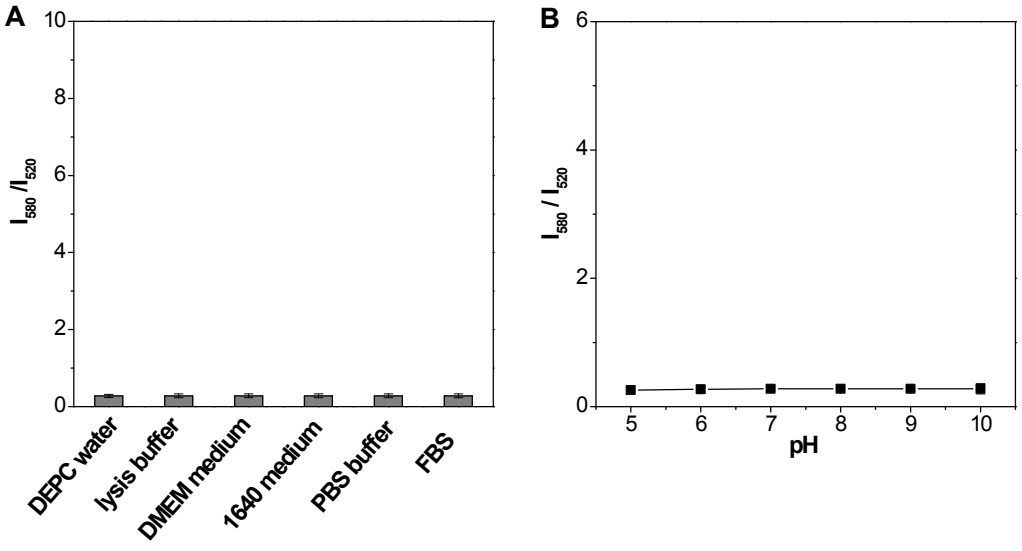

Figure S3. Stability of the strategy. Equal amount of TP and MB were co-incubated with different media including DEPC water, lysis buffer, DMEM medium, 1640 medium, PBS buffer, FBS or reaction buffer with different pH values from 5.0 to 10.0 at 37 °C for 2 h, and fluorescent spectra were measured. (A) Fluorescence intensity ratio (I_{580}/I_{520}) in various media. (B) Fluorescence intensity ratio (I_{580}/I_{520}) with different pH values. The error bars represent standard deviation (± SD) from three measurements.

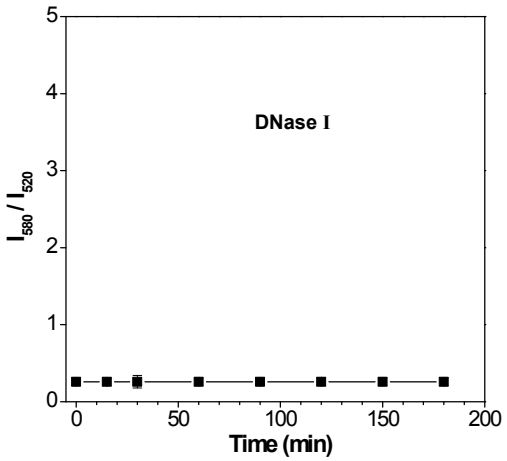

Figure S4. Fluorescence intensity ratio (I_{580}/I_{520}) for DNase I. The DNA nuclease DNase I was co-incubated with equal amount of TP and MB in PBS buffer at 37 °C for 3 h, and the time-dependent fluorescent spectra were measured. The error bars represent standard deviation (± SD).

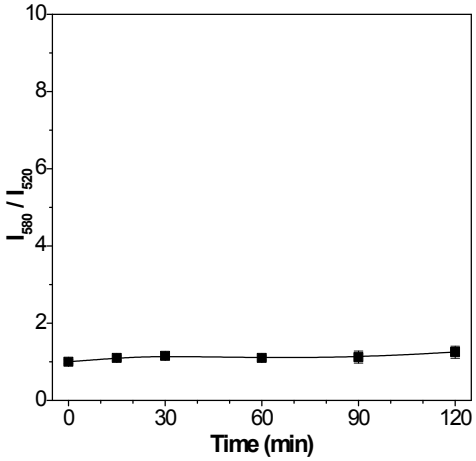

Figure S5. Control experiments for telomerase activity of the strategy with mismatched DNA sequences. Time-dependent fluorescent enhancements of the strategy with mismatched DNA sequences in HeLa lysate. 10 μL of TP (10 μM) with mismatched DNA sequences and 10 μL of MB (10 μM) were mixed with 4 μL dNTPs (10 mM), 20 μL 10× TRAP buffer, and 136 μL DEPC water. The mixture was co-incubated with 10 μL of HeLa lysate. (TP strand: 5′-FAM-AATCCGTCGAGCAGAGAA-3′. The mismatched bases that differ from those in TP strand are marked in red.)

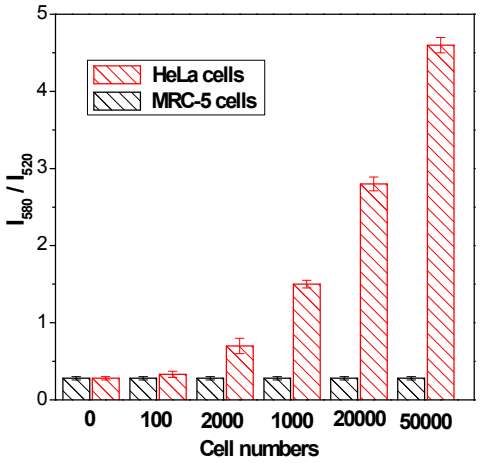

Figure S6. Telomerase activity in tumuor HeLa cell lysates and normal MRC-5 cell lysates with increasing cell number. The error bars represent standard deviation from three measurements.

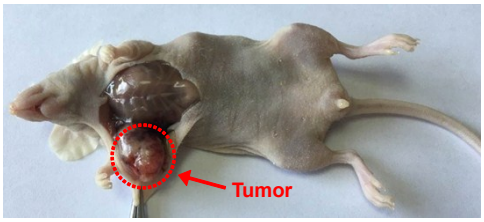

Figure S7. Image of an athymic nude mouse with cervical tumour model. The picture was taken by a camera under natural light.