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Supporting Information For

A seesaw ratiometric probe for dual-spectrum imaging and detection of telomerase activity in single living cells

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Experimental Section:

Chemicals and materials.

Chloroauricacid ($HAuCl_4.3H_2O$) and MTT were purchased from Sigma-Aldrich (St. Louis, MO). The Human TE Elisa Kits was purchased by Shanghai Kang lang Biotech Co., Ltd. RPMI and RPMI 1640 Medium Modified purchased by HyCloneTM. LysoTracker Green DND-26 purchased by Yeasen Biotech Co., Ltd. All chemicals employed were of analytical grade and were used without further purification. Double-distilled deionized water was used throughout the experiments. The cells were obtained from Tumor Marker Research Center, Cancer Hospital of the Chinese Academy of Medical Sciences (Beijing, China). The oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China); and the sequences are listed in Table S1 and others materials are listed in supporting information.

Table S1. Oligonucleotide sequence used in this experiment.

Oligonucleotides Name	Sequence	Description
H1	GCTGAAGGGTTAGGGTTTCAGC	5'-Cy3, 3'-SH
H2	CCTAACCCTAACCCTAACTCTG GTCGACGGATTTTTTTTTAATCC GTCGACCAGAGTT	5'-Rox

Equipments.

The transmission electron microscopy (TEM) images were captured with a JEM 1200EX transmission electron microscope (JEOL, Japan). UV/Vis absorption spectra were obtained

with a Cary 50 UV/Vis-NIR spectrophotometer (Varian, USA). The cells were disrupted with Scientz-IID ultrasonic cell disruptor, (Scientz, Ningbo China). The SERS analysis was performed in a Renishaw Invia Raman spectrometer (RamLab-010) at an excitation wavelength of 633 nm (Renishaw, England). The wave number accuracy was ±1 cm^{-1}. Fluorescence imaging was performed using a Leica TCS SP5 inverted confocal microscope (Leica, Germany).

Preparation of the gold nanoparticles (AuNPs).

The traditional citrate reduction method [1] was used to synthesize the gold nanoparticles (AuNPs). After immersing in the nitric acid solution for 24 h, the distilling flask and the condenser pipe were then rinsed by deionized water before used. Next, 100 ml 0.01% $HAuCl_4$ solution was stirred and heated continuously to reach its boiling point. Then 1.5 mL 1% trisodium citrate solution was added when the solution reached the boiling point and the color of the solution was changed from a primrose yellow to a wine red a few minutes later. To ensure the complete reduction reaction, the AuNPs solution need to be refluxed for another 30 min and cooled to the room temperature. The gold colloidal solutions were then stored in brown bottle at 4 °C temperature for the later experiment.

Preparation of the telomerase solution.

We used original human telomerase extract (80 IU/L). Telomerase solutions with different concentrations from 4×10^{-4} to 4×10^{-12} IU were diluted by the telomerase diluent agent to prepare for detecting telomerase in vitro and stored on the refrigerator at 4 °C.

Preparation of Functionalized SR Probe: To prepare functionalized SR probe, the H1 DNA (1 μM, 50 μL) were annealed at 95 °C water bath for 5 min and cooled with ice bath before used. Next, fifty microliters of a solution containing of the annealed H1 DNA was left in 1 mL AuNPs solution (20 nm) for 24 h in a constant temperature vibrator. Then, the thiol-modified H1 strands were immobilized on the surfaces of the AuNPs via a covalent gold−thiol bond. Subsequently, 200.0 μL of 0.05 M NaCl was blended with the mixture, and then 200.0 μL of 0.1 M NaCl was added after 6 hours to enhance the stability of the SR probe. After 6 hours, the H1-AuNPs complex were centrifuged at 12000 rpm for 30 min and resuspended in annealed H2 DNA (1 μM, 50 μL) for another 2 h. Afterward, the preparation of the SR probe was determined by measuring the Raman signals of Rox and Cy3 at 633 nm

laser.

Polyacrylamide gel electrophoresis.

First, the amount of DNA (1×10^{-5} M) need to be calculated according to the formula $10^{-5}\times V\times M=0.2$ ng. Therein, V represented the bulk of the DNA (1×10^{-5} M) and M represented the molecular weight of the DNA. Later, the bulk of the DNA solution need to be added to 5 μL with PBS solution, annealed at 95 °C for 5 min and cooled at 4 °C before used. Second, 100 mL 50×TAE (Tris, $Na_2EDTA\cdot 2H_2O$, glacial acetic acid and deionized water) was prepared. Next, 17.5 % polyacrylamide gel (40 % gel, deionized water, 50 × TAE, 10 % APS and TEMED) was prepared. Later, the gel need to be mingled adequately and added into the glass from the corner until the glass was filled with the gel completely. Besides, the electrophoretic comb need to be inserted to the gel to form the lane and the gel need to be frozen for 2 h. Next, the electrophoretic comb was pulled out, and the 1×TAE was poured into the electrophoretic pool and 110 V voltage was inputted for 10 min previously. Then, 5 μL DNA and 1 μL 6× loading buffer were mingled and 5 μL mixture was drawn to the lane, 180 V voltage was inputted for 3 min and 125 V voltage was inputted for 90 min until the bright bands were reached at the 3/4 of the gel. Last, the gel was dyed for 1 h and imaged through UV.

The preparation of cell lysate-A and cell lysate:

The cell lysate-A was prepared as follows: MCF-7 cells were collected in the cell culture medium in an EP tube and centrifuged at 1000 rpm for 5 min, and washed with PBS buffer. The cells were broken with the ultrasonic cell disruptor and centrifuged at 10000 rpm for 10 min, the supernatant was left (including GSH, H_2O_2, Cys and other biothiols), and the precipitates (including cell fragments, proteins, organelles and other macromolecules) were discarded. Then telomerase solutions were added respectively. The cell lysate is prepared as follows: the different numbers of cells were disrupted with ultrasonic cell disruptor, and finally dispersed into 30 μL of 0.01 M PBS buffer.

SERS analysis for telomerase in homogeneous solution:

1 μL different telomerase solutions and 5 μL dNTPs were left in the SR probe solution to react with the telomerase primer in H2 for 2 h at 37 °C. The final results were gained from the average of ten parallel Raman spectra at different detection sites, and all the tests were taken

in triplicate. The standard deviation of the experiment is represented by error bars.

Fluorescence imaging of the MCF-7 cells: After 4 h cell incubation, the extracellular SR probes were removed by washing the cells with PBS solution for three times to reduce the background fluorescence. Ultimately, the cells were imagined with the confocal laser scanning microscope with 40 × objective. The excitation sources were 514 nm and the fluorescence emission of the Lyso tracker, Cy3 and Rox was collected from 520-560, 560-600 and 600-640 nm of band-pass filter.

SERS imaging of the MCF-7 cells: After 4 h incubation time, the MCF-7 cells were imagined with the Renishaw Invia Raman spectrometer (Renishaw, England). 633 nm HeNe laser (10 % laser power) was utilized to irradiate the cells and the detection ranges of Raman signals were set from 600 cm^{-1} to 1700 cm^{-1} with 50 × objective.

Fluorescence imaging of the probe escaping from lysosome.

MCF-7 cells were incubated with 0.5 mL culture medium containing the given amount of probe at 37 °C for varying time. After washing three times with 1×PBS, the cells were incubated with fresh RPMI 1640 medium containing 75 nM lysosome traker (Lyso@tracker Green) for 60 min followed by imaging.

Fluorescence imaging of telomerase inhibition experiments

3′-Azido-3′-deoxythymidine (AZT) was used as an inhibitor for telomerase activity, MCF-7 cells were incubated with 0.5 mL culture medium containing 1 mM AZT solution for 2 h. After washing three times with 1×PBS. the AZT treated MCF-7 cells and untreated cells were incubated with the SR probe solution for 4 h.

Result and discussion

The characteristic of the SR probe.

The transmission electron microscope (TEM) was used to detect the characterization of the nanophase materials such as the diameter shape and the dispersibility. The AuNPs in our work was characterized by TEM and the UV visible spectrum and the results are shown in Figure S1. The diameter of the AuNPs is 20 nm and shows a good spherical dispersibility. Besides, the DNA stands, H1,H2 (5.0 μM), Au NPs, Au NP complex were measured through a Cary 50 UV/Vis-NIR spectrophotometer. According to the previous reports, the ultraviolet

characteristic peaks of DNA and AuNPs were 260 nm and 520 nm, respectively. In addition, the ultraviolet characteristic peaks of the Cy3 and Rox were 500-550 nm and 550-600 nm. The ultraviolet characteristic peaks are also shown in the Fig. S1 and proved that the SR probe was prepared successfully.

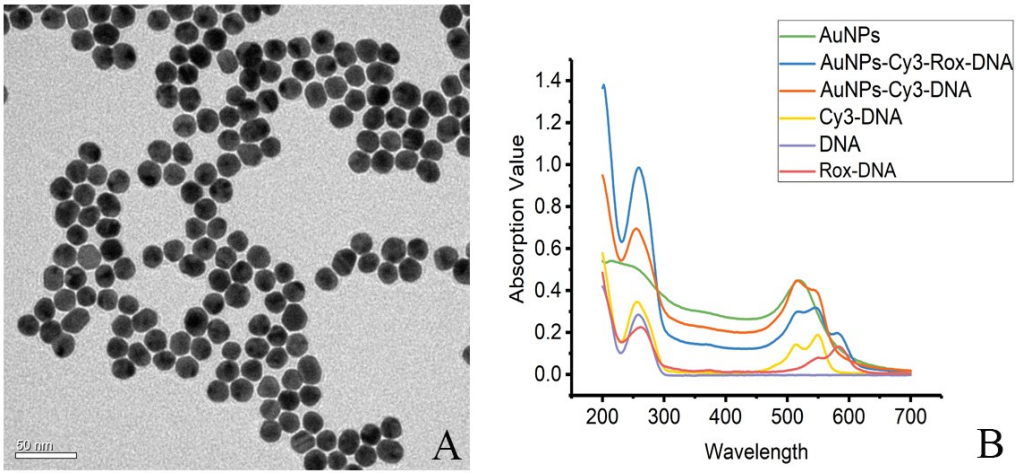

Fig. S1 (A) TEM image of the AuNPs (B) UV spectra of the SR probe.

Raman spectra of the reactant and the product.

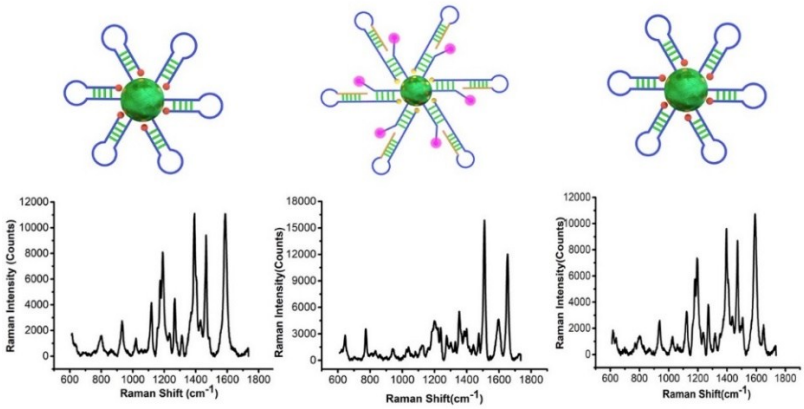

Fig. S2 The Raman spectra of the reactant and the product.

Investigation of the feasibility of the experiment.

In Fig. S3, 1499 cm^{-1} and 1586 cm^{-1} are the characteristic peak of Rox and Cy3, respectively. An obvious change of Raman signal was occurred only in the present of the telomerase, and in the presence of different concentration of telomerase, the reverse changes of the dual Raman signals are observed in Fig. S4, indicating that this method had a high recognition capacity for the telomerase.

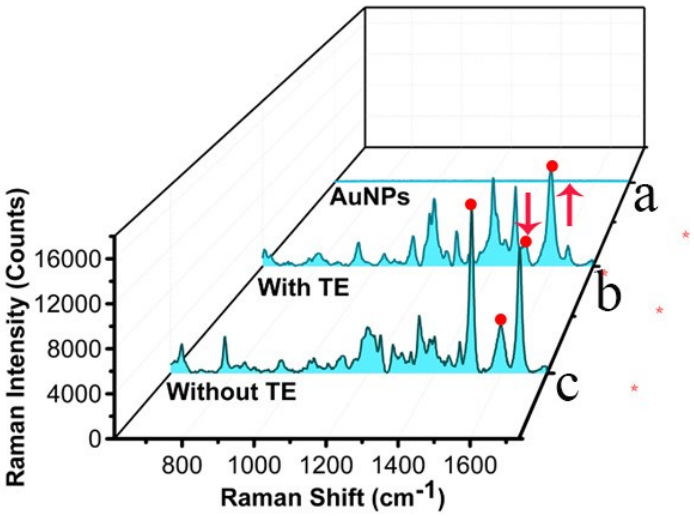

Fig. S3 SERS spectra obtained from the detection of telomerase (TE), (a) AuNPs (b) In the presence of telomerase; (c) In the absence of telomerase.

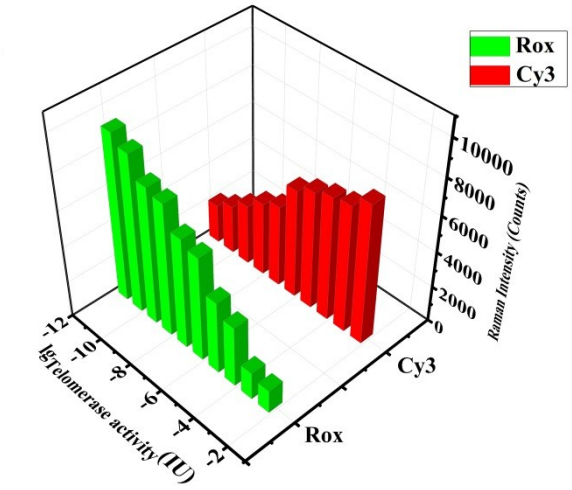

Fig. S4 The Raman signal reverse changes of Cy3 and Rox trigged by telomerase with different concentrations.

A study of this SR probe using 12 % native polyacrylamide gel electrophoresis (PAGE) further confirmed the above results. An obvious change of Raman signal was occurred only in the present of the telomerase, indicating that this method had a high recognition capacity for the telomerase. Besides, four different bright bands (P1, P2, P3 and P4) were observed clearly in Fig.S5. The bright band P1 and P2 in the lane 1 and lane 2 was the hairpin DNA H1 and the hairpin DNA H2. There was only one band in the lane 1 and 2. P2 was higher than P1 due to the different bases in the hairpin DNA H1 and H2. The bright band P3 was higher than P1 and P2 in the lane 3, indicating that the hybridization reaction between H1 and H2 was

occurred in the line 3. And the highest band P4 was observed in the lane 4. The P4 in the lane 4 represented the hexamer telomeric repeats sequence (TTAGGG)n triggered by telomerase. Combined with the above results, the feasibility of the experiment is proved successfully.

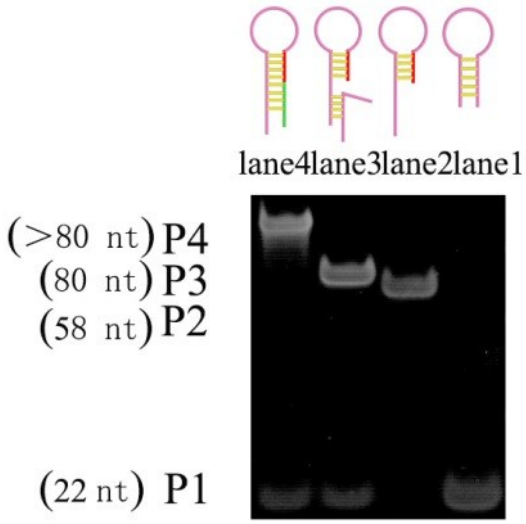

Fig. S5 Polyacrylamide gel electrophoresis characterization of the synthesis of the SR probe and the reaction process.

Optimization of the reaction pH.

The pH of the reaction solutions strongly influence DNA hybridization and are the two most important parameters for optimization. Therefore, we investigated the intensity of the Raman signal under different pH conditions. The influence of pH values ranging from 5.0 to 9.0 on the Raman signal intensity produced by 4×10^{-6} IU telomerase is shown in Fig. S6. The Raman intensity ratio reached a maximum at pH 7.4. Therefore, we selected pH 7.4 as the optimum pH value.

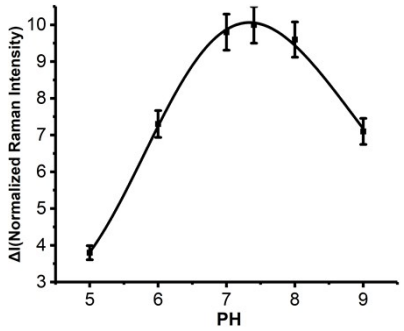

Fig. S6 The influence of pH of the reaction on the intensity ratios of the Raman signal in response to 4 $\times 10^{-6}$ IU telomerase.

Optimization of the reaction time.

The reaction time was investigated. Fig. S7 shows the changes in the SERS signals generated by performing the experiments at different time intervals. The results revealed that the Raman intensity ratio increased rapidly as the incubation time increased and reached a plateau after 120 min. Therefore, we deduced that 120 min was the best incubation time for the assay.

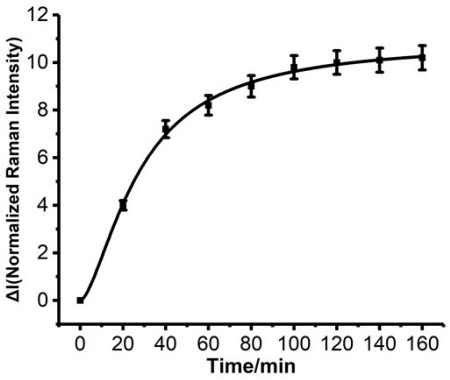

Fig. S7 Effect of reaction time on the intensity ratios of the signal in response to 4×10^{-6} IU telomerase.

Raman imaging of the SR probe.

When MCF-7 cells were incubated with the SR probe for 4 hours, the SERS signal of the SR probe were collected at different time points, and the Raman imaging of SR probes in the MCF-7 cells was obtained. In the present of the intracellular telomerase, the Raman signal of Cy3 (red) was off-on, while the Raman signal of Rox (green) was on-off. Therefore, as shown in Fig. S8, the Raman signal of Rox decreased with the increase of reaction time, while the Raman signal of Cy3 increased, which proved that SR probe can react with the intracellular telomerase .

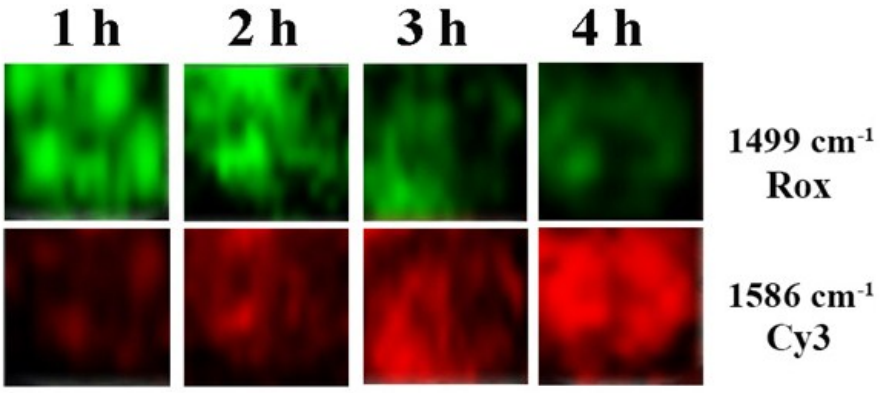

Fig. S8 The Raman imaging of the SR probe.

Investigation of the efficiency of the probe escaping from lysosome.

In Fig.S9, with the incubation time of 5 min, the fluorescence intensity of Cy3 was high (red) in the cell membrane and the lysosome traker (yellow) was also high in the cytoplasm. On the contrary, when the incubation time was 60 min, the fluorescence intensity of the lysosome traker was weaker due to the probes released from lysosome and the fluorescence intensity of Cy3 was also high in the cytoplasm. These above results demonstrated that the SR probe could be suitable for the detection of telomerase activity with high escape efficiency from lysosome.

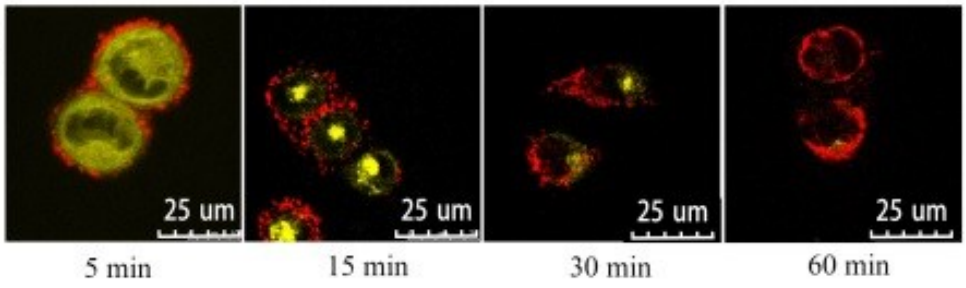

Fig. S9 Laser scanning confocal microscopy fluorescence images of MCF-7 cells incubated with 50 μL of SR Probe and lysosome tracker (Green) for 1 h (scale bar =25 μm).

Investigation of telomerase inhibition.

The fluorescence image of MCF-7 cell are shown in Fig.S10. The higher Cy3 and lower Rox fluorescence intensity of the AZT treated cells are observed, comparing to untreated cells, indicating the inhibition of AZT toward intracellular telomerase activity.

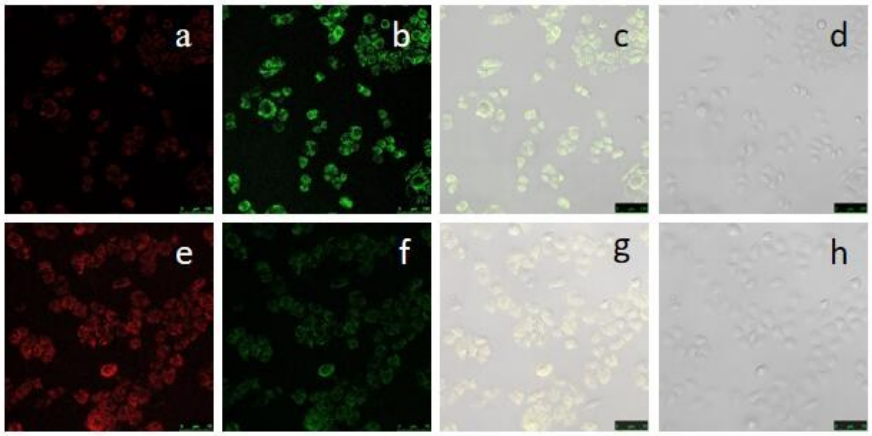

Fig. S10 Fluorescence images(from left to right: the images of Cy3 signal, the images of Rox signal, the overlapping images, bright field images) of untreated MCF-7 cell (a-d)and AZT treated cell(e-h), incubated with 50 μL of SR Probe for 4 h.

Investigate the stability and cytotoxicity of the SR probe.

The time-dependent dynamic SERS signals of the SR probe were observed. As shown in Fig. S11, the Raman intensity of Cy3 and Rox was changed rapidly at the beginning of the incubation time. And with the incubation time prolonged, the dynamic variation of the SERS intensity decreased gradually. At the end of 5 h, the SERS intensity reached a plateau and was not changed distinctly, indicted that the SR probe contained a high resistance of the complex environment degradation in the living MCF-7 cells.

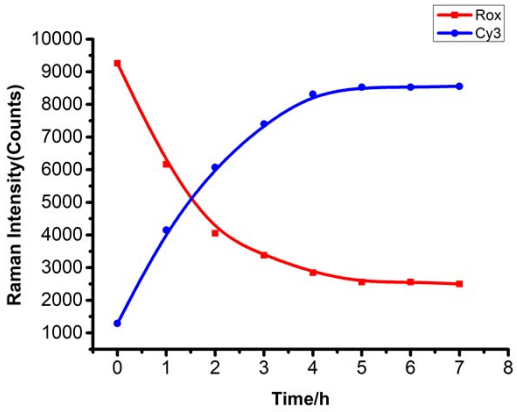

Fig. S11 Raman intensities with the increasing reaction time.

The MTT assay was executed to investigate the cytotoxicity of the SR probe, the results are shown in Fig.S12. The MCF-7 cells maintained 96.5 % in 10 h, and the MCF-7 cells remained 94.4 % after 40 h, indicating a low cytotoxicity of the SR probe.

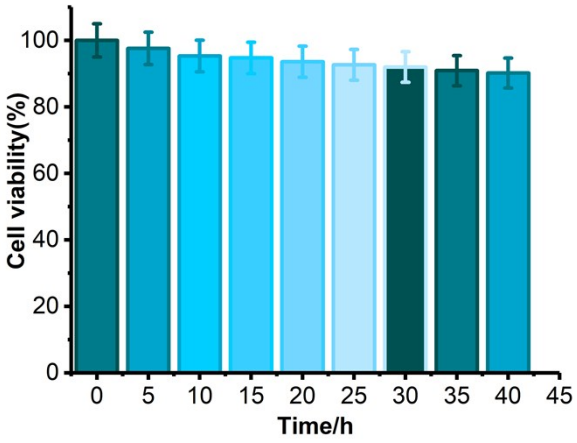

Fig. S12. Viability of MCF-7 cells after incubation for different times.

Table S2. The comparison of different methods for telomerase activity detection

Method	Probe	Detection sample	The description of the following methods
SERS detection[2]	copper phthalocyanine	intracellular telomerase activity	prepared EG-TiO_2 nanocomposite SERS platform using copper phthalocyanine (CuPc) as a recognition probe.
SERS detection[3]	telomeric substrate (TS) primers, folding into G-quadruplex structures, and intercalation of methylene blue	telomerase activity from various cancer cell lines	Constructed the TS primer-attached nanogap-rich Au NWs, through the elongation of TS primers, folding into G-quadruplex structures, and intercalation of methylene blue.
SERS detection[4]	a pyramid probe, using Cy5 as the SERS reporter.	intracellular telomerase activity	prepare gold nanoparticle pyramids, in which four single-stranded DNA molecules, which could be complemented by the cyanine 5 (Cy5) modified reporter sequence and telomerase primer (TP) to construct SERS probes.
SERS detection[5]	polystyrene (PS) coated SERS tags	cell imaging and vivo imaging	PS coated SERS tags.
SERS-colorimetry[6] detection	Au dimer-based probe	telomerase activity both in cell extracts and in the urine of patients	an Au dimer-based probe was assembled and telomerase activity was reflected according to the color variations of solution and the Raman intensity of Raman reporter.
SERS-colorimetry[7]	Au nanoparticle -	telomerase	Au nanoparticle modified

detection	based probe	activity in cell extracts	with Raman molecules and telomeric repeat complementary oligonucleotide are employed as the colorimetric-SERS bifunctional reporting nanotag, while magnetic nanoparticles functionalized with telomerase substrate oligonucleotide are used as the capturing substrate.
This method SERS-fluorescence detection	seesaw ratiometric (SR) probe	telomerase activity in single living cell	using the target triggered DNA structure switching, two different dye tags were utilized to produce opposite dual-signal changes. Fluorescence imaging can achieve a direct and rapid visualization about dynamic uptake and interaction of the SR probe and telomerase. Meanwhile, using dual-signal reverse changes, the measure of SERS dual-signal ratio affords high sensitivity for the detection of telomerase activity in single living cell.

Table S3. The comparison of different dual mode methods for telomerase activity detection

Method	Probe	Sensitivity	The linear range
SERS-colorimetry[6] detection	Au dimer-based probe	Lowest detection limit: 6.1 × 10^{-15} IU (20 μL detection sample solution, in cell extracts and in the	1.1 × 10^{-14} IU to 2.8 × 10^{-11} IU

		urine of patients)	
SERS-colorimetry[7] detection	Au nanoparticle - based probe	10 cells/mL (cell extracts)	10 cells to 1000 cells
This method SERS-fluorescence detection	seesaw ratiometric (SR) probe	The lowest detection amout in PBS buffer: 4×10^{-12} IU(in 50 μL detection sample solution); The lowest detection amout in vivo: single living cell	The SERS detetcion: 4×10^{-12} to 4×10^{-4} IU The ratiometric detection: 4×10^{-7} to 4×10^{-4} IU 4×10^{-12} to 4×10^{-8} IU

Reference:

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