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Supporting Information

Intracellular MicroRNA Imaging Using Telomerase-Catalyzed FRET Ratioflares with Signal Amplification

Liman Xian,[a] Haoying Ge,[a] Feng Xu,[a] Ning Xu,[a] Jiangli Fan,[ab] Kun Shao[a] and Xiaojun Peng*[ab]

[a] State Key Laboratory of Fine Chemicals, Dalian University of Technology, High-tech District, Dalian 116024, P. R. China

[b] Shenzhen Research Institute, Dalian University of Technology, Nanshan District, Shenzhen 518057, P. R. China

*** E-mail: pengxj@dlut.edu.cn.**

1. Preparation of telomerase-catalyzed FRET ratioflares.

The AuNPs were synthesized using the sodium citrate reduction method. Before the experiments, all glassware was cleaned in aqua regia (HCl/HNO_3, 3:1), rinsed with H_2O, and then oven-dried. Next, 100 mL of 0.01% $HAuCl_4$ was heated to boiling with vigorous stirring and 3.5 mL trisodium citrate (1%) was added under stirring. Then the solution color turned from pale yellow to colorless and finally to burgundy. Boiling was continued for an additional 10 min. After the heating source was removed, the colloid was stirred until the solution reached room temperature. Then it was filtered through a 0.45 μm Millipore membrane filter. Transmission electron microscopy (TEM) images indicated the particle sizes are 13 ± 2 nm. The prepared AuNPs were stored at 4 ℃.

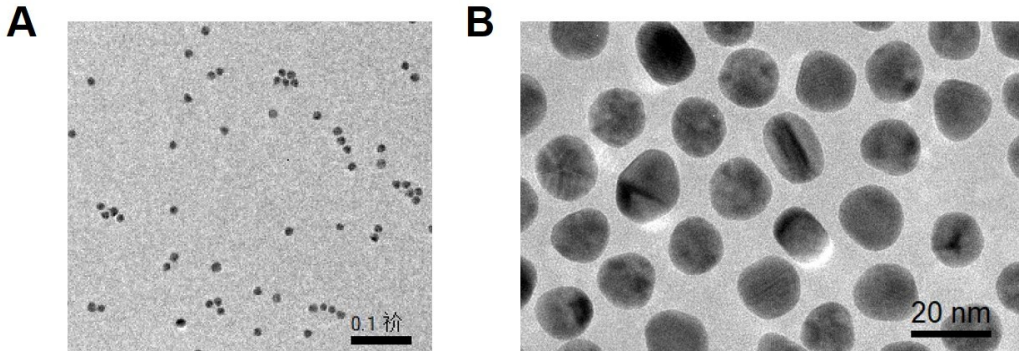

Figure S1. Characterization of AuNPs and DNA-AuNPs. (A) TEM micrograph of 13 ± 2 nm AuNPs (scale bar = 0.1 μm). (B) TEM micrograph of 13 ± 2 nm AuNPs (scale bar = 20 nm).

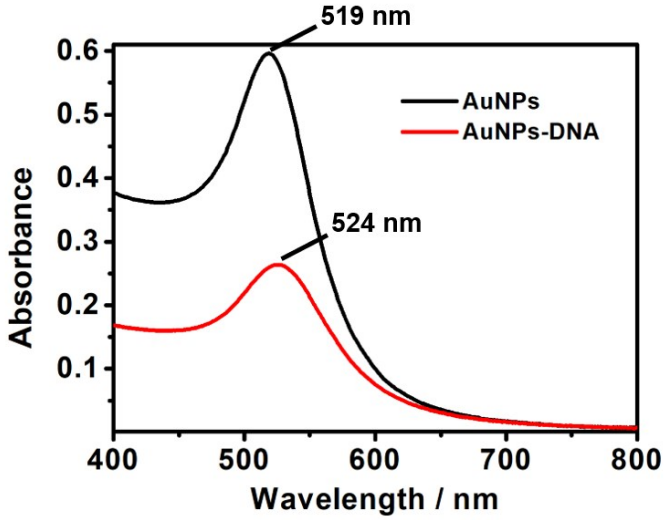

Figure S2. Absorption spectra of AuNPs (black line) and DNA functionalized AuNPs (red line).

After reduced by Tris (2-carboxyethyl) phosphine hydrochloride (TCEP·HCl), thiol-modified oligonucleotides (capture probes) were added to gold colloids at a concentration of 3 μM of oligonucleotide per 1 mL of 10nM colloid and shaken overnight. After 16 hours, Phosphate buffer (0.1 M; pH = 7.4) was added to the mixture to achieve a 0.01 M phosphate concentration, and aliquots of sodium chloride solution (2.0 M) were added to the mixture over an eight-hour period to achieve a final sodium chloride concentration of 0.3 M. The solution containing the functionalized particles was centrifuged (13,000 rpm, 20 min) and resuspended in phosphate buffered saline (PBS; 137 mM NaCl, 10 mM Phosphate, 2.72 mM KCl, pH 7.4, Hyclone) three times to produce the purified AuNPs used in all subsequent experiments. Purified oligonucleotide functionalized AuNPs were suspended to a concentration of 10 nM in PBS containing 1 μM of the detection probes. The mixture was heated to 75 ℃, slowly cooled to room temperature, and stored in the dark for at least 12 hours to allow hybridization. Finally, the solution containing the telomerase-catalyzed FRET ratioflares was centrifuged (13000 rpm, 30 min) and resuspended in PBS for the following experiments. The concentration of the particles was determined by measuring their extinction at 524 nm ($\varepsilon = 2.7 \times 10^8$ $L \cdot mol^{-1} \cdot cm^{-1}$). [1]

DNA sequences loaded on AuNPs were quantitated according to the published protocol. [2] Mercaptoethanol (20 mM) was added to the ratioflare solution, which was incubated overnight with shaking at room temperature. Released DNA probes were then separated via centrifugation and the fluorescence was measured with a fluorescence spectrometer. The fluorescence with a 543 nm excitation wavelength was converted to molar concentrations of DNAs by interpolation from a standard linear calibration curve that was prepared with known concentrations of TAMRA labeled reporter with same conditions. The average and standard deviation were obtained by 3 parallel experiments in each trial. The result reveals each AuNP contains approximately 63 detection probes.

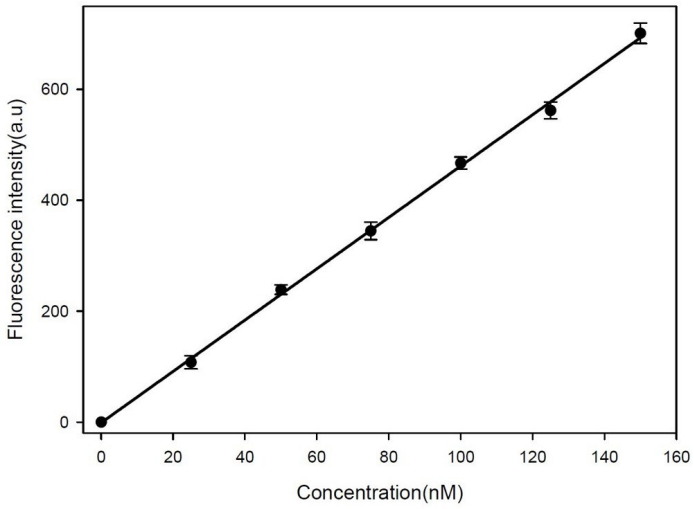

Figure S3. Evaluation of Amounts of detection probes on each AuNP. Standard linear calibration curve of fluorescence signal against the concentration of TAMRA labeled detection probes.[2] The fluorescence of released DNA from AuNP was converted to molar concentrations of DNAs by interpolation from the above standard linear calibration curve. The excitation wavelength was 543 nm and the emission wavelength was 580 nm. The error bars represent the standard deviations.

2. Fluorescence assay of target microRNA using the telomerase-catalyzed FRET ratioflares

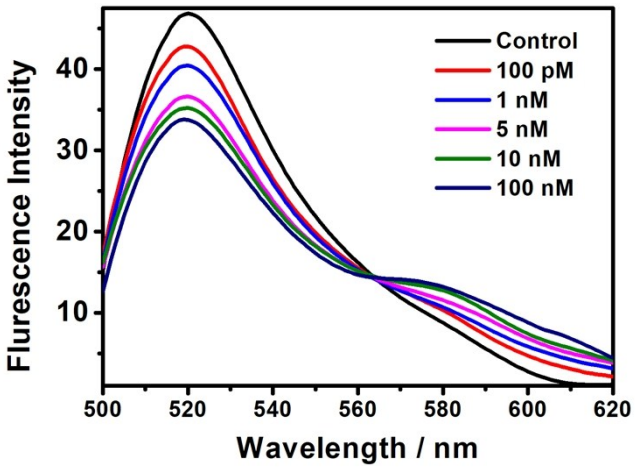

Figure S4. Fluorescence spectra determination at various DNA template concentrations from 100 pM to 100 nM when telomerase was absent.

3. Telomerase extraction

The cells were collected in the exponential phase of growth, and 5×10^7 million cells were dispensed in a 1.5 mL EP tube, washed twice with ice-cold phosphate buffered saline (pH 7.4) solution, and resuspended in 200 μL of ice-cold CHAPS lysis buffer (10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.5% (w/v) CHAPS, 10% (v/v) glycerol, 0.1 mM PMSF). The cells were incubated for 30 min on ice and then centrifuged for 20 min (16 000 rpm, 4 °C). Without disturbing the pellet, the cleared lysate was carefully transferred to a fresh RNase-free tube, flash frozen, and stored at −80 °C before use.

A standard addition method was used for quantification of telomerase activity in HeLa cell extract using ELISA kit. The ELISA kit analysis was performed with the extracts of EGCG-treated cells through a standard curve method. 50 μL of cell extracts were firstly added in the wells of ELISA plate followed by incubation at 37 °C for 30 min. The plate was then washed with PBS and added with 50 μL labeling reagent (from the kit) to incubate at 37 °C for 30 min. Afterward, the medium was removed, and 50 μL of color development agent A and 50 μL of color development agent B (from the kit) were added to vibrate for 10 min at 37 °C. 50 μL of stop buffer (from the kit) was finally added to each well to stop the color reaction, and the absorbance was measured at 450 nm on a microplate reader to obtain the telomerase activity in the extracts. The standard curve was obtained and shown in figure S5. The telomerase activity we extracted from HeLa cells before was calculated to be 11.5 IU/L.

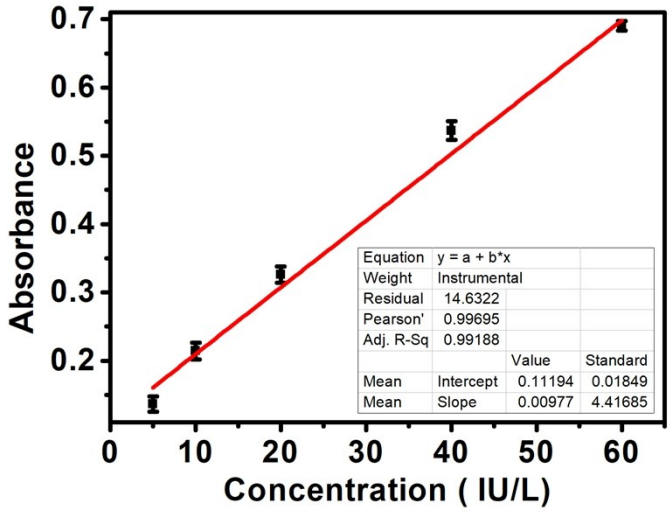

Figure S5. The standard curve depends on different telomerase activity obtained from ELISA kit standard method.

4. Cell Culture and staining.

4.1 Cell viability experiment

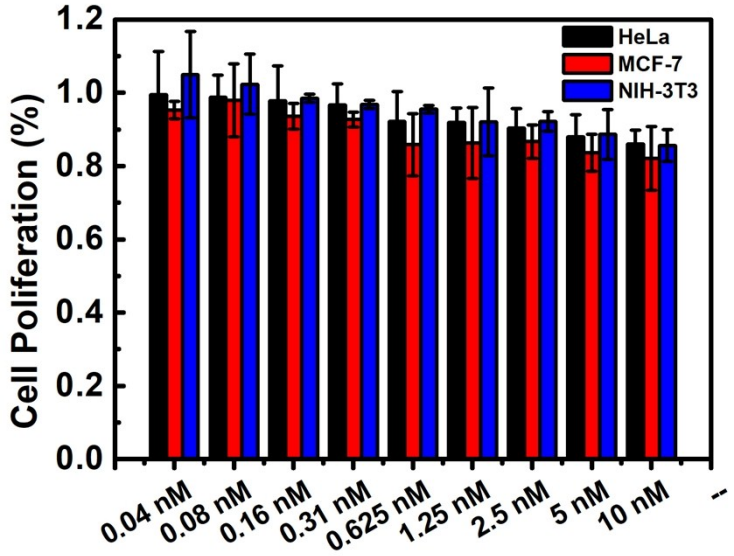

Figure S6. MTT experiment of HeLa, MCF-7 and NIH-3T3 cells with telomerase-catalyzed FRET ratioflares for 24 h.

4.2 Live-cell detection of telomerase-catalyzed FRET ratioflares by microscopy

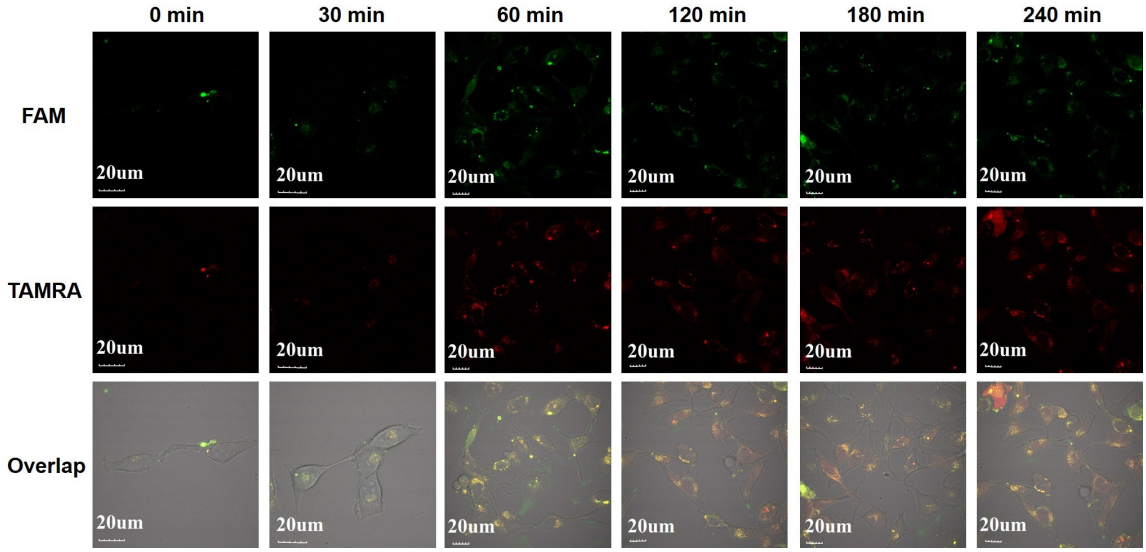

Figure S7. Time-dependent fluorescence intensity of FRET ratioflares in HeLa cells. At 120 min, the TAMRA fluorescence was brilliant, and the intensities did not increase when the incubation times were increased further in HeLa cells.

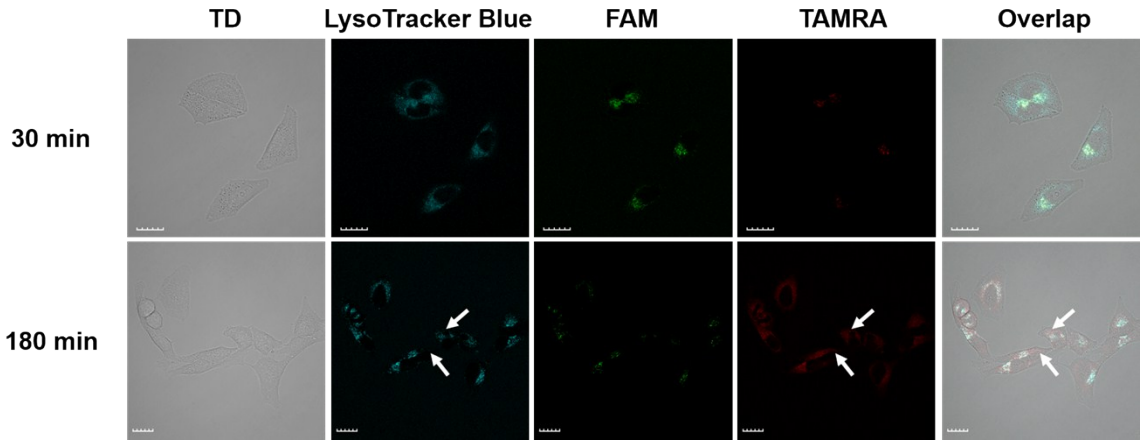

Figure S8. Representative One-photon confocal fluorescence images of HeLa cells after incubation with telomerase-catalyzed FRET ratioflares for 30 min and 180 min show the endosome/lysosome escape, respectively. Here LysoTracker Blue was used as a probe to label intracellular endosome and lysosomes.

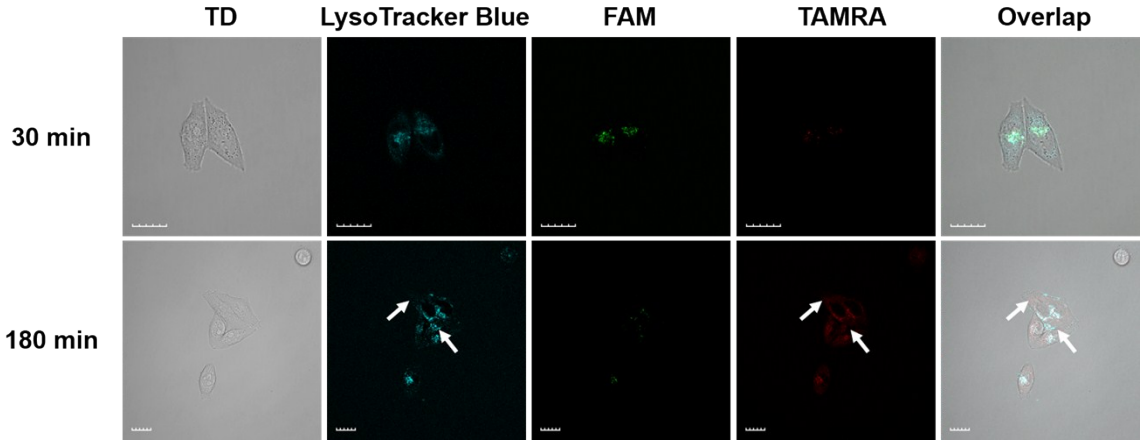

Figure S9. Representative One-photon confocal fluorescence images of MCF-7 cells after incubation with telomerase-catalyzed FRET ratioflares for 30 min and 180 min show the endosome/lysosome escape, respectively. Here LysoTracker Blue was used as a probe to label intracellular endosome and lysosomes.

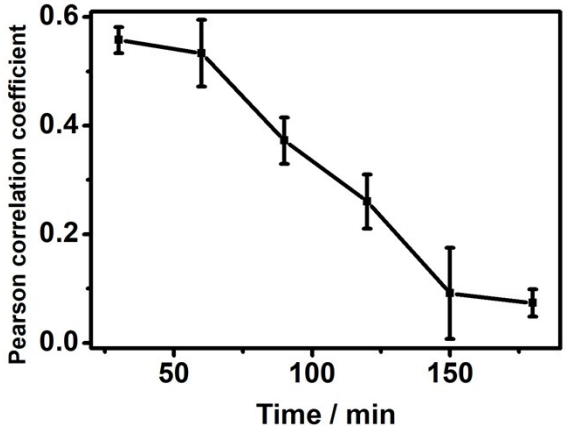

Figure S10. Quantification (Pearson correlation coefficient) of co-localization of telomerase-catalyzed FRET ratioflares in lysosomes over incubation time (HeLa cells).

4.3 Analysis of average fluorescence intensity

The images were post-processed with Olympus FV10-ASW3.0 viewer software. To improve the statistical accuracy, the average fluorescence intensity in cells can be determined from the quantitative analysis of the regions of interest (ROIs) at different locations.

4.4 Inhibit telomerase activity experiment

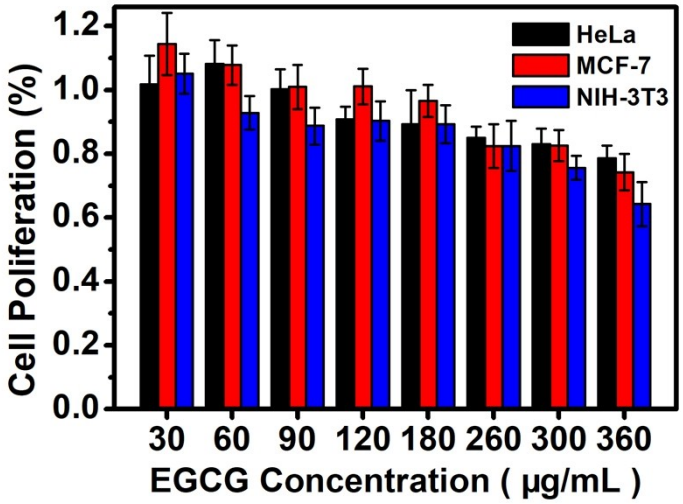

Figure S11. MTT experiment of HeLa, MCF-7 and NIH-3T3 cells with EGCG for 48 h.

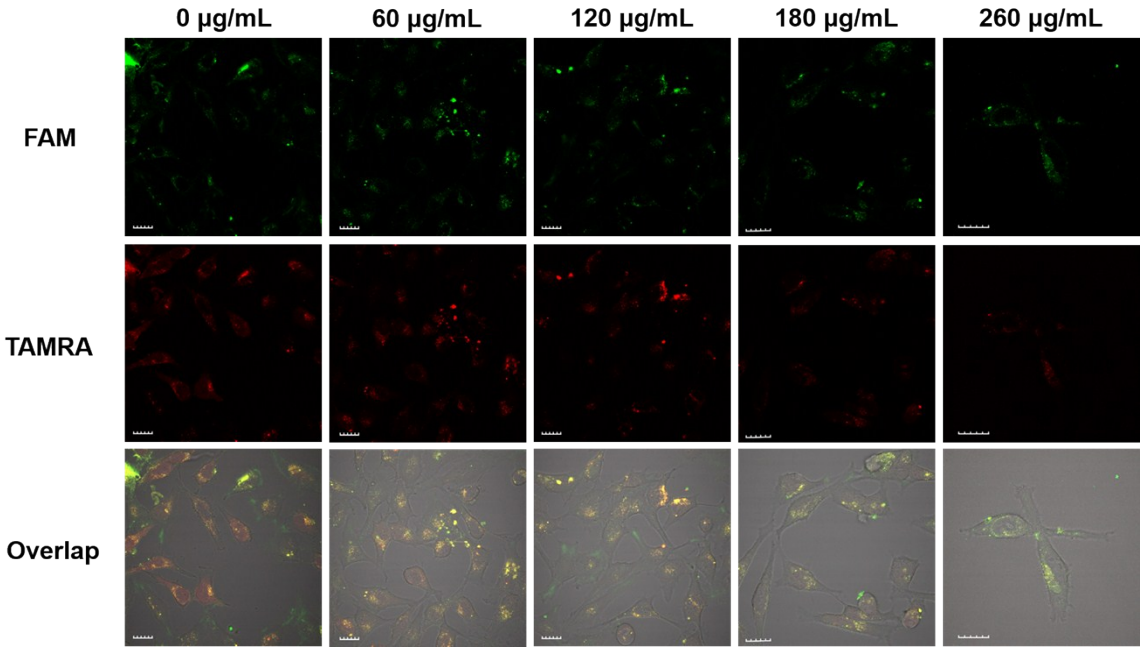

Figure S12. One-photon confocal fluorescence imaging of miR-21 in HeLa cells after incubation with different concentration of EGCG for 48 h.

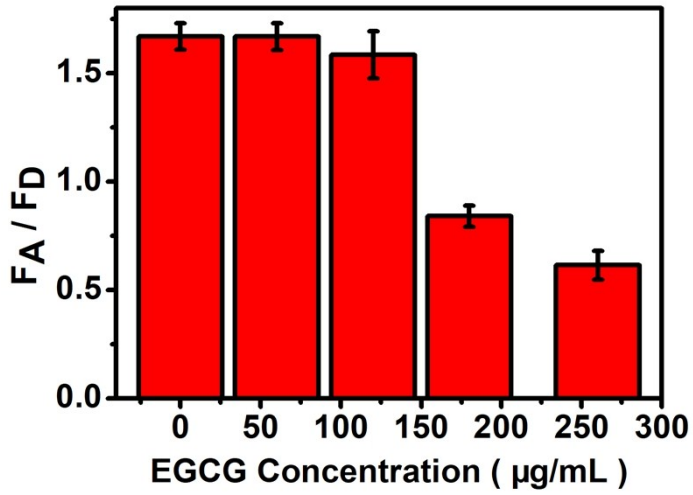

Figure S13. Average fluorescence intensity ratio between FAM channel and TAMRA channel after incubating with EGCG.

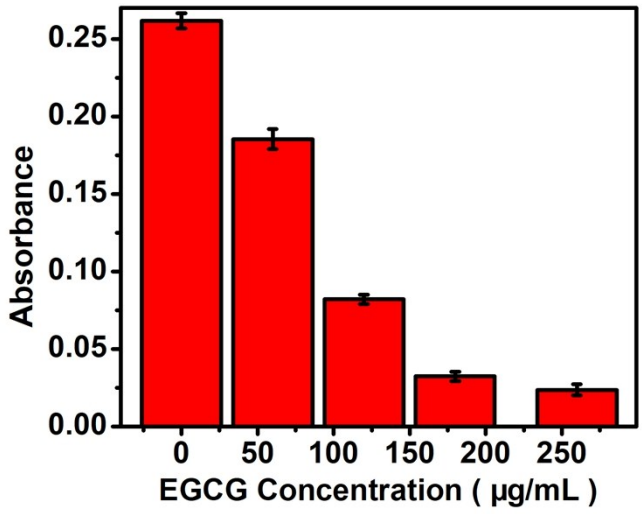

Figure S14. Absorbance of telomerase activity in HeLa cells after incubation with different concentration of EGCG for 48 h through ELISA Kit analysis.

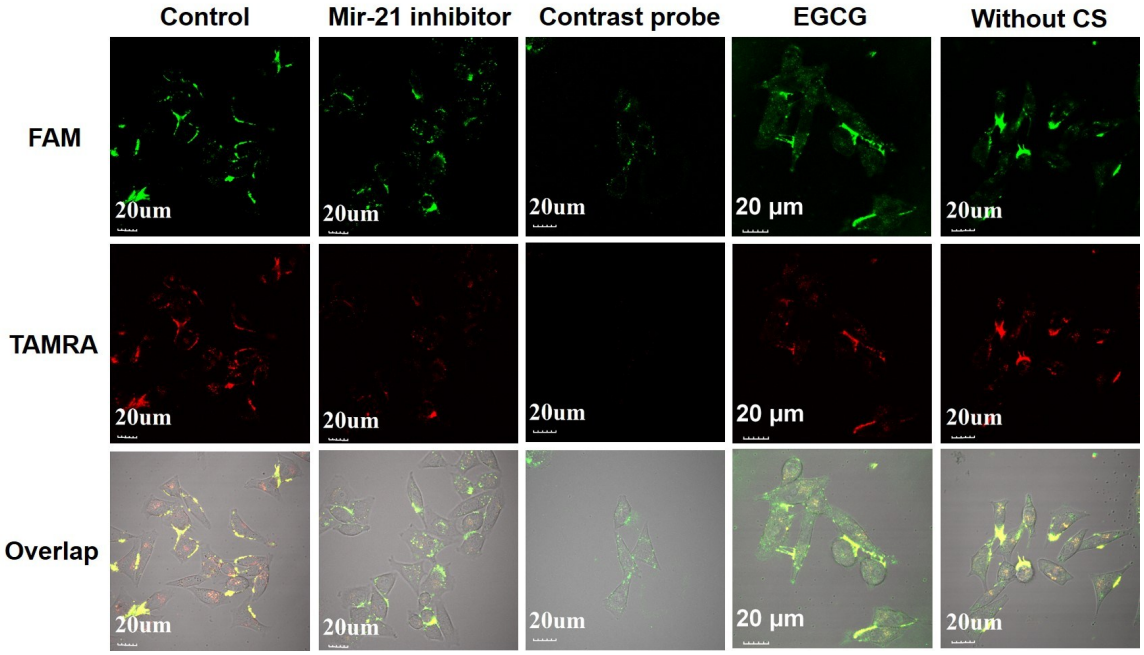

Figure S15. One-photon confocal fluorescence imaging of miR-21 in MCF-7 cells after incubation with with anti-miR-21 sequence, contrast probes, EGCG and without **CS**.

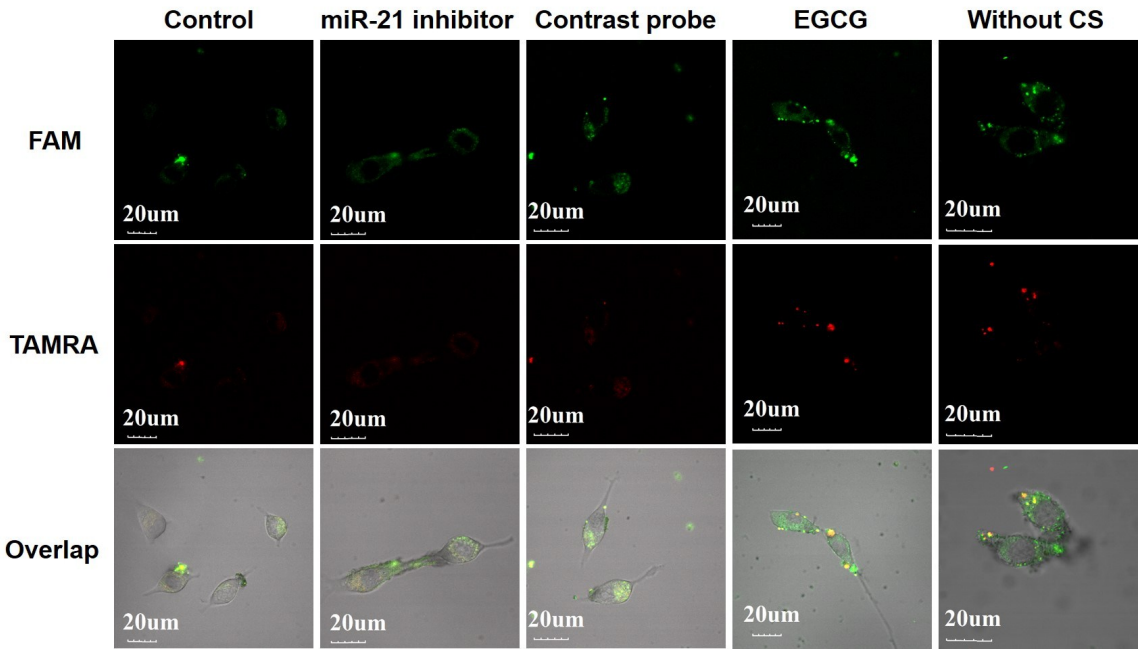

Figure S16. One-photon confocal fluorescence imaging of miR-21 in NIH-3T3 cells after incubation with with anti-miR-21 sequence, contrast probes, EGCG and without **CS**.

References

[1] Y. W. Cao, R. C. Jin and C. A. Mirkin, *J. Am. Chem. Soc.*, 2001, **123**, 7961.

[2] Y. J. Yang, J. Huang, X. H. Yang, K. Quan, H. Wang, L. Ying, N. L. Xie, M. Ou and K. M. Wang, *J. Am. Chem. Soc.*, 2015, **137**, 8340.