Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Gate-Voltage Modulated Electrostatic Interaction between Probe and Channel Material of FET Biosensors for Detecting Telomerase

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1. Experimental Procedures

1.1 Materials:

Ultrapure water from Millipore filtration system (Millipore, Bedford, MA, United States) source was used for the experiments. Indium (III) nitrate hydrate ($In(NO_3)_3 \bullet xH_2O$), (3-aminopropyl)trimethoxysilane (APTMS), trimethoxy(propyl)silane (PTMS), 3-maleimidobenzoic acid N-hydroxysuccinimide ester (MBS) and 1-dodecanethiol were purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd. The deoxynucleotide solution mixture (dNTPs), RNase inhibitor, DEPC-treated water, and 1× CHAPS lysis buffer were purchased from Sangon Biotech (Shanghai, China; DEPC = diethylpyrocarbonate).

1.2 Fabrication of In_2O_3 FET:

Firstly, the $In(NO_3)_3$ thin films were fabricated through spinning-coating 1 mM $In(NO_3)_3 \bullet xH_2O$ solutions with 4000 rpm for 60 s on a 300 nm-thick SiO_2/heavily doped silicon wafers. Then, the In_2O_3 thin films were fabricated through prebaking at 150 °C for 10 min, and thermal annealing at 350 °C for 3 h. The interdigitated device with sie-gate electrodes (80 μm width channel distance) was fabricated through standard photolithography (MDA-400M) followed by depositing 10 nm Ti /40 nm Au on In_2O_3 thin film (Angstrom Engineering).

1.3 Fabrication of side-gate FET biosensors:

Briefly, spacer molecules of mixed (3-aminopropyl)triethoxysilane (APTMS) and (trimethoxy(propyl)silane (PTMS) solutions with 1:9 v/v ratio were decorated on In_2O_3 surfaces by thermally evaporation at 40 °C for 1 h, then 1-dodecanethiol (DDSH) molecules incubated in 1 mM ethanolic solutions were modified to passivate Au electrodes for 1 h. The 1 mM linker molecules of 3-maleimidobenzoic acid N-hydroxysuccinimide ester (MBS) in a 1:9 (v/v) mixture of dimethyl sulfoxide and phosphate buffer saline (PBS) were decorated on spacer molecules for 30 min. The decorated FET devices were immersed in 1 μM thiolated telomerase primer solutions for about 18 h, then followed by rinsing again with deionized water and dried by blowing dry N_2 gas. The sequences of the TS used in the study are as follows: 5'-$HS(CH_2)_6$TTTTTTAATCCGTCGAGCAGAGTT-3'.

1.4 Characterization of FET biosensors:

The height of telomerase primer before and after telomerase extension is characterized by atomic force microscopy (AFM). We molecularly labeled a quencher group black hole quencher 1 (BHQ1) and fluorescence group hexachlorofluorescein (HEX) respectively at the both ends of quenching fluorescence sequence (QFS). The emission of HEX (λ_{max} = 556 nm) and the absorption of BHQ1 (λ_{max} = 534 nm) have a high degree of overlap. QFS contains TS sequence and three repeated units TTAGGG, which is used to simulate the telomerization process. Fluoresence and quencher groups labeled at both ends are used to indicate the variation of distance between 3'-end and 5'-end under different gate voltage conditions. The result of extended ssDNA by telomerase was verified by the nondenaturing polyacrylamide gel electrophoresis analysis.

1.5 Cell culture:

Hepatoma cells (HepG2, obtained from China Center for Type Culture Collection, Wuhan, Hubei, China) and cervical cancer cells (HeLa, obtained from China Center for Type Culture Collection, Wuhan, Hubei, China) were cultured in 1640 (GIBCO) medium with 1% penicillin streptomycin (PS, 10000 IU penicillin and 10 000 μg/mL streptomycin, Multicell) and 10% fetal calf serum (FCS, Shanghai Yuanye Biotechnology Co. LTD) in a culture flask at 37 °C in a humidified atmosphere containing 5% CO_2. Breast cancer cells (MCF-7, obtained from China Center for Type Culture Collection, Wuhan, Hubei, China) were cultured similarly in Dulbecco's modified Eagle's medium (DMEM, Multicell). human lung fibroblast cells (HLF, obtained from China Center for Type Culture Collection, Wuhan, Hubei, China) were cultured similarly in minimum essential medium (MEM/EBSS, 1×, HyClone).

1.6 Telomerase extraction:

Cultured cells were first suspended in 1× CHAPS lysis buffer to make the concentrate 5000 cells/μL and incubated on ice for 30 min. Then, the mixture was centrifuged at 12 000 g for 20 min at 4 °C. Finally, the supernatant was transferred, aliquoted, and stored at −80 °C.

1.7 Telomerase Extension Reaction:

The telomerization reaction was performed by using TS modified FET, in the presence of dNTP (2 mM), RNase inhibitor (1 U/μL) and telomerase solution (20 mM Tris-HCl buffer, pH 7.8, 1.5 mM $MgCl_2$, consisted of telomerase extracts from the specified number of canner cells) at 37 °C for 40 min.

1.8 Telomerase Detection by FET biosensors:

Polydimethylsiloxane (PDMS) wells were used to separate PBS buffer solution on top of individual FET electrodes. The electrical measurements were performed on low temperature analytical probe stations (CRX-6.5K, Lake Shore) with a semiconductor parameter analyzer (4200-SCS, Keithley). The transfer curves with 0 to 0.8 V gate voltages and 10 mV drain voltage were obtained. At the same time, the real-time measurements were held at 0.4 V gate voltages and 0.01 V drain voltage.

2. Figures and Tables

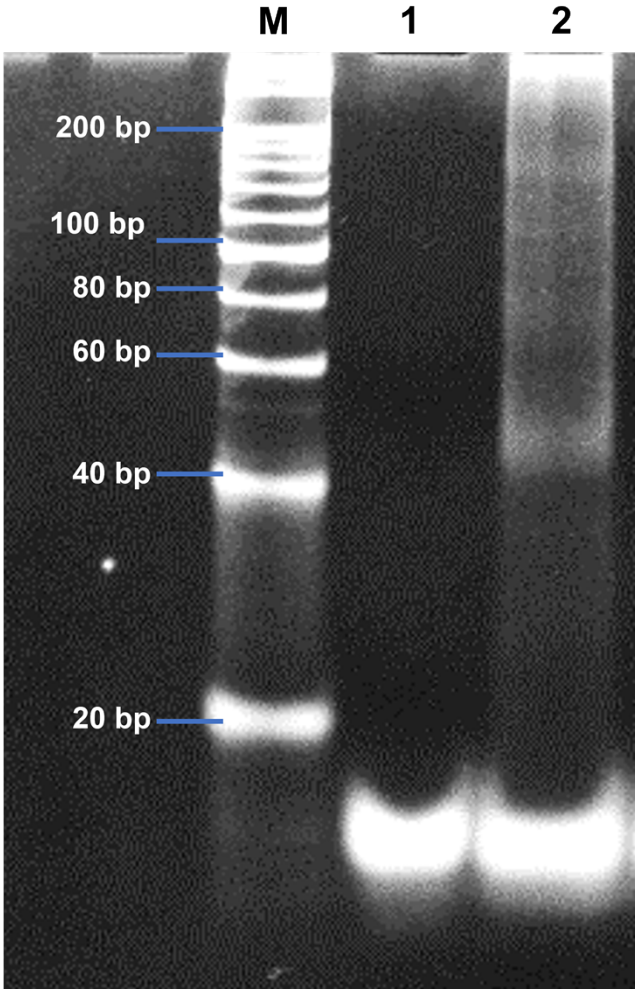

Fig. S1. Nondenaturing PAGE analysis of telomerization process: DNA ladder marker (lane M), TS primer in the absence (lane 1) and presence (lane 2) of telomerase extracts from 20000 HepG2 cells.

Table S1. Oligonucleotides used in this study.

Name	Sequence (5'to3')	Length (nt)	Purify method
TP	5'-HS$(CH_2)_6$TTTTTTAATCCGTCGAGCAGAGTT-3'	24	HPLC
QFS	5'-HS$(CH_2)_6$TTTTTTAA/iBHQ1dT/CCGTCGAGCAGAG TTTTAGGGTTAGGGTTAGGGTTAGGG-(HEX)	42	HPLC

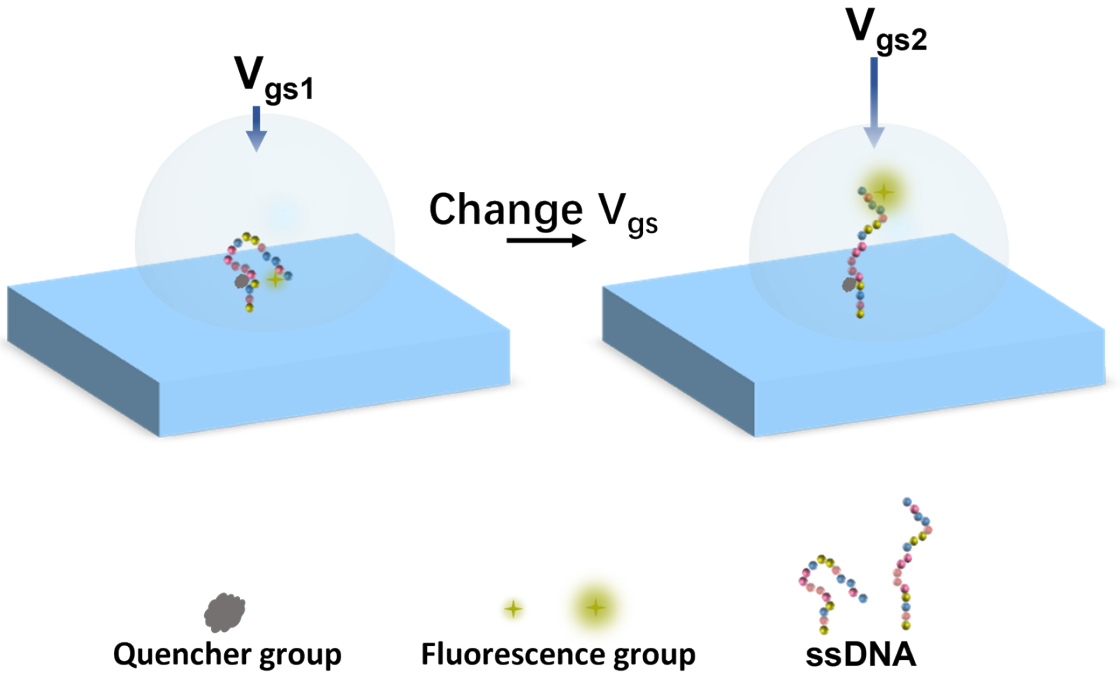

Fig. S2. Testing the changes of fluorescent intensity generated by the variation of the distance between the fluorescence group and quenching group under different gate voltage.

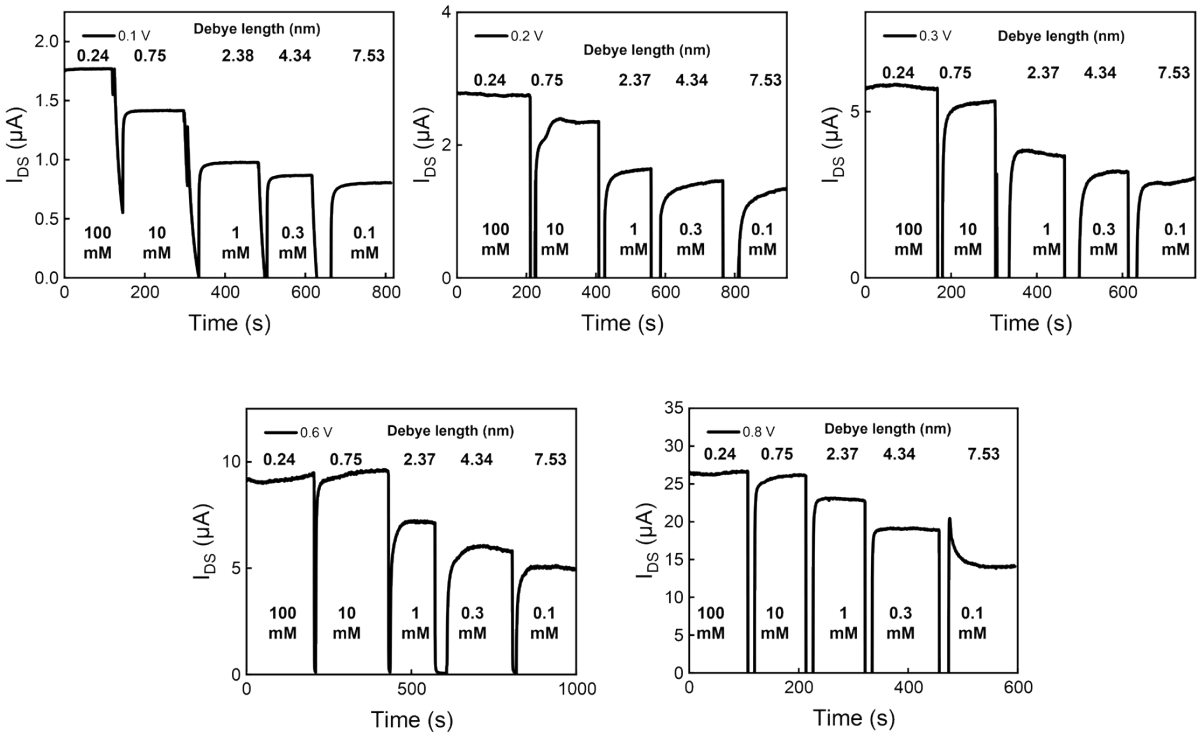

Fig. S3. Debye length modulation. Time domain device response after telomerization process under different gate voltage, followed by PBS buffer exchange to 100, 10, 1, 0.3 and 0.1 mM.

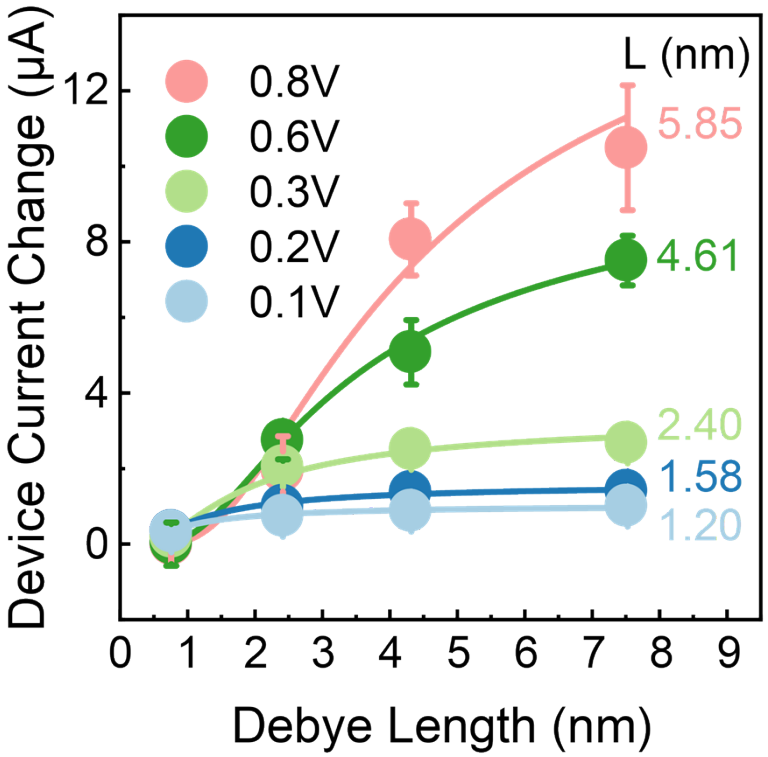

Fig. S4. Nonlinear fit of device signal change versus the debye length according to formula 2 in the main text. Fitting parameters are L=1.20, 1.58, 2.40, 4.61 and 5.85 nm while gate voltage is 0.1, 0.2, 0.3, 0.6 and 0.8V.

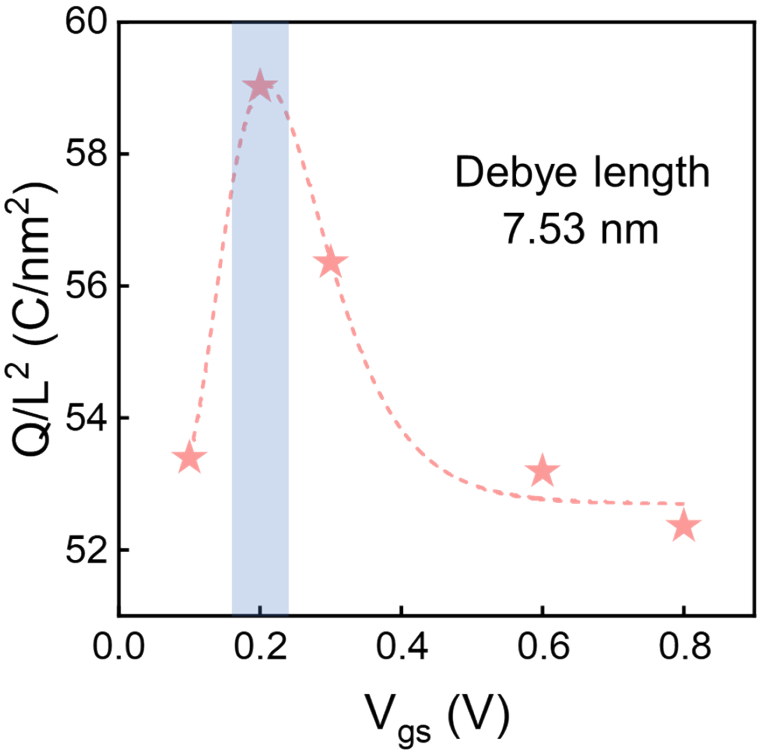

Fig. S5. The repulsive interaction between the ssDNA probe and the In_2O_3 channel under the interaction of gate voltage while Debye length up to 7.53 nm.

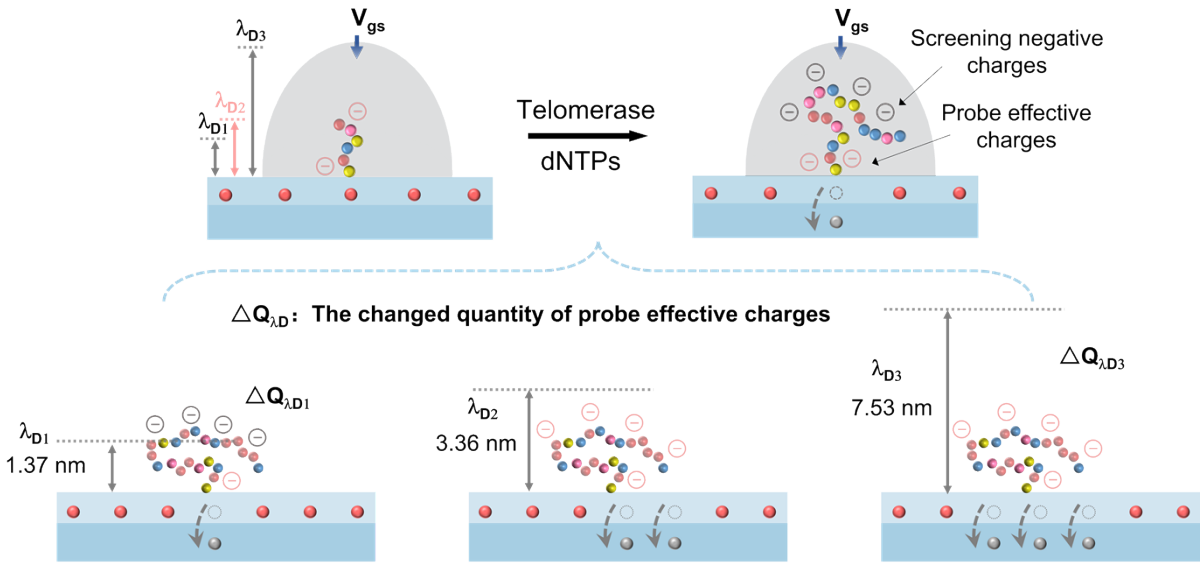

Fig. S6. The efficient charge of ssDNA while detecting telomerase under different debye length conditions (1.37 nm, 33.6 nm, and 7.53 nm).

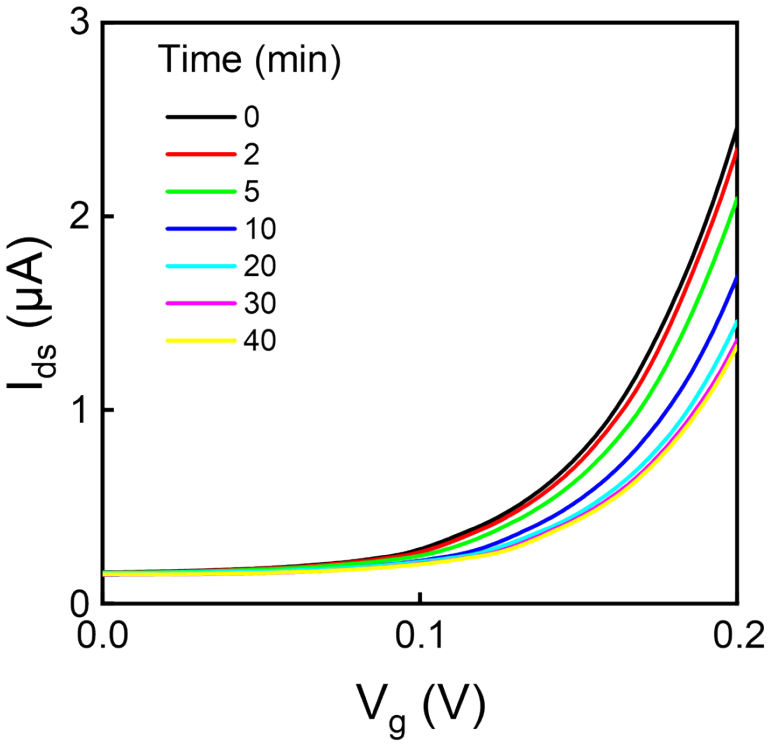

Fig. S7. The current change while detecting HepG2 telomerase under different time of telomerization process (2, 5, 10, 20, 30, and 40 min).

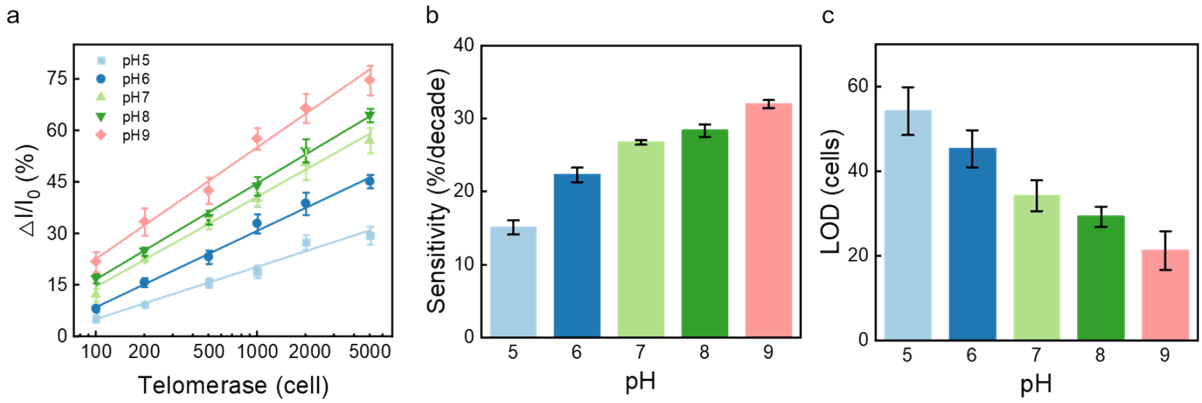

Fig. S8. The linear range (a), sensitivity (b), and LOD (c) of detecting telomerase under different pH (5-9).

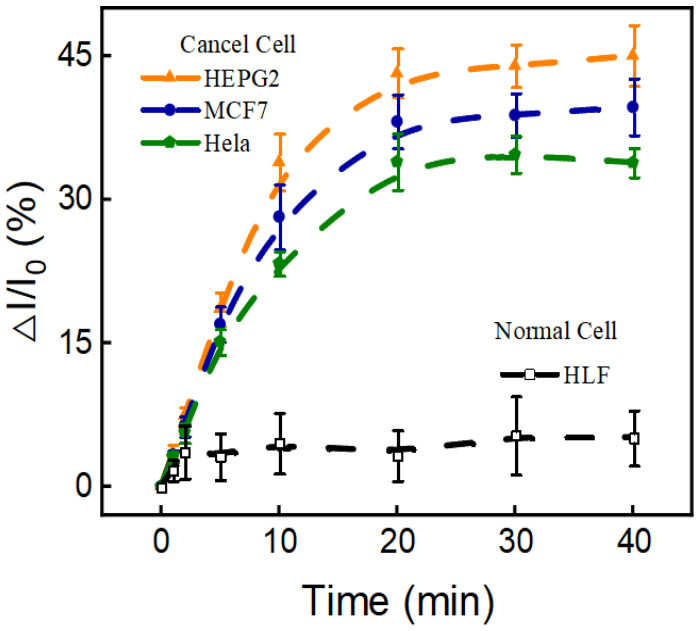

Fig. S9. Due to the lack of telomerase activity in normal cells, the sample of HLF do not show obvious change in current signal even incubation time was extended to 40 minutes.

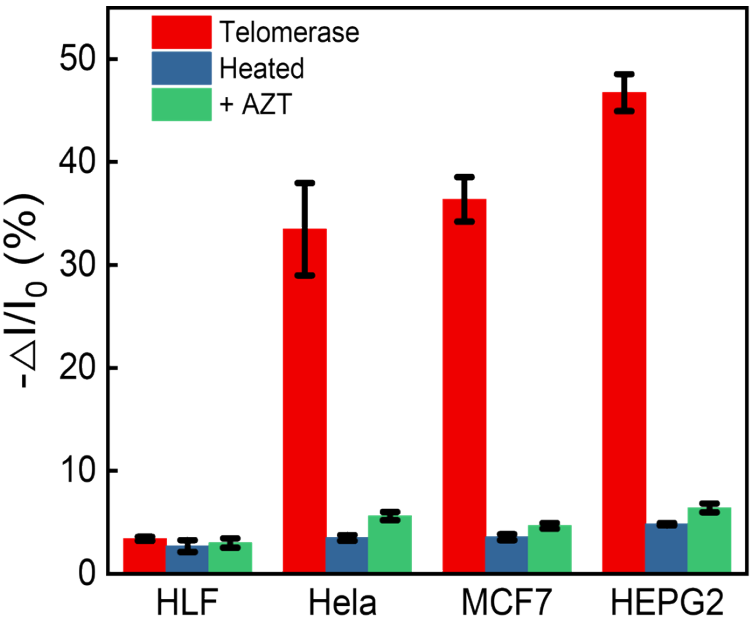

Fig. S10. The current changes of detecting telomerase from HLF, Hela, MCF7 and HepG2 and the comparison with the results after being heated at 95 °C and inhibited by AZT.

3. References

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