Supporting Information

Detecting telomerase activity at the single-cell level using a CRISPR-Cas12a-based chip

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Experimental procedures

1. Materials and chemicals

All oligonucleotides were synthesized and HPCL-purified by Sangon Biotech (Shanghai, China) Co., Ltd. Lab Cas12a (Cpf1) and NEB 2.1 buffer were purchased from New England Biolabs Inc. (Ipswich, USA). Ultrapure water was purified through a Milli-Q system (Millipore, Synergy). The mixture nucleotides (dNTPs), HEPES buffer, phenylmethyl-sulfonyl fluoride (PMSF) and NP-40 lysis buffer were purchased from Sangon Biotech (Shanghai, China). Bovine serum albumin (BSA) was obtained from Sigma-Aldrich, Inc. (St. Louis, MO). N,N-Dime-thylformamide (DMF), $FeCl_3 \cdot 6H_2O$, ethylene glycol, tetraethyl orthosilicate (TEOS), and glutaraldehyde were purchased from Macklin (Shanghai, China). Polydimethylsiloxane (PDMS, Sylgard 184) was purchased from Dow Corning Corp. (Midland, MI, USA). Methanol, ethanol, concentrated ammonia aqueous solution and other inorganic salt reagents were obtained from HUSHI (Shanghai, China). PBS buffer (0.1 M NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4 and 1.5 mM KH_2PO_4, pH 7.4) was prepared in-house.

2. Telomerase extension reaction

MSNPs-TS were washed several times with ultrapure water (DNase/RNase-free, Sterile) before use and extended in 1× TRAP buffer (20 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 63 mM KCl, 0.05% (v/v) Tween 20, 1 mM EGTA) containing 2 mM dNTP, 0.02 mg/mL BSA, and 10 μL of telomerase extracts by incubating for 40 min at 30 °C. Afterward, the MSNPs-TS-extension probes were obtained by halting the reaction through multiple washes with HEPES buffer, facilitated by a magnet, and subsequently subjected to blocking with 1% BSA.

3. Cell culture and telomerase extraction

The human normal breast cell line (MCF-10A cells), human lung cancer cell line (A549 cells), mouse melanoma cell line (B16 cells) and mouse breast carcinoma cell line (4T1 cells) were obtained from the Institute of Chinese Academy of Medical Sciences (Shanghai, China). The different cancer cell lines were cultivated in Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. All cell cultures were meticulously maintained at a constant temperature of 37 °C in a 5% CO_2 humidified atmosphere. For telomerase extraction, the NP-40 method was employed. Initially, 1,000,000 cells were collected into a DNase/RNase-free microfuge tube and subjected to centrifugation (1000 rpm, 5 min), involving several washing steps. Immediately after removal of the supernatant, a mixture of NP-40 and 1% PMSF (1 mL) was added and the cells were lysed on ice for 30 min. Afterwards, the cells were centrifuged at 4 °C (12000 rpm, 20 min) and the resulting supernatant was aliquoted and promptly frozen at -80 °C for further analysis.

4. Conventional TRAP assays and polyacrylamide gel electrophoresis analysis

A total of 10 μL of telomerase extracts were introduced into a reaction mixture comprising 1× TRAP buffer, 2 mM dNTP, 0.02 mg/mL BSA, and 2 ng/μL TS.[1] The mixture was incubated at 30 °C for a duration of 40 min.[1] Then the primer mix including 0.02 ng/μL ACX (5'-GCGCGGCTTACCCTTACCCTTACCCTT ACC-3'), 0.02 ng/μL NT (5'- ATCGCTTCTCGGCCTTTT-3') and 0.0005 attomol/μL TSNT (5'-

AATCCGTCGAGCAGAGTTAAAAGGCCGAGAAGCGAT-3') template and 2 U of Taq DNA polymerase were added to the resulting solution for internal standard. PCR was carried out with the following program: 95 °C for 5 min; 30 cycles at 95 °C for 30 s, 55 °C for 1 min, and 72 °C for 2 min; 72 °C for 10 min; and 4 °C. Finally, the PCR products were analyzed through polyacrylamide gel electrophoresis (PAGE) containing 12% acrylamide. Samples (10 μL) mixed with loading buffer (2 μL) were loaded onto polyacrylamide gel in 1× TBE buffer. Electrophoresis was conducted at 200 V for a duration of 1.5 to 2 h, followed by staining with Gel Red for 20 min before visualization using a gel imaging analysis system.

5. Telomerase quantification

The quantification of telomerase extracts was conducted through a procedure analogous to the determination of specific cell quantities via step dilution. Specifically, 1,000,000 cells were collected and subsequently lysed in a mixture consisting of 1 mL of NP-40 and 1% PMSF. For the quantification process, 100 μL of telomerase extract, representing an equivalent of 100,000 cells, was diluted with 900 μL of the NP-40 and 1% PMSF mixture. Currently, 100 μL of telomerase extension corresponds to an equivalence of 10,000 cells in the assay protocol.

6. Supplementary Figures

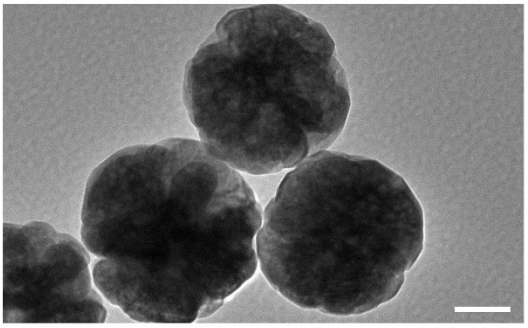

Fig. S1 TEM image of the Fe_3O_4 nanoparticles. Scale bar represents 100 nm.

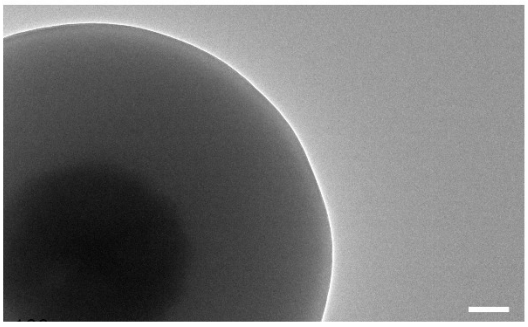

Fig. S2 TEM image of the Fe_3O_4@SiO_2 nanoparticles. Scale bars denotes 100 nm.

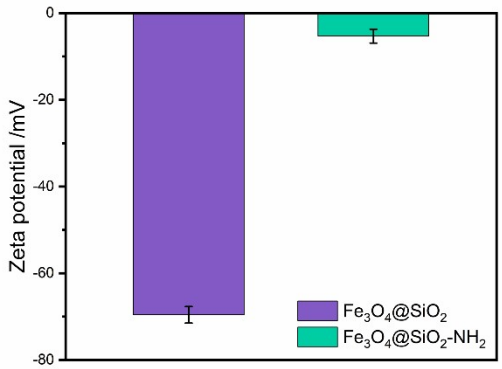

Fig. S3 Zeta potential of Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2-NH_2.

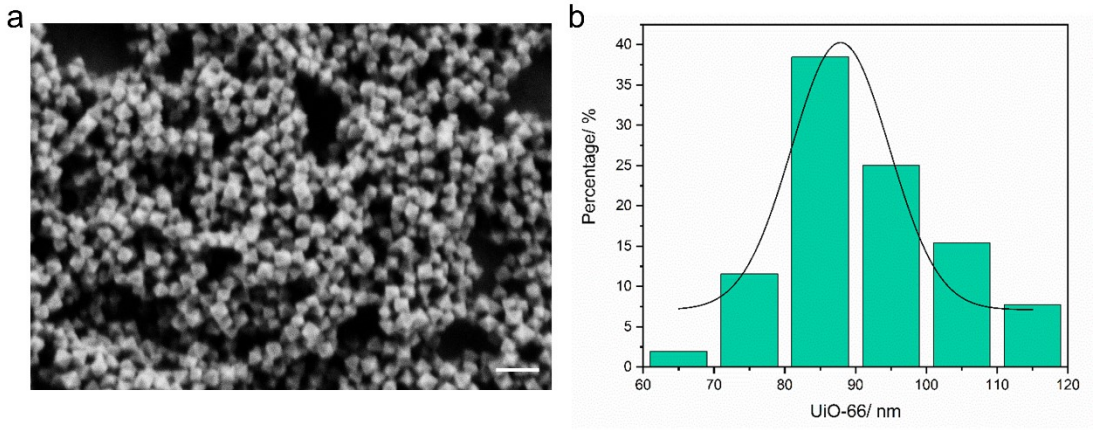

Fig S4 (a) SEM image and (b) particle size distribution of UiO-66. Scale bar is equal to 200 nm.

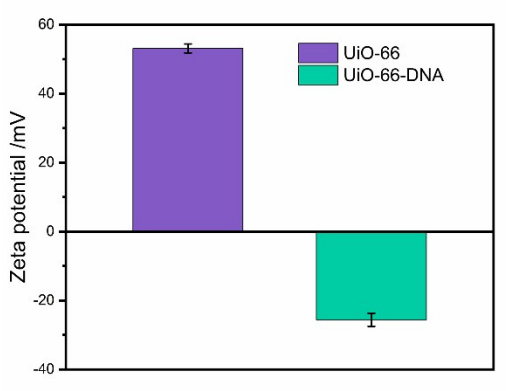

Fig. S5 Zeta potential of UiO-66 and DNA-functionalized UiO-66 nanoparticles.

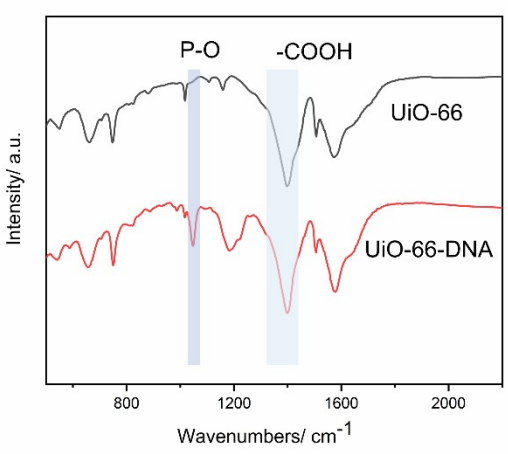

Fig. S6 FTIR spectra of UiO-66 and DNA functionalized-UiO-66 nanoparticles.

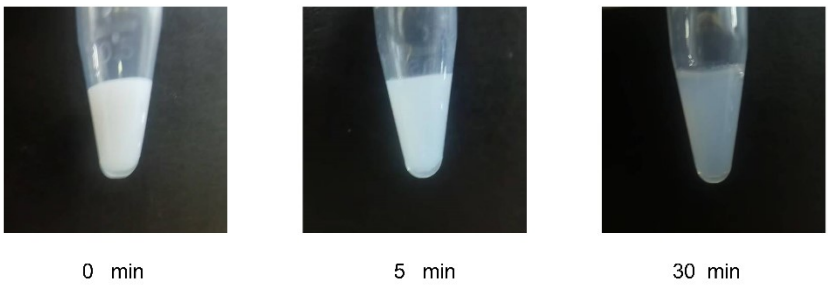

Fig. S7 Picture of UiO-66 solution treated with 8 mM phosphate at various time intervals (0, 5, and 30 min, from left to right).

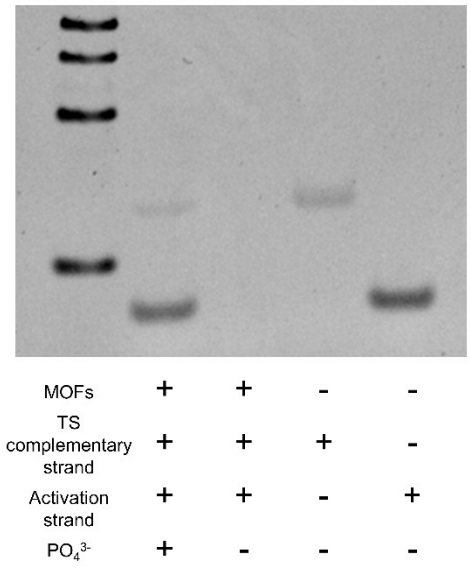

Fig. S8. PAGE showed that DNA was released from UiO-66. From left to right: UiO-66-DNA treated by phosphate, UiO-66-DNA, TS complementary strand, Activation strand.

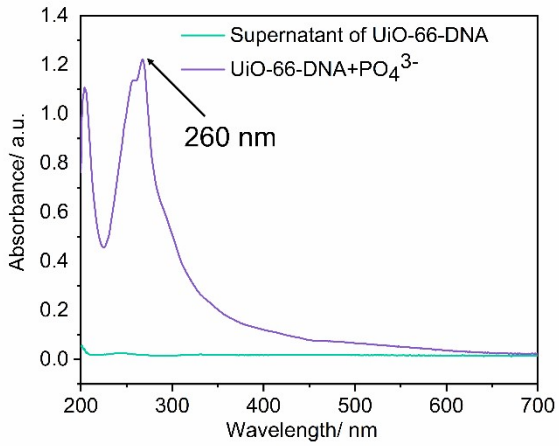

Fig. S9 UV-vis absorption spectra of suspension of UiO-66-DNA (The UiO-66-DNA solution was first centrifuged, and then the supernatant was taken) and DNA releasing process from UiO-66 in buffer (pH 7.4, 0.1 M NaCl).

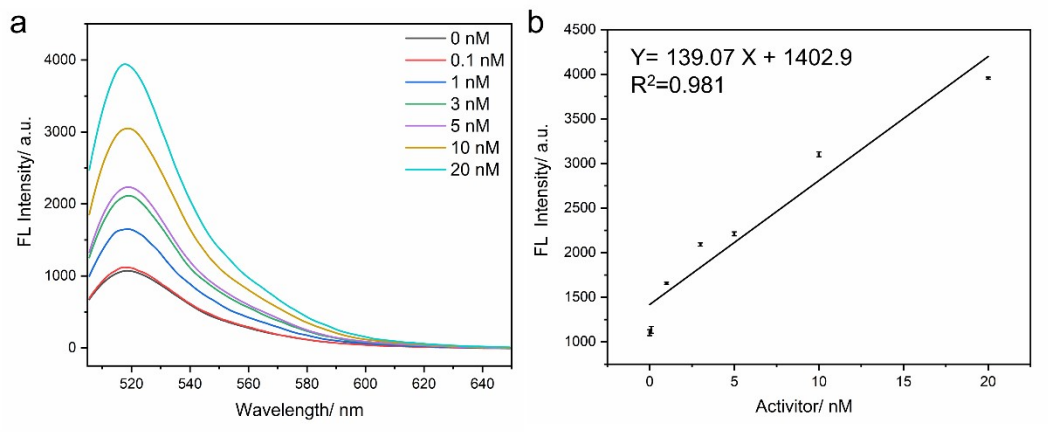

Fig. S10 (a) Fluorescence spectra at different concentrations of activators between 0 and 20 nM. (b) Linear relationship between the fluorescence intensity (at 520 nm) and the concentrations of activators. The data are represented as mean ± SD of triplicate samples.

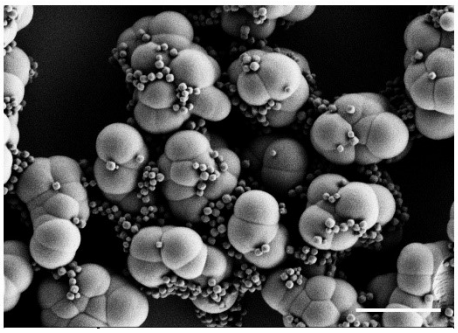

Fig. S11 SEM image of the binding capability of the UiO-66-DNA probe with the MSNPs-TS-extension probe. Scale bar represents 2 μm.

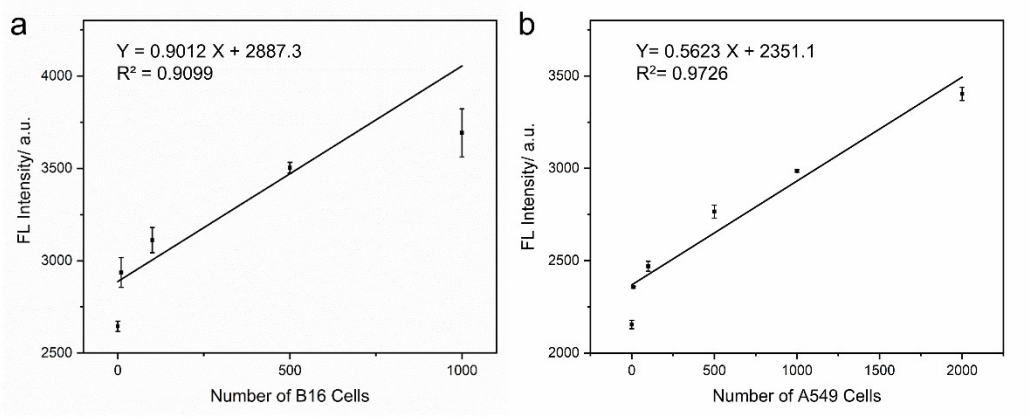

Fig. S12 Linear relationship between the fluorescence intensity (at 520 nm) and the number of (a) B16 cells and (b) A549 cells

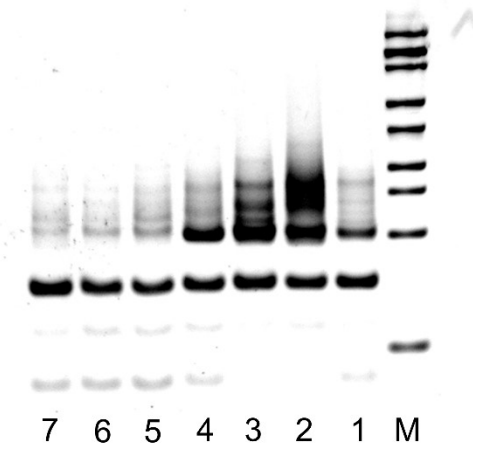

Fig. S13 Conventional PAGE image analysis of extension products using A549 cells: DNA ladder marker (M), heated-inactive control (lane 1), telomerase extracts from 10,000 cells (lane 2), 5,000 cells (lane 3), 2000 cells (lane 4), 1,000 cells (lane 5), 100 cells (lane 6), and 10 cells (lane 7).

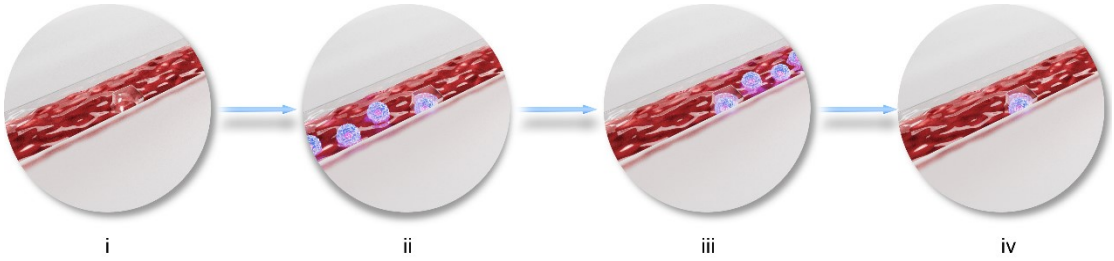

Figure S14 Process of single-cell capture in the microfluidic chip. (i) Microchannel in its initial state. (ii) Introduction of cells for capture. (iii) Removal of excess cells. (iv) Retention of single cells.

7. Supplementary table

Table S1 Oligonucleotides used in this study.

Name	Sequence (5'-3')

TS complementary strand	P-TTTTTTTTTCCCTAACCCTAACCCTAACCCTAACCCTAA
MSNPs-TS probe	NH2 C6-TTTTTTTTTTTTTAATCCGTCGAGCAGAGTT
FQ reporter	FAM-TTAATT-BHQ
NT	ATCGCTTCTCGGCCTTTT
ACX	GCGCGGCTTACCCTTACCCTTACCCTAACC
TSNT	AATCCGTCGAGCAGAGTTAAAAGGCCGAGAAGCGAT
TS	AATCCGTCGAGCAGAGTT
Activation strand	P-TTTTTTTTTGGCCAGTACCTCATGGAT
crRNA	UAAUUUCUACUAAGUGUAGAUAUCCAUGAGGUACUGGCCAA

Table S2 Comparison of different single-cell detection techniques.

Aspect	Stem-loop primer-mediated exponential amplification[2]	Fluorescent amplification method[3]	Fluorescent polarization strategy[4]	Quantum dot-based electrochemical biosensor[5]	Microfluidics-assisted MOF-DNA barcode-amplified CRISPR-Cas12a assay
Detection efficiency	High	High	High	High	High
Specificity	Excellent	Excellent	Excellent	Excellent	Excellent
Sensitivity	Single cell (unclear about the process of single-cell capture.)	Single cell (through dilution strategies)	Single cell (through dilution strategies)	Single cell (through the calculation of LOD values)	True Single cell (lysing single cells on the chip and then detecting telomerase activity)

Table S3 Comparison of the two methods.

Aspect	Microfluidics-assisted MOF-DNA barcode-amplified CRISPR-Cas12a assay	TRAP method
Detection cost	Low, does not require PCR equipment	High, requires PCR equipment
Sensitivity	Detects single cells	Detects several dozen cells (depending on cell viability)
Specificity	Excellent	Slightly lower
Operation steps	Relatively simple	Complex and time-consuming
Application scope	Applicable to various DNA and protein detection, including cancer-related biomarkers	More limited in scope
Detection time	Short (around 3 hours)	Half a day or more

Equation

The limit of detection defined by IUPAC is calculated as follows:

$$x_L = x_{b1} + k s_{b1}$$

where x_L is the limit of detection, x_{b1} is the blank mean, k is the constant data related to confidence (usually k = 3 is taken, and the confidence interval is 99.6% atthis time), and s_{b1} is the standard deviation of blank value.

References

1. Herbert, B.-S.; Hochreiter, A. E.; Wright, W. E.; Shay, J. W. *Nature Protocols*, 2006, **1**, 1583-1590.
2. Wang, H.; Wang, H.; Liu, C.; Duan, X.; Li, Z. *Chemical Science*, 2016, **7**, 4945-

4950.

3. Gao, Y.; Xu, J.; Li, B.; Jin, Y. *Biosensors and Bioelectronics*, *2016*, **81**, 415-422.

4. Gao, Y.; Xu, J.; Li, B.; Jin, Y. *ACS Applied Materials & Interfaces*, 2016, **8**, 13707-13713.

5. Li, C.-c.; Hu, J.; Lu, M.; Zhang, C.-y. *Biosensors and Bioelectronics*, 2018, **122**, 51-57.