Supplementary Information

PAM-Less Conditional DNA Substrates Leverage *trans*-Cleavage of CRISPR-Cas12a for Versatile Live-Cell Biosensing

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Materials and methods

Materials. All the oligonucleotides listed in supplementary table 1-8 were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai). dNTP mixture and 3'-Azido-3'-deoxythymidine (AZT, ≥98%) were purchased from Sigma-Aldrich (St. Louis, U.S.A.). Dithiothreitol (DTT, ≥99%), heparin sodium (from porcine intestinal, ≥150 U/mg), ATP (≥99%), CTP (≥99%), GTP (≥99%), UTP (≥99%), and egtazic acid-glycol ether diamine tetraacetic acid (EGTA, ≥97%) were purchased from Sangon Biotech Co., Ltd. (Shanghai). CHAPS lysis buffer was purchased from Millipore (Bed-ford, MA). Fetal bovine serum (FBS), trypsin, Dulbecco's Modified Eagle's Medium (DMEM), and RPMI-1640 were purchased from Invitrogen (Gibco, USA). Oligomycin (≥98%), Lipo8000™ transfection reagent (Lipo8000), and Cell Counting Kit-8 (CCK-8) were purchased from Beyotime Biotechnology (Shanghai). RNAprep pure Cell/Bacteria Kit was purchased from Tiangen Biotech Co., Ltd. (Beijing), *Evo M-MLV* RT Mix Kit and SYBR® Green Premix *Pro Tag* HS qPCR Kit were purchased from Accurate biology (Changsha). All the solutions were prepared with DEPC water.

The components of the buffer solutions used in the experiment are listed as follows. 10× telomerase extension buffer: 200 mM Tris-HCl (pH 8.3), 15 mM $MgCl_2$, 630 mM KCl, 0.5% (v/v) Tween-20, and10 mM EGTA; 5× HEPES buffer: 200 mM HEPES (pH 7.4), 500 mM NaCl, and100 mM $MgCl_2$; 5× Tris buffer: 100 mM Tris-HCl (pH 7.5), 700 mM NaCl, and 25 mM KCl; 5× CRISPR cleavage buffer: 100 mM Tris-HCl (pH 7.5), 500 mM KCl, 25 mM $MgCl_2$, 5% (v/v) glycerol, 5 mM DTT and 250 μg/mL heparin.

Protein expression and purification. The coding sequence of LbCas12a was optimized and synthesized by Sangon Biotech Co. Ltd. (Shanghai, China) based on the gene sequence of plasmid pET-LbCpf1-2NLS (Addgene, #102566), and then cloned into pET28a to obtain the expression vector. The recombinant plasmid was transformed into *E. coli.* BL21 (Rosetta), and a single clone was cultured at 37 °C overnight in a 5 mL liquid Luria-Bertani (LB) medium. Then 1% (v/v) of the culture was inoculated into 250 mL of fresh liquid LB and cultured at 37°C with shaking at 220 rpm until reaching the logarithmic growth phase. After that, 0.2 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce protein expression at 16 °C for 16 hours. Cells were harvested by centrifugation and resuspended in 50 mL lysis buffer (50 mM Tris-HCl, pH 8.0, and 1.5 M NaCl). Then, the cells were lysed by sonication at 4 °C, and the supernatants were subsequently loaded on Ni-NTA agarose chromatography (ÄKTA, GE) to obtain purified LbCas12a protein. Finally, the buffer was exchanged into the desalination buffer (20 mM Tris-HCl, pH 8.0, 600 mM NaCl, 2 mM DTT, and 10% (v/v) glycerol) by a desalination column. The purified protein was analyzed by SDS-PAGE and quantified by the improved Bradford protein assay dye reagent kit. Finally, the protein was stored at –80 °C for later use.

Cell culture. Human cervical cancer cells (HeLa), human breast cancer cells (MCF-7) and human embryonic kidney cells (293T) were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine and 1% antibiotic-antimycotic (100 units/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of Amphotericin B) at 37 °C in a humidified atmosphere containing 5% CO_2. Human non-small cell lung cancer cells (A549) and human hepatocytes cells (LO2) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine and 1% antibiotic-antimycotic at 37 °C in a humidified atmosphere containing 5% CO_2. The number of cells was measured by an automated cell counter (Countstar, Shanghai, China).

Total RNA extraction and quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Total RNA was isolated from LO2, MCF-7, A549, HeLa and 293T cells using RNAprep pure Cell/Bacteria Kit (Tiangen

Biotech Co., Ltd. (Beijing) following the manufacturer's protocol. The concentration of total RNA was determined by Synergy™ Mx multimode microplate reader Receives (BioTek, USA).

For the qRT-PCR analysis of miRNA-21 expression level, cDNA samples were synthesized by reverse-transcription from 0.5 μg of total cellular RNA with 0.1 μM RT-primer using *Evo M-MLV* RT Mix Kit (reverse transcriptase, Accurate biology, Changsha) on an S1000™ Thermal Cycler (Bio-Rad Laboratories, Inc., USA) according to the manufacturer's instructions. PCR was performed with SYBR® Green Premix *Pro Tag* HS Qpcr Kit (Accurate biology, Changsha) on a QuantStudio 7 Flex (Invitrogen Life Technologies, Carlsbad, California, USA). The 20 μL of solution contain 2 μL of cDNA sample, 0.4 μL of forward primer (10 μM), 0.4 μL of reverse primer (10 μM), 10 μL of 2×SYBR® Green Pro Tag HS Premix, and 7.2 μL of RNase free water. The PCR was performed in the following conditions: 95˚C for 30 s, 40 cycles of 95˚C for 5 s and 60˚C for 30 s.

For the qRT-PCR analysis of TERT mRNA, cDNA samples were synthesized by reverse-transcription from 0.5 μg total cellular RNA using *Evo M-MLV* RT Mix Kit according to the manufacturer's instructions. The PCR conditions were the same as the PCR for miRNA-21.

Telomerase extraction. 1×10^6 cells in the exponential growth phase were collected, washed twice with ice-cold PBS (pH 7.4), and centrifugated at 2000 rpm for 5 min at 4 °C. The cells were resuspended in 200 μL of ice-cold CHAPS lysis buffer and incubated on ice for 30 min, then centrifuged at 12000 rpm for 20 min at 4 °C. Finally, the supernatant was collected and stored at -80 °C for further experiment.

Nucleic acid hybridization. All the nucleic acid strands were dissolved in DEPC-treated water and quantified by the absorbance at 260 nm by a Synergy™ Mx multimode microplate reader Receives (BioTek, USA). All the dsDNA were prepared by the hybridization TS (or truncated TS) with NTS in a molar ratio of 1:1.2 in annealing buffer (20 mM Tris-HCl, pH 7.5, 150 mM KCl, and 2.5 mM $MgCl_2$). The mixture was incubated at 95 °C for 5 min, followed by gradient cooling to 25 °C. $pcDNA_{ATP}$ was produced by mixing TS_{ATP}, NTS_{ATP} and $Blocking_{ATP}$ in a molar ratio of 1:1.2:1.5 in the annealing buffer with the same annealing procedure. Similarly, $pcDNA_{miRNA}$ was prepared by mixing TS_{miRNA}, NTS_{miRNA} and $Blocking_{miRNA}$ in a molar ratio of 1:1.2:3.

***In vitro* fluorescence measurements.** For the construction of pDNA, reactions were initiated by mixing 100 nM LbCas12a/gRNA complexes with 50 nM dsDNA substrate and 1.0 μM FQ reporters in CRISPR cleavage buffer with a total volume of 20 μL. The reactions were carried out at 37°C for 2 hours, and the fluorescence emission intensity at 520 nm was read on QuantStudio 7 Flex (Invitrogen Life Technologies, Carlsbad, California, USA) at 488 nm excitation every 30 seconds.

For the *in vitro* telomerase activity assay, cell lysates from different numbers of cells were incubated with a mixture containing 1.0 μM $pcDNA_{Tel}$ and 300 μM dNTPs in telomerase extension buffer with a total volume of 10 μL at 37 °C for 1 hour. Then, 100 nM LbCas12a/gRNA complexes and 1.0 μM FQ reporters mixed in CRISPR cleavage buffer were added with a final volume of 20 μL. The reactions were carried out at 37°C for 2 hours, and the fluorescence emission intensity at 520 nm was read on QuantStudio 7 Flex (Invitrogen Life Technologies, Carlsbad, California, USA) at 488nm excitation every 30 seconds. For control experiments, telomerase in the cell lysates was deactivated by heating at 95 °C for 10 min. For the *in vitro* telomerase activity inhibition experiment, cell lysates containing 1000 cells were pre-incubated with various concentrations of AZT for 12 hours at 37 °C before the telomerase activity assay mentioned above.

For the *in vitro* ATP assay, 375 nM $pcDNA_{ATP}$ was incubated with various concentrations of ATP at 37 °C for 15 min in HEPES buffer with a total volume of 10 μL. Then 2 μL of ATP reaction product was mixed with 100

nM LbCas12a/gRNA complexes and 1 μM FQ reporters in CRISPR cleavage buffer with a final volume of 20 μL. The reactions were carried out at 30 °C for 2 hours, then the fluorescence intensity at 520 nm and fluorescence emission spectroscopy scanning from 500 nm to 600 nm were read on Synergy™ Mx multimode microplate reader Receives (BioTek, USA) at 488nm excitation.

For the *in vitro* miRNA assay, 100 nM $pcDNA_{miRNA}$ was incubated with various concentrations of miRNA-21 at 37 °C for 30 min under in Tris buffer with a total volume of 10 μL. Then 2 μL of miRNA-21 reaction product was incubated with 100 nM LbCas12a, 100 nM gRNA and 1 μM FQ reporters in CRISPR cleavage buffer with a final volume of 20 μL. The reactions were carried out at 30 °C for 2 hours, then the fluorescence intensity at 520 nm and fluorescence emission spectroscopy scanning from 500 nm to 600 nm were read on Synergy™ Mx multimode microplate reader Receives (BioTek, USA) at 488nm excitation. For miRNA-122, the reaction conditions were the same as miRNA-21.

Laser scanning confocal imaging of intracellular targets. For the verification of *trans*-cleavage activity of LbCas12a in the cytoplasm, different cell lines (HeLa, A549, MCF-7 and LO2) were plated on confocal dishes in 200 μL culture medium at 37 °C for 24 hours. Then, 100 nM wild-type DNA substrate was transfected into cells with lipofectamine 8000 in 100 μL culture medium. After 3 hours, the medium was removed and cells were washed three times with ice-cold PBS. Then, 50 nM Cas12a/gRNA complexes and 1.0 μM FQ reporters were co-transfected into cells by Lipofectamine 8000 with a 100 μL total volume for 5 hours. The medium was removed again and cells were washed three times with ice-cold PBS, and 200 μL of fresh medium was added to the confocal dish before fluorescent confocal imaging.

The fluorescence intensity of each cell was quantified using ImageJ software. The data represents the normalized average intensity value of n cells in each group relative to the experimental group. For the *in vivo* telomerase activity sensing, HeLa cells were treated with the same steps as the above-mentioned *trans*-cleavage activity verification experiment except for the replacing of wild-type DNA substrate with 100 nM $pcDNA_{Tel}$ or 100 nM mimic $pcDNA_{Tel}$. For *in vivo* telomerase activity inhibition assay, HeLa cells were plated on confocal dishes in 200 μL culture medium at 37 °C for 24 hours. After washing with ice-cold PBS for three times, cells were incubated with different amounts of AZT for another 24 hours and then directly perform the telomerase activity sensing experiment illustrated above.

For ATP and miRNA live-cell sensing, A549 cells or LO2 cells were plated on a confocal dish in 200 μL culture medium at 37 °C for 24 hours. 50 nM Cas12a/gRNA complexes, 100 nM pcDNA and 1 μM FQ reporters were transfected to cells with Lipofectamine 8000 in culture medium with a 100 μL total volume. After 5 hours, the medium was removed and cells were washed three times with ice-cold PBS, and 200 μL of fresh medium was added to the confocal dish before fluorescent confocal imaging. For in vivo ATP inhibition assay, A549 cells were plated on confocal dishes in 200 μL culture medium at 37 °C for 24 hours. After washing with ice-cold PBS for three times, cells were incubated with 3.0 μg/mL oligomycin for 3 hours and then directly perform the ATP sensing experiment illustrated above.

Table S1. Oligonucleotides for exploring the ability of bubble DNA substrates on Cas12a activation.

Oligonucleotides		Sequences
TTTA	[a]TS	3’-CTGCTGTTTTGAAATCTAGCAATGCGATTGATACT-5’
	[b]NTS-0 nt	5’-GACGACAAAACTTTAGATCGTTACGCTAACTATGA-3’
	[c]NTS-1 nt	5’-GACGACAAAACTTTACATCGTTACGCTAACTATGA-3’
	NTS-2 nt	5’-GACGACAAAACTTTACTTCGTTACGCTAACTATGA-3’
	NTS-6 nt	5’-GACGACAAAACTTTACTAGCATACGCTAACTATGA-3’
	NTS-8 nt	5’-GACGACAAAACTTTACTAGCAATCGCTAACTATGA-3’
	NTS-10 nt	5’-GACGACAAAACTTTACTAGCAATGCCTAACTATGA-3’
	NTS-20 nt	5’-GACGACAAAACTTTACTAGCAATGCGATTGATACT-3’
gRNA		5’-UAAUUUCUACUAAGUGUAGAUGAUCGUUACGCUAACUAUGA-3’
FQ reporter		5’-F-TTATT-Q-3’

[a]The PAM complementary sequences on TS are underlined.

[b]The PAM sequences on NTS are underlined in bold.

[c]The nonmatched sites are shown in red.

Table S2. Oligonucleotides for the construction of pDNA.

Oligonucleotides		Sequences
AAAT	[a]TS	3’-CTGCTGTTTTGTTTACTAGCAATGCGATTGATACT-5’
	[b]NTS-0 nt	5’-GACGACAAAACAAATGATCGTTACGCTAACTATGA-3’
	[c]NTS-6 nt	5’-GACGACAAAACAAATCTAGCATACGCTAACTATGA-3’
AGAT	TS	3’-CTGCTGTTTTGTCTACTAGCAATGCGATTGATACT-5’
	NTS-0 nt	5’-GACGACAAAACAGATGATCGTTACGCTAACTATGA-3’
	NTS-6 nt	5’-GACGACAAAACAGATCTAGCATACGCTAACTATGA-3’
AGAG	TS	3’-CTGCTGTTTTGTCTCCTAGCAATGCGATTGATACT-5’
	NTS-0 nt	5’-GACGACAAAACAGAGGATCGTTACGCTAACTATGA-3’
	NTS-6 nt	5’-GACGACAAAACAGAGCTAGCATACGCTAACTATGA-3’
TAAT	TS	3’-CTGCTGTTTTGATTACTAGCAATGCGATTGATACT-5’
	NTS-0 nt	5’-GACGACAAAACTAATGATCGTTACGCTAACTATGA-3’
	NTS-6 nt	5’-GACGACAAAACTAATCTAGCATACGCTAACTATGA-3’
TAAC	TS	3’-GGGATTGCTAGCAATGCGATTGATCAT-5’
	NTS-0 nt	5’-CCCTAACGATCGTTACGCTAACTATGA-3’
	NTS-6 nt	5’-CCCTAACCTAGCATACGCTAACTATGA-3’
TGAT	TS	3’-CTGCTGTTTTGACTACTAGCAATGCGATTGATACT-5’
	NTS-0 nt	5’-GACGACAAAACTGATGATCGTTACGCTAACTATGA-3’
	NTS-6 nt	5’-GACGACAAAACTGATCTAGCATACGCTAACTATGA-3’
CAAT	TS	3’-CTGCTGTTTTGGTTACTAGCAATGCGATTGATACT-5’
	NTS-0 nt	5’-GACGACAAAACCAATGATCGTTACGCTAACTATGA-3’
	NTS-6 nt	5’-GACGACAAAACCAATCTAGCATACGCTAACTATGA-3’
CCCT	TS	3’-CTGCTGTTTTGGGGACTAGCAATGCGATTGATACT-5’
	NTS-0 nt	5’-GACGACAAAACCCCTGATCGTTACGCTAACTATGA-3’
	NTS-6 nt	5’-GACGACAAAACCCCTCTAGCATACGCTAACTATGA-3’
CGAT	TS	3’-CTGCTGTTTTGGCTACTAGCAATGCGATTGATACT-5’
	NTS-0 nt	5’-GACGACAAAACCGATGATCGTTACGCTAACTATGA-3’
	NTS-6 nt	5’-GACGACAAAACCGATCTAGCATACGCTAACTATGA-3’
GAAT	TS	3’-CTGCTGTTTTGCTTACTAGCAATGCGATTGATACT-5’
	NTS-0 nt	5’-GACGACAAAACGAATGATCGTTACGCTAACTATGA-3’
	NTS-6 nt	5’-GACGACAAAACGAATCTAGCATACGCTAACTATGA-3’
GCTA	TS	3’-ACTATTCGATCTAGCAATGCGATTGATACT-5’
	NTS-0 nt	5’-GAAGCTAGATCGTTACGCTAACTATGA-3’
	NTS-6 nt	5’-GAAGCTACTAGCATACGCTAACTATGA-3’
GGTT	TS	3’-CGTCCAACTAGCAATGCGATTGATACT-5’
	NTS-0 nt	5’-GCAGGTTGATCGTTACGCTAACTATGA-3’
	NTS-6 nt	5’-GCAGGTTCTTCCATACGCTAACTATGA-3’

[a]The PAM complementary sequences on TS are underlined.

[b]The PAM sequences on NTS are underlined in bold.

[c]The nonmatched sites are shown in red.

Table S3. Oligonucleotides for PAM integrality.

Oligonucleotides		Sequences
PAM integrality	[a]TS	3’-CTGCTGTTTTGGGGACTAGCAATGCGATTGATACT-5’
	[b]TS-P1	3’-CTGCTGTTTTGGGGTCTAGCAATGCGATTGATACT-5’
	TS-P2	3’-CTGCTGTTTTGGGCTCTAGCAATGCGATTGATACT-5’
	TS-P3	3’-CTGCTGTTTTGGCCTCTAGCAATGCGATTGATACT-5’
	TS-P4	3’-CTGCTGTTTTGCCCTCTAGCAATGCGATTGATACT-5’
	[c]NTS	5’-GACGACAAAACCCCTCTAGCATACGCTAACTATGA-3’

[a]The PAM complementary sequences on TS are underlined.

[b]The nonmatched sites are shown in red.

[c]The PAM sequences on NTS are underlined in bold.

Table S4. Oligonucleotides used in the PAGE experiment.

Oligonucleotides		Sequences
cis-cleavage	[a]FAM-TS	3’-F-CTGCTGTTTTGAAATCTAGCAATGCGATTGATACTCCCGACAGAC-5’
	[b]NTS-0 nt	5’- GACGACAAAACTTTAGATCGTTACGCTAACTATGAGGGCTGTCTG-3’
	[c]NTS-1 nt	5’- GACGACAAAACTTTACATCGTTACGCTAACTATGAGGGCTGTCTG-3’
	NTS-2 nt	5’- GACGACAAAACTTTACTTCGTTACGCTAACTATGAGGGCTGTCTG-3’
	NTS-6 nt	5’- GACGACAAAACTTTACTAGCATACGCTAACTATGAGGGCTGTCTG-3’
trans-cleavage	ssDNA-FAM	5’-GACGACAAAACTTTAGATCGTTACGCTAACTATGAGGGCTGTCTGTGGAATGC TA-F-3’

[a]The PAM complementary sequences on TS are underlined.

[b]The PAM sequences on NTS are underlined in bold.

[c]The nonmatched sites are shown in red.

Table S5. Oligonucleotides for the construction of $pcDNA_{Tel}$.

Oligonucleotides	Sequences
Truncated TS_{Tel}	3’- TTGAGACGAGCTGCCTAACG-5’
[a]Extended TS_{Tel}	3’-ATTGGGATTGGGATTGAGACGAGCTGCCTAACG-5’
[b, c]NTS_{Tel}	5’- AACCCTAA**CCCT**TCGAGAGCTCGACGGATTGC-3’
Mimic TS	3’- TAGCCGTCGACCGTGGCGGTACTAGT-5’
Mimic NTS	5’-ACGCAGG**TTTC**TAGCCGAGCTGGCACCGCCATGATCA-3’
$gRNA_{Tel}$	5’-UAAUUUCUACUAAGUGUAGAUAACUCUGCUCGACGGAUUGC-3’

[a]The PAM complementary sequences on TS are underlined.

[b]The PAM sequences on NTS are underlined in bold.

[c]The nonmatched sites are shown in red.

Table S6. Sequences of the oligonucleotides in $pcDNA_{ATP}$.

Oligonucleotides	Sequences
$Blocking_{ATP}$	5’-AGAACCTGGGGGAGTATTGCGGAGGAAGGTTGATCGT-3’
[a]TS_{ATP}	3’- TTCCAACTAGCAATGCGATTGATACT-5’
[b, c]NTS_{ATP}	5’- CCCA**GGTT**CTTCCATACGCTAACTATGA-3’
Mimic $blocking_{ATP}$	5’-AGAACCTGGTTAAGTATTGCGGAGTAAGGTTGATCGT-3’

[a]The PAM complementary sequences on TS are underlined.

[b]The PAM sequences on NTS are underlined in bold.

[c]The nonmatched sites are shown in red.

Table S7. Sequences of the oligonucleotides in $pcDNA_{miRNA}$.

Oligonucleotides	Sequences
miRNA-21	3’- AGUUGUAGUCAGACUAUUCGAU-5’
$Blocking_{miRNA-21}$	5’-AGTAGCTTCAACATCAGTCTGATAAGCTAGATCGT-3’
[a]$TS_{miRNA-21}$	3’- ACTATTCGATCTAGCAATGCGATTGATACT-5’
[b, c]$NTS_{miRNA-21}$	5’- GAA**GCTA**CTTCCATACGCTAACTATGA-3’
miRNA-122 (DNA)	3’- TGTTTGTGGTAACAGTGTGAGGT-5’
$Blocking_{miRNA-122}$	5’-AGTGGAGTACAAACACCATTGTCACACTCCAGATCGT-3’
$TS_{miRNA-122}$	3’- AGTGTGAGGTCTAGCAATGCGATTGATACT-5’
$NTS_{miRNA-122}$	5’- TAC**TCCA**CTTCCATACGCTAACTATGA-3’

[a]The PAM complementary sequences on TS are underlined.

[b]The PAM sequences on NTS are underlined in bold.

[c]The nonmatched sites are shown in red.

Table S8. Sequences of the primer sequences in qRT-PCR and TRAP assay.

Oligonucleotides	Sequences
RT-primer for miRNA-21	5’-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCAACA-3’
Forward primer for miRNA-21	5’-GCCCGCTAGCTTATCAGACTGATG-3’
Reverse primer for miRNA-21	5’-GTGCAGGGTCCGAGGT-3’
Forward primer for TERT mRNA	5’-TTCCGCAGAGAAAAGAGGGC-3’
Reverse primer for TERT mRNA	5’-CACGCTGAACAGTGCCTTCAC-3’
Forward primer for GAPDH	5’-CAACTCACTCAAGATTGTCAGCAA-3’
Reverse primer for GAPDH	5’-GGGATGGACTGTGGTCATGA-3’
[a]TRAP-TS primer	5’-AATCCGTCGAGCAGAGTT-3’
[b]TRAP-ACX	5’-GCGCGGCTTACCCTTACCCTAACC-3’
[c]TRAP-TSNT	5’-AATCCGTCGAGCAGAGTTAAAAGGCCGAGAAGCGAT-3’
[d]TRAP-NT	5’-ATCGCTTCTCGGCCTTTT-3’

[a]The primer for telomerase as well as the forward primer for extension product and internal standard.

[b]The reverse primer for extension product.

[c]The internal standard control.

[d]The reverse primer for internal standard.

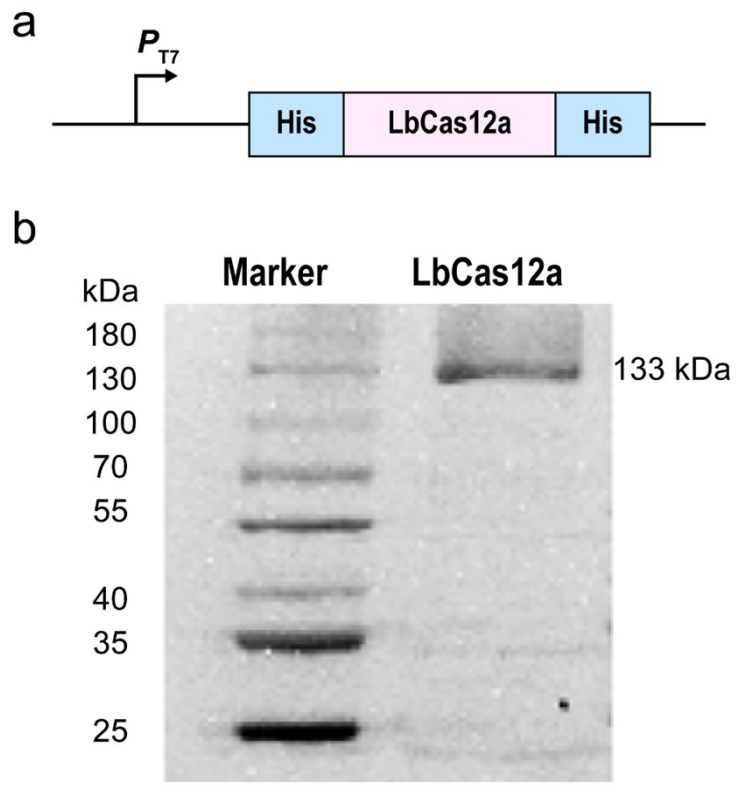

Fig. S1. Expression of LbCas12a. (a) The vector for the expression of LbCas12a. (b) Analysis of the purified LbCas12a by SDS-PAGE.

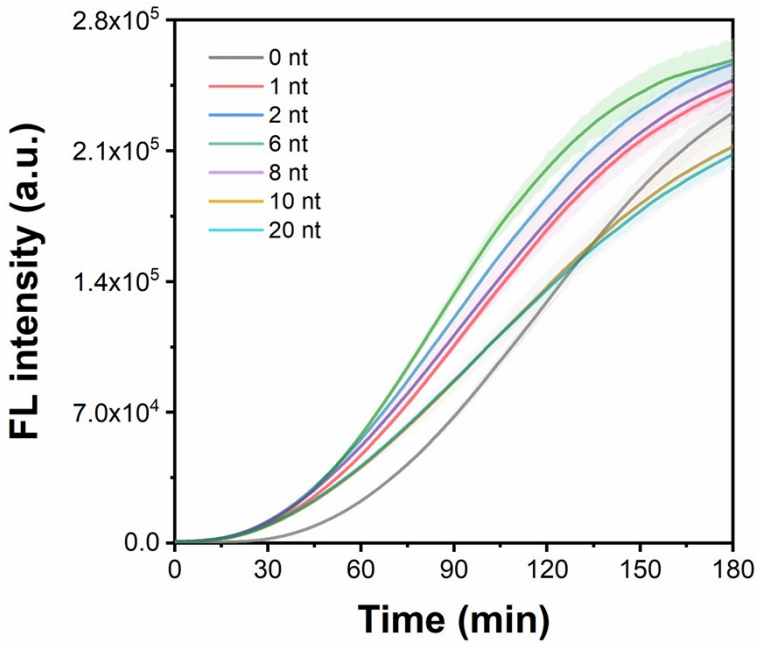

Fig. S2. Analysis of the *trans*-cleavage activity of LbCas12a triggered by dsDNA substratescontaining different numbers of nonmatched sites. LbCas12a, 100 nM; gRNA, 100 nM; wild-type DNA targets or dsDNA substrates with a bubble, 50 nM; FQ reporter, 1.0 μM. FL, fluorescence; a.u., arbitrary units.

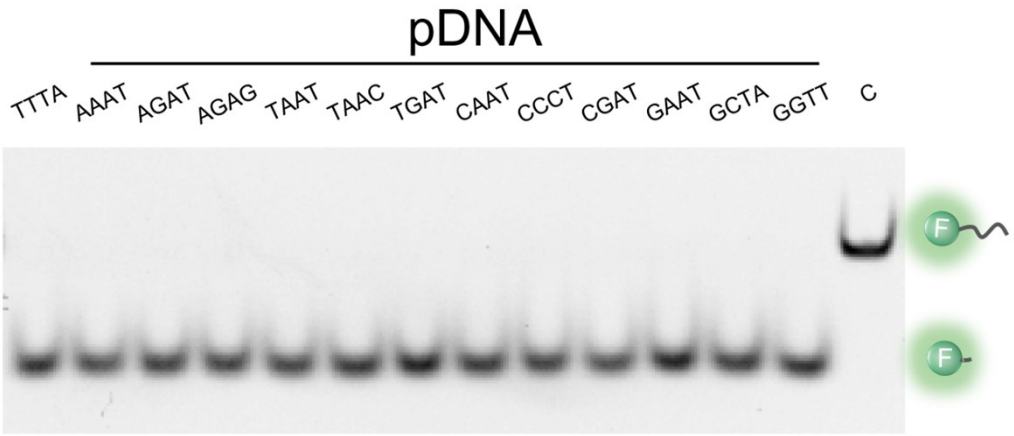

Fig. S3. Analysis of the *trans*-cleavage activities of LbCas12a activated by pDNA. LbCas12a, 100 nM; gRNA, 100 nM; each pDNA, 50 nM; ssDNA-FAM, 1.0 μM; reaction time, 2 h.

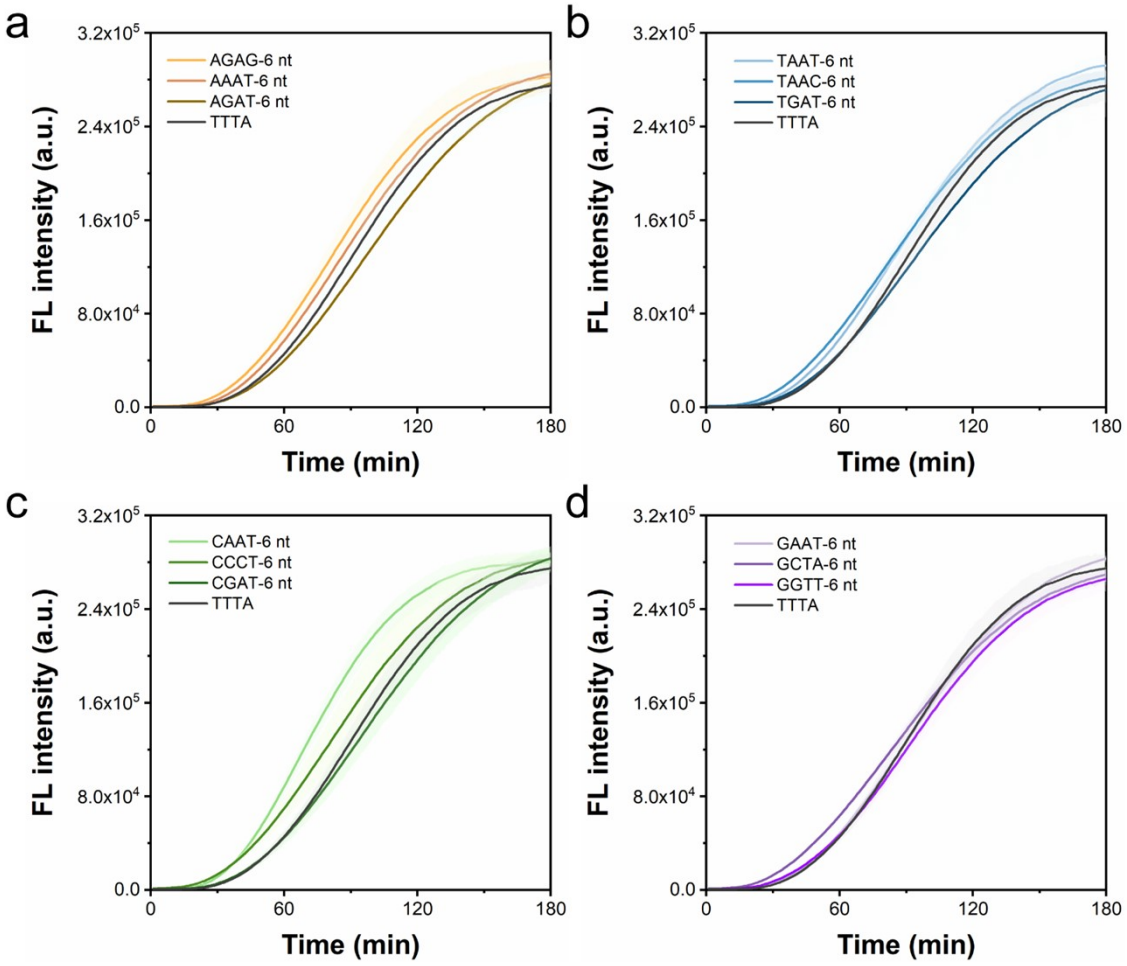

Fig. S4. Fluorescence kinetic analysis of *trans*-cleavage of FQ reporters by LbCas12a activated by bubble DNA substrates. LbCas12a, 100 nM; gRNA, 100 nM; wild-type DNA targets or dsDNA substrates with a bubble, 50 nM; FQ reporter, 1.0 μM.

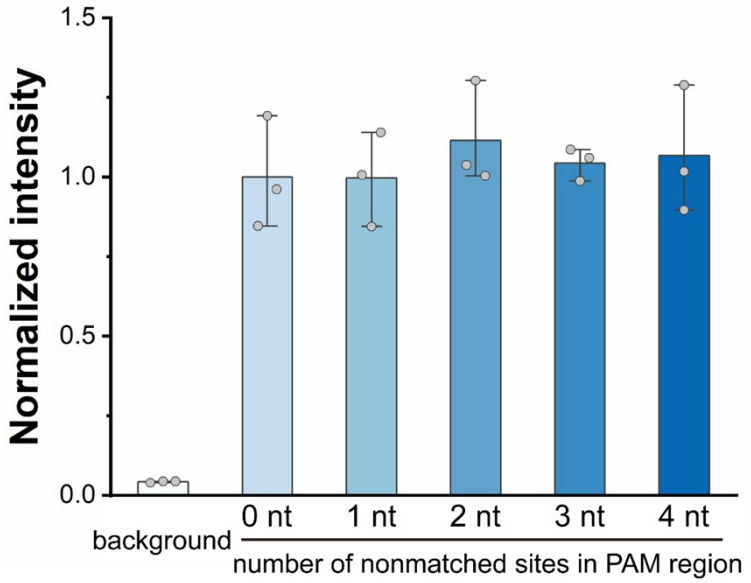

Fig. S5. Effect of the number of nonmatched sites in PAM region of pDNA on the activation of LbCas12a's *trans*-cleavage activity. LbCas12a, 100 nM; gRNA, 100 nM; each PAM-less DNA target, 50 nM; FQ reporter, 1.0 μM.

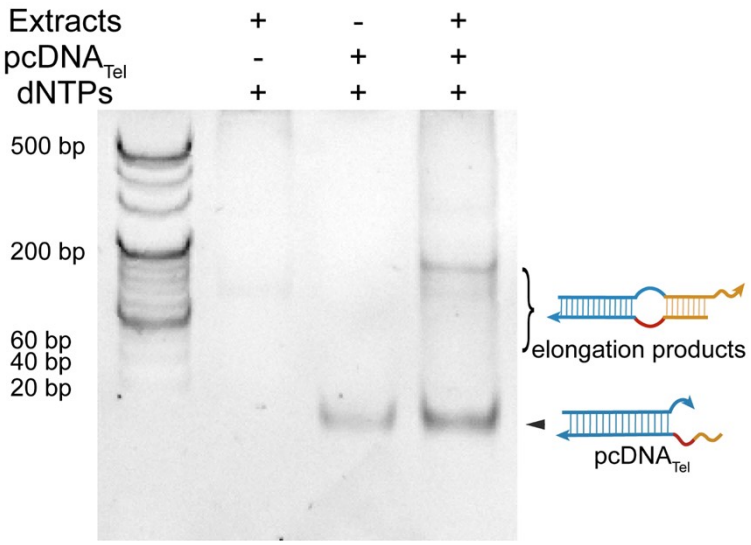

Fig. S6. Analysis of telomerase-catalyzed elongation by native PAGE. Lane 1, DNA marker. Extract, telomerase extract from 5×10^4 Hela cells; pcDNAT$_{Tel}$, 250 nM; dNTPs, 0.5 mM.

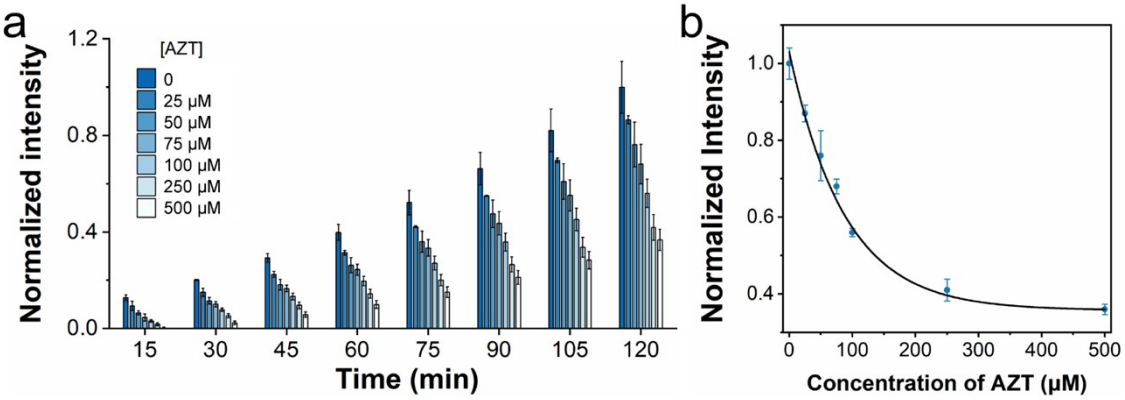

Fig. S7. pcDNA-based Cas12a assay for the detection of telomerase pretreated with AZT. (a) Real-time fluorescence intensity of pcDNA-based Cas12a assay in response to telomerase extracts of 1000 HeLa cells pretreated with various concentrations of AZT. (b) Fluorescence response curve of pcDNA -based Cas12a assay in response to telomerase extracts pretreated with various concentrations of AZT. LbCas12a, 100 nM; gRNA, 100 nM; pcDNA $_{Tel}$, 50 nM; FQ reporter, 1.0 μM.

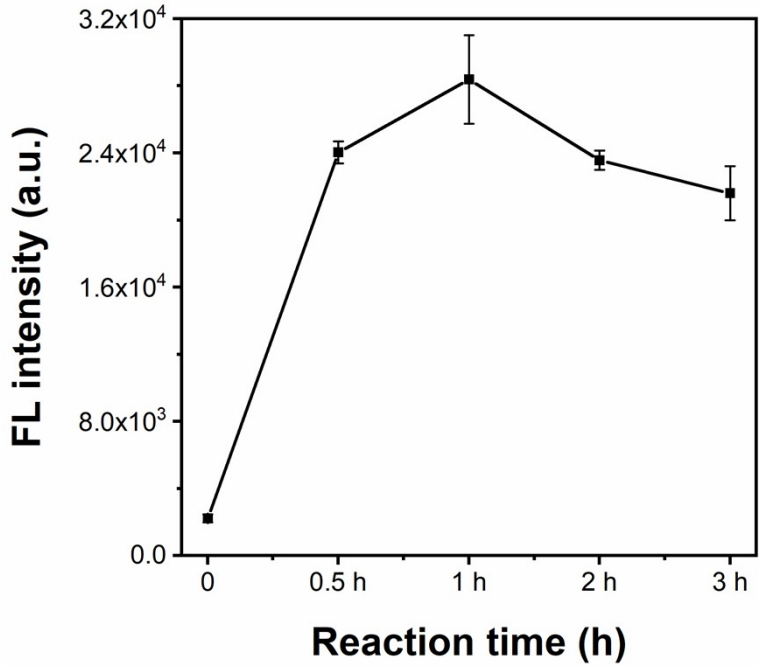

Fig. S8. Effect of the extension reaction time on the fluorescence response in telomerase assay. LbCas12a, 100 nM; gRNA, 100 nM; pcDNA $_{Tel}$, 50 nM; FQ reporter, 1.0 μM.

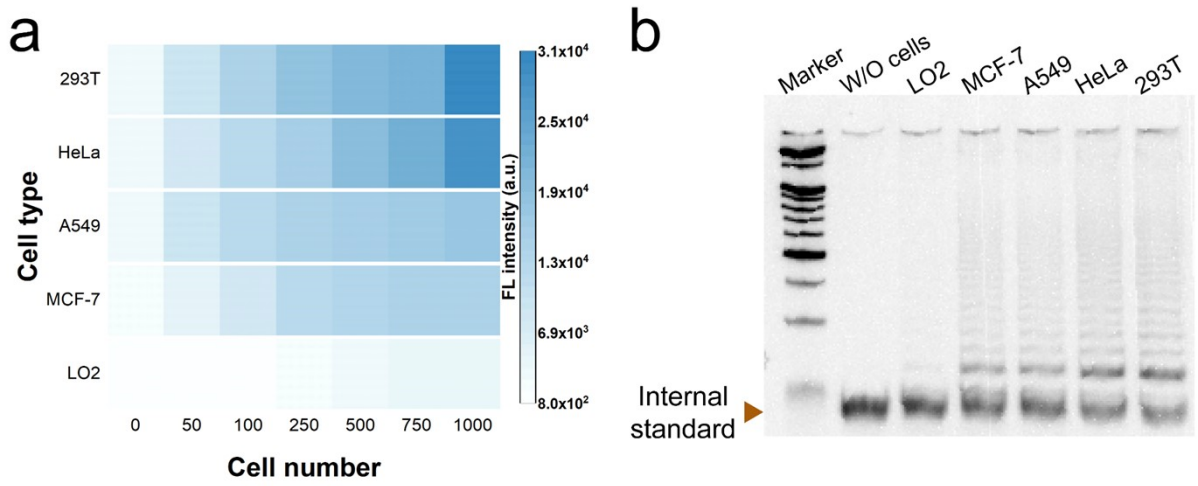

Fig. S9. (a) Heat map of fluorescence intensity of pcDNA-based Cas12a assay responds to telomerase from the lysates of different cell lines. (b) Analysis of telomerase extracts from different cell lines by TRAP assay. Cell number, 1000; LbCas12a, 100 nM; gRNA, 100 nM; $pcDNA_{Tel}$, 50 nM; FQ reporter, 1.0 μM; incubation time, 2 hours.

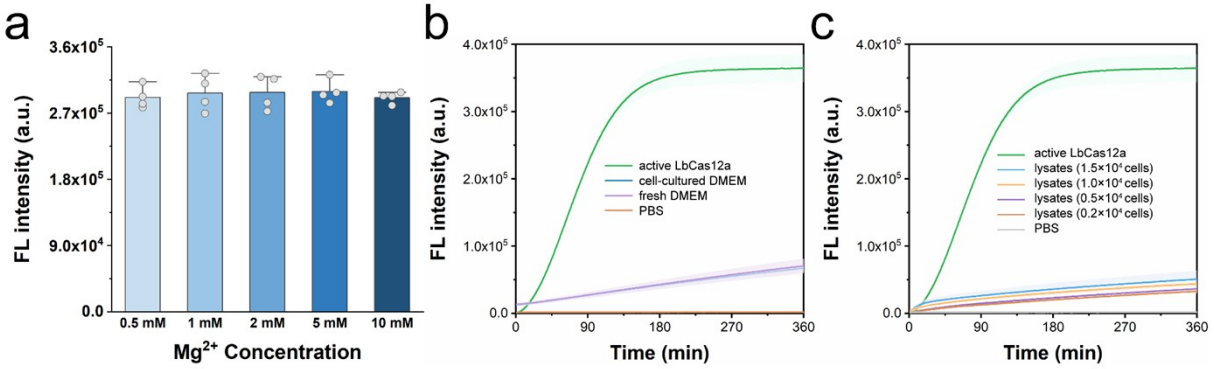

Fig. S10. Evaluation of the stability of FQ reporter in biological samples. (a) Analysis of the effects of Mg^{2+} concentration on *trans*-cleavage activity of LbCas12a. (b) The influence of cell-cultured and fresh DMEM medium on the stability of FQ reporter. (c) The influence of cell lysates from different numbers of cells on the stability of FQ reporter. LbCas12a, 100 nM; gRNA, 100 nM; wild-type DNA targets, 50 nM; FQ reporter, 1.0 μM. FL, fluorescence; a.u., arbitrary units.

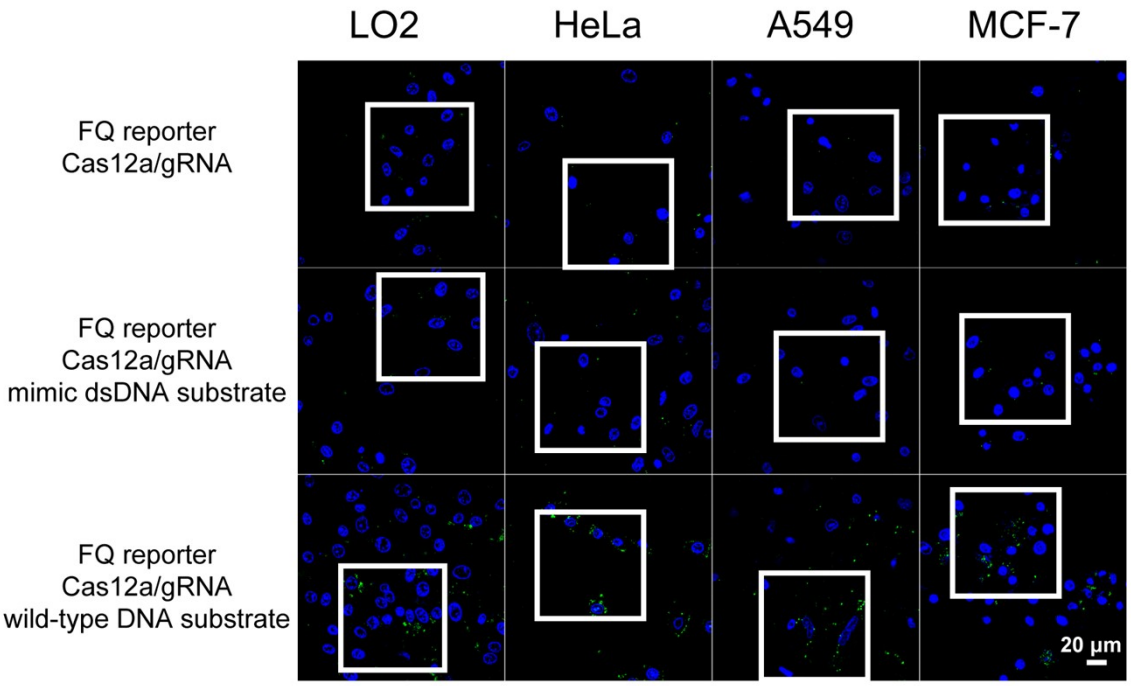

Fig. S11. CLSM images of *trans*-cleavage of FQ reporters by LbCas12a in different cell lines with indicated treatments. (i) LbCas12a/gRNA and FQ reporter; (ii) LbCas12a/gRNA, FQ reporter and random dsDNA; (iii) LbCas12a/gRNA, FQ reporter and wild-type DNA substrate. LbCas12a, 50 nM; gRNA, 50 nM; wild type DNA substrate, 100 nM; FQ reporter, 1.0 μM; Scale bar, 20 μm.

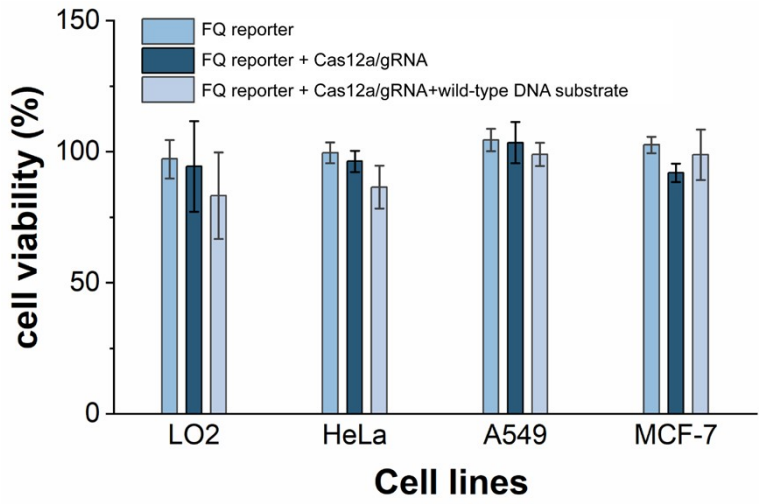

Fig. S12. Effect of the components of pcDNA-based Cas12a assay on cell viability. Cell viability of different cells by the standard Cell Counting Kit-8 (CCK-8) assay under the experimental conditions of the *trans*-cleavage activity of LbCas12a in the cytoplasm. LbCas12a, 50 nM; tran, 50 nM; wild-type DNA substrate, 100 nM; FQ reporter, 1.0 μM.

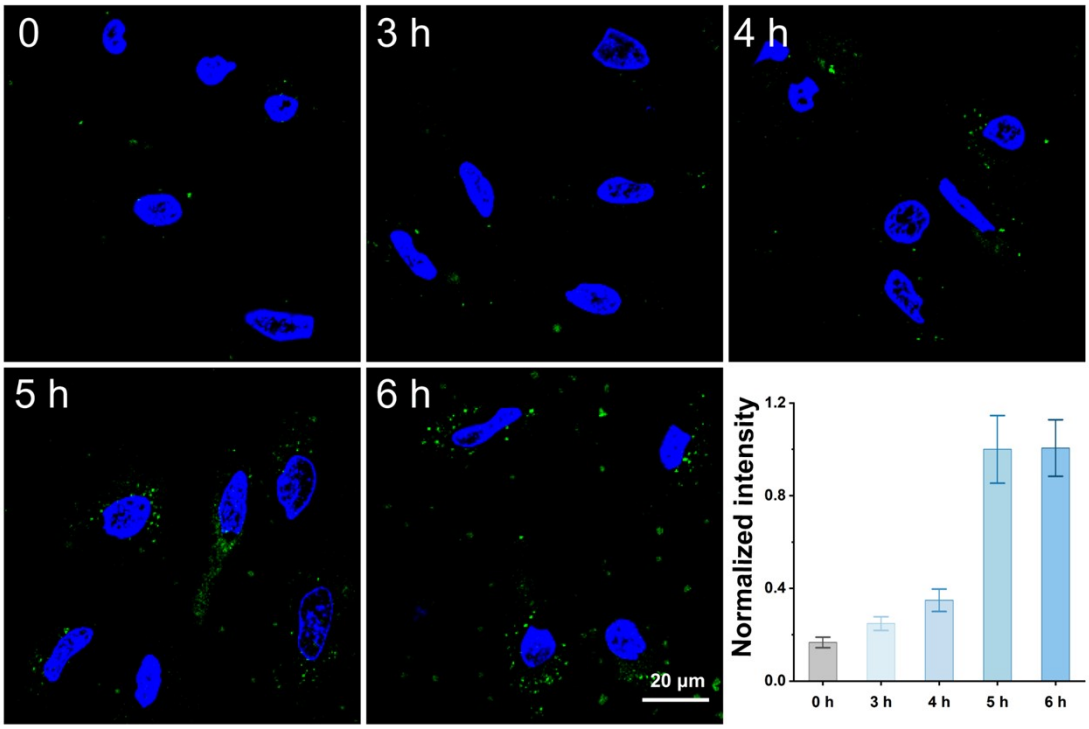

Fig. S13. Exploring the appropriate time of pcDNA-based Cas12a assay for in vivo telomerase sensing. CLSM images and corresponding semi-quantitative fluorescence intensities of pcDNA -based Cas12a assay in response to telomerase in living cell. LbCas12a, 50 nM; gRNA, 50 nM; pcDNA, 100 nM; FQ reporter, 1.0 μM. Scale bar, 20 μm. Data represent means ± SD (n = 50, 42, 53, 53, 43 cells, from left to right).

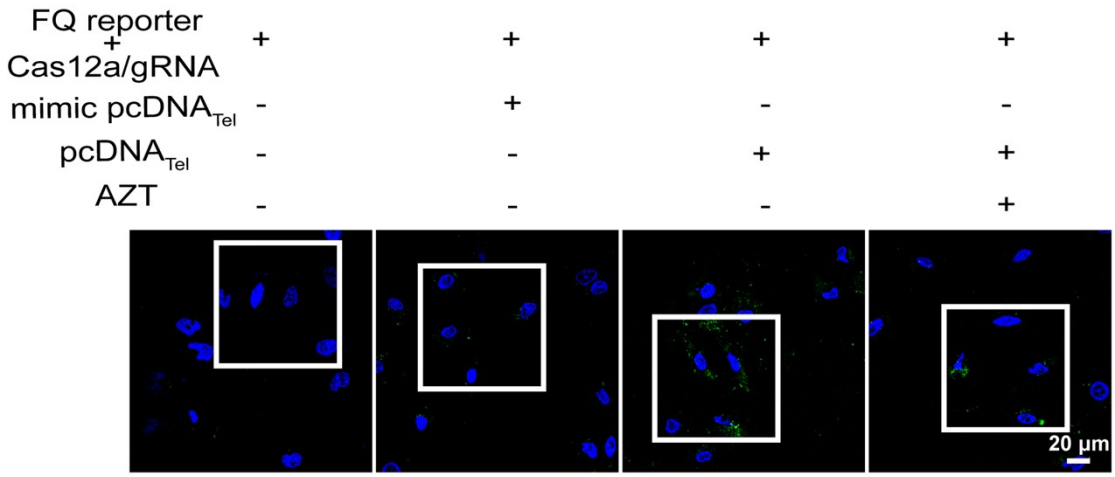

Fig. S14. The CLSM images of the Cas12a assay for *in situ* sensing of telomerase activity in HeLa cells. LbCas12a, 50 nM; gRNA, 50 nM; random dsDNA or pcDNA$_{Tel}$, 100 nM; FQ reporter, 1.0 μM; AZT, 200 μM. Scale bar, 20 μm.

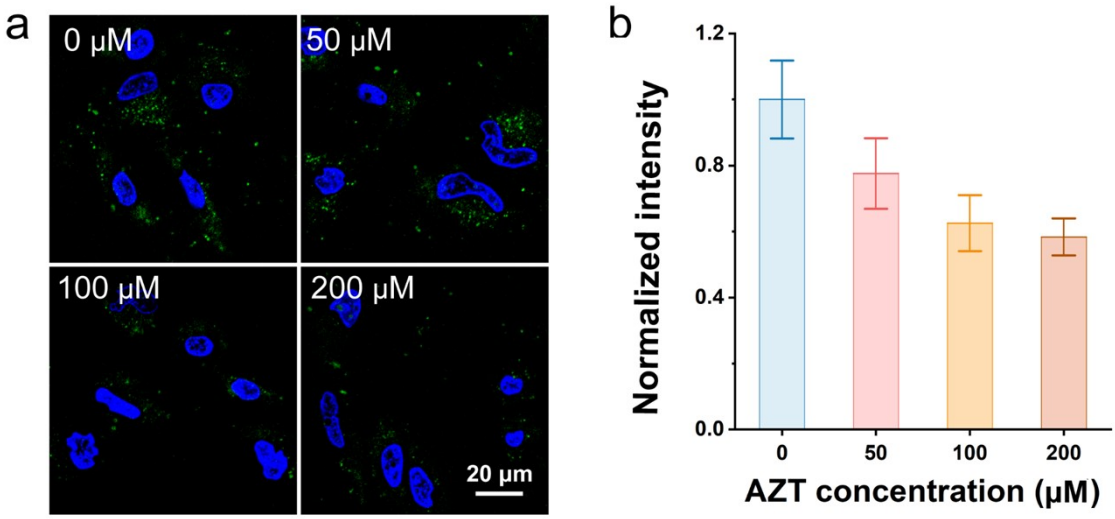

Fig. S15. pcDNA-based Cas12a assay for *in vivo* sensing of telomerase pretreated with AZT. (a) CLSM images and (b) corresponding normalized fluorescence of the Cas12a assay for the sensing of telomerase activity in HeLa cells pretreated with different concentrations of AZT. LbCas12a, 50 nM; gRNA, 50 nM; $pcDNA_{Tel}$, 100 nM; FQ reporter, 1.0 μM. Scale bar, 20 μm. Data represent means ± SD (n = 42, 39, 40, 53 cells, from left to right).

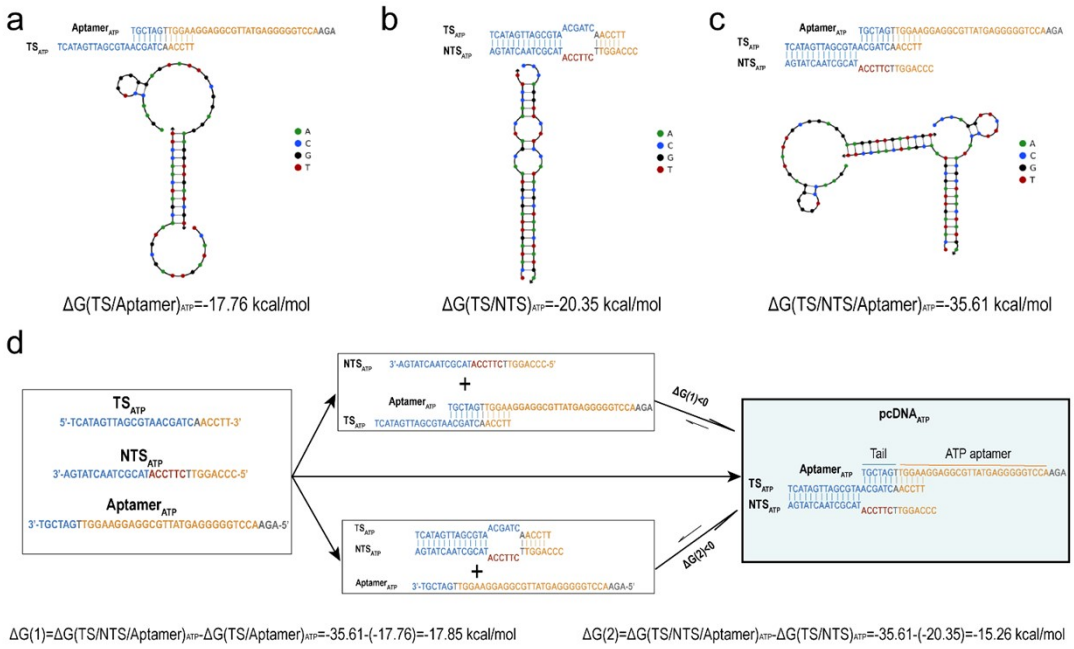

Fig. S16. The design of $pcDNA_{ATP}$. (a-c) Standard free energy of nucleic acid hybridization complexes predicted by NUPACK (http://www.nupack.org/) (1). (d) General design schematics of sequences and prediction of reaction direction based on ΔG calculation. ΔG is calculated at 37°C.

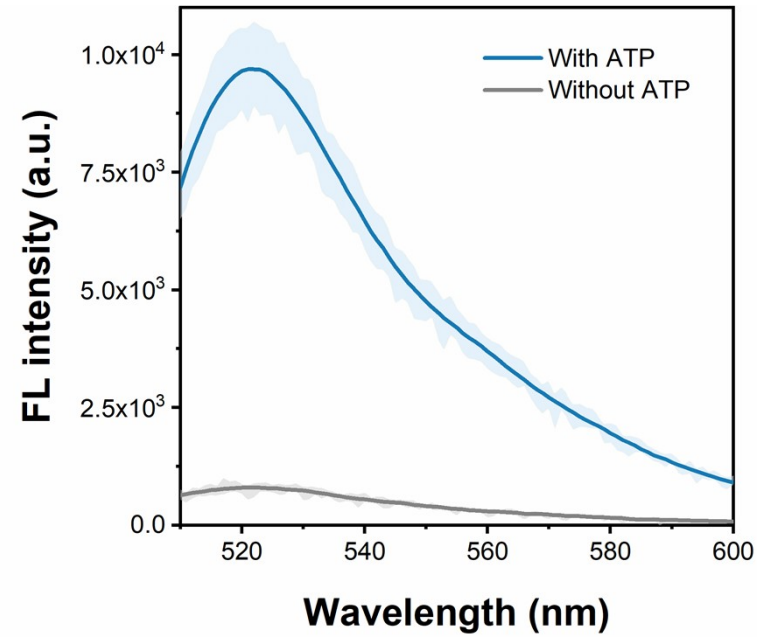

Fig. S17. Fluorescence emission spectra of $pcDNA_{ATP}$-based Cas12a assay in response to ATP. LbCas12a, 100 nM; gRNA, 100 nM; $pcDNA_{ATP}$, 500 nM; FQ reporter, 1.0 μM; ATP, 1.0 mM.

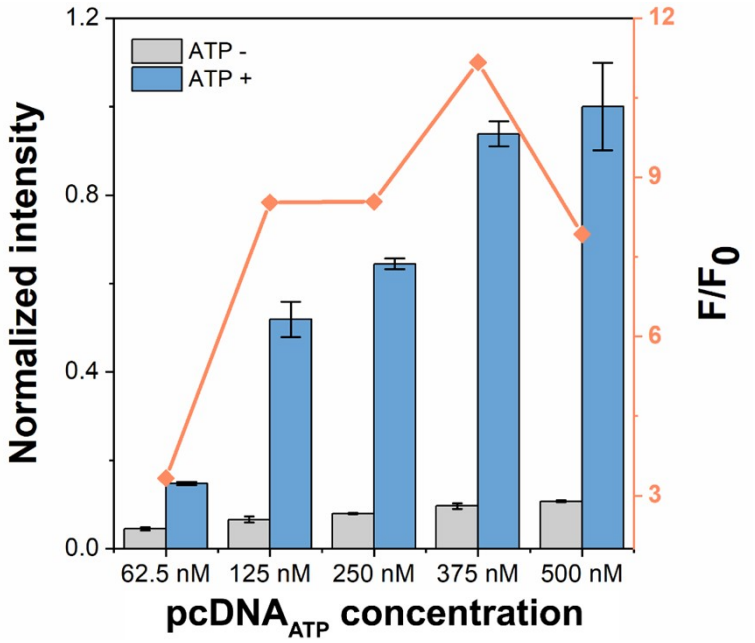

Fig. S18. Optimization of the $pcDNA_{ATP}$ concentration in $pcDNA_{ATP}$-based Cas12a assay. Effect of the concentrations of $pcDNA_{ATP}$ on the fluorescence response signals and background signals in the assay of ATP. LbCas12a, 100 nM; gRNA, 100 nM; FQ reporter, 1.0 μM; ATP, 1.0 mM.

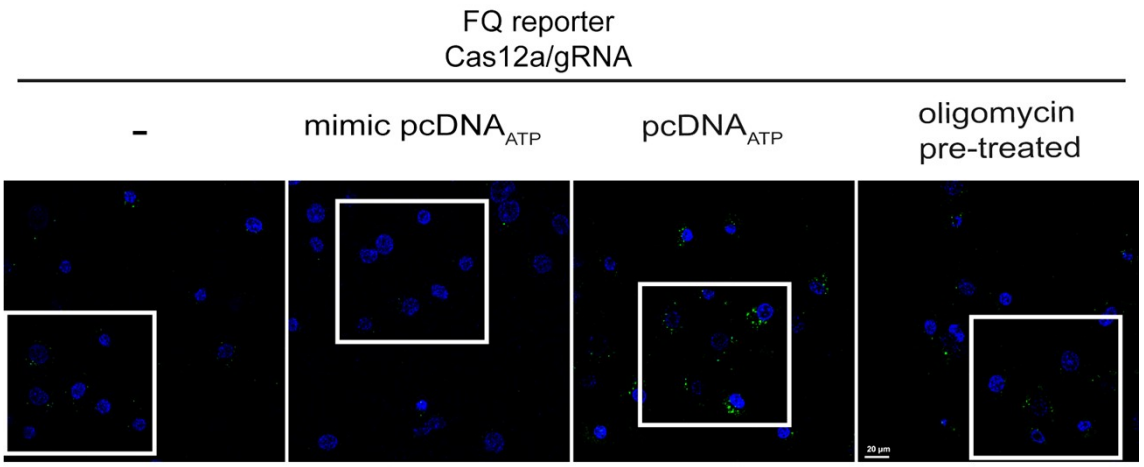

Fig. S19. CLSM images of the sensing of intracellular ATP in HeLa cells using the pcDNA-based Cas12a assay. LbCas12a, 50 nM; gRNA, 50 nM; mimic pcDNA$_{ATP}$ or pcDNA$_{ATP}$, 100 nM; FQ reporter, 1.0 μM. Scale bar, 20 μm.

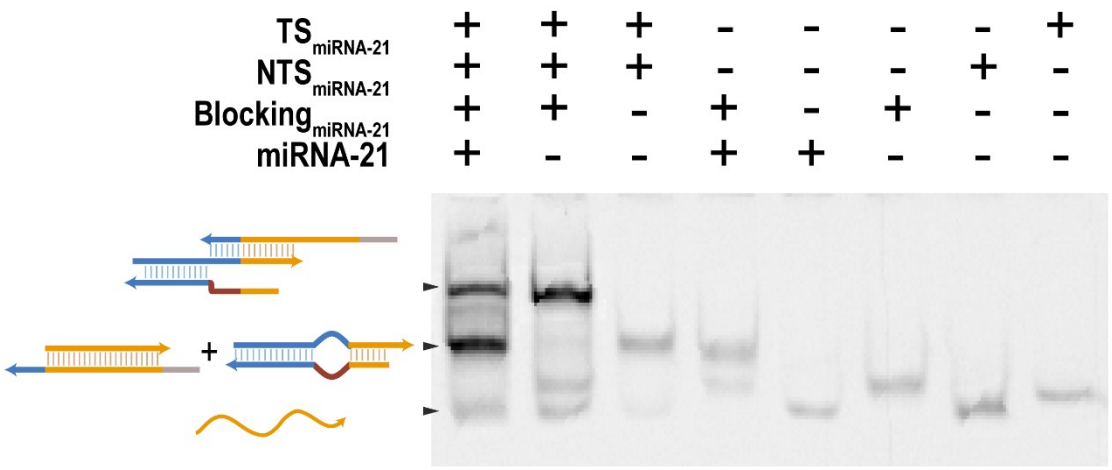

Fig. S20. Verification of the responsiveness of pcDNA$_{miRNA-21}$ by native PAGE. Loading volume, 10 μL; the concentration of ssDNA strands or hybridized strands, 300 nM; ATP, 1.0 mM.

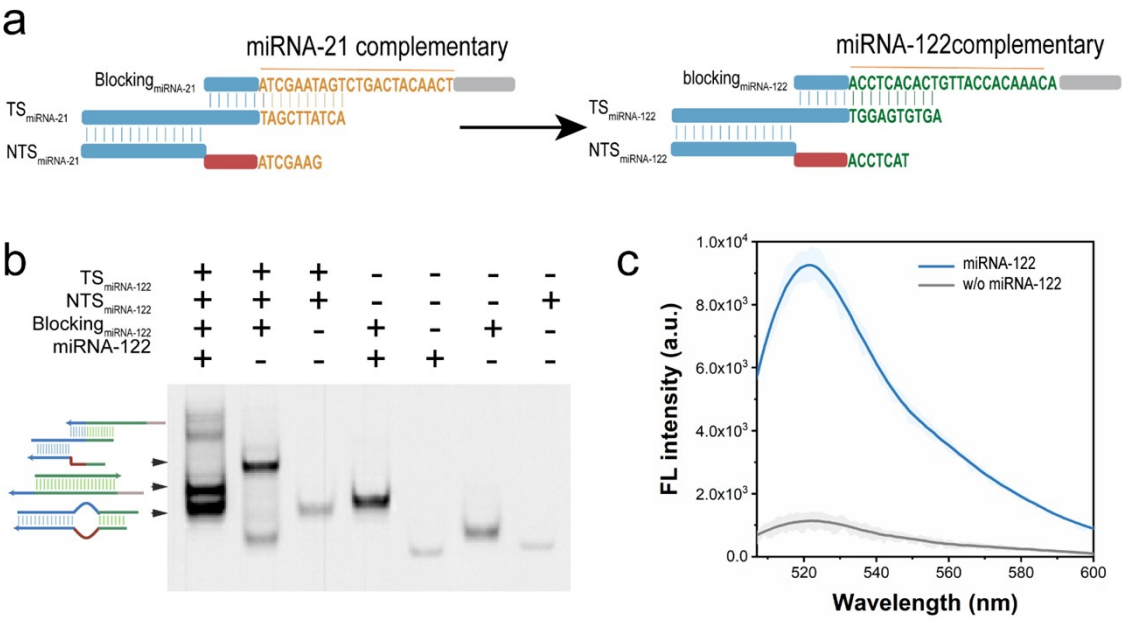

Fig. S21. pcDNA-based Cas12a assay for miRNA-122 sensing. (a) Schematic diagram of the design of $pcDNA_{miRNA\text{-}122}$. (b) Analysis of the responsiveness of $pcDNA_{miRNA\text{-}122}$ by native PAGE. Loading volume, 10 μL; the concentration of ssDNA strands or hybridized strands, 300 nM; miRNA-122 (DNA), 1 μM. (c) Fluorescence emission spectra of pcDNA-based Cas12a assay in response to miRNA-122. LbCas12a, 100 nM; gRNA, 100 nM; FQ reporter, 1.0 μM; $pcDNA_{miRNA\text{-}122}$, 100 nM; miRNA-122 (DNA mimic), 500 nM. The possible reasons for the discrepancy between Fig. S21b and Fig. S20 are as follows: (1). There are two distinct bands of reaction products in lane 1 of Fig. S21b, but two overlapping bands in Fig. S20. Compared with the $blocking_{miRNA\text{-}21}$ sequence in Fig. S20, the $blocking_{miRNA\text{-}122}$ sequence in Fig. S21 contains one more oligonucleotide; thus, the migration rate of $blocking_{miRNA\text{-}122}$/miRNA-122 duplex is slower than that of $blocking_{miRNA\text{-}21}$/miRNA-21 duplex. At the same time, the sequences of $TS_{miRNA\text{-}122}$/$NTS_{miRNA\text{-}122}$ and $TS_{miRNA\text{-}21}$/$NTS_{miRNA\text{-}21}$ have almost no difference in length. Therefore, there is some discrepancy between the bands of lane 1 in Fig. S21b and S20. (2). Two bands with slow migration rates at the top of lane 1 in Fig. S21b might be attributed to the byproducts from the intermolecular crosslinks between the reaction products.

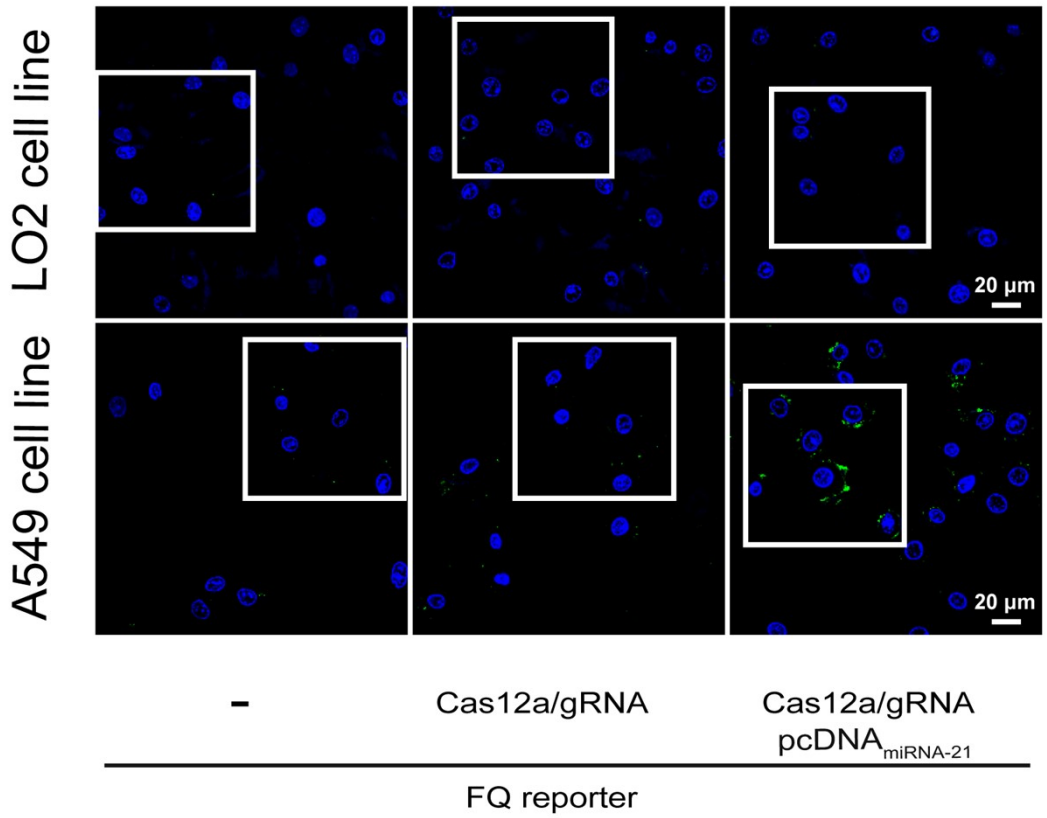

Fig. S22. CLSM images of the sensing of endogenous miRNA-21 in living cells using the pcDNA-based Cas12a assay. LbCas12a, 50 nM; gRNA, 50 nM; $pcDNA_{miRNA\text{-}21}$, 100 nM; FQ reporter, 1.0 μM. Scale bar, 20 μm.

Reference

1. R. M. Dirks, J. S. Bois, J. M. Schaeffer, E. Winfree and N. A. Pierce, *SIAM Rev.,* 2007, **49,** 65-88.