Electronic Supplementary Information (ESI)

A DNA-Tetrahedron-Based Molecular Computation Device for Logic Sensing of Dual MicroRNAs in Living Cells

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Experimental Section

Chemicals and Materials.

All DNA and RNA strands were synthesized and HPLC purified by Sangon Biotechnology Co., Ltd. (Shanghai, China), and the sequences are listed in Table S1.

All aqueous solutions were prepared using DEPC-treated ultrapure water (⩾18 MΩ, Milli-Q, Millipore). Lipofectamine 3000 was obtained from Invitrogen (Thermo Fisher Scientific, USA). 6× loading buffer and SYBR Gold were purchased from TaKaRa Biotechnology Co., Ltd (Dalian, China). All the cells we used were obtained from the Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cell medium DMEM basic (1×) was obtained from GIBCO (USA).

Apparatus.

All buffer pH measurements were performed with an Orion 3 Star pH meter (Thermo Scientific, USA). The gel electrophoresis was imaged on an Azure c600 (Azure Biosystems, USA). Centrifugation was performed with a Beckman Coulter Allegra 25R centrifuge (Brea, CA, USA). All fluorescence measurements were carried out on a F7000 (Hitachi, Japan) spectrofluorometer with a temperature controller. All cells were incubated by using a Thermo FORMA-3111 CO2 incubator (ThermoFisher, USA). The hydrodynamic size was measured by the Zetasizer Nano ZS (Malvern). Confocal laser scanning microscopy (CLSM) studies were performed using an Olympus IX-70 inverted microscope with an Olympus FluoView 500 confocal scanning system. The flow cytometry analysis was performed using a Gallios machine (Beckman Coulter, USA). MTT assay were performed on a RT 6000 microplate reader.

Preparation and Characterization of the DT-Based Device.

The DT-based device consists of the DT scaffold and three functional toes. The DT scaffold were self-assembled according to previous reports[26,27] with slight modifications by annealing, as well as the double helix functional toes, including A/ B/ C, C21/B* and C122/ C*. In brief, to assemble the DT scaffold, four customized oligonucleotide strands (P1, P2, P3, and P4) were mixed in equal molar ratio in TM buffer (20 mM Tris, 50 mM $MgCl_2$, pH = 8.0). The stock solutions, which had final concentrations of 2 μM, were heated to 95°C for 5 min and then cooled on ice in 1 min, and finally stored at 4 °C for at least 4 h. Similarly, for the synthesis of A/B/C-DT, seven customized single-stranded oligonucleotide strands (P1, P2, P3, P4, A, B, and C) were mixed in equal molar ratio in TM buffer (20 mM Tris, 50 mM $MgCl_2$, pH = 8.0). The stock solutions, which had final concentrations of 2 μM, were heated to 95°C for 5 min and then cooled on ice in 1 min, and finally stored at 4 °C for at least 4 h. Then we mixed the A/B/C-DT with C21/B* and C122/C*, which these two different recognition toes could be selectively loaded on the bottom face by strand hybridization to finally form the DT-based device. All these sequences can be seen in **Table S1**. Successful formation of the DT scaffold and DT-based device was verified by use of 6% native-PAGE and DLS. The strand displacement process was verified by use of 12% native-PAGE. For native-PAGE, the 10 μL DNA solution was mixed with 2 μL 6×loading buffer and 2 μL SYBR Gold, and then run on a 6% (or 12%) native polyacrylamide gel. The electrophoresis was conducted in 1× TBE buffer at a constant voltage of 100 V for 2 h. The DLS characterization of the DT scaffold and DT-based device was performed using diluted sample.

Stability Assay of the DT-Based Device.

To evaluate the nuclease resistance ability of the DT-based device, 10% FBS, which was significantly higher than what could exist in a cellular milieu, was used to digest the DT-based device. The simple dsDNAs were used as the control sample. The samples were incubated with 10% FBS for different times (0, 1, 2, 3, 4, and 6 h). Then all the samples were collected to perform gel electrophoresis analysis. Native polyacrylamide gel analysis was conducted using a 6% (or 12%) native polyacrylamide gel in 1 × TBE (Tris borate EDTA) buffer, respectively. After the samples were added, the gels were run at a constant voltage of 100 V for 2 h.

In Vitro Fluorescence Experiments.

All fluorescence measurements were performed using a F7000 (Hitachi, Japan) spectrofluorometer with a temperature controller. Fluorescence studies proved the signaling property of the DT-based device. Each single free strand (B*, C*, miR-21, miR-122) was respectively added to 100 nM DT-based device in TM buffer, and the mixture was incubated at 37 °C for 3 hours. The fluorescence spectra of the mixture were collected from 500 to 650 nm with 488 nm excitation in a 200 μL quartz cuvette. All experiments were repeated at least three times. For the sensitivity test, different concentrations of target miR-21 was respectively added to 100 nM DT-based device in TM buffer, add an excess of another target. The mixture was incubated at 37 °C for 3 hours. Then, do the same for miR-122. The measuring processing was the same as above. For the selectivity test, the completely correct target inputs (miR-21, miR-122),

single-base-mismatched miR-21 or miR-122 inputs (SM-21, miR-122), (miR-21, SM-122), let 7 family miRNAs (let 7a and let 7b) and no target inputs (0, 0) were added into the DT-based device with a final concentration of 100 nM reacted 3 h at 37 °C. The measuring processing was the same as above.

Cell Culture and Transfection.

The Huh7, HeLa, HEK293T cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37 °C in a humidified 5% CO_2-containing atmosphere. The cells were grown to 80% confluence for 24 h before transfection with probes. The cell density was determined using a hemocytometer. Transfection assays were performed according to the manufacturer's protocol. Briefly, when using 35 mm glass dishes or 6-well plates, transfection was carried out using 3 μL lipofectamine 3000 and certain concentrations of probes in 300 μL of Opti-Mem, then diluted to 1000 μL with DMEM and incubated at 37 °C for 4 h.

Confocal Fluorescence Imaging.

Various cells were seeded into a confocal dish for 24 h at 37 °C and then incubated with 200 mL culture medium containing 100 nM DT-based device for for 6 h or various times (for time optimization) at 37 °C. After washing three times with PBS, the fluorescence of cells was visualized from 505 to 525 nm with the excitation wavelength of 488 nm for FAM. All cells were observed under an Olympus IX-70 inverted microscope with an Olympus FluoView 500 confocal scanning system. The fluorescence images were presented after processing using Image Pro plus 6.0 software and ImageJ version 1.38× software. For the CLSM experiments after treatment with synthetic miRNA or antisense inhibitor oligodeoxyribonucleotides, the cells were first transfected and incubated with miR-21 mimics (200 nM), miR-122 mimics (200 nM), anti-miR-21(200 nM) or anti-miR-122(200 nM) for 2 h. One group of cells without treatment served as the control. Other steps were performed in the same way as described above.

Flow Cytometry Assay.

Huh-7 cells and the other two negative cells were grown to 80% in 6-well plates under the above-described conditions. The cells were washed with PBS, then transfected and incubated in fresh medium with extra adding 100 nM synthetic miRNA for artificially changing the intracellular expression level of miR-21 and miR-122 experiments. After 3 h incubation, the cells were washed with PBS, and the cells were cultured with the DT-based device (100 nM). After 6 h incubation, the cells were washed with PBS (pH=7.4) three times and detached from the culture plate by trypsin–EDTA solution. Next, the suspended cell solution was centrifuged at 2000 rpm for 4 min and washed with PBS three times. Finally, the cells were resuspended in PBS for flow cytometric analysis on a Beckman Coulter Gallios machine at 488 nm excitation.

Cell Co-Localization Assays.

The Huh7 cells were seeded in a 35 mm confocal dish and incubated for 24 h. After washing three times with PBS, the cells were incubated with the 100 nM DT-based device in a 1 mL volume of culture medium containing 10% FBS for 6 h. After

additional washing with PBS, the cells were treated with 5 μg mL^{-1} Hoechst-33342 for 10 min. After incubation, the cells were washed twice with 1 mL of D-PBS and subjected to confocal microscope imaging. Excitation of Hoechst was carried out at λ= 405 nm and emissions were collected in the blue channel. The excitation of FAM was carried out at λ=488 nm and the emissions of FAM were recorded in green channel.

Cytotoxicity Assay.

To investigate the cytotoxicity, a standard MTT assay was operated. Huh-7 cells were seeded in 96-well plates at a density of 1×10^6 cells per well and a total volume of 200 μL per well. Plates were then maintained at 37 °C in 5% CO_2 atmosphere for 24 h. Afterward, the cells were treated with three concentrations of DT-based device (0, 50, 100 nM) for 6, 12, 18, and 24 h, respectively, and 100 μL MTT solutions were then added to each well for 4 h. After removing the remaining MTT solution, 150 μL DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 490 nm with a RT 6000 microplate reader.

qRT-PCR.

Total cellular RNAs were extracted from Huh-7 cells, Hela cells, and HEK293T cells respectively, using Trizol reagent (Sangon Co. Ltd., Shanghai, China) according to the manufacturer's instructions. Real-time PCR reverse transcription was carried out with SG Fast qPCR Master Mix (2X) (BBI) on a Stepone plus qRT-PCR system (ABI). U6 was used as an internal control for miRNA detection. The relative expression level can be estimated by the values of $2^{-\Delta\Delta Ct}$. The primers used in this experiment were described in Table S1. All reactions were performed in triplicate.

Supporting Tables:

Oligo	Sequences (5'-3')
P1	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGA GAAGAGCCGCCATAGTA
P2	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATG CGAGGGTCCAATACAAAGTTTTTTTTTTTTTTT TTTT
P3	GGTGGTGGTGGTTGTGGTGTTTTTTCAGACTTAGGAATGTG CTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
P4	TATCTCATCTCATCTATCATCTATCAACTGCCTGGTGATAAA ACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
B	TGGAGTTAAAGATCAGGGAACACATAAACATCAACA
A	FAM-GTTAGATGTTAGTTTCACGAAGACAATGAT
C	TAGATGATAGATGAGATGAGATATGTTTATGTGTTCCCTG ATCTTTAACTCCAATCATTGTCTTCGTGAAACTAACATCT AAC-BHQ1
C21	AAAACACCACAACCACCACCACCACATCAACATCAGTCT GATAAGCTA
B*	ATCAGACTGATGTTGATGTTTATGTGTTCCCTGATCTTTAA CTCCA
C122	CAAACACCATTGTCACACTCCAATCAAAAAAAAAAAAAA AAAAA ACTTT
C*	GTTAGATGTTAGTTTCACGAAGACAATGATTGGAGTGT GACAATGG
Anti-miR-21	U*C*A*ACAUCAGUCUGAUAAGC*U*A*
miR-21	U*A*G*CUUAUCAGACUGAUGUU*G*A*
Anti-miR-122	C*A*A*ACACCAUUGUCACACUC*C*A*
miR-122	U*G*G*AGUGUGACAAUGGUGUU*U*G*
SM-21	UAACUUAUCAGACUGAUGUUGA
SM-122	UGGAGUGUGAGAAUGGUGUUUG
Let-7a	UGAGGUAGUAGGUUGUAUAGUU
Let-7b	UGAGGUAGUAGGUUGUGUGGUU
miR-21 forward primer	ACACTCCAGCTGGGTAGCTTATCAGACTG
miR-21 reverse primer	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGT
miR-122 forward primer	GCCGTGGAGTGTGACAATGGT
miR-122 reverse primer	GTGCAGGGTCCGAGGT
U6 forward	CTCGCTTCGGCAGCACA
U6 reverse	AACGCTTCACGAATTTGCGT

DNA Tetrahedron were self-assembled by P1, P2, P3 and P4.

The * represents phosphorothioate modification.

Table S1. Oligonucleotide sequences used in this work.

Supporting Figures:

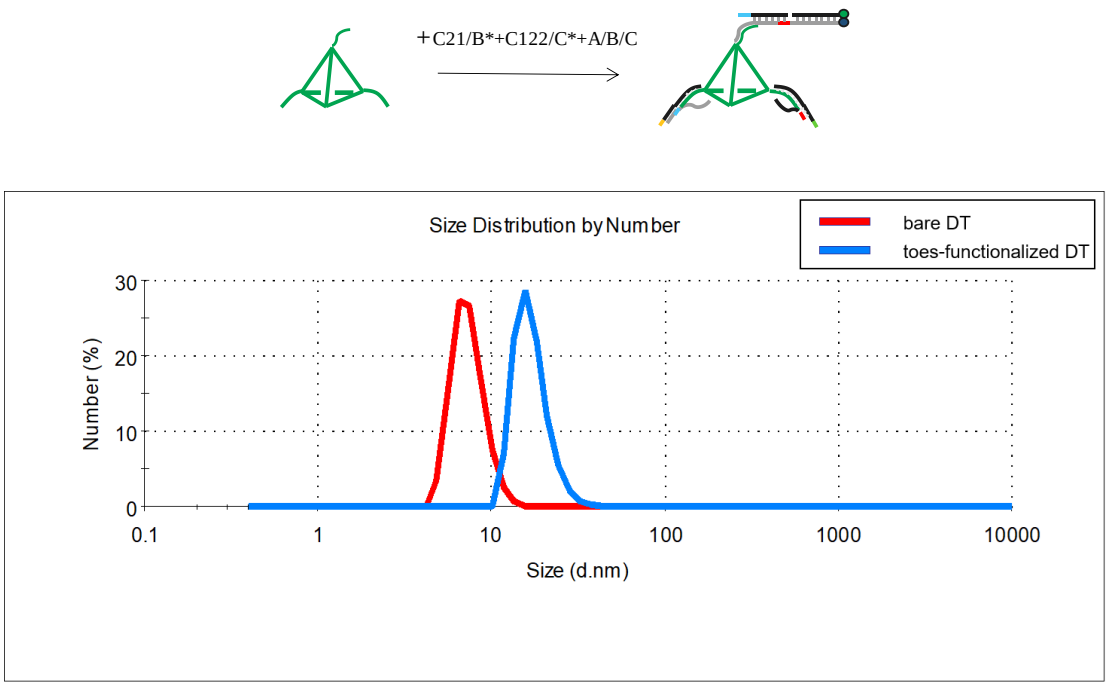


Figure S1. DLS analysis of the size between the bare DT and the toes-functionalized DT.

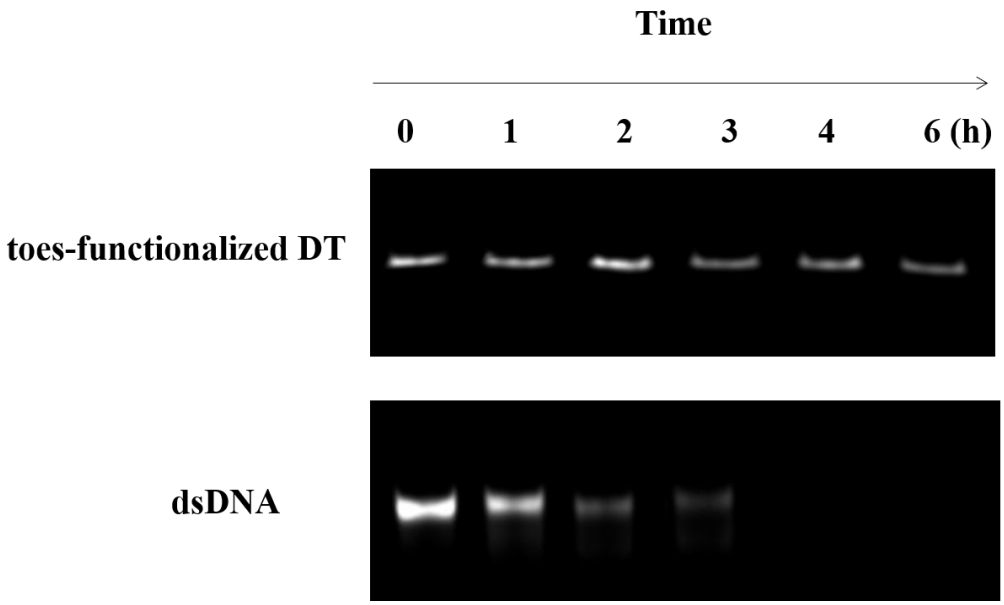


Figure S2. Native PAGE analysis of degradation between the toes-functionalized DT and dsDNA in 10% FBS.

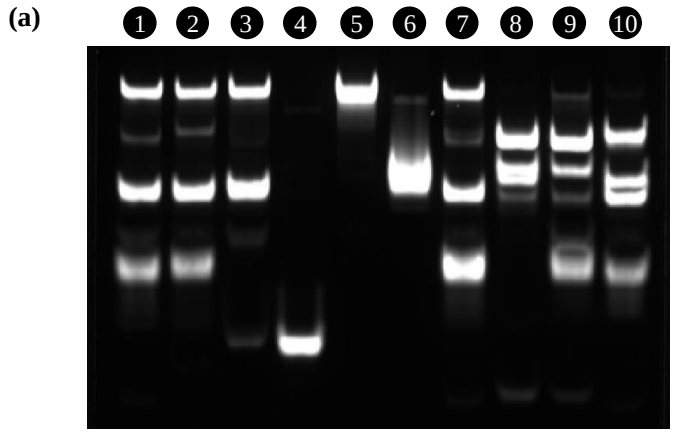
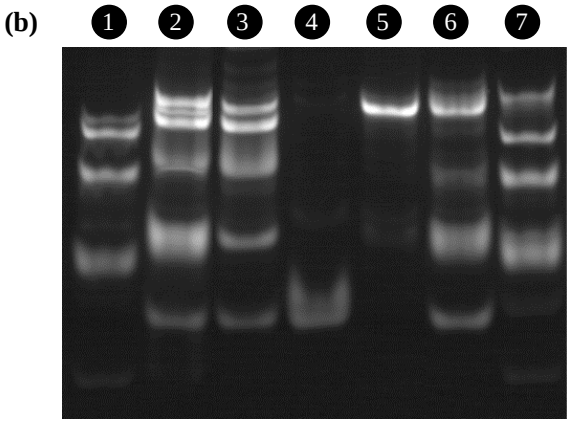


Figure S3. The strand displacement processes.

(a) The strand displacement processes were verified by use of 12% native-PAGE.
Lane 1: miRNA21+C21/B*+C*+A/B/C.
Lane 2: B*+miRNA122+C122/C*+A/B/C.
Lane 3: B*+C*+ A/B/C.
Lane 4: A.
Lane 5: C*/C.
Lane 6: B*/B.
Lane 7: miRNA21+C21/B*+miRNA122+C122/C*+A/B/C.
Lane 8: C21/B* +C122/C*+A/B/C.
Lane 9: C21/B*+miRNA122+C122/C*+A/B/C.
Lane 10: C21/B*+miRNA21+C122/C*+A/B/C.



(b) The strand displacement processes were verified by use of 15% native-PAGE.
Lane 1: miRNA21+C21/B*+A/B/C.
Lane 2: miRNA21+C21/B*+C*+A/B/C.
Lane 3: B*+miRNA122+C122/C*+A/B/C.
Lane 4: A.
Lane 5: C*/C.
Lane 6: miRNA122+C122/C*+A/C.
Lane 7: miRNA21+C21/B*+miRNA122+C122/C*+A/B/C.

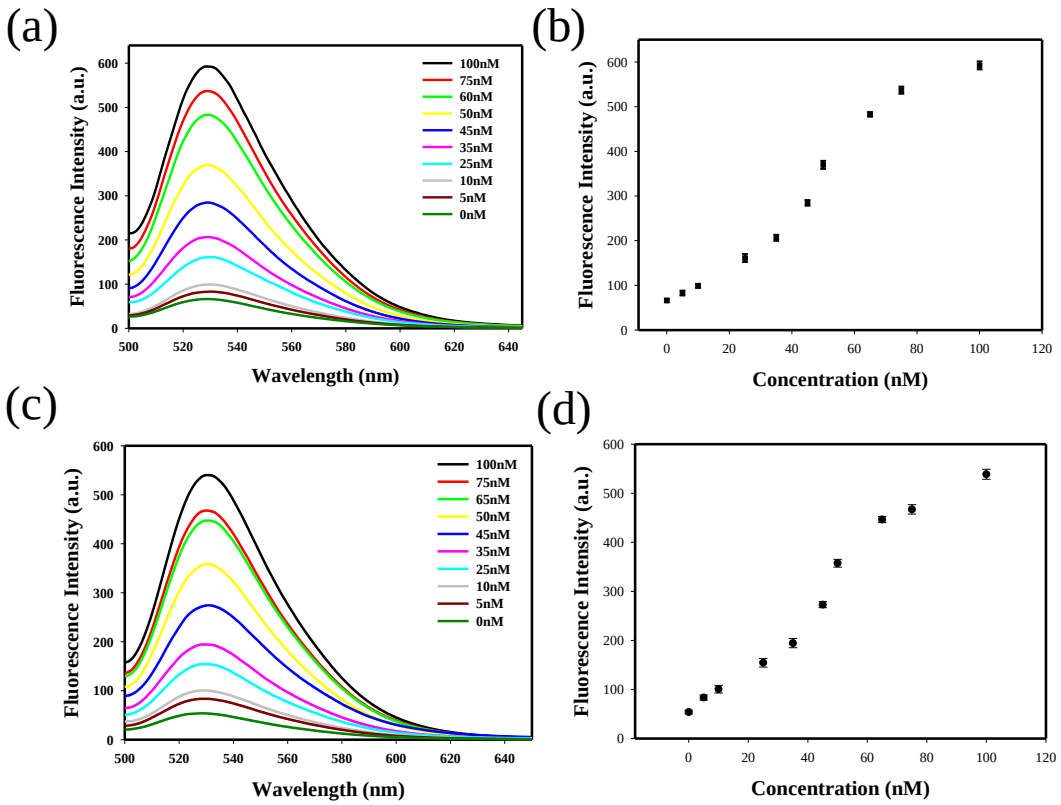


Figure S4. Response of the DT-based device to different concentrations of target miR-21 (a and b) and miRNA-122 (c and d). The detection limits (LOD) of the DT-based device were calculated to be 2.4 nM for miRNA-21 and 2.8 nM for miRNA-122 at 3σ, respectively. The data error bars indicate mean ± SD (n = 3).

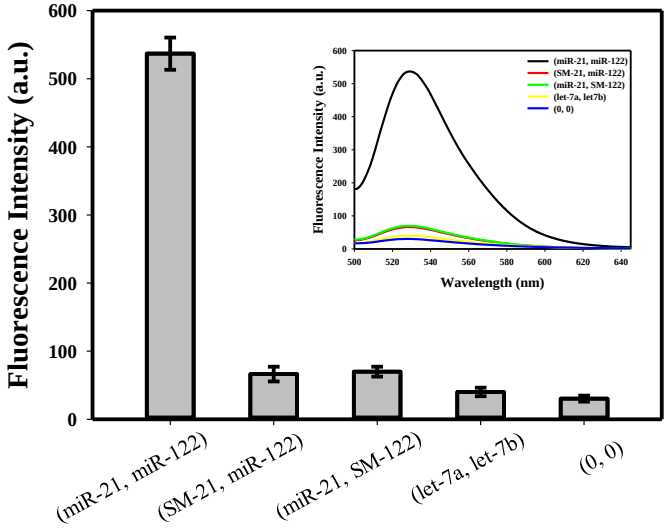


Figure S5. Specificity of the DT-based device for several miRNAs inputs. The specificity of the DT-based device was evaluated by detecting five different miRNA sequences, including completely correct target inputs (miR-21, miR-122), single-base-mismatched miR-21 or miR-122 inputs (SM-21, miR-122), (miR-21, SM-122), let 7 family miRNAs (let 7a and let 7b) and no target inputs(0, 0). Error bars were estimated from three replicate measurements.

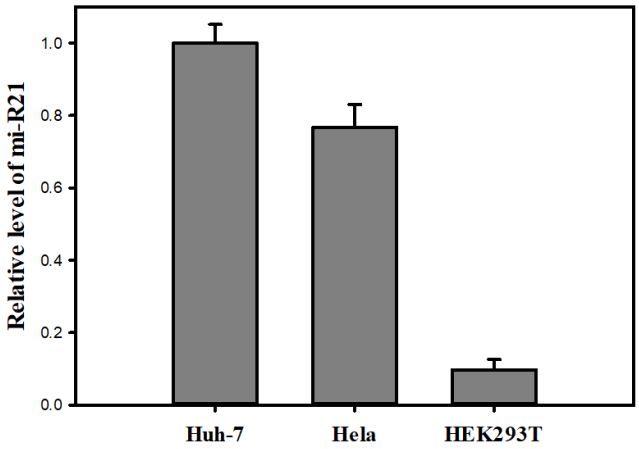


Figure S6. RT-qPCR analysis of miR-21. RT-qPCR analysis of relative expression levels of miR-21 in Huh-7 cells, Hela cells and HEK293T cells, respectively.

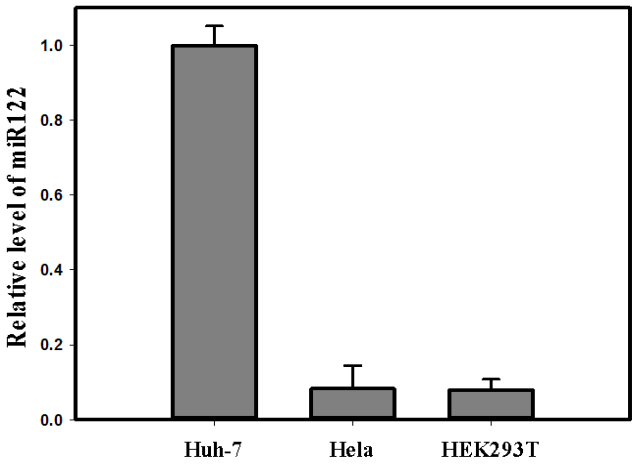


Figure S7. RT-qPCR analysis of miR-122. RT-qPCR analysis of relative expression levels of miR-122 in Huh-7 cells, Hela cells and HEK293T cells, respectively.

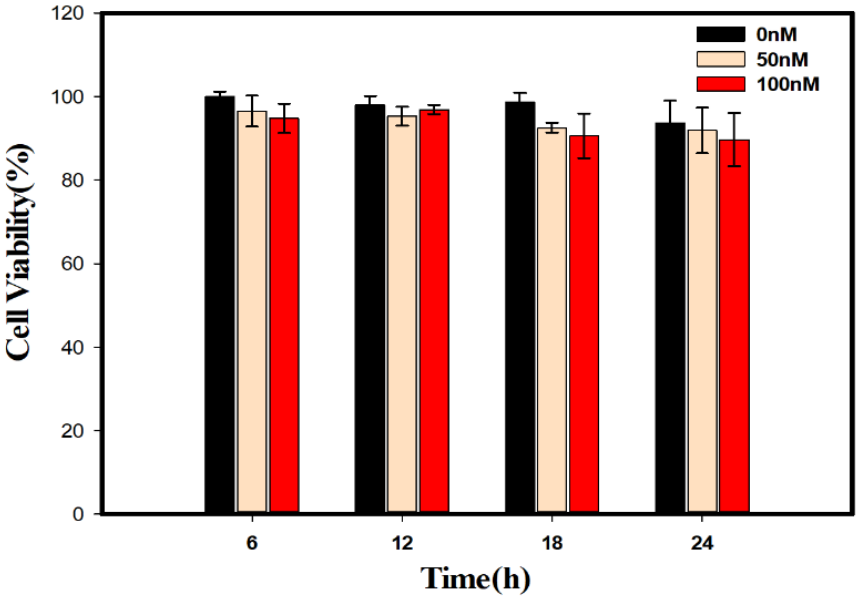


Figure S8. Cell viability assay (MTT). Huh 7 cells were incubated with different concentrations of the DT-based logic device for 6 h,12 h,18 h and 24 h.

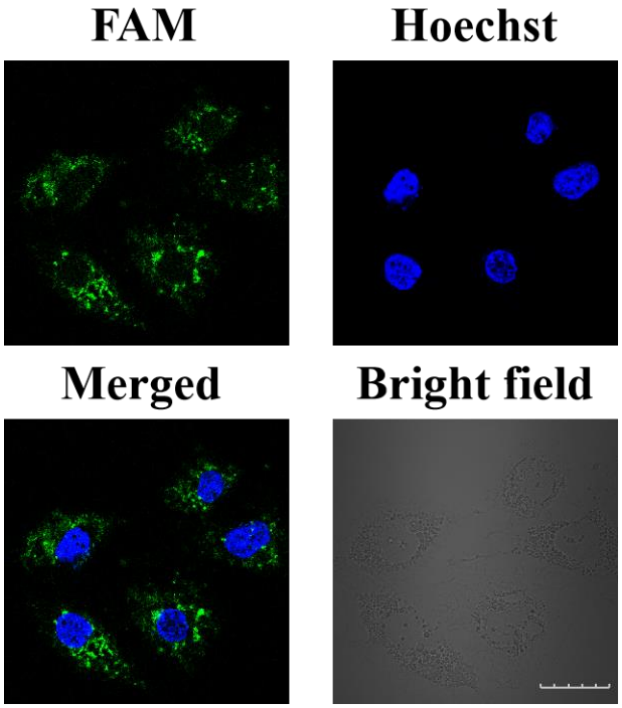


Figure S9. Fluorescence colocalization analysis. Co-localization analysis of the DT-based logic device by confocal microscopy. The huh7 cell nucleus was counterstained with Hoechst 33342 (blue). Scale bars were 20 μm.

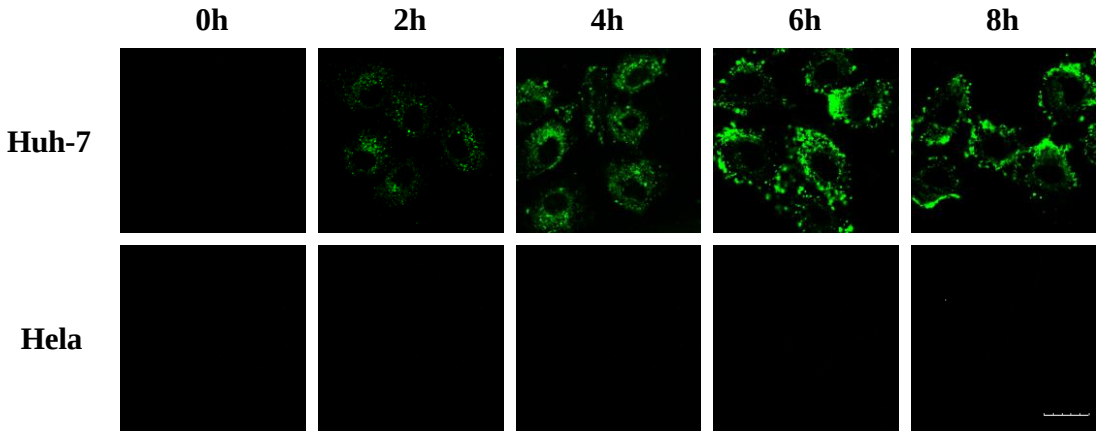


Figure S10. Real-time fluorescence imaging of the DT-based device in Huh-7 cells and Hela cells. The excitation wavelength was 488 nm, and the images were collected in the range of 505−525 nm. Scale bar is 20 μm.