Electronic Supplementary Information (ESI)

A DNAzyme Cascade for Amplified Detection of Intracellular miRNA

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Experimental Section

Chemicals and Materials.

SYBR Gold was obtained from Invitrogen (USA). Lipofectamine 3000 and Opti-MEM were purchased from Invitrogen (Thermo Fisher Scientific, USA). Cell culture media DMEM was gained from Thermo Scientific HyClone (USA). All cell lines were obtained from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All the chemicals were of analytical grade and used without further treatment. All aqueous solutions were prepared using DEPC-treated ultrapure water (≥18 MΩ, Milli-Q, Millipore). All oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and purified by high-performance liquid chromatography (HPLC). The sequences of the oligonucleotides are listed in **Table S1**.

Apparatus.

All the fluorescence measurements were carried out at 37 ^{o}C in a Hitachi F-7000 fluorescence spectrometer (Japan). All cells were incubated by using a Thermo FORMA 3111 CO_2 incubator (Thermo Fisher, USA). All buffer pH measurements were performed with Orion 3 Star pH meter (Thermo Scientific, USA). Confocal laser scanning microscopy (CLSM) studies were acquired using an Olympus IX-70 inverted microscope with an Olympus FluoView 500 confocal scanning system. The flow cytometry analysis of cells was performed on a Gallios machine (Beckman Coulter, USA).

Gel Electrophoresis Experiments.

Gel electrophoresis analysis was performed using native polyacrylamide gel electrophoresis (PAGE) in 1×TBE buffer. S1 and S2 were separately added to the 20 mM Tris-HCl buffer (137 mM NaCl, 10 mM Mg^{2+}, pH 7.4) heated to 95 ^{o}C, maintained 5 min, then cooled to room temperature slowly to form hairpin structure. For the upstream DNAzyme circuit, 1 μM S1 and 0.4 μM miR-141 were added to the Tris-HCl buffer and incubated for 1 h at 37 ^{o}C. For the downstream DNAzyme circuit, 1 μM S2 and 0.4 μM initiator were mixed with 1 μM S3 incubated for 1 h. For the DNAzyme cascade reaction, 1 μM S1, 1 μM S2 and 0.4 μM miR-141 were mixed

with 1 μM S3 and incubated for 1 h. The electrophoresis was performed at a constant potential of 80 V for 2 h after loading 10 μL of each sample into the lanes.

Fluorescence Experiments.

Fluorescence experiments were carried out with excitation wavelength at 488 nm and emission between 510 nm and 650 nm. The slit width was set to be 5 nm for the excitation and 5 nm for the emission. For the downstream initiator detection, the 100 nM S2 and 120 nM S3 were incubated with increasing concentrations of initiator (10 pM, 100 pM, 200 pM, 500 pM, 800 pM, 1 nM, 10 nM, 50 nM, 100 nM). In kinetic study of downstream DNAzyme circuit, 100 pM, 1 nM, 10 nM, 100 nM initiators were employed, the data was recorded every 10 min. When miR-141 used as input, DNA probes (100 nM S1, 100 nM S2 and 120 nM S3) were incubated with different concentration miR-141. After reaction for 1 h at 37 ^{o}C, and the fluorescence spectra was taken. For kinetic assay, fluorescence intensities were tested after incubating with miR-141 (100 pM, 500 pM, 1 nM) for 100 min. For selectivity test, a certain concentration of miR-141, miR-21, miR-429 and let-7d stock solution were added into the probes with a final concentration of 50 nM. The measuring processing was as the same as above.

Stability Assay.

To prove nuclease degradation of the S3, we mixed 0.8 μM S3 (without any modification) and 3' inverted thymidine (Ti) modified S3 with 20% fetal bovine serum and incubated for 0 h, 1 h, 2 h, 3 h at 37 °C, respectively. Then, 10 μL mixture was mixed with 2 μL loading buffer and 2 μL SYBR Gold (6×), followed by 12% PAGE in 1×TBE buffer running at 80 V for 2 h and analyzed by gel imaging system. Then, 50 nM miR-141 was analyzed by the DNA probes (100 nM S1, 100 nM S2 and 120 nM S3) in complex biological fluids, e.g., diluted 10 %, 20 % and 50% serum buffer. After incubation for 1 h, and the fluorescence spectra was taken. The fluorescence of FAM was collected between 510 nm and 650 nm by use of the maximal excitation wavelength at 488 nm.

Cell Culture.

LoVo (colon cancer cell line), 22Rv1 (prostate carcinoma cell line), HeLa (cervical

cancer cell line) and SMMC-7721 (hepatocellular carcinoma cell line) were cultured in DMEM medium supplemented with 13% fetal bovine serum, 100 U/mL of 1% antibiotics penicillin/streptomycin, and incubated at 37 ^{o}C in a humidified atmosphere of 5% CO_2 and 95% air.

Confocal Fluorescence Images.

All cell lines were cultured in DMEM medium supplemented for 36 h to reach 70-80%. Transfection assays were performed according to manufacturer's protocol. Briefly, 125 μL Opti-MEM supplemented with 3.0 μL lipofectamine 3000 were mixed with 125 μL Opti-MEM containing 100 nM S1, 100 nM S2, 120 nM S3 for 15 min at room temperature. Next, the cells were incubated with the 1 mL mixture for 1 h at 37 ^{o}C, and washed with PBS (pH 7.4) three times before imaging. In control experiment, three groups of LoVo cells were transfected with DNAzyme cascade system (100 nM non-cleavable S1, 100 nM S2, 120 nM S3), non-cleavable probe (100 nM non-cleavable S1, 100 nM S2, 120 nM S3) and inactive probe (100 nM inactive S1, 100 nM S2, 120 nM S3). Untreated LoVo cells were used as the control group. After incubation for 1 h, the cells were washed and imaged. To detect the dynamic changes of miR-141 in cells, two groups of LoVo cells were pretreated with 300 nM anti-miR-141 and 300 nM miR-141 mimics for 2 h, respectively, the without treatment served as the control. All cells were observed under an Olympus IX-70 inverted microscope with an Olympus FluoView 500 confocal scanning system, and a 515 nm bandpass filter was used for fluorescence detection. The excitation wavelength was 488 nm, and the images were collected in the range of 505-525 nm.

Flow Cytometry Analysis.

LoVo cells and SMMC-7721 cells were cultured in culture media for 36 h to reach 80-90%. After transfection with the mixture of 100 nM S1, 100 nM S2 and 120 nM S3 for 1 h, the cells were washed with PBS (pH 7.4) three times and detached from culture plate by Trypsin-EDTA solution. Next the suspended cell solution was centrifuged for 4 min at 2,000 g and washed three times. Finally, the cells were resuspended in PBS for flow cytometry analysis on Beckman Coulter Gallios machine under 488 nm excitation.

RT-qPCR.

Total cellular RNAs was extracted from LoVo, 22Rv1, HeLa and SMMC-7721 cells, respectively, using Trizol reagent (Sangon Co.Ltd., Shanghai, China) according to the manufacturer's instructions. The cDNA samples were prepared by using the reverse transcription (RT) reaction with AMV First Strand cDNA Synthesis Kit (BBI, Toronto, Canada). qPCR analysis of miRNA was performed with SG Fast qPCR Master Mix(2X) (BBI), according to the indicated protocol on a LightCycler480 Software Setup (Roche). The relative expression of miR-141 was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers used in this experiment were described as follow:

miR-141 forward: ACACTCCAGCTGGGTAACACTGTCTGGT

miR-141 reverse: CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGC CATCTTT

U6 forward: CTCGCTTCGGCAGCACA

U6 reverse: AACGCTTCACGAATTTGCGT

Table S1. Oligonucleotide sequences used in this work.

Name	Sequence（5′-3′）
S1	GGATCAAAAACATCTTTACACT*rA*GTCTTTTTTTTTGA TCCGAGCCGGACGAAGTGACAGTGTT
Non-cleavable S1	GGATCAAAAACATCTTTACACTAGTCTTTTTTTTTGA TCCGAGCCGGACGAAGTGACAGTGTT
Inactive S1	GGATCAAAAACATCTTTACACT*rA*GTCTTTTTTTTTGA TCCGGCCCGGACGAAGTGACAGTGTT
S2	GGAGATAGTTCAGCGTAAAGATGTTTTTGATCCGAA CTATCTCCGAGCCGGTCGAAAACTAAGA
S3	FAM-TCTTAGTT*rA*GGATAGTT-BHQ-1-CTi
S3*	AAAAAAAAAATCTTAGTT*rA*GGATAGTTC
Initiator	GGATCAAAAACATCTTTACACT
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-429	UAAUACUGUCUGGUAAAACCGU
let-7d	AGAGGUAGUAGGUUGCAUAGUU
miR-141 mimics	T*A*A*CACTGTCTGGTAAAGAT*G*G*
anti-miR-141	C*C*A*TCTTTACCAGACAGTGT*T*A*

The *rA* denotes RNA base. Non-cleavable S1 is in which the *rA* base at the cleavage site is replaces with a deoxyribose base. The Ti in S3 sequence represents an inverted thymidine at the 3’ position. The * in miR-141 mimics and anti-miR-141 represent phosphorothioate modification.

Supporting Figures:

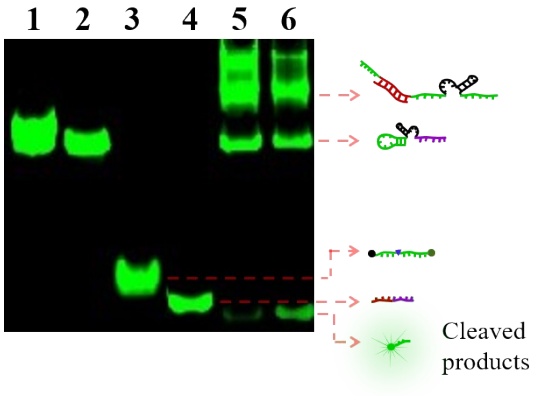

Figure S1. Gel electrophoresis image of the whole DNAzyme cascade system. Lane 1: S1; Lane 2: S2; Lane 3: S3; Lane 4: miR-141; Lane 5: S1 + S2 + S3 + miR-141; Lane 6: S1 + S2 + S3 + miR-141 + Mg^{2+}.

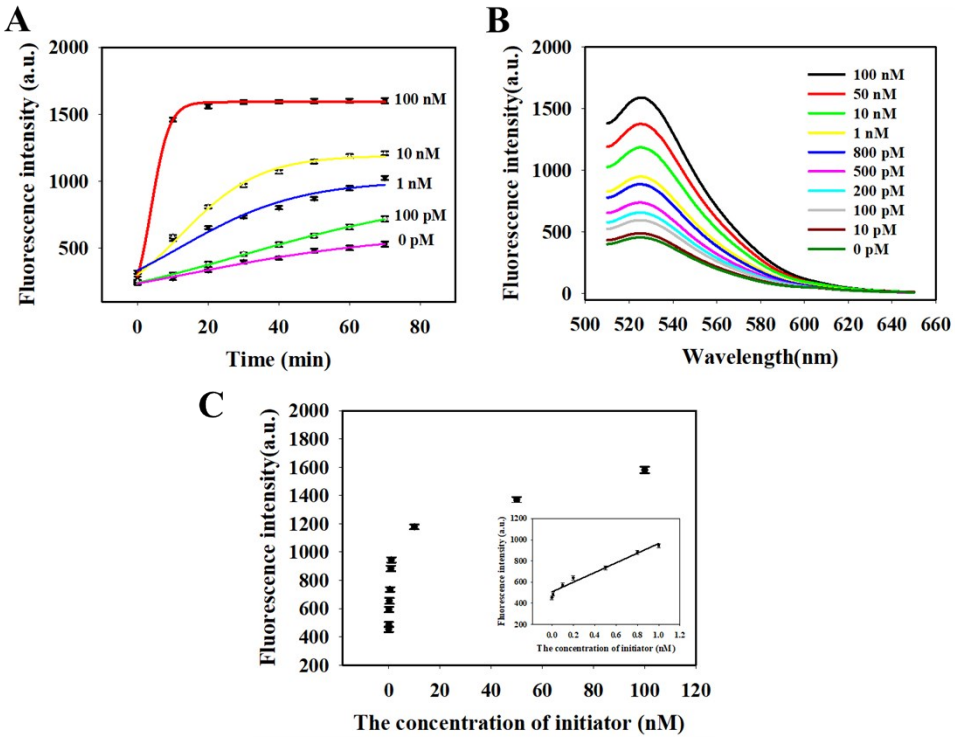

Figure S2. Fluorescence performances of the downstream DNAzyme circuit *in vitro*. (A) Kinetic and (B) fluorescence emission spectra of downstream DNAzyme circuit with various concentration of initiator, and (C) corresponding calibration curves. Inset: linear response at initiator concentrations lower than 1 nM. The error bars indicate mean ± SD (n = 3).

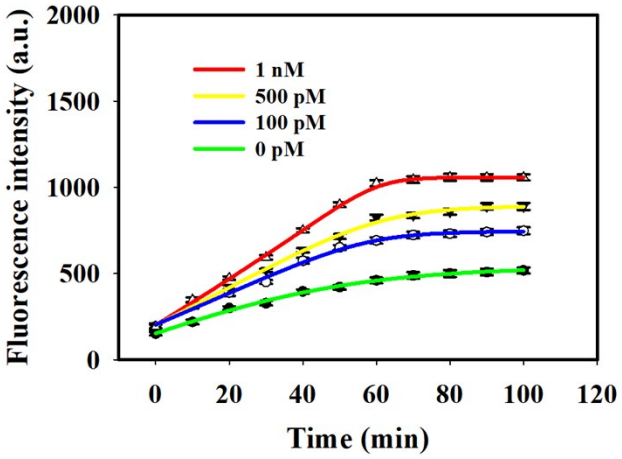

Figure S3. Kinetics of the DNAzyme cascade amplification system with different concentrations of miR-141. The error bars indicate mean ± SD (n = 3).

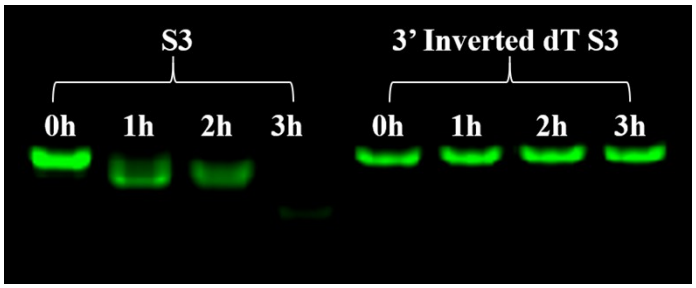

Figure S4. Stability of 3'-3' dT modified DNA strand. S3 and 3' inverted dT modified S3 incubated in 20% fetal bovine serum at 37 ^{o}C for different time and imaged by 12% PAGE.

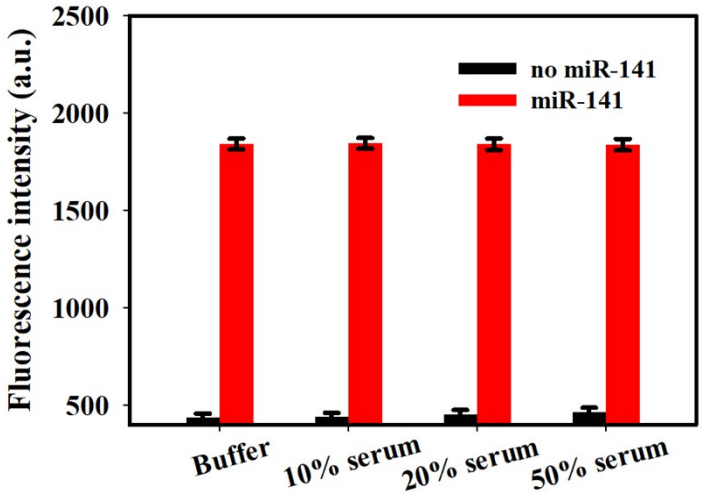

Figure S5. Bar representation of the fluorescence intensity changes of the 50 nM miR-141-analyzing DNAzyme cascade circuit in different serum solutions. The error bars indicate mean ± SD (n = 3).

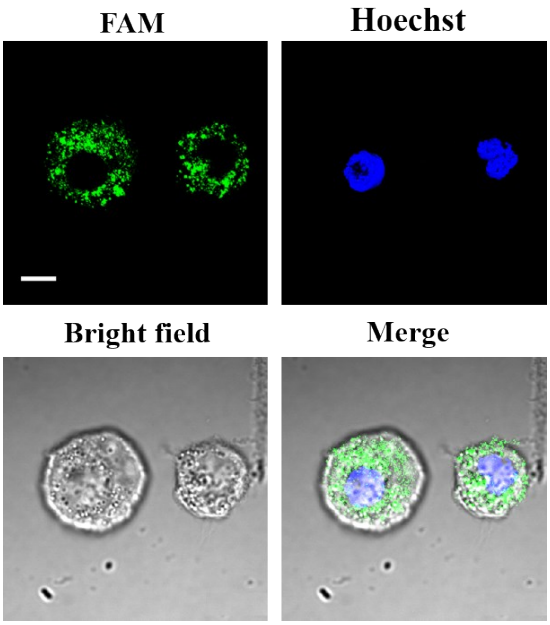

Figure S6. Fluorescence colocalization experiments of the DNAzyme cascade system with LoVo cells for 1 h at 37 °C. The cell nucleus was stained with Hoechst. The excitation wavelength was 488 nm, and the images were collected in the range of 505-525 nm. Scale bar is 10 μm.

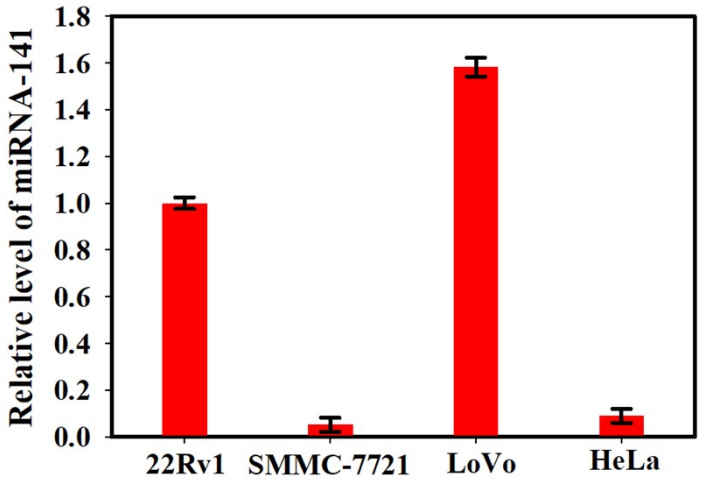

Figure S7. qRT-PCR analysis of relative expression levels of miR-141 in four different cells.

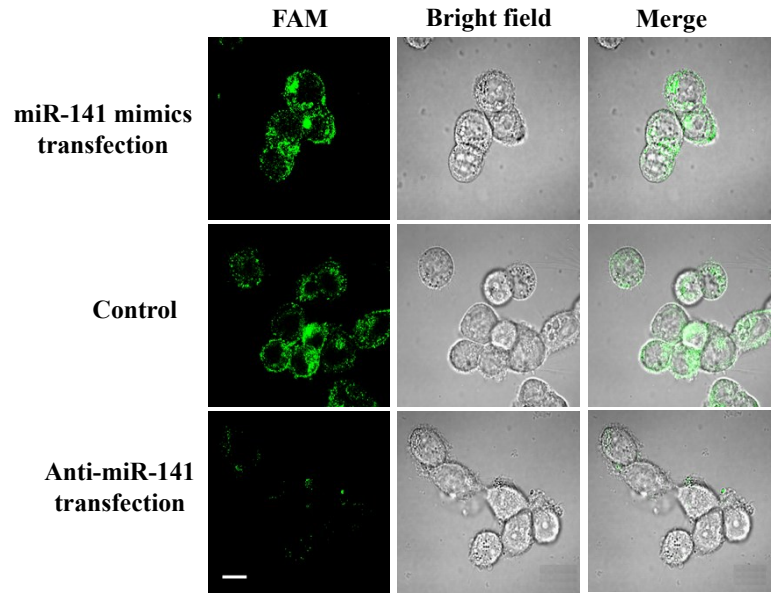

Figure S8. Confocal fluorescence images of LoVo cells incubated with the DNAzyme cascade system after being transfected with miR-141 mimics and anti-miR-141 for 1 h. Untreated LoVo cells were used as the control group. Scale bar is 10 μm.