Supporting Information

An Intelligent Demethylase-Driven DNAzyme Sensor for Highly Reliable Metal Ions Imaging in Living Cells

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Experimental Section

Reagents and materials.

DNA oligonucleotides were synthesized and HPLC-purified by TaKaRa Biotechnology Co. Ltd. (Dalian, China). All the DNA sequences were list in **Table S1**. Hydrogen peroxide (H_2O_2) was bought from Aladdin Reagents (Shanghai, China). Adenosine triphosphate (ATP), glutathione (GSH), glucose, alkaline phosphatase (ALP), α-KG, and zinc chloride ($ZnCl_2$) were bought from Sigma-Aldrich (MO, USA). FTO demethylase (DMase) was bought from Abcam. Polynucleotide kinase (PNK), CpG Methytransferase (M.SssI), and dam Methytransferase (Dam MTases) were bought from New England Biolabs (Ipswich, MA, USA). Bovine Serum Albumin (BSA) was bought from Genview (USA). *N, N, N', N'*-tetrakis (2-pyridylmethyl) ethylenediamine (TPEN) was bought from Macklin (Shanghai, China). RIPA cell lysis buffer and Hoechst 33342 were obtained from Beyotime Institute of Biotechnology (Shanghai, China). Lipofectamine 3000 transfection reagent was bought from Invitrogen (Carlsbad, CA). Dulbecco's Modified Eagle Medium (DMEM) was bought from HyClone (Logan, Utah, U.S.A.). MCF-7 cells and L02 cells were bought from Shanghai Institutes for Biological Sciences (SIBS). MCF-10A and HepG2 cells were bought from Procell Life Science & Technology Co., Ltd.

Fluorescence assay

For the activation of the m^6A caged-DNAzyme mediated by FTO demethylase (DMase), 250 nM DMase and 500 nM m^6A caged-DNAzyme were incubated in 20 μL reaction buffer (50 mM HEPES, pH 7.5, 100 μM α-KG, 100 μM Ascorbate, 50 μM $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ and 1 mM TCEP) for 1 h at room temperature. Then the reaction of FTO-mediated demethylation process was quenched by heating for 15min at 95 ºC. For the fluorescence assay of DMase-activated DNAzyme, 50 nM caged-DNAzyme (pretreated with DMase) and 200 nM substrate were incubated in 200 μL buffer containing 10 mM HEPES, 200 mM NaCl, 100 μM $ZnCl_2$. The fluorescence changes were measured at a fixed excitation of 488 nm and the emission spectra were collected from 505 to 650 nm.

Polyacrylamide gel electrophoresis assay

100 nM DNAzyme or DMase-treated caged-DNAzyme and 1 μM substrate were reacted in HEPES buffer (10 mM, 200 mM NaCl, 100 μM $ZnCl_2$) at 37 °C for 2 h. Then 10

μL of the sample was mixed with 2 μL 6 × loading buffer and then loaded into 20% denatured polyacrylamide gel. The PAGE was implemented at 100 V for 1 h, and later stained with GelRed and imaged by FluorChem FC3 (Protein-Simple, USA) under 365 nm UV irradiation.

Cell culture and imaging analysis

MCF-7 cells, L02 cells and HepG2 cells were cultured in DMEM with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. MCF-10A cells were cultured in a complete growth medium. All these cells were cultured at 37 °C with 5% CO_2. Cells were seeded into 35 mm confocal dishes with 1.0 mL DMEM medium for 24 h. Then cells were treated with 20 μM $ZnCl_2$ or TPEN for 0.5 h, and next the medium was replaced with 400 μL Opti-MEM medium containing 4 μL lipo 3000 and 200 nM DMADz system. After 4 h incubation, the cells were incubated with Hoechst 33342 for 12 min, and then washed by PBS for three times before CLSM imaging.

For the downregulation of DMase, MCF-7 cells were transfected with 80 nM siRNA by using lipo 3000. After transfection with 12 h, the culture medium was replaced with fresh medium and further cultivated for another 12 h. Then cells were harvested for the following experiments.

Flow cytometry analysis

MCF-7 cells, MCF-10A cells L02 cells, and HepG2 cells were seeded and cultured in 12-well plates for 24 h. Then the cells were incubated with DMADz system for different times, and then detached from culture dishes using Trypsin-EDTA solution. After being centrifuged at 1400 rpm for 4 min, the cells were resuspended with 300 μL PBS for flow cytometry analysis. Fluorescence was determined by recording 10000 cells, and the data were analyzed by FlowJo software.

Table S1. The DNA sequences used to construct the DMADz system

No.	Sequence (5'→3')
DNAzyme	GTCATTCTCCGAGCCGGTCGAAAACAGTCA
Caged-DNAzyme	GTCATTCTCCGA^{Me}GCCGGTCGAAAACAGTCA
Mutant caged-DNAzyme	GTCATTCTCCGA^{Me}TCCGGTCTAAAACAGTCA
FAM-Substrate	FAM-TGACTGTTrAGGAATGAC
Substrate strand	**FAM**-TGACTGTTrAGGAATGAC-**BHQ**
FTO-silencing siRNA	UCUCACAAGCAGCGGCUAUUU

rA: ribonucleotide, A^{Me}: m^{6}A-modified nucleotides, blue nucleotide: mutant base.

Fluorescence spectra of the DMase-driven DNAzyme system

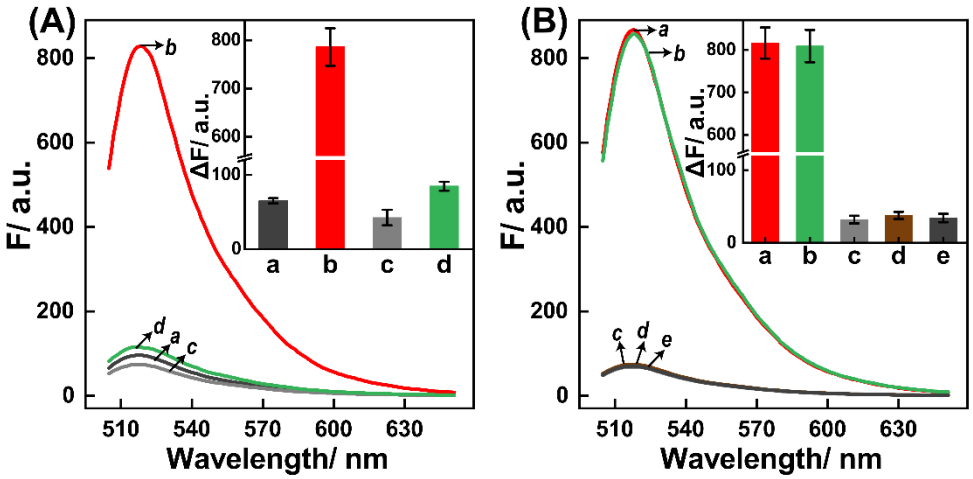

Figure S1. **(A)** Fluorescence spectra of the intact DNAzyme or m^6A-modified DNAzyme for sensing Zn^{2+} ions (100 μM). (a) Dz, (b) Dz + Zn^{2+}, (c) cDz, (d) cDz + Zn^{2+}, (λ_{ex} = 480 nm; λ_{em} = 520 nm). The system was composed of 50 nM intact DNAzyme or 50 nM m^6A-caged DNAzyme and 200 nM substrate. **(B)** Fluorescence spectra of (a) intact Dz, (b) DMase-treated cDz, (c) the heat-deactivated DMase-treated cDz, (d) intact cDz, and (e) DMase-treated mutant cDz. Error bars were acquired from n=3 experiments.

Sensing selectivity of DMADz against different analytes

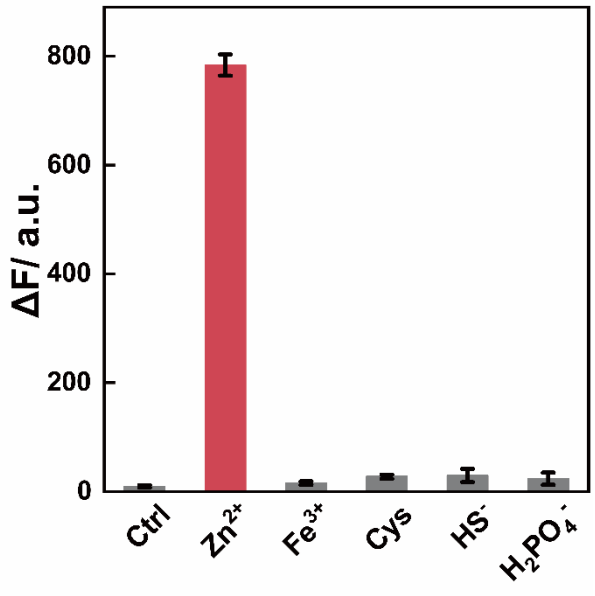

Figure S2. Sensing selectivity of DMADz to different analytes (100 μM). Error bars were acquired from n=3 experiments.

Optimization of reaction temperature

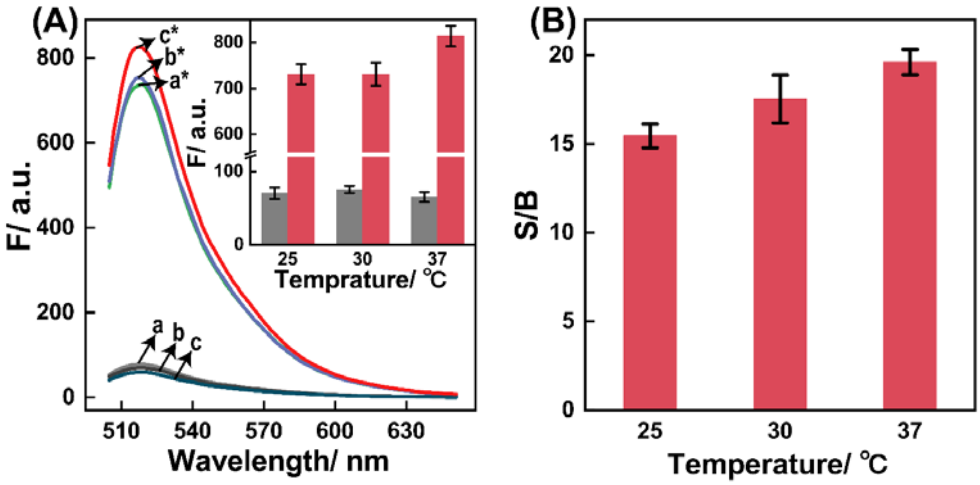

Figure S3. **(A)** Fluorescence spectra of the DMADz system treated at different temperatures: 25 °C, 30 °C, 37 °C. (a) 0 μM Zn^{2+} at 25 °C, (b) 0 μM Zn^{2+} at 30 °C, (c) 0 μM Zn^{2+} at 37 °C, (a') 100 μM Zn^{2+} at 25 °C, (b') 100 μM Zn^{2+} at 30 °C, (c') 100 μM Zn^{2+} at 37 °C. Inset: Fluorescence intensity changes of the DMADz system treated at 25 °C, 30 °C, 37 °C. **(B)** The signal-to-background ratio of the DMADz system treated at 25 °C, 30 °C, 37 °C. Error bars were acquired from n=3 experiments.

Optimization of dosage and incubation time of DMase

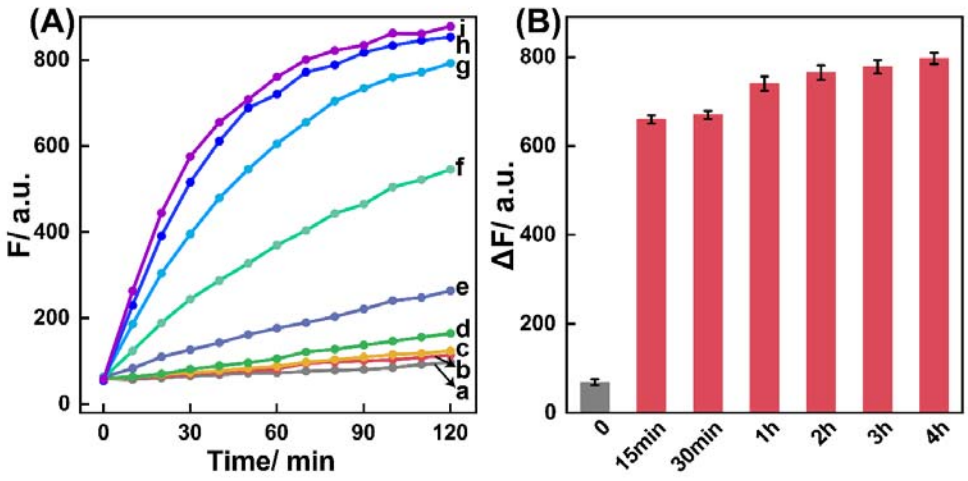

Figure S4. **(A)** Time-dependent fluorescence intensity of the caged DNAzyme responding to different concentrations of DMase. (a) 0, (b) 5 nM, (c) 10 nM, (d) 25 nM, (e) 50 nM, (f) 100 nM, (g) 200 nM, (h) 250 nM, (i) 400 nM. The concentration of the caged DNAzyme was fixed at 500 nM. **(B)** Fluorescence intensity changes of the caged DNAzyme with different incubation time of DMase. Error bars were acquired from n=3 experiments.

Validation of the specific DMase-activated responsiveness

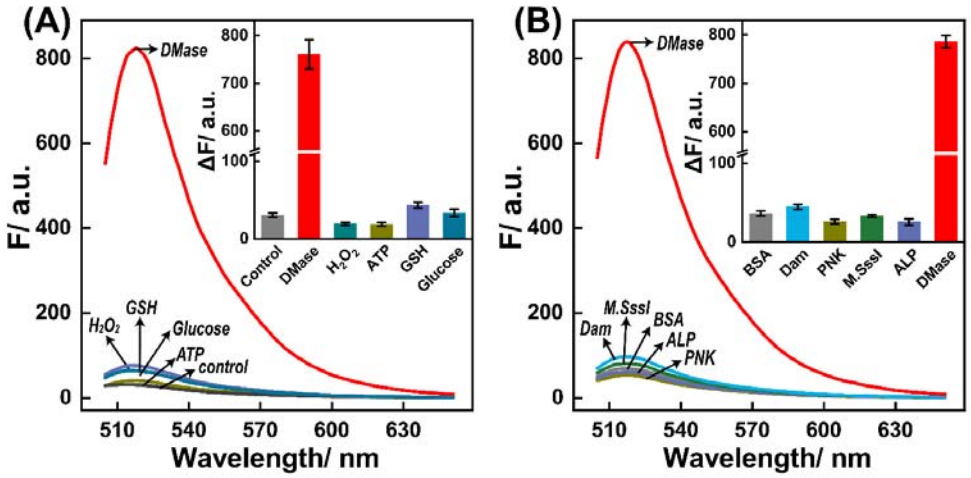

Figure S5. **(A)** Fluorescence spectra of the responsiveness of the caged DNAzyme corresponding to 250 nM DMase, 100 μM H_2O_2, 5 mM ATP, 5 mM GSH, 2 mM Glucose. Inset: Fluorescence intensity changes of the different treatments. **(B)** Fluorescence spectra of the responsiveness of the caged DNAzyme corresponding to 250 nM DMase, 50 μg/mL BSA, 20 U/mL Dam, 20 U/mL PNK, 20 U/mL M. SssI, 20 U/mL ALP. Inset: Fluorescence intensity changes of the different treatments. Error bars were acquired from n=3 experiments.

Stability of the DMase-activated DNAzyme in cell lysate

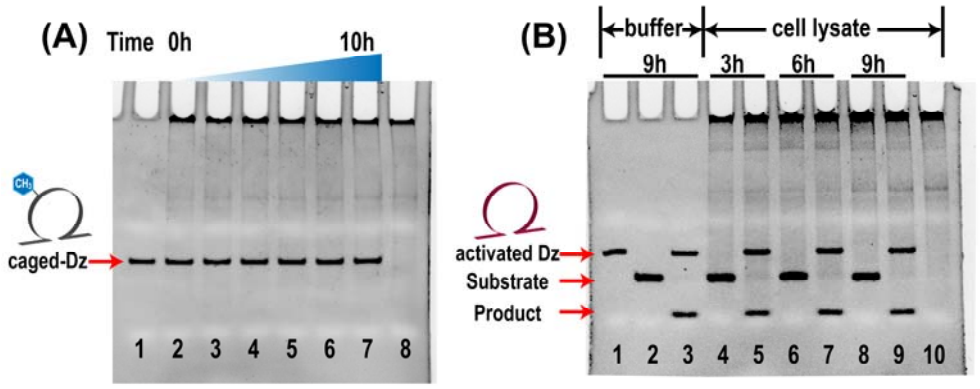

Figure S6. **(A)** Stability of the caged-DNAzyme in MCF-7 cell lysate at different times (from lane 2 to lane 7: 0 h, 2 h, 4 h, 6 h, 8 h, 10 h). Lane 1 was the caged-DNAzyme in HEPES buffer, lane 8 was the control of cell lysate. **(B)** Stability of the DMase-treated caged-DNAzyme and substrate strand in MCF-7 cell lysate at different times. Lane 1-3: samples in HEPES buffer, lane 4-9: samples in cell lysate, lane 10 was the control of cell lysate. All samples were treated with 100 μM Zn^{2+}.

Optimization of incubation time of the DMase-driven DNAzyme system

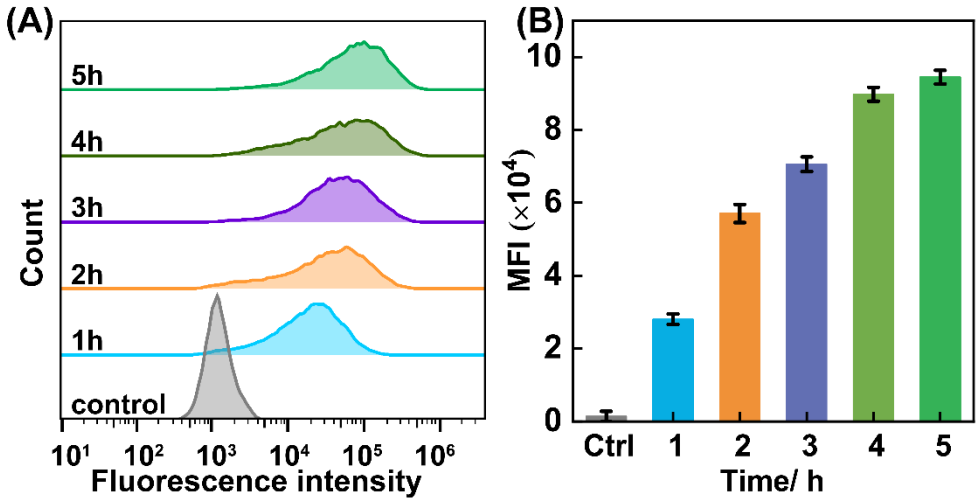

Figure S7. **(A)** Flow cytometry analysis of the DMADz system treated MCF-7 cells at different incubation times (1h, 2h, 3h, 4h, 5h). **(B)** Mean fluorescence intensity of the flow cytometry results as shown in **(A)**. Error bars were acquired from n=3 experiments.

The influence of Zn^{2+} ions to the DMase-mediated demethylation process

The interference of Zn^{2+} ions to the DMase-mediated demethylation process was explored to investigate the feasibility of the DMADz system in cell system. Different concentrations of Zn^{2+} ions were incubated with FTO DMase for 30min before the DMase-mediated m^6A demethylation process. As shown in the **Figure S8**, there is a negligible effect on the activity of DMase when the concentration of Zn^{2+} ions was in 1-20 μM, while the demethylation activity of the DMase was a bit affected with the increasing concentration of Zn^{2+} ions. Thus, the low concentrations of Zn^{2+} ions have little influence on the activity of DMase, demonstrating that the weak interference of the supplementary Zn^{2+} ions to the cellular FTO DMase and the feasibility of the DMADz sensor applied in cell system.

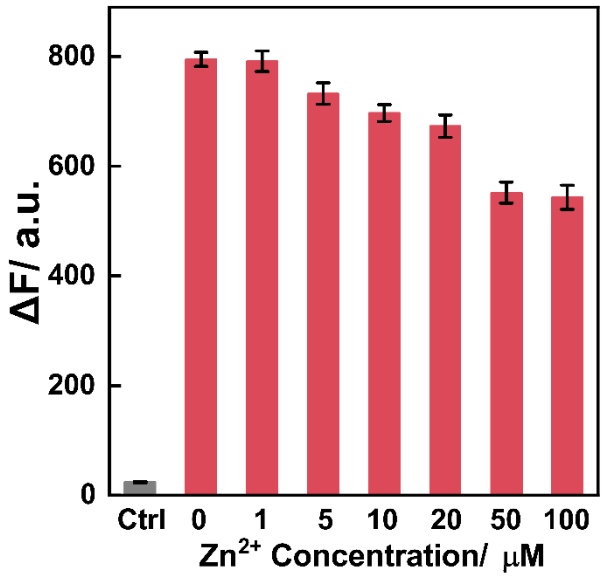

Figure S8. The influence of different concentrations of Zn^{2+} ions on the DMase-mediated demethylation process. Error bars were acquired from n=3 experiments.

Flow cytometry analysis of cells with different treatments

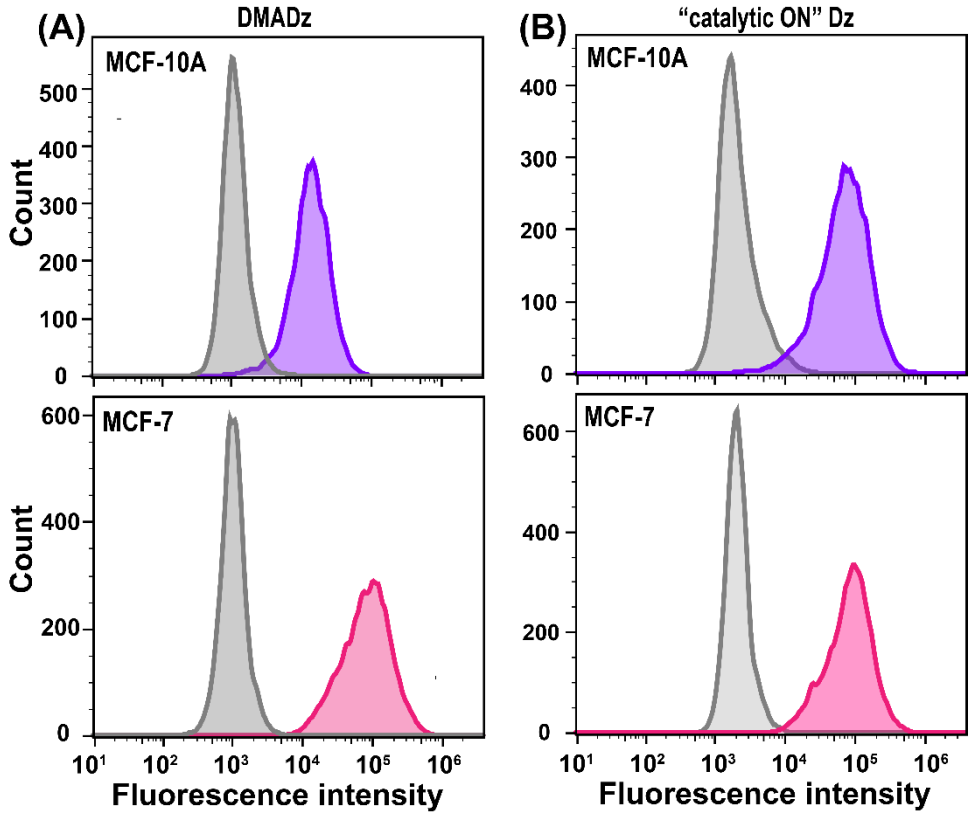

Figure S9. **(A)** Flow cytometry analysis of different cells treated with DMADz system. **(B)** Flow cytometry analysis of different cells treated with "catalytic ON" Dz system.

Cell-selective Zn^{2+}-sensing ability of DMADz system

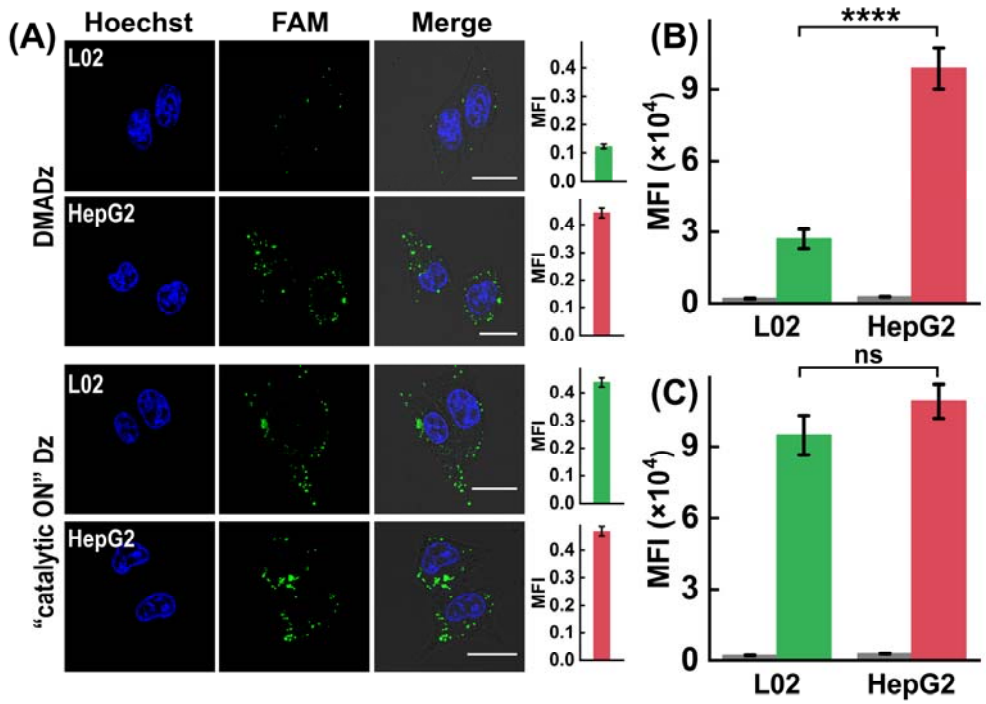

Figure S10. **(A)** CLSM images of L02 cells and HepG2 cells treated with DMADz system or "catalytic ON" Dz system. The corresponding statistical histogram of mean fluorescence intensity was shown aside. **(B)** Mean fluorescence intensity of flow cytometry results of L02 cells and HepG2 cells treated with DMADz system. **(C)** Mean fluorescence intensity of flow cytometry results of L02 cells and HepG2 cells treated with "catalytic ON" Dz system. ****$p < 0.0001$ (t tests), ns, no significance. Scale bar=20 μm.

Table S2. Comparison of different fluorescence methods for Zn^{2+} detection

System	Detection range (μM)	Detection limit (μM)	Ref.
Two-photon DNAzyme-based fluorescent probes for imaging metal ions	0-1000	1	[1]
Enzyme-activatable reconstructed DNAzyme sensor for cell-selective imaging of metal ions	0-100	0.6	[2]
DNAzyme-based fluorescent sensor for detecting metal ions in blood serum	0-200	2.96	[3]
DNAzyme-functionalized porous carbon nanospheres for imaging microRNA-21 and zinc ions	2-100	0.43	[4]
A ratiometric probe based on FRET for imaging of cellular metals	5-100	1.54	[5]
Purine-based fluorescent sensors for imaging zinc ions in HeLa cells	2-100	2	[6]
Endogenous demethylase-driven DNAzyme sensor for site-specifically metal-ion-sensing	0-100	0.4	This work

Reference

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