Supporting Information for

Hollow microporous organic network-based nanoprobe for imaging tumor-associated mRNA in living cells

Zhi Yan,[a,b] Yi-Wen Li,[a] Rui-Jia Yang,[a] Yuan-Yuan Cui,[a] Lei-Liang Zhang,[b,*] Cheng-Xiong Yang[a,*]

[a] *School of Pharmaceutical Sciences & Institute of Materia Medica, Medical Science and Technology Innovation Center, Shandong First Medical University & Shandong Academy of Medical Sciences, 250117, Jinan, Shandong, China*

[b] *Department of Clinical Laboratory Medicine, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Jinan, Shandong, China*

E-mail: cxyang@nankai.edu.cn

1. Chemicals and Materials.

The tetrakis(4-ethynylphenyl)methane (TEPM) was provided by Chengdu Xinhong Material Technology Co., Ltd. (Chengdu, China). 1,1′-Bis(4-bromophenyl)-[4,4′-bipyridine]-1,1′-diiumchloride (BBDC) were purchased from Jilin Academy of Science-Yanshen Technology Co., Ltd. (Jilin, China). Acetonitrile (ACN), ammonia solution ($NH_3 \cdot H_2O$, 30%), methanol (MeOH), *N,N*-dimethylformamide (DMF), ethanol (EtOH), and dichloromethane were provided by Fuyu Chemical Co., Ltd. (Tianjin, China). Copper(I) iodide (CuI) was obtained from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). Bis(triphenylphosphine)palladium dichloride ($Pd(PPh_3)_2Cl_2$, >98 %) was purchased from Saen Chemical Technology Co., Ltd. (Shanghai, China). 2,2′-Azobis(2-methylpropionitrile) (AIBN, 99%) was provided by Aladdin Chemistry Co. (Shanghai, China). The Cell Counting Kit-8 (CCK-8) and 4% paraformaldehyde was purchased from Labgic Technology Co. Ltd. (Beijing, China). Dimethyl sulfoxide (DMSO, >99.8%), phosphate buffered saline (PBS, pH = 5.0, pH = 7.4), trypsin-EDTA solution, penicillin/streptomycin mixture were obtained from Solarbio Bioscience & Technology Co. Ltd. (Beijing, China). Sepantronium bromide (YM155) was purchased from Beyotime Institute of Biotechnology (Shanghai, China). DNA oligonucleotides were synthesized by Sangon Biotechnology Co., Ltd (Shanghai, China).

Fluorescence emission spectra were obtained on the Hitachi F-7000 Fluorescence spectrophotometer. N_2 adsorption-desorption isotherms were obtained using an ASAP 2460 specific surface area and pore size analyzer (Micromeritics,

USA). Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a Thermo Scientific Nicolet iS10 (Shimadzu, Japan). Scanning electron microscopy (SEM) images were recorded using a MIRA LMS (TESCAN, Czech Republic) instrument. High-resolution transmission electron microscopy (TEM) images were obtained using a JEOL JEM 2100 (JEOL, Japan). Fluorescence imaging of survivin mRNA in living cells was carried out on ZEISS LSM900 confocal fluorescence microscope.

2. Synthesis of H-MON/CF@Survivin

2.1 Synthesis of SiO_2

SiO_2 nanoparticles were synthesized following previously reported method with minor modifications [1]. Typically, tetraethyl orthosilicate (TEOS, 6 mL) was dissolved in 150 mL of ethanol under vigorous stirring at room temperature for 30 min. Subsequently, 7.5 mL of water and a specified volume of ammonia solution ($NH_3 \cdot H_2O$) were added dropwise, and the mixture was stirred continuously for 12 h. The resulting products were collected by centrifugation and repeatedly washed with ethanol to remove any residual reactants. The final solid was dried in a vacuum oven at 70 ℃ for 6 h to obtain SiO_2 nanoparticles. By varying the volume of $NH_3 \cdot H_2O$ added, the particle sizes of SiO_2 were effectively tuned to 75 nm (with 5 mL $NH_3 \cdot H_2O$), 120 nm (6 mL), 160 nm (7.5 mL), 260 nm (8 mL), and 320 nm (8.5 mL).

2.2 Synthesis of MON@SiO_2

SiO_2 (150 mg), Pd $(PPh_3)_2Cl_2$ (16.8 mg), CuI (4.4 mg), triethylamine (15 mL), and toluene (15 mL) were combined in a flask. The mixture was ultrasonicated for 30 min, after which 1,1′-Bis(4-bromophenyl)-[4,4′-bipyridine]-1,1′-diiumchloride (BBDC, 258 mg) and tetrakis(4-ethynylphenyl)methane (TEPM, 100 mg) were added and the reaction was allowed to proceed at 80 ℃ under stirring for 10 h. Upon cooling to room temperature, the yellow crude product was collected by centrifugation (8000 rpm, 5 min) and sequentially washed with a methanol/DMF mixture (1:1 v/v, 100 mL) and dichloromethane (20 mL). The final product, denoted as MON@SiO_2, was obtained after drying in a vacuum oven at 70 ℃ for 6 h.

2.3 Synthesis of H-MON

MON@SiO_2 (50 mg) was dispersed in 100 mL of NaOH solution (2 M). The suspension was stirred at room temperature for 24 h, after which the product was collected by centrifugation (8000 rpm, 5 min). The solid was subsequently rinsed repeatedly with ethanol and ultrapure water until the supernatant reached neutral pH. The resulting product was dried at 70 ℃ overnight to obtain H-MON.

2.4 Synthesis of H-MON/CF

H-MON/CF was synthesized via two sequential post-synthetic modifications starting from H-MON. The first step involved a thiol-yne click reaction to produce H-MON/C. Typically, H-MON (100 mg), Cys (100 mg), 2,2’-azobis(2-methylpropionitrile) (AIBN, 88 mg), and toluene (7.5 mL) were combined in a dried three-necked flask under a N_2 atmosphere. The mixture was heated to 100 ℃ and

maintained for 24 h. The crude product, H-MON/C, was collected by centrifugation (8000 rpm, 5 min) and washed with a methanol/DMF mixture (1:1 v/v, 100 mL).

In the second step, H-MON/CF was prepared through an amide condensation reaction between the amino groups on the grafted Cys and the carboxyl groups on Folic acid (FA). Specifically, FA (100 mg) and N, N'-dicyclohexylcarbodiimide (DCC, 60 mg) were dissolved in dimethylsulfoxide (DMSO, 10 mL) in a dried three-necked flask under N_2 protection. The solution was stirred at room temperature for 1 h, after which H-MON/C (50 mg) was added, and stirring continued at room temperature for 24 h. The resulting crude H-MON/CF was collected by centrifugation (8000 rpm, 5 min), washed three times with a methanol/DMF mixture (1:1 v/v, 100 mL each), followed by three washes with dichloromethane (30 mL each). The final product was obtained after drying overnight under vacuum.

2.5 Preparation of H-MON/CF@Survivin

The Cy5-labeled survivin recognition sequences was added into the aqueous solution of HMON/CF and fully mixed under dark. After shaking for 5 h, H-MON/CF@Survivin was collected by centrifugation and washed with water for three times.

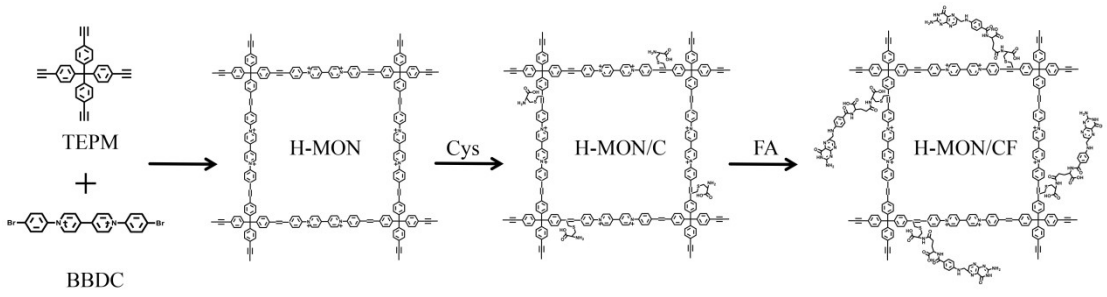

Fig. S1 Scheme for the fabrication of H-MON/CF.

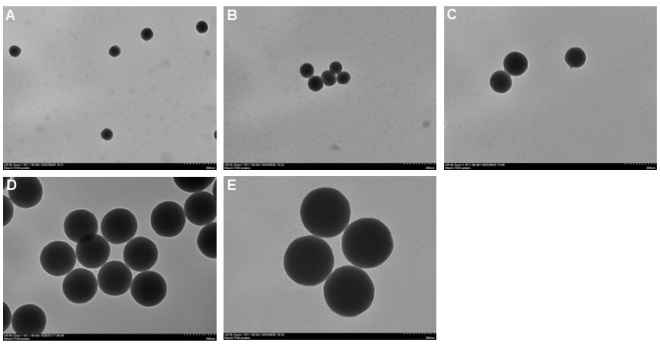

Fig. S2 TEM of SiO_2 in different diameters: (A) 75 nm, (B) 120 nm, (C) 160 nm, (D) 260 nm, and (E) 320 nm.

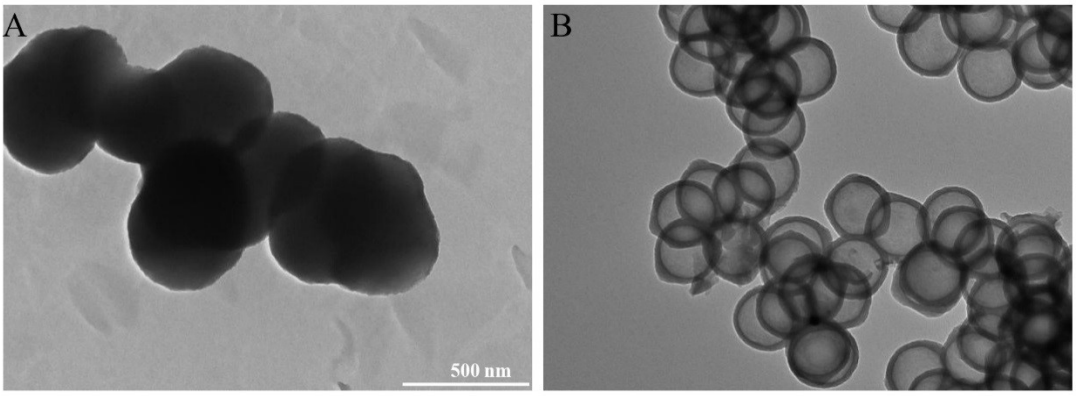

Fig. S3 TEM images of solid MON (A) and H-MON(B).

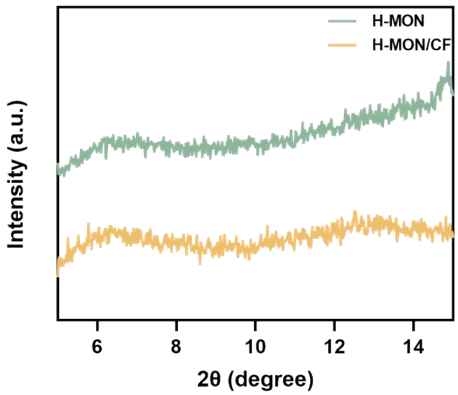

Fig. S4 The XRD patterns of H-MON and H-MON/CF.

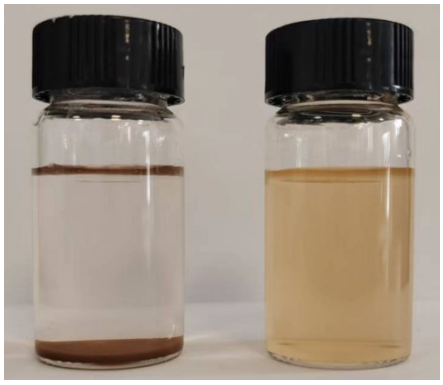

Fig. S5 Photos of solid MON (left) and H-MON/CF (right) dispersed in water.

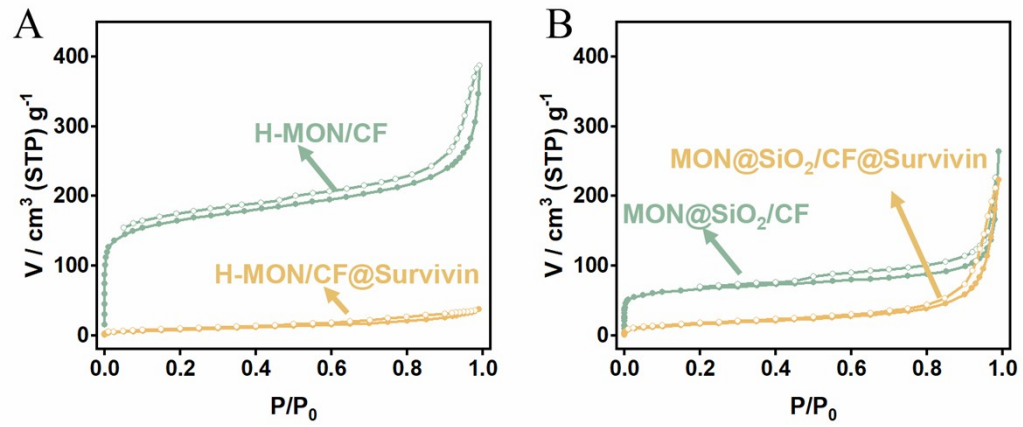

Fig. S6 N_2 adsorption-desorption isotherms of H-MON/CF and H-MON/CF@Survivin (A), N_2 adsorption-desorption isotherms of MON@SiO_2/CF and MON@SiO_2/CF@Survivin (B).

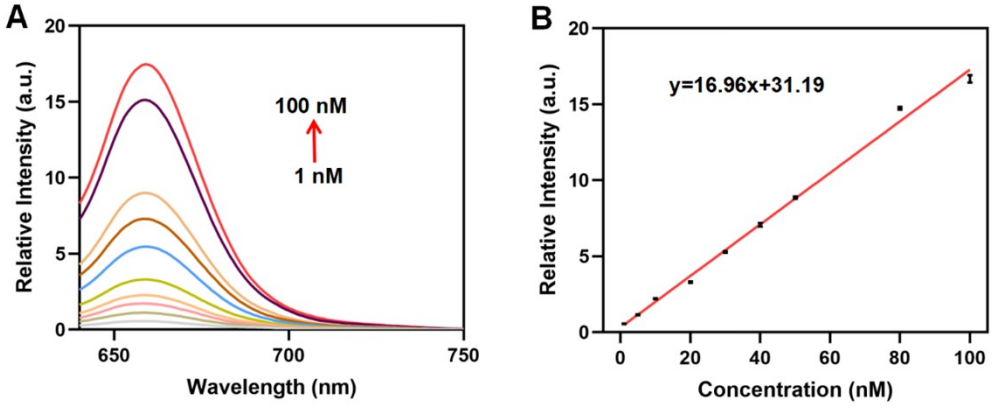

Fig. S7 Fluorescence spectra of Cy5-labeled survivin (A) and fluorescence standard curves of Cy5-labeled survivin (B).

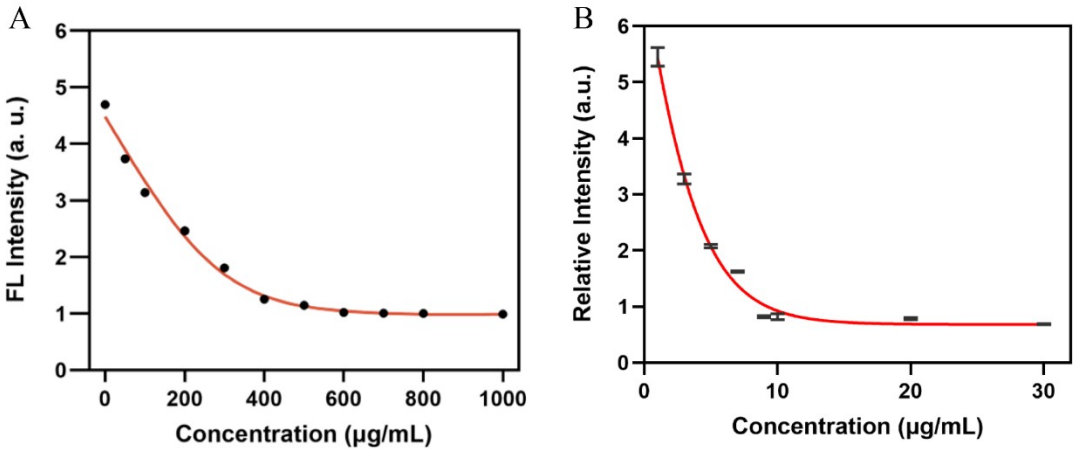

Fig. S8 Fluorescence intensities of Cy5-labeled surviving recognition DNA sequences treated with different concentrations of MON@SiO_2 (A) and H-MON (B).

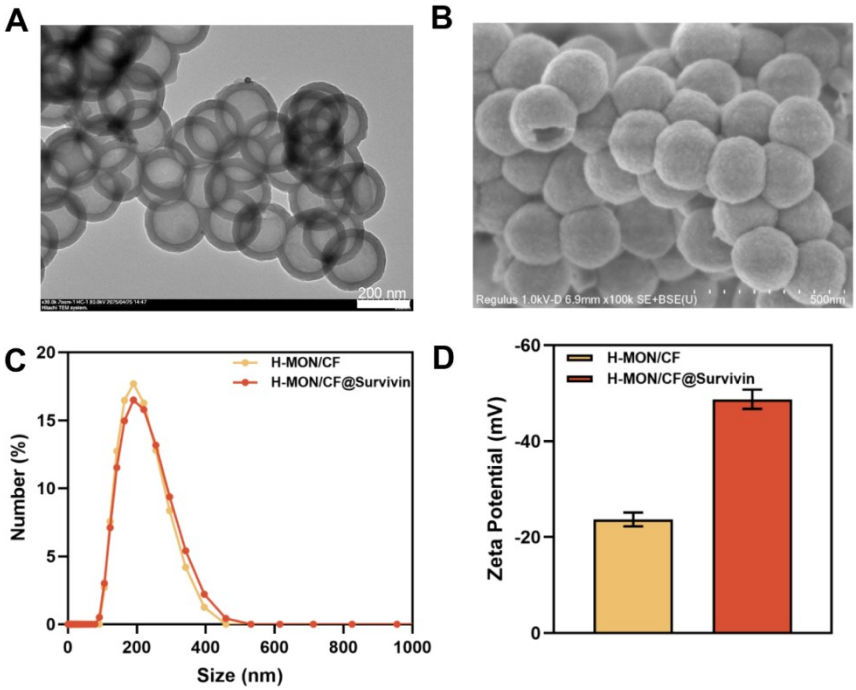

Fig. S9 TEM (A) and SEM (B) images of H-MON/CF@Survivin; (C) DLS distributions of H-MON/CF and H-MON/CF@Survivin; (D) zeta potentials of H-MON/CF and H-MON/CF@Survivin.

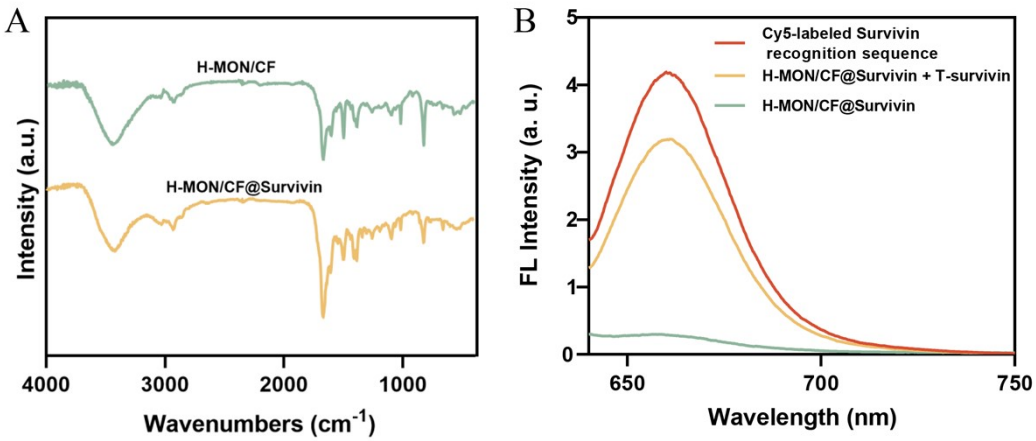

Fig. S10 (A) FT-IR spectra of H-MON/CF and H-MON/CF@Survivin. (B) Fluorescence curves of Cy5-labeled Survivin, H-MON/CF@Survivin, and the fluorescence recovery of H-MON/CF@Survivin in the presence of survivin mRNA.

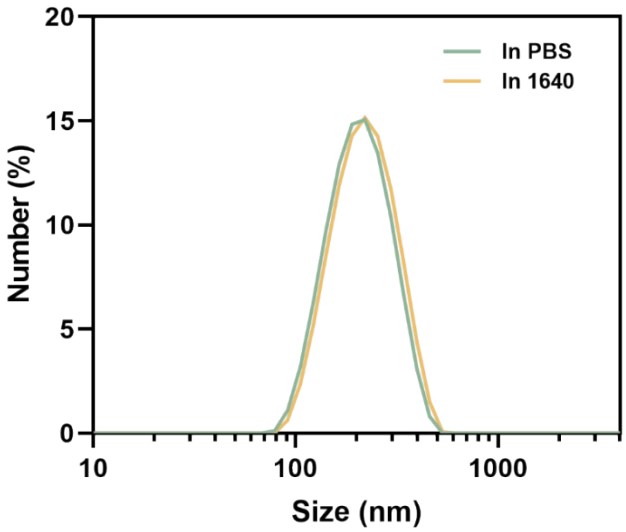

Fig. S11 DLS characterization of H-MON/CF@Survivin in different solutions.

3. Characterization of H-MON/CF@Survivin for recognizing Survivin mRNA.

3.1 Hybridization Experiment

Different concentrations (0-30 nM) of the survivin nucleic acid target were introduced into solutions of H-MON/CF@Survivin (5 μg/mL). After incubation at 37

℃ for 20 min, the Cy5 fluorescence signal in each solution was subsequently recorded using a fluorescence spectrophotometer.

3.2 Specificity Experiment

To assess the selectivity of H-MON/CF@Survivin for survivin mRNA, we tested its interaction with various species, including different nucleic acid targets (survivin mRNA, TK1 mRNA, mRNA-c-myc, miRNA-T21, and miRNA-T221), ions (Cu^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, Mn^{2+}, and Na^{+}) and amino acids (Cys, GSH, Phe, Arg, Lys, Tyr, His, and Thr). After incubation with H-MON/CF@Survivin at 37 ℃ for 20 min, the fluorescence signal of Cy5 in the solutions was detected using a fluorescence spectrophotometer.

3.3 Calculation of DNA Loading Density

A series of Cy5-survivin solution with concentrations ranging from 1-100 nM were prepared and their fluorescence spectra were collected to draw the fluorescence standard curve of Cy5-labeled survivin.

For comparing the DNA loading densities on H-MON/CF, the free mRNA in the supernatants were collected by centrifugal separation of H-MON/CF, then the unloaded mRNA was detected with a fluorescence spectrophotometer and its concentration was calculated based on the fluorescence standard curve method, and the mRNA loading densities of different methods were further calculated using following formula: $LD = (n_{Total\ mRNA}-n_{unloaded\ mRNA})/m_{H\text{-}MON}$

3.4 Storage Stability

To compare the storage stability of H-MON/CF@Survivin, the nanoprobes were dispersed in PBS and 1640 solutions, the fluorescence intensity of Cy5 in the solutions were recorded with a fluorescence spectrophotometer for five days.

3.5 Fluorescence Imaging of Survivin mRNA in MCF-7 Cells

MCF-7 cells were typically seeded in a 22 mm cover glass-bottom culture dish at a density of 5×10^4 cells/mL. After 24 h, the culture medium was removed, three groups of experiments were designed and performed:

For the control group, cells were treated with fresh medium.

For the H-MON group, cells were treated with H-MON (5 μg/mL) for 2 h.

For the H-MON@Survivin group, cells were treated with H-MON@Survivin (5 μg/mL) for 2 h.

For the H-MON/CF@Survivin group, cells were treated with H-MON/CF@Survivin (5 μg/mL) for 2 h.

Before fluorescence imaging of each group, the medium was removed and washed with fresh medium three times. Finally, the fluorescence images of MCF-7 cells were acquired with confocal fluorescence microscopy and red channel for survivin.

3.6 Fluorescence imaging of survivin mRNA after treatment by YM155 in MCF-7 cells

MCF-7 cells were typically seeded in a 22 mm cover glass-bottom culture dish at a density of 5×10^4 cells/mL. After 24 h, the culture medium was removed, three

groups of experiments were designed and performed: in the control group, cells were treated with H-MON/CF@Survivin for 2 h, and then, in the model group, cells were pretreated with YM155 (5, 10 nM) for 48 h and then treated with H-MON/CF@Survivin for 2 h.

Before fluorescence imaging of each group, the medium was removed and washed with fresh medium three times. Finally, the fluorescence images of MCF-7 cells were acquired with confocal fluorescence microscopy and red channel for survivin.

3.7 Confocal Fluorescence Imaging

MCF-10A and MCF-7 cells were selected as models to evaluate the mRNA imaging capability of the nanoprobes. These cells were firstly cultured in confocal dishes and rinsed with serum-free medium prior to treatment. Subsequently, the nanoprobes, dispersed in serum-free medium at a final concentration of 10 μg/mL, were introduced and incubated with the cells for 2 h. After incubation, the cells were washed three times with PBS and subjected to imaging using a laser scanning confocal microscope.

Before fluorescence imaging of each group, the medium was removed and washed with fresh medium three times. Finally, the fluorescence images of MCF-7 cells were acquired with confocal fluorescence microscopy and red channel for survivin.

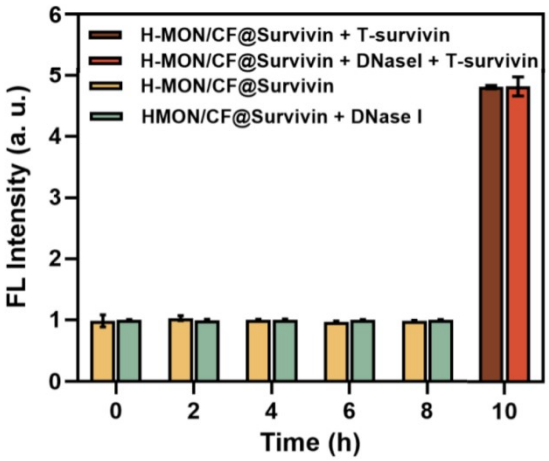

Fig. S12 Relative fluorescence intensity changes of H-MON/CF@Survivin in the presence of DNase I and the survivin target.

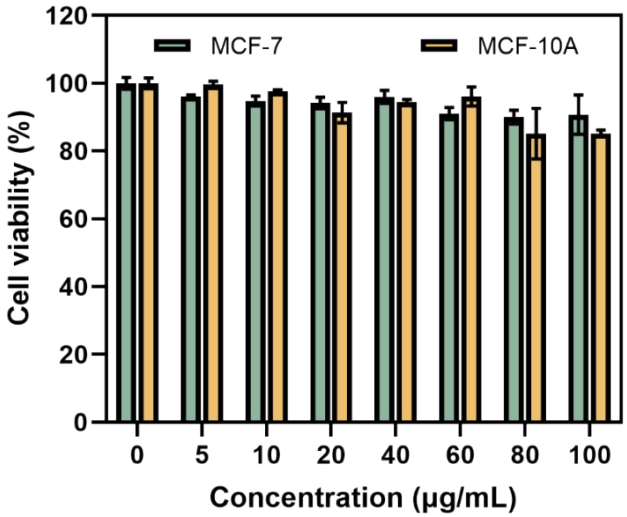

Fig. S13 The cell viability of MCF-7 cells and MCF-10A cells after incubating with different concentrations of H-MON/CF@survivin.

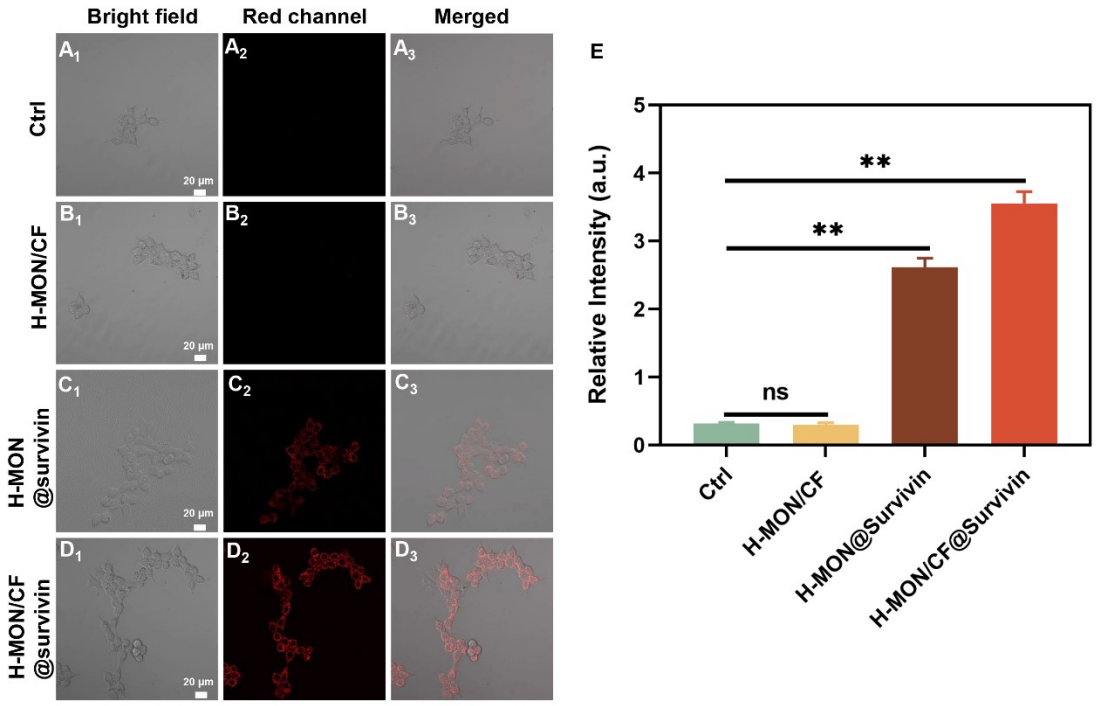

Fig. S14 Confocal fluorescence imaging in MCF-7 cell. (A-D) The cell treated with PBS H-MON/CF, H-MON@Survivin, and H-MON/CF@Survivin for 2h, respectively. (E) The relative intensities of cell. The values are the mean ± SD for n = 3 fields of cells, **P < 0.01. Scale bar: 20 μm.

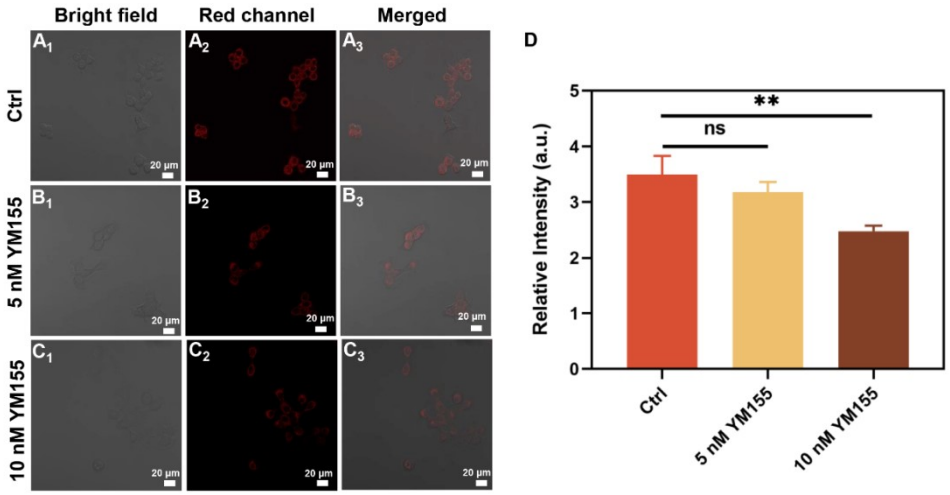

Fig. S15 Confocal fluorescence imaging YM155-induced downregulation of endogenous survivin mRNA in MCF-7 cell. (A-C) Cells were pretreated with

different concentrations of YM155 (0, 5, and 10 nM) for 48 h, followed by coincubation with H-MON/CF@Survivin for 2 h. (D) The relative intensities of cell. The values are the mean ± SD for n = 3 fields of cells, **P < 0.01. Scale bar: 20 μm.

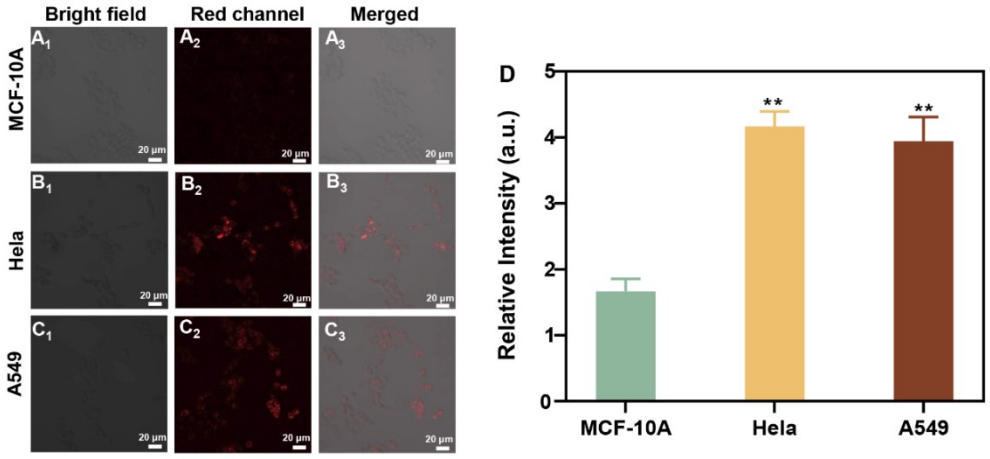

Fig. S16 Confocal fluorescence imaging in MCF-10A (A), A549 (B) and Hela (C) after treated with H-MON/CF@Survivin for 2 h. (D) The relative intensities of cell. The values are the mean ± SD for n = 3 fields of cells, **P < 0.01. Scale bar: 20 μm.

Table S1 Comparison of different nanoprobes for Survivin mRNA loading

Target	Platform	Loading capacity	LOD	Response time	Reference
ATP	COF	5 nM/mg	DNA: 3.7nM ATP:25-200 μM	10 min	[1]
Survivin mRNA	COF	0.572 nM/mg	-	30 min	[2]
Survivin mRNA	COF	Survivin mRNA: 46.06 nM/mg	-	30 min	[3]
Survivin mRNA MUC1	COF	survivin mRNA: 0.106 nM/mg MUC1: 0.120 nM/mg	Survivin mRNA: 0.4 nM MUC1: 0.01 μg/mL	-	[4]
ATP	MOF	0.66 nM/mg	4.24 μM	-	[5]
CEA	COF	No Report	0.005 ng/mL	40 min	[6]
c-myc	COF	0.695 nM/mg	2 nM	20 min	[7]
TK1 mRNA	COF	0.97 nM/mg	0.427 nM	30 min	[8]
Survivin mRNA TK1 mRNA	COF	Survivin mRNA: 0.5936 nM/mg TK1 mRNA: 0.6003 nM/mg	Survivin mRNA: 18.3 nM TK1 mRNA: 6.8 nM	30 min	[9]
$VEGF_{165}$	COF	50 nM/mg	20.9 fg/mL	-	[10]
Survivin mRNA	MON	5.5 μM/mg	0.9 nM	20 min	This Work

Table S2. Sequences of DNA employed in this work.

Oligonucleotide	Sequence (5'-3')
Cy5-labeled survivin recognition sequence	Cy5-TAGAGATGCGGTGGTC
mR-survivin	GACCACCGCATCTCTA
miR-221	AGCTACATTGTCTGCTGGGTTC
miR-T21	TAGCTTATCAGACTGATGTTGA
mR-TK1	AAGTATGCCAAAGACACTCGC
mR-c-myc	CTCAACGTTACGTTCAC

4. References

1. S. W. Gao, N. Li, Y. Y. Cui and C. X. Yang, *J. Chromatogr. A.* 2024, **1736**, 465419.

2. W. Li, C. X. Yang, X. P. Yan, *Chem. Commun.*, 2017, 53, 11469.

3. P. Gao, M. Z. Wang, Y. Y. Chen, W. Pan, P. Zhou, X. Y. Wan, N. Li, B. Tang. *Chem. Sci.*, 2020, **11**, 6882.

4. P. Gao, J. Q. Yin, M. Z. Wang, R.Y. Wei, W. Pan, N. Li, B. Tang. *Anal. Chem.* 2022, **94**, 13293-13299.

5. P. Gao, R. Y. Wei, Y. Y. Chen, X. H. Liu, J. Zhang, W. Pan, N. Li, B Tang. *Anal. Chem.* 2021, **93**, 13734-13741.

6. X. X. Shi, H. Xu, Y. N. Wu, Y. M. Zhao, H. M. Meng, Z. H. Li, L. B. Qu, J. *Anal. Test.*, 2021,**5**,165-173.

7. S. J. Chen, H. X. Yan, Q. K. Tang, Y. W. Gu, J. Zhang, Y. W. Yang, H. L. Wang, Z. S. Qian, L. Li, L. H. Guo, Y. B. Zeng, *Microchem. J.*, 2025, **215**, 114197.

8. P. Gao, K. Tang, R. X. Lou, X. H. Liu, R.Y. Wei, N. Li, B. Tang. *Anal. Chem.* 2021, **93**, 12096-12102.

9. P. Gao, X. Y. Shen, X. H. Liu, Y. Y. Chen, W. Pan, N. Li, B. Tang, *Anal. Chem.* 2021, **93**, 11751-11757.

10. P. Gao, X. Y. Shen, X. H. Liu, B. J. Cui, M.Z. Wang, X. Y. Wan, N. Li, B. Tang, *Anal. Chem.* 2021, **13**, 41498-41506.

11. J. Cui, L. Kan, Z. Z. Li, L. Y. Yang, M. H. Wang, L. H. He, Y. F. Lou, Y. L. Xue, Z. H. Zhang, *Talanta*, 2021, **228**, 122060.