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Electronic Supplementary Information

A COF-Based Anti-Interference Nanoplatform for Intracellular Nucleic Acid Imaging

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Reagents and Materials. 5,10,15,20-tetrakis(4-aminophenyl)-21H,23H-porphine (Tph) and 2,5-dihydroxyterephthalaldehyde (Dha) were obtained from Changchun Third Party Pharmaceutical Technology Co. Ltd. Deoxyribonuclease I (DNase I), bovine serum albumin (BSA), glutathione (GSH), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), were purchased from Solarbio Science and Technology (Beijing, China). Graphene oxide (GO) was purchased from Nanjing XFNANO Materials Tech. Co., Ltd (Nanjing, China). Hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 4H_2O$, 99.99%), $NaBH_4$, trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), NaCl and $MgCl_2$ were purchased from China National Pharmaceutical Group Corporation (Shanghai, China). DNA oligonucleotides were synthesized and purified by Sangon Biotechnology Co., Ltd (Shanghai, China) and the sequences of these oligonucleotides are shown in Table S1. 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) was purchased from Sigma Chemical Company. Confocal dish was purchased from Cellvis, Mountain View, CA. The HepG2 and HL-7702 cells were purchased from Procell (Wuhan, China). All the other chemical reagents were of analytical grade and used without further purification.

Apparatus. Fourier infrared spectrometer (Nicolet iS50 FT-IR) was used to characterize the infrared spectrum. Powder X-ray diffraction (XRD) pattern was obtained on a Rigaku SmartLab SE X-Ray Powder Diffractometer with Cu Kα line focused radiation (λ = 1.5405 Å). Transmission electron microscopy (TEM, HT7700, Japan) was carried out to characterize the morphology of the nanoparticles. UV-vis spectroscopy was achieved with UV-1700 (Shimadzu, Japan). Fluorescence spectra were obtained using a FLS-980 Edinburgh Fluorescence Spectrometer with a Xenon lamp. The absorbance was measured in a microplate reader (Synergy 2, Biotek, USA) for the MTT assay. Confocal fluorescence imaging studies were performed using a TCS SP8 confocal laser scanning microscope (Leica, Germany). Imaging flow cytometry was accomplished on Amnis ImageStream MarkII (Merck Millipore, Seattle, WA). Centrifuge 5424 R (Eppendorf, Germany) was employed to collect nanoparticles from solutions.

Preparation of COF NPs. The porphyrin COF was prepared by mixing Dha (10 mg, 0.06 mmol) and Tph (20.3 mg, 0.03 mmol) in dichlorobenzene/butyl alcohol/6 M acetic acid (5/5/1, v/v/v, 1.7 mL). After sonication for 10 min, the mixture was degassed in a Pyrex tube (20 mL) through freeze-pump-thaw cycles for three times and then sealed off. The tube was heated 3 days at 120 °C. After that, the product was collected and washed with 20 mL of THF, acetone for three times. The COF NPs was prepared by disperse COF into an agate mortar and manual grinded for 120 min and further collected via differential centrifugation (4000 rmp for 10 min, collect the supernatant to remove large COF, and 10000 rmp, 20 min, collect the precipitation to obtain the nanoscale COF NPs). The obtained COF NPs were redispersed in H_2O for further use.

Fluorescence spectra of Tph and COF NPs. Tph ethanol solution and COF NPs aqueous solution were prepared, the concentration of Tph were fixed to be 1 μg/mL. The fluorescence properties of Tph and COF NPs were further recorded with a fluorescence spectrophotometer.

Preparation of COF-TK1. COF-TK1 was prepared by directly mixing Cy5-labeled recognition sequence solution with COF NPs. For optimizing the concentration ratio, the recognition sequence (50 nM) was mixed with different concentration of COF NPs (0-400 μg/mL). the obtained solutions were further stirred for 15 min and then the fluorescence intensities of the solutions were recorded with fluorescence spectrophotometer.

Preparation of GO-TK1. GO-TK1 was prepared by directly mixing Cy5-labeled recognition sequence solution with GO solution. For optimizing the concentration ratio, the recognition sequence (50 nM) was mixed with different concentration of GO (0-30 μg/mL). the obtained solutions were further stirred for 15 min and then the fluorescence intensities of the solutions were recorded with fluorescence spectrophotometer.

Preparation of Au-TK1. 13 nm AuNPs were prepared by injecting 1.8 mL of trisodium citrate (1 %) solution into 100 mL of boiling HAuCl4 (0.01 %) solution under vigorous stirring. The solution color changed from pale yellow to colorless and finally to burgundy, then the solution was kept boiling for another 10 min and cooled to obtain the AuNPs solution. Au-TK1 was prepared by adding TCEP activated TK1 molecular beacon into a solution of AuNPs (1 nM) and shaken for 12 h. Then SDS (10 %) was mixed with the solution to reach a final concentration of 0.1 %. The phosphate buffer (0.1 M, pH 7.4) containing 1 M NaCl was added to the mixture over an eight-hour period to achieve 0.01 M phosphate concentration and 0.1 M NaCl final concentration. The solution was further aged for 48 h at room temperature. Then Au-TK1 was acquired by centrifugation (13500 g, 30 min) and resuspended in PBS buffer for three times. Finally, the Au-TK1 solution was stored at 4 °C after sterilizing by a 0.22 μm acetate syringe filter.

Storage stability. To evaluate the storage stability of the nanoplatforms, COF-TK1, GO-TK1 and Au-TK1 were dispersed in PBS and 1640 solutions, the fluorescence properties of the solutions were recorded for a week.

Kinetics Assay. TK1 target was added into the COF-TK1 (120 μg/mL), GO-TK1 (10 μg/mL) and Au-TK1 (1 nM) buffer solutions, then the fluorescence signal of the solution at different timepoints were recorded with a fluorescence spectrophotometer.

TK1 Detection and Specificity Experiments. To evaluate the detection performance and specificity of COF-TK1, GO-TK1 and Au-TK1, different targets including T-mRNA survivin, T-TK1, T-miR-T21 and T-miR221 were added into the solutions. After 30 min's incubation, the fluorescence properties of the solutions were recorded with a fluorescence spectrophotometer.

Nuclease Assay. To evaluate the nuclease stability of COF-TK1, GO-TK1 and Au-TK1, DNase I was incubated with the probe directly. At different timepoints of 1, 2, 3, 4 and 5 h, the fluorescence property of the solution was detected and compared with the original solution. At 5.5 h, target survivin was further added into the two solutions and the fluorescence intensities were recorded after 30 min.

Anti-interference effects. To evaluate the anti-interference effects of COF-TK1, GO-TK1 and Au-TK1. BSA (1 mg/mL) or the TK1 target were added into the solution of COF-TK1 and GO-TK1 solutions, the fluorescence intensities were recorded for 4 h. GSH (5mM) or TK1 target were added into the COF-TK1 and Au-TK1 solutions, and the fluorescence intensities were recorded for 8 h.

MTT assay. To investigate the biocompatibility of the COF-TK1, GO-TK1 and Au-TK1, MTT assay was carried out. HepG2 cells were dispersed within 96-well microtiter plates at 37 °C for 24 h. After that, different concentrations of COF-TK1, GO-TK1 and Au-TK1 were incubated with the cells for 24 h. Then, the MTT solution (150 μL, 0.5 mg/mL in PBS) was further added to each well for another 4 h. After removing the remaining MTT medium, 150 μL of DMSO was added to each well. The absorbance was measured at 490 nm with microplate reader.

Confocal fluorescence imaging. For evaluating the cell imaging effect of the nanoplatforms, HepG2 and HL-7702 cells were cultured in confocal dishes. Then COF-TK1, GO-TK1 and Au-TK1 dissolved in culture medium was added into the dishes (the concentration of DNA recognition

sequence was same) and further incubated for 4 h. Finally, the cells were washed with PBS and further imaged on a laser scanning confocal microscope. To further compare the anti-interference effect of the probes, HL-7702 cells cultured in confocal dishes were preincubated with GSH (5 mM), BSA (1 mg/mL) or the both. Then the cells were incubated with different probes for 4 h, and then washed with PBS for confocal imaging.

Flow cytometry analysis. The cell imaging and anti-interference effects of the nanoplatforms were also quantitative analyzed by flow cytometry analysis. The cells cultured in the culture dishes were treated with the same conditions for confocal fluorescence imaging. Then the cells were digested and washed with PBS for three times. Finally, the cells were analyzed on a flow cytometer.

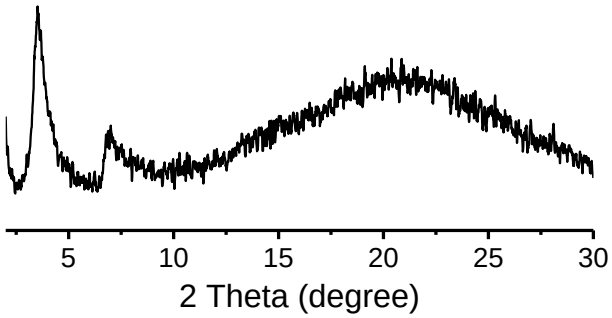

Figure S1. The PXRD pattern of the COF NPs.

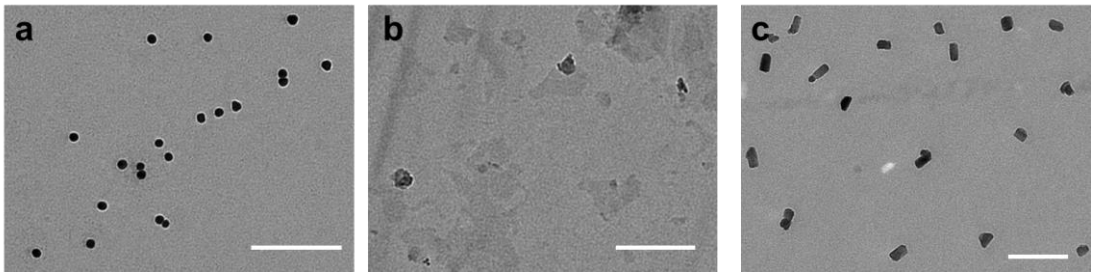

Figure S2. TEM image of (a) AuNPs, (b) GO, and (c) COF NPs, scale bars: 200 nm.

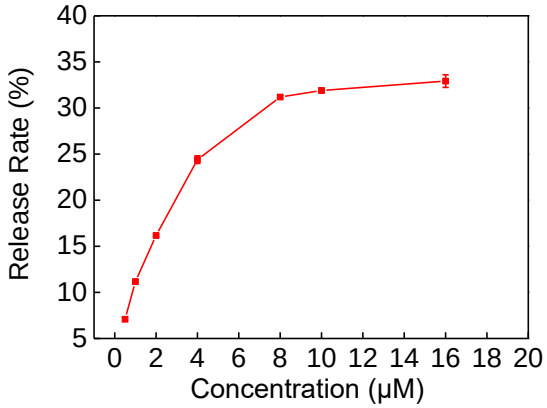

Figure S3. DNA release profiles of COF-TK1 in the presence of excessive non-dye labeled TK1 ssDNA.

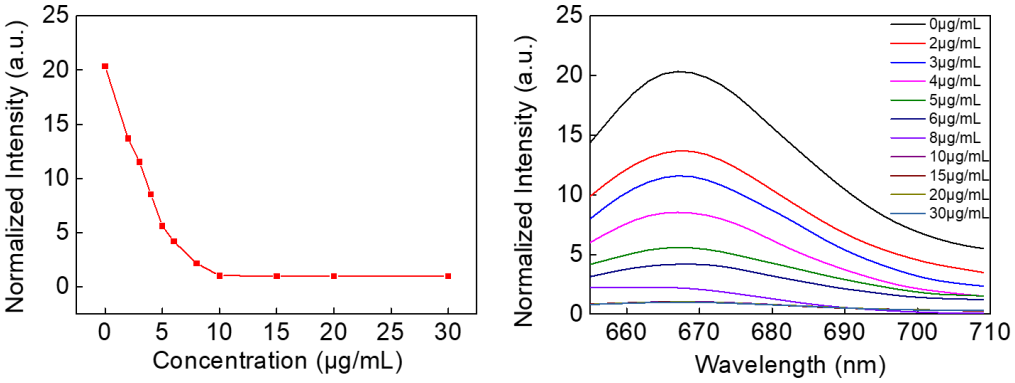

Figure S4. Left: fluorescence quenching of Cy5-labeled TK1 recognition sequence by different concentrations of GO. Right: fluorescence spectra of Cy5-labeled TK1 recognition with different concentrations of GO.

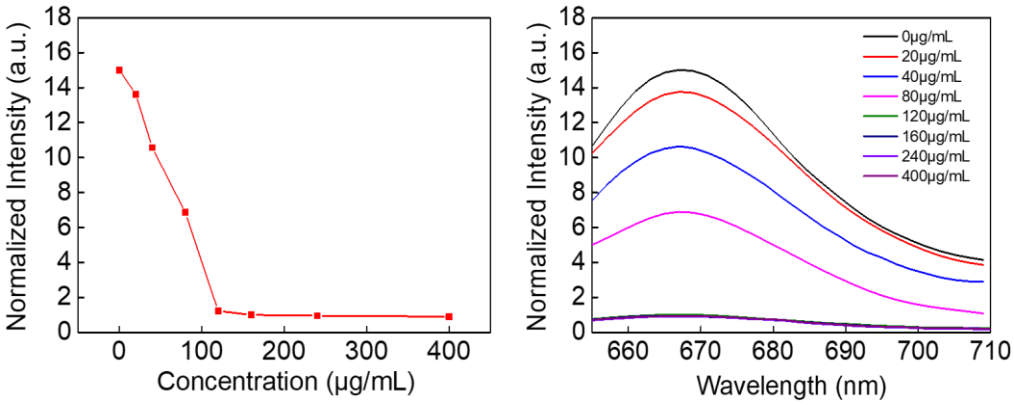

Figure S5. Left: fluorescence quenching of Cy5-labeled TK1 recognition sequence by different concentrations of COF NPs. Right: fluorescence spectra of Cy5-labeled TK1 recognition with different concentrations of COF NPs.

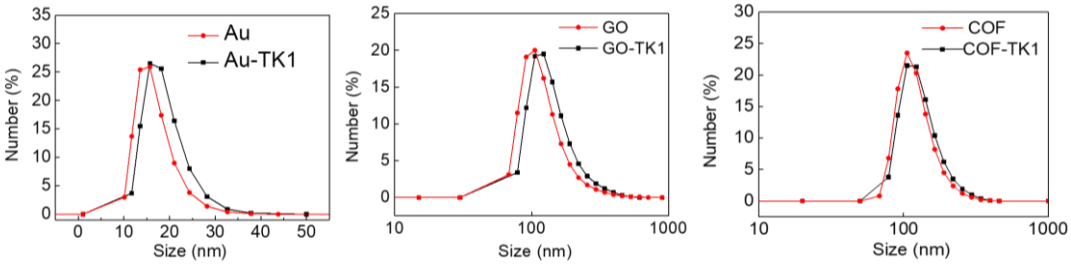

Figure S6. DLS size distribution of Au/Au-TK1, GO/GO-TK1, and COF/COF-TK1.

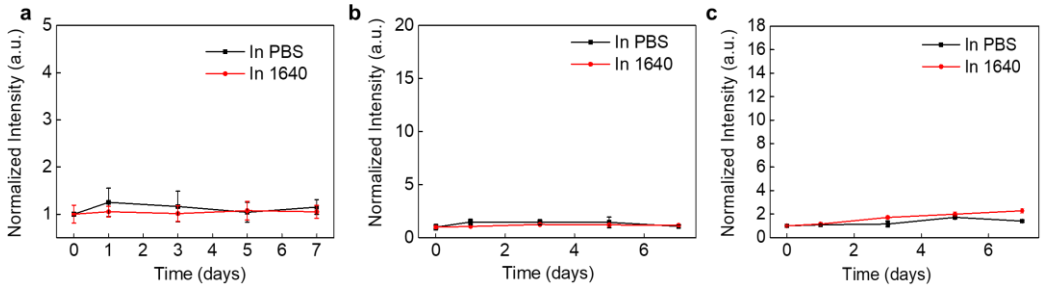

Figure S7. The relative fluorescence intensity changes of (a) Au-TK1, (b) GO-TK1, and (c) COF-TK1 in PBS or 1640 buffer for 0-7 d.

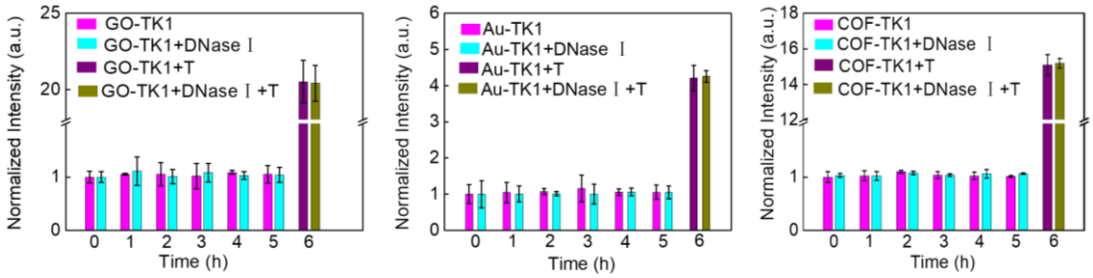

Figure S8. The anti-DNase I effect of three nanoplatforms. Relative fluorescence intensity changes of Au-TK1, GO-TK1 and COF-TK1 in the presence of DNase I and the TK1 target (T).

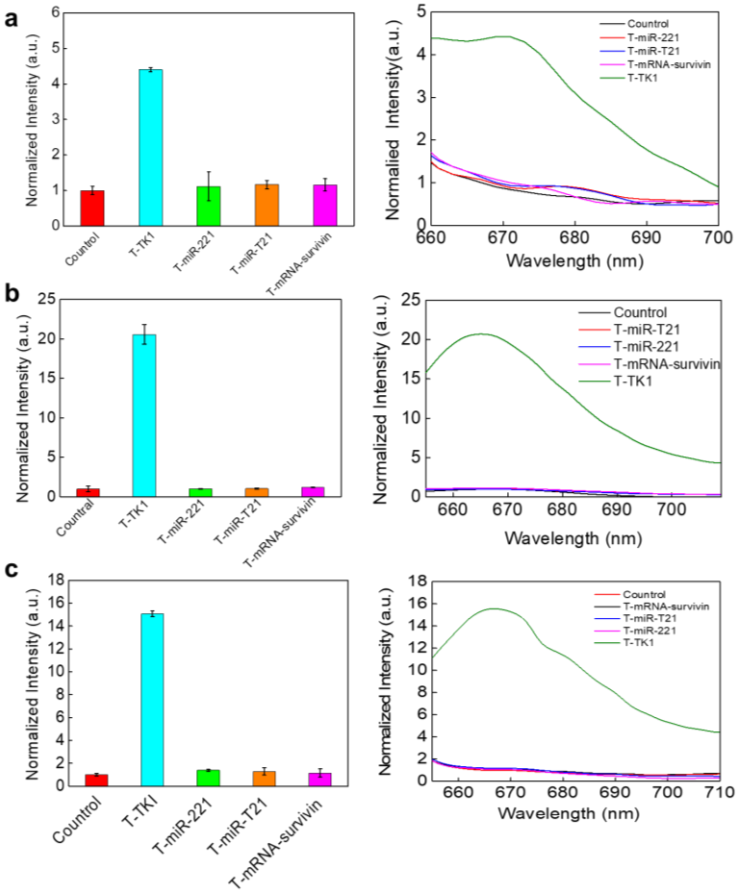

Figure S9. The fluorescence signal recovery of (a) Au-TK1, (b) GO-TK1 and (c) COF-TK1 in the presence of different targets. The left column are the normalized fluorescence intensities, the right column are representative fluorescence spectra.

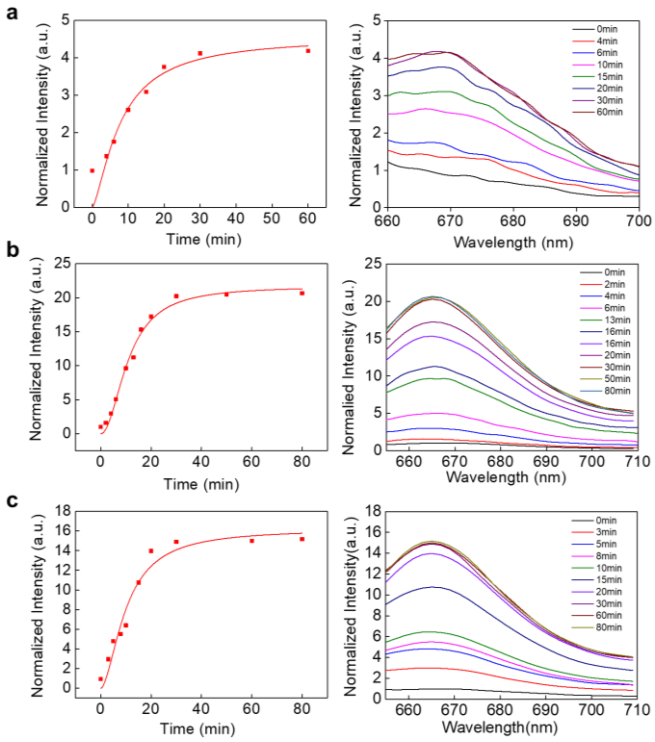

Figure S10. Time-dependent fluorescence changes of (a) Au-TK1, (b) GO-TK1, and (c) COF-TK1 in the presence of the TK1 target. The left column are the normalized fluorescence intensities, the right column are the representative fluorescence spectra.

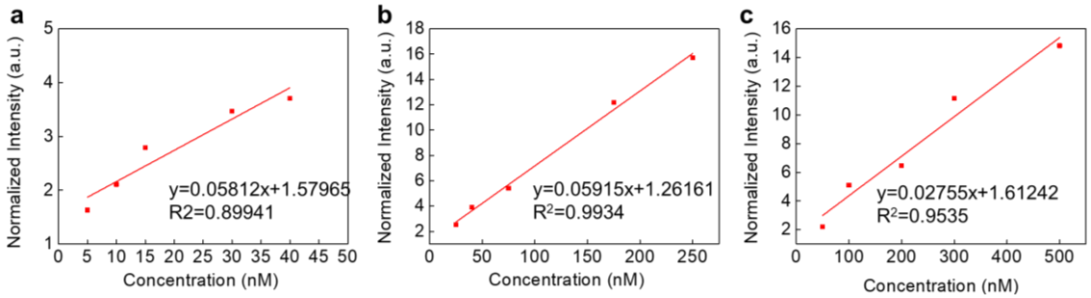

Figure S11. The standard linear calibration curves of fluorescence intensities versus target concentrations for (a) Au-TK1, (b) GO-TK1 and (c) COF-TK1.

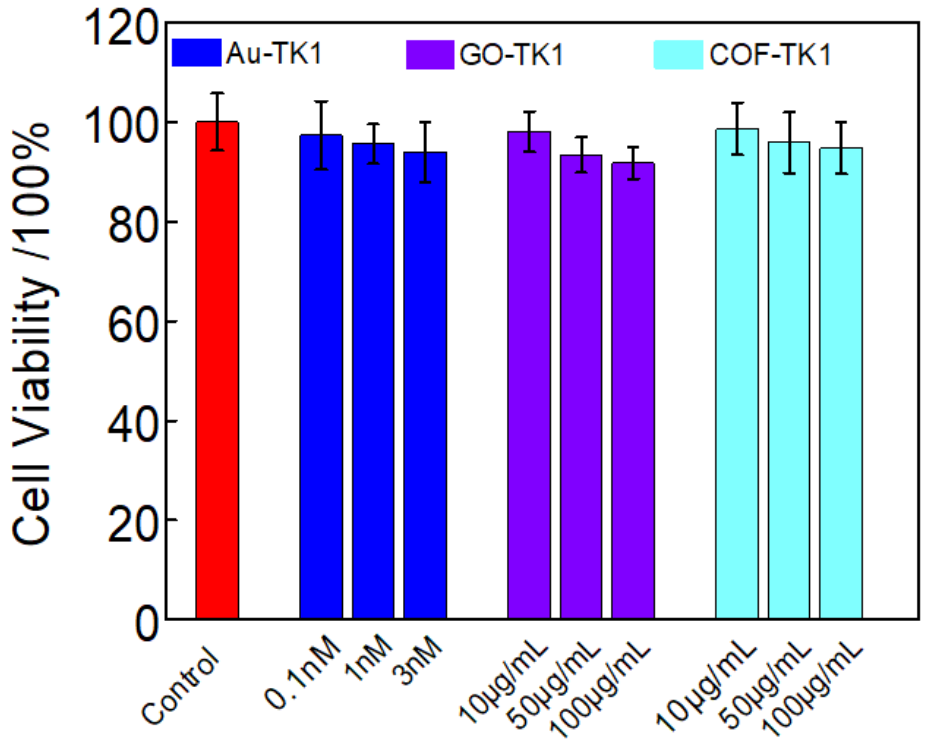

Figure S12. Cell viabilities of HepG2 cells after incubation with different concentrations of Au-TK1, GO-TK1, and COF-TK1 for 24 h.

Table S1. Oligonucleotide sequences employed in this work.

Oligonucleotide	**Sequence** (5′-3′)
TK1 recognition sequence	Cy5- GCGAGTGTCTTTGGCATACTT
TK1 ssDNA	GCGAGTGTCTTTGGCATACTT
TK1 molecular beacon	Cy5-ACGACGCGCGAGTGTCTTTGGCATACTTCGTCGTAAAAAA-SH
T-TK1	AAGTATGCCAAAGACACTCGC
T-miR-221	AGCTACATTGTCTGCTGGGTTTC
T-miR-T21	TAGCTTATCAGACTGATGTTGA
T- mRNA survivin	GACCACCGCATCTCTA