Electronic Supplementary Material

R11 peptides can promote the molecular imaging of spherical nucleic acids for bladder cancer margin identification

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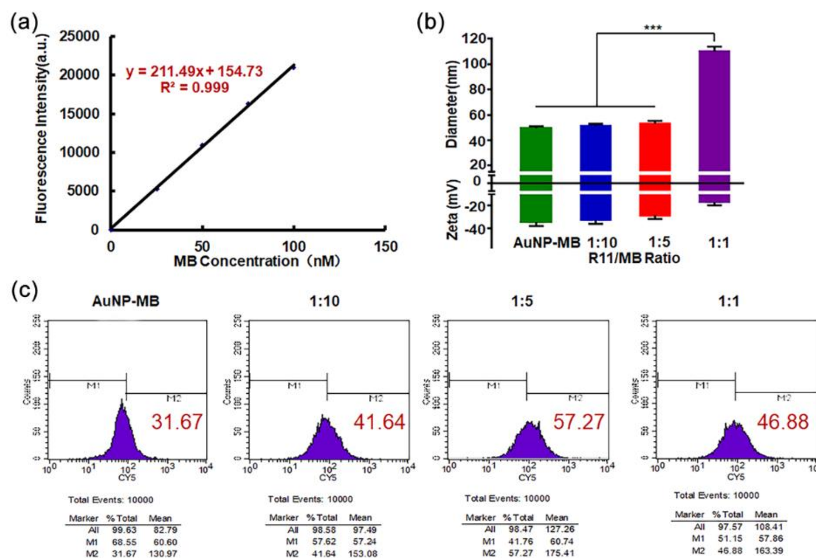

Figure S1 (a) The calibration standard curve of MB concentration. (b) The diameter and zeta potential of AuNP-MB and AuNP-MB@R11 with different N/P ratios of 1:10, 1:5 and 1:1. (c) The mean fluorescence intensity data from flow cytometry of 253J cells treated with AuNP-MB and AuNP-MB@R11 with different R11/MB ratios of 1:10, 1:5 or 1:1, respectively.

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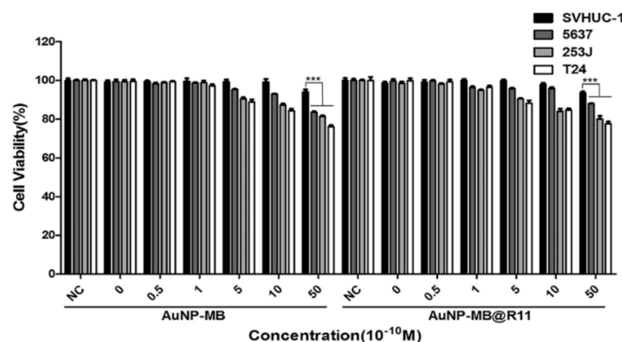

Figure S2 The cytotoxicity in T24, 253J, SVHUC-1, and 5637 cell lines incubated with 1 nM AuNP-MB or AuNP-MB@R11 using MTT.

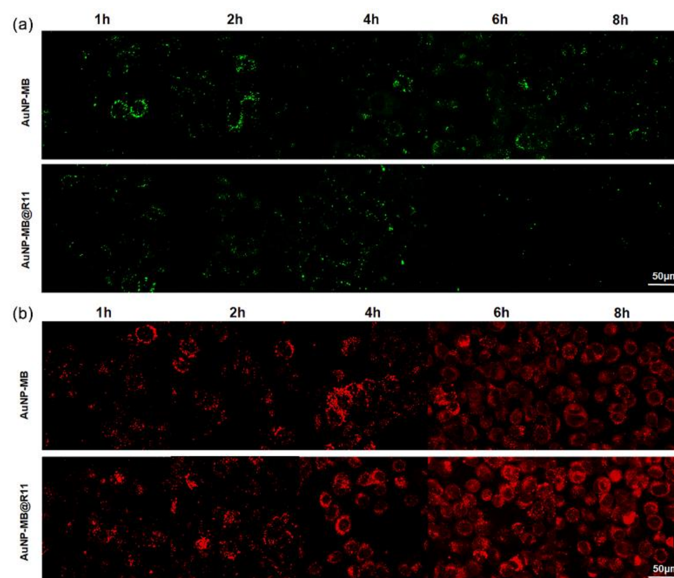

Figure S3 (a) 5637 Cells treated with AuNP-MB and AuNP-MB@R11 for 1, 2, 4, 6, 8 h were stained in lysotracker for the visualization of lysosome in green. (b) 5637 Cells treated with AuNP-MB and AuNP-MB@R11 for 1, 2, 4, 6, 8 h for the visualization of AuNP-MB and AuNP-MB@R11 in red.

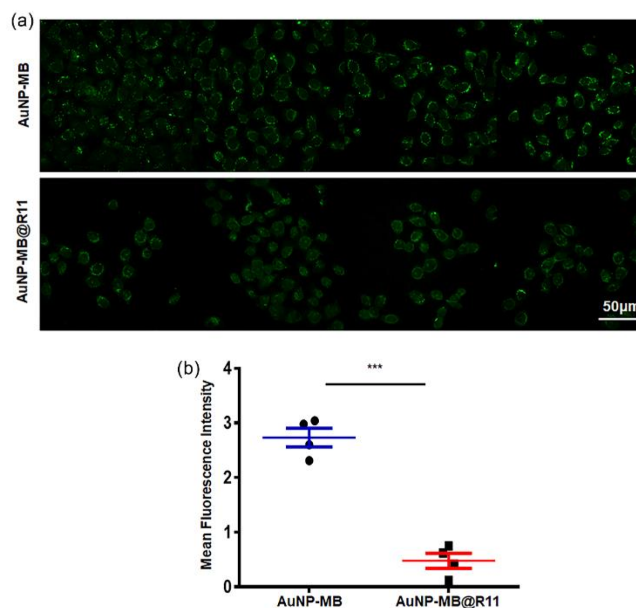

Figure S4 (a) 5637 Cells treated with AuNP-MB and AuNP-MB@R11 for 6 h were stained in lysotracker for the visualization of lysosome. (b) The relative fluorescence density of 5637 Cells treated with AuNP-MB and AuNP-MB@R11 for 6 h. All data were calculated and compared by the image processing software Image J. Significance was attributed for $P<0.001$.

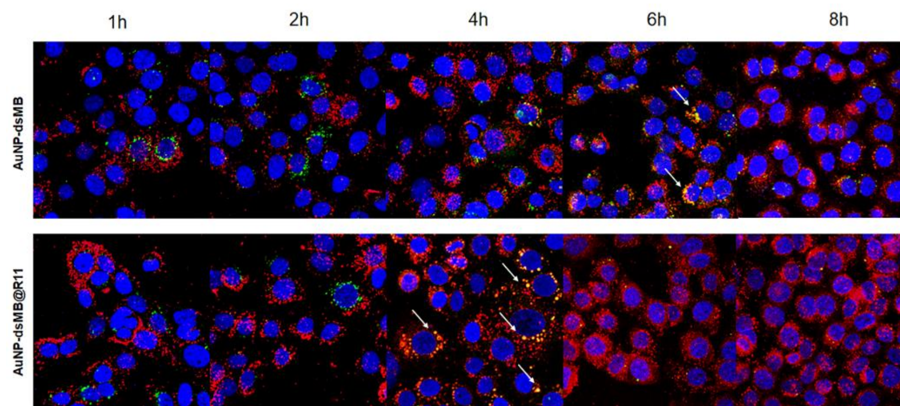

Figure S5 5637 cells treated for 1, 2, 4, 6, 8 h were stained with DAPI for the visualization of the cell nuclei in blue, Cy5 for the visualization of AuNP-dsMB and AuNP-dsMB@R11 in red and lysotracker for the visualization of lysosome in green.

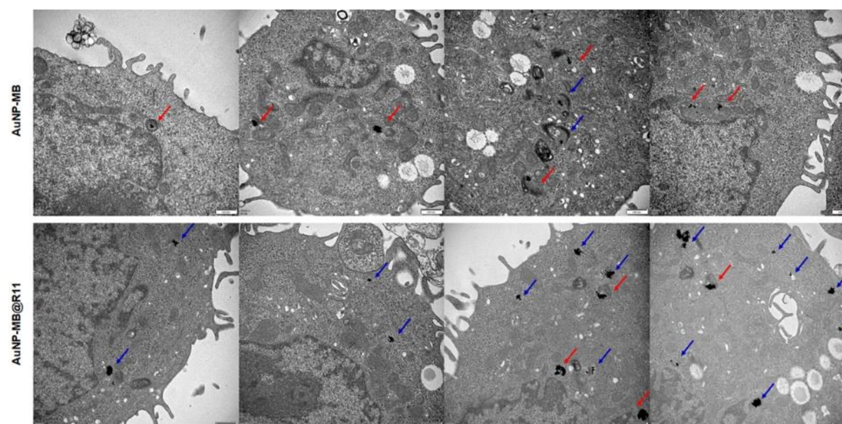

Figure S6 TEM of 5637 cells treated with AuNP-MB and AuNP-MB@R11 for 6 h. Red arrows represented the nanoparticles in endo/lysosome and blue arrows represented those in cytoplasm.

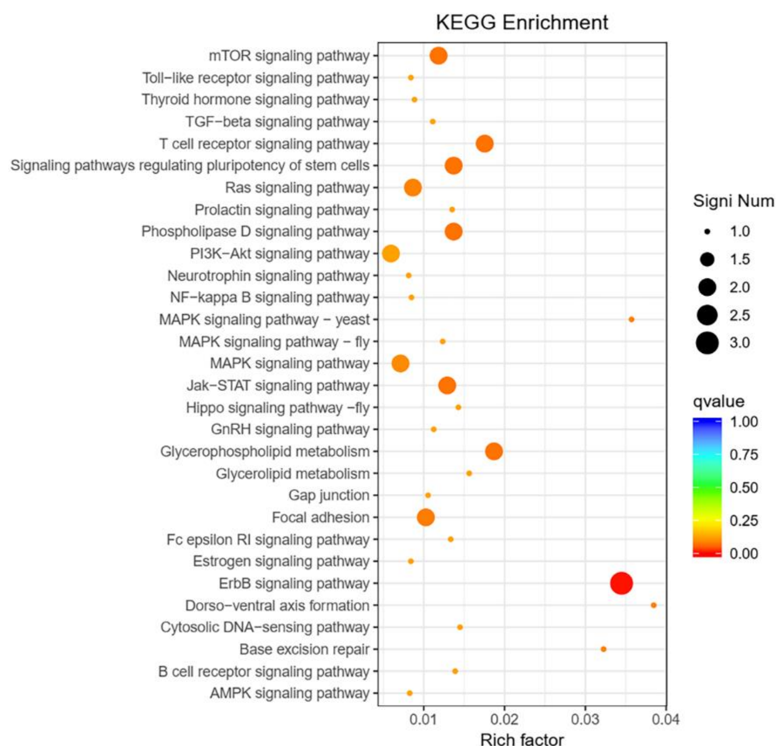

Figure S7 Different signaling pathways between cells treated with AuNP-MB or AuNP-MB@R11 for 4 h based on KEGG analysis. The vertical axis represents the pathway annotation information, and the horizontal axis represents the Rich Factor corresponding to the function (the number of different genes divided by the number of total genes). The QValue is represented by the color of the dot. The number of different genes contained in each function is represented by the size of the dot.

Table S1 Sequences of peptides and oligonucleotides used in this study

Name	Sequence
R11 Peptide	N-Gly-R-R-R-R-R-R-R-R-R-R-R-C
MB	5'Cy5-CGACG GAGAAAGGGCTGCCA CGTCG-3'SH
dsMB	5'Cy5-CGACG GAGAAAGGGCTGCCA CGTCG-3'SH 3'-CTC TTT CCCGACGGT-5'
Target sequence	5'- TGGCAGCCCTTTCTC -3'
Single-base(C) MS	5'- TGGCCGCCCTTTCTC -3'
Single-base(T) MS	5'- TGGCTGCCCTTTCTC -3'
Single-base(G) MS	5'- TGGCGGCCCTTTCTC -3'
The primer of Survivin mRNA, forward	5'-AGGACCACCGCATCTCTACAT -3'
The primer of Survivin mRNA, reverse	5'-AAGTCTGGCTCGTTCTCAGTG -3'
The primer of GAPDH mRNA, forward	5'-GGAGCGAGATCCCTCCAAAAT -3'
The primer of GAPDH mRNA, reverse	5'-GGCTGTTGTCATACTTCTCATGG -3'

Experimental Section

1.1 Reagents and Material

DNA oligonucleotides were synthesized and purified by Sangon Biological Technology (Shanghai, China). The MBs are modified by Cy5 at the 5' end and thiol at the 3' end. R11 peptides were synthesized and purified by GL Biochem Ltd. (Shanghai, China). The sequences of these oligonucleotides and peptides are shown in Table S1. Hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 4H_2O$, 99.99%), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP-HCl), dithiothreitol (DTT) and dimethyl sulphoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, USA); deoxyribonucle-ase I (DNase I), the PrimerScript RT reagent kit and SYBR Green Master Mix were purchased from TAKARA Biotechnology Co. (Dalian, China), and trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), Sodium dodecylsulfate (SDS), NaCl, Na_2HPO_4, and NaH_2PO_4 were purchased from China National Pharmaceutical Group Co. (Shanghai, China). TRIzol and MTT reagent was purchased from Life Technologies Co. (Carlsbad, USA). Lysotracker was purchased from ThermoFisher Co. (Shanghai, China). All the chemicals were of analytical grade and were used without further purification. Ultrapure water (18.2 MΩ) was used through-out the experiments.

1.2 Preparation of AuNP-MB and AuNP-MB@R11

The gold nanoparticles with average diameter of 23 nm were synthesized by the method developed by Frans (1973). Gold nanoparticles were used to obtain pure and sterilized stock solutions consisting of the nanoprobe resuspended in phosphate buffer (pH=7.0, 0.01 M) with a concentration of 1 nM at 4 °C.Then 20 μl of the above Cy5-labeled nanoprobe and 80 μl H_2O was added into 100 μl of 1 M DTT. The mixture was incubated at room temperature with shaking overnight, followed by centrifugation at 15000 rpm to remove any gold precipitate. 150 μl of the supernatant was placed in a 96-well plate and the fluorescence signals (excitation: 646 nm; emission filter: 667 nm) were measured using a Multi-Mode Microplate Reader (SynergyMX; Bio-Tek Instruments Inc., Winooski, USA). The concentration of oligonucleotide was determined by comparing to a calibration standard curve. Based on the concentration of oligonucleotide above, different concentration of R11 peptides with R11/MB ratios of 1:1, 1:5 and 1:10 were added to the nanoprobe solution. Then the mixtures were incubated at room temperature for 2 h to obtain a series of nanoprobes with different concentrations, which were stored at 4 °C for further research.

1.3 Physicochemical Characteristic

The morphology of AuNP were observed using transmission electron microscopy (TEM) (Model H7650; Hitachi Ltd., Tokyo, Japan). Ultraviolet-visible light (UV-vis) absorption spectra were recorded on a spectrophotometer (Beckman DU800; Beckman Coulter Inc., Atlanta, USA), using disposable polyacryl cuvettes. The size distribution and Zeta potential were analyzed by Zeta-sizer (Malvern Zeta-sizer; Malvern Ins. Ltd., Malvern, UK) at 25 °C in water.

1.4 Cell Viability Assay

Seeded in four 96-well plate at a population of 5000 cells per well, SVHUC-1, T24, 253J, 5637 cells were incubated with a serious concentration of 0, 0.5, 1, 5, 10, 50×10^{-10} M of AuNP-MB and AuNP-MB@R11 for 24 h. After that, 20 µl of MTT stock solutions (5 mg/mL MTT in PBS) mixed with 180µl of complete DMEM or 1640 medium was added into each well of cells. After 3 h, 120 µl of DMSO was further added into each well followed by thorough removing the supernatant. Then the absorbable was measured by a microplate reader at a wavelength of 490nm after the 96-well plates were shaken for 10 min. Cell viability was expressed as a percentage relative to the control gropes after subtraction of the background absorbance.

1.5 Real-time Quantitative PCR

Real-time quantitative PCR was performed to evaluate the expression of survivin mRNA. SVHUC-1, T24, 253J, 5637 cells were seeded into 6-well plates and cultured in growth medium overnight. Then the medium was replaced with DMEM media containing AuNP-MB and AuNP-MB@R11 (1 nM) respectively. After 12 h, the total RNA was extracted using TRIzol reagent following the manufacture's protocol. Subsequently, cDNA was synthesized using a PrimerScipt RT reagent kit (Takara Bio). In addition, the relative levels of the target gene mRNA transcript were measured using RT-PCR (C1000 Thermal Cycler; Bio-Rad Laboratories Inc., Hercules, USA). All of the samples were run in triplicate and normalized to GAPDH.

1.6 Transmission Electron Microscope (TEM)

To prepare microtome samples of cells, 5637 cells were incubated with AuNP-MB and AuNP-MB@R11 (1 nM) for 6 h. After that, cells were carefully washed with sterile PBS twice and transferred into 1.5 mL eppendorf tubes. Collected cell pellets were resuspended in a solution of 4 vol % paraformaldehyde and 1.25 vol % glutaraldehyde and were fixed overnight. Then, samples were postfixed in a 2 vol % osmium tetroxide solution for 45 min. After this, cells were fully dehydrated and embedded in epoxy resin. Ultrathin sections of 70 nm were cut and poststained with uranyl acetate and lead citrate. Cell samples were analyzed by TEM at 100 kV.