Supporting information

Radionuclide-labeled Gold Nanoparticles for Nuclei-targeting Internal Radio-immunity Therapy

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Experiments and Methods

Materials. Chemical agents: Chloroauric acid ($HAuCl_4$, AR), l-amino-9-octadecene ($CH_3(CH_2)_7CH=CH(CH_2)_7CH_2NH_2$, oleylamine (OM), tert-Butylamine borane (C_4H_4BN), amino-poly (ethylene glycol)-thiol ($HS\text{-}PEG_{2000}\text{-}NH_2$, MW = 2000 Da), 1,3,4,6-Tetrachloro-3α, 6α-diphenylg lycouril ($C_{16}H_{10}C_{l4}N_4O_2$, Iodogen), dichloromethane (CH_2Cl_2), 3-(4-hydroxybenzene)-succinimide propionate ($C_{13}H_{13}NO_5$, Bolton-Hunter reagent), dimethylsulfoxide (DMSO), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimine (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich Chemical Co., Ltd.. Trichloromethane ($CHCl_3$) was obtained from Sinopharm Chemical Reagent Co., Ltd.. $Na^{131}I$ solution was provided by Shanghai Xinke Pharmaceutical Co., Ltd.. All above agents were of analytical grade for direct use without extra purification. **Bioreagents:**

Phosphate buffered saline (PBS), dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were supplied by GIBCO (Grand Island, New York, USA). Deionized water was obtained from a Milli-Q system (Health Force Bio-meditech Holdings Ltd.). Cell counting kit-8 (CCK-8) and reactive oxygen species (ROS) assay kit was provided by Shanghai Yeasen Biotech Co., Ltd.. γ-H2AX kit and all antibodies for immunohistochemical analysis, Polymerase Chain Reactions (PCRs) and western blotting assay were supplied by Wuhan servisebio technology Co., Ltd. All antibodies for *in vivo* flow cytometry were provided by Cell Signaling Technology Inc., Beverly, MA, USA. Fluorescein isothiocyanate (FITC), 4',6-diamidino-2-phenylindole (DAPI), paraformaldehyde and glutaraldehyde were purchased from Sigma-Aldrich Chemical Co., Ltd.. TAT peptide (Met-Asp-Pro-Val-Asp-Pro-Asn-Iie-Glu, Mw = 1029.1) and Annexin V-FITC/PI apoptosis detection kit were furnished from Sangon Biotech Co., Ltd., Shanghai. Giemsa stain was provided by Sinopharm Chemical Reagent Co., Ltd..

Synthesis of PEGylated AuNPs and AuNPs@PEG-TAT. $HAuCl_4$ (0.5 g) was dispersed in $CHCl_3$ (100 mL), and then added with oleylamine (15 mL). The solution changed to be clearly orange-red under ultrasonication, and was magnetically stirred under the protection of nitrogen at 25 °C. Next, tert-butylamine borane (0.25 g) was mixed for another 20 min stirring. The action was terminated with the addition of ethanol (200 mL), followed by centrifugation at 12,000 revolutions per minute (rpm) for 10 min and finally dissolved in $CHCl_3$ (50 mL).

For PEG modification of AuNPs, HS-PEG_{2000}-NH_2 (60 mg) was added into AuNPs solution (50 mL), and adequately mixed with supersonic treatment before stirring overnight. Therewith, $CHCl_3$ was removed by rotary evaporation under room temperature, and the collection was washed by ethanol (50 mL) and dried in a lyophilizer. Finally, the obtained production was re-suspended in ethanol solution (50 mL) and stored at 4 °C for further use.

To make TAT peptide grafted onto the surface of amino-functionalized AuNPs, TAT (200 μg, 0.2 mmol), EDC (38 mg) and NHS (57 mg) were blended in deionized water (3 mL) and stirred for 5 min, referring to the dosage in a published study.[1] The pre-synthesized AuNPs (1 mL, 2 mg mL^{-1}) was dropwisely added into the reaction

system for continuous stir upto 24 h at room temperature. After eliminating the excess TAT by centrifugation and washing twice in water, the final TAT-grafted AuNPs (AuNPs-TAT) nanoparticles were dispersed in deionized water (3 mL) for next experiments.

Characterization of AuNPs and AuNPs-TAT. Morphology of AuNPs was characterized by transmission electron microscope (TEM, JEOL-200 CX) operated at 200 kV. X-ray diffraction (XRD) patterns were performed utilizing a Rigaku D/MAX-2250V diffractometer, under Cu Kα (λ = 0.154056 nm) radiation at a scanning speed of 10° min^{-1} in 2θ range of 20 – 70°. The size distribution of AuNPs and AuNPs-TAT were measured by dynamic light scattering (DLS) using a Nanotrac Wave II Q Nanoparticle Size Analyzer (Microtrac, America). Fourier transform infrared (FT-IR) spectra of AuNPs and AuNPs-TAT was obtained by a Bruker TENSOR II FTIR Spectrometer using KBr pellets. Characteristic UV-vis absorption spectra of AuNPs under different concentrations (1000 ppm, 500 ppm, 250 ppm) was acquired by an UV-vis spectrophotometer (UV-3600, Shimadzu, Japan).

^{131}I labeling rate and stability tests. ^{131}I was labeled through a standard Iodogencatalyzed method:[2] Firstly, Iodogen was predissolved in dichloromethane (0.5 mg mL^{-1}), and each Eppendorf (EP) microcentrifuge tube coated with the iodogen solution (0.1 mL, 50 μg) was subpackaged and dried by a drying oven heating up to 60 °C for 2 h. For radiolabeling, Bolton-Hunter reagent (4 μg, 1 mg mL^{-1}, in DMSO) and Na^{131}I solution (1.5 mCi) were sequentially added into an Iodogen tube and were gently shaken for 15 min. The system was then trisected and each part of ^{131}I (500 μCi) was separately added into AuNPs (100 μg, 2 mg mL^{-1}), TAT (10 μg, 1 mg mL^{-1}, in PBS) or equal AuNPs-TAT, respectively. The subsequent reaction lasted for 1 h under the condition of vibration at room temperature, during which the reaction tubes containing AuNPs were intermittently oscillated every 20 min to avoid sedimentation.

For testing the labeling capacity and stability, radio thin layer chromatography (radio-TLC, Mini-scan, B-MS-1000F, Eckert & Ziegler radiaopharma, Inc., MA, USA) with a γ-detector was applied by using Whatman chromatography paper and 0.9%

NaCl solution as the stationary and mobile phase. The in vitro stability of ^{131}I labeled AuNPs, TAT and AuNPs-TAT were tested by co-culturing with PBS, DMEM and serum (80 μL for each) at 37 °C for different periods of time (1.5 h, 3 h, 5 h, 10 h, 15 h, 20 h, 25 h).

Cytotoxicity of AuNPs and AuNPs-TAT. The anthropogenic colon carcinoma (HCT116) cell line was supplied by BoGoo BioTech Co., Ltd., Shanghai, and cultured in high-glucose DMEM supplemented with 10% heat-inactivated FBS in a humidified incubator containing 5% CO_2 at 37 °C. Cells were subcultured every three days after the medium was replaced by fresh complete medium.

The cell toxicity was accessed via the standard CCK-8 method. Briefly, HCT116 cells were seeded into four 96-well plates (1.6×10^4 cells per well) with complete medium (200 μL per well, five replication wells) and sustained overnight at 37 °C. Then, the culture medium was replaced with fresh DMEM containing different concentrations of AuNPs or AuNPs-TAT (AuNPs doubled from 7.8 μg mL^{-1} to 1000 μg mL^{-1}) and went on with additional 24 h and 48 h incubating, respectively. At 3 h before each test, CCK-8 solution (15 μL) was added to each well. Finally, the absorbance value was detected with a iMark™ microplate reader (Bio-Rad, Hercules, CA, USA) at the wavelength of 450 nm, and the cell viability was calculated as: Cell viability (%) = absorbance of experimental group/absorbance of control group × 100%.

Cellular uptake behaviors of AuNPs and AuNPs-TAT. In order to confirm the targeting ability of TAT for cell nucleus, confocal laser scanning microscopy (CLSM) was firstly applied. HCT116 cells were grown on coverslips in six confocal dishes (10^5 cells per dish) to adhere for 24 h. Meanwhile, FITC (2 μL, 1 mg mL^{-1}, dissolved with DMSO) was added to AuNPs and AuNPs-TAT solution (with equal dosage of AuNPs for 300 μg) at 12 h post stirring, and continued to react for another 12 h in dark. The obtained FITC-AuNPs and FITC-AuNPs-TAT was dispersed in DMEM (1 mL per confocal plate) and co-cultivated with HCT116 cells for 2 h, 4 h and 8 h, respectively. Hereafter, cells were rinsed with PBS to remove un-internalized nanoparticles and fixed with 4% paraformaldehyde at 37 °C for 10 min. Cell nucleus were then stained with DAPI (200 μL, dilution 1 : 10, PBS) for 15 min in dark, followed by PBS washing for two times.

Finally, the fixed cells were imaged by a confocal fluorescence microscope (Olympus FV 1000; Olympus, Tokyo, Japan), with FITC and DAPI excited at 633 nm and 488 nm, respectively. Moreover, to avoid the false positive results caused by overlap of the scanning slices, Z-stack projection views containing sequential images along the z-axis of a representative cell were collected by utilizing a fast 3D volume reconstruction algorithm, with the layer thickness of 5 μm.

To further visualize the subcellular localization of nanoparticles, bio-TEM scanning was performed. HCT116 cells grown tobe anchorage-dependent in a six-well cell culture plate (10^5 cells per well) were co-cultivated with AuNPs and AuNPs-TAT (with equal AuNPs concentration at 500 μg mL^{-1}) at 37 °C. Then, the solution was removed at the given time point (2 h, 4 h and 8 h post AuNPs or AuNPs-TAT added) and electron microscopy fixative (2.5% glutaraldehyde) was quickly added for another 1 h incubation in dark. Subsequently, the cells were collected by cell scraper, centrifugated at 2000 rpm for 5 min and fixed again, followed by dehydration, osmosis, embedding and slicing (60 – 80 nm), and finally observed by a bio-TEM system (Hitachi, HT7700, 80.0 kv).

CCK-8 assay of ^{131}I labeled AuNPs, TAT and AuNPs-TAT. HCT116 cells were seeded in sextuplicate (1.6×10^4 cells in 100 μL of DMEM per well) for 24 h adherent growth. Cells were then re-cultivated in FBS-free medium added with free ^{131}I, ^{131}I-TAT, ^{131}I-AuNPs or ^{131}I-AuNPs-TAT at different concentrations (0, 100, 500 and 1000 μCi mL^{-1}) for 24 h at 37 °C, with corresponding dosage ratio of TAT : AuNPs : ^{131}I at 1 : 10 : 50. HCT116 cells viability was determined by CCK-8 assay as described above.

Cellular experiments. Basically, all therapeutic experiments on cellular level except for clone formation assay were conducted based on adherent cells in logarithmic growth phase pre-seeded in six-well culture plates (5×10^5 cells in 1mL DMEM per well) for 24 h at 37 °C. After attachment, the culture medium was discarded, and cells were washed with PBS and re-incubated in FBS-free medium containing the following substances: DMEM alone (control), free Na^{131}I, ^{131}I-TAT, ^{131}I-AuNPs or ^{131}I-AuNPs-TAT, with equivalent usage of ^{131}I (500 μCi mL^{-1}), TAT (10 μg) and AuNPs (100 μg mL^{-1}). After 24 h drug treatments, cells from five groups (three repeated

samples for each group) were immediately harvested by trypsinization, precipitated by centrifugation, resuspended in PBS (50 μL) and stored at 4 °C in EP tubes (1.5 mL) for the following tests (name this as method A).

Cell apoptosis by flow cytometry. As with method A, HCT116 cells (approximately 10^6 per sample) harvested from five groups were resuspended in binding buffer (100 μL) and co-cultured with Annexin V-FITC (5 μL) and propidium iodide (PI, 5 μL) for 15 min in the dark at 25 °C. Separably, cells at early or late stage of apoptosis were distinguished by Annexin V-FITC (green staining, excitation at λex = 488 nm, emission at λem = 525 nm) and PI (red staining, λex = 535 nm, λem = 617 nm). The fluorescence images were analyzed *via* a flow cytometry analyzer (ImageStream mkII, Merck-Millipore, Seattle, WA, USA) after diluted with 400 μL binding buffer. All data were quantified using the IDEAS™ Software (Millipore).

Intracellular ROS detection by flow cytometry. Firstly, 2',7'-Dichlorodi-hydrofluorescein diacetate (DCFH-DA) (1 mL, 10 μM, 1 : 1000 dilution in DMEM) was dropped into the test tubes containing nearly 10^6 cells treated as method A. HCT116 cells were further incubated for 30 min at 37 °C in dark, and were shook up every 5 min to fully expose to the probes. Later on, the excessive probes that escaped from being internalizated by cells were washed away by DMEM. Finally, intracellular ROS level was monitored by flow cytometry (in accordance with above apoptosis test), and quantified according to the fluorescence intensity of DCF (λex = 480 nm, λem = 525 nm), which was the final production of nonfluorescent DCFH-DA after ROS oxidation.

DSBs detection by immunofluorescence analysis. Incubated HCT116 cells as above method (A). After this, 4% paraformaldehyde was used to fix the cells (10^6 per sample in 1.5 mL EP tube) for 20 min. Then, cells were rinsed twice with PBS, harvested by centrifugation (6000 rpm, 6 min) and smeared on coverslips. Afterward, cells were permeabilized with 1% of Triton X-100 (100 μL) in PBS at room temperature for 20 min, followed by thrice PBS washing before blocked with 3% bovine serum albumin (BSA) for 30 min at room temperature. With the BSA removed, a primary antibody, anti-phospho-H2AX antibody (diluted 1 : 200 in PBS) was added for co-incubation overnight at 4 °C. After PBS washing and discarded, cells were

incubated with a secondary antibody, Alexa Fluor 488 goat anti-rabbit IgG (diluted 1 : 400; Servicebio, UK) for another 50 min in dark. The nuclei were counterstained with DAPI for 10 min in dark after rinsed twice by PBS. Finally, cells were thoroughly washed, mounted and visualized with a fluorescence microscope (Nikon Eclipse CI, Japan), with the exciting and emitting light for DAPI at 380 nm and 420 nm, and for FITC-IgG at 480 nm and 530 nm, respectively. Quantitative characterization based on the integral optical density (IOD) of images was performed via the Image-pro plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA). Moreover, the number of γ-H2AX foci in cells were counted mannually, and the value of foci per cell was calculated to illustrate the DSBs damage.

Western blotting analysis. In accordance with method A, HCT116 cells (10^6 per sample) with different treatments were collected. Total cell proteins were extracted by lysing the cells with RIPA buffer (250 μL) supplemented with phosphatase inhibitor cocktail, followed by a microcentrifuge (12,000 rpm at 4 °C for 10 min) after 30 minutes on ice, and total protein lysates were obtained from the supernate. Then, samples were suspended in 10% SDS loading buffer with the ratio of 4 : 1 and boiled for 5 min. Next, proteins were resolved by SDS-polyacrylamide gel electrophoresis and transferred onto a 0.22 μm-thick polyvinylidene fluoride (PVDF) membrane. Afterwards, the obtained membranes were blocked with 0.5% skimmed milk (diluted in 0.5% TBST) on rockers for 1 h at room temperature, and incubated overnight at 4 °C with the primary antibodies of 1 : 750 diluted anti-BAX, anti-Caspase 3, anti-cytochrome c (Cyt c) and anti-ki-67, and 1 : 2000 diluted anti-Bcl-2. After trice washing by TBST (5 min per time), the membranes were probed with corresponding secondary antibodies conjugated with HRP (dilution 1 : 3000) for 30 min before another thrice TBST washing. Moreover, anti β-actin antibody was adopted to test the β-actin protein as loading control. In the end, western blot was performed under chemiluminescence condition using a Epson scanner (Perfection V300, China) and the intensity of bands was quantified by the alphaEaseFC software (Alpha Innotech, San Leandro, Calif).

Cell clone formation assay. To access the influence of various formations on cells

proliferation, HCT116 cells at the logarithmic phase were trypsinized and made into single-cell suspension with FBS-free culture medium. Then, cells of varied counts (100, 200, 500, 1000, 2000) were sorted on a MoFlo XDP Cell Sorter (Beckman Coulter, USA) using a streaming sorting technique, and planked identically into four six-well plates with complete medium for 24 h incubation at 37 °C. Later, the medium was discarded, and the four plates were respectively exposed to DMEM containing ^{131}I, ^{131}I-TAT, ^{131}I-AuNPs or ^{131}I-AuNPs-TAT, each with growing doses of ^{131}I (0, 5, 10, 20 and 40 μCi) for treating increasing number of cells. After 24 h treatment, the solution was replaced by complete medium for continued cell cultivation, with a regular interval of 3 days for medium refreshing. The cultivation was terminated until cell clones were visible to the naked eyes after 14 days post treatments, and then the culture medium was cleaned away by PBS. For clone assay, cells were fixed by 75% ethanol (1.5 mL per well) for 10 min, and stained by 0.5% Giemsa (1.5 mL per well) for 10 min right after the ethanol was removed. Finally, wells were thoroughly cleaned and dried before recorded by a digital camera (Canon 5D Mark III), and the cell colonies with more than 50 cells were counted manually. The whole experiment was performed in triplicate for reliable results. Cell colony efficiency (CE) was determined by the percentage of formed clonies in inoculated cells, and cell surviving fraction (SF) was calculated as the ratio of CE in experimental groups to non-irradiated control group. The sensitivity enhancement ratio (SER) of each therapeutic group to ^{131}I group was also calculated.

Animal modal. All animal studies and experimental procedures were approved by the Committee on Ethics of Biomedicine, Second Military Medical University, and performed following Regulation on the Administration of Laboratory Animals (2017 Revision), which was authorized by Ministry of Science and Technology of the People´s Republic of China. For in vivo experiments, female BALB/c nude mice (5 weeks aged, 18 – 20 g weight) were obtained from Shanghai Jihui Biological Technology Co. Ltd., and raised in specific pathogen-free (SPF) environment with free access to sufficient water and food. For tumor-bearing models, 5×10^6 HCT116 cells suspended in PBS (50 μL) were implanted subcutaneously into the root of right upper

limb of each mouse. At day 10 post inoculation, mice with tumors of about 0.6 cm in maximum length (approximately 90 – 140 mm^3 in volume) were picked out for further operations.

Further, to make a deeper exploration for the immunologic effect in this work, another group of immunocompetent BALB/c mice (Female, 4 weeks) were raised, and subcutaneously implanted with murine CT26 colon cancer cells (BoGoo BioTech Co., Ltd., Shanghai) to build immunocompetent tumor-bearing mice model for further immunological research.

***In vivo* tumor imaging and therapy.** The selected nude mice were randomly divided into five groups after intratumorally injected with DMEM only (control, 10 μL) or DMEM containing free $Na^{131}I$, ^{131}I-TAT, ^{131}I-AuNPs or ^{131}I-AuNPs-TAT, at the equal dosage of ^{131}I (500 μCi) corresponding to AuNPs (100 μg) and TAT (10 μg) per mouse with a multi-point injective method (three uniformly distributed sites in each tumor) by using 10 μL injectors.

For intratumoral drug metabolism, SPECT/CT scanning (Symbia T16, Siemens, Germany) was conducted with the following parameters: for SPECT: pinhole collimator, aperture 6 mm, matrix 128 × 128, energy peak 40 keV, window width 20%, collection counts 5×10^5; for CT: voltage 135 kV, current 40 mA, pitch 1.0, reconstruction thickness 1 mm. The nude mice were firstly anesthetized with 3% pentobarbital (50 – 75 μL per mouse) through intraperitoneal injection and then imaged at different time intervals (2 h, 6 h, 12 h, 24 h, 48 h, 96 h, 168 h) post-injection (P.I.), with ensured sober condition between two scans. Afterward, the radioactivity counts of ^{131}I in tumor and thyroid were measured on the planar images by drawing a 3D region of interest (ROI) for the given mouse, and the maximum uptake value of ^{131}I tracer was recorded.

To evaluate the anti-tumor efficacy of drugs in nude mice, all mice in the five groups (n = 5 for each group) were measured for the maximum tumor length (L) and width (W) by a vernier caliper every three days within 18 days P.I., along with photographing by a digital camera (Canon 5D Mark III). Tumor volumes (V) were calculated ($V = L \times W^2 \times 2^{-1}$) and the relative tumor volumes ($V\ V_0^{-1}$) were given after

divided by the initial volume (V_0) at 0 d P.I.. Tumor volumes in control group were allowed to reach maximum 1000 mm^3 in consideration of the care regulations. Simultaneously, to confirm the security of drugs for mice bodily functions, mice weights were also recorded at the time of tumor measuring. Moreover, to evaluate the long-term influence of various drugs, the survival time of mice in five groups (seven mice per group) was observed until day 60 P.I..

Also, all immunocompetent tumor-bearing BALB/c mice were photographed to record the tumor size at the end of different treatments.

Pathological and immunological experiments. After 18 days of treatment, the random nude mice from each group were sacrificed by overdose anesthetics, and tumors on the mice were surgically excised, fixed in 10% formalin, processed into paraffin sections (3 μm thick), and stained by Haematoxylin and Eosin (H&E) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), aimed at evaluating the pathological morphology changes and apoptosis degree in tumor tissues.

For H&E staining, briefly, tumor slices were mounted on glass slides, dewaxed in dimethylbenzene, dehydrated in graded ethanol, and rinsed with deionized water. Then, the slices were firstly stained by hematoxylin (staining nucleus into blue) for 3 – 5 min, differentiated, washed back to blue, and then dyed with eosin (staining cytoplasm into red) for 5 min after dehydrated in gradient alcohol. Finally, the slices were subjected to ethanol and dimethylbenzene clearing, mounted on neutralresinsize, and detected under an optical microscopy (Nikon Eclipse E100).

For TUNEL assay, the tumor slices mounted on glass slides were firstly dewaxed and dehydrated as done in H&E staining. Then, the tissue was retrieved by proteinase K and permeabilized, and the catalyzer TdT in combination with FITC-labeled dUTP (at the ratio 1 : 9) were added for 2 h staining at 37 °C, before the incubation in hydrogen peroxide (3%) for 15 min in dark. Next, the slices were incubated for 30 min with converter-POD in a humidified box followed by the DAB chromogenic reaction. Furtherly, the tumor sections were counterstained with hematoxylin, differentiated in hydrochloric acid alcohol, and returned to blue

ammonia solution. After dehydration in gradient alcohol, vitrification in dimethylbenzene and mounting on neutralresinsize, all slides were finally checked by an optical microscope (CIC, XSP-C204) under 480 nm excitation and 530 nm emission wavelength, and cells exhibiting brown intracellular particles were considered as positive staining.

For immunological verifications, first, tumor tissue collected from the nude mice was tested. Initially, immunohistochemical staining was conducted to examine the tumor infiltration of immune cells including B cells, Natural Killer (NK) cells, macrophage cells, myeloid-derived suppressor cells (MDSCs) and dendritic cell (DCs), immune cytokines including interleukin-2 (IL-2), IL-12, interferon-gamma (IFN-γ) and tumor necrosis factor (TNF-α), as well as the proliferation index (ki-67) and transcription factor (snail). In detail, similar to most steps in TUNEL assay, the tumor slices were firstly dewaxed and dehydrated, and then retrieved by antigen retrieval buffer (EDTA, pH 9.0) before the 25 min incubation in 3% hydrogen peroxide. Next, after the antigen was blocked with 3% BSA for 30 min at room temperature, the sections were first incubated with PBS diluted rabbit anti-CD19 (1 : 200), anti-NK1.1 (1 : 100), anti-iNOS (1 : 500), anti-CD11b (1 : 500), anti-CD11c (1 : 200), anti-IL-2 (1 : 2000), anti-IL-12 (1 : 400), anti-INF-γ (1 : 200), anti-TNF-α (1 : 100), anti-ki-67 (1 : 100) and anti-snail (1 : 400) antibodies overnight at 4 °C and then incubated for 50 min at room temperature with corresponding secondary antibodies, goat anti-rabbit IgG in combination with streptavidin-HRP (1 : 200 dilution). Finally, the slides were colored, restained, dehydrated, mounted and imaged as done in TUNEL assay, and the expression abundance of antigens was semiquantitatively analyzed by calculating the integrated optical density (IOD) value using the software Image-pro plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA).

Intratumoral immune cytokines from nude mice were tested again by Polymerase Chain Reactions (PCRs). First, the tumor tissue was grinded, and the total RNA was excised with a Trizol Reagent and diluted to a proper concentration of 200 ng μL^{-1}. Then, reverse transcription reactions were performed using a RevertAid First Strand cDNA Synthesis Kit according to the manufacturer's instructions. Next, to analyze IL-2,

IL-12, IFN-γ and TNF-α expression *in vivo*, rat PCR primers were re-designed as follows: IL-2 (188 bp), sense 5'-AAGATGAACTTGGACCTCTGCGG-3' and antisense 5'-CCATCTCCTCAGAAAGTCCACCA-3'; IL-12 (231 bp), sense 5'-ACAAAGGAGGCGAGACT CTGA-3' and antisense 5'-CACATGTCACTGCCCGAGA-3'; IFN-γ (251 bp), sense 5'-CTCAAGTGGCATAGATGTGGAAG-3' and antisense 5'-TGACCTCAAACTTGGCAATACTC-3'; TNF-α (172 bp), sense 5'-GTCCCCAAAGGGATGAGAAGT-3' and antisense 5'-GGCAC CACTAGTTGGTTGTCT-3'. PCRs were carried out in a total volume of 12.5 μL of qPCR Mix containing 7.5 μM (2.0 μL) of PCR primers, 2.5 μL of reverse transcription product and 8.0 μL of ddH_2O. Later, RNA was amplified by PCRs with primers with initial denaturation at 95 ˚C for 5 min, then thermocycling for 40 cycles at 95 ˚C for 15 sec and 60 ˚C for 60 sec, followed by melting curve based on temperature rising from 75 ˚C to 95 ˚C at the rate of 1 ˚C every 20 sec. Finally, with GAPDH as a house-keeping gene, the expression level of IL-2, IL-12, IFN-γ and TNF-α were semi-quantitatively analyzed by using ABI 7300 real-time fluorescent quantitative PCRs.

Further, immunological experiments were performed on immunocompetent BALB/c mice. First, fluorescence flow cytometry was conducted to test the same immune cells with nude mice, and additional T cells. In detail, tumors extracted from different groups were treated by ProteoExtract subcellular fractionation kit and made into single-cell suspension (1×10^6 cells mL^{-1}), and were added into the blank, compensation control and all sample tubes. Then, cells were labeled with the superficial fluorescent antibodies, including CD45-APC-Cy7, CD3-BV605, CD4-BV421, CD8-AF700, CD45r-PE-Cy5, CD49b-FITC, CD11b-BB700, F4/80-PE-CF594, CD11c-APC and Gr-1-BV510. After washed by 1 × Perm/Wash™ buffer, incubated with Fixation/Permeabilization solution (0.25 mL) and resuspended in 1 × Perm/Wash™ buffer (100 μL), cells were labeled with CD206-PE-Cy7. Finally, cells were washed, centrifuged, resuspended and tested by a flow cytometry (BD Accuri C6, Heidelberg, Germany). The analysis of cellular lineages was based on informed biased gating strategies. In detail, $CD45^+$ live singlets were first identified, and then stratified to identify $CD3^+$ T cells, $CD3^-CD45R^+$ B cells, $CD3^-CD49b^+$ NK cells and $CD3^+CD49b^+$ NKT cells. Remaining cells recognized as non-T non-B gate were further manually

identified by gating on $CD11b^+F4/80^+$ Macrophage, $CD11b^+Gr\text{-}1^+$ MDSCs and $CD11b^+CD11c^+$ DCs. $CD3^+$ T cells were segregated by CD4 and CD8 to obtain Tc and Th cells. M1 macrophages was identified on the basis of CD206 and CD11c expression as $CD11b^+F4/80^+CD206^-CD11c^+$. Additionally, samples were evaluated by a dimensionality reduction (embedding) algorithm by using the flowjo10.6.2 software, based on t-Distributed Stochastic Neighbor Embedding (t-SNE) analysis using the Python implementation from https://github.com/DmitryUlyanov/Multicore-TSNE.

Intratumoral immune cytokines from immunocompetent BALB/c mice were tested by PCRs, and experimental details were consistent with the PCRs in nude mice.

Statistical analysis. In this article, all data were presented as mean ± standard deviation (s.d.) with at least three independent replicates throughout. Statistical significance was determined by two independent sample t-test. Asterisks indicate significant differences (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$).

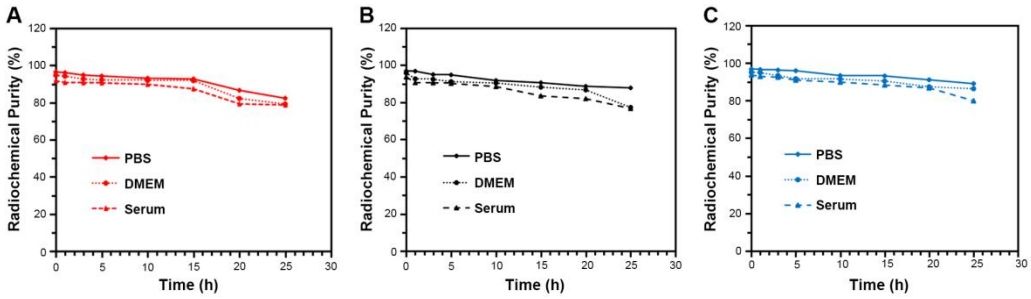

Figure S1. *In vitro* stability test of (A) ^{131}I-AuNPs-TAT, (B) ^{131}I-AuNPs and (C) ^{131}I-TAT fabricated in PBS, DMEM and BSA at 37 °C obtained within 25 h after labeling.

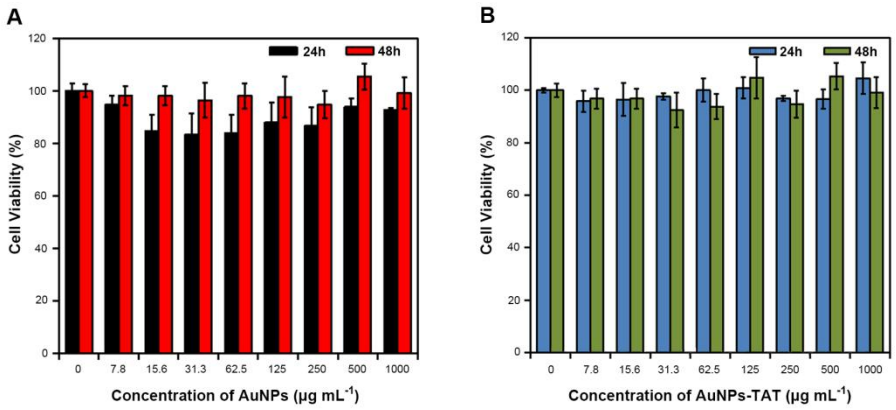

Figure S2. Cytotoxicity assessment by CCK-8 assay for HCT116 cells incubated with different concentrations of (A) AuNPs and (B) AuNPs-TAT for 24 and 48 hours, respectively. The experiments were performed in quintuplicate in 96-well microplates, and data were in mean ± s.d..

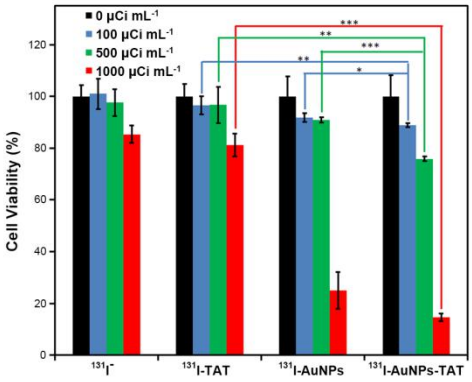

Figure S3. HCT116 cells viability after 24 h incubation with ^{131}I, ^{131}I-TAT, ^{131}I-AuNPs and ^{131}I-AuNPs-TAT with various ^{131}I concentrations (0, 100, 500 and 1000 μCi mL^{-1}). The experiments were performed in quintuplicate in 96-well microplates. Data were in mean ± s.d.. Two sample t-test was used for statistical information.

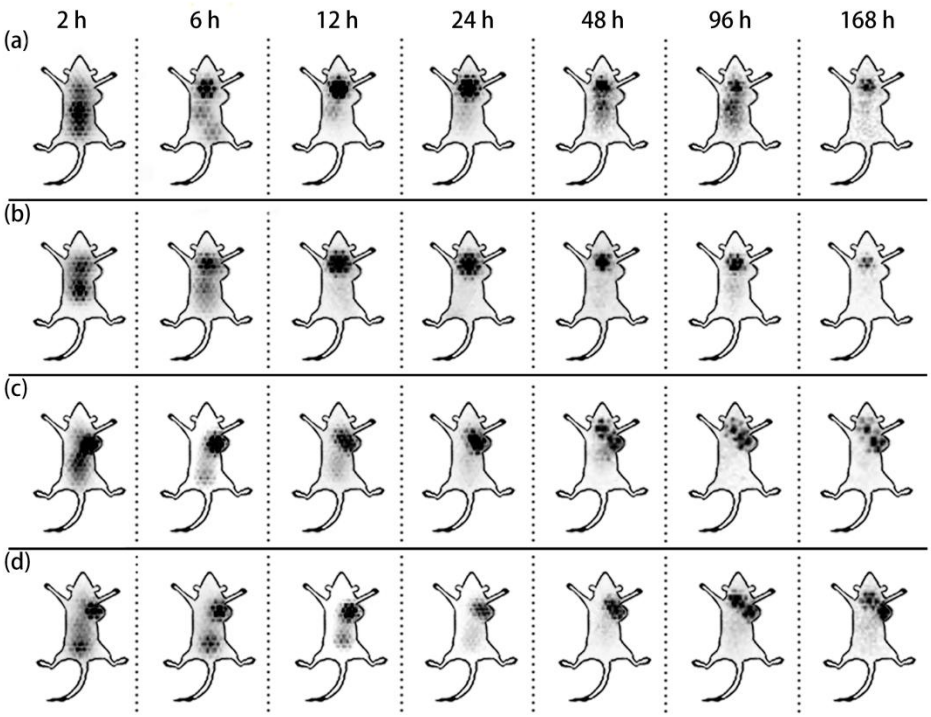

Figure S4. *In vivo* SPECT planar scanning of nude mice at various time points post intratumoral injection of (a) free ^{131}I, (b) ^{131}I-TAT, (c) ^{131}I-AuNPs and (d) ^{131}I-AuNPs-TAT.

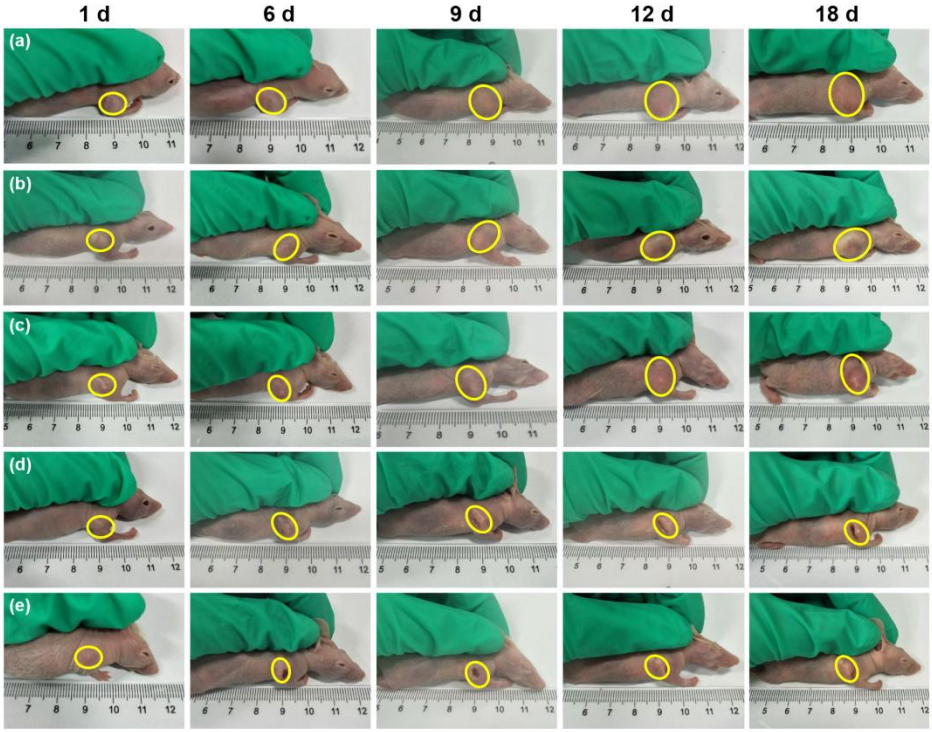

Figure S5. Typically digital photographs of HCT116 tumor-bearing nude mice on 1 d, 6 d, 9 d, 12 d and 18 d post intratumoral injection of (a) PBS, (b) Na^{131}I solution, (c) ^{131}I-TAT, (d) ^{131}I-AuNPs and (e) ^{131}I-AuNPs-TAT. Tumors were highlighted with solid yellow circles.

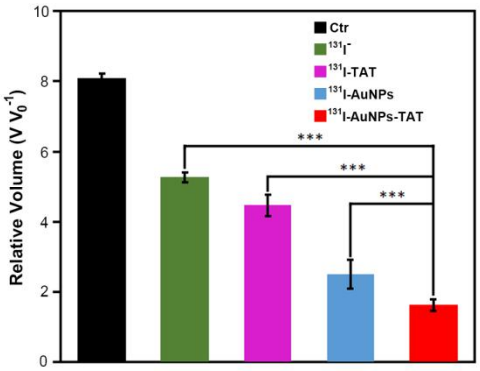

Figure S6. Relative tumor volume ($V\ V_0^{-1}$) of nude mice was measured on day 18 P.I.. Two sample t-test was used for statistical information. Asterisk represents significant difference. (***$P < 0.001$).

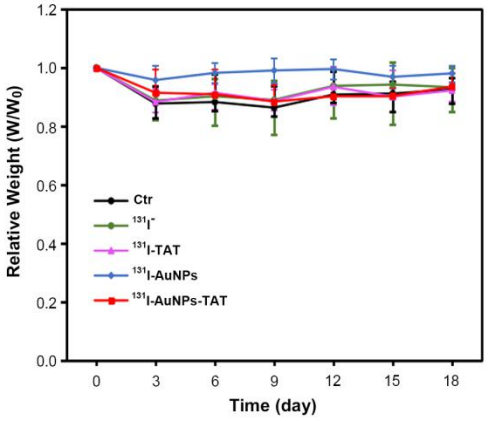

Figure S7. Time-course body weight (W) record of nude mice within 18 days post various treatments (W_0 indicates the initial weight at 0 d P.I.). n = 5, mean ± s.d..

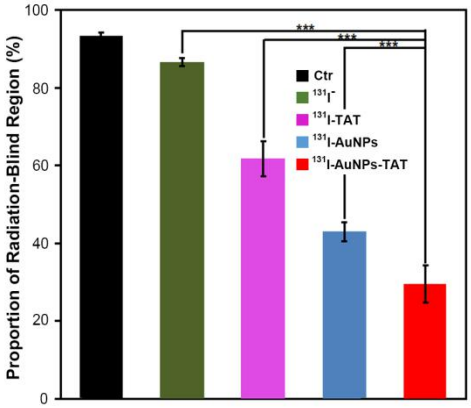

Figure S8. Quantitative comparison of the area of radiation-blind regions among different nude mice groups based on H&E staining. n = 3 sections per group. Two sample t-test was used for statistical information. Asterisk represents significant difference (***$P < 0.001$).

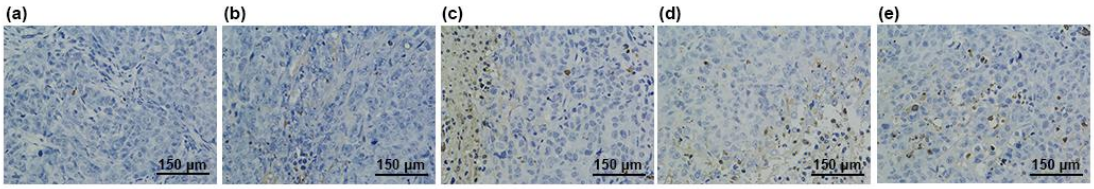

Figure S9. Representative TUNEL staining images (original magnification 200) of HCT116 tumor tissues harvested from nude mice at 18 day P.I. of (a) PBS only (Control), (b) $Na^{131}I$ solution, (c) ^{131}I-TAT, (d) ^{131}I-AuNPs and (e) ^{131}I-AuNPs-TAT. n = 3 sections per group.

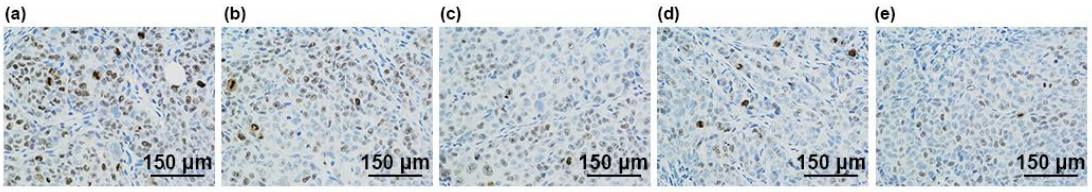

Figure S10. Typical immunohistochemistry images of ki-67 in tumor slices collected from nude mice in (a) PBS only (Control), (b) $Na^{131}I$ solution, (c) ^{131}I-TAT, (d) ^{131}I-AuNPs and (e) ^{131}I-AuNPs-TAT group, at 18 d P.I. (original magnification 400).

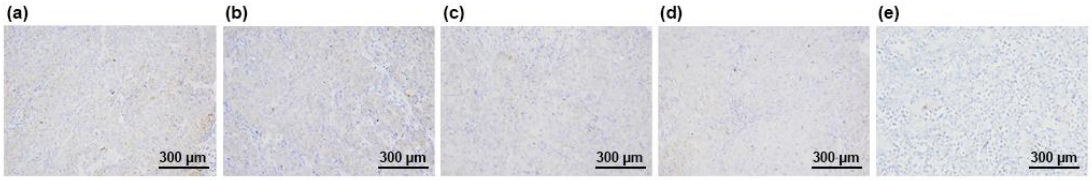

Figure S11. Typical immunohistochemistry images of snail in tumor slices collected from nude mice in (a) PBS only (Control), (b) $Na^{131}I$ solution, (c) ^{131}I-TAT, (d) ^{131}I-AuNPs and (e) ^{131}I-AuNPs-TAT group, at 18 d P.I. (original magnification 200).

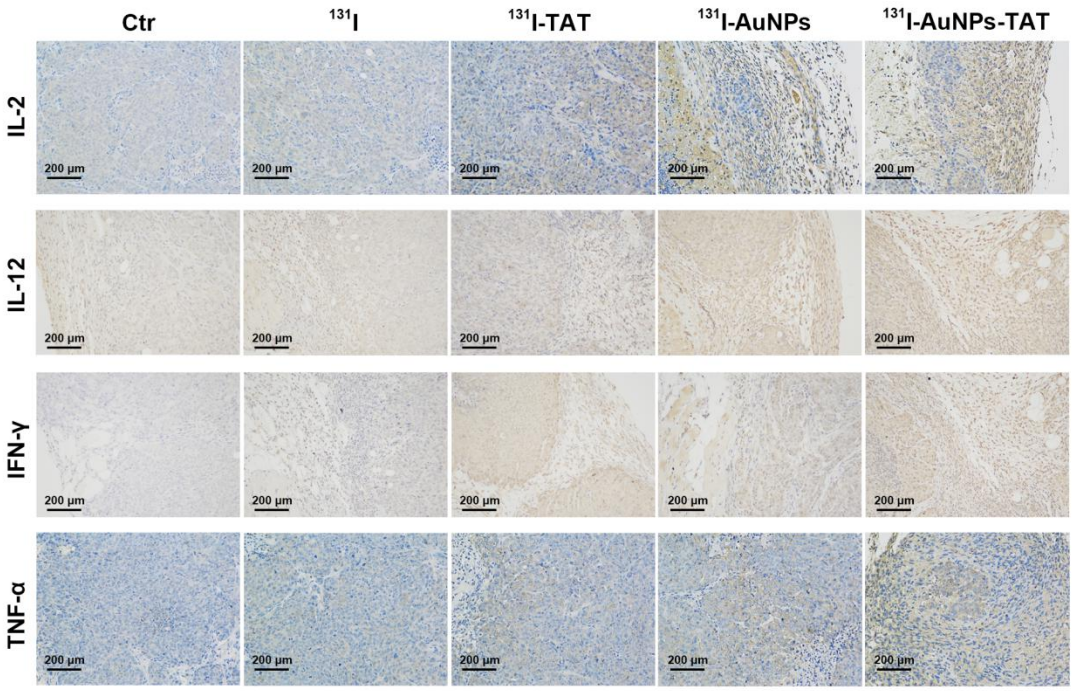

Figure S12. Typical immunohistochemistry images of cytokines in tumors collected from nude mice at 18 d after different treatments (original magnification 200).

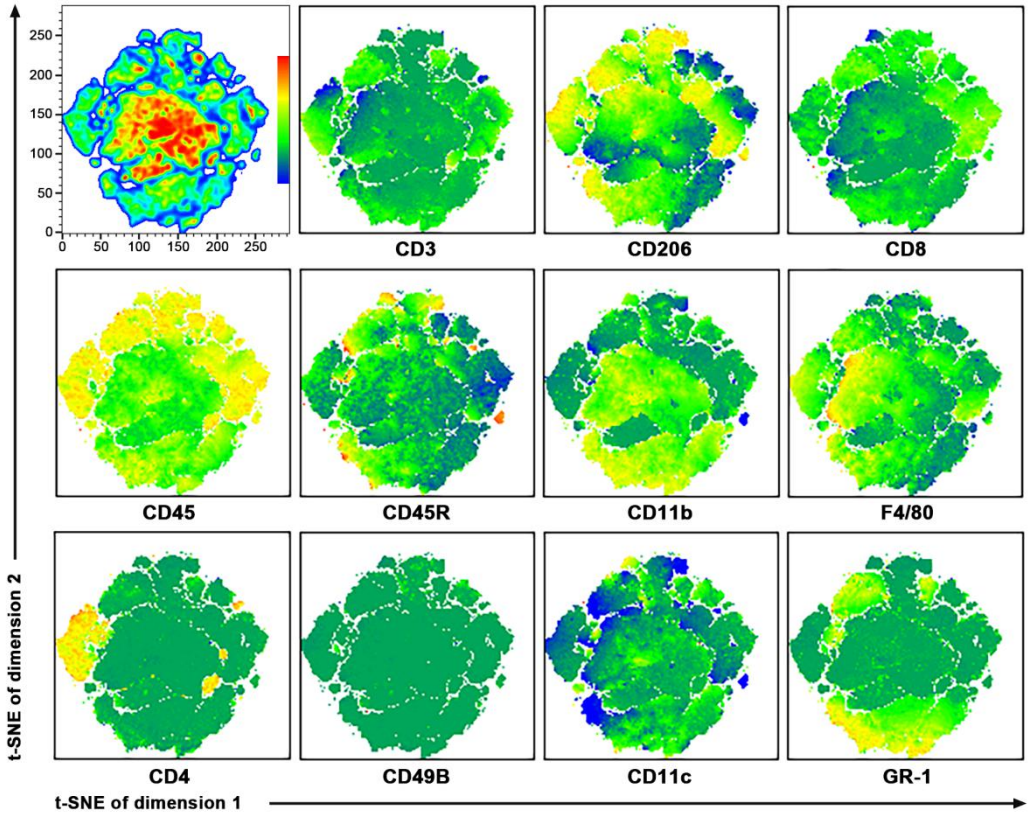

Figure S13. The t-SNE map showing tumour-infiltrating immune antibodies integrated across all colorectal tumour samples from immunocompetent BALB/c mice.

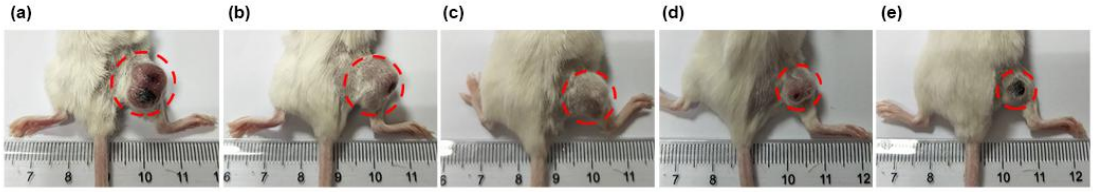

Figure S14. Typically digital photographs of CT26 tumor-bearing BALB/c mice on 14 d post intratumoral injection of (a) PBS, (b) $Na^{131}I$ solution, (c) ^{131}I-TAT, (d) ^{131}I-AuNPs and (e) ^{131}I-AuNPs-TAT. Tumors were highlighted with dotted red circles.

Video S1. Confocal fluorescence view recorded at the equatorial planes in Z-direction of cell, for further confirmation of TAT-mediated AuNPs into the cell nuclei. The reconstruction thickness is 5 μm.

Video S1.mp4

References

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2 G. Sun, T. Wang, X. Li, D. Li, Y. Peng, X. Wang, G. Jia, W. Su, C. Cheng, J. Yang and C. Zuo, *Adv. Healthc. Mater.,* 2018, **7**, 1800375–1800384.