Supporting Information

Controllable synthesis of barium carbonate nano- and microparticles for SPECT and CT imaging

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1. Materials

1.1. For particles synthesis

Barium chloride dihydrate ($BaCl_2 \times 2H_2O$, M_W = 147.01, ≥99%), anhydrous sodium carbonate (Na_2CO_3, M_W = 105.99, ≥99.5%), and poly(acrylic acid) (PAA) were obtained from Sigma-Aldrich and used without further purification. Purified water with a specific resistivity higher than 18.2 MΩ cm^{-1} from a three-stage Milli-Q Plus 185 purification system was used.

1.2. For radiolabeling

Tin(II) chloride dihydrate ($SnCl_2 \times 2H_2O$, 98%) was purchased from Merck (Germany). Hydrochloric acid (> 35%, TraceSELECT) was purchased from Honeywell-Fluka (USA). Sodium hydroxide (BioXtra, ≥98%) and TWEEN® 80 were purchased from Sigma-Aldrich (Germany).

1.3. For in vitro experiment

Alpha Minimum Essential Medium (Alpha-MEM) was purchased from Biolot, Russia. Phosphate-buffered saline (PBS) and UltraGlutamine I were purchased from Lonza, Switzerland. Fetal bovine serum (FBS) was obtained from HyClone, USA. Trypsin-EDTA solution was purchased from Capricorn Scientific, Germany. AlexaFluor 488 (AF488), propidium iodide (PI, M_w = 668.39, ≥94%), and calcein acetoxymethyl (Calcein AM) were purchased from Sigma-Aldrich.

1.4. For in vivo experiment

C57BL/6 mice 6-8 weeks old, a sterile syringe with a removable 29 G needle (Vogt Medical, Germany), isoflurane (Baxter, USA), zolazepam hydrochloride (Zoletil, Virbac, France), xylazine hydrochloride (Rometar, Bioveta, a.s., Czech Republic), and phosphate buffered saline (Sigma-Aldrich, Germany) were used.

1.5. For histological analysis

Formaldehyde, paraffin, 1% hydrochloric acid solution, methanol, potassium hexacyanoferrate (II) trihydrate, and PBS (phosphate buffered saline) were obtained from Thermo Fisher Scientific (USA). Ehrlich's hematoxylin was purchased from Labiko (Russia). Eosin Y (M_W = 647.89) was obtained from Sigma-Aldrich (Germany). Glycergel mounting medium was obtained from Agilent Dako (USA).

2. Synthesis of $BaCO_3$ particles

The synthesis of $BaCO_3$ particles was carried out via a series of **reactions** (**I-V**), and the influence of various parameters such as the concentration of $BaCl_2$ and Na_2CO_3 solutions, solvent molar ratios, pH, and the addition of PAA was investigated and presented in **Table S1**. The details of the different protocols used are outlined below:

For instance, 0.1 M of either $BaCl_2$ aqueous solution (**Reactions I, II**) or $BaCl_2$–ethylene glycol (EG) solution (**Reactions III-V**) (molar ratio of H_2O:EG = 2.5:1) were premixed with 3 mg/mL of PAA to form a mixture (20 mL) that was further incubated for 60 min. Then 0.1 M of Na_2CO_3 aqueous solution (**Reactions I, II**) or 0.1 M of Na_2CO_3 with 3 mg/mL PAA (molar ratio of H_2O:EG = 2.5:1) (**Reactions III - V**) to form another mixture (20 mL) and stirred for another 60 min. The reactions were stirred either at room temperature (**Protocols II-V**) or at 80°C (**Protocol I**) for a total reaction time of 60 min. The pH of the Na_2CO_3-PAA-EG solution mixture was adjusted to 3-5, using 750 μL of 1 M HCl before it was added to the $BaCl_2$-PAA-EG solution mixture, only in **Reaction V**. The resulting suspensions were washed three times with distilled water, centrifuged at 13000 rpm for 3 min, and suspended in 1 mL of 0.9% NaCl solution.

Table S1. Optimization of synthesis parameters for size reduction and morphology improvement of $BaCO_3$ particles.

*Reaction	Solvent/ Ratio of solvents (v/v)	PAA **pre-incubation with $BaCl_2$	PAA **pre-incubation with Na_2CO_3	Temperature (°C)	***pH value
I	H_2O	✔	×	25	9-11
II	H_2O	✔	×	80	8-10
III	EG: H_2O = 2.5:1	×	✔	25	11-12
IV	EG: H_2O = 2.5:1	✔	✔	25	9-10
V	EG: H_2O = 2.5:1	✔	✔	25	3-5

*** Concentrations** of PAA, $BaCl_2$, and Na_2CO_3 were 0.1 M, and they were added at a molar ratio of 3:1:1.

** **Pre-incubation time** for both salts were 60 min.

*** **pH value** during the addition of PAA to 0.1 M Na_2CO_3 - EG solution.

3. Structural characterization of $BaCO_3$ particles

3.1. Scanning and transmission electron microscopy

Geometry and size of the developed $BaCO_3$ particles (**$BaCO_3$ MPs** and **$BaCO_3$ NPs**) were characterized by scanning electron microscopy (SEM, Quanta 200, FEI, Netherlands) with an accelerating voltage of 10 kV and a high-resolution transmission electron microscope (TEM, Zeiss Libra 200F, Carl Zeiss, Germany) equipped with a field emission emitter and an OMEGA energy filter. Before the measurements, 1 μL of each type of $BaCO_3$ particles were dispersed in water and then dropped on a coverslip. The samples were dried for 10 min and then measured by SEM and TEM. An X-MAX energy-dispersive X-ray detector (Carl Zeiss, Germany) was used to analyze the elemental composition of the particles.

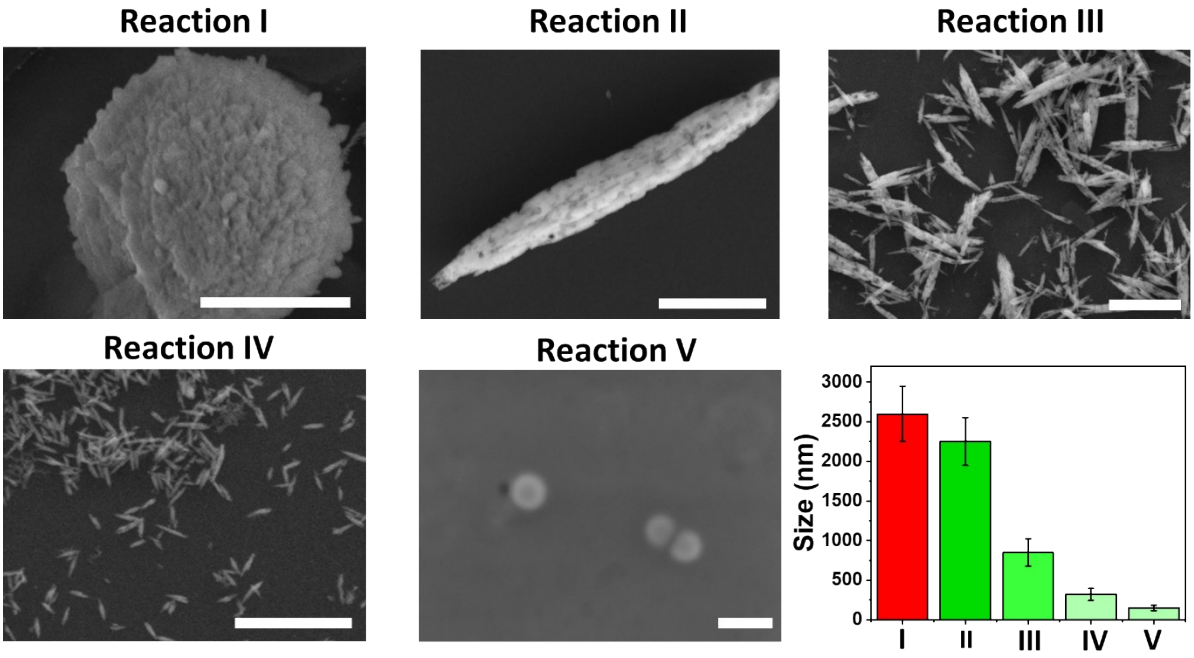

Figure S1. SEM images of the final products obtained with the developed protocols and the corresponding average sizes for **reaction I**, **reaction II**, **reaction III**, **reaction IV**, and **reaction V**. Scale bar = 1 μm (**reaction I – IV)** and 200 nm **(reaction V)**. The results are shown as an average value ± standard deviation (n = 3).

3.2. Powder X-Ray diffraction (PXRD)

The crystalline phase of $BaCO_3$ particles (**$BaCO_3$ MPs** and **$BaCO_3$ NPs**) was studied using PXRD analysis. Diffraction patterns of $BaCO_3$ were recorded on a Shimadzu 7000-maxima X-ray diffractometer with a 2 kW characteristic CuKα (Kα1 λ = 1.54059 Å, angular range 2θ = 5°-60°) X-ray radiation source and Bragg-Brentano goniometer geometry. The angular resolution during the analysis was 0.01 deg at a scanning speed of 1 deg/min.

3.3. Fourier-transform infrared (FTIR) spectroscopy

The Fourier transform infrared spectra were obtained by Shimadzu IRTracer-100 combined with Specac Quest, a single-reflection ATR accessory, by scans in the range from 400 to 4000 cm^{-1} with a resolution of 1 cm^{-1}.

3.4. Dynamic light scattering (DLS)

Zetasizer Nano ZS90 analyzer (Malvern, USA) equipped with a 633 nm HeNe laser was used to measure the hydrodynamic diameters (D_h) of the $BaCO_3$ particles. For this, 1 mg of particles was diluted 500 times in water (25 °C and pH 7.4) and measured.

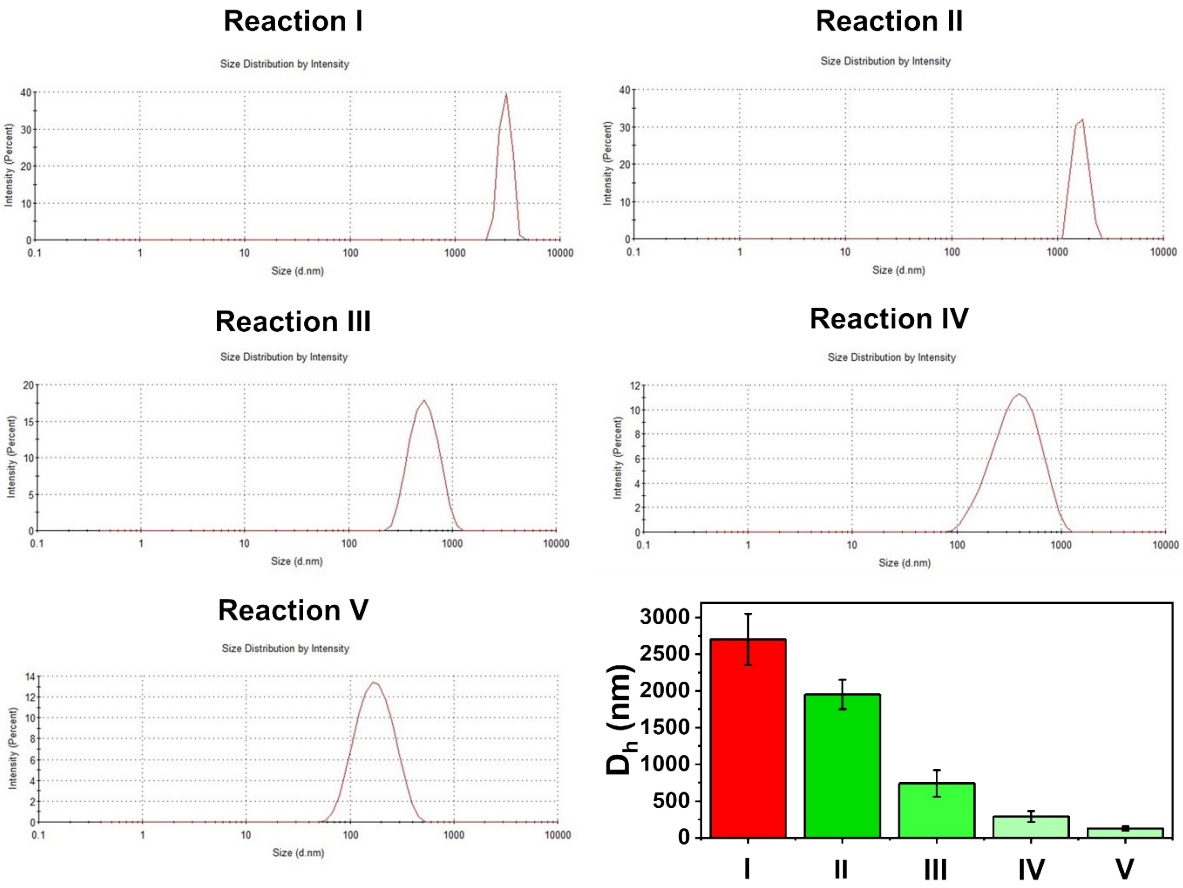

Figure S2. DLS of the final products obtained with the developed protocols and the corresponding average sizes for **reaction I**, **reaction II**, **reaction III**, **reaction IV**, and **reaction V**. The results are shown as an average value ± standard deviation (n = 3).

4. *In vitro* stability of $BaCO_3$ particles

Analysis of the hydrodynamic diameter (D_h) of **$BaCO_3$ MPs** and **$BaCO_3$ NPs** was carried out as a part of the *in vitro* stability assay. For this, **$BaCO_3$ MPs** and **$BaCO_3$ NPs** were incubated in 0.9% NaCl and 0.1% BSA at pH 3, pH 6.5 and pH 10. The D_h change was analyzed at different moments of time (1, 2, 3, 4, 8 and 14 d). The samples were then measured using a BKwinner803 Photon Correlation DLS Nano Particle Size Analyzer (Jinan Winner Particle Instrument Stock Co., Ltd., China).

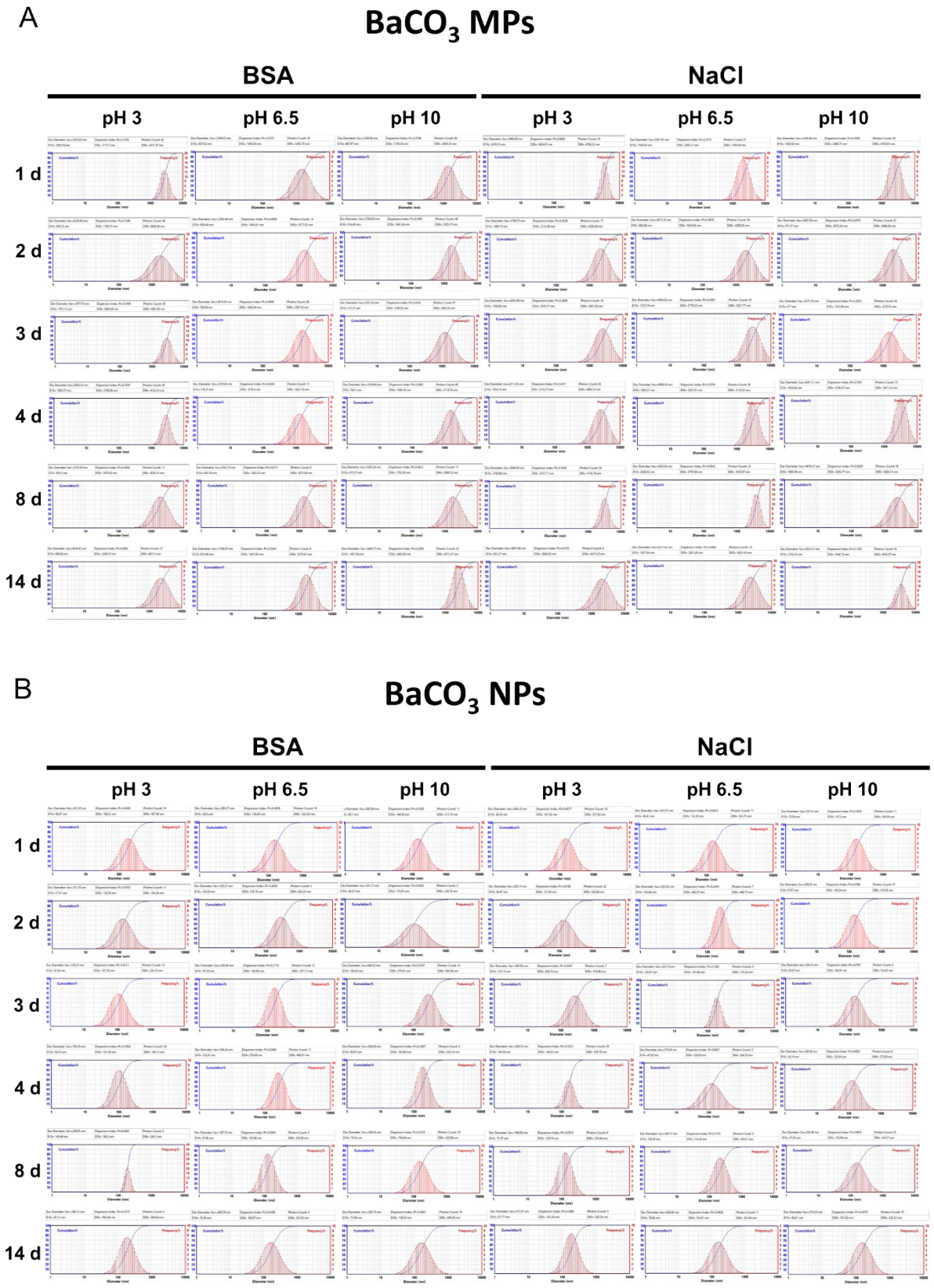

Figure S3. Hydrodynamic diameters (D_h) of **$BaCO_3$ MPs** (A) and **$BaCO_3$ NPs** (B) incubated in 0.9% NaCl and 0.1% BSA at pH 3, pH 6.5 and pH 10 during 14 d.

5. X-ray attenuation analysis

We analyzed X-ray attenuation properties of **$BaCO_3$ MPs**, **$BaCO_3$ NPs**, and **Iohexol**. For this, **$BaCO_3$ MPs**, **$BaCO_3$ NPs**, and **iohexol** were added to a 24-well plate at different concentrations from 0.625 to 20 mg/mL. Biograph mCT40 (Siemens) was used to obtain a contrast gradient. The obtained images were then analyzed with Siemens Biograph mCT (software version syngo MI.PET/CT 2012A). Finally, the percentage of X-ray attenuation was plotted versus the measured concentration of **$BaCO_3$ MPs**, **$BaCO_3$ NPs**, and **iohexol**.

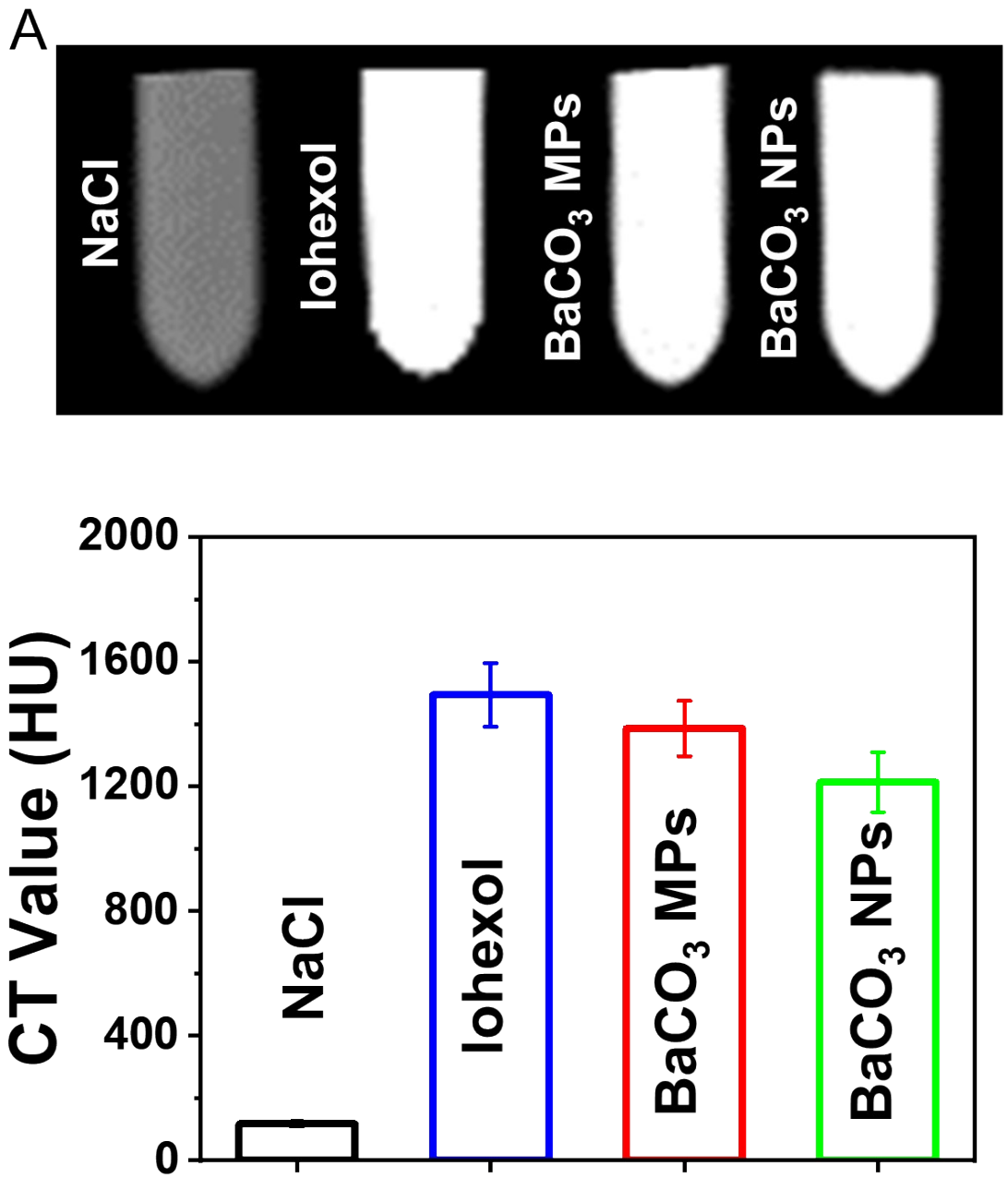

Figure S4. *In vitro* CT images of **$BaCO_3$ MPs** and **$BaCO_3$ NPs** (A) with corresponding measured HU values (B) at the concentration of particles of 20 mg/mL. As negative and positive controls, 0.9% NaCl solution and **iohexol** (20 mg/mL) were used, respectively. The results are shown as an average value ± standard deviation (n = 3).

6. Fluorescent labeling of $BaCO_3$ particles

6.1. Fluorescence labeling of bovine serum albumin (BSA)

BSA was fluorescently labeled with Cy5 dye using the method described previously[1]. Briefly, 144 mg of BSA was added to 40 mL of PBS (pH 7.4) to a final concentration of 3.85 mg/mL. Further, 10 μg of Cy5-dye was resuspended in 4.4 mL of DMSO. Afterwards, the dye solution was mixed with BSA (the final BSA concentration was 7 mg/mL) and incubated for 24 h at 4 °C. Then, the unbound dye was removed with a dialysis procedure[2]. For this, the resulting solution was poured into a dialysis bag, which was then placed in a beaker, and stirred for 3 d (500 rpm, 4 °C). The water with unreacted dye was changed every 24 h.

6.2. Fluorescence labeling of $BaCO_3$ MPs

To obtain Cy5-fluorescent labeled **$BaCO_3$ MPs**, the co-precipitation method was used. For this, 20 mL of $BaCl_2 \times 2H_2O$ (0.1 M) was premixed with 3 mg/mL of PAA solution during 1 h. Then, 1 mL of Cy5-BSA complex was added to the solution. Next, 20 mL of Na_2CO_3 solution (0.1 M) was added to the reaction. The components were stirred together at room temperature for 60 min. The resulting Cy5-fluorescent labeled particles were washed multiple times with Milli-Q water and purified by centrifugation for 3 min at 4000 rpm after the reaction. Finally, the pellets were obtained and stored for further investigations.

6.3. Fluorescence labeling of $BaCO_3$ NPs

To obtain Cy5-fluorescent labeled **$BaCO_3$ NPs**, the co-precipitation method was used. For this, 20 mL of $BaCl_2$ solution (0.1 M) was mixed with 3 mg/mL of PAA solution during 1 h. Then, 1 mL of Cy5-BSA complex was added to the solution. Next, 20 ml of 0.1 M Na_2CO_3 with 3 mg/mL PAA in EG solution was added to the reaction at a molar ratio of 2.5:1. The components were stirred together at room temperature for 60 min. The resulting Cy5-fluorescent labeled **$BaCO_3$ NPs** was washed multiple times with Milli-Q water and purified by centrifugation for

3 min at 13000 rpm after the reaction. Finally, pellets were obtained and stored for further investigations.

7. Cell culture

Fibroblast (MEF), chronic myelogenous leukemia (K562) and monocytic (THP-1) cell lines were used for *in vitro* cell studies. AlphaMEM and RPMI-1640 supplemented with 10 vol% v/v FBS (HyClone, USA), additional 2 mM UltraGlutamine I, 100 IU/mL penicillin, and 0.1 mg/mL streptomycin (Biolot Russia). The cell culture was maintained in a sterile humidified atmosphere containing 95% air and 5% CO_2 at 37°C. Additionally, murine melanoma cell lines (B16-F10 cells) were obtained from the American Type Culture Collection and were used to form tumor models. Cells were cultured in AlphaMEM supplemented with 10% of vol. FBS and additional 2 mM UltraGlutamine I. The cell culture was maintained in a sterile humidified atmosphere containing 95% air and 5% CO_2 at 37°C.

8. Cellular uptake

To visualize cellular uptake of **$BaCO_3$ MPs** and **$BaCO_3$ NPs** by B16-F10, the cells were seeded into 24-well plates (1×10^5 per well). Next day, the Cy5-labeled **$BaCO_3$ MPs** and **$BaCO_3$ NPs** were added to the cells at different concentrations (0.5- 2 mg/mL), and the cells were left overnight. Next day, the cells were fixed, then, their cytoskeletons were stained with phalloidin conjugated with AF488, and the cell nucleus were stained with PI. For this, cells were washed 2 times with PBS and permeabilized with 10 vol% of formalin for 40 min. Afterwards, the cells were washed again with PBS, and 1 μM of AF488 was added. The MEF cells were left for another 1 h. After that, 20 μM of PI was added for 20 min. Afterwards, the cells were washed again with PBS. Cellular uptake of **$BaCO_3$ MPs** and **$BaCO_3$ NPs** was visualized using a confocal laser scanning microscope (CLSM) Leica TCS SP8 (Germany). To visualize the cell cytoskeletons, an argon laser emitting at 488

nm was used. To visualize the cell nucleus, a helium neon laser emitting at 543 nm was used. To visualize Cy5-labeled **$BaCO_3$ MPs** and **$BaCO_3$ NPs,** a helium neon laser emitting at 633 nm was used. The confocal pinhole was set to 1 Airy unit, and the images were taken with an HC PL FLUOTAR 10x/0.30 PH2 Objective.

9. Cell viability

Cell viability was assessed using a LIVE/DEAD assay. For this, 10^5 cells (MEF, THP-1 and K562) per well were seeded in a 24-well plates. Then, the **$BaCO_3$ MPs**, **$BaCO_3$ NPs**, and **iohexol** were added to the wells at the concentrations from 0 to 2 mg/mL. As a positive control, cells without the particles were used. The cells were further incubated at 37°C for 24 h. After the incubation, cells were gently washed once with a pre-warmed (37°C) solution of 1x PBS, and then stained with calcein AM and propidium iodide (PI) to reveal living (calcein AM)/dead (PI) cells. For this, 3 μM of calcein AM and 5 μM of PI were diluted in warm (37°C) 1x PBS and added to cells, and then the cells were incubated for 30 min at 37°C. Wells without cells were used to determine the level of the background signal. After staining, cells were immediately placed under a CLSM. An argon laser emitting at 488 nm was used to detect calcein AM (live cells). A helium-neon laser emitting at 543 nm was used to detect PI (dead cells). The images were taken with an Objective HC PL FLUOTAR 10x/0.30. The experiment was carried out three times. The images with living and dead cells were then analyzed with FIJI open-source image analysis software. Finally, the percentage of living cells was plotted versus the added concentration of **$BaCO_3$ MPs**, **$BaCO_3$ NPs** and **iohexol**.

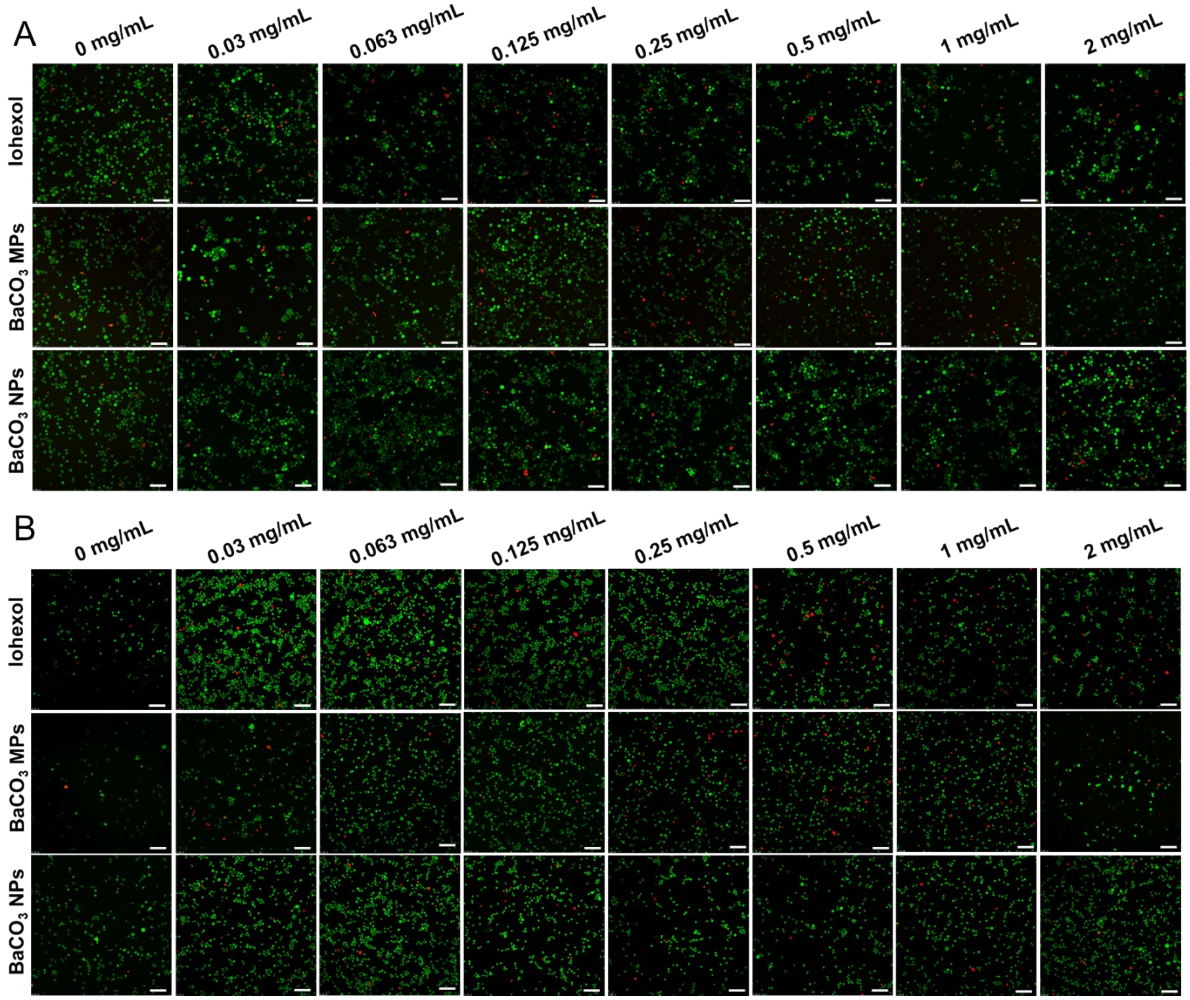

Figure S5. CLSM images of K562 (A) and THP-1 (B) cells incubated with **$BaCO_3$ MPs**, **$BaCO_3$ NPs** and **Iohexol** after co-staining with calcein AM (green) and PI (red). Scale bar = 100 μm.

10. Hemolysis assay

Hemolysis assay was performed to study the influence of **$BaCO_3$ MPs** and **$BaCO_3$ NPs** on human red blood cells (RBCs)[3]. For this, 1 mL of 3.8% sodium citrate solution was added to 10 mL of blood to avoid blood clotting. Then, blood was added to a 7 mL centrifuge tube containing 7 mL of ficoll solution (density 1.077). Next, the reaction solution was centrifuged for 30 min at 312 x g. As a result of the interaction of ficoll and blood, the RBCs sank to the bottom of the tube, separating from the plasma. The resulting cell pellet was washed in a sodium phosphate buffer (pH 7.2) five times. Then, the human RBCs were diluted to a

final concentration (5%, v/v) in PBS. **$BaCO_3$ MPs** and **$BaCO_3$ NPs** in PBS were further added to human RBCs at different concentrations (0.125, 0.25, 0.5, 1, 2, and 4 mg/mL) to a final volume of 1 mL. Afterwards, the human RBCs were incubated for 2 h, then centrifuged for 5 min at 300 x g. The quantitative determination of hemoglobin concentration in the supernatant was carried out using a spectrophotometer at an absorption wavelength of 540 nm. As a positive control, a buffer for lysis of erythrocytes was added to the human RBCs, and PBS was added as a negative control. The percentage of hemolysis was calculated using the formula:

$$\text{Hemolysis assay}(\%) = \frac{d_{\text{sample}} - d_{\text{negative control}}}{d_{\text{positive control}} - d_{\text{negative control}}} \times 100\%$$

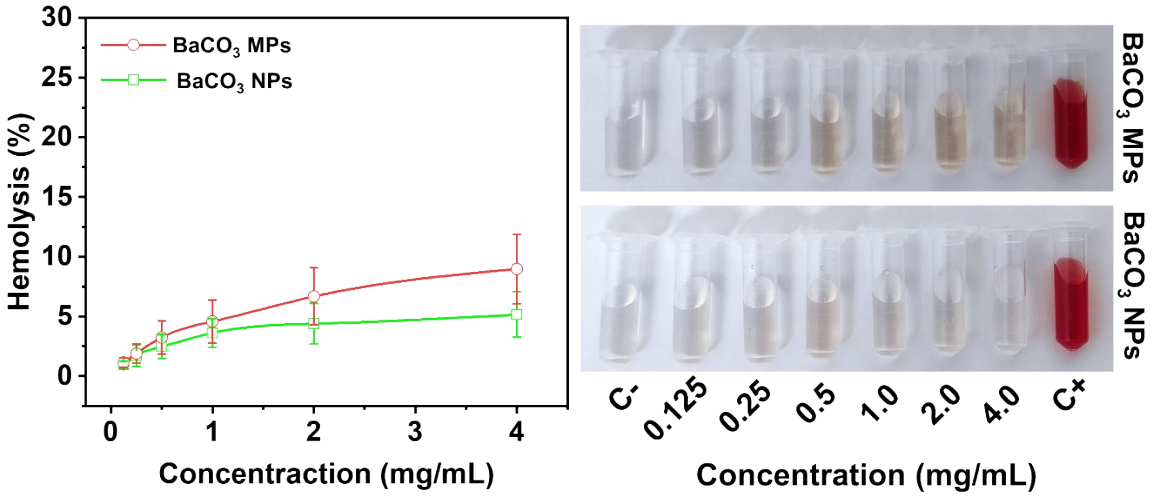

Figure S6. Relative rate of hemolysis (%) in human RBCs upon incubation with suspension of **$BaCO_3$ MPs** and **$BaCO_3$ NPs** at different concentrations (0.125-4 mg/mL) with corresponding digital images of the hemolysis assay performed with **$BaCO_3$ MPs** and **$BaCO_3$ NPs**. It should be noted that in **Figure S6** positive and negative controls were the same for **$BaCO_3$ MPs** and **$BaCO_3$ NPs**. The results are shown as average value ± standard deviation (n = 4).

11. Radiolabeling of $BaCO_3$ MPs and $BaCO_3$ NPs

The **$BaCO_3$ MPs** and **$BaCO_3$ NPs** were obtained as described above. Then, 900 μL of $SnCl_2$ (10 mg/mL, 2% HCl solution in 0.9% NaCl) and 1 μL of Tween 80 were added to the dried particles (10 mg). The suspension was carefully resuspended, and 100 μL (10 MBq) of $^{99m}TcO_4^-$ (solution in 0.9% NaCl) was added to the resulting suspension. The samples were incubated in a thermomixer for 1 h (900 rpm, 25 °C). Then, the pH of the reaction was gently adjusted to 6.5-7 with 0.5 M NaOH. The radiolabeled particles were washed 3 times (12.000 rpm, 5 min) and resuspended in 1 mL of 0.9% NaCl solution.

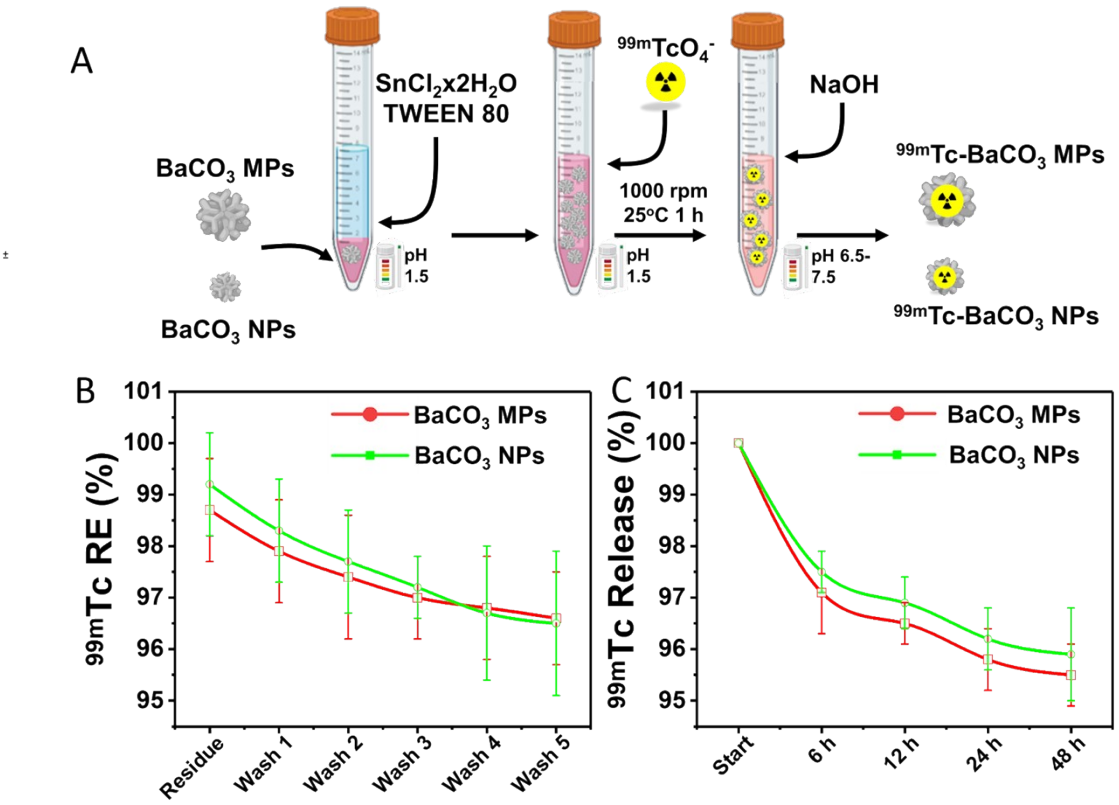

Figure S7. Radiolabeling of ^{99m}Tc with **$BaCO_3$ MPs** and **$BaCO_3$ NPs**: (A) Schematic illustration of ^{99m}Tc incorporation into **$BaCO_3$ MPs** and **$BaCO_3$ NPs** (B) Radiolabeling efficiency (RE, %) of ^{99m}Tc-labeled **$BaCO_3$ MPs** and **$BaCO_3$ NPs**. (C) Retention efficiency of ^{9m}Tc-labeled **$BaCO_3$ MPs** and **$BaCO_3$ NPs**. The results are shown as average value ± standard deviation (n = 4).

12. Animals

Healthy male and female C57BL/6 mice (6–8 weeks old, 18–22 g) were used for *in vivo* experiments. The animals were obtained from the St. Petersburg center of laboratory animals "Rappolovo" of the National Research Center "Kurchatov Institute" in Russia. The mice were housed in plastic cages in specific pathogen-free conditions at 23 °C and humidity of 40-60% in a 12-h light/dark cycle for at least 14 d, with free access to food and water.

All the animal experiments were conducted after the approval by the veterinary office of the Russian Research Center of Radiology and Surgical Technologies and its local ethics committee. All the methods were performed in accordance with the relevant guidelines and regulations.

13. Tumor establishment

In order to establish the tumors in C57BL/6 mice (males, 8-week-old, 18-22 g), the B16-F10 melanoma cells were used. Briefly, melanoma cells were harvested in the exponential growth phase by trypsinization. Then, the cells were washed twice with ice-cold phosphate-buffered saline (PBS) and resuspended in a PBS solution. Afterwards, the concentrated and counted cells were promptly subcutaneously injected (50 μL with concentration $1x10^6$ cells/mL) into the right leg of C57BL/6 mice (0 d). Seven days after the tumor establishment (7 d), the mice were checked, and animals with a sufficient size of the tumor (approximately 0.05 ± 0.01 cm^3) were utilized for further experiments.

14. X-ray and computer tomography (CT) imaging

Biograph mCT40 (Siemens) and GE Definium 8000 system with dual GE digital detectors (GE Healthcare) were used to investigate biodistribution and contrast properties of $BaCO_3$ particles *in vivo.* For this, **$BaCO_3$ MPs**, **$BaCO_3$ NPs** and **iohexol** were intratumorally or intravenously (100 mg/kg) injected in C57BL/6 mice with subcutaneous tumors. For this, mice were previously immobilized using

Zoletil (0.8 mL/kg) and Rometar (0.6 mL/kg). Applied settings for the CT imaging were the following: tube voltage (120 V) and current (160 mA), focal spot size (0.6 x 0.7 mm/7º), reconstructed slice widths (0.6 mm), slice increment (10 mm), and energy window (435-650 keV). The 3D images were then obtained using RadiAnt DICOM Viewer software. Settings for GE Definium 8000 system were the following: line frequency (50 Hz), line impedance (0.125 ohms, 440 V, 80 kW), and inrush current (1000 Amps). The signal intensity of organs and tumors was processed using DICOM Editor and ImageJ software. The signal intensity was represented in a.u.

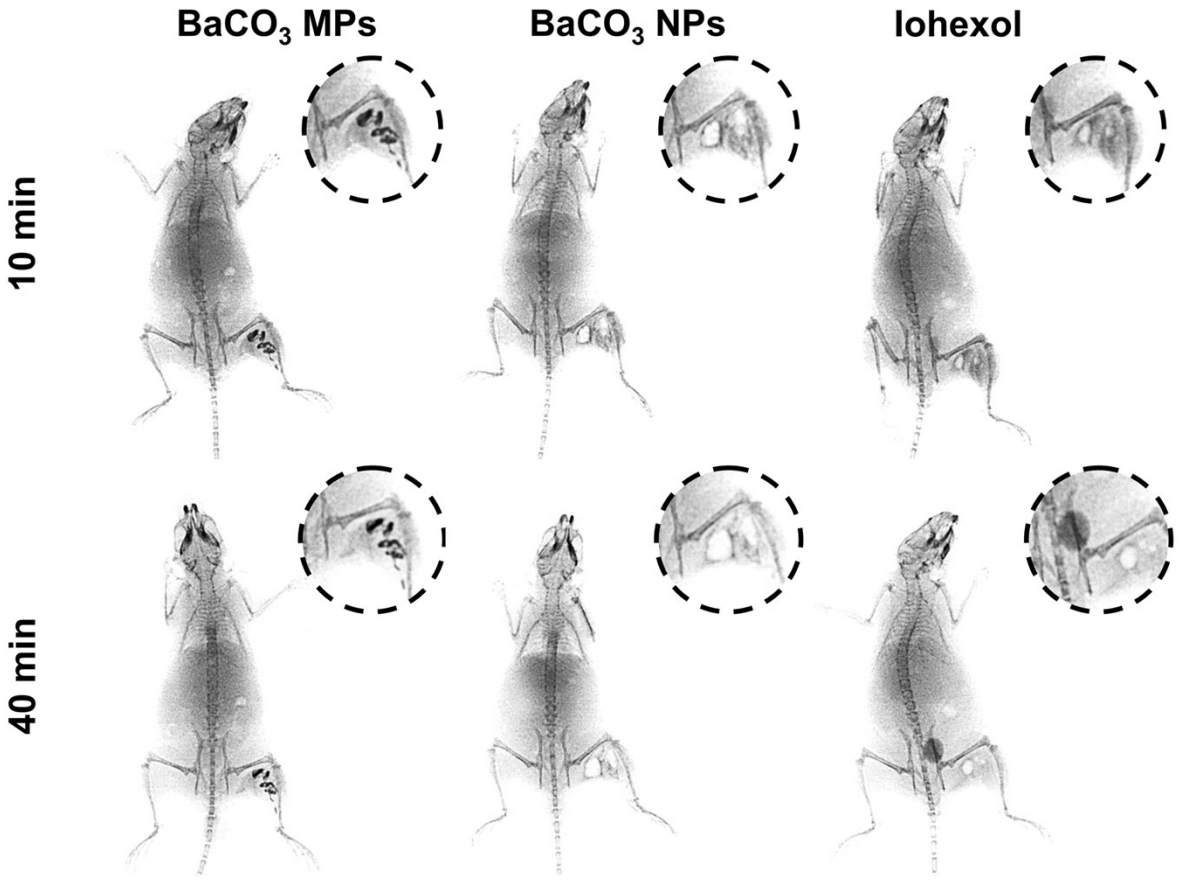

Figure S8. X-Ray imaging obtained using GE Definium 8000 system after intratumoral injection of **$BaCO_3$ MPs**, **$BaCO_3$ NPs** and **iohexol** into tumor-bearing C57BL/6 mice at different time points.

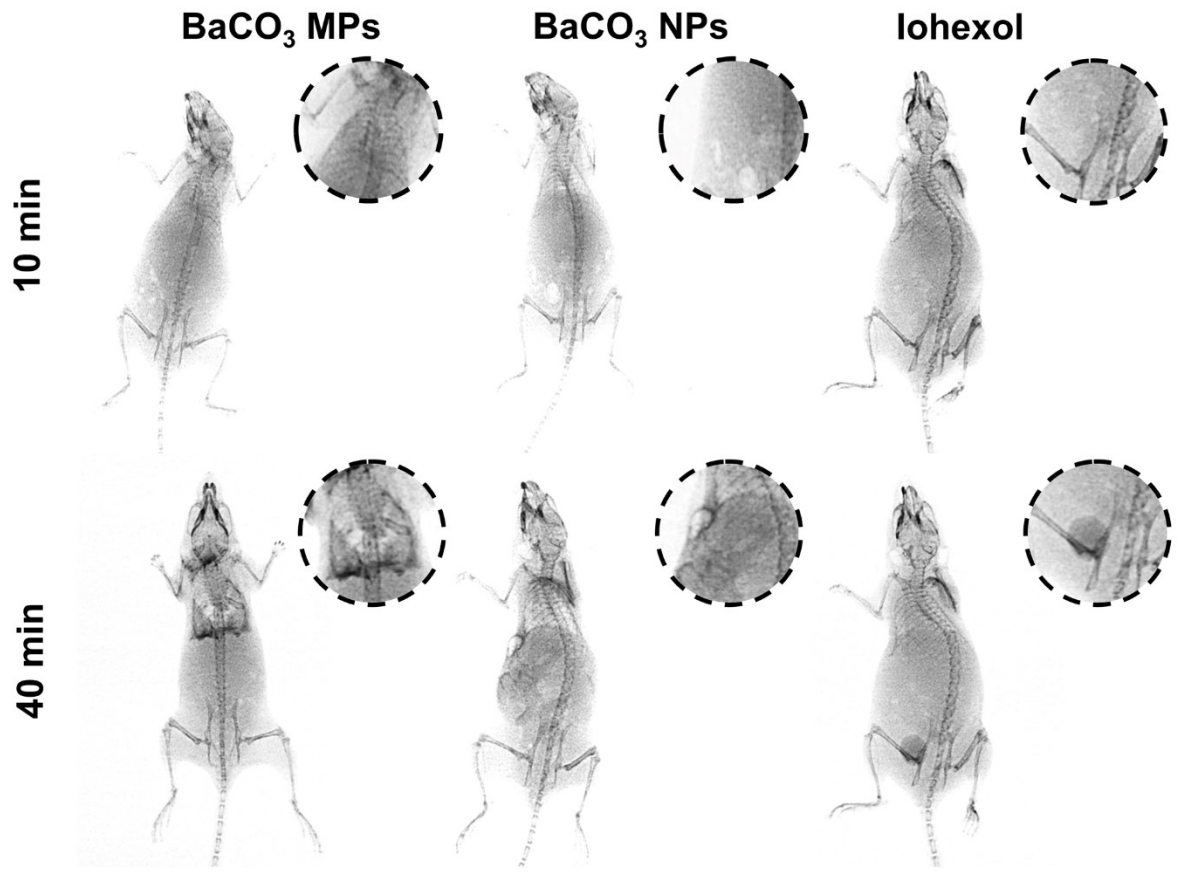

Figure S9. X-Ray imaging obtained using GE Definium 8000 system after intravenous injection of **$BaCO_3$ MPs**, **$BaCO_3$ NPs** and **iohexol** into tumor-bearing C57BL/6 mice at different time points.

15. SPECT imaging

The biodistribution of the radiolabeled **$BaCO_3$ MPs** and **$BaCO_3$ NPs** was additionally investigated using SPECT Discovery NM 630 (GE Healthcare). C57BL/6 mice with subcutaneous tumors were divided into two groups randomly. Each group was injected with radiolabeled **$BaCO_3$ MPs** and **$BaCO_3$ NPs** at the same dosage (10 MBq, 100 mg/kg). After intratumoral and intravenous injection of **$BaCO_3$ MPs** and **$BaCO_3$ NPs**, the mice were immobilized with Zoletil 0.8 mL/kg, Rometar 0.6 mL/kg and further visualized using SPECT, by measuring primary gamma radiation at 155 keV. Visualization was performed at different time points (1, 24, 48 h). To visualize the anatomy of mice and localization of

SPECT signals, digital outlines of mice were used for further data formation. The SPECT images were superimposed on the outlines and analyzed using an image editor with a merge function. After imaging, the mice were sacrificed, and the main organs (the lungs, liver, heart, spleen, and kidneys) and tumors were extracted for radiometry and histological analysis. The signal intensity was represented in a.u.

16. Fluorescence imaging

For fluorescence imaging, mice with stable subcutaneous tumor formation (0.05 ± 0.01 cm^3) were euthanized after 7 d of tumor-induced cells injection by cervical dislocation according to the guidelines approved by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and local ethics committee. For the organ harvesting, the animal limbs were fixed with needles in the preparation tray, surface treatment of the wool with an antiseptic solution was carried out, the xiphoid process was captured, and the skin was incised, through which the necessary organs were extracted and washed of blood. The organs (the heart, lungs, liver, spleen, and kidneys) and tumors were isolated and analyzed by an *in vivo* fluorescence imaging system (IVIS Lumina II, PerkinElmer Inc, USA). After biodistribution analysis of **$BaCO_3$ MPs** and **$BaCO_3$ NPs,** the organs were placed in formalin for the subsequent histology analysis. The fluorescence intensity of *ex vivo* organs and tumors were processed using DICOM Editor and ImageJ software. The fluorescent signals were represented in a.u.

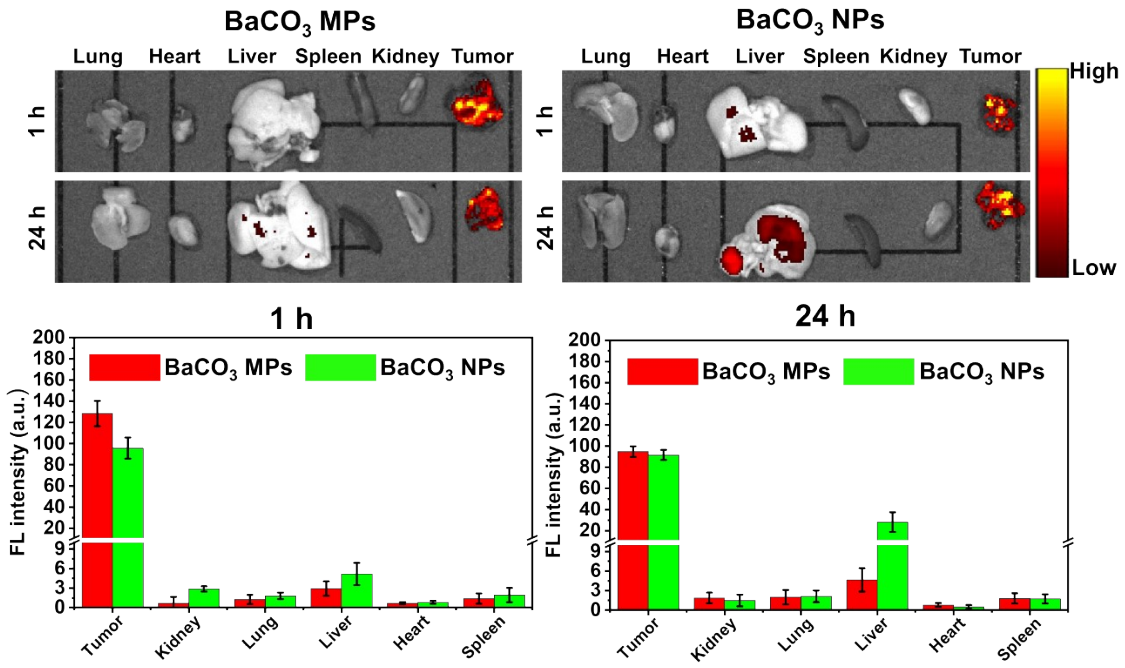

Figure S10. *Ex vivo* fluorescence imaging of major organs (lungs, heart, liver, spleen, and kidneys) and tumor after intratumoral injection of Cy5-labeled **$BaCO_3$ MPs** and **$BaCO_3$ NPs** with corresponding quantitative assessment of their biodistribution. Time of observation was 1 h and 24 h, respectively. The results are shown as an average value ± standard deviation (n = 3).

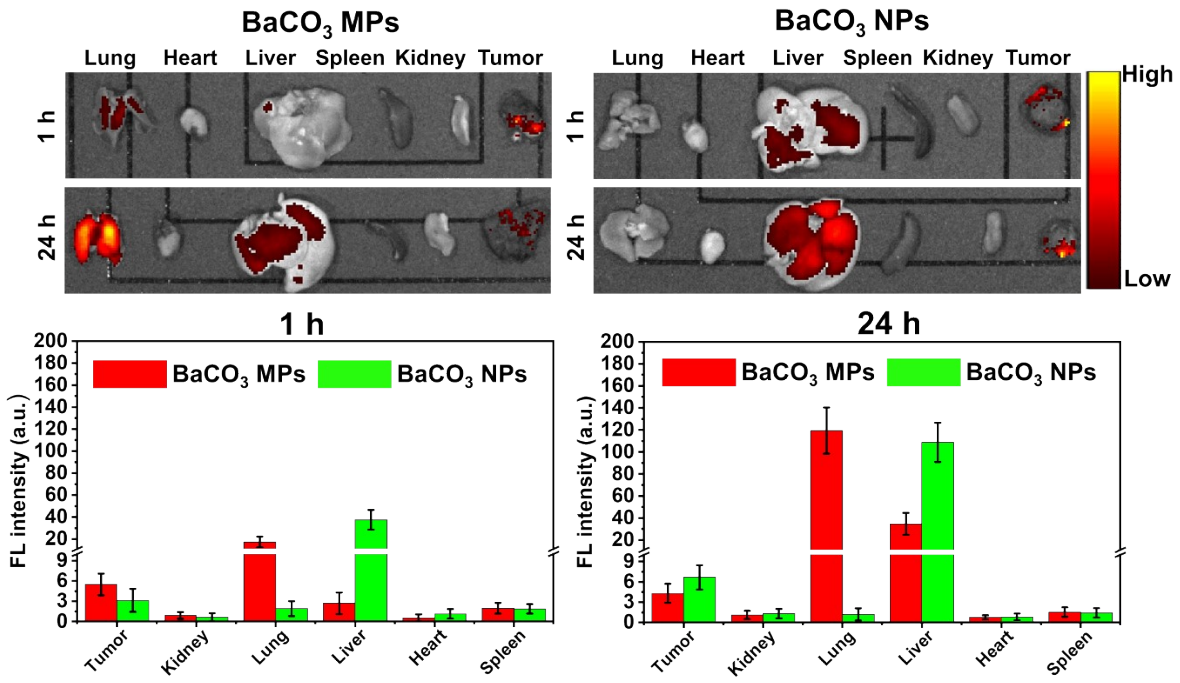

Figure S11. *Ex vivo* fluorescence imaging of major organs (lungs, heart, liver, spleen, and kidneys) and tumor after intravenous injection of Cy5-labeled **$BaCO_3$ MPs** and **$BaCO_3$ NPs** with corresponding quantitative assessment of their biodistribution. Time of observation was 1 h and 24 h, respectively. The results are shown as an average value ± standard deviation (n = 3).

17. Radiometry of organs

TRIATHLER portable spectrometric radiometer (Hidex Oy, Finland) was used to perform direct radiometry of main organs of tumor-bearing C57BL/6 mice investigate distribution of radiolabeled **$BaCO_3$ MPs** and **$BaCO_3$ NPs** in tumor-bearing C57BL/6 mice using direct radiometry analysis. For this, radiolabeled **$BaCO_3$ MPs** and **$BaCO_3$ NPs** were injected intratumorally and intravenously using the same dose (10 MBq, 100 mg/kg). The animals were sacrificed after 24 h after the administration. The organs (the heart, liver, lungs, spleen, and kidneys) and tumors were removed from the body, placed in plastic tubes and weighed. The total radioactivity for each tested sample was calculated as the percentage of the injected activity adjusted by the weight of tissues (% ID/g).

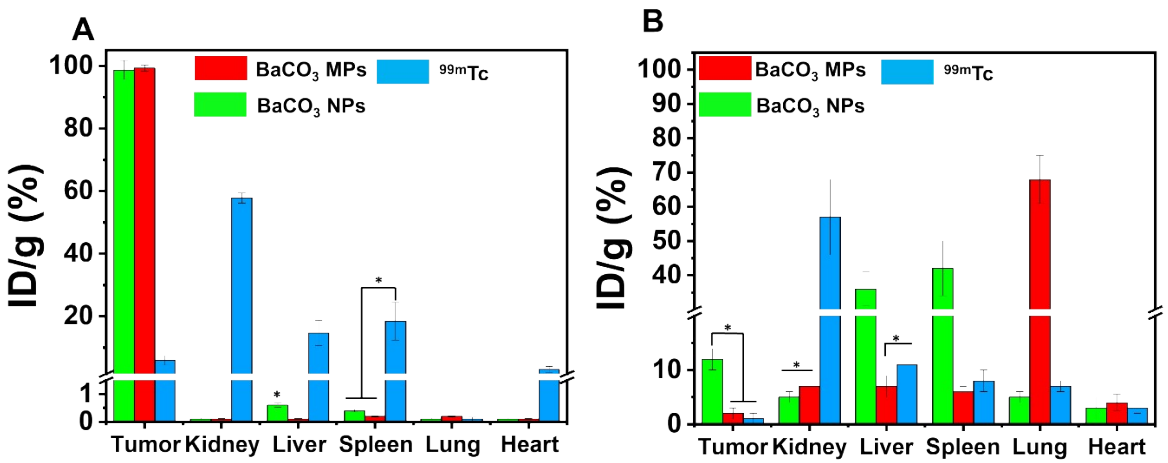

Figure S12. *Ex vivo* radiometry analysis of major organs (the lungs, heart, liver, spleen, and kidneys) and tumor after intratumoral injection (A) and intravenous injection (B) of ^{99m}Tc-labeled **$BaCO_3$ MPs** and **$BaCO_3$ NPs**. Time of observation was 24 h. The results are shown as an average value ± standard deviation (n = 3). Symbol * indicates $p < 0.05$.

18. Histological analysis

18.1. Fluorescent staining

Biopsy samples from the examined organs (the liver, kidneys, spleen, heart, and lungs) and tumors were isolated, gently washed in PBS, and placed in 10% of neutral buffered formalin for no more than 24 h. After fixation, histological processing and staining were carried out according to the following procedure. First, the specimens were dehydrated in isopropanol series on the Leica TP1020 Tissue Processor (Leica) and embedded in paraffin blocks using an embedding station Microm EC350-1 (Thermo Scientific) with HISTOMIX paraffin (Biovitrum). Second, the obtained histological sections were cut into 4-5 μm-thick slices using HM 340E Rotary Microtome (Thermo Scientific) and placed on glass slides.

Following this, the samples were placed in a solution of PI (1 mg/mL) for 1 h, which provides further visible blue fluorescent signal from the colored cell nuclei.

The sections were then washed twice in PBS solution and fixed with glycergel according to the standard protocol. The fixed samples were then visualized under CLSM (objective HC PL FLUOTAR 10x/0.30).

Finally, the resulting histology slides were scanned with a histological scanner Aperio AT2 (Leica) and processed to obtain images using NDP.view2 Viewing software (Hamamatsu Photonics, Japan). Images of the liver, spleen, lungs and tumor slice samples are presented in **Figure 7**. Images of the heart and kidney samples are presented in **Figure S13**.

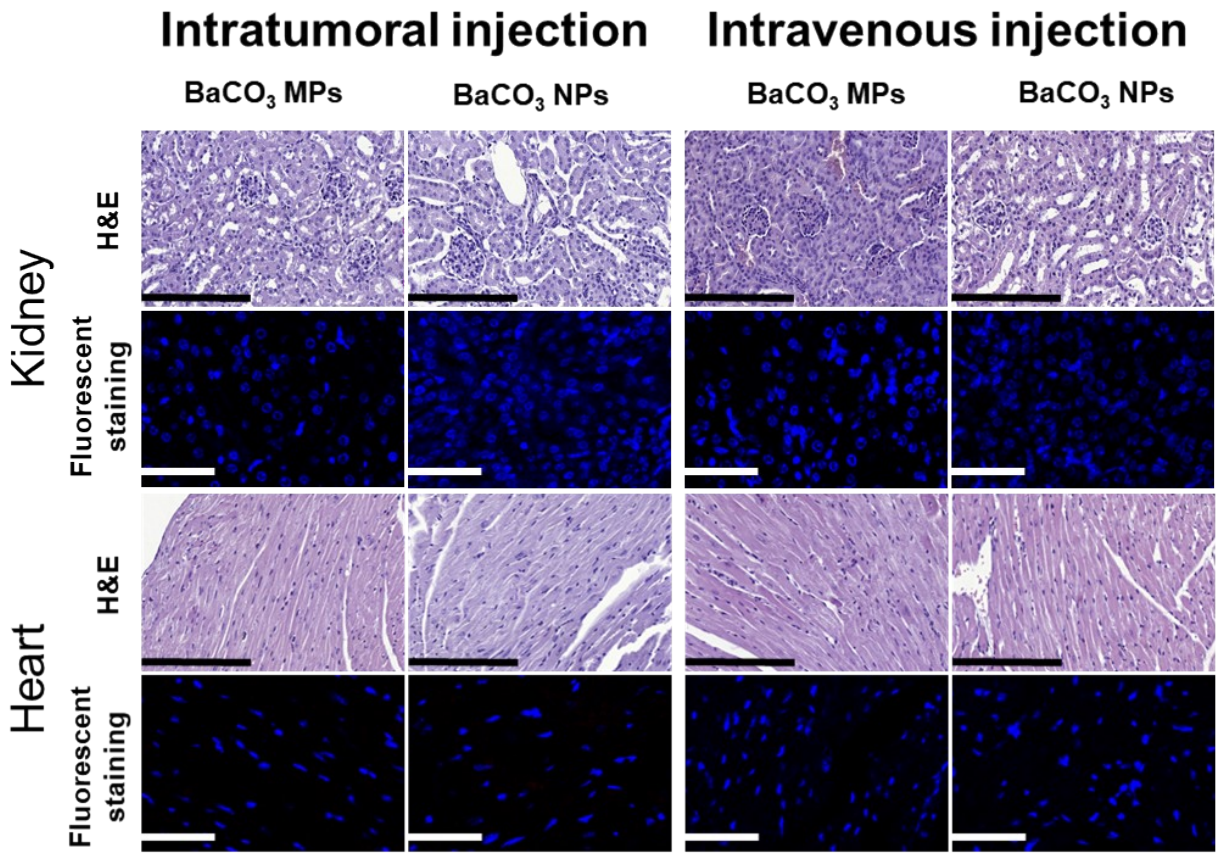

Figure S13. Histological analysis (fluorescent staining) of B16-F10 tumor-bearing mice after intratumoral and intravenous injection of **$BaCO_3$ MPs** and **$BaCO_3$ NPs**. Images of selected organ tissues (the heart and kidneys) and tumor were taken 24 h after the injection of **$BaCO_3$ MPs** and **$BaCO_3$ NPs** (100 mg/kg). Scale bars for H&E = 200 μm and for fluorescent imaging = 50 μm.

18.2. H&E staining

To examine the pathological events and confirm there are no adverse effects of **$BaCO_3$ MPs** and **$BaCO_3$ NPs**, all the mice were sacrificed, and the main organs were harvested. Biopsy samples were taken from the main organs (the liver, kidneys, spleen, heart, and lungs) and tumors for histological analysis. The organs were fixed in 10% formalin and subjected to further histopathological examinations. The samples were then dehydrated with an isopropanol solution on a Leica TP1020 Tissue Processor. Then, the specimens were embedded in paraffin blocks using an embedding station (Microm EC 350–1, Thermo Scientific), and serial sections of 4-5 μm thickness were cut using a microtome (HM 340E Rotary Microtome, Thermo Scientific). After that, the obtained slices were transferred to distilled water, and Ehrlich's hematoxylin solution was added. After 5 min of staining the sections were transferred to distilled water and washed for 3 min. Then, the samples were transferred to a 1% solution of hydrochloric acid diluted in 70% ethanol for 3 sec. Next, the samples were again washed in pure distilled water for 20 min. Afterwards, the samples were transferred to a 1% aqueous solution of eosin for 1-2 min. The stained sections were washed with distilled water and dehydrated with 96% ethanol. Clarification was performed with a mixture of carbol-xylene for 1 min. Finally, the samples were fixed in glycergel. The obtained samples were analyzed with light microscopy and scanned with a histological scanner Aperio AT2 (Leica). Afterwards, samples were processed to obtain images using NDP.view2 Viewing software (Hamamatsu Photonics, Japan).

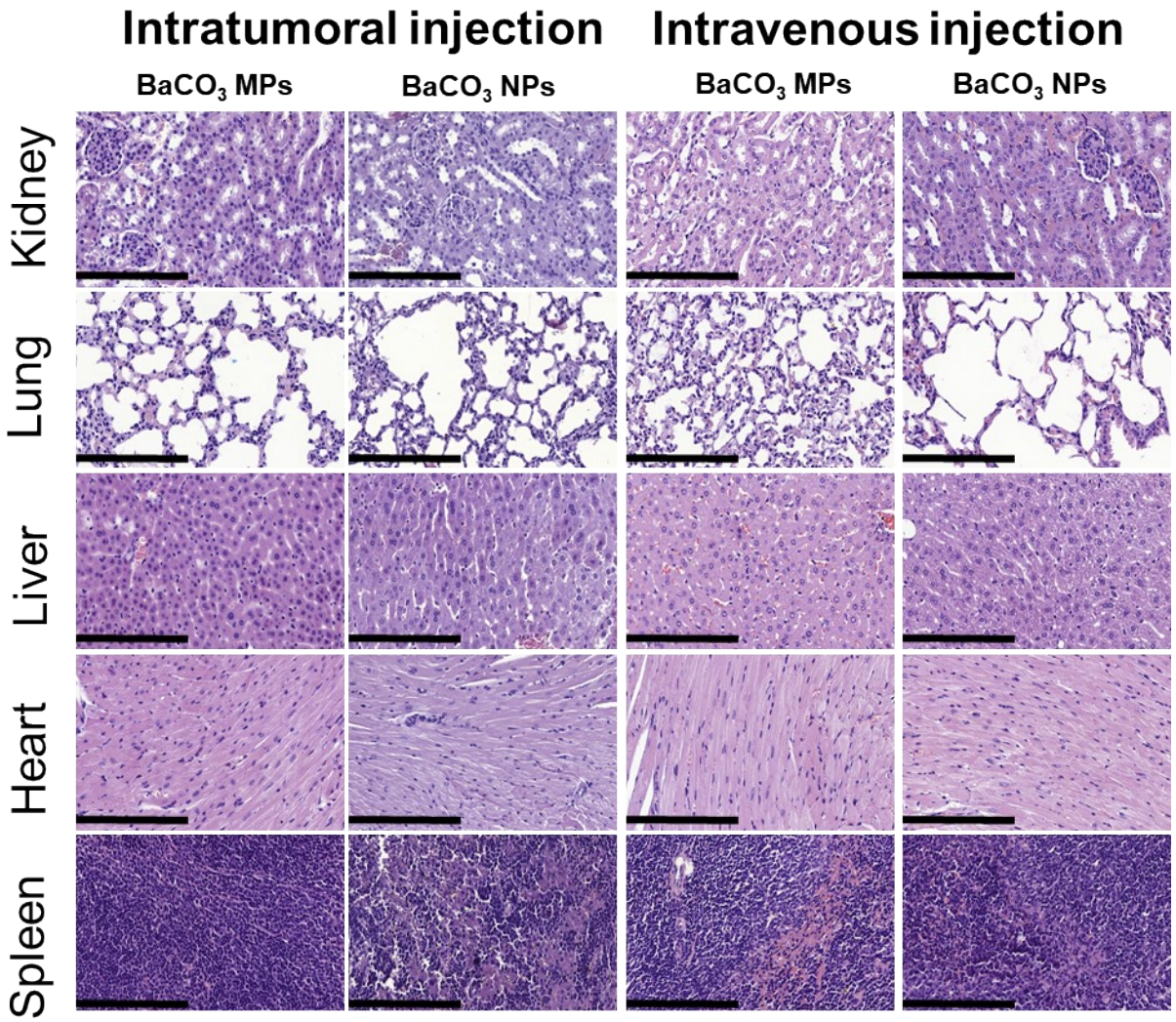

Figure S14. Histological H&E analysis of B16-F10 tumor-bearing mice after intratumoral and intravenous injection of **$BaCO_3$ MPs** and **$BaCO_3$ NPs** on the 5th d post-injection. Scale bars = 200 μm.

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