Materials and Methods

Cell Lines and Animal Model

All animal studies were conducted under a protocol approved by the University of Southern California Institutional Animal Care and Use Committee. The human prostate cancer cell line (PC3) and human colorectal cancer cell line (HT29) were obtained from American Type Culture Collection and cultured under recommended conditions. Tumor models were established in 4- to 6-wk-old male (for PC3) or female (for HT29) athymic nude mice obtained from the Division of Laboratory Animal Medicine (DLAM) at UNC. Cells were used for in vitro and in vivo experiments when they reached 80%–90% confluence. To generate the mouse tumor model, 4- to 6-wk-old nu/nu mice were each subcutaneously inoculated with (5–10) $\times 10^6$ tumor cells, suspended in 100 μL of PBS, at the front flank. The tumor sizes were monitored every other day and the mice were used for in vivo studies when the tumor reached 4-6 mm in diameter (typically at 3–5 wk after inoculation of the cancer cells).

Synthesis of Cy5.5-MAb159 and Cy5.5-hIgG

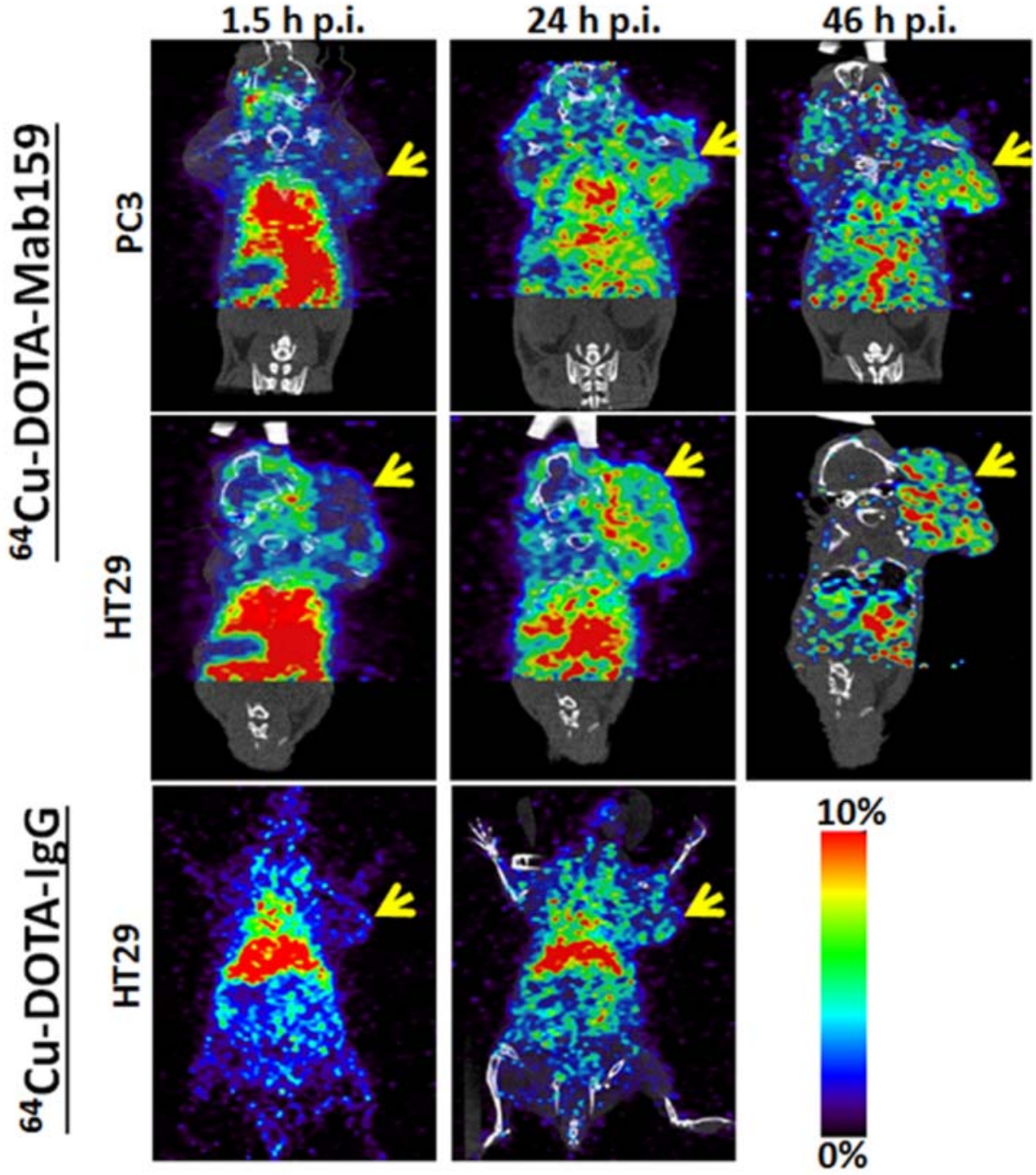
An aqueous solution of MAb159 or hIgG (350 μg) was mixed with Cy5.5-NHS in 0.1 M borate buffer (pH 8.5). The molar ratio of antibody to Cy5.5-NHS was 1 to 2.4. After incubation overnight at 4°C, the reaction mixture was passed through a PD-10 column and eluted with PBS solution. Cy5.5-MAb159 or Cy5.5-hIgG was collected; the absorbance at 280 nm and 673 nm of Cy5.5-MAb159 and Cy5.5-hIgG was measured with Thermo BioMate 3S spectrophotometers (Thermo Fisher Scientific Inc.). The dye-to-antibody molar ratio was calculated according to the manufacturer's instructions.

Immunofluorescence Staining of HT 29 and PC3 Tumor Tissues

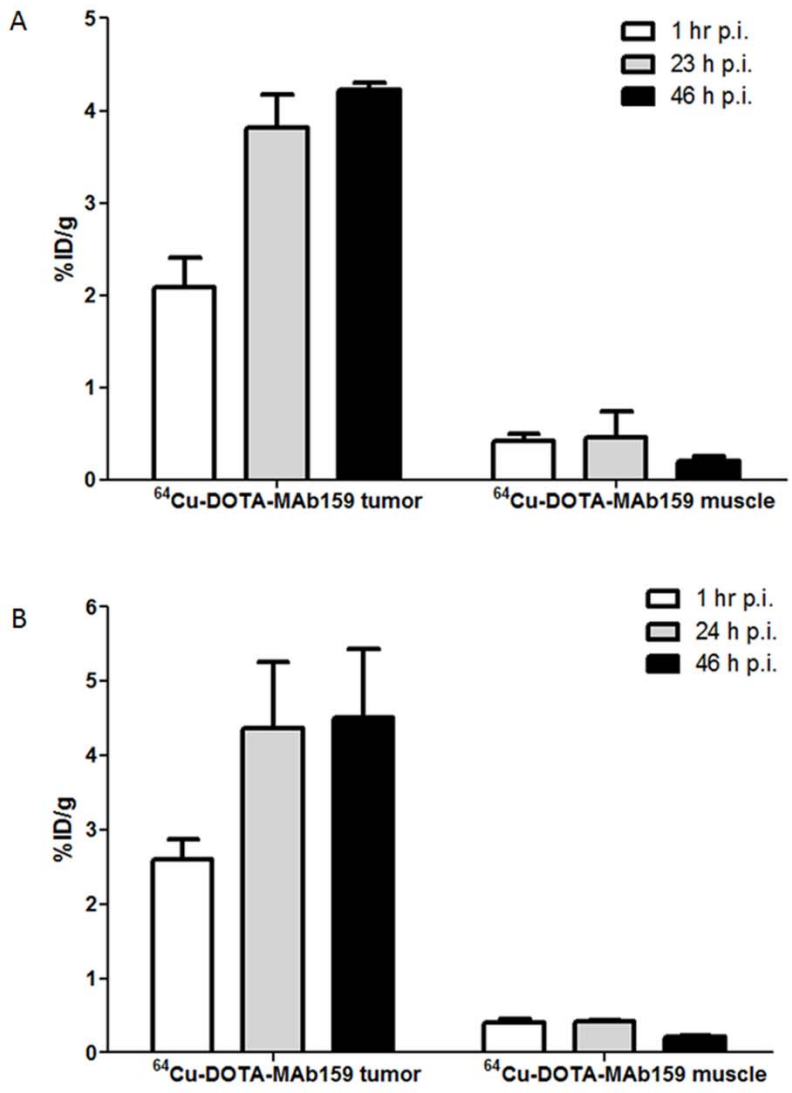
For HT29 and PC3 tumors, Cy5.5-MAb159 or Cy5.5-hIgG was injected into each tumor-bearing mouse via the tail vein. At 48 h after injection, the mice were euthanized and the tumors were dissected, embedded in optimal-cutting-temperature compound (Sakura Finetek) and cut into 8-μm sections. Frozen sections were fixed in ice-cold acetone for 5 min, blocked with 10% normal horse serum (Gibco) for 20 min, and then incubated with primary anti-CD31 antibody at room temperature for 30 min. After PBS buffer washing, the sections were incubated with secondary antibody for 30 min at room temperature. Subsequently, the slides were covered with EverBrite Mounting Medium containing DAPI and observed under a Zeiss LSM 710 laser scanning microscope.

Cellular Assays in Gemcitabine-Treated HT29 Cells

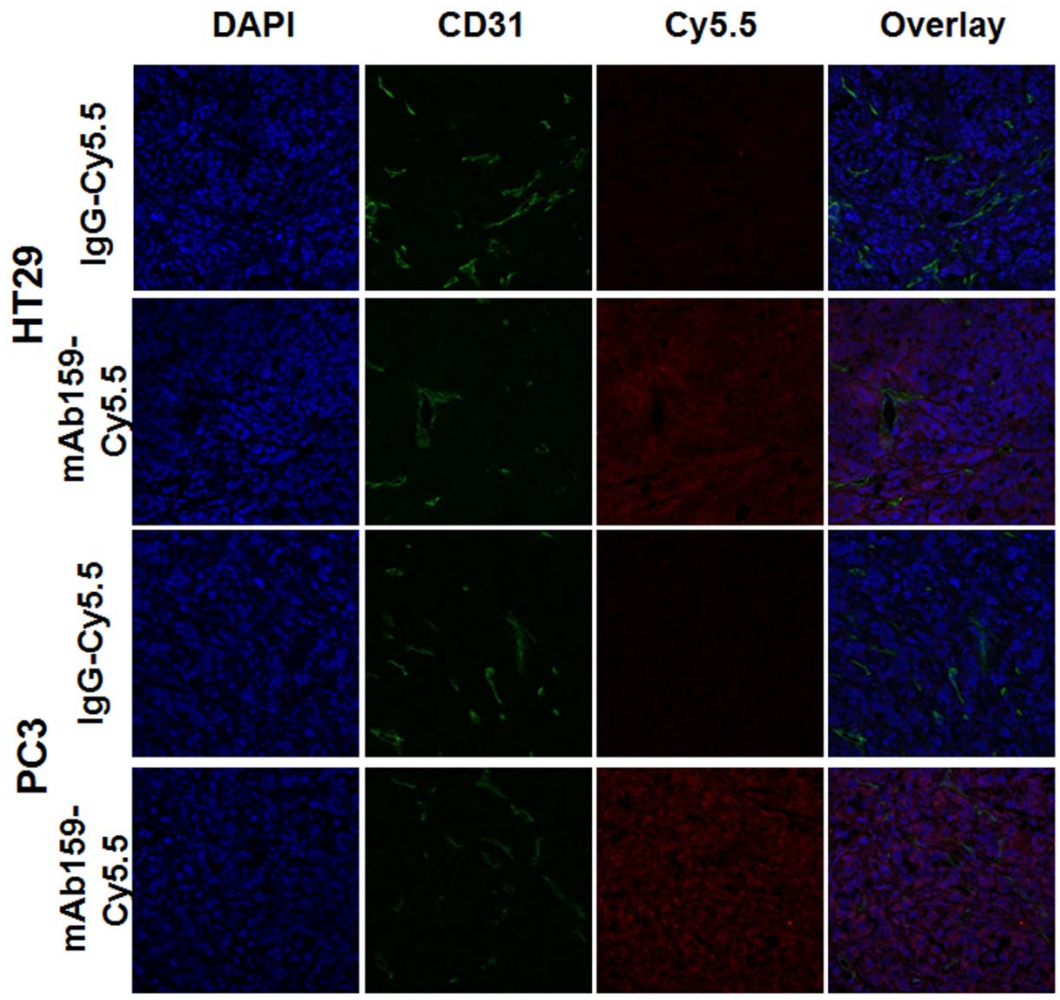
HT29 cells were treated with 50 μM of gemcitabine at 37°C for 24 h. The cells were rinsed briefly with PBS and used for the experiments. To test GRP78-related cell uptake of ^{64}Cu-DOTA-MAb159 or ^{64}Cu-DOTA-IgG, 37 kBq (1 μCi) of ^{64}Cu-DOTA-MAb159 or ^{64}Cu-DOTA-IgG were incubated with as-prepared HT29 cells at room temperature for 120 min, and cells were rapidly washed 3 times with ice-cold PBS and lysed with 0.1N NaOH/0.1% SDS. The cell lysates were measured for radioactivity on a gamma counter. To test the GRP78 expression level in gemcitabine-treated HT29 cells, the HT29 cells were first incubated with PBS containing 10% goat serum for 20 min at room temperature and then were incubated with Cy5.5-MAb159 or Cy5.5-hIgG for 30 min. The slides were covered with EverBrite Mounting Medium containing DAPI and observed under a Zeiss LSM 710 laser scanning microscope. In both experiments, untreated cells were included as a control.



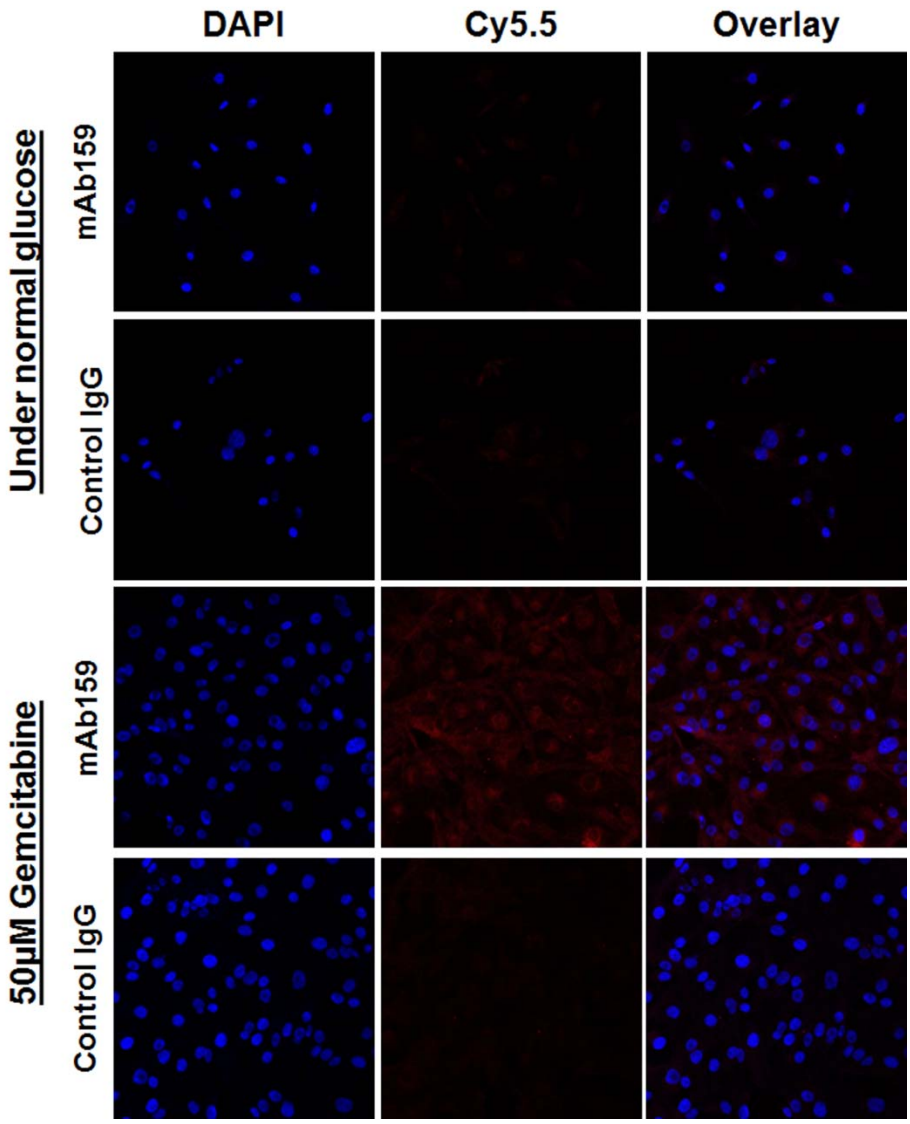
Supplemental Figure 1: Decay-corrected whole-body coronal small animal PET/CT scans of mice bearing PC-3 (top) and HT29 (middle) tumors. Images were obtained at 1.5, 24, and 46 h after injection of ^{64}Cu-DOTA-MAb159. ^{64}Cu-DOTA-hIgG (bottom) control was performed in HT29 tumor model at 1.5, 24, and 46 h after injection. Arrows indicate tumors.



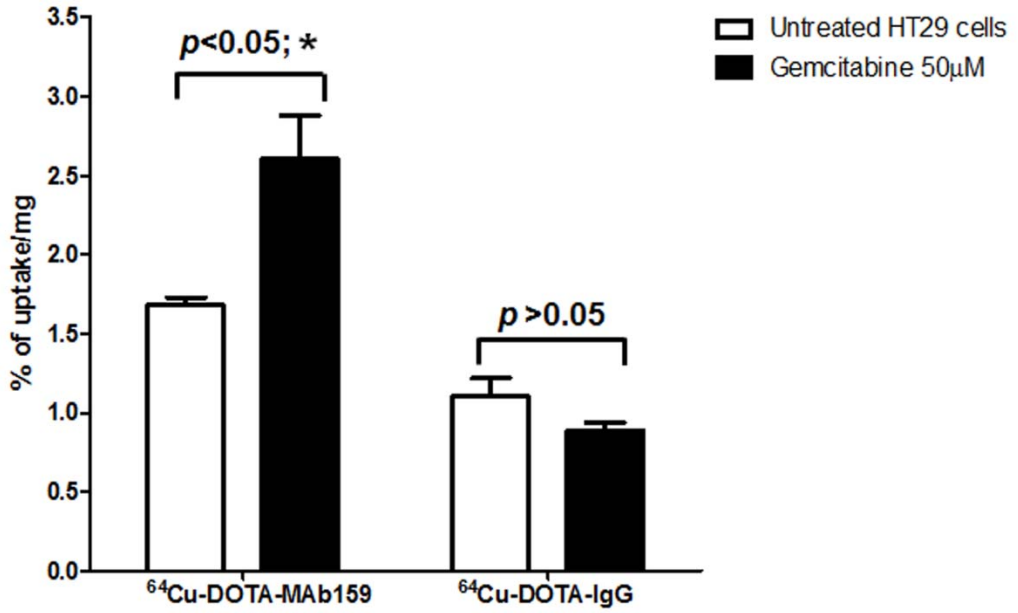
Supplemental Figure 2. Quantitative PET analysis of radioactivity in tumor and muscle of mice bearing PC-3 tumors (A) or HT29 tumors (B) after injection of ^{64}Cu-DOTA-MAb159.



Supplemental Figure 3. Antibody distribution analysis on PC3 and HT29 tumor sections at 48 h after injection of hIgG-Cy5.5 or MAb159-Cy5.5 (red). The tumor sections were also stained for DAPI (blue) and CD31-positive blood vessels (green).



Supplemental Figure 4. GRP78 staining before and after gemcitabine (50 μM) treatment. Gemcitabine treatment resulted in an increased binding of MAb159-Cy5.5 to HT29 cells, while control treatment showed only minimal binding of MAb159-Cy5.5. In contrast, gemcitabine treatment did not change the binding of hIgG-Cy5.5.



Supplemental Figure 5. The effect of gemcitabine on cell uptake of ^{64}Cu-DOTA-MAb159. Cell uptake of ^{64}Cu-DOTA-MAb159 increased from 1.68% ± 0.09% to 2.61% ± 0.47% because of gemcitabine treatment ($P < 0.05$). In contrast, cell uptake of ^{64}Cu-DOTA-IgG remained unchanged (1.10% ± 0.21% for untreated cells vs. 0.89% ± 0.10% for treated cells, $P > 0.05$).