**Additional file 1: Supplementary materials and methods**

**Preparation of $^{64}$Cu-cyclam-RAFT-c(-RGDfK-)₄ ($^{64}$Cu-RaftRGD) and $^{64}$Cu-diacetyl-bis (N⁴-methylthiosemicarbazone) ($^{64}$Cu-ATSM)**

For preparation of $^{64}$Cu-RaftRGD, 1 nmol of cyclam-RAFT-c(-RGDfK-)₄ (molecular weight: 4119.6) in 20 μL of dimethyl sulfoxide was mixed with 148 MBq of $^{64}$CuCl₂ in 20 μL of ammonium citrate buffer (100 mM, pH 5.5) and incubated at 70 °C for 10 min. The radiolabeling efficiency was assessed by reversed-phase high-performance liquid chromatography (solvent A, 0.1% trifluoroacetic acid in H₂O; solvent B, 0.1% trifluoroacetic acid in acetonitrile; flow rate = 1.3 mL/min, linear gradient, 5 to 100% solvent B in 15 min; Cosmosil 5C₁₈-MS-II column, 4.6 ID × 150 mm [Nacalai Tesque, Inc., Kyoto, Japan]). The analysis was performed on a Waters chromatography system (Nihon Waters K.K., Tokyo, Japan) equipped with a Waters 1525 binary pump, Waters 2489 dual absorbance detector, and radiation detection system (Ludlum Model 44-10 γ-scintillator and Model 2200 Scaler/Ratemeter, Ludlum Measurements, Inc., Sweetwater, Texas). The relative radioactivity was expressed in millivolts (mV).

For preparation of $^{64}$Cu-ATSM, 1 nmol of H₂ATSM (molecular weight: 261.37) in 1 μL of dimethyl sulfoxide was mixed with 7.4 MBq of $^{64}$CuCl₂ in 1 μL of ammonium citrate buffer and incubated at room temperature for 15 min. The radiolabeling efficiency was determined by silica gel thin-layer chromatography (silica gel 60; Merck, Darmstadt, Germany) using ethyl acetate as a mobile phase.
Radioactivity levels on the thin-layer chromatography plates were analyzed using a bioimaging analyzer (FLA-7000; Fujifilm, Tokyo, Japan).

**Autoradiography and fluorescence imaging**

The excised tumors were embedded in Tissue-Tek OCT compound (Sakura Finetek, Tokyo, Japan), and frozen by immersion in n-hexane precooled at −80 °C. Frozen sections (10 µm thick) were then made, air-dried, and kept in the dark. For autoradiography, after overnight exposure of the tumor sections to an imaging plate (BAS-MS 2040, Fujifilm) at −80 °C, the plate was scanned using a bioimaging analyzer (FLA-7000) for determination of intratumoral radioactivity distribution. The sections were then stored at −80 °C until the radioactivity decayed to negligible levels, after which the sections were fixed with 2% paraformaldehyde at room temperature for 10 min, mounted with mounting agent (Dapi-Fluoromount-G™; SouthernBiotech, Birmingham, AL) containing 4′,6-diamidino-2-phenylindole for nucleus staining, and observed for intratumoral Cy5.5 fluorescence distribution. An adjacent autoradiographed section was fixed in cold acetone at −20 °C for 10 min, stained with a rat anti-mouse CD31 monoclonal antibody (1:1500 dilution; BD Biosciences, Bedford, MA), and visualized using Alexa Fluor 594-conjugated goat anti-rat antibody (1:200 dilution; Invitrogen, Camarillo, CA). After autoradiography and fluorescence imaging, the slides were immersed in phosphate-buffered saline at 4 °C for a couple of days to remove the coverslips, and then stained with hematoxylin and eosin (HE). Fluorescence and HE images of the whole-
tumor sections were acquired using a fluorescence microscope (BZ-9000, Keyence, Osaka, Japan) or the Odyssey CLx near-infrared fluorescence imaging system (LI-COR Biotechnology, Lincoln, NE) as indicated.

**Histological study of tumor proliferation**

The excised tumors were embedded in Tissue-Tek OCT compound and frozen by immersion in \( n \)-hexane precooled at \(-80^\circ C\). Frozen sections (10 \( \mu \)m thick) were cut and stored at \(-80^\circ C\) until the radioactivity decayed to negligible levels. The sections were fixed with 4\% paraformaldehyde at room temperature for 15 min, incubated with a rabbit anti-human Ki67 antibody (SP6, 1:500 dilution; Abcam, Cambridge, UK) followed by a peroxidase-labeled polymer-conjugated goat anti-rabbit immunoglobulin (Dako, Glostrup, Denmark), and visualized with the chromogen diaminobenzidine. Nuclear counterstaining was carried out with hematoxylin.

**Hematology and hepatorenal functions**

Examination of the hematological and hepatorenal functions was performed as previously described (1). For hematology, 10 \( \mu \)L of tail vein blood was examined in a hematology analyzer (Celltac \( \alpha \) MEK-6458, Nihon Kohden, Tokyo, Japan) for measurement of white blood cell count (WBC, \( 10^2/\mu \)L), red blood cell count (RBC, \( 10^6/\mu \)L), platelet count (PLT, \( 10^9/\mu \)L), hemoglobin concentration (HGB, g/dL), hematocrit value (HCT, %), and red blood cell indices, including mean
cell volume (MCV, fL), mean cell hemoglobin (MCH, pg), and mean cell hemoglobin concentration (MCHC, g/dL). For hepatorenal function test, the blood collected by cardiac puncture was examined in a blood chemistry analyzer (FDC7000V, Fujifilm) for determination of the levels of blood urea nitrogen (BUN, mg/dL), creatinine (CRE, mg/dL), glutamate oxaloacetate transaminase (GOT, U/L), glutamate pyruvate transaminase (GPT, U/L), gamma-glutamyl transpeptidase (GGT, U/L), and alkaline phosphatase (ALP, U/L).

Supplementary reference