Supplemental Information for

Anti-tumor Effect of Integrin Targeted ^{177}Lu-3PRGD$_2$ and Combined Therapy with Endostar

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SUPPLEMENTAL INFORMATION

Materials

The bifunctional chelator 1,4,7,10-tetraazadodecane- *N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA) was purchased from Macrocyclics, Inc. (Dallas, TX). 1-Ethyl-3-[3-(dimethylamino)-propyl] carbodiimide (EDC), N-hydroxysulfono-succinimide (SNHS), and Chelex 100 resin (50-100 mesh) were purchased from Sigma-Aldrich (St. Louis, MO). Water and all buffers were passed through a Chelex 100 column (1×15 cm) before use in DOTA conjugation and radiolabeling procedures to ensure that aqueous buffers were metal free. $^{177}LuCl_3$ solutions were obtained from Perkin-Elmer (Norwalk, CT). PEG_4-E[PEG_4-c(RGDfK)]$_2$ ($3PRGD_2$) were obtained from the Peptides International, Inc. (Louisville, KY).

Cell culture and animal model

U87MG cells were cultured in low glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) at 37°C in a humidified atmosphere with 5% CO_2. Female BALB/c nude mice (4~5 weeks of age) were purchased from the Department of Experimental Animal, Peking University Health Science Center. U87MG tumor model was established by subcutaneous injection of 2×10^6 U87MG tumor cells into the right thighs. Since the shorter particle range of ^{177}Lu leads to better absorption in smaller tumors, when the tumor volume reached 50~60 mm^3 (10~12 days after inoculation), the U87MG tumor-bearing nude mice were used for radionuclide therapy studies. When the tumor volume reached 150~200 mm^3 (16~18 days after inoculation), the U87MG tumor-bearing nude mice

were used for biodistribution and gamma imaging studies. All animal experiments were performed in accordance with guidelines of Institutional Animal Care and Use Committee of Peking University.

Histopathology and immunohistochemistry

IHC was performed using rat anti-mouse CD31 (1:100; BD Biosciences) antibody and Ki67 (1:200; Boisynthesis Biotechnology Co, Beijing, China). Briefly, frozen U87MG tumor tissue slices (7-μm thickness) were fixed with ice-cold acetone, rinsed with PBS and blocked with 10% BSA for 30 min at room temperature. The slices were incubated with rat anti-mouse CD31 (1:100; BD Biosciences) antibody for 1 h at room temperature and then visualized with FITC-conjugated goat anti-rat secondary antibodies (1:200; Jackson Immuno-Research Laboratories, West Grove, PA).

Endostar (s.c.) treatment groups were selected to perform the CD31 and Anti-NG2 chondroitin Sulfate Proteoglycan (1:200； Millipore) IHC staining on treatment day 0, 5, 7 and 14. Briefly, frozen U87MG tumor tissue slices (7-μm thickness) were fixed with ice-cold acetone, rinsed with PBS and blocked with 10% BSA for 30 min at room temperature. The slices were incubated with rat anti-mouse CD31 (1:100; BD Biosciences) antibody or Anti-NG2 chondroitin Sulfate Proteoglycan (1:200； Millipore) for 1 h at room temperature and then visualized with FITC-conjugated goat anti-rat secondary antibodies (1:200; Jackson Immuno-Research Laboratories, West Grove, PA).

Table S1. Selected Biodistribution and T/NT data of ^{177}Lu-3PRGD$_2$ in U87MG tumor mice.

Biodistribution of ^{177}Lu-3PRGD$_2$ in U87MG tumor mice. (Mean ± SD, n = 4)					
	1 h	4 h	24 h	72 h	Blocking (1 h)
Blood	0.37 ± 0.27	0.04 ± 0.01	0.06 ± 0.09	0.08 ± 0.09	0.18 ± 0.03
Heart	0.50 ± 0.18	0.36 ± 0.09	0.17 ± 0.05	0.09 ± 0.07	0.13 ± 0.02
Liver	1.47 ± 0.33	1.16 ± 0.21	0.40 ± 0.19	0.14 ± 0.02	0.28 ± 0.03
Spleen	1.17 ± 0.39	1.11 ± 0.25	0.89 ± 0.23	0.18 ± 0.08	0.23 ± 0.01
Lung	1.31 ± 0.35	0.74 ± 0.08	0.30 ± 0.03	0.06 ± 0.04	0.41 ± 0.05
Kidney	4.18 ± 1.08	3.13 ± 0.59	1.22 ± 0.83	0.76 ± 0.17	2.65 ± 0.39
Intestine	5.16 ± 0.48	4.15 ± 1.02	1.90 ± 1.43	0.44 ± 0.43	0.92 ± 0.25
Stomach	2.72 ± 0.60	1.47 ± 0.53	0.83 ± 0.22	0.13 ± 0.10	0.54 ± 0.06
Bone	0.57 ± 0.17	0.37 ± 0.16	0.28 ± 0.15	0.06 ± 0.05	0.22 ± 0.08
Muscle	0.64 ± 0.63	0.31 ± 0.06	0.30 ± 0.20	0.15 ± 0.05	0.15 ± 0.04
Brain	0.08 ± 0.01	0.05 ± 0.02	0.04 ± 0.03	0.01 ± 0.01	0.03 ± 0.02
Tumor	6.03 ± 0.65	4.62 ± 1.44	3.55 ± 1.08	1.22 ± 0.18	0.36 ± 0.08
T/LI	4.19 ± 0.54	3.96 ± 0.96	9.43 ± 1.80	8.84 ± 1.65	
T/KI	1.49 ± 0.24	1.51 ± 0.49	2.34 ± 0.45	1.63 ± 0.15	
T/IN	1.03 ± 0.25	1.19 ± 0.51	2.35 ± 0.84	4.25 ± 4.77	
T/MU	9.45 ± 3.85	16.21 ± 8.25	16.62 ± 9.38	8.83 ± 3.30	
T/BR	81.5 ± 13.6	130.3 ± 90.1	154.6 ± 126.2	178.2 ± 142.7	

Table S2 WBC Variations on Different Days after Injection of ^{177}Lu-3PRGD$_2$

Doses	Post-injection Day				
	2	**6**	**9**	**13**	**16**
111 MBq	52.86 ± 7.76	56.54 ± 9.56	47.31 ± 18.45	52.15 ± 16.05	96.93 ± 19.32
74 MBq	66.86 ± 12.98	61.18 ± 32.51	52.25 ± 28.46	59.57 ± 28.14	99.76 ± 32.41
37 MBq	67.92 ± 33.83	67.59 ± 15.55	65.42 ± 32.05	93.86 ± 37.80	105.84 ± 11.41
0 MBq	98.91 ± 12.59	106.61 ± 30.76	110.04 ± 22.19	119.25 ± 24.06	115.16 ± 24.00

Note: Data are expressed as the percentage of original values.

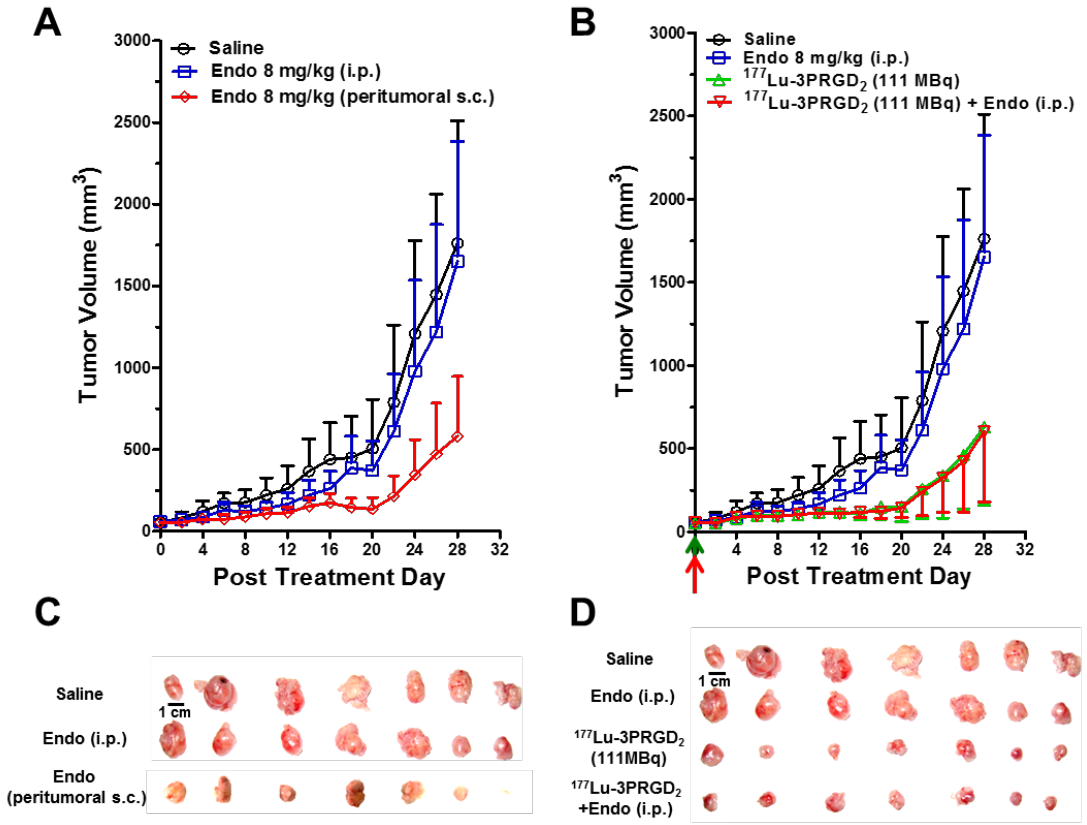

Figure S1. (A) Anti-angiogenic therapy of established U87MG tumors in nude mice with saline, Endostar (8 mg/kg, intraperitoneal injection) or Endostar (8 mg/kg, peritumoral subcutaneous injection). (B) Tumor pictures of groups above in (A) at the end of treatment. (C) Combined therapy of established U87MG tumors in nude mice with saline, Endostar (8 mg/kg intraperitoneal injection), ^{177}Lu-3PRGD$_2$ (111 MBq), and ^{177}Lu-3PRGD$_2$ (111 MBq) + Endostar (8 mg/kg intraperitoneal injection). (D) Tumor pictures of groups above in (C) at the end of treatment. Volume of tumors in each treatment group was measured and expressed as a function of time (means ± SD, n = 7 per group).

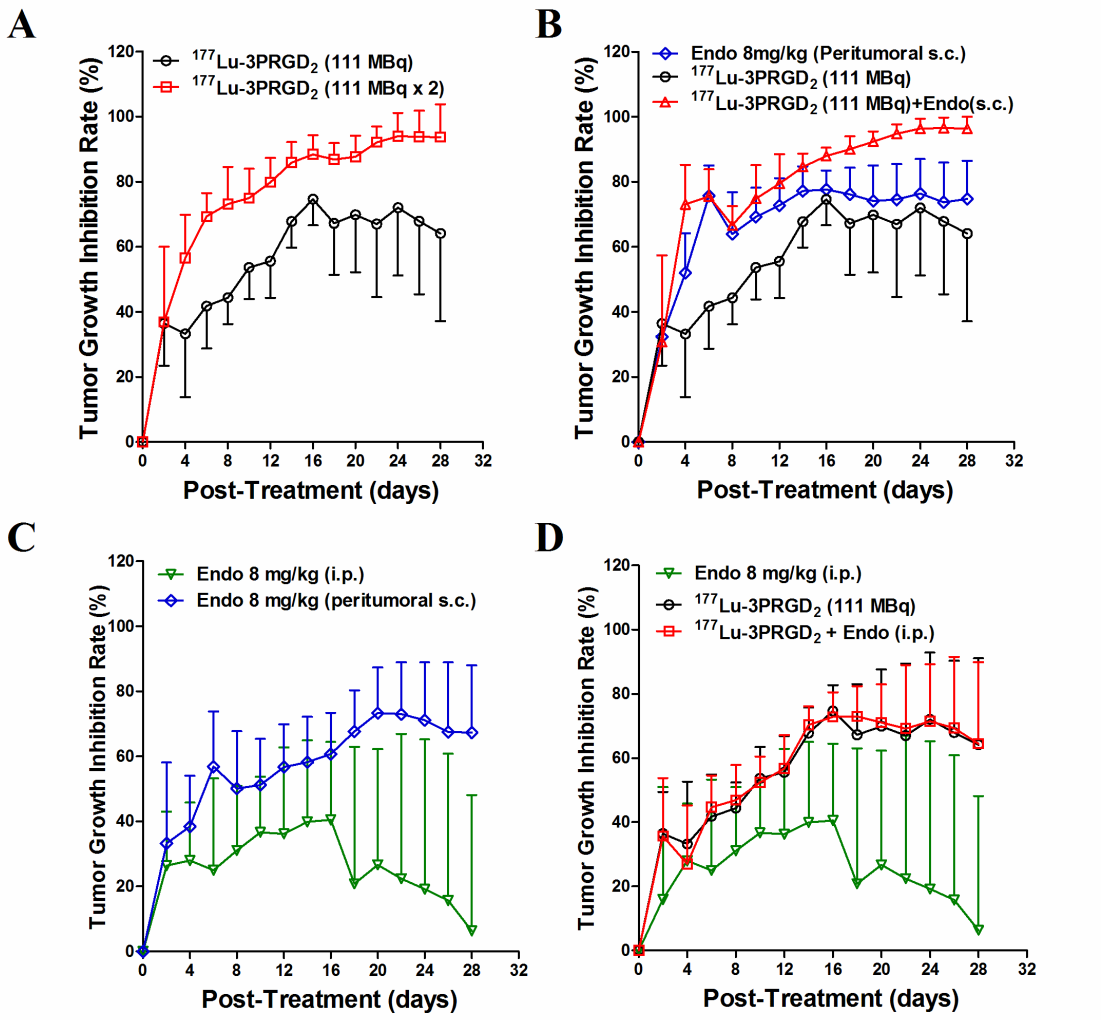

Figure S2. Tumor growth inhibition curve of treatment groups compared to untreated control group. (A) Tumor growth inhibition curve of treatment groups administrated with ^{177}Lu-3PRGD$_2$ (111 MBq), or ^{177}Lu-3PRGD$_2$ (111 MBq × 2). (B) Tumor growth inhibition curve of treatment groups administrated with Endostar (8 mg/kg, peritumoral subcutaneous injection), ^{177}Lu-3PRGD$_2$ (111 MBq day 0), Endostar (8 mg/kg, peritumoral subcutaneous injection) + ^{177}Lu-3PRGD$_2$ (111 MBq day 0). (C) Tumor growth inhibition curve of treatment groups administrated with Endostar (8 mg/kg, intraperitoneal injection) or Endostar (8 mg/kg, peritumoral subcutaneous injection). (D) Tumor growth inhibition curve of treatment groups administrated with Endostar (8 mg/kg, intraperitoneal injection), ^{177}Lu-3PRGD$_2$ (111 MBq day 0), or ^{177}Lu-3PRGD$_2$ (111 MBq day 0) + Endostar (8 mg/kg, intraperitoneal injection).

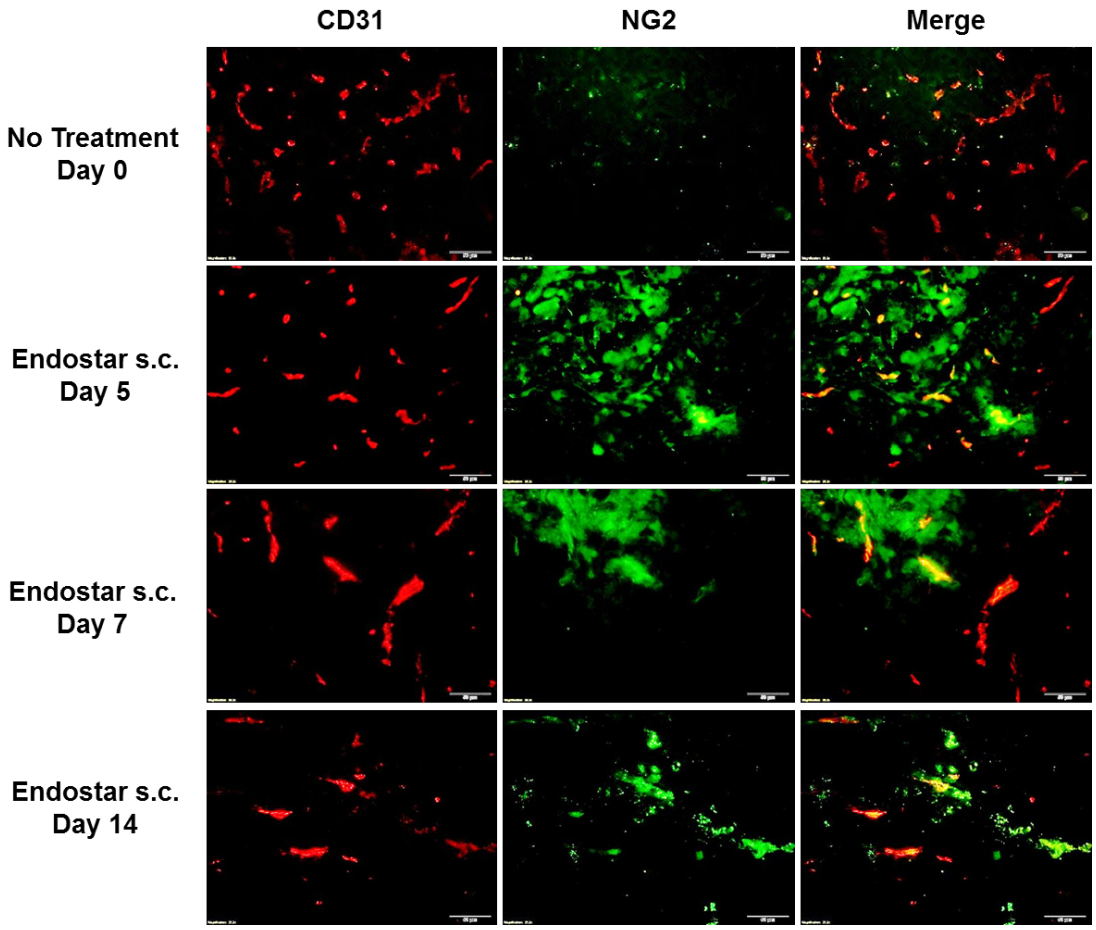

Figure S3. CD31 (red) and NG2 (green) staining of the frozen U87MG tumor tissue slices from the tumor-bearing nude mice treated with Endostar (8 mg/kg, peritumoral subcutaneous injection) on the day 0, 5, 7 and 14.

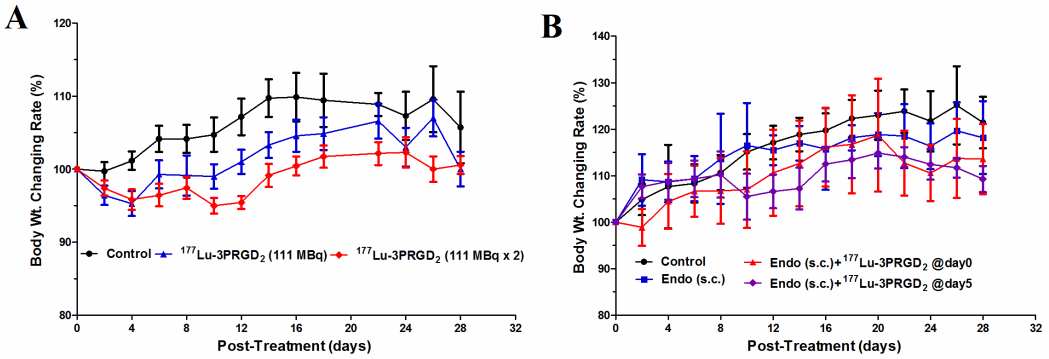

Figure S4. Body weight changing rate of established U87MG tumors in nude mice. (A) Tumor mice were administrated with saline, ^{177}Lu-3PRGD$_2$ (111 MBq), or ^{177}Lu-3PRGD$_2$ (111 MBq × 2). (B) Tumor mice were administrated with saline, Endostar (8 mg/kg, peritumoral subcutaneous injection), Endostar (8 mg/kg, peritumoral subcutaneous injection) + ^{177}Lu-3PRGD$_2$ (111 MBq day 0), or Endostar (8 mg/kg, peritumoral subcutaneous injection) + ^{177}Lu-3PRGD$_2$ (111 MBq day 5).