Supporting Information

Methods and Materials

General

Chemicals were purchased from Sigma Aldrich (St. Louis, MO) except when indicated. *p*-Benzyl-isothiocyanato-desferroxamine was obtained from Macrocyclics, Inc. (Dallas, Texas). Chemicals were used without further manipulations unless stated. Metal-free ultrapure water (>18.2 MΩ·cm, Milli-Q, Millipore, Billerica, MA) was obtained by allowing Chelex 100 resin (Bio-Rad Laboratories, Hercules, CA) to soak overnight at a ratio of 5g resin per 100 ml water as stated by the manufacturer. For dose calibration measurements, a Capintec CRC-55tR (Capintec, Ramsey NJ) calibrated for Zr-89 was utilized. Activity quantification as counts-per-minute (cpm) was obtained through a Perkin Elmer Wizard^2 2480 automatic gamma counter with an energy window of 800-1000 keV normalized for Zr-89 (909 keV). Radiochemical purity and yields were determined via a Bioscan AR-2000 radioTLC plate reader equipped with Winscan version 3.13 software. Silica-gel impregnated glass-fiber instant thin-layer chromatography paper (ITLC-SG, Varian Inc.) was used for ITLC analysis with 50 mM DTPA, pH 7 as the mobile phase. PD10 desalting columns containing Sephadex G-25 M pre-packed resins were purchased from GE Healthcare, UK. Centrifugal columns having a 10 kDa molecular weight cut-off were purchased from Millipore Corp. (Billerica, MA).

Cell lines

All tissue culture manipulations were conducted under a laminar flow hood using aseptic technique. Colo205-luc2 colorectal cancer cells (Bioware Ultra Caliper Life Sciences, Hopkinton, MA) were grown as adherent monolayers with RPMI 1640 (ATCC, Manassas, VA) supplemented with 10% fetal bovine serum (FBS, heat inactivated). DMS79 small cell lung (CRL-2049), and BxPC3 (CRL-1687) pancreas carcinoma were all purchased from ATCC. DMS79 cells were grown as suspended aggregates in RPMI 1640 media supplemented with 10% FBS and L-glutamine. The melanoma cell line SK-MEL-28 was propagated with Eagle's Minimum Essential Medium and 10% FBS while BxPC3 cells were cultured in RPMI1640 with 10% FBS. All cells were grown in a 5% CO2 atmosphere at 37 °C with bi-weekly serial passages upon reaching confluence. Adherent cells were harvested with 0.25% Trypsin and 0.53 mM EDTA in Hank's Buffered Salt Solution (HBSS) with no calcium or magnesium present. The cells were counted using Vi-cell cell viability counter (Beckman Coulter, Fullerton, CA).

Animal models

All animals were treated according to guidelines set by the Institutional Animal Care and Use Committee. Female CB17SC-F severe combined immunodeficient (SCID) mice with restricted flora (Taconic Farms, Inc., Hudson, NY) and female athymic (nu/nu) mice (Harlan Laboratories) were quarantined for acclimatization in MSKCC animal facility for 1 week prior to xenografting. These mice were freely given access to food and water at all times. Tumors were induced on the hind legs by subcutaneous (s.c.) injection of 2-3×10^6 million cells of the cell lines mentioned above in a 200 μL suspension of 1:1 media: BD Matrigel Basement Membrane Matrix (BD

Sciences, Bedford, MA). Palpable tumors developed approximately five weeks for the DMS79 cancer line and 2-3 weeks for the rest of the other malignancies after implantation.

Preparation of 5B1-DFO conjugate

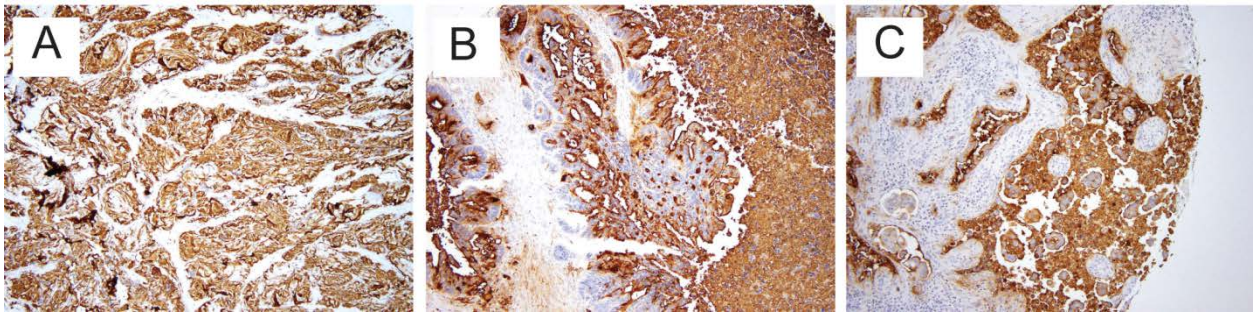
A volume of 200 μL containing 1.24 mg (7.8 nmol) of 5B1 (6.2 mg/ml in saline) was added onto a 1.5 ml tube. The pH of the protein solution was adjusted to ~ 8.9-9.1 using 0.1 M sodium carbonate. A four-fold equivalence of DFO-Bz-SCN (23.5 μg or 31.2 nmol in 20 μL DMSO) was added to the vial. The reaction was incubated at 37 °C for 45 minutes with occasional mild stirring. Subsequent purification using a PD10 size exclusion column removed any unreacted DFO-Bz-SCN and DMSO with 0.9% saline as the mobile phase. Through Lindmo assays, the immunoreactivity was determined to be moderately retained at ~ 72.4±1.1%.

Zr-89 Radiolabeling

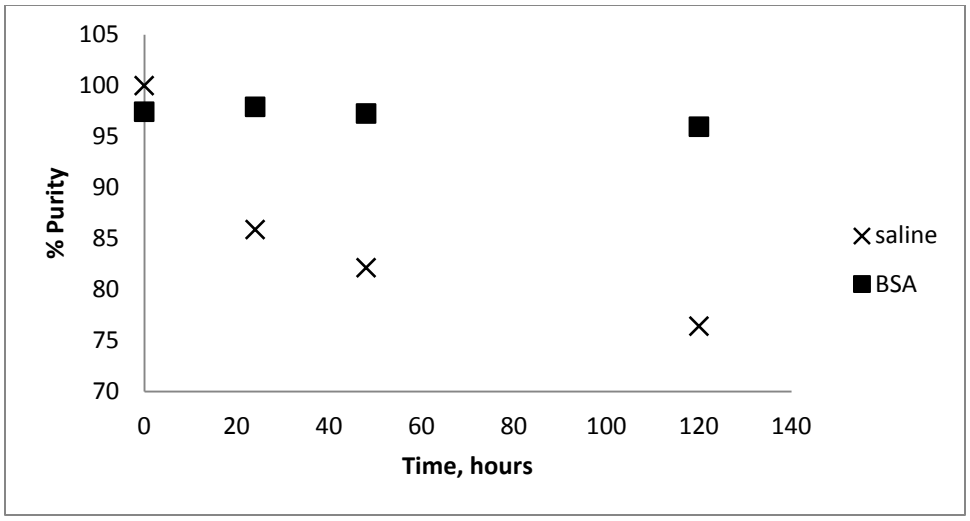
The production of Zr-89 was made by a proton irradiation of a solid yttrium foil target on an EBCO TR19/9 variable beam energy cyclotron (Ebco Industries Inc., British Columbia, Canada) using methods published previously (*1*). After end-of-bombardment, Zr-89 was isolated from the target by passing through a column of hydroxamate modified resin essentially capturing the isotope of interest. Elution was done by washing the column with 1 M oxalic acid resulting in a >99.99% radionuclidic and radiochemical purity and an effective specific activity of 195-497 MBq/μg (5.28-13.4 mCi/μg) (*1, 2*). Typical radiolabeling conditions involve the following protocols. A solution of ^{89}Zr-oxalate (~4 mCi) was added into a 1.5 ml vial and the pH was adjusted to ~ 7.0-7.2 with 1 M Na_2CO_3. Effervescence was observed as the neutralization process resulted in CO2 evolution. After the pH was adjusted and equilibrium was reached, 84 μL (323 μg) of 5B1-DFO (3.86 mg/ml) in saline was added. The reaction was incubated at room temperature with intermittent mild shaking. After 1-2 h, the reaction was quenched with approximately 50 μL of 50 mM DTPA (pH~7). Crude radiolabeling yields were determined to be >95% using iTLC with the Zr-89 5B1 complex remaining at the origin (R_f=0.30) while the free Zr-89 is found at the solvent front (R_f=0.65). Purification of ^{89}Zr-5B1 was performed using PD10 size exclusion column with saline as the eluent. The final radiochemical purity was >99% based on iTLC analysis.

1. Holland JP, Sheh Y, Lewis JS. Standardized methods for the production of high specific-activity zirconium-89. *Nucl Med Biol.* 2009;36:729-739.

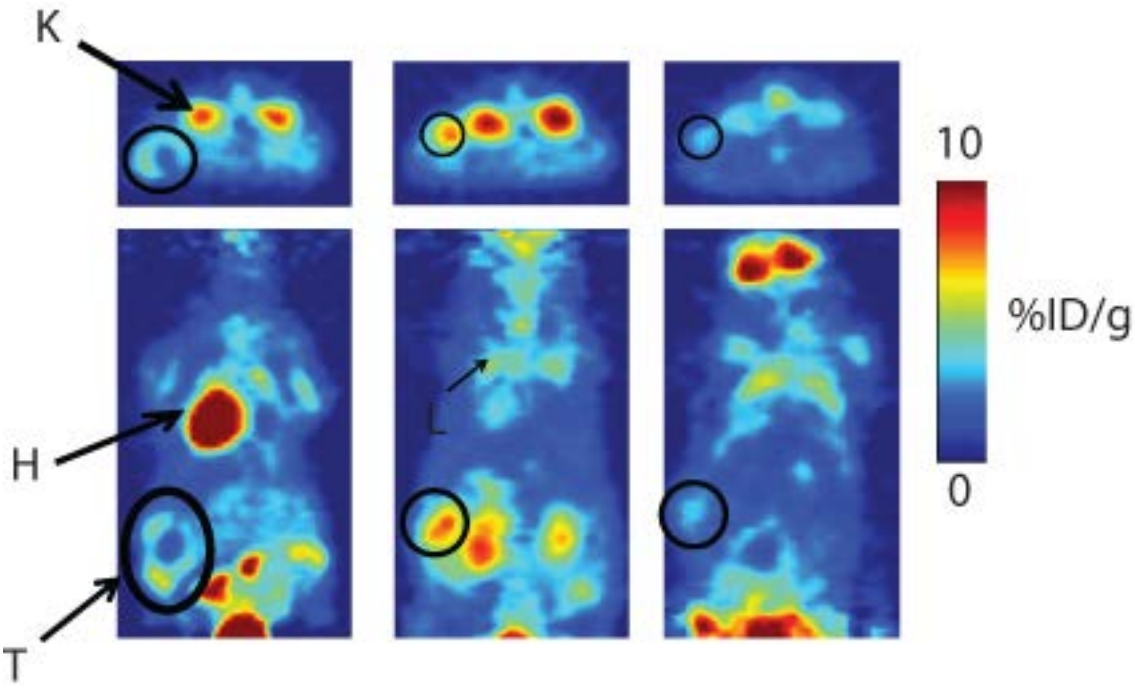
2. Verel I, Visser GW, Boellaard R, Stigter-van Walsum M, Snow GB, van Dongen GA. 89Zr immuno-PET: comprehensive procedures for the production of 89Zr-labeled monoclonal antibodies. *J Nucl Med.* 2003;44:1271-1281.



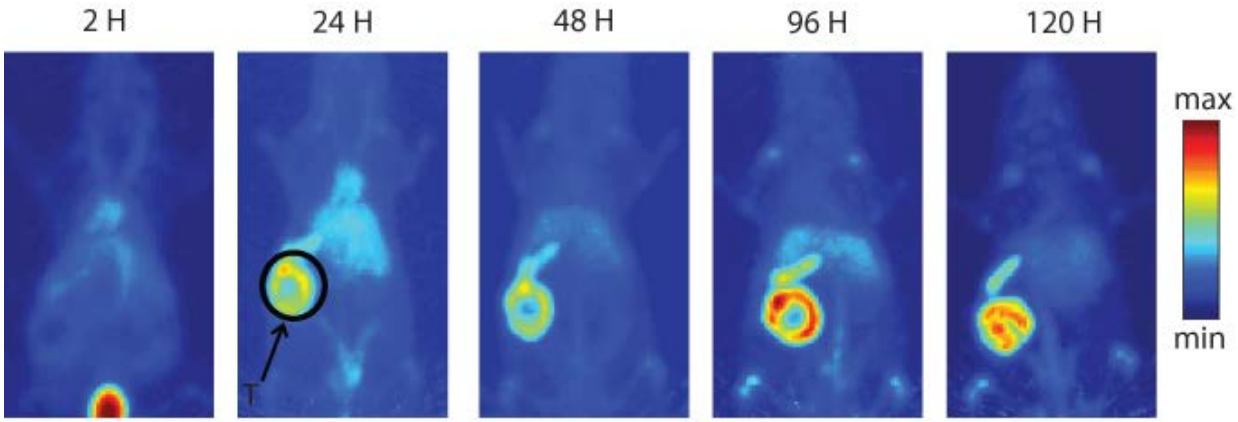
Supplemental Figure 1. 5B1 immunohistochemistry staining on Urinary bladder, mucinous adenocarcinoma, stage IV (A); Ovary, metastatic carcinoma from colon (B); and Lymph node, metastatic carcinoma , IIIA (C).



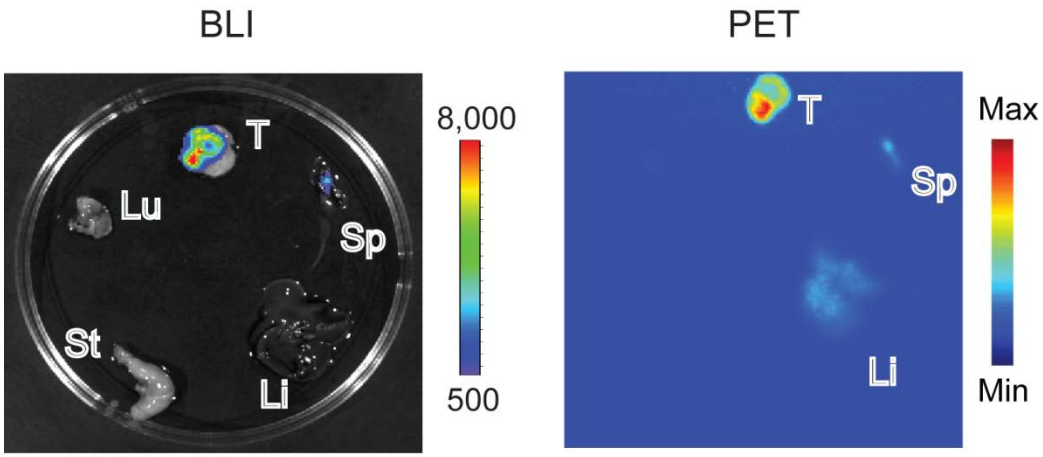
Supplemental Figure 2. Stability curve showing remaining % bound Zr-89 in saline and 1% BSA solutions at 37 °C over five days.



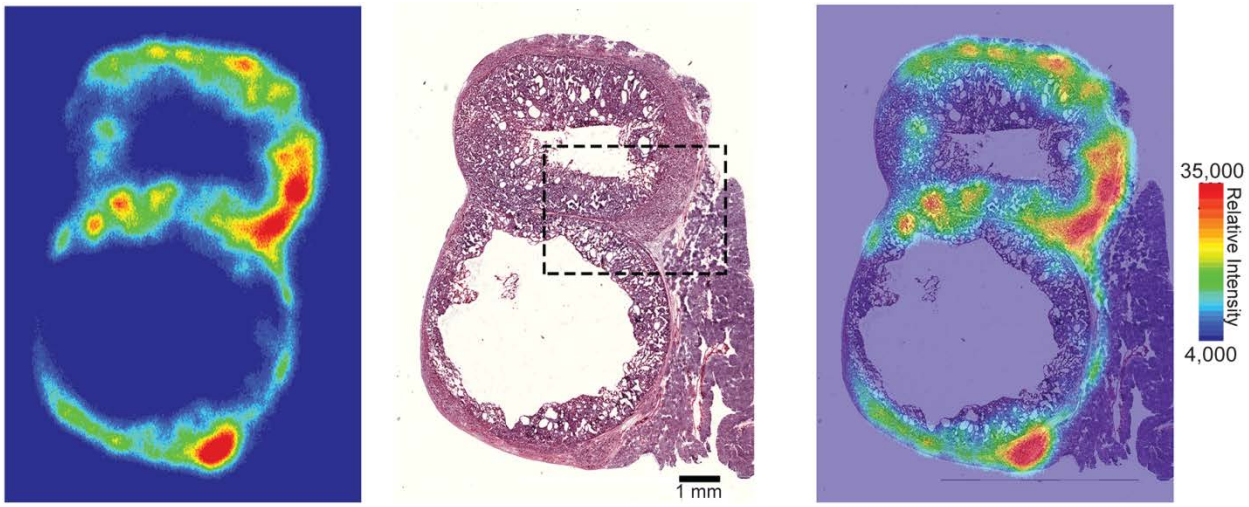
Supplemental Figure 3. Representative planar images of mice bearing orthotopic BxPC3 pancreas xenografts with FDG as the radiotracer show moderate tumor (T) detection with non-specific binding in normal tissues particularly in the kidneys (K), heart (H) and lungs (L).



Supplemental Figure 4. Serial PET maximum intensity projection images of mice bearing BxPC3 pancreas tumor xenografts transplanted in the pancreas with ^{89}Zr-5B1 as the PET tracer acquired from 2-120 h post-intravenous injection. The tumor (T) is remarkably delineated by ^{89}Zr-5B1.



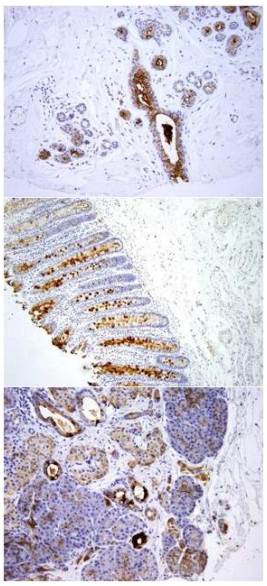
Supplemental Figure 5. BLI (left) and PET (right) imaging of BxPC3 pancreatic tumor (T) and normal tissues (i.e. spleen=Sp, liver=Li, stomach=St, lungs=Lu).



Supplemental Figure 6. Digital Autoradiography (left), histologic staining (center) and co-registration (right) of a tumor section obtained from an orthotopic BxPC3 tumor.

Supplemental Table 1: Survey of 5B1 binding to normal tissues. Most tissues were negative, except selected [1] breast ductal cells (top), [2] sigmoid colon goblet cells (middle), and [3] pancreas exocrine cells (bottom).

Normal Tissue	Stain
Brain	neg
Breast	+ [1]
Colon	+ [2]
Kidney	neg
Liver	neg
Lung	neg
Lymph node	neg
Muscle	neg
Pancreas	+ [3]
Placenta	neg
Skin	neg
Spleen	neg
Stomach	neg



Supplemental Table 2: Staining of Pancreatic ductal adenocarcinomas with 5B1.

IHC 5B1	Stage	Age	Sex	Histology
neg	II	71	M	moderately differentiated
pos++	III	68	M	moderately differentiated
neg	III	64	F	moderately differentiated
pos++	III	46	M	moderately differentiated
pos++	III	54	M	moderately differentiated
pos++	III	40	M	moderately differentiated
pos+/-	IVA	66	M	moderately differentiated
pos++	IVA	45	M	moderately differentiated
poor tissue	IVA	64	F	moderately differentiated
pos++	IVA	69	M	poorly differentiated

Supplemental Table 3: Biodistribution of ^{89}Zr-5B1 administered via lateral tail vein in female SCID mice bearing DMS79 small cell lung cancer xenografts.

Tissue	24 h n=5	48 h n=5	120 h n=5	24 h block n=5	24 h IgG n=5
Blood	11.04 ± 2.14	6.29 ± 0.32	2.63 ± 0.53	14.38 ± 4.24	11.47 ± 1.35
Tumor	28.16 ± 0.45	16.18 ± 8.75	9.36 ± 2.09	14.53 ± 8.60	6.08 ± 2.91
Heart	3.76 ± 0.20	3.33 ± 0.63	1.55 ± 0.27	4.84 ± 0.88	4.50 ± 1.84
Lungs	9.13 ± 1.64	5.94 ± 0.76	3.01 ± 0.69	11.28 ± 1.72	9.93 ± 0.94
Liver	2.59 ± 1.33	2.09 ± 1.19	1.30 ± 0.74	4.58 ± 2.23	2.69 ± 0.88
Spleen	4.29 ± 0.77	4.16 ± 0.66	2.81 ± 0.57	4.51 ± 1.36	3.10 ± 0.73
Stomach	1.14 ± 0.38	1.12 ± 0.62	0.17 ± 0.09	1.50 ± 0.44	0.97 ± 0.31
Sm.intestine	1.23 ± 0.40	0.96 ± 0.21	0.21 ± 0.04	1.76 ± 0.39	1.19 ± 0.10
Lg.intestine	0.50 ± 0.03	0.81 ± 0.34	0.17 ± 0.04	0.57 ± 0.07	0.52 ± 0.27
Kidneys	3.17 ± 1.33	2.57 ± 0.96	1.78 ± 0.99	1.51 ± 0.73	5.78 ± 0.98
Bone	5.86 ± 1.19	5.47 ± 1.80	6.16 ± 1.34	6.59 ± 1.81	3.55 ± 0.93
Muscle	0.63 ± 0.12	0.55 ± 0.17	0.31 ± 0.22	0.86 ± 0.09	0.50 ± 0.28
Tumor/Blood	3.15 ± 0.64	5.19 ± 1.86	6.22 ± 2.62		
Tumor/Muscle	30.50 ± 7.80	58.60 ± 17.88	32.08 ± 7.47		
Tumor/Liver	17.39 ± 8.77	13.44 ± 5.57	12.99 ± 3.56		
Tumor/Kidney	15.29 ± 2.52	6.37 ± 0.27	9.10 ± 1.00		

Supplemental Table 4: Biodistribution of ^{89}Zr-5B1 in selected organs of female SCID Mice bearing Colo205-luc tumors

Tissue	24 h					48 h
	10 ug	25 ug	50 ug	block	IgG	25 ug
	n=4	n=3	n=4	n=3	n=3	n=3
Blood	2.75 ± 0.33	6.74 ± 3.26	5.54 ± 0.89	1.62 ± 0.26	12.29 ± 3.97	0.98 ± 0.63
Tumor	5.50 ± 0.40	9.60 ± 2.11	8.96 ± 4.66	1.07 ± 0.65	3.94 ± 1.93	4.02 ± 2.00
Heart	1.84 ± 0.82	3.36 ± 1.92	2.74 ± 1.52	0.67 ± 0.14	3.87 ± 1.02	0.90 ± 0.24
Liver	14.30 ± 0.59	8.55 ± 6.40	10.90 ± 5.40	18.98 ± 3.25	7.61 ± 0.04	11.89 ± 2.01
Spleen	11.59 ± 4.17	6.24 ± 1.80	7.41 ± 2.90	3.12 ± 0.77	7.68 ± 5.70	13.30 ± 0.72
Stomach	0.40 ± 0.26	0.43 ± 0.11	0.81 ± 0.21	0.35 ± 0.14	0.54 ± 0.08	0.39 ± 0.29
Sm. intestine	0.90 ± 0.90	0.43 ± 0.15	0.91 ± 0.29	0.25 ± 0.02	0.71 ± 0.15	0.62 ± 0.12
L. intestine	0.95 ± 0.73	0.87 ± 0.28	0.82 ± 0.27	0.71 ± 0.44	0.89 ± 0.38	0.89 ± 0.93
Kidney	2.08 ± 0.48	2.57 ± 0.54	2.06 ± 0.82	1.09 ± 0.55	12.84 ± 0.87	2.05 ± 1.45
Muscle	0.73 ± 0.17	0.81 ± 0.48	0.44 ± 0.21	2.43 ± 0.08	0.64 ± 0.20	3.66 ± 1.01
Bone	5.68 ± 0.83	1.92 ± 1.30	3.25 ± 1.12	0.29 ± 0.19	3.34 ± 0.95	0.32 ± 0.15
Tumor/Blood	2.03 ± 0.36	1.42 ± 0.86	1.73 ± 1.08		0.31 ± 0.07	1.40 ± 0.46
Tumor/Muscle	7.90 ± 2.38	13.80 ± 5.22	24.99 ± 9.95		6.15 ± 2.02	17.47 ± 10.63
Tumor/Liver	0.39 ± 0.04	1.82 ± 1.51	0.69 ± 0.50		0.77 ± 0.43	1.00 ± 0.72
Tumor/Kidney	2.77 ± 0.87	3.93 ± 1.57	4.38 ± 1.76		0.42 ± 0.20	3.07 ± 1.85

Supplemental Table 5: Biodistribution of ^{89}Zr-5B1 administered via lateral tail vein in female SCID mice bearing BxPC3 pancreas cancer xenografts.

Tissue	24 h n=4	48 h n=4	120 h n=4	24 h block n=5	24 h IgG n=5
Blood	18.04 ± 1.87	13.76 ± 1.31	9.33 ± 1.19	19.76 ± 5.55	23.65 ± 1.09
Tumor	84.73 ± 12.28	75.38 ± 20.71	114.07 ± 23.09	51.97 ± 25.64	11.22 ± 1.63
Heart	7.32 ± 1.61	5.51 ± 0.38	4.00 ± 0.85	8.37 ± 2.58	9.28 ± 1.24
Lungs	13.04 ± 3.87	12.77 ± 0.77	8.59 ± 3.60	16.87 ± 3.11	16.34 ± 3.83
Liver	5.13 ± 1.99	6.94 ± 2.09	6.70 ± 3.73	5.56 ± 2.10	4.48 ± 1.75
Spleen	11.50 ± 2.80	19.09 ± 7.13	19.84 ± 7.90	7.11 ± 1.67	7.25 ± 1.52
Stomach	1.59 ± 0.65	1.14 ± 0.73	1.52 ± 0.36	1.67 ± 0.79	1.04 ± 0.36
Sm. Intestines	1.81 ± 1.03	1.60 ± 0.31	1.83 ± 0.33	1.73 ± 0.64	1.49 ± 0.26
L. Intestines	1.29 ± 0.57	0.86 ± 0.24	1.21 ± 0.55	1.53 ± 0.59	1.15 ± 0.41
Kidney	5.74 ± 1.52	6.73 ± 2.20	6.95 ± 2.82	8.67 ± 2.42	22.53 ± 7.40
Bone	7.27 ± 2.66	6.13 ± 3.31	9.52 ± 1.44	8.01 ± 2.00	6.37 ± 1.60
Muscle	1.66 ± 0.48	1.39 ± 0.44	0.82 ± 0.36	1.71 ± 0.23	1.38 ± 0.41
Pancreas	2.78 ± 0.66	1.73 ± 0.80	0.96 ± 0.39	2.66 ± 0.98	2.96 ± 1.17
Tumor/Blood	4.71 ± 0.56	7.59 ± 2.41	11.15 ± 0.94		
Tumor/Muscle	51.82 ± 18.98	58.95 ± 28.66	178.27 ± 29.28		
Tumor/Liver	10.11 ± 3.84	11.62 ± 2.20	26.41 ± 11.67		
Tumor/Kidney	15.11 ± 5.50	9.60 ± 1.49	15.43 ± 4.97		
Tumor/Pancreas	27.30 ± 4.11	41.08 ± 10.39	148.39 ± 19.13		