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 soaking in Chelex 100 resin (Bio-Rad Laboratories, Hercules, CA) overnight at a ratio of 5g resin per 100 ml water as indicated in the manufacturer's instructions. For dose calibration measurements, a Capintec CRC-55tR (Capintec, Ramsey NJ) calibrated for Zr-89 was utilized (Calibration # 465). Quantification of radioactivity in 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. Vivaspin 500 centrifugal filters with a 10 kDa MWCO was obtained from GE Healthcare (Amersham, UK).

^{89}Zr 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 (~1 mCi) was added into a 1.5 ml vial containing 100 µL metal-free water. The pH was adjusted to ~ 7.0-7.2 with 1 M Na_2CO_3. Effervescence was observed as the neutralization process resulted in CO_2 evolution. After the desired pH was obtained, 125-140 µg (Mb-DFO: 1.56-1.75 nmol; Cys-Db-DFO: 2.5 – 2.8 nmol) minibody/diabody-DFO was added. The reaction was incubated at room temperature with intermittent mild shaking. After 1-1.5 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 labeled proteins remaining close to the origin (R_f=0.30) while the free Zr-89 is found near the solvent front (R_f=0.65). Purification of ^{89}Zr-protein was performed using a PD10 size exclusion column with saline as eluent and further concentrated with Vivaspin 500(MWCO: 10 kDa) centrifugal filter. The final radiochemical purity was >99% based on iTLC analysis.

SDS Gel Electrophoresis

20 µg of each sample was diluted in 20 µl of saline and mixed in a 1:1 ratio with Tris-Glycine SDS Sample Buffer (Invitrogen). After boiling at 95° C for 10 minutes, the samples were run on a 4-20% Tris-Glycine Gel (Invitrogen) with Tris-Glycine SDS Running Buffer (Invitrogen) at 150 V for 45 minutes. The gel was washed with 100 mL of ultrapure water for 15 minutes, then developed using Simple Blue Sage Stain (Invitrogen) for 1 hour and washed over night with 100 mL of ultrapure water (**SI Fig. 1**).

Supplemental Table 1. Biodistribution of ^{89}Zr-Mb administered via lateral tail vein in male athymic nude mice bearing PSMA(+) LNCaP prostate xenografts.

Tissue	1 h			4 h			12 h			24 h		
	n=4			n=4			n=4			n=3		
Blood	14.21	±	2.52	6.64	±	2.62	3.34	±	0.71	1.99	±	0.73
Tumor	2.29	±	0.49	4.71	±	0.98	6.12	±	2.03	12.12	±	3.61
Heart	4.50	±	0.86	2.48	±	0.78	2.13	±	0.53	1.57	±	0.45
Lungs	4.25	±	1.76	2.36	±	1.33	1.78	±	0.80	1.98	±	1.10
Liver	2.26	±	0.72	1.47	±	0.68	2.74	±	0.40	5.06	±	2.57
Spleen	1.52	±	0.35	1.33	±	0.66	1.47	±	0.18	1.77	±	0.96
Stomach	0.24	±	0.10	0.17	±	0.09	0.39	±	0.26	0.73	±	1.14
Sm. Intestines	1.39	±	0.62	0.50	±	0.23	0.48	±	0.08	0.54	±	0.21
L. Intestines	0.10	±	0.03	0.78	±	0.20	1.36	±	0.62	0.53	±	0.31
Kidney	6.98	±	1.74	6.58	±	1.54	6.29	±	0.94	10.67	±	4.42
Bone	3.78	±	1.10	3.39	±	0.59	2.77	±	0.69	1.55	±	0.17
Muscle	0.45	±	0.10	0.39	±	0.17	0.30	±	0.04	0.43	±	0.20
Tumor/Blood	*0.17*	±	*0.06*	*0.81*	±	*0.36*	*1.75*	±	*0.33*	*5.93*	±	*1.97*
Tumor/Liver	*1.05*	±	*0.28*	*2.63*	±	*0.09*	*1.88*	±	*0.58*	*1.89*	±	*0.20*
Tumor/Kidneys	*0.35*	±	*0.13*	*0.65*	±	*0.04*	*0.80*	±	*0.35*	*0.90*	±	*0.21*
Tumor/Muscle	*6.13*	±	*0.53*	*11.35*	±	*3.86*	*19.37*	±	*5.48*	*26.81*	±	*7.94*

Supplemental Table 2. Biodistribution of ^{89}Zr-Mb administered via lateral tail vein in male athymic nude mice bearing PSMA(-) PC3 prostate xenografts.				
Tissue	**1 h**	**4 h**	**12 h**	**24 h**
	n=4	**n=4**	**n=4**	**n=4**
Blood	13.98 ± 1.46	6.94 ± 0.98	2.76 ± 0.44	1.14 ± 0.26
Tumor	2.28 ± 0.40	2.10 ± 0.34	1.67 ± 0.52	2.36 ± 0.31
Heart	3.95 ± 0.96	2.40 ± 0.24	1.66 ± 0.26	1.40 ± 0.43
Lungs	6.23 ± 2.22	1.72 ± 0.47	1.39 ± 0.60	1.32 ± 0.81
Liver	2.83 ± 1.16	1.78 ± 0.22	3.21 ± 1.31	3.07 ± 1.71
Spleen	2.24 ± 0.78	1.18 ± 0.24	1.69 ± 0.79	1.22 ± 0.27
Stomach	0.32 ± 0.05	0.47 ± 0.28	0.44 ± 0.14	0.08 ± 0.03
Sm. Intestines	1.60 ± 1.01	0.80 ± 0.43	0.39 ± 0.13	0.31 ± 0.14
L. Intestines	0.39 ± 0.31	0.99 ± 0.14	1.51 ± 1.58	0.24 ± 0.09
Kidney	8.18 ± 3.11	4.83 ± 0.96	3.59 ± 2.14	7.96 ± 4.46
Bone	2.81 ± 0.26	1.60 ± 0.39	1.45 ± 0.83	1.31 ± 0.63
Muscle	0.42 ± 0.12	0.21 ± 0.04	0.42 ± 0.29	0.13 ± 0.03
Tumor/Blood	*0.16 ± 0.01*	*0.30 ± 0.01*	*0.61 ± 0.20*	*2.13 ± 0.35*
Tumor/Liver	*0.81 ± 0.38*	*1.12 ± 0.09*	*0.54 ± 0.12*	*0.96 ± 0.46*
Tumor/Kidneys	*0.28 ± 0.13*	*0.44 ± 0.35*	*0.46 ± 0.24*	*0.36 ± 0.17*
Tumor/Muscle	*5.68 ± 1.46*	*11.86 ± 3.49*	*7.31 ± 3.24*	*18.10 ± 2.56*

Supplemental Table 3. Competitive binding with 200 µg and 500 µg non-radioactive Mb and huJ591.

Tissue	12 h p.i. (200 µg Mb)	12 h p.i. (500 µg Mb)	24 h p.i. (500 µg huJ591)
	n=6	n=4	n=4
Blood	4.08 ± 0.99	3.66 ± 0.68	2.76 ± 1.18
Tumor	3.73 ± 1.27	3.82 ± 0.70	4.98 ± 2.38
Heart	1.94 ± 0.55	2.06 ± 0.48	2.17 ± 0.58
Lungs	2.42 ± 0.98	1.22 ± 0.29	1.75 ± 0.93
Liver	4.24 ± 1.84	3.43 ± 1.21	3.49 ± 2.02
Spleen	1.60 ± 0.63	1.47 ± 0.65	2.08 ± 1.06
Stomach	0.34 ± 0.18	0.24 ± 0.12	0.32 ± 0.15
Sm. Intestines	0.52 ± 0.17	0.50 ± 0.23	0.45 ± 0.26
L. Intestines	0.47 ± 0.14	0.51 ± 0.23	0.95 ± 0.91
Kidney	9.60 ± 5.14	16.72 ± 6.14	8.61 ± 4.77
Bone	1.37 ± 0.81	2.98 ± 0.69	2.84 ± 1.46
Muscle	0.36 ± 0.23	0.36 ± 0.06	0.44 ± 0.14

Supplemental Table 4. Biodistribution of ^{89}Zr-Cys-Db administered via lateral tail vein in male athymic nude mice bearing PSMA(+) LNCaP prostate xenografts.

Tissue	1 h			4 h			12 h			24 h			12 h Block		
	n=4			n=4			n=5			n=4			n=4		
Blood	15.40	±	4.23	4.44	±	0.55	1.71	±	0.43	0.59	±	0.08	1.59	±	0.18
Tumor	4.08	±	1.00	6.91	±	2.94	12.26	±	2.54	6.53	±	0.98	5.64	±	1.75
Heart	5.50	±	1.03	3.42	±	0.95	2.61	±	0.52	1.79	±	0.06	2.81	±	0.20
Lungs	7.35	±	1.95	3.13	±	1.17	1.97	±	0.58	0.93	±	0.24	1.23	±	0.10
Liver	8.61	±	0.80	5.40	±	2.10	5.70	±	2.79	5.54	±	1.65	5.96	±	1.69
Spleen	2.63	±	0.40	2.54	±	1.02	2.50	±	1.57	1.75	±	0.62	1.94	±	0.83
Stomach	0.37	±	0.03	0.59	±	0.39	0.80	±	0.38	0.59	±	0.19	0.92	±	0.40
Sm. Intestines	2.90	±	1.06	1.26	±	0.36	0.95	±	0.38	0.74	±	0.08	0.95	±	0.27
L. Intestines	0.47	±	0.22	1.57	±	0.69	1.21	±	0.40	0.77	±	0.33	1.03	±	0.33
Kidney	13.26	±	5.92	16.86	±	4.43	13.77	±	1.94	15.47	±	8.09	13.36	±	1.79
Bone	4.48	±	1.82	4.25	±	1.54	3.69	±	0.63	1.57	±	0.52	5.09	±	2.95
Muscle	0.72	±	0.17	0.68	±	0.18	0.47	±	0.09	0.43	±	0.12	0.44	±	0.09
Tumor/Blood	*0.27*	±	*0.03*	*1.24*	±	*0.12*	*4.93*	±	*0.89*	*11.19*	±	*2.16*			
Tumor/Liver	*0.53*	±	*0.08*	*1.25*	±	*0.18*	*0.82*	±	*0.18*	*1.23*	±	*0.27*			
Tumor/Kidneys	*0.46*	±	*0.17*	*0.50*	±	*0.08*	*0.84*	±	*0.23*	*0.53*	±	*0.30*			
Tumor/Muscle	*4.90*	±	*0.87*	*10.08*	±	*2.31*	*15.55*	±	*3.49*	*12.86*	±	*1.72*			

Supplemental Table 5. Biodistribution of ^{89}Zr-Cys-Db administered via lateral tail vein in male athymic nude mice bearing PSMA(-) PC3 prostate xenografts.				
Tissue	**1 h**	**4 h**	**12 h**	**24 h**
	n=5	**n=4**	**n=5**	**n=4**
Blood	10.39 ± 2.19	3.50 ± 0.41	0.96 ± 0.12	0.39 ± 0.12
Tumor	2.36 ± 0.48	3.64 ± 1.53	2.75 ± 0.51	3.44 ± 0.85
Heart	3.52 ± 0.98	2.98 ± 0.38	2.51 ± 0.26	2.10 ± 0.53
Lungs	5.14 ± 1.80	1.44 ± 0.11	1.15 ± 0.65	1.19 ± 0.47
Liver	5.05 ± 3.60	4.53 ± 1.32	5.39 ± 2.81	2.70 ± 0.37
Spleen	2.19 ± 1.10	1.72 ± 0.52	1.20 ± 0.36	0.93 ± 0.23
Stomach	0.52 ± 0.17	0.54 ± 0.24	0.77 ± 0.33	0.18 ± 0.09
Sm. Intestines	1.38 ± 0.50	0.94 ± 0.26	0.55 ± 0.23	0.40 ± 0.13
L. Intestines	1.15 ± 0.44	0.76 ± 0.13	0.92 ± 0.31	0.45 ± 0.09
Kidney	7.29 ± 2.61	12.74 ± 5.63	9.13 ± 2.68	9.10 ± 1.35
Bone	3.36 ± 0.80	3.60 ± 0.51	2.51 ± 1.70	1.81 ± 0.66
Muscle	0.82 ± 0.51	0.73 ± 0.25	0.54 ± 0.24	0.29 ± 0.05
Tumor/Blood	*0.24 ± 0.08*	*1.01 ± 0.34*	*2.87 ± 0.43*	*9.18 ± 1.76*
Tumor/Liver	*0.67 ± 0.44*	*0.89 ± 0.53*	*0.63 ± 0.31*	*1.06 ± 0.52*
Tumor/Kidneys	*0.36 ± 0.16*	*0.34 ± 0.21*	*0.32 ± 0.10*	*0.39 ± 0.15*
Tumor/Muscle	*3.62 ± 1.77*	*5.28 ± 2.19*	*5.68 ± 1.86*	*12.53 ± 5.08*

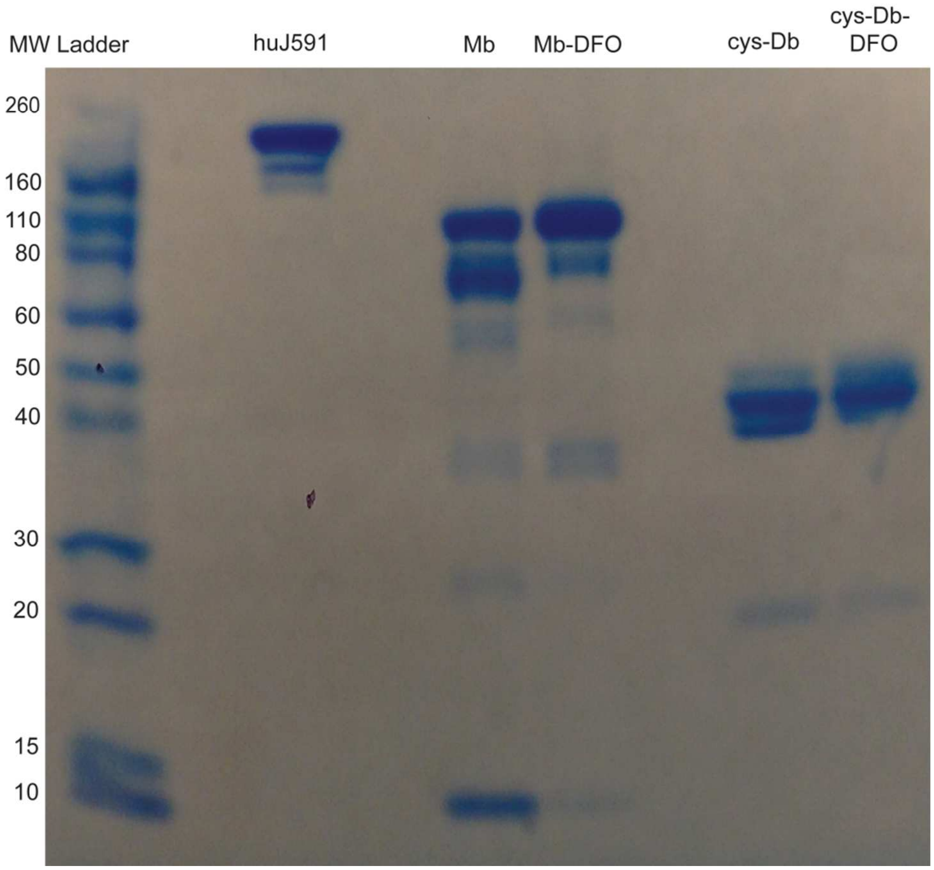
SI Table 6. Tumor uptake expressed as %ID/g (mean ± S.D.) of all three radiotracers obtained from VOIs.			
Time, h	**^{89}Zr-Mb**	**^{89}Zr-Cys-Db**	**^{89}Zr-huJ591**
1	3.23 ± 0.96	5.58 ± 1.09	2.94 ± 0.66
4	6.16 ±1.43	8.19 ± 1.41	10.21 ±1.95
12	6.85 ± 0.87	9.84 ± 2.54	15.84 ± 1.79
24	7.93 ± 0.67	9.17 ± 1.48	26.57 ± 3.50

SI Table 7. Blood clearance of all three radiotracers obtained from heart VOIs expressed as %ID/g (mean ± S.D.).

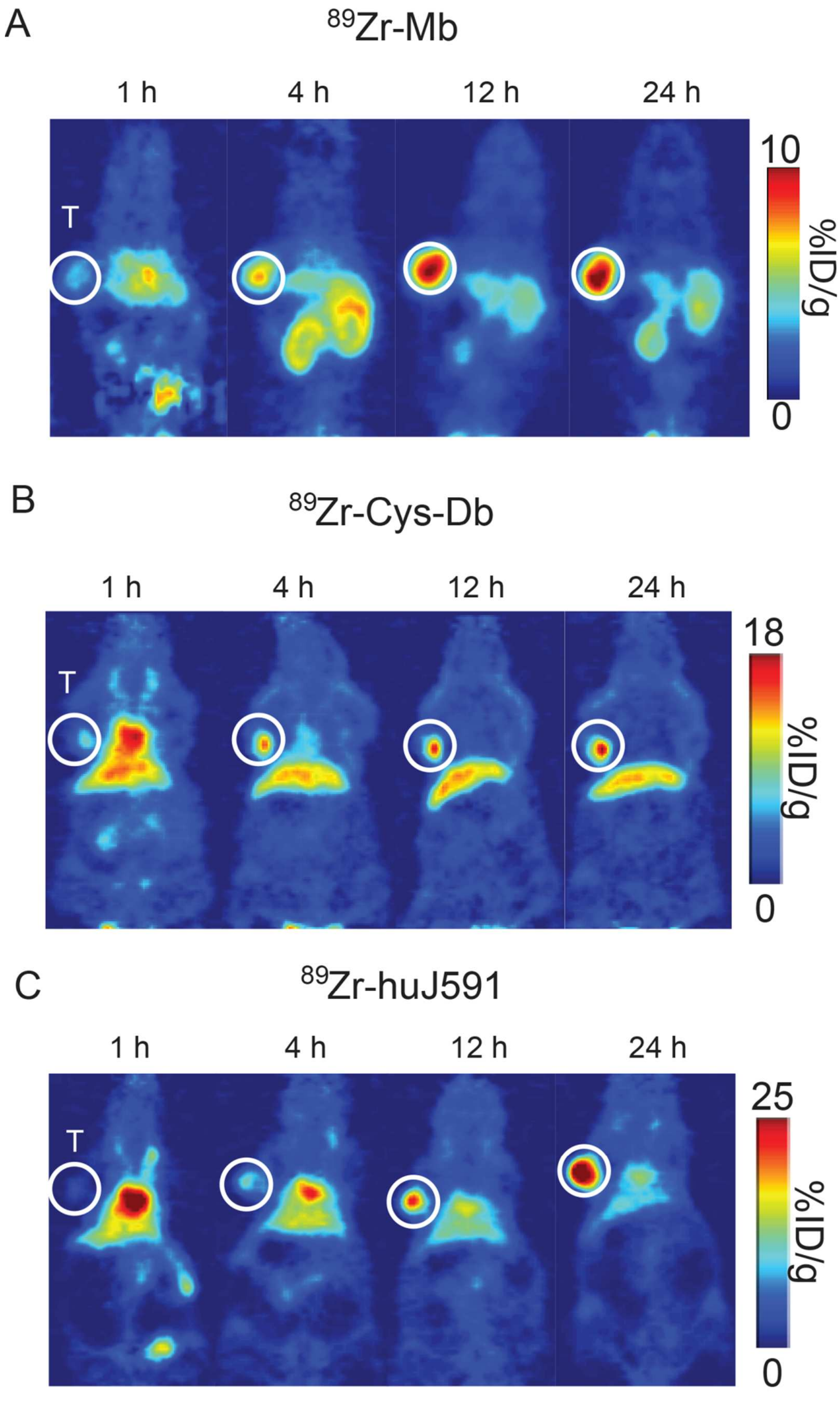
Time, h	^{89}Zr-Mb	^{89}Zr-Cys-Db	^{89}Zr-huJ591
1	20.86 ± 3.39	13.19 ± 2.70	25.49 ± 2.11
4	13.90 ± 3.06	5.90 ± 1.10	21.66 ±1.91
12	5.06 ± 2.84	2.74 ± 0.61	16.36 ± 1.57
24	3.26 ± 1.20	2.00 ± 0.18	12.13 ± 1.26

SI Table 8. Comparison of tumor-to-muscle ratios of all radiotracers.			
Time, h	**^{89}Zr-Mb**	**^{89}Zr-Cys-Db**	**^{89}Zr-huJ591**
1	2.53 ± 0.34	3.48 ± 0.48	2.92 ± 0.21
4	4.90 ± 0.96	5.17 ± 1.09	6.69 ± 1.79
12	10.04 ± 3.70	6.09 ± 0.85	7.87 ± 1.63
24	9.59 ± 3.68	6.89 ± 1.15	11.02 ± 2.30

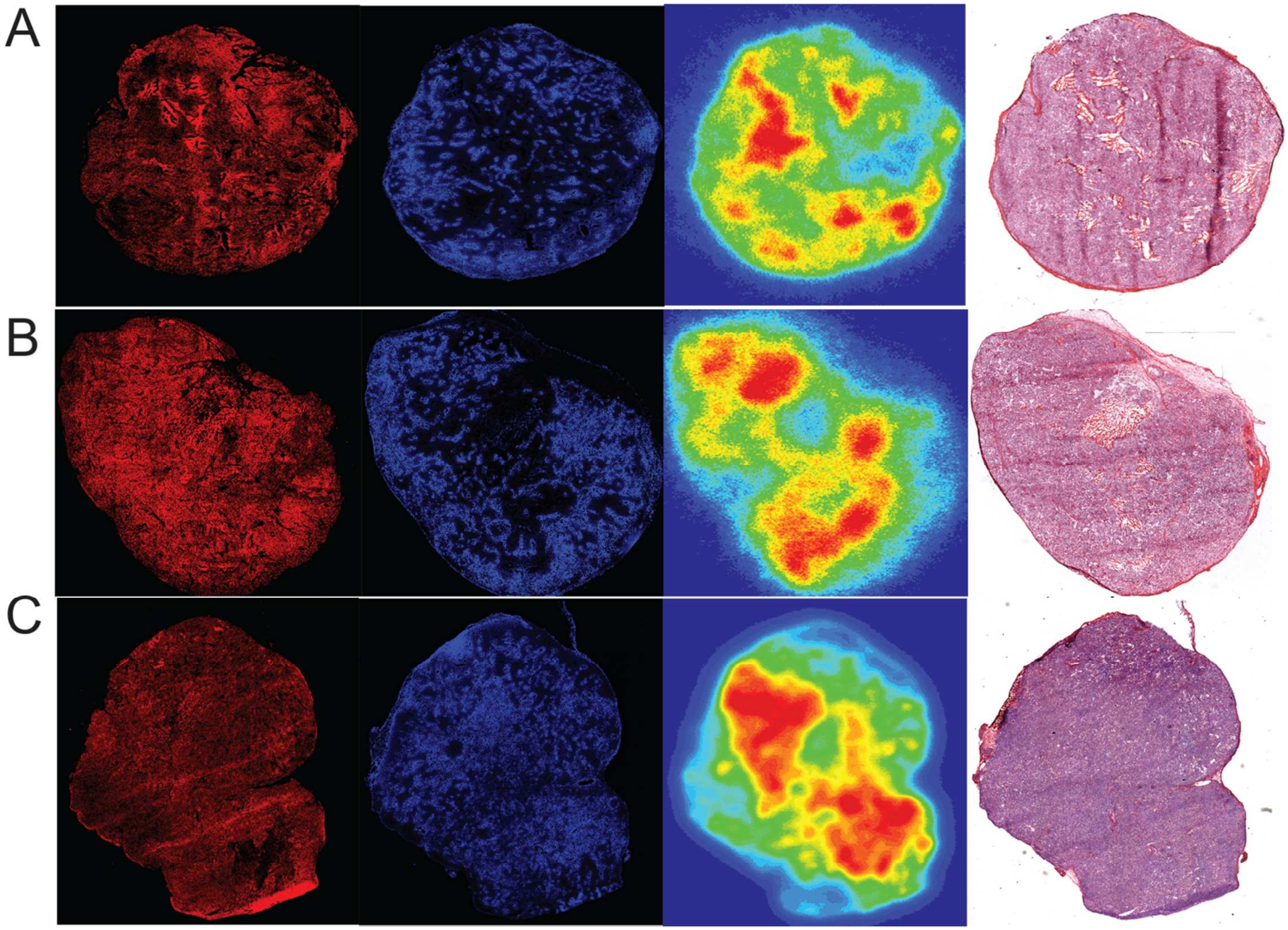
SI Table 9. Comparison of tumor-to-blood (heart) ratios of all radiotracers.			
Time, h	**^{89}Zr-Mb**	**^{89}Zr-Cys-Db**	**^{89}Zr-huJ591**
1	0.15 ± 0.03	0.43 ± 0.09	0.12 ± 0.02
4	0.41 ± 0.02	1.40 ± 0.22	0.47 ± 0.09
12	1.67 ± 0.81	3.58 ± 0.19	0.98 ± 0.19
24	2.65 ± 0.90	4.91 ± 0.26	2.22 ± 0.46



SI Figure 1. SDS PAGE gel electrophoresis of huJ591, DFO-derivatized and unmodified Mb and Cys-Db.



SI Figure 2. Planar sections of serial PET images obtained with ^{89}Zr-Mb (A), ^{89}Zr-Cys-Db (B) and ^{89}Zr-huJ591 from 1-24 h post-injection.



SI Figure 3. Autoradiography and Histology. Hoechst 33342 (blue), PSMA staining (red), autoradiographs and H&E stain of (A) ^{89}Zr-Mb and (B) ^{89}Zr-Cys-Db and (C) ^{89}Zr-huJ591.

References:

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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.* Aug 2003;44(8):1271-1281.