Supplementary Figures and Tables

CD146-targeted immunoPET and NIRF imaging of hepatocellular carcinoma with a dual-labeled monoclonal antibody

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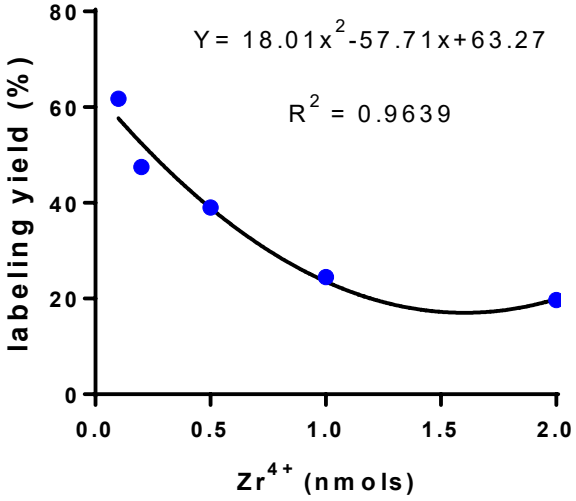

Figure S1. Isotopic dilution experiment showing the dependency between labeling yield and ^{89}Zr specific activity. The estimated number of deferoxamine per antibody was 1.3.

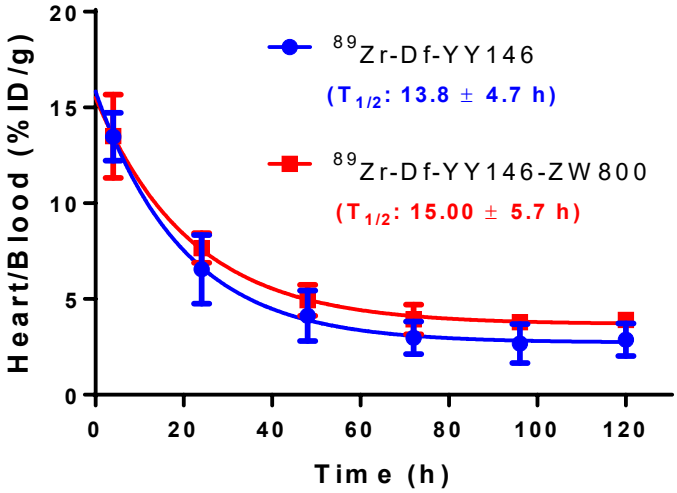

Figure S2. Time activity curves derived from the heart of mice injected with ^{89}Zr-Df-YY146 or ^{89}Zr-Df-YY146-ZW800. Single exponentials were fitted to the data to determine the blood circulation half-life. No statistical difference was observed between the half-life of the tracers.

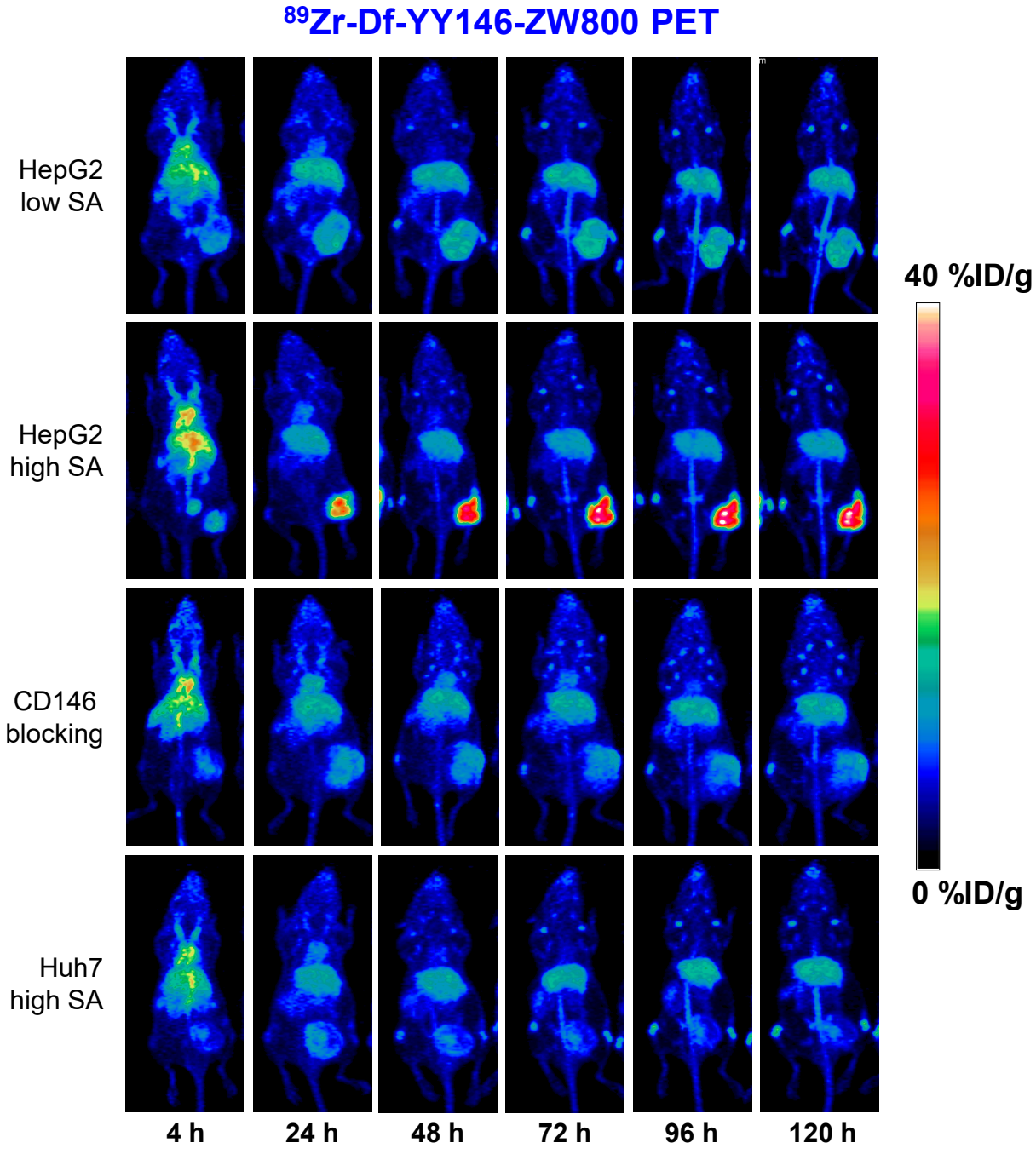

Figure S3. MIPs of sequential PET images acquired at 4, 24, 48, 72, 96, and 120 after intravenous injection of 5.6-7.4 MBq of ^{89}Zr-Df-YY146-ZW800 into tumor bearing athymic nude mice. Mice bearing HepG2 tumors were injected with low SA (18.5 GBq/µmol; first row) or high SA (29.6 GBq/µmol; second row) of ^{89}Zr-Df-YY146-ZW800, and with YY146 (50 mg/kg) 24 h prior the administration of the high SA tracer (third row). The bottom group corresponds to Huh7 tumors bearing mice injected with high SA ^{89}Zr-Df-YY146-ZW800.

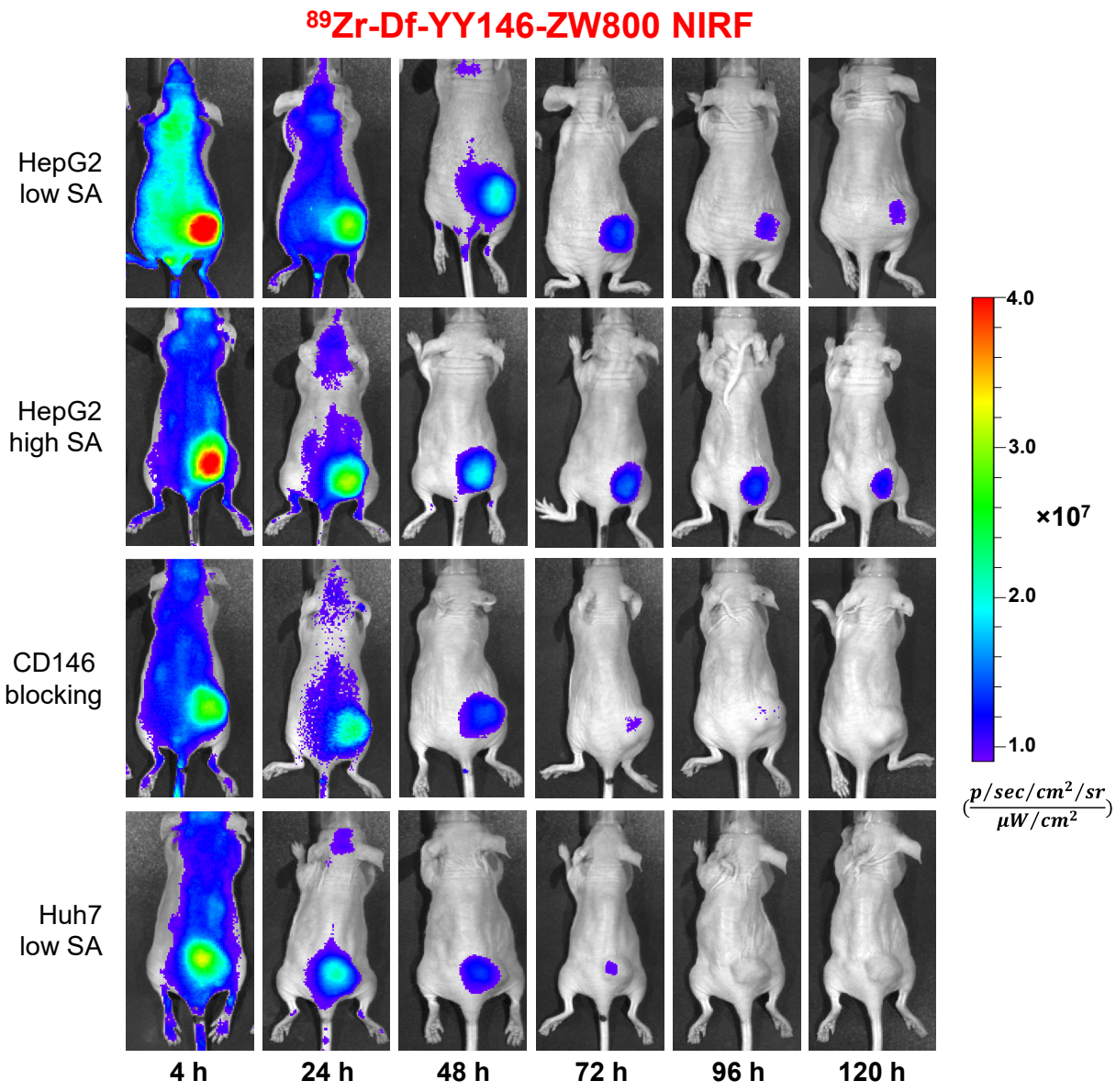

Figure S4. Sequential NIRF images acquired immediately after PET scanning (Figure S3). Mice bearing HepG2 (CD146+) tumors were injected with 250 pmols (first row), 400 pmols (second row), or 250 pmols of ^{89}Zr-Df-YY146-ZW800 plus YY146 (50 mg/kg) 24 h before NIRF tracer injection (third row). The last group of mice bearing Huh7 (CD146-) tumors was given 250 pmols of ^{89}Zr-Df-YY146-ZW800 (fourth row).

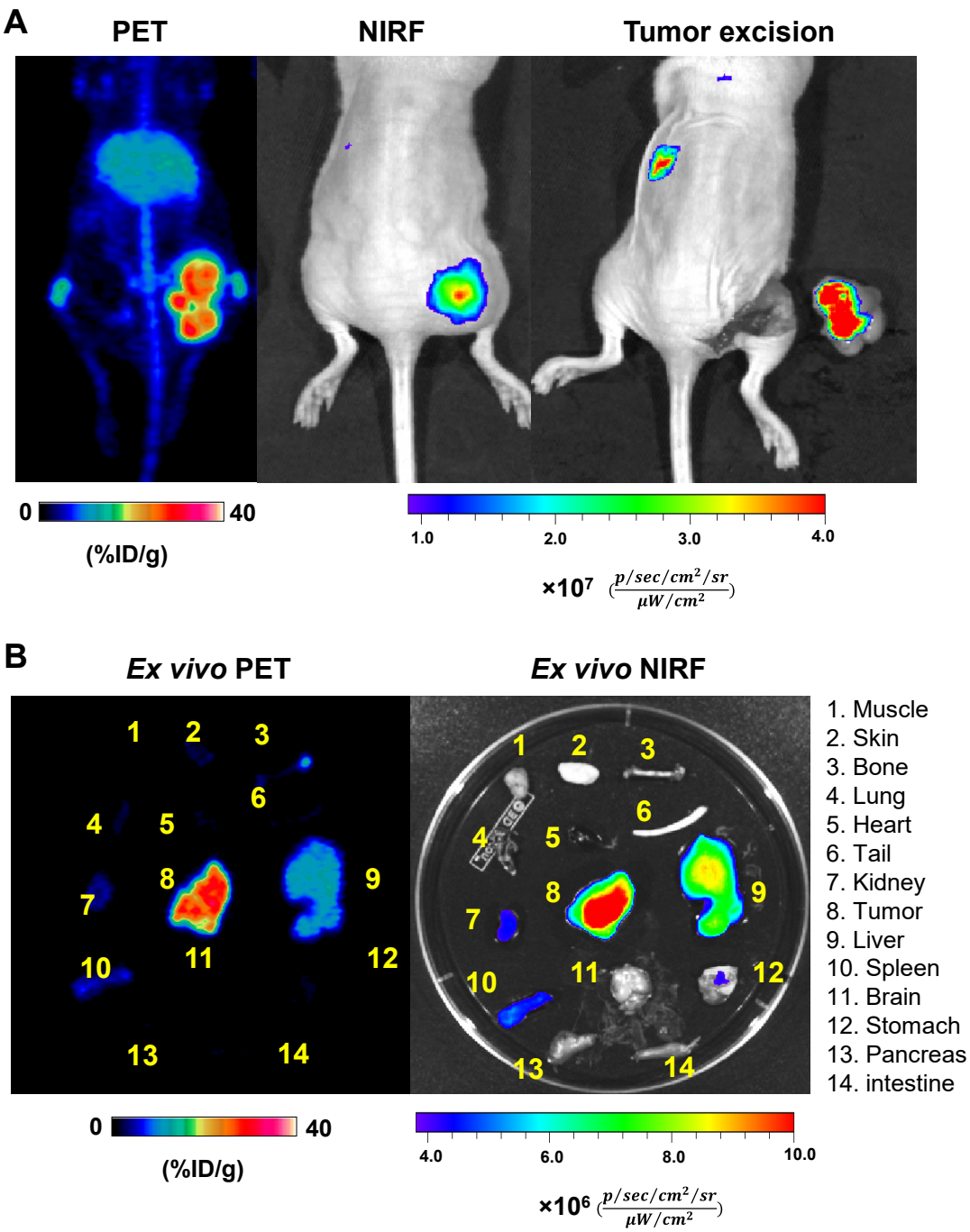

Figure S5. *In vivo* and e*x vivo* PET/NIRF imaging correlation at 120 h post injection of ^{89}Zr-Df-YY146-ZW800. **A)** CD146-positive HepG2 tumors were detected by PET, NIRF, and *ex vivo* after fluorescently guided resection. **B)** Major organs were excised and imaged *ex vivo* by both PET and NIRF imaging modalities. Excellent qualitative correlation was observed in terms of signal intensity and distribution between PET and fluorescence images.

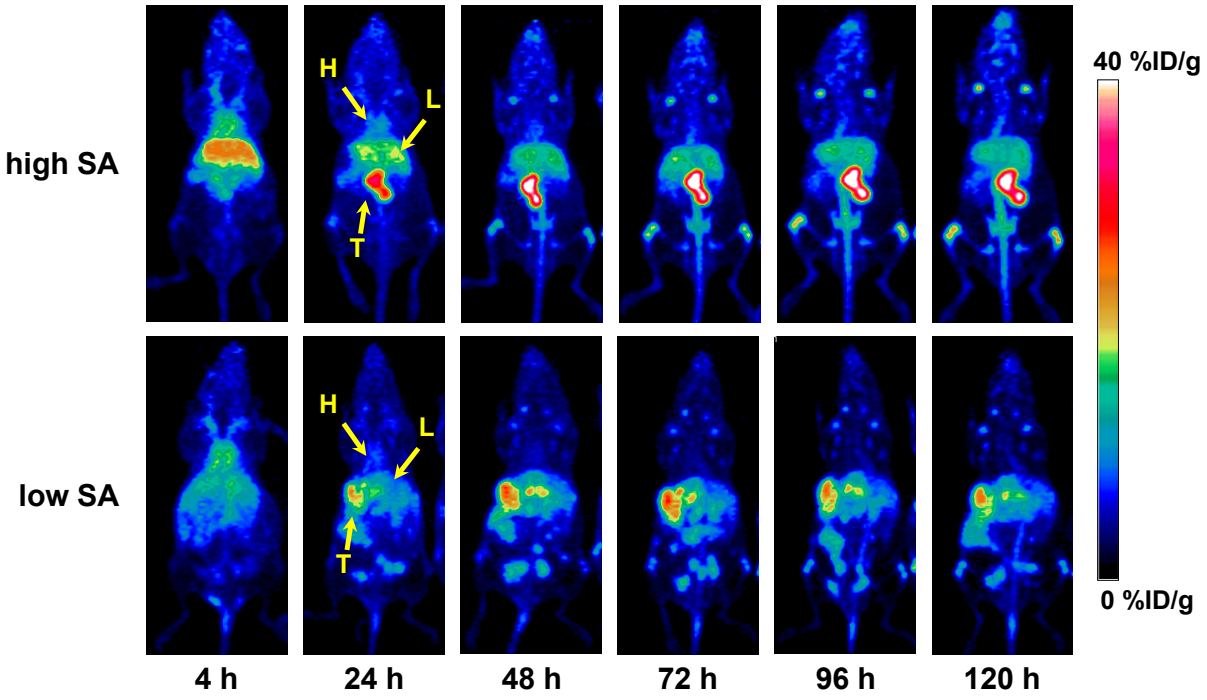

Figure S6. Longitudinal *in vivo* PET imaging of orthotopic HepG2 tumor-bearing mice. Representative MIPs PET images show the biodistribution of ^{89}Zr-Df-YY146-ZW800 in mice administered high (29.6 GBq/μmol; top row) or low (18.5 GBq/μmol; bottom row) SA of the tracer. In both cases HepG2 tumors were successfully visualized. Yellow arrow point to tissues with prominent uptake: H, heart; L, liver; T, tumor.

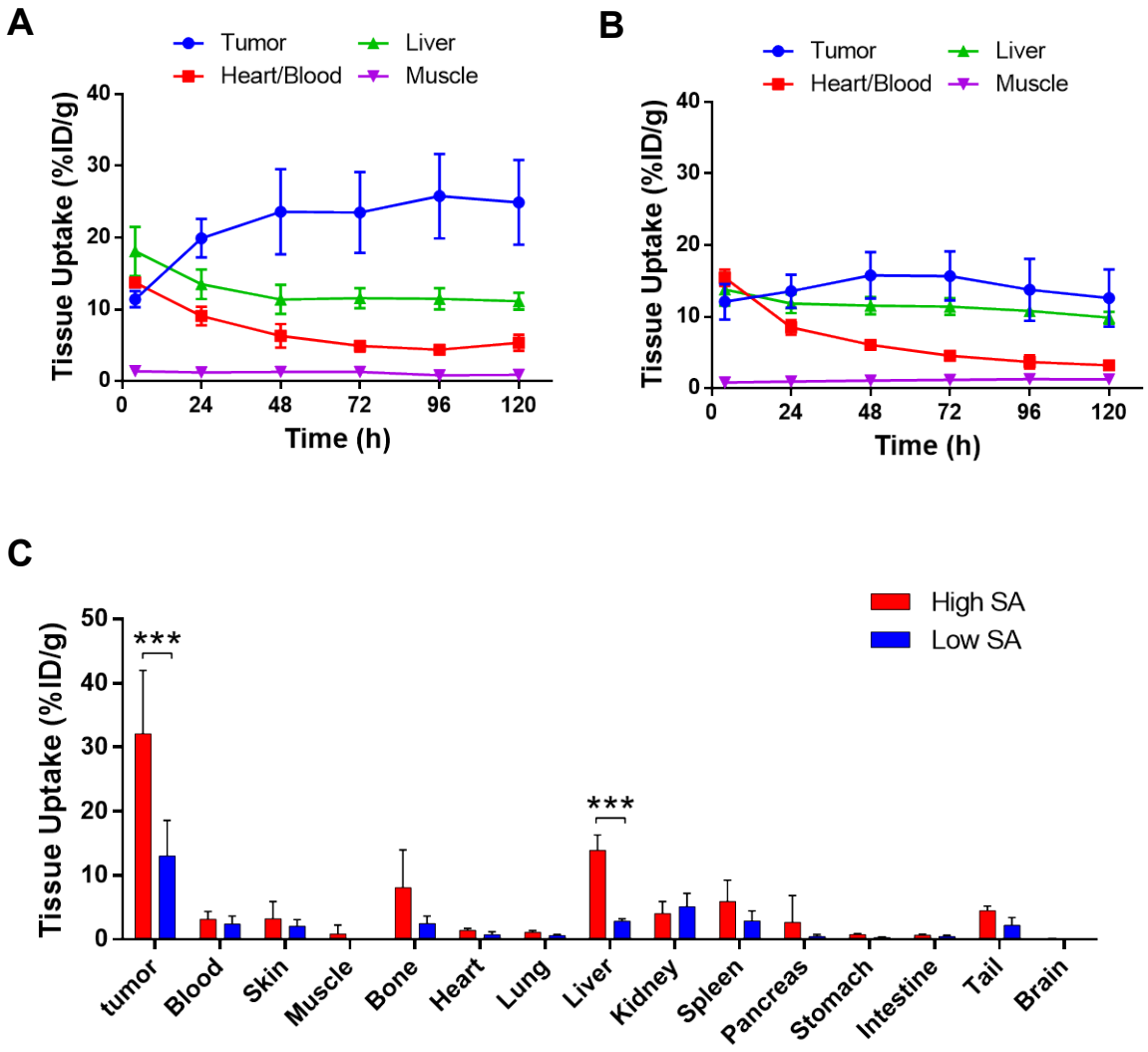

Figure S7. Quantitative analysis of *in vivo* PET data and *ex vivo* biodistribution. Time-activity curves derived from heart/blood, liver, muscle, and tumor of mice bearing HepG2 orthotopic xenografts after injection of **(A)** high SA (29.6 GBq/µmol; n=3) or **(B)** low SA (18.5 GBq/µmol; n=3) ^{89}Zr-Df-YY146-ZW800. A significant decrease in tumor uptake was noted in mice injected a low SA tracer. **C)** *Ex vivo* biodistribution data at 120 h after ^{89}Zr-Df-YY146-ZW800 administration. Mice were sacrificed following the last PET/NIRF scan and major tissues collected, weighed, and counted; tissue uptake in reported as %ID/g ± SD (*** P<0.0001).

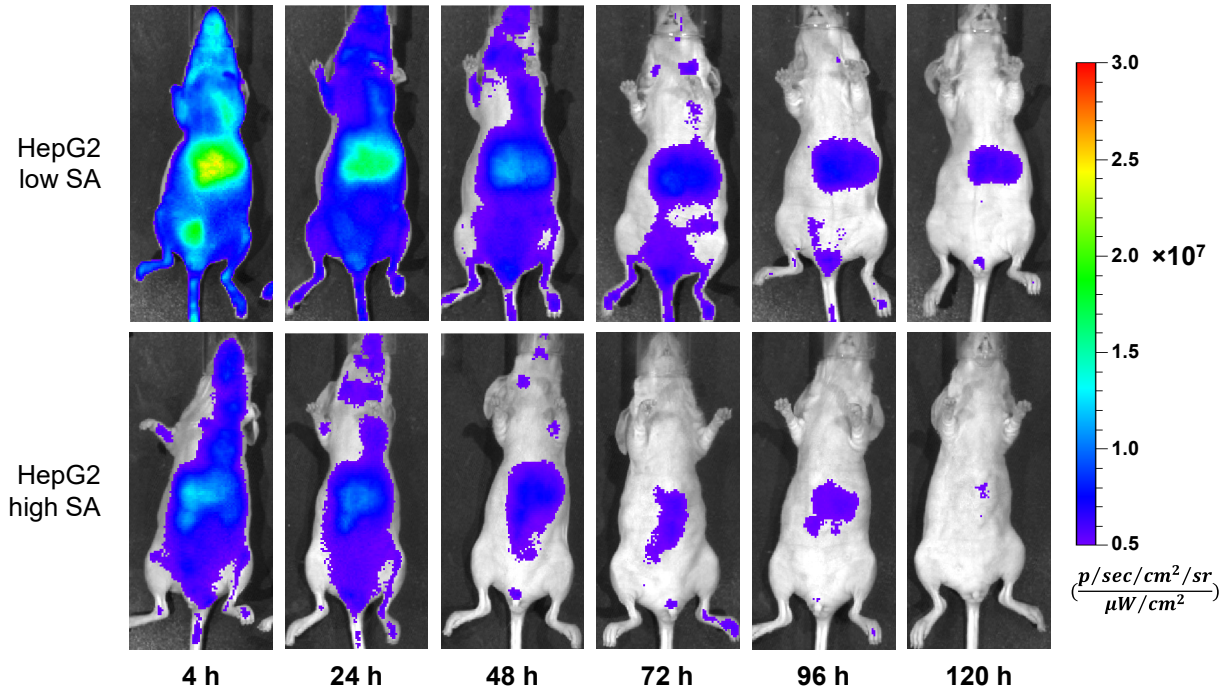

Figure S8. Longitudinal in vivo NIRF imaging of orthotopic tumors. HepG2 tumor-bearing mice were administers 400 pmol (top row; n=3) or 250 pmol (bottom row) of ^{89}Zr-Df-YY146-ZW800. Orthotopic tumors were not readily visualized due to poor penetration of excitation/emission light. A much higher background fluorescence was observed in mice injected 400 pmol of the tracer.

Table S1. Longitudinal quantitative PET data of ^{89}Zr-Df-YY146 and ^{89}Zr-Df-YY146-ZW800 biodistribution in tumor-bearing mice.

Organ/Tissue	Mean (%ID/g) ± SD					
	4 h p.i.	24 h p.i.	48 h p.i.	72 h p.i.	96 h p.i.	120 h p.i.
Subcutaneous Xenografts						
^{89}Zr-Df-YY146 (n=4)						
HepG2	10.40 ± 1.73	24.60 ± 1.42	28.60 ± 5.21	31.65 ± 7.15	29.85 ± 6.61	30.23 ± 6.93
Huh7	2.33 ± 0.46	3.60 ± 0.53	3.70 ± 1.33	3.35 ± 1.00	2.30 ± 0.88	2.60 ± 0.83
Heart/Blood	13.48 ± 1.25	6.55 ± 1.79	4.13 ± 1.31	2.98 ± 0.84	2.68 ± 1.02	2.88 ± 0.84
Liver	10.00 ± 1.80	6.20 ± 1.58	6.15 ± 1.82	5.55 ± 1.22	5.93 ± 1.65	5.60 ± 1.78
Muscle	0.96 ± 0.36	0.79 ± 0.39	0.91 ± 0.48	0.51 ± 0.22	0.69 ± 0.36	0.48 ± 0.43
^{89}Zr-Df-YY146 + CD146 blocking (n=3)						
HepG2	5.73 ± 0.92	7.55 ± 0.49	8.20 ± 0.58	8.20 ± 0.59	8.23 ± 0.48	8.38 ± 0.49
Huh7	1.80 ± 0.75	3.35 ± 0.54	3.10 ± 2.25	3.60 ± 1.62	2.83 ± 1.46	3.28 ± 0.74
Heart/Blood	15.75 ± 1.77	8.73 ± 1.08	6.63 ± 1.16	5.43 ± 1.31	4.65 ± 1.20	3.53 ± 0.62
Liver	9.15 ± 1.58	7.58 ± 0.61	7.65 ± 0.33	7.38 ± 0.29	7.93 ± 0.29	7.88 ± 0.42
Muscle	1.15 ± 0.06	1.40 ± 0.41	1.53 ± 0.46	1.40 ± 0.41	1.10 ± 0.28	1.10 ± 0.45
^{89}Zr-Df-YY146-ZW800 high SA (n=3)						
HepG2	8.03 ± 1.71	16.57 ± 2.25	22.00 ± 3.03	22.07 ± 2.70	23.53 ± 2.55	23.87 ± 2.97
Heart/Blood	13.50 ± 2.18	7.67 ± 0.78	4.93 ± 0.81	3.93 ± 0.78	3.80 ± 0.26	3.90 ± 0.10
Liver	13.97 ± 1.55	10.63 ± 0.97	10.10 ± 1.04	10.30 ± 1.18	10.37 ± 1.22	10.20 ± 1.23
Muscle	0.83 ± 0.29	1.33 ± 0.06	0.70 ± 0.30	0.80 ± 0.17	0.83 ± 0.31	0.80 ± 0.17
^{89}Zr-Df-YY146-ZW800 low SA (n=5)						
HepG2	8.78 ± 3.11	13.72 ± 4.13	15.00 ± 3.56	15.54 ± 3.13	15.20 ± 3.96	15.12 ± 4.35
Heart/Blood	13.68 ± 1.21	6.50 ± 2.00	5.10 ± 1.35	4.16 ± 0.91	3.44 ± 0.96	3.80 ± 0.55
Liver	15.52 ± 3.12	10.66 ± 1.91	10.22 ± 1.69	10.34 ± 2.22	9.52 ± 1.94	9.64 ± 1.90
Muscle	1.12 ± 0.33	1.44 ± 0.30	1.46 ± 0.47	0.98 ± 0.20	0.98 ± 0.33	0.74 ± 0.18
^{89}Zr-Df-YY146-ZW800 + CD146 blocking (n=4)						
HepG2	5.60 ± 0.63	7.63 ± 1.78	7.90 ± 1.50	8.13 ± 0.99	7.90 ± 0.81	7.88 ± 0.83
Heart/Blood	15.98 ± 0.98	9.60 ± 1.43	7.58 ± 1.45	6.65 ± 1.57	5.38 ± 1.62	4.45 ± 1.41
Liver	12.95 ± 2.07	9.68 ± 1.61	9.90 ± 1.87	10.30 ± 2.30	10.15 ± 2.05	10.45 ± 2.21
Muscle	0.80 ± 0.57	1.33 ± 0.25	1.10 ± 0.14	1.25 ± 0.37	1.10 ± 0.08	0.95 ± 0.31
^{89}Zr-Df-YY146-ZW800 high SA (n=3)						
Huh7	5.40 ± 0.57	6.25 ± 0.07	6.40 ± 0.99	6.10 ± 0.57	5.20 ± 1.13	5.05 ± 0.64

Heart/Blood	13.40 ± 2.12	7.75 ± 0.07	5.15 ± 0.49	3.95 ± 0.21	3.25 ± 0.35	3.80 ± 0.85
Liver	13.45 ± 2.19	10.75 ± 0.35	9.40 ± 0.14	10.50 ± 0.42	10.85 ± 1.34	10.70 ± 0.85
Muscle	1.10 ± 0.00	1.40 ± 0.28	1.40 ± 0.14	1.05 ± 0.07	1.10 ± 0.00	0.90 ± 0.42
Orthotopic Xenografts						
^{89}Zr-Df-YY146-ZW800 high SA (n=4)						
HepG2	11.43 ± 1.12	19.93 ± 2.69	23.63 ± 5.94	23.53 ± 5.63	25.80 ± 5.90	24.93 ± 5.91
Heart/Blood	13.75 ± 0.57	9.08 ± 1.30	6.33 ± 1.63	4.90 ± 0.79	4.40 ± 0.29	5.35 ± 1.14
Liver	18.10 ± 3.40	13.53 ± 2.07	11.40 ± 2.00	11.58 ± 1.39	11.48 ± 1.46	11.15 ± 1.16
Muscle	1.38 ± 0.33	1.23 ± 0.33	1.30 ± 0.22	1.28 ± 0.56	0.80 ± 0.16	0.88 ± 0.15
89Zr-Df-YY146-ZW800 low SA (n=5)						
HepG2	12.10 ± 2.47	13.58 ± 2.32	15.78 ± 3.25	15.70 ± 3.41	13.78 ± 4.31	12.62 ± 3.99
Heart/Blood	15.46 ± 1.13	8.52 ± 1.04	6.08 ± 0.37	4.56 ± 0.76	3.68 ± 0.93	3.22 ± 0.71
Liver	13.82 ± 2.28	11.84 ± 1.32	11.54 ± 1.20	11.42 ± 1.18	10.80 ± 0.63	9.88 ± 0.82
Muscle	0.82 ± 0.26	0.96 ± 0.11	1.08 ± 0.13	1.18 ± 0.24	1.28 ± 0.08	1.24 ± 0.11

Values are reported as %ID/g (mean ± SD).

Table S2. *Ex vivo* biodistribution of ^{89}Zr-Df-YY146 and ^{89}Zr-Df-YY146-ZW800, 120 h after iv administration into tumor-bearing nude mice.

	^{89}Zr-Df-YY146		^{89}Zr-Df-YY146-ZW800					
	S.c. xenografts model		S.c. xenografts model				Orthotopic model	
Tissue	HepG2/Huh7 (n=4)	CD146 blocking (n=3)	HepG2 High SA (n=3)	CD146 blocking (n=4)	HepG2 Low SA (n=5)	Huh7 high SA (n=3)	High SA (n=4)	Low SA (n=5)
HepG2	30.82 ± 5.73	7.81 ± 1.65	20.35 ± 2.10	8.62 ± 2.05	10.16 ± 0.14	NA	13.01 ± 5.57	32.10 ± 9.88
Huh7	2.28 ± 1.57	3.14 ± 0.84	NA	NA	NA	4.58 ± 0.42	NA	NA
Blood	1.99 ± 0.72	4.59 ± 1.00	2.22 ± 0.34	7.62 ± 0.44	2.29 ± 1.27	3.19 ± 0.49	2.37 ± 1.26	3.13 ± 1.24
Skin	1.57 ± 1.04	3.06 ± 0.75	2.14 ± 1.28	4.09 ± 1.73	1.80 ± 0.10	1.38 ± 0.57	2.05 ± 1.05	3.24 ± 2.69
Muscle	0.55 ± 0.11	0.37 ± 0.15	0.13 ± 0.02	0.22 ± 0.04	0.19 ± 0.07	0.19 ± 0.02	0.12 ± 0.02	0.87 ± 1.36
Bone	5.01 ± 1.62	1.47 ± 0.34	2.27 ± 1.08	2.32 ± 0.82	3.49 ± 1.32	3.53 ± 0.13	2.48 ± 1.15	8.06 ± 5.91
Heart	1.06 ± 0.25	1.70 ± 0.25	0.85 ± 0.23	2.23 ± 0.74	1.20 ± 0.15	1.57 ± 0.06	0.72 ± 0.46	1.43 ± 0.28
Lung	1.92 ± 0.38	1.29 ± 0.31	0.69 ± 0.09	3.51 ± 1.99	1.51 ± 0.24	2.12 ± 0.08	0.64 ± 0.13	1.08 ± 0.32
Liver	7.08 ± 1.97	8.89 ± 0.42	9.44 ± 1.36	13.15 ± 3.61	15.87 ± 2.08	13.21 ± 3.17	2.83 ± 0.41	13.93 ± 2.37
Kidney	4.69 ± 0.88	5.20 ± 0.79	5.19 ± 1.45	5.79 ± 2.22	4.32 ± 1.00	4.72 ± 0.12	5.12 ± 2.06	4.03 ± 1.89
Spleen	3.90 ± 1.13	3.06 ± 0.93	4.29 ± 1.68	4.18 ± 0.37	5.69 ± 1.03	5.72 ± 0.22	2.91 ± 1.53	5.92 ± 3.33
Pancreas	0.43 ± 0.17	0.44 ± 0.08	0.39 ± 0.30	0.77 ± 0.21	0.36 ± 0.01	0.25 ± 0.03	0.52 ± 0.28	2.65 ± 4.18
Stomach	0.65 ± 0.12	0.45 ± 0.10	0.31 ± 0.09	0.60 ± 0.17	0.71 ± 0.20	1.15 ± 0.97	0.31 ± 0.09	0.79 ± 0.13
Intestine	0.99 ± 0.55	0.67 ± 0.23	0.57 ± 0.10	0.65 ± 0.10	0.62 ± 0.02	0.83 ± 0.20	0.48 ± 0.18	0.69 ± 0.12
Tail	3.42 ± 1.04	2.28 ± 0.40	3.11 ± 0.71	3.16 ± 0.60	3.08 ± 0.33	2.91 ± 1.01	2.20 ± 1.20	4.52 ± 0.71
Brain	0.08 ± 0.02	0.10 ± 0.02	0.07 ± 0.07	0.15 ± 0.08	0.07 ± 0.00	0.15 ± 0.03	0.07 ± 0.05	0.12 ± 0.04

Values are reported as %ID/g (mean ± SD).