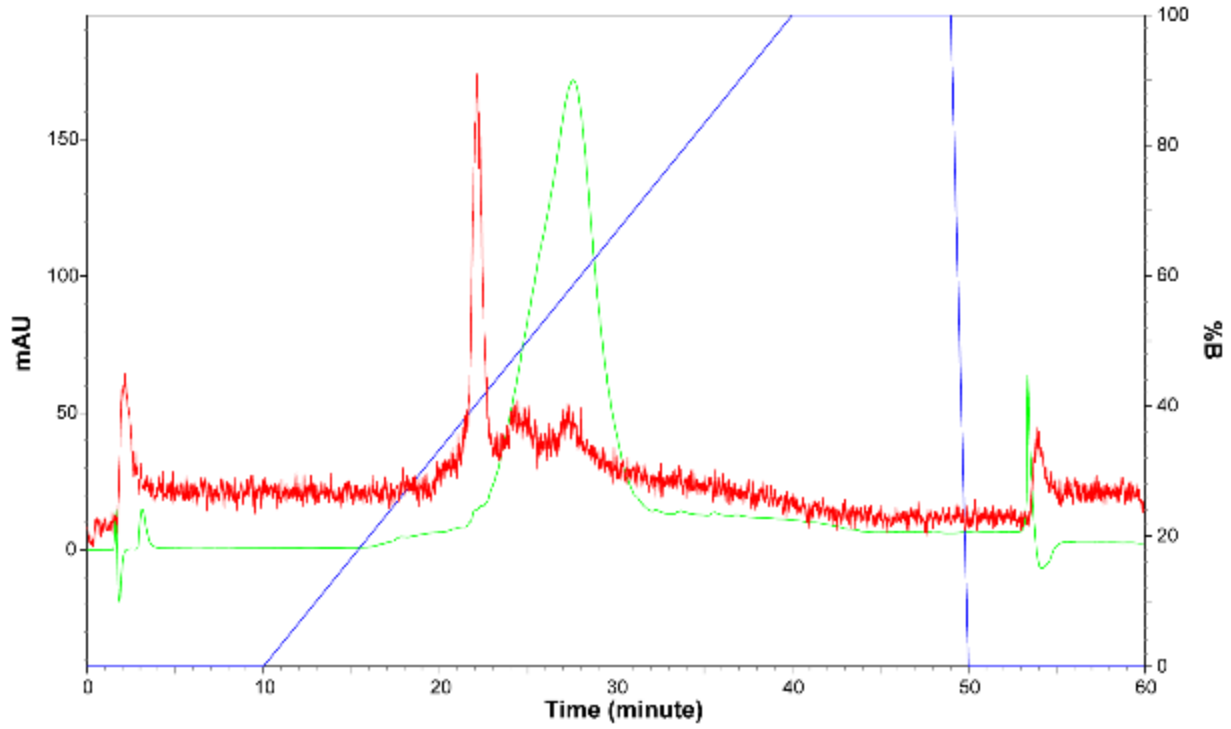
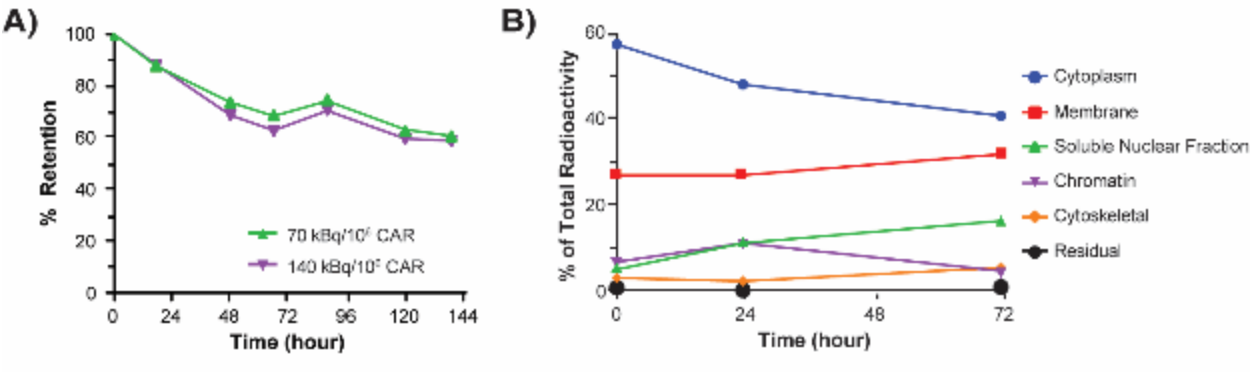
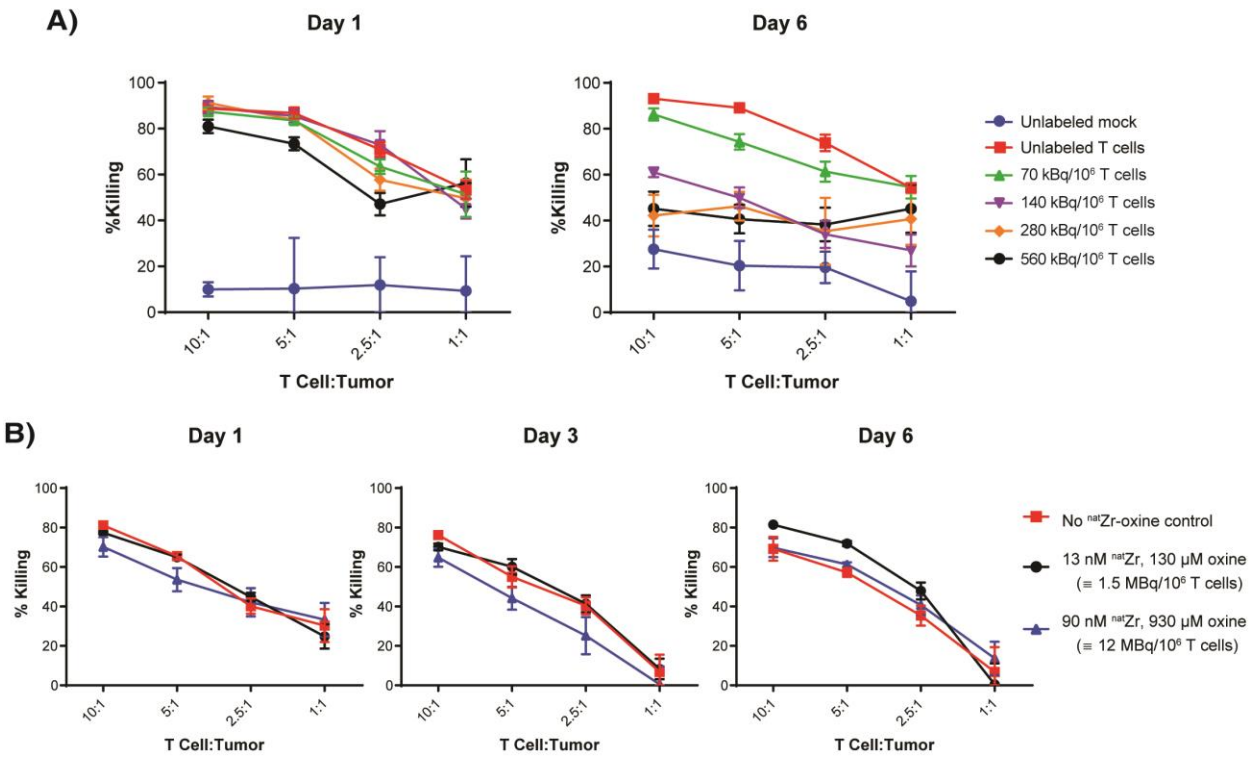


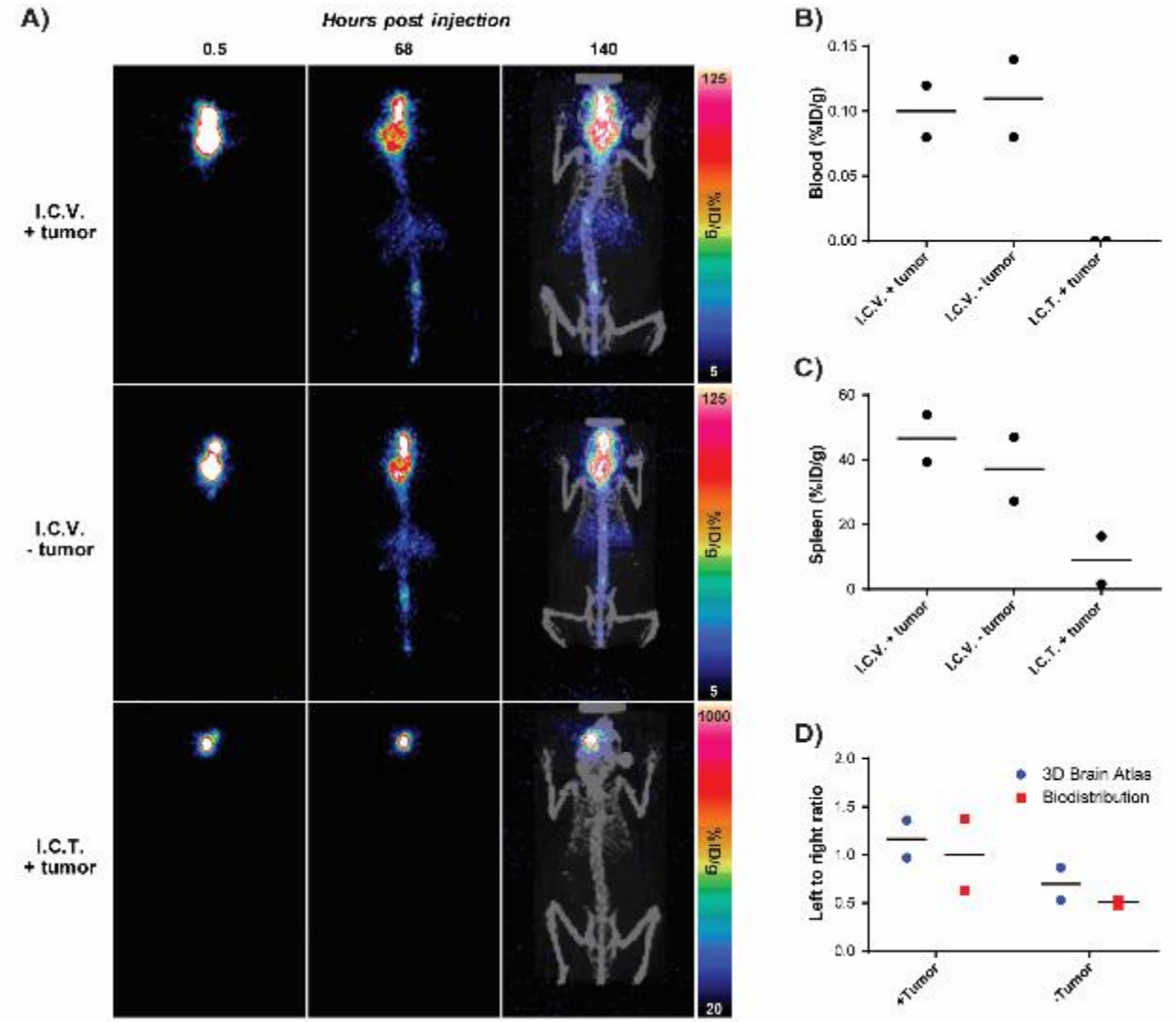
Supplemental Figure 1. Chromatographic analysis of ^{89}Zr-oxine complex formation. After incubation for 15 minutes at room temperature, ^{89}Zr-oxine was injected on a C18 RP-HPLC. ^{89}Zr-oxine radioactivity (red) eluted at 22 minutes with a corresponding UV_{254nm} peak for oxine (green) while free ^{89}Zr eluted from the column at 2 minutes. By comparing the heights of the first peak (2 min) to the eluted peaks (20 to 30 min), the calculated radiolabeling efficiency was 82%. The solvent gradient (blue) was 0 to 100 % acetonitrile plus 0.1% TFA.



Supplemental Figure 2. ^{89}Zr-oxine is retained in CAR T cells. **A)** IL13Rα2-CAR T cells were labeled with 70 or 140 kBq per million cells and incubated in T cell media at 5% CO_2, 37 °C for 6 days. Radioactivity retention values are expressed as the percent radioactivity associated with the cell pellet (% retention = cell pellet radioactivity / total radioactivity) (n = 3). **B)** IL13Rα2-CAR T cells were labeled at 70 kBq per million cells, cultured for 0, 24 and 72 hours, and the radioactivity in the subcellular fractions was counted and expressed as a percentage of the total cell radioactivity.



Supplemental Figure 3. Effects of ^{89}Zr-oxine labeling on CAR T cell cytotoxicity are dose and time-dependent. After labeling for 30 minutes in HBSS at 37 ˚C and culturing in standard culture conditions, IL13Rα2-CAR T cells were incubated with PBT030-2 ffLuc+ cells for 7 hours at a ratio of 10, 5, 2.5, and 1 T cell to 1 tumor cell. **A)** The cytotoxicities of ^{89}Zr-oxine labeled CAR cells were compared to unlabeled CAR and mock T cells. **B)** IL13Rα2-CAR T cells were incubated with an equivalent preparation of ^{nat}Zr-oxine and compared to an unlabeled control. Percent killing was determined as the loss of luminescence in comparison to an experimental maximum (no effector cells) (mean ± S.E.; n = 6).



Supplemental Figure 4. Systemic distribution of intraventricularly injected CAR T cells and cross hemisphere tumor trafficking. A) Representative PET (0 and 68 hours) and PET/CT (140 hours) maximum intensity projections of NSG mice with or without PBT030-2 ffLuc+ tumors following intratumoral (I.C.T.) and intraventricular (I.C.V.) administration of ^{89}Zr-oxine labeled IL13Rα2-CAR T cells (70 kBq/10^6 cells). **B-C)** Mean radioactive counts (%ID/g) in the blood and spleen of mice at 140 hours. **D)** Mean ratios of the left and right hemisphere tissue counts (red) compared to the quantification of the left and right cortexes of PET images using the 3D Brain Atlas tool (blue) at 140 hours. Mice were injected with ^{89}Zr-oxine labeled CAR T cells in the right hemisphere and tumors were implanted in the left frontal cortex.

SUPPLEMENTAL TABLE 1

Biodistribution of ^{89}Zr-oxine labeled IL13Rα2-CAR T cells in NSG mice with and without PBT030-2 ffLuc+ tumors at 150 h

	%ID*						%ID/g*					
Treatment location	Right Ventricle				Left Intratumoral		Right Ventricle				Left Intratumoral	
Tumor-bearing	No		Yes				No		Yes			
	MS#1	MS#2	MS#3	MS#4	MS#5	MS#6	MS#1	MS#2	MS#3	MS#4	MS#5	MS#6
Organ												
Blood†	0.03	0.02	0.02	0.02	0.00	0.00	0.14	0.08	0.12	0.08	0.00	0.00
Heart	0.03	0.04	0.02	0.04	0.00	0.00	0.17	0.23	0.13	0.21	0.00	0.00
Lung	0.10	0.08	0.07	0.06	0.01	0.03	0.46	0.36	0.34	0.28	0.06	0.15
Liver	13.8	13.0	12.4	12.4	2.54	7.58	7.52	10.2	10.5	10.3	2.06	5.87
Stomach‡	0.04	0.06	0.05	0.05	0.02	0.01	0.06	0.07	0.08	0.06	0.04	0.02
Spleen	1.21	1.20	0.98	1.34	0.07	0.71	27.3	47.0	39.3	53.9	1.60	16.3
Small Intestine‡	0.21	0.21	0.19	0.12	0.18	0.08	0.11	0.13	0.13	0.09	0.13	0.07
Large Intestine‡	0.17	0.15	0.13	0.15	0.05	0.09	0.10	0.09	0.09	0.10	0.05	0.08
Kidneys	0.40	0.39	0.38	0.37	0.28	0.28	0.81	0.92	0.84	0.88	0.70	0.66
Muscle	0.01	0.02	0.03	0.02	0.15	0.00	0.05	0.12	0.16	0.09	0.96	0.00
Bone	0.52	0.55	0.67	0.50	0.71	0.68	4.21	2.76	4.95	4.00	5.02	4.05
Carcass	1.69	1.61	0.31	0.31	2.43	2.45	0.73	0.52	0.08	0.08	0.48	0.70
Stifle	0.58	0.40	0.66	0.50	0.52	0.57	5.94	4.62	7.00	4.58	3.15	4.50
Vertebrae	3.43	3.88	2.79	3.03	1.19	0.90	9.42	7.03	7.86	8.19	3.92	2.89
Cerebellum	9.23	7.81	6.00	6.98	0.08	0.14	31.2	60.2	46.3	53.3	0.49	0.62
Right Cerebrum	3.86	8.88	4.20	6.54	0.70	2.69	11.4	63.1	29.9	49.3	2.72	7.97
Left Cerebrum	2.06	4.30	5.78	4.14	56.0	37.9	6.08	30.0	40.3	27.1	219	119
Olfactory Bulbs	2.18	2.82	1.33	3.37	1.01	25.6	36.8	180	53.9	124	20.6	518
Left to Right Cerebrum (Biodistribution)	*0.53*	*0.48*	*1.38*	*0.63*	*79.8*	*14.1*						
Left to Right Cortex (3D Brain Atlas)	*0.85*	*0.52*	*1.43*	*0.93*	*16.1*	*6.11*						

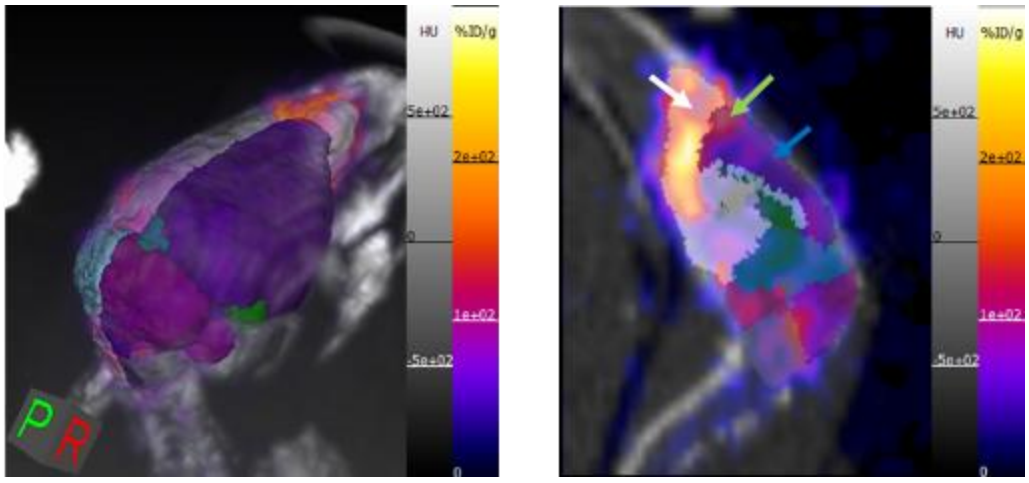
*Values were obtained from individual tissue gamma counts.
†200 μL of blood sampled from cardiac puncture.
‡Includes contents.

SUPPLEMENTAL TABLE 2

Biodistribution of ^{89}Zr-oxine labeled PSCA-CAR T cells in NSG mice with PC3-PSCA tumors at 170 h

Treatment type (intravenous)	%ID*						%ID/g*					
	^{89}Zr-oxine only		^{89}Zr-oxine Mock		^{89}Zr-oxine CAR		^{89}Zr-oxine only		^{89}Zr-oxine Mock		^{89}Zr-oxine CAR	
	MS #1	MS #2	MS #3	MS #4	MS #5	MS #6	MS #1	MS #2	MS #3	MS #4	MS #5	MS #6
Organ												
Blood†	0.06	0.06	0.02	0.02	0.03	0.02	0.28	0.28	0.08	0.08	0.13	0.11
Heart	0.17	0.22	0.04	0.03	0.05	0.05	1.24	1.42	0.33	0.28	0.38	0.34
Lung	0.45	0.49	0.31	0.36	0.80	0.58	2.28	2.25	1.48	1.85	4.04	2.98
Liver	11.6	12.1	44.3	45.4	54.1	58.7	11.0	10.5	39.9	44.3	46.8	50.3
Gall Bladder	0.01	0.01	0.02	0.03	0.01	0.02	0.44	1.27	0.96	1.47	0.60	1.24
Spleen	0.33	0.25	5.16	5.39	5.46	6.22	8.32	6.58	211	195	232	154
Stomach‡	0.20	0.23	0.13	0.05	0.09	0.10	0.31	0.34	0.16	0.13	0.20	0.13
Small Intestine‡	0.57	0.81	0.30	0.24	0.39	0.33	0.47	0.58	0.19	0.17	0.28	0.24
Large Intestine‡	0.33	0.49	0.13	0.18	0.21	0.23	0.36	0.49	0.14	0.22	0.21	0.16
Kidneys	0.89	1.02	1.44	1.29	1.44	1.78	2.83	2.93	4.05	4.38	3.95	5.30
Muscle	0.10	0.11	0.02	0.04	0.04	0.03	0.68	0.60	0.15	0.22	0.18	0.15
Tumor	0.73	0.52	0.08	0.17	0.48	0.70	1.69	1.61	0.31	0.27	2.43	2.45
Femur	2.09	2.37	0.77	0.53	0.67	0.54	13.7	14.0	0.71	0.68	4.81	4.22
Carcass	50.3	47.8	13.0	5.33	13.5	13.1	3.02	2.61	5.11	3.89	0.70	0.73
Stifle	1.96	2.06	0.62	1.83	0.61	0.55	19.9	14.8	3.05	2.59	5.92	4.20
Vertebrae	3.78	3.79	1.31	0.02	1.17	1.02	12.9	9.46	0.08	0.08	3.06	1.88
Tumor to Blood							*6.00*	*5.70*	*3.69*	*3.40*	*18.3*	*21.8*
Tumor to Muscle							*2.49*	*2.69*	*2.06*	*1.23*	*13.5*	*16.0*

*Values were obtained from individual tissue gamma counts.
†200 µL of blood sampled from cardiac puncture.
‡Includes contents.



Supplemental Figure 5. Co-registering VivoQuant 3D Brain Atlas to anatomical CT and corresponding PET regions of interest. Left panel: Representative fitting of the VivoQuant 3D Brain Atlas to anatomical CT scans. Right panel: a representative sagittal section with 3D Brain Atlas overlays showing ^{89}Zr-labeled CAR T cell PET signal (%ID/g in pseudocolor) within the tumor (green arrow), located in the cortex (blue arrow and purple region), and posterior to the olfactory bulb (white arrow and beige region).