

SUPPLEMENTAL TABLE 1. Summary of Tracer Specifications

Tracer QC	⁶⁴ Cu-DOTA-HAC-PD1	⁶⁴ Cu-NOTA-HAC-PD1	⁶⁸ Ga-NOTA-HAC-PD1	⁶⁴ Cu-NOTA-HACA-PD1	⁶⁸ Ga-NOTA-HACA-PD1	⁶⁸ Ga-DOTA-HACA-PD
Chelates/Tracer	0.92±0.01	0.74±0.22	0.74±0.22	0.54±0.23	0.54±0.23	0.43±0.10
Chemical Purity	> 86.2%	>90.2%	87.5 %	> 85.2%	> 85.2%	> 91.7%
Radiochemical Purity	> 98%	>98%	>88%	> 87.7%	> 87.8%	> 87.4%
SA	4843.8 MBq/μmol	6199.5 MBq/μmol	1832.9 MBq/μmol	5822.32 MBq/μmol	1455.6 MBq/μmol	2162.9 MBq/μmol
Groups	hPD-L1+ (n=4) hPD-L1- (n=7) Blocking (n=4) Dual (n=5)	hPD-L1+(n=1) Dual (n=2)	hPD-L1+(n=2) hPD-L1- (n=2)	Dual (n=6)	Dual (n=4)	Dual (n=3)
Imaging	1,2,4,18,24h	1h	1h	1h	N/A	1h
BIOD	1, 24h	1h	1h	1h	1h	1h

SUPPLEMENTAL TABLE 2. ImmunoPET Tracer Biodistribution Single Tumor Model

Organ %ID/g (Mean ± SD)	Cu ⁶⁴ -DOTA- HAC-PD1 (n=3)	Cu ⁶⁴ -DOTA- HAC-PD1 (n=3)	Cu ⁶⁴ -DOTA- HAC-PD1 (n=2, Blocking)	Cu ⁶⁴ -NOTA- HAC-PD1 (n=1)	⁶⁸ Ga-NOTA- HAC-PD1 (n=2)	⁶⁸ Ga-NOTA- HAC-PD1 (n=2)
Tumor +	2.1 ± 1.6	NA	0.9 ± 0.0	3.0 ± 0.0	0.9 ± 0.3	NA
Tumor-	NA	1.2 ± 0.9	NA	NA	NA	0.6 ± 0.0
Muscle	2.0 ± 1.7	0.5 ± 0.1	1.2 ± 0.4	0.7 ± 0.0	0.5 ± 0.2	0.5 ± 0.2
Blood	0.5 ± 0.2	0.3 ± 0.0	1.8 ± 1.8	0.6 ± 0.0	0.2 ± 0.1	0.4 ± 0.2
Heart	1.4 ± 0.5	1.3 ± 0.2	1.7 ± 1.3	2.2 ± 0.0	1.1 ± 0.0	0.8 ± 0.5
Lungs	0.9 ± 0.4	0.9 ± 0.1	2.5 ± 1.5	1.2 ± 0.0	0.7 ± 0.2	0.5 ± 0.3
Liver	16.3 ± 1.7	17.1 ± 1.0	15.5 ± 2.3	15.6 ± 0.0	4.4 ± 0.8	4.4 ± 0.0
Spleen	9.2 ± 0.8	3.4 ± 1.2	8.7 ± 9.1	5.3 ± 0.0	2.8 ± 0.1	2.4 ± 0.6
Pancreas	2.6 ± 1.0	2.2 ± 1.0	5.8 ± 0.6	3.5 ± 0.0	0.7 ± 0.4	1.1 ± 0.0
Stomach	1.9 ± 0.7	2.7 ± 0.1	8.5 ± 3.1	1.7 ± 0.0	1.0 ± 0.1	1.0 ± 0.2
SI	2.4 ± 0.9	2.7 ± 1.4	2.8 ± 1.3	1.2 ± 0.0	0.7 ± 0.1	0.7 ± 0.0
LI	3.0 ± 1.4	1.5 ± 0.7	3.6 ± 0.5	1.0 ± 0.0	1.4 ± 0.8	2.0 ± 0.5
Kidney	73.7 ± 48.5	84.8 ± 44.0	43.6 ± 0.9	76.2 ± 0.0	67.3 ± 2.1	49.7 ± 9.1
Bone	1.4 ± 0.7	1.3 ± 0.8	0.6 ± 0.2	1.6 ± 0.0	0.7 ± 0.6	0.6 ± 0.3
BM	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Skin	0.9 ± 0.3	0.9 ± 0.7	0.3 ± 0.2	0.5 ± 0.0	0.7 ± 0.1	0.4 ± 0.1
Brain	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

SUPPLEMENTAL TABLE 3. ImmunoPET Tracer Biodistribution Dual Tumor Model

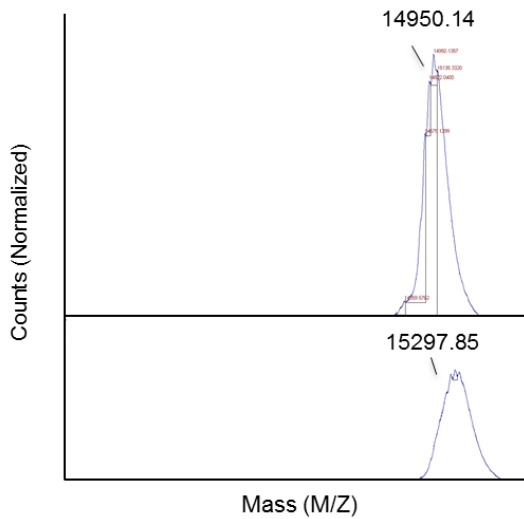
Organ %ID/g (Mean ± SD)	⁶⁴ Cu-DOTA- HAC-PD1 (n=3)	⁶⁴ Cu-NOTA- HAC-PD1 (n=2)	⁶⁴ Cu-NOTA- HACA-PD1 (n=6)	⁶⁸ Ga-NOTA- HACA-PD1 (n=4)	⁶⁸ Ga-DOTA- HACA-PD1 (n=3)
Tumor +	1.8 ± 0.2	4.2 ± 0.8	2.7 ± 1.1	3.8 ± 1.6	2.8 ± 1.5
Tumor-	0.9 ± 0.7	3.5 ± 1.7	0.8 ± 0.4	1.7 ± 1.3	0.8 ± 0.1
Muscle	0.3 ± 0.1	1.3 ± 0.4	0.3 ± 0.1	0.4 ± 0.3	0.2 ± 0.1
Blood	0.3 ± 0.0	1.2 ± 0.3	1.4 ± 0.3	8.7 ± 4.7	3.7 ± 0.9
Heart	1.8 ± 0.3	11.7 ± 10.5	0.9 ± 0.2	2.3 ± 0.8	1.4 ± 0.5
Lungs	0.5 ± 0.2	2.3 ± 1.1	2.0 ± 1.1	3.0 ± 1.7	1.2 ± 1.1
Liver	19.8 ± 2.5	17.0 ± 5.9	7.4 ± 4.7	8.1 ± 0.2	3.3 ± 0.2
Spleen	4.0 ± 3.1	5.5 ± 1.4	1.4 ± 0.4	3.5 ± 0.6	0.2 ± 0.2
Pancreas	1.3 ± 0.3	3.6 ± 1.1	1.4 ± 1.5	1.2 ± 0.4	0.5 ± 0.3
Stomach	1.2 ± 1.0	3.4 ± 0.8	1.7 ± 0.6	2.1 ± 0.5	1.1 ± 0.4
SI	2.0 ± 1.2	3.5 ± 1.9	1.8 ± 0.9	2.1 ± 1.2	0.7 ± 0.7
LI	1.1 ± 0.6	6.2 ± 1.4	1.6 ± 0.3	4.0 ± 0.5	1.0 ± 0.9
Kidney	113 ± 15.7	55.6 ± 8.3	96.2 ± 15.5	20.9 ± 15.4	55.8 ± 13.4
Bone	2.0 ± 0.7	1.5 ± 1.6	0.9 ± 0.4	1.5 ± 0.9	0.6 ± 0.3
BM	0.1 ± 0.0	0.1 ± 0.0	1.0 ± 2.3	0.0 ± 0.0	0.1 ± 0.0
Skin	0.7 ± 0.4	1.2 ± 0.9	0.4 ± 0.2	0.7 ± 0.2	0.5 ± 0.2
Brain	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.2

SUPPLEMENTAL TABLE 4. Tumor +: Background Ratios Dual Tumor Model

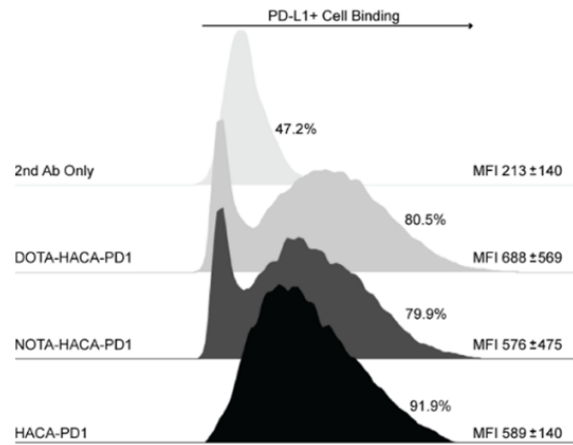
Organ (Mean ± SD)	⁶⁴ Cu-DOTA- HAC-PD1 (n=3)	⁶⁴ Cu-NOTA- HAC-PD1 (n=2)	⁶⁴ Cu-NOTA- HACA-PD1 (n=6)	⁶⁸ Ga-NOTA- HACA-PD1 (n=4)	⁶⁸ Ga-DOTA- HACA-PD1 (n=3)
Tumor +	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Tumor-	4.2 ± 4.8	1.3 ± 0.4	4.6 ± 3.8	2.6 ± 0.9	3.5 ± 2.0
Muscle	6.8 ± 2.5	3.4 ± 0.5	10.3 ± 7.5	12.3 ± 3.9	15.2 ± 9.6
Blood	6.4 ± 1.5	3.7 ± 0.2	2.0 ± 0.9	0.5 ± 0.1	0.8 ± 0.5
Heart	1.0 ± 0.1	0.6 ± 0.6	3.5 ± 2.1	1.6 ± 0.5	2.4 ± 1.6
Lungs	4.1 ± 1.5	1.9 ± 0.6	1.7 ± 1.1	1.9 ± 2.1	6.3 ± 7.0
Liver	0.1 ± 0.0	0.3 ± 0.0	0.5 ± 0.4	0.4 ± 0.2	0.9 ± 0.5
Spleen	0.7 ± 0.4	0.8 ± 0.1	2.1 ± 1.1	1.0 ± 0.3	52.0 ± 61.1
Pancreas	1.5 ± 0.6	1.2 ± 0.2	6.8 ± 9.3	3.5 ± 2.4	7.8 ± 5.7
Stomach	2.2 ± 1.3	1.2 ± 0.1	2.0 ± 1.3	1.8 ± 0.6	2.9 ± 1.9
SI	1.2 ± 0.7	1.3 ± 0.5	2.1 ± 1.5	2.6 ± 1.94	9.2 ± 7.8
LI	2.4 ± 1.8	0.7 ± 0.0	1.9 ± 0.9	0.9 ± 0.4	5.2 ± 4.1
Kidney	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.5 ± 0.7	0.1 ± 0.0
Bone	1.0 ± 0.5	5.0 ± 4.6	3.8 ± 2.3	3.4 ± 2.4	6.8 ± 6.9
BM	31.7 ± 21.1	53.6 ± 16.2	73.6 ± 98.1	119.8 ± 50.0	54.6 ± 38.9
Skin	3.1 ± 2.0	4.5 ± 2.7	7.5 ± 3.2	5.8 ± 2.6	5.9 ± 2.6
Brain	31.1 ± 8.8	41.3 ± 10.1	29.4 ± 12.8	28.9 ± 11.5	68.2 ± 75.0

* Tumor: Background Ratios are calculated for each individual mouse and the reported value is the mean ± SD for the cohort.

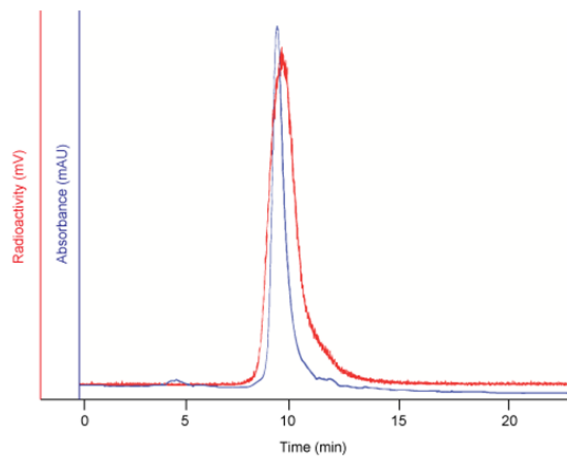
A. Chelates/Binder



B. Immunoreactivity

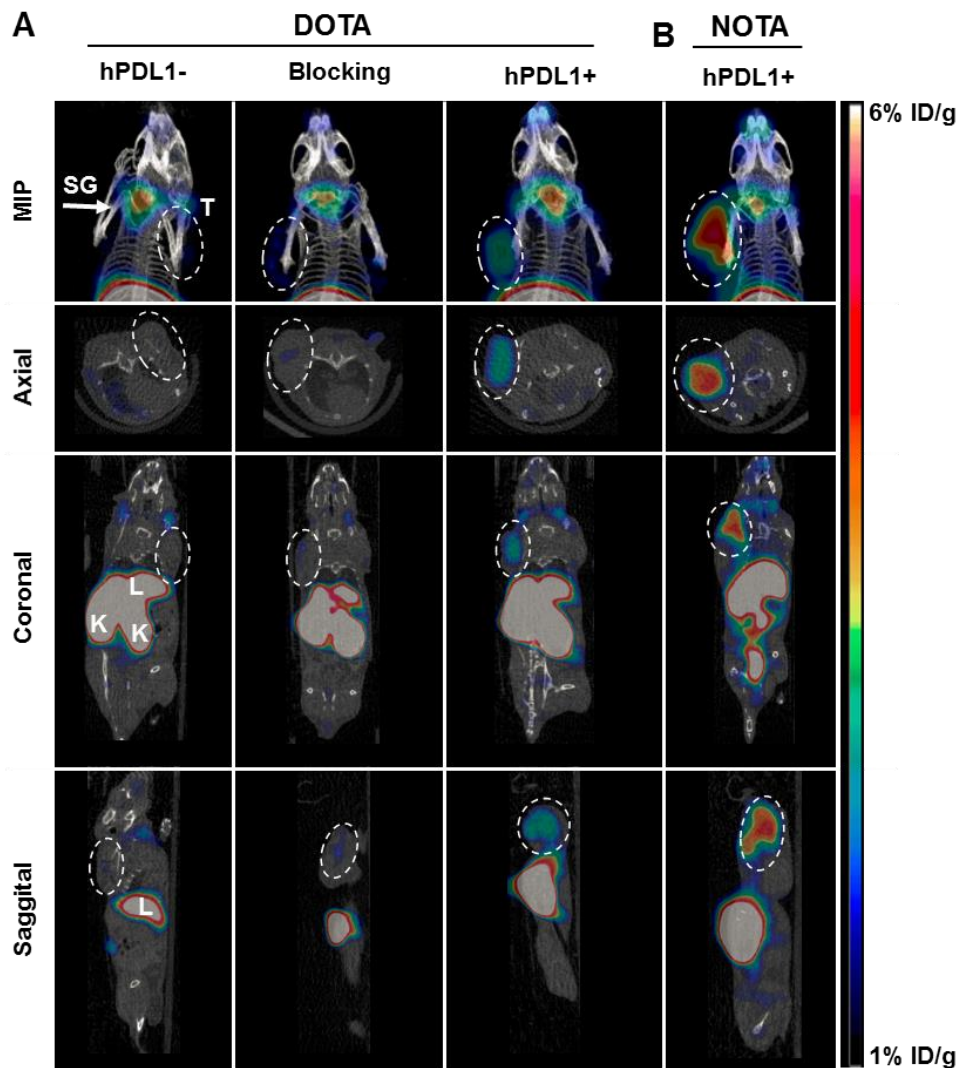


C. Radiochemical purity



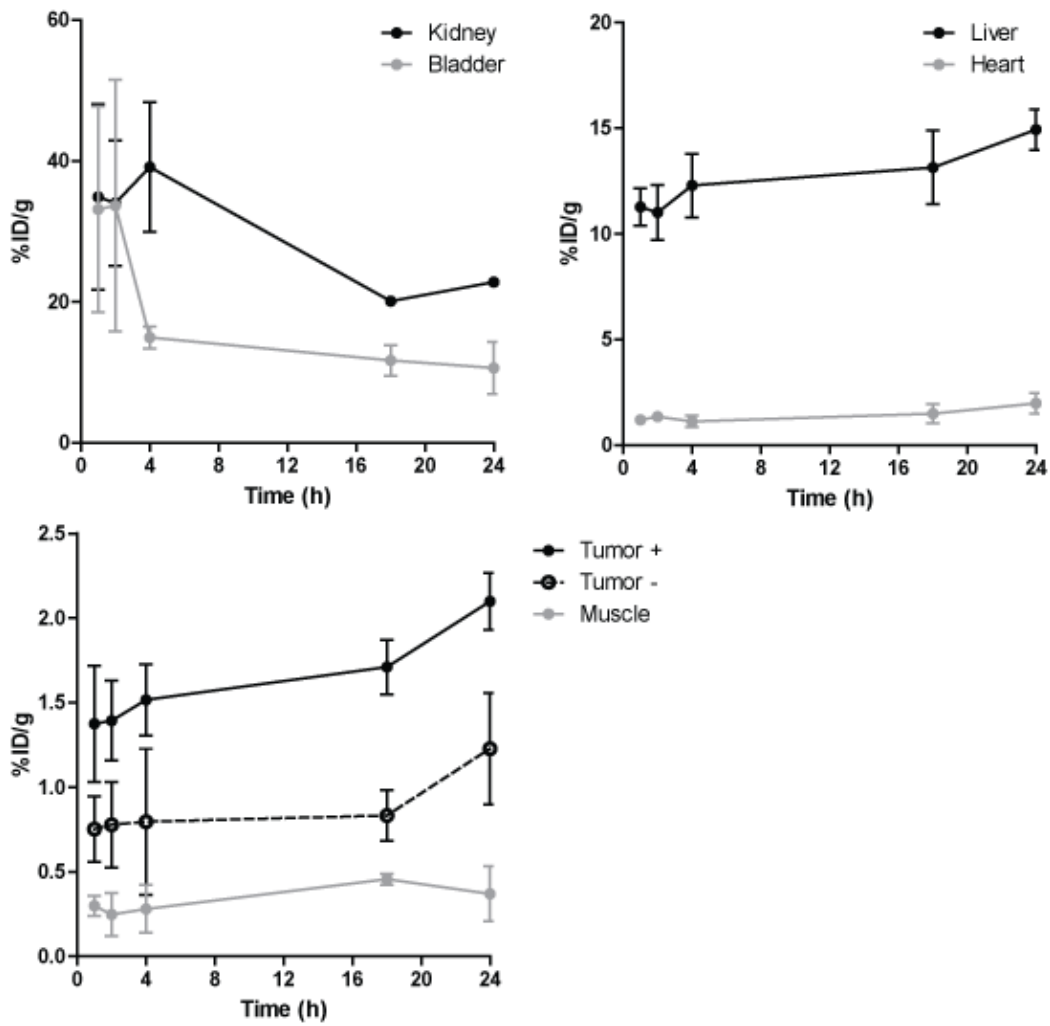
Quality control workflow

Supplemental Figure 1. Tracer Quality Control. Representative schematics of: (A) Mass spec determination of number of chelates/ binder. (B) FACS assessment of drop in immunoreactivity after chelation [MFI: median fluorescent intensity] Conjugates were tested for their binding ability on hPD-L1 expressing CT26 cells. Each of the conjugates (200 pM, 100µL) were placed in triplicate vials containing CT26-hPD-L1 cells (3×10^5) and kept on ice for 1 h. Cells were washed three times after 1 h with 1%PBSA to remove unbound HACA conjugates. After washing, each of the wells received 100 µL of anti-His-tag-APC antibody for FACS staining and were incubated on ice in the dark. After 1 h cells were washed three times and analyzed by FACS. (C) HPLC assessment of purity and specific activity.

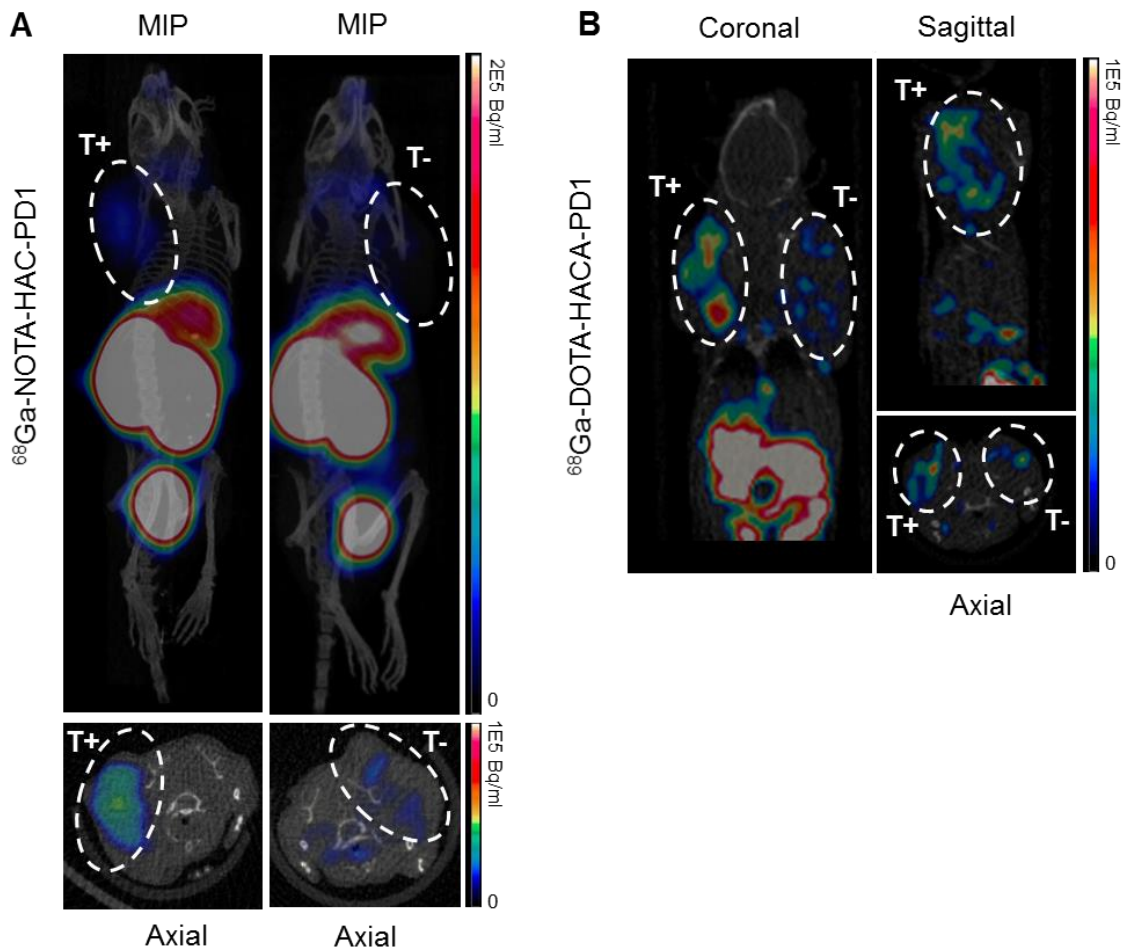


Supplemental Figure 2. Human PD-L1 Immune Checkpoint Imaging with ^{64}Cu Variants in a Single Tumor Model.

MicroPET imaging of hPD-L1 with ^{64}Cu -DOTA-HAC (A), or ^{64}Cu -NOTA-HAC (B). From left to right, PET-CT images one hour post injection of tracer (200 $\mu\text{Ci}/25\mu\text{g}/200\mu\text{l}$) in NSG mice bearing subcutaneous CT26 hPD-L1(-) tumor, CT26 hPD-L1(+) blocked tumor, or CT26 hPD-L1(+) tumor. Blocking was performed with 500 $\mu\text{g}/200\mu\text{l}$ of unlabeled HAC-PD-1, 2 hours prior to PET tracer. The immunoPET tracer exhibits rapid and highly specific hPD-L1+ tumor uptake which is ideal for potential clinical imaging applications. T-tumor, Li-liver, K-kidneys, SG-salivary glands. Scale bar indicates uptake from 1-6 %ID/g.



Supplemental Figure 3. Static PET scans at 1, 2, 4, 18 and 24hrs p.i. of ^{64}Cu -DOTA-HAC-PD1 in NSG mice bearing dual tumors.



Supplemental Figure 4. Human PD-L1 Immune Checkpoint Imaging with ^{68}Ga Variants. MicroPET imaging of hPD-L1 with $^{68}\text{Ga-NOTA-HAC}$ (A), or $^{68}\text{Ga-DOTA-HACA}$ (B) in single and dual tumor bearing mice 1h P.I. respectively. Dashed line demarcates tumor. MIP, Maximum intensity projection; T+, hPD-L1+; T-, hPD-L1-.

Supplemental Video 1. ^{64}Cu -DOTA-HAC-PD1 MIP 1h p.i. (~50 $\mu\text{Ci}/10\ \mu\text{g}/200\ \mu\text{l}$) of NSG mouse bearing dual subcutaneous tumors in shoulders (right = hPD-L1+; left = hPD-L1-). Colors represent 1%(blue) – 6%(red) ID/g.

Supplemental Video 2. ^{64}Cu -NOTA-HAC-PD1 MIP 1h p.i. (~50 $\mu\text{Ci}/10\ \mu\text{g}/200\ \mu\text{l}$) of NSG mouse bearing dual subcutaneous tumors in shoulders (right = hPD-L1+; left = hPD-L1-). Colors represent 1%(blue) – 6%(red) ID/g.

Supplemental Video 3. ^{64}Cu -NOTA-HACA-PD1 MIP 1h p.i. (~50 $\mu\text{Ci}/10\ \mu\text{g}/200\ \mu\text{l}$) of NSG mouse bearing dual subcutaneous tumors in shoulders (right = hPD-L1+; left = hPD-L1-). Colors represent 1%(blue) – 6%(red) ID/g.