Supporting Information for: Linear Desferrichrome-linked silicon-rhodamine antibody conjugate enables targeted multimodal imaging of HER2 in vitro and in vivo.

Shin Hye Ahn, Daniel Thach, Brett A. Vaughn, Vincent M. Alford, Alyssa N. Preston, Scott T. Laughlin and Eszter Boros*

ORCID ID: 0000-0002-4186-6586 (Eszter Boros)

Department of Chemistry, Stony Brook University, 100 Nicolls Road, Stony Brook, NY 11790, United States

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Table S1. Absorbance and calculated I values of each concentration of SiR-Ph-NCS

Concentration (nmoles/mL)	Absorbance	I ($=I_0 10^{-edc}$)
1	0.02	2.0
5	0.11	11.0
10	0.24	24.0
20	0.64	64.0
30	0.98	98.0
40	1.15	115.0
50	1.58	158.0

For SiR-LDFC-trastuzumab, absorbance at 630 nm = 0.10, and IgG concentration = 0.599 mg/mL (1.03 fluorophores per antibody).

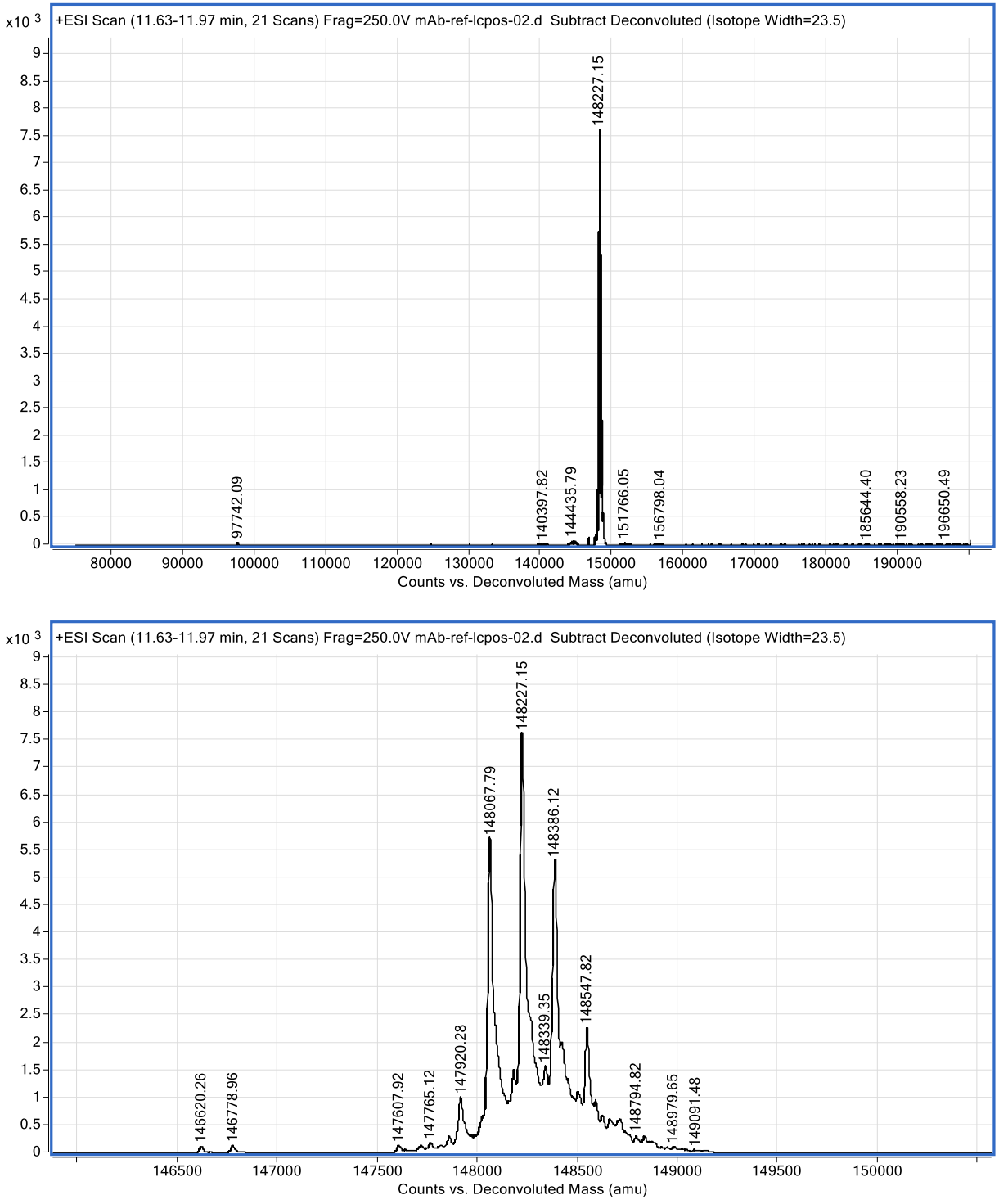

Figure S1. Deconvolution of extracted mass spectra of trastuzumab from Rt = 11.72', ESI+ chromatogram (top), and its expansion (bottom).

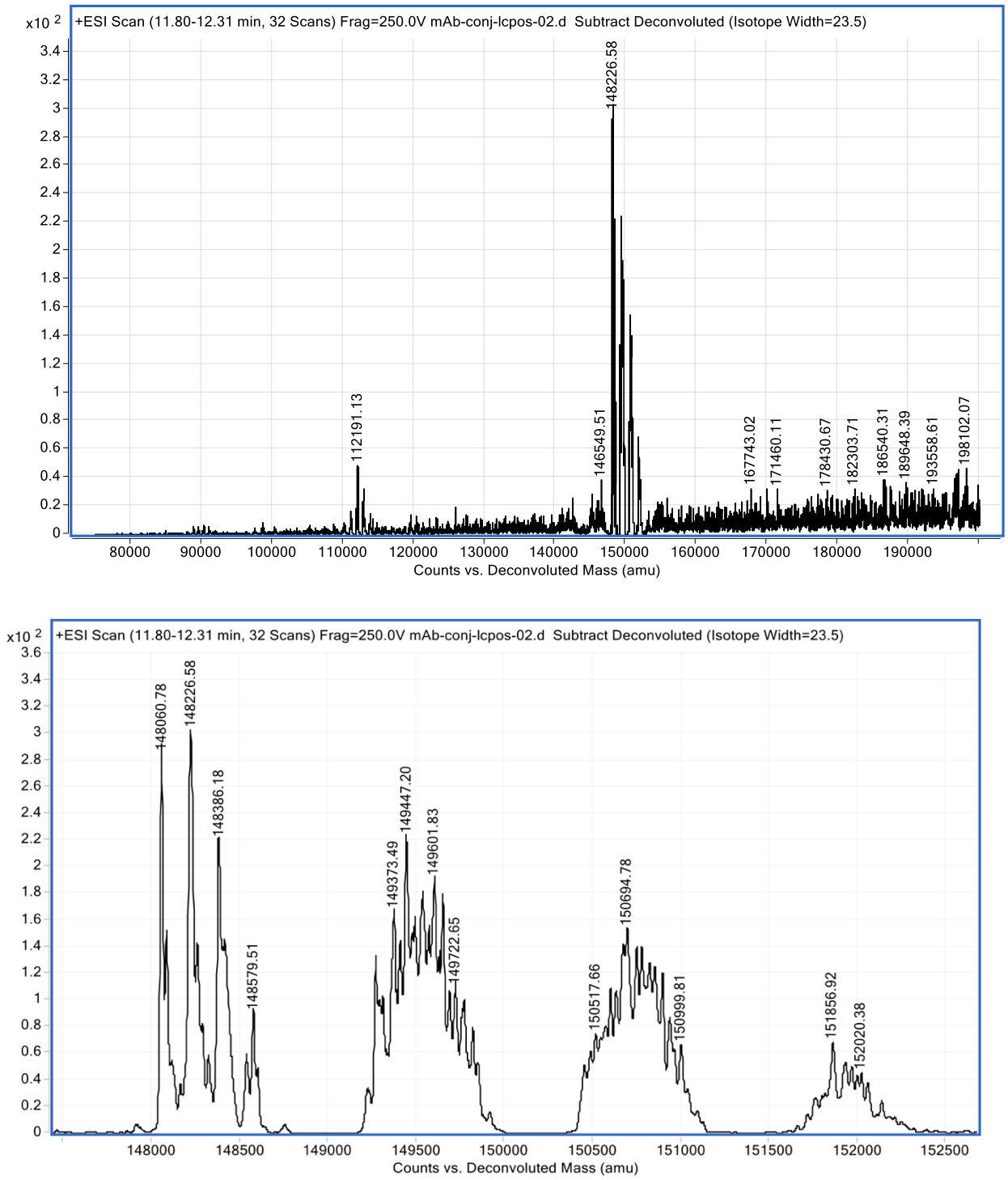

Figure S2. Deconvolution of extracted mass spectra of SiR-LDFC-trastuzumab from Rt= 11.95', ESI^+ chromatogram (top), and its expansion (bottom).

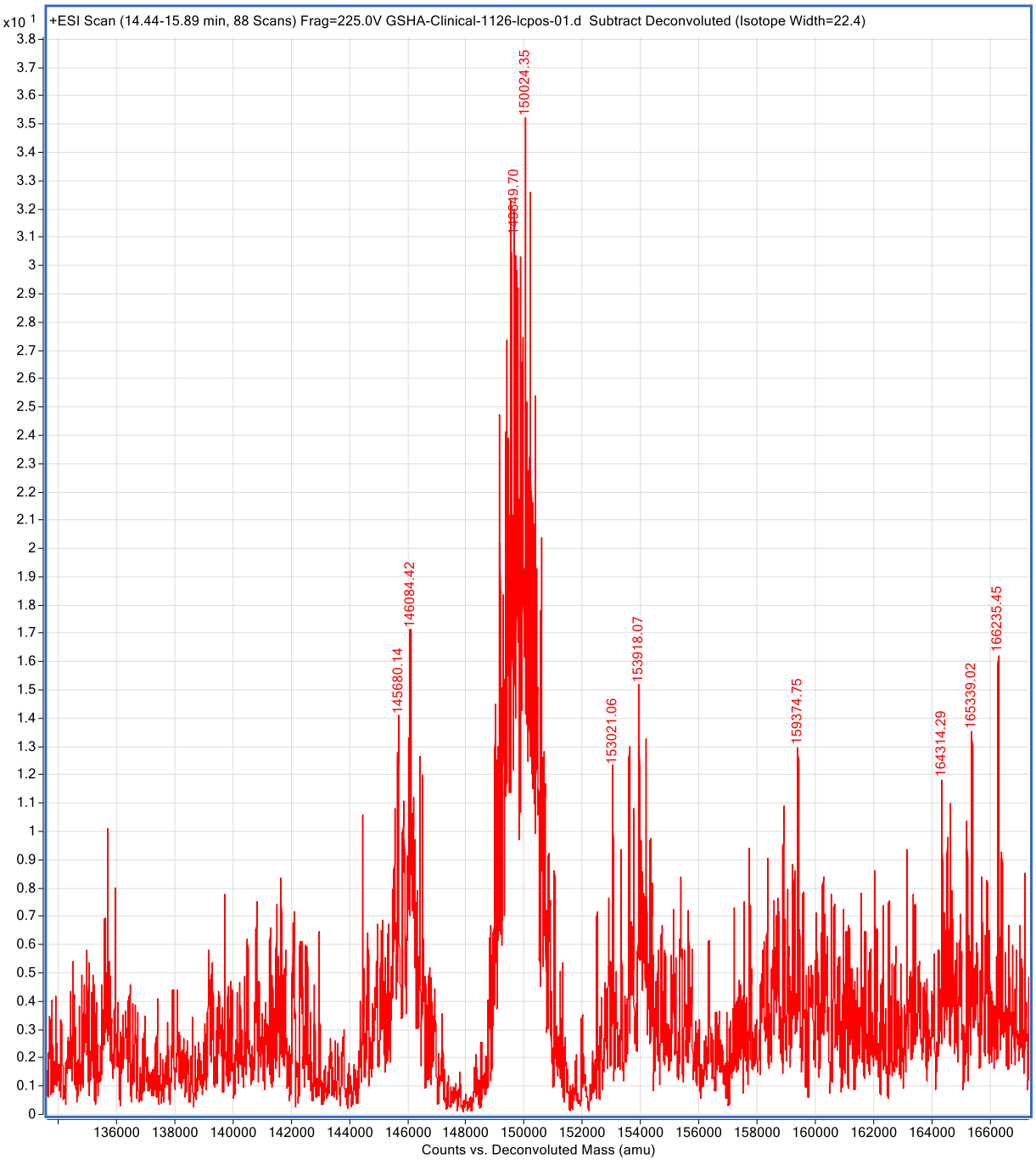

Figure S3. Deconvolution of extracted mass spectra of DFO-trastuzumab from ESI$^+$, TIC chromatogram, Rt = 15.02'.

Quantification of fluorophore-antibody ratio from ESI-TOF MS/MS data:

For comparison with fluorophore-antibody ratio calculated from the calibration curve, the area under each peak was quantified, and divided by the sum of area of all peaks. These values were then used to calculate the weighted average mass of the antibody conjugate. The difference between the weighted average (149618 kDa) and the reference antibody (148226 kDa) was divided by the molar mass of SiR-LDFC-NCS to yield a ratio of 1.09 fluorophores per antibody.

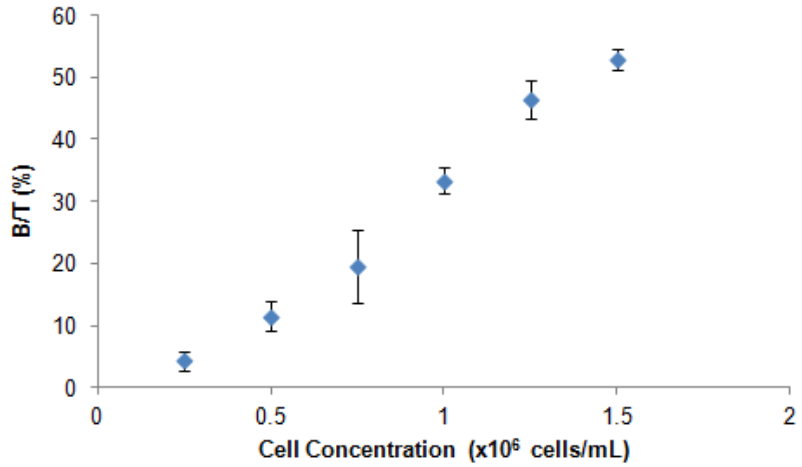

Figure S4. Determination of the immunoreactivity of ^{89}Zr-SiR-LDFC-trastuzumab. Trace amounts of ^{89}Zr-SiR-LDFC-trastuzumab were incubated with increasing concentration of SKOV3 cells (n=3).

Table S2. Each microcentrifuge tube containing SKOV-3 cells were counted for activity after incubation with ^{89}Zr-SiR-LDFC-trastuzumab, to determine the immunoreactivity (n=3).

Concentration (x10^6 cells/mL)	Total Activity (counts)	Bound Activity (counts)	Bound/Total (%)
0.25	18859	819±280	4.34
0.5		2163±455	11.5
0.75		3686±1092	19.
1		6297±417	33.4
1.25		8747±594	46.4
1.5		9973±310	52.9

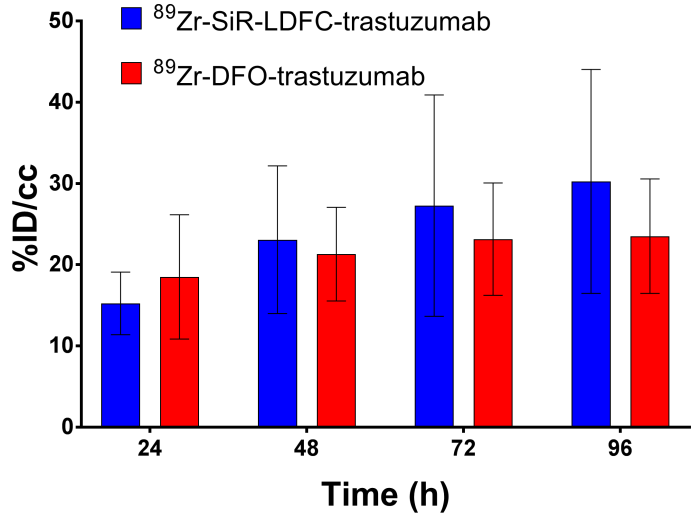

Figure S5. ROI analysis of tumoral uptake of ^{89}Zr-SiR-LDFC-trastuzumab and ^{89}Zr-DFO-trastuzumab in SKOV3 subcutaneous tumor-bearing mice (n=4) at 24, 48, 72 and 96 h p.i.

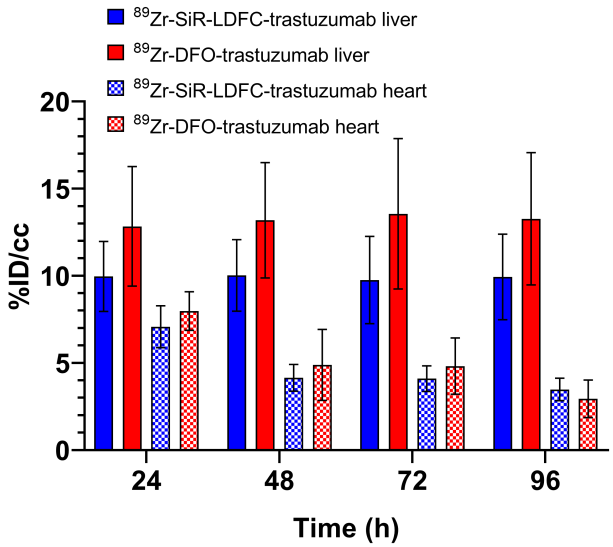

Figure S6. ROI analysis of liver and heart uptake of ^{89}Zr-SiR-LDFC-trastuzumab and ^{89}Zr-DFO-trastuzumab in SKOV3 subcutaneous tumor-bearing mice (n=4) at 24, 48, 72 and 96 h p.i.

Table S3. ROI analysis of uptake of ^{89}Zr-SiR-LDFC-trastuzumab and ^{89}Zr-DFO-trastuzumab in SKOV3 subcutaneous tumor-bearing mice (n=4) at 24, 48, 72 and 96 h p.i.

Time (h)	^{89}Zr-SiR-LDFC-trastuzumab (%ID/cc)			^{89}Zr-DFO-trastuzumab (%ID/cc)		
	Tumor	Liver	Heart	Tumor	Liver	Heart
24	15.3±3.9	10.0±2.0	7.1±1.2	18.5±7.6	12.8±3.4	8.0±1.1
48	23.1±9.1	10.0±2.0	4.1±0.8	21.3±5.8	13.2±3.3	4.9±2.0
72	27.3±13.6	9.8±2.5	4.1±0.7	23.1±6.9	13.6±4.3	4.8±1.6
96	30.3±13.8	9.9±2.5	3.5±0.6	23.5±7.1	13.2±3.8	2.9±1.1

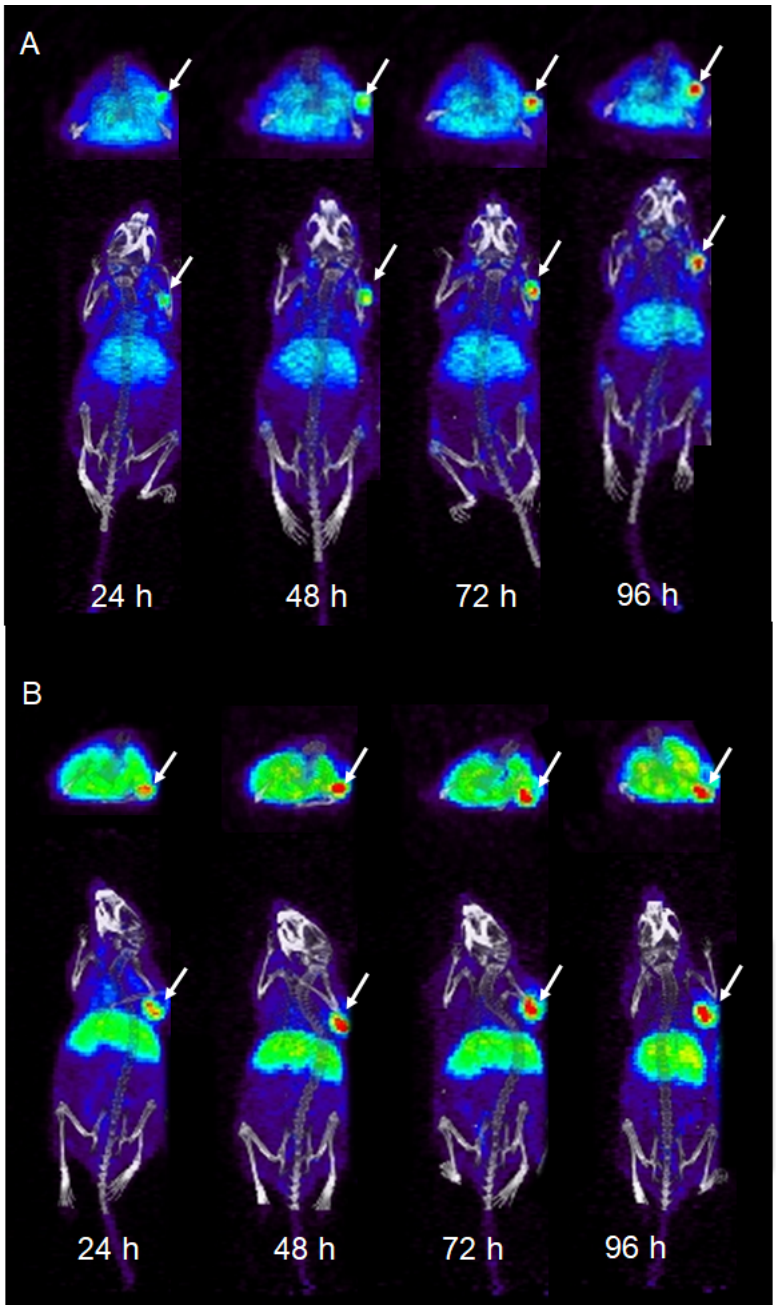

Figure S7. Maximum intensity projection ^{89}Zr-SiR-LDFC-trastuzumab (**A**) and ^{89}Zr-DFO-trastuzumab (**B**) PET/CT images in SKOV-3 subcutaneous tumor-bearing mice (n=4) at 24, 28, 72 and 96 hours p.i. Tumor indicated by white arrow.

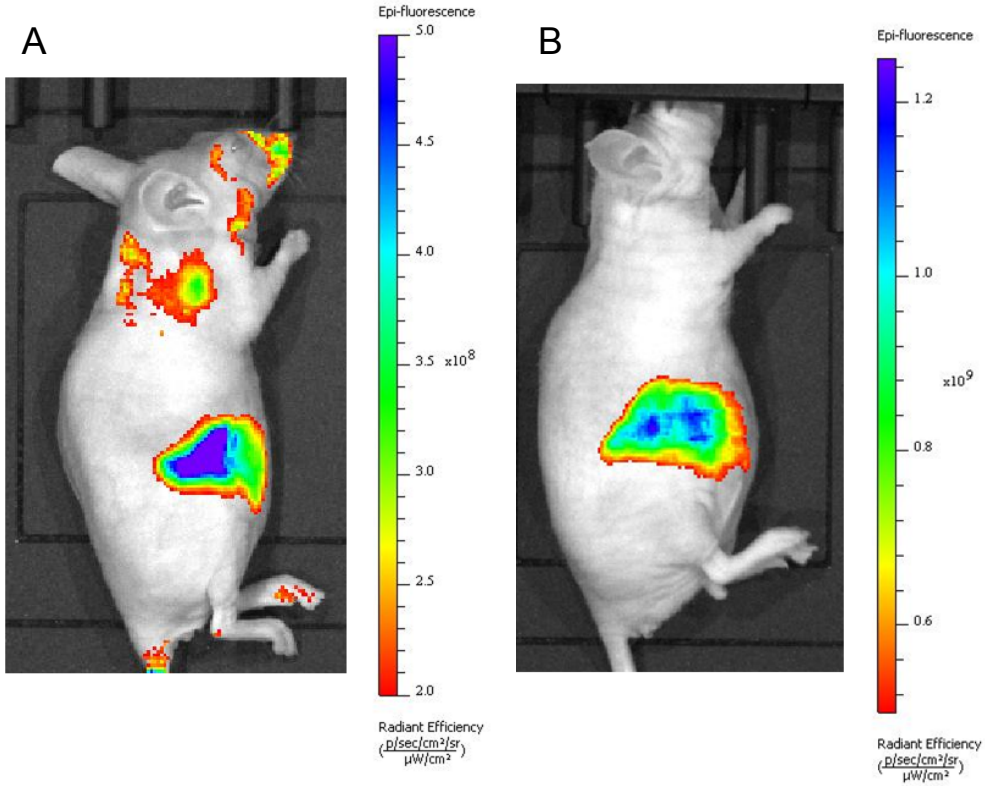

Figure S8. Fluorescence image of (**A**) SKOV3 tumor-bearing mouse injected with ^{89}Zr-SiR-LDFC-trastuzumab at 96 h p.i. and (**B**) male athymic nude mouse with no fluorophore injection.