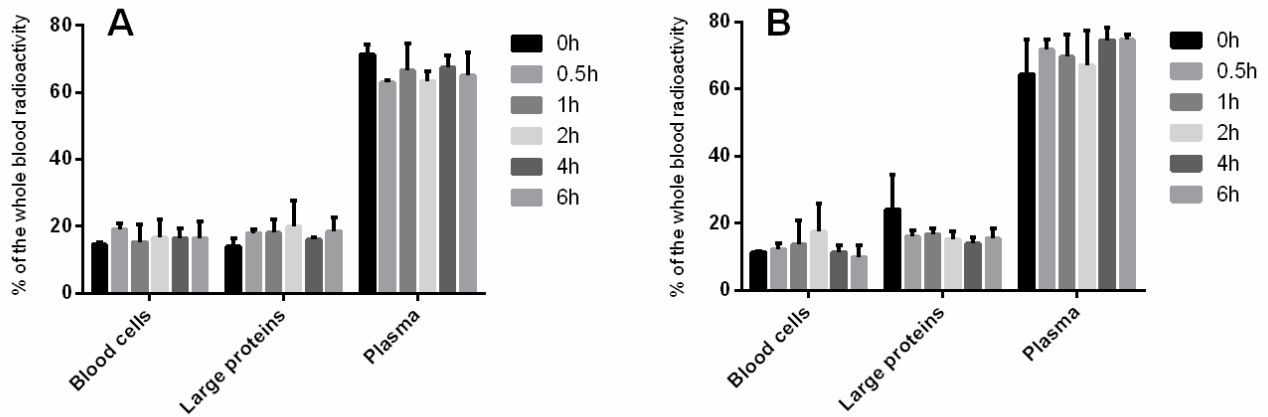


Supplemental Figure 1. Evaluation of thermal stability of A1 (A) and C6 (B) sdAbs by circular dichroism from 25°C to 80°C.

	RadioChemical Purity (%)		Lipophilicity
	After Radiolabeling	6 h	Log <i>P</i>
^{99m} Tc-A1	99.7±0.1	99.2±0.1	-1.8±0.5
^{99m} Tc-C6	99.1±0.8	98.8±0.5	-2.3±0.8

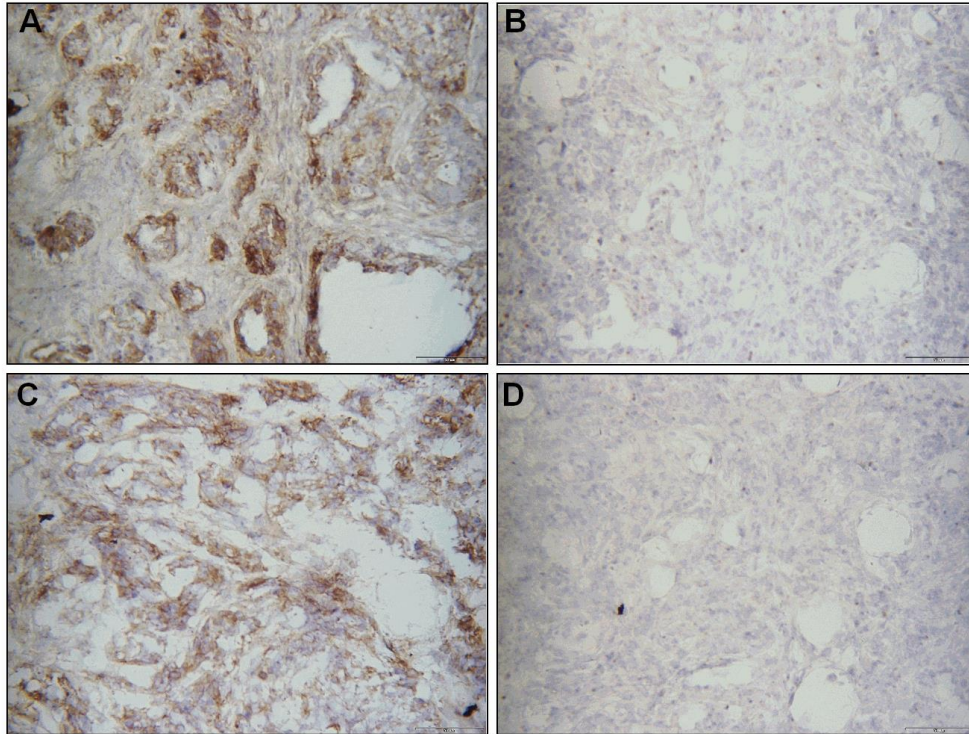
Supplemental Table 1. Radiochemical purity and partition coefficient (Log *P*) of ^{99m}Tc-A1 and ^{99m}Tc-C6. Data are presented as mean ± Standard Deviation (n = 3).



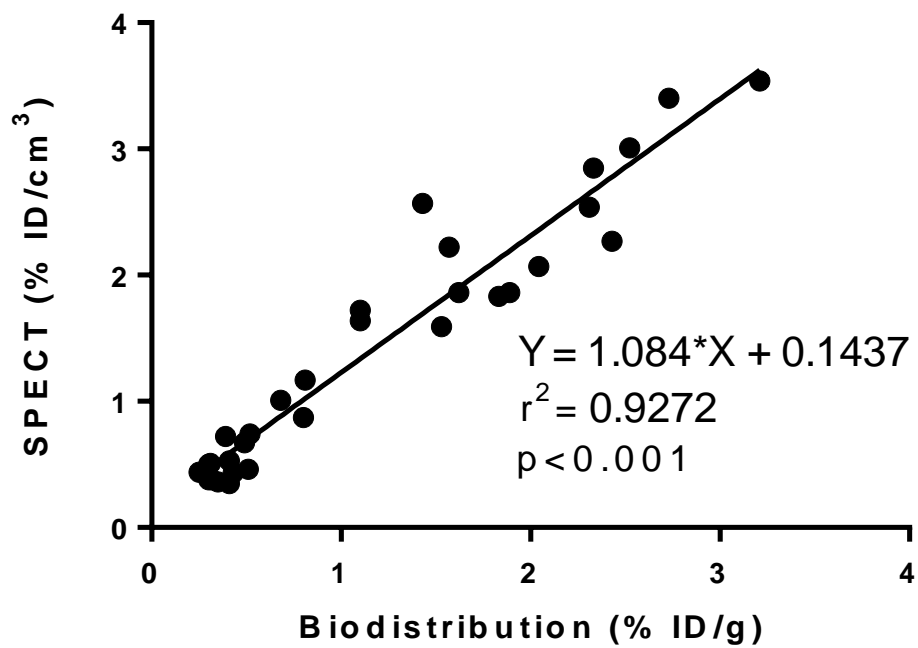
Supplemental Figure 2. *In vitro* blood distribution patterns of $^{99m}\text{Tc-A1}$ (A) and $^{99m}\text{Tc-C6}$ (B) following incubation with whole human blood. Results were expressed as a % of whole blood radioactivity (mean \pm Standard Deviation, n=3).

Time	0h	0.5h	1h	2h	4h	6h
^{99m} Tc-A1	99.8±0.1	99.8±0.1	99.7±0.1	99.2±0.6	98.7±0.3	98.8±0.5
^{99m} Tc-C6	99.6±0.1	99.8±0.1	99.2±0.5	98.6±0.4	98.2±0.3	98.0±0.2

Supplemental Table 2. *In vitro* stability of ^{99m}Tc-A1 and ^{99m}Tc-C6 in human blood. The zero time point refers to the stability observed immediately following contact with human blood. Results are expressed as a % of intact radiolabeled sdAb in the remaining plasma fraction. Experiments were performed in triplicate.



Supplemental Figure 3. Determination of mesothelin expression by ex vivo immunohistochemistry on HCC70 (A, C) or MDA-MB-231 (B, D) tumors, performed with commercial antibody (a, b) or A1 sdAb (c, d). Representative image (x20) of HCC70 (A, C) or MDA-MB-231 tumor (B, D) with mesothelin staining. HCC70 and MDA-MB-231 tumors were fixed using acetone for 10 minutes at -20 °C and 10- μ m thick cryosections were obtained. Immunohistochemistry was performed using A1 sdAb (20 μ g/mL) or commercial anti-mesothelin antibody polyclonal anti-mesothelin (0.5 μ g/mL, Boster immunoleader). For the A1 assay, tumors were then incubated with a rabbit anti-histidine antibody (1/200, anti-6x His tag® antibody, ab9108 Abcam). In both cases, the revelation was performed using a biotinylated antibody goat anti-Rabbit IgG (1/500, BA-1000, Vector), followed by DAB coloration (Vector).



Supplemental Figure 4. Correlation between tumor uptake assessed by SPECT and biodistribution.