Supporting Information

Development of ^{99m}Tc-Radiolabeled Nanosilica for Targeted Detection of HER2-Positive Breast Cancer

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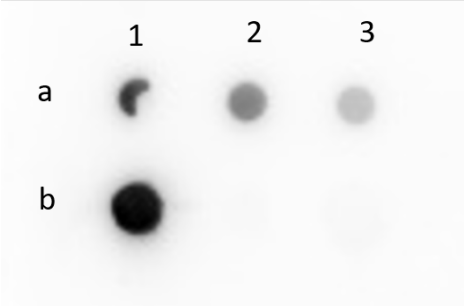

Figure S1. The digitized image of the Dot Blot analysis performed on SiNP-TZ (b1) compared to SiNP (b2) and core-shell NPs (b3). Dots from (a1) to (a3) represent standard amount of TZ (150, 75, 37 ng, respectively).

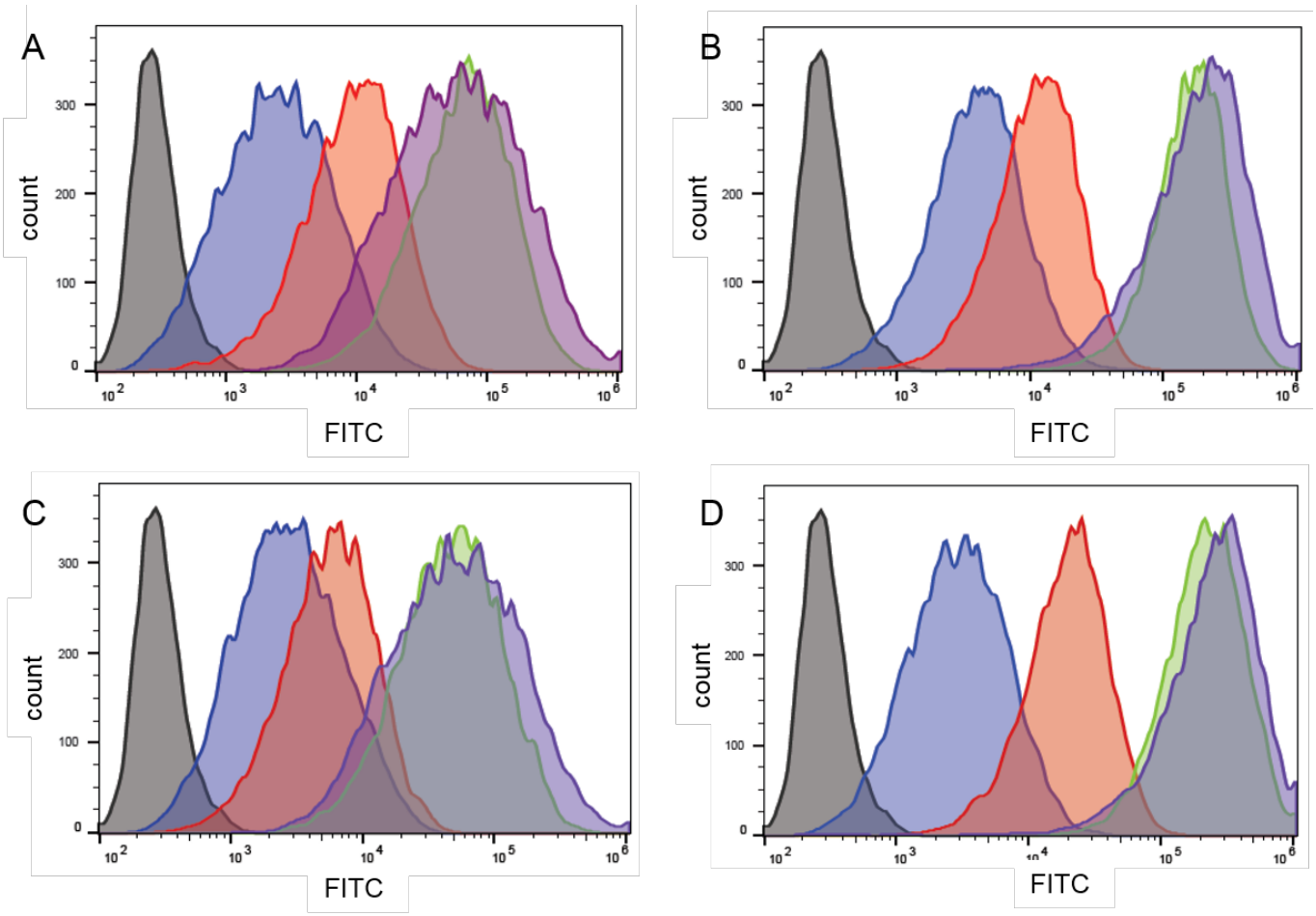

Figure S2. Assessment of binding specificity by flow cytometry. $HER2^+$ SK-BR-3 cells were incubated with 50 mg/mL of A) SiNP, B) SiNP-TZ, C) SiNP-NTA and D) SiNP-NTA-TZ for 20 min (blue), 1 h (red), 4 h (green) and 24 h (violet). Untreated cells were used as negative control (grey) to set the autofluorescence of the cells.

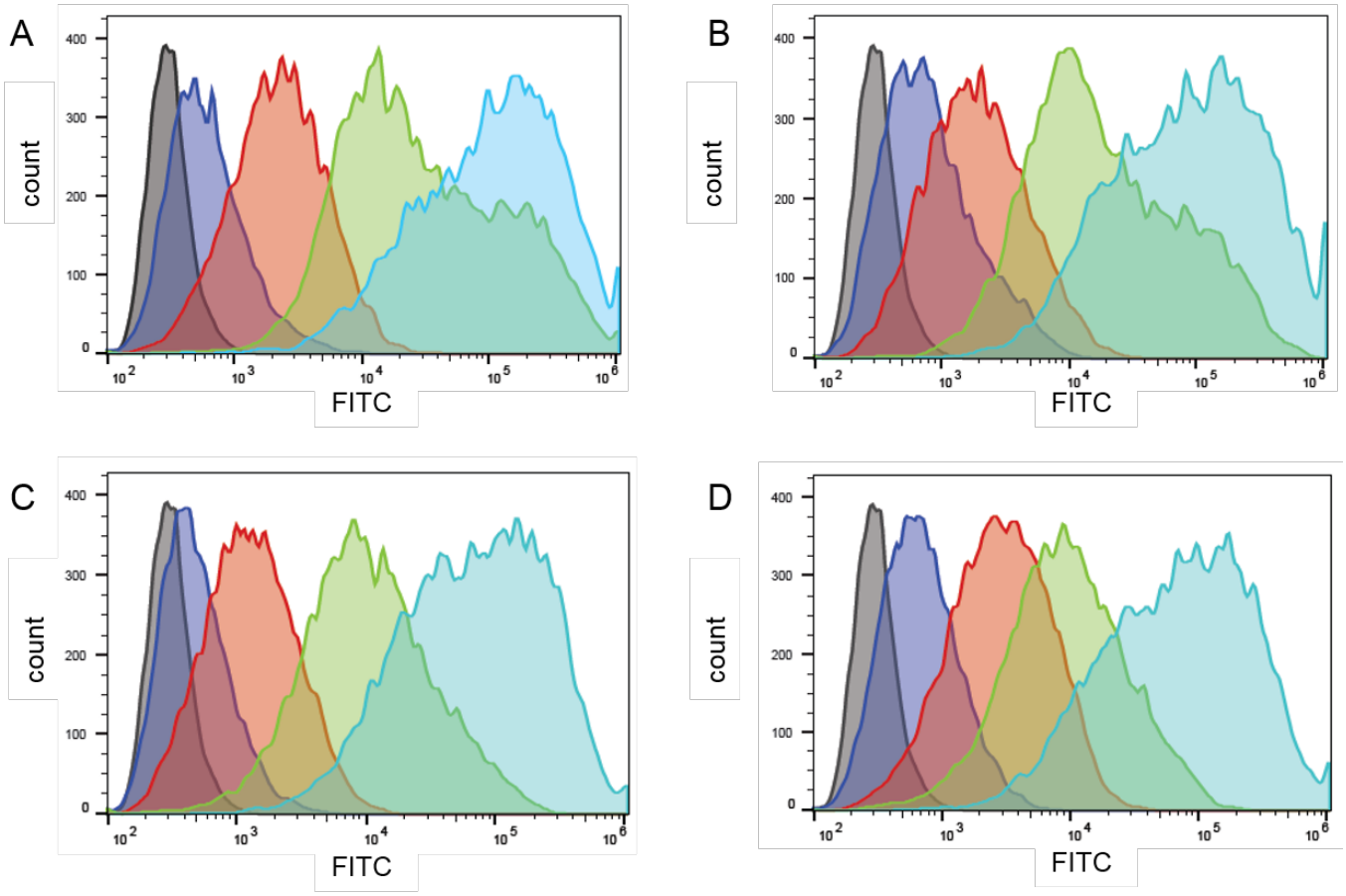

Figure S3. Assessment of binding specificity by flow cytometry. HER2– MDA-MB-468 cells were incubated with 50 mg/mL of A) SiNP, B) SiNP-TZ, C) SiNP-NTA and D) SiNP-NTA-TZ for 20 min (dark blue), 1 h (red), 4 h (green) and 24 h (light blue). Untreated cells were used as negative control (grey) to set the autofluorescence of cells.

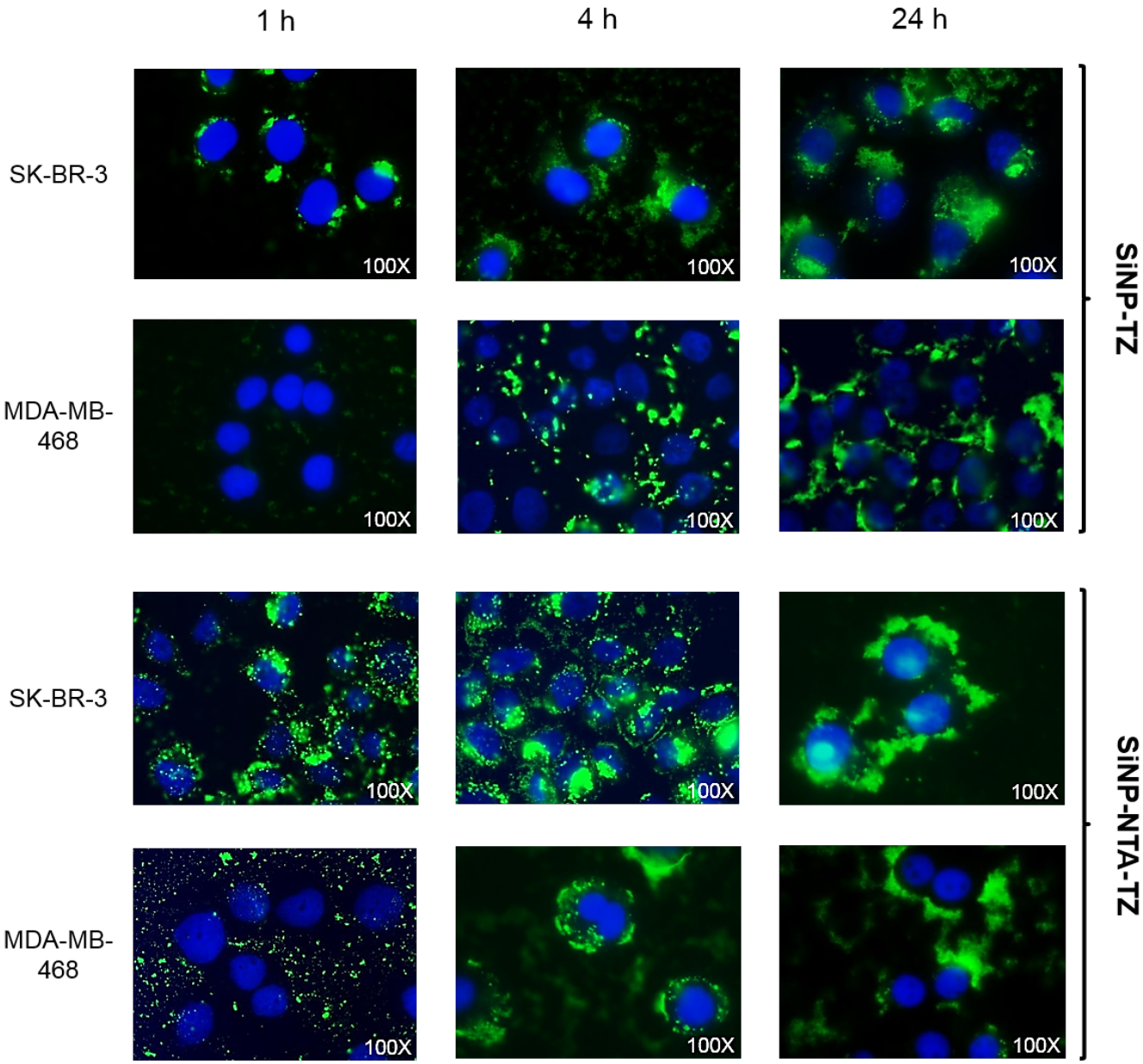

Figure S4. Specificity binding evaluation by fluorescence microscopy. SK-BR-3 and MDA-MB-468 cells were grown on coverslips for 24 h and then exposed for 1, 4 and 24 h to 50 µg/ml of FITC-labeled (green) SiNPs, functionalized with Hc-TZ. Nanoparticles were engineered with or without nitrilotriacetic acid chelating linker (NTA), for evaluate its contribution on targeting capability. Nuclei were stained with Hoechst (blue).

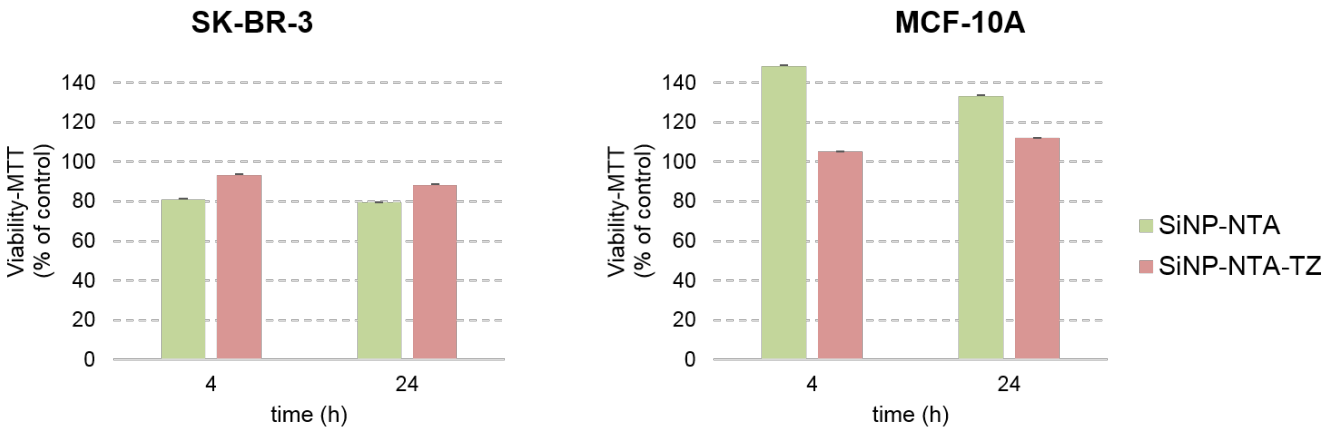

Figure S5. MTT test was replicated at 4 and 24 h in non-transformed mammary epithelial MCF-10A cell line, ($HER2^{-}$) in comparison to SK-BR-3 breast cancer cell line ($HER2^{+}$), incubated with SiNP-NTA and/or SiNP-NTA-TZ.

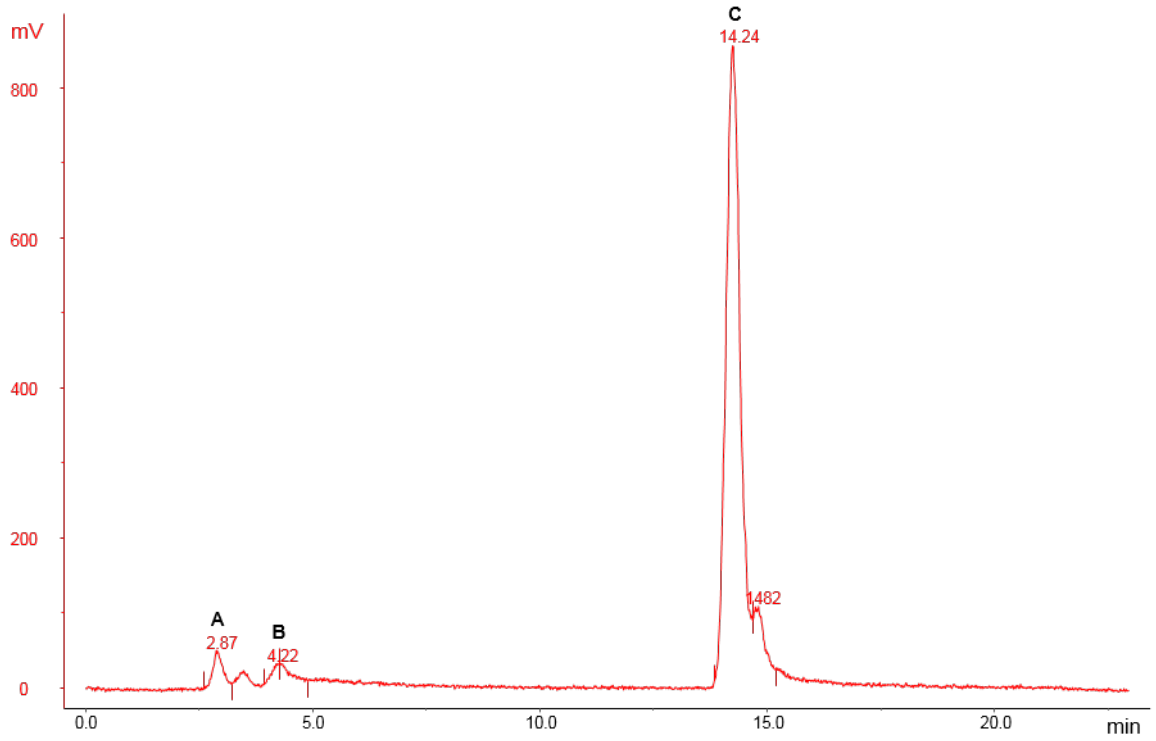

Figure S6. Radiochemical yield of ^{99m}Tc-Tricarbonyl core labeling HisTag, after 50 min incubation: Pertechnetate $[^{99m}TcO_4]^-$ (A); ^{99m}Tc-Tricarbonyl core $[^{99m}Tc(CO)_3]^+$ (B); ^{99m}Tc-Tricarbonyl-HisTag complex (C).

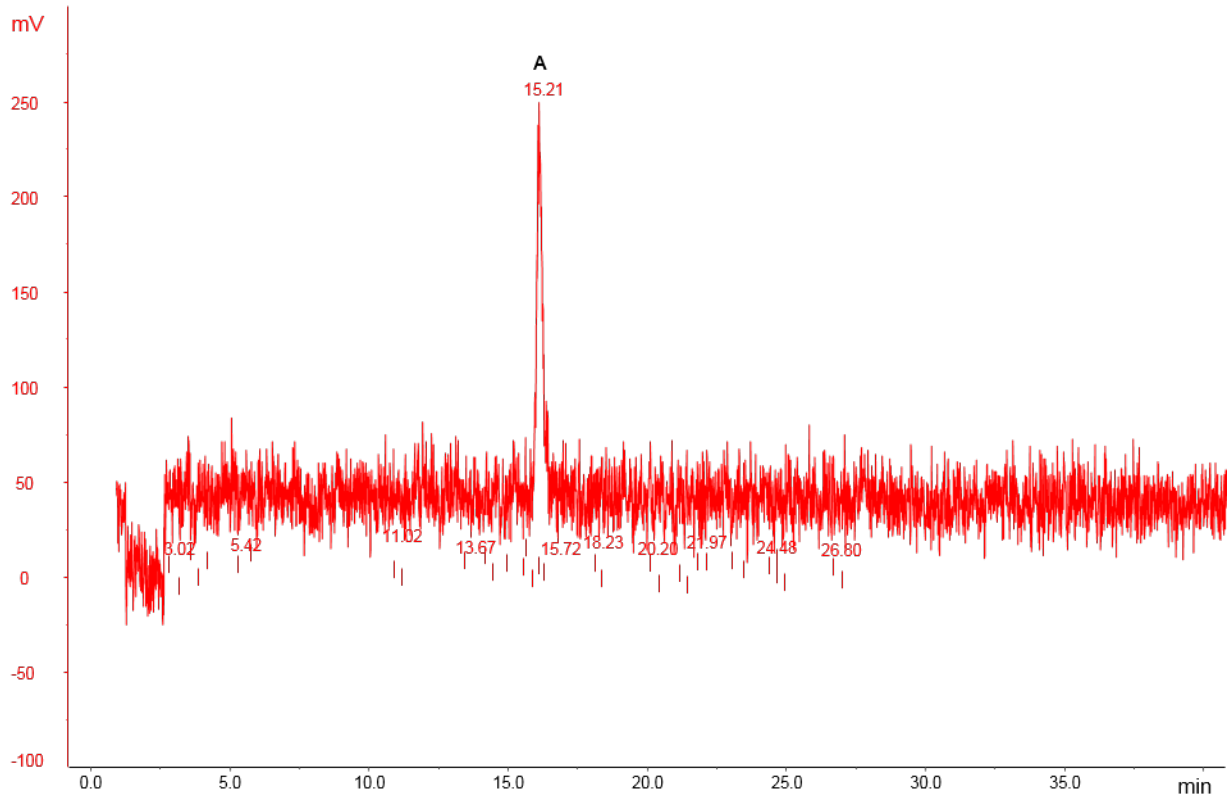

Figure S7. Stability of ^{99m}Tc-Tricarbonyl-HIS-TAG complex after 24 hr EOS, in aqueous solutions over a broad pH range (pH 2-12): ^{99m}Tc-Tricarbonyl-HisTag complex (A).

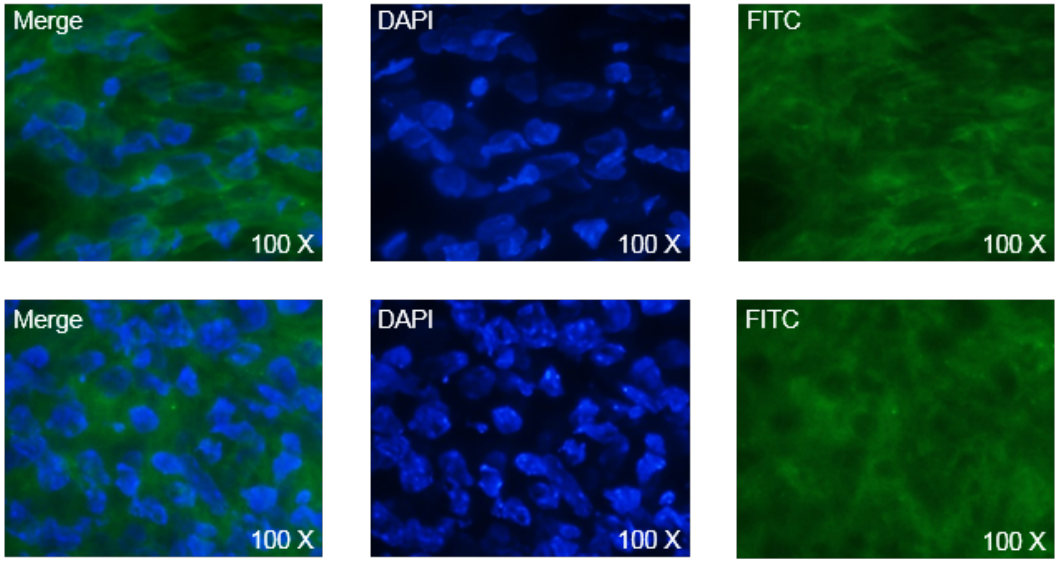

Figure S8. *Ex vivo* fluorescence microscopy acquisition on SK-BR-3 tumor cryosections collected at 24 h post-injection of SiNP-NTA (top) and SiNP-NTA-TZ (bottom). Merge images represent the colocalization of SiNPs (green) and reporting nuclei (blue).

Tissue	At 4 h		At 6 h		At 24 h	
	(n=3)		(n=3)		(n=4)	
(%ID/g)	SiNP-NTA	SiNP-NTA-TZ	SiNP-NTA	SiNP-NTA-TZ	SiNP-NTA	SiNP-NTA-TZ
Blood	0.84 ± 0.14	1.27 ± 0.24	0.88 ± 0.21	1.05 ± 0.15	0.43 ± 0.06	0.52 ± 0.11
Heart	0.32 ± 0.07	0.44 ± 0.01	0.46 ± 0.13	0.33 ± 0.13	0.22 ± 0.02	0.99 ± 1.53
Lung	0.89 ± 0.13	2.31 ± 0.79	1.09 ± 0.12	1.38 ± 0.64	0.47 ± 0.07	0.35 ± 1.28
Liver	58.6 ± 10.9	36.3 ± 7.13*	44.6 ± 34.9	13.2 ± 11.7	7.83 ± 2.08	9.14 ± 5.34
Spleen	6.48 ± 0.49	6.96 ± 1.00	4.98 ± 4.68	2.62 ± 3.68	0.82 ± 0.20	2.42 ± 1.89
Stomach	0.44 ± 0.17	0.46 ± 0.28	0.52 ± 0.21	0.42 ± 0.16	0.33 ± 0.05	0.25 ± 0.12
Kidney	13.3 ± 1.55	5.52 ± 0.04*	51.6 ± 59.8	6.83 ± 1.36	14.3 ± 2.02	5.19 ± 1.57*
Intestine	0.72 ± 0.18	1.44 ± 0.88	1.99 ± 1.22	0.43 ± 0.17	0.35 ± 0.07	0.26 ± 0.06
Tumor	0.52 ± 0.08	0.67 ± 0.30	1.06 ± 0.43	0.35 ± 0.07	0.77 ± 0.28	0.32 ± 0.07
Tiroyd	0.53 ± 0.11	0.67 ± 0.19	0.54 ± 0.12	0.44 ± 0.08	0.40 ± 0.09	0.35 ± 0.11
Muscle	0.31 ± 0.02	0.19 ± 0.03*	0.38 ± 0.29	0.18 ± 0.05	0.26 ± 0.08	0.13 ± 0.03

Table S1. Radioactivity distribution after ^{99m}Tc-labeled SiNP-NTA or SiNP-NTA-TZ injection. Radioactivity concentration is expressed as percentage of injected dose per gram of tissue (%ID/g). Values are expressed as mean ±S.D. of three rats for each time point. (*$p<0.05$ vs. SiNP-NTA)