Supporting Information for:

In Vivo Targeted Cancer Imaging, Sentinel Lymph Node Mapping and Multi-Channel Imaging with Biocompatible Silicon Nanocrystals

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Hydrodynamic diameter measurement by dynamic light scattering:

TEM of formaldehyde-fixed micelle-encapsulated Si QDs showed spherical aggregates of crystalline particles with 50 to 120 nm overall diameter. The hydrodynamic diameter measured by dynamic light scattering was 60 to 160 nm. Figure S1 A shows the MSiQD size distribution from dynamic light scattering (DLS) measurements. The particles have a mean hydrodynamic radius of 50 nm. The formulations used for most experiments were in this radius range. Figure S1 B shows the size distribution of MSiQD-cRGD particles used for tumor targeting. The particles in this particular sample have a mean hydrodynamic radius of 55 nm. Figure S1 C shows the particle size distribution in a sample from which larger particles have been centrifuged out, for use in the SLN mapping experiments. These particles have a mean radius of 15 nm. The smaller particles are more effective for lymph node mapping.

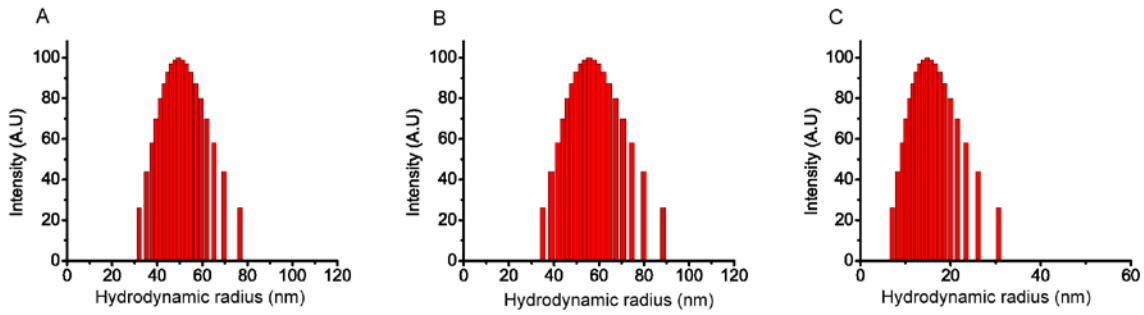

Figure S1. Dynamic light scattering (DLS)-based size distribution of micelle encapsulated silicon quantum dots used for sentinel lymph node mapping.

Si-QD absorbance spectra:

Absorbance spectra were measured for particles with peak emission at 590 nm, 630 nm and 725 nm and plotted to estimate the band gap. The indirect band gaps estimated by absorbance were 3.5 eV, 3.09 eV and 2.55 eV, respectively. The shift in

band gap based on absorbance corresponds to the shift in emission wavelength, but the emission energies are significantly lower (2.1, 2.0, and 1.7 eV).

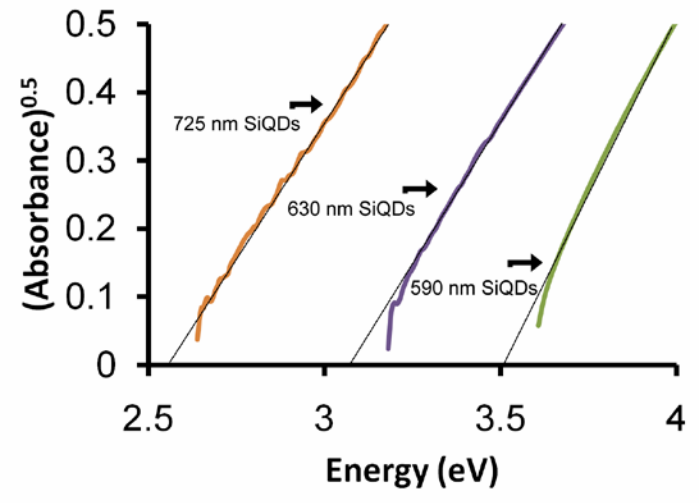

Figure S2. Absorbance of Si QDs of different sizes, plotted such that the intersection with the horizontal axis gives the approximate band gap.

All Si nanocrystal formulations exhibit minimal cytotoxicity in vitro

A variety of formulations of Si QDs are of interest for bioimaging, particularly targeted imaging where surface functional groups are needed for attachment of targeting ligands. It would not be practical or humane to conduct in vivo studies for all of these formulations at the present time. Instead, the in vitro cell viability (MTS) assay was used to evaluate the cytotoxicity of various types of Si QDs for a particular cell line of interest. Cytotoxicity studies of CdSe, CdTe, and CdSe/ZnS QDs with different sizes and surface coatings are well documented in the literature(1-2). However, similar studies have not been performed for silicon nanoparticles, possibly due to the challenges in fabricating high quality Si QDs. Figure S3 shows the in vitro cytotoxicity effects of

un-etched silicon particles and etched silicon particles of different sizes on human pancreatic cancer (Panc-1) cell line at 24 and 48 hours post-treatment. The average diameter of the un-etched particles is estimated to be 10 nm. Four sizes of etched particles were used, with estimated average diameters of 2, 2.5, 3 and 4 nm, respectively. These particles are hereafter referred to as green, yellow, orange, and red emitting Si QDs. Figure S3 shows that cells treated with both un-etched and etched silicon particles maintained greater than 80% viability even at particle concentration as high as 70 µg/mL. Below this concentration, cellular metabolic activity did not differ significantly from the control experiment. This low cytotoxicity suggests that one can use concentrations of these Si nanocrystals up to 70 µg/mL for in vitro studies.

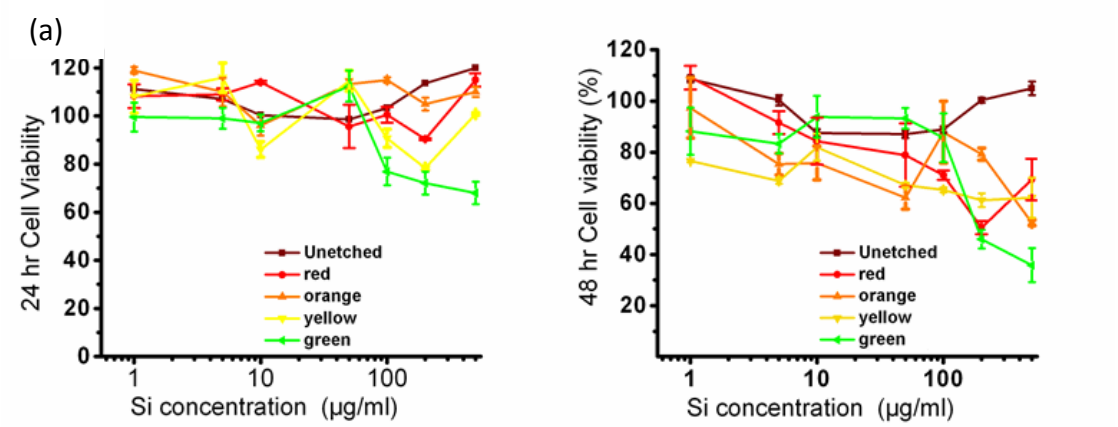

Figure S3. Cytotoxicity data for Panc-1 cells treated with "bare" unetched silicon quantum dots and etched silicon quantum dots. MTS assays illustrating percentage cell viability (relative to nontreated cells being arbitrarily assigned 100% viability) upon exposing the cells to different concentrations of the silicon particles for (a) 24 and (b) 48 hours.

Recently, we have reported the preparation of water-dispersible and biocompatible Si nanocrystals by encapsulating them in phospholipid micelles(3). These nanoparticles were observed to maintain their photoluminescence for as long as several months. Phospholipid micelles were used to enable stable dispersion of Si nanocrystals in water,

creating a hydrophilic shell with PEG groups protruding out from the particle surface. Previously, it was reported that micelle-encapsulated CdSe/ZnS QDs, with a single QD per micelle, can be prepared with a size of 20 nm(4). In comparison, our methods resulted in larger micelles where each micelle contains many QDs, probably with 20 to 50 QDs per micelle. Multiple QDs in a micelle may provide for more sensitive detection in biological systems than single QDs would, thus increasing the sensitivity for in vitro and in vivo imaging. We have previously demonstrated that these bioconjugates can be used as contrast agents for labeling cells, and have demonstrated their use for in vivo imaging above. Figure S4 shows fluorescence microscopy images (Nuance, CRI, 400 nm short-pass excitation filter, 600 nm long-pass emission filter) of RAW macrophage cells labeled with phospholipid micelle-encapsulated Si QDs, with the QDs primarily distributed in the cytoplasm. Local spectral analysis of the overall cell staining by the particles confirms that the luminescence signal is indeed from the Si QDs. It is worth mentioning that no significant nuclear damage was observed upon treatment with the QDs. This result further confirms their non-toxicity and ability to serve as probes for biomedical applications.

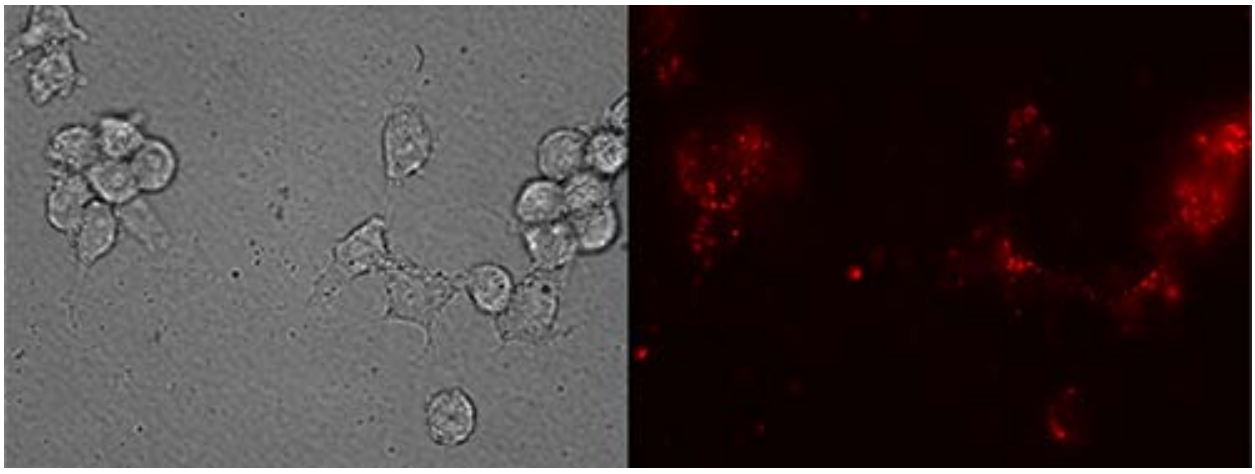

Figure S4. Confocal image of RAW cells treated with phospholipid-PEG micelle-encapsulated Si QDs. The panel on the left displays the transmission images, and the corresponding fluorescence image is shown in the right panel.

To probe the potential toxicity of the micelle-encapsulated Si QDs with different surface chemistry on the micelle exterior, MTS assays were again carried out. In this study, the potential cytotoxic effects of amine, carboxyl, folate and transferrin-functionalized phospholipid-PEG encapsulated Si QDs were systematically investigated. The basic rationale for choosing these four types of functionalized micelle-encapsulated Si QDs for this study is because these terminal functional groups are commonly used for bioconjugation and receptor mediated targeting purposes. Overall, amine, carboxyl, folate and transferrin-functionalized phospholipid-PEG encapsulated red emitting Si QDs revealed no cytotoxic effects to the treated cells at concentrations of 100 μg/mL for both 24 and 48 hours post-treatment, as shown in Figure S5. However, the metabolic activity of the cells was affected when these concentrations were higher than 110 μg/mL, and the cell viability values reached 70% for 24 hours and 60% for 48 hours of treatment when compared to non-treated controls. The low cytotoxicity of the particles was attributed to the effective protection of hydrophobic Si QDs by the biocompatible micelle encapsulation as well as the inherent biocompatibility of silicon.

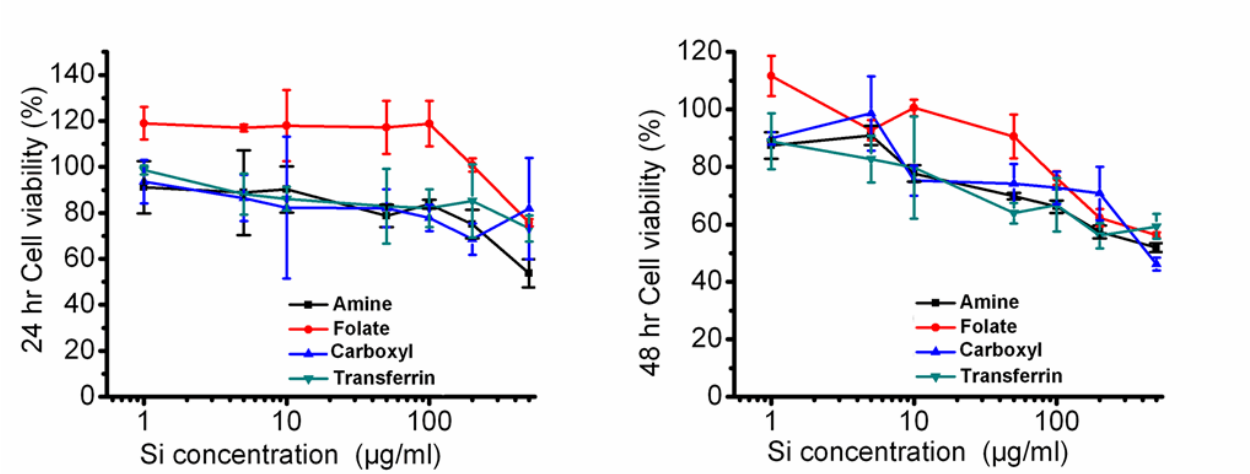

Figure S5. Cytotoxicity studies of Panc-1 cells treated with phospholipid-PEG micelle-encapsulated silicon quantum dots with different functional groups on the micelle exterior. MTS assays illustrating percentage cell viability (relative to non-treated cells assigned 100% viability) upon exposing the cells to different concentrations of micelle-encapsulated silicon quantum dots for (a) 24 and (b) 48 hours. Concentrations are based on the mass of Si, not including the mass of the phospholipid.

Changes in biodistribution for targeted MSiQDs

Figure S6 shows images (ordinary illumination and fluorescence) of organs harvested from tumor xenograft mice (shown in Fig. 2 of the manuscript). These illustrate that the uptake of MSiQD-cRGD in the tumor of the mouse treated with targeted particles was accompanied by a decrease in the concentration of SiQDs in the liver of that mouse, in comparison to the control mouse treated with non-targeted MSiQD, which showed no uptake in the tumor and higher concentration in the liver.

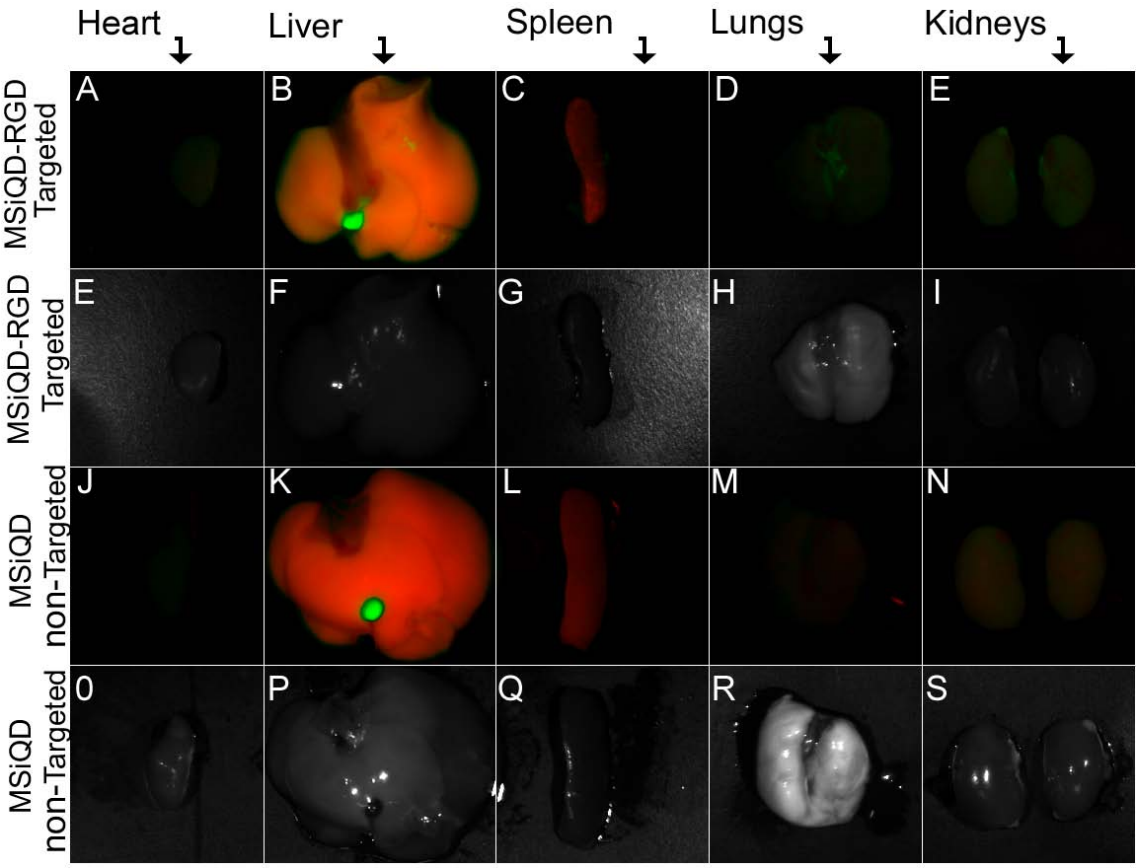

Figure S6. Organ Images of Panc-1 tumor bearing mice injected with targeted and non targeted formulations of silicon quantum dots. Luminescent (A-E) and ordinary illumination (E-I) images of vital organs of a mouse injected with MSiQD-cRGD and sacrificed 40 hours post injection. Luminescent (J-N) and ordinary illumination (0-S) images of vital organs of a mouse injected with methoxy terminated MSiQD and sacrificed 40 hours post injection.

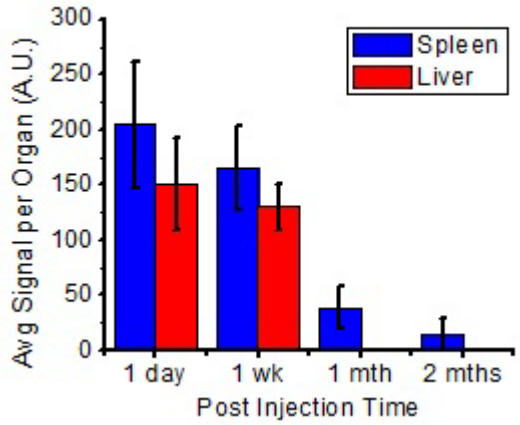

Figure S7. Luminescence clearance from spleen and liver of mice injected with MSiQDS. The graph shows data for up to 2 months post injection. (n=3 for each data point).

References for Supporting Information:

1. Derfus AM, Chan WCW, & Bhatia SN (2004) Probing the Cytotoxicity of Semiconductor Quantum Dots. *Nano Lett.* 4(1):11-18.
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4. Dubertret B*, et al.* (2002) In Vivo Imaging of Quantum Dots Encapsulated in Phospholipid Micelles. *Science* 298(5599):1759-1762.