Supplementary Data

In vivo Multiple Color Lymphatic Imaging Using Upconverting Nanocrystals.

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Supplementary figure 1. Dynamic light scattering (DLS) measurements were performed on a $Delsa^{TM}$ Nano C system (Beckman Coulter). Hexane solutions of nanocrystals with concentration of about 0.2mg/ml were used. DLS data for the as-synthesized nanocrystals confirm the TEM results, showing an average particle size of 20.6-nm, seen in figure 1.

DLS data on the as-injected, functionalized nanocrystal suspension is not currently available, however based on previously published works^{1,2}, we surmise a surface PEG coating of ~15-20-nm in thickness, yielding an approximate final particle size of 50-60-nm in diameter.

In order to eliminate the aggregation or contamination, injected solution was safely passed through 0.22mm-filter (Millipore, Bedford, MA) immediately before injection with minimal loss, which was measured by the spectral absorbance with a UV-Vis system (8453 Value UV-Visible Value System; Agilent Technologies, Santa Clara, CA).

- 1. Z. Liu, C. Davis, W. Cai, L. He, X. Chen, H. Dai. Proc. Natl. Acad. Science, 2008, 105, 1410-1415.
- 2. Torchilin, et.al. Eur J Pharm Biopharm. 2006, 62(3), 235-240.



Supplementary figure 2. (a) Room temperature upconversion emission spectra of NaYF4:Yb/Er (green) and NaYF4:Yb/Tm (blue) nanocrystals under 980nm excitation.
(b) Powder X-ray diffraction pattern of the NaYF4:Yb/Er nanocrystals.



Supplementary figure 3. Comparative single shot fluorescence lymphatic imaging obtained at 550 nm after injection of either the green upconverting nanocrystal (980 nm excitation) or the quantum dot 545 (468 nm excitation). No background is observed with the green upconverting nanocrystal. In contrast, due to the autofluorescence, the body structures are clearly shown in the image obtained with the quantum dot 545.