## **Electronic Supplementary Information**

## Multilayered Semiconducting Polymer Nanoparticles with Enhanced NIR Fluorescence for Molecular Imaging in Cells, Zebrafish and Mice

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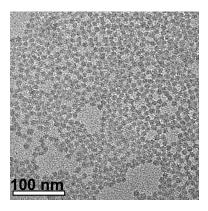
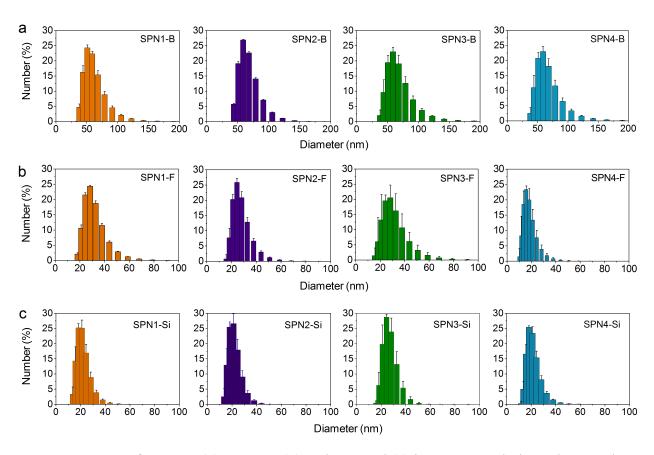
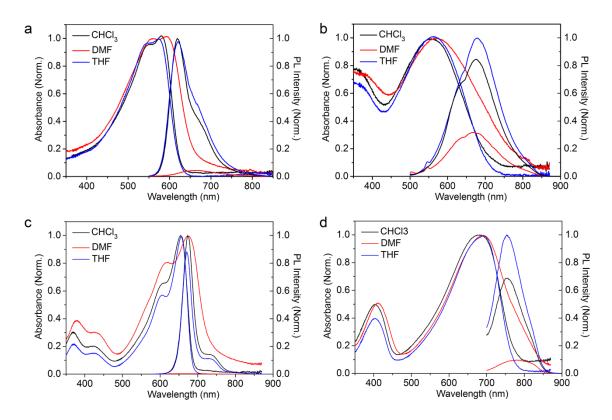


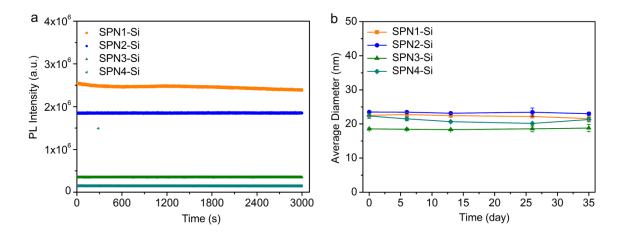
Figure S1. TEM of SPNs-Si: SPN4-Si.



**Figure S2.** DLS of SPNs-B (a), SPNs-F (b) and SPNs-Si (c) in aqueous solution. The error bars represent standard deviations of three separate measurements.

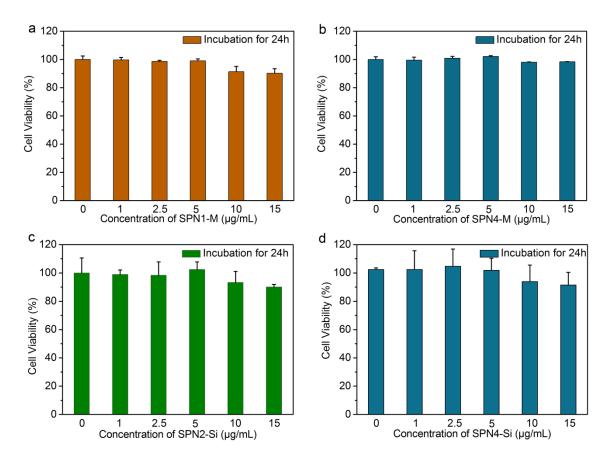


**Figure S3.** UV–Vis absorption and fluorescence spectra of SP1 (**a**), SP2 (**b**), SP3 (**c**), SP4 (**d**) in CHCl<sub>3</sub>, THF and DMF. The concentration of SPs is 1.35, 2.25, 2.82 and 2.1 μg/mL, respectively. The excitation wavelengths are 530, 470, 590, and 620 nm for SP1, SP2, SP3 and SP4, respectively.

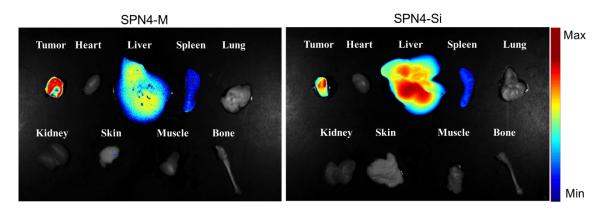


**Figure S4.** (a) Fluorescence intensities of SPNs-Si as a function of time under continuous illumination up to 50 min. (b) The hydrodynamic diameters of SPNs-Si as a function of storage time. The concentrations of SPN1-Si, SPN2-Si, SPN3-Si, SPN4-Si are 1, 1.7, 1.2, and 0.9

μg/mL, respectively.



**Figure S5**. Cell viability of HeLa cells after incubation with SPN1-M (a), SPN4-M (b), SPN2-Si (c), SPN4-Si (d) solution at different concentrations for 24 h.



**Figure S6.** *Ex vivo* fluorescence imaging of major organs and tumor tissues from mice treated with SPN4-M or SPN4-Si. The mice were sacrificed at 24 h post-injection.