Supporting Information for

Tumor-Activatable Clinical Nanoprobe for Cancer Imaging

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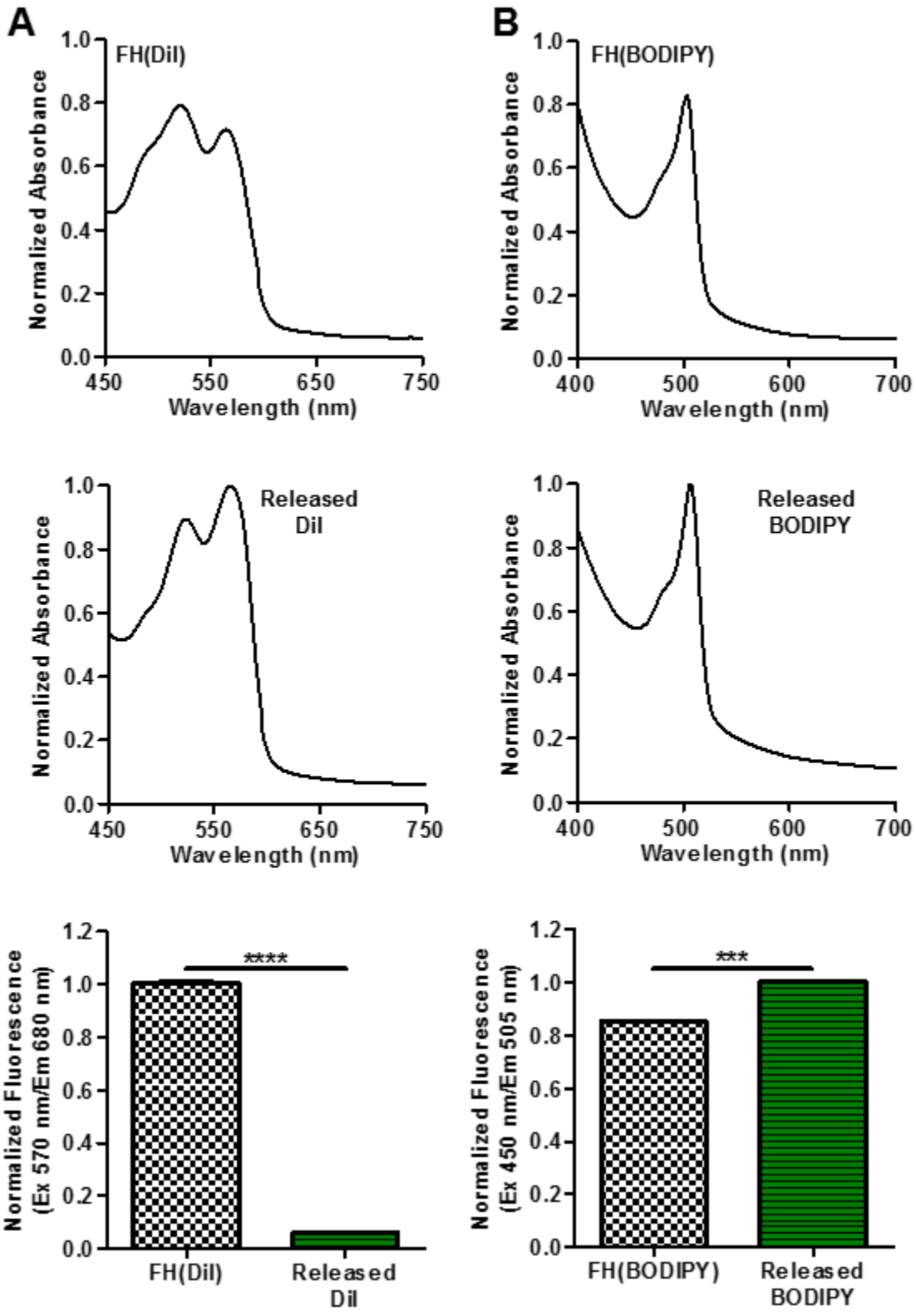

Figure S1. Optical properties of dye-loaded FH and released dyes. Absorbance spectra of entrapped (top) and released (middle) dyes were compared. In addition, the fluorescence of encapsulated and released dyes were compared (bottom). For the absorbance graphs, the maximum absorbance value of released or encapsulated dye was normalized to 1.0, and all other values were adjusted proportionally. For DiI, RhB and BODIPY, absorbance of FH can be observed around 450 nm. For the fluorescence graphs, fluorescence values

were normalized to the largest fluorescence value. Graphs describe behavior of DiI (A), BODIPY (B), RhB (C), IR-820 (D) and ICG (E).

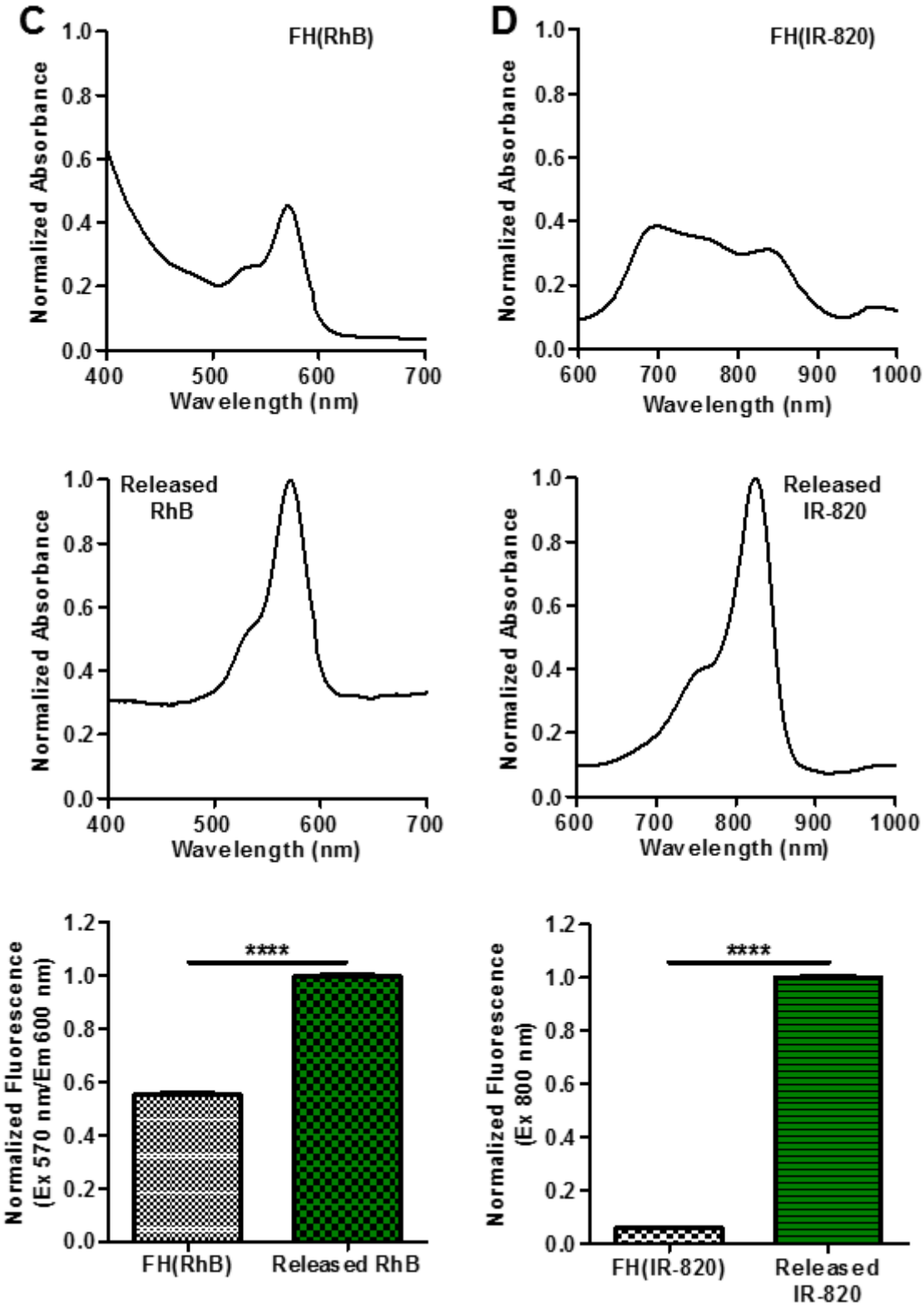

Figure S1 (continued).

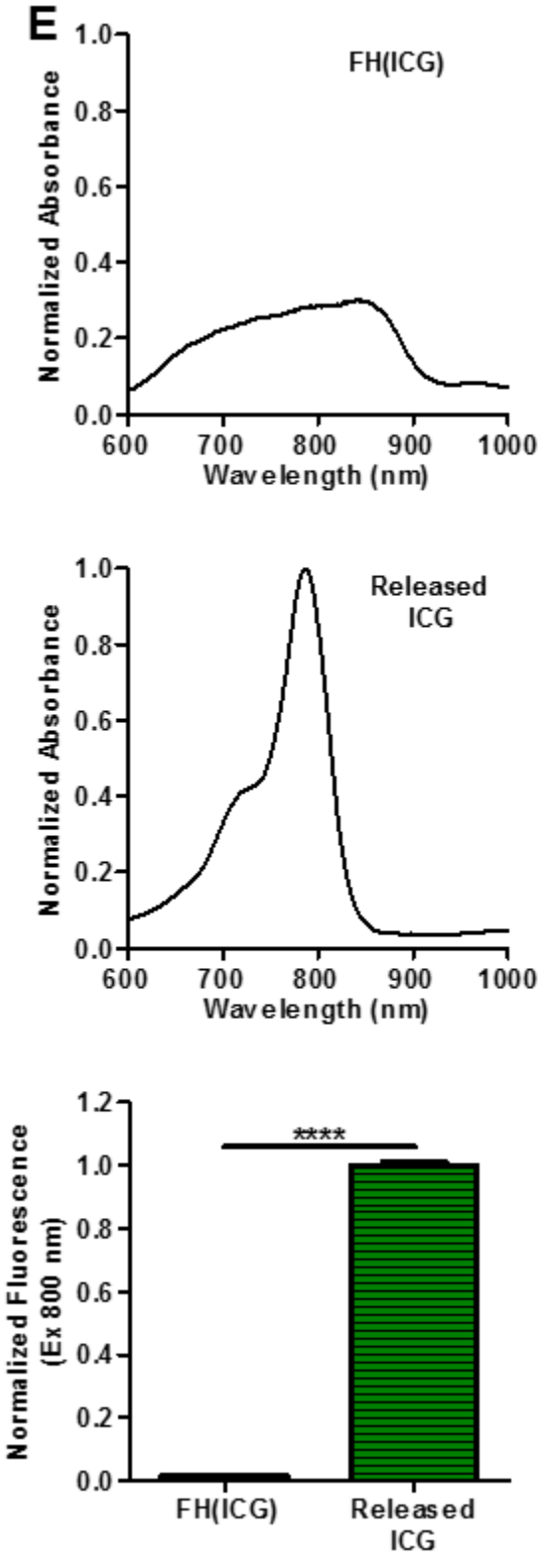

Figure S1 (continued).

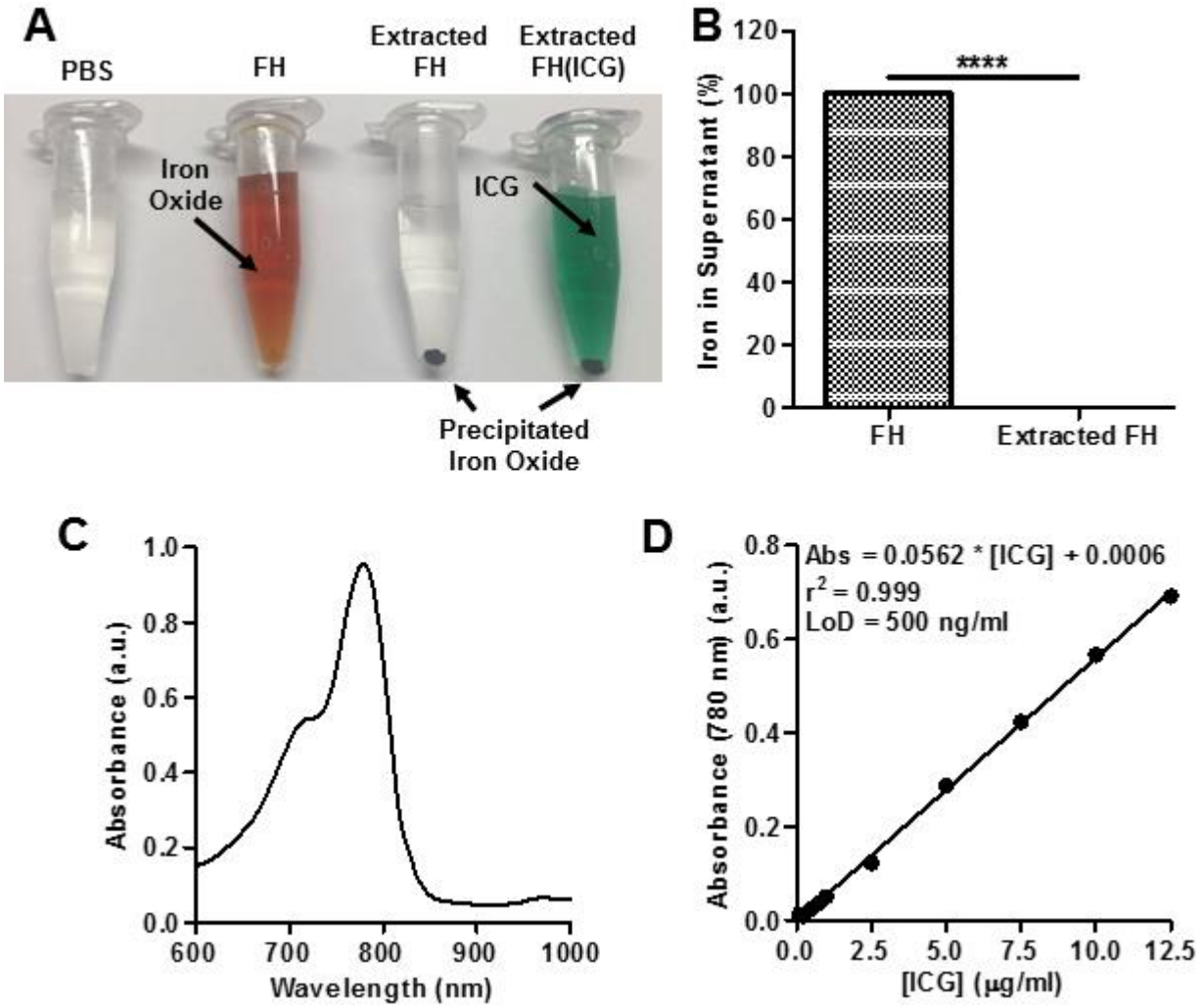

Figure S2. Development of ICG quantification method using iron precipitation. Images of PBS, FH, extracted FH solution and extracted FH(ICG) solution (A). Absorbance-based quantification (450 nm) of iron remaining in supernatant following precipitation (B). Absorbance spectrum of extracted ICG (C) and calibration curve of ICG (D).

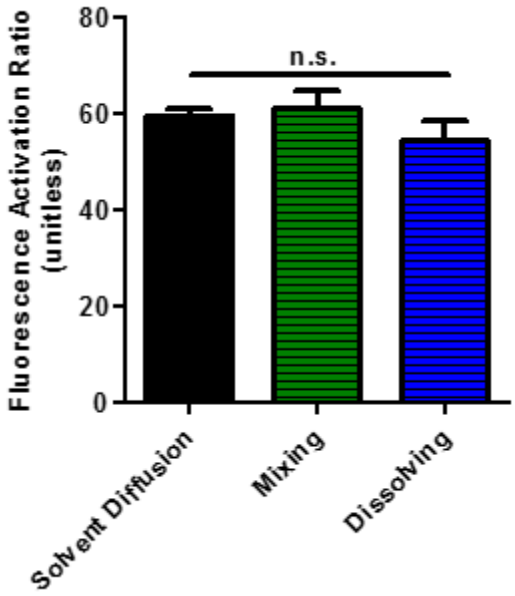

Figure S3. Fluorescence activation ratio comparison between FH(ICG) that was prepared by various methods. FH(ICG) has similar fluorescence activation ratios regardless of the preparation method.

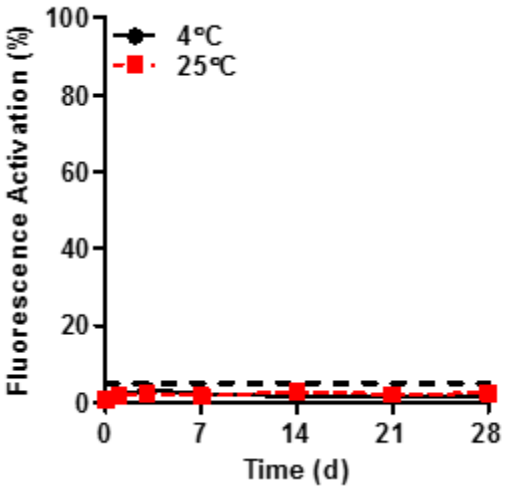

Figure S4. Fluorescence quenching of FH(ICG) during storage. The fluorescence of FH(ICG) was measured over time and normalized to fluorescence of released ICG. The dotted line represents a 5% fluorescence of FH(ICG), which was selected as the threshold for fluorescence activation.

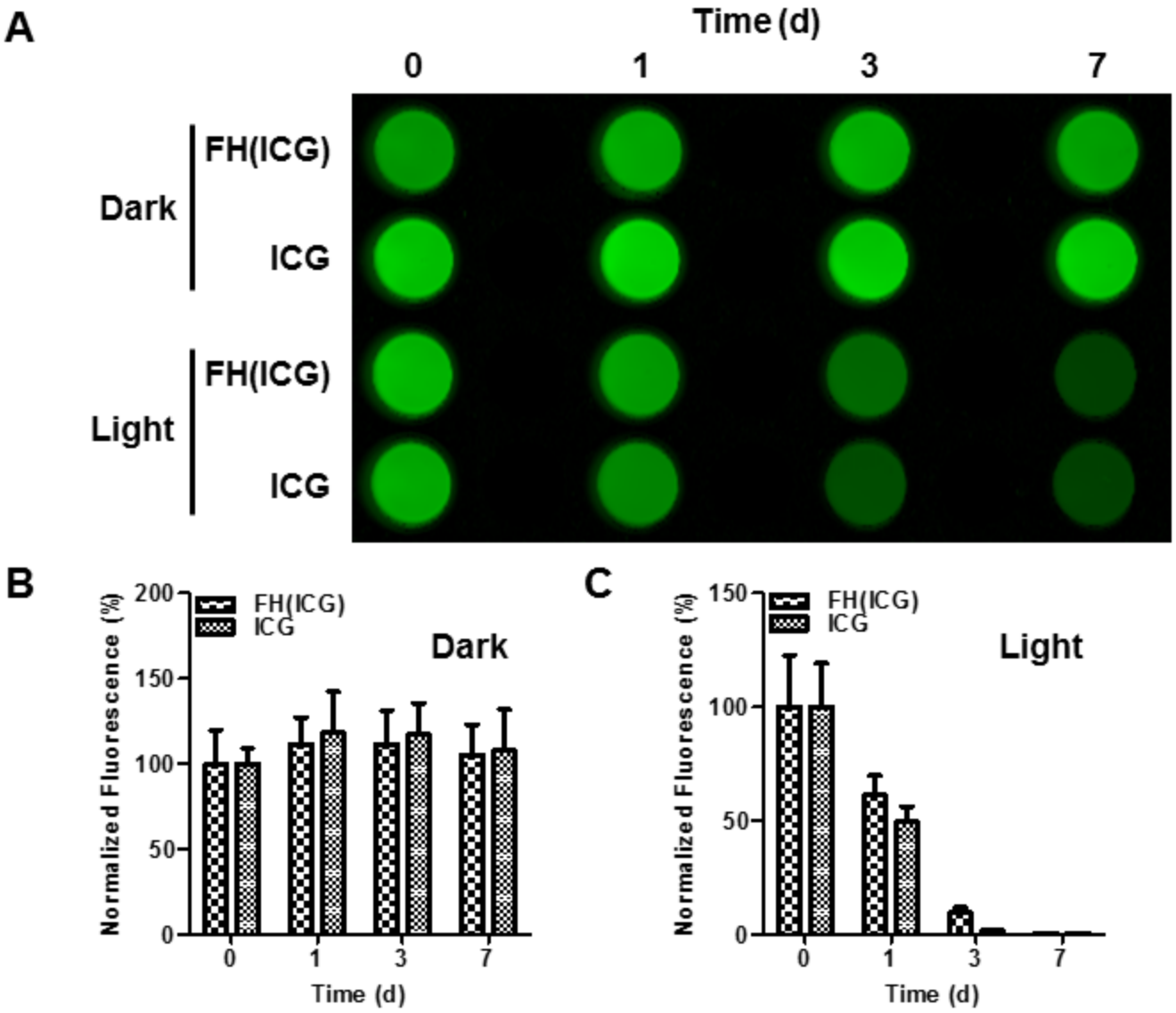

Figure S5. Fluorescence stability of FH(ICG). Fluorescence of FH(ICG) and ICG stored in the dark at 4°C or the light at 25°C (A). For FH(ICG) samples, fluorescence was quantified after diluting the samples with equal volume of DMSO. Quantification of FH(ICG) and ICG fluorescence for dark (B) and light samples (C).

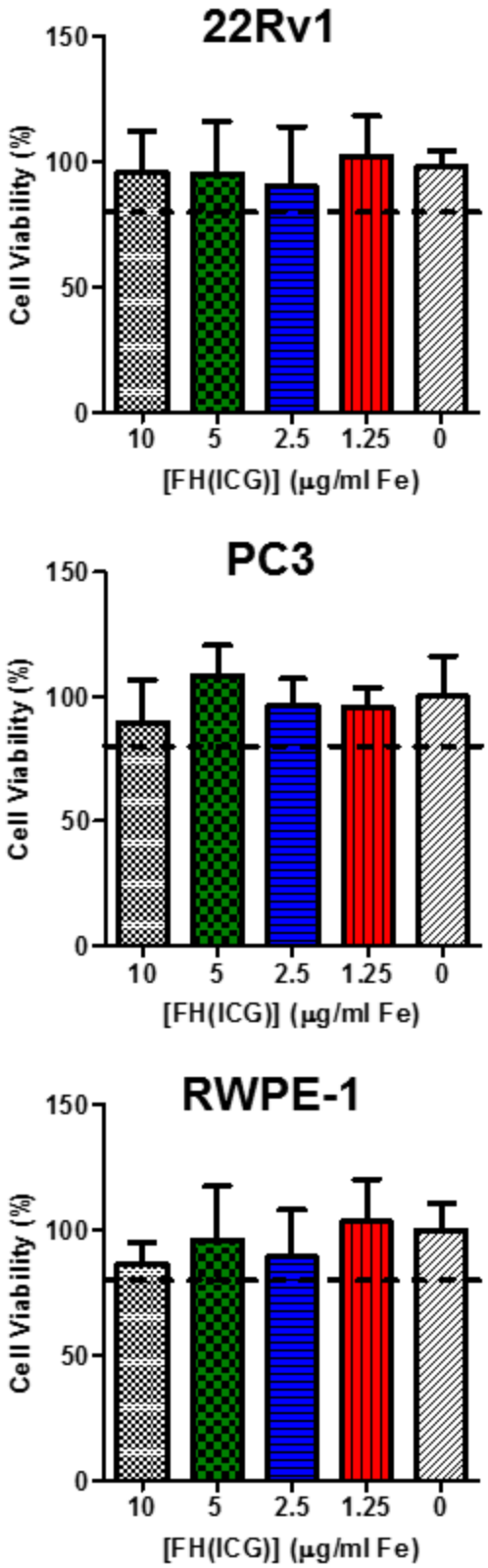

Figure S6. Cell viability of FH(ICG)-treated cells. Cells were treated with FH(ICG) containing 10, 5, 2.5 1.25 or 0 µg/ml iron, and 3.33, 1.67, 0.83, 0.42 or 0 µg/ml ICG, respectively, for 72 h. The dotted line indicates 80% viability, which was selected as a threshold for toxicity.

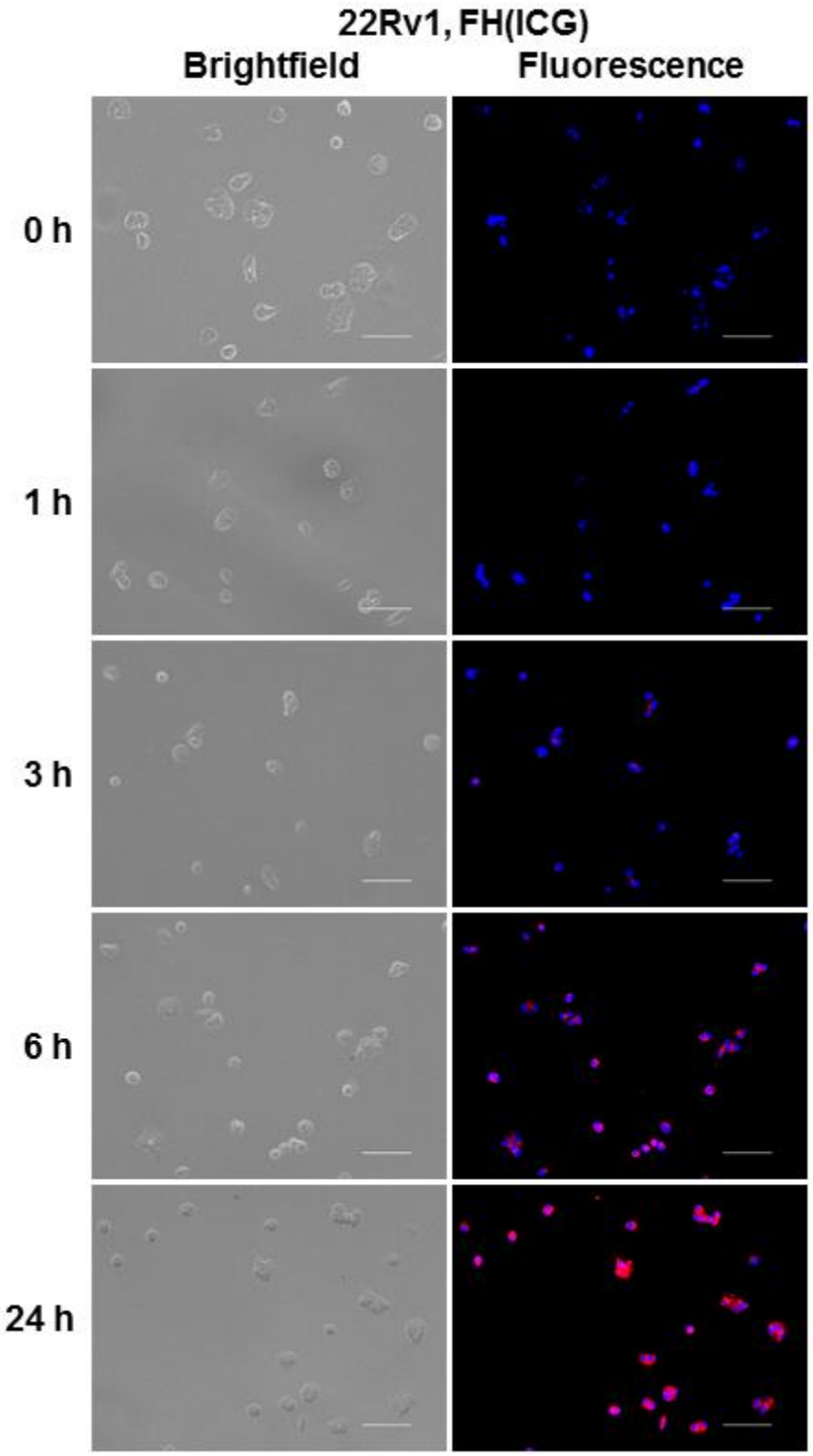

Figure S7. Microscope images of 22Rv1 cells treated with FH(ICG) for various amounts of time. Brightfield and fluorescence images were taken at 20x magnification. One image is shown for each timepoint. In fluorescence images, blue represents nuclei and red represents ICG. Scale bars are 100 μm.

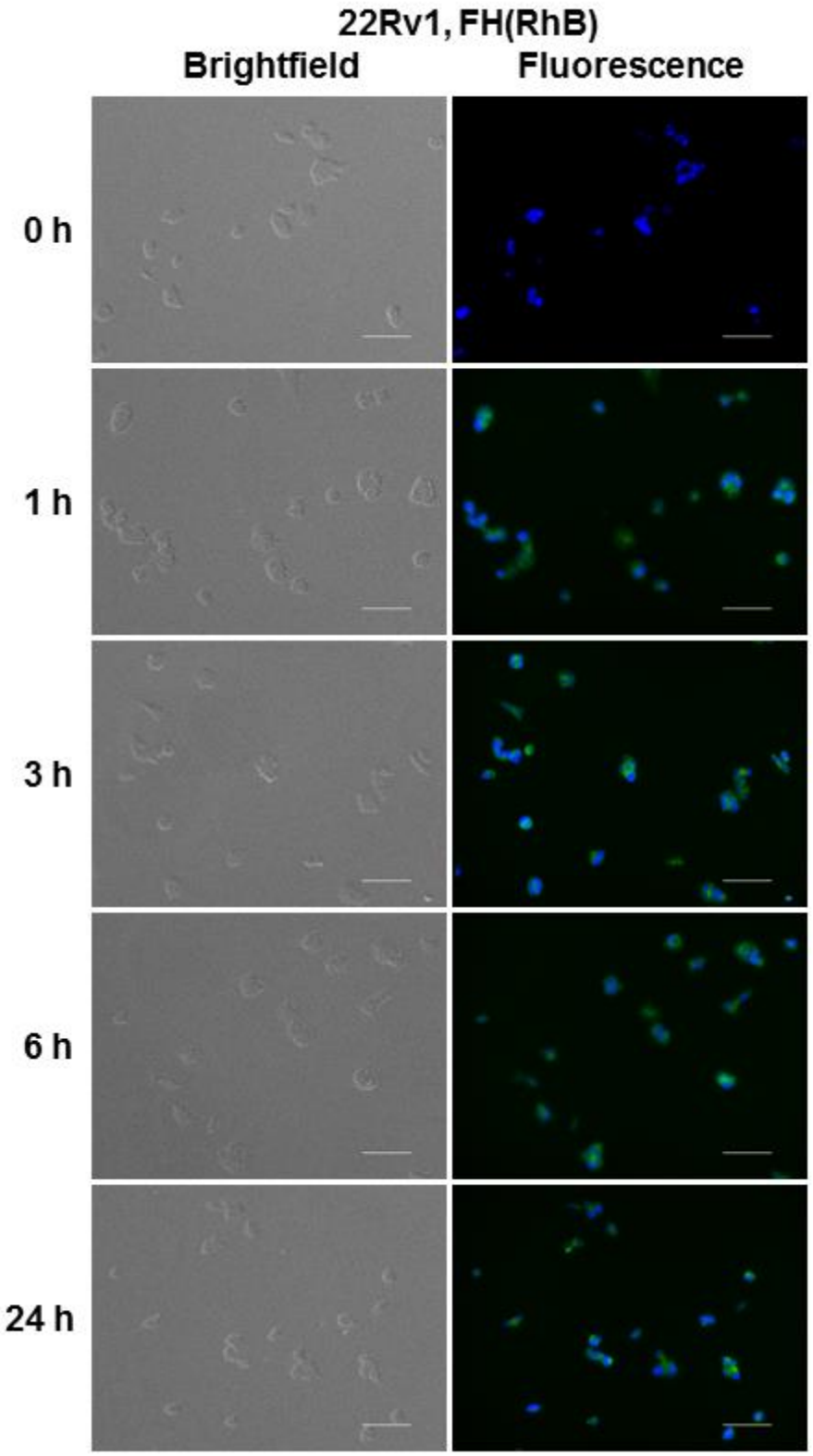

Figure S8. Microscope images of 22Rv1 cells treated with FH(RhB) for various amounts of time. Brightfield and fluorescence images were taken at 20x magnification. One image is shown for each timepoint. In fluorescence images, blue represents nuclei and green represents RhB. Scale bars are 100 μm.

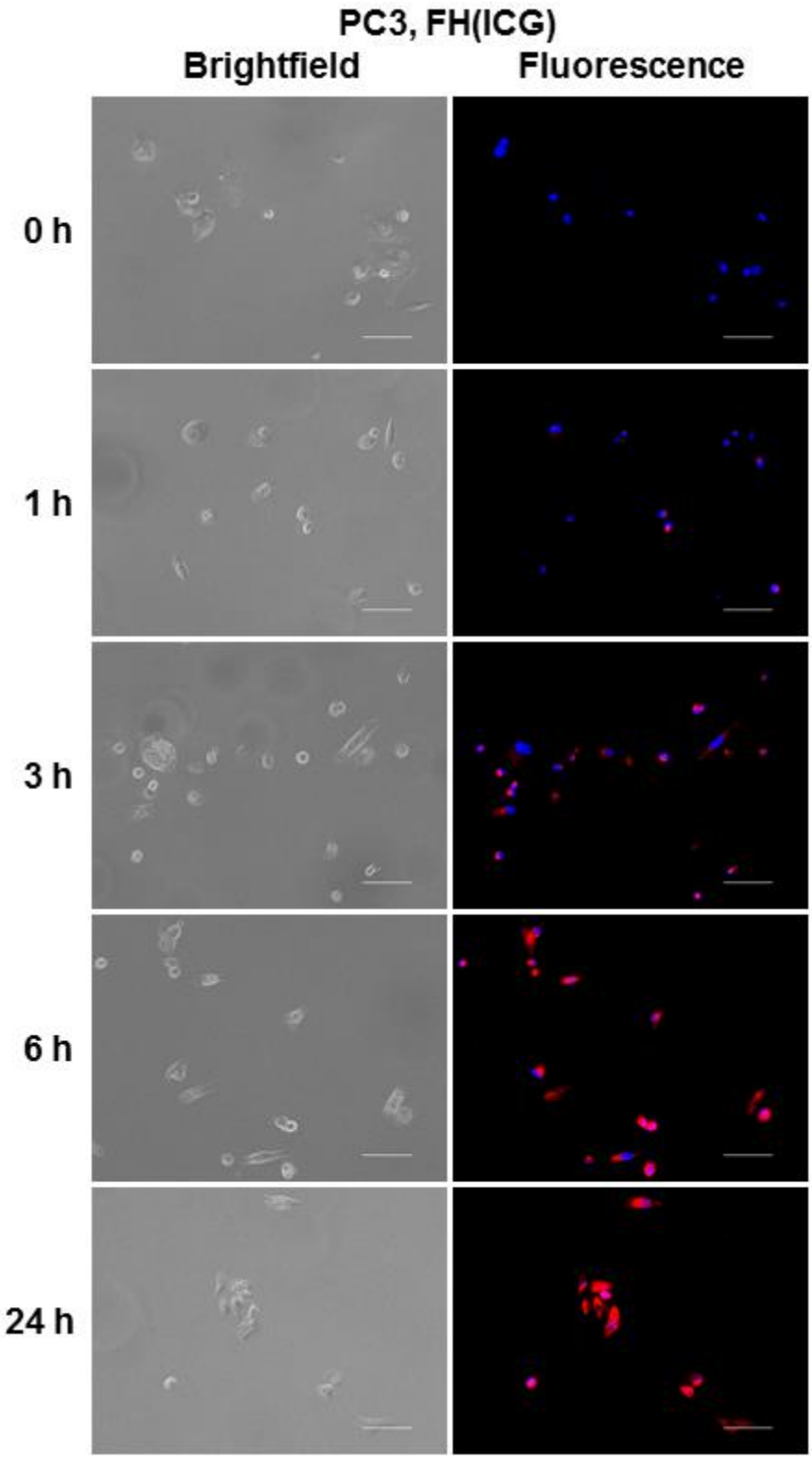

Figure S9. Microscope images of PC3 cells treated with FH(ICG) for various amounts of time. Brightfield and fluorescence images were taken at 20x magnification. One image is shown for each timepoint. In fluorescence images, blue represents nuclei and red represents ICG. Scale bars are 100 μm.

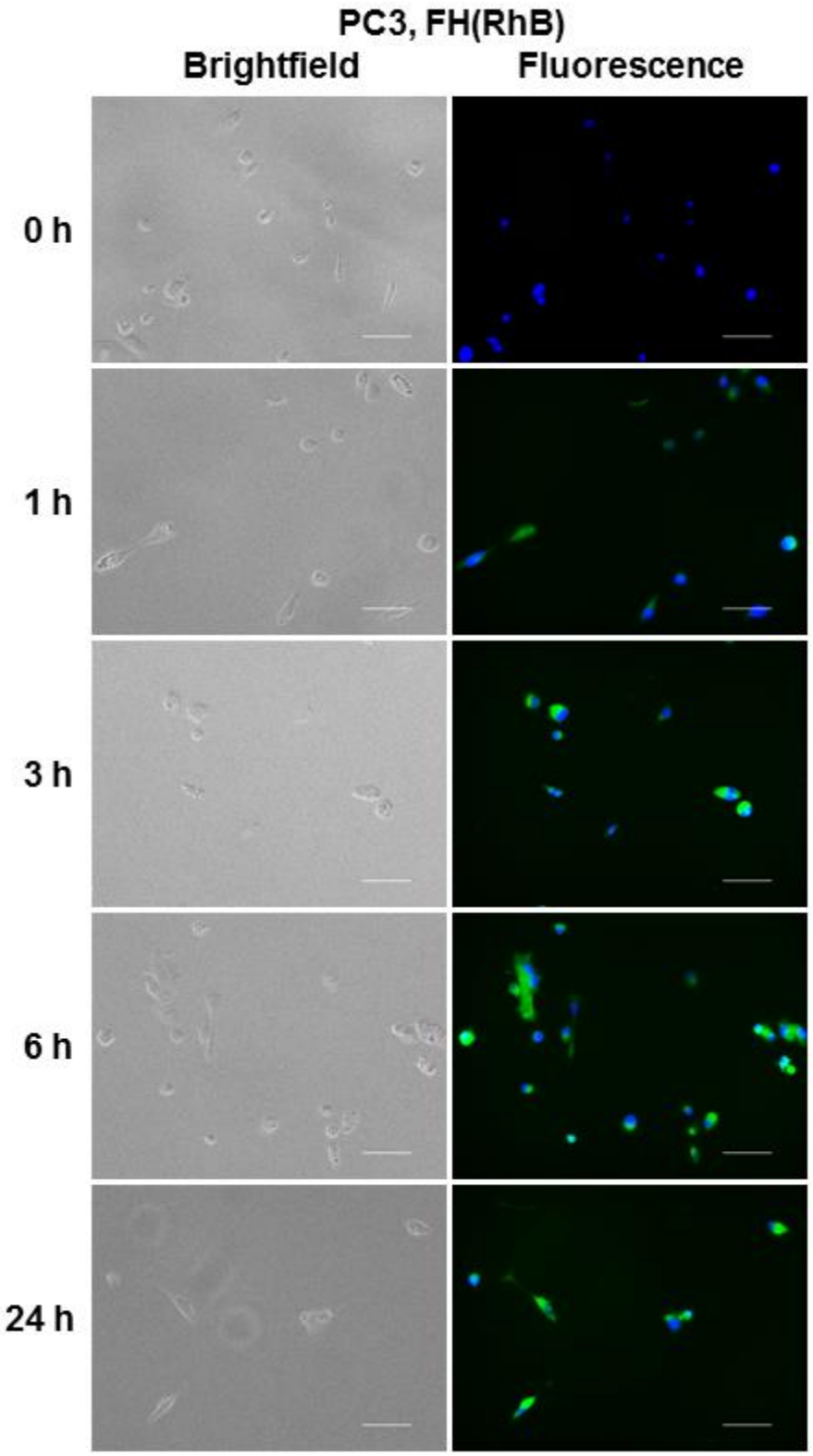

Figure S10. Microscope images of PC3 cells treated with FH(RhB) for various amounts of time. Brightfield and fluorescence images were taken at 20x magnification. One image is shown for each timepoint. In fluorescence images, blue represents nuclei and green represents RhB. Scale bars are 100 μm.

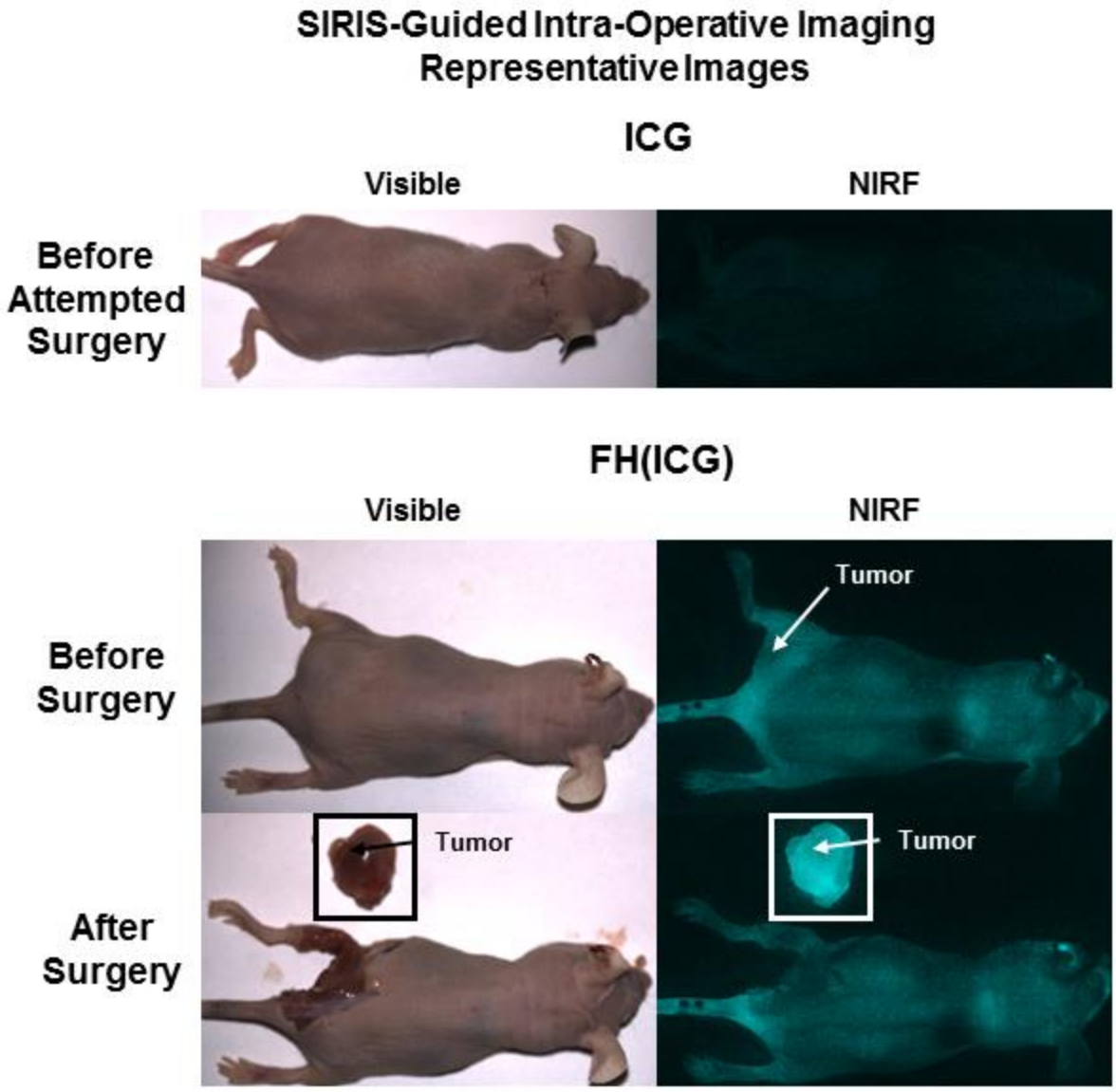

Figure S11. Representative images from SIRIS-guided fluorescence-based mock intraoperative surgery using FH(ICG). Tumor was identified on the left flank of the mouse, and the tumor was removed with guidance from tumor fluorescence. Tumor was identified and removed, and no residual fluorescence remained at the tumor site. Surgery was not performed with ICG because the tumor could not be visualized by fluorescence.