Development of tumor-targeted indocyanine green-loaded Ferritin Nanoparticles for intraoperative detection of cancers

Supporting Information

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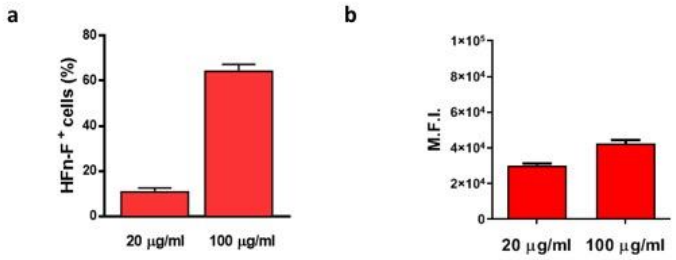

Figure S1: HFn-F binding in MCF-10A cells expressed as % of HFn-F^+ cells (a) and as Mean Fluorescence Intensity (b; M.F.I.) after incubation with 20 and 100 μg/ml of HFn-F.

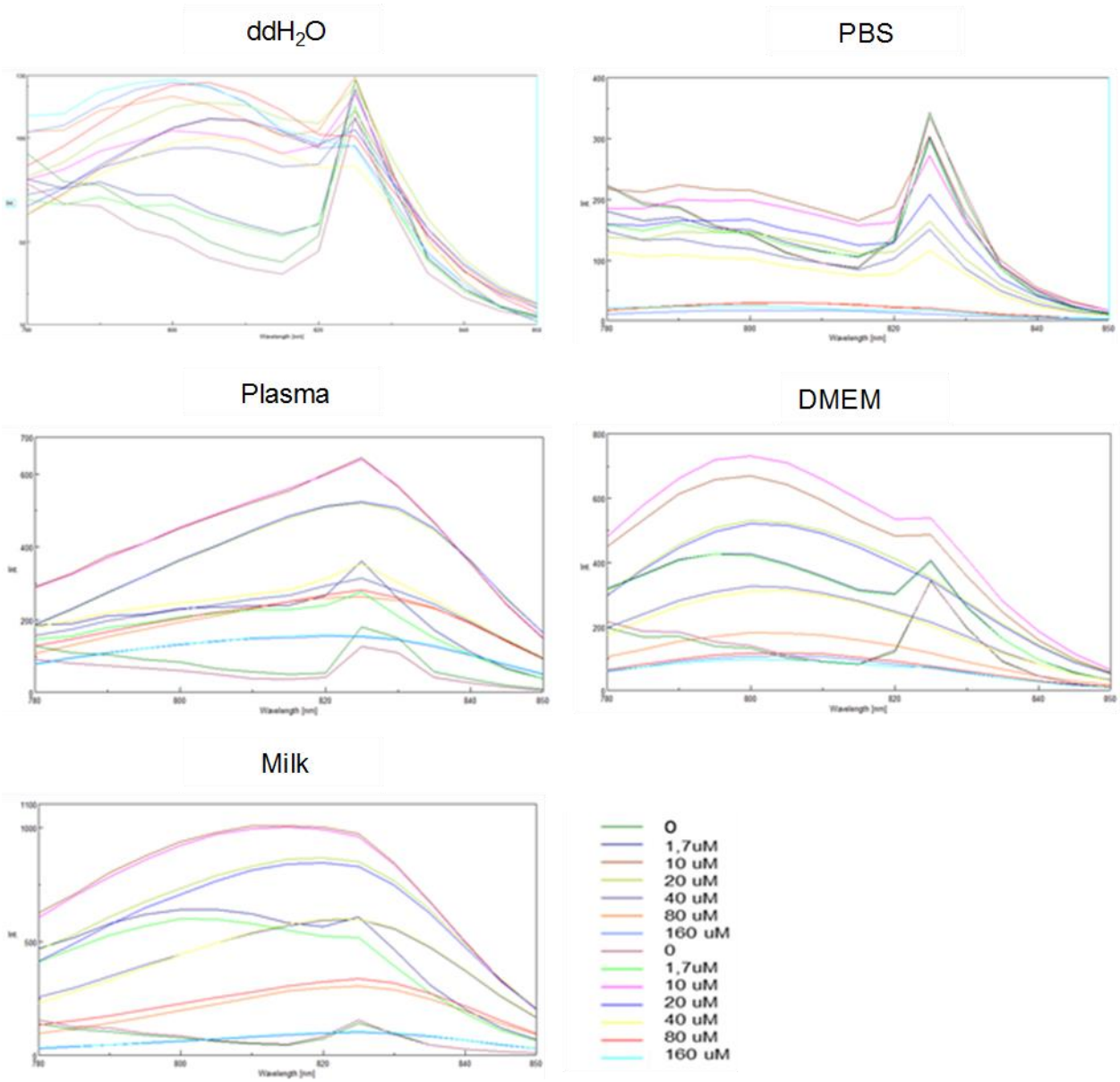

Figure S2. Fluorescence emission of ICG at different concentrations in testing media. The profiles display different behaviors using different media.

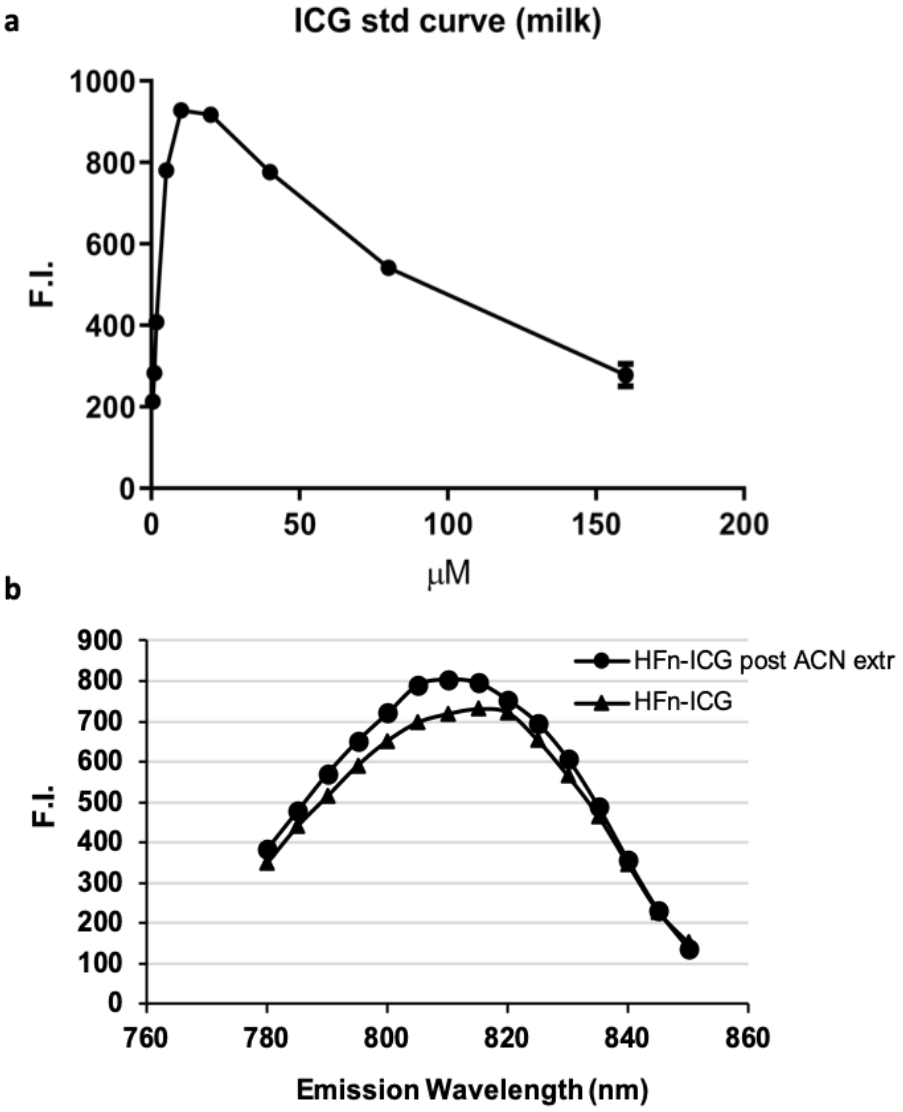

Figure S3. a) Standard fluorescence curve of free ICG diluted in milk used to quantify the encapsulation efficiency in HFn-ICG nanoformulates. Fluorescence of ICG is directly proportional up to a concentration of 10 μM. Then it reaches a plateau and it starts decaying, probably due to the molecule self-quenching. b) Analysis of fluorescence intensity of HFn-ICG after dye extraction with acetonitrile (ACN). The peak shifts from 820 nm to 805 and the fluorescence intensity is increased due to the release of the dye.

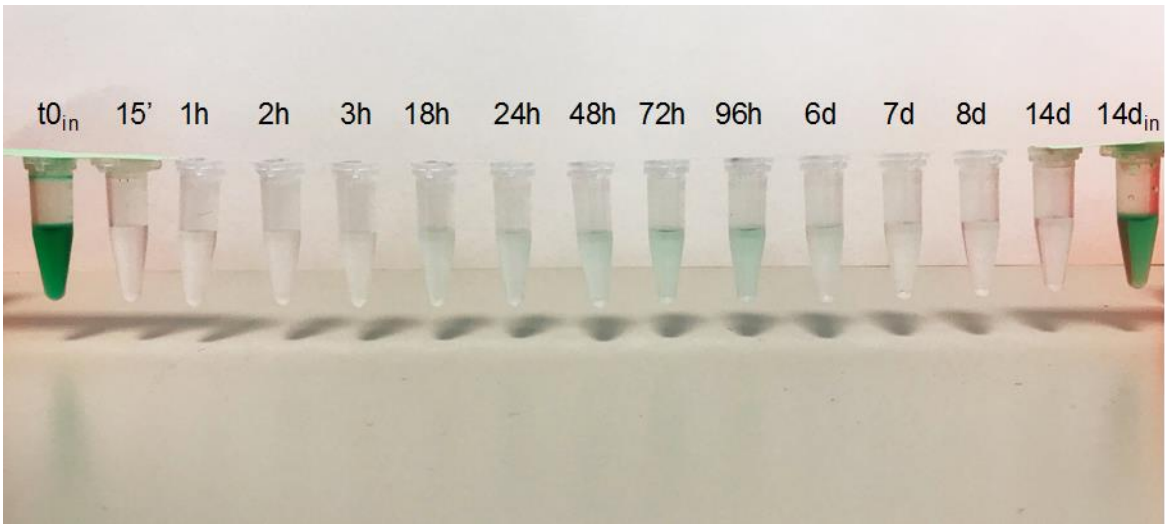

Figure S4. ICG release from HFn-ICG. The release of the dye was evaluated qualitatively by dialysis. A picture of the sample loaded into the dialysis tube was obtained at the beginning of the analysis ($t0_{in}$). This was compared with the samples recovered from outside the membrane at each time point and with the sample collected from inside at the end of the incubation. Photograph courtesy of Sevieri M.

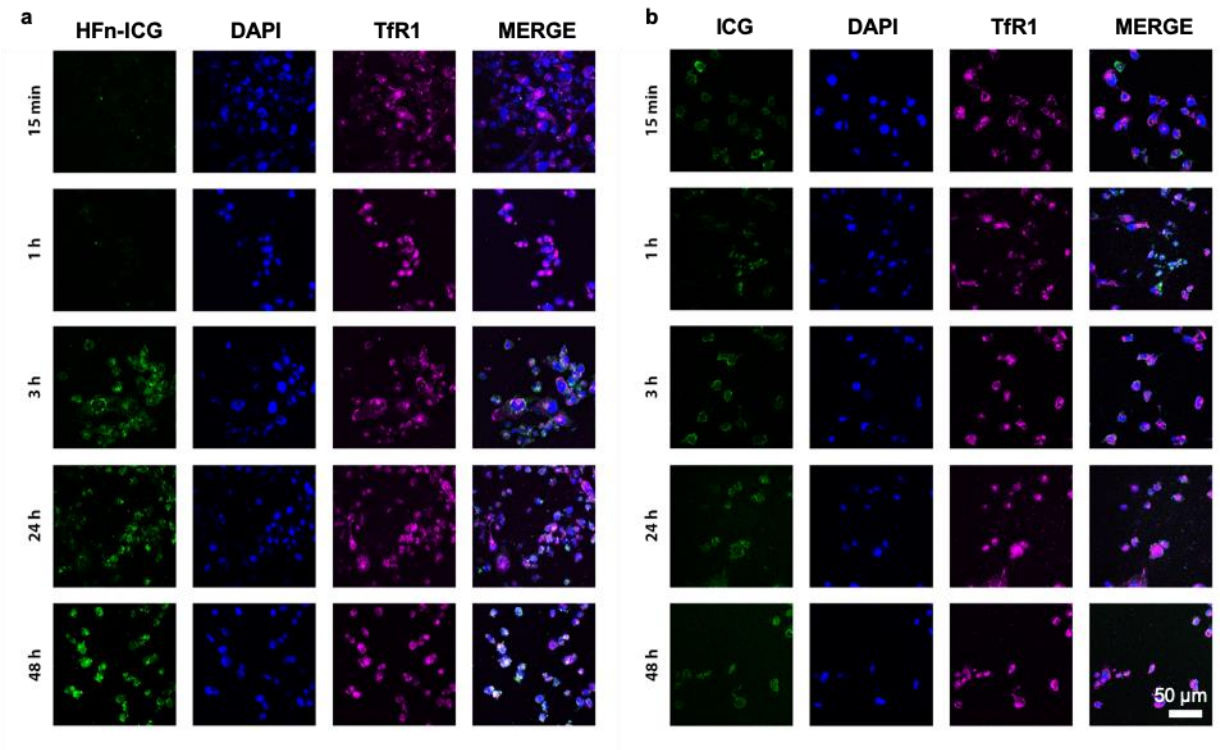

Figure S5. Colocalization of HFn-ICG and TfR1. Confocal images of MDA-MB 231 cells incubated 15 min, 1, 3, 24, 48h at 37 °C in complete cell culture medium with HFn-ICG or free ICG (green; 50 µg/mL; panel a and b, respectively). Nuclei were stained with DAPI (blue). TfR1 was recognized with anti-TfR1 antibody (Abcam) and labelled with an anti-rabbit secondary antibody conjugated with Alexa Fluor 488. Scale bar is valid for all inserts and is equal to 50 µm.

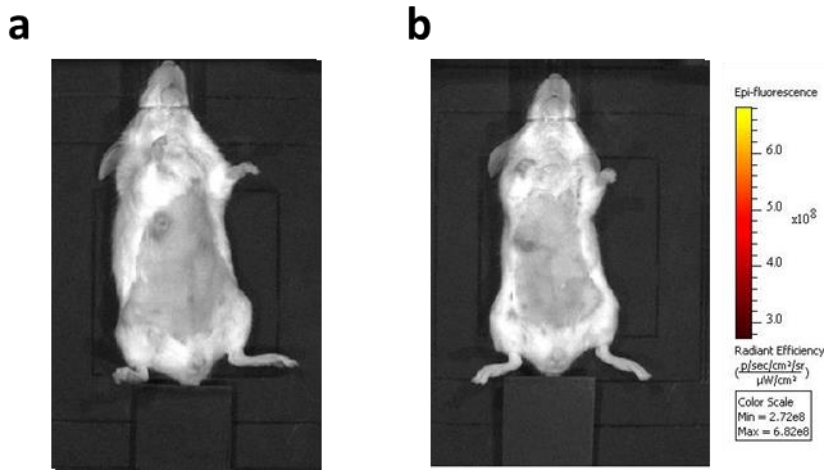

Figure S6. *In vivo* Tumor targeting of HFn-ICG and ICG. The *in vivo* targeting of HFn-ICG (a, and ICG (b) was evaluated in tumor bearing mice, 24h after I.V. administration by IVIS Lumina II. No ICG fluorescence was detected in either HFn-ICG or ICG treated mice. Color scale expressed as total radiant efficiency ($\times 10^8$), n=3.