

## Supporting Information

### **Near-infrared dye IRDye800CW-NHS coupled to Trastuzumab for near-infrared II fluorescence imaging in tumor xenograft models of HER-2-positive breast cancer**

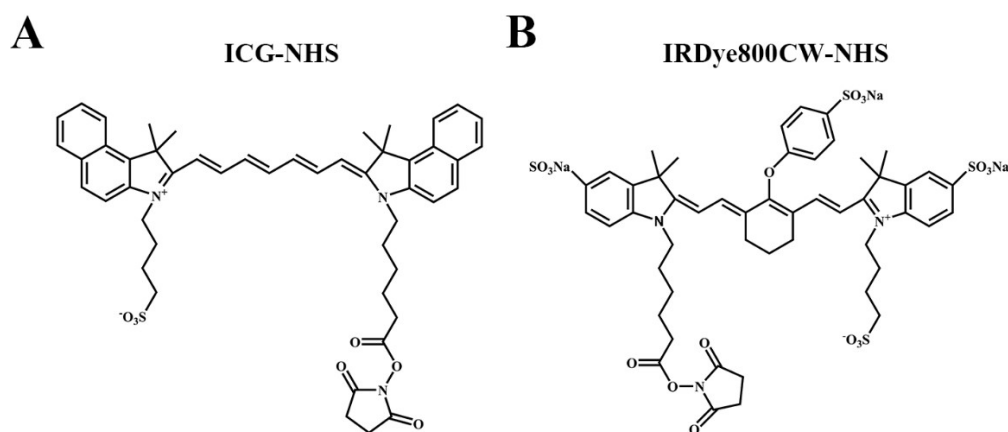
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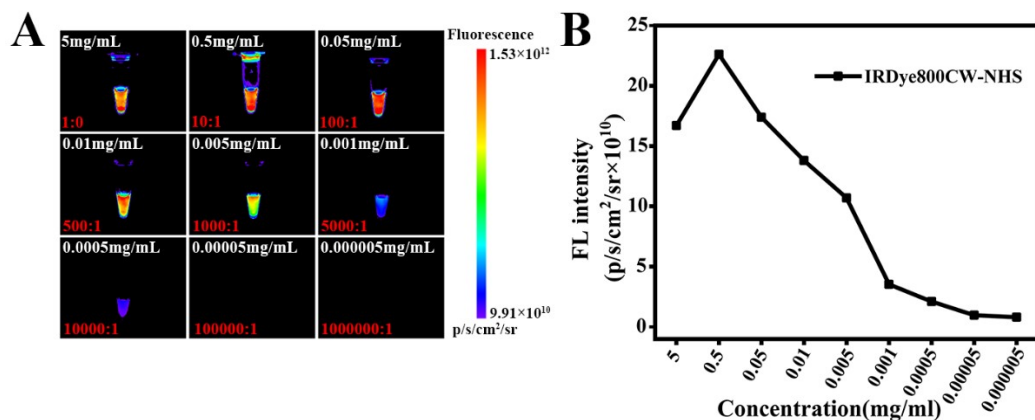
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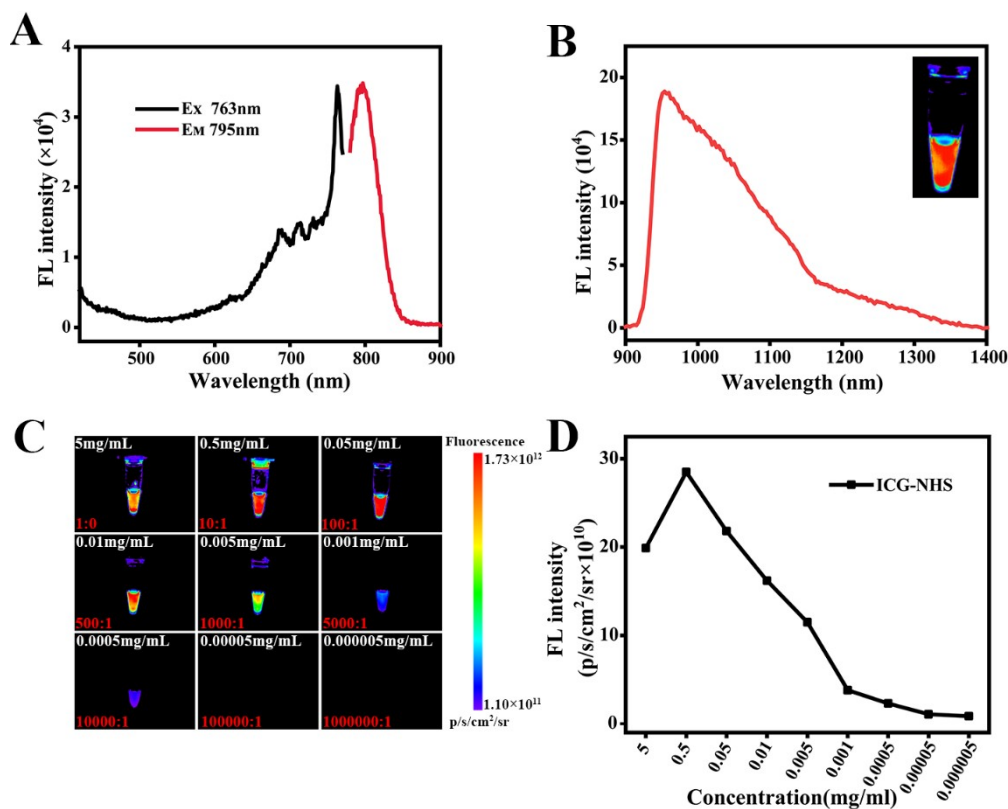
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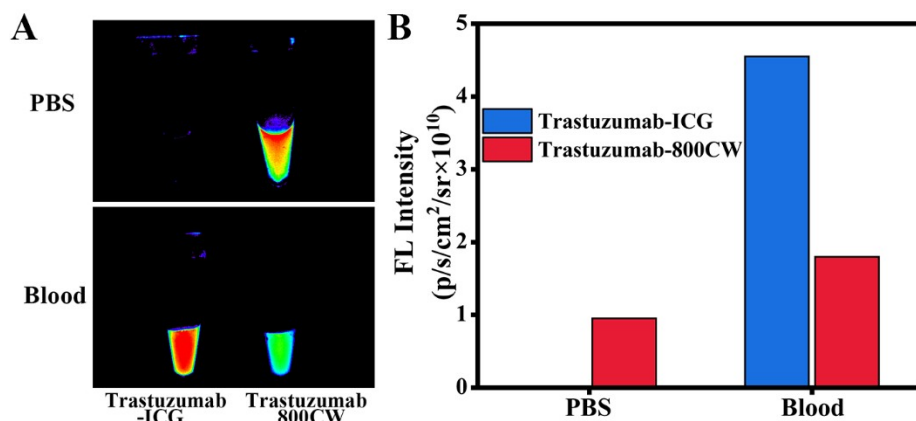
**Figure S1** (A) Structure of ICG-NHS. (B) Structure of IRDye800CW-NHS.



**Figure S2** (A) Under same grey levels, fluorescence imaging images of different concentrations of IRDye800CW-NHS in the NIR-II. (B) Relationship between different concentrations of IRDye800CW-NHS and fluorescence intensity.



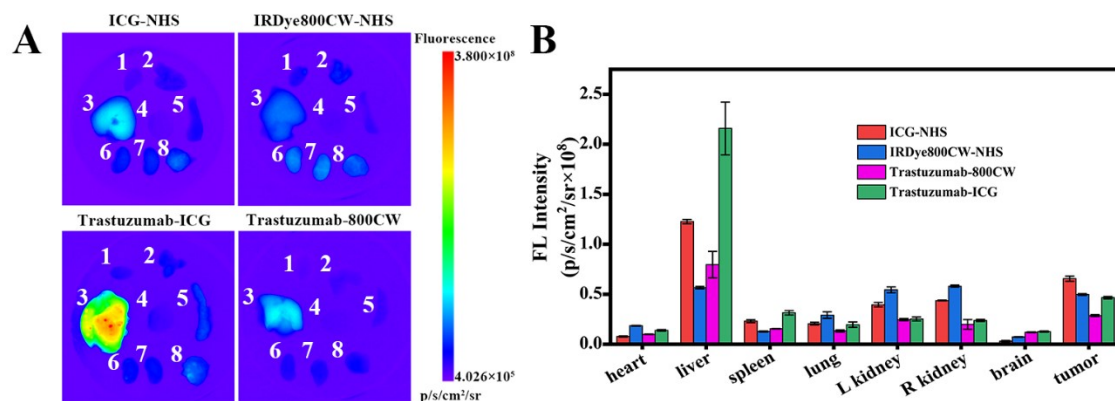
**Figure S3** Optical properties of ICG-NHS. (A) Fluorescence excitation and emission spectra of ICG-NHS in the NIR-I. (B) Fluorescence spectra of ICG-NHS in the NIR-II (Laser excitation at 780 nm), inset is the fluorescence image of the NIR-II under the InGaAs camera. (C) Under same grey levels, fluorescence imaging images of different concentrations of ICG-NHS in the NIR-II. (D) The relationship between the different concentrations of ICG NHS versus fluorescence intensity.



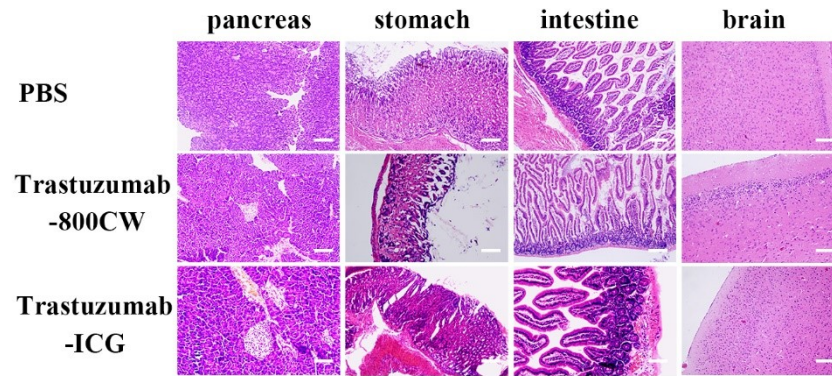
**Figure S4** (A) NIR-II imaging of Trastuzumab-ICG and Trastuzumab-800CW (50  $\mu\text{mol L}^{-1}$ ) in PBS and bovine blood, respectively. (B) Trastuzumab-ICG and Trastuzumab-800CW NIR-II imaging intensities in PBS and bovine blood, respectively.



**Figure S5** Bright field image of tumor location in a subcutaneous xenograft tumor model.



**Figure S6** In vitro imaging and probe distribution of ICG-NHS, IRDye800CW-NHS, Trastuzumab-800CW and Trastuzumab-ICG (The concentration of the dyes is 215  $\mu\text{mol L}^{-1}$  and the concentration of the couplers is 50  $\mu\text{mol L}^{-1}$ , dissolved in PBS with pH 7.4) in HER-2-negative MDA-MB-231 tumor-bearing nude mice. (A) Under same grey levels, imaging of tumor and major organs in vitro NIR-II after 72 h (1: heart, 2: lung, 3: liver, 4: brain, 5: spleen, 6: left kidney, 7: right kidney, 8: tumor). (B) Residual fluorescence intensity of the tumor region and major organs after 72 h.



**Figure S7** H&E staining analysis of HER-2-positive tumor-bearing nude mice injected with PBS, Trastuzumab-800CW and Trastuzumab-ICG at day 14 for pancreas, stomach, intestine and brain (scale bar: 100  $\mu$ m).