Supplementary Materials for

Molecular imaging of tumor-infiltrating macrophages in a preclinical mouse model of breast cancer

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Supplementary Materials and Methods

Preparation of DyLight755-trastuzumab conjugate

Anti-human epidermal growth factor receptor 2 (HER2) monoclonal antibody trastuzumab was mixed with DyLight755-NHS (Pierce, Rockford, IL) in bicarbonate buffer (pH = 9.0) at a 6:1 molar ratio of DyLight755-NHS:trastuzumab. After incubation at room temperature for 1 h, the DyLight755-trastuzumab conjugate, denoted as Dye755-anti-HER2, was purified using a PD-10 desalting column (GE Healthcare, Piscataway, NJ). The degree of labeling (dye:protein ratio) for Dye755-anti-HER2 was calculated to be 3.9 based on UV measurements of A_{280} and A_{755}.

In vivo simultaneous NIRF imaging of Dye-anti-CD206 and Dye755-anti-HER2

The A549 tumor-bearing nude mouse model was established by inoculating 5×10^6 tumor cells subcutaneously into the right front flanks of female BALB/c nude mice (4–5 weeks of age; Department of Laboratory Animal Science, Peking University). For the in vivo NIRF imaging study, three A549 tumor-bearing nude mice were intravenously injected with a mixture of 0.2 nmol Dye-anti-CD206 and 0.2 nmol Dye755-anti-HER2. At 24 h postinjection, the mice were subjected to in vivo NIRF imaging using the Dylight680 spectrum (excitation = 675 nm, emission = 720 nm) and the Dylight755 spectrum (excitation = 745 nm, emission = 800 nm), respectively.

HER2 and CD206 overlay staining

HER2-specific Rhodamine-trastuzumab was prepared by conjugating trastuzumab with Rhodamine-NHS, followed by PD-10 column purification. Frozen A549 tissue slices from tumor-bearing nude mice were incubated with rat anti-mouse CD206 antibody (BioLegend, San Diego, CA) for 1 h at room temperature. After washing with PBS, the slices were incubated with both Rhodamine-trastuzumab (2 μg/mL) and fluorescein isothiocyanate (FITC)-conjugated anti-rat secondary antibody. After a final wash with PBS, the slices were examined under a confocal fluorescence microscope.

Supplementary Figures

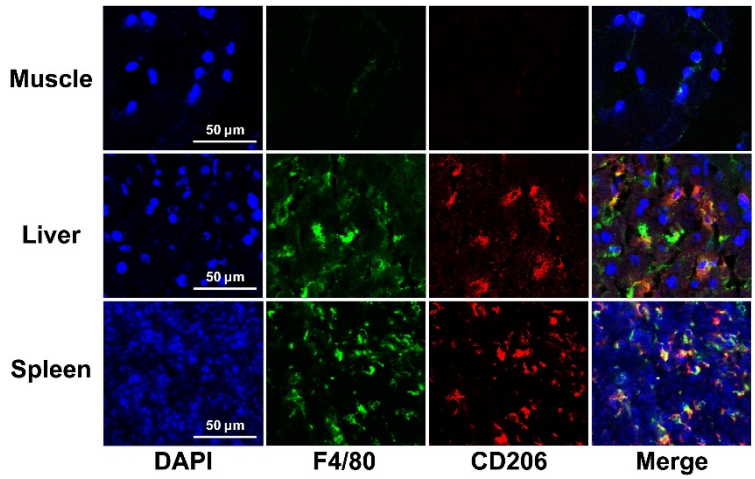

Figure S1. Overlay immunofluorescence staining of F4/80 and CD206 in the muscle, liver, and spleen of normal BALB/c mice.

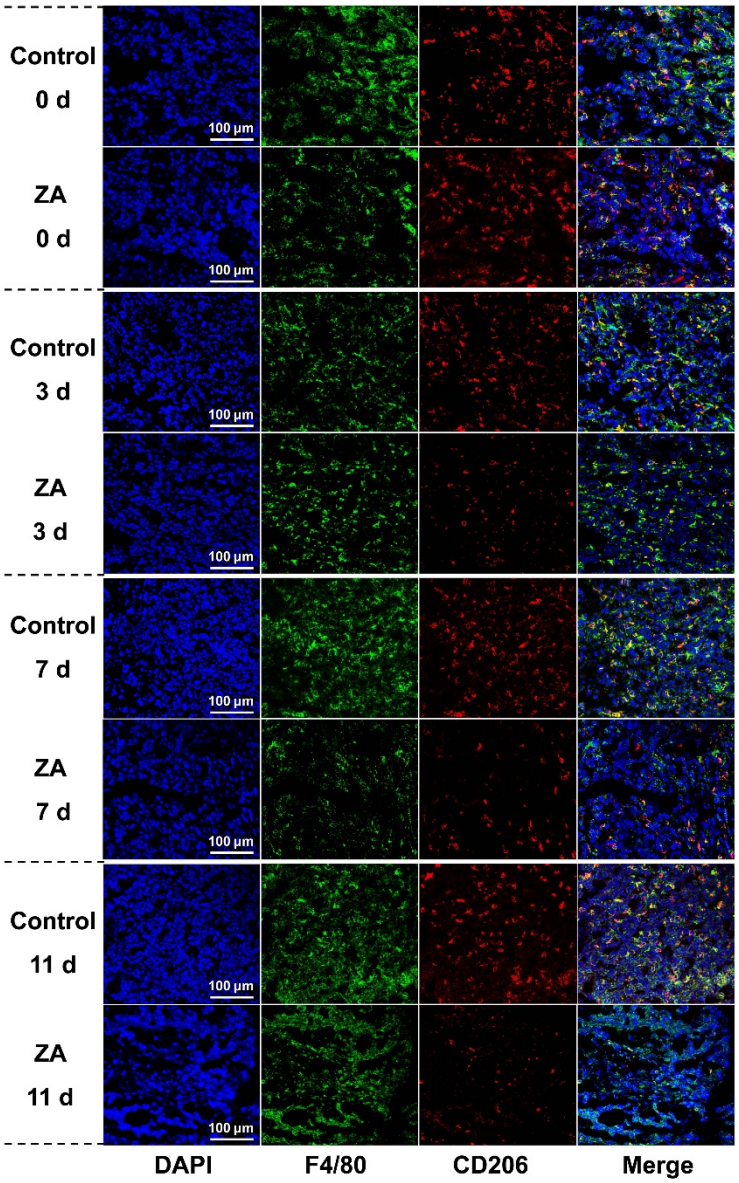

Figure S2. Overlay immunofluorescence staining of murine CD206 and F4/80 in 4T1 tumor tissues on days 0, 3, 7, and 11 after zoledronic acid (ZA; 150 μg/kg in PBS daily for 7 days) or PBS treatment (control).

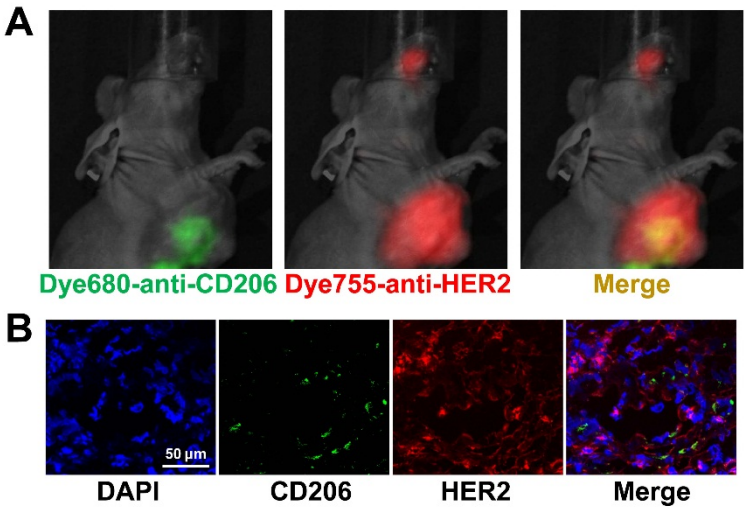

Figure S3. (A) Representative in vivo two-color NIRF images of 4T1 tumor-bearing mice at 24 h after injection of the mixture of Dye-anti-CD206 (Dye680-anti-CD206) and Dye755-anti-HER2. The images indicated that HER2 (imaged by Dye755-anti-HER2; red) were nearly homogenously distributed within the tumor, whereas the tumor-infiltrating macrophages (imaged by Dye680-anti-CD206; green) were located mainly in the tumor center. (B) Immunofluorescence staining demonstrated that there was no overlay between HER2 (expressed on tumor cells) and CD206 (expressed on tumor-infiltrating macrophages) in A549 tumor tissue.