Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary data for

A novel NIR-II probe for improved tumor-targeting NIR-II imaging

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Materials and characterization

All chemical reagents were obtained from the commercial suppliers and used without further purification. The ^{1}H and ^{13}C NMR spectra were acquired on Bruker 400 MHz magnetic resonance spectrometers. Data for ^{1}H NMR spectra are reported as follows: chemical shifts are reported as δ in units of parts per million (ppm) relative to chloroform-d (δ 7.26, s); coupling constants are reported as a *J* value in Hertz (Hz); multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets) and m (multiplet); the number of protons (n) for a given resonance is indicated nH based on the spectral integration values. MALDITOF-MS spectra were obtained by an AB SCIEX 4700 TOF/TOF System. UV-vis was tested with a Shimadzu Model UV-1700 spectrometer. Photoluminescence (PL) spectra were taken by a fluorescence spectrometer (F900, Edinburgh Instruments Ltd.) NIR-II in vivo imaging was performed on a small animal imaging system with fiber-coupled 808 nm laser system.

Cell line and animal model.

4T1 cells was obtained from the Type Culture Collection of the Chinese Academy of Sciences and culture media was obtained from Invitrogen Co. (Carlsbad, CA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin at 37°C and 5% CO_2. The 4T1 tumor model were established by subcutaneous injection of 4T1 (~5 × 10^6 in 100 μL of PBS) into the front flank of female athymic nude mice. The mice were subjected to imaging studies when the tumor volume reached 300-600 mm^3. Animal experiments were performed according to a protocol approved by the Jining Medical University Institutional Animal Care and Use Committee.

***In Vivo* NIR-II fluorescence imaging.**

Before conducting experiments, 2% isoflurane in oxygen was used to anesthetize mice in prone position. Three mice were used during parallel experiments to collect all the in vivo data. To study the imaging ability, mice were imaged before dot injection to record the background signal. After mice were intravenously injected with IR-RGD (2 mg/Kg), the NIR-II fluorescence imaging with thermoelectric cooled InGaAs camera

was used to collect images over different time points, respectively. 808 nm continuous irradiation (50 mW/cm^2) was used as the light source in the NIR-II fluorescence imaging system with exposure time of 50 ms equipped with 1100 nm long-pass filter.

Synthesis details

Synthesis and Characterization of **IR-820**. The compound 1 (690 mg, 2.0 mmol), compound 2 (172 mg, 1.0 mmol) and AcONa (328 mg, 4.0mmol) were added to Ac_2O (10 mL) and the mixture was stirred at 80 ^oC under vigorous stirring with a maintained positive pressure of nitrogen. The reaction mixture was further stirred for 10 h at this temperature, and then cooled to room temperature. The solvent was removed by evaporation and the residues was purified by flash column chromatographyon silica gel to afford pure sample (799 mg, 58%) as a solid. ^{1}H NMR (600 MHz, DMSO) δ 8.37 (d, *J* = 14.0 Hz, 2H), 8.30 (d, *J* = 8.4 Hz, 2H), 8.08 (dd, *J* = 14.8, 8.5 Hz, 4H), 7.82 (d, *J* = 8.8 Hz, 2H), 7.66 (t, *J* = 7.5 Hz, 2H), 7.52 (t, *J* = 7.4 Hz, 2H), 6.42 (d, *J* = 14.2 Hz, 2H), 4.36 (s, 4H), 2.78 (s, 4H), 2.56 (t, *J* = 7.3 Hz, 4H), 1.96 (s, 12H), 1.90 (d, *J* = 6.0 Hz, 7H), 1.81 (dd, *J* = 14.1, 7.1 Hz, 4H).^{13}C NMR (151 MHz, DMSO) δ 173.76, 147.83, 142.52, 140.27, 134.04, 131.94, 130.93, 130.39, 128.22, 127.92, 126.80, 125.45, 122.75, 112.36, 101.79, 51.15, 44.44, 27.50, 26.85, 26.40, 22.96, 20.99. ESI-MS [Calcd. for $C_{46}H_{50}ClN_2O_6S_2^-$: 825.29, found: m/z 825.40].

Synthesis and Characterization of **IR-RGD**. **IR-820** (4.2 mg, 5 μmol) was dissolved in 0.5 mL of DMSO. c(RGDfc) (3.9 mg, 5 μmol) was dissolved in 0.5 mL of de-gassed 0.1% sodium ascorbate (w/v) in phosphate buffer-saline (PBS) and added to the reaction solution. The reaction was stirred at RT for 2 h. The reaction mixture was monitored by analytical HPLC, which was performed using Symmetry C-18 columns from YMC (3 μm, 150 × 4.6 mm i.d.). The mobile phases (A) demineralized water and (B) acetonitrile were acidified to pH 3 with trifluoroacetic acid. Gradient elution was performed as follows: 10% of B, 0-3 min;10-90% of B, 3-12 min; 90% of B, 12-16 min; 90-10% of B, 16-18min; 10% of B, 18-20min. The product was purified by Pro-

HPLC. Lyophilization of the purified material gave 3.28 mg (48%) of **IR-RGD**. MALDI-TOF [Calcd. for $C_{70}H_{83}N_{10}O_{13}S_3^+$: 1369.54, found: m/z 1369.5411].

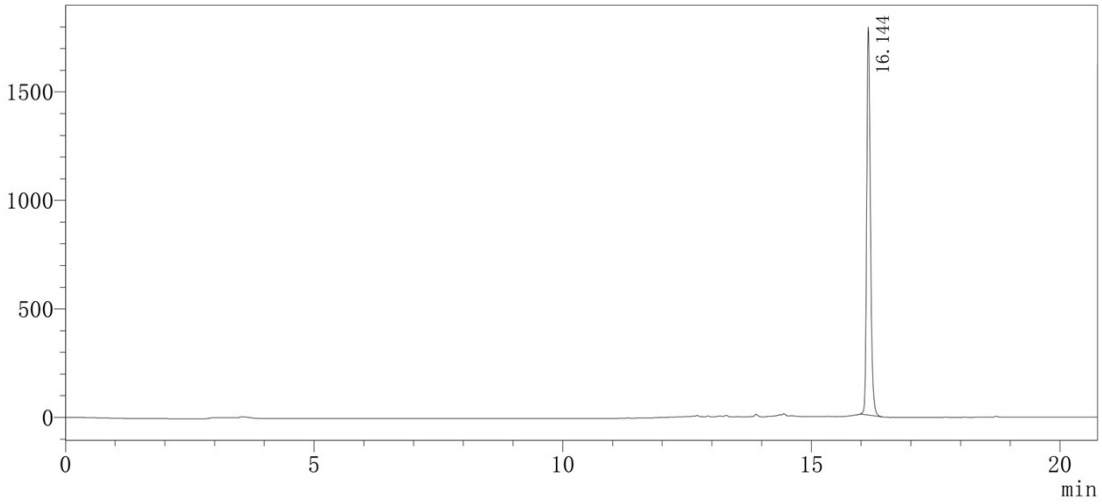

Figure S1. HPLC analysis of compound IR-820.

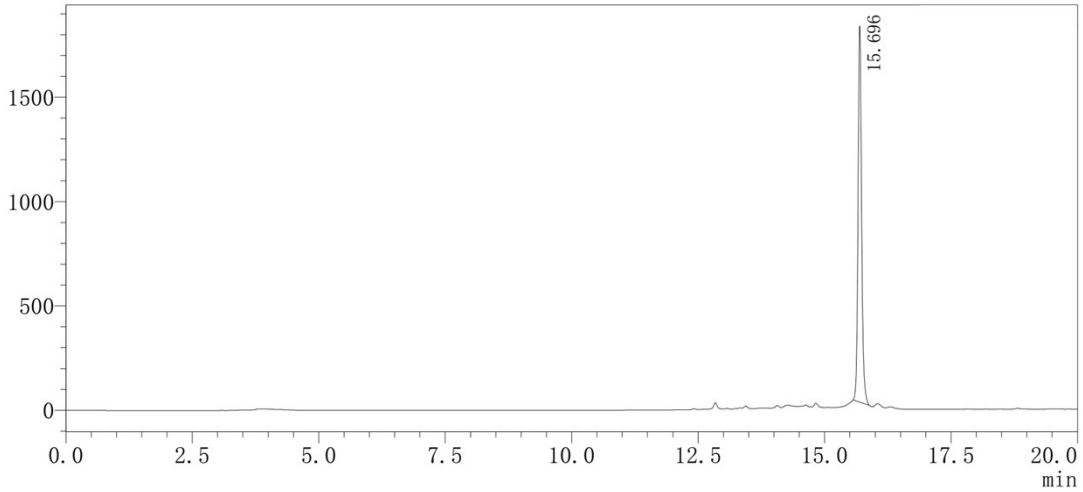

Figure S2. HPLC analysis of compound IR-RGD.

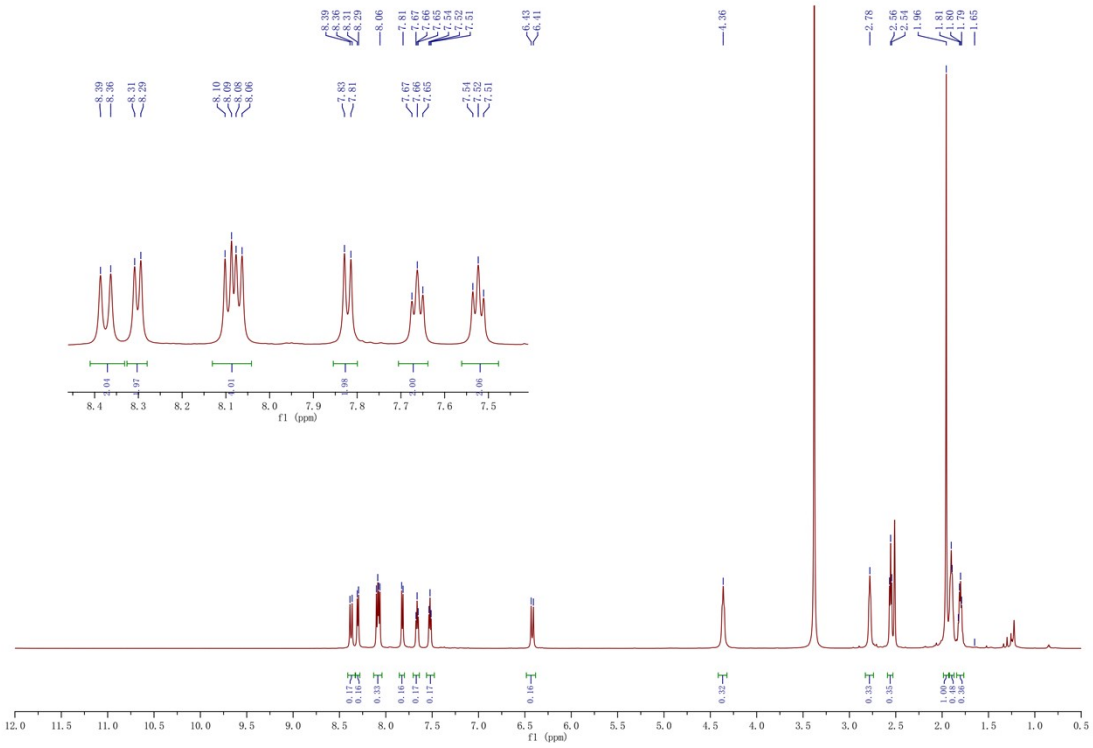

Figure S3. ^{1}H NMR spectroscopy of compound of IR-820.

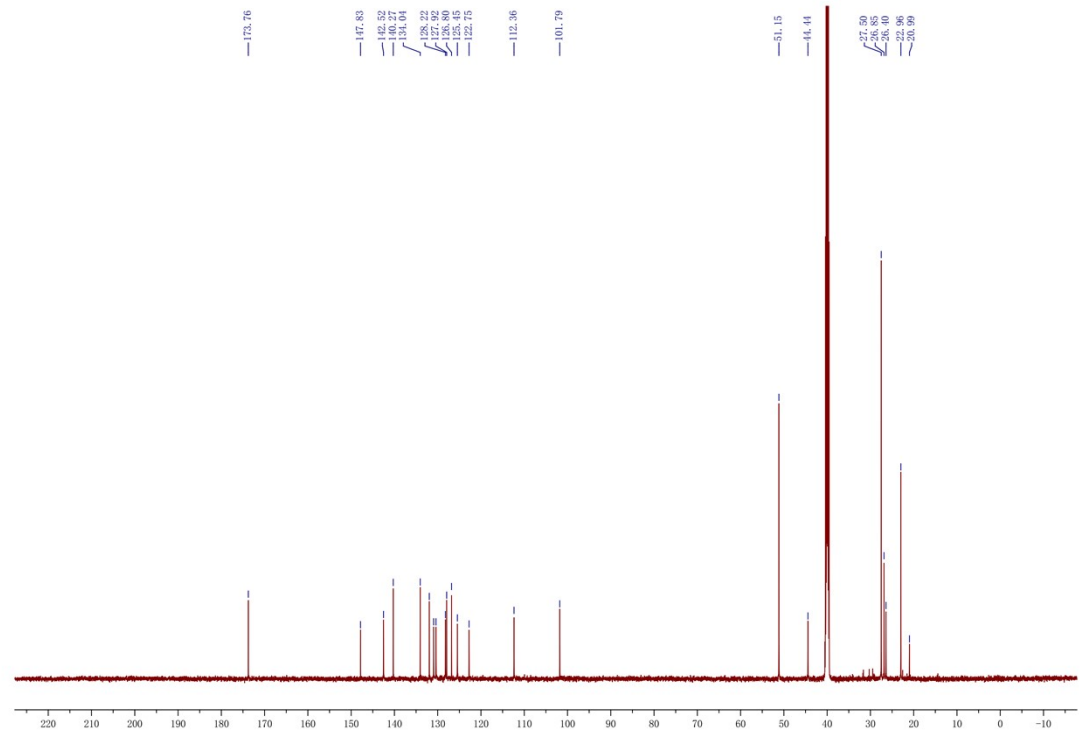

Figure S4. ^{13}C NMR spectroscopy of compound of IR-820.

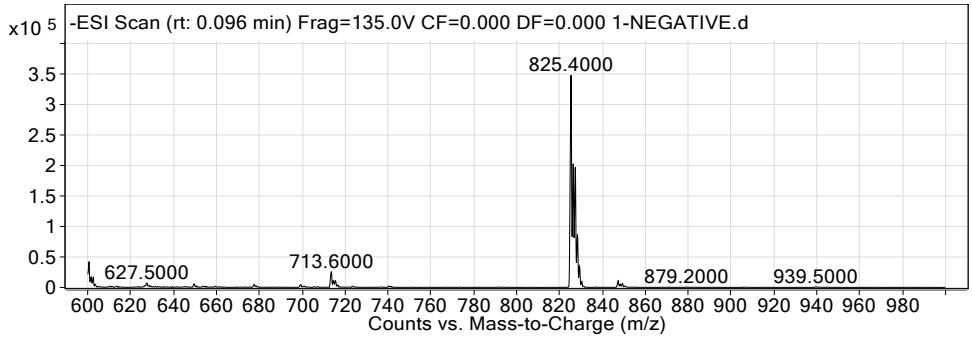

Figure S5. MS spectroscopy of compound IR-820.

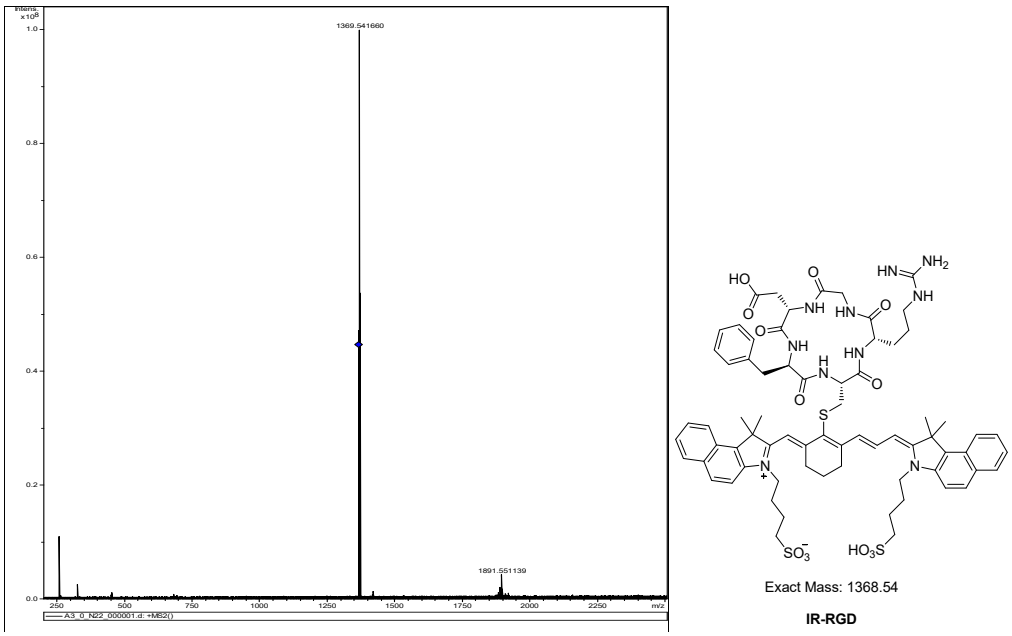

Figure S6. MALDI-TOF-MS measurement of **IR-RGD.**

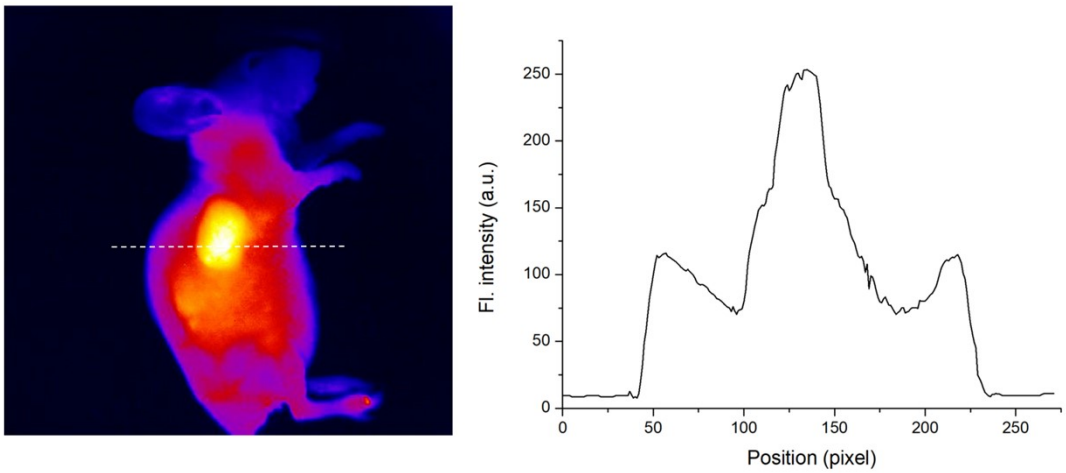

Figure S7. The line profile of the fluorescence intensity.

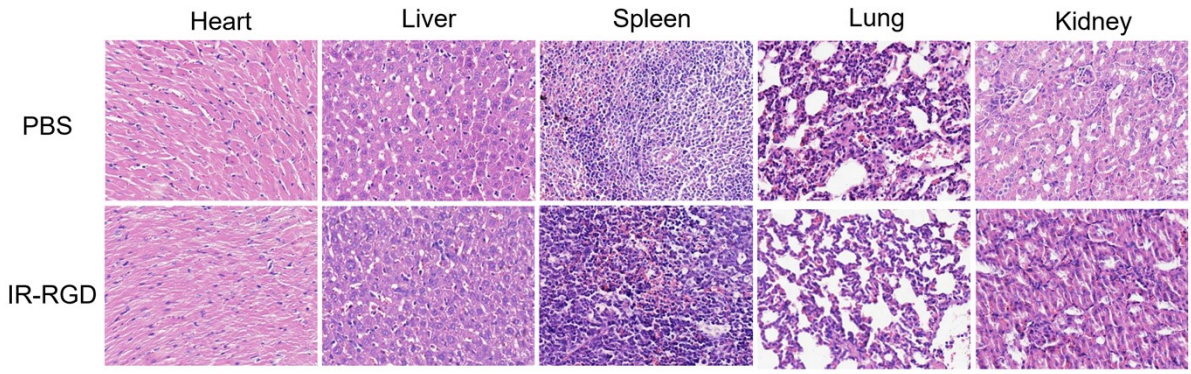

Figure S8. Histological H&E staining for main organs (liver, spleen, kidney, lung and heart) of the mice intravenously administrated with PBS and IR-RGD.

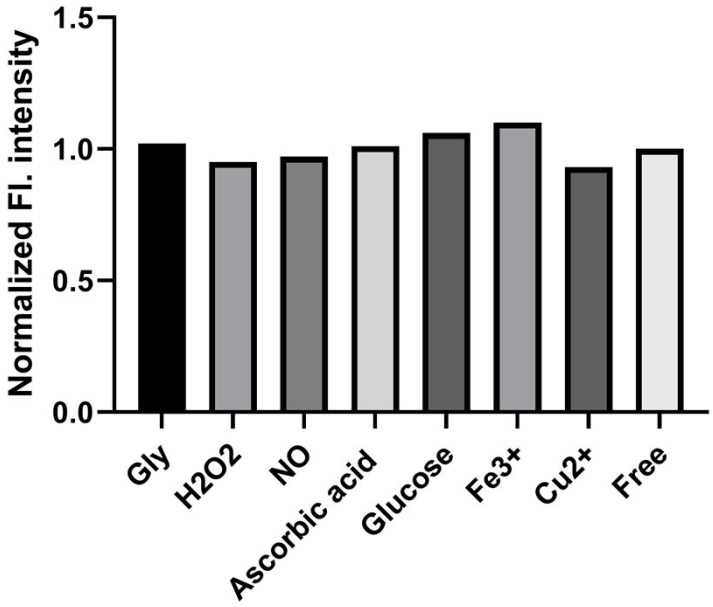

Figure S9. Fluorescence change with Glycine, H_2O_2, NO, Ascorbic acid, Glucose, Fe^{3+} and Cu^{2+} (10 μM for each)

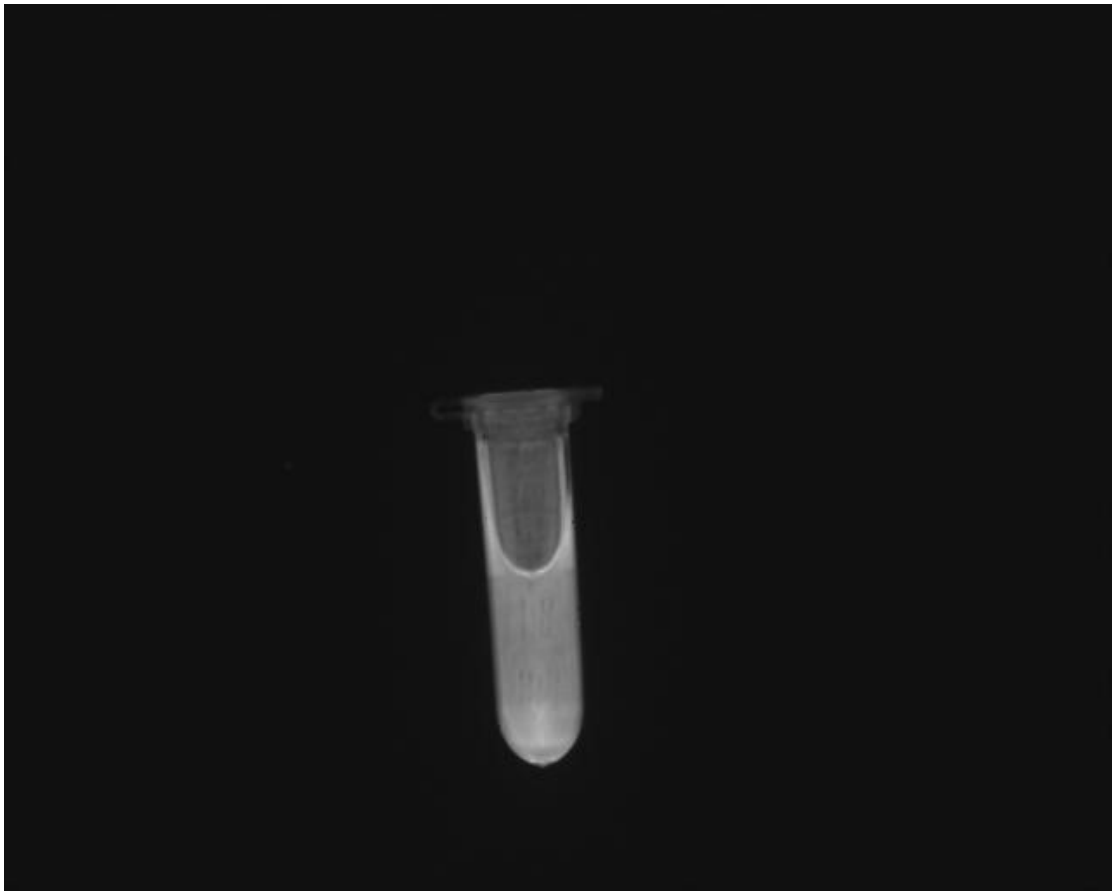

Figure S10.NIR-II imaging of 10 micro molar IR-RGD solution at 10 mW/cm^2 excitation

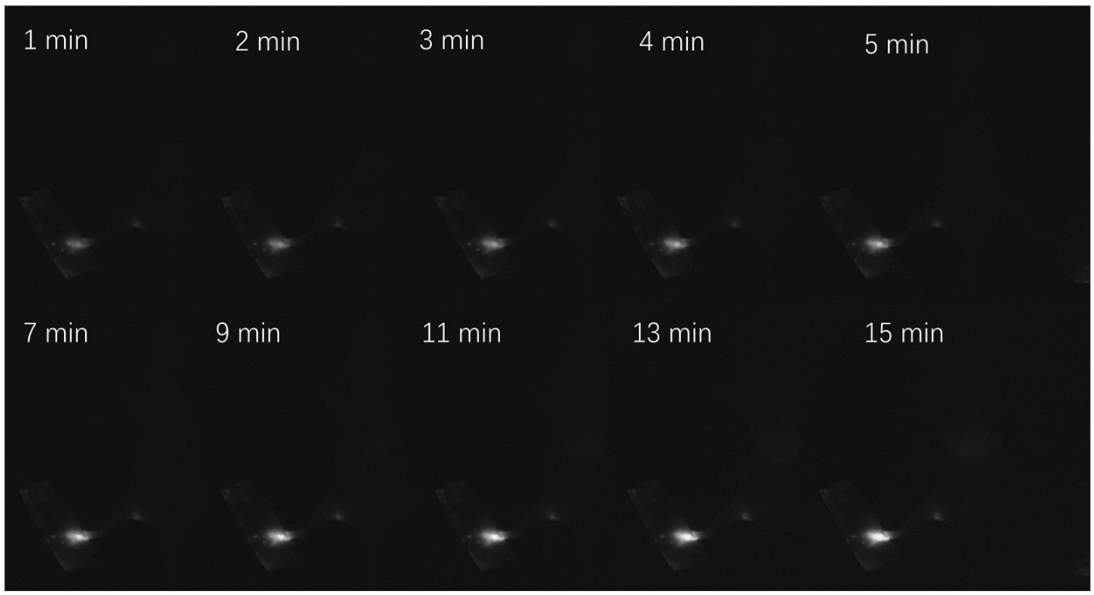

Figure S11. Lymph node NIR II imaging.