Supporting Information

Self-Illuminating *in vivo* Lymphatic Imaging Using a Bioluminescence Resonance Energy Transfer Quantum Dot Nano-Particle

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Preparation for the streptavidin-conjugated BRET-Qdot655 ([BRET-Qdot655]-SA)

The preparation of BRET-Qdot conjugated with streptavidin has not been previously described. These triplex conjugates are commercially available from Zymera Inc. (San Jose, CA). Briefly, the preparation of BRET-Qdot conjugates with streptavidin ([BRET-Qdot655]-SA) is done using carboxylated Qdots655 (Invitrogen) by a proprietary conjugation protocol developed by Zymera. The [BRET-Qdot]-SA conjugates were purified by spin filtration using a 100K Amicon spin filter (Millipore Corporation). The [BRET-Qdot]-SA conjugates (500 nM) are in 10 mM Tris buffer (pH 7.4) and are stored at 4o C. These conjugates have on average 5 streptavidin units per BRET-Qdot.

Supplementary figures

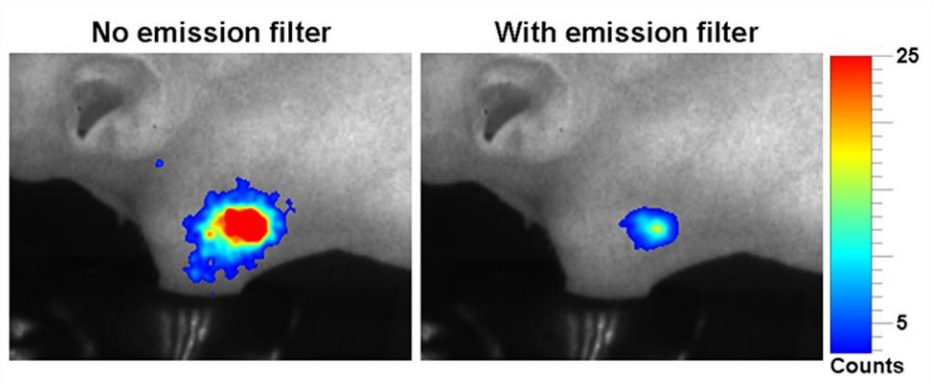

Figure S1.

BRET lymphatic images acquired with or without emission filter (655 / 40 nm band pass filter) 30 min after coelenterazine injections. BRET signal decreases by 79.5 ± 0.5 % (n=5, average ± SD) with emission filter, but lymph nodes of interest are still depicted by BRET imaging.

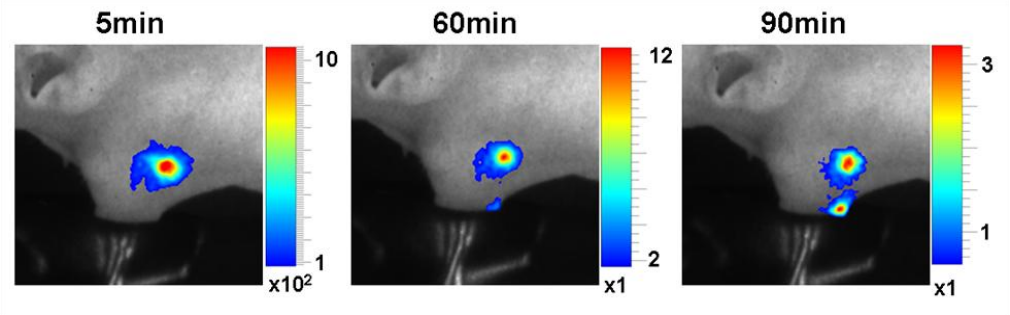

Figure S2.

Serial BRET lymphatic images of same mice receiving BRET-Qdot655 injection (10 pmol) at left paw followed by coelenterazine injection (10 μg) at the opposite side of same paw. Prolonged depiction of the lymph node of interest is achieved up to 90min (n=3).

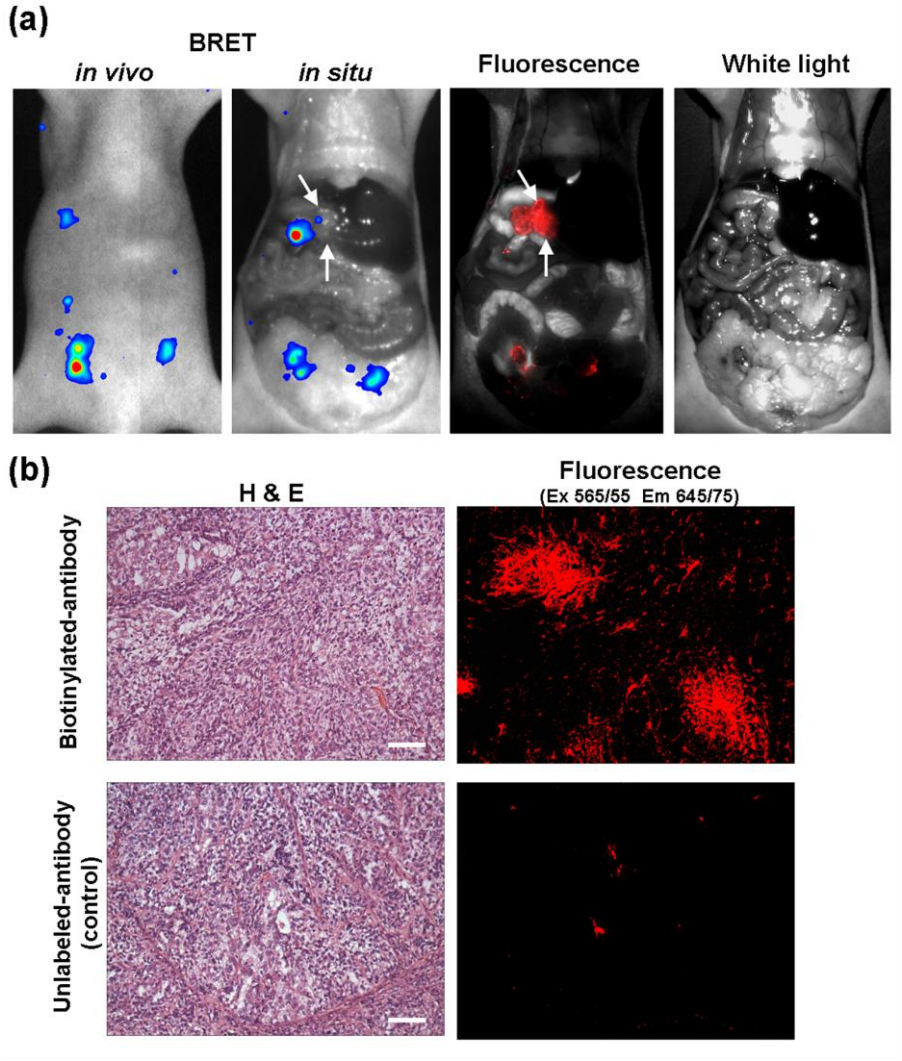

Figure S3

Target cancer imaging employing the pretargeting dosing method with a streptavidin-conjugated BRET-Qdot655 ([BRET-Qdot655]-SA). Mice bearing intraperitoneal SKOV3 tumors, human ovarian cancer cell line over-expressing HER2 receptor, received i.p. 50-μg biotinylated trastuzumab (anti-HER2 monoclonal antibody) 48 hrs prior to imaging, and received i.p. 10-pmol [BRET-Qdot655]-SA 24 hrs prior to

imaging. After i.v. injection of 10-μg coelenterazine, BRET and fluorescence imaging were performed. BRET images show that BRET signal can be localized in the target tumors (a). However, the discrepancy between BRET and Qdot signal are observed (arrows). Fluorescence microscopic images confirm accumulations of [BRET-Qdot655]-SA in a tumor nodule, while minimal accumulations are detected in a control tumor nodule, which received same amount of non-labeled trastuzumab with same protocol instead of biotinylated-trastuzumab (b). Bars on H&E images are 100 μm.

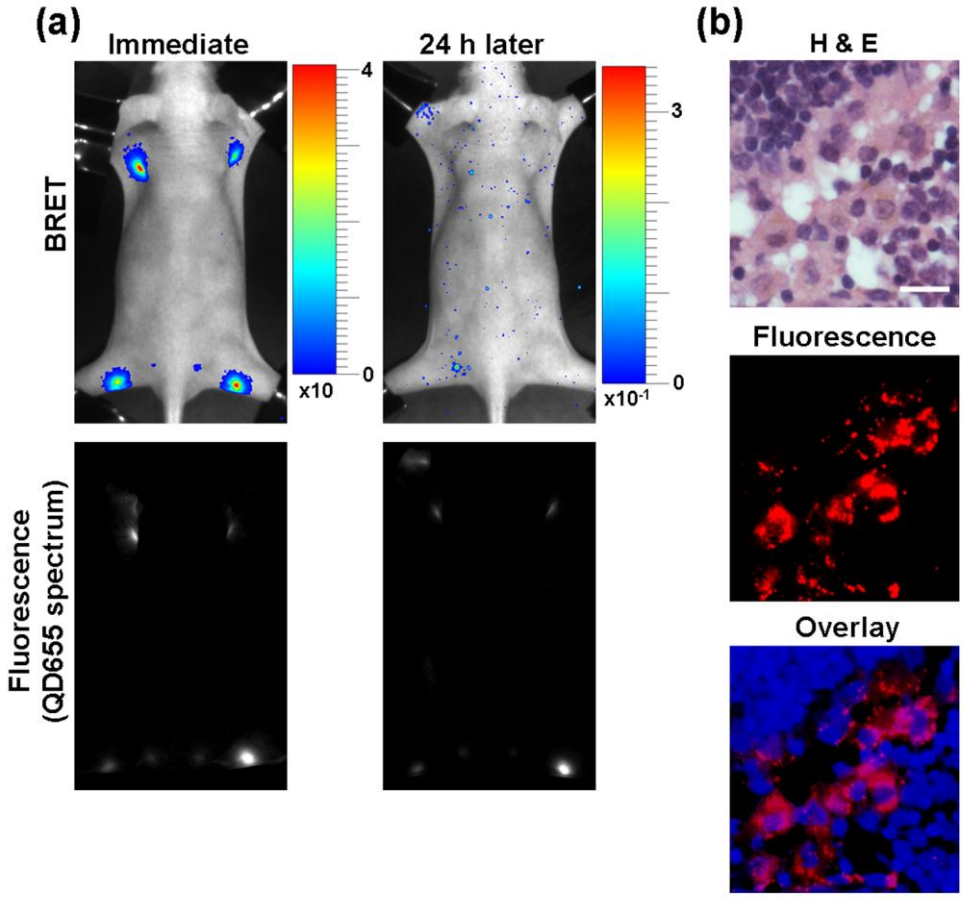

Figure S4.

Serial BRET and fluorescence lymphatic images of same mice receiving BRET-Qdot655 injection at all four paws. Although fluorescence signals in lymph nodes are identified at 24 h, no BRET signal can be detected in a lymph node. Fluorescence microscopic images of the resected lymph node at 24 h demonstrate internalization of BRET-Qdot655 particles in the cells (b). Bar on H&E image is 25 μm.