Supporting Information

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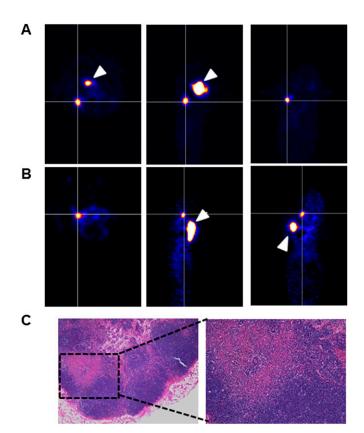


Fig. S1. (A) Representative ¹⁸F-AIF-NEB PET images of axillary LNs in the orthotropic breast cancer model (left to right, transaxial, sagittal, and coronal images). Arrowheads indicate primary tumors. (*B*) Representative ¹⁸F-AIF-NEB PET images of a cervical LN in the orthotropic breast cancer model (left to right, transaxial, sagittal, and coronal images). Arrows indicate tumor-draining axillary LNs, and arrowheads indicate primary tumors. (*C*) H&E staining of axillary LNs. No apparent tumor metastasis was identified.

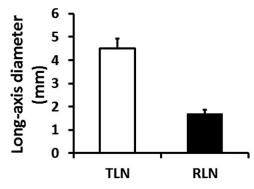


Fig. S2. The average long-axis diameter of the left LN measured by MRI is significantly larger than that of the right one.

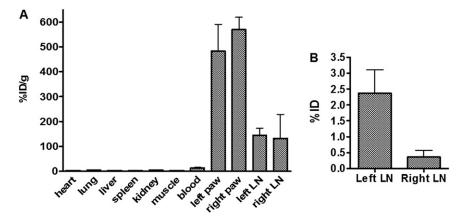


Fig. S3. Ex vivo biodistribution after local injection of ¹⁸F-AIF-NEB in a tumor metastasis model. The 5×10^5 fLuc⁺ murine breast cancer 4T1 cells in 30 µL of PBS were injected into the left hock area of Balb/C mice (n = 5). Four weeks after tumor inoculation, 0.37 MBq (10 µCi) of ¹⁸F-AIF-NEB in 20 µL of saline was injected into both the left and right footpads of each mouse. At 30 min after tracer injection, all mice were killed and the major organs and LNs from both sides were harvested for radioactivity measurement using a gamma counter. (*A*) The majority of the radioactivity injected remained at the injection sites (paws) and popliteal LNs. No difference in %ID/g between the left LN and right LN was found. (*B*) There is significantly more radioactivity accumulation in the left popliteal LNs (tumor-draining side) than the contralateral LNs due to the much enlarged tumor-draining popliteal LNs (P < 0.05). The result is consistent with the PET image-based quantification.

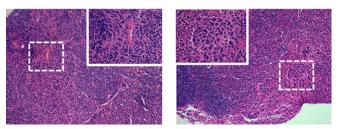


Fig. S4. H&E staining found micrometastasis foci inside some of the tumor-draining LNs at 4 wk after inoculation of Fluc⁺ 4T1 cells via hock injection.

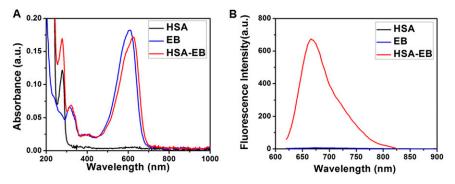


Fig. S5. Absorbance (A) and fluorescence emission (B) of EB with and without albumin. EB showed a strong absorbance peak at 620 nm with or without albumin. EB is almost not fluorescent without albumin. However, with albumin, EB showed a strong fluorescence emission peak at 680 nm.