

## Supplementary Information

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### Increased precision of orthotopic and metastatic breast cancer surgery guided by matrix metalloproteinase activatable near-infrared fluorescence probes

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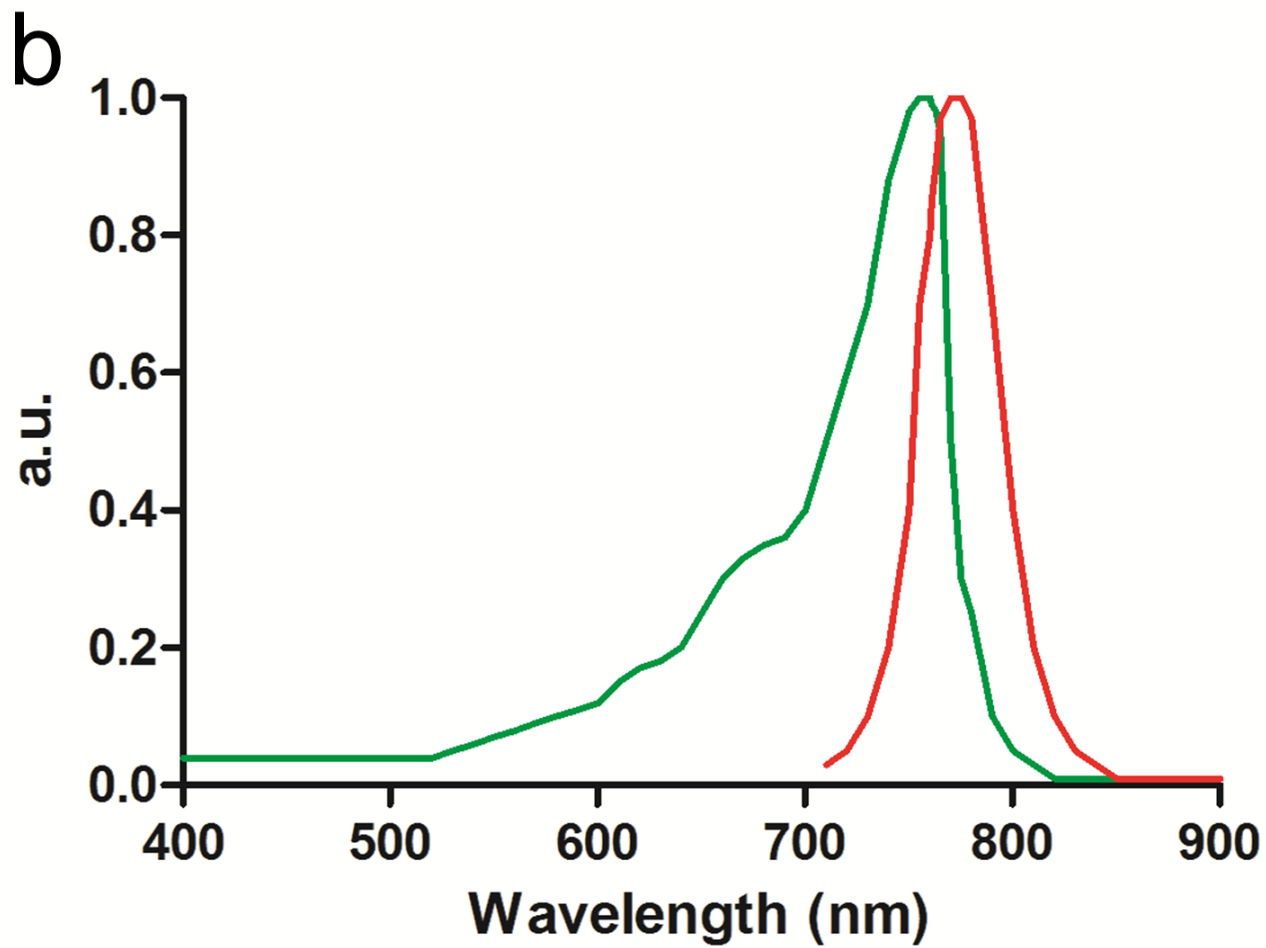
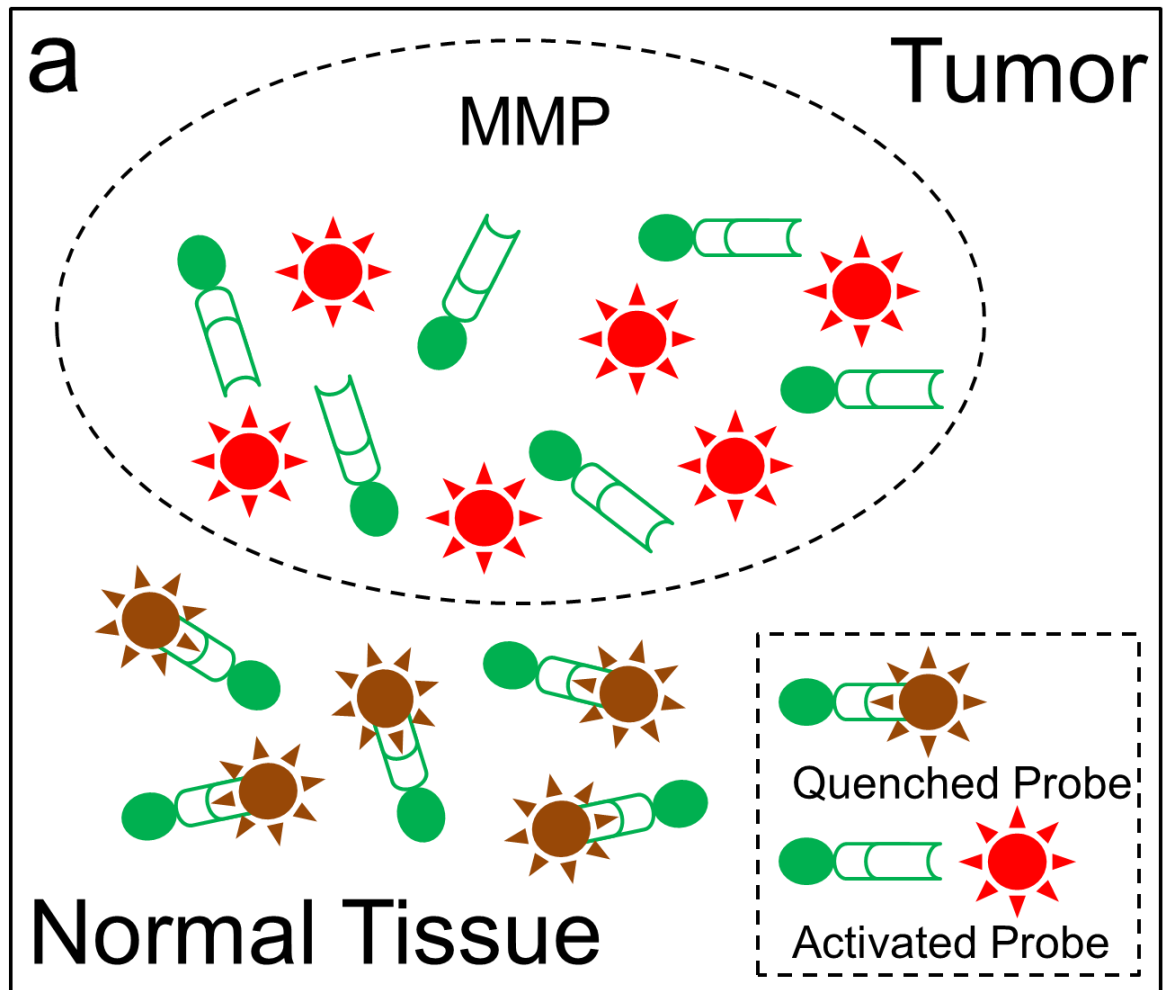
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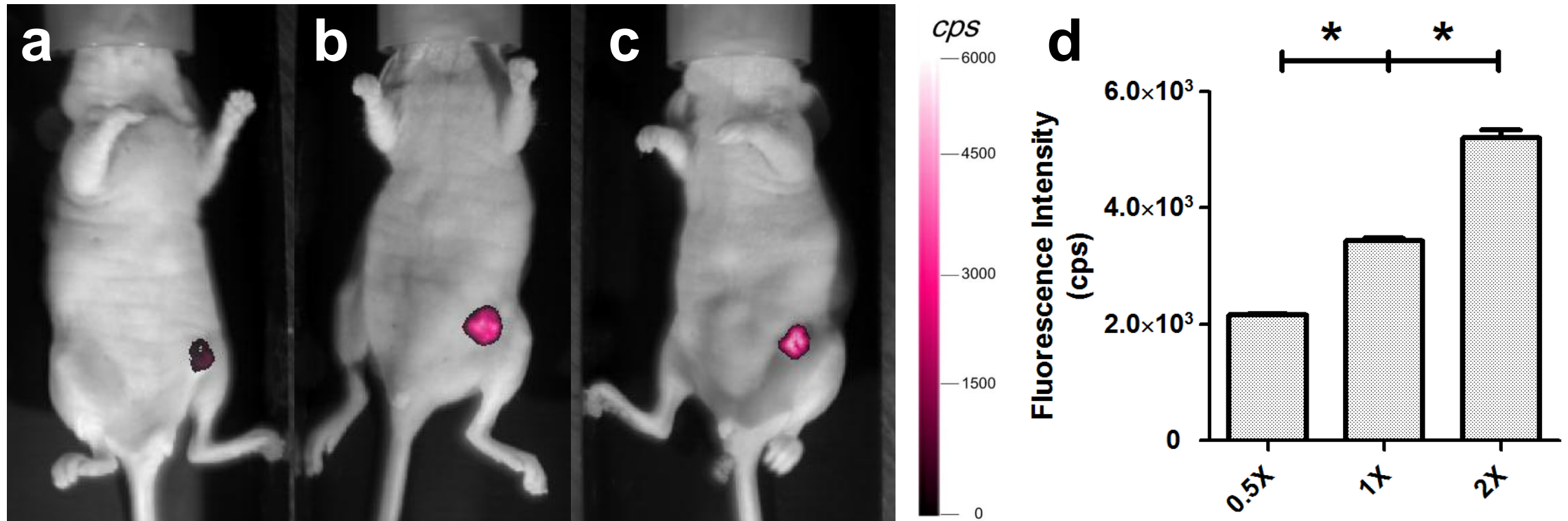
Dr. Zhongliang Wang, Email: [wangzl@xidian.edu.cn](mailto:wangzl@xidian.edu.cn)

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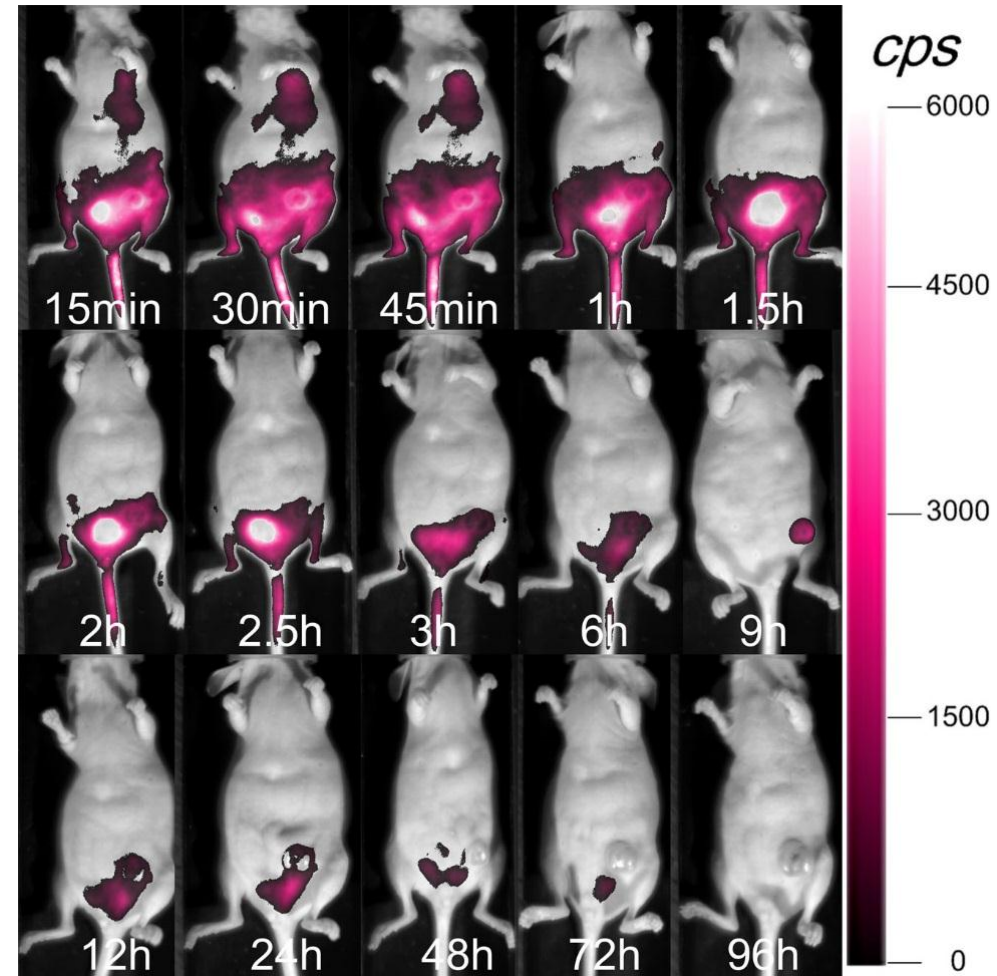
Dr. Jie Tian, E-mail: [tian@ieee.org](mailto:tian@ieee.org); [jie.tian@ia.ac.cn](mailto:jie.tian@ia.ac.cn)



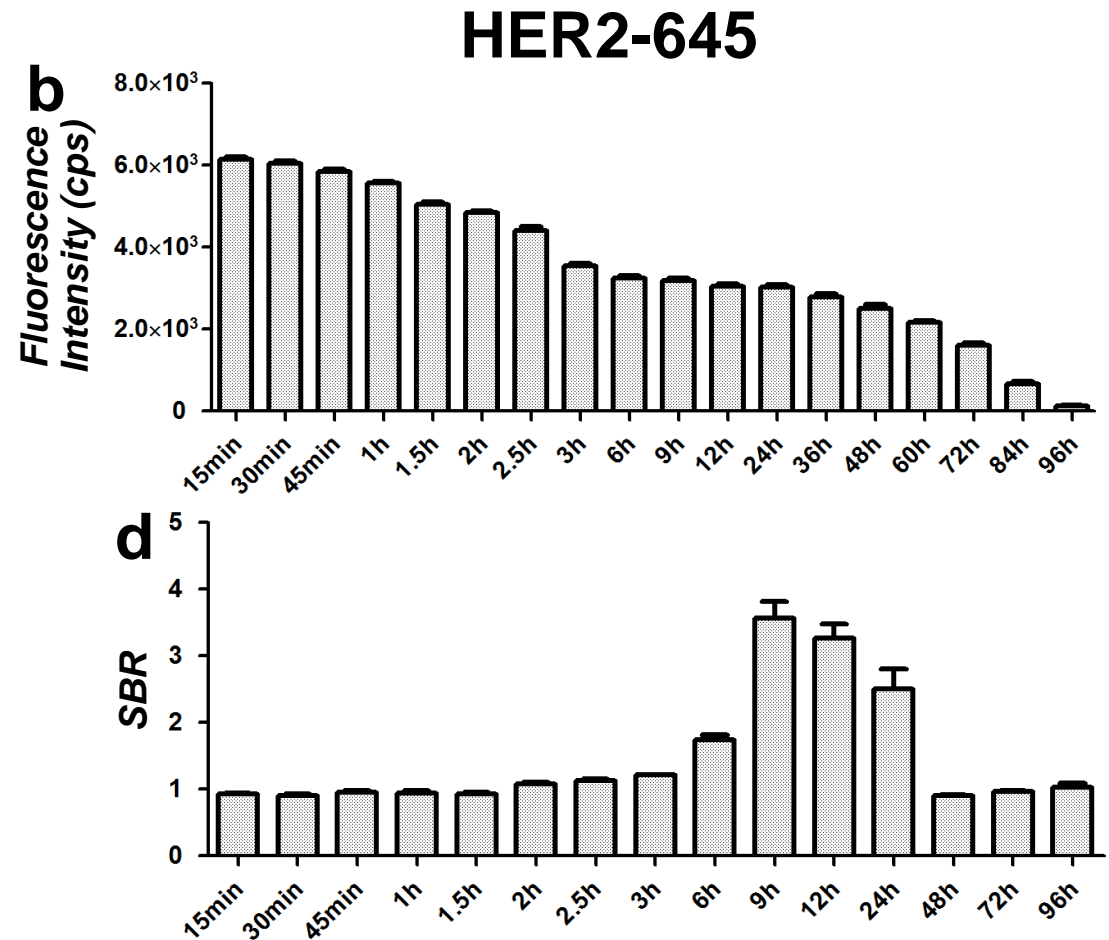
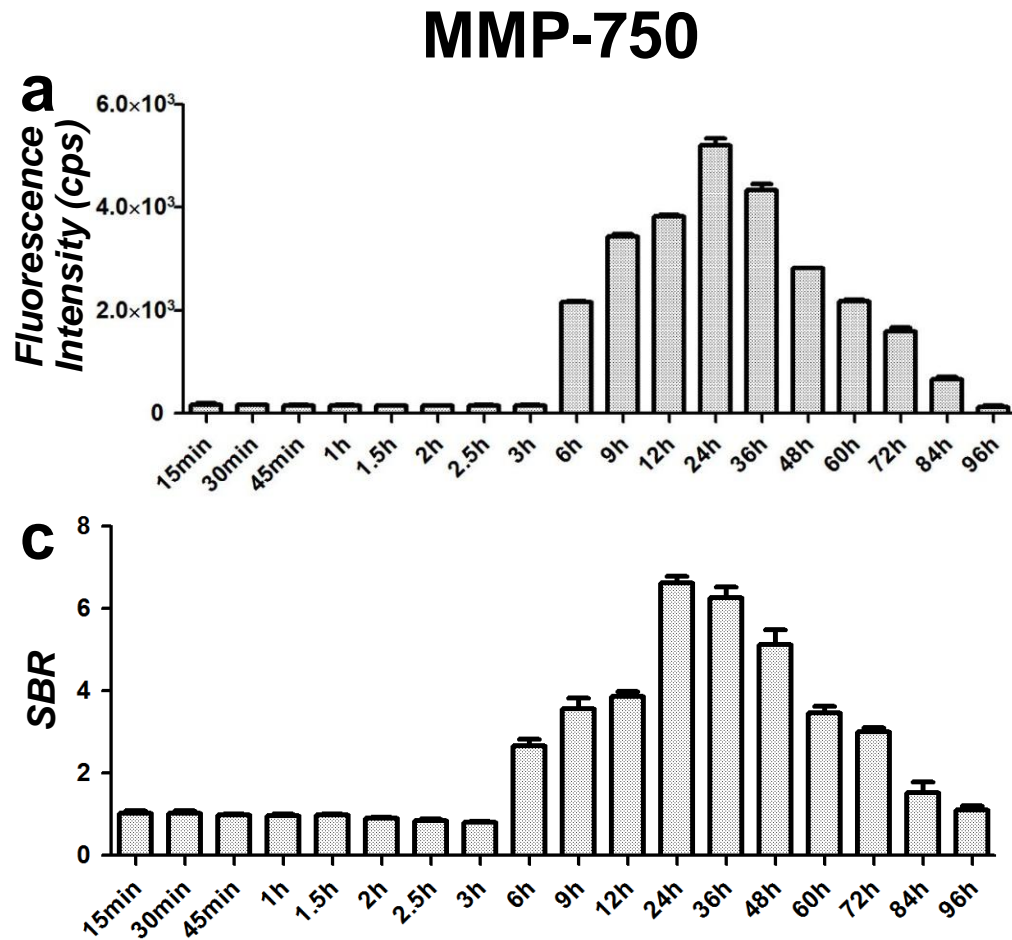
**Figure S1.** Schematic diagram of the probe targeting property. (a) The tumor area was shown in the dotted line. When the smart MMP-750 probe was injected into the normal tissues, the fluorescence was quenched as shown in brown color. When the smart probe reached to the MMP-overexpressing tumor area, the probe was activated by the cleavage of MMP enzyme and emitted NIR fluorescence. In this case, the excitation and emission spectra of activated MMP-750 probe were shown in the Figure S1b. ( $\lambda_{Ex} = 749$  nm,  $\lambda_{Em} = 775$  nm).



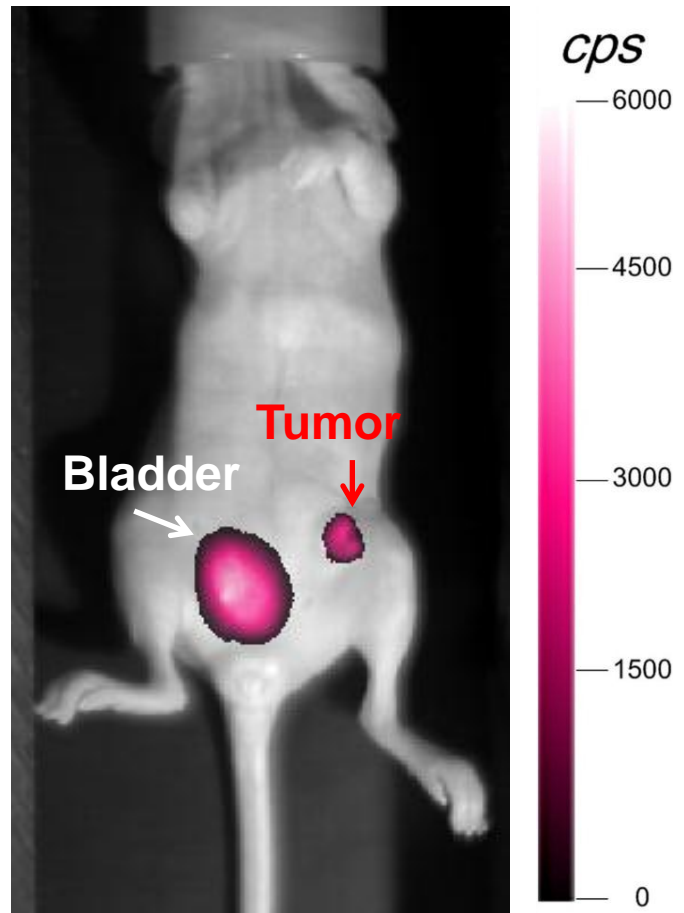
**Figure S2.** Fluorescence Molecular Imaging of MMP-750 probe 24 h after IV injection into the orthotopic breast tumor models. (a) half dosage (1 nmol, 100  $\mu$ L), (b) recommended dosage (2 nmol, 100  $\mu$ L), and (c) double dosage (4 nmol, 100  $\mu$ L). (d) The quantified analysis of the tumor area, which showed statistics significance ( $p < 0.05$ ) between different groups. Mean and standard deviation were derived from three independent experiments.



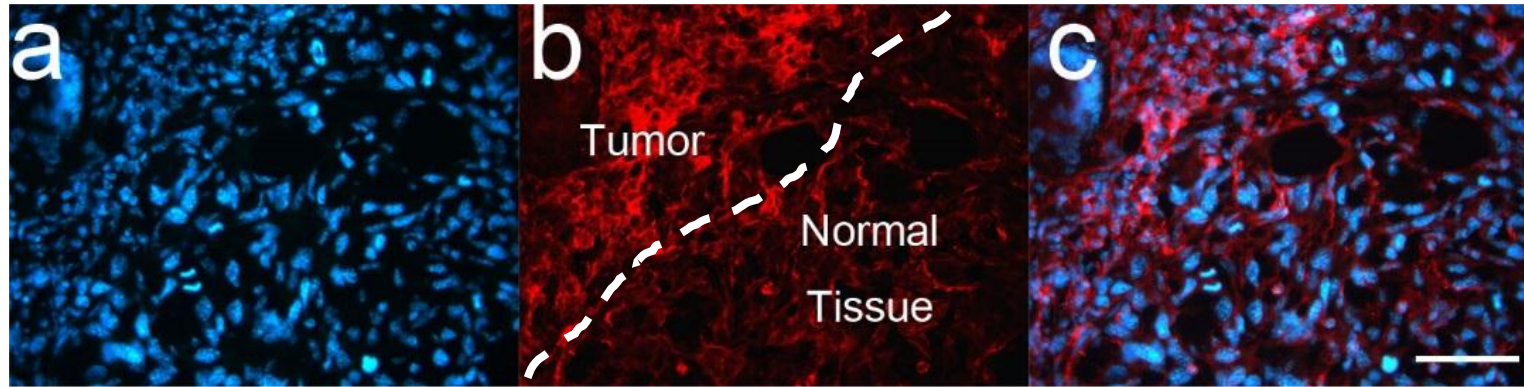
**Figure S3.** Biodistribution of the HER2-645 probe *in vivo* at different time points after IV injection using the Berthold equipment.



**Figure S4.** Quantification of the in vivo biodistribution of the MMP-750 and HER2-645 probes at different time points. The quantification of fluorescence intensity in tumor areas with MMP-750 probe (a) and HER2-645 probe (b). Signal to background ratio (SBR) analysis of MMP-750 probe (c) and HER2-645 probe (d).



**Figure S5.** Intra-operative FMI-guided BCS results using HER2-645 probe.



**Figure S6.** HER2 immunofluorescence staining on orthotopic tumor. (a) DAPI, (b) HER2 immunofluorescence, and (c) overlay. scale bar = 50  $\mu\text{m}$ .