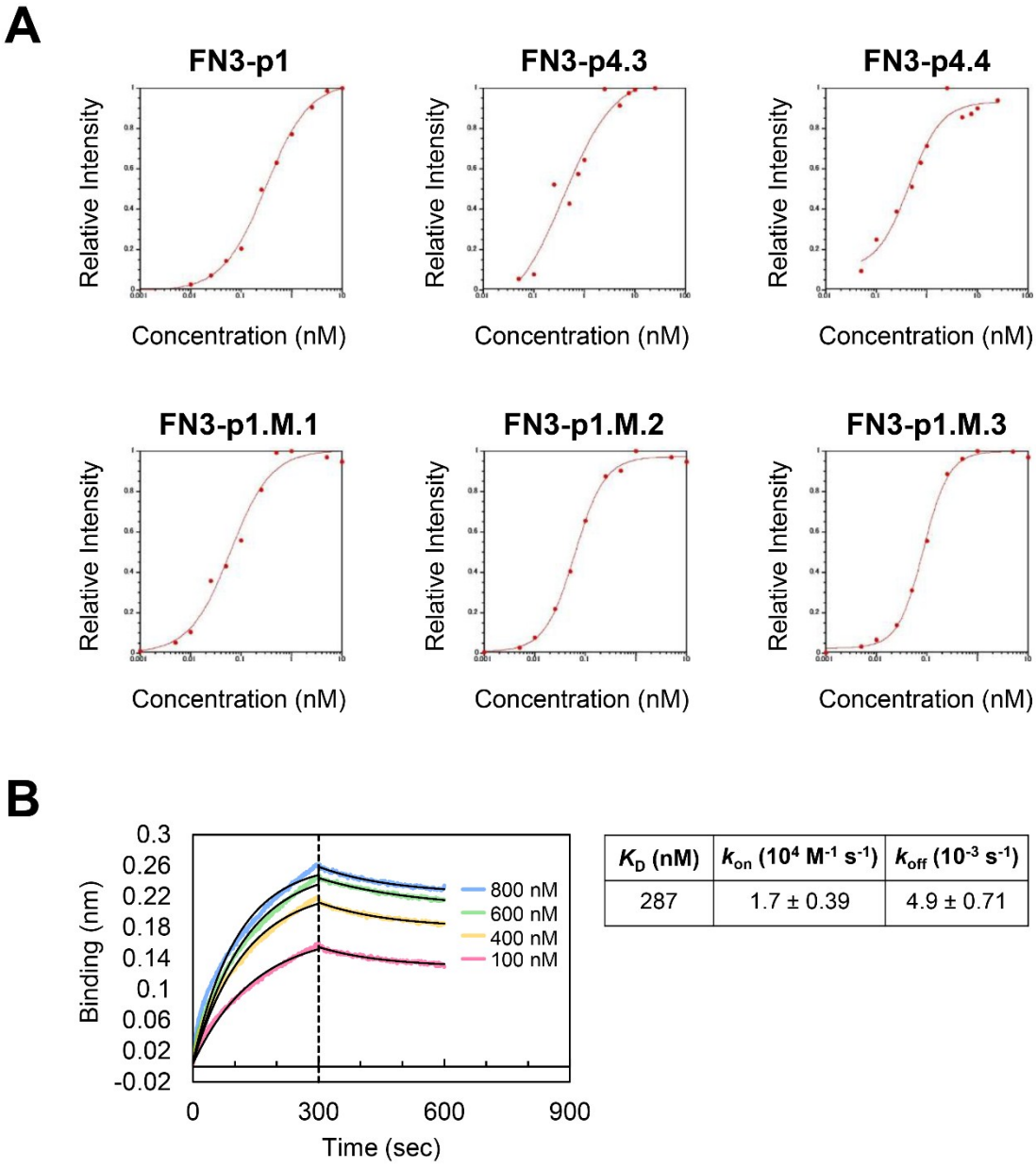
Electronic Supplementary Information

Strategic design to create HER2-targeting proteins with target-binding peptides immobilized on a fibronectin type III domain scaffold



K_D (nM)	k_{on} (10^4 M^{-1} s^{-1})	k_{off} (10^{-3} s^{-1})
287	1.7 ± 0.39	4.9 ± 0.71

Figure S1. Affinity evaluation by ELISA (A) and biolayer interferometry (B). (A) HER2-Fc was immobilized on ELISA plates and exposed to various concentrations of FN3-p1, FN3-p4.3, FN3-p4.4, FN3-p1.M.1, FN3-p1.M.2, or FN3-p1.M.3 proteins with His-tag. Binding was detected with an HRP-conjugated anti-His tag antibody. The results are representative of three experiments. (B) Biotinylated HER2-Fc was immobilized on streptavidin biosensors and exposed to several concentrations of HBP-FLAP. The representative sensorgrams from three experiments (left) and binding kinetics (right) were shown.

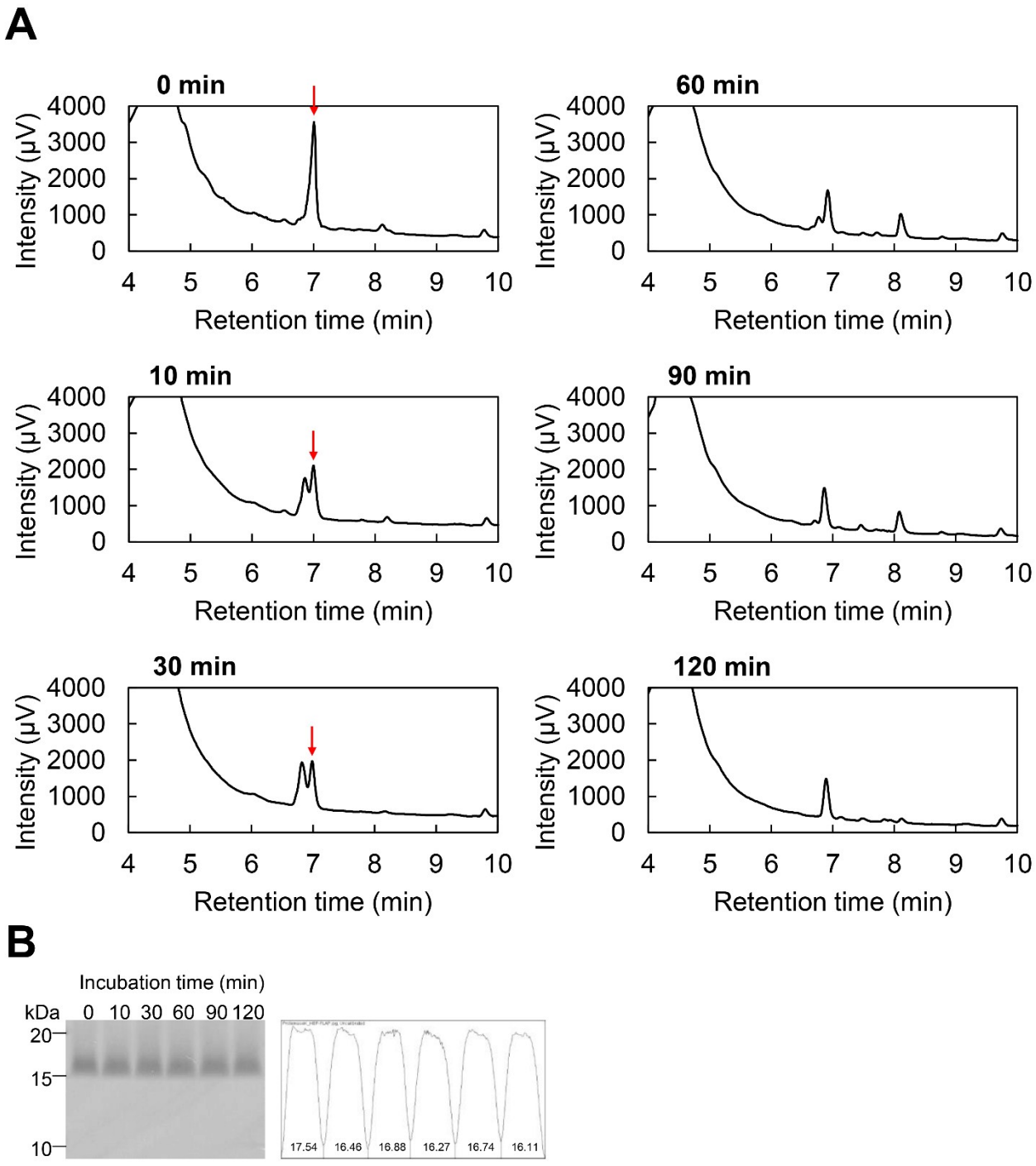


Figure S2. Proteinase K digestion. (A) HPLC analysis of p1.M.2 peptide digestion by proteinase K. C-terminally elongated peptides appeared at 7 min (red arrow) in retention time. (B) SDS-PAGE analysis (left panel) and a measurement of band intensity (right panel) of FN3-p1.M.2 treated with proteinase K at the indicated times.

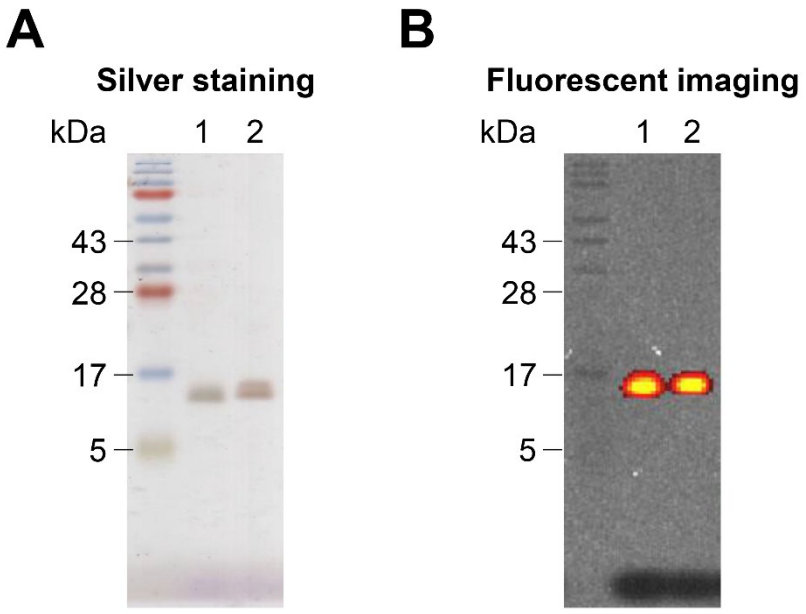


Figure S3. Preparation of *in vivo* imaging probes. SDS-PAGE analysis of HBP-FLAP-IR800 (1) and FN3-IR800 (2) by silver staining (A) and fluorescent imaging (B).