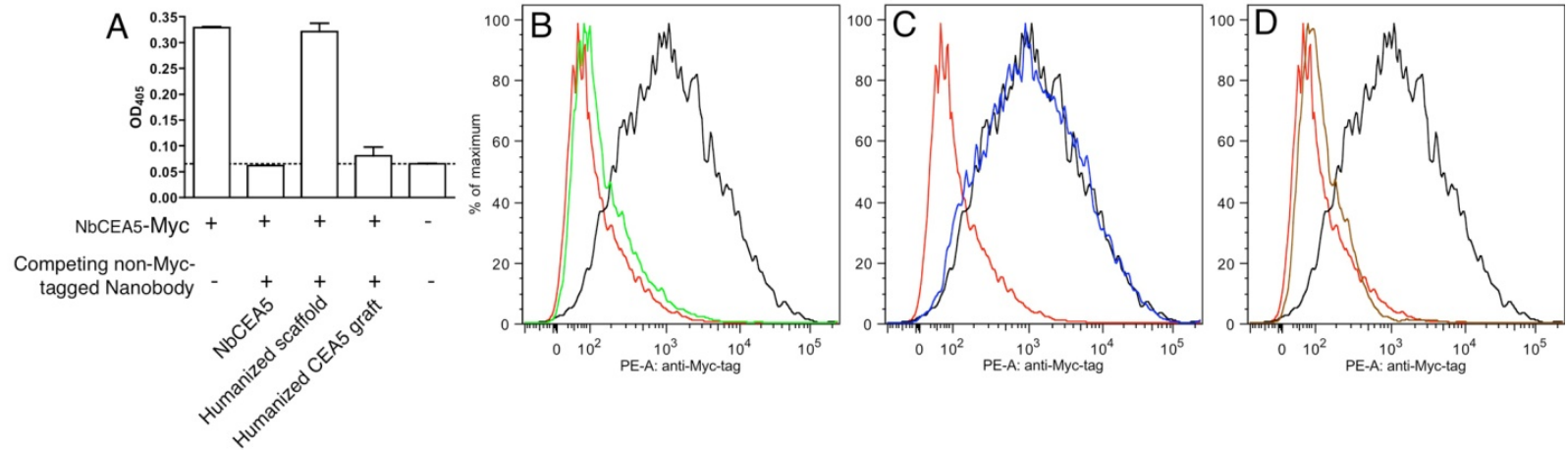
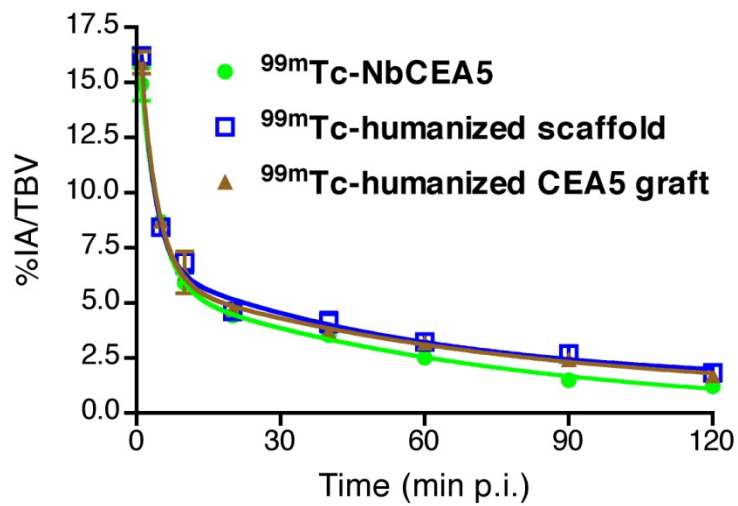


SUPPLEMENTAL FIGURE 1: Polyacrylamide gel electrophoresis of His₆-tagged Nanobodies NbCEA5 (1-3), humanized scaffold (4-6) and humanized CEA5 graft (7-9) to visualize the purity of the Nanobodies after the three successive purification steps: in *E. coli* periplasmic extracts (1,4,7), after affinity chromatography (2,5,8) and after additional Size Exclusion Chromatography (3,6,9). kDa, kilo-dalton; M.W., molecular weight.



SUPPLEMENTAL FIGURE 2: A. Competition study using ELISA: binding of Myc-tagged NbCEA5 to immobilized CEA protein is blocked by a 10-fold excess of non-Myc-tagged Nanobodies NbCEA5 and humanized CEA5 graft, but not by the humanized scaffold; B-D. Binding of Myc-tagged NbCEA5 (black lines) to CEA-expressing LS174T cells is blocked by a 10-fold excess of non-Myc-tagged Nanobodies NbCEA5 (B, green line) and humanized CEA5 graft (D, brown line), but not by humanized scaffold (C, blue line), as determined by flow cytometry. The red lines in B-D are background staining (no Nanobody added). OD₄₀₅, optical density at 405 nm.



SUPPLEMENTAL FIGURE 3: All i.v. injected ^{99m}Tc -labeled Nanobodies are cleared equally fast from the blood. Blood clearance curves are presented as mean of percent injected activity (%IA) per total blood volume (TBV) over time \pm SD (n=2).