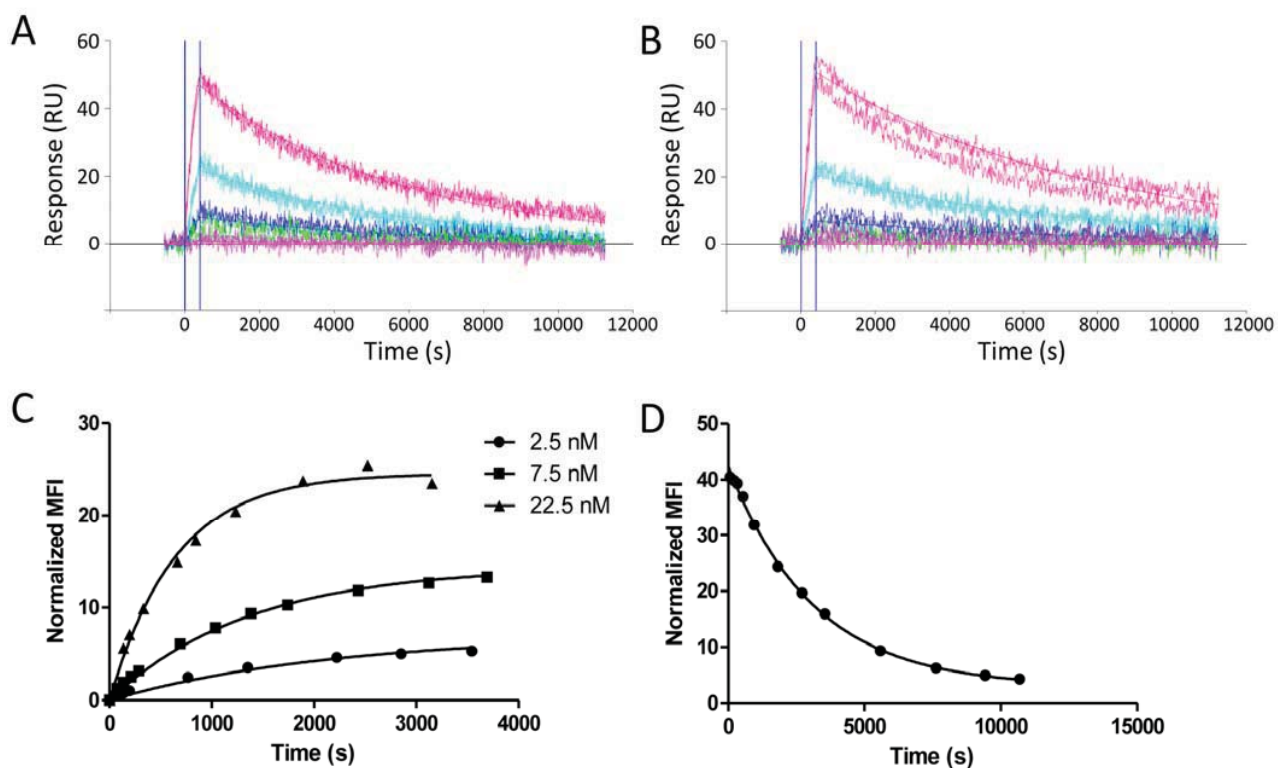


**A Novel Fusion Toxin Derived from an EpCAM-specific Designed Ankyrin Repeat  
Protein has Potent Anti-tumor Activity**

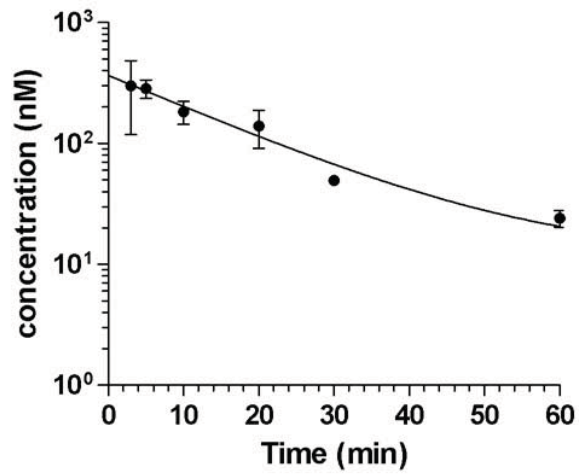
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Zangemeister-Wittke <sup>\*1,4</sup>

Supplementary Figure 1



**Supplementary Figure 1.** Determination of the EpCAM-binding affinity of Ec4 (A) and Ec4-ETA<sup>®</sup> (B) by SPR measurements. Enzymatically biotinylated EpCAM was immobilized on a neutravidin chip and increasing concentrations of Ec4 or Ec4-ETA<sup>®</sup> (0.31 nM, 1 nM, 3.16 nM, 10 nM, 31.6 nM) were assayed, each in duplicate. Using a global fit, Ec4 revealed an association rate constant of  $(1.1 \pm 0.03) \cdot 10^5 \text{ M}^{-1} \text{ s}^{-1}$  and a dissociation rate constant of  $(1.8 \pm 0.002) \cdot 10^{-4} \text{ s}^{-1}$ , yielding a  $K_D$  of  $1.7 \pm 0.006 \text{ nM}$ . For Ec4-ETA<sup>®</sup>, the association rate constant was determined as  $(6.2 \pm 0.03) \cdot 10^4 \text{ M}^{-1} \text{ s}^{-1}$  and the dissociation rate constant as  $(1.3 \pm 0.002) \cdot 10^{-4} \text{ s}^{-1}$ , giving rise to a  $K_D$  of  $2.2 \pm 0.01 \text{ nM}$ . The association (C) and dissociation (D) of fluorescently labeled Ec4 to MCF-7 cells was monitored by flow cytometry. A  $k_a$  of  $(5.5 \pm 0.3) \cdot 10^4 \text{ M}^{-1} \text{ s}^{-1}$  and a  $k_d$  of  $(3.2 \pm 0.1) \cdot 10^{-4} \text{ s}^{-1}$  result in a  $K_D$  of  $5.8 \pm 0.4 \text{ nM}$ .

Supplementary Figure 2



**Supplementary Figure 2.** Clearance of Ec4-ETA". Mice were injected i.v. via the tail vein with 30  $\mu$ g Ec4-ETA". At selected time points after injection (3, 5, 10, 20, 30 and 60 min) blood samples were drawn. The concentration of Ec4-ETA" in serum was determined by ELISA and fit to a single exponential decay function with plateau. Data are means of 2 to 4 mice per group and bars indicate SD.