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

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Supplementary Figure 1. Determination of the EpCAM-binding affinity of Ec4 (A) and Ec4-ETA" (B) by SPR measurements. Enzymatically biotinylated EpCAM was immobilized on a neutravidin chip and increasing concentrations of Ec4 or Ec4-ETA" (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 $1.7 \pm 0.006$ nM. For Ec4-ETA", 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 $2.2 \pm 0.01$ 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 $5.8 \pm 0.4$ nM.
Supplementary Figure 2. Clearance of Ec4-ETA". Mice were injected i.v. via the tail vein with 30 µg 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.