Supplementary Material

Multivalent adaptor proteins specifically target NK cells carrying a universal chimeric antigen receptor to ErbB2 (HER2)-expressing cancers

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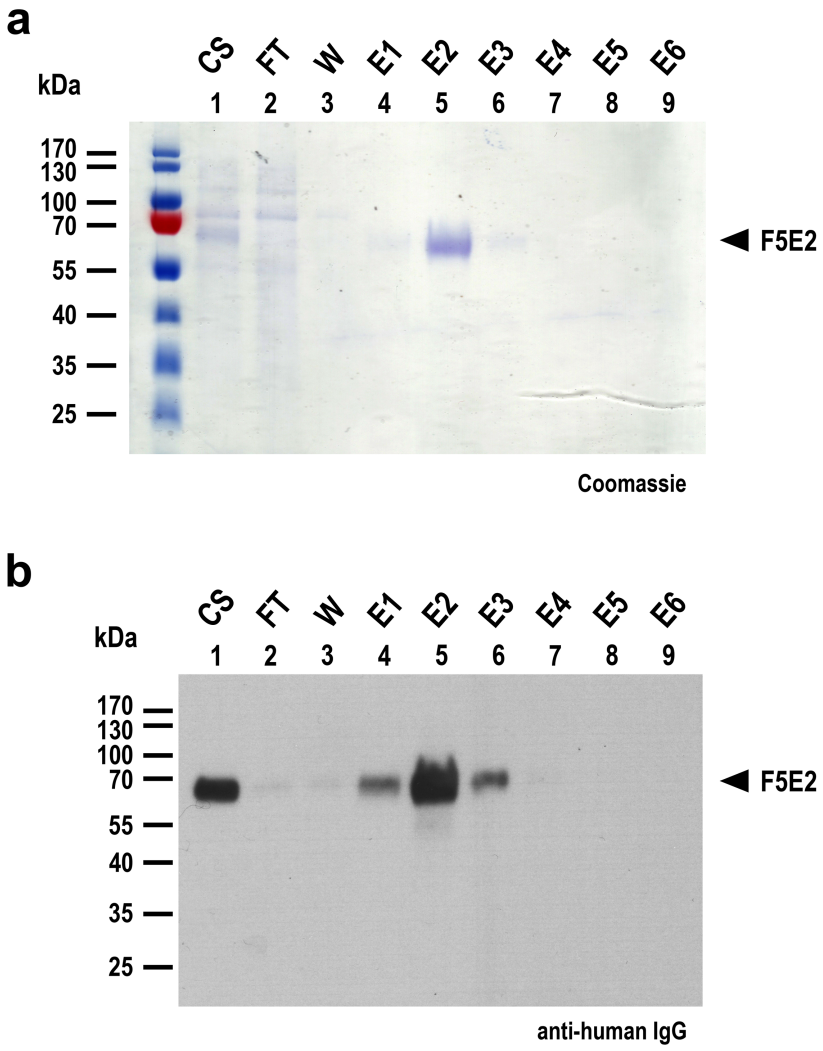
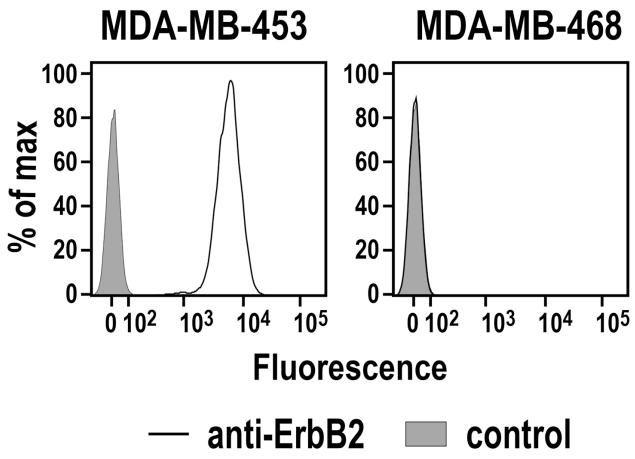
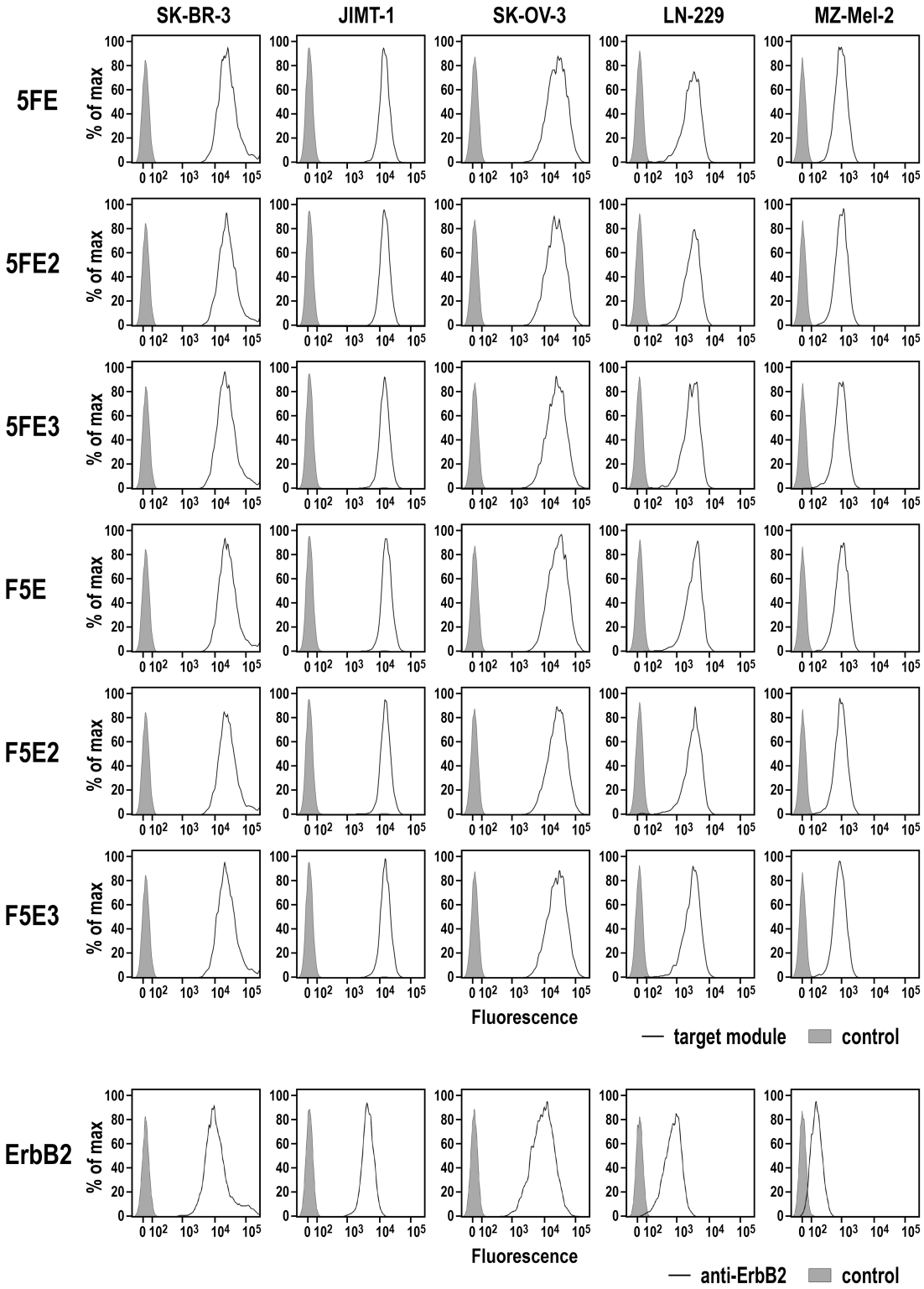


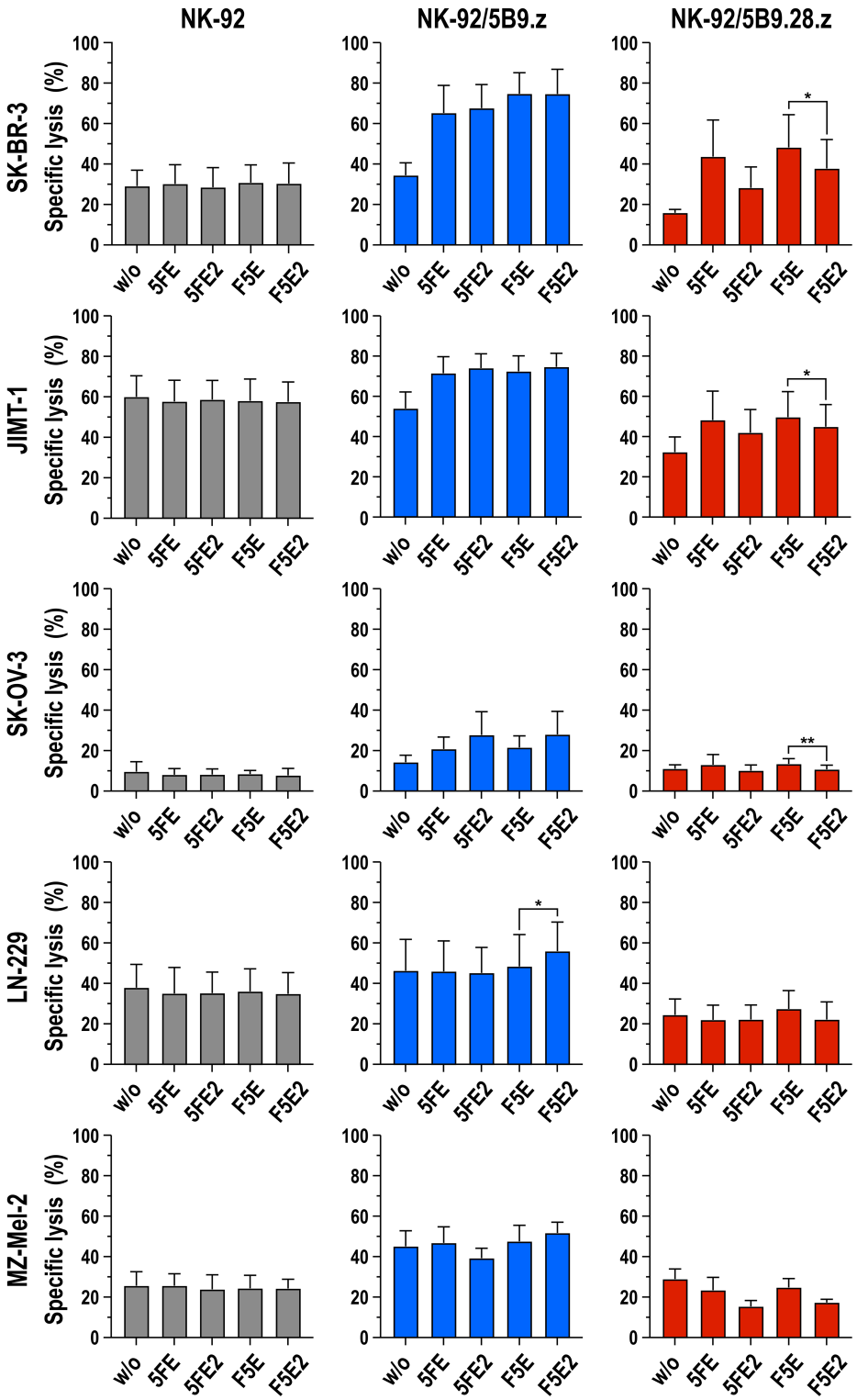
Supplementary Fig. 1 Purification of bispecific target modules. Recombinant TM proteins were expressed as soluble proteins in Expi293F cells and purified from culture supernatants by Protein G affinity chromatography as exemplarily shown for TM F5E2. **a** SDS-PAGE analysis of culture supernatant (CS), flow through (FT), wash fraction (W) and elution fractions (E) 1 to 6 under reducing conditions followed by Coomassie staining. **b** Immunoblot analysis with HRP-conjugated anti-human IgG antibody.



Supplementary Fig. 2 ErbB2 surface expression of MDA-MB-453 and MDA-MB-468 breast carcinoma cells was analyzed by flow cytometry with directly labeled anti-ErbB2 antibody (solid lines). An isotype-matched irrelevant antibody was included as control (filled areas).



Supplementary Fig. 3 Upper panels: Binding of purified target modules to ErbB2-expressing SK-BR-3 and JIMT-1 breast carcinoma cells, SK-OV-3 ovarian carcinoma cells, LN-229 glioblastoma cells and MZ-Mel-2 melanoma cells was investigated by flow cytometry with anti-human IgG antibody (solid lines). Cells stained with secondary antibody in the absence of a TM served as control (filled areas). Lower panels: For comparison, cells were stained with directly labeled anti-ErbB2 antibody (solid lines) or an isotype-matched control antibody (filled areas).



Supplementary Fig. 4 Differential effects of ErbB2-specific target modules on the cytotoxic activity of UniCAR-expressing NK-92/5B9.z (blue bars) and NK-92/5B9.28.z cells (red bars) against SK-BR-3 and JIMT-1 breast carcinoma, SK-OV-3 ovarian carcinoma, LN-229 glioblastoma and MZ-Mel-2 melanoma cells were investigated after co-incubation of effector and target cells in the absence or presence of 0.32 nM of target modules 5FE, 5FE2, F5E or F5E2 at an E/T ratio of 5:1 for 3 hours. Parental NK-92 cells were included for comparison (gray bars).