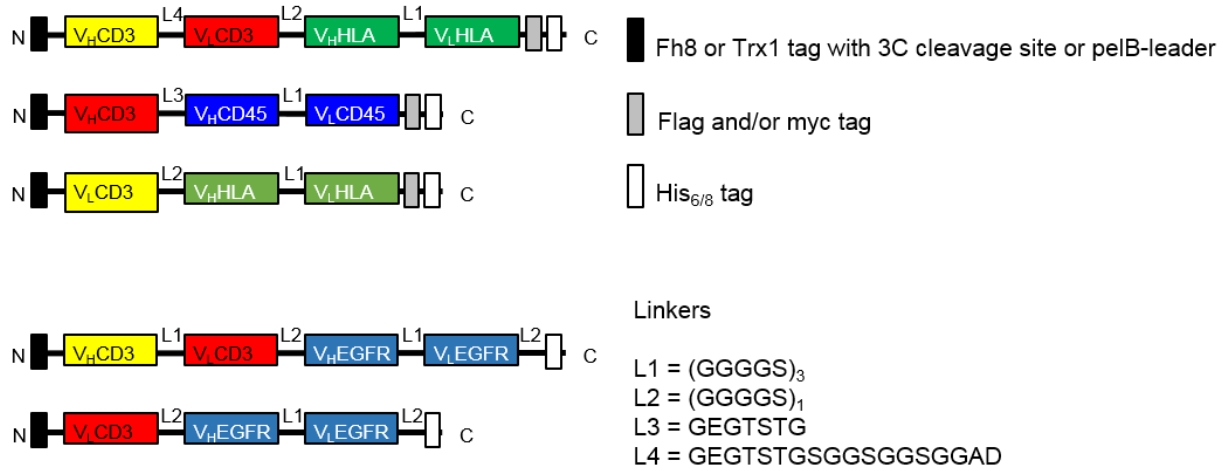
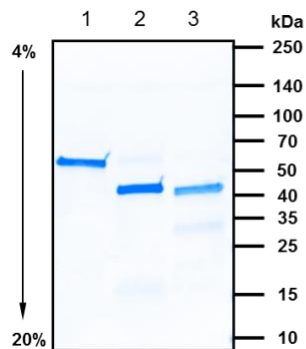


Supplementary Information:

a



b



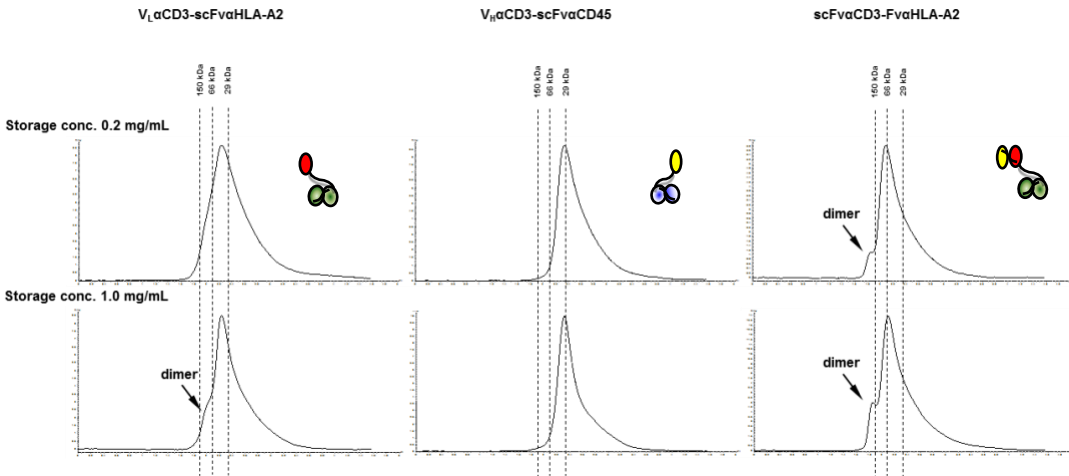
c

construct	Total amount [mg]/10l	Purity [%]
scFvαCD3-scFvαHLA-A2	2.6	93
V _H αCD3-scFvαCD45	2.5	86
V _L αCD3-scFvαHLA-A2	1.5	64

Supplementary Figure 1. (a) Schematic representation of the constructs used in this study. **(b)** SDS-PAGE analysis of hemibody constructs. 1 μg of scFvαCD3-scFvαHLA-A2 (1), V_HαCD3-scFvαCD45 (2) and V_LαCD3-scFvαHLA-A2 (3) SEC-purified construct was subjected to gradient SDS-PAGE under reducing conditions followed by Coomassie staining, a typical profile is shown

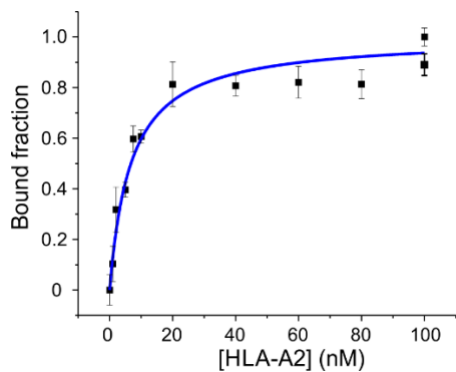
(c) Table summarizing yields of SEC-purified constructs. Protein amounts are from *E. coli* broth with an average OD₆₀₀ of 5.0.

Supplementary Figure 2: Long term stability.



SEC purified constructs were stored at the given concentrations over a period of one month at 2-8°C in diluent buffer. Subsequently, samples were analyzed by size exclusion chromatography (SEC). SEC conditions: Sample volume loaded on column = 50 μL, Column = Superdex200 Increase 5/150 GL, running buffer = diluent buffer, flow rate = 0.4 mL/min, temperature = RT, diluent buffer = 50mM Na phosphate pH7.5, 300 mM NaCl, typical profiles are shown.

Supplementary Figure 3



Binding isotherm of VLαCD3-scFvαHLA-A2/HLA-A2 interaction: : Diffusion time constants measured by FCS of 1nM VLαCD3-scFvαHLA-A2 in presence of increasing concentration of

HLA-A2 antigens, normalized to the fraction of bound V_LαCD3-scFvαHLA-A2. The blue line is a fit to the data using the conventional model for a bi-molecular binding isotherm.

Supplementary Table 1: Estimated group size for the NSG mouse model.

Group size for experiments in the NSG mouse model:

Mice per group	5	6	7	8	9	10
Effectsize ¹	2.0	1.8	1.6	1.5	1.4	1.3
SD fraction ²	0.49	0.56	0.61	0.66	0.71	0.75

¹mean difference in standard deviation (SD) units detectable with 80% power at 2-sided 0.05 level of significance with 2-sample t-test;
²SD as a fraction of biologically meaningful difference (inverse of effect size).

The sample size to achieve statistically significant data for this model was calculated based on an estimated effect size of 2 according to Ms. Katherine Guthrie, Fred Hutchinson Cancer Research Center, Seattle, USA, using GPOWER 3.1.2 ¹.

References for Supplementary Figures and Material

1. Erdfelder, E., Faul, F., & Buchner, A. GPOWER: A general power analysis program. *Behavior Research Methods, Instruments, & Computers* **28**, 1-11. (1996).