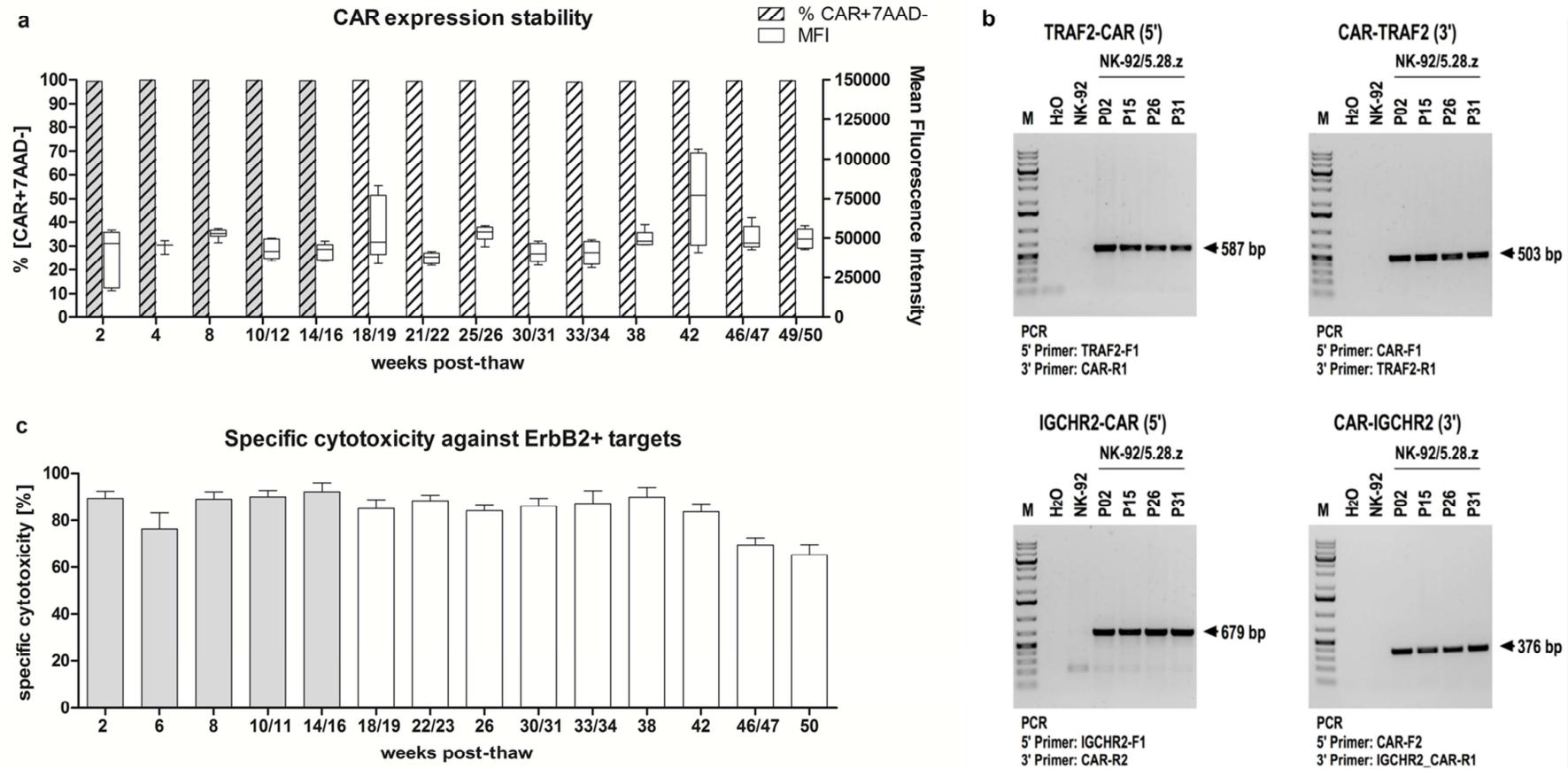


**Supplementary figure 1 Comparison of NK-92/5.28.z cell growth in two variants of X-Vivo 10 media containing transferrin from two different sources**  
 Black dots represent proliferation rates in X-Vivo 10 medium containing recombinant transferrin at indicated time points. Black squares represent X-Vivo 10 containing human holo-transferrin.



**Supplementary figure 2 Stability of transgene (CAR) expression and functionality of NK-92/5.28.z cells in a large-scale long-term maintenance culture**  
**(a)** CAR expression analysis was performed up to week 50 with the cells derived from 3 representative vials thawed from master cell bank. Data are presented as a % of CAR+7AAD- cells (hatched bars) and as a MFI (box with whiskers: min to max; Mean  $\pm$  SEM). **(b)** PCR analysis of vector integration sites in cell clone NK-92/5.28.z (#78) at different time points of continuous expansion. The genomic DNA was isolated after 1 week (passage 2, P2), 7.5 weeks (passage 15, P15), 13.5 weeks (passage 26, P26) and 16.5 weeks (passage 31, P31). Genomic DNA of unmodified parental NK-92 cells and reactions without addition of genomic DNA (H2O) served as controls. **(c)** Specific cytotoxicity against ErbB2+ targets was tested with the cells derived from 3 representative vials thawed from master cell bank up to week 50 using EuTDA killing assay. Data shown as Mean  $\pm$  SEM. Grey bars shown in the figure a and c represent three-month period of maintenance culture.

**Supplementary table 1** Impact of different serum substitutes in GMP-compliant, albumin containing culture media supplemented with 500 U/ml of IL-2 on NK-92/5.28.z cells proliferation

Culture medium	Human serum substitute	Doubling time [h]	Maximal fold expansion	Maximal concentration/ml in batch culture [ $\times 10^5$ ]
X-Vivo 10 w/o Phenol red and Gentamycin containing recombinant Transferrin (Lonza, #BE02-055Q)	5 % of heat inactivated human plasma	28.84 ± 0.5	24.97 ± 0.65	12.49 ± 0.32
	5 % of human platelet lysate 1	35.6 ± 1.38	21.37 ± 1.01	10.7 ± 0.52
	5 % of human platelet lysate 2	37.48 ± 1.89	18.07 ± 3.04	9.01 ± 1.52
	serum-free culture w/o acclimation	83.11 ± 3.08	5.3 ± 0.18	2.65 ± 0.09
	serum-free culture post-acclimation	47.37 ± 2.75	20.4 ± 1.71	10.2 ± 0.85
X-Vivo 10 w/o Phenol red and Gentamycin (Lonza, #BE04-743Q)	5 % of heat inactivated human plasma	34.39 ± 0.63	15 ± 0.6	7.5 ± 0.3
CellGro SCGM (CellGenix)	5 % of heat inactivated human plasma	33.29 ± 0.2	21.23 ± 2.34	10.62 ± 1.17
	serum-free culture	90.97 ± 5.47	3.03 ± 0.2	1.52 ± 0.1

Grey field indicates optimal formulation and respective growth parameters. Results shown as Mean ± SEM

**Supplementary table 2** Cytokine profile of PMA (50 ng/ml)/Ionomycin (500 ng/ml) stimulated target cells

Target cell line	Soluble factor	Concentration [pg/ml]
MDA-MB-453	IL-8	9.93 ± 1.2
MDA-MB-468	IL-6	247 ± 30.09
	IL-8	635 ± 27.53
	TNF	6.64 ± 2.72
K562	GM-CSF	286.61 ± 76.55
	IL-6	235.26 ± 64.34
	IL-8	524.61 (n=1)
	TNF	311.92 ± 47.92

Results shown as Mean ± SEM