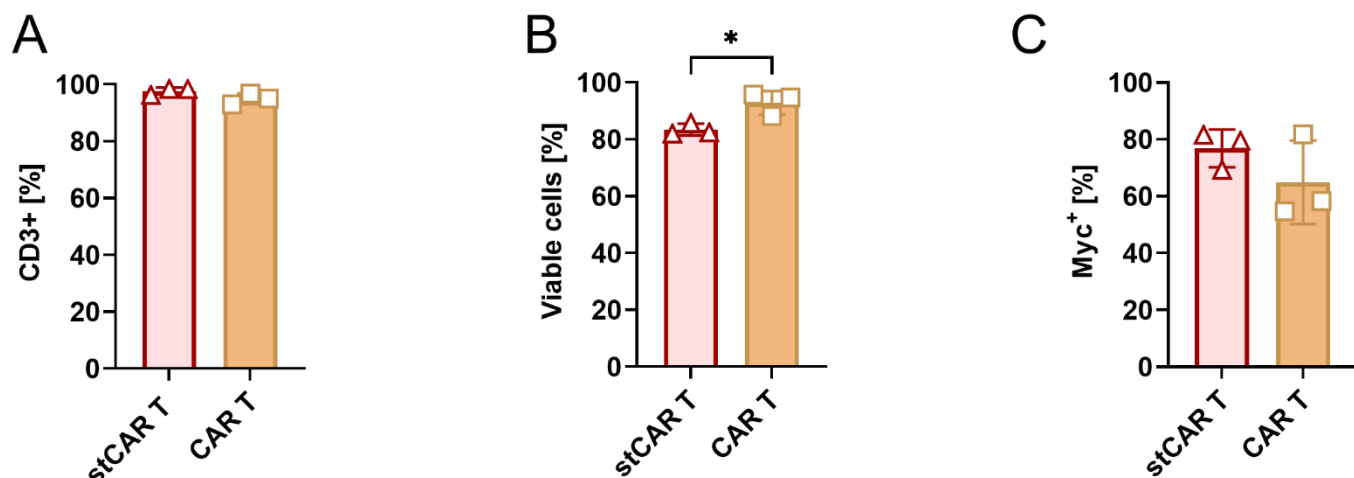


Rapid cytokine release syndrome caused by short-term CAR T cells in a preclinical mouse model

Appendix

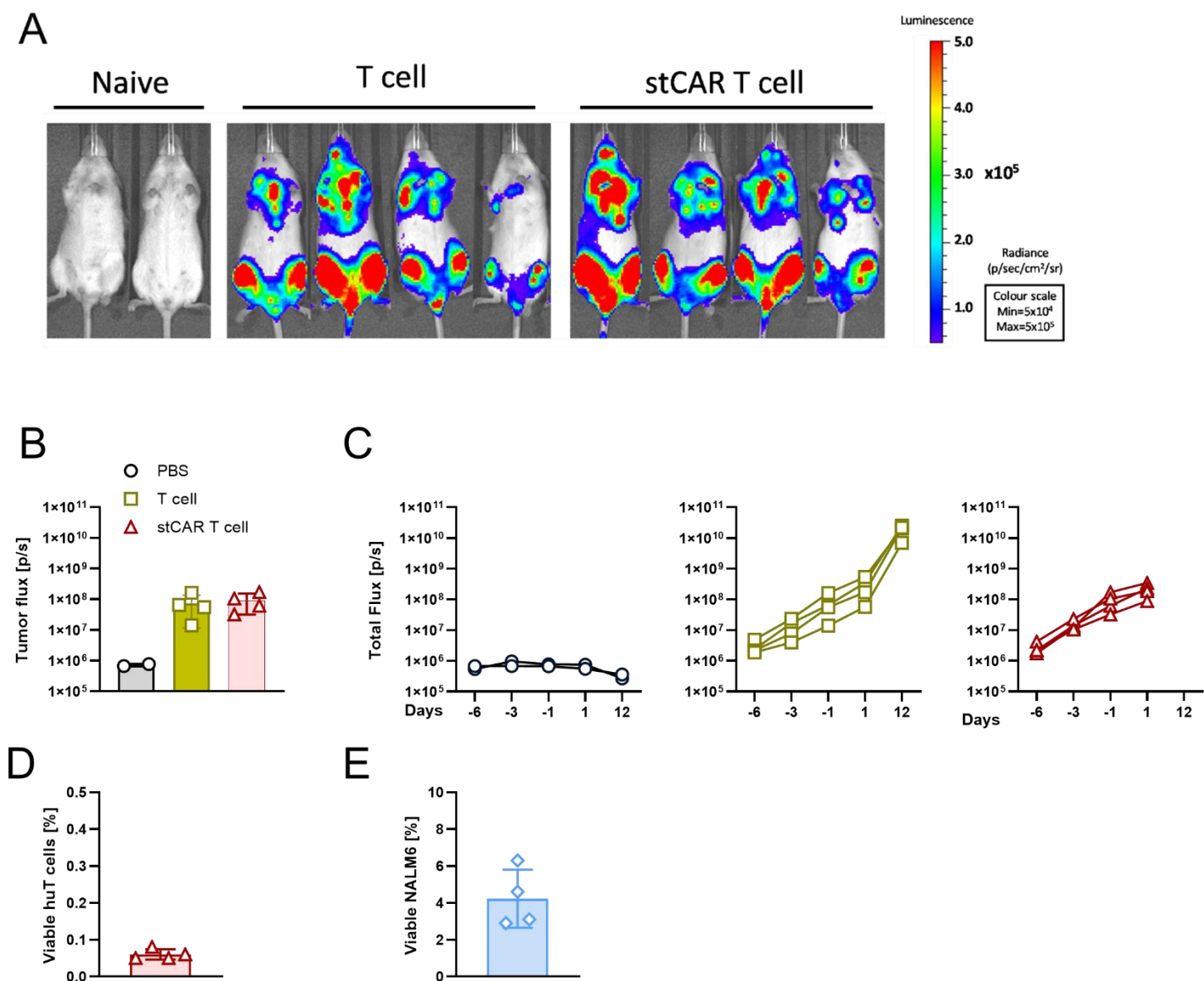
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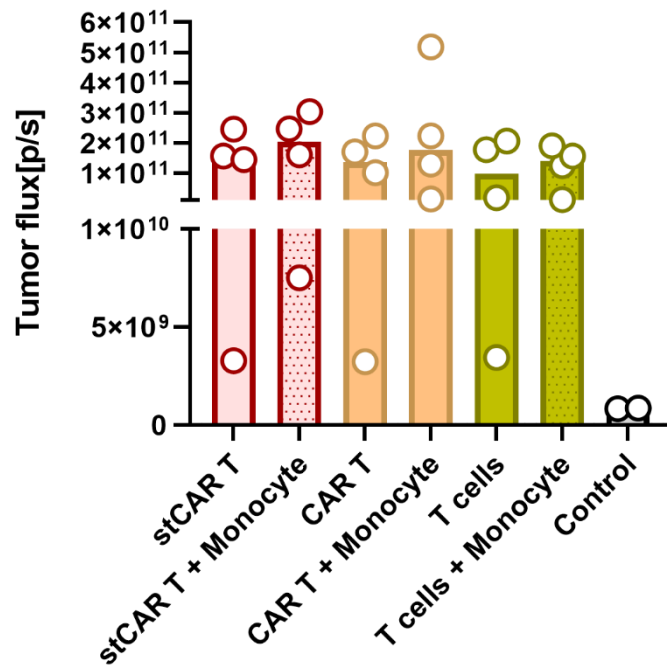
Appendix Figure S1: Properties of short-term and conventional CAR T cells.

Short-term CAR T (stCAR T) cells and CAR T cells were generated from human PBMCs. T cell frequencies **(A)** and cell viabilities **(B)** were determined by flow cytometry. Mean values and standard deviations are shown for three donors. **(C)** Expression of CAR detected after over night co-culture with NALM6 target cells as determined by myc tag detection by flow cytometry. Statistics were determined by unpaired t-test with indicated significant $p = 0.0235$.



Appendix Figure S2: Tumor cell engraftment in NSG-SGM3 mice.

Tumor burden in NSG-SGM3 mice engrafted with EBFP and luciferase expressing NALM6 tumor cells was determined by *in vivo* bioluminescence imaging (IVIS). Mice without tumor cell engraftment (PBS) were used to determine background signals. **(A)** Tumor burden one day prior to stCAR T cell and T cell treatment are shown as IVIS images of the ventral side of each mouse with a color scale for tumor signal intensity. **(B)** Bar diagrams summarize quantification of the tumor flux with a single data point for each mouse including mean and standard deviation of the group. **(C)** The kinetics of the tumor flux over time is shown for each mouse represented by one line. Percentages of human T cells **(D)** and Nalm6 cells **(E)** in the bone marrow. n = 2 (PBS), 4 (T cell), 4 (stCAR T cell).



Appendix Figure S3: Supplemenatry data to Fig. 4

Tumour burden was assessed by measuring luciferase activity on day 9 after NALM6 cell injections using IVIS. Based on the measured signals, animals were assigned to the different groups to achieve equal tumour burden between groups.

Appendix Table S1: P-values of statistically significant pair-wise comparisons for the levels of neutrophils in the groups of Fig. EV3B

Comparisons	p value¹
stCART wo target vs. stCART+mono	0.0006
stCART vs. stCART+mono	0.0056
stCART+mono vs. CART	0.0008
stCART+mono vs. CART+mono	0.0167
stCART+mono vs. T cells	<0.0001
stCART+mono vs. T cells +mono	<0.0001
stCART+mono vs. PBS	<0.0001

¹as determined by two-way ANOVA Šídák's multiple comparisons test

Appendix Table S2: Detailed information about antibodies used in the study

	Antibody	Clone	Fluorochrome	Concentration	Volume	Company	Order NO
1	FcR-blocking	-	-		1µL	Miltenyi Biotec	130-059-901
2	CD45	5B1	VioBlue	900 µg/µl	0.5µL	Miltenyi Biotec	130-092-880
		2D1	BV510	0.005 µg/µl	0.5µL	BioLegend	368526
3	CD3	BW264/56	PerCP	375.0 µg/µl	0.5µL	Miltenyi Biotec	130-113-131
		HIT3a	BV605	0.1 µg/µl	0.5µL	BD Bioscience	564712
5	CD14	REA599	APC	25µg/µl	0.5µL	Miltenyi Biotec	130-110-520
		Tuk	Percp	25µg/µl	0.5µL	Miltenyi Biotec	130-094-969
6	CD4	L200	PE	0.003 µg/µl	0.5µL	BD Bioscience	550630
		VIT-4	PerCp/ VioBlue	500µg/µl 200µg/µl	0.5µL	Miltenyi Biotec	130-113- 779/219
7	CD8	RPA-T8	BV786	0.003 µg/µl	0.5µL	BD Bioscience	555369
		BW135/80	APC/PE		0.5µL	Miltenyi Biotec	130-113-154
8	VSV-G	8G5F11	unlabeled	0.1 µg/µl	0.1µL	Kerafest	EB0010
9	anti-mouse IgG	polyclonal	AF647	1.4mg/ml	0.5µL	Jackson ImmunoResearch	115-605-164
10	CD45RA	T6D11	VioBlue	450µg/µl	0.5µL	Miltenyi Biotec	130-113-360
11	CD62L	145/15	Pe-Vio 770	37.5µg/µl	0.5µL	Miltenyi Biotec	130-113-621
12	LAG-3	REA351	VioBlue	100µg/µl	0.5µL	Miltenyi Biotec	130-118-549
13	PD1	PD1.3.1.3	PE-Vio770	225µg/µl	0.5µL	Miltenyi Biotec	130-117-698
14	TIM-3	7D3	PE	0.1 µg/µl	1µL	BD Bioscience	563422
15	Ly-6G	REA126	PE-Vio770	10µg/µl	1µL	Miltenyi Biotec	130-107-977
16	CD11b	M1/70	Percp	0.2 µg/ml	0.5µL	eBioscience	45-0112-80
17	CD11C	N4/18	VioBlue	0.2 µg/ml	0.5µL	eBioscience	15-0114-82
18	anti-Myc	SH1- 26e7.1.3	FITC	500µg/µl	0.5µL	Miltenyi Biotec	130-116-485