Fig. S1

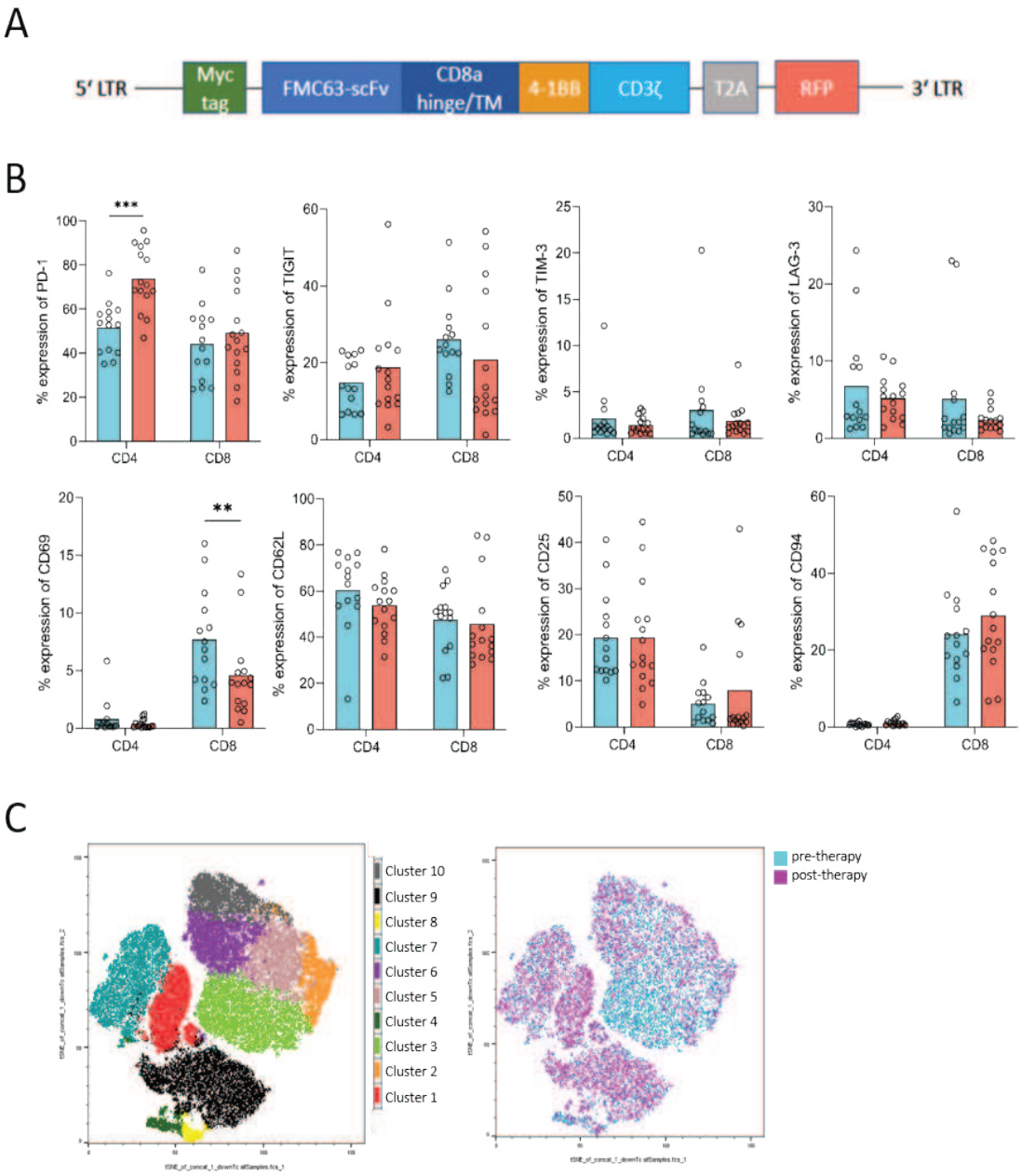

Fig. S1: Previous chemotherapy exposure alters T cell composition of B-NHL patients. (A) Map of the second-generation CAR construct used for functional testing. (B) Expression of PD-1, TIGIT, TIM-3, LAG-3, CD69, CD62L, CD25 and CD94 on both cohorts. (C) t-SNE of the tested flow cytometry panel.

Fig. S2

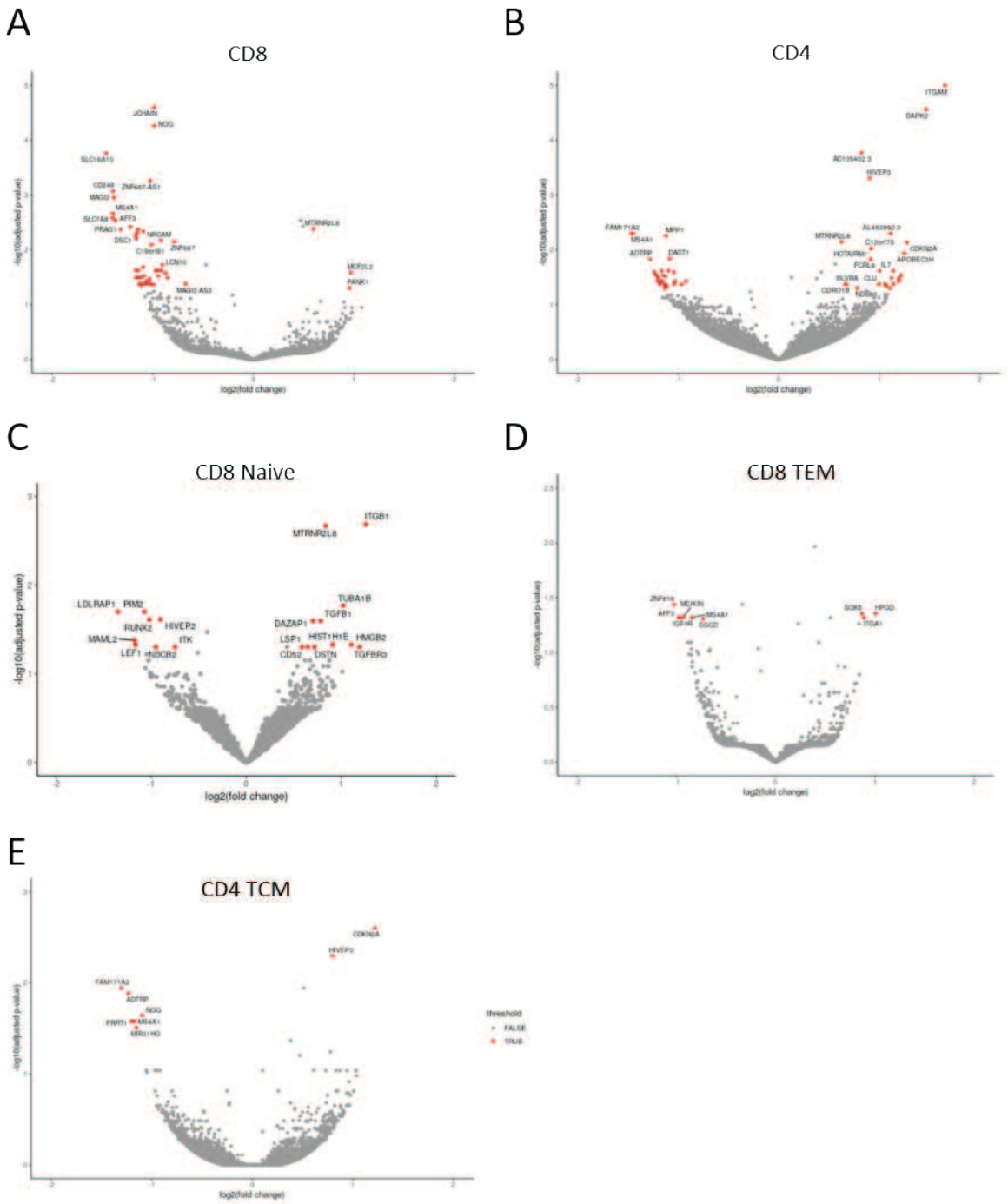

Fig. S2: Single cell RNA sequencing uncovers changes in gene expression of treatment-exposed T cells. Volcano plots of top up- and down-regulated genes in (A) CD8 T cells, (B) CD4 T cells, (C) CD8 Naïve T cells, (D) CD8 EM T cells and (E) CD4 CM T cells.

Fig. S3

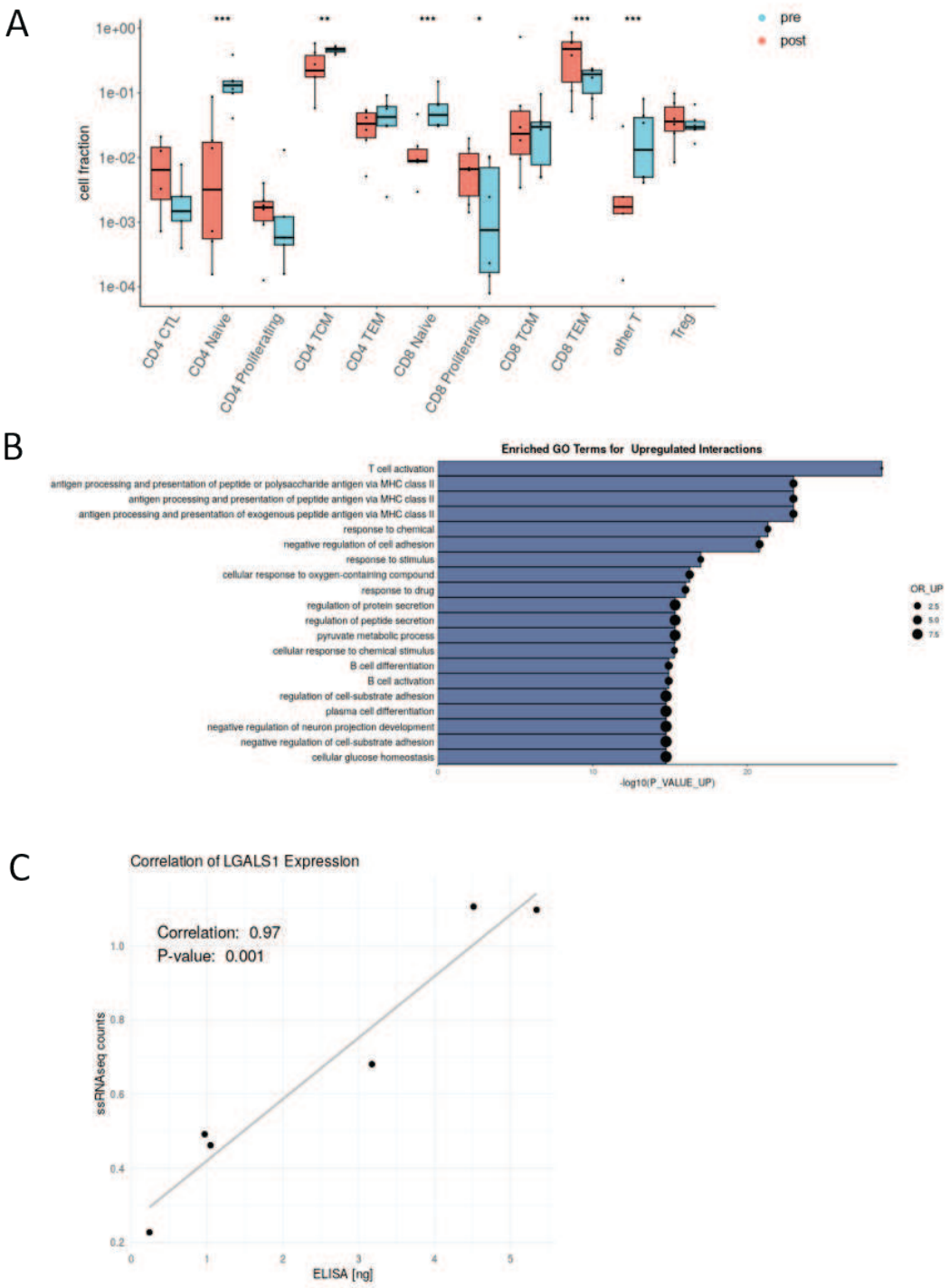

Fig. S3: Single cell RNA sequencing uncovers changes in gene expression of treatment-exposed T cells. (A) Comparison of cell type distributions between pre- and post-therapy T cells. t-tests were used for statistical significance. (B) Gene ontology analysis on the top upregulated interactions of post-therapy T cells. (C) Correlation of RNA counts obtained by single cell RNA sequencing and protein level detected via ELISA for Galectin-1.

Fig. S4

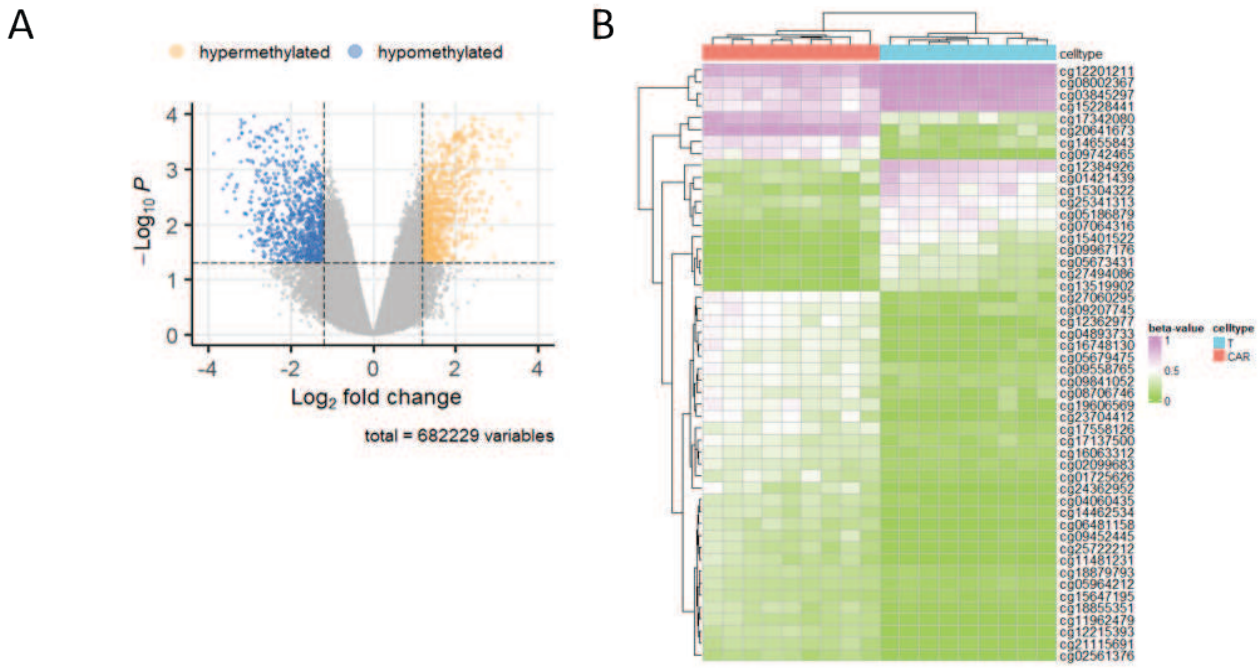

Fig. S4: Epigenetic changes occurring in T cells during CAR T cell manufacturing. (A) Volcano plot of hyper- and hypomethylated CpGs in a paired analysis of T cells vs CAR T cells. (B) Hierarchical clustering depicting the 50 top differentially methylated CpGs between T cells and CAR T cells.

Fig. S5

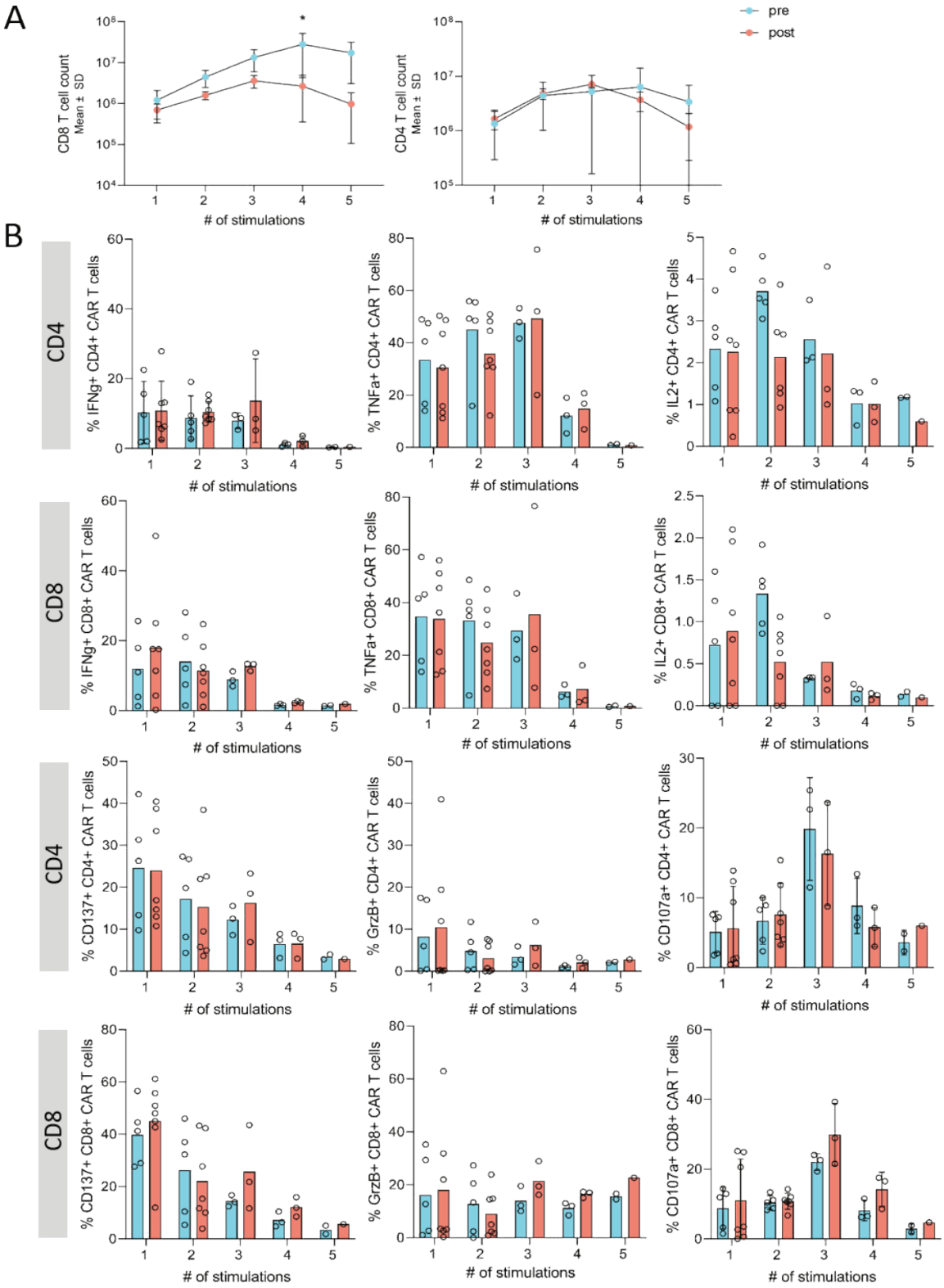

Fig. S5: Pre-therapy T cells generate a more effective CAR T product. (A) expansion of CD8 and CD4 T cells during the repetitive kill assay. (B) Expression of a number of markers 24h after antigen stimulus.

Fig. S6

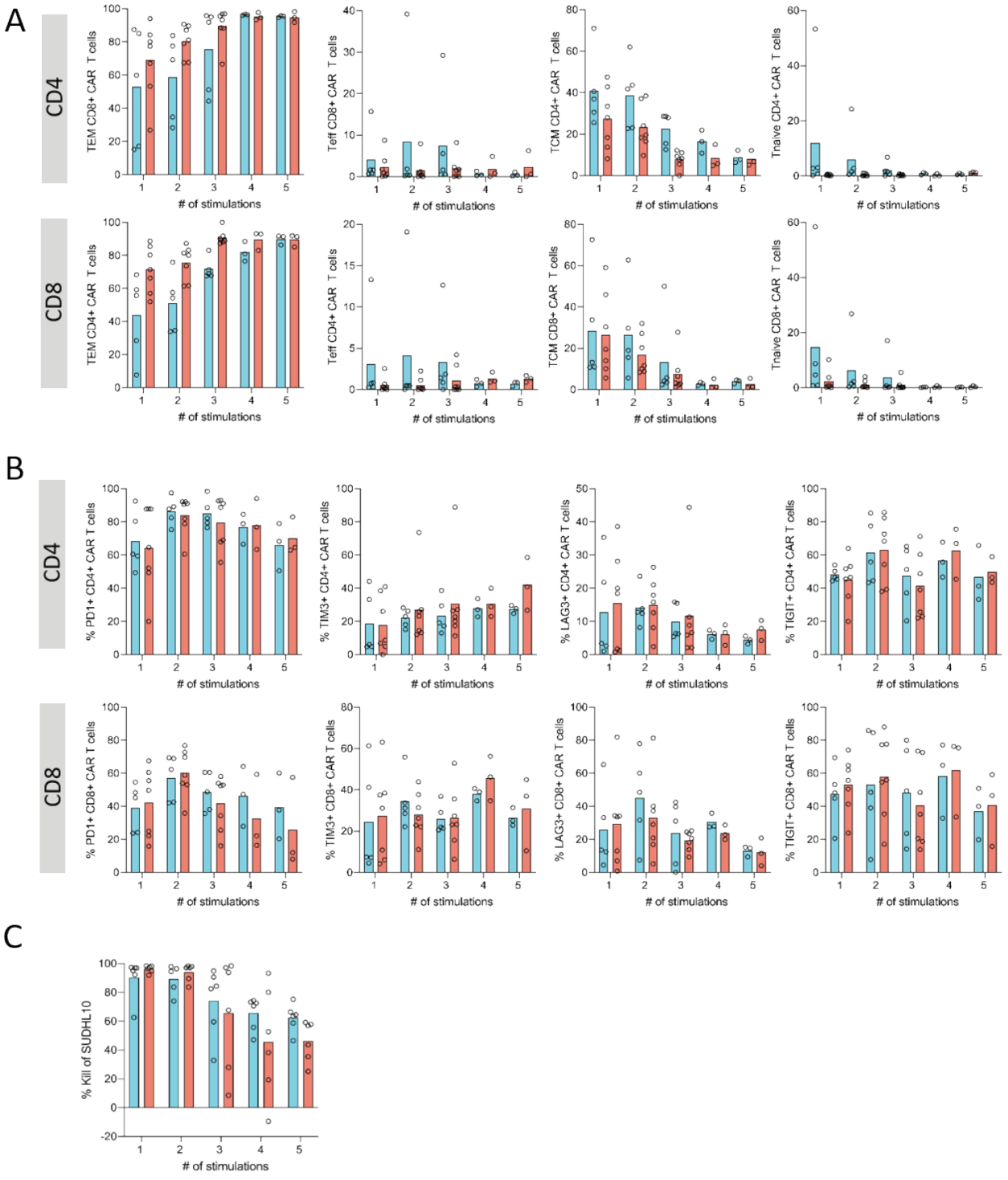

Fig. S6: Pre-therapy T cells generate a more effective CAR T product. (A) Phenotype distribution and (B) expression of a number of markers 24h after antigen stimulus. (C) Kill of SUDHL-10 target cell line in 5 repetitive stimulations. Kill is defined as the reduction in tumor cells relative to the start of the experiment.

ID	Cohort	Disease	Sex	Age	Immunochemotherapy	Time since	Assays performed
1	pre	DLBCL	M	45			A, B, C, D
2	pre	DLBCL	M	66			A, B, C, D
3	pre	DLBCL	M	37			A, B, C, D
4	pre	DLBCL	M	87			A, B, C
5	pre	DLBCL	M	65			A, B, C
6	pre	DLBCL	M	60			A, B, C, E
7	pre	FL	M	44			A, C, F
8	pre	FL	F	69			A, C, F
9	pre	FL	M	47			A, C, F
10	pre	DLBCL	F	22			A, C, D
11	pre	PMBCL	M	57			A, C, D
12	pre	FL	F	58			A, F
13	pre	MCL	M	57			A, F
14	pre	PMBCL	M	33			A
15	pre	FL	M	69			F
16	pre	DLBCL	F	53			F
17	pre	DLBCL	F	57			F
18	pre	DLBCL	M	78			F
19	pre	DLBCL	M	86			F
20	pre	DLBCL	F	56			F
21	post	DLBCL	M	47	R-Benda, R-CHOP, Pola-BR	< 3 months	A, B, C, D
22	post	DLBCL	F	59	R-Benda, R-CHOP, Tafa-Len	< 3 months	A, B, C, D
23	post	DLBCL	M	71	R-CHOP, R-DHAP	< 3 months	A, B, C, D
24	post	DLBCL	M	52	R-CHOP, R-DHAP, Pola-BR	< 3 months	A, B, C
25	post	DLBCL	F	70	R-CHOP, B-ALL protocol > 55 years	11 months	A, B, C
26	post	DLBCL	M	71	R-CHOP, R-GDP, R-Pola-BR	< 3 months	A, B, C
27	post	FL/DLBCL	M	59	R-CHOP, O-Benda, R2, R-DHAP/ICE	< 3 months	A, C, F
28	post	FL	M	61	R-CHOP, R-Benda	10 years	A, C, D
29	post	DLBCL	F	79	R-CHOP, R-GemOx	15 months	A, F
30	post	DLBCL	F	81	R-CHOP, Pola-BR	< 3 months	A, F
31	post	MCL	M	50	R-CHOP/DHAP, R-Benda, Nivo-ICE, Ibr, alloHSCT, R-Ibr, Zanu	< 3 months	A, F
32	post	FL	F	50	O-CHOP, R-DHAP, autoHSCT, R-CHP, O-Lena	< 3 months	A, F
33	post	DLBCL	M	47	R-CHOP-MTX	4 months	A, F
34	post	FL	F	76	R-COEP, R-GemOx, Pix	< 3 months	A
35	post	DLBCL/FL	M	51	R-CHOP, R-DHAO/DHAOx, Pola-BR	< 3 months	A
36	post	MCL	F	67	R-CHOP/DHAP, autoHSCT, Ibr/Ibr+Ven	< 3 months	A
37	post	DLBCL	M	73	R-CHOP, R-DHAOx, Pola-BR	< 3 months	C
38	post	FL	F	63	R-Benda, R-CHOP, R2, R-CHOP	< 3 months	C
39	post	DLBCL	M	74	R-CHOP, Pola-BR	< 3 months	C
40	post	FL	M	74	R-Benda/CVP, Mosu-Len, Obi-Zanu	< 3 months	F
41	post	DLBCL	F	60	R-CHOP	9 months	F
42	post	MCL	M	74	R-CHOP/DHAP, autoHSCT, Ibr, alloHSCT		F
43	post	FL	M	54	R-CHOP	3 years	F
44	post	FL	M	62	R-CHOP	5 months	F

Table S1: Sample overview. List of the patient data of samples used for each assay. Abbreviations are explained on the following page.

Assays:

Flow cytometry T cells: A

Single cell sequencing T cells: B

DNA methylation profiling T cells: C

DNA methylation profiling CAR T cells: D

Functional Assays CAR T: E

Immunochemotherapy:

R-CHOP = R: Rituximab, C: Cyclophosphamide, H: Doxorubicin, O: Vincristine (Oncovin), P: Prednisolone

R-COEP = R: Rituximab, C: Cyclophosphamide; O: Vincristine (Oncovin), E: Etoposide, P: Prednisolone

R-CHP = R: Rituximab, C: Cyclophosphamide, H: Doxorubicin, P: Prednisolone

R-CVP = R: Rituximab, C: Cyclophosphamide, V: Vincristine, P: Prednisolone

R-GemOx = R: Rituximab, Gem: Gemcitabine, Ox: Oxaliplatin

Pola-BR = Pola: Polatuzumab, B: Bendamustine, R: Rituximab

R-DHAP = R: Rituximab, D: Dexamethasone, HA: High-dose Cytarabine (Ara-C), P: Cisplatin (Platinum)

R-DHAOx = R: Rituximab, D: Dexamethasone, HA: High-dose Cytarabine (Ara-C), Ox: Oxaliplatin

Tafa-Len = Tafa: Tafasitamab, Len: Lenalidomide

R-GDP = G: Gemcitabine, D: Dexamethasone, P: Cisplatin (Platinum)

O-Benda = O: Obinutuzumab, Benda: Bendamustine

R2 = R: Rituximab, R: Lenalidomide (Revlimid)

ICE = I: Ifosfamide, C: Carboplatin, E: Etoposide

O-Lena = O: Obinutuzumab, Lena: Lenalidomide

alloHSCT = allogeneic Hematopoietic Stem Cell Transplantation

autoHSCT = autologous Hematopoietic Stem Cell Transplantation

Ibr = Ibrutinib

MTX = Methotrexate

Mosu = Mosunetuzumab

Nivo = Nivolumab

Pix = Pixantrone

Ven = Venetoclax

Zanu = Zanubrutinib

B-ALL = B cell Acute Lymphoblastic Leukemia

Table S2

up in post-therapy samples	down in post-therapy samples
SOX5	THNSL2
SP140	HIVEP2
PARD6G	AL355516.1
MOSPD3	MRC2
NEU4	IGHA1
AL133477.2	AL162253.2
HOTAIRM1	RAPGEF4
AC105402.3	TGFA
AL512625.1	ZNF667
HLA-B	PRAG1
CD70	DBH-AS1
ZNF683	LCN10
XIST	DSC1
OASL	C19orf81
FDFT1	LTBP3
LGALS1	AL078604.4
TBX20	AC107884.1
AL450992.3	MEIKIN
HIVEP3	EPB41L5
SMC4	CELSR1
APOBEC3H	ITGA6
AC131097.3	ANKRD55
RGS9	LINC01258
MTRNR2L8	MPP1
BFSP1	LINC01954
CTSW	DYNC2LI1
RORB	SSTR3
RIBC2	AC090204.1
DOCK5	MAP3K14-AS1
DPY19L1	AL161644.1
ITGAM	ZNF667-AS1
CDKN2A	SARDH
ARHGAP10	AP000446.1
CDKN2B-AS1	LINC02246
	MAML2
	DBNDD1
	LINC00920
	EBF4
	NRCAM
	CCR10
	GRB10

	GNAI1
	AC104806.2
	PBX1
	NUPR2
	DACT1
	GNG7
	LINC01281
	ADTRP
	WNK3
	ADGRA3
	MS4A1
	AP005380.1
	AL163932.1
	LAMC3
	CELA1
	GPA33
	AKR1C1
	CDK5R1
	RGL1
	IGKC
	AP000547.3
	C14orf132
	CTDSPL
	TPCN1
	TSHZ2
	AC010896.1
	AC113143.3
	PRRT1
	KCNIP4
	ZNF223
	ALDH7A1
	SPATA32
	LINC02752
	MARCH1
	ZSCAN23
	MMP11
	ACTN1-AS1
	NOG
	AL158151.1
	CCDC110
	IQCK
	MMP28
	IGF1R

	NMNAT3
	SLC7A8
	RGS18
	DPYSL4
	ADD2
	AC011726.3
	B4GALNT4
	SGCD
	KLHL29
	KRT73
	EDAR
	AC092574.2
	AC007342.4
	AC092645.2
	WDR31
	AL109930.1
	Z98745.2
	TP53TG3D
	AJAP1
	JCHAIN
	SDK1
	SH3BGRL2
	MAN1C1
	TMEM272
	AC006333.2
	SFRP5
	STAP1
	CEBPE
	CAMKK1
	ACTN1
	NKILA
	IRAIN
	CNNM1
	TMIGD2
	CALCB
	C9orf24
	AL022724.3
	AC011484.1

Table S2: Significantly up- and down-regulated genes in T cells of patients post-therapy compared to pre-therapy. DEGs with an adj.p<0.05 and absolute logFC>0.58.