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## **Supplemental Information**

**Efficacy and Safety of CD28- or 4-1BB-Based**

**CD19 CAR-T Cells in B Cell**

**Acute Lymphoblastic Leukemia**

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## Supplementary Information

**Table S1. Statistical analysis of the bioluminescent intensity at the tumor region of mice treated with CD28 CAR-T (28z) or 4-1BB CAR-T (bbz) at the dose of  $1 \times 10^6$  cells/mouse.**

	Saline		bbz		28z	
	Mean	SD	Mean	SD	Mean	SD
Day 0	7.01E+03	1.15E+03	7.67E+03	1.21E+03	7.47E+03	4.11E+03
Day 5	8.53E+04	4.56E+04	8.04E+04	1.37E+04	7.60E+03	4.23E+03
Day 7	1.93E+04	9.60E+04	4.97E+03 <sup>##*</sup>	1.61E+03	2.49E+04 <sup>##</sup>	1.41E+04
Day 14	1.69E+06	1.19E+06	1.26E+03 <sup>##*</sup>	5.12E+02	1.54E+05 <sup>#</sup>	8.23E+04
Day 17	1.15E+07	6.84E+06	8.02E+03 <sup>##*</sup>	3.07E+03	1.51E+06 <sup>#</sup>	1.20E+06
Day 21	3.16E+07	5.42E+06	3.24E+03 <sup>##*</sup>	2.69E+03	9.99E+06 <sup>#</sup>	9.30E+06

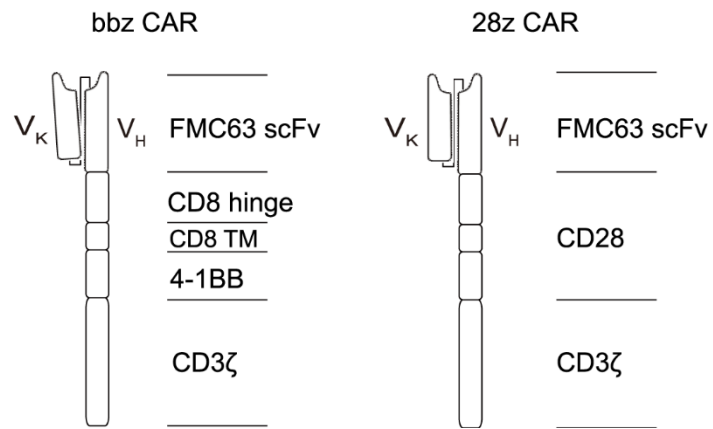
#,  $P < 0.05$  compared with saline group; ##,  $P < 0.01$  compared with saline group; \*,  $P < 0.05$  compared with 28z group.

**Table S2. Characteristics of patients infused with CD28- or 4-1BB-based CAR-T cells**

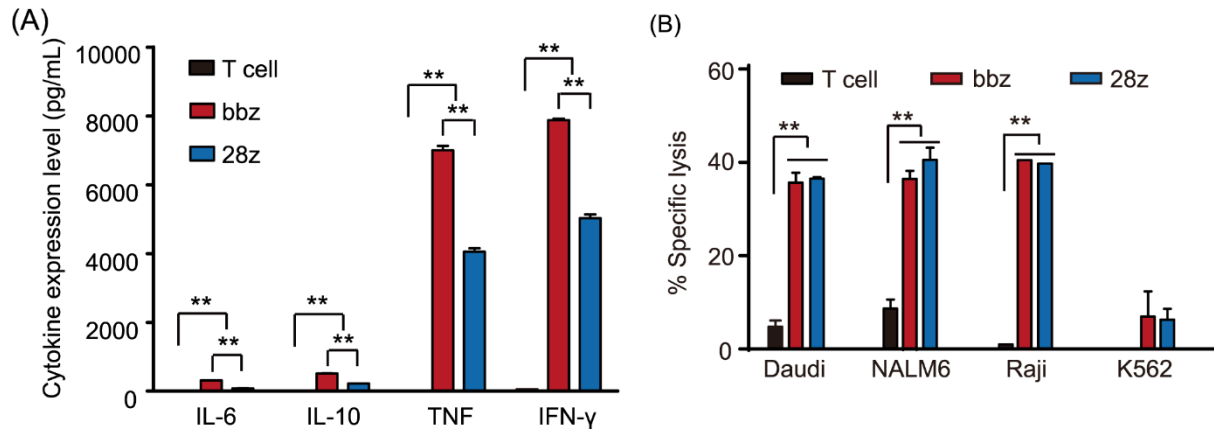
Characteristics	No. (%) of patients	
	28z	bbz
<b>Gender</b>		
Male	11(61)	11(61)
Female	7(39)	7(39)
<b>Age, year</b>		
0-13	12(67)	10(56)
≥14	6(33)	8(44)
<b>Disease status</b>		
Relapsed after HSCT	5(28)	2(12)
Relapsed after chemotherapy	12(67)	15(83)
Primary refractory	1(5)	1(5)
<b>Complex karyotypes</b>		
Yes	10(56)	9(50)
No	5(28)	8(44)
Unknown	3(16)	1(6)
<b>BCR-ABL fusion gene</b>		
Yes	12(66)	6(33)
No	3(17)	12(67)
Unknown	3(17)	0(0)
<b>Tumor burden</b>		
<70%	12(67)	16(89)
≥70%	6(33)	2(11)

**Table S3. The percentage of B cells in the peripheral blood of patients treated with CD28 CAR-T or 4-1BB CAR-T cells.**

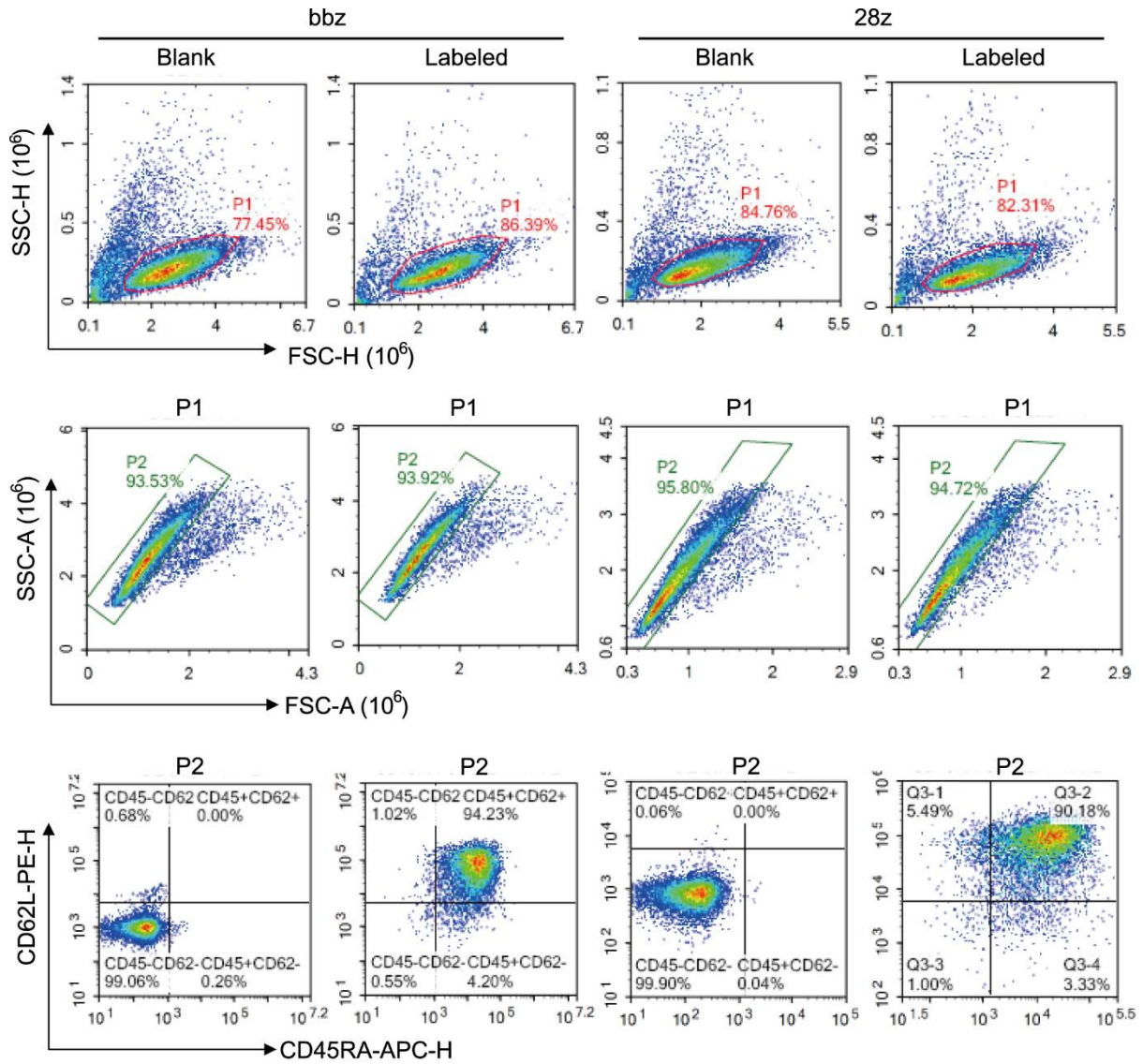
	Patient No.	Baseline	D4	D7	D10	D14	D21
28z	A01	NA	1.05	BMDL	BMDL	BMDL	BMDL
	A02	1.48	0.06	0.46	NA	BMDL	BMDL
	A03	19.09	24.8	20.2	1.22	0.13	BMDL
	A04	0.02	0.20	0.61	0.43	NA	BMDL
	A05	BMDL	BMDL	BMDL	NA	BMDL	BMDL
	A06	NA	BMDL	0.96	NA	BMDL	BMDL
	A07	3.27	1.03	BMDL	NA	0.02	NA
	A10	5.96	3.67	4.06	0.18	BMDL	BMDL
bbz	B01	BMDL	1.10	2.08	NA	0.01	BMDL
	B02	4.20	0.05	NA	BMDL	BMDL	BMDL
	B03	NA	BMDL	BMDL	NA	BMDL	NA
	B04	0.41	0.14	0.2	NA	NA	BMDL
	B05	NA	BMDL	BMDL	NA	NA	NA
	B06	NA	BMDL	BMDL	NA	NA	NA
	B07	0.67	BMDL	BMDL	NA	NA	NA
	B08	1.98	33.5	37.1	BMDL	NA	NA



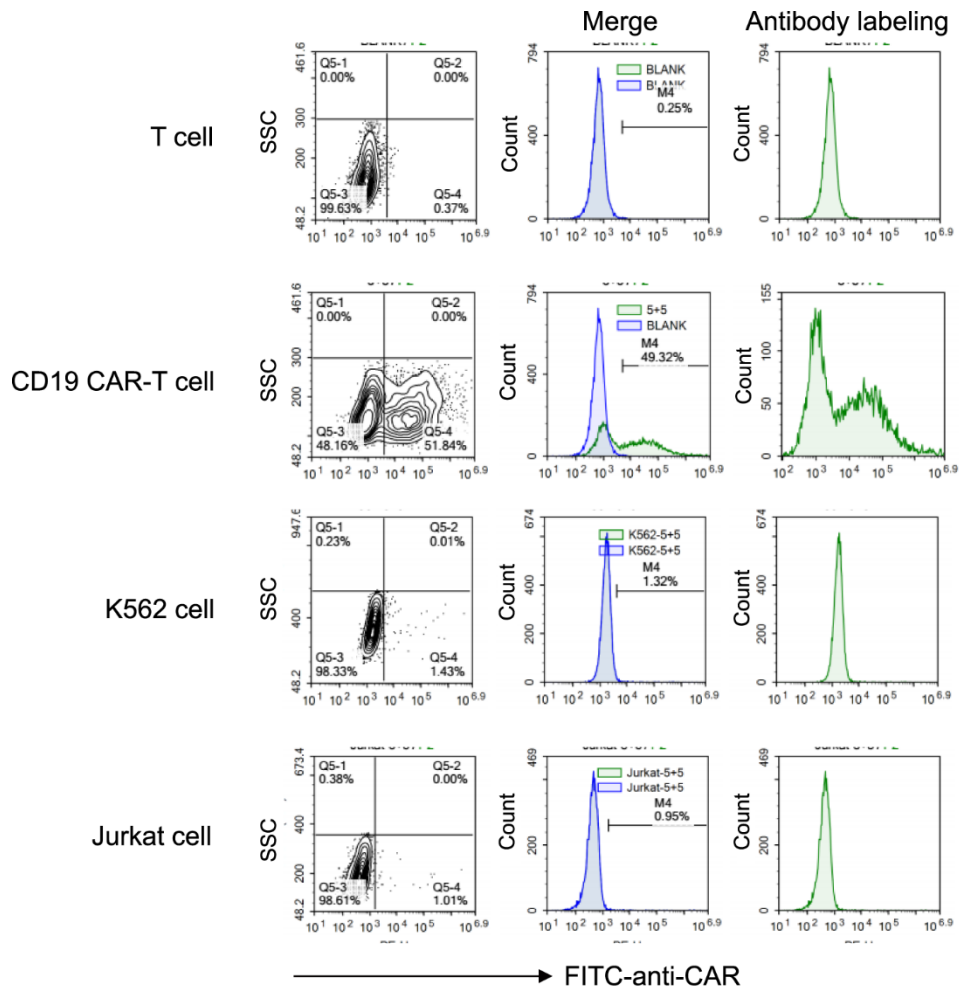
**Figure S1. Schematic illustration of 4-1BB CAR (bbz CAR) and CD28 CAR (28z CAR) compositions.**



**Figure S2. The activation and killing efficiency of CD28- and 4-1BB-based CAR-T cells *in vitro*.** (A) The expression level of cytokines after CAR-T cells or T cells were co-cultured with Daudi cells. \*\*,  $P < 0.01$ . (B) The killing efficiency of CAR-T cells when co-cultured with indicated target cells at the E: T ratio of 5:1. Tumor cells including Daudi, NALM6, Raji, and K562 cells that were incubated with T cells with the equivalent number of CAR-T cell group served as controls. \*\*,  $P < 0.01$ . 28z, CD28 CAR-T cell; bbz, 4-1BB CAR-T cell.



**Figure S3. Representative FACS plots of 4-1BB CAR-T (bbz) or CD28 CAR-T (28z) cells that were stained with CD45RA-APC and CD62L-PE antibodies. Blank, cells without antibody labeling; labeled, cells that were labeled with CD45RA-APC and CD62L-PE antibodies.**



**Figure S4. FACS plots showing the specificity of FITC-anti-CAR.** The FITC-anti-CAR antibody was incubated with T cell, CD19-specific CAR-T cell, K562 cell, or Jurkat cell, and fluorescence intensity was analyzed using flow cytometry. Only CD19 CAR-T cell showed FITC positive signal. Merge means the merged FACS plots of cells with (green) or without (blue) antibody incubation.