



Research article

**An effective approach for targeting lymphoma and leukemia cell lines
with a novel Tan-CAR (CD30/CD20) T cell**

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Supplementary

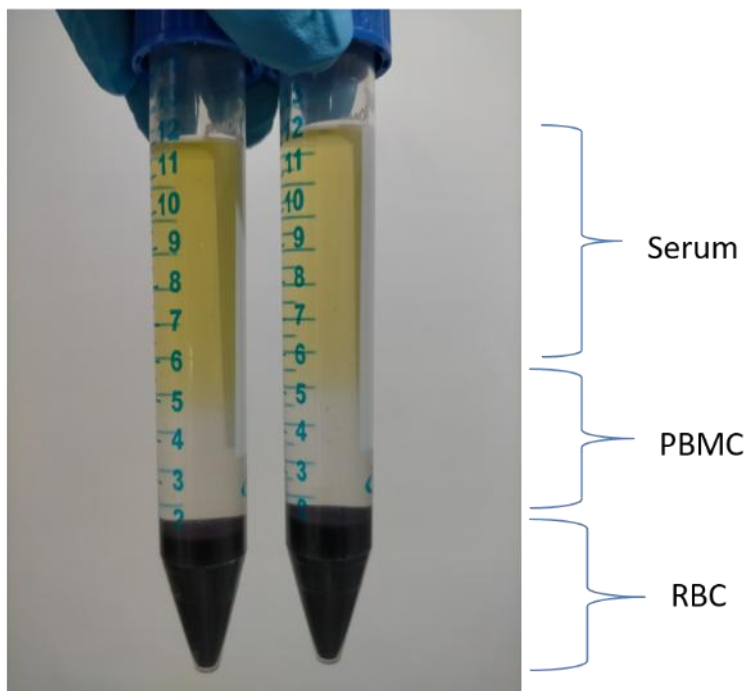


Figure S1. Ficoll density gradient method used for the separation of PBMC from blood sample.

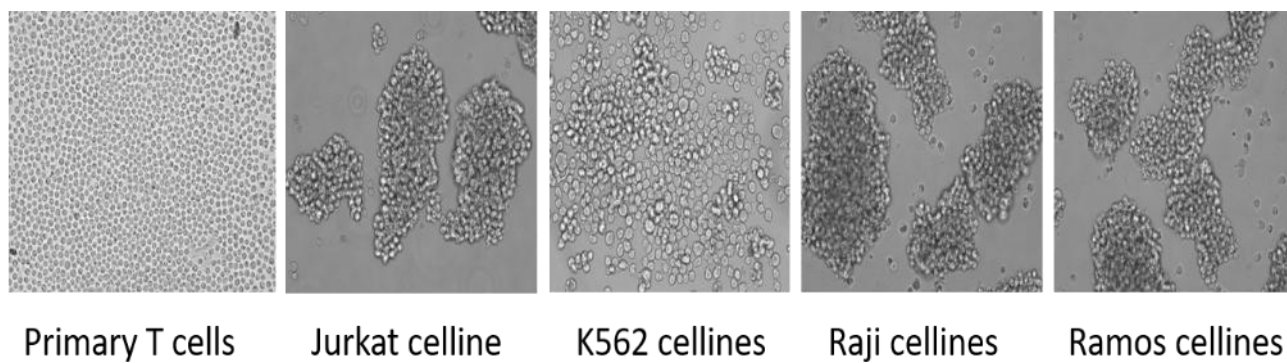


Figure S2. Cell images of different cell types used for this study.

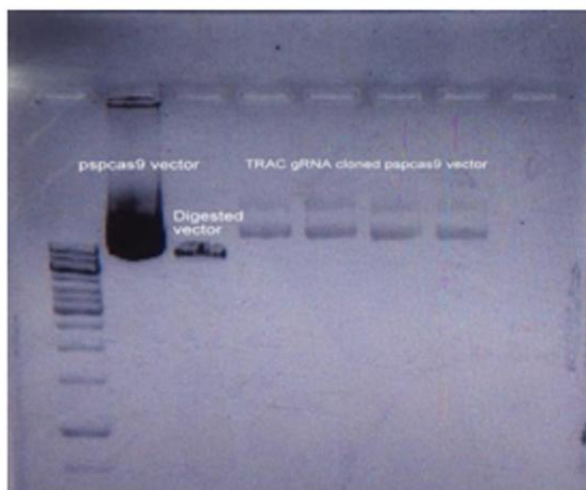
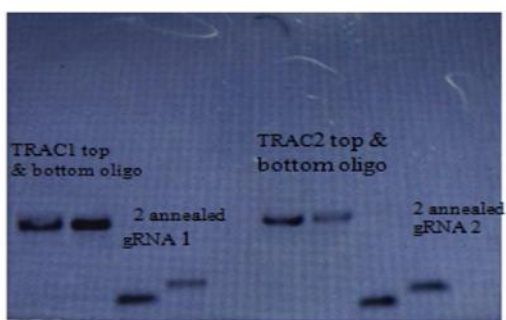
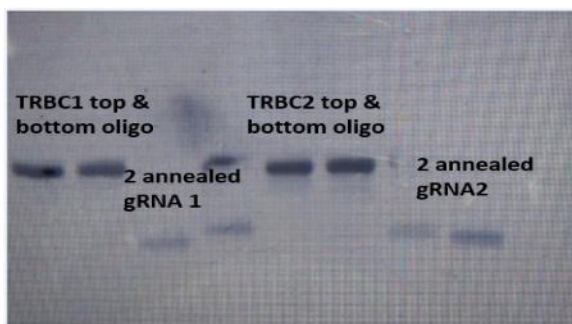


Figure S3. TRAC and TRBC gRNA cloning into PSCAS9 plasmid.



3. TRAC gRNA for knockout TCR gene



4. TRBC gRNA for knockout TCR gene

Figure S4. TRAC1 and TRAC2 Top and Bottom gRNA annealed, TRBC1 and TRBC2 Top and Bottom gRNA annealed.

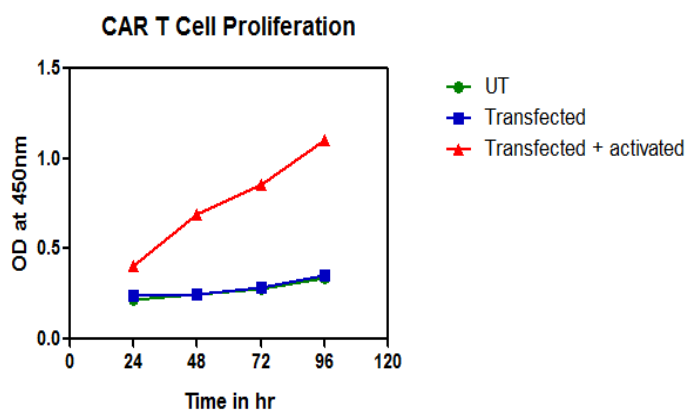


Figure S5. Analysis of T cell proliferation with constant number of cells and different cell samples in an increasing time frame upto 96hr.

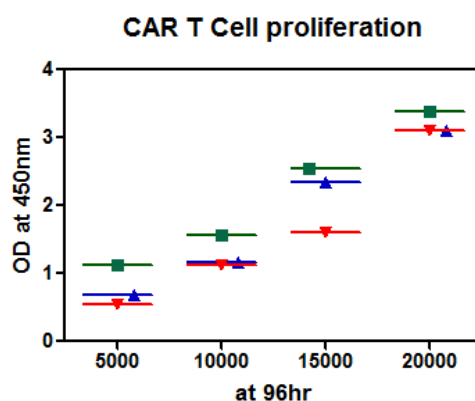


Figure S6. Comparative study on proliferation of T cells with increasing number of cell population and different T-cell samples.

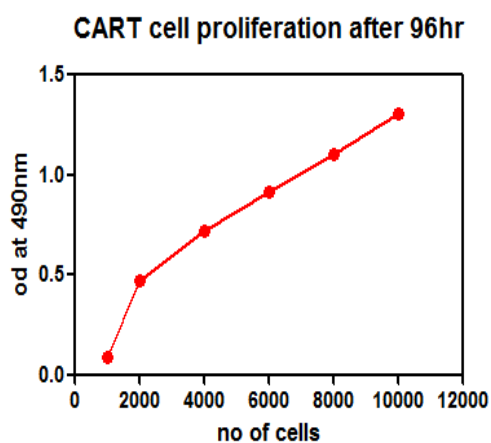
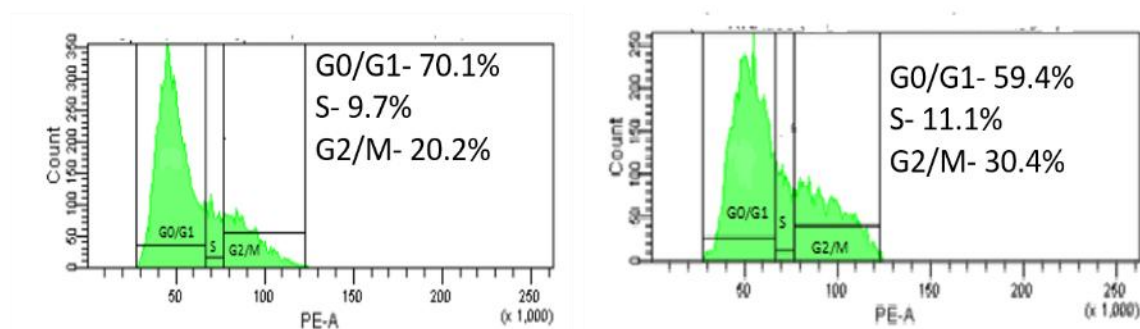


Figure S7. CAR T cell proliferation test with increasing number of cells incubated for 96hr.



1. CART cell proliferation without supplements 2. CART T cell expansion with supplements

Figure S8. Cell cycle analysis before and after using supplements for 96hr.

Table S1. TCR knockout gRNA.

Guide RNA	TOP STRAND	BOTTOM STRAND
1 TRAC/gRNA1	CACCGTCTCTCAGCTGGTACACGGC	AAACGCCGTGTACCAGCTGAGAGAC
2 TRAC/gRNA2	CACCGTTCGGAACCCAATCACTGAC	AAACGTCAGTGATTGGGTTCCGAAC
3 TRBC/gRNA1	CACCGAACAAGGTGTTCCACCCG	AAACCGGGTGGGAACACCTTGTTTC
4 TRBC/gRNA2	CACCGCTGTCAAGTCCAGTTCTAC	AAACGTAGAACTGGACTTGACAGC

Table S2. CAR, TRAC, TRBC gene primers for PCR and RT-PCR.

Gene	FORWARD	REVERSE
1 CAR (PCR)	GAGATTGTGTTGACACAGAGCCCC	CTGCATGTGGAGAGCATCGTATG
2 CAR(RT-PCR)	TGGACAAGGCACAAAAGTCG	GTATCGCCGTTACCAGGGTA
3 TRAC (PCR)	ATATCCAGAACCCTGACCCTG	TCA GCT GGA CCA CAG CCG
4 TRBC (PCR)	AGGACCTGAACAAGGTGTTCC	GTAGTAGGGCCCATTGACCAC



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