Supplementary Information

T cell receptor β-chain-targeting chimeric antigen receptor T cells against T cell malignancies

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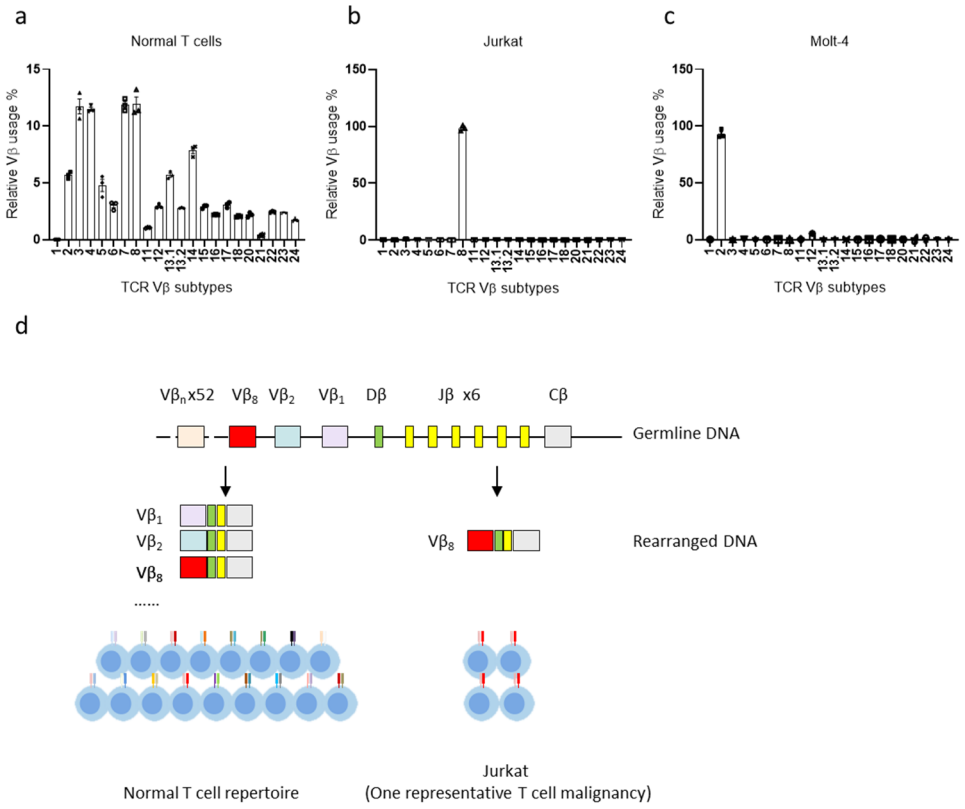
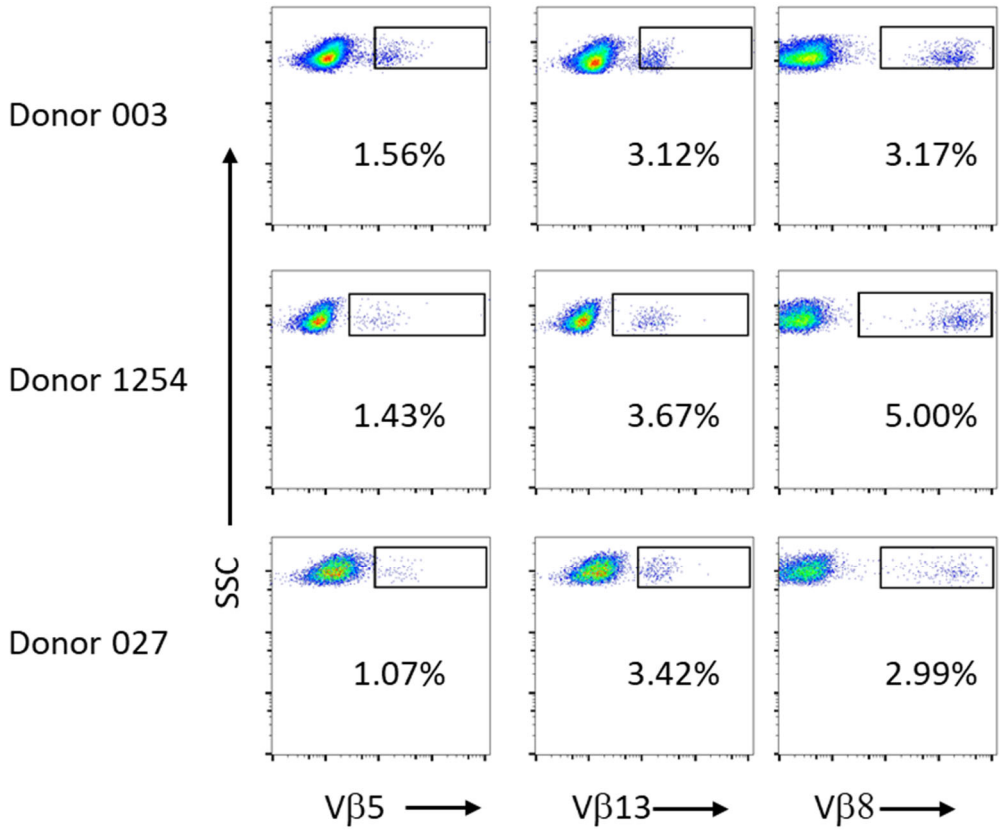
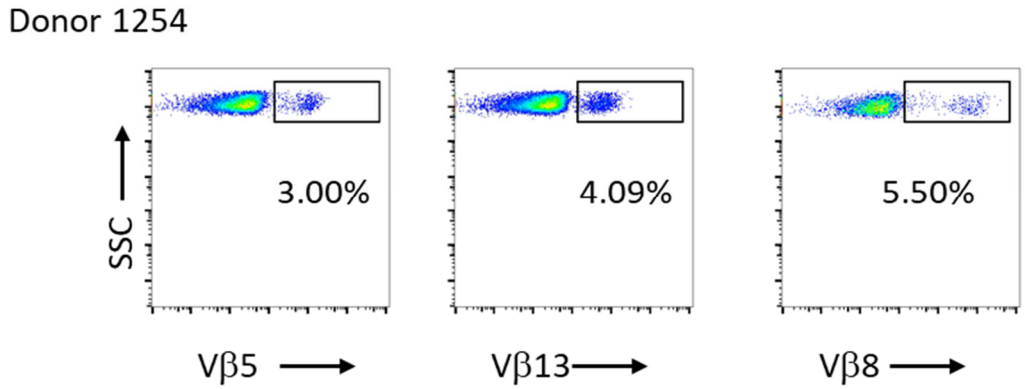
Supplemental Data for Figures:



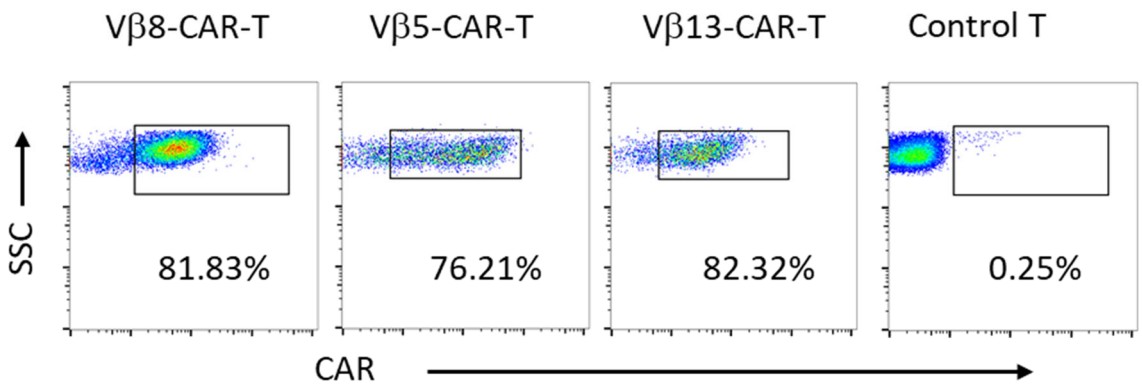
Supplementary Figure 1. TCR Vβ usage in normal and malignant T cells. **a-c** Real-time quantitative PCR analysis of TCR Vβ mRNA-expression levels (n = 3 biologically independent samples/group) in normal T cells from PBMCs (**a**), Jurkat T cell leukemia cells (**b**) and Molt-4 cells (**c**). **d** Schematic representation of different TCR Vβ usage in normal T cell repertoire and malignancy. The data shown represented the mean ± SEM. Representative results of one from three replicate experiments were shown in panels **a-c**. The cartoons in this figure were created with BioRender.com.



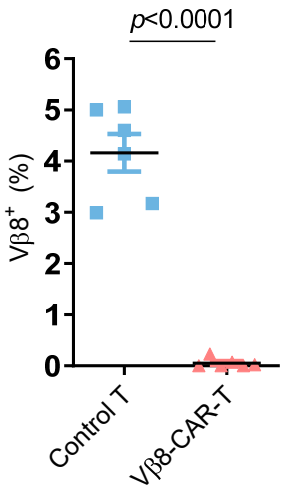
Supplementary Figure 2. TCR Vβ specific antibody recognized a small portion of normal T cells. Normal T cells of peripheral blood of indicated donors were stained with commercially available anti-Vβ5, Vβ13, or Vβ8 antibody and analyzed by flow cytometry.



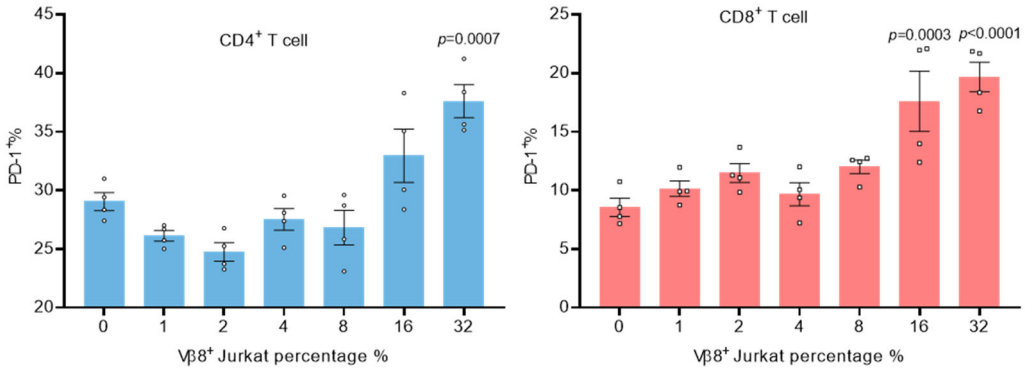
Supplementary Figure 3. Home-made recombinant TCR Vβ specific antibody recognized a small portion of normal T cells. Normal T cells of peripheral blood of indicated donor were stained with home-made recombinant anti- Vβ5, Vβ13, or Vβ8 antibody and analyzed by flow cytometry.



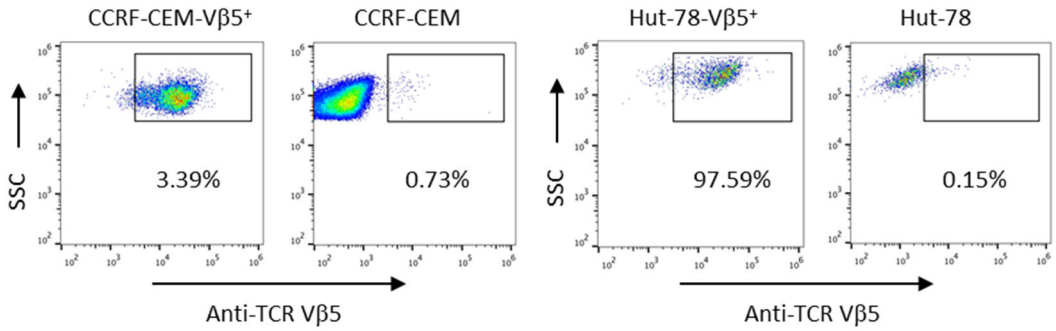
Supplementary Figure 4. CAR expression of different TCR Vβ specific CAR-T cells. Mock T cells or indicated CAR-T cells were stained with anti-mouse Fab antibody and analyzed by flow cytometry.



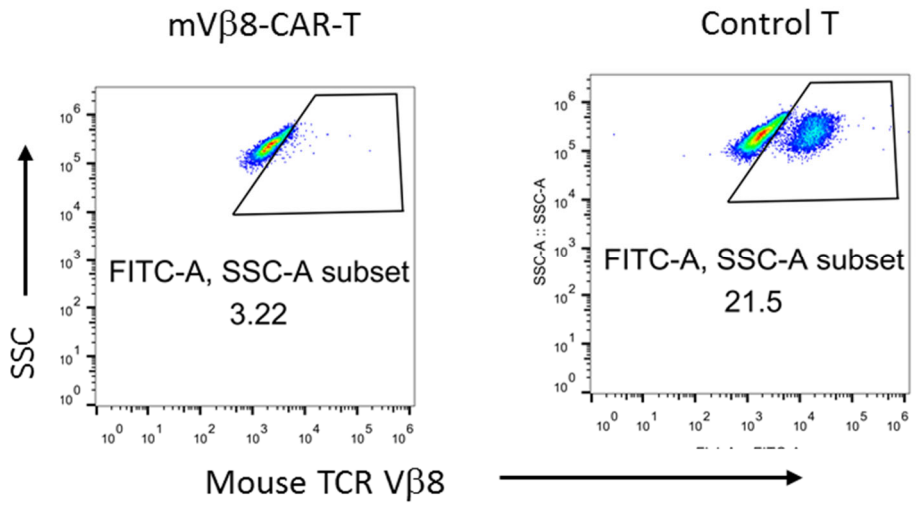
Supplementary Figure 5. Vβ8 expression in different donor-derived CAR-T cells. Control T cells and Vβ8-CAR-T cells from donor #228, #1254, #Z003, #108, #024 and #027 were stained with anti-TCR Vβ8 antibody and analyzed by flow cytometry (n = 6 biologically independent samples/group). The data shown represented the mean ± SEM. Statistical significances were determined by two-sided unpaired t-tests. The normality of the data was confirmed by Shapiro–Wilk test.



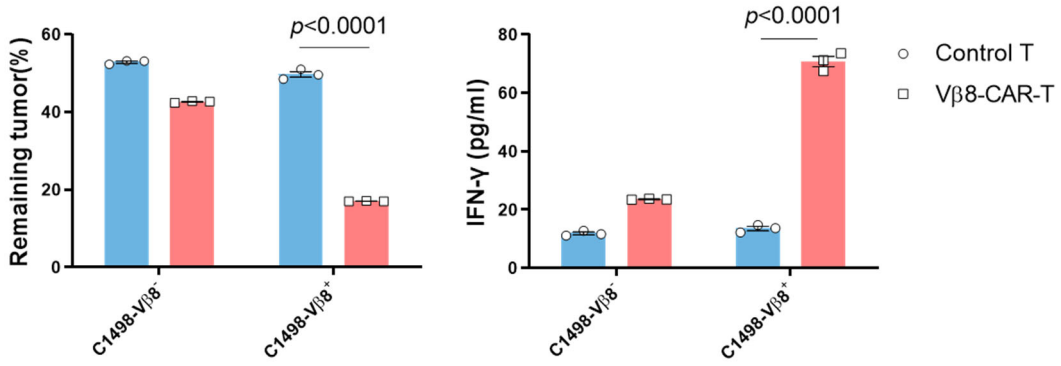
Supplementary Figure 6. CAR-T cell exhaustion analysis. Vβ8-CAR-T cells were cultured with indicated percentage of Jurkat cells. The expression of PD-1 were analyzed by flow cytometry (n = 4 biologically independent samples/group). The data shown represented the mean ± SEM. Statistical significances were determined by two-sided, one-way ANOVA with Dunnett's multiple comparisons correction.



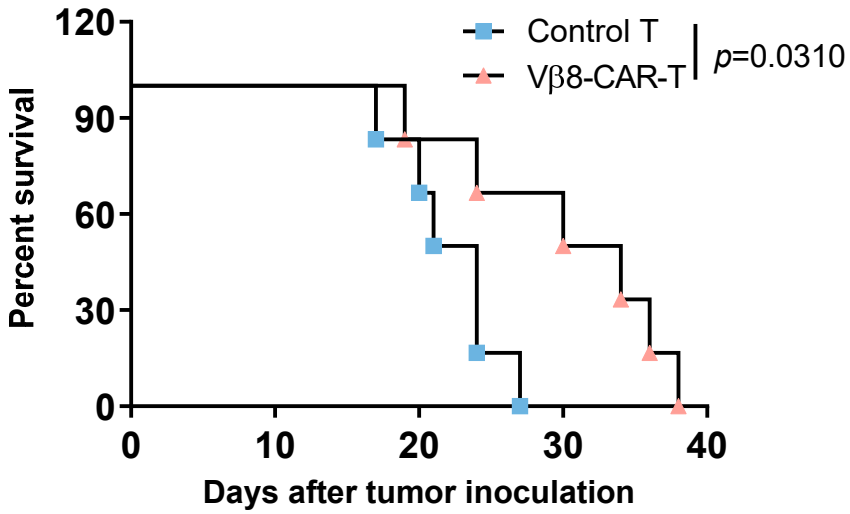
Supplementary Figure 7. Construction of TCR Vβ5 expressing CCRF-CEM and Hut-78 cells. Parental and TCR Vβ5 expressed CCRF-CEM and Hut-78 cells were stained with anti-TCR Vβ5 antibody and analyzed by flow cytometry.



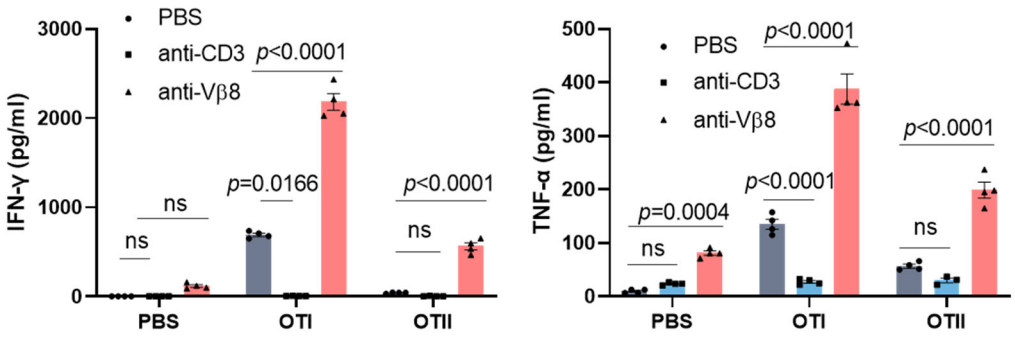
Supplementary Figure 8. Mouse Vβ8-CAR-T cells specifically targeted Vβ8^{+} primary mouse T cells. Control T cells or mVβ8-CAR-T cells were stained with anti-mouse Vβ8 antibody and analyzed by flow cytometry.



Supplementary Figure 9. Mouse Vβ8-CAR-T cells specifically killed Vβ8$^+$ tumor cells. Un-transduced T cells or Vβ8-CAR-T cells were co-cultured with C1498-Vβ8$^-$ or C1498-Vβ8$^+$ cells in triplicate wells at a CAR-T: C1498 cell ratio of 2:1 for 24 h (n = 3 biologically independent samples/group). The indicated cytokines in the supernatants were analyzed by CBA assay. The data shown represented the mean ± SEM. Statistical significances were determined by two-sided, two-way ANOVA with sidaks multiple comparisons correction.



Supplementary Figure 10. Mouse Vβ8-CAR-T cells prolonged survival of $V\beta8^+$ tumor bearing mice (n = 6 mice/group). NSG mice were intravenously inoculated with 2.5×10^5 C1498-$V\beta8^+$ tumor cells. Four days later, tumor-bearing mice were treated with 3×10^6 un-transduced or mouse Vβ8-CAR-T cells. Kaplan–Meier OS curves were shown for the inoculated mice. Statistical significances were determined by the two-sided Mantel–Cox test. Representative results of one from two replicate experiments were shown.



Supplementary Figure 11. T cell-mediated immunity after TCR Vβ8^{+} cell depletion. WT B6 mice were intraperitoneally injected with 200 μg of anti-CD3 or anti-TCR Vβ8 antibodies, or PBS on days -2 and 5. The mice were vaccinated with 10 μg of the DEC-OVA fusion protein and 100 μg of an anti-CD40 antibody as an adjuvant on day 0. On day 12, splenocytes were stimulated with OT-I or OT-II peptides. Two days later, indicated cytokines ware analyzed by CBA assay (n = 4 biologically independent samples/group). The data shown represented the mean ± SEM. Statistical significances were determined by two-sided, one-way ANOVA with Dunnett's multiple comparisons corrections. ns, not significant. Representative results of one from two replicate experiments were shown.