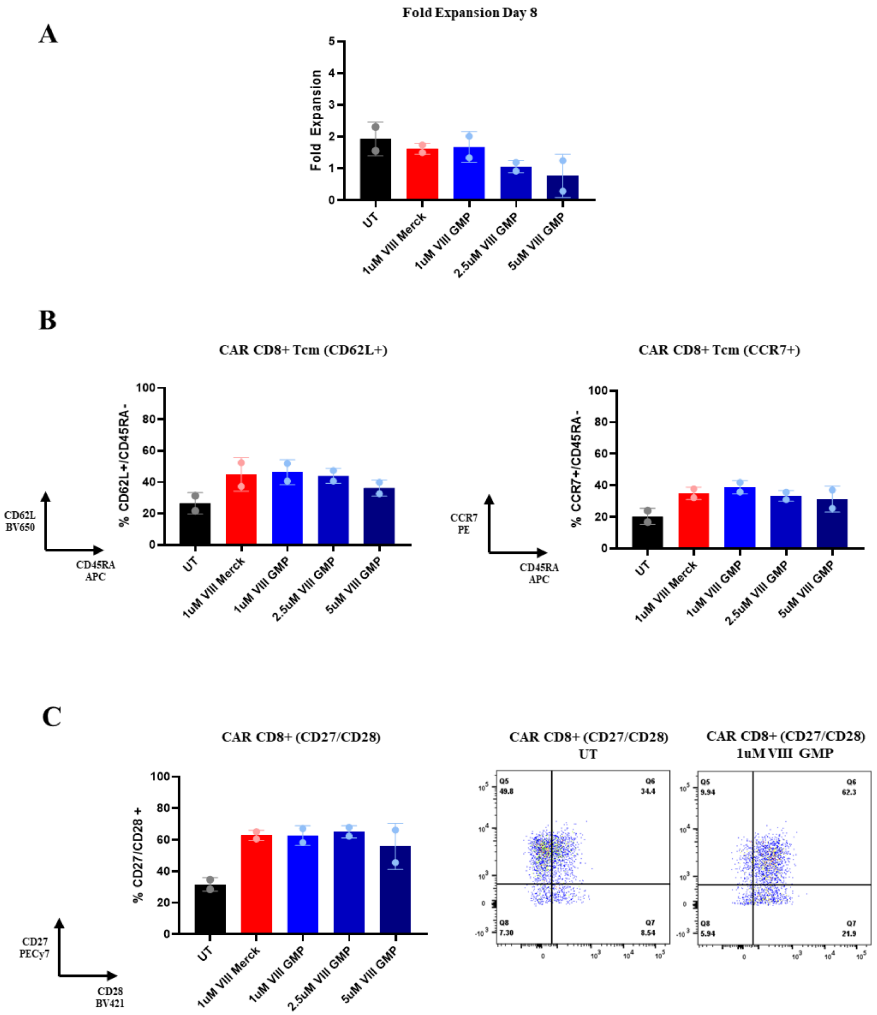
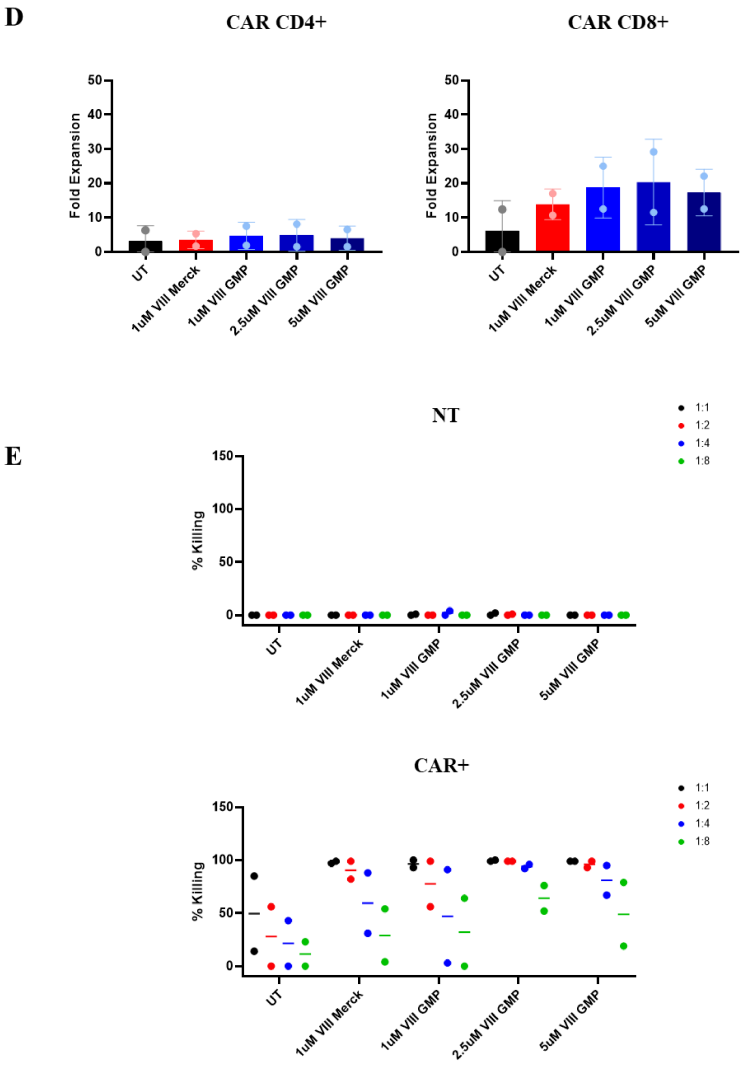
Supplementary Figure 1



Supplementary Figure 1

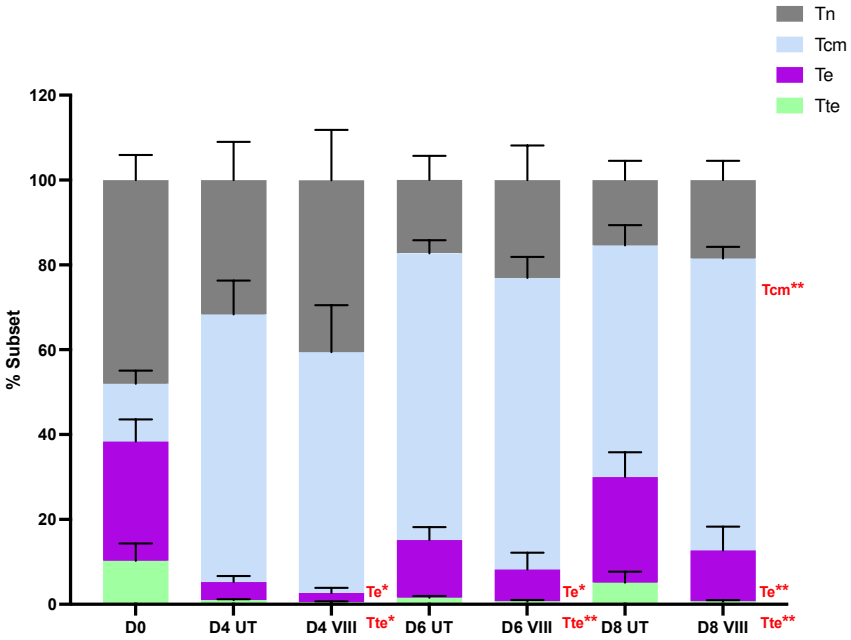


Supplementary Figure 1. Comparison of VIII (Merck) vs VIII-GMP (Ardena) and titration of VIII-GMP.

(A) Total T-cell fold expansion by the end of manufacture at Day 8 **(B)** % Tcm subset in CAR CD8s at Day 8, determine by flowcytometry as (CD62L+/CD45RA-) and (CCR7+/CD46RA-). **(C)** Extended phenotyping depicting % CD27+/CD28+ cells characterised by flowcytometry. Representation flowcytometry plots from one donor in the UT and 1µM VIII-GMP are depicted to the right. **(D)** CD4/CD8 CAR fold expansion following a 7-day co-culture with irradiated RAJI-19WT

target cell lines. **(E)** Graphs depicting % killing of RAJI-19GFP target cells post rechallenge of NT or CAR T-cells in a 72-hour killing assay. Results from all conditions were normalised to the untreated non-transduced (NT) condition at each E:T ratio. All data sets compare 1µM VIII-Merck and 1µM VIII-GMP. VIII GMP is further titrated to 2.5µM and 5µM at n=2 per condition ± SD and/or individual data points.

Supplementary Figure 2

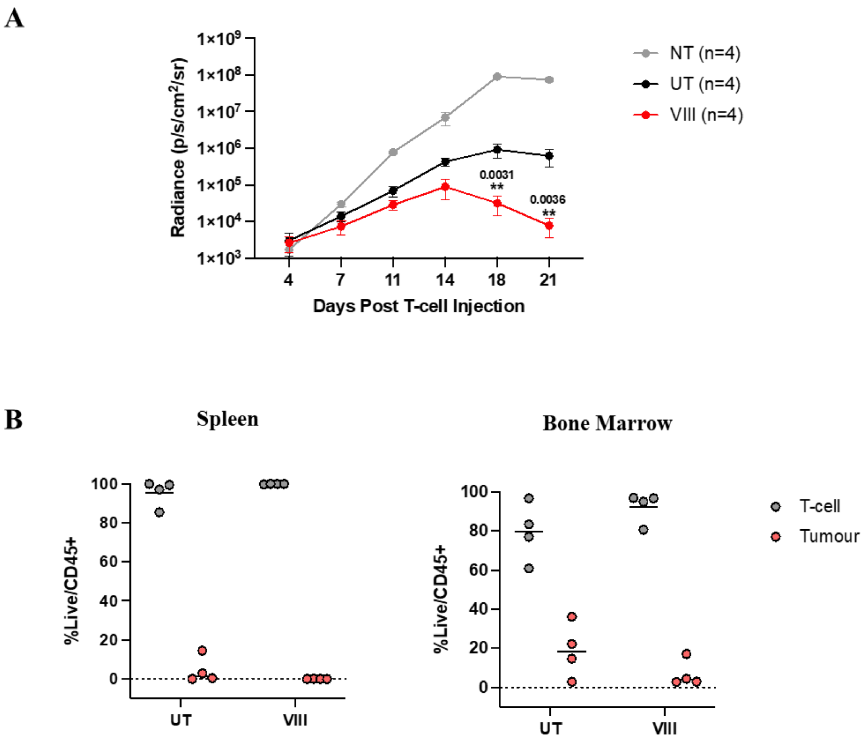


Supplementary Figure 2. Summary of CAR-T phenotype throughout manufacture.

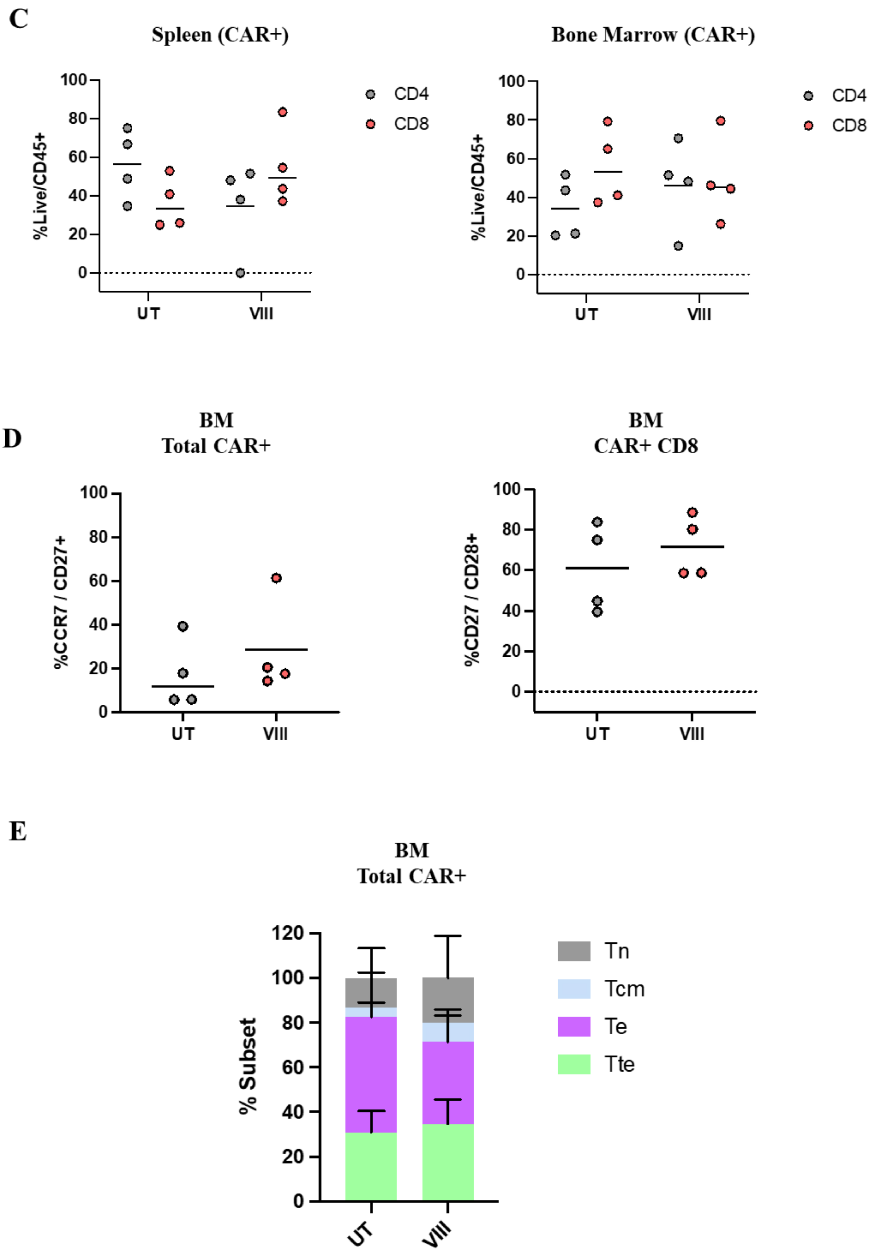
Graphical representation of Tn (CCR7+/CD45RA+), Tcm (CCR7+/CD45RA-), Te (CCR7-/CD45RA-) and Tte (CCR7-/CD45RA+) subsets in total CAR T-cells determined by flowcytometry, ± SD at baseline and throughout manufacture. Statistical comparisons were made against paired UT sample in each subset at matched timepoints, n=6. Two tailed Mann-Whitney U test, ns $P>0.05$, *$P<0.05$ and ** $P<0.01$.

Method: Systemic leukaemia was established via intravenous injection of $5x10^5$ NALM6-FLUC followed by $5x10^5$ non-transduced (NT) or CAR T-cells 4 days later. Tumour burden was measured bi-weekly via bioluminescent imaging (BLI) using the IVIS spectrum in-vivo imaging system (Perkin Elmer) following intraperitoneal (IP) injection of 2mg D-luciferin in 200µl PBS. Photon emission from NALM6 cells was measured as photons/sec/cm2/steradian. Mice were humanely euthanised Day 21 and spleen/bone marrow samples were harvested in cold Hanks Balanced Salt Solution (HBSS) (Sigma Aldrich) for downstream analysis. Spleens were gently pressed over a 70µm and subsequently a 30µm cell strainer to dissociate. Bone marrow was flushed with PBS from femoral shafts over a 70µm and subsequently a 30µm cell strainer. CountBright™ beads were added to samples to determine absolute cell numbers, and flowcytometry was performed on the BD LSRFortessa™ and analysed using FlowJo v10 software.

Supplementary Figure 3



Supplementary Figure 3

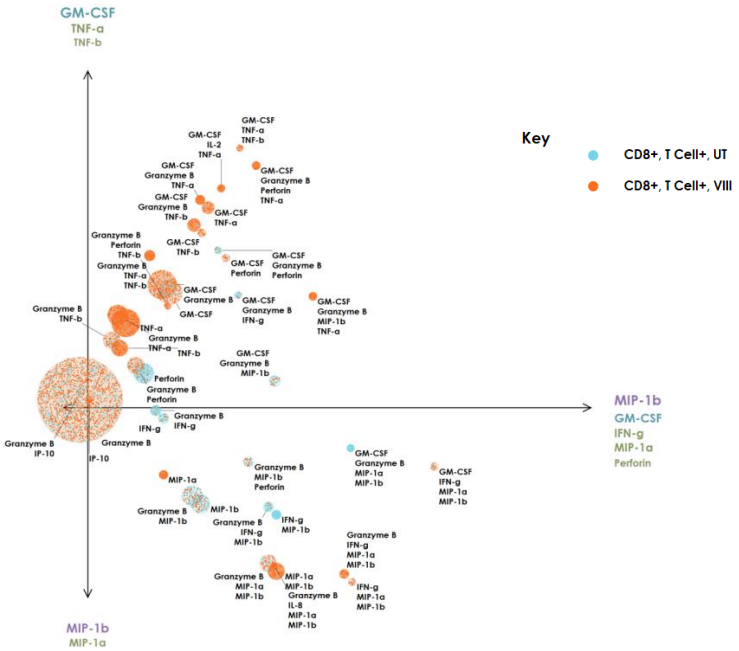


Supplementary Figure 3. In-vivo model and CAR T-cell analysis from mouse spleen and bone marrow at Day 21.

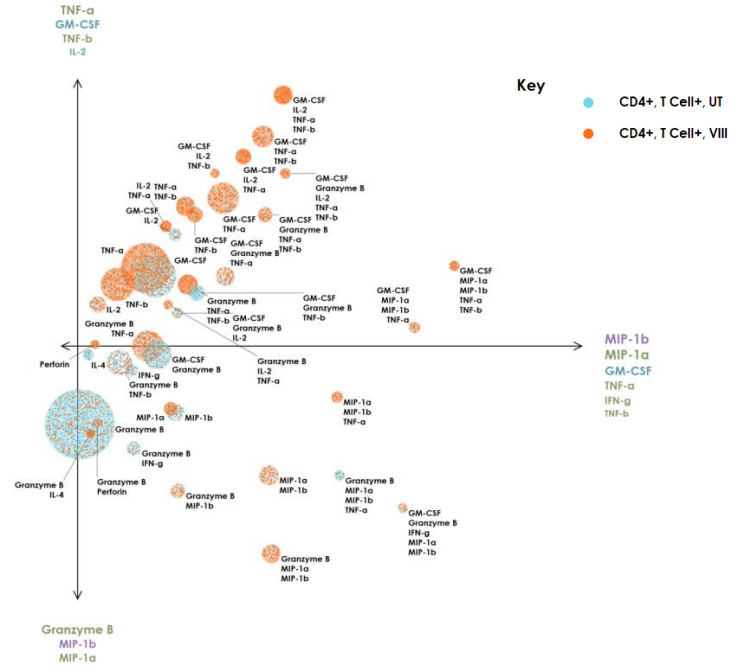
(A) Tumour burden measured by bioluminescent imagining (BLI) in NALM6 tumour established mice treated with non-transduced (NT) T-cells or CAR T-cells manufactured with (VIII) or without (UT) ± SEM **(B)** Residual percentages of total T-cell and tumour cells in mouse spleen and bone marrow at Day 21, depicting individual data points **(C)** Percentage of CAR+ CD4/8 in spleen and bone marrow at Day 21 in NALM6 tumour established mice treated with UT/VIII CAR T-cells, depicting individual data points. **(D)** Frequencies of CCR7+/CD27+ in total CAR T-cells and CD27+/CD28+ in CD8 CAR T-cells in bone marrow of mice treated with UT/VIII CAR T-cells, depicting individual data points. **(E)** Graphical representation of Tn (CCR7+/CD45RA+), Tcm (CCR7+/CD45RA-), Te (CCR7-/CD45RA-) and Tte (CCR7-/CD45RA+) subsets in total CAR T-cells from bone marrow determined by flowcytometry, ± SD. **(A-E)** Cells derived from one healthy donor, n=4 mice per group. **(A)** Two-way ANNOVA corrected for multiple comparisons by Tukey's test on log transformed data, ns $P>0.05$ and ** $P<0.01$. **(B-C)** Two-way ANNOVA corrected for multiple comparisons by Bonferroni's test, ns $P>0.05$. **(D-E)** Two tailed Mann-Whitney U test, ns $P>0.05$.

Supplementary Figure 4

CAR CD8+ PAT PCA



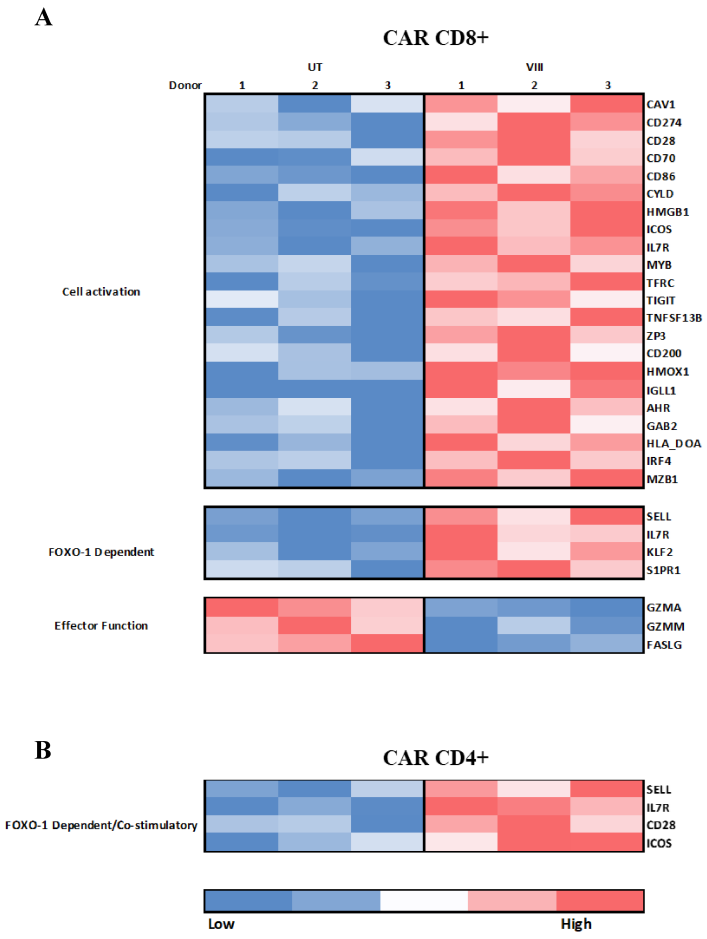
CAR CD4+ PAT PCA



Supplementary Figure 4. PAT PCA of polyfunctional profiles.

Polyfunctionality Activity Topography (PAT), Principal Component Analysis (PCA) of CD4 and CD8 T-cell subsets demonstrating primary polyfunctional profiles where radius is proportional to secretion frequency. Determined using Isoplexis™ platform, following 20-hour 2:1 RAJI-19WT:CAR stimulation, n=4.

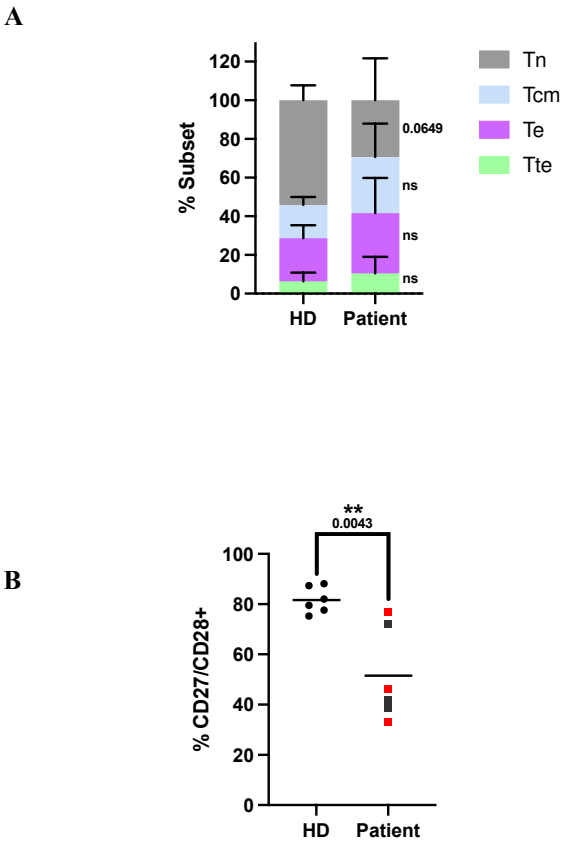
Supplementary Figure 5



Supplementary Figure 5. CAR T-cell transcriptome signature

(A-B) Heat map representation of transcripts specific to cell activation, FOXO1 and effector function in CD8 CAR T-cells and FOXO1/co-stimulatory transcripts in CD4 CAR T-cells. Colour represents low to high transcript expression. Rows are specific to each gene and columns highlights expression in each donor following UT/VIII manufacture. Only significantly differentially expressed pathways and genes are included in figures at a $p<0.05$ cut off.

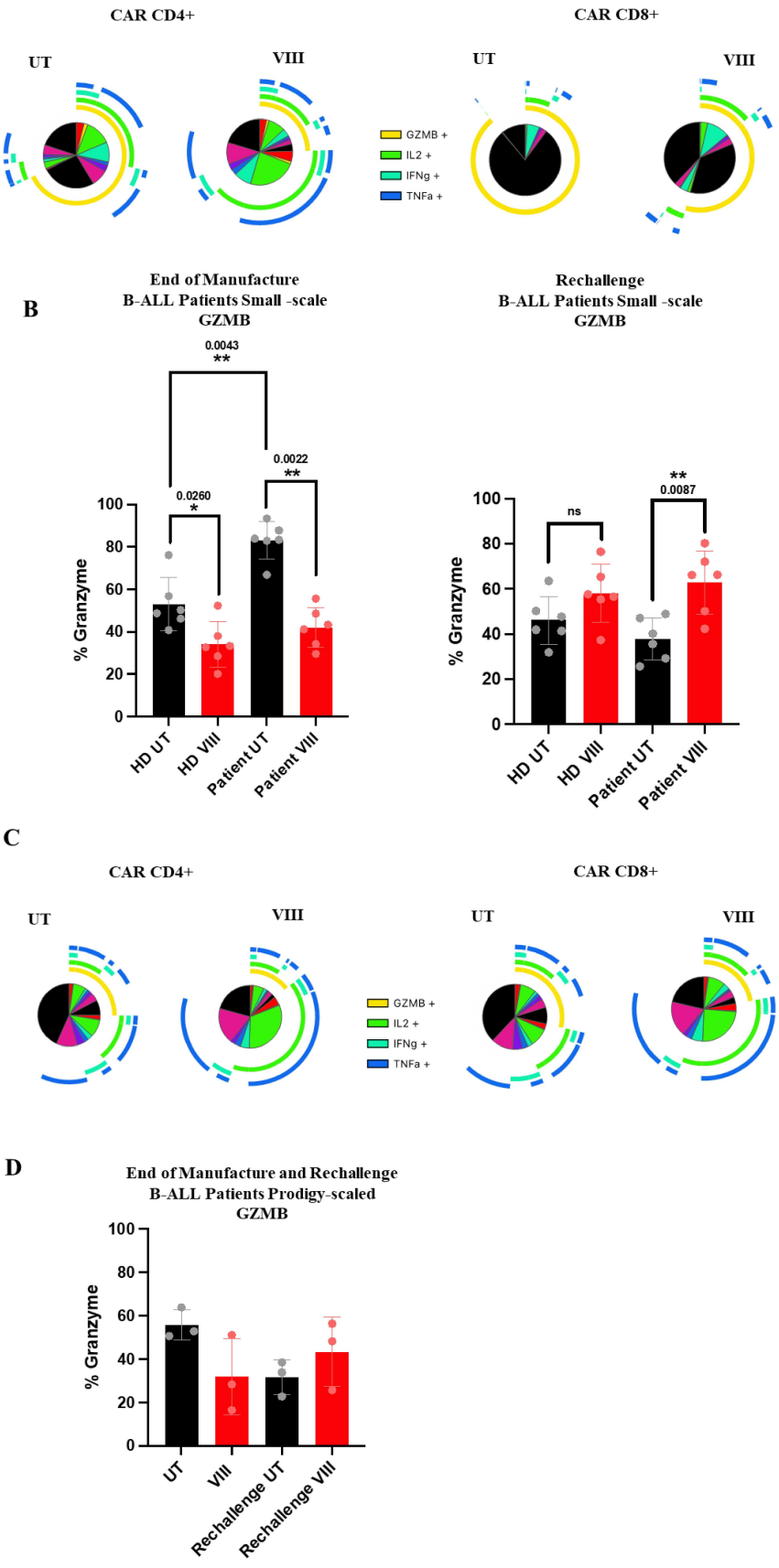
Supplementary Figure 6



Supplementary Figure 6. Phenotype analysis of baseline T-cells derived from healthy donor and B-ALL patients.

(A) Graphical representation of Tn (CCR7+/CD45RA+), Tcm (CCR7+/CD45RA-), Te (CCR7-/CD45RA-) and Tte (CCR7-/CD45RA+) subsets in total T-cells from healthy donor (HD) and B-ALL patients at baseline determined by flowcytometry, ± SD. **(B)** Percentage CD27+/CD28+ in total T-cells from healthy donor (HD) and B-ALL patients at baseline, depicting individual data points. Black squares in patient group represent patients in remission and red squares represent patients with CD19+ relapse. **(A-B)** n=6, Two tailed Mann-Whitney U test, ns $P>0.05$ and ** $P<0.01$.

Supplementary Figure 7



Supplementary Figure 7. Intracellular cytokine production

(A) Pie chart of proportion of intracellular cytokines measured by flowcytometry following RAJI-19WT stimulation in B-ALL patient CAR-T manufactured at small-scale. Arcs represent proportion of single or polyfunctional secretion. **(B)** Percentage of total CAR-T producing GZMB in healthy donor (HD) or B-ALL patient in small-scale assessments at the end of manufacture or at rechallenge using CAR-T co-cultured with RAJI-19WT targets 7-days prior, ± SD. (**C**) Pie chart of proportion of intracellular cytokines measured by flowcytometry following RAJI-19WT stimulation in B-ALL patient CAR-T manufactured at Prodigy scale. Arcs represent proportion of single or polyfunctional secretion. **(D)** Percentage of total CAR-T producing GZMB in B-ALL patient Prodigy-scale assessments at the end of manufacture or at rechallenge using CAR-T co-cultured with RAJI-19WT targets 7-days prior, ± SD. **(A-B)** n=6. **(C)** n=3 **(B)** Two tailed Mann-Whitney U test, ns $P>0.05$. *$P<0.05$ and ** $P<0.01$. **(D)** n=3, Two tailed Mann-Whitney U test, ns $P>0.05$.