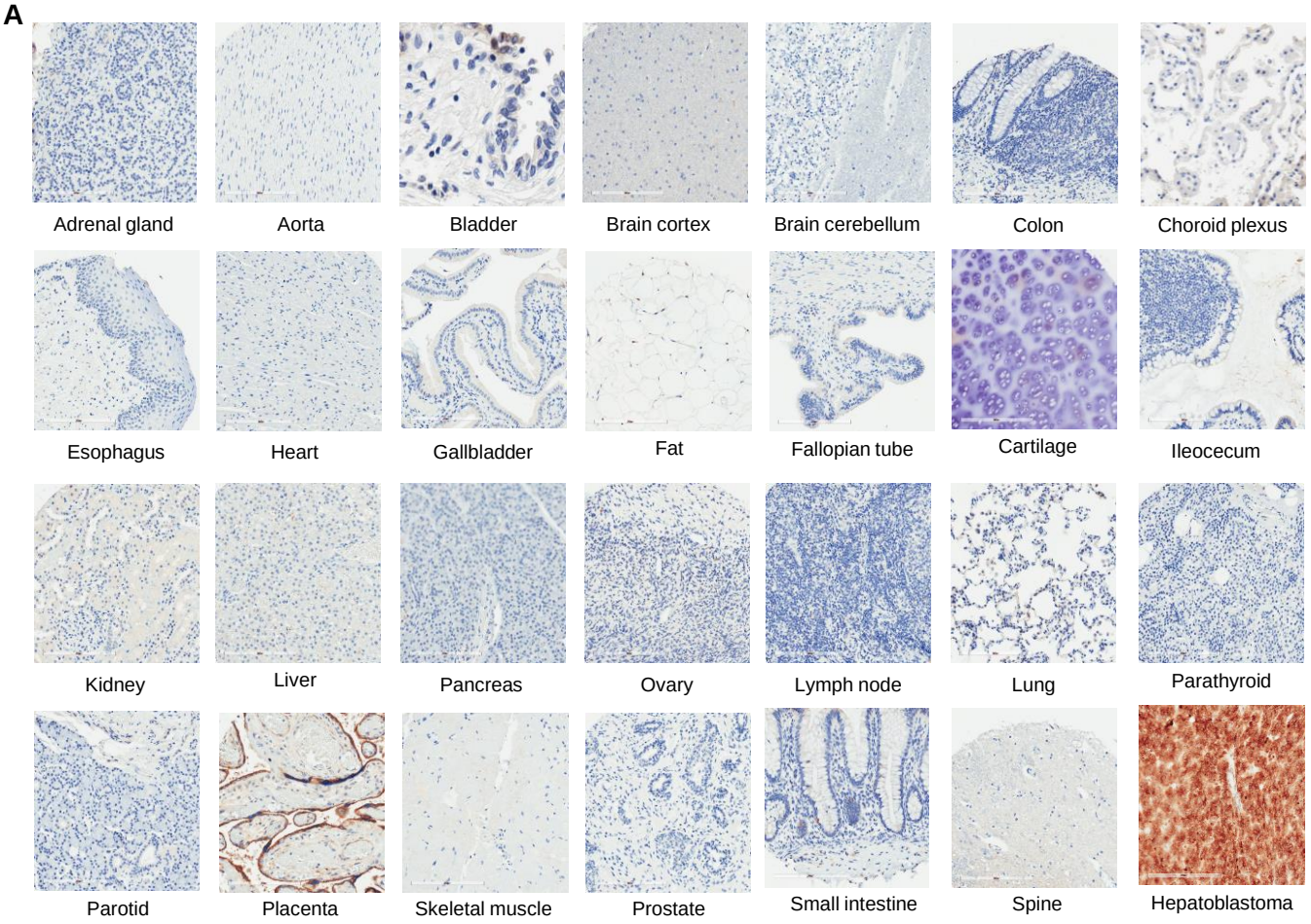
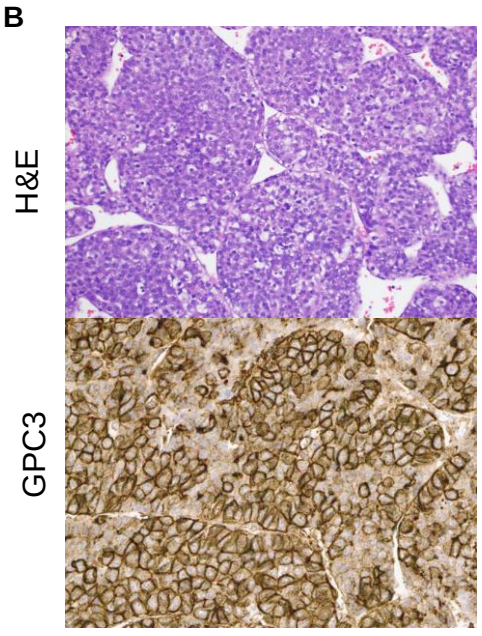
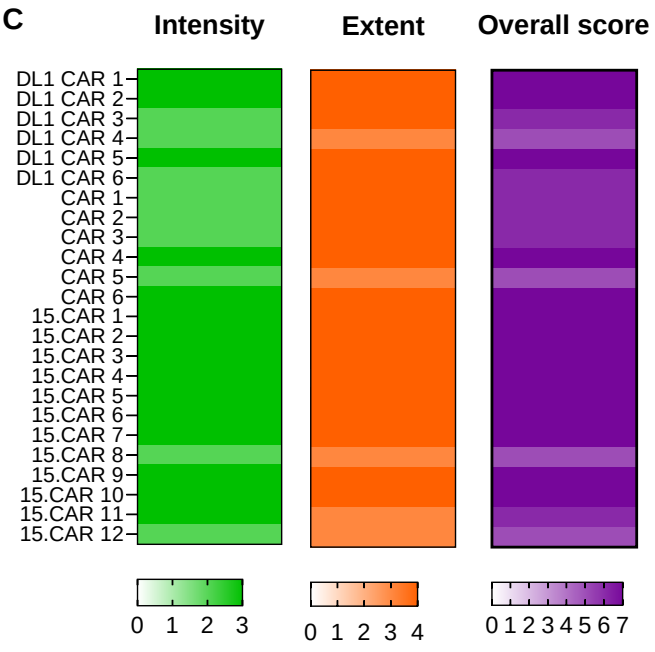
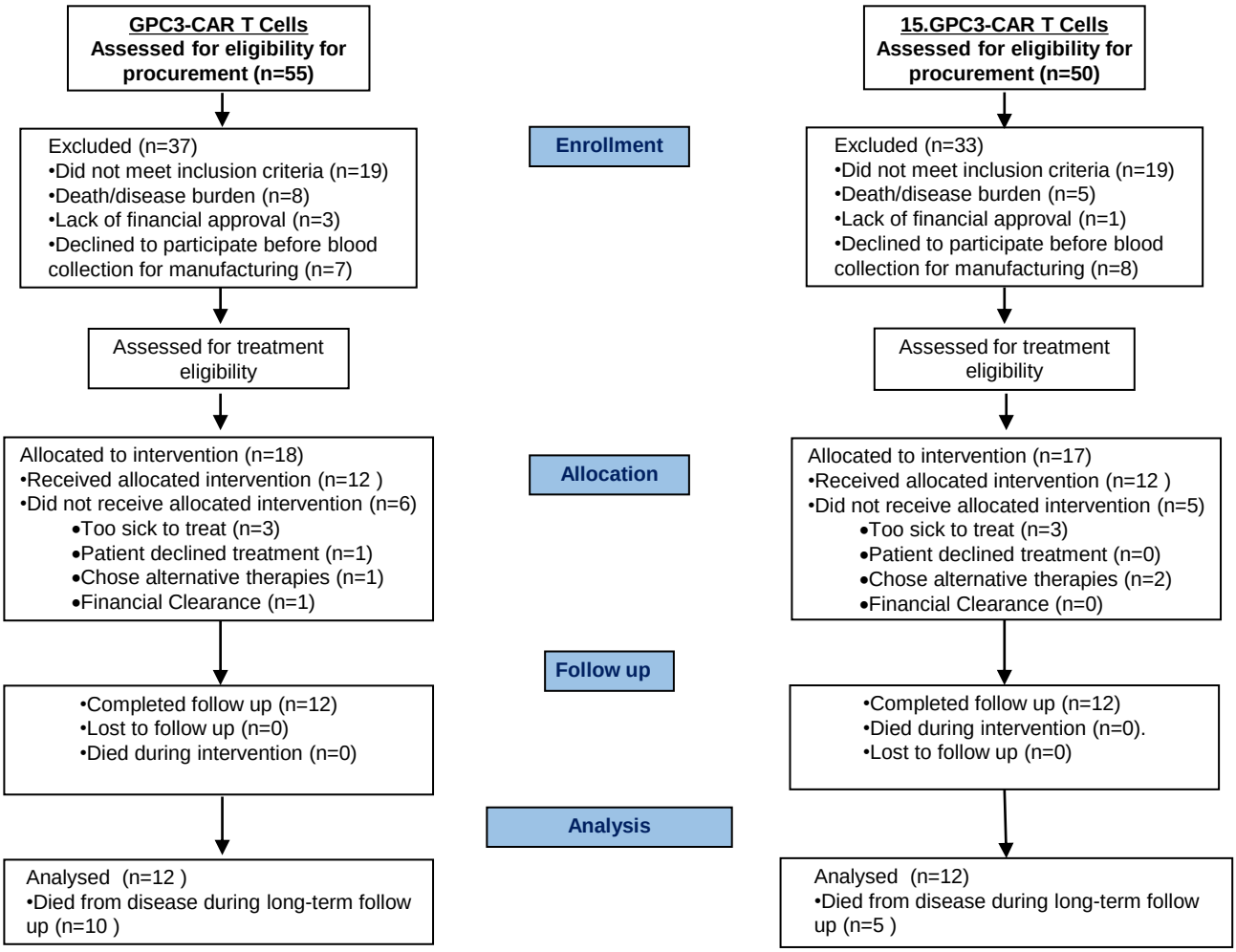


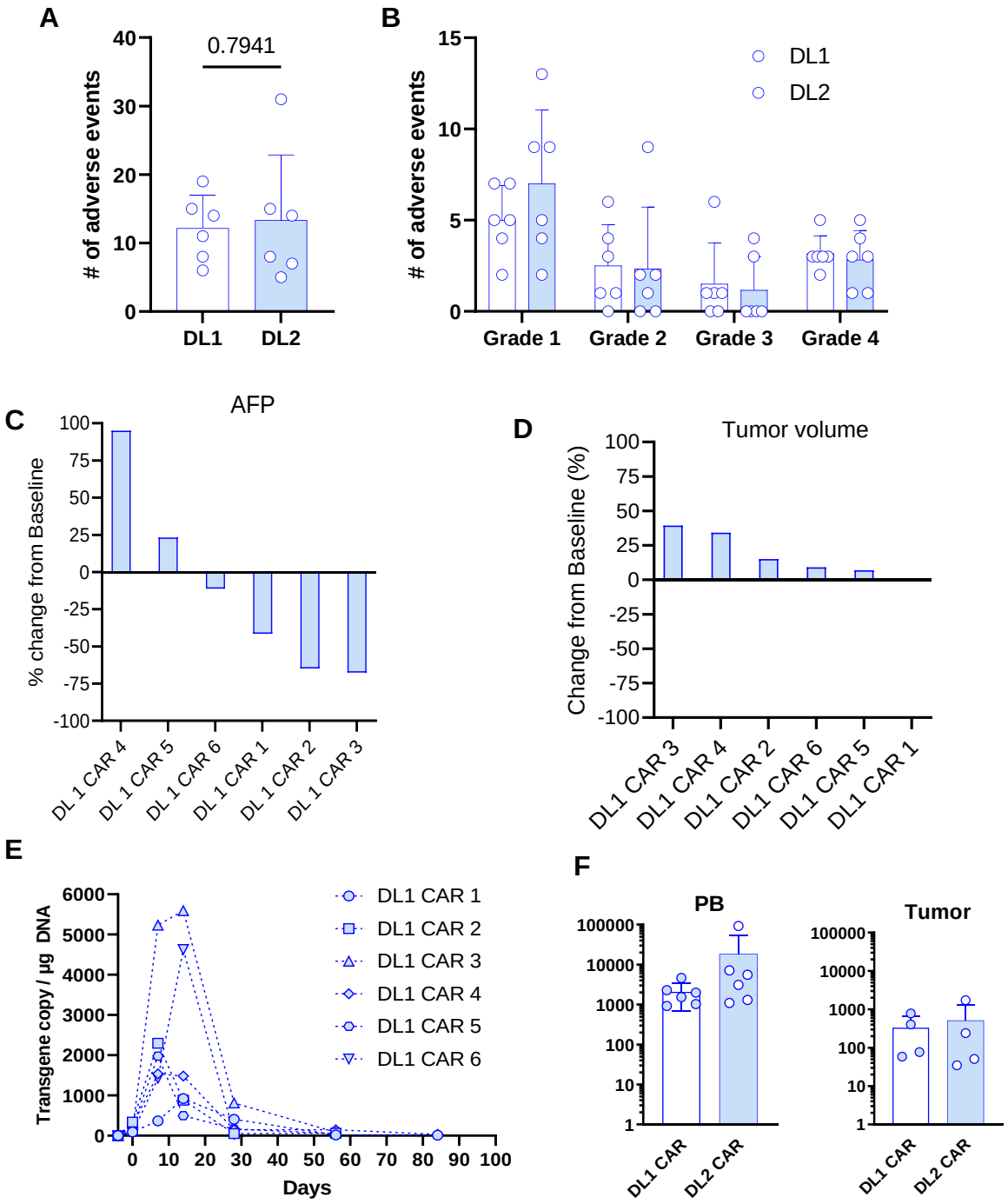




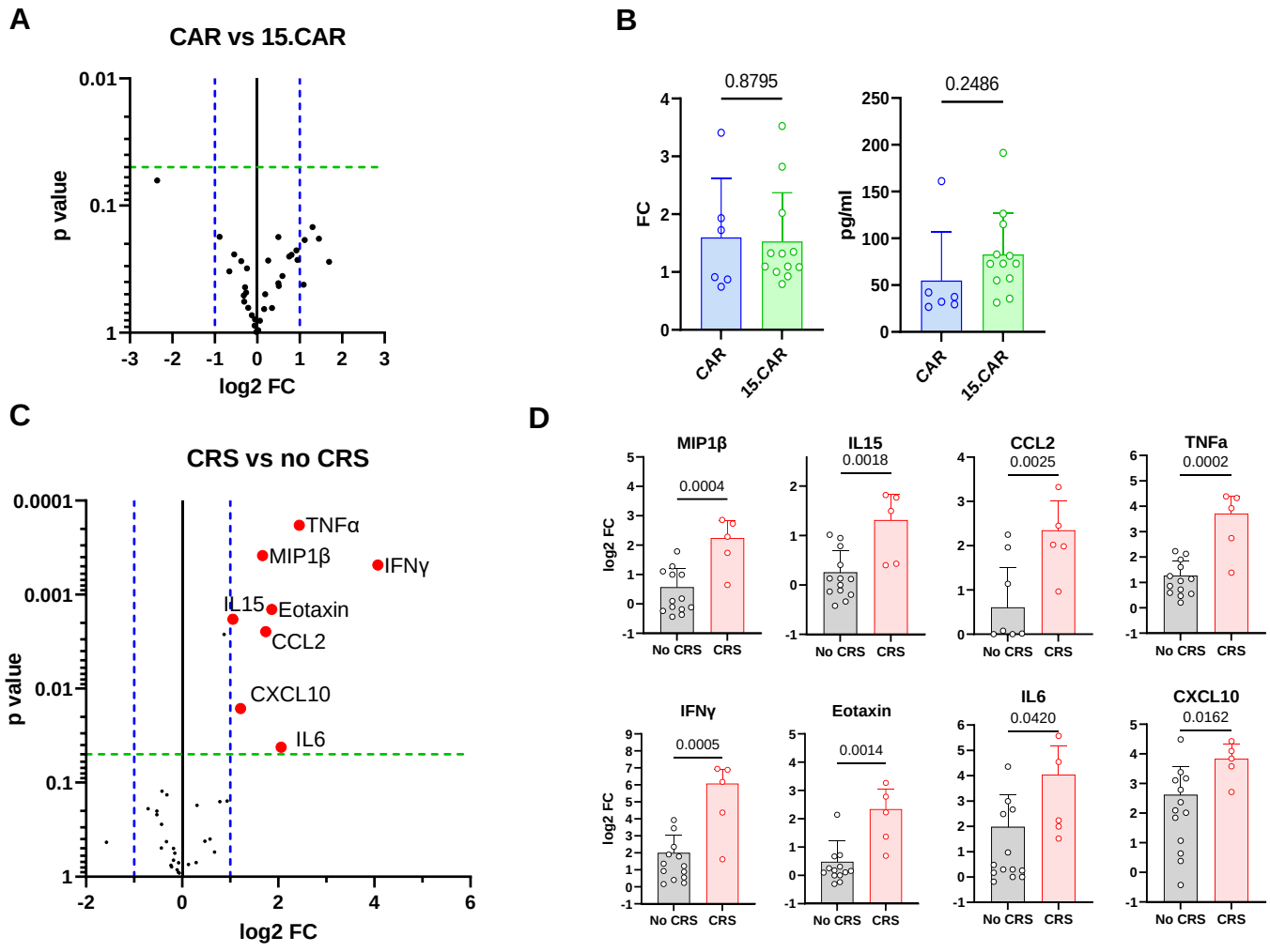
Extended data figure 1: Assessment of GPC3 expression: GPC3 expression was measured by Immune-histochemistry. **A**. Expression of GPC3 in pediatric tissue array – samples from hepatoblastoma and placenta were used as positive controls*(19)*. **B**. Example of an enrollment sample from Patient 15.CAR 2. **C.** Intensity, extent and cumulative GPC3 expression scores for enrolled patients as previously described *(20)*.



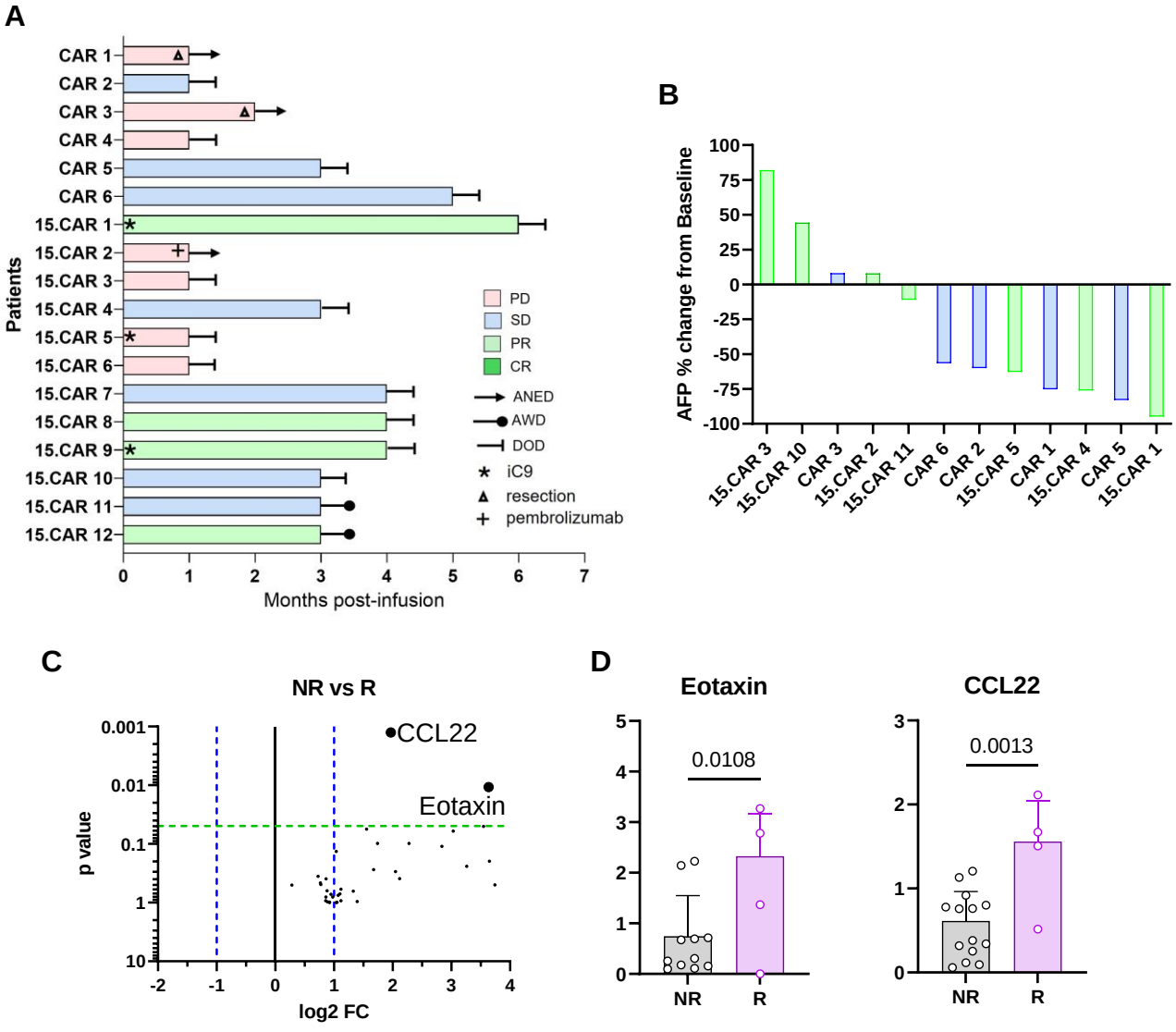
Extended data figure 2. Patient accrual and enrollment: Consort diagram summarizing patients referred, enrolled, and treated on GPC3- and 15.GPC3-CAR T cell studies.



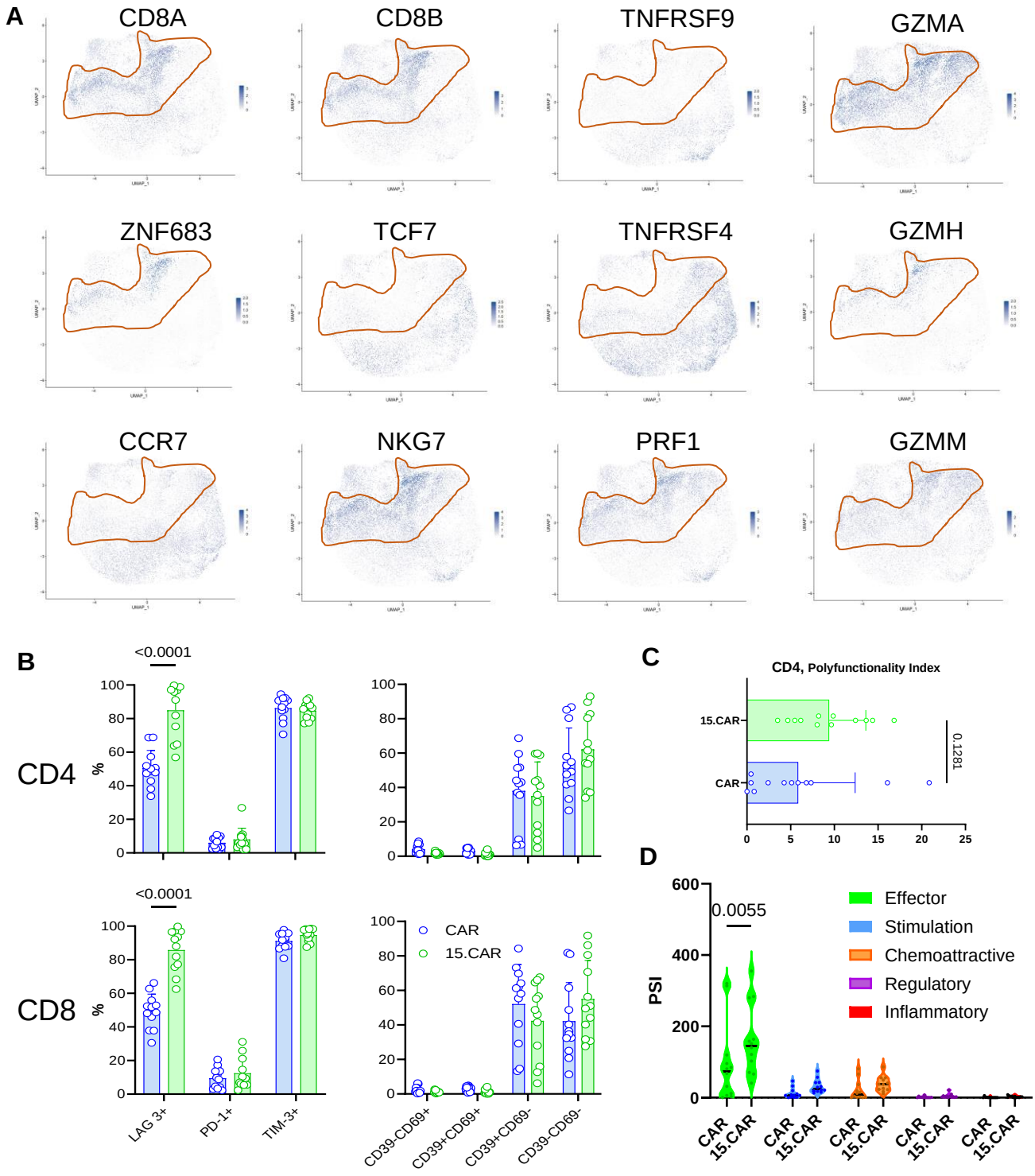
Extended data figure 3: Safety, antitumor activity and peripheral blood and tumor kinetics of patients treated with CAR T cells DL1 and DL2: A. Total number adverse events and **B**. Number of adverse events for each patients treated at 1 x 10^7 / m^2 (DL1, n=6) and 3 x 10^7 / m^2 (DL2, n=6). **C**. Change in serum AFP levels in patients treated at DL1 dose of GPC3-CAR T cells. **D**. Change in tumor volume of these patients. **E**. Peripheral blood transgene copy numbers at indicated timepoints for patients treated at DL1 with GPC3-CAR T cells. **F**. Comparison of transgene copy numbers in PB (left) and tumor (right) of GPC3-CAR T cell levels treated on DL1 and DL2. Comparisons by two-tailed, unpaired T test and two-way ANOVA with Sidac correction. Data represented as mean ± SD.



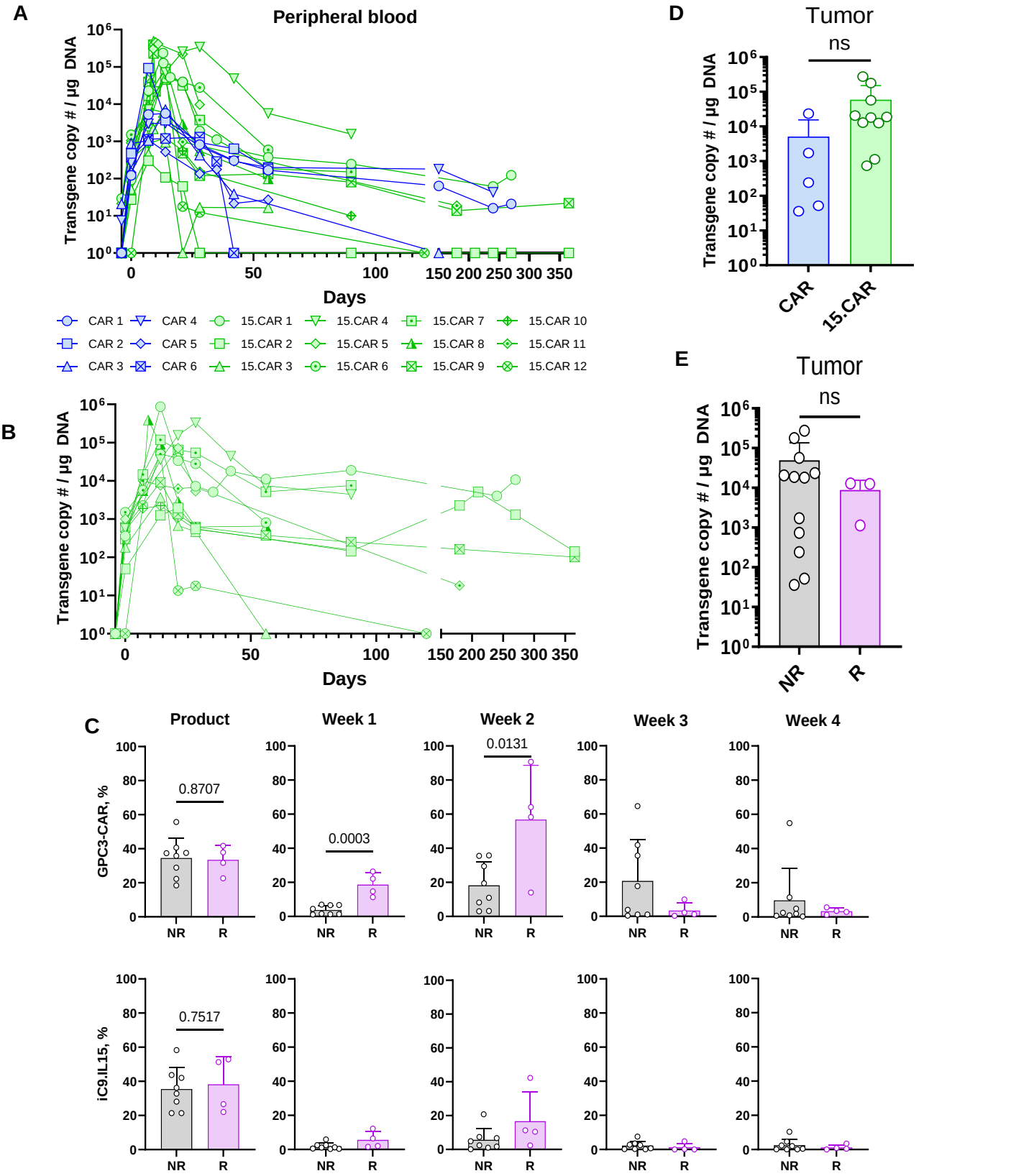
Extended data figure 4. Serum cytokine and chemokine kinetics post-infusion. Levels of chemokines and cytokines were quantified on Day -4, Day 0 and weekly until Day 28. Fold change (FC) was calculated from Day 0 (baseline) to assess changes dependent on CAR T and 15.CAR T cell infusions. **A**. Comparison of FC from baseline to peak concentration for all measured analytes. **B**. Fold change and peak expansion concentration of IL15 in CAR vs 15.CAR treated patients. **C-D**. Differentially expressed cytokines in patients with (n=5) and without CRS (n=13). Overview of all measured analytes (C) and individual cytokines with at least two-fold, statistically significant increase (D). Two-tailed, unpaired T test. Data represented as mean ± SD.



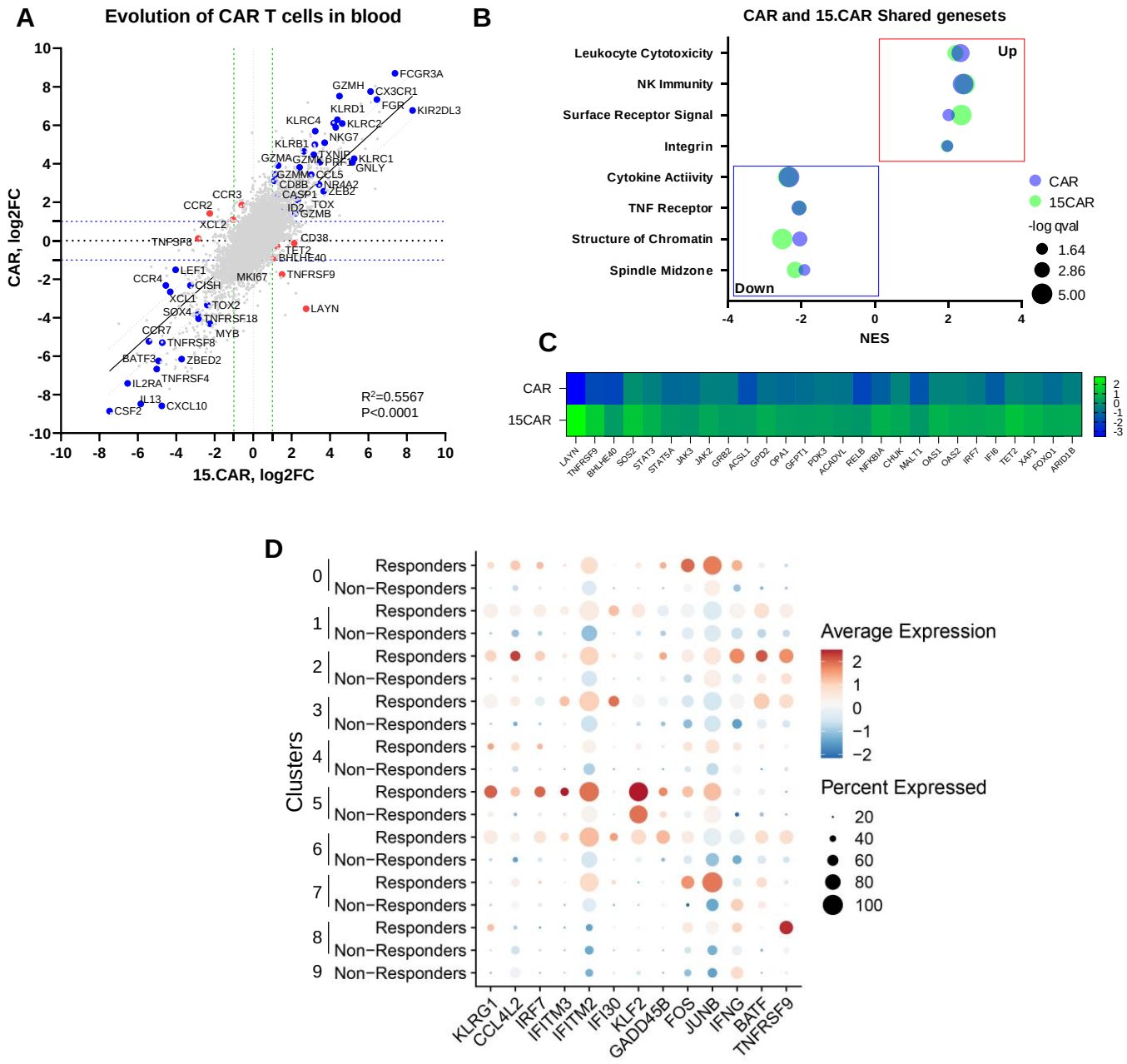
Extended data figure 5. Antitumor response characteristics in patients treated with CAR and 15.CAR T cells: **A**. Long term outcome of treated patients with additional treatments shown for those with Alive with no evidence of disease (ANED). Patients needing the iC9 safety switch indicated. AWD: alive with disease. DOD: Died of disease. **B**. Serum alpha-feto protein (AFP) was measured in the CLIA certified clinical laboratory before and after CAR T cell infusions. Waterfall plot representing changes in AFP concentration from baseline in patients with AFP secreting tumors. **C**. Differentially expressed cytokines in non-responders (NR) vs responders (R) according to RECIST criteria. **D**. Comparison of individual cytokines with at least two-fold, statistically significant increase. Two tailed, unpaired T test. Data represented as mean ± SD.



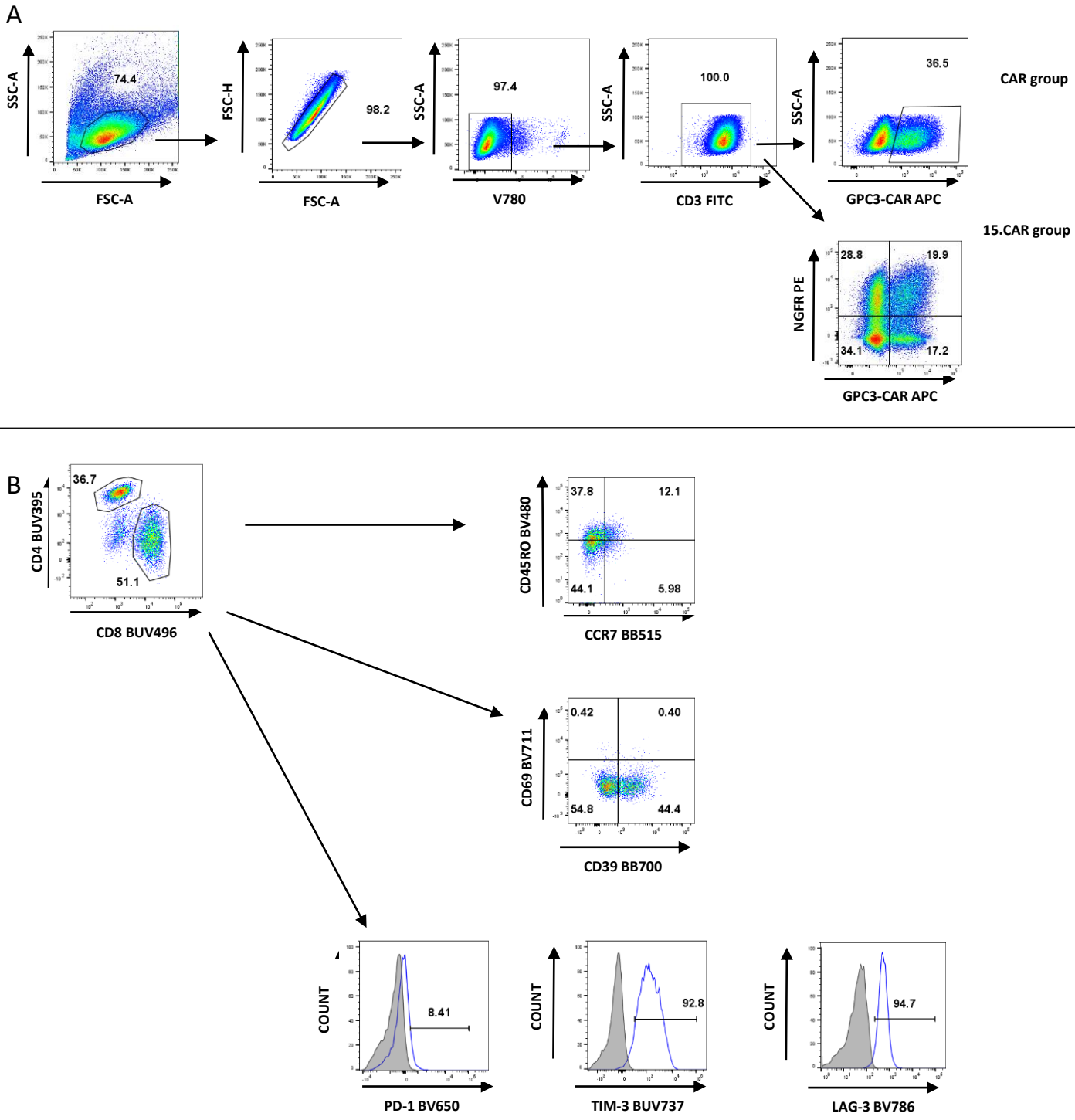
Extended data figure 6. Gene expression, cell surface marker phenotype and polyfunctionality of CAR vs 15.CAR T cell infusion products. A. UMAP projections showing indicated genes for individual cells after combining data from all CAR and 15.CAR T cell infusion products. Region outlined with orange corresponds to 15.CAR enriched cells.**.** Cells were stained for expression of indicated cell surface markers. **B**. CD4 (top) and CD8 (bottom) positive CAR T cells' expression of exhaustion (LAG3, PD1, TIM3) markers and proportion of CD39/CD69 subsets. Comparison by two-way ANOVA with Sidac correction for multiple comparisons. **C-D**. Cells were evaluated by the Isoplexis, single cell cytokine detection system. (C). Polyfunctionality index of CD4 subset and (D). Polyfunctionality strength index of the indicated products. Two-way ANOVA with Šídák correction. Data represented as mean ± SD.



Extended data Fig 7. Expansion, persistence and trafficking of CAR vs 15.CAR T cells post-infusion. Expansion and persistence of infused cell populations were quantified with qPCR. **A-B**. Transgene copy numbers for the GPC3-CAR (**A**) and iC9.NGFR.IL15 (**B**). **C**. Comparison of CAR and iC9.NGFR.IL15 transgene expression in non-responder (NR) vs responder (R) products and peripheral blood samples at indicated timepoints by flow cytometry. **D-E**. GPC3-CAR transgene frequencies in tumor biopsies in CAR vs 15.CAR (C) and in R vs NR groups (D). Two-tailed, unpaired T test. Data represented as mean ± SD.



Extended data Fig 8. Gene expression evolution in CAR and 15.CAR T cells post-infusion. The transcriptomic profile of Infusion products and peripheral blood CAR and 15.CAR T cells (A-C) or infusion products and tumor infiltrating 15.CAR T cells (D) were interrogated with single cell RNA sequencing. Differentially expressed genes (DEGs) for indicated groups were determined by comparing the product and post-infusion samples. **A.** DEGs from infusion product to peripheral blood represented by log2 fold change (log2FC) in CAR (y axis) vs 15.CAR (x axis) T cells. **B**. Selected gene sets enriched in CAR vs 15.CAR T cells in PB. **C**. Heatmap representing a subset of DEGs from the pre-infusion product to PB comparison in CAR vs. IL15.CAR. **D**. Selected cluster specific DEGs in 15.CAR T cells captured in tumors post-infusion. Cluster 9 contains only Non-responder cells.



Extended data Fig 9: Gating strategy for product and peripheral blood CAR T cell phenotyping. Patient-derived and product samples were processed and stained with indicated fluorochrome-conjugated antibodies. A) After gating on live cells and removing duplets, the T cell population was defined as CD3+ and the evaluated for CAR and IL-15 expression based on Anti Fab APC and NGFR PE, respectively. B) Manufactured products were further characterized based on gating strategy in A. The CAR+ subset was further analyzed to determine CD4/CD8, memory, and exhaustion markers.