Supplementary Materials

Materials and Methods

PBMCs from healthy donors

Peripheral blood was collected from healthy volunteer donors aged 18-26. Mononuclear cells were isolated from peripheral blood using CPT tube (BD) gel-barrier centrifugation. Purified $CD8^+$ populations were obtained by positive selection using MACS magnetic microbead sorting (Miltenyi) of the entire PBMC isolate, while $CD4^+$ cells were positively selected from the CD8-deplete fraction. Bulk PBMCs and purified $CD4^+$ and $CD8^+$ cells were cryopreserved in CryoStor (Sigma).

TCR spectratyping

Cryopreserved FP $CD8^+$ and $CD4^+$ cells were screened for clone-specific expansion using the TCR Vβ Repertoire Kit (BD Biosciences). Analysis of 24 Vβ segments was performed by incubating cells resuspended in PBS with the recommended volume of each of the eight TCR Vβ antibody mixes provided with the kit and a CD3 mAB (BD Biosciences) for 20 minutes at room temperature. Cells were washed and fixed in 2% paraformaldehyde prior to acquisition on the LSRFortessa. Samples were analyzed using FlowJo software and the quantity of each Vβ subset was reported as a percentage of the total $CD3^+$ population.

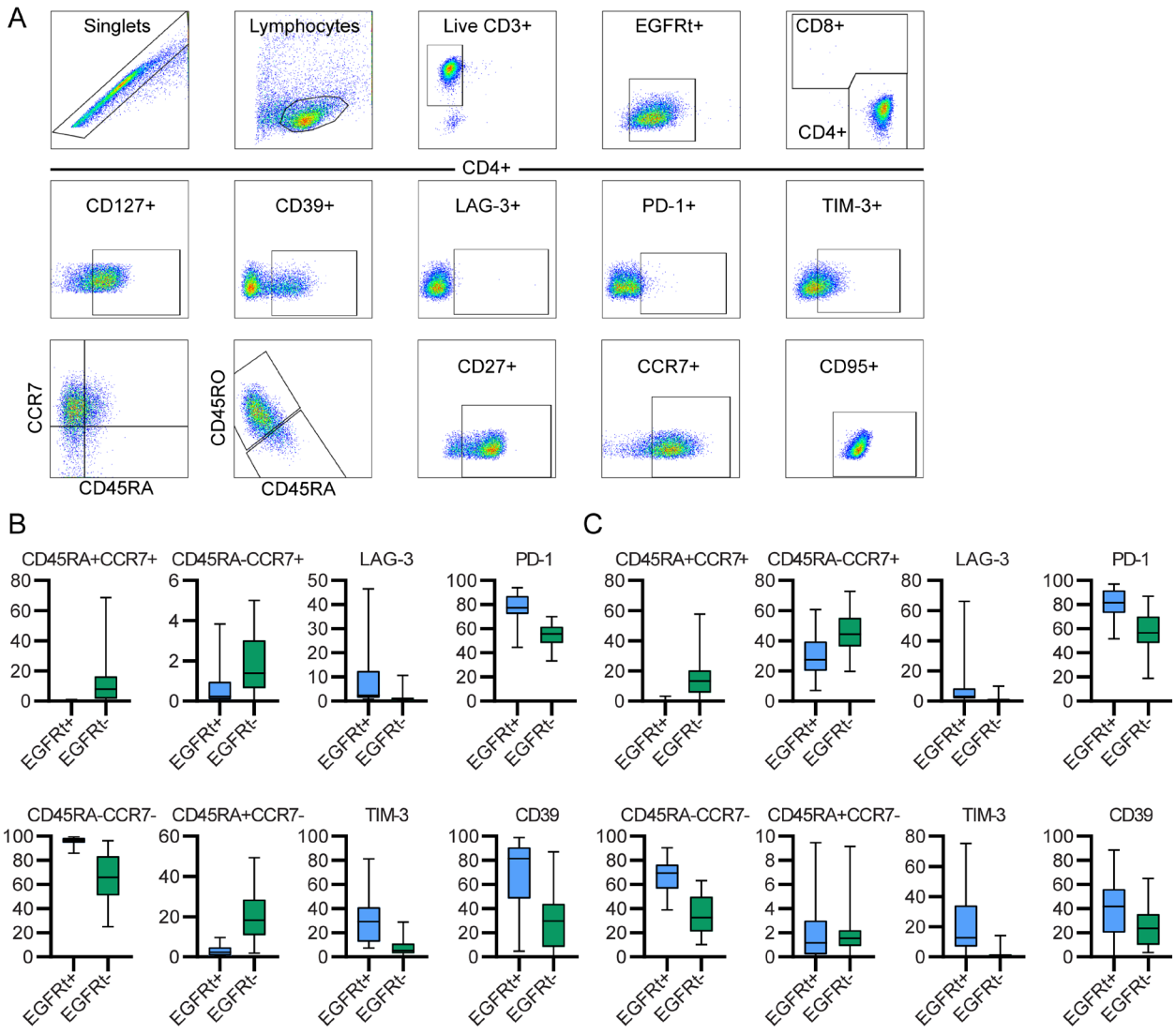

Fig. S1: Phenotypic analysis of EGFRt$^+$ cells at peak expansion. A. Representative gating of CD4$^+$ FP. B-C. Box plots of CD8$^+$ (B) and CD4$^+$ (C) EGFRt$^+$ (blue) and EGFRt$^-$ (green) phenotype at peak expansion (D10-D14). Bar represents the median, box represents the 25% and 75% quartiles, and error bars represent the range.

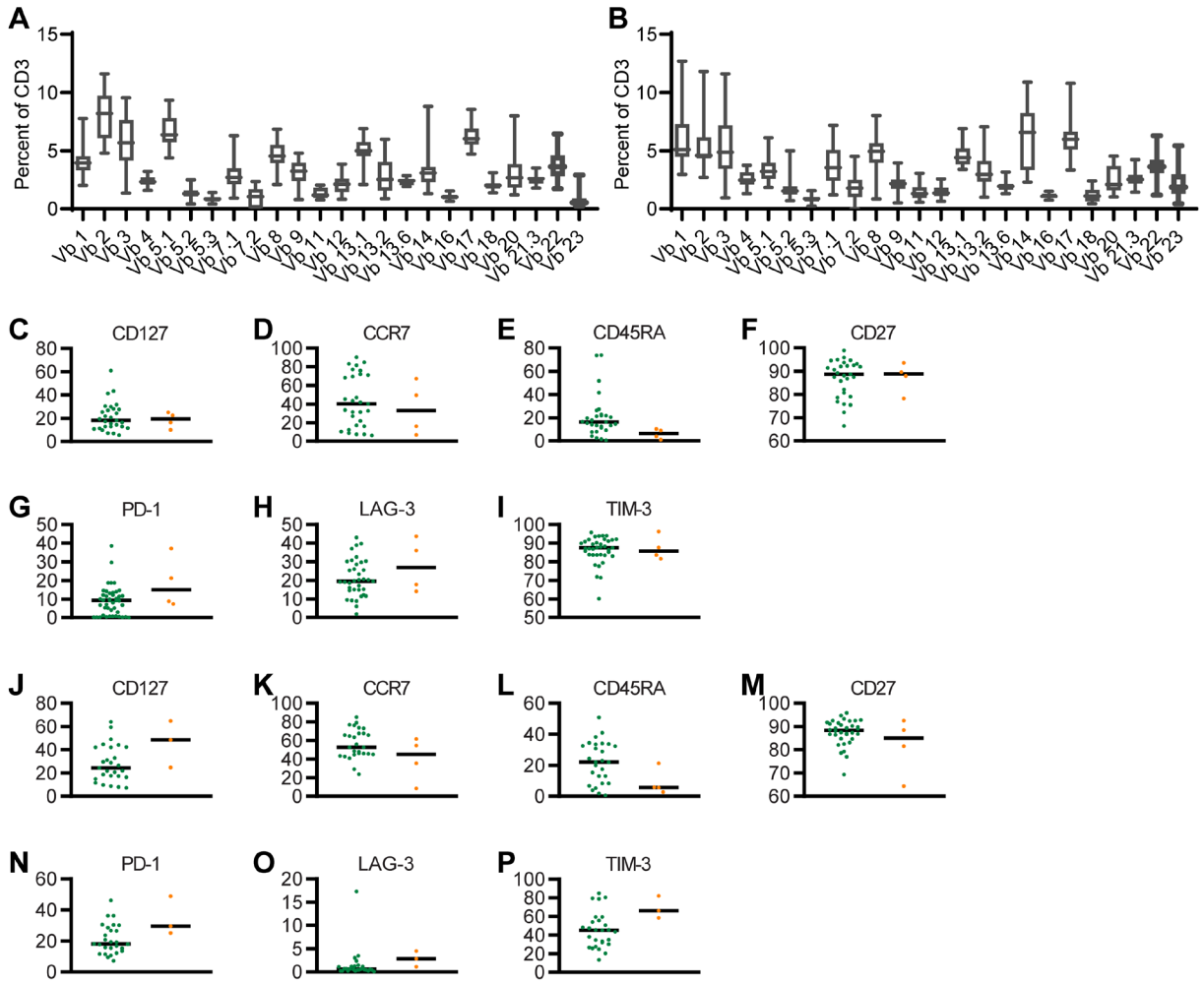

Fig. S2: Phenotypic analysis of EGFRt+ final product cells in dysfunctional and functional groups. A-B. Spectratype of CD8+EGFRt+ FP (A) and CD4+EGFRt+ FP (B). C-I. Dot plots of CD8+EGFRt+ FP cells expressing (C) CD127, (D) CCR7, (E) CD45RA, (F) CD27, (G) PD-1, (H) LAG-3 and (I). J-P. Dot plots of CD4+EGFRt+ FP cells expressing (J) CD127, (K) CCR7, (L) CD45RA, (M) CD27, (N) PD-1, (O) LAG-3 and (P) TIM-3. Green circles: functional response, orange circles: dysfunctional response. Bar represents the median.

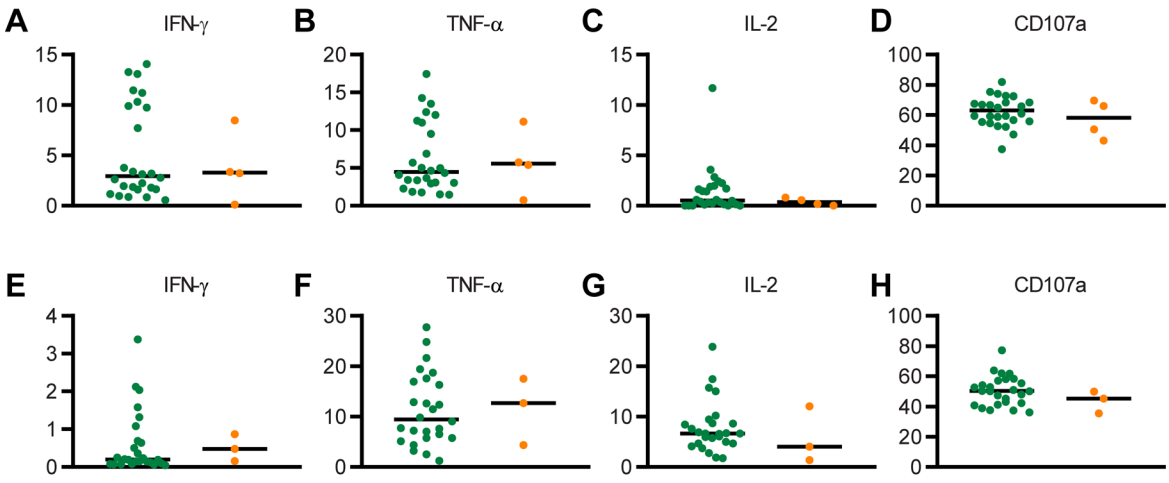

Fig. S3: Functional analysis of EGFRt$^+$ final product cells in dysfunctional and functional groups. A-D. Dot plots of the percentage of CD8$^+$EGFRt$^+$ FP cells secreting IFN-γ (A), TNF-α (B), IL-2 (C) and CD107a (D) following antigen-specific stimulation. E-H. Dot plots of the percentage of CD4$^+$EGFRt$^+$ FP cells secreting IFN-γ (E), TNF-α (F), IL-2 (G) and CD107a (H) in the functional (green) and dysfunctional (orange) groups. Bar represents the median.

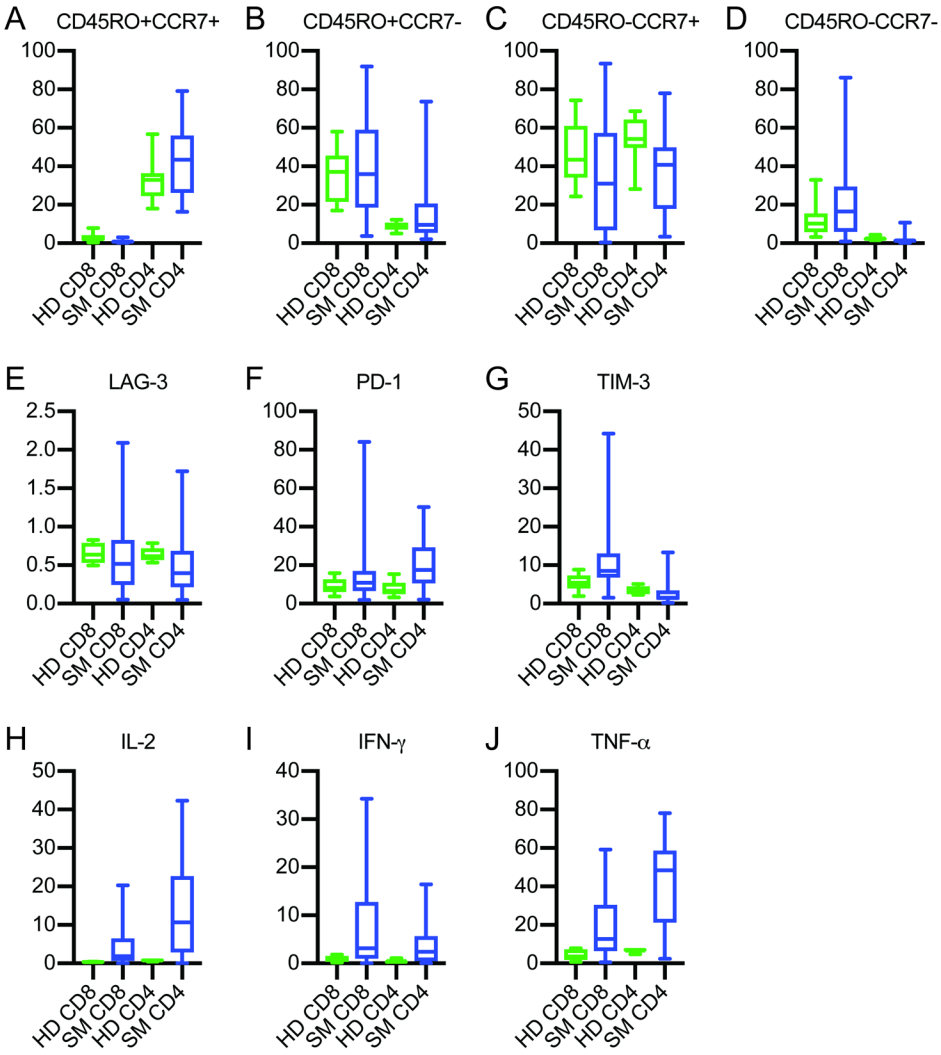

Fig. S4: Phenotypic analysis of subject T cells. A-D. Box plots of $CD8^+$ (left) and $CD4^+$ (right) cells from healthy donor PBMC (green) and subject starting material (blue) expressing $CD45RO^+CCR7^+$ (A), $CD45RO^+CCR7^-$ (B), $CD45RO^-CCR7^+$ (C), $CD45RO^-CCR7^-$ (D). E-G. Box plots of $CD8^+$ (left) and $CD4^+$ (right) cells expressing LAG-3 (E), PD-1 (F) and TIM-3 (G) from healthy donor PBMC (green) and subject starting material (blue). H-J. Box plots of $CD8^+$ (left) and $CD4^+$ (right) cells secreting IL-2 (H), IFN-γ (I) and TNF-α (J) in response to CD3/CD28 stimulation from healthy donor PBMC (green) and subject starting material (blue). Bar represents the median, box represents the 25% and 75% quartiles, and error bars represent the range.

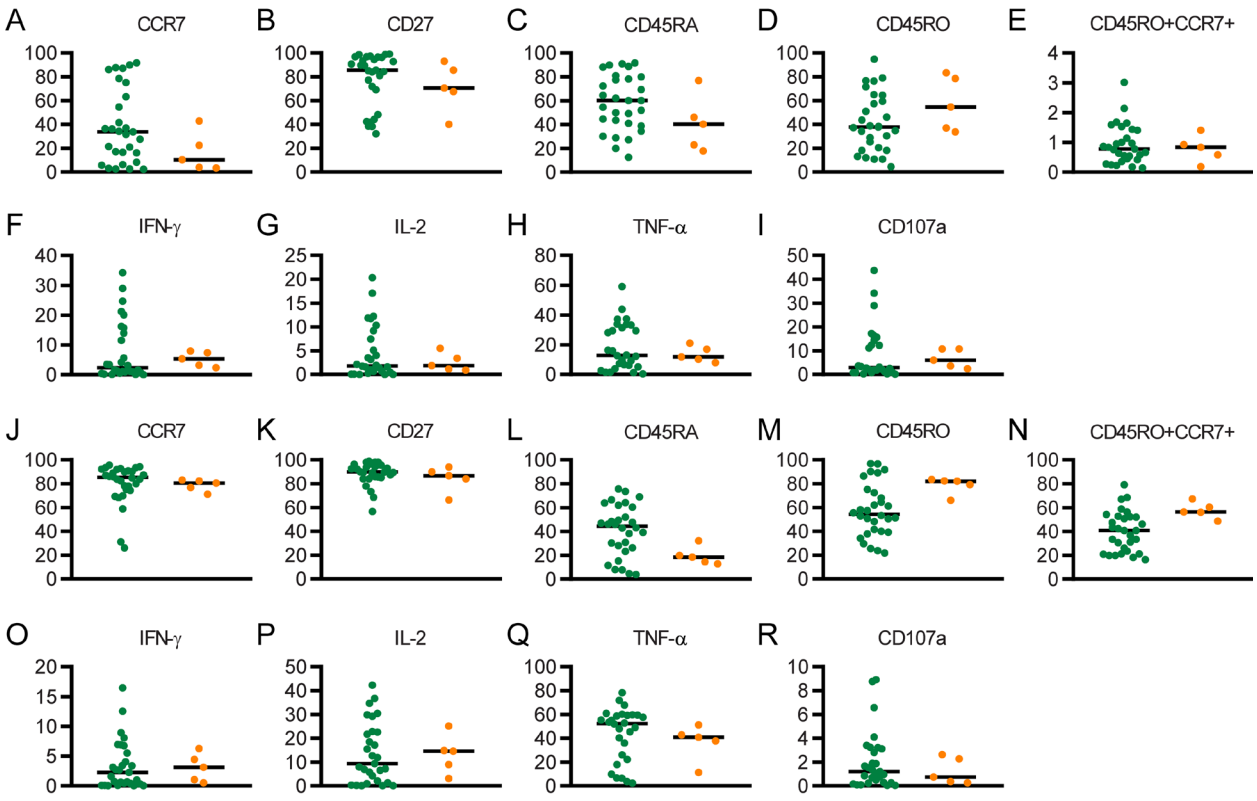

Fig. S5: Phenotypic and functional analysis of starting material cells in dysfunctional and functional groups. A-E. Dot plots of $CD8^+$ starting material cells expressing CCR7 (A), CD27 (B), CD45RA (C), CD45RO (D) and $CD45RO^+CCR7^+$ (E). F-I Dot plots of $CD8^+$ starting material cells secreting IFN-γ (F), IL-2 (G), TNF-α (H) and CD107a (I) in response to CD3/CD28 stimulation. J-N. Dot plots of $CD4^+$ starting material cells expressing CCR7 (J), CD27 (K), CD45RA (L), CD45RO (M) and $CD45RO^+CCR7^+$(N) F-I Dot plots of $CD4^+$ starting material cells secreting IFN-γ (N), IL-2 (O), TNF-α (P) and CD107a (Q) in response to CD3/CD28 stimulation. Green circles: functional response, orange circles: dysfunctional response. Bar represents the median.

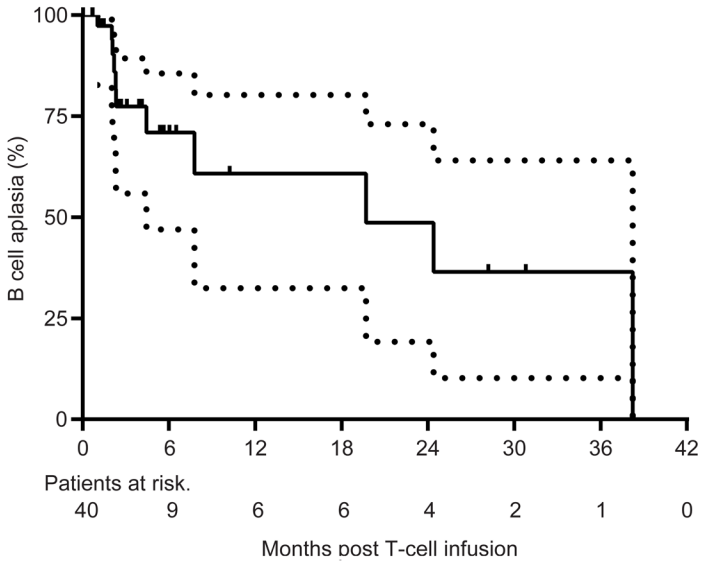

Fig. S6: B cell aplasia duration. Kaplan-Meier of B cell aplasia (BCA) of all subjects that achieve BCA (n=40). Median follow-up was 26.2 months. Dotted line represents 95% confidence.

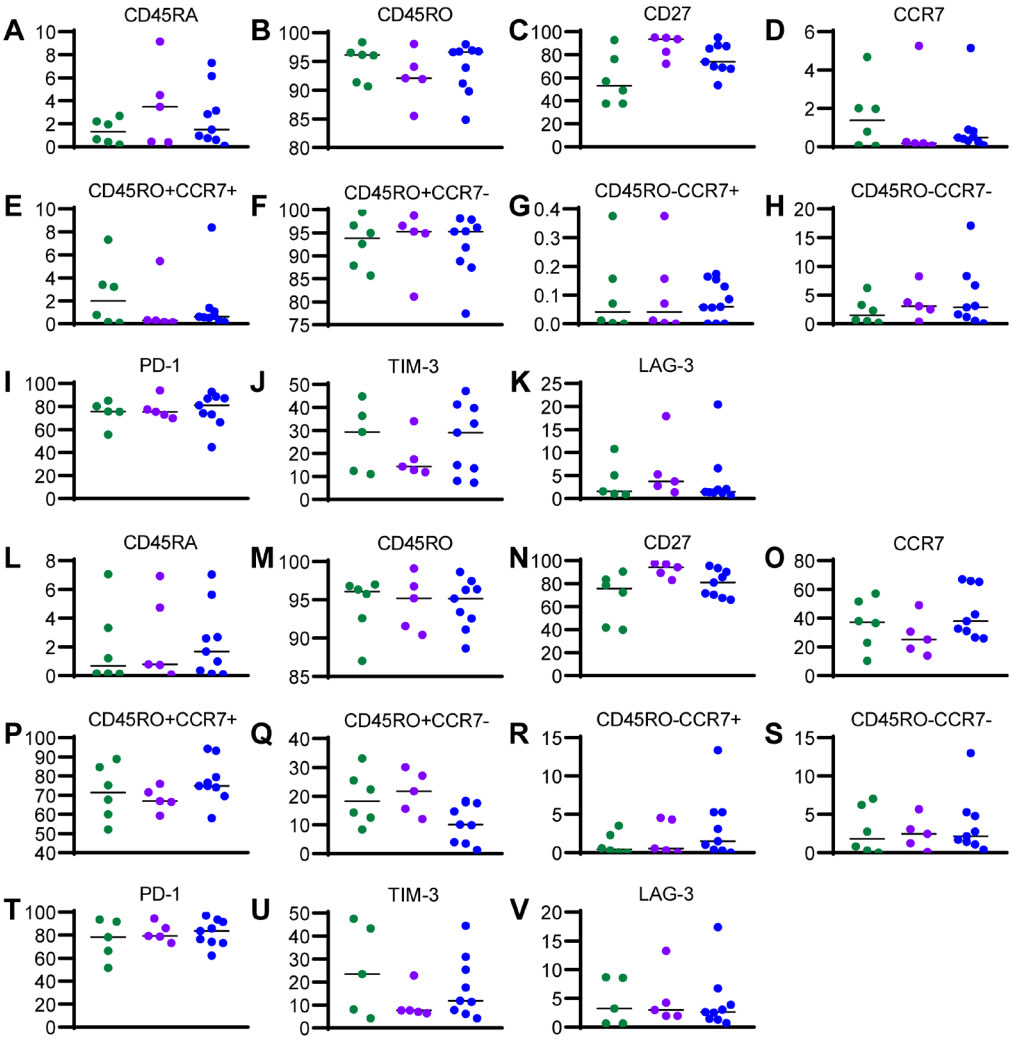

Fig. S7: Phenotypic analysis of EGFRt$^+$ cells at peak expansion in shortBCA, mediumBCA and longBCA groups. A-O. Dot plots of CD8$^+$EGFRt$^+$ cells expressing CD45RA (A), CD45RO (B), CD27 (C), CCR7 (D), CD45RO$^+$CCR7$^+$ (E), CD45RO$^+$CCR7$^-$ (F), CD45RO$^-$CCR7$^+$ (G), CD45RO$^-$CCR7$^-$ (J), PD-1 (I), TIM-3 (J) and LAG-3 (K). L-V. Dot plots of CD4$^+$EGFRt$^+$ cells expressing CD45RA (L), CD45RO (M), CD27 (N), CCR7 (O), CD45RO$^+$CCR7$^+$ (P), CD45RO$^+$CCR7$^-$ (Q), CD45RO$^-$CCR7$^+$ (R), CD45RO$^-$CCR7$^-$ (S), PD-1 (T), TIM-3 (U) and LAG-3 (V). Bar represents the median. Green circles: longBCA, purple circles: mediumBCA, blue circles: shortBCA.

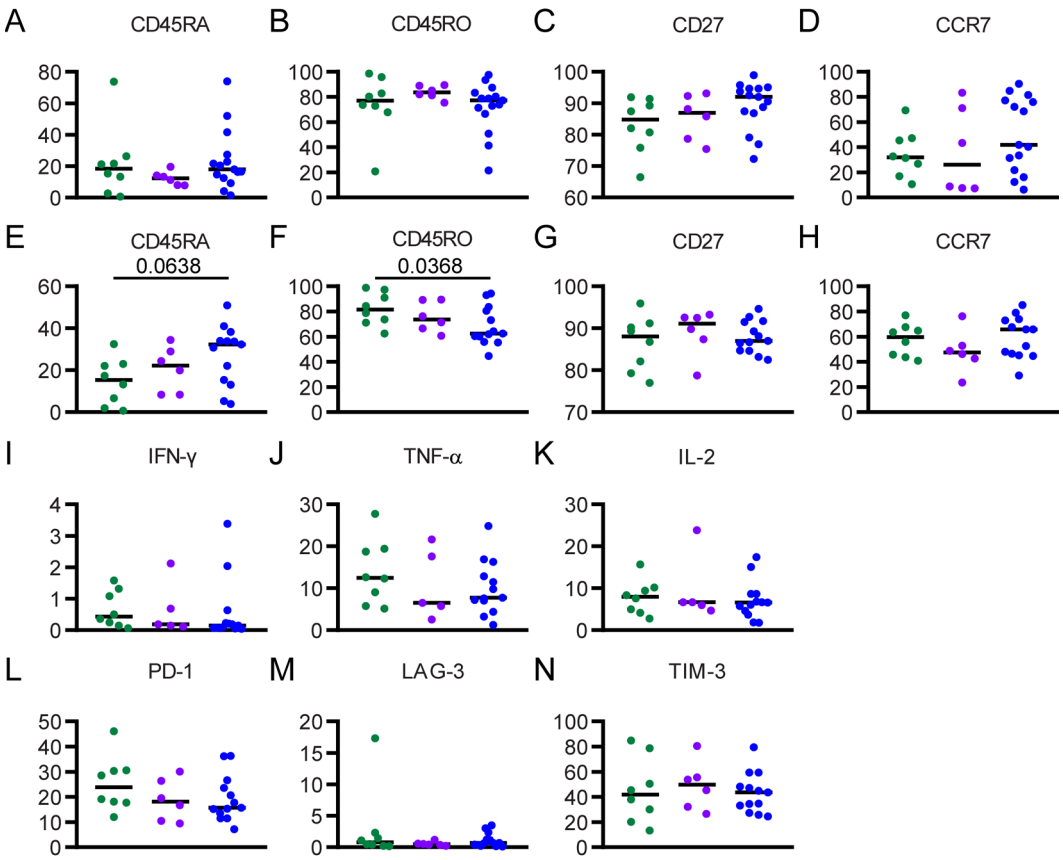

Fig. S8: Phenotypic and functional analysis of EGFRt⁺ final product cells in BCA groups.

A-D. Percentage of CD8⁺EGFRt⁺ FP cells expressing CD45RA (A), CD45RO (B), CD27 (C) and CCR7 (D). E-H. Percentage of CD4⁺EGFRt⁺ FP cells expressing CD45RA (E), CD45RO (F), CD27 (G) and CCR7 (H). I-J. Percentage of CD4⁺EGFRt⁺ FP cells secreting IFN-γ (I), IL-2 (J), TNF-α (K) in response to antigen-specific stimulation. L-N. Percentage of CD4⁺EGFRt⁺ FP cells expressing PD-1 (L), LAG-3 (N) and TIM-3(O). Bars represent the median, p values calculated using a Mann-Whitney test. Green circles: longBCA, purple circles: mediumBCA, blue circles: shortBCA. Bar represents the median.