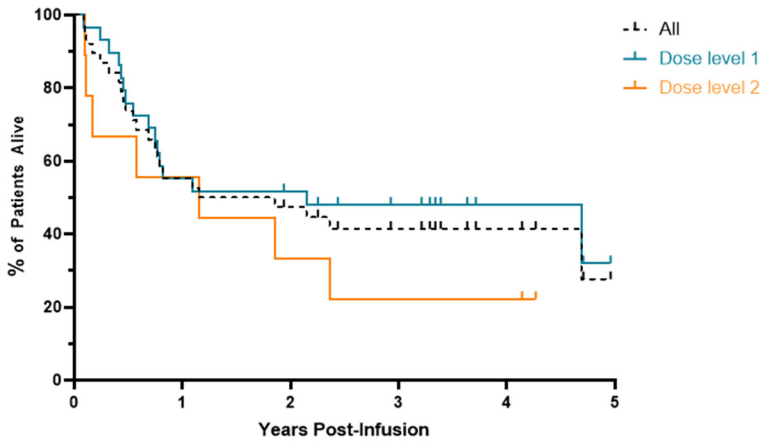
Extended data

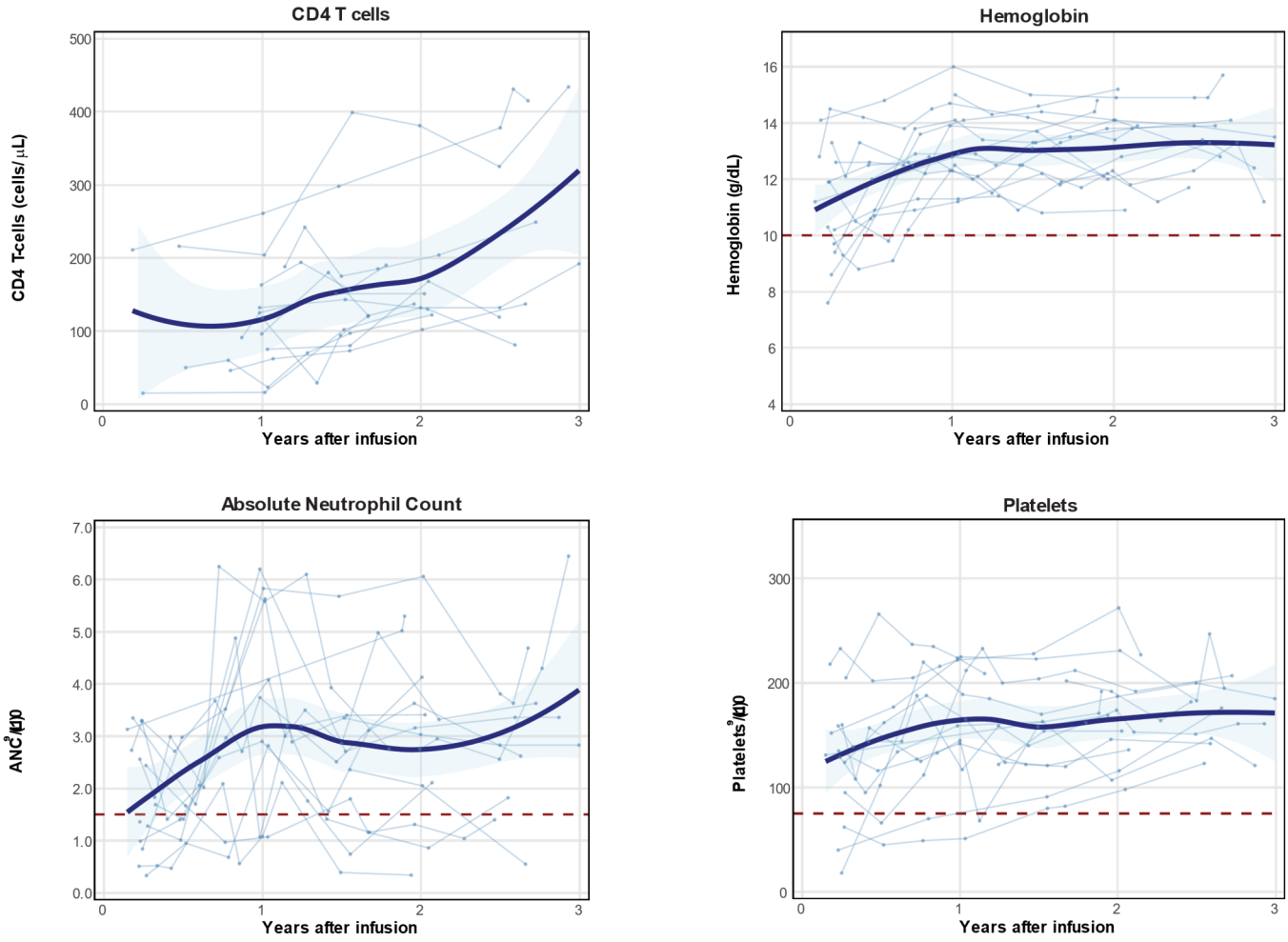
Extended data Figure 1 Survival outcomes by dose level and immunologic recovery post-infusion

(A) Kaplan-Meier curves showing overall survival (OS) stratified by CAR22 dose level. Patients treated at dose level 1 (1 million CAR+ T cells/kg, n=29) demonstrated a median OS of 25.7 months compared to 13.8 months for those treated at dose level 2 (3 million CAR+ T cells/kg, n=9). Vertical ticks indicate censored observations (B) Longitudinal analysis of hematological parameters in peripheral blood over 36 months post-infusion, including CD4 T cell counts, Hemoglobin levels, Absolute neutrophil count (ANC) and Platelet counts. For all panels, a generalized additive model (GAM) was employed for data smoothing and trend visualization (solid line), with individual patient measurements shown as data points. The shaded area represents the 95% confidence interval of the GAM prediction. Red dashed lines indicate thresholds for grade 2 hematotoxicity according to CTCAE criteria

(A)



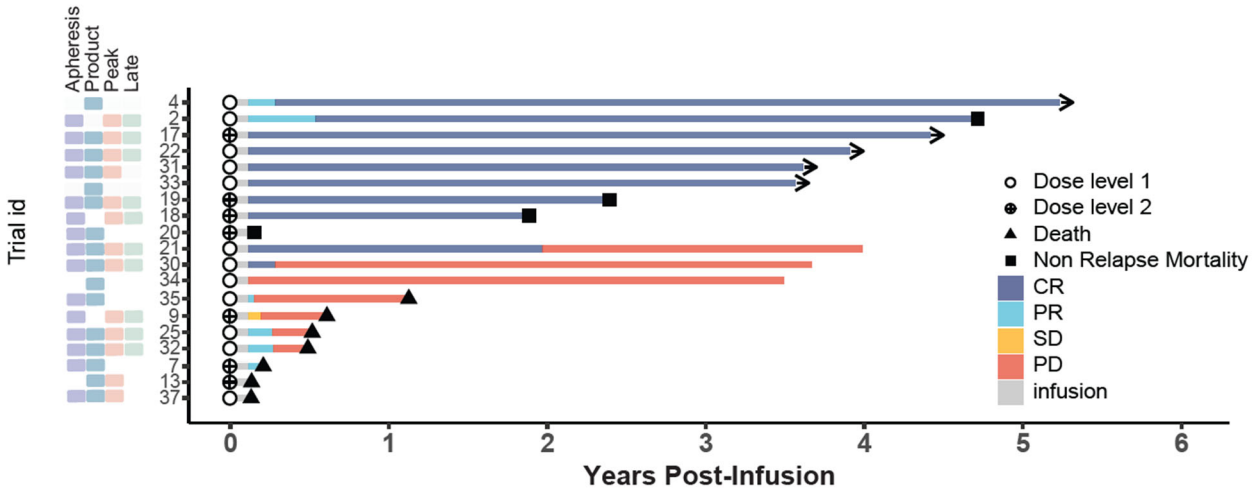
(B)


CD4 T cells
CD4 T-cells (cells/ μL)
Years after infusion
Hemoglobin
Hemoglobin (g/dL)
Years after infusion
Absolute Neutrophil Count
ANC (10^9/L)
Years after infusion
Platelets
Platelets (10^9/L)
Years after infusion

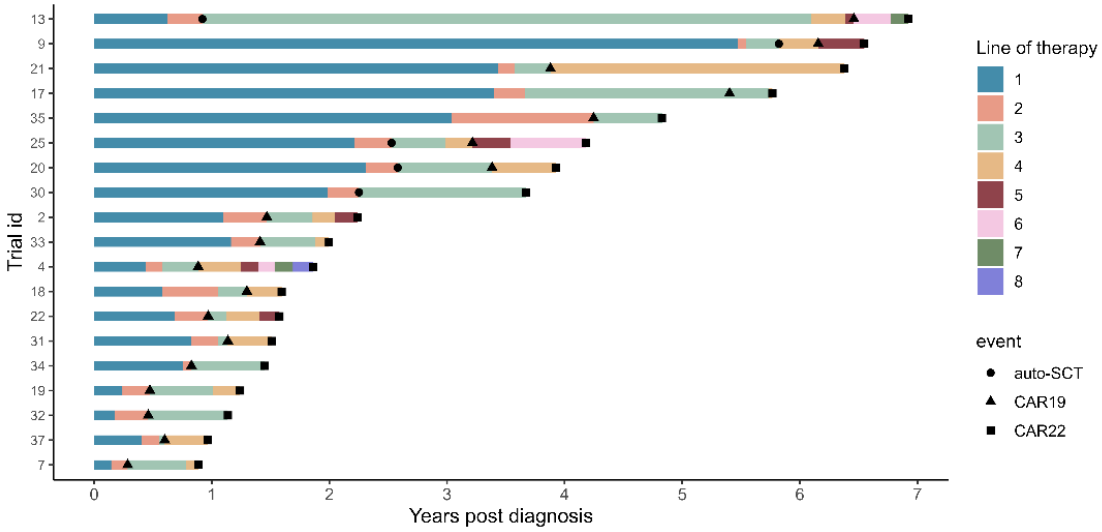
Extended data figure 2 Longitudinal Characterization of Immune Cell Composition and T Cell Dynamics Associated with Clinical Response to CAR-T Cell Therapy

(A) Longitudinal patient sampling timeline and clinical outcomes visualization showing treatment response duration and overall survival for individual patients included in single-cell RNA sequencing (scRNA-seq) and T cell receptor sequencing (scTCR-seq) analyses. (B) Response duration to prior lines of therapy, a median of four prior lines of therapy was applied (C) Gene expression heatmap of canonical markers used for T cell subset identification, aggregated by pseudobulk analysis. (D) CD4/CD8 T cell ratio quantified by scRNA-seq, stratified by timepoint, response status, and CAR mRNA detection. (E) Temporal dynamics of CD4+ and CD8+ CAR T cell populations following infusion as measured by flow cytometry. (F) B3GAT1 (CD57) expression pseudobulked per patient at timepoint apheresis, plotted in relation time since CAR19 infusion (G) Predicted CD57 protein expression levels across T cell subsets as a marker of terminal differentiation and senescence (H) Relative distribution of immune cell types at baseline stratified by clinical response status at 3 months post-infusion. (I) Pre-treatment lymphocyte-to-monocyte ratio (LMR) in peripheral blood measured prior to lymphodepleting chemotherapy, stratified by clinical response status at 3 months post-treatment. (J)Flow cytometry analysis of CAR T products (n=38) showing distribution of naïve & central memory cells compared to effector and TEMRA cells (K) T Stemness scores (based on combined SELL, CCR7, CD28, IL7R, LEF1 and TCF7 expression) across CD4+ and CD8+ CAR T cells

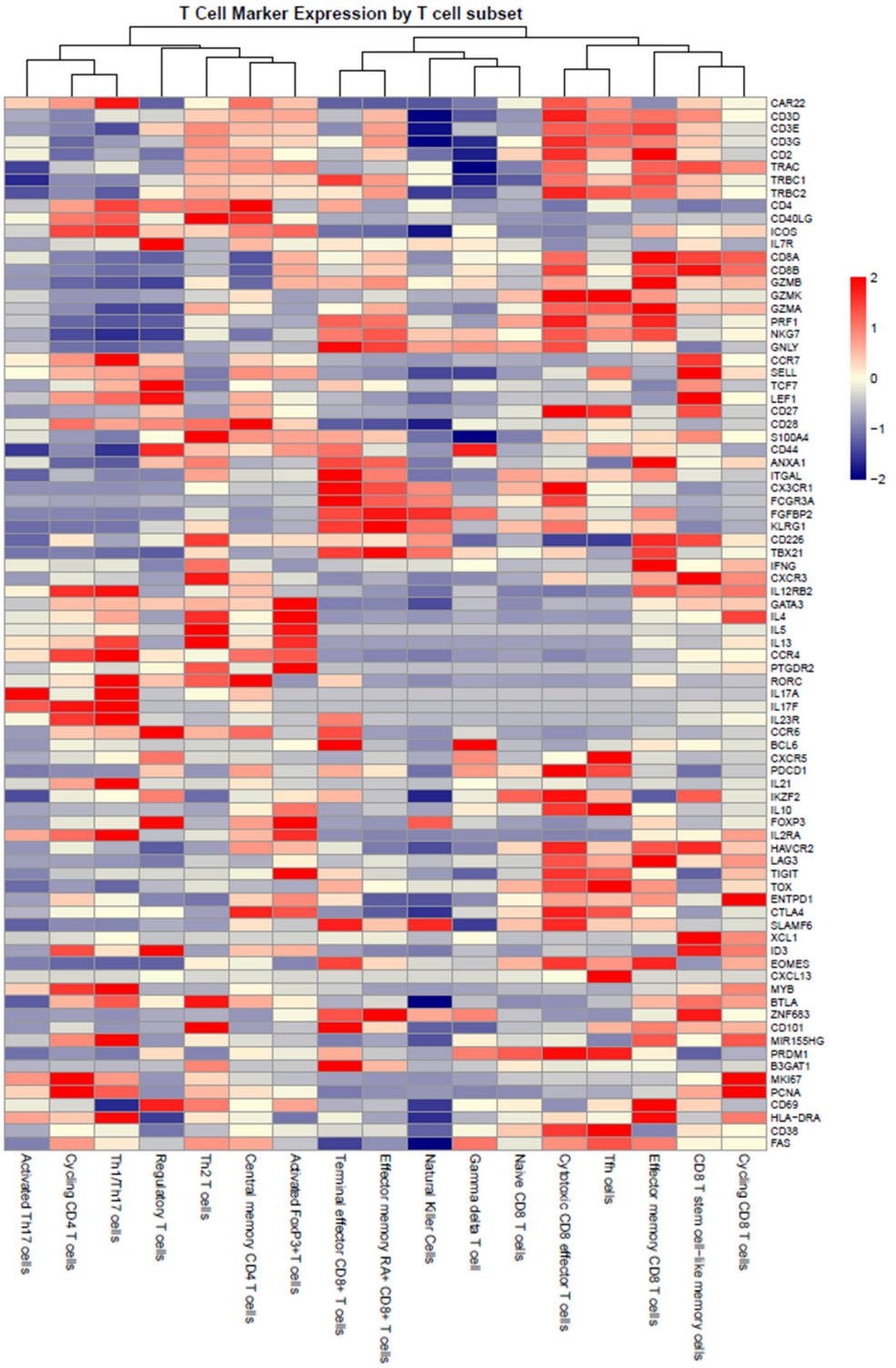
(A)



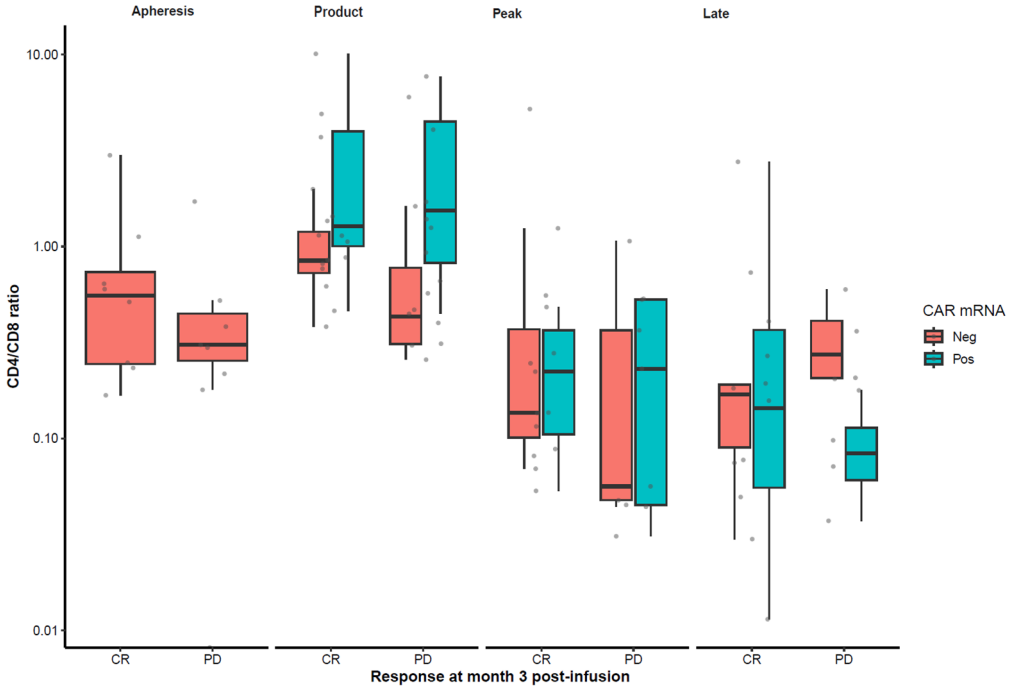
(B)


Trial id
13
9
21
17
35
25
20
30
2
33
4
18
22
31
34
19
32
37
7
0
1
2
3
4
5
6
7
Years post diagnosis
Line of therapy
1
2
3
4
5
6
7
8
event
auto-SCT
CAR19
CAR22

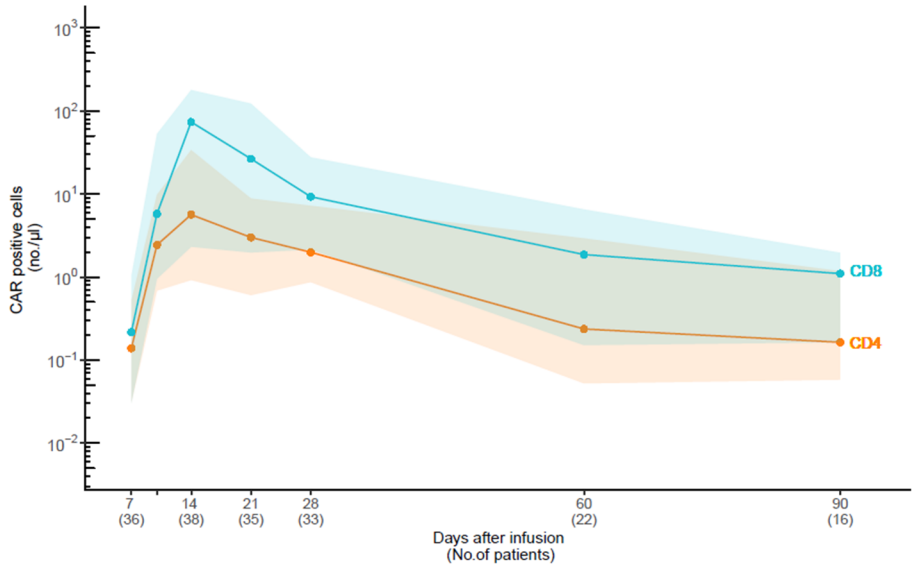
(C)


T Cell Marker Expression by T cell subset
CAR22
CD3D
CD3E
CD3G
CD2
TRAC
TRBC1
TRBC2
CD4
CD40LG
ICOS
IL7R
CD8A
CD8B
GZMB
GZMK
GZMA
PRF1
NKG7
GNLY
CCR7
SELL
TCF7
LEF1
CD27
CD28
S100A4
CD44
ANXA1
ITGAL
CX3CR1
FCGR3A
FGFBP2
KLRG1
CD226
TBX21
IFNG
CXCR3
IL12RB2
GATA3
IL4
IL5
IL13
CCR4
PTGDR2
RORC
IL17A
IL17F
IL23R
CCR6
BCL6
CXCR5
PDCD1
IL21
IKZF2
IL10
FOXP3
IL2RA
HAVCR2
LAG3
TIGIT
TOX
ENTPD1
CTLA4
SLAMF6
XCL1
ID3
EOMES
CXCL13
MYB
BTLA
ZNF683
CD101
MIR155HG
PRDM1
B3GAT1
MKI67
PCNA
CD69
HLA-DRA
CD38
FAS
2
1
0
−1
−2
Activated Th17 cells
Cycling CD4 T cells
Th1/Th17 cells
Regulatory T cells
Th2 T cells
Central memory CD4 T cells
Activated FoxP3+T cells
Terminal effector CD8+ T cells
Effector memory RA+ CD8+ T cells
Natural Killer Cells
Gamma delta T cell
Naive CD8 T cells
Cytotoxic CD8 effector T cells
Tfh cells
Effector memory CD8 T cells
CD8 T stem cell−like memory cells
Cycling CD8 T cells

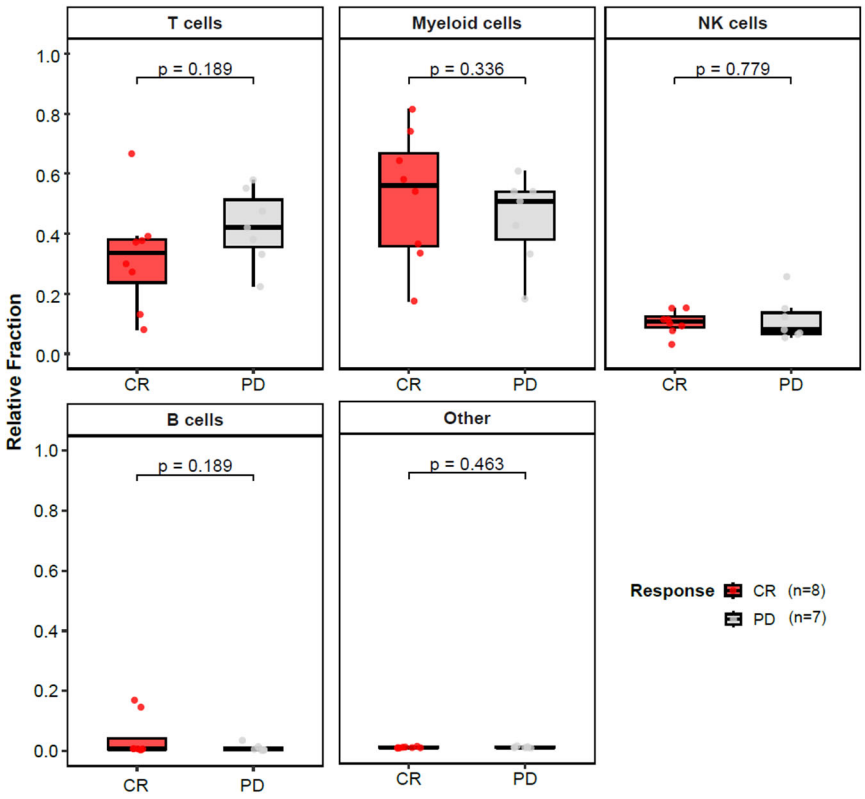
(D)


Apheresis
Product
Peak
Late
10.00
1.00
0.10
0.01
CD4/CD8 ratio
CR
PD
Response at month 3 post-infusion
CAR mRNA
Neg
Pos

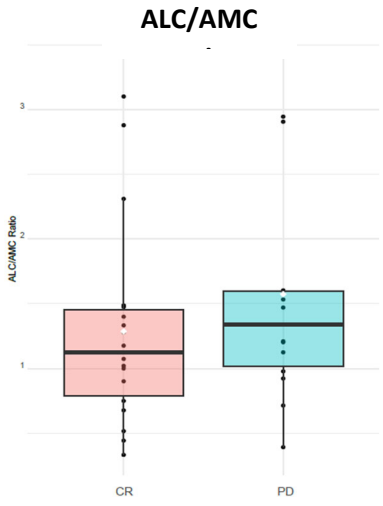
(E)


CAR positive cells (no./μl)
CD8
CD4
7 (36)
14 (38)
21 (35)
28 (33)
60 (22)
90 (16)
Days after infusion (No.of patients)

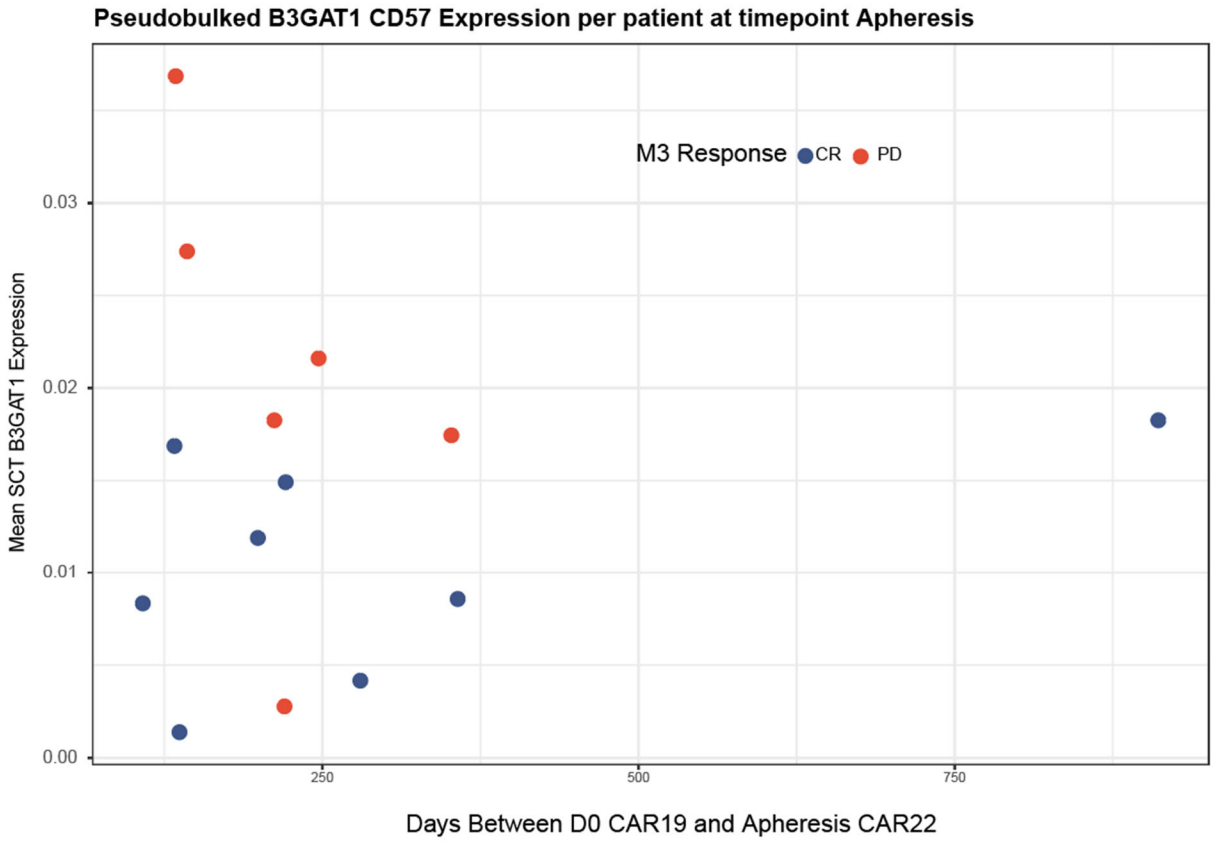
(F)


T cells
Myeloid cells
NK cells
B cells
Other
p = 0.189
p = 0.336
p = 0.779
p = 0.189
p = 0.463
Relative Fraction
1.0
0.8
0.6
0.4
0.2
0.0
CR
PD
Response
CR (n=8)
PD (n=7)

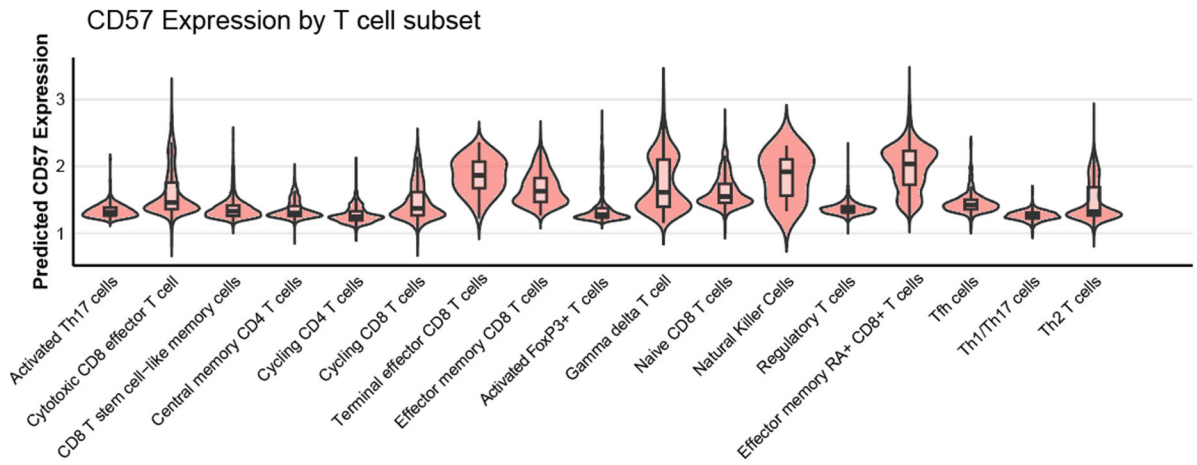
(G)


ALC/AMC
ALC/AMC Ratio
3
2
1
CR
PD

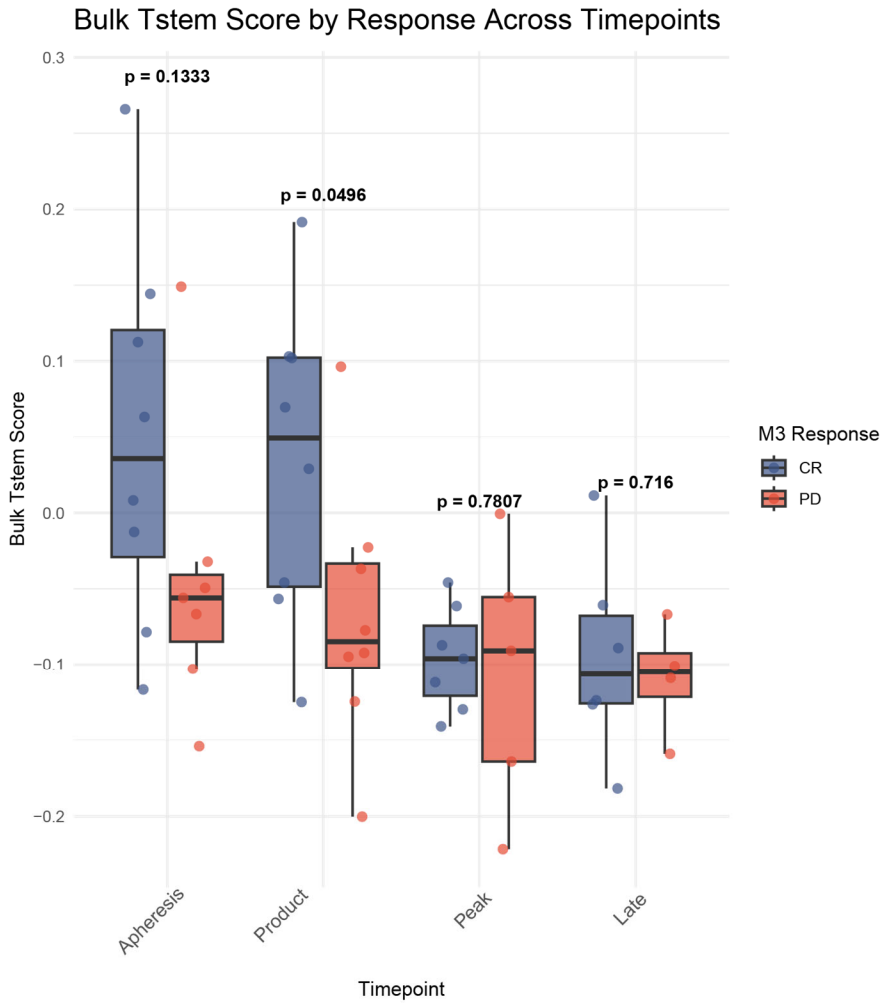
(H)


Pseudobulked B3GAT1 CD57 Expression per patient at timepoint Apheresis
M3 Response CR PD
Mean SCT B3GAT1 Expression
0.03
0.02
0.01
0.00
250
500
750
Days Between D0 CAR19 and Apheresis CAR22

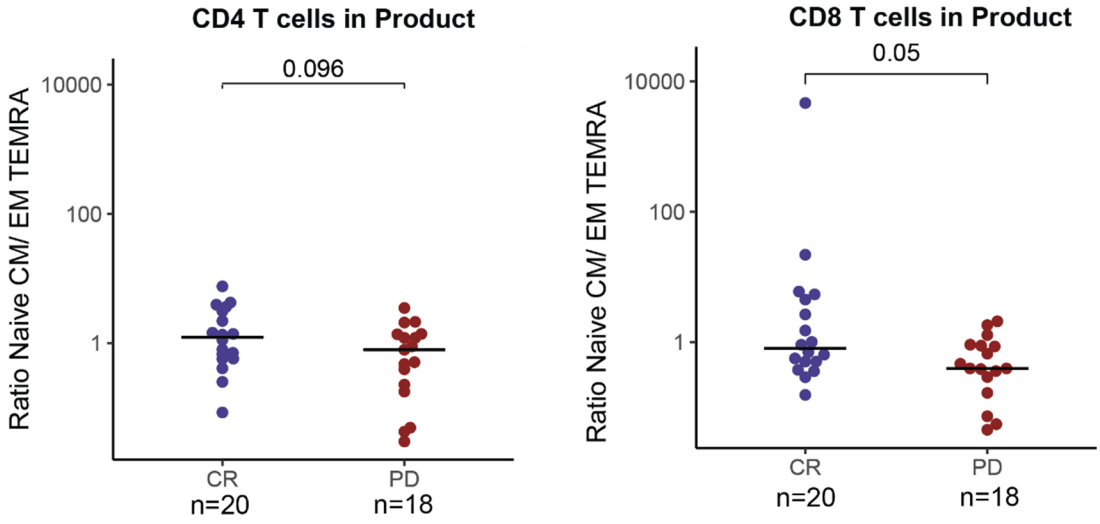
(I)


CD57 Expression by T cell subset
Predicted CD57 Expression
3
2
1
Activated Th17 cells
Cytotoxic CD8 effector T cell
CD8 T stem cell–like memory cells
Central memory CD4 T cells
Cycling CD4 T cells
Cycling CD8 T cells
Terminal effector CD8 T cells
Effector memory CD8 T cells
Activated FoxP3+ T cells
Gamma delta T cell
Naive CD8 T cells
Natural Killer Cells
Regulatory T cells
Effector memory RA+ CD8+ T cells
Tfh cells
Th1/Th17 cells
Th2 T cells

(J)


Bulk Tstem Score by Response Across Timepoints
p = 0.1333
p = 0.0496
p = 0.7807
p = 0.716
Bulk Tstem Score
0.3
0.2
0.1
0.0
−0.1
−0.2
Apheresis
Product
Peak
Late
Timepoint
M3 Response
CR
PD

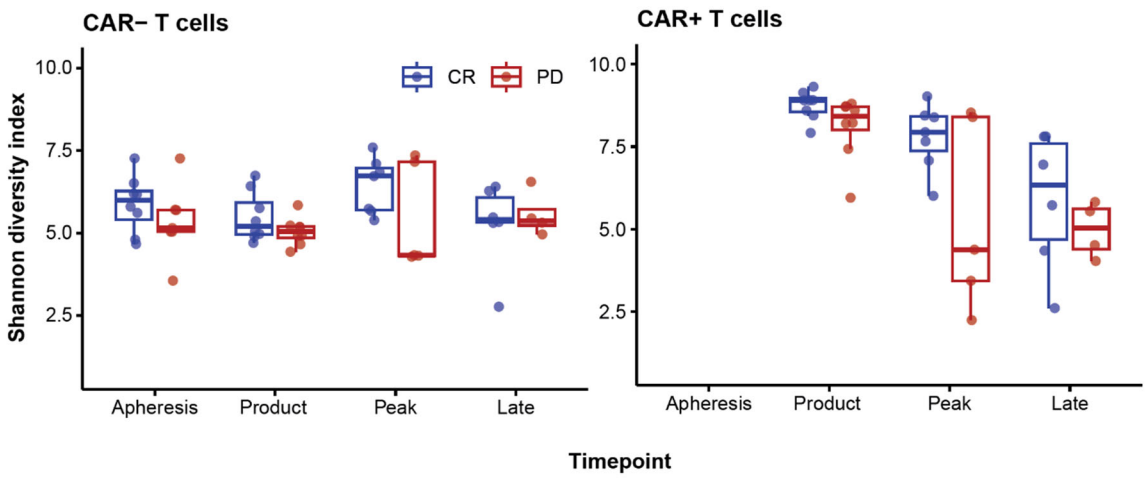
(K)


CD4 T cells in Product
0.096
Ratio Naive CM/ EM TEMRA
10000
100
1
CR
n=20
PD
n=18
CD8 T cells in Product
0.05
Ratio Naive CM/ EM TEMRA
10000
100
1
CR
n=20
PD
n=18

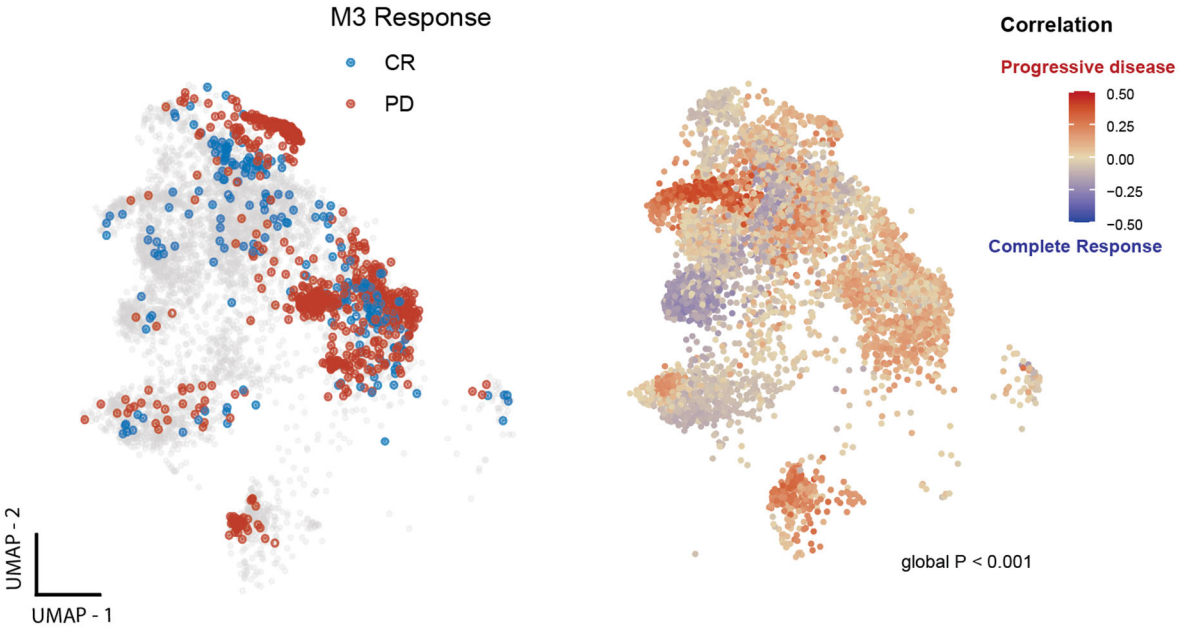
Extended data figure 3 TCR repertoire by response

(A) TCR diversity as measured by Shannon entropy index across timepoints, stratified by month 3 clinical response status and CAR transgene expression (B) UMAP visualization with the top 10 most abundant clonotypes per patient highlighted at peak timepoint, stratified by response. Covarying neighborhood analysis reveals regions with high relative abundance associated with treatment response

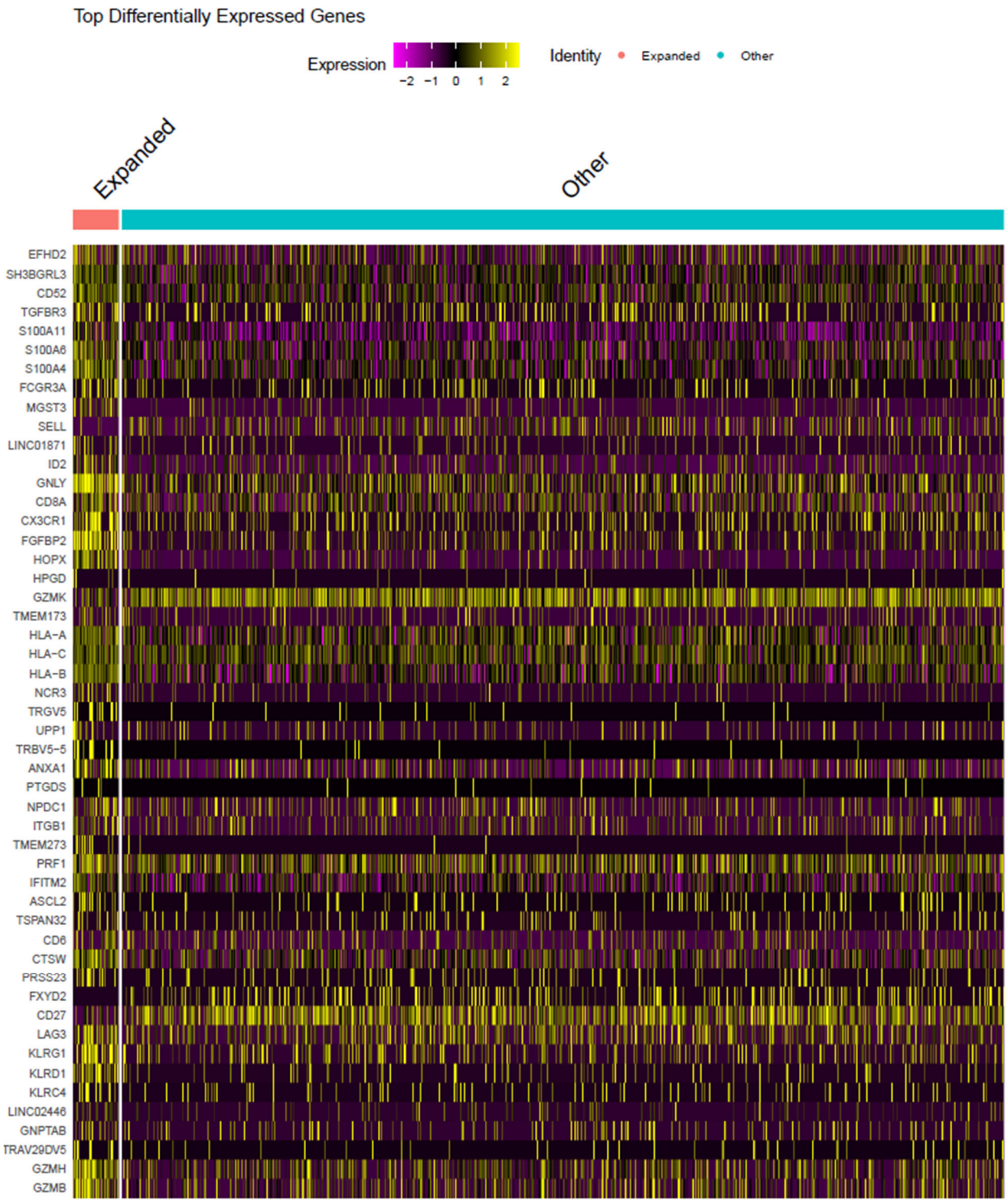
(A)



(B)



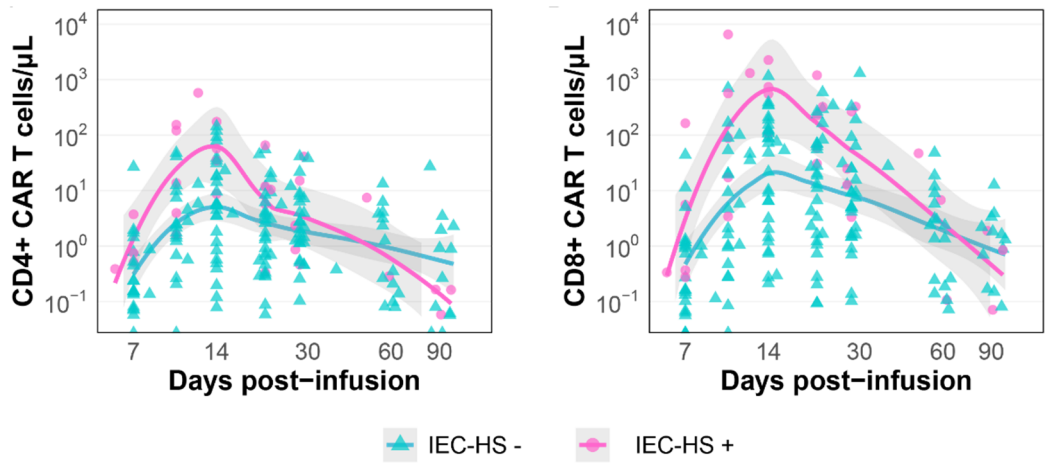
(C)


Top Differentially Expressed Genes
Expression
−2 −1 0 1 2
Identity
Expanded
Other
Expanded
Other
EFHD2
SH3BGRL3
CD52
TGFBR3
S100A11
S100A6
S100A4
FCGR3A
MGST3
SELL
LINC01871
ID2
GNLY
CD8A
CX3CR1
FGFBP2
HOPX
HPGD
GZMK
TMEM173
HLA-A
HLA-C
HLA-B
NCR3
TRGV5
UPP1
TRBV5-5
ANXA1
PTGDS
NPDC1
ITGB1
TMEM273
PRF1
IFITM2
ASCL2
TSPAN32
CD6
CTSW
PRSS23
FXYD2
CD27
LAG3
KLRG1
KLRD1
KLRC4
LINC02446
GNPTAB
TRAV29DV5
GZMH
GZMB

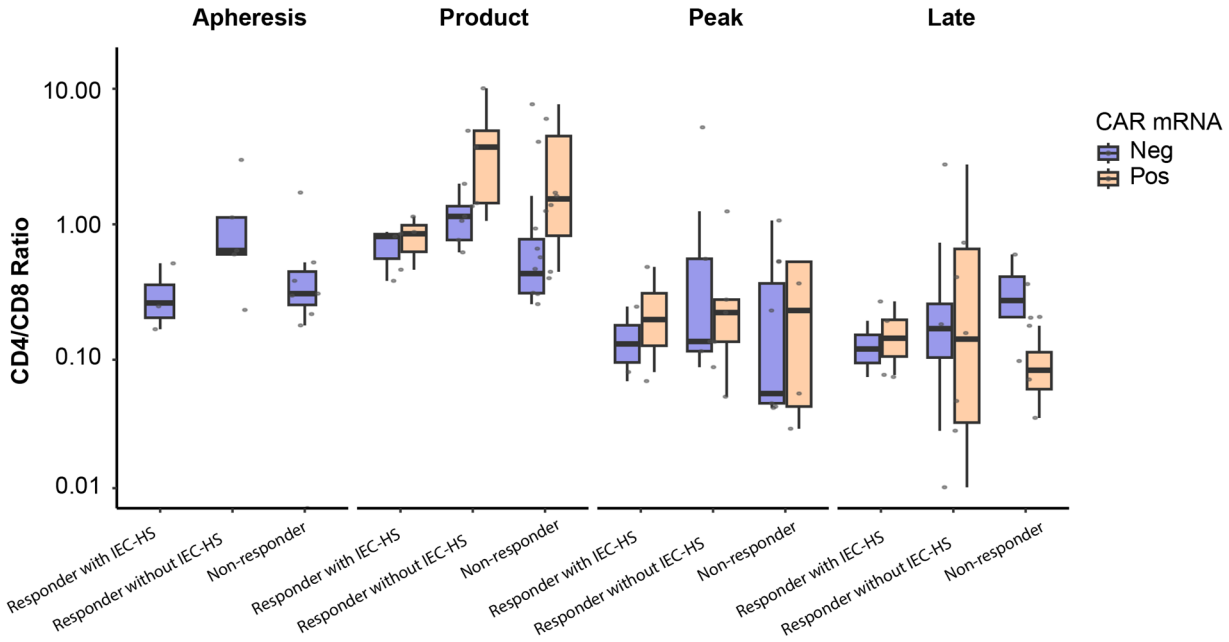
Extended data figure 4 Immune Cell Dynamics and Transcriptional Profiles Associated with Immune Effector Cell-Associated Hematopoietic Syndrome (IEC-HS) in CAR-T Cell Therapy

(A) Post-infusion dynamics of CD4+ and CD8+ T cell expansion measured by flow cytometry in all treated patients, stratified by IECHS development status (B) CD4/CD8 T cell ratio quantified by scRNA-seq, stratified by timepoint, response and IEC-HS status, and CAR mRNA detection (C) Clonotype frequency and density at peak expansion in responding patients with and without IEC-HS, highlighted the region as identified by covarying neighborhood analysis (D) Single-cell RNA sequencing analysis of myeloid cell subset composition in apheresis material, stratified by subsequent IECHS development status (E) Type I interferon gene expression profiles across myeloid cell subsets in apheresis material (F) Gene Set Enrichment Analysis (GSEA) showing hallmark pathways upregulated in myeloid cells from patients who later developed IECHS

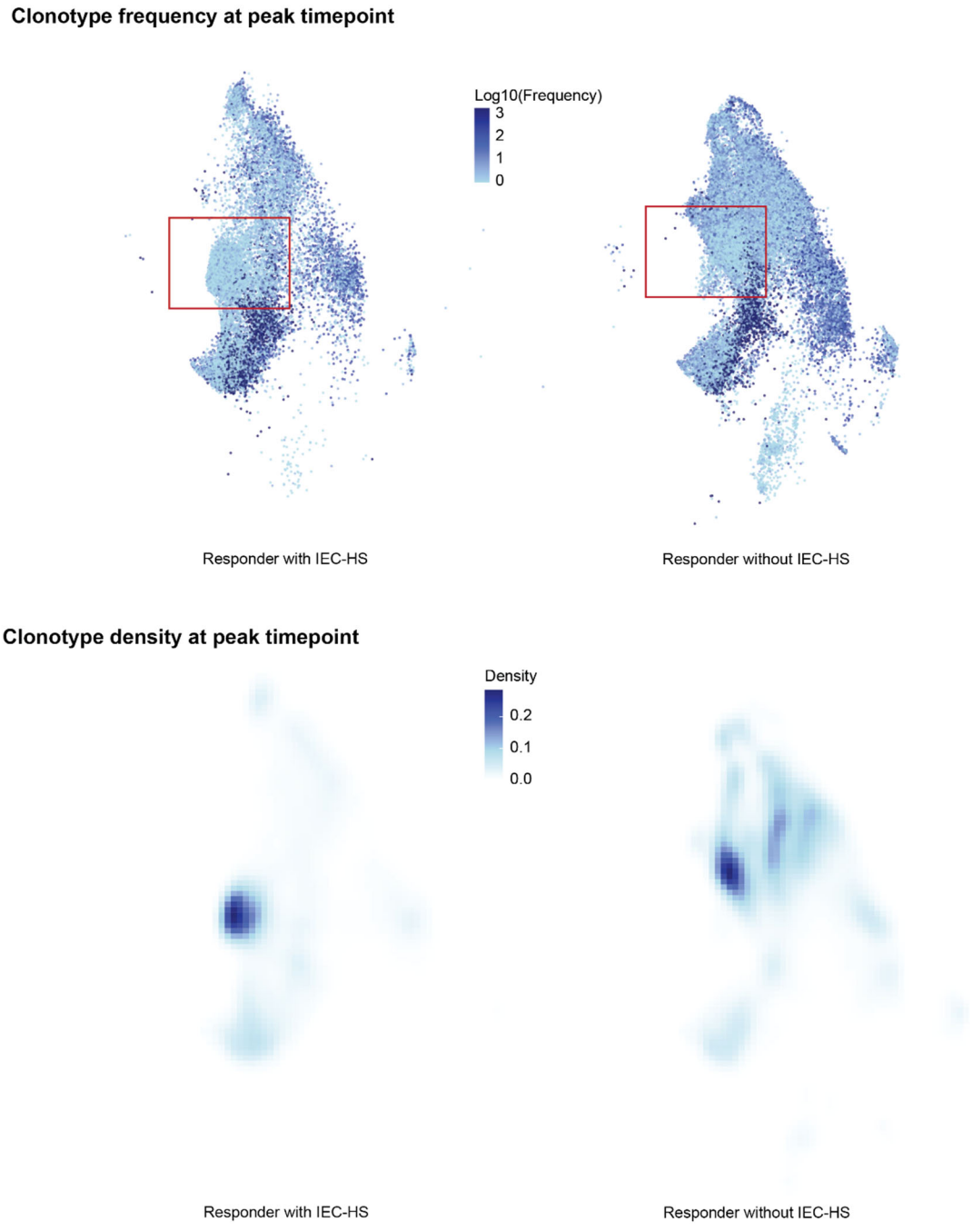
(A)



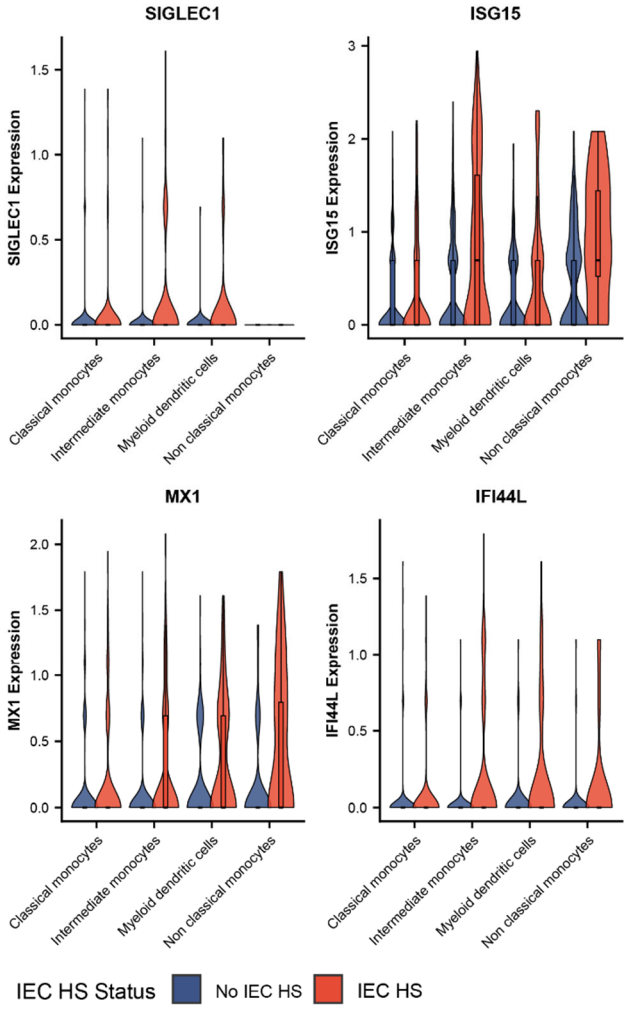
(B)


Apheresis
Product
Peak
Late
CD4/CD8 Ratio
10.00
1.00
0.10
0.01
CAR mRNA
Neg
Pos
Responder with IEC-HS
Responder without IEC-HS
Non-responder

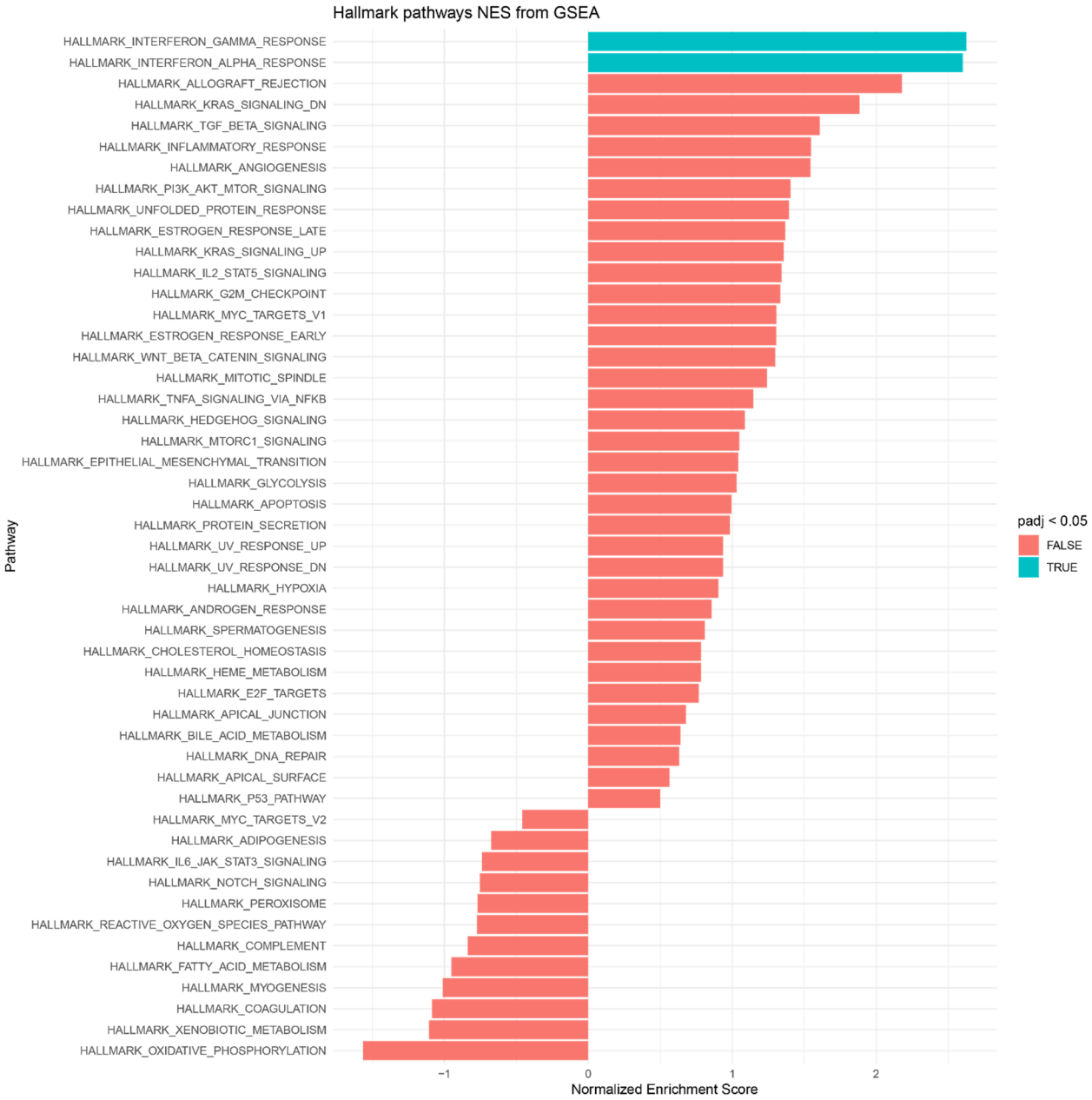
(C)


Clonotype frequency at peak timepoint
Log10(Frequency)
3
2
1
0
Responder with IEC-HS
Responder without IEC-HS
Clonotype density at peak timepoint
Density
0.2
0.1
0.0
Responder with IEC-HS
Responder without IEC-HS

(D)


SIGLEC1
ISG15
MX1
IFI44L
SIGLEC1 Expression
ISG15 Expression
MX1 Expression
IFI44L Expression
Classical monocytes
Intermediate monocytes
Myeloid dendritic cells
Non classical monocytes
IEC HS Status
No IEC HS
IEC HS

(E)


Hallmark pathways NES from GSEA
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_INTERFERON_ALPHA_RESPONSE
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_KRAS_SIGNALING_DN
HALLMARK_TGF_BETA_SIGNALING
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_ANGIOGENESIS
HALLMARK_PI3K_AKT_MTOR_SIGNALING
HALLMARK_UNFOLDED_PROTEIN_RESPONSE
HALLMARK_ESTROGEN_RESPONSE_LATE
HALLMARK_KRAS_SIGNALING_UP
HALLMARK_IL2_STAT5_SIGNALING
HALLMARK_G2M_CHECKPOINT
HALLMARK_MYC_TARGETS_V1
HALLMARK_ESTROGEN_RESPONSE_EARLY
HALLMARK_WNT_BETA_CATENIN_SIGNALING
HALLMARK_MITOTIC_SPINDLE
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_HEDGEHOG_SIGNALING
HALLMARK_MTORC1_SIGNALING
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_GLYCOLYSIS
HALLMARK_APOPTOSIS
HALLMARK_PROTEIN_SECRETION
HALLMARK_UV_RESPONSE_UP
HALLMARK_UV_RESPONSE_DN
HALLMARK_HYPOXIA
HALLMARK_ANDROGEN_RESPONSE
HALLMARK_SPERMATOGENESIS
HALLMARK_CHOLESTEROL_HOMEOSTASIS
HALLMARK_HEME_METABOLISM
HALLMARK_E2F_TARGETS
HALLMARK_APICAL_JUNCTION
HALLMARK_BILE_ACID_METABOLISM
HALLMARK_DNA_REPAIR
HALLMARK_APICAL_SURFACE
HALLMARK_P53_PATHWAY
HALLMARK_MYC_TARGETS_V2
HALLMARK_ADIPOGENESIS
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_NOTCH_SIGNALING
HALLMARK_PEROXISOME
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY
HALLMARK_COMPLEMENT
HALLMARK_FATTY_ACID_METABOLISM
HALLMARK_MYOGENESIS
HALLMARK_COAGULATION
HALLMARK_XENOBIOTIC_METABOLISM
HALLMARK_OXIDATIVE_PHOSPHORYLATION
Pathway
−1
0
1
2
Normalized Enrichment Score
padj < 0.05
FALSE
TRUE

Extended data table 1

Baseline demographic and clinical characteristics of all treated patients and scRNAseq cohort

Baseline demographic and clinical characteristics of all treated patients		
Characteristics	**All patients (n=38)**	**scRNAseq cohort (n=19)**
Age		
Median - year [range]	65 [25 - 84]	65 [25-76]
≥ 65yr - no. (%)	19 (50)	9 (47)
Female sex - no. (%)	17 (45)	9 (47)
ECOG performance status score of 0-1 - no. (%) §	38 (100)	19 (100)
Prior therapies		
Median number of lines [range]	4 [3-8]	4 [3-8]
Relapse after CAR T-cell therapy - no. (%) ¥	37 (97)	18 (95)
Disease status at infusion		
Median tumor burden - mm^2 [range] ∫	1705 [252-11165]	1805 [252-8971]
Elevated Lactate Dehydrogenase level - no. (%) ^	32 (84)	15 (79)
Elevated Ferritin level - no. (%) ^	16 (42)	9 (47)
Elevated CRP level - no. (%) ^	27 (71)	13 (68)
Toxicity post CAR T infusion, any grade		
CRS	36 (95)	18 (95)
ICANS	4 (11)	1 (5)
IEC-HS	5 (13)	4 (21)

§ Eastern Cooperative Oncology Group (ECOG) performance-status scores are assessed on a 5-point scale, with a score of 0 indicating no symptoms and higher scores indicating greater disability. A score of 1 indicates that the patient is ambulatory but restricted from strenuous activity.

CRS= Cytokine Release Syndrome,ICANS =immune effector cell-associated neurotoxicity syndrome; IEC-HS=immune effector cell-associated HLH-like syndrome