Safety and efficacy of the combination of copanlisib and nivolumab in patients with Richter's transformation or transformed non-Hodgkin lymphoma: results from a phase I trial

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Received: November 11, 2024.
Accepted: June 20, 2025.
Early view: July 3, 2025.

https://doi.org/10.3324/haematol.2024.286945

Supplement

Supplemental Methods

Additional Inclusion/Exclusion Criteria

Additional inclusion criteria include Eastern Cooperative Oncology Group (ECOG) performance status ≤2 and adequate organ function. Patients were excluded if they required chronic use of steroids, had prior allogeneic stem cell transplant, or had prior checkpoint inhibitor therapy within 2 years or at any time to treat RT.

Sample Size, Power, Accrual Rate, and Study Duration

The 3+3 dose escalation scheme will be utilized, with 3 to 6 patients evaluated at each dose level. It is expected that a maximum of 12 patients will be enrolled to the dose discovery phase, with 6 patients treated at MTD. Protocol amendment dated 08/04/21 increased the expected total number of participants from 15 to 21 participants. As of 08/04/21, eleven participants were enrolled in the dose-finding portion of the study, of which 8 participants were treated at the MTD (6 evaluable). Therefore, up to ten additional patients will be enrolled in the expansion cohort, for a total of up to 16 evaluable participants treated at the MTD. There is >90% chance that any AE event with 11% or greater incidence is observed at least once among 21 patients. As of 08/15/22, 21 patients have been accrued. Protocol amendment dated 08/17/22 further increased the total number of participants from 21 to 27 by adding 6 more patients with FL transformation to the expansion cohort, to better evaluate outcomes in this subset. With 6 more accruals, there will be a total of 12 patients with FL transformation treated on the study. The total number of patients treated at MTD will be increased to 24. The chance of observing at least 1 occurrence of a particular AE event among 24 patients at MTD is at least 92% if the true AE incidence is 10%+.

The study will accrue over approximately 4 years, with a total study duration of up to 7 years.

Correlative Studies

Patient samples. Peripheral blood samples were obtained from patients on study (at baseline, cycle 1 day 1 and cycle 6 day 1 prior to administration of study drugs and at end of treatment). Peripheral blood mononuclear cells (PBMCs) were isolated using standard Ficoll-Hypaque technique (Amersham). Red blood cells were lysed using ACK buffer (Thermo Fisher Scientific).

Flow cytometry. Flow cytometry was performed as previously.[1] For cytokine staining, equal numbers of tumor (or human PBMC cells) were cultured in vitro for 5 hours in the presence of 50 ng/mL phorbol 12-myristate 13-acetate (PMA) (Sigma Aldrich), 1 µg/mL ionomycin (Sigma Aldrich) and 5 µg/mL BFA (Biolegend). Data were acquired on BD Fortessa and analyzed with FlowJo software V10 (Oregon, USA).

Single-cell RNA-seq (scRNA) data collection and processing. scRNA-seq samples were obtained from a total of 5 patients at baseline (prior to treatment), Cycle 2 Day 1 (C2D1), and Cycle 6 Day 1 (C6D1). For each sample, the unfiltered feature-barcode matrix was generated by

processing multiplexed FASTQ files with the Cell Ranger v.7.1.0 'cellranger multi' command, using default parameters and the prebuilt GRCh38 genome reference for scRNA-seq. All subsequent analyses were performed using Seurat v.4.4.0.[2]

Cell demultiplexing was achieved using HTOdemux function from the Seurat package, where the negative cluster was defined based on minimal non-zero expression. A series of quality control filters were applied to remove barcodes falling into any of the following categories: (1) too few total transcript counts (< 500); (2) potential debris with too few expressed genes (< 500); (3) potential doublets with an excessively high number of expressed genes (> 5,000); and (4) potential dead cells or cells exhibiting signs of stress and apoptosis, indicated by a high proportion of mitochondrial gene expression (> 10% of total transcript counts).

A Seurat object was constructed for each sample using the unfiltered feature-barcode matrix. Each sample underwent scaling and normalization using Seurat's 'SCTransform' function to correct for batch effects, with the parameter vars.to.regress = c('percent.mito') and default settings for other parameters. Merged analyses and subsequent cell/sample subsetting also employed the same scaling and normalization procedures. Clustering was performed using the original Louvain algorithm with the top 50 principal components, as implemented in the 'FindNeighbors' and 'FindClusters' functions (resolution = 0.8). Uniform Manifold Approximation and Projection (UMAP) was generated using the RunUMAP function with the same 10 principal components used in clustering.

scRNA-seq cell-type annotation. Main cell types were assigned to each cluster by manually reviewing the expression of a comprehensive set of marker genes. All annotations were performed by a single researcher to ensure consistency.

Differential gene expression and pathway analysis. Differential gene expression analysis was conducted using two-tailed Wilcoxon rank-sum tests via Seurat's FindMarkers function, comparing cells from different cell types or treatment groups. Volcano plots were employed to visualize differentially expressed genes (DEGs) in each cell type or treatment group. All differential expression analyses utilized the 'SCT' assay. Hallmark gene sets from MSigDB (v.7.5.1)[3] were used for gene set over-representation analysis.

Pseudo-bulk differential gene expression analysis. For pseudo-bulk differential gene expression analysis, gene counts were aggregated (summed) for each sample and cell type. Differential expression was assessed using the DESeq2 R package (v.1.38.2),[4] with patient ID included as a covariate in the design formula to account for the paired design. The moderated stat-statistics from 'DESeq' test were used as a preranked gene list for Gene set enrichment analysis (GSEA), performed with the fgsea R package (v.1.24.0).

Supplemental Table 1. Management of Immune Related Toxicities including Dose Modifications

Recommended Dose Modification by Toxicity Grade

Diarrhea/Colitis	**Management/Next Dose** ***Copanlisib***	**Management/Next Dose** ***Nivolumab***
≤Grade 1 (defined as an increase of < 4 stools/day over baseline)	*Continue at current dose*	*Continue at current dose*
Grade 2 (defined as an increase of 4-6 stools/day over baseline)	*Continue at current dose*	*Hold until diarrhea resolves or improves to ≤ Grade 1. Resume at the same dose level*
Grade 3 (defined as ≥ 7 stools/day over baseline or hospitalization due to diarrhea)	*Hold until resolved to ≤ Grade 1. Resume at one dose level lower.*	*Hold until resolved to ≤ Grade 1. Resume at the same dose level*
Grade 4 (defined as life threatening diarrhea)	*Hold until diarrhea resolves to < Grade 1. Resume at one dose level lower.*	*Permanently discontinue*
Recommended management: • ≤ Grade 1: Treat with loperamide • Grade 2: Consider treatment with loperamide. If the diarrhea does not resolve within 24 hours, consider treatment budesonide or prednisolone at 1mg/kg with a taper. • Grade 3: Treat with budesonide, and consider prednisolone at 1 mg/kg with a quick taper. • Grade 4: Treat with budesonide or prednisolone at 1 mg/kg with a taper. Further management to be done per Coutre et al.[5]		

Transaminitis	**Management/Next Dose** ***Copanlisib***	**Management/Next Dose** ***Nivolumab***
Grade 1 (defined as AST/ALT ≤ 3 x ULN)	*Continue at current dose*	*Continue at current dose*
Grade 2 (defined as ALT/AST > 3-5 x ULN)	*Continue at current dose. Monitor LFT levels weekly.*	*Continue at current dose. Monitor LFT levels weekly.*
Grade 3 (defined as ALT/AST > 5-20 x ULN)	*Hold and monitor LFT levels weekly until resolved to Grade 1. Resume at one dose level lower and titrate upward as tolerated.*	*Hold and monitor LFT levels weekly until resolved to Grade 1. Resume at current dose.*
Grade 4 (defined as > 20 x ULN)	*Hold and monitor LFT levels weekly until resolved to Grade 1. Resume at one dose level lower. Titration is not allowed.*	*Permanently discontinue*
Recommended management: LFTs will be monitored every 2 weeks for the first 3 months of treatment, then every 4 weeks for the following 3 months, and every 1-3 months thereafter throughout the entirety of the study.		

Non-infectious Pneumonitis	**Management/Next Dose** ***Copanlisib***	**Management/Next Dose** ***Nivolumab***
Grade 1 (defined as asymptomatic; clinical or diagnostic observation; radiographic changes only)	*Hold and evaluate every 2-3 days for development of symptoms. Resume at the same dose level once resolved.*	*Hold and evaluate every 2-3 days for development of symptoms. Resume at the same dose level once resolved.*
Grade ≥ 2 (defined as symptomatic; intervention indicated; limiting instrumental ADLs)	*Permanently discontinue*	*Permanently discontinue*
Hypophysitis	**Management/Next Dose**	**Management/Next Dose**

	Copanlisib	***Nivolumab***
Grade 1	*Continue at current dose.*	*Hold dose, if Grade ≤1 can resume at next cycle*
Grade 2	*Continue at current dose monitor hypothalamus function weekly.*	*Hold dose, if Grade ≤1 can resume at next cycle*
Grade 3	*Per DLT Guidelines above*	*Hold dose, if Grade ≤1 can resume at next cycle*
Grade 4	*Per DLT Guidelines above*	*Permanently discontinue*
Adrenal Insufficiency	**Management/Next Dose** ***Copanlisib***	**Management/Next Dose** ***Nivolumab***
Grade 1	*Continue at current dose.*	*Hold dose, if Grade ≤1 can resume at next cycle*
Grade 2	*Continue at current dose monitor adrenal function weekly.*	*Hold dose, if Grade ≤1 can resume at next cycle*
Grade ≥ 3	*Per DLT Guidelines above*	*Permanently discontinue*
Type 1 Diabetes Mellitus	**Management/Next Dose** ***Copanlisib***	**Management/Next Dose** ***Nivolumab***
Grade 1	*Continue at current dose.*	*Hold dose, if Grade ≤1 can resume at next cycle*
Grade 2	*Continue at current dose. Monitor glucose weekly.*	*Hold dose, if Grade ≤1 can resume at next cycle*
Grade 3	*Per DLT Guidelines above*	*Hold dose, if Grade ≤1 can resume at next cycle*
Grade 4	*Per DLT Guidelines above*	*Permanently discontinue*
Dermatitis	**Management/Next Dose** ***Copanlisib***	**Management/Next Dose** ***Nivolumab***
Grade 1	*Continue at current dose.*	*Hold dose, if Grade ≤1 can resume at next cycle*
Grade 2	*Continue at current dose ;reassess weekly.*	*Hold dose, if Grade ≤1 can resume at next cycle*

Grade 3	*Per DLT Guidelines above*	*Hold dose, if Grade ≤1 can resume at next cycle*
Grade 4	*Per DLT Guidelines above*	*Permanently discontinue*
Encephalitis	**Management/Next Dose** ***Copanlisib***	**Management/Next Dose** ***Nivolumab***
New onset moderate or severe neurologic signs or symptoms	*Per DLT Guidelines above*	*Hold dose, if Grade ≤1 can resume at next cycle*
Immune-mediated encephalitis	*Permanently discontinue*	*Permanently discontinue*
≥Grade 3 infection	*Hold until resolution*	*Hold until resolution*
Any grade Pneumocystis jiroveci pneumonia (PJP)	Hold copanlisib, and treat infection if confirmed. Resume copanlisib at the same dose with PJP prophylaxis	Hold nivolumab and treat infection if confirmed. Resume at the same dose

Supplemental Table 2. Comprehensive List of AEs.

Attributable AEs (possibly or higher), any grade:

	N (%)
Diarrhea	12 (44%)
Anemia	10 (37%)
Fatigue	10 (37%)
Alkaline phosphatase increased	10 (37%)
Neutrophil count decreased	10 (37%)
Platelet count decreased	10 (37%)
Hyperglycemia	10 (37%)
Nausea	9 (33%)
Hypertension	9 (33%)
Aspartate aminotransferase increased	8 (30%)
Alanine aminotransferase increased	7 (26%)
Lymphocyte count decreased	7 (26%)
White blood cell decreased	7 (26%)
Rash maculopapular	6 (22%)
Fever	5 (19%)
Abdominal pain	5 (19%)
Hyponatremia	4 (15%)
Hypomagnesemia	3 (11%)
Muscle cramp	3 (11%)
Blood bilirubin increased	3 (11%)
Chills	3 (11%)
Infusion related reaction	2 (7%)
Hypokalemia	2 (7%)
Hypophosphatemia	2 (7%)
Vomiting	2 (7%)
Hypercalcemia	2 (7%)
Dizziness	2 (7%)
Lung infection	2 (7%)
Hypoalbuminemia	2 (7%)
Arthralgia	2 (7%)
Hypoglycemia	1 (4%)
Myalgia	1 (4%)
Erythema chest/abdomen	1 (4%)
Febrile neutropenia	1 (4%)
Generalized edema	1 (4%)
Hypocalcemia	1 (4%)
Headache	1 (4%)
Sinus tachycardia	1 (4%)
Hyperthyroidism	1 (4%)

	N (%)
Esophagitis	1 (4%)
EOSINOPHILIC ESOPHAGITIS	1 (4%)
Colitis	1 (4%)
Dyspnea	1 (4%)
Malaise	1 (4%)
Pneumonitis	1 (4%)
Skin ulceration	1 (4%)
Septic Shock	1 (4%)
Creatinine increased	1 (4%)
Constipation	1 (4%)
Dyspepsia	1 (4%)
Flatulence	1 (4%)
Gastroesophageal reflux disease	1 (4%)
INTERMITTENT VOMITING	1 (4%)
Lymphocyte count increased	1 (4%)
Hyperphosphatemia	1 (4%)
Hematuria	1 (4%)
Enterocolitis infectious	1 (4%)
Epistaxis	1 (4%)
Rash acneiform	1 (4%)
Eosinophilia	1 (4%)
Dysgeusia	1 (4%)
Dysuria	1 (4%)
Urinary frequency	1 (4%)
Cognitive disturbance	1 (4%)

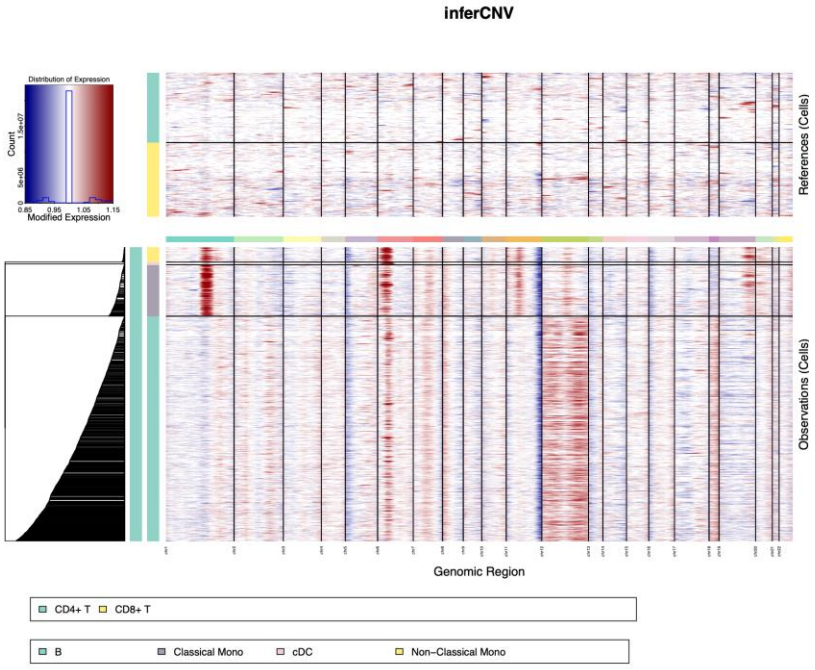
All attributable grade 3+ AEs:

	N (%)
Neutrophil count decreased	6 (22%)
Lymphocyte count decreased	4 (15%)
Hypertension	3 (11%)
Anemia	3 (11%)
Platelet count decreased	3 (11%)
Abdominal pain	3 (11%)
White blood cell decreased	2 (7%)
Lung infection	2 (7%)
Diarrhea	2 (7%)
Hyperglycemia	2 (7%)
Hypokalemia	1 (4%)
Febrile neutropenia	1 (4%)
EOSINOPHILIC ESOPHAGITIS	1 (4%)

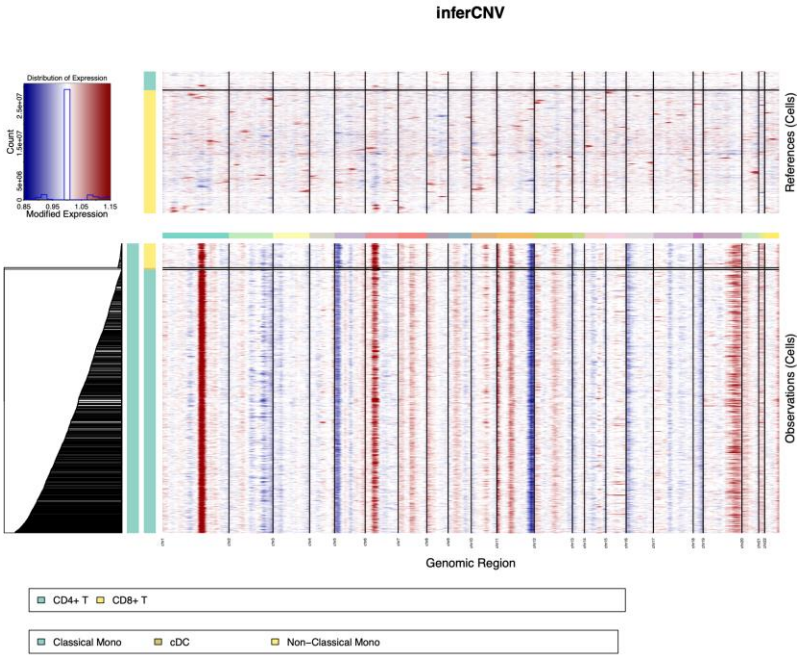
	N (%)
Fatigue	1 (4%)
Colitis	1 (4%)
Pneumonitis	1 (4%)
Rash maculopapular	1 (4%)
Alkaline phosphatase increased	1 (4%)
Septic Shock	1 (4%)
Hypophosphatemia	1 (4%)
Alanine aminotransferase increased	1 (4%)

Supplemental Figure 1. *(A)* Copy number variation (CNV) analysis of single-cell gene expression using inferCNV. The heatmaps depict chromosomal alterations in responders (R) and non-responders (NR). Reference cells, including CD8+ T cells, NK cells and CD4+ T cells, show minimal variation, serving as a baseline for CNV assessment.

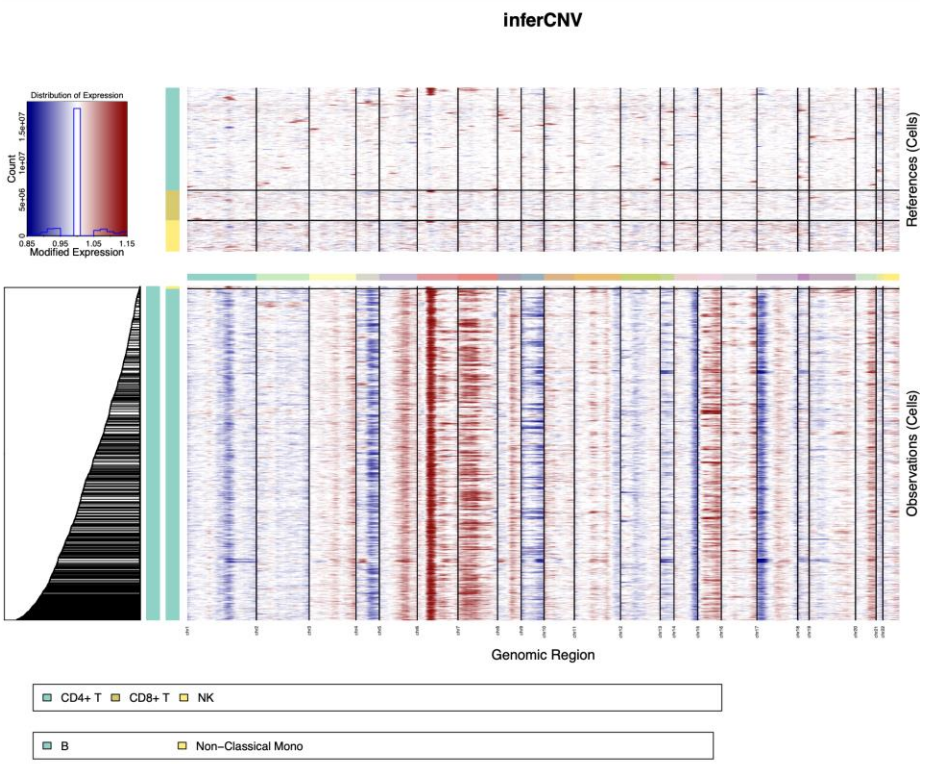
Sample 9 (R)



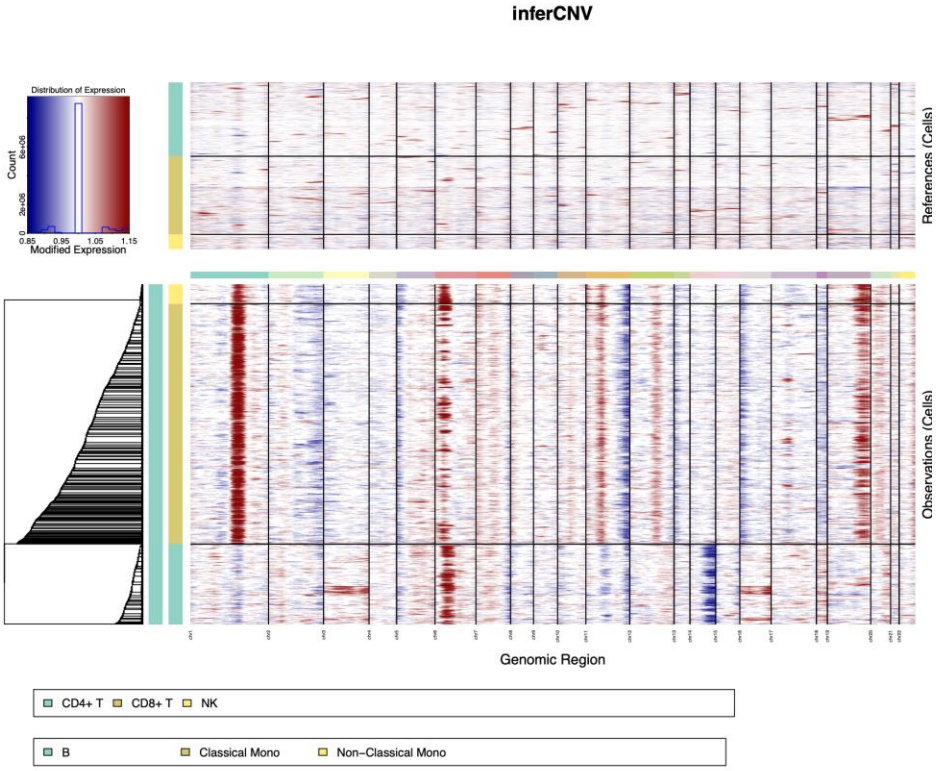
Sample 10 (R)



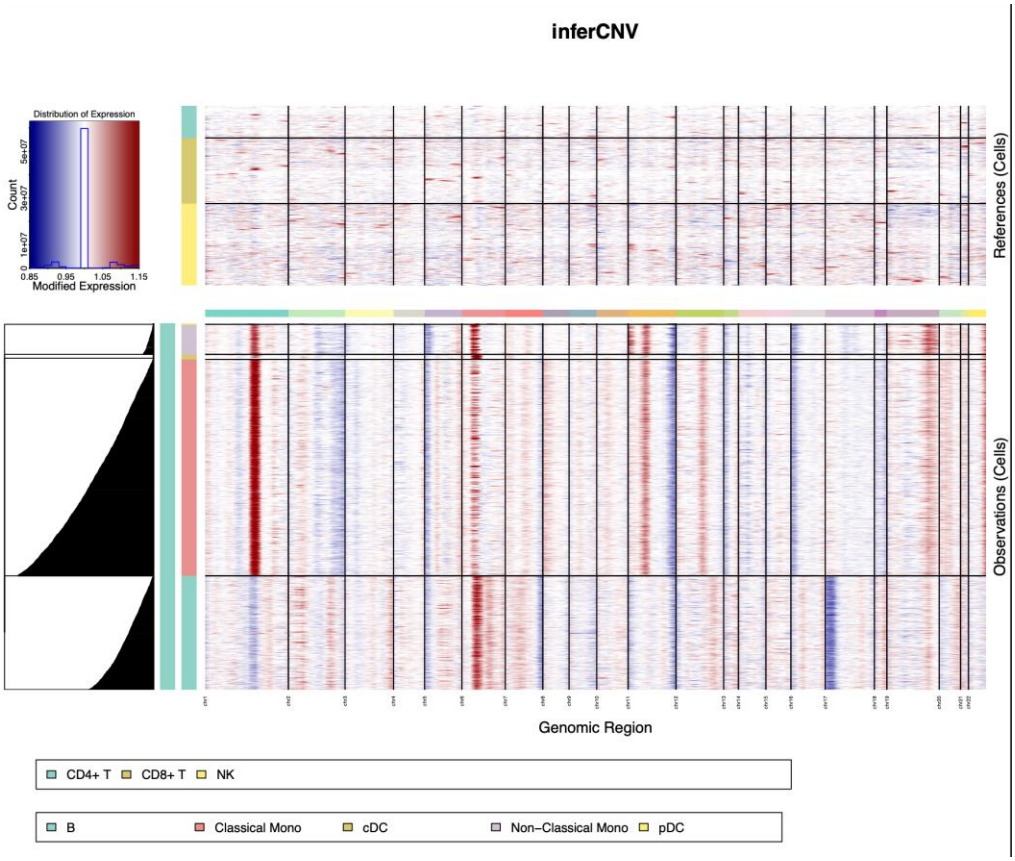
Sample 13 (NR)



Sample 14 (NR)

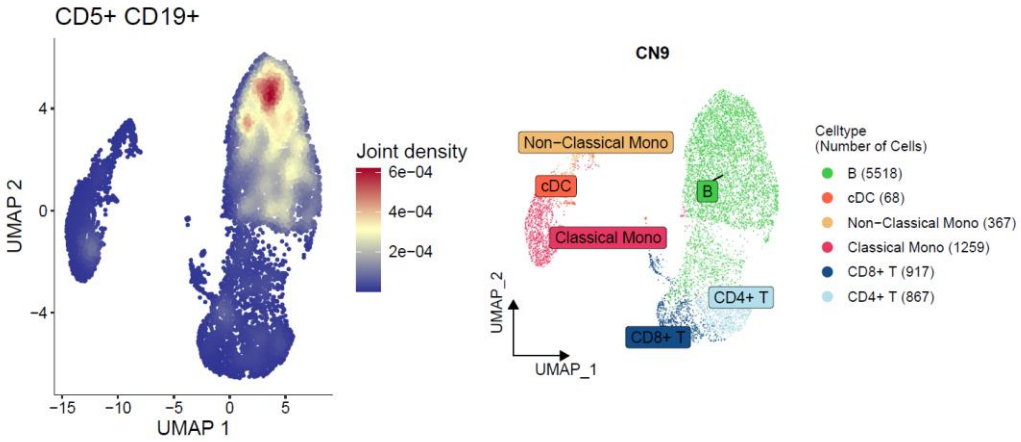


Sample 15 (NR)

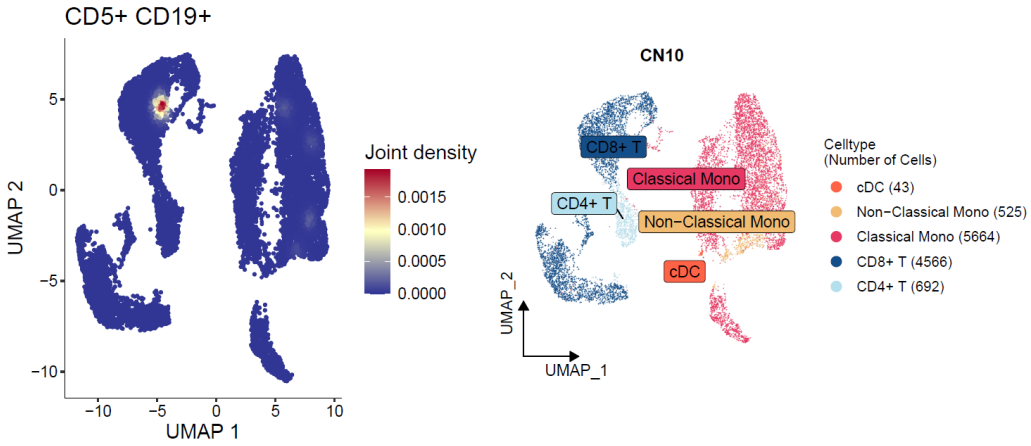

inferCNV
Distribution of Expression
Count
Modified Expression
References (Cells)
Observations (Cells)
Genomic Region
CD4+ T
CD8+ T
NK
B
Classical Mono
cDC
Non−Classical Mono
pDC

Supplemental Figure 1. *(B)* Co-expression of CD5 and Cd19 genes as determined by scRNA-Seq. Cells with highest co-expression are highlighted in red.

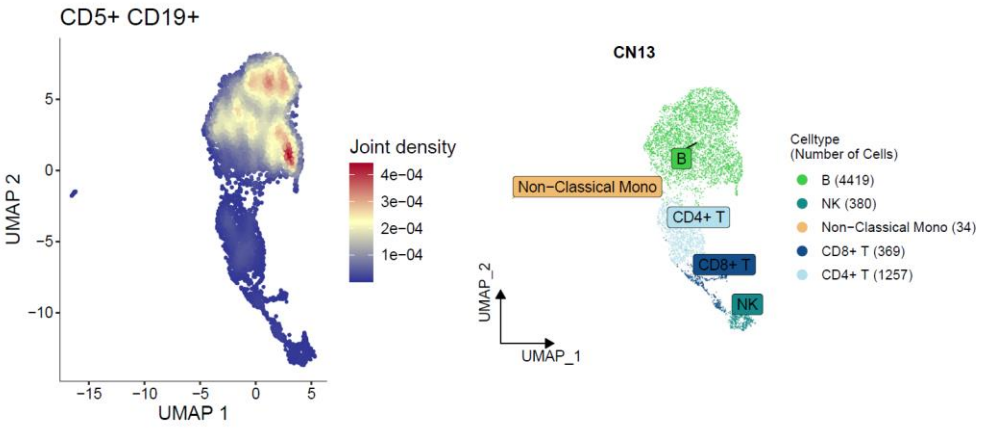
Sample 9 (R)



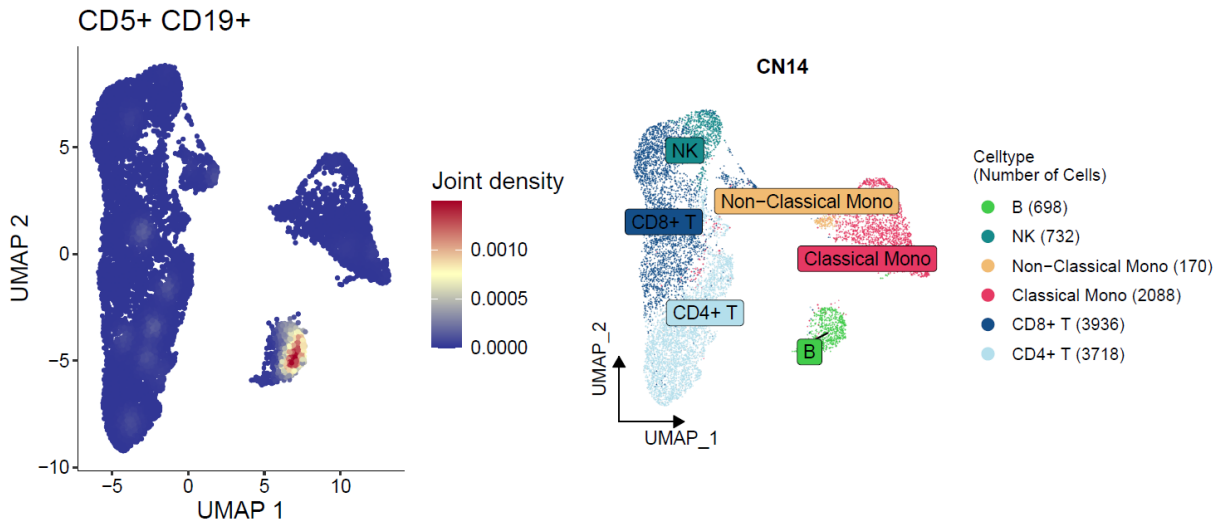
Sample 10 (R)



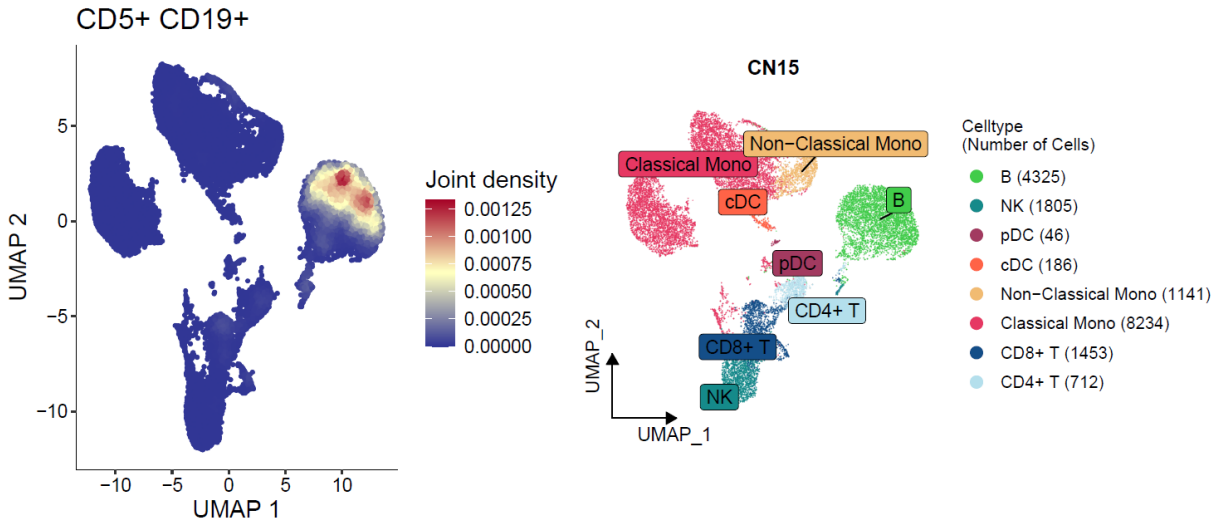
Sample 13 (NR)



Sample 14 (NR)

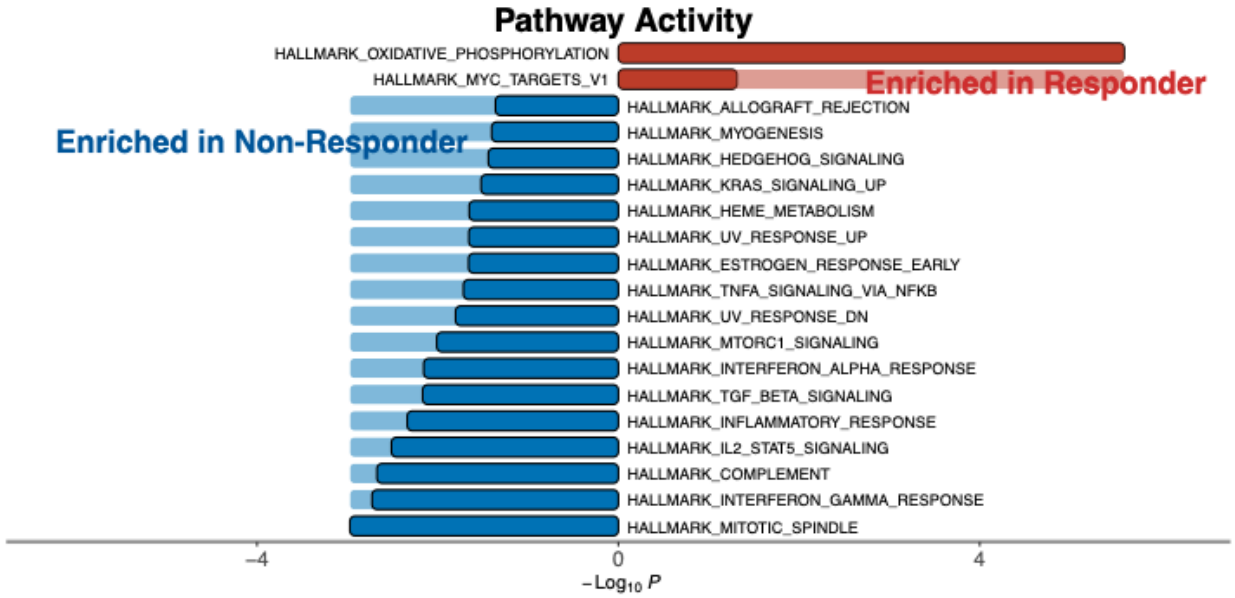


Sample 15 (NR)

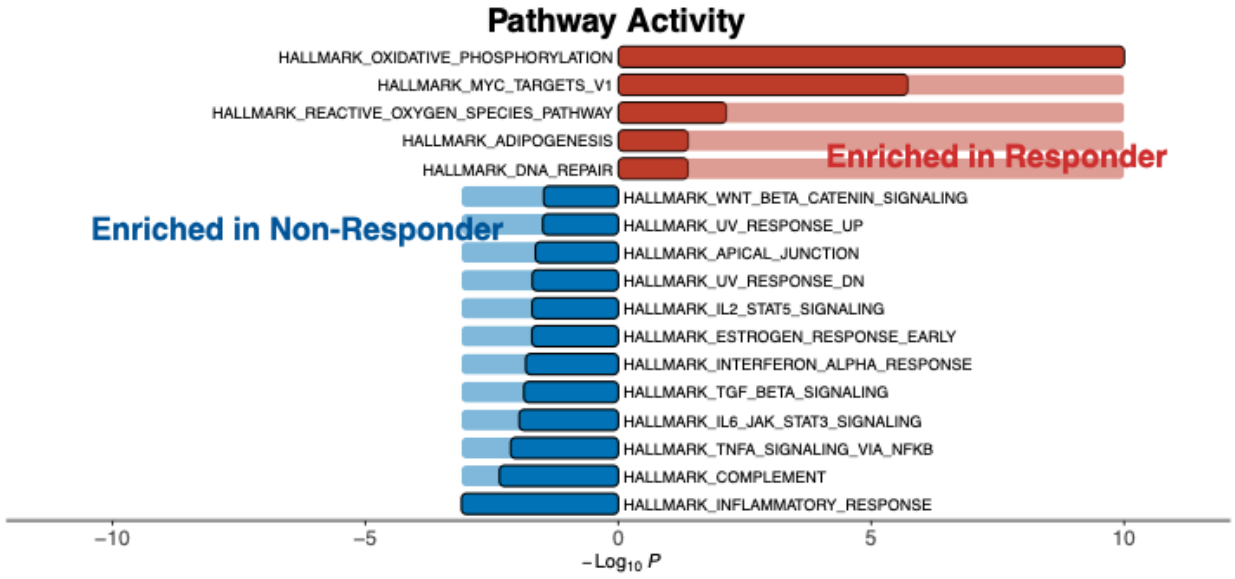


Supplemental Figure 2. Pathway activity analysis of different cell types at the baseline timepoint, comparing responders versus non-responders. Pathways enriched in responders are shown in red, while those enriched in non-responders are displayed in blue. The x-axis represents the statistical significance (-log10 P-value) of pathway enrichment.

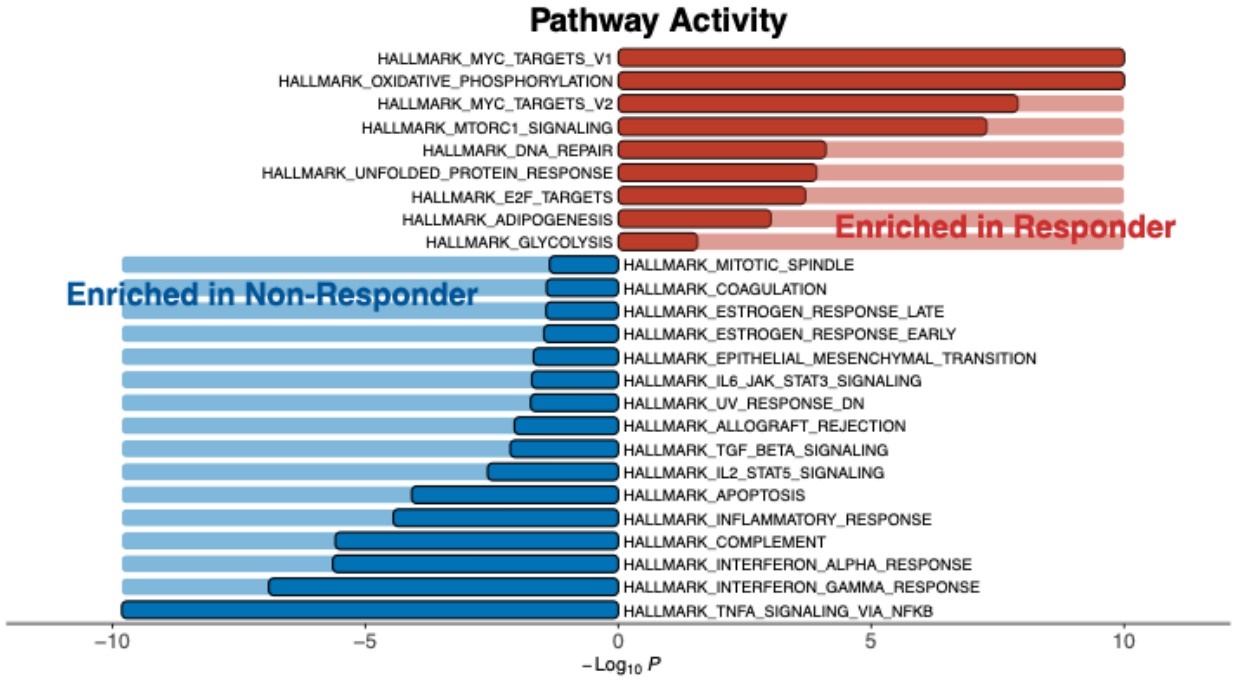
CD8+ T-cells



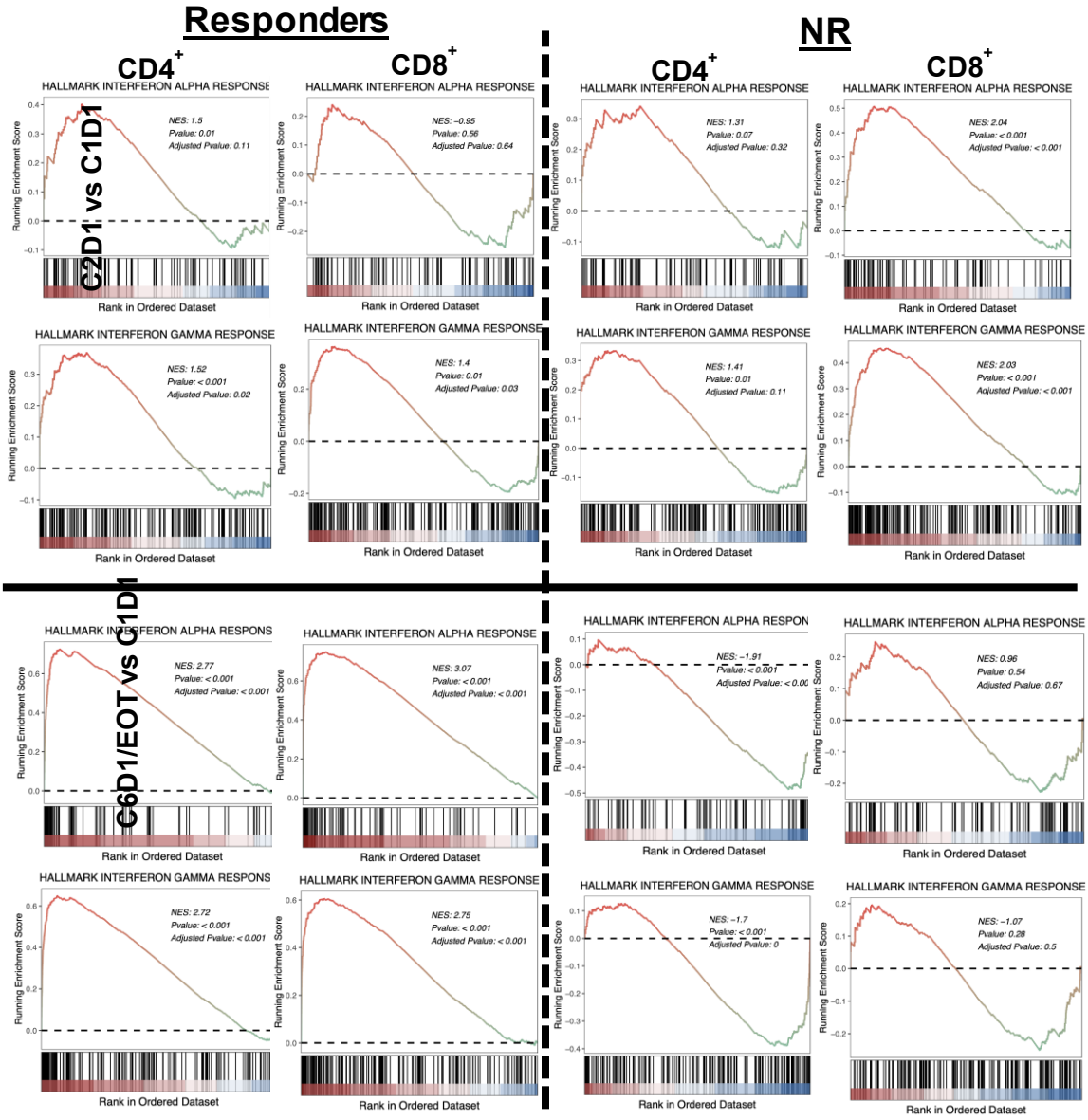
CD4+ T-cells



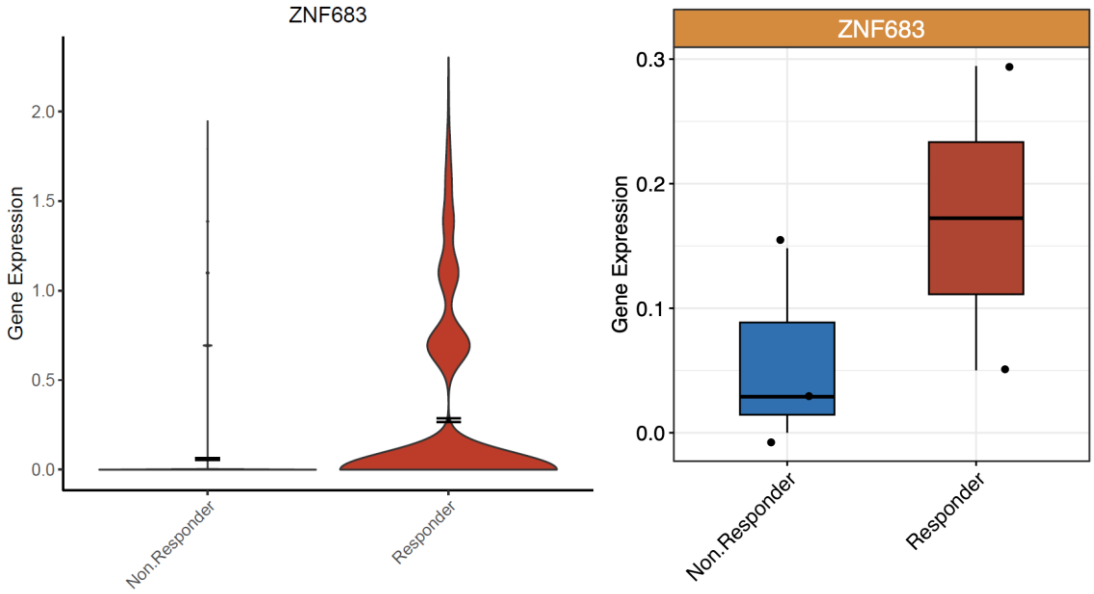
B-cells


Pathway Activity
HALLMARK_MYC_TARGETS_V1
HALLMARK_OXIDATIVE_PHOSPHORYLATION
HALLMARK_MYC_TARGETS_V2
HALLMARK_MTORC1_SIGNALING
HALLMARK_DNA_REPAIR
HALLMARK_UNFOLDED_PROTEIN_RESPONSE
HALLMARK_E2F_TARGETS
HALLMARK_ADIPOGENESIS
HALLMARK_GLYCOLYSIS
Enriched in Responder
Enriched in Non-Responder
HALLMARK_MITOTIC_SPINDLE
HALLMARK_COAGULATION
HALLMARK_ESTROGEN_RESPONSE_LATE
HALLMARK_ESTROGEN_RESPONSE_EARLY
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_UV_RESPONSE_DN
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_TGF_BETA_SIGNALING
HALLMARK_IL2_STAT5_SIGNALING
HALLMARK_APOPTOSIS
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_COMPLEMENT
HALLMARK_INTERFERON_ALPHA_RESPONSE
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_TNFA_SIGNALING_VIA_NFKB
−10
−5
0
5
10
−Log10 P

Supplemental Figure 3. Pseudo-bulk GSEA pathway enrichment analysis of signaling pathways in T cell subpopulations. Enrichment plots for IFN-α and IFN-γ signaling pathways in $CD4^+$ and $CD8^+$ T cells at C2D1 and C6D1 compared to baseline for both responders and non-responders (NR); NES, normalized enrichment score.



Supplemental Figure 4. ZNF683 gene expression at the baseline in CD8+ T cells in responders and non-responders. Left graph displays single-cell RNA sequencing (scRNA) violin plots, illustrating the distribution of ZNF683 expression in individual cells. The right graph presents pseudo-bulk expression analysis, summarizing expression levels per group.



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