Cell Reports Medicine, Volume *4*

Supplemental information

Single-cell RNA sequencing reveals distinct T cell populations in immune-related adverse events of checkpoint inhibitors

Shoiab Bukhari, Brian S. Henick, Robert J. Winchester, Shalom Lerrer, Kieran Adam, Yevgeniya Gartshteyn, Rohan Maniar, Ziyan Lin, Alireza Khodadadi-Jamayran, Aristotelis Tsirigos, Mary M. Salvatore, Galina G. Lagos, Steven L. Reiner, Matthew C. Dallos, Matthen Mathew, Naiyer A. Rizvi, and Adam Mor

A

Patient Key	Clinical	Age	Gender	Tumor	Stage	irAE type	irAE grade	Steroids	Weeks to onset
P1	Arthritis	49	Female	NSCLC	4	Musculoskeletal	3	Systemic	4-6
P2	Arthritis	62	Female	NSCLC	4	Musculoskeletal	2	Systemic	4-6
P3	Arthritis	53	Female	NSCLC	3	Musculoskeletal	2	Intra-articular	4-6
P4	Thyroditis	73	Male	NSCLC	4	Endocrine	3	None	N/A
P5	Thyroiditis	70	Male	NSCLC	4	Endocrine	3	None	N/A
P6	Thyroiditis	76	Male	NSCLC	4	Endocrine-Renal	2	Systemic	N/A
P7	Arthritis	65	Female	NSCLC	4	Musculoskeletal	2	Systemic	4-6
P8	Pneumonitis	75	Male	NSCLC	4	Pulmonary	2	Systemic	4-6
P9	no irAE	75	Female	NSCLC	4	none	0		No irAE
P10	Pneumonitis	51	Female	NSCLC	4	Pulmonary	3	Systemic	4-6
P11	Pneumonitis	55	Male	NSCLC	4	Pulmonary	2	Systemic	4-6
P12	Pneumonitis	72	Male	NSCLC	4	Pulmonary	2	Systemic	4-6
P13	Pneumonitis	58	Male	Prostate	N/A	Pulmonary	3	Systemic	4-6
P14	Pneumonitis	74	Male	Prostate	N/A	Pulmonary	3	Systemic	4-6
P15	Pneumonitis	52	Female	NSCLC	4	Pulmonary-Endocrine-Renal	3	Systemic	4-6
P16	Neuritis	65	Female	NSCLC	4	Neuro-Gastrointestinal	3	Systemic	
P17	no irAE	67	Male	NSCLC	4	none	0	None	No irAE
P18	no irAE	40	Female	HNSCC	4	none	0	None	No irAE
P19	no irAE	74	Male	HNSCC	4	none	0	None	No irAE
P20	no irAE	77	Male	NSCLC	4	none	0	None	No irAE
P21	no irAE	81	Male	NSCLC	4	none	0	None	No irAE
P22	no irAE	68	Female	NSCLC	4	none	0	None	No irAE
P23	no irAE	69	Male	Prostate	N/A	none	0	None	No irAE
P24	no irAE	66	Male	Prostate	N/A	none	0	None	No irAE

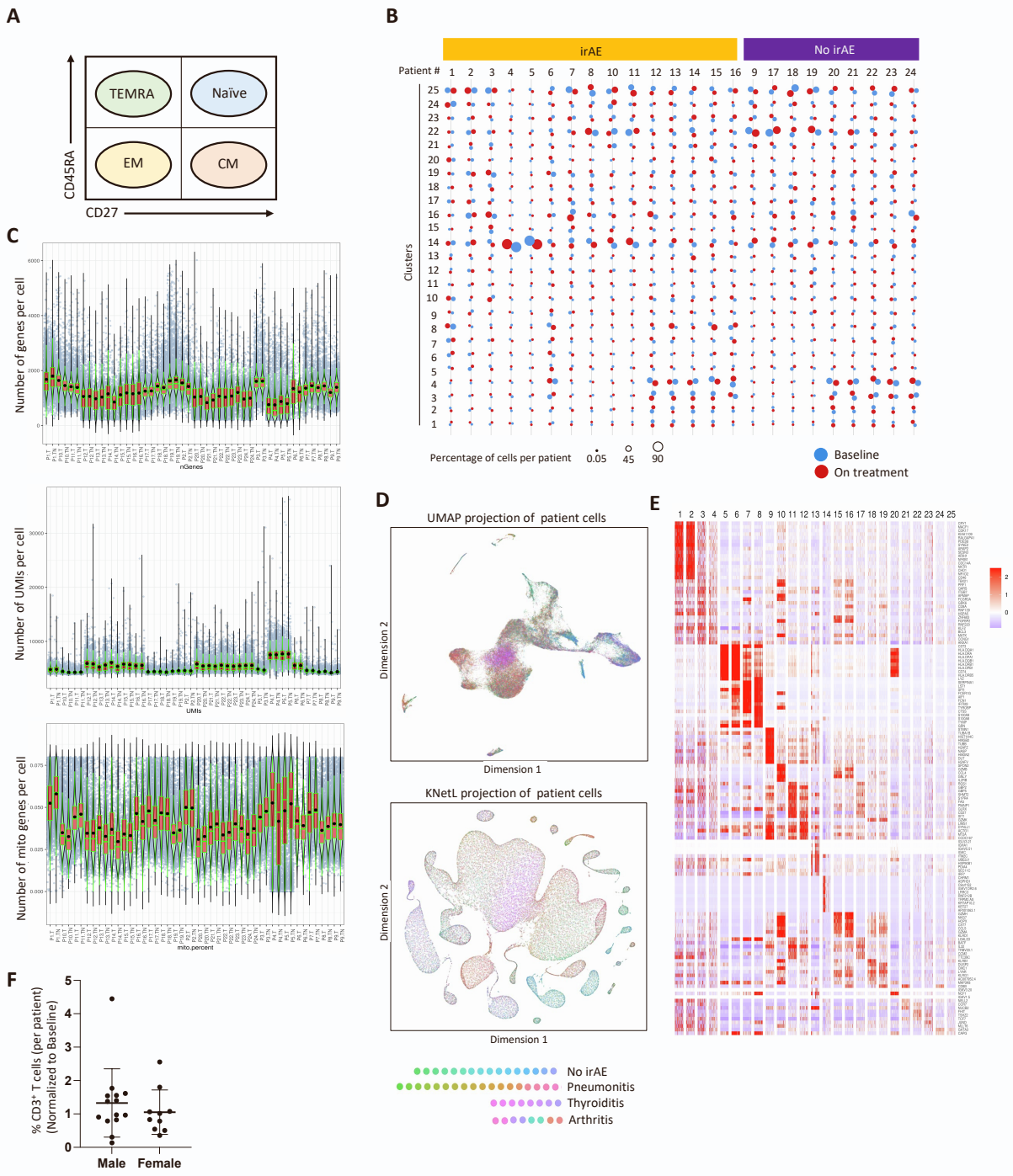
B

Patients	irAEs group	
Age (average)		70 years
Gender (n)	Male	62
	Female	73
Tumor type	Lung	46
	GU	37
	Melanoma	25
	Other	27
Agent (ICIs)	Pembrolizumab	28
	Nivolumab	41
	Atezolizumab	21
	Durvalumab	2
	Combination	43
irAEs type	Joints	58
	Skin	16
	Neurologic	16
	Endocrine	20
	Pulmonary	14
	Other	26
irAEs grade		2.7
Time to onset		57 days
Total number of patients		**135**

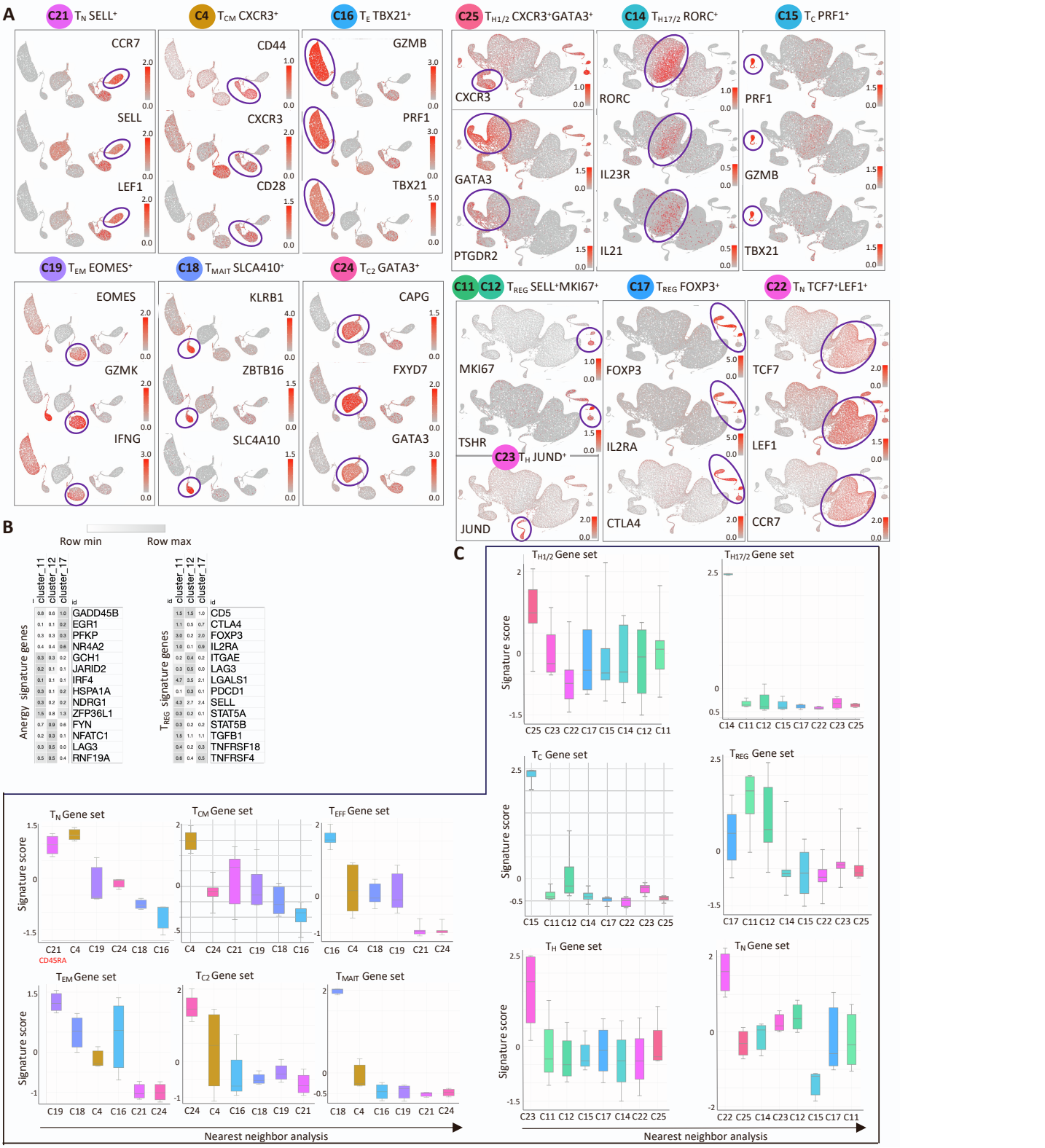
Table S1. Clinical characterization of the patients enrolled to the study, related to figure 1.

CD8+ T cells annotation				CD4+ T cells annotation			
Subset	Surface Markers	Intracellular/Transcription factors	Cytokines	Subset	Surface Markers	Intracellular/Transcription factors	Cytokines
Naïve	SELL			**TH1**	KLRD1	STAT1	TNF
	IL7R				IFNGR1	STAT4	LTA
	CCR7				CXCR3	TBX21	IFNG
	CD45RA				CXCR6		IL2
	CD27				CCR1		
					CCR5		
Effector	IL2RA	TBX21	IFNG		IL12RB1		
	TNFRSF8		IL2		IL18R1		
	CD69		PRF1		TNFSF11		
	TNFRSF4		GZMB / GZMA		HAVCR2		
	ICOS		TNFA				
	KLRG1		CCL3				
	HAVCR2		CCL4	**TH2**	CXCR4	BATF	IL4
			CCL5		CCR4	GATA3	IL5
					CCR8	IRF4	IL13
					PTGDR2	STAT6	AREG
					HAVCR1		
Effector Memory	CD44	EOMES	GZMK		IL17RB		
	CD45RO /CD45RA-	TBET	IFNG++		IL33		
	CD62L low		TNFA++				
	CD127 high		PRF1				
	KLRG1 high		IL2				
	CCR7 low			**TH17**	IL1R1	AHR	CSF2
					KLRB1	BATF	IL17A
					CCR4	MAF	IL17AF
Central Memory	CD45RO/CD45 RA-	TBET	IFNG		CCR6	NFKBIZ	IL17F
	CD62L low	EOMES	IL2		IL21R	IRF4	IL21
	CD127 ++		TNFA		IL12RB1	RORA	IL22
	CCR7 low					RORC	
	CD27					STAT3	
	CD28						
	CXCR3 mid			**CD4 Cytotoxic**		TBX21	PRF1
							GZMB
MAIT cells	KLRB1	SLC4A1					GZMA
		ZBTB16					
				TREG	CTLA4	FOXP3	
Regulatory	CD57	B3GAT1			IL2RA		
	CD28-	FOXP3					
	KLRG1++	IKAROS					
	PD1	EGR1		**TFH**	CXCR3	BATF	IL21
	LAG3	EGR2			CXCR5	BCL6	
	HLADR				ICOS	MAF	
					PDCD1	IFR4	
						STAT3	
TRMs	CD69+	ITGA1					
	CD103+/-	ITGAE					
	CD101	SELPLG					
	CD49a	Hobit					
	PD1	Blimp1					
	CXCR6	Runx3					
	CLA	Notch/RBPj					
	CCR8						

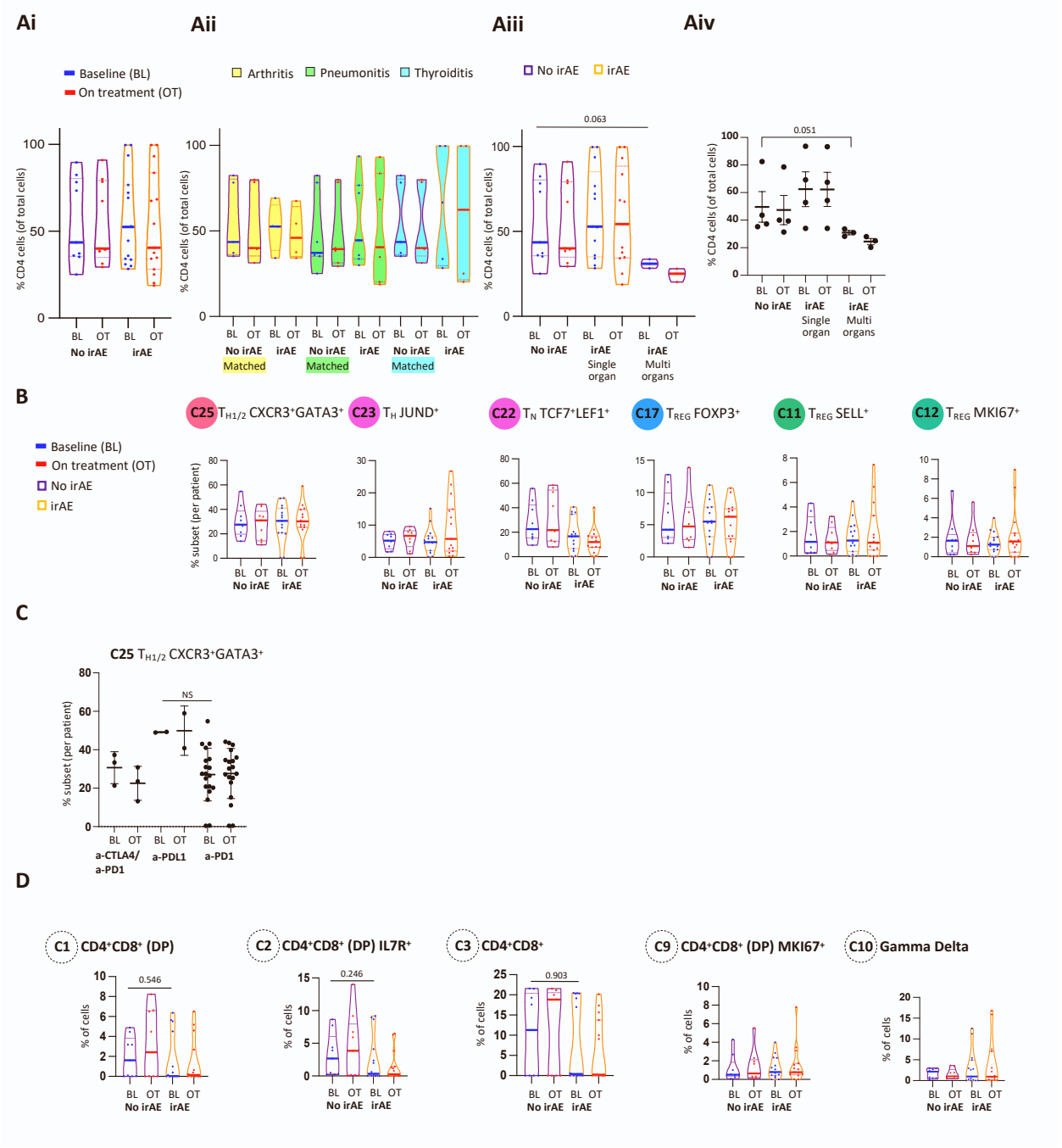
Table S2. T cell subset markers, related to figure 2.



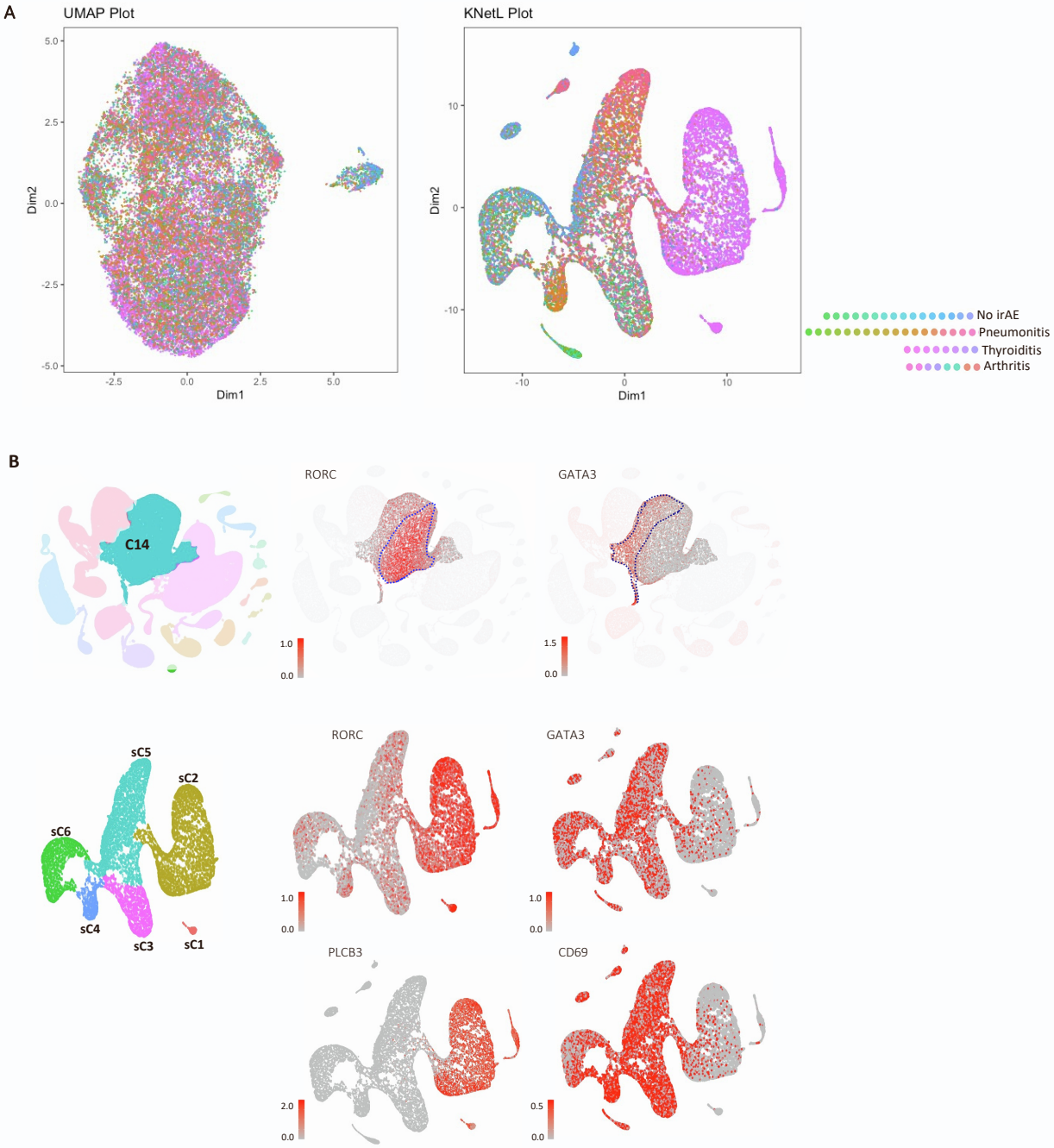
Supplemental figure 1. T cells annotation and characterization, related to figure 2. (A) T cells were annotated into Naïve, central memory (CM), effector memory (EM), terminally differentiated (TEMRA) maturation subsets based on expression levels of CD27 and CD45RA. (B) Qualitative representation of T cell abundances at baseline and on treatment within each cluster per patient using Beeswarm map. (C) Quality control bar plots showing the number of genes, UMI, mito genes per cell. (D) UMAP and KNetL projection of cells contributed by each patient. (E) Heatmap representation of cluster defining marker genes and other cluster associated genes. (F) Dot plot representing the total T cell distribution in males compared to females. Statistical significance: T test, unpaired Mann-Whitney, P-value, Exact-two-tailed, the center lines denote the mean of SEM. T (on treatment) TN (baseline).



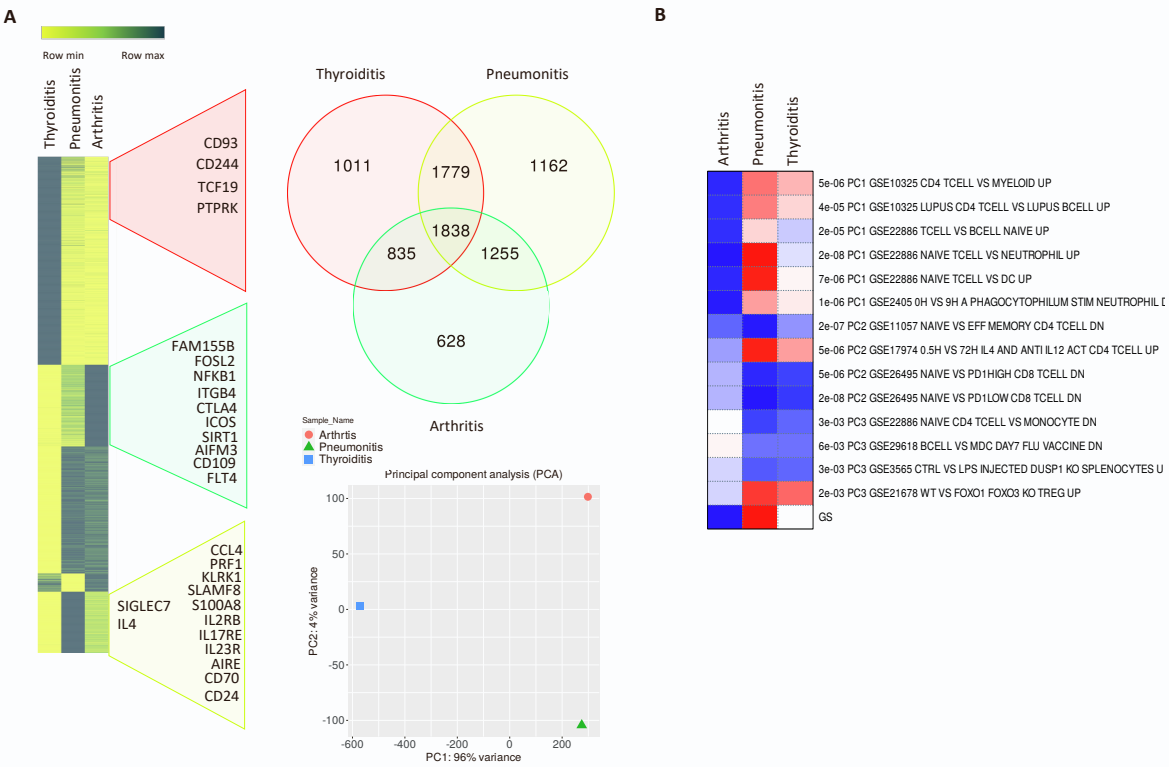
Supplemental figure 2. Gene markers of T cell subsets, related to figure 2. (A) KNetL plots projecting the RNA expression of marker genes indicated in red. Each red dot represents a cell. (B) Heatmap representation of RNA expression of anergy and T_{REG} marker genes sets. (C) Bar graphs indicting the marker gene sets score for each subset cluster, further resolved in the basis of nearest between the marker gene sets.



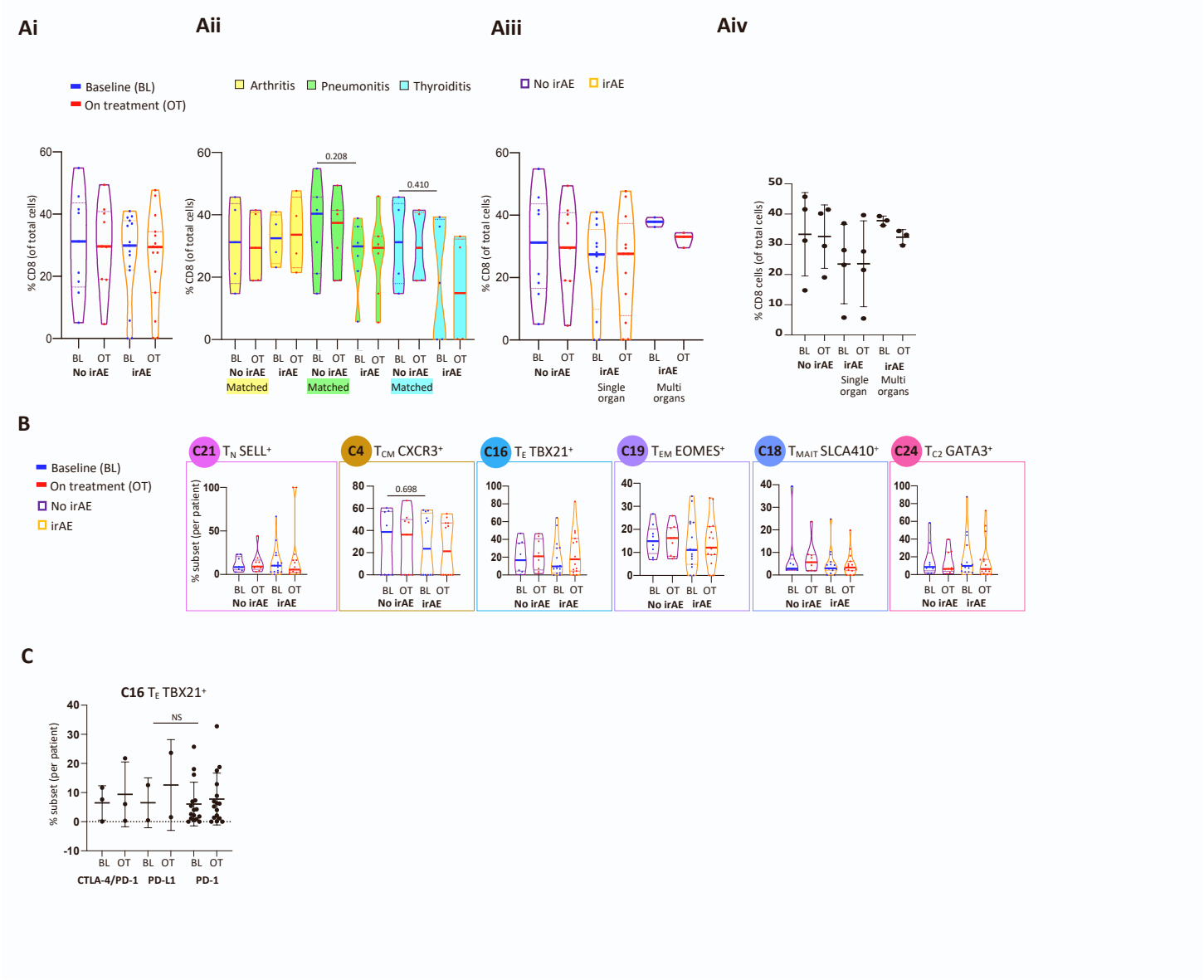
Supplemental figure 3. Characterization of CD4 T cells, related to figure 3. Percentages distribution of subset of CD4 T cells between no irAE and the different irAE groups of patients at baseline and on treatment (A). Percentages of CD4 specific clusters among patients with and without irAEs and baseline and on treatment with ICIs (B). Percentages of cells of cluster 25 based on the specific type of ICIs used (C). Double positive and gamma delta T cells (D).



Supplemental figure 4. Characterization of the T cells in cluster 14, related to figure 4. (A) UMAP and KNetL projections of cells from sub-clustering of meta-cluster 14. (B) KNetL projection of marker gene RORC, GATA3, PLCB3, and CD69.

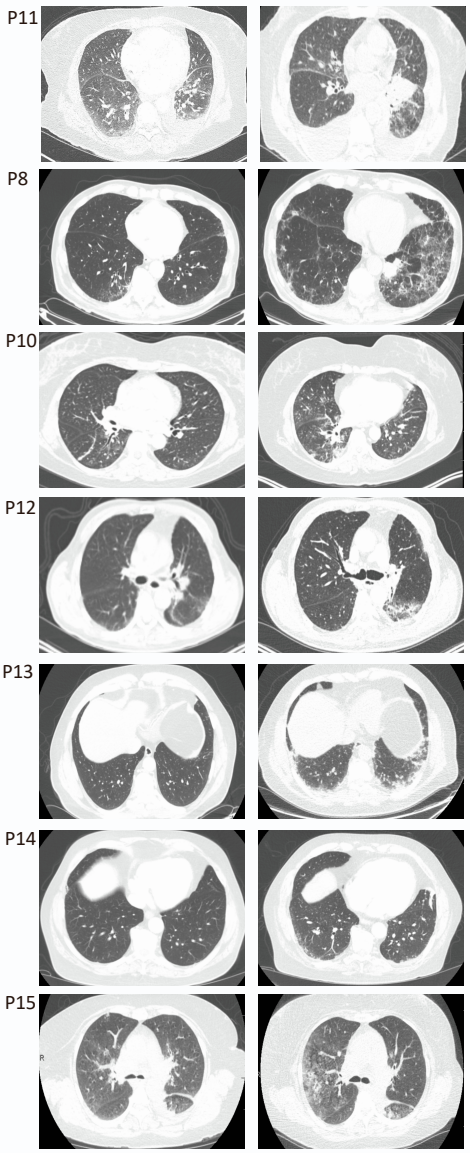


Supplemental figure 5. Organ specific toxicities and association with gene expression, related to figure 4. Heat map, Venn diagram, and PCA plot of the different genes that characterize patient with arthritis, thyroiditis and pneumonitis irAEs (A). Comparison of organ specific irAEs genes and published GSE studies (B).



Supplemental figure 6. Characterization of CD8 T cells, related to figure 5. Percentages of CD8 cell clusters among patients with different types of irAEs (A). Percentages of specific clusters of cells of patients with and without irAEs. Percentages of subset of patients treated with different types of ICIs (C).

A



B

Patient No.	Pneumonitis Pattern	Tumor type	irAE grade	Size Dominant nodule (mm)	Location of nodule
P8	CHP	NSCLC	2	26	LUL
P10	CHP	NSCLC	3	77	RLL
P11	CHP	NSCLC	2	37	RLL
P12	OP	NSCLC	2	18	LLL
P13	OP	Prostate	3	0	NA
P14	OP	Prostate	3	0	NA
P15	OP	NSCLC	3	67	LLL

Supplemental figure 7. CT scans of pneumonitis patients at baseline and on treatment, related to figure 6. CT scan images (A). Clinical characterization of irAE patients that developed pneumonitis (B).