**Supplemental Figure 1: Heterogeneous immune responses in Head and Neck Cancer.** **(A)** Whole-slide scan image by Phenochart for patient CUHN041 (poorly infiltrated, shown in Figure 1A). Blue rectangle 1 represents a region of tumor invasive margin while blue rectangle 2 represents a region of tumor core in the CUHN041 sample. **(B)** Whole-slide scan image by Phenochart for patient CUHN024 (highly infiltrated, shown in Figure 1A). Blue rectangle 1 represents a region of tumor invasive margin while blue rectangle 2 represents a region of tumor core in the CUHN024 sample. Scale bars indicate $800\mu m$. **(C-F)** A223 tumors grow heterogeneously when different cell numbers were injected into C57BL/6J mice at **(C)** 1 million per flank (n=24); **(D)** 500,000 per flank (n=20); **(E)** 15,000 per cheek (n=4); **(F)** 5,000 per cheek (n=5). **(G-H)** No tumor rejection was observed in other commonly used HNC cell lines. **(G)** B4B8 cells were injected into WT Balb/c mice at 1 million per cheek (n=9). **(H)** LY2 cells were injected into WT Balb/c mice at 1 million per cheek (n=15).

Supplemental Figure 2A-K

L

% of CD8 CD244+ vs Tumor Volume — $R^2 = 0.3041$

% of CD8 CD69+ vs Tumor Volume — $R^2 = 0.3409$

% of CD8 CD122^{high} vs Tumor Volume — $R^2 = 0.5004$

% of CD8 Ly6C+ vs Tumor Volume — $R^2 = 0.2988$

M Original Cohort Survival

N Second Cohort Survival
- Rechallenged
- Naive

O Second Cohort: Naive
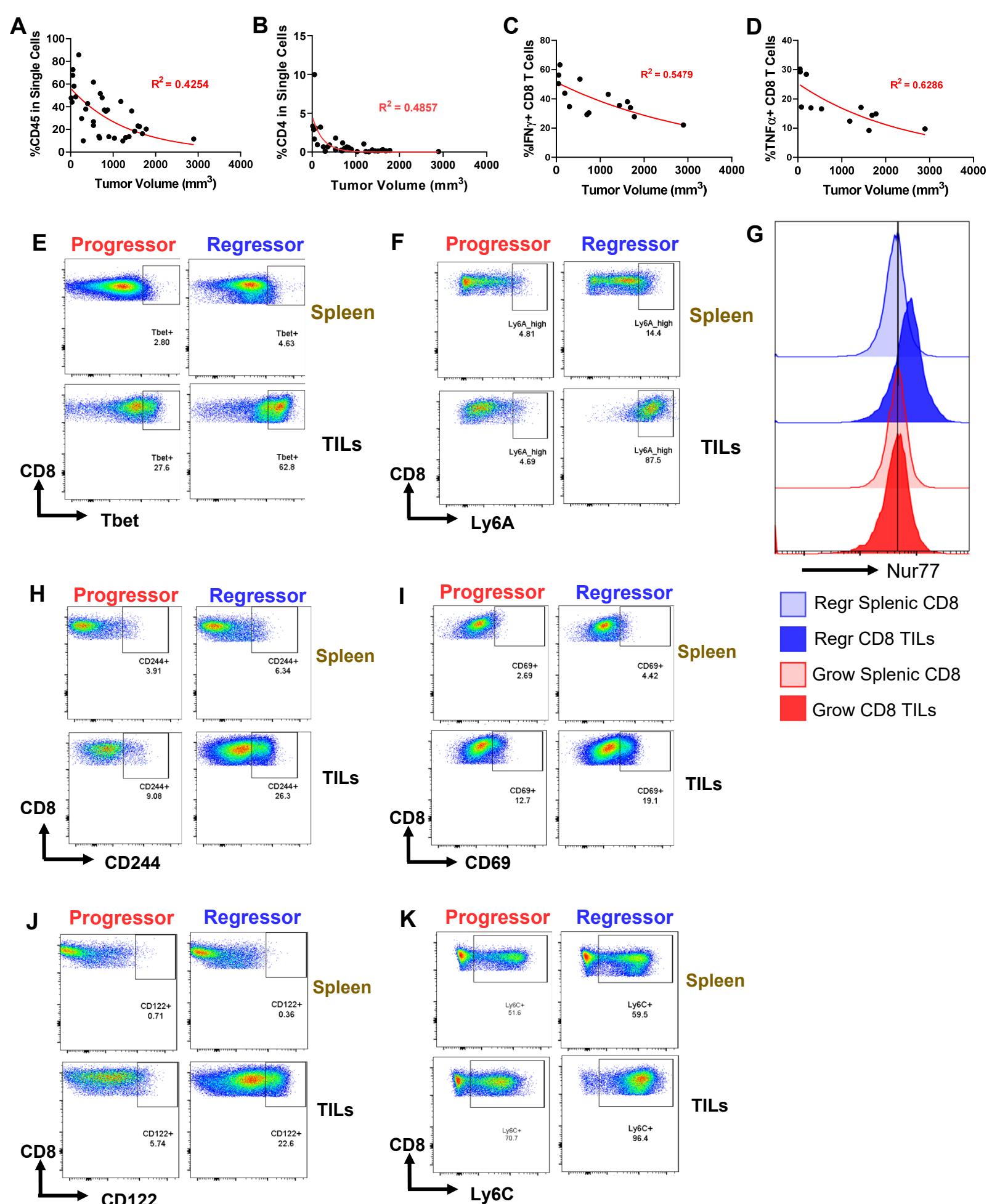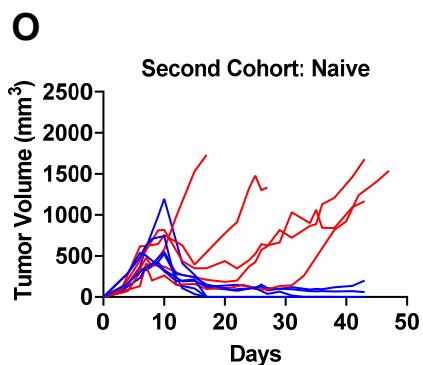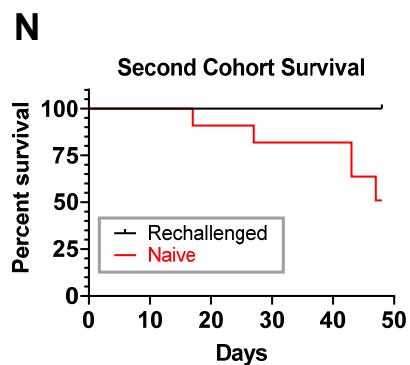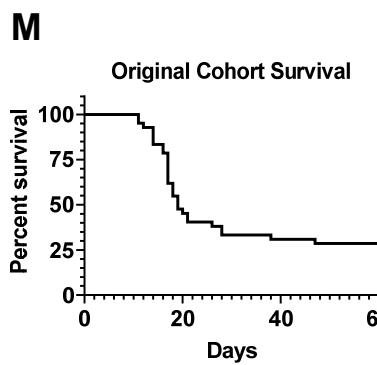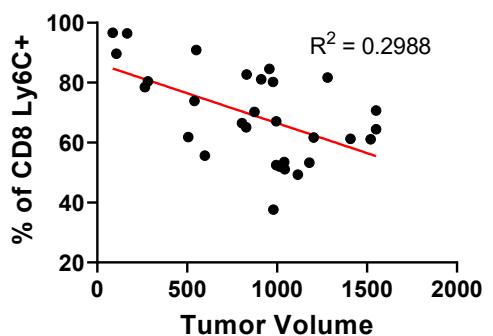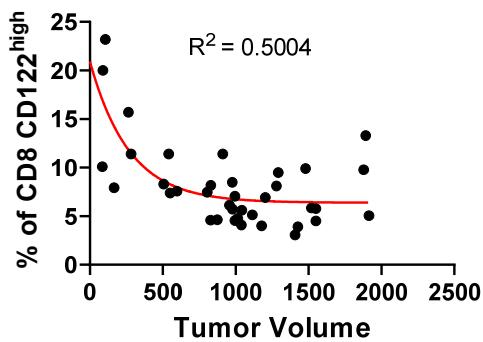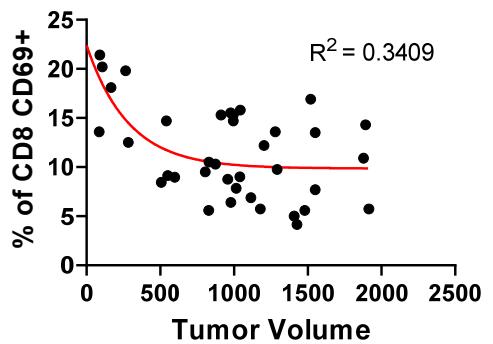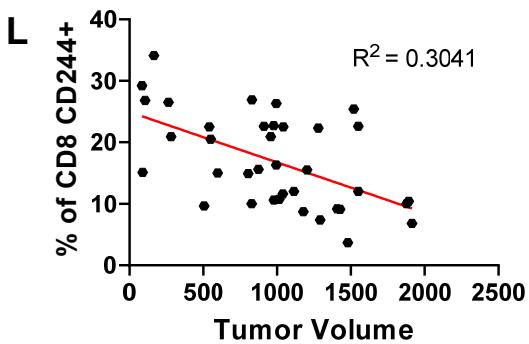
Supplemental Figure 2L-O

Supplemental Figure 2: Tumor regression correlates to immune response. A223 tumors were implanted in WTB6 mice, measured for tumor volume (mm^3) and then analyzed by flow cytometry. **(A)** Correlation of the percentage of $CD45^+$ cells in the single cell gate with tumor volume (mm^3) ($R^2 = 0.4254$) (n=34; pooled from 8 independent experiments). **(B)** Correlation of the percentage of $CD4^+$ cells in the single cell gate with tumor volume ($R^2 = 0.4857$) (n=34; 8 independent experiments). **(C)** Correlation of the percentage of $IFN\gamma^+$ cells within $CD8^+$ T cells with tumor volume ($R^2 = 0.5479$) (n=14, 3 independent experiments). **(D)** Correlation of the percentage of $TNF\alpha^+$ cells within $CD8^+$ T cells with tumor volume ($R^2 = 0.6286$) (n=12, 2 independent experiments). **(E-K)** Several activation markers are differentially expressed in CD8 TILs in Progressors vs. Regressors. Splenic CD8 T cells from Progressors or Regressors are shown as controls. A higher percentage of Regressor CD8 TILs express T-bet **(E),** Ly6A **(F)**, CD244 **(H),** CD69 **(I),** CD122 **(J),** Ly6C **(K)** than Progressor TILs or splenic controls. **(G)** Regressor CD8 TILs express a higher level of Nur77 than Progressor TILs or splenic controls. **(L)** A223 tumors were implanted in WT B6 mice, measured for tumor volume (mm^3) and then analyzed by flow cytometry. **(L, upper left)** Correlation of the percentage of $CD244^+$ cells within $CD8^+$ cells with tumor volume (mm^3) ($R^2 = 0.3041$) (n=37; pooled from 2 independent experiments). **(L, upper right)** Correlation of the percentage of $CD69^+$ cells within $CD8^+$ cells with tumor volume ($R^2 = 0.3409$) (n=37; pooled from 2 independent experiments). **(L, lower left)** Correlation of the percentage of $CD122^{high}$ cells within $CD8^+$ cells with tumor volume ($R^2 = 0.5004$) (n=37; pooled from 2 independent experiments). **(L, lower right)** Correlation of the percentage of $Ly6C^+$ cells within $CD8^+$ cells with tumor volume ($R^2 = 0.2988$) (n=30; 1 independent experiment). **(M)** Survival of WT B6 mice upon injection with A223 tumors ("Original Cohort") (n=38) shows the typical ~25% survival. **(N and O)** Regressors develop memory responses against A223 tumors. **(N)** 9 regressors from the original cohort were challenged again with A223 tumors ("Second Cohort") and all of the rechallenged mice survived (100%). In parallel, age-matched naïve WT B6 mice were challenged with A223 tumors for the first time (n=11); however, only about 50% of naïve mice survived. **(O)** Tumor growth curves of naïve mice (n=11) as shown in (N).

A Top Clonotypes in Each Sample — Log10 (Percent)

B Top CDR3α's in Each Sample — Log10 (Percent)

C Top CDR3β's in Each Sample — Log10 (Percent)

More common in Progressors / More common in Regressors

D Consensus TCRα CDR3

E Consensus TCRβ CDR3

F GLIPH Group 7 · GLIPH Group 8 · GLIPH Group 9 · GLIPH Group 10

Relative Ratio of % in **Progressors** to % in **Regressors**

|	In Progressors	In Regressors
Progressor Clonotypes	31	3
Regressor Clonotypes	5	44

****

G

Supplemental Figure 3

Supplemental Figure 3: Top TCR clonotypes and TCR specificity groups appear to be mutually exclusive between regressing and progressing TILs. **(A)** Heatmap of top TCR clonotypes that are >1% in any given sequenced TIL sample (including GT2, GT3, RT1, RT2 and RT3), sorted by descending abundance (%) in GT3, then descending abundance in GT2, then ascending abundance in RT1, then ascending abundance in RT2, then ascending abundance in RT3. Heatmap cells are colored according to the log10 of the percent in each sample. Many commonly used TCR clonotypes are only detected in one recipient mouse with only a few exceptions, indicating highly individualized anti-tumor immune responses. **(B)** Heatmap of top TCRα CDR3 sequences (CDR3α) (>1% in any given sample), sorted by average abundance in progressing TILs vs. average abundance in regressing TILs. **(C)** Heatmap of top TCRβ CDR3 sequences (CDR3β) (>1% in any given sample), sorted by average abundance in progressing TILs vs. average abundance in regressing TILs. **(D and E)** CDR3 sequences for TCRα and TCRβ regressor clonotypes were analyzed for a consensus sequence. Regressor CDR3α sequences ranged from 12 to 16 amino acids (AAs), with 16 AAs being the most abundant. Regressor CDR3β sequences ranged from 12 to 16 AAs, with 15 AAs being the most abundant. **(D)** Consensus AA sequence for top TCRα CDR3 regions in Regressors (>1% in a regressor sample) (AA-length=16). Unique sequences were scaled by their abundance in samples (ex: a sequence averaging in 5% of samples would be counted 5 times, while a sequence averaging in 20% of samples would be counted 20 times) and plotted using ggseqlogo. **(E)** Consensus AA sequence for top TCRβ CDR3 regions in Regressors (>1% in a regressor sample) (AA-length=15). Unique sequences were scaled by their abundance in samples and plotted using ggseqlogo. **(F)** Network plots of GLIPH Groups 7, 8, 9, and 10. Each node represents a TCRβ CDR3 AA sequence, and each line represents a global similarity to another CDR3 sequence. Node sizes represent overall abundance in samples and nodes are colored based on the relative ratio between their percent in progressing samples (**red**) versus their percent in regressing samples (**blue**) where purple is a sequence shared between progressing and regressing samples. Relative ratio is calculated as (% in Progressors)/(% in Progressors + % in Regressors). **(G)** Statistical analysis for progressor vs regressor clonotypes. Clonotypes were designated as Progressor Clonotypes or Regressor Clonotypes by overall abundance in either group (See Figure 4C for more common in Progressors vs more common in Regressors). The difference between two groups was analyzed by Fisher's Exact Test to evaluate whether the clonotypes were exclusive to Progressors or Regressors or shared. 31 Progressor Clonotypes were observed in Progressors only, whereas 3 were observed also in Regressors. 44 Regressor Clonotypes were observed in Regressors only, whereas 5 were observed also in Progressors. (**** $p < 0.0001$).

A

RT1 TRA V-J Gene Usage

RT2 TRA V-J Gene Usage
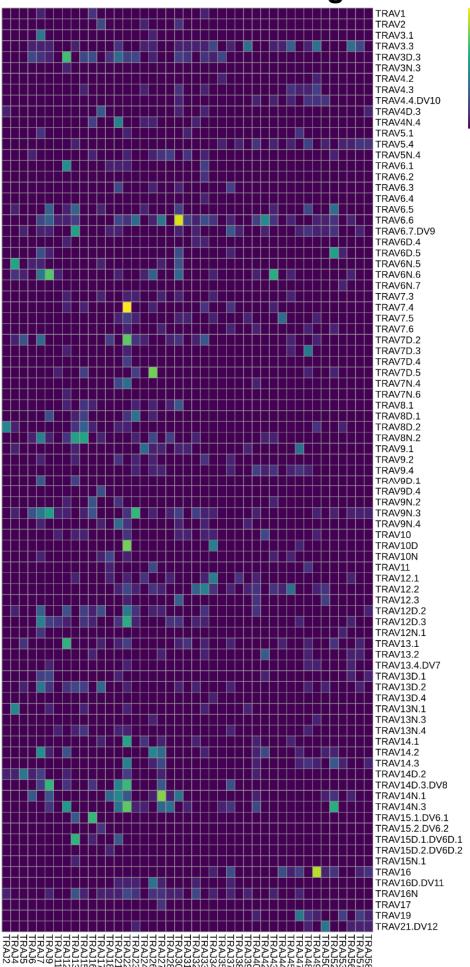
RT3 TRA V-J Gene Usage
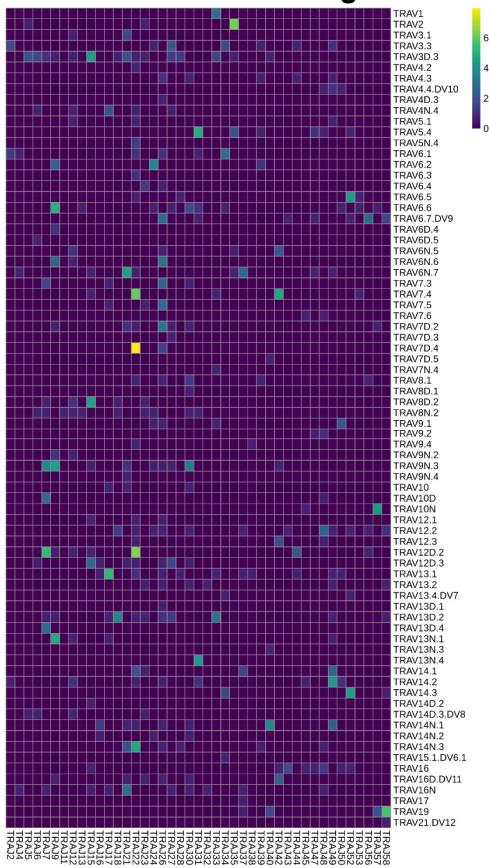
GT2 TRA V-J Gene Usage

GT3 TRA V-J Gene Usage

Supplemental Figure 4A

B
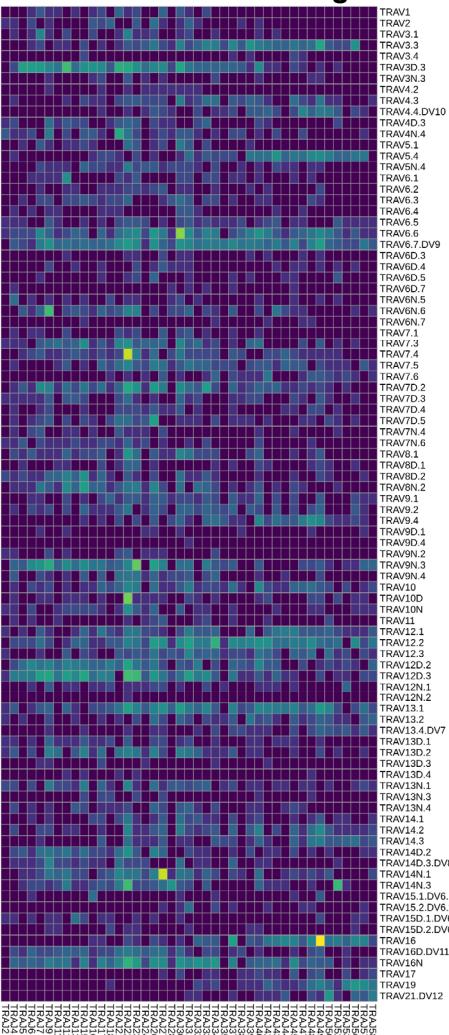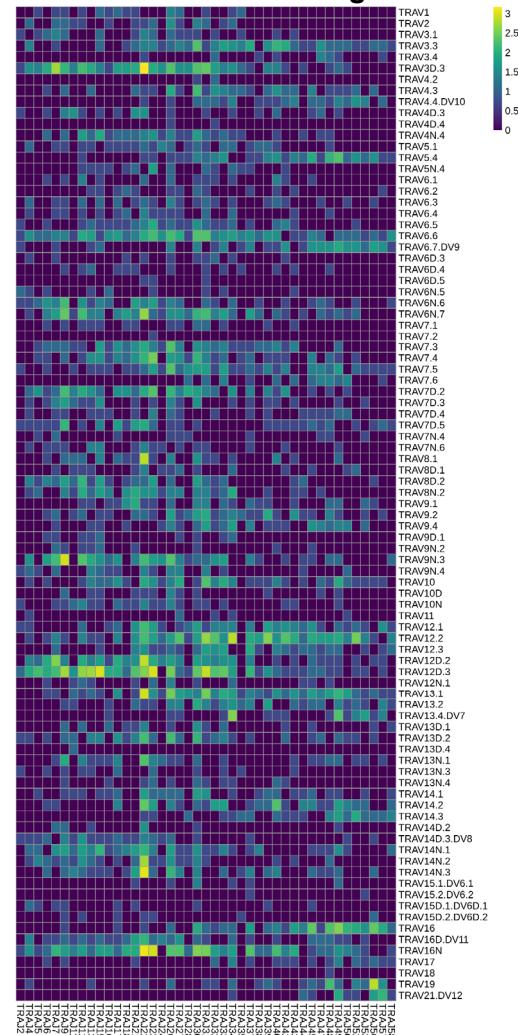
Supplemental Figure 4: V-J gene usage in 8 samples sequenced for TCR including TIL and splenic controls. (A) TCRα V-J gene usage combinations in all T cells of each TIL sample (5 in total, RT1, RT2, RT3, GT2 and GT3), colored by log(# of cells). **(B)** TCRα V-J gene usage combinations in all T cells of each spleen sample (3 in total, RS2, RS3 and GS3), colored by log(# of cells).
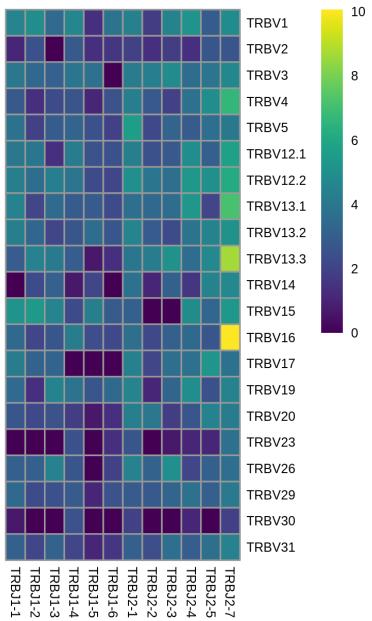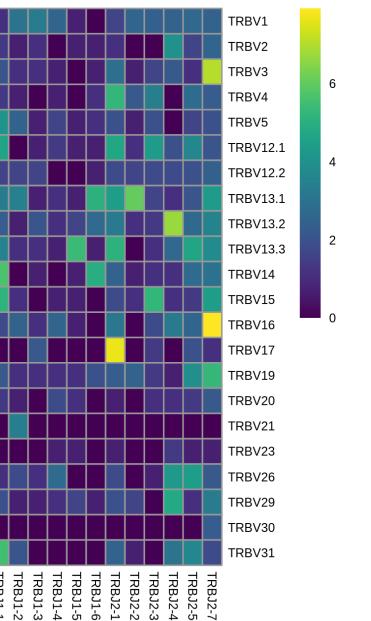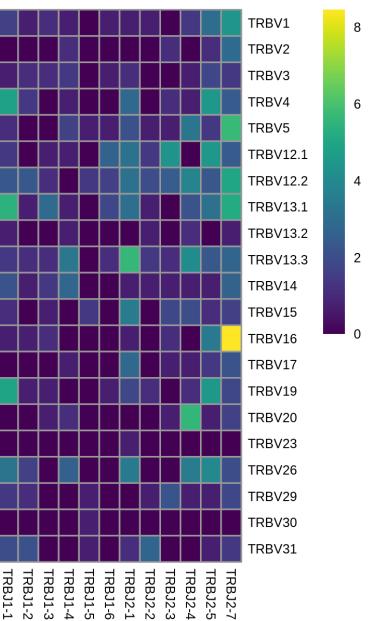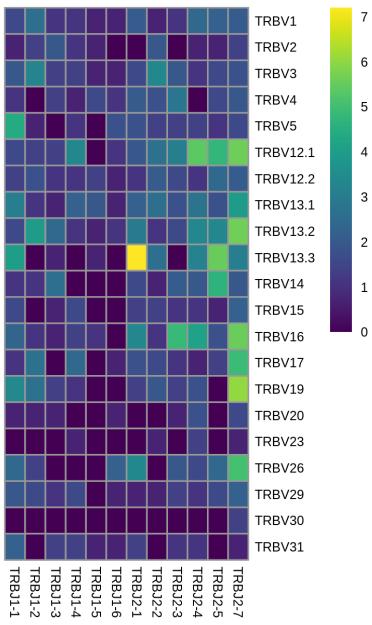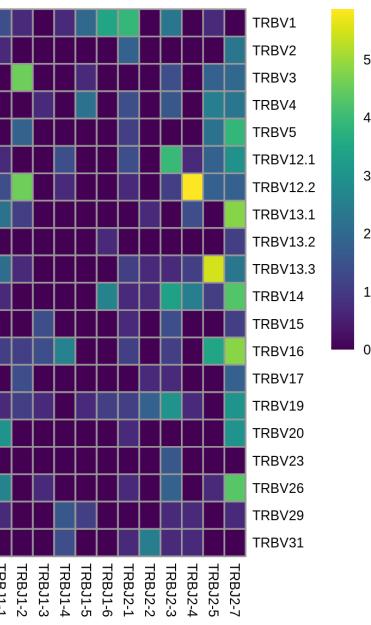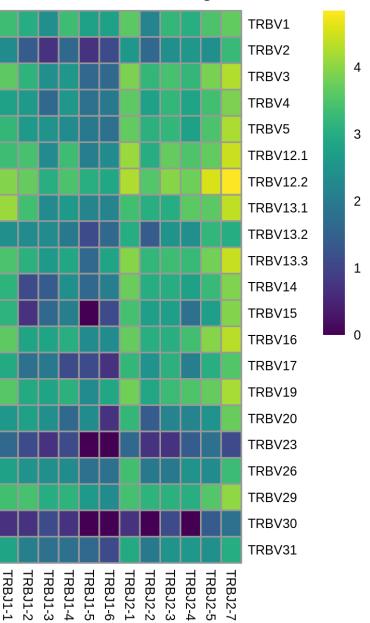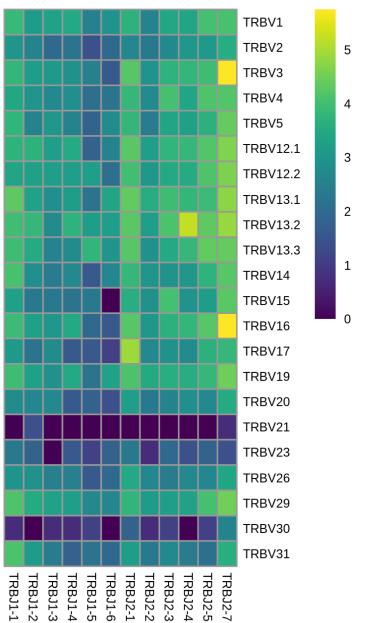
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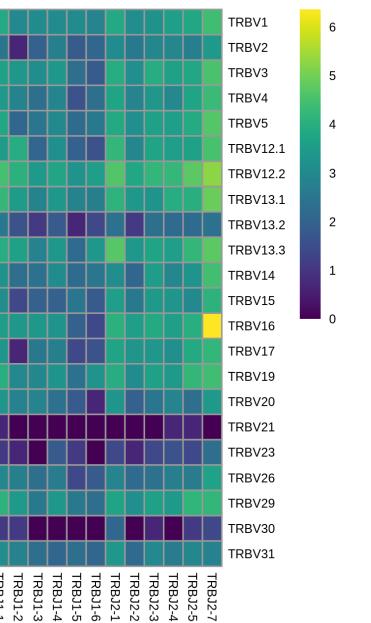
Supplemental Figure 5: TCRβ Gene Usage in 8 Samples sequenced for TCR including 5 TIL samples and 3 splenic samples. TCRβ V-J gene usage combinations in all T cells of each sample, colored by log(# of cells).

A WTB6 Spleen

B WTB6 TRA V-J Gene Usage

C WTB6 TRB V-J Gene Usage
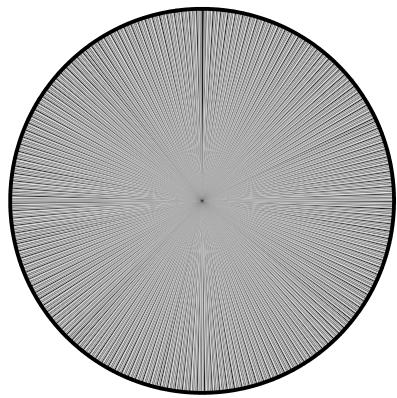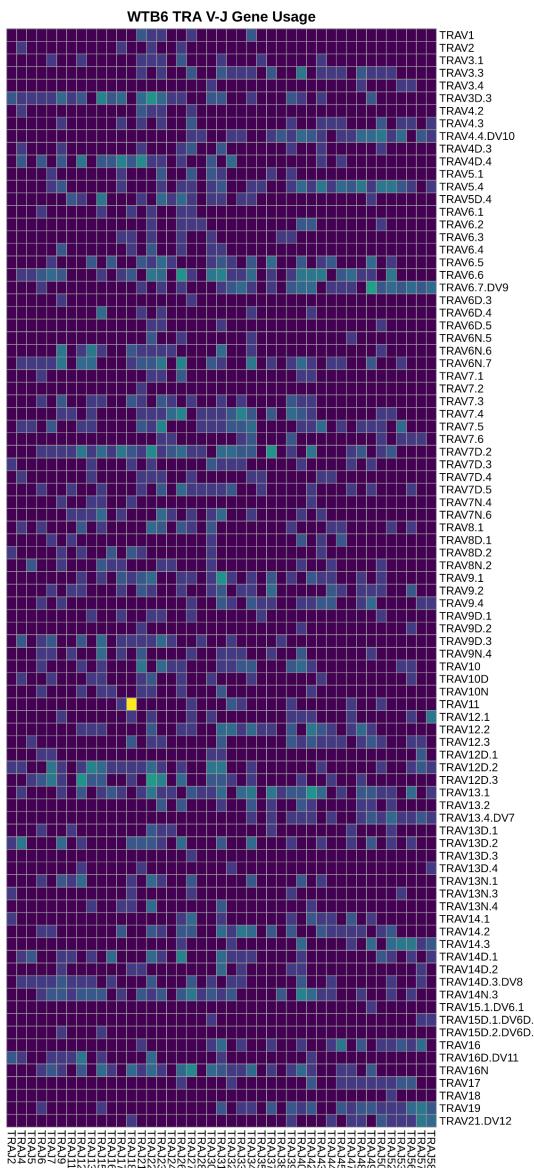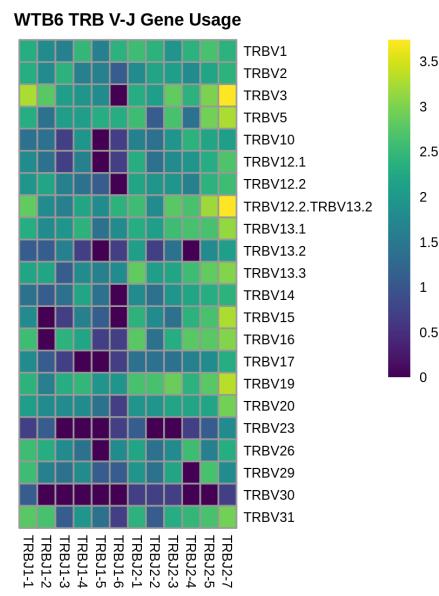
Supplemental Figure 6: Non-Tumor Bearing WT B6 Repertoire. Publicly available data from a non-tumor-bearing C57BL/6J mouse were downloaded from 10×Genomics, listed under the "Splenocytes from C57BL/6 mice, 10k cells (v2)" dataset. VDJ data were analyzed in the same manner as our 8 samples. **(A)** TCR clonotype distribution in 1435 T cells in a WT B6 spleen. Cells containing the same TCR (one "clonotype") are shown as a single pie slice representing the percent of these cells in the entire sample. **(B)** TCRα V-J gene usage combinations in 1435 T cells in a WT B6 spleen, colored by log(# of cells). **(C)** TCRβ V-J gene usage combinations in 1435 T cells in a WT B6 spleen, colored by log(# of cells).
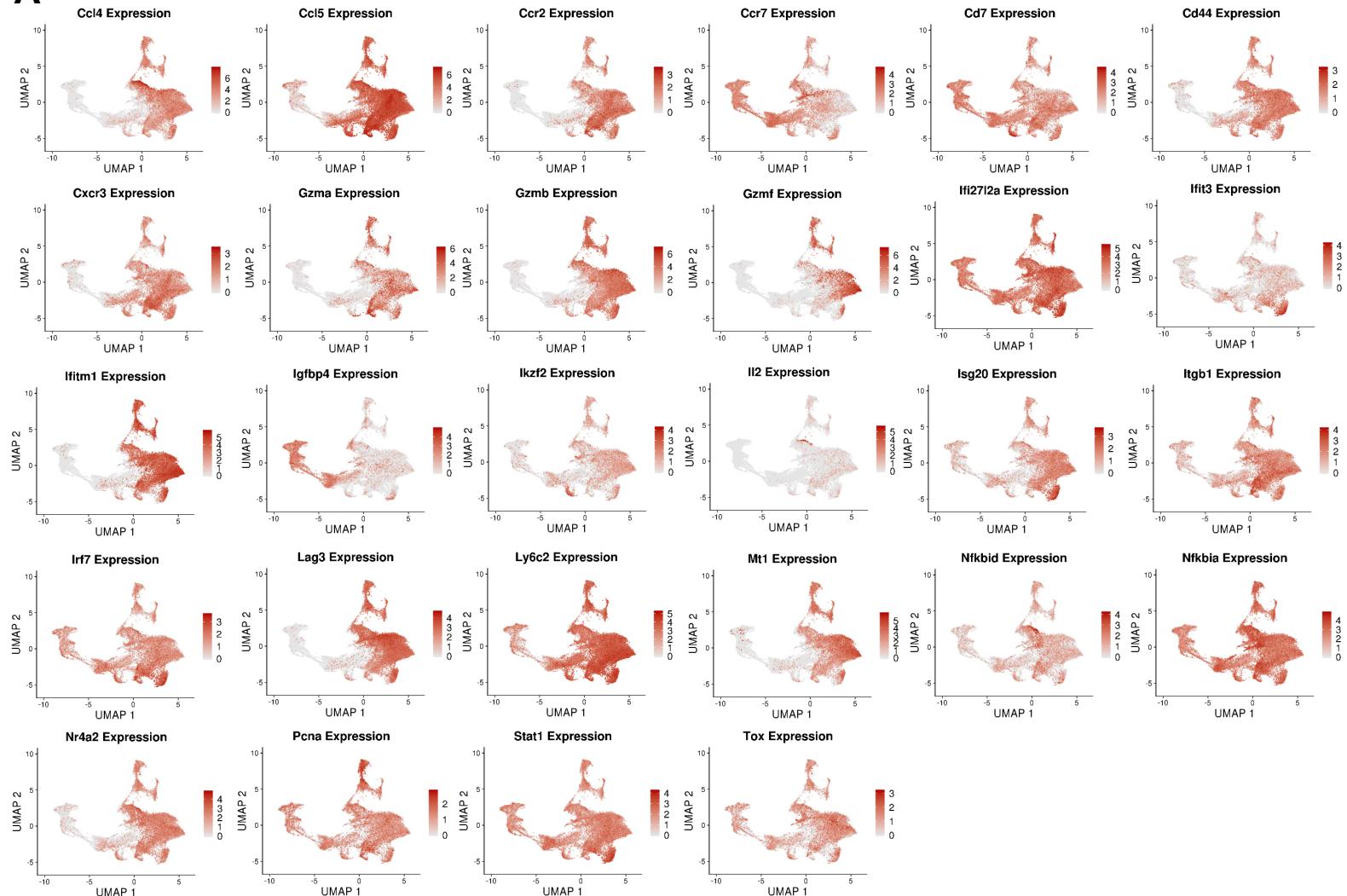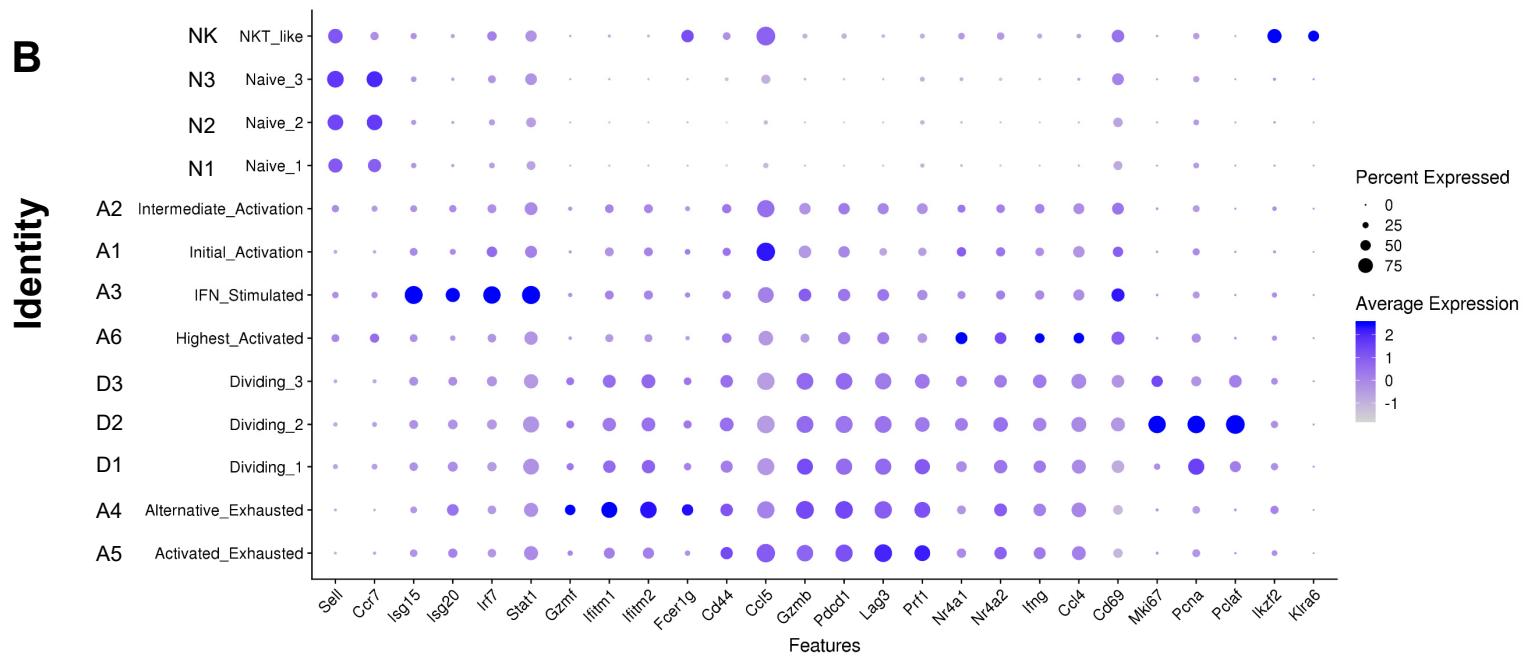
Supplemental Figure 7A-B: Differential expression of T cell activation genes in different clusters. (A) >41,000 cells from 9 samples (Grow1-TIL, Regr1-TIL, Grow2-TIL, Regr2-TIL, Regr2-Spln, Grow3-TIL, Regr3-TIL, Grow3-Spln, Regr2-Spln) were clustered together using UMAP, and colored based on normalized expression of a given gene (gray = little to no expression; red = high expression). 28 UMAPs were shown for 28 representative T cell activation genes. **(B)** Dot plot of representative genes defining each cluster in the UMAP.

Supplemental Figure 7A, B
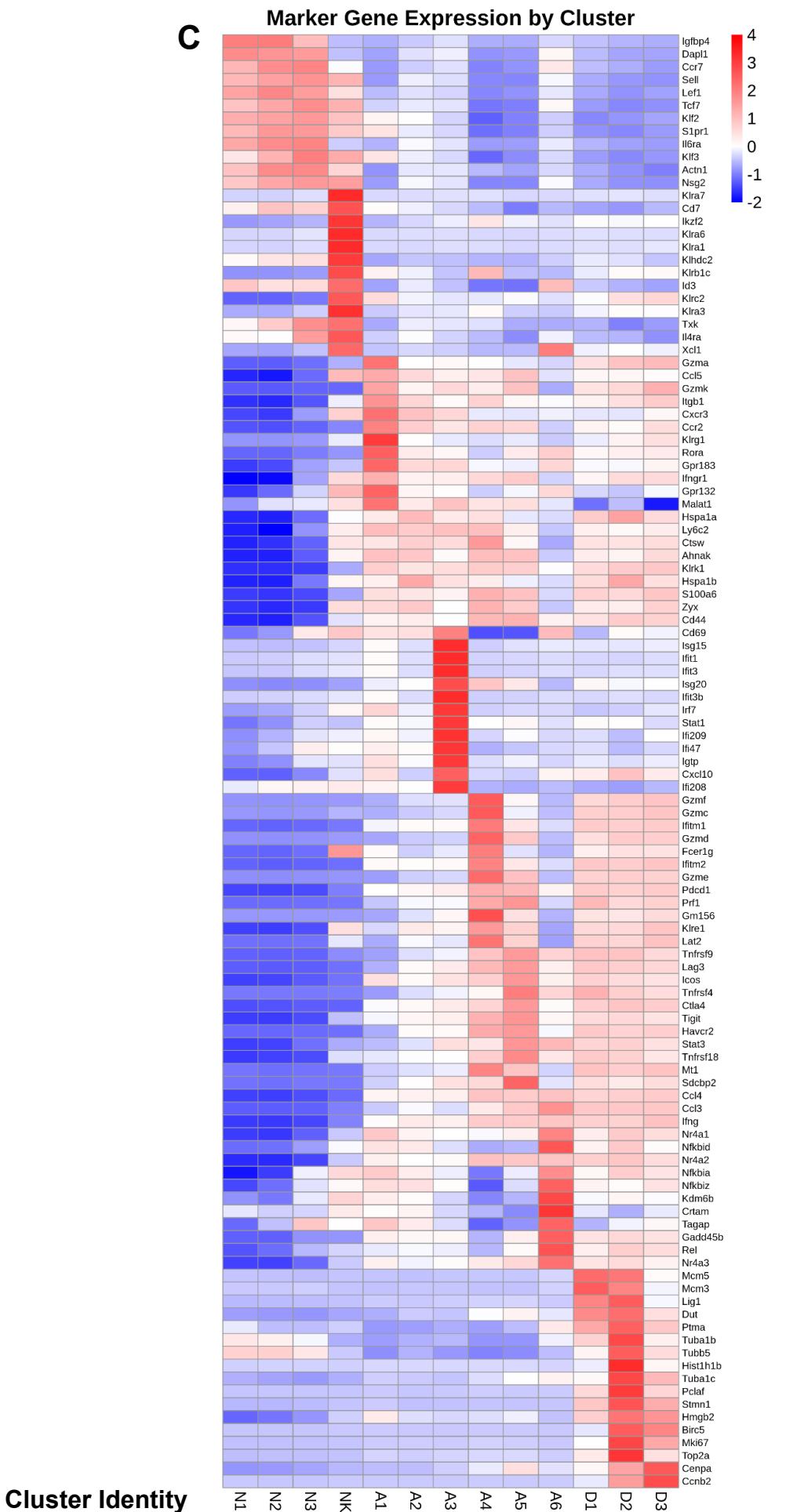
Supplemental Figure 7C: Differential expression of T cell activation genes in different clusters. (C) "Marker" genes for each cluster (as defined by FindMarkers in Seurat) are shown in a heatmap plotting the average expression of all cells in each cluster. Color designations are scaled by row.
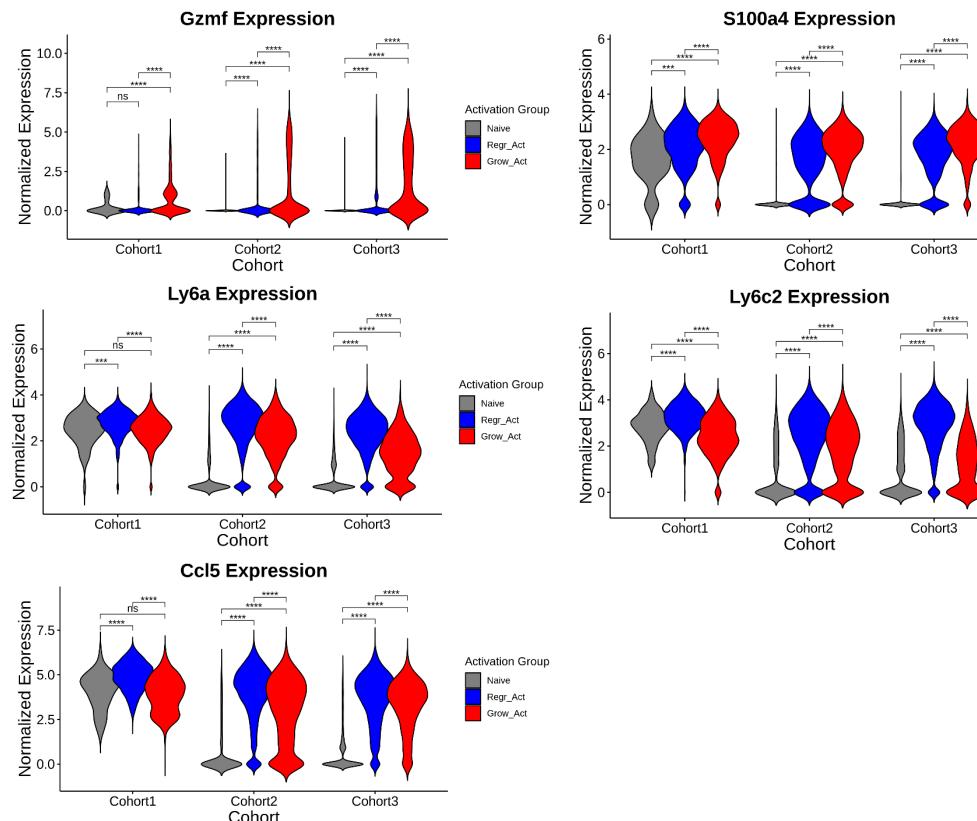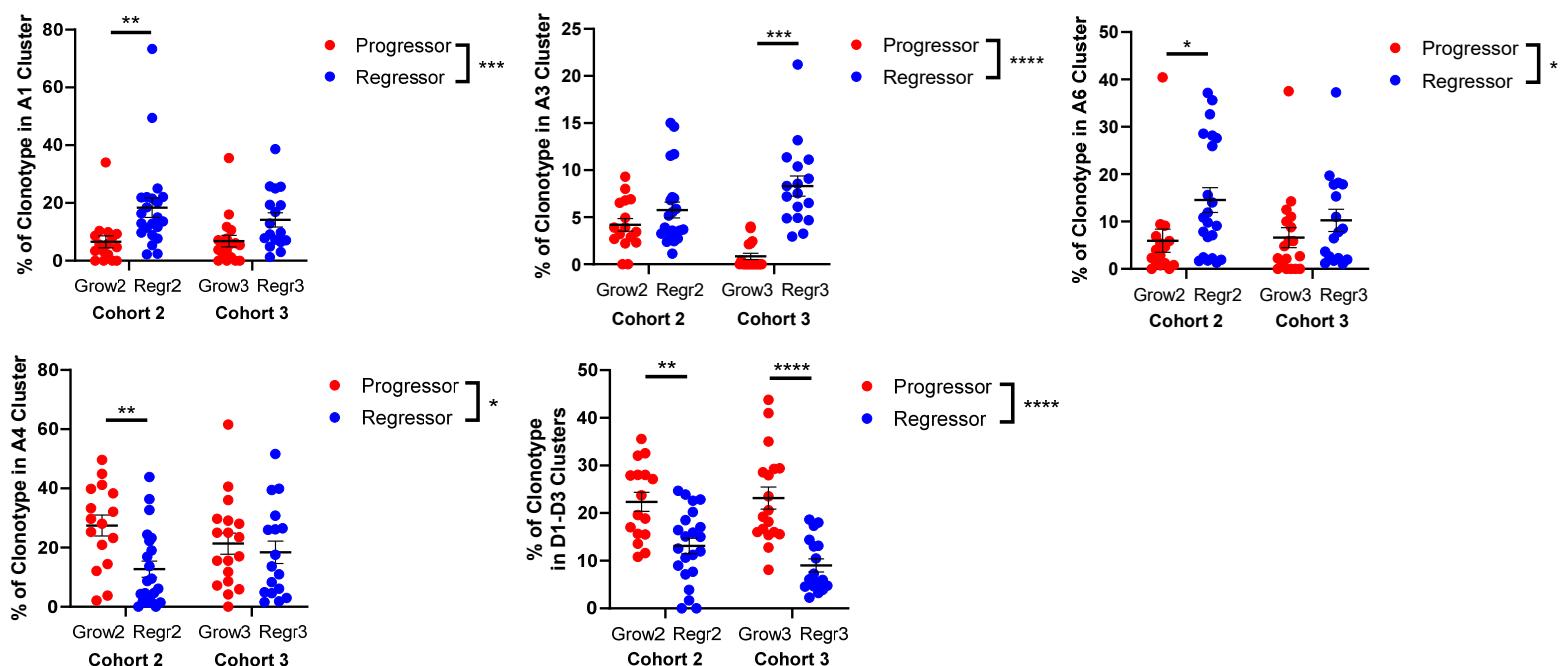
Supplemental Figure 8A-B: Reanalysis by Cohort. Data shown in Figures 5G and 6B were analyzed in order to account for differences between sequencing cohorts. **(A)** Gene expression violin plots from Figure 5G are alternatively shown by separating cells into their three respective sequencing cohorts (Cohort 1 = Grow1 and Regr1; Cohort 2 = Grow2 and Regr2; Cohort 3 = Grow3 and Regr3). Representative genes upregulated in growing or regressing activated TILs (residing in one of the 6 activated clusters: A1-A6) versus naïve T cells (residing in N1-N3). Grow-Act = activated clusters in growing samples; Regr-Act = activated clusters in regressing samples; Naïve = naïve clusters in all samples. Differences were evaluated using two-way ANOVA for Activation group (Regr_Act, Grow_Act, Naïve) and for Cohort. Variation by Activation Group, Cohort, and Interaction were all statistically significant (p<0.0001). Tukey's multiple comparison of means tests were conducted between each Activation group and were all statistically significant (adjusted p-value = 0). **(B)** Data from Figure 6B are alternatively shown by separating clonotypes into their respective samples (Grow2, Regr2, Grow3, Regr3) which come from two sequencing cohorts (Cohort 2 = Grow2 and Regr2; Cohort 3 = Grow3 and Regr3). Clusters that are differentially occupied by progressing and regressing clonotypes are quantified with dot plots with a black line indicating the mean. Differences were evaluated using two-way ANOVA for Progression group (Progressor vs. Regressor) and for Cohort. Variations by Progression group were statistically significant as indicated on the right. Sidak's multiple comparison tests were conducted to compare samples within each cohort, with significance indicated above. (*p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001)

C

Tsc22d3 Expression

Gzmf Expression

Hspa1a Expression

Hist1h2ap Expression

D

Ly6a Expression

Ccl5 Expression

Mt1 Expression

Ly6c2 Expression
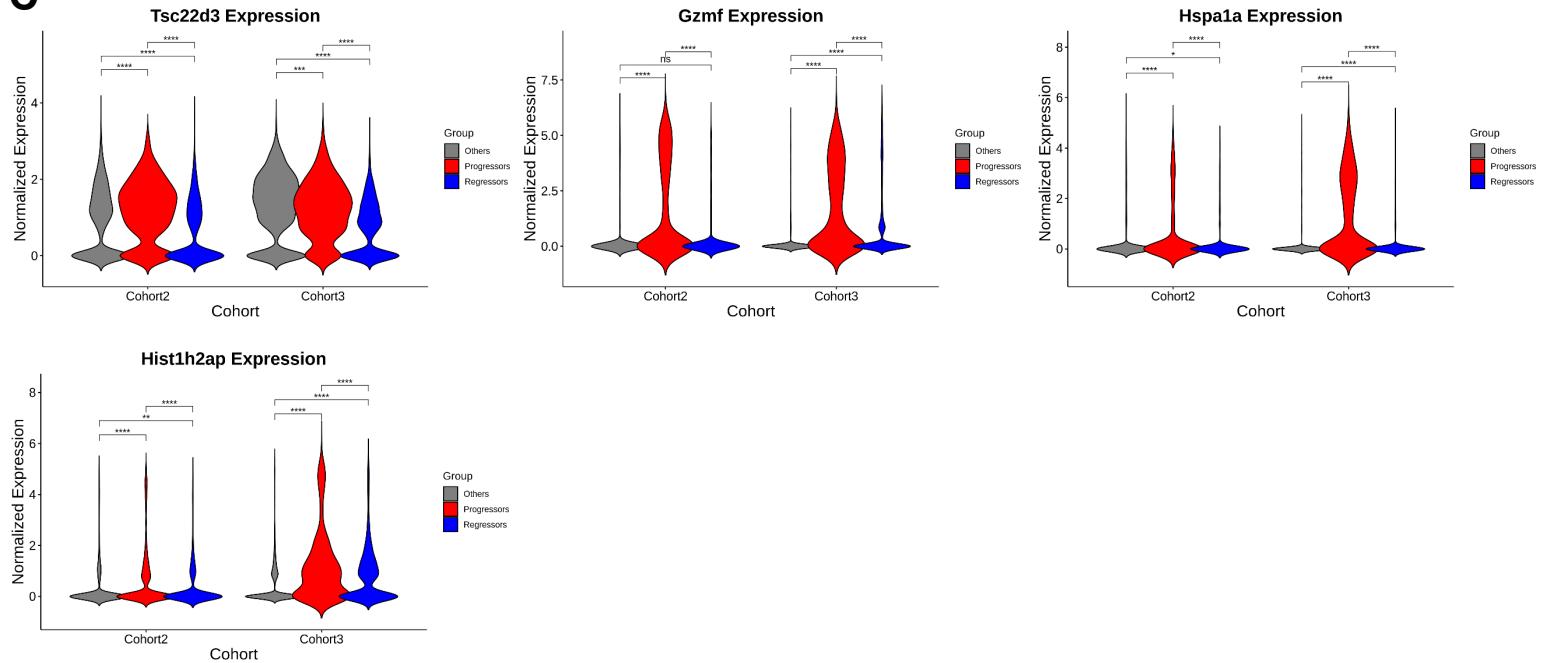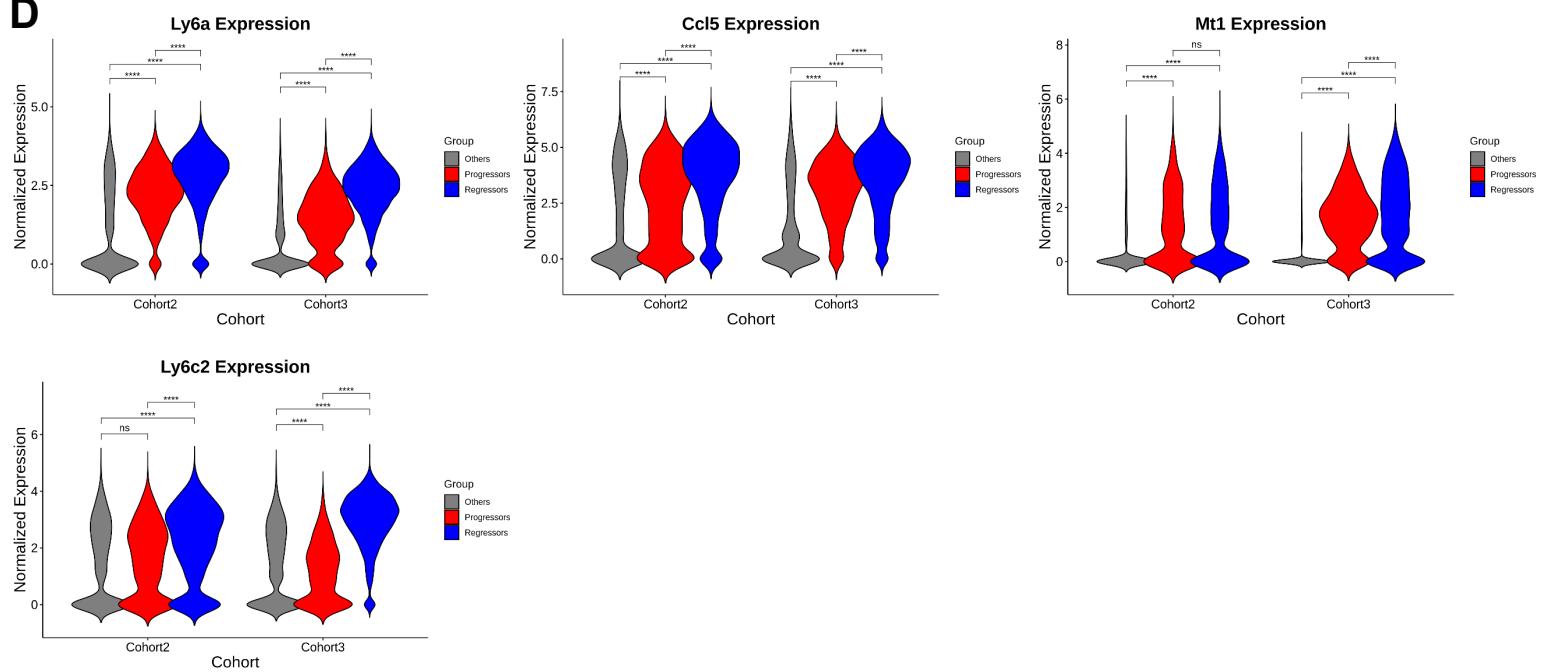
Supplemental Figure 8C-D: Reanalysis by Cohort. Data shown in Figures 7D and 7E are alternatively shown by separating cells of top clonotypes into their two respective sequencing cohorts (Cohort 2 = Grow2 and Regr2; Cohort 3 = Grow3 and Regr3). The normalized gene expression for all cells in each category is shown. Differences were evaluated using two-way ANOVA for Progression group (Progressor vs. Regressor) and for Cohort. **(C)** Violin plots of genes more highly expressed in Progressor top TCR clonotypes from Figure 7D. **(D)** Violin plots of genes more highly expressed in Regressor top TCR clonotypes from Figure 7E. Variation by Activation Group, Cohort, and Interaction were all statistically significant (p<0.0001). Tukey's multiple comparison of means tests were conducted between each Activation group and were all statistically significant (adjusted p-value = 0). (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Supplemental Table 1. 18 samples sequenced by Single-cell TCR and Single-cell RNA sequencing.

Sample	Abbrev.	Mouse	Tissue	Sorted cells	Chemistry	# Cells
Regressing TIL #1 (3')	Regr1-TIL	Regressor #1	Tumor	Live	3'	1655
Progressing TIL #1 (3')	Grow1-TIL	Progressor #1	Tumor	Live	3'	1790
Regressing TIL #1 (5')	Regr1-5p-TIL	Regressor #1	Tumor	CD8+, Live	5'	44389
Regressing TIL #1 (VDJ)	RT1	Regressor #1	Tumor	CD8+, Live	VDJ	22045
Regressing TIL #2 (5')	Regr2-TIL	Regressor #2	Tumor	CD8+, Live	5'	7832
Regressing TIL #2 (VDJ)	RT2	Regressor #2	Tumor	CD8+, Live	VDJ	5634
Progressing TIL #2 (5')	Grow2-TIL	Progressor #2	Tumor	CD8+, Live	5'	4262
Progressing TIL #2 (VDJ)	GT2	Progressor #2	Tumor	CD8+, Live	VDJ	2599
Regressing Spleen #2 (5')	Regr2-Spln	Regressor #2	Spleen	CD8+, Live	5'	6598
Regressing Spleen #2 (VDJ)	RS2	Regressor #2	Spleen	CD8+, Live	VDJ	5268
Regressing TIL #3 (5')	Regr3-TIL	Regressor #3	Tumor	CD8+, Live	5'	7276
Regressing TIL #3 (VDJ)	RT3	Regressor #3	Tumor	CD8+, Live	VDJ	5940
Progressing TIL #3 (5')	Grow3-TIL	Progressor #3	Tumor	CD8+, Live	5'	1727
Progressing TIL #3 (VDJ)	GT3	Progressor #3	Tumor	CD8+, Live	VDJ	1548
Regressing Spleen #3 (5')	Regr3-Spln	Regressor #3	Spleen	CD8+, Live	5'	6020
Regressing Spleen #3 (VDJ)	RS3	Regressor #3	Spleen	CD8+, Live	VDJ	5096
Progressing Spleen #3 (5')	Grow3-Spln	Progressor #3	Spleen	CD8+, Live	5'	3939
Progressing Spleen #3 (VDJ)	GS3	Progressor #3	Spleen	CD8+, Live	VDJ	3847

Supplemental Table 1: 18 Samples Sequenced by Single-Cell-RNA Sequencing. 3 progressing tumors and 3 regressing tumors were removed from 6 mice, tumors were digested, and cells were flow-sorted. The first cohort of mouse tumors (Regressor #1 and Progressor #1) were both sorted for Live, small-FSC cells to capture bulk tumor-infiltrating cells and each was subjected to 3' library prep, and then separately, Regressor #1 was also sorted for Live CD8+ cells and subjected to 5' library prep for RNA expression and TCR VDJ. The second cohort of mouse tumors (Regressor #2 and Progressor #2) were both sorted for Live CD8+ cells and subjected to 5' library prep for RNA expression and TCR VDJ. The spleen from Regressor #2 was also sorted for Live CD8+ cells and subjected to 5' library prep for RNA expression and TCR VDJ. The third cohort of mouse tumors (Regressor #3 and Progressor #3) were both sorted for Live CD8+ cells and subjected to 5' library prep for RNA expression and TCR VDJ. The spleens from Regressor #3 and Progressor #3 were also sorted for Live CD8+ cells and subjected to 5' library prep for RNA expression and TCR VDJ. To fit with the subsequent cohorts' samples of CD8 T cells, the 3' RNA-sequenced samples (Regr1-TIL, Grow1-TIL) were filtered during analysis to include only CD8 T cell clusters. The 5'/TCR sample from Regressor #1 was overloaded with cells (~50,000) so while ~22,000 cells had complete TCR VDJ regions sequenced, the RNA expression data was not deep enough and deemed not useful for analysis. # Cells in the last column represents the number of cells used after filtering for CD8 T cells with appropriate RNA expression (see details in Methods).

Supplemental Table 2: Detailed clonotype information for top 10 TCR clones in each sample.

Sample	TCRα CDR3	TCRαV	TCRαJ	TCRβ CDR3	TCRβV	TCRβD	TCRβJ	TCRα CDR3 #2	TCRαV #2	TCRαJ #2	% of Sample
Regr1-TIL	CAASEHASSGSWQLIF	TRAV7-4	TRAJ22	CASSLEGTGGYEQYF	TRBV16	TRBD2	TRBJ2-7	NA	NA	NA	5.9
	CAASEGASSGSWQLIF	TRAV14N-1	TRAJ22	CASSLEGTGGYEQYF	TRBV16	TRBD2	TRBJ2-7	NA	NA	NA	4.4
	CAASSSNYNVLYF	TRAV10	TRAJ21	CASSLEGTGGYEQYF	TRBV16	TRBD2	TRBJ2-7	NA	NA	NA	4.1
	CAASSSNYNVLYF	TRAV10	TRAJ21	CASSVTGGSYEQYF	TRBV13-3	TRBD2	TRBJ2-7	NA	NA	NA	4
	CAASEGASSGSWQLIF	TRAV14N-1	TRAJ22	CASSLEGTGGYEQYF	TRBV16	TRBD2	TRBJ2-7	NA	NA	NA	3.7
	CAASSSNYNVLYF	TRAV10	TRAJ21	CASSLEGTGGYEQYF	TRBV16	TRBD2	TRBJ2-7	NA	NA	NA	2.6
	CAASEHASSGSWQLIF	TRAV7-4	TRAJ22	CASSLEGTGGYEQYF	TRBV16	TRBD2	TRBJ2-7	NA	NA	NA	2.4
	CAASMVASSGSWQLIF	TRAV10N	TRAJ22	CASSLEGTGGYEQYF	TRBV16	TRBD2	TRBJ2-7	NA	NA	NA	2.2
	CALSDPGYQNFYF	TRAV12-2	TRAJ49	CASSLEGTGGYEQYF	TRBV16	TRBD2	TRBJ2-7	NA	NA	NA	2
	CAASEHASSGSWQLIF	TRAV7-4	TRAJ22	CASSVTGGSYEQYF	TRBV13-3	TRBD2	TRBJ2-7	NA	NA	NA	1.8
Regr2-TIL	CAMREGHGTGYQNFYF	TRAV16	TRAJ49	CASSLERTGGYEQYF	TRBV3	TRBD2	TRBJ2-7	NA	NA	NA	13.3
	CAAGGTNTGKLTF	TRAV14N-1	TRAJ27	CASGDRQNTLYF	TRBV13-2	None	TRBJ2-4	NA	NA	NA	6.3
	CAVSMNNYAQGLTF	TRAV7D-5	TRAJ26	CASSDGGSTGQLYF	TRBV13-1	TRBD1	TRBJ2-2	NA	NA	NA	5.3
	CAASSYASSGSWQLIF	TRAV10D	TRAJ22	CASSLEGTGGYEQYF	TRBV16	TRBD2	TRBJ2-7	NA	NA	NA	4.3
	CALSGSMGYKLTF	TRAV6N-6	TRAJ9	CASSDRGPNNQAPLF	TRBV13-3	TRBD1	TRBJ1-5	NA	NA	NA	2.4
	CAASNMGYKLTF	TRAV14D-3-DV8	TRAJ9	CASSPDWNYAEQFF	TRBV4	TRBD2	TRBJ2-1	NA	NA	NA	2.2
	CAASSSSGSWQLIF	TRAV14D-3-DV8	TRAJ22	CASRLGGYEQYF	TRBV19	TRBD2	TRBJ2-7	NA	NA	NA	2.1
	CAVMYNQGKLIF	TRAV9N-3	TRAJ23	CASSFWGAETLYF	TRBV15	TRBD2	TRBJ2-3	NA	NA	NA	2.1
	CAASEHASSGSWQLIF	TRAV7-4	TRAJ22	CASSLEGTGGYEQYF	TRBV16	TRBD1	TRBJ2-7	NA	NA	NA	2.1
	CALSRSNNNNAPRF	TRAV6N-6	TRAJ43	CASSDPGNYAEQFF	TRBV13-3	None	TRBJ2-1	NA	NA	NA	2
Regr3-TIL	CAASEPPSGSWQLIF	TRAV7D-4	TRAJ22	CASSLEAVSSYEQYF	TRBV16	None	TRBJ2-7	NA	NA	NA	42.1
	CAAGALGTGSKLSF	TRAV19	TRAJ58	CASSQDWNEQYF	TRBV5	None	TRBJ2-7	NA	NA	NA	4.8
	CALSEDYSNNRLTL	TRAV12D-2	TRAJ7	CGAGLVQNTLYF	TRBV20	None	TRBJ2-4	NA	NA	NA	3.4
	CALGTNSAGNKLTF	TRAV13-1	TRAJ17	CASSDAEVFF	TRBV13-1	None	TRBJ1-1	NA	NA	NA	3.2
	CAASEHASSGSWQLIF	TRAV7D-4	TRAJ22	CASSLEAVSSYEQYF	TRBV16	None	TRBJ2-7	CAASEPPSGSWQLIF	TRAV7D-4	TRAJ22	2.8
	CALSPSASSGSWQLIF	TRAV12D-2	TRAJ22	CASSLEGTGGYEQYF	TRBV16	None	TRBJ2-7	NA	NA	NA	2.8
	CAASDGNRIFF	TRAV5-4	TRAJ31	CASSRGRDTEVFF	TRBV19	None	TRBJ1-1	NA	NA	NA	2.2
	CASSNMGYKLTF	TRAV13N-1	TRAJ9	CASSSTAANTEVFF	TRBV4	None	TRBJ1-1	NA	NA	NA	2
	CALGSNMGYKLTF	TRAV6-6	TRAJ9	CASSGQGNYAEQFF	TRBV13-3	None	TRBJ2-1	NA	NA	NA	1.9
	CAASGTSGSWQLIF	TRAV14N-3	TRAJ22	CASGDAQGEQYF	TRBV12-2	None	TRBJ2-7	NA	NA	NA	1.8
Grow2-TIL	CALGSNMGYKLTF	TRAV6-6	TRAJ9	CASSDQGNYAEQFF	TRBV13-3	TRBD1	TRBJ2-1	NA	NA	NA	15.4
	CALGSNMGYKLTF	TRAV6-6	TRAJ9	CASSGQGNYAEQFF	TRBV13-3	TRBD1	TRBJ2-1	CAVTPGGYKVVF	TRAV7D-5	TRAJ12	7.2
	CAASVSSGSWQLIF	TRAV14D-3-DV8	TRAJ22	CASRLGGYEQYF	TRBV19	TRBD2	TRBJ2-7	CALADTNAYKVIF	TRAV6-6	TRAJ30	5.9
	CAVSTDYSNNRLTL	TRAV9N-3	TRAJ7	CASGDEGGRFEQYF	TRBV13-2	TRBD2	TRBJ2-7	NA	NA	NA	5.0
	CAVSAHNNNAPRF	TRAV3D-3	TRAJ43	CASSDGGGQDTQYF	TRBV13-3	TRBD2	TRBJ2-5	CAVSTHSGGSNAKLTF	TRAV9-4	TRAJ42	4.8
	CVLTLDTNAYKVIF	TRAV9-2	TRAJ30	CASSHLGGQNTLYF	TRBV12-1	TRBD2	TRBJ2-4	NA	NA	NA	3.9
	CAASEHASSGSWQLIF	TRAV7D-4	TRAJ22	CASSLEGTGGYEQYF	TRBV16	TRBD1	TRBJ2-7	NA	NA	NA	2.5
	CAPPNQGGRALIF	TRAV13D-2	TRAJ15	CASSRDKDTQYF	TRBV14	None	TRBJ2-5	NA	NA	NA	1.8
	CATDDQGGRALIF	TRAV8D-2	TRAJ15	CASSLSGQGGEQYF	TRBV26	TRBD1	TRBJ2-7	NA	NA	NA	1.6
	CAASGTGANTGKLTF	TRAV14-3	TRAJ52	CASSPGTGGYEQYF	TRBV12-1	TRBD1	TRBJ2-7	NA	NA	NA	1.5
Grow3-TIL	CAASASSGSWQLIF	TRAV14N-3	TRAJ22	CASGDQGGQNTLYF	TRBV12-2	None	TRBJ2-4	NA	NA	NA	18.7
	CAMERANTNKVVF	TRAV13-4-DV7	TRAJ34	CASSLTGGQDTQYF	TRBV13-3	None	TRBJ2-5	NA	NA	NA	11.1
	CALNSNTNKVVF	TRAV12-2	TRAJ34	CASSDDEQYF	TRBV13-1	None	TRBJ2-7	NA	NA	NA	7.6
	CATDNSGTYQRF	TRAV8N-2	TRAJ13	CASSLVTKNSDYTF	TRBV3	None	TRBJ1-2	NA	NA	NA	5.9
	CATDEQGGRALIF	TRAV8N-2	TRAJ15	CASSHTGTGGYEQYF	TRBV26	None	TRBJ2-7	NA	NA	NA	4.2
	CALGEVMHNVLYF	TRAV6-6	TRAJ21	CASSLGSYEQYF	TRBV14	None	TRBJ2-7	NA	NA	NA	4.2
	CAASELASSGSWQLIF	TRAV7-4	TRAJ22	CASSLEGTGGYEQYF	TRBV16	None	TRBJ2-7	NA	NA	NA	3.6
	CALNRNNYAQGLTF	TRAV6N-6	TRAJ26	CTCSANLVNYAEQFF	TRBV1	None	TRBJ2-1	NA	NA	NA	3
	CATDAQGGRALIF	TRAV8D-2	TRAJ15	CASGGGTGRANSDYTF	TRBV12-2	None	TRBJ1-2	NA	NA	NA	2.8
	CAARDDSGYNKLTF	TRAV5N-4	TRAJ11	CASSPTGGARDTQYF	TRBV13-3	None	TRBJ2-5	CALGDLNYNVLYF	TRAV6N-7	TRAJ21	1.9
Regr2-Spl	CAMREGHGTGYQNFYF	TRAV16	TRAJ49	CASSLERTGGYEQYF	TRBV3	TRBD2	TRBJ2-7	NA	NA	NA	2.12
	CAAGGTNTGKLTF	TRAV14N-1	TRAJ27	CASGDRQNTLYF	TRBV13-2	None	TRBJ2-4	NA	NA	NA	1.32
	CAASSYASSGSWQLIF	TRAV10D	TRAJ22	CASSLEGTGGYEQYF	TRBV16	TRBD2	TRBJ2-7	NA	NA	NA	0.71
	CAVMYNQGKLIF	TRAV9N-3	TRAJ23	CASSFWGAETLYF	TRBV15	TRBD2	TRBJ2-3	NA	NA	NA	0.48
	CARGGANTGKLTF	TRAV14N-3	TRAJ52	CASSETGGQDTQYF	TRBV13-3	TRBD2	TRBJ2-5	NA	NA	NA	0.46
	CAASDPASSGSWQLIF	TRAV7-4	TRAJ22	CASSLEGTGGYEQYF	TRBV16	TRBD2	TRBJ2-7	NA	NA	NA	0.32
	CALSGSMGYKLTF	TRAV6N-6	TRAJ9	CASSDRGPNNQAPLF	TRBV13-3	TRBD1	TRBJ1-5	NA	NA	NA	0.27
	CAVSMNNYAQGLTF	TRAV7D-5	TRAJ26	CASSDGGSTGQLYF	TRBV13-1	TRBD1	TRBJ2-2	NA	NA	NA	0.21
	CAAASSGSWQLIF	TRAV7D-2	TRAJ22	CASNSGGTEVFF	TRBV14	TRBD1	TRBJ1-1	NA	NA	NA	0.16
	CAASASSGSWQLIF	TRAV14N-3	TRAJ22	CASGDQGGQNTLYF	TRBV13-2	TRBD1	TRBJ2-4	NA	NA	NA	0.16
Regr3-Spl	CALSPSASSGSWQLIF	TRAV12D-2	TRAJ22	CASSLEGTGGYEQYF	TRBV16	None	TRBJ2-7	NA	NA	NA	4.71
	CAASEPPSGSWQLIF	TRAV7D-4	TRAJ22	CASSLEAVSSYEQYF	TRBV16	None	TRBJ2-7	NA	NA	NA	2.32
	CAASDPNSGGSNAKLTF	TRAV7-4	TRAJ42	CASSGTGYSYEQYF	TRBV13-1	None	TRBJ2-7	NA	NA	NA	0.44
	CAASNNNYAQGLTF	TRAV7D-2	TRAJ26	CASSLDWGGYEQYF	TRBV16	None	TRBJ2-7	NA	NA	NA	0.37
	CALGMSNYNVLYF	TRAV6N-7	TRAJ21	CASSEGGGSYAEQFF	TRBV13-3	None	TRBJ2-1	NA	NA	NA	0.29
	CAAGHTGNYKYVF	TRAV19	TRAJ40	CASSDRGEVQDTQYF	TRBV13-3	None	TRBJ2-5	NA	NA	NA	0.23
	CAASEHASSGSWQLIF	TRAV7-4	TRAJ22	CASSLEGTGGYEQYF	TRBV16	None	TRBJ2-7	CIVTDIGQTGFASALTF	TRAV2	TRAJ35	0.21
	CALGSNMGYKLTF	TRAV6-6	TRAJ9	CASSGQGNYAEQFF	TRBV13-3	None	TRBJ2-1	NA	NA	NA	0.19
	CAASENNYAQGLTF	TRAV7D-2	TRAJ26	CASSFDWGGYEQYF	TRBV16	None	TRBJ2-7	NA	NA	NA	0.15
	CASSNMGYKLTF	TRAV13N-1	TRAJ9	CASSSTAANTEVFF	TRBV4	None	TRBJ1-1	NA	NA	NA	0.15
Grow3-Spl	CAMERANTNKVVF	TRAV13-4-DV7	TRAJ34	CASSLTGGQDTQYF	TRBV13-3	None	TRBJ2-5	NA	NA	NA	0.12
	CAASEHGNYNQGKLIF	TRAV7-4	TRAJ23	CASSQGRGLGYEQYF	TRBV5	None	TRBJ2-7	NA	NA	NA	0.09
	CAASAGAASLGKLQF	TRAV14-1	TRAJ24	CASSRPRDEQYF	TRBV17	None	TRBJ2-7	NA	NA	NA	0.06
	CAASATNTGKLTF	TRAV14N-3	TRAJ27	CASSINWGGADEQYF	TRBV19	None	TRBJ2-7	NA	NA	NA	0.06
	CAASRDSGYNKLTF	TRAV7D-2	TRAJ11	CASSQVGLGSNTGQLYF	TRBV5	None	TRBJ2-2	NA	NA	NA	0.06
	CALKSNMGYKLTF	TRAV6N-6	TRAJ9	CASSDARYEQYF	TRBV13-3	None	TRBJ2-7	NA	NA	NA	0.06
	CALPSSFSKLVF	TRAV9N-4	TRAJ50	CASSDEGGANSDYTF	TRBV13-1	None	TRBJ1-2	NA	NA	NA	0.06
	CAMREVNYGNEKITF	TRAV16D-DV11	TRAJ48	CASRLGGRYAEQFF	TRBV29	None	TRBJ2-1	NA	NA	NA	0.06
	CATGSSNTNKVVF	TRAV8N-2	TRAJ34	CASSQDPGQLNSDYTF	TRBV5	None	TRBJ1-2	NA	NA	NA	0.06
	CAVRENYGSSGNKLIF	TRAV3-4	TRAJ32	CASSLDRGEVFF	TRBV16	None	TRBJ1-1	NA	NA	NA	0.06

Supplemental Table 2. Detailed clonotype information for top 10 TCR clones in each sample. CD8 T cells from each sample were grouped into clones by identical nucleotide sequence of the CDR3 regions of TCRα and TCRβ chains. The top 10 TCR clones by abundance in each sample are shown, with their corresponding CDR3 AA sequences, V, D, J gene usage, and percent (% = the number of cells in each clone / the number of total cells sequenced for a given sample). Occasionally, some clones contain two TCRα chains, which is plausible since allelic exclusion does not operate efficiently for the TCRα chain.

Supplemental Table 3: Shared TCR clonotypes in different TIL samples.

Clonotype (TCRα_TCRβ CDR3)	Shared Clonotype #	n.Regr1	n.Grow2	n.Grow3	n.Regr2	n.Regr3	Samples	#Samples
CAASEHASSGSWQLIF_CASSLEGTGGYEQYF	Shared Clonotype 1	2046	81	0	108	7	RT1-RT2-RT3-GT2	4
CALSDHTGANTGKLTF_CASSLDWGQDTQYF			2	1	6	66	RT2-RT3-GT2-GT3	4
CAVSMSNMGYKLTF_CASSLGLGGAETLYF			7	13	33	0	RT2-GT2-GT3	3
CAASEPPSGSWQLIF_CASSLEAVSSYEQYF			0	1	0	2137	RT3-GT3	2
CAASASSGSWQLIF_CASGDQGGQNTLYF	Shared Clonotype 2		0	249	100	0	RT2-GT3	2
CAVSMNNYAQGLTF_CASSDGGSTGQLYF			1	0	225	0	RT2-GT2	2
CALGSNMGYKLTF_CASSGQGNYAEQFF	Shared Clonotype 3		53	0	0	102	RT3-GT2	2
CAASGTGANTGKLTF_CASSPGTGGYEQYF			69	8	0	0	GT2-GT3	2
CALGEDSGTYQRF_CASKQNQDTQYF			1	0	52	0	RT2-GT2	2
CAIAFNSAGNKLTF_CASSDAEQFF			1	0	8	0	RT2-GT2	2
CVLGDHTGANTGKLTF_CASSRDWGQDTQYF			0	1	8	0	RT2-GT3	2
CAVSMSNMGYKLTF_CASSPGLGGAETLYF			0	0	2	2	RT2-RT3	2
CAASASSGSWQLIF_CASSRDWGGGQNTLYF			1	0	0	1	RT3-GT2	2

Supplemental Table 3: Shared TCR clonotypes in different TIL samples. TIL Samples with TCR VDJ information in the UMAP (Regr2-TIL, Grow2-TIL, Regr3-TIL, Grow3-TIL) were evaluated for any clonotypes that are shared between samples. The 13 shared clonotypes are shown. For each TCR clonotype, values are shown for the number of cells in each of the 4 TIL samples. 3 of the shared clonotypes were chosen based on the number of samples (Shared Clonotype 1 = 3 samples on the UMAP; 4 samples if including RT1; Shared Clonotype 2 = 2 samples; Shared Clonotype 3 = 2 samples) and on whether there were at least 50 cells of that clonotype in more than one sample. As noted in Supplemental Table 1, the The 5'/TCR sample from Regressor #1 was overloaded with cells (~50,000) so while ~22,000 cells had complete TCR VDJ regions sequenced, the RNA expression data was not deep enough and deemed not useful for analysis.

Supplemental Table 4: TCRβ CDR3 sequences analyzed by GLIPH cluster into top 10 Groups.

Group	Consensus	Sequence	GT2	GT3	RT1	RT2	RT3
1	CASSL(E)GTGGYEQYF	CASSLEPVYAEQFF	0	0	0	0.258	0
		CASSLELGGLEQYF	1.898	0	0	0.117	0
		CASSLEPGSYEQYF	0	0.067	0	0	0
		CASSLERTGGYEQYF	0	0	0	14.678	0
		CASSTGTGGYEQYF	0	0	0.013	0	0
		CASSPGTGGFEQYF	0	0	0.004	0	0
		CASSLGTGGPEVFF	0	0	0.022	0	0
		CASSPGTGVYEQYF	0	0	0	0.047	0
		CASSLGTGGYEQYF	0	0	0.004	0	0
		CASSLEGSGGYEQYF	0	0	0.004	0	0
		CASSLEGTGSYEQYF	0	0	0	0.211	0
		CASSLGTGGWEQYF	0	0	0.009	0	0
		CASSLEPVFAEQFF	0.276	0	0	0	0
		CASSLEPGDSYEQYF	0	0	0.027	0	0
		CASSLEPGENTLYF	0	0	0	0.023	0
		CASSLGLGGHEQYF	0	0	0.009	0	0
		CASSAGTGGYEQYF	0	0.202	0.004	0	0
		CASSMGTGGYEQYF	0	0	0.009	0	0
		CASSLEGTGGYERYF	0	0	0.004	0	0
		CASSPGTGGFTQYF	0	0	0	0	0.018
		CASSLGLGGYEQYF	0	0	0.049	0	0
		CASSLEPGSSYEQYF	0	0	9.411	0	0
		CASSLGTGVYEQYF	0	0	0.013	0	0
		CASSPGTGGYEQYF	2.358	0.539	0	0	0
		CASSLEGTGDYEQYF	0	1.618	0	0	0
		CASSFGTGGYEQYF	0	0	0	0.047	0
		CASSLEPGGAYEQYF	0	0	0.031	0	0
		CASSLEPGGSQNTLYF	0	0	0.022	0	0
		CASSLELGGYEQYF	0	0	0.218	0	0
		CASSSGTGGYEQYF	0	0	0.356	0	0
		CASSLEPLYEQYF	0	0	0	0.023	0
		CASSLEPVSNERLFF	0	0.607	0	0	0
		CASSLEGTGGYEQYF	3.582	3.709	48.788	10.645	6.092
		CASSLEPTGGYEQYF	0	0	0	1.290	0
		CASSHGTGGYEQYF	0	0	0.018	0	0
		CASSLEGTGGHEQYF	0	0	0.004	0	0
		CASSLEGTGEYEQYF	0	0	0.004	0	0
		CASSLELGGREQYF	0.765	0	0	0.047	0
		CASSLGTGVSEQYF	0	0	0.018	0	0
2	CASSLEAVSSYEQYF	CASSLEAVSSYEQYF	0	0.067	0	0	49.429
		CAISLEAVSSYEQYF	0	0	0	0	0.018
3	CASSDQGNYAEQFF	CASSGQGNYAEQFF	10.165	0	0	0	1.904
		CASSDQGNYAEQFF	18.647	0	0	0	0
		CASSDPGNYAEQFF	0	0	0	2.462	0
4	CASGDQGCQNTLYF	CASGDQGGQNTLYF	0.031	19.150	0	3.259	0
		CASGDQGCQNTLYF	0.031	0	0	0	0
5	CASSLTGGQNTLYF	CASSLWGGQDTQYF	0	0	0.013	0	0
		CASSPTGVGNTLYF	0	0	0.018	0	0
		CASSGTGGQNTLYF	0	0	0	0	0.163
		CASSLAGDGNTLYF	0	0	0.022	0	0
		CASSDGLGQDTQYF	0	0	0	0.047	0
		CASSLAGDQNTLYF	0.031	0	0	0	0
		CASSGTGGGNTLYF	0	0.067	0	0	0
		CASSLAGGGNTLYF	0	0	0.004	0	0
		CTSSPTGVGNTLYF	0	0	0.004	0	0
		CASSLTGGVDTQYF	0	0	0.004	0	0
		CASSATGGQDTQYF	0	0.539	0	0	0
		CAWSLTGGGNTLYF	0	0	0.004	0	0
		CASSTLGGQDTQYF	0	0	0.018	0	0
		CASSEGGGQDTQYF	0	0	0.004	0	0
		CASSLTGGQDTQYF	0	11.126	0	0	0
		CASSGTGGQDTQYF	0	0.135	0	0	0.073
		CASSALGGQDTQYF	0.031	0	0	0	0
		CASSETGGQDTQYF	0	0	0.076	1.383	0
		CASSLTGVGNTLYF	0	0	0.013	0	0
		CASSRTGGGNTLYF	0.031	0	0	0	0
		CASSDGGGQDTQYF	5.879	0	0	0	0
6	CASSVTGGSYEQYF	CASSRTGGAYEQYF	0	0	0	0	0.018
		CANSVTGGSYEQYF	0	0	0.004	0	0
		CASSCTGGAYEQYF	0	0	0	0	0.018
		CASSRTGGSYEQYF	0	0	0	0.023	0
		CASSRSGGSYEQYF	0	0	0	0	0.018
		CASNVTGGSYEQYF	0	0	0.004	0	0
		CASSVTGGSYEQYF	0	0	12.920	0	0
7	CASSDGEQFF	CASSTGEQYF	0.031	0	0	0	0
		CASSLGEVFF	0	0	0	0.023	0
		CASSDGRQFF	0	0	0	0.023	0
		CASSLEEVFF	0	0	0.018	0	0
		CASSDAEVFF	0	0	0	0	3.246
		CASSDAEQFF	0.092	0	0	0.211	0
		CASSLHEQYF	0	0	0.044	0	0
		CASSDGEQYF	0	0	0.009	0	0
		CASSDDEQYF	0	7.620	0	0	0
		CASSDGEVFF	0	0	0	0	0.018
8	CGAGTGGYEQYF	CGARTGGYEQYF	0	0.607	0	0	0
		CATGTGGYEQYF	0	0	0	0.023	0
		CGAKTGGYEQYF	0	0	0.040	0	0
		CSVGTGSYEQYF	0	0	0.018	0	0
		CASRLGGYEQYF	7.716	0	0.004	2.720	0
9	CASGDVQNTLYF	CASGDVQNTLYF	0.276	0	0	0	0
		CASGDRQNTLYF	0	0	0	6.987	0
		CGAGLVQNTLYF	0	0	0	0	3.772
10	CASSLGGYEQYF	CAWSLPNTEVFF	0.153	0	0	0	0
		CASSDLGTEQFF	0	0.067	0	0	0
		CASSDAHTEVFF	0	0.337	0	0	0
		CASSDANTEVFF	0	0	0.018	0	0
		CASSDGGTEVFF	0.153	0	0	0	0
		CASSDRNTEVFF	0	0	0.004	0	0
		CASSVTGYEQYF	0	0	0.004	0	0
		CASSNRGREQYF	0.031	0	0	0	0
		CASSSGYTEVFF	0	0	0	0	0.018
		CASSLGPYEQYF	0	0	0.009	0	0
		CASSPGQYEQYF	0	0	0.036	0	0
		CASSLGSYEQYF	0	4.181	0	0	0
		CASSLRVYEQYF	0	0	0	0	0.036
		CASSVRGYEQYF	0	0	0	0.023	0
		CASSDGGGEVFF	0.245	0	0	0.258	0
		CAWSPGHYEQYF	0	0	0	0	0.018
		CASSLGNTEVFF	0.031	0	0	0	0
		CASSLPGVEQYF	0	0.067	0	0	0
		CASSLVLYEQYF	0.031	0	0	0	0
		CASSPRGYEQYF	0	0	0.022	0	0
		CASSFGGREQYF	0	0	0	0.023	0
		CASSPGHYEQYF	0	0	0	0.023	0
		CASSLKGREVFF	0	0	0.018	0	0
		CASSLPGTEVFF	0	0	0	0.023	0
		CASSSGQTEVFF	0	0	0.018	0	0
		CASSLGGREQYF	0	0	0.058	0	0
		CASSQGGYEQYF	0	0	0.009	0	0
		CASSLGNYEQYF	0	0	0.004	0	0
		CASSVRGREQYF	0.031	0	0	0	0
		CASSLRPYEQYF	0	0	0.018	0	0
		CASSLGYTEVFF	0	0	0.018	0	0
		CASSLTSYEQYF	0	0	0.004	0	0
		CASSHRGREQYF	0	0	0	0	0.036
		CASSLVSYEQYF	0	0	0.004	0	0
		CASSDGNTEVFF	0.092	0	0	0	0
		CASSPGNTEVFF	0	0	0.013	0	0
		CASSLQGTEVFF	0	0	0.009	0	0
		CASSHGGYEQYF	0	0.270	0	0	0
		CASSIRNTEVFF	0	0	0.004	0	0
		CASSLVPYEQYF	0.031	0	0	0	0
		CASSSGNTEVFF	0	0	0.009	0	0
		CASSIPGTEVFF	0	0	0	0	0.018
		CASSFRGREQYF	0	0	0.018	0	0.163
		CASSPGGYEQYF	0.061	0	0	0.023	0
		CASSDVGGEQYF	0.031	0	0	0	0
		CASSIGGREQYF	0	0	0.013	0	0
		CASSLGQYEQYF	0	0	0.067	0	0
		CASSDRGREQYF	0	0	0.004	0	0
		CASSLGRYEQYF	0	0	0.187	0	0

Supplemental Table 4: TCRβ CDR3 sequences analyzed by GLIPH clustering into top 10 groups. All TCRβ CDR3 sequences from 5 TIL samples (GT2 = Progressing TIL #2; GT3 = Progressing TIL #3; RT1 = Regressing TIL #1; RT2 = Regressing TIL #2; RT3 = Regressing TIL #3) were analyzed using the GLIPH algorithm. Specificity groups were ordered based on the sum of the abundance of each sequence in the specificity group in each of the 5 samples, to capture the most expanded clonotypes in the dataset and their related sequences. The 10 specificity groups with the highest sum of sequence abundances are shown. CDR3 sequences within these top 10 GLIPH groups are shown with their percent in each TIL sample (% = the number of a unique CDR3 sequence / the number of total CDR3 sequences in any given sample).

Supplemental Table 5: Differential gene expression between progressing TILs and naïve T cells or between regressing TILs and naïve T cells

Gene	Progressing Fold Change	Progressing Adjusted P-value	Regressing Fold Change	Regressing Adjusted P-value
Ccl4	8.70	2.52E-02	12.42	1.86E-05
Gzmf	15.63	3.34E-05	1.00	NA
Ifitm1	8.60	7.99E-06	8.03	9.48E-01
Gzmb	7.46	3.92E-07	8.24	1.05E-04
Lag3	6.42	7.19E-13	6.35	4.61E-07
Pdcd1	6.54	6.22E-11	6.06	6.64E-06
Klrc1	4.94	4.66E-08	5.96	2.39E-09
Tnfrsf9	5.76	2.54E-13	5.02	2.49E-04
Rgs16	5.31	8.10E-05	4.76	1.76E-02
Ccl5	3.53	1.00E-01	6.50	4.74E-09
S100a4	5.25	5.10E-11	3.99	6.70E-05
S100a6	8.11	7.10E-09	1.00	NA
Bhlhe40	4.46	2.96E-09	4.22	7.12E-05
Lgals3	4.06	2.85E-02	4.33	3.18E-07
Ccl3	3.89	1.44E-03	4.39	3.39E-03
Lgals1	4.44	5.42E-16	3.77	7.96E-06
Anxa2	4.40	2.17E-09	3.74	1.80E-01
Cxcr6	3.57	3.14E-08	4.52	4.47E-09
Ifng	3.89	1.94E-01	3.82	2.16E-01
Mt1	3.38	2.84E-02	4.32	1.79E-01
Bcl2a1d	3.53	4.62E-13	3.70	2.72E-06
Bcl2a1b	3.52	6.54E-07	3.66	1.31E-03
Klrk1	3.49	4.05E-04	3.52	4.15E-04
AW112010	2.97	8.86E-06	3.98	2.14E-05
Icos	2.96	2.99E-01	3.91	3.75E-02
Litaf	3.37	4.95E-05	3.36	3.69E-04
Sh2d2a	3.46	6.26E-04	3.22	6.08E-02
Serpinb9	3.48	2.68E-08	3.10	4.71E-05
Ctla4	3.17	3.27E-03	3.40	5.70E-03
Serpinb6b	3.71	2.31E-05	2.71	1.29E-02
Capg	3.68	9.92E-06	2.66	1.20E-01
Nkg7	2.72	1.19E-07	3.22	1.72E-07
Prf1	2.76	8.73E-05	3.08	7.90E-04
Klrd1	2.81	2.38E-03	3.01	3.84E-04
Id2	2.76	4.88E-02	2.93	3.48E-03
Nr4a2	2.53	1.79E-02	3.10	3.72E-04
Il2rb	2.89	1.41E-06	2.69	8.43E-02
Gzma	2.70	9.60E-01	2.82	6.33E-01
Srgn	2.65	1.25E-04	2.85	2.74E-04
Ly6a	1.00	NA	4.43	3.54E-04
S100a11	2.61	1.62E-03	2.73	1.47E-02
Hif1a	2.64	2.24E-03	2.69	4.84E-04
Aldoa	2.53	1.63E-04	2.73	6.38E-04
Ctla2a	3.03	3.24E-01	2.20	4.18E-01
Vim	3.21	1.60E-04	1.92	2.57E-02
Ndfip1	2.53	1.51E-04	2.54	4.02E-03
Dusp5	2.09	6.54E-02	2.95	9.11E-04
Havcr2	2.57	1.35E-04	2.46	1.36E-02
Sdf4	2.59	4.47E-09	2.40	6.10E-04
AC163354.1	2.43	5.14E-05	2.54	2.66E-04
Ifitm2	3.85	1.32E-01	1.00	NA
Tpi1	2.43	5.98E-03	2.24	7.29E-02
Ptms	2.30	8.80E-02	2.33	3.49E-01
Glrx	2.13	1.89E-01	2.45	4.81E-02
Tnfrsf18	2.35	2.55E-06	2.16	3.63E-01
Nr4a1	1.99	5.31E-02	2.46	4.10E-01
Irf8	2.31	4.52E-02	2.13	4.66E-02
Sub1	2.00	1.04E-12	2.42	2.70E-10

Supplemental Table 5: Differential gene expression between progressing TILs vs. naïve, or between regressing TILs vs. naïve T cells. Cells in activated clusters of the UMAP (A1-A6) from either progressing TIL samples (n = 3200 cells) or regressing TIL samples (n = 7590 cells) were compared to naïve T cell clusters (N1-N3) from all samples (n = 7155 cells) using Seurat's FindConservedMarkers function to identify differentially expressed genes, controlled for cohort. Compared to naïve T cells, genes most upregulated in progressing TILs and in regressing TILs are shown.

Supplemental Table 6: Differential gene expression between either cells with Progressor Top Clonotypes or cells with Regressor Top Clonotypes, compared to Other Clonotypes in spleens.

Gene	Progressing Fold Change	Progressing Adjusted P-value	Regressing Fold Change	Regressing Adjusted P-value
Gzmf	9.04	8.41E-302	1.00	NA
Ifi27l2a	-2.65	4.18E-123	1.30	7.70E-01
Ly6c2	-1.38	7.37E-02	2.12	1.10E-85
Hspa1a	4.22	4.63E-56	1.00	NA
Isg15	-1.46	4.19E-09	1.49	3.59E-31
Tsc22d3	1.03	7.26E-07	-1.65	1.27E-54
Bst2	-1.43	1.24E-24	1.18	4.42E-05
Irf7	-1.40	9.40E-13	1.19	9.70E-05
Zfp36	1.05	4.19E-35	-1.53	2.32E-48
Zbp1	-1.17	8.90E-01	1.41	7.79E-55
Stat1	-1.22	6.52E-01	1.35	6.55E-50
Ms4a6b	-1.29	4.46E-17	1.28	2.06E-41
Gstp1	1.38	8.42E-39	-1.17	3.22E-10
Cxcr3	-1.31	2.09E-11	1.23	1.59E-20
Ifi209	-1.33	7.37E-05	1.16	5.13E-04
Ifi214	-1.25	4.63E-04	1.20	2.11E-09
Ifi213	-1.32	3.79E-11	1.12	4.56E-03
Igtp	-1.22	5.29E-01	1.20	5.54E-24
Hist1h2ap	3.30	1.70E-53	1.00	NA
Ccl4	2.77	1.25E-174	4.81	1.76E-249
Mt1	4.21	2.04E-302	5.90	0.00E+00
Gzmd	2.66	4.75E-115	1.00	NA
Ly6a	1.60	7.42E-144	3.11	0.00E+00
Gzme	2.37	2.94E-128	1.00	NA
Ccl5	1.00	NA	2.35	2.45E-287
Gzmc	2.29	1.74E-182	1.00	NA
Serpinb9b	2.17	2.77E-102	1.00	NA
Vim	2.86	0.00E+00	1.72	4.77E-80
Nrgn	2.13	4.04E-246	1.00	NA
Sv2c	2.31	0.00E+00	1.27	7.77E-24
Gzmb	3.70	0.00E+00	4.69	0.00E+00
AW112010	2.07	1.87E-262	3.05	0.00E+00
Hspa1b	1.98	1.30E-57	1.00	NA
Fcer1g	1.97	1.15E-41	1.00	NA
Rbm3	1.93	3.16E-270	1.00	NA
Icos	2.14	1.17E-272	3.06	0.00E+00
S100a4	3.50	0.00E+00	2.62	1.05E-268
Capg	3.15	0.00E+00	2.27	6.74E-150
Dusp5	1.52	3.10E-144	2.40	0.00E+00
Cxcr6	2.15	1.28E-286	3.01	0.00E+00
Serpinb6b	2.98	0.00E+00	2.16	1.26E-226
Gm156	1.80	6.90E-103	1.00	NA
Serinc3	1.78	5.50E-205	1.00	NA
Ccr2	1.00	NA	1.76	1.06E-100
Ccl3	2.57	2.06E-100	3.28	2.04E-90
Klrc1	2.54	1.96E-297	3.24	0.00E+00
Ms4a4b	1.00	NA	1.68	9.03E-302
Hilpda	1.58	1.56E-104	2.26	1.13E-205
Pclaf	1.67	5.26E-52	1.00	NA
Nr4a1	1.35	4.42E-42	1.99	1.64E-190
H2-K1	1.01	2.04E-03	1.64	3.01E-243
Nkg7	1.95	9.74E-290	2.58	0.00E+00

Supplemental Table 6: Differential gene expression between either cells with Progressor Top Clonotypes or cells with Regressor Top Clonotypes, compared to Other Clonotypes in spleens. Cells were grouped into clonotypes based on the paired amino acid sequences of their CDR3α and CDR3β regions. Clonotypes that made up >1% of a regressor sample were classified as "Regressor Top Clonotypes" (6990 cells from 33 clonotypes), clonotypes that made up >1% of a progressor sample were classified as "Progressor Top Clonotypes" (2533 cells from 27 clonotypes), and cells that made up <1% of a spleen sample were classified as "Other Clonotypes" (10363 cells from 10154 clonotypes). Progressor Top Clonotypes (left) were compared to Other Clonotypes, or Regressor Top Clonotypes were compared to Other Clonotypes (right) using Seurat's FindConservedMarkers function (controlling for cohort) to identify differentially expressed genes; the most differentially expressed genes by fold change are shown.

Supplemental Table 7: Differential gene expression between cells with progressor top TCR clonotypes and cells with regressor top TCR clonotypes

Upregulated Genes in Regressor Top Clonotypes

Gene	Adjusted p-value	Fold Change	%Expressing in Regressors	%Expressing in Progressors
Ifi27l2a	4.82E-31	3.45	0.575	0.326
Ly6c2	8.98E-45	2.92	0.8915	0.7105
Isg15	1.78E-40	2.16	0.392	0.195
Ccl5	9.67E-28	2.00	0.9395	0.8785
Ly6a	1.08E-84	1.94	0.9545	0.91
Samhd1	1.19E-64	1.91	0.671	0.526
Xist	2.16E-11	1.83	0.7445	0.4265
Ccl4	5.93E-02	1.74	0.704	0.7425
Ccr2	3.47E-20	1.73	0.4395	0.2105
Ms4a4b	2.09E-124	1.71	0.9935	0.9915
Bst2	2.27E-31	1.69	0.588	0.495
H2-Q7	2.83E-37	1.68	0.944	0.9275
Irf7	7.62E-27	1.66	0.42	0.272
Ms4a6b	1.14E-93	1.65	0.9515	0.924
Zbp1	7.25E-35	1.65	0.541	0.403
Stat1	7.67E-44	1.65	0.75	0.689
Shisa5	9.06E-52	1.64	0.9945	0.9905
H2-K1	6.19E-66	1.63	1	0.9985
Ltb	4.32E-23	1.62	0.8685	0.852
Cxcr3	1.04E-33	1.60	0.347	0.1515
Rtp4	1.14E-25	1.57	0.26	0.0945
Dusp5	3.64E-36	1.57	0.8185	0.7665
Dynll1	7.76E-19	1.57	0.9055	0.869
Slfn2	1.26E-25	1.54	0.827	0.7775
Ifi209	1.74E-09	1.54	0.502	0.3645
Ifit1	1.55E-18	1.52	0.146	0.0445
Ifi214	1.09E-13	1.50	0.356	0.1585
Isg20	4.13E-02	1.50	0.511	0.447
Ifi47	4.91E-22	1.50	0.639	0.6
Emb	8.79E-03	1.49	0.4885	0.4205
AW112010	5.17E-27	1.48	0.9935	0.998
Nr4a1	1.41E-02	1.48	0.518	0.4625
Ifi213	2.04E-14	1.47	0.3485	0.1855
Npc2	9.30E-21	1.47	0.911	0.91
Slfn1	5.76E-14	1.46	0.441	0.322
Igtp	4.35E-09	1.46	0.449	0.364
Vsir	1.45E-09	1.46	0.6015	0.518
Tgtp2	2.10E-13	1.46	0.3275	0.225
Tgif1	1.05E-14	1.44	0.4725	0.3795
Itgb7	5.57E-09	1.44	0.3485	0.217
Icos	9.25E-29	1.43	0.911	0.912
Ypel3	3.24E-09	1.43	0.804	0.779
Itgb1	9.94E-06	1.42	0.6365	0.6195
Epsti1	3.06E-08	1.41	0.883	0.892
Mt1	6.77E-01	1.40	0.6585	0.7335
Gm4070	7.02E-17	1.40	0.4205	0.305
Ubald2	4.88E-15	1.40	0.791	0.802
Cxcr6	1.34E-12	1.40	0.9345	0.935
Ifi208	1.08E-08	1.39	0.3065	0.1895
Cd53	4.15E-45	1.39	0.914	0.903
Gabarapl2	4.58E-31	1.38	0.8555	0.838

Upregulated Genes in Progressor Top Clonotypes

Gene	Adjusted p-value	Fold Change	%Expressing in Progressors	%Expressing in Regressors
Hspa1a	2.92E-47	4.56	0.375	0.084
Gzmf	1.27E-52	3.38	0.527	0.2185
Hist1h2ap	1.57E-12	2.29	0.515	0.3825
Jun	4.50E-19	2.13	0.6685	0.412
Hspa1b	1.30E-46	2.03	0.337	0.0555
Sv2c	2.35E-131	1.82	0.6195	0.2215
Nrgn	1.69E-103	1.70	0.5575	0.2215
Tsc22d3	2.39E-74	1.69	0.8395	0.535
Rbm3	5.27E-87	1.68	0.9985	0.9735
Vim	1.36E-109	1.66	0.9895	0.912
Gstp1	4.31E-55	1.62	0.945	0.8325
Dnajb1	1.53E-08	1.61	0.715	0.5395
Serpinb9b	1.64E-07	1.54	0.2015	0.1115
Tuba1b	2.10E-17	1.53	0.7195	0.5335
Tubb5	3.47E-23	1.49	0.8675	0.6825
Serinc3	5.50E-48	1.45	0.7655	0.4655
Cirbp	5.85E-29	1.45	0.767	0.4375
Stmn1	2.64E-12	1.45	0.3195	0.1605
Rpl35a	5.66E-60	1.43	0.999	0.9945
Ddit4	4.92E-36	1.43	0.6495	0.4015
Il18r1	1.21E-31	1.42	0.6815	0.344
Itgav	2.75E-42	1.41	0.7295	0.4315
Rpl17	1.35E-73	1.39	0.997	0.9895
Arsb	8.01E-65	1.39	0.772	0.4805
Capg	1.29E-16	1.39	0.926	0.747
Rpl10-ps3	1.03E-66	1.38	0.994	0.972
Csf1	2.48E-33	1.38	0.6015	0.329
Serpinb6b	1.48E-35	1.38	0.88	0.7125
Rpl3	4.62E-137	1.36	0.9995	0.9935
Ctla2a	5.31E-26	1.36	0.8745	0.748
Cd160	2.98E-26	1.36	0.6445	0.359
Rps26	2.67E-56	1.36	0.9995	0.998
Gstm5	6.61E-77	1.35	0.2605	0.052
Il18rap	3.87E-26	1.35	0.6185	0.32
Ndrg1	1.67E-62	1.35	0.3755	0.1255
Rps6	1.96E-42	1.35	0.993	0.969
Klrb1c	1.27E-48	1.35	0.2065	0.0295
Bcl2	1.60E-37	1.34	0.7175	0.4565
S100a4	4.53E-33	1.34	0.9605	0.861
Ube2c	5.99E-25	1.34	0.2155	0.093
Rpl21	2.59E-37	1.34	0.9995	0.997
Ubl3	2.28E-16	1.34	0.7985	0.52
Hist1h2ae	1.47E-14	1.33	0.2515	0.147
Cbx3	9.00E-22	1.32	0.624	0.3285
Rpl28	2.64E-76	1.32	0.9985	0.997
Rpl35	8.88E-72	1.32	0.9995	0.996
Hnrnpa1	3.85E-25	1.31	0.902	0.7495
Rps29	1.36E-86	1.31	1	0.9975
Slc25a4	2.00E-30	1.30	0.813	0.57
Rps17	1.72E-16	1.30	0.9565	0.873
Pclaf	2.61E-14	1.30	0.23	0.0995

Supplemental Table 7: Differential gene expression between cells with progressor top TCR clonotypes and cells with regressor top TCR clonotypes. Cells were grouped into clonotypes based on the paired amino acid sequences of their CDR3α and CDR3β regions. Clonotypes that made up >1% of a regressor sample were classified as "Regressor Top Clonotypes" (6990 cells from 33 clonotypes) and clonotypes that made up >1% of a progressor sample were classified as "Progressor Top Clonotypes" (2533 cells from 27 clonotypes). Regressor Top Clonotypes were compared to Progressor Top Clonotypes (left) or the reverse (right) using Seurat's FindConservedMarkers function (controlling for cohort) to identify differentially expressed genes; the most differentially expressed genes by fold change are shown.

Supplemental Table 8: Flow Cytometry Antibodies and MSI antibodies used in the study.

Antigen	Fluorophore	Company	Product #	Clone	Dilution
Flow Cytometry					
Mouse CD3	PE	Biolegend	100307	145-2C11	1:200
Mouse CD4	BV421	Biolegend	100563	RM4-5	1:200
Mouse CD4	BV650	Biolegend	100545	RM4-5	1:200
Mouse CD8a	BV711	Biolegend	100747	53-6.7	1:200
Mouse CD45	BUV395	BD Bioscience	564279	30-F11	1:200
Mouse CD69	PE/Cy5	Biolegend	104509	H1.2F3	1:200
Mouse CD122	PerCP-Cy5.5	Biolegend	123211	TM-β1	1:200
Mouse CD223 (LAG3)	PE	Biolegend	125207	C9B7W	1:200
Mouse CD244	BV605	BD Bioscience	740345	2B4	1:200
Mouse CD279 (PD-1)	APC/Cy7	Biolegend	135223	29F.1A12	1:200
Mouse IFNγ	PE	eBioscience	12-7311-41	XMG1.2	1:250
Mouse Ly6A/E (Sca-1)	BV421	Biolegend	108127	D7	1:200
Mouse Ly6C	BV421	Biolegend	128031	HK1.4	1:200
Mouse Nur77	PerCP-eFluor710	eBioscience	46-5965-82	12.14	1:100
Mouse Tbet	PE/Cy7	Biolegend	644823	4B10	1:100
Mouse TCRβ	APC	Biolegend	109211	H57-597	1:200
Mouse TNFα	APC	Biolegend	506307	MP6-XT22	1:250
Live/Dead	Green (488)	Invitrogen	L23101	--	1:1000
Multispectral Immunofluorescence					
Human Pan-Cytokeratin	none	Dako	M351501-2	AE1/AE3	1:500
Human CD20	none	Abcam	ab9475	L26	1:400
Human CD8	none	Dako	M710301-2	C8/144B	1:100