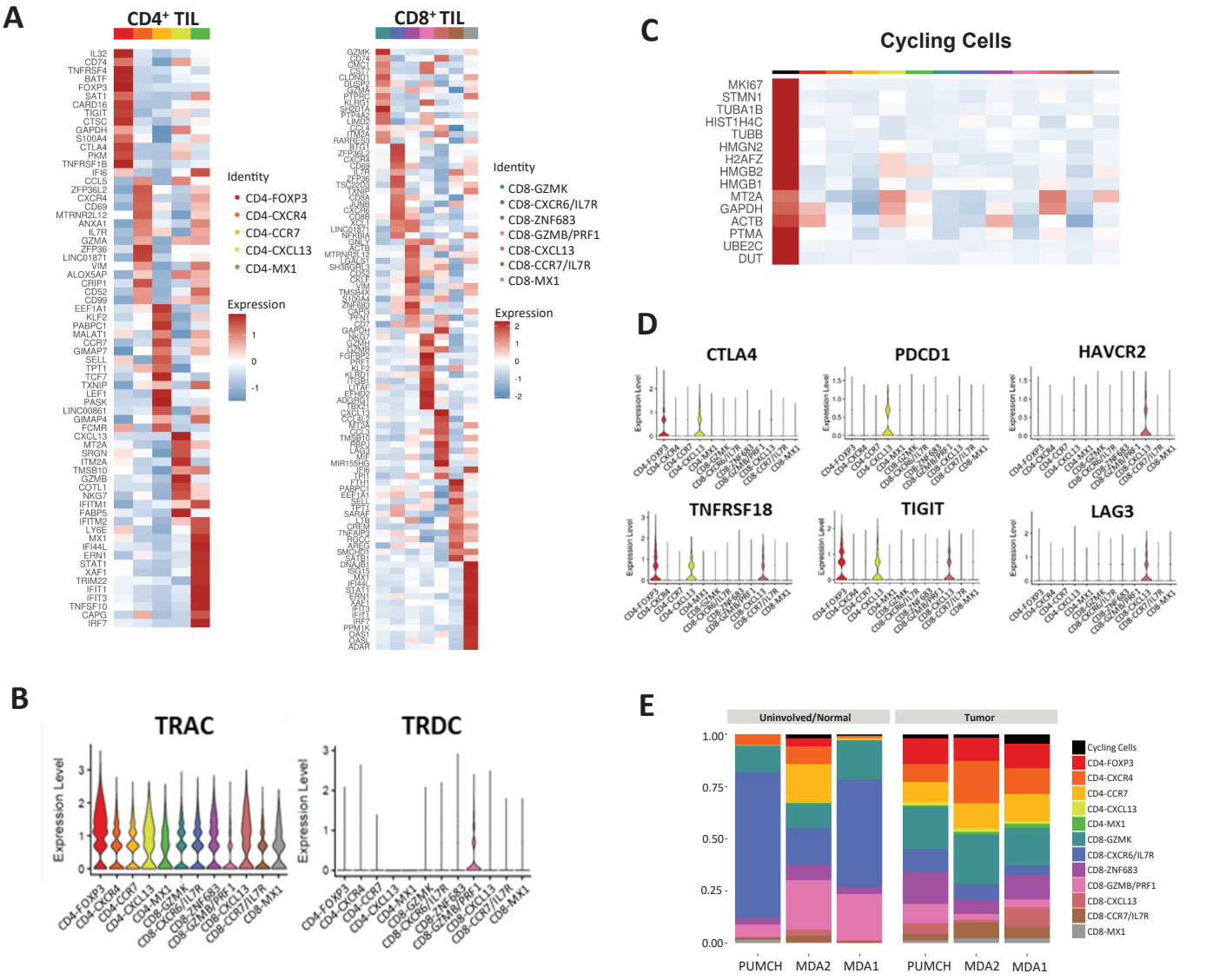
SUPPLEMENTARY FIGURES S2-S11

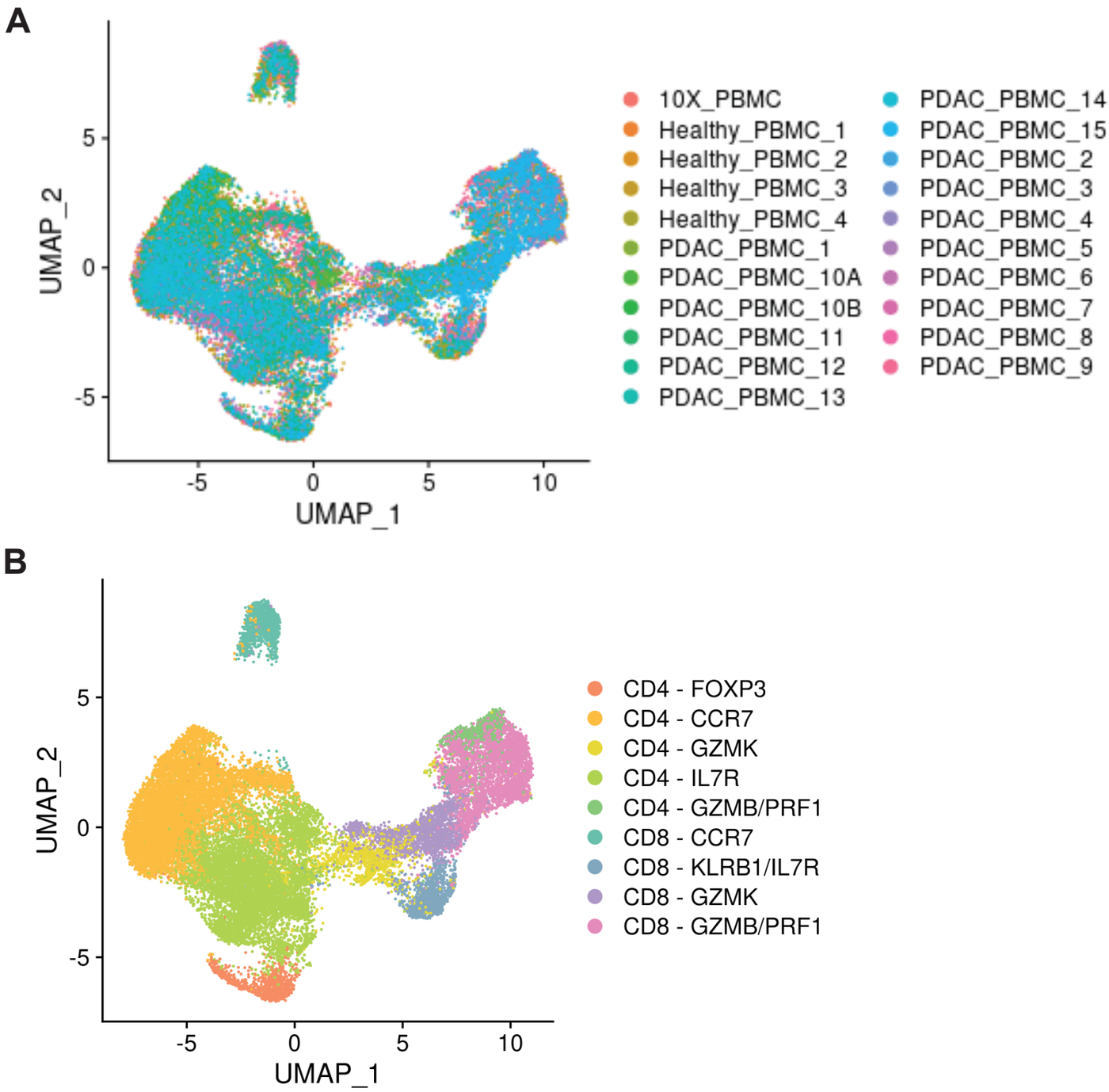
Single cell sequencing reveals trajectory of tumor-infiltrating lymphocyte states in pancreatic cancer

Supplementary Figure S2



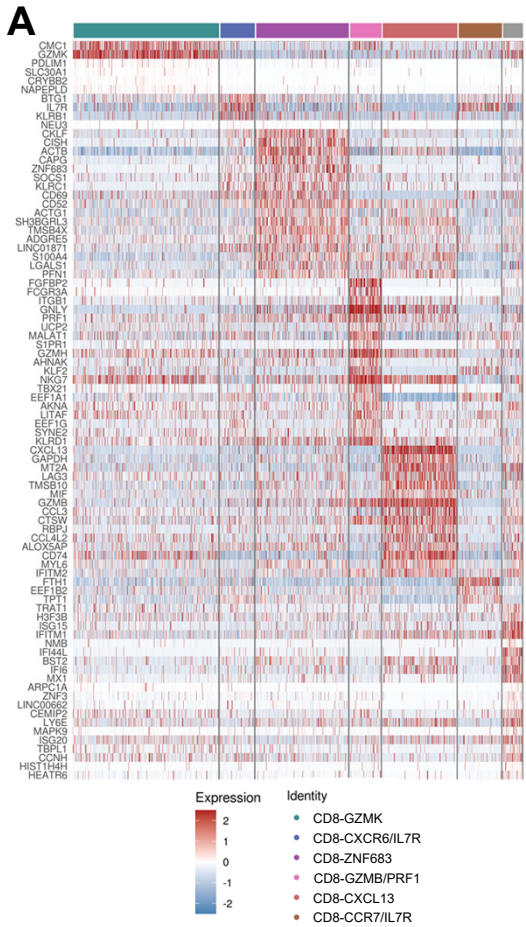
Supplementary Figure S2. (A) Heatmap of top differentially expressed genes for $CD4^+$ and $CD8^+$ TIL. The average gene expression across all cells within each cell state is displayed. The color gradient indicates higher to lower relative expression as it moves from red to blue. **(B)** Violin plot showing expression of TRAC and TRDC across all TIL cell state. Each cell state is color coded according to the color assigned to each population. **(C)** Heatmap of top differentially expressed genes for the cycling cell population. The average gene expression across all cells within each cell state is displayed. The color gradient indicates higher to lower relative expression as it moves from red to blue. **(D)** Violin plots showing relative expression of selected immune checkpoint genes for each cell state. **(E)** Overall composition for each cohort for uninvolved and tumor samples, with individual cell states indicated by colored segments.

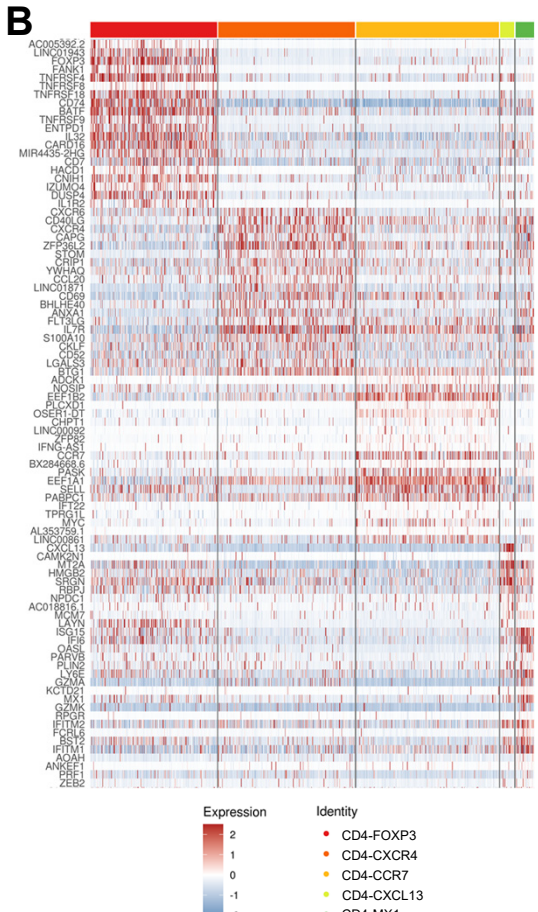
Supplementary Figure S3



Supplementary Figure S3. The uniform manifold approximation and projection (UMAP) of single T-cells from publicly available data sets of healthy and patient peripheral blood mononuclear cells (PBMC) samples, color coded by **(A)** cohort/patient and **(B)** transcriptomic cell state. Each dot is a single cell and is colored according to dataset/patient and transcriptomic cell state respectively. **(C)** The degree to which the top 20 genes (ranked by adjusted p-value) in the TIL cell states (tissue clusters) from the full data set (MDA1, MDA2, and PUMCH) overlap with the those in the PBMC data sets (PBMC cell state) are visualized in a gene overlap matrix. Blue indicates a high degree of overlap, and beige indicates a low degree.

Supplementary Figure S4

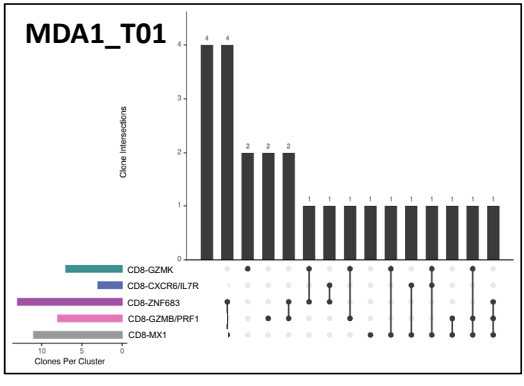


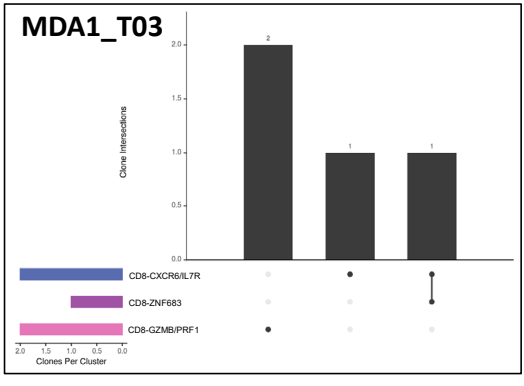


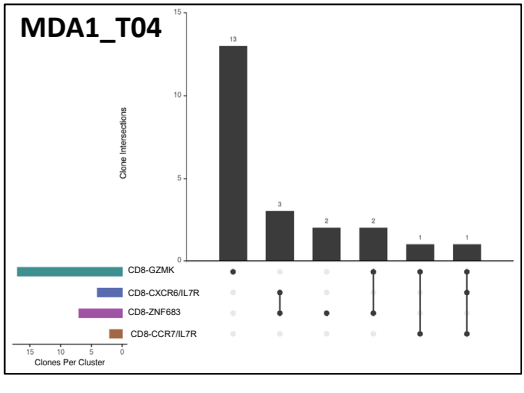
Supplementary Figure S4. Heatmap of unsupervised clustering of **(A)** $CD8^+$ tumor-infiltrating lymphocyte (TIL) and **(B)** $CD4^+$ TIL for the MDA1 data set shows top differentially expressed genes for each cell state.

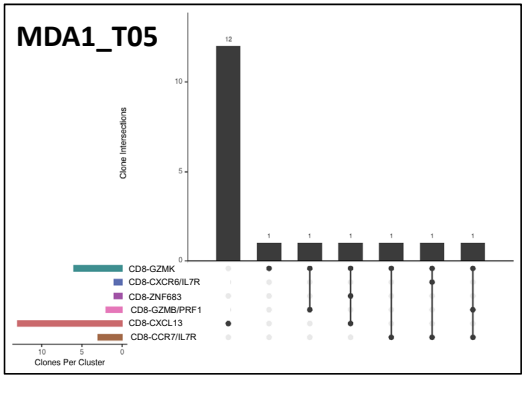
Supplementary Figure S5

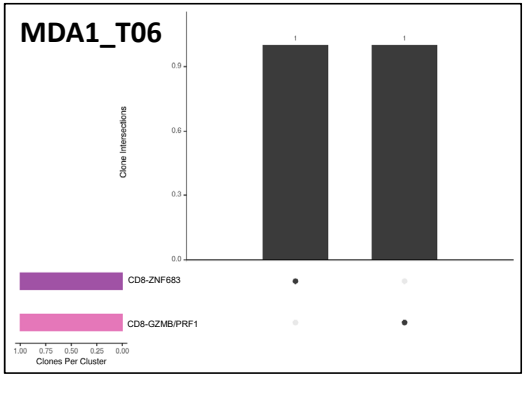
CD8 fresh

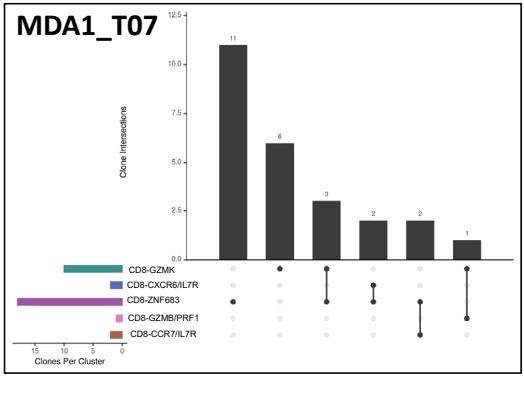








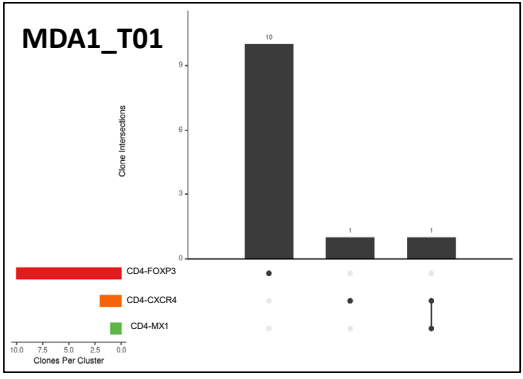


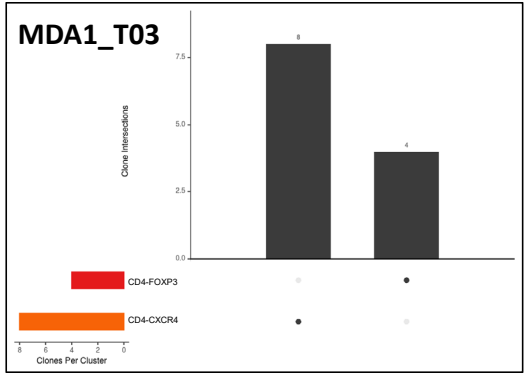


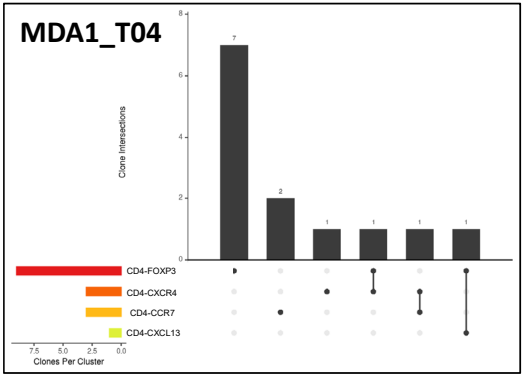
Supplementary Figure S5. Individual patient upset plots for $CD8^+$ tumor-infiltrating lymphocyte (TIL) that show the degree to which T-cell receptor (TCR) clones are shared between multiple cell states. The vertical, black bars indicate the number of times an intersection is detected. Underneath each bar, the clusters in which at least one expanded clonotype (>2 cells) is detected are symbolized by a solid, black circle. If clones are found in multiple cell states, a line is drawn between those cell states. If there is no sharing, only a single black dot is shown. The horizontal bars colored by transcriptomic cell state indicate the number of TCR clones per cell state. Plots and clusters are shown only for samples that had expanded $CD8^+$ TIL clonotype.

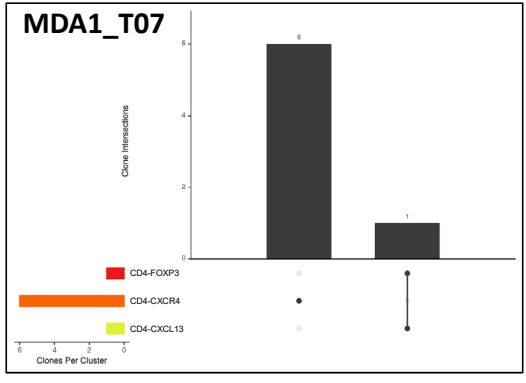
Supplementary Figure S6

CD4 fresh



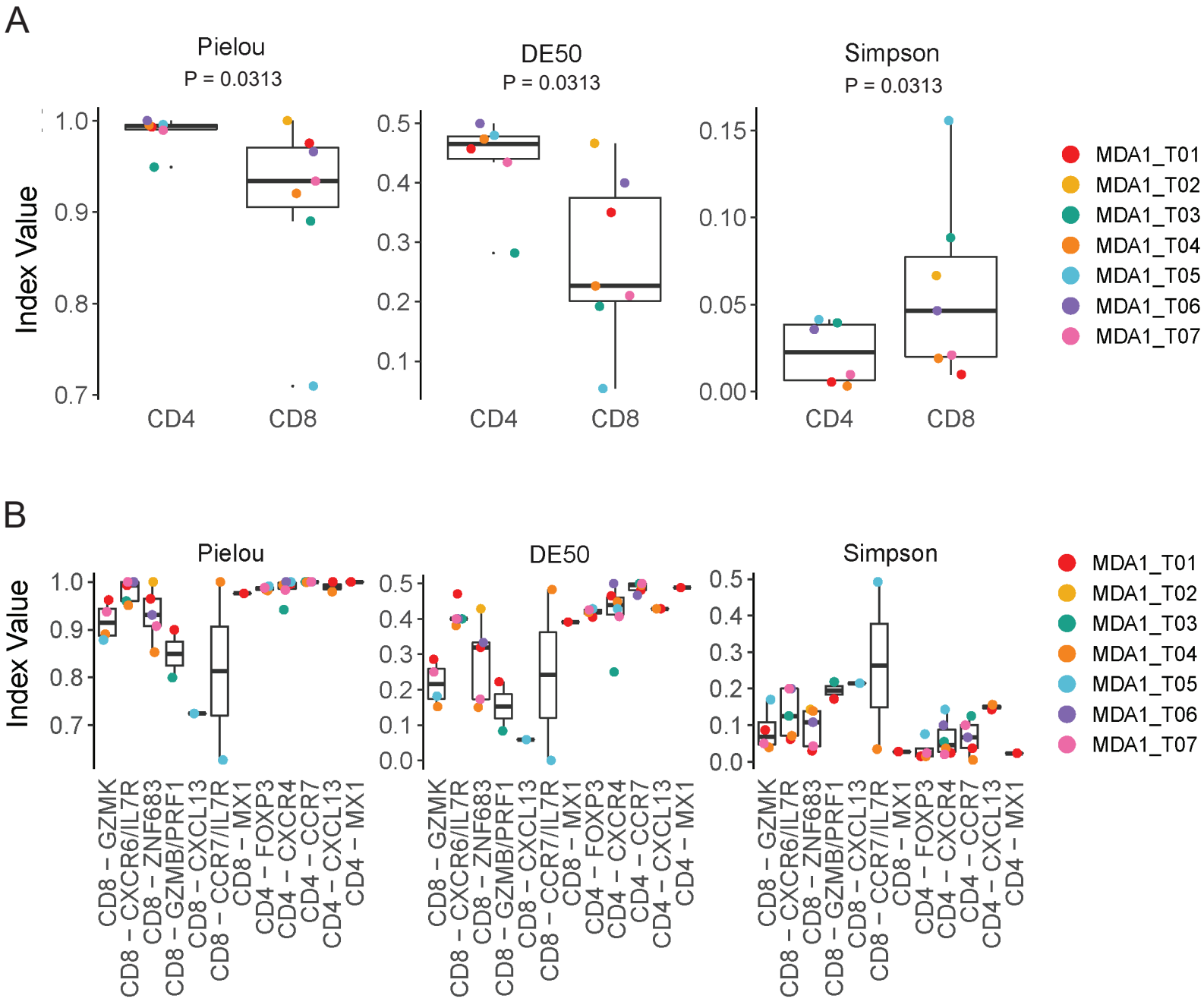






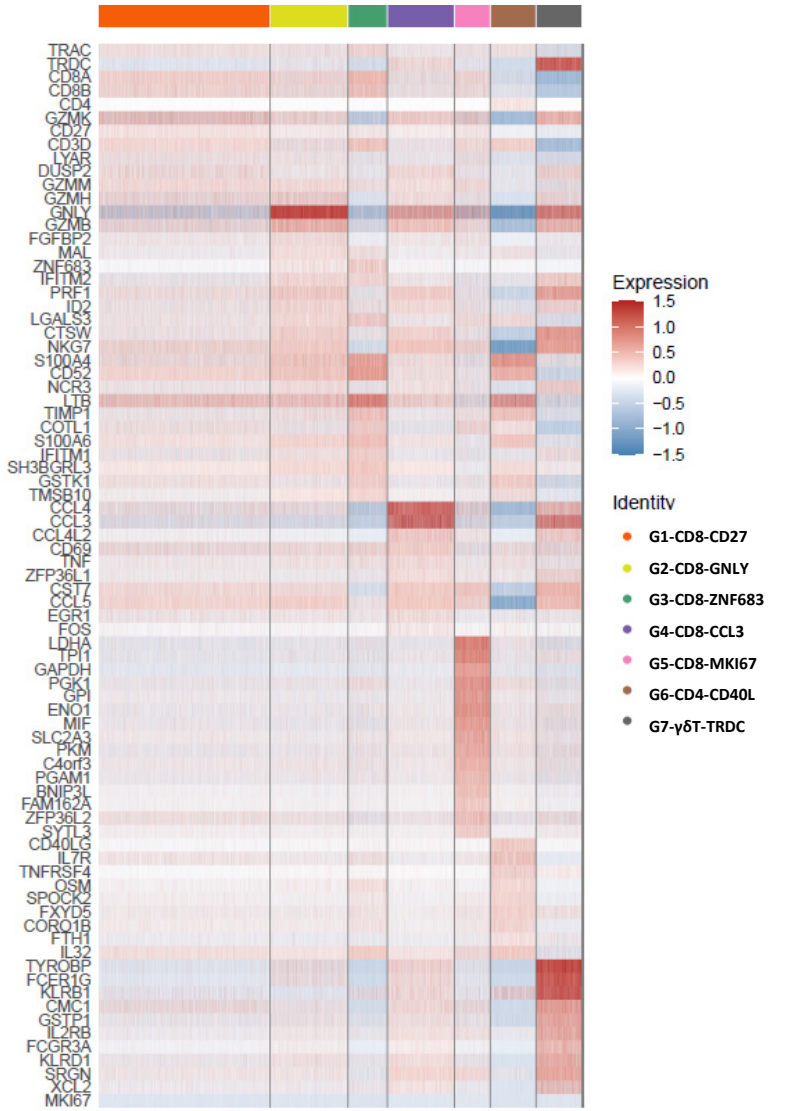
Supplementary Figure S6. Individual patient upset plots for $CD4^+$ tumor-infiltrating lymphocytes (TIL) that show the degree to which T-cell receptor (TCR) clones are shared between multiple cell states. The vertical, black bars indicate the number of times an intersection is detected. Underneath each bar, the clusters in which at least one expanded clonotype (>2 cells) is detected are symbolized by a solid, black circle. If clones are found in multiple cell states, a line is drawn between those cell sates. If there is no sharing, only a single black dot is shown. The horizontal bars colored by transcriptomic cell state indicate the number of TCR clones per cell state. Plots and clusters are shown only for samples that had expanded $CD4^+$ TIL clonotype.

Supplemental Figure 7



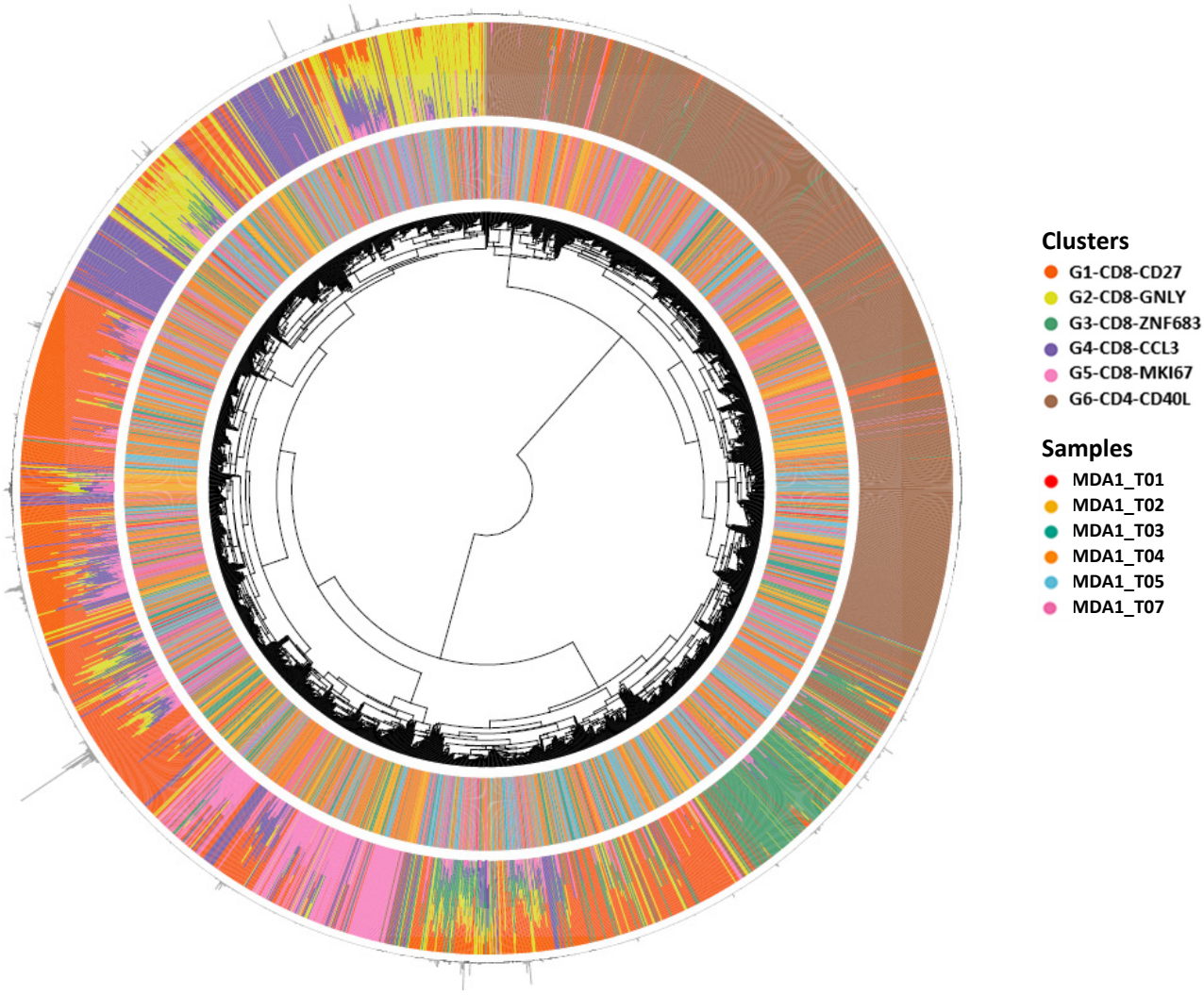
Supplementary Figure S7. (A-B) The Pielou index (left panel), DE50 index (middle pannel), and Simpson diversity index (right panel) were applied to measure clonal evenness and diversity within (A) the bulk CD4 and CD8 TIL for each patient and (B) for each cell state within the CD8 and CD4 populations for each patient. Only those samples with ≥5 cells detected for a cell state are displayed.

Supplementary Figure S8



Supplementary Figure S8. Heatmap of unsupervised clustering of $CD3^+$ tumor-infiltrating lymphocytes (TIL) for the cultured TIL products shows top differentially expressed genes for each cell state.

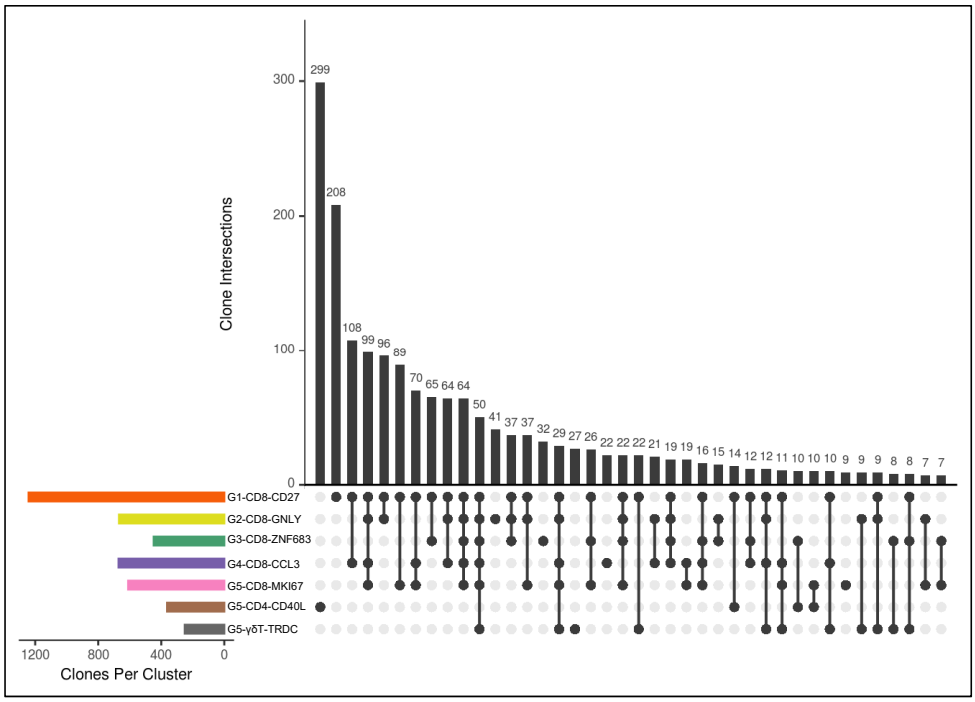
Supplementary Figure S9



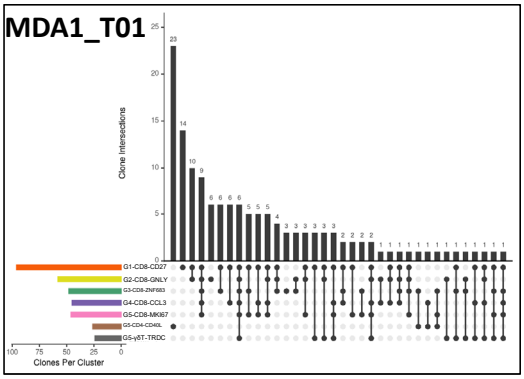
Supplementary Figure S9. Circos plot for cultured $CD3^+$ tumor-infiltrating lymphocytes combining the T-cell receptor (TCR) sequencing data with the transcriptomic data. The outer ring indicates TCR frequency; the adjacent, middle ring is colored by the transcriptomic state assignment; the inner ring is colored by sample. Each bar represents one clonotype.

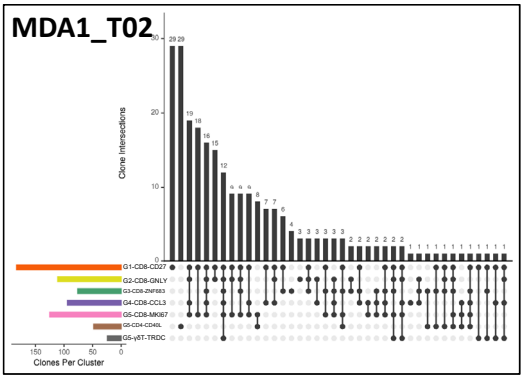
Supplementary Figure S10

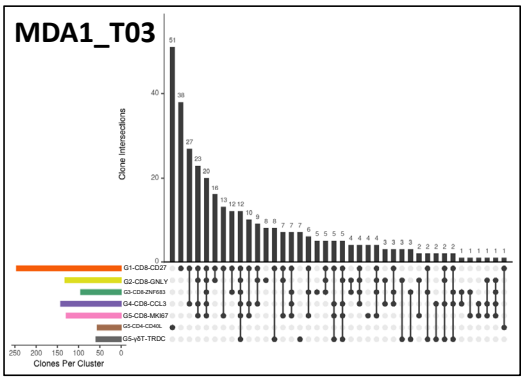
A

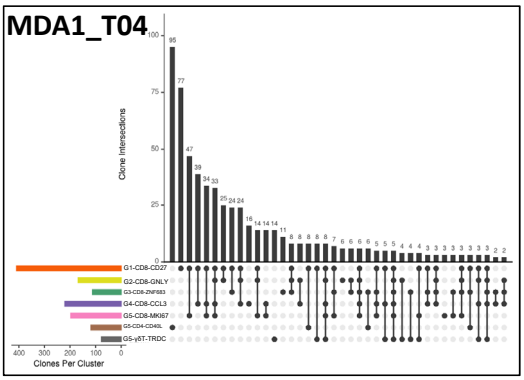


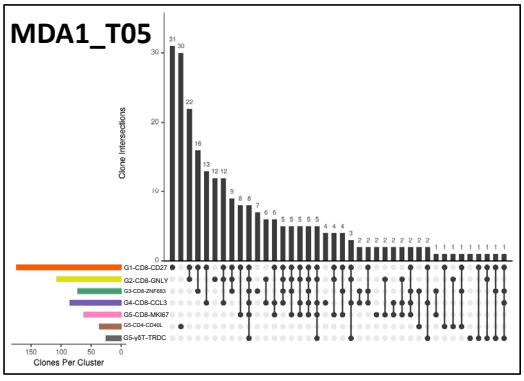
B

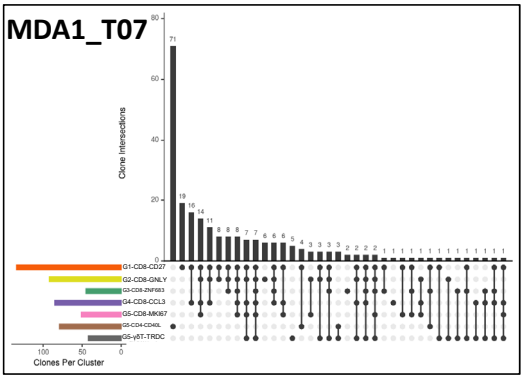






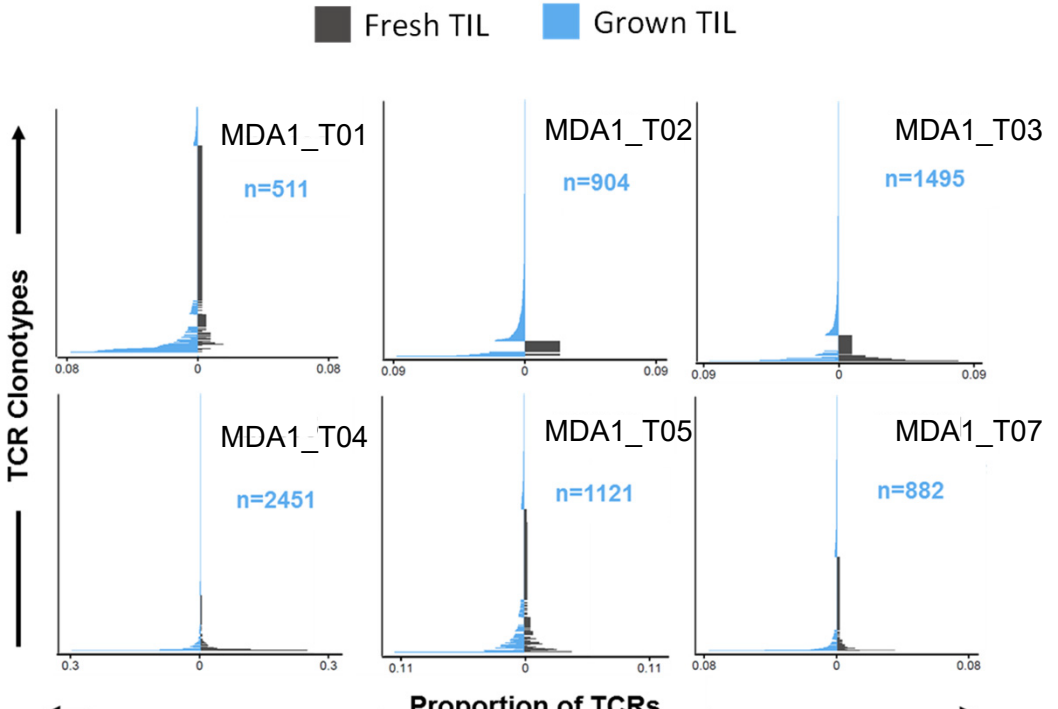






Supplementary Figure S10. Upset plots for all cultured CD3+ tumor-infiltrating lymphocytes (TIL) **(A)** and broken down by individual patient **(B)** that show the degree to which T-cell receptor (TCR) clones are shared between multiple cell states. The vertical, black bars indicate the number of times an intersection is detected. Underneath each bar, the cell states in which at least one expanded clonotype (>2 cells) is detected are symbolized by a solid, black circle. If clones are found in multiple cell states, a line is drawn between those cell states. If there is no sharing, only a single black dot is shown. The horizontal bars colored by transcriptomic cluster indicate the number of TCR clones per cell state. MDA1_T06 cultured TIL were not available for sequencing.

Supplementary Figure S11



Supplementary Figure S11. Comparison of the frequency of TIL clonotypes found in the fresh PDAC tissue (black) and the grown culture (blue). The number of TIL is shown for the grown culture but not for the fresh TIL as there is no way to know the number of TIL clones in the piece of PDAC tissue used for culture.