**Table S1. Patient characteristics of tumor samples used for TIL sequencing in this study.** All patients are included, both archival (Fig. 1-2, fig. S4) and prospective (Fig. 3), along with complete four-digit complete HLA class I and HLA class II typing.

**Table S2.** **Cell counts from each patient within each cluster from archival scRNA analysis.** Samples included are those from Fig. 1A.

**Table S3. Markers of each cluster from archival scRNA analysis.** Significantly upregulated and downregulated DEGs from clusters 0-11 (Fig. 1) are included. Genes are sorted by average log-fold change.

**Table S4. Gene signatures and cluster markers used for single cell gene set enrichment analysis.** Tab 1: published gene signatures included in this study. Tab 2: gene signatures derived from DEGs in clusters identified in Fig. 1A as well as NeoTCR4 and NeoTCR8 signatures, with NeoTCR-enriched cluster signatures (Cluster 1 and Cluster 6) and NeoTCR4 and NeoTCR8 signatures are labeled in bold red text.

**Table S5. Clustered Correlation Matrix of each cluster and NeoTCR4 and NeoTCR8 signatures with scRNA gene signatures from other studies.** Correlation values (Pearson’s r) of scGSEA scores for cells from Fig. 1, compared both to 107 published signatures, 12 clusters described in Fig. 1, and newly described NeoTCR4 and NeoTCR8 signatures. Red: Positive correlation, Blue: Negative Correlation. NeoTCR4 and NeoTCR8 columns are labeled in bold red text.

**Table S6. Public Viral TCRs and cell barcodes found in archival TIL samples.** Cells expressing TCRs with CDR3β matching public TCRs against viruses influenza A, CMV, and EBV are shown.

**Table S7. Summary of previously known, predicted, and prospectively validated NeoTCRs from archival and independent 4 patients in this study.** TCRs are labeled according to CD4 or CD8 derivation and annotated with reactivity data.Tab 1 shows TCRs included in the retrospective portion of the study (Fig. 2, fig. S4). Categories within the TCRs include “known” (reactivity data obtained prior to this study) and “newly identified” (reactivity identified as part of this study). 8 previously assessed TCR clones that tested negative to candidate neoantigens are shown from tumor 4323 (“Dominant non-reactive TCRs,” Fig. 2B). Also included are clone counts within each cluster. Tab 2 shows TCRs included in the prospective portion of the study (Fig. 3) along with clone counts in the prospective TIL scRNA. Tab 3 shows CDR3 sequences of TCRs targeting driver genes utilized or identified in this study, including the HLA restriction of the neoantigens.

**Table S8. DEGs of NeoTCR-expressing cells within Fig. 2.** Tab 1 shows NeoTCR4 DEGs, Tab 2 shows NeoTCR8 DEGs, and Tab 3 shows NeoTCR DEGs from patients who received prior immune checkpoint blockade (ICB). NeoTCR4 DEGs are those genes significantly up- or down-regulated in 261 CD4-derived NeoTCRs compared to all other cells (Fig. 2E, bottom right). NeoTCR8 DEGs consist are genes significantly up- or down-regulated in 281 CD8-derived NeoTCRs compared to all other cells (Fig. 2E, bottom left). Tab 3 shows those genes differentially expressed in NeoTCR-expressing cells from patients who received prior ICB (4261, 4298, 4342) compared to other cells from those samples.

**Table S9. Upstream Regulators identified from Ingenuity Pathway Analysis (IPA) for top genes from the NeoTCR signatures.** NeoTCR4 and NeoTCR8 signatures were analyzed within IPA for identification of putative regulators. Genes whose downstream targets are enriched in the NeoTCR signatures are potentially upstream regulators. Tab 1 shows upstream regulators of NeoTCR4 signature and Tab 2 shows upstream regulators of NeoTCR8 signature.

**Table S10. Putative NeoTCR signature comparisons.** Versions of potential NeoTCR4 and NeoTCR8 signatures were generated of various lengths (all are shown in tab 1) and compared by AUC analysis for their ability to call known CD4 and CD8 NeoTCRs (tab 2 showing AUC values on archival samples (Fig. 2 and fig. S4). Tab 3 shows the sensitivity and specificity of gene sets derived from known dysfunctional or effector genes *ENTPD1*, *CXCL13*, *PDCD1*, *ITGAE*, *TIGIT*, *TOX*, *LAG3*, *HAVCR2*, and *GZMK*. Tab 4 shows the sensitivity and specificity of putative NeoTCR signatures that omit the known dysfunctional/effector genes. In all tabs, versions of NeoTCR4 and NeoTCR8 used throughout the study are labeled in bold red text

**Table S11. Tumor-associated antigen (TAA) expression within tumors from prospective specimens 4393, 4394, 4400, 4421.** Log2TPM values are shown for the expression of 301 candidate TAA genes within the prospective tumor samples. Genes that were included in TCR screening for 4393 and 4400 are highlighted in orange.

**Table S12. AUC analysis of NeoTCR identification by study-derived and previously published signatures.** AUC values are shown for each signature’s sensitivity and specificity in calling CD4 NeoTCRs in archival specimens (CD4 training), CD8 NeoTCRs in archival specimens (CD8 training), CD4 NeoTCRs in prospective specimens (CD4 validation), CD8 NeoTCRs in prospective specimens (CD8 validation). Scoring with random gene signatures (Random1 and Random2) as well as scoring of public viral TCRs (public viral training) are included as controls. NeoTCR4 and NeoTCR8 rows are labeled in bold red text.