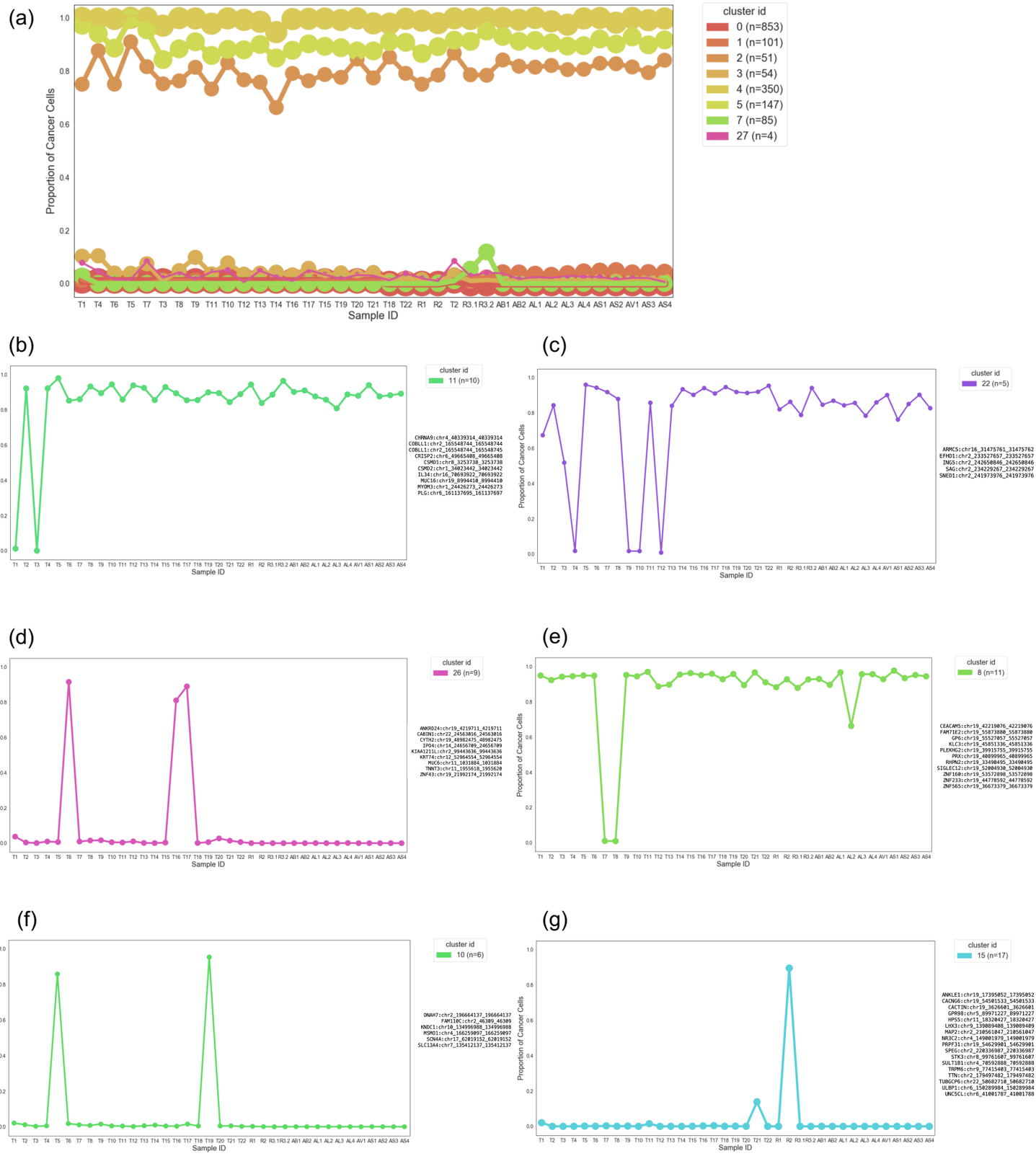
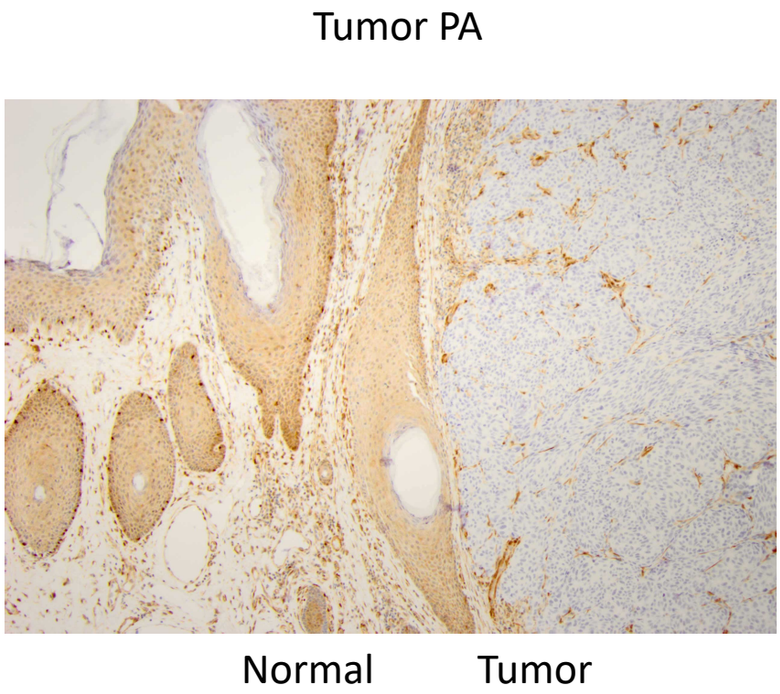
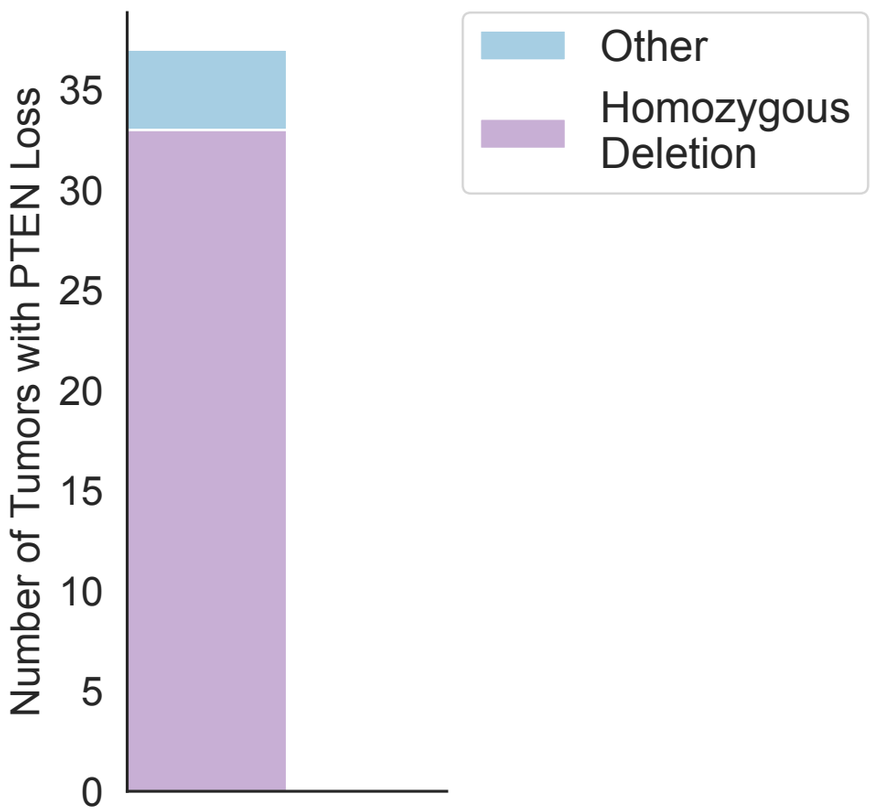
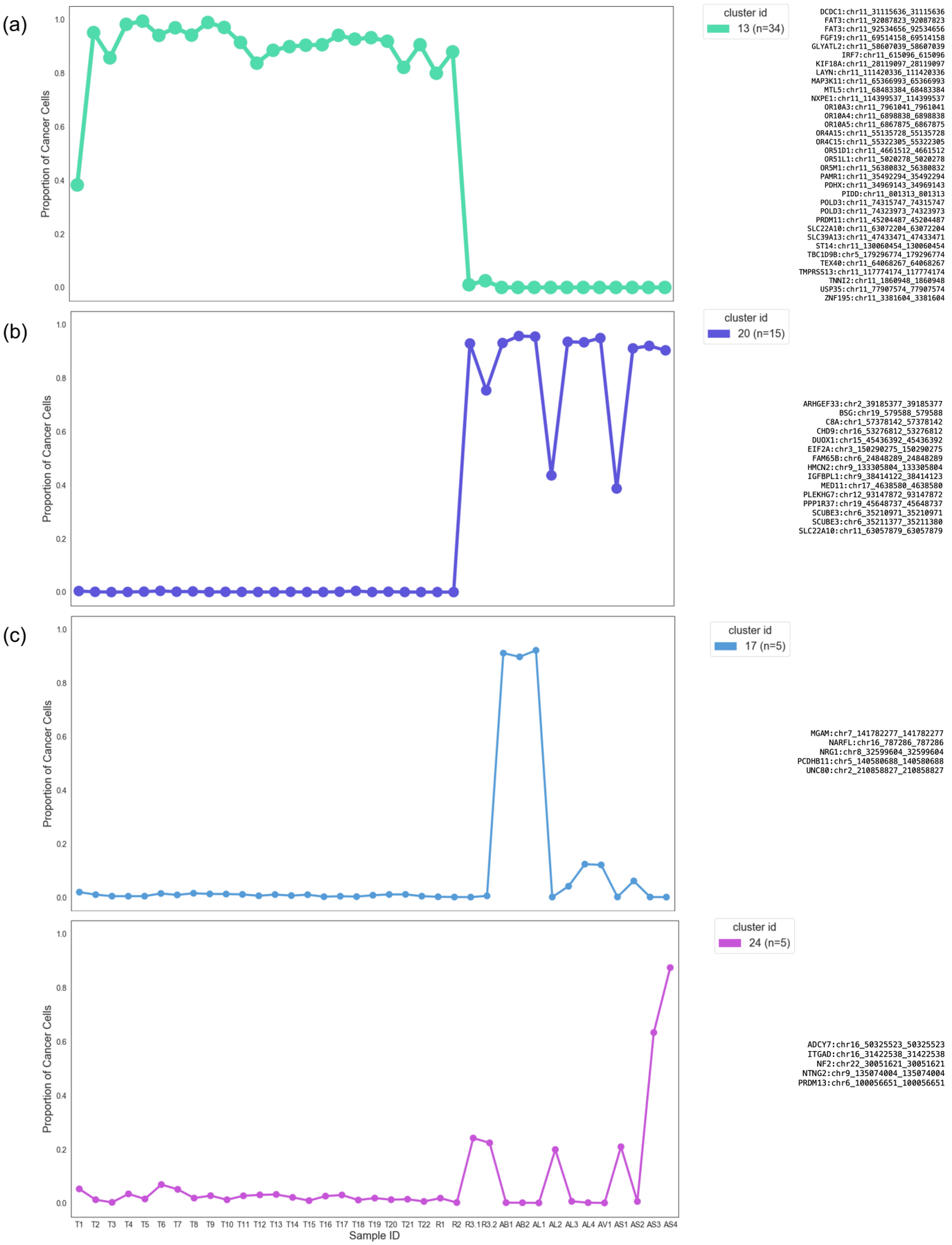


Supplementary Data Figure 1. Pyclone Analyses and Mutational Clusters.
(a) Mutational Clusters with High Numbers of Mutations. Clusters 2, 4, 5 likely represent mutations that are clonal and shared between all tumors. Clusters 0, 1, 3, 7, 27 represent mutations that are mostly found in one tumor at low CCF and may be enriched in artifacts. (b) **Mutational Cluster Defining Lineage 0**. The only tumors missing this cluster are T1 and T3. Notably, mutations in this cluster are located on different chromosomes, suggesting the common ancestor of T1 and T3 branched from the other tumors at an earlier point before these mutations were acquired. These mutations were manually reviewed and showed no evidence of artifact. (c) **Mutational Cluster Defining Lineage 2**. This pattern demonstrates the loss of mutations in a common ancestor of the tumors in Lineage 2 (T4, T9, T10, T12), consistent with deletion of a segment of Chromosome 2q. (d) **Mutational Cluster Defining Lineage 4**. This pattern demonstrates the gain of mutations in a common ancestor of the three tumors in Lineage 4 (T6, T16, T17). (e) **Mutational Cluster Loss Defining Lineage 5**. The only tumors missing this cluster are T7 and T8. Notably, all mutations in this cluster are in Chr19q, and the two tumors missing this cluster (T7, T8) also have a corresponding LOH in chromosome 19q, suggesting loss of 19q in a common ancestor. (f) **Mutational Cluster Defining Lineage 6**. This pattern demonstrates the gain of mutations in a common ancestor of the two tumors in Lineage 6 (T5, T19). (g) **Mutational Cluster Defining Early Resistant Brain Metastasis**. This cluster represents the acquired mutations distinguishing the brain metastasis (R2) from the other tumors. These mutations, 10 of which have a multiplicity of ~2 and 7 of which have a multiplicity of ~1, also allow for the timing of a genome doubling event unique to this tumor in lineage 3. These mutations were manually reviewed and showed no evidence of artifact.

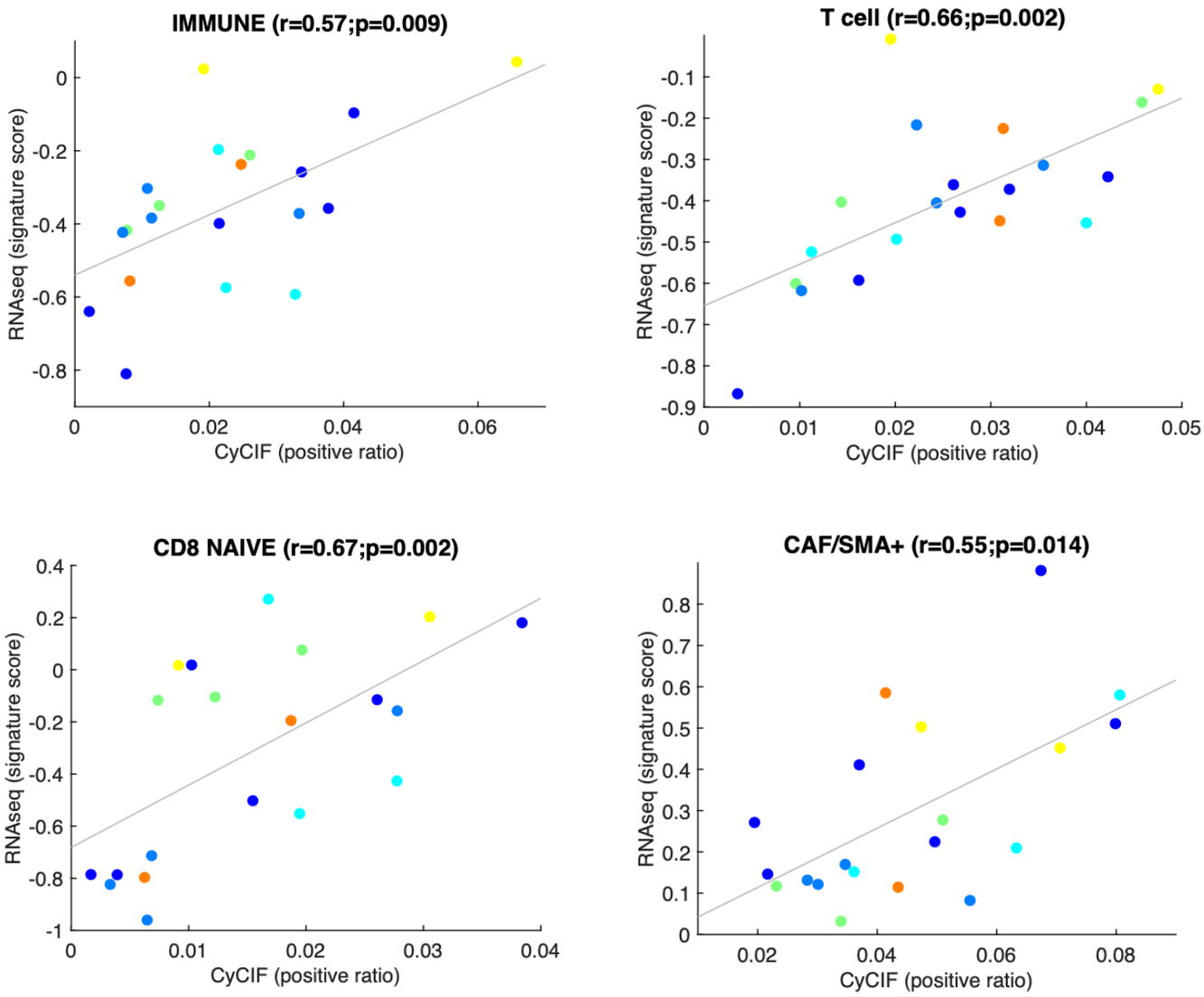




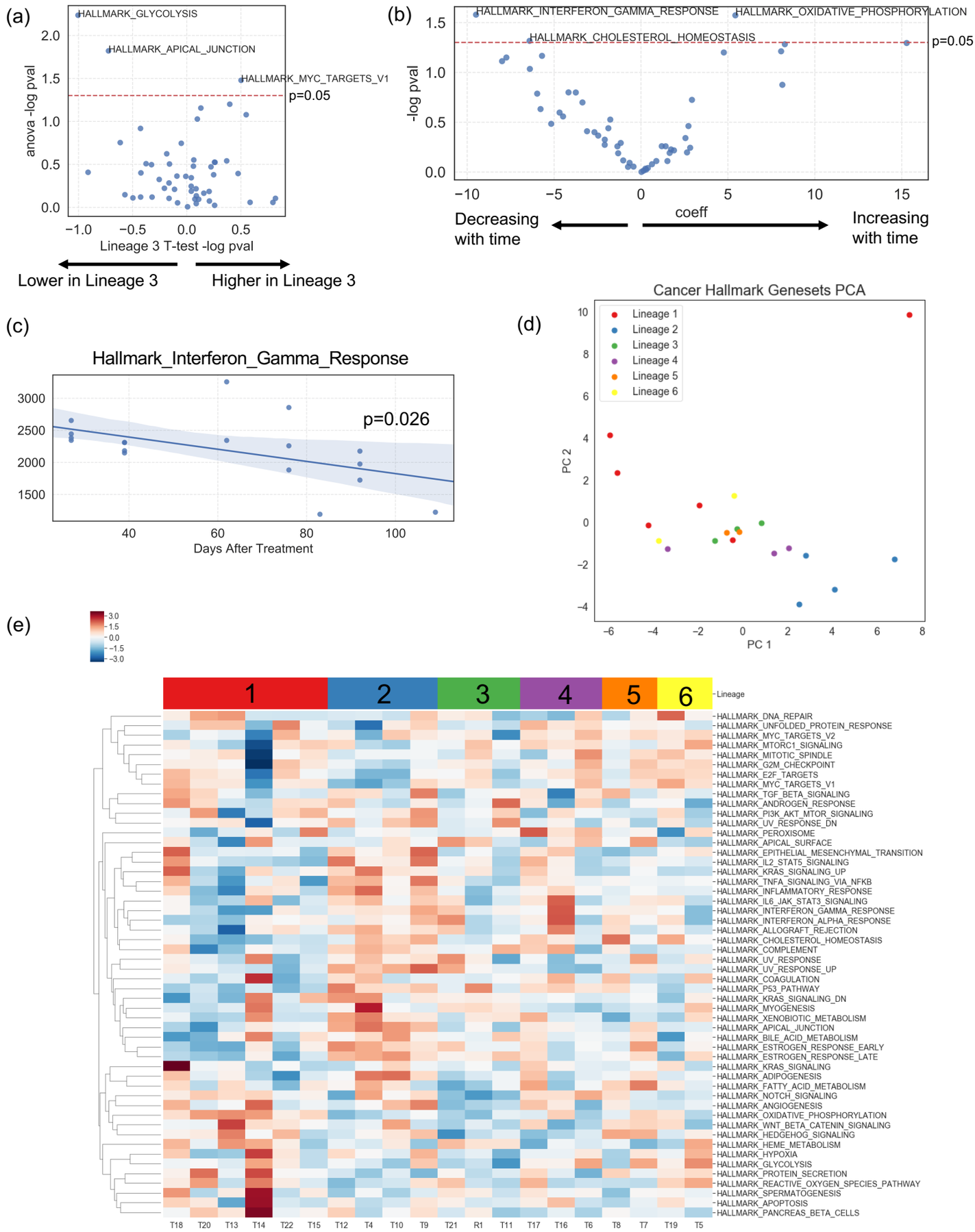
Supplementary Data Figure 2. Tumor PTEN expression by IHC. Top: a representative slide showing loss of PTEN expression by IHC in the tumor, compared to normal PTEN expression in non-tumor tissue. Genetically 33/37 tumors were found to have homozygous deletion in *PTEN*, with a LOH in *PTEN* in the remaining 4 tumors, and all 19 tumors tested for PTEN IHC expression were negative including 2 tumors (T6, T7) without homozygous deletion inferred genetically.



Supplementary Data Figure 3. Mutational Clusters in Late Resistant Samples. (a) Mutational Cluster Defining Lineage 3 Late Resistant Samples. This pattern demonstrates the loss of mutations in a common ancestor of 13 of the analyzed tumors in Lineage 3 (the late-emerging resistant lesions R3.1 and R3.2 and the 11 post-autopsy lesions). These mutations are all found in chromosome 11, with a corresponding LOH in chromosome 11. **(b) Mutational Cluster Defining Lineage 3 Late Resistant Samples**. This pattern demonstrates the gain of mutations in a common ancestor of 13 of the analyzed tumors in Lineage 3 (the late-emerging resistant lesions R3.1 and R3.2 and the 11 post-autopsy lesions). These mutations are scattered across the genome and upon manual review, this cluster was found to be under-clustered, with a few of the 15 mutations absent in AL2 and AS1, explaining the lower cluster CCF overall for this cluster for these samples. **(c) Mutational Clusters Defining Phylogeny of Late Resistant and Post-Autopsy Samples**. This pattern demonstrates the gain of mutations in a subset of tumors (upper figure). Upon manual review of individual mutations, cluster 17 was found to be under-clustered, with NARFL mutations present in AB1, AB2, AL1, AL4, AV1, AL3, and AS2, and NRG1 present in AB1, AB2, AL1, AL4, AV1, and the other three mutations only in AB1, AB2, and AL1, explaining the lower cluster CCF overall for this cluster for these samples. For the cluster 24 mutations, we see a similar phenomenon where 2 (PRDM13 and ITGAD) are present in R3.1, R3.2, AL2, AS1, AS3 and AS4 and the remaining 3 are present only in AS3 and AS4 (upon manual review).

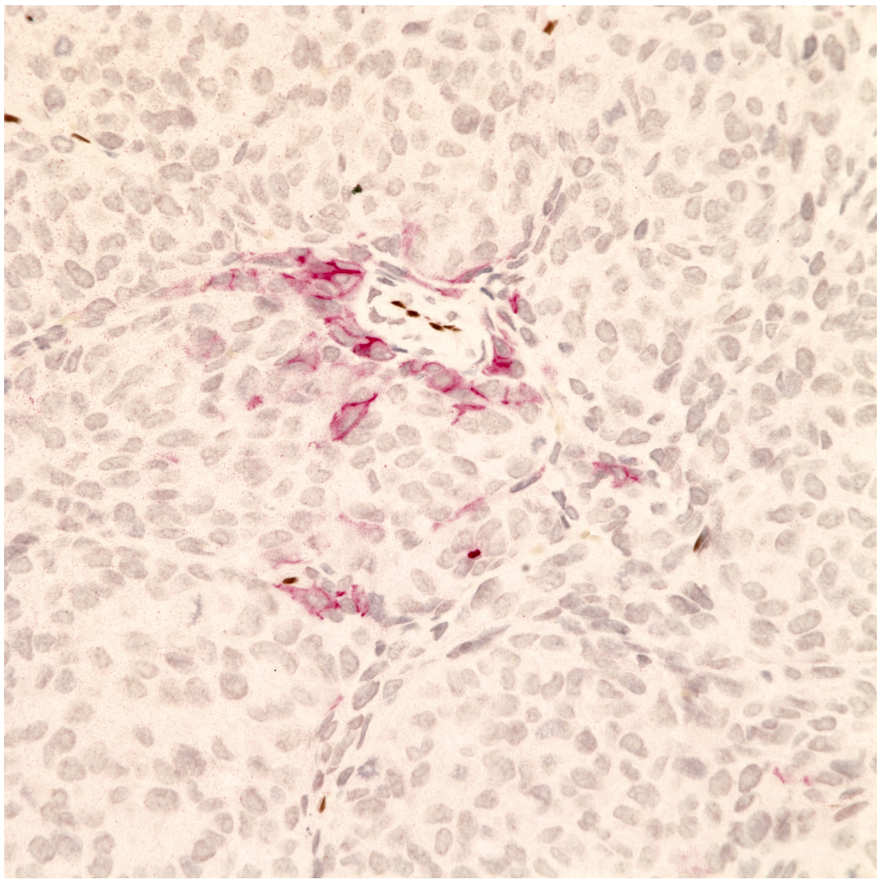


Supplementary Data Figure 4. Integrated Analysis of Immune Signatures from RNA and Protein Expression Data. Correlation between selected immune cell signature scores calculated from bulk RNAseq TPM and proportion of cells from CyCIF (multiplex IF).

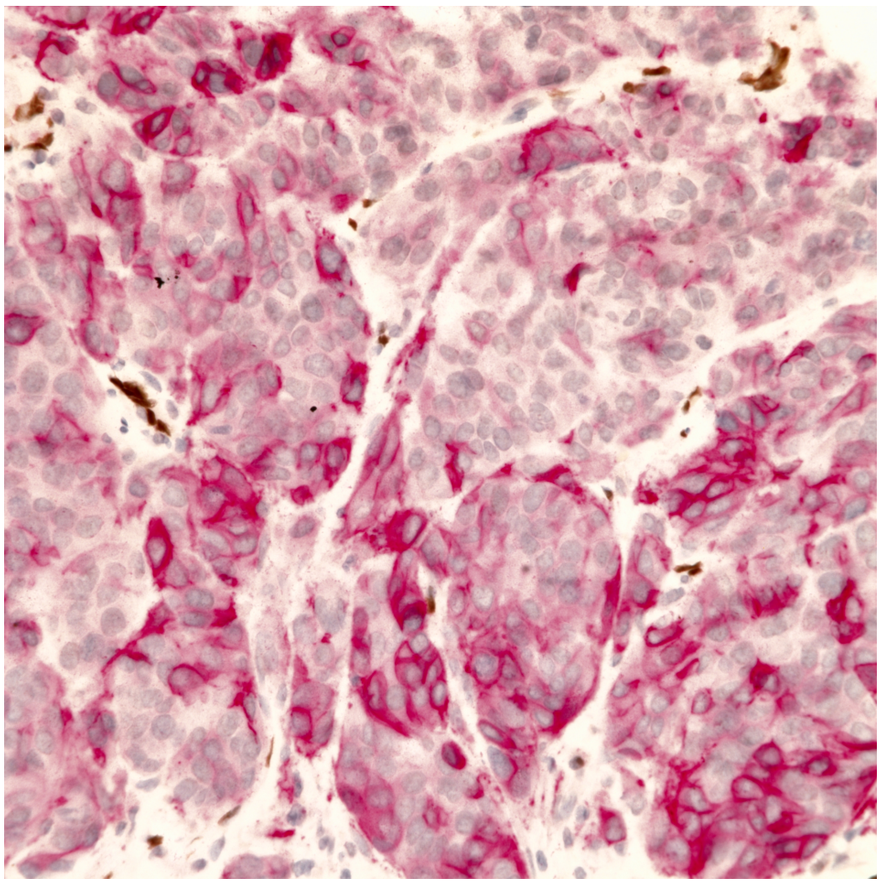


Supplementary Data Figure 5. Cancer Hallmark Pathway Activity by Lineage and Time. Single-sample GSEA on trancriptome data was used to infer an activity score across 50 cancer hallmark genesets for each tumor sample (n=20 samples) (a) **Association of lineages with hallmark genesets**. Each dot represents a hallmark geneset. The y-axis represents the ANOVA p-value of the association between lineage and geneset activity, and the x-axis is the T-test p-value of Lineage 3 tumor geneset activity vs other tumors not in Lineage 3. The red line represents p = 0.05. (b) **Association of hallmark geneset activity with time.** Tumors from D27-D109 were evaluated for a linear association with time. (c) **Activity of interferon gamma response pathway over time.** Each dot represents a tumor; inferred IFN-gamma activity decreases over time (p = 0.026). **Global Cancer Hallmark Geneset Activity by Lineage. (d) PCA of Hallmark Geneset Activity of Tumors by Lineage.** Prinicipal component analysis was applied to hallmark geneset scores and tumors plotted by their principal component (PC) 1 and 2 scores. (e) **Heatmap of Hallmark Geneset Activity by Tumor and Lineage**. Each column is a tumor, and each heatmap entry is the normalized (mean = 0, standard deviation = 1) value of the geneset activity, and the tumors are ordered by lineage. Lineage 2 appears to have consistently higher immune related genesets including interferon-gamma and alpha response, IL6_JAK_STAT3_Signaling, TNFA signaling, and inflammatory response.

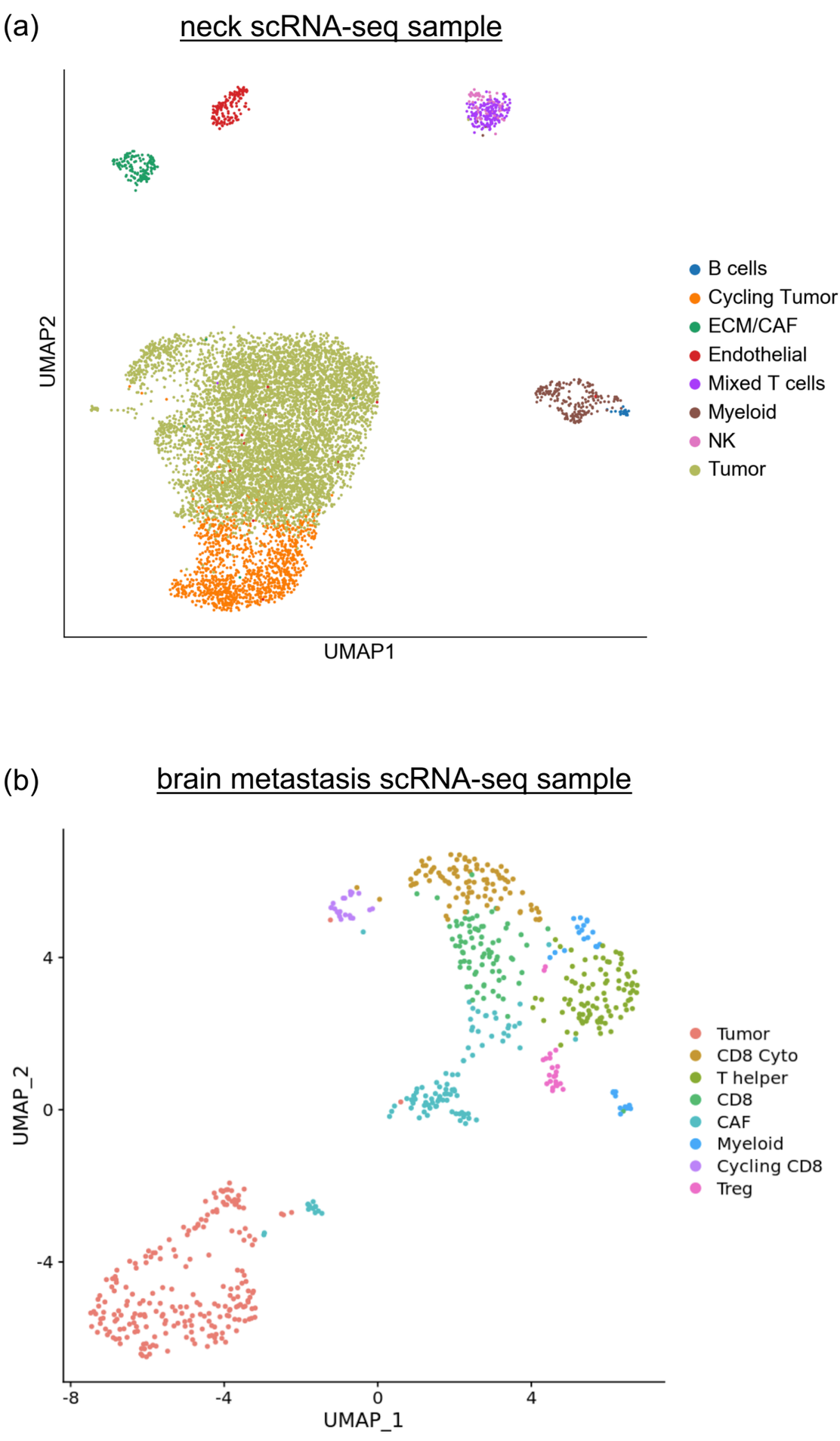
(a)



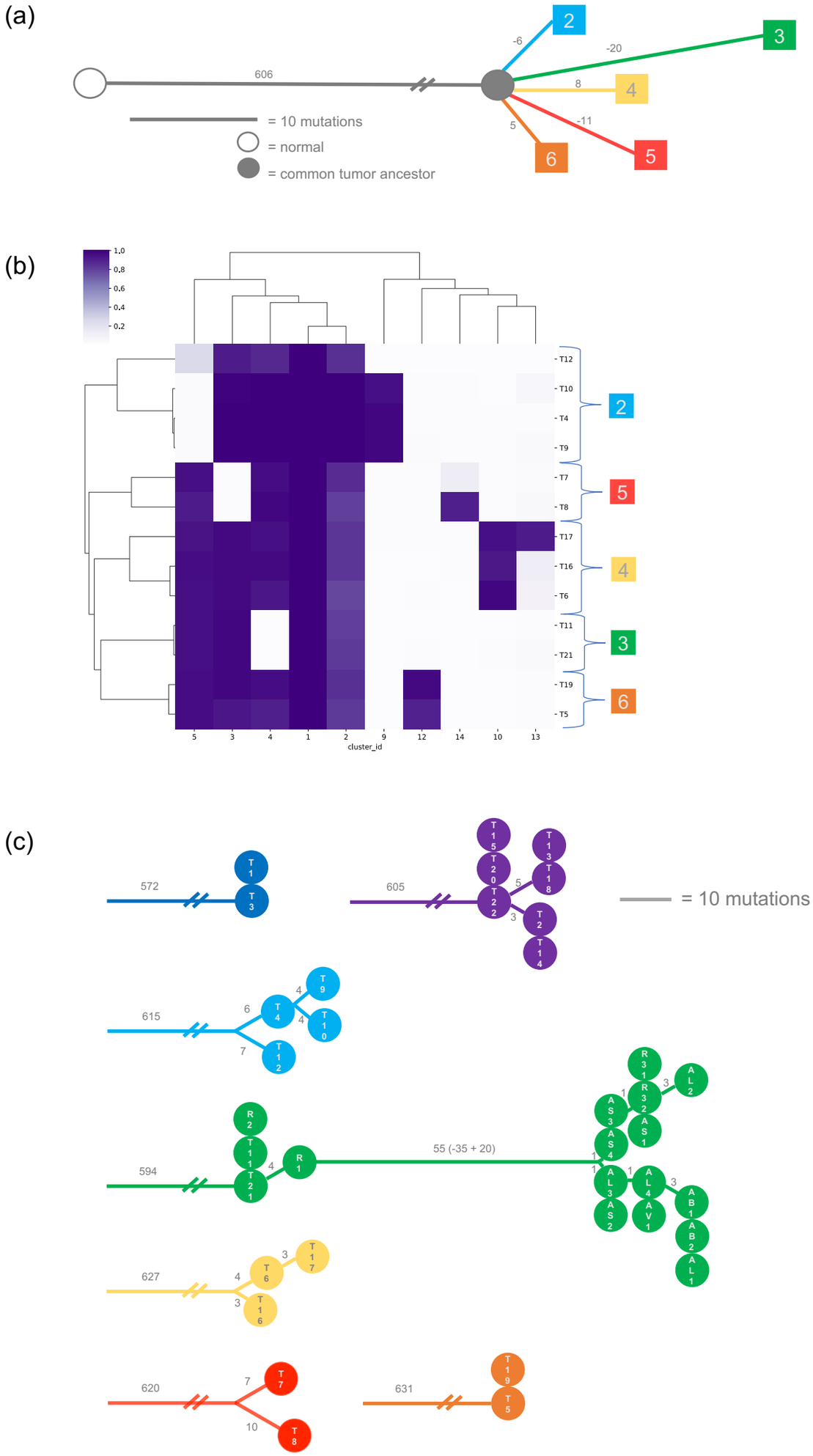
(b)



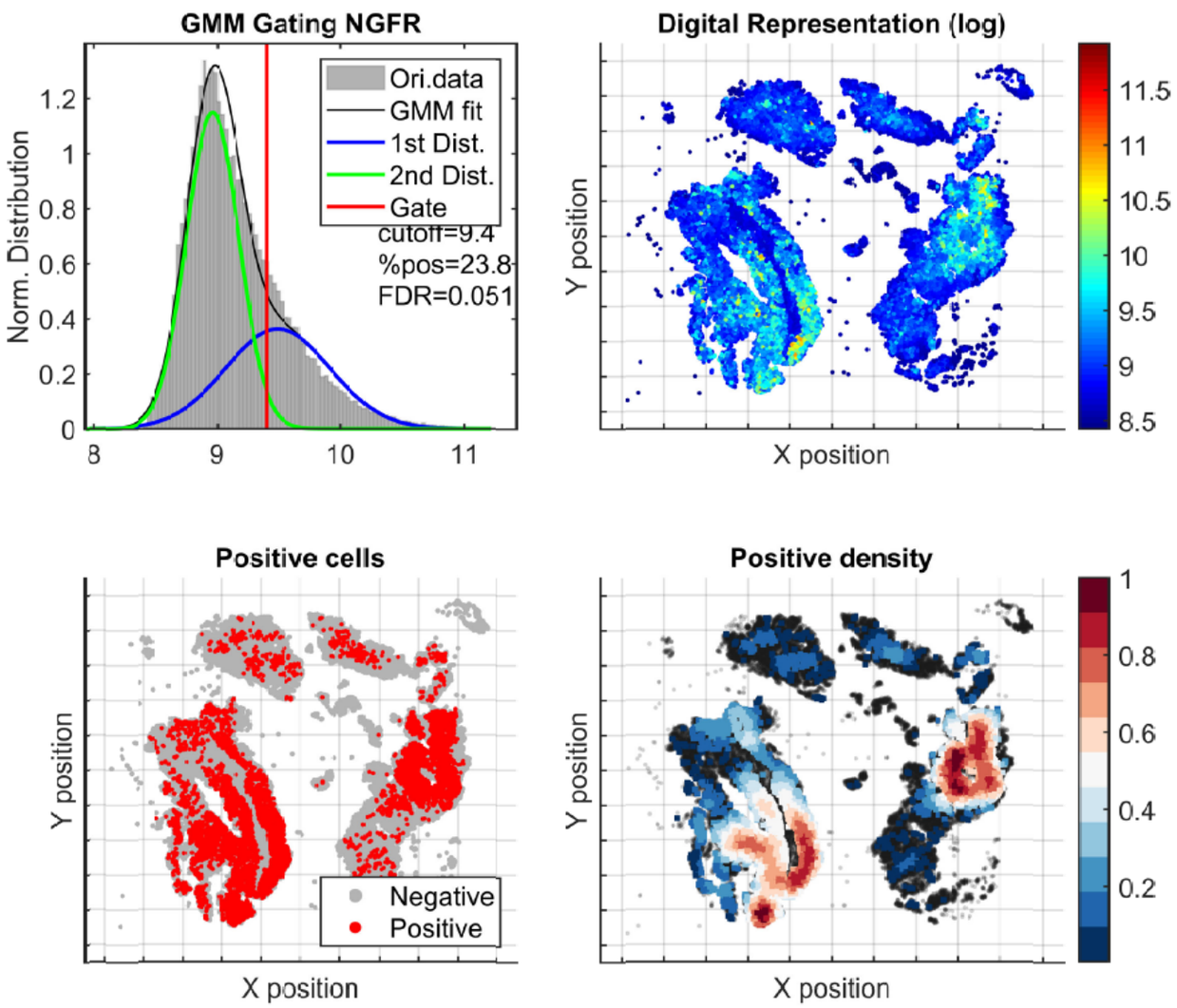
Supplementary Data Figure 6. Immunohistochemistry staining of melanoma samples. (a) NGFR (red) and ERG (endothelial stain, brown) show NGFR+ melanoma cells arranged in a pseudovascular pattern independent from endothelial cells. (b) NGFR (red) and ERG (endothelial stain, brown) show a different pattern of NGFR+ melanoma cells distinct from endothelial cells in a separate part of the tumor.



Supplementary Data Figure 7. scRNA-seq of Cell Types in the Microenvironment of Two Patient Samples. UMAP plots demonstrating clusters of cells from patient 98 (a) neck scRNA-seq 10X sample and the (b) brain Smart-seq scRNA-seq sample



Supplementary Data Figure 8. Phylogeny using PhylogicNDT. (a) phylogenetic tree of lineage 2, 3, 4, 5, and 6 derived from phylogicNDT results of representative tumors from lineage 2, 3, 4, 5, and 6. (b) hierarchically clustered heatmap of inferred cancer cell fractions (CCFs) for each mutation cluster (columns) for each tumor (rows) from selected 5 lineages calculated by phylogicNDT, re-deriving the same 5 different lineages as the pyclone results. (c) individual phylogenies for individual tumors from each lineage based on lineage-specific phylogicNDT results (lineage 0: dark blue; lineage 1: purple; lineage 2: light blue; lineage 3: green; lineage 4: yellow; lineage 5; red, lineage 6: orange)



Supplementary Data Figure 9. Illustration of GMM 1D gating for t-CyCIF data.