DEFINING TRANSCRIPTIONAL PHENOTYPES AND HETEROGENEITY ACROSS BILIARY TRACT CANCERS AND MATCHED PDX MODELS

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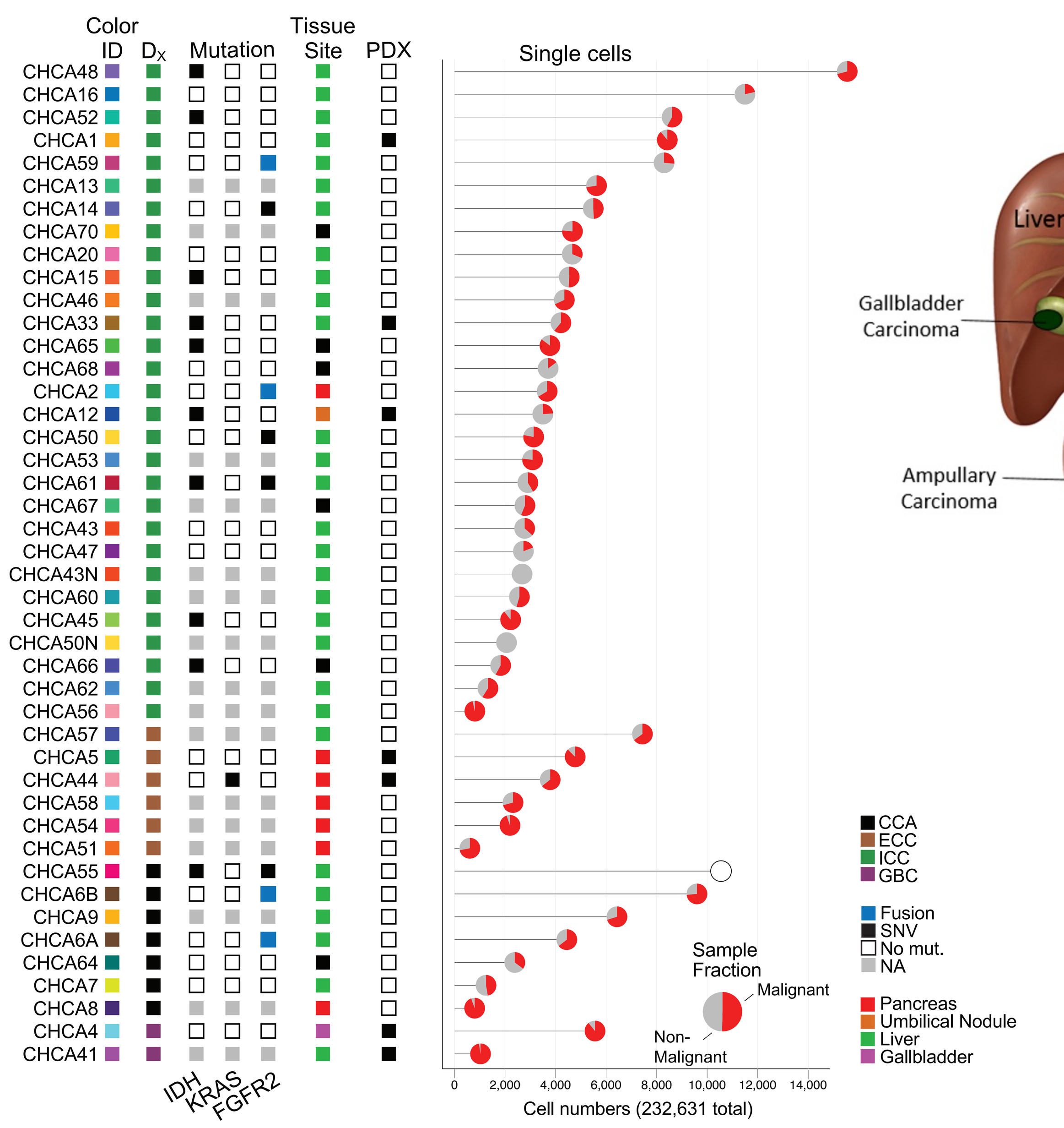
Biliary tract cancers (BTC) are an aggressive family of malignancies characterized by significant heterogeneity and poor patient outcomes. While numerous efforts have characterized BTCs genomically, few studies have focused on the RNA classification of primary BTCs at single-cell resolution. Here, we present our work to identify conserved malignant cell RNA expression states and benchmark PDX model systems against their primary patient counterparts.

Methods: We performed probe-based single-cell RNA sequencing (scRNA-seq; 10X Genomics Flex) on a cohort of BTC resection specimens including intra- and extra-hepatic cholangiocarcinoma and gall bladder carcinoma. A subset of these samples had matched PDX models that were also analyzed with scRNA-seq.

Results: We successfully captured a total of 232,631 patient cells across both malignant and non-malignant compartments. Using non-negative matrix factorization (NMF), we identified multiple conserved malignant cell RNA expression states, some of which were unique to specific disease subtypes (e.g., intra- versus extra-hepatic cholangiocarcinoma). Cross-correlation analysis comparing these single-cell programs to literature-curated gene sets demonstrated overlaps with major states described in prior bulk RNA-seq datasets but also revealed several new gene expression programs. We benchmarked PDX model systems against their corresponding patient tumors and observed variable preservation of clinical states in models.

Clinical and genomic features of the study cohort



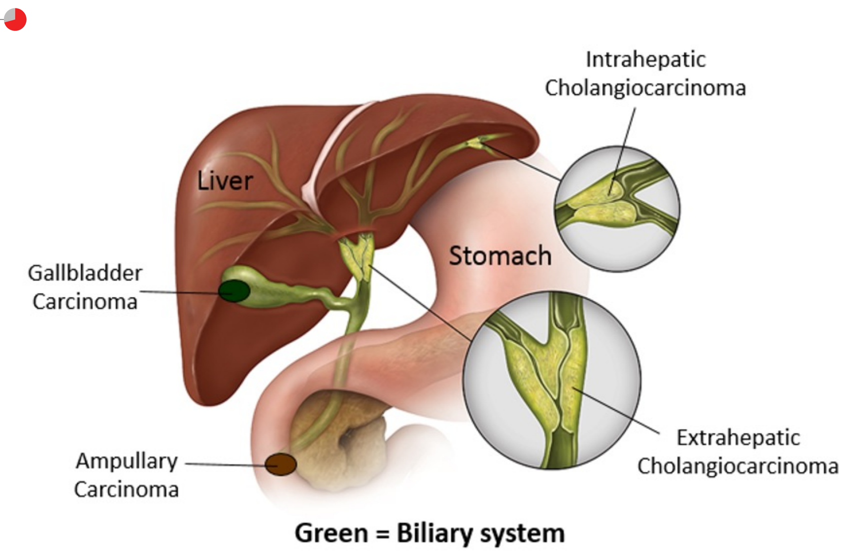


Figure 1. Clinical and genomic features of the study cohort. Mutations were assessed using OncoPanel. The number of single cells captured per biopsy, along with the proportion of malignant and non-malignant cells, is shown on the right.

Conclusions

We are continuing to interrogate this dataset to identify transcriptional programs associated with specific BTC genotypes. In future efforts, we plan to relate our dissociative scRNA-seq findings to spatial transcriptomic measurements across clinical BTC tissue microarrays. We anticipate that these high-resolution maps of BTC tumors and paired patient models will provide a blueprint for understanding how BTC transcriptional states shape malignant cell behaviors and will uncover new approaches to therapeutically target this challenging set of cancers.

Funding sources

NCI/NIH, Dana-Farber Cancer Institute Philanthropic Funds, DFCI Hale Family Center for Pancreatic Cancer Research, Microsoft, Project Ex Vivo

Transcriptional states in patient tumors

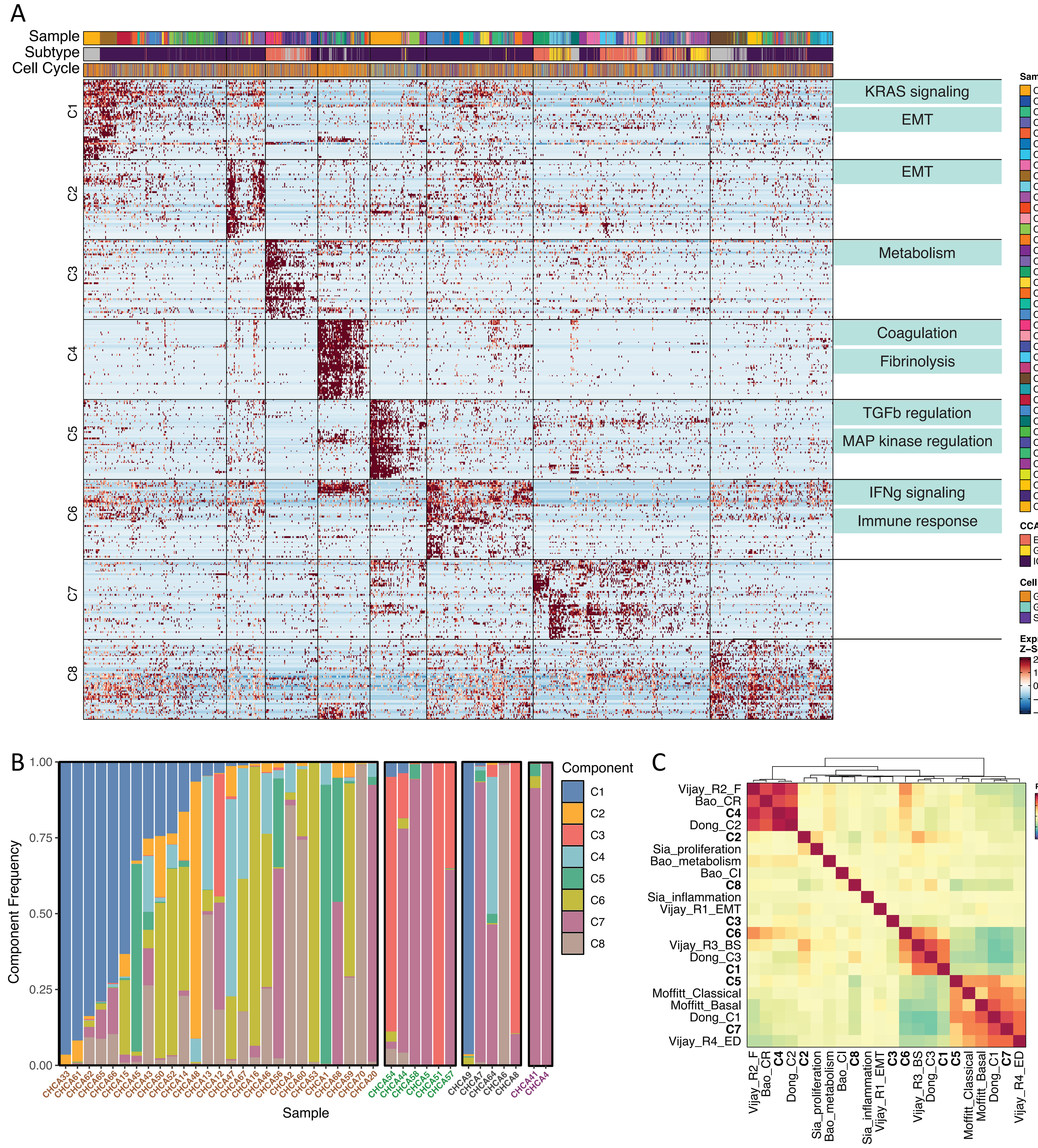


Figure 2. Transcriptional states in patient tumors.
(A) NMF-derived expression signatures in patient samples. Rows represent the top 50 genes for each NMF program; columns are ordered by NMF loading.
(B) Fractional contribution of each NMF program (color fill) within each patient sample.
(C) Cross-correlation analysis comparing each patient-derived single cell's signature score across NMF programs and literature-curated gene sets.

States in PDX models

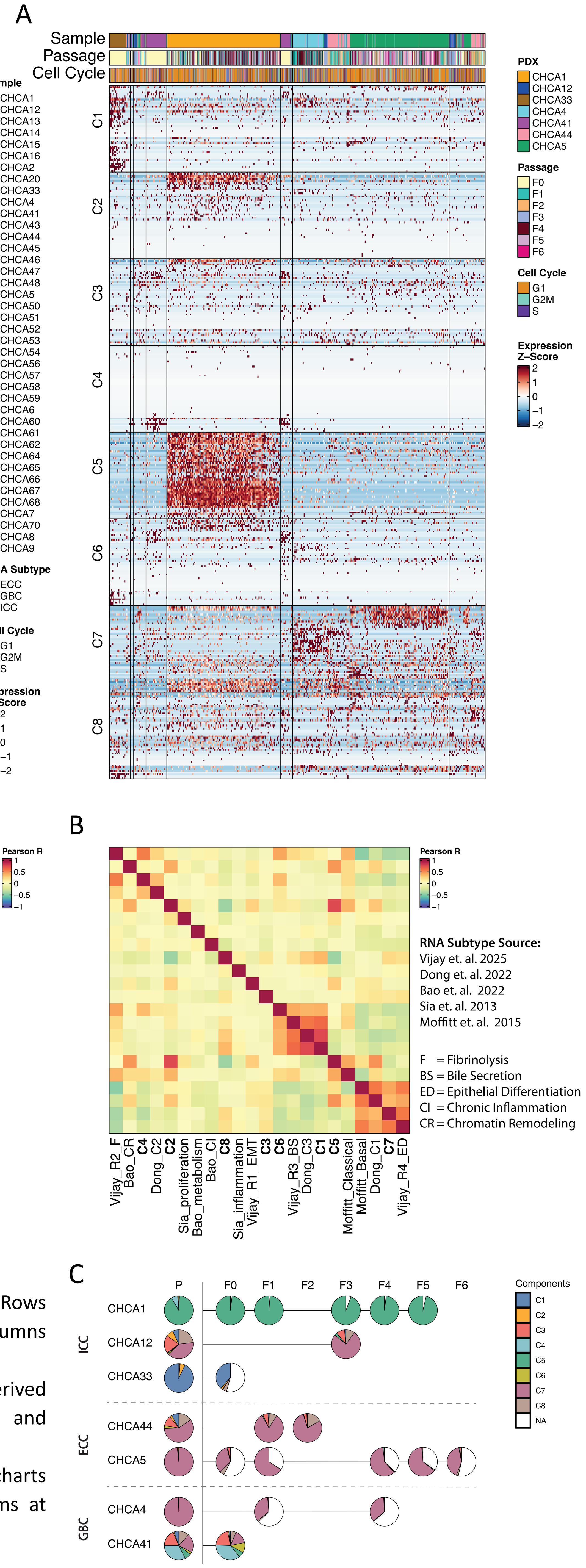


Figure 3. States expressed in PDX models.
(A) NMF-derived expression signatures in PDX models. Rows represent the top 50 genes for each NMF program; columns are ordered by program signature scores.
(B) Cross-correlation analysis comparing each PDX-derived single cell's signature score across NMF programs and literature-curated gene sets.
(C) Swimmer's plot depicting PDX state evolution. Pie charts indicate the fraction of cells expressing NMF programs at each passage.