Mapping the Tumor Immune Microenvironment in intrahepatic, Perihilar and Distal Cholangiocarcinoma

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INTRODUCTION

• Visualization of the cellular heterogeneity and spatial architecture of the tumor microenvironment is becoming increasingly important for understanding the mechanisms underlying immunotherapeutic response and resistance

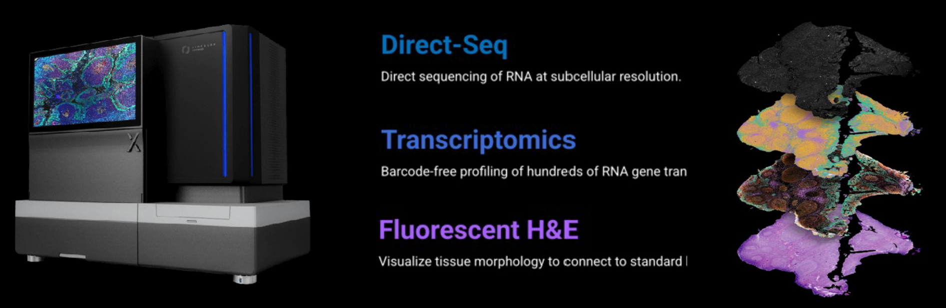
• Anatomical subtypes of CCA differ in their embryonic origin, carcinogenic mechanisms, molecular landscape, and treatment response — yet current systemic therapies are not tailored to these distinctions.

• Direct comparisons of TME spatial organization across distinct anatomical CCA subtypes are lacking — addressing this gap could yield meaningful clinical insights.

METHODS

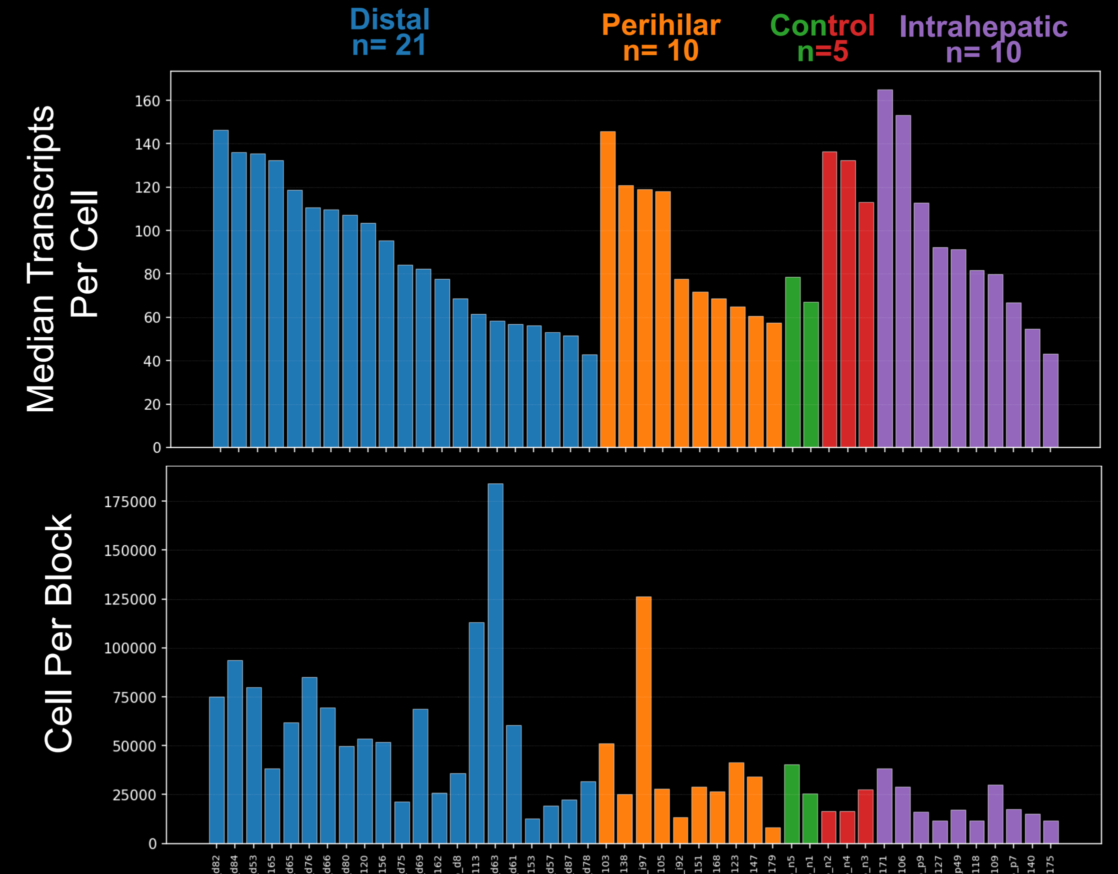
• FFPE tumor tissues spanning the three anatomical CCA subtypes — distal (dCCA, n=21), perihilar (pCCA, n=10), and intrahepatic (iCCA, n=10) — were profiled alongside matched non-tumor controls (n=5).

• Tissues were processed on the G4X Spatial Sequencer (Singular Genomics), an integrated spatial multi-omics platform delivering 500-plex in situ transcriptomics with a custom CCA-tailored panel, alongside fluorescent H&E, at subcellular resolution across a 4.5 × 4.5 mm imaging area.



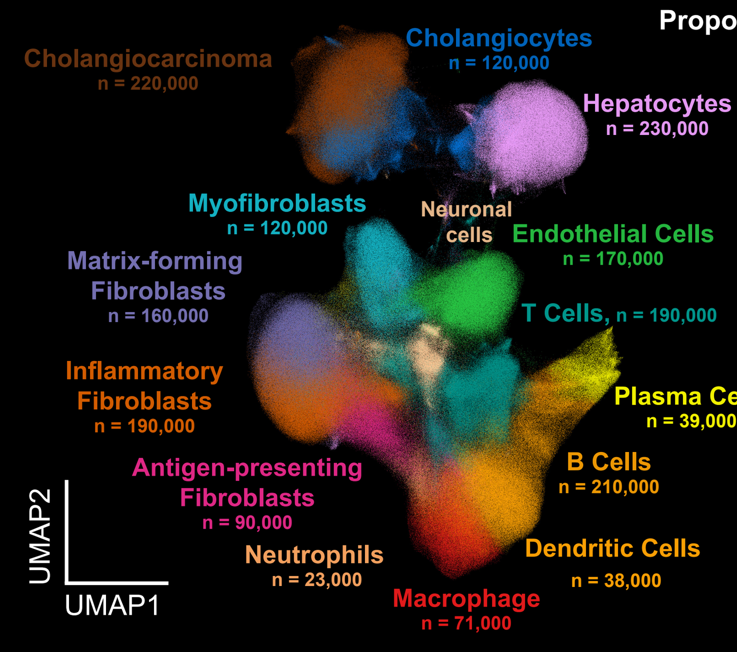
RESULTS

Quality Control Metrics

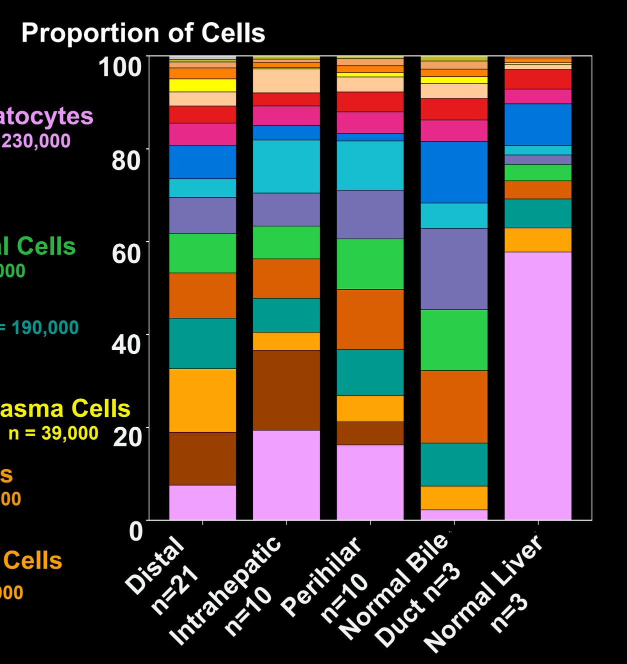


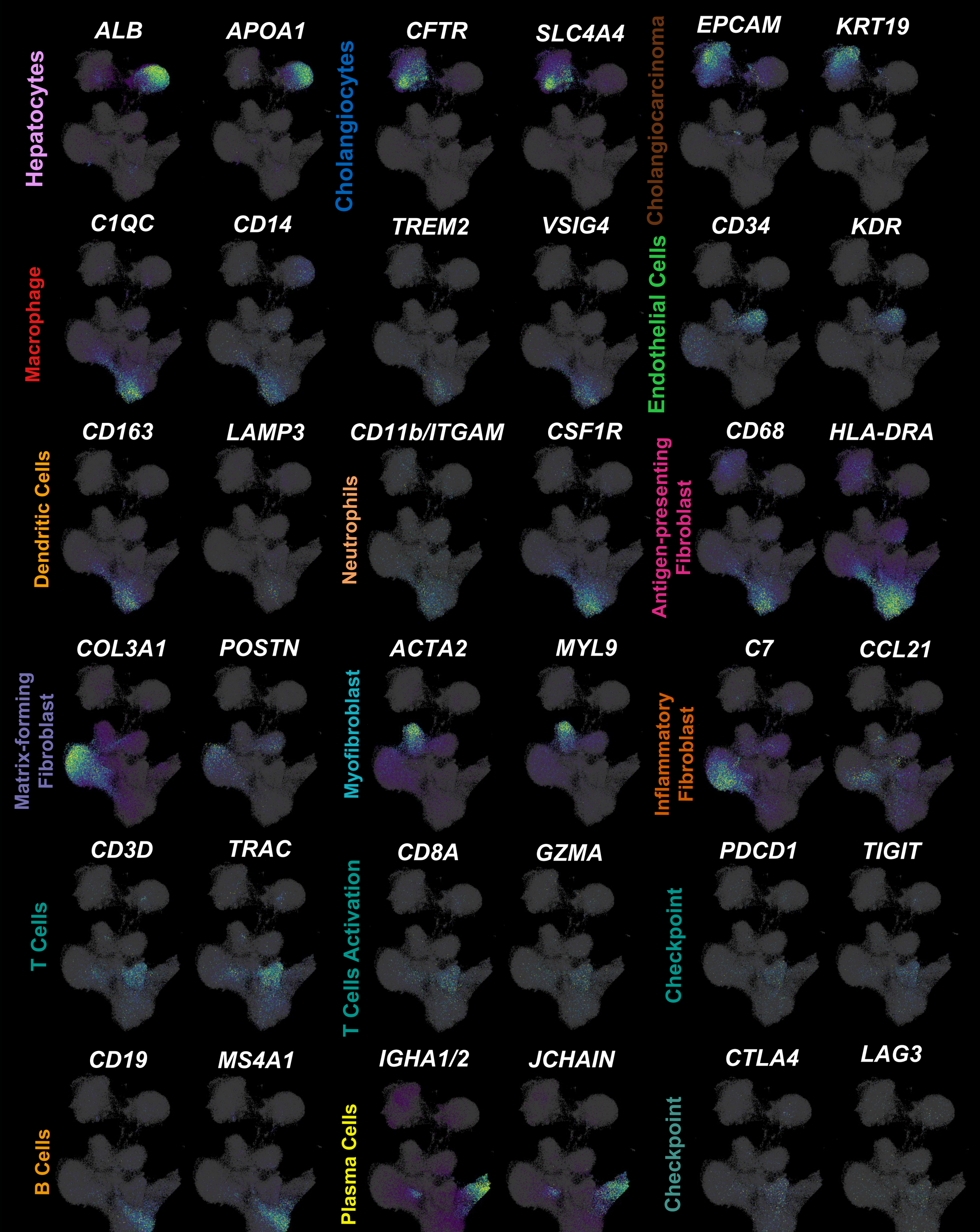
SINGLE CELL & NICHE ANALYSIS of CHOLANGIOCARINOMA MICROENVIRONMENT

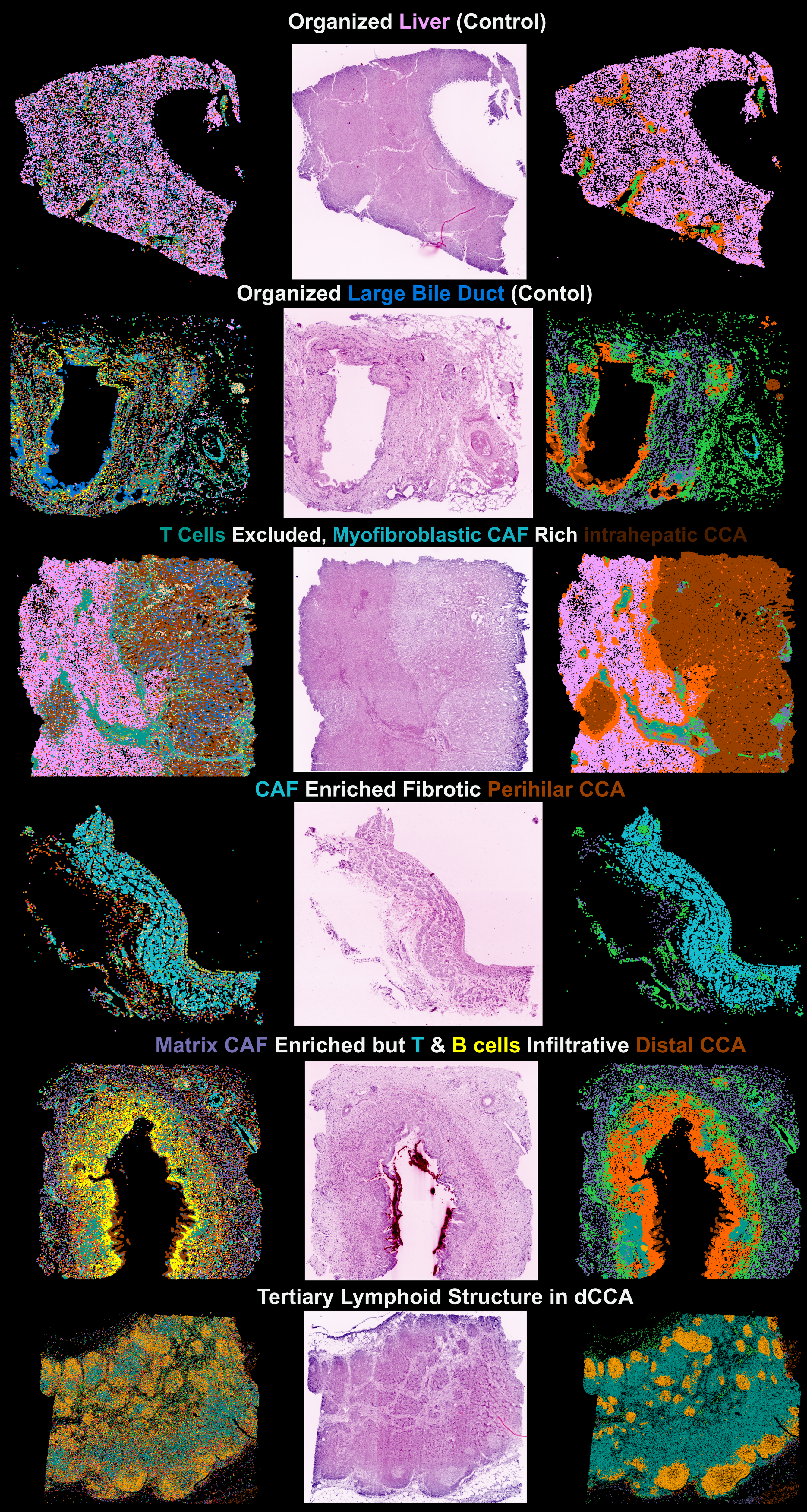
2 Million Cells, 41 CCA Samples & 5 Controls



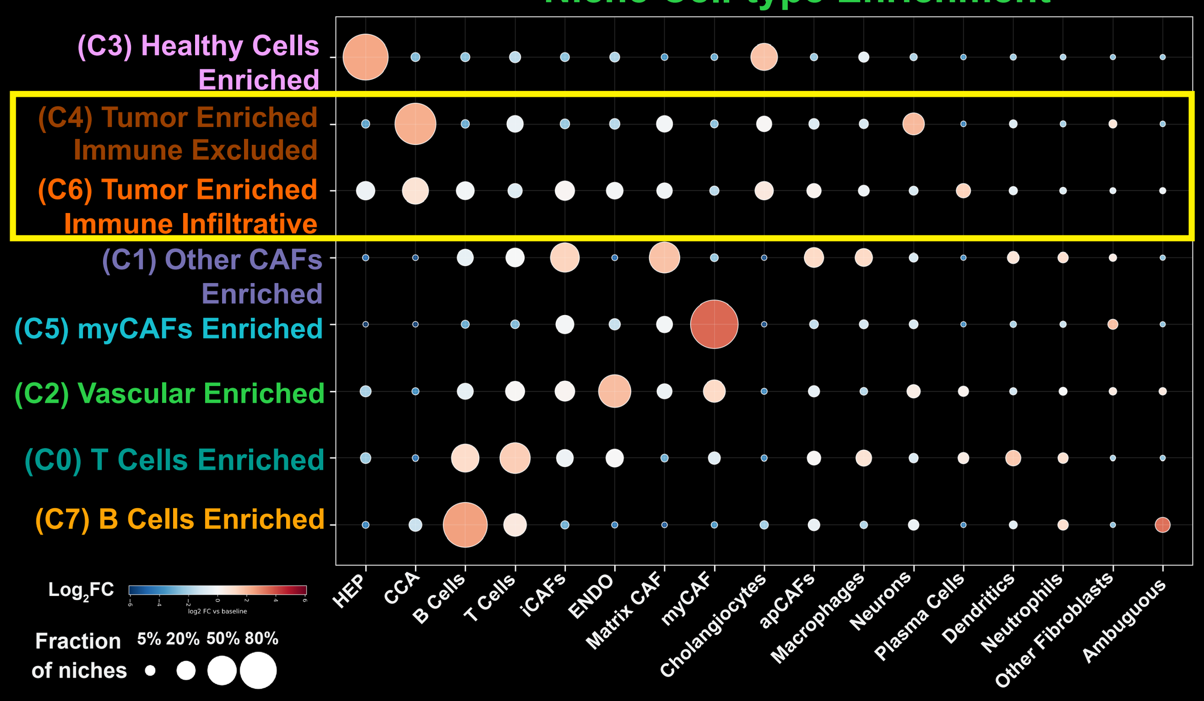
↑ B/Plasma cells in dCCA & ↑ T cells / myCAF in iCCA/pCCA



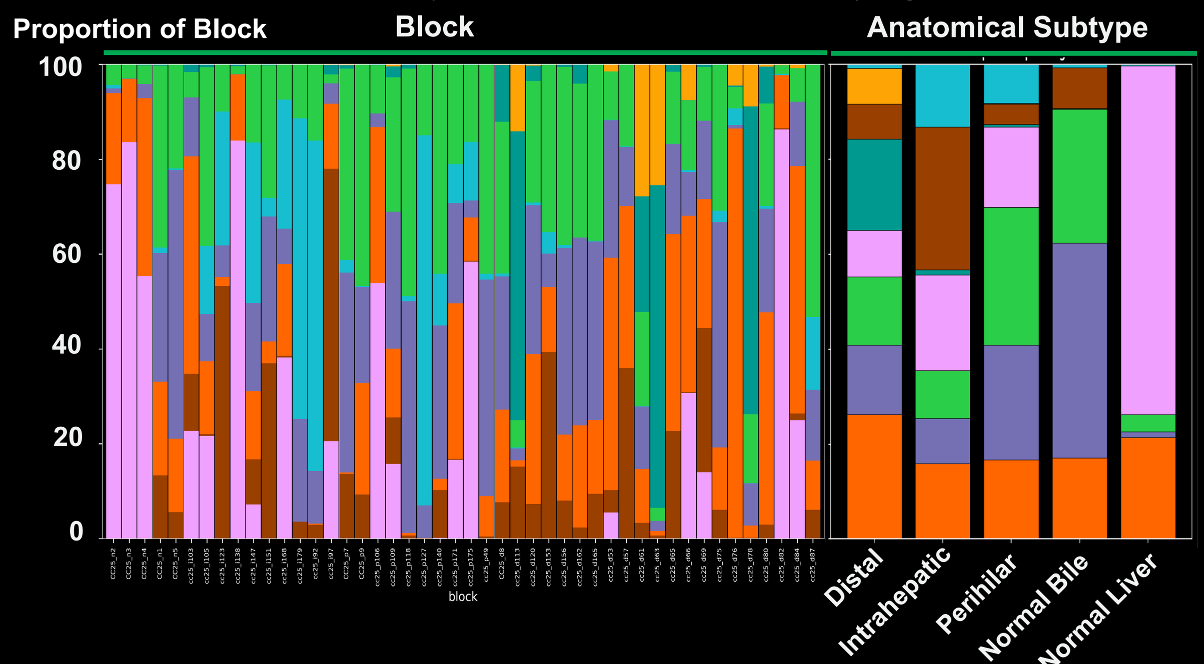




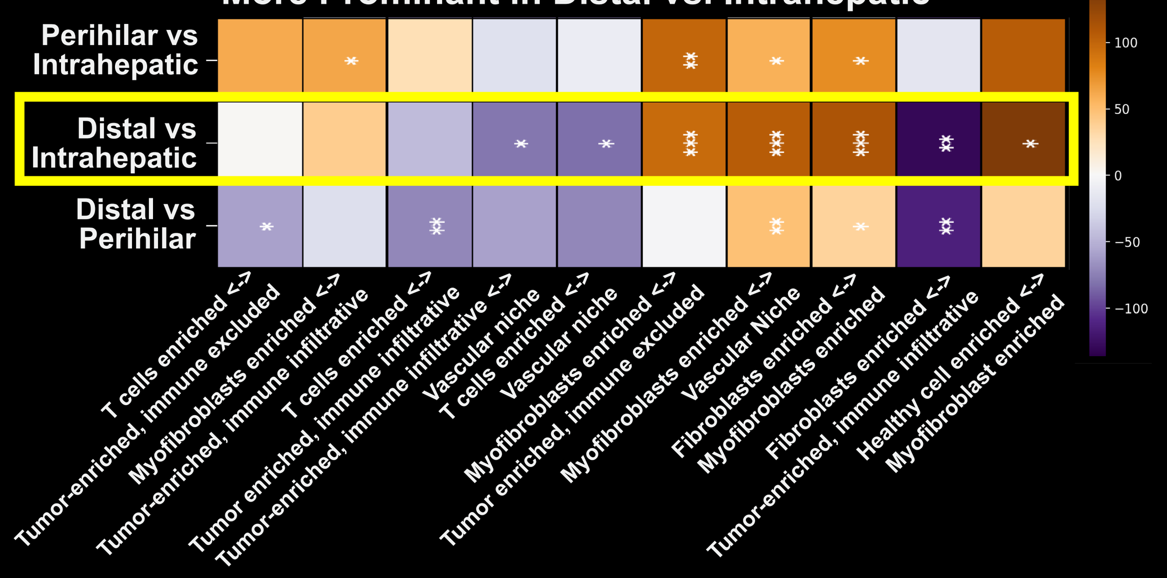
Niche Cell-type Enrichment



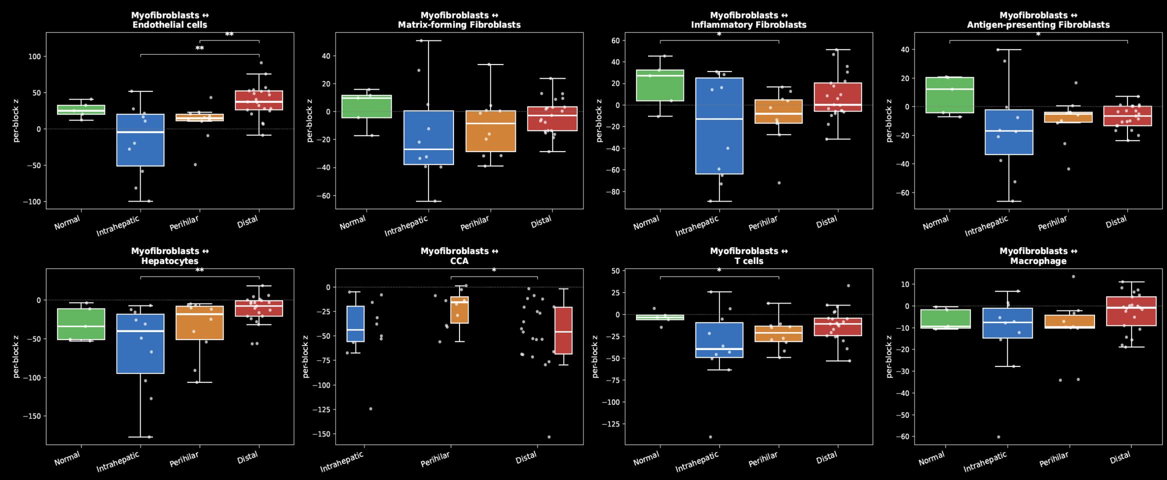
Niche Composition - ↑ Immune Niche in dCCA, ↑ myCAF in i/pCCA



Differential Niche Neighborhoods - More Prominant in Distal vs. Intrahepatic



Cell Level - Neighborhood analysis



SUMMARY & FUTURE STEPS

• 2 Million cells were interrogated across the 41 iCCA FFPE and 5 normal control samples, with the expression of 500+ genes captured, enabling us to characterize the CCA TMEfrom diverse subtype in high resolution and granularity

• Anatomical subtype displayed a distinct TME architecture: dCCA was enriched in B/plasma cells and harbored tertiary lymphoid structures (TLS) with an immune-infiltrated phenotype; iCCA showed an immune-excluded pattern; pCCA was dominated by myofibroblastic CAFs (myCAFs) and T cells, forming a fibrotic barrier.

• Niche-level analysis revealed cellular neighborhoods that were differentially enriched across subtypes indicating that anatomical origin shapes the immune and stromal landscape.

• Future work will focus on multi-regional analysis to analyse the tumor heterogeneity and define recurring patterns of TME with correlation to treatment response and clinical outcome

PATIENT FRIENDLY SUMMARY

• Cholangiocarcinoma (bile duct cancer) can start in three different parts of the biliary tree — inside the liver (intrahepatic), at the liver's exit (perihilar), or closer to the intestine (distal). Although these are often treated the same way, they may actually behave as distinct diseases.

• Every tumor is a mixture of cancer cells and many non-cancerous cells. Some non-cancerous cells help the body fight the tumor; others — or even the same cells in a different state — protect the cancer and block treatment from working. Together they make up the tumor microenvironment, which is central to whether therapy succeeds.

• Using a new technology that maps millions of cells without disturbing the tissue, we built a detailed atlas of each subtype and found they look strikingly different:

-Distal tumors contained organized clusters of immune cells resembling mini lymph nodes — a sign of active anti-tumor immunity.

-Intrahepatic tumors largely locked immune cells out, a pattern linked to treatment resistance.

-Perihilar tumors were filled with fibroblasts that build thick walls around the cancer, shielding it from immune attack.

• These differences suggest that immunotherapy and other treatments may need to be tailored to where the cancer arose — and our map points to specific cell types that could be targeted to make therapy work better for each subtype.

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