

PhenoCycler (CODEX) Ultra-high Plex Single Cell Imaging of the Liver Immune Landscape to Understand the Effect of Aging and High Fat Diet on development of Hepatocellular Carcinoma.

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BACKGROUND

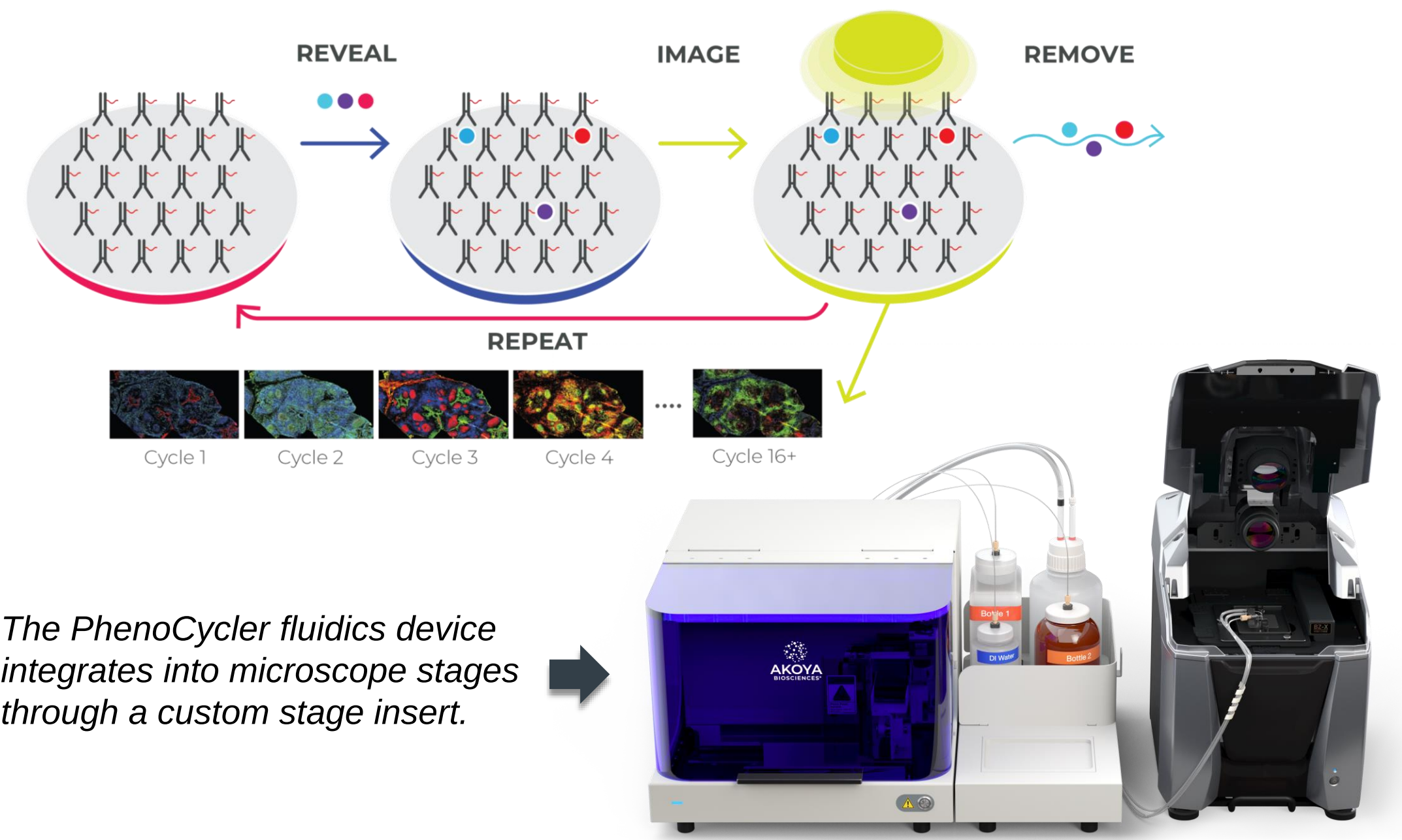
Hepatocellular carcinoma (HCC) is an emerging health crisis due to rapidly increasing incidence rates and an overall 5-year survival rate of 18%. Consequently, there is an urgent need for new therapies and better preventive strategies. Aging and fatty liver are two of the biggest risk factors for HCC, and with ever-increasing aging and obese populations, uncovering the role of aging and fatty liver in the evolution of HCC has become imperative. Aging and obesity are well known to be characterized by immune dysregulation leading to persistent inflammation, and a dysregulated immune microenvironment is also a common theme shared by multiple hallmarks of HCC. In this study, we aim to assess the immune microenvironment of aged mice with fatty livers by performing spatial phenotyping of distinct immune signatures which can potentially predispose hepatocytes to HCC. To address this, we used Akoya's ultra-high multiplexed PhenoCycler (formerly CODEX) imaging system to interrogate 31 different biomarkers in liver samples at the single cell level. Specifically, we compared the liver immune microenvironment of mice within 2 different treatment groups (old mice on a high fat diet (HFD) and young mice on a normal diet (ND)) to the immune landscape of liver tumor tissue. Our goal is to identify distinct immune phenotypes present in both, liver tumor tissues and livers of HFD mice, compared to ND livers – to better understand how immune dysregulation can predispose an aged and fatty liver to HCC.

METHODS

CODEX® Technology makes use of reversible hybridization between the barcodes of CODEX® antibodies and their complementary oligos conjugated to fluorescent dyes (Reporters) to reveal tens of CODEX® antibodies sequentially

STAIN: Fresh-frozen mouse liver tissues were stained with 31 oligo-barcoded CODEX antibodies in a single step.

IMAGE: Antibodies were visualized with fluorescent CODEX® reporters via iterative reveal-image-remove cycles. For each cycle, up to three CODEX® Reporters, each with a spectrally-distinct dye, are applied to the stained tissue to the corresponding barcode. This process is repeated until all antibodies have been imaged.



ANALYZE: Tissues were imaged with a Keyence BZ-X700/800 series epifluorescence imaging system. and data were analyzed using QuPath (Quantitative Pathology & Bioimage Analysis, <https://qupath.github.io/>) Software and CytoMAP (Gerner Lab, Department of Immunology, University of Washington, Seattle, WA).

RESULTS

Figure 2. Dramatic expansion of immunologically rich 'germinal centers' and CD24+ hepatocyte progenitors adjacent to ducts in aged HFD mice.

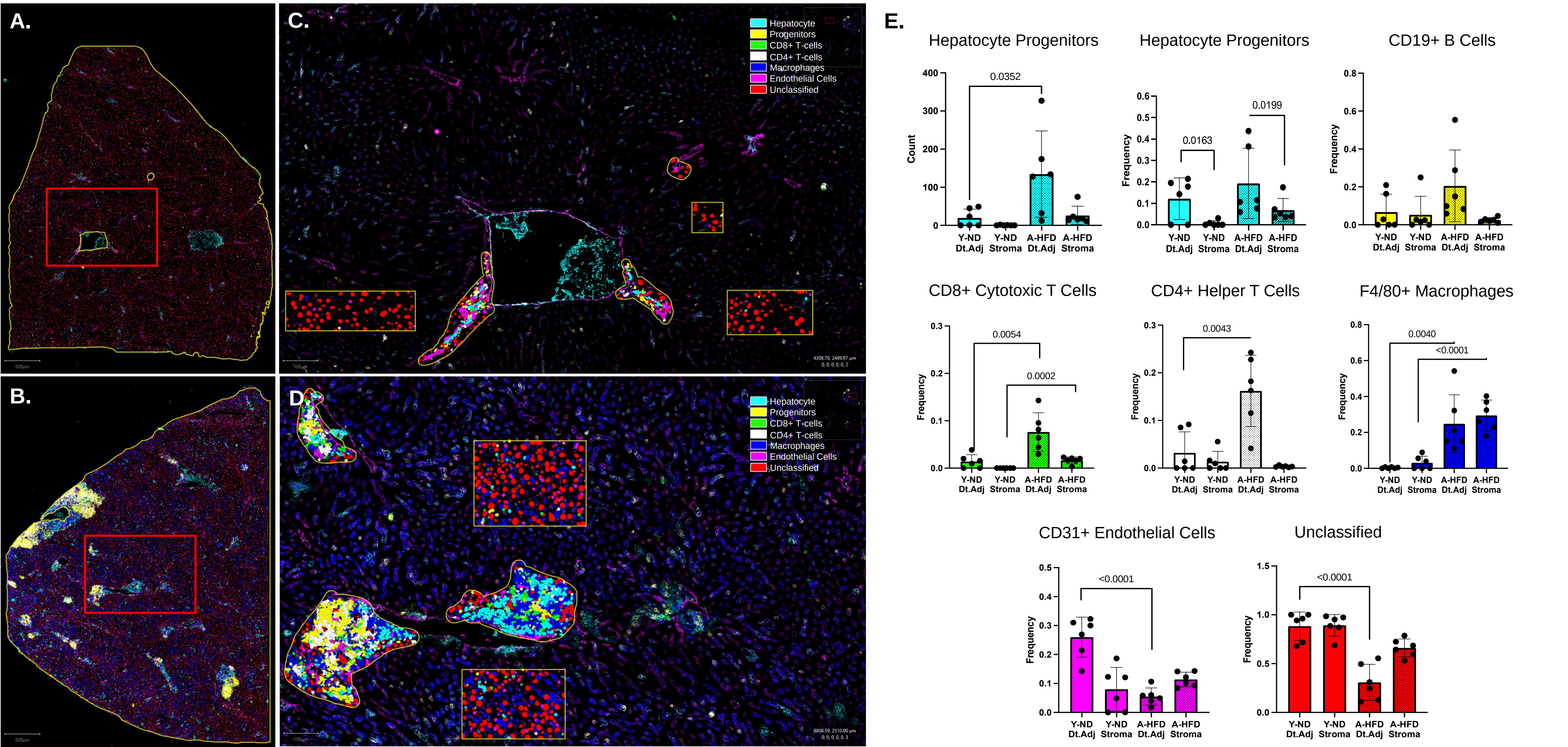


Figure 1. T-cells from HFD liver are phenotypically exhausted.

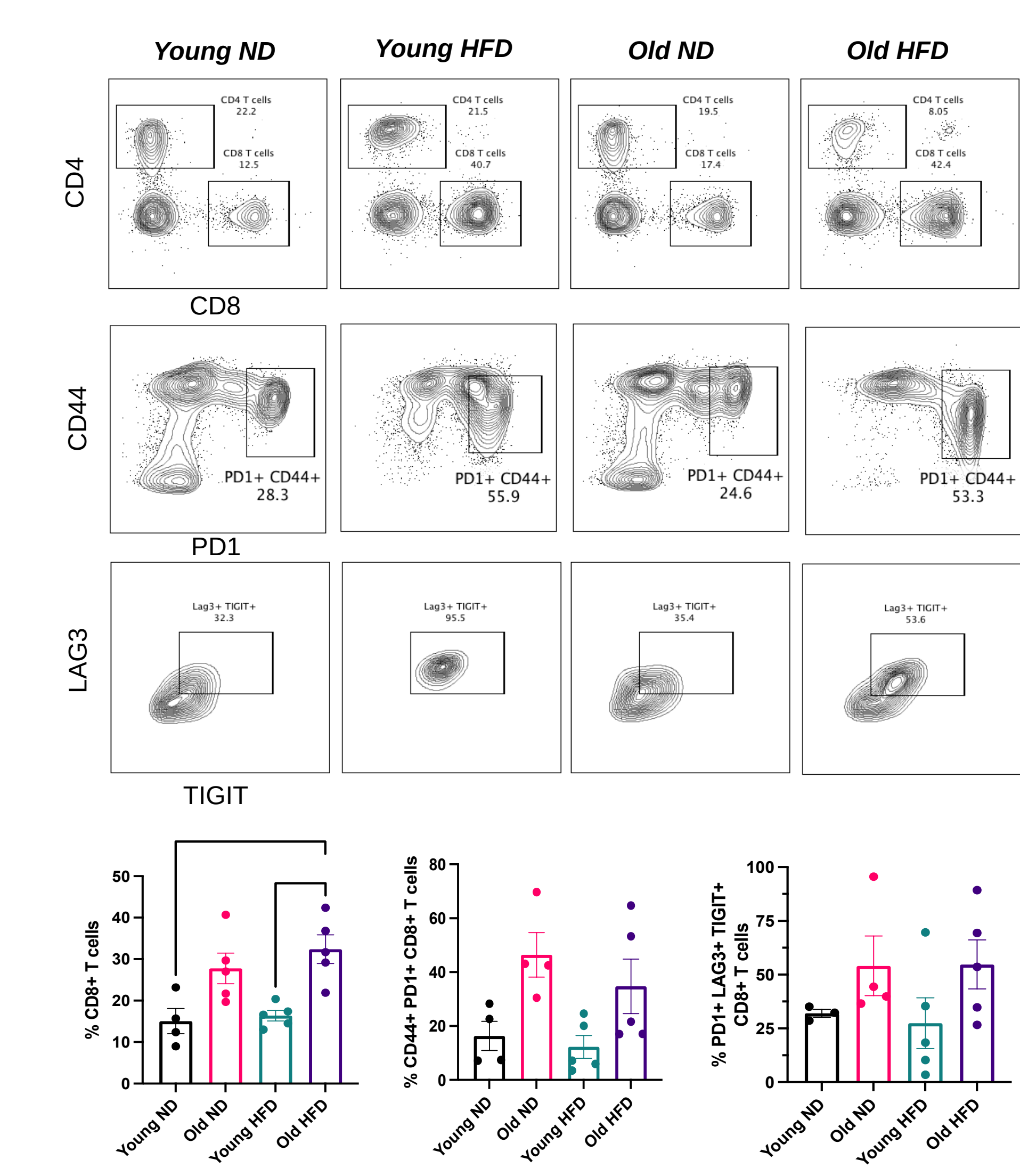


Figure 1: T cells from HFD liver are phenotypically exhausted. Flow Cytometry on T cells from young and old mice livers with Normal Diet(ND) or High Fat Diet(HFD). immunophenotyping the aged HFD and young normal liver T cells, show an increase in the total number of CD8+ T cells and an increase in markers of exhaustion in the aged liver

Figure 2: Dramatic expansion of immunologically rich 'germinal centers' and CD24+ hepatocyte progenitors adjacent to ducts in Aged High Fat Diet Liver. A. Young normal and C. Aged HFD livers. C,D. zoomed in view of the nodules in young vs aged HFD respectively and single overlays of the markers listed in the legend E. The regions in the yellow outline were used for the quantification of cell types in duct adjacent nodules and liver stroma in aged HFD vs young normal.

Figure 3. Expansion of immune cells in liver cancer in both germinal centers and liver stroma.

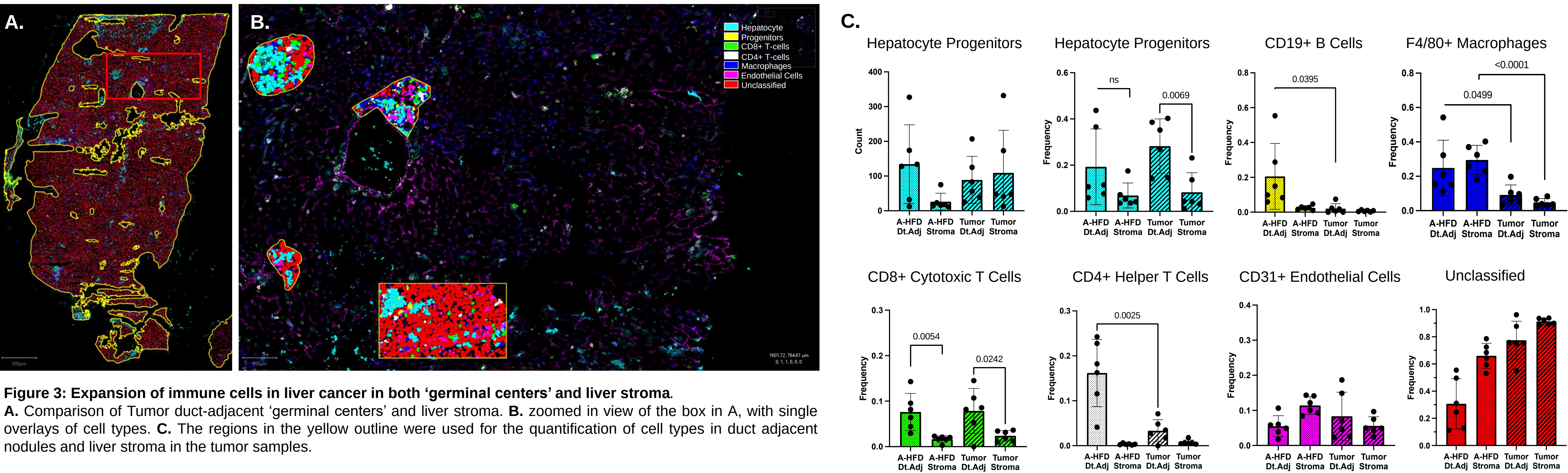


Figure 3: Expansion of immune cells in liver cancer in both 'germinal centers' and liver stroma. A. Comparison of Tumor duct-adjacent 'germinal centers' and liver stroma. B. zoomed in view of the box in A, with single overlays of cell types. C. The regions in the yellow outline were used for the quantification of cell types in duct adjacent nodules and liver stroma in the tumor samples.

CONCLUSIONS

These data demonstrate a significant expansion of immunologically rich clusters of cells that resemble germinal centers observed in livers of aged mice fed with HFD and in HCC.

Aged livers and HCC tumors appeared highly similar in immuno-composition and further support the notion of aging and fatty liver disease as a precursor to HCC.

We further investigated the immune cell landscape in young compared to aged fatty livers and found exhaustion of otherwise functional T cells. These data suggest an attempt to counteract exhaustion by expanding germinal centers, opening potential therapeutic avenues to stimulate germinal centers to induce anti-tumoral immunity before the onset of HCC.