Background Hepatocellular Carcinoma (HCC) is considered a prototype of inflammation-derived cancer arising from chronic liver injury. The cellular composition of the HCC tumor immune microenvironment (TiME) has a major impact on cancer biology as the TiME influences tumor initiation, progress, and response to therapy. Mucosal-associated invariant T (MAIT) cells can represent the most abundant T cell subtype in the human liver and are assigned crucial roles in regulating immunity and inflammation in the context of infection, albeit their role in HCC remains elusive.

Methods Study design is displayed in figure 1. High-dimensional flow cytometry (n=37) and scRNA sequencing (n=8) was used to analyze MAIT cell phenotypic changes in patient tissue samples. Highly-multiplexed immunofluorescence microscopy was used to quantify immune cell infiltration in paired human HCC samples. We developed and validated a 37-plex antibody panel and applied CODEX technology to simultaneously profile in situ expression of 37 proteins at sub-cellular resolution in n=15 HCC patient samples using whole slide scanning. We established an image analysis pipeline using a machine learning (ML) algorithm (S³-CIMA) to quantify the MAIT cell interaction network at the HCC invasive front. Murine models of orthotopic HCC using transgenic mouse strains were used for in vivo validation, a co-culture system using PD-L1 and CSF1R blocking strategies or depletion of TAMs using LysmCre x Csf1rLsL-DTR transgenic mice increased MAIT cell infiltration (p<0.05) within the HCC TiME and show previously underappreciated heterogeneity as seen by scRNA-seq. CODEX imaging revealed the distinct cellular composition of the MAIT neighborhood in human HCC tissue. This allowed for in-depth characterization of cellular interaction networks underlying the MAIT neighborhood in human HCC tissue. S³-CIMA, a novel ML method, to analyze our spatially nuanced interactions between MAITs and PD-L1+CSF1R+ TAMs localized in the adjacent (non-tumor) liver as key regulatory elements of MAIT cell dysfunction.

Results Hepatic MAIT cells in n=37 patient samples are characterized by impaired infiltration (p<0.001) into tumor lesions, decreasing dysfunction (e.g. upregulation of PD-1 (p<0.05) & reduced IFN-γ (p<0.01) within the HCC TiME and show previously underappreciated heterogeneity as seen by scRNA-seq. CODEX imaging revealed the distinct cellular composition of the MAIT neighborhood in human HCC tissue. This allowed for in-depth characterization of cellular interaction networks underlying the MAIT cell dysfunction in HCC. S³-CIMA, a novel ML method, to analyze our spatially resolved immune cell atlas of human liver cancer identified interactions of CSF1R+PD-L1+ tumor-associated macrophages (TAMs) and MAIT cells localized in the adjacent (non-tumor) liver as key regulatory elements of MAIT cell dysfunction. Finally, perturbation of this detrimental cell-cell interaction using PD-L1 and CSF1R blocking strategies or depletion of TAMs using LysmCre x Csf1rLsL-DTR transgenic mice increased MAIT cell infiltration (p<0.05) into murine HCC lesions and reinvigorated the cytotoxic MAIT cell phenotype (p<0.01).

Conclusions This work provides evidence that MAIT antitumor immunity and response to ICB therapies relies on organized, spatially nuanced interactions between MAITs and PD-L1+CSF1R+ TAMs within the tumor immune microenvironment.