Characterization of HBV and co-infection with HDV and HIV through spatial transcriptomics

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Supplemental Material

Supplementary table 1. ROI annotations

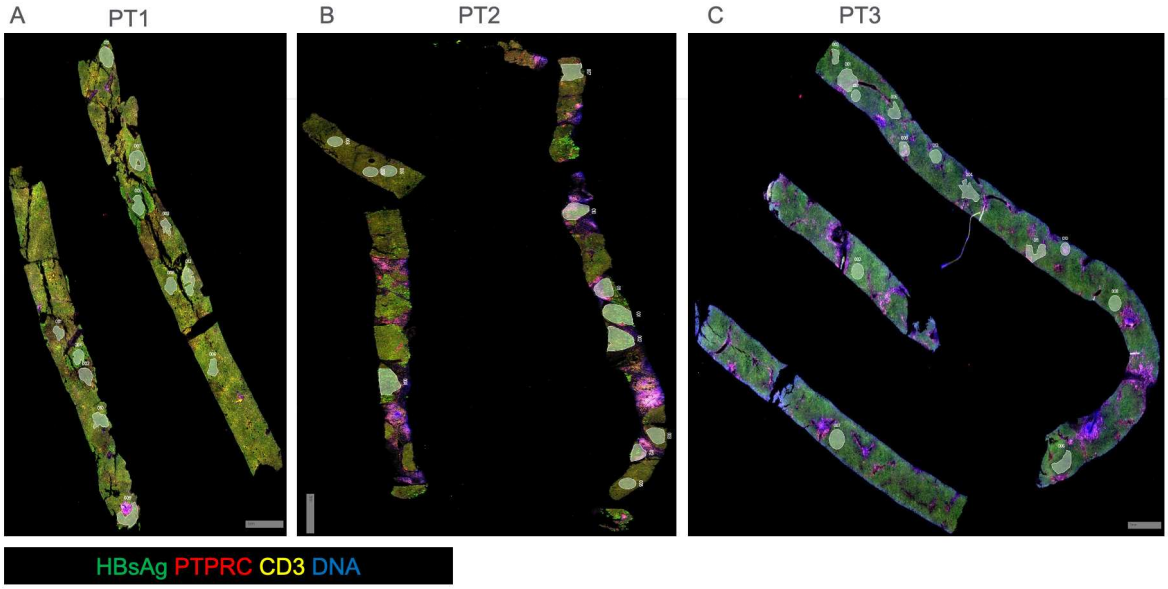
Supplementary table 2. Differential gene expression (DEG) of immune cell and HBsAg presence in ROIs

Supplementary table 3. Gene Ontology Biological Processes (GO.BP) Pathways of DEG in immune ROI

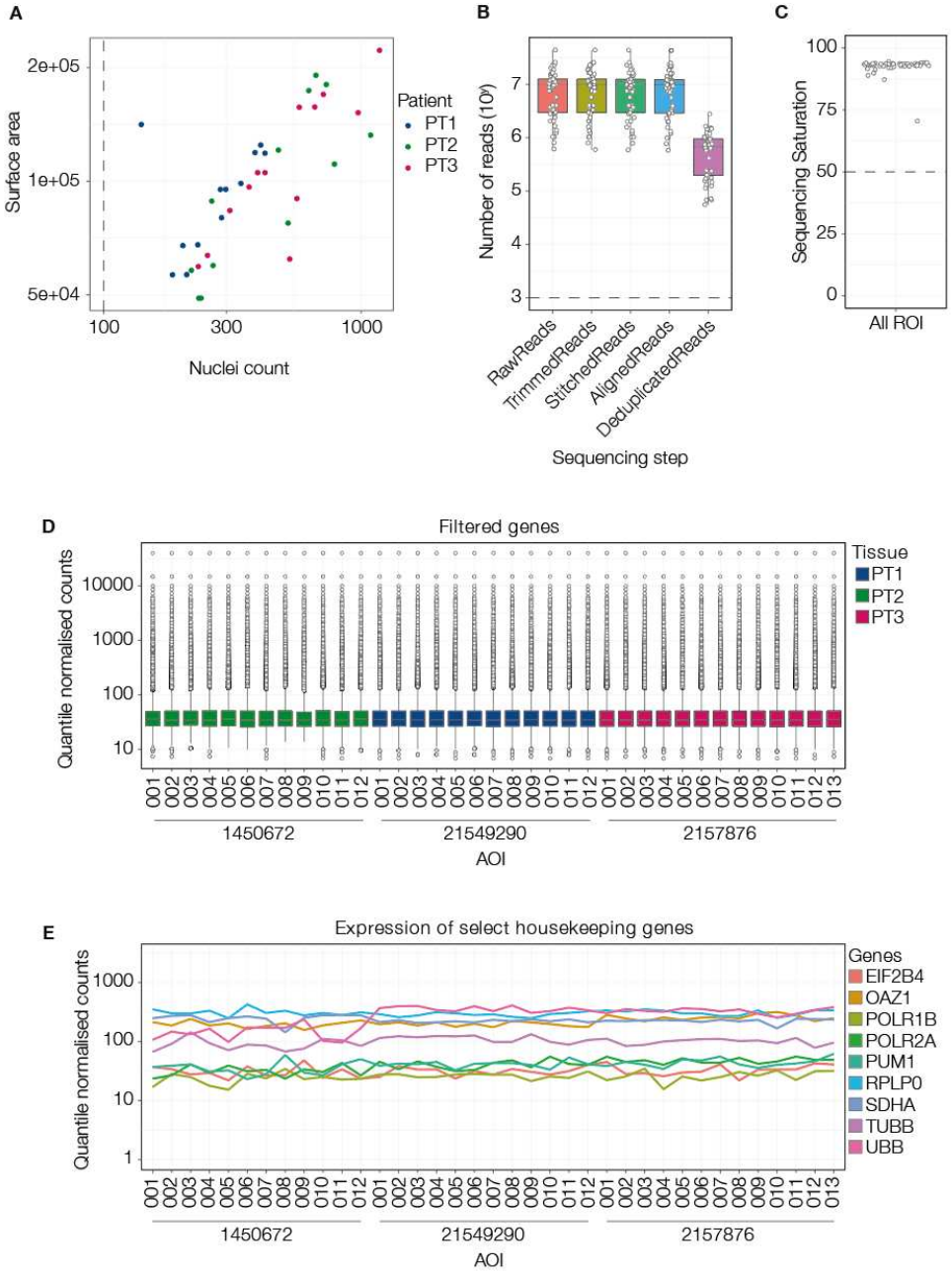
Supplementary table 4. WGCNA Modules and Genes

Cross A, *et al. eGastroenterology* 2024; 2:e100067. doi: 10.1136/egastro-2024-100067

Supplementary Figures

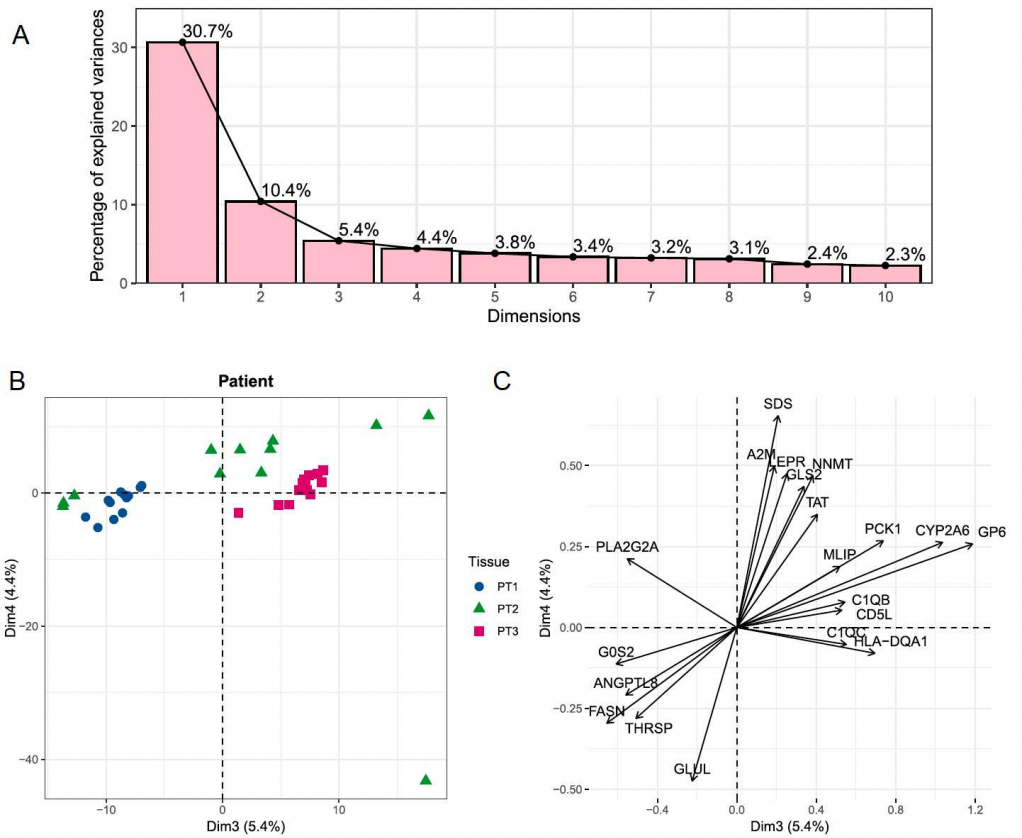


Supplementary figure 1: Multiplex immunofluorescent images and all selected ROIs for each patient tissue analysed. PT1- HBV (A), PT2 – HBV/HDV (B) and PT3- HIV/HBV (C). Morphology markers shown HBsAg (Green), PTPRC (CD45 – Red), CD3 (Yellow) and DNA dye (Blue). A geometric strategy was employed for selection of ROIs, which were segmented on the basis of HBsAg staining and CD45 staining to capture the presence of the wider immune cell infiltrate.

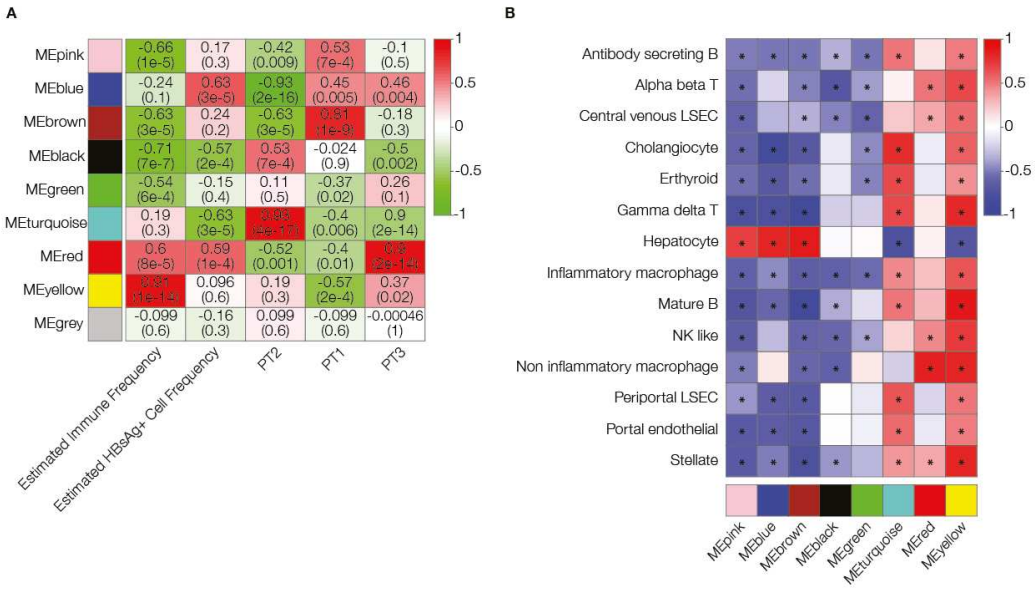


Supplementary figure 2: Quality metrics of sequencing and gene normalisation. The surface area and estimated nuclei count characteristics of the 37 regions of interest (ROI) are shown (A). All ROI passed manufacturer recommended thresholds for read counts as

raw, trimmed, stitched, aligned and deduplicated reads (B). Sequencing saturation of all ROI were above NanoString recommendations of 50% (C). The distribution of the 9672 detectable genes after quantile normalization (D) and consistency of select housekeeping gene expression post-normalisation is shown (E).



Supplementary figure 3: Principal Component Analysis (PCA) dimensions. (A) PCA analysis indicating the percentage contribution from each dimension. The third and fourth dimensions produced by principal component analysis annotated by (B) biopsy and (C) top contributing genes.



Supplementary figure 4: Characterization of WCGNA modules of co-expressed genes in HBV biopsy tissue. The correlation between module eigengene expression and region of interest (ROI) traits, such as estimated immune cell frequency per ROI group, estimated HBsAg+ cell frequency per ROI group and tissue identity is shown (A). P values are shown in brackets. The correlation between module eigengene expression and the relative abundance of cells, derived from cell deconvolution methods, is shown in (B). * indicates $P < 0.05$.