



# Chromatin profiling from formalin-fixed paraffin-embedded (FFPE) samples for biomarker discovery

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## THE PROBLEM: Patient-derived FFPE tissues hold a wealth of potential information but are challenging to leverage for genomics

- **Billions** of banked FFPE samples could enable biomarker discovery & drug development
  - Leveraging these for transcriptional profiling is key to understanding gene regulation in disease
- **RNA-seq is the standard tool for studying gene expression, but it has major limitations:**
  - Fails on most archival FFPE material due to heavily degraded RNA (Figure 1A)
  - Requires poly(A) or hybridization capture that adds costs and misses key transcripts (Figure 1B)
  - Is blind to enhancer activity – key regulatory features that dictate cell identity
- **ATAC-seq is emerging, but has major constraints:**
  - Requires 5-10X more tissue than a routine FFPE section
  - Does not provide insights into transcriptional activity

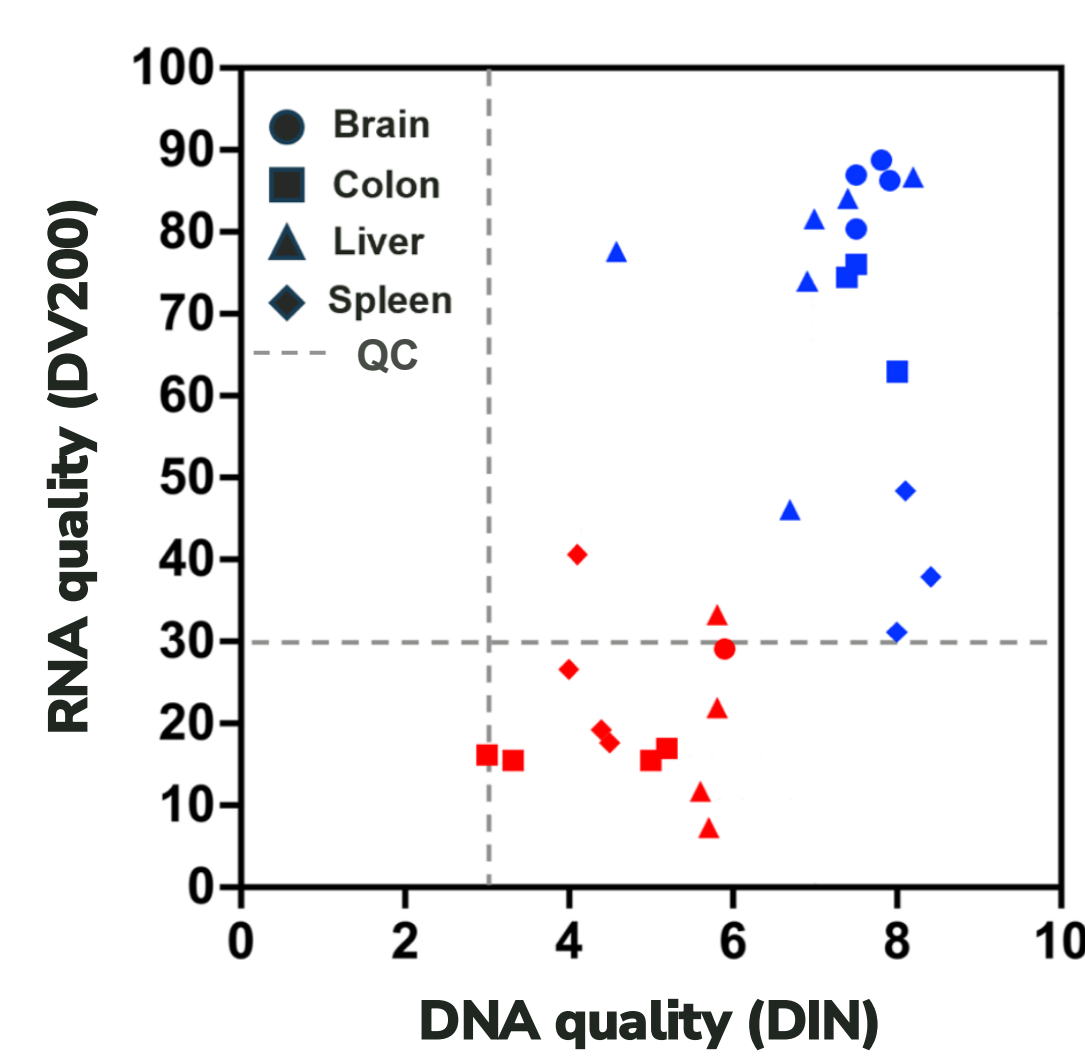
## IDEA: Target RNA Polymerase II (RNAPII) bound loci for fragmentation

- ✓ 52-heptad repeats in the C-Terminal Domain (CTD)
- ✓ Leverage phosphoforms to target transcriptionally active genes
- ✓ No lysines = no damage from FFPE fixation

## IMPACT: Robust signal amplification

- ✓ Comprehensive readout of transcription + active regulatory elements
- ✓ Targets stable DNA

### A RNA More Labile Than DNA in FFPE vs. Frozen Tissue (Charles River + EpiCypher)



### B Histone HyperTx in Cancer Missed by RNA-seq (Zheng et al., PNAS 2025)

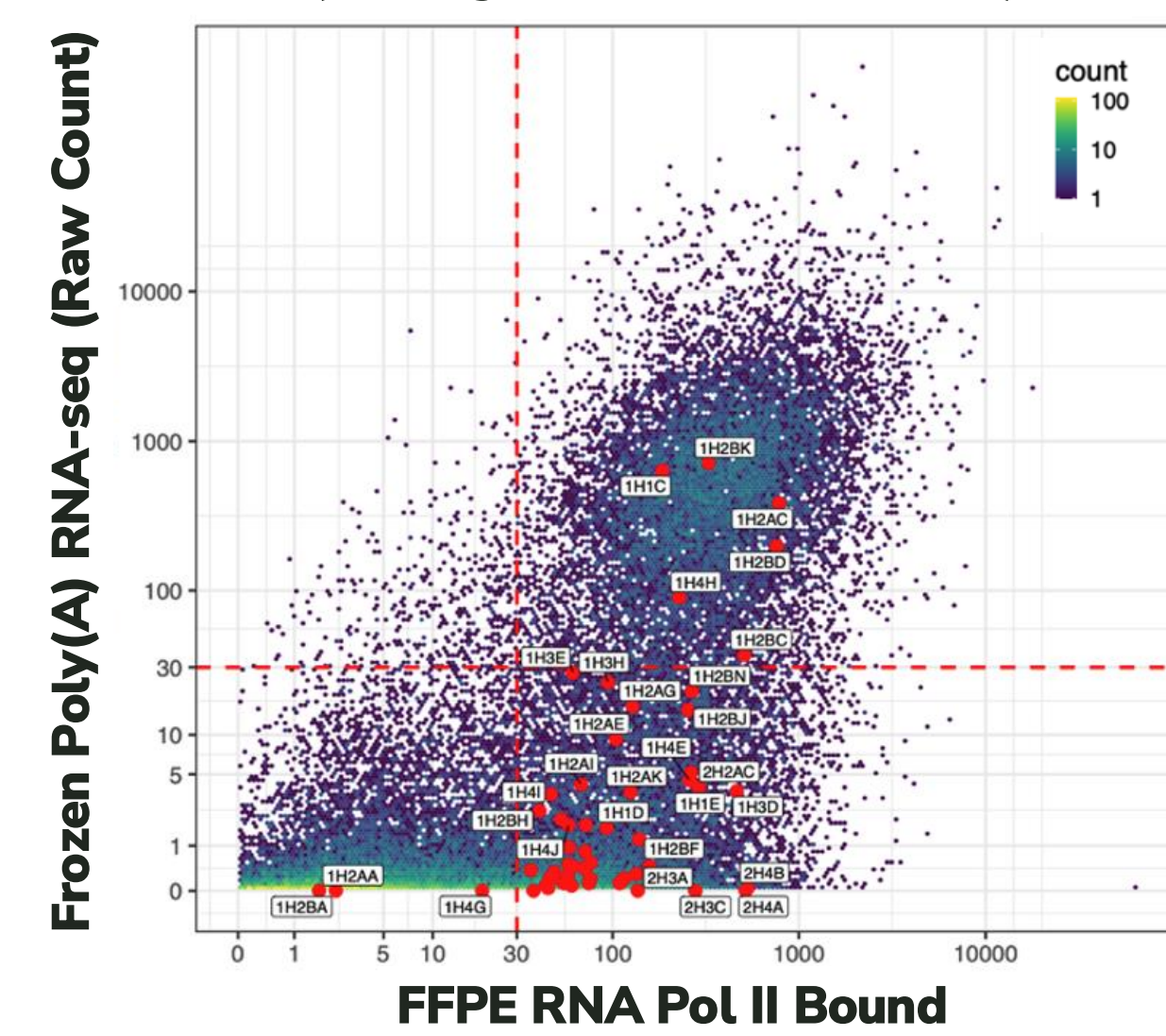


Figure 1. Targeting RNA Polymerase II-bound DNA has potential to uncover a wealth of regulatory information from archival FFPE. (A) FFPE-RNA is lower quality compared to FFPE-DNA from matched mouse tissue. Dashed lines indicate quality thresholds for DNA (DIN >3) and RNA (DV200 >30). (B) Zheng et al. (PMID: 40314975) leveraged CUT&Tag-FFPE, a DNA-based approach to identify genomic loci actively occupied by RNAPII to identify regions of hypertranscription unique to cancer. Matched RNA-seq data failed to capture these since histone mRNA is not poly(A)-tailed.

## THE SOLUTION: A novel DNA-based assay to comprehensively profile transcriptional activity with greater stability and resolution

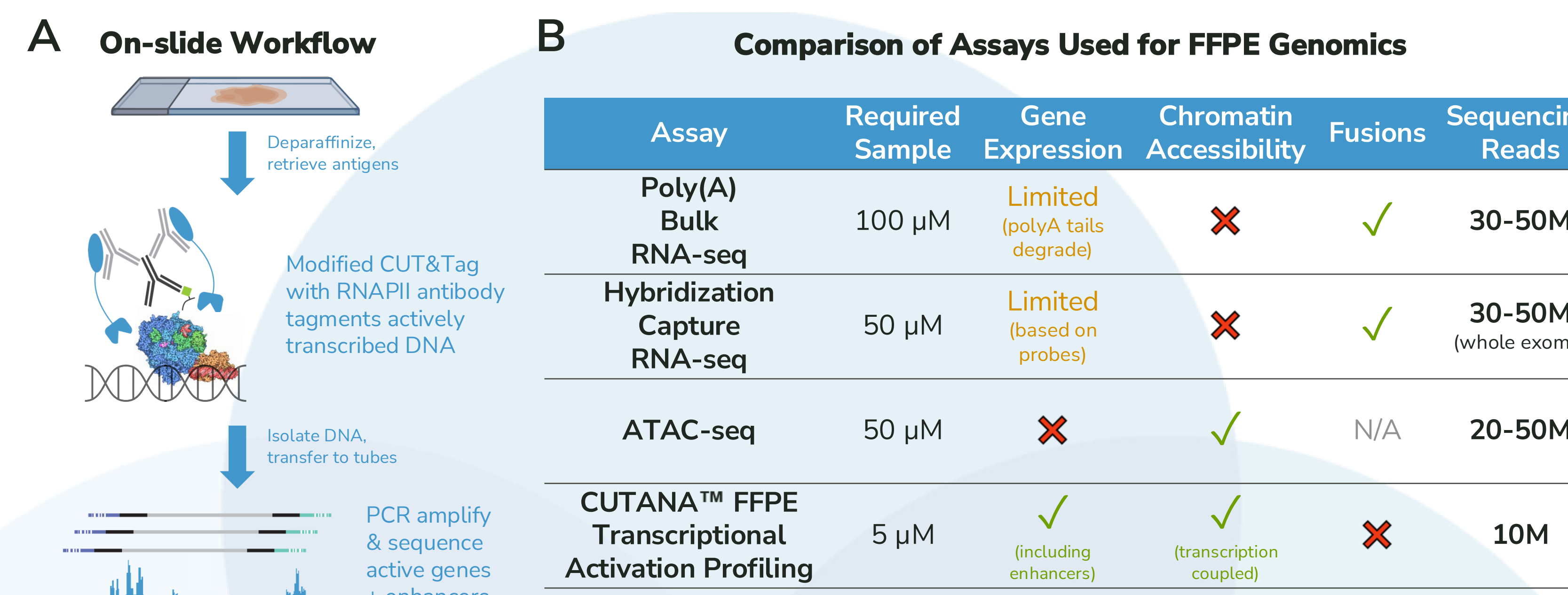


Figure 2. By concentrating transposase at RNAPII-bound genes (A), CUTANA™ FFPE offers distinct advantages over alternative genomic approaches in FFPE (B), including requiring less material, lower sequencing depth, and greater overall coverage of transcriptional activity (exception: detection of fusion transcripts).

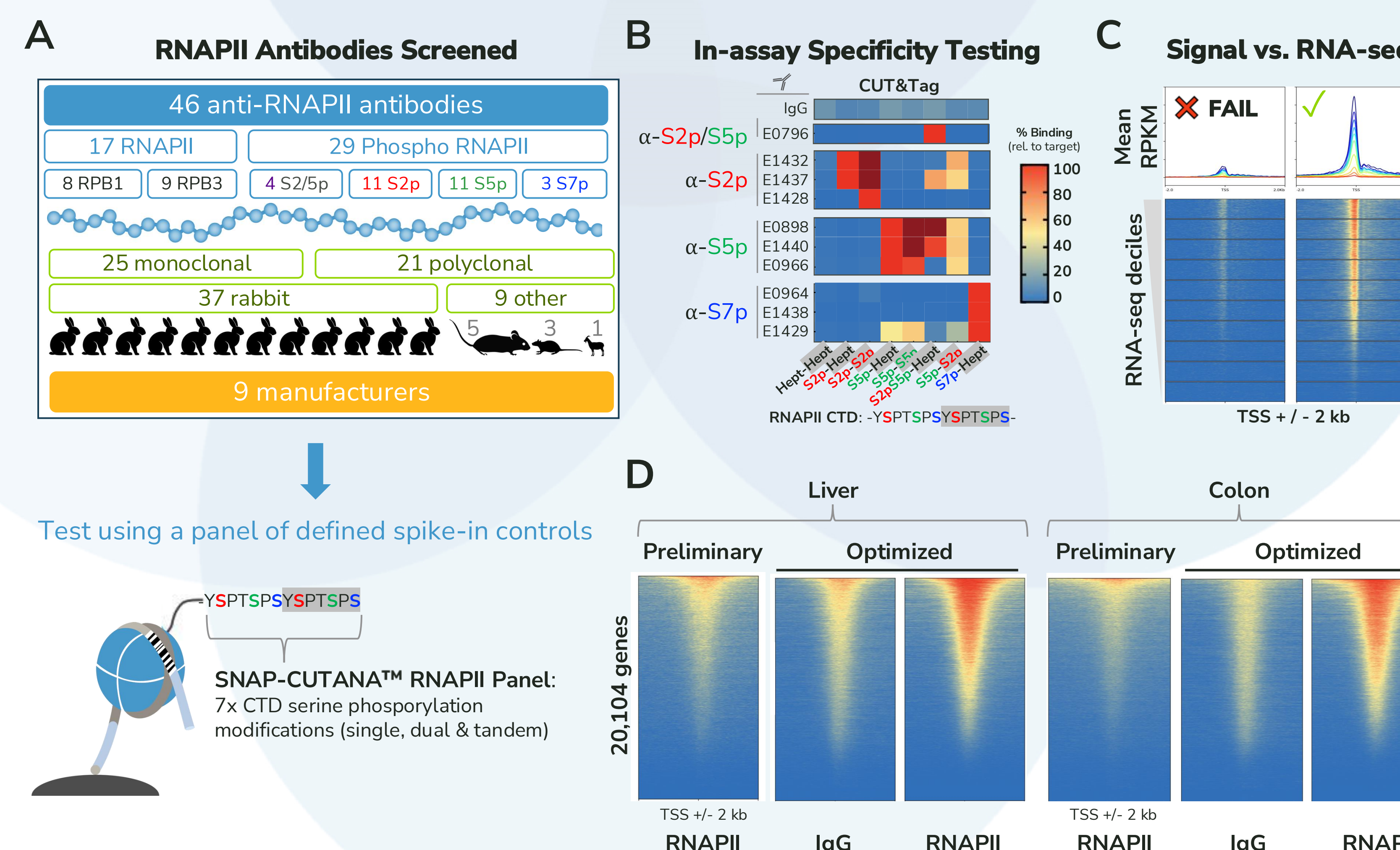


Figure 3. CUTANA™ FFPE assay development involved a rigorous approach to reagent selection and workflow optimization. (A-C) Screen for best-in-class RNAPII antibodies used defined controls, genome-wide signal assessment, and RNA-seq benchmarking. (D) Optimized on-slide workflow resulted in robust profiles from FFPE material.

## ASSAY BENCHMARKING: CUTANA™ FFPE outperforms ATAC-seq in signal strength and transcriptional relevance

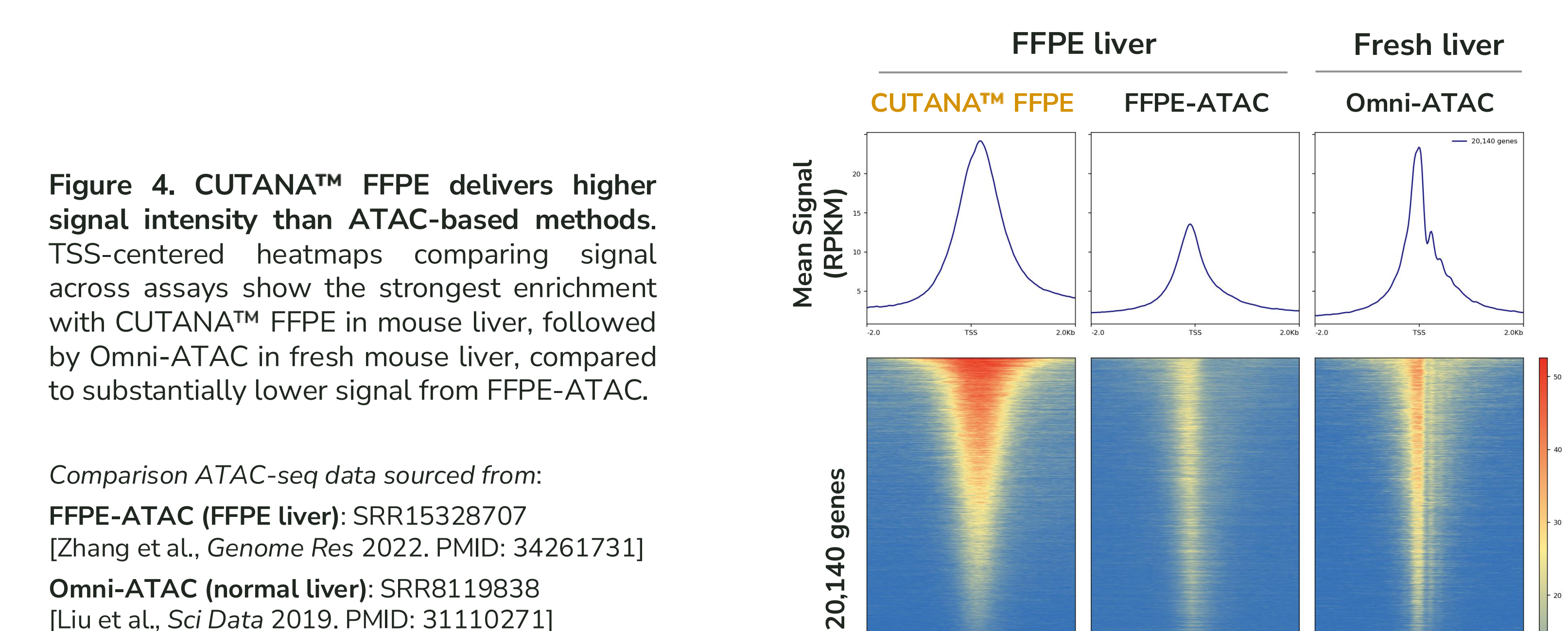


Figure 4. CUTANA™ FFPE delivers higher signal intensity than ATAC-based methods. TSS-centered heatmaps comparing signal across assays show the strongest enrichment with CUTANA™ FFPE in mouse liver, followed by Omni-ATAC in fresh mouse liver, compared to substantially lower signal from FFPE-ATAC.

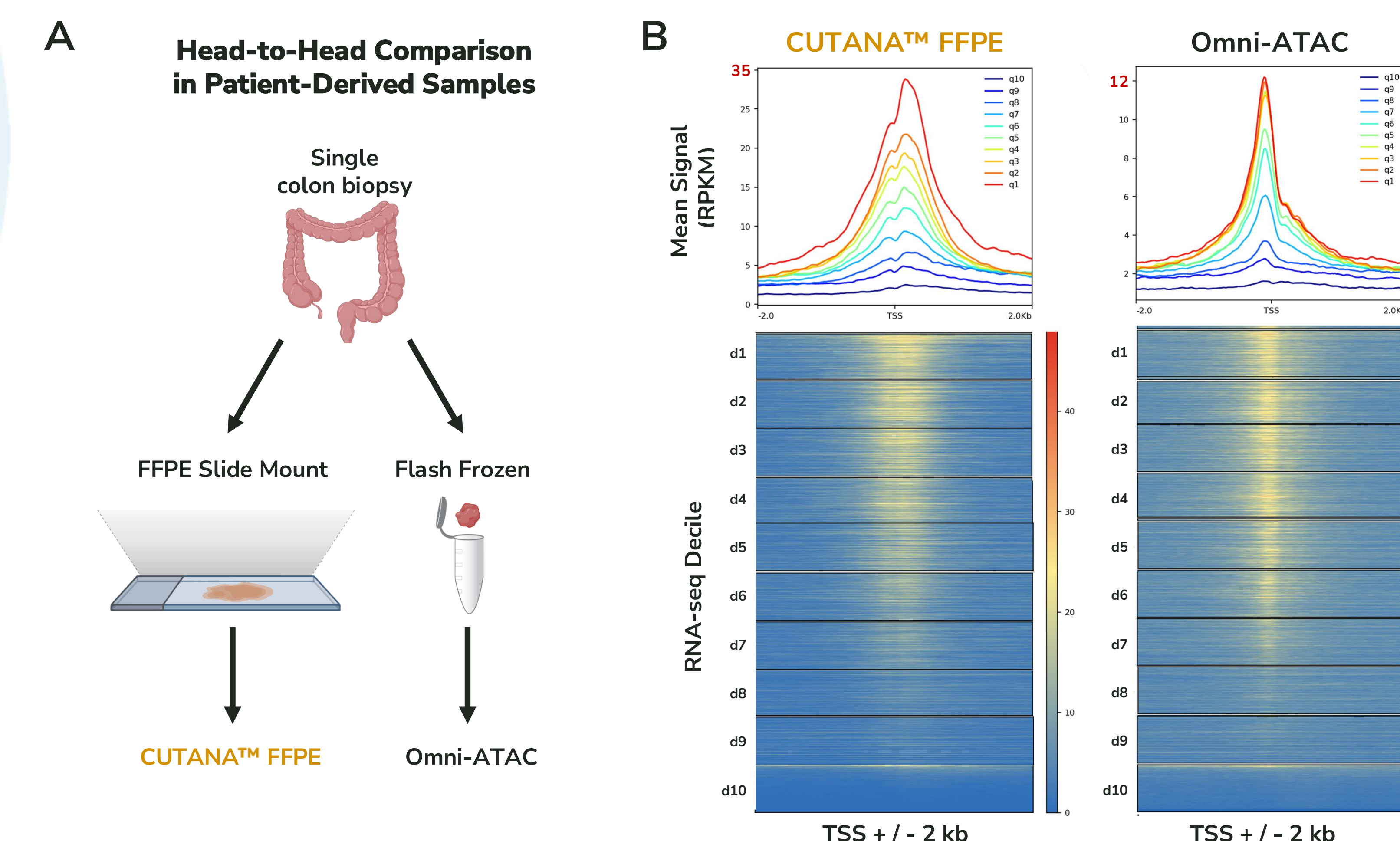


Figure 5. CUTANA™ FFPE is transcription-coupled; ATAC-seq is not. (A) Controlled benchmarking experiment was conducted using tissue derived from a single human colon biopsy. (B) Metagene profiles centered on the TSS for human colon FFPE sections, with genes ranked into deciles by RNA-seq expression level. Unlike Omni-ATAC, CUTANA™ FFPE resolves clear signal stratification across all ten deciles (see in particular top deciles), confirming that RNAPII targeting quantitatively couples the readout to active transcription. Samples, ATAC-seq, and RNA-seq data courtesy of the Sheikh laboratory, UNC.

## Unlock transcriptional and regulatory biology from FFPE – without the limits of RNA

### CUTANA™ FFPE resolves tissue-specific transcriptional dynamics with the precision of RNA-seq

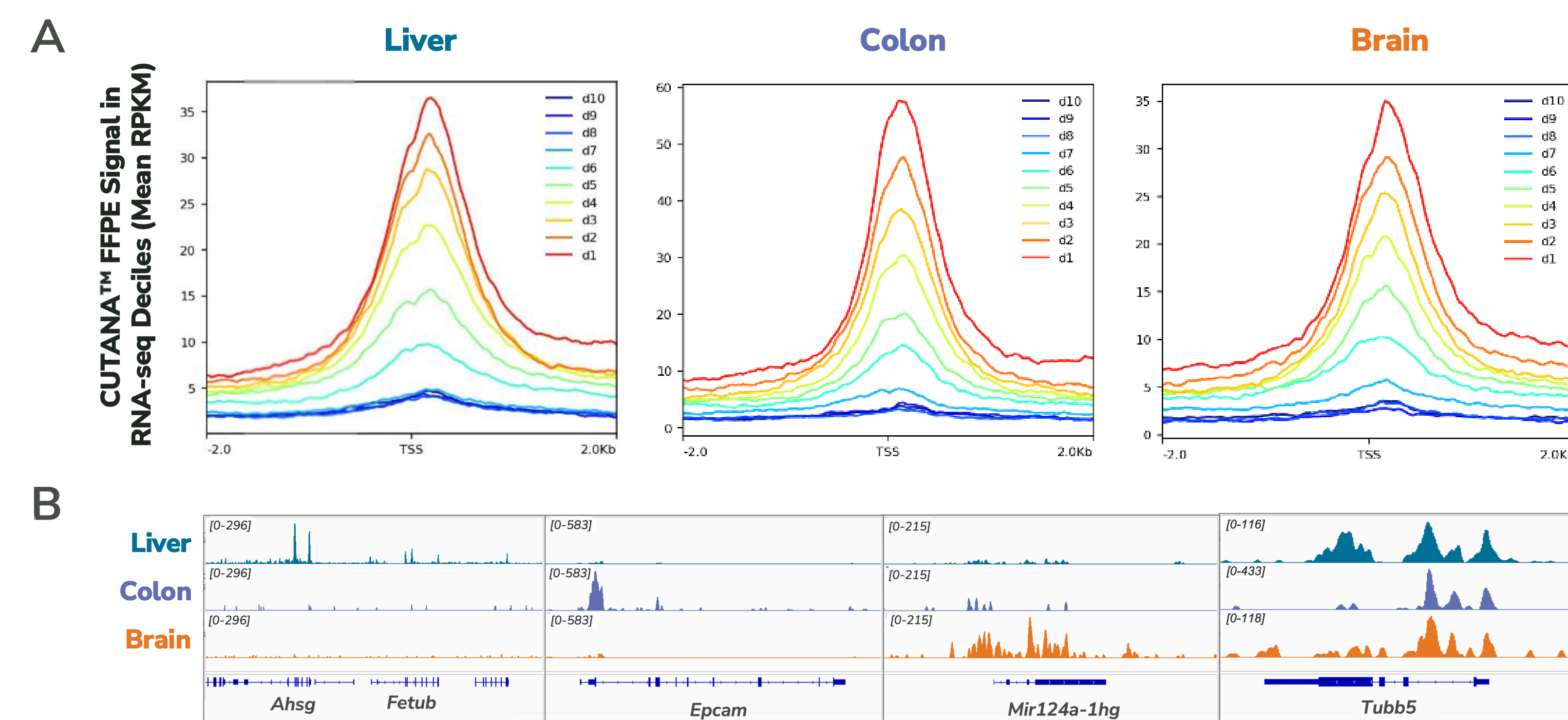


Figure 6. CUTANA™ FFPE readily identifies tissue-specific genes. (A) Metagene profiles centered on transcription start sites (TSS) demonstrate quantitative coupling between CUTANA™ FFPE and RNA-seq across liver, colon, and brain sections. (B) Genome browser tracks show RNAPII occupancy at tissue-specific genes in liver (Ahsg), colon (Epcam), and brain (Mir124a-1hg), alongside a ubiquitously expressed housekeeping gene (Tubb5) with signal across all tissues. These patterns indicate that the assay captures biologically meaningful, tissue-specific transcriptional activity.

### CUTANA™ FFPE identifies putative prognostic biomarkers from FFPE

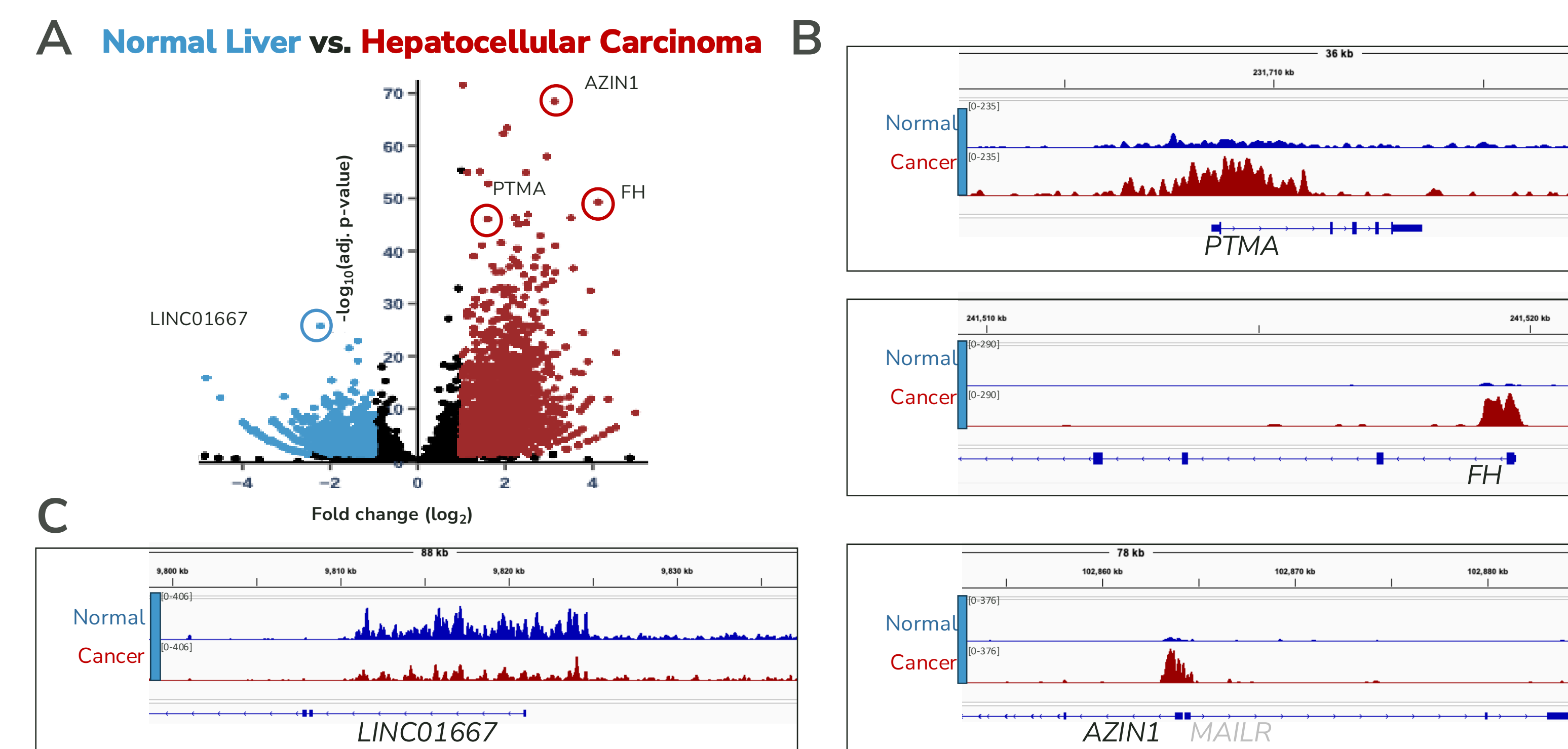


Figure 7. CUTANA™ FFPE resolves transcriptional changes in hepatocellular cancer. (A) Volcano plot showing genes with differential RNAPII abundance between patient-derived FFPE tumor and FFPE tumor-adjacent normal liver tissue. (B) Gene browser view of genes known to be overexpressed in hepatocellular cancer types (circled red in the volcano plot; PMIDs: 25865690, 37982952). (C) Gene browser track showing LINC01667 expression in normal vs. cancer tissue, a lncRNA correlated with poor prognosis (PMID: 34458133) that may be missed by FFPE RNA-seq.

### CUTANA™ FFPE in action

- >200 samples tested
  - Human & mouse tissues: breast, colon, brain, liver, kidney, lung, lymph
  - Healthy and disease: cancer, immune disorders, intestinal disease
- >70% overall success rate (quality data) vs. ~40% for RNA-seq
  - 5 µm slides (vs. 50 µm for RNA-seq)
  - 30-year retrospective (1995-2025)
  - NO restrictions on collection methods
- 100% success with curated cohort
  - Rigorously controlled collection methods
  - Patient intestinal biopsies
  - PI: Shehzad Sheikh (UNC)

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