

# Integrating Spatial Open Chromatin and DNA Methylation Profiling with Spatial ATAC-TAPS+

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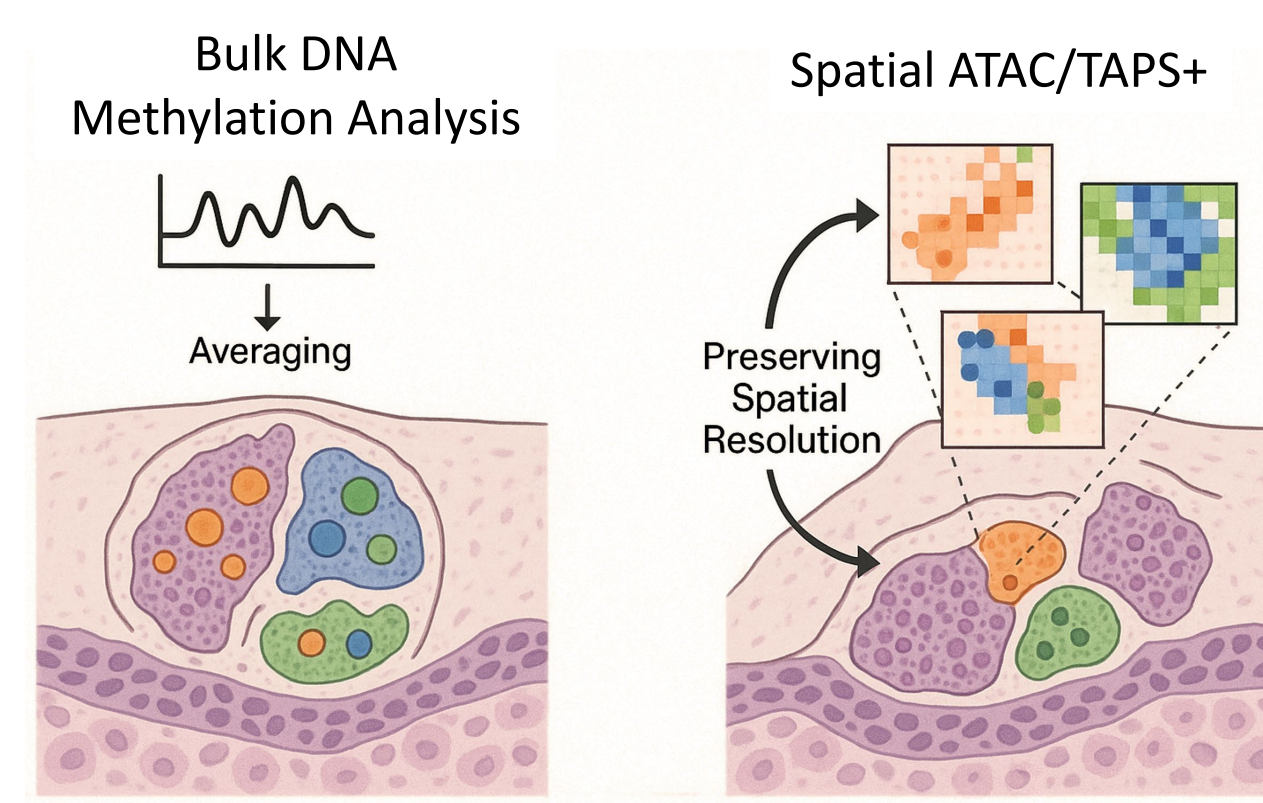
## 1. Technology and Validation

### Bulk Methylation Masks Spatial Regulatory Heterogeneity

DNA methylation profiling is a cornerstone of tumor classification, prognostication, and biomarker discovery, particularly in brain cancers where methylation signatures guide molecular subtyping and clinical decisions.

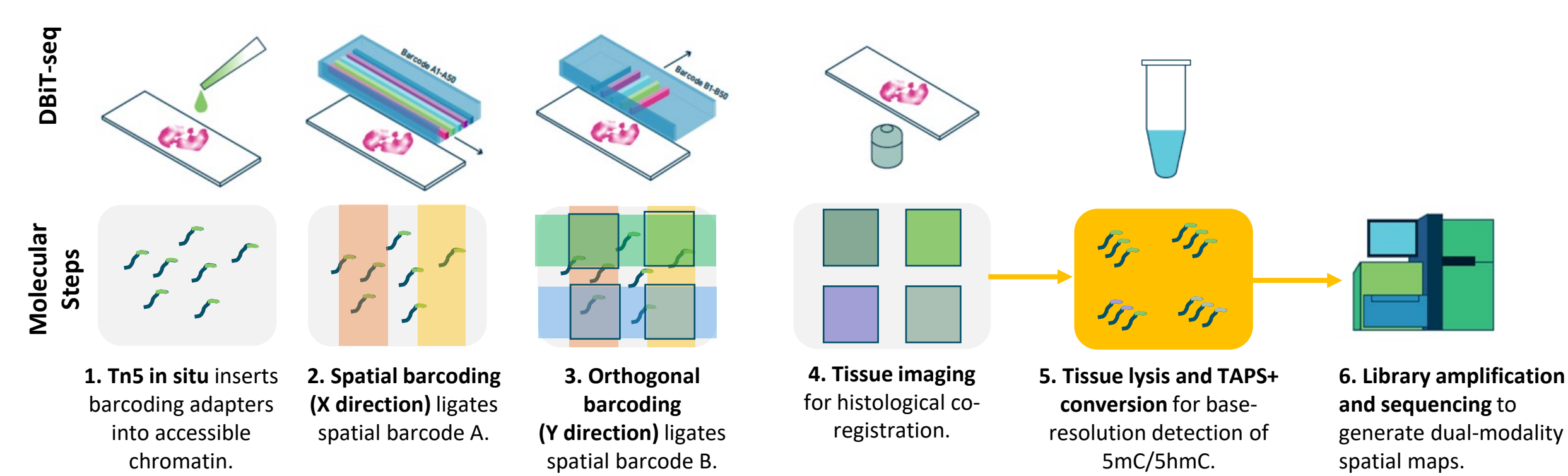
However, conventional bulk approaches average signals across heterogeneous tissue regions, obscuring spatially restricted epigenetic states that drive tumor progression, therapy resistance, and microenvironmental interactions. Subclones, invasive fronts, and resistant niches often occupy specific anatomical locations but become invisible once tissue architecture is lost.

Spatially resolved DNA methylation profiling preserves tissue context while capturing cytosine modification states, enabling direct linkage of regulatory programs to histology and local cellular environments. This approach reveals epigenetic heterogeneity in situ, providing a more accurate view of tumor biology and therapeutic vulnerability.



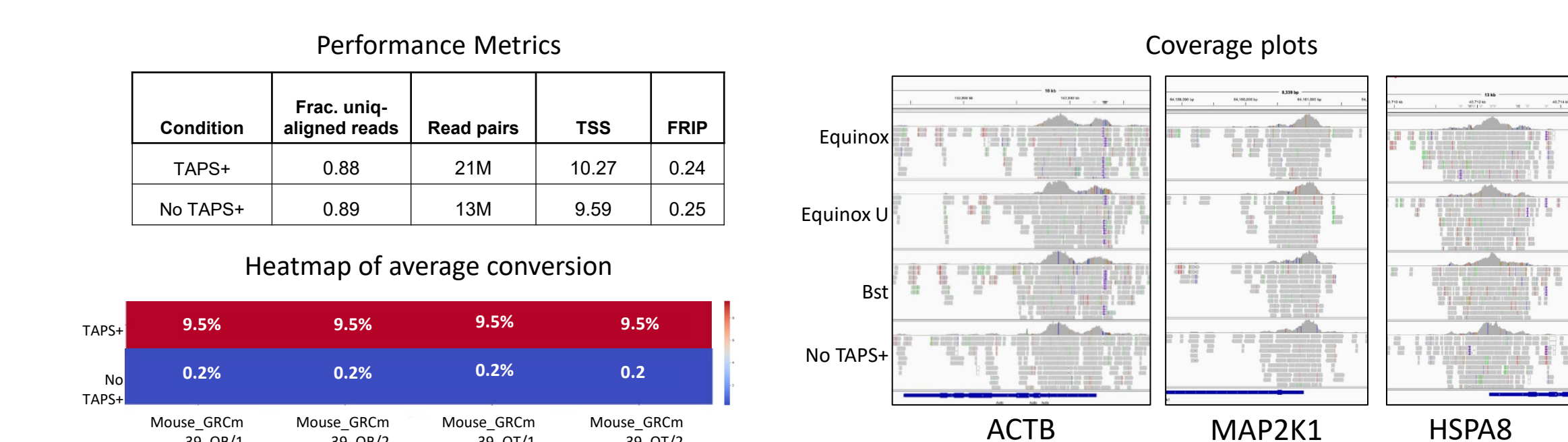
**Figure 1.1. Tumor classification relies on bulk DNA methylation signatures that average across heterogeneous regions.**

### Spatial ATAC-TAPS+: Co-Registered Accessibility and Methylation in One Tissue Section



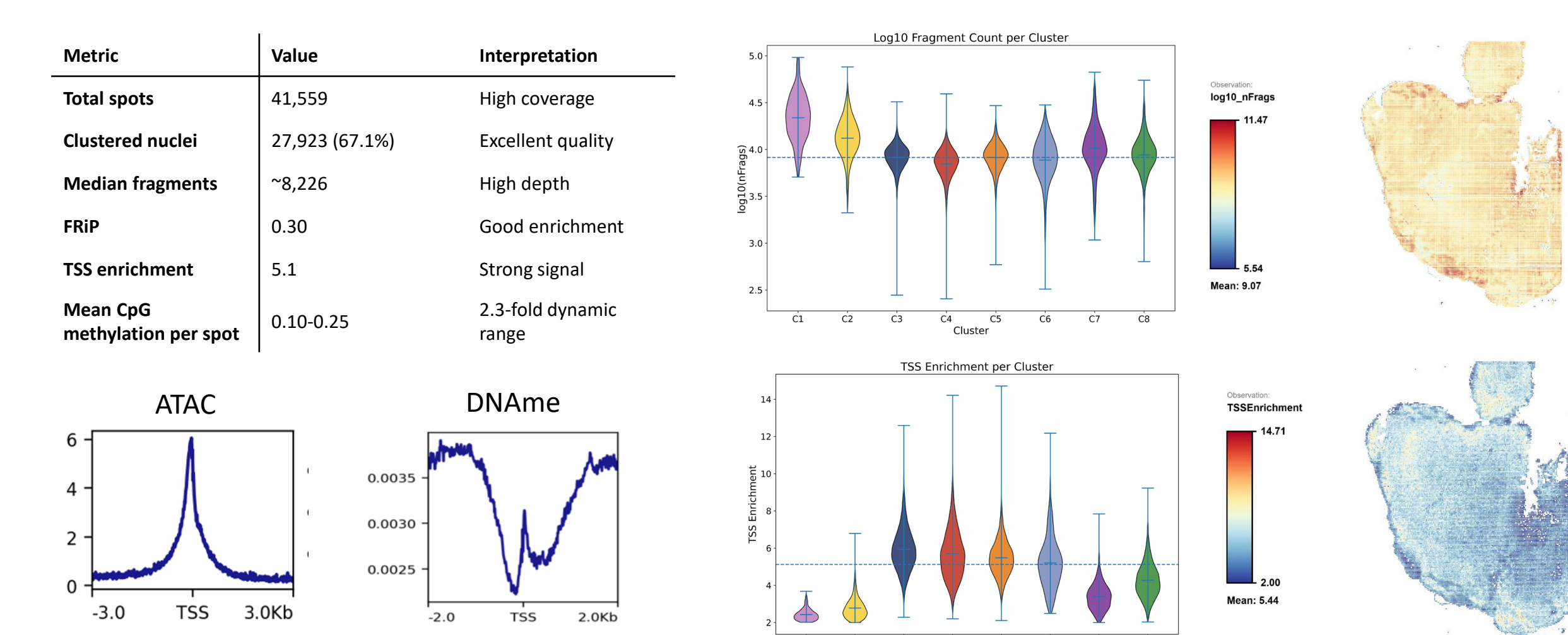
**Figure 1.2. Spatial ATAC-TAPS+ workflow.** In situ fragmentation is followed by orthogonal microfluidic barcoding, tissue imaging, and Watchmaker's TAPS+ conversion prior to library preparation. For this study, libraries were sequenced on the Ultima Genomics platform to enable deep coverage for integrated accessibility and methylation profiling. A modular analysis pipeline generates co-registered accessibility and methylation maps.

### TAPS+ Conversion Preserves ATAC Library Quality



**Figure 1.3. TAPS+ conversion preserves spatial ATAC library integrity.** Fragment size distribution, FRIP, and TSS enrichment remain stable before and after conversion, confirming compatibility of TAPS+ chemistry with spatial ATAC libraries.

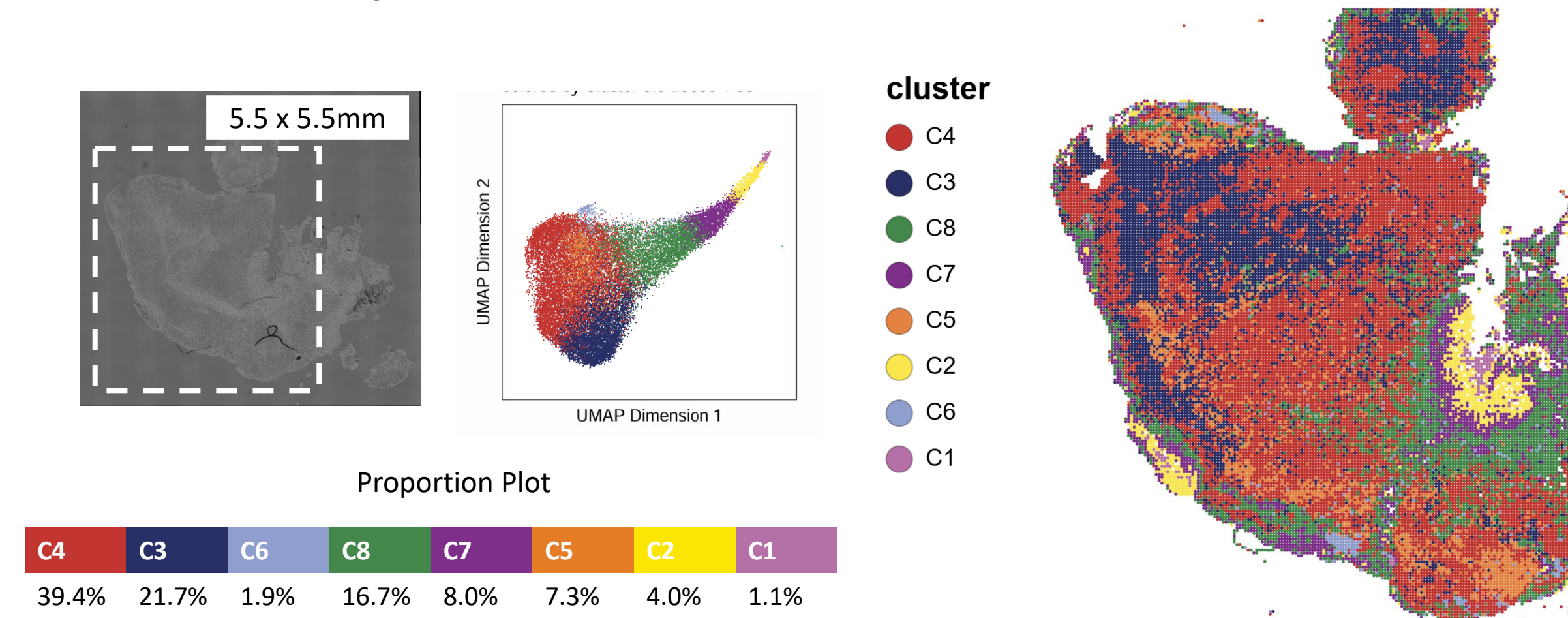
### Spatial ATAC-TAPS+ Produces High-Quality Accessibility and Methylation Data



**Figure 1.4. Human medulloblastoma profiling demonstrates robust chromatin accessibility and CpG methylation measurement.** TSS enrichment coincides with methylation depletion, consistent with canonical promoter architecture.

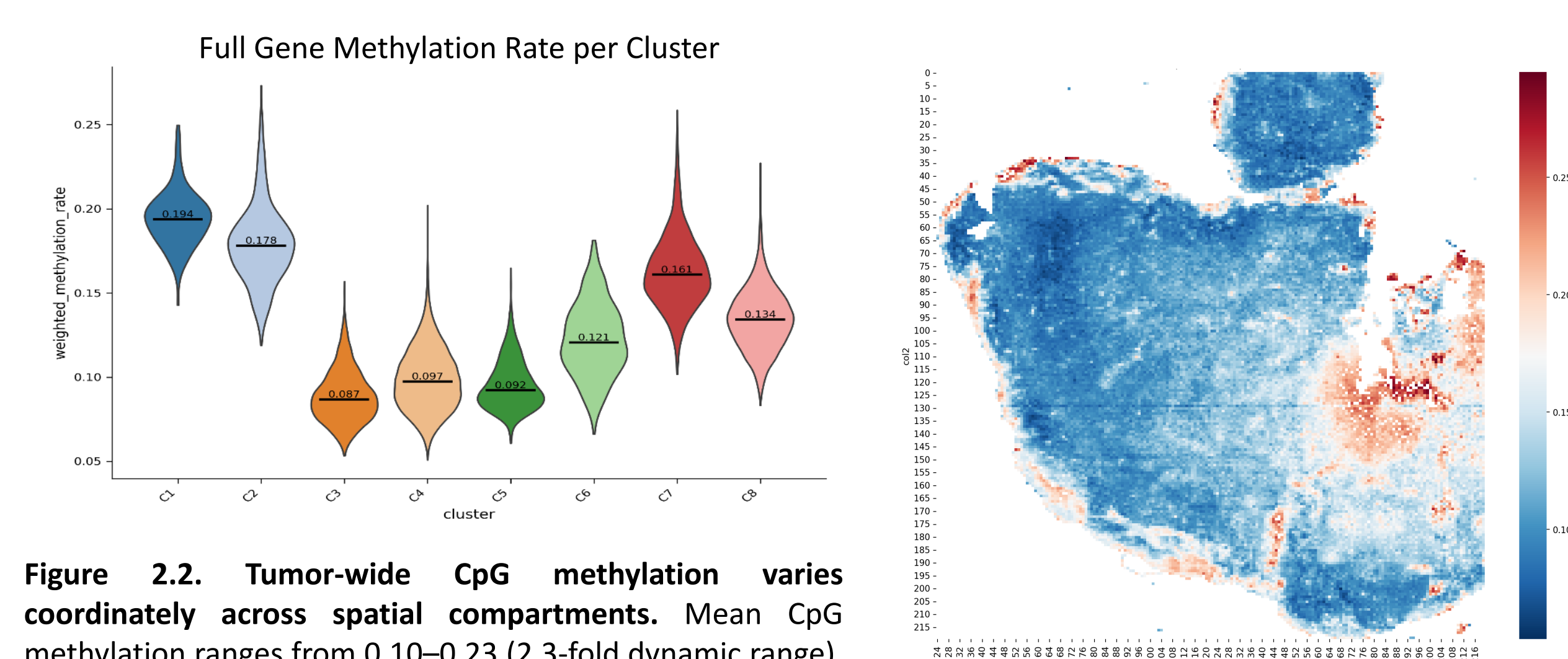
## 2. Results & Performance

### Spatial ATAC-TAPS+ Resolves Eight Distinct Epigenetic Compartments in Human Medulloblastoma



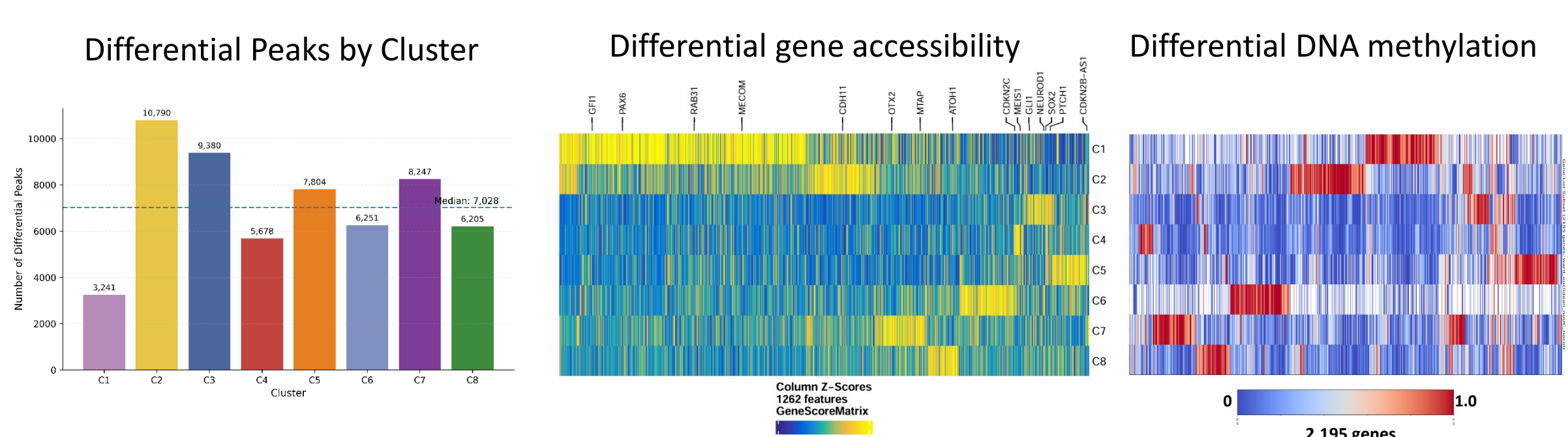
**Figure 2.1. Eight spatially coherent epigenetic compartments are resolved.** Unsupervised clustering identifies distinct regulatory states across 27,923 nuclei at 10 μm resolution. Compartments are spatially organized, indicating stable regulatory segregation rather than intermixed clones.

### Spatial DNA Methylation Exhibits Coordinated Tumor-Wide Variation



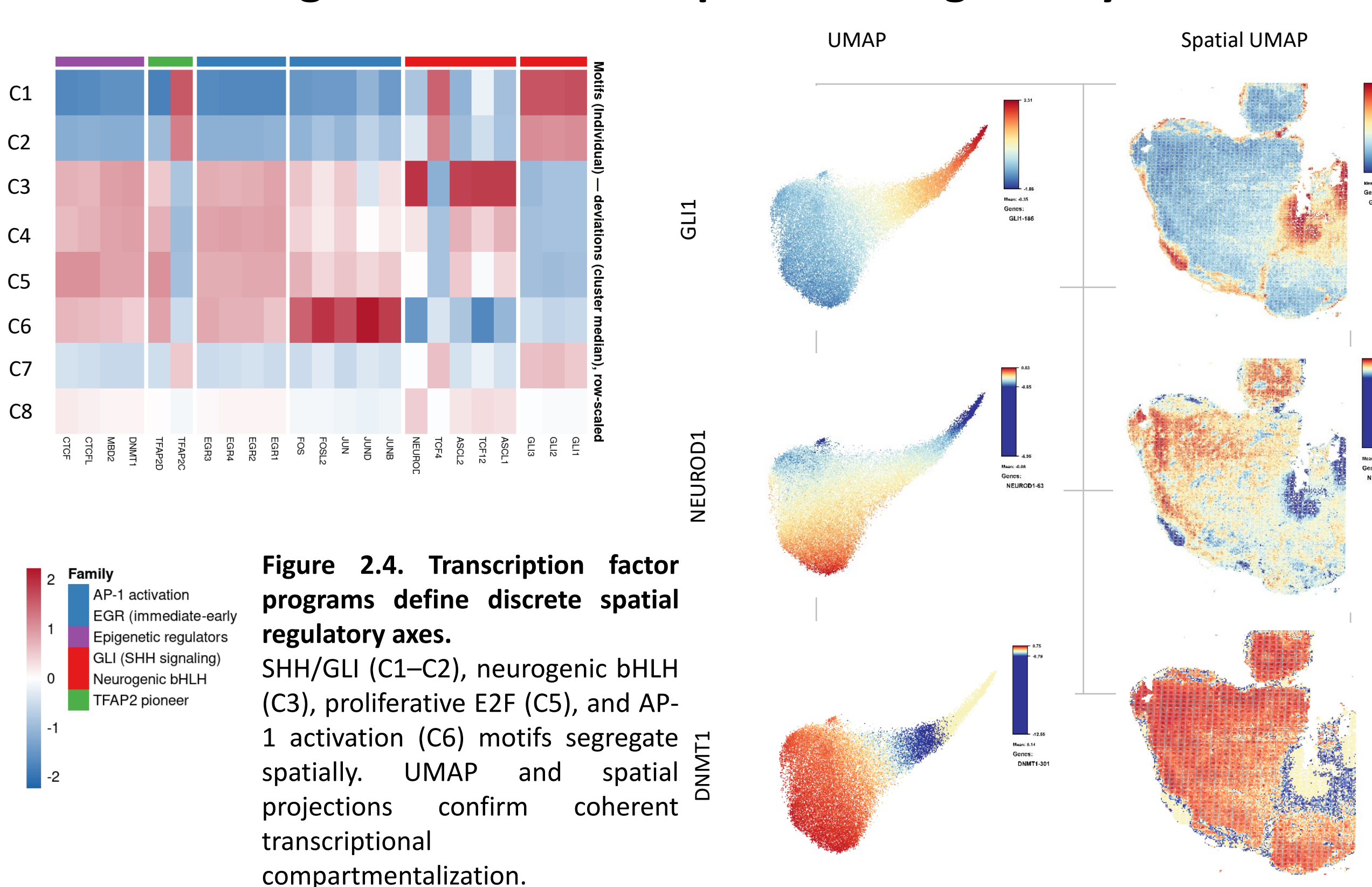
**Figure 2.2. Tumor-wide CpG methylation varies coordinately across spatial compartments.** Mean CpG methylation ranges from 0.10–0.23 (2.3-fold dynamic range). Methylation maps align with tumor morphology, demonstrating structured epigenetic organization.

### Coordinated Accessibility and Methylation Divergence Defines Stable Regulatory Compartments



**Figure 2.3. Accessibility and methylation diverge coordinately across compartments.** Clusters contain 6–11K enriched regulatory loci and differential methylation across more than 2000 genes. Heatmaps demonstrate stable regulatory segregation rather than technical bias.

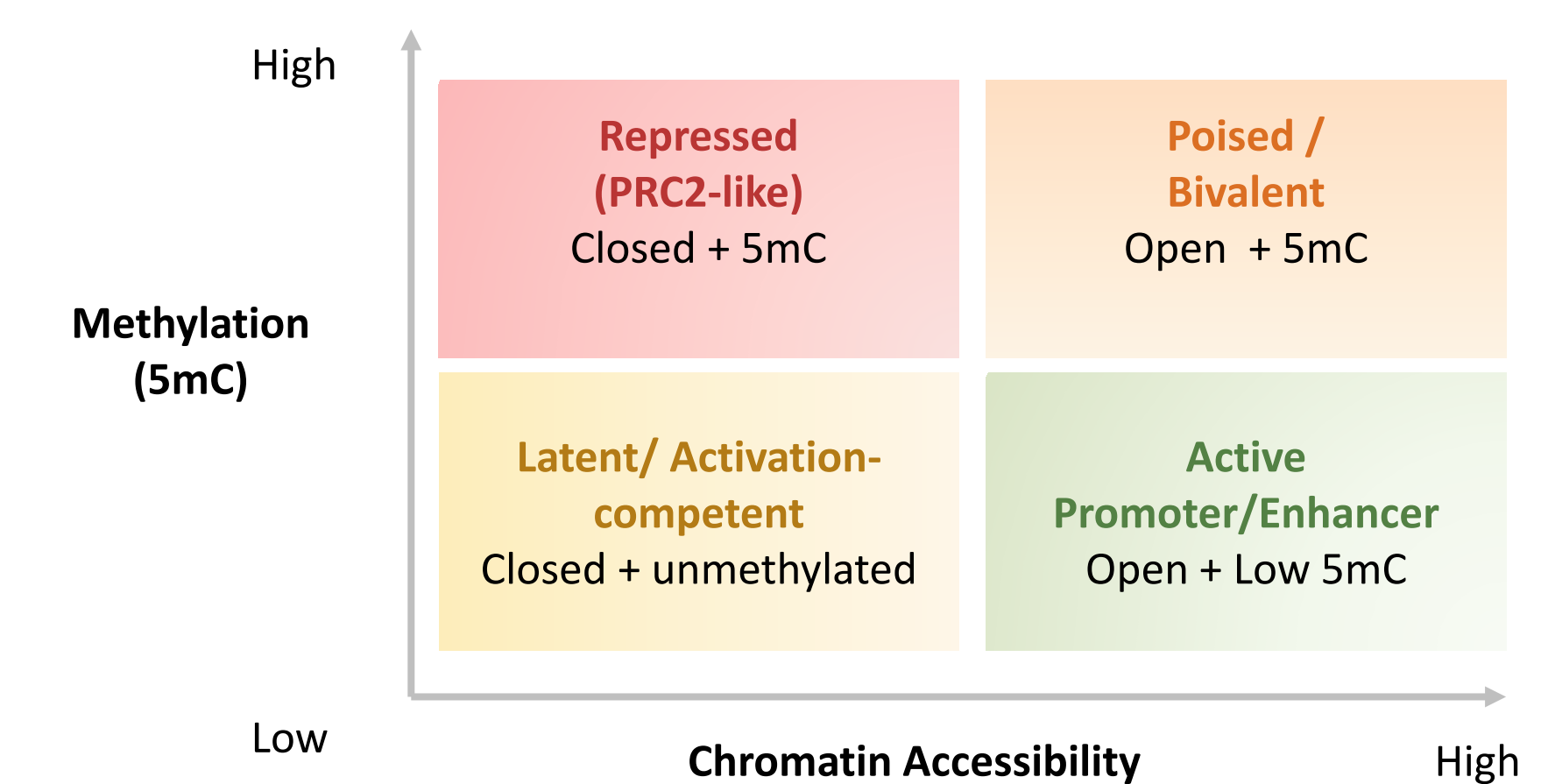
### Motif Programs Define Interpretable Regulatory States



**Figure 2.4. Transcription factor programs define discrete spatial regulatory axes.** SHH/GLI (C1–C2), neurogenic bHLH (C3), proliferative E2F (C5), and AP-1 activation (C6) motifs segregate spatially. UMAP and spatial projections confirm coherent transcriptional compartmentalization.

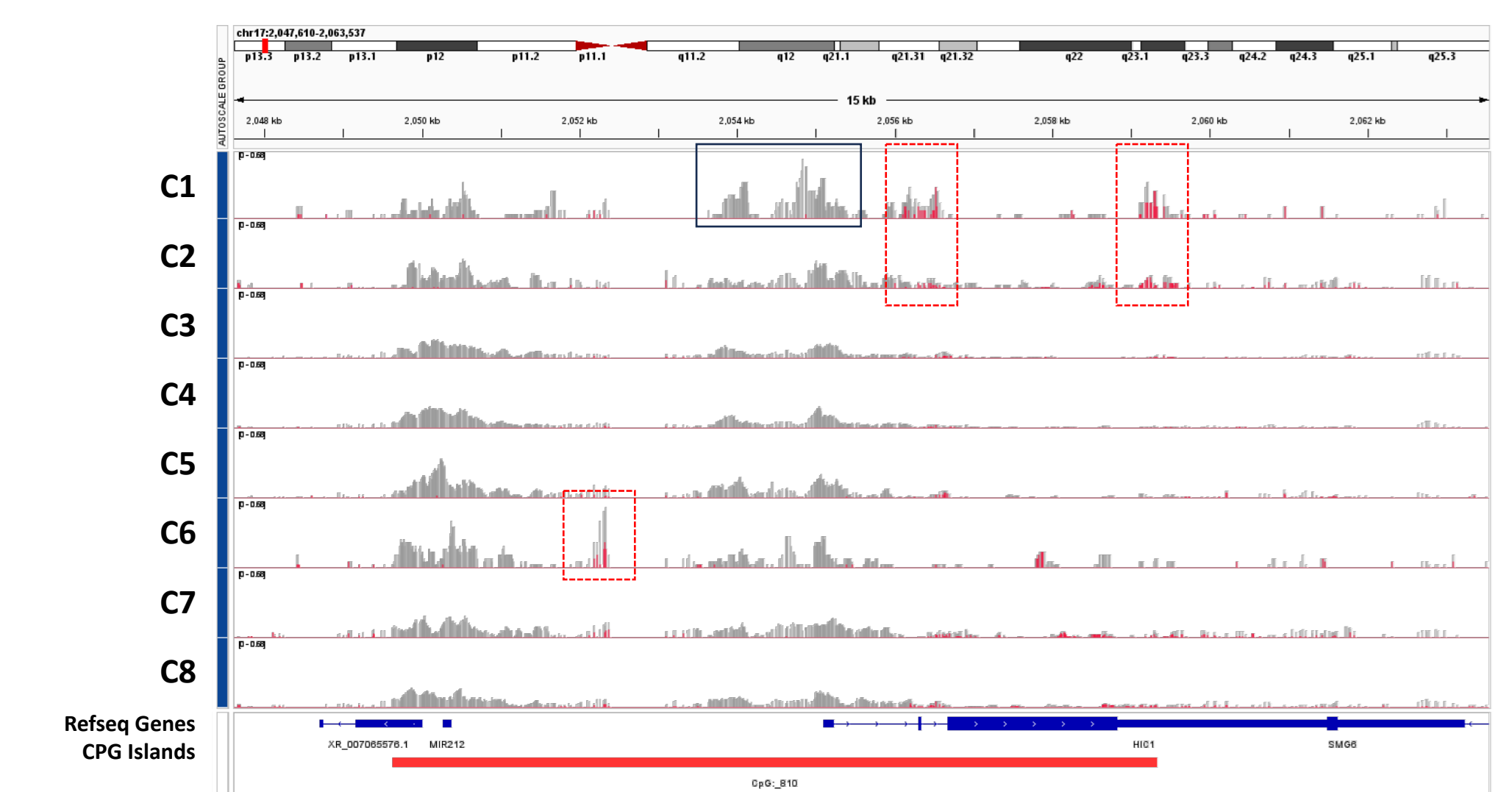
## 3. Regulatory Interpretation & Biological Insights

### Dual-Modality States Enable direct Regulatory Interpretation



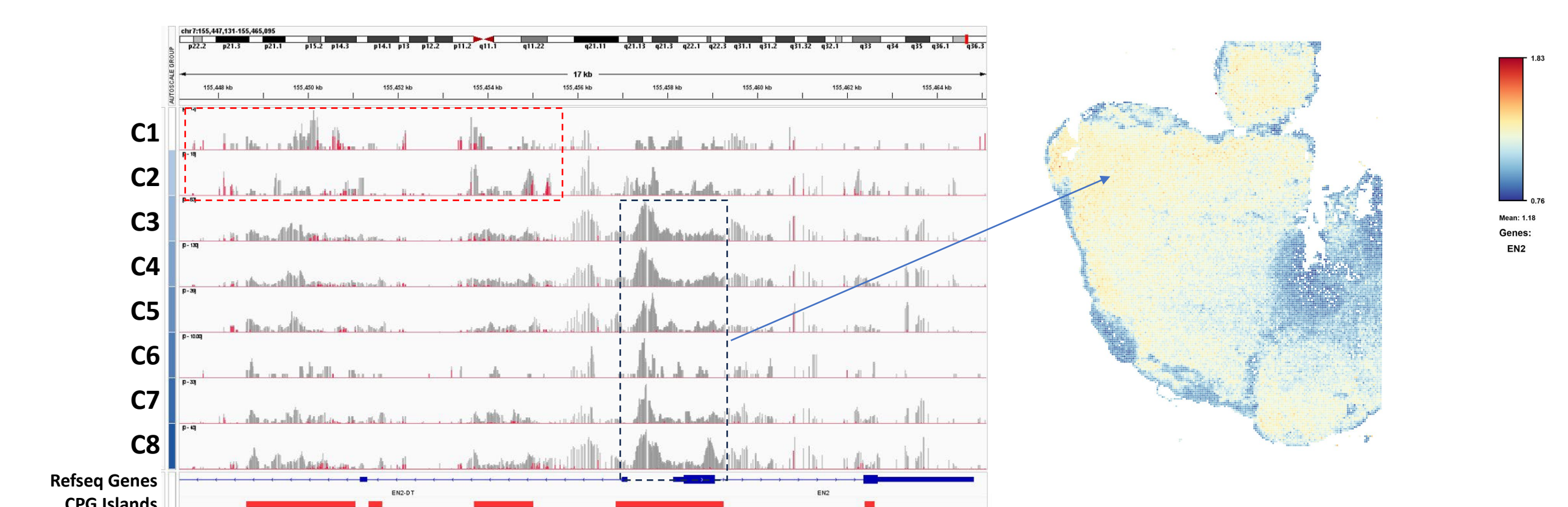
**Figure 3.1. Integrated accessibility and 5mC define functional chromatin states.** Open/low-5mC regions mark active promoters and enhancers, open/5mC regions reflect poised states, closed/5mC regions are repressed (PRC2-like), and closed/unmethylated loci represent latent states. This framework enables direct interpretation of spatial epigenetic architecture.

### Cluster-Specific DNA Methylation Distinguishes Regulatory States



**Figure 3.2. Cluster-resolved DNA methylation at the HIC1 locus (chr17).** Modified/Total (TAPS+) tracks across eight spatial clusters reveal focal and CpG island-associated hypermethylation within the HIC1 regulatory region. Select clusters exhibit strong enrichment of methylation at promoter-proximal and CpG island segments (highlighted), while others remain comparatively hypomethylated. These patterns distinguish regulatory compartments within the tissue and demonstrate that spatial DNA methylation provides orthogonal resolution beyond chromatin accessibility alone.

### Spatially Resolved DNA Methylation Reveals Regulatory Compartmentalization at EN2



**Figure 3.3. Cluster-resolved TAPS+ tracks at the EN2 locus reveal focal CpG island hypermethylation enriched in specific spatial clusters (highlighted).** Spatial projection confirms that differential methylation maps to discrete tissue compartments, demonstrating that DNA methylation provides regulatory resolution beyond chromatin accessibility alone.

## Future Directions

#### Expand to FFPE Archival Tissue

Adapting Spatial ATAC-TAPS+ to formalin-fixed paraffin-embedded material will unlock retrospective cohort studies and enable direct integration with existing clinical biobanks, including WHO-classified brain tumor collections where bulk methylation array data already exists for comparison.

#### Multi-Sample Cohort Profiling

Profiling matched primary and recurrent tumor pairs will enable direct tracking of clonal epigenetic evolution, identifying resistance-associated regulatory niches that emerge under therapeutic pressure and linking spatial compartment shifts to clinical outcome.

#### Integration with Spatial Transcriptomics

Co-registration of Spatial ATAC-TAPS+ with spatial RNA data from adjacent sections will link regulatory state directly to gene expression output, providing a complete spatial multi-omic map of tumor cell identity and epigenetic state within the same tissue architecture.