

# Advanced multiomic sequencing technologies reveal complex crosstalk between chromatin proteins and DNA methylation in neurodevelopment

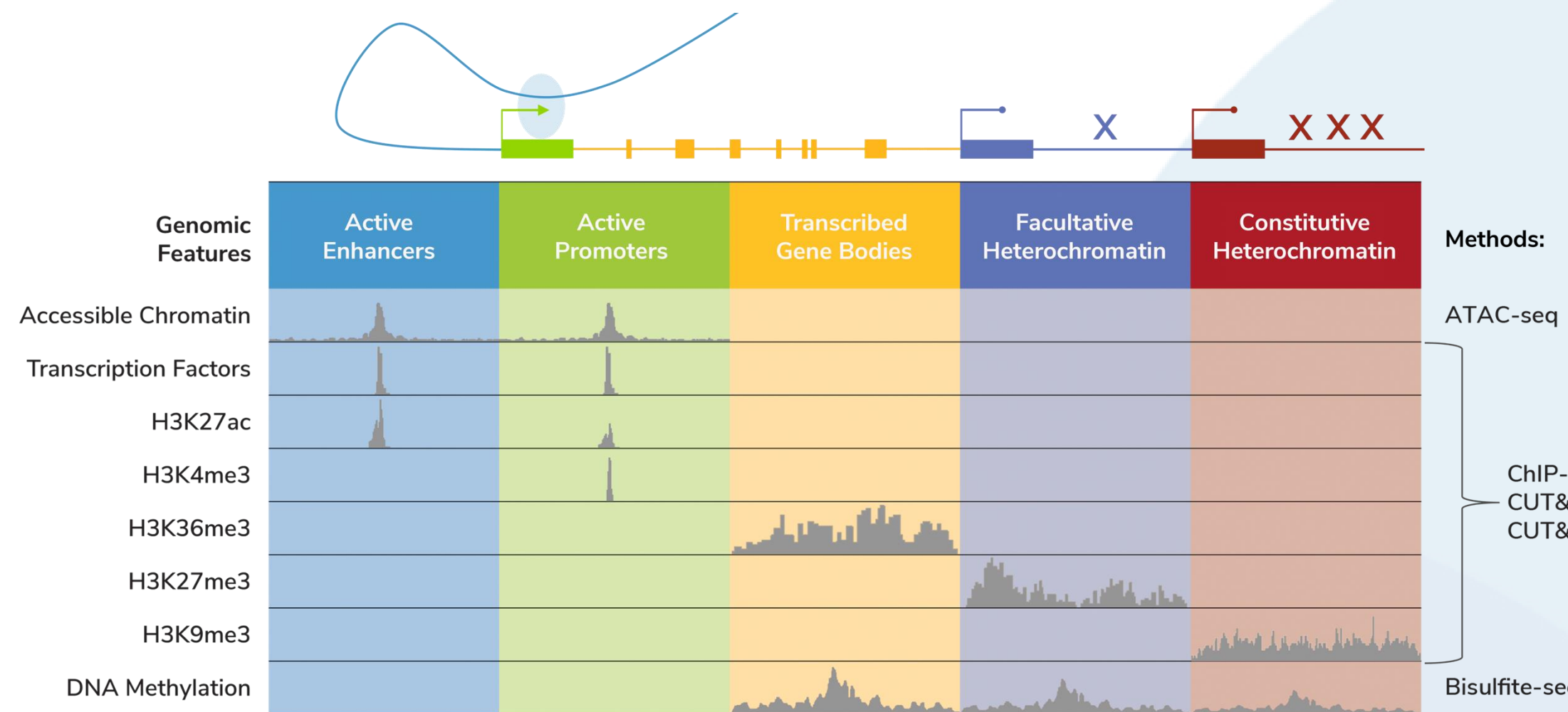


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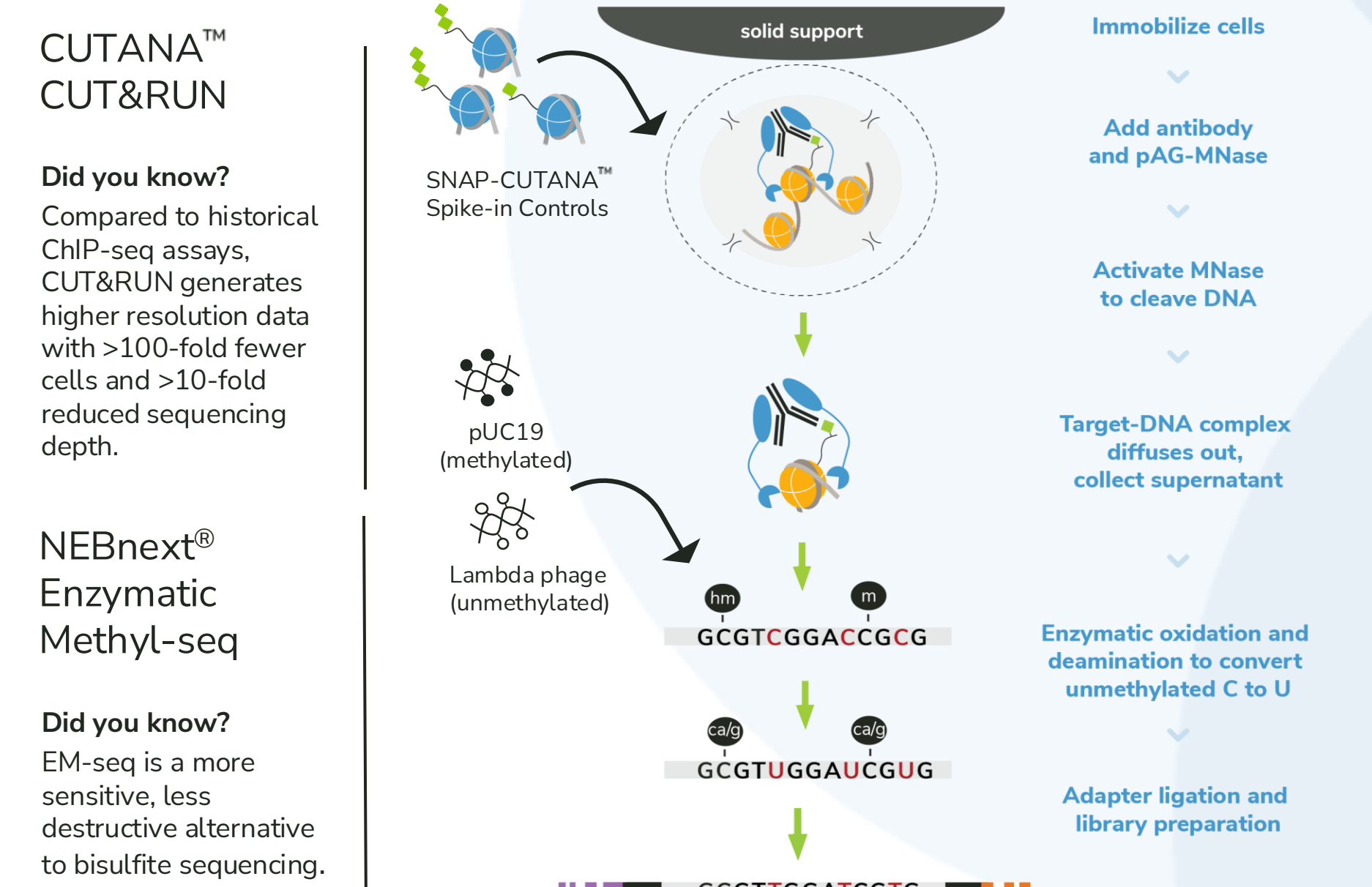
## Gene expression is controlled by complex molecular crosstalk between DNA methylation and chromatin proteins

- **Precise regulation** of DNA methylation (DNAm) and chromatin proteins is critical to gene expression and healthy pre- and post-natal development
- DNAm and chromatin proteins are **associated with specific genomic features** (Figure 1)
- **Studies of DNAm - chromatin protein crosstalk** are limited to low-resolution or indirect methods
- **Improved technologies** are needed to directly resolve DNAm - chromatin protein crosstalk



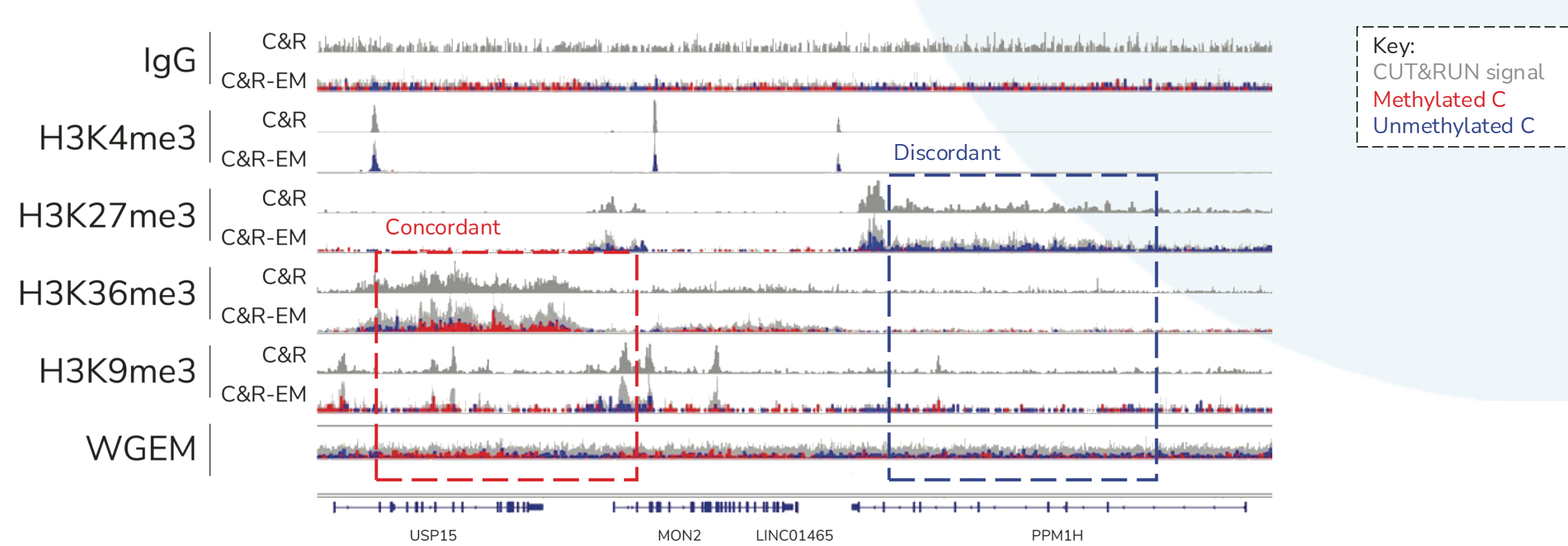
**Figure 1.** Chromatin proteins and DNAm define genomic features and reveal important regulatory mechanisms governing gene expression.

## CUT&RUN-EM is a powerful multiomic assay to simultaneously profile DNAm and chromatin proteins



**Figure 2.** CUT&RUN-EM integrates EpiCypher CUT&RUN with NEB EM-seq to deliver a direct multiomic assay enabling the simultaneous profiling of DNAm and chromatin proteins. CUT&RUN releases antibody-bound chromatin into solution, which is easily separated from bead-immobilized cells, thus eliminating a major source of background. Isolated DNA is enzymatically converted, distinguishing unmethylated and methylated cytosines in next-generation sequencing data. Defined nucleosome and DNA controls enable assay standardization.

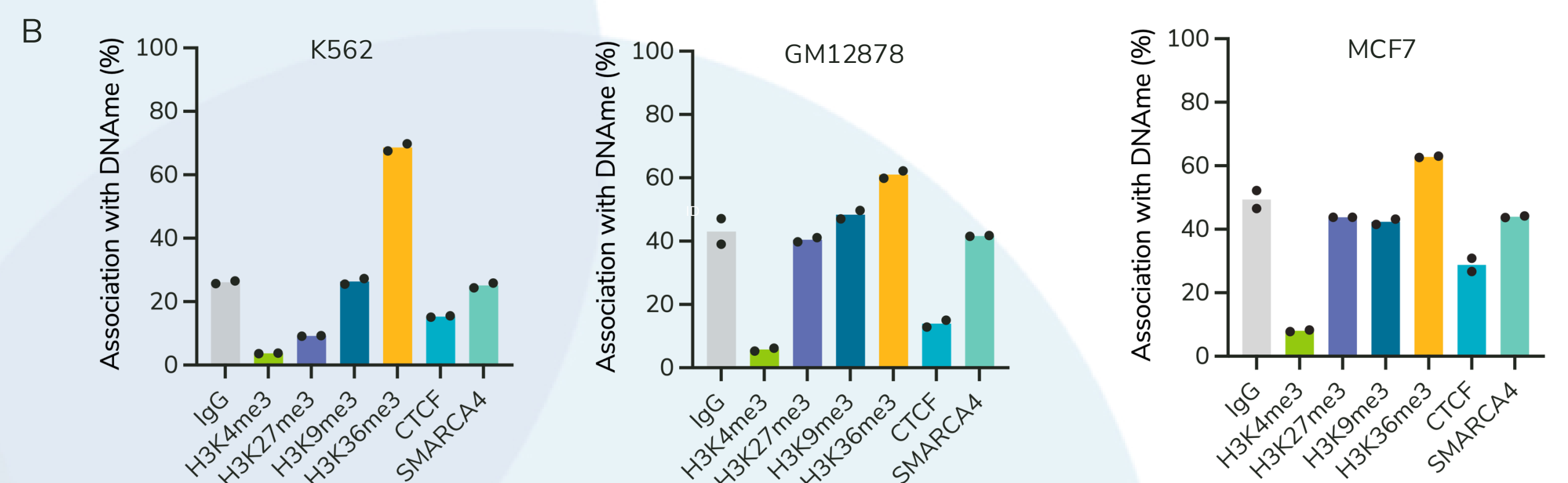
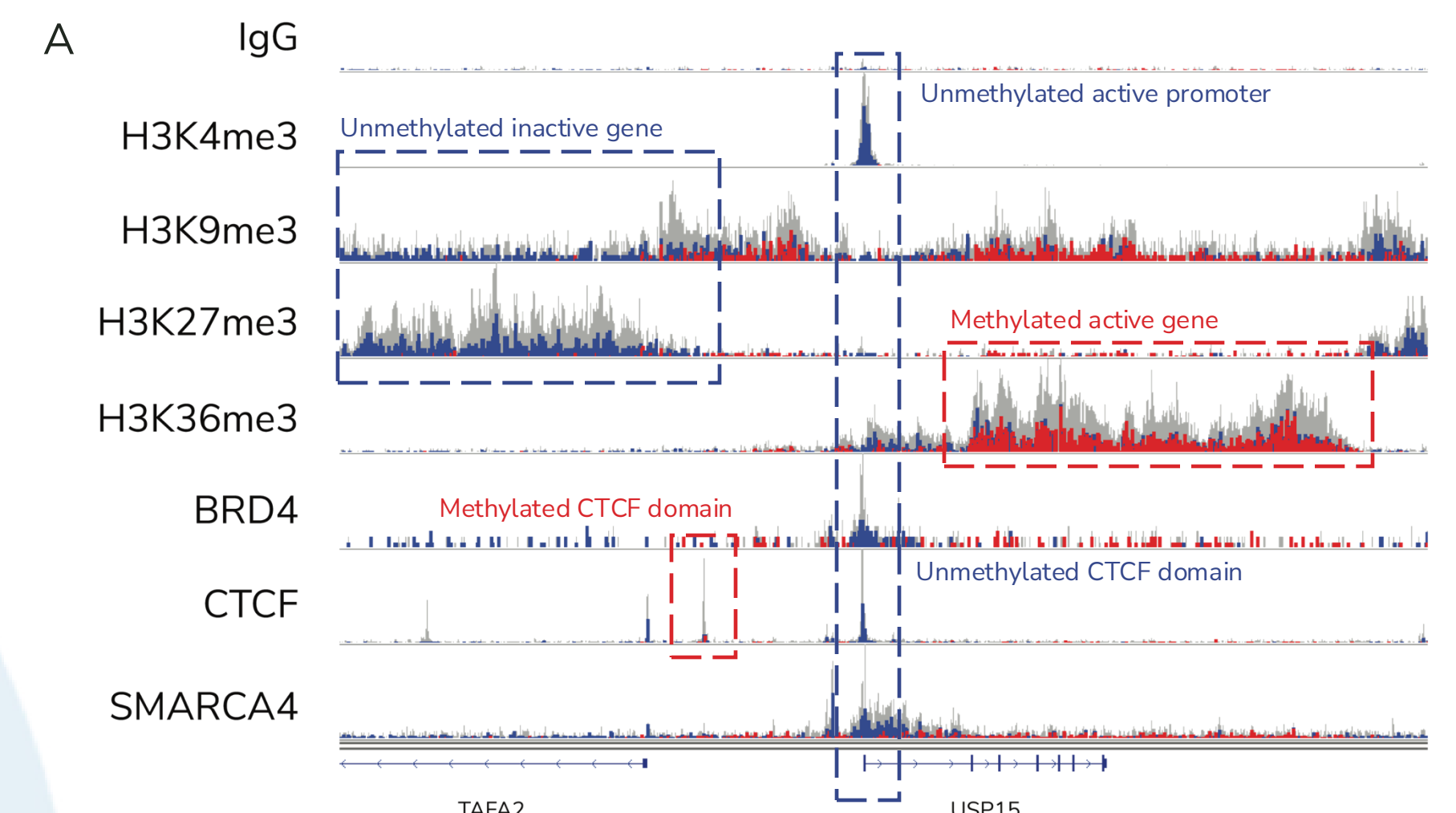
## One assay, countless insights: benchmarking CUT&RUN-EM to CUT&RUN and EM-seq



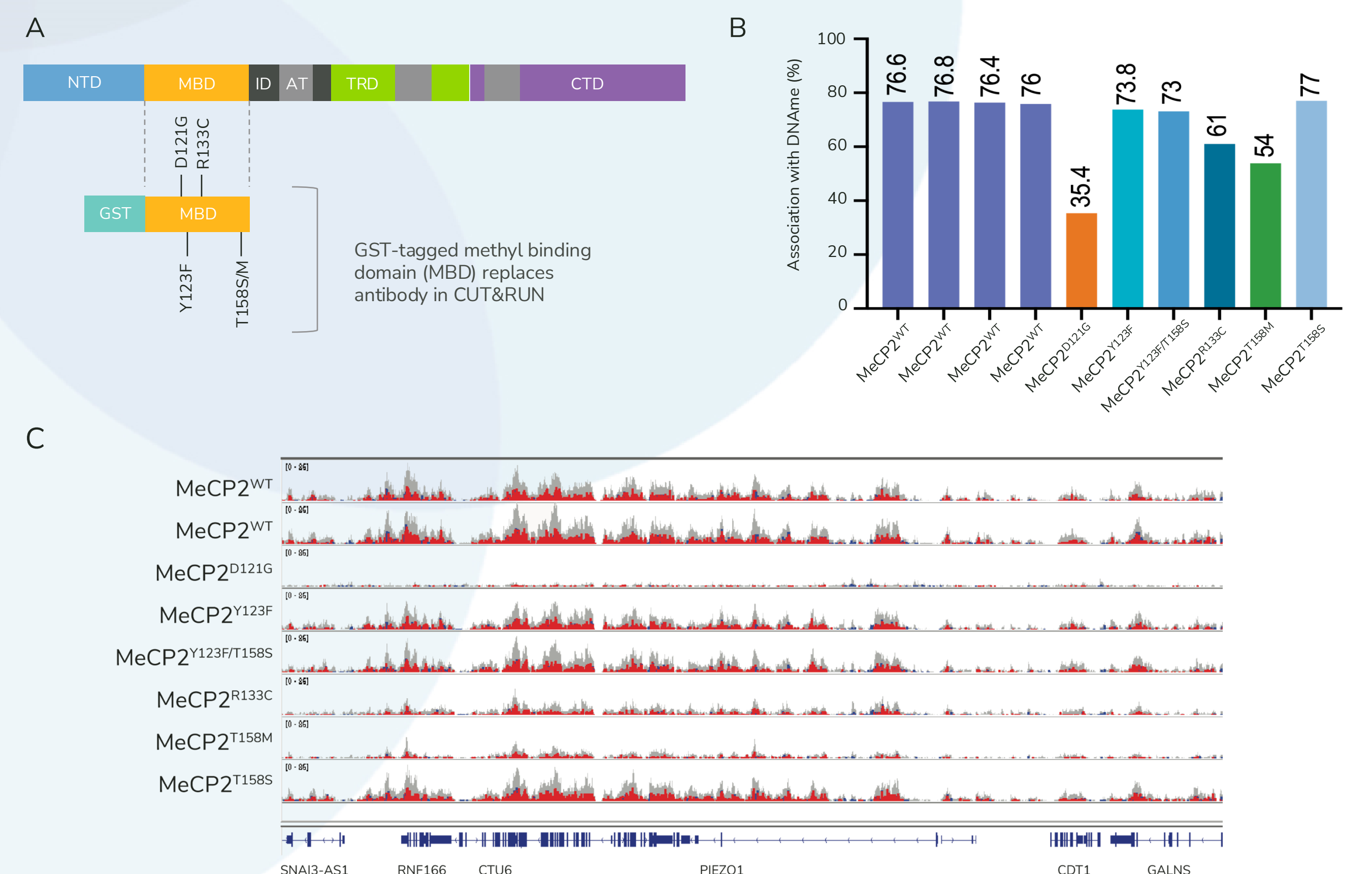
**Figure 3.** Genome browser tracks comparing CUT&RUN (C&R), CUT&RUN-EM (C&R-EM), and whole genome EM-seq (WGEM) in K562 cells. Histone PTM-defined loci are concordant (red box) or discordant (blue box) from DNA methylation signal, highlighting the power of CUT&RUN-EM to characterize multiple epigenomic features. IgG a negative control.

## CUT&RUN-EM reveals distinct DNAm profiles at defined genomic features

**Figure 4.** Genome browser tracks (A) and percent methylation analysis (B) of CUT&RUN-EM datasets are consistent with known biological functions of various targets (e.g. unmethylated H3K4me3 promoters, methylated H3K36me3 gene bodies). (B) Chromatin protein association with DNAm varies across cell lines, highlighting the utility of CUT&RUN-EM to provide deeper gene regulatory insights than CUT&RUN or EM-seq alone. Black dots represent technical replicates.



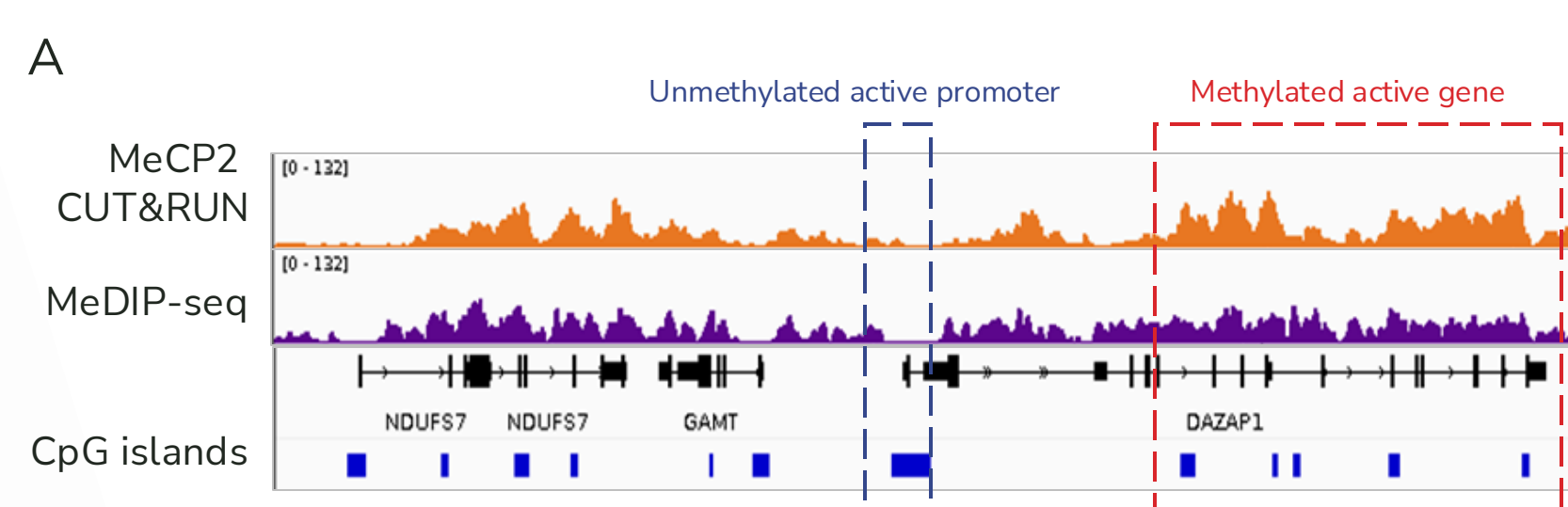
## CUT&RUN-EM provides direct multiomic insights into patient-identified Rett syndrome alleles



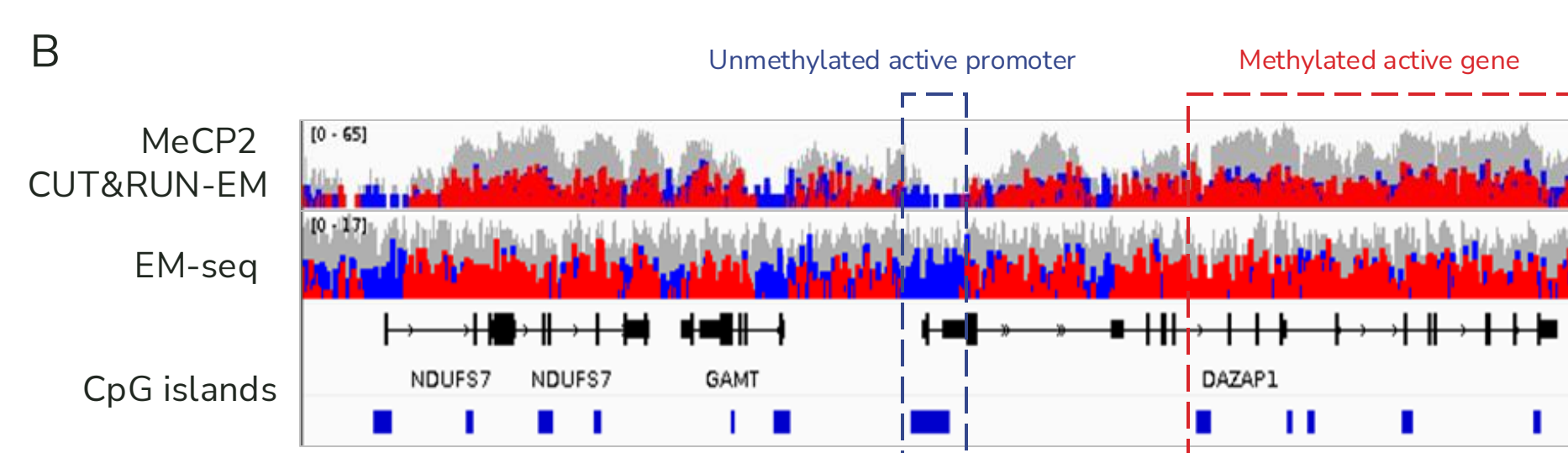
**Figure 5.** Employing MeCP2 as a detection reagent in CUT&RUN-EM provides multiomic insights into patient-identified Rett syndrome alleles. (A) Schematic of MeCP2 protein structure showing the location of the methyl binding domain (MBD). The location of four clinically-relevant Rett syndrome variants are indicated. Wildtype and variant MeCP2 MBDs were subcloned and GST-tagged for use as detection reagents in CUT&RUN-EM experiments, described herein. (B) MeCP2 MBD variants display varying levels of DNAm binding in K562 cells. Percent methylation calculated as the fraction of EM-converted base pairs in CUT&RUN-EM. (C) CUT&RUN-EM genome browser tracks visualize diminished localization of GST-MeCP2 alleles to DNAm in K562 cells.

## Ultra-sensitive, low-cost DNA methylation profiling with MeCP2 CUT&RUN-EM

### MeCP2 CUT&RUN provides massive gains vs. MeDIP-seq



### Combine with EM-seq for base-pair resolution DNAm profiling



### Capture 83% of DNAm with 34X less sequencing



**Figure 6.** The use of MeCP2 as a DNA methylation sensor in CUT&RUN provides multiple strategies for DNA methylation profiling. (A) Validation of GST-MeCP2 MBD in CUT&RUN (orange), using 500k K562 cells and 10M sequencing reads. To examine DNA methylation enrichment capabilities, MeCP2 CUT&RUN was benchmarked against commonly used methyl-DNA immunoprecipitation (MeDIP-seq) assays, shown in purple. MeCP2 showed high concordance with MeDIP-seq at >5-fold reduced sequencing depth. (B) MeCP2 was used in CUT&RUN-EM to enrich methylated DNA and provide a base-pair resolution readout of DNA methylation (K562 cells). Whole-genome EM-seq was used to benchmark data, which required >100M reads. Notably, MeCP2 CUT&RUN-EM generates similar DNA methylation profiles, while only requiring 16M reads and 500k cells. (C) Genome-wide targeting of DNAm-rich areas using MeCP2 CUT&RUN-EM greatly reduces sequencing cost while maintaining sensitivity.

### Conclusions

- CUT&RUN-EM provide a wholistic view of DNAm and chromatin protein **interplay a single workflow**
- CUT&RUN-EM can be leveraged to **gain mechanistic insights** into patient-derived genetic variants
- MeCP2 CUT&RUN-EM provides a **base pair resolution, low-cost alternative** to genome-wide DNAm assays

### Download poster

