Multi-omics analysis of *BRPF1* mutations in rare disease

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Background

Bromodomain and PHD finger-containing protein 1 (*BRPF1*) is an epigenetic 'reader' and scaffolding protein that forms a complex with the lysine acetyltransferases *KAT6/B* and accessory proteins to facilitate histone acetylation at **H3K23, H3K14, and H3K9**, increasing chromatin accessibility and gene expression.

De novo, pathogenic mutations in *BRPF1* result in a rare neurodevelopmental disorder, **Intellectual Developmental Disorder with Dysmorphic Facies and Ptosis** (IDDDFP, OMIM#617333).

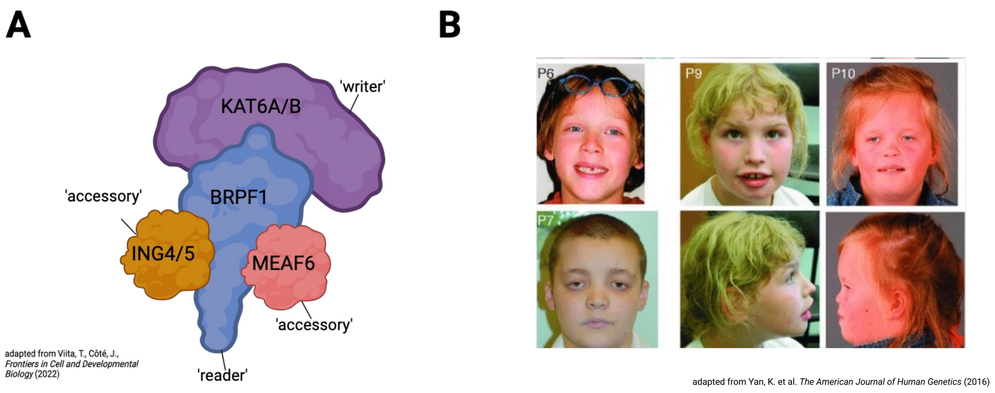
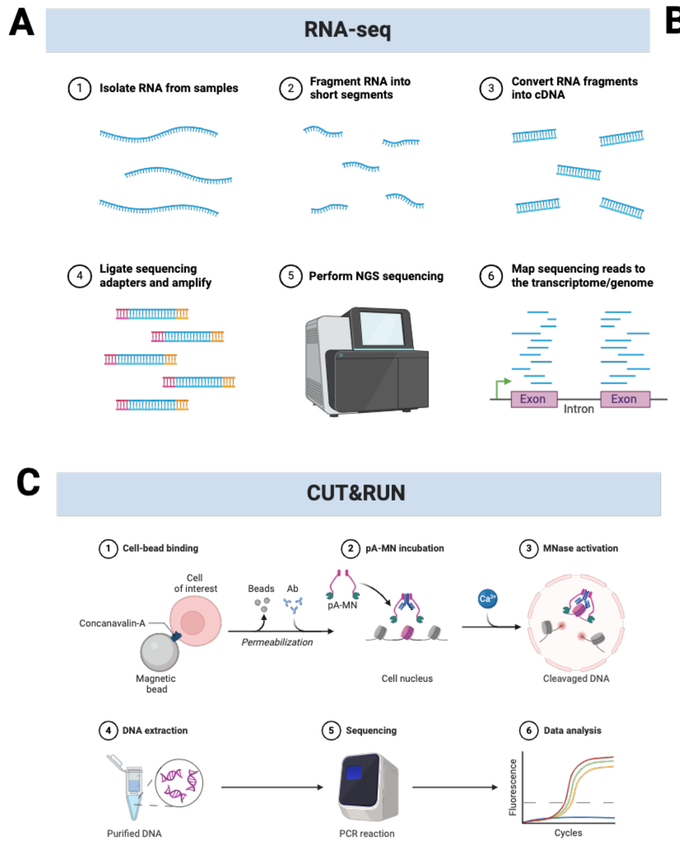


Figure 1: BRPF1 facilitates lysine acetyltransferase (KAT) activity at histone and non-histone proteins. A) As a chromatin reader, BRPF1 forms a tetrameric complex with KAT6A/B and accessory proteins to facilitate histone acetylation at H3K23, H3K14, and H3K9. B) Germline mutations in *BRPF1* result in a rare neurodevelopmental disorder known as Intellectual Developmental Disorder with Dysmorphic Facies and Ptosis (IDDDFP). Approximately 60 cases of IDDDFP are reported in the literature.

The specific molecular mechanism linking *BRPF1* mutations to disease is not well understood. To this end, we developed clonal BRPF1-reporter Schwann cell models that recapitulate pathogenic mutations in *BRPF1* endogenously: p.Q96*, p.Y713*, and p.C998Afs*73. These lines were subsequently used to profile the effect of *BRPF1* mutations on the epigenome and transcriptome via multi-omics assays such as ATAC-seq, RNA-seq, and CUT&RUN.

Methods



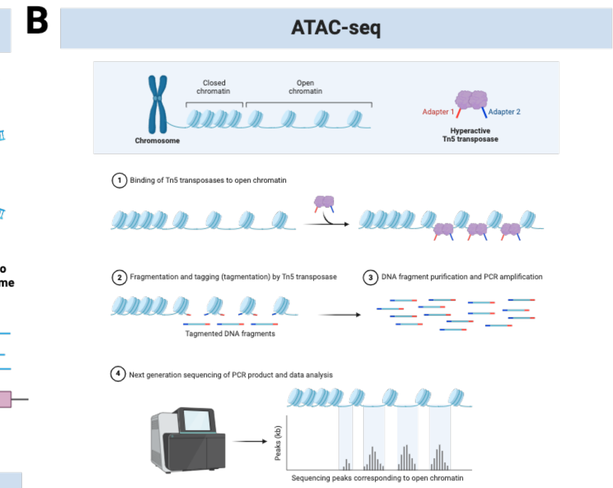


Figure 3: Omics assays to profile the effects of *BRPF1* mutations on the epigenome and transcriptome.
We developed cell models to study the effects of three *BRPF1* mutations on the epigenome and transcriptome in an immortalized Schwann cell line. A) RNA-seq to identify differences in gene expression between *BRPF1* wild type and mutated samples. B) ATAC-seq to profile differential chromatin accessibility at genomic loci. C) CUT&RUN to assess BRPF1-DNA binding and possible co-localization with histone H3 acetylation sites.

BRPF1 mutations dysregulate the epigenome and transcriptome

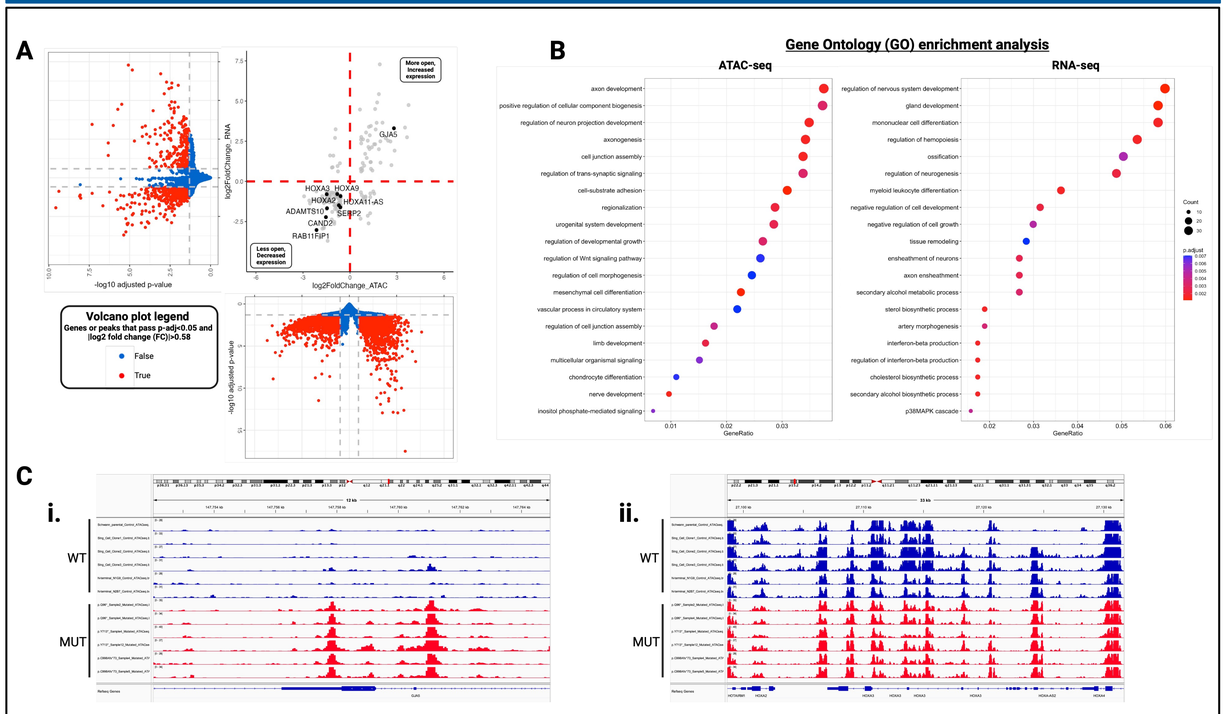


Figure 4: *BRPF1* mutations drive dysregulation of expression and transcription of *HOXA* cluster genes and other genes associated with key developmental pathways. Epigenomic data from *BRPF1*-mutated samples compared to controls shows regulation of *HOXA* cluster genes and *GJA5*. A) Integration of chromatin accessibility (ATAC-seq, X-axis) and gene expression (RNA-seq, Y axis) data derived from *BRPF1*-HiBiT Schwann cells that were edited to reflect pathogenic mutations in *BRPF1* (controls n=6, cases n=6). Differentially expressed peaks or genes are marked in red (Bonferroni p-adj<0.05 and |log2 fold change (FC)|>0.58). 155 unique targets were dysregulated across both datasets (center). Genes associated with the *HOXA* cluster and other key developmental pathways are labeled. B) ATAC-seq and RNA-seq gene ontology highlights enrichment of genes related to development and cell signaling. C) (i) bigWig coverage tracks for ATACseq across *GJA5*. (ii) bigWig coverage tracks for ATACseq across the *HOXA* cluster.

Validation of H3K9ac and H3K14ac antibodies for CUT&RUN

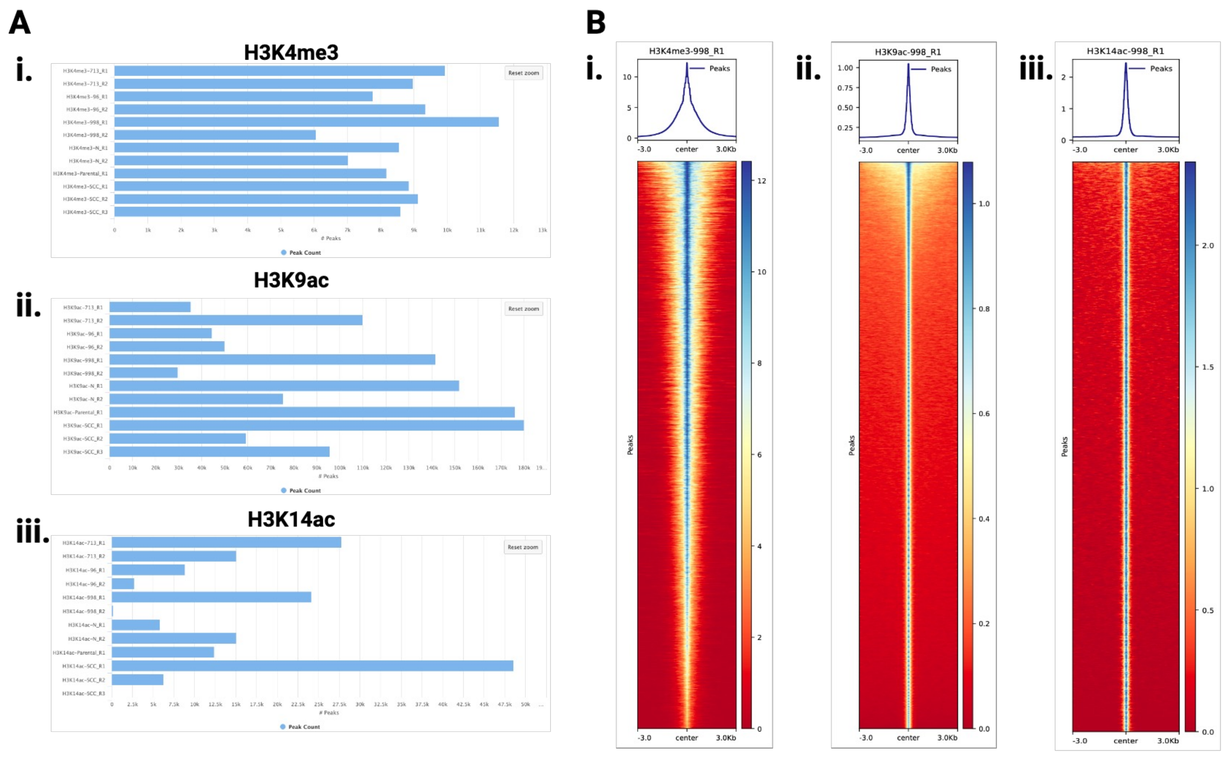


Figure 7: Validation of H3K9ac and H3K14ac antibodies for CUT&RUN. A) Total peak count for the H3K4me3 (positive control), H3K9ac, and H3K14ac antibodies relative to IgG antibody (negative control) (i, ii, iii, respectively). B) Tornado plots demonstrating the average signal intensity across the transcription factor start sites (TSS). Example tornado plots in the the Schwann cell line with the *BRPF1* mutation p.C998Afs*73 assessing the H3K4me3, H3K9ac, and H3K14ac antibodies (i, ii, iii, respectively).

Conclusions and Future Directions

- *BRPF1* mutations result in epigenetic and transcriptional dysregulation of the *HOXA* cluster of genes, *SEPR2*, and *GJA5*, involved in key developmental pathways.
- Pilot assay validates H3K9ac and H3K14ac antibodies for CUT&RUN.
- *BRPF1* mutations alter H3K9ac and H3K14ac at *HOXA3,* suggesting impaired reader function.
- Future directions include the optimization of additional antibodies for CUT&RUN.

BRPF1 mutations alter H3K9ac and H3K14ac at *HOXA3*

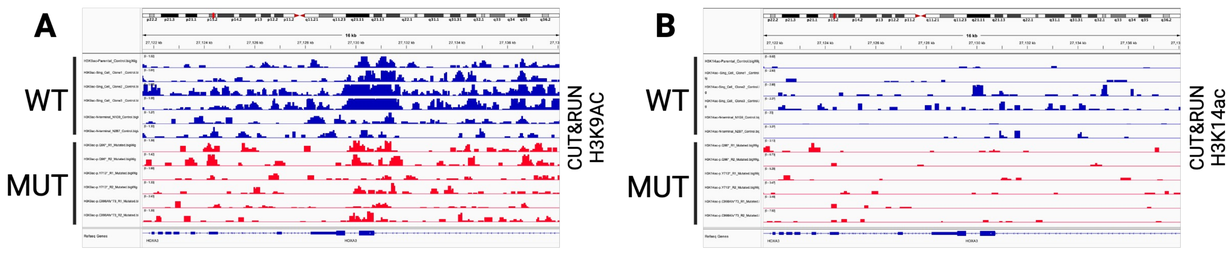


Figure 8: Pilot CUT&RUN assay identifies altered H3K9 and H3K14 acetylation at *HOXA3* upon *BRPF1* mutation. A) BigWig coverage tracks for CUT&RUN H3K9ac antibody bound control and mutated samples across *HOXA3.* B) BigWig coverage tracks for CUT&RUN H3K14ac antibody bound control and mutated samples across *HOXA3.*

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