Figure legends

Figure 1. Global methylation dysregulation, but not global hydroxymethylation dysregulation, is associated with tumor type and grade. A) Cumulative proportion of 5-hmC and 5-mC in tumors and non-tumor tissue. **B)** Methylation dysregulation index of 5-hmC and 5-mC by tumor type and **D)** grade. Gray segments indicate median MDI values. Differences in MDI were calculated using the Kruskal-Wallis test. **C)** Correlation between 5-hmC MDI and 5-mC MDI calculated using Spearman rank correlation. Linear regression line is indicated by the blue line. 95% confidence interval indicated by gray bands.

Figure 2. Adjusting for proportions of cell types of interest reduces the number of differentially hydroxymethylated and methylated CpGs across tumor types compared to non-tumor pediatric brain tissue. The number of differentially hydroxymethylated (hmC) and methylated (mC) CpGs under q-value < 0.05 threshold in **A)** astrocytoma (ATC), **B)** embryonal tumors (EMB), **C)** ependymoma (EPN), and **D)** glioneuronal/neuronal tumors (GNN) compared to non-tumor pediatric brain tissue. X-axes indicate each cell type proportion included in the model. Each model, even the 'unadjusted' model includes sex and age at diagnosis in the linear model. **E)** Venn diagram of the differentially hydroxymethylated CpGs among the different tumor types.

Figure 3. Hypo-hydroxymethylation of CpGs is associated with changes in gene expression. Association between differentially hydroxymethylated CpG beta coefficients and log2 fold changes in gene expression for **A)** astrocytoma, **B)** embryonal tumors, **C)** ependymoma, and **D)** glioneuronal/neuronal tumors. Red points indicate significantly differentially expressed genes. Shapes indicate genomic context of CpGs.

Figure 4. 5-hmC is altered in cell type-specific and tumor type-specific manner. Cell type associated differentially **A)** hydroxymethylated and **B)** methylated CpGs in each tumor type. Venn diagram of shared differentially hydroxymethylated CpGs in **C)** neuronal-like cell types and **D)** progenitor-like cell types across the four tumor types. **ATC**: Astrocytoma; **EMB**: Embryonal tumors; **EPN**: Ependymoma; **GNN**: Glioneuronal/neuronal tumors.

Figure 5. Cell type-specific differential hydroxymethylation tumor type-specific. Enrichment of differentially hydroxymethylated CpGs at specific genomic contexts by tumor type and direction of differential methylation.

Figure 6. Alterations in hydroxymethylation are associated with cell type-specific changes in gene expression. A) Summary heatmap of changes in gene expression in the gene sets with differentially hydroxymethylated CpGs per cell type. The number of differentially hydroxymethylated CpGs associated with **B)** *HDAC4* and **C)** *IGF1R* in each genomic context across the different tumor types in neuronal-like cell types and progenitor-like cell types. Blue bars indicate the number of hydroxymethylated CpGs that are decreased in the tumors. Yellow bars indicate the number of hydroxymethylated CpGs that are increased in the tumors. **D)** Gene expression levels of *HDAC4* and *IGF1R* for each cell type across the tumor types and non-tumor tissue.

Supplementary figure legends

Supplementary Figure 1. Tumor purity differences by **A)** tumor type and **B)** grade.

Supplementary Figure 2. Methylation dysregulation index from 5-hmC and 5-mC by **A)** tumor type and **B)** grade without outliers. Gray segments indicate median MDI values. Differences in MDI were calculated using the Kruskal-Wallis test. **C)** Correlation between 5-hmC MDI and 5-mC MDI calculated using Spearman rank correlation. The linear regression line is indicated by the blue line. 95% confidence interval indicated by gray bands.

Supplementary Figure 3. Tumor purity is not associated with MDI. Correlation between tumor purity and **A)** 5-mC MDI and **B)** 5-hmC MDI calculated using Spearman rank correlation. The linear regression line is indicated by the blue line. 95% confidence interval indicated by gray bands.

Supplementary Figure 4. A) Results from principal component analysis of cell type proportions from single nuclei RNA-seq of pediatric central nervous system tumors and non-tumor pediatric brain tissue. **B)** Comparison of each tumor type's proportions per cell type against the proportions found in non-tumor pediatric brain tissue. Each point indicates a sample. Differences in proportions are calculated using Wilcoxon signed-rank test.

Supplementary Figure 5. Volcano plots of differential **A)** 5-hmC CpGs and **C)** 5-mC CpGs in astrocytoma in the cell type proportion unadjusted model. Volcano plots of differential **B)** 5-hmC CpGs and **D)** 5-mC CpGs in astrocytoma in the cell type proportion adjusted model. Labeled # of CpGs on the left of each plot are CpGs with decreased methylation in tumors compared to non-tumor tissue. Labeled # of CpGs on the right of each volcano plot are CpGs with increased methylation in tumors compared to non-tumor tissue. Red points indicate statistically significant differential CpGs under the q-value < 0.05 threshold.

Supplementary Figure 6. Volcano plots of differential **A)** 5-hmC CpGs and **C)** 5-mC CpGs in embryonal tumors in the cell type proportion unadjusted model. Volcano plots of differential **B)** 5-hmC CpGs and **D)** 5-mC CpGs in astrocytoma in the cell type proportion adjusted model. Labeled # of CpGs on the left of each plot are CpGs with decreased methylation in tumors compared to non-tumor tissue. Labeled # of CpGs on the right of each volcano plot are CpGs with increased methylation in tumors compared to non-tumor tissue. Red points indicate statistically significant differential CpGs under the q-value < 0.05 threshold.

Supplementary Figure 7. Volcano plots of differential **A)** 5-hmC CpGs and **C)** 5-mC CpGs in ependymoma in the cell type proportion unadjusted model. Volcano plots of differential **B)** 5-hmC CpGs and **D)** 5-mC CpGs in astrocytoma in the cell type proportion adjusted model. Labeled # of CpGs on the left of each plot are CpGs with decreased methylation in tumors compared to non-tumor tissue. Labeled # of CpGs on the right of each volcano plot are CpGs with increased methylation in tumors compared to non-tumor tissue. Red points indicate statistically significant differential CpGs under the q-value < 0.05 threshold.

Supplementary Figure 8. Volcano plots of differential **A)** 5-hmC CpGs and **C)** 5-mC CpGs in glioneuronal/neuronal tumors in the cell type proportion unadjusted model. Volcano plots of differential **B)** 5-hmC CpGs and **D)** 5-mC CpGs in astrocytoma in the cell type proportion adjusted model. Labeled # of CpGs on the left of each plot are CpGs with decreased methylation in tumors compared to non-tumor tissue. Labeled # of CpGs on the right of each volcano plot are CpGs with increased methylation in tumors compared to non-tumor tissue. Red points indicate statistically significant differential CpGs under the q-value < 0.05 threshold.

Supplementary Figure 9. A) Number of differentially expressed genes unadjusted and adjusted for cell type proportions for each tumor type. Venn diagram of the genes with significant **B)** increased and **C)** decreased expression in the tumor types. **D)** Pathways associated with shared genes with increased expression in the tumors. Only pathways under q-value < 0.05 are shown. **E)** Pathways associated with shared genes with decreased expression in the tumors. Only pathways under q-value < 0.05 are shown.

Supplementary Figure 10. Volcano plot of differential expression test in the **A)** cell type proportion unadjusted and **B)** cell type proportion adjusted model comparing astrocytoma and non-tumor brain tissue. **C)** Pathways associated with the differential expression in astrocytoma.

Supplementary Figure 11. Volcano plot of differential expression test in the **A)** cell type proportion unadjusted and **B)** cell type proportion adjusted model comparing embryonal tumors and non-tumor brain tissue. **C)** Pathways associated with the differential expression in embryonal tumors.

Supplementary Figure 12. Volcano plot of differential expression test in the **A)** cell type proportion unadjusted and **B)** cell type proportion adjusted model comparing ependymoma and non-tumor brain tissue. **C)** Pathways associated with the differential expression in ependymoma.

Supplementary Figure 13. Volcano plot of differential expression test in the **A)** cell type proportion unadjusted and **B)** cell type proportion adjusted model comparing glioneuronal/neuronal tumors and non-tumor brain tissue. **C)** Pathways associated with the differential expression in glioneuronal/neuronal tumors.

Supplementary Figure 14. Association between changes in 5-mC and gene expression for embryonal tumors. Red points indicate significantly differentially expressed genes.

Supplementary Figure 15. A) Cell type-specific differentially hydroxymethylated and methylated CpGs in astrocytoma. Venn diagram of differentially **B)** hydroxymethylated and **C)** methylated CpGs in neuronal-like cell types (NEU) and progenitor-like cell types (PROG).

Supplementary Figure 16. A) Cell type-specific differentially hydroxymethylated and methylated CpGs in embryonal tumors. Venn diagram of differentially **B)** hydroxymethylated and **C)** methylated CpGs in neuronal-like cell types (NEU) and progenitor-like cell types (PROG).

Supplementary Figure 17. A) Cell type-specific differentially hydroxymethylated and methylated CpGs in ependymoma. Venn diagram of differentially **B)** hydroxymethylated

and **C)** methylated CpGs in neuronal-like cell types (NEU) and progenitor-like cell types (PROG).

Supplementary Figure 18. A) Cell type-specific differentially hydroxymethylated and methylated CpGs in glioneuronal/neuronal tumors. Venn diagram of differentially **B)** hydroxymethylated and **C)** methylated CpGs in neuronal-like cell types (NEU) and progenitor-like cell types (PROG).

Supplementary Figure 19. Venn diagram of **A)** hypomethylated and **B)** hypermethylated CpGs in neuronal-like cell types across tumor types. Venn diagram of **C)** hypomethylated and **D)** hypermethylated CpGs in progenitor-like cell types across tumor types.

Supplementary Figure 20. Venn diagram of genes with differentially hydroxymethylated CpGs in **A)** astrocytomas, **B)** embryonal tumors, **C)** ependymomas, and **D)** glioneuronal/neuronal tumors.

Supplementary Figure 21. Boxplot of enrichment scores of genes with differentially hydroxymethylated CpGs per cell type in astrocytoma and non-tumor brain tissue. Comparison between tumor and non-tumor was made with Wilcoxon rank test.

Supplementary Figure 22. Boxplot of enrichment scores of genes with differentially hydroxymethylated CpGs per cell type in embryonal tumors and non-tumor brain tissue. Comparison between tumor and non-tumor was made with Wilcoxon rank test.

Supplementary Figure 23. Boxplot of enrichment scores of genes with differentially hydroxymethylated CpGs per cell type in ependymoma and non-tumor brain tissue. Comparison between tumor and non-tumor was made with Wilcoxon rank test.

Supplementary Figure 24. Boxplot of enrichment scores of genes with differentially hydroxymethylated CpGs per cell type in glioneuronal/neuronal tumors and non-tumor brain tissue. Comparison between tumor and non-tumor was made with Wilcoxon rank test.

Table legends.

Table 1. Subject demographics

Table 2. Summary measures of global averages and MDI of 5-hmC and 5-mC

Table 3. Genomic context of dhmCpGs in **A**) *HDAC4* and **B**) *IGF1R* for each tumor type.

Table 4. Comparison of the number of differentially hydroxymethylated CpGs in *HDAC4* and *IGF1R* identified by bulk tissue EWAS and CellDMC for each tumor type.

Supplementary table legends.

Supplementary Table 1. Distribution of samples with varying molecular characterization available for analysis.

Supplementary Table 2. Association between tumor purity, grade with **A)** 5-hmC MDI and **B)** 5-mC MDI, determined by linear regression.

Supplementary Table 3. The number of differentially expressed genes with differentially hydroxymethylated CpGs identified by bulk tissue epigenome-wide association study. Categorized by the direction of gene expression change and the genomic context of the differentially hydroxymethylated CpGs for each tumor type.

Supplementary Table 4. The number of nuclei from single nuclei RNA-seq that were included in the CellDMC analysis.

Supplementary Table 5. The number of differentially hydroxymethylated CpGs per tumor type identified by CellDMC. Categorized by the change in the level of hydroxymethylation and cell type of association.