**Supplemental Figure 1: Genomic predictors of survival are recapitulated in an external CBTN cohort.** Kaplan-Meier survival curves of patients comparing survival in patients from PNOC003 (n=28; blue) and CBTN (n=22; red) cohorts (**A);** in patients with *TP53*mut (n=15; red) versus *TP53*wt (n=7; blue) in H3K27M-altered tumors from CBTN cohort (**B);** in patients with 10del (n=8; red) versus 10wt (n=13; blue) in H3K27M-altered tumors from CBTN cohort (**C)**; and in patients with combinations of *TP53*mut/10del (n=14; red), *TP53*mut/10wt (n=20; blue), and *TP53*wt/10wt (n=14; green) in H3K27M-altered tumors from the combined PNOC003 and CBTNcohorts (**D)**. **E,** Hazard ratio analysis of both PNOC003 and CBTN cohorts evaluating impact of *TP53*mut and 10del as independent biomarkers of survival (n=49 events).CBTN, Children’s Brain Tumor Network; wt, wildtype; mut, mutant; del, deletion.

**Supplemental Figure 2: *PDGFRA* and *PTEN* alterations associate with worse survival within H3K27-altered, *TP53*mut DIPG.** Kaplan-Meier survival curves and log-rank P-values for H3K27-altered**,** *TP53* mutant DIPG patients stratified by *PDGFRA* (**A**) and *PTEN* (**B**) alteration status. mut, mutant.

**Supplemental Figure 3: Post-radiation tumor volume and size correlates with somatic alterations.** Association between somatic driver gene status (left column) and change in estimated tumor volume (middle column) and tumor size measured by anterior-posterior (AP) and transverse (TR) dimensions (right column) post-RT.

**Supplemental Figure 4: *TP53*mut associates with CIN in both PNOC003 and CBTN cohorts.** Volcano plots comparing *TP53*mut versus *TP53*wt and CIN overall, chromosome losses (“CIN (loss)”), and chromosome gains ((“CIN (gain)”) PNOC003 cohort (n=30) (**A**) and CBTN cohort (n=21) (**B**). CIN, chromosome instability; CBTN, Children’s Brain Tumor Network; wt, wildtype; mut, mutant.

**Supplemental Figure 5: Methylation profiling confirms diffuse midline glioma, H3K27M-mutant diagnosis in previously identified H3wt tumor.** Methylation profiling report with copy number variation profile of P-04 demonstrating tumor segregates most closely with diffuse midline glioma, H3K27M mutant with calibration score of 0.96. wt, wildtype.

## Supplemental Figure 6: Decrease in H3K27M-mutant plasma ctDNA post-radiation correlates with longer PFS/OS. Kaplan-Meier survival curves comparing PFS and OS between patients with decreased (blue) versus stable/increased (red) plasma H3K27M ctDNA at pre- and post-radiation timepoints, inclusive of patients without markedly elevated ctDNA levels at diagnosis (A-E) and with markedly elevated ctDNA levels at diagnosis (F, G). PFS, progression-free survival; OS, Overall survival; dec, decrease; inc, increase.

## Supplemental Figure 7: Tumor and derived cell lines demonstrate similar global chromosome-level alterations. Methylation profiles comparing tumor (left column) and tumor derived cell lines (right column), patient IDs in middle column.

## Supplemental Figure 8: Genetic alterations in PNOC003 tumors differ spatially and temporally at time of diagnosis, progression, and autopsy, while maintaining driver alterations. Oncoprint representation of driver somatic mutations performed on tumors at biopsy, progression, and autopsy, as applicable. Patients arranged in column groupings and genes are labelled in rows. Percentages on right column represent proportion of patients in cohort with alteration, listed highest to lowest. WES, whole exome sequencing; WGS, whole genome sequencing; RNAseq, RNA sequencing; TMB, tumor mutation burden.