**Supplementary Figure Legends**

**Figure S1. A,** Representative IHC images of BMI-1 staining in DIPG patient tumors and their matched normal tissue. Images are different areas of the same section. **B,** tSNE plot of scRNAseq data from the Filbin-Cohort representing correlations between different samples and **C,** relative *BMI-1* expression. **D,** Correlation of *Bmi-1* expression and stemness score in murine DIPG tumor and control brain stem.

**Figure S2. A,** Scheme representing the experiment design of PTC596 treatment, drug wash-off and time points to evaluate PTC596 activity by immunoblot analysis of BMI-1, H2AK119Ub (Day 4) and cell growth by WST-1 assay (Days 8 and 11) in HFF, CCHMC-DIPG-1 and CCHMC-DIPG-2 cells. β-actin and total H2A served as loading control. Error bars represent the SD from triplicates. **B,** CCHMC-DIPG-1 cells treated with PTC596 (100 nM) were quantified for the number of γH2AX foci/cell at the indicated time points. **C**, Representative IF images and cell cycle analysis (with the indicated % of cells) of CCHMC-DIPG-2 cells treated with PTC596 (100 nM) for 24 hrs and 48 hrs. DNA damage (γH2AX in green) and apoptosis (cleaved caspase-3 in red) were evaluated and their respective quantification. DAPI (blue) represent nuclei. Error bars represent the SD from 4 different fields (n=150-400 cells/field). **D**, Immunoblot analysis of H3 S10-P in DIPG cells treated with indicated doses of PTC596 for 24 hrs. Total H3 served as loading control. **E,** Representative IF-FISH images of metaphase spreads with centromere probe (red) and γH2AX (green) in CCHMC-DIPG-1 and SU-DIPG-IV cells treated with PTC596 (60 nM and 30 nM respectively) for 48 hrs. DAPI (blue) indicates chromosomes. White arrowheads indicate chromosomes with PSCS.

**Figure S3. A**, Soft agar assay of an additional cell line, CCHMC-DIPG-1, to assess sensitization to IR when treated with PTC596 and its respective quantification. Error bars represent the SD from two independent experiments run in triplicate. **B,** Relative cell growth calculated by WST-1 assay at Day 7 used to calculate the CI values in figure 4C. Three independent experiments were performed and data from one experiment is shown as a representative result. Error bars represent the SD from triplicates.

**Figure S4.** Immunoblot analysis of BMI-1 and H2AK119Ub after treatment of DIPG cells with PTC596 and EPZ011989 at Days 6 and 8 as represented in figure 5D.

**Figure S5.** Survival probability of mice post-drug withdrawal starting from drug holiday. *P* values are indicated, \**p*<0.05.

**Supplementary Table S1.** Histone mutation and BMI-1 expression (log2 foldchange) of patient tumor section used for IHC in figure S1A.

**Supplementary Table S2.**  IC50(nM) of PTC596 in cell lines treated for 72hrs.