Online Resource 1

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Recurrent *KBTBD4* small in-frame insertions and absence of *DROSHA* deletion or *DICER1* mutation differentiate pineal parenchymal tumor of intermediate differentiation (PPTID) from pineoblastoma

Contents:

Supplementary Methods

Whole-exome sequencing analysis

Tumor tissue was macrodissected from formalin-fixed, paraffin-embedded blocks for the 8 patients. Genomic DNA was extracted from this macrodissected tumor tissue using the QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer's protocol. Genomic DNA was also extracted from matched normal tissues (peripheral blood, n=4; skeletal muscle, n=2; skin, n=1; uninvolved brain parenchyma, n=1) using the QIAamp DNA Blood Midi Kit (Qiagen) or QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer's protocol. Multiplex library preparation was performed using the KAPA Hyper Prep Kit (Roche) according to the manufacturer's specifications using 250 ng of sample DNA. Hybridization-capture of pooled libraries was performed using the NimbleGen SeqCap EZ Human Exome Probes v3.0 Bait Library (Roche). Captured libraries were sequenced as paired-end 100 base pair reads on a HiSeq 2500 instrument (Illumina). Sequence reads were mapped to the reference human genome build GRCh37 (hg19) using Sentieon DNAseq [1]. Deduplication and recalibration of reads was performed using Sentieon DNAseq [1]. Somatic variant detection including single nucleotide variants and insertions/deletions was performed with Sentieon TNscope [2]. Sequencing metrics were determined using Picard CalculateHsMetrics and Picard CollectInsertSizeMetrics [3]. Variant annotation was performed with Annovar [4]. Single nucleotide variants and insertions/deletions were visualized and verified using Integrated Genome Viewer [5]. Copy number analysis was performed using CNVkit [6] and visualized using Nexus Copy Number (BioDiscovery).

High-confidence somatic nonsynonymous mutations were defined as those single nucleotide variants and insertions/deletions fulfilling the following criteria: 1) greater than 10 variant reads in the tumor sample, 2) greater than 10% variant allele frequency in the tumor sample, 3) greater than 10 total reads in the normal sample, 4) less than 2 variant reads in the normal sample, 5) less than 2% variant allele frequency in the normal sample, and 6) variants within the coding sequence or splice sites of well-

annotated protein coding genes that are predicted to result in variation of the encoded amino acid sequence (*i.e.* excluding synonymous variants).

Supplementary References

1. Sentieon. Sentieon DNAseq. https://www.sentieon.com/products/

2. Sentieon. Sentieon TNscope. https://www.sentieon.com/products/

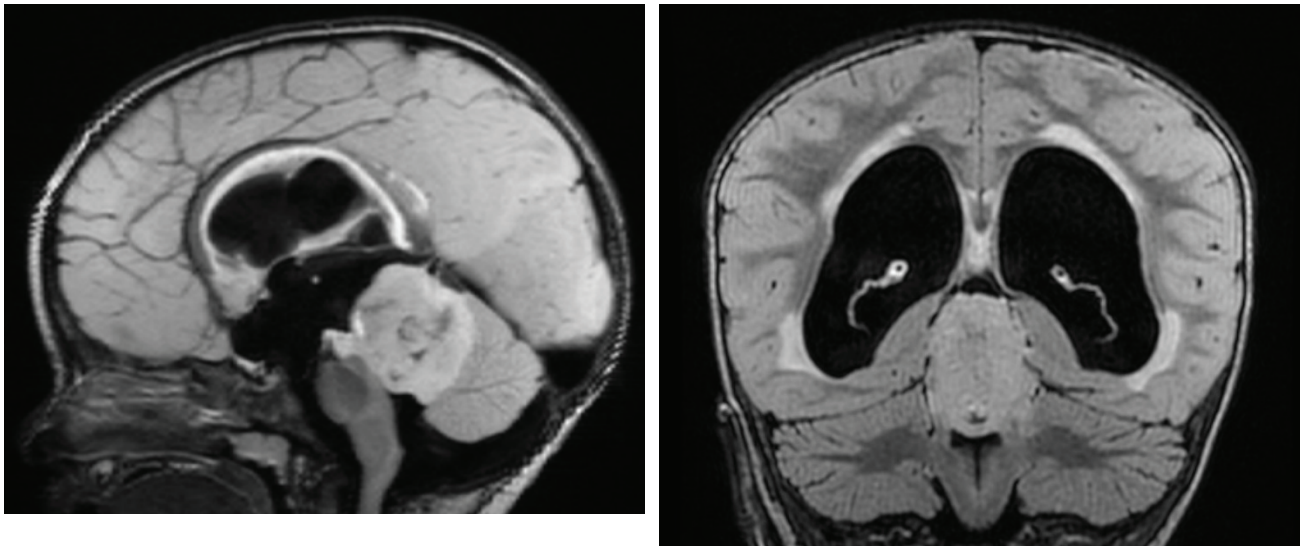
3. Broad Institute. Picard. https://broadinstitute.github.io/picard/

4. Yang H, Wang K (2015) Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR. Nat Protoc 10:1556-1566

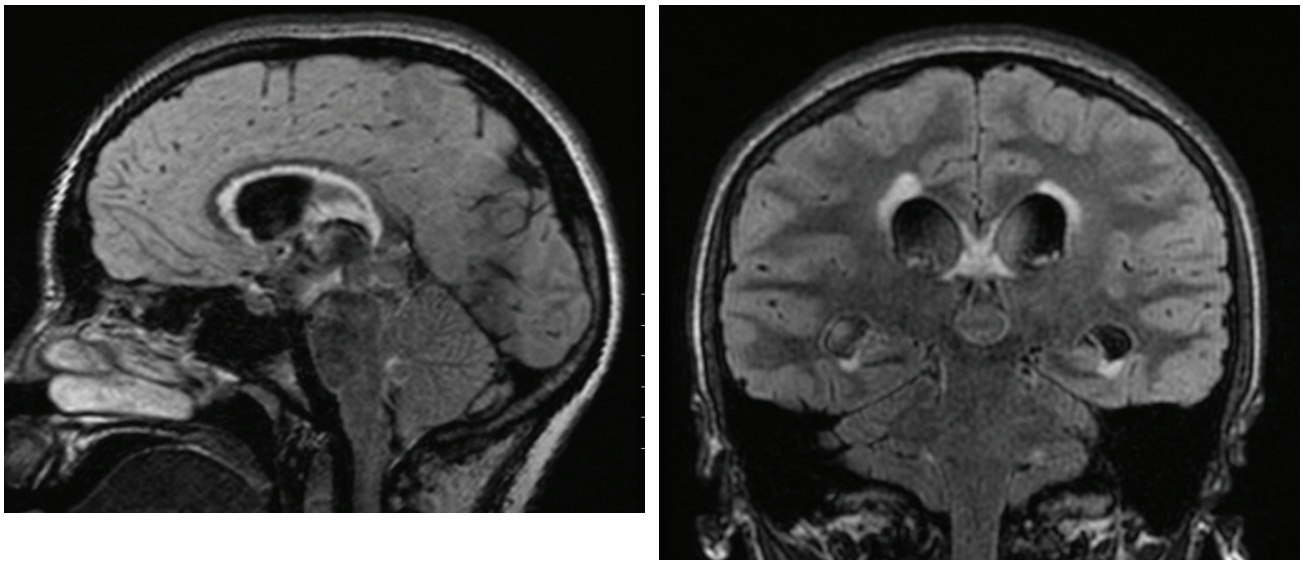
5. Thorvaldsdottir H, Robinson JT, Mesirov JP (2013) Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief Bioinform 14:178-192

6. Talevich E, Shain AH, Botton T, Bastian BC (2014) CNVkit: Genome-wide copy number detection and visualization from targeted sequencing. PLoS Comput Biol 12:e1004873

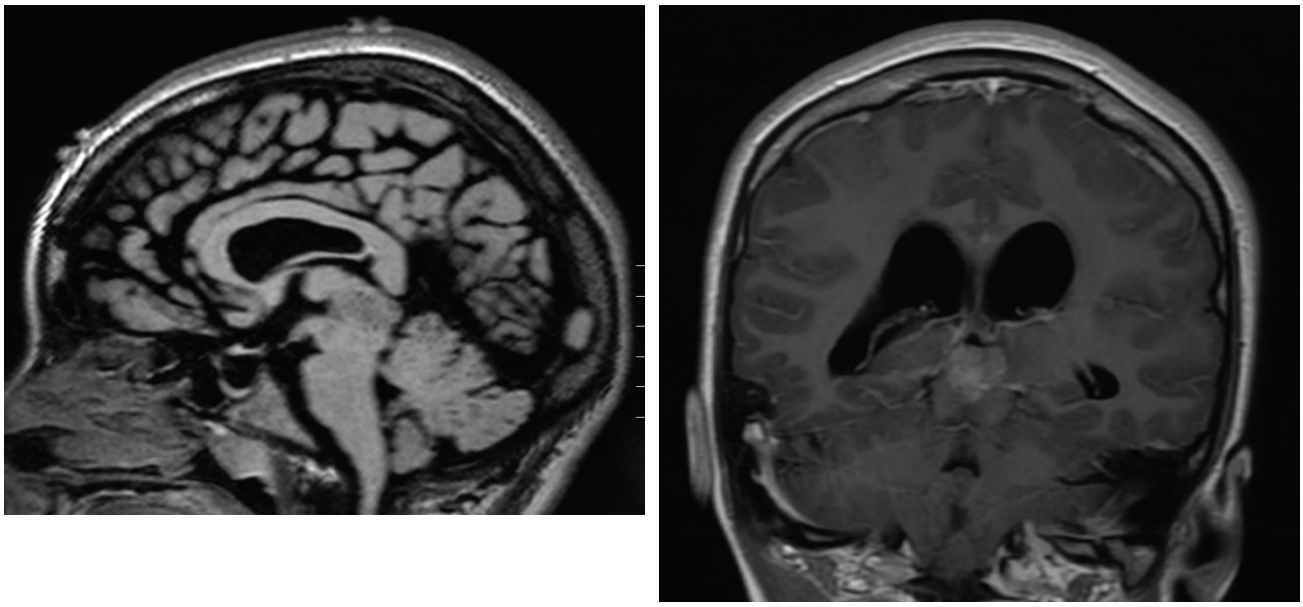
PPT #1, 1 y/o M, pineoblastoma, DICER1 p.E1813D + p.V1080fs



PPT #3, 13 y/o F, pineoblastoma, DROSHA homozygous deletion

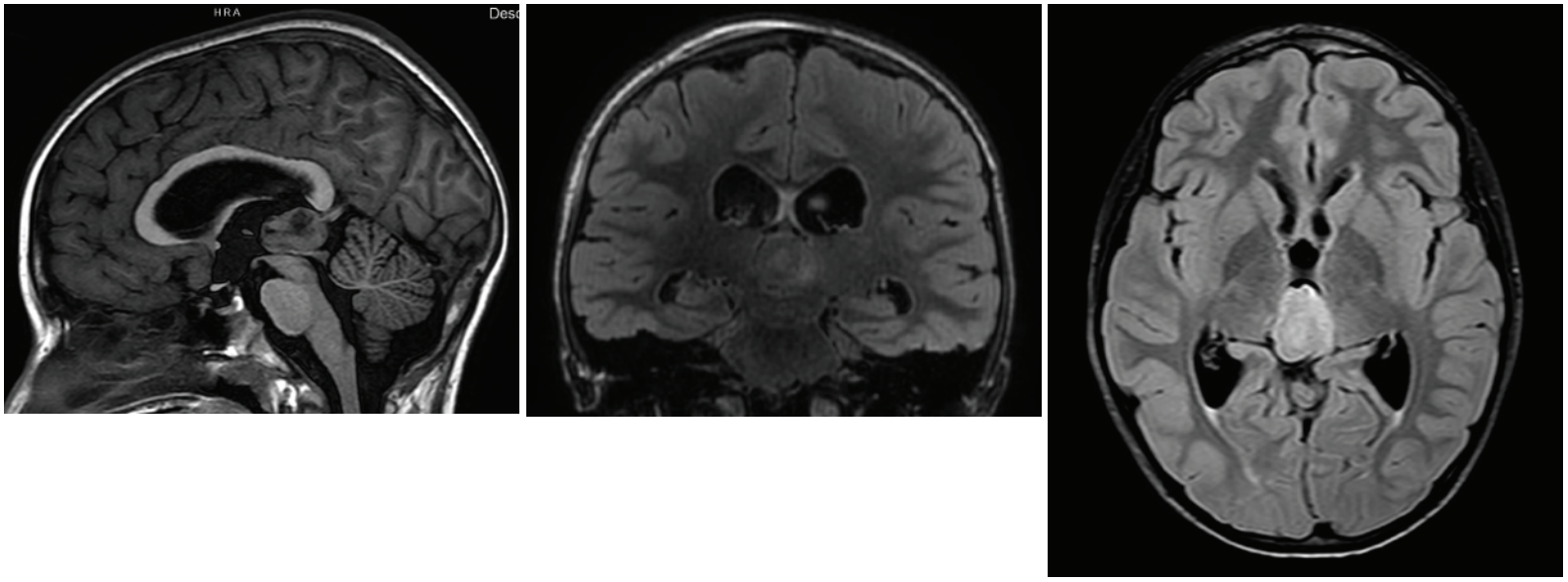


PPT #4, 16 y/o F, pineoblastoma, DROSHA homozygous deletion

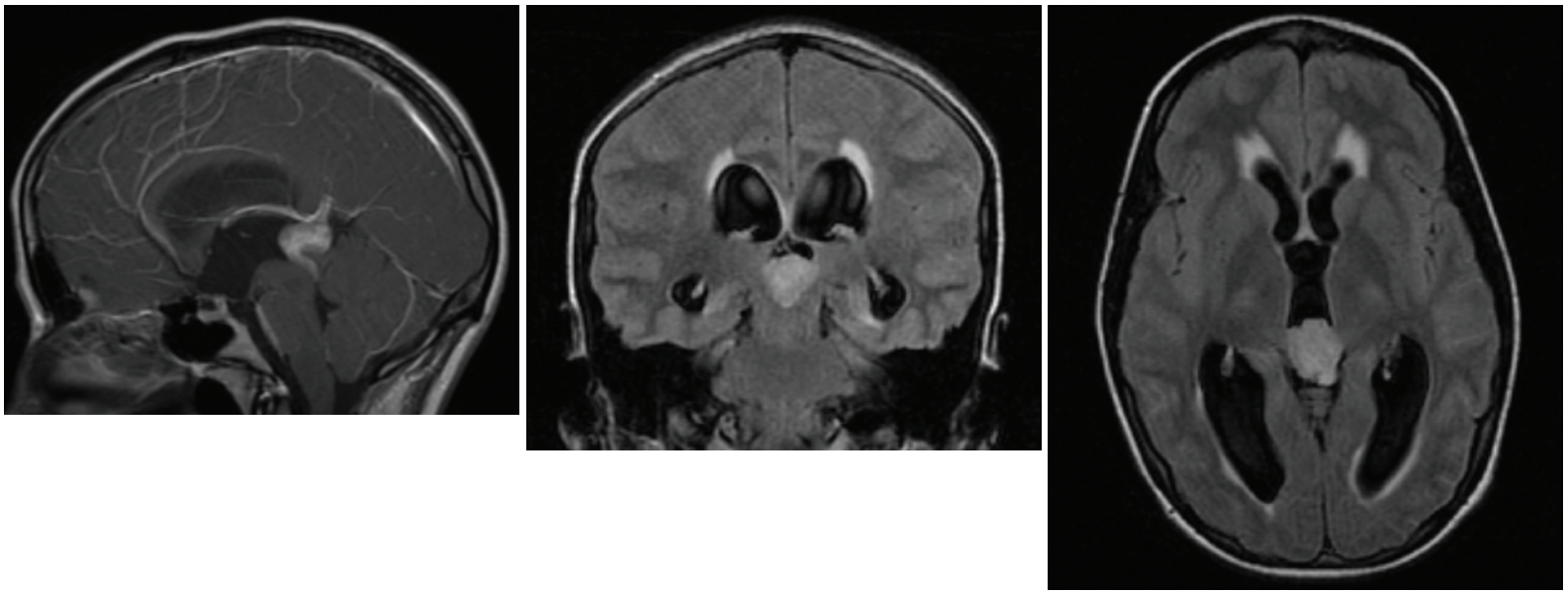


Supplementary Figure 1. Pre-operative imaging features of the eight patients with pineal parenchymal tumors that were studied by whole exome sequencing.

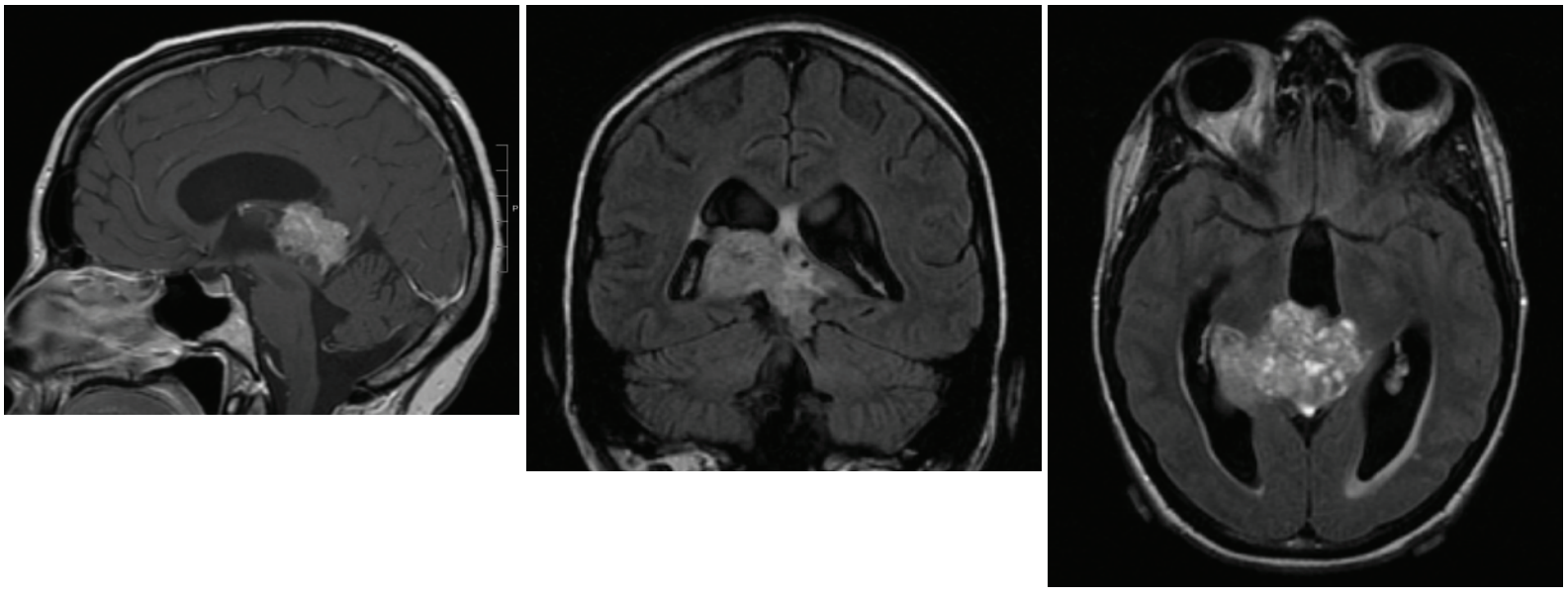
PPT #5, 9 y/o M, PPITD, KBTBD4 p.R313delinsPRR



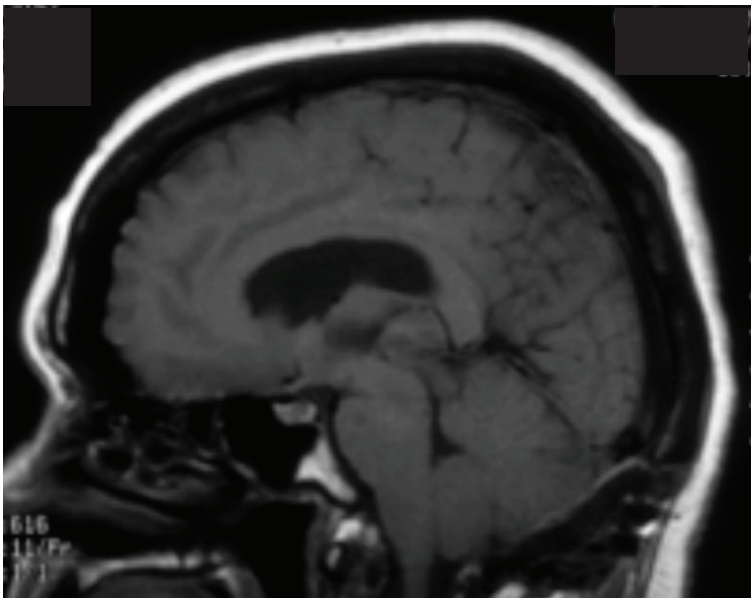
PPT #6, 18 y/o F, PPTID, KBTBD4 p.R313delinsPRR

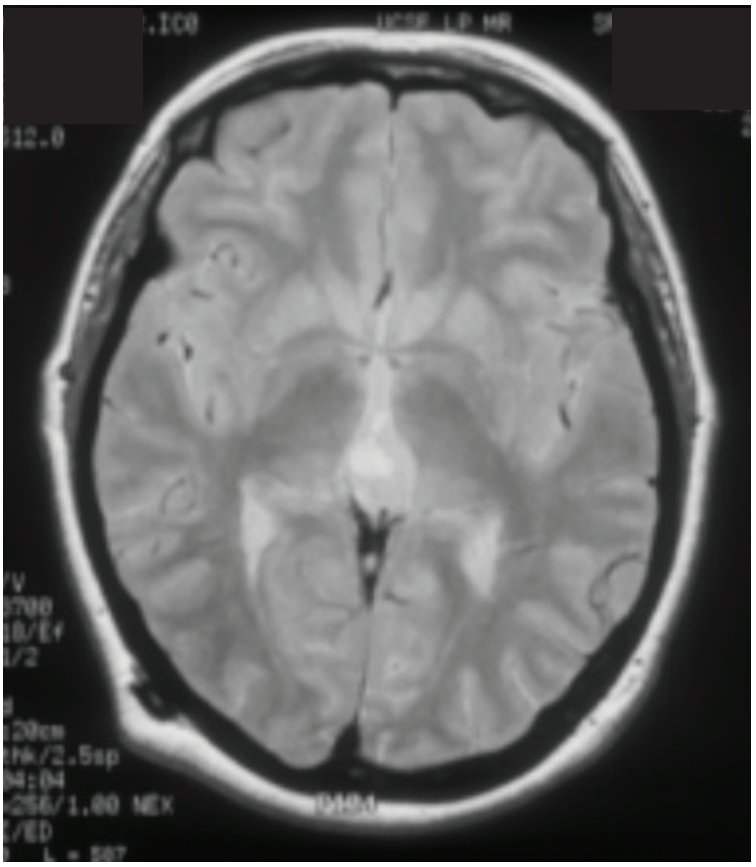


PPT #7, 27 y/o F, PPTID, KBTBD4 p.R313delinsPRR

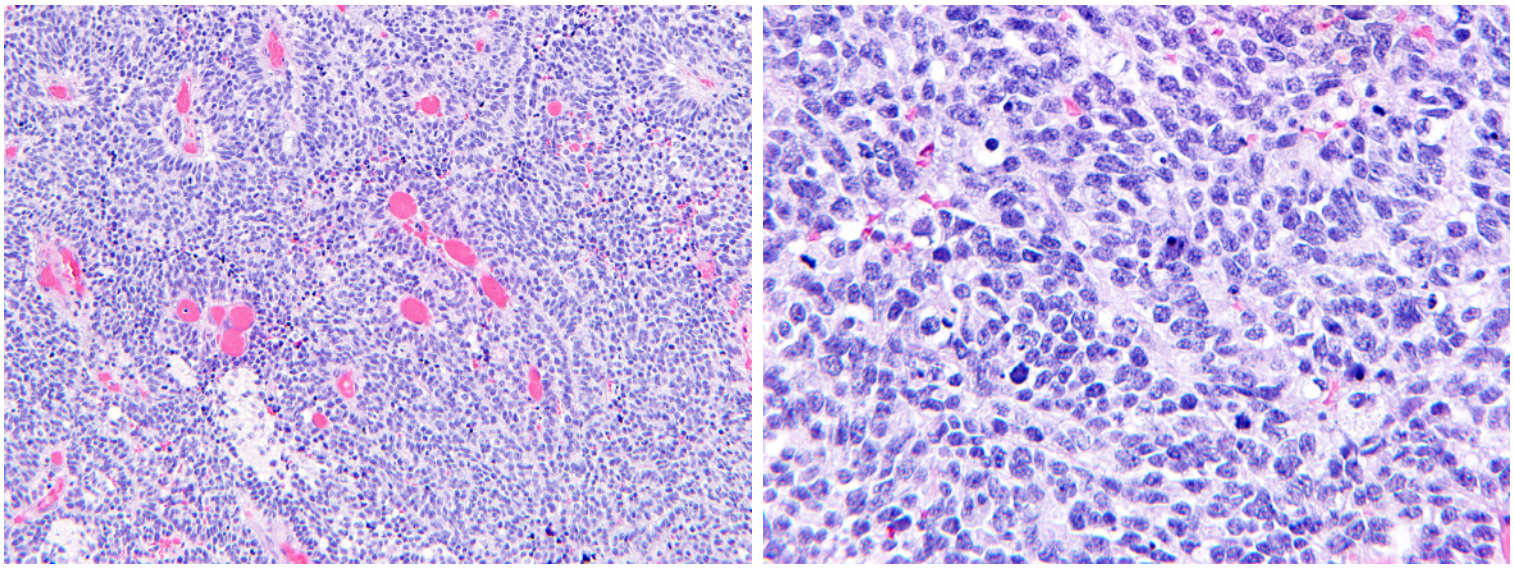


PPT #8, 55 y/o F, pineocytoma, wildtype DICER1, DROSHA, and KBTBD4

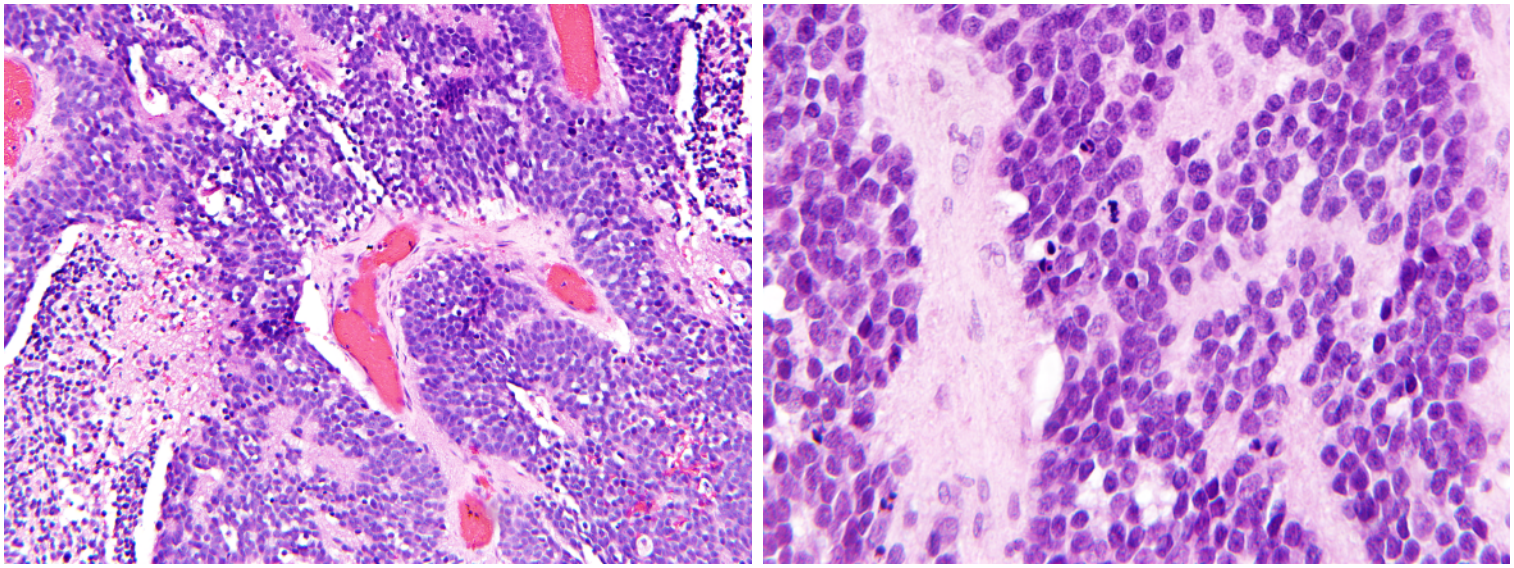




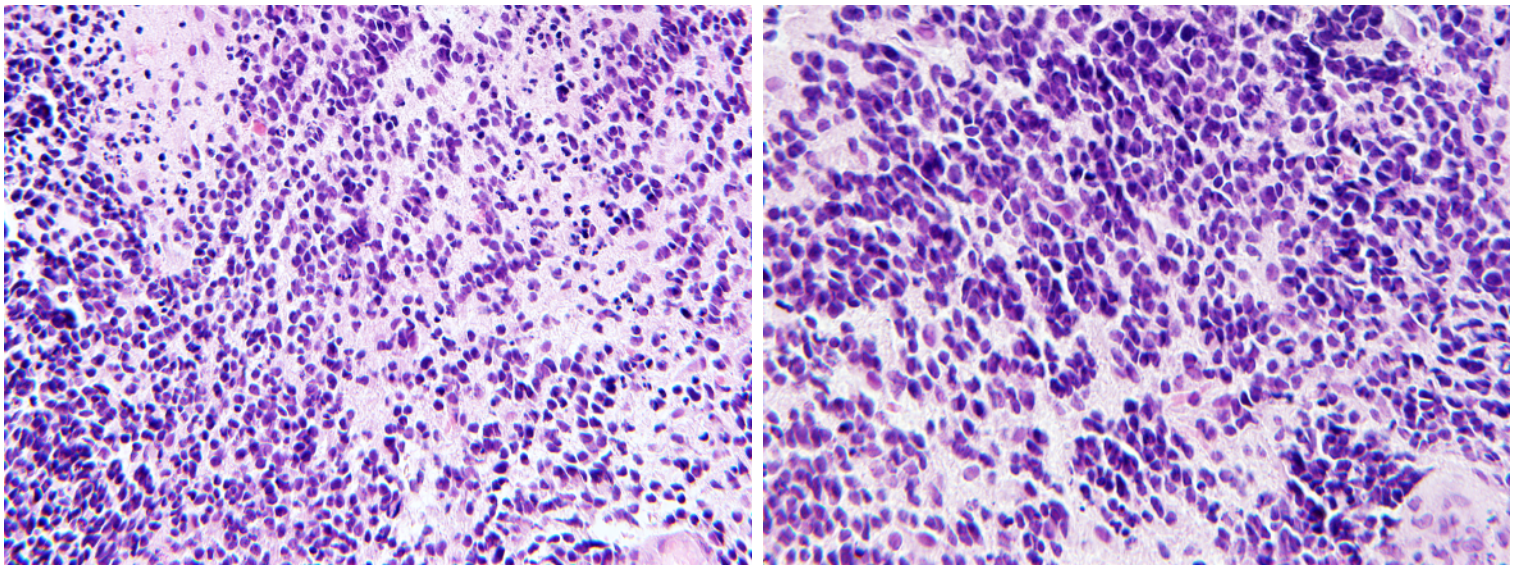
PPT #1, 1 y/o M, pineoblastoma, DICER1 p.E1813D + p.V1080fs



PPT #2, 17 y/o M, pineoblastoma, DICER1 p.D1734fs + LOH

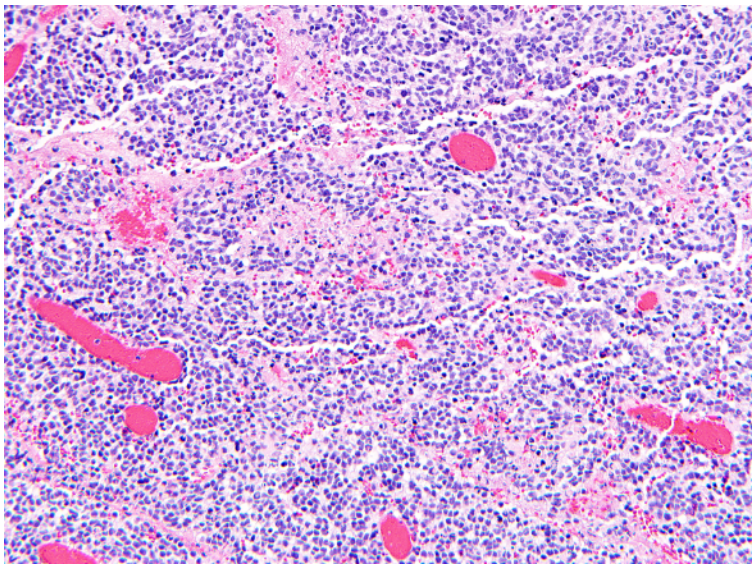


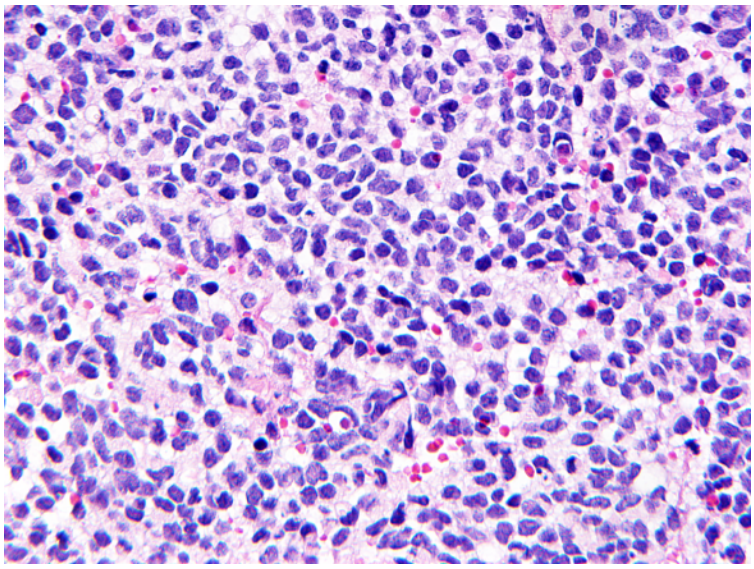
PPT #3, 13 y/o F, pineoblastoma, DROSHA homozygous deletion



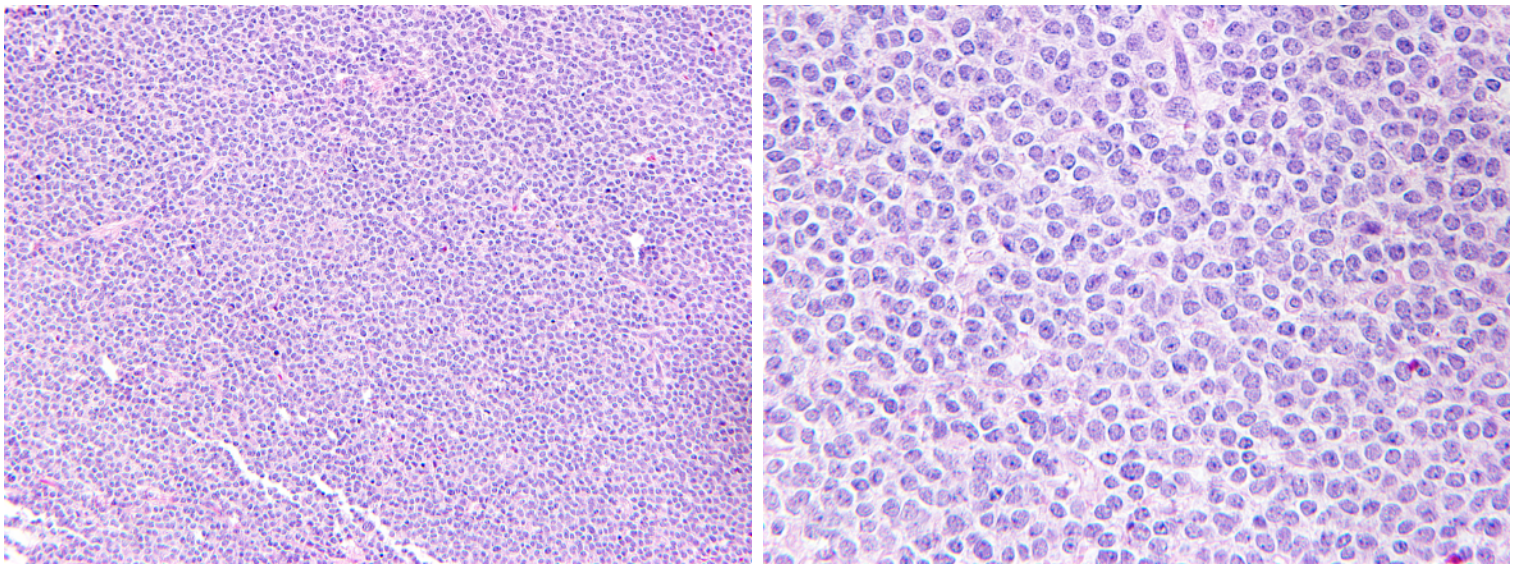
Supplementary Figure 2. Histologic features of the four pineoblastoma cases.

PPT #4, 16 y/o F, pineoblastoma, DROSHA homozygous deletion

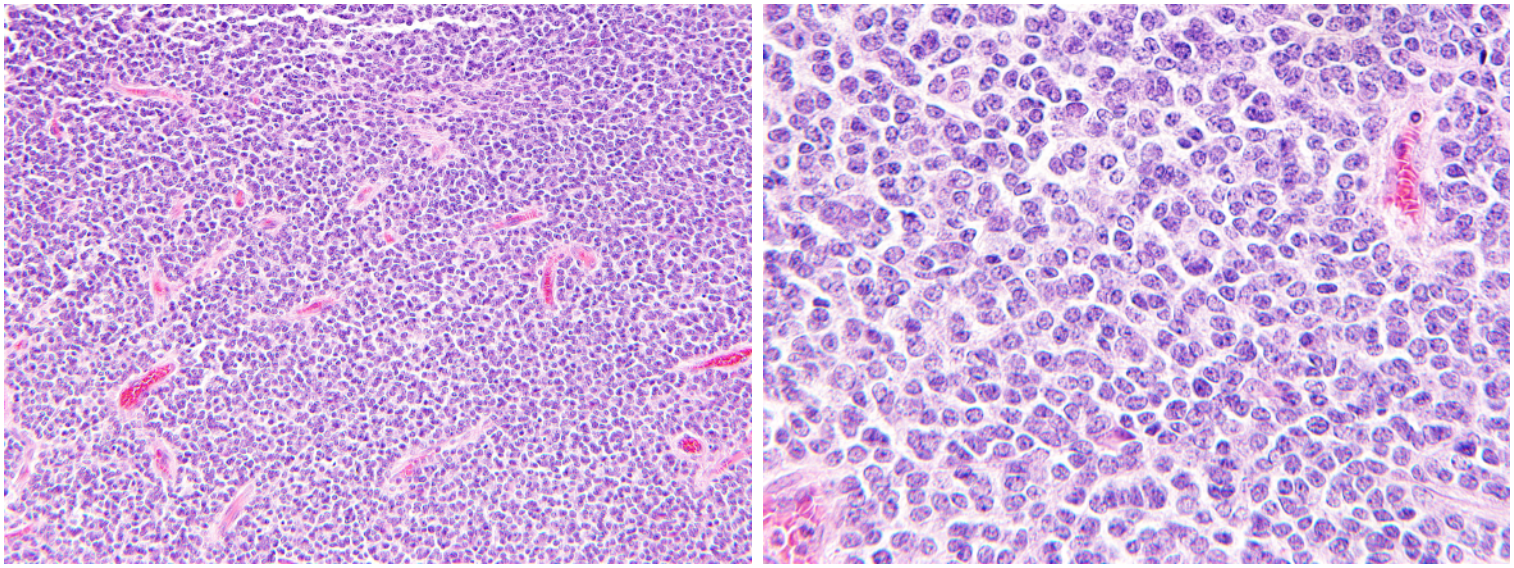




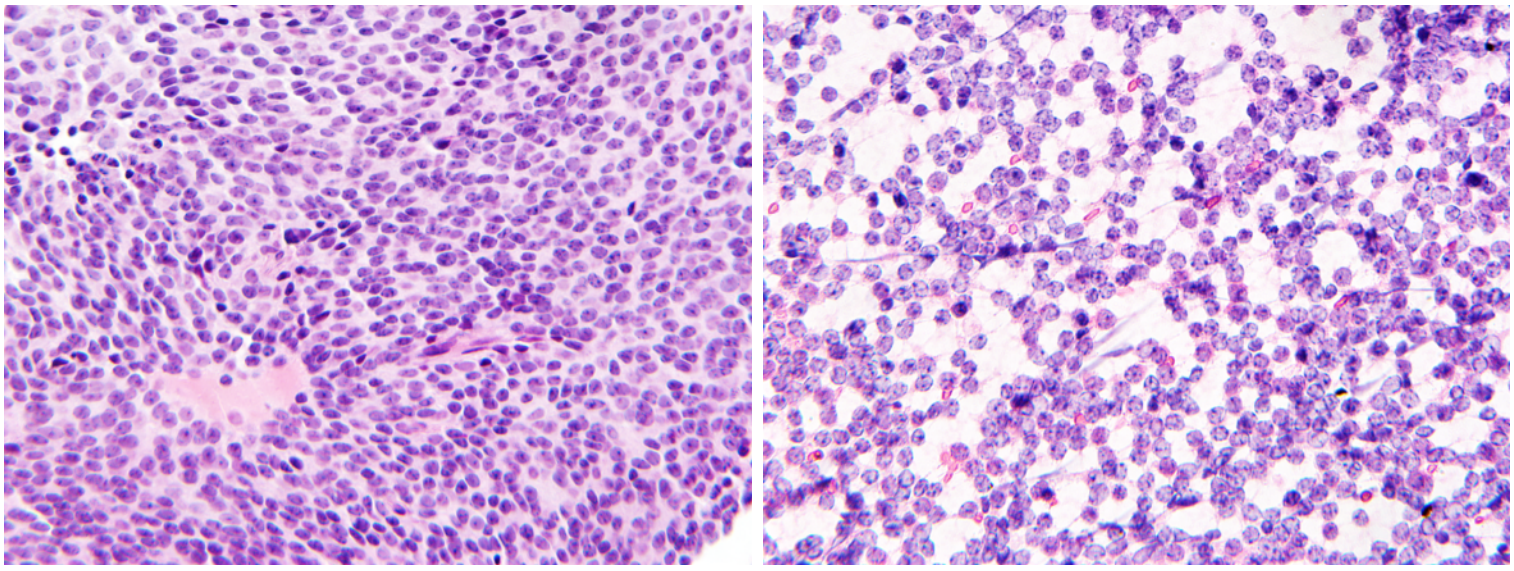
PPT #5, 9 y/o M, PPTID, KBTBD4 p.R313delinsPRR



PPT #6, 18 y/o F, PPITD, KBTBD4 p.R313delinsPRR

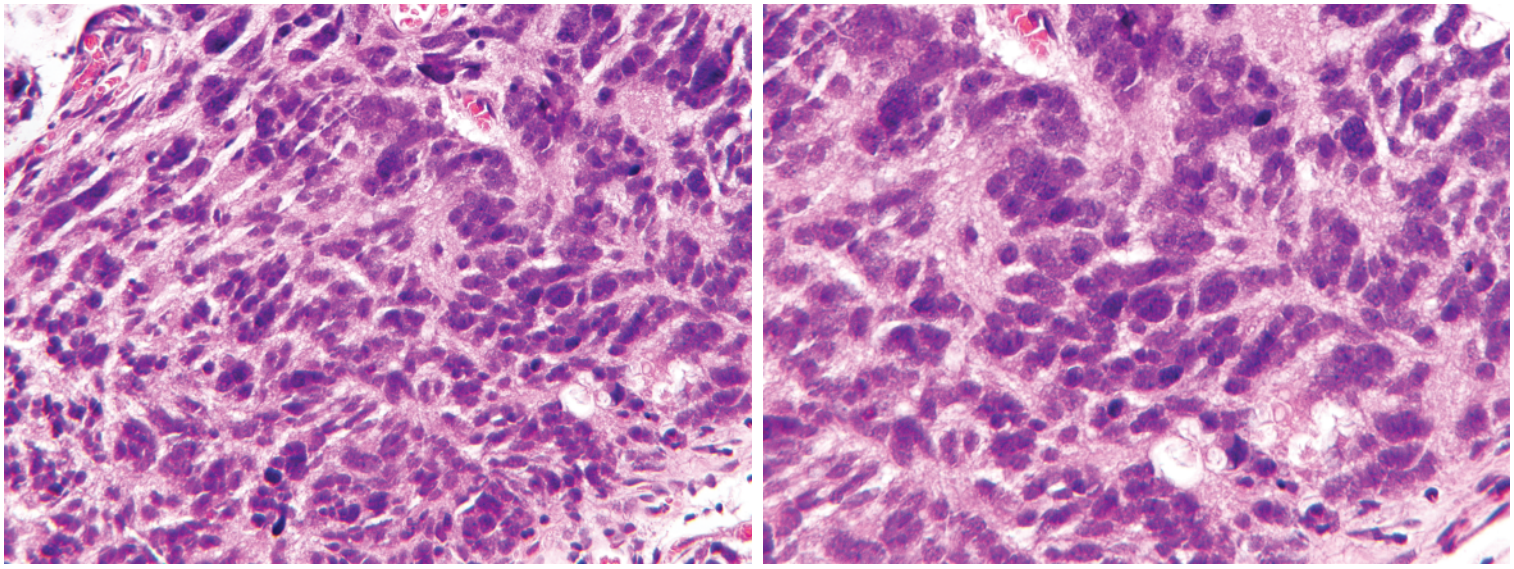


PPT #7, 27 y/o F, PPTID, KBTBD4 p.R313delinsPRR

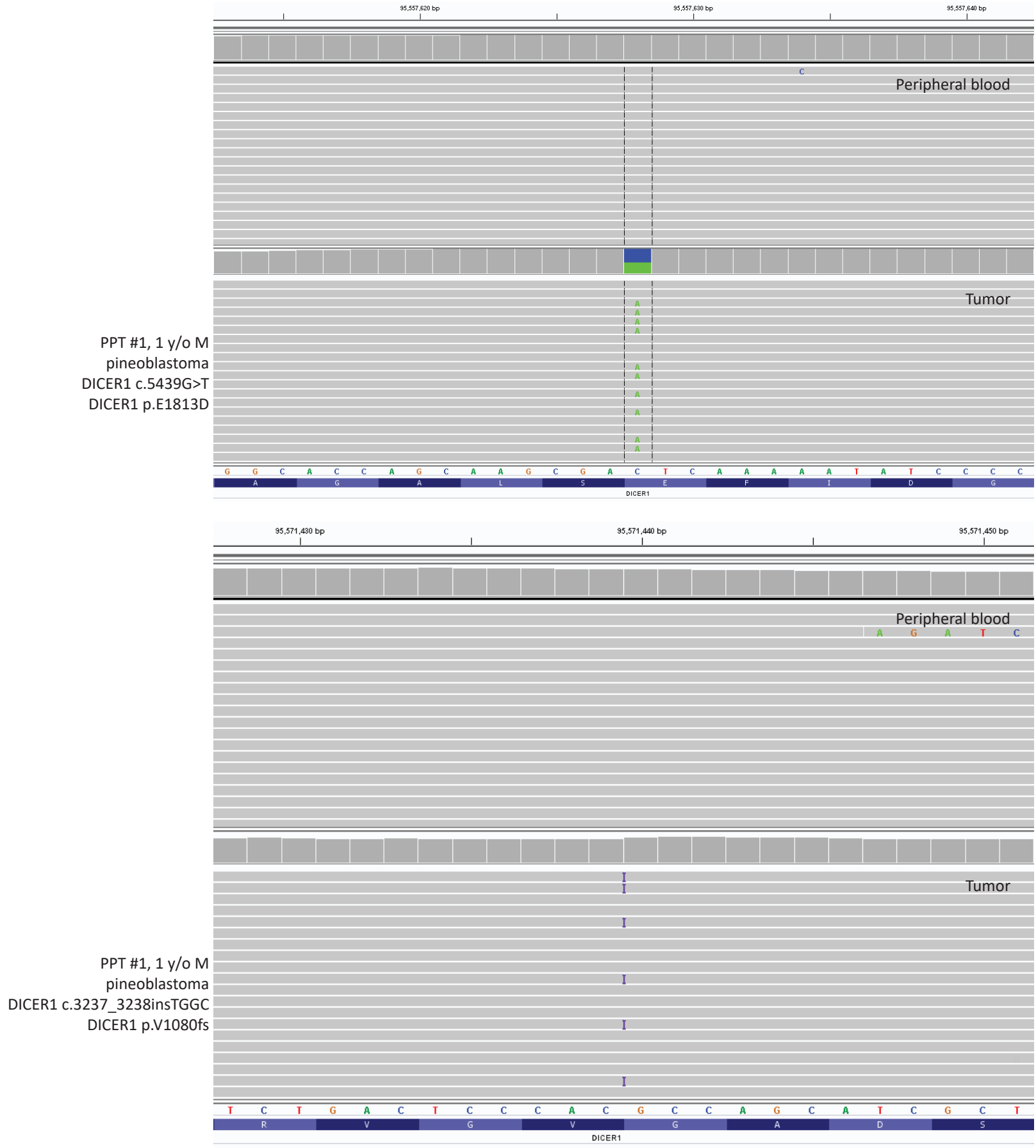


Supplementary Figure 3. Histologic features of the three PPTID cases.

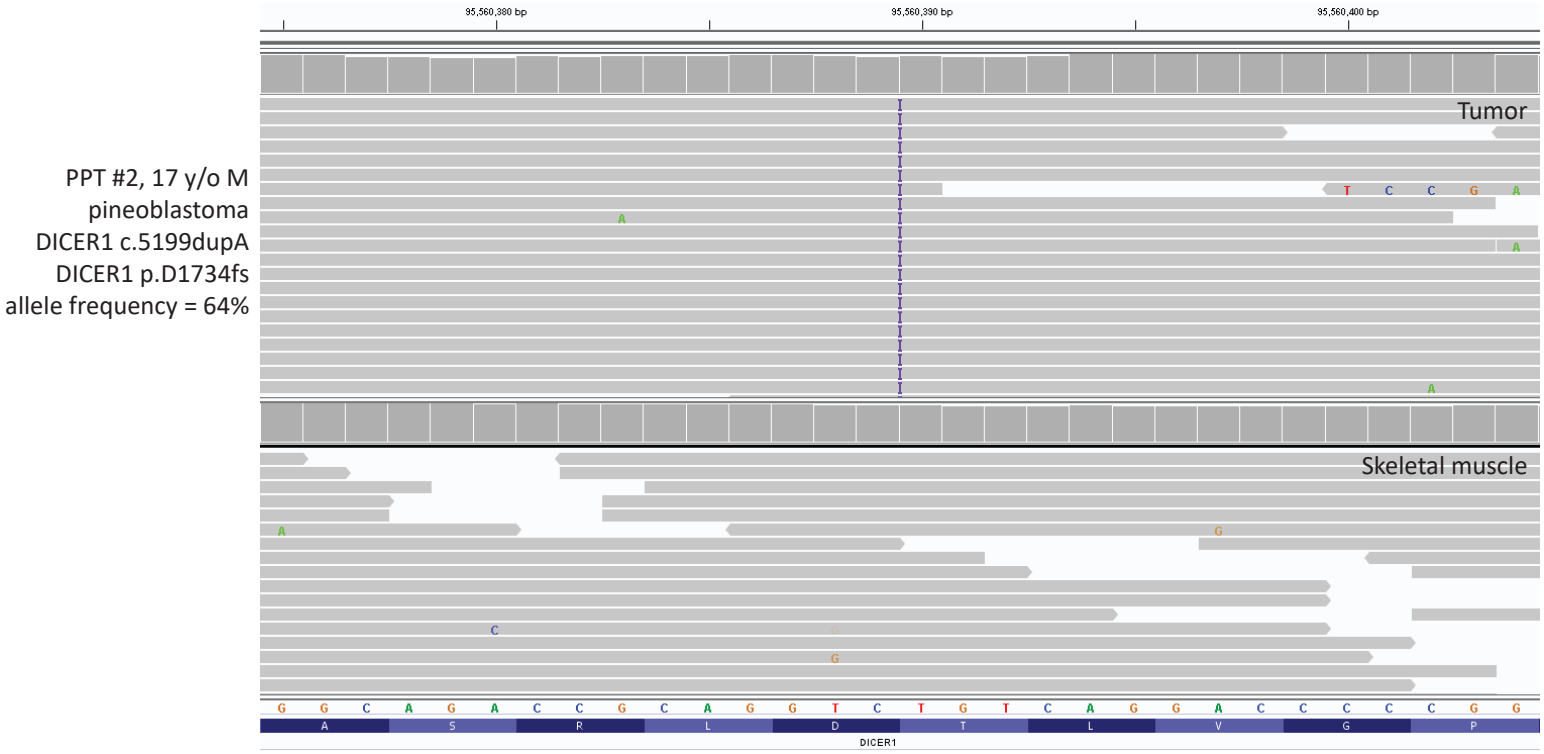
PPT #8, 55 y/o F, pineocytoma, wildtype DICER1, DROHSA, and KBTBD4

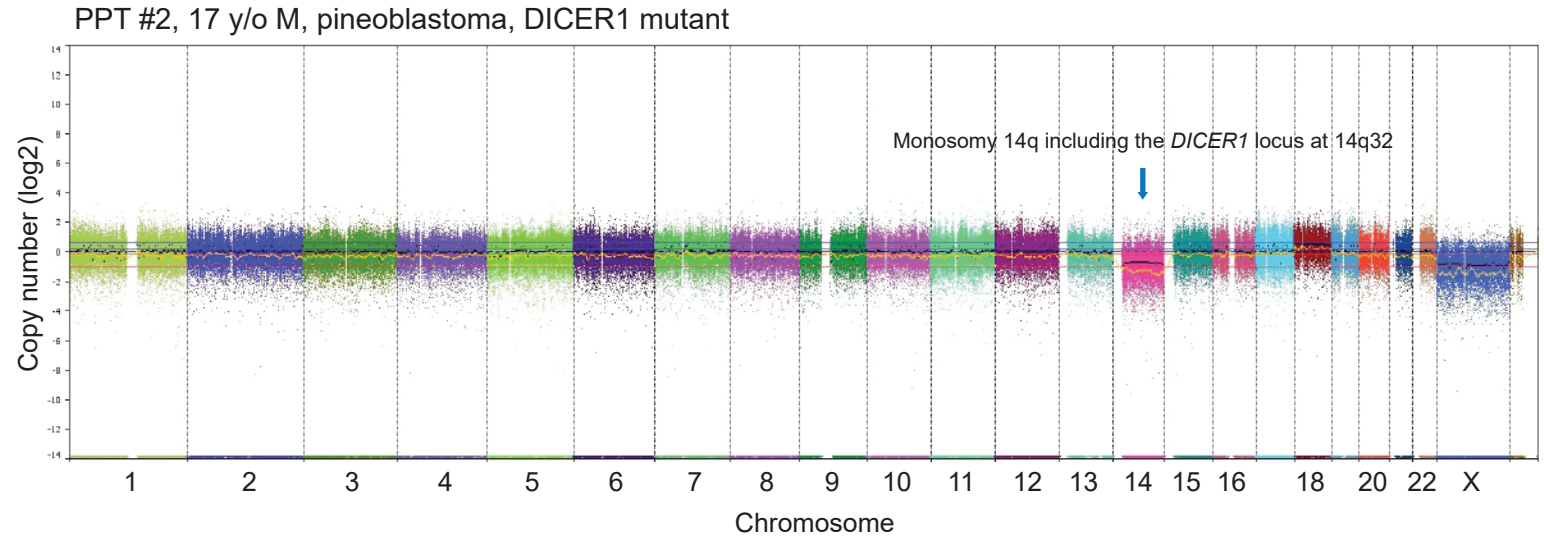


Supplementary Figure 4. Histologic features of the one pineocytoma case.

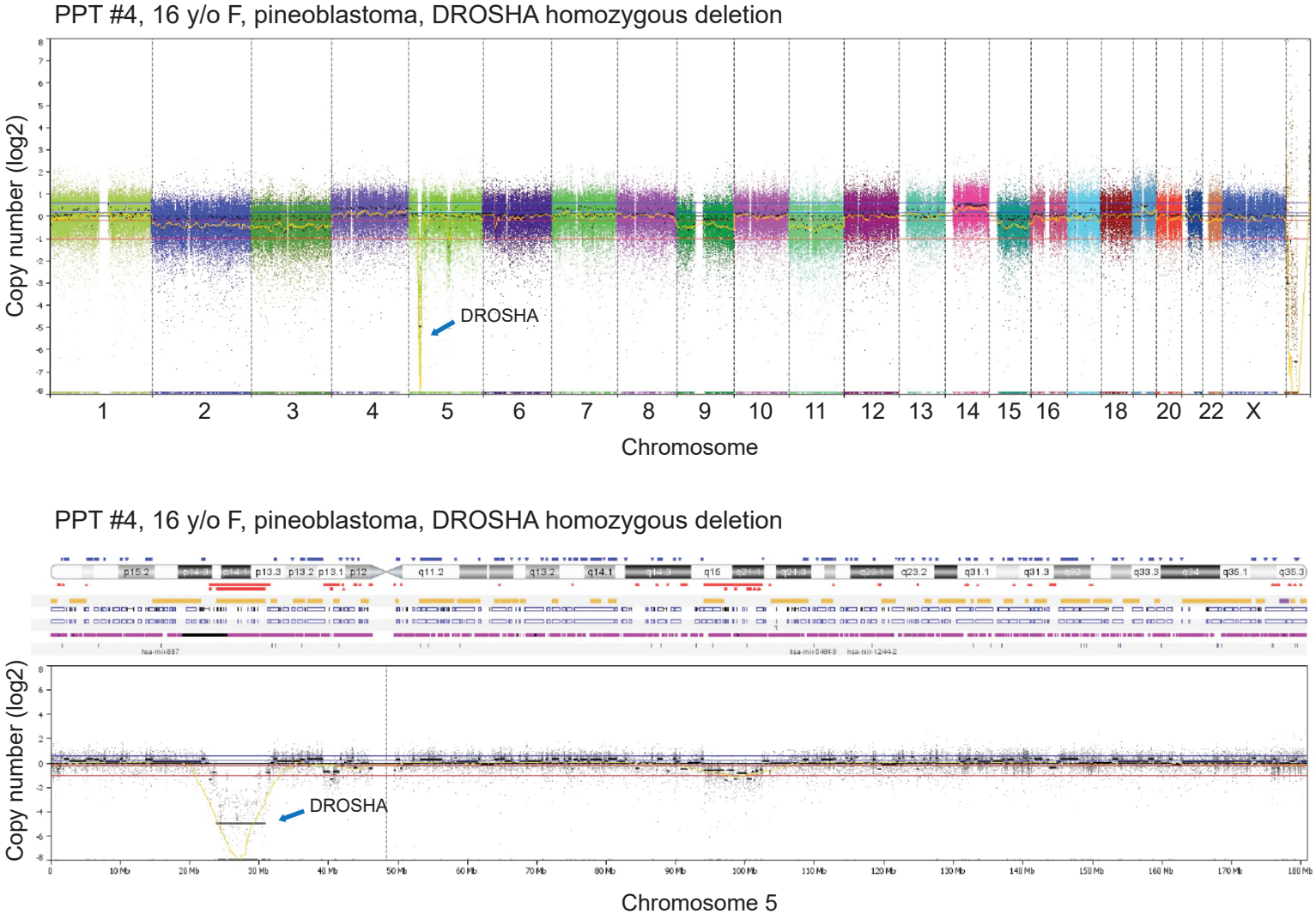


Supplementary Figure 5. Snapshots from the Integrated Genome Viewer of the biallelic somatic *DICER1* mutations present in pineoblastoma PPT #1. Reference transcript NM_177438.

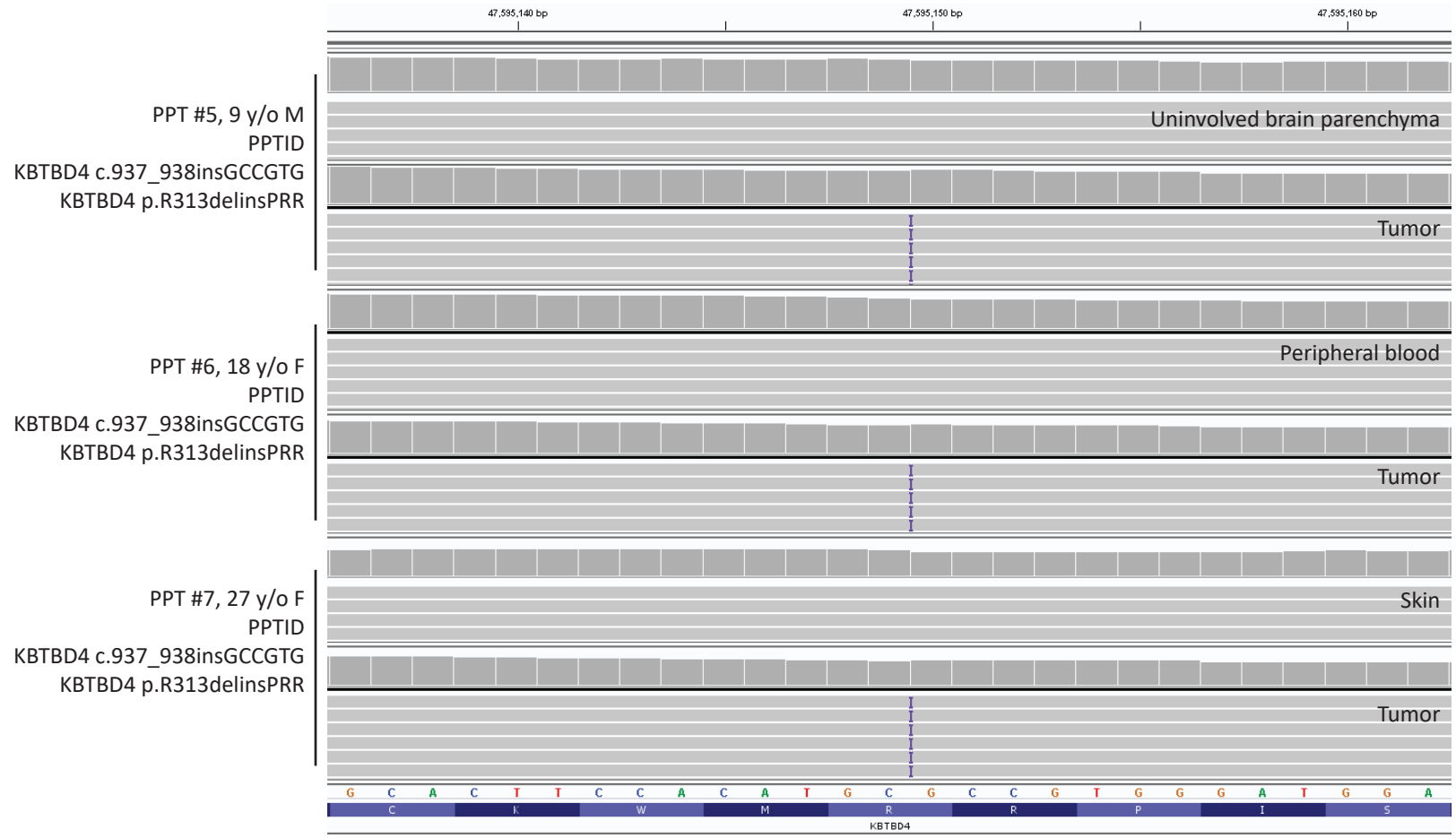




Supplementary Figure 6. Somatic *DICER1* p.D1734fs mutation with loss of the remaining wildtype allele in pineoblastoma PPT #2. Snapshot from the Integrated Genome Viewer (top) showing the somatic *DICER1* c.5199dupA, p.D1734fs mutation that is present at a hemizygous allele frequency of 64% due to loss of chromosome 14q that is eliminating the remaining wildtype allele. Reference transcript NM_177438. Genome-wide chromosomal copy number plot (bottom) showing the monosomy 14q event that is eliminating the remaining wildtype *DICER1* allele in the tumor.



Supplementary Figure 7. Copy number plots for the whole genome (top) and chromosome 5 (bottom) for the pineoblastoma PPT #4 showing the focal homozygous deletion of *DROSHA* at 5p13.



Supplementary Figure 8. Snapshots from the Integrated Genome Viewer of the identical somatic *KBTBD4* p.R313delinsPRR small in-frame insertion present in each of the three cases of PPTID. Reference transcript NM_018095.