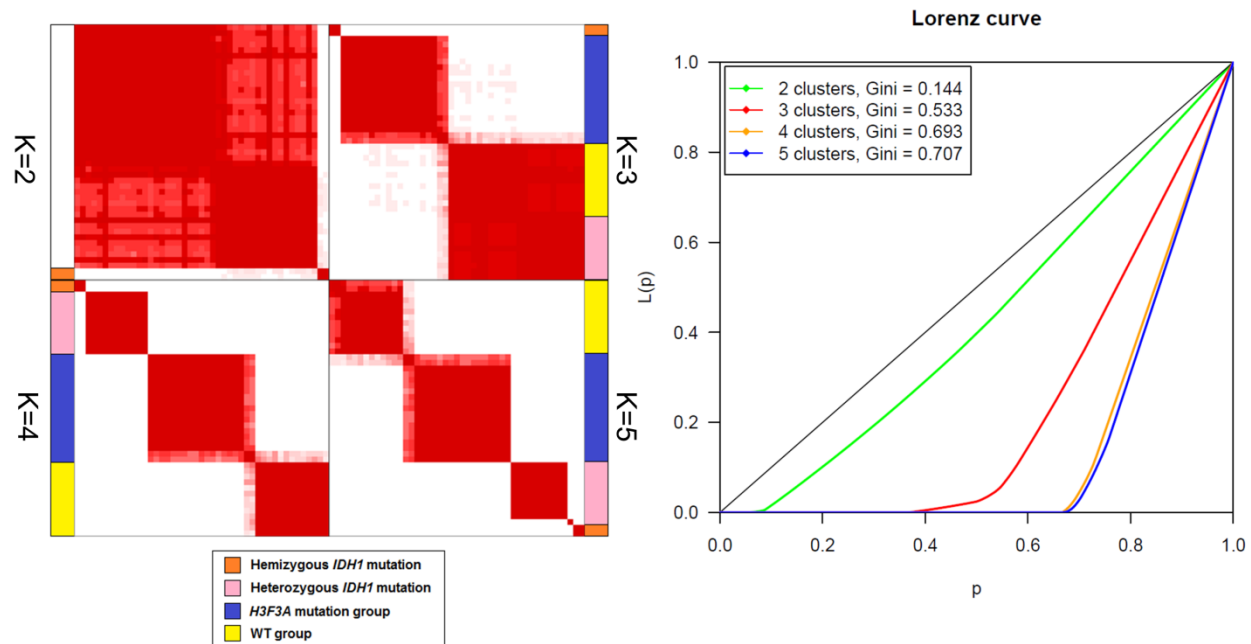
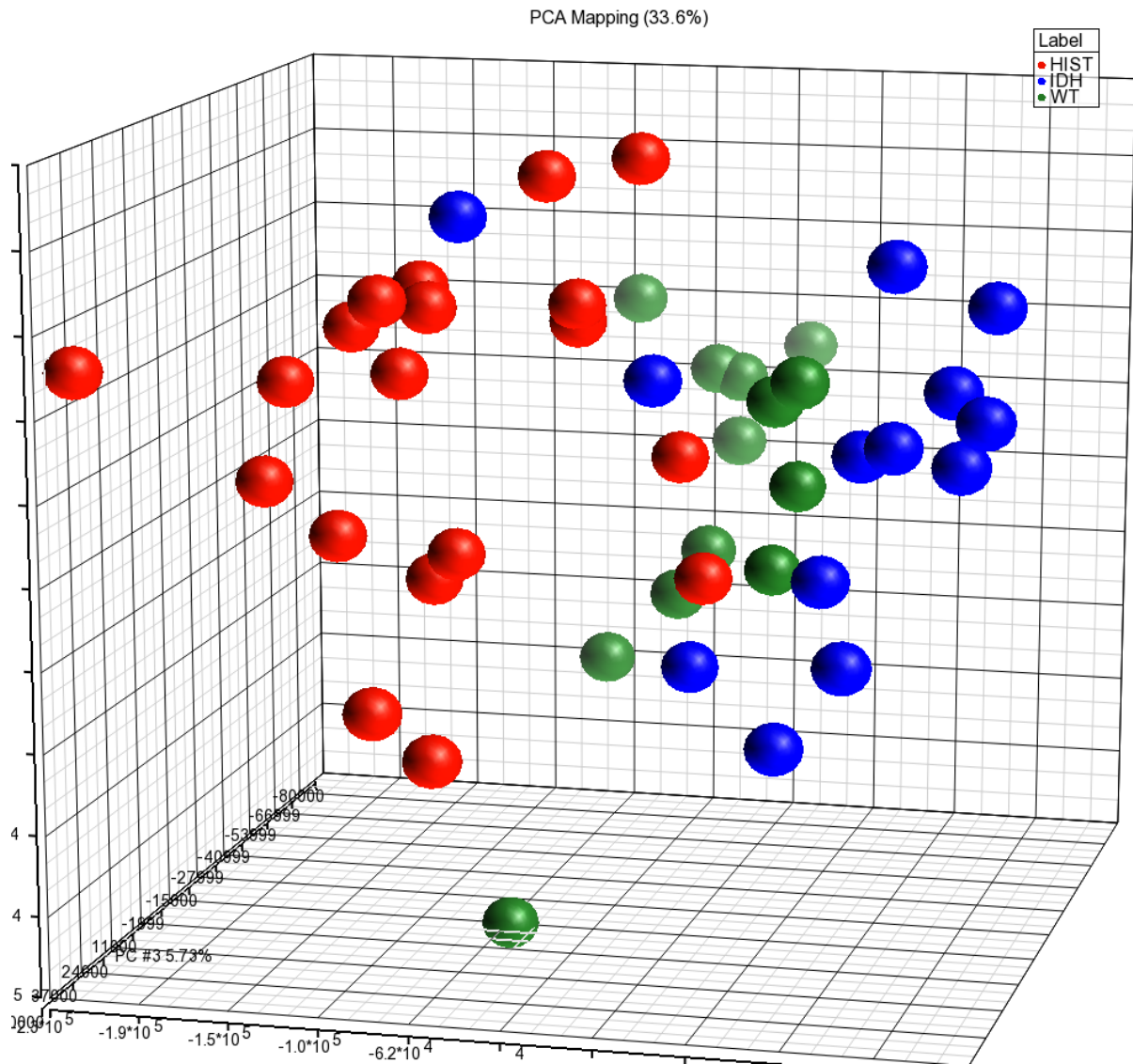


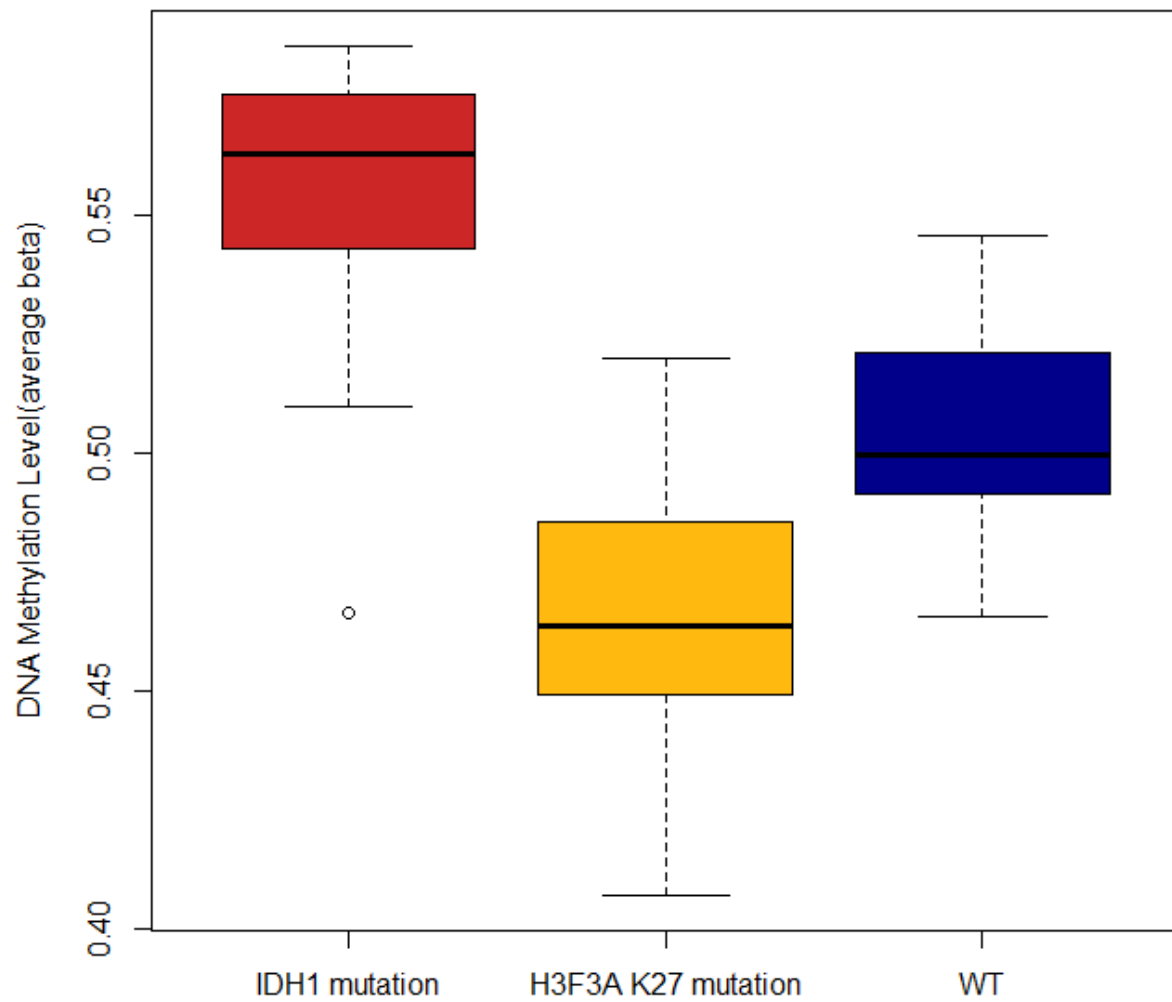
Supplementary Figure 1 Kaplan-Meier survival curves of brainstem patients. (a) Comparison between *PPM1D* mutation vs. *PPM1D* WT patients. (b) Comparison between *PPM1D* mutation vs. *TP53* mutation patients. (c) Comparison between *PPM1D/H3F3A* mutations vs. *TP53/H3F3A* mutations patients.



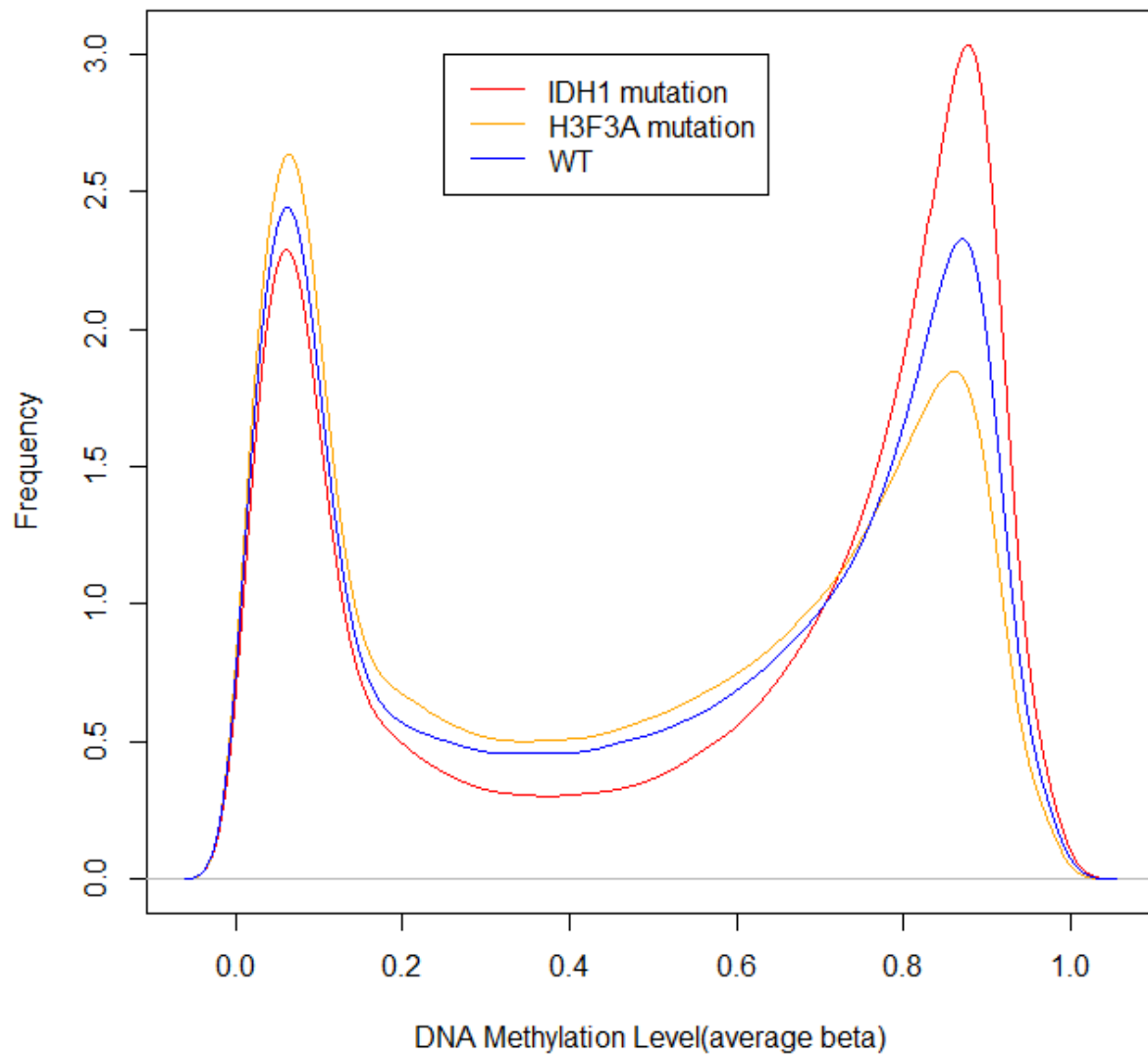
Supplementary Figure 2 Consensus clustering by methylation beta-value and Lorenz curve. Our three groups could be mapped almost perfectly to the *IDH1* and *H3F3A* mutation status. We also noted that hemizygous *IDH1* mutation samples could be differentiated from heterozygous *IDH1* mutation samples.



Supplementary Figure 3 Principal component analysis for our 45 samples. *H3F3A* mutation (HIST), *IDH1* mutation (IDH) and neither mutation (WT) could be differentiated by DNA methylation level.



Supplementary Figure 4 Boxplot for DNA methylation level for sample averages. *IDH1* mutation group shows hypermethylated pattern while *H3F3A* mutation group shows hypomethylated pattern.



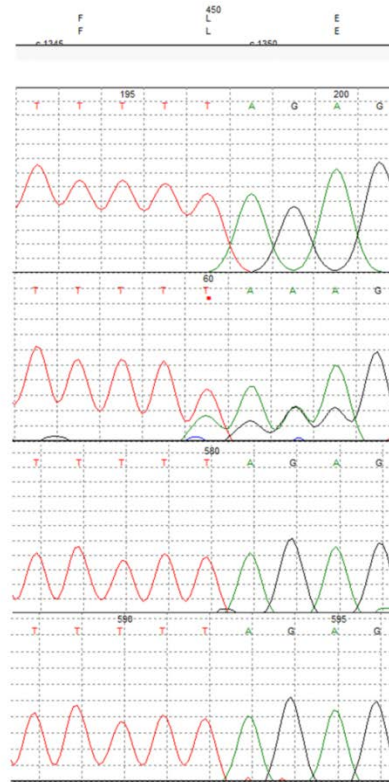
Supplementary Figure 5 Density plot for probes of DNA methylation level (beta-value) in different groups. *IDH1* mutation group (red) shows hypermethylated pattern and *H3F3A* (K27) mutation group (orange) shows hypomethylated pattern.

NM_003620
(reference sequence)

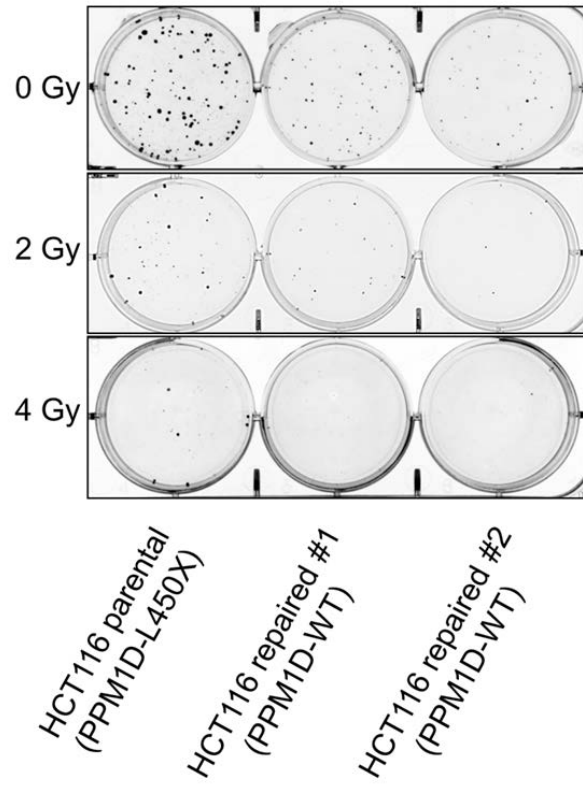
HCT116 parental
(PPM1D-L450X)

HCT116 repaired #1
(PPM1D-WT)

HCT116 repaired #2
(PPM1D-WT)



Supplementary Figure 6 Electropherograms of HCT116 parental, repaired #1, and repaired #2. Electropherograms demonstrate repair of the heterozygous frameshift mutation to a wild type *PPM1D* sequence in these lines.



Supplementary Figure 7 Colony formation assay HCT116 parental, repaired #1, and repaired #2. Colony formation was assessed in the absence of IR, and under 2 Gy, or 4 Gy IR. Representative stained colony formation plates are shown.

Supplementary Table 1 Summary table of the 50 cases included in this genomic study

Patient ID	Gender	Age	Histopathology	Grade	Location	Sequencing
234	female	30	Astrocytoma	III	medulla and C2	WES, TSS
235	male	31	Oligodendroglioma	III	right medulla	WES, TSS
237	female	44	Oligoastrocytoma	II	pons and medulla	WES, TSS
238	male	25	GBM	IV	thalamus, pons, medulla and cerebellum	WES, TSS
239	female	42	Oligoastrocytoma	III	right pons and medulla	WES, TSS
240	female	10	Oligoastrocytoma	III	right pons	WES, TSS
241	female	23	Astrocytoma	III	left midbrain and left thalamus	WES, TSS
242	female	49	Oligoastrocytoma	III	left pons	WES, TSS
243	male	30	Oligoastrocytoma	III	midbrain	WES, TSS
244	male	38	Oligoastrocytoma	II	pons and medulla	WES, TSS
245	male	45	Oligoastrocytoma	II	midbrain,pons,medulla and cerebellum	WES, TSS
246	male	16	Astrocytoma	II	pons	WES, TSS
247	male	25	GBM	IV	pons and medulla	WES, TSS
313	male	20	GBM	IV	brainstem	WES, TSS
248	male	48	Oligodendroglioma	III	left thalamus	WES, TSS
249	female	24	Oligoastrocytoma	III	left thalamus	WES, TSS
250	male	11	Astrocytoma	III	right thalamus	WES, TSS
251	male	42	Astrocytoma	III	right thalamus	WES, TSS
252	female	53	Oligoastrocytoma	II	left thalamus + midbrain	WES, TSS
254	male	12	Oligodendroglioma	III	right thalamus	WES, TSS
255	female	5	Oligoastrocytoma	II	left thalamus + midbrain	WES, TSS
257	male	27	Astrocytoma	II	bilateral thalamus, mainly in right	WES, TSS
258	male	41	GBM	IV	right thalamus	WES, TSS
259	male	24	GBM	IV	right thalamus, third and left ventricle	WES, TSS
260	male	15	Astrocytoma	II	left thalamus	WES, TSS
261	female	22	GBM	IV	left thalamus	WES, TSS
BST-2	female	10	Astrocytoma	II	left pons	TSS
19	male	22	Oligoastrocytoma	II	right pons and right medulla	TSS
8	male	47	GBM	IV	pons	TSS
4	male	6	Astrocytoma	II	medulla and C7	TSS
6	female	21	Astrocytoma	II	pons	TSS
13	male	46	Oligoastrocytoma	II	left pons	TSS
16	female	38	Astrocytoma	II	right pons and right medulla	TSS
2	male	41	Oligoastrocytoma	II	pons and medulla	TSS
BST-14	female	38	Oligoastrocytoma	II	pons	TSS
BST-15	female	32	Astrocytoma	II	pons	TSS
BST-17	male	31	Oligoastrocytoma	II	midbrain and the third ventricle	TSS
BST-20	female	10	Astrocytoma	II	pons	TSS
3	male	12	Astrocytoma	II	medulla	TSS
9	male	6	Astrocytoma	II	pons	TSS
11	female	11	Oligoastrocytoma	II	medulla	TSS
12	male	29	Astrocytoma	III	brainstem	TSS
14	female	40	Astrocytoma	II	pons and medulla	TSS

18	male	27	Astrocytoma	II	pons and medulla	TSS
24	female	8	Astrocytoma	III	pons and medulla	TSS
1	male	14	Oligoastrocytoma	II	right thalamus	TSS
10	male	47	GBM	IV	right thalamus	TSS
15	female	5	Astrocytoma	III	right thalamus	TSS
21	male	23	GBM	IV	left thalamus	TSS
23	female	44	GBM	IV	right thalamus	TSS

#WES: Whole Exome Sequencing, TSS: Targeted Sanger Sequencing

Supplementary Table 2 Summary table of Whole Exome Sequencing Statistics

Case		Number of somatic sequence alterations identified	Number of somatic copy number alterations identified	Sequenced Bases Mapped to Genome(G)	Sequenced Bases Mapped to Target Regions(G)	Fraction of Sequenced Bases Mapped to Target Regions	Bases in target regions with at least 10 reads(M)	Fraction of bases in target regions with at least 10 reads	Average Number of Total High Quality Sequences at Each Base	Average Number of Distinct High Quality Sequences at Each Base
234	Tumor	30	75	9.9	5.2	52.58%	50.19	92.41%	89.14	79.6
	Normal			9.23	5.22	56.55%	50.72	93.40%	94.37	82.61
235	Tumor	24	55	9.84	5.27	53.49%	50.39	92.79%	90.69	79.57
	Normal			8.3	4.62	55.63%	50.09	92.23%	83.62	75.13
237	Tumor	27	0	8.03	4.37	54.49%	50.17	92.38%	73.95	66.03
	Normal			9.51	4.98	52.32%	50.6	93.17%	89.13	78.16
238	Tumor	29	150	10.9	6.3	57.91%	47.28	87.06%	109.01	68.39
	Normal			9.12	5.02	55.02%	50.57	93.12%	89.87	77.26
239	Tumor	31	0	9.5	5.55	58.40%	50.68	93.32%	95.49	84.07
	Normal			7.16	4.25	59.25%	50.15	92.35%	76.61	66.21
240	Tumor	19	0	9.62	5.56	57.73%	50.85	93.63%	95.39	85.8
	Normal			9.01	5.24	58.19%	50.75	93.45%	94.69	82.06
241	Tumor	15	0	9.97	5.48	54.92%	50.46	92.92%	94.03	80.58
	Normal			7.33	4.16	56.73%	49.97	92.01%	74.93	68.76
242	Tumor	69	0	8.83	4.78	54.14%	50.64	93.25%	81.1	71.37
	Normal			11.13	6.71	60.30%	51.1	94.10%	120.99	97.04
243	Tumor	26	1	8.59	4.78	55.66%	50.66	93.29%	82.09	74.07
	Normal			8.03	4.58	57.07%	50.23	92.50%	83.08	74.16
244	Tumor	20	0	9.29	4.86	52.27%	50.79	93.53%	83.65	77.13
	Normal			9.15	5.23	57.11%	50.76	93.47%	94.62	83.49
245	Tumor	25	0	9.83	5.57	56.63%	50.73	93.41%	95.86	82.24
	Normal			8.48	4.86	57.27%	50.29	92.61%	87.99	75.57
246	Tumor	27	11	9.19	5.09	55.36%	49.99	92.05%	87.61	76.83
	Normal			8.09	4.59	56.68%	50.76	93.48%	82.48	72.13
247	Tumor	21	0	9.35	5.05	53.98%	49.86	91.82%	73.86	64.99
	Normal			7.99	4.42	55.34%	49.64	91.41%	73.32	63.55
248	Tumor	34	14	10.11	5.44	53.79%	50.08	92.23%	79.32	69.82
	Normal			8.92	4.8	53.79%	50.18	92.39%	79.38	69.04
249	Tumor	30	126	7.95	4.38	55.11%	49.99	92.05%	65.24	60.46
	Normal			11.34	7.02	61.93%	50.98	93.88%	120.49	100.49
250	Tumor	19	40	6.59	3.64	55.15%	48.66	89.60%	55.07	51.19
	Normal			8.03	4.59	57.21%	50.04	92.14%	77.19	67.9
251	Tumor	23	35	8.87	4.98	56.16%	49.45	91.07%	77.77	61.76
	Normal			8.42	4.68	55.58%	49.53	91.21%	80.65	70.75
252	Tumor	42	98	8.77	5.03	57.37%	49.37	90.91%	78.38	66.08
	Normal			8.61	4.72	54.83%	49.29	90.76%	81.56	61.34
254	Tumor	15	39	7.29	4.2	57.53%	49.84	91.78%	62.28	56.32
	Normal			9.37	5.18	55.24%	49.47	91.09%	88.1	63
255	Tumor	4	0	7.79	4.11	52.68%	49.42	91.00%	59.48	54.12
	Normal			9.61	5.37	55.87%	50.21	92.47%	89.93	80.29

257	Tumor	37	14	8.53	5.07	59.46%	50.32	92.66%	78.64	68.18
	Normal			7.04	3.98	56.53%	49.24	90.67%	68.53	62.41
258	Tumor	53	102	9.95	6.52	65.58%	51.04	93.98%	102.45	83.65
	Normal			8.37	4.82	57.67%	49.68	91.48%	83.37	74.53
259	Tumor	17	0	13.16	7.11	54.03%	51.38	94.62%	108.68	97.98
	Normal			10.47	5.71	54.52%	50.65	93.26%	97.63	84.28
260	Tumor	23	5	14.34	8.64	60.27%	51.54	94.90%	131.42	72.96
	Normal			11.71	6.85	58.54%	51.31	94.48%	117.5	100.23
261	Tumor	25	7	9.56	5.79	60.61%	50.49	92.98%	88.32	71.42
	Normal			11.13	6.78	60.87%	51.17	94.22%	115.79	99.11
313	Tumor	23	NA	20.15	12.12	60.14%	52	96%	193	127
	Normal			17.98	10.92	60.71%	52.03	96%	191	116

Supplementary Table 5 Summary table of Sanger sequencing of validation for randomly selected mutations from whole exome sequencing

Number of Mutations Selected for Validation	106
Number of Successful PCR Amplification	91
Successful Rate of PCR	86%
<hr/>	
Number of Confirmed Mutations	81
Sensitivity of Whole Exome Sequencing	89%

Supplementary Table 6 Summary and Statistical Analysis of patients age, groups by different gene mutations

	median	p-value
<i>IDH1</i> MUT (n=8)	42.5	0.00555 (t-test)
<i>IDH1</i> WT (n=42)	24	
<i>H3F3A</i> MUT (n=27)	24	0.1731 (t-test)
<i>H3F3A</i> WT (n=23)	32	
<i>TP53</i> MUT (n=32)	26	0.6321 (t-test)
<i>TP53</i> WT (n=18)	24	
<i>PPM1D</i> MUT/ <i>H3F3A</i> MUT in brainstem (n= 6)	25.5	0.4787 (Wilcoxon rank sum test)
<i>TP53</i> MUT/ <i>H3F3A</i> MUT in brainstem (n=9)	25	

Supplementary Table 7 Survival and treatment information for brainstem glioma patients

Case ID	Histopathology	Grade	Overall Survival (Month)	Survival Status (1=death, 0=living)	Treatment
BST-2	Astrocytoma	II	11.25	1	Surgery + Chemotherapy
234	Astrocytoma	III	2	1	Surgery
19	Oligoastrocytoma	II	0.5	1	Surgery
235	Oligodendroglioma	III	1	1	Surgery
8	GBM	IV	4	1	Surgery
4	Astrocytoma	II	27.3	0	Surgery
237	Oligoastrocytoma	II	28.3	0	Surgery
238	GBM	IV	13	1	Surgery
6	Astrocytoma	II	29.05	0	Surgery
13	Oligoastrocytoma	II	26.3	0	Surgery
16	Astrocytoma	II	32.05	0	Surgery + Radiotherapy
239	Oligoastrocytoma	III	0.75	1	Surgery
240	Oligoastrocytoma	III	5	1	Surgery + Radiotherapy
2	Oligoastrocytoma	II	13.75	0	Surgery + Radiotherapy
241	Astrocytoma	III	31.8	0	Surgery + Concurrent chemoradiotherapy + adjuvant chemotherapy
242	Oligoastrocytoma	III	32.3	0	Surgery + Concurrent chemoradiotherapy
243	Oligoastrocytoma	III	29	1	Surgery + Concurrent chemoradiotherapy + adjuvant chemotherapy
244	Oligoastrocytoma	II	33.05	0	Surgery + Radiotherapy
BST-14	Oligoastrocytoma	II	33.8	0	Surgery + Radiotherapy
BST-15	Astrocytoma	II	14	1	Surgery
245	Oligoastrocytoma	II	35.3	0	Surgery + Concurrent chemoradiotherapy + adjuvant chemotherapy
BST-17	Oligoastrocytoma	II	18	1	Surgery + Concurrent chemoradiotherapy + adjuvant chemotherapy
246	Astrocytoma	II	17	1	Surgery
247	GBM	IV	2	0	Surgery
BST-20	Astrocytoma	II	27.3	0	Surgery
3	Astrocytoma	II	5	1	Surgery
9	Astrocytoma	II	18.3	0	Surgery
11	Oligoastrocytoma	II	17	0	Surgery + Radiotherapy
12	Astrocytoma	III	0.25	0	Surgery
14	Astrocytoma	II	3	0	Surgery
18	Astrocytoma	II	20.3	1	Surgery + Concurrent chemoradiotherapy
24	Astrocytoma	III	10.75	1	Surgery
313	GBM	IV	18	0	Surgery + Radiotherapy

Supplementary Table 9 Primers used for generating the truncating mutations (PPM1D472 and PPM1D540)

	Forward	Reverse
PPM1D472	5'-GGAATGGCGGGGCTGTACTCGCTG-3'	5'-GGGTTATGGATCTTTTGAGGGTATGACTAC-3'
PPM1D540	5'-GGAATGGCGGGGCTGTACTCGCTG-3'	5'-GGGCTATTCTAATGTCCTTTTAAAGTTTGTGG-3'