

Figure S1. EGFRvIII and GAPDH amplification plots with various dilution of RNA concentrations. RNA 0.01 μg (A) and 0.1 μg (B) was used in reverse transcription reaction, then quantitative PCR was performed for EGFRvIII and GAPDH, respectively. EGFRv, epidermal growth factor receptor variant.

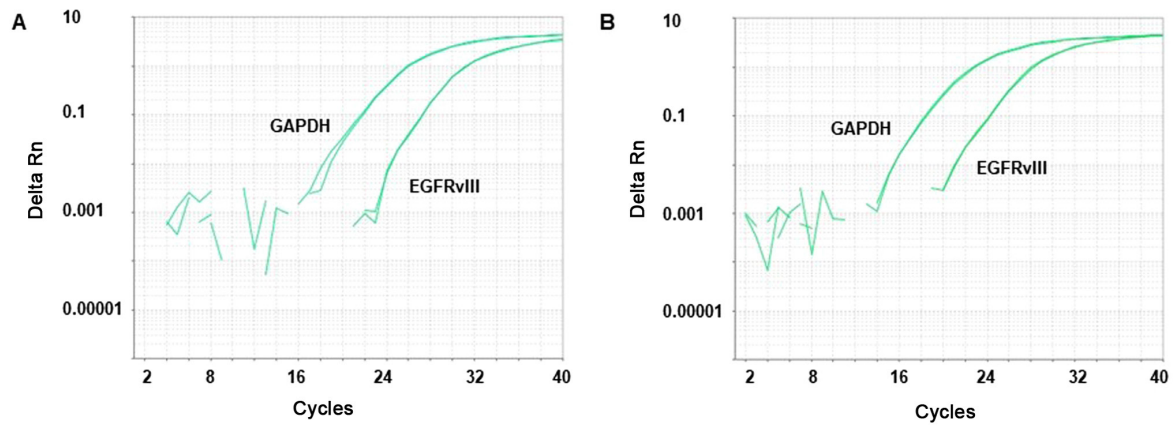


Figure S2. EGFRvIII and GAPDH amplification plots with various dilution of RNA concentrations. RNA 0.25 μg (A) and 0.5 μg (B) was used in reverse transcription reaction, then the quantitative PCR was performed for EGFRvIII and GAPDH, respectively. EGFRv, epidermal growth factor receptor variant.

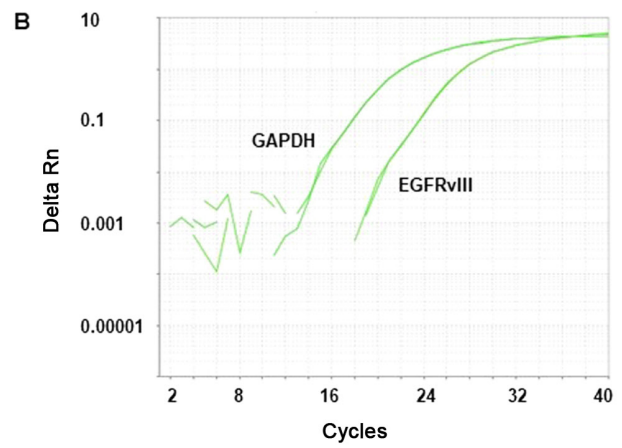
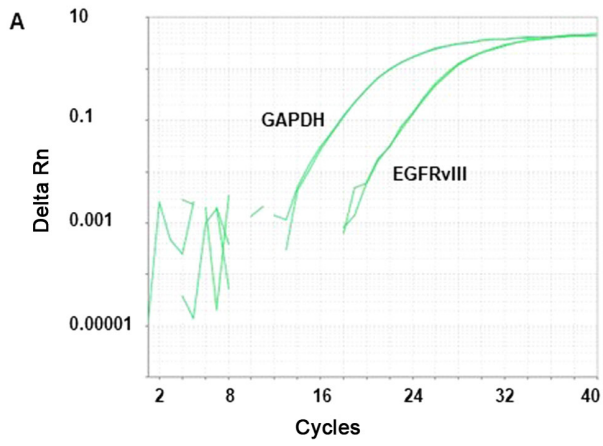


Figure S3. EGFRvIII and GAPDH amplification plots with RNA from positive control cells and K-562 cells RNA (negative control). (A) 1.0 μg RNA from positive control cells, and in (B) 1.0 μg RNA from K-562 cells (negative control) was used in reverse transcription reaction, then quantitative PCR was performed for EGFRvIII and GAPDH, respectively. EGFRv, epidermal growth factor receptor variant.

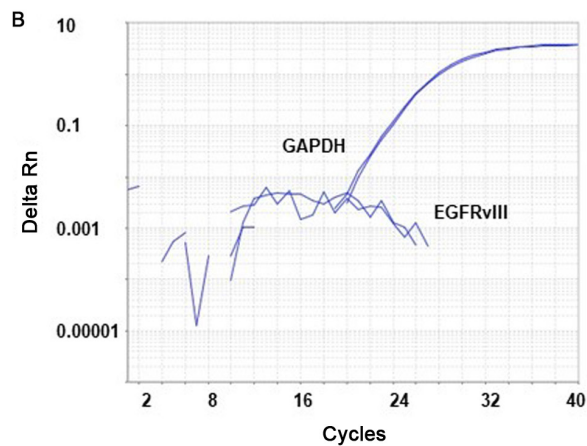
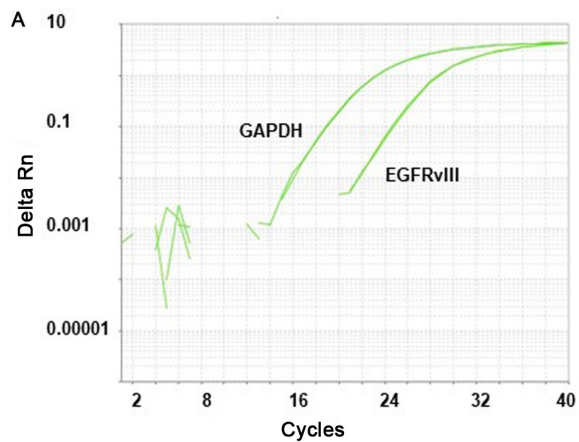


Figure S4. EGFRvIII and GAPDH amplification plots with DNA from (positive control cells) and DNA from (negative control cells). (A) 0.1 μ g DNA from positive control cells, and (B) 0.1 μ g DNA from negative control cells was directly used for quantitative PCR for EGFRvIII and GAPDH, respectively. EGFRv, epidermal growth factor receptor variant.

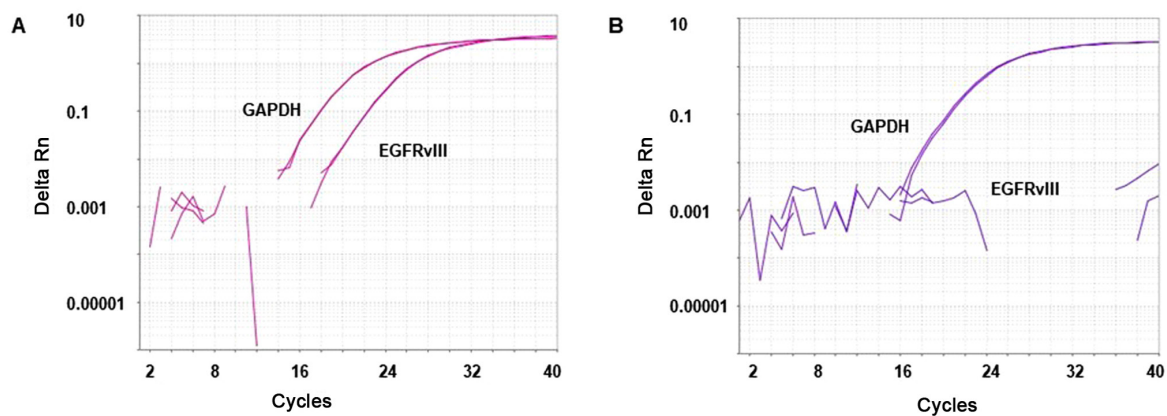


Figure S5. Verification EGFRvIII transcript by PCR using known positive and negative controls. PCR products of 238 bp (red arrow) are shown for positive samples (lanes 1-10) and in lanes 11-19 no EGFRvIII-specific PCR product formed by negative controls. The forward primer is a junctional primer spanning exons 1-8 (CAGTATTGATCGGGAGAGCC) and amplifies only the exon 2-7 deletion mutant of EGFR (EGFRvIII). Lane denoted as (M) is a DNA ladder. EGFRv, epidermal growth factor receptor variant.

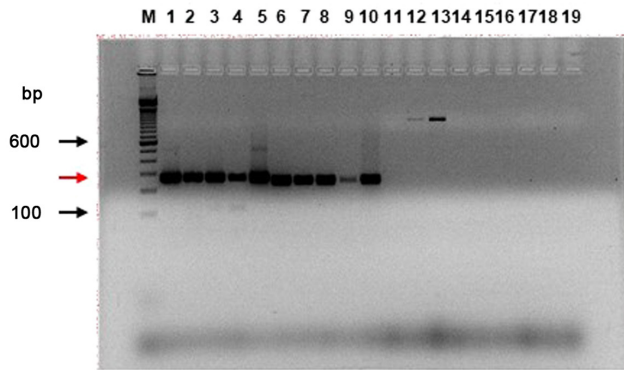


Table SI. Primers used for RT-qPCR and Sanger sequencing.

Gene and primer name	Primer sequence	Product (bp)	Assay
EGFRvIII (F)	5'-GGCTCTGGAGGAAAAGAAAGGTAATT-3'	96	RT-qPCR
EGFRvIII (R)	5'-CCGTCTTCCTCCATCTCATAGC-3'		
EGFRvIII TaqMan-probe.	6-FAM-5'-TGACAGATCACGGCTC-MGBNFQ-3'	217	RT-qPCR
GAPDH (F)	5'-GAAGGTGAAGGTCGGAGTC-3'		
GAPDH (R)	5'-GATGGGATTTCCATTGATGAC-3'		
GAPDH TaqMan-probe.	VIC-5'-TCACCAGGGCTGCTT-MGBNFQ-3'	238	Sequencing
EGFRvIII seq (F)	5'-CAGTATTGATCGGGAGAGCC-3'		
EGFRvIII seq (R)	5'-GCCCTTCGCACTTCTTACAC-3'	213	Sequencing
IDH1 (F)	5'-TGAAAACGGCCAGTTGAGAAGAGGGTTGAGGAGTT-3'		
IDH1 (R)	5'-CAGGAAACAGCTATGACCAACATGCAAATCACATTATTGCC-3'	288	Sequencing
IDH2 (F)	5'-TGAAAACGGCCAGTGGGTTCAAATTCTGGTTGAA-3'		
IDH2 (R)	5'-CAGGAAACAGCTATGACCTAGGCGAGGAGCTCCAGT-3'		Sequencing
M-13 (F)	5'-TGAAAACGGCCAGT-3'		
M-13 (R)	5'-CAGGAAACAGCTATGACC-3'		Sequencing

RT-qPCR, reverse transcription-quantitative PCR; F, forward; R, reverse; IDH, isocitrate dehydrogenase; EGFRv, epidermal growth factor receptor variant.

Table III. EGFRvIII Expression in GBM tumors of Saudi Arabian population.

Lab code	Sex	Age, years	Diagnosis	EGFRvIII RT-qPCR
46-N	F	38	GBM grade-IV	POS
47-N	M	61	GBM grade-IV	NEG
51-N	M	73	GBM grade IV	NEG
53-N	M	57	GBM grade-IV	NEG
55-N	F	30	GBM grade-IV	POS
56-?	M	71	GBM	POS
59-N	M	60	GBM grade-IV	POS
62-N	M	62	GBM Recurrent	POS
63-N	M	61	GBM	NEG
68-N	F	40	GBM grade-IV	NEG
69-N	F	32	GBM grade-IV	NEG
82-N	M	69	GBM grade-IV	NEG
HALK-2	M	45	GBM	POS
HALK-3	M	56	GBM	POS
HALK-4	F	8	Low grade glioma	POS
HALK-5	F	37	GBM	NEG
HALK-8	M	37	GBM	POS
HALK-9	M	65	GBM	POS
HALK-12	M	17	High grade glioma	POS
HALK-16	M	50	GBM	NEG
HALK-17	M	58	GBM	POS
HALK-18	F	35	GBM	NEG
HALK-21	F	30	Favor GBM	POS
HALK-22	M	49	GBM	POS
HALK-23	F	59	GBM	POS
HALK-24	M	60	GBM	NEG
HALK-27	M	59	GBM	POS
HALK-29	F	54	GBM	POS
HALK-31	F	64	GBM	POS
HALK-32	M	50	GBM	POS
HALK-33	M	43	GBM	POS
HALK-34	F	12	GBM	POS
HALK-39	M	45	GBM	POS
HALK-40	F	48	Low grade glioma	NEG
HALK-41	M	51	GBM	NEG
HALK-47	M	60	GBM	NEG
HALK-48	M	62	GBM	NEG
KAMC-3	M	48	GBM	POS
KAMC-12	F	26	GBM	NEG
KAMC-15	F	15	GBM	POS
KAMC-17	M	23	GBM	POS
KAMC-18	F	47	Glioma	NEG
KAMC-19	M	43	Glioma	NEG
KAMC-20	F	62	Glioma	NEG
KAMC-22	M	21	GBM	POS
KAMC-25	F	45	Glioma	NEG
KAMC-26	M	31	GBM	NEG
KAMC-27	M	35	GBM	NEG
KAMC-34	F	48	GBM	POS
KAMC-35	F	51	GBM	NEG
KAMC-36	F	26	GBM	NEG
KAMC-40	M	29	GBM	NEG
KAMC-44	M	50	GBM	NEG
KAMC-47	F	65	GBM	NEG
KAMC-49	F	58	GBM	POS

EGFRv, epidermal growth factor receptor variant; RT-qPCR, reverse transcription-quantitative PCR; GBM, glioblastoma; F, female; M, male; NEG, negative; POS, positive.

Table III. EGFRvIII expression in astrocytoma of Saudi Arabian population.

Lab code	Sex	Age, years	Diagnosis	EGFRvIII RT-qPCR
42-N	F	4	Pilocytic astrocytoma grade-I	NEG
71-N	M	35	Astrocytoma grade-II	NEG
84-N	M	9	Pilocytic astrocytoma	NEG
85-N	M	8	Pilocytic astrocytoma	NEG
88-N	F	12	Pilocytic astrocytoma	NEG
HALK-7	M	6	Pilocytic astrocytoma	NEG
HALK-11	M	1	Diffuse astrocytoma	POS
HALK-13	M	7	Pilocytic astrocytoma	NEG
HALK-20	F	11	Pilocytic astrocytoma	NEG
HALK-28	F	4	Pilocytic astrocytoma	NEG
HALK-36	M	13	Pilocytic astrocytoma	NEG
HALK-37	M	5	Pilocytic astrocytoma	NEG
HALK-42	F	4	Pilocytic astrocytoma	NEG
KAMC-8	M	56	Astrocytoma	NEG
KAMC-29	M	15	Astrocytoma	NEG
KAMC-31	M	28	Astrocytoma	NEG
KAMC-33	M	36	Pilocytic astrocytoma	POS
KAMC-37	M	58	Pilocytic astrocytoma	POS
KAMC-45	M	50	Anaplastic astrocytoma	NEG
KAMC-50	F	9	Astrocytoma	POS
KAMC-51	M	6	Astrocytoma	POS
KAMC-53	M	12	Anaplastic astrocytoma	NEG

EGFRv, epidermal growth factor receptor variant; RT-qPCR, reverse transcription-quantitative PCR; F, female; M, male; NEG, negative; POS, positive.

Table SIV. EGFRvIII expression in oligodendroglioma tumors of Saudi Arabian population.

Lab code	Sex	Age, years	Diagnosis	EGFRvIII RT-qPCR
50-N	F	6	Oligodendroglioma grade-II	NEG
HALK-6	F	36	Anaplastic oligodendroglioma	NEG
HALK-10	F	17	Anaplastic oligodendroglioma	NEG
HALK-14	M	39	Oligodendroglioma	NEG
HALK-15	M	6	Anaplastic oligodendroglioma	POS
HALK-25	F	25	Oligodendroglioma	POS
HALK-30	F	51	Oligodendroglioma	NEG
HALK-35	M	33	Anaplastic oligodendroglioma	NEG
HALK-44	F	27	Anaplastic oligodendroglioma	NEG
KAMC-2	M	67	Oligodendroglioma	NEG
KAMC-16	F	23	Anaplastic oligodendroglioma	POS
KAMC-41	M	42	Oligodendroglioma	POS
KAMC-46	M	33	Anaplastic oligodendroglioma	NEG

EGFRv, epidermal growth factor receptor variant; RT-qPCR, reverse transcription-quantitative PCR; F, female; M, male; NEG, negative; POS, positive.

Table SV. EGFRvIII expression in brain tumors of Saudi Arabian population.

Lab code	Sex	Age, years	Diagnosis	EGFRvIII RT-qPCR
9-N	M	10	Medulloblastoma	NEG
40-N	F	59	Meningioma grade-I	NEG
43-N	M	24	Fibrillary astrocytoma grade-II	NEG
44-N	F	3	Choroid plex papilloma grade-II	NEG
45-N	M	66	Meningioma grade-II	NEG
49-N	M	35	Craniopharyngioma	NEG
52-N	M	10	Medulloblastoma grade-IV	NEG
54-N	F	14	Medulloblastoma	POS
60-N	F	2	Rhabdoid tumor	NEG
61-N	M	7	Medulloblastoma	NEG
70-N	F	3	Ependymoma grade-I	NEG
83-N	M	25	Ependymoma grade-II	NEG
86-N	M	50	Synovial sarcoma	NEG
87-N	F	61	Meningioma	NEG
HALK-1	F	18	Ganglioglioma, atypical	POS
HALK-19	F	40	Gliosarcoma	NEG
HALK-26	F	16	Ganglioglioma	NEG
HALK-38	F	27	Ganglioglioma	NEG
HALK-43	F	25	Ganglioglioma	NEG
HALK-45	F	38	Gliosarcoma	NEG
HALK-46	M	32	Ganglioglioma	NEG
KAMC-1	M	50	Metastatic adenocarcinoma	NEG
KAMC-5	M	55	Hemangioblastoma	NEG
KAMC-9	F	56	Craniopharyngioma	NEG
KAMC-11	F	60	Reactive gliosis	POS
KAMC-21	M	67	Medulloblastoma	NEG
KAMC-28	F	39	Ependymoma	POS
KAMC-43	M	87	Gliosarcoma	NEG
KAMC-48	M	21	Gemistocytic astrocytoma	NEG

EGFRv, epidermal growth factor receptor variant; RT-qPCR, reverse transcription-quantitative PCR; F, female; M, male; NEG, negative; POS, positive.

Table SVI. Association between IDH1 and IDH2 mutations with EGFRvIII status in brain tumors.

Serial number	Lab code	Diagnosis	IDH1	EGFRvIII
1	HALK-27	GBM	c.395G>A; p. (Arg132His)	POS
2	KAMC-12	GBM	c.395G>A; p. (Arg132His)	POS
3	63-N	GBM	c.352C>T; p. (Pro118Ser)	NEG
4	47-N	GBM grade-IV	c.356G>A; p. (Arg119Gln)	NEG
5	51-N	GBM grade-IV	c.315C>T; p. (Gly105Gly)	NEG
6	KAMC-47	GBM	c.395G>A; p. (Arg132His)	NEG
7	HALK-41	GBM	c.395G>A; p. (Arg132His)	NEG
8	HALK-48	GBM	c.395G>A; p. (Arg132His)	NEG
9	HALK-39	Low grade glioma	c.369A>G; p. (Gly123Gly) ^c	POS
10	KAMC-27	GBM	c.395G>A; p. (Arg132His)	NEG
11	KAMC-15 ^b	GBM	c.395G>A; p. (Arg132His)	POS
12	KAMC-20 ^b	Glioma	c.395G>A; p. (Arg132His)	NEG
13	KAMC-44 ^b	GBM	c.395G>A; p. (Arg132His)	NEG
14	62-N	GBM	c.395G>A; p. (Arg132His)	POS
15	50-N	Oligodendroglioma grade II	c.395G>A; p. (Arg132His)	NEG
16	HALK-10	Anaplastic Oligodendroglioma	c.395G>A; p. (Arg132His)	NEG
17	HALK-25	Oligodendroglioma	c.395G>A; p. (Arg132His)	POS
18	HALK-30	Oligodendroglioma	c.395G>A; p. (Arg132His)	NEG
19	HALK-44	Anaplastic oligodendroglioma	c.395G>A; p. (Arg132His)	NEG
20	KAMC-41	Oligodendroglioma	c.395G>A; p. (Arg132His)	POS
21	KAMC-2	Oligodendroglioma	c.395G>A; p. (Arg132His)	NEG
22	HALK-35	Anaplastic oligodendroglioma	c.395G>A; p. (Arg132His)	NEG
23	HALK-11	Diffuse astrocytoma	c.395G>A; p. (Arg132His)	POS
24	KAMC-33	Pilocytic astrocytoma	c.395G>A; p. (Arg132His)	POS
25	KAMC-37	Pilocytic astrocytoma	c.395G>A; p. (Arg132His)	POS
26	KAMC-50	Astrocytoma	c.395G>A; p. (Arg132His)	POS
27	KAMC-51	Astrocytoma	c.395G>A; p. (Arg132His)	POS
28	71-N ^b	Astrocytoma grade-II	c.395G>A; p. (Arg132His)	NEG
29	49-N	Craniopharyngioma	c.472C>T; p. (Pro158Ser) ^a	NEG
30	KAMC-28	Ependymoma grade-II	c.395G>A; p. (Arg132His)	POS
31	83-N	Ependymoma grade-II	c.395G>A; p. (Arg132His)	NEG
32	HALK-19	Gliosarcoma	c.395G>A; p. (Arg132His)	NEG
33	61-N ^b	Medulloblastoma	c.315C>T; p. (Gly105Gly)	NEG
34	54-N	Medulloblastoma	c.380C>T; p. (Pro127Leu)	POS
35	HALK-46	Ganglioglioma	c.395G>A; p. (Arg132His)	NEG
36	HALK-45	Gliosarcoma	c.395G>A; p. (Arg132His)	NEG

^aNovel IDH2 mutation; ^bthese tumors also contain IDH2 mutations; ^cnovel IDH1 mutation. EGFRv, epidermal growth factor receptor variant; RT-qPCR, reverse transcription-quantitative PCR; F, female; M, male; GBM, glioblastoma; NEG, negative; POS, positive.