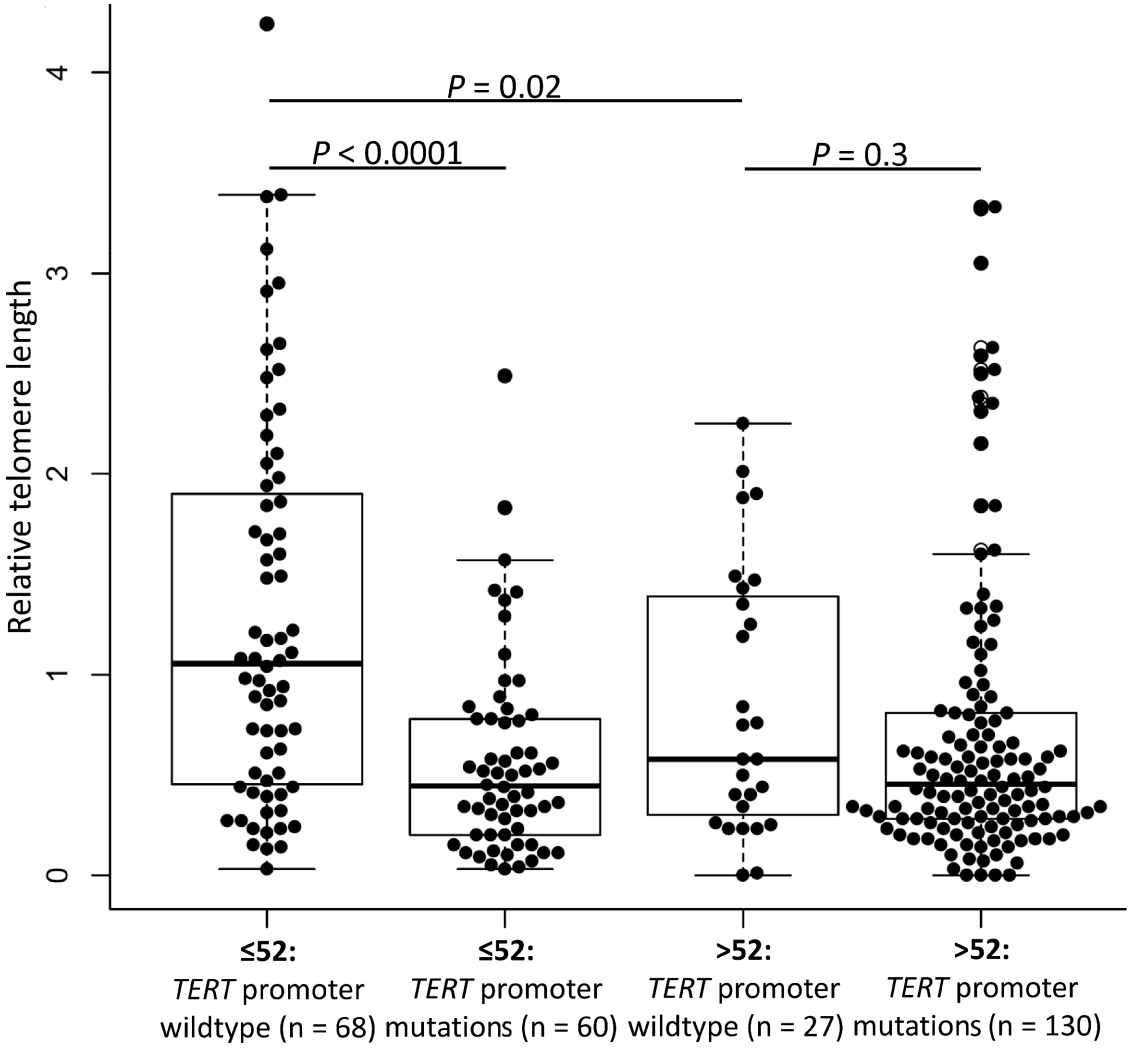
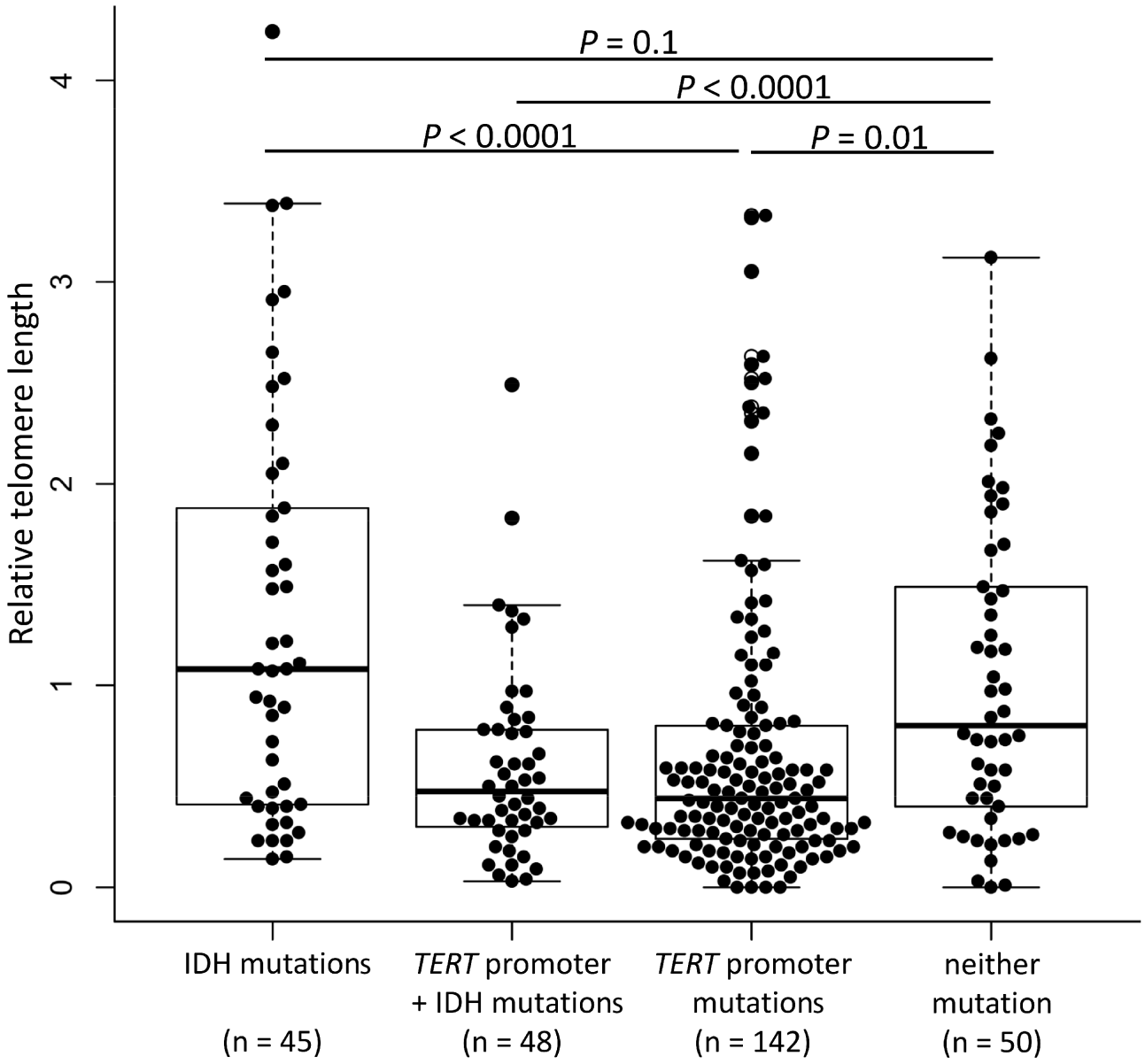
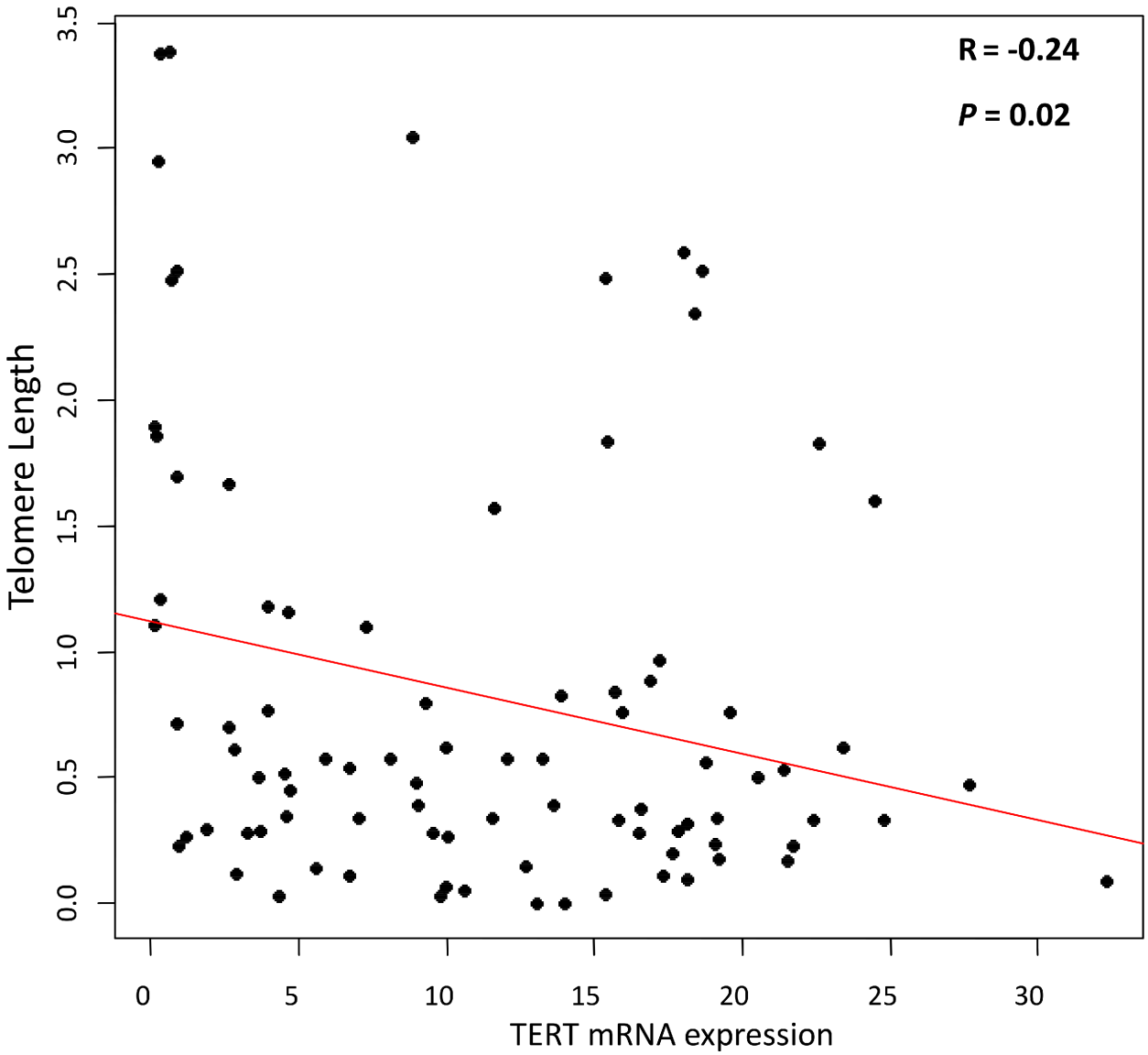
SUPPLEMENTARY FIGURES AND TABLES



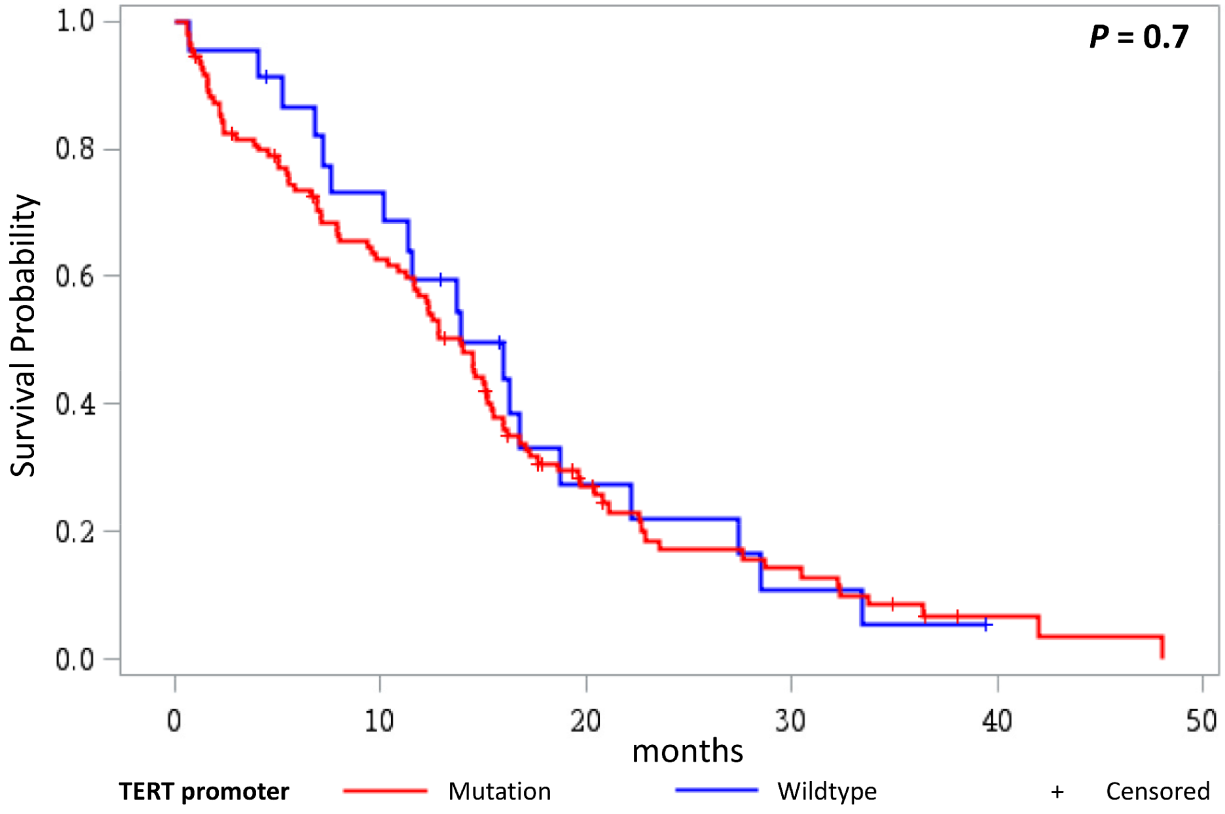
Supplementary Figure 1: Telomere lengths in glioma tumors stratified according to age. Relative telomere length in gliomas without and with *TERT* promoter mutations, stratified according to median age of the patients. Experiments were carried out in triplicate and box plots represent mean ± s.e.m. *P*-values were determined by *t*-test.



Supplementary Figure 2: Relative telomere lengths in glioma samples according to mutation status of IDH and the *TERT* promoter. Comparison of relative telomere lengths in gliomas with and without mutations in *IDH* and the *TERT* promoter, respectively. Experiments were carried out in triplicate and box plots represent mean ± s.e.m. *P*-values were determined by *t*-test.

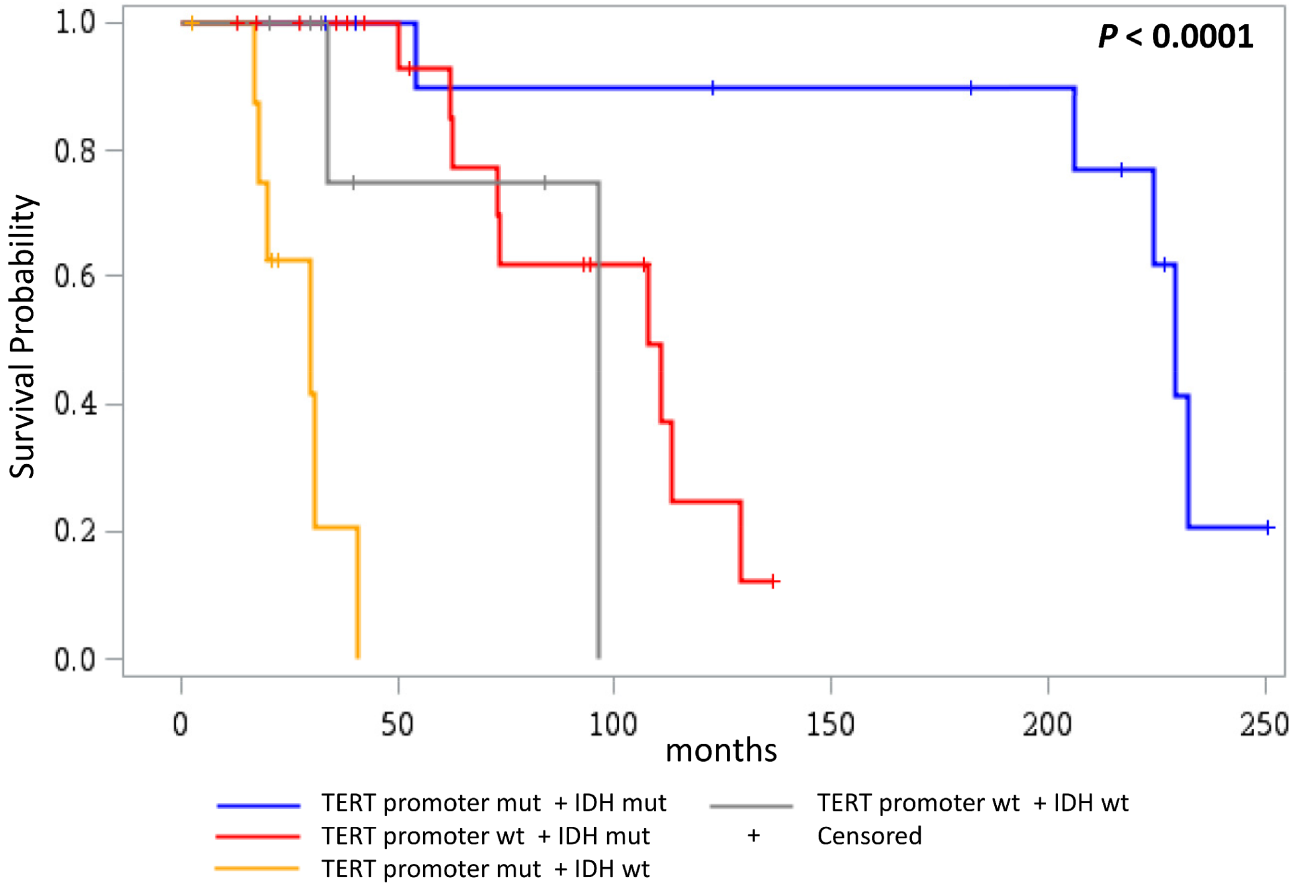


Supplementary Figure 3: Correlation between telomere length and TERT mRNA expression. Investigation of correlation using linear regression between relative telomere length and relative TERT mRNA expression for tumors were both values were available (n = 88). P-values were determined by ANOVA.



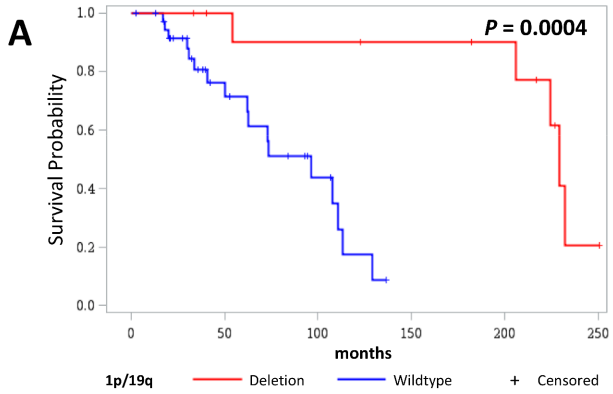
	Total	Failed	censored
TERTmut	111	91	20
TERTwt	23	19	4
Total	134	110	24

Supplementary Figure 4: Overall survival in patients with primary glioblastoma according to *TERT* promoter status.

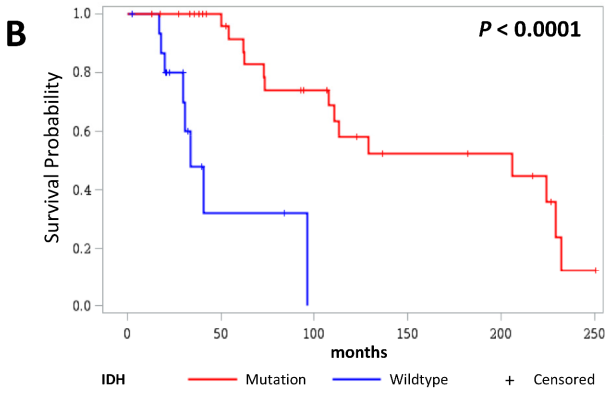


	Total	Failed	censored
TERTmut + IDHmut	13	5	8
TERTwt + IDHmut	21	9	12
TERTmut + IDHwt	9	6	3
TERTwt + IDHwt	7	2	5
Total	50	22	28

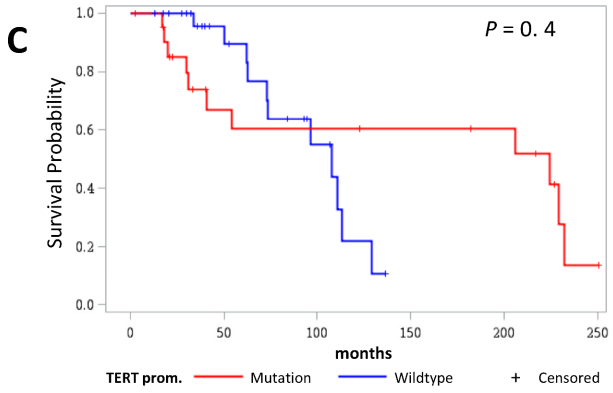
Supplementary Figure 5: Overall survival according to combined status of *TERT* promoter and *IDH* mutation in patients with astrocytoma.



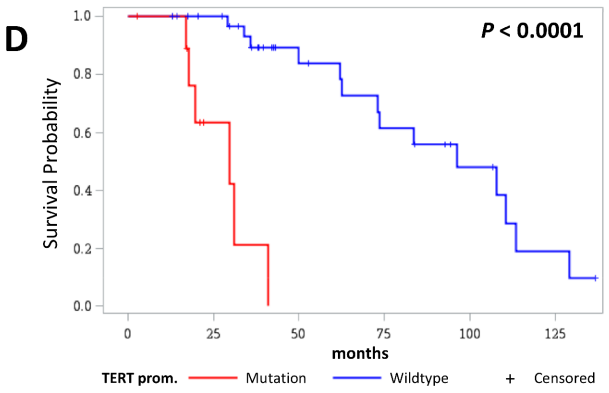
1p/19q	Total	Failed	censored
wt	38	17	21
deletion	12	5	7
Total	50	22	28



IDH	Total	Failed	censored
wt	16	8	8
mut	34	14	20
Total	50	22	28

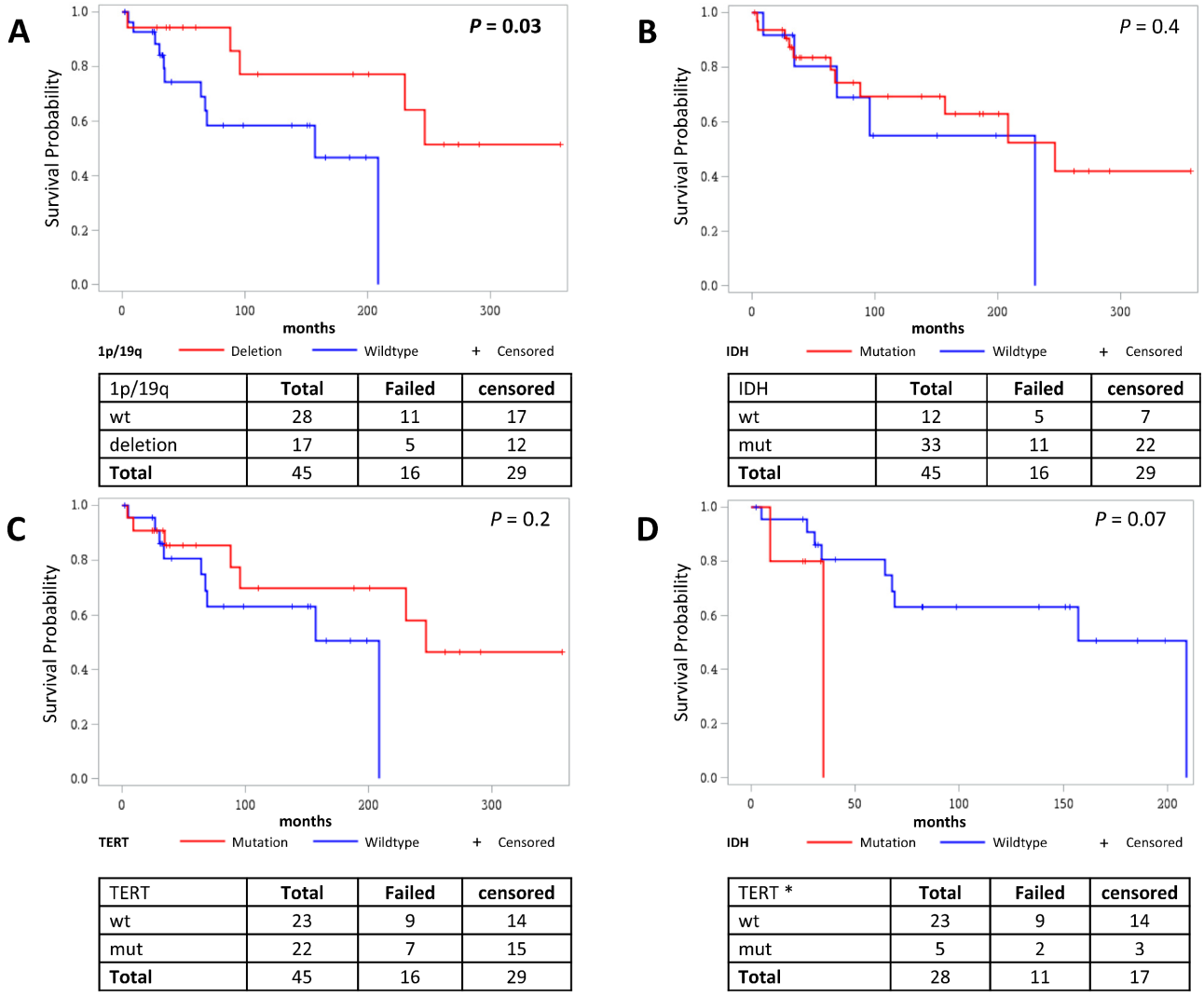


TERT	Total	Failed	censored
wt	28	11	17
mut	22	11	11
Total	50	22	28



TERT *	Total	Failed	censored
wt	28	11	17
mut	10	6	4
Total	38	17	21

Supplementary Figure 6: Overall survival in patients with astrocytoma according to alterations in the *TERT* promoter, *IDH* and at 1p/19q. Kaplan Meier analysis of differences in overall survival in astrocytoma patients stratified according to **(A)** co-deletions in 1p/19q, **(B)** *IDH* mutations and **(C)** *TERT* promoter mutations. **(D)** To assess the effect of *TERT* promoter mutations without the influence of 1p/19q co-deletions, patients that carried the co-deletions were removed from the analysis and survival analysis stratified by *TERT* promoter mutations was repeated. *Analysis of survival of patients with *TERT* promoter mutations and without 1p/19q co-deletions.



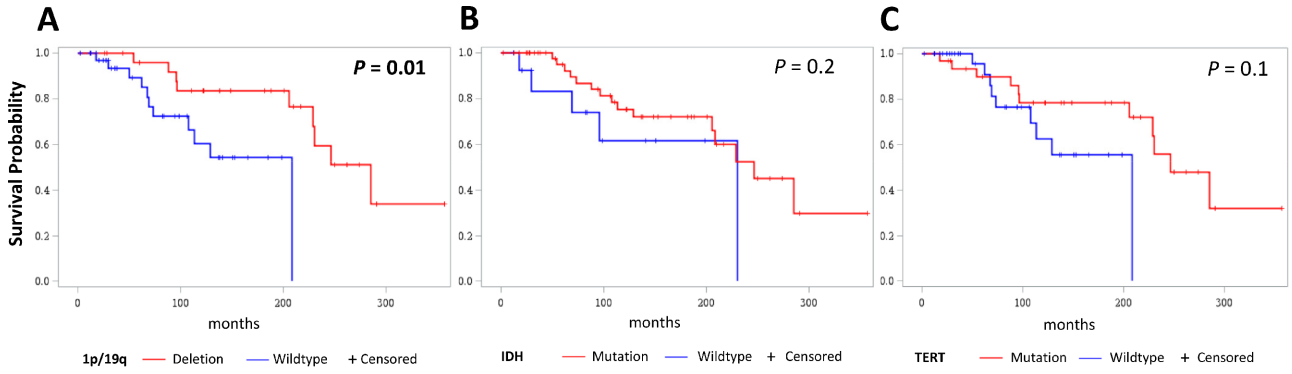
1p/19q	Total	Failed	censored
wt	28	11	17
deletion	17	5	12
Total	45	16	29

IDH	Total	Failed	censored
wt	12	5	7
mut	33	11	22
Total	45	16	29

TERT	Total	Failed	censored
wt	23	9	14
mut	22	7	15
Total	45	16	29

TERT *	Total	Failed	censored
wt	23	9	14
mut	5	2	3
Total	28	11	17

Supplementary Figure 7: Overall survival in patients with oligoastrocytoma according to alterations in the *TERT* promoter, *IDH* and at 1p/19q. Kaplan Meier analysis of differences in overall survival in patients with oligoastrocytoma stratified according to **(A)** co-deletions in 1p/19q, **(B)** *IDH* mutations and **(C)** *TERT* promoter mutations. **(D)** To assess the effect of *TERT* promoter mutations without the influence of 1p/19q co-deletions, patients that carried the co-deletions were removed from the analysis and survival analysis stratified by *TERT* promoter mutations was repeated. *Analysis of survival of patients with *TERT* promoter mutations and without 1p/19q co-deletions.

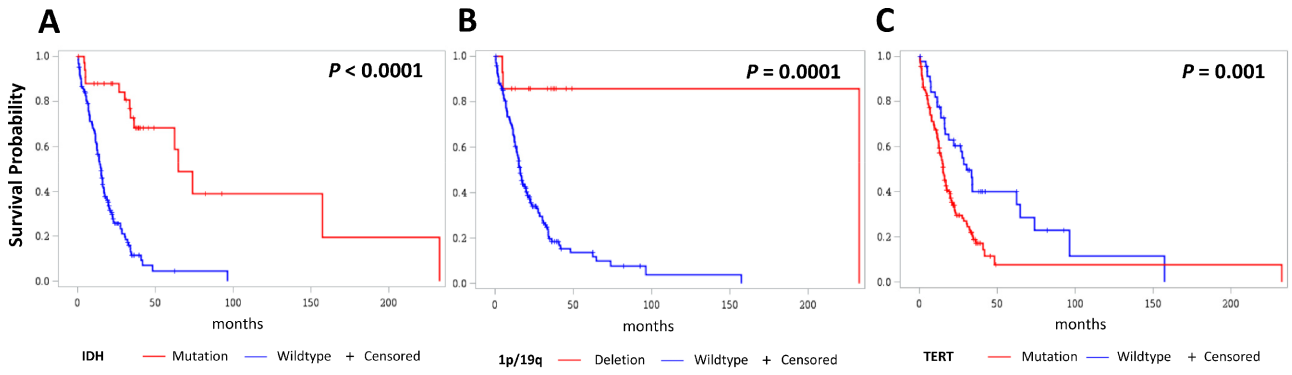


1p/19q	Total	Failed	censored
wt	36	11	25
deletion	28	9	19
Total	64	20	44

IDH	Total	Failed	censored
wt	14	5	9
mut	50	15	35
Total	64	20	44

TERT	Total	Failed	censored
wt	33	9	24
mut	31	11	20
Total	64	20	44

Supplementary Figure 8: Overall survival in patients with low grade gliomas according to alterations in the *TERT* promoter, *IDH* and at 1p/19q. Kaplan Meier analysis of differences in overall survival in patients with low grade gliomas (grade II), including astrocytomas, oligoastrocytomas and oligodendrogliomas. For survival analysis patients were stratified according to **(A)** co-deletions in 1p/19q, **(B)** *IDH* mutations and **(C)** *TERT* promoter mutations. In **(C)** *TERT* promoter mutations seem to confer a better survival, however, this association is due to co-occurrence with 1p/19q co-deletions. In low grade gliomas only three patients harboured *TERT* promoter mutations without 1p/19q co-deletions in their tumors.

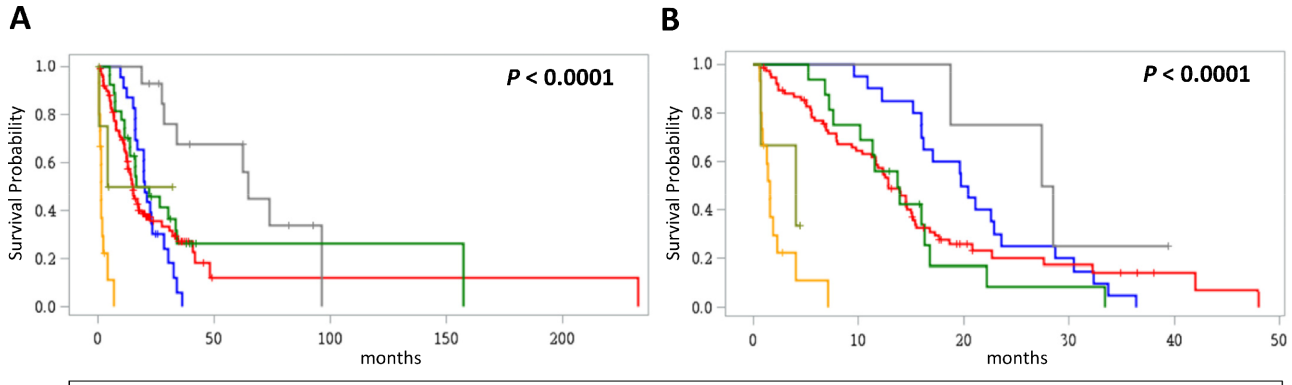


IDH	Total	Failed	censored
wt	151	116	35
mut	34	14	20
Total	185	130	55

1p/19q	Total	Failed	censored
wt	171	127	44
deletion	14	3	11
Total	185	130	55

TERT	Total	Failed	censored
wt	139	101	38
mut	46	29	17
Total	185	130	55

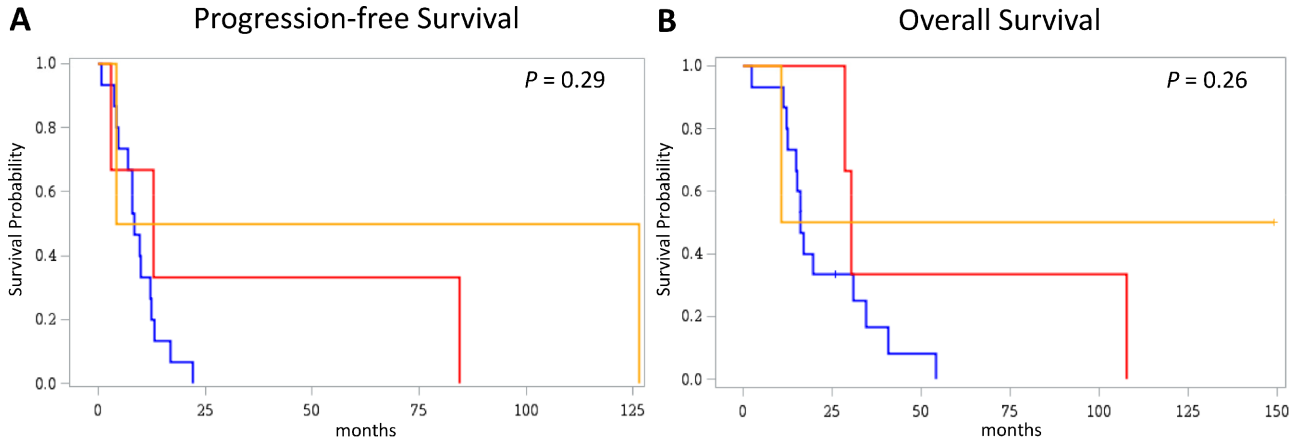
Supplementary Figure 9: Overall survival in patients with high grade gliomas according to alterations in the *TERT* promoter, *IDH* and at 1p/19q.



TERT	Treatment Group		Total	Failed	censored
mut	none	(1)	15	13	2
mut	TMZ/PC	(2)	101	67	34
mut	TMZ+other	(3)	23	21	2
wt	none	(1)	5	2	3
wt	TMZ/PC	(2)	27	19	8
wt	TMZ+other	(3)	14	8	6
Total			185	130	55

TERT	Treatment Group		Total	Failed	censored
mut	none	(1)	15	13	2
mut	TMZ/PC	(2)	76	58	18
mut	TMZ+other	(3)	20	20	0
wt	none	(1)	3	2	1
wt	TMZ/PC	(2)	16	14	2
wt	TMZ+other	(3)	4	3	1
Total			134	110	24

Supplementary Figure 10: Overall survival in glioma patients according to different treatment regimens. Kaplan Meier analysis of overall survival in glioma patients receiving different treatment regimens and additionally stratified according to their *TERT* promoter mutational status. Patients were stratified into three groups, where group 1) did not receive any treatment, group 2) received radiotherapy alone or in combination with PC or TMZ and group 3) received TMZ and radiotherapy combined with a third adjuvant. Survival analysis then was performed according to the different treatment groups and the TERT promoter mutational status in **(A)** the complete set of glioma patients and **(B)** patients with primary glioblastomas.



	TERT promoter	Total	Failed	censored
Primary - recurrence	mut/mut	15	15	0
Primary - recurrence	wt/wt	3	3	0
Primary - recurrence	mut/wt	2	2	0
Total		20	20	0

	TERT promoter	Total	Failed	censored
Primary - recurrence	mut/mut	15	14	1
Primary - recurrence	wt/wt	3	3	0
Primary - recurrence	mut/wt	2	1	1
Total		20	18	2

Supplementary Figure 11: Survival analysis in patients with primary tumors and recurrences. Analysis of **(A)** progression-free and **(B)** overall survival in patients for whom information about their primary glioma tumor and corresponding recurrence was available. Patients were grouped according to *TERT* promoter mutations, where both primary and recurrence carried *TERT* promoter mutations or both lesions did not carry the mutations. A third group consisted of the two patients that showed *TERT* promoter mutations in their primary tumors but were wildtype in corresponding recurrences.

Supplementary Table 1: Distribution of clinical parameters and mutations in different histological classifications

	All	Astrocytomas	Oligoastrocytomas	Oligodendrogliomas	Primary Glioblastomas
	***n* = 303**	***n* = 56**	***n* = 55**	***n* = 27**	***n* = 165**
Gender male female	192 111	38 18	34 21	13 14	107 58
Age ≤ 55 > 55	152 151	48 8	47 8	19 8	38 127
Grade low (II) high (III + IV)	78 225	33 23	34 21	11 16	0 165
IDH wildtype mutation	203 100	19 37	16 39	11 16	157 8
9p21 wildtype mutation *missing*	138 119 *46*	30 17 *9*	34 13 *8*	16 9 *2*	58 80 *27*
Deletions at 1p and/or 19q wildtype deletion *missing*	185 74 *44*	34 15 *7*	23 24 *8*	11 14 *2*	117 21 *27*
1p/19q codeletions wildtype codeletion *missing*	214 45 *44*	39 10 *7*	28 19 *8*	11 14 *2*	136 2 *27*

Supplementary Table 2: Association of *TERT* promoter mutations with mutations in IDH1/2 and deletions at 9p21 and 1p19q in different glioma subtypes

	Astrocytomas		Oligoastrocytomas		Oligodendrogliomas		Primary Glioblastomas	
	***n* = 56**		***n* = 55**		***n* = 27**		***n* = 165**	
	***TERT* promoter**		***TERT* promoter**		***TERT* promoter**		***TERT* promoter**	
	wildtype	**mutation**	**wildtype**	**mutation**	**wildtype**	**mutation**	**wildtype**	**mutation**
IDH wildtype	10	9	8	8	7	4	28	129
mutation	24	13	21	18	1	15	5	3
	OR = 0.60; 95% CI 0.20 – 1.85; *P* = 0.37		OR = 0.86; 95% CI 0.27 – 2.75; *P* = 0.8		OR = 26.25; 95% CI 2.46 – 280.20; ***P* = 0.001**		OR = 0.13; 95% CI 0.03 – 0.58; ***P* = 0.002**	
9p21 Wildtype	21	9	18	16	5	11	16	42
mutation	7	10	5	8	2	7	10	70
	OR = 3.33; 95% CI 0.96 – 11.54; ***P* = 0.05**		OR = 1.80; 95% CI 0.49 – 6.64; *P* = 0.4		OR = 1.60; 95% CI 0.24 – 10.57; *P* = 0.6		OR = 2.67; 95% CI 1.11 – 6.42; ***P* = 0.03**	
1p and/or 19q Wildtype	26	8	18	5	7	4	22	95
deletion	3	12	5	19	0	14	4	17
	OR = 13.0; 95% CI 2.92 – 57.85; ***P* = 0.0002**		OR = 13.70; 95% CI 3.38 – 55.32; ***P* < 0.0001**		OR = 4.50; 95% CI 1.90 – 10.68; ***P* = 0.0004**		OR = 0.98; 95% CI 0.30 – 3.22; *P* = 1.0	
1p/19q wildtype	29	10	23	5	7	4	26	0
codeletion	0	10	0	19	0	14	110	2
	OR = 2.00; 95% CI 1.29 –3.10; *P* < 0.0001		OR = 4.80; 95% CI 2.20 – 10.47; ***P* < 0.0001**		OR = 4.50; 95% CI 1.90 – 10.68; ***P* = 0.0004**		OR = 1.2; 95% CI 1.0 – 1.04; *P* = 0.5	

P –values were derived from χ^2-test and considered statistically significant if < 0.05.
Bold font indicates statistical significance.

Supplementary Table 3: Multivariate Model in complete set of Gliomas grouped according to *TERT* promoter and *IDH* status

Parameter		*P*	HR	95% CI	
Group	*TERT+IDH*	**0.03**	**0.22**	**0.08**	**0.59**
Group	*IDH*	0.72	1.14	0.56	2.36
Group	*TERT*	0.78	0.93	0.55	1.56
Age		**< .0001**	**1.04**	**1.02**	**1.06**
Grade	III	**< .0001**	**5.22**	**2.41**	**11.33**
Grade	IV	**< .0001**	**32.92**	**12.89**	**84.04**
CDKN2A/B	Deletion	0.43	1.15	0.81	1.64
Treatment - Group	PC	0.23	1.79	0.7	4.61
Treatment - Group	RT	0.06	1.75	0.99	3.12
Treatment - Group	TMZ+ot	0.72	0.93	0.61	1.41
Treatment - Group	none	**< .0001**	**5.61**	**2.94**	**10.72**

Supplementary Table 4: Multivariate Model in Astrocytomas grouped according to *TERT* promoter and *IDH* status

Parameter		*P*	HR	95% CI	
group	*TERT+IDH*	0.02	0.02	0.001	0.54
group	*IDH*	0.42	0.35	0.03	4.33
group	*TERT*	**0.02**	**37.67**	**1.63**	**870.82**
Age		0.11	1.06	1.00	1.13
Grade	III	0.23	0.35	0.07	1.91
Grade	IV	0.58	0.40	0.01	10.89
CDKN2A/B	Deletion	**0.01**	**6.11**	**1.47**	**25.37**
Treatment - Group	PC	1.00	0.00	0.00	-
Treatment - Group	RT	0.24	7.38	0.27	201.46
Treatment - Group	TMZ+ot	0.25	2.69	0.50	14.47
Treatment - Group	none	0.99	0.00	0.00	-

Supplementary Table 5: Multivariate Model in Astrocytomas

Parameter		*P*	HR	95% CI	
TERT promoter	Mutation	**0.02**	**37.65**	**1.63**	**869.91**
IDH	Mutation	0.42	0.35	0.03	4.32
1p/19q co-deletion	Deletion	**0.002**	**0.002**	**0.00**	**0.09**
CDKN2A/B	Deletion	**0.01**	**6.12**	**1.48**	**25.37**
Age		0.11	1.06	0.99	1.13
Grade	III	0.22	0.35	0.07	1.90
Grade	IV	0.58	0.40	0.01	10.88
Treatment – Group*	RT	0.24	7.38	0.27	201.53
Treatment – Group*	TMZ+ot	0.25	2.69	0.50	14.48

*excluded from analysis: treatment types: PC (n = 1), None (n = 7)

Supplementary Table 6: Multivariate Model in Oligoastrocytomas

Parameter		*P*	HR	95% CI	
TERT promoter	Mutation	0.62	2.05	0.12	35.50
IDH	Mutation	0.27	0.16	0.01	3.98
1p/19q co-deletion	Deletion	0.54	0.57	0.09	3.75
CDKN2A/B	Deletion	**0.05**	**0.19**	**0.04**	**1.02**
Age		0.10	1.06	0.99	1.14
Grade	III	**0.02**	**16.45**	**1.64**	**165.01**
Treatment - Group*	PC	0.60	0.68	0.16	2.84
Treatment - Group*	RT	0.33	0.30	0.03	3.46
Treatment - Group*	TMZ+ot	0.20	0.27	0.04	1.96

*excluded from analysis: treatment types: None (n = 4)

Supplementary Table 7: Multivariate Model in Oligoastrocytomas (Exclusion of 1p/19q co-deletions)

Parameter		*P*	HR	95% CI	
TERT promoter	Mutation	0.75	1.90	0.04	89.68
IDH	Mutation	0.82	0.67	0.02	19.88
CDKN2A/B	Deletion	0.47	0.50	0.07	3.37
Age		0.24	1.07	0.96	1.19
Grade	III	0.12	11.37	0.53	242.59
Grade	IV	0.32	0.34	0.04	2.88
Treatment - Group	RT	0.79	1.60	0.05	48.49
Treatment - Group	TMZ+ot	0.34	0.40	0.06	2.65

Supplementary Table 8: Multivariate Model in Low Grade Gliomas*

Parameter		*P*	HR	95% CI	
TERT promoter	Mutation	0.03	0.24*	0.07	0.87
IDH	Mutation	0.74	0.79	0.20	3.14
CDKN2A/B	Deletion	0.23	0.20	0.65	6.03
Age		**0.02**	**1.08**	**1.01**	**1.14**
Treatment - Group	PC	0.53	0.60	0.12	2.94
Treatment - Group	RT	0.26	0.26	0.04	1.86
Treatment - Group	TMZ+ot	1.21	1.21	0.28	5.18

**HR could not be calculated when 1p19q was included, resulting in a misleadingly negative HR for TERT promoter mutations*

Supplementary Table 9: Multivariate Model in High Grade Gliomas

Parameter		*P*	HR	95% CI	
TERT promoter	Mutation	0.16	1.49	0.86	2.59
1p/19q co-deletion	Deletion	**0.003**	**0.09**	**0.02**	**0.44**
IDH	Mutation	0.67	0.84	0.37	1.89
CDKN2A/B	Deletion	0.61	0.91	0.62	1.33
Treatment - Group	PC	0.66	0.76	0.23	2.53
Treatment - Group	RT	0.28	1.39	0.76	2.53
Treatment - Group	TMZ+ot	0.22	0.75	0.47	1.19
Treatment - Group	none	**< .0001**	**9.58**	**4.99**	**18.40**
Age		**< .0001**	**1.05**	**1.03**	**1.06**

Supplementary Table 10: Comparison of genetic alterations in primary glioma tumors and matched recurrences

Primary tumor	*CDKN2A/B*	1p/19q	*IDH*	*TERT* promoter	rs2853669	Histology at primary diagnosis
Recurrence						Histology final
BT-121	deletion[a]	partial 1p deletion[m]	wt	−124C > T	TT	glioblastoma WHO grade IV
BT-121R1	deletion[a]	partial 1p deletion[m]	wt	−124C > T	TT	glioblastoma WHO grade IV
BT-123	deletion[a]	co-deleltion 1p19q[d]	c.395G > A	−124C > T	CC	oligoastrocytoma WHO grade III
BT-123R1	deletion[a]	co-deleltion 1p19q[d]	c.395G > A	−124C > T	CC	oligoastrocytoma WHO grade III
BT-123R2	deletion[a]	co-deleltion 1p19q[d]	c.395G > A	−124C > T	CC	glioblastoma WHO grade IV
BT-154	wt	wt	wt	−124C > T	TT	glioblastoma WHO grade IV
BT-154R1	wt	wt	wt	−124C > T	TT	glioblastoma WHO grade IV

(*Continued*)

Primary tumor / Recurrence	*CDKN2A/B*	1p/19q	*IDH*	*TERT* promoter	rs2853669	Histology at primary diagnosis / Histology final
BT-159	deletion[a]	wt	wt	−124C > T	TT	oligoastrocytoma WHO grade III
BT-159R1	deletion[a]	wt	wt	−124C > T	TT	oligoastrocytoma WHO grade III
BT-159R2	deletion[a]	wt	wt	−124C > T	TT	glioblastoma WHO grade IV
BT-177	deletion[a]	wt	wt	−124C > T	CT	oligoastrocytoma WHO grade III
BT-177R1	deletion[a]	wt	wt	−124C > T	CT	glioblastoma WHO grade IV
BT-185	wt	wt	wt	wt	TT	glioblastoma WHO grade IV
BT-185R1	wt	wt	wt	wt	TT	glioblastoma WHO grade IV
BT-200	wt	wt	wt	−124C > T	CC	glioblastoma WHO grade IV
BT-200R1	wt	wt	wt	−124C > T	CC	glioblastoma WHO grade IV
BT-215	wt	wt	c.395G > A	wt	CT	anaplastic oligoastrocytoma WHO grade III
BT-215R1	wt	wt	c.395G > A	wt	CT	glioblastoma WHO grade IV
BT-220	wt	wt	c.395G > A	wt	CT	glioblastoma WHO grade IV
BT-220R1	wt	wt	c.395G > A	wt	CT	glioblastoma WHO grade IV
BT-222	deletion[a]	wt	wt	−124C > T	CT	glioblastoma WHO grade IV
BT-222R1	deletion[a]	wt	wt	−124C > T	CT	glioblastoma WHO grade IV
BT-264	wt	wt	wt	−124C > T	CT	glioblastoma WHO grade IV
BT-264R1	wt	wt	wt	−124C > T	CT	glioblastoma WHO grade IV
BT-266	wt	wt	wt	−124C > T	TT	glioblastoma WHO grade IV
BT-266R1 (BT364)	wt	wt	wt	−124C > T	TT	glioblastoma WHO grade IV
BT-43	deletion[a]	wt	wt	−146C > T	TT	glioblastoma WHO grade IV
BT-43R1	deletion[a]	wt	wt	−146C > T	TT	glioblastoma WHO grade IV
BT-62	wt	wt	wt	−146C > T	TT	glioblastoma WHO grade IV
BT-62R1	wt	wt	wt	−146C > T	TT	glioblastoma WHO grade IV
BT-92	deletion[a]	partial 1p deletion[k]	wt	−124C > T	CT	glioblastoma WHO grade IV
BT-92R1	deletion[a]	partial 1p deletion[k]	wt	−124C > T	CT	glioblastoma WHO grade IV
BT-163	deletion[a]	wt	wt	−124C > T	CT	glioblastoma WHO grade IV
BT-163R1	wt	wt	wt	wt	CT	glioblastoma WHO grade IV; necrosis, single tumor cells
BT-171	deletion[a]	wt	c.395G > A	−124C > T	CC	oligodendroglioma WHO grade II
BT-171R1	wt	wt	wt	wt	CC	anaplastic oligoastrocytoma, WHO grade III
BT-261	deletion[a]	wt	wt	−124C > T	CT	glioblastoma WHO grade IV
BT-261R1	wt	wt	wt	−124C > T	CT	glioblastoma WHO grade IV
BT-288	wt	wt	wt	−146C > T	CT	glioblastoma WHO grade IV

(*Continued*)

Primary tumor / Recurrence	*CDKN2A/B*	1p/19q	*IDH*	*TERT* promoter	rs2853669	Histology at primary diagnosis / Histology final
BT-288R1	wt	partial 19q deletion[h]	wt	−146C > T	CT	glioblastoma WHO grade IV
BT-56	deletion[a]	wt	wt	−124C > T	TT	anaplastic astrocytoma WHO grade III.
BT-56R1	wt	wt	wt	−124C > T	TT	glioblastoma WHO grade IV

[a]comprises MLPA probes for CDKN2A-3, CDKN2A-2a, CDKN2A-1, CDKN2B-2, CDKN2B-1b
[d]comprises MLPA probes for 1 p arm: TNFRSF14–8, TP73–1, PARK7–7, MFN2–10, WNT4–2, PTAFR-4, PRDX1–7, PRDX1–2c, FAF1–4, CDKN2C-1, CDKN2C-3a, PPAP2B-2, MIR101–1–1, FUBP1–8, GTF2B-2, DPYD-1, NRAS-5, NOTCH2–5; 19 q arm: CCNE1–11, PDCD5–1, UPK1A-4, UPK1A-7, TGFB1–3, CIC-10, ZNF296–2, PPP1R15A-2, BAX-4, CHMP2A-4, CHMP2A-2
[h]comprises MLPA probes for TGFB1–3, CIC-10, ZNF296–2, PPP1R15A-2, BAX-4, CHMP2A-4, CHMP2A-2
[k]comprises MLPA probes for PARK7–7, CDKN2C-1, CDKN2C-3a
[m]comprises MLPA probes for PRDX1–7, PRDX1–2c, FAF1–4, CDKN2C-1, CDKN2C-3a

Supplementary Table 11: PCR conditions and primer sequences

Gene	$MgCl_2$ concentration and additives	Size (bp)	Primer sequence	Annealing temperature
TERT promoter: –27 to –286				
forward reverse	2 mM $MgCl_2$; 5% Glycerol	260	5′CCCACGTGCGCAGCAGGAC3′ 5′CTCCCAGTGGATTCGCGGGC3′	60°C
IDH1				
forward reverse	1.5 mM $MgCl_2$; 5% Glycerol	286	5′TGGCGTCAAATGTGCCACTATC3′ 5′ACACATACAAGTTGGAAATTT CTGGGC3′	53°C
IDH2				
forward reverse	1.5 mM $MgCl_2$; 5% Glycerol	373	5′AGGACCCCCGTCTGGCTGTG3′ 5′CGGCCCGGTCTGCCACAAAG3′	61°C
TERT mRNA expression				
forward (TERT exon 3) reverse (TERT exon 4)	2.5 mM $MgCl_2$	145	5′CGGAAGAGTGTCTGGAGCAA3′ 5′GGATGAAGCGGAGTCTGGA3′	60°C
Telomere length measurement				
telg telc	-	79	5′ACACTAAGGTTTGGGTTTGGGTTTGGGTTTG GGTTAGTGT3′ 5′TGTTAGGTATCCCTATCCCTATCCCTATCCCTA TCCCTAACA3′	59°C
ALB				
albugcr2 albdgcr2	-	98	5′CGGCGGCGGGCGGCGCGGGCT GGGCGGCC ATGCTTTTCAGCTCT GCAAGTC3′ 5′GCCCGGCCCGCCGCGCCCGTCCCGCCGAGC ATTAAGCTCTTTGG CAACGTAGGTTTC3′	85°C