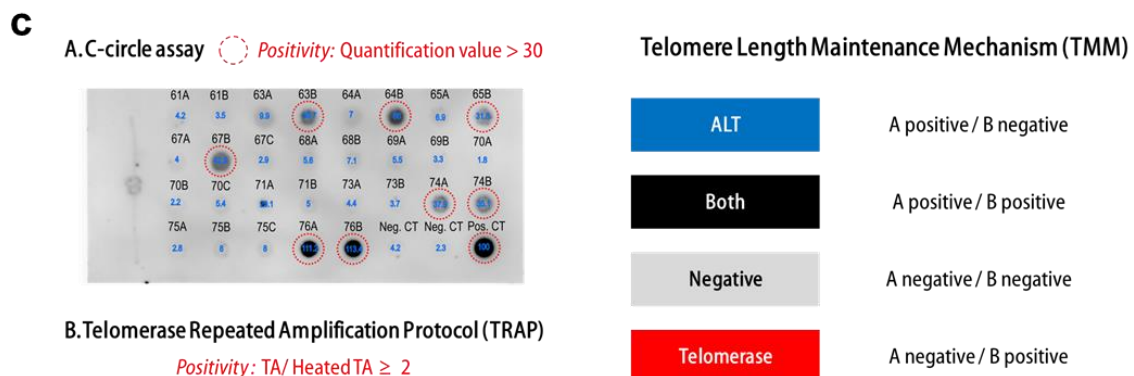
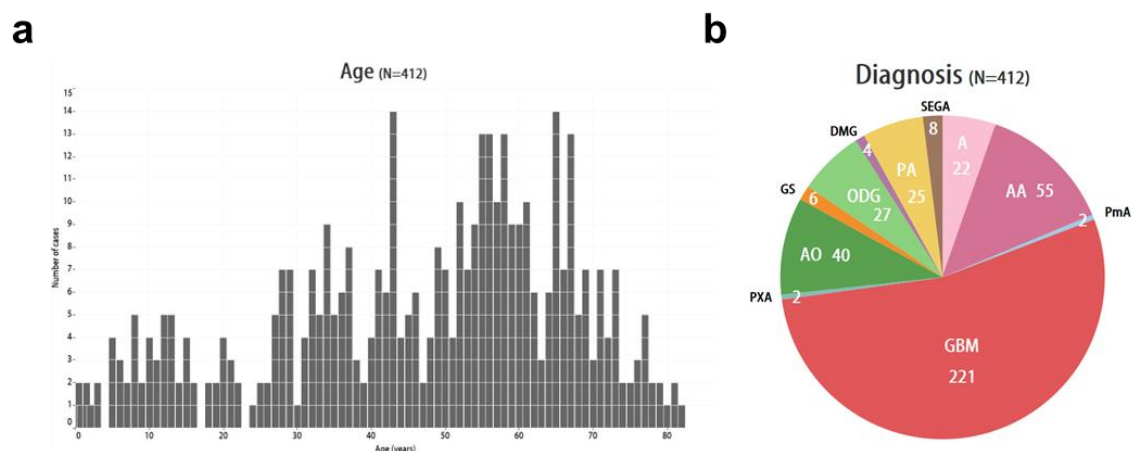


Figure S1 a. Distribution of patients according to age (ranging from 6 months to 82 years). **b.** Pie chart showing the distribution of histological diagnoses among 412 glioma patients. **c.** Telomerase maintenance mechanism (TMM) groups were defined based on relative telomerase activity (RTA) and c-circle assay (CCA) results. The result of CCA was considered positive if the quantified relative value of the blot intensity >30. RTA was calculated by dividing telomerase activity by heated telomerase activity in the telomerase repeated amplification protocol (TRAP). The Telomerase group includes patients with evident telomerase activity (RTA ≥ 2) without any ALT activity (negative CCA), while the ALT group includes patients without evident telomerase activity (RTA < 2) and with ALT activity (positive CCA). The Both group included patients with evident telomerase activity and ALT (RTA ≥ 2 and positive CCA), while the Negative group included patients with limited evidence of telomerase activity and ALT (RTA < 2 and negative CCA). **d.** The subdistribution of histological diagnosis among TMM groups. **e.** The subdistribution of TMM groups among gliomas grouped according to histological diagnosis. (GBM; glioblastoma, GS; gliosarcoma, DMG; diffuse midline glioma, AA; anaplastic astrocytoma, A; astrocytoma, AODG; anaplastic oligodendroglioma, ODG; oligodendroglioma, PA; pilocytic astrocytoma, PXA; pleomorphic xanthoastrocytoma, SEGA; subependymal giant cell astrocytoma)



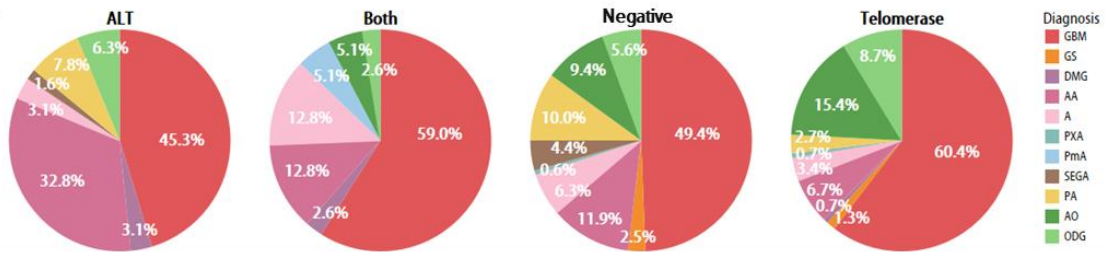
	1	2	3	4	5	6	7	8	9	10	11	12
A	TS8.0	TS8	TS8.0S									
B	S1.0	S1	S1.0S									
C	S2.0	S2	S2.0S									
D	S3.0	S3	S3.0S									
E	S4.0	S4	S4.0S									
F	S5.0	S5	S5.0S									
G	S6.0	S6	S6.0S									
H	S7.0	S7	S7.0S									

Standard curve:
TS8: TS8, Control template, telomerase-specific hybridization buffer
TS8.0: Lysis buffer, telomerase-specific hybridization buffer
TS8.0S: TS8, Control template, Internal Standard (IS)-specific hybridization buffer
Samples:
S1...: Samples, telomerase-specific hybridization buffer
S1.0...: Heat-treated samples, telomerase-specific hybridization buffer
S1.0S...: Samples, Internal Standard (IS)-specific hybridization buffer

$$RTA = \frac{(AS - AS_0)/AS_{IS}}{(ATS8 - ATS8_0)/ATS8_{IS}} \times 100$$

AS: absorbance of sample
AS₀: absorbance of heat- or RNase-treated sample
AS_{IS}: absorbance of Internal Standard (IS) of the sample
ATS8: absorbance of Control template (TS8)
ATS8₀: absorbance of Lysis buffer
ATS8_{IS}: absorbance of Internal Standard (IS) of the Control template (TS8)

d



e

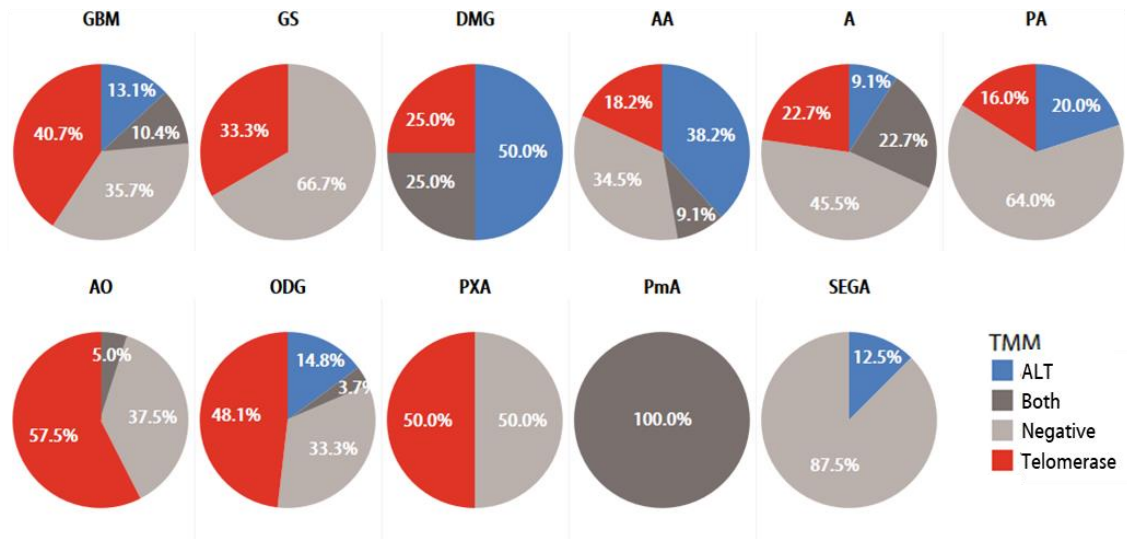


Figure S2 The genetic and clinical characteristics of glioma patients in each TMM group. Note that the *TERTp* and *ATRX* loss are mutually exclusive but are not closely correlated with the TMM. **a.** Telomerase group (n=149). **b.** Negative group (n=160). **c.** ALT group (n=64). **d.** Both group (n=39)

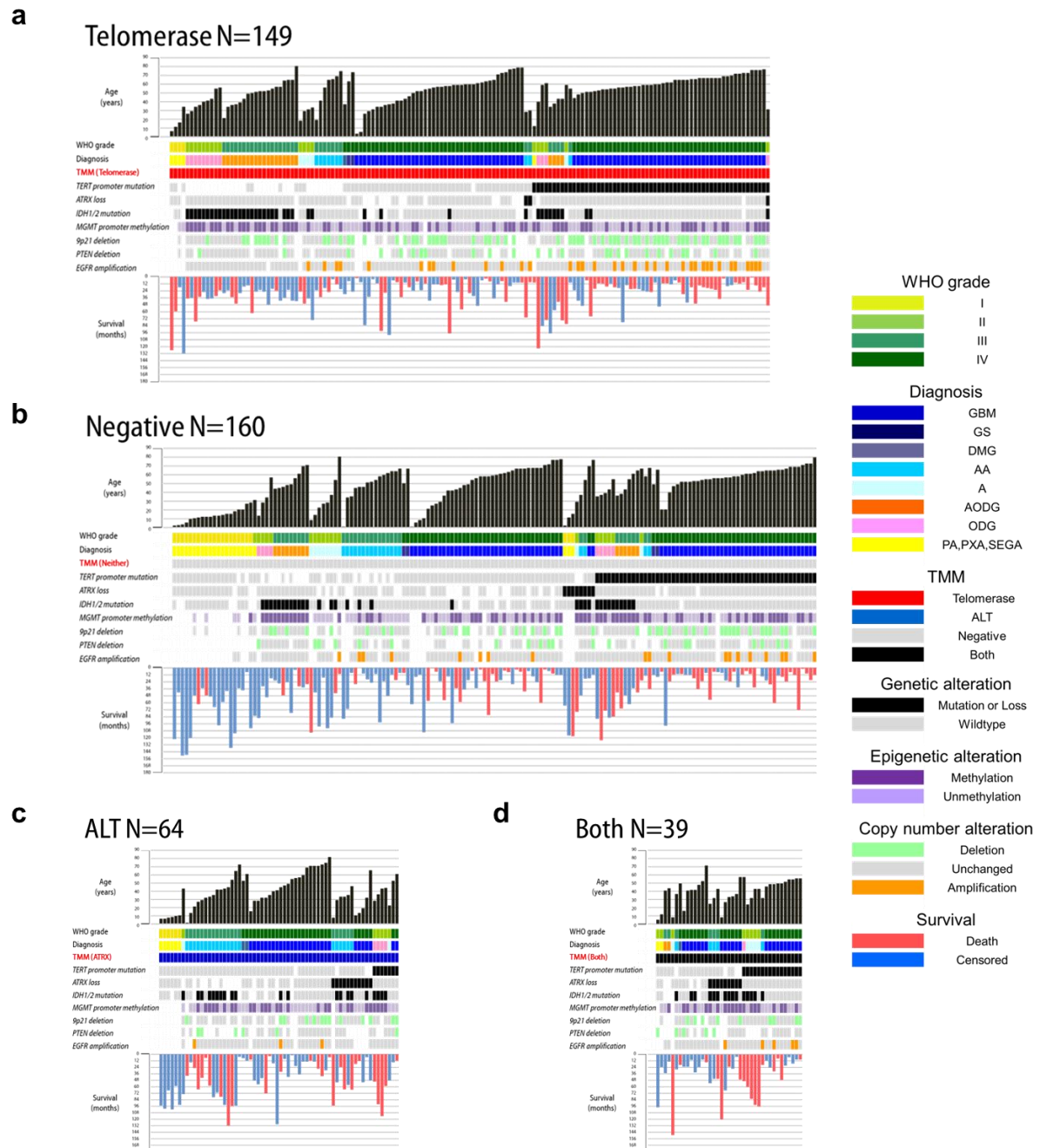


Figure S3 a. TMM status according to *TERTp* and *ATRX* loss statuses. The correlogram shows a strong positive correlation between the ALT group and *mutATRX/wtTERTp* or *wtATRX/wtTERTp* gliomas and a strong negative correlation between the ALT group and *wtATRX/mutTERTp* gliomas. The Both group had a strong positive correlation with *mutATRX/wtTERTp* gliomas and a negative correlation with *wtATRX/wtTERTp* gliomas. The Telomerase group had a strong negative correlation with *mutATRX/wtTERTp* gliomas and a positive correlation with *wtATRX/mutTERTp* gliomas. Note that the Negative group had no significant correlation with the *TERTp* or *ATRX* status. There was a single unusual case harboring mutation in both *TERTp* and *ATRX*. The color intensity and size of the circle are proportional to the correlation coefficients. Positive correlations are displayed in blue, while negative correlations are displayed in red (Pearson residuals, chi-squared = 36.848, df = 9, p<0.001). **b.** TMM status according to *IDH1* and *IDH2* mutation status. The correlogram indicates that *mutIDH* has strong positive correlations with the ALT and Both groups and a strong negative correlation with the Negative group (Pearson residuals, chi-squared = 7.9992, df = 3, p=0.046). **c.** TMM, *ATRX*, *EGFR*, *PTEN* status according to *TERTp* mutation status. 32.8% and 10.9% *TERTp* mutation samples had *EGFR* amplification and *PTEN* deletion respectively, 7.9% and 5.6% *TERTp* wild type samples had *EGFR* amplification and *PTEN* deletion respectively. *TERTp* mutation samples were mostly classified as Telomerase group with some Negative and Both, *TERTp* wild type samples had all 4 TMM groups.

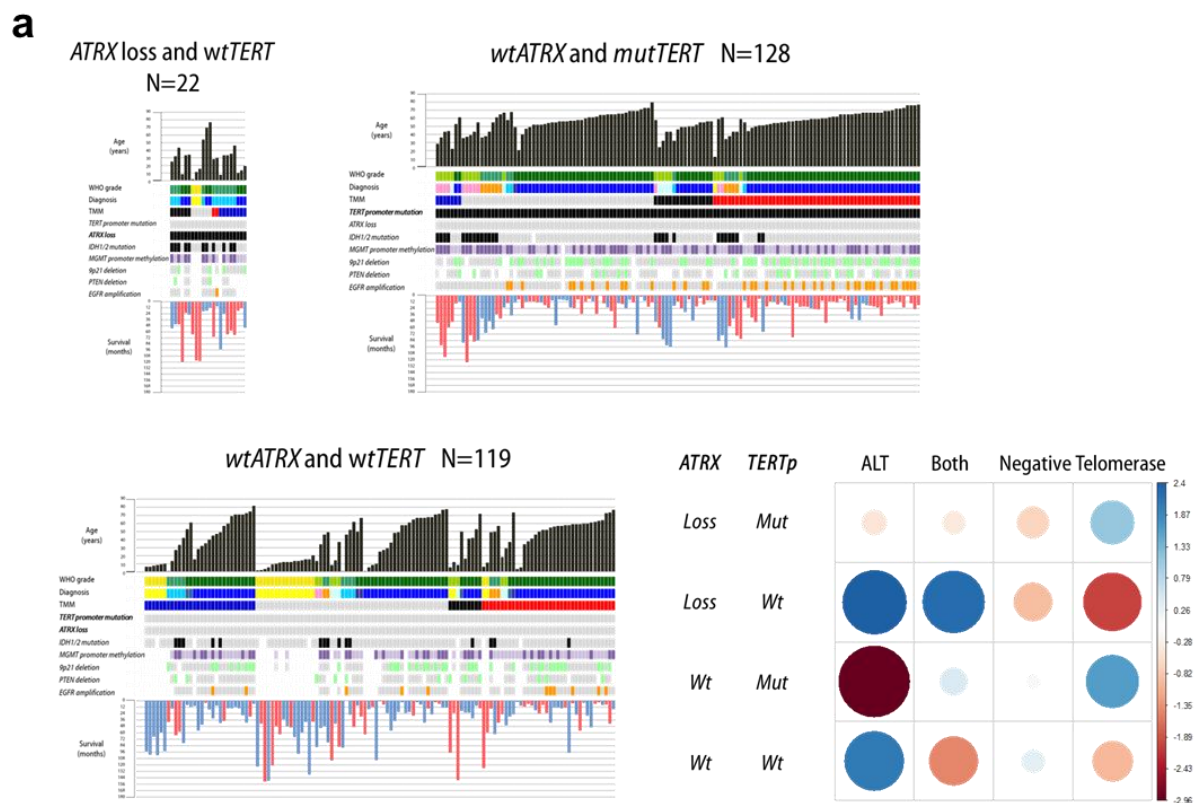


Figure S4 Comparison of age distributions in TMM groups stratified according to major signature gene status (Student's t-test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). **a.** *TERT* promoter. **b.** *ATRX*. **c.** *IDH*. **d.** *MGMT* promoter. (Mut; mutation, WT. wild-type, M; methylated, U; unmethylated)

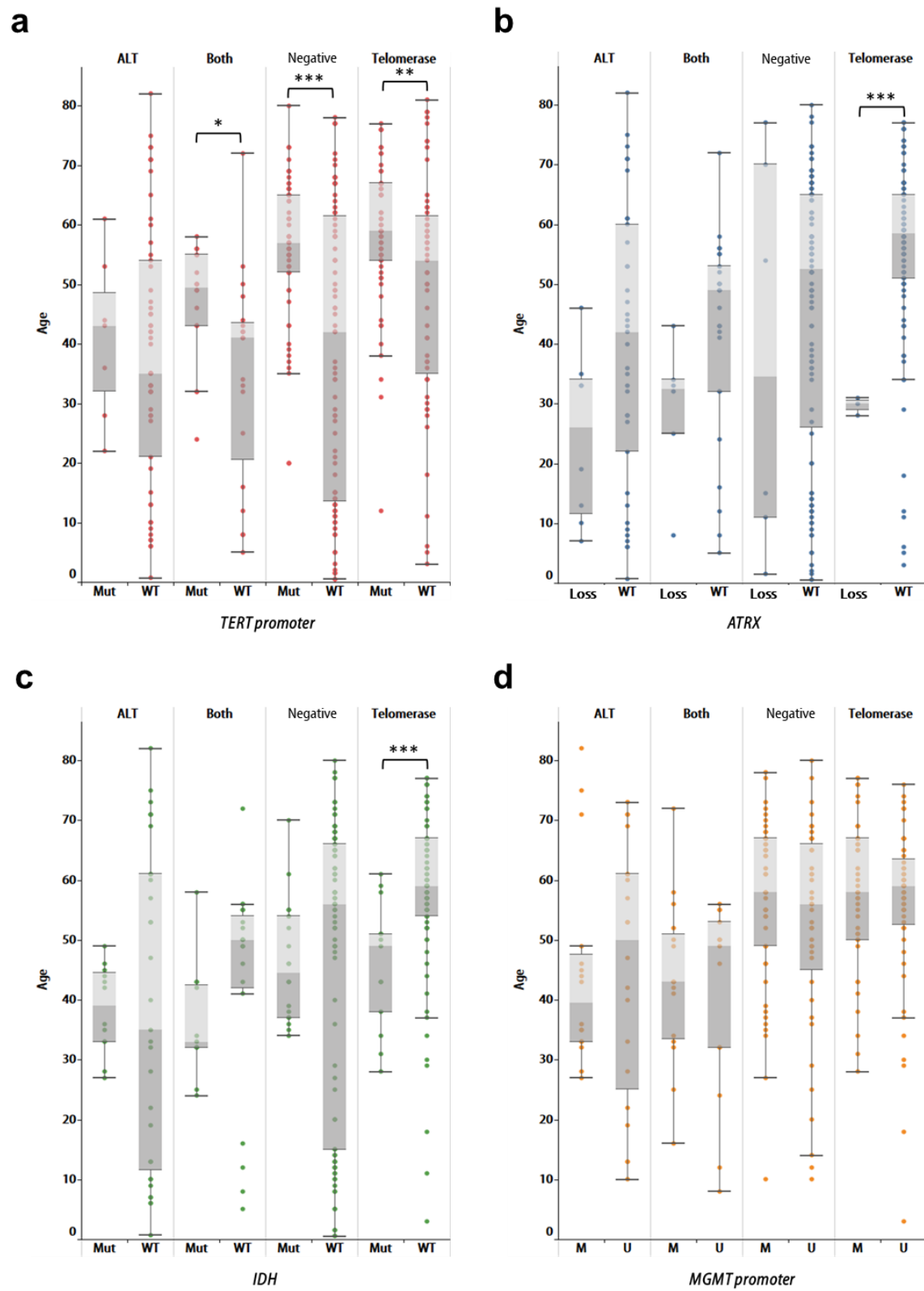


Figure S5 a. Relative telomerase activity (RTA) trends with age. More patients with detectable RTA and fewer patients with undetectable RTA are observed with increasing age. **b.** Age-dependent RTA distributions among TMM groups and WHO grades.

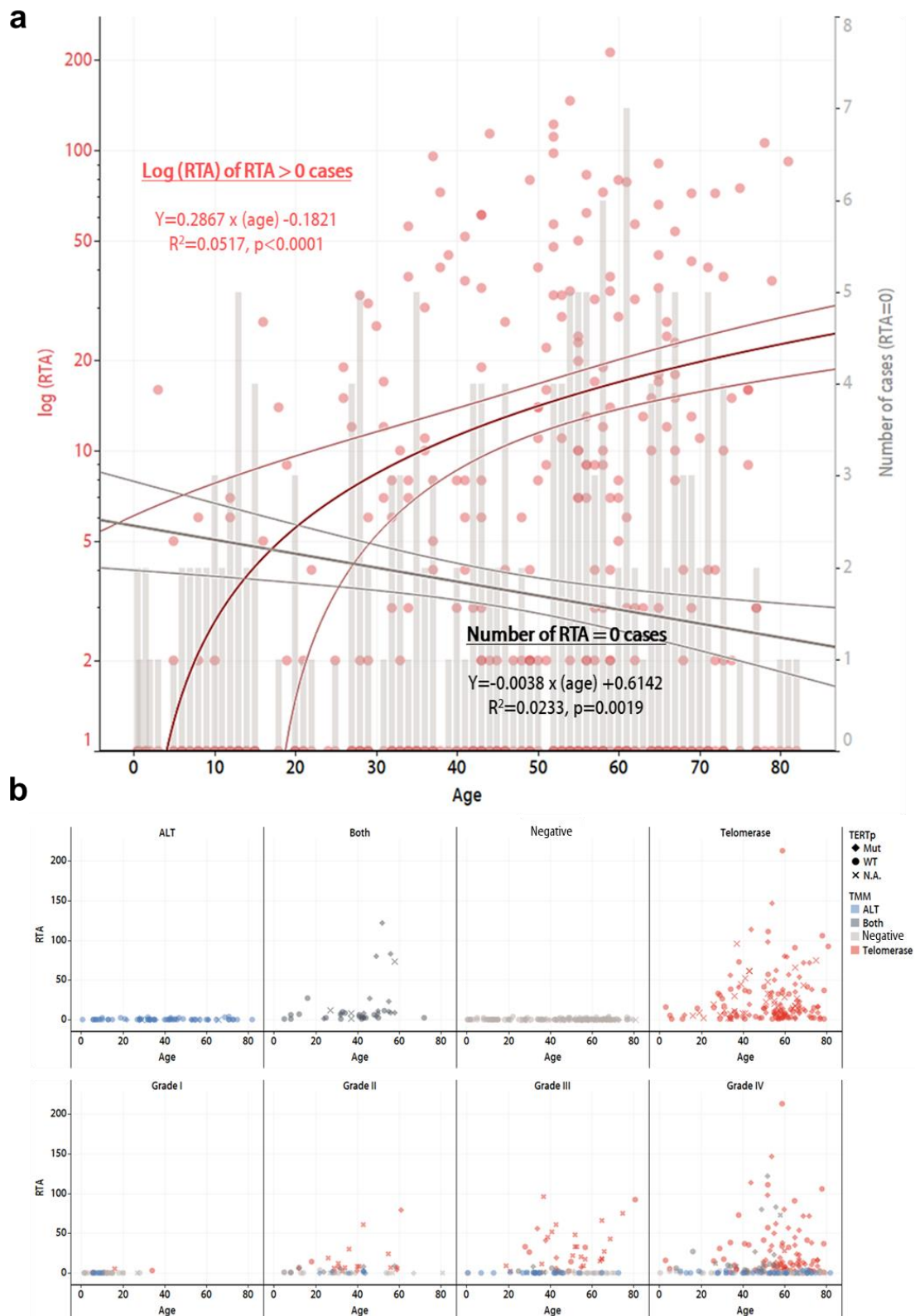
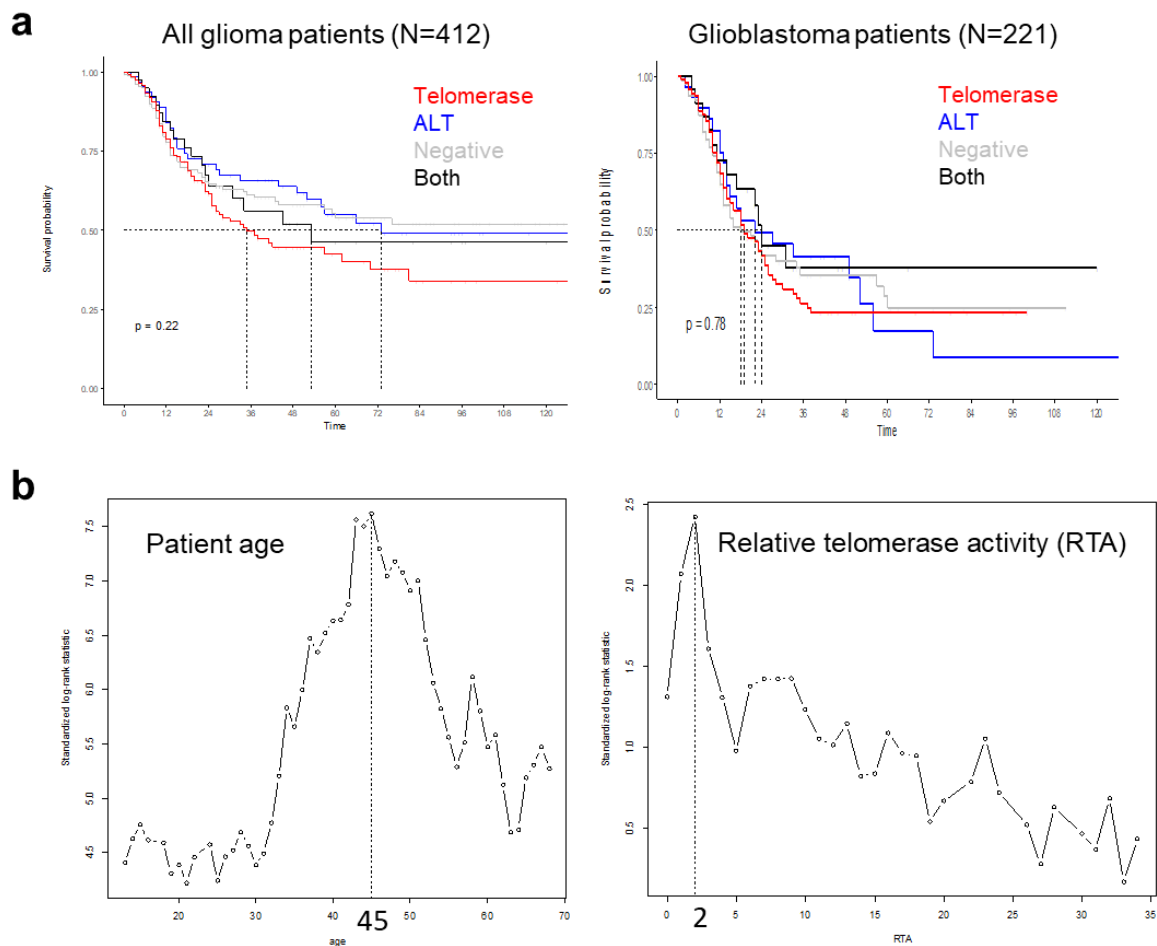
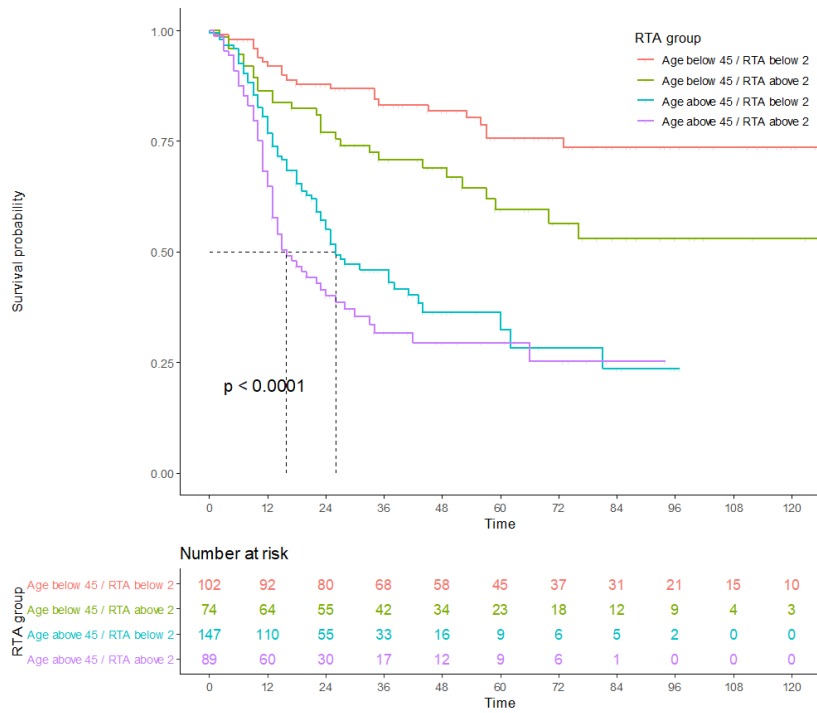


Figure S6 a. Kaplan-Meier curve of overall survival (OS) in all glioma patients stratified by TMM group ($p=0.22$). No significant differences in OS were observed among the TMM groups. **b.** Optimal cutoffs of age (age 45 years) and relative telomerase activity (RTA 2) for OS were determined by means of maximally selected log-rank statistics. **c.** The application of optimal age and RTA cutoffs for defining prognostic groups of glioma patients was confirmed by Kaplan-Meier analysis ($p<0.0001$). **d.** Cox proportional hazards model of TMM, age, RTA, grade and IDH mutation showing significant low risk in $RTA \leq 2$ and age below 45 groups.



c



d

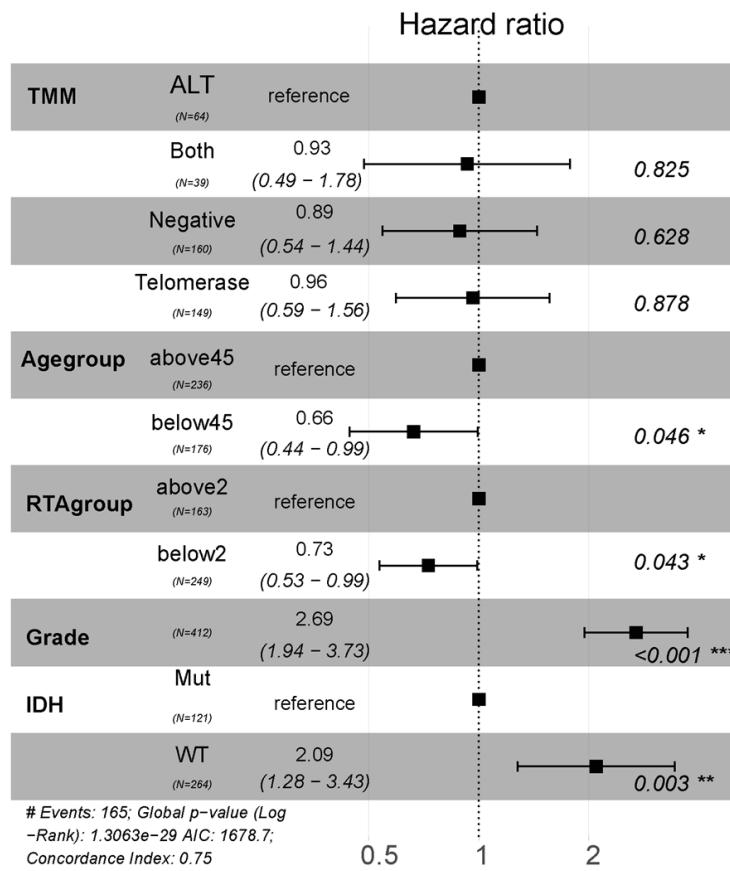
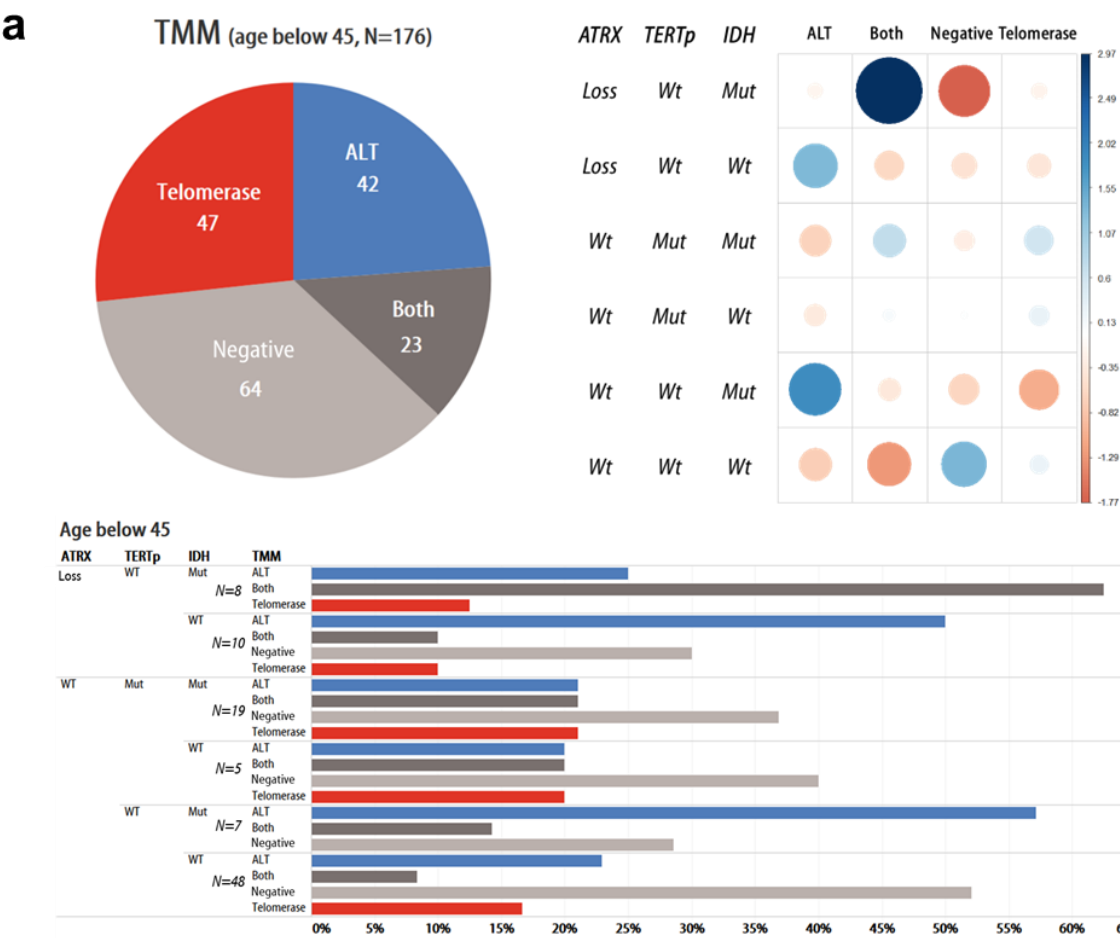


Figure S7 The distribution of TMM groups in age subsets, additionally stratified by major genetic signatures in glioma and their correlogram. The color intensity and size of the circle are proportional to the correlation coefficients. **a.** Subset of patients aged younger than 45 (Pearson residuals, chi-squared = 25.269, df = 15, p=0.04644). **b.** Subset of patients aged older than 45 (Pearson residuals, chi-squared = 28.18, df = 15, p=0.02047)



b

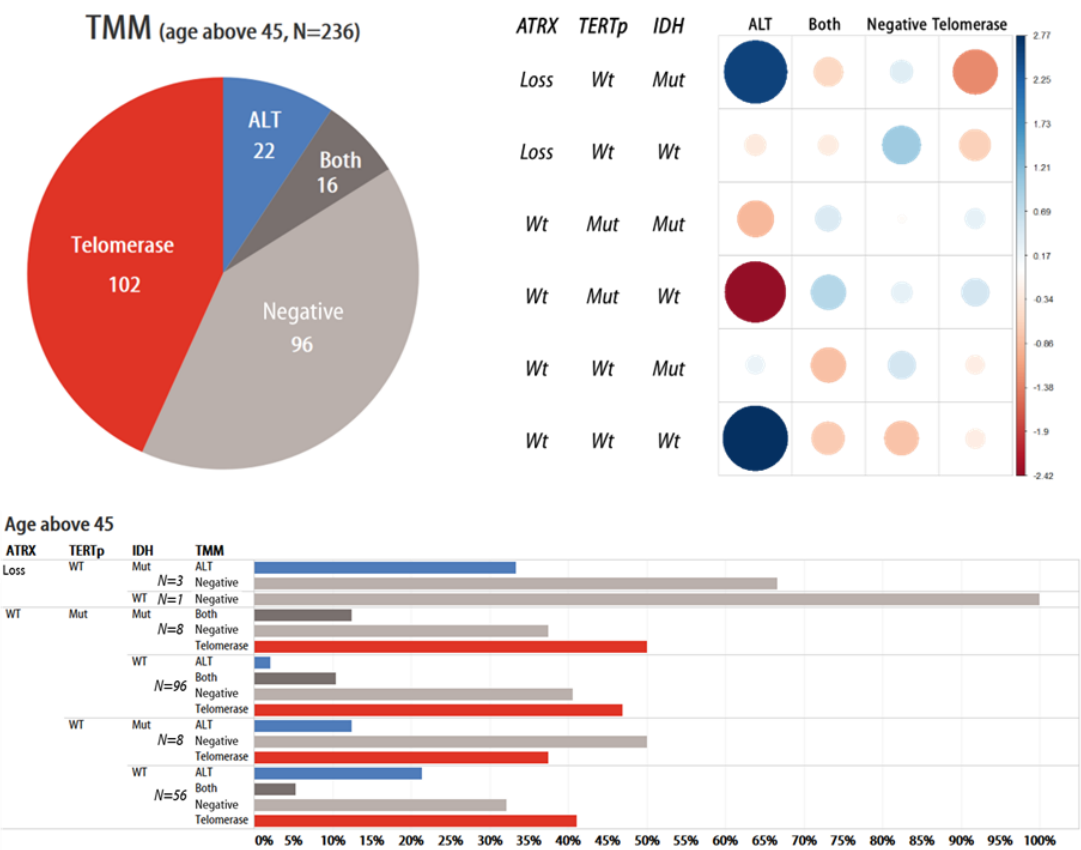


Figure S8 PCA plot showing three distinct clusters of 24 GBM samples according to 53 TMM-related genes. Clusters 1, 2 and 3 comprise Negative, Telomerase and Telomerase-like Negative, and ALT and ALT-like Negative samples, respectively

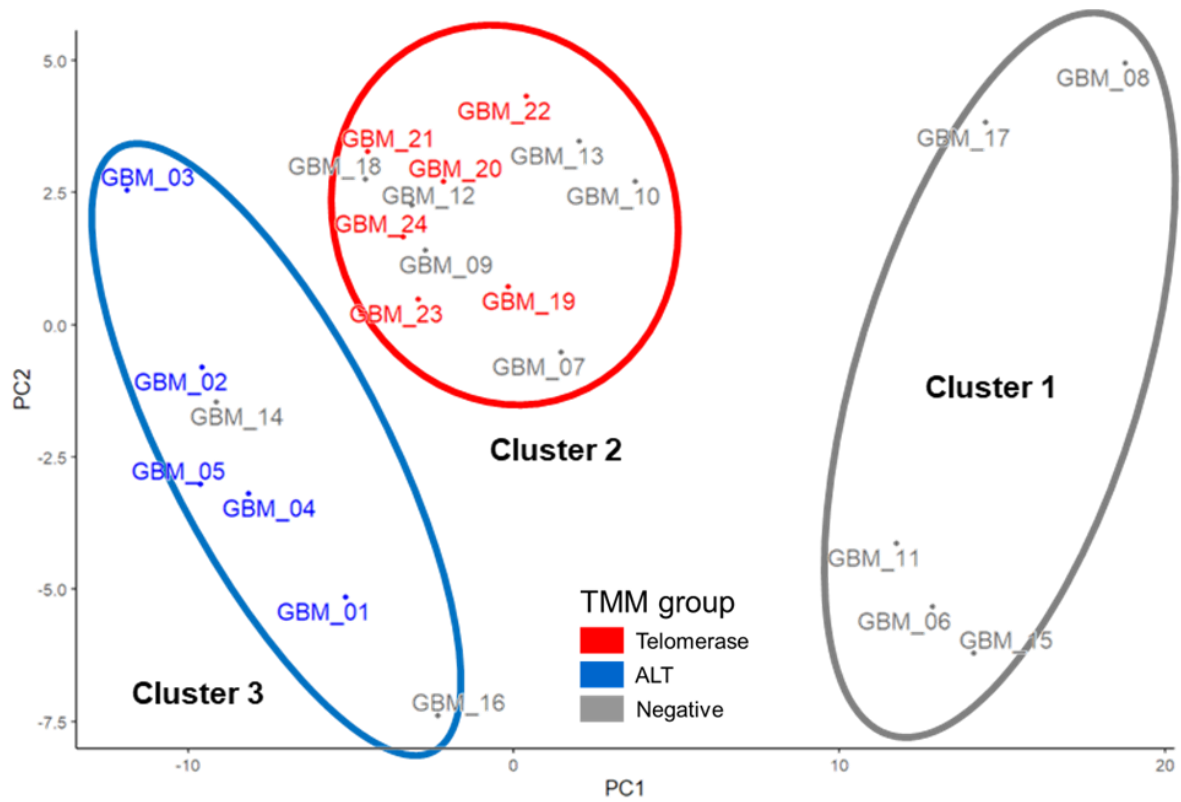
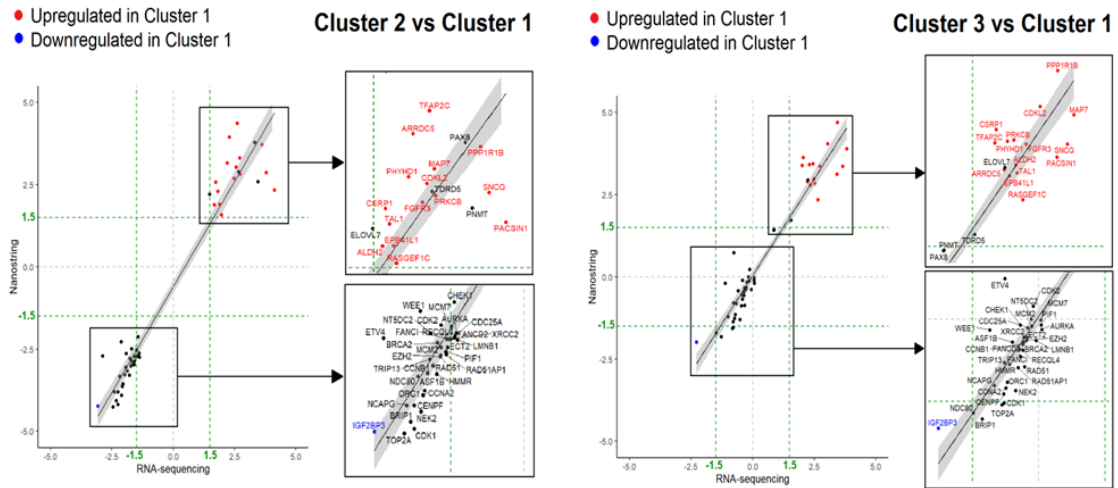


Figure S9 NanoString validation of Negative-specific genes. **a.** Correlation plot of RNA-seq data with NanoString data for Cluster 1 vs Clusters 2 and 3 showing 16 genes that were validated as Negative-specific. **b.** Box plots of NanoString expression data for 16 Negative-specific genes that were significantly differentially expressed in Cluster 1 compared with Clusters 2 and 3

a



b

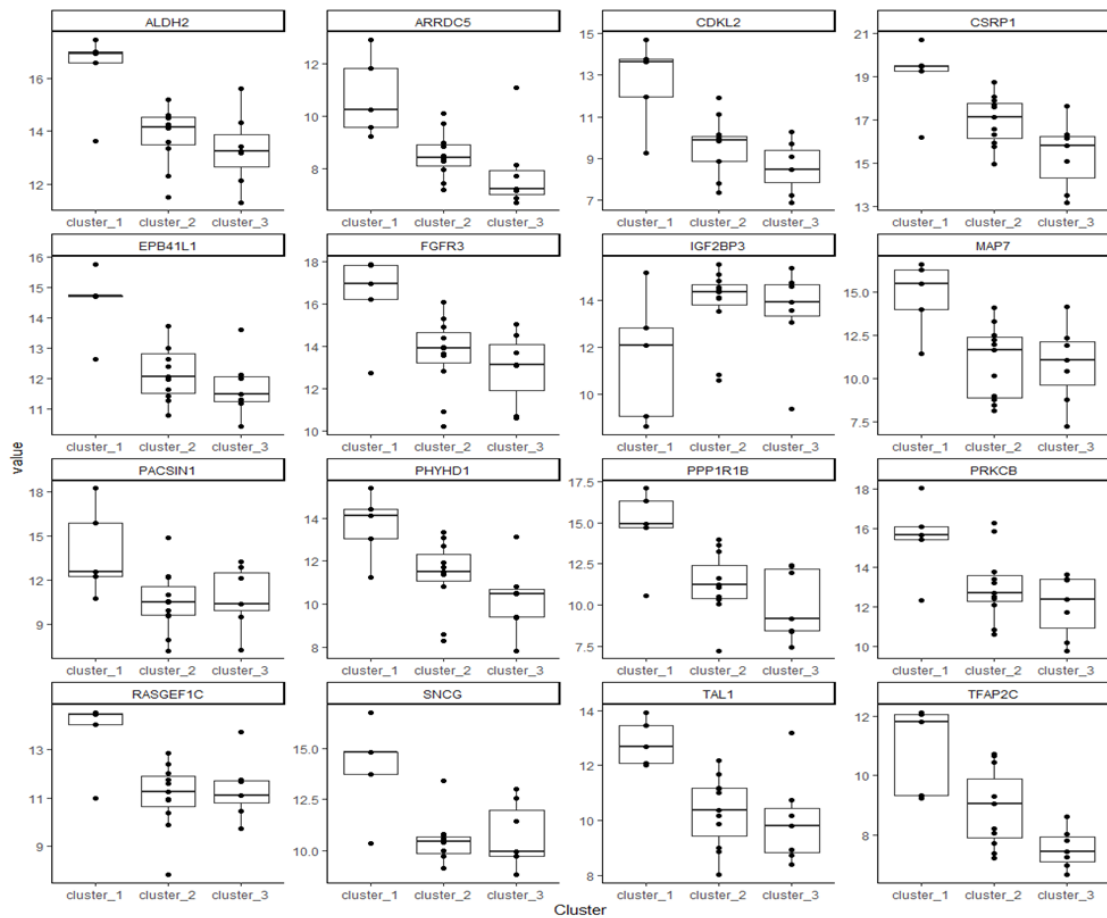


Figure S10 Detailed dot plot of the GSEA preranked analysis showing the gene set names in each significant functional group. Cell cycle- and cell division-related genes were downregulated in the Negative group compared to the Telomerase and ALT groups

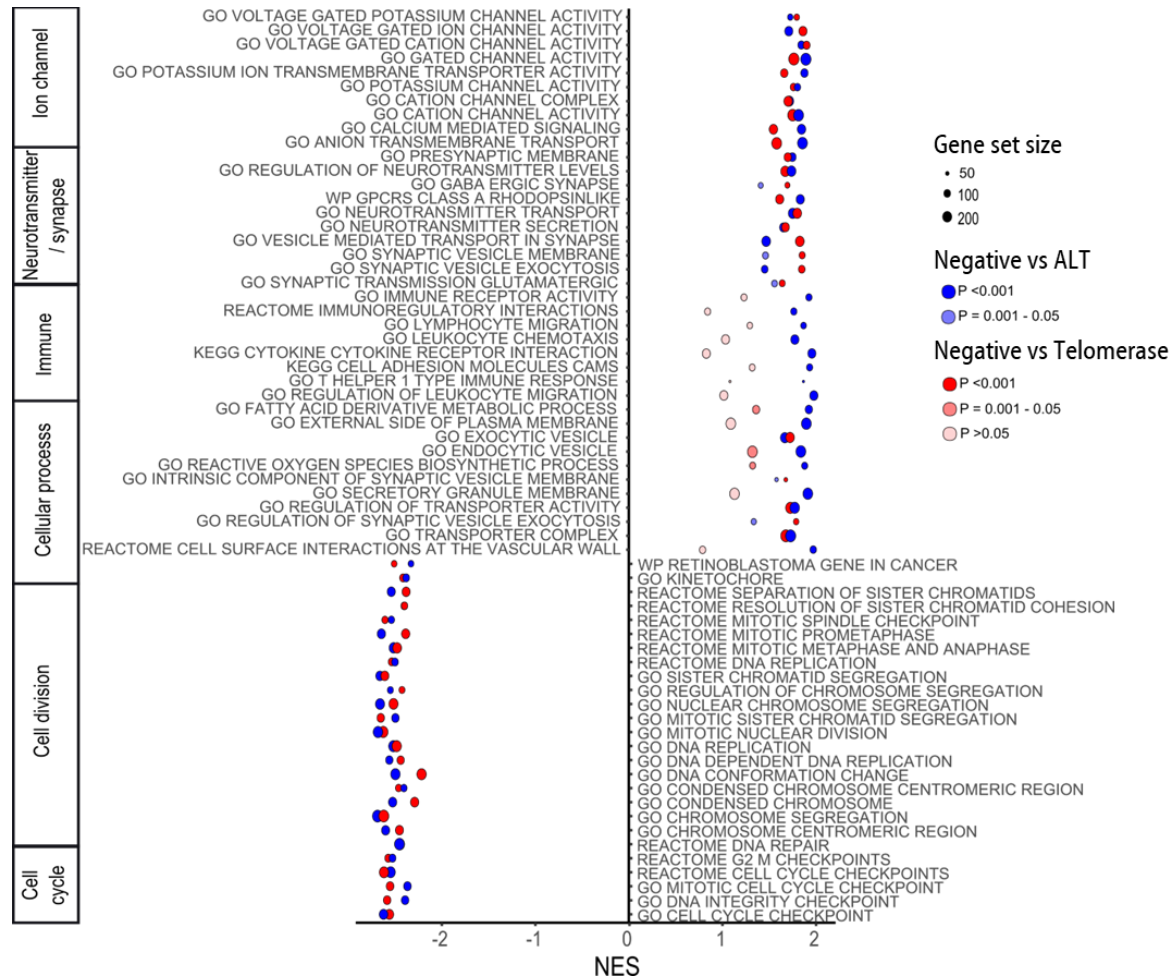


Figure S11 Comparison of the proliferation index (Ki-67) of glioblastoma tissue samples (n=221) among TMM groups (ANOVA with the Bonferroni correction T-test; * $p<0.05$, ** $p<0.01$). The Both group had a significantly higher Ki-67 index than the other groups

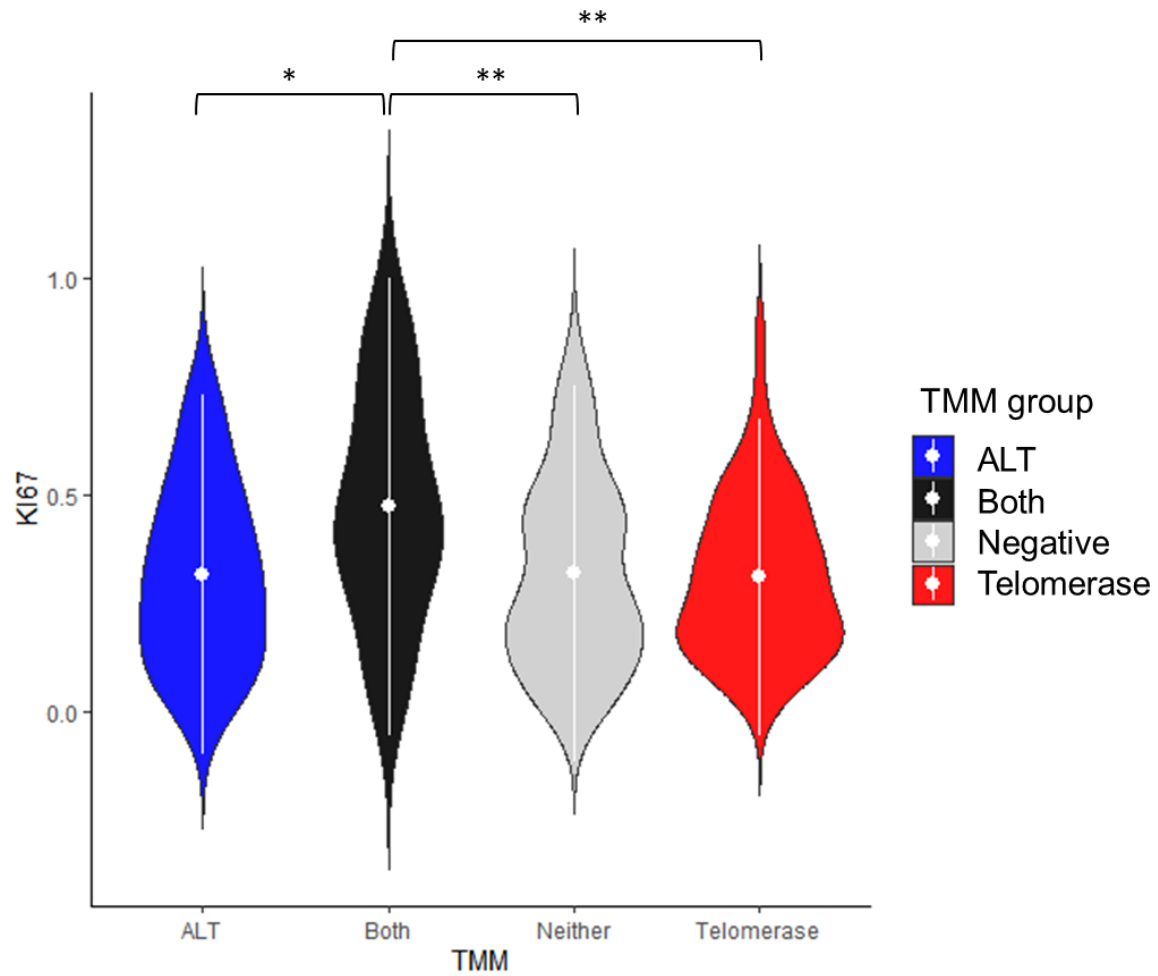


Figure S12 a. Summary of 64 glioma patients with longitudinal samples. Abbreviations indicate the histological diagnosis (O, oligodendroglioma; AO, anaplastic oligodendroglioma; A, astrocytoma; AA, anaplastic astrocytoma; GBM, glioblastoma; GS. i). **b.** Distribution of recurrence intervals between the initial tumor and first recurrence (interval 1) and between the first and second recurrences (interval 2)

a

Primary	Recurrent1	Recurrent2	Number of patients
O → O			2
O → AO			5
AO → AO	AO → (AO)		4 (1)
A → GBM			1
AA → AA	AA → (AA)		4 (1)
AA → GBM	GBM → (GBM)		6 (1)
GBM → GBM	GBM → (GBM)		40 (2)
GBM → GS			1
GS → GS			1

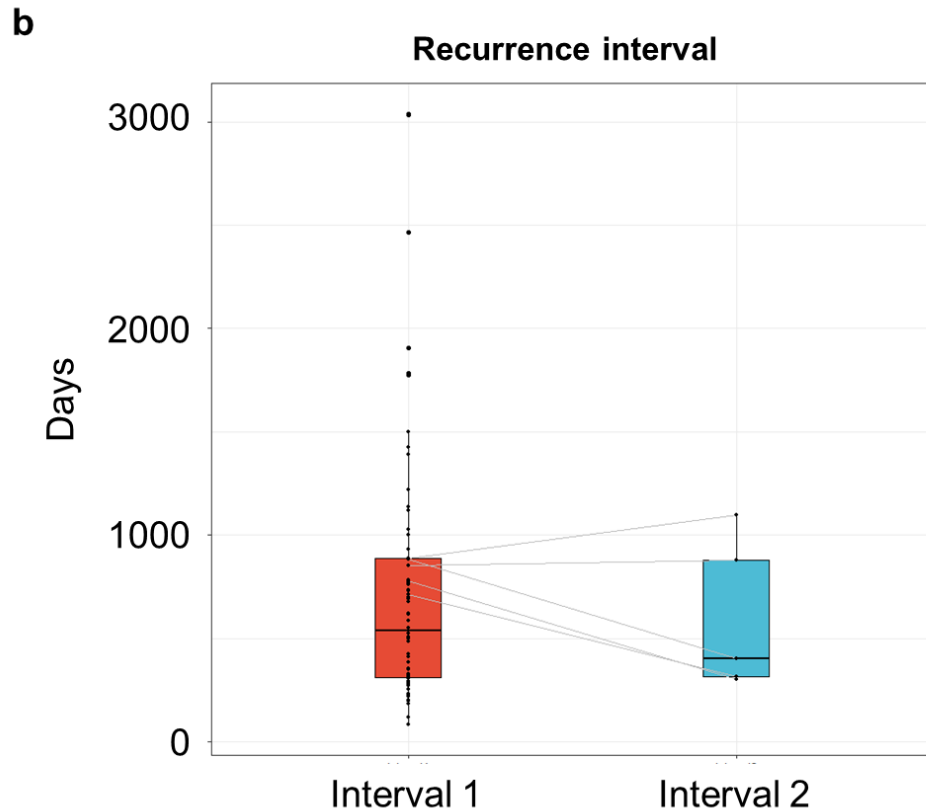


Figure S13 Distribution of patients with RTA changes according to the initial treatment protocol.

