Table S1 Two significantly survival-related methylation sites in training dataset.

| Probe ID | Chromosomal location | Gene symbol | CGI coordinate | Feature type | P value<sup>a</sup> | Coef.<sup>b</sup> | P value<sup>b</sup> |
|----------|----------------------|-------------|----------------|--------------|-----------------|----------------|----------------|
| cg003901 | chr12:1322659 53–132265954 | GALNT9 | chr12:1322659 2–132266198 | Island | 5.69E-13 | -6.733 | 2.16E-08 |
| cg195988 | chr13:1006739 38–100673939 | TMTC4 | chr13:10067407 5–100675469 | N_Shore | 1.36E-09 | -7.902 | 5.22E-08 |

<sup>a</sup> in univariate Cox regression analysis;  
<sup>b</sup> in multivariate Cox regression analysis;

Table S2 Results for Cox regression models of the two-CpG site signature and clinical factors as covariates.

| Variables | Univariate Cox model |  |  |  | Multivariate Cox model |  |  |  |
|-----------|----------------------|---|---|---|-------------------------|---|---|---|
|           | HR | 95% CI | P-value | HR | 95% CI | P-value |
| Two-CpG signature | 2.38 | 2.02-2.80 | <0.0001 | 2.354 | 1.97-2.81 | <0.0001 |
| Age       | 1.059 | 1.04-1.07 | <0.0001 | 1.053 | 1.04-1.07 | <0.0001 |
| Two-CpG signature | 2.38 | 2.02-2.80 | <0.0001 | 2.479 | 2.01-2.93 | <0.0001 |
| Gender    | 0.925 | 0.65-1.32 | 0.665 | 0.657 | 0.45-0.95 | 0.024 |
| Two-CpG signature | 2.38 | 2.02-2.80 | <0.0001 | 2.153 | 1.82-2.55 | <0.0001 |
| WHO grade | 3.277 | 2.22-4.83 | <0.0001 | 2.209 | 1.47-3.31 | <0.0001 |
| Two-CpG signature | 2.38 | 2.02-2.80 | <0.0001 | 2.373 | 2.01-2.79 | <0.0001 |
| Histologic subtype | 0.761 | 0.60-0.97 | 0.026 | 0.915 | 0.73-1.15 | 0.448 |
| Two-CpG signature | 2.38 | 2.02-2.80 | <0.0001 | 2.372 | 1.59-3.53 | <0.0001 |
| IDH status | 5.521 | 2.06-14.79 | 0.0006 | 4.108 | 1.49-11.33 | 0.006 |

Table S3 The ROC results of two-DNA methylation signature and other known biomarkers in the TCGA validation cohort.

| Signature         | AUC | 95% CI of AUC | P value<sup>a</sup> | Type     | P value<sup>b</sup> | Ref |
|-------------------|-----|---------------|---------------------|----------|---------------------|-----|
| Two-DNA methylation | 0.908 | 0.84–0.97 | 2.72E-09 | Methylation | This study |
| 21-mRNA           | 0.923 | 0.86–0.99 | 7.63E-10 | Protein coding | 0.624 | <sup>1</sup> |
| Seven-mRNA        | 0.716 | 0.59–0.85 | 1.66E-03 | Protein coding | 0.005 | <sup>2</sup> |
| Gene/AI   | Sensitivity | Specificity | $P$-value | Coding Type | $p$-value |
|----------|-------------|-------------|----------|-------------|-----------|
| Six-mRNA | 0.776       | 0.65–0.90   | 6.30E-05 | Protein     | 0.037     |
| Four-MRNA| 0.833       | 0.71–0.95   | 1.00E-06 | Protein     | 0.137     |
| Three mRNA | 0.826     | 0.70–0.94   | 2.00E-06 | Protein     | 0.122     |
| MGMT     | 0.507       | 0.37–0.64   | 0.924    | Protein     | < 0.001   |
| PD-1     | 0.726       | 0.60–0.85   | 1.03E-03 | Protein     | 0.005     |
| PTEN     | 0.695       | 0.62–0.77   | 3.00E-05 | Protein     | < 0.001   |
| NFKB     | 0.653       | 0.52–0.79   | 0.026    | Protein     | < 0.001   |
| SHOX2    | 0.789       | 0.67–0.91   | 2.70E-05 | Protein     | 0.043     |
| SERPINA5 | 0.841       | 0.75–0.93   | 7.20E-07 | Protein     | 0.125     |
| TIMP1    | 0.835       | 0.72–0.95   | 1.05E-06 | Protein     | 0.140     |
| NAMPT    | 0.838       | 0.74–0.94   | 8.95E-07 | Protein     | 0.121     |
| GRN      | 0.671       | 0.54–0.80   | 0.013    | Protein     | 0.001     |
| SERPINE1 | 0.779       | 0.67–0.89   | 5.00E-05 | Protein     | 0.025     |
| six-CpG signature | 0.947 | 0.88–1     | 7.01E-11 | Methylation | 0.802 |
| MGMT     | 0.822       | 0.72–0.92   | 3.00E-06 | Methylation | 0.078     |
| NDRG2    | 0.854       | 0.75–0.96   | 2.39E-07 | Methylation | 0.194     |
| PTEN     | 0.805       | 0.67–0.93   | 9.00E-06 | Methylation | 0.079     |
| PD-1     | 0.814       | 0.69–0.94   | 5.00E-05 | Methylation | 0.090     |
| cg12434587 | 0.63     | 0.47–0.78   | 0.058    | Methylation | 0.001     |
| cg12981137 | 0.695    | 0.56–0.83   | 0.004    | Methylation | 0.003     |
| cg27151711 | 0.799    | 0.66–0.93   | 1.30E-05 | Methylation | 0.077     |
| cg16523424 | 0.834    | 0.72–0.95   | 1.00E-06 | Methylation | 0.137     |
| cg04791822 | 0.780    | 0.64–0.92   | 4.40E-05 | Methylation | 0.053     |
| cg15509705 | 0.819    | 0.70–0.94   | 3.00E-06 | Methylation | 0.097     |
| Gender   | 0.504       | 0.37–0.64   | 9.58E-01 | Clinical    | < 0.001   |
| Age      | 0.835       | 0.73–0.94   | 1.00E-06 | Clinical    | 0.005     |
| Grade    | 0.607       | 0.48–0.74   | 0.118    | Clinical    | < 0.001   |
| IDH1     | 0.809       | 0.56–1.00   | 5.40E-02 | Clinical    | 0.225     |
| Subtype  | 0.554       | 0.41–0.70   | 4.68E-01 | Clinical    | < 0.001   |
| Radiation therapy | 0.581 | 0.44–0.72   | 2.76E-01 | Clinical    | < 0.001   |
| Family history of cancer | 0.521 | 0.35–0.70   | 8.11E-01 | Clinical    | < 0.001   |

* a. in ROC analysis;
* b. in the statistical comparison (Z-test) between AUC value of corresponding signature and the two-DNA methylation signature.
Table S4: The ROC results of two-DNA methylation signature and other known biomarkers in the GSE104293 validation cohort.

| Signature           | AUC   | 95% CI of AUC | P value\(^a\) | Type                | P value\(^b\) | Ref |
|---------------------|-------|---------------|----------------|---------------------|----------------|-----|
| Two-DNA methylation | 0.736 | 0.62–0.85     | 1.81E-04       | Methylation         |                | This study |
| \textit{six-CpG signature} | 0.686 | 0.56–0.81     | 0.064          | Methylation         | 0.284          | 13   |
| MGMT                | 0.634 | 0.50–0.76     | 0.042          | Methylation         | 0.111          | 6    |
| NDRG2               | 0.719 | 0.60–0.83     | 0.001          | Methylation         | 0.145          | 14   |
| PTEN                | 0.53  | 0.38–0.67     | 0.649          | Methylation         | 0.137          | 15   |
| PDCD1               | 0.589 | 0.45–0.72     | 0.176          | Methylation         | 0.270          | 7    |
| cg12434587          | 0.629 | 0.50–0.75     | 0.049          | Methylation         | 0.031          | 16   |
| cg12981137          | 0.641 | 0.51–0.77     | 0.031          | Methylation         | 0.280          | 16   |
| cg27151711          | 0.63  | 0.48–0.78     | 0.048          | Methylation         | 0.130          | 11   |
| cg16523424          | 0.68  | 0.54–0.81     | 0.006          | Methylation         | 0.419          | 11   |
| cg04791822          | 0.563 | 0.42–0.70     | 0.339          | Methylation         | 0.015          | 11   |
| cg15509705          | 0.681 | 0.54–0.82     | 0.006          | Methylation         | 0.054          | 11   |
| Gender              | 0.542 | 0.42–0.66     | 0.485          | Clinical factor     | 0.011          |     |
| Age                 | 0.529 | 0.40–0.65     | 0.629          | Clinical factor     | 0.009          |     |
| Radiation therapy   | 0.558 | 0.44–0.68     | 0.34           | Clinical factor     | 0.018          |     |
| MGMT status         | 0.551 | 0.43–0.67     | 0.401          | Clinical factor     | 0.017          |     |

\(^a\) in ROC analysis;  
\(^b\) in the statistical comparison (Z-test) between AUC value of corresponding signature and the two-DNA methylation signature.
Figure S1. Workflow for the construction and validation of the DNA methylation prognostic signature.
Figure S2. Kaplan–Meier and ROC analyses of LGG patients in different sex groups. (A) Kaplan–Meier estimates of the patients’ OS for low- and high-risk patient, and the OS differences between two groups were determined by Log-rank test; (B) ROC curves show the sensitivity and specificity of the signature in predicting the OS of patients.
Figure S3. Kaplan–Meier and ROC analyses of LGG patients with different WHO grades. (A) Kaplan–Meier analysis with Log-rank test was performed to estimate the differences in OS between the low- and high-risk patients. (B) ROC curves of the signature were used to demonstrate the sensitivity and specificity in predicting the OS of patients.
Figure S4. Kaplan–Meier and ROC analyses of LGG patients with different histologic. (A) Kaplan–Meier estimates of the patients’ OS for low- and high-risk patient in different stage cohorts, and the OS differences between two groups were determined by Log-rank test; (B) ROC curves show the sensitivity and specificity of the signature in predicting the OS of patients.
Figure S5. Kaplan–Meier and ROC analyses of LGG patients with IDH1 mutation and wild-type. (A) Kaplan–Meier analysis with Log-rank was performed to estimate the differences in OS between the low- and high-risk patients. (B) ROC curves of the signature were used to demonstrate the sensitivity and specificity in predicting the OS of patients.
Figure S6. Kaplan–Meier and ROC analyses of LGG patients received adjuvant radiation therapy or not, respectively. (A) Kaplan–Meier analysis with Log-rank test was performed to estimate the differences in OS between the low- and high-risk patients. (B) ROC curves of the signature were used to demonstrate the sensitivity and specificity in predicting the OS of patients.
Figure S7. Kaplan–Meier and ROC analyses of LGG patients have family history of cancer. (A) Kaplan–Meier analysis with Log-rank was performed to estimate the differences in OS between the low- and high-risk patients. (B) ROC curves of the signature were used to demonstrate the sensitivity and specificity in predicting the OS of patients.
Figure S8. Correlation between two-DNA methylation risk score and ICB immunotherapy-related signature.

Figure S9. (A) KEGG and (B) Reactome pathway enrichment analysis result for genes that interacted with two genes in tour signature. The numbers of genes were represented by the length of the bars or the size of the dots, and the color of the bars/dots corresponds to the p-value according to legend.
Figure S10. (A-B) Correlation between the expression of the genes and their methylation levels was evaluated for each gene through the Pearson’s correlation test. Reported P values are two sided. (C-D) The expression of genes in pan-cancer.
Figure S11. Correlation between the expression of biomarkers from the two-DNA methylation prognostic biomarker and immune cell infiltration level in LGG.

Figure S12. (A) Kaplan–Meier analysis with Log-rank was performed to estimate the differences in OS between the G-CIMP + and G-CIMP- patients. Patients with G-CIMP+ had a favorable prognosis. (B) The violin plot of two-CpG site signature risk scores in G-CIMP + and G-CIMP- patients. Mann-Whitney U test was used to estimate the differences.
Figure S13. Kaplan–Meier and ROC analyses of individual DNA methylation in the training cohort. (A) Kaplan–Meier analysis with Log-rank was performed to estimate the differences in OS between the low- and high-risk patients. (B) ROC curves of the individual methylation signature were used to demonstrate the sensitivity and specificity in predicting the OS of patients.
Figure S14. Kaplan–Meier and ROC analyses of individual DNA methylation in the TCGA validation cohort. (A) Kaplan–Meier analysis with Log-rank test was performed to estimate the differences in OS between the low- and high-risk patients. (B) ROC curves of the individual methylation signature were used to demonstrate the sensitivity and specificity in predicting the OS of patients.
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