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## Supplemental Information

# Comprehensive analysis to identify DLEU2L/TAOK1 axis as a prognostic biomarker in hepatocellular carcinoma

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**Table. S1. Immunohistochemistry of PTEN in Human Protein Atlas database.**

| No. | Tissue type | ID | Age | Gender | Staining |
|---|---|---|---|---|---|
| 1 | normal | 1720 | 67 | male | low |
| 2 | normal | 3402 | 54 | female | low |
| 3 | tumor | 879 | 70 | male | not detected |
| 4 | tumor | 2766 | 73 | female | not detected |

## Table. S2. Univariate analysis of overall survival in HCC patients stratified based on clinical characteristics.

| Factor | Variable | N | DLEU2L Expression (Median) | P value | TAOK1 Expression (Median) | P value | Overall survival | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Months (Median) | 95% CI (mean) | P value (Log-rank test) |
| Age | ≥60 | 200 | 0.012 | 0.863 | 1.907 | 0.463 | 52.63 | 58.00-74.86 | 0.272 |
| | <60 | 169 | 0.012 | | 2.019 | | 46.33 | 58.20-76.71 | |
| Gender | Male | 248 | 0.011 | 0.046 | 1.866 | 0.01 | 47.67 | 58.00-72.60 | 0.257 |
| | Female | 121 | 0.014 | | 2.15 | | 52.67 | 58.48-81.88 | |
| Diameter | >5 | 94 | 0.018 | **0.031** | 2.072 | **0.029** | 34.33 | 43.98-67.49 | **<0.0001** |
| | ≤5 | 272 | 0.011 | | 1.907 | | 52.54 | 63.64-78.29 | |
| Lymph-node metastasis | Negative | 251 | 0.011 | 0.161 | 2.019 | 0.004 | 48.92 | 64.09-80.31 | 0.071 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Positive | 4 | 0.044 | | 3.5 | | 54.38 | -133.6 | |
| | Unknown | 113 | 0.013 | | 1.807 | | 49.67 | 46.57-64.47 | |
| Distant metastasis | Negative | 265 | 0.011 | 0.159 | 1.999 | 0.742 | 48.75 | 62.61-78.37 | 0.003 |
| | Positive | 4 | 0.003 | | 2.107 | | 2.257 | -38.27 | |
| | Unknown | 100 | 0.013 | | 1.887 | | 50.96 | 49.42-67.85 | |
| TNM stage | I–II | 255 | 0.011 | **0.048** | 1.887 | **0.032** | 50.67 | 63.71-79.07 | **<0.0001** |
| | III–IV | 90 | 0.019 | | 2.082 | | 34.17 | 43.61-68.27 | |
| | Unknown | 24 | 0.012 | | 2.037 | | 61.29 | 42.62-77.94 | |
| BMI | <18.5 | 21 | 0.015 | 0.893 | 1.955 | 0.653 | 48.92 | 35.11-108.1 | 0.093 |
| | 18.5-23.9 | 136 | 0.011 | | 2.068 | | 39.04 | 47.95-66.00 | |
| | 24-27.9 | 82 | 0.011 | | 1.889 | | 53.63 | 65.82-95.92 | |
| | ≥28 | 94 | 0.012 | | 1.83 | | 59.25 | 62.70-86.87 | |

**Table. S3. Univariate and Multivariate analysis (Cox regression model) of DLEU2L in HCC patients.**

| Factor | Univariate Cox | | | Munivariate Cox | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | P value (Log-rank test) | HR | 95% CI | P value (Log-rank test) |
| Age | 1.216 | 0.857-1.725 | 0.273 | | | |
| Gender | 1.227 | 0.861-1.748 | 0.258 | | | |
| TNM stage | 1.791 | 1.415-2.269 | 0 | | | |
| Diameter | 2.208 | 1.605-3.037 | 0 | 2.14 | 1.553-2.949 | 0 |
| Lymph-node metastasis | 1.231 | 1.025-1.479 | 0.026 | | | |
| Distant metastasis | 1.273 | 1.058-1.531 | 0.01 | 1.276 | 1.060-1.536 | 0.01 |
| BMI | 1.034 | 0.880-1.216 | 0.683 | | | |
| DLEU2L expression (high/low) | 1.572 | 1.110-2.226 | 0.011 | 1.369 | 0.965-1.943 | 0.078 |

**Table. S4. Univariate and Multivariate analysis (Cox regression model) of TAOK1 in HCC patients.**

| Factor | Univariate Cox | | | Munivariate Cox | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | P value (Log-rank test) | HR | 95% CI | P value (Log-rank test) |
| Age | 1.216 | 0.857-1.725 | 0.273 | | | |
| Gender | 1.227 | 0.861-1.748 | 0.258 | | | |
| TNM stage | 1.791 | 1.415-2.269 | 0 | | | |
| Diameter | 2.208 | 1.605-3.037 | 0 | 2.245 | 1.634-3.085 | 0 |
| Lymph-node metastasis | 1.231 | 1.025-1.479 | 0.026 | | | |
| Distant metastasis | 1.273 | 1.058-1.531 | 0.01 | 1.325 | 1.099-1.597 | 0.003 |
| BMI | 1.034 | 0.880-1.216 | 0.683 | | | |
| DLEU2L expression (high/low) | 1.537 | 1.085-2.177 | 0.016 | 1.622 | 1.141-2.306 | 0.007 |

## Table. S5. Correlation analysis betweenTAOK1 and biomarkers of Dendritic cell, Th1, Th2, Th17, and Treg in HCC in GEPIA.

| **Description** | Gene markers | LIHC | |
|---|---|---|---|
| | | Cor | P value |
| Dendritic cell | BDCA-4 (NRP1) | 0.51 | 0 |
| Th1 | STAT1 | 0.34 | 2e-13 |
| Th2 | STAT6 | 0.47 | 0 |
| Th17 | STAT3 | 0.63 | 0 |
| Treg | STAT5B | 0.65 | 0 |

## Table. S6. Correlation analysis between TAOK1 and biomarkers of immune cells using TIMER.

| **Description** | Gene markers | LIHC | |
|---|---|---|---|
| | | Cor | Pvalue |
| CD8+ T cell | CD8A | -0.059 | 0.607 |
| | CD8B | -0.123 | 0.279 |
| T cell (general) | CD3D | -0.074 | 0.518 |
| | CD3E | -0.169 | 0.137 |
| | CD2 | -0.202 | 0.075 |
| B cell | CD19 | 0.027 | 0.815 |
| | CD79A | -0.16 | 0.158 |
| Monocyte | CD86 | -0.022 | 0.845 |
| | CD115 (CSF1R) | -0.128 | 0.261 |
| TAM | CCL2 | -0.06 | 0.601 |
| | CD68 | 0.041 | 0.716 |
| | IL10 | -0.009 | 0.938 |
| M1 Macrophage | INOS (NOS2) | 0.144 | 0.204 |
| | IRF5 | 0.118 | 0.298 |
| | COX2 (PTGS2) | 0.213 | 0.059 |
| M2 Macrophage | CD163 | 0.119 | 0.295 |
| | VSIG4 | -0.051 | 0.655 |
| | MS4A4A | -0.062 | 0.587 |
| Neutrophils | CD66b (CEACAM8) | -0.247 | **0.028** |
| | CD11b (ITGAM) | -0.031 | 0.783 |

| | | | |
|---|---|---|---|
| | CCR7 | 0.022 | 0.849 |
| Natural killer cell | KIR2DL1 | -0.08 | 0.483 |
| | KIR2DL3 | -0.147 | 0.195 |
| | KIR2DL4 | -0.179 | 0.114 |
| | KIR3DL1 | 0.026 | 0.819 |
| | KIR3DL2 | -0.228 | **0.043** |
| | KIR3DL3 | -0.152 | 0.181 |
| | KIR2DS4 | 0.018 | 0.872 |
| Dendritic cell | HLA-DPB1 | -0.044 | 0.697 |
| | HLA-DQB1 | -0.076 | 0.505 |
| | HLA-DRA | -0.044 | 0.698 |
| | HLA-DPA1 | -0.076 | 0.507 |
| | BDCA-1 (CD1C) | 0.02 | 0.863 |
| | **BDCA-4 (NRP1)** | 0.449 | ******* |
| | CD11c (ITGAX) | 0.111 | 0.328 |

| | | | |
|---|---|---|---|
| Th1 | T-bet (TBX21) | -0.103 | 0.366 |
| | STAT4 | 0.059 | 0.603 |
| | **STAT1** | 0.405 | ** |
| | IFN-γ (IFNG) | 0.042 | 0.714 |
| | TNF-α (TNF) | -0.12 | 0.294 |
| Th2 | GATA3 | 0.14 | 0.217 |
| | **STAT6** | 0.498 | *** |
| | STAT5A | 0.266 | **0.018** |
| | IL13 | 0.193 | 0.089 |
| Tfh | BCL6 | 0.373 | ** |
| | IL21 | NA | NA |
| Th17 | **STAT3** | 0.594 | *** |
| | IL17A | 0.106 | 0.35 |
| Treg | FOXP3 | 0.118 | 0.298 |
| | CCR8 | 0.256 | **0.023** |
| | **STAT5B** | 0.461 | *** |
| | TGFβ (TGFB1) | 0.145 | 0.202 |
| T cell exhaustion | PD-1 (PDCD1) | -0.127 | 0.267 |
| | CTLA4 | -0.069 | 0.547 |
| | LAG3 | -0.089 | 0.436 |
| | TIM-3 (HAVCR2) | 0.044 | 0.699 |
| | GZMB | -0.339 | * |

*P<0.01, ** P<0.001, *** P<0.0001.

## Supplemental figure and legends.

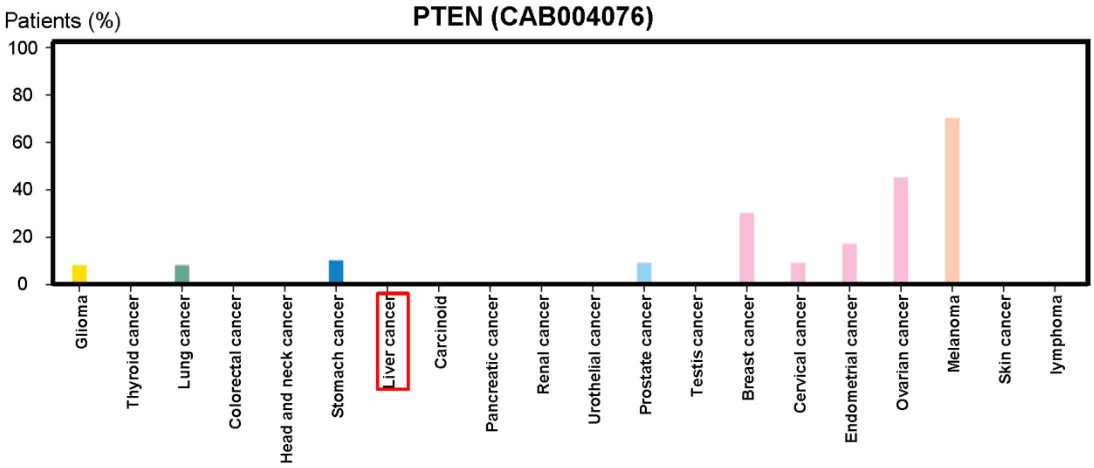

**Fig. S1. Expression distribution expression of PTEN in various normal tissues.**

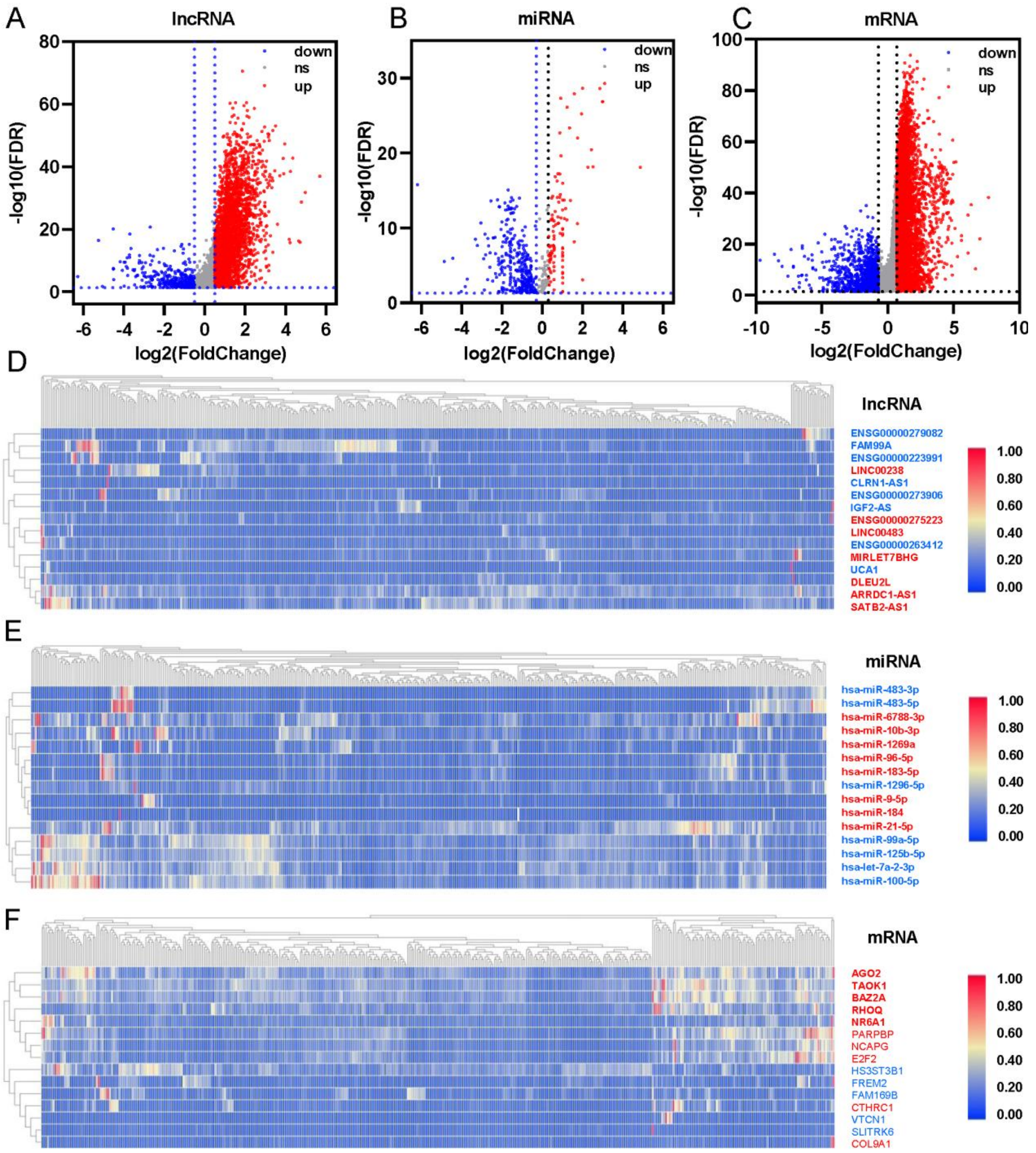

**Fig. S2. Volcano plots and heatmap plots of DElncRNAs, DEmiRNAs, and DEmRNAs between HCC samples and adjacent nontumor samples.** Red indicates upregulated genes, and blue indicates downregulated genes. The volcano plots described (A) 3371DElncRNAs (| log2Fold- Change| > 0.5 and adjusted p value < 0.05), (B) 422DEmiRNAs (|log2FoldChange|>0.3 and adjusted p value < 0.05), and (C) 8294DEmRNAs (| log2FoldChange| > 0.7 and adjusted p value < 0.05). (D-F) The horizontal axis of the heatmap denotes the samples, and the vertical axis of heatmap denotes 15 significant DEGs.

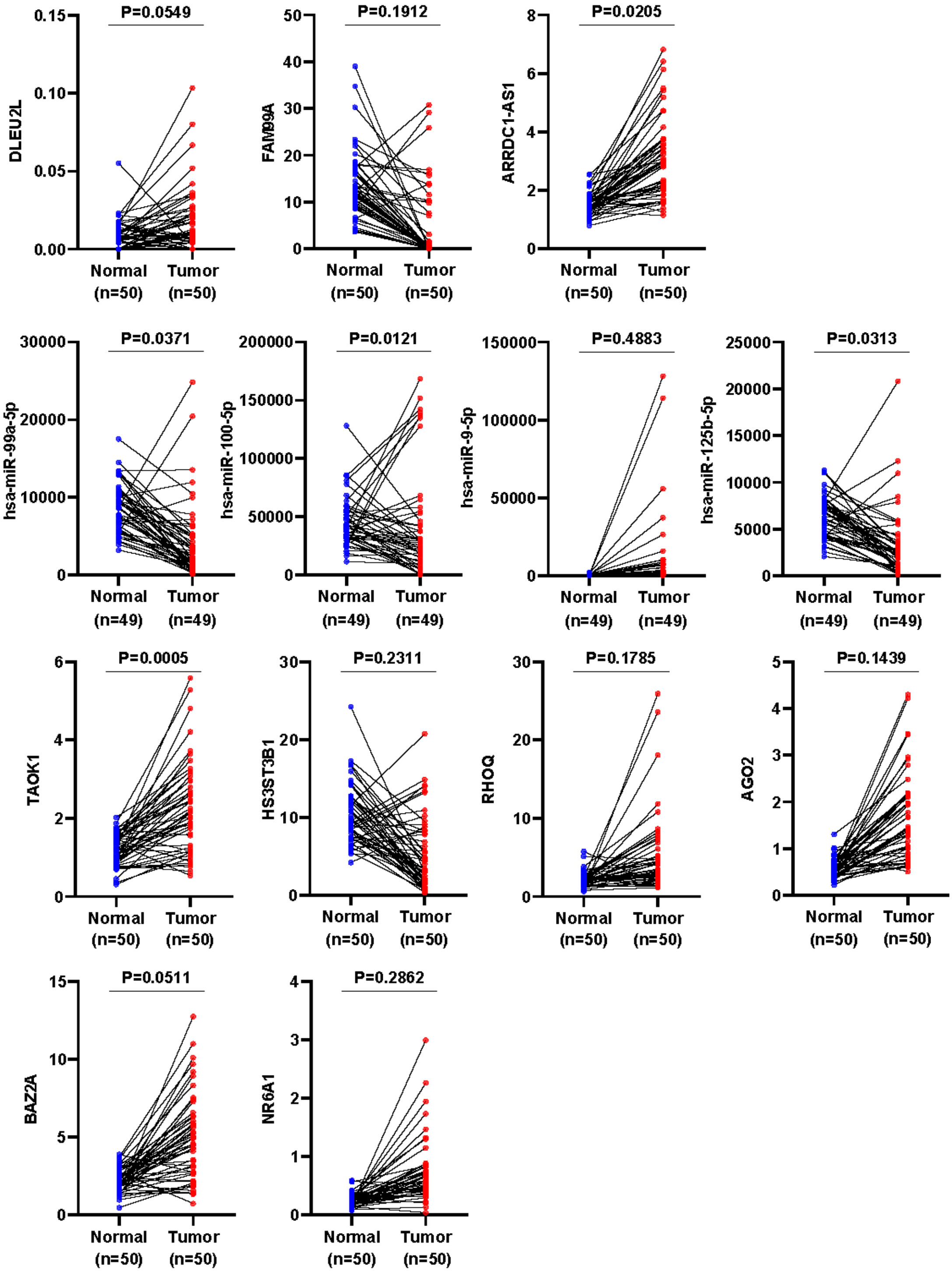

**Fig. S3. The distribution of 13 hub-RNAs expression value from the triple regulatory network in 50 paired HCC tissues.**

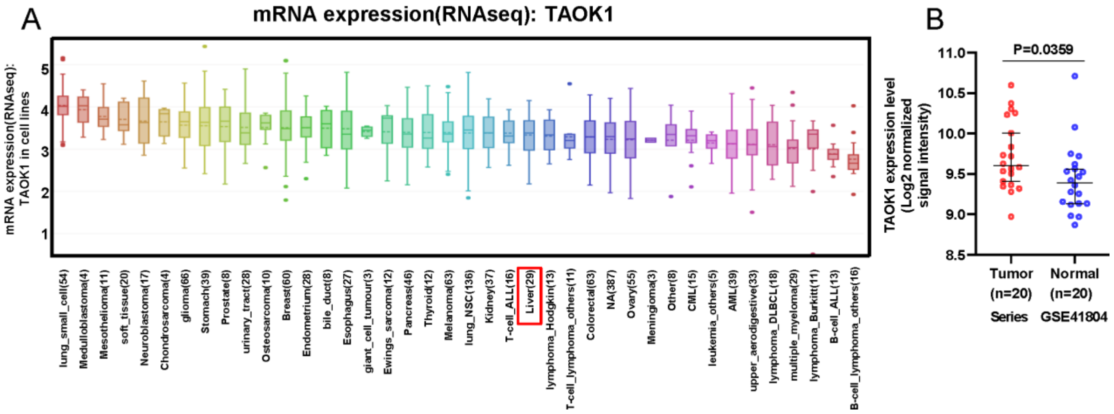

**Fig. S4. Verify the expression of TAOK1.** (A) Expression distribution of TAOK1 in pan-cancer cell lines. (B)The expression level of TAOK1 in the GSE41804 HCC cohort of 20 paired HCC samples.

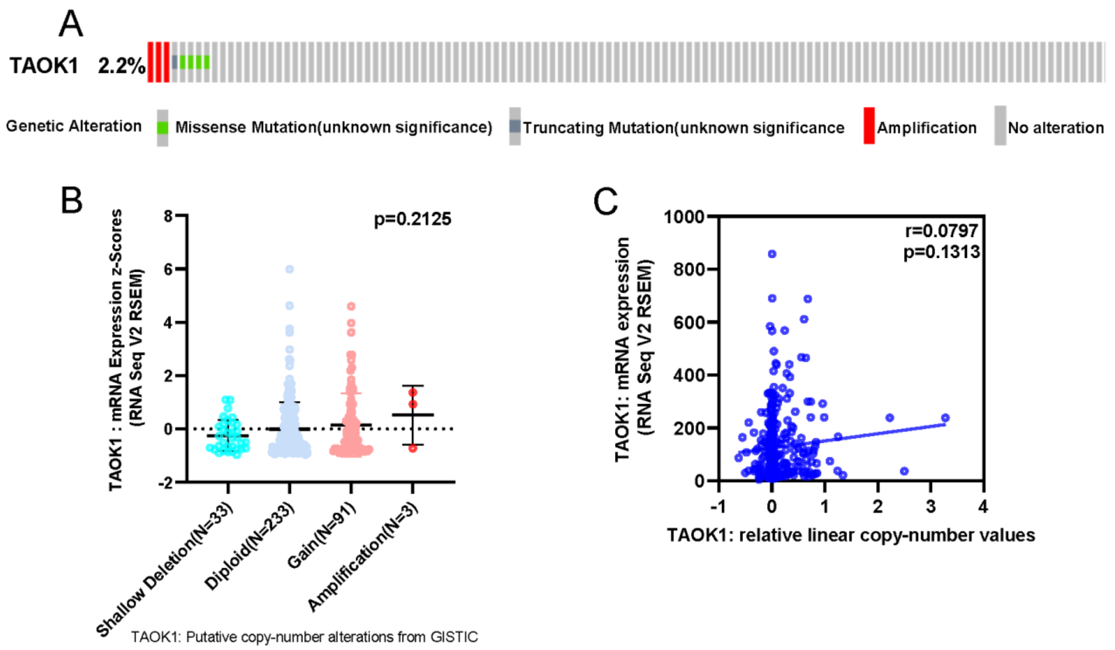

**Fig. S5. The mutation status of TAOK1 in HCC.** (A) The distribution of TAOK1 genomic alterations in the TCGA HCC dataset is shown on the cBioPortal OncoPrint plot. The correlation analysis between TAOK1 copy number and mRNA expression are shown in point plot (B) and correlation plot (C).

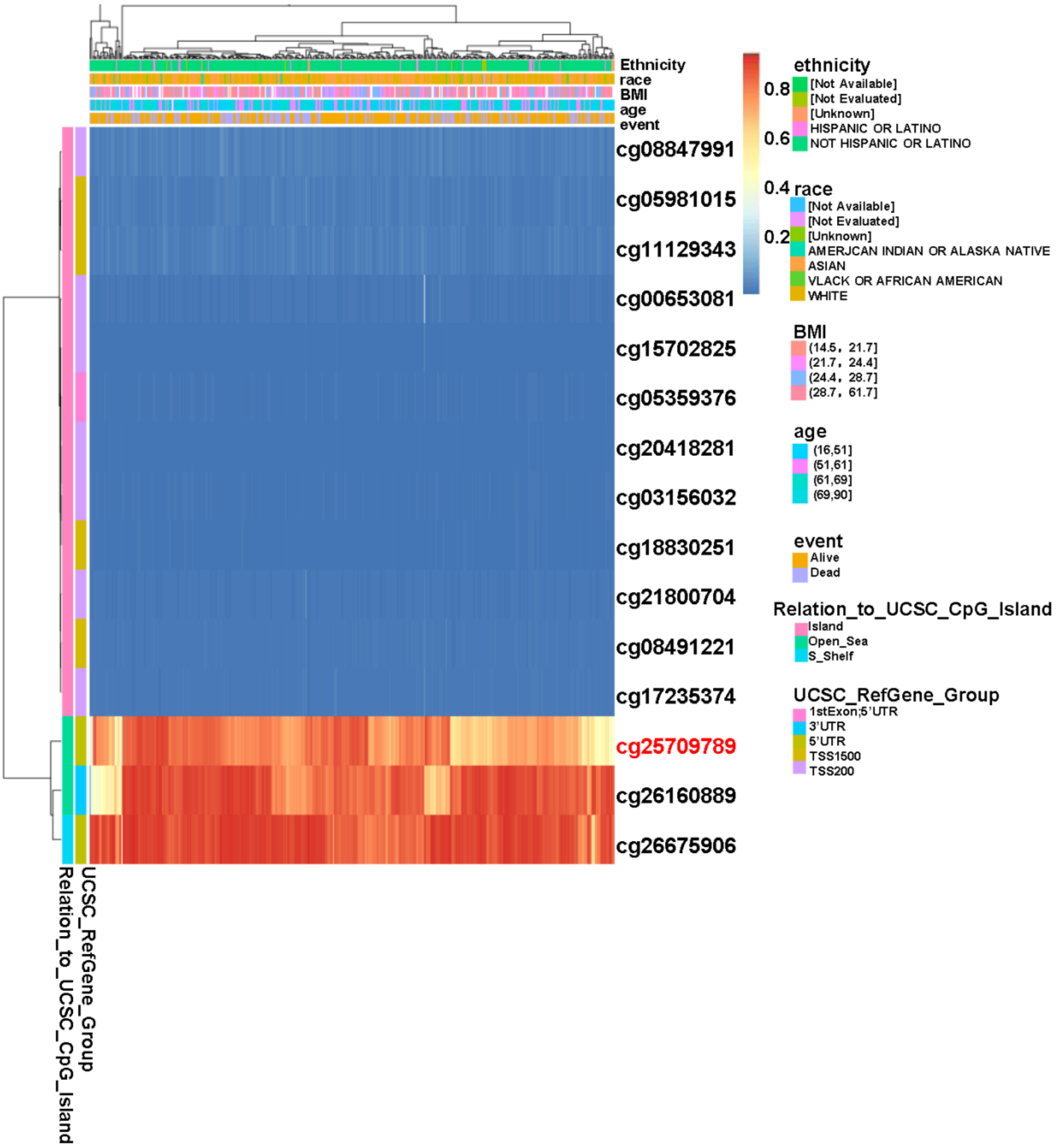

**Fig. S6. Different methylated regions associated with TAOK1.** Different methylated regions associated with TAOK1 were presented by heatmap using MethSurv.

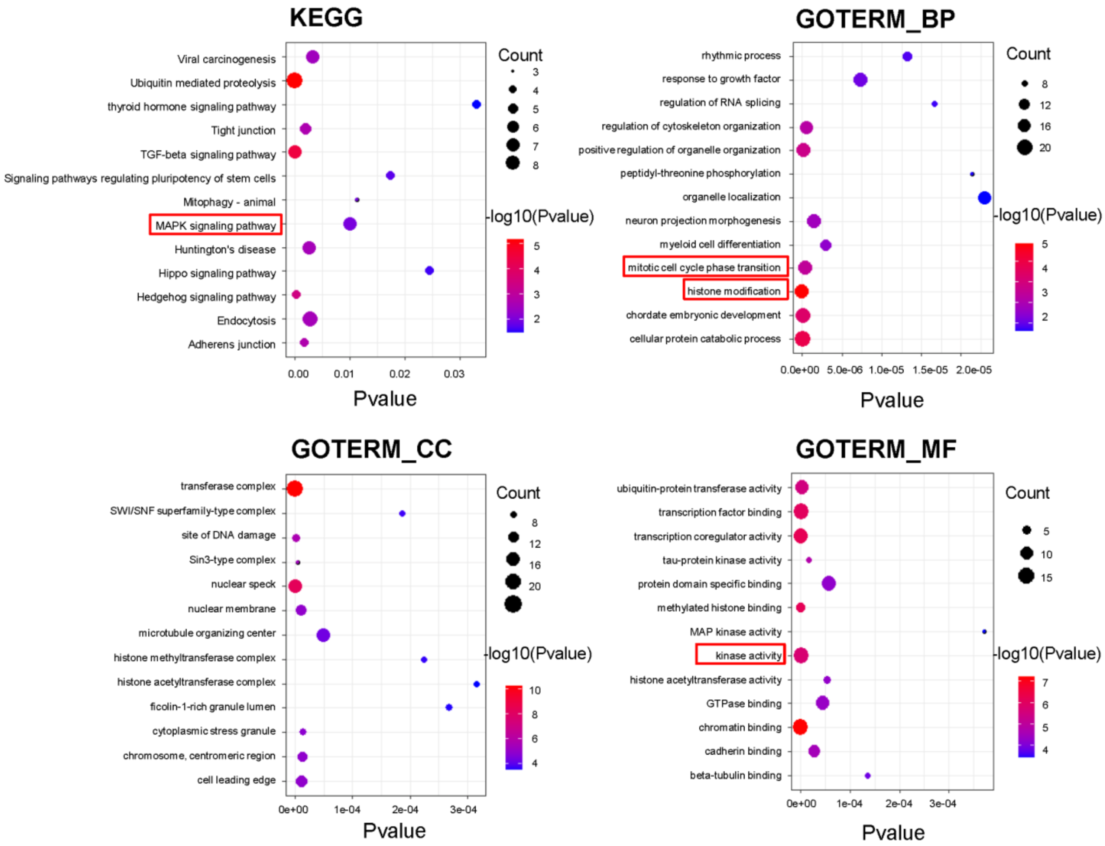

**Fig. S7. Functional enrichment analysis of TAOK.** Functional enrichment analysis (including GO and KEGG) of TAOK associated genes in HCC.