# DNAJC10 Correlates with Tumor Immune Characteristics and Predicts the Prognosis of Glioma Patients

**Feng Liu**
The Second Affiliated Hospital of Nanchang University
**Zewei Tu**
The Second Affiliated Hospital of Nanchang University
**Junzhe Liu**
The Second Affiliated Hospital of Nanchang University
**Xiaoyan Long**
East China Institute of Digital Medical Engineering
**Bing Xiao**
The Second Affiliated Hospital of Nanchang University
**Hua Fang**
The Second Affiliated Hospital of Nanchang University
**Kai Huang**
The Second Affiliated Hospital of Nanchang University
**Xingen Zhu** ( ndefy89006@ncu.edu.cn )
The Second Affiliated Hospital of Nanchang University

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# Abstract

**Background:** A role of DNAJC10 has been reported in several cancers, but its function in glioma is not clear. The purpose of this study was to investigate the prognostic role and the underlying functions of DNAJC10 in glioma.

**Methods:** Reverse transcription and quantitative polymerase chain reaction and western blotting were performed to quantify the relative DNAJC10 mRNA and protein expressions of clinical samples. Wilcoxon rank sum tests were used to compare DNAJC10 expression between or among glioma subgroups with different clinicopathological features. The overall survival (OS) rates of glioma patients with different DNAJC10 expression were compared with the Kaplan-Meier method (two-sided log-rank test). The prognosis-predictive accuracy of the DNAJC10 was evaluated by time-dependent receiver operating characteristic (ROC) curves. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes annotations were conducted using the “clusterProfiler” package. Single-sample gene set enrichment analysis was used to estimate immune cell infiltrations and immune-related function levels. The independent prognostic role of DNAJC10 was determined by univariate and multivariate Cox regression analyses. A DNAJC10-based nomogram model was established using multivariate Cox regression in the R package “rms.”

**Results:** Higher DNAJC10 expression was observed in gliomas. It was upregulated in tumors with higher World Health Organization grade, isocitrate dehydrogenase wild-type status, 1p/19q non-co-deletion, and methylguanine-DNA methyltransferase unmethylated gliomas. Patients with gliomas with higher DNAJC10 expression had poorer prognoses than those with low-DNAJC10 gliomas. The predictive accuracy of 1/3/5-year OS of DNAJC10 was stable and robust using a time-dependent ROC model. Functional enrichment analysis recognized that T cell activation and T cell receptor signaling were enriched in higher DNAJC10 gliomas. Immune cell and stromal cell infiltrations, tumor mutation burden, copy number alteration burden, and immune checkpoint genes were also positively correlated with glioma DNAJC10 expression. A DNAJ10-based nomogram model was established and showed strong prognosis-predictive ability.

**Conclusion:** Higher DNAJC10 expression correlates with poor prognosis of patients with glioma and is a potential and useful prognostic biomarker.


# Background

Glioma is the most fatal intracranial malignancy and the most common primary brain tumor in adults; it is classified into four grades (I-IV) by the World Health Organization (WHO) [1, 2]. WHO grades II (diffuse low-grade) and III (intermediate-grade) gliomas are defined as lower-grade gliomas (LGGs), while WHO IV gliomas (glioblastoma, GBM) have been paid more attention by cancer researchers because of their resistance to traditional treatment strategies [2, 3]. Gliomas are highly invasive, resistant to drug therapy, likely to recur, and carry a high mortality rate, and the mainstream therapy is surgical resection in

combination with chemotherapy and/or radiotherapy [4]. Despite treatment improvements in recent decades, glioma is still a difficult to treat cancer that is often fatal due to its intricate chemoresistance mechanisms and intratumor heterogeneity; the curative effect and survival time of patients with glioma patients remain unsatisfactory [5, 6]. It is therefore necessary to identify effective molecular biomarkers and treatment targets for this devastating disease.

DNAJC10 (DnaJ heat shock protein [HSP] family member C10), also named ERDJ5 or PDIA19, encodes an endoplasmic reticulum (ER)-localized protein that forms part of the ER-associated degradation (ERAD) complex involved in identifying and degrading misfolded proteins [7]. It functions as an ER co-chaperone by reducing incorrect disulfide bonds in intracellular misfolded glycoproteins. The role of ER stress (ERS) in cancer has been increasingly studied since ER-associated proteins were found to play important roles in cancer initiation and progression [8]. Among these, DNAJC10 has been associated with neuroblastoma, colorectal cancer, and prostate cancer [9–11]. A network analysis of HSP family members by Sun et al. [12] showed that DNAJC10 was a prognostic factor of glioma, but that study lacked systemic evidence from multiple cohorts. Here we performed a systemic and multi-cohort prognostic analysis of DNAJC10 to assess its role in glioma.

We collected data from 3 independent glioma cohorts (TCGA [n = 507], CGGAseq1 [n = 628], and CGGAseq2 [n = 309]) and 12 clinical samples and found that the mRNA and protein expressions of DNAJC10 were upregulated in glioma samples compared with normal brain tissue (NBT) in datasets and clinical samples. Moreover, DNAJC10 expression level was significantly associated with clinicopathological features of gliomas including WHO grade, isocitrate dehydrogenase (IDH) mutation status, 1p/19q co-deletion status, and O(6)-methylguanine-DNA methyltransferase (MGMT) methylation status. Survival analysis with the Kaplan-Meier method revealed that glioma patients with higher DNAJC10 expression had shorter overall survival (OS) time and a lower OS rate in all three independent glioma cohorts. Importantly, DNAJC10 expression predicted the OS of patients with glioma in a time-dependent receiver operating characteristic (ROC) curve model. We also performed differential expression analysis between low- and high-DNAJC10 expression gliomas to identify differentially expressed genes (DEGs) related to DNAJC10. The top 1000 up- and down-regulated DEGs were subjected to Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Tumor Hallmarks (Gene Set Enrichment Analysis [GSEA]) analyses. DNAJC10 expression correlated with most immune characteristics, immune and stromal scores, tumor mutation burden (TMB), copy number alteration (CNA) burden, and the expression of 12 immune checkpoint genes (ICPGs). Finally, uni- and multivariate Cox regression analyses were carried out to determine the independent prognostic role of DNAJC10 expression, and a nomogram model was established based on DNAJC10 expression level, WHO grade, and 1p/19q co-deletion status to better predict the clinical outcomes of patients with glioma.

## Methods

# Data acquisition and processing

The level-3 mRNA expression profiles (AffyU133a platform) and relevant clinicopathological and survival data of glioma patients from The Cancer Genome Atlas (TCGA) dataset were obtained from the UCSC Xena repository (https://xenabrowser.net/), and the mRNA expression data (Illumina HiSeq platform) and clinical information of the two CGGA-seq cohorts were downloaded from the Chinese Glioma Genome Atlas (CGGA) dataset (http://www.cgga.org.cn/). All \data contained in the three RNA-seq expression matrixes were downloaded in FPKM (Fragments Per Kilobase Million) format, and the FPKM data were transferred to TPM (Transcripts Per Kilobase Million) format for subsequent analysis.

Single-cell RNA-seq (scRNA-seq) dataset GSE84465 was downloaded from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) website [13]. The processing method for scRNA-seq data was carried out as described previously [14].

# Gene expression profiling interactive analysis (GEPIA) online analysis

Given the lack of sufficient NBT mRNA expression data (control group) in the three glioma cohorts, comparison analysis was conducted between NBT (data from the GTEx dataset) and glioma samples (LGG and GBMs from TCGA dataset) in the GEPIA website (http://gepia.cancer-pku.cn/) [15]. $P < 0.05$ was used as cut-off value to judge the statistically significant differences in DNAJC10 expression between NBT (n = 207) and gliomas (LGG, n = 518; GBM, n = 163). The format of DNAJC10 expression data was transformed to log2(TPM + 1) to fit the Gaussian distribution.

# Patient/sample inclusion criteria

Glioma patients who meet the following criteria were included in this study: (1) glioma samples with mRNA expression data; (2) patients survived longer than 30 days from the day of diagnosis (OS > 30 days); and (3) WHO grade information.

# Clinical sample collection

The three clinical NBTs and nine glioma samples included in our study were collected from inpatients who underwent surgical excision in the Neurosurgery Department of The Second Affiliated Hospital of Nanchang University between 2019 and 2021. The 12 clinical samples consisted of 3 WHO grade II, 3 WHO grade III, 3 WHO IV gliomas, and 3 non-neoplastic samples (collected from intractable epilepsy patients). The tumor and NBT samples were frozen in liquid nitrogen and stored at -80°C until use. The study was approved by the Medical Ethics Committee of The Second Affiliated Hospital of Nanchang University. Sample acquisition and utilization were performed in accordance with the approved guidelines. Informed consent was obtained from each inpatient.

# Cell culture and immunofluorescence microscopy

To monitor the intracellular localization of DNAJC10, U87 glioma cells grown on coverslips were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 60 min then incubated in 0.3% Triton X-100 in PBS for 15 min. Then the cells were washed with PBS and blocked in 5% goat serum for 1 h

before they were incubated with anti-DNAJC10 (1:50, 13101-1-AP, Proteintech) rabbit polyclonal antibody at 4°C overnight (> 12 h). Alexa Fluor 488–conjugated Goat Anti-Rabbit IgG H&L (1:200, ab150077, Abcam, Cambridge, UK) was used as the secondary antibody. Next, U87 glioma cells were incubated with 10 μg/mL DAPI (C0065, Solarbio, Beijing, China) for 30 s in the dark. Finally, the cells were washed three times with PBS and visualized using a fluorescence microscope (Nikon, Tokyo, Japan).

# Reverse transcription and quantitative polymerase chain reaction (RT-qPCR)

To detect DNAJC10 mRNA expression levels in clinical non-neoplastic and glioma samples, we acquired total RNA from each sample using RNA TRIzol reagent (Invitrogen, Carlsbad, CA, USA). According to the manufacturer instructions, cDNA synthesis was conducting using a Reverse Transcription Kit (Guangzhou Ribobio Co., Ltd, Guangzhou, China). RT-qPCR analysis was conducted on the Lightcycler 480 Real-Time PCR System (Roche Lifescience, Penzberg, Germany). Relative DNAJC10 mRNA expression levels were calculated using the $2^{-\Delta\Delta CT}$ method, and the corresponding glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA expression level was used as an internal control. The DNAJC10 and GAPDH primers were as follows: DNAJC10 forward 5'-CTCCGAAATCAAGGCAAGAGG-3', and reverse 5'-ACCCTTCTTTTACACCAGTGC-3' [16]; GAPDH forward 5'-GGCTGAGAACGGGAAGCTTGTCAT-3', and reverse 5'-CAGCCTTCTCCATGGTGGTGAAGA-3' [17].

# Western blot and antibodies

Total protein from each sample was extracted using radioimmunoprecipitation assay lysis buffer containing fresh 1% phenylmethyl sulfonyl fluoride freshly. Then, equal protein quantity (15 μg) of each sample was determined by bicinchoninic acid assay (KeyGEN Biotech, Nanjing, China) and were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Next, proteins in the SDS-PAGE gels were transferred to polyvinylidene difluoride membranes (Millipore, Burlington, MA, USA). The membranes were blocked with 10% skim milk for 1 h at room temperature and then incubated with anti-DNAJC10 (1:1000, 13101-1-AP, Proteintech, Rosemont, IL, USA) rabbit polyclonal antibody and anti-GAPDH rabbit polyclonal antibody (1:4000, 10494-1-AP, Proteintech) at 4°C overnight (more than 12 h). The blots were then incubated with secondary horseradish peroxidase-conjugated antibody (Affinipure Goat Anti-Rabbit IgG, 1:4000, SA00001-2, Proteintech) for approximately 2 h at room temperature. The bands were developed with enhanced chemiluminescence reagent (32106, Thermo Fisher Scientific, Waltham, MA, USA) reagents using GV6000M (GelView 6000pro, Guangzhou Biolight Biotechnology Co., Ltd., Guangzhou, China). The gray intensities of protein bands were measured with ImageJ software (National Institutes of Health, Bethesda, MD, USA) and standardized to the GAPDH intensity.

# Single-sample GSEA (ssGSEA)

ssGSEA was used to quantify the abundance of immune cell infiltration or immune function in the glioma tumor microenvironment (TME) [18]. Twenty-nine gene sets for annotating each immune-cell infiltration and function were obtained from the Molecular Signature Database (MSigDB, https://www.gsea-msigdb.org/). We calculated 29 enrichment scores for each glioma sample using corresponding immune-

related gene sets from the ssGSEA algorithm, and the relative scores represent the immune cell infiltration abundance or immune function levels.

# Functional enrichment annotation analysis

Before performing functional enrichment annotation analysis, glioma samples were divided into low- and high-DNAJC10 subgroups. Then “limma” R package [19] was used to identify DEGs between low- and high-DNAJC10 gliomas through the whole transcriptome range. Genes with adjusted $P < 0.001$ were defined as DEGs, and the top 1000 up- and down-regulated genes were used to perform GO and KEGG annotations using the “clusterProfiler” R package [20], respectively. GSEA of hallmarks enriched in high-DNAJC10 gliomas was conducted using “GSEA” software (version 4.0.1) [21].

# Statistics

Wilcoxon rank sum tests were performed to compare DNAJC10 expression levels between various glioma subgroups with different clinicopathological features. The Kaplan-Meier method (two-side log-rank test) was applied to assess survival differences between glioma subgroups with distinct DNAJC10 expression levels, and the most statistically significant cutoff value was chosen using the “survminer” package (“surv-cutpoint” function). The prognostic predictive ability of DNCJC10 mRNA expression was assessed by time-dependent ROC curves (“timeROC” package [22]), and the area under the curve (AUC) was used as the comparable index. Student’s t-tests were applied to determine the different levels of these immune-related factors (including immune score, stromal score, TMB, CNA burden, 29 immune-related features, and 12 ICPGs) between the low- and high-DNAJC10 glioma subgroups. Uni- and multivariate Cox regression were performed in all three independent glioma cohorts to evaluate the independent prognostic role of DNAJC10. The R package “rms” was used to establish the nomogram model based on the multivariate Cox regression analysis results. C-index and calibration plots were used to assess the predictive ability of the nomogram. R programming language (version 3.6.1) was the statistical analysis tool.

## Results

# DNAJC10 is upregulated in glioma tissue

To explore aberrant DNAJC10 expression in gliomas, we conducted comparison analysis with the GEPIA webtool and found that DNAJC10 mRNA levels were upregulated in both LGG (n = 518) and GBM (n = 163) samples from the TCGA database compared with NBT samples from the GTEx database (n = 207) (Fig. 1A). We then analyzed DNAJC10 expression in the single-cell RNA data to visualize the expression distribution among different intra-glioma cell; the results showed that DNAJC10 was most highly expressed in GBM cells and immune cells (Fig. 1B). To visualize DNAJC10 protein in glioma cells, we performed immunofluorescence assays in the U87 glioma cell line. DNAJC10 was mainly localized in the cytoplasm and cell membrane (Fig. 1C). To investigate aberrant DNAJC10 expression at the protein level, we obtained glioma DNAJC10 immunohistochemistry (IHC) images from the Human Protein Atlas (HPA,

https://www.proteinatlas.org/) and observed higher DNAJC10 protein expression in high-grade gliomas compared to LGGs (Fig. 1D). To further verify this finding, RT-qPCR was used to quantify DNAJC10 mRNA expression in clinical samples, and the results confirmed that DNAJC10 mRNA was upregulated in glioma samples compared with NBT (Fig. 1E). Finally, we detected DNAJC10 protein expression in 12 clinical samples using western blot. Consistent with the RT-qPCR results, DNAJC10 protein was overexpressed in glioma samples in a WHO grade-dependent fashion (Fig. 1F).

# DNAJC10 correlates the clinicopathological features of gliomas

Given the aberrantly high levels of DNAJC10 in gliomas, we analyzed different DNAJC10 expression levels between/among different clinicopathological features. The heatmap in Fig. 2A shows the associations of DNAJC10 expression (ordered from low to high) with age, sex, WHO grade, histological classification, IDH mutation status, 1p/19q co-deletion status, and MGMT methylation status in the TCGA cohort. The results from the three independent glioma cohorts showed that DNAJC10 expression was greatest in higher WHO grade gliomas (Fig. 2B), which was consistent with the IHC results from the HPA dataset. High DNAJC10 expression in glioma tissue was also significantly associated with MGMT unmethylated status (Fig. 2C), IDH wild-type status (Fig. 2D), and 1p/19q non-co-deletion status (Fig. 2E).

# Higher DNAJC10 expression in glioma tissue is associated with poor patient prognosis

To better illustrate the clinical significance of aberrant expression of DNAJC10, we performed survival analysis (Kaplan-Meier method) and generated ROC curves to evaluate the prognostic role and predictive power of DNAJC10 levels in patients with glioma. The survival curve analysis results indicated that for all three cohorts, glioma patients with higher DNAJC10 expression survived for shorter times than the lower expression group (Fig. 3A-C). The ROC curves indicated that DNAJC10 levels had a robust and stable prognosis predictive ability for OS in patients with gliomas; the AUCs to predict the 1/3/5-year OS rates were 0.728/0.777/0.690 in TCGA, 0.540/0.585/0.625 in CGGA-seq1, and 0.642/0.728/0.78 in CGGA-seq2 (Fig. 3D-F). Moreover, subgroup survival analysis revealed that DNAJC10 overexpression was associated with shorter OS in patients with both LGGs (Fig. 3G) and GBMs (Fig. 3H) in all three glioma cohorts.

# Functional enrichment analysis of DNAJC10 in gliomas

To further clarify the underlying functions, pathways, and tumor hallmarks associated with DNAJC10 levels, we performed differential expression analysis between the low- and high-DNAJC10 subgroups to identify DEGs associated with DNAJC10 expression (false discovery rate < 0.05) in the TCGA glioma cohort. The top 1000 up-regulated (positive with DNAJC10) and down-regulated (negative with DNAJC10) DEGs were subjected to GO and KEGG analyses, respectively. The GO results of up-regulated DEGs showed that elevated DNAJC10 was strongly correlated with cytokine and receptor activities (molecular function); extracellular matrix, plasma membrane, and receptor complexes (cellular component); and T cell, leukocyte, and lymphocyte activation (biological process) (Fig. 4A). Down-regulated genes were

mainly enriched in channel activities (molecular function); synaptic membrane and transporter complexes (cellular component); and signal release, ion transmembrane transport, and G-protein-coupled receptor signaling pathway (biological process) (Fig. 4B). The KEGG pathway analysis indicated that top 1000 up-regulated DEGs were enriched in cytokine-cytokine receptor interaction, chemokine signaling pathway, cell adhesion molecules, JAK-STAT signaling pathway, T cell receptor signaling pathway, and IL-17 signaling pathway (Fig. 4C), and top 1000 down-regulated DEGs were enriched in neuroactive ligand-receptor interaction, calcium signaling pathway, cAMP signaling pathway, GABAergic synapse, Glutamatergic synapse, and synaptic vesicle cycle (Fig. 4D). Next, GSEA was performed to judge the significance of enriched tumor hallmarks in the high-DNAJC10 glioma subgroup compared with the low-DNAJC10 glioma subgroup. The results revealed that the following hallmarks were significantly enriched in high-DNAJC10 gliomas: Complement, Epithelial-Mesenchymal Transition, IL-2-STAT5 Signaling, Hypoxia, Coagulation, TNFA signaling via NFκB, Interferon gamma response, Inflammatory response, Allograft rejection, and PI3K-AKT-mTOR signaling.

# DNAJC10 correlates with tumor immune characteristics

The GO and KEGG results of revealed a potential correlation between DNAJC10 and T cell activation and T cell receptor signaling, which motived us to investigate the associations between DNAJC10 and tumor immune characteristics. ssGSEA was used to calculate the immune score, stromal score, and the 29 immune-related characteristic scores. The heatmap ordered by DNAJC10 expression showed that most immune-related characteristics were significantly associated with DNAJC10 expression, except the mast cells and T follicular helper scores (Fig. 5A). Then the immune score, stromal score, TMB, and CNA burden were compared between the low- and high-DNAJC10 subgroups. All four were significantly increased in high-DNAJC10 subgroup gliomas (Fig. 5B-E). Furthermore, 12 reported ICPG expression levels were compared between the subgroups, and the results showed that all 12 were overexpressed in high-DNAJC10 gliomas compared with low-DNAJC10 gliomas (Fig. 5F).

# Cox regression analysis and nomogram establishment

To evaluate the independent prognostic role of DNAJC10 expression in gliomas, we first performed univariate Cox regression analysis to assess the prognostic abilities of DNAJC10 expression levels and other clinicopathological factors (age, sex, WHO grade, IDH mutation status, 1p/19q co-deletion status, and MGMT methylation status). The results showed that higher age, WHO grade, and DNAJC10 expression level were risk factors in all three glioma cohorts; IDH mutation, 1p/19q co-deletion, and methylated MGMT were protective factors in gliomas; and sex was excluded in the subsequent multivariate Cox regression analysis due to a lack of significance (Fig. 6A). Age, WHO grade, IDH mutation status, 1p/19q status, MGMT status, and DNAJC10 expression level were included in multivariate Cox regression analysis, but only WHO grade, 1p/19q status, and DNAJC10 expression level were independent prognostic factors in all three glioma cohorts (Fig. 6A).

To assess the potential of DNAJC10 as a biomarker in clinical predictive applications, we established a clinical nomogram model using the three independent prognostic factors (WHO grade, 1p/19q status, and

DNAJC10 expression level) identified in the multivariate Cox regression model using the TCGA training cohort (Fig. 6B). To evaluate the nomogram model's accuracy, C-indexes were calculated for each cohort (TCGA: 0.837; CGGA-seq1: 0.648; CGGA-seq2: 0.675) to test nomogram robustness. Calibration curves indicated that the DNAJC10-based nomogram could accurately predict the 1/3/5-year OS rates of glioma patients (Fig. 6C-E).

# Discussion

Glioma cells in the TME survive despite challenges like hypoxia, metabolic dysregulation, immune surveillance, and chronic inflammation. To survive in such harsh conditions, glioma cells must maintain intracellular and intercellular homeostasis [23]. DNAJC10 is a member of the HSP and protein disulfide isomerase families that is an important factor in the ERS response to help degrade misfolded proteins, refold proteins, and secreting cytokines. It was reported to have an important role in several cancers, but its mechanical impact in glioma is still uncertain. In these research, we systematically assessed the potential role of DNAJC10 in gliomas using several bioinformatic and experimental methods.

Public glioma datasets and collected clinical samples were used to measure the relative expressions of DNAJC10 mRNA and protein levels in gliomas compared with NBT samples. Several bioinformatic datasets (GTEx, TCGA, CGGA, and HPA) were used to determine relative DNAJC10 expression levels in glioma tissue. The results revealed that both mRNA and protein levels were upregulated in gliomas, which were confirmed by qRT-PCR and western blot assays of clinical samples. Notably, DNAJC10 expression increased with WHO grade, suggesting that DNAJC10 might be associated with glioma malignancy.

Kaplan-Meier curves analyses of the three glioma cohorts from public datasets revealed that DNAJC10 mRNA expression could be used to divide glioma patients into long- and short-OS subgroups. The subgroup analysis showed that DNAJC10 mRNA expression had prognostic ability in patients with LGGs and GBMs. Conversely, DNAJC10 was found to be a protective factor or cancer suppressor in breast cancer and neuroblastoma [11, 16]. However, these conclusions are based on data from three retrospective independent glioma cohorts, and they should be investigated in future prospective studies.

DEGs between low- and high-DNAJC10 gliomas were identified to perform functional analyses to elucidate the potential functions of DNAJC10 in glioma. T cell activation, T cell complex, and T cell signaling pathway were annotated in the high-DNAJC10 glioma subgroup, suggesting that DNAJC10 might play a role in cancer immunity. The ssGSEA algorithm was used to calculate immune score, stromal score, immune cell infiltrations, or immune-related functions of each glioma sample in the TCGA cohort. Immune/stromal scores represent the infiltrations of immune/stromal cells in tumor tissues, and the results indicated that gliomas with higher DNAJC10 expression had higher immune scores, stromal scores, TMB, CNA burden, and ICPG expression levels. This indicates that DNAJC10 expression level might represent the immune infiltration status of gliomas, but the potential correlations and causality need additional evidence and further exploration.

Ojore et al. used mass spectrometry to identify the substrate proteins of ERdj5 in the HT1080 cell line [24] and showed that secreted proteins (e.g., epidermal growth factor-containing fibulin-like protein 1, laminin-5 beta3, transforming growth factor-beta, fibronectin, laminin subunit gamma, collagen alpha-3[VI], stanniocalcin-1, laminin B2, laminin B1, etc.) might undergo disulfide modification by ERdj5. This information could provide useful clues for further investigation of potential mechanisms of DNAJC10 in cancer progression.

# Conclusion

Our study identified mRNA biomarkers and assessed the prognostic utility by coupling bioinformatic methods and clinicopathologic samples. DNAJC10 mRNA expression was upregulated in gliomas and strongly correlated with clinicopathological features. Specifically, glioma patients with higher DNAJC10 expression had poorer prognosis, and its levels showed stable predictive accuracy in predicting the 1/3/5-year OS of glioma patients. In functional annotation analysis, DNAJC10 expression significantly correlated with glioma immune infiltration, suggesting that it could affect the functions of substrate proteins that DNAJC10 catalyzes intracellularly. Finally, we established a nomogram to improve the predictive accuracy of DNAJC10 that could be applied in clinical practice.

# Abbreviations

TCGA: The Cancer Genome Atlas;

CGGA: Chinese Glioma Genome Atlas.

LGG: lower-grade glioma;

GBM: glioblastoma;

WHO: World Health Organization;

MGMT: O(6)-methylguanine-DNA methyltransferase;

IDH: isocitrate dehydrogenase;

NBT: normal brain tissues;

ERS: endoplasmic reticulum stress;

ROC: receiver operating characteristic;

AUC: area under curves;

DEG: differential expression gene;

GO: Gene Ontology;

KEGG: Kyoto Encyclopedia of Genes and Genomes;

ssGSEA: single-sample gene set enrichment analysis;

TMB: Tumor mutation burden;

CNA: Copy Number Alteration;

## Declarations

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# Authors' contributions

XG and KH designed the research topic and content. FL, ZW and JZ performed the bioinformatical and statistical analysis, figures designs and manuscript writing. XY, BX and HF did the data and sample collecting and processing. All authors contributed to the article and approved the submitted version of manuscript.

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# Availability of data and materials

The original unprocessed data used in our work is stored in the Chinese Glioma Genome Atlas (CGGA; http://www.cgga.org.cn/) and the University of California, Santa Cruz Xena browser (UCSC Xena; https://xenabrowser.net/ datapages/).

# Ethics approval and consent to participate

This research was approved by the Medical Ethics Committee of the Second Affiliated Hospital of Nanchang University, and patients involved gave informed consent. We confirmed that all the experiment

protocol for involving human data was in accordance with the guidelines of Declaration of Helsinki in the manuscript.

# Consent for publication

All authors approve the publication of this work.

# Competing interests

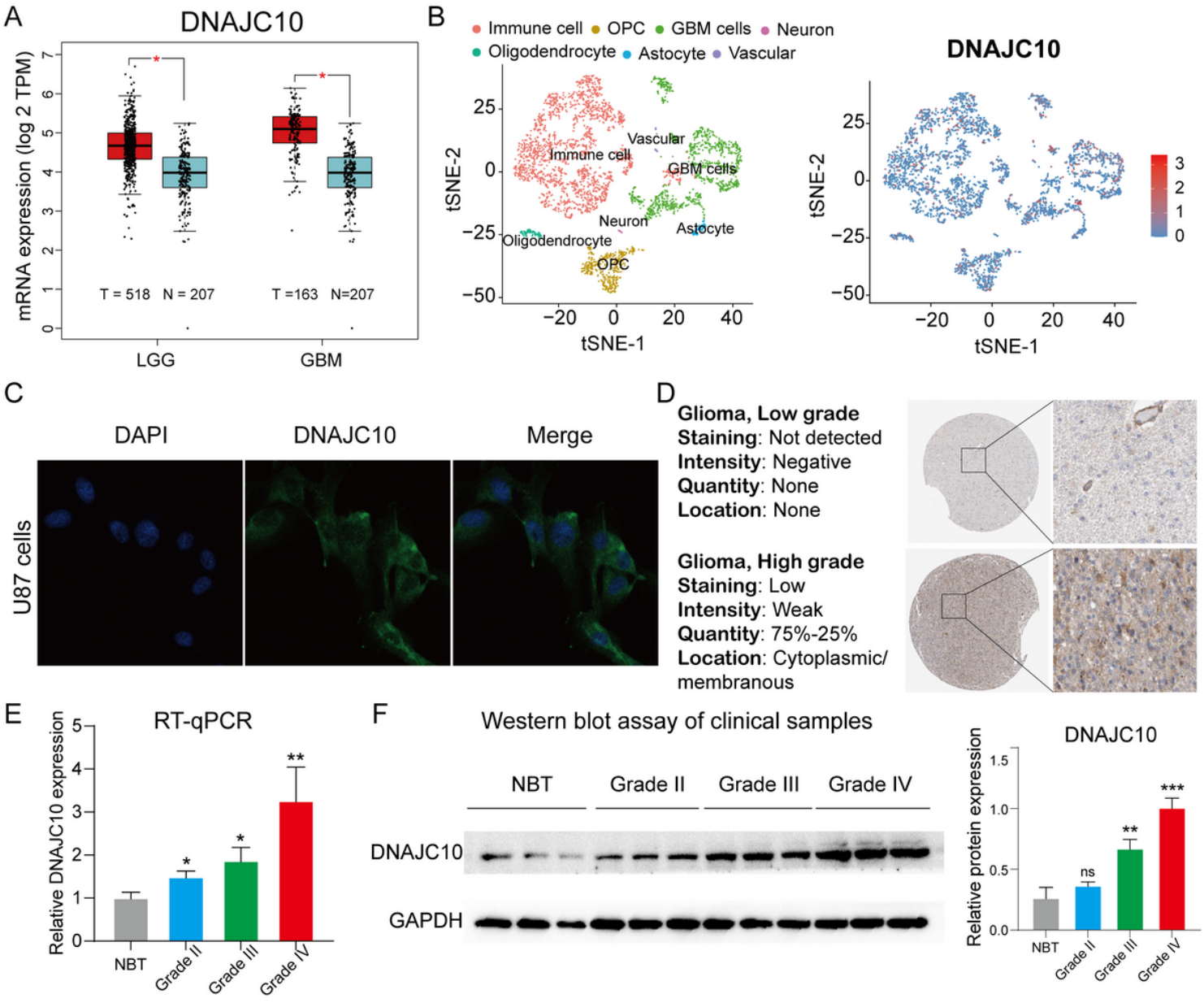
No competing interests exist.

## References


1. Killock D: **CNS cancer: molecular classification of glioma**. *Nat Rev Clin Oncol* 2015, **12**(9):502.
2. Cancer Genome Atlas Research N, Brat DJ, Verhaak RG, Aldape KD, Yung WK, Salama SR, Cooper LA, Rheinbay E, Miller CR, Vitucci M *et al*: **Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas**. *N Engl J Med* 2015, **372**(26):2481–2498.
3. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH *et al*: **The somatic genomic landscape of glioblastoma**. *Cell* 2013, **155**(2):462–477.
4. Van Meir EG, Hadjipanayis CG, Norden AD, Shu HK, Wen PY, Olson JJ: **Exciting new advances in neuro-oncology: the avenue to a cure for malignant glioma**. *CA Cancer J Clin* 2010, **60**(3):166–193.
5. Jiang T, Mao Y, Ma W, Mao Q, You Y, Yang X, Jiang C, Kang C, Li X, Chen L *et al*: **CGCG clinical practice guidelines for the management of adult diffuse gliomas**. *Cancer Lett* 2016, **375**(2):263–273.
6. Jiang T, Nam DH, Ram Z, Poon WS, Wang J, Boldbaatar D, Mao Y, Ma W, Mao Q, You Y *et al*: **Clinical practice guidelines for the management of adult diffuse gliomas**. *Cancer Lett* 2021, **499**:60–72.
7. Ushioda R, Hoseki J, Araki K, Jansen G, Thomas DY, Nagata K: **ERdj5 is required as a disulfide reductase for degradation of misfolded proteins in the ER**. *Science* 2008, **321**(5888):569–572.
8. Xu S, Sankar S, Neamati N: **Protein disulfide isomerase: a promising target for cancer therapy**. *Drug Discov Today* 2014, **19**(3):222–240.
9. Fasano E, Serini S, Piccioni E, Toesca A, Monego G, Cittadini AR, Ranelletti FO, Calviello G: **DHA induces apoptosis by altering the expression and cellular location of GRP78 in colon cancer cell lines**. *Biochim Biophys Acta* 2012, **1822**(11):1762–1772.
10. Misra UK, Pizzo SV: **Modulation of the unfolded protein response in prostate cancer cells by antibody-directed against the carboxyl-terminal domain of GRP78**. *Apoptosis* 2010, **15**(2):173–182.
11. Thomas CG, Spyrou G: **ERdj5 sensitizes neuroblastoma cells to endoplasmic reticulum stress-induced apoptosis**. *J Biol Chem* 2009, **284**(10):6282–6290.

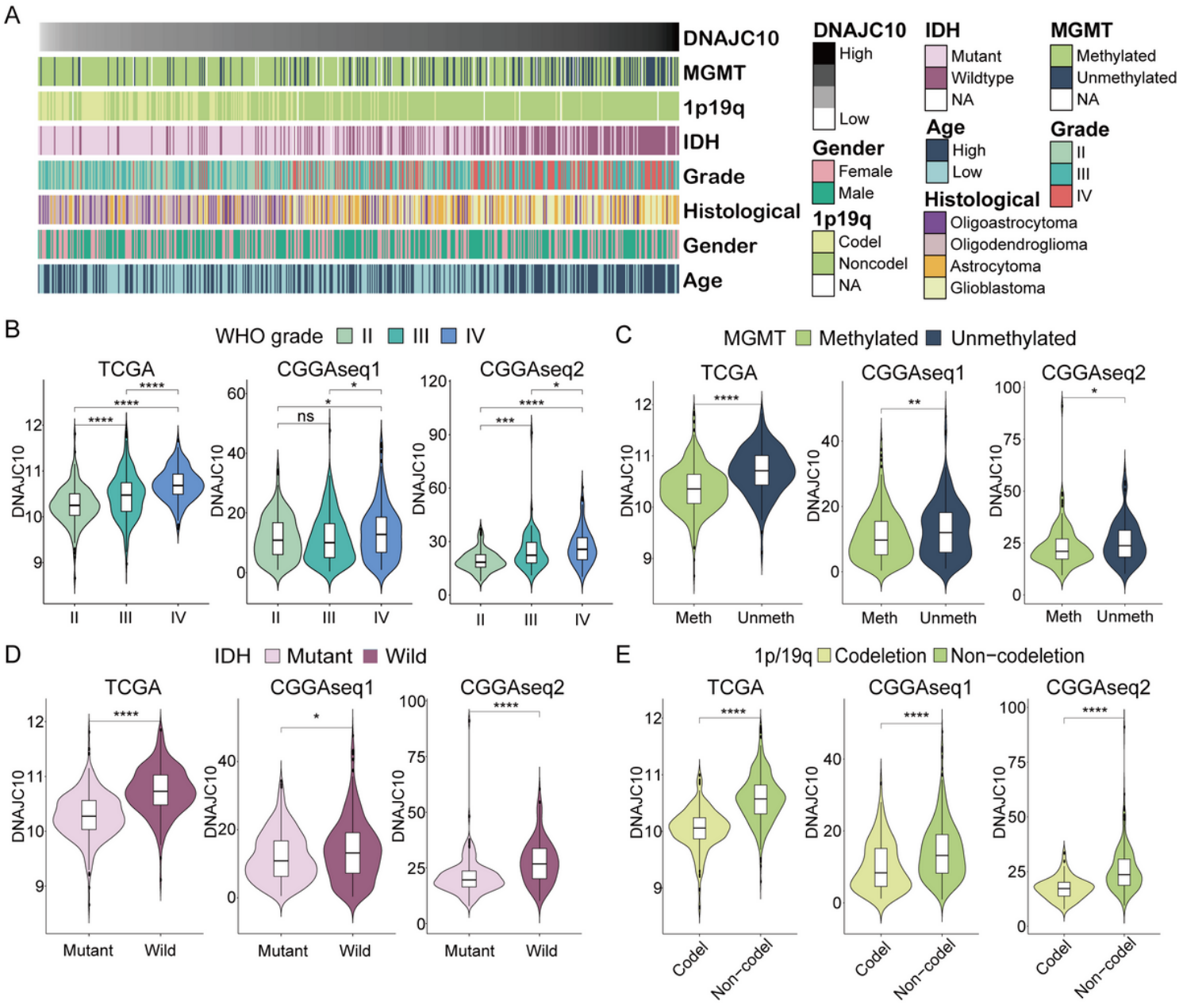
12. Sun H, Zou HY, Cai XY, Zhou HF, Li XQ, Xie WJ, Xie WM, Du ZP, Xu LY, Li EM *et al*: **Network Analyses of the Differential Expression of Heat Shock Proteins in Glioma**. *DNA Cell Biol* 2020, **39**(7):1228–1242.
13. Darmanis S, Sloan SA, Croote D, Mignardi M, Chernikova S, Samghababi P, Zhang Y, Neff N, Kowarsky M, Caneda C *et al*: **Single-Cell RNA-Seq Analysis of Infiltrating Neoplastic Cells at the Migrating Front of Human Glioblastoma**. *Cell Rep* 2017, **21**(5):1399–1410.
14. Wang Z, Guo X, Gao L, Wang Y, Ma W, Xing B: **Glioblastoma cell differentiation trajectory predicts the immunotherapy response and overall survival of patients**. *Aging (Albany NY)* 2020, **12**(18):18297–18321.
15. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z: **GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses**. *Nucleic Acids Res* 2017, **45**(W1):W98-W102.
16. Acun T, Senses KM: **Downregulation of DNAJC10 (ERDJ5) is associated with poor survival in breast cancer**. *Breast Cancer* 2020, **27**(3):483–489.
17. Gur-Dedeoglu B, Konu O, Bozkurt B, Ergul G, Seckin S, Yulug IG: **Identification of endogenous reference genes for qRT-PCR analysis in normal matched breast tumor tissues**. *Oncol Res* 2009, **17**(8):353–365.
18. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, Schinzel AC, Sandy P, Meylan E, Scholl C *et al*: **Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1**. *Nature* 2009, **462**(7269):108–112.
19. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK: **limma powers differential expression analyses for RNA-sequencing and microarray studies**. *Nucleic Acids Res* 2015, **43**(7):e47.
20. Yu G, Wang LG, Han Y, He QY: **clusterProfiler: an R package for comparing biological themes among gene clusters**. *OMICS* 2012, **16**(5):284–287.
21. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES *et al*: **Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles**. *Proc Natl Acad Sci U S A* 2005, **102**(43):15545–15550.
22. Blanche P, Dartigues JF, Jacqmin-Gadda H: **Estimating and comparing time-dependent areas under receiver operating characteristic curves for censored event times with competing risks**. *Stat Med* 2013, **32**(30):5381–5397.
23. Qi Y, Liu B, Sun Q, Xiong X, Chen Q: **Immune Checkpoint Targeted Therapy in Glioma: Status and Hopes**. *Front Immunol* 2020, **11**:578877.
24. Oka OB, Pringle MA, Schopp IM, Braakman I, Bulleid NJ: **ERdj5 is the ER reductase that catalyzes the removal of non-native disulfides and correct folding of the LDL receptor**. *Mol Cell* 2013, **50**(6):793–804.

# Figures



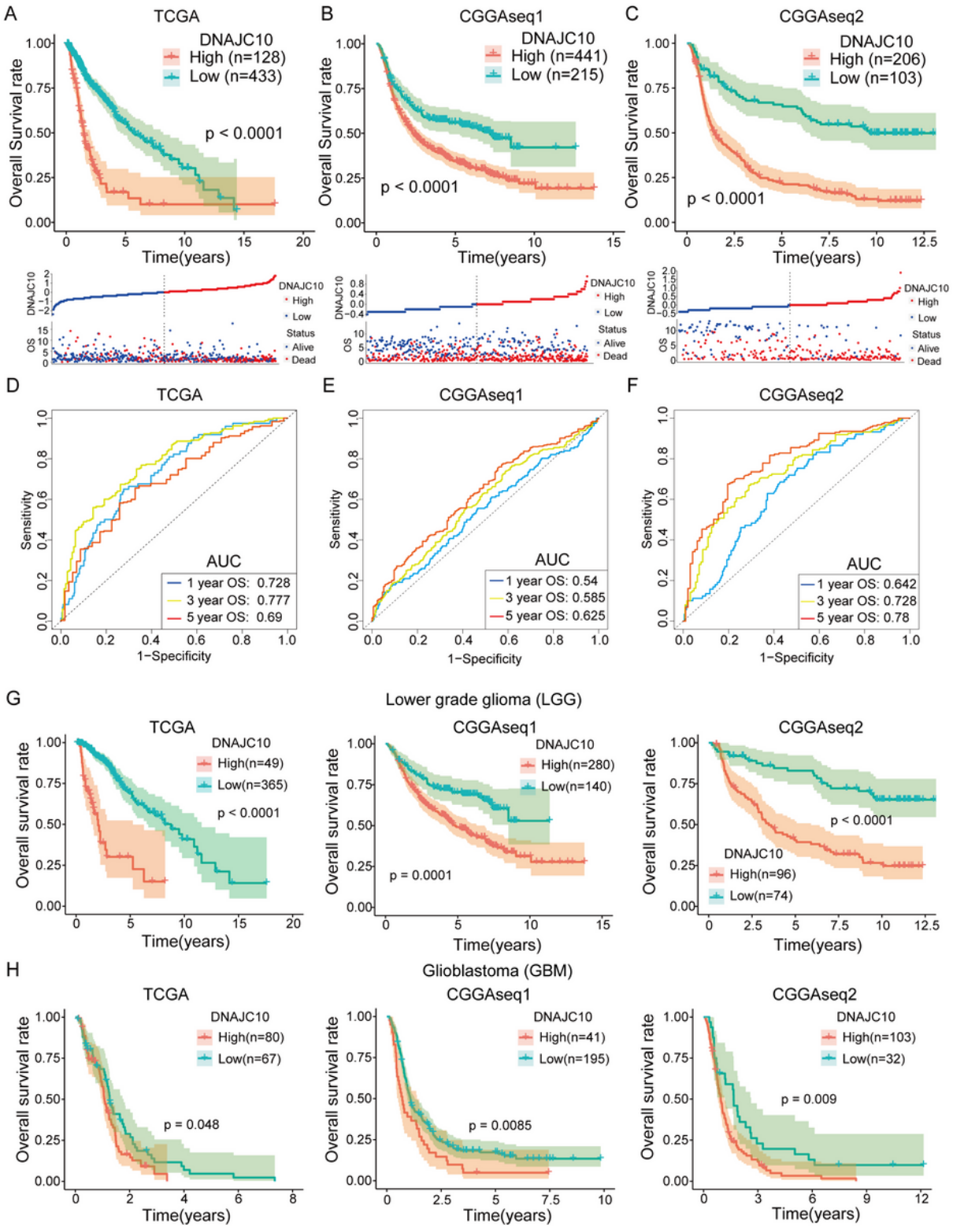

## Figure 1

(A) Boxplots of the unbalanced DNAJC10 expression levels between normal brain tissue (GTEx dataset) and gliomas (TCGA dataset) obtained from the GEPIA website. (B) All 7 kinds of cells were marked according to the annotations of published work, and the expression distribution of DNAJC10 in scRNA-seq data is represented (The color from blue to red indicate the expression level increasing). (C) The protein distribution of DNAJC10 in U87 cells. (D) Protein expression comparisons between immunohistochemical specimens of DNAJC10 in glioma (Data obtained from the Human Protein Atlas database). (E) Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) to quantify the mRNA expression levels of 12 clinical samples. (F) West blot assay was used to quantify the DNAJC10 protein levels of 12 clinical samples.



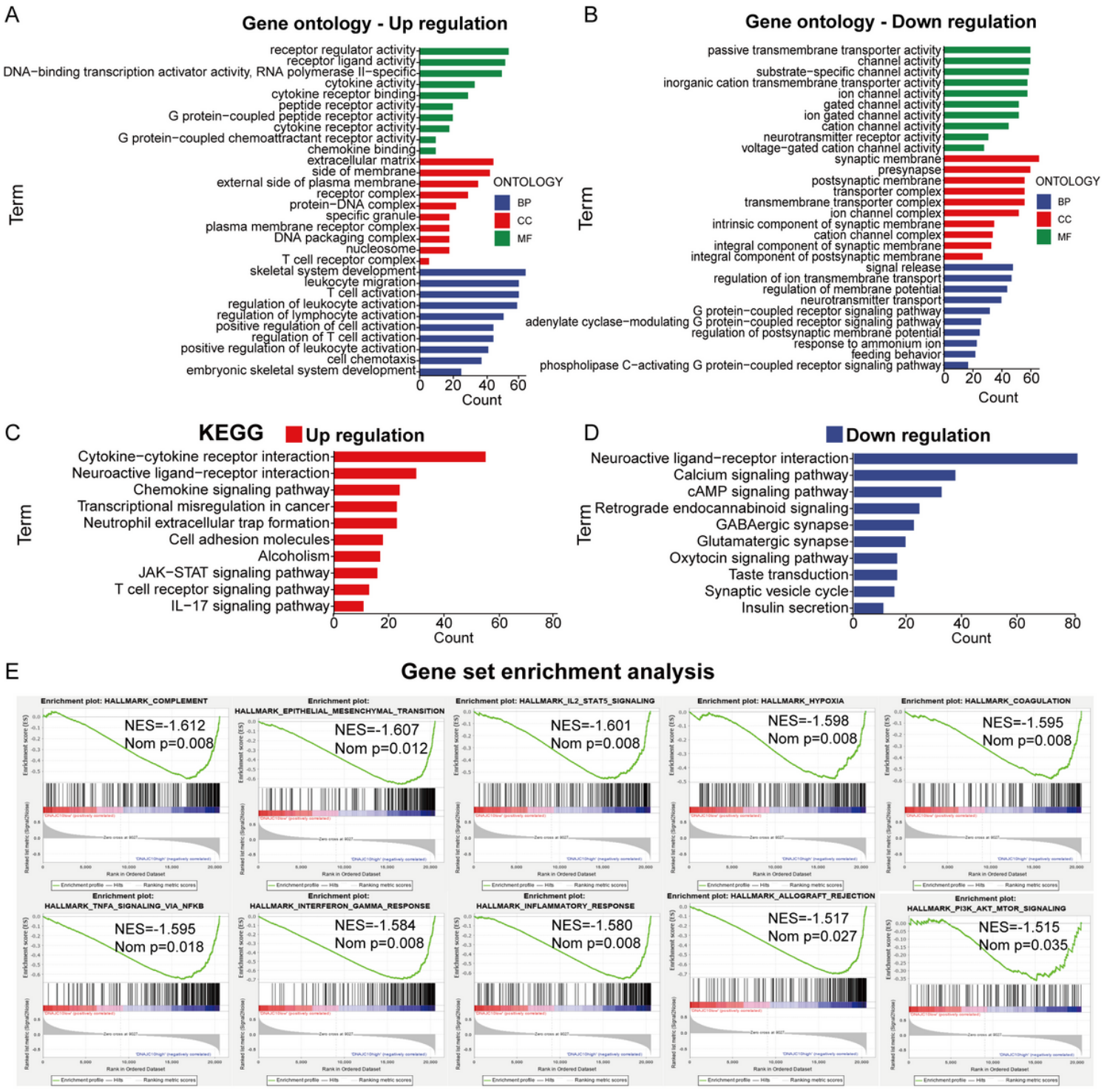

## Figure 2

(A)The heatmap represents the distribution associations of clinical indicators ordered by DNAJC10 expression level. (B-E) The DNAJC10 mRNA expression levels were significantly associated with the WHO grade (B), MGMT methylation status (C), IDH mutation status (D) and 1p/19q codeletion status (E).



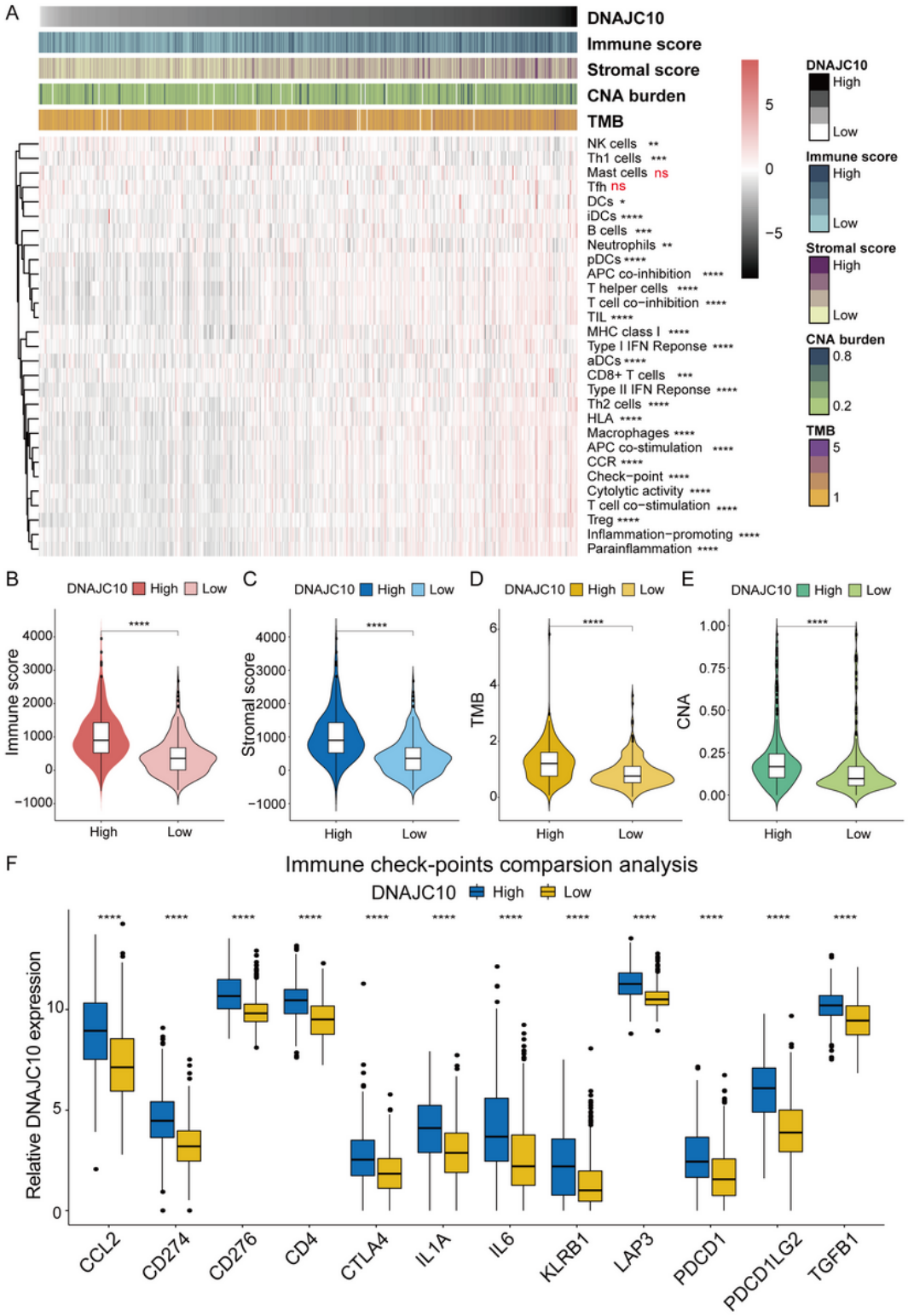

## Figure 3

(A-C) Kaplan-Meier survival curves indicated that glioma patients with higher DNAJC10 expression level showed shorter survival time and rate in three independent glioma cohorts (TCGA, CGGAseq1 and CGGAseq2). (D-F) Receiver operator characteristic (ROC) curves showed the DNAJC10 was a robust and stable prognostic indicator in glioma patients. (G-H) The prognostic role of DNAJC10 remained strong in lower-grade gliomas (G) and glioblastoma (H).



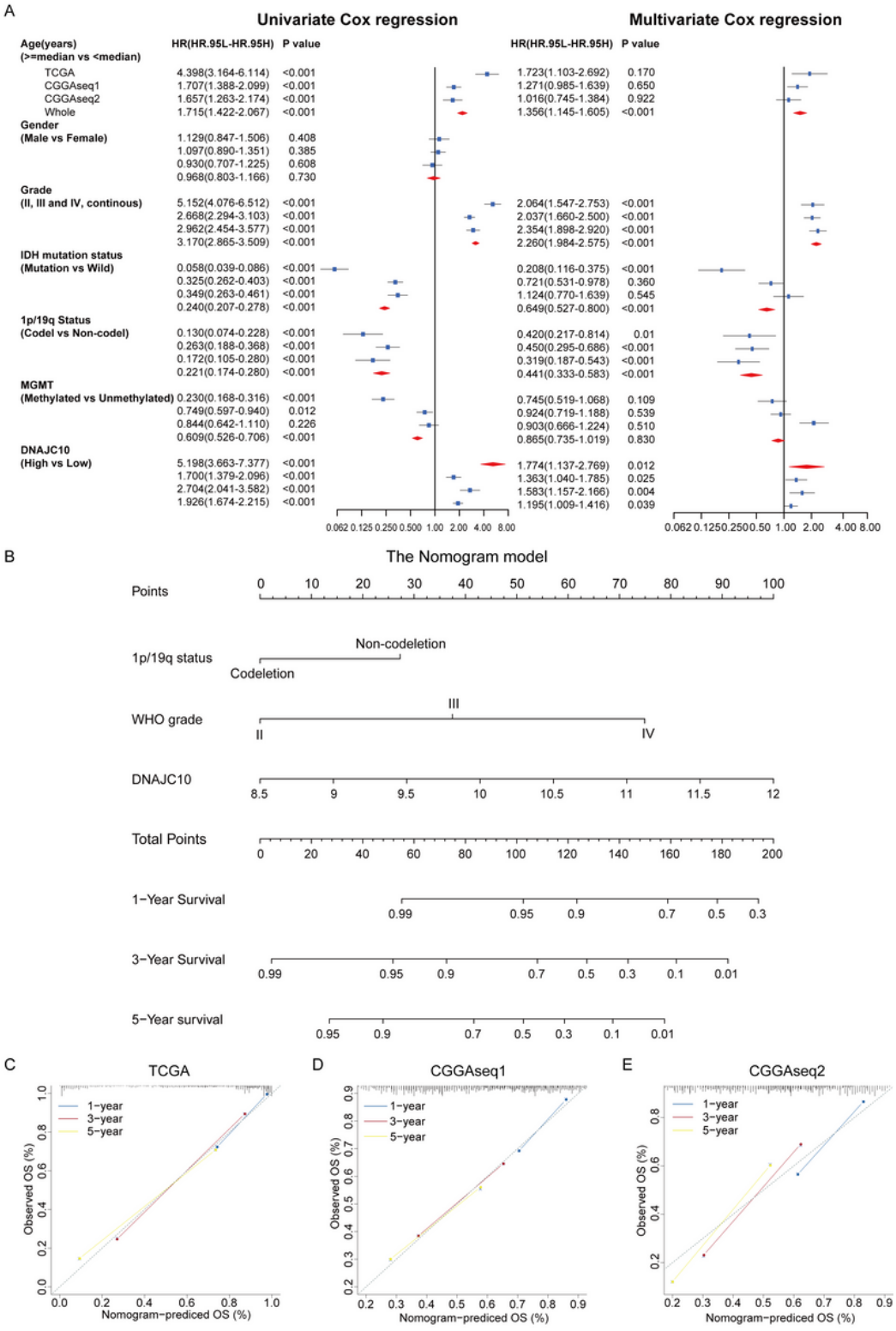

## Figure 4

(A-B) Gene ontology (GO) analysis showed the terms enriched in glioma patients with high DNAJC10 expression (A) and low expression (B). (C-D) Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis showed the terms enriched in glioma patients with high DNAJC10 expression (C) and low expression (D). (E) Gene set enrichment analysis (GSEA) indicated the 10 tumor hallmarks enriched in glioma patients with high DNAJC10 expression.




## Figure 5

(A) The heatmap showed the immune infiltration and function levels distribution according to the DNAJC10 expression level from low to high. (B-E) The violin plots showed that higher DNAJC10 expression was associated with higher immune score (B), stromal score (C), TMB (D) and CNA burden (E). (F) Comparison analysis of 12 immune check-point genes between low- and high-DNAJC10 expression gliomas.




## Figure 6

(A) Univariate and multivariate Cox regression results of DNAJC10 and other clinicopathological factor of glioma patients. (B) Establishment of the DNAJC10-based nomogram model. (C-E) 1/3/5-year OS calibration curves indicate the predictive robustness of the DNAJC10-based nomogram prognostic model in the TCGA (C), CGGAseq1 (D) and CGGAseq2 (E) glioma cohorts.