

# The Prognostic Value of Risk Score Based on Immunogenomic Landscape Analysis in Glioma

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## Research article

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# Abstract

**Background:** Glioma is a lethal intracranial tumor, and inflammation plays an important role in the initiation and development of glioma. Hence, there is an urgent need to conduct a bioinformatics analysis of immune-related genes (IRGs) for glioma. The present study aims to explore the association of the risk score with clinical outcomes and predict the prognosis with glioma.

**Methods:** In The Cancer Genome Atlas (TCGA) database, 462 low grade glioma (LGG) samples and 166 glioblastoma (GBM) samples were reviewed, and IRGs correlated with the prognosis were selected by performing a survival analysis and establishing a Cox regression model. The potential molecular mechanism of these IRGs were also explored with assistance of computational biology. The risk score based on seven survival-associated IRGs was determined with the help of the multivariable Cox analysis, the patients were divided into two subgroups according to their risk score.

**Results:** It was found that these differentially expressed IRGs were involved with the cytokine-cytokine receptor through functional enrichment analysis. The risk score based on the seven IRGs (SSTR5, CXCL10, CCL13, SAA1, CCL21, CCL27 and HTR1A) performed well in predicting patient's the overall survival (OS), and correlated with age, 1p/19q codeletion status, IDH status, and WHO grades, both in the training (TCGA) datasets and the validation ((Chinese Glioma Genome Atlas) CGGA) datasets. The risk score also could reflect infiltration through several types of immune cells.

**Conclusions:** This present study screened some IRGs associated with the patient's clinical characteristic and prognosis, connect to the immune repertoire, demonstrated the importance of the risk score as a promising biomarker for estimating the clinical prognosis of glioma.

## Background

Glioma is a common and lethal intracranial tumor, which can cause great harm to a person's health [1, 2]. Glioblastoma (GBM) is defined as grade IV glioma, and is the most aggressive type among all of grades [3]. The standard treatment for glioma patients includes maximum surgical resection, radiotherapy and chemotherapy [4–6]. Despite the intervention of these comprehensive regimens, these patients still received bad clinical outcomes: the 5-year survival rate remains at less than 5% and the median survival is only 14 months [7, 8]. Hence, glioma has become one of the most challenging malignancies in the world. Through the development of omics studies, the understanding of the potential molecular mechanisms of glioma has made great progress. However, a number of problems still needs to be further explored, and the identification of the specific molecular markers of glioma may bring opportunities to precisely predict the prognosis and choose a suitable personal medicine for the treatment of glioma.

The human immune system has an important ability to regulate the potential molecular mechanisms of tumors, and a number of evidences have proven the signature of IRGs in the complex regulatory network [9, 10]. Recently, studies on immunotherapy have provided a new perspective to effectively and safely treat some lethal tumors [11, 12]. However, the molecular mechanism of the immunology in glioma still remains unclear, especially its immunogenomic effect [13]. At present, researchers can quickly identify crucial

biomarkers for tumor monitoring and surveillance due to the development of gene expression databases. For example, Li et al investigated the potential molecular mechanisms of IRGs in non-squamous non-small cell lung cancer, which developed an immune signature to create a new individualized treatment [14]. In addition, Lin et al also comprehensively explored the prognostic value of IRGs in papillary thyroid cancer, and found some IRGs that were closely correlated to the clinical prognosis of patients [15]. However, the clinical relevance of IRGs in glioma remains unclear and needs further exploration.

In the present study, the clinical relevance of IRGs in glioma was demonstrated by using the RNA sequencing data from TCGA datasets and was verified in CGGA datasets. The differentially expressed IRGs were selected, survival analysis and the Cox regression model were used to identify the risk score relevant to the seven survival-associated IRGs in these glioma patients. Furthermore, bioinformatics analyses were conducted to investigate the underlying biological functions of IRGs in glioma. In conclusion, the present study provides a novel insight that may have great promotion in the immunotherapy of glioma patients.

## Methods

### Data of glioma samples and IRGs acquisition

The transcriptome RNA-sequencing data and corresponding clinicopathological data of glioma were downloaded from the TCGA datasets (<https://portal.gdc.cancer.gov/>) and the CGGA datasets (<http://www.cgga.org.cn/>), which contained data from 628 (TCGA) and 298 (CGGA) glioma samples. We screened the differentially expressed and survival-related genes in TCGA datasets, and the data of IRGs were through the Immunology Database and Analysis Portal (ImmPort) database (<https://immport.niaid.nih.gov>) [16]. A variety of IRGs supported by ImmPort were a strong basis of immunology research, and provided a number of IRGs for the tumor study. The IRGs downloaded from the ImmPort website had a significant bearing on the initiation and development of immune activity.

### Selection of differentially expressed and survival-related IRGs

In order to identify the IRGs associated with glioma, differentially expressed IRGs between LGG and GBM were selected using the R language software (<http://bioconductor.org/packages/edgeR/>) [17]. Then, a univariate Cox regression analysis of the expressions of IRGs and prognosis of patients in TCGA was performed. Hence, we demonstrated that all differentially expressed IRGs were survival-related IRGs in glioma.

### Molecular characteristics of Hub IRGs

IRGs with clinical applications as hub IRGs were identified, and the clinical values were further systematically explored. The protein-protein interaction (PPI) network, which was based on data obtained from the string online database (<https://stringdb.org/>) [18], which was constructed to determine the interactions between these differentially expressed IRGs. The result of the PPI network was displayed by Cytoscape v3.6.1 [19].

Functional enrichment analyses were also conducted to investigate the underlying biological roles of these differentially expressed IRGs and hub IRGs in glioma. In order to explore molecular mechanisms of these IRGs, the Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.7 (<http://David.abcc.ncifcrf.gov/>) [20], via the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were used to analyze these differentially expressed IRGs [21], and performed the functional and pathway enrichment analyses.  $P < 0.05$  was set as the cutoff criterion.

Since transcription factors (TFs) with significant molecular functions to directly up-regulate and down-regulate the gene expression, it is essential to determine the potential molecular biological functions of TFs to regulate these hub IRGs. Cistrome Cancer is a database that contains 318 TFs, and is a significant tool for computational and experimental tumor research [22]. TFs correlated to the OS were extracted to construct the regulatory network with 20 hub IRGs.

## Development of the immune-related prognostic risk score

To further screening for the functions of these genes, hub IRGs were submitted for multivariate Cox regression analysis, with selecting seven IRGs as independent prognostic indicators to determine the risk score. The risk score was defined using the formula as follows [23]:

$$\text{Risk score} = \sum \text{Coef}_i * x_{ini} = 1.$$

This formula was used to calculate a risk score for each patient both in the training (TCGA) and validation (CGGA) datasets. In order to divided the glioma samples, high risk and low risk groups were established using the median risk score. The prognostic value of the risk score was assessed in patients with different grade of glioma both in the training datasets and validation datasets.

The TIMER online database has been used to visualize and analyze the abundance of infiltrating immune cells in tumors, which integrates a number of samples, including 32 kinds of tumors from TCGA datasets [24]. Furthermore, the TIMER database also has been used to research the infiltration level of six types of immune cells in tumors. Hence, the immune infiltrate levels of glioma patients were downloaded from the TIMER database, and the relationship between the risk core and immune cell infiltration was explored in present study.

## Statistical analysis

The R language v 3.6.1 (<https://www.rproject.org/>) was mainly used to perform the statistical analysis. In addition, the R language v 3.6.1 was used to conduct gene functional enrichment analyses, and determine the areas under the curves (AUC) of the survival, ROC curve, KM survival analysis [25]. Furthermore, the nomogram and risk score were also formulated using the R package. Differences among clinical outcomes were test using independent t-tests. A P-values < 0.05 was considered statistically significant.

## Results

# Differential expression of IRGs

A total of 676 differentially expressed genes were finally identified using the R language v 3.6.1, which included 392 over-expressed and 284 lowly-expressed genes (Figs. 1A and 1B). In addition, 91 differentially expressed IRGs were also selected, which included 66 over-expressed and 25 lowly-expressed genes (Figs. 1C and 1D). Furthermore, the cytokine-cytokine receptor interaction was the most frequently implicated in these differentially expressed IRGs in the KEGG pathways (Fig. 2A). “Behavior”, “extracellular region”, and “cytokine activity”, were the most frequent biological terms among cellular component, biological process, and molecular function (Table 1).

Table 1  
GO analysis of differentially expressed IRGs

ID	Description	PValue	Count
Biological process			
GO:0007610	behavior	1.35E-21	30
GO:0006935	chemotaxis	1.72E-19	20
GO:0042330	taxis	1.72E-19	20
GO:0006955	immune response	4.87E-18	31
GO:0007626	locomotory behavior	1.63E-17	22
GO:0006952	defense response	2.71E-16	28
GO:0007267	cell-cell signaling	1.62E-15	27
Molecular function			
GO:0005125	cytokine activity	4.73E-30	28
GO:0008009	chemokine activity	3.51E-17	13
GO:0042379	chemokine receptor binding	8.19E-17	13
GO:0008528	peptide receptor activity, G-protein coupled	1.58E-09	11
GO:0001653	peptide receptor activity	1.58E-09	11
GO:0005179	hormone activity	1.73E-08	10
GO:0042277	peptide binding	3.95E-07	11
GO:0019955	cytokine binding	4.27E-06	8
GO:0008083	growth factor activity	5.81E-06	9
GO:0008188	neuropeptide receptor activity	0.00182191	4
Cellular component			
GO:0005576	extracellular region	5.82E-27	60
GO:0005615	extracellular space	2.68E-25	39
GO:0044421	extracellular region part	4.17E-22	41
GO:0005887	integral to plasma membrane	0.003102973	18
GO:0031226	intrinsic to plasma membrane	0.003912249	18
GO:0042105	alpha-beta T cell receptor complex	0.027565322	2

IRGs, Immune-related genes; GO, Gene Ontology

ID	Description	PValue	Count
GO:0042101	T cell receptor complex	0.080461603	2
IRGs, Immune-related genes; GO, Gene Ontology			

## Selection of survival-associated IRGs

Since the OS is crucial for clinical diagnosis and treatment, we focused our efforts on identifying the potential molecular biomarkers which could affect the prognosis or assist in the diagnosis of diseases. In the present study, to extract survival-associated IRGs, we selected differentially expressed IRGs were related to the OS ( $P < 0.05$ ). Finally, all differentially expressed IRGs were crucially related to the OS in glioma patients after screening (Additional file 2: table S2).

## Identification of Hub IRGs

The PPI network analysis proved the 20 hub genes among these survival-associated IRGs (Figs. 2C and 2D). We found that the inflammatory pathway was the most often enriched by these hub IRGs, the chemokine signaling pathway and cytokine-cytokine receptor interaction was the most frequently implicated in these hub IRGs in the KEGG pathways (Fig. 2B). “cell”, “immune response”, and “C-C chemokine receptor activity”, were the most frequent biological terms among cellular component, biological process, and molecular function (Table 2). A forest plot demonstrated 8 hub IRGs were lowly-expressed and 12 hub IRGs were over-expressed in glioma samples (Fig. 2E). Owing to the significant clinical value of these IRGs, we embarked on a comprehensive exploration of their molecular characteristics. Moreover, genetic alterations (mutation and copy number change) of these hub IRGs were examined and the frequencies were very low ( $< 0.8\%$ ) in glioma (Fig. 3), demonstrating that the different expressions of these Hub IRGs in glioma were not caused by genetic alterations.

Table 2  
GO analysis of 20 IRGs

ID	Description	PValue	Count
Biological process			
GO:0006955	immune response	2.27E-08	7
GO:2000406	positive regulation of T cell migration	4.73E-05	3
GO:0006954	inflammatory response	5.98E-04	4
GO:0006935	chemotaxis	8.75E-04	3
GO:0060326	cell chemotaxis	0.001416464	3
GO:0032467	positive regulation of cytokinesis	0.033206267	2
GO:0007186	G-protein coupled receptor signaling pathway	0.084203345	2
Cellular component			
GO:0005623	cell	0.007592404	3
GO:0005615	extracellular space	0.054033918	4
GO:0016021	integral component of membrane	0.054979621	10
GO:0005576	extracellular region	0.079108856	3
Molecular function			
GO:0016493	C-C chemokine receptor activity	5.06E-05	3
GO:0004966	galanin receptor activity	0.004433	2
GO:0004994	somatostatin receptor activity	0.005539	2
IRGs, Immune-related genes; GO, Gene Ontology			

## TF-based Network with Hub IRGs

In order to explore the regulatory biomolecular function of these hub IRGs that corresponded to the clinical outcomes, the potential molecular mechanisms of survival-associated IRGs were researched. The expression of 318 TFs were detected, and 9 differentially expressed TFs between the GBM and LGG samples were selected (Figs. 4A and 4B). Among these 9 differentially expressed TFs, all of them were correlated with the OS of glioma patients, 8 TFs were up-regulated while 1 TF was down-regulated in glioma samples (Fig. 4C). Furthermore, a gene regulatory framework was established based on the 9 TFs and 20 hub IRGs (Fig. 4D). The regulatory relationships between these survival-associated TFs and hub IRGs were illustrated by the regulatory TF-based network.

# The risk scores associated with clinicopathological features

The expression of seven IRGs were selected to determine the risk score based on the minimum criteria, and the coefficients obtained from the least absolute shrinkage and selection operator (LASSO) in TCGA dataset. Then, the risk score was used to separate the glioma samples into high-risk and low-risk groups with differentially clinicopathological features (Fig. 5). The risk score played a crucial role in the identification of glioma patients, according to the potential differential clinical characteristics ( $P < 0.05$ ) (Fig. 6A). In addition, the ROC curve suggested a very good predictive performance for the risk score based on IRGs in predicting glioma patient clinical outcomes ( $AUC = 0.885$ , Fig. 6B). It was also found that the risk score which was as a prognostic factor in different grades of glioma ( $P < 0.05$ ) (World Health Organization [WHO] grade II, and III and GBM) (Figs. 6C-6E). Furthermore, the univariate Cox regression analysis and the multivariate Cox regression analysis demonstrated that the risk score could become an independent prognostic predictor after other parameters were adjusted, including age, grade, gender, IDH status and 1p/19q code1 status both in the training dataset (TCGA) (Fig. 7A, Fig. 7C) and the validation dataset (CGGA) (Fig. 7B, Fig. 7D). Further, we also found the risk score could be a prognostic factor in all grade glioma and GBM groups in CGGA dataset ( $P < 0.05$ ) (Figs. 7E-7F). This conclusion confirmed the risk score derived from seven IRGs could be considered as an independent prognosis predictor in glioma patients. The clinical outcomes of seven survival-associated IRGs were also explored both in the training dataset (TCGA) (Table 3) and the validation dataset (CGGA) (Additional file 3: Table S3). We also examined the relationships between the risk score and each clinical characteristic, including age, grade, gender, IDH status and 1p/19q code1 status. The risk score was significantly higher in old age, 1p/19q non-code1, IDH-wildtype and WHO  $\geq$ , no difference was observed in gender (Fig. 8B) in both the TCGA (Figs. 8A-8E) and CGGA (Additional file 1: Fig.S1) datasets. In order to determine whether the risk score could reflect the GBM immune microenvironment status, the relationships between the risk score and infiltration level of immune cells were described (Figs. 9A-9F).

Table 3

The relationship between the expression of seven IRGs and the clinical characteristics in TCGA dataset

Genes	Grade (IV/ II-III)		Gender (Male/Female)		Age ( $\geq 60/\leq 60$ )		1p19q (Codel/Non-Codel)		IDH (Mutant/Wildtype)	
	t	P	t	P	t	P	t	P	t	P
SSTR5	16.801	< 0.001	0.374	0.708	5.264	< 0.001	3.6	< 0.001	8.937	< 0.001
CXCL10	-21.867	< 0.001	-1.168	0.243	-7.15	< 0.001	-6.213	< 0.001	-11.806	< 0.001
CCL13	-6.43	< 0.001	-1.366	0.172	-2.85	< 0.001	-2.873	< 0.001	-5.311	< 0.001
SAA1	-19.463	< 0.001	-1.714	0.087	-7.822	< 0.001	-8.771	< 0.001	-13.266	< 0.001
CCL21	6.234	< 0.001	0.982	0.327	1.252	0.212	1.7385	0.084	3.026	0.003
CCL27	-9.846	< 0.001	-2.319	0.021	-3.91	< 0.001	-5.389	< 0.001	-8.141	< 0.001
HTR1A	19.106	< 0.001	0.82	0.412	7.262	< 0.001	4.268	< 0.001	9.806	< 0.001

IRGs, Immune-related genes; TCGA, The Cancer Genome Atlas;

## Discussion

Although it has been confirmed that IRGs play an important role in tumorigenesis and tumor progression, there is still a need to make a comprehensive genome-wide profiling research to determine the relationship between molecular biological functions and patient clinical characteristics [26]. The integrated analysis in the present study provides a better understanding of the clinical characteristics of IRGs and clarifies the molecular biological functions of IRGs. Several IRGs have played an important role in the initiation and development of glioma, which may act as crucial biomarkers to assist in the diagnosis and treatment of patients. Furthermore, the risk score based on the seven survival-associated IRGs was used to measure the infiltration level of six type immune cells and assessed the OS of glioma patients.

Although biological technology and equipment have greatly progressed present, the underlying specific molecular mechanisms of IRGs in glioma still remains unclear. In previous studies, some researchers had explored the differential expression of several IRGs among different grades of glioma, and provided a novel and comprehensive perspective in the mechanism of glioma progression in the genetic molecule level [27, 28]. However, a system analysis has not been performed on the characteristics of IRGs in glioma, to date. Hence, the study of the tumor immune microenvironment is a crucial part for investigations on the immunotherapy of glioma.

Since alterations to the genome were associated with the invasive characteristics of tumor cells, focus was given on this analysis to clarify the potential relationships between the immunogenomic profile and immune microenvironment in tumors, which might connect with patient's OS. The gene functional enrichment analysis in the present study demonstrated that these differentially expressed IRGs were mainly enriched in cytokine-cytokine receptor interactions and chemokine signaling pathways. The chemokine system actively participates in the development of central nervous system tumors and is involved in the pathogenesis of glioma. Furthermore, chemokines and their receptors were interrelated with chemotaxis, leukocyte infiltration, invasiveness and the promotion of glioma cell proliferation. The bioinformatics analysis demonstrated that the upregulation of IRGs could promote the initiation and development of glioma through the influence of some inflammatory cells and pathways.

In order to explore potential molecular mechanisms of IRGs, TFs regulated the network with IRGs were constructed to determine whether several crucial TFs could up-regulate or down-regulate the present hub IRGs. HOXC11, HOXC9, PAX3, ELF5, GATA4, HNF4A, HOXA9 and HOXB13 occupied a prominent position in this network. To date, no literature has been reported on the molecular biological functions of HOXC11, HOXC9, ELF5 and HNF4A in regulating the initiation of glioma, and the present study has provided limited information on the functions of other TFs in regulating glioma [29–31].

The risk score was chosen as a tool to monitor the immune microenvironment and suggested the OS in glioma patients. In addition, seven survival-associated IRGs (SSTR5, CXCL10, CCL13, SAA1, CCL21, CCL27 and HTR1A) were selected by the LASSO to calculate the risk score in TCGA dataset. Considering that the underlying molecular mechanism corresponding to the potential clinical value of these seven survival-associated IRGs, among these genes, SSTR5 is downregulated in glioma and inhibits glioma cell proliferation [32], while CXCL10 and CCL20 is upregulated and promotes glioma cell proliferation [33–34]. SAA2 can increase migration and invasion behaviors of glioma cell [35]. No literature has been reported on the molecular biological functions of HTR1A in glioma at present. Overall, these studies provided little information on the molecular biological functions of these survival-associated IRGs in glioma.

The nomogram has been extensively applied to calculate the clinical risk factors and predict clinical prognosis in many tumors, which has shown favorable effects [36, 37]. Hence, the risk score was connected with the clinical characteristics (grade, gender, age, IDH status, 1p/19q code), and a nomogram was constructed to prove that the risk score has a great performance in predicting the OS of glioma patients. In addition, the nomogram could also predict the 5-year survival rate in the differential risk score groups via the KM survival analysis. According to the nomogram established in the present study, the risk score based on seven survival-associated IRGs were demonstrated as an independent prognostic factor, which may provide new evidences for the treatment of glioma.

In order to explore the tumor-immune interactions, it is necessary to characterize the immune infiltration landscape. The relationship between risk score and level of immunocyte infiltration was established to determine the regulation mechanism of the immune microenvironment in GBM. In the present study, we demonstrated the infiltrations level of B\_ cell and Neutrophil were evidently positively correlated with the risk score, while CD\_4 T cell infiltration level was significantly negatively correlated with the risk score. These

results indicated that the higher infiltration levels of B\_ cell, Neutrophil and lower CD\_4 T cell might be observed in high-risk patients.

These results suggested that immune cells played an important role in the pathogenesis of glioma, and it was also confirmed that the risk score could be regarded as a potential predictor for monitoring the infiltration level of immune cells. Previously, Ge J et al demonstrated that glioma patients had significantly upregulated frequencies of CD4 + cells, when compared to healthy controls [38]. Sokratous G et al suggested that the infiltration level of cytotoxic T cell in glioma could importantly affect the clinical prognosis [39]. However, the biological function of these immune cells in glioma has not been fully and comprehensively explored. The analysis in the present study can provide novel insights for solving several problems, and be a foundation for more in-depth and high-quality researches in the treatment of glioma patients.

There were still some limitations in the present research. First, the samples did not contain information about the excision scope of glioma that connected to a patient's prognosis. Hence, the collection of more thorough and comprehensive information needs to be explored in the future. In addition, without verifying with an independent cohort, the credibility of these present research results remains to be challenged. Furthermore, the lack of an in vitro or in vivo experiment was also a limitation of the present study.

## Conclusions

In the future, certain problems still need to be explored. For example, the potential relationship between immunogenomics and proteomics needs to be further explored to clarify the immunologic varieties in glioma. Furthermore, the relationships between disturbed immuno-genomes and premalignant lesions also need to be deeply explored. In the present study, it demonstrates that IRGs play a significant role in the initiation and progression of glioma, and that the risk score is closely correlated to the clinical prognosis of patients. The present study provides a perspective that can help establish new and effective immunotherapeutic approaches for glioma.

## Abbreviations

IRGs: Immune-related genes; TCGA: The Cancer Genome Atlas; LGG: Low grade glioma; GBM: Glioblastoma; CGGA: Chinese Glioma Genome Atlas; ImmPort: Immunology Database and Analysis Portal; ROC: Receiver operating characteristic; KM: Kaplan-Meier; PPI: Protein-protein interaction; DAVID: Database for Annotation, Visualization and Integrated Discovery; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; TFs: Transcription factors; AUC: Areas under the curves; ROC: Receiver operating characteristic; LASSO: Least absolute shrinkage and selection operator; WHO: World Health Organization.

## Declarations

### Acknowledgments

Not applicable.

## Author's contributions

HL and CT were responsible for the conception and design. KH and MW were responsible for data collection and analysis. MY and SL drafted the part of methods and results. XZ drafted and revised this article. All authors reviewed, edited, and approved the final manuscript.

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## Availability of data and materials

Data for this study were obtained from the TCGA and CGGA datasets.

## Ethics approval and consent to participate

Because patient data in the TCGA and CGGA datasets were de-identified, signed informed consent was waived in this study.

## Consent to publish

Not applicable.

## Competing interests

The authors declare no competing interest with study.

## References

1. Chai R, Zhang K, Wang K, et al. A novel gene signature based on five glioblastoma stem-like cell relevant genes predicts the survival of primary glioblastoma. *J Cancer Res Clin Oncol*. 2018; 144 (3): 439-447.
2. Jiang T, Mao Y, Ma W, et al. CGCG clinical practice guidelines for the management of adult diffuse gliomas. *Cancer Lett*. 2016; 375 (2): 263–273.
3. Jouvett A, Scheithauer BW, Kleihues P. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol*, 2007 (2); 114: 97-109.
4. Chang CH, Liu WT, Hung HC, et al. Synergistic inhibition of tumor growth by combination treatment with drugs against different subpopulations of glioblastoma cells. *BMC Cancer*. 2017; 17 (1): 905.
5. Peeken JC, Goldberg T, Pyka T, et al. Combining multimodal imaging and treatment features improves machine learning-based prognostic assessment in patients with glioblastoma multiforme. *Cancer Med*. 2019; 8 (1): 128-136.
6. Stavrinou P, Kalyvas A, Grau S, et al. Survival effects of a strategy favoring second-line multimodal treatment compared to supportive care in glioblastoma patients at first progression. *J Neurosurg*. 2018; 1: 1-6.

7. Czarnek N, Clark K, Peters KB, et al. Algorithmic three-dimensional analysis of tumor shape in MRI improves prognosis of survival in glioblastoma: a multi-institutional study. *J Neurooncol.* 2017; 132(1): 55-62.
8. Capellades J, Puig J, Domenech S, et al. Is a pretreatment radiological staging system feasible for suggesting the optimal extent of resection and predicting prognosis in glioblastoma? An observational study. *J Neurooncol.* 2018; 137 (2): 367-377.
9. Fridman WH, Zitvogel L, Sautès-Fridman C, et al. The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol.* 2017,14 (12): 717-734.
10. Mizukoshi E, Kaneko S. Immune cell therapy for hepatocellular carcinoma. *J Hematol Oncol.* 2019; 12 (1): 52.
11. Popovic A, Jaffee EM, Zaidi N. Emerging strategies for combination checkpoint modulators in cancer immunotherapy. *J Clin Invest.* 2018; 128 (8): 3209–3218.
12. Carter BW, Halpenny DF, Ginsberg MS, et al. Immunotherapy in Non-Small Cell Lung Cancer Treatment: Current Status and the Role of Imaging. *J Thorac Imaging.* 2017; 32 (5): 300–312.
13. Ampie L, Woolf EC, Dardis C. Immunotherapeutic advancements for glioblastoma. *Front Oncol.* 2015 5:12.
14. Li B, Cui Y, Diehn M, et al. Development and Validation of an Individualized Immune Prognostic Signature in Early-Stage Nonsquamous Non-Small Cell Lung Cancer. *JAMA Oncol.* 2017; 3 (11): 1529-1537.
15. Lin P, Guo YN, Shi L, et al. Development of a prognostic index based on an immunogenomic landscape analysis of papillary thyroid cancer. *Aging (Albany NY).* 2019; 11 (11): 480-500.
16. Bhattacharya S, Dunn P, Thomas CG, et al. ImmPort, toward repurposing of open access immunological assay data for translational and clinical research. *Sci Data.* 2018; 5: 180015.
17. Dai Z, Sheridan JM, Gearing LJ, et al. edgeR: a versatile tool for the analysis of shRNA-seq and CRISPR-Cas9 genetic screens. *F1000Res.* 2014; 3: 95.
18. Qian Z, Li Y, Fan X, et al. Molecular and clinical characterization of IDH associated immune signature in lower-grade gliomas. *Oncoimmunology.* 2018; 7 (6): e1434466.
19. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* 2019; 47 (D1): 607-613.
20. Reimand J, Isserlin R, Voisin V, et al. Pathway enrichment analysis and visualization of omics data using g: Profiler, GSEA, Cytoscape and EnrichmentMap. *Nat Protoc.* 2019; 14 (2): 482-517.
21. Dennis G Jr, Sherman BT, Hosack DA, et al. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol.* 2003; 4 (5): P3.
22. Zhao Z, Bai J, Wu A, et al. Co-LncRNA: investigating the lncRNA combinatorial effects in GO annotations and KEGG pathways based on human RNA-Seq data. *Database (Oxford).* 2015;10: 2015.
23. Li S, Wan C, Zheng R, et al. Cistrome-GO: a web server for functional enrichment analysis of transcription factor ChIP-seq peaks. *Nucleic Acids Res.* 2019; 47 (8): 206-11.

24. Li T, Fan J, Wang B, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. *Cancer Res.* 2017; 77 (21): 108-110.
25. Kamarudin AN, Cox T, Kolamunnage-Dona R. Time-dependent ROC curve analysis in medical research: current methods and applications. *BMC Med Res Methodol.* 2017; 17 (1): 53.
26. Johnston MJ, Nikolic A, Ninkovic N, et al. High-resolution structural genomics reveals new therapeutic vulnerabilities in glioblastoma. *Genome Res.* 2019; 29 (8): 1211-22.
27. Wu DM, Wang S, Wen X, et al. Long noncoding RNA nuclear enriched abundant transcript 1 impacts cell proliferation, invasion, and migration of glioma through regulating miR-139-5p/ CDK6. *J Cell Physiol.* 2019; 234 (5): 5972-5987.
28. Wang R, Wei J, Li Z, et al. Bioinformatical analysis of gene expression signatures of different glioma subtypes. *Oncol Lett.* 2018; 15 (3): 2807-2814.
29. Misuraca KL, Hu G, Barton KL, et al. A Novel Mouse Model of Diffuse Intrinsic Pontine Glioma Initiated in Pax3-Expressing Cells. *Neoplasia.* 2016; 18 (1): 60-70.
30. Xu Y, Xu W, Lu T, et al. miR-126 affects the invasion and migration of glioma cells through GATA4. *Artif Cells Nanomed Biotechnol.* 2017;45(1):1-7.
31. Xiong Y, Kuang W, Lu S, et al. Long noncoding RNA HOXB13-AS1 regulates HOXB13 gene methylation by interacting with EZH2 in glioma. *Cancer Med.* 2018; 7 (9): 4718-4728.
32. Barbieri F, Pattarozzi A, Gatti M, et al. Differential efficacy of SSTR1, -2, and -5 agonists in the inhibition of C6 glioma growth in nude mice. *Am J Physiol Endocrinol Metab.* Nov 2009; 297 (5): E1078-1088.
33. Maru SV, Holloway KA, Flynn G, et al. Chemokine production and chemokine receptor expression by human glioma cells: role of CXCL10 in tumour cell proliferation. *J Neuroimmunol.* Aug 13 2008;199(1-2):35-45.
34. Zhai H, Heppner FL, Tsirka SE. Microglia/macrophages promote glioma progression. *Glia.* Mar 2011; 59 (3): 472-485.
35. Knebel FH, Albuquerque RC, Massaro RR, Maria-Engler SS, Campa A. Dual effect of serum amyloid A on the invasiveness of glioma cells. *Mediators Inflamm.* 2013;2013:509089.
36. Chen T, Xu L, Ye L, et al. A new nomogram for recurrence-free survival prediction of gastrointestinal stromal tumors: Comparison with current risk classification methods. *Eur J Surg Oncol.*2019; 45:1109-1114.
37. Chen J, Fang A, Chen M, et al. A novel inflammation-based nomogram system to predict survival of patients with hepatocellular carcinoma. *Cancer Med.* 2018; 7 (6): 5027-5035.
38. Ge J, Zhao L, Li G, et al. Cytotoxic CD4(+) T cells are correlated with better prognosis in Han Chinese grade II and grade III glioma subjects and are suppressed by PD-1 signaling. *Int J Neurosci.* 2017; 127 (5): 386-395.
39. Sokratous G, Polyzoidis S, Ashkan K. Immune infiltration of tumor microenvironment following immunotherapy for glioblastoma multiforme. *Hum Vaccin Immunother.* 2017; 13 (11): 2575–2582.

## Supplementary Information

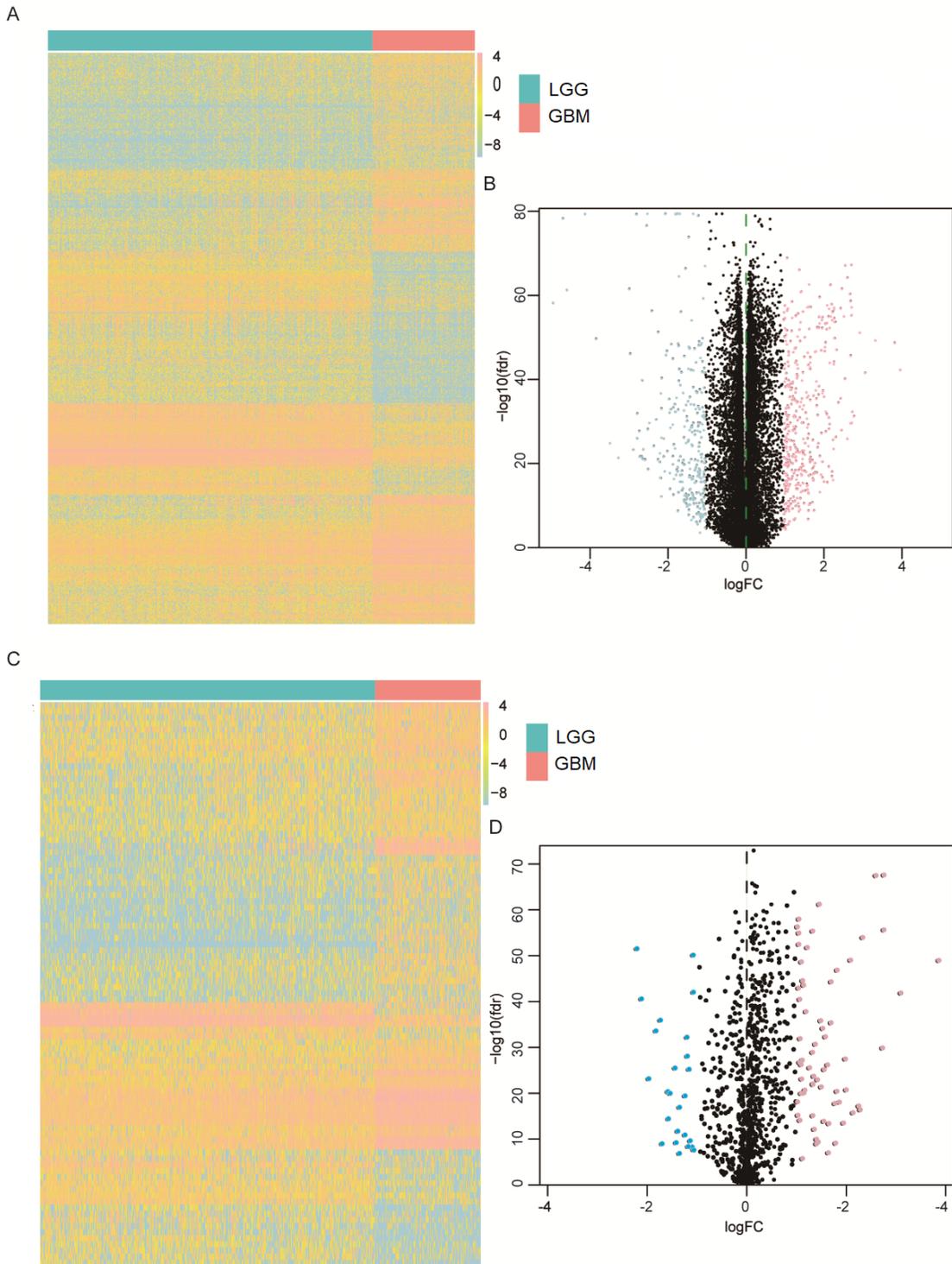
**Additional file 1: Fig S1.** The relationship between the risk score and the clinical characteristics in CGGA datasets. Age (A); gender (B); 1p/19q code status (C); IDH status (D); grade (E).

**Additional file 2: Table S1.** Clinicopathological features of patients included in this study.

**Additional file 3: Table S2.** General characteristics of survival-associated IRGs.

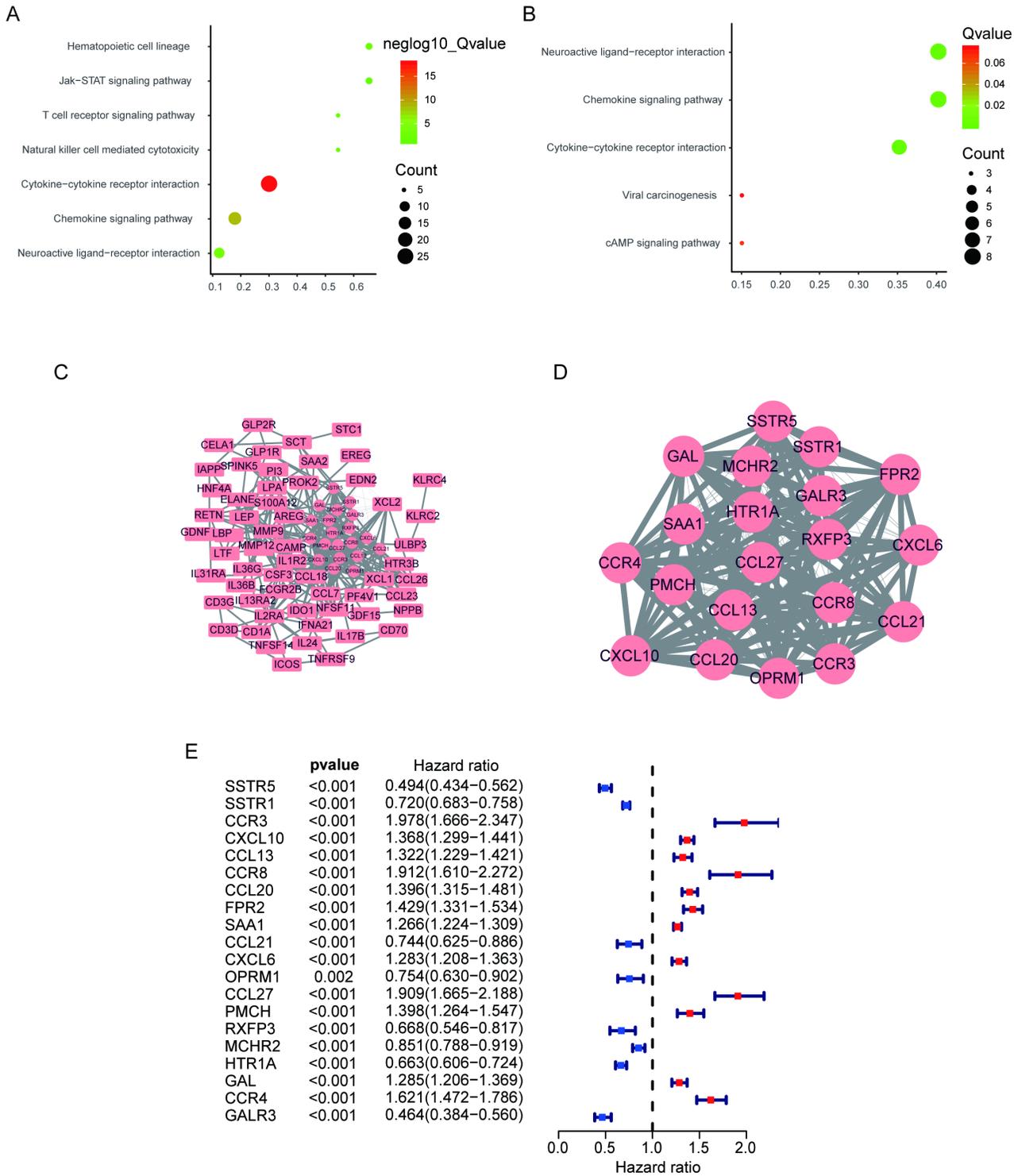
**Additional file 4: Table S3.** The relationship between the expression of the seven IRGs and the clinical characteristics in CGGA dataset.

## Figures



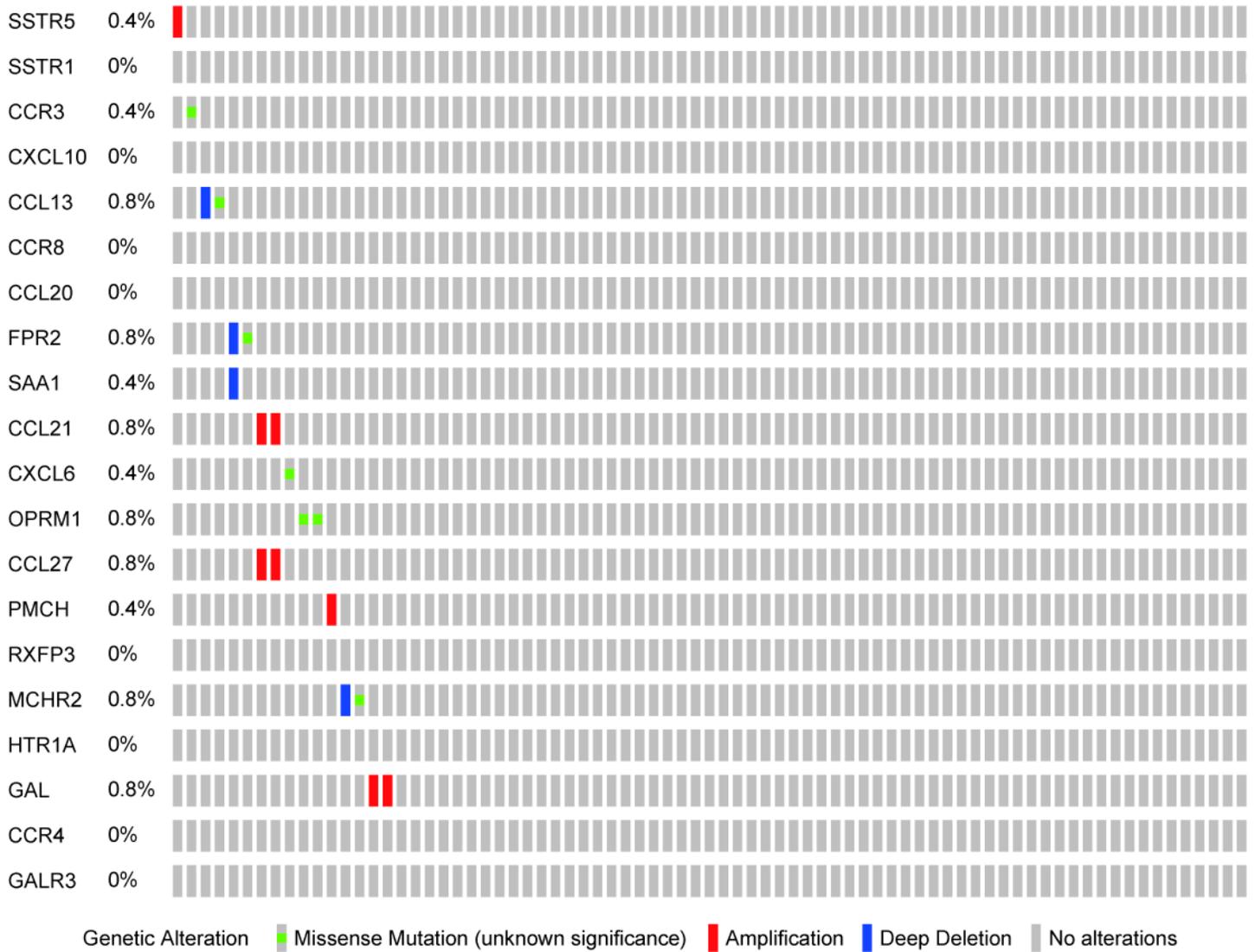
**Figure 1**

Selection differentially expressed IRGs. The differentially expressed genes were selected by constructing the Heatmap (A) and volcano plot (B) between LGG and GBM. The differentially expressed IRGs were also selected using the heatmap (C) and volcano plot (D).



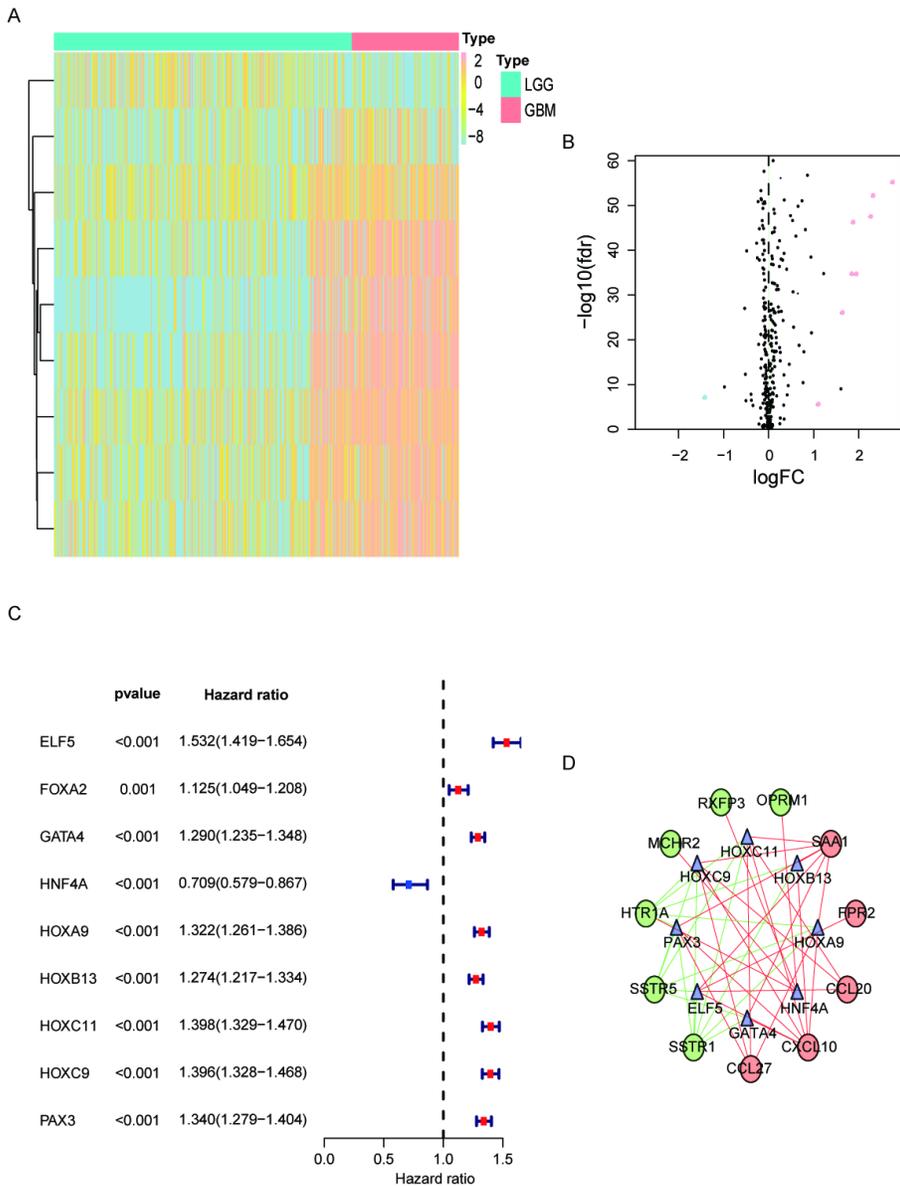
**Figure 2**

Expression profiles of survival-associated IRGs. The KEGG pathways of these survival-associated IRGs (A) and hub IRGs (B). The PPI network of 91 survival-associated IRGs (C) and 20 hub IRGs (D). The forest plot showing the prognostic values of hub IRGs (E).



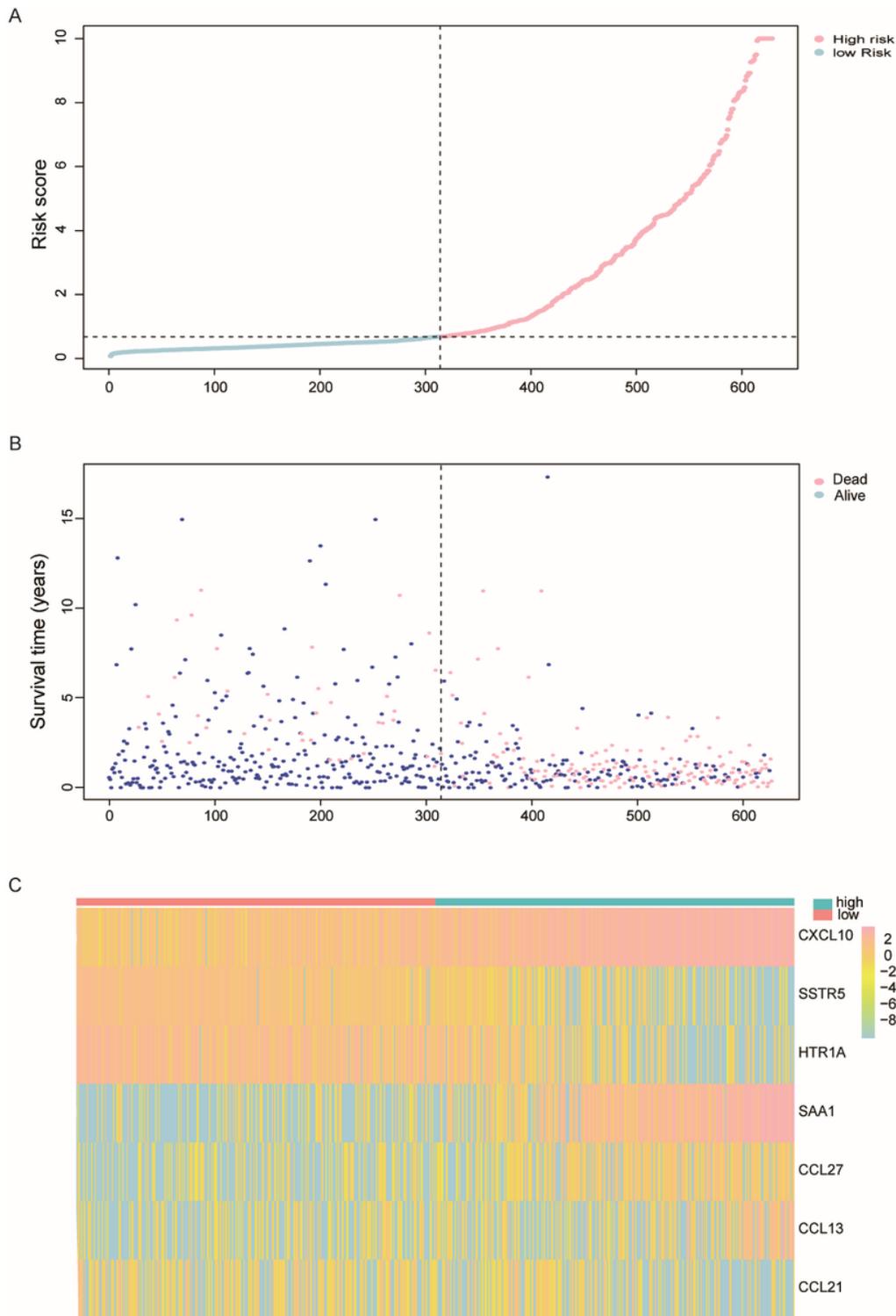
**Figure 3**

Genetic changes of 20 hub IRGs. The frequencies of genetic change (mutation or coy number change) of the 20 hub genes were very low ( $\leq 0.8\%$ ) in glioma samples.



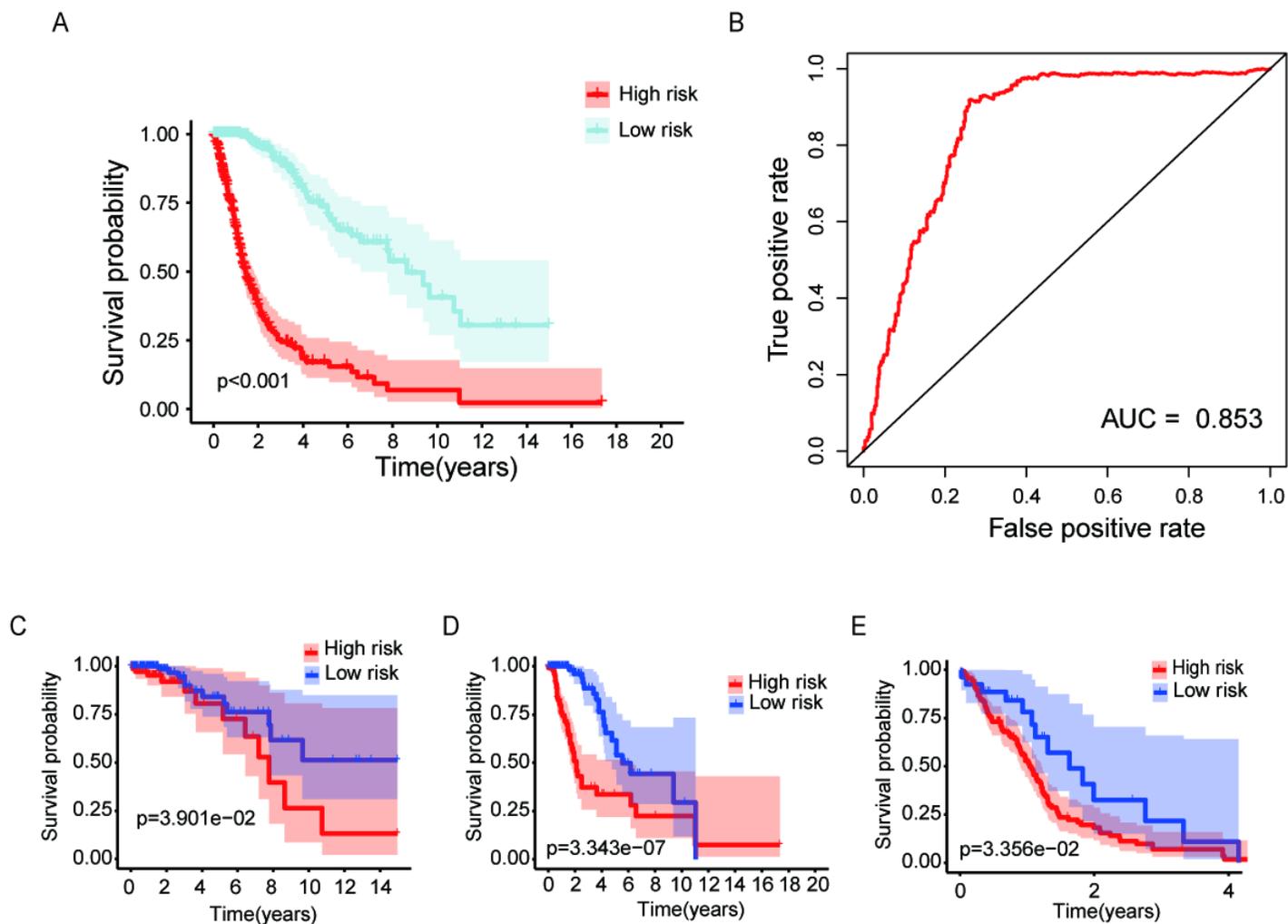
**Figure 4**

TF-based network with hub IRGs. Differentially expressed TFs were selected using a heatmap (A) and volcano plot (B). The forest plot showing the prognostic values of these differentially expressed TFs. The network based on potential regulatory mechanisms between survival-associated TFs and hub IRGs.



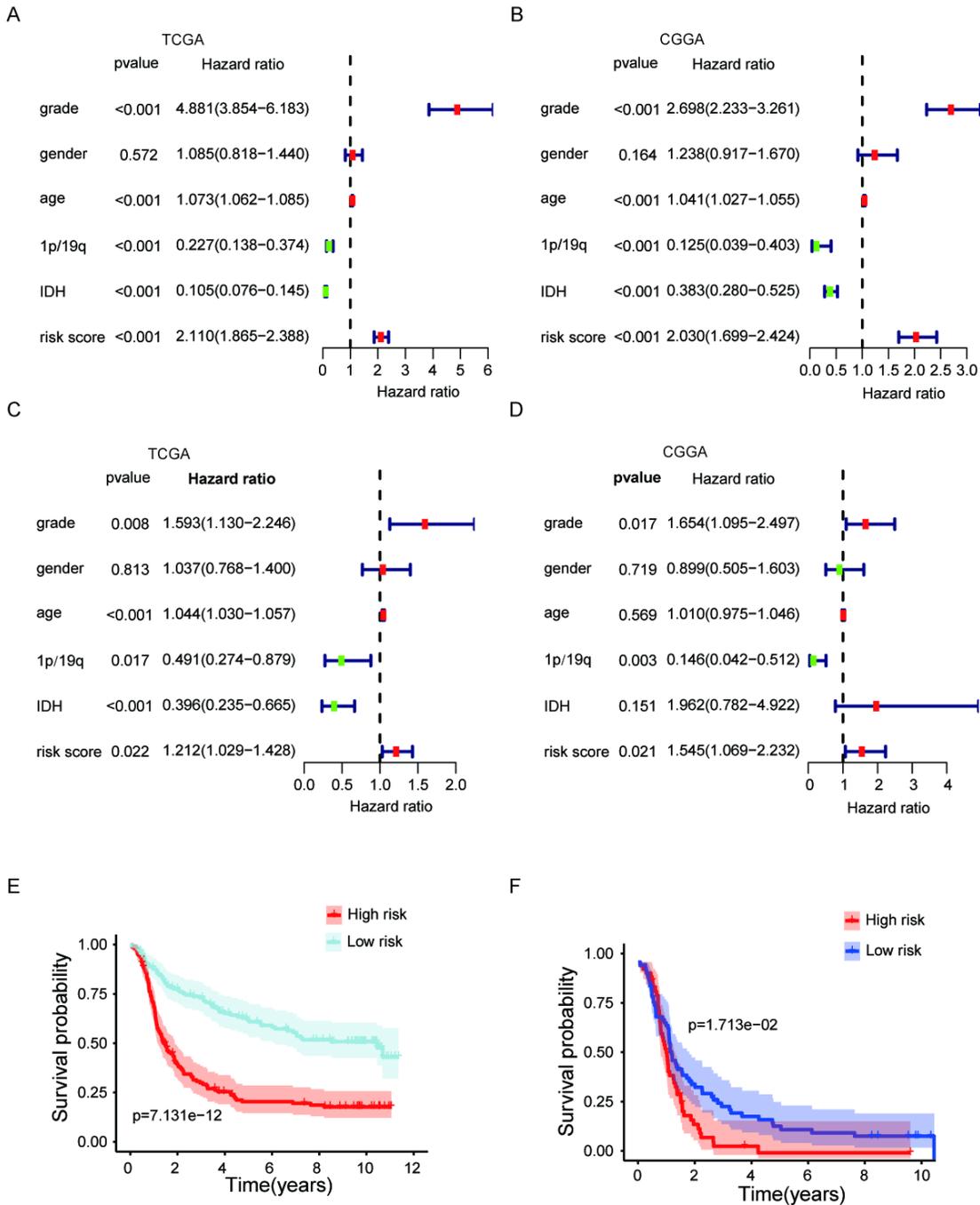
**Figure 5**

The risk score based on the seven IRGs. The distribution of seven IRGs and rank of the risk score for each patient (A). Survival status of patients in the high-risk and low-risk groups (B). The expression levels of the seven IRGs in the heatmap (C).



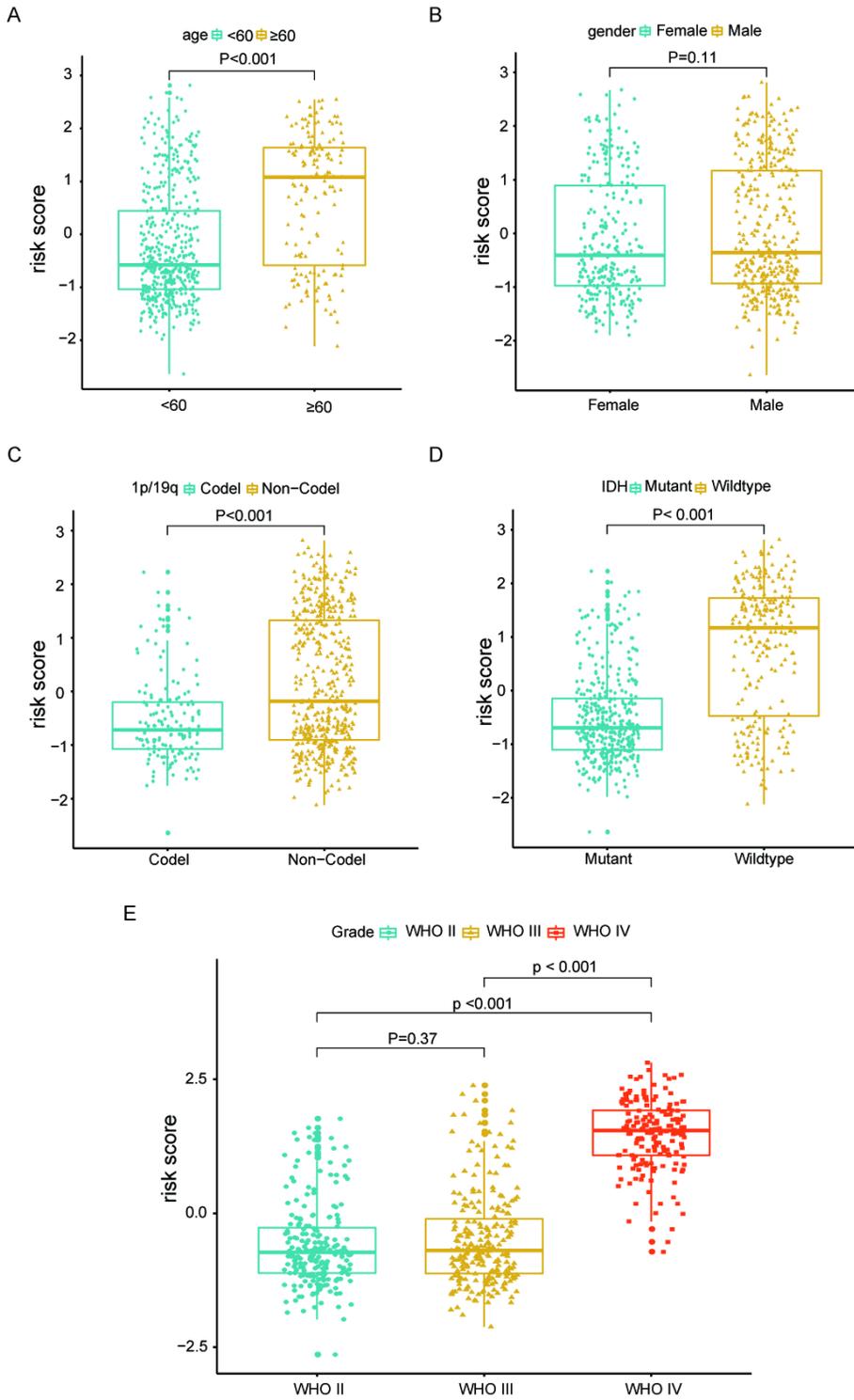
**Figure 6**

The nomogram based on the risk score for predicting the OS in TCGA datasets. The survival analysis for predicting the clinical prognosis (A). The area under the curve of AUCs was 0.885 (B). The subgroup survival analysis performed in 2-grade glioma (C), 3-grade glioma (D), glioblastoma (E).



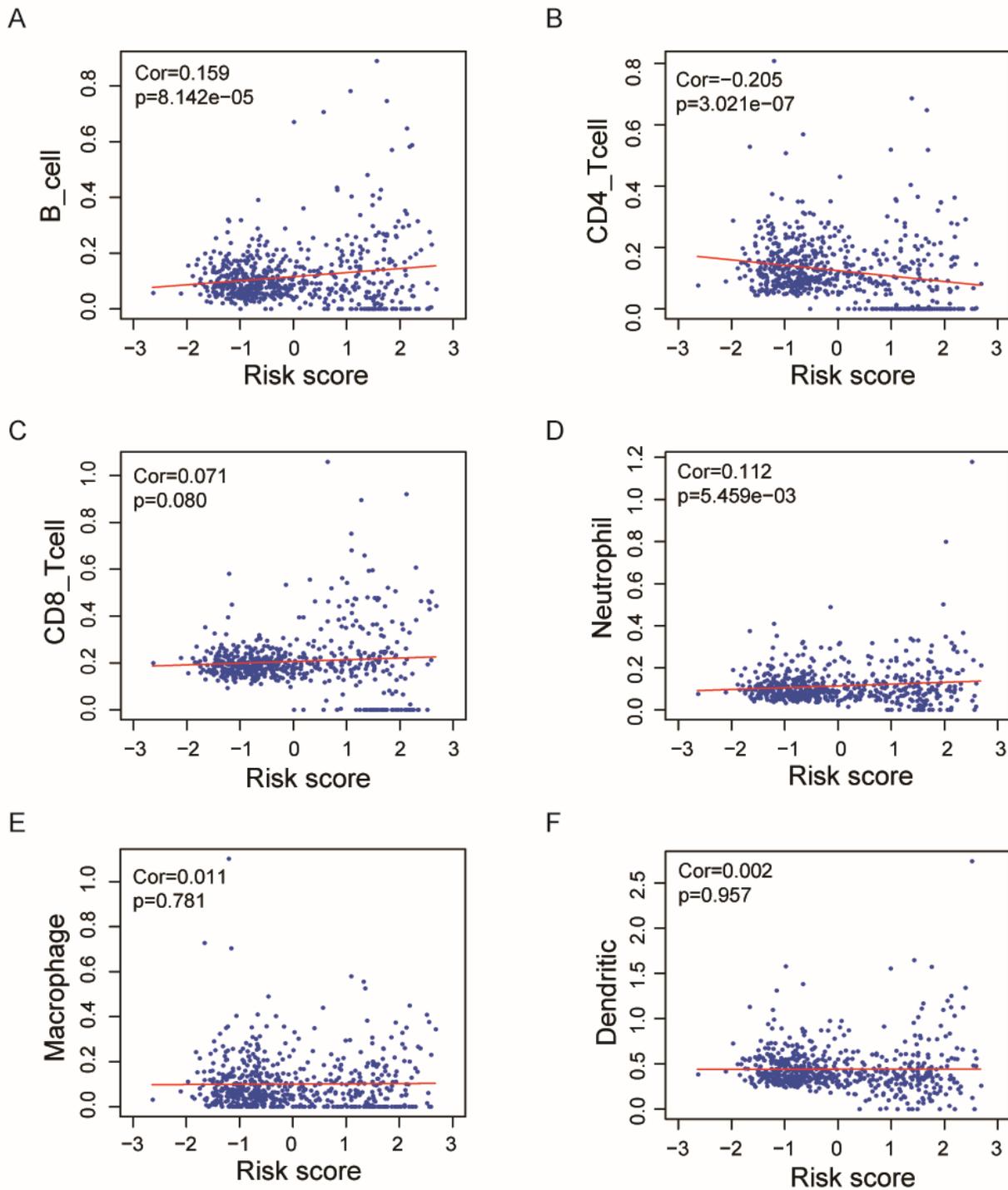
**Figure 7**

Relationship between clinicopathological features and the OS. Univariate and multiple Cox regression analysis of the association between clinicopathological factors and OS in the TCGA (A and B) and CGGA (C and D) datasets; survival analysis for predicting the clinical prognosis (E) and performed in glioblastoma (F) in CGGA datasets.



**Figure 8**

The relationship between the risk score and the clinical characteristics in TCGA datasets. Age (A); gender (B); 1p/19q codel status (C); IDH status (D); grade(E).



**Figure 9**

The relationships between the risk score and infiltration level of the six types immune cells. B\_ cell (A); CD\_4 T cell (B); CD8\_ T cell (C); Neutrophil (D); Macrophage (E); Dendritic (F).

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS2.docx
- FigureS1.tif
- TableS1.docx
- TableS3.docx