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Title: Comprehensive analysis of pyroptosis-associated in molecular classification, immunity and prognostic of glioma

Running head: Pyroptosis and glioma

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Abstract

Integrative analysis was performed in the Chinese Glioma Genome Atlas and The Cancer Genome Atlas to describe the pyroptosis-associated molecular classification and prognostic signature in glioma. Pyroptosis-related genes were used for consensus clustering and to develop a prognostic signature. The immune statuses, molecular alterations and clinical features of differentially expressed genes were analyzed among different subclasses and risk groups. A lncRNA-miRNA-mRNA network was built, and drug sensitivity analysis was used to identify small molecular drugs for the identified genes. Glioma can be divided into two subclasses using 30 pyroptosis-related genes. Cluster 1 displayed high immune signatures and poor prognosis as well as high immune-related function scores. A prognostic signature based on 15 pyroptosis-related genes of the CGGA cohort can predict the overall survival of glioma and was well validated in the TCGA cohort. Cluster 1 had higher risk scores. The high-risk group had high immune cell and function scores and low DNA methylation of pyroptosis-related genes. The differences in pyroptosis-related gene mutations and somatic copy numbers were significant between the high-risk and low-risk groups. The ceRNA regulatory network uncovered the regulatory patterns of different risk groups in glioma. Nine pairs of target genes and drugs were identified. In vitro, CASP8 promotes the progression of glioma cells. Pyroptosis-related genes can reflect the molecular biological and clinical features of glioma subclasses. The established prognostic signature can predict prognosis and distinguish molecular alterations in glioma patients. Our comprehensive analyses provide valuable guidelines for improving glioma patient management and

individualized therapy.

Keywords: Glioma, pyroptosis, prognostic signature, Tumor immunity, clinical nomogram

INTRODUCTION

Gliomas are the most common types of primary tumors in the central nervous system and one of the most devastating tumors(1). At present, the main treatment methods of glioma are surgical resection, radiotherapy, chemotherapy or chemoradiotherapy(2). Although great efforts have been made to improve glioma treatment, the prognosis of glioma patients remains poor(3). One of the main reasons is that the molecular mechanism is still not fully understood. Therefore, the exploration and research of the underlying mechanism of gliomas and identification of potential treatment targets followed by application in clinical practice have important theoretical and practical significance.

Pyroptosis is one of the pathways involved in programmed cell death, such as apoptosis, ferroptosis, necroptosis, and autophagy.(4) Cookson et al. first used pyroptosis to describe the caspase-1-dependent pattern of cell death found in macrophages(5). Pyroptosis, distinct from apoptosis and necrosis, contributes to a range of human diseases as a new mechanism of cell death. Pyroptosis is a proinflammatory form of programmed cell death that is dependent on the activity of caspase acid-specific proteases(6). In the coupling of the amino-terminal and carboxy-terminal linkers of gasdermin D (GSDMD) by caspases, the latter is displaced onto the membrane and perforated, inducing moisture penetration, cell swelling and the release of inflammatory

factors, which is followed by pyroptosis(7). A previous study reported that pyroptosis plays an important role in immunity and diseases. Pyroptosis can promote the death of damaged cells during infection and acts as an alarm signal for the recruitment of immune cells to the site of infection to promote the removal of pathogens, thus effectively protecting the body(8). In recent years, its role in tumorigenesis and cancer development has been studied comprehensively. Various regulators have been reported to be involved in the process of pyroptosis and play pivotal roles in the progression of tumors, such as hepatocellular carcinoma, lung cancer, and breast cancer(9-11). However, few studies have investigated the role of pyroptosis in glioma, and comprehensive analyses of pyroptosis regulators in glioma, their correlation with clinical characteristics and their prognostic value have not been reported.

In the present study, we first outlined the molecular subtypes of gliomas based on pyroptosis-related genes in the CGGA dataset and described the clinical and molecular characteristics and immune status of each subclass. Then, we developed a prognostic signature of pyroptosis-related genes based on the CGGA cohort, validated this prognostic signature in the TCGA cohort. Furthermore, we explored the clinical and molecular patterns, including immune infiltration, somatic copy number alterations, mutations, and DNA methylation, and established a lncRNA-miRNA-mRNA regulatory network. Finally, we explored the correlation between small molecular drugs and the identified prognostic signature genes. Our comprehensive analyses provide new insight into the functions of pyroptosis in the initiation, development, and progression of glioma.

MATERIALS AND METHODS

Data source

We downloaded the genomic data, copy number alteration, methylation and clinical data of glioma patients from the CGGA (http://www.cgga.org.cn/) and TCGA databases (https://portal.gdc.cancer.gov/). Additional gene-centric RMA-normalized gene expression profiles and drug response data of over 1000 cancer cell lines were accessed from the Genomics of Drug Sensitivity in Cancer (GDSC) database (https://www.cancerrxgene.org/downloads). Immune-associated data, including immune cells and immunophenoscores, were downloaded from TCIA (https://tcia.at/home). Thirty-three pyroptosis-related genes were defined from a previous publication and are provided in Table S1(12-15).

Identification of glioma subclasses and Gene set variation analysis

We identified the optimal clustering number visualizing consensus matrix, tracking plot, and cumulative distribution function plot. In addition, a T-distributed stochastic neighbor embedding-based approach was used to validate the clustering in glioma patients. We calculated the enrichment scores for every sample using the GSVA R package.

Development and validation of a prognostic signature

We developed a pyroptosis-related prognostic signature based on the CGGA training cohort. Twenty differentially expressed genes with $P<0.05$ were entered into LASSO Cox regression, which identified potential genes for the prognostic signature in the CGGA training cohort. Then, we calculated the risk score for each sample of the CGGA

111 and TCGA validation cohorts using the obtained regression coefficient in the CGGA
112 training cohort: risk score =coef1*$gene_1$ expression+$coef_2$* $gene_2$
113 expression+…$coef_n$*$gene_n$ expression. The CGGA and TCGA samples were divided
114 into a high-risk group and a low-risk group based on the median risk score. Receiver
115 operating characteristic curves were plotted to evaluate the 1-year, 2-year, and 3-year
116 sensitivity and specificity of the prognostic signature. We also established a prognostic
117 nomogram to evaluate the clinical value of the prognostic signature. Calibration
118 analysis of the prognostic predictive value of the nomogram was carried out.

119 **Functional enrichment analysis, estimation of tumor stem cell-like properties and**
120 **immune infiltration**

121 Gene Ontology and KEGG pathway analyses were performed using the "clusterProfiler"
122 package. We used single-sample gene set enrichment analysis (ssGSEA) to estimate the
123 enrichment score of stem cell-like properties (RNAss, DNAss) and the TME (stromal
124 score, immune score, and ESTIMATE score) in the TCGA cohort because the CGGA
125 dataset did not provide such data. The immune-related cell and function scores were
126 also calculated for each sample (downloaded from https://www.gsea-msigdb.org/).

127 **Somatic copy number alteration, mutation, and DNA methylation analysis**

128 Based on the risk groups in the TCGA cohort, we compared the somatic copy number
129 alteration, mutation, and DNA methylation levels between the high-risk and low-risk
130 groups using the "limma" R package.

131 **Construction of a ceRNA network and drug sensitivity**

132 To further explore the transcriptome regulation network of different risk groups, we

used Cytoscape version 3.8.2 to establish a lncRNA-miRNA-mRNA regulatory network. We explore the correlation between small molecular drugs and the identified prognostic signature genes using Pearson correlation analysis |R|>0.25 and P>0.05 were considered significant.

Verification of experiments in vitro

We further performed the Western blot, cell migration assays, cell scratchy assays, and clonogenic assays to verify the present finding. We selected the CASP8 to validate the molecular function because CASP8 showed significant differences between normal tissue and GBM or LGG, and the elevated expression is associated with poor prognosis. The details of experiments process in vitro were supplied in Additional file 1.docx.

Statistical analysis

The log-rank test was used to compare the survival curves of Kaplan-Meier analysis. The hazard ratio (HR) and 95% confidence interval (CI) of each gene and clinical parameters were calculated when univariate and multivariate Cox regression were applied. All analyses were achieved using R software version 4.0. A two-sided P value <0.05 was considered significant unless otherwise specified.

RESULTS

Identification of glioma subclasses

The flow chart of the data analysis is presented in Figure 1A. From two CGGA RNA-seq datasets, we obtained 1018 samples of gene expression data and further identified 30 pyroptosis-related genes based on MAD>0.5. The gene symbols and descriptions of the 30 pyroptosis-associated genes used for classification are listed in the Additional

file 2: Table S1. We first explored the interactions among these genes using PPIs (Figure 1B), and the PPI network indicated that CASP8, CASP4, CASP1, NLRP3, NLRP1 and NLRC4 are hub genes. The correlation circle plot of the 30 genes is presented in Figure 1C (red: positive correlation; green: negative correlation). We identified the optimal k value as 2 by estimating the comprehensive correlation coefficient. Therefore, we divided the glioma samples into two different subclasses: cluster 1 and cluster 2. For the optimal k value (k=2), the consensus matrix showed a relatively sharp and clear boundary, indicating stable and robust clustering (Figure 1D). To verify the subclass stability, we further performed t-sensitivity PCA and found that a two-dimensional t-sensitivity distribution supported subtype clustering (Figure 1E). The consensus clustering for each sample is listed in Additional file 2: Table S2. The Kaplan-Meier analysis indicates that the median survival time was significantly shorter in cluster 2 than in cluster 1 (MST: 1.87 vs. 6.92 years, $P<0.001$, Figure 1F). This result indicated that the two subclasses had distinct prognostic patterns.

Correlation of glioma subclasses with pyroptosis-related genes

Two subclasses were obtained based on pyroptosis-related genes. To explore the pathway enrichment for the two subclasses, we performed GSVA by transforming the expression data from a gene-by-sample matrix to a gene set by two subclasses. Then, differential pathways were enriched in the two subclasses. Compared with cluster 1, the GSVA results indicated that cluster 2 had 182 kinds of significantly differential signaling pathways (Additional file 2: Table S3). The upregulated pathways were associated with immune-related pathways, such as autoimmune, allograft rejection,

177 graft versus host disease, primary immunodeficiency, antigen processing and
178 presentation. Some signaling pathways, such as the cytosolic DNA sensing pathway,
179 NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, and
180 metabolism-related pathways, were also significantly enriched. The significantly
181 downregulated pathway was long-term potentiation (Figure 2A).

182 **Clinical characteristics and transcriptomes of glioma subclasses**

183 We explored the correlation of subclasses with clinical characteristics (Figure 2B).
184 Compared with patients in cluster 2 with a favorable prognosis, patients in cluster 1
185 tended to have GBM (P<0.001), WHO grade IV (P<0.001), a higher proportion of
186 age >41 years, 1p19q non-codeletion status (P<0.001), and IDH wildtype status
187 (P<0.001). Sex, PRS type and radiotherapy status were not associated with the
188 molecular subclasses (P>0.05). For the pyroptosis-related genes except CASP9,
189 significant differential expression was observed in the two clusters. Among these
190 differentially expressed genes, all genes were upregulated in cluster 1 and
191 downregulated in cluster 2 (Figure 2B). We also compared the differences in pyroptosis-
192 related genes in patients with different histologies, grades, IDH mutation statuses, and
193 1p19q statuses. Compared with the LGG group, the GBM group had one upregulated
194 gene (AIM2) and 21 downregulated genes (Additional file 3: Figure S1A). Twenty-one
195 DEGs were found for grade, and their expression increased with increasing WHO grade
196 (P<0.005, Additional file 3: Figure S1B). For IDH status, 25 DEGs were found
197 (Additional file 3: Figure S1C). Thirty pyroptosis-related DEGs were found for 1p191
198 status (Additional file 3: Figure S1D).

199 We further performed differential expression analysis between cluster 1 and cluster 2.
200 A total of 392 DEGs were found, 18 genes were upregulated, and 372 genes were
201 downregulated in cluster 2 (Additional file 2: Table S4). GO and KEGG enrichment
202 analyses were performed for all DEGs (Additional file 2: Table S5 and Table S6). A
203 total of 874 differentially expressed functions were enriched, including 709 biological
204 processes, 95 cellular components and 70 molecular functions. The top 30 enrichment
205 results are presented in Additional file 3: Figure S2. Most of these functions were
206 associated with immunity. In addition, 56 pathways were also identified in the KEGG
207 analysis (Additional file 3: Figure S3), and the top five pathways were phagosome,
208 Staphylococcus aureus infection, tuberculosis, complement and coagulation cascades,
209 and human T-cell leukemia virus 1 infection.

210 **Correlation of glioma subclasses with immune status**

211 To explore the tumor heterogeneity between the two subclasses, we investigated the
212 immune cell and immune function differences. Compared with cluster 2, cluster 1 had
213 higher aDC, CD8+ T cell, DC, iDC, macrophage, mast cell, neutrophil, NK cell, pDC,
214 T helper cell, Tfh cell, Th2 cell, TIL, and Treg levels (all $P<0.001$, Additional file 3:
215 Figure S4A). Similarly, cluster 1 had higher immune function scores than cluster 2,
216 including APC coinhibition, APC costimulation, CCR, checkpoint, cytolytic activity,
217 HLA, inflammation promotion, MHC class I, parainflammation, T cell coinhibition,
218 type I IFN response and type II IFN response (all $P<0.001$, Additional file 3: Figure
219 S4B).

220 **Development of a pyroptosis-related prognostic signature in glioma**

Initially, we performed univariate Cox regression to identify the correlations of the 30 pyroptosis-related genes with OS (Additional file 3: Figure S5A) in the CGGA cohort. In total, 20 pyroptosis-related genes were identified as associated with the overall survival of glioma patients. The Kaplan-Meier plot indicated that high expression of CASP3, CASP4, CASP5, CASP6, CASP8, ELANE, GSMAD, IL6, NLRP3, NOD1, NOD2, PLCG1, PRKACA, PYCARD, and SCAF11 was associated with poorer OS in glioma. Using 20 prognostic pyroptosis-related genes, we developed a prognostic signature by performing LASSO regression in the CGGA training cohort (Additional file 3: Figure S5B and S5C). Fifteen of the 20 prognostic genes were used to develop the risk signature. We calculated the risk score for each sample using the regression coefficients of the 15 genes (Additional file 2: Table S7). Glioma patients with risk scores greater than the median value were divided into a high-risk group, and the others were divided into a low-risk group. Compared with the low-risk group, the high-risk group was more likely to have GBM ($P<0.001$), a higher WHO grade ($P<0.001$), recurrence ($P<0.001$), older age ($P<0.001$), IDH wildtype status ($P<0.001$), 1p19q non-codeletion status ($P<0.001$), and a history of chemotherapy ($P<0.001$). The heatmap showed the association between the risk group and clinical parameters and differentially expressed genes of the high- and low-risk groups (Additional file 3: Figure S5D). Furthermore, we found that glioma patients belonging to cluster 1, patients with a poor prognosis, patients with GBM, patients with WHO grade IV patients with 1p19q non-codeletion status and patients with IDH wildtype status had higher risk scores (all $P<0.001$, Additional file 3: Figure S6).

The Kaplan-Meier analysis showed that the high-risk group had a significantly poorer OS than the low-risk group (Figure 3A-3 B). Univariate Cox regression indicated that the risk score was positively associated with OS in glioma (HR=3. 105, 95% CI: 2.681–3.596, P<0.001, Figure 3C). Multivariate Cox regression suggested that the risk score was an independent unfavorable prognostic predictor in glioma (HR=1.685, 95% CI: 1.392–2.039, P<0.001, Figure 3D). In addition, PRS type, tumor grade, and age were positively associated with OS. However, chemotherapy, wildtype IDH status, and 1p19q status were negatively associated with OS in the CGGA training cohort. The PCA plot indicated that patients in different risk groups were separated into obviously different clusters (Figure 3E). Time-dependent receiver operating characteristic analysis was performed to evaluate the predictability of the prognostic model. Our results showed that the AUCs at 1 year, 2 years, and 3 years were 0.717, 0.784 and 0.773 (Figure 3F), respectively. We further compared the OS status among different histology, IDH status, 1p19q codeletion status, and grade subgroups. The results showed that the OS of the high-risk group was still poorer than that of the low-risk group (Additional file 3: Figure S7, all P<0.001).

External validation of the pyroptosis-related prognostic signature in glioma

To further validate the prognostic value of the pyroptosis-related gene model, we also calculated the risk score of glioma patients in the TCGA cohort using the regression coefficients of the CGGA cohort. The Kaplan-Meier analysis indicated a significant correlation of the high-risk group with worse OS than the low-risk group (Figure 4A-4C). Univariate Cox regression showed that the risk score was significantly associated

with OS in the TCGA cohort (HR=2.084, 95% CI: 1.890-2.297, P<0.001, Figure 4D). In multivariate Cox regression, the risk score was also an independent prognostic indicator (HR=1.425, 95% CI: 1.247–1.629 P<0.001, Figure 4E). The PCA plot validated the high- and low-risk distribution of all glioma patients based on the TCGA cohort. Furthermore, the AUCs of the risk score were 0.844 at 1 year, 0.863 at 2 years, and 0.874 at 3 years (Figure 4F).

Prognostic prediction models

To further evaluate the clinical prediction value of the prognostic signature, we constructed a prognostic nomogram model based on multivariate Cox regression analysis that included all clinical parameters in the CGGA cohort The calibration curves indicated that the clinical nomogram model could precisely predict the 1-year, 3-year and 5-year OS of glioma patients (C-index=0.799). The predictive accuracy of this nomogram was well validated in the TCGA cohort (C-index=0.841, Figure 5).

Functional enrichment and immune infiltration analyses based on the prognostic signature

We further explored the underlying biological functions that define the survival of glioma patients. We first performed DEG analysis between the high-risk and low-risk groups and then annotated the functions of the DEGs in terms of biological processes, cellular components, and molecular functions using GO enrichment and KEGG pathways. We identified 338 DEGs in the CGGA cohort (Additional file 2: Table S8) and 2600 DEGs in the TCGA cohort (Additional file 2: Table S9). The GO enrichment and KEGG pathway analyses indicated that the CGGA and TCGA cohorts shared some

287 enrichment results, such as extracellular matrix organization, extracellular structure
288 organization, immune response, ECM-receptor interaction, and cell adhesion molecules
289 (Figure 6A-6D).
290 We also explored the differences in immune cells and immune functions based on the
291 risk score in the CGGA (Figure 6E and Figure 6G) and TCGA datasets (Figure 6F and
292 Figure 6H). As shown in the box plots, the immune cell score showed a similar trend in
293 the CGGA and TCGA datasets. All immune cell scores were significantly upregulated
294 in the high-risk group. The immune function differences of the different risk groups
295 were the same in the CGGA and TCGA datasets (all $P<0.001$). All immune function
296 scores were significantly upregulated in the high-risk group. Significant expression
297 levels were also observed among different immune subtypes, which indicated that the
298 glioma prognosis risk could be associated with immune status (Additional file 3: Figure
299 S8). We also explored the correlation of the expression of target genes with cancer stem
300 cell-like properties (RNAss, DNAss) and the TME (stromal score, immune score, and
301 ESTIMATE score). We found that PCG1 was negatively associated with RNAss, the
302 stromal score, the immune score, and the ESTIMATE score. SCAF11 was only
303 negatively associated with DNAss. The rest of the genes showed positive correlations
304 with RNAss, DNAss and the stromal, immune and ESTIMATE scores (Additional file
305 3: Figure S9).

306 **Molecular alterations of pyroptosis-related genes based on the prognostic**
307 **signature**

308 Molecular alterations of pyroptosis-related genes were also evaluated based on

309 histology in the TCGA dataset. NLRP2, NLRP7, and PLCG1 were the only gene
310 alterations in LGG, and NLRP3, NLRP7, NLRP2, SCAF11, NOD1, PLCG1, NLRP1,
311 and CASP1 were gene alterations in GBM. All gene alterations were within 2% (Figure
312 7). The somatic copy number alteration analysis indicated significant differences
313 among the pyroptosis-related genes. Among these genes, the copy variation number
314 was significantly increased in GPX4, NLRP7, NLRP2, CASP3, CASP6, IL1B, CASP8,
315 IL6, AIM2, NLRP4, NLRP3, PRKACA, ELANE, SCAF11, CASP9, NOD1, and
316 PLCG1 and was significantly decreased in GSDMB, GSDMD, NLRP1, CASP9,
317 TIRAP, CASP1, CASP4, NOD2, CASP5, PYCARD, GSMDC, GSMDA, and IL18 in
318 the high-risk group. The DNA methylation levels of the pyroptosis-related genes were
319 also compared. The results showed that the overall DNA methylation levels were
320 significantly decreased in the high-risk group and increased in the low-risk group.

321 **Construction of a ceRNA network based on the prognostic signature**

322 A ceRNA network was constructed based on the differentially expressed mRNAs,
323 lncRNAs and miRNAs between the high-risk and low-risk groups in the TCGA dataset.
324 We identified 763 downregulated mRNAs, 1176 upregulated mRNAs, 116
325 downregulated lncRNAs, 132 upregulated lncRNAs (Additional file 2: Table S10), 47
326 downregulated miRNAs and 71 upregulated miRNAs (Additional file 2: Table S11).
327 Finally, 39 mRNAs (28 upregulated and 11 downregulated), 26 lncRNAs (15
328 upregulated and 15 downregulated) and 14 miRNAs (13 upregulated and 1
329 downregulated) were included in the ceRNA network (Figure 8). The Kaplan-Meier
330 curves suggested that 13 lncRNAs (positive correlation: AC025211.1, AC068643.1,

331 GDNF-AS1, and LINC00519; negative correlation: ADH1L1-AS2, CRNDE,
332 FAM181A-AS1, HOTAIRM1, MCF2L-AS1, MIR210HG, NEAT1, SLC6A1, and
333 SNHG9; Additional file 3: Figure S10), 41 mRNAs (Additional file 2: Table S12 and
334 Additional file 3: Figure S11) and 8 miRNAs (miR-21, miR-155, miR-200a, miR-216a,
335 miR-221, miR-222, miR-429, and miR-503; Additional file 3: Figure S12) were
336 associated with OS in glioma patients.

337 **Drug sensitivity analysis**

338 To identify potential target drugs, we performed correlations of the identified prognostic
339 signature genes with drugs. We identified 257 pairs of significant gene-drug
340 correlations (Additional file 2: Table S13). There were 9 pairs with correlation
341 coefficients >0.5 or <-0.5.

342 ELANE-hydroxyurea, ELANE-cyclophosphamide, CASP3-nelarabine, NOD2-
343 imiquimod, NLRP3-rebimastat, ELANE-ABT-199, ELANE-imexon, and NOD2-
344 isotretinoin showed drug sensitivity. PRKACA-cobimetinib showed drug resistance
345 (Figure 9).

346 **CASP8 promotes the progression of glioma cells**

347 We selected the CASP8 to validate the molecular function because CASP8 showed
348 significant differences between normal tissue and GBM or LGG in GTEx database
349 (Additional file 3: Figure S13). We firstly detected the expression of CASP8 in glioma
350 cell lines using the Western blot analysis, and found CASP8 is the most highly
351 expressed in LN299 cell. We built the CASP8-si LN229, H4 and U87 cells of glioma.
352 The qPCR indicated mRNA level of CASP8 is significantly down-regulated in U87 and

LN229 cells. Furthermore, the silence of CASP8 expression inhibited the cell migration ability (Figure 10). The clonogenic assay also showed that the number of clonogenicity of U87 and LN229 cells were significantly suppressed after knockout of CASP8. These results suggested that CASP8 promotes the progression of glioma cells.

DISCUSSION

The traditional histologic-based classification has some limitations, although this classification system has been updated several times over the years and serves clinicians well. One of the primary limitations is interobserver variability(16). A previous study reported that the concordance for reviewing a case is only approximately 50% among different neuropathologists, especially for astrocytic glioma versus oligodendroglioma(17). The development of genomics has allowed us to better understand the differences in prognosis and molecular features and promote effective treatment in glioma subclasses based on molecular features. Using 30 pyroptosis-related genes, we divided glioma patients into two subtypes. Significant overall survival differences were observed between cluster 1 and cluster 2. GSVA indicated that cluster 1 was enriched in some immune-related pathways. Cluster 1 and cluster 2 showed absolute differences in immune cells and immune functions. The infiltration levels of all kinds of immune cells, except Th1 cells, were higher in cluster 1, which had a poor prognosis than in cluster 2. Cluster 1 also showed more significant trends in some main immune function levels, such as immune checkpoints, inflammation promotion, par inflammation. A recent study reported that pyroptosis presents antitumor immune function in tumors, namely, pyroptosis-induced inflammation triggers robust antitumor

immunity and can synergize with checkpoint blockade(18). Moreover, some key pathways were also highly enriched in cluster 1, such as the NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, and cytosolic DNA sensing pathway, which were reported to be involved in glioma progression(19-21). These results indicated that pyroptosis-related genes divided glioma patients into two-dimensional distributions well.

We established a prognostic signature based on 15 pyroptosis-related genes. This prognostic signature was well validated in an external independent cohort, and in terms of its predictability, AUCs of 0.844, 0.863, and 0.874 were achieved for 1, 2, and 3 years, respectively, which showed its high discernibility. Combining clinical features and the risk score of the 15 genes, we developed a nomogram for clinical application. The CGGA and TCGA datasets showed high consistency. These results indicated that the prognostic signature based on pyroptosis-related genes has high clinical value.

The signature genes were involved in two biological mechanisms of pyroptosis. The assembly of inflammasome bodies is the initial step of the classical pyroptosis pathway. The inflammasome is a macromolecular protein complex in the cytoplasm necessary for the occurrence of inflammation and can recognize dangerous signaling molecules such as bacteria and viruses. The inflammasome is mainly composed of pattern recognition receptors (PRRs), apoptosis-associated speck-like protein (ASC) and pro-caspase-1 precursors(22). PRRs are receptor proteins responsible for recognizing different signal stimuli in cells. They are mainly composed of nucleotide-binding oligomerization domain-like receptor protein (NLRP) 1, NLRP3, nucleotide-binding

oligomerization domain-like receptor protein C4 (NLRC4), absent in melanoma 2 (AIM2) and other components(15). ASC is an adaptor protein that is mainly composed of the N-terminal pyrindomain (PYD) and the C-terminal caspase activation and recruitment domain (CARD)(23). Procaspase-1 is an effector molecule that can specifically cleave GSDMD after activation. After the danger signal sensor NLR1, NLRP3 or AIM2 recognizes the danger signal molecule, the N-terminal PYD is combined with the N-terminal PYD of the adaptor protein. ASC then recruits Caspase-1 through the interaction of the CARDCARD domain to complete the assembly of the inflamed body(24). This method of cell death mediated by Caspase-1 is called the classical pathway of pyroptosis(25). The non-classical pathway of pyrolysis is mainly mediated by Caspase-4, Caspase-5 and Caspase-11. After cells are stimulated by bacterial LPS, Caspases-4, -5, and -11 directly bind to bacterial LPS and are activated(26). Activated Caspases-4, -5, and -11 specifically cleave GSDMD and release the intramolecular inhibition of the GSDMD-N domain(27). The combination of the GSDMD-N-terminus and cell membrane phospholipids causes cell membrane pore formation, cell swelling and rupture and induces cell pyrolysis; the GSDMD-N-terminus can also activate Caspase-1 by activating the NLRP3 inflammasome(28). Activated Caspase-1 stimulates the maturation of IL-18 and IL-1β precursors, and IL-18 and IL-1β are secreted to the outside of the cell and amplify the inflammatory response. Yang et al found that in the nonclassical pathway that relies on Caspase-11, gap junction protein-1 (Pannexin-1) can be cleaved, and the cleavage of Pannexin-1 can activate its own channel and release ATP, which induces pyrolysis(29). Lamkanfi et al

found that in the nonclassical pathway that relies on Caspase-11, Pannexin-1 cleavage can also activate the NLRP3 inflammasome, which in turn activates Caspase-1 and induces the occurrence of pyroptosis(30). According to the results, mutations of pyroptosis-related genes are mainly attributed to the classical pathway of pyrolysis. More research is needed to validate the molecular mechanisms.

Based on the risk score, we classified glioma patients into high- and low-risk groups to discriminate clinical outcomes. We further explored the molecular features between the high- and low-risk groups. The functional enrichment analysis results were similar in the TCGA and CGGA datasets, and the same pathways appeared in the two datasets, such as ECM-receptor interaction, GABAergic synapse, focal adhesion, and extracellular matrix organization. The immune cells and immune functions showed similar trends: immune cell and functional scores were higher in the high-risk group. The clinical features showed that cluster 1 had a higher risk score and poorer prognosis than cluster 2. The results indicated that the classification was accurate and validated in the risk model. Furthermore, we compared the gene alterations, CNVs, and DNA methylation levels. Significantly different levels were observed, which reflected the different molecular features of the different risk groups. The ceRNA network identified several key lncRNA-miRNA-mRNA regulatory networks: FAM181A-AS1-miR-21-(CPEB3, SAIB1, BLC7A, MAP2K3, JAG1, TGFBI, FAM46A, SPRY2, and CALD1). The survival analysis further suggested the regulatory correlation: elevated FAM18A-AS1 and miR-21 were associated with poor prognosis in glioma, and low expression of BCL7A, SATB1 and CPEB3 was associated with favorable prognosis. Previous

experiments have reported the promoting role of miR-21 in glioma(31), and upregulation of SATB1 and CPEB3 is associated with the development and progression of glioma(32, 33). The drug sensitivity analysis indicated that NOD2, ELANE, CASP3, and PYCARD showed sensitivity to small molecular drugs, and PRKACA, IL6, and NLLRP3 showed resistance to some drugs. It was reported that the inhibition of the NLRP3 inflammasome by beta-hydroxybutyrate can suppress the migration of glioma cells(34). These results may provide some guidelines for clinical practice.

The present study indicated that pyroptosis-related genes can be used to classify glioma patients into two subclasses based on different molecular features and clinical characteristics. The established prognostic model based on 15 pyroptosis-related genes not only predicted the prognosis of glioma patients but also reflected the molecular alterations, immune infiltration statuses, and stem cell-like properties of different risk groups. The classification based on the risk score of prognostic signature genes revealed a lncRNA-miRNA-mRNA regulatory network. The correlation of signature genes with drug sensitivity may provide a rationale for clinical applications. Finally, our study provides a new understanding of pyroptosis in the development and progression of glioma and contributes new important insights for promoting glioma treatment strategies.

Authors' contributions

ZZL designed this study and directed the research group in all aspects, including planning, execution, and analysis of the study. LS drafted the manuscript. YYL, NL, YJZ, QZ collected the data. LZZ provided the statistical software, performed the data

analysis, YYL arranged the Figures and Tables. SLF revised the manuscript. All authors have read and approved the final version of the manuscript.

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Conflict of Interest Statement

The authors declare that they have no competing interests

Data Availability Statements

All data can be download from TCGA database (https://portal.gdc.cancer.gov/) and CGGA (http://www.cgga.org.cn/)

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Figure legends

Figure 1 Identification of glioma subclasses using consensus clustering method in the CGGA dataset. **(A)** Flow chart of the study. **(B)** PPI network indicating the interactions among pyroptosis-related genes (interaction score=0.7). **(C)** The circle plot of correlation among pyroptosis-related genes (green line: negative correlation, red line: positive correlation). **(D)** Consensus matrix method clustering using 30 pyroptosis-related genes. **(E)** PCA analysis showed the distribution of two glioma subclasses in the CGGA dataset. **(F)** Overall survival curve of two clusters in the cohort.

Figure 2 Characteristics of patients in cluster 1 and cluster 2 in CGGA cohort. **(A)** Heatmap of gene set variation analysis of the pyroptosis-related genes from cluster 1 and cluster 2. **(B)** Heatmap showed the correlations between two subclasses and clinical characteristics and differentially expressed pyroptosis-related genes in the CGGA

596 cohort.

597 **Figure 3** Establishment of a pyroptosis-related gene prognostic signature in the CGGA
598 cohort. **(A)** Kaplan-Meier curves for OS of patients in high- and low-risk group in
599 CGGA Cohort. **(B)** Distribution of risk score of all patients of CGGA cohort, and
600 Patients' survival time distribution. **(C)** Forest plot of univariate cox regression between
601 risk score and prognosis of glioma. **(D)** Forest plot of multivariate cox regression of
602 between risk score and prognosis of glioma. **(E)** PCA plot for signature genes based on
603 risk score group. **(F)** ROC curves showed the predictive efficiency of risk score at 1-
604 year, 2-year, 3-year point.

605 **Figure 4** External validation of a pyroptosis-related gene prognostic signature in the
606 TCGA cohort. **(A)** Kaplan-Meier curves for OS of patients in high- and low-risk group
607 in TCGA Cohort. **(B)** Distribution of risk score of all patients of TCGA cohort and
608 Patients' survival time distribution of TCGA cohort. **(C)** Forest plot of univariate cox
609 regression between risk score and prognosis of glioma in TCGA cohort. **(D)** Forest plot
610 of multivariate cox regression of between risk score and prognosis of glioma in TCGA
611 cohort. **(E)** PCA plot for signature genes based on risk score group in TCGA cohort. **(F)**
612 ROC curves showed the predictive efficiency of risk score at 1-year, 2-year, 3-year
613 point in TCGA cohort.

614 **Figure 5** Establishment and validation of nomogram model based on prognostic
615 signature genes. (A) Nomogram model established in the CGGA cohort. (B) The 1-year
616 calibration curves in the CGGA cohort. (C) The 3-year calibration curves in the CGGA
617 cohort. (D) The 5-year calibration curves in the CGGA cohort. (E) The 1-year

618 calibration curves in the TCGA cohort. (F) The 3-year calibration curves in the TCGA
619 cohort. (G) The 5-year calibration curves in the TCGA cohort.
620 **Figure 6** Functional enrichment and immune status analysis. **(A)** Barplot of enrichment
621 analysis based on prognostic-related signature genes in CGGA cohort. **(B)** Bubble plot
622 of enrichment analysis based on prognostic-related signature genes in CGGA cohort.
623 **(C)** Barplot of enrichment analysis based on prognostic-related signature genes in
624 TCGA cohort. **(D)** Bubble plot of enrichment analysis based on prognostic-related
625 signature genes in TCGA cohort. **(E)** Boxplot showed the ssGSEA scores for immune
626 cells based on risk group in CGGA cohort. **(F)** Boxplot showed the ssGSEA scores for
627 immune cells based on risk group in TCGA cohort. **(G)** Boxplot showed the ssGSEA
628 scores for immune pathways based on risk group in CGGA cohort. **(H)** Boxplot showed
629 the ssGSEA scores for immune pathways based on risk group in TCGA cohort
630 **Figure 7** Molecular alterations of pyroptosis-related genes in TCGA dataset. **(A)** The
631 mutations frequencies in low-risk group. **(B)** The mutations frequencies in high-risk
632 group. **(C)** Somatic copy number alteration based on risk groups. **(D)** DNA methylation
633 expression based on risk groups.
634 **Figure 8** The ceRNA network based on risk groups in TCGA dataset (red: up-regulation.
635 blue: down-regulation).
636 **Figure 9** Drug sensitivity analysis for identified prognostic-related genes based on
637 TCGA dataset (Top 16). **(A)** NOD2 and isotretinoin. **(B)** ELANE and Imexon. **(C)**
638 ELANE and ABT-199. **(D)** NLRP3 and Rebimastat. **(E)** NOD2 and Imuiquimod. **(F)**
639 CASP3 and Nelarabine. **(G)** ELANE and Cyclophosphamid. **(H)** ELANE and

Hydroxyurea. **(I)** PRKACA and Cobimetinib. **(J)** PRKACA and Rapamycin. **(K)** ELANE and Nandrolone. **(L)** PRKACA and Temsirolimus. **(M)** NOD2 and Eleschomol. **(N)** IL6 and geldanamycin. **(O)** IL6 and Lenvatinib. **(P)** PYCARD and Cyclophospharr

Figure 10 CASP8 promotes progression of glioma cells. **(A)** The expression of CASP8 protein in human HA and glioma cell lines. **(B)** The western blot of CASP8 in U87, U1251, H4 cell lines after siRNA. **(C)** The mRNA expression level of CSAP8 in U87 and U251 after siRNA. **(D and E)** The scratch assay of CASP8-si in U87 and U251 cell lines. **(F and G)** Transwell assay of CASP8-si U87 and U251 cell lines. (H and I) The clonogenic assay of CASP8 in U87 and U251 cell lines.

Supplementary materials legends

Additional file 1: The details of experiments process in vitro

Additional file 2: Table S1-S12.xlsx

Table S1 The 30 pyroptosis associated genes used for classification

Table S2 Glioma classification pattern

Table S3 GSVA enrichment analysis between these distinct pyroptosis-regulated clusters

Table S4 The result of differential expression analysis (Cluster 2 vs Cluster 1)

Table S5 Functional enrichment analyses of subclass differentially expressed genes (Cluster 2 vs Cluster 1)

Table S6 Pathway enrichment analysis of differentially expressed genes from two subclasses

Table S7 15 identified pyroptosis-related signature genes in prognostic model

662 **Table S8** Differentially expressed genes from CGGA based on risk score

663 **Table S9** Differentially expressed genes from TCGA based on risk score

664 **Table S10** Differentially expressed lncRNA from TCGA based on risk score

665 **Table S11** Differentially expressed mir-RNA from TCGA based on risk score

666 **Table S12** Prognosis-related genes in the ceRNA network

667 **Table S13** Results of drug sensitivity based on 15 pyroptosis-related prognostic

668 signature genes

669 **Additional file 3:**

670 **Figure S1** Comparisons of different clinical parameters for pyroptosis-related genes.

671 **(A)** LGG and GBM. **(B)** WHO II vs WHOIII vs WHO IV. **(C)** IDH: mutations vs

672 wildtyp. **(D)**1p19_status: codel vs non-codel.

673 **Figure S2** Barplot of GO enrichment analysis for differentially expressed genes based

674 on subclasses.

675 **Figure S3** KEEG pathways analysis for differentially expressed genes based on

676 subclasses.

677 **Figure S4** Correlation of glioma subclasses with immune infiltration. **(A)** Immune cells.

678 **(B)** immune function

679 **Figure S5** Identification of 215 genes risk signature for OS by LASSO regression in

680 the CGGA cohort. (A) Forest plot of univariate cox regression of OS for 30 pyroptosis-

681 related genes. (B) Cross-validation for tuning parameters selection int the LASSO

682 regression. (C) LASSO regression of the 15 OS-related genes. (D) Heatmap showed

683 the association between risk group and clinical parameters and differentially expressed

genes of high- and low-risk group.

Figure S6 Boxplot of risk score among different clinical characteristics. (A) Subclasses: Cluster 1 vs Cluster 2. (B) Outcomes: Dead vs Alive. (C) Histology: GBM vs LGG. (D) Grade: WHO II vs WHO III vs WHO IV. (E) 1p19q status: Codeletion vs non-codeletion. (F) IDH status: Mutant wildtype.

Figure S7 Subgroup analysis of OS based on risk score. **(A)** LGG. **(B)** GBM. **(C)** IDH wildtype. **(D)** IDH mutation. **(E)** 1p9ql non-codel. **(F)** 1p9ql codel. **(G)** WHO II. **(H)** WHO III. **(I)** WHO IV

Figure S8 Comparisons of 15 signature genes among different immune subtype.

Figure S9 Correlation of expression of 15 signature genes with cancer stem cell-like properties (RNAss, DNAss) and TME (Stromal score, Immune score, and ESTIMATE Score. (A) RNAss. (B) DNAss. (C) Stromal score. (D) Immune score. (E) ESTIMATE score.

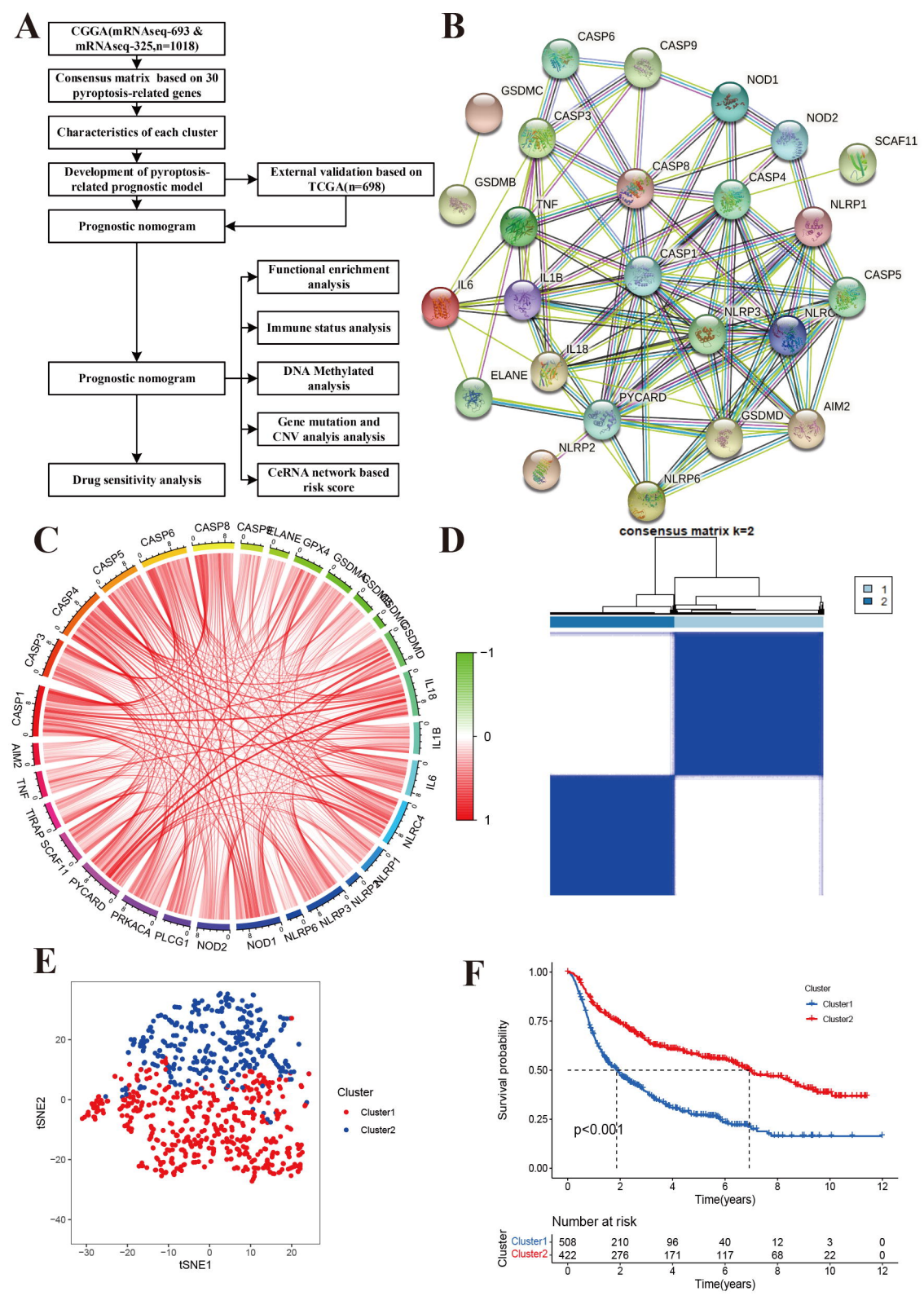
Figure S10 Kaplan-Meier curves of lncRNAs for OS in the ceRNA network. **(A)** AC025211.1. **(B)** AC068643.1. **(C)**ADH1L1-AS2. **(D)** CRNDE. **(E)** FAM181A-AS1. **(F)** GDNF-AS1. **(G)** HOTAIRM1. **(H)** LINC00519 **(I)** MCF2L-AS1. **(J)** MIR210HG. **(K)** NEAT1 **(L)**SLC6A1. **(M)** SNHG9.

Figure S11 Forest plot of mRNAs for OS in the ceRNA network

Figure S12 Kaplan-Meier curves of mir-RNAs for OS in the ceRNA network. **(A)** mir-21. **(B)** mir-155. **(C)** mir-200a. **(D)** mir-216a. **(E)** mir-221. **(F)** mir-222. **(G)** mir-429. **(H)** mir-503.

Figure S13 The expression levels of identified prognostic genes between tumor and

normal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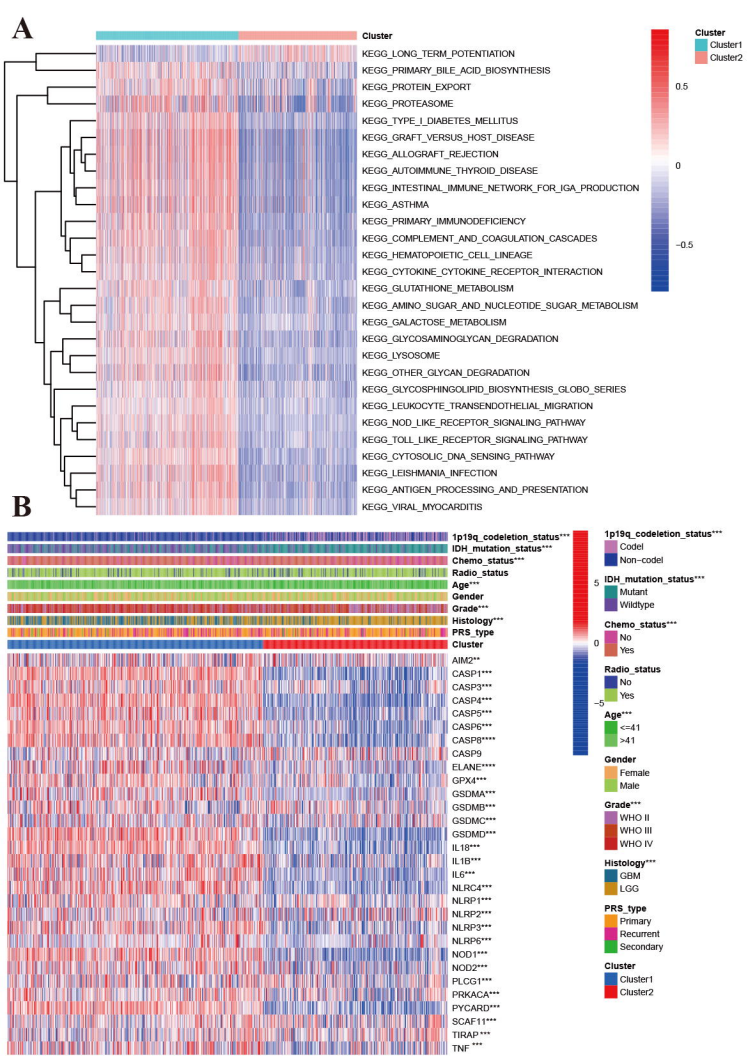
CGGA(mRNAseq-693 & mRNAseq-325,n=1018)
Consensus matrix based on 30 pyroptosis-related genes
Characteristics of each cluster
Development of pyroptosis-related prognostic model
External validation based on TCGA(n=698)
Prognostic nomogram
Prognostic nomogram
Functional enrichment analysis
Immune status analysis
DNA Methylated analysis
Gene mutation and CNV analyis analysis
CeRNA network based risk score
Drug sensitivity analysis
B
CASP6
CASP9
GSDMC
NOD1
CASP3
NOD2
SCAF11
CASP8
GSDMB
CASP4
TNF
NLRP1
CASP1
CASP5
IL6
IL1B
NLRP3
IL18
ELANE
PYCARD
GSDMD
AIM2
NLRP2
NLRP6
C
CASP6
CASP8
CASP5
ELANE
GPX4
CASP4
CASP3
CASP1
AIM2
TNF
TIRAP
SCAF11
PYCARD
PRKACA
PLCG1
NOD2
NOD1
NLRP6
NLRP3
NLRP2
NLRP1
NLRC4
IL6
IL1B
IL18
GSDMD
D
consensus matrix k=2
1
2
−1
0
1
E
tSNE2
tSNE1
Cluster
Cluster1
Cluster2
F
Survival probability
Time(years)
Cluster
Cluster1
Cluster2
p<0.001
Number at risk
Cluster1 508 210 96 40 12 3 0
Cluster2 422 276 171 117 68 22 0
Time(years)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Cluster
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KEGG_PRIMARY_BILE_ACID_BIOSYNTHESIS
KEGG_PROTEIN_EXPORT
KEGG_PROTEASOME
KEGG_TYPE_I_DIABETES_MELLITUS
KEGG_GRAFT_VERSUS_HOST_DISEASE
KEGG_ALLOGRAFT_REJECTION
KEGG_AUTOIMMUNE_THYROID_DISEASE
KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION
KEGG_ASTHMA
KEGG_PRIMARY_IMMUNODEFICIENCY
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES
KEGG_HEMATOPOIETIC_CELL_LINEAGE
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION
KEGG_GLUTATHIONE_METABOLISM
KEGG_AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_METABOLISM
KEGG_GALACTOSE_METABOLISM
KEGG_GLYCOSAMINOGLYCAN_DEGRADATION
KEGG_LYSOSOME
KEGG_OTHER_GLYCAN_DEGRADATION
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_GLOBO_SERIES
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY
KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY
KEGG_LEISHMANIA_INFECTION
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION
KEGG_VIRAL_MYOCARDITIS
Cluster
Cluster1
Cluster2
0.5
0
−0.5
B
1p19q_codeletion_status***
IDH_mutation_status***
Chemo_status***
Radio_status
Age***
Gender
Grade***
Histology***
PRS_type
Cluster
AIM2**
CASP1***
CASP3***
CASP4***
CASP5***
CASP6***
CASP8****
CASP9
ELANE****
GPX4***
GSDMA***
GSDMB***
GSDMC***
GSDMD***
IL18***
IL1B***
IL6***
NLRC4***
NLRP1***
NLRP2***
NLRP3***
NLRP6***
NOD1***
NOD2***
PLCG1***
PRKACA***
PYCARD***
SCAF11***
TIRAP***
TNF ***
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0
−5
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Codel
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IDH_mutation_status***
Mutant
Wildtype
Chemo_status***
No
Yes
Radio_status
No
Yes
Age***
<=41
>41
Gender
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WHO III
WHO IV
Histology***
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Recurrent
Secondary
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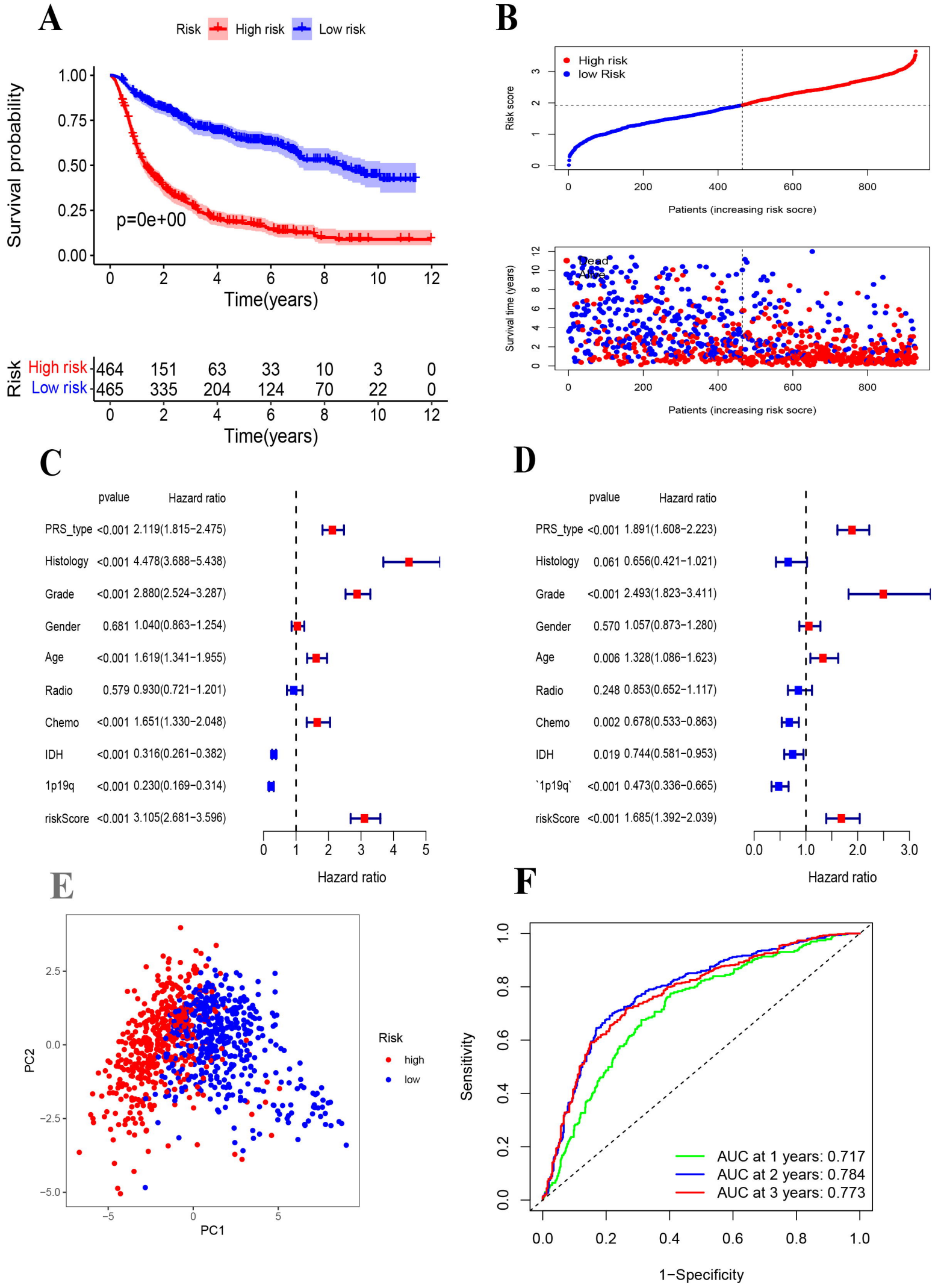
Cluster1
cluster2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Risk
High risk
Low risk
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0.50
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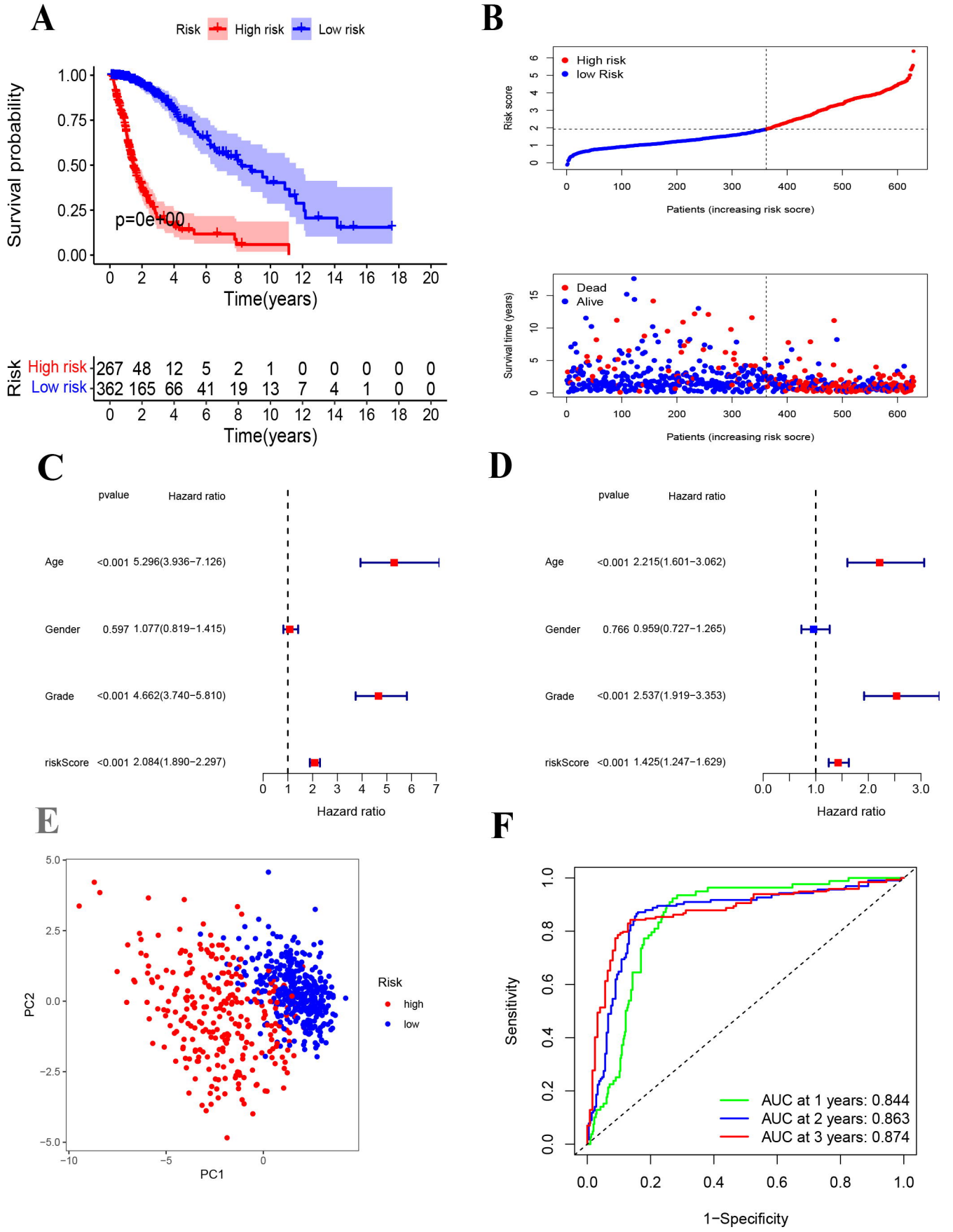
4
6
8
10
12
Time(years)
Risk
High risk 464 151 63 33 10 3 0
Low risk 465 335 204 124 70 22 0
Time(years)
B
High risk
low Risk
Risk score
Patients (increasing risk socre)
Dead
Alive
Survival time (years)
Patients (increasing risk socre)
C
pvalue
Hazard ratio
PRS_type <0.001 2.119(1.815−2.475)
Histology <0.001 4.478(3.688−5.438)
Grade <0.001 2.880(2.524−3.287)
Gender 0.681 1.040(0.863−1.254)
Age <0.001 1.619(1.341−1.955)
Radio 0.579 0.930(0.721−1.201)
Chemo <0.001 1.651(1.330−2.048)
IDH <0.001 0.316(0.261−0.382)
1p19q <0.001 0.230(0.169−0.314)
riskScore <0.001 3.105(2.681−3.596)
Hazard ratio
D
pvalue
Hazard ratio
PRS_type <0.001 1.891(1.608−2.223)
Histology 0.061 0.656(0.421−1.021)
Grade <0.001 2.493(1.823−3.411)
Gender 0.570 1.057(0.873−1.280)
Age 0.006 1.328(1.086−1.623)
Radio 0.248 0.853(0.652−1.117)
Chemo 0.002 0.678(0.533−0.863)
IDH 0.019 0.744(0.581−0.953)
`1p19q` <0.001 0.473(0.336−0.665)
riskScore <0.001 1.685(1.392−2.039)
Hazard ratio
E
PC2
PC1
Risk
high
low
F
Sensitivity
1−Specificity
AUC at 1 years: 0.717
AUC at 2 years: 0.784
AUC at 3 years: 0.773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Low risk
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0.50
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0.00
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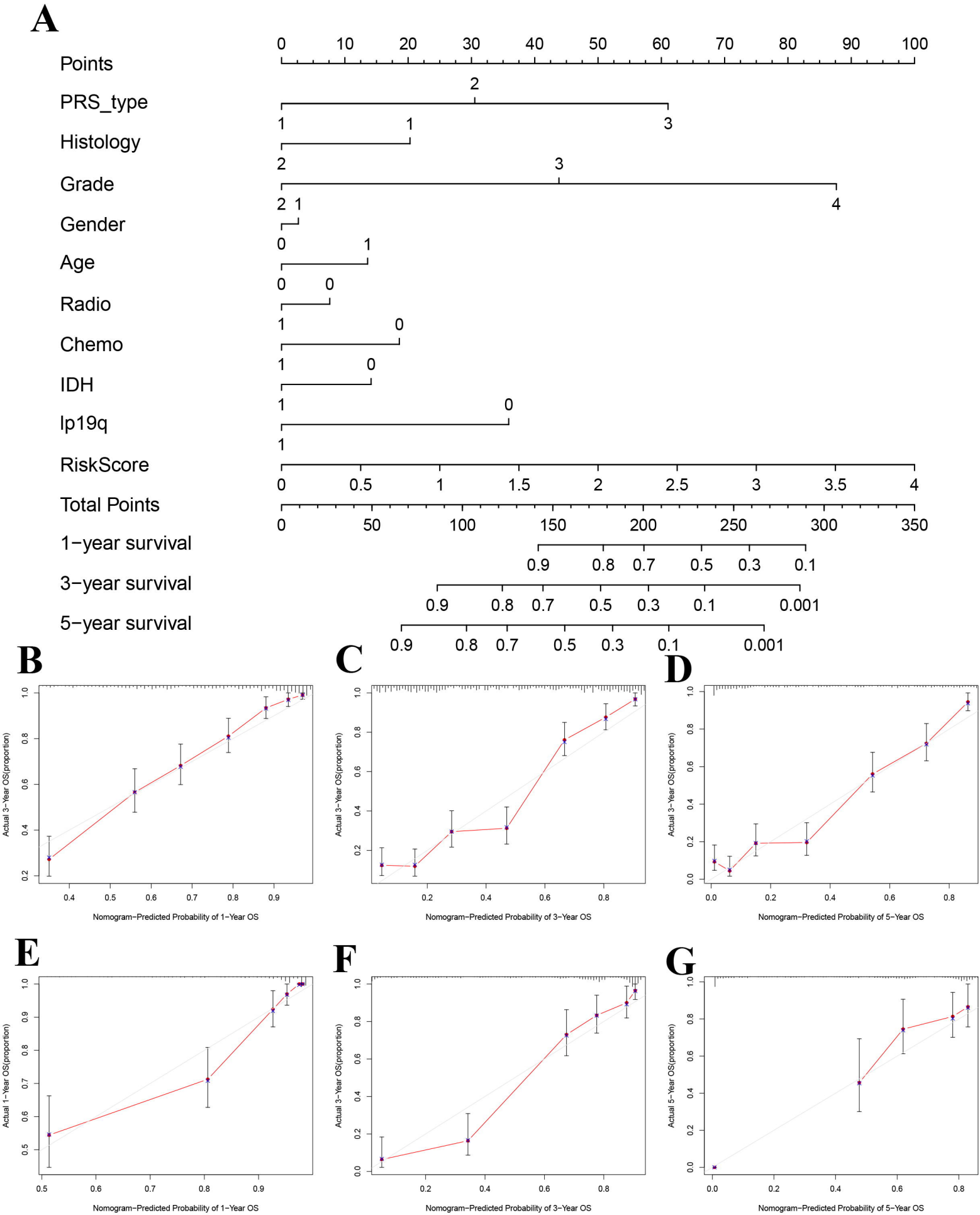
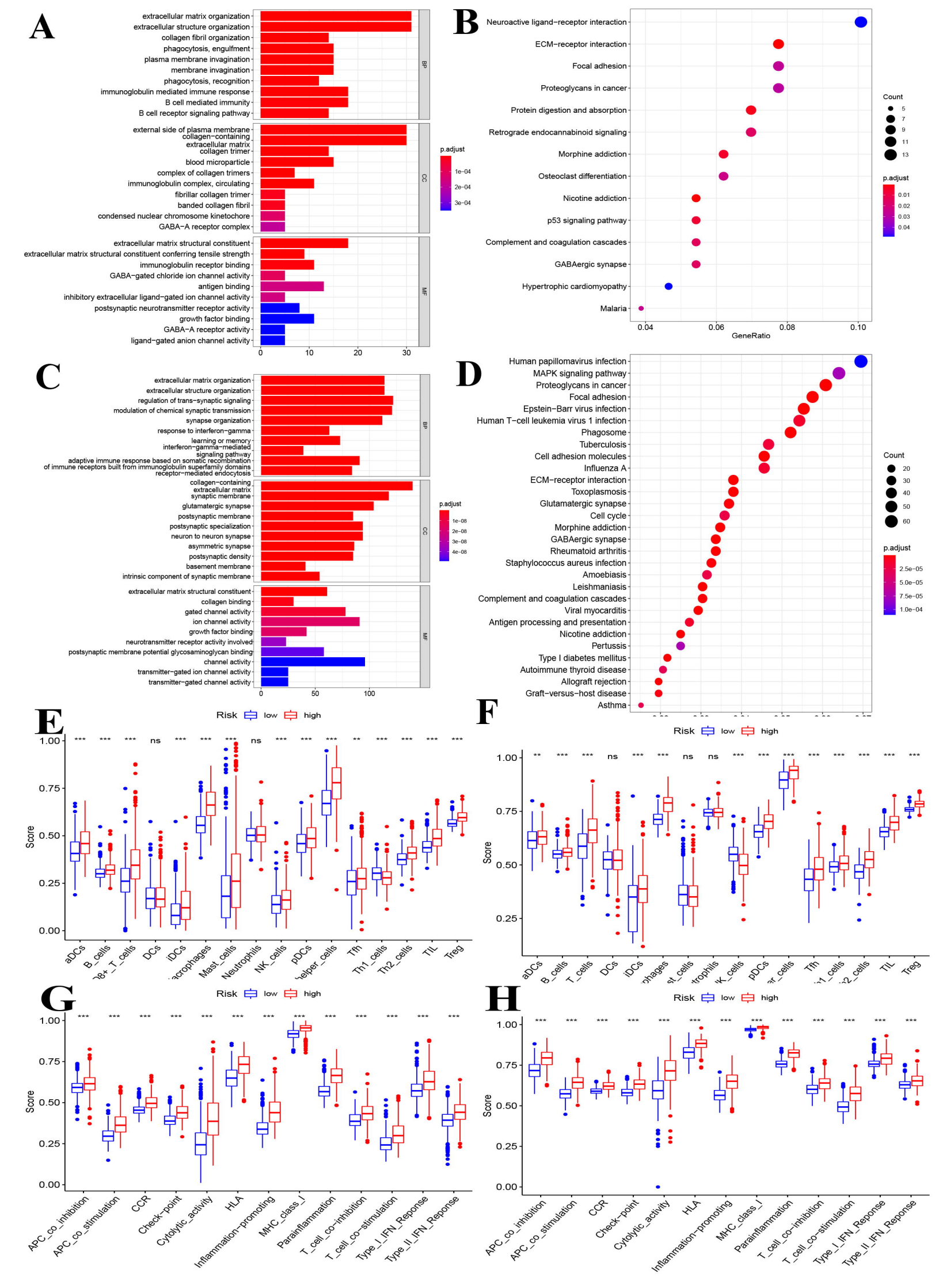
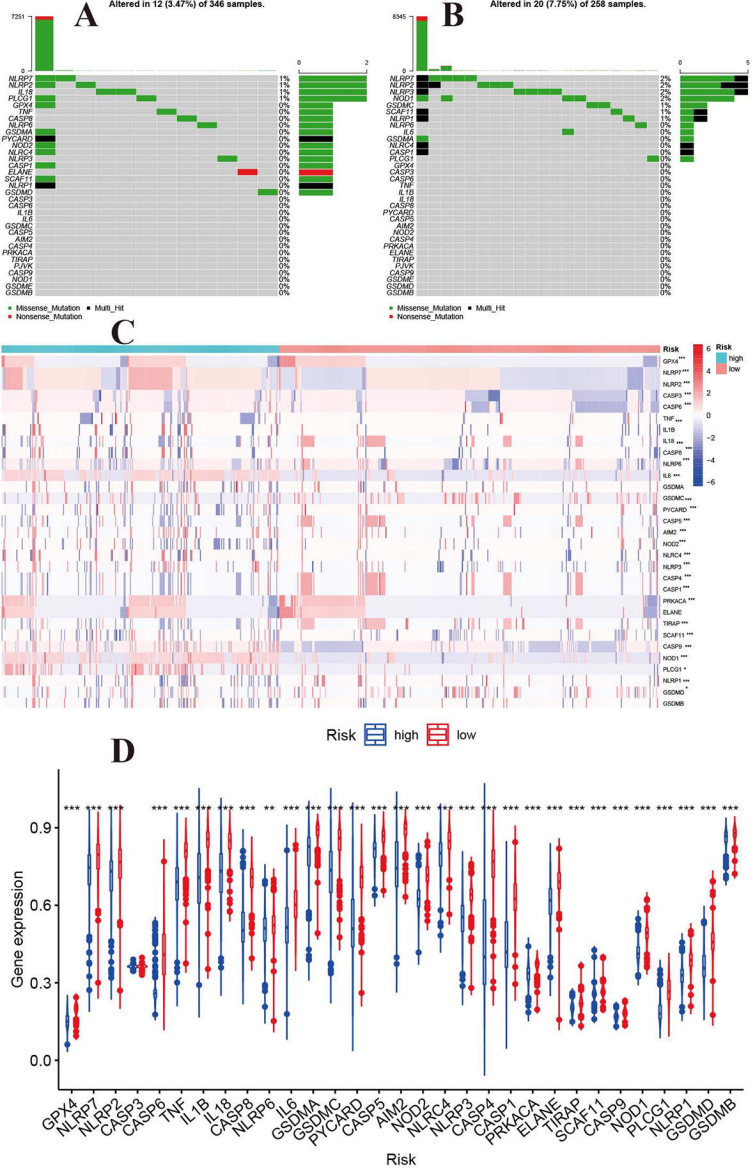
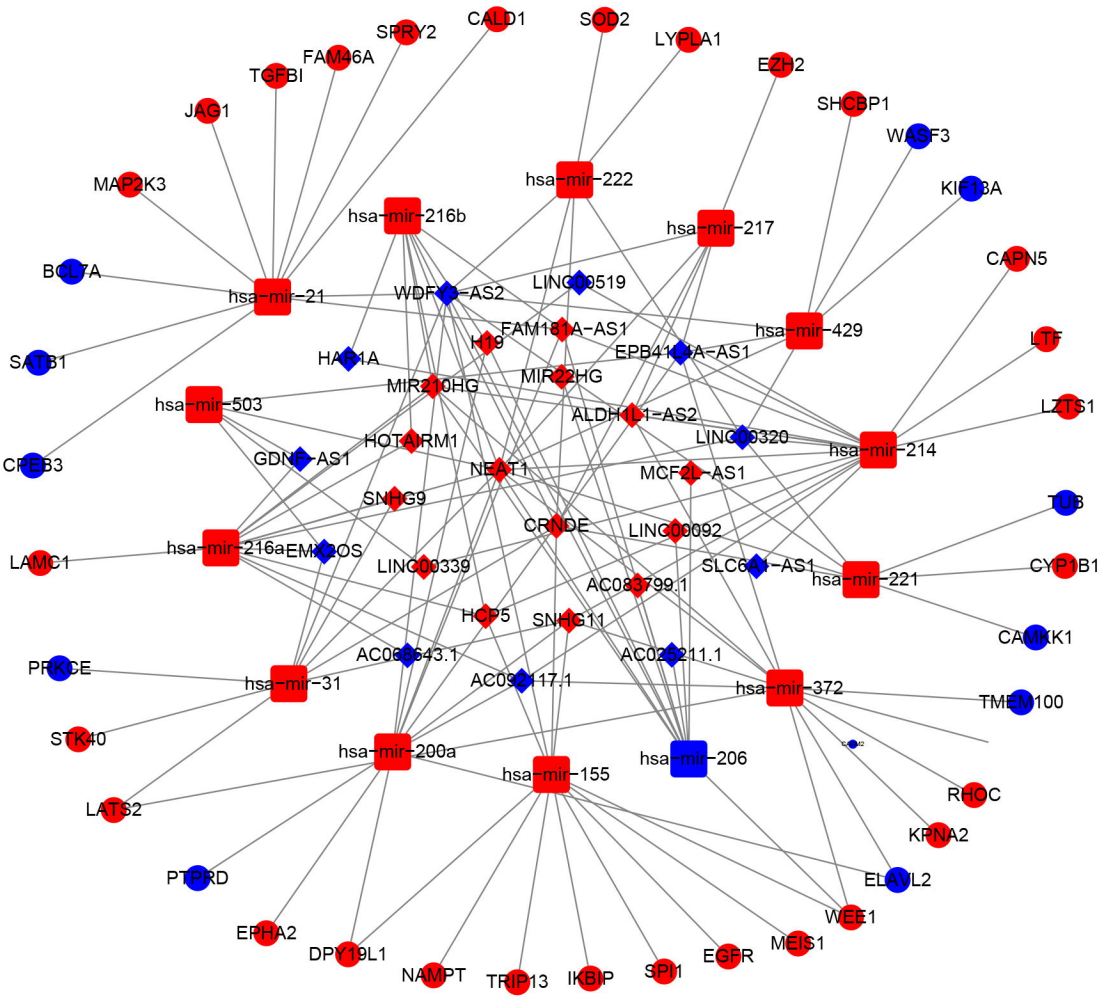
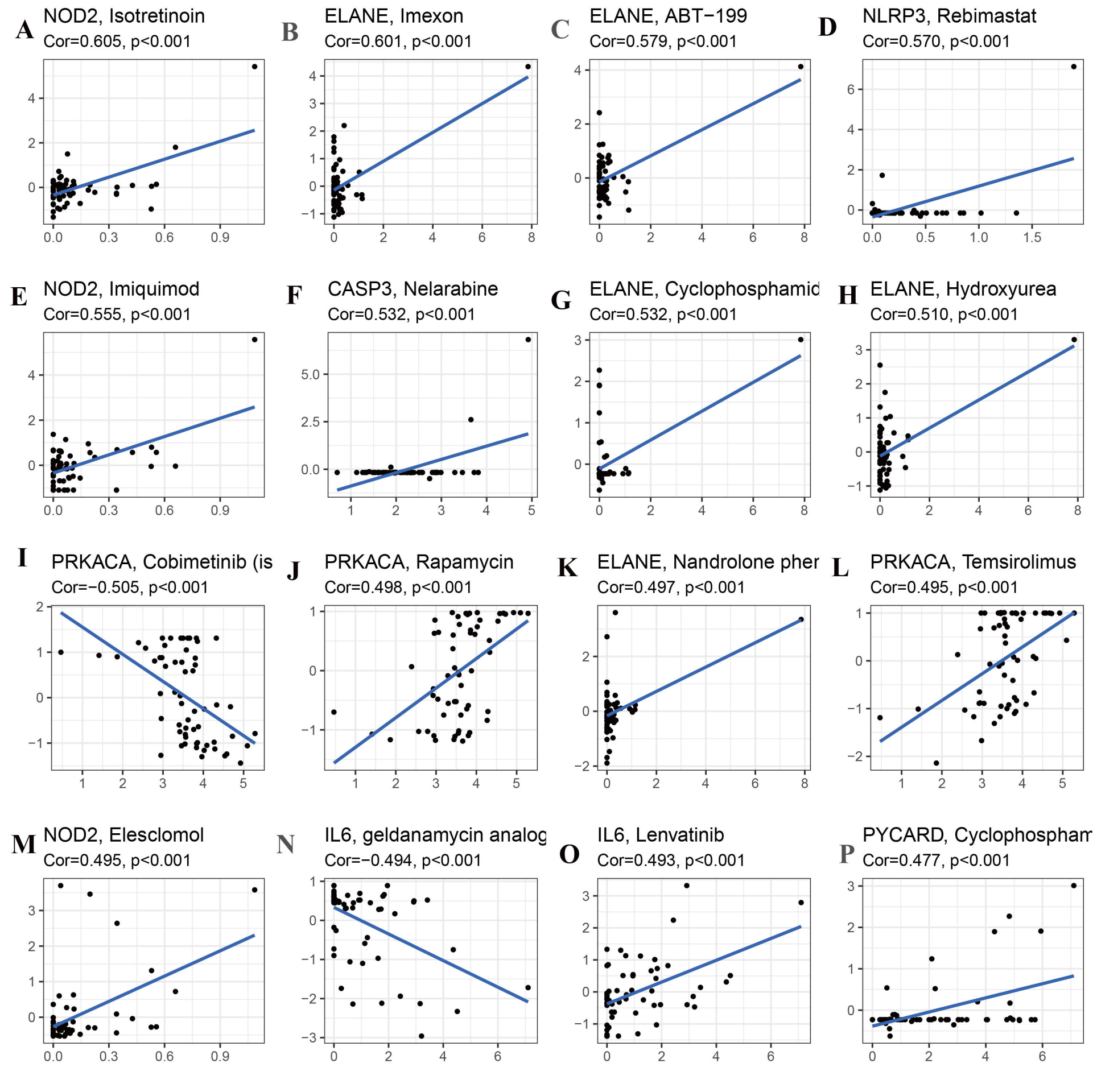
Time(years)
Risk
High risk 267 48 12 5 2 1 0 0 0 0 0
Low risk 362 165 66 41 19 13 7 4 1 0 0
0 2 4 6 8 10 12 14 16 18 20
Time(years)
B
High risk
low Risk
Risk score
0 1 2 3 4 5 6
0 100 200 300 400 500 600
Patients (increasing risk socre)
Dead
Alive
Survival time (years)
0 5 10 15
0 100 200 300 400 500 600
Patients (increasing risk socre)
C
pvalue
Hazard ratio
Age <0.001 5.296(3.936−7.126)
Gender 0.597 1.077(0.819−1.415)
Grade <0.001 4.662(3.740−5.810)
riskScore <0.001 2.084(1.890−2.297)
0 1 2 3 4 5 6 7
Hazard ratio
D
pvalue
Hazard ratio
Age <0.001 2.215(1.601−3.062)
Gender 0.766 0.959(0.727−1.265)
Grade <0.001 2.537(1.919−3.353)
riskScore <0.001 1.425(1.247−1.629)
0.0 1.0 2.0 3.0
Hazard ratio
E
5.0
2.5
0.0
−2.5
−5.0
PC2
−10 −5 0
PC1
Risk
high
low
F
Sensitivity
0.0 0.2 0.4 0.6 0.8 1.0
AUC at 1 years: 0.844
AUC at 2 years: 0.863
AUC at 3 years: 0.874
0.0 0.2 0.4 0.6 0.8 1.0
1−Specificity

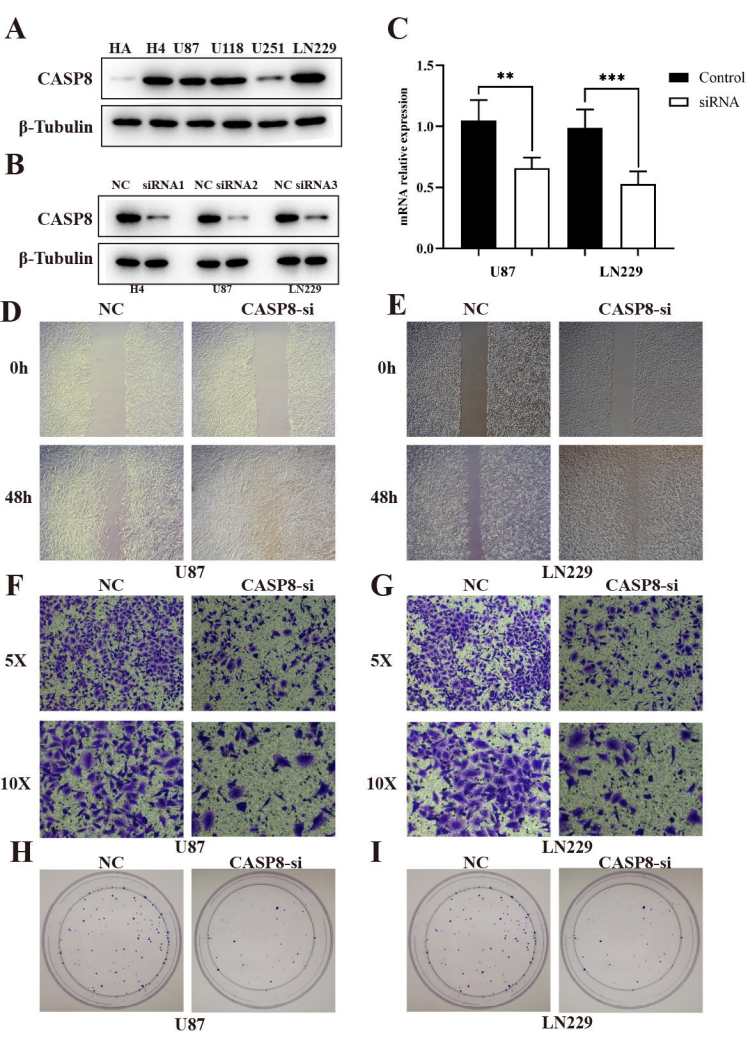

A
Points
PRS_type
Histology
Grade
Gender
Age
Radio
Chemo
IDH
1p19q
RiskScore
Total Points
1−year survival
3−year survival
5−year survival
B
C
D
E
F
G
Actual 3−Year OS(proportion)
Nomogram−Predicted Probability of 1−Year OS
Nomogram−Predicted Probability of 3−Year OS
Nomogram−Predicted Probability of 5−Year OS
Actual 1−Year OS(proportion)
Actual 5−Year OS(proportion)


A
extracellular matrix organization
extracellular structure organization
collagen fibril organization
phagocytosis, engulfment
plasma membrane invagination
membrane invagination
phagocytosis, recognition
immunoglobulin mediated immune response
B cell mediated immunity
B cell receptor signaling pathway
BP
external side of plasma membrane
collagen-containing extracellular matrix
collagen trimer
blood microparticle
complex of collagen trimers
immunoglobulin complex, circulating
fibrillar collagen trimer
banded collagen fibril
condensed nuclear chromosome kinetochore
GABA-A receptor complex
CC
extracellular matrix structural constituent
extracellular matrix structural constituent conferring tensile strength
immunoglobulin receptor binding
GABA-gated chloride ion channel activity
antigen binding
inhibitory extracellular ligand-gated ion channel activity
postsynaptic neurotransmitter receptor activity
growth factor binding
GABA-A receptor activity
ligand-gated anion channel activity
MF
p.adjust
1e-04
2e-04
3e-04
0
10
20
30
B
Neuroactive ligand-receptor interaction
ECM-receptor interaction
Focal adhesion
Proteoglycans in cancer
Protein digestion and absorption
Retrograde endocannabinoid signaling
Morphine addiction
Osteoclast differentiation
Nicotine addiction
p53 signaling pathway
Complement and coagulation cascades
GABAergic synapse
Hypertrophic cardiomyopathy
Malaria
0.04
0.06
0.08
0.10
GeneRatio
Count
5
7
9
11
13
p.adjust
0.01
0.02
0.03
0.04
C
extracellular matrix organization
extracellular structure organization
regulation of trans-synaptic signaling
modulation of chemical synaptic transmission
synapse organization
response to interferon-gamma
learning or memory
interferon-gamma-mediated signaling pathway
adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains
receptor-mediated endocytosis
BP
collagen-containing extracellular matrix
synaptic membrane
glutamatergic synapse
postsynaptic membrane
postsynaptic specialization
neuron to neuron synapse
asymmetric synapse
postsynaptic density
basement membrane
intrinsic component of synaptic membrane
CC
p.adjust
1e-08
2e-08
3e-08
4e-08
extracellular matrix structural constituent
collagen binding
gated channel activity
ion channel activity
growth factor binding
neurotransmitter receptor activity involved
postsynaptic membrane potential glycosaminoglycan binding
channel activity
transmitter-gated ion channel activity
transmitter-gated channel activity
MF
0
50
100
D
Human papillomavirus infection
MAPK signaling pathway
Proteoglycans in cancer
Focal adhesion
Epstein-Barr virus infection
Human T-cell leukemia virus 1 infection
Phagosome
Tuberculosis
Cell adhesion molecules
Influenza A
ECM-receptor interaction
Toxoplasmosis
Glutamatergic synapse
Cell cycle
Morphine addiction
GABAergic synapse
Rheumatoid arthritis
Staphylococcus aureus infection
Amoebiasis
Leishmaniasis
Complement and coagulation cascades
Viral myocarditis
Antigen processing and presentation
Nicotine addiction
Pertussis
Type I diabetes mellitus
Autoimmune thyroid disease
Allograft rejection
Graft-versus-host disease
Asthma
Count
20
30
40
50
60
p.adjust
2.5e-05
5.0e-05
7.5e-05
1.0e-04
E
Risk low high
Score
aDCs
B_cells
CD8+_T_cells
DCs
iDCs
Macrophages
Mast_cells
Neutrophils
NK_cells
pDCs
T_helper_cells
Tfh
Th1_cells
Th2_cells
TIL
Treg
F
Risk low high
Score
aDCs
B_cells
T_cells
DCs
iDCs
Macrophages
Mast_cells
Neutrophils
NK_cells
pDCs
T_helper_cells
Tfh
Th1_cells
Th2_cells
TIL
Treg
G
Risk low high
Score
APC_co_inhibition
APC_co_stimulation
CCR
Check-point
Cytolytic_activity
HLA
Inflammation-promoting
MHC_class_I
Parainflammation
T_cell_co-inhibition
T_cell_co-stimulation
Type_I_IFN_Reponse
Type_II_IFN_Reponse
H
Risk low high
Score
APC_co_inhibition
APC_co_stimulation
CCR
Check-point
Cytolytic_activity
HLA
Inflammation-promoting
MHC_class_I
Parainflammation
T_cell_co-inhibition
T_cell_co-stimulation
Type_I_IFN_Reponse
Type_II_IFN_Reponse


A
Altered in 12 (3.47%) of 346 samples.
7251
NLRP7 1%
NLRP2 1%
IL18 1%
PLCG1 1%
GPX4 1%
TNF 0%
CASP8 0%
NLRP6 0%
GSDMA 0%
PYCARD 0%
NOD2 0%
NLRC4 0%
NLRP3 0%
CASP1 0%
ELANE 0%
SCAF11 0%
NLRP1 0%
GSDMD 0%
CASP3 0%
CASP6 0%
IL1B 0%
IL6 0%
GSDMC 0%
CASP5 0%
AIM2 0%
CASP4 0%
PRKACA 0%
TIRAP 0%
RIPK 0%
CASP9 0%
NOD1 0%
GSDME 0%
GSDMB 0%
Missense_Mutation
Multi_Hit
Nonsense_Mutation
B
Altered in 20 (7.75%) of 258 samples.
8345
NLRP7 2%
NLRP2 2%
NLRP3 2%
NOD2 2%
GSDMC 1%
SCAF11 1%
NLRP1 1%
NLRP6 0%
IL6 0%
GSDMA 0%
NLRC4 0%
CASP1 0%
PLCG1 0%
GPX4 0%
CASP3 0%
CASP6 0%
TNF 0%
IL1B 0%
IL18 0%
CASP8 0%
PYCARD 0%
CASP5 0%
AIM2 0%
NOD2 0%
CASP4 0%
PRKACA 0%
ELANE 0%
TIRAP 0%
RIPK 0%
CASP9 0%
GSDME 0%
GSDMD 0%
GSDMB 0%
Missense_Mutation
Multi_Hit
Nonsense_Mutation
C
Risk
high
low
GPX4***
NLRP7***
NLRP2***
CASP3***
CASP6***
TNF***
IL18
IL1B***
CASP8***
NLRP6***
IL6***
GSDMA
GSDMC***
PYCARD***
CASP5***
AIM2***
NOD2***
NLRC4***
NLRP3***
CASP4***
CASP1*
PRKACA***
ELANE
TIRAP***
SCAF11***
CASP9***
NOD1***
PLCG1**
NLRP1***
GSDMD*
GSDMB
D
Risk
high
low
Gene expression
0.9
0.6
0.3
0.0
GPX4
NLRP7
NLRP2
CASP3
CASP6
TNF
IL1B
IL18
CASP8
NLRP6
IL6
GSDMA
GSDMC
PYCARD
CASP5
AIM2
NOD2
NLRC4
NLRP3
CASP4
CASP1
PRKACA
ELANE
TIRAP
SCAF11
CASP9
NOD1
PLCG1
NLRP1
GSDMD
GSDMB
Risk


SPRY2
CALD1
SOD2
LYPLA1
FAM46A
TGFBI
EZH2
JAG1
SHCBP1
WASF3
MAP2K3
hsa-mir-222
KIF13A
hsa-mir-216b
hsa-mir-217
CAPN5
BCL7A
hsa-mir-21
LINC00519
WDFY3-AS2
FAM181A-AS1
H19
hsa-mir-429
LTF
SATB1
HAP1A
EPB41L4A-AS1
MIR210HG
MIR22HG
hsa-mir-503
ALDH1L1-AS2
LZTS1
HOTAIRM1
LINC00320
hsa-mir-214
CPEB3
GDNF-AS1
NEAT1
MCF2L-AS1
SNHG9
TUB
CRNDE
LINC00092
LAMC1
hsa-mir-216a
EMX2OS
LINC00339
SLC6A1-AS1
CYP1B1
hsa-mir-221
AC083799.1
HCP5
SNHG11
CAMKK1
AC068643.1
AC025211.1
PRKCE
hsa-mir-31
AC092117.1
hsa-mir-372
TMEM100
STK40
hsa-mir-200a
hsa-mir-206
hsa-mir-155
RHOC
LATS2
KPNA2
PTPRD
ELAVL2
WEE1
EPHA2
MEIS1
DPY19L1
EGFR
NAMPT
TRIP13
IKBIP
SPI1


A
NOD2, Isotretinoin
Cor=0.605, p<0.001
B
ELANE, Imexon
Cor=0.601, p<0.001
C
ELANE, ABT−199
Cor=0.579, p<0.001
D
NLRP3, Rebimastat
Cor=0.570, p<0.001
E
NOD2, Imiquimod
Cor=0.555, p<0.001
F
CASP3, Nelarabine
Cor=0.532, p<0.001
G
ELANE, Cyclophosphamid
Cor=0.532, p<0.001
H
ELANE, Hydroxyurea
Cor=0.510, p<0.001
I
PRKACA, Cobimetinib (is
Cor=−0.505, p<0.001
J
PRKACA, Rapamycin
Cor=0.498, p<0.001
K
ELANE, Nandrolone pher
Cor=0.497, p<0.001
L
PRKACA, Temsirolimus
Cor=0.495, p<0.001
M
NOD2, Elesclomol
Cor=0.495, p<0.001
N
IL6, geldanamycin analog
Cor=−0.494, p<0.001
O
IL6, Lenvatinib
Cor=0.493, p<0.001
P
PYCARD, Cyclophospham
Cor=0.477, p<0.001


A
HA
H4
U87
U118
U251
LN229
CASP8
β-Tubulin
B
NC
siRNA1
NC siRNA2
NC siRNA3
CASP8
β-Tubulin
H4
U87
LN229
C
mRNA relative expression
1.5
1.0
0.5
0.0
**
***
Control
siRNA
U87
LN229
D
NC
CASP8-si
0h
48h
U87
E
NC
CASP8-si
0h
48h
LN229
F
NC
CASP8-si
5X
10X
U87
G
NC
CASP8-si
5X
10X
LN229
H
NC
CASP8-si
U87
I
NC
CASP8-si
LN229