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The role of *NPC2* gene in glioma was investigated based on bioinformatics analysis

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Glioblastoma (GBM) is one of the most malignant primary brain tumors in adults. The *NPC2* gene (Niemann–Pick type C intracellular cholesterol transporter 2) is a protein-coding gene with a lipid recognition domain. The *NPC2* gene was found to be significantly increased in gliomas (LGG and GBM), and it is now thought to be a risk factor. COX analysis demonstrated that *NPC2* was a significant risk factor for glioma. Functional enrichment analysis of genes that were differentially expressed between high and low expression groups revealed that genes were primarily enriched in the regulation of trans-synaptic signaling, Retrograde endocannabinoid signaling and other pathways. According to the findings of the immunoinfiltration investigation, the *NPC2* gene and macrophage, DC, etc. have a strong positive association. In addition, patients with high *NPC2* expression had higher levels of immune cell expression. Medication sensitivity research revealed that *NPC2*'s differential expression had some bearing on patients' medication sensitivity. There was a strong correlation between the prognosis of glioma patients and the gene sets *NUDT*19 and *NUME*. In brief, the *NPC2* gene was identified to be a possible biomarker of glioma, and preliminary analysis was done on the role of the *NPC2* gene in immunological microenvironment of glioma.

Glioblastoma (GBM) is a highly malignant primary brain tumor prevalent in adults, representing 57% of all gliomas and 48% of all primary malignant central nervous system (CNS) tumors[1]. It exhibits an infiltrative and expansive growth pattern[2,3]. The World Health Organization (WHO) classifies GBM as the highest grade (IV) among CNS tumors[4,5]. The current standard treatment for GBM involves maximal safe surgical resection, followed by concurrent radiation therapy and adjuvant chemotherapy[6–9]. Despite advancements in multimodal therapies for GBM, including surgery, radiation, systemic treatments (chemotherapy, targeted therapy), and supportive care, the effectiveness of these approaches remains limited. The median survival rate is only 15 months, with a generally poor overall prognosis, resulting in low long-term survival rates[10,11]. Moreover, GBM patients experience progressive decline in neurological function and quality of life, which significantly impacts patients, caregivers, and families. Enhancing the treatment and survival duration, as well as improving the quality of life for GBM patients, poses a significant challenge[12]. Therefore, it is crucial to study the regulatory mechanisms of GBM malignant progression, explore early GBM biomarkers, and develop early diagnosis, treatment, and prognosis strategies for GBM.

The *NPC2* gene is a gene responsible for encoding a protein that possesses a domain specialized in recognizing lipids. Extensive research has indicated its involvement in both lipid metabolism and the innate immune pathway. Previous investigations have provided evidence that *NPC2* plays a pivotal role in governing the transportation of cholesterol within the late endosomal/lysosomal system[13]. The pivotal function of *NPC2* lies in its role as the principal carrier for facilitating the removal of cholesterol from lysosomes. This process holds significant importance in terms of promoting the infiltration of immune cells and enhancing immune surveillance[14]. In recent times, numerous investigations have directed their attention towards examining the significance of *NPC2* in the context of cancer. These studies have yielded noteworthy findings, demonstrating that the *NPC2* protein exhibits elevated expression levels in various types of cancer, including breast cancer, colorectal cancer, thyroid cancer, and lung cancer. The recruitment of matrix macrophage lineage cells is hindered by *NPC2*, a protein secreted by malignant anterior lung tumors, as uncovered by Kamata et al.[14].

In their pioneering study, Wei et al. initiated an investigation into the prognostic significance of the interplay between *NPC2* and GBM, revealing *NPC2* as a viable candidate for a prognostic biomarker in GBM[15]. Nevertheless, the precise biological function of *NPC2* in the onset, progression, and relapse of GBM, especially in

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relation to the tumor immune microenvironment, remains elusive. Consequently, this present study undertook a comprehensive analysis of the *NPC2* gene expression in glioblastoma and its association with the immune microenvironment, utilizing cutting-edge bioinformatics methodologies. These findings carry substantial clinical implications for the treatment and prognosis of GBM.

Materials and methods

Data sources

In this study, RNA-seq data and clinical information (age, sex, tumor stage, tumor grade, IDH mutation status, 1p/19q deletion status, MGMT promoter status, chemotherapy status, and radiotherapy status) for glioma (including LGG and GMB) were downloaded from the TCGA and CGGA public databases (Tables S1, S2). Dataset GSE68848 with 95 LGG samples, 228 GMB samples, and 28 control samples was obtained from the GEO database. In addition, normal brain tissue samples were downloaded from the GTEx database.

Expression and prognosis of *NPC2* gene in pan-cancer

The "limma" package was utilized to analyze the difference in *NPC2* expression between cancer and paracancer tissues, and univariate cox regression was applied to analyze the survival prognosis of the *NPC2* gene in pan-cancer[16]. The "ggplot" package was used to plot the level of *NPC2* expression in various normal tissues.

Analysis of expression level of *NPC2* gene in glioma

Boxplot was applied to show the expression of *NPC2* gene in LGG, GMB and normal control samples in GSE68848 dataset, and the expression level of *NPC2* gene in glioma patients and control samples (glioma vs control) in TCGA dataset. And the Cell-PLoc 2.0 program was employed to identify *NPC2*'s protein subcellular localisation (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/).

Expression analysis of *NPC2* gene in different clinical subgroups of glioma

In the TCGA-glioma and CGGA datasets, boxplots were utilized to demonstrate the association between *NPC2* expression and multiple clinicopathological characteristics. Age, sex, tumor stage, Grade, IDH mutation status, 1p/19q deletion status, MGMT promoter status, chemotherapy status, and radiotherapy status were among the clinicopathological characteristics associated with glioma[17]. TCGA-glioma samples were classified into groups with high or low expression based on the median level of *NPC2* gene expression. The distribution disparities were compared using the Chi-square test.

Prognostic ability analysis of *NPC2* gene and clinicopathological characteristics

In the TCGA-glioma database, the Kaplan–Meier curve was used to examine the association between the *NPC2* gene and the prognosis of gliomas, including analyses of overall survival (OS) and progression-free survival (PFS). In addition, clinicopathological characteristics and *NPC2*'s potential for independent prognostication were investigated using univariate and multivariate Cox regression analysis. The prognostic value was further validated using the Receiver operating characteristic (ROC) curve, and an effective biomarker is one with an area under the curve (AUC) larger than 0.8.

Screening and functional enrichment analysis of differentially expressed genes

With screening parameters of p-adjust 0.05 and logFC $|>1$, the "DESeq2" R software was utilized to evaluate differentially expressed genes (DEGs) between high and low *NPC2* expression groups in glioma samples from the TCGA-glioma database. The analytic results were then decriminalized using a volcano map. Then, GO and KEGG functional enrichment analysis was performed using the R-package "clusterprofiler", and GSEA was used for single gene functional enrichment analysis of *NPC2*.

Exploring the mechanism influencing the expression of *NPC2* gene

The cBioPortal database was employed to evaluate the mutation of the *NPC2* gene in TCGA-glioma samples, the expression of the *NPC2* gene under copy number variation, and the relationship between the copy number of *NPC2* and mRNA expression level. Using the MetSurv tool, DNA methylation levels in the *NPC2* gene and the prognostic value of CpG in the *NPC2* gene were examined.

Drug sensitivity analysis and PPI network design

In order to assess the variations in drug sensitivity between the high/low *NPC2* groups, 198 anti-cancer medications were employed. To attempt to find the genes that interact with *NPC2*, the Search Tool for the Retrieval of Interacting Genes (STRING) database was employed, leaving the study of experimental validation and co-expression interactions.

Validation of the gene expression by quantitative real-time PCR

The quantitative real-time polymerase chain reaction (qRT-PCR) was used to verify the expression of *NPC2* in cells (SVGP12 and DBTRG) and tissues (cancer and normal). The DBTRG is a type of human glioblastoma cell, while SVGP12 represents human astrocytes. TRIZol (Thermo Fisher, Shanghai, CN) was used to extract total RNA and reverse transcribe mRNA into cDNA. qPCR was performed using SureScript-First-strand-cDNA-synthesis-kit (Servicebio, WuHan, CN).

Immunohistochemical (IHC) staining assays

The study involved the collection of 10 normal and 10 tumor samples from XX hospital for immunohistochemical experiments aimed at investigating differences in *NPC2* expression. Initially, paraffin sections with a thickness of 5 μm were meticulously prepared and subjected to a series of steps including deaffinity treatment, rehydration, blocking, antigen extraction, and cooling to room temperature. Following this, primary antibodies were applied and left to incubate overnight at 4 °C. Subsequently, secondary antibodies were introduced, followed by three washes with PBS. Procedures such as DAB staining, counterstaining, and fixation were then carried out. Evaluation involved selecting five random fields under a microscope, where the presence of brown particles in the cell nucleus and cytoplasm was considered indicative of positivity. The percentage of positive cells was graded on a scale from 0 (no positive cells) to 4 (> 75% positive), with an Immunoreactive Score (IRS score) calculated as the product of staining intensity and the percentage of positive cells.

Results

Expression and prognosis of *NPC2* gene in pan-cancer

Systematically examined the *NPC2* gene's expression in all types of cancer, we discovered that it was expressed in all of them (Fig. 1A), and that the LGG, GBM, SKCM, THCG, and other cancers had significantly higher levels of expression (Fig. 1B). *NCP2* gene was revealed to be a significant risk factor in GBM, HNSC, LGG, and STAD and to be negatively associated or even irrelevant in many cancers according to prognostic analysis (Fig. 1C).

We also investigated into the expression of the *NPC2* gene in gliomas. In accordance with the pattern of TCGA patients and GTEx normal samples, the expression of *NPC2* gene of GBM and LGG patients in the GSE68848 dataset was considerably greater than that of Normal patients (Fig. 1D,E). The significance of the *NPC2* gene in glioma disease was made clear by all of these findings, so we performed additional research.

Expression analysis of *NPC2* gene in different clinical subgroups of glioma

We examined differences in gene expression in various clinical categories to further investigate the potential association between the *NPC2* gene and glioma. Analysis of the difference of *NPC2* gene expression in different types of gliomas showed that the expression level of *NPC2* gene was higher in GBM, recurrent astrocytoma (rA), recurrent anaplastic astrocytoma (rAA) and recurrent GBM (rGBM) (Fig. 2A). The findings demonstrated that the expression of *NPC2* gene was significantly higher in WHO IV than in WHO III and II patients (Fig. 2B), and patients with wild-type IDH mutation status under various grade subtypes had considerably higher *NPC2* gene expression (Fig. 2C,D). Patients with various subtypes of the 1p/19q co-deletion status showed significantly

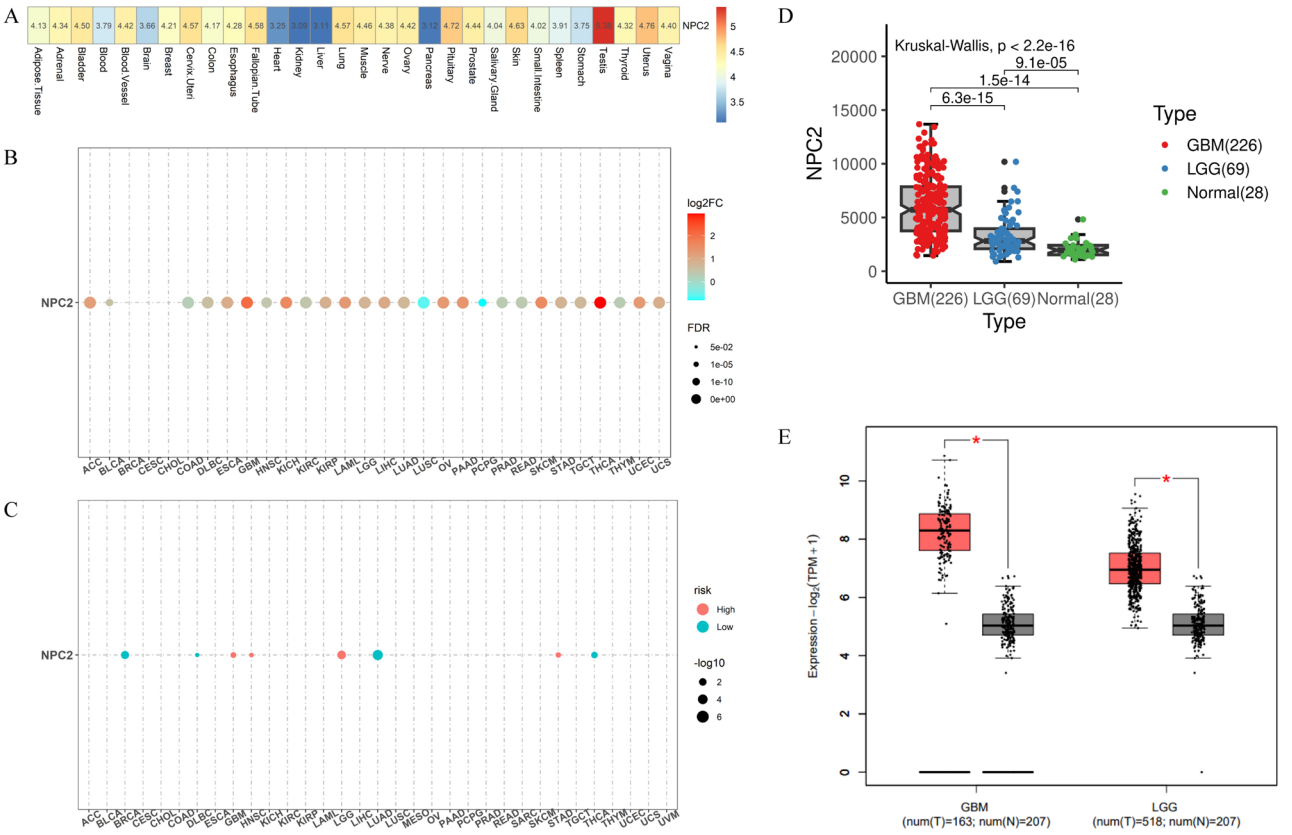

Figure 1. Expression and prognosis of NPC2 in pan-cancer. (**A**) Expression of NPC2 in normal tissues; (**B**) Differential expression of NPC2 in pan-cancer; (**C**) Pan-cancer prognosis analysis of NPC2; (**D**)Expression of NPC2 in GEO dataset; (**E**) Expression of NPC2 in TCGA and GTEx integrated database.

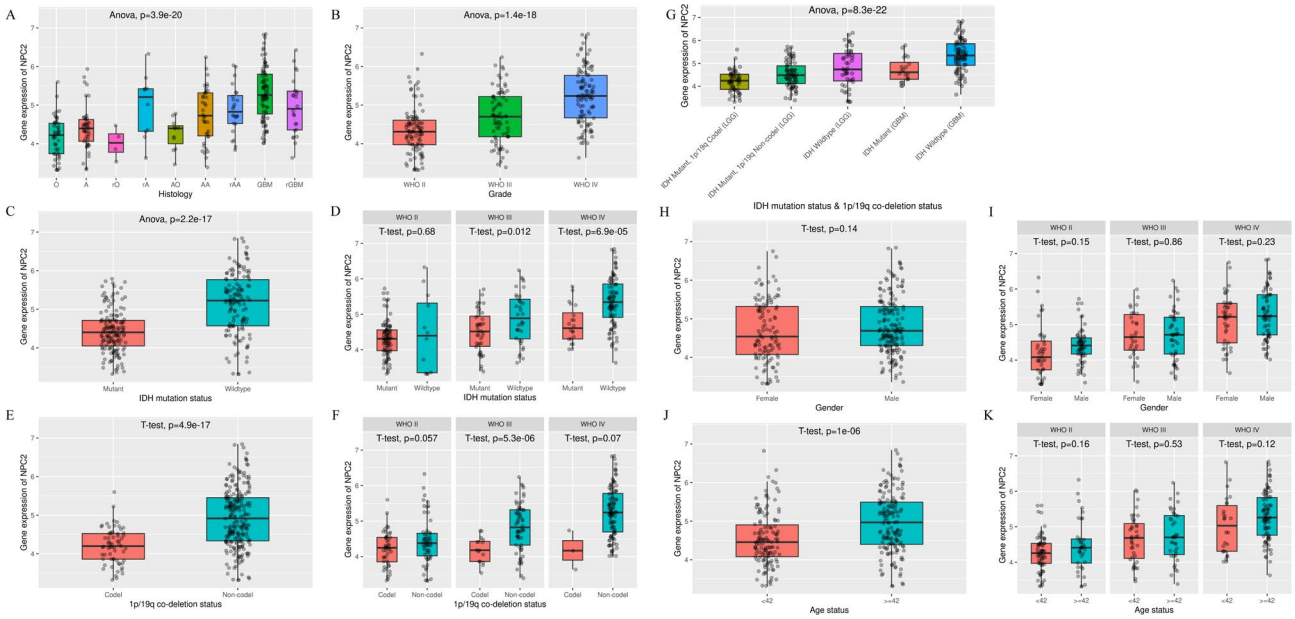

Figure 2. Expression of NPC2 in different clinical molecular subtypes.

varied gene expression ($P < 0.001$), and *NPC2* gene was highly expressed in both WHO III and IV 1p/19q co-deletion Non-codle patients (Fig. 2E,F). Overall, patients with GBM IDH wildtype showed increased *NCP2* gene expression (Fig. 2G). Additionally, male patients and patients older than 42 had greater gene expression levels (Fig. 2H–K). In the traditional subtypes, the sample numbers of patients in the high and low *NPC2* groups was basically the same, indicating that *NPC2*-based patient grouping is an independent glioma typing strategy (Table 1).

Expression level of *NPC2* and prognosis of glioma patients

In the TCGA dataset, the K-M survival curve revealed that patients with glioma, such as GBM and LGG, with high *NPC2* expression had considerably worse prognoses (Fig. 3A–C). Univariate and multivariate COX

Characteristics	Low expression of NPC2	High expression of NPC2	P
n	152	152	
Gender, n (%)			1
Female	57	58	
Male	95	94	
Age, n (%)			5.53E−05
< 42	51 (16.7%)	87 (28.6%)	
> = 42	101 (33.3%)	65 (21.4%)	
PRS_type			0.04085
Primary	100 (32.9%)	119 (39.1%)	
Recurrent	37 (12.2%)	21 (6.9%)	
Secondary	15 (4.9%)	12 (3.9%)	
Grade			1.26E−13
WHO II	10 (6.25%)	78 (25.65%)	
WHO III	38 (12.5%)	35 (11.51%)	
WHO IV	95 (31.25%)	39 (12.82%)	
IDH_mutation			9.99E−13
Mutant	50 (16.44%)	113 (37.17%)	
Wildtype	102 (33.55%)	39 (12.83%)	
X1p19q_codel			1.39E−07
Codel	12 (3.95%)	50 (16.44%)	
Non-codel	140 (46.05%)	102 (33.55%)	

Table 1. Correlation between NPC2 expression with clinicopathologic features of glioma.

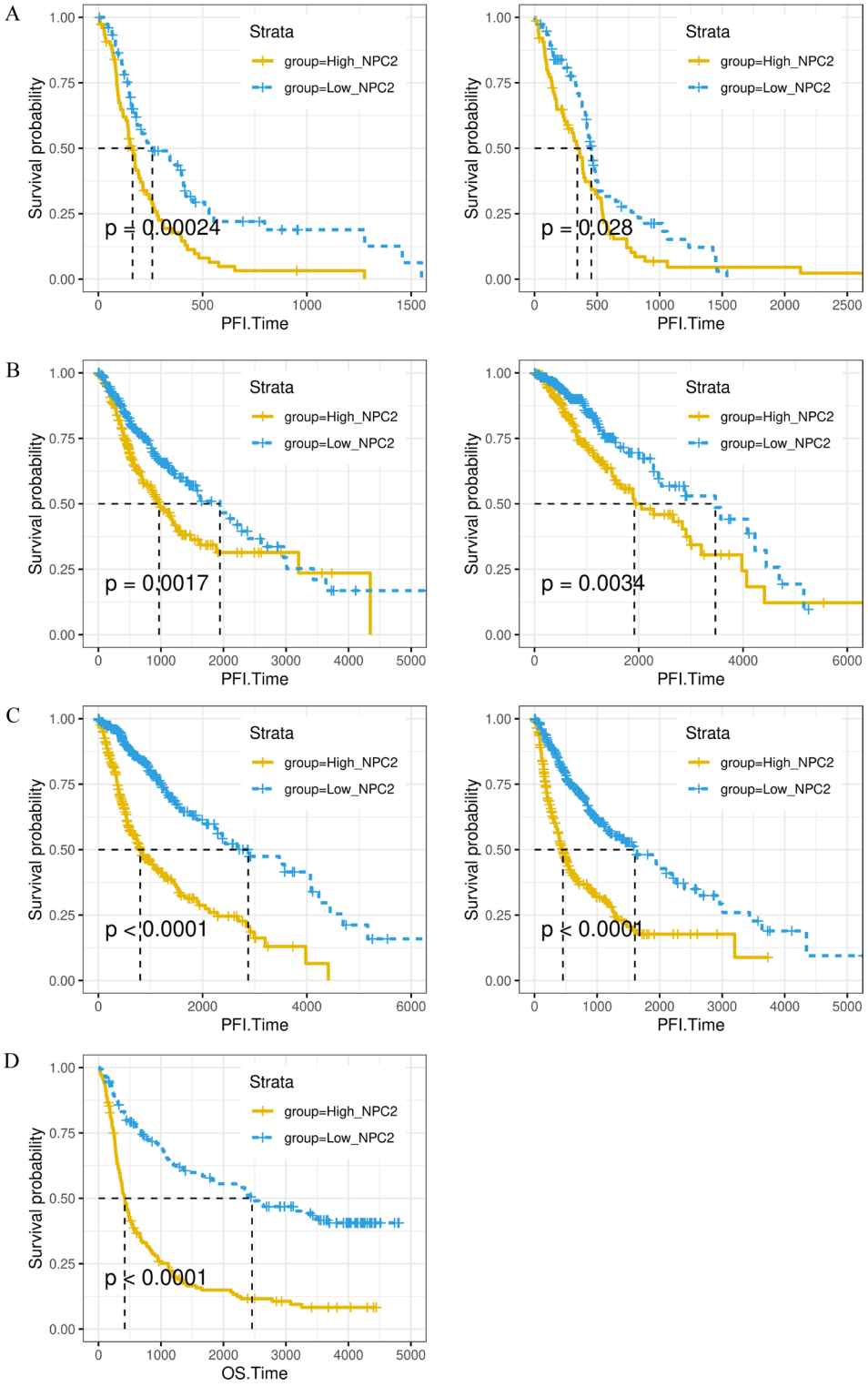

Figure 3. Relationship between NPC2 expression and prognosis of glioma patients. (**A**) Prognosis analysis of NPC2 in TCGA-GBM; (**B**) Prognosis analysis of NPC2 in TCGA-LGG; (**C**)Prognostic analysis of NPC2 in TCGA_GBM and TCGA_LGG combined data; (**D**) Association of NPC2 with glioma prognosis in the CGGA database.

analyses also confirmed that *NPC2* was a significant risk factor in gliomas (Tables 2 and 3). Additionally, patient K-M curves from the CGGA dataset demonstrated that low-expression *NPC2* samples had considerably better prognoses than high-expression samples, supporting our findings (Fig. 3D).

Analysis of prognostic value of *NPC2* gene

After TCGA-glioma patients (AUC = 0.697) and patients with various clinical features were subjected to ROC curve analysis, it was discovered that *NPC2* showed significant prognostic and predictive value in glioma patients (Fig. 4A–F). The AUC values of IDH mutation status, X1p/19q codel-status, PRS, age, Grade were 0.772, 0.764, 0.543, 0.644 and 0.745, respectively (Fig. 4B–F). In addition, the CGGA database results also confirm our findings (Fig. 4G).

Screening and functional enrichment analysis of differentially expressed genes

With screening parameters of p-adjust 0.05 and logFC | > 1, the R program "DESeq2" was utilized to screen for DEGs in the high and low *NPC2* expression groups of TCGA-glioma samples (Fig. 5A). According to GO enrichment analysis, DEGs were particularly enriched in the regulation of trans-synaptic signaling, modulation of chemical, synaptic membrane, channel activity, and other processes (Fig. 5B). According to KEGG functional enrichment analysis, DEGs were primarily implicated in pathways such as retrograde endocannabinoid signaling and neuroactive ligand-receptor interaction (Fig. 5C). Figure 5D,E shows heat maps of *NPC2* DNA methylation in GBM and LBB (using probes cg11740921, cg21915100) analyzed based on the MetSurv database.

Relationship between *NPC2* gene and immune infiltration in glioma patients

We showed the relationship between the *NPC2* gene and immune cells in Fig. 6A,B as a heat map and scatter diagram, respectively. The findings suggested that the *NPC2* gene was substantially connected with Neutrophil, Macrophage, and DC in various forms of glioma. In instance, there were significant correlation between LGG and macrophage (0.81), as well as between macrophage and LGG (0.70) and GBM (0.70). Analysis of the immune cell level differences between the high and low *NPC2* expression groups revealed that the levels of activated B cells and the other 14 immune cells were considerably greater in the high expression group (Fig. 6C).

Drug sensitivity analysis

This study analyzed the differences in drug sensitivity to anticancer drugs in patients with high-low *NPC2* expression groups. These results indicated that the differential expression of *NPC2* had a certain effect on the drug sensitivity of patients, indicating that *NPC2* could provide some guidance for the drug use of patients with glioma (Fig. 7A–F).

Interaction gene set analysis

The String database was used to search out *NPC2* interacting genes, including *LDB3*, *GM2A*, *NUDT19*, *SLC9A3R2*, *GRN*, *NME2*, *CFB*, *NPC1* and *RRAGA* (Fig. 8A). And these genes were mainly related to DNA damage inhibit, hormone ER activate and other pathways in glioma (Fig. 8B). Moreover, genes *NUDT19* and

	p value	HR
PRS.type	4.2e−13	0.35 (0.26–0.46)
Age	0.00024	1.7 (1.3–2.2)
Gender	0.66	0.94 (0.72–1.2)
Grade	2.4e−20	0.18 (0.12–0.26)
IDH.mutation.status	2.6e−13	2.8 (2.1–3.7)
X1p19q.codel.status	1.2e−12	5.9 (3.6–9.6)
NPC2	1.1e−15	0.31 (0.24–0.42)

Table 2. Unifactor cox analysis.

	p value	HR
PRS.typelow_PRS	1.4e−07	0.44 (0.33–0.6)
Age > 42	7.8e−01	1 (0.78–1.4)
Gender male	8.3e−01	0.97 (0.73–1.3)
Gradelow_Grade	3.4e−09	0.29 (0.2–0.44)
IDH.mutation.status whidtype	1.1e−01	1.3 (0.94–1.8)
X1p19q.codel.status Non-codel	3.6e−07	3.9 (2.3–6.6)
NPC2 Low_NPC2	4.6e−03	0.64 (0.48–0.87)

Table 3. Multivariate cox analysis.

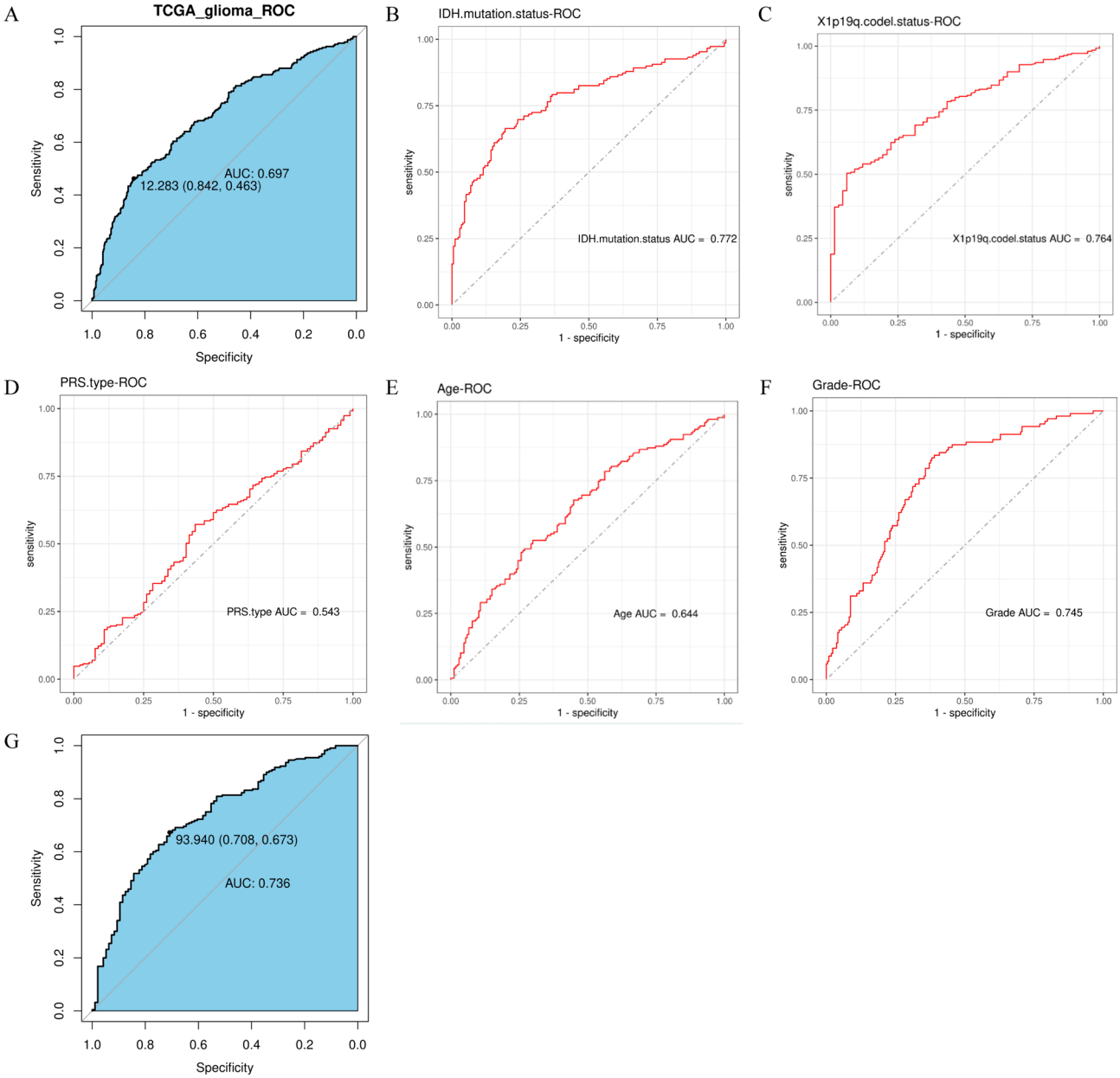

Figure 4. Diagnostic value analysis of NPC2. (**A**) Diagnostic value of NPC2 in TCGA database; (**B**–**F**) Diagnostic value of NPC2 in different clinical features of glioma; (**G**) Diagnostic value of NPC2 in CGGC database.

*NME*2 were significantly positively correlated with the prognosis of LGG (Fig. 8C). And the expression of *SLC*9*A*3*R*2, *CFB* and *LDB*3 genes were strong negative correlation with methylation level in LGG (Fig. 8D). By analyzing the correlation between *NPC2* interacting gene set methylation and patient survival in glioma patients, it was found that the methylation of *NPC*2, *NUDT*19 and *CFB* was significantly correlated with the prognosis of LGG patients (Fig. 8E).

Validation of the expression of *NPC2*

The expression of *NPC2* gene in cells and tissues verified by qRT-PCR showed that the expression of *NPC2* gene in DBTRG cells was significantly higher than that of AVGP12, and the expression of *NPC2* gene in cancer tissues was significantly higher (Fig. 9A,B). Additionally, the expression of NPC2 protein in tumor samples was significantly higher than that in normal samples based on the results of IHC (Fig. 9C).

Discussion

GBM is a common malignant tumor in adults with poor prognosis and high recurrence rate[18]. In recent years, with the wide application of high-throughput sequencing technology in the field of cancer, we have more understanding of the mechanism of the occurrence and development of GBM. In order to further understand

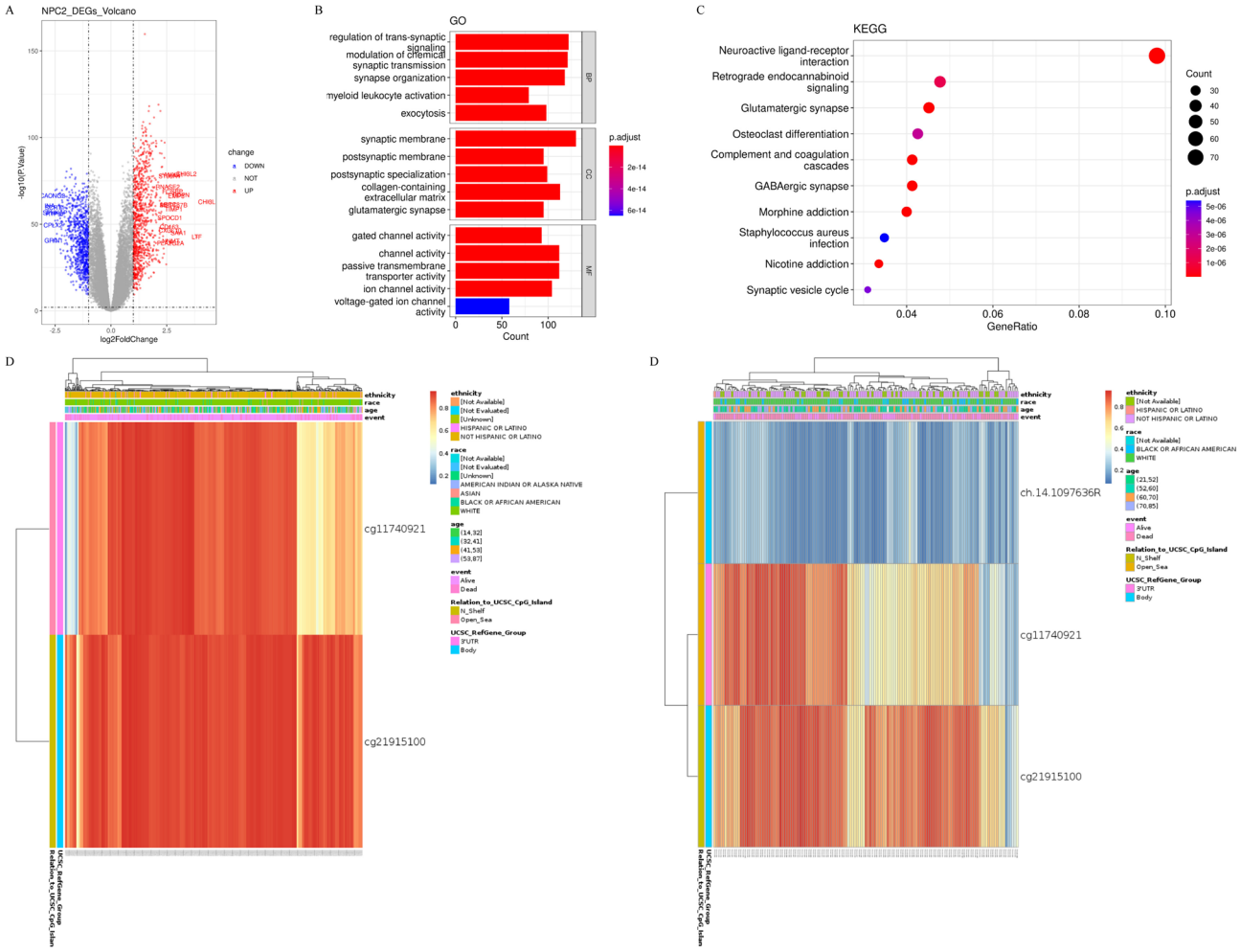

Figure 5. Functional enrichment analysis of NPC2 in TCGA-glioma database. (**A**) Volcano plot; (**B**) GO enrichment analysis; (**C**) KEGG enrichment analysis; (**D**) Methylation levels of NPC2 in GBM; (**E**) Methylation levels of NPC2 in LBB.

the role of different genes in the occurrence and development of GBM and to find new therapeutic targets, it is very important to develop reliable prognostic markers. However, there are few reports about the relationship between *NPC2* and the prognosis of GBM. Our study was designed to explore the role and prognostic value of *NPC2* in the occurrence and development of GBM.

The *NPC2* gene has a long 13.5 kb, which consists of five exons and is located at position 24.3 on the long arm of human chromosome 14. Its coding protein *NPC2* (NPC intracellular cholesterol transporter 2) is a protein coding gene containing a lipid recognition domain, which has been proved to play an important role in biological processes such as cholesterol metabolism[19]. Some studies have found that the mutation of this gene is associated with thyroid cancer[20], gastric cancer[21] and Niemann–Pick disease[22]. In order to further clarify the relationship between *NPC2* gene and GBM and to guide the prognosis of GBM. We systematically studied the expression of *NPC2* gene in all types of cancers, and found that it was expressed in all types of cancers, and the expression levels of LGG, GBM, SKCM and THC were significantly higher than those in other tumors. According to prognostic analysis, *NCP2* gene is an important risk factor in GBM, hNSC, LGG and STAD. We confirmed the high expression of *NPC2* in GBM by immunohistochemistry and PCR experiment. Therefore, we further studied the expression of *NPC2* gene in gliomas. According to the comparison between TCGA patients and normal samples in GTEx, the *NPC2* gene expression in GBM and LGG patients in GSE68848 data set was significantly higher than that in normal patients. All these findings clearly demonstrate the importance of *NPC2* gene in glioma diseases. Therefore, we further studied the differences of gene expression among different clinical types in order to further study the potential relationship between *NPC2* gene and glioma. We found that the expression level of *NPC2* gene was higher in GBM, Ra, RAA and rGBM. In WHOIV, the expression of *NPC2* gene was significantly higher than that in patients with WH O III and II. And the expression of *NPC2* gene in patients with wild type IDH mutation was significantly higher than that in patients with WHOIV under different subtypes. Overall, patients with GBMIDH wild type showed increased expression of *NCP2* gene. In addition, male patients and patients over 42 years of age had higher levels of gene expression, which was consistent with reports that age was identified as a prognostic factor for GBM[23]. We confirmed that *NPC2* is an important risk factor for glioma by Kmurm survival curve and univariate and multivariate COX analysis. In addition, the Kmuri

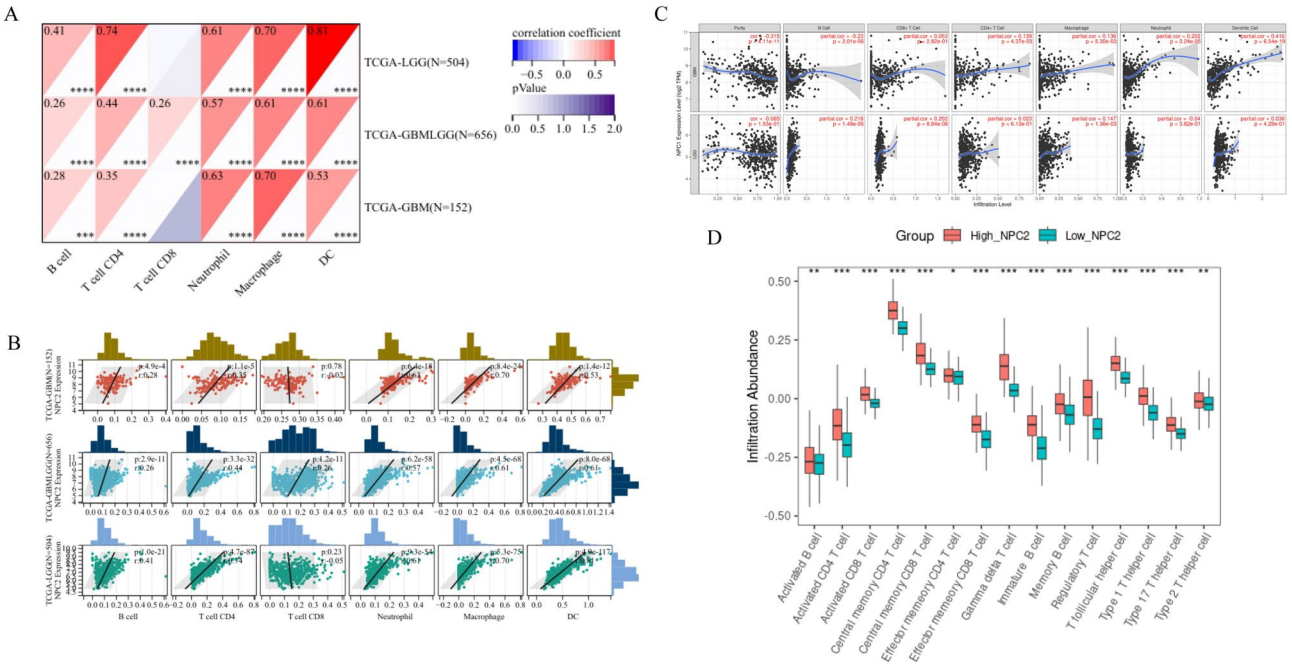

Figure 6. Immunoinfiltration analysis. (**A**) Correlation heatmap of NPC2 and immune cells; (**B**) Scatter plot of correlation between NPC2 and immune cells; (**C**) Differences in immune cell levels between patients with high and low NPC2 expression.

M curve of patients with CGGA dataset shows that low expression *NPC2* samples have a better prognosis than high expression samples, which is consistent with the previous conclusion. After analyzing the ROC curve of patients with glioma and patients with different clinical characteristics, it is found that *NPC2* has significant prognostic and predictive value for patients with glioma.

In order to further understand the specific mechanism and related pathways of *NPC2* in the occurrence and development of GBM. According to GO enrichment analysis, we found that DEGS is especially abundant in trans-synaptic signal regulation, chemical regulation, synaptic membrane, channel activity and so on. Among them, chemical regulation, synaptic membrane and channel activity play an important role in tumorigenesis, development and immunity[24–26], which is consistent with our findings. Through KEGG function-rich analysis, we found that DEGS is mainly involved in retrograde endogenous cannabinoid signal transduction and neuroactive ligand-receptor interaction, but this finding has not been studied in the literature. In addition, we found that *NPC2* gene is closely related to neutrophils, macrophages and DC in different forms of gliomas by immune infiltration analysis. For example, there is a significant correlation between LGG and macrophages, and between macrophages and LGG and GBM. The level of activated B cells and 14 other immune cells in the group with high expression of *NPC2* was quite high. They may promote GBM cell growth and invasion by creating an immunosuppressive microenvironment[27]. Through drug sensitivity analysis, we found that the differential expression of *NPC2* has a certain impact on the drug sensitivity of patients, indicating that *NPC2* can provide some guidance for the use of drugs in patients with glioma. We speculate that *NPC2* may be a new immune-related target for prognosis and treatment of GBM.

In short, we found and verified the negative correlation between the expression of *NPC2* gene and the prognosis of GBM. As an independent prognostic index of GBM, it provides a potential molecular target for clinical treatment of gliomas. However, there are also shortcomings in this study. Although we verified the expression of *NPC2* by qPCR and immunohistochemistry, constructing animal or cellular experiments for further validation is very necessary. And there is no in-depth exploration of the mechanism of related molecules in this study. In the future, we will explore the important role of NPC gene in the occurrence and development of glioma.

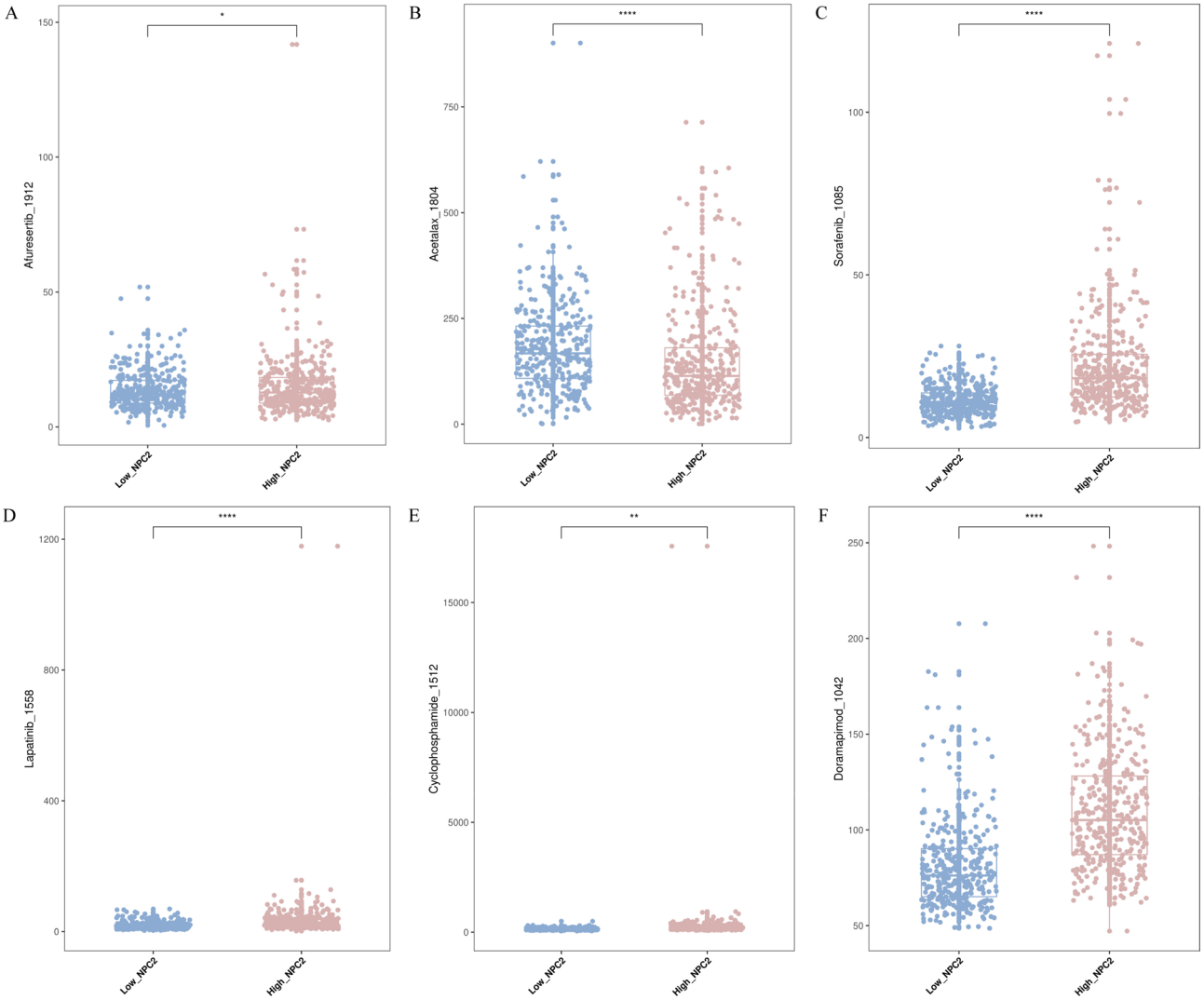

Figure 7. Drug sensitivity analysis of patients in the NPC2 expression group of the TCGA-glioma database to anticancer drugs.

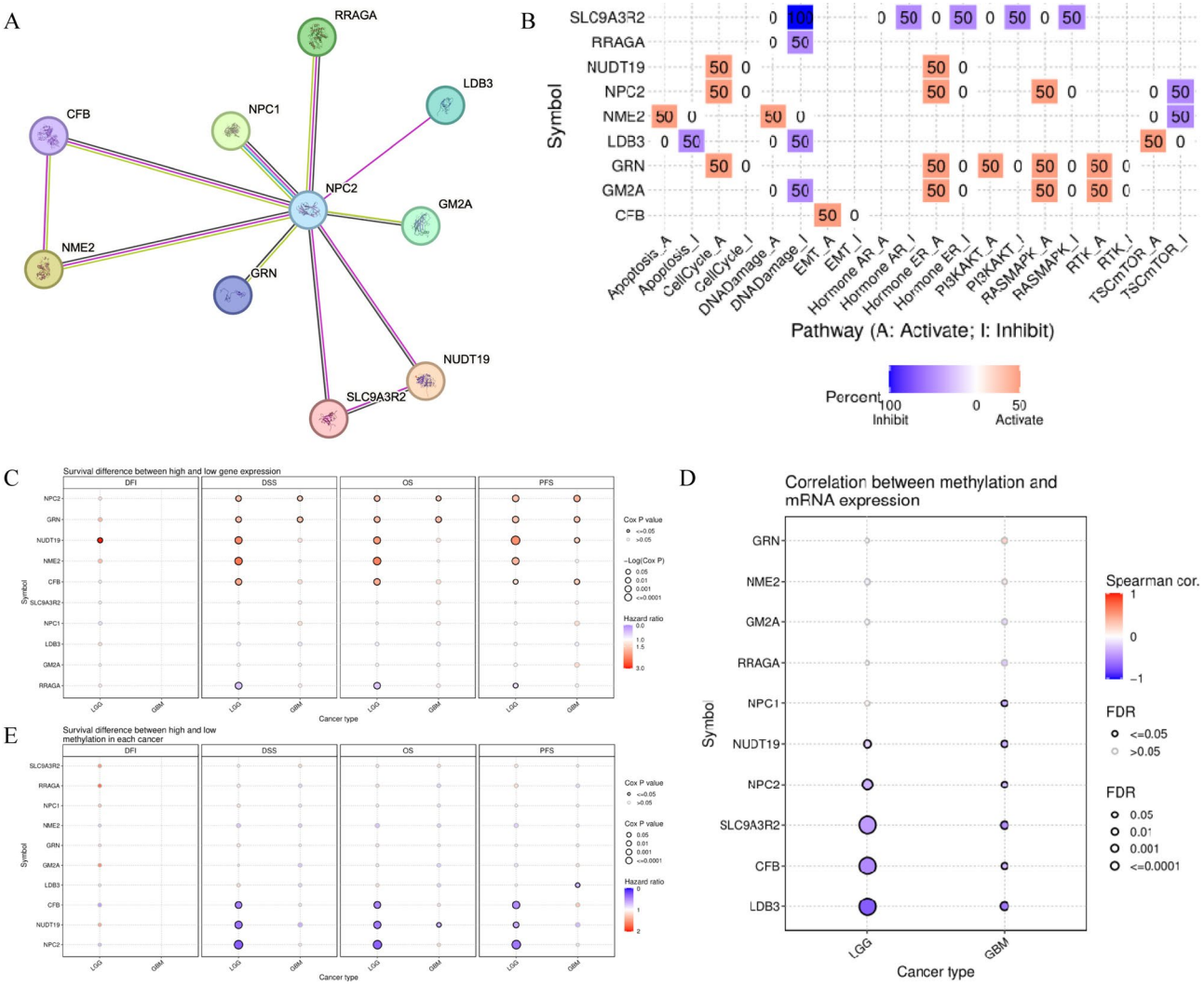

Figure 8. Analysis of genes interacting with NPC2. (**A**) PPI network construction using string database to obtain NPC2 expression interaction gene set; (**B**) Correlation analysis between NPC2 expression interacting gene set and patient prognosis; (**C**) Correlation between expression of NPC2 interacting gene set and methylation in glioma patients; (**D**) Correlation between methylation and patient survival.

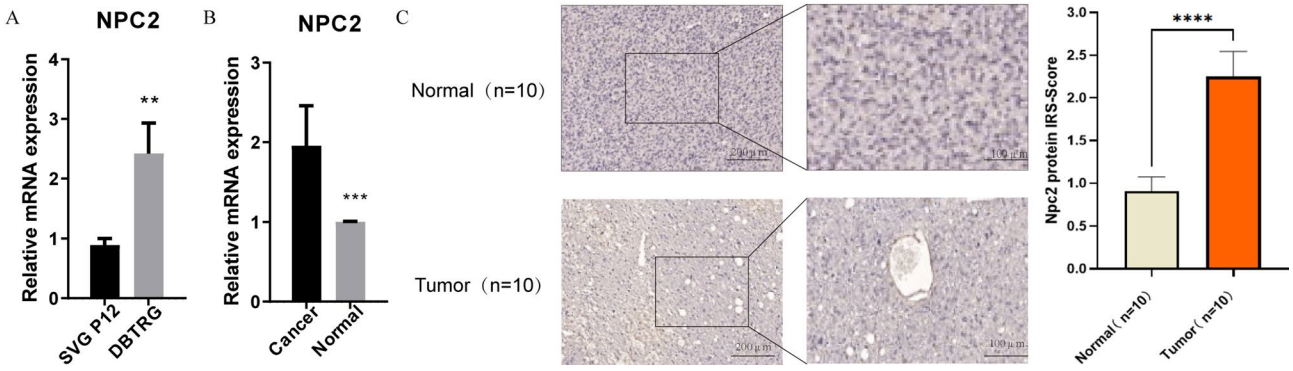

Figure 9. The expression of *NPC2* was verified by RT-qPCR. (**A**) The differential expression of *NPC2* in tissues (cancer and normal) was detected by RT-qPCR; (**B**) The differential expression of *NPC2* in cells (SVGP12 and DBTRG) was detected by RT-qPCR. (**C**) The differential expression of NPC2 in tissues was detected by IHC.

Data availability

The datasets analyzed for this study can be found in the TCGA-glioma (http://www.cancer.gov/tcga), CGGA-glioma (http://www.cgga.org.cn/), GEO (https://www.ncbi.nlm.nih.gov/geo/), GTEx (https://gtexportal.org/home).

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Author contributions

ZGW and ZGM is the co-first author. WD design of the work; ZGW acquisition analysis, WD interpretation ofdata; WD the creation of new software used in the work; ZGW, ZGM, and WD have drafted and guided the work or substantively revised it. All authors read and approved the final manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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