

# Identification of therapeutic targets and prognostic biomarkers among STAT family in glioblastoma

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## Primary research

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# Abstract

**Background:** Glioblastoma (GBM) is the most common and aggressive primary brain malignancies with high incidence and mortality. The aberrant activation of STAT signaling was confirmed to result in tumor pathogenesis and progress by regulating cell cycle, cell survival, and immune response. **Methods:** The clinical significance of and regulation network of STAT family in GBM were explored with several web applications or database. **Results:** The level of STAT1/3/5A/5B/6 were increased in GBM while STAT4 level was decreased. GBM patients with high expression of STAT1/2/3/5A/6 and low expression of STAT4/5B had a worse overall survival. Among the STAT family, STAT 4 and STAT6 were the top two frequently mutated genes. Correlation suggested a low to moderate correlation among STAT family. STAT family were also involved in the activation or inhibition of the famous cancer related pathways. Immune infiltrates analysis suggested that STAT5A level showed significantly correlated with the abundance of immune cells and the level of immune gene biomarkers. GO functions and KEGG pathways analysis revealed that STAT5A was involved in immune response-regulating signaling pathway, neutrophil and lymphocyte mediated immunity, single-stranded DNA binding, cytokine-cytokine receptor interaction, NOD-like receptor signaling pathway, NF-kappa B signaling pathway, and TNF signaling pathway. Moreover, we also identified several Kinase and transcription factor targets of STAT5A in GBM. **Conclusions:** Our results revealed the therapeutic targets, prognostic biomarkers and regulation network of STAT family in GBM, laying the foundation for further studies about STAT family in therapy and prognosis of GBM.

## 1. Background

Glioblastoma (GBM) is the most common and aggressive primary brain malignancies, accounting for about 45.6% of brain malignancies [1]. In 2020, an estimated of 23,890 patients would be initially diagnosed with brain malignancies in America alone [2]. No significant progress was made in the standard of care in the past of two decades [3]. Moreover, the prognosis of GBM patients was poor, with a median overall survival less than 20 months and 5-year-survival of 5% [4, 5]. The exploration and identification of novel biomarkers for the prognosis and therapy, and a combination of current treatments, are the most promising strategies for the management of GBM.

Signal transducer and activator of transcription (STAT) signaling pathways are crucial for the function, proliferation, and apoptosis of immune cells, such as CD8<sup>+</sup> cells and dendritic cells [6]. The aberrant activation of STAT signaling was confirmed to result in tumor pathogenesis and progress by regulating cell cycle, cell survival, and immune response [7, 8]. STAT family consist of STAT1-4, STAT5A/5B, and STAT6. Previous studies revealed that STAT1 acted as an anti-oncogene in GBM under hypoxia and inhibited angiogenesis [9]. STAT3 interacted with FOXM1 could confer radio-resistance in GBM [10]. These evidences demonstrated the vital function of that STAT in the occurrence and progression of GBM. However, the potential application value and mechanism of STAT family in therapy and prognosis of GBM still not be clarified.

In this study, we performed a systematic exploration about the level and prognostic value of STAT family in GBM. Besides, single nucleotide variation (SNV), correlation, cancer-related pathway, drug sensitivity, Immune infiltrates, and function analysis of STAT family in GBM were also conducted. Our study may reveal the potential application value and mechanism of STAT family in therapy and prognosis of GBM.

## **2. Materials And Methods**

### **2.1 Oncomine**

The Oncomine ([www.oncomine.org/](http://www.oncomine.org/)) is a web application for translational bioinformatics services [11]. It contains 700 + independent datasets, which could help researcher interrogate gene expression profiles, and biological interactions. Using Oncomine web application with the  $P < 0.05$  and fold-change = 2, STAT family mRNA level in GBM were detected.

### **2.2. GEPIA**

GEPIA ([www.gepia.cancer-pku.cn/index.html](http://www.gepia.cancer-pku.cn/index.html)) is web application developed by Zefang Tan et al. of Peking University. Various customizable functions, including tumor/normal differential expression analysis and correlation analysis of STAT family in GBM, were explored by GEPIA [12]. Pearson correlation coefficient was utilized on the The Cancer Genome Atlas (TCGA) GBM dataset with a P-value of 0.05.

### **2.3 PrognoScan**

PrognoScan (<http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html>), a public database, could performed meta-analysis of the prognostic value of targets [13]. The significance of STAT family in the prognosis of GBM patients was explored with PrognoScan.

### **2.4. TIMER**

TIMER ([www.cistrome.shinyapps.io/timer/](http://www.cistrome.shinyapps.io/timer/)) is web application developed by Li et al. for immune infiltrates analysis [14]. Various customizable functions, including immune cell infiltrates and immune gene biomarker correlation analysis of STAT family in GBM, were explored by TIMER. Table 3 showed the immune gene biomarkers and their corresponding immune cells, which had been referred in previous studies[15–17].

Table 3

Correlation analysis between STAT5A and gene biomarkers of immune cells in glioblastoma

Description	Gene biomarkers	TIMER		CCGA	
		Cor	P-value	Cor	P-value
CD8 + T cell	CD8A	0.159	0.0501	0.4	***
	CD8B	0.275	***	0.46	***
T cell (general)	CD3D	0.355	***	0.54	***
	CD3E	0.492	***	0.59	***
	CD2	0.393	***	0.6	***
B cell	CD19	0.242	**	0.58	***
	CD79A	0.079	0.332	0.5	***
Monocyte	CD86	0.473	***	0.62	***
	CD115(CSF1R)	0.563	***	0.57	***
TAM	CCL2	0.494	***	0.48	***
	CD68	0.554	***	0.7	***
	IL10	0.666	***	0.38	***
M1 Macrophage	INOS (NOS2)	-0.077	0.343	0.24	***
	IRF5	0.487	***	0.64	***
	COX2(PTGS2)	0.584	***	0.18	***
M2 Macrophage	CD163	0.47	***	0.55	***
	VSIG4	0.401	***	0.56	***
	MS4A4A	0.436	***	0.58	***
Neutrophils	CD66b (CEACAM8)	-0.076	0.348	–	–
	CD11b (ITGAM)	0.627	***	0.66	***
	CCR7	0.269	***	0.39	***
Natural killer cell	KIR2DL1	0.113	0.166	–	–
	KIR2DL3	0.171	*	–	–
	KIR2DL4	0.356	***	–	–
	KIR3DL1	0.167	*	–	–
	KIR3DL2	0.099	0.222	–	–
	KIR3DL3	0.114	0.162	–	–
	KIR2DS4	0.158	0.0507	–	–
Dendritic cell	HLA-DPB1	0.509	***	0.55	***
	HLA-DQB1	0.348	***	0.23	***
	HLA-DRA	0.459	***	0.55	***
	HLA-DPA1	0.44	***	0.47	***
	BDCA-1(CD1C)	0.207	*	0.29	***
	BDCA-4(NRP1)	0.405	***	0.38	***
	CD11c (ITGAX)	0.277	***	0.64	***

Description	Gene biomarkers	TIMER		CCGA	
		Cor	P-value	Cor	P-value
Th1	T-bet (TBX21)	0.006	0.939	0.46	***
	STAT4	0.294	***	0.23	***
	STAT1	0.259	**	0.42	***
	IFN-g (IFNG)	0.068	0.402	–	–
	TNF-a (TNF)	0.237	**	0.39	***
Th2	GATA3	0.399	***	0.55	***
	STAT6	0.553	***	0.69	***
	STAT5A	–	–	–	–
	IL13	-0.188	*	0.35	***
Tfh	BCL6	0.124	0.126	0.27	***
	IL21	-0.052	0.525	–	–
Th17	STAT3	0.348	***	0.39	***
	IL17A	-0.028	0.733	–	–
Treg	FOXP3	0.395	***	0.44	***
	CCR8	0.297	***	–	–
	STAT5B	0.051	0.531	0.17	***
	TGFb (TGFB1)	0.481	***	0.69	***
T cell exhaustion	PD-1 (PDCD1)	0.186	*	0.43	***
	CTLA4	0.396	***	0.47	***
	LAG3	0.001	0.992	0.47	***
	TIM-3 (HAVCR2)	0.411	***	–	–
	GZMB	0.316	***	0.46	***

## 2.5 CGGA

CGGA (the Chinese Glioma Genome Atlas, [www.cgga.org.cn](http://www.cgga.org.cn)) is a database for exploring brain tumors with 2,000 samples from Chinese cohorts [18]. We used “mRNAseq\_693” of CGGA to explore the prognostic value of STAT family, and their correlation with immune gene biomarkers in GBM.

## 2.6 GSCALite

GSCALite ([www.bioinfo.life.hust.edu.cn/web/GSCALite/](http://www.bioinfo.life.hust.edu.cn/web/GSCALite/)) a web-based platform developed by Liu et al. for gene set cancer analysis [18]. Various customizable functions, including SNV, cancer pathway activity, and drug analysis of STAT family in GBM, were explored by GSCALite.

## 2.7. LinkedOmics

LinkedOmics ([www.linkedomics.org](http://www.linkedomics.org)) contains multi-omics data and clinical data of 11 158 patients from TCGA project, which could performed translational bioinformatics services [19]. Various customizable functions, including correlated gene analysis, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, kinase and transcription factor-target analysis were explored by LinkedOmics with the TCGA GBM sample (N = 153). Pearson Correlation and Gene Set Enrichment Analysis were performed with a p-value < 0.05.

## 2.8. GeneMANIA

GeneMANIA ([www.genemania.org](http://www.genemania.org)) is a prediction server, which could be used for gene prioritization and predicting gene function [20]. The customizable functions, including kinase LCK and transcription factor ELF1 network, were explored with GeneMANIA by constructing a PPI network.

## 3. Results

### 3.1. Expression and prognosis analysis of STAT family in GBM.

Oncomine and GEPIA was allowed to performed expression analysis of STAT family in GBM. As listed in Fig. 1 and Table 1. A gene expression profiling revealed an up-regulation of STAT1 in tumor tissues with a fold change of 3.136, and a p-value of 1.58E-10 [21]. Another study also revealed an up-regulation of STAT1 in tumor tissues with a fold change of 2.701, and a p-value of 1.69E-5 [22]. A total of two datasets suggested that STAT3 was increased in GBM with the fold change of 2.076 and 2.270, respectively [23, 24]. Data of Lixin et al. suggested that STAT5A and STAT5B was increased in GBM (STAT5A: fold change = 2.255; STAT5A: fold change = 2.953) [24]. We observed that the expression of STAT6 were significantly upregulated GBM, and the fold change was 2.103 ( $p = 0.004$ ). Results from GEPIA indicated an increased level of STAT1, STAT3, and STAT5A, and a decreased level of STAT4 in GBM tissues compared with brain tissues (Fig. 2A).

Table 1  
The mRNA levels of STAT family in glioblastoma (ONCOMINE).

STATs	Fold Change	P value	t-test	Reference
STAT1	3.136 2.701	1.58E-10 1.69E-5	9.074 6.363	PMCID: PMC556127 PMID: 12894235
STAT2	NA	NA	NA	NA
STAT3	2.076 2.270	2.81E-7 2.30E-10	11.595 8.047	PMID: 16204036 PMID: 16616334
STAT4	NA	NA	NA	NA
STAT5A	2.255	7.46E-4	7.909	PMID: 16616334
STAT5B	2.953	4.61E-9	7.559	PMID: 16616334
STAT6	2.103	0.004	4.798	PMID: 16697959

We then performed prognosis analysis of STAT family in GBM. The results from Prognoscan were listed in Table 2. We observed that GBM patients with high expression of STAT1(HR [95% CI]: 1.55 [1.11–2.16],  $p = 0.010313$ ), and STAT2 (HR [95% CI]: 1.55 [1.11–2.16],  $p = 0.018909$ ) had a worse overall survival.

Moreover, another two datasets demonstrated that GBM patients with high expression of STAT4(HR [95% CI]: 0.25 [0.09–0.70], P = 0.007913), and STAT5B (HR [95% CI]: 0.74 [0.46–1.19], P = 0.012642) had a better overall survival. The data from CGGA were utilized to verify the results, which revealed that GBM patients with high expression of STAT1 (p = 0.00035), STAT2 (p = 0.00032), STAT3 (p = 0.016), STAT5A (p < 0.0001) and STAT6 (p = 0.0086) had a worse overall survival (Fig. 2B).

Table 2  
The prognostic value of STAT family in glioblastoma (PrognoScan).

STATs	Dataset	Endpoint	Cox p-value	HR [95% CI]
STAT1	GSE4412-GPL96	Overall Survival	0.010313	1.55 [1.11–2.16]
STAT2	MGH-glioblastoma	Overall Survival	0.017809	7.49 [1.42–39.59]
STAT3	NA	NA	NA	NA
STAT4	GSE7696	Overall Survival	0.007913	0.25 [0.09–0.70]
STAT5A	NA	NA	NA	NA
STAT5B	MGH-glioblastoma	Overall Survival	0.012642	0.74 [0.46–1.19]
STAT6	NA	NA	NA	NA

### 3.2. SNV, correlation, cancer-related pathways, and drug sensitivity analysis of STAT family in GBM.

As shown in Fig. 3A, missense mutation was the most common variant classification and C > T was the most common SNV class. Among the STAT family, STAT 4 and STAT6 were the top two frequently mutated genes (Fig. 3A). Correlation analysis of STAT family demonstrated a low to moderate correlation among STAT family (Fig. 3B).

Figure 3C showed the result of STAT family in the famous cancer related pathways. The STAT family played significant functions in GBM mainly by activating apoptosis pathway, EMT pathway, hormone ER pathway, RAS/MAPK pathway, and RTK pathway, and inhibiting cell cycle pathway, DNA damage response pathway, hormone AR pathway, and P13K/AKT pathway (Fig. 3C). The results of drug sensitivity analysis were shown in Fig. 4. GBM patients with low expression of STAT5B show resistance to 59 drugs or small molecules. Furthermore, GBM patients with low expression of STAT5A show resistance to 42 drugs or small molecules (Fig. 4).

### 3.3. Immune infiltrates analysis of STAT5A in GBM.

Above results suggested that STAT5A expression was increased in GBM and acted as potential prognostic biomarker. Moreover, STAT5A was involved in the activation and inhibition of famous cancer related pathways, and drug resistance. Thus, STAT5A may play a significant role the pathogenesis and

progress, and we select it for further analysis. We next analyzed the correlation between STAT5A and immune infiltrates in GBM because of the important role of STAT family in immune response [25, 26]. As presented in Fig. 5, we observed that STAT5A level showed significantly correlated with the abundance of B cell ( $p = 1.91e-02$ ), CD4 + T cells ( $p = 9.55e-03$ ), Macrophage ( $p = 1.64e-04$ ), Neutrophils ( $p = 6.63e-04$ ) and Dendritic cells ( $p = 1.60e-16$ ).

The results of immune gene biomarkers correlation analysis revealed that STAT5A expression showed strong correlation gene biomarkers expression in GBM, which were presented in Table 3. For gene biomarkers of CD8 + T cell, data of TIMER revealed that CD8B expression was positively associated with STAT5A expression, and data of CGGA revealed that CD8A and CD8B expression were positively associated with STAT5A expression. Moreover, for gene biomarkers of T cell (CD3D, CD3E, CD2), Monocyte (CD86 and CD115), and TAM (CCL2, CD68, IL10), and M2 Macrophage (CD163, VSIG4, MS4A4A), data of TIMER and CGGA suggested a strong correlation between STAT5A expression and all the gene biomarkers expression. When analyzed gene biomarkers of Natural killer cell, we observed that the level of KIR2DL3, KIR2DL4, and KIR3DL1 were positively correlated with STAT5A expression in TIMER. In TIMER and CGGA, eight gene biomarkers of Dendritic cell were strongly associated with STAT5A expression. Similarly, the level of STAT4, STAT1, TNF, GATA3, STAT6 and IL13 were positively correlatively with STAT5A level. Two gene biomarkers (FOXP3 and TGFB1) of Treg and three gene biomarkers (PD-1, CTLA4 and GZMB) of showed strong correlation with STAT5A expression. Therefore, STAT5A may act as target for the immunotherapy of GBM.

### **3.4. Enrichment analysis of STAT5A in THCA.**

Figure 6A showed the genes were significantly correlated with STAT5A in GBM. Among these genes, the top 50 most significant genes positively (Fig. 6B) and negatively (Fig. 6C) associated with STAT5A were also extracted. Moreover, Supplementary Fig. 1 showed the top three genes positively associated with STAT5A, which were TMEM199 ( $cor = 0.6939$ ,  $p = 2.721e-23$ ), FES ( $cor = 0.6883$ ,  $p = 8.324e-23$ ), and SLC24A6 ( $cor = 0.6829$ ,  $p = 2.414e-22$ ). Enrichment analyses of STAT5A, including GO function and KEGG pathways, were conducted. The data of GO function analysis in Fig. 6D-6F suggested that the functions of STAT5A in GBM mainly enriched in immune response-regulating signaling pathway, neutrophil and lymphocyte mediated immunity, regulation of immune effector process, immunological synapse, cytokine receptor binding, single-stranded DNA binding, and immunoglobulin binding. Furthermore, the data of KEGG pathway in Fig. 6G and Fig. 6H suggested that the functions of STAT5A in GBM mainly enriched in cytokine-cytokine receptor interaction, NOD-like receptor signaling pathway, NF-kappa B signaling pathway, TNF signaling pathway, and Th17 cell differentiation.

<b>Complement and coagulation cascades</b>
Pertussis
Th17 cell differentiation
TNF signaling pathway
Hematopoietic cell lineage
Lysosome
NF-kappa B signaling pathway
Osteoclast differentiation
NOD-like receptor signaling pathway
Cytokine-cytokine receptor interaction

### 3.5. Kinase and transcription factor target network analysis of STAT5A in GBM.

The kinase and transcription factor target networks of STAT5A in GBM were also explored. The results were listed in Table 4. The data suggested kinase LCK, SYK, LYN, HCK, and TTK as the most five significant kinase network targets. With the construction of a PPI network, we found that kinase LCK target network were involved in the regulation of immune response, antigen receptor-mediated signaling pathway, T cell activation and receptor signaling pathway (Fig. 7A). As for transcription factor target, the most five significant targets were V\$ELF1\_Q6, V\$IRF\_Q6, V\$PEA3\_Q6, V\$PU1\_Q6, and V\$NERF\_Q2. With the construction of a PPI network, we found that kinase LCK target network were involved in the regulation of immune response, regulation of lymphocyte and leukocyte activation, and B cell activation (Fig. 7B).

Table 4

The kinase and transcription factor targets of STAT5A in glioblastoma (LinkedOmics).

Enriched Category	Geneset	LeadingEdgeNum	FDR
Kinase Target	Kinase_LCK	27	0
	Kinase_SYK	18	0
	Kinase_LYN	24	0
	Kinase_HCK	11	0
	Kinase_TTK	5	0
Transcription Factor Target	V\$ELF1_Q6	86	0
	V\$IRF_Q6	76	0
	V\$PEA3_Q6	91	0
	V\$PU1_Q6	56	0.00014 0.00015
	V\$NERF_Q2	64	

## 4. Discussion

The STAT family could mediate various biological processes, including cellular immunity, invasion, differentiation, and apoptosis [27]. Activated by diverse cytokines, STAT signaling played a central role in immunity, cell death, and cancer pathogenesis and progression [8]. Extensive studies have revealed that STAT family exert important functions in cancers, such as hepatocellular carcinoma. However, the study about the all the STAT family in GBM has never been performed. So, our study was carried out.

Expression analysis revealed that the level of STAT1/3/5A/5B/6 were increased in GBM while STAT4 level was decreased. GBM patients with high expression of STAT1/2/3/5A/6 and low expression of STAT4/5B had a worse overall survival. These results were consistent with previous studies. Balaram et al also found that STAT1 is upregulated in GBM and correlated with a worse prognosis [28]. Another study suggested STAT3 as a potential biomarker for the prognosis of GBM [29]. Therefore, we suggested that STAT family may act as potential prognostic biomarkers in GBM.

Cancer-related pathways analysis revealed that The STAT family played significant functions in GBM mainly by activating apoptosis pathway, EMT pathway, hormone ER pathway, RAS/MAPK pathway, and RTK pathway, and inhibiting cell cycle pathway, DNA damage response pathway, hormone AR pathway, and P13K/AKT pathway. Adetola et al also revealed that STAT3 inhibition induced apoptosis in cancer cells [30]. In hepatocellular carcinoma, STAT1 was also supposed as tumor-suppressor, and induced G0/G1 cell cycle arrest and apoptosis of tumor cell [31]. Therefore, STAT family may regulate the pathogenesis and progress of GBM via these pathways.

In our study, we also observed that STAT5A level showed significantly correlated with the abundance of immune cells and the level of immune gene biomarkers. Actually, these immune cells and immune gene biomarkers had been reported to be therapy target and play a significant role in various types of cancer. Immune cells, including dendritic cells and CD8 + cytotoxic T lymphocytes, could function as promising targets for the therapy of bone metastasis [32]. In breast cancer, CD8 + T cells and regulatory T cells were

suggested as the reliable prognostic biomarker [33]. CTLA4 and PD-1 had been suggested as immune checkpoint for cancer immunotherapy [34]. Therefore, we suggested STAT5A as potential therapeutic target for cancer immunotherapy.

We next performed GO functions and KEGG pathways analysis, which revealed that STAT5A was involved in immune response-regulating signaling pathway, neutrophil and lymphocyte mediated immunity, single-stranded DNA binding, cytokine-cytokine receptor interaction, NOD-like receptor signaling pathway, NF-kappa B signaling pathway, and TNF signaling pathway. Interestingly, the functions and pathways were associated with immune response and the carcinogenesis and progression. Fernando et al. suggested NOD-like Receptors as important players and targets in the interface between innate immunity and cancer [35]. Previous study found that TNF signaling pathway was involved in the regulation of proliferation and invasion in cervical carcinoma [36]. Single-stranded DNA-binding proteins are supposed as the key of the integrity of the genome[37].

Single-stranded DNA-binding were also involved in cell-cycle checkpoint activation following DNA damage in cancer [37]. Therefore, STAT5A may affect the pathogenesis and progress of GBM via these signaling pathways.

There is no gainsaying that some limitations were found in our study. First, the sample size in our study was relatively small, and it would be better if a larger cohort study were performed. Moreover, our study was short of clinicopathological features analysis.

## 5. Conclusion

In conclusion, our study revealed the expression and prognostic value of STAT family. Immune infiltrates analysis suggested that STAT5A level showed significantly correlated with the abundance of immune cells and the level of immune gene biomarkers. Enrichment analysis revealed that STAT5A was involved in immune response-regulating signaling pathway, cytokine-cytokine receptor interaction, NOD-like receptor signaling pathway, and TNF signaling pathway. Our results revealed the clinical significance of and regulation network of STAT family in GBM, laying the foundation for further studies about STAT family in therapy and prognosis of GBM.

## Abbreviations

GBM: Glioblastoma, STAT: Signal transducer and activator of transcription, SNV: single nucleotide variation, TCGA: The Cancer Genome Atlas, GO: Gene Ontology, KEGG: Kyoto Encyclopedia of Genes and Genomes

## Declarations

**Ethics approval and consent to participate**

Not applicable.

### **Consent for publication**

Not applicable.

### **Availability of data and materials**

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

### **Competing interests**

The authors declare that they have no competing interests.

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### **Authors' contributions**

Chenglin Li and Yanyu Wu were responsible for the design of the study and the writing the manuscript. <sup>1</sup>, Yanfei Zhou, Hanshun Deng, Yuanshen Ye, and Shuizhen Zhao were responsible for data analysis work. Shangnan Liang, Shirong Cai, Jincal Lin, and Yaolong Tang were responsible for the edit of the manuscript. All authors read and approved the final manuscript.

## **References**

1. Wirsching H-G, Galanis E, Weller M (2016) Glioblastoma. *Handb Clin Neurol* 134:381-397. doi:10.1016/B978-0-12-802997-8.00023-2
2. Siegel RL, Miller KD, Jemal A (2020) Cancer statistics, 2020. *CA Cancer J Clin* 70 (1):7-30. doi:10.3322/caac.21590
3. Broekman ML, Maas SLN, Abels ER, Mempel TR, Krichevsky AM, Breakefield XO (2018) Multidimensional communication in the microenvirons of glioblastoma. *Nat Rev Neurol* 14 (8):482-495. doi:10.1038/s41582-018-0025-8
4. Alexander BM, Cloughesy TF (2017) Adult Glioblastoma. *J Clin Oncol* 35 (21):2402-2409. doi:10.1200/JCO.2017.73.0119
5. Sasmita AO, Wong YP, Ling APK (2018) Biomarkers and therapeutic advances in glioblastoma multiforme. *Asia Pac J Clin Oncol* 14 (1):40-51. doi:10.1111/ajco.12756
6. Saleiro D, Plataniias LC (2015) Intersection of mTOR and STAT signaling in immunity. *Trends in immunology* 36 (1):21-29. doi:10.1016/j.it.2014.10.006

7. Arumuggam N, Bhowmick NA, Rupasinghe HP (2015) A Review: Phytochemicals Targeting JAK/STAT Signaling and IDO Expression in Cancer. *Phytotherapy research : PTR* 29 (6):805-817. doi:10.1002/ptr.5327
8. Dong Z, Chen Y, Yang C, Zhang M, Chen A, Yang J, Huang Y (2019) STAT gene family mRNA expression and prognostic value in hepatocellular carcinoma. *OncoTargets and therapy* 12:7175-7191. doi:10.2147/ott.S202122
9. Zhang Y, Jin G, Zhang J, Mi R, Zhou Y, Fan W, Cheng S, Song W, Zhang B, Ma M, Liu F (2018) Overexpression of STAT1 suppresses angiogenesis under hypoxia by regulating VEGF-A in human glioma cells. *Biomed Pharmacother* 104:566-575. doi:10.1016/j.biopha.2018.05.079
10. Maachani UB, Shankavaram U, Kramp T, Tofilon PJ, Camphausen K, Tandle AT (2016) FOXM1 and STAT3 interaction confers radioresistance in glioblastoma cells. *Oncotarget* 7 (47):77365-77377. doi:10.18632/oncotarget.12670
11. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, Barrette TR, Anstet MJ, Kincead-Beal C, Kulkarni P, Varambally S, Ghosh D, Chinnaiyan AM (2007) Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia* 9 (2):166-180. doi:10.1593/neo.07112
12. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z (2017) GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Research* 45 (W1):W98-W102. doi:10.1093/nar/gkx247 %J Nucleic Acids Research
13. Mizuno H, Kitada K, Nakai K, Sarai A (2009) PrognoScan: a new database for meta-analysis of the prognostic value of genes. *BMC Med Genomics* 2:18-18. doi:10.1186/1755-8794-2-18
14. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, Li B, Liu XS (2017) TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. *Cancer research* 77 (21):e108-e110. doi:10.1158/0008-5472.Can-17-0307
15. Siemers NO, Holloway JL, Chang H, Chasalow SD, Ross-MacDonald PB, Voliva CF, Szustakowski JD (2017) Genome-wide association analysis identifies genetic correlates of immune infiltrates in solid tumors. *PloS one* 12 (7):e0179726. doi:10.1371/journal.pone.0179726
16. Danaher P, Warren S, Dennis L, D'Amico L, White A, Disis ML, Geller MA, Odunsi K, Beechem J, Fling SP (2017) Gene expression markers of Tumor Infiltrating Leukocytes. *Journal for immunotherapy of cancer* 5:18. doi:10.1186/s40425-017-0215-8
17. Sousa S, Maatta J (2016) The role of tumour-associated macrophages in bone metastasis. *Journal of bone oncology* 5 (3):135-138. doi:10.1016/j.jbo.2016.03.004
18. Jia D, Li S, Li D, Xue H, Yang D, Liu Y (2018) Mining TCGA database for genes of prognostic value in glioblastoma microenvironment. *Aging (Albany NY)* 10 (4):592-605. doi:10.18632/aging.101415
19. Vasaikar SV, Straub P, Wang J, Zhang B (2017) LinkedOmics: analyzing multi-omics data within and across 32 cancer types. *Nucleic Acids Research* 46 (D1):D956-D963. doi:10.1093/nar/gkx1090 %J Nucleic Acids Research

20. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT, Maitland A, Mostafavi S, Montojo J, Shao Q, Wright G, Bader GD, Morris Q (2010) The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Research* 38 (suppl\_2):W214-W220. doi:10.1093/nar/gkq537 %J Nucleic Acids Research
21. Liang Y, Diehn M, Watson N, Bollen AW, Aldape KD, Nicholas MK, Lamborn KR, Berger MS, Botstein D, Brown PO, Israel MA (2005) Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. *Proc Natl Acad Sci U S A* 102 (16):5814-5819. doi:10.1073/pnas.0402870102
22. Shai R, Shi T, Kremen TJ, Horvath S, Liao LM, Cloughesy TF, Mischel PS, Nelson SF (2003) Gene expression profiling identifies molecular subtypes of gliomas. *Oncogene* 22 (31):4918-4923. doi:10.1038/sj.onc.1206753
23. Bredel M, Bredel C, Juric D, Harsh GR, Vogel H, Recht LD, Sikic BI (2005) Functional network analysis reveals extended gliomagenesis pathway maps and three novel MYC-interacting genes in human gliomas. *Cancer Res* 65 (19):8679-8689. doi:10.1158/0008-5472.CAN-05-1204
24. Sun L, Hui A-M, Su Q, Vortmeyer A, Kotliarov Y, Pastorino S, Passaniti A, Menon J, Walling J, Bailey R, Rosenblum M, Mikkelsen T, Fine HA (2006) Neuronal and glioma-derived stem cell factor induces angiogenesis within the brain. *Cancer Cell* 9 (4):287-300. doi:10.1016/j.ccr.2006.03.003
25. Waldmann TA, Chen J (2017) Disorders of the JAK/STAT Pathway in T Cell Lymphoma Pathogenesis: Implications for Immunotherapy. *Annual review of immunology* 35:533-550. doi:10.1146/annurev-immunol-110416-120628
26. Yan Z, Gibson SA, Buckley JA, Qin H, Benveniste EN (2018) Role of the JAK/STAT signaling pathway in regulation of innate immunity in neuroinflammatory diseases. *Clinical immunology (Orlando, Fla)* 189:4-13. doi:10.1016/j.clim.2016.09.014
27. Groner B, von Manstein V (2017) Jak Stat signaling and cancer: Opportunities, benefits and side effects of targeted inhibition. *Molecular and cellular endocrinology* 451:1-14. doi:10.1016/j.mce.2017.05.033
28. Thota B, Arimappamagan A, Kandavel T, Shastry AH, Pandey P, Chandramouli BA, Hegde AS, Kondaiah P, Santosh V (2014) STAT-1 expression is regulated by IGFBP-3 in malignant glioma cells and is a strong predictor of poor survival in patients with glioblastoma. *J Neurosurg* 121 (2):374-383. doi:10.3171/2014.4.JNS131198
29. Rodrigues BR, Queiroz-Hazarbassanov N, Lopes MH, Bleggi-Torres LF, Suzuki S, Cunha IW, Sanematsu P, Martins VR (2016) Nuclear unphosphorylated STAT3 correlates with a worse prognosis in human glioblastoma. *Pathol Res Pract* 212 (6):517-523. doi:10.1016/j.prp.2016.03.001
30. Shodeinde A, Ginjupalli K, Lewis HD, Riaz S, Barton BE (2013) STAT3 Inhibition Induces Apoptosis in Cancer Cells Independent of STAT1 or STAT2. *J Mol Biochem* 2 (1):18-26
31. Chen J, Wang H, Wang J, Huang S, Zhang W (2015) STAT1 inhibits human hepatocellular carcinoma cell growth through induction of p53 and Fbxw7. *Cancer Cell Int* 15:111-111. doi:10.1186/s12935-

015-0253-6

32. Luo G, He Y, Zhao Q, Yu X (2017) Immune Cells Act as Promising Targets for the Treatment of Bone Metastasis. *Recent Pat Anticancer Drug Discov* 12 (3):221-233. doi:10.2174/1574892812666170606123113
33. Meng S, Li L, Zhou M, Jiang W, Niu H, Yang K (2018) Distribution and prognostic value of tumor-infiltrating T cells in breast cancer. *Mol Med Rep* 18 (5):4247-4258. doi:10.3892/mmr.2018.9460
34. Byun DJ, Wolchok JD, Rosenberg LM, Girotra M (2017) Cancer immunotherapy - immune checkpoint blockade and associated endocrinopathies. *Nat Rev Endocrinol* 13 (4):195-207. doi:10.1038/nrendo.2016.205
35. Velloso FJ, Trombetta-Lima M, Anschau V, Sogayar MC, Correa RG (2019) NOD-like receptors: major players (and targets) in the interface between innate immunity and cancer. *Biosci Rep* 39 (4):BSR20181709. doi:10.1042/BSR20181709
36. Li M, Ren C-X, Zhang J-M, Xin X-Y, Hua T, Wang H-B, Wang H-B (2018) The Effects of miR-195-5p/MMP14 on Proliferation and Invasion of Cervical Carcinoma Cells Through TNF Signaling Pathway Based on Bioinformatics Analysis of Microarray Profiling. *Cell Physiol Biochem* 50 (4):1398-1413. doi:10.1159/000494602
37. Wu Y, Lu J, Kang T (2016) Human single-stranded DNA binding proteins: guardians of genome stability. *Acta Biochim Biophys Sin (Shanghai)* 48 (7):671-677. doi:10.1093/abbs/gmw044

## Supplementary Figure 1

Supplementary Fig 1. The three most significant genes correlated with STAT5A in GBM.

## Figures

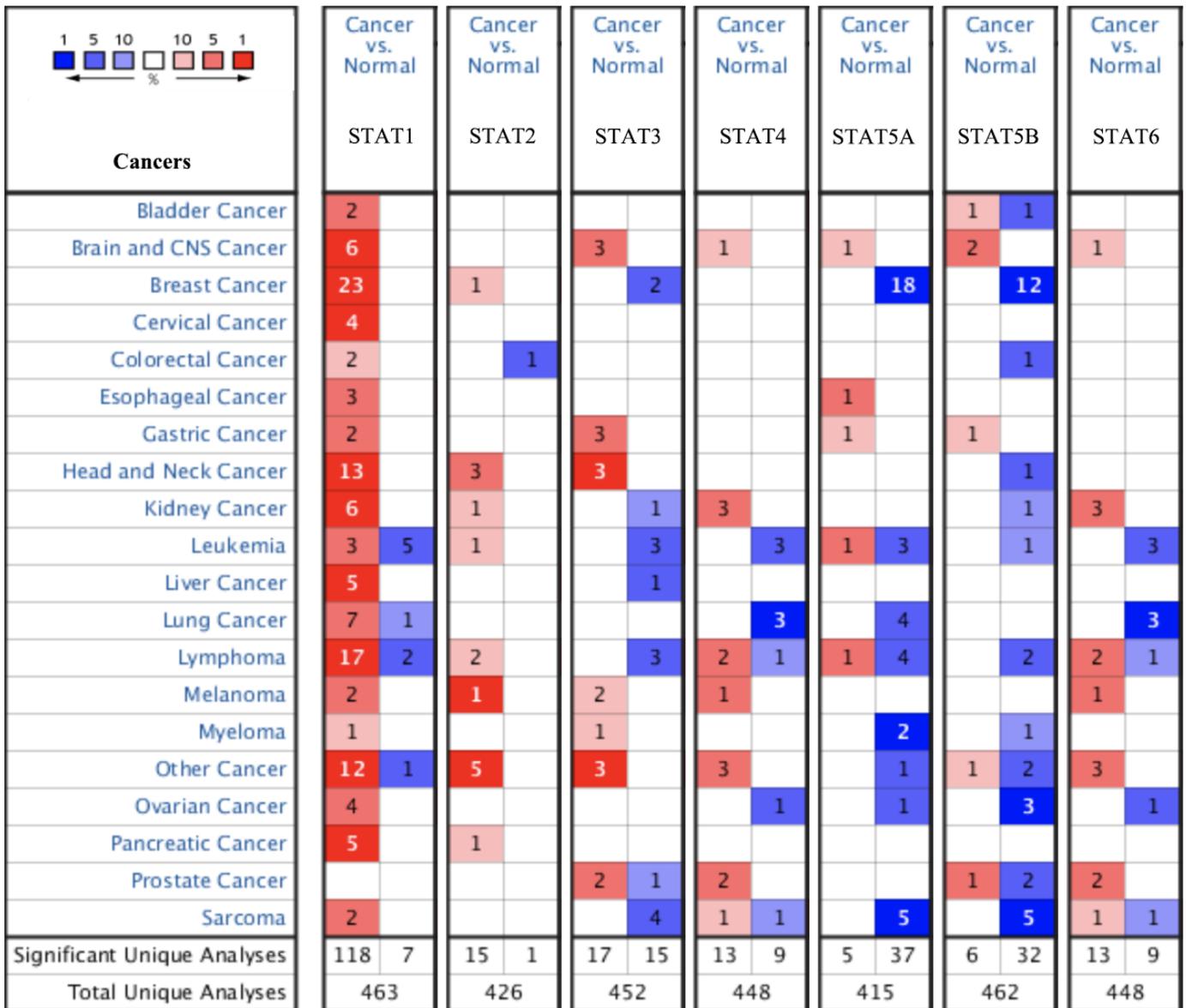
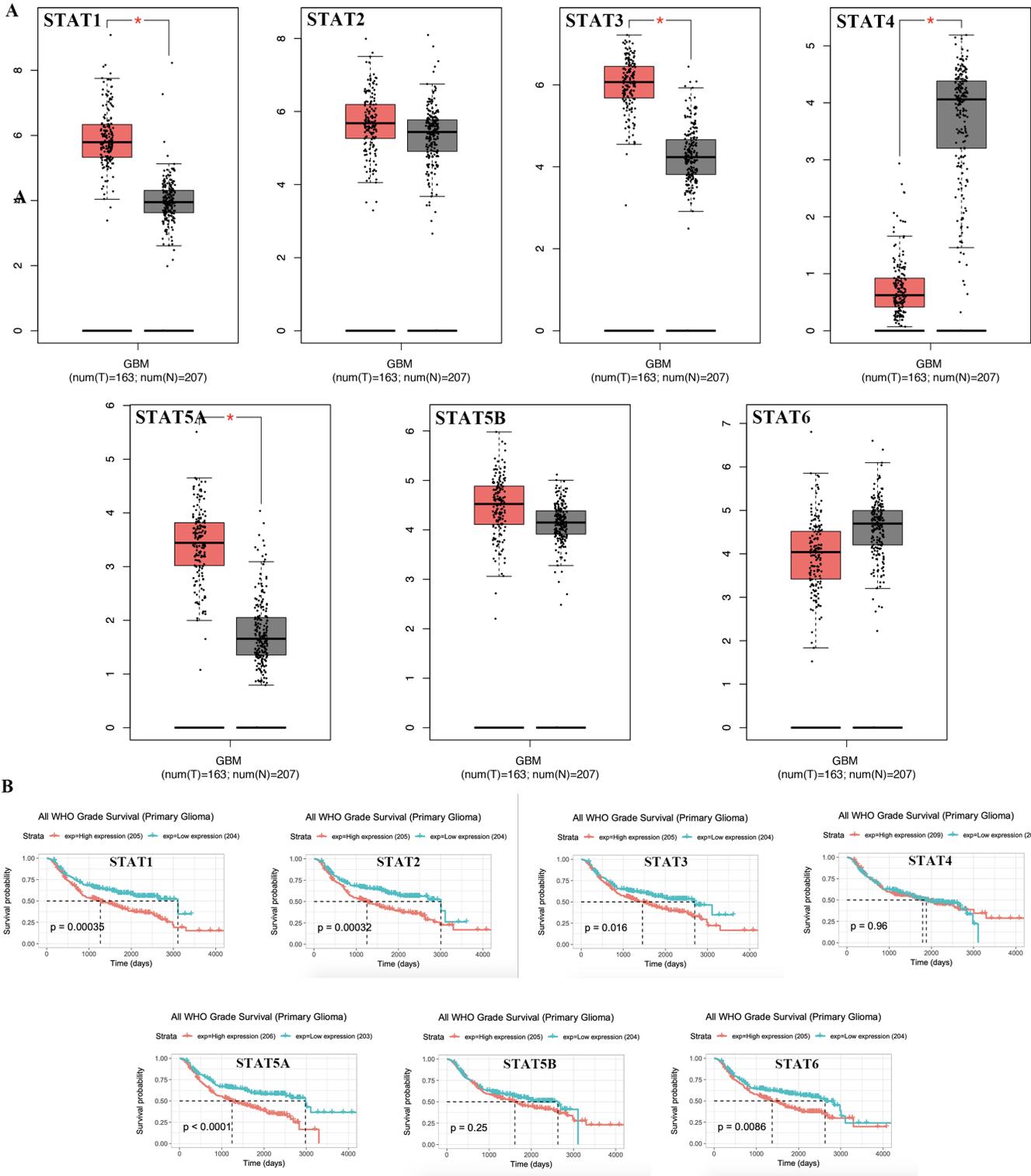


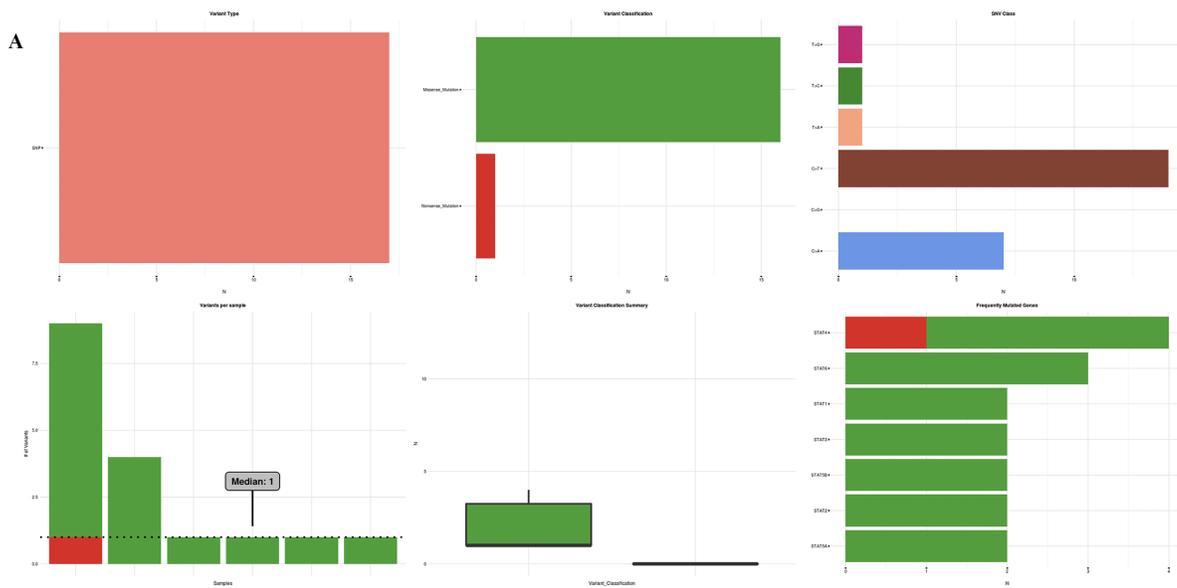
Figure 1

STAT family expression in GBM.



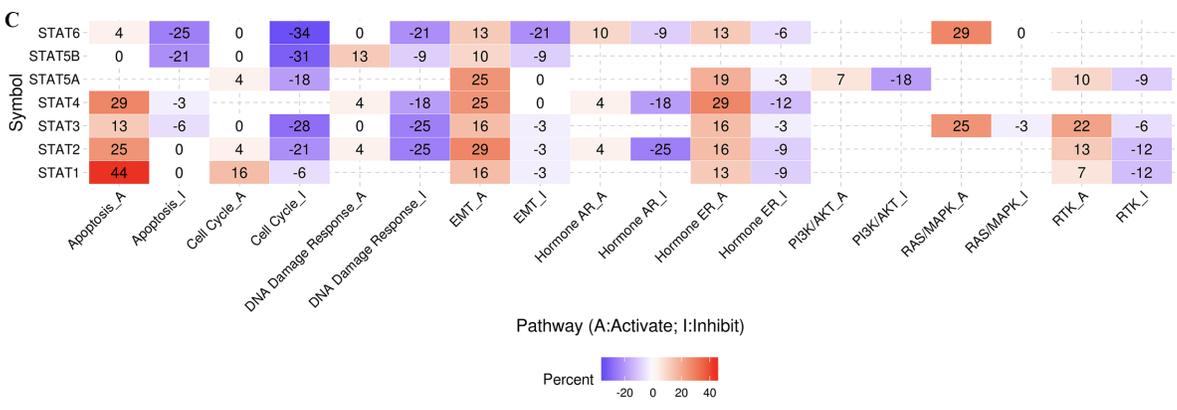
**Figure 2**

Expression and prognosis analysis of STAT family in GBM. (A) The level of STAT family in GBM tissues and brain tissues. (B) The prognostic value of STAT family in GBM. \* $p < 0.05$ .



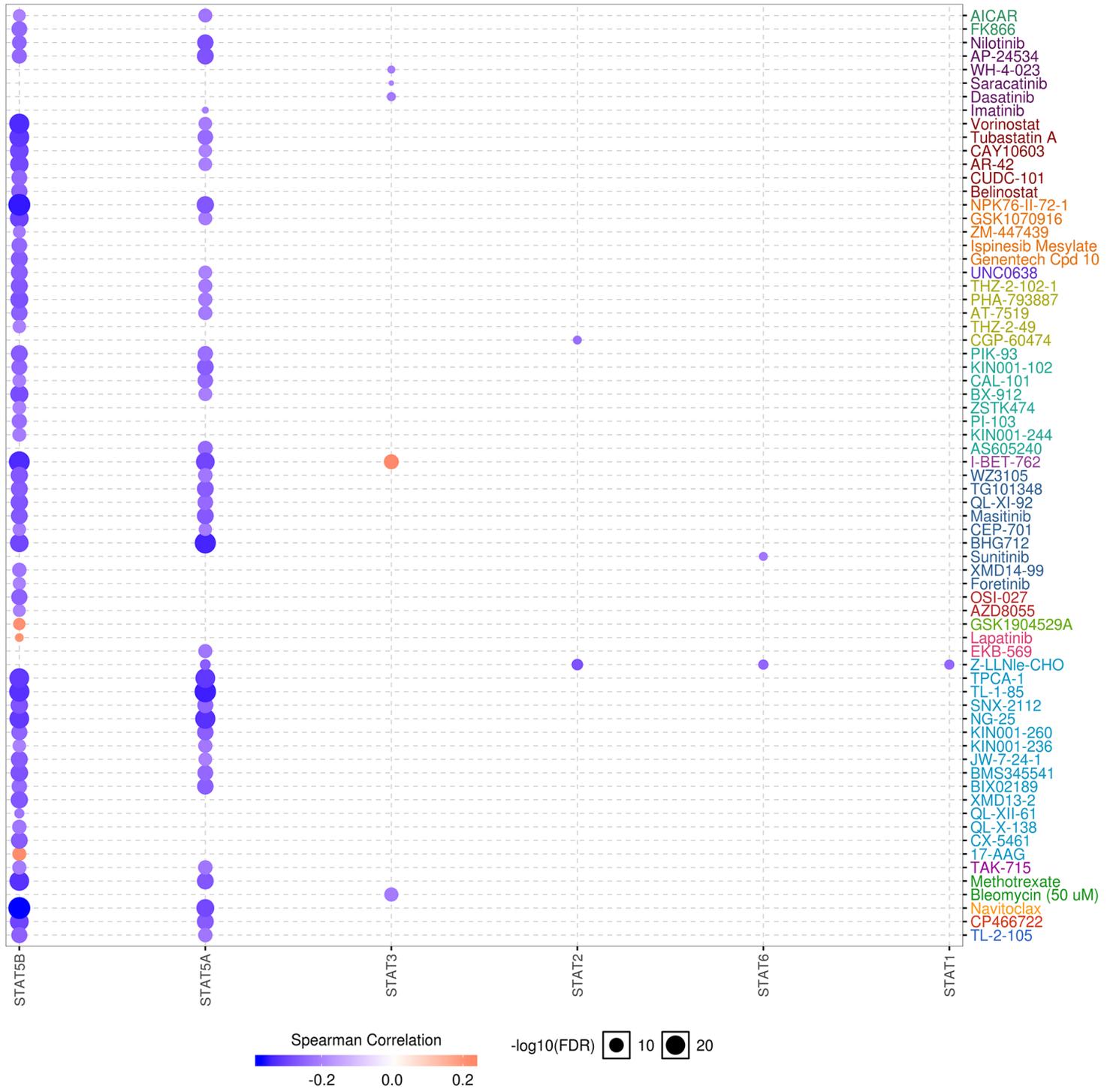
**B**

	<i>STAT1</i>	<i>STAT2</i>	<i>STAT3</i>	<i>STAT4</i>	<i>STAT5A</i>	<i>STAT5B</i>	<i>STAT6</i>
<i>STAT1</i>	1.000	0.710	0.300	-0.002	0.340	0.230	0.023
<i>STAT2</i>	0.710	1.000	0.330	0.096	0.420	0.350	0.300
<i>STAT3</i>	0.300	0.330	1.000	-0.010	0.540	0.610	0.220
<i>STAT4</i>	-0.002	0.096	-0.010	1.000	0.170	0.064	0.280
<i>STAT5A</i>	0.340	0.420	0.540	0.170	1.000	0.350	0.540
<i>STAT5B</i>	0.230	0.350	0.610	0.064	0.350	1.000	0.055
<i>STAT6</i>	0.023	0.300	0.220	0.280	0.540	0.055	1.000



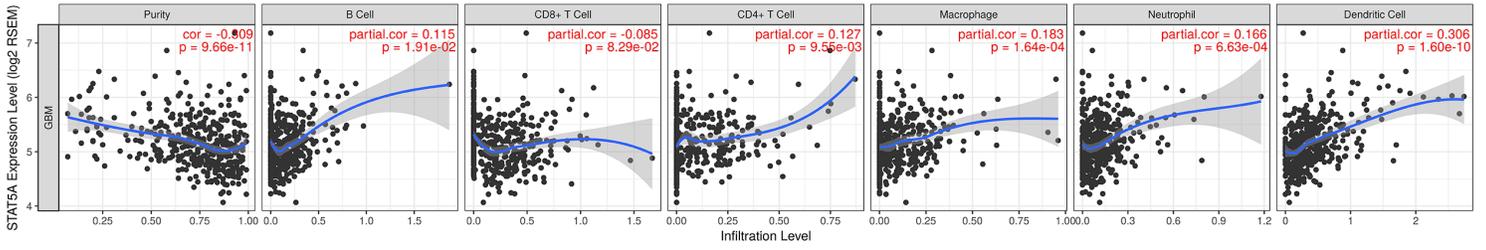
**Figure 3**

SNV, co-expression and cancer related pathway analysis of STAT family in GBM. (A) SNV analysis of STAT family in GBM. (B) Correlation heat map of STAT family in GBM. (C) The role of STAT family in the cancer related pathways



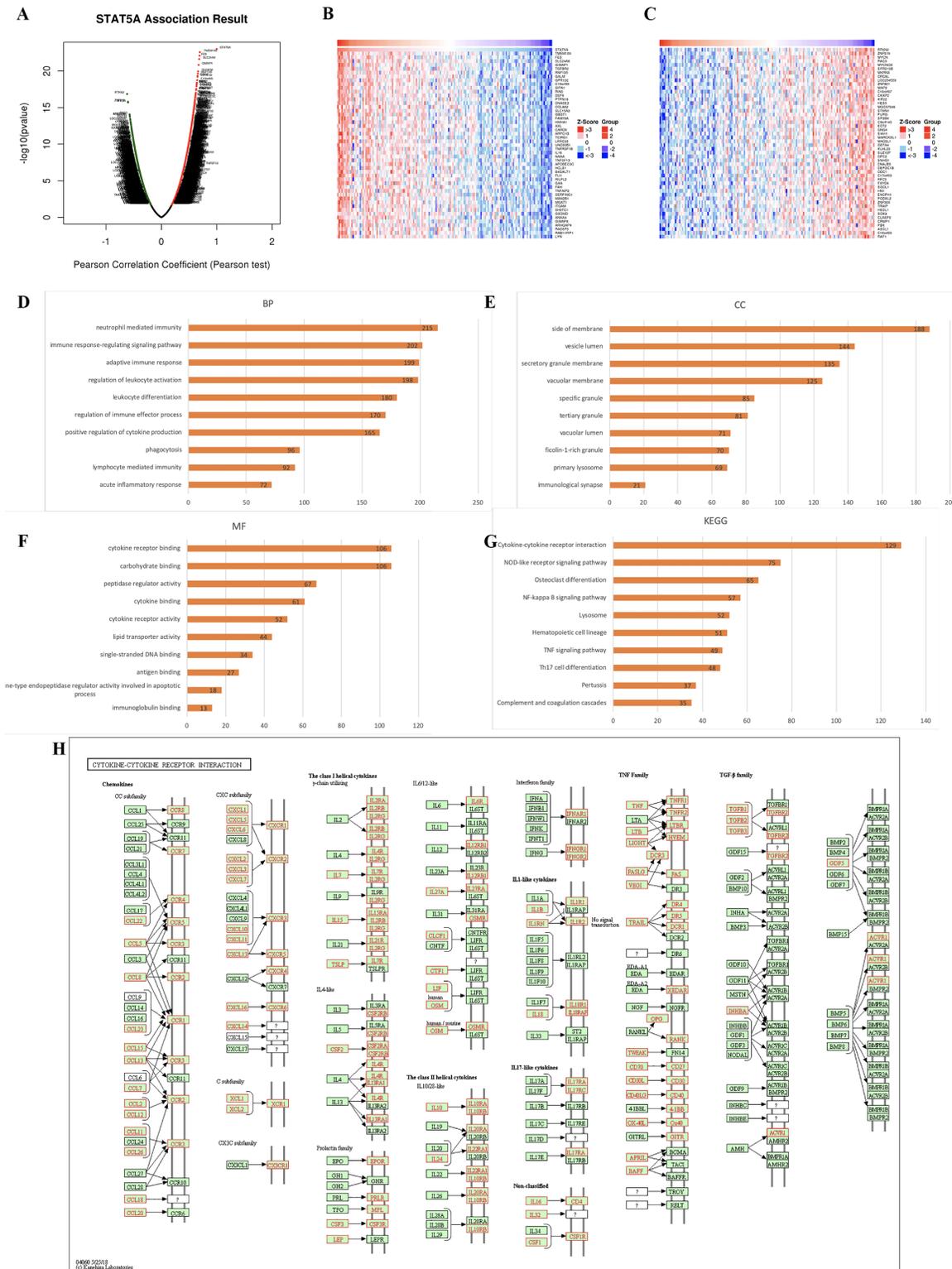
**Figure 4**

The drug sensitivity analysis of STAT family in GBM. The negative correlation means that low expression of gene is resistant to the drug, vice versa.



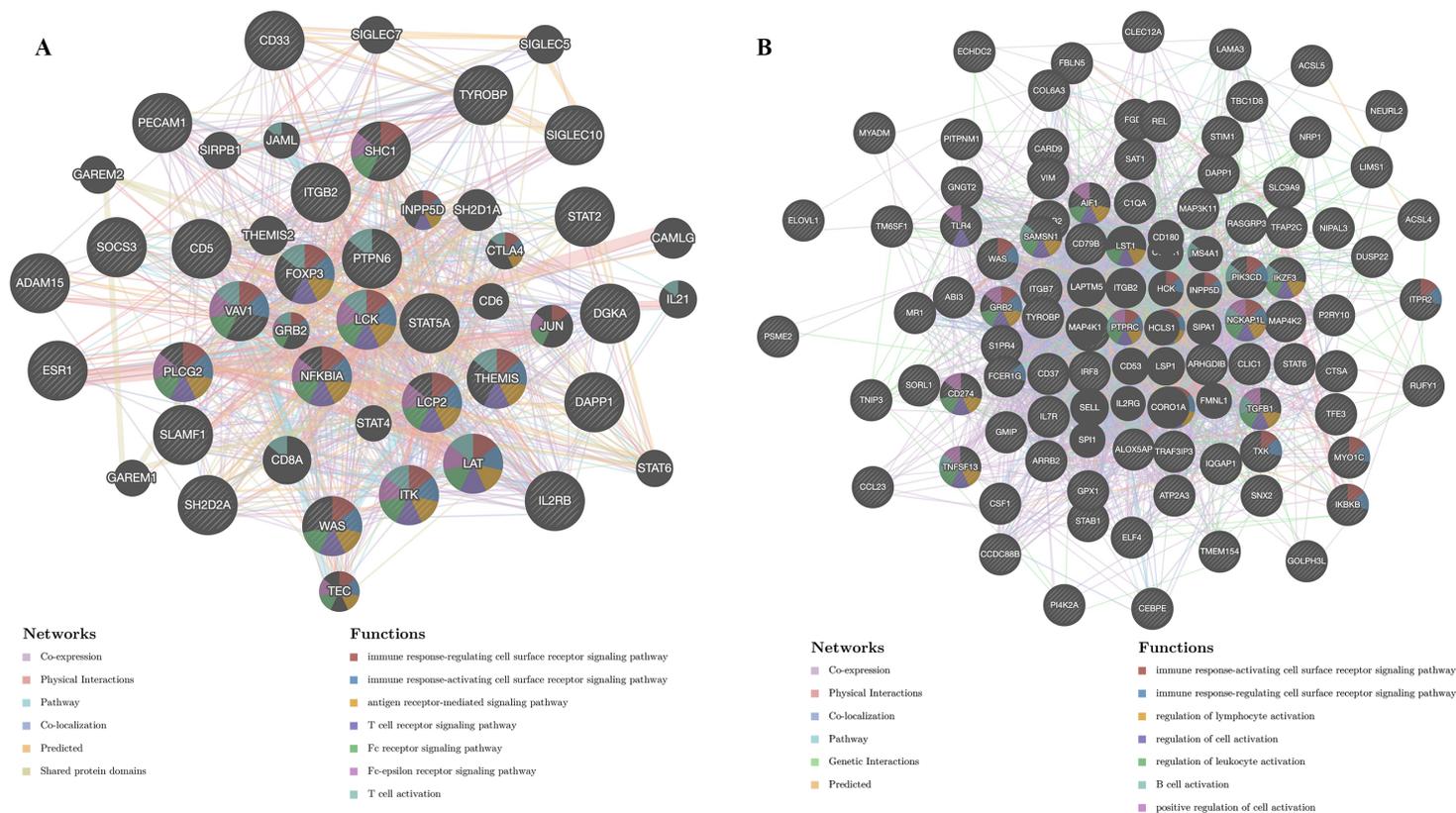
**Figure 5**

Immune cell infiltration analysis of STAT5A in GBM.



**Figure 6**

Enrichment analysis of STAT5A in GBM. (A) The genes associated with STAT5A in GBM. (B) The 50 most significant genes showing positive correlation with STAT5A in GBM. (C) The 50 most significant genes showing negative correlation with STAT5A in GBM. (D-F) GO analysis. (G) KEGG pathway analysis. (H) KEGG pathway annotations of cytokine-cytokine receptor interaction.



**Figure 7**

PPI network of kinase LCK and transcription factor ELF1 networks. (A) The applied bioinformatics methods and the biological functions of the gene sets of kinase LCK networks. (B) The applied bioinformatics methods and the biological functions of the gene sets of transcription factor ELF1 networks.

## Supplementary Files

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- [SupplementaryFig1.tif](#)