# Identification of RNA Binding Protein Signature to Predict Overall Survival for Bladder Cancer

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**Research**

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# Abstract

**Background:** Bladder urothelial cancer (BLCA) is the 10th most common and 13th most deadly cancer globally. RNA binding proteins (RBPs) were reported to participate in the occurrence and progression of varieties of diseases, including malignant tumors. However, the role of RBPs in BLCA is rarely reported.

**Methods:** We downloaded the transcriptome sequence gene expression data and the related clinical data from the cancer genome atlas (TCGA) database, and identified the differently expressed RBPs between BLCA and normal tissues. We explored the biological function by gene ontology (GO) enrichment and the Kyoto Encyclopedia of Genes and Genomes database (KEGG) enrichment analysis. 388 key RBPs were obtained by protein-protein interaction (PPI). We constructed a prognostic risk model, explored the risk score related to BLCA, and validated the results both in the test group and HPA database.

**Results**: In total, 388 differently expressed RBPs were discovered. Five prognostically relevant hub RBPs (YARS, EFTUD2, OAS1, DARS2, TRIM71) were used to construct a prognostic model based on multiple Cox analysis. We found that the overexpression of YARS, EFTUD2, DARS2, and TRIM71 were unfavorable prognostic factors for BLCA patients. Furthermore, we validated the results by the TCGA cohort and the Human Protein Atlas (HPA) database.

**Conclusion**: We identified five RNA binding protein signatures to predict the overall survival for BLCA patients, which provide new insights for the pathogenesis and treatment of BLCA.


# Background

Bladder urothelial cancer (BLCA) is the 10th most common and 13th most deadly cancer globally, and over 430,000 men and women are newly diagnosed worldwide every year, leading to a sizeable societal burden[1, 2]. Non-metastatic BLCA is classified into non-muscle invasive bladder cancers (NMIBCs) and Muscle-invasive bladder cancers (MIBCs). NMIBCs represent approximately 70% of newly diagnosed bladder cancer[3]. MIBCs account for approximately 20% of new diagnoses of bladder cancer. Despite radical cystectomy, approximately 50% of patients with MIBCs are diagnosed with distant metastasis [2, 4]. Pelvic lymphadenectomy is an integral part of curative surgery for high-risk NMIBCs and MIBCs[5]. Patients with metastatic disease have a 5-year survival under 5% in the US [4]. It is critical to developing effective treatments for BLCA. The heterogeneous molecular mechanism and biological behavior of BLCA can promote the development of precision medicine of BLCA and provide novel therapeutic targets [6]. Furthermore, BLCA patients with the same tumor stage may have different outcomes due to individual differences. Therefore, exploring precise and useful biomarkers is required to predict the prognosis of BLCA.

With the development of gene sequencing, vast amounts of non-coding RNA were found, and it has been verified that non-coding RNA played a critical role in cell function [7]. Thus, the concept of RNA binding proteins (RBPs) was proposed, and RBPs were referred to proteins that were expressed distinctly in cells and participated in the function of different kinds of RNA [8]. Stefanie Gerstberger and her coworkers had

identified 1542 RBPs in the human genome and analyzed their interactions with RNA [7]. They found that nearly 50% of RBPs played essential roles in mRNA metabolic pathways, 11% constituted ribosomal proteins, and the rest acted in ncRNA metabolic processes. Therefore, RBPs were essential for the normal physiological function of cells in humans. Recently, it has been reported that RBPs were associated with the initiation and progression of many diseases. RBPs could regulate epidermal progenitors at a posttranscriptional level [9]. Besides, RBPs participated in the regulation of amino acid levels by regulating SLC mRNA abundance [10].

Moreover, several studies showed that the abnormality of RBPs was related to lung cancer [11], glioma [12], colorectal cancer [13]and hepatocarcinoma [14]. It was reported that human ribosomal protein S3 (RPS3) served as a variety of novel pro-tumorigenic RBP in hepatocarcinoma, and RPS3 could bind to the 3’ UTR of SIRT1 mRNA in hepatocarcinoma, resulting in the stable expression of the oncogene SIRT1 [14]. More importantly, a novel small molecule for hepatocarcinoma based on the RPS3/SIRT1 pathway was identified. Therefore, RBPs provide a novel insight for cancer treatment. However, the roles RBPs in BLCA have not been reported yet. Research on Algorithm based on the cancer genome atlas (TCGA) and computers is a new method for exploring the changes and significance of tumor histology and behavior, which has been applied to the treatment and prognosis of bladder cancer[15, 16]. Moreover, mRNAs were applied for the rapid diagnosis of bladder carcinoma[17]. Therefore, the roles RBPs in BLCA were explored with an Algorithm based on TCGA and computers in this work.

In this study, we downloaded data from TCGA database, obtained differently expressed RBPs between normal samples and BLCA samples, and performed gene ontology (GO) enrichment and The Kyoto Encyclopedia of Genes and Genomes database (KEGG) enrichment analysis to explore the function of differently expressed RBPs. We explored hub differently expressed RBPs by protein-protein interaction (PPI) network and investigated their potential impact on BLCA patients' survival by constructing a prognostic model based on RBPs as independent risk factors. Finally, we verified the reliability and authenticity of the outcomes with the test model and HPA database.

# Methods

### Data sources

The transcriptome sequence gene expression data of 430 cases (tumor samples, 411; normal samples, 19 cases) in FPKM format and related clinical data in XML format were downloaded from the TCGA database (https://cancergenome.nih.gov). The genes of RBPs were downloaded from https://portal.gdc.cancer.gov. In this work, all of the analytical processes of RBPs in BLCA were shown in Figure 1figure1.tif.

### Identification of differently expressed RBPs

In order to select differently expressed RBPs (DERBPs), the expression data of BLCA tissue and normal bladder tissue was assessed in an R environment (version Rx64 3.6.2). DERBPs were obtained with the

following criteria: |log2FC|≥ 1 and FDR < 0.05. Limma was applied to correct data, and the Wilcoxon test was performed on data [18]. Heat map and Volcano map of differently expressed RBPs were generated with the “pheatmap” package.

### Biological enrichment analysis of DERBPs

In order to explore the biological function of DERBPs, we carried out GO enrichment analysis and KEGG enrichment analysis in R environment with packages “colorspace” “clusterProfiler” [19], “enrichplot”, “ggplot2” , “org.Hs.eg.db”, “ggplot2”, “stringi”. Under the condition of simultaneous $P$ 0.05 and $q$ 0.05, the enrichment analysis was recognized as significant. In particular, the $q$ value is the *p-value* corrected with the “limma” package.

### PPI network constructions

We constructed the PPI network through online STRING and polished it with Cytoscape Version 3.6.1. The key modules and genes were screened using Molecular Complex Detection (MCODE) plug-in and showed with Cytoscape. Nodes whose confidence in the interactive relationship was more significant than 0.95 were selected to construct the network.

### Identifying prognostic differently expressed RBP genes.

To evaluate the relationship between RBPs and the prognosis of BLCA patients, univariate Cox regression analysis was performed successively, and RBPs related to survival ($P$ 0.01) were screened. In the univariate Cox regression analysis, the “survival” package was employed in the R environment.

### Constructing RBP gene-associated prognostic model

Patients in TCGA dataset were randomly divided into train groups and test groups at a ratio of 5:5. DERBPs with $P$ 0.05 were selected to carry out multivariate Cox regression analysis in the training group. Subsequently, we calculated the individualized risk score with coefficients and constructed RBP gene-associated prognostic model (RGPM). The risk score for every BLCA patient was obtained based on the following formula [20].

$$\text{Risk score} = \beta_{RNA1} \times \text{exprgene}_{RNA1} + \beta_{RNA2} \times \text{exprgene}_{RNA2} + \ldots + \beta_{RNAn} \times \text{exprgene}_{RNAn}$$

In the formula, the Risk score represents the RGPM based risk signature calculate by RGPM, β represents the regression coefficient of each gene, RNAn refers to the five key RBP genes involved in RGPM.

### Prognostic analysis based on RGPM.

To further assess BLCA patients’ prognosis, the patients with BLCA in the training group were subdivided into a low-risk group and a high-risk group according to the median risk score of RGPM. The “survival” package was used to carry out survival analysis of patients in the low-risk and high-risk groups, the “survminer” package was employed to visualize the data, and the “pheatmap” package was used for

analyzing the status of individuals in the R environment. Receiver operating characteristic (ROC) curve, risk curve and the survival status of individual BLCA patients were obtained according to the RGPM.

### Internal validation of the RGPM-based risk signature in the test group

To validate our RGPM model predictions' accuracy, internal validation of the RGPM-based risk signature in the test group was performed. The risk score formula was applied in the test group, and the risk score was calculated. We conducted survival analysis according to the method described in building RGPM.

### Verifying the expression of RBP proteins in BLCA tissue

We downloaded pathological slice images of five hub RBPs expression levels on The Human Protein Atlas (HPA) online database (http://www.proteinatlas.org/) [21].

### Nomogram construction

Nomograms were reported to evaluate the overall survival of cancer patients [22]. We constructed a nomogram with the multivariate Cox analysis results related to hub RBPs. Total nomogram scores were utilized to predict the 1-, 2-, and 3- year OS in BLCA patients.

## Results

### Identification of differentially expressed RBPs in BLCA patients

We explored the role of RBPs in the prognosis of BLCA patients in our study (Figure 1). The transcriptome sequence gene expression data of 430 cases (tumor samples, 411; normal samples, 19 cases) in FPKM format and related clinical data in XML format were downloaded from the TCGA database, and the data was processed with R software packages, then DERPBs were discovered. Based on the previous studies, we examined the expression of 1542 RBPs in TCGA [7]. As shown in Figure 2figure2.tif, 388 DERPBs were displayed ($P$<0.05, |log2FC| >1.0). Among 388 differently expressed RBPs, 219 were upregulated, and 169 were downregulated.

### GO/KEGG enrichment analysis of DERBPs

To explore the potential roles which the DERBPs played in bladder urothelial cancer, GO enrichment and KEGG enrichment analysis were conducted. The results obtained from GO enrichment analysis displayed that the DERBPs were closely associated with RNA splicing, RNA catabolic process, ncRNA processing, catalytic activity acting on RNA (Figure 3Afigure3.tif). The KEGG enrichment analysis results showed that the DERBPs had a close connection with RNA transport, spliceosome, and mRNA surveillance pathway (Figure 3Bfigure3.tif).

### Key module screening from PPI network

To further explore the function the DERBPs played in bladder urothelial cancer, the PPI network was built with online analysis tools STRING (Version 11) and visualized with Cytoscape (Version 3.7.2). All hub differently expressed RBPs with the number of nodes above ten were shown in Figure 4Afigure4.tif. To further investigate and acquire possible key modules, the co-expression network was constructed with the MODE tool. As shown in Figure 4Bfigure4.tif, 388 key DERBPs were obtained and participated in RNA splicing, RNA catabolic process, ncRNA processing, catalytic activity acting on RNA, RNA transport, spliceosome and mRNA surveillance pathway.

### Identification of prognostic DERBPs.

In order to build RBP gene-associated prognostic model (RGPM), univariate Cox regression analysis was carried out, and then 11 hub RBPs which were associated with the prognosis of BLCA patients were discovered (*P*0.01). The results were shown in Figure 5Afigure5.tif. Six high-risk genes and five low-risk genes were screened.

### Construction of RBP gene associated prognostic model (RPGM).

To constructing RBP gene associated prognostic model, multiple Cox regression analysis was performed, and coefficients of each hub RBP gene were obtained. As shown in Figure 5B, YARS, EFTUD2, TRIM71, and DARS2 were significantly associated with BLCA patients' survival. All of them were negative prognostic factors. OAS1 was positively related to the prognosis of BLCA patients. Subsequently, we constructed RGPM with five hub RBPs base on multiple univariate analyses. According to the following formula, the risk score of each bladder patients were calculated: Risk score = (0.6573* Exp YARS) + (0.6510* Exp EFTUD2) + (-0.1756* Exp OAS1) + (0.3804 * Exp DARS2) + (0.6038* Exp TRIM71).

### Survival analysis of RBP scores.

In order to evaluate the predictive ability of RGPM, a survival analysis of RBP scores was carried out. The RBP gene risk score for each BLCA patient was calculated based on the RGPM. In the training group, BLCA patients were subdivided into low-risk groups and high-risk groups according to the median risk score. As shown in figure 6Afigure6.tif, patients in low-risk groups have a better survival probability. Then, a time-dependent ROC analysis was performed in order to assess the predictive ability further. Figure 6Bfigure6.tif showed that the area under the ROC curve (AUC) of our risk score model was 0.784. This indicated that the risk score model could be applied significantly. The expression heat map, survival status of patients, and risk score of the signature classified in the low-risk and high-risk groups are shown in Figure 6Cfigure6.tif. These results above illustrated that the prognosis-related genetic risk score model has moderate sensitivity and specificity. In general, survival analysis, time-dependent ROC analysis, and risk score analysis were performed in the training group (Figure 6figure6.tif).

### Validation of the RGPM-based risk signature.

To validate the accuracy of the RGPM, we validated the RGPM in the test group at first. A similar analysis performed in the training group was executed in the test group (Figure 7figure7.tif). The results in Figure

7figure7.tif showed that patients in the low-risk group had a better prognosis than that in the high-risk group, which was consistent with results obtained from the training group. Subsequently, we validated the differential expression of five hub RBPs in normal tissue and BLCA tissue. We sought the results of immunohistochemistry of five hub RBPs from the HPA database. As shown in Figure 8Afigure8.tif, the expression of YARS, OAS1, EFTUD2, DARS2, and TRIM71 were higher in BLCA than normal tissues. The results of immunohistochemistry further confirmed the accuracy of our RGPM. In general, all of the results from the internal validation of our RGPM in the test group and the HPA database illustrated the RBP gene-associated prognostic model's accuracy.

### Construction of a hub RBP-based prognostic nomogram

To better assess the impact of the five hub RBPs on the survival of BLCA patients, a nomogram based on the multivariate Cox analysis of five hub RBPs was constructed (Figure 8Bfigure8.tif). Moreover, we could clearly estimate the patient's 1-year survival rate, 2-year survival rate, and 3-year survival rate from the nomogram. Figure 8 figure8.tif showed that as the risk scores (YARS, EFTUD2, DARS2, TRIM71) increased, the 1-year, 2-year, and 3-year overall survival of BLCA patients declined OAS1 was positively related to the overall survival of BLCA patients. The risk nomogram was consistent with our above results, which confirmed the risk nomogram's prognostic value.

## Discussion

BLCA is the 10th most common and 13th most deadly cancer globally, and the incidence of BLCA is increasing every year [1, 2]. Despite standardized treatment, patients with BLCA still relapse. The heterogeneous molecular mechanism and biological behavior of BLCA can promote the development of precision medicine of BLCA and provide novel therapeutic targets [6]. Therefore, exploring precise and useful biomarkers is required to predict the prognosis of BLCA.

Since 1542 RBPs in humans and their interactions with RNA had been reported in 2014 [7], emerging studies have focused on the relationship between RBPs and disease. Despite the rapid development of medicine, tumors are still difficult to cure. Recently, it was reported that the abnormality of RBPs is related to the prognosis of lung cancer [11], glioma [12] and liver cancer [22]. As far as we know, the relation between RBPs and BLCA was not reported previously. In the present study, we downloaded data from the TCGA database. Based on the TCGA database data, 1542 RBPs in humans were analyzed, and 388 differently expressed RBPs between BLCA tissue and normal tissue were identified.

The potential function of the differently expressed RBPs played in BLCA was explored, GO and KEGG analysis was conducted, and 388 differently expressed RBPs were acquired via the PPI network. The results showed that the differently expressed RBPs were closely associated with RNA splicing, RNA catabolic process, ncRNA processing, catalytic activity acting on RNA. As reported previously, RBPs could interact with RNA, affecting the function of RNA, causing changes in biological behavior. Post-transcriptional gene regulation (PTGR) participated in regulating the balance of cell metabolism and the behavior of cells by RNA [7]. Marco Tripodi and his co-workers reported that the RBPs could control

microRNA sorting as a component of the hepatocyte exosomes [23]. Moreover, the cold-shock domain-containing cold-stress response protein (CIRBP), which belongs to the glycine-rich RNA-binding protein family, could modulate cancer and inflammation [24]. The results from KEGG pathways analysis showed that the differently expressed RBPs were also had a close connection with RNA transport, spliceosome, and mRNA surveillance pathway.

Three hundred eighty-eight differently expressed RBPs were acquired via the PPI network. We explored their prognostic significance by univariate Cox regression analysis, and 11 prognostic related hub RBPs were obtained. Among 11 prognostic related hub RBPs, TIA1, RPP21, GEMIN7, OAS1, and APOBEC3H were favorable prognostic factors in bladder urothelial cancer YARS, EFTUD2, GARS, XPO5, DARS2, and TRIM71 were unfavorable for the prognosis of BLCA patients. Then we built a prognostic risk model based on coefficients from multivariate Cox regression analysis and identified five prognostic related hub RBPs, the overexpression of YARS, EFTUD2, DARS2, and TRIM71 were unfavorable prognostic factors in BLCA. To validate the differential expression of five hub RBPs in normal tissue and BLCA tissue, we sought immunohistochemistry of five hub RBPs from the HPA database. The expression of YARS, OAS1, EFTUD2, DARS2, and TRIM71 was higher in BLCA than normal tissues. The risk nomogram was constructed further to confirm the prognostic value of the prognostic risk model. As the risk scores (YARS, EFTUD2, DARS2, TRIM71) increased, the 1-year, 2-year, and 3-year overall survival of BLCA patients declined, and OAS1 was positively related to the overall survival of BLCA patients. The risk nomogram further confirmed the prognostic value of our prognostic risk model. Consisted with our results, it has been reported that TIA1 [25], OAS1 [26–28], APOBEC3H [29] and YARS had a particular association with the initiation, progression, and treatment effect of BLCA patients. YARS (tyrosyl-tRNA synthetase) catalyzed the binding of tyrosine to its cognate tRNA. It functioned as an oncogenic protein and could promote gastric cancer progression by PI3K/AKT signal pathway [30]. OAS1 was a part of the 2'-5'-oligoadenylate synthetases (OAS) family, and high expression of OAS1 was associated with the poor prognosis of breast cancer patients [31]. The HPA database results showed that OAS1 was a favorable prognostic factor in BLCA but unfavorable to liver cancer patients. OAS1 was a variety of immune-related genes and was related to the prognosis of bladder urothelial cancer [26]. The E3-ubiquitin ligase TRIM71 as a novel mutant p53-binding protein could suppress ovarian cancer initiation and progression through degrading mutant p53 [32]. Although the exact mechanism of five hub RBPs influencing BLCA was unknown, they did have a close connection with the biological behavior of bladder urothelial cancer. However, there were still some limitations in our study: (1). Our data was completely downloaded from the TCGA database and HPA database. The data and clinical characteristics in the database derived from different institutions, and the standards of selecting each sample were probably different, so that selection bias could exist in statistics. (2). The number of our samples was relatively less to construct the predicted model. (3.) We validated our results with our samples from the TCGA database. In future, we plan to design new research based on the experiment from cell level, animal level, and clinical research to verify our results further.

# Conclusions

In general, by constructing a risk prediction model and verifying our model's accuracy, we identified five hub RBPs that were probably related to the initiation, progression, and prognosis of BLCA. Despite the limits in this research, we confirmed that five RNA binding proteins could predict the overall survival of BLCA patients, which provides new insights for the pathogenesis and treatment of BLCA.

# Declarations


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### Authors' contributions

Dan-Xia Li and Wen-Kai Han designed the study protocol. Wen-Kai Han analyzed the data and performed statistical analysis. Kai Che completed the figures. Dan-Xia Li wrote the article and Hai-Tao Niu made revisions. Dan-Xia Li and Wen-Kai Han contributed equally to this work. All authors approved the final version of the article


### Acknowledgements

Not applicable.


### Ethics approval and consent to participate

There were no cell, tissue, or animal studies. No ethical requirements are involved. Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

All the data and materials are available.

### Competing interests

The authors declare that they have no conflict of interests and no competing financial interest exists.

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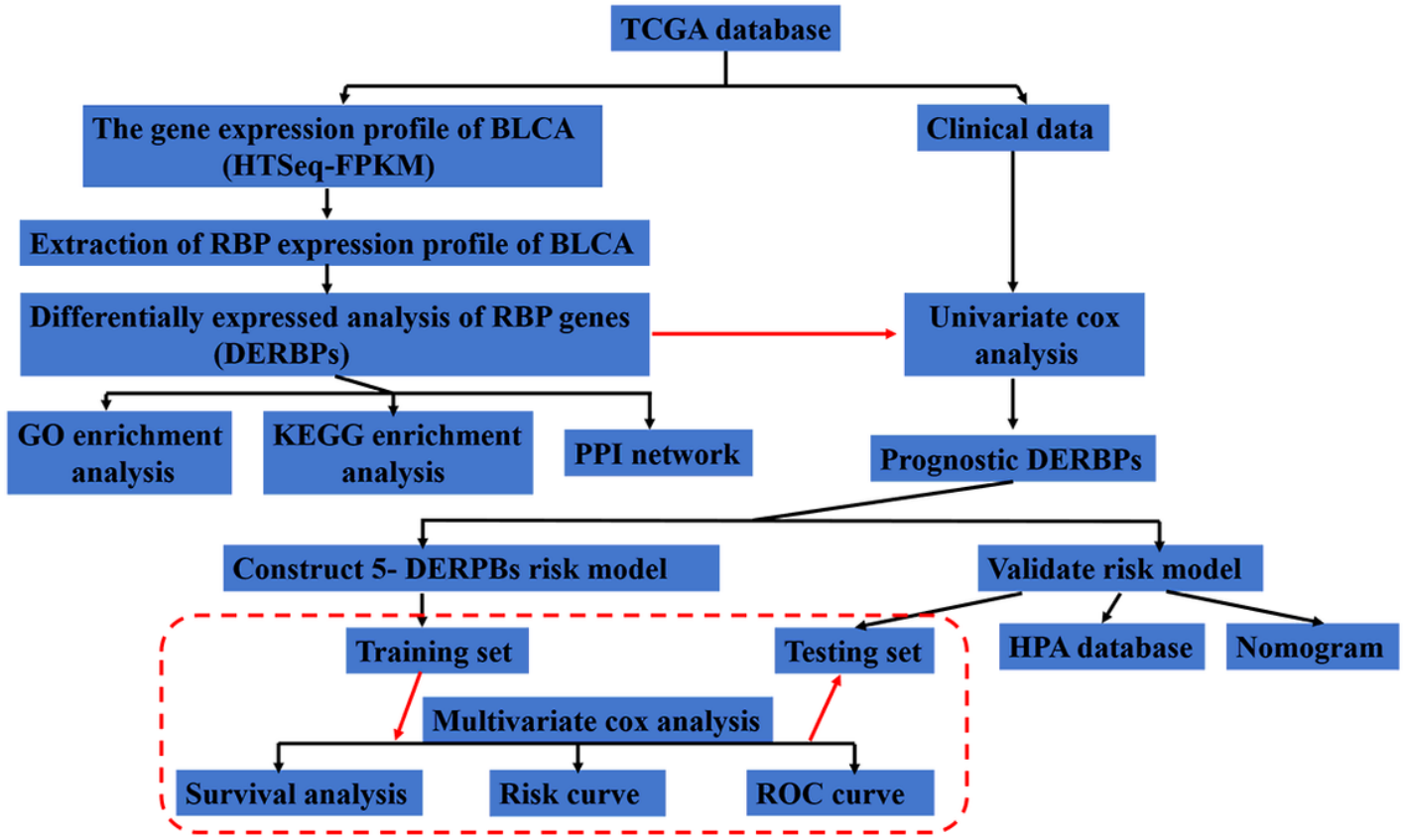
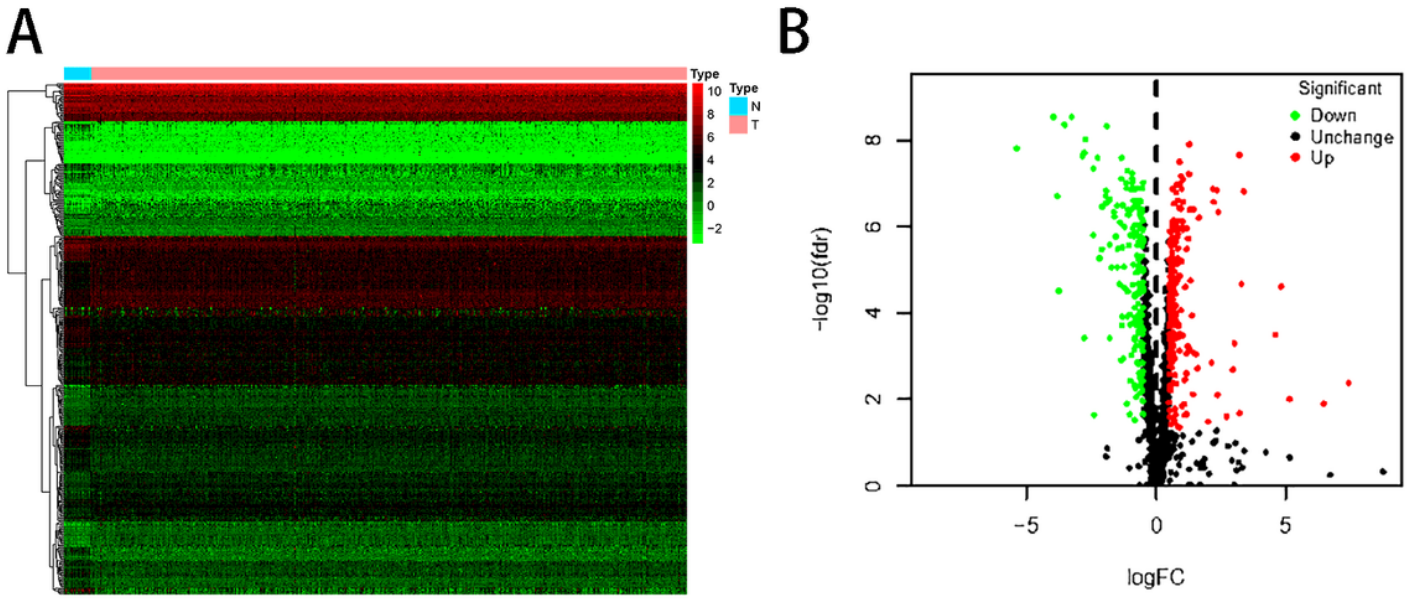
# Figures



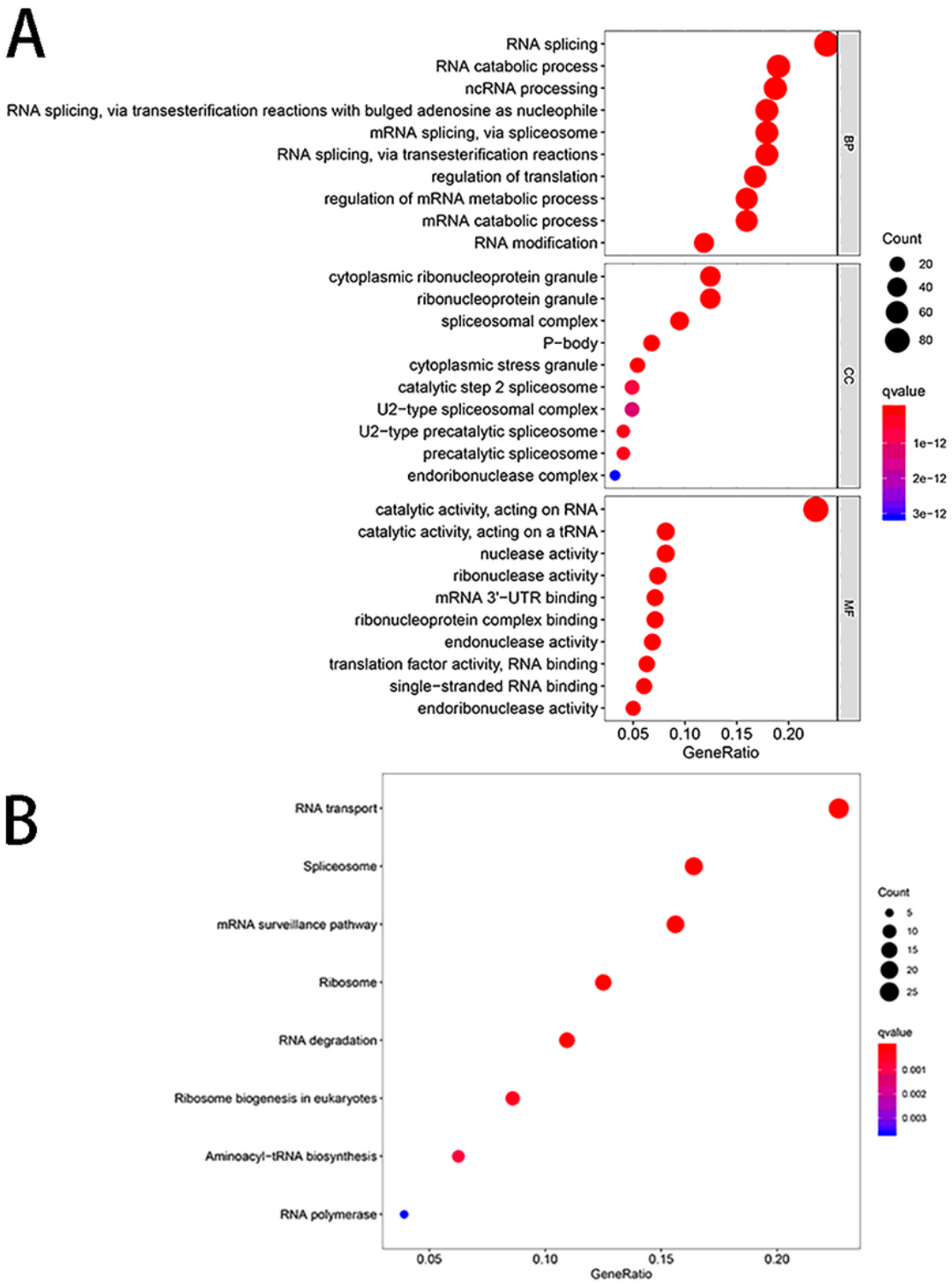

## Figure 1

Flow chart of analyzing the RBPs in BLCA.



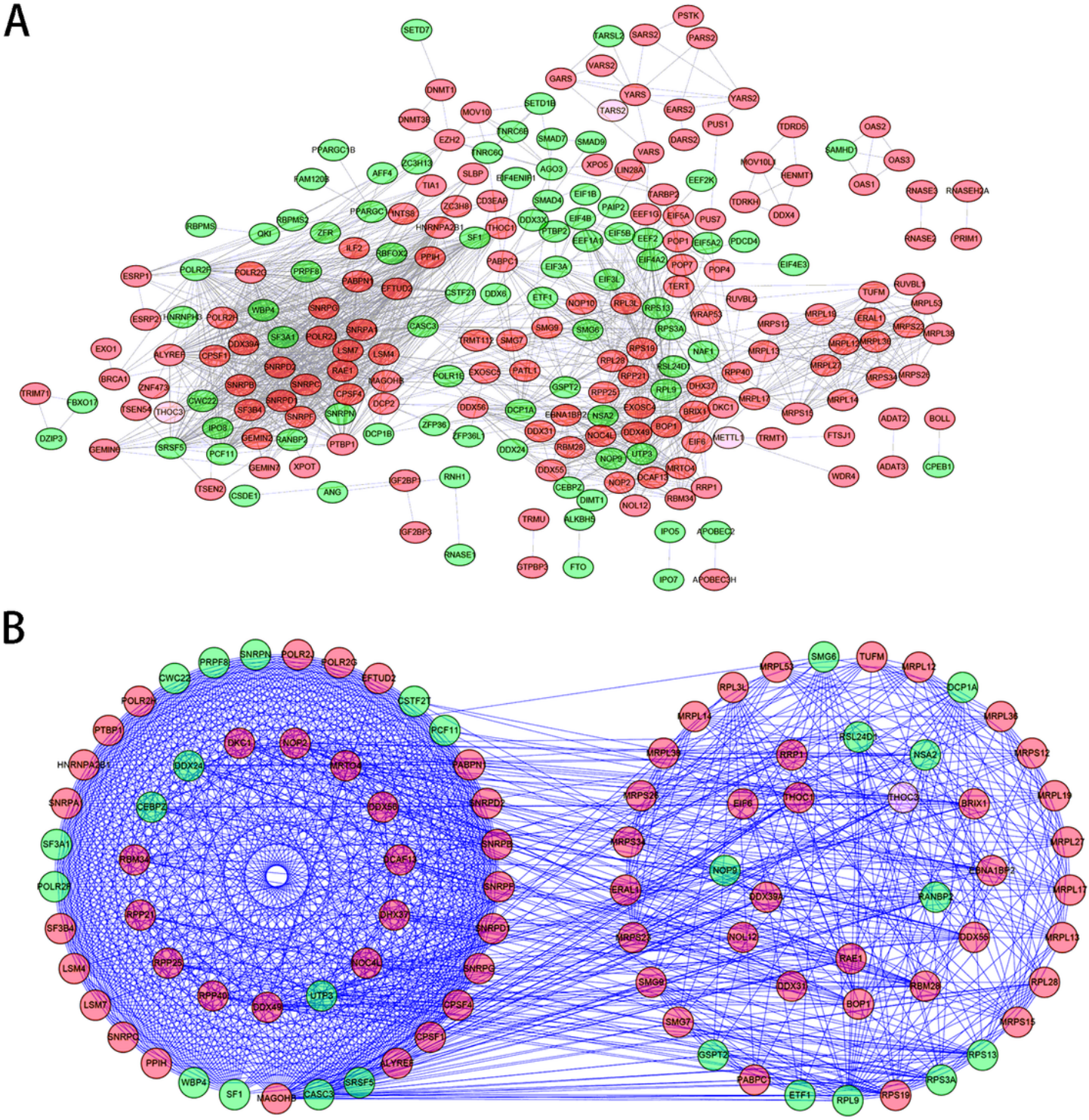

## Figure 2

The differentially expressed RBPs in BLCA. (A) Heat map; (B) Volcano plot.



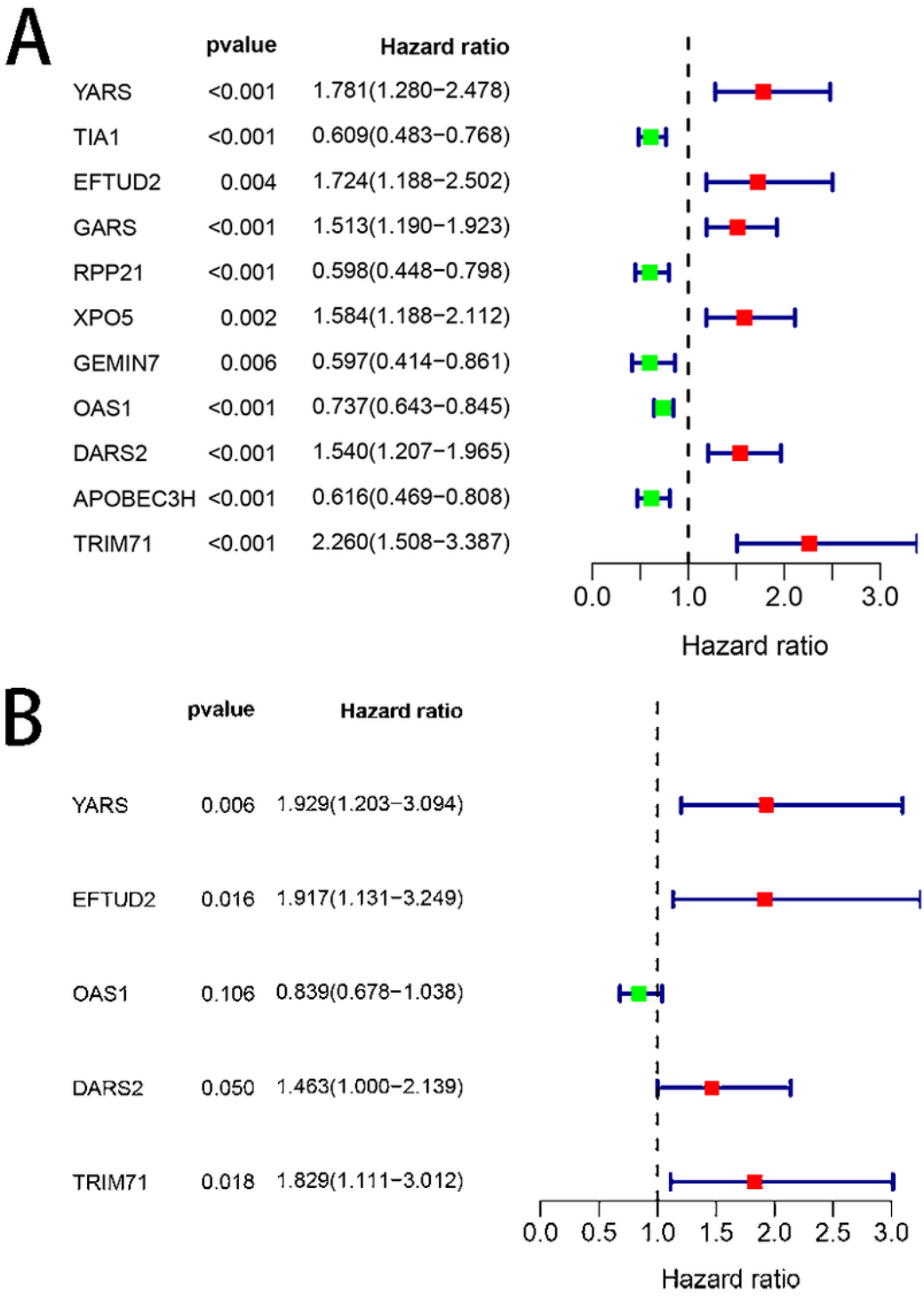

## Figure 3

Function enrichment analysis of the differently expressed RBPs. (A) Gene ontology (GO) enrichment analysis; (B) The Kyoto Encyclopedia of Genes and Genomes database (KEGG) pathway.



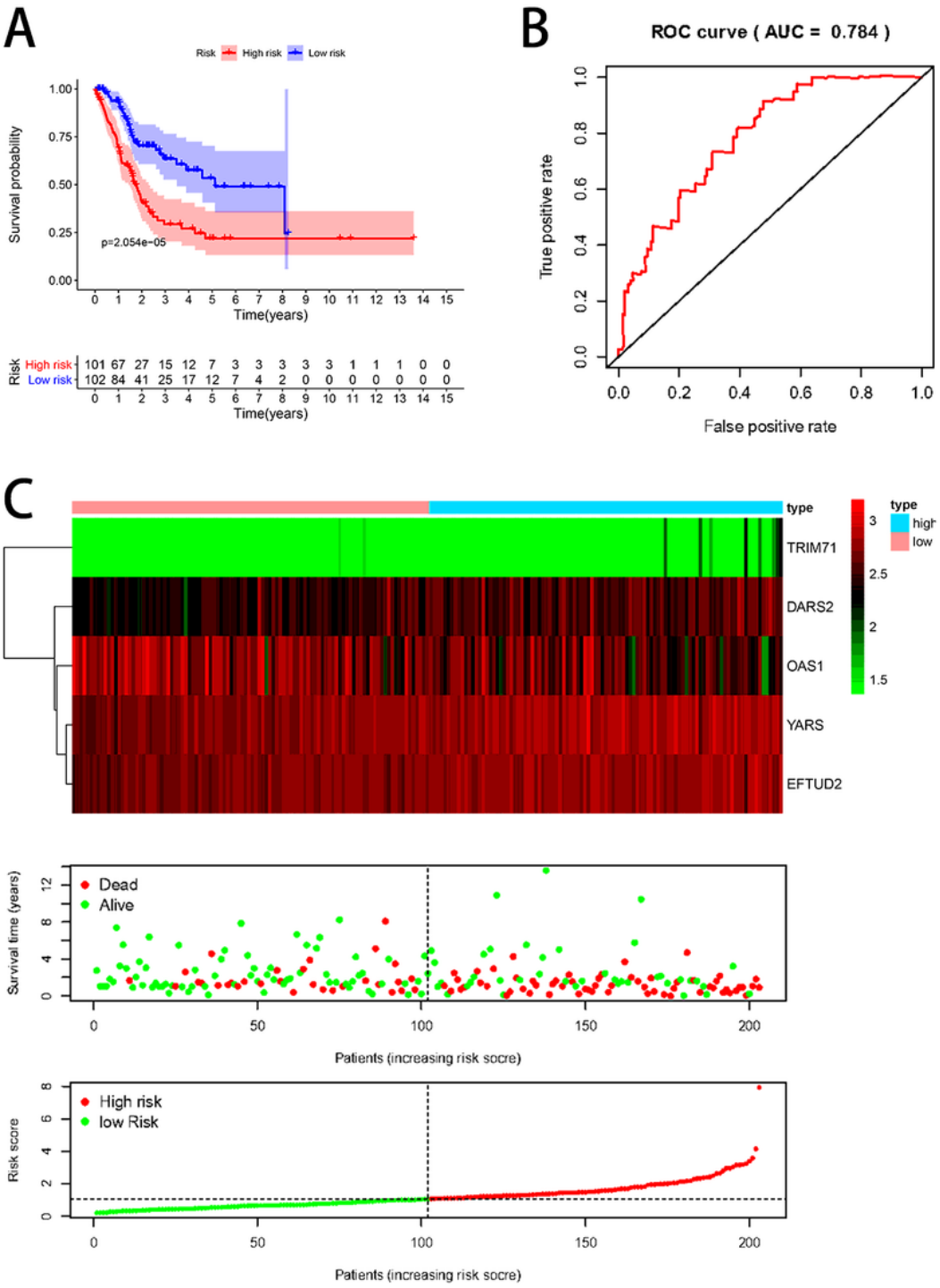

## Figure 4

Screening key RBPs. (A) Protein-protein interaction network of differently expressed RBPs; (B) Critical modules from PPI network. The green represents the down regulated RBPs; the red represents the up regulated RBPs.



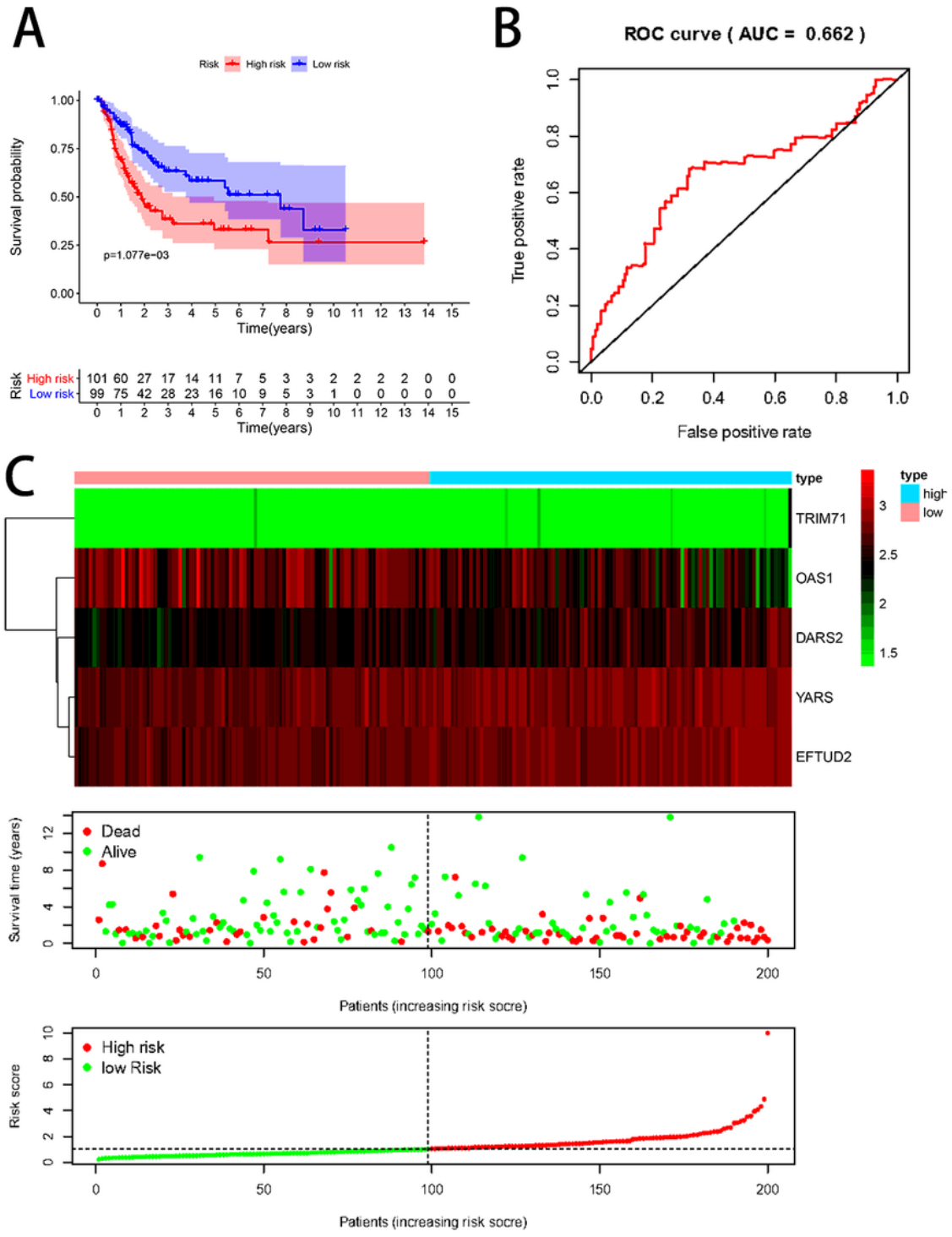

## Figure 5

A. Univariate Cox regression analysis of RBPs in bladder urothelial cancer. B. Multivariate Cox regression analysis of RBPs in bladder urothelial cancer.



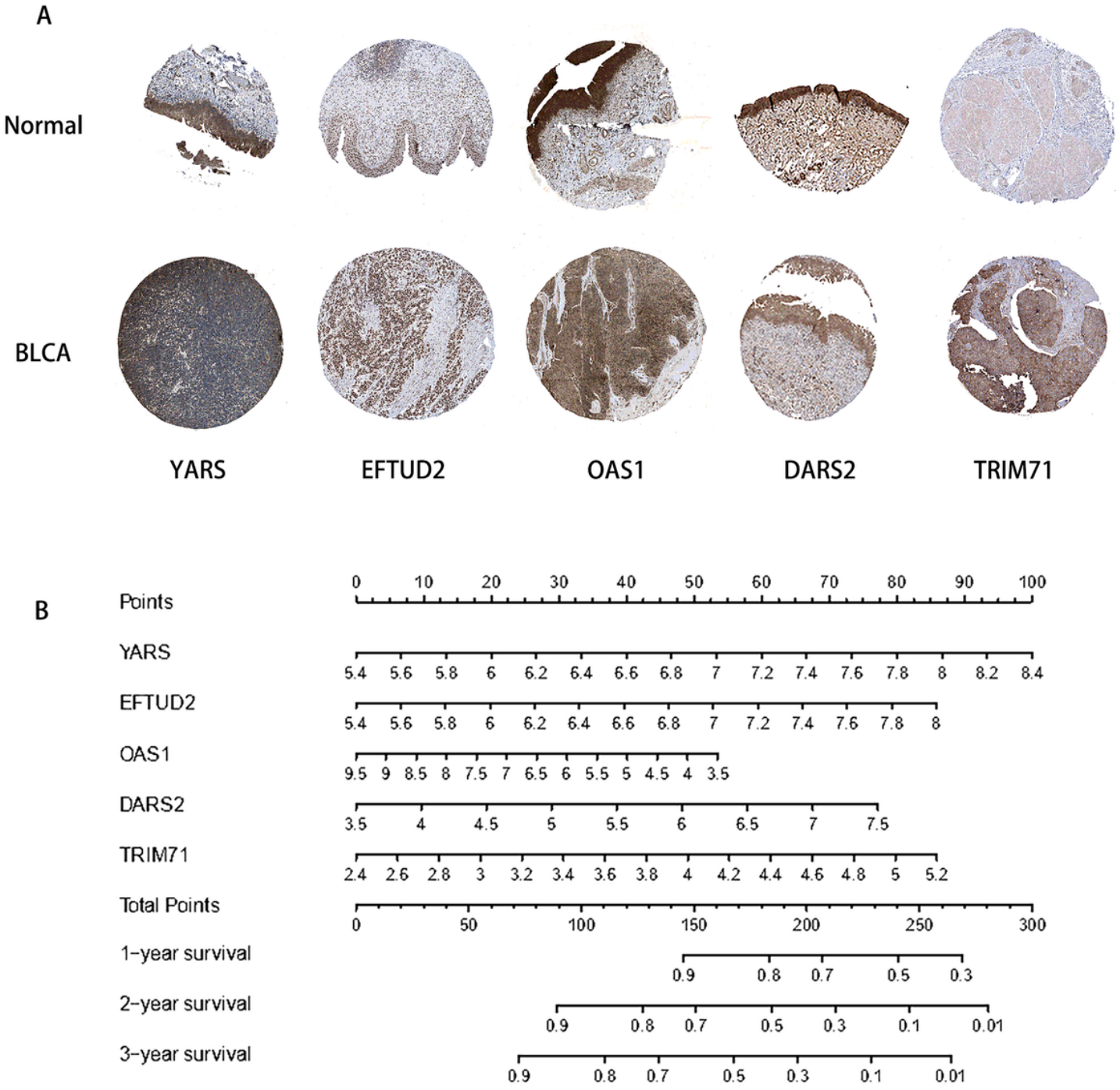

## Figure 6

Prognostic model of five hub RBPs in BLCA in train groups. (A) Survival curves of BLCA patients in low-risk group and high-risk group; (B) ROC curves for predicting overall survival based on risk score; (C) Heatmap of five hub RBPs, overall survival status, risk score distribution.




## Figure 7

Prognostic model of five hub RBPs in BLCA in test groups. (A) Survival curves of BLCA patients in low-risk group and high-risk group; (B) ROC curves for predicting overall survival based on risk score; (C) Heatmap of five hub RBPs, overall survival status, risk score distribution.




## Figure 8

A. Validation of the expression of five hub RBPs in normal bladder tissue and BLCA in the HPA database.
B. Nomogram for predicting the prognosis of BLCA based on five hub HBPs in the TCGA cohort.