

Comprehensive Analysis of the Expression and Prognosis for E2Fs in Human Cervical Cancer

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Research

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Abstract

Background: Accumulating evidence was found that E2Fs are a family of transcription factors that are aberrantly expressed or multi-activated in malignant tumors and are found in a variety of tumorigenesis. However, the expression pattern and exact role of E2Fs in cervical cancer (CC) tumorigenesis and progression have not been elucidated.

Methods: In the study arrangement, we examined the expression and prognostic value of E2Fs in CC patients using multiple databases, including ONCOMINE, GEPIA, cBioPortal, Metascape, and Kaplan-Meier mapper.

Results: E2F1/2/3/8 is upregulated in CC patients, with E2F1 mRNA levels upregulated with better OS correlation. E2F3/8 has an even worse RFS difference and could presumably be used as a CC therapeutic target.

Conclusion: These results suggest that E2F1/2/3/8 can exist as a marker for CC. However, more experimental results are needed to confirm this inference.

Background

The family of E2F transcription factors (E2Fs) is an important part of the normal cell cycle process [1] and has functions such as promoting cell proliferation [2], cell differentiation [3], apoptosis [4] and DNA repair [5]. The E2Fs family mainly includes E2F1~E2F8 cytokines. Currently, great progress has been made in the study of the family of nuclear transcription factors E2Fs. The protein structure of E2Fs mainly includes a dimeric domain and a highly conserved DNA domain [6]. E2Fs bind to dimeric proteins through the dimeric domain to form heterodimeric proteins, which have the ability to bind to specific DNA after entering the nucleus. Highly conserved DNA-binding domains can bind to specific DNA sequences and have specific effects on the transcription of target proteins [7]. It has been found that the oncogenic effects of E2F1-E2F3 transcription factors can be detected in a variety of human tumors [8,9], such as liver cancer, bladder cancer, and retinoblastoma in patients with E2F1 or E2F3 transcription factors. Expression of the E2Fs family has also been detected in patients with other tumors such as glioblastoma, lung cancer, ovarian cancer, breast cancer, and gastric cancer [10]. Many studies have shown that E2F1 mRNA overexpression has been detected in a large number of gastric cancer patients [11]. Studies on colon cancer have shown that overexpression of E2F4 is a key factor in promoting colon cancer [12]. Studies related to hepatocellular carcinoma have found that E2F1 expression is positively correlated with apoptosis [13]. Cervical cancer (CC) is a common gynecologic malignancy that poses a serious threat to women's health. Its incidence ranks first among female cancers, and with the change in people's lifestyles, its incidence is on the rise and is becoming younger [14]. Although the HPV vaccine can interfere with the course of cervical cancer [15], widespread access will take time. Currently, patients have a huge burden in the early stages of treatment due to the lack of clear and effective biomarkers for CC. Studies have shown that the positive expression rate of E2F1 is significantly higher in cervical cancer

patients than in controls [16], suggesting that E2F1 promotes cancer during the development of cervical cancer [17], suggesting that HPV may promote the dissociation of E2F1 and accelerate the development of cancer [18]. According to the results of clinical trials, the E2Fs family can be expressed as biomarkers of CC, and although the specific mechanism is still unclear at this stage, in this study, we performed a comprehensive database analysis based on the relationship between extended nuclear E2Fs family transcription factors and CC, and attempted to elucidate the relationship between the eight E2Fs subtypes and the onset and progression of CC.

Materials And Methods

Oncomine database analysis

ONCOMINE (www.oncomine.org) is an open source online cancer genotyping platform that effectively facilitates the expression and analysis of genome-wide research [19]. The transcriptional expression levels of E2F family members in CC were analyzed using ONCOMINE platform. The threshold limit is as follows: P-value=0.05; A fold - change = 1.5; and the data type is all. Each gene of Cancer comparisons were performed using sample and normal control data sets.

GEPIA database analysis

Gene-expression Profiling Interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn/>) is a geographic analysis based on transcriptional expression data in cancer, containing 9736 tumor data and 8587 genomic profiles of normal samples. The GEPIA database provides key interactive and customizable functions, including differential expression analysis, analysis mapping, correlation analysis, patient survival analysis, similar gene testing, and dimensionality reduction analysis [20].

TCGA and CBioPortal Analysis

The cBioPortal for cancer genomics (<http://www.cbioportal.org/>) is affiliated with the Memorial Sloan Kettering Cancer Center and provides information regarding the integrative analysis of complex cancer genomics and clinical profiles from 105 cancer studies in the TCGA pipeline [21]. The frequency of E2F family gene alterations (amplification, deep deletion, and missense mutations), copy number variance obtained from Genomic Identification of Significant Targets in Cancer (GISTIC), and mRNA expression z-scores (RNA Seq V2 RSEM) were assessed using the cBioPortal for Cancer Genomics database and TCGA [22]. OncoPrint, overall survival (OS), or disease-free survival (DFS) plotter were obtained according to the online instructions in c-BioPortal.

Functional Enrichment Analysis

GeneMANIA (<http://www.genemania.org>) is a user-friendly website that provides information for protein and genetic interactions, pathways, co-expression, colocalization, and protein domain similarity of submitted genes [23]. KOBAS (<http://kobas.cbi.pku.edu.cn/>) is a widely used gene set enrichment (GSE) analysis tool [24]. Its 1.0 and 2.0 versions were released in 2005 and 2011, respectively. The first two versions have been cited 1335 times by SCI, covering 5945 species and containing relevant knowledge. KOBAS has two important modules: the comment module and the enrich module. For the annotation module, it takes a list of genes as input, including ids or sequences, and generates annotations for each gene based on multiple databases about pathways, diseases, and gene ontologies [25]. The enrichment module can take any one of gene list or gene expression data as input, and generate the enrichment gene set, corresponding name, P-value or enrichment probability and enrichment score according to the results of various methods. Bioconductor 3.8 includes 1649 software packages, 360 experimental data packages, 941 annotation packages, and 23 workflows. KEGG data were used to analyze the metabolic pathways of gene products in cells and the functions of these gene products, and p-value < 0.01 was selected.

The Kaplan–Meier Plotter Analysis

The Kaplan-Meier Plotter (www.kmplot.com) database is an online computing platform for gene microarray expression analysis and survival information analysis [26]. The database contains more than 5000 samples to evaluate the effects of multiple genes or genomes on expression in cancer [27-30]. In this study, Kaplan-Meier was used to detect the expression level of E2F family members in the prognosis of CC patients. The overall Survival (OS) and relapse-free survival (RFS) of patients with CC were determined by dividing the patient samples into two groups based on median expression (high vs. low expression) and assessing using a Kaplan–Meier survival plot, with a hazard ratio with 95% confidence intervals and log rank p-value. The HR with 95% CI and the log-rank P-value was calculated in each Kaplan–Meier survival plot and the cutoff of log-rank P-value was defined as 0.05.

Results

Transcription levels of E2Fs in patients with CC

Eight E2F family members have been identified in human genome, and their mRNA expression levels in human cancer have been determined using the ONCOMINE database. The results are presented in Figure 1 and Table 1. ONCOMINE analysis revealed that the expression of E2F1, E2F2, E2F3, E2F7, and E2F8 is up-regulated in cervical cancer patients. The transcription levels of E2F1 were significantly higher in patients with CC in three datasets. In Scotto's dataset, E2F1 was overexpressed in cervical squamous cell carcinoma compared with that in the normal samples, with a fold change of 3.640 and p-value of 1.23E-10. In Zhai's dataset, E2F1 was overexpressed in cervical squamous cell carcinoma epithelia with a fold change of 1.624 and p-value of 2.39E-06. The transcription levels of E2F2 were significantly higher in patients with CC in two datasets. In the Biewenga's statistics, the fold change of mRNA expression of E2F2 in cervix serous carcinoma was 2.190 and p-value of 5.831E-6. In Zhai's dataset, E2F2 was

upregulated in cervix serous carcinoma with a fold change of 3.296 and p-value of 3.25E-10. The mRNA levels of E2F3 in cervical cancer (fold change = 4.546 and p-value = 8.31E-13) and cervical squamous cell carcinoma (fold change = 3.252 and p-value = 2.91E-09) were significantly higher than those in the normal samples in Pyeon's and Scotto's datasets. The transcriptional levels of E2F7 in cervical cancer (fold change = 9.390 and p-value = 3.02E-15) were significantly different from those in the normal samples in Pyeon's dataset. A similar trend was found for E2F8 in Zhai's and Scotto's datasets: the mRNA levels of E2F8 in cervical squamous cell carcinoma epithelia (fold change = 2.557 and p-value = 9.81E-05) and cervical squamous cell carcinoma (fold change = 2.557 and p-value = 1.91E-05) were significantly higher than those in the normal samples. In addition, no significant difference in E2F4, E2F5, and E2F6 mRNA expression was found between CC and normal controls, according to ONCOMINE analysis.

Table 1

The transcription levels of E2F family members in between different types of CC and cervical normal tissues(ONCOMINE).

	Type of Cervical Cancer versus Normal Cervical Tissue	Fold Change	p Value	t Test	Source and/or Reference
E2F1	Cervical Squamous Cell Carcinoma	3.640	1.23E-10	7.886	Scotto Cervix 2 statistics[31]
	Cervical Squamous Cell Carcinoma Epithelia	1.624	2.39E-6	6.212	Zhai Cervix statistics[32]
E2F2	Cervical Squamous Cell Carcinoma	2.190	5.83E-6	8.117	Biewenga Cervix statistics[33]
E2F3	Cervical Squamous Cell Carcinoma Epithelia	3.296	3.25E-10	9.625	Zhai Cervix statistics[32]
	Cervical Cancer	4.546	8.31E-13	10.055	Pyeon Multi-cancer statistics[34]
	Cervical Squamous Cell Carcinoma	3.252	2.91E-9	6.950	Scotto Cervix 2 statistics[31]
E2F4	NA	NA	NA	NA	NA
E2F5	NA	NA	NA	NA	NA
E2F6	NA	NA	NA	NA	NA
E2F7	Cervical Cancer	9.390	3.02E-15	12.346	Pyeon Multi-cancer statistics[34]
E2F8	Cervical Squamous Cell Carcinoma Epithelia	2.557	9.81E-5	4.719	Zhai Cervix statistics[32]
	Cervical Squamous Cell Carcinoma	2.557	1.91E-5	4.646	Scotto Cervix 2 statistics[31]

Relationship between the mRNA levels of E2Fs in patients with CC

We compared the transcription expression of E2F family members between CC and normal tissues by using the GEPIA dataset (Figure 2). The results showed that the mRNA expression levels of E2F1, E2F2, E2F3, E2F7, and E2F8 were significantly higher in CC tissues than in normal cervical tissues, whereas the transcription expression levels of E2F4, E2F5 and E2F6 were not significantly different between CC and normal tissues. By using the GEPIA dataset, we also analyzed the relationship between the transcription levels of E2Fs and the tumor stage of patients with CC, E2F1, E2F2, E2F3, E2F4, E2F5, E2F6, E2F7, and E2F8 groups did not significantly differ (Figure 3).

Co-expression and interaction analyses of E2Fs at the gene and protein levels in patients with CC

At the genetic level, GeneMANIA was used to perform correlation analysis on E2F family members (Figure 4A). The results showed that E2F1 and E2F8, E2F3 and E2F8, E2F7 and E2F8 were co-expressed. A relationship was found in the co-localization of E2F1 and E2F3 with E2F3 and E2F2. In addition, it was found that E2F2 and E2F5 are related in genetic interaction. In addition, E2f1 and E2F2, E2F4, E2F6, E2F4 and E2F5, E2F3 and E2F7 also have the same pathway. There is a physical interaction between E2f7 and E2F8, E2F1 and E2F6.

Through STRING analysis, the interaction between members of the E2F gene family was identified at the protein expression level. In terms of co-expression, text mining and protein homology, E2F1 interacts with E2F2, E2F4, and E2F8, while E2F7 interacts with E2F8. The normal physiological functions of related targets are all related to cell cycle, cell apoptosis and proliferation. The detailed results are shown in Figure 4B. Enrichment analysis of E2F family members revealed that E2F family members are associated with cell cycle, cellular senescence, chronic myeloid leukemia, and other biological pathway signals.

Sequence alterations in E2Fs affect Os and DFs in patients with CC

Alteration frequency of E2F mutations in CC was analyzed using cBioPortal. A total of 607 patients from two datasets of cervical squamous cell carcinoma were analyzed. Among the 2 CC datasets analyzed, alterations ranging from 11.11% to 10.74% were found for the gene sets submitted for analysis (Figure 5A). As was shown in Figure 5B, high mutation rate of E2Fs was observed in CC patients. In the 297 sequenced CC patients, genetic alteration was found in 166 CC patients and the mutation rate was 56%. The percentages of genetic alterations in E2F family members for CC varied from 5 to 18% for individual genes based on the TCGA Pancancer dataset. The mutation rates of E2F1, E2F2, E2F3, E2F4, E2F5, E2F6, E2F7 and E2F8 were 18%, 8%, 13%, 13%, 9%, 12%, 6%, 5%, respectively (Figure 5B). Next, we analyzed

genetic alteration and their associations with OS and DFS of CC patients, results from Kaplan-Meier plot and log-rank test showed that genetic alteration in E2Fs was associated with OS (Figure 5C, $p=0.110$) and DFS (Figure 5D, $p=0.0450$) of CC patients. These results implied that genetic alteration of E2Fs could affect CC patients' prognosis.

Prognostic Values of E2Fs in Patients With CC

We used Kaplan–Meier plotter to further determine the prognostic values of the mRNA expression of E2Fs correlated with OS and RFS in all CC patients ($P<0.05$). The Kaplan-Meier survival curves are shown in Figure 6 and Figure 7. First, we analyzed the relationship between the combined mRNA expression of all E2Fs and the prognosis of CC patients. The survival curves (Figure 6A) revealed that a higher level of combined E2F expression predicts a better OS in CC, while CC patients with higher mRNA levels of E2Fs were found to have poorer RFS (Figure 7A). Next, we focused on the relationship between the mRNA expression levels of individual E2F members and the prognosis of CC patients. As shown in Figure 6B–I, the increased mRNA levels of E2F1 and E2F2 were strongly associated with better OS. Additionally, Figure 7B–I shows that transcription levels of E2F3 and E2F8 were negatively associated with RFS in CC patients. The remaining E2F family members were not related with OS or RFS in CC.

Discussion

Cervical cancer is one of the most common gynecological malignant tumors, and its incidence ranks the third among female malignant tumors in the world. Cervical cancer is more common in middle-aged women [36]. The E2F transcription factor family has been shown to be associated with cell cycle in vivo and to have significant effects on apoptosis, and proliferation. It has been confirmed that E2F overexpression is closely related to the manifestations of various malignant tumors, such as the occurrence and development of liver cancer and ovarian cancer [37-40]. However, the expression of different E2F family members and their exact role in cervical cancer are unclear. In this study, we attempted to systematically investigate mRNA expression, gene change, correlation, potential function, and prognostic value of different E2Fs in CC patients.

E2F1 is one of the classic transcription factor, on cell cycle regulation in vivo, the function such as cell proliferation, apoptosis, autophagy has significant influence. In recent years, the study found that in patients with a variety of malignant tumors in vivo detection of E2F1 overexpression, in cervical cancer research results show that the E2F1 may promote the miR - 136 in cervical cancer cells show lower expression, raised its expression inhibits cell proliferation, promote apoptosis, accelerate the course of cervical cancer [41-44]. In our study, ONCOMINE and GEPIA datasets showed that the expression of E2F1 was up-regulated in CC patients, and E2F1 expression was associated with the clinical characteristics of CC patients. By using Kaplan–Meier plotter, we found that E2F1 RNA expression level was increased, which was associated with better OS in all CC patients. E2F2 regulates many cellular processes, such as

cell cycle, DNA synthesis, proliferation, and tumorigenesis [45-48]. According to the current research results, the reduced activity of E2F2 can be used as a targeted HPV link to inhibit cervical cancer [49]. In our report, the expression of E2F2 in cervical cancer tissues was higher than that in normal tissues. Although, E2F2 expression was not correlated with tumor stage in patients with breast cancer, a high E2F2 expression was significantly correlated with better OS in all of the patients with cervical cancer.

Recent studies have also shown that E2F3 is positively correlated with the regulation of cell cycle in organisms and it has an effect on biological functions such as cell proliferation and apoptosis [50-52]. Evidence points out that targeted therapy of cervical cancer can be achieved by reducing E2F3 [53]. There is also evidence that lncRNA TTN-AS1 is involved in the progression of cervical cancer cells by regulating the miR-573-E2F3 axis [54], which provides a new perspective for our study of therapeutic strategies for cervical cancer cells. Similarly, in the present study, we demonstrated significantly higher expression of E2F3 in CC tissues and poorer RFS in cervical cancer patients, so we consider E2F3 as another therapeutic target for CC patients.

E2F4 transcription factor is a key factor in cell apoptosis and cell cycle. E2F4 is found in many diseases. E2F4 is also overexpressed in cervical cancer lesions. However, a recent study pointed to evidence that HAND2-AS1 recruits the transcription factor E2F4 to the C16orf74 promoter region and down-regulates C16orf74 expression to suppress cervical cancer development [55]. Although there was no significant up- or down-regulation of E2F4 expression levels in CC, further studies on the role of E2F4 in CC are needed, considering the little evidence available.

E2F5 is an important member of the E2F family. It has growth inhibitory properties and has been observed in several solid cancers, such as osteosarcoma[56], colon cancer [57], and breast cancer [58]. E2F5 has been proven to be a landmark indicator of prostate cancer [59]. The current research also shows that increasing the barrier to E2F5 can reduce the carcinogenic activity of HPV and affect the transformation of cervical cancer [60]. However, mRNA expression levels of E2F5 did not have prognostic values in CC patients according to our study.

E2F6 is an important factor that regulates mitotic events in the retinoblastoma-related gene (RB / E2F) pathway. The downstream genes that E2F6 can regulate are divided into activation and inhibition methods, which involve cell cycle regulation, growth and apoptosis, proliferation and Important physiological functions such as differentiation. E2F6 can inhibit DNA damage-induced apoptosis [61-63]. In recent years, it has been found to be related to hypoxia [64]. The expression of E2F6 mRNA and protein in cells is regulated by oxygen concentration. E2F6 mRNA and/or protein expression is reduced under hypoxia. However, the expression of E2F6 in cervical tumor tissues has not been reported yet.

E2F7 is an atypical E2F family member that acts as a transcriptional repressor of E2F target genes, thereby contributing to cell cycle arrest for DNA repair and genomic integrity [65-66]. E2F7 is related to the manifestations of multiple cancers, and studies have shown that it can promote the proliferation and migration of breast cancer cells [67]. The overexpression of E2F7 protein also shows a correlation with the development of gastric cancer [68]. In addition, studies have shown that the long non-coding RNA

NEAT1 regulates the miR-889-3p / E2F7 axis by activating the PI3K / AKT pathway, thereby accelerating the cell process of cervical cancer [69]. Excessive activation of E2F7 accelerates the transformation of cervical cancer. Although, ONCOMINE and GEPIA datasets revealed that the expression of E2F7 was up-regulated in human CC, the Prognostic Values of E2F7 were not related with OS or RFS in CC.

The transcription factor E2F8 is an important regulator of the cell cycle, and the unrestricted activation of the dependent transcription of the E2F family is considered to be an important driving force for tumor formation and progression. Studies have shown that E2F8 plays an important role in embryonic development and cell cycle control by inhibiting E2F1 [70-71]. However, it is not yet known whether E2F8 is involved in the progression of cervical cancer. In the near future, if it is proved to knock down E2F8 in cervical cancer cell lines, E2F8 can effectively induce the expression of epithelial-mesenchymal transition (EMT) markers [72]. Compared with patients with low E2F8 expression, cervical cancer patients with high E2F8 expression have higher FIGO staging and recurrence rates. In conclusion, studies have shown that E2F8 is highly correlated with progression-free survival in patients with cervical cancer. In our study, E2F8 had higher mRNA expression and worse RFS in cervical cancer compared with normal tissues, which seemed consistent with the role of E2F3 as an oncogene.

More and more evidences show that the eight members of E2F family interact with each other to affect cell cycle, cell proliferation, apoptosis and carcinogenesis. We also analyzed the coexpression and correlation of E2F family at gene and protein levels, the results show that in the topological heterogeneity analysis of targets associated with family genes, MSH2, E2F1, and E2F2 had high degree values, and existing studies confirmed that the positive expression of MSH2 in cervical tissues showed a significant positive correlation with the degree of pathological differentiation of cervical squamous cell carcinoma patients, and the increased expression of MSH2 was closely related to the development of cervical cancer, and to some extent reflected the malignancy of cervical cancer [73]. From the enrichment analysis results HPV is the leading cause of cervical cancer, but does not necessarily convert to malignancy [74]. It has been shown that high-risk HPV subtypes can act directly on the tumor suppressor proteins p53 and PRb, which can inhibit the two pathways that regulate the cell cycle and promote the malignancy of CC; TGF- β / smads signaling pathway, as an important negative regulatory system of epithelial cell proliferation, is believed to control cell proliferation and induction of apoptosis, and is the central segment of the body's tumor suppression system. Alteration of TGF- β 1 is an important mechanism in the formation of HPV-related cervical cancer. Although we have recognized the importance of E2F family interaction in the pathogenesis and development of OC, the specific molecular mechanism of E2F family interaction remains to be further studied. Our study adds to the growing evidence regarding the complexity of the E2F family members and their associated signaling pathways, which offer clues into the rational development of multi-targeted and E2F-mediated targeted therapy.

To further clarify the genetic alteration, potential function, and carcinogenic mechanism of the E2F family members, we calculated the percentages of genetic alterations in E2F family members for CC and found that they varied from 5 to 18% for individual genes based on TCGA Pancancer dataset. Further, cases

with alterations in one of the query gene had worse OS and DFS than those without any alterations in the query genes, although the difference of OS was not statistically significant.

Conclusion

In this study, we systematically analyzed the expression, genetic alterations, genetic correlates, potential functions, and prognostic value of E2Fs in cervical cancer. The heterogeneity and complexity of the molecular biology of cervical cancer were well understood. Our results suggest that the increased expression of E2F1, 2, 3 and 8 in cervical cancer tissues may play an important role in the development of CC.

Abbreviations

CC: cervical cancer; KEGG Kyoto Encyclopedia of Genes and Genomes GO Gene Ontology GEPIA Gene-expression Profiling Interactive Analysis OS overall survival DFS disease-free survival GSE gene set enrichment RFS survival survival.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

SZ, YQY. These authors contributed equally to this work. SZ and YQY participated in the design of this study. ZJW and YJ carried out the study and collected important back- ground information. SZ and YQY drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data were available on request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Analysis Type by Cancer	Cancer vs. Normal		Cancer vs. Normal		Cancer vs. Normal		Cancer vs. Normal							
	<i>E2F1</i>	<i>E2F2</i>	<i>E2F3</i>	<i>E2F4</i>	<i>E2F5</i>	<i>E2F6</i>	<i>E2F7</i>	<i>E2F8</i>						
Bladder Cancer	2	2	3										3	
Brain and CNS Cancer		3	1	2		2	15	1			4	1	4	
Breast Cancer	9	16	1	6			8				9	1	6	
Cervical Cancer	2	1	3								1		2	
Colorectal Cancer	7		6	7	4		16	2	6		14		1	
Esophageal Cancer	2		1	5							1			
Gastric Cancer		1		9		1					2	1		
Head and Neck Cancer	1			10		1	1				4			
Kidney Cancer	1			2	2									2
Leukemia	4	4		4	1	4	1		4	4		1	2	7
Liver Cancer	1		2	5		1	1				1		4	
Lung Cancer	5		6	14	1		8		1		3		6	
Lymphoma	2		1	2	2	4		2	5				1	4
Melanoma	1	1		3										
Myeloma							1	1						
Other Cancer	3		2	9	2	1	1		2		3		2	1
Ovarian Cancer	2			5			1						3	
Pancreatic Cancer	1			4			1				3	1	3	
Prostate Cancer		1	1	2	1		1	2	6		1		2	
Sarcoma	3		1	2	5				2	1			3	1
Significant Unique Analyses	46	9	33	16	94	8	14	7	65	15	10	1	46	5
Total Unique Analyses	449		403		438		455		457		206		376	

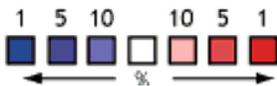


Figure 1

The transcription levels of E2F family members in different types of cancers (ONCOMINE). The graph shows the numbers of datasets with statistically significant mRNA over-expression (red) or down-regulated expression (blue) of the target gene. The threshold was designed with following parameters: p-value of 0.05 and fold change of 1.5

Analysis Type by Cancer	Cancer vs. Normal		Cancer vs. Normal		Cancer vs. Normal		Cancer vs. Normal									
	<i>E2F1</i>	<i>E2F2</i>	<i>E2F3</i>	<i>E2F4</i>	<i>E2F5</i>	<i>E2F6</i>	<i>E2F7</i>	<i>E2F8</i>								
Bladder Cancer	2	2	3										3			
Brain and CNS Cancer		3	1	2		2	15	1				4	1	4		
Breast Cancer	9	16	1	6			8					9	1	6		
Cervical Cancer	2	1	3									1		2		
Colorectal Cancer	7		6	7	4		16	2	6			14		1		
Esophageal Cancer	2		1	5								1				
Gastric Cancer		1		9		1						2	1			
Head and Neck Cancer	1			10		1	1					4				
Kidney Cancer	1			2	2									2		
Leukemia	4	4		4	1	4	1		4	4			1	2	7	
Liver Cancer	1		2	5			1	1				1		4		
Lung Cancer	5		6	14	1		8		1			3		6		
Lymphoma	2		1	2	2	4		2	5			1		4		
Melanoma	1	1		3												
Myeloma							1	1								
Other Cancer	3		2	9	2	1		1		2		3		2	1	
Ovarian Cancer	2			5				1						3		
Pancreatic Cancer	1			4				1				3	1	3		
Prostate Cancer		1	1	2	1		1	2	6		1			2		
Sarcoma	3		1	2	5				2	1				3	1	
Significant Unique Analyses	46	9	33	16	94	8	14	7	65	15	10	1	46	5	43	10
Total Unique Analyses	449		403		438		455		457		206		256		376	

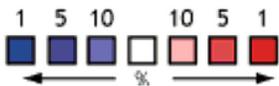


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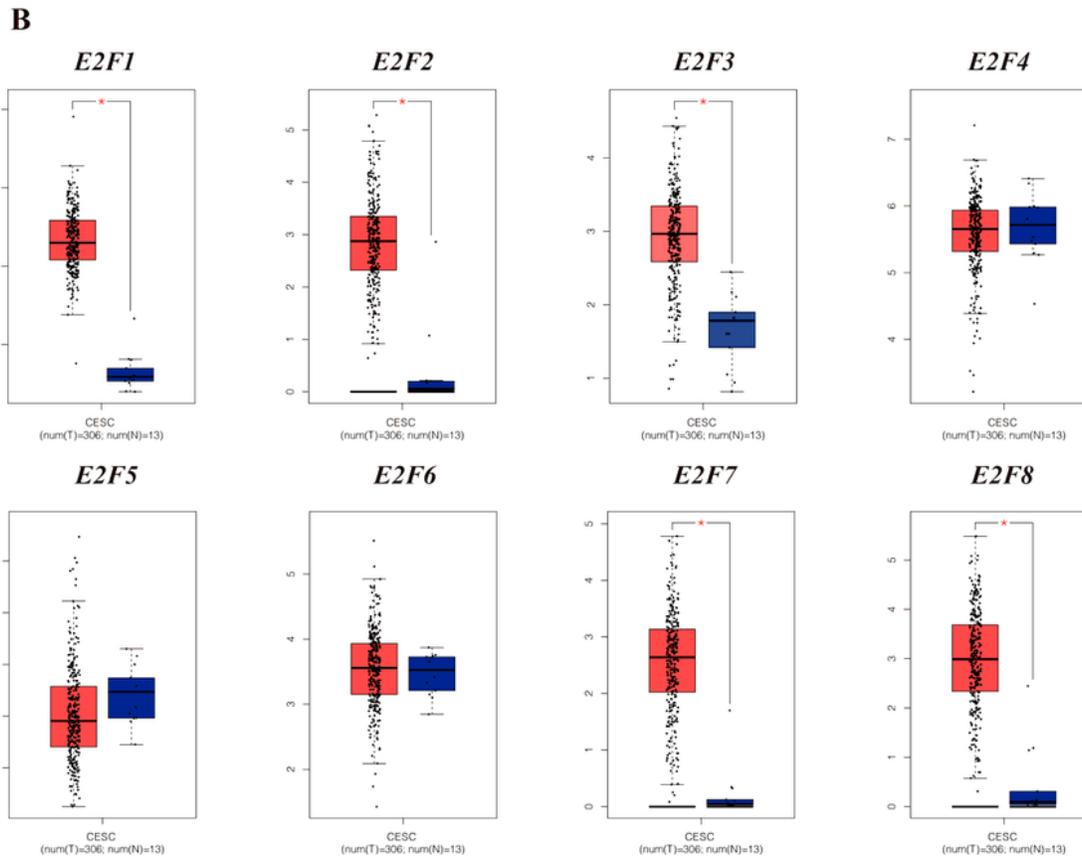
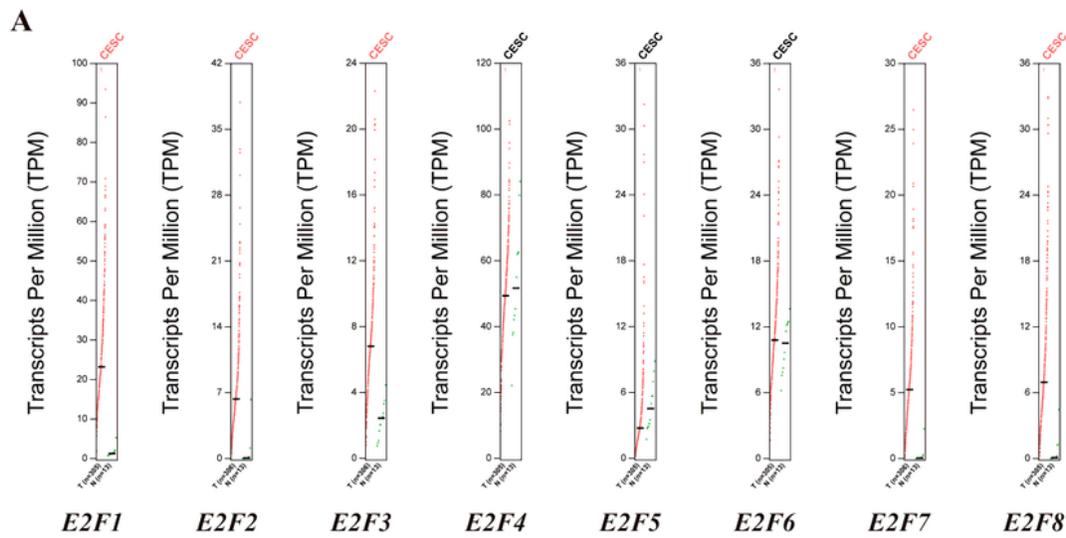


Figure 2

The expression of E2F family members in CC patients (GEPIA). Scatter diagram and box plots derived from gene expression data for GEPIA comparing the expression of a specific E2F family member in CC tissue and normal tissues; the p-value was set at 0.05. The mRNA expression pattern of E2F1, E2F2, E2F3, E2F4, E2F5, E2F6, E2F7, and E2F8 between CC and normal tissues. *Indicate that the results are statistically significant.

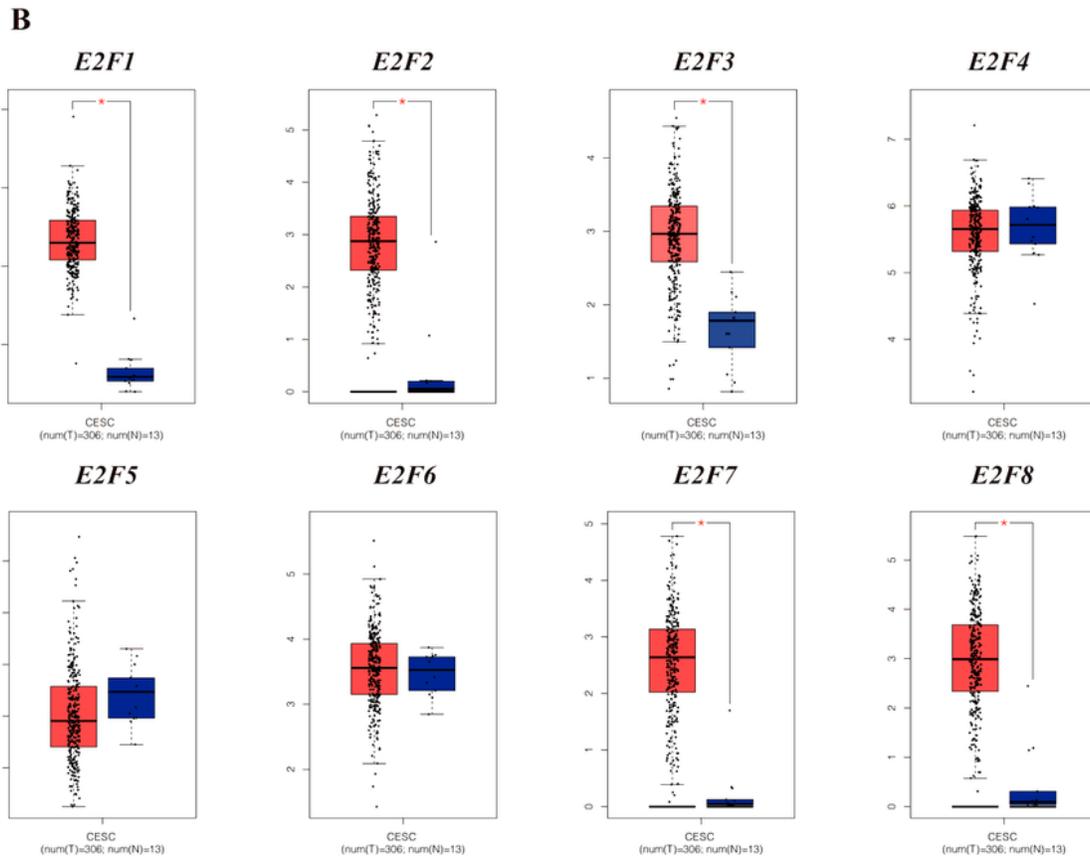
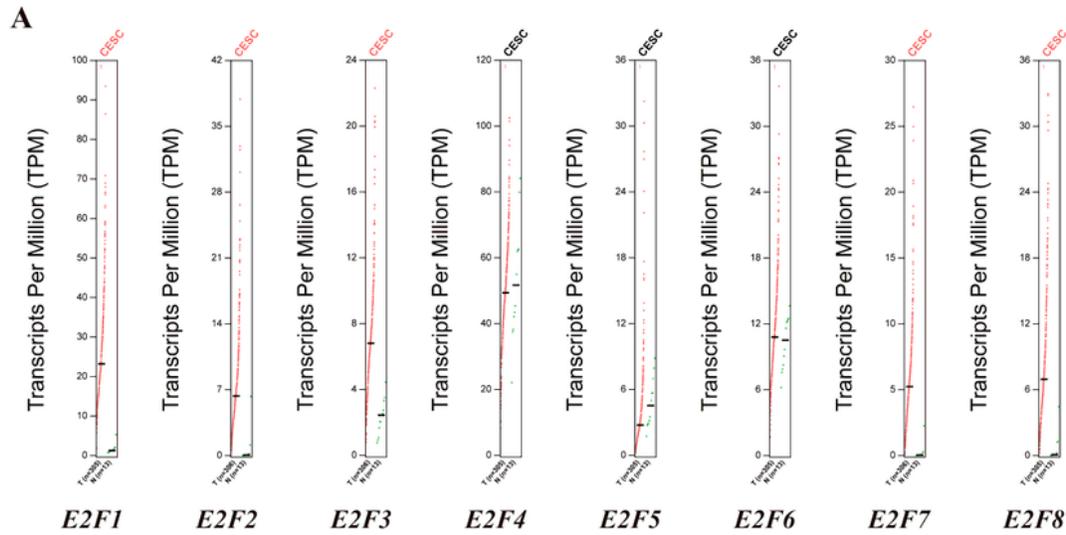


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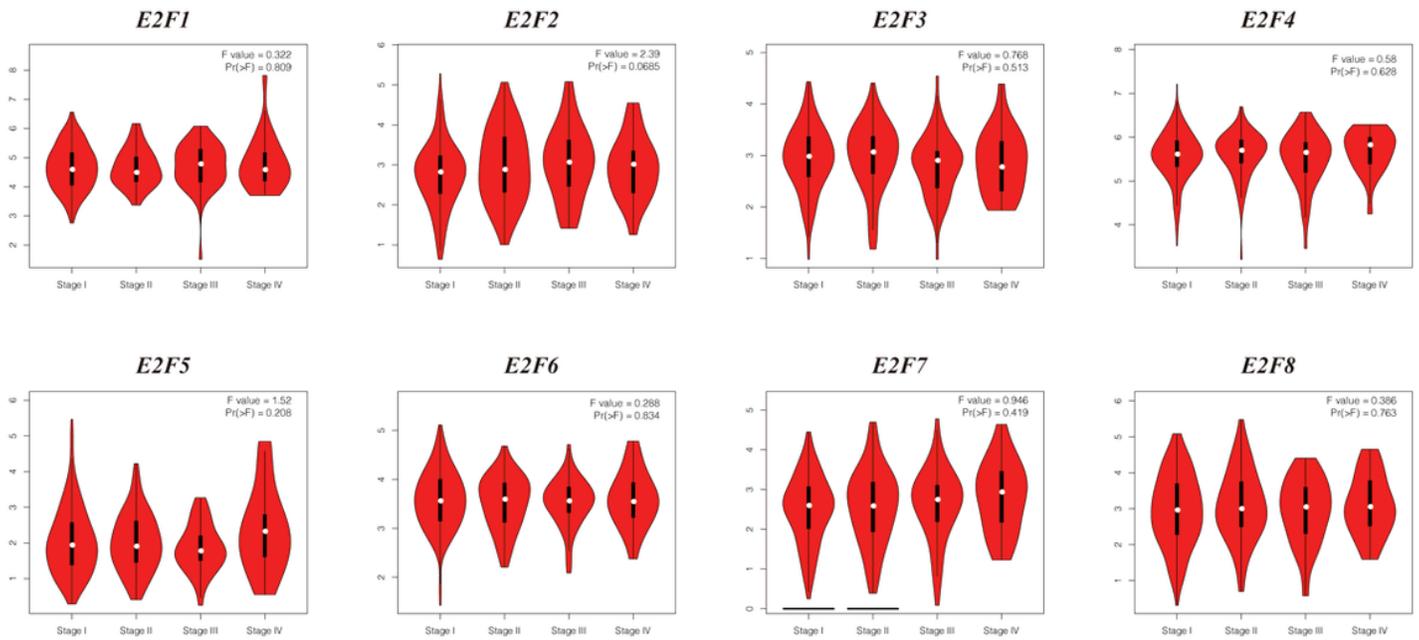


Figure 3

Correlation between E2F expression and tumor stage in CC patients (GEPiA). Violin plot derived from correlation between the expression of a specific E2F family member and tumor stage in patients with CC; the p-value was set at 0.05. The distribution of E2F1, E2F2, E2F3, E2F4, E2F5, E2F6, E2F7, and E2F8 mRNA expression correlated with tumor stage.

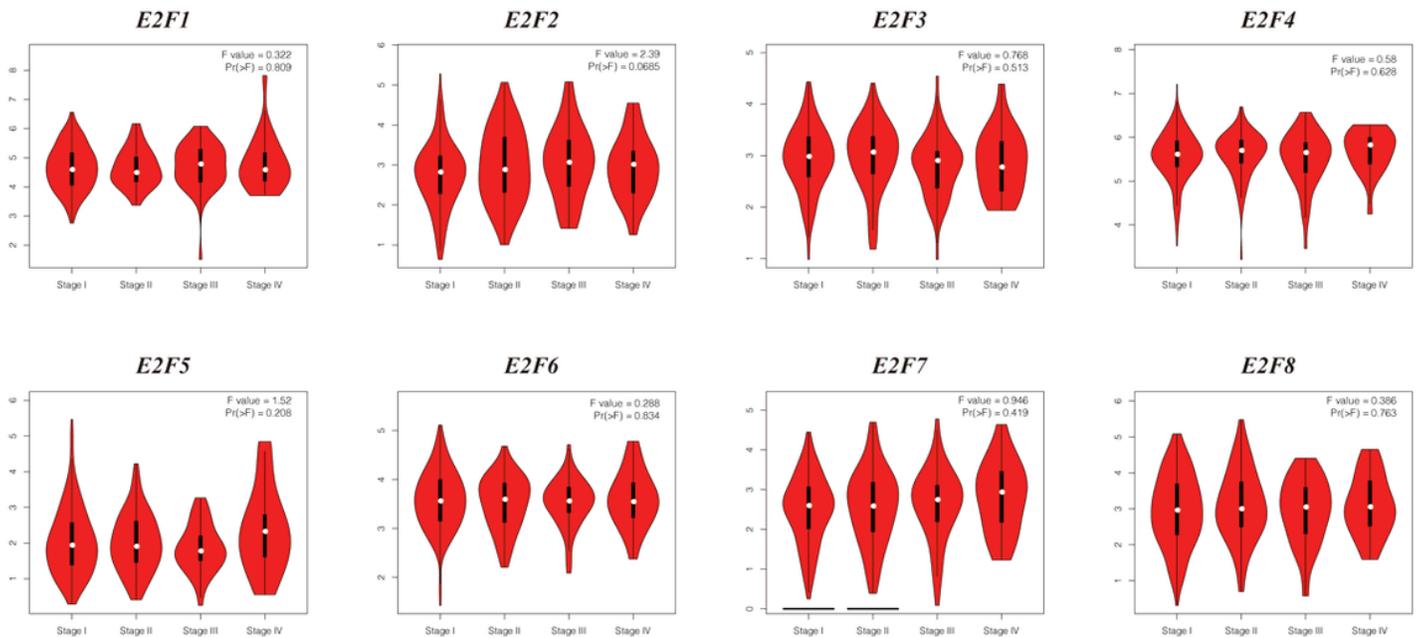


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Correlation between E2F expression and tumor stage in CC patients (GEPIA). Violin plot derived from correlation between the expression of a specific E2F family member and tumor stage in patients with CC; the p-value was set at 0.05. The distribution of E2F1, E2F2, E2F3, E2F4, E2F5, E2F6, E2F7, and E2F8 mRNA expression correlated with tumor stage.

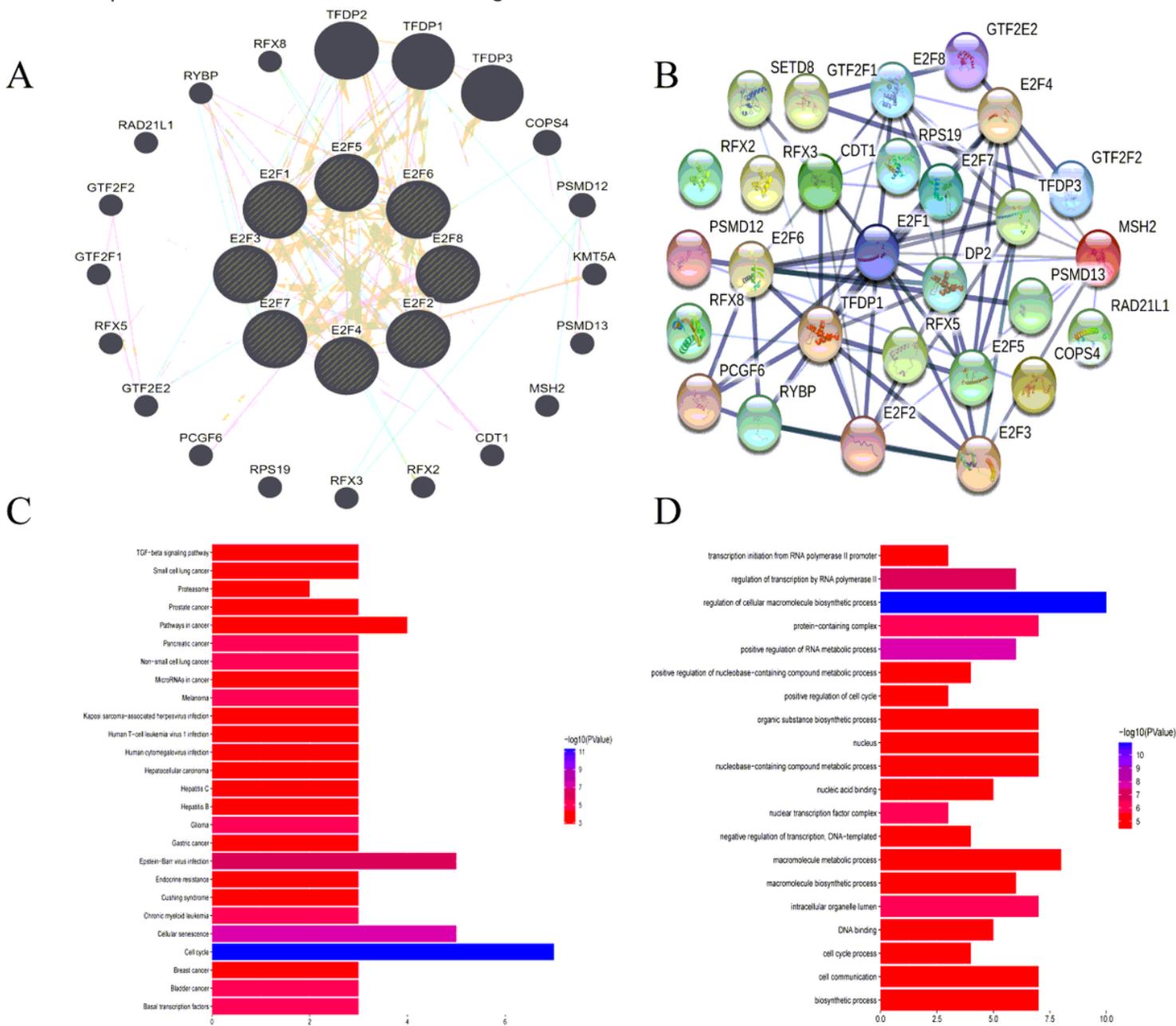


Figure 4

The enrichment analysis of E2F family members and neighboring genes in CC

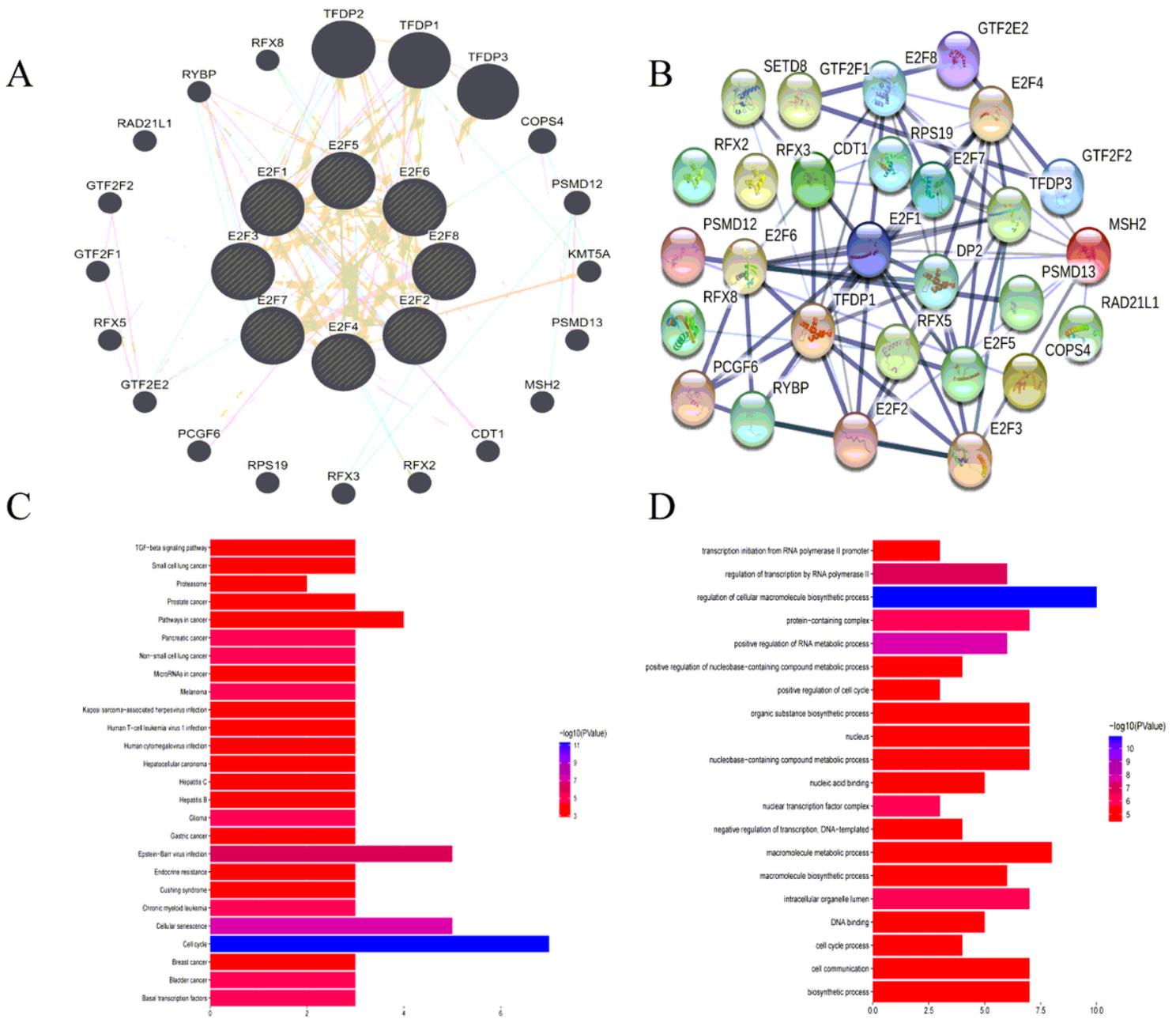


Figure 4

The enrichment analysis of E2F family members and neighboring genes in CC

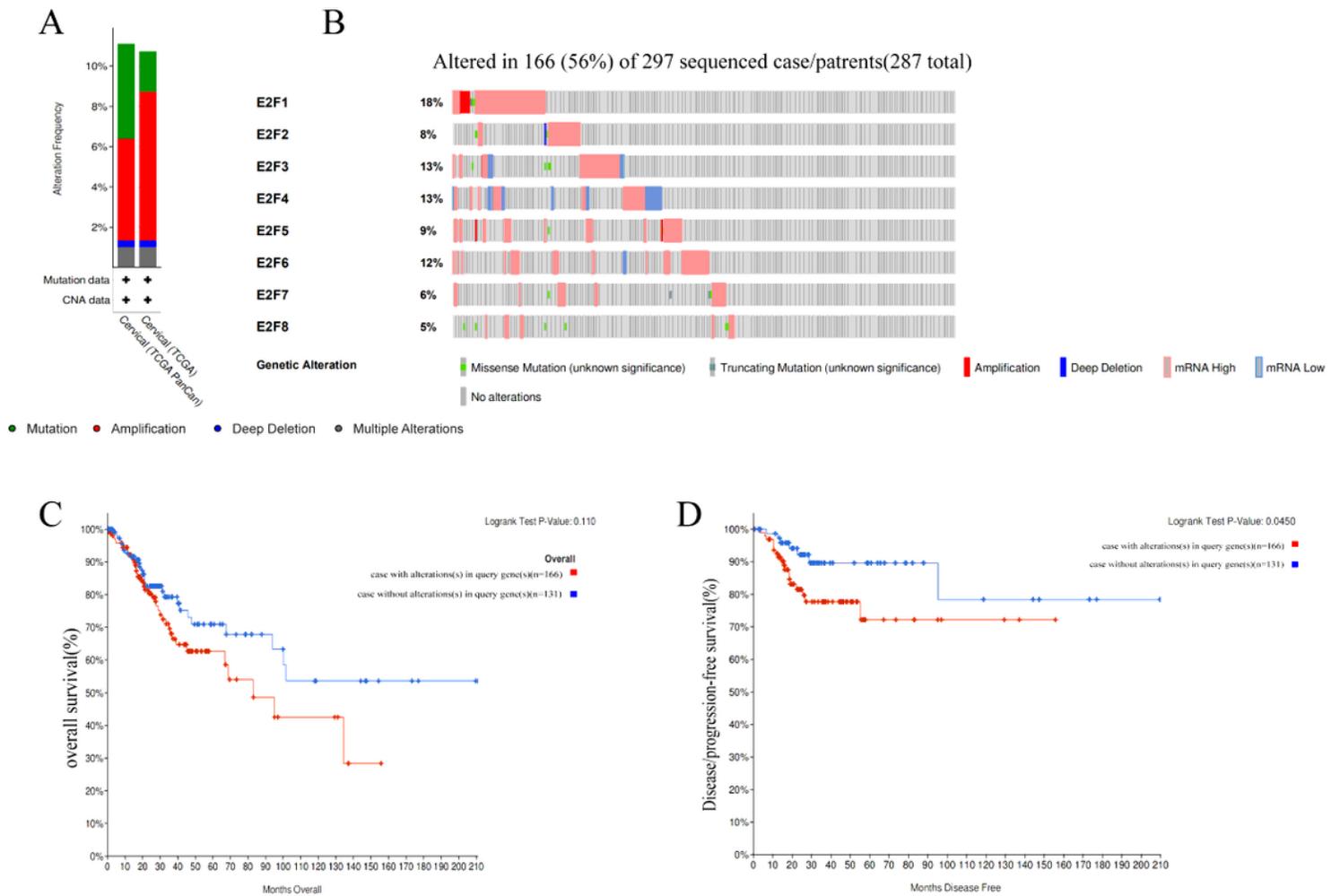


Figure 5

Alteration frequency of E2F family members and neighbor gene network in CC (cBioPortal). (A) Summary of alterations in E2F family members. (B) OncoPrint visual summary of alteration on a query of E2F family members. (C) Kaplan-Meier plots comparing OS in cases with/without E2F family member gene alterations. (D) Kaplan-Meier plots comparing disease-free survival (DFS) in cases with/without E2F family member alterations.

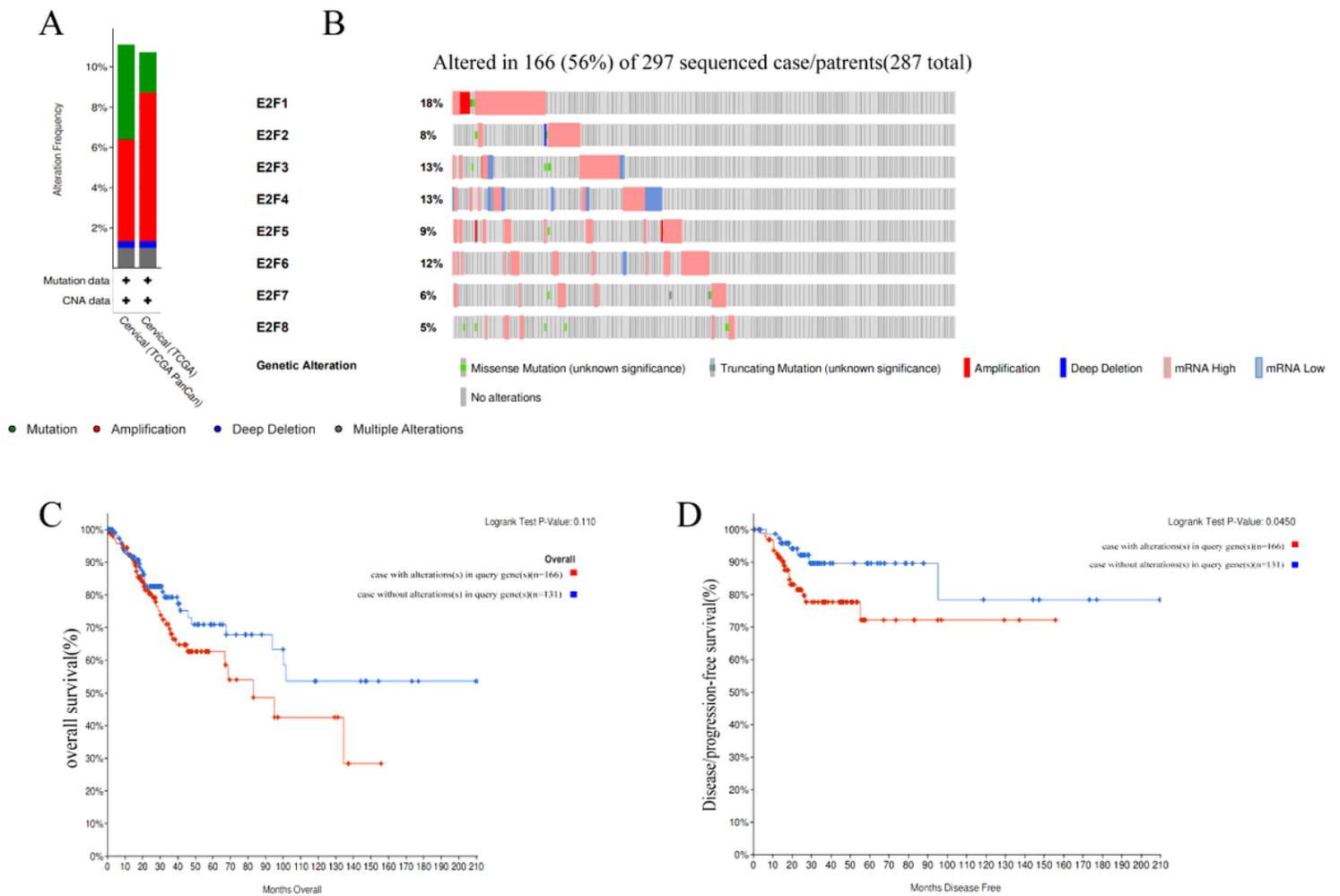


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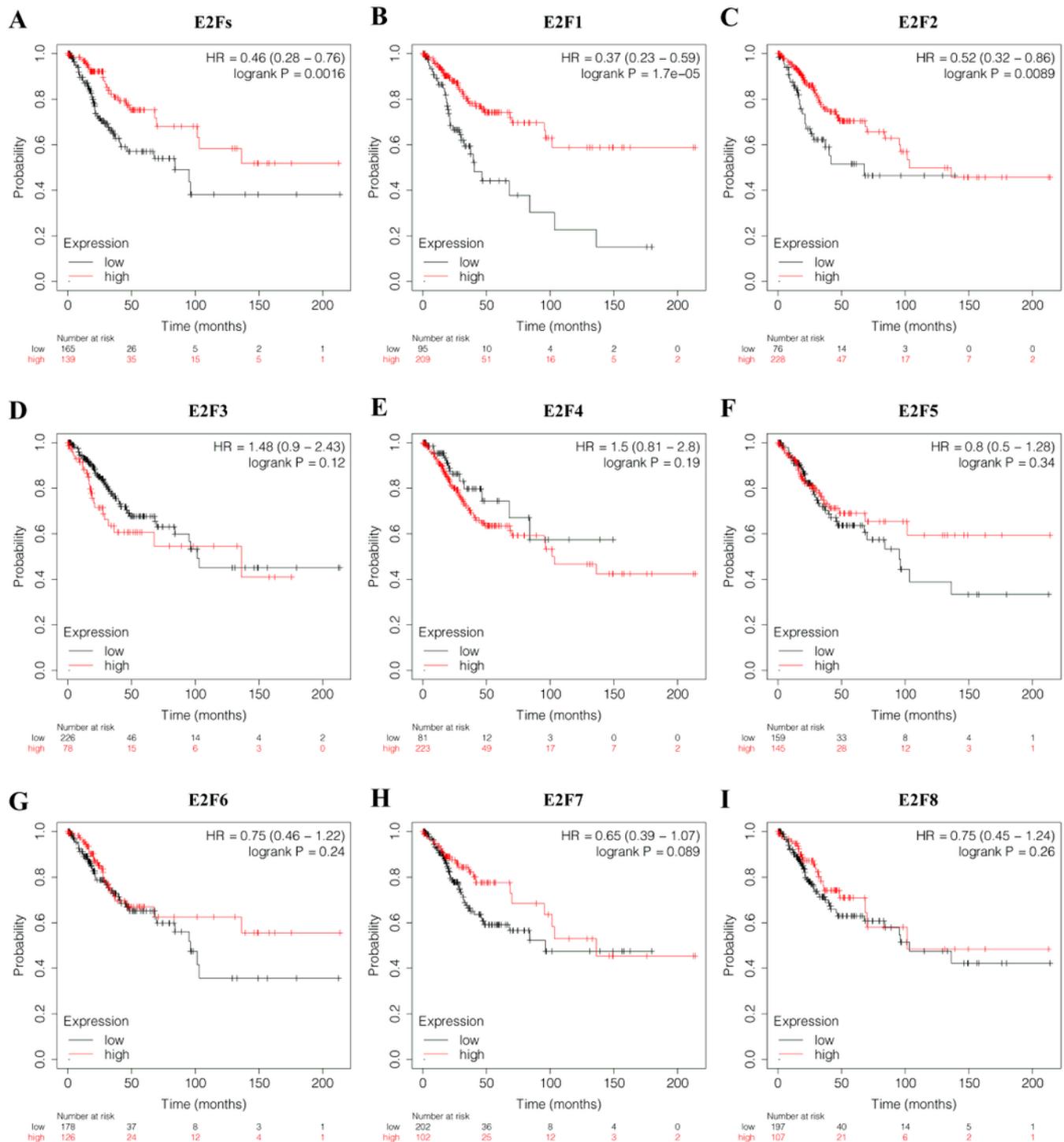


Figure 6

The prognostic value of mRNA levels of E2Fs in CC patients (OS in Kaplan-Meier Plotter). (A) The prognostic values of all E2Fs correlated with OS in CC patients. (B-I) The prognostic values of individual E2F members correlated with OS in CC patients.

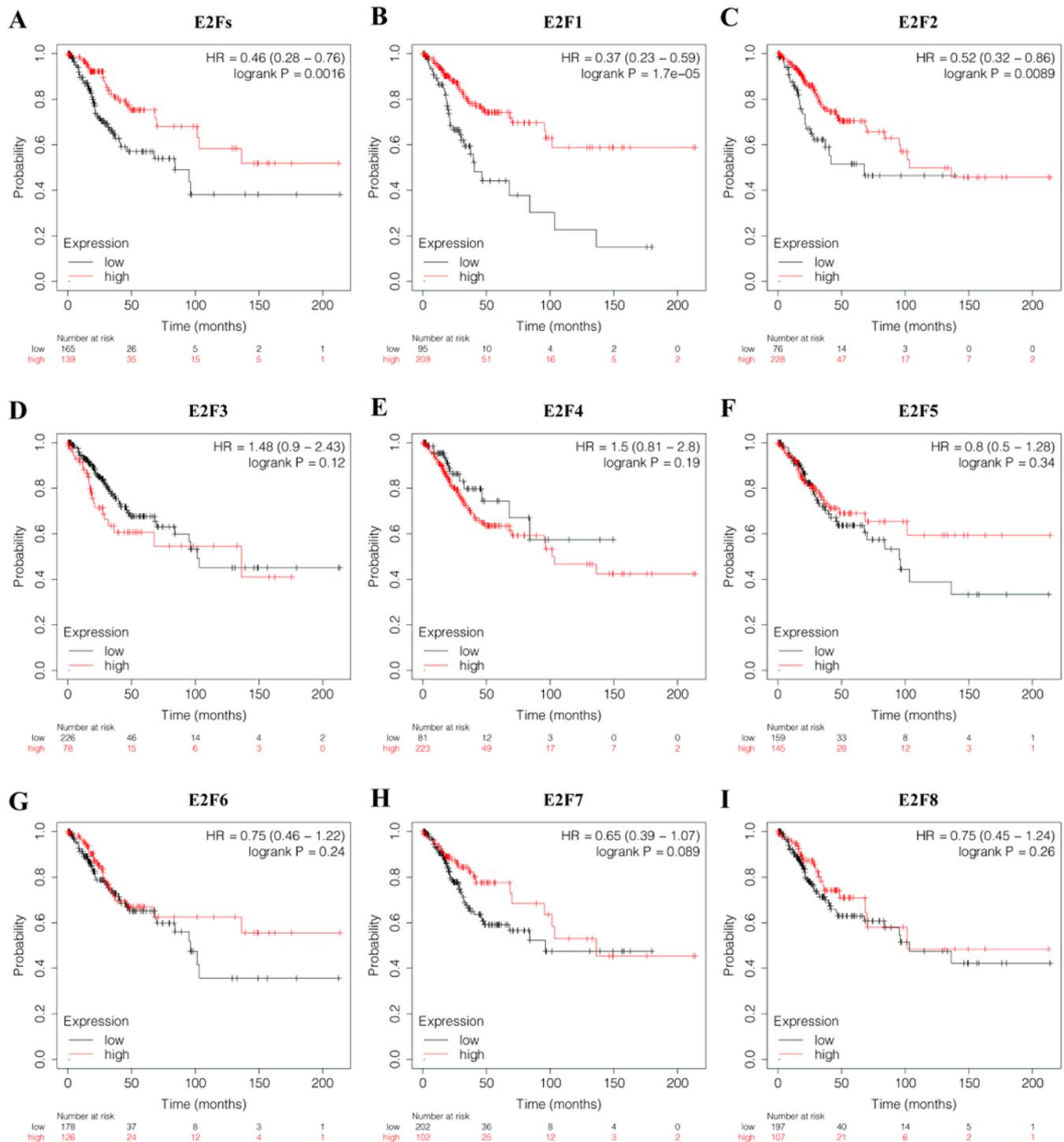


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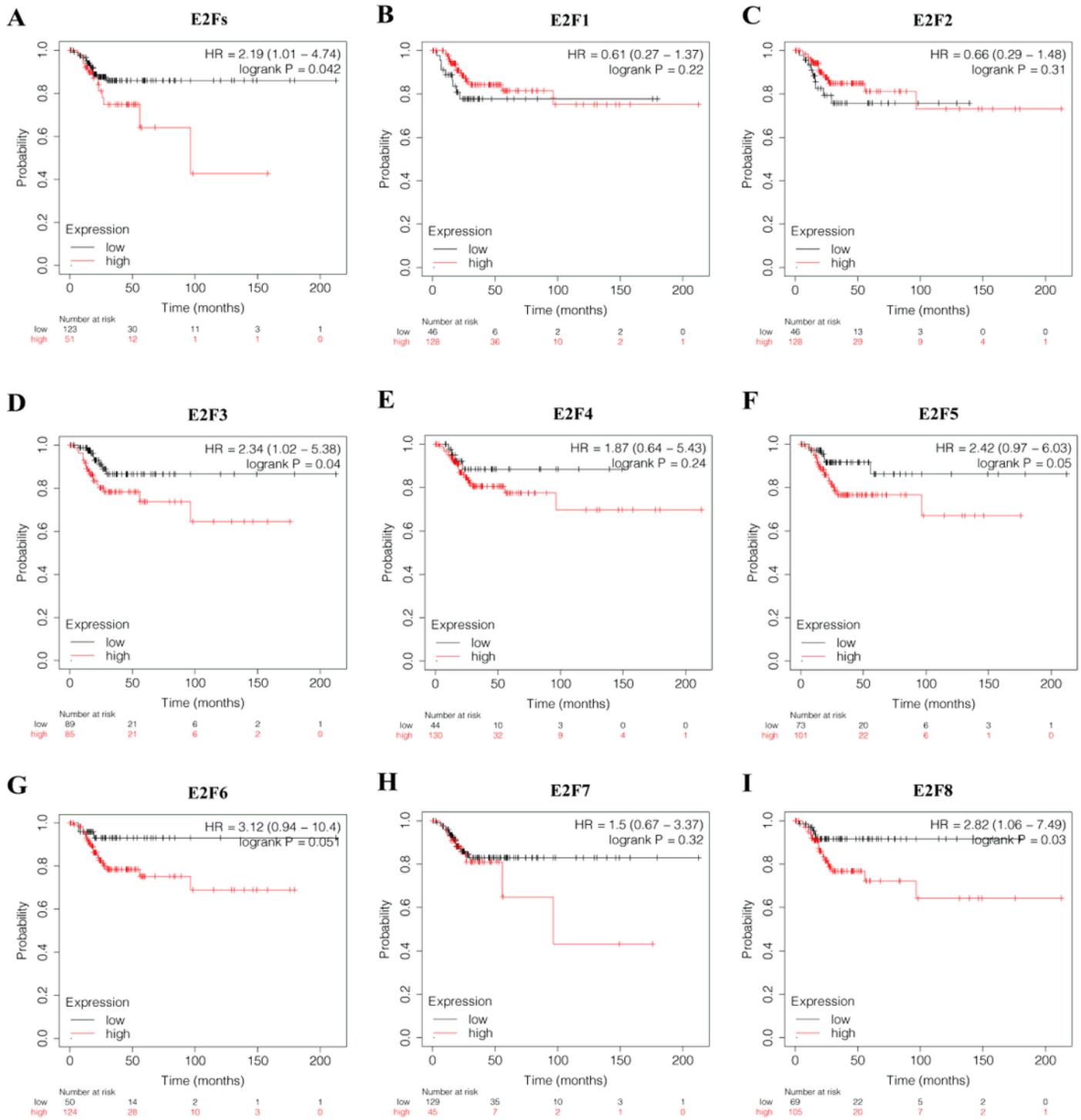


Figure 7

The prognostic value of mRNA levels of E2Fs in CC patients (RFS in Kaplan-Meier Plotter). (A) The prognostic values of all E2Fs correlated with RFS in CC patients. (B-I) The prognostic values of individual E2F members correlated with RFS in CC patients.

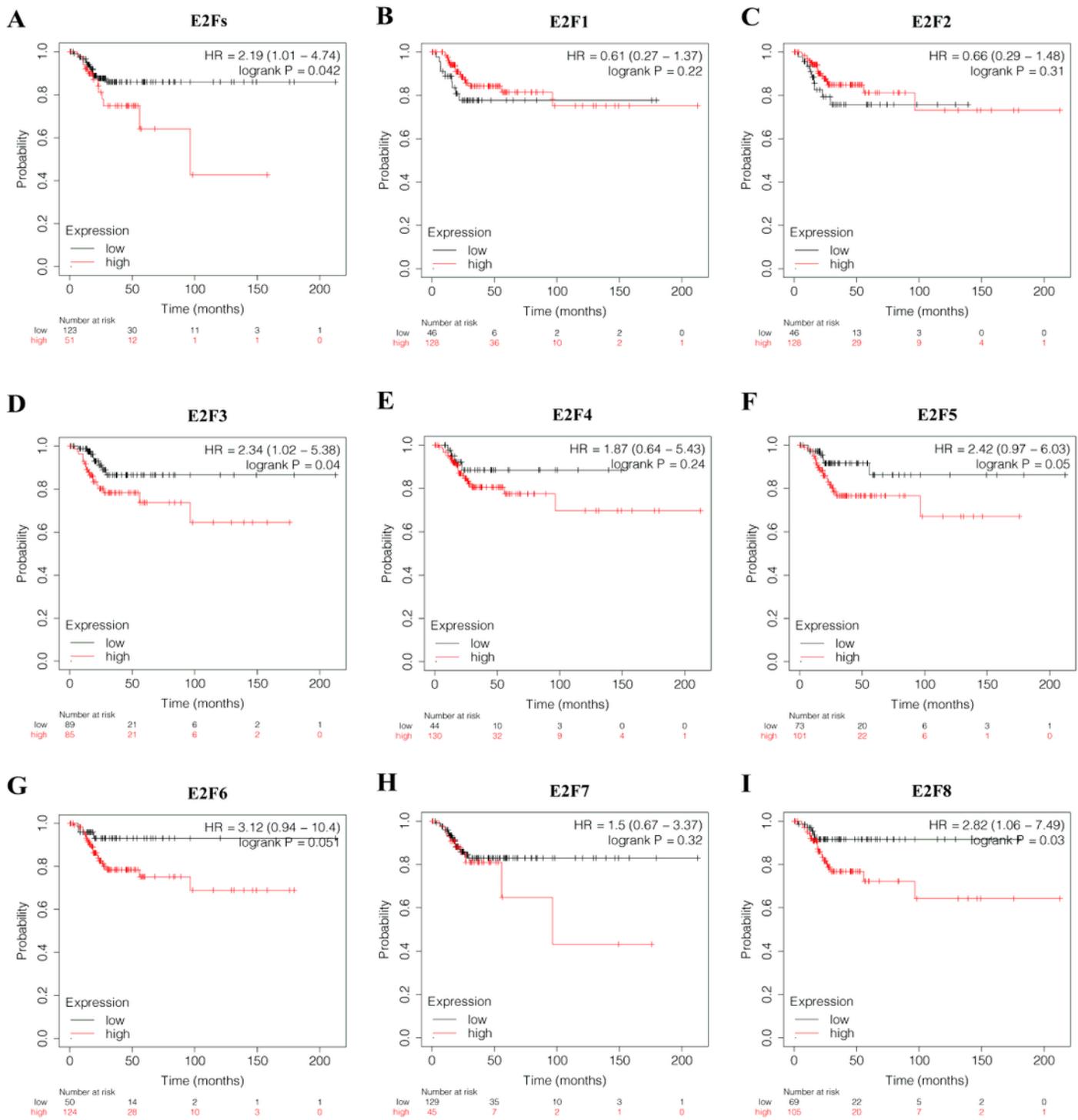


Figure 7

The prognostic value of mRNA levels of E2Fs in CC patients (RFS in Kaplan-Meier Plotter). (A) The prognostic values of all E2Fs correlated with RFS in CC patients. (B-I) The prognostic values of individual E2F members correlated with RFS in CC patients.