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DATABASE ANALYSIS

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Prognostic Value of E2F Transcription Factor Expression in Pancreatic Adenocarcinoma

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Pancreatic adenocarcinoma (PAAD) is one of the deadliest types of cancer. In the early stages, patients often have atypical symptoms, making diagnosis difficult. The prognosis of diagnosed patients is very poor and treating PAAD is challenging. Therefore, determining reliable risk factors related to PAAD development is critical for improving patient prognosis. E2F family transcription factors (TFs) are essential regulators of DNA synthesis and cell cycle progression in eukaryotic cells, and they have been identified as prognostic biomarkers associated with multiple cancer types. However, further research is necessary to establish the prognostic relevance of these TFs in PAAD patients.

Material/Methods: We assessed PAAD patient transcriptional and outcome data using the TIMER, ONCOMINE, STRING, GEPIA, cBioPortal, Kaplan-Meier Plotter, GSCALite, and starBase databases.

Results: PAAD tumor tissues exhibited increased expression of E2F1/3/5/7/8 relative to that in normal tissues, while the expression of E2F2/3/6/8 was associated with a more advanced tumor stage. Survival analyses indicated that PAAD patients expressing higher levels of E2F1/2/3/7/8 exhibited shorter overall survival (OS) and disease-free survival (DFS) than patients expressing lower levels of these TFs. In addition, E2F4 and E2F6 overexpression was associated with poorer DFS and OS, respectively. We also found that the expression of E2Fs was significantly correlated with immune infiltrates, including CD8+ T cells, CD4+ T cells, B cells, dendritic cells, neutrophils, and macrophages.

Conclusions: Our study may provide new insights into the optimal choice of immunotherapy and promising novel targets for therapeutic intervention in PAAD patients.

Keywords: E2F Transcription Factors • Pancreatic Adenoma • Prognosis

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/933443

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Background

Pancreatic adenocarcinoma (PAAD) is among the deadliest forms of cancer, with a 5-year survival rate that is under 10% [1]. PAAD incidence has risen in recent years, and because patients with early-stage disease do not exhibit reliable symptoms, it is rarely diagnosed until it is advanced, at which point patient prognosis is very poor. Identifying prognostic biomarkers associated with PAAD patient outcomes is thus vital to better guide patient management efforts and to aid in the formulation of novel therapeutics. E2F family transcription factors (TFs) serve as critical regulators of eukaryotic cell proliferation. Mammals express 8 different EF2 family proteins (E2F1-8) that are broadly classified as transcriptional activators (E2F1-E2F3a) or transcriptional repressors (E2F3b-E2F8) [2] and control biological activities such as cell cycle progression, DNA damage responses, cell death, and differentiation in a context-specific manner [3]. E2F TF expression patterns have been shown to be dysregulated in many cancers, including ovarian cancer [4], breast cancer [5], bladder cancer [6], prostate cancer [7], lung adenocarcinoma [8], and PAAD [9].

In an effort to elucidate novel prognostic biomarkers related to PAAD patient outcomes, we employed a series of bioinformatics tools and databases to systematically assess E2F expression patterns in PAAD patients. Through this approach, we explored the relationships between these TFs and patient survival while additionally clarifying their potential regulatory roles in cells to guide future studies of PAAD patient treatment.

Material and Methods

Ethics Statement

The Academic Committee of Dalian Medical University approved this study in accordance with the Declaration of Helsinki. As all the datasets were derived from previously published literature, informed consent for research use was previously provided.

ONCOMINE Analysis

The ONCOMINE (https://www.oncomine.org/resource/main.html) database compiles gene chip data pertaining to a large collection of tumor types, enabling data mining related to tumor-related transcription profiles [10]. The mRNA levels of E2F family members in clinical tumor samples in the ONCOMINE database were compared to the levels in normal control samples using *t* tests, with significance thresholds of $P<0.01$ and fold-change (FC) >2.

TIMER Analysis

The online TIMER tool (http://timer.cistrome.org/) enables systematic analyses of immune cell populations associated with 23 cancers compiled in The Cancer Genome Atlas (TCGA) [11]. Herein, the TIMER tool was utilized to assess E2F family TF expression in a range of tumor types. The relationship between the transcription level of E2Fs and the level of immune cell infiltration in PAAD was evaluated.

GEPIA Analysis

The GEPIA webserver (http://gepia.cancer-pku.cn/) enables the standardized analysis of RNA-seq data pertaining to 9736 tumors and 8587 normal samples derived from the TCGA and GTEx databases, allowing users to compare differential gene expression and patient survival outcomes, calculate correlations between gene expression patterns, conduct dimensionality reduction analyses, and to assess other gene-related information of interest [12]. Herein, GEPIA was utilized to assess the differential expression E2F family TFs in tumors and normal samples, with $P<0.05$ and $|\log2FC| > 1$ as the threshold for statistical significance.

Kaplan-Meier Plotter Analysis

The Kaplan-Meier Plotter database (www.kmplot.com) compiles cancer-related gene expression and survival outcome data [13,14]. Herein, this database was used to examine the association between E2F family protein expression in PAAD patients and the overall survival (OS) and disease-free survival (DFS) of these patients as assessed using Kaplan-Meier curves following patient stratification into high- and low-expression groups for each gene of interest based upon median E2F expression levels in a given patient cohort. This tool provided *P* values, median mRNA expression levels, hazard ratios (HRs), and 95% confidence intervals (CIs) corresponding to these analyses.

TCGA Data and cBioPortal

The TCGA is a compilation of pathological and sequencing-related data associated with 30 cancer types [15]. The Provisional TCGA PAAD dataset incorporates data from 186 cases, and this database was selected for analyses of E2F expression using cBioPortal (http://www.cbioportal.org/) [16,17]. Each gene was analyzed for factors such as mutational profiles, copy number variations (CNVs) from GISTIC, mRNA expression Z-scores (RNA-Seq V2 RSEM), and protein expression Z-scores (RPPA). In addition, coexpression and network analyses were performed based on the instructions provided by cBioPortal.

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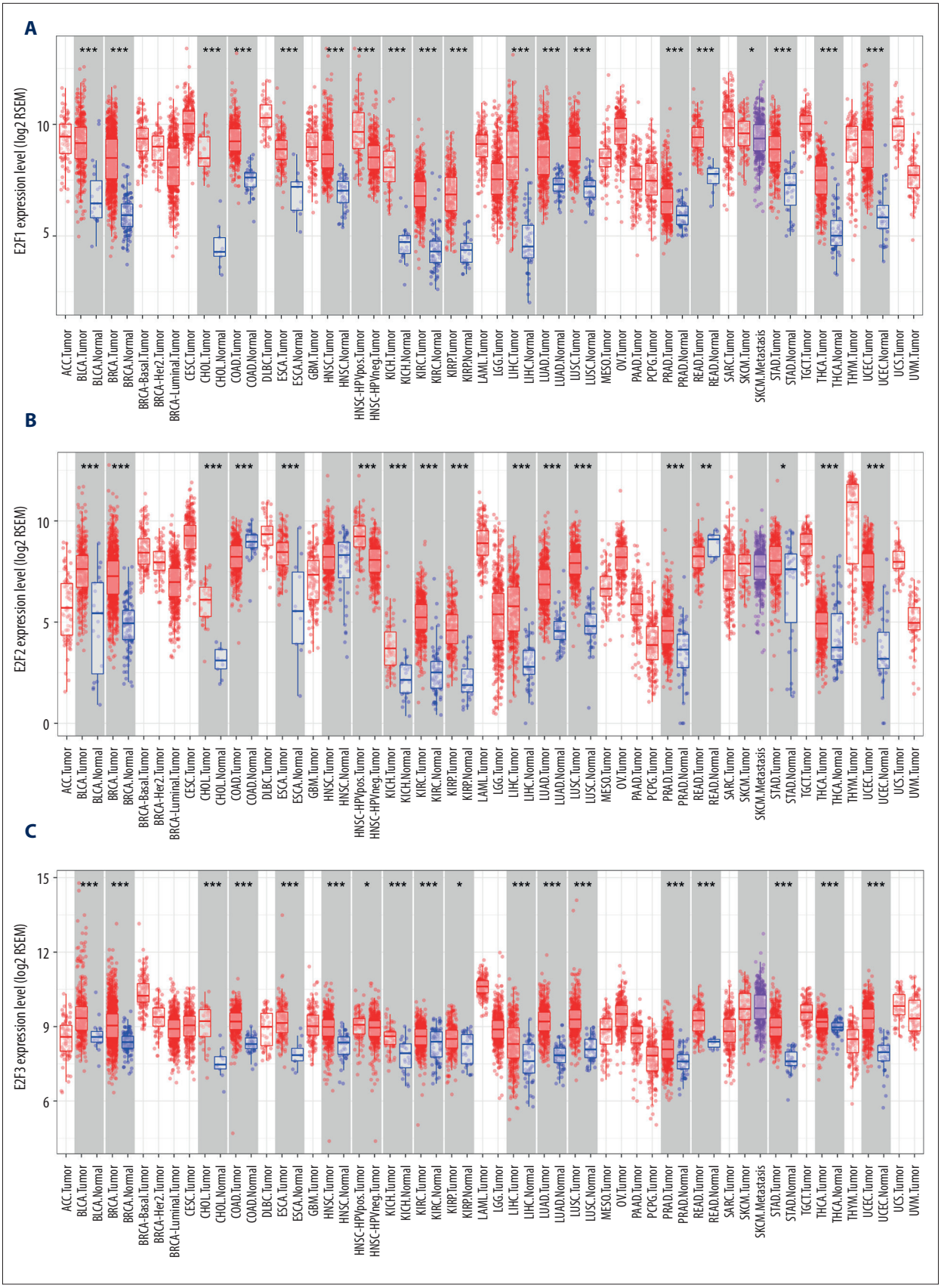

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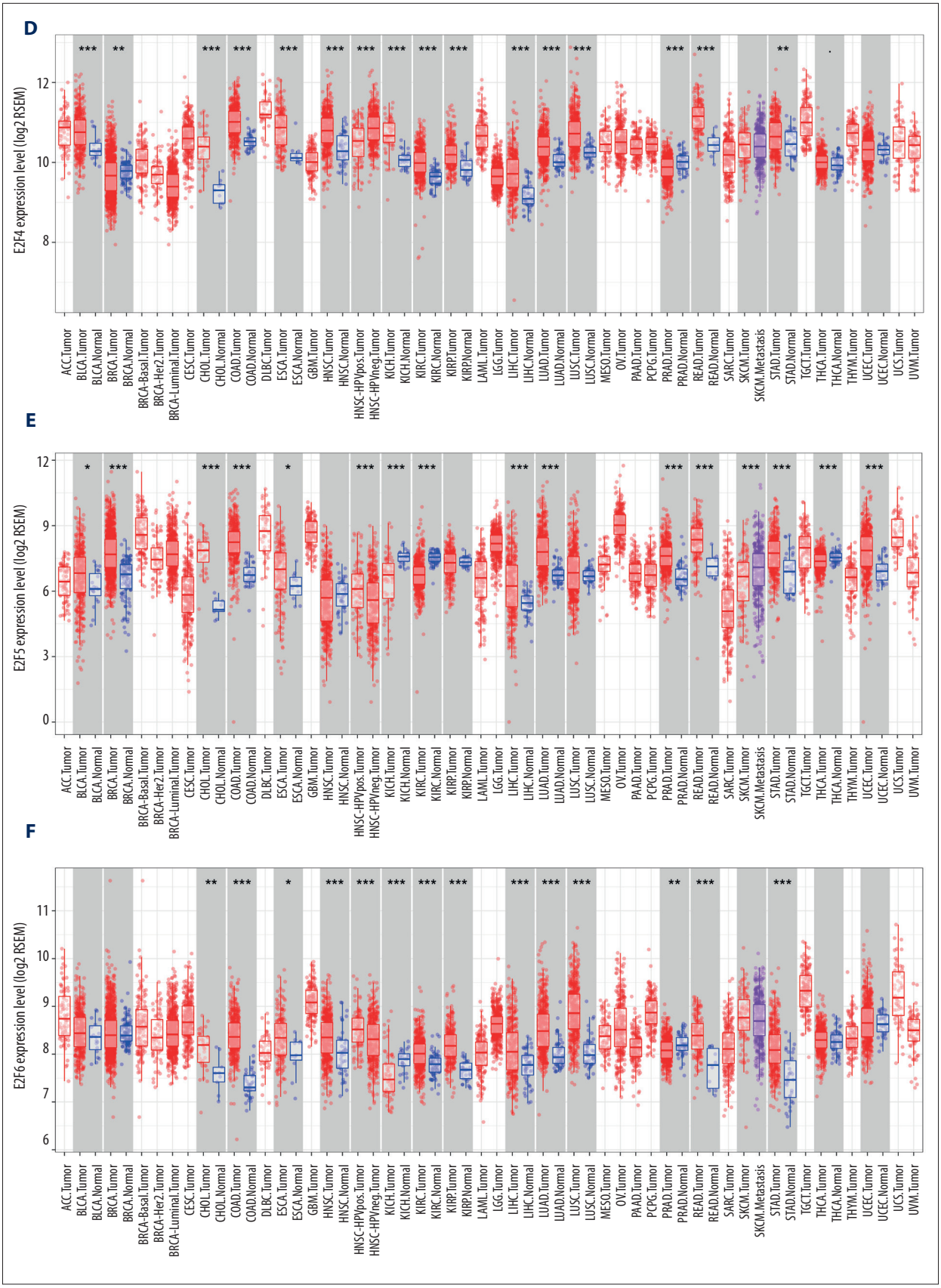
D
E2F4 expression level (log2 RSEM)
E
E2F5 expression level (log2 RSEM)
F
E2F6 expression level (log2 RSEM)
ACC.Tumor
BLCA.Tumor
BLCA.Normal
BRCA.Tumor
BRCA.Normal
BRCA-Basal.Tumor
BRCA-Her2.Tumor
BRCA-Luminal.Tumor
CESC.Tumor
CHOL.Tumor
CHOL.Normal
COAD.Tumor
COAD.Normal
DLBC.Tumor
ESCA.Tumor
ESCA.Normal
GBM.Tumor
HNSC.Tumor
HNSC.Normal
HNSC-HPVpos.Tumor
HNSC-HPVneg.Tumor
KICH.Tumor
KICH.Normal
KIRC.Tumor
KIRC.Normal
KIRP.Tumor
KIRP.Normal
LAML.Tumor
LGG.Tumor
LIHC.Tumor
LIHC.Normal
LUAD.Tumor
LUAD.Normal
LUSC.Tumor
LUSC.Normal
MESO.Tumor
OV.Tumor
PAAD.Tumor
PCPG.Tumor
PRAD.Tumor
PRAD.Normal
READ.Tumor
READ.Normal
SARC.Tumor
SKCM.Tumor
SKCM.Metastasis
STAD.Tumor
STAD.Normal
TGCT.Tumor
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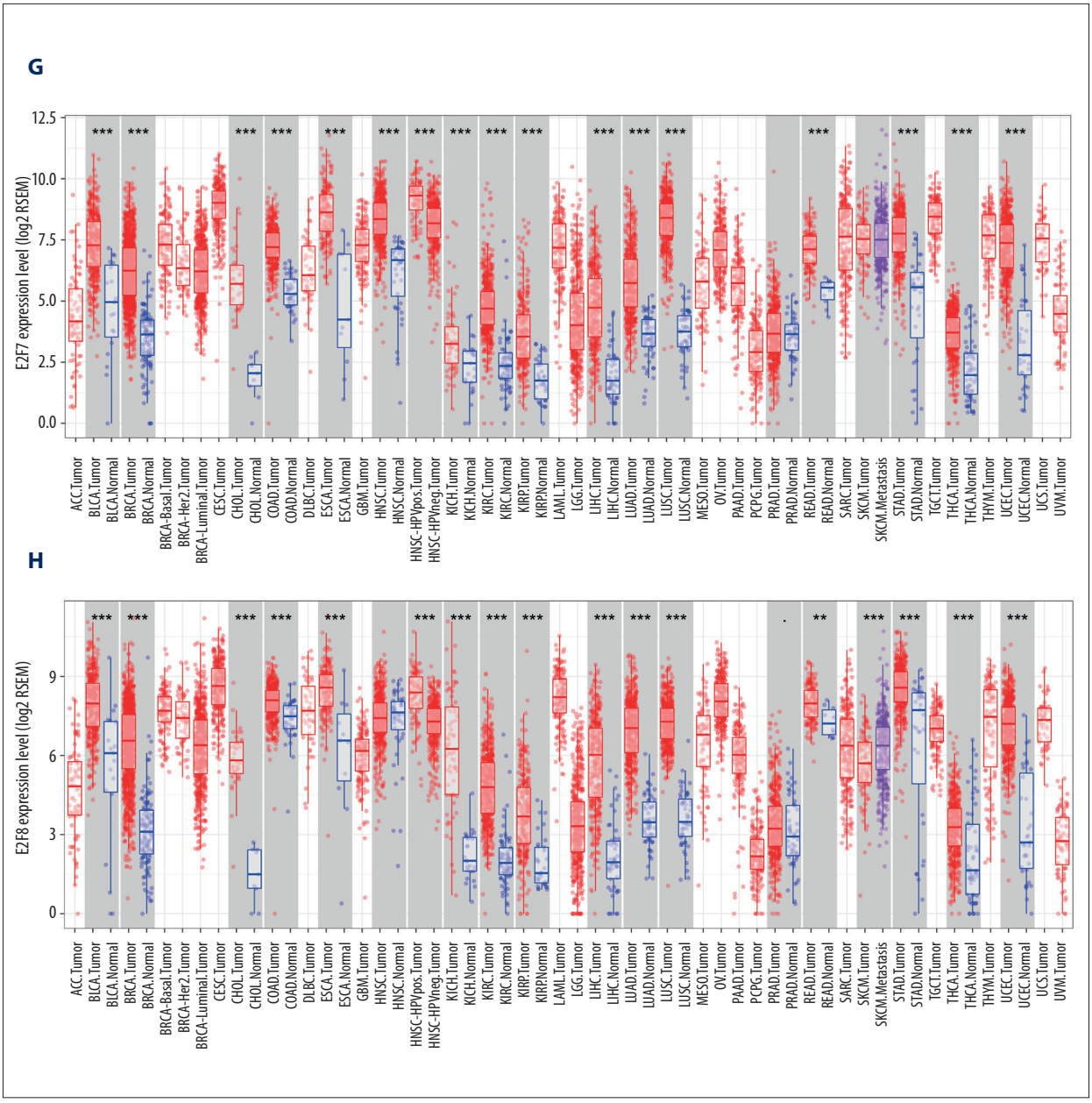
THYM.Tumor
UCEC.Tumor
UCEC.Normal
UCS.Tumor
UVM.Tumor



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I

Analysis type by cancer	Cancer vs Normal E2F1		Cancer vs Normal E2F2		Cancer vs Normal E2F3		Cancer vs Normal E2F4		Cancer vs Normal E2F5		Cancer vs Normal E2F6		Cancer vs Normal E2F7		Cancer vs Normal E2F8	
Bladder cancer	3	2	2	3	8		2	3	5	5	2		2		6	
Brain and CNS cancer	2	12	5	7	10	3	2	11	24	3	3	1	5	3	8	1
Breast cancer	22	1	23	5	18	2	9	13	21	2	10	12	20	5	20	1
Cervical cancer	5		2	2	5		2	2	1	2	1	1	2	1	4	
Colorectal cancer	20	1	2	22	21		15	4	25	7	7		20	1	16	
Esophegal cancer	6	2		3	8		2	2	6	1	2		1	1	1	3
Gastric cancer	10		2	1	12		2	2	6	1	3	1	7	1	3	
Head and neck cancer	13	2	1	9	17	1	6	15	12	9	1		9	5	5	2
Kidney cancer	4	5	4	3	7	1	8	3	6	5			1	2	4	4
Leukemia	9	11	2	9	7	10	9	12	14	10		2	2	4	3	13
Liver cancer	3	2	3	3	8			5	4	3			1	1	6	
Lung cancer	14	4	10	4	18		16	4	15		5		5		12	
Lymphoma	19	1	9	3	13	7	19	6	18	19	4	2	5	8	4	2
Melanoma	1	2	1		3		3		2	1			1			
Myeloma	2	3	2	1	4	1	2	2	2	2					1	
Other cancer	13	6	8	3	15	8	2	5	10	6	2		9	7	6	3
Ovarian cancer	8		5		11	1	10		11	1	2	1	4	1	11	
Pancreatic cancer	1	3		2	6	1		4	3	2			4	3	5	
Prostate cancer	3	3	5	3	10	1	3	6	16		1	2	1		5	2
Sarcoma	12	1	2	5	11		1	7	5	8	1				10	2
Significant unique analyses	169	61	88	87	211	34	111	105	206	86	44	22	99	43	128	32
Total unique analyses	355		309		344		361		363		112		162		282	

Figure 1. Expression levels of the E2F family of proteins in different cancers. (**A-H**) E2F family TF expression levels in different cancer types and corresponding normal tissues as assessed with the TIMER database. (**I**) The transcriptional expression of E2F family members in different cancers and corresponding normal tissues as assessed with the ONCOMINE database.

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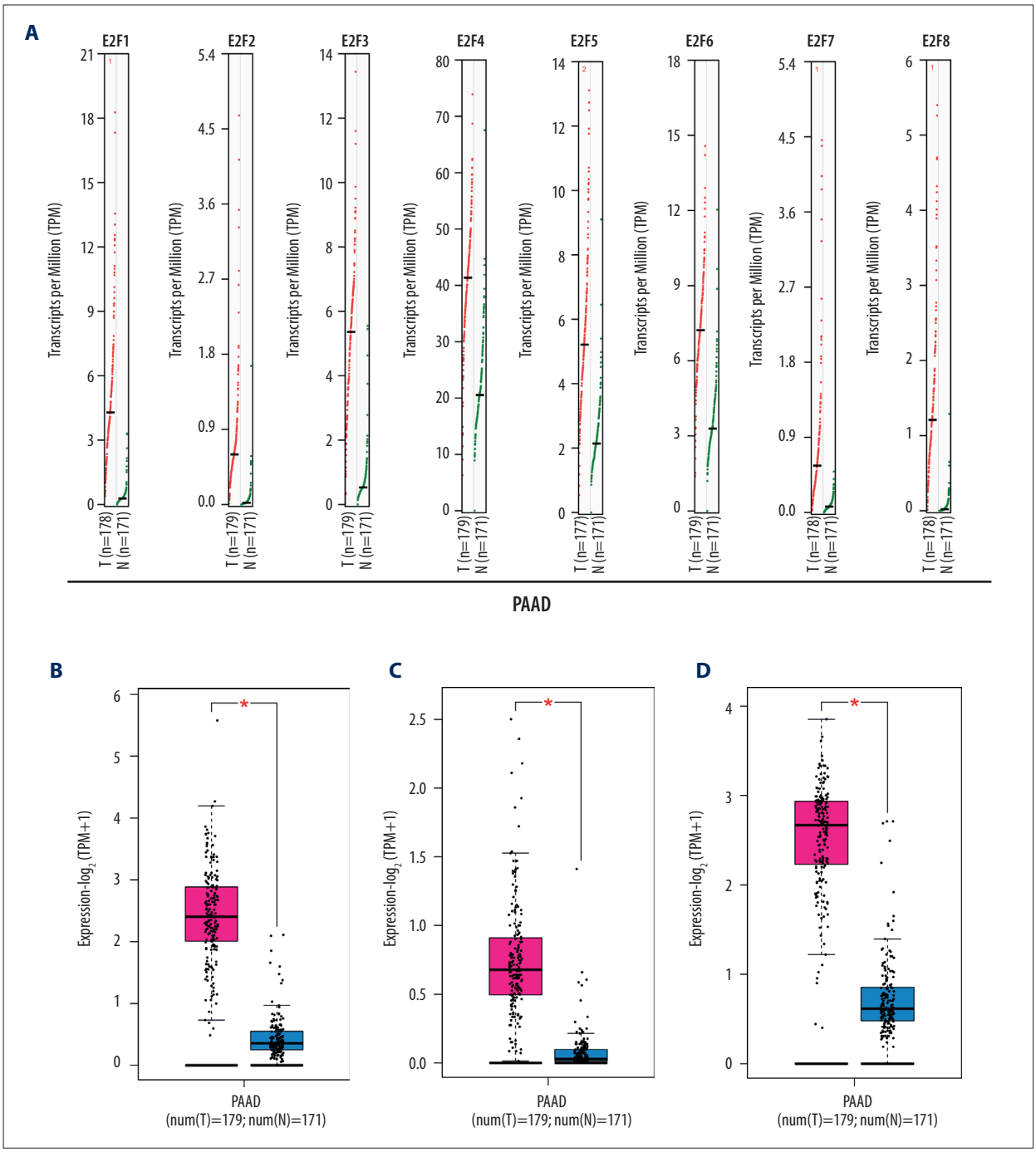
A
E2F1
E2F2
E2F3
E2F4
E2F5
E2F6
E2F7
E2F8
Transcripts per Million (TPM)
T (n=178)
N (n=171)
T (n=179)
N (n=171)
T (n=177)
N (n=171)
PAAD
B
C
D
Expression-log2 (TPM+1)
PAAD
(num(T)=179; num(N)=171)

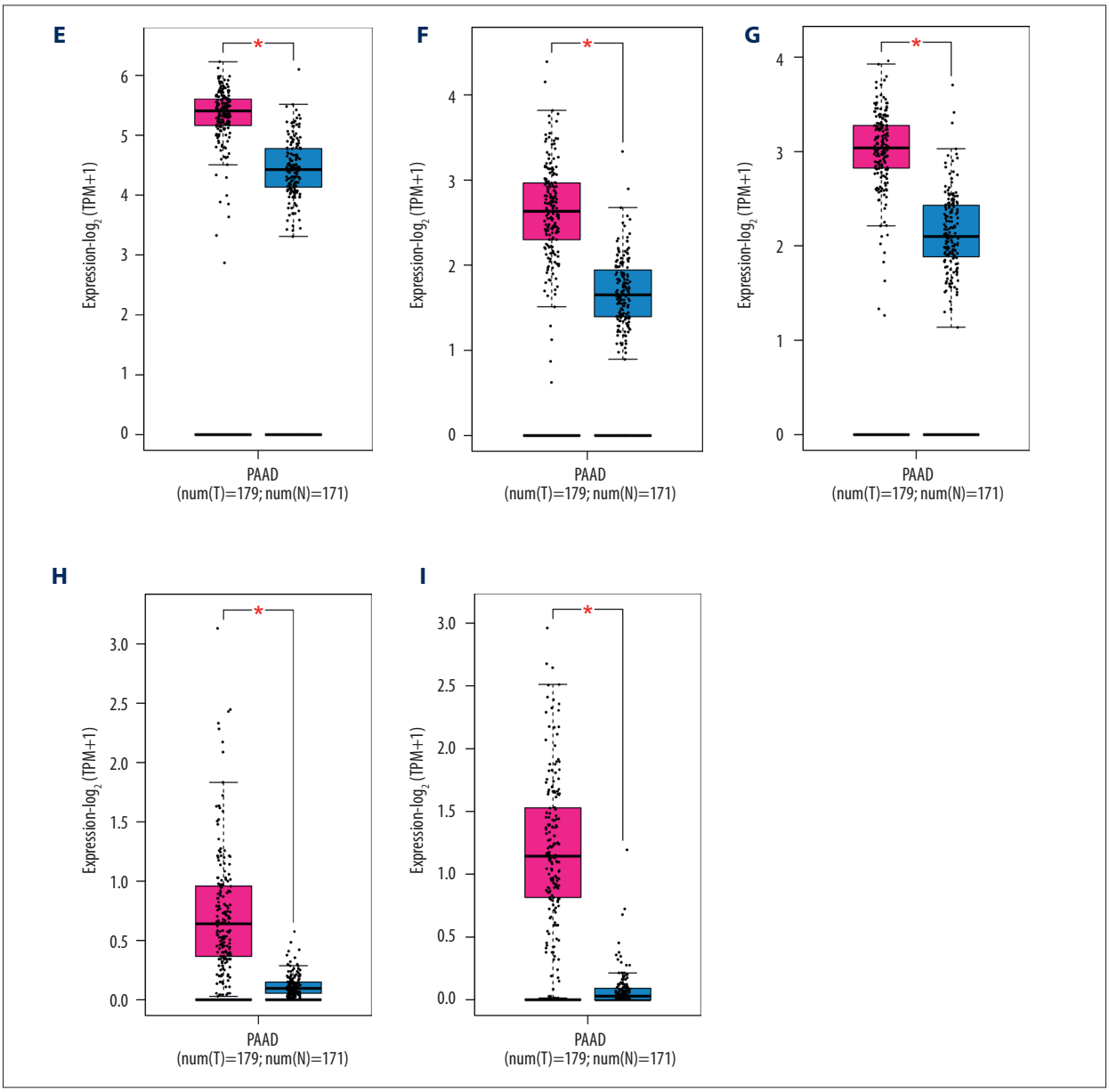

Figure 2. GEPIA-based assessment of E2F protein expression levels in PAAD. (**A**) Differential E2F protein expression in PAAD and normal tissue samples was assessed using the GEPIA database. (**B-I**) Box plots were used to demonstrate differences in E2F expression between PAAD and normal tissues in the GEPIA database.

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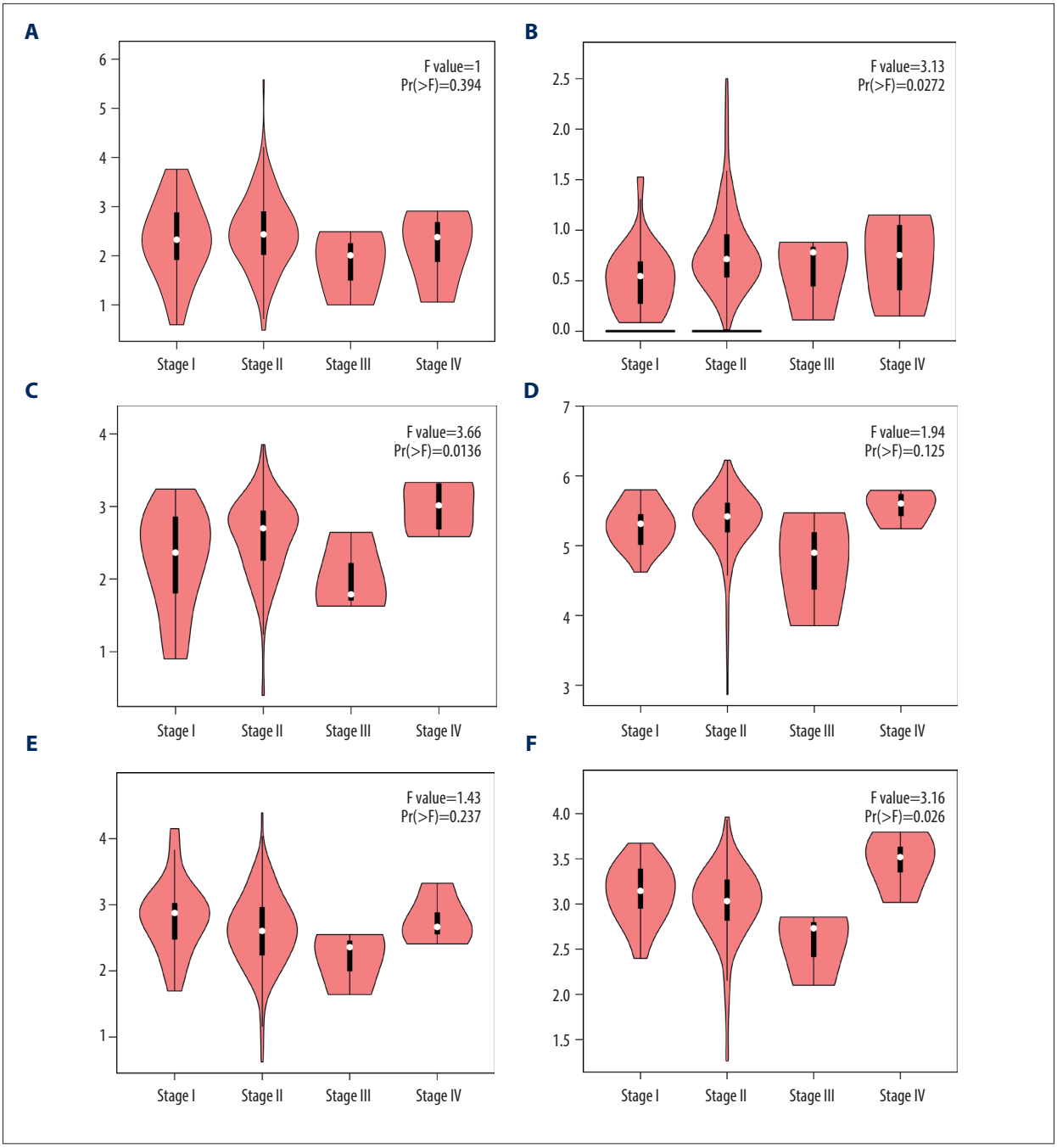

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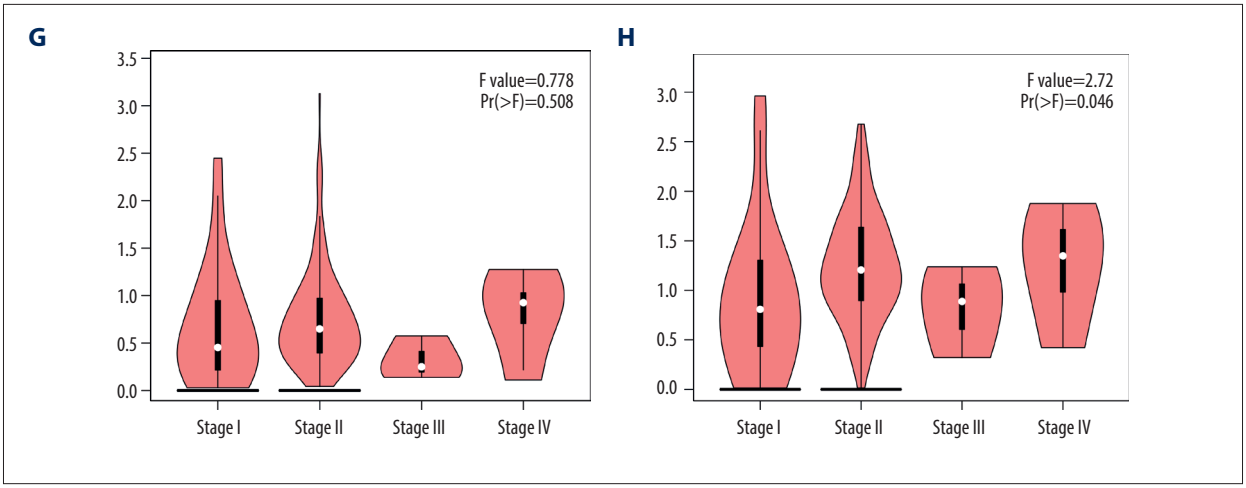

Figure 3. (**A-H**) Correlation between the expression of E2F family proteins and PAAD tumor stage as assessed using GEPIA.

Protein–Protein Interaction (PPI) Network and Path Analyses

The STRING database (v 10.5; https://string-db.org/) was employed to generate a PPI network for E2F family proteins [18], with 30 putative E2F-interacting proteins additionally being identified. The FUNRICH website was used to assess the potential functions of the genes included in the above PPI networks, with the Cytoscape ClueGo plug-in being used to conduct GO and KEGG enrichment analyses of these genes. $P<0.05$ served as the significance threshold for these analyses.

GSCALite Analysis

The interactive GSCALite tool (http://bioinfo.life.hust.edu.cn/web/GSCALite/) enables users to conduct genomic analyses of particular cancers and to visualize correlations, gene expression data, and related information in a flexible manner [19]; available reporting information included differential gene expression, OS, CNVs, single-nucleotide variations, normal tissue expression levels, miRNA regulation, methylation, and drug sensitivity data. Herein, this tool was utilized to examine relationships between E2F family members and cancer-related pathways and to assess how these relationships were associated with tumor sensitivity to a range of anticancer drugs. PAAD patient OS and DFS were also analyzed with the Oncolnc website (http://www.oncolnc.org/).

StarBase Analysis

The starBase database (http://starbase.sysu.edu.cn/agoClipRNA.php?source=lncRNA&flag) [20] was employed to assess miRNA/lncRNA regulatory relationships in PAAD, with the results of these analyses then used to construct ceRNA networks and to perform coexpression analyses. Differentially expressed lncRNAs and mRNAs associated with PAAD were submitted to starBase, and Kaplan-Meier analyses with log-rank tests were used to assess the prognostic relationship between these lncRNAs, associated mRNAs, and PAAD patient OS, with $P<0.05$ and $|\log2FC| > 0.5$ as the significance criteria.

Human Protein Atlas (HPA)

Images of immunohistochemistry (IHC) staining for PAAD and normal tissues were collected from the HPA (https://www.proteinatlas.org/), which applies transcriptomics and proteomics to provide different atlases based on tissue type, cell type, and pathology.

Results

Assessment of E2F Family Gene Expression in PAAD Patients

To explore the differential expression of the 8 known E2F family TFs in PAAD patients, we used the TIMER database to compare relative expression levels of E2F1-8 in normal tissues and PAAD tumors using the TCGA dataset (**Figure 1A-1H**). This analysis revealed that all 8 of these E2F family members were upregulated in certain tumors, with E2F3 being significantly upregulated in pancreatic cancer patients in 6 datasets, E2F7 and E2F8 significantly upregulated in pancreatic cancer patients in 4 and 5 datasets, respectively, and E2F1 and E2F5 upregulated in pancreatic cancer patients (**Figure 1I**). IHC of E2F expression in PAAD was studied using the HPA (**Supplementary Figure 1**). The protein levels of E2F1 (**Supplementary Figure 1A**) were increased in PAAD tissues compared with paracancerous tissues (**Supplementary Figure 1B**).

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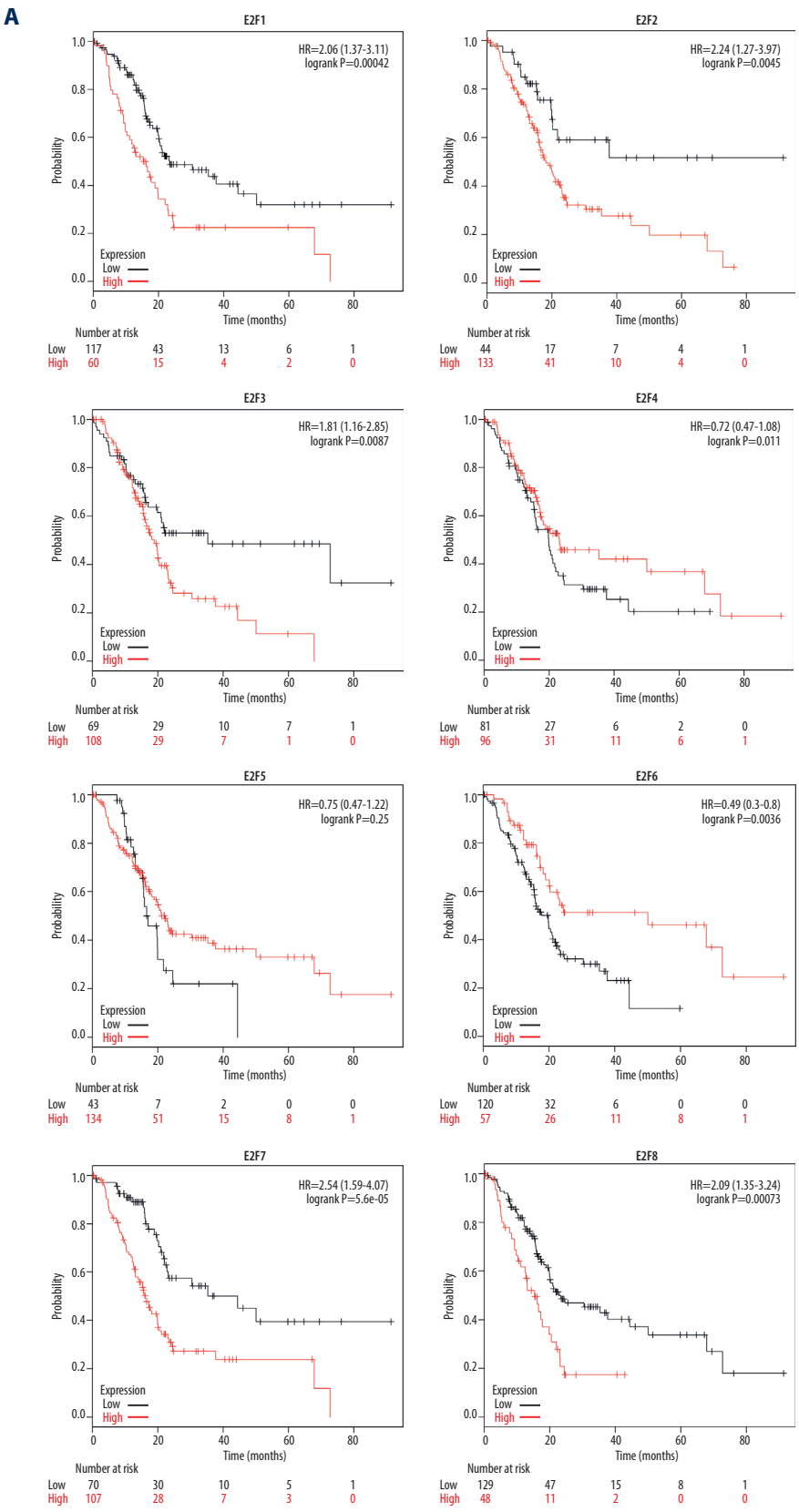

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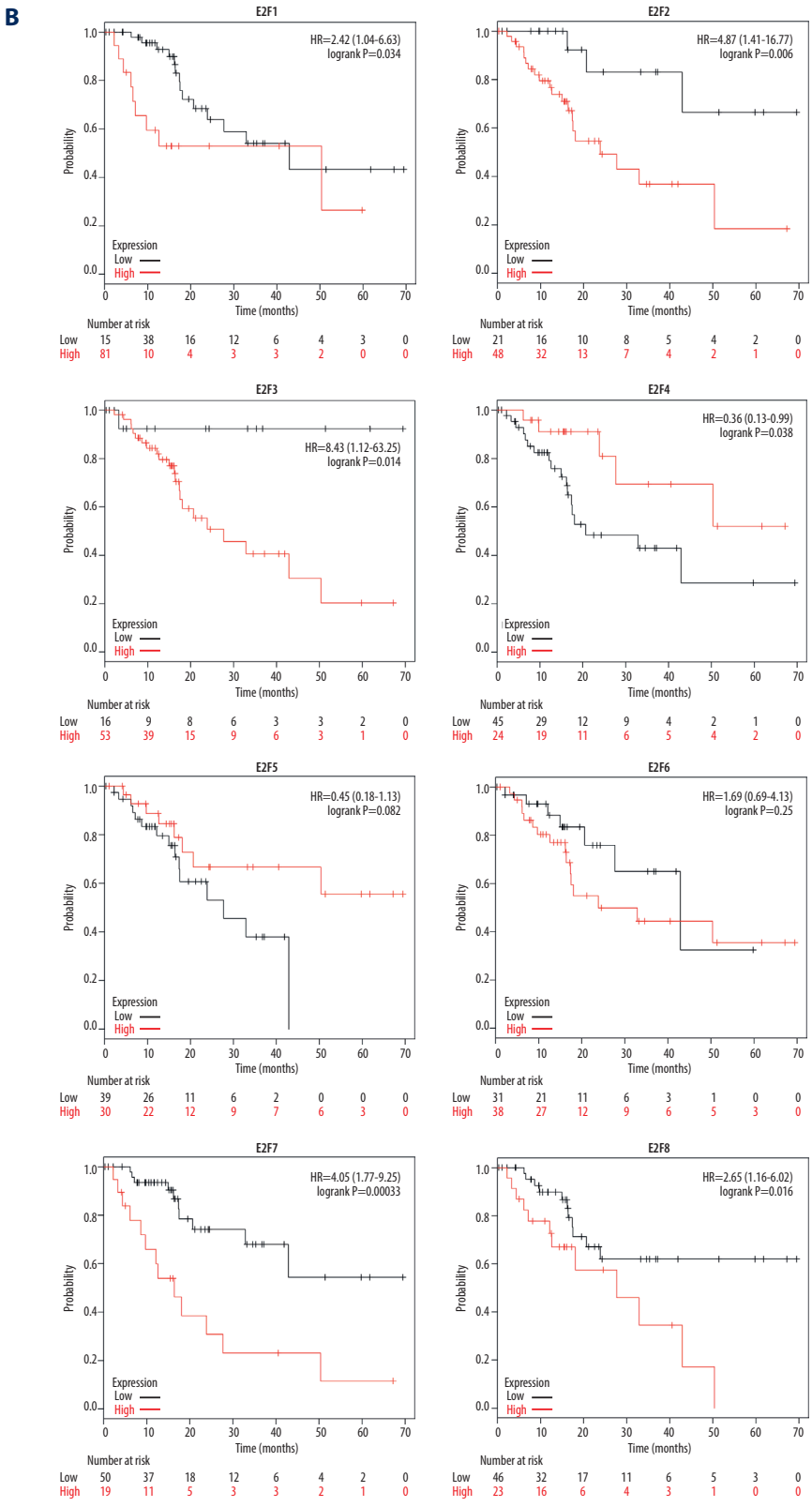

Figure 4. (**A, B**) Evaluation of the prognostic relevance of E2F family TF mRNA expression levels in PAAD patients, as assessed using the Kaplan-Meier Plotter tool.

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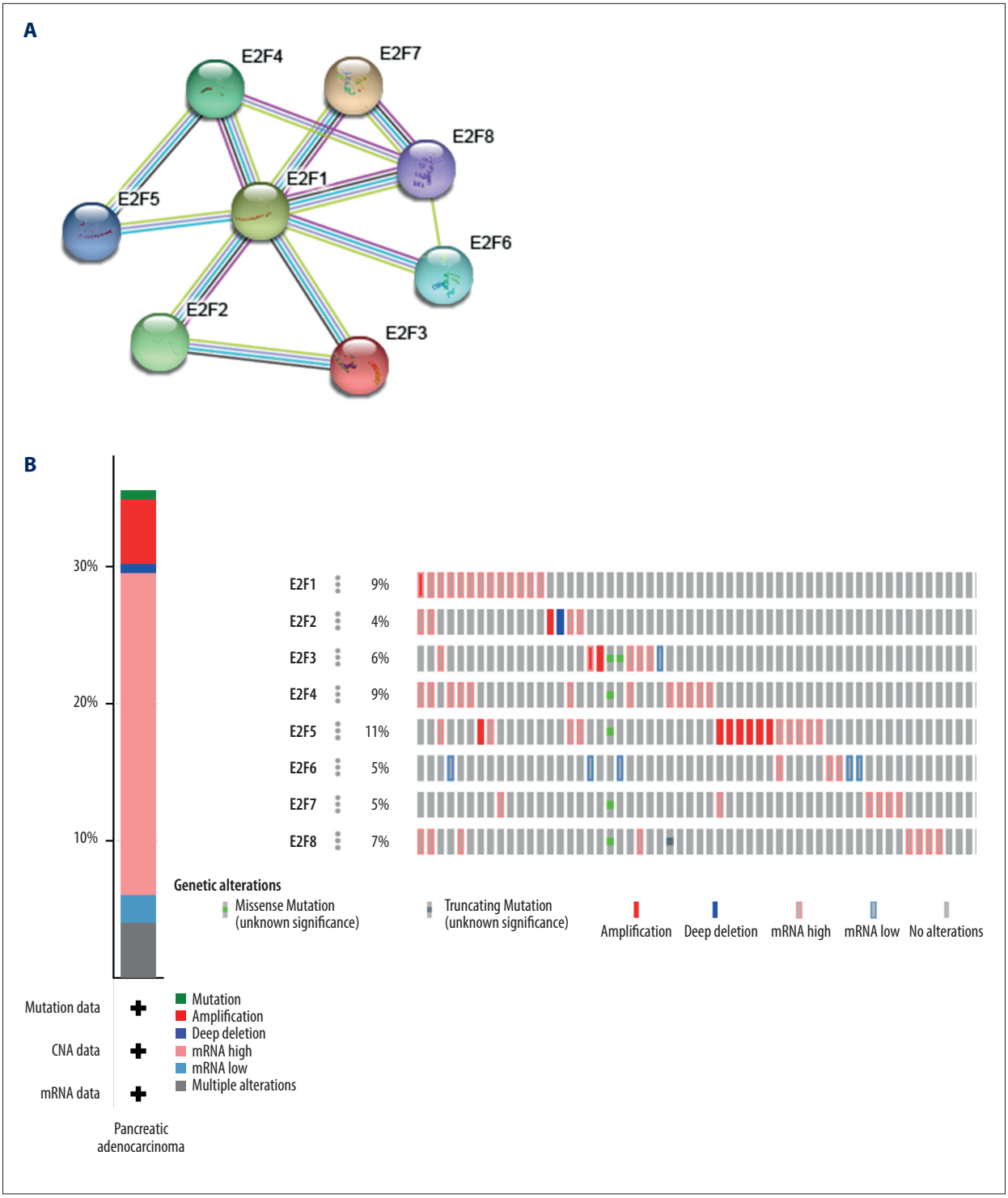

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C

	E2F1	E2F2	E2F3	E2F4	E2F5	E2F6	E2F7	E2F8
E2F1	1	0.62	0.12	0.28	0.11	0.052	0.42	0.4
E2F2	0.62	1	0.18	0.38	0.25	0.092	0.15	0.55
E2F3	0.12	0.18	1	0.53	0.33	0.49	0.36	0.36
E2F4	0.28	0.38	0.53	1	0.36	0.5	0.26	0.47
E2F5	0.11	0.25	0.33	0.36	1	0.54	0.16	0.27
E2F6	0.052	0.092	0.49	0.5	0.54	1	0.14	0.2
E2F7	0.42	0.15	0.36	0.26	0.16	0.14	1	0.29
E2F8	0.4	0.55	0.36	0.47	0.27	0.2	0.29	1

D

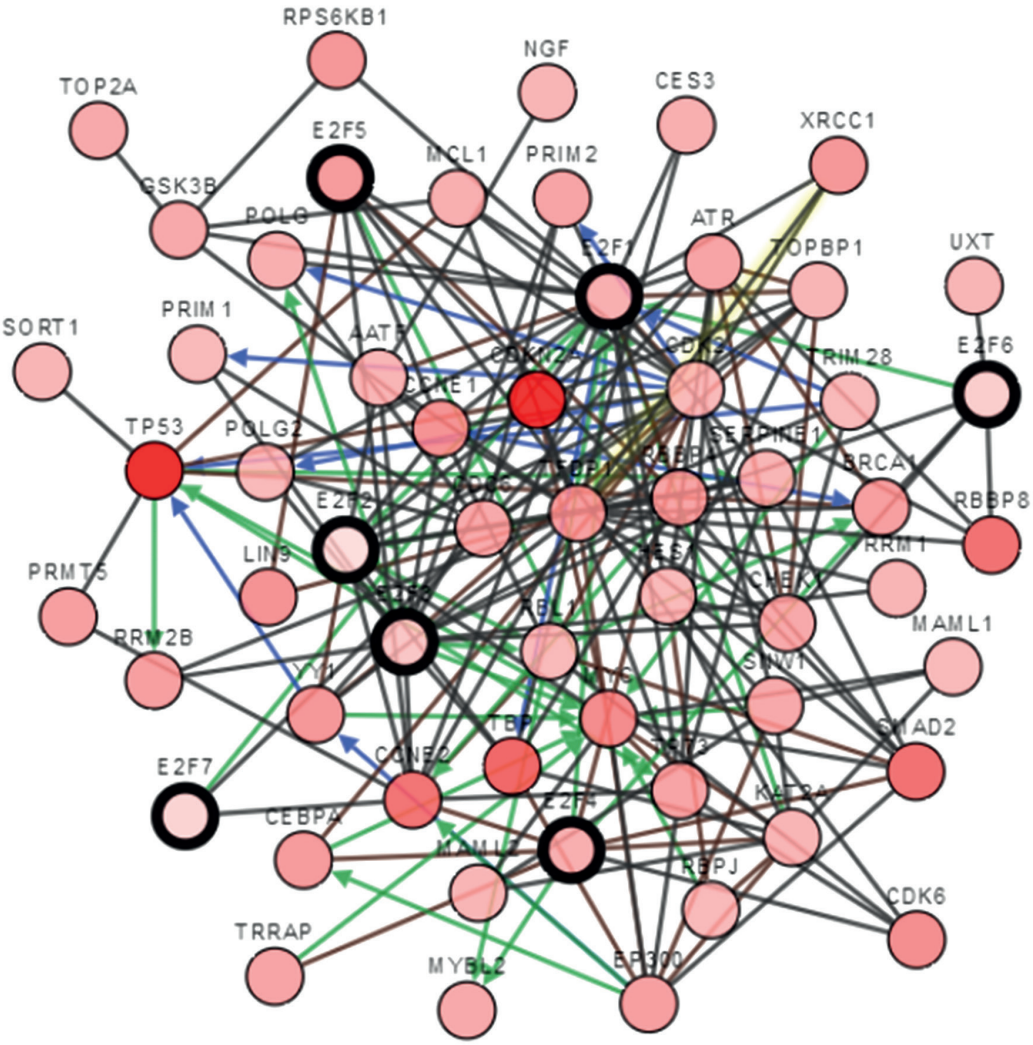

Figure 5. Functional analysis of E2F family members. (**A**) A PPI network of E2F family TFs expressed in PAAD was constructed with the STRING tool. (**B**) An analysis of E2F family protein expression and mutations in PAAD patients was conducted using cBioPortal. (**C**) The GEPIA database was used to conduct an E2F family member coexpression analysis in PAAD. (**D**) An E2F pathway analysis was performed using cBioPortal.

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Associations Between E2F Gene Expression and the Clinicopathological Characteristics of PAAD Patients

Next, we explored the differences in E2F expression levels in PAAD and normal tissues using the GEPIA dataset, revealing that all 8 E2F family members (E2F1-8) were expressed at significantly higher levels in PAAD tissues relative to normal pancreatic tissues (**Figure 2**). We additionally examined the association between these E2F family members and tumor stage, revealing that E2F2/3/6/8 expression levels were significantly associated with tumor stage, whereas E2F1/4/5/7 expression levels were not (**Figure 3**).

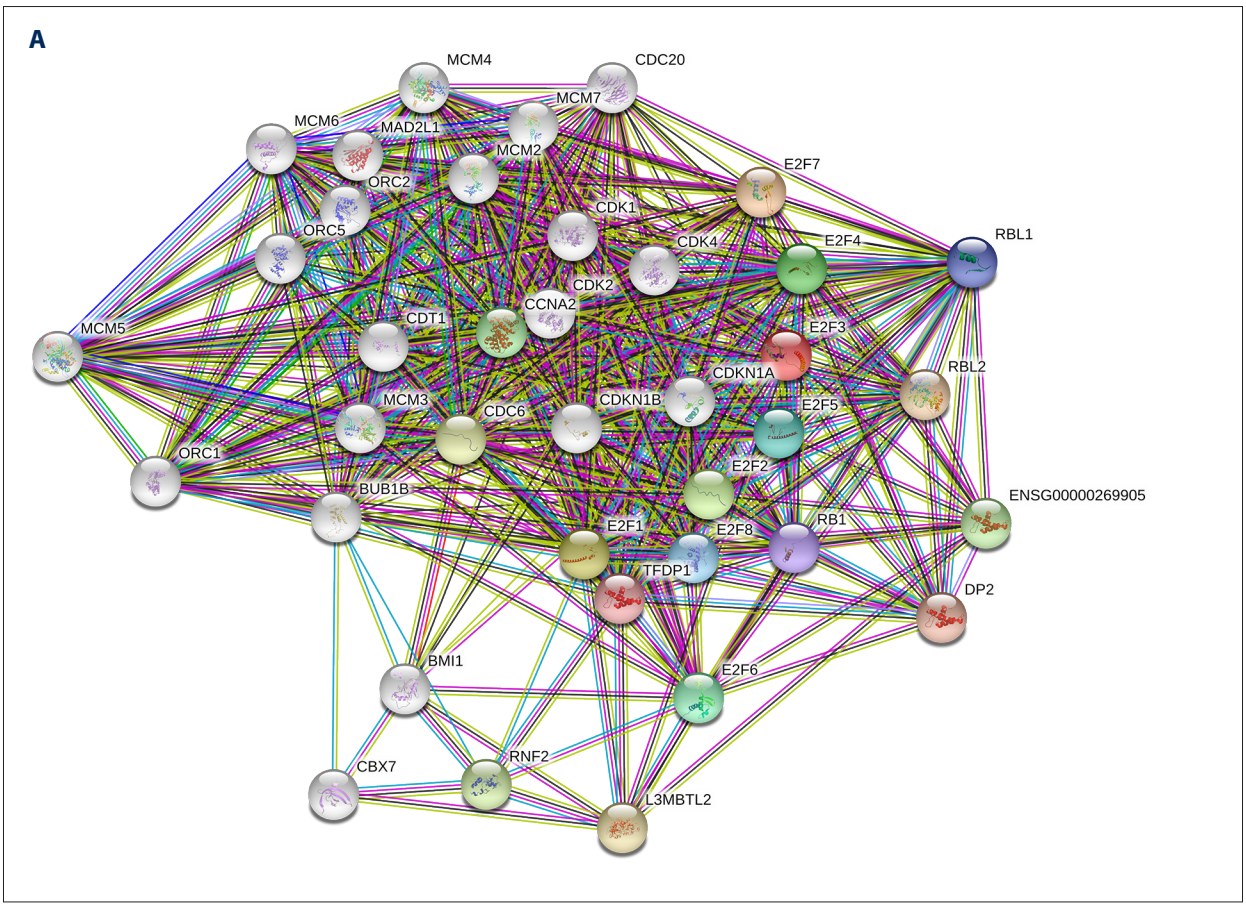
Upregulation of E2F1/2/3/7/8 and Downregulation of E2F4/5 are Associated with Better PAAD Patient Prognosis

We then examined the association between E2F expression levels and PAAD patient survival using the Kaplan-Meier Plotter tool and corresponding log-rank tests, which indicated that higher levels of E2F1/2/3/7/8 and lower levels of E2F6 expression were significantly associated with OS ($P<0.05$) (**Figure 4A**), while increased E2F1/2/3/7/8 mRNA levels and decreased E2F4 mRNA levels were significantly related to DFS ($P<0.05$) (**Figure 4B**). Specifically, higher E2F1/2/3/7/8 levels were associated with poorer OS and DFS, whereas lower E2F4 and E2F6 levels were associated with poorer DFS and poorer OS, respectively.

Predicted Functional Roles of E2F Family TFs and Related Genes in PAAD Patients

We next constructed PPI networks using the STRING tool to evaluate potential interacting proteins with E2F family members (**Figure 5A**). In addition, alterations in E2F proteins were assessed with the cBioPortal tool by analyzing 186 PAAD samples (TCGA, Provisional; **Figure 5B**) [16]. The GEPIA tool was then used to conduct analyses of E2F family TFs following Pearson's correlation. The following significant positive correlations were identified through this analysis: E2F1 with E2F2, E2F7, and E2F8; E2F2 with E2F1 and E2F8; E2F3 with E2F4 and E2F6; E2F4 with E2F3, E2F6, and E2F8; E2F5 with E2F6; E2F6 with E2F3, E2F4, and E2F5; E2F7 with E2F1; and E2F8 with E2F1, E2F2, and E2F4 (**Figure 5C**). A pathway analysis of these E2F family members was then conducted with the cBioPortal tool (**Figure 5D**).

We then expanded these analyses by constructing PPI networks incorporating 30 E2F-related proteins (**Figure 6A, Table 1**).



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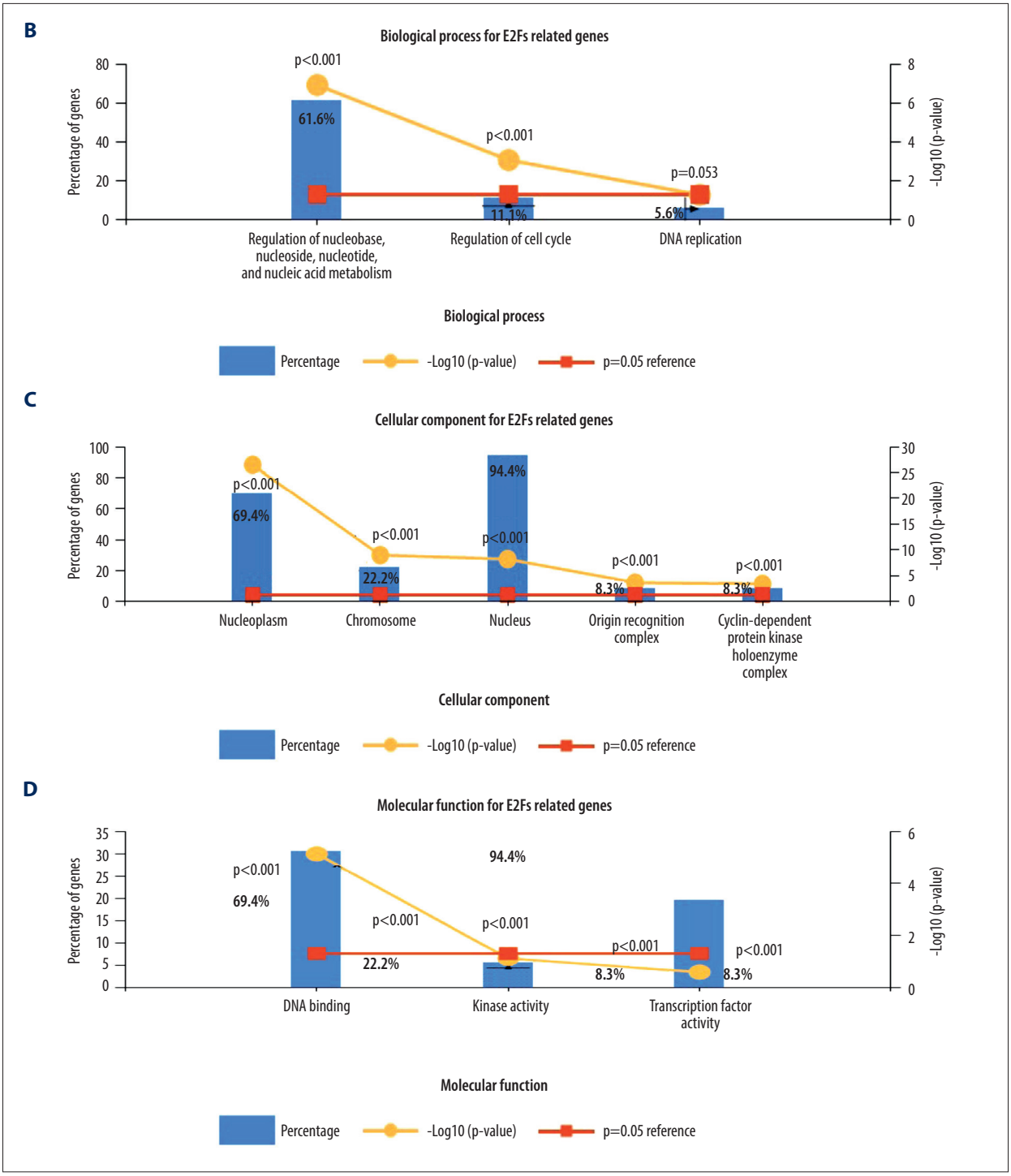

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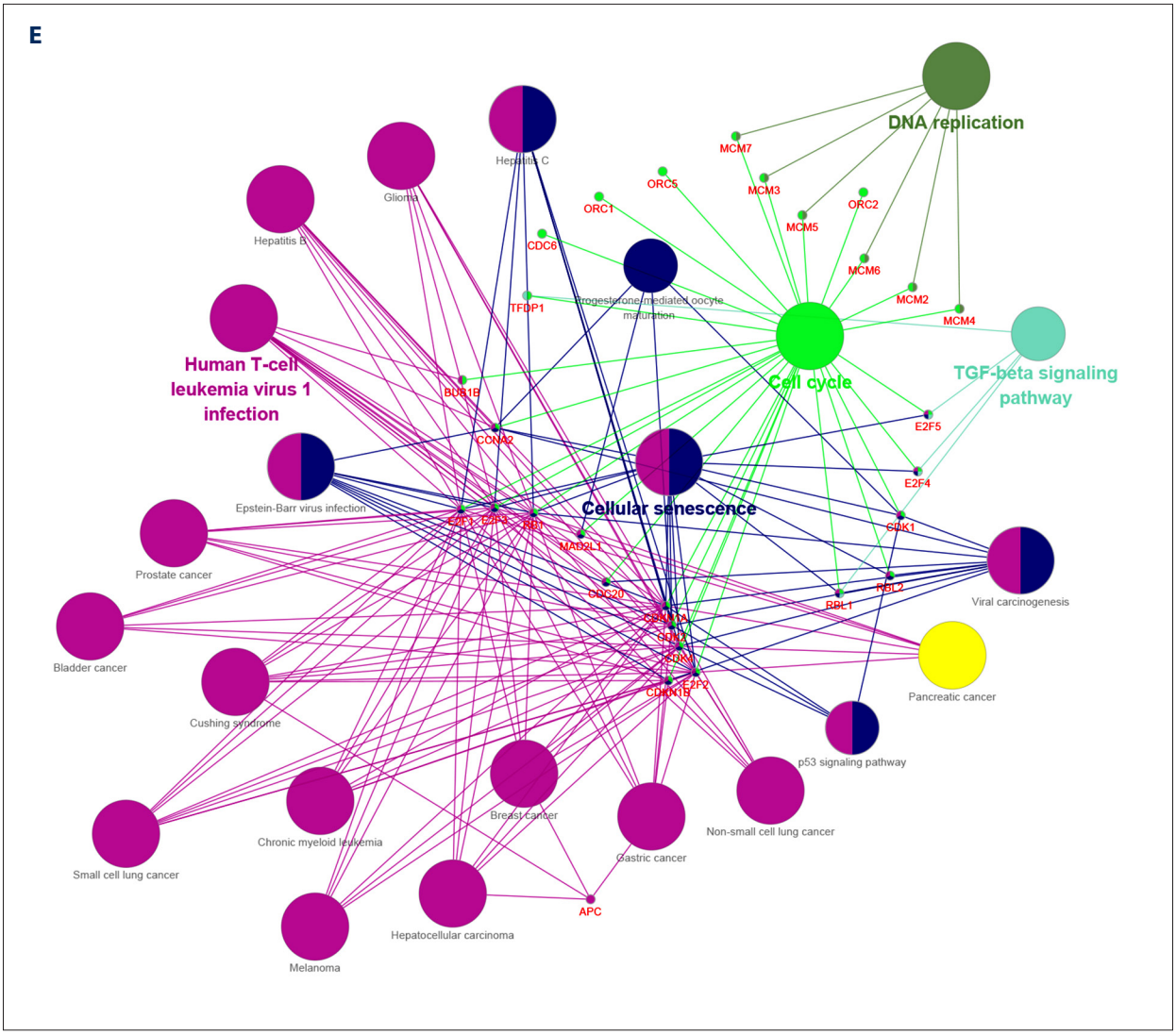

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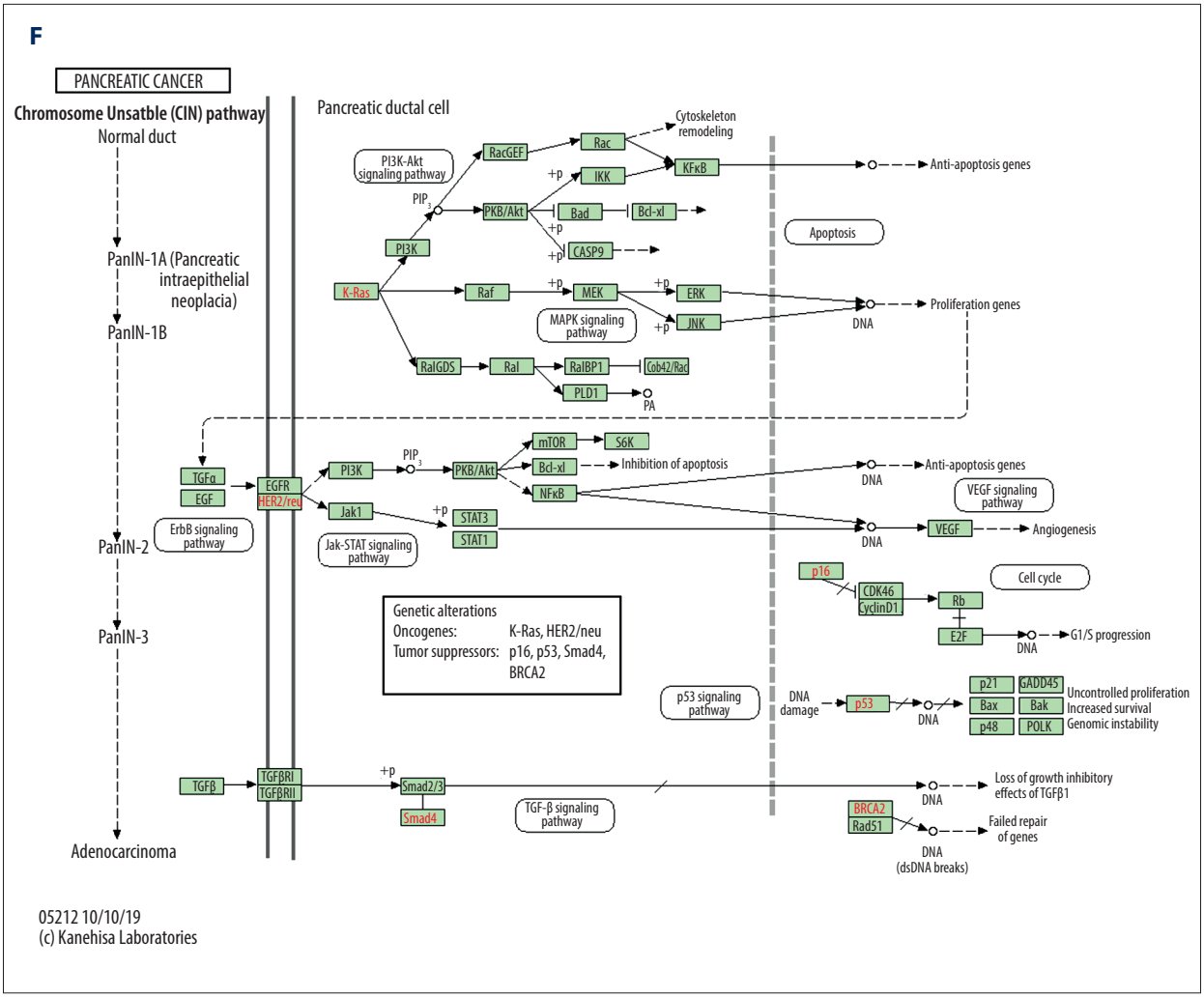

Figure 6. Functional analyses of E2F-related proteins in PAAD. (**A**) E2Fs and the top 30 E2F-related interacting proteins were incorporated into a PPI network generated using the STRING tool. (**B-D**) GO enrichment analyses for these 38 E2F family members and related proteins were conducted with the FUNRICH tool. (**E**) KEGG pathway enrichment analyses for these 38 E2F family members and related proteins were conducted using the ClueGo plug-in in Cytoscape software. (**F**) KEGG pathways regulated by E2F family members.

Table 1. The top 30 E2F-related proteins identified using the STRING tool.

E2Fs related genes	Genes name
E2Fs family genes (n=8)	E2F1, E2F2, E2F3, E2F4, E2F5, E2F6, E2F7, E2F8
Non-E2Fs family genes (n=30)	MCM3, CDT1, CCNA2, TFDP1, L3MBTL2, RNF2, ORC2, CDK1, BUB1B, BMI1, ORC1, MCM5, RBL1, CDK4, ENSG00000269905, ORC5, CDKN1B, CDC20, CDK2, CBX7, CDC6, MCM7, DP2, MCM4, CDKN1A, MCM6, RB1, MCM2, RBL2, MAD2L1

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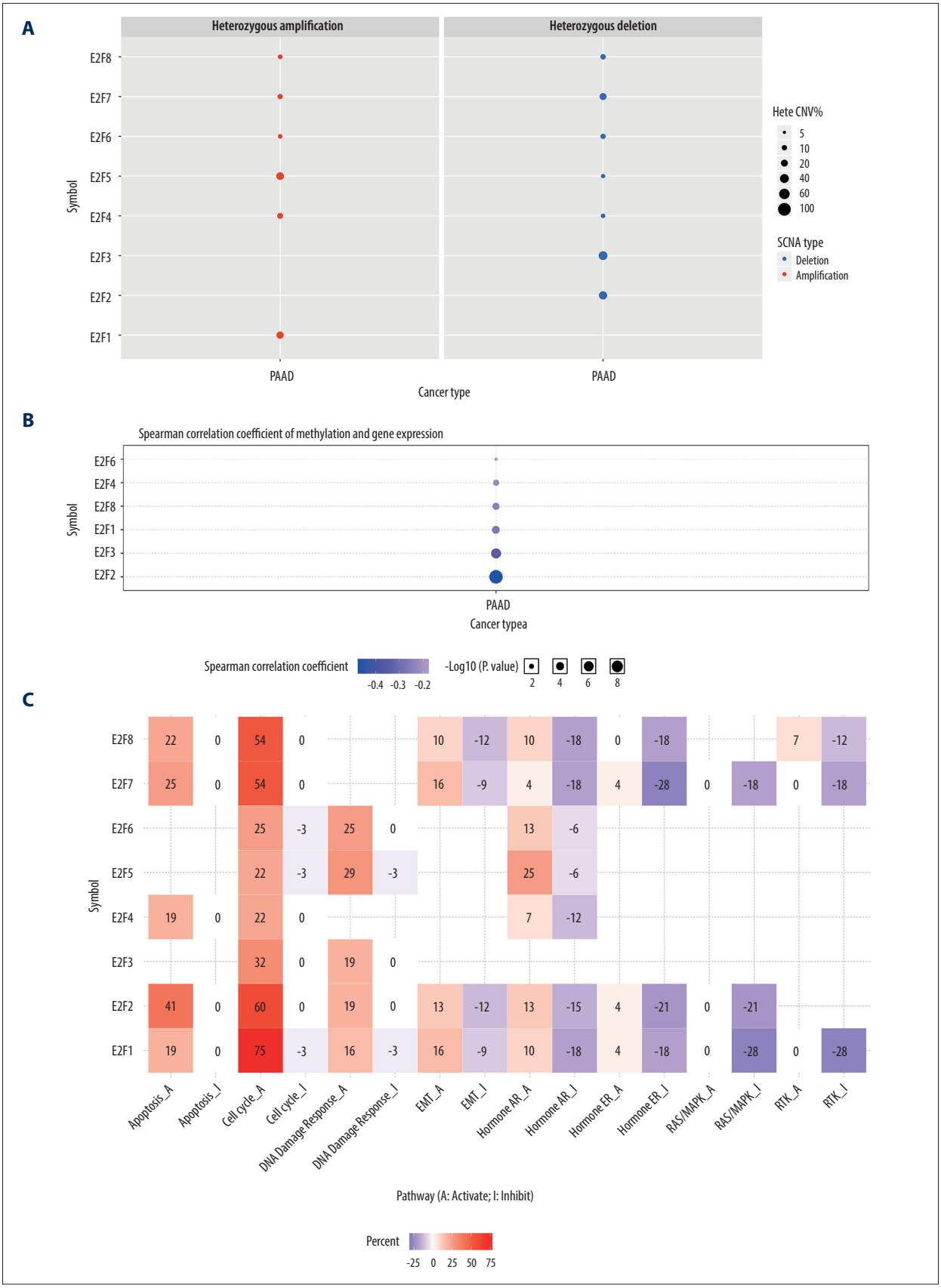

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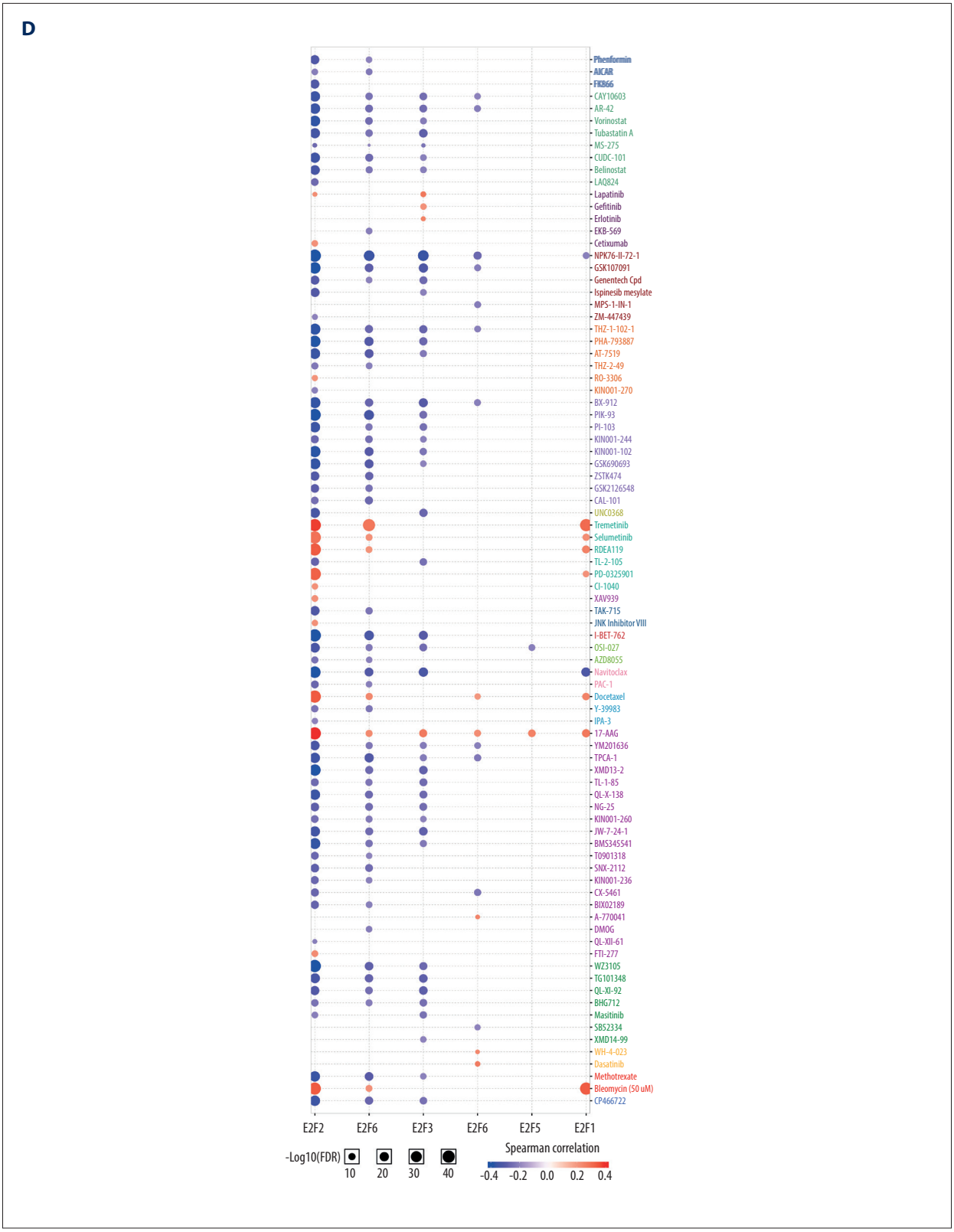

Figure 7. Mutations and functions of E2F family members as analyzed using GSCALite. (**A**) CNV analysis of E2F family members. (**B**) Correlations between the methylation of E2F family members and their expression in PAAD. (**C**) E2F family member-related pathway activity in PAAD. (**D**) The role of E2F family members in the context of drug sensitivity.

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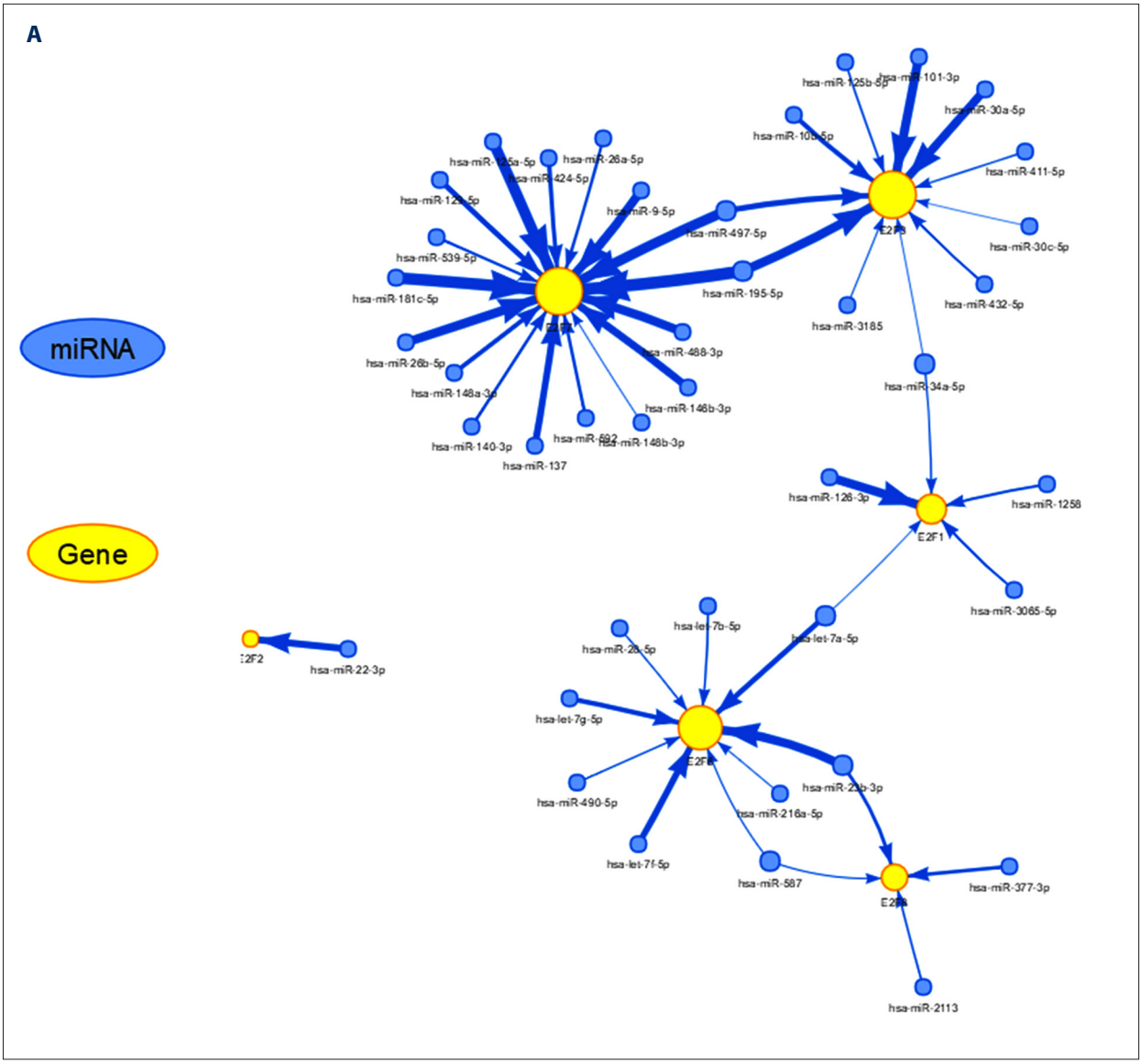

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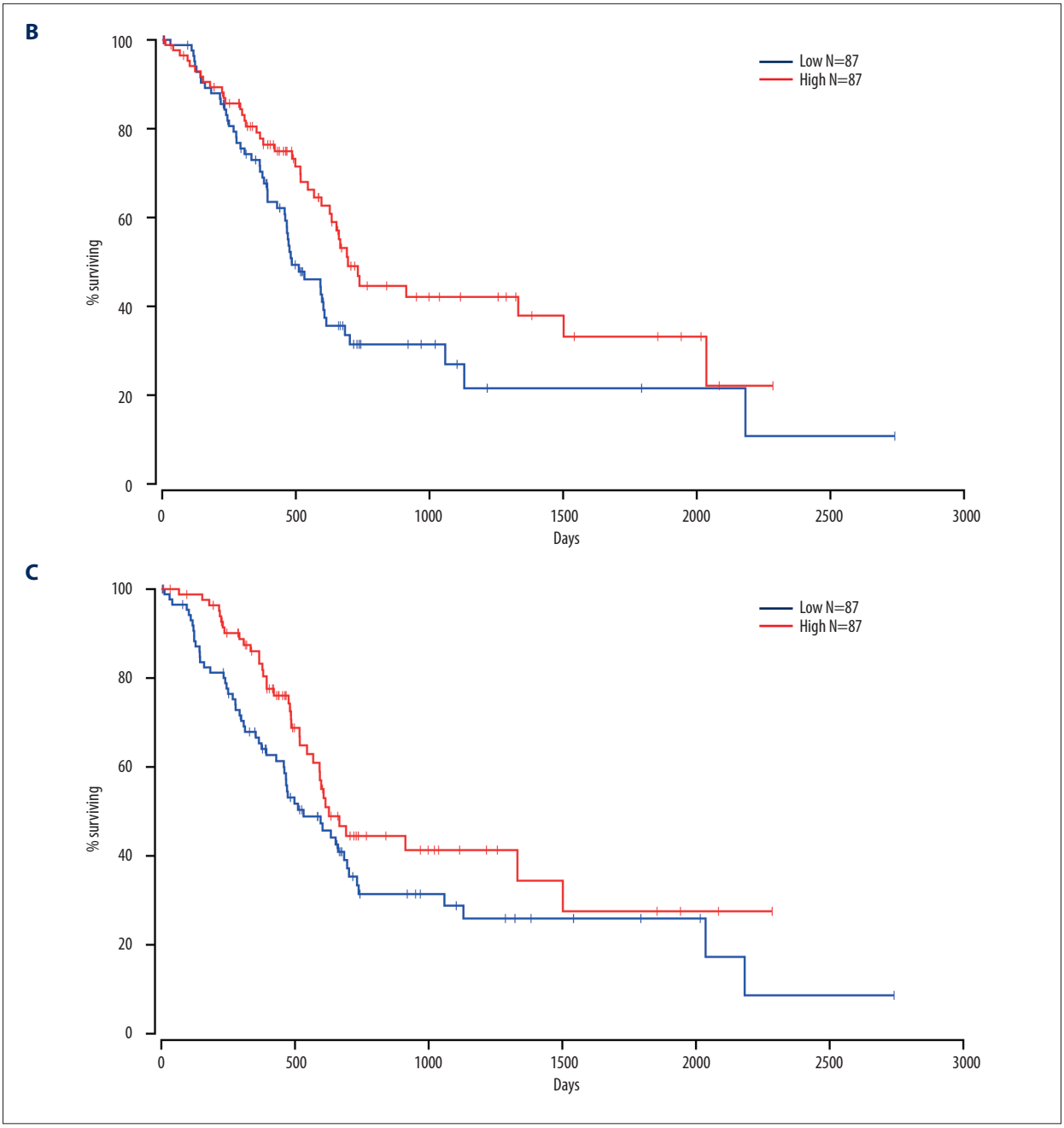

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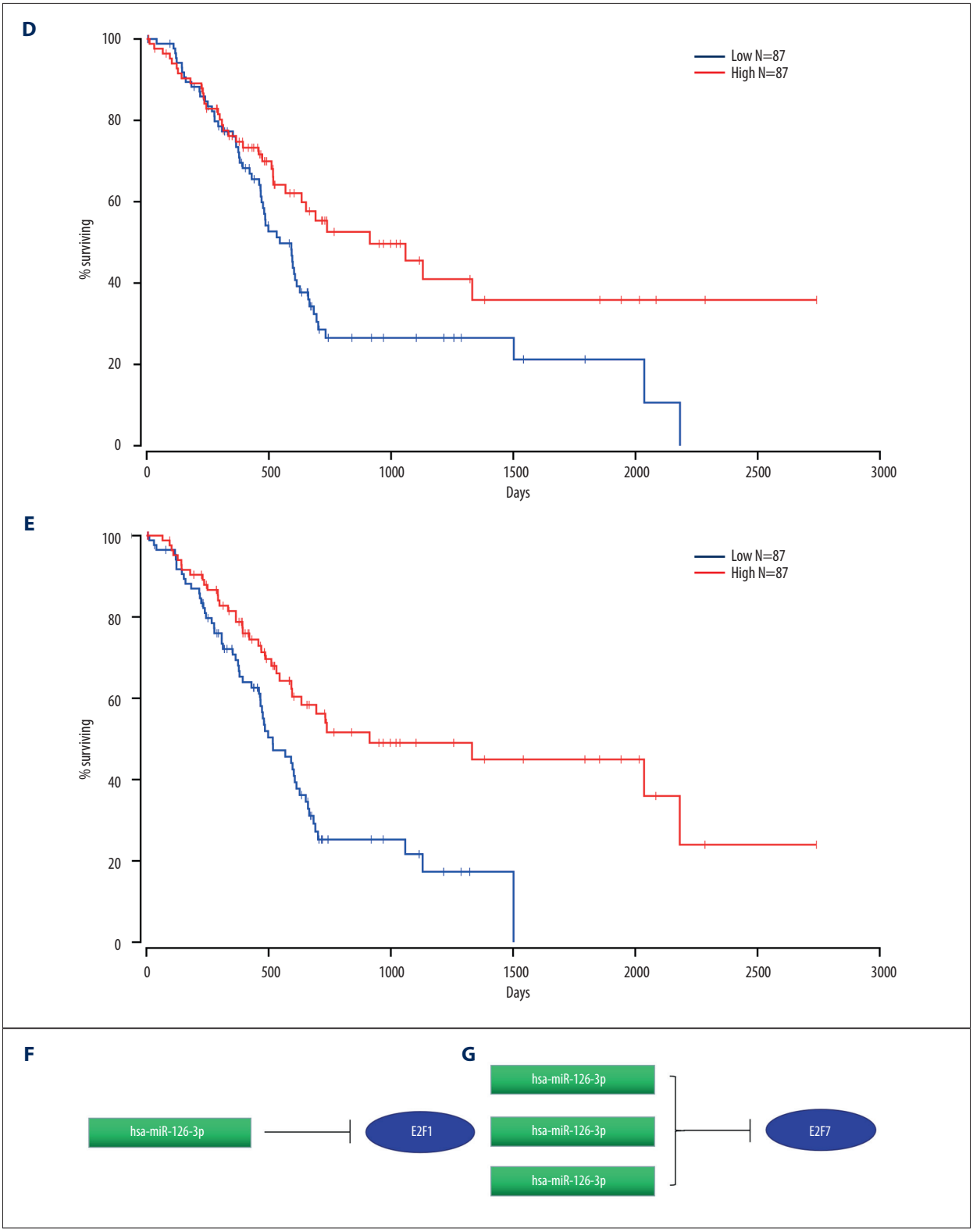

Figure 8. Prognostic analyses of E2F gene-related miRNAs. (**A**) Survival outcomes associated with miRNAs predicted to regulate E2F family members were assessed with the GSCALite tool. (**B, F**) Survival outcomes associated with E2F1-related miRNAs were assessed with Oncolnc. (**C-E, G**) Survival outcomes associated with E2F7-related miRNAs were assessed with Oncolnc.

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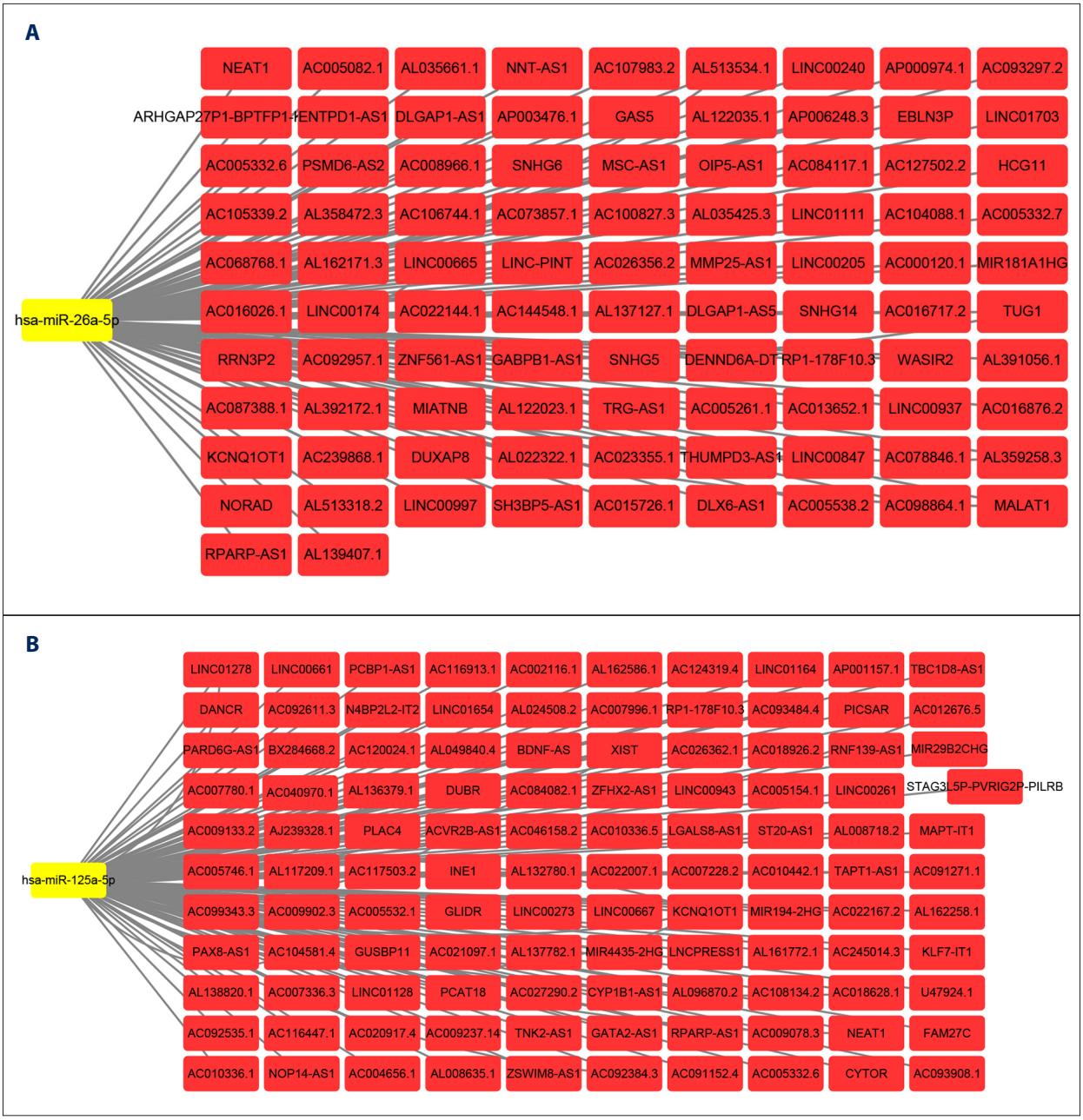

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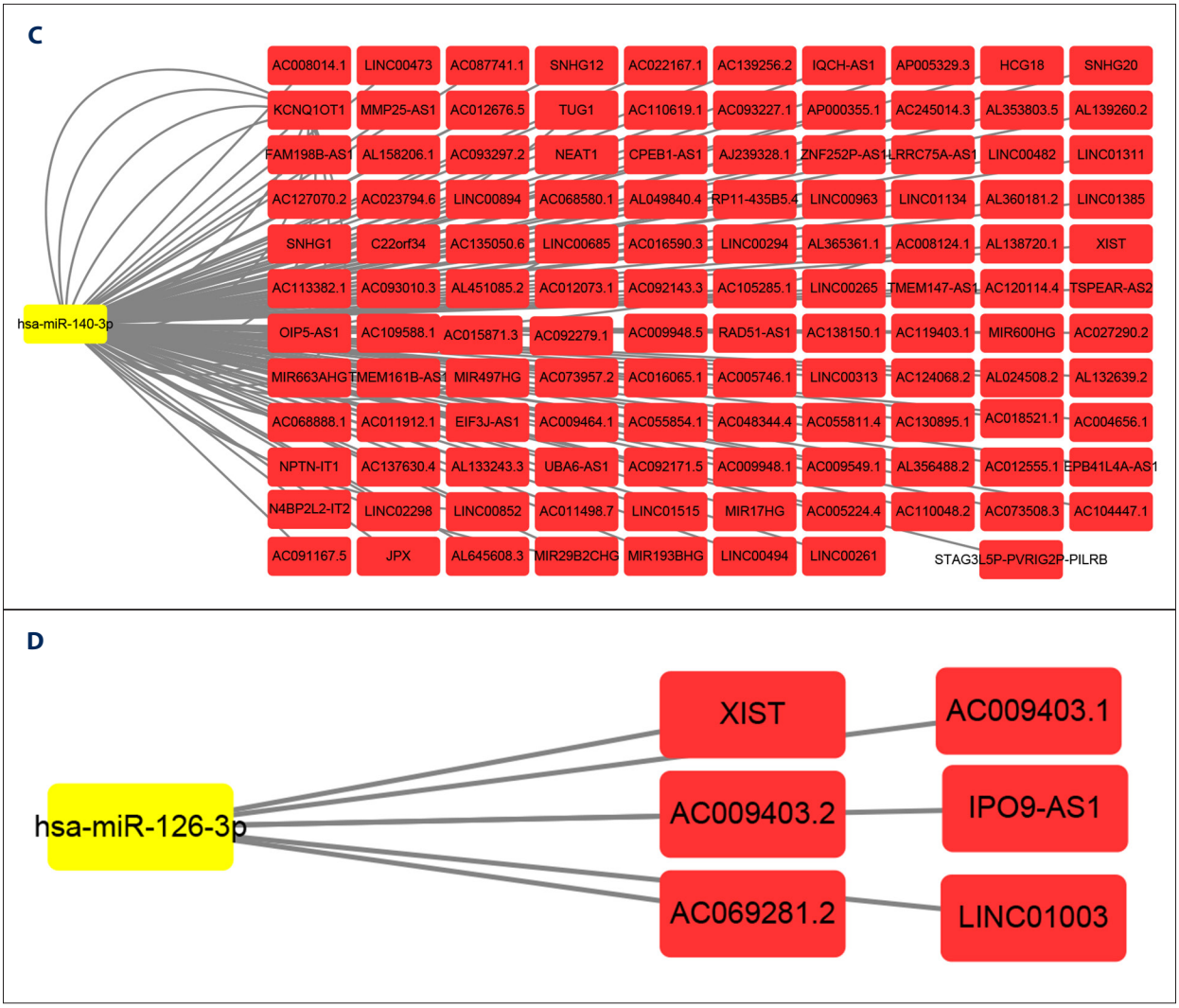

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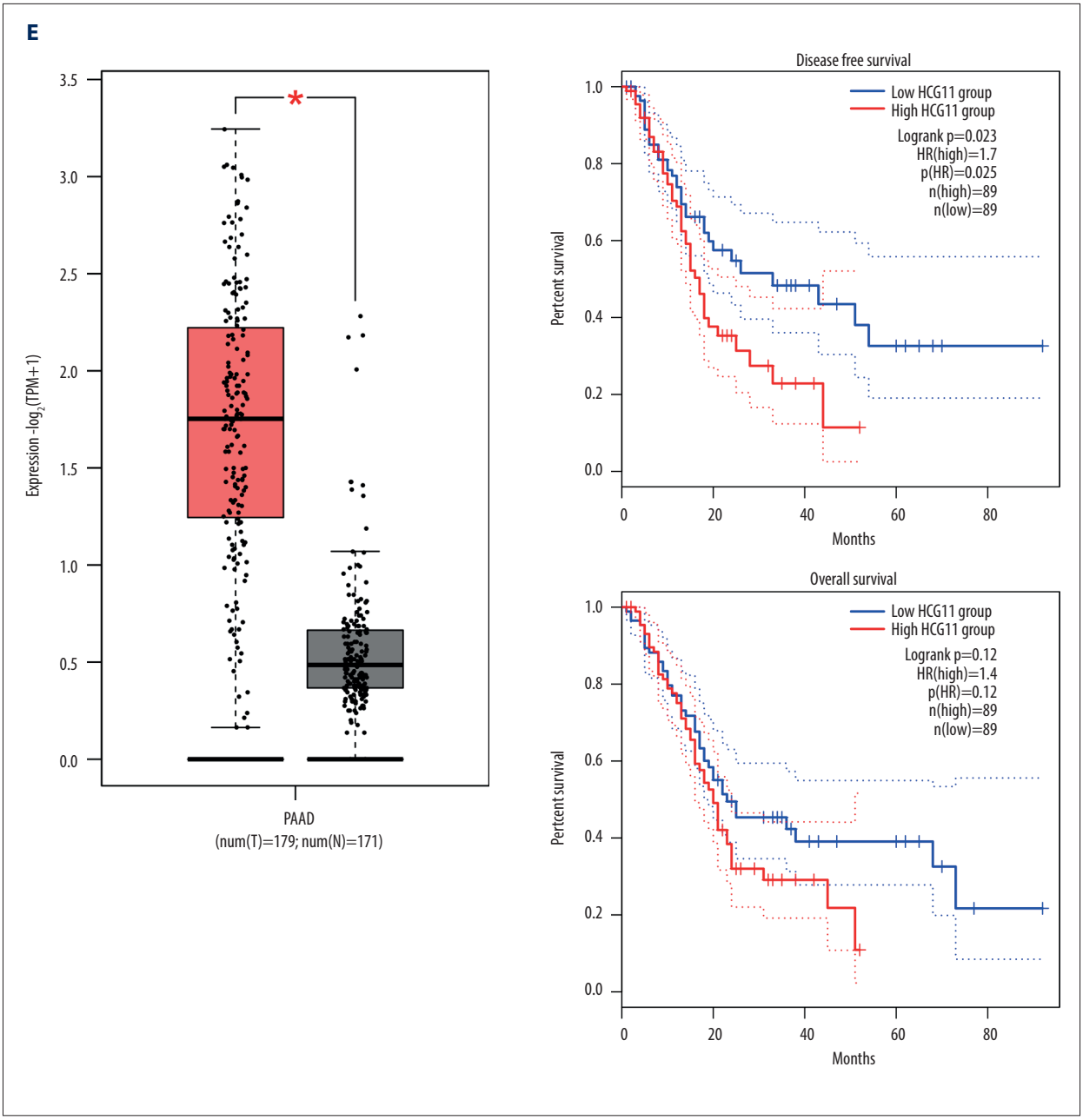

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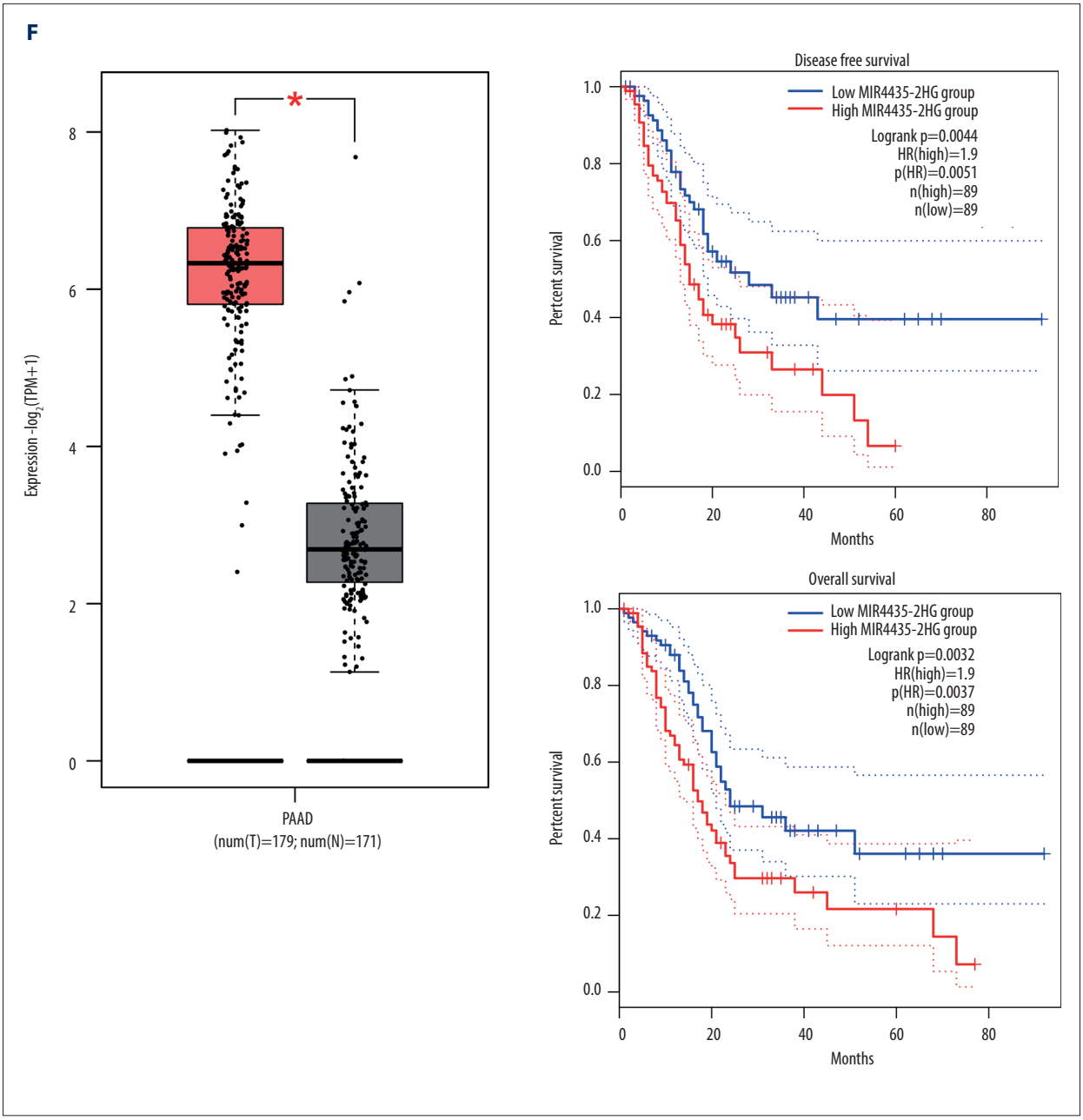

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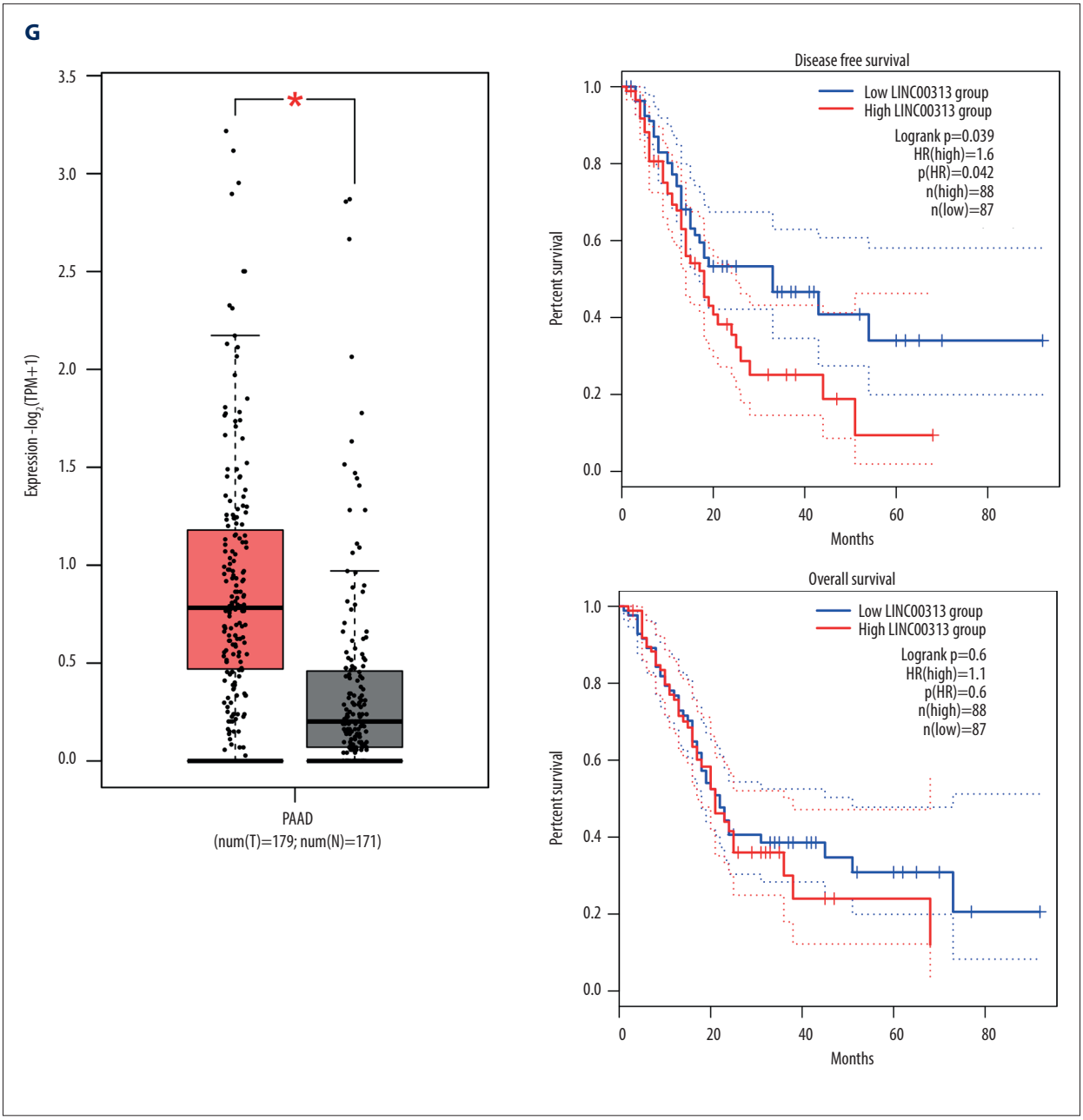

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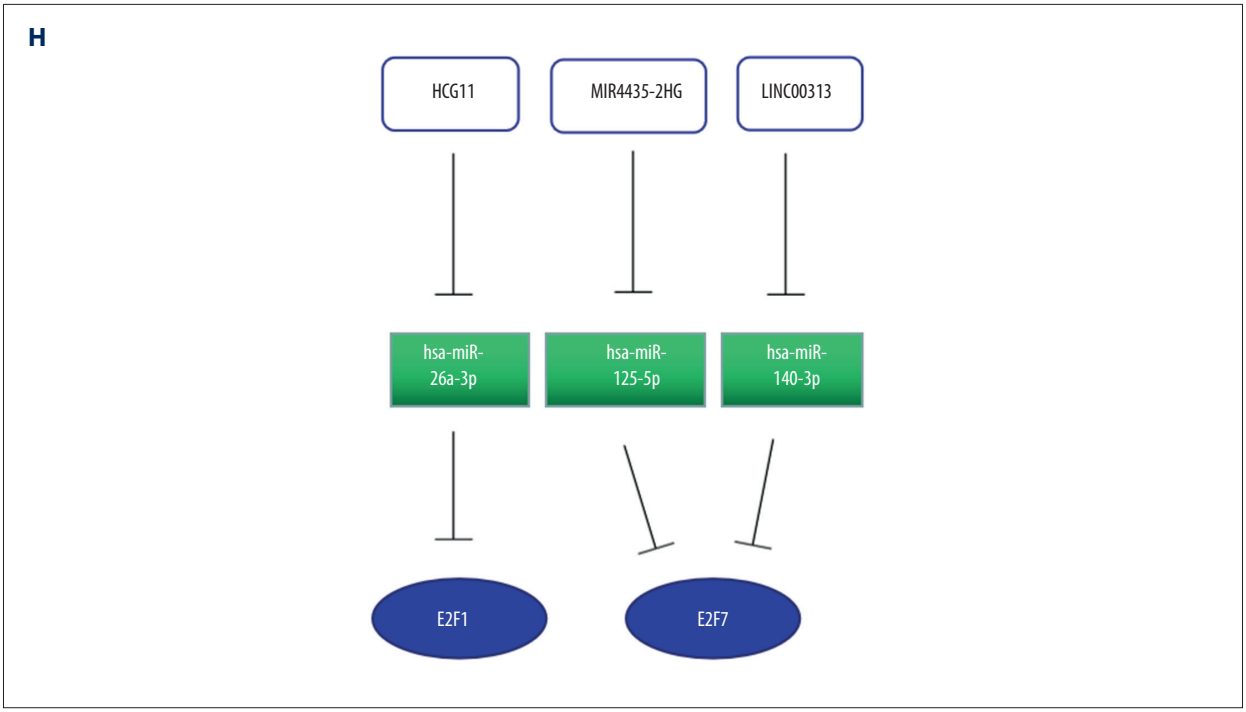
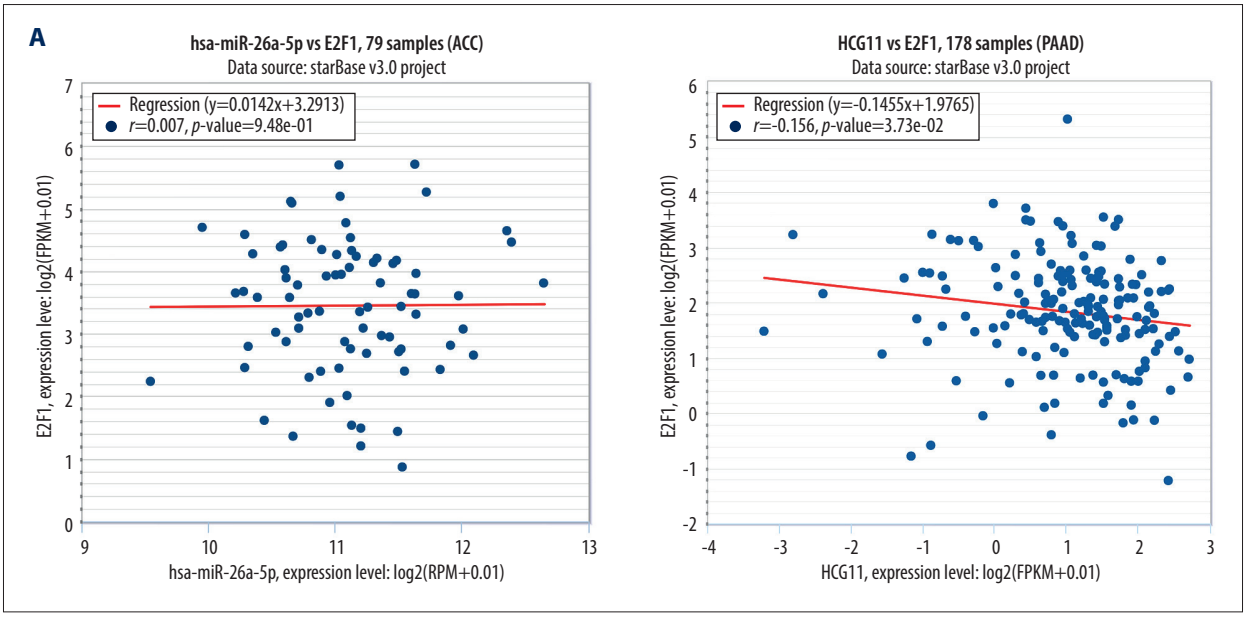


Figure 9. Associations between ceRNA networks and PAAD patient outcomes. (**A-D**) The Cytoscape tool was used to predict lncRNAs upstream of specific miRNAs of interest. (**E-G**) The prognostic relevance of putative upstream lncRNAs was assessed. (**H**) Putative ceRNA (lncRNA/miRNA/mRNA) regulatory relationships identified in the present analysis.



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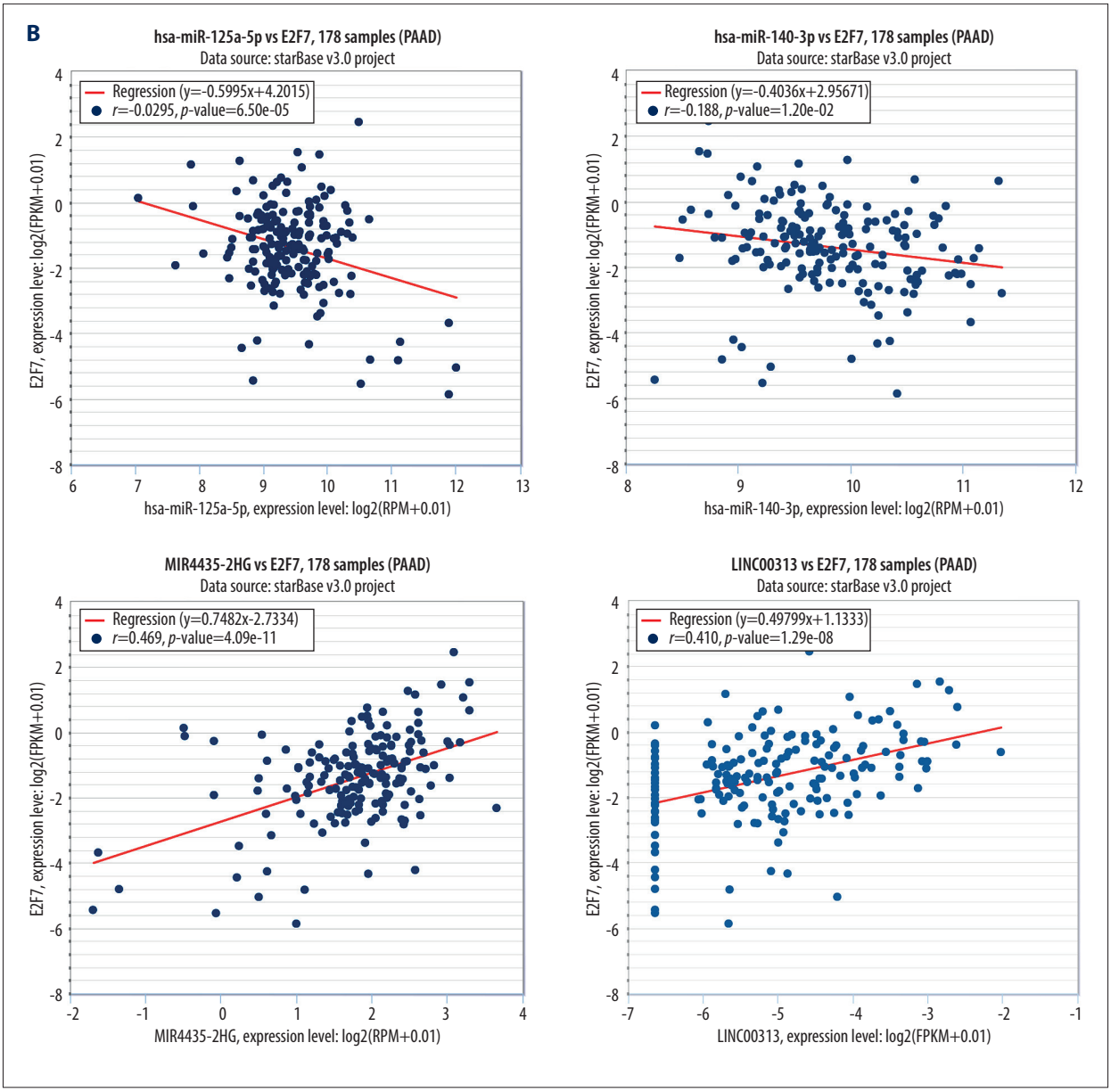

Figure 10. Coexpression analyses of associations between E2F family members and related noncoding RNAs. (**A**) Coexpression relationship analysis of E2F1/miRNA26a-5p/HCG11 was conducted using the starBase tool. (**B**) A coexpression relationship analysis of E2F7 and related noncoding RNAs was conducted using the starBase tool.

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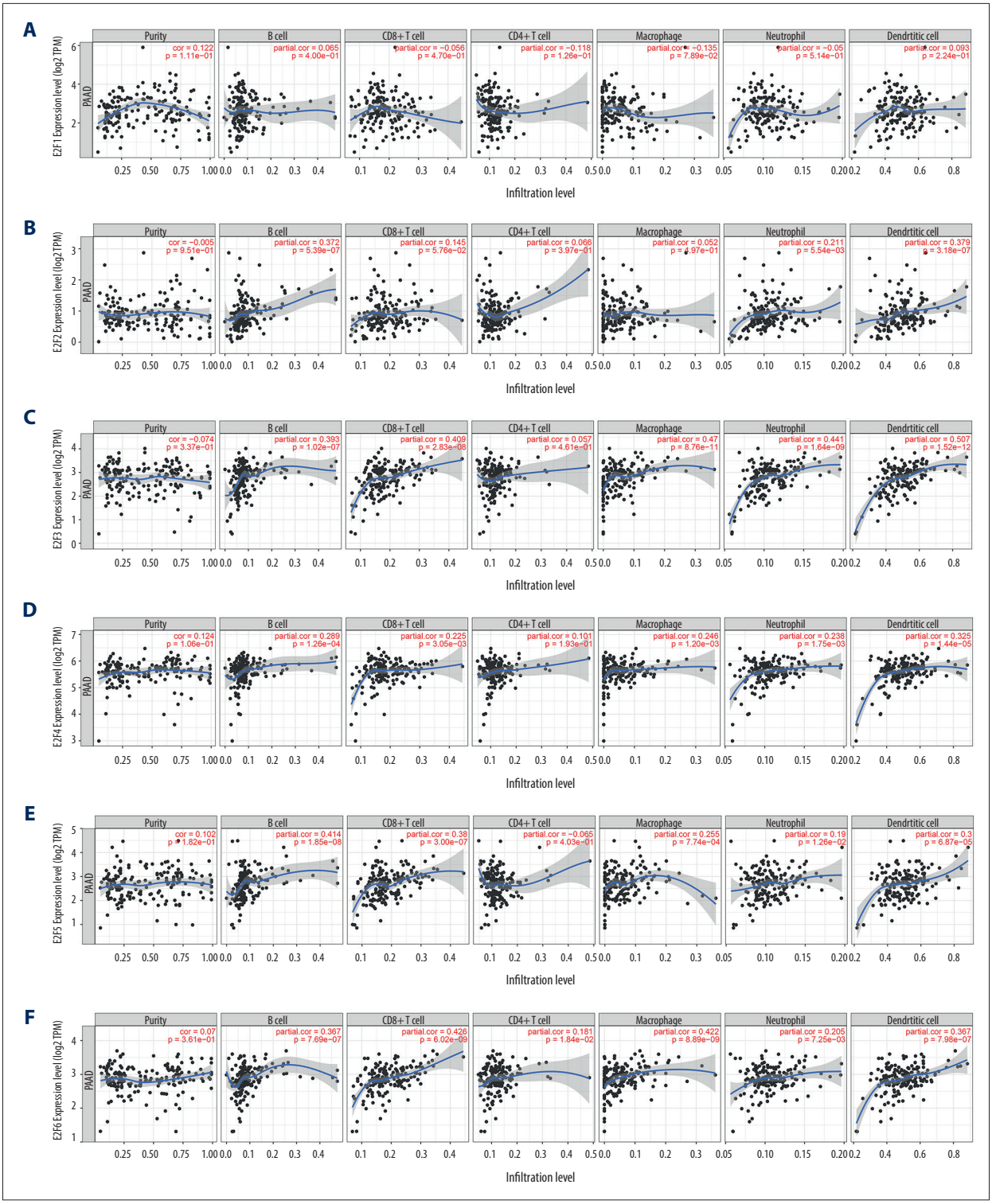

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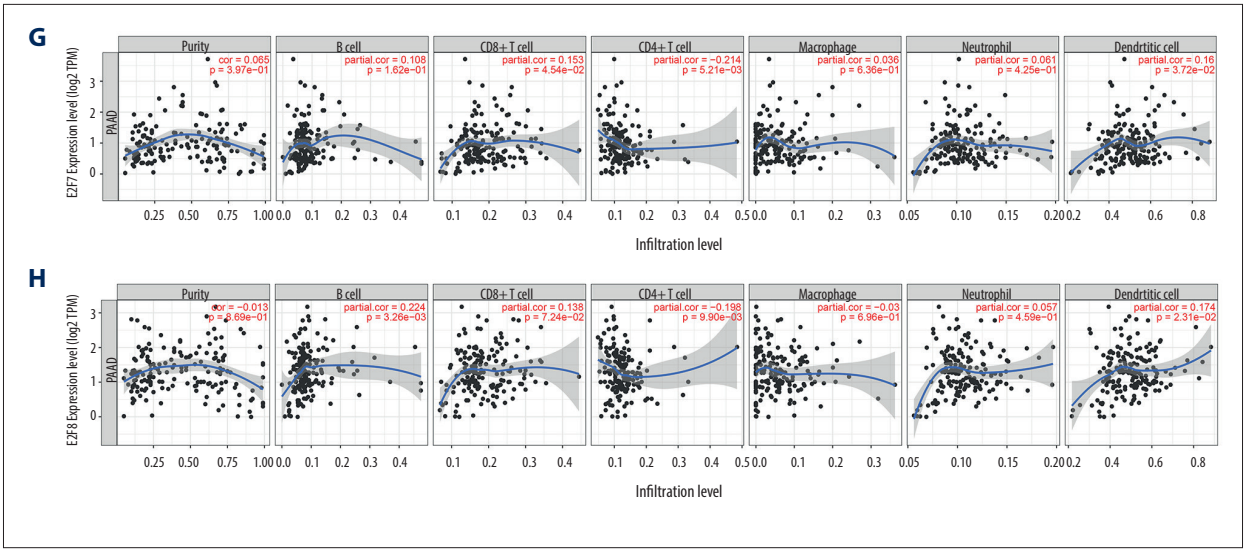

Figure 11. The relationship between E2F expression levels and immune cell infiltration in PAAD (TIMER). The correlation between the abundance of immune cells and the expression of E2F1 (**A**), E2F2 (**B**), E2F3 (**C**), E2F4 (**D**), E2F5 (**E**), E2F6 (**F**), E2F7 (**G**), and E2F8 (**H**) in PAAD.

GO enrichment analyses of the 38 genes included in the above PPI network were then conducted using the FUNRICH tool (https://david.ncifcrf.gov/), which assessed the enrichment of these genes in particular biological processes (BPs), cellular components (CCs), and molecular functions (MFs) (**Figure 6B-6D**). Enriched BPs included regulation of nucleobase, nucleoside, nucleotide, and nucleic acid metabolism and regulation of cell cycle; enriched CCs included the nucleoplasm, chromosome, nucleus, origin recognition complex, and cyclin-dependent protein kinase holoenzyme complex; and enriched MFs included DNA binding. Enriched KEGG pathways associated with these 38 E2F family members and related genes were additionally identified (**Figure 6E**), revealing that they are associated with the cell cycle signaling pathway (**Figure 6F**).

Assessment of the Mechanistic Roles of E2F Family Members and Associated ceRNA Network Construction

To explore the mechanisms and prognostic relevance of E2F family members in PAAD patients, we leveraged the GSCALite tool to assess E2F CNVs and found that E2F1/5 amplification and E2F2/3 deletion were significantly associated with PAAD patient gene expression (**Figure 7A**). We also observed that methylation status was negatively correlated with the expression of the analyzed E2F family TFs in PAAD (**Figure 7B**). We then explored the functional importance of these E2F family members in a range of cancer-associated pathways, the results of which revealed that they are involved in cyclin activation, apoptotic pathway regulation, and DNA damage responses. Specific E2F proteins were able to inhibit the hormone AR and RAS/MAPK pathways (**Figure 7C**). These findings suggested that specific E2F-related genomic abnormalities have value in the context of screening for therapeutic efficacy and clinical responses to certain medications. In the drug sensitivity analyses, E2F2/3/8 expression levels were negatively correlated with drug resistance (**Figure 7D**).

Next, we sought to establish an miRNA-related regulatory network corresponding to PAAD by identifying upstream miRNAs associated with PAAD patient survival using GSCALite (**Figure 8A**), with further evaluation of the prognostic value with the Oncolnc website (http://www.oncolnc.org/). This approach identified 4 relevant miRNAs, including E2F1-related miR-126-3p, and overexpression of miR-126-3p was linked to a better patient prognosis ($P<0.05$) (**Figure 8B**). The other 3 miRNAs, miR-26a-5p, miR-140-3p, and miR-125a-5p, were associated with the expression of E2F7, with its overexpression linked to better patient outcomes ($P<0.05$) (**Figure 8C-8E**). Using starBase, we predicted upstream lncRNA regulators of these miRNAs (**Figure 9A-9D**). The GEPIA database indicated that the lncRNAs HCG11, MIR4435-2HG, and LINC00313 were all expressed at high levels and were closely linked to PAAD patient OS and DFS (**Figure 9E-9G**), which enabled us to construct a putative E2F-related ceRNA regulatory network for PAAD (**Figure 9H**). Coexpression analyses indicated that E2F1 was negatively correlated with HCG11 expression in PAAD patients (**Figure 10A**), and E2F7 expression was positively correlated with the levels of MIR4435-2HG and LINC00313 but negatively correlated with the expression of miR-140-3p and miR-26a-5p (**Figure 10B**).

The Transcription Level of E2Fs and Various Levels of Immune Infiltration in PAAD

To elucidate the relationship between E2Fs and the inflammatory response and immune cell infiltration, we used the TIMER

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database (http://timer.cistrome.org/) for analysis; the results of which are shown in **Figure 11A** and indicate that the level of E2F expression is associated with the level of immune infiltration in PAAD. We found that E2F2 expression was positively correlated with the infiltration of B cells, neutrophils, and dendritic cells (**Figure 11B**). E2F3/4/5/6 expression was positively correlated with the infiltration of B cells, CD8+ T cells, macrophages, neutrophils, and dendritic cells (**Figure 11C-11F**), and E2F7 expression was positively correlated with the infiltration of CD8+ T cells and dendritic cells (**Figure 11G**). E2F8 expression was positively correlated with the infiltration of B cells and dendritic cells but was negatively correlated with the infiltration of CD4+ T cells (**Figure 11H**).

Discussion

The dysregulation of E2F family TFs has been reported in many tumor types [4-9], and the advent of novel high-throughput transcriptomic technologies has emphasized the importance of studying the functional roles of these TFs as regulators of PAAD oncogenic progression through mechanisms associated with a range of noncoding RNAs. Indeed, lncRNA/miRNA/mRNA signaling and the related regulatory mechanisms have been extensively documented in multiple cancer types [21], and the roles of these RNAs in PAAD must be clarified to better establish key diagnostic and prognostic biomarkers related to this cancer type. Herein, we conducted novel analyses of the expression and molecular roles of E2F family TFs in PAAD and explored their associations with patient outcomes by analyzing lncRNA/miRNA/mRNA signaling axes via a series of bioinformatics approaches.

E2F1 was the first identified E2F family member, and it can control the G1/S-phase transition by regulating DNA replication and controlling its own expression in a feedback loop [22]. We observed significant E2F1 overexpression in PAAD cells, which is known to promote oncogenesis and tumor cell proliferation [23]. While ONCOMINE and TCGA analyses indicated that PAAD tissues expressed higher levels of E2F1 than the healthy tissues did, this TF was not associated with clinical characteristics in PAAD patients. Despite this, higher E2F1 expression was associated with poorer patient OS and DFS. Constructed ceRNA networks further identified miR-126-3p to be related to E2F1 expression and poor patient prognosis, with HCG11 identified as an upstream lncRNA predicted to regulate miR-126-3p expression and to be negatively correlated with E2F1 expression.

E2F2 is also a key regulator of PAAD onset and progression, with miR-214-5p having previously been identified as an oncogenic regulator of this TF in PAAD [24]. Herein, we did not detect any significant difference in E2F2 expression between PAAD and normal tissues, but we did observe a significant relationship between the expression of this TF and PAAD tumor stage. Higher levels of E2F2 expression were associated with worse PAAD patient OS and DFS, and GSCALite analyses suggested that E2F2 expression levels are positively correlated with drug resistance.

E2F3 was previously identified as a key regulator of aggressive behaviors in prostate cancer and was reported to be related to tumor stage, grade, and proliferation in bladder cancer [25]. Sun et al [26] determined that the lncRNA NEAT1 can drive the progression of NSCLC by functioning as a miR-377-3p ceRNA to promote E2F3 upregulation. Consistent with these prior reports, we detected high levels of E2F3 expression in PAAD tissues relative to control tissue samples, with this expression being correlated with tumor stage. High E2F3 expression was also associated with worse PAAD patient OS and DFS and was positively correlated with drug resistance.

The E2F4 transcriptional repressor can suppress the proliferation of lung cancer cells [27]. We did not detect any significant change in E2F4 expression when comparing PAAD and normal tissue samples, and the expression of this TF was unrelated to PAAD patient tumor stage. However, high E2F4 levels were correlated with better DFS in PAAD patients but were unrelated to OS. These results are in line with the ability of E2F4 to function as a tumor suppressor.

E2F5 has been shown to be expressed at high levels in glioblastoma and prostate cancer [28,29]. While we observed increased E2F5 expression in PAAD tissues compared to normal tissues, it was unrelated to PAAD patient tumor stage or survival outcomes.

E2F6 is known to function as an oncogene in lung cancer, and miR-424 can suppress its expression and consequently disrupt A549 cell proliferation and migration [29,30]. We observed no significant difference in E2F6 expression in PAAD, although it was related to patient tumor staging. Additionally, no association between E2F6 expression and patient prognosis was detected.

E2F7 is a tumor suppressor gene encoded on chromosome 12q21.2 that controls cell cycle progression [31]. We observed significantly increased E2F7 expression in PAAD patient tumors relative to control tissues, and higher expression of this TF was associated with worse PAAD patient OS and DFS. However, the expression of E2F7 was unrelated to other clinical characteristics of PAAD patients. Three total miRNAs were identified as having a relationship with E2F7 (miR-140-3p, miR-26a-5p, and miR-125a-5p), and their overexpression was related to poor patient outcomes. Using the starBase tool, we additionally identified MIR4435-2HG and LINC00313 as putative upstream lncRNAs that were positively correlated with E2F7 expression in

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PAAD, and the expression of this TF was negatively correlated with the expression of miR-26a-5p and miR-140-3p.

E2F8 can block the activity of other E2F family TFs and bind in a dominant-negative fashion to the cyclin D1 promoter [32], but it has not been studied in the context of PAAD. We found that PAAD tumors exhibited increased E2F8 expression relative to that in normal tissues and that the expression of this TF was related to tumor stage. In addition, higher E2F8 expression was linked to poorer OS and DFS in PAAD patients, and E2F8 levels were positively correlated with drug resistance.

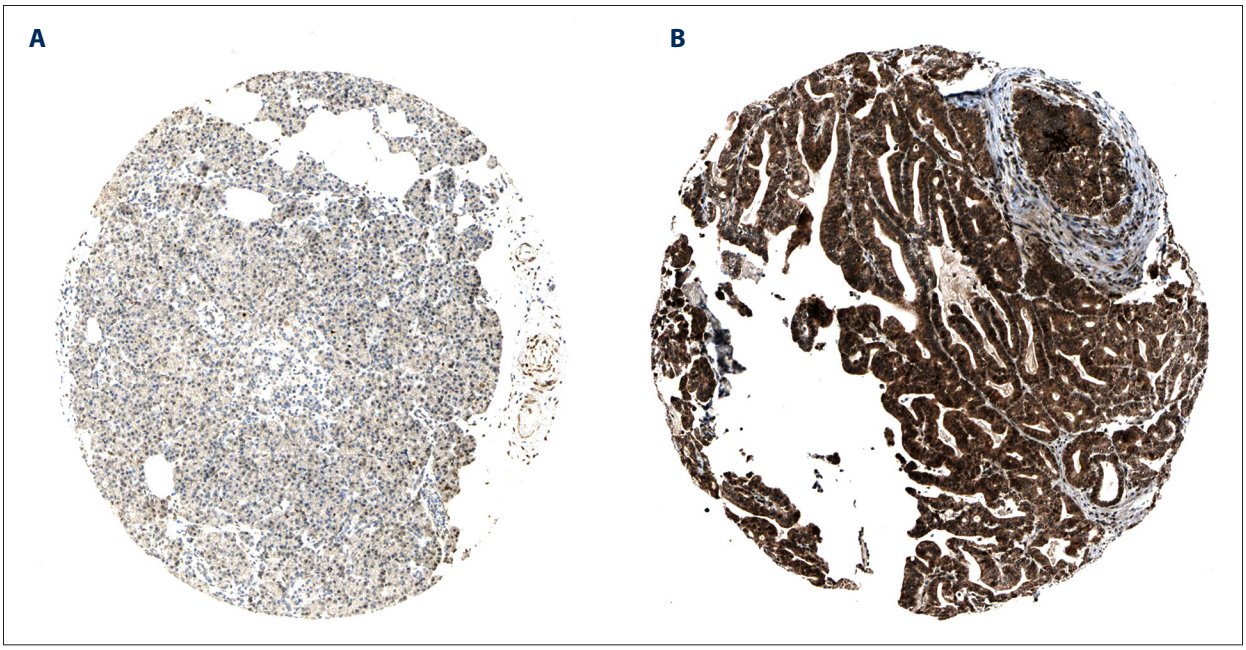
Conclusions

In summary, we observed marked upregulation of E2F1-8 in PAAD, suggesting that these TFs play key roles in the onset and progression of this deadly form of malignancy. Specifically, we determined that E2F1/2/3/7/8 may represent viable prognostic biomarkers of PAAD patient survival, whereas E2F2/3/6/8 levels were significantly related to tumor stages in these patients. As such, E2F2/3/8 may represent promising targets for PAAD treatment, although additional research will be essential to confirm the validity of these results and the utility of E2Fs as therapeutic targets or prognostic biomarkers. The expression of E2Fs and the infiltration of the 6 immune cell types in PAAD suggests that E2Fs may be involved in the regulation of PAAD tumor immunity. This indicates that E2Fs not only can be used as a prognostic indicator of patients with PAAD but also reflect the patients' immune status.

Declaration of Figure Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

Supplementary Data



Supplementary Figure 1. The protein levels (https://www.proteinatlas.org/) of E2F1 (**A**) were increased in PAAD tissues compared with paracancerous tissues (**B**).

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