SUMO-Activating Enzyme Subunit 1 (SAE1) is A Promising Diagnostic Cancer Metabolism Biomarker of Hepatocellular Carcinoma

Jiann Ruey Ong
Taipei Medical University Shuang Ho Hospital Ministry of Health and Welfare
Oluwaseun Adebayo Bamodu
Taipei Medical University Shuang Ho Hospital Ministry of Health and Welfare
Nguyen Viet Khang
Taipei Medical University Hospital
Yen-Kuang Lin
Taipei Medical University Hospital
Chi-Tai Yeh
Taipei Medical University Shuang Ho Hospital Ministry of Health and Welfare
Wei-Hwa Lee
Taipei Medical University Hospital
Yih-Giun Cherng (stainless@s.tmu.edu.tw)
Taipei Medical University Hospital https://orcid.org/0000-0002-0201-4380

Research

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Abstract

Background: Hepatocellular carcinoma (HCC) is one of the most diagnosed malignancies and a leading cause of cancer-related mortality globally. This is exacerbated by its highly aggressive phenotype, and limitation in early diagnosis and effective therapies. The SUMO-activating enzyme subunit 1 (SAE1) is a component of a heterodimeric small ubiquitin-related modifier that plays a vital role in SUMOylation, a post-translational modification involving in cellular events such as regulation of transcription, cell cycle and apoptosis. Reported overexpression of *SAE1* in glioma in a stage-dependent manner suggests it probable role in cancer initiation and progression.

Methods: In this study, hypothesizing that SAE1 is implicated in HCC metastatic phenotype and poor prognosis, we analyzed the expression of *SAE1* in several cancer databases.

Results: Here, we demonstrated that *SAE1* is overexpressed in HCC samples compared to normal liver tissue, and this observed *SAE1* overexpression is stage and grade-dependent and associated with poor survival. The receiver operating characteristic analysis of *SAE1* in TCGA-LIHC patients (n=421) showed an AUC of 0.925, indicating an excellent diagnostic value of *SAE1* in HCC. Our protein-protein interaction analysis for SAE1 showed that SAE1 interacted with and activated oncogenes such as PLK1, CCNB1, CDK4 and CDK1, while simultaneously inhibiting tumor suppressors including PDK4, KLF9, FOXO1 and NEDD4. Immunohistochemical staining and clinicopathological correlate analysis of SAE1 in our TMU-SHH HCC cohort (n =54) further validated the overexpression of SAE1 in cancerous liver tissues compared with 'normal' paracancerous tissue, and high SAE1 expression was strongly correlated with metastasis and disease progression.

Conclusion: In conclusion, the present study demonstrates that SAE1 is a targetable molecular biomarker with high potential diagnostic and prognostic implications for patients with HCC.

Highlights

(i) The SUMO-activating enzyme subunit 1 (SAE1) is a component of a heterodimeric small ubiquitin-related modifier.

(ii) SAE1 is implicated in HCC metastatic phenotype and poor prognosis in liver cancer.

(iii) SAE1 interacted with and activated oncogenes such as PLK1, CCNB1, CDK4 and CDK1.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most commonly diagnosed cancer and ranks as the third commonest cause of cancer-related mortality, accounting for more than 700,000 fatalities in the world, annually [1]. The major risk factors for HCC include chronic infection of hepatitis B and C viruses (HBV and HCV), cirrhosis, alcohol abuse and non-alcoholic fatty liver disease (NAFLD) [2].

Hepatocarcinogenesis is characterized by dysregulated activation and/or expression of relevant genes in/on the hepatocytes, with resultant oncogene upregulation and tumor suppressor downregulation [3]. The last 5 decades has been characterized by discovery several biomarkers for diagnosis of HCC, including the α-fetoprotein (AFP), AFP-L3 (a heteroplast of AFP), des-γ-carboxyprothrombin (DCP), α-L-fucosidase (AFU), golgi protein 73 (GP73), osteopontin (OPN) and carbohydrate antigen 19 – 9 (CA19-9), which is globally regarded as diagnostic serological biomarkers for diagnosis of HCC patients. However, due to the clonal evolution, intratumoral and interpatient heterogeneity of HCC [4, 5], like AFP, the diagnostic validity and clinical applicability of all these serological biomarkers remain debatable, especially considering their sub-optimal diagnostic specificity and sensitivity for early detection of HCC [6, 7].

Similarly, histochemical biomarkers of HCC including glypican-3 (GPC-3), hepatocyte paraffin 1 (Hep Par 1), heat shock protein 70 (HSP70), glutamine synthetase (GS), arginase-1 (Arg-1), cytokeratin 7 and 19 (CK7 and CK19) are also plagued with same weakness in spite of their overall strength [8, 9]. Against the background of this diagnostic challenge, the discovery of a biomarker with high and reliable diagnostic and prognostic accuracy and validity remain an unmet need in hepato-oncology clinics. Thus, the exploration for such biomarker in the present study; with the ultimate aim of proffering a therapeutic target, as well as improving the accuracy of diagnosis and efficacy of treatment modality in patients with HCC.

The disease course and progression of HCC is facilitated by altered cellular gene expression with dysregulated metabolism and pathophysiological signaling pathways [3–5]. SUMOylation, a post-translational modification that entails addition of small ubiquitin-like modifier (SUMO) groups to target proteins, is involved in numerous cellular events including transcriptional regulation, protein stability, cell cycle and apoptosis [10]. Upregulated expression of SAE1 (SUMO-activating enzyme subunit 1), an essential heterodimeric SUMO-activating effector of SUMOylation, has been implicated in the tumorigenesis and progression of several human malignancies, including in glioma [11], gastric cancer [12], and more broadly, in Myc-driven carcinomas [13, 14], however, the biological roles of SAE1 in HCC remains underexplored.

In the present study, hypothesizing that SAE1 is implicated in HCC metastatic phenotype and poor prognosis, we investigated the variability of *SAE1* expression in several cancer databases and its probable implication in HCC progression. Results presented herein indicate that compared to normal liver samples, SAE1 is overexpressed in HCC, associated with the enhanced metastatic phenotype, disease progression, and poor prognosis of patients with HCC; Thus, indicating that SAE1 possesses reliable and clinically-relevant diagnostic value and is a potential novel biomarker of prognosis for HCC.

2. Materials And Methods

2.1 HCC samples and cohort characterization

Clinical samples of patients with HCC were retrieved from the HCC tissue archive of the Taipei Medical University - Shuang-Ho Hospital (TMU-SHH), New Taipei, Taiwan. After exclusion of cases with incomplete clinical information and insufficient sample for biomedical assays, only 54 clinical samples were used in the present study. This study was approved by the Institutional Human Research Ethics Review Board (TMU-JIRB No. 201302016) of Taipei Medical University.

2.2 Data acquisition and statistical analysis of HCC

The raw gene expression data of *SAE1* and related genes obtained by RNA sequencing (RNA-seq) along with clinical data were downloaded from the freely-accessible Genotype-Tissue Expression (GTEx) (https://gtexportal.org/), the Cancer Genome Atlas (TCGA) (https://xenabrowser.net/) and the Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) databases. All data were visualized and analyzed using the GraphPad Prism version 8.0.0 for Windows, (GraphPad Software, San Diego, California USA, www.graphpad.com). Hazard ratios obtained from the analysis of overall and progression-free survival curves in various TCGA databases were visualized using forest plots. STRING version 11.0 (https://string-db.org/) was used for visualization of protein-protein interaction network and functional enrichment analysis.

2.3 Immunohistochemistry

Standard immunohistochemical (IHC) staining and the quantitation of the staining were performed as previously described [15]. Briefly, after de-waxing of the 5 µm-thick sections using xylene and re-hydration with ethanol, endogenous peroxidase activity was blocked using 3% hydrogen peroxide. This was followed by antigen retrieval, blocking with 10% normal serum, and incubation of the sections with anti-SAE1 antibody (1:500; #ab185552, Abcam, Cambridge, UK) overnight at 4 °C, followed by goat anti-rabbit IgG (H + L) HRP-conjugated secondary antibody (1:10,000; #65-6120, Thermo Fisher Scientific Inc., Waltham, MA, USA). As chromogenic substrate, Diaminobenzidine (DAB) was used, and the stained sections were counter-stained with Gill's hematoxylin (Thermo Fisher Scientific, Waltham, MA, USA). The univariate and multivariate analyses were done using the Cox proportional hazards regression model.

2.4. Statistical Analysis

All assays were performed at least thrice in triplicate. Values are expressed as the mean ± standard deviation (SD). Comparisons between groups were estimated using Student's t-test for cell line experiments or the Mann-Whitney U-test for clinical data, Spearman's rank correlation between variables, and the Kruskal-Wallis test for comparison of three or more groups. The Kaplan-Meier method was used for the survival analysis, and the difference between survival curves was tested by a log-rank test. Univariate and multivariate analyses were based on the Cox proportional hazards regression model. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 20 (IBM, Armonk, NY, USA). A p-value < 0.05 was considered statistically significant.

3. Results

3.1 Gene expression profile of *SAE1* in Pan-Cancer cohort

To examine the expression of *SAE1* in various tissue types, we analyzed expression data of samples (n = 17382) derived from non-disease tissues (n = 54) obtained from 948 donors using the Genotype-Tissue Expression (GTEx) project (GTEx Analysis Release V8 (dbGaP Accession phs000424.v8.p2) [16]. The lowest expression of *SAE1* was observed in liver (*n* = 110), pancreas (*n* = 167), kidney (*n* = 27) and pituitary (*n* = 107), in increasing order of magnitude, while testis (*n* = 165) and bone marrow (*n* = 70) exhibited the highest *SAE1* expression levels (Fig. 1A). Further exploring the *SAE1* mRNA levels in paired tumor - non-tumor samples from patients with one of 18 different cancer types using the Cancer Genome Atlas (TCGA) datasets, we observed that *SAE1* was significantly more expressed in liver hepatocellular carcinoma (n = 371), compared to their normal tissue counterparts (n = 50) (Figs. 1B **and C**). The upregulation of *SAE1* expression was also found in several other cancer types, including lung squamous cell carcinoma (LUSC, *n* = 553), colon adenocarcinoma (COAD, *n* = 327), head and neck squamous cell carcinoma (HNSC, *n* = 534), kidney chromophobe (KICH, *n* = 91), breast invasive carcinoma (BRCA, *n* = 1211), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC, *n* = 306) and uterine corpus endometrial carcinoma (UCEC, *n* = 211) (Figs. 1B **and C**).

3.2 *SAE1* is overexpressed in HCC and associated with disease progression.

Having demonstrated that *SAE1* is significantly more expressed in HCC compared with the non-tumor samples (~ 1.1-fold, p < 0.0001) **(**Fig. 2A**)**, to minimize probable experimental design-based bias, we excluded unpaired cases (n = 321) and analyzed the expression of *SAE1* in only cases with paired tumor - non-tumor samples (n = 100). Our results indicate that regardless of excluded cases, the median expression of *SAE1* mRNA remained significantly upregulated in the tumor samples (~ 1.1-fold, p < 0.0001) **(**Fig. 2B**)**. Probing for probable role of *SAE1* in disease progression, we demonstrated that *SAE1* expression increased with HCC stage, as evidenced by higher expression in advanced stages than in the early stages or non-tumor (stages II > stage I > > non-tumor) (p < 0.0001), indicating the increased expression of *SAE1* is tumorigenic and stage-dependent **(**Fig. 2C**)**. Supporting the results above, our analysis of four other HCC cohort datasets downloaded from the Gene Expression Omnibus (GEO): GSE36376 (Park 2012, *n* = 433), GSE64041 (Makowska 2014, *n* = 120), GSE14520 (Wang 2009, *n* = 445), GSE76297 (Wang 2015, *n* = 304) showed that *SAE1* was significantly overexpressed in all these four datasets **(**Fig. 2D**)**, further confirming the overexpression of the gene in TCGA-LIHC. Concordantly, we demonstrated that the expression of *SAE1* increased as histologic grade increased (p < 0.0001), was equivocal for gender, and mildly higher in patients aged < 60 (**Supplementary Figures S1A-C**). More so, *SAE1* expression in T1 < T2 < T3 < T4 (p = 0.0009), mildly higher in N1 and M1 compared with N0 (p = 0.255) and M0 (p = 0.682), respectively (**Supplementary Figures S1D-F**). Expectedly, patients with residual tumor (R1 and R2) has higher expression of *SAE1* compared with R0 (p = 0.958), and statistically significant upregulation of *SAE1* was observed in the deceased compared to those alive (p = 0.025) and equivocal for radiation therapy **(Supplementary Figures S1G-I)**. These results indicate the overexpression of *SAE1* in HCC, and its association with disease progression in a stage- and grade-dependent manner.

3.3 The overexpression of *SAE1* is associated with metastasis and poor prognosis in patients with HCC.

The eligible subjects in the TMU-SHH HCC cohort were aged from 25 to 85 with median age of 58.24 for patients with high SAE1 (*n* = 25) and 61.14 for those with low SAE1 (*n* = 29). 9 (16.67%) were male and 45 (83.33%) were female. Analyzed clinicopathological data, including patients' demographic (age and gender) and biochemical profile (α-fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigens 125 and 199 (CA125 and CA199), lymph node metastasis, tumor stages and survival status) are summarized in Table 1. Furthermore, employed the Cox proportional hazard model for clinicopathological analysis of *SAE1* expression, along with disease-specific risk factors, including age, gender, α-fetoprotein (AFP) and metastasis in the TMU-SHH HCC cohort (*n* = 54). Results of both univariate and multivariate analyses revealed that high SAE1 protein expression level is strongly associated with metastasis (Table 2). Corroborating the findings from big data analysis, IHC staining of samples from our TMU-SHH HCC cohort (*n* = 54) showed a 1.85-fold upregulated expression of SAE protein in the cancerous HCC compared to the non-tumor para-cancer liver tissue ($p < 0.0001$) (Fig. 3A). Probing for clinical relevance of the observed high expression of SAE1 protein, using the Kaplan-Meier curve for survival analysis, we demonstrated that compared to patients with low SAE1 expression (n = 20), those with high SAE1 expression (n = 18) exhibited worse overall survival ((HR (95%CI): 5.578 (1.250–24.890); p = 0.024)) (Fig. 3B). These results indicate that overexpression of *SAE1* is associated with metastasis and poor prognosis in patients with HCC.

Table 1
Patient clinicopathological characteristics of TMU-SHH HCC cohort.

Clinicopathological variable	**High SAE1 (*n* = 25)**		**Low SAE1 (*n* = 29)**		***p*-value**
Gender (%)					
Male	7	28	2	6.9	0.088
Female	18	72	27	93.1	
Tumor stage (%)					
I + II	18	72	14	48.3	0.021*
III + IV	7	28	15	51.7	
Metastasis (%)					
M0	18	72	10	48.3	0.036*
M1	7	28	19	51.7	
Age (%)					
≤ 65	17	68	16	55.2	0.494
> 65	8	32	13	44.8	
AFP (%)					
< 400	20	80	19	65.5	0.379
≥ 400	5	20	10	34.5	
SAE1 (%)					
< 350	25	100	0	0	< 0.001*
≥ 350	0	0	29	100	
Survival status (%)					
Survived	19	90.5	12	52.2	0.014*
Expired	2	9.5	11	47.8	
* *p*-value < 0.05					

Table 2
Univariate and multivariate analysis of SAE1 expression in TMU-SHH cohort

Clinicopathological variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Gender Male vs Female	2.050	0.262–16.017	0.4937	0.575	0.063–5.278	0.6244
Age (≤ 65 vs > 65)	1.008	0.960–1.057	0.7532	0.968	0.922–1.017	0.1944
AFP < 400 vs ≥ 400	2.375	0.725–7.782	0.1533	1.053	0.290–3.821	0.9378
Metastasis M0 vs M1	10.258	2.206–47.701	0.0030*	11.500	2.014–65.667	0.0060*
Q-Score < 300 vs ≥ 300	1.026	1.002–1.051	0.0319*	1.025	1.000-1.049	0.0468*
* *p*-value < 0.05						

3.4 *SAE1* is a reliable diagnostic and prognostic biomarker for HCC

To evaluate the diagnostic and prognostic validity of *SAE1* in HCC, we performed a survival analysis of the *SAE1*expression-stratified TCGA-LIHC dataset using the Kaplan-Meier plots and receiver operating characteristic (ROC) curves. We demonstrated that patients with high *SAE1* expression exhibited worse overall survival (OS) (HR = 1.873, $p = 0.0004$), disease-specific survival (DSS) (HR = 2.070, $p = 0.0016$), and progression-free survival (PFS) (HR = 1.809, $p < 0.0001$) over a follow-up period of 10 years (Figs. 4A-C). In addition, we found that patient with advanced stage HCC exhibited worse OS compared with those in early stage (stage III/IV *vs* I/II: $p < 0.0001$) (**Supplementary Figure S2A**). Furthermore, from our intergroup analysis of *SAE1* expression in HCC *vs* normal liver for diagnostic implication, the area under the ROC curve (AUC) was 0.925 (Youden's J = 0.71, SE = 0.01, $p < 0.0001$) (Fig. 4D), with hazard ratios and 95% confidence intervals of 1.873 (1.321–2.656) and 1.809 (1.345–2.434) for OS and PFS, respectively (Figs. 4E **and F**). More so, compared with *SAE1* expression in non-tumor, the AUCs for *SAE1* expression in patients with stages I, II, III, and IV were 0.92 ($p < 0.0001$), 0.93 ($p < 0.0001$), 0.94 ($p < 0.0001$), and 1.00 ($p = 0.0003$), respectively (**Supplementary Figures S2B-F**).

3.5 *SAE1* upregulates oncogenic effectors of cell cycle progression while downregulating FOXO1-associated tumor suppressing signaling

To unravel the underlying molecular mechanism of already documented *SAE1*- associated hepatocarcinogenesis, we probed for genes concomitantly upregulated or suppressed when *SAE1* is upregulated, and SAE1-dependent protein-protein interaction (PPI). Using the STRING database (https://string-db.org) for visualization of probable network of SAE1-associated functional proteins in humans, we found that SAE1 exhibited strong interaction with SUMO proteins such as SAE2 (also called UBA2), SUMO-conjugating enzyme E2I (UBE2I/UBC9) and SUMO specific peptidase 1 (SENP1), neural precursor cell-expressed developmentally down-regulated protein 8 (NEDD8), ubiquitin-conjugating enzyme E2M (UBE2M), RAN GTPase-activating protein 1 (RANGAP1), RAN binding protein 2 (RANBP2), RWD domain containing protein 3 (RWDD3), cullin-4A (CUL4A), cullin-5 (CUL5), cullin-associated NEDD8-dissociated protein 1 (CAND1), RING-box protein 1 (RBX1), S-phase kinase-associated protein 1/2 (SKP1/2), and defective in cullin neddylation 1 domain-containing 1 (DCUN1D1) protein (Fig. 5A). Furthermore, we used the cBioPortal for Cancer Genomics (https://www.cbioportal.org/) for identification of genes with significant positive or negative correlation with *SAE1* in the TCGA-LIHC cohort. Our results showed that *SAE1* is strongly co-expressed with the cell cycle-related oncogenes *PLK1* ($r = 0.64$, $p < 0.0001$), *CCNB1* ($r = 0.64$, $p < 0.0001$), *CDK4* ($r = 0.58$, $p < 0.0001$) and *CDK1* ($r = 0.58$, $p < 0.0001$) (Fig. 5B), but inversely related to tumor suppressor genes *PDK4* ($r=-0.47$, $p < 0.0001$), *KLF9* ($r=-0.47$, $p < 0.0001$), *FOXO1* ($r=-0.42$, $p < 0.0001$) and *NEDD4* ($r=-0.41$, $p < 0.0001$) (Fig. 5C). These findings suggest that *SAE1* upregulates oncogenic effectors of cell cycle progression while downregulating FOXO1-associated tumor suppressing signaling.

4. Discussion

Hepatocarcinogenesis entails alteration of cellular gene expression with consequent loss of benignity, acquisition of malignant phenotype and enhancement of the aggressiveness of the resultant cancerous liver cells [3]. Previous studies have shown that ubiquitylation and SUMOylation are significantly enhanced in HCC [14]. Against the background of reported implication of *SAE1* in SUMOylation and oncogenesis in several malignancies, including glioma and gastric cancer [11, 12], and aiming to validate its clinical validity and applicability as a reliable diagnostic and/or prognostic biomarker, the present study explored the expression and of *SAE1*, an indispensable molecular effector of SUMOylation [17], by probing and analyzing clinicopathological data from our in-house HCC cohort and several HCC databases.

In this study, we demonstrate that *SAE1 is* differentially expressed in normal and cancerous tissues, including in paired normal liver and HCC samples. More so, we provided evidence that the enhanced expression of SAE1 is both grade- and stage-dependent, indicating a probable role for SAE1 in enhanced onco-aggressiveness and disease progression in patients with HCC. This is consistent with reports demonstrating that SUMOylation-dependent transcriptional sub-programming is required for Myc-driven tumorigenesis, and more so implicating SAE1 in the progression of human glioma and gastric cancer through the activation of SUMOylation-mediated oncogenic signaling pathways [11–13].

Also, clinically relevant, we demonstrated that the overexpression of SAE1 is associated with poor prognosis, as evident in the shorter overall or disease-specific, and relapse-free survival time of patients with high expression of SAE1 in our HCC cohort and freely accessible larger HCC cohorts. These findings are corroborated by recent report that the expression of key components of the SUMO-involved regulatory network including enhanced UBE21 and SAE1 gene expression levels were strongly linked to poor prognosis in HCC [18] and that the sumoylation pathway is associated with adverse clinical outcome for patients with multiple myeloma [19].

In addition, we demonstrated that underlying the oncogenic and HCC-promoting activity of SAE1 was its ability to upregulate oncogenic effectors of cell cycle progression while downregulating FOXO1-associated tumor suppressing signaling. This is consistent contemporary knowledge that loss of FOXO1 promotes tumor growth and metastasis [20], and accruing evidence that SUMOs such as SAE1 are essential for the regulation of several cellular processes, including transcriptional regulation, transcript processing, genomic replication and DNA damage repair, where efficiency or inefficiency of the later determines initiation of mitosis or delayed mitotic entry, S-phase arrest, and altered cell cycle progression [21–23]; this has significant implication for diseases such as cancer, and suggests that SAE1 is a potential therapeutic target for patients with HCC.

More interestingly, we provided some evidence that SAE1 is a reliable diagnostic biomarker for HCC, with the differential expression of SAE1 in paired normal liver and HCC samples exhibiting an AUC of 0.9252. Similarly, the prognostic relevance of SAE1 expression was shown with stage dependent AUCs ranging from 0.9091 for stage 1 to 1.00 for stage IV, and KM plots indicating worse clinical outcome for patients with high SAE1 expression compared to their counterparts with low SAE1 expression. These findings are clinically valid and statistically relevant considering that the ROC curve is a vital tool in disease diagnostics and prognostics, especially where the evaluation of a biomarker's discriminatory ability is being carried out or for validation of diagnostic and/or prognostic tests. The AUC is the most widely used accuracy index of overall discriminatory power for biomarker identification and validation, such that higher AUC values indicate higher discriminability of a diagnostic or prognostic biomarker or test [24, 25].

In conclusion, the present study demonstrates that SAE1 is a targetable SUMO-related molecular biomarker with high potential diagnostic and prognostic implications for patients with HCC.

Declarations

Ethics approval and consent to participate

This study was conducted in a cohort of patients with HCC cancer at Taipei Medical University Shuang-Ho Hospital, Taipei, Taiwan. The study was reviewed and approved by the institutional review board (TMU-JIRB: 201302016).

Consent for publication

The authors declare that they have no potential financial competing interests that may in any way, gain or lose financially from the publication of this manuscript at present or in the future. Additionally, no non-financial competing interests are involved in the manuscript.

Authors' contributions: Jiann Ruey Ong: Study conception and design, collection and assembly of data, data analysis and interpretation, and manuscript writing. Chi-Tai Yeh, Oluwaseun Adebayo Bamodu, Nguyen Viet Khang, Yen-Kuang Lin, Wei-Hwa Lee: Data analysis and interpretation. Yih-Giun Cherng: Study conception and design, data analysis and interpretation, final manuscript approval. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed in the current study are publicly accessible as indicated in the manuscript.

Conflict of Interests: The authors declare that they have no potential financial competing interests that may in any way, gain or lose financially from the publication of this manuscript at present or in the future. Additionally, no non-financial competing interests are involved in the manuscript.

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Supplementary Data

Figure S1. The overexpression of *SAE1* is associated with metastasis and poor prognosis in patients with HCC**.** Boxplots showing the mRNA expression levels of *SAE1* according to histologic grade **(A)**, genders **(B)**, age at initial pathologic diagnosis **(C)**, TNM classification **(D-F)**, residual tumor **(G)**, vital status **(H)** and radiation therapy **(I)**.

Figure S2. *SAE1* is a reliable diagnostic and prognostic biomarker for HCC. **(A)** Kaplan-Meier curves of overall survival according to HCC pathologic stages. **(B-E)** ROC analysis of *SAE1* expression in non-tumor versus tumor in stages I, II, III and IV. **(F)** ROC analysis of *SAE1* expression characterized by various clinicopathological factors.

Figures

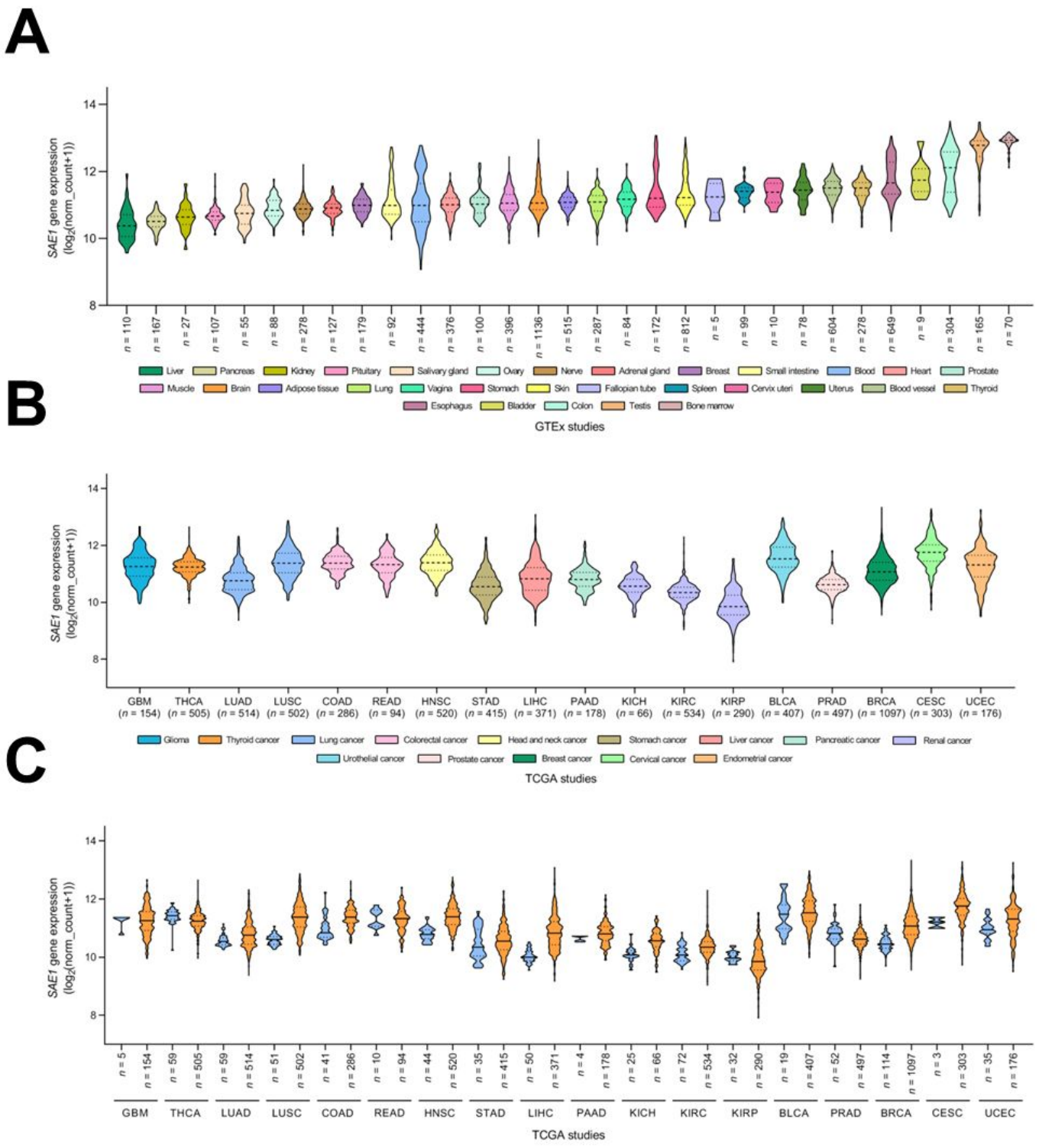
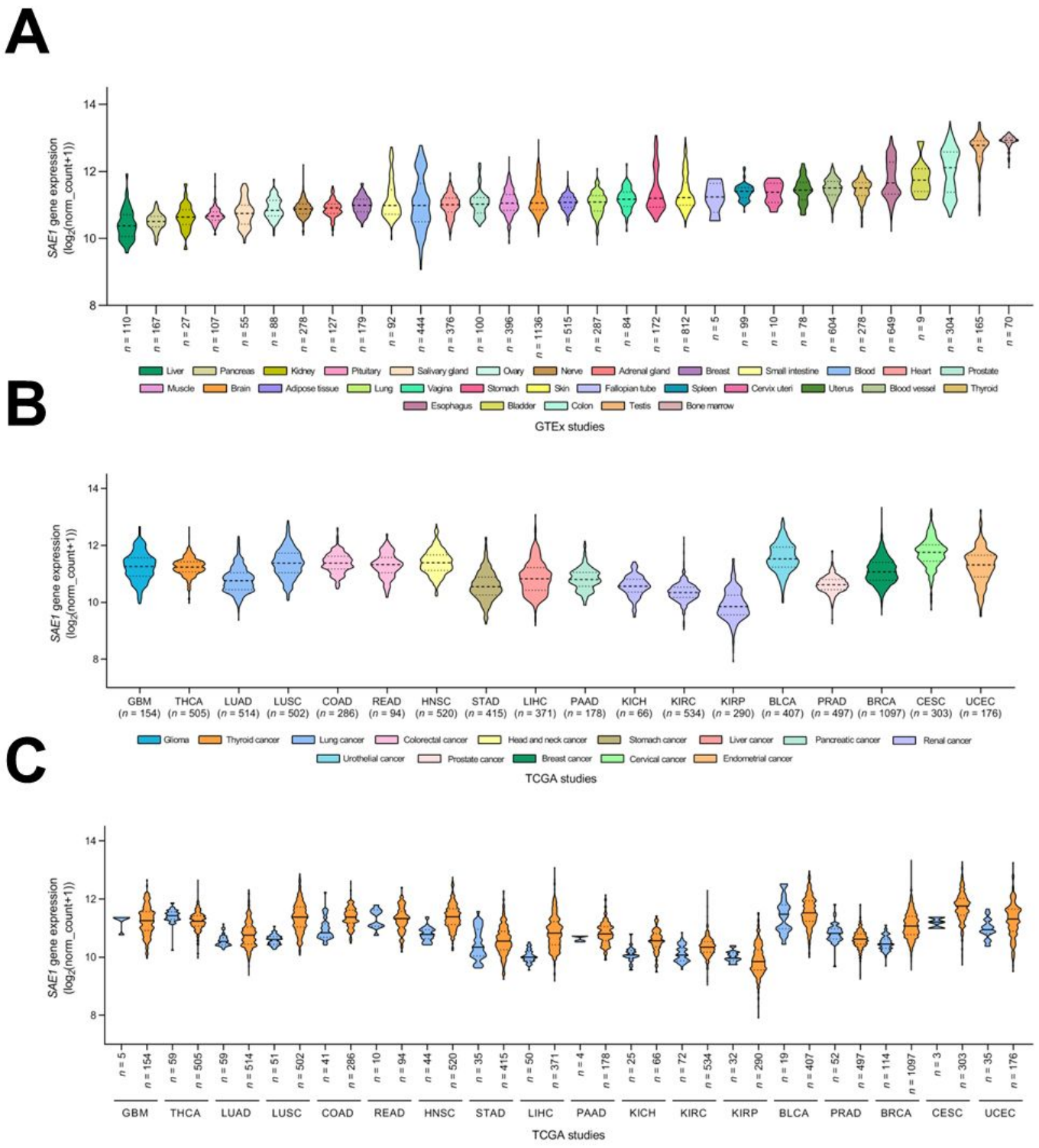


Figure 1

Gene expression profile of SAE1 in Pan-Cancer cohort. Violin plots showing the mRNA expression levels of SAE1 in different human tissue types according to GTEx database (A), and in various human cancer types according to TCGA database (B). SAE1 expression levels in adjacent tumor (labeled in blue) and tumor samples (labeled in orange) according to TCGA database (C).



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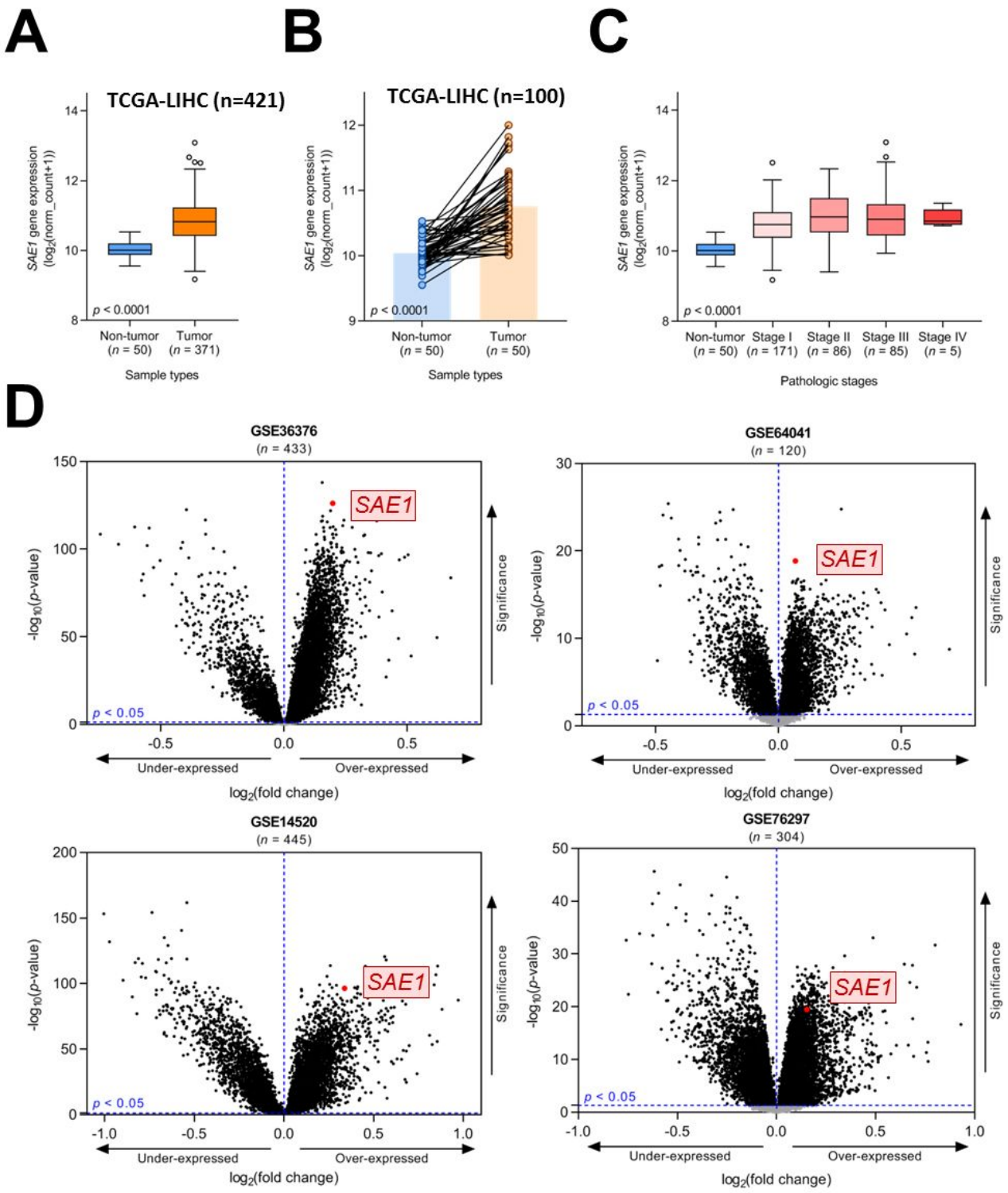
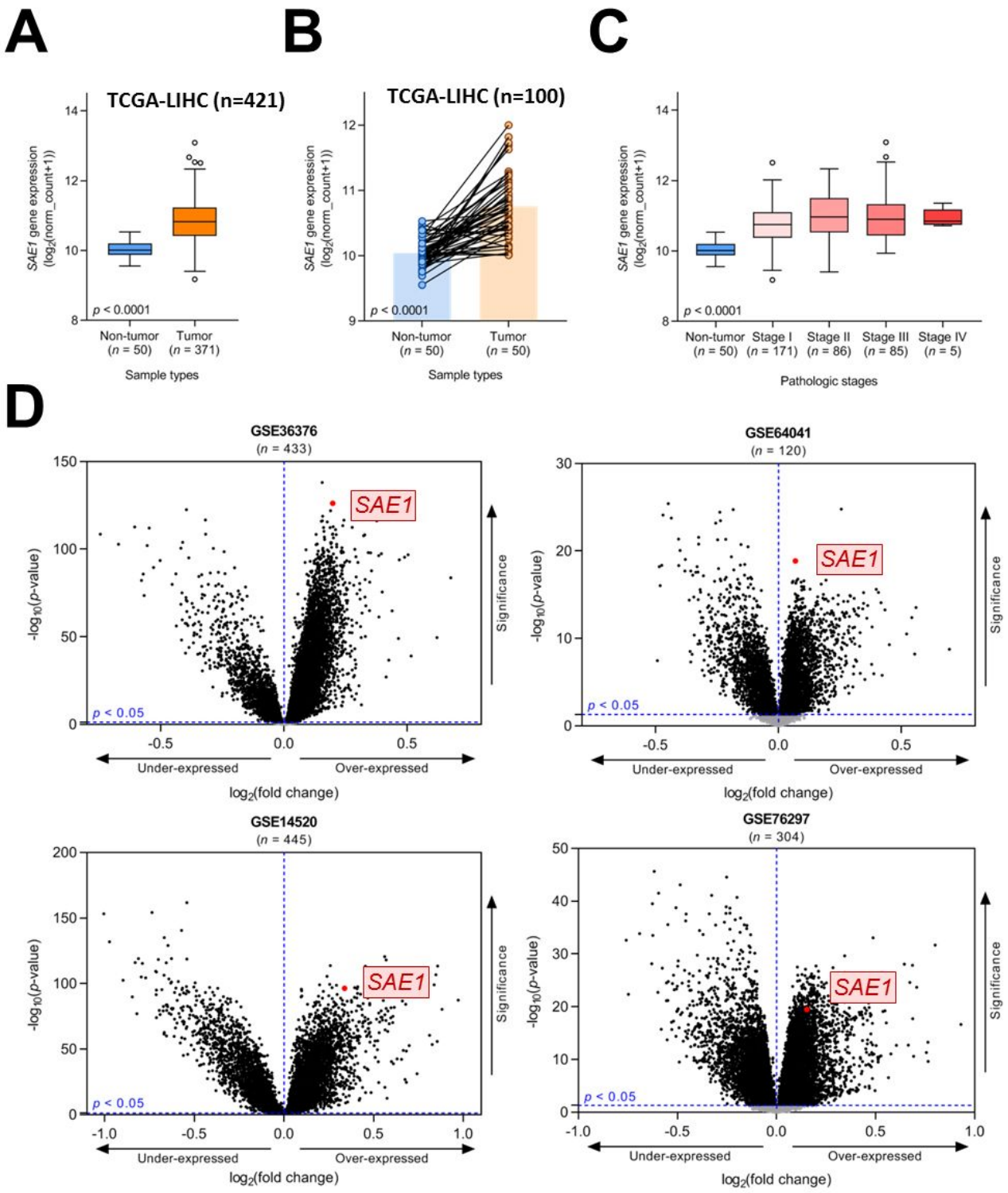


Figure 2

SAE1 is overexpressed in HCC and associated with disease progression. (A) Boxplot showing the mRNA expression levels of SAE1 in HCC and non-HCC samples according to TCGA-LIHC database, (B) The expression of SAE1 in the same patients, (C) Boxplot showing the SAE1 expression grouped by pathologic stages, (D) Volcano plots indicating SAE1 upregulated according to GEO databases (GSE36376, GSE64041, GSE14520 and GSE76297)



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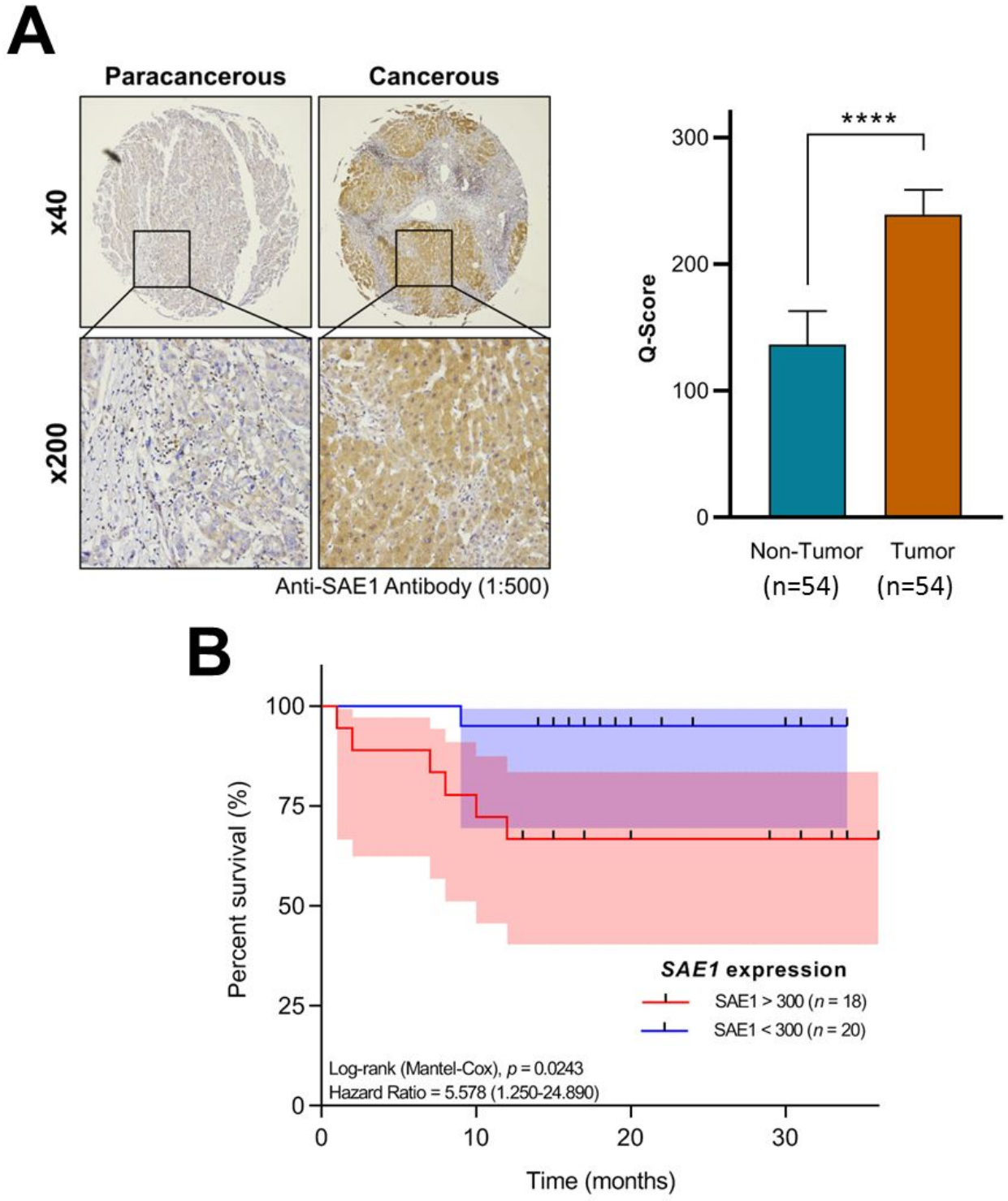
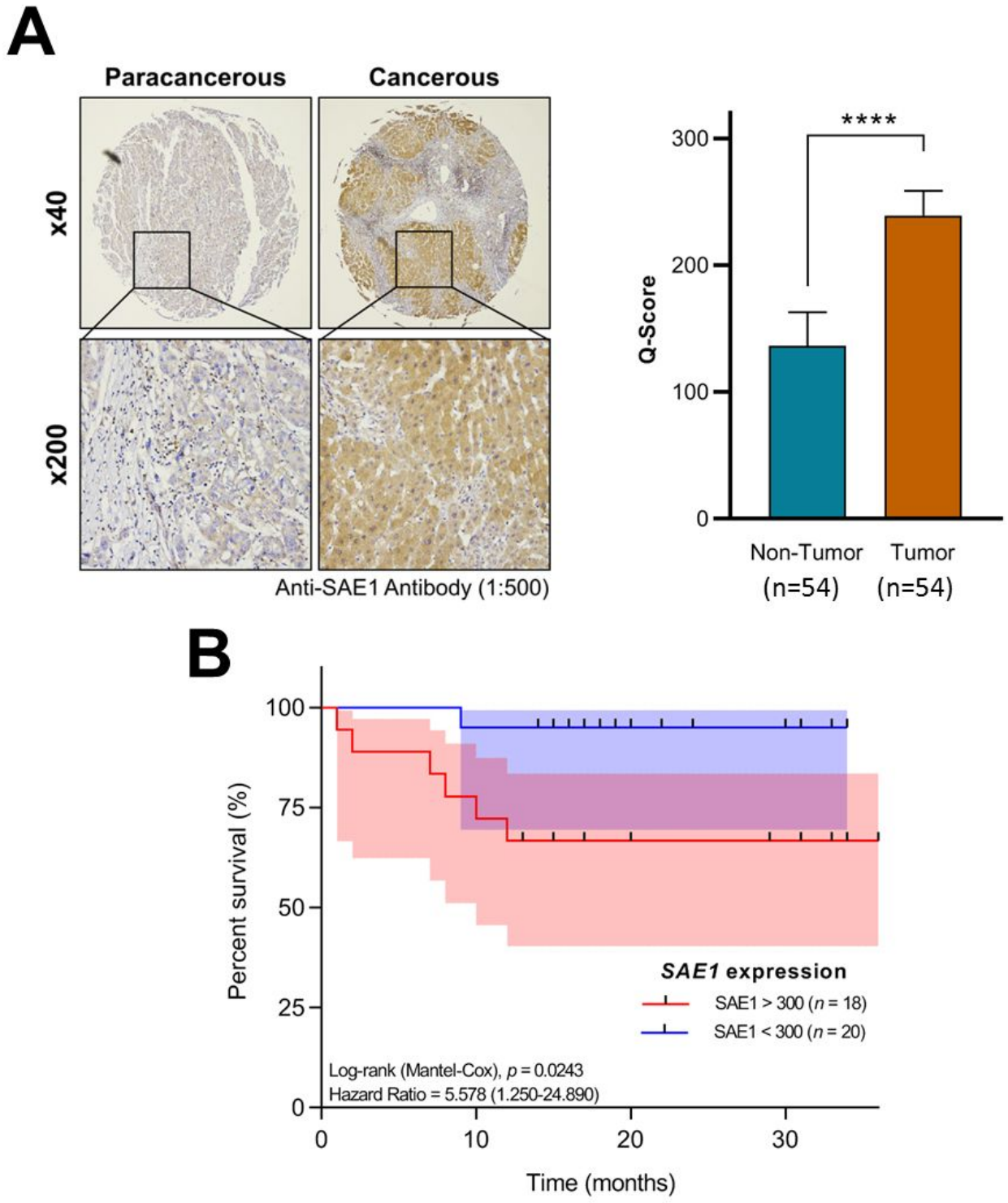


Figure 3

The overexpression of SAE1 is associated with metastasis and poor prognosis in patients with HCC. (A) Representative IHC photo-image (left) and graphical quantitation (right) of SAE1 protein expression in paracancerous and cancerous tissues. (B) Kaplan-Meier curve of overall survival according to SAE1 protein levels of TMU-SHH HCC cohort.



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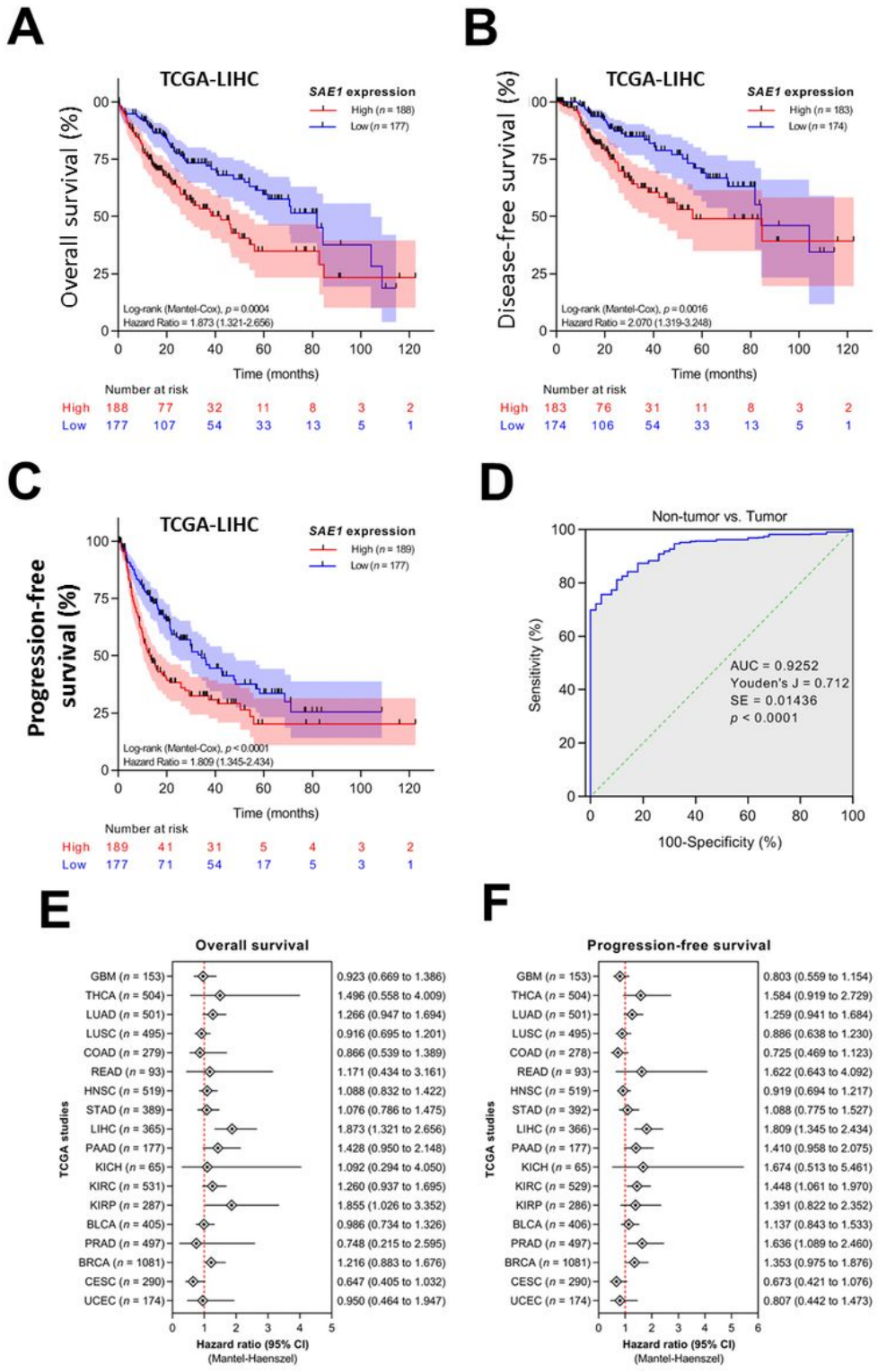
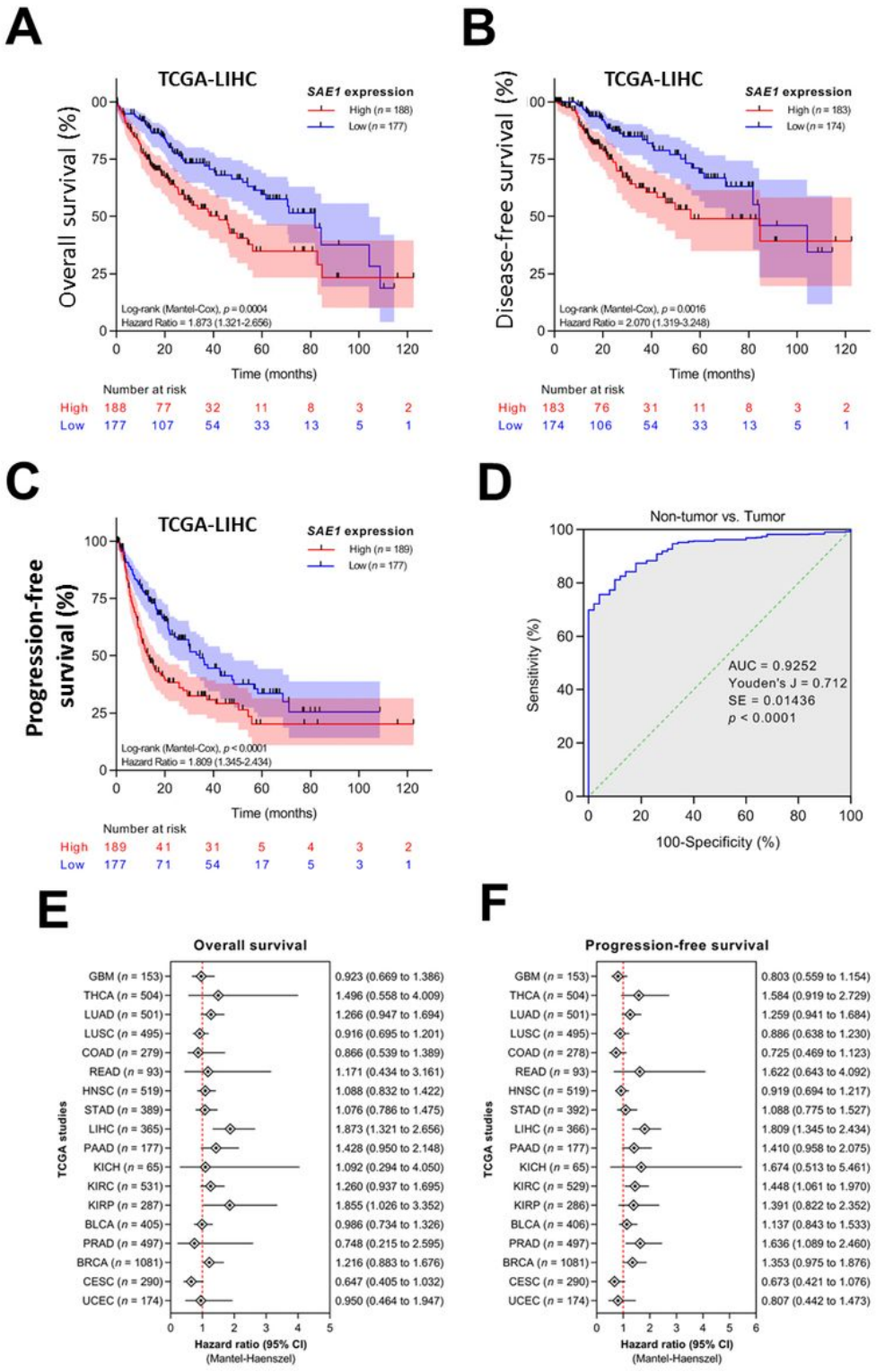


Figure 4

SAE1 is a reliable diagnostic and prognostic biomarker for HCC. (A-C) Kaplan-Meier curves of overall survival, disease-specific survival and progression-free interval for HCC patients according to TCGA LIHC database. (D) ROC analysis of SAE1 expression in non-tumor versus tumor. (E-F) Forest plot showing hazard ratio estimates and 95% confidence intervals according to TCGA studies.



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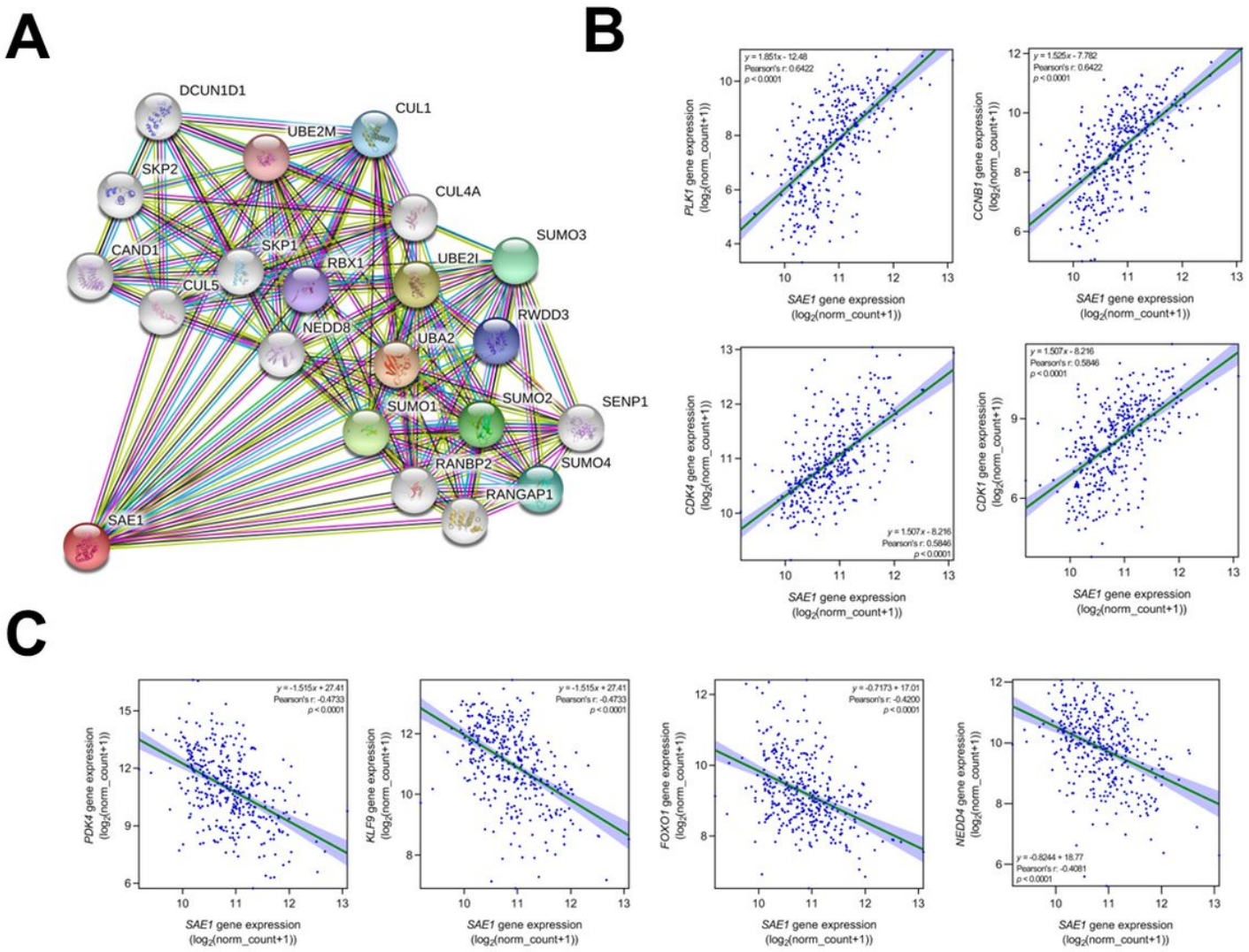

Figure 5

SAE1 upregulates oncogenic effectors of cell cycle progression while downregulating FOXO1-associated tumor suppressing signaling. (A) The SAE1-involved protein-protein interaction network constructed by STRING database, (B) The expression relationship between SAE1 and oncogenes/tumor suppressor genes.

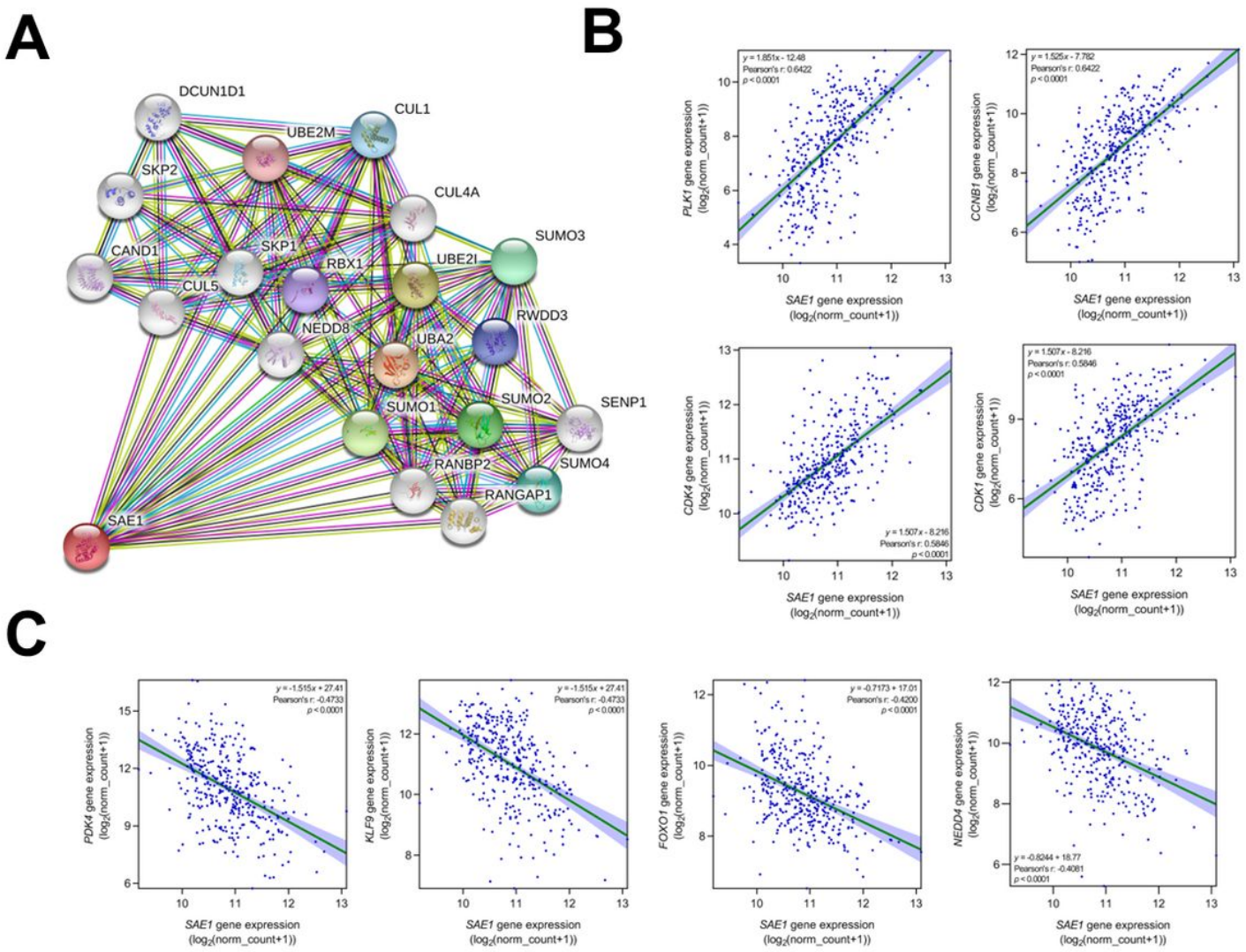

Figure 5

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Supplementary Files

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