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*Original Article*

# Coiled-coil domain-containing protein 58 (CCDC58) is a novel prognostic biomarker correlated with mitochondrial functions in hepatocellular carcinoma

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**Abstract:** Background: Recent researches found that mitochondrial functions were substantially involved in tumor progression, whereas the particular mechanism is unrecognized. Coiled-Coil Domain-Containing Protein 58 (CCDC58), one of the mitochondrial matrix import factors, acts as a novel regulator or stabilizer involved in mitochondrial protein import machinery. Whether and how an up-regulation of CCDC58 causes poor prognosis of patients in Hepatocellular Carcinoma (HCC) still required further researches. Methods: Tumor immune estimation resource (TIMER), Hepatocellular Carcinoma Database (HCCDB) and UALCAN databases were utilized to explore the expression level in diverse types of tumors compared with normal tissues. The prognostic potential of CCDC58 mRNA was evaluated via the Kaplan-Meier plotter, Gene Expression Profiling Interactive Analysis (GEPIA) and the Human Protein Atlas (HPA) databases. Corresponding clinicopathological factors were analyzed in Kaplan-Meier plotter. According to the median of mRNA expression levels of CCDC58, we divided The Cancer Genome Atlas (TCGA) data of HCC patients into two groups, highly expressed one and lowly expressed one, so as to perform the enrichment analyses of Gene Oncology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Protein-Protein Interaction (PPI) Network was constructed by STRING site and the co-expressed genes were functionally enriched. Immunohistochemistry was adopted to detect protein expression of CCDC58 in HCC patients. Results: This study indicated that CCDC58 protein expression level was obviously higher in HCC than that in paired paracancerous tissues. The up-regulated CCDC58 mRNA is prone to poor prognosis of patients in HCC through various indexes, such as overall survival (OS), disease-free survival (DFS), disease-specific survival (DSS), relapse-free survival (RFS) and progression-free survival (PFS). Additionally, univariate and multivariate Cox regression analyses suggested that CCDC58 could be viewed as an independent risk factor for HCC patients. The expression of CCDC58 is associated with 28 GO terms related to mitochondria and 5 KEGG pathways including oxidative phosphorylation. The PPI network revealed 10 interactive proteins about constituent components of mitochondria. Conclusions: These findings demonstrated CCDC58 to be a potential diagnostic and prognostic biomarker in HCC and correlated with mitochondria acting on tumor biosynthesis and energy production. It is reliable for CCDC58 to be targeted to design novel treatments for HCC patients.

**Keywords:** Coiled-coil domain-containing protein 58, hepatic cell carcinoma, prognosis, mitochondria

## Introduction

Hepatocellular carcinoma (HCC), as the fourth most common cause of cancer-related death, contributes much to the worldwide cancer burden [1]. The well-established risk factors for HCC include Hepatitis B or C virus infections, alcohol consumption, metabolic syndrome, diabetes, obesity and nonalcoholic fatty liver disease [2, 3]. The high rates of relapse and metastasis of HCC lead to poor prognosis. Additionally, during the progression of HCC, many biomarkers play the crucial role in tumor growth and invasion [4]. Therefore, early detection of high-risk patients, exploration of novel sensitive biomarkers and investigations of the

potential molecular mechanisms remain prerequisites for improving the clinical outcomes.

Mitochondria play key roles in the energy production and biosynthesis of tumor cells. It was once generally believed that the growth of tumor tissues depended on the overactive glycolysis mechanism in the cytoplasm. However, with the in-depth study of tumor metabolism advancing, such exact tumorigenic cell populations were discovered reliant heavily on mitochondrial respiration [5]. The oxidative phosphorylation (OXPHOS) in mitochondria of tumor cell was proved to facilitate tumor tissue growth. Mitochondrial deletion and OXPHOS deficiency significantly caused the limitation of tumorigenic potential [6] and improvement of the cytotoxic drugs' sensitivity of tumor cells [7]. Novel strategies have been designed based on targeting mitochondrial function, such as OXPHOS [8]. Tumorigenic biomarkers are necessarily required.

Since mitochondria dysfunction was reported to promote HCC [9], to better predict the prognosis of HCC patients, we identified a total of 11 mitochondria related genes that may significantly influence the outcome of HCC patients. These 11 genes were then submitted to least absolute shrinkage and selection operator (LASSO) analysis to construct the prognostic model. We finally established a mitochondria-related prognostic signature with 6 genes. The area under the curve (AUC) values of the model were 0.731, 0.708, and 0.716 at 1, 2 and 5 years. Since the immunohistochemistry (IHC) revealed that only CCDC58 was upregulated in HCC compared with the paracancerous tissue, we further explored the importance of CCDC58 in HCC.

CCDC58, also known as Mix23, is an intermembrane space protein [10]. It was demonstrated to be a novel regulator or stabilizer in the process of effective proteins import into the mitochondrial matrix, and in particular in temperature-sensitive Tim17 mutants, the scarcity of Mix23 issues in the synthetic growth defect [11]. Besides, CCDC58 was found to be correlated with the mitochondrial single-nucleotide polymorphisms, an essential impact factor in Acquired Immune Deficiency Syndrome (AIDS) progression [12]. As a coiled-coil domain-containing protein, CCDC58 also functioned in the inner mitochondrial membrane [13]. However, the expression levels and the potential function of CCDC58 in tumorigenesis remain uncertain.

This study aimed at investigating the diagnosis and prognosis potential of CCDC58 in HCC. In detail, Tumor Immune Estimation Resource (TIMER), Hepatocellular Carcinoma Database (HCCDB) and UALCAN database were utilized to ascertain whether CCDC58 was overexpressed in HCC compared with the adjacent tissue. Furthermore, UALCAN also contributed to exploring the expression profiles of CCDC58 in terms of corresponding clinicopathologic features of HCC. To analyze its prognosis potential, we had access to Kaplan-Meier plotter and Interactive Analysis (GEPIA) databases. To figure out the function of biological macromolecules under the potential mechanism, we employed GSEA and explored the co-expressed genes of CCDC58 via Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). For further understanding of the related functions and mechanism, we established Protein-Protein Interaction (PPI) Network to explore the function-related proteins. Eventually, as a proof of protein expression levels of CCDC58, IHC was employed as soon as we obtained tissues of HCC patients and the paired adjacent tissues. On the one hand, our conclusion of CCDC58 whose expression level was apparently increased in HCC, shed light on its vital role in the HCC diagnosis. On the other hand, we reveal the prognosis significance of CCDC58 as new targets and strategies for HCC treatment.

## Methods

### *HCCDB analysis*

The CCDC58 mRNA expression in HCC was identified from the HCCDB database (http://lifeome.net/database/hccdb), a one-stop online resource, curating 15 public datasets that cover approximately 4000 clinical samples [14]. It integrates data from the Gene Expression Omnibus (GEO), Liver Hepatocellular Carcinoma Project of The Cancer Genome Atlas (TCGA-LIHC) and Liver Cancer - RIKEN, JP Project from International Cancer Genome Consortium (ICGC LIRI-JP). And the 4D metric it defined is conducive to comprehensively understanding co-expression networks in cancerous tissue, adjacent liver tissue and normal liver tissue. Subscribers also can conveniently obtain

graphical results from computational analyses involving differential expression analysis, survival analysis, and co-expression analysis.

*UALCAN analysis*

UALCAN (http://ualcan.path.uab.edu) integrates data from TCGA level 3 RNA-seq and clinical data from 31 cancer types [15]. It is an online tool to analyze gene expression profiles, as well as in various tumor sub-groups based on individual cancer stages, tumor grade, race or other clinicopathologic features.

*Immunohistochemistry (IHC)*

Formalin-fixed HCC patients' tissue samples were embedded in paraffin. Tissue sections were made and baked at 59°C for 1 h for deparaffinage. Dealing with inactivation and antigen-repair treatment, sections were then stained with CCDC58 antibody (1:100, Thermofisher, P5A-61587) overnight at 4°C and secondary antibodies were incubated for 1 h at room temperature. Next, phosphate buffered saline (PBS) was utilized to wash the sections and hematoxylin to repeatedly stain. Subsequently, sections were covered and observed by microscope. The scoring criterion was multiplying the score of staining intensity and the score of positive area. The staining intensity score was categorized as negative (score = 0), weak (score = 1), moderate (score = 2) and strong (score = 3), while the positive area scoring was based on the staining percentage of the tumor cells in range of 0-100. If different staining intensities were observed in the same slice, the H score should be the sum of the products of staining intensity and corresponding area. As a result, the H score ranges from 0 to 300. Furthermore, the calculated H score was applied for statistical analyses as low expression and high expression divided by the median.

*Kaplan-Meier plotter and GEPIA analysis*

The Kaplan-Meier plotter contributed to identifying the prognostic value of CCDC58 and expression in liver, breast, lung, prostate, gastric and ovarian cancer. In particular, it is testified towards the tool that overexpression of CCDC58 correlates to the clinical prognosis in HCC with different clinicopathological factors. Hazard ratios (HR) and log-rank *P*-values were taken as parameters of group cutoff: median; hazards ratio: yes; 95% confidence interval: yes [16].

The GEPIA database (http://gepia.cancer-pku.cn/), a universally available online database, is emerged as an analyzing tool for RNA sequencing expression data from TCGA and the GTEx projects [17]. The data comes from 9,736 tumors and 8,587 normal samples. The website also had functions in generate survival curves of overall survival (OS) and recurrence-free survival (RFS), which is on account of the log-rank test and the Mantel-Cox test.

*TIMER database analysis*

The Tumor Immune Estimation Resource (TIMER) (http://timer.comp-genomics.org/), known as a consolidated database for immune infiltration analysis, contains more than 10,000 samples of 32 tumor types from The Cancer Genome Atlas (TCGA). The database aimed at determining the abundance of tumor infiltrates based on gene expression [18]. We obtained different expression levels of CCDC58 mRNA in various types of cancers via the DiffExp module with default parameters. The correlation remained unapparent between CCDC58 mRNA and infiltration of various tumor immune cells, including B cells, $CD8^+$ T cells, dendritic cells, T cells (general), TAMs, M1 macrophages, M2 macrophages, monocytes, neutrophils, natural killer cells, T helper cells (Th), regulatory T cells (T(reg) cells), follicular helper T cells (Tfh), and exhausted T cells.

*Gene set enrichment analysis (GSEA)*

369 Liver hepatocellular carcinoma cases downloaded from TCGA database were separated into two groups based on the median value of CCDC58 mRNA expression level. In order to determine potential hallmark and signal pathway of HCC, GSEA analysis was performed using the GSEA software version 4.1. In detail, we selected gene matrix collections including h.all.v7.4.symbols.gmt, c2.cp.kegg.v7.4symbols.gmt and c5.go.v7.4symbols.gmt that contained three sub-collections: biological process (BP), molecular function (MF), and cellular component (CC) adopted from The Molecular Signatures Database (MSigDB). The permutations terms were set to 1,000 times, for "phenotype" and other parameters default

values. The remarkably significant enrichment gene sets were displayed with plots. The screening threshold was set at FDR q-value < 25% and nominal *P*-value < 5%.

*PPI network*

STRING (https://string-db.org) is a public online database for establishing PPI network construction with retrieved and screened hub genes [19]. The medium confidence is set at 0.4 in minimum. The edges connected nodes in the plot represent the associations of the pair. The PPI network of CCDC58 was built with 11 genes after excluding the irrelevant ones. The correlations and the potential functions of the genes were validated via GO analysis.

*Clinical samples*

57 pairs of HCC tumor tissues (C) and matched adjacent tissues (N) were obtained through hepatectomy from 57 patients that were histologically diagnosed with resectable HCC in Changhai Hospital. This study was approved by the Ethics Committee of Changahi Hospital affiliated to Naval Medical University. Inclusion criteria: 1. Patients were histologically diagnosed with HCC. 2. Patients were 18-60 years old. Exclusion criteria: 1. Patients were also diagnosed with other malignant tumors. 2. Patients were also diagnosed with severe autoimmune diseases or acquired immune diseases.

*Statistical analysis*

Part of the statistical analysis and the visualization work was accomplished using R program, such as GO and KEGG pathway analysis. The comparative results of immunohistochemistry were analyzed by Mann-Whitney test. The survival curve was depicted with Kaplan-Meier method in the way of log-rank test. And for shedding light on the relationship between CCDC58 expression and the OS or PFS in HCC patients with various clinical characteristics, we utilized univariate and multivariate Cox regression. A value of $P < 0.05$ was considered statistically significant.

## Results

*Construction of a prognostic panel composed of 6 mitochondria-related genes' signature*

Since mitochondria dysfunction was reported to promote HCC [9], to better predict the prognosis of HCC patients, we identified 115 genes that may be related to the prognosis of HCC using GEPIA ($P < 0.05$). Depending on subcellular localization, a list of 1,136 mitochondria-located genes were downloaded from MitoCarta3.0 [20] (https://www.broadinstitute.org/) (Supplementary Table 1). After preliminary screening, a total of 11 genes related to oxidative stress were identified. **Figure 1A** shows the Venn diagram of screening. These 11 genes - Coiled-Coil Domain-Containing Protein 58 (CCDC58), Mitochondrial Carrier 1 (MTCH1), RNA Pseudouridine Synthase D3 (RPUSD3), Translocase of Inner Mitochondrial Membrane 23 (TIMM23), Mitochondrial Inner Membrane Protein MPV17 (MPV17), MutY DNA Glycosylase (MUTYH), Ubiquinol-Cytochrome C Reductase Hinge Protein (UQCRH), Deoxythymidylate Kinase (DTYMK), Mitochondrial Ribosomal Protein L11 (MRPL11), member of RAS oncogene family (RAB24) and Acyl-CoA Thioesterase 7 (ACOT7) were submitted to LASSO analysis to construct the prognostic model (**Figure 1B**). The independent variable's trajectory was explored in **Figure 1C**, and 10-fold cross-validation was used to analyze the CI under each lambda, as shown in **Figure 1D**. We finally established a mitochondria-related prognostic signature with 6 genes, including CCDC58, TIMM23, MPV17, MUTYH, UQCRH, and DTYMK (**Figure 1E**). The area under the curve (AUC) values of the model were 0.731, 0.708, and 0.716 at 1, 2 and 5 years (**Figure 1F**). Since the IHC revealed that only CCDC58 was upregulated in HCC compared with the paracancerous tissue, we further explored the importance of CCDC58 in HCC.

*The mRNA expression levels of CCDC58 in hepatocellular carcinoma and other cancers*

The TIMER online database was utilized to analyze the differential expression levels of CCDC58, and compare multiple cancers with matched normal tissues in TCGA. In consequence, we found that CCDC58 was overexpressed in liver hepatocellular carcinoma (LIHC) and various cancers such as bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), stomach adenocarcinoma (STAD), and uterine corpus endometrial carcinoma (UCEC), while lowly expressed in thyroid carcinoma

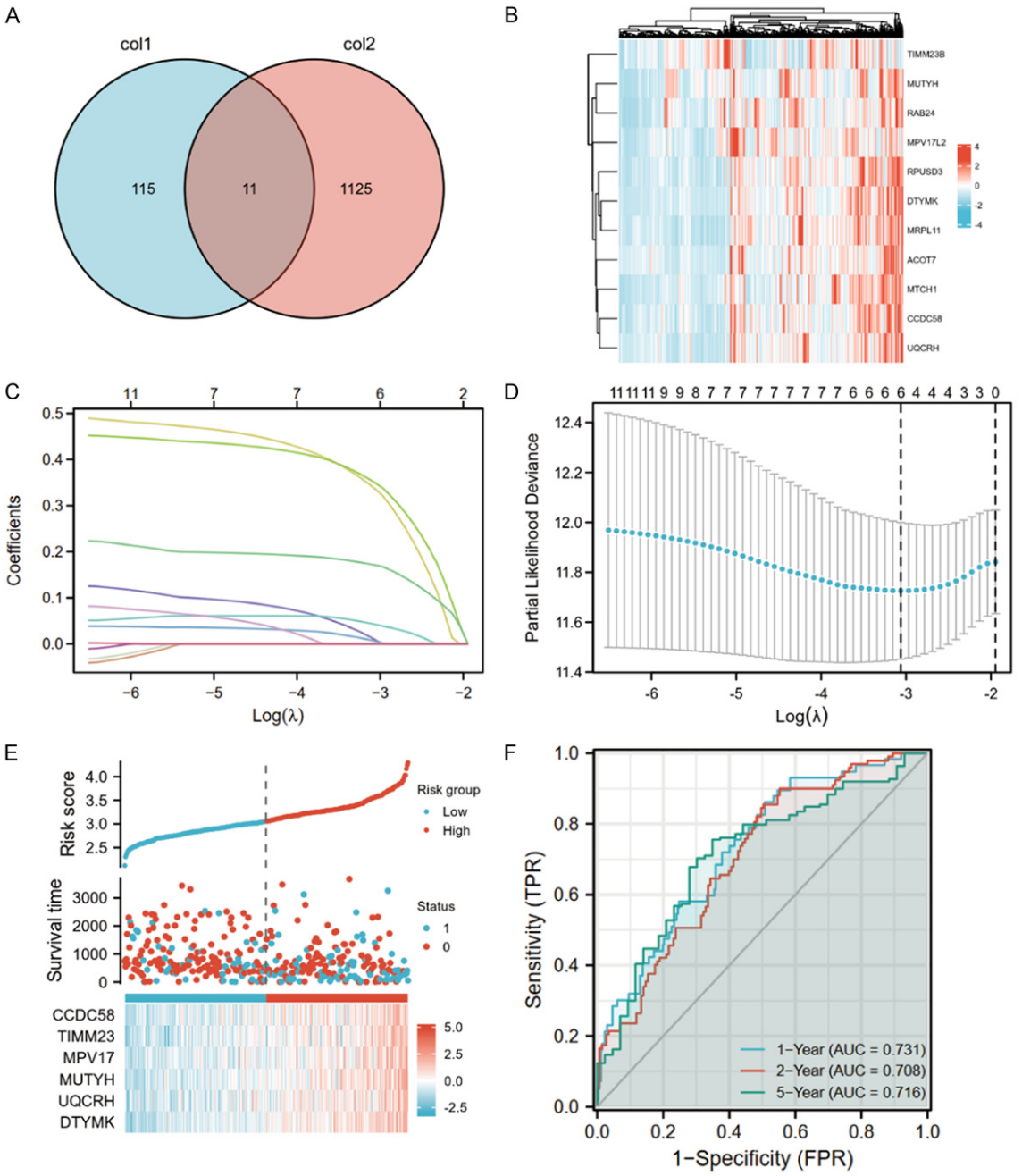
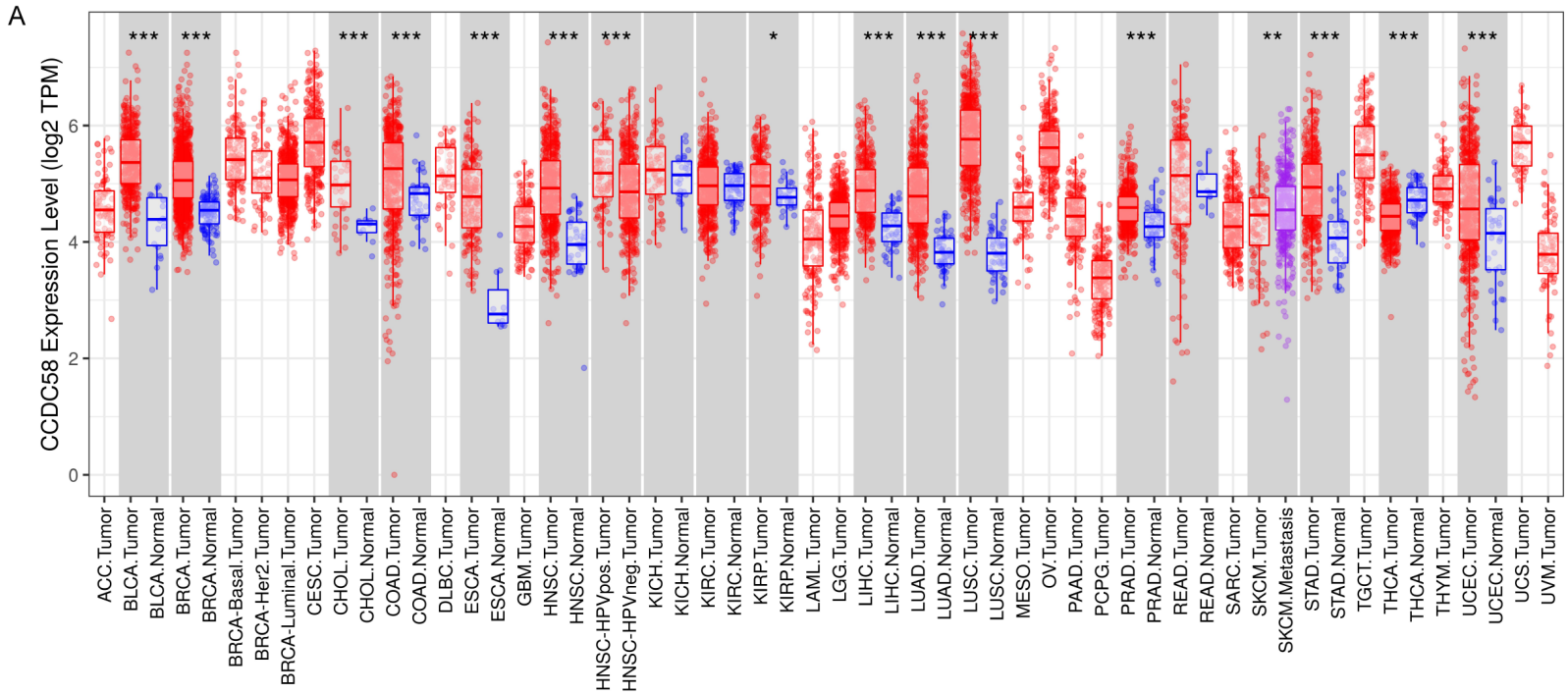



**Figure 1.** Prognostic modal of 6 mitochondria-related genes' signature. (A) Venn plot and (B) heat plot of HCC prognostic-related genes and mitochondria-related gene. (C) Individual gene's trajectory. The horizontal axis represents the log value of the gene lambda, and the vertical axis represents the independent gene's coefficient. (D) CIs with different values of lambda. (E) Prognostic modal of 6 mitochondria-related genes' signature. (F) Receiver operating characteristic (ROC) curve of 6 mitochondria-related genes' signature prediction.

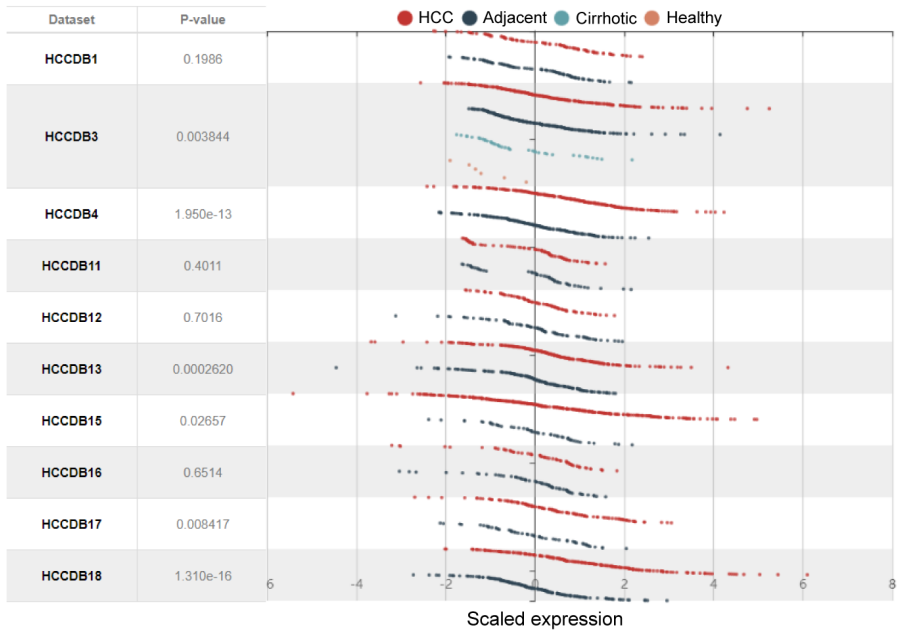
(THCA) ($P < 0.05$) (**Figure 2A**). Consequently, higher expression of CCDC58 in HCC tissue compared with the adjacent tissue was observed in most datasets (6/10), such as HCCDB3, HCCDB4, HCCDB13, HCCDB15, HCCDB17 and HCCDB18 (**Figure 2B**). Additionally, it was validated that CCDC58 differentially expressed in HCC specimens in TCGA based on sample types, individual cancer stages, patient's race and tumor grade. In detail, as is depicted in **Figure 2C-F**, CCDC58 mRNA levels were obviously higher in tumor and adjacent

The prognostic value of CCDC58 in hepatocellular carcinoma



B

| Dataset | P-value |
|---|---|
| HCCDB1 | 0.1986 |
| HCCDB3 | 0.003844 |
| HCCDB4 | 1.950e-13 |
| HCCDB11 | 0.4011 |
| HCCDB12 | 0.7016 |
| HCCDB13 | 0.0002620 |
| HCCDB15 | 0.02657 |
| HCCDB16 | 0.6514 |
| HCCDB17 | 0.008417 |
| HCCDB18 | 1.310e-16 |

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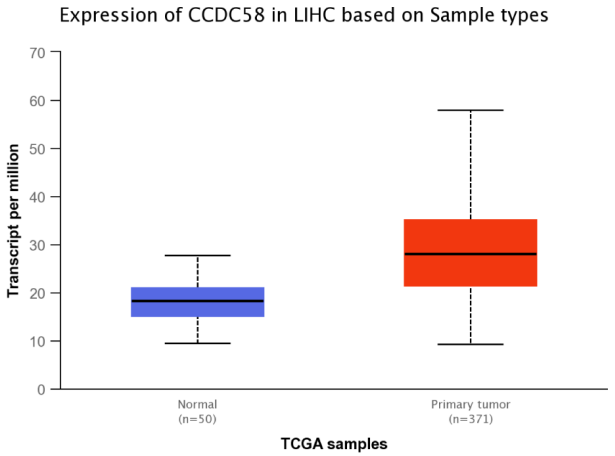


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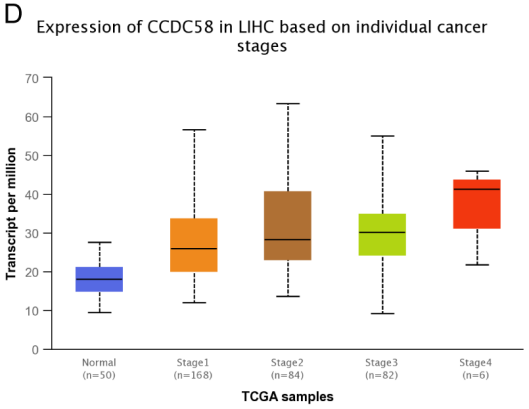


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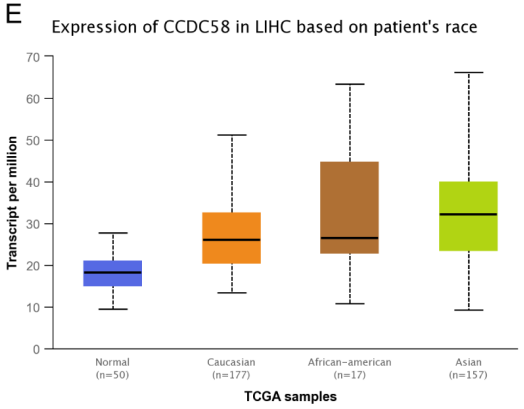


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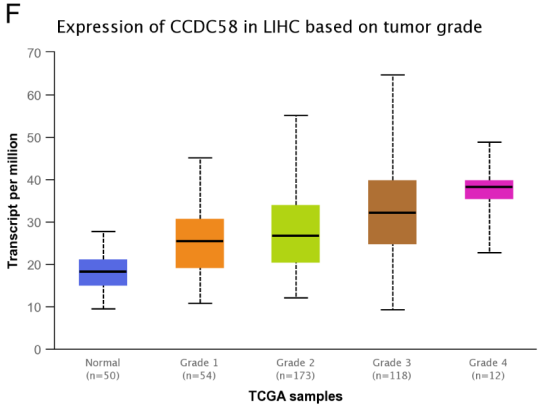

**Figure 2.** The expression levels of CCDC58 were analyzed via three databases. (A) Human CCDC58 expression levels in diverse cancer types were determined by Tumor Immune Estimation Resource (TIMER) site (*P < 0.05, **P < 0.01, ***P < 0.001). (B) The expression of CCDC58 in Hepatocellular Carcinoma Database (HCCDB) datasets including 10 datasets. And CCDC58 transcription in subgroups of HCC patients, stratified in terms of cancer stage, tumor grade and other criteria in UALCAN database. Box-whisker plots showing that the expression of CCDC58 was positively associated with sub groups, like (C) CCDC58 in normal and Liver hepatocellular carcinoma (LIHC) individuals; (D) tumor stage 1, 2, 3 or 4; (E) race containing African American, Caucasian and Asian; (F) tumor grade 1, 2, 3 or 4. * represents P < 0.05, ** represents P < 0.01, *** represents P < 0.001.

tissues. The consequence reflected incremental increase in the CCDC58 expression levels with higher individual cancer stage and tumor grade. Moreover, we further explored the levels of CCDC58 deoxyribonucleic acid (DNA) copy in HCC in virtue of HCCDB database, a specific HCC database including 12 different HCC datasets. According to the results from TIMER, HCCDB and UALCAN, we reach the conclusion that CCDC58 is a potential diagnostic biomarker for HCC patients.

*High expression of CCDC58 corresponded with poor prognosis of hepatocellular carcinoma*

There are several prognostic monitoring indexes and the common ones refer to overall survival (OS), disease-free survival (DFS), disease-specific survival (DSS), relapse-free survival (RFS) and progression-free survival (PFS). We utilized the indexes to testify the prognosis value of CCDC58 in HCC. Hence, CCDC58 was found to be a poor prognostic factor for liver cancer patients in the Kaplan-Meier plotter database, which is based on Affymetrix microarrays (**Figure 3A-D**; OS HR [95% CI] = 1.56 [1.11-2.21], P = 0.01; RFS HR [95% CI] = 1.51 [1.07-2.13], P = 0.017; DSS HR [95% CI] = 1.68 [1.08-2.61], P = 0.02; PFS HR [95% CI] = 1.37 [1.01-1.87], P = 0.045). Consequently, we further assessed whether CCDC58 expression was associated with poor prognosis via GEPIA database whose sequencing data were from TCGA (**Figure 3E**, OS, P = 1.7E-7; **Figure 3F**, DFS, P = 0.0062). As is shown, the trend was apparently observed that high CCDC58 expression level predicts lack of survival benefit in HCC patients. Additionally, we evaluated the survival rate of other cancer types with high expression of CCDC58 mRNA. According to the analyses in Kaplan-Meier database, high CCDC58 caused poor overall survival in lung cancer, poor relapse-free survival in breast cancer, poor progression free survival in ovarian cancer, while well first progression survival, overall survival and post progression survival in gastric cancer (Supplementary Figure 1).

*The correlation between CCDC58 expression and clinical characteristics of HCC patients*

To better understand the clinical relevance of CCDC58 expression in HCC, we investigated the corresponding characteristics of HCC patients in the Kaplan-Meier plotter databases. As is shown in **Table 1**, high CCDC58 expression could bring about both worse OS and PFS in patients without alcohol consumption (OS: HR = 1.75, P = 0.016; PFS: HR = 1.60, P = 0.034) and without hepatitis viral infection (OS: HR = 1.87, P = 0.014; PFS: HR = 1.74, P = 0.018). Particularly, overexpression of CCDC58 negatively impacted OS and PFS in stage 3 patients (OS: HR = 2.24, P = 0.0064; PFS: HR = 2.16, P = 0.0052), stage 2+3 patients (OS: HR = 2.29, P = 0.029; PFS: HR = 1.61, P = 0.018), stage 3+4 patients (OS: HR = 2.23, P = 0.0049; PFS: HR = 2.11, P = 0.0056) and OS in stage 1 patients (HR = 2.34, P = 0.008), PFS in stage 2 (HR = 1.98, P = 0.049). It was demonstrated that high CCDC58 expression shortened the survival time of the HCC patients. Curiously, whether there was vascular invasion or not, CCDC58 expression and PFS showed the same negative association. Therefore, the analyses of clinical features implied that the application of CCDC58 mRNA as a prognostic indicator should not be divorced from individual patient's condition.

*CCDC58 was related to several mitochondria-related functions and pathway in HCC*

Mitochondria were deemed as pivotal in cell metabolism, whose dysfunction was suspected to be involved in tumorigenesis and tumor progression [21]. The oxidative phosphorylation occurred in mitochondria promoted tumor growth. In order to figure out the correlation between CCDC58 expression and mitochondria, we conducted GO term and KEGG pathway analyses using GSEA. Among the results of GO and KEGG analyses, those involved in variations of CCDC58 expression were notably significant ones correlated with mitochondria and

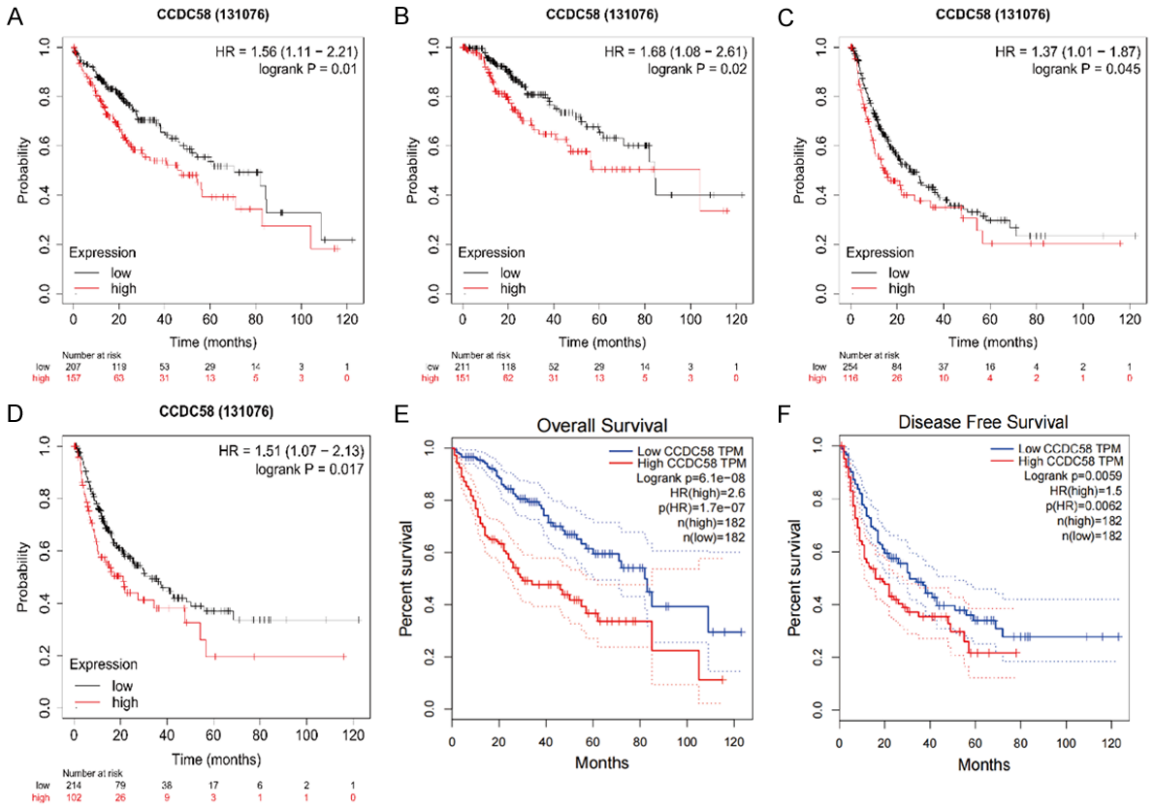


**Figure 3.** Evaluating the correlation between CCDC58 expression and prognostic survival in HCC patients through Kaplan-Meier plotter. (A) Overall survival, n = 364; (B) Disease-specific survival, n = 362; (C) Progression-free survival, n = 370; (D) Relapse-free survival, n = 316. OS (E) and DFS (F) analyses in Gene Expression Profiling Interactive Analysis (GEPIA) site with 364 paired samples from The Cancer Genome Atlas (TCGA) database.

the respiratory chain. The GO aspects were respectively displayed in **Figure 4A-C**. The enriched BP contained: positive regulation of release of cytochrome C from mitochondria; oxidative phosphorylation; mitochondrial cytochrome C oxidase assembly; protein targeting to mitochondrion; protein localization to mitochondrion; mitochondrial organization, transport, respiratory chain complex assembly, fusion, calcium iON homeostasis, transmembrane transport, morphogenesis, cytochrome C oxidase assembly; protein import into mitochondrial matrix and so on. In the meanwhile, the CC and MF terms were involved in intrinsic component of mitochondrial membrane (including the inner and the outer), inner mitochondrial membrane protein complex, ATPase regulator and activator activity and ligase activity forming carbon oxygen bonds. Besides, the gene sets were enriched in oxidative phosphorylation (ES = 0.699, normal *P*-value = 0.002, FDR q-value = 0.051) by KEGG analyses, as shown in **Figure 4D**, **4E**. Lastly, the enriched results from GSEA reflected that CCDC58 participated in various functions and pathways related to mitochondria. Besides, two cancer related pathways: cell cycle pathway (ES = 0.460, normal *P*-value = 0.082, FDR q-value = 0.340) and P53 signaling pathway (ES = 0.324, normal *P*-value = 0.166, FDR q-value = 0.597) were also enriched, which partly explained the poor prognosis of CCDC58 high expression patients.

*Establishment of the PPI network*

Next, we depicted a plot of PPI network using STRING database so as to shed light on the potential mechanisms of CCDC58. Strong interactions were detected between CCDC58 and ATP synthase peripheral stalk subunit F6 (ATP5J), mitochondrial ribosomal protein L39 (MRPL39), mitochondrial ribosomal protein L35 (MRPL35), single stranded DNA binding

**Table 1.** Correlation of Coiled-Coil Domain-Containing Protein 58 (CCDC58) mRNA expression levels and clinical prognosis in hepatocellular carcinoma (HCC) patients with diverse clinicopathological attributions via Kaplan-Meier plotter

| Clinicopathological characteristics | Overall survival (n = 364) | | | Progression-free survival (n = 370) | | |
|---|---|---|---|---|---|---|
| | N | Hazard ratio | *P*-value | N | Hazard ratio | *P*-value |
| Sex | | | | | | |
| Female | 118 | 1.55 (0.80-2.98) | 0.190 | 121 | 1.71 (0.99-2.96) | 0.051 |
| Male | 246 | 1.86 (1.17-2.98) | **0.008** | 249 | 1.27 (0.87-1.86) | 0.220 |
| Vascular invasion | | | | | | |
| None | 203 | 1.37 (0.82-2.30) | 0.230 | 205 | 0.62 (0.40-0.99) | **0.041** |
| Yes | 90 | 1.97 (0.79-4.90) | 0.14 | 92 | 2.07 (1.16-3.70) | **0.011** |
| Race | | | | | | |
| white | 181 | 1.81 (1.14-2.87) | **0.011** | 184 | 1.46 (0.96-2.22) | 0.072 |
| Asian | 155 | 1.59 (0.82-3.09) | 0.17 | 157 | 1.58 (0.96-2.59) | 0.069 |
| Stage | | | | | | |
| 1 | 170 | 2.34 (1.23-4.47) | **0.008** | 171 | 0.66 (0.40-1.10) | 0.106 |
| 2 | 83 | 0.67 (0.29-1.54) | 0.34 | 85 | 1.98 (0.99-3.95) | **0.049** |
| 1+2 | 253 | 1.75 (1.07-2.85) | **0.025** | 256 | 1.31 (0.88-1.95) | 0.190 |
| 3 | 83 | 2.24 (1.24-4.06) | **0.006** | 85 | 2.16 (1.24-3.77) | **0.005** |
| 2+3 | 166 | 1.68 (1.05-2.68) | **0.029** | 170 | 1.61 (1.08-2.41) | **0.018** |
| 3+4 | 87 | 2.23 (1.26-3.96) | **0.005** | 90 | 2.11 (1.23-3.61) | **0.006** |
| 4 | 5 | - | - | 5 | - | - |
| Grade | | | | | | |
| 1 | 55 | 4.81 (1.64-14.12) | **0.002** | 55 | 2.05 (0.93-4.52) | 0.071 |
| 2 | 174 | 1.67 (0.93-2.99) | 0.084 | 177 | 1.22 (0.75-1.99) | 0.428 |
| 3 | 118 | 1.58 (0.86-2.89) | 0.134 | 121 | 0.76 (0.45-1.27) | 0.300 |
| 4 | 12 | - | - | 12 | - | - |
| Alcohol consumption | | | | | | |
| yes | 115 | 1.79 (0.92-3.49) | 0.084 | 117 | 1.69 (0.91-3.15) | 0.093 |
| none | 202 | 1.75 (1.10-2.77) | **0.016** | 205 | 1.60 (1.03-2.48) | **0.034** |
| Hepatitis virus | | | | | | |
| yes | 150 | 1.91 (0.99-3.70) | 0.051 | 153 | 0.63 (0.40-1.01) | 0.053 |
| none | 167 | 1.87 (1.13-3.09) | **0.014** | 169 | 1.74 (1.10-2.75) | **0.018** |
| AJCC | | | | | | |
| 1 | 180 | 2.09 (1.14-3.81) | **0.014** | 181 | 0.64 (0.39-1.05) | 0.072 |
| 2 | 90 | 0.61 (0.27-1.38) | 0.230 | 93 | 2.09 (1.03-4.22) | **0.037** |
| 3 | 78 | 2.25 (1.23-4.12) | **0.007** | 80 | 1.74 (0.99-3.08) | 0.053 |
| 4 | 13 | - | - | 14 | - | - |
| Sorafenib treat | | | | | | |
| treated | 29 | 4.72 (1.48-15.04) | 0.082 | 30 | 2.16 (0.97-4.81) | 0.053 |

Bold values indicate P < 0.05.

protein 1 (SSBP1), VHL binding protein 1 (VBP1), poly(rC) binding protein 2 (PCBP2), guanine nucleotide-binding protein subunit beta-2-like 1 (GNB2L1), TIA1 cytotoxic granule associated RNA binding protein like 1 (TIAL1), cyclin G associated kinase (GAK), and mitochondrial transcription rescue factor 1 (C6orf203) proteins (**Figure 5A**). Consequently, enrichment analysis was performed for the further interactive exploration of these proteins. The results from cellular components analysis provided a reliable basis for these protein connected with mitochondria. In detail, they are enriched in mitochondrion, mitochondrial protein complex,

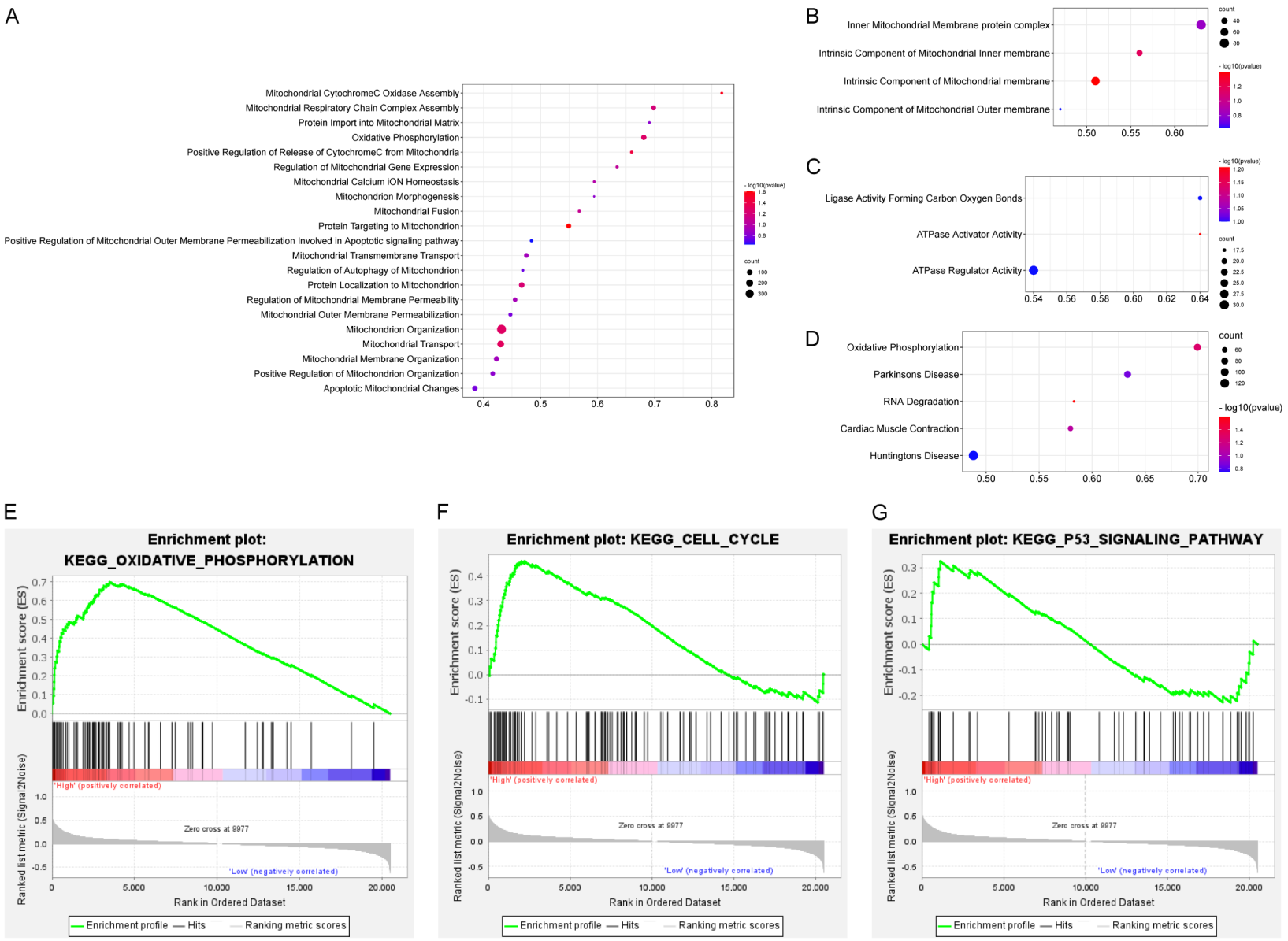


**Figure 4.** The significantly enriched Gene Ontology (GO) annotations and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways correlated with mitochondria of CCDC58 in HCC: (A) biological processes, (B) cellular components, (C) molecular functions, (D) KEGG pathways, (E) oxidative phosphorylation pathway, (F) Cell Cycle pathway and (G) P53 signaling pathway.

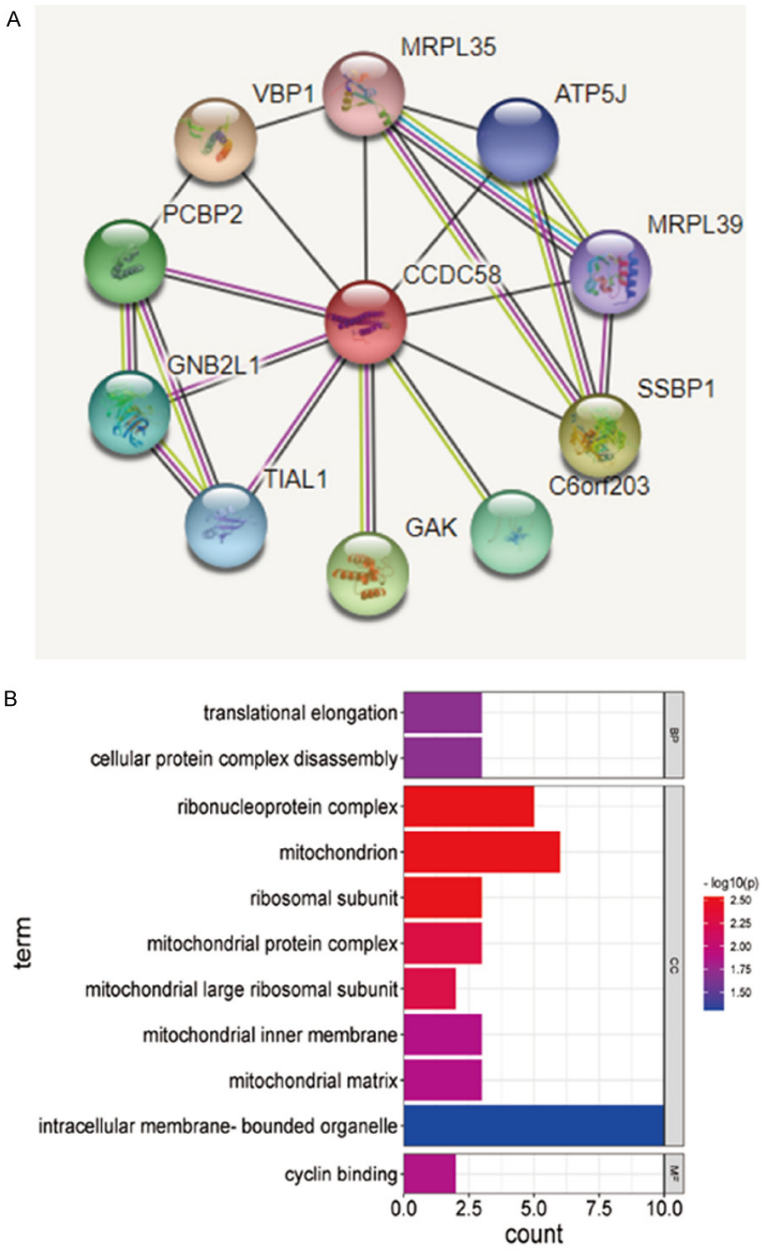


**Figure 5.** A. Protein-Protein Interaction Network (PPI) of CCDC58 in HCC contracted via STRING site. B. GO enrichment analyses of the interacting genes in HCC.

mitochondrial large ribosomal subunit, mitochondrial matrix, mitochondrial inner membrane (**Figure 5B**).

*The protein expression level of CCDC58 verified in HCC clinical samples*

In this single-center study of 57 HCC patients, 9 patients died of disease progression until this research was finished, so the data were not enough to analyze the OS and PFS, while the tumor stage, metastasis, AFP expression and Ki67 expression were compared between the high CCDC58 expression group and low CCDC58 expression group. Immunohistochemistry staining was performed to assess the CCDC58 protein expression. In accord with the previous bioinformatic analysis, as the **Figure 6A-D** depicted, the CCDC58 protein was expressed much higher in HCC tissues (**Figure 6A**) than the adjacent tissues (**Figure 6B**). We observed a much stronger dot-like cytoplasmic staining that gathered at mitochondria in HCC cells, which coincided with the result from another method, indirect immunofluorescence microscopy, in the human protein atlas. Moreover, the more positive intensity of immunostaining was prone to appearing in HCC tissues with higher grade, which indicated that the tumor tissue with low degree of differentiation (**Figure 6C**) was stained stronger than the well-differentiated tissue (**Figure 6D**) to a certain extent. We visualized the IHC results in heat map and gained the same conclusion of differential expression of CCDC58 in HCC ($P < 0.001$) (**Figure 6E**). High CCDC58 expression group showed an increased proportion of higher tumor grade (**Figure 6F**). However, we didn't observe significant difference in metastasis (**Figure 6G**), AFP expression (**Figure 6H**) and percentage of Ki67 expression (**Figure 6I**) between high CCDC58 expression group and low CCDC58 expression group.

## Discussion

Hepatocellular carcinoma (HCC), with the second highest mortality rate, posed a substantial threat to public health globally [22]. Merely

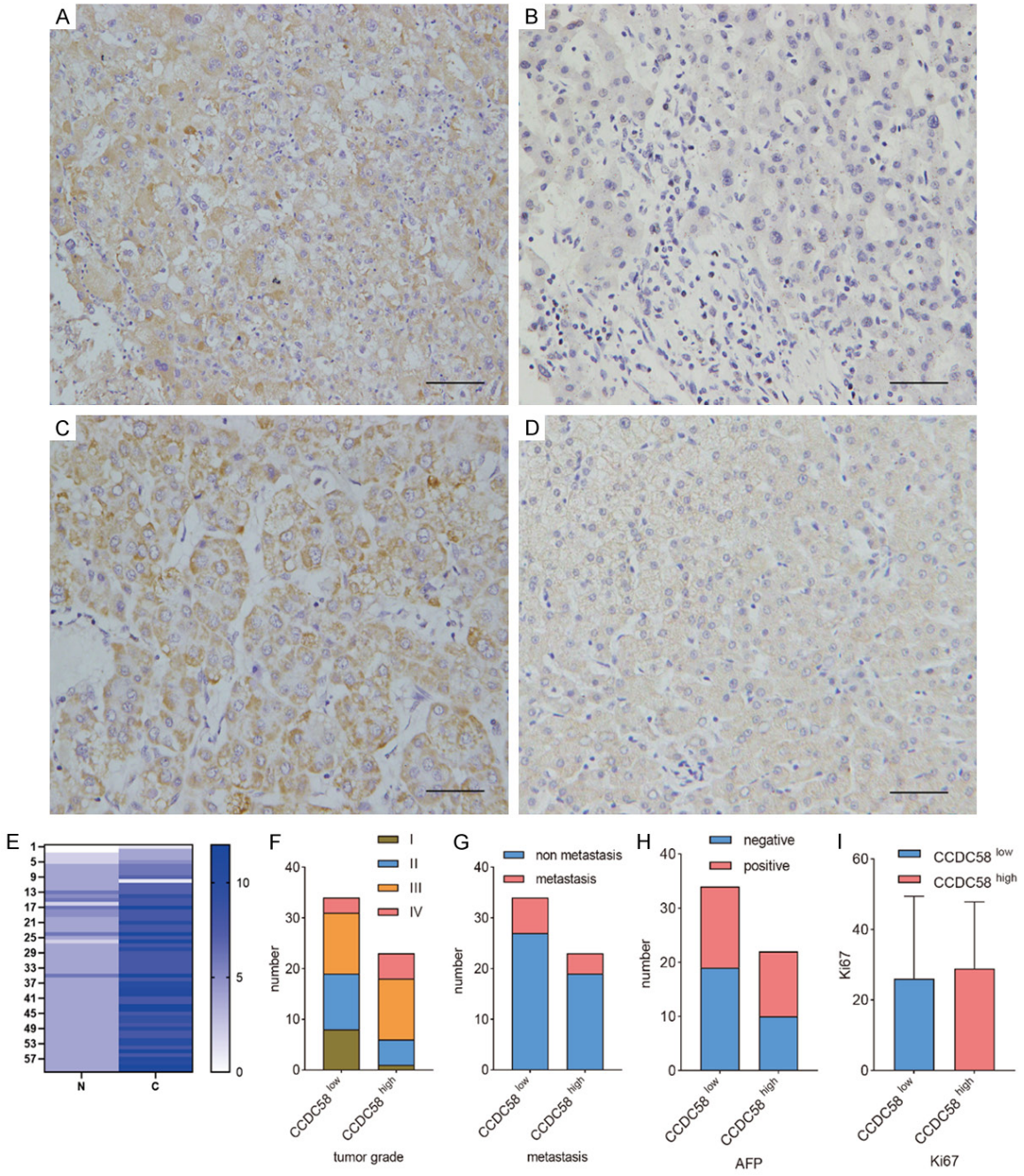


**Figure 6.** Immunohistochemistry staining of HCC tumor tissues (A), matched adjacent tissues (B), low differentiation HCC tumor tissue (C) and high differentiation HCC tumor tissue (D). Bar = 100 μm. (E) Immunohistochemistry results visualized in heat map. (F) Tumor grade, (G) metastasis, (H) AFP expression and (I) percentage of Ki67 expression between high CCDC58 expression group and low CCDC58 expression group.

20% HCC patients obtained prognostic benefits owing to early diagnosis and timely treatment, while the rest missed the opportunities of tumor resection due to advanced-stage diagnosis [23]. In this study, a mitochondria-related prognostic signature with 6 genes, including CCDC58, TIMM23, MPV17, MUTYH, UQCRH, DTYMK were identified to be an independent biomarker for predicting the outcome of HCC patients. Since the CCDC58 was the only gene upregulated on protein level, we decided to further explore the function of CCDC58 in HCC.

Using bioinformatics analysis methods, we identified CCDC58 as a novel potential diagnostic and prognostic biomarker for HCC patients. In particular, our results provide novel insights for understanding the potential role of CCDC58 in tumor mitochondria function.

We identified the expression level of CCDC58 in HCC via HCCDB and UALCAN database based on independent datasets from GEO and TCGA datasets. Consistent differential expression of CCDC58 was observed in both databases and we found that CCDC58 mRNA was up-regulated in HCC in contrast to normal tissues. The phenomenon was validated by IHC with 57 pairs of clinical samples. The expression of CCDC58 in other types of cancer was examined through TIMER analysis. Besides LIHC, CCDC58 was also highly expressed in BLCA, BRCA, CHOL, COAD, ESCA, HNSC, LUSC, PRAD, STAD and UCEC, while lowly expressed in THCA.

Additionally, it was testified whether the CCDC58 expression inversely affected prognosis utilizing diverse monitoring indexes in Kaplan-Meier, GEPIA and Atlas database. Here, in line with our expectations: high transcription levels of CCDC58 were obviously correlated with the OS and DSS of HCC patients, reflecting that the high expression of CCDC58 is harmful to the prognosis of HCC patients and might weaken the patients' capability of fighting against other diseases. Moreover, closely connecting with the PFS and RFS respectively, up-regulation of CCDC58 remains a hint at promoting severe deterioration and impacting survival rate of patients treated with multiple therapies. To explore the potential effects of CCDC58 expression on tumor progression, the clinicopathological parameters were further deliberated. UALCAN analysis indicated that higher cancer stages and tumor grades tended to be accompanied with higher CCDC58 expression, whereas there was none of apparent prognostic correlations in Kaplan-Meier. It might be attributed to the scarce samples in stage 4 and grade 4. Astonishingly, high CCDC58 expression led to worse prognosis of patients without alcohol consumption or hepatitis viral infection, which is opposite to current acknowledged reports [24]. And, concerning the few research about CCDC58 in the occurrence and development of various types of tumors, we comprehensively investigated the prognosis value of CCDC58 overexpression and found decreased survival rate in lung, breast, and ovarian cancers but increased survival rate in gastric cancer. Now that CCDC58 is possible to be a potential prognostic biomarker, deeper research may well be required to elucidate the functional mechanism.

According to previous researches, since CCDC58 is such an intermembrane space protein [25], it is possible to interact with other coiled-coil molecules based on its specific structure [26]. CCDC58 was reported to participate in a variety of mitochondrial behaviors. For instance, CCDC58 acts as a regulator or stabilizer in the process of effective protein imported into the mitochondrial matrix [25]. It was also found that CCDC58 is connected with mitochondrial single-nucleotide polymorphisms, a vital factor in the progression of AIDS [12]. And consistent with the study of other coiled-coil domain-containing proteins [13], we found CCDC58 functional in the inner mitochondrial membrane towards GO analyses. Current researches focused on mitochondria whose functionality is deemed to be critically required for tumor anabolism [27]. Despite the fact that tumor tissues growth was universally acknowledged to rely on the overactive glycolysis mechanism in the cytoplasm, the OXPHOS in mitochondria was recently identified to accelerate tumor growth [28-30]. In prostate cancer, the OXPHOS in primary tumors reached high levels in progression until an advanced stage [31]. Besides, OXPHOS may also influence the efficacy of certain anti-tumor medicine. A part of breast cancer patients resist to metformin through increased OXPHOS gene transcription [32]. Targeting OXPHOS may present a promising tumor remedy through regulating mitochondrial function. According to previous research, the mitochondrial disfunction and/or blocking OXPHOS reduced tumorigenic potential [33, 34]. Co-Targeting OXPHOS also increased the efficacy of radio-immunotherapy, which provided insight into exploration of new combination therapy [35]. The first clinical trial targeting OXPHOS by IM156 (a biguanide mitochondrial protein complex 1 inhibitor) in refractory advanced solid tumors achieved stable disease (SD) in 32% of patients [36]. However, in another phase I trial, a small-molecule complex I inhibitor, IACS-010759, targeting OXPHOS in advanced solid tumors and acute myeloid leu-

kemia, induced elevated blood lactate and neurotoxicity, and the narrow therapeutic index and dose-limiting toxicities underlined the importance of strict medication dosage [37].

In our study, CCDC58 was indicated to be involved in the OXPHOS pathway in KEGG analysis, by which it likely acts on cytochrome C oxidase, a principal regulator OXPHOS, owing to the results from GO analysis [38]. Concretely speaking, the expression of CCDC58 mRNA could affect the cytochrome C release from mitochondria, cytochrome C oxidase assembly and other mitochondrial biological functions such as protein targeting to mitochondrion, protein localization to mitochondrion, mitochondrial organization, transport, respiratory chain complex assembly, fusion, calcium iON homeostasis, transmembrane transport, morphogenesis and protein import into mitochondrial matrix. Furthermore, the STRING analysis shows the correlated proteins which were similar to CCDC58 dealing with mitochondrial membrane components. The direct co-expressed gene MRPL35 was found to play a key role in cytochrome C oxidase assembly [39]. And the co-expressed gene the mitochondrial SSBP1 was speculated to participate in mitochondrial DNA replication [40]. Here, the crosstalk between CCDC58 and mitochondria offered a potential target of CCDC58 for novel therapies in the future. Nevertheless, since we carried out this study on the basis of preliminary data and hypothesis-generating prospects, the concrete mechanisms of CCDC58 expression referring to mitochondrial functions and OXPHOS is still required for further researches. In summary, our study has demonstrated that elevated CCDC58 expression level is relevant to poor prognosis in HCC. CCDC58 expression may play a role in mitochondrial functions in the way of regulating the oxidative phosphorylation process. It could serve as a prognosis biomarker for HCC.

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## Disclosure of conflict of interest

None.

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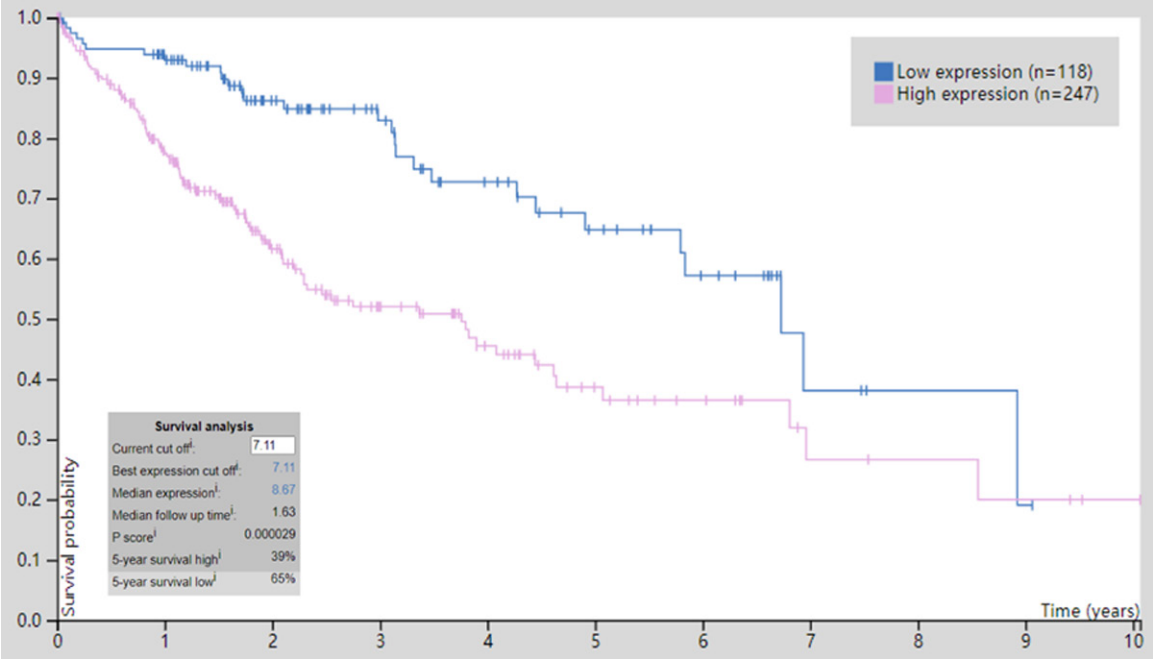


**Supplementary Figure 1.** Evaluating the correlation between CCDC58 expression and overall survival in liver cancer patients through Kaplan-Meier plotter.