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ZYG11B participates in the modulation of colorectal cancer cell proliferation and immune infiltration and is a prognostic biomarker

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Abstract

Propose The biological function of ZYG11B is still unclear, and few studies on ZYG11B in colorectal cancer were reported. The purpose of our research is to detect the biological functions of ZYG11B in colorectal cancer through The Cancer Genome Atlas (TCGA) database online and the vitro cell experiments.

Methods The information of ZYG11B in colorectal cancer were downloaded from TCGA database. The bioinformatics analysis has been utilized to examine the expression, functional enrichment, association of immune, clinicopathological characteristics and diagnostic prognostic value. CCK-8 and apoptosis assays have been utilized to identify the abilities of progression and apoptosis. The abilities of invasion and migration were detected by transwell assay.

Results In contrast to adjacent normal tissues, colorectal cancer tissues exhibited a notably diminished expression of ZYG11B ($p < 0.001$). Further examination through functional enrichment analysis unveiled the enrichment of various pathways associated with tumor proliferation and apoptosis. The implementation of CCK-8 and apoptosis assays validated the suppressive impact of ZYG11B on the progression of colorectal cancer cells ($p < 0.001$). A significant positive correlation was found between ZYG11B and Tcm and T helper cells ($R \geq 0.3$, $p < 0.001$). Moreover, the expression of ZYG11B demonstrated a prominent presence among subjects without previous experience of colon polyps ($p < 0.05$), devoid of lymphatic infiltration ($p < 0.01$), and age ≤ 65 years ($p < 0.01$). Additionally, ZYG11B exhibited higher expression levels among patients diagnosed with colorectal adenocarcinoma ($p < 0.05$). Following the analysis of survival prognosis, it became evident that increased ZYG11B expression correlated with enhanced survival rates ($p < 0.01$) and the ability to accurately forecast the prognosis and survival of COAD/READ patients.

Conclusion ZYG11B plays a tumor suppressive role in the proliferation process of colorectal cancer and may have a broad application prospect in the diagnosis and prognosis evaluation of colorectal cancer with more study.

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Introduction

Colorectal cancer (CRC) is a serious and deadly form of cancer. The global incidence of cancer amounted to around 9.96 million newly diagnosed cases in 2020, with colorectal cancer accounting for 1.93 million cases, ranking third in terms of prevalence [1]. Additionally, colorectal cancer caused 940,000 deaths in 2020, ranking second among all cancer [1]. The high occurrence and fatality rates of CRC place a significant burden on society. Approximately 20% of patients diagnosed with colorectal cancer develop metastases, while around 50% of individuals initially diagnosed with localized disease eventually develop metastases. The effective clinical management of these patients remains a complex medical and social challenges [2]. Recognizing the complexity of early-stage colorectal carcinogenesis, there has been a shift towards molecular profiling-based stratified treatment approaches for colorectal cancer [3].

ZYG11B functions as the substrate adapter component within the E3 ubiquitin ligase complex known as ZYG11B-CUL2-Elongin BC across the Gly/N-degron pathway [4]. It acts redundantly alongside ZER1 to selectively target substrates that possess N-terminal glycine degrons for destruction by the proteasome pathway. The Cullin 2-RING E3 Ubiquitin ligase (CRL2) complex's N-terminal glycine degron receptors demonstrate the ability to recognize these particular substrates [5]. The presence of glycine N-terminal degrons is particularly prevalent at sites of caspase cleavage and contributes to the removal of proteolytic fragments generated during apoptosis [5]. Despite this knowledge, the precise molecular mechanism by which ZYG11B operates in colorectal cancer (CRC) remains unidentified.

Our study conducted bioinformatics analysis to detect what kinds of roles the ZYG11B plays to evalue the prognosis of colon adenocarcinoma/rectum adenocarcinoma (COAD/READ), by using the clinical data and RNA sequencing acquired from TCGA. ZYG11B expression levels of mRNA have been observed to be elevated in COAD/READ compared with normal tissue, and its potential role in the tumorigenesis of COAD/READ was investigated. Furthermore, a correlation analysis has been conducted to assess the correlation among ZYG11B and various clinicopathological characteristics. Finally, we evaluated the ROC curve and prognosis of ZYG11B, suggesting that it may played some functions for the diagnosis and treatment of COAD/READ. But, the more functions of ZYG11B in COAD/READ and the practical significance in clinical remains to be discovered. Our investigation provides our valuable viewpoints about the mechanisms of COAD/READ tumorigenesis and highlights the significance of ZYG11B within this disease.

Materials and methods

Data processing and ethical declaration

The TCGA database (https://portal.gdc.cancer.gov/) was used to obtain RNA-seq data and clinical data of 11,093 generic cancer subjects and 689 cases of patients with COAD/READ, including 50 cases of colorectal adenocarcinoma cases and paired adjacent tissues. The data that was obtained by downloading was in the format of HTSeq- Fregments Per Kilobase per Million (FPKM) at level 3, which was then transformed into Log2 format for upcoming investigation. GEO database (Gene Expression Omnibus) was used to obtain clinical data of 155 cases of patients with COAD/READ. The TCGA and GEO database, which is publicly accessible, adheres to specific guidelines and ensures the acquisition of signed informed consents prior to collection of data.

Differentially expressed genes analysis

The patients in the COAD/READ database were categorized into 2 distinct groups, high and low expression, relied on the expression of ZYG11B gene median level. A negative binomial generalized linear model has been utilized with the R package DESeq2 (version 1.26.0) for determining the differentially expressed genes (DEGs) between these two groups. A double-tail hypothesis test was performed [6]. The expression of genes was converted into $\log_2$ value. And the Log_2 value of differential gene expression >1 and adjusted $p<0.05$ were set as the threshold. Displaying the findings through volcano map requires the application of the R package ggplot2 (version 3.3.3).

Functional enrichment analysis of ZYG11B related differential genes

Using the Metascape database2 and an online tool, a functional enrichment analysis of ZYG11B-associated differential genes was conducted [7]. This analysis outcomes were displayed using the ggplot2 package (version 3.3.3). The clusterProfiler software (version 3.14.3) was utilized to conduct Gene Set Enrichment Analysis (GSEA) [8]. The gene set utilized in the investigation was c2.cp.v7.2.symbols.gmt, which was acquired from MSigDB. A total of 261 clusters have been determined, exhibiting a threshold of false discovery rate (FDR) below 0.25 and reported as $p<0.05$. To investigate protein-protein interactions (PPI), STRING database3 was utilized [9].

Cell lines

The American Type Culture Collection (ATCC) provided the human normal colorectal epithelial cell line NCM460 and the human CRC cell lines (HCT15, RKO, Lovo, HCT116, and HCT8). To authenticate their identity, short tandem repeat analysis was conducted utilizing the AmpF/STR Identifier Kit provided by Applied Biosystems. The cells underwent culturing in accordance with established protocols, utilizing Dulbecco's Modified Eagle Medium (DMEM) enriched wih 10% fetal bovine serum (FBS) obtained from Gibco. The culture media also contained 1% streptomycin, 1% penicillin-streptomycin, and 1% non-essential amino acids (NEAAs) sourced from Invitrogen. Incubation was carried out at a temperature of 37 °C within a humidified environment enriched via 5% CO_2.

Plasmid construction, RNA interference ectopic expression and transfection

The expression vector pcDNA3.1 from Tsingke Biotechnology Co, Ltd. (China) was used for synthesizing and cloning the full-length complementary cDNAs of ZYG11B. RNA interference from Tsingke Biotechnology was used for knocking down ZYG11B. According to the protocol, CRC cells were transfected with the plasmid vectors and RNA interference using jetPRIME (Polyplus-transfection, USA). The sequence of ZYG11B plasmid in Additional file 1 and RNA interference are listed in Additional file 2 Table S1.

qRT-PCR

The GeneJET RNA Purification Kit (Thermo Fisher Company, USA) was utilized for the extraction of total RNA. Subsequently, the RNAs extracted from cells were reverse-transcribed utilizing the Takara Reverse Transcription Kit (Japan). The SYBR Green Master Mix (Yeasen, China) was utilized for quantitative real-time PCR analysis in a real-time quantitative PCR system (Bio-Rad, USA). The mRNA expression levels of different gene sets were quantified relative to GAPDH mRNA. Tsingke Biotechnology Co., Ltd. (China) synthesized all the primers used in this research, and Additional file 2 Table S2 contains a comprehensive list of these primer sequences.

Apoptosis assay

To assess apoptosis, the apoptosis detection kit known as Annexin V-fluorescein isothiocyanate (FITC) generated by Sigma-Aldrich was used. The HCT8 overexpressed , HCT15 downregulated ZYG11B and negative control cells were digested, centrifuged and then 200 μL HCT-8 and HCT15 cells suspension was added to 1 mL cold PBS, and the cells were suspended by gently shaking, then underwent centrifugation at 1000 rpm for a duration of 10 min at 4 ℃, and the supernatant was eliminated. After repeating the above step twice, the cells underwent re-suspension in 200 μL of labeled buffer. The cells were inoculated with 10 μL Annexin V-FITC. Gently mixed and react at normal temperature without light for 15 min. Cells were tested immediately on the flow cytometer (BD Biosciences) after adding 300 μl labeled buffer solution.

EDU assay

Cells were cultured in a 24-well plate and treated with 250 μL of EdU medium (Ribobio, China). The incubation procedure was operated following the manufacturer's instructions.

Cell progression

The cellular progression has been estimated utilizing the Cell Counting Kit - 8 (GK10001, GLPBIO). In a concise manner, the cells underwent inoculation at 1×10^3 per well in 96 - well plates overnight before treatment as desired. Cell Counting Kit-8 (10 μL) and DMEM (100 μL) have been supplemented in every well and subjected to incubation for a period of 2 h before recording luminescence on the SkanIt Software (Thermo). The measurements were continuous for 6 days.

Cells that had been treated were subsequently seeded in 96 - well plates at 1×10^3 per well. Afterwards, the cells were incubated for 24, 48, 72, 96, and 120 h. Cell progression underwent evaluation using Cell Counting Kit 8 (CCK-8, Kumanoto, Japan) based on the manufacturer's guidelines through measuring the absorbance at 450 nm by Thermo Fisher Scientific microplate reader.

Transwell assays

The invasion/migration abilities of the cells have been evaluated utilizingtranswell assays in 24-well transwell chambers (Falcon, USA) that were coated/not coated with Matrigel (Corning, USA). The cells in suspension (2×10^5 cells/mL) were then reseeded on the apical side of the chamber utilizing 200 μl of media devoid of serum. A volume of 600 μL of media comprising 20% fetal bovine serum (FBS) was introduced into the basolateral side of the chamber. Following the incubation period at a temperature of 37 °C with 5% CO_2, the outer membrane underwent fixation using 95% ethanol and subsequently stained with a 4 g/mL crystal violet solution for a duration of 10 min. Using a phase-contrast microscope (Olympus, Tokyo, Japan), the number of invading/migrating cells were then determined.

Correlation of ZYG11B and immune cell infiltration within CRC

The immunoinfiltration matrix data was analyzed using correlation analysis to investigate the correlation between ZYG11B and immunoinfiltration. The findings were then

visualized using the lollipop chart in the ggplot2 package. The R package ssGSEA [version 1.46.0] can be utilized for running the ssGSEA algorithm [10] was employed to determine immune infiltration. 24 kinds of immune cells markers from immunity article [11] were used to calculate immune infiltration. Furthermore, we employed the Tumor Immune Single-cell Hub 2 (TISCH2) database, accessible at http://tisch.comp-genomics.org/home/, to conduct an analysis of tumor immune single-cells. This research aimed to examine the expression of ZYG11B in various cell types within CRC.

Nomogram analysis and calibration curves

Survival package [3.3.1] was used to test the proportional risk hypothesis and Cox regression analysis was performed. Nomogram related models and Calibration curves were developed and displayed utilizing rms package [6.3-0]. Nomogram analysis was performed using RNA-seq data in TPM format and clinical data extracted from the TCGA and GEO databases.

Statistical analysis

Data was analyzed by R package 4.2.1 and SPSS20.0. Independent sample t-test has been employed to analyze the statistical difference among 2 groups. Wilcoxon test was utilized to assess the two groups' variances. The FRD of the expression of different mRNAs in GSEA were corrected by Benjamini-Hochberg method. Sensitivity and specificity of the prognostic model were evaluated using the ROC curve. To examine the relationship between gene expressions, we utilized the Spearman correlation test. To compare the survival curves, both the Logrank test and Kaplan-Meier method have been utilized. The comparison of CCK-8 growth curve data was tested by Bonferroni method of two-factor analysis of variance. The risk prediction ability of ZYG11B was evaluated by Cox regression analysis. $p<0.05$ was reported as statistically significant.

Results

ZYG11B expression profiles in multiple kinds of malignancies and associated DEGs in colorectal cancer cells

ZYG11B expression has been analyzed within multiple forms of cancer utilizing data from the TCGA database. ZYG11B showed significant differential expression among the 21 types of cancer ($p<0.05$) (Fig. 1A), and it was found to be lowly expressed in COAD and READ ($p<0.05$) (Fig. 1A). Within TCGA COAD/READ tissues, the expression of ZYG11B was significantly lower compared to healthy donors ($p<0.001$, Fig. 1B) both and paired neighboring normal tissues ($p<0.001$, Fig. 1C). A group of 698 patients has been divided into 2 groups, high and low expression group, relied on the median value of ZYG11B expression in COAD and READ. The mRNA expression levels underwent comparison in these two groups, resulting in the identification of 252 DEGs (67 overexpressed and 185 underexpressed, Fig. 1D and E) within the overexpression group ($|\log2FC>1|$, $p<0.05$).

Functional enrichment analysis of DEGs correlated with ZYG11B within colorectal cancer

The website "Metascape" (https://metascape.org/gp/index.html#/main/step1) was conducted to assess ZYG11B-correlated DEGs function enrichment in COAD/READ patients. According to Fig. 2A, we found that several tumor proliferation related mechanisms underwent enrichment, such as Insulin-like Growth Factor Binding Proteins (IGFBPs) (R-HSA-381426), G protein-coupled receptor signaling pathway (GO: 0007188), and negative modulation of cell differentiation (GO: 0045596). The GSEA results showed that the ZYG11B -related DEGs in clusters were significantly associated with mitosis and tumor proliferation related clusters in COAD/READ (Fig. 2B-G). Additionally, PPI analysis showed that ZYG11B mediated the ubiquitination of target proteins and (Fig. 2H) including CUL2 (Cullin-2), RBX1 (E3 ubiquitin-protein ligase RBX1), COMMD1 (COMM domain-containing protein 1), ASB4 (Ankyrin repeat and SOCS box protein 4), LRR1 (Leucine-rich repeat protein 1), cell cycle progression such as RBX1, and inhibited cell proliferation such as GLMN (Glomulin). Hence, ZYG11B plays important roles within the functions of cell cycle, cell progression and ubiquitination of target proteins in CRC.

ZYG11B suppresses the progression of colorectal cancer cells

To ascertain the negative impact of ZYG11B on development of CRC cells, RNA was extracted from NCM460, HCT15, HCT8, RKO, LOVO and HCT116 cells to detect the expression of ZYG11B. qRT-PCR findings revealed that the expression of ZYG11B was relatively low within HCT8 cells and was relatively high within HCT15 cells (Fig. 3A). The overexpressed ZYG11B plasmid was transfected in HCT8 cells (Figure S1A) and RNA interference was transfected in HCT15 cells to knockdown ZYG11B ($p<0.001$) (Figure S1B). The CCK8 assay demonstrated that the upregulation of ZYG11B inhibited the growth of HCT8 cells (Fig. 3B) and downregulation of ZYG11B promoted the growth of HCT15 cells (Fig. 3C), and the findings from the EdU assay and apoptosis assay indicated that ZYG11B hindered cell death in CRC cells. (Fig. 3D, E, Figure S1C). In order to determine the impact of ZYG11B on metastasis, invasion and migration experiments confirmed that ZYG11B did not affect cell metastasis (Figure S1D). In conclusion, ZYG11B inhibited

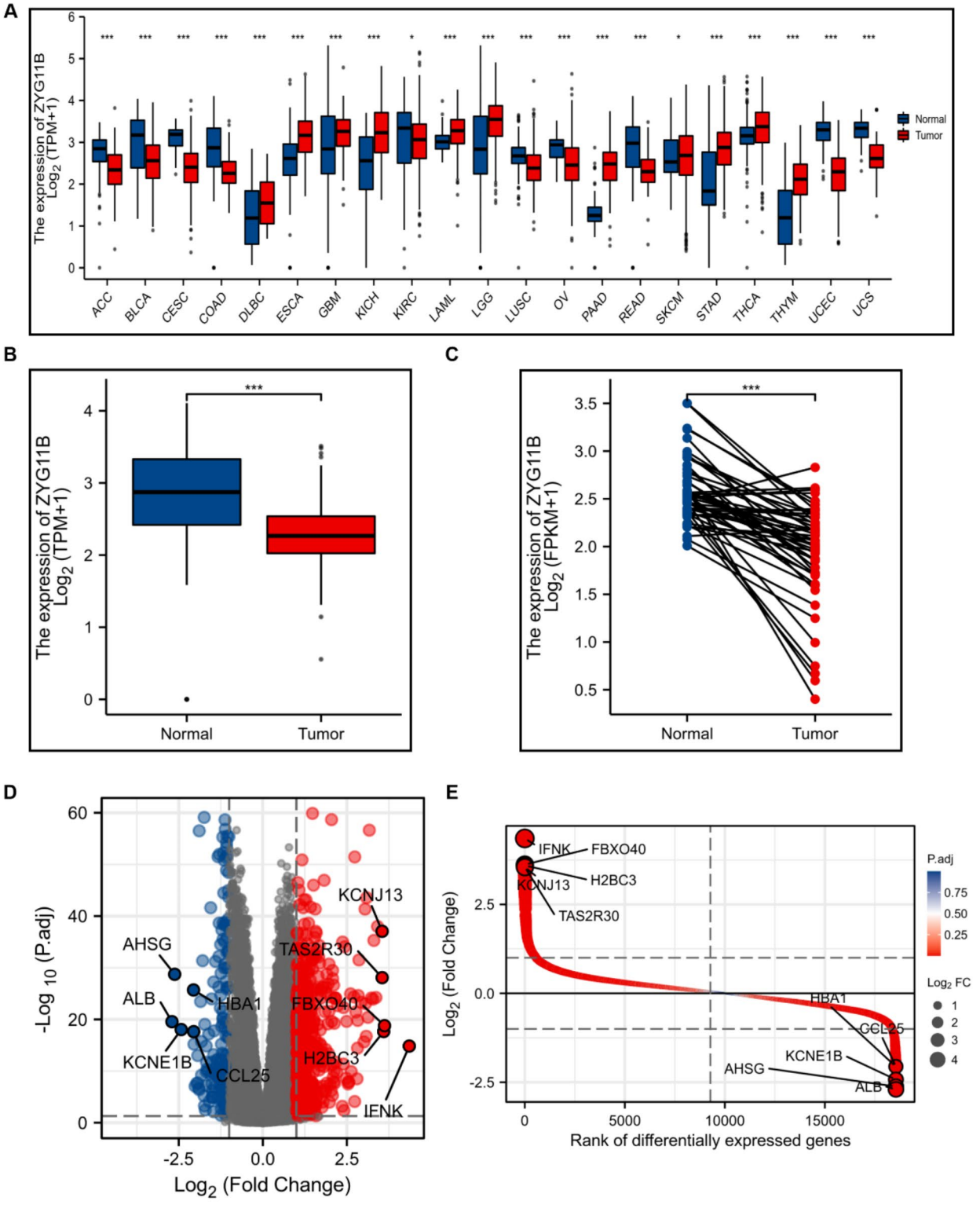

Fig. 1 ZYG11B mRNA expression profiles in CRC and pan-cancer. (**A**) ZYG11B expression in pan-cancer relied on TCGA dataset. * $p < 0.05$; *** $p < 0.001$. (**B**) The contrast of ZYG11B expression between COAD/READ and unpaired healthy tissues. (**C**) The comparison of ZYG11B expression among COAD/READ and paired normal tissues. Volcano Plot (**D**) and Rank of differentially expressed genes (**E**) of ZYG11B -related DEGs among high- and low-ZYG11B expression groups depending on the median ZYG11B value

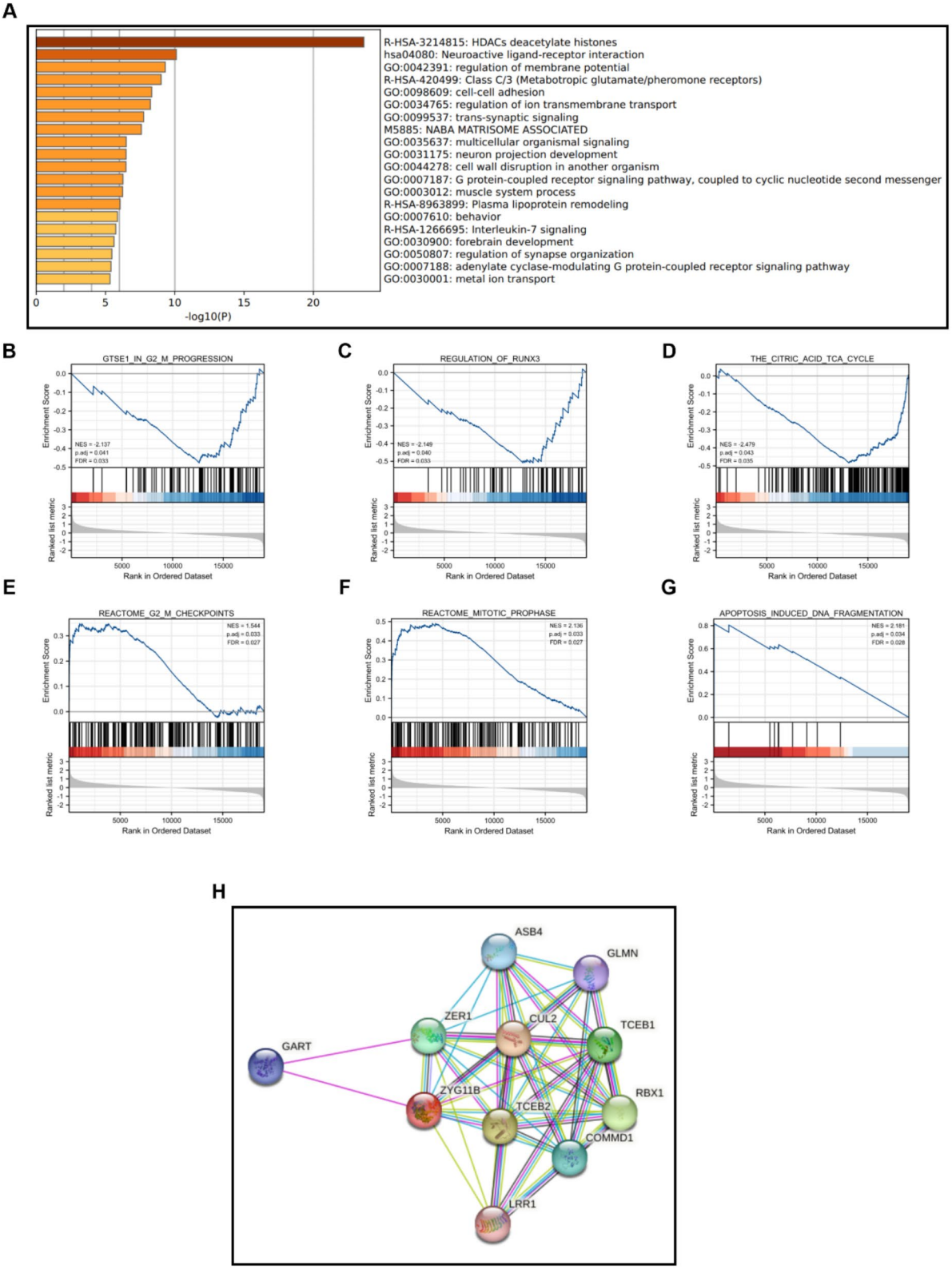

Fig. 2 Analysis of functional enrichment of DEGs associated with ZYG11B. (**A**) The top 20 pathways related to DEGs functional annotation among groups having high and low ZYG11B expression were identified by the Metascape database. (**B-G**) GSEA analysis among high- and low- ZYG11B expression groups. (**H**) PPI analysis revealed potential interacting protein with ZYG11B

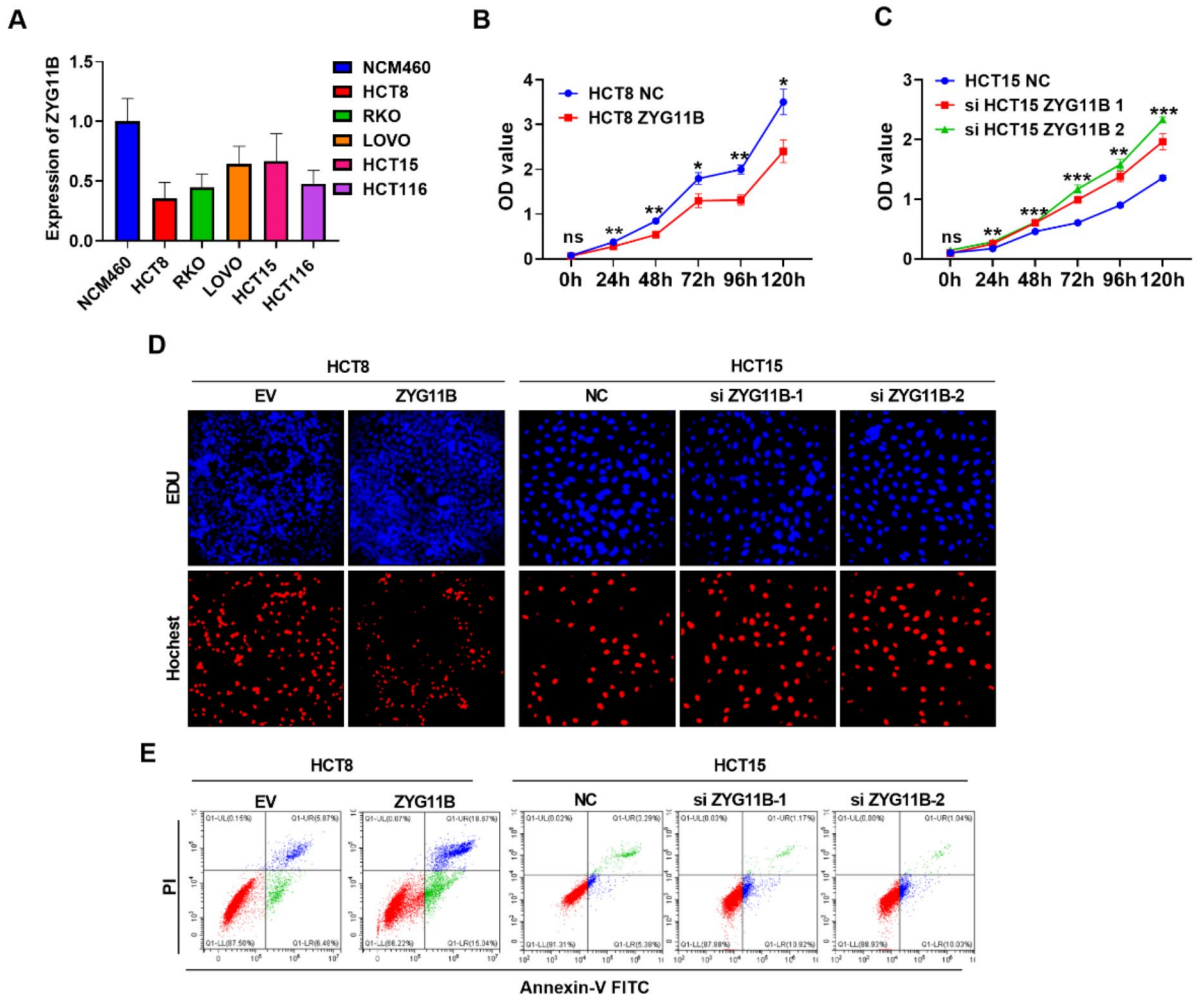

Fig. 3 The biological functions of ZYG11B in CRC cells. (**A**) qRT-PCR identification of ZYG11B expression within NCM460, HCT8, RKO, LOVO, HCT15, HCT116 cells. (**B**), (**C**) CCK-8 assay confirmed the effect of ZYG11B on the proliferation of HCT8 and HCT15 cells. (**D**) EdU assay used to confirm the impact of ZYG11B on the proliferation of HCT8 and HCT15 cells. (**E**) Apoptosis assay used to confirm the impact of ZYG11B on the proliferation of HCT8 and HCT15 cells. ns, no significance; * $p < 0.05$; ** $p < 0.01$

abilities of proliferation and apoptosis but did not affect abilities of metastasis.

Association of ZYG11B and immune cell infiltration in CRC

Throughout the current investigation, we analyzed the connection among ZYG11B and immune cells in CRC. Our findings, as depicted in Fig. 4A, indicate a statistically significant correlation between ZYG11B and the majority of immune cells investigated. Notably, Tcm and T helper cells demonstrated a statistically significant association with ZYG11B expression ($R \geq 0.3$, $p < 0.001$) (Fig. 4B). Moreover, we assessed the levels of infiltration by Tcm and T helper cells in both the high expression and low expression groups of ZYG11B. The results revealed that upregulation of ZYG11B may potentially facilitate T helper cells and Tcm infiltration (Fig. 4C-D). Afterwards, we evaluated the expression of ZYG11B at the single-cell level on tumor microenvironment (TME) through TISCH2. The results revealed that ZYG11B mainly expressed in T cells, endothelia, fibroblasts, myofibroblastsa based on CRC_GSE108989 and CRC_GSE166555 database. According to the above results, ZYG11B can contributed to the regulation of immune cell infiltration and tumor immune microenvironment.

Relationship between the expression of ZYG11B and clinicopathological characteristics in COAD/READ individuals

Throughout the current investigation, we examined the significance and activity of ZYG11B expression in COAD/READ patients depending on data acquired from the TCGA database. Through an analysis of ZYG11B

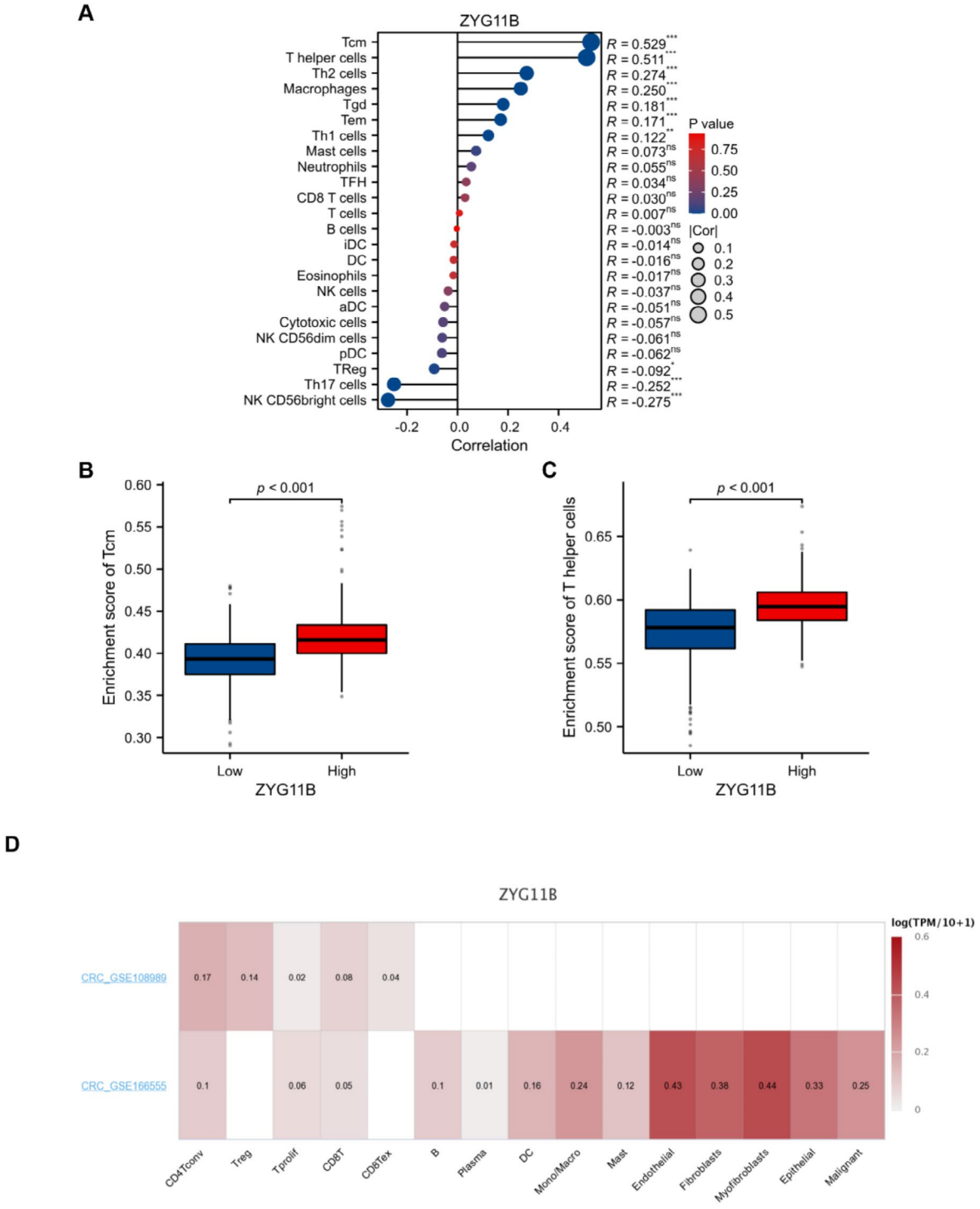

Fig. 4 The association among the expression of ZYG11B and immune cells infiltration in COAD/READ. (**A**) Spearman's correlation coefficient was utilized to detect the relationship between the expression profiles of ZYG11B and 24 immune cell types infiltration levels. (**B-C**) The contrast among the highest associated immune cells infiltration levels in the high- and low-PDE2A expression groups, which include Tcm cells (**B**) and T helper cells (**C**). (**D**) The evaluation of ZYG11B expression at the single-cell level on TME through TISCH2. Tcm, T central memory; Th, T helper cells; Th1, type 1 Th cells; Th2, type 2 Th cells; Tem, T effector memory; Tgd, T gamma delta; Tfh, T follicular helper; iDCs, immature DCs; DCs, dendritic cells; aDCs, activated DCs; NK, natural killer; pDCs, plasmacytoid DCs; Treg, regulatory T cells; Th17, type 17 Th cells. *** $p < 0.001$

expression levels in relation to patients' clinical characteristics, we made several interesting findings. Firstly, ZYG11B exhibited high levels of expression in patients who had no previous history of colon polyps (Fig. 5A). Additionally, patients with no lymphatic infiltration also showed elevated ZYG11B expression levels (Fig. 5B). Furthermore, we observed that ZYG11B expression was particularly high in patients below the age of 65 (Fig. 5C). Lastly, patients diagnosed with colorectal adenocarcinoma also exhibited increased levels of ZYG11B expression (Fig. 5D). The results suggest that ZYG11B may be closely related to some clinical characteristics of patients with COAD/READ.

Diagnostic predictive significance of ZYG11B in COAD/READ patients

In order to conduct the clinical evaluation of ZYG11B, the diagnostic significance of ZYG11B in COAD/READ was confirmed using the ROC curve. The AUC of 0.764 indicated a significantly high sensitivity and specificity of ZYG11B in diagnosing COAD/READ (Fig. 6A). We used the GEO database validation set (GSE103479) to confirm the similar diagnostic value of ZYG11B in COAD/

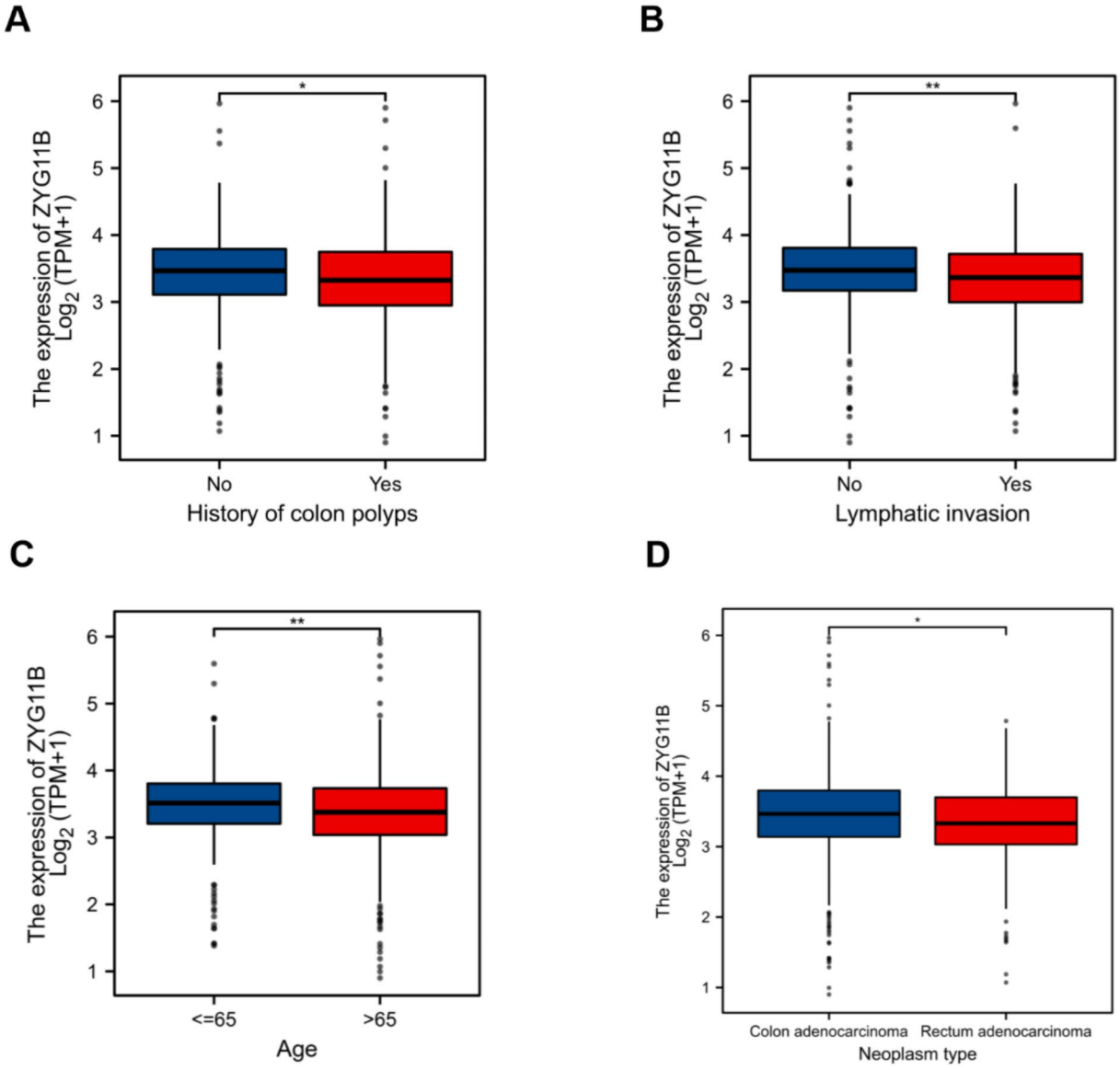

Fig. 5 Association between ZYG11B Expression and Clinicopathological Characteristics. The associations among ZYG11B expression and history of colon polyps (**A**), lymphatic infiltration (**B**), age (**C**), and location of adenocarcinoma (**D**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

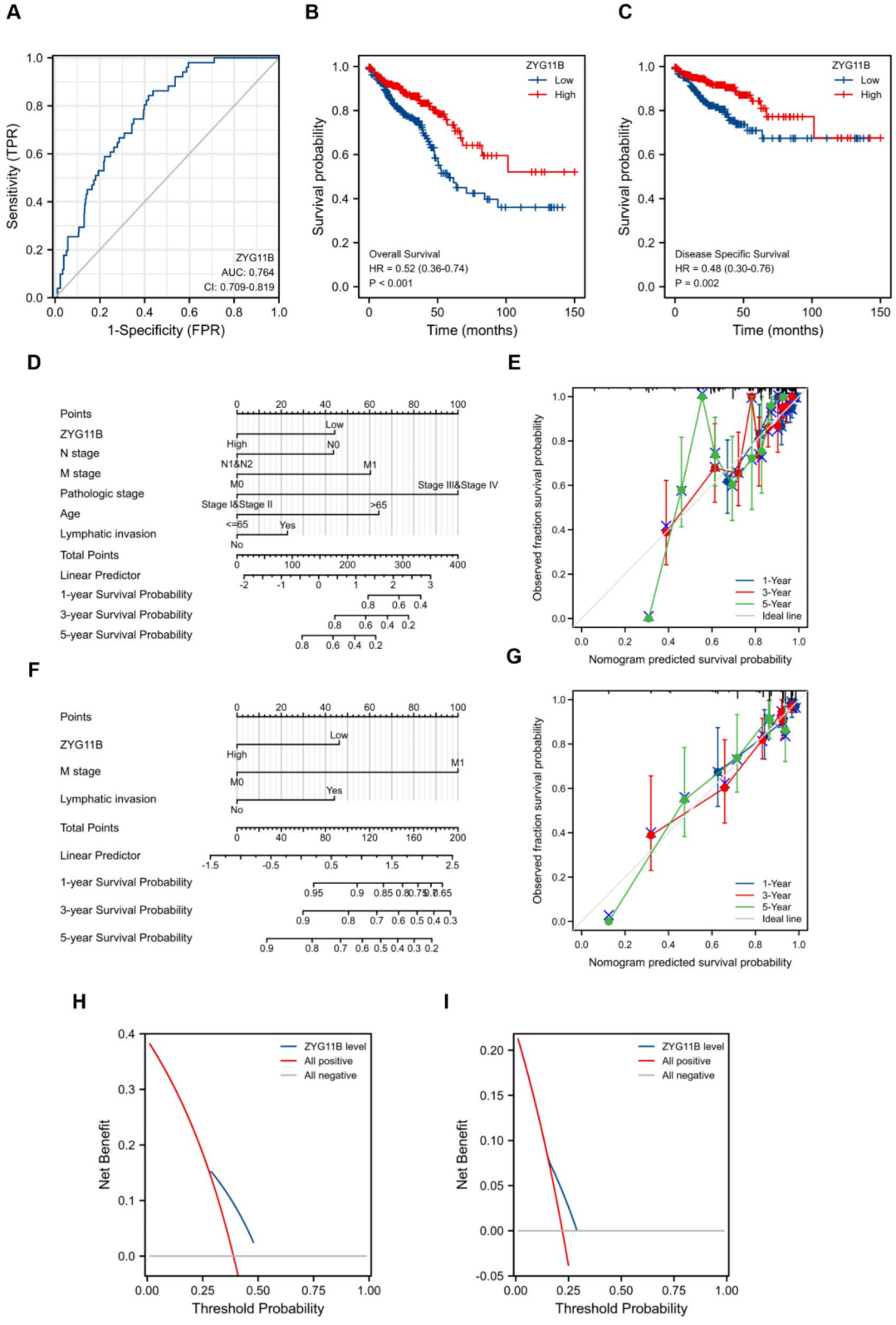

Fig. 6 Diagnostic prognostic values of ZYG11B in COAD/READ patients. ROC curve analysis has been conducted to evaluate the capacity of ZYG11B in diagnosing COAD/READ utilizing database of the TCGA (**A**). To compare the overall survival (**B**) and disease-specific survival (**C**) between the groups with high and low expression of ZYG11B, Kaplan-Meier analyses were carried out. Nomograms incorporating ZYG11B expression were constructed to establish risk scoring models for 1-, 3-, and 5-year (OS) (**D-F**) and (DSS) (**F-G**). (**H-I**) DCA for the expression of ZYG11B prediction models in OS (**H**) and DSS (**I**)

READ (Figure S2A). Additionally, the survival analysis using Kaplan-Meier curves demonstrated that overexpression of ZYG11B has been correlated with enhanced disease-specific survival (DSS) and overall survival (OS) across COAD/READ individuals. Nevertheless, there was no significant variation detected in the progression-free interval (PFI) (Fig. 6B-C), the similar results are verified in the GEO database (GSE103479) (Figure S2B-C). The findings obtained from the calibration curve exhibited an excellent prognostic ability of the nomogram in estimating survival probability among COAD/READ patients (Fig. 6D-G). Furthermore, the 5-year Decision Curve Analysis (DCA) depicted the predictive significance of ZYG11B in predicting OS (Fig. 6H) and DSS (Fig. 6I) in COAD/READ patients. Collectively, the expression of ZYG11B serves as a primary predictive factor for long-term survival prognosis in COAD/READ individuals.

Discussion

CRC possesses one of the highest rates of morbidity and mortality among all kinds of cancers and most patients are initially diagnosed with advanced stage, so exploring potential targets for diagnosis and treatment is a current research focus. There are few studies on the functions of ZYG11B. The investigation demonstrated that ZYG11B employs its armadillo (ARM) repeats to create narrow and profound cavities, thereby predominantly interacting with the initial four residues of Gly/N-degrons [12]. But, the precise functions and mechanisms of ZYG11B in CRC have not yet been comprehensively elucidated.

Our results indicate that ZYG11B expression within TCGA COAD/READ tissues exhibited significant reduction contrasted to adjacent normal tissues. The main biological functions of ZYG11B were centered around the cell progression, cell cycle, and the ubiquitination of target proteins. Additionally, in vitro cell experiments demonstrated that ZYG11B inhibited the proliferation and apoptosis of CRC cells while not affecting their invasive and migratory capacities. The previous results suggested the contribution of ZYG11B in mediating the modulation of CRC autophagy through its interaction with LINC01871 [13]. Functional enrichment analysis highlighted the role of ZYG11B in regulating IGFBPs, which showed varying impacts on tumor progression depending on the specific subtypes. For instance, in hepatocellular carcinoma (HCC), IGFBP3 exhibited tumor growth inhibition by inducing IGFBP3 promoter hypermethylation through reactivation of genes with demethylating agents and/or histone deacetylase suppressors. Conversely, IGFBP-2 promoted cell invasion and migration in glioma by interacting with its ligand, the invasion inhibitory protein 45 (IIp45), leading to nuclear import and promoting angiogenesis through the activation of VEGF transcription. Moreover, PPI analysis revealed close associations between ZYG11B and ubiquitin-related as well as cell proliferation-related proteins, further supporting the likelihood of ZYG11B's involvement in the regulation of tumor proliferation.

According to our analysis in TCGA database, ZYG11B expression in COAD/READ exhibited significant reduction contrasted to neighboring healthy tissues and the biological functions of ZYG11B mainly focused on cell cycle, cell proliferation and ubiquitination of target proteins. Additionally, vitro cell experiments demonstrated that ZYG11B could weaken the abilities of proliferation and apoptosis, but did not affect the abilities of invasion and migration in CRC cells. Previous study revealed that the ZYG11B involved in mediating the regulation of CRC autophagy by LINC01871 [13]. Functional enrichment analysis showed that ZYG11B involved in regulating IGFBPs. IGFBPs played different roles in the progression of tumors based on different subtypes [14]. IGFBP3 has the capabilities to induce the hypermethylation of its own promoter and the inhibition of tumor growth in hepatocellular carcinoma (HCC) through reactivation of genes, accomplished by demethylating agents and/or histone deacetylase suppressors [15, 16]. On the other side, through its interaction with the IGFBP-2 ligand invasion inhibitory protein 45 (IIp45), IGFBP-2 modulated the effect of cell migration and invasion on glioma [17] and induced nuclear import to promote angiogenesis through activation of VEGF transcription [18]. What's more, PPI assay demonstrated that ZYG11B exhibit a robust correlation with ubiquitin-related and cell proliferation related proteins, which further confirmed that ZYG11B may be participated in the modulation of tumor progression.

TCGA and TISCH2 analysis demonstrated that ZYG11B induced the infiltration of Tcm and T helper cells and mainly expressed in T cells, endothelia, fibroblasts, myofibroblastsa in COAD/READ patients confirming the functions of regulation on immune cell infiltration and tumor immune microenvironment. Tcm cells could possess the capacity to generate elevated cytokine quantities, exhibit enhanced cytotoxic efficacy, and demonstrate a superior capability in eliminating established tumors. What's more, Tcm persisted longer than Tem cells in vivo [19]. T helper cells which act as intermediate processes in immune responses, contain a variety of subtypes and different subtypes play different roles in tumor progression. Tregs can disturb the immune response against tumors in carcinogenesis, resulting in the growth and spread of various cancers. This disruption can occur through contact-dependent mechanisms like programmed cell death 1 (PD-1) or contact-independent mechanisms like IL-2 sequestration [20]. In melanoma, the underlying mechanisms by which T helper 9 cells enhance antitumor immunity involve stimulating mast cells and enhancing their cytotoxic function. This

stimulation contributes to the initiation and recruitment of dendritic cells expressing CC-chemokine receptor 6 (CCR6+) and helps induce the response of cytotoxic T lymphocytes (CTL). It also enhances the role of natural killer (NK) cells and allows direct killing of tumor cells [21, 22]. Additionally, a study revealed that ZYG11B can modulate the secretion of cGAMP and following interferon and inflammatory cytokines transcription [22]. This regulation occurs by increasing the binding affinity of cGAS-DNA, promoting cGAS-DNA condensation, and stabilizing the condensed complex. Additionally, high expression of ZYG11B in the endothelia, fibroblasts and myofibroblastsa suggests that ZYG11B may play roles in tumor immunoregulation but the functions of ZYG11B in tumor immunity need to be further explored.

Through analysing the expression of ZYG11B in TCGA database, ZYG11B was highly expressed in patients with no history of colon polyps, no lymphatic infiltration, ≦65 years old, or patients with colorectal adenocarcinoma, suggesting that ZYG11B may play protective functions in the progression of CRC. The AUC of ZYG11B was 0.764 and patients with high ZYG11B could have longer OS and DSS, the aforementioned outcomes revealed that ZYG11B could be recruited as a convincing factor for CRC diagnosis. What's more, calibration curve and DCA curve analysis futher confirmed the diagnostic prognostic value. However, there was some limitations in our study. In the future, relevant experiments in vivo and in vitro on potential mechanism and biological functions need to be further verified and we will future utilize other databases or clinical samples to verify our conclusions.

Conclusions

In brief, our research outcomes have exposed the initial evidence regarding the clinical prognostic significance and partial roles of ZYG11B in COAD/READ. Our discoveries significantly suggest that ZYG11B has the potential to impede tumor growth and modulate immune responses, making it a promising biomarker for predicting treatment response and prognosis in COAD/READ individuals. Nonetheless, to comprehend the underlying mechanisms and biological ramifications of ZYG11B, additional *in vivo and in vitro* studies are required.

Abbreviations

TCGA	The Cancer Genome Atlas
CRC	Colorectal cancer
COAD/READ	Colon adenocarcinoma/rectum adenocarcinoma
FPKM	Fregments Per Kilobase per Million
DEGs	Differentially expressed genes
GSEA	Gene Set Enrichment Analysis
FDR	False discovery rate
PPI	Protein-protein interactions
ATCC	The American Type Culture Collection
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal bovine serum
NEAAs	Non-essential amino acids
FITC	Annexin V-fluorescein isothiocyanate
TISCH2	Tumor Immune Single-cell Hub 2
BLCA	Bladder Cancer
BRCA	Breast Cancer
ACC	Adrenocortical Cancer
COAD	Colon Cancer
GBM	Glioblastoma
CESC	Cervical Cancer
ESCA	Esophageal Cancer
DLBC	Large B-cell Lymphoma
KIRC	Kidney Clear Cell Carcinoma
KICH	Kidney Chromophobe
LAML	Acute Myeloid Leukemia
LGG	Lower Grade Glioma
LUAD	Lung Adenocarcinoma
LUSC	Lung Squamous Cell Carcinoma
PRAD	Prostate Cancer
OV	Ovarian Cancer
READ	Rectal Cancer
STAD	Stomach Cancer
SKCM	Melanoma
THYM	Thymoma
UCEC	Endometrioid Cancer
THCA	Thyroid Cancer
UCS	Uterine Carcinosarcoma
IGFBPs	Insulin-like Growth Factor Binding Proteins
CUL2	Cullin-2
RBX1 E3	Ubiquitin-protein ligase RBX1
COMMD1	COMM domain-containing protein 1
ASB4	Ankyrin repeat and SOCS box protein 4
LRR1	Leucine-rich repeat protein 1
TME	Tumor microenvironment
Tcm	T central memory
Th	T helper cells
Th1	Type 1 Th cells
Th2	Type 2 Th cells
Tem	T effector memory
Tgd	T gamma delta
Tfh	T follicular helper
iDCs	Immature DCs
DCs	Dendritic cells
aDCs	Activated DCs
NK	Natural killer
pDCs	Plasmacytoid DCs
Treg	Regulatory T cells
Th17	Type 17 Th
DSS	Disease-specific survival
OS	Overall survival
PFI	Progression-free interval
DCA	Decision Curve Analysis

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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Author contributions

JC.Z and MM.C contribute equally to this work. JC.Z and MM.C wrote the manuscript. JY.L checked the statistical methods in this article. YJ.G and J.Z approved the submitted version and have agreed both to be personally

accountable for the author's own contribution. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

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Competing interests
The authors declare no competing interests.

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