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RESEARCH ARTICLE

Multi-omics Profiling Nominates Caspase-6 (*CASP6*) as a Candidate Prognostic Biomarker in Kidney Renal Clear Cell Carcinoma

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Abstract Caspase (CASP) family members have been proposed as pharmacodynamic biomarkers for monitoring anticancer responses, yet their prognostic relevance across malignancies remains incompletely characterized. The present investigation systematically assessed the prognostic potential of seven proapoptosis-associated caspase genes (*CASP2*/*3*/*6*/*7*/*8*/*9*/*10*) across pan-cancer cohorts using integrative analysis of TIMER2.0, PrognoScan, and GEPIA2 databases. The screening identified *CASP6* and *CASP9* as candidate survival-associated genes; elevated *CASP6* expression correlated with favorable outcomes in kidney renal clear cell carcinoma (KIRC) and colon adenocarcinoma, while high *CASP9* levels were associated with improved survival in kidney renal papillary cell carcinoma (KIRP). TIMER2.0 immune events analysis revealed *CASP6*-associated immune microenvironment remodeling in KIRC, characterized by coordinated infiltration of B cells, $CD4^+$ T cells, and myeloid populations. UALCAN methylation profiling demonstrated tumor-specific promoter hypermethylation of *CASP6* in KIRC and *CASP9* in KIRP, which is strongly correlated with transcriptional downregulation. cBioPortal analysis identified missense mutations (*CASP6* E16G/K238R in colon adenocarcinoma; *CASP9* S133F in KIRP) classified as variants of uncertain significance. Through multi-omics integration, *CASP6* emerged as a candidate transcriptional biomarker in KIRC, with expression levels showing potential associations with therapeutic response probabilities. These findings suggest that caspase expression signature may serve as complementary tool for prognostic stratification in renal malignancies.

Keywords: caspase, anticancer, prognostic potential, *CASP6*, kidney renal clear cell carcinoma

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Introduction

Corresponding to programmed cell death mechanism, apoptosis is a critical regulatory process for eliminating malignant cells including neoplastic populations. The caspase (CASP) family comprises initiator caspases (-2, -8, -9, -10) responsible for apoptosis initiation and executioner caspases (-3, -6, -7) implementing apoptotic demolition [1]. Caspase-2 exhibits functional pleiotropy beyond apoptosis,

participating in tumor suppression, senescence modulation, and regulation of cell division [2, 3]. Caspase-3 exists as an inactive precursor during apoptosis, requiring cleavage by initiator caspases for activation. The activated enzyme orchestrates both pyroptotic and apoptotic pathways to exert tumoricidal effects [4, 5]. Caspase-6 demonstrates functional versatility by initiating PANoptosis (pyroptosis/apoptosis/necroptosis convergence), with demonstrated host-protective activity against influenza A viral pathogenesis [6]. Caspase-7, a multifunctional regulator of programmed cell death, not only coordinates inflammatory and apoptotic pathways but also transiently preserves plasma membrane integrity [7]. Caspase-8 functions as a master molecular rheostat, dynamically regulating apoptosis-necroptosis-pyroptosis cross-talk and maintaining tissue homeostasis through developmental and adult stages [8]. As the principal initiator of the intrinsic apoptotic pathway, caspase-9 integrates diverse cellular stress signals [9, 10], with BMP4-mediated granulosa cell survival attributed to caspase-9 downregulation [11]. Caspase-10 initiates apoptosis via apoptosis-inducing complex FADDosome, promoting TRAF2-dependent cFLIPL degradation and subsequent caspase-8 activation [12].

Given the critical role of apoptotic signaling in oncotherapy, caspase expression profiling has emerged as a pharmacodynamic biomarker for evaluating anticancer therapeutics. *In vivo* experiments show that *Ganoderma lucidum* spore oil demonstrates antitumor efficacy against MDA-MB-231 cell growth of breast cancer and tumorigenesis through *CASP3* upregulation-mediated apoptosis [13]. *Gastrodia elata Blume* exhibits chemopreventive potential in human HT29 colon cancer cells via *CASP3* induction [14]. *Anacyclus pyrethrum* extract markedly upregulates *CASP3* transcriptional activation, arrests cell cycle (G1 phase) and stimulates apoptosis in colorectal carcinoma model [15]. The condensed tannin weimaining, derived from *Fagopyrum cymosum*, suppresses 4T1-luc2 breast cancer growth via caspase-3-dependent apoptosis [16]. *Nigella Sativa* exerts dose- and time-dependent apoptosis-promoting effects in MCF-7 breast cancer cells through coordinated upregulation of *CASP3*/*8*/*9* [17]. With fragmented genomic DNA, parthenolide from *Tanacetum parthenium* inhibits cervical (SiHa) and breast (MCF-7) cancer cell proliferation by concentration-dependent approach, and elevates *CASP3*/*6*/*9* expression [18]. *Lithospermum erythrorhizon*-derived shikonin suppresses A549 lung adenocarcinoma growth via G1-phase arrest and *CASP3*/*7* mRNA upregulation [19]. Curcumin, a yellow pigment from *Curcuma longa* [20], demonstrates therapeutic efficacy against advanced breast epithelial cancer through *CASP9* activation in MCF-10F cells [21].

Previous studies have established that BCL-2 family proteins are the principal regulators of mitochondrial outer membrane permeabilization, a critical commitment step in the intrinsic apoptotic pathway, whereas caspases function as the downstream executioners of apoptosis [22]. Given their role as the final common pathway of apoptosis, focusing on caspases enables a direct assessment of the apoptotic execution capacity of tumor cells. Importantly, while BCL-2 family-mediated apoptosis is primarily governed by protein-protein interactions and post-translational modifications [23], caspase transcriptional changes may more directly reflect the execution phase of apoptosis and thus correlate more closely with patient prognosis. However, tumorigenesis cannot be universally attributed to defective caspase activation, as apoptotic dysregulation represents only one facet of oncogenic transformation [24]. Modern cancer treatments require efficacy in both tumor burden diminution and enduring prognostic enhancement [25]. This study systematically evaluates whether transcriptional upregulation of seven caspase family members (*CASP2*/*3*/*6*/*7*/*8*/*9*/*10*) correlates with therapeutic response and survival outcomes across malignancies, potentially establishing caspase expression signatures as cancer prognostic biomarkers.

Materials and Methods

TIMER-based analysis of caspase expression and immune infiltration

Across The Cancer Genome Atlas (TCGA) malignancies, gene expression profiles of seven caspase family members (*CASP2*/*3*/*6*/*7*/*8*/*9*/*10*) were quantitatively assessed running TIMER2.0 (accessed 5 March 2025) [26]. Following this, the TIMER2.0 algorithm was employed to quantify the infiltration levels of six immune cell subsets (B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells), thereby assessing the correlation between caspase expression and immune infiltration patterns across various cancer types (accessed 17 March 2025).

Survival analysis using PrognoScan and GEPIA2 platforms

Prognostic correlations were investigated through two complementary approaches including PrognoScan (accessed 8 March 2025) and GEPIA2 (accessed 14 March 2025). PrognoScan permits a meta-analysis of clinically annotated microarray datasets [27]. This study calculated the prognostic

value of caspase expression in multiple cancer cohorts. GEPIA2 incorporates TCGA tumor samples with Genotype-Tissue Expression (GTEx) normal tissue data for survival analysis [28], utilizing RNA-seq data to establish caspase-survival relationships through Kaplan-Meier methodology.

Genomic alteration profiling via cBioPortal

The cBioPortal (accessed 18 March 2025) was executed to characterize caspase-associated somatic mutation profiles, including variant classification (e.g. missense) and genomic localization across pan-cancer datasets. Multidimensional oncogenomic data from a series of cancer studies were analyzed to identify mutation hotspots and mutation type [29]. Missense variants identified from cBioPortal were evaluated for functional impact using Functional Analysis through Hidden Markov Models (FATHMM) with the cancer-associated variant prediction mode [30]. Applying the default threshold of -0.75, scores below this cutoff were considered potentially cancer-associated, whereas scores above were classified as neutral/benign.

Promoter methylation analysis using UALCAN

UALCAN served to compare caspase's promoter methylation levels between primary tumor and matched normal tissue across diverse tumor subdivisions classified with features of clinicopathology and patient attribute using TCGA transcriptome and clinical patient data [31]. Secondary analyses stratified findings based on cancer stage, patient demographics (age, weight), tumor grade, and gender (accessed 14 March 2025).

Statistical framework

Survival outcomes were analyzed via log-rank testing for multiple comparisons. Immune correlation matrices were generated using Spearman's coefficient. Inter-group comparisons employed Student's t-tests. All analyses utilized a statistical significance threshold of $p = 0.05$.

Results

Differential caspase expression across malignancies

TIMER2.0 analysis quantified mRNA expression of seven caspase family members (*CASP2*/*3*/*6*/*7*/*8*/*9*/*10*) across more than twenty TCGA tumor types (Fig. 1A). Comparative analysis revealed significant transcriptional downregulation ($p < 0.05$) relative to normal tissue baselines in 1) *CASP2* within KICH, THCA, 2) *CASP3* within COAD, READ, 3) *CASP6* within COAD, KICH, KIRC, 4) *CASP7* within COAD, KICH, KIRP, LUAD, LUSC, READ, 5) *CASP8* within BRCA, KICH, 6) *CASP9* within UCEC, THCA, READ, PCPG, KICH, KIRC, KIRP, COAD, 7) *CASP10* within UCEC, READ, PRAD, LUSC, KICH, KIRP, COAD, BRCA (Fig. 1B). KICH demonstrated the most frequent caspase suppression, followed by COAD (Fig. 1C).

Prognostic significance of caspase expression

Based on above cleaning cancer types, PrognoScan hazard ratio (HR) analysis identified 26 significant associations ($p < 0.05$) between caspase expression and overall survival (OS) across five malignancies including breast, colorectal, lung, prostate cancers and renal cell carcinoma (Fig. 2A). *CASP7* showed no prognostic correlation ($p > 0.05$), while *CASP3* and *CASP9* exposed adverse associations with HR more than 1 (Fig. 2A, B). Protective associations ($HR < 1$, $p < 0.05$) pinpointed four caspase genes (*CASP2*/*6*/*8*/*10*). Specifically, high expression of *CASP2* (OS HR 0.70, 95% CI = 0.50 ~ 0.98), *CASP8* (OS HR 0.63, 95% CI = 0.42 ~ 0.95), and *CASP10* (OS HR 0.54, 95% CI = 0.38 ~ 0.78) in breast cancer corresponded to a better prognosis (Fig. 2B). In colorectal cancer, high expression of *CASP8* (OS HR 0.33, 95% CI = 0.12 ~ 0.90) and *CASP10* (OS HR 0.34, 95%CI = 0.14 ~ 0.86, OS HR 0.32, 95%CI = 0.12 ~ 0.90, OS HR 0.08, 95%CI = 0.01 ~ 0.54, OS HR 0.11, 95%CI = 0.02 ~ 0.80, correspondingly) were coupled with a favorable prognosis, as well as the expression of *CASP2* (OS HR 0.23, 95% CI = 0.06 ~ 0.95), *CASP6* (OS HR 0.35, 95% CI = 0.13 ~ 0.96), *CASP8* (OS HR 0.26, 95% CI = 0.15 ~ 0.45), and *CASP10* (OS HR 0.21, 95%CI = 0.08 ~ 0.55, OS HR 0.51, 95%CI = 0.31 ~ 0.84, OS HR 0.65, 95%CI = 0.45 ~ 0.95, OS HR 0.72, 95%CI = 0.52 ~ 1.00, sequentially) in lung cancer (Fig. 2B).

Survival validation in TCGA and GTEx cohorts

GEPIA2 analysis confirmed favorable prognostic events with high level expression of *CASP6* in COAD ($p < 0.01$) and KIRC ($p < 0.05$), likewise *CASP9* in KIRP ($p < 0.05$) (Fig. 3A-C). *CASP7* and *CASP9* separately presented weak positive correlations with survival prognosis in COAD (Fig. 3D, E) and UCEC (Fig. 3F). Conversely, high expression levels of *CASP2* was adverse prognosticator in KICH

patients (Fig. 3G). Additional survival curves were placed in Figure S1. These outcome supported that raised expression of *CASP6* in COAD and KIRC, and of *CASP9* in KIRP responded to favorable prognosticator (HR < 1, p < 0.05).

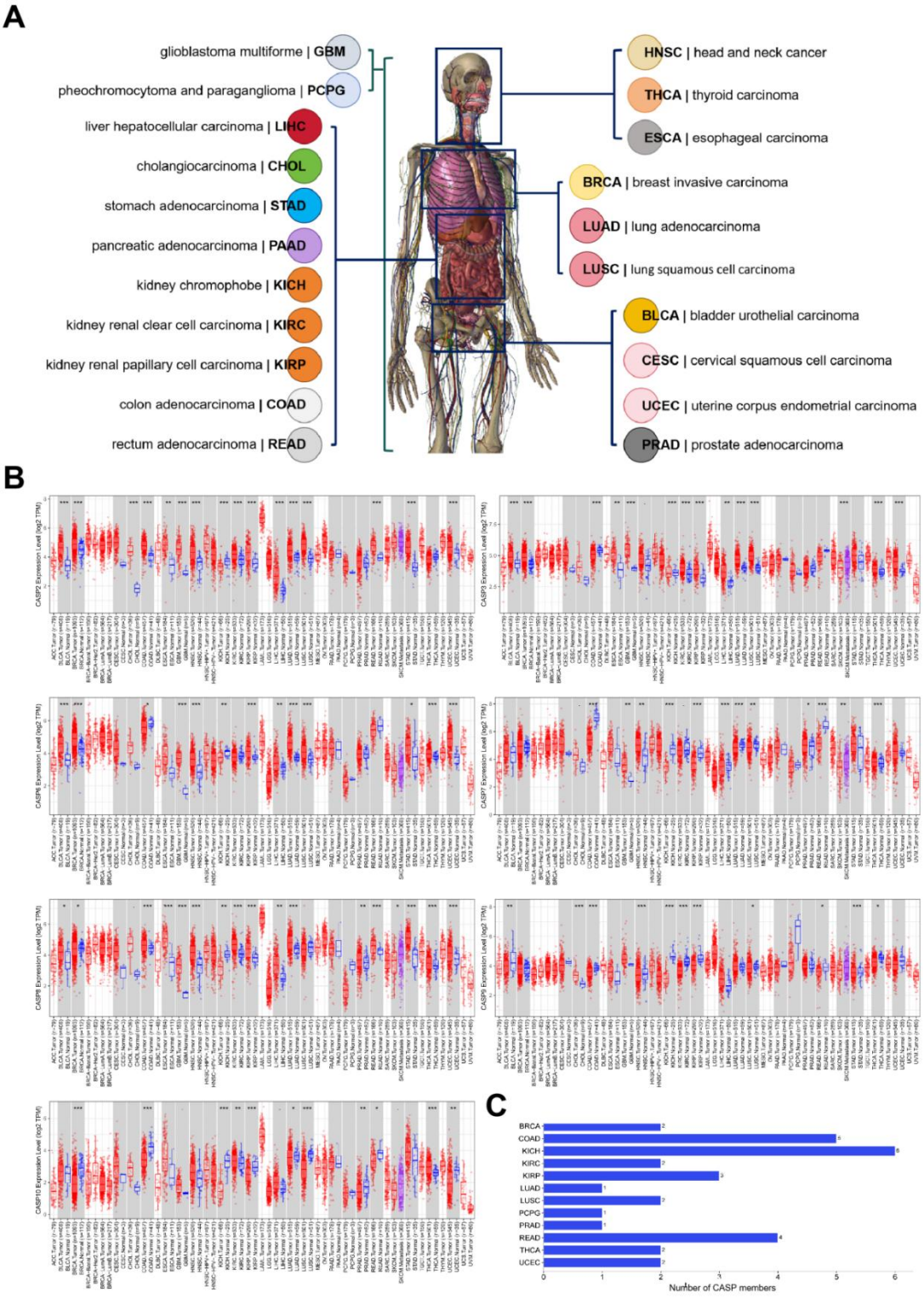


Figure 1. Differential caspase mRNAs in various cancers by TIMER2.0 databases. **A** TCGA tumor types associated with mRNA expression of 7 caspase family members. **B** Differential expression of *CASP2*, *3*, *6*, *7*, *8*, *9*, *10* (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). **C** Number of qualified caspase (CASP).

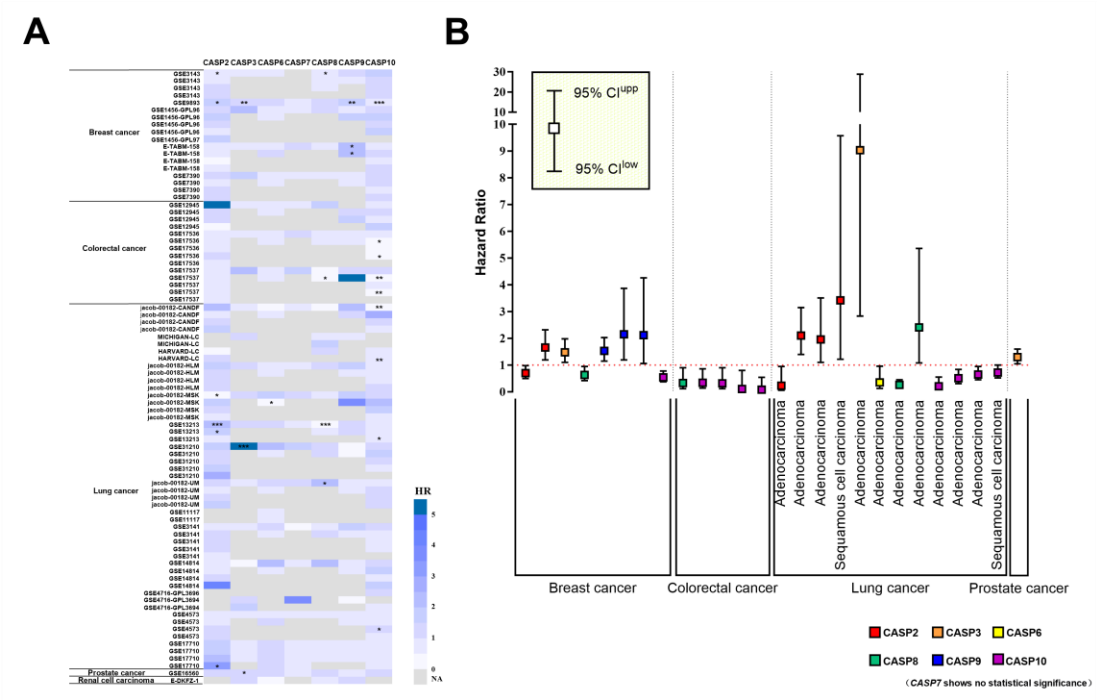


Figure 2. Prognosis analysis under overall survival using PrognoScan. **A** *CASP2*, *3*, *6*, *7*, *8*, *9*, *10* expression related with hazard ratio in different cancer datasets. HR indicates hazard ratio (*p < 0.05, ** p < 0.01, *** p < 0.001). **B** Statistics on prominent hazard ratio with 95%CI.

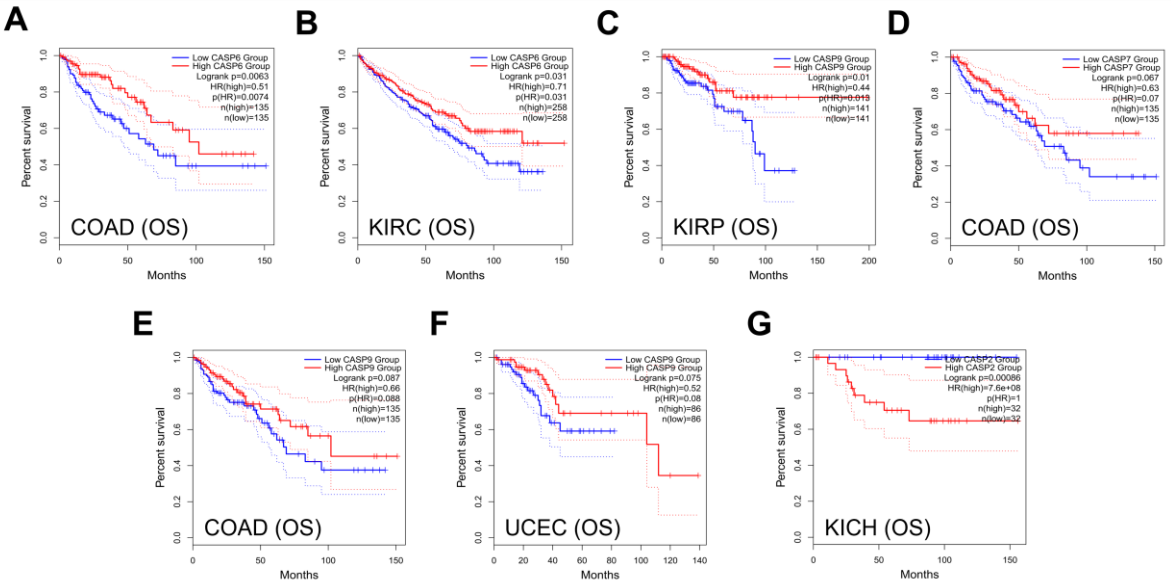


Figure 3. Association between gene expression levels and overall survival across various cancer types **A** COAD with *CASP6*, **B** KIRC with *CASP6*, **C** KIRP with *CASP9*, **D** COAD with *CASP7*, **E** COAD with *CASP9*, **F** UCEC with *CASP9*, and **G** KICH with *CASP2.* OS refers to overall survival. COAD, KIRC, KIRP, UCEC, and KICH note colon adenocarcinoma, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, uterine corpus endometrial carcinoma, kidney chromophobe in turn.

Immune microenvironment correlations

TIMER2.0 unveiled that expression level of *CASP6* was a negative correlation to the infiltration levels with neutrophils (ρ = -0.23, p < 0.001), macrophages (ρ = -0.204, p < 0.001), and dendritic cells (ρ

= -0.17, p < 0.01) in COAD (Fig. 4A). Instead in KIRC, *CASP6* expression represented a pronounced positive correlation with infiltration levels of neutrophils (ρ = 0.232, p < 0.001), CD4$^+$ T cells (ρ = 0.209, p < 0.001), dendritic cells (ρ = 0.198, p < 0.001), macrophages (ρ = 0.143, p < 0.01), and B cells (ρ = 0.104, p < 0.05) (Fig. 4B). Only CD4$^+$ T cells (ρ = 0.245, p < 0.001) exposed active immune associations for *CASP9* in KIRP, being statistically significant (Fig. 4C).

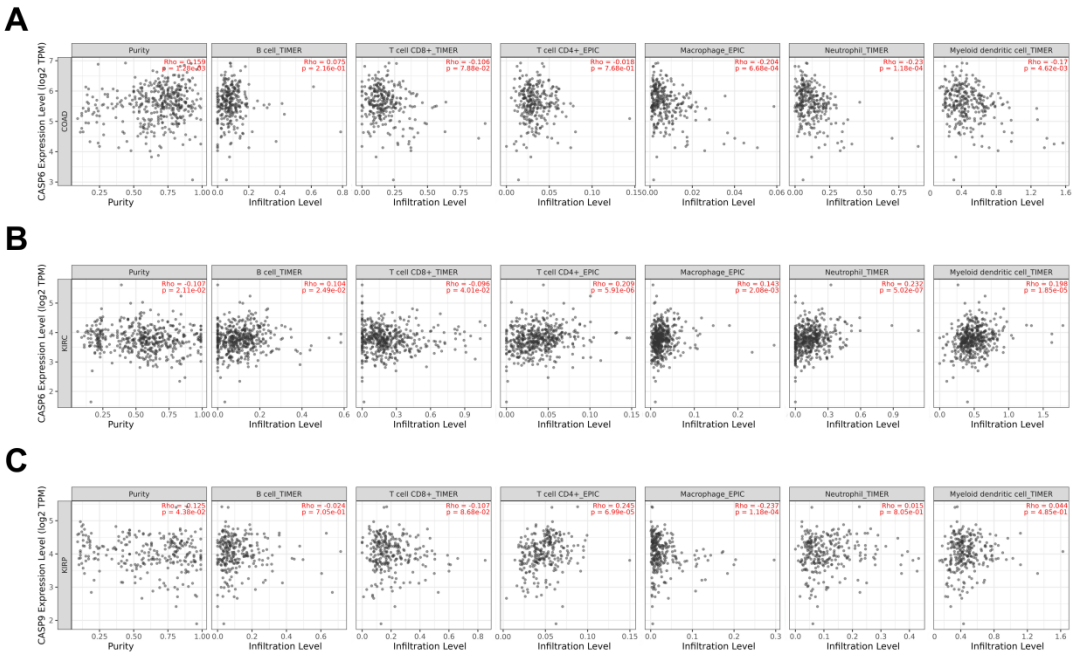


Figure 4. Immune infiltration and gene expression under overall survival in **A** COAD and **B** KIRC with *CASP6*, and in **C** KIRP with *CASP9*. COAD, KIRC, and KIRP denote colon adenocarcinoma, kidney renal clear cell carcinoma, and kidney renal papillary cell carcinoma, respectively.

Genomic alteration landscape

Two missense mutations (E16G and K238R) in *CASP6* were defined as unknown significance in COAD (Vasaikar: n = 110 [32]) through cBioPortal (Fig. 5A). Wild-type status was observed in *CASP6* of KIRC (Guo: n = 98 [33]; Sato: n = 106 [34]; Gerlinger: n = 78 [35]; TCGA: n = 1496) (Fig. S2). A missense mutation (S133F) in *CASP9* remained undefined characteristic in KIRP (TCGA: n = 576) (Fig. 5B). To further assess the potential functional impact of the three VUS (*CASP6* E16G/K238R and *CASP9* S133F), in silico prediction was performed using FATHMM. All three variants received scores above the default cancer-associated threshold (-0.75), E16G (2.23), K238R (1.74), and S133F (2.25), suggesting that these missense substitutions are predicted to be neutral/benign with regard to cancer association.

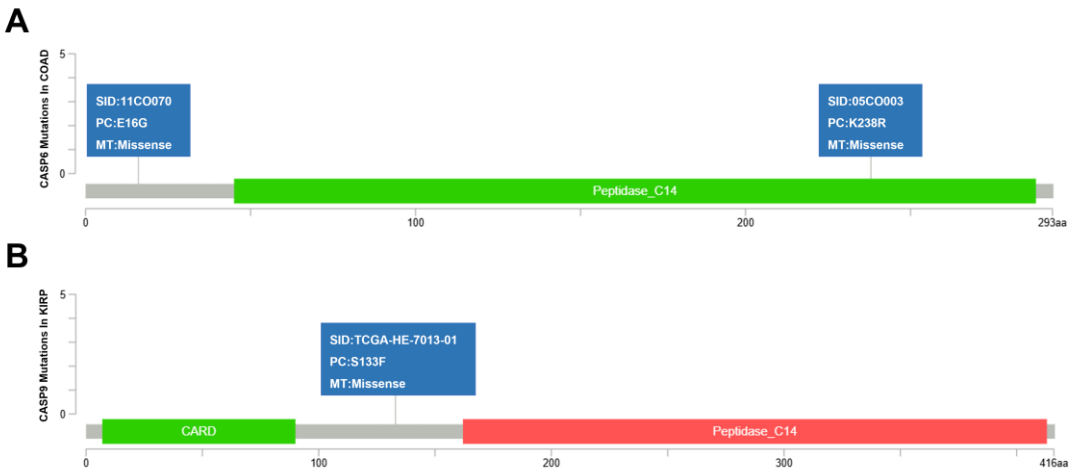


Figure 5. *CASP6* and *CASP9* mutation severally in **A** COAD and **B** KIRP. COAD stands for colon adenocarcinoma. KIRP refers to kidney renal papillary cell carcinoma. SID, PC and MT indicate separately sample ID, protein change and mutation type.

Epigenetic regulation patterns

CASP6 promoter presented significant hypermethylation ($p < 0.05$) in KIRC (Fig. 6A-E), including primary tumor, individual cancer stage from 1 to 4, age from 41 to 80 years old, tumor grade from 1 to 4, male and female, rather than in COAD by using UALCAN (Fig. S3). *CASP9* attended to significant high level of promoter methylation ($p < 0.05$) in primary tumor, cancer stage from 1 to 3, age from 21 to 80 years old, normal weight, extreme weight and obese, male and female of KIRP (Fig. 6F-J).

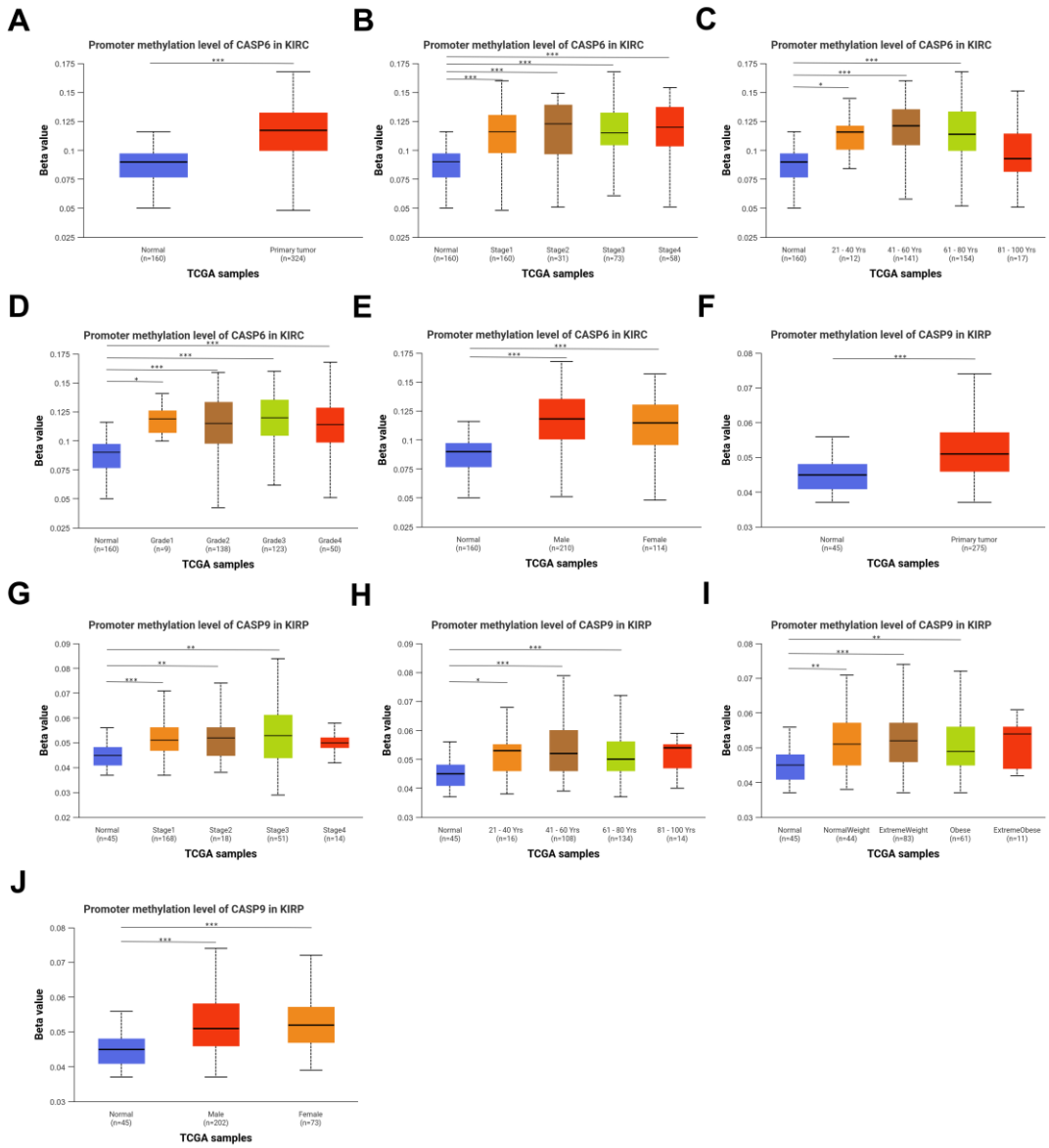


Figure 6. Promoter hypermethylation of *CASP6* in **A-E** KIRC and of *CASP9* in **F-J** KIRP (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). KIRC is kidney renal clear cell carcinoma. KIRP is kidney renal papillary cell carcinoma.

Discussion

The pan-cancer transcriptional profiling revealed significant downregulation of seven caspase family members (*CASP2*/*3*/*6*/*7*/*8*/*9*/*10*) across malignancies compared to normal counterparts. Systematic screening identified tumor-type specific caspase suppression patterns including *CASP2*-KICH/THCA, *CASP3*-COAD/READ, *CASP6*-COAD/KICH/KIRC, *CASP7*-COAD/KICH/KIRP/LUAD/LUSC/READ, *CASP8*-BRCA/KICH, *CASP9*-UCEC/THCA/READ/PCPG/KICH/KIRC/KIRP/COAD, *CASP10*-UCEC/READ/PRAD/LUSC/KICH/KIRP/COAD/BRCA, with KICH and COAD exhibiting most frequent suppression patterns. *CASP8* and *CASP10* emerged as favorable prognostic indicators in colorectal cancer (HR < 1, p < 0.05) through PrognoScan hazard ratio analysis. Paradoxically, COAD tumors exhibited elevated *CASP8* expression versus normal tissues, while *CASP10* expression failed to stratify survival outcomes. GEPIA2 validation confirmed the prognostic utility of *CASP6* in COAD and KIRC, as well as *CASP9* in KIRP.

We subsequently employed TIMER2.0 to investigate immune contexture associations with *CASP6* expression in COAD/KIRC and *CASP9* in KIRP. Building upon forge bonds between immune infiltration patterns and clinical outcomes [36, 37], our analysis revealed distinct tumor microenvironment profiles. *CASP6* expression was positive correlations with adaptive immune participants comprising B cells, $CD4^+$ T cells, and myeloid populations in KIRC, whereas being negatively related with immunosuppressive subsets in COAD. A plausible explanation for this opposing correlation lies in the context-dependent regulation exerted by *CASP6*-mediated pyroptosis, which operates differently across distinct tumor microenvironments. In COAD, caspase-6 triggers pyroptosis by cleaving GSDMC and upregulates the chemokine CXCL2, which specifically recruits myeloid-derived suppressor cells [38]. Conversely, in KIRC, caspase-6, as a core component of PANoptosis, activates the NLRP3 inflammasome and promotes the maturation and release of IL-1β and IL-18, thereby enhancing innate immune responses and facilitating the recruitment of effector immune cells, which underlies the positive correlation observed [39, 40]. Furthermore, studies in liver inflammation models have demonstrated that caspase-6 modulates macrophage NEK7/NLRP3 inflammasome function via the RIPK1-IκBα axis, and notably, the regulatory effect of caspase-6 on RIPK1 is not invariant, as caspase-6 deficiency can either induce RIPK1 upregulation or promote its degradation under different pathophysiological conditions [41]. Comparatively, the significant relation between *CASP9* expression and immune infiltration only corresponded to $CD4^+$ T cells in KIRP. Prior study has reported that tumor-related immune cell may be indicative for favourable prognoses in patients [42]. Deficient memory B cells or growing amount of M0 macrophages in tumors had certain relevance with the inferior prognosis of early-stage lung adenocarcinoma [43]. Dendritic cell immunotherapy can drive tumor infiltration by lymphocytes, which may have beneficial effect on clinical outcome [44]. Gene mutation type is one of the most momentous elements influencing gene expression level [45]. Somatic *CASP6* missense mutations have been documented in gastrointestinal carcinomas [46]. In the present research, genomic analysis identified two *CASP6* missense variants (E16G/K238R) in COAD and one *CASP9* missense variant (S133F) in KIRP, all initially classified as VUS. FATHMM-based prediction indicated that all three are neutral/benign (scores above -0.75). Nevertheless, whether these variants contribute to the reduced *CASP6*/*CASP9* expression observed in these tumors, or merely represent passenger mutations, warrants further investigation through functional assays.

DNA methylation, an essential performance of epigenetic modification of genome, exerts a critical influence on disease genesis and advancement [47]. The average methylation rates of gene promoter are inversely coupled with gene transcript levels [48]. For instance in human pulmonary carcinoma H1299 cells, dCas9-DNMT3a system detects that CpG island is hypermethylated about thirty percent in SMARCA2 promoter, and SMARCA2 transcription level is reduced by nearly three folds [49]. Moreover, previous study indicates that elevated methylation of the *CASP9* promoter in KIRP reduces gene expression [50]. In our work, DNA methylation profiling demonstrated tumor-specific promoter hypermethylation of *CASP6* in KIRC and *CASP9* in KIRP. This epigenetic silencing mechanism aligns with existing observations in SMARCA2. However, given that promoter hypermethylation was not observed in COAD despite reduced *CASP6* expression, additional mechanisms may contribute to *CASP6* dysregulation in this tumor type. Notably, histone deacetylation is a well-established mechanism for transcriptional repression. Histone deacetylases can be recruited to specific gene loci via methyl-CpG-binding domain proteins or other chromatin-modifying complexes, leading to chromatin compaction and gene silencing [51].

These findings collectively emphasize the necessity for rigorous biomarker validation when interpreting caspase expression profiles in oncological contexts. The functional dichotomy observed across caspase-2 isoforms, exemplified by Casp-2L's pro-survival versus Casp-2S's anti-apoptotic activities, underscores the limitations of bulk transcriptional analysis [52]. To be specific, the short isoform of caspase-2 is involved in several anti-apoptotic pathways, and in human B lymphoma Namalwa cells, the long isoform of caspase-2 as an endogenous apoptosis inhibitor postpones DNA fragmentation caused by TNF-alpha and avoids caspase cascade activation [53]. Besides apoptosis-promoting substrates activation, *CASP3* triggers a negative reaction in which COOH-terminal PS2 polypeptide antagonizes the apoptotic behavior [54]. In a screening of primary breast tumor specimens obtained from patients undergoing surgical resection, analysis reveals that around 25% of both tumor tissues and histologically normal adjacent peritumoral tissues exhibit no detectable reduction in caspase-3 mRNA levels or protein expression [55]. The experiment on inflammation-induced tumorigenesis discovers that apoptosis and cellular proliferation are unaffected from *CASP6* levels in mouse colon, suggesting that *CASP6* is not expected to participate in colitis-related tumors [56]. *CASP6*'s null proapoptotic impact in colitis-associated tumors contrasts with its prognostic utility in KIRC, highlighting context-dependent functionality. Caspase-8 suppresses oncogenic transformation independent of apoptotic signaling [57], complicating its biomarker interpretation. Caspase-8 isoform 5, 6, 7, 8 may restrict the proapoptotic activity due to deficiency of catalytic site [58, 59], while caspase-8

promotes metastasis *in vivo* by tyrosine-kinase-dependent interaction with components of focal adhesion complex [60]. For caspase-10's V410I variant and pro-domain-only polypeptides, the former one impairs caspase-10 capability inducing apoptosis [61], while the latter one demonstrates NF-κB-mediated survival advantages [62, 63], confounding expression-outcome correlations. In mammalian cells, caspase-10g as a kind of caspase-10 splice variants only stimulates slight apoptosis with overexpression [64]. Herein our results align with increasing evidence of caspase pleiotropy. Although apoptosis-related *CASP2*/*3*/*6*/*7*/*8*/*9*/*10*, prognostic analyses indicated that *CASP6* emerged as a candidate predictor supported by survival evaluation in KIRC, being implicated in immune microenvironment remodeling and epigenetic silencing via promoter hypermethylation.

Conclusions

Through systematic pan-cancer investigation of seven apoptosis-associated caspases (*CASP2*/*3*/*6*/*7*/*8*/*9*/*10*) at expressive and prognostic levels, this multi-omics study revealed their limited utility as standalone biomarkers for therapeutic efficacy assessment. Notably, *CASP6* demonstrated translational potential for KIRC treatment monitoring, a finding corroborated by multidimensional validation incorporating survival stratification, immune contexture profiles and methylation dynamics. This specificity contrasts with other caspases' functional ambiguities. In addition, given the exploratory nature of this bioinformatics-based study, it should be emphasized that our findings are derived from transcriptomic and DNA methylation data and do not constitute protein-level validation. The utility of *CASP6* as a prognostic biomarker in KIRC therefore warrants further corroboration through direct assessment of its protein expression, enzymatic activity, and post-translational modification status in tumor tissues, as well as validation in prospective cohort studies and functional experiments.

Conflicts of Interest

The authors declare no conflicts of interest regarding this publication.

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Supplementary material

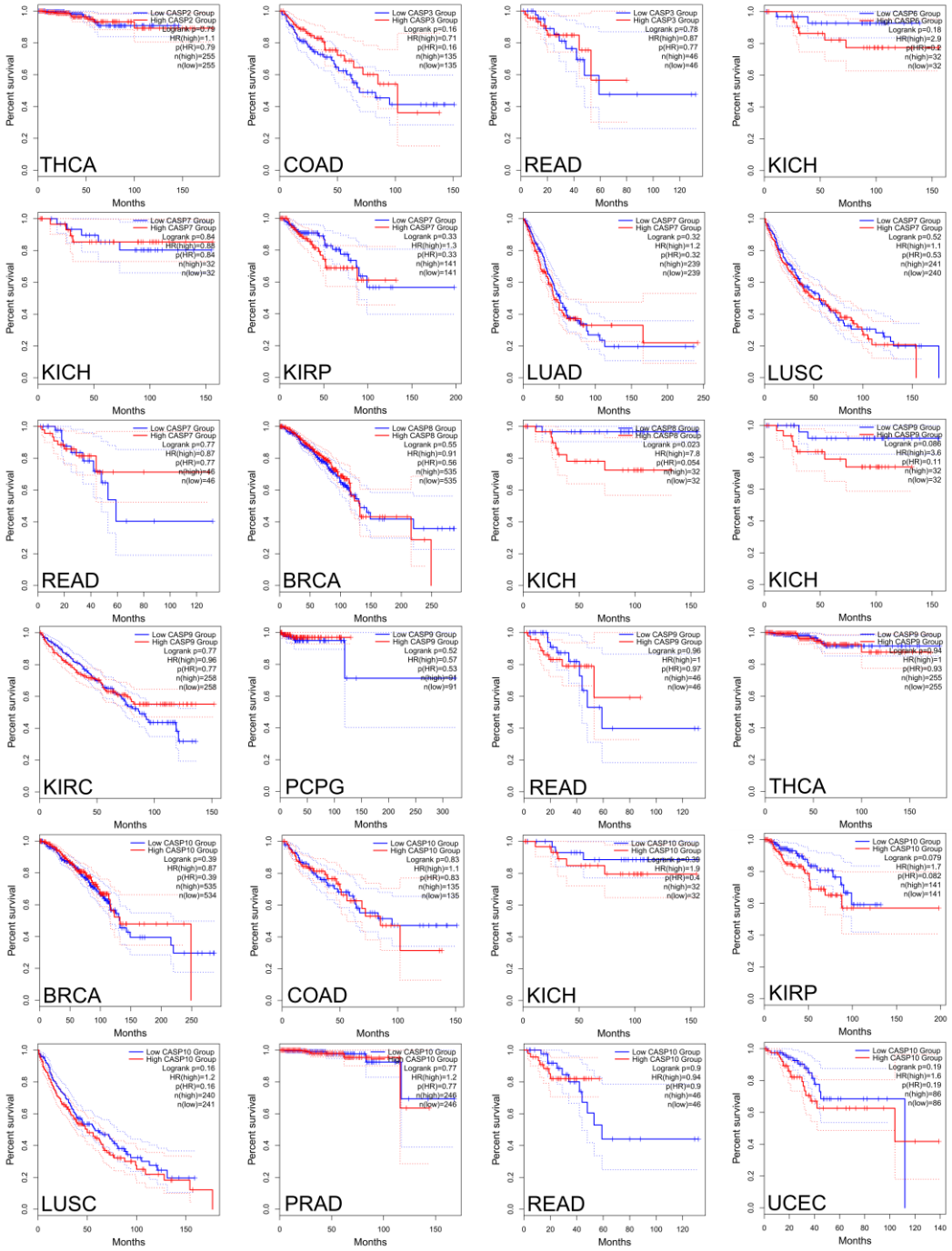


Figure S1. Association between caspase gene expression and prognosis for overall survival.

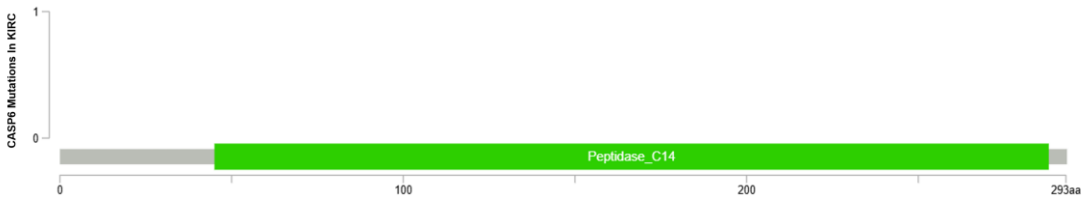


Figure S2. Undiscovered *CASP6* mutation in kidney renal clear cell carcinoma (KIRC).

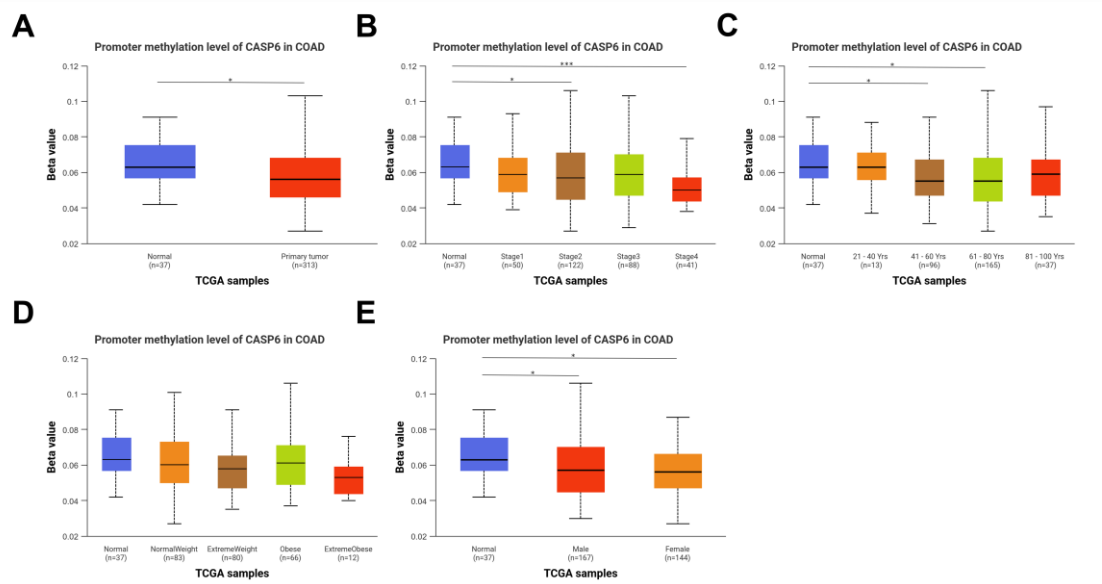


Figure S3. Promoter methylation level of *CASP6* in colon adenocarcinoma (COAD) (*$p < 0.05$, ***$p < 0.001$).