NSUN6 as a Key RNA Methylation Regulator in Colon Cancer

Qian Zhao
Zhengzhou University First Affiliated Hospital https://orcid.org/0000-0002-8732-0175
Zhao Sun
Zhengzhou University First Affiliated Hospital
Hongyi Li
Zhengzhou University First Affiliated Hospital
Jingyi Li
Zhengzhou University First Affiliated Hospital
Fengmei Chen
Zhengzhou University First Affiliated Hospital
Muxi Wang
Zhengzhou University First Affiliated Hospital
Hongqiang Jiang
Zhengzhou University First Affiliated Hospital
Tongwen Sun
Zhengzhou University First Affiliated Hospital
Zhangsuo Liu
Zhengzhou University First Affiliated Hospital
Yang Fu
Zhengzhou University First Affiliated Hospital
Jing-Hua Yang (jyang@zzu.edu.cn)
Zhengzhou University First Affiliated Hospital

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Abstract

Background

RNA methylation plays an important role in epigenetic modifications, which involves in the regulation of RNA processing, stability and translation. While the cross-talking and the mechanism of multi-type RNA methylation in colon cancer needs further investigation. Here we report the comprehensive analysis of the RNA methylation regulators for N6-methyladenosine, N1-metylasdenosine and 5-methylcytosine in the tumorigenesis and progression of colon cancer.

Methods

The mRNA expression and genetic mutation of 40 RNA modification regulators in patients with colon cancer were obtained from TCGA and GEO database. Consensus clustering is applied to classify the patients based on the regulator expression. The patients with different survival rates are examined for their gene mutations, biological pathways, and immune properties. The regulators with the highest vimp score are identified to be important for survival using the improved random forest analysis method. Biopsy samples from 108 patients are used to validate the key regulators using tissue microarray and specific antibodies. Finally, the mechanism underlying the methylation regulators is further examined by GSVA and correlation analysis.

Results

The patients are clearly clustered into two groups with different survivals. The patients with the worse survival are correlated with dysregulated KRAS signaling, hypoxia and immune responses. They are also prone to increased stromal scores and myeloid-derived suppressor cells. Markedly, m5C methyltransferase NSUN6 is identified as the most significant regulator for prognosis. The upregulated expression of NSUN6 is further proved in 108 patients by immunohistochemistry and tissue array, suggesting its association with better prognosis and lower tumor staging. Bioinformatic analysis indicates that NSUN6 enhances the expression of E3 ubiquitin ligase FBXW7, activates ubiquitin-proteolysis and suppresses oncogene expression.

Conclusions

Patients with colon cancer are clearly clustered into different survivals by RNA methylation regulators, and the worse prognosis is relevant to dysregulated KRAS signaling, hypoxia and immune response. NSUN6 is found to prolong the survival and strongly correlated with FBXW7 that reduces the oncogene expression and activates the ubiquitin-proteolysis.

1. Background

Colon cancer is the most frequent cancer of gastrointestinal tract with poor prognosis leading to the fourth cause of mortality in cancer globally(Dekker et al. 2019, Siegel et al. 2020). The most common pathological type is the colon adenocarcinoma, which is associated with malignant epithelial tumor. Though colonoscopy increases the initial diagnosis of colorectal cancer, some patients are at risk of the lymphatic and distant metastasis with poor prognosis(Messersmith 2019). Several biological factors like diet, microorganisms as well as their metabolites are associated with CRC carcinogenesis(O"Keefe and Stephen 2016). It is recently reported that the new biological discoveries and therapeutic treatments are unraveled by integrating the multi-omics data(2019). Exploring the molecular mechanisms of colon cancer relevant to poor prognosis thus becomes a vital task.

RNA methylation is one of the genomic epigenetic modifications that is involved in the many biological processing, such as RNA processing, RNA nuclear export, translation, stability and RNA-protein interactions. Several types of RNA methylation are found in messenger RNAs (mRNAs), non-coding RNAs (ncRNAs), transfer RNA (tRNAs) and small nuclear RNAs (snRNAs), including N6-methyladenosine (m6A), 5-methylcytosine (m5C), N1-metylasdenosine (m1A) and 2-O-methylation (2'OMe)(Shi et al. 2020). These modification types are usually reversible and dynamic, and controlled by a series of regulatory proteins known as 'writers', 'readers' and 'erasers'. Generally, RNA modifications are connected in vivo. It is demonstrated that RNAs can be modified by multiple modifications and some regulators can also take part in multiple methylation processes. For example, YTH domain family proteins can be readers both in m6A and m1A modifications(Dai et al. 2018, Wang et al. 2015).

The regulation of RNA methylation is closely related to diseases especially tumorigenesis and cancer development(Boo and Kim 2020, e et al. 2020, Jonkhout et al. 2017). The molecular function of m6A modification in colon cancer has been discussed in colon cancer. Regulators such as WTAP, METTL3, FTO and IGF2BP2 are known as oncogenes or tumor suppressors, which are involved in the WTAP-WT1-TBL1, METTLE3-miR-1246-SPRED2, FTO-miR-1266, and LINRIS-IGF2BP2-MYC signaling axis(Deng et al. 2019, Peng et al. 2019, Shen et al. 2018, Zhang et al. 2015, Zhu et al. 2020). The regulators can also be the predictors for survival. For instance, HNRNPC and YTHDF1 are relevant to the survival of colon cancer (Tao et al. 2019). Generally, different expression pattern of RNA methylation regulators leads to different biological type, which devotes to precise medicine. The various m6A expression patterns have been demonstrated to have different molecular types, which have different response to PD-1 immunotherapy in gastric cancer(Zhang Bo et al. 2020). Thus, it could be helpful for investigating the RNA modification pattern in colon cancer by integrating the major RNA methylation regulators due to their complex interactions.

Notably, RNA methylation regulators especially for m5C and m1A are less characterized in colon cancer. NSUN6, belonging to the family of Nop2/Nol1/SUN domain (NSUN) proteins, is previously identified as a m5C methyltransferase by mediating the methylation of C72 of tRNACys and tRNAThr(Haag et al. 2015, Liu et al. 2017). Recently, NSUN6 is reported to enhance the stability and translation of target mRNA,

which have the consensus sequence motif CTCCA(Selmi et al. 2021). The further analysis shows that NSUN6 could be a tumor suppressor in specific cancers(Selmi et al. 2021, Yang et al. 2021). In comparison with other family proteins, the mRNA level of NSUN6 has the opposite tread of tumor grade and stage in gastrointestinal cancer(Xiang S. et al. 2020).

In this study, a panel of the major RNA methylation regulators are used to classify patients with colon cancer. The two clusters are different in several biological pathways and immune response. Those regulators are analyzed in association with the prognosis and the NSUN6 is identified as the most influential regulator by machine learning model. The tissue array assay is applied to confirm the protein level of NSUN6 in tumor and adjacent tissues and investigate the impact of survival in colon cancer. The potential mechanism of NSUN6 as a tumor suppressor in colon cancer is also recognized.

2. Materials And Methods

2.1. Datasets and RNA modification regulators

The colon cancer transcriptome profilers from TCGA and GEO database are retrieved by TCGAbiolinks and GEOquery. RNA sequencing data from TCGA database is in form of FPKM value which is subsequently transformed into TPM format, whereas the data form GEO datasets is directly used for analysis. Patients who exhibited incomplete survival data (overall survival) are excluded in this study. The regulators comprised of writers, erasers as well as readers are collected from a recent high quality review(e et al. 2020). Three major types of RNA modifications (m6A, m5C and m1A) are selected in our study and the details are shown in Tab. A1. Their correlations are calculated by Pearson correlation method, the r values more than 0.4 indicated the high positive correlation. The interactions are analyzed by the String (https://string-db.org). The hub genes are identified through the ‘ClusteringCoefficient’ method in cytoHubba.

2.2. Classification of colon adenocarcinoma patients

The similarity of patients is calculated by k-means algorithm based on the normalized RNA regulators TPM value. The clusters are established via the consensusClusterPlus (version 1.53.0) package using the parameters (Item = 0.8, max = 9, resampling = 1000, method = “km”, distance = “Eroup”). The optional cluster number is evaluated by using the cumulative distribution function curve. In addition, PCA (Principal component analysis) and PLS-DA (Partial least squares discrimination analysis) are conducted to visualize the variations among the subtypes.

2.3. Gene mutation and pathway enrichment analysis

The gene mutation data obtained from TCGA database are summarized by using mutation types as well as the frequency of RNA modification regulators, then the mutation data between the subgroups are compared together. The survival outcomes of patients with the wild type and mutation type regulators are also compared. GSVA (Gene Set Variation Analysis) implemented a non-parametric unsupervised method to estimate the variation in each sample’s specific biological process activity(Hnzelmann et al. 2013). In

this study, the GSVA and GSEA methods (Gene set enrichment analysis) determine the variations among the subgroups. The reference biological pathway datasets included c2.cp.kegg.v7.2.symbols.gmt, c5.go.bp.v7.2.symbols.gmt and h.all.v7.4.symbols.gmt, which are downloaded from the MisgDb database. Besides, GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis are also carried out to explore the different potential biological pathway in subgroups.

2.4. Estimation of immune scores, stromal scores and immune cells

The ESTIMATE algorithm is used to evaluate immune cell infiltration, stromal content as well as tumor purity(Yoshihara et al. 2013). The relative abundance of each immune cell is determined by ssGSEA (single-sample gene-set enrichment analysis) algorithm. The scores are then normalized to compare with each subgroup using the limma R package. The immune infiltration gene set, containing the natural killer T cells, activated CD8 T cells, as well as macrophages, is obtained from a published study(Charoentong et al. 2017). The association between the regulators and immune cells are computed via the Spearman correlation analysis.

2.5. Survival analysis and single gene meta-analysis

Datasets is collected in agreement with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidance. The COAD patients are obtained from TCGA and GEO database without overall survival information. For single gene survival analysis, the gene is divided into two groups by cutoff value, then the Kaplan-Meier curves are used to describe the survival differences between the subgroups. The overall survival is used to evaluate the patient's outcomes and he log-rank test is used to detect the significance. RandomForestSRC (R package), is combined with the improved random forest algorithm to analyze the survival data. Gimp scores, calculated via the 'vim function in RandomForestSRC package, are used to evaluate the importance of the regulators. For meta-analysis, the mRNA expression level is scaled in each dataset, and the HR (Hazard ratio) together with 95% CI is calculated by univariate Cox regression analysis. We used the 'mutagen function from R meta package to achieve the combined HR.

2.6. Immunohistochemistry and Immunostaining evaluation

The tissue microarray which has 104 colon cancer tissues and 80 adjacent normal colon tissues is purchased from Shanghai Outdo Biotech Co., Ltd. (Shanghai, China). Paraffin sections are deparaffinized and rehydrated with xylene and alcohol, subjected to antigen retrieval by citric acid antigen retrieval buffer, and blocked in 5% bovine serum albumin to prevent nonspecific antibody binding. Sections are incubated overnight at 4°C with rabbit anti-NSUN6 antibody (Bioss) which is used at a dilution of 1: 500 in phosphate-buffered saline (PBS), followed by incubation with anti-rabbit secondary antibody conjugated to horseradish peroxidase for 50 min. 3,3'-diaminobenzidine (DAB) is applied as a chromogen for visualization of immunoreaction. Then tissue sections are lightly counterstained with hematoxylin, washed, and mounted with glass coverslips. At last, visualize staining of tissue under a microscope, acquisitive and analysis image. The expression score of IHC, defined as the staining intensity multiplied

by the percent tumor positive area, is semi-quantitatively determined by the H-score (0–300). The H-score of NSUN6 is calculated by the multiplication of the intensity score (0, no staining; 1, light brown; 2, brown; 3, dark brown.) by the area of expression (0–100%). The H-score is scaled before survival analysis. The significance of association between the protein level and clinical characteristics is evaluated by Chi square test or exact Fisher test.

2.7. The potential mechanism of NSUN6 in colon cancer

The biological pathway scores are first calculated by GSVA method, and the pathways whose score are closely to NSUN6 values are regarded as the potential pathway of NSUN6. Then, we identify the co-expression genes by Pearson relative analysis method. The overlap gene between selected the specific pathway and genes is fully evaluated in validated datasets. The gene fasta is retrieved from NCBI database. TIMER2, is a web server involved in public gene expression analysis. We use this tool to scan the gene expression level between the tumor and normal tissue besides assessing the survival outcomes in pan-cancer.

2.8. Statistical analysis

The DEGs (different expression genes) are determined using the limma R package, whereby the threshold of adjust P value is < 0.05. The cut-off value for each gene is determined by the 'surv-cutpoint' R function in the survminer R package, which could identify the maximum rank statistic. Two-side p values < 0.05 are statistically significant. All the data analyses are conducted in R 4.0.2 software. The tools of data analysis and visualization are summarized in Tab. A2.

3. Results

3.1. Two patient clusters identified by RNA methylation regulators

521 tissues from patients who diagnosed with colon adenocarcinoma are obtained from TCGA database. After excluding the patients who exhibited incomplete survival data, 453 patients are included in our cohort. A panel of 40 regulators for m6A, m1A and m5C methylations (Tab. S1) are analyzed by consensus clustering analysis. These patients are clearly categorized into two groups (Fig. 1a): 242 patients with worse prognosis (cluster 1) and 211 patients with better prognosis (cluster 2, p = 0.022). The best consensus cluster number is 2 (Fig. 1b, c) as detailed in Fig. S1. By PLS-DA analysis, the two clusters are also distinguished properly by the expression of RNA methylation regulators (Fig. 1d). These regulators are also differently expressed in the tumor and normal tissues (Fig. S2a). Correlation analysis demonstrates that HNRNPA21 is positively correlated with METTL14, TRMT10A and HNRBPC (Fig. S2b). String and intensive analysis indicate that NSUN2 and NSUN6 are hub proteins (Fig. S2c, d).

3.2. Different mutations of the regulators in the two patient clusters

Patients without mutation information are excluded in further analysis. RNA methylation regulators are often mutated in colon cancer with frequencies ranging from 0 to 7%. Among them, the m5C erasers such as TET3 and TET1 exhibit the highest rate for missense, nonsense, and frame shift mutations (Fig. 2a). High mutation frequencies in ZFHX4 and CSMD3 are found in the patients with worse prognosis whereas the mutations in RYR2 and OBSCN are often associated in better prognosis (Fig. 2c, d). In comparison, METTL16 is considered as a m6A writer and found with low but distinguishable mutations in patients with different prognosis (Fig. 2b). Furthermore, the patient cluster with worse prognosis tends to have a mutated YTHDF3 (Fig. 2e).

3.3. Different biology pathways in the two patient clusters

Notably, in the patients with worse prognosis, carcinogenic processes and immune activation including ECM receptor interaction, KRAS signaling pathway, inflammatory response and mast cell differentiation are enriched (Fig. 3a). GSVA analysis shows that the cell substrate adhesion is enriched in the cluster with bad prognosis (Fig. 3c), while GO analysis indicates that the O-glycan pathway is different in the two clusters (Fig. 3e). Besides, the DNA replication pathway, CRD mediated mRNA stabilization pathway, spliceosome pathway, the KRAS signing pathway, hypoxia and ECM receptor interaction are vital to cancer research. GSEA analysis indicates that the immune response score is higher in the patient cluster with worse prognosis (Fig. 3b). Thus, we apply ssGSEA and ESTIMATE algorithms to determine the degree of immune infiltration and the relative content of each immune cell. The results showed that no significant difference for immune infiltrating cell types (Fig. S3a) in subgroups. The cell composition of the MDSC, NK T cell, effector memory CD8 T cell and CD56dim NK cells (Fig. S3c-g) in cluster 1 are higher that cluster 2. Meanwhile, the stromal score is increased in the patient cluster with worse prognosis (Fig. S3a).

3.4. RNA methylation regulators strongly associated with prognosis

Increased expression of RRP8, TRMT6, TMT10B, NSUN6, ALKBH5, IGF2BP1, YTHDF2 and IGF2BP2 is associated with worse prognosis. On the contrary, increased expression of ALYREF, DNMT2, YBX1 and YTHDC2 is correlated with better survival outcome (Fig. 4).

3.5. The controversial influence of NSUN6 mRNA level in prognosis

Results above suggest that various regulators are related to prognosis. To identify the most influence regulators for deep analysis, we used the random forest to select the regulator (NSUN6) according to the highest vimp scores (Fig. 5a). NSUN6 gene are validated by external datasets from GEO database; however, those results are not consensus with the TCGA database. The external validated GEO datasets included GSE17536, GSE72968 and GSE39582, the Kaplan-Meier curves showed the opposite outcomes

compared to TCGA (Fig. 5a-e). The mRNA expression value is scaled in each dataset, the result of univariate Cox analysis is shown in Tab. A3. The I2 (71%) indicated that a high heterogeneity existed in multi-datasets. A combined HR based on random effect model, the HR with 95%CI (0.98, 0.82–1.19) suggests that the mRNA level of NSUN6 is not sufficient to judge the survival of patients (Fig. 5f).

3.6. Increased NSUN6 protein level leads to good prognosis.

The protein levels of NSUN6 in human colon cancer specimens (180 samples) are examined by immunohistochemistry (IHC) analysis. The results showed that NSUN6 is major localized to the cytoplasm, and is over-expressed by in colon tumor tissues (Fig. 6a and 6c). The immunostaining status of tissue array is consensus with HPA database (Fig. 6b). The results of paired T test confirmed that both mRNA and protein level of NSUN6 is over-expressed in colon cancer (Fig. 6d). Patients with high expression of NSUN6 had obvious survival advantage (Fig. 6f). The further analysis showed the patients with increased NSUN6 tended to had the lower AJCC stage (Fig. 6e and Table 1). Besides, the associations between NSUN6 and clinical characteristic are evaluated (Table 1). In spite of the statics significance, three patients suffered tumor metastasis in low NSUN6 group whereas no patients are observed in high NSUN6 group.

Table 1
The relationship between protein level of NSUN6 and clinical characteristics.

variables	Protein level of NSUN6 (n)		X^2 value	
	Group low	Group high		P value
Age			0	1
< 65	12	20		
>= 65	24	40		
Gender			0.98	0.32
Female	29	25		
Male	18	39		
Metastasis			2.81	0.09
No	35	64		
Yes	3	0		
Pathological grade			0.06	0.97
	2	4		
	18	32		
	17	28		
AJCC stage			8.87	0.03*
	0	4		
	19	40		
	16	20		
	3	0		

3.7. The potential mechanism of NSUN6 in colon cancer

The patients are ordered by mRNA level of NSUN6, then we calculate the correlation with GSVA scores. After analysis, we find that some pathways are more active in the high expression NSUN6 group, such as the ubiquitin proteolysis, RNA spliceosome pathway. Markedly, the KRAS signing pathway, adipogenesis, myogenesis and hypoxia pathway of those patients are inhibited (Fig. 7a). The results suggest that NSUN6 involved or regulated those pathways to function in colon cancer. Meanwhile, we identify FBXW7 as a co-expressed gene of NSUN6, which is confirmed in multi-datasets (Fig. 7d-g). What' more, FBXW7 is the only gene by intersecting the co-expression genes and ubiquitin proteolysis pathway (Fig. 7b). Moreover, FBXW7 has the CTCCA motif, which is necessary for NSUN6 to function of mRNA m5C

(Fig. 7c). In conclusion, NSUN6 could enhance the mRNA level of FBXW7 by m5C methylation to slow down the tumor progression.

3.8. The mRNA level of NSUN6 and the association with prognosis in pan-cancer

Notably, NSUN6 expression varies in different cancers. Thus, differential expression and survival analysis is further conducted to assess the role of NSUN6 in pan-cancers. NSUN6 is highly expressed in multiple cancers such as COAD (Colon adenocarcinoma), ESCA (Esophageal carcinoma), HNSC (Head and Neck squamous cell carcinoma), KIRC, KIRP, LUAD, PRAD, READ, STAD (Stomach adenocarcinoma), LUSC (Lung squamous cell carcinoma) (Fig. 8a). The univariate Cox regression analysis and Kaplan-Meier analysis showed that the effect of NSUN6 on cancer is diversified. The preferred prognosis is found in BLCA, HNSC-HPV+, PAAD and SKCM.

4. Discussion

In this study, regulations and interaction networks of 40 methylation regulators are analyzed in patients with colon cancer. By constructing protein-protein networks among the regulators, the m5C writers such as NSUN2 and NSUN6 are found to be hub proteins critical in the interaction network. For gene mutation, both TET3 and TET1 exhibit the highest mutation frequency (7%). In addition, the mutations in these regulatory factors will impact on patients' prognosis, and the wild type YTDHC1 has a better survival performance than mutation type. In support, experimental evidence demonstrates that the NSUN2 acts as an oncogene through promoting gastric cancer development by repressing p57Kip2 in an m5 C-dependent manner(Mei et al. 2020). NSUN2-specific sites are marked by a 3'TCCA motif, which are differ to the CTCCA motif of NSUN6(Huang et al. 2019, Selmi et al. 2021).

By consensus clustering over the mRNA level of regulators, the patients with colon adenocarcinoma are categorized into two clusters with different prognosis. Gene mutations are similar in two clusters, then the different biological pathways are investigated. The results suggest that these patients with worse prognosis usually have more active hypoxia, EMT signaling pathway, KRAS signaling pathway and NF-κB signing pathway, which are associated with tumor progression(Kikuchi et al. 2009, Xiang X. and Shah 2012, Ye et al. 2015). NF-κB (Nuclear Factor-kappa B), an ubiquitous transcription factor, regulates cytokines and cytokine receptors involving in inflammatory and immune response(Baldwin 1996). In colon cancer, the NF-κB signaling pathway plays a very important role in cell proliferation, angiogenesis, metastasis and drug resistance(Soleimani et al. 2020). The GSEA results showed that the immune response is active in patients with worse prognosis. Notably, immune cells are the important contents of tumor microenvironment, which are keys for immune cells therapy(Grivennikov et al. 2010, Weber et al. 2020). On this basis, the myeloid-derived suppressor cells (MDSC) are active ($p < 0.001$) in patients with worse prognosis, which apparently exert the suppressive effect on other immune cells(Gabrilovich and Nagaraj 2009). Additionally, the hypoxia, EMT signaling pathway together with the high proportion of

stromal could suppress the positive of the immune response, which explains the worse prognosis(Gamradt et al. 2021, Palazón et al. 2012, Romeo et al. 2019).

The RNA methylation regulators are closely related to prognosis. The impact of regulators on survival and use the random forest to select the key regulator. The results show that NSUN6 has the highest 'vimp' score, which indicates the importance for prognosis. However, the results of TCGA and GEO database are not consistent. Thus, meta-analysis is performed to achieve the combined HR and 95%CI (0.98,0.82–1.19). Tissue microarray assay results show that the patients with the high protein level of NSUN6 have a better prognosis. Particularly, increased NSUN6 protein level leads to the lower tumor stage and less tumor metastasis. This result agrees with previous experiments that NSUN6 is a tumor suppressor by functioning as a m5C writer in pancreatic and breast cancer cells(Selmi et al. 2021, Yang et al. 2021). In conclusion, the NSUN6 writer is regarded as a protective protein in colon cancer.

In addition, FBXW7 is found to be one of co-expression genes of NSUN6 and the result is validated by multi-datasets. FBXW7, a member of the F-box protein family, is a receptor subunit for Skp1/Cullin/F-box protein E3 ubiquitin ligases and functions as a tumor suppressor in colon cancer through reducing the oncogenes by ubiquitin-proteolysis pathway(Akhoondi et al. 2007, Fukushima et al. 2012). In this study, the high NSUN6 level is positively associated with ubiquitin-proteolysis pathway and also negatively associated with adipogenesis, myogenesis, hypoxia and KRAS signaling pathway. Therefore, our study provides evidence that NSUN6 as a m5C writer enhances the mRNA level of FBXW7 in colon cancer and can be developed as prognostic biomarker for patients with colon cancer. The function of NSUN6 needs to be further explored experimentally.

5. Conclusions

In colon cancer, patients can be divided into two groups with different survival by the expression of RNA methylation regulators including m6A, m5C and m1A. The patients with worse prognosis have the dysregulated biological pathways relevant to KRAS signaling pathway, hypoxia pathway and MDSC immune cells. Among those regulators, NSUN6 is the most important predictor and has been validated by tissue array. NSUN6 is proposed to improve the mRNA level of FBXW7 that reduces the oncogenes by activating the ubiquitin-proteolysis pathway, and that patients with colon cancer would benefit from the increased protein level of NSUN6.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Shanghai Outdo Biotech Company, the member of National Human Genetic Resources Sharing Service Platform (shanghai, China). The present study was performed in accordance with the ethical standards of The Institutional and National Research Committee.

Consent for publication

All authors have read and approved the content and agree to submit for consideration for publication in the journal.

Availability of data and materials

The data can be obtained from TCGA database (https://www.cancer.gov/tcga) and GEO database.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

Conceptualization, Zhangsuo Liu, Yang Fu and Jing-Hua Yang; Formal analysis, Qian Zhao, Zhao Sun, Hongyi Li, Jingyi Li and Jing-Hua Yang; Funding acquisition, Yang Fu and Jing-Hua Yang; Investigation, Qian Zhao, Fengmei Chen and Tongwen Sun; Project administration, Tongwen Sun, Zhangsuo Liu, Yang Fu and Jing-Hua Yang; Resources, Zhao Sun, Fengmei Chen, Hongqiang Jiang, Yang Fu and Jing-Hua Yang; Software, Qian Zhao, Zhao Sun and Jingyi Li; Validation, Zhao Sun, Hongyi Li and Muxi Wang; Visualization, Qian Zhao and Hongyi Li; Writing – original draft, Qian Zhao and Zhao Sun; Writing – review & editing, Yang Fu and Jing-Hua Yang.

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Figures

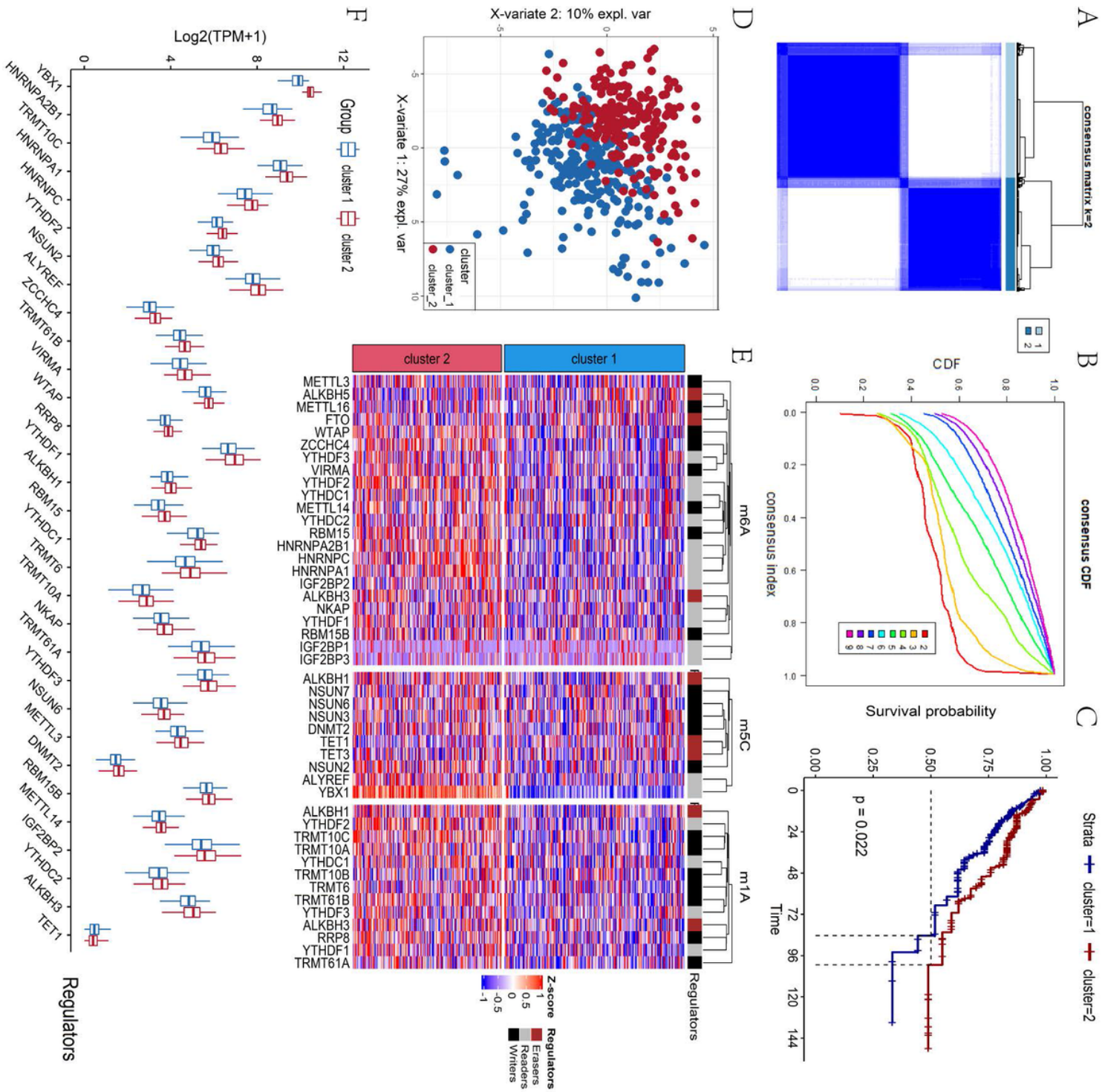

Figure 1

Two patient clusters with different prognosis by m6A, m1A and m5C methylation regulators. a and b. The best number of clusters using the consensus clustering method. c. Kaplan-Meier survival analysis of the two groups: cluster 2 has the better prognosis than cluster 1 (p = 0.022). d. The PLS-DA analysis of the two clusters. e. Gene expression level of different regulators in each sample. f. Significantly different regulators by limma R package and the boxplot of each regulator in two groups.

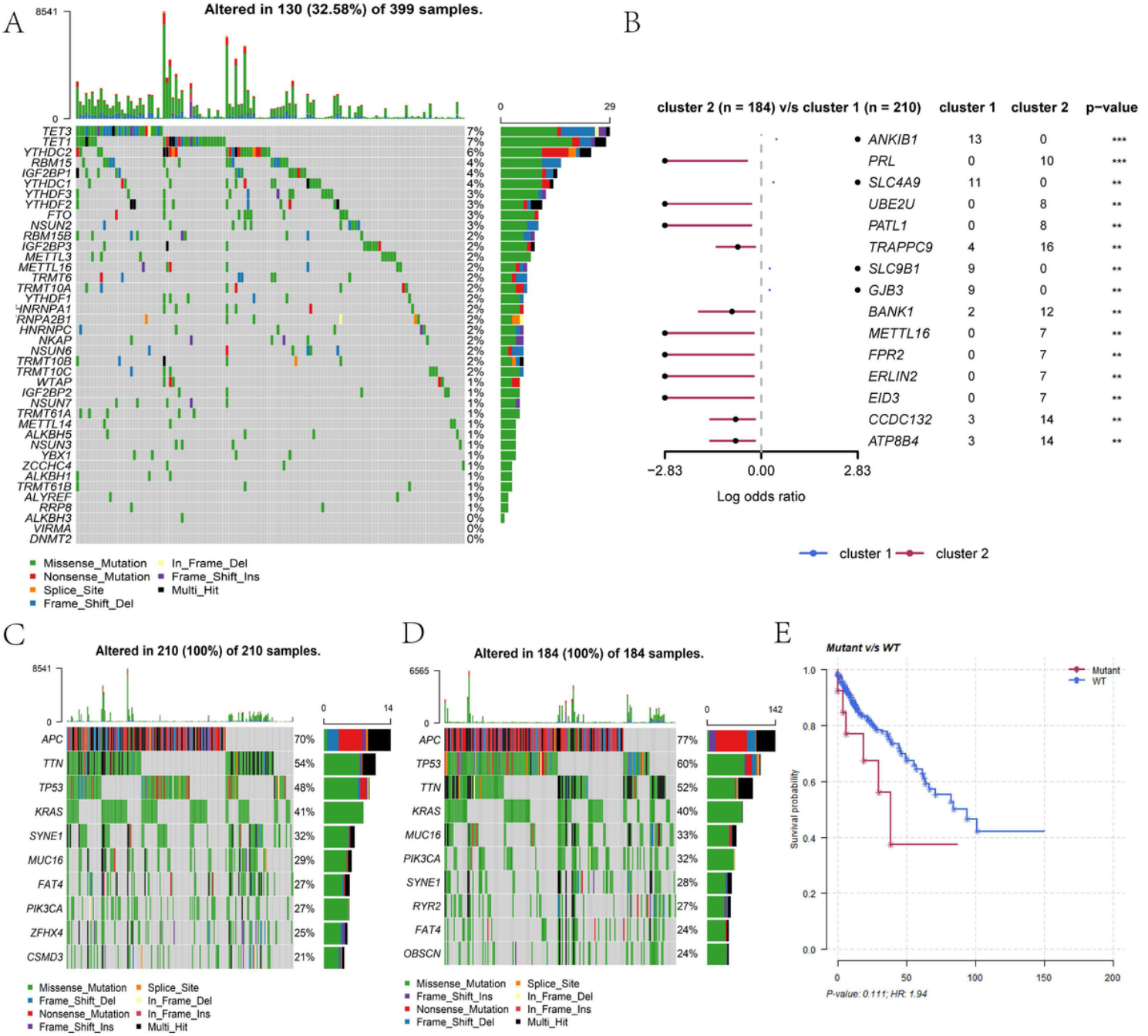

Figure 2

Mutation associated with the regulators in the two patient clusters. a. Landscape of the regulator mutations in colon cancer. b. Different frequencies of gene mutation in the two patient clusters. c and d. The TOP 10 mutation genes and their mutation types in the two patient clusters. e. The Kaplan-Meier survival curve of wild and mutant type of YTHDF3.

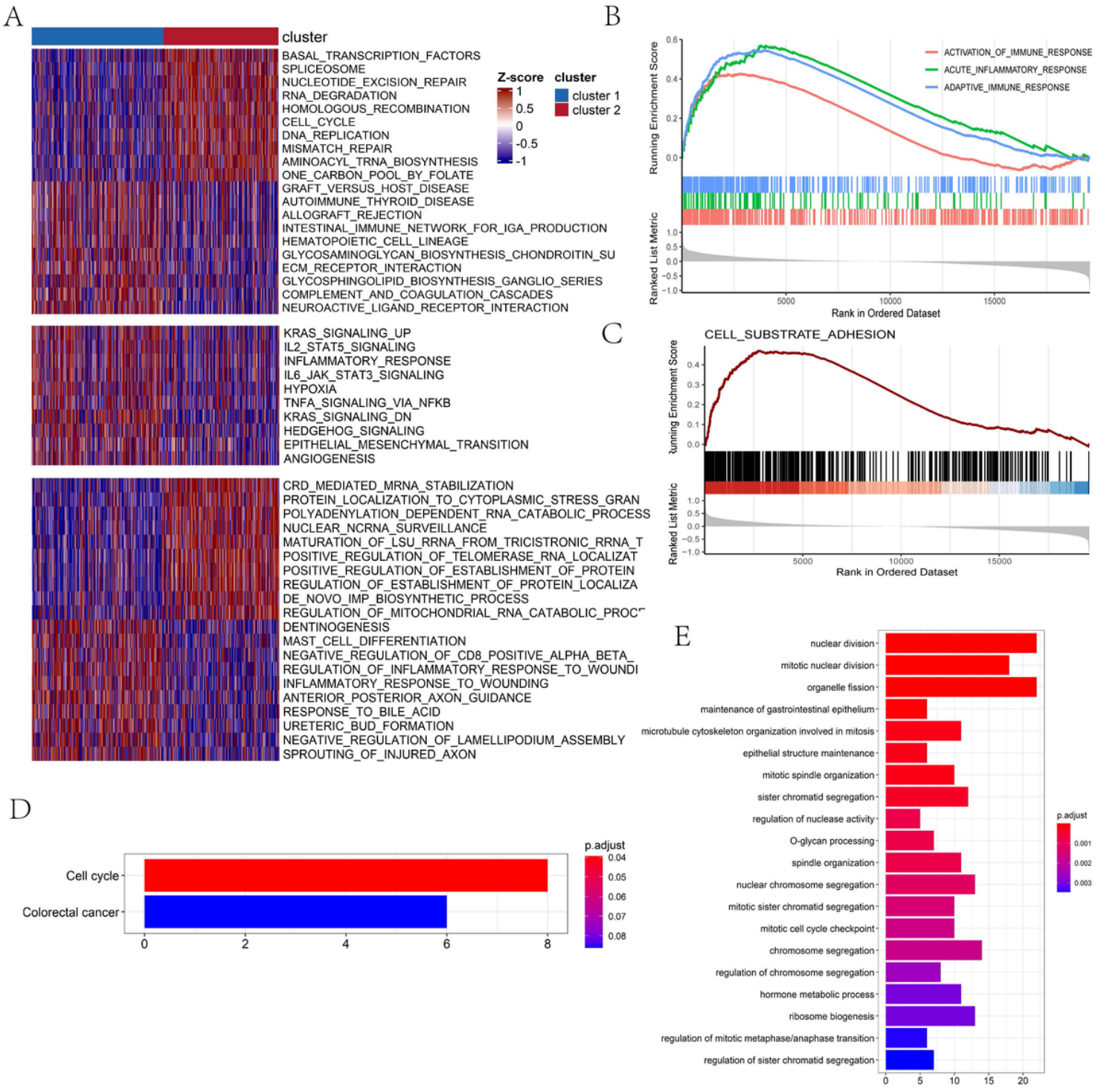

Figure 3

The difference of biological pathway in the two clusters. a. GSVA analysis: the preferable pathways for different patient clusters. Blue, the cluster with bad prognosis; Red, the cluster with better prognosis. b. The immune response is more active in the cluster with bad prognosis. c. The cell substrate adhesion pathway is enriched in the cluster with bad prognosis. d. KEGG analysis: cell cycle is different in the two clusters. e. GO analysis: biological pathways are enriched in epithelium maintenance and cell division.

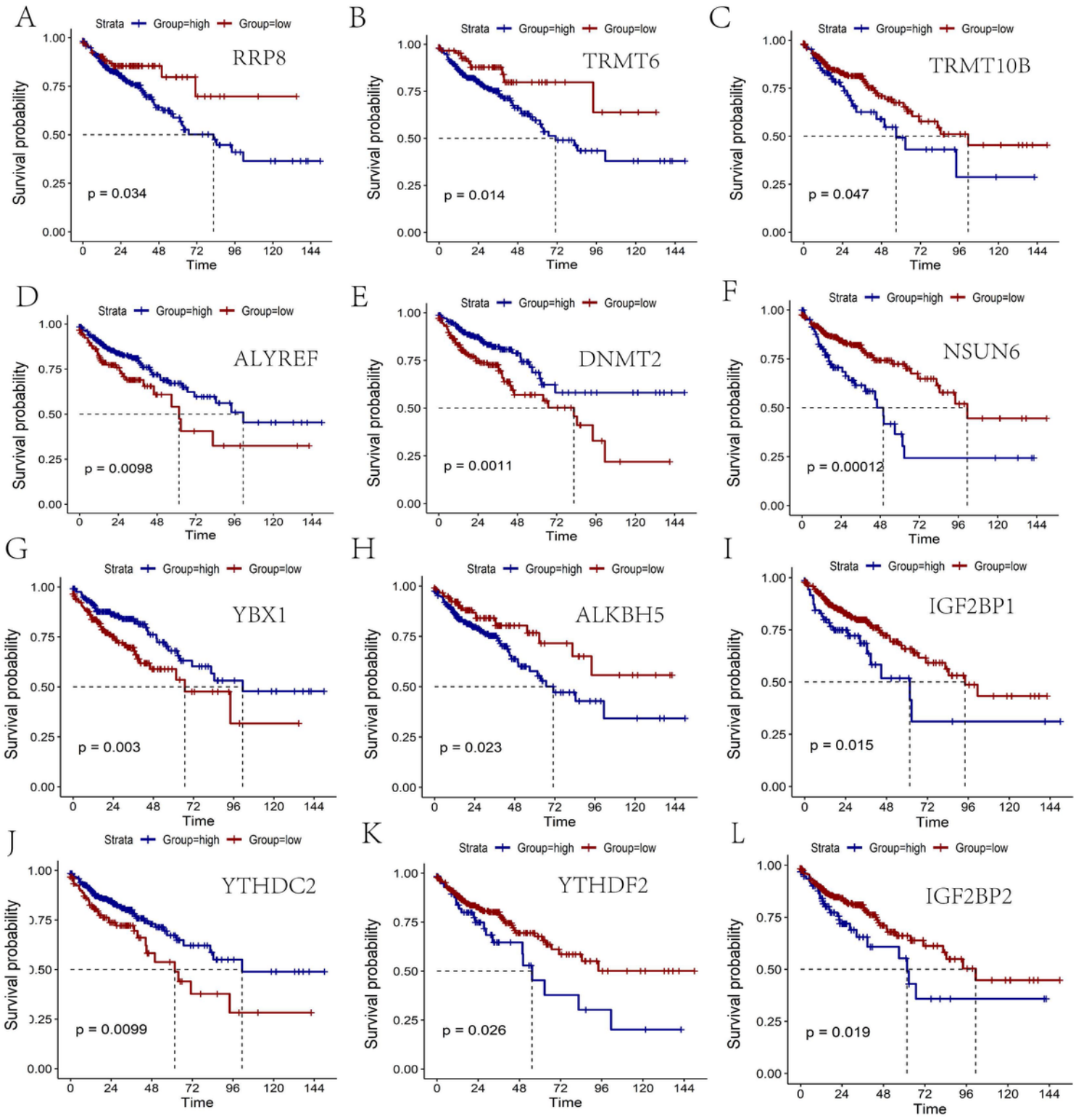

Figure 4

The Kaplan-Meier survival curves of RNA modification regulators. a –l. The Kaplan-Meier survival curves of RRP8, TRMT6, TRMT10B, ALTYREF, DNMT2, NSUN6, YBX1, ALKBH5, IGF2BP1, YTHDC2, YTHDF2 and IGF2BP2. The cut off values are decided by the survminer R package.

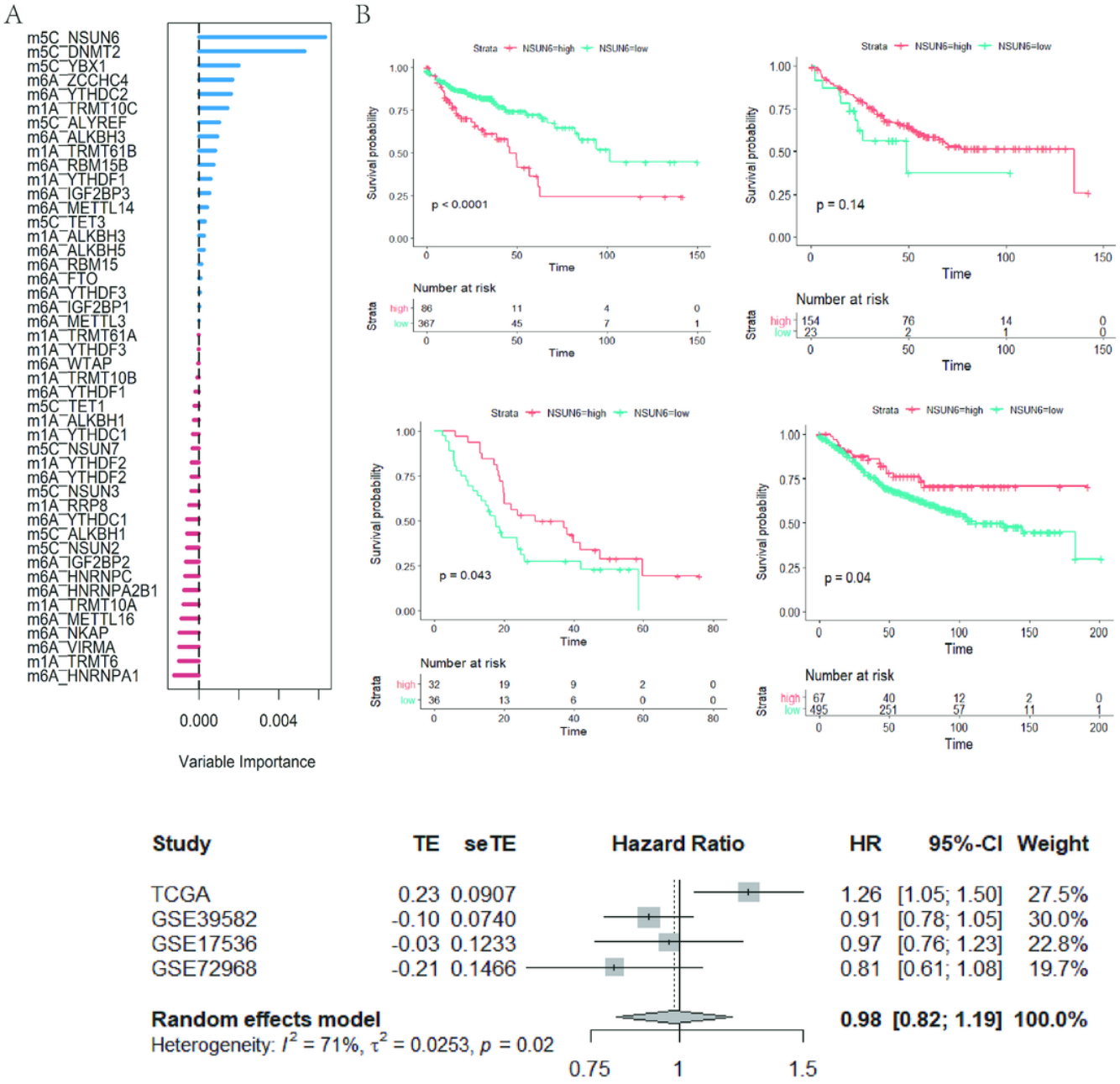

Figure 5

The controversial association between the mRNA level of NSUN6 and prognosis. a. The vimp scores of the regulators based on the random forest model. b-e. The Kaplan-Meier curves of NSUN6 based on TCGA, GSE17536, GSE72698 and GSE39582 dataset, respectively. The subgroups are determined by cut-off values which are calculated based on each dataset via survminer R package. f. The meta-analysis result of NSUN6 by integrating the results of all above databases.

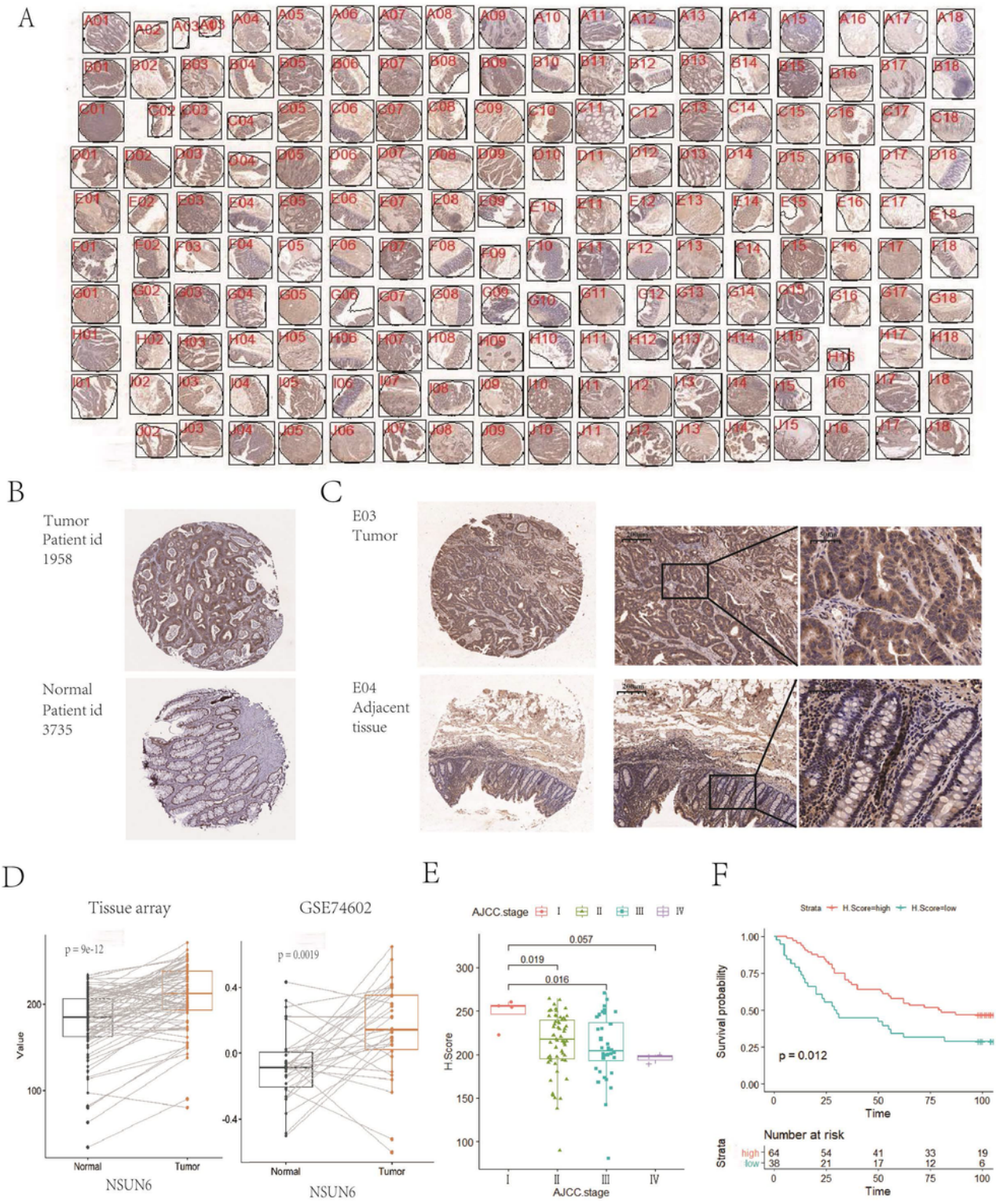

Figure 6

The tissue array assay demonstrated that NSUN6 is a protective protein in colon cancer. a. The full scan of the immunohistochemistry, which contained 104 tumors and 80 adjacent normal tissues. b. The immunohistochemistry of colon cancer from the HPA database. c. The typical immunostaining image of NSUN6 in tumor and paired adjacent tissue, the right panel is showed the details. d. NSUN6 is high expressed both in mRNA and protein level. The paired T test of normal and tumor tissue both in tissue

array (left) and GSE74602 dataset (right). e. The protein level of NSUN6 in different AJCC 7th stage. f. The Kaplan-Meier curve of NSUN6 based on tissue array.

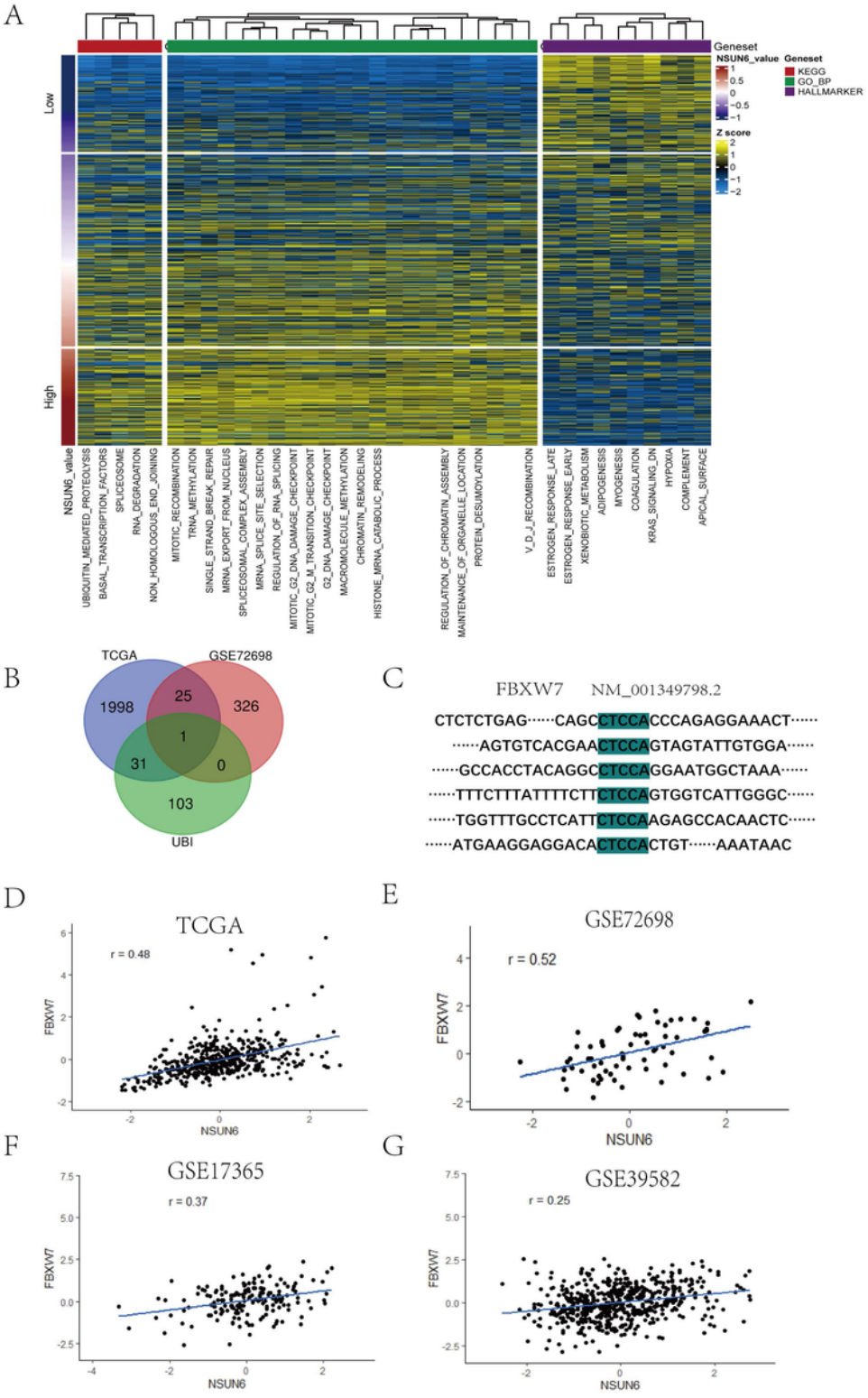

Figure 7

NSUN6 as a tumor suppressor probably by enhancing the mRNA level of FBXW7. a. The heatmap showed the relationship between NSUN6 and activation of biological pathways. b. The intersection of the co-expression gene of NSUN6 in two cohorts and the gene of ubiquitin proteolysis pathway. c. The gene

fasta of FBXW7 and the motif of NSUN6 is labeled as color green. d-g. The Pearson correction analysis of NSUN6 and FBXW7 in four datasets. All results indicated NSUN6 and FBXW7 had the closely co-expression relationship.

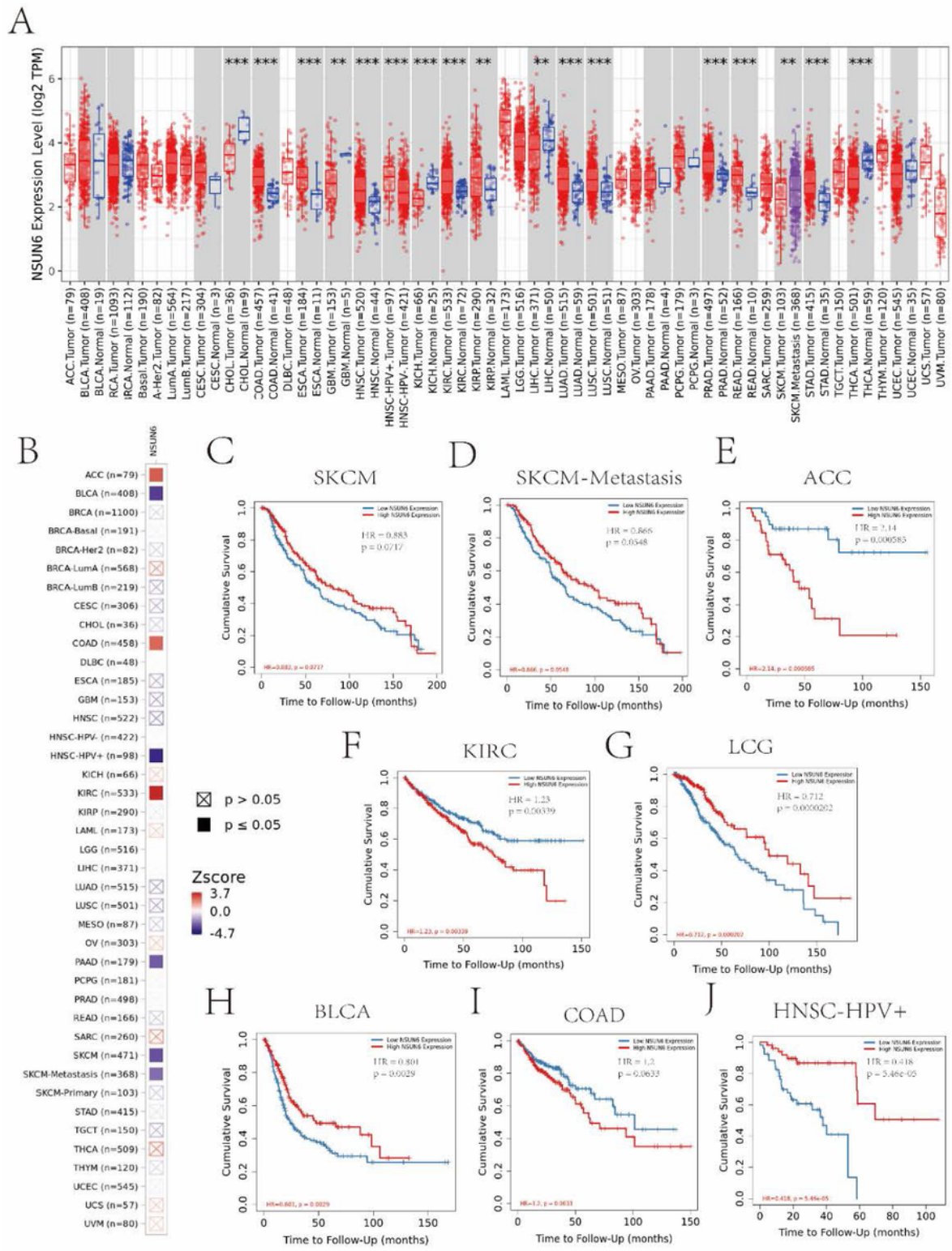

Figure 8

The mRNA level of NSUN6 and the survival analysis in pan-cancer. a. Expression of NSUN6 in different tumor types. Red, tumor tissues; blue, normal tissues; *, significance. b. The Cox regression analysis of

NUSN6 in pan-cancer. The HRs are scaled as Z-scores. c-j. The Kaplan-Meier curves of NSUN6 in pan-cancer. The cancer types are selected by statistics significance.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure.pdf
- SupplementaryTable.docx