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Identification of Necroptosis-related Subtypes, Construction of a Prognosis Model, and Characterization of Immune Infiltration in Colon Cancer.

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Abstract

Background: Necroptosis is a type of programmed necrosis that is participating in the pathology of many diseases. However, the potential mechanisms of action and biological functions of necroptosis-related genes (NRGs) in tumor cells are poorly understood.

Methods: We explored alterations in NRGs in 1147 colon cancer samples and assessed their expression from three separate public datasets. Based on the expression of 67 NRGs we divided the patients into two subtypes. Differentially expressed genes (DEGs) associated with prognosis were then screened and the samples were divided into two new gene subtypes based on their expression. The NRG_score was constructed to predict overall survival (OS) and its accuracy in predicting the prognosis of colon cancer patients was tested. Therefore, in order to improve the clinical practice function of NRG_score, a highly accurate multi-factor nomogram was produced.

Results: We observed that NRGs expression was associated with patient age, gender, grading, prognosis and immune cell infiltration characteristics. We identified 301 necroptosis subtype-related prognostic genes. In addition, we constructed the NRG_score that can accurately predict the prognosis of colon cancer patients.

Conclusion: Our established NRG_score was significantly correlated with immune cell infiltration, somatic cell mutation, cancer stem cell (CSC) index and sensitivity to drugs associated with colon cancer. These researches will contribute to our further understanding of NRGs in colon cancer. More importantly, it provides direction for evaluating the prognosis of colon cancer patients and for exploring more useful immunotherapy options.

Introduction

Colon cancer is recognized as a highly malignant tumor and one of the most serious diseases in the world[1]. In China, colon cancer is the top five malignant tumors with the highest mortality rate[23]. Surgery is recognized as the most effective method of treating early-stage colon cancer[4]. However, the high mortality rate of this disease is related to poor diagnosis in its early stage. When colorectal cancer is diagnosed at an early stage, the survival rate of most patients is significantly improved after it is detected[5]. Therefore, it is essential to explore valid and accurate biological markers for risk assessment and potential therapeutic targets in colon cancer.

Necroptosis is a novel form of cellular necrosis that regulates RIPK1 and RIPK3 in tumor cells to enhance CD8+directed antitumor cell immune processes[6]. Necroptosis has a mechanism to direct the death of other cells and can be used to counteract the apoptotic resistance of tumor cells, and is therefore increasingly being considered as an excellent strategy for the treatment of malignant tumors. It was reported that in mice, a necrotic tumor cell cellmimicry nanovaccine induced NKG2D+ and CD8+ leukocyte expansion to enhance anti-tumor properties[7]. In addition, necroptosis can generate an

immunosuppressive tumor microenvironment to promote the biological behavior of cancer, which also suggests us that necroptosis is a potential therapeutic target in colon cancer[8].

Our study divided 1,147 colon cancer patients into two subtypes based on the expression levels of 67 NRGs. We then screened for differentially expressed genes (DEGs) in the two groups of necroptosis subtypes, and based on the expression of DEGs, patients were divided into two new gene subtypes. Ultimately, we created a prognostic scoring model to predict OS and infer the effectiveness of immunotherapy in colon cancer patients.

Materials And Methods

Datasets sources

The map of our research work is shown in Figure S1. Gene expression (fragments per kilobase million, FPKM) and clinical data related to prognosis of colon cancer were obtained from the Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) and The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer. gov/) databases. Two GEO colon cancer cohorts (GSE17536 and GSE39582) and TCGA cohorts were downloaded for subsequent biological analyses. Combine the three sets of colon cancer data sets, and use the “Combat” algorithm to eliminate the batch effect of the samples. We deleted the sample data with no OS information. Therefore, the data of 1147 colon cancer patients (Table S1) were used for subsequent research. The clinical information related to prognosis included age, gender, tumor stage, overall survival follow-up time, and survival status.

Consensus clustering analysis of NRGs

According to previous research with necroptosis, we finally collected the profile of 67 NRGs (Table S2). The “ConsensusClusterPlus” package in R was used to consensus unsupervised clustering analysis. Then we classify samples into different subtypes based on the expression of NRGs expression. In order to observe the difference of NRGs in different subtypes, we employed the hallmark gene set gene set (c2. cp.kegg.v7.2) of the MSigDB (http://www.broad.mit.edu/ gsea/msigdb/) database for gene set variation analysis (GSVA). We then analyzed the potential relationship between two different subtypes and 22 immune cell types.

Relationship between subtypes with the clinicopathological characteristics and prognosis of colon cancer

To verify the clinical value of the two subtypes, we studied the potential relationship between the two subtypes, clinical features, and prognosis. The clinical characteristics of the sample include age, gender and tumor stage. Moreover, we used the “survival” and “survminer” packages in R to generate a survival curve to compare the differences between the two subtypes.

Screening and functional enrichment of necroptosis-related EDGs

Application of the R software limma package to screen for DEGs between necroptosis subtypes (fold-change of 1.5; $p<0.05$). To further investigate the potential functions and biological enrichment pathways of DEGs associated with necroptosis subtypes. Functional and pathway enrichment analysis of DEGs using the “clusterprofiler” package of R software.

Construction and assessment of prognostic models and nomogram

Firstly, a one-way Cox regression approach was applied to DEGs to identify genes associated with OS in colon cancer. Secondly, patients were divided into different gene subtype groups based on the expression of genes screened for association with OS in colon cancer. Finally, colon cancer patients were randomly and equally divided into training and test groups, and the NRG_score associated with necroptosis was constructed using the training group data. In addition, LASSO analysis and multivariate Cox statistical analysis were used to select the best prognostic features by the “glmnet” package of the R software.

The NRG_score model equation is calculated as follows:

$$NRG\_score = \Sigma(Expi * coefi)$$

The formulae Coefi and Expi indicate the risk factors and expression of genes associated with OS in colon cancer, respectively. Patients in the training set were divided into high and low risk groups based on the median NRG_score, with risk scores below the median NRG_score being the low-risk group and above being the high-risk group. Follow-up survival analysis was then performed. Then, the “ggplot2” package of R software was applied to perform principal component analysis (PCA) on the samples in the two risk groups. In a similar way, samples from the test group were classified and biologically analyzed, and the receiver operating characteristic (ROC) curves were produced to verify the accuracy of the NRG_score.

To improve the clinical usefulness of the NRG_score, we constructed a nomogram by using risk scores and clinical data by the “rms” package of R software. In the nomogram system, each clinical indicator has a corresponding score and the sum of all clinical indicators scores for each patient is the total clinical score for the patient[9].

Immune cell infiltration, CSC Index, mutation and drug susceptibility analysis

In order to identify potential differences between high and low risk groups in colon cancer, we used the CIBERSORT method to investigate the relationship between 22 immune cells and genes that make up the

NRG_score, and applied boxplots to observe differences in the expression of different risk groups in immune cell species. Then, we explored the potential association between high and low risk groups and the CSC index. In addition, based on the mutation data downloaded from the TCGA database, we explored two groups of cellular mutations using the “maftool” package of R software. Finally, the “pRRophetic” package was applied to study the semi-inhibitory concentration (IC50) values of drugs normally used in colon cancer and to observe the sensitivity of the drugs between the two groups.

Statistical analyses

Statistical analyses were all performed using R version 4.1.2 and $p < 0.05$ was considered statistically significant.

Results

Genetic and copy number alterations of NRGs in colon cancer

Our research contains 67 NRGs. Biological analysis of the frequency of somatic mutations in these NRGs showed that the incidence of mutations in colon cancer was significantly increased (Figure 1a). 46.12% of the 399 colon cancer patient samples had mutations. Of these, BRAF was the most mutation-prone gene (15%), followed by ATRX (8%), while four NRGs (TNFRSF1B, CDKN2A, TNFSF10 and ID1) were not mutated.

We next examined copy number alterations of somatic in these NRGs and identified copy number variations (CNV) that were prevalent in all 67 NRGs. Of these, CNV was generally increased for MYC, ID1, ZBP1, CD40, SPATA2, and STAT3, while CNV was reduced for TARDBP, TNFRSF1B, TLR3 and FAS (Figure. 1b). Figure 1c shows the position of CNV alterations in the NRGs on the respective chromosomes. We further explored the genetic differences between colon cancer tissues and normal tissues and showed that the expression levels of most NRGs showed significant differences. NRGs with low copy number variation frequencies, such as FAS and TLR3, were lowly expressed in colon cancer samples compared to normal samples, whereas NRGs with high copy number variation frequencies, such as MYC and SPATA2, were highly expressed in colon cancer samples (Figure. 1d), and based on the results, it was hypothesized that CNV may affect the gene expression of NRGs. Nevertheless, there were also differences in the expression of some NRGs between colon cancer samples and normal samples that were not associated with CNV gain or deletion. Therefore, gene expression levels are not only regulated by CNV[10]. Other factors can also be involved in regulating gene expression, such as DNA methylation[11]. Our study suggests that NRGs have a potential function in the development of colon cancer.

Identification of necroptosis subtypes in colon cancer

For the purpose of analysis, the expression pattern of NRGs in the biology of colon cancer, we used 1147 samples from 3 downloaded colon cancer datasets (TCGA-COAD, GSE39582 and GSE17536) for subsequent analysis in our research. Table S3 demonstrates the prognostic value of the 67 NRGs in the univariate Cox regression and Kaplan-Meier analysis in the colon cancer sample, filtered at $p < 0.05$. Subsequently, the necrotic apoptosis network demonstrated the linkage between NRGs and their prognostic value for patients with colon cancer (Figure 2a and Table S4).

To further characterize the expression pattern of NRGs in colon cancer, according to the expression of 67 NRGs, we applied a clustering algorithm to classify the colon cancer samples. The results demonstrate that dividing all samples into two subtypes ($k = 2$), A ($n = 521$) and B ($n = 624$), produces the smallest experimental error (Figure 2b). PCA analysis showed striking differences in the expression of NRGs between the A and B subtypes (Figure 2c). The survival curve demonstrates that the survival rate of subtype A samples is lower than that of subtype B samples over a long period of time (Figure 2d). In addition, significant differences in the NRGs expression of the two subtypes and clinical features can be seen in the NRGs expression heat map (Figure 2e).

GSVA enrichment and Immune cell infiltration analysis in distinct subtypes

GSVA enrichment analysis revealed significant differences in immune pathway enrichment between subtypes A and B. Significantly different pathways included natural killer cell-mediated cytotoxicity, T and B cell receptor signaling pathway, Toll-like receptor signaling pathways, ECM receptor interaction, antigen processing and presentation and leukocyte trans endothelial migration (Figure 3a; Table S5). Subsequently, we applied the CIBERSORT algorithm to analyze the relationship between A and B subtypes in 22 immune cell types. The results showed that the expression of the two subtypes A and B was significantly different in the immune cell species (Figure 3b). Among the 19 immune cells that showed significant differences ($p < 0.001$), subtype B showed lower expression of immune infiltration compared to subtype A.

Identification of gene subtypes based on DEGs

To further investigate the biological behavior of necroptosis, we screened 599 necroptosis subtype-related DEGs and performed biological function and pathway enrichment analysis (Figure 4a-b). These necroptosis subtype-related DEGs are markedly enriched in modules of biological processes associated with leukocytes (Figure 4a). The main enrichment pathways of DEGs are also immune-related as shown by the results of KEGG analysis (Figure 4b). This suggests a significant role for necroptosis in immune regulation. We then applied a univariate Cox analysis to screen 599 necroptosis subtype-related genes for prognostic value ($p < 0.05$), and we finally identified 301 genes associated with survival for subsequent analysis (Table S6). To further explore the relationship between necroptosis and biological processes,

consensus clustering analysis was applied to divide the samples into two gene subtypes, A and B, based on the expression of 301 prognostic genes (Figure 4c). Survival curve results show that patients with gene subtype B have lower OS than those with gene subtype A ($p < 0.001$; Figure 4d). Furthermore, most of the genes were highly expressed in the necrotrophic apoptotic gene subtype B. (Figure 4e). As expected, the two necroptosis-related gene subtypes appeared to differ significantly in the expression results of NRGs (Figure 4f).

Construction and assessment of prognostic models and nomogram

Based on the necroptosis subtype-related 301 prognostic genes, we constructed NRG_score. Figure 5a shows the distribution of samples across two subtypes of necroptosis, two gene subtypes and high and low risk groups. In the first place, we applied the “caret” package from R to divide the sample randomly and equally into training (n = 568) groups and test (n = 567) groups. LASSO analysis and multivariate Cox statistical analysis were used to select the best prognostic features for 301 necroptosis prognostic genes. We then performed a LASSO regression model analysis and finally screened for 13 genes associated with OS, including PNMA1, CXCL13, PDGFRL, GREM1, SLC2A3, FABP4, HOXC6, NT5E, IL7R, CXCL11, IL1R2, NOX1 and CKMT2 (Figure S2). Based on the results of the analysis, the NRG_score model equation is as follows:

Risk score = (0.2101* expression of PNMA1) + (−0.1008* expression of CXCL13) + (−0.4855* expression of PDGFRL) + (0.1179* expression of GREM1) + (0.2148* expression of SLC2A3) + (0.1958* expression of FABP4) + (0.1632* expression of HOXC6) + (0.1925* expression of NT5E) + (−0.2543* expression of IL7R) + (−0.1110* expression of CXCL11) + (−0.2111* expression of IL1R2) + (−0.1159* expression of NOX1) + (−0.1153* expression of CKMT2).

Based on the risk scores of the samples, we visualized the distribution of each sample. In typing according to necroptosis-related DEGs, gene subtype B had a significantly higher NRG_score than gene subtype A (Figure 5b). But in typing according to NRGs subtype A had a higher NRG_score than subtype B (Figure 5c). Next, we divided the patients in the training group into two groups based on the median NRG_score, with patients with a risk score below the median NRG_score being classified as low-risk group (n = 284) and those with a risk score above the median NRG_score being classified as high-risk group (n = 284). Figure 5d-e shows that as NRG_score increases, the number of patients in the high-risk group gradually increases and the chance of recurrence of colon cancer gradually rises. Survival curve results suggest that OS is significantly higher in patients with low NRG_score than in those with high NRG_score ($p < 0.001$; Figure 5f). In addition, we produced ROC curves for NRG_score and Figure 5g shows that the accuracy of predicting patients by NRG_score at 1, 3 and 5 years was 0.764, 0.759 and 0.735 respectively.

Subsequently, we selected data from the test (n = 567) group to validate the prognostic accuracy of the NRG_score. In the same way as in the previous study, we divided the patients in the training group into a high-risk group (n = 272) and a low-risk group (n = 295) based on the median value of NRG_scores. The results showed that the number of patients in the high-risk group tended to rise as the NRG_score increased (Figure 6a-b). The results of the survival curve reveal that the prognosis of patients in the low-risk group is significantly better than that of the high-risk group ($p < .001$; Figure 6c). The predictive accuracy of the ROC curve for the validation group was 0.707, 0.679 and 0.692 for 1-, 3- and 5 years respectively, indicating that the NRG_score is an excellent predictor of survival in patients with colon cancer (Figure 6d).

To improve the clinical usefulness of the NRG_score, we constructed a nomogram by using risk scores and clinical data (Figure 6e). The factors affecting prediction include, gender, age, risk score and patient stage. Subsequently, the calibration plots show that the nomogram we constructed are functionally similar to the ideal prognostic model (Figure 6f).

Monitoring of immune cell checkpoints between the high and low risk groups

We applied the CIBERSORT algorithm to observe the relationship between risk score and immune cell abundance. Testing of 22 human immune cells showed the risk score was positively correlated with M2 macrophages, M0 macrophages and neutrophils and negatively correlated with M1 macrophages, plasma cells, activated memory CD4 + T cells, testing memory CD4 + T cells and CD8 + T cells (Figure 7a). Low-risk group significantly correlated with immune score; high-risk group correlated with stromal score (Figure 7b). Subsequently, we assessed the association of 13 genes in the NRG_score with immune cell infiltration. It is clearly observed that most of the genes are significantly associated with immune cells (Figure 7c).

CSC Index, mutation and drug susceptibility analysis of NRG_score

We explored the potential correlation between NRG_score and CSC index in colon cancer. Based on the results of the study, we conclude that the CSC index ($R = -0.24$, $p < 0.001$) decreases as the NRG_score increases, indicating that colon cancer cells with lower risk scores have lower levels of cell differentiation (Figure 8a). We then explored mutations between the two risk groups in the TCGA-COAD mutation dataset. The gene with the highest mutation rate is APC, and all of the genes shown in the graph are mutated to varying degrees (Figure 8b-c). We next explored differences in the sensitivity of chemotherapeutic agents for colon cancer in high and low risk groups. We observed that patients in the high-risk group were more sensitive to shikimic, while those in the low-risk group were more sensitive to

gemcitabine and Cyclopamine In conclusion, the findings point to a significant correlation between NRGs and drug sensitivity (Figure 8d-f).

Discussion

Previous studies have shown that necroptosis plays a crucial role in the biological behavior of many kinds of malignancies[12]. In addition, necroptosis was considered to be a promising method for the elimination of tumor cells[13]. There have been studies reporting a potential relationship between necroptosis and colonic inflammation as well as colon cancer[14]. However, the relationship between necroptosis-related genes and colon cancer prognosis, and the role of their molecular mechanisms are far from being fully elucidated. We classified colon cancer patients into two subtypes, A and B, based on the expression of 67 NRGs. In contrast, subtype B has a better OS than subtype A. The two subtypes also differed markedly in terms of immune pathway activation, with subtype A showing high expression in T and B cell receptor signaling pathway, and the Toll-like receptor signaling pathways and natural killer cell-mediated cytotoxicity. We then screened for differentially expressed genes (DEGs) in two sets of necroptosis subtypes and classified patients into two new gene subtypes based on the expression of DEGs. For this purpose, we created a NRG_score containing 13 genes to predict OS and infer the effectiveness of immunotherapy in colon cancer patients. To improve the clinical usefulness of NRG_score, we constructed a high-accuracy nomogram. Patients can be divided into high and low risk groups based on the median value of NRG_scores. Significant differences in clinical characteristics, prognosis, cytosolic mutations, immune cell infiltration, CSC index and drug sensitivity were found between the different risk groups. This demonstrates that the NRG_scores we have created are not only effective in predicting the prognosis of colon cancer patients but also offer a new pathway for the treatment of colon cancer.

The tumor microenvironment is significantly relevant to the development of cancer cells and to drug resistance[15]. Components of the tumor microenvironment associated with cancer cells include the extracellular matrix (ECM), tumor-infiltrating immune cells (TIIC), lymphocytes, and blood vessels[16]. Immune cells are the main cellular components of the tumor microenvironment, such as lymphocytes and granulocytes, which can be involved in the coordination of cancer cells to promote inflammatory processes[17]. In addition, TIIC in the tumor microenvironment has been reported to have a predictive role in cancer prognosis[18]. In our study a significant difference between high and low risk groups in the immune score can be seen. We infer that low NRG_score is associated with immune activation, while high NRG_score is associated with immune suppression. In addition, the 13 genes involved in the composition of the NRG_score showed significant correlations in most of the 22 TIICs. This suggests that NRGs have a crucial role in the biology of colon cancer. It has been demonstrated that memory T cells promote immune defense against colon cancer[19]. The number of T cells is higher in colon cancer than in paracancerous tissue and is positively correlated with patient prognosis[202122]. In our study, the NRG_score was negatively correlated with the degree of infiltration of CD4+ and CD8+ T cells, which is consistent with the results of previous studies.M1 macrophages and M2 macrophages have been shown

to correlate with malignancy. M1 macrophages have an inhibitory effect on tumor cells[23]. M2 macrophages can use their immunosuppressive function to promote matrix remodeling, which can be beneficial to tumor cell development[(23)24] . Moreover, increased density of M2 macrophages was positively correlated with metastasis in colon cancer[2526]. It has also been shown that high infiltration of M2 macrophages is associated with a poorer prognosis in patients with colon cancer[27]. After exploration we can observe that with increasing NRG_score M1 macrophage infiltration gradually decreases, while M2 macrophage infiltration gradually increases, which is consistent with the results of previous experimental studies. Up to now, the role of neutrophils in tumors has not been well elucidated. Some studies suggest that neutrophils promote the metastatic function of cancer cells, but some scholars believe that neutrophils have an anti-tumor metastatic function[282930]. In our study, the NRG_score and the degree of neutrophil infiltration were positively correlated, but this does not prove that neutrophils have a role in promoting colon cancer metastasis, and further research is needed to draw specific conclusions. Plasma cells have been proven to inhibit the development of cancer[3132]. In addition to producing antibodies, plasma cells can also influence T cells by producing cells and immune factors for anti-tumor purposes[33]. It has been shown that plasma cells or plasma cell-related genes are associated with a satisfactory prognosis for lung[34], colon[35], breast[36] or other malignancies[37]. In fact, in recent years of pan-cancer studies, the characteristic expression of plasma cell-associated genes has been recognized as a positive prognostic influence in tumor types[38]. Our analysis showed a high infiltration of plasma cells in the low NRG_score, which is consistent with previous findings. This research has some limitations. Firstly, our samples are drawn from public databases, the data are all retrospective. Hence studies may be subject to selection bias. Secondly, the clinical variables we obtained lacked treatment, recurrence or other important factors which could have had an impact on the prognosis of the patients studied. Finally, we need clinical trials to prove the accuracy of the study.

Conclusion

We have explored the potential mechanisms of NRGs in colon cancer to modulate the tumor immune microenvironment, clinical features and prognosis. This study demonstrates the potential value of NRGs in tumor development and clinical practice, and provides new directions for immunotherapy in colon cancer.

Declarations

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None.

Authors' contributions

YQ designed the research and wrote the manuscript and preformed the bioinformatics analysis. YQ and LY analyzed the data and confirmed the authenticity of all the raw data generated during the study. JC, GZ and XJ contributed to the critical reading and correction of the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Ethics committee approval was obtained from the Institutional Ethics Committee of North China University of Science and Technology Affiliated Hospital (approval no. 2021211A) to the commencement of the study laid down in the 1964 Declaration of Helsinki. Written informed consent was provided by all the participants.

Consent for publication

All personnel involved in this research have agreed to publish.

Competing interests

The authors declare that they have no competing interests.

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Figures

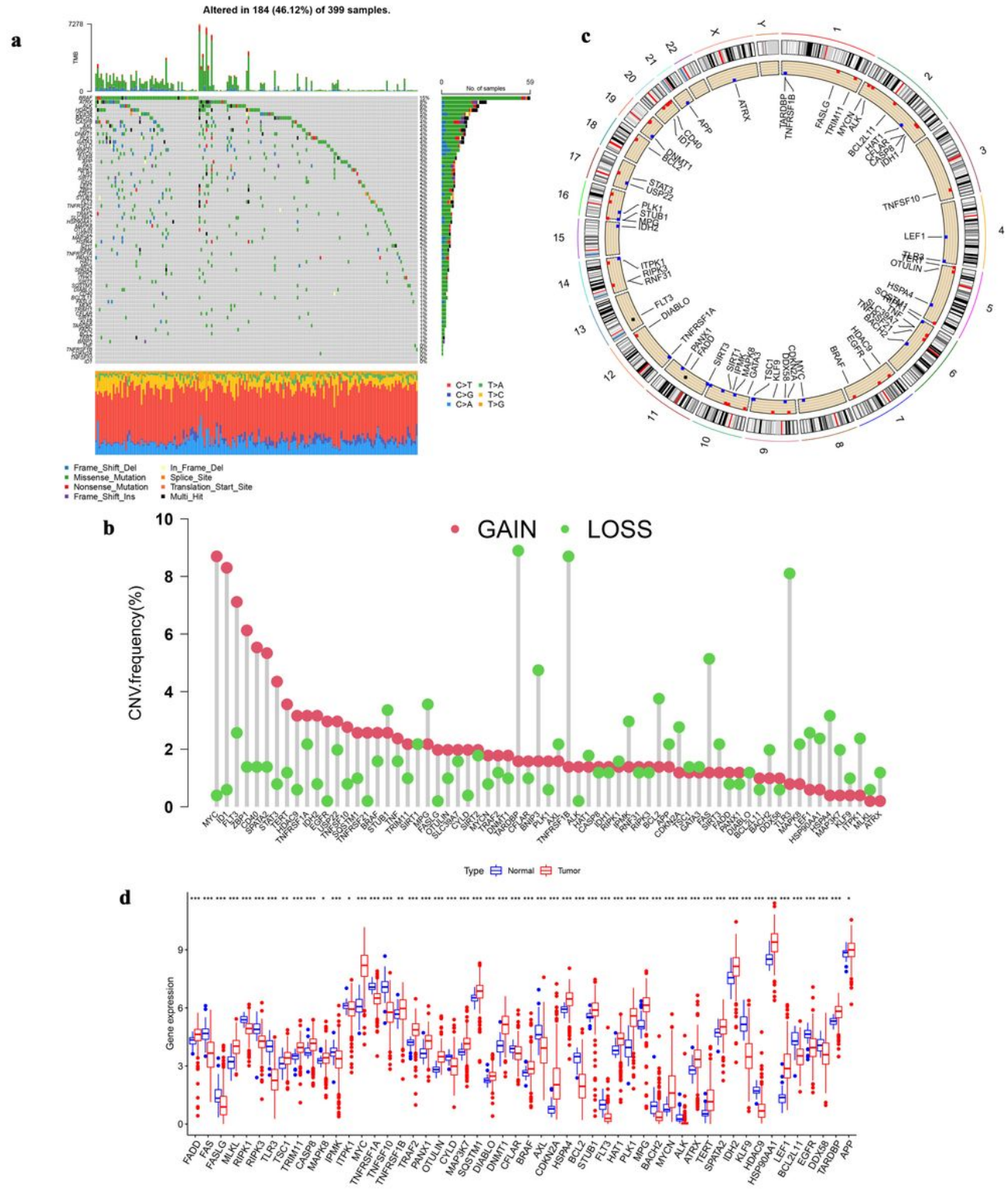

Figure 1

Genetic and transcriptional alterations of NRGs in colon cancer. (a) Mutation frequencies of 67 NRGs in 399 and 184 patients with COAD, respectively, from the TCGA cohort. (b) Frequencies of CNV gain, loss, and non-CNV among NRGs. (c) Locations of CNV alterations in NRGs on 23 chromosomes. (d) Expression distributions of 67 NRGs between normal and colon cancer tissues. NRGs, necroptosis-related genes; COAD, colon adenocarcinoma; TCGA, The Cancer Genome Atlas; CNV, copy number variant.

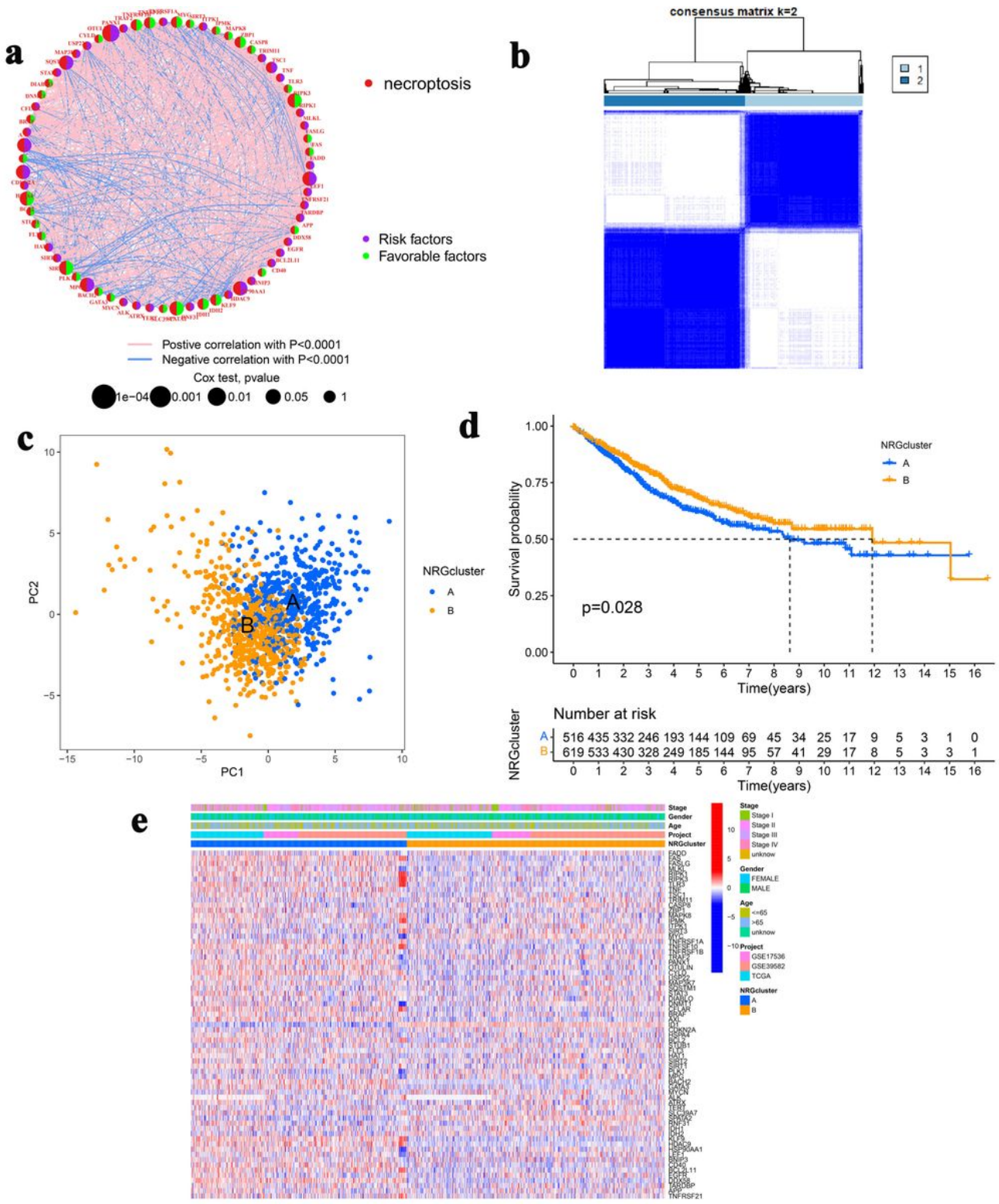

Figure 2

NRG subtypes and clinicopathological and biological characteristics of two distinct subtypes of samples divided by consistent clustering. (a) Interactions among NRGs in colon cancer. The line connecting the NRGs represents their interaction, with the line thickness indicating the strength of the association between NRGs. Green and pink represent negative and pink positive correlations, respectively. (b) Consensus matrix heatmap defining two clusters (k = 2) and their correlation area. (c) PCA analysis

showing a remarkable difference in transcriptomes between the two subtypes. (d) Univariate analysis showing 67 NRGs related to the OS time. (e) Differences in clinicopathologic features and expression levels of NRGs between the two distinct subtypes. PRG, necroptosis-related gene; PCA, principal components analysis; OS, overall survival.

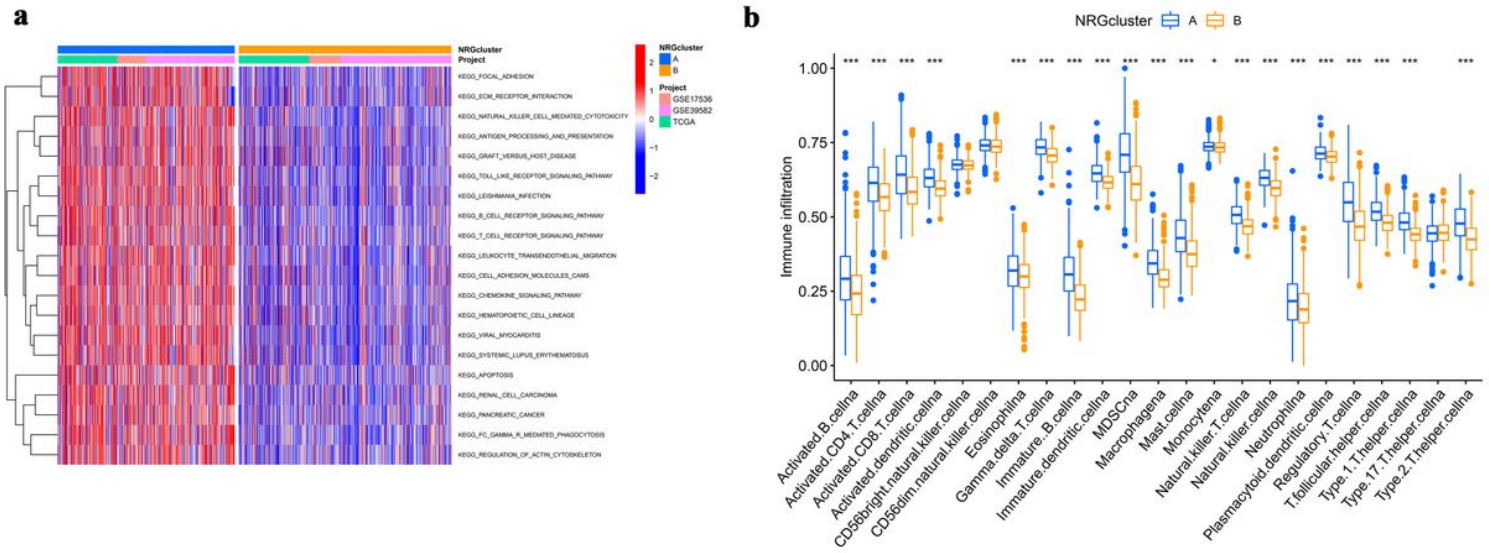

Figure 3

Correlations of tumor immune cell microenvironments and two subtypes. (a) GSVA of biological pathways between two distinct subtypes, in which red and blue represent activated and blue inhibited pathways, respectively. (b) Abundance of 22 infiltrating immune cell types in the two subtypes.

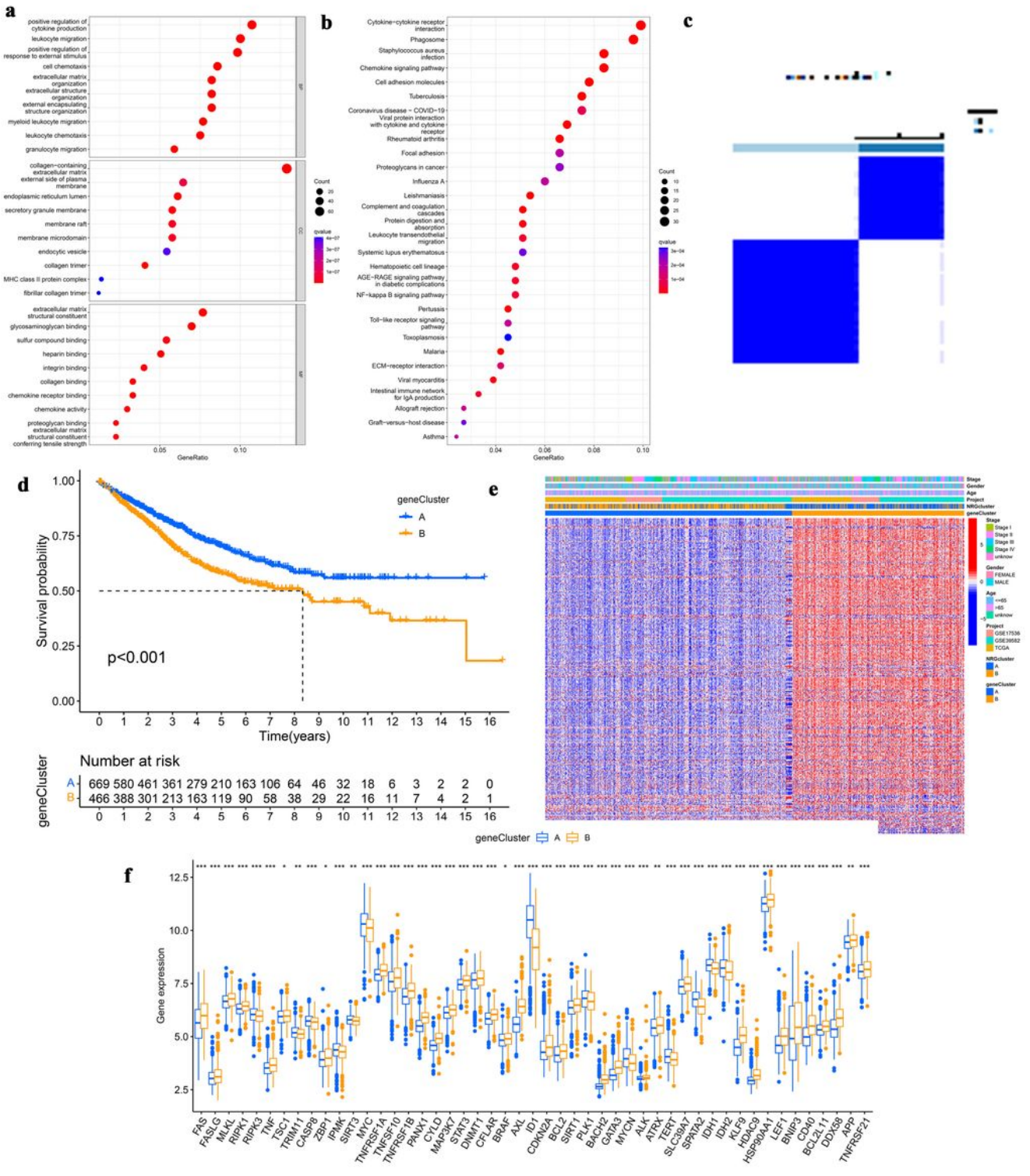

Figure 4

Identification of gene subtypes based on DEGs. (a-b) GO and KEGG enrichment analyses of DEGs among two necroptosis subtypes. (c) Identification of gene subtypes based on DEGs among two necroptosis subtypes in colon cancer cohort. (d) Kaplan–Meier curves for OS of the two gene subtypes ($p < 0.001$). (e) Relationships between clinicopathologic features and the two gene subtypes. (f) Differences in the

expression of 67 NRGs among the two gene subtypes. DEGs, differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; NRGs, necroptosis-related genes.

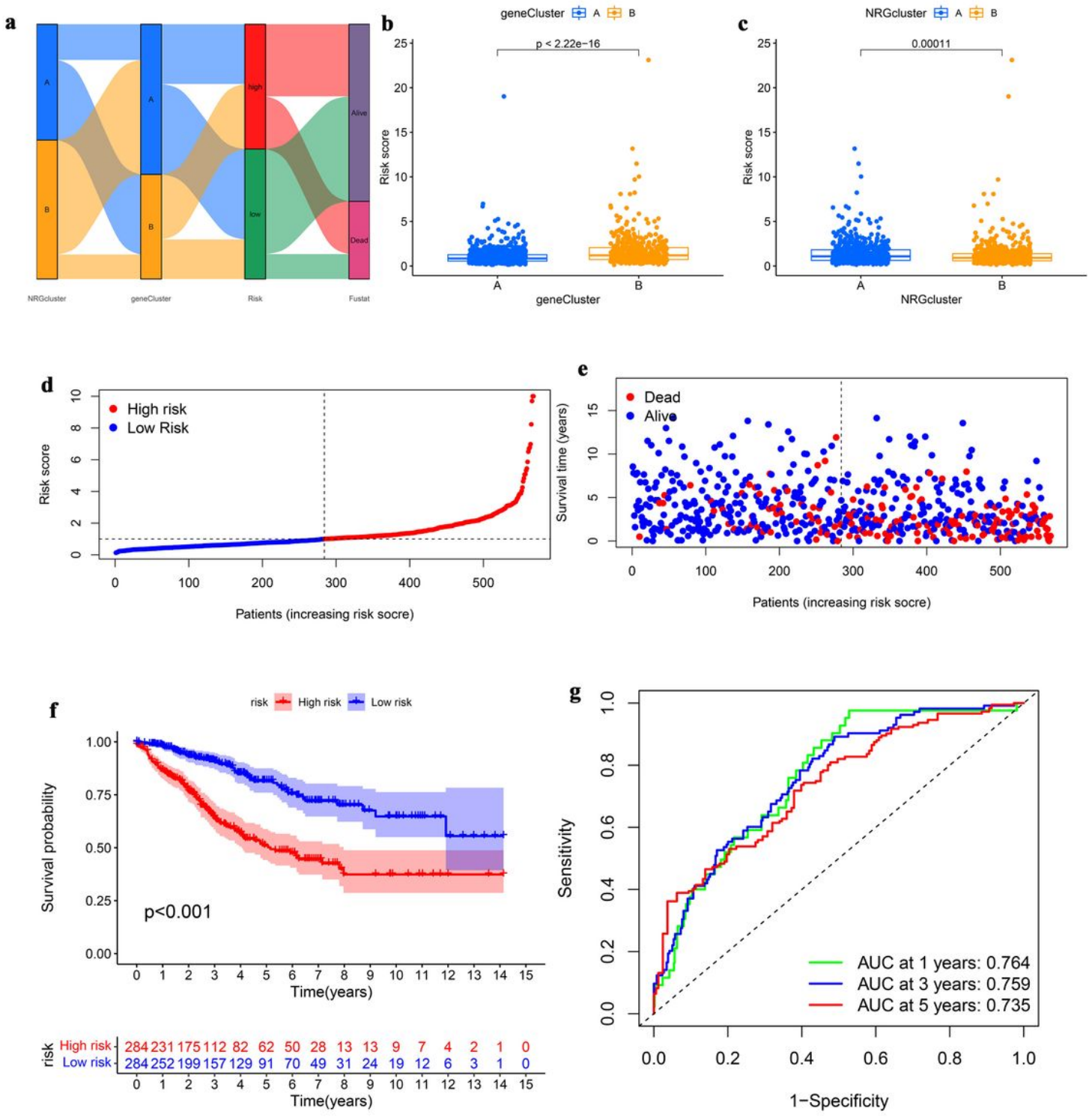

Figure 5

Construction of the NRG_score in the training set. (a) Alluvial diagram of subtype distributions in groups with different NRG_scores and survival outcomes. (b) Differences in NRG_score between gene subtypes.

(c) Differences in NRG_score between necroptosis subtypes. (d-e) Ranked dot and scatter plots showing the NRG_score distribution and patient survival status. (f) Kaplan–Meier analysis of the OS between the two groups. (g) ROC curves to predict the sensitivity and specificity of 1-, 3-, and 5-year survival according to the NRG_score.

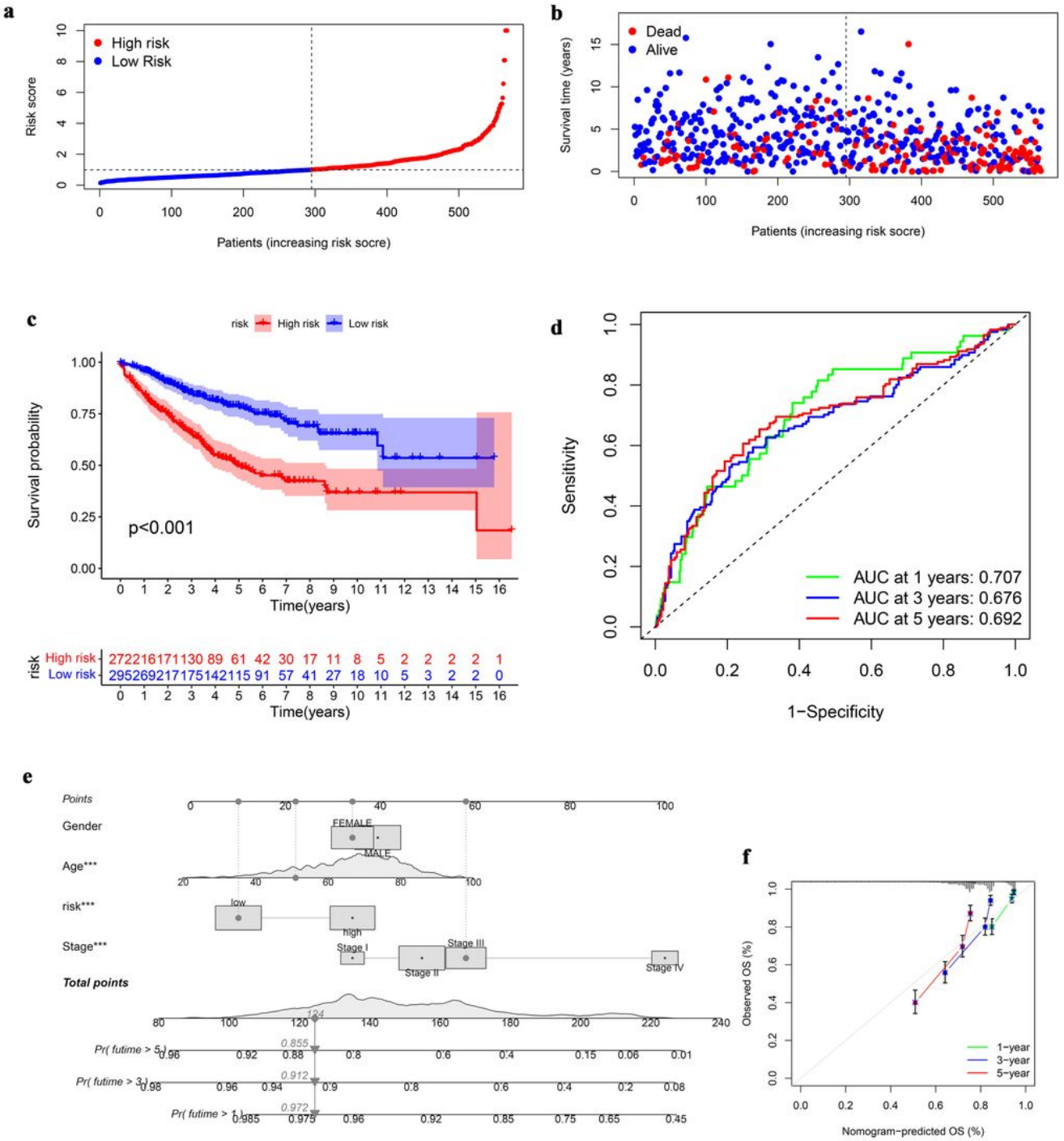

Figure 6

Validation of the NRG_score in the test set. (a-b) The ranked dot plot indicates the NRG_score distribution and scatter plot presenting the patients' survival status. (c) Kaplan–Meier analysis of the OS between the two groups. (d) ROC curves to predict the sensitivity and specificity of 1-, 3-, and 5-year survival according to the NRG_score. (e) Nomogram for predicting the 1-, 3-, and 5-year OS of colon cancer patients. (f) Calibration curves of the nomogram for predicting.

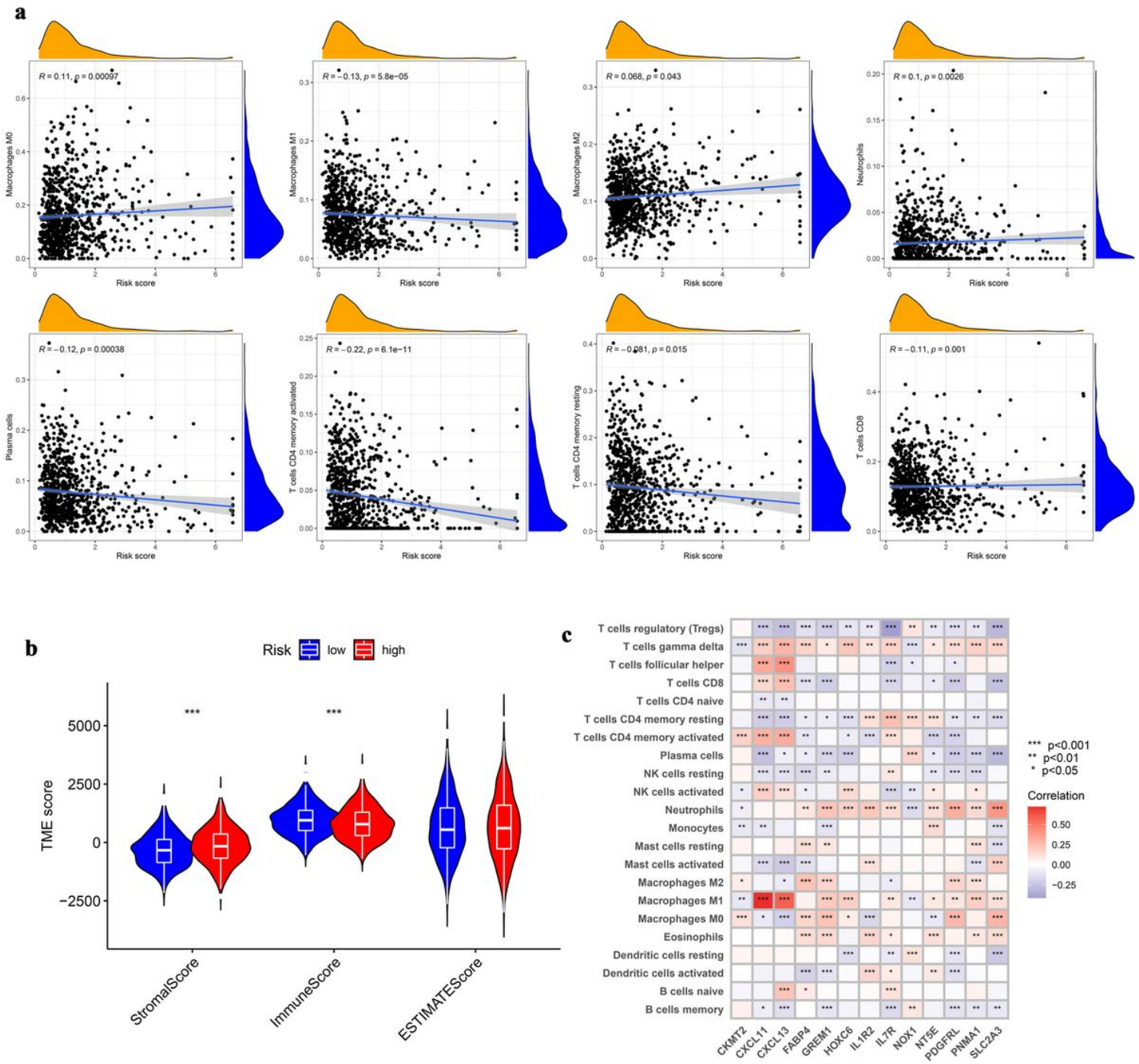

Figure 7

Evaluation of the tumor microenvironment and checkpoints between the two groups. (a) Correlations between NRG_score and immune cell types. (b) Correlations between NRG_score and both immune and stromal scores. (c) Correlations between the abundance of immune cells and thirteen genes in the proposed model.

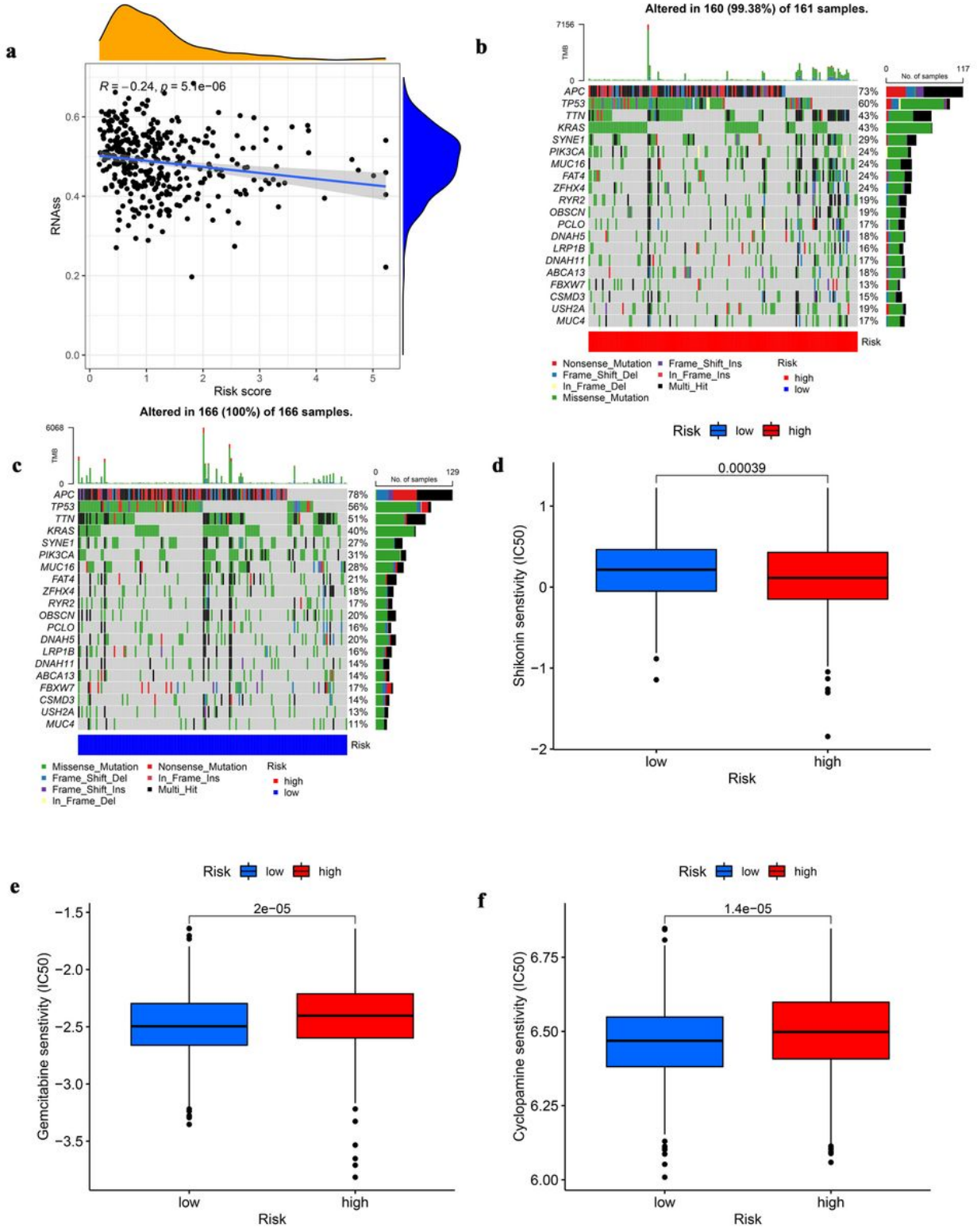

Figure 8

Comprehensive analysis of the NRG_score in colon cancer. (a) Relationships between NRG_score and CSC index. (b-c) The waterfall plot of somatic mutation features established with high and low NRG_scores. (d-f) Relationships between NRG_score and chemotherapeutic sensitivity. CSC, cancer stem cell.

Supplementary Files

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- FigureS1.jpg
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- SupplementaryTables.xlsx