

Analysis of multi-omics differences in left-side and right-side colon cancer

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Research

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Abstract

Background:Colon cancer is one of the common tumors of digestive tract. Studies of left-side colon cancer(LCC) and right-side colon cancer(RCC) show that these two subtypes had different prognosis, outcomes, and clinical response to chemotherapy. Therefore,it is necessary to explore the necessity of clinical classification of anatomic subtypes about colon cancer.

Methods:We selected the transcriptome data, clinical information and somatic mutation data of colon cancer patients from the the Cancer Genome Atlas(TCGA)database portal.The transcriptome data included 390 colon cancer patients(172 LCC samples and 218 RCC samples),and the somatic mutation data included 142 LCC samples and 187 RCC samples.By conducting a multi-omics analysis of the LCC and RCC from the four aspects of clinical characteristics, immune microenvironment , transcriptomic differences and mutation differences, so as to compare the expression and prognosis difference of LCC and RCC.We are the first to construct prognostic signatures respectively for LCC and RCC respectively.The prognostic signatures is validated by internal testing set, complete set and external testing set(GSE39582).Additionally we also verified the independent prognostic value of the signature.

Results:Clinical characteristics analysis results show that RCC had a significantly worse prognosis than LCC.Analysis the immune microenvironment analysis shows that RCC was more immune infiltration than LCC.The results of differential gene analysis showed that there were 360 differential expressed genes,with 142 up genes in LCC and 218 up genes in RCC.Correlation analysis of mutated genes showed that the expression of mutated genes in RCC was negatively correlated, while the expression of mutated genes in LCC was positively correlated, and the mutation frequency of RCC was generally higher than that of LCC.Meanwhile, our 4-mRNA LCC and 6-mRNA RCC prognostic signatures are highly predictive and can be used as independent prognostic factors.

Conclusion:The clinical classification of anatomic subtypes of colon cancer is of great significance for its early diagnosis and prognostic risk assessment.Our study provides directions for individualized treatment of left and right colon cancer.

Background

Colon cancer is one of the most common cancers in the world and it is also the second leading cause of cancer-related deaths in the United States[1]. For a long time, it was widely believed that the accurate location of the tumor deserves no attention for the reason that the accurate location itself would not affect patients' survival.

However, in the past decade, the differences between LCC and RCC have received a great quantity of attention[2]. Embryonic origin could be a good perspective to explain this disease[3]. As we all known,the orgin of RCC comes from the midgut, which includes the cecum, ascending colon, and hepatic flexure. In contrast, the orgin of LCC comes from the hindgut, which includes the splenic flexure, descending colon and sigmoid colon.

Because of the different prognosis, outcomes, and clinical response to chemotherapy between LCC and RCC, it has received increasing attention. In some investigations, it has been reported that LCC is associated with a better prognosis compared with RCC[4]. A recent systematic review noted that many studies have identified their differences in epidemiology, clinical presentation, pathology, and genetic mutations through anatomical subsites[5].

Most of the studies indicated that the patients with RCC showed lower survival rate compared with LCC[6]. But to this day, the data are still controversial. Weiss Jennifer M and co-workers demonstrated that when analysis was adjusted for multiple patient, disease, comorbidity, and treatment variables, no overall difference in 5-year mortality was seen between LCC and RCC[7].

Therefore, further analysis about LCC and RCC is supposed to be done. This study conducted a multi-omics analysis of the LCC and RCC from the four aspects of clinical characteristics, immune microenvironment, transcriptomic differences and mutation differences, so as to discuss the necessity of clinical classification of the anatomic subtypes of colon cancer.

Method And Data

Data downloading

First, we downloaded transcriptome data, clinical information and somatic mutation data of colon cancer patients from the Cancer Genome Atlas (TCGA) database portal (<https://portal.gdc.cancer.gov>). The transcriptome data included 390 colon cancer patients (172 LCC samples and 218 RCC samples), and the somatic mutation data included 329 colon cancer patients (142 LCC samples and 187 RCC samples). According to the research of Jiang Yimei et al, the LCC consists of the descending colon, sigmoid colon, and splenic flexure of colon and the RCC consists of the ascending colon, cecum, and hepatic flexure of colon[8]. Then, we used `genecode.v22.annotation` (<https://www.gencodegenes.org/>) to comment transcriptional data downloaded from TCGA database.

Clinical Analysis in LCC and RCC

In order to analyze the difference between LCC and RCC in terms of age, gender, pT, pN, pM, pStage and survival, we used R to classify the data and then we used Pearson's Chi-square (χ^2) test to calculate the difference of clinical characteristics between LCC and RCC. What's more, we compared the survival rates of LCC and RCC in different clinical subtypes using 'survival' package in R.

Immune microenvironment in LCC and RCC

In order to explore the differences between LCC and RCC in the immune microenvironment, we got immune-related gene set with 29 immune cell types and immune-related functions from previous studies[8]-[9][10][11][12][13]. We used single sample gene set enrichment analysis (ssGSEA) algorithm to obtain the scores of 29 immune cell types and immune-related functions with 'GSVA' package in R[14]. And we visualized the result using 'pheatmap' package in R[15]. For further analyzing the

difference in immune microenvironment between LCC and RCC, we used 'estimate' package in R to calculate the immune score, stromal score, ESTIMATE score and tumor purity. Then we compared the differences between the two groups through Mann-Whitney U test. What's more, the expression level of human leukocyte antigen (HLA) gene family, immune check-point genes and the abundance of Immune cell infiltration were compared in LCC and RCC. We obtained the abundance of Immune cell infiltration used CIBERSORT[16].

Screening of Differential gene in LCC and RCC

Through $|\log_2 \text{fold change}(\text{LogFC})| > 1$ and false discovery (FDR) < 0.05 , we used Wilcoxon-test to identify the difference expression of mRNAs in LCC and RCC. The Visualization through the heatmap and volcano diagram. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment process of differential genes was realized through the Database for Annotation, Visualization and Integrated Discovery (DAVID) database (<https://david.ncifcrf.gov/>)[17]. The top 20 biological processes (BP) of GO enrichment analysis[18]. were demonstrated by circle diagram, while the top 15 KEGG pathways[19] were demonstrated by bubble diagram.

Screening of Prognostic mRNAs in LCC and RCC by univariate COX analysis

All the different genes were included in the further study. We used 'survival' package in R with $P < 0.005$ to identify the prognostic mRNAs in LCC and RCC respectively by univariate COX[20]. There were too many prognostic genes associated with RCC. For subsequent analysis, We used LASSO regression algorithm with penalty term to delete genes with multicollinearity.

Construction and verification of the prognosis signature and validation of prognostic models

Prognostic genes related of LCC and RCC were included in the further study, We randomly divided TCGA LCC patients into training set and internal testing set according to 1:1 ratio, and established a 4-mRNA LCC prognosis model through multivariate COX regression analysis with noose penalty[21]-[22]. We used the same method to establish a 6-mRNA RCC prognosis signature[23]-[24][25]. Through constructing risk score, the samples was divided into two groups with the median of risk score. We judged the effect of the model by plotting the Kaplan-Meier (KM) curve and receiver operating characteristic (ROC) curve[26]. GSE39582 data set which was download from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE39582>)[27], is used as an external validation set to validate the model by plotting the Kaplan-Meier (KM) curve. The GSE39582 included 562 colon cancer samples (342 LCC samples and 220 RCC samples) and the survival information in accordance with the GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array) (Table S1). For further verify the model effect, We performed an independent prognostic analysis of the risk score in total TCGA set. The risk score was calculated as[28]:

$$Riskscore = \sum_{i=1}^N (Exp_i \times Coef)$$

with N representing the number of signature genes, Exp_i representing the gene expression levels, and Coef representing the estimated regression coefficient value from the Cox proportional hazards analysis.

Single gene mutation Analysis in LCC and RCC

On the JAVA8 platform, we used Perl scripts to calculate the mutation frequency with number of variants/the length of exons for each sample[29]. According to the location of colon cancer, the samples were divided into two groups, and Mann-Whitney test[30] was used to compare the TMB difference between two groups. For visualization, we used 'maftools' package[31] and performed Fisher's exact test in pairs between top 25 mutated genes to analyze the mutational exclusion and co-occurrence. We also used 'oncoplot' in R to visualize the top 30 mutated genes of 142 LCC samples and 187 RCC samples to produce waterfall plots. Then we chose 'ggplot2' and 'boxplot' package to visualize the classification and frequency of mutation types, frequency of variant types, frequency of SNV classes, tumor mutation burden in specific samples and the top 10 mutated genes in LCC and RCC.

Results

The difference of clinical characteristics in LCC and RCC

The information of LCC and RCC in TCGA database and the chi-square test results of clinical characteristics can be seen in Table 1. After separating the data of LCC and RCC, we classified the data from the aspects of stage, T, N, M and age. The Kaplan-Meier (KM) curve was used to compare the survival differences of different clinical characteristics between the two groups. Finally the result prompted that RCC has a worse prognosis than LCC, which was also manifested stage III-IV, T3-4, N1-2 (Figure 1a-d). Although there was no statistical difference between M1 and Age > 65 subgroups, the survival rate of RCC was still worse than that of LCC (Figure 1e-f).

Immune microenvironment landscape between LCC and RCC

Through ssGSEA algorithm, 29 types of immune cells and their functions were enriched in each sample, and then we obtained the immune score, stromal score, ESTIMATE score and tumor purity. By observing the heatmap, we found that the RCC had a higher immune invasion than the LCC (Figure 2a). Comparing the scores of the two groups, we found that only the immune scores had significant differences (Figure 2b). By further comparing the expression levels of HLA gene family and immune checkpoint-related genes and the abundance of immune cell infiltration, we reconfirmed the idea that RCC has higher immune infiltration than LCC (Figure 2c-d). Therefore, this result suggests that the right side of colon cancer is more immune than the left which might provide a new direction for the treatment of colon cancer.

Differential genes analysis between LCC and RCC

Wilcoxon-test was used to extract differential mRNAs. After that, we obtained 360 differential genes were obtained, which included 218 up genes in RCC and 142 up genes in LCC (Figure 3a-b). All of the differential genes were included in Table S2. Through DAVID database, All the differential expressed genes were enriched by biological processes of GO and pathways of KEGG (Table S3, Table S4). The top 20 biological processes of GO enrichment analysis were demonstrated by circle diagram, while the top 15 KEGG pathways were demonstrated by bubble diagram (Figure 3c-d). And 'associative learning', 'arachidonic acid secretion' and 'anterior/posterior pattern specification' were the top 3 biological process. The differential expressed genes were significantly enriched in the 'Steroid hormone biosynthesis' pathway.

Univariate COX screening of prognostic genes in LCC and RCC

Under the condition of $P < 0.005$, we respectively screened the genes related to the prognosis of LCC and RCC by univariate Cox in the LCC and RCC patients. We obtained 9 genes related with prognosis in LCC and 22 genes related with prognosis in RCC (Table 2-3). In order to avoid model overfitting, we performed LASSO regression with penalty term on RCC to solve the multicollinearity problem again by dimension reduction, and finally obtained 12 genes related with prognosis in RCC (Figure S2).

Construction of prognosis signature in LCC and RCC

TCGA LCC patients were divided into training set and internal testing set according to 1:1 ratio. Then through multivariate COX regression analysis with noose penalty, we established a 4-mRNA LCC prognosis signature and 6-mRNA RCC prognosis signature.

In 4-mRNA LCC prognosis signature, risk score was calculated as: $C1orf105 * 0.458 + FAM132B * 1.703 + TNNT1 * 0.130 + RSPO4 * 0.268$ (Table 4). With the median of risk score (0.622) in training set, patients were assigned to the high risk or low risk group. Patients with high risk score was had significantly worse survival rate than those with low-risk scores ($P = 0.046$, Figure 4a). Furthermore, the AUC of the risk score for 1-year, 2-year, 3-year and 5-year OS were 75.1%, 81.0%, 86.0% and 90.4% respectively (Figure 4b). The survival status, risk scores and gene expression data of LCC patients in the training group are illustrated in Figure 4c-e. RSPO4, FAM132B and TNNT1 were highly expressed in the high risk group, while C1orf105 was low-expressed in the high risk group.

And in 6-mRNA RCC prognosis signature, risk score was calculated as: $OFCC1 * 4.834 + KLRG2 * 0.195 + PAX5 * 0.461 + SYNGR3 * 0.096 + SLC22A31 * 1.232 + CCDC160 * 0.368$ (Table 5). With the median of risk score (0.689) in training set, patients were assigned to the high risk or low risk group. Patients with high risk score was had significantly worse survival rate than those with low-risk scores (0.012, Figure 5a). Furthermore, the AUC of the risk score for 1-year, 2-year, 3-year and 5-year OS were 77.6%, 71.4%, 67.0% and 79.2% respectively (Figure 5b). The survival status, risk scores and gene expression data of RCC patients in the training group are illustrated in Figure 5c-e. All of the 6 genes were highly expressed in the high risk group.

Validation of the prognosis signature in LCC and RCC

To validate the prognosis signature, its prognostic accuracy was further assessed in three independent cohorts, including the testing set, the total TCGA data set and the GSE39582 data set.

In 4-mRNA LCC prognosis signature, the OS in high risk group was significantly worse than low risk group in the testing set ($P = 0.016$, Figure 6a), and the predicted 1-year, 2-year, 3-year and 5-year OS was 73.1%, 76.0%, 77.9% and 70.0%, respectively (Figure 6b). And the total TCGA set also validated the prognostic accuracy of the signature ($P = 0.014$, Figure 6c), with respective AUCs of 73.2%, 77.6%, 82.0% and 79.3% for 1-year, 2-year, 3-year and 5-year OS (Figure 6d).

In 6-mRNA RCC prognosis signature, the OS in high risk group was significantly worse than low risk group in the testing set ($P = 0.042$, Figure 7a), and the predicted 1-year, 2-year, 3-year and 5-year OS was 77.0%, 75.4%, 68.9% and 64.6%, respectively (Figure 7b). And the total TCGA set also validated the prognostic accuracy of the signature ($P = 0.002$, Figure 7c), with respective AUCs of 76.0%, 71.8%, 66.3% and 71.8% for 1-year, 2-year, 3-year and 5-year OS (Figure 7d).

What's more, the GSE39582 data set shown the same conclusion in 3-mRNA LCC prognosis signature ($P = 0.185$) and 6-mRNA RCC prognosis signature ($P = 0.25$) ($P = 0.018$, Figure 6e, $P = 0.025$, Figure 7e). The survival status, risk scores and gene expression data of LCC and RCC patients in the testing set and total TCGA set are illustrated in Figure S2, Figure S3.

The prognosis signature confers additional prognostic power for LCC and RCC patients

Clinical characteristics including pStage ($P < 0.001$), pN ($P < 0.001$), pM ($P = 0.004$) and the riskscore ($P < 0.001$) were closely associated with patient survival in LCC (Figure 8a), and pStage ($P < 0.001$), pT ($P < 0.001$), pN ($P < 0.001$), pM ($P < 0.001$), Age ($P = 0.013$) and riskscore were closely associated with patient survival in RCC (Figure 8b). Multivariate Cox regression analysis further showed that the our signature is an independent prognostic indicator for OS both in LCC and RCC (Figure 8c-d, Table S5-6).

Single gene mutation landscape in LCC and RCC

Among all of these mutations which included missense mutation, delectation, nonsense mutation, splice site, insertion, translation start site and nonstop mutation, missense mutation is the most obvious. In addition, we also found that single nucleotide poly-morphism (SNP) are more frequent than insertions or deletion and the most common single nucleotide variants (SNV) was C > T. Also, the number of altered bases in each sample was counted which showed mutation types in box plot. Finally, the top 10 mutated genes in LCC and RCC were exhibited with ranked percentages (Figure S4, Figure S5). We used Mann-Whitney test to compare the TMB of LCC and RCC, and the results showed that the RCC had higher TMB (Figure 9a). Mutation information of each sample in LCC and RCC was exhibited in waterfall plot (Figure 9b-c), which showed that the mutation frequency of RCC is generally higher than that of LCC. The correlation analysis of top 25 mutated genes in LCC and RCC respectively through maftools package

showed that the expression of mutated genes in RCC was mostly negatively correlated, while that in LCC was mostly positively correlated and the results were more obvious than that in RCC(Figure 9d-e).

Discussion

Colon cancer is one of the most common malignant tumors of the digestive system. With the continuous improvement of living standards and dietary habits of Chinese residents in recent years. The incidence of habitual or dietary changes is on the rise year by year[32]. According to the primary swelling of colon cancer of tumour place different, can divide colonic cancer into left-side colon cancer and right-side colon cancer. The primary site of the left-side colon included the splenic flexure, descending colon and sigmoid colon. And the right-side colon included the cecum, ascending colon, and hepatic flexure. The literature [33] shows that the prognosis of left colon cancer is better than that of right colon cancer, and the survival rate is higher. Therefore, further analysis of the classification of clinical subtypes of colon cancer is necessary.

We first isolated LCC and RCC data and used Kaplan-Meier(KM) curve to compare the survival differences of patients with different clinical characteristics between the two groups. Analysis of clinical features in this study showed that RCC had a worse prognosis than LCC, and was also shown in Stage III+Stage IV, T3+T4, N1+N2, M1, Age>65. This conclusion was also confirmed in the study of Ulanja MB, et al[34]. But different from his study, we also conducted multi-omics analysis on the immune microenvironment, differential gene analysis and single gene mutation of LCC and RCC respectively.

By using the ssGSEA algorithm, we obtained scores for 29 immune cell types and immune-related functions. We found a higher immune infiltrate in the RCC than the LCC. This is consistent with the results of Zhang L et al. on the immune microenvironment landscape in different tumor Location[35]. To further investigate the differences in the degree of immune infiltration between the two groups, we calculated the immune score, stroma score, and tumor purity. Only the immune score was significantly different between the two groups, and the RCC presented with a higher immune score. This is consistent with previous reports[36]. The same is true for HLA family genes and immune checkpoint related gene expression and immune cell infiltration abundance. These results suggest that the RCC is more immune infiltration than the LCC, providing a new direction for immune-related treatment of colon cancer.

From the above results and the clinicopathological analysis of the right hemicolon by Chen Wei et al[37], it is reasonable to believe that LCC and RCC may be tumors of different properties and have different carcinogenic mechanisms. Therefore, LCC and RCC were screened by differential genes and established relevant prognostic signature respectively. With $|\text{LogFC}| > 1$ and $\text{FDR} < 0.05$, we obtained 360 differential genes (218 up genes in RCC and 142 up genes in LCC). By univariate COX regression and multivariate COX regression with noose penalty, we have constructed 4-mRNA LCC prognostic signature and 6-mRNA RCC prognostic signature. The model has been verified by internal testing set, complete set and external testing set. Also the model has been validated as an independent prognostic indicator. It is understood that we are the first to establish prognostic signatures for both left-side and right-side colon cancers, which have

been validated internally and externally. This provides the basis for personalized treatment of left and right colon cancer.

According to previous studies, RCC has a more pronounced mutation landscape than LCC[38]. In order to further explore the differences between LCC and RCC at the mutation level, we used Mann-Whitney test to compare the TMB of LCC and RCC respectively. The results show that RCC has high TMB. Correlation analysis of top 25 mutated genes in LCC and RCC showed that the expression of mutated genes in RCC was mostly negatively correlated, while the expression of mutated genes in LCC was mostly positively correlated, and the results were more significant than that of RCC. These results suggest that the classification of clinical subtypes of colon cancer may be of great significance for the determination of clinical diagnosis and treatment direction in the future.

Conclusion

In the results of the multi-omics analysis of LCC and RCC, we can observe their significant differences in clinical characteristics, immune microenvironment, transcriptomic differences and single gene mutation differences, suggested that the difference in gene expression can be analyzed and divided into different clinical subtypes to help the early clinical diagnosis and prognosis of colon cancer, which is convenient to provide individualized treatment and prognostic evaluation basis for patients with left and right colon cancer[39]. And 4-mRNA LCC prognostic signature and 6-mRNA RCC prognostic signature can provide basis for personalized treatment of colon cancer. Although our research has some reference value, our study still needs further clinical validation.

Abbreviations

LCC:left-side colon cancer;RCC:right-side colon cancer;TCGA:The Cancer Genome Atlas;ssGSEA:single sample gene set enrichment analysis;HLA:human leukocyte antigen;DAVID:the Database for Annotation, Visualization and Integrated Discovery;GO:Gene Ontology;KEGG:Kyoto Encyclopedia of Genes and Genomes;K-M:Kaplan-Meier;ROC:receiver operating characteristic;GEO:the Gene Expression Omnibus;OS:Overall survival;SNP:single nucleotide poly-morphism;SNV:single nucleotide variants.

Declarations

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Authors' contributions

QGJ, JZDM, YYH, YSL and MYY designed the study. YYH and JZDM collected the mRNA transcriptome data, single gene mutation data and clinical information from TCGA. YYH, JZDM and YSL performed

analyses on TCGA data. QGJ and TYL performed statistical analyses. YSL and MYY wrote the manuscript. QGJ, JZDM, TYL and YYH reviewed and revised the manuscript. All authors read and approved the finally manuscript.

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Data Availability Statement

All analyzed data are accessible online, and the results of this article are included within the article as well as in additional files.

TCGA:<https://portal.gdc.cancer.gov>

GSE39582:<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE39582>

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Conflict of Interest Statement

The authors confirm that there are no conflicts of interest.

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Tables

Table 1 Clinical features for the COAD patients in the LCC and RCC in TCGA

Parameters	LCC patients (n = 172)	RCC patients (n = 218)	χ^2	P value
Age,y			7.814	0.005
≤65	85	76		
>65	87	142		
Gender			0.035	0.852
Male	89	116		
Female	83	102		
pT			0.263	0.608
T1-2	37	41		
T3-4	135	176		
unknow	0	1		
pN			3.022	0.082
N0	93	138		
N1-2	79	80		
pM			2.099	0.147
M0	126	162		
M1	31	25		
unknow	15	31		
pStage			2.934	0.087
Stage I-II	89	130		
Stage III-IV	81	81		
unknow	2	7		
Survival			5.122	0.024
Alive	144	163		
Dead	26	55		
unknow	2	0		

Abbreviations:LCC:Left-side colon cancer;RCC:Right-side colon cancer;TCGA:The Cancer Genome Atlas; χ^2 :Chi-square value

Table 2 The prognostic mRNAs by univariable Cox analysis in LCC

mRNA	HR	95%CI		P value
		Low	High	
C1orf105	1.412	1.154	1.729	0.001
OSR1	10.074	2.908	34.898	<0.001
FAM132B	3.310	1.597	6.858	0.001
WNT7A	1.750	1.212	2.525	0.003
FDCSP	1.083	1.026	1.144	0.004
SMTNL2	1.317	1.109	1.564	0.002
FCER2	1.446	1.144	1.828	0.002
TNNT1	1.114	1.050	1.182	<0.001
RSPO4	1.230	1.074	1.410	0.003

Abbreviations: LCC:Light-side colon cancer;mRNA:messenger RNA; CI:confidence interval; HR:hazard ratio.

Table 3 The prognostic mRNAs by univariable Cox analysis in RCC

mRNA	HR	95%CI		P value
		Low	High	
LMX1A	3.964	1.712	9.176	0.001
COLGALT2	1.165	1.070	1.270	0.000
SNCB	2.748	1.537	4.912	0.001
OFCC1	48.830	6.787	351.331	0.000
FABP7	4.518	1.896	10.770	0.001
PAX4	1.130	1.045	1.223	0.002
KLRG2	1.253	1.124	1.398	<0.001
PAX5	1.347	1.126	1.612	0.001
PCDH8	166.438	7.157	3870.700	0.001
HS6ST3	4.836	1.677	13.953	0.004
SYNGR3	1.116	1.045	1.191	0.001
CHST6	1.266	1.111	1.443	<0.001
SLC22A31	1.635	1.324	2.020	<0.001
NEUROD2	1.877	1.299	2.712	0.001
TCAP	1.791	1.250	2.566	0.001
GREB1L	2.482	1.369	4.502	0.003
FCER2	1.118	1.042	1.198	0.002
SLC7A10	2.237	1.370	3.653	0.001
APLP1	1.087	1.029	1.147	0.003
RSPO4	1.479	1.216	1.799	<0.001
INSM1	1.038	1.012	1.064	0.004
CCDC160	1.436	1.200	1.718	<0.001

Abbreviations: RCC:Right-side colon cancer;mRNA:messenger RNA; CI:confidence interval; HR:hazard ratio.

Table 4 Multivariate Cox regression modeling in LCC

id	coef	HR	95%CI		P value
			Low	High	
C1orf105	0.458	1.581	1.134	2.205	0.007
FAM132B	1.703	5.492	1.390	21.693	0.015
TNNT1	0.130	1.139	1.026	1.265	0.015
RSP04	0.268	1.307	1.087	1.572	0.004

Abbreviations: LCC:Light-side colon cancer;CI:confidence interval; HR:hazard ratio.

Table 5 Multivariate Cox regression modeling in RCC

id	coef	HR	95%CI		P value
			Low	High	
OFCC1	4.834	125.723	8.492	1861.210	<0.001
KLRG2	0.195	1.215	1.011	1.461	0.038
PAX5	0.461	1.586	1.201	2.095	0.001
SYNGR3	0.096	1.101	1.007	1.204	0.035
SLC22A31	1.232	3.428	1.599	7.350	0.002
CCDC160	0.368	1.444	1.213	1.720	<0.001

Abbreviations: RCC:Right-side colon cancer;CI:confidence interval; HR:hazard ratio.

Figures

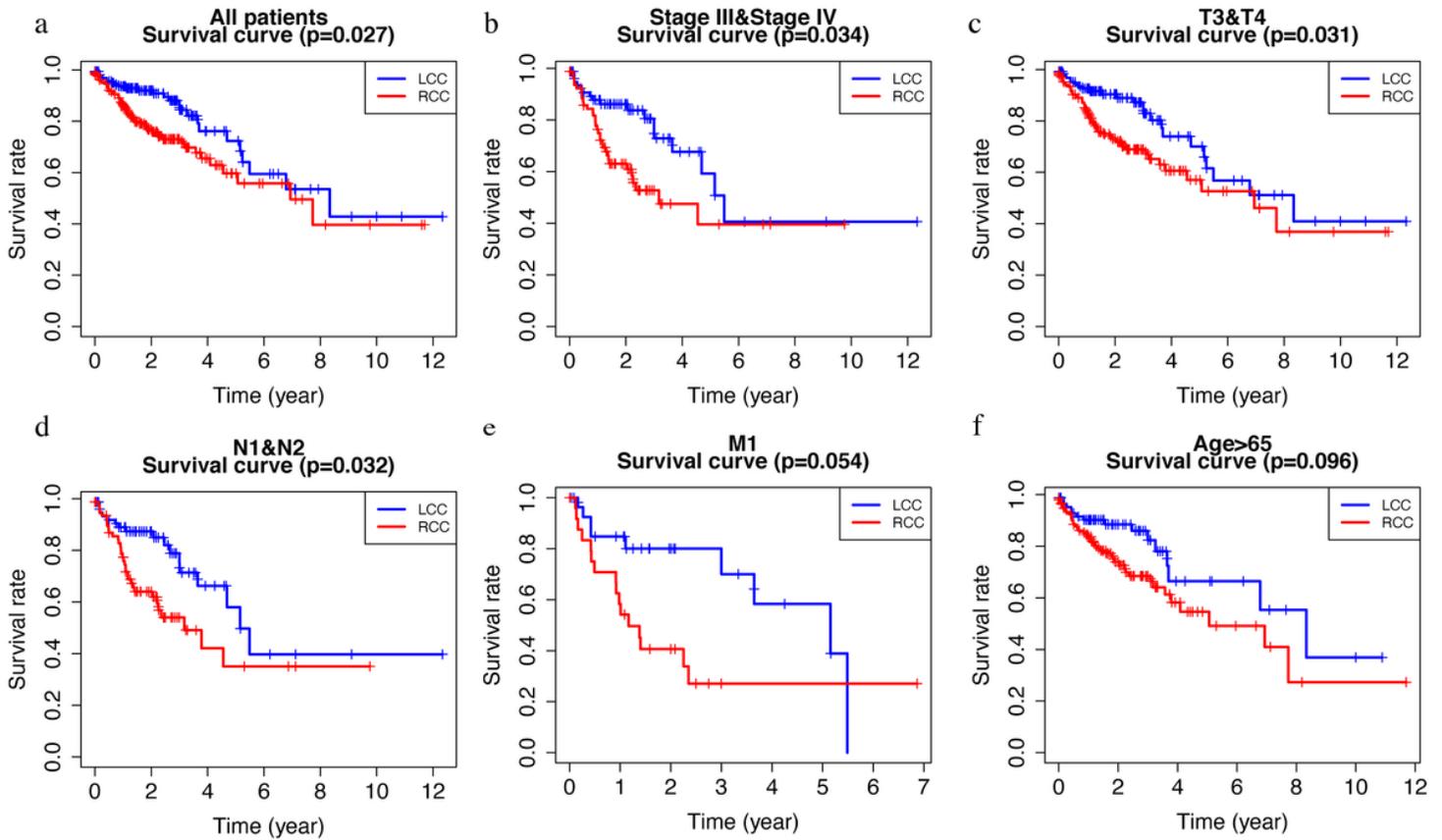


Figure 1

Comparison of survival rates of LCC and RCC in different clinical subtypes. Survival analysis of different clinical characteristics including (a)all patients,(b)stage III&IV,(c)T3&T4,(d)N1&N2,(e)M1,(f)age>65

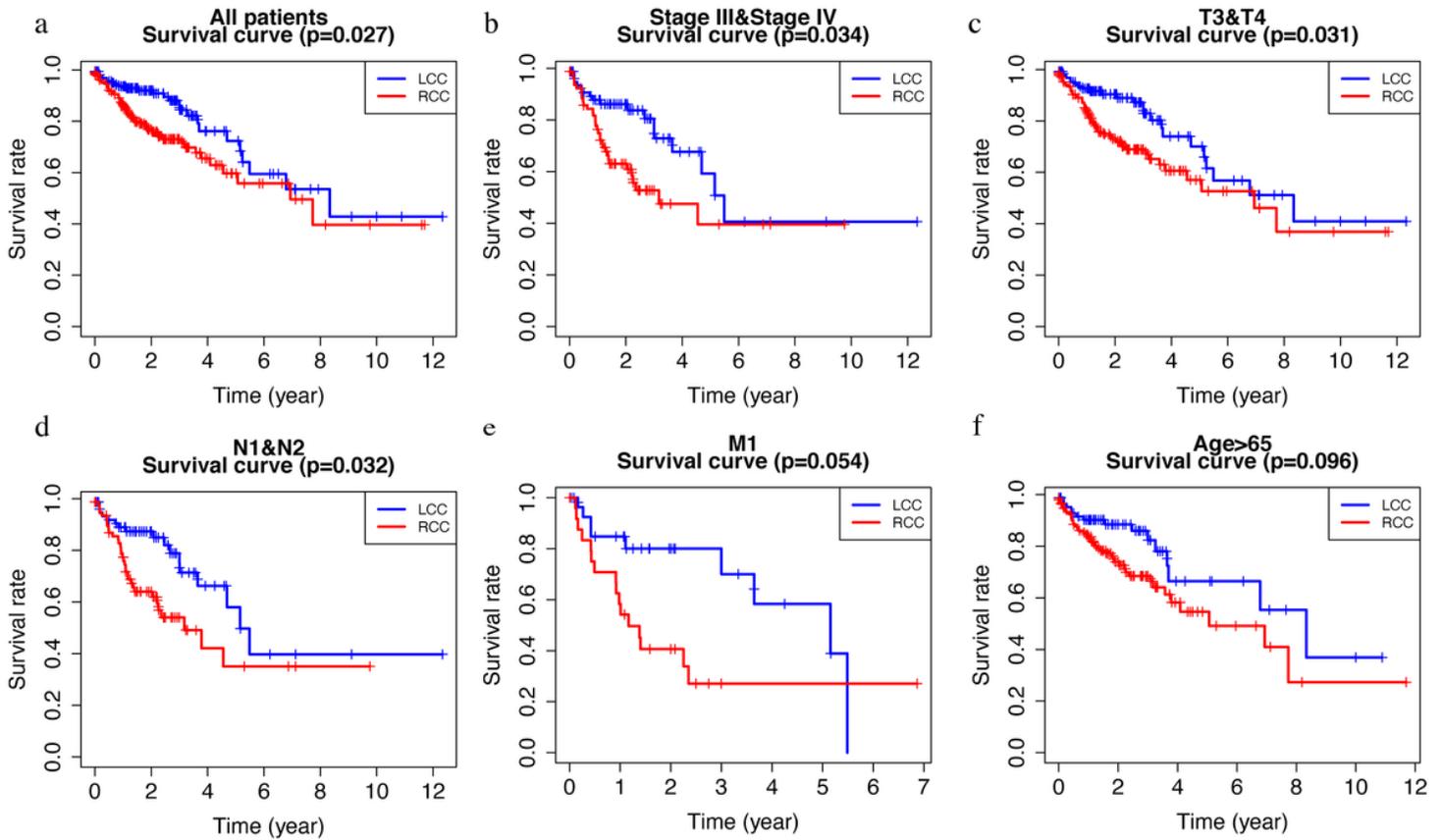


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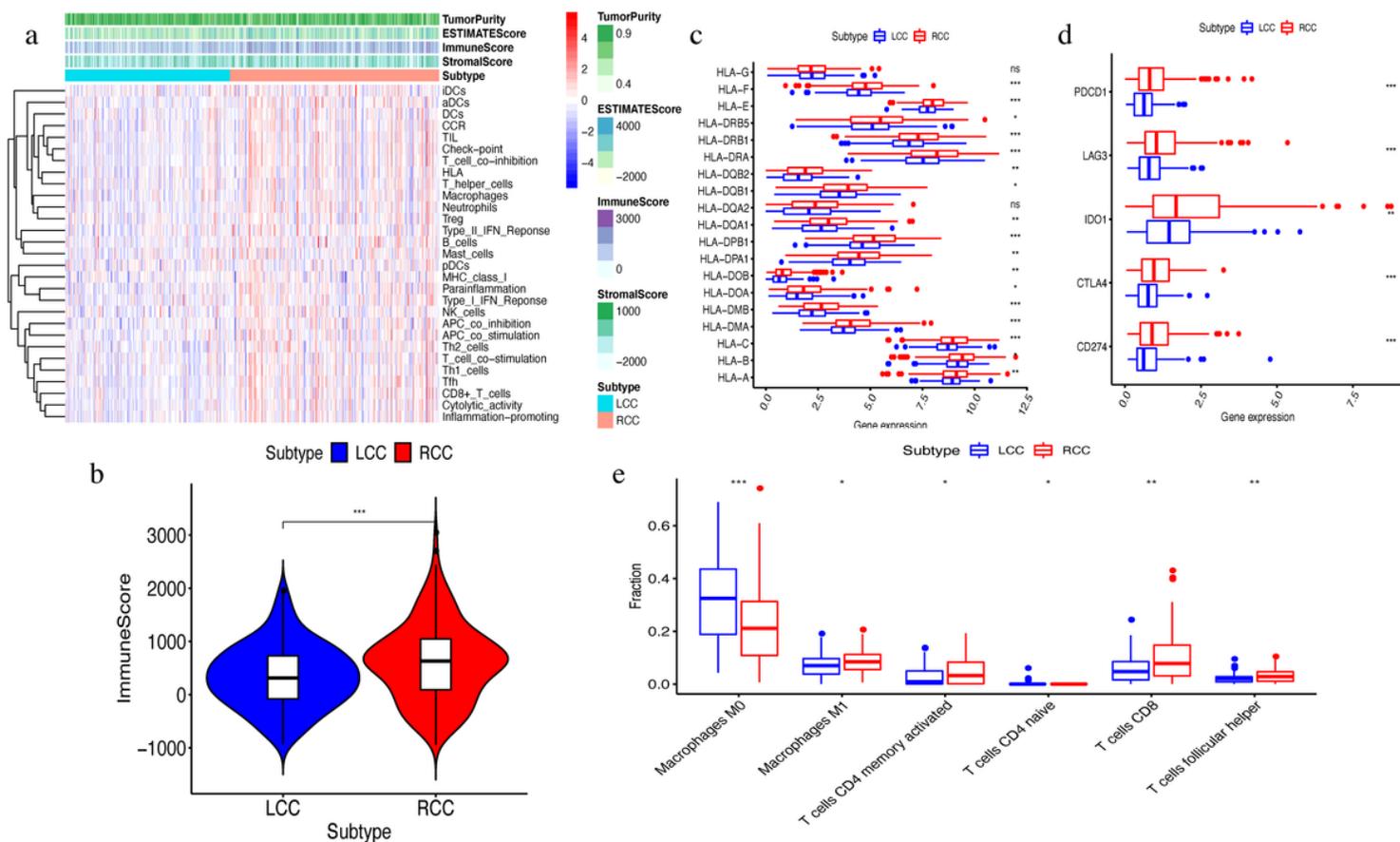


Figure 2

Exploration and validation the differences of immune microenvironment between LCC and RCC. Through ssGSEA,29 immune-related gene sets were enriched, including immune cells and immune processes. (a)The heat map is also included the tumor purity, ESTIMATE score, immune score and stromal score. (b)Variance analysis of the immune score between LCC and RCC. (c)The expression levels of HLA gene family in samples from LCC and RCC. (d)The expression levels of immune checkpoint genes (PDCD1,LAG3,IDO-1,CTLA4,CD274) in samples from LCC and RCC. (e)The abundance of 6 types of infiltrating immune cells in samples from LCC and RCC. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

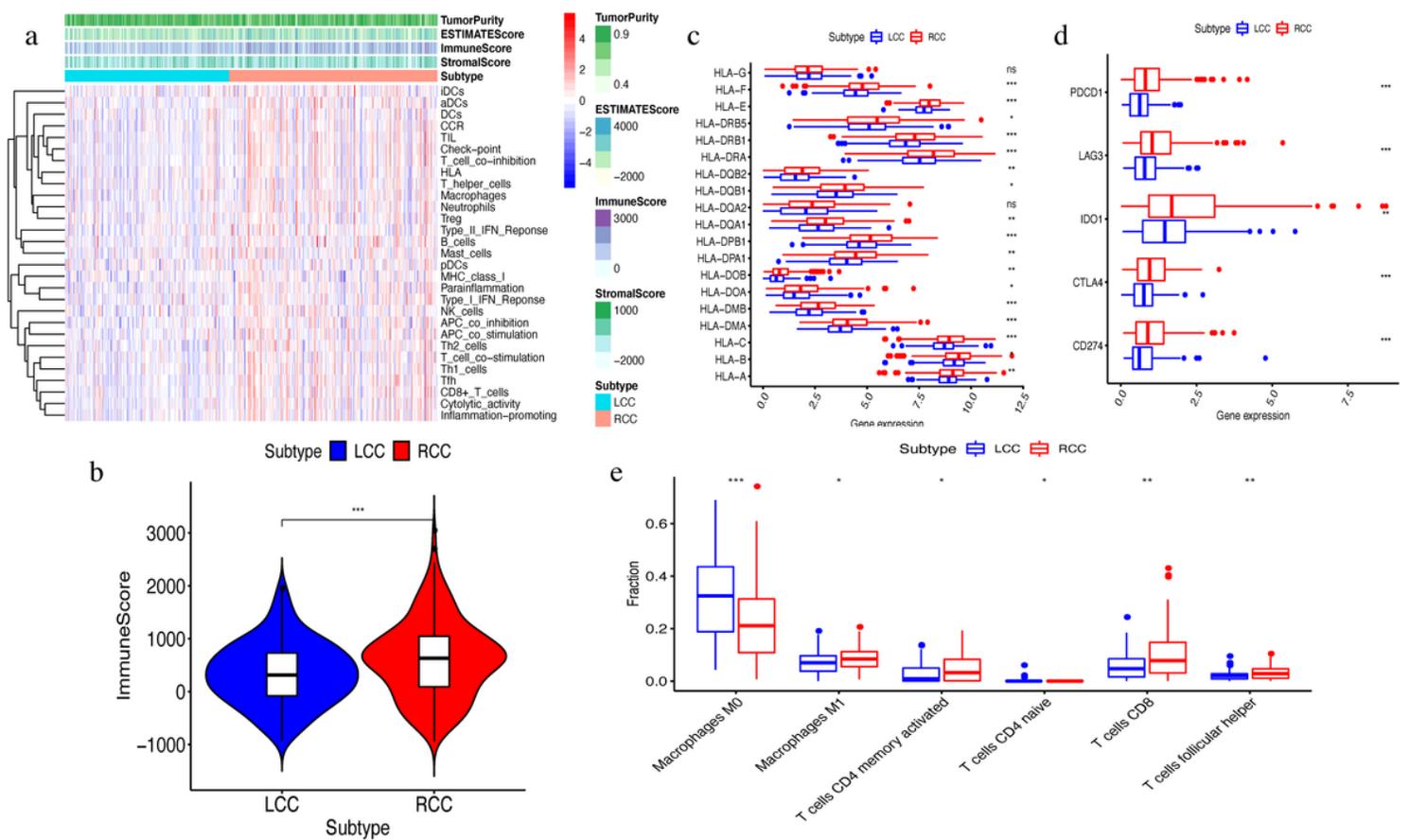


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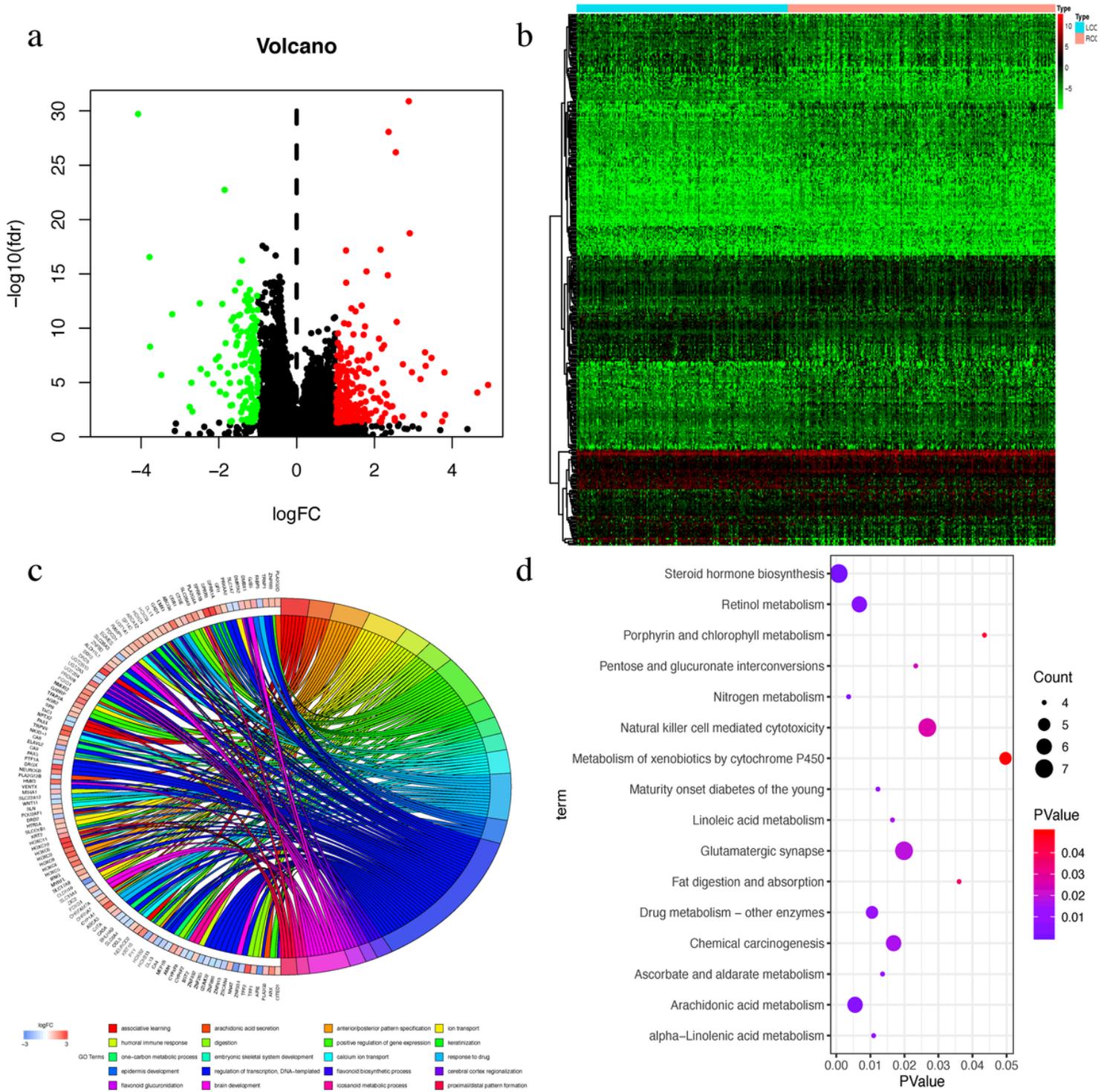


Figure 3

The Differentially expressed mRNAs in LCC and RCC. (a)Volcano plot for differential expressed mRNAs in LCC and RCC. Green dots represent up-regulated genes in LCC, while red dots represent up-regulated genes in RCC. (b)Heatmap of differential expressed mRNAs between LCC and RCC. (c)Circle diagram demonstrated the top 20 biological processes of GO enrichment analysis. (d)Bubble diagram demonstrated the top 15 KEGG pathways.

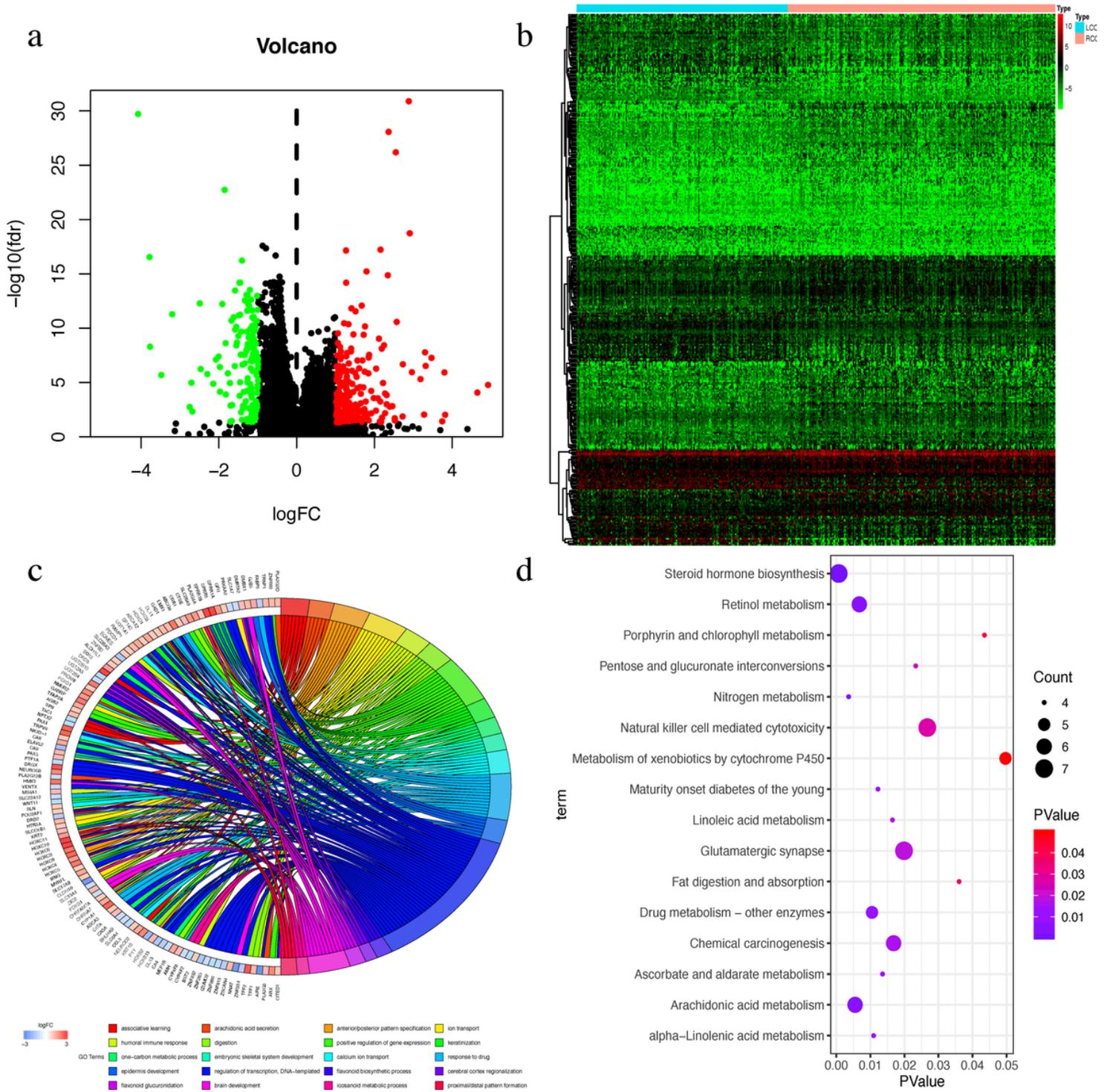


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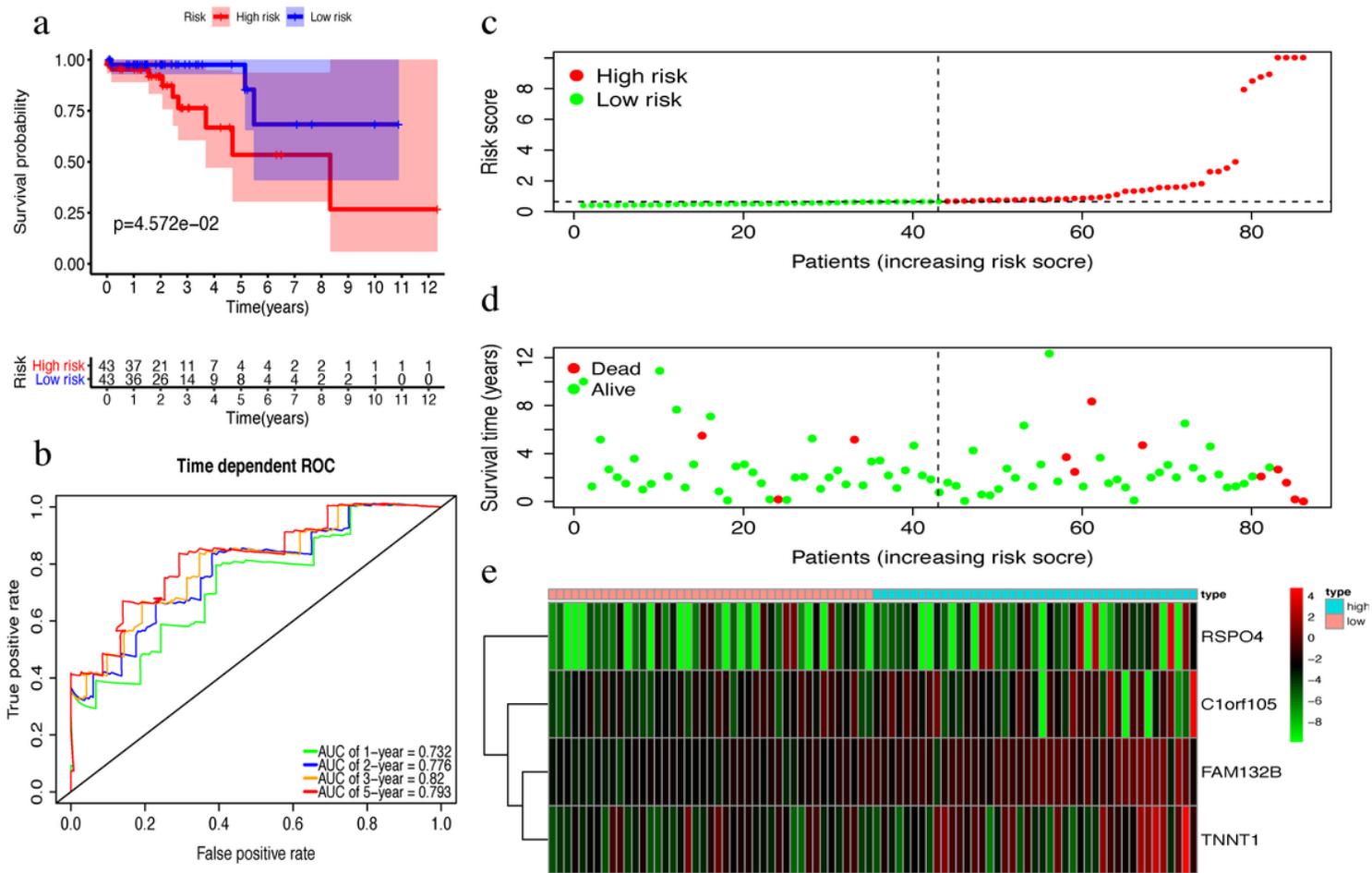


Figure 4

Construction of the prognostic model in the training group of LCC (a)The Kaplan-Meier(K-M) survival curves in the training set, (b)Time-dependent ROC curves in the training set at 1-year,2-year,3-year and 5-year. (c)The survival status of LCC patients in the training group.Green dots represent the patient is still alive, while red dots represent the patient has dead. (d)Risk scores of LCC patients in the training group.Green dots represent the patient assigned to the low risk group, while red dots represent the patient assigned to the high risk group. (e)mRNAs expression levels of four mRNA LCC prognosis signature in the training group.

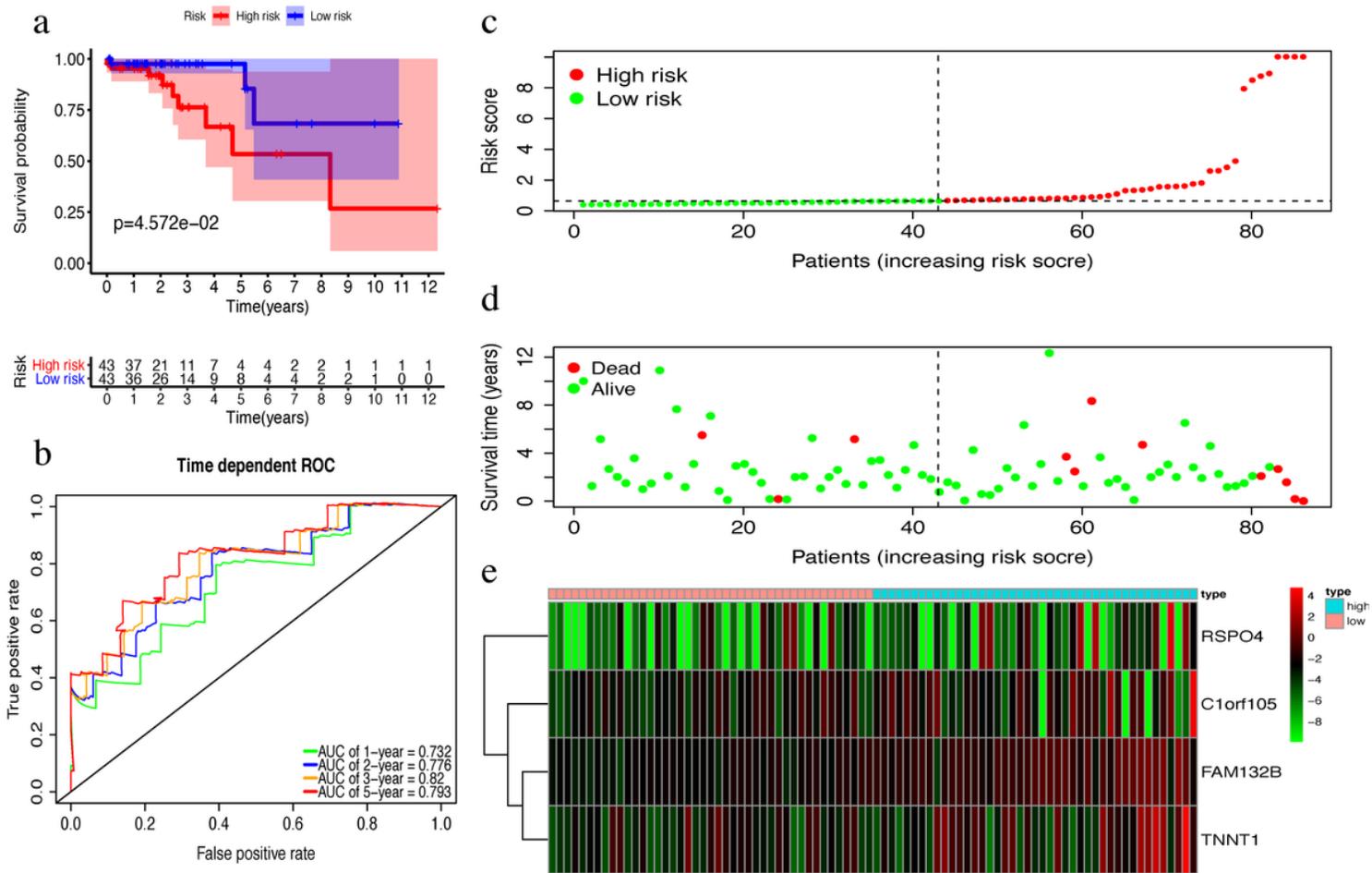


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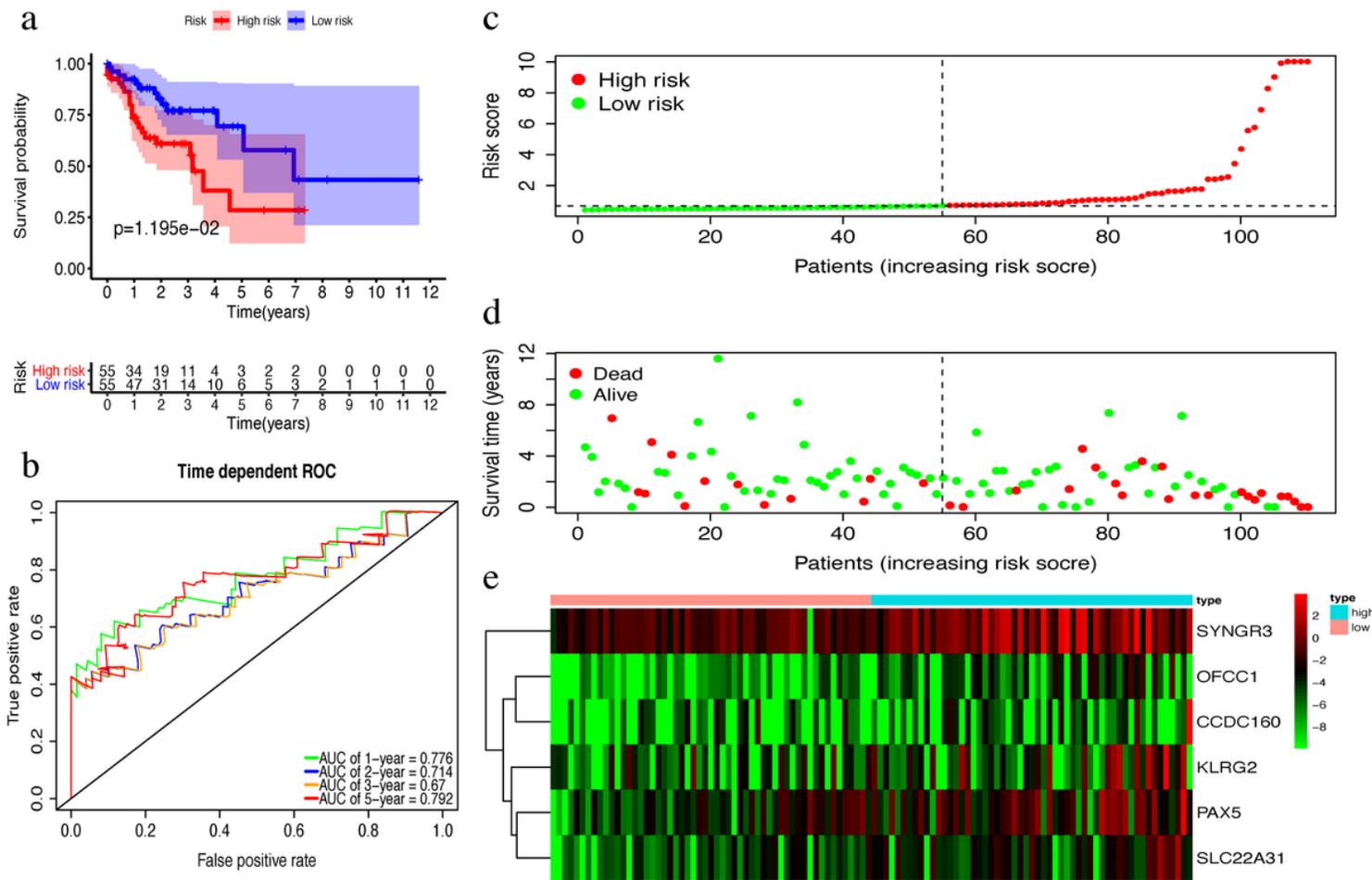


Figure 5

Construction of the prognostic model in the training group of RCC (a)The Kaplan-Meier(K-M) survival curves in the training set (b)Time-dependent ROC curves in the training set at 1-year,2-year,3-year and 5-year. (c)The survival status of RCC patients in the training group.Green dots represent the patient is still alive, while red dots represent the patient has dead. (d) Risk scores of RCC patients in the training group.Green dots represent the patient assigned to the low risk group, while red dots represent the patient assigned to the high risk group. (e)mRNAs expression levels of six mRNA LCC prognosis signature in the training group.

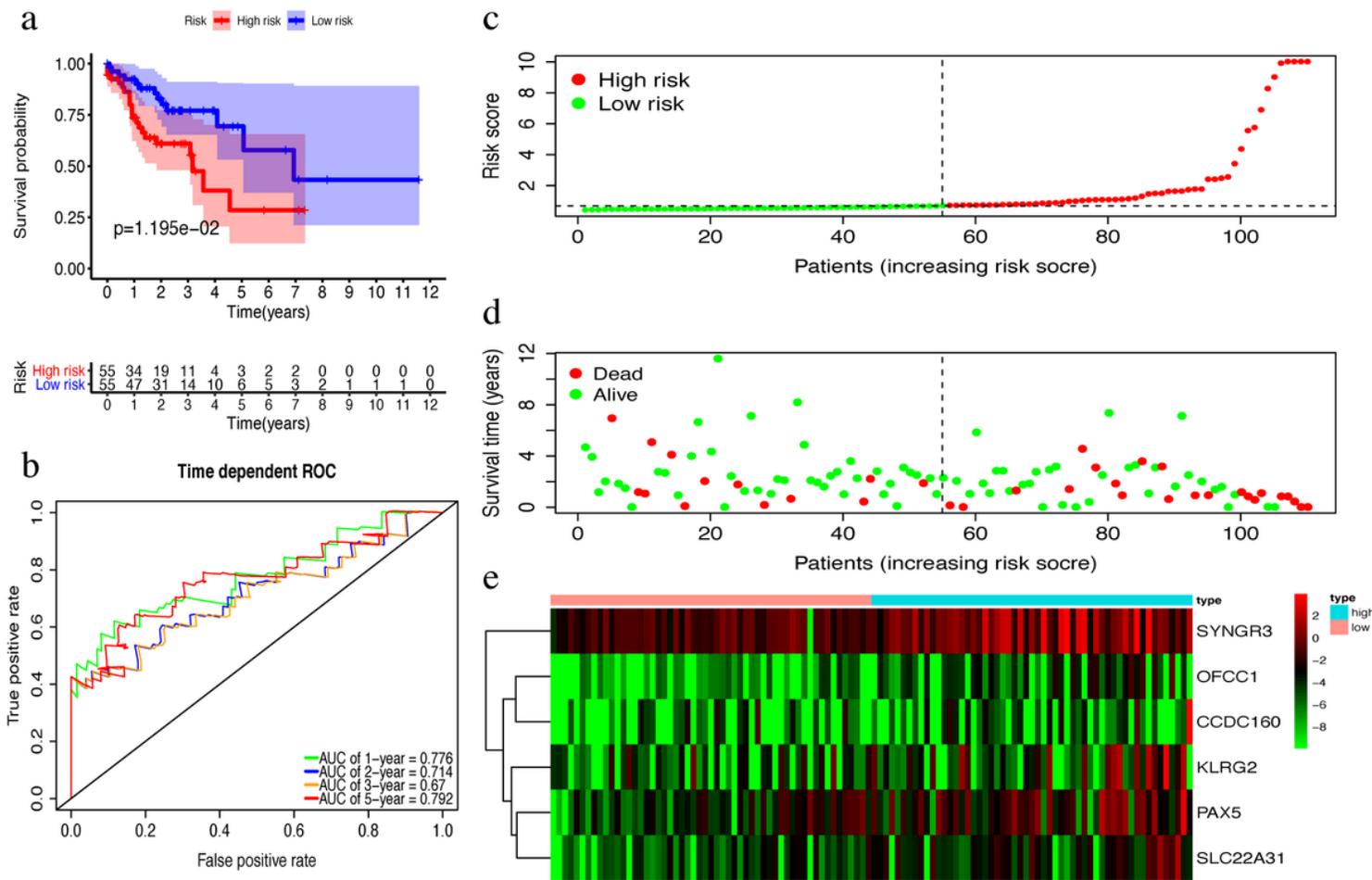


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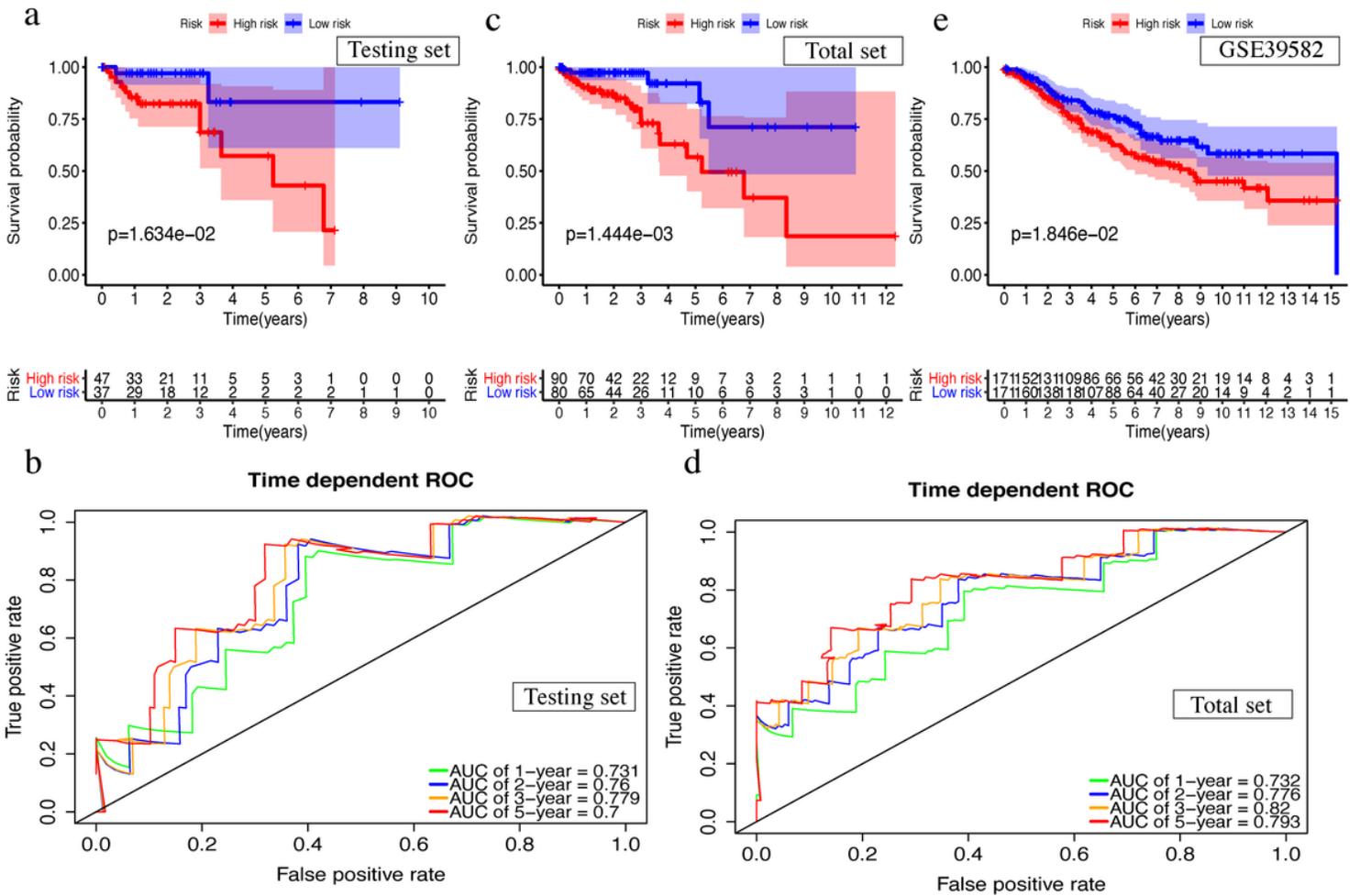


Figure 6

Validation of the prognostic signature of LCC (a,c,e)The Kaplan-Meier(K-M) survival curves in the testing set,the total set and GSE39582 (b,d)Time-dependent ROC curves in the testing set and the total set at 1-year,2-year,3-year and 5-year.

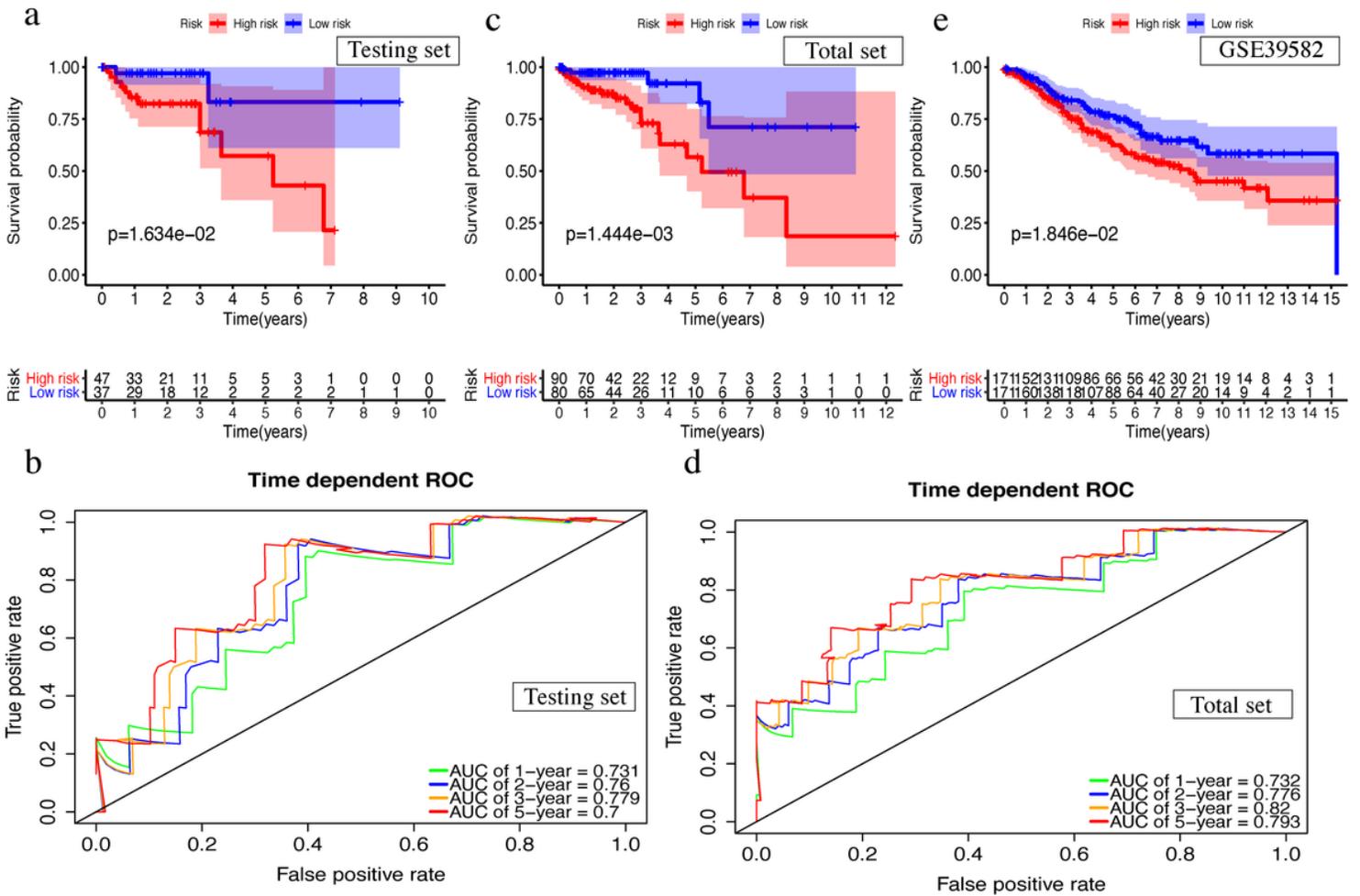


Figure 6

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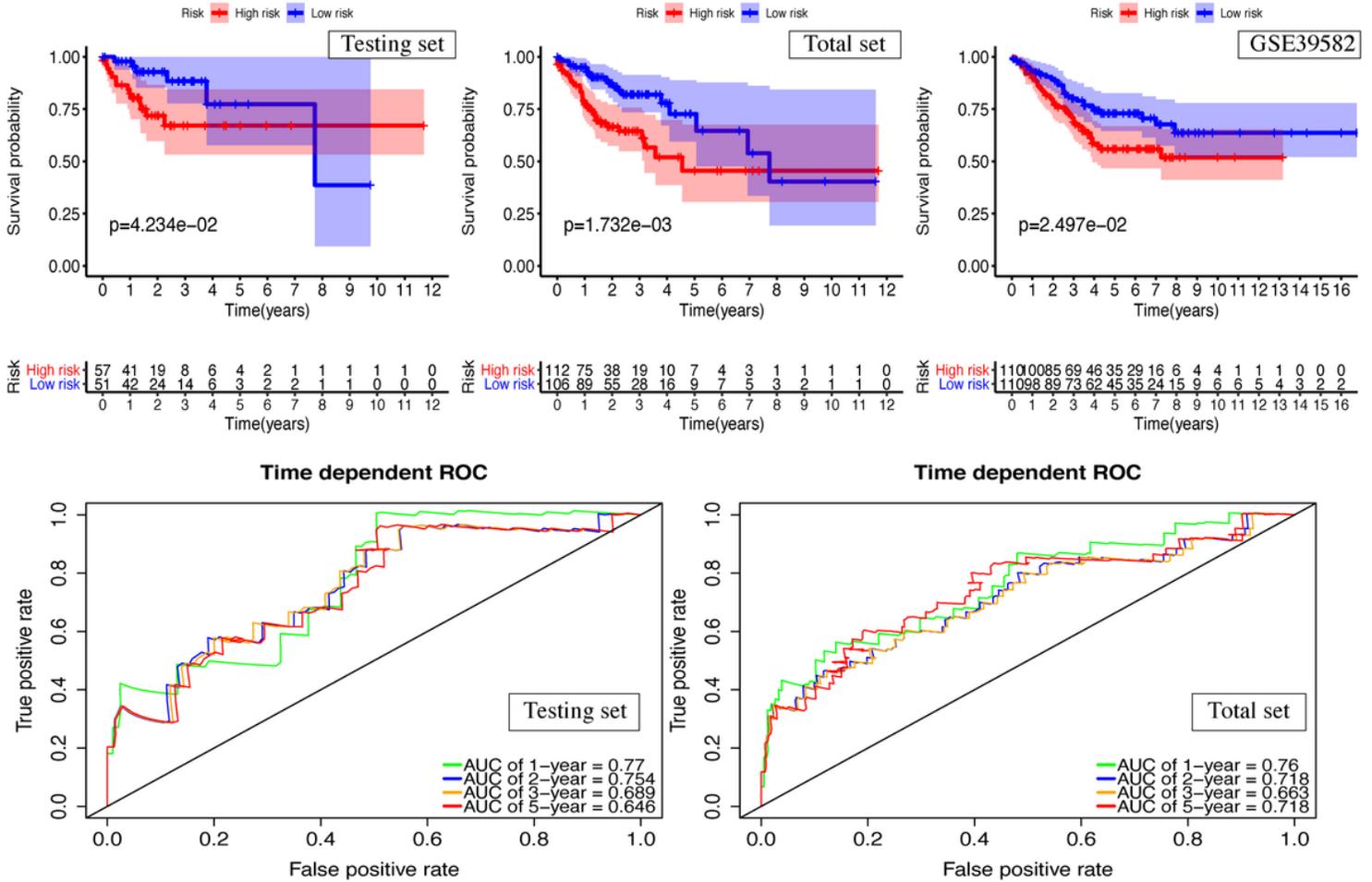


Figure 7

Validation of the prognostic signature of RCC (a,c,e) The Kaplan-Meier(K-M) survival curves in the testing set, the total set and GSE39582 (b,d) Time-dependent ROC curves in the testing set and the total set at 1-year, 2-year, 3-year and 5-year.

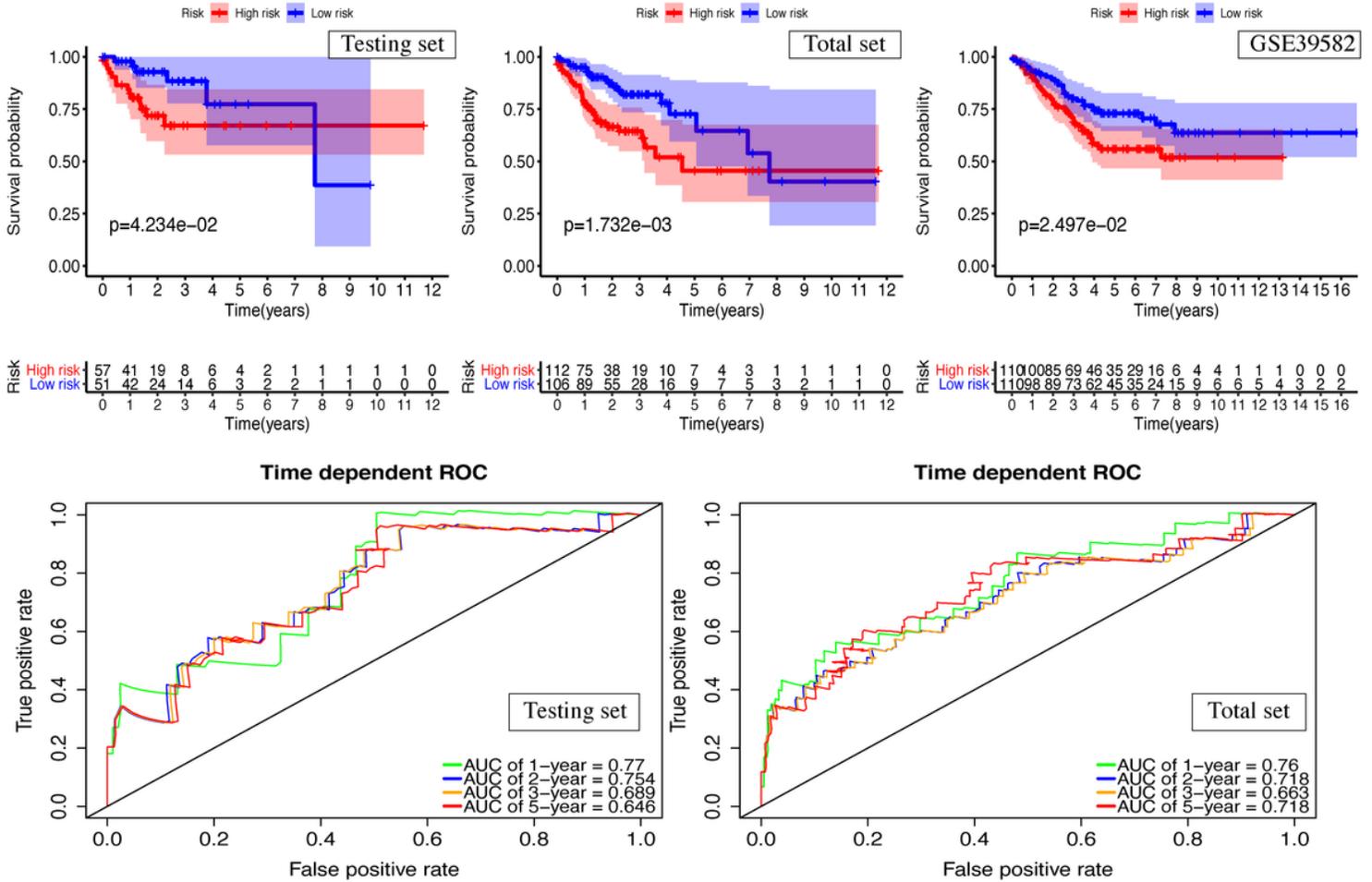


Figure 7

Validation of the prognostic signature of RCC (a,c,e) The Kaplan-Meier(K-M) survival curves in the testing set, the total set and GSE39582 (b,d) Time-dependent ROC curves in the testing set and the total set at 1-year, 2-year, 3-year and 5-year.

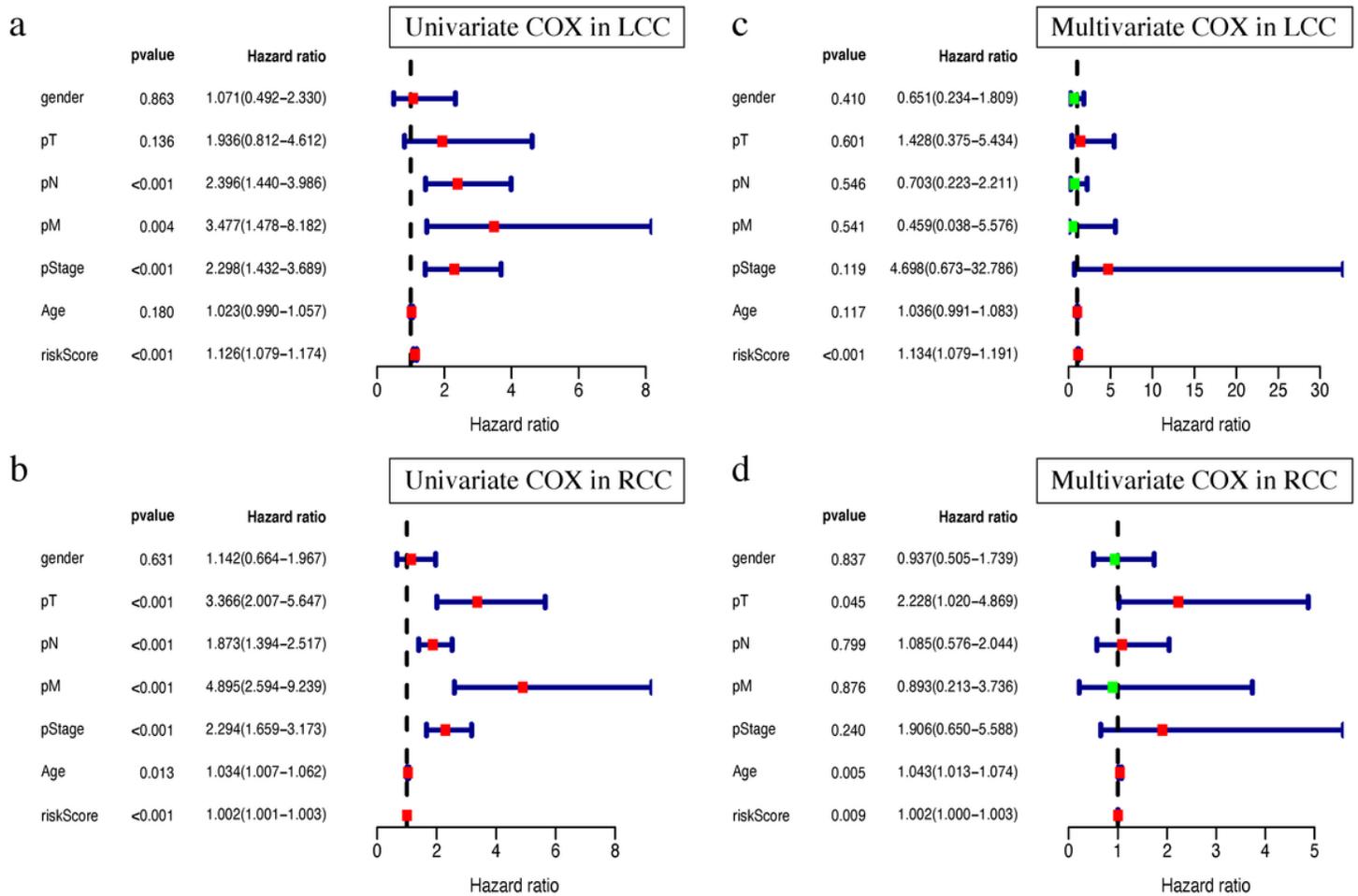


Figure 8

Independent prognostic analysis of two prognostic signatures (a)Univariate COX analysis of LCC prognostic signatures and clinical characteristics. (b)Univariate COX analysis of RCC prognostic signatures and clinical characteristics. (c)Multivariate COX analysis of LCC prognostic signatures and clinical characteristics. (d)Multivariate COX analysis of RCC prognostic signatures and clinical characteristics.

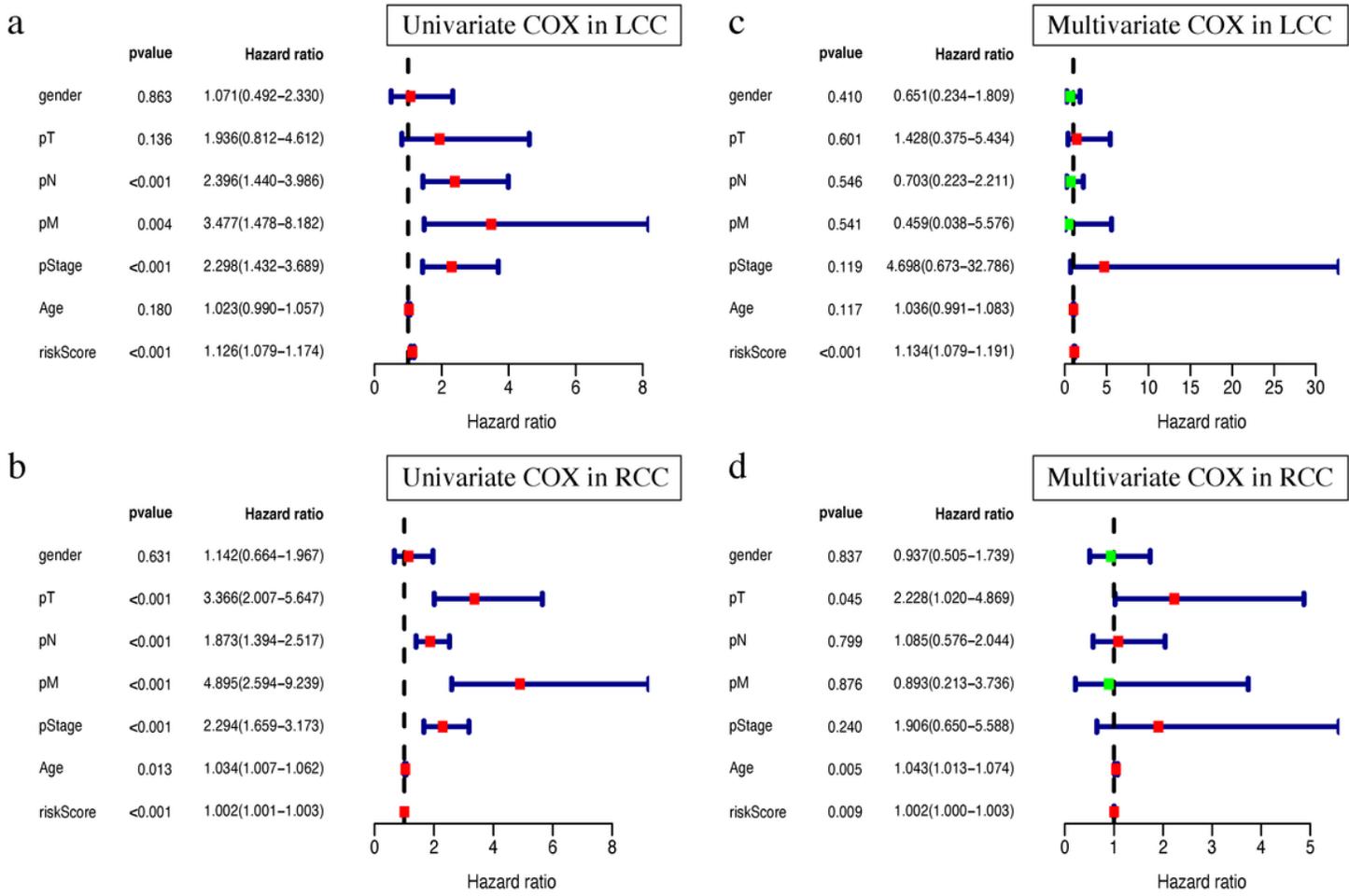


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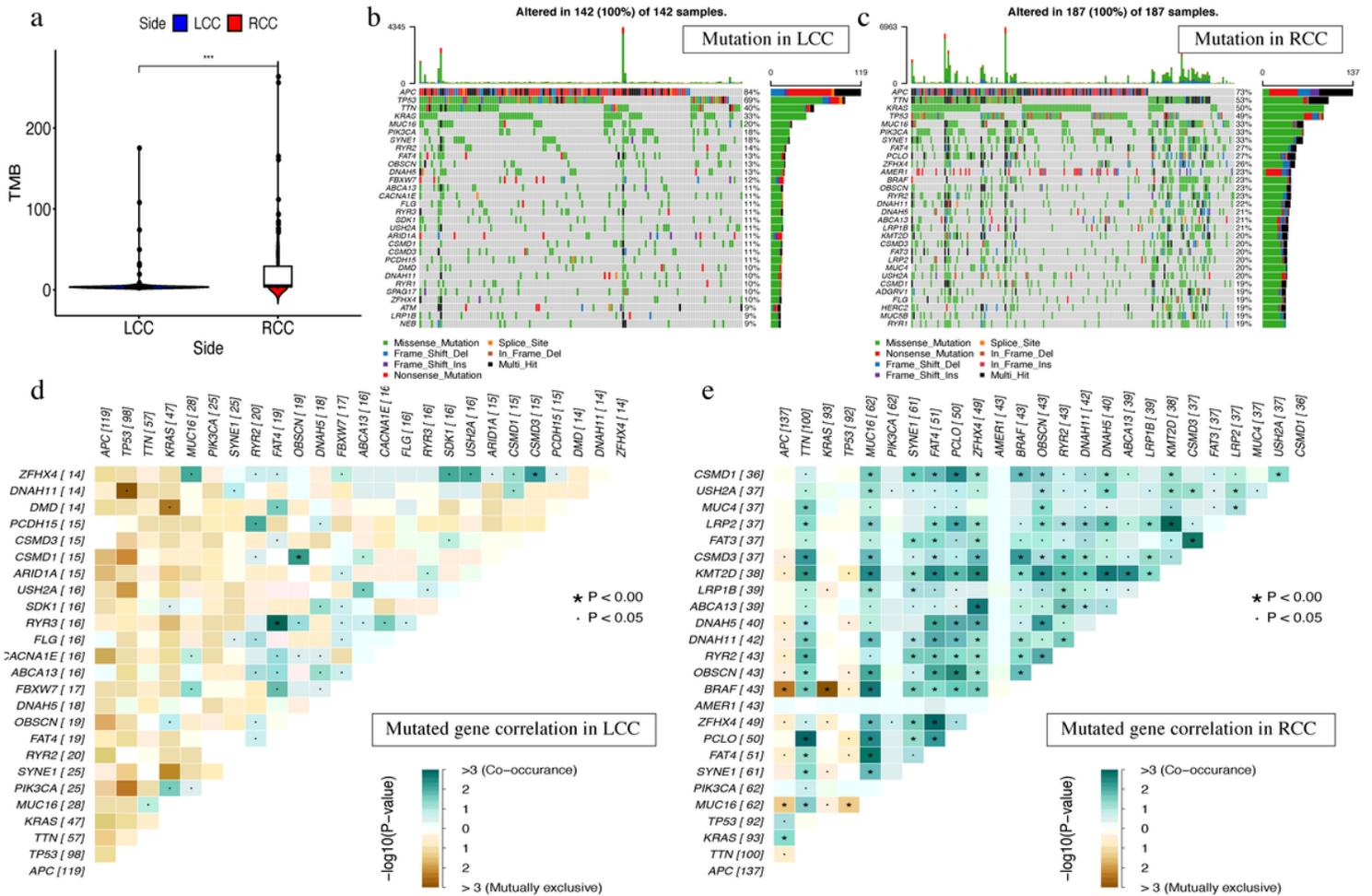


Figure 9

The landscape of single gene mutation in 142 LCC samples and 187 RCC samples. (a) The TMB of samples from two immune subgroups (*P<0.05, **P<0.01, ***P<0.001) (b)Waterfall plot displayed the top 30 frequently mutated genes in LCC. (c)Waterfall plot displayed the top 30 frequently mutated genes in RCC. (d)The coincident and exclusive associations across mutated genes in LCC. (e)The coincident and exclusive associations across mutated genes in RCC.

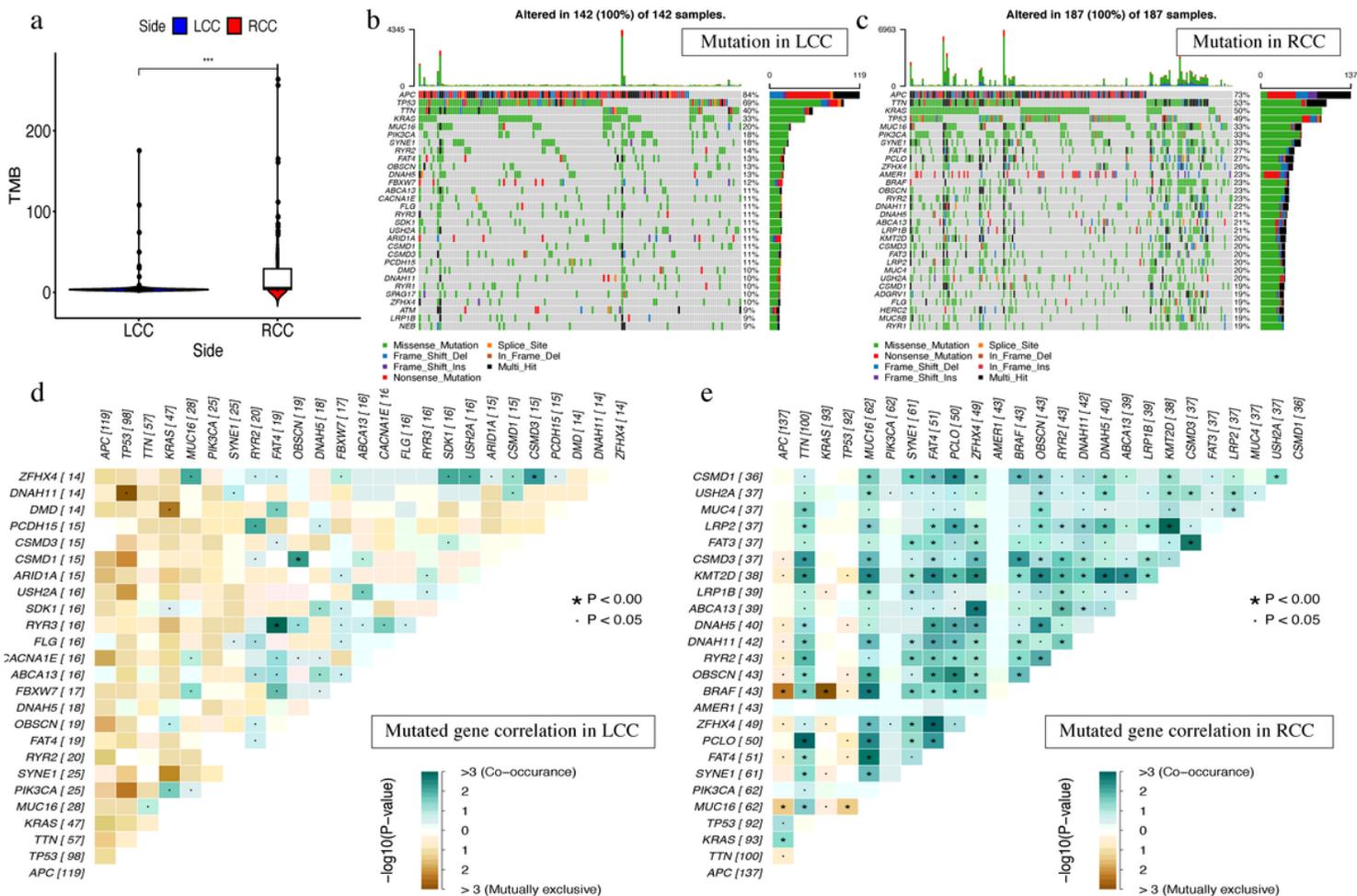


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