Original Article

Diagnostic genes and immune infiltration analysis of colorectal cancer determined by LASSO and SVM machine learning methods: a bioinformatics analysis

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Background: Genetic factors account for approximately 35% of colorectal cancer risk. The specificity and sensitivity of previous diagnostic biomarkers for colorectal cancer could not meet the need of clinical application. The expanding scale and inherent complexity of biological data have encouraged a growing use of machine learning to build informative and predictive models of the underlying biological processes. The aim of this study is to identify diagnostic genes of colorectal cancer by using machine learning methods.

Methods: The GSE41328 and GSE106582 data sets were downloaded from the Gene Expression Omnibus (GEO) database. The gene expression differences between colon cancer and normal tissues were analyzed. The key colorectal cancer genes were screened and validated by Least Absolute Shrinkage and Selection Operator (LASSO) and Support Vector Machine (SVM) regression. Immune cell infiltration and the correlation with the key genes in patients with colon cancer were further analyzed by CIBERSORT.

Results: Eleven key genes were identified as biomarkers for colon cancer, namely *ASCL2*, *BEST4*, *CFD*, *DPEPCFD*, *FOXQ1*, *TRIB3*, *KLF4*, *MMP7*, *MMP11*, *PYY*, and *PDK4*. The mean area under the receiver operating characteristic (ROC) curve (AUC) of all 11 genes for colon cancer diagnosis were 0.94 with a range of 0.91–0.97. In the validation set, the expression of the 11 key genes was significantly different between colon cancer and normal subjects ($P<0.05$) and the mean AUCs were 0.82 with a range of 0.70–0.88. Immune cell infiltration analyses demonstrated that the relative quantity of plasma cells, T cells, B cells, NK cells, MO, M1, Dendritic cells resting, Mast cells resting, Mast cells activated, and Neutrophils in the tumor group were significantly different to the normal group.

Conclusions: *ASCL2*, *BEST4*, *CFD*, *DPEPCFD*, *FOXQ1*, *TRIB3*, *KLF4*, *MMP7*, *MMP11*, *PYY*, and *PDK4* were identified as the key genes for colon cancer diagnosis. These genes are expected to become novel diagnostic markers and targets of new pharmacotherapies for colorectal cancer.

Keywords: Diagnostic genes; immune infiltration; colorectal neoplasms; machine learning

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Introduction

Colorectal cancer has a relatively high incidence and mortality, with a gradually increasing incidence in recent years. According to statistics, it is the third most common cancer in the United States after breast cancer (prostate cancer for men) and lung cancer (1). Although advances in colonoscopy can provide early detection of colorectal cancer, and radiotherapy, chemotherapy, immunotherapy, and other treatment methods have improved the five-year survival rate to 65% (2), the annual number of deaths remains high at 52,980, accounting for 8.7% (52,980/608,570) (3) of all cancer deaths. Moreover, colorectal cancer in China is ranked among the top five diseases (4), and given that genetic factors account for approximately 35% of colorectal cancer risk (5) it is essential to study its pathogenesis further. The quantification of gastrointestinal tumor risk should be combined with clinical and molecular data to allow an accurate phenotypic assessment and genetic diagnosis (6). Several biomarkers have been identified recently for the diagnosis of colorectal cancer such as secretin receptor (SCTR) gene methylation (7), tRNA-derived small RNAs (tDRs) (8), long non-coding RNAs (lncRNAs) (9), and TMEM236 gene (10). However, the specificity and sensitivity of these diagnostic biomarkers for colorectal cancer could not meet the need of clinical application (11).

With advancing research, the recent focus of interest has shifted to the tumor microenvironment (TME) (12). The tumor microenvironment (the internal environment in which tumor cells produce and live) includes not only the tumor cells themselves but also peripheral fibroblasts, immune and inflammatory cells, and other various cells. Meanwhile, the cellular interstitium, microvessels, and biomolecules infiltrated in nearby areas are characterized by hypoxia, chronic inflammation, and immunosuppression (13,14). The involvement of immune cells in the development of cancer has also been reported by many studies (15). The expanding scale and inherent complexity of biological data have encouraged a growing use of machine learning to build informative and predictive models of the underlying biological processes (16). In this study, potential colorectal cancer diagnostic genes were screened by using machine learning methods. To construct a more accurate diagnostic signature, we employed two most commonly used traditional machine learning methods, Least Absolute Shrinkage and Selection Operator (LASSO) and Support Vector Machine (SVM) algorithms (16,17). Additionally, immune cell infiltration was investigated by CIBERSORT analysis (18) to observe the correlation between key genes and infiltrating immune cells to identify new biomarkers for colorectal cancer diagnosis and subsequent treatment. We present the following article in accordance with the STARD reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-536/rc).

Methods

Study design

This is a bioinformatics analysis study and the potential colorectal cancer diagnostic genes were screened by using machine learning methods. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Download, standardization, and integration of data

Two datasets—GSE41328 and GSE106582—were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). GSE41328 and GSE106582 are gene expression datasets of 20 cases of colorectal cancer tissues and 194 cases of normal tissues, respectively. The data were normalized and merged with the R software packages "limma" and "Sva" as the training set. GSE110225 is a gene expression dataset of 60 cases of colorectal adenocarcinoma and normal tissues, which was used as the validation set for key gene differences.

Differential gene analysis

The gene expression differences between colon cancer and normal tissues were analyzed, and the screening threshold for differentially expressed genes (DEGs) was |logFC|=2 adj.P.Val. Filter <0.05. Volcano maps and heat maps were drawn with the "ggplot2" and "pheatmap" software packages. Ggplot2 (19) and pheatmap are R software packages in R language that can visualize gene expression in normal and tumor groups (20).

Gene Ontology (GO) annotation, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway clustering, Disease Ontology (DO), cluster analysis, and Gene Set Enrichment Analysis (GSEA) of differential genes

Gene cluster or enrichment analysis was employed to

cluster various functions, pathways, and disease-associated genes, using the R software packages "org.Hs.eg.db" and "enrichplot". Each GO annotation consists of a gene and the corresponding GO term, which mainly includes three aspects: molecular functions (MF), biological process (BP), and cellular components (CC) (21). The KEGG database is a bioinformatics database established in 1995 by the Kanehisa laboratory at the Bioinformatics Center, Kyoto University, Japan. It is now an important bioinformatics knowledge base for integrating and interpreting large-scale molecular datasets generated by genome sequencing and other high-throughput experimental techniques. The most central database is the KEGG PATHWAY and KEGG ORTHOLOGY database (22). The KEGG clustering pathway of differential genes was applied in this study. DO analysis is a simple analysis of genetic disease enrichment (23), which was performed by using the "DOSE" R software package. The condition for the GO entries annotation, KEGG pathway, and disease analysis in this study was adj.P.Val. Filter <0.05. GSEA enrichment analysis was performed on the results of the KEGG and GO analyses by the data sets c2.cp.kegg.v7.4.symbols.gmt and c5.go.v7.4.symbols.gmt, respectively.

Screening and validation of the key colon cancer genes by LASSO and SVM regression

LASSO and SVM regression are two machine learning methods commonly used to screen variables (24,25); two regressions of the selected differential genes were intersected as key diagnostic colorectal cancer genes in this study. The diagnostic ability of the key colorectal cancer genes was examined using the area under the receiver operating characteristic (ROC) curve (AUC). Using gene set GSE110225 as the training set, the differences in the expression of the key tumor genes and their diagnostic ability for colon cancer were observed. A value of 0.75 was deemed as useful discrimination performance of AUC (26).

Analysis of immune cell infiltration in patients with colon cancer

CIBERSORT is a tool for deconvolving the expression matrix of immune cell subtypes based on the principle of linear support vector regression of genes. Immune cell infiltration was estimated by RNA-Seq data first published in *Nature Methods* in 2015 (27), which is currently the most commonly used analytical tool for immune cell infiltration estimation (18). The relative quantity of infiltrating immune cells in patients with colon cancer and the correlation between immune cells and the key diagnostic colorectal cancer genes were analyzed by CIBERSORT in this study. The correlation between the 11 key genes and immune cells was represented by a lollipop chart.

Statistical analysis

The gene expression differences between colon cancer and normal tissues were screened by LASSO and SVM regression based on machine learning method. The diagnostic performance of the genes was assessed using AUC. The distribution of the differentially expressed genes was shown by heatmaps. A two-tailed P value <0.05 was considered as statistical significance. All the statistical analyses were performed by using R software (Version 4.1.1).

Results

Analysis results of expressed genes in colon cancer tissues and normal tissues

The datasets were downloaded, merged, and normalized, followed by gene difference analysis, as shown in *Figure 1*. Filtered with the condition of |logFC|>2, the DEG analysis revealed 60 differentially expressed genes between colon cancer tissues and normal tissues, including 43 downregulated and 17 upregulated genes (*Figure 2A*). In colorectal cancer patients, the upregulated genes included *CLDN1*, *FOXQ1*, and *TRIB3*, and the downregulated genes included *CA1*, *CLCA4*, and *AQP8*. The specific results are shown in *Figure 2B*.

GO annotation, clustering KEGG pathway, and GSEA analysis of the differential genes

GO annotation and KEGG pathway clustering analyses were performed on the differential genes (*Figure 3A*,*3B*). The GO annotation demonstrated that the BPs of the key genes were clustered in 'extracellular matrix organization', 'extracellular encapsulating structure organization', and 'collagen metabolic process'; the cellular components clustered in the 'apical part of cell', 'cell membrane projection', and 'cluster of actin-based cell projections'; the molecular functions clustered in 'metallopeptidase activity', 'oxidoreductase activity', and 'cyclase regulator activity'; KEGG showed that the key genes clustered in 'bile

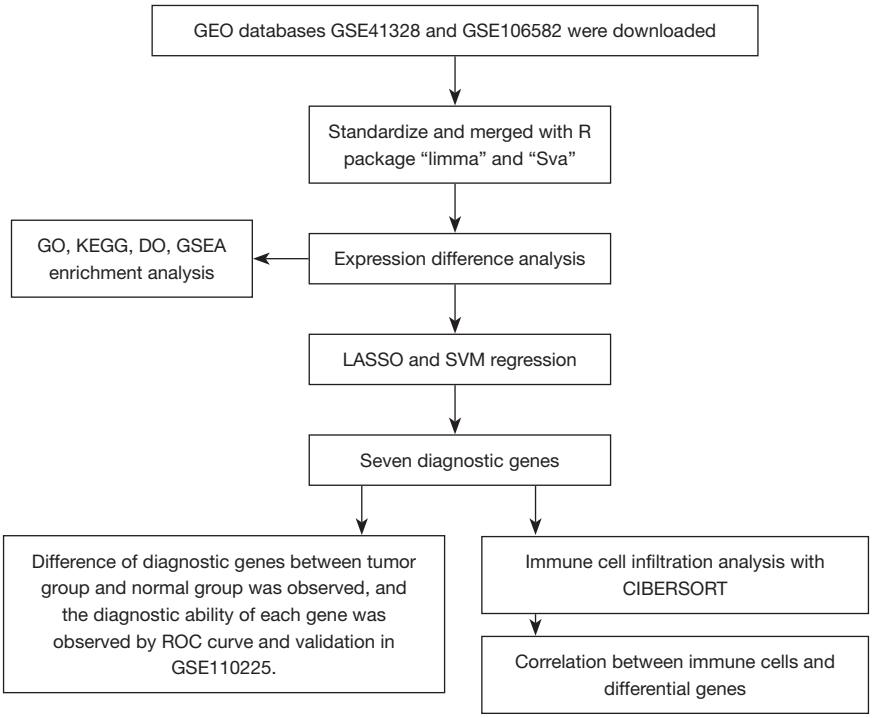


Figure 1 The study flowchart. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DO, disease ontology; GSEA, Gene Set Enrichment Analysis; LASSO, Least Absolute Shrinkage and Selection Operator; SVM, Support Vector Machine; ROC, receiver operating characteristic.

secretion', 'nitrogen metabolism', and 'retinol metabolism' on the pathway. The GO annotation and KEGG results were further analyzed by GSEA, and in colorectal cancer patients the pathway clustered in *KEGG-CELL-CYCLE*, *KEGG-DNA-REPLICATION*, and *KEGGPROTEEASOME*. GO entries were clustered in *GOBP_CHROMOSOME_SEGREGATION*, *GOBP_DNA_CONFORMATION_CHANGE*, and *GOBP_DNA_REPAIR*. The specific results are shown in Figure S1.

Screening and validation of the key colon cancer genes by LASSO and SVM regression

LASSO and SVM regression identified 14 and 19 genes associated with a diagnosis of colorectal cancer, respectively, for which the intersection was taken (see *Table 1*). Eventually, 11 genes were screened as the key diagnostic genes for colorectal cancer (*Figure 4A-4C*), including *ASCL2*, *BEST4*, *CFD*, *DPEPCFD*, *FOXQ1*, *KLF4*, *MMP7*, *MMP11*, *PYY*, *PDK4*, and *TRIB3*. The AUCs of the 11 key genes associated with colon cancer were 91.4%, 96.0%, 93.2%, 91.6%, 97.3%, 96.6%, 97.2%, 95.5%, 93.6%, 95.3%, and 97.4%, respectively (*Figure 4D*). The 11 key genes were significantly different in expression in colorectal cancer and normal tissues in the validation set GSE110225 ($P<0.05$), with *ASCL2*, *DPEPCFD*, *FOXQ1*, *MMP7*, *MMP11*, and *TRIB3* being highly expressed in colon cancer patients (*Figure 5*). The AUCs of the 11 key diagnostic genes for colorectal cancer in the validation set were 70.6%, 86.2%, 84.1%, 82.0%, 85.5%, 86.2%, 88.6%, 77.9%, 84.1%, 81.3%, and 83.0%, respectively (*Figure 6*).

Analysis of immune-infiltrating cells

The relative quantity of immune cells in colon cancer tissues and normal tissues (*Figure 7A*), the correlation between infiltrating immune cells (*Figure 7B*), and the difference in the quantity of infiltrating immune cells between the two groups (*Figure 7C*) were analyzed by CIBERSORT. For the infiltrating immune cells, macrophages MO were negatively correlated with plasma cells, T cells CD4 memory were negatively correlated with Mast cells resting, which were positively correlated with Mast cells activation, with correlation coefficients of –0.62, –0.59, –0.58, and 0.50,

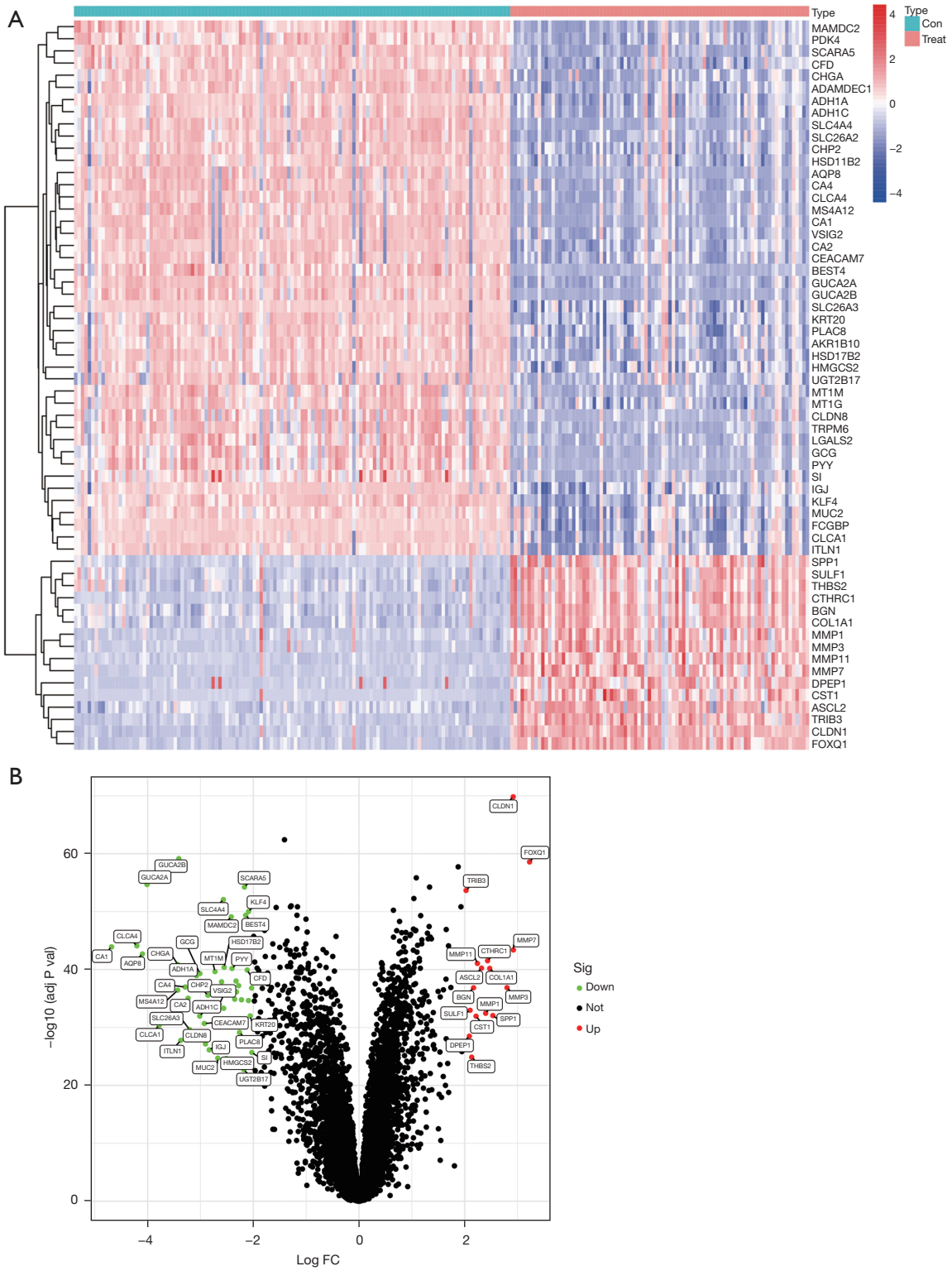


Figure 2 Differential gene expression between colon cancer tissues and normal tissues. (A) Heat map of differential gene expression. (B) Volcano map of the upregulation and downregulation of the top 50 differential genes in colon cancer.

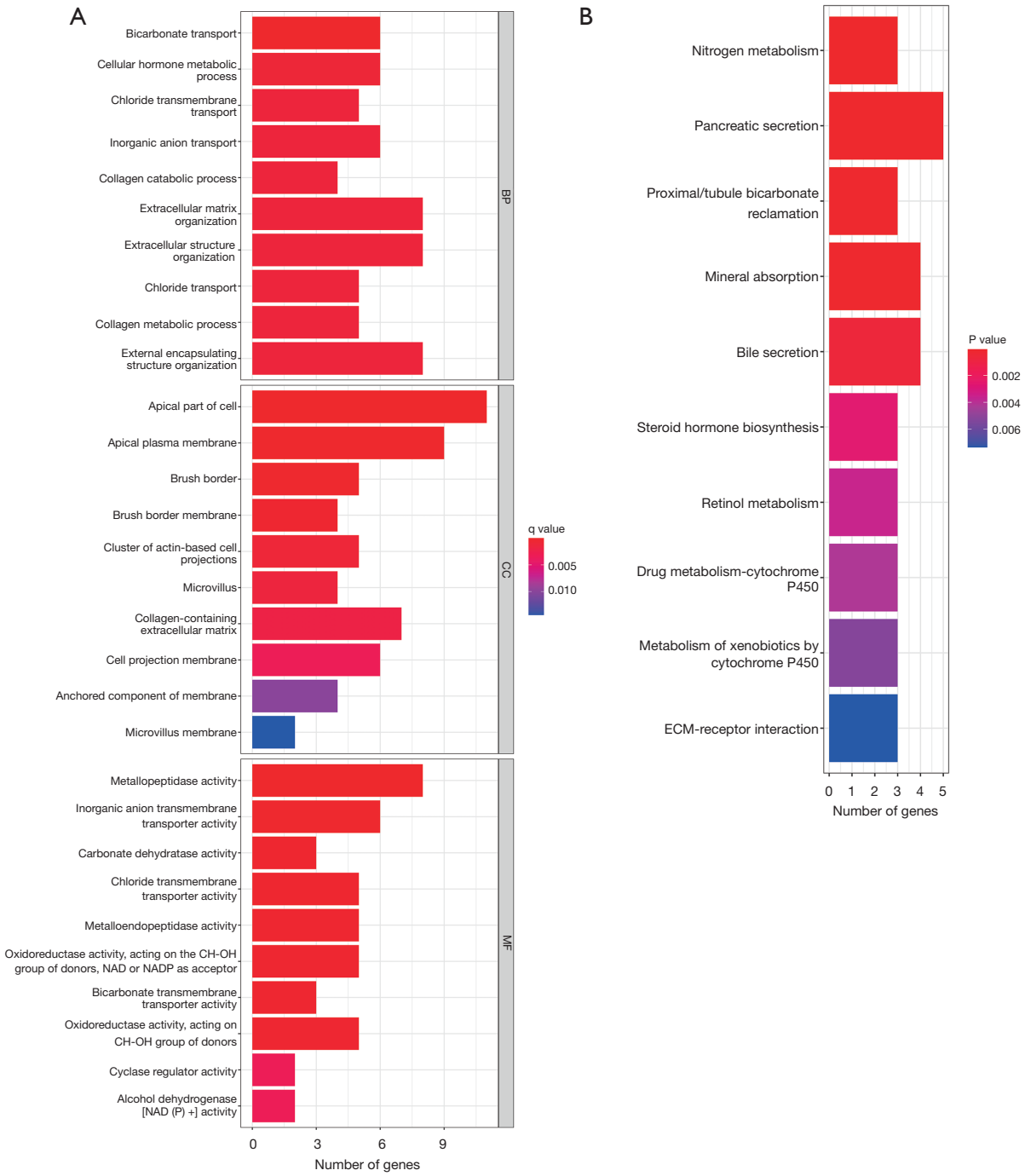


Figure 3 Differential gene GO annotation and KEGG analysis results between colorectal cancer tissues and normal tissues. (A) GO annotation results; (B) KEGG pathway analysis results. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

respectively; Mast cells resting were negatively correlated with Mast cells activated, with a correlation coefficient of –0.55; The cells with different quantity of infiltrating immune cells between the two groups were plasma cells, T cells, B cells naive, NK cells resting, macrophages MO, M1, Dendritic cells resting, Mast cells resting, Mast cells activation, and Neutrophils. Genes upregulated in colorectal cancer tissues *ASCL2*, *DPEPCFD*, *FOXQ1*,

Table 1 The 11 intersection genes of LASSO and SVM regression

Different genes of LASSO regression	Different genes of SVM regression	Intersection genes
FOXQ1	*CLDN1*	*FOXQ1*
TRIB3	*TRIB3*	*TRIB3*
KLF4	*FOXQ1*	*KLF4*
BEST4	*SCARA5*	*BEST4*
MMP7	*MAMDC2*	*MMP7*
MMP11	*ASCL2*	*MMP11*
ASCL2	*BEST4*	*ASCL2*
PYY	*CST1*	*PYY*
CFD	*GUCA2B*	*CFD*
MT1M	*KLF4*	*PDK4*
PDK4	*MMP7*	*DPEP1*
LGALS2	*MMP11*	
SPP1	*GUCA2A*	
ADH1C	*DPEP1*	
DPEP1	*CFD*	
IGJ	*CTHRC1*	
	PYY	
	PDK4	
	CA2	

LASSO, Least Absolute Shrinkage and Selection Operator; SVM, Support Vector Machine.

MMP7, *MMP11*, and *TRIB3* were associated with B cell naive, natural killer (NK) cells resting, macrophages MO, and M1 immune cells (*Figure 8*).

Discussion

DEG screening and GO, KEGG, and GSEA enrichment analyses were performed in this study. In total, 60 DEGs were selected, including 17 upregulated and 43 downregulated genes. The results of the GO analysis showed that DEGs were involved in 'extracellular matrix organization', 'extracellular encapsulation structural organization', 'collagen metabolic process', 'cell apex', 'cell projection membrane population', 'actin-based cell projection', 'metallopeptidase activity', 'oxidoreductase activity' and 'cell cycle regulator'. The KEGG pathway enrichment analysis showed correlations in bile secretion, nitrogen metabolism, retinol metabolism, and extracellular matrix (ECM)-receptor interaction pathways. These pathways may play an important role in tumor immune escape, adhesion, degradation, motility, and proliferation processes (28), and their role in other cancers has been demonstrated: ECM has been shown to be upregulated in prostate cancer tissues (29), and the ECM-receptor interaction pathway was involved in the invasion and metastasis of gastric cancer (30). Additionally, a recent study on glioblastoma, the most lethal adult brain tumor, showed that the pathological features of abnormal neovascular development, diffuse tumor cell infiltration, and interactions between ECM and the glioblastoma microenvironment were important factors in disease progression (31). It was notable that the immune-related functions were found in the KEGG pathway. These results suggested that DEGs are highly relevant to the immune system, confirming our hypothesis. They may prevent immune cells from attacking cancer cells and promote immune escape to induce tumor progression and metastasis. Our GSEA enrichment analysis showed the involvement of cell cycle and DNA replication processes, suggesting that cell cycle checkpoint inhibitors or cycle arrest may be effective in treating colorectal cancer.

This is the first study to combine LASSO and Support Vector Machine-Recursive Feature Elimination (SVM-RFE) algorithms to identify and validate key biomarkers of colorectal cancer in a test set. Finally, 11 key genes were identified, including *ASCL2*, *BEST4*, *CFD*, *DPEPCFD*1, *FOXQ1*, *KLF4*, *MMP*7, *MMP11*, *PYY*, *PDK4*, and *TRIB3*. The AUC value was >0.91 for all 11 key genes in the training set. However, the AUC only reached about 0.7 in the validation set, which indicates that while the constructed model has a robust validation performance, its test performance needs further improvement.

Achaete scute-like-2 (*ASCL2*) is a key downstream molecule of the Wnt/β-catenin signaling pathway; it is a basic helix-loop helical transcription factor homolog found in enterocytes that may play an indispensable role in the maintenance of intestinal stem cell effects (32). It has been shown that *ASCL2* expression leads to tumor growth arrest through miRNA-302b-mediated conditional reprogramming cells (CRC) progenitor cells and induces miR-200 expression, which further promotes the plasticity of epithelial-mesenchymal transition-mesenchymal-epithelial transition (EMT-MET) through transcriptional mechanisms (33). Downregulating *ASCL2* can also promote apoptosis by enhancing autophagy in colorectal cancer cells (34).

The Bestrophin (BEST) family are newly discovered

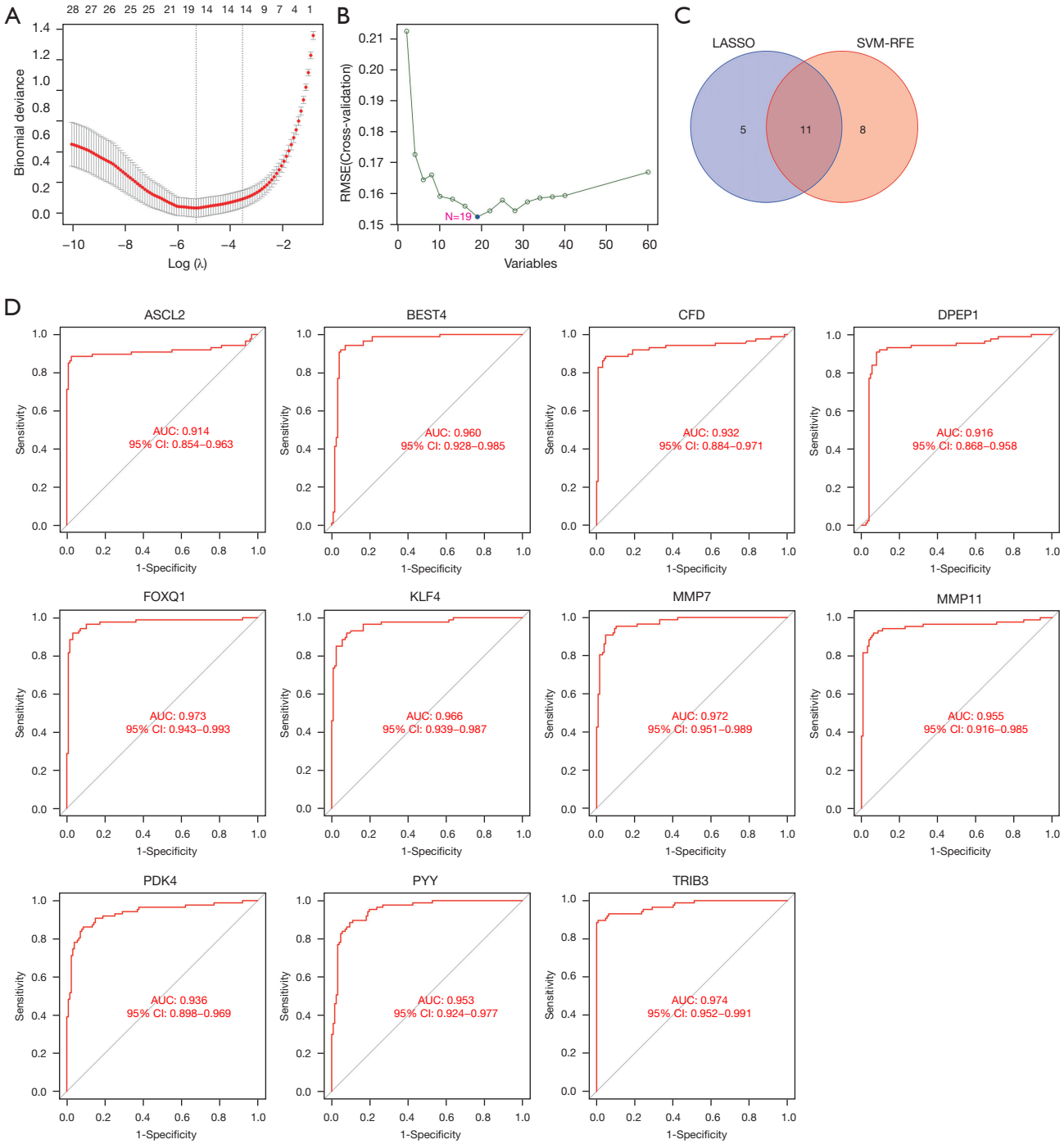


Figure 4 Key genes obtained after the intersection of the LASSO and SVM regressions: (A) number of genes screened by LASSO regression; (B) number of genes screened by SVM regression; (C) Venn diagram of the intersection of the two regression methods; (D) ROC curve of 11 key genes for colorectal cancer diagnosis in the training set (GSE41328 and GSE106582). LASSO, Least Absolute Shrinkage and Selection Operator; SVM-RFE, Support Vector Machine-Recursive Feature Elimination; ROC, receiver operating characteristic.

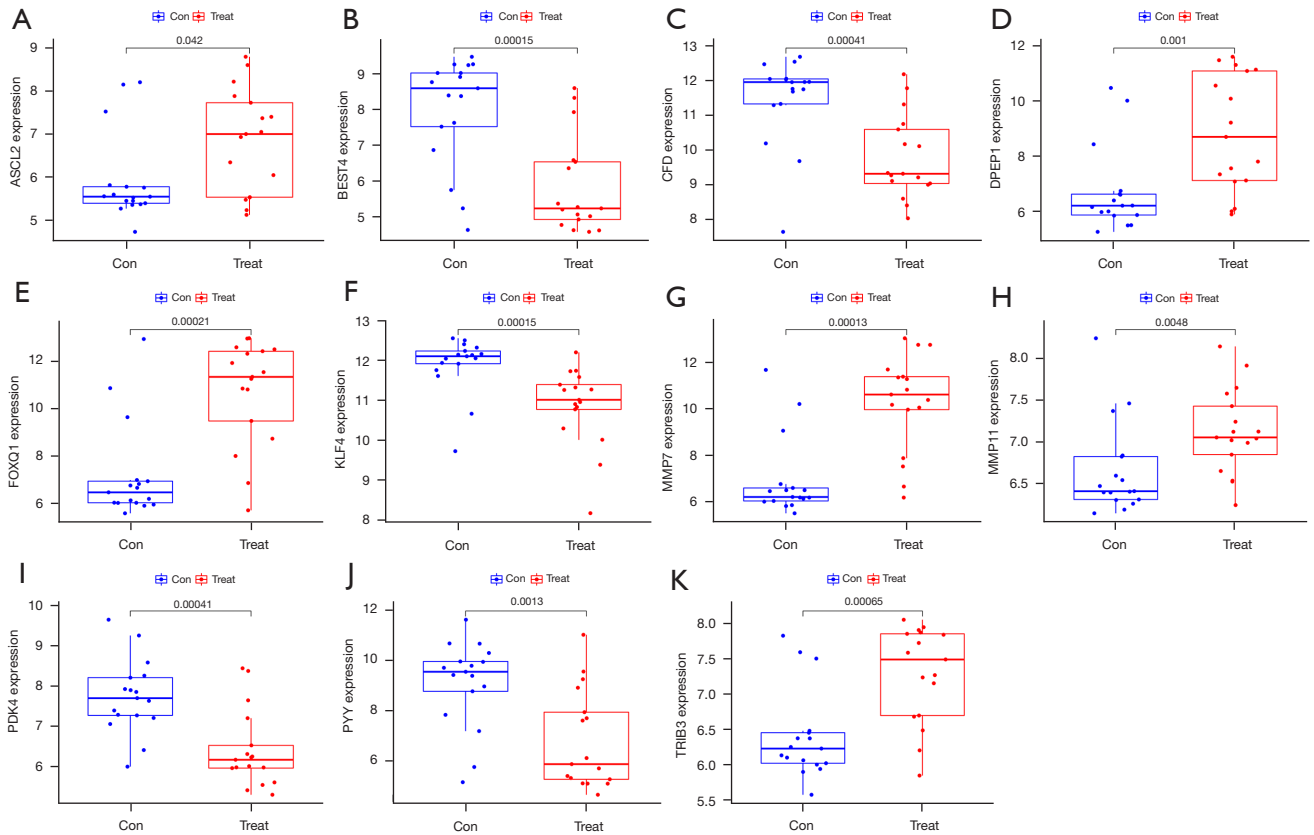


Figure 5 Bar plots showing the expression differences of the 11 key genes in colorectal cancer and normal tissues from the validation set (GSE110255).

genes encoding ion channels that can function as Cl channels, HCO3 channels, or voltage-gated Ca2^{+} channels. *BEST4* is mainly expressed within the human colon (35). *BEST4* expression has been shown to be upregulated in clinical colorectal cancer samples, and its high expression level has been associated with advanced TNM stage, lymph node metastasis, and poor survival, with a potential oncogenic role in colorectal carcinogenesis and metastasis through modulation of *BEST4*/PI3K/Akt signal transduction (36).

Currently, there are few reports about CFD (Complement Factor D). Lipoprotein (Complement Factor D) is an adiponectin, which is mainly secreted by adipocytes. This effect is mediated by C3a, a downstream product of adiponectin, which is produced in the replacement pathway of the Complement system (37). In the literature, *CFD* acts as an enhancing dose for tumor proliferation and cancer stem cell (CSC) properties in breast cancer. The role of *CFD* in colorectal cancer remains to be further explored (38).

Dipeptidase (*DPEPCFD*) 1 is a zinc-dependent metalloprotease underlying glutathione and leukotriene metabolism. In colorectal cancer samples, *DPEPCFD* 1 expression was significantly increased in tumor tissue samples, and an elevated *DPEPCFD*1 mRNA expression was associated with positive lymph node metastasis (39). Additionally, *DPEPCFD*1 was demonstrated to promote the proliferation of colon cancer cells *in vitro* and *in vivo* through a *DPEPCFD1/MYC* positive feedback loop (40). The enrichment in the ECM-receptor interaction pathway found by GSEA in this study was similar to that reported in the literature. *DPEPCFD*1 has also been shown to play a role in rectal cancer metastasis by inhibiting the leukotriene D4 signaling pathway and increasing E-cadherin expression (41).

FOXQ1 belongs to the FOX transcription factor superfamily, characterized by a conserved binding of 110 amino acids responsible for DNA binding involved in tumor proliferation, apoptosis, migration, and invasion (42). Knockdown of FOXQ induces inhibition of cell

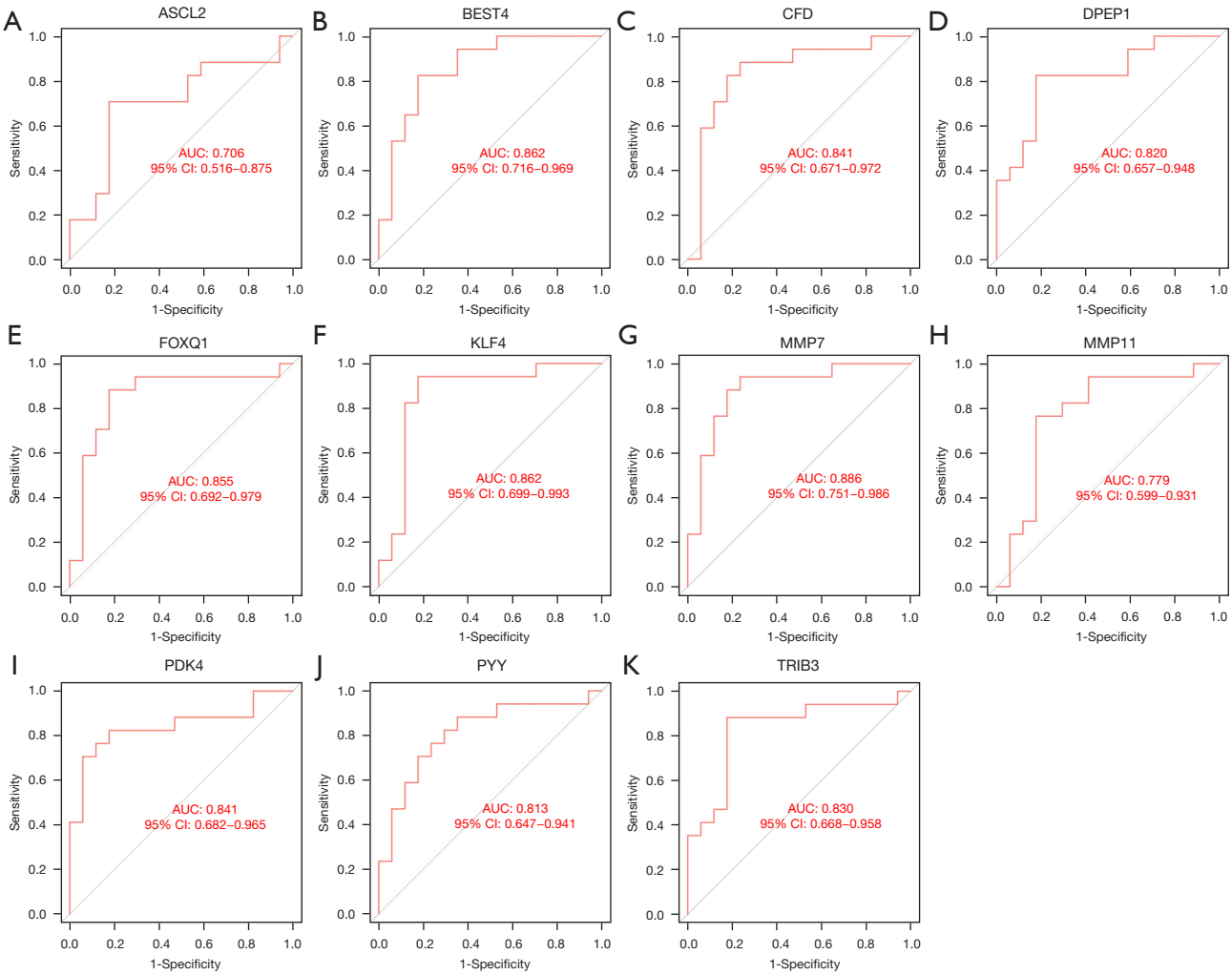
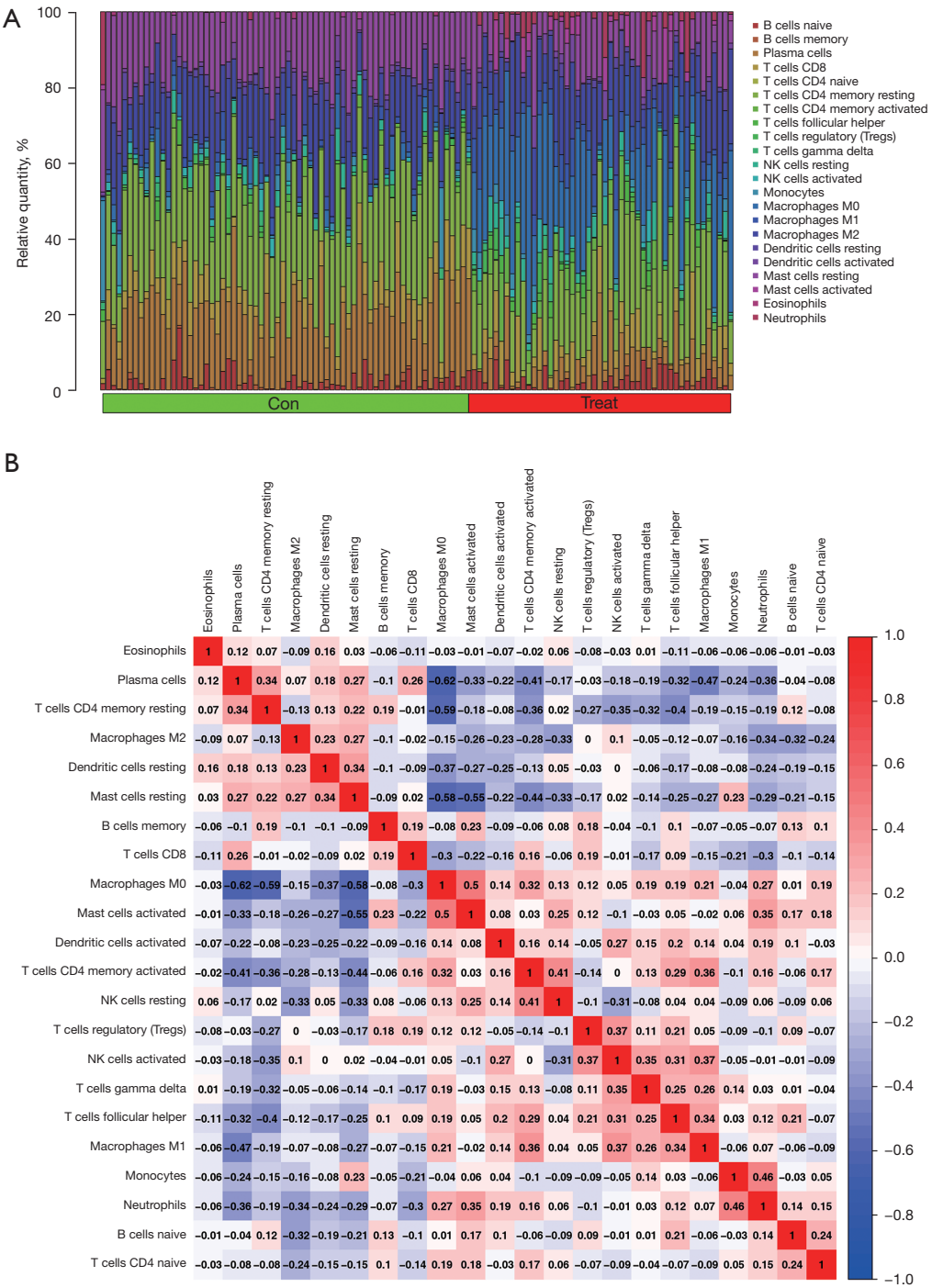


Figure 6 ROC curve of 11 key genes in the validation set (GSE110255) for colorectal cancer diagnosis. ROC, receiver operating characteristic.

proliferation, as well as migration, and invasion of colorectal cancer cells (43).

KLF4 family members are expressed in many cell lineages and play crucial roles in development, metabolism, and multipotency. Their dysregulation is highly involved in the development of human diseases, including cancer, and they play an important role in regulating intestinal epithelial homeostasis (44). *KLF4* promotes tumor development by epigenetic modification, and the increased expression of miR-29a has been shown to promote colorectal cancer metastasis by directly targeting *KLF4* to regulate MMP2/E-cad (45). Furthermore, *KLF4* protein expression has been shown to correlate significantly with colorectal cancer differentiation in clinical specimens by immunohistochemistry, and downregulation of *KLF4* expression may contribute to poor tumor differentiation (46).

Matrix metalloproteinase (MMP) is an enzyme component that degrades extracellular matrix proteins and promotes cancer invasion and metastasis. MMPs have been studied in serum and tissues, and an increased expression of specific MMPs has been associated with poor prognostic parameters (47). Wu *et al.* found that *MMP7* expression was associated with colorectal cancer metastasis and poor prognosis (48). In addition, it was found that *MPC1* mediated *MMP7* activation of the Wnt/β-catenin pathway by promoting β-catenin nuclear translocation after silencing (49). *MMP11* has also been shown to be associated with poor prognosis in gastric cancer (50).



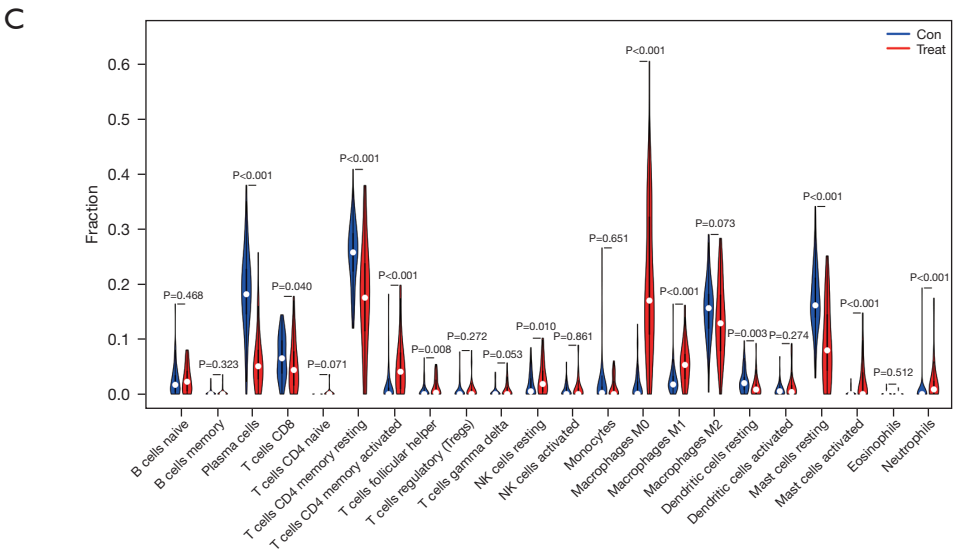


Figure 7 Results of immune cell infiltration analysis. (A) Relative quantity of 22 immune cells in normal tissues and colon cancer tissues; (B) correlation heat map of infiltrating immune cells in colon cancer tissues; (C) differential analysis of the relative quantity of 22 immune cells in normal and colon cancer tissues.

Peptide YY, originally isolated from the pig intestine, is restricted to endocrine cells in the colon. A comprehensive bioinformatics analysis exploring the clinical value of primary CRC biomarkers found that *PYY* was a core gene (51). However, it has been concluded that *PYY* is unlikely to be involved in the development and growth of colorectal cancer. Whether this conclusion remains valid should be confirmed by subsequent experimental validation (52).

Pyruvate Dehydrogenase Kinase (PDK) generates four kinase families in humans. *PDK4* is mainly expressed in muscle and affects glucose consumption during metabolism (53). It has been reported in the literature that when *PDK4* was stably inhibited, colorectal cancer cell migration and invasion were reduced, and apoptosis was increased. *PDK4* also reduced the expression of vimentin, hypoxia-inducible factor-1 (HIF-1), and vascular endothelial growth factor A (VEGFA) (54).

Tribbles pseudokinase 3 (*TRIB3*) contains a substrate-binding domain. However, it lacks the conserved catalytic amino acid motif required for kinase activity (55). Recent studies have shown that *TRIB3* is a crucial oncoprotein associated with many different types of cancer, including hepatocellular carcinoma, colorectal cancer, and gastric cancer (56-58).

To quantify the relative proportion of infiltrating immune cells in colorectal cancer gene expression profiles, immune cell infiltration can be calculated using the bioinformatics algorithm CIBERSORT, which is increasingly used to estimate immune cell infiltration because of its good performance (27). In this study, CIBERSORT was used to investigate the role of immune cell infiltration in colorectal cancer. Our analysis found differences in immune cells between the colorectal cancer and control groups. The differences were found among plasma cells, T cells CD8, T cells CD4 memory, T cells CD4 memory resting, T cells CD4 memory activated, T cells follicular helper, NK cells resting, Macrophages M0, Macrophages M1, Dendritic cells resting, Dendritic cells activated, Mast cells resting, Mast cells activated, and Neutrophils. Additionally, the expression of T cells CD4 activated, T cells follicular helper, NK cells resting, Macrophages M0, Macrophages M1, Mast cells activated, and Neutrophils were higher in the colorectal cancer group. We also studied the relationship between the expression of key genes in colorectal cancer and immune cells to provide new clinical guidance for cancer diagnosis.

Using comprehensive bioinformatics and machine learning algorithms, the genomic landscape of colorectal cancer and its correlation with immune cell infiltration was elucidated in this study. A total of 11 prognosis-associated key genes were found to play pleiotropic roles in the TME of colorectal cancer. These central genes are involved in the formation of the immune microenvironment and could

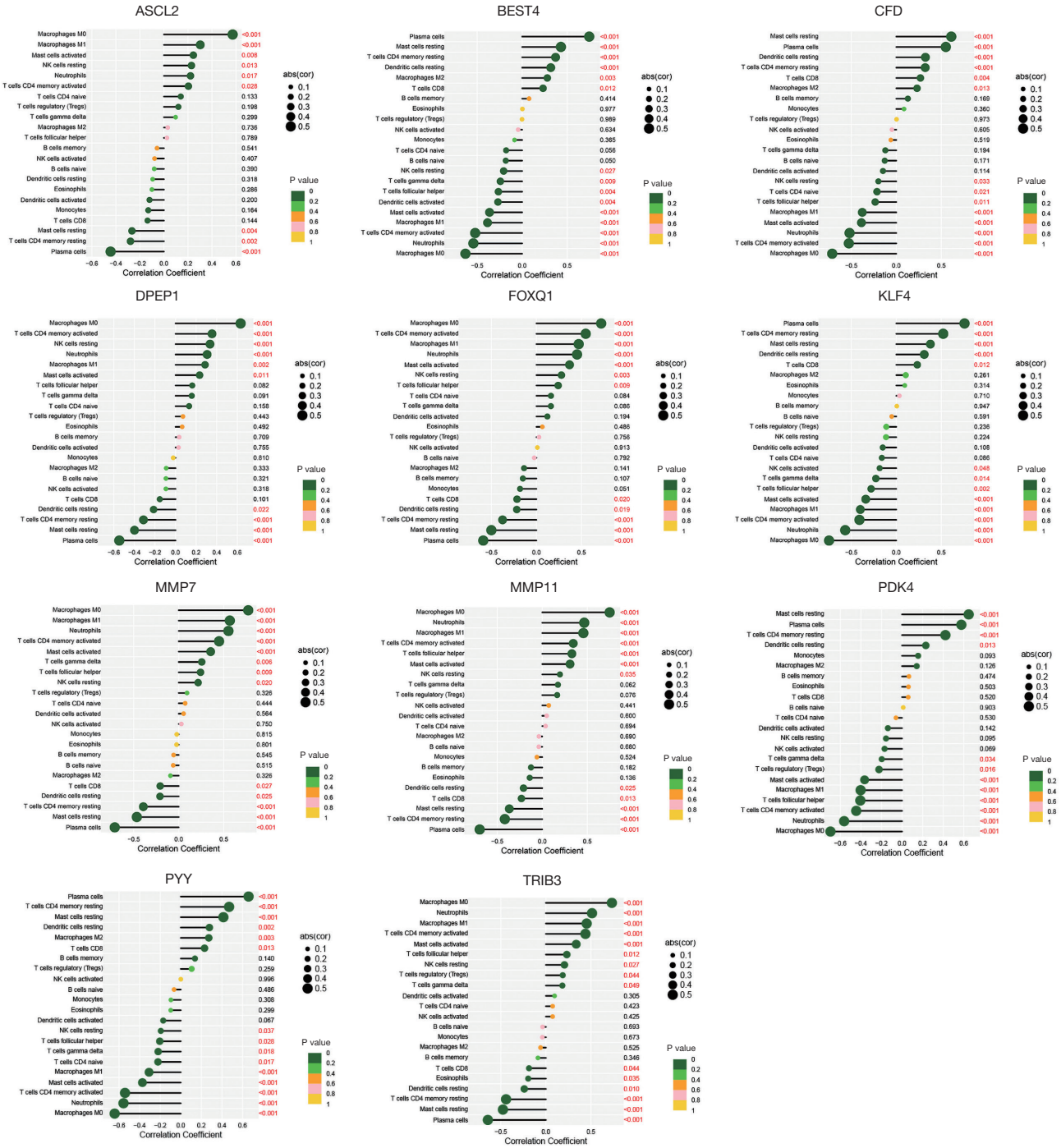


Figure 8 Lollipop chart of the correlation between 11 key genes and immune cells. The left ordinate of the figure indicates immune cells, and the right ordinate indicates the P value of the correlation analysis, where P<0.05 is represented by red, the head size and length of the lollipop indicates the size of the correlation coefficient. The direction of the head on the left side of 0 indicates a positive correlation, whereas the right side of 0 indicates a negative correlation.

represent potential therapeutic targets. Further experiments on the current findings based on retrospective datasets and clinical specimens should be performed to validate our results.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-536/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-536/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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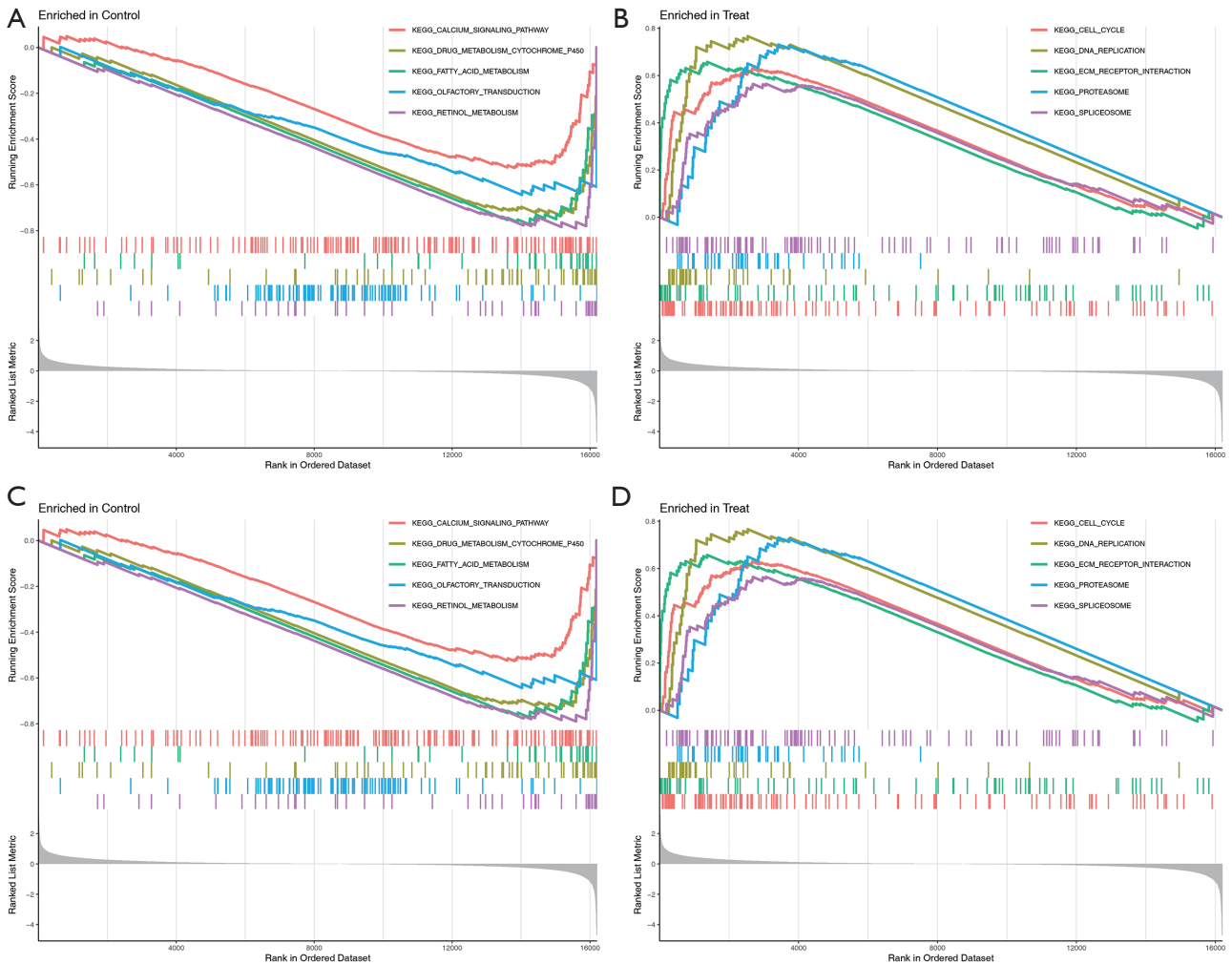

Figure S1 The results of the GSEA analysis for the GO annotation and KEGG pathway analysis. GSEA, Gene Set Enrichment Analysis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

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