Observational Study

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# CD3D and CD247 are the molecular targets of septic shock

Qian Yang, MD[a], Zhijuan Feng, MD[b], Danyang Ding, MD[c], Chunbo Kang, MD[c,*]

**Abstract**

Septic shock is a serious systemic disease with circulatory failure and abnormal cell metabolism caused by sepsis. However, the relationship between CD3D and CD247 and septic shock remains unclear. The septic shock datasets GSE33118 and GSE142255 profiles were generated from the gene expression omnibus databases GPl570, GPl17586. Differentially expressed genes (DEGs) were screened and weighted gene co-expression network analysis was performed. The construction and analysis of protein–protein interaction (PPI) network, functional enrichment analysis, gene set enrichment analysis (GSEA) were performed. Gene expression heat map was drawn. Immune infiltration analysis was performed. Comparative toxicogenomics database (CTD) analysis were performed to find the disease most related to the core gene. Targets can was used to screen miRNAs regulating the hub DEGs. 467 DEGs were identified. According to the gene ontology analysis, they were mainly enriched in the regulation of immune response, cell activation, signaling receptor activity, enzyme binding. Kyoto encyclopedia of genes and genomes analysis showed that they were mainly enriched in the TCR signaling pathway, Fc epsilon RI signaling pathway. GSEA showed that the DEGs were mainly enriched in immune response regulation, cell activation, TCR signaling pathway, Fc epsilon RI signaling pathway. Positive regulation of Fc receptor signaling pathway, PID IL12 2 pathway, immune response was observed in go enrichment items in the enrichment items of metascape. PPI networks got 5 core genes. Gene expression heat map showed that 5 core genes (CD247, Lck, cd3e, cd3d, ITK) were lowly expressed in the sepsis shock samples and highly expressed in the normal samples. CTD analysis showed that 5 core genes (CD247, Lck, cd3e, cd3d, ITK) were found to be associated with hemorrhage and necrosis. Low expression of cd3d, CD247 was observed in septic shock, and the lower the level of cd3d, CD247, the worse the prognosis.

**Abbreviations:** CTD = comparative toxicogenomics database, DEGs = differentially expressed genes, GO = gene ontology, GSEA = gene set enrichment analysis, IL = interleukin, KEGG = Kyoto encyclopedia of genes and genomes, PPI = protein–protein interaction, TCR = T cell receptor, TOM = topological overlap matrix, WGCNA = weighted gene co-expression network analysis.

**Keywords:** bioinformatics, CD247, CD3D, prognosis, septic shock

## 1. Introduction

Septic shock is a severe systemic inflammatory response syndrome.[1] The prevalence increases with age, and the elderly and immunocompromised patients are more likely to develop septic shock.[2] Mortality increases with the degree of multiorgan failure.[3] The pathogen may be any bacterium, fungus, virus, or parasite. Pathogen infection causes activation of the immune system, releasing a large number of inflammatory factors and mediators, leading to systemic inflammatory response syndrome and multiple organ dysfunction. Septic shock is characterized by systemic inflammatory response, hypotension, dysfunction of multiple organs, rapid progression and high mortality, and difficulties in treatment.[4,5] Systemic symptoms with rapid progression, the earliest symptoms include fever, chills, fatigue, etc, with consciousness disturbance, rapid heart rate, blood pressure and so on as the disease progresses. Hypotension and shock will appear dizziness, syncope, palpitations, shortness of breath, skin dampness, limbs syncope and other symptoms. Rgan dysfunction may be present. Symptoms of infection include diarrhea, vomiting, skin redness, etc.[6,7] Pathological features include inflammatory response, coagulopathy, tissue hypoxia, and multiple organ failure.[8–10] Septic shock is a severe infectious disease that causes unconsciousness, multiple organ failure, bleeding, infection, and has a high lethality. The cause of septic shock remains unclear, and genetic factors, chromosomal abnormalities, and gene fusions may contribute to the disease. Therefore, it is particularly important to deeply investigate the molecular mechanisms underlying septic shock.

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*The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.*

*The Ethics Committee of Beijing Rehabilitation Hospital, Capital Medical University approved the study.*

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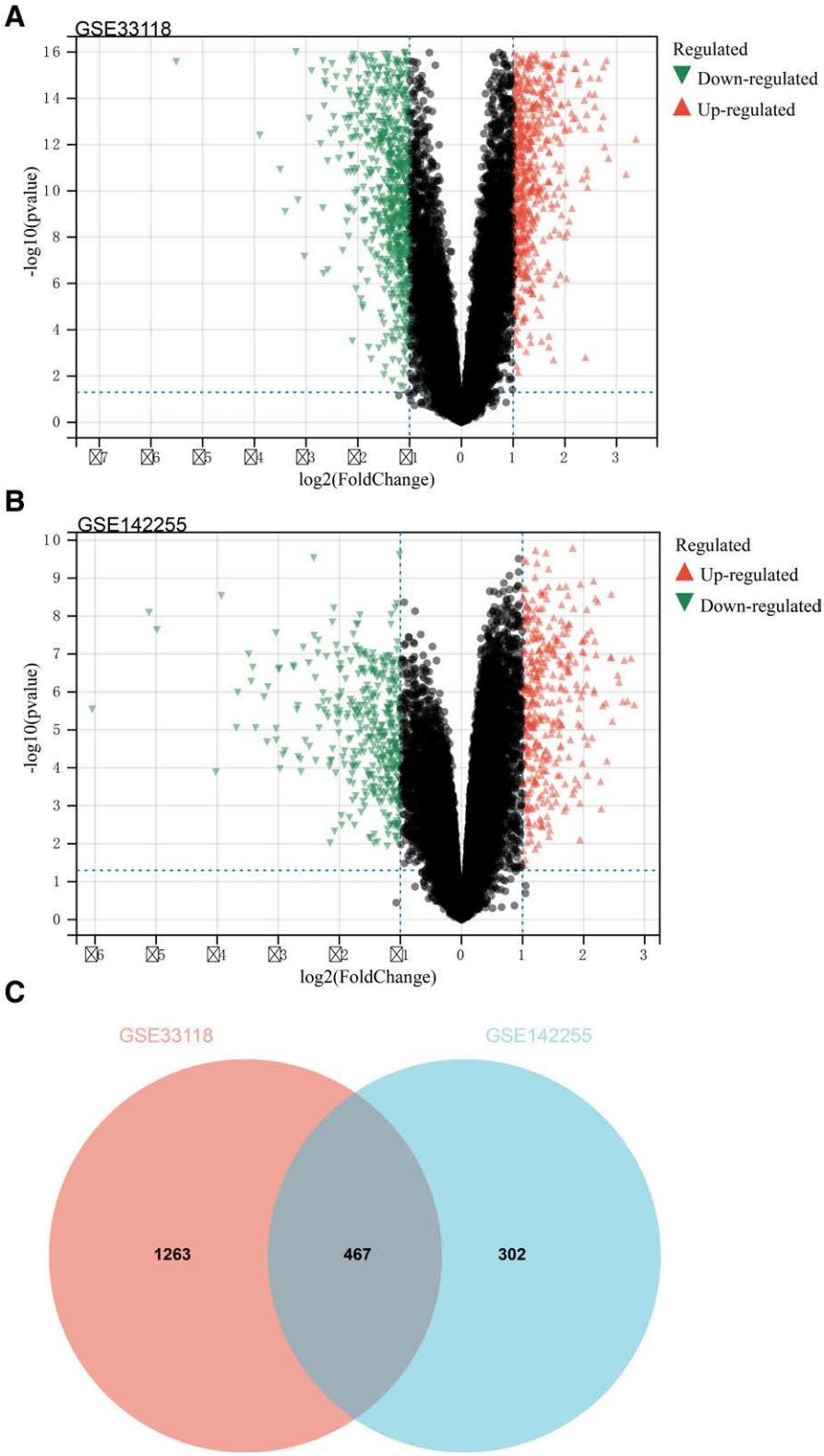


**Figure 1.** Analysis of differentially expressed genes (DEGS). (A) GSE33118 (B) GSE142255 (C) The intersection of DEGs in the 2 data sets was taken by Venn diagram, and 467 DEGs were obtained.

Bioinformatics is an interdisciplinary field that involves computer science, mathematics, biology, and statistics. The development of bioinformatics technology has greatly assisted biological research, accelerating the interpretation and understanding of biomolecules such as genomes, proteins, and metabolomes. Bioinformatics technology includes sequence analysis, structure analysis, functional prediction, systems biology, genomics, and proteomics. With the development of high-throughput sequencing technology and the reduction of costs, a large amount of biological information is stored

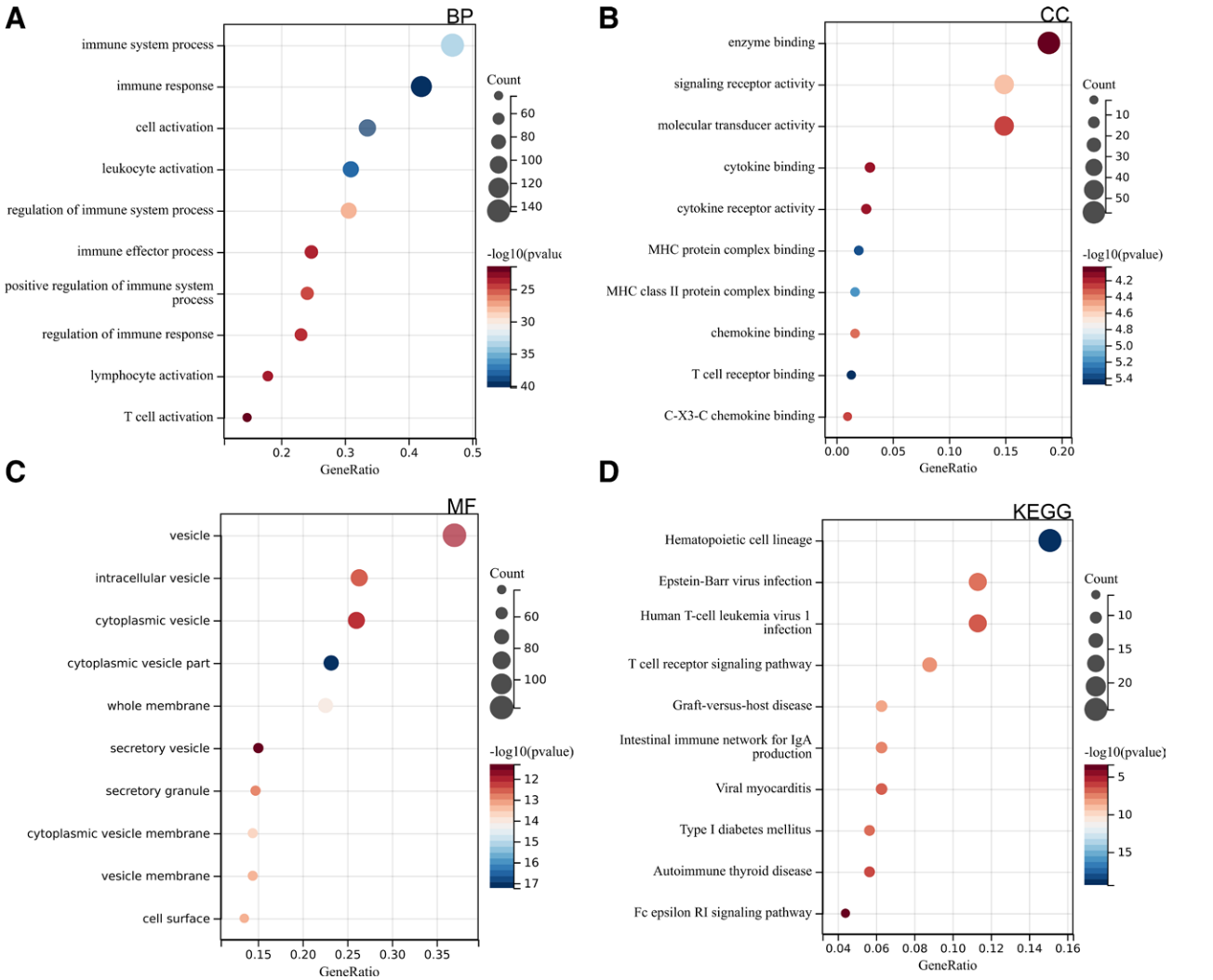


**Figure 2.** Functional enrichment analysis. (A) BP, (B) CC, (C) MF, (D) Kyoto encyclopedia of genes and genomes (KEGG).

in public databases. Bioinformatics technology is constantly evolving, allowing for more efficient and accurate interpretation of biological information. The advantages of bioinformatics technology are mainly reflected in its efficiency, accuracy, visualization, and reproducibility.

However, the relationship between cd3d, CD247 and septic shock is still unclear. Therefore, in this paper, we aimed to use bioinformatics techniques to mine the core genes between septic shock and normal tissues, as well as perform enrichment analysis, pathway analysis. Public datasets were used to validate the significant roles of cd3d, CD247 in septic shock. And the basal cell experiment was applied to verify it.

## 2. Method

### *2.1. Septic shock dataset*

In this study, the septic shock datasets GSE33118 and GSE142255 profiles were generated from the gene expression omnibus databases GPl570, GPl17586 (http://www.ncbi.nlm.nih.gov/geo/) downloaded from. GSE33118 included 20 septic shock and 42 normal blood samples, and GSE142255 included 8 septic shock and 7 normal blood samples. Differentially expressed genes (DEGs) for identifying septic shock.

### *2.2. Screening of DEGs*

We first log 2 transformed the GSE33118 and GSE142255 datasets, respectively, performed multiple linear regression using the lmfit function, and finally obtained the significance of difference for each gene by calculating the adjusted t-statistic, adjusted F-statistic, and log ratio of differential expression based on empirical Bayes adjustment with standard error approaching a common value. After that, the differential genes of GSE33118 and GSE142255 were intersected to obtain DEGs.

### *2.3. Weighted gene co expression network analysis (WGCNA)*

First, we calculated the median absolute deviation for each gene by using the gene expression matrix from GSE33118, excluding the top 50% of genes with the minimum mad, removing outlier genes and samples by using the good samples genes method from the R package WGCNA, and further used WGCNA to construct a scale free co expression network. Specifically, first, a Pearson correlation matrix and average linkage method were performed for all pairs of genes, and then, Using the power function a_ mn = |C_ mn|^ β A weighted adjacency matrix was constructed (C ð u Mn = Pearson correlation between gene Þ m and gene Þ n; a Þ u Mn = adjacency

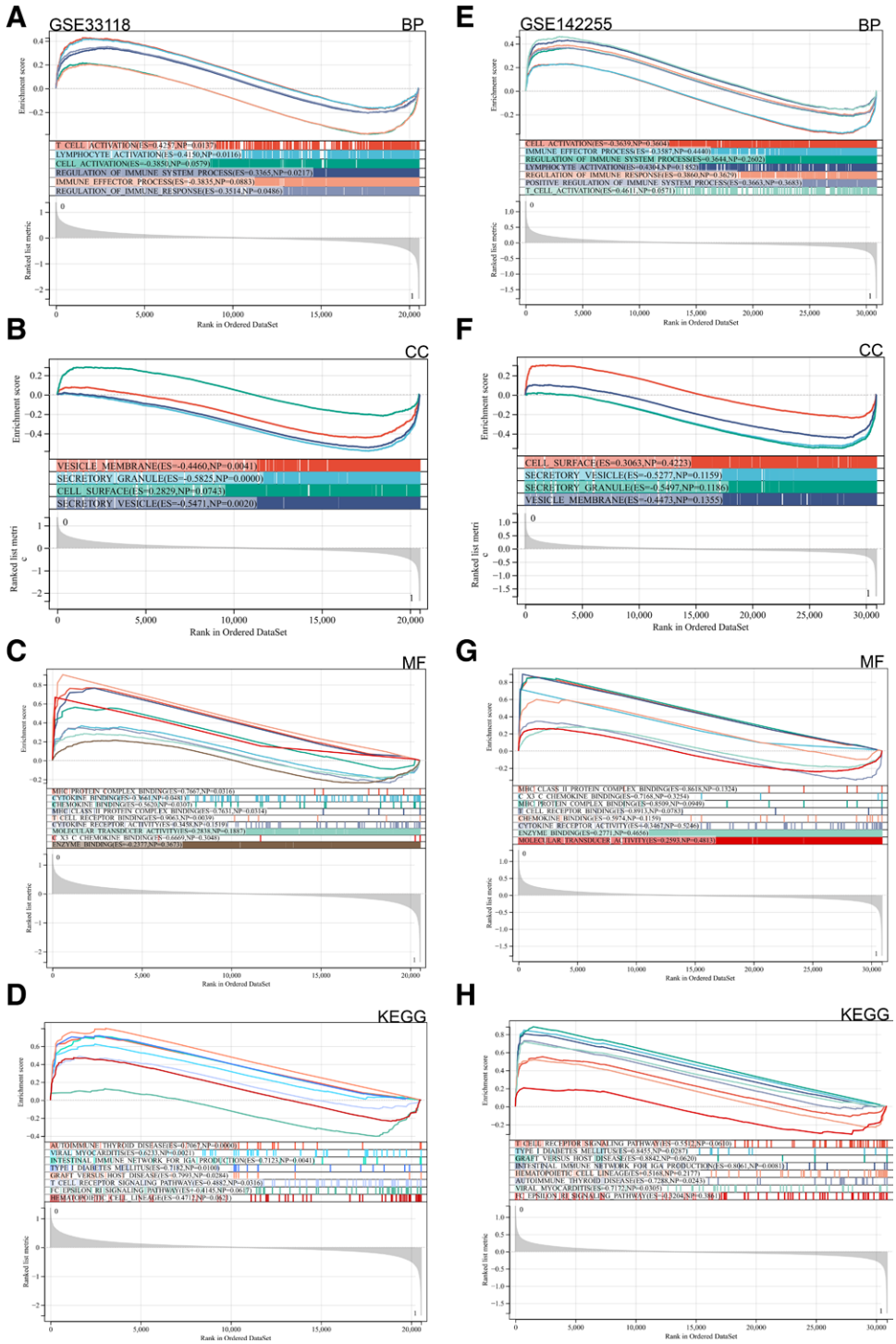


**Figure 3.** Gene set enrichment analysis (GSEA). (A–D) GSE33118, (E–H) GSE142255.

between gene m and gene N). β is a soft threshold parameter that can emphasize strong correlations among genes and attenuate the effects of weak and negative correlations. After selecting a power of 10, the adjacency was converted to a topological overlap matrix (TOM) that can measure the network connectivity of 1 gene, defined as the sum of its adjacency to all other genes, for the network gene ratio, and calculate the corresponding dissimilarity (1-TOM). To classify genes with similar expression profiles into gene modules, average linkage hierarchical clustering was performed according to a TOM based dissimilarity metric with a minimum size (gene set) of 30 for the gene dendrogram. Set the sensitivity as: 3. To further analyze the modules, we calculated the dissimilarity of module eigengenes, selected a cut line for the module dendrogram, and merged some modules. In addition, we incorporated modules with a distance of <0.25, and it is noteworthy that the gray module was considered as the set of genes that could not be assigned to any module.

### *2.4. Construction and analysis of protein-protein interaction (PPI) networks*

String database (http://string-db.org/) is designed to collect, score, and integrate all publicly available sources of

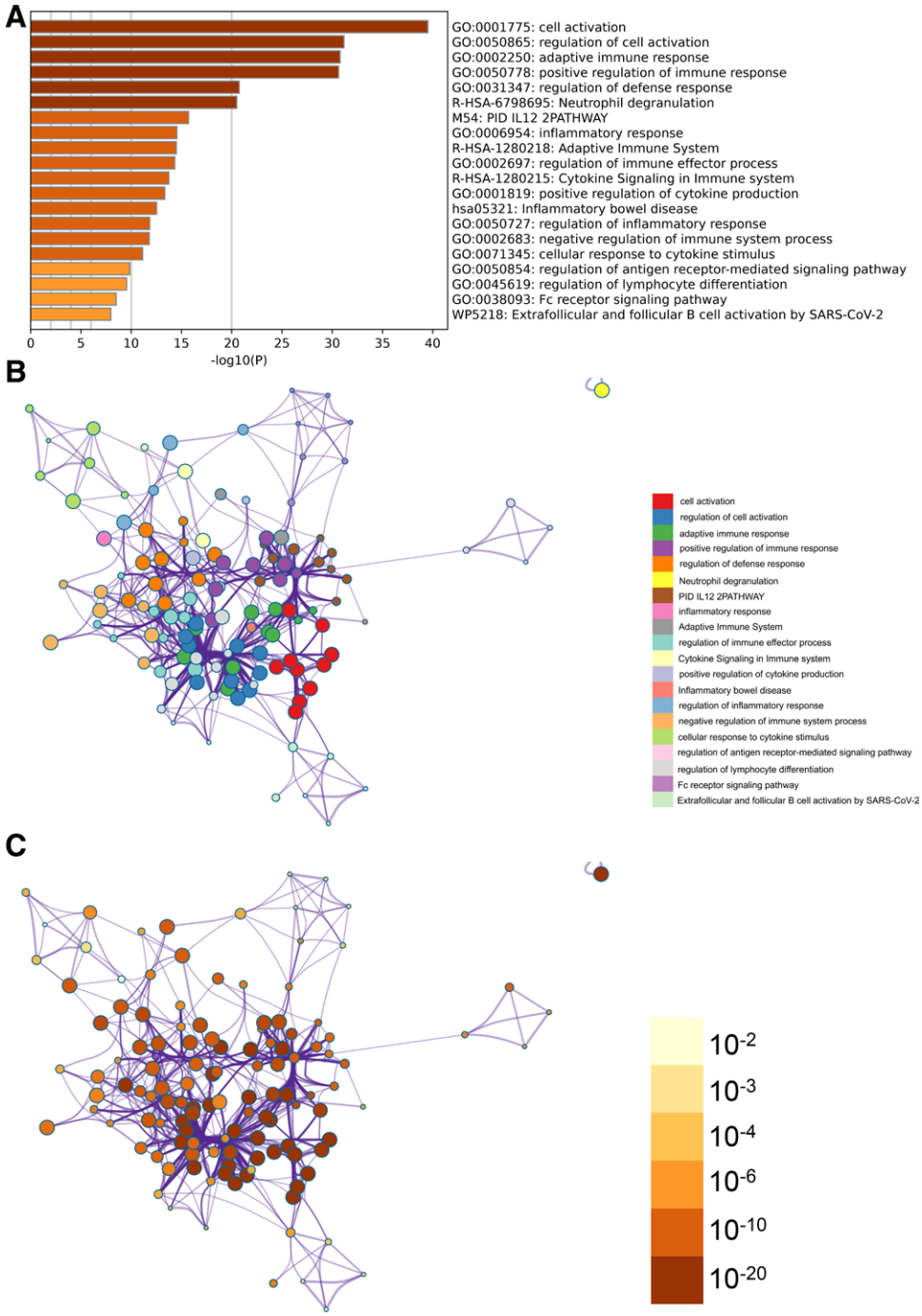


**Figure 4.** Metascape enrichment analysis. (A) Positive regulation of Fc receptor signaling pathway, PID IL12 2 pathway, immune response was observed in go enrichment items in the enrichment items of metascape. (B) Output the enrichment network colored by enrichment terms. (C) Output the enrichment network colored by *P* value.

protein-protein interaction information and complement these with computational predictions. In this study, the differential gene list was entered into the string database to construct a PPI network of predicted core genes (confidence level > 0.4). Cytoscape software can provide biologists with biological network analysis and 2-dimensional (2D) visualization. In this study, the PPI network formed by string database was visualized and predicted core genes by Cytoscape software. First, we imported the PPI network into Cytoscape software to calculate the best correlated ten genes by 3 algorithms, and after visualization, we derived a core gene list by taking the intersection of the Venn diagrams.

### *2.5. Functional enrichment analysis*

Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) analysis are computational methods for evaluating gene functions and biological pathways. The list of differential genes screened by the Venn diagram was entered into the KEGG rest API (https://www.kegg.jp/kegg/rest/keggapi.html). The latest KEGG pathway gene annotations were acquired to map genes into the background set for enrichment analysis using the R package cluster profiler (version 3.14.3) to obtain the results of gene set enrichment. GO annotations of genes from the R package org.hs.e.g.db (version 3.1.0) were also used to map genes into the background set as background, setting a minimum

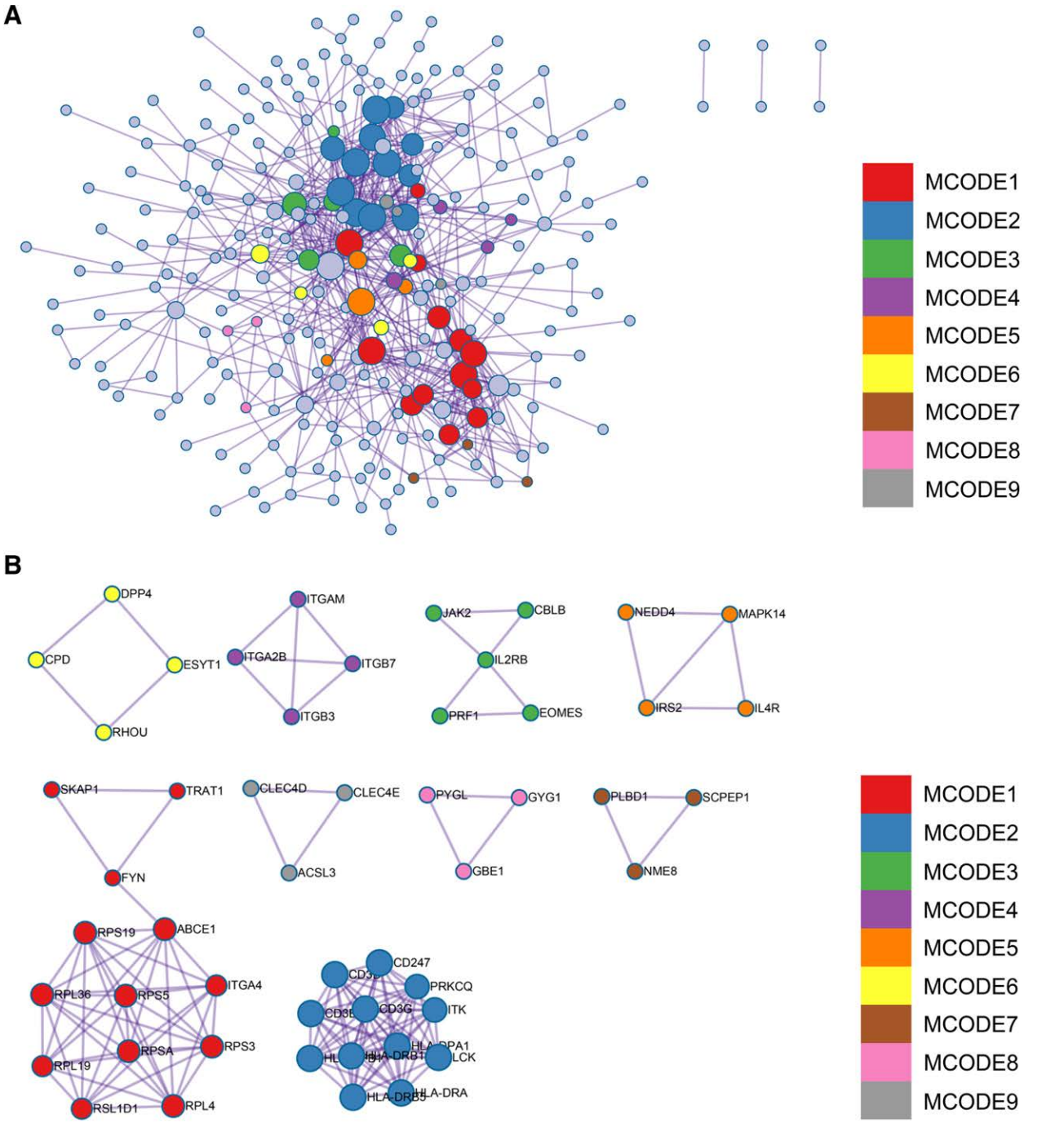


**Figure 5.** Metascape enrichment analysis. Visualize the association and confidence of each enrichment item.

gene set of 5, a maximum gene set of 5000, *P* value of <.05 and a FDR of <.25 were considered statistically significant measures.

In addition, the metascape database can provide a comprehensive resource for gene list annotation and analysis and visualize exports. We used metascape (http://metascape.org/gp/index.html) database, performed functional enrichment analysis of the differential gene list described above, and exported.

### *2.6. GSEA*

For the gene set enrichment analysis (GSEA), we selected candidate genes from the GSEA (DOI: 10.1073/pnas.0506580102, http://software.broadinstitute.org/gsea/index.jsp) website, GSEA software (version 3.0) was obtained. The samples were divided into 2 groups on the basis of septic shock and normal blood, and the molecular signatures database (DOI: 10.1093/bioinformatics/btr260, http://www.gsea-msigdb.org/gsea/downloads.jsp). The c2.cp.kegg.v7.4.symbols.gmt subset was downloaded to evaluate relevant pathways and molecular mechanisms based on gene expression profiles and phenotypic grouping, setting a minimum gene set of 5, a maximum gene set of 5000, 1000 resampling, *P* value of <.05 and a FDR of <.25 were considered statistically significant. GO and KEGG analyses were performed genome-wide. Formulated by GSEA.

### *2.7. Gene expression heatmap*

We used the R package heatmap to visualize the expression differences of core genes between septic shock and normal blood

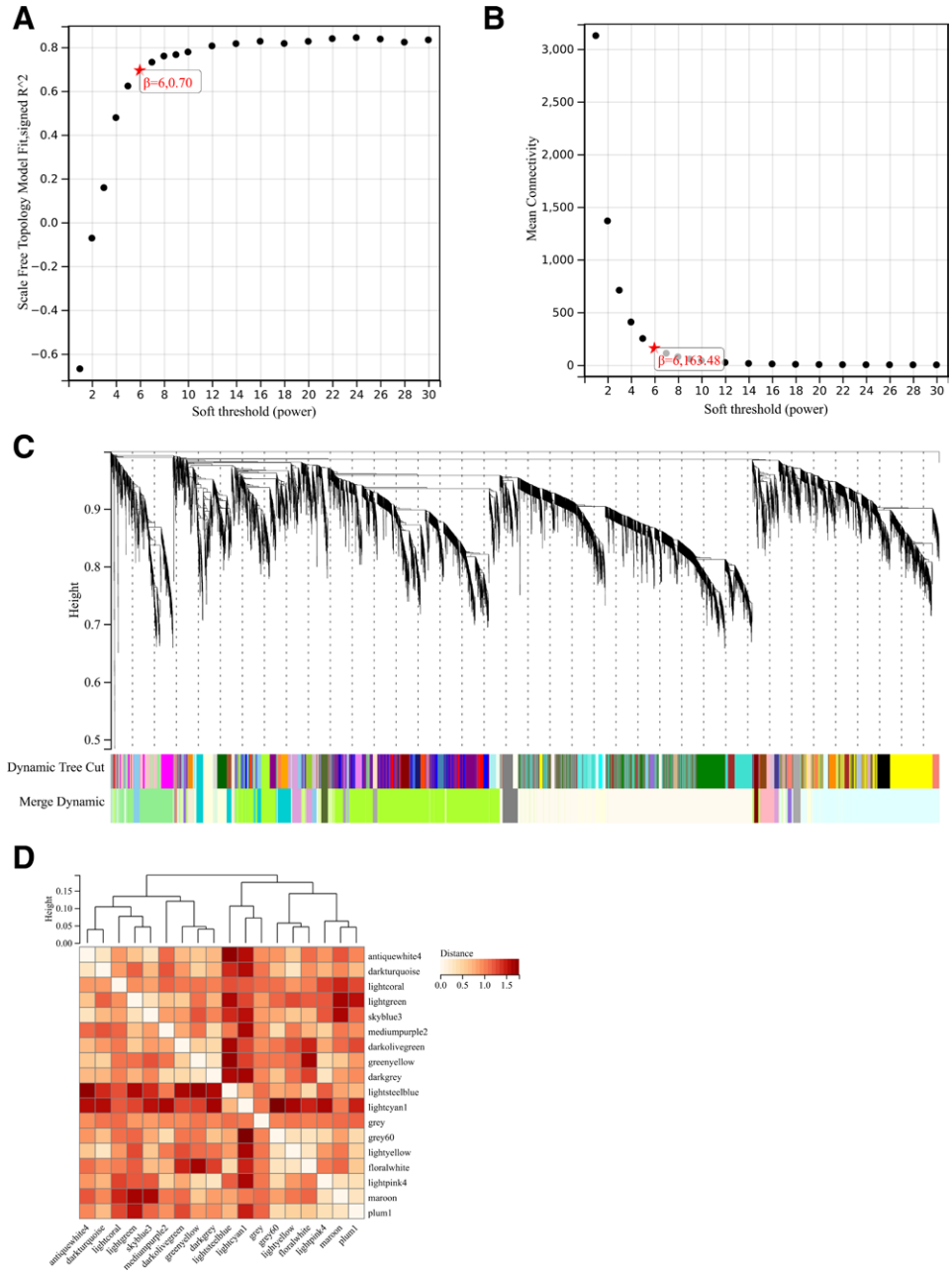


**Figure 6.** Weighted gene co-expression network analysis (WGCNA). (A) β = 6, 0.70. (B) β = 6, 163.48. (C) A hierarchical clustering tree of all genes was constructed, and 18 significant modules were generated. (D) The interactions between these modules were analyzed.

samples by plotting the expression amounts of core genes in the gene expression matrix of GSE33118 and GSE142255, respectively, which were found by the 3 algorithms in the PPI network. Heatmap showing the expression of core genes in dataset GSE33118 and dataset GSE142255.

### *2.8. Immune infiltration analysis*

CIBERSORT (http://CIBERSORT.stanford.edu/) is a very commonly used method to calculate immune cell infiltration, and the lm22 gene file was used to define 22 immune cell subsets. We applied an integrated bioinformatics approach to analyze the gene expression matrix of GSE33118 and GSE142255 using the cibersort package, respectively, and deconvoluted the expression matrix of immune cell subtypes using the principle of linear support vector regression to estimate the abundance of immune cells, while using confidence $P <$ .05 as a cutoff criterion to select samples with sufficient confidence.

### *2.9. CTD analysis*

The CTD (comparative toxicogenomics database) integrates data on the interaction between chemicals, genes, functional phenotypes, and diseases, which can greatly facilitate the study of disease-related environmental exposure factors and potential mechanisms of action of drugs. We entered the core genes into the CTD website to find the most relevant diseases to the core genes and drew an expression difference radar plot for each gene with Excel.

### *2.10. miRNA*

Targetscan (https://www.targetscan.org/) is an online database for prediction analysis of miRNAs and target genes. In our study, targetscan was used to screen miRNAs regulating the hub DEGs.

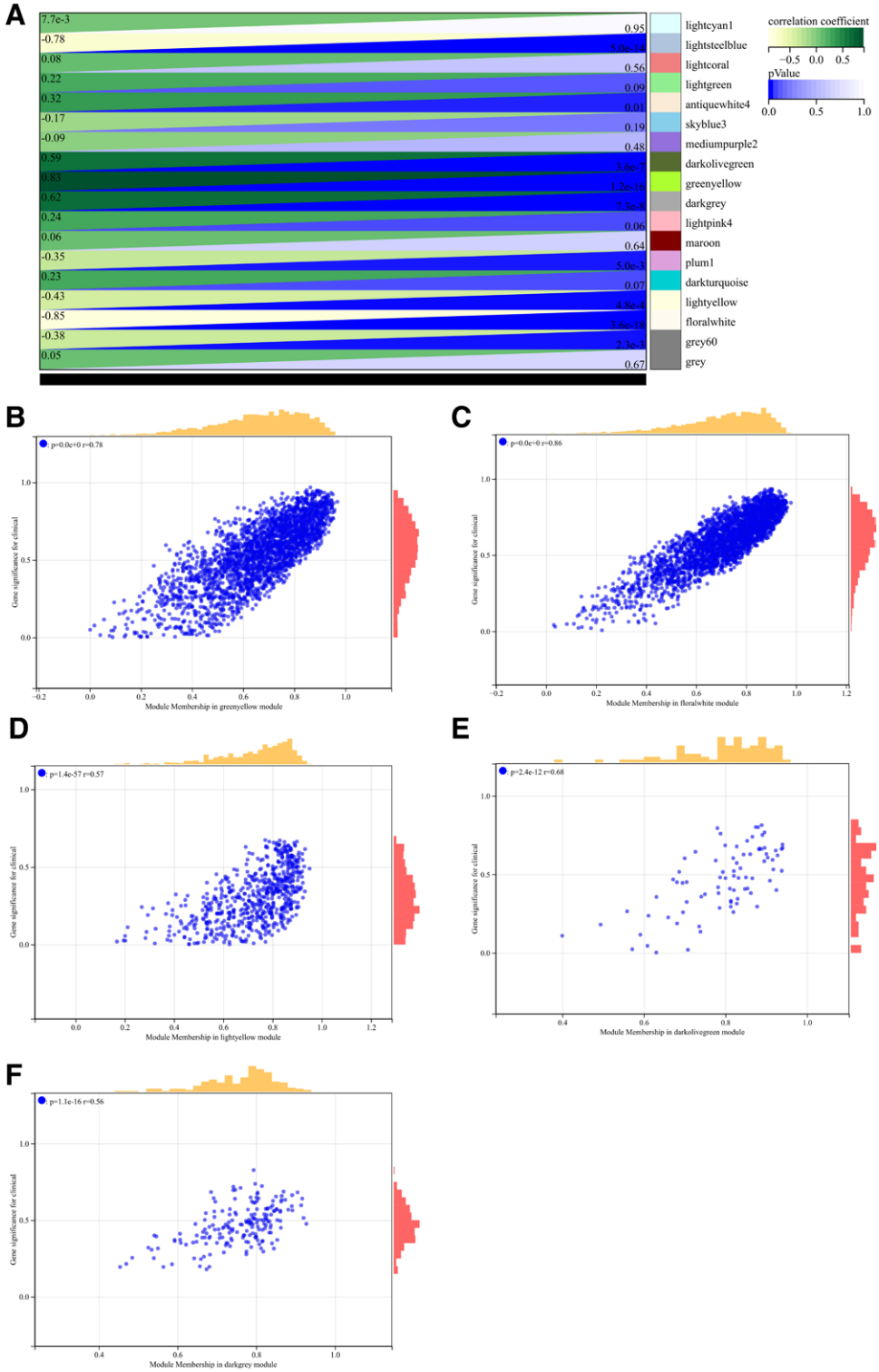


**Figure 7.** Weighted gene co-expression network analysis (WGCNA). (A) Generated module to phenotype correlation heatmaps. (B–F) GS to MM correlation scatter plots for the associated hub genes.

## 3. Result

### *3.1. Analysis of differentially expressed genes*

In this study, following the set cutoff values, we identified DEGs with gene expression matrices for GSE33118 (Fig. 1A) and GSE142255 (Fig. 1B), respectively, and then intersected the DEGs of the 2 datasets using a Venn diagram, finally resulting in a total of 467 DEGs (Fig. 1C).

### *3.2. Functional enrichment analysis*

***3.2.1. DEGs functional enrichment analysis.*** We then performed GO and KEGG analysis on these DEGs, and according to GO analysis, they were mainly enriched in the regulation of immune response, cell activation, signaling receptor activity, enzyme binding (Fig. 2A–C), and KEGG analysis showed that they were mainly enriched in the T cell

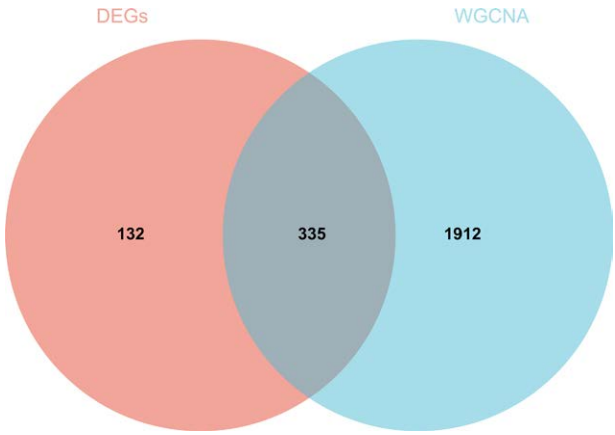


**Figure 8.** WGCNA. Plotted and intersected the Venn diagram with the differential genes screened by WGCNA with differentially expressed genes (DEGs). WGCNA = weighted gene co-expression network analysis.

receptor (TCR) signaling pathway, Fc epsilon RI signaling pathway (Fig. 2D).

### 3.3. GSEA analysis

In addition, we performed GSEA enrichment analysis genome-wide, aiming to find possible enrichment terms in non DEGs and validate the results for DEGs. Intersection of enriched terms with GO KEGG enriched terms of DEGs as shown in, the enrichment results of dataset GSE33118 (Fig. 3A–D) with those of GSE142255 (Fig. 3E–H) showed that the DEGs were mainly enriched in immune response regulation, cell activation, TCE signaling pathway, Fc epsilon RI signaling pathway.

### 3.4. Metascape enrichment analysis

Positive regulation of Fc receptor signaling pathway, PID IL12 2 pathway, immune response was observed in go enrichment items in the enrichment items of metascape (Fig. 4A), meanwhile we also output the enrichment network colored by enrichment terms and *P* value to visualize the association and confidence of each enrichment item (Figs. 4B, C and 5).

### 3.5. WGCNA

The choice of soft threshold power is an important step in WGCNA analysis. Network topology analysis was performed to determine soft threshold power. The soft threshold power in the WGCNA analysis was set to 9, which was the lowest power for a scale-free topology fit index of 0.9 (Fig. 6A and B). A hierarchical clustering tree of all genes was constructed, and 18 significant modules were generated (Fig. 6C). The interactions between these modules were then analyzed (Fig. 6D). And generated module to phenotype correlation heatmaps (Fig. 7A) and GS to MM correlation scatter plots for the associated hub genes (Fig. 7B–F).

We also plotted and intersected the Venn diagram with the differential genes screened by WGCNA with DEGs (Fig. 8).

### 3.6. Construction and analysis of protein-protein interaction (PPI) networks

We selected the differential genes enriched on the most relevant enriched items from the enrichment analysis to be input into the string online database as a differential gene list to construct a PPI network, which was analyzed by Cytoscape software (Fig. 9A). Core gene clusters were obtained (Fig. 9B), then different algorithms were used to identify the hub genes and make a Venn diagram and obtain the union as the core genes (Fig. 9F), MCC, MNC, degree algorithm was adopted to identify the core genes (Fig. 9C–E), finally we got 5 core genes (CD247, Lck, cd3e, cd3d, ITK).

### 3.7. Gene expression heatmap

We visualized and made heat maps to visualize the expression of the core genes in the sepsis shock datasets GSE33118 (Fig. 10A) and GSE142255 (Fig. 10B), and we found that 5 core genes (CD247, Lck, cd3e, cd3d, ITK) were lowly expressed in the sepsis shock samples and highly expressed in the normal samples, which led to the speculation that they may have inverse regulatory effects on sepsis shock.

### 3.8. Immune infiltration analysis

We used the cibersort package to analyze the gene expression matrix of GSE33118 and GSE142255, respectively, with GSE33118 results in Figure 11 and GSE142255 results in Figure 12.

At 95% confidence, the proportion results of immune cells of the full gene expression matrix (Figs. 11A and 12A) and the immune cell expression heatmap in the dataset (Figs. 11B and 12B) were obtained, and the correlation analysis was also performed on infiltrating immune cells, resulting in a plot of co expression patterns among immune cell components (Figs. 11C and 12C).

### 3.9. CTD analysis

In this study, we input the hub gene list into the CTD website to search for diseases associated with core genes, improving the understanding of gene disease association. Five core genes (CD247, Lck, cd3e, cd3d, ITK) were found to be associated with hemorrhage and necrosis (Fig. 13).

### 3.10. Prediction and functional annotation of miRNAs associated with hub genes

In this study, we input the hub gene list into targetscan to search for relevant miRNAs, improving the understanding of gene expression regulation (Table 1). We found that the related miRNAs of CD247 gene were hsa-mir-330-5p, hsa-mir-326; The related miRNAs of Lck genes are hsa-mir-325-3p; The related miRNAs of cd3d gene are hsa-mir-7153-5p, hsa-mir-146a-5p, hsa-mir-146b-5p; The related miRNAs of ITK gene are hsa-mir-155-5p

## 4. Discussion

Septic shock is a severe infectious disease that causes multiple organ failure, shock, impaired consciousness, bleeding, infectious risk, poor prognosis, and is highly lethal and harmful. Septic shock is a systemic inflammatory response syndrome due to severe infection, in which invasion of infectious material causes activation of the immune system and tumor necrosis factor release from immune cells- α (tumor necrosis factor- α). Inflammatory mediators such as interleukin-1 β (IL-1 β) and (IL-6), which will further activate immune cells and trigger more inflammatory responses.[11–13] The release of inflammatory mediators will activate immune cells such as macrophages and neutrophils, which will aggregate to the site of infection and the systemic circulatory system to release more inflammatory mediators and oxygen free radicals, etc, leading to tissue damage and organ dysfunction.[14,15] Septic shock causes dysregulation of the immune system, including impaired T and B cell function, altered activity of macrophages, and aberrant activation of the complement system, which all contribute to decreased immune

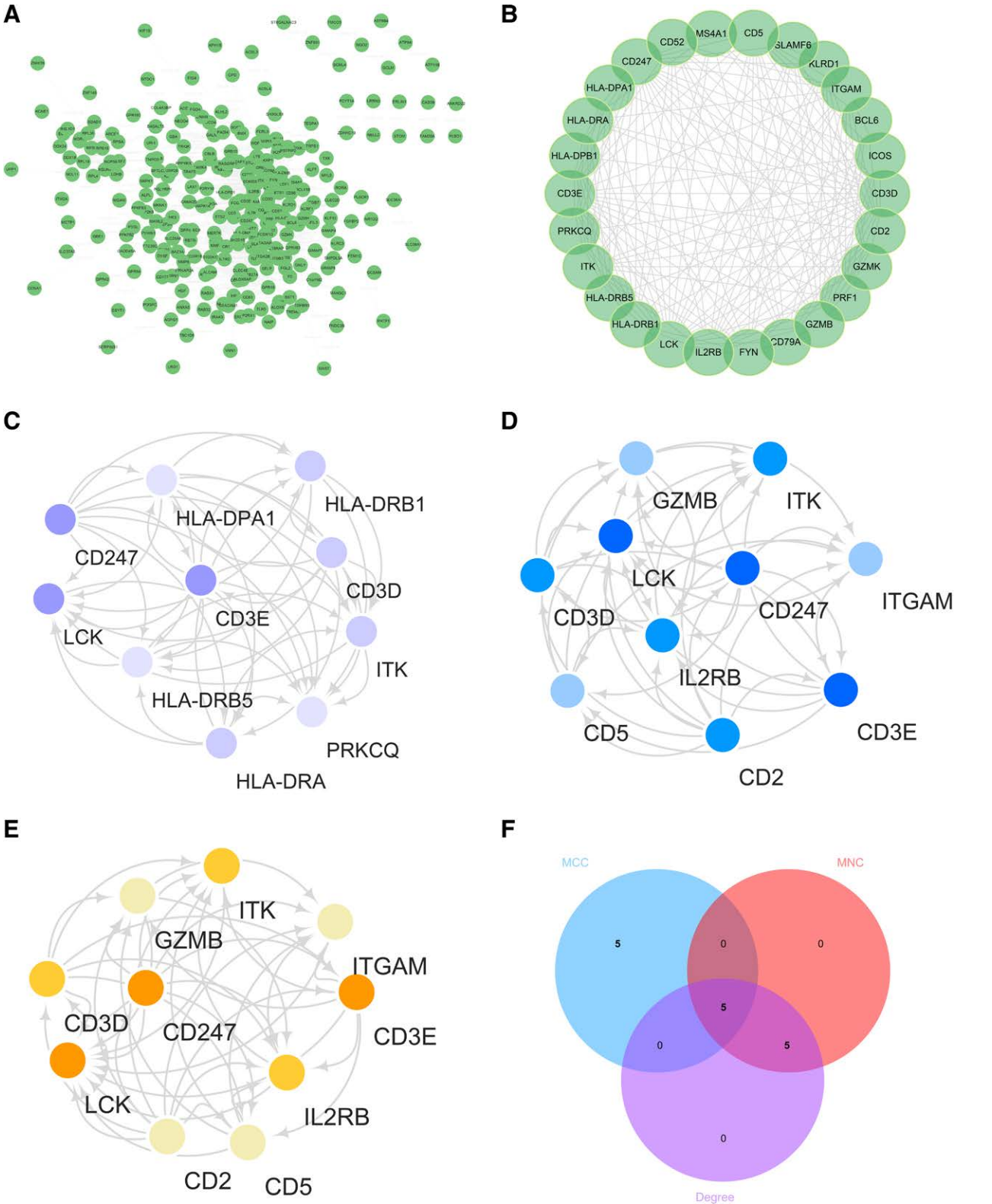


**Figure 9.** Construction and analysis of protein-protein interaction (PPI) networks. (A) The PPI network. (B) Core gene clusters were obtained. (C) MCC algorithm was adopted to identify the core genes. (D) MNC algorithm was adopted to identify the core genes. (E) Degree algorithm was adopted to identify the core genes. (F) Venn diagram and obtain the union as the core genes.

function and exacerbated inflammatory responses.[16–18] Many bacterial infections produce endotoxins, such as lipopolysaccharide, which activate immune cells, causing activation of the immune system and aggravation of inflammatory responses.[19] Septic shock causes dysfunction of vascular endothelial cells, including altered expression of cell adhesion molecules, altered function of vascular tone regulation, and so on, which can affect blood circulation and tissue perfusion and aggravate inflammatory responses and organ damage. Anti-inflammatory drugs such as glucocorticoids and NSAIDs can inhibit the

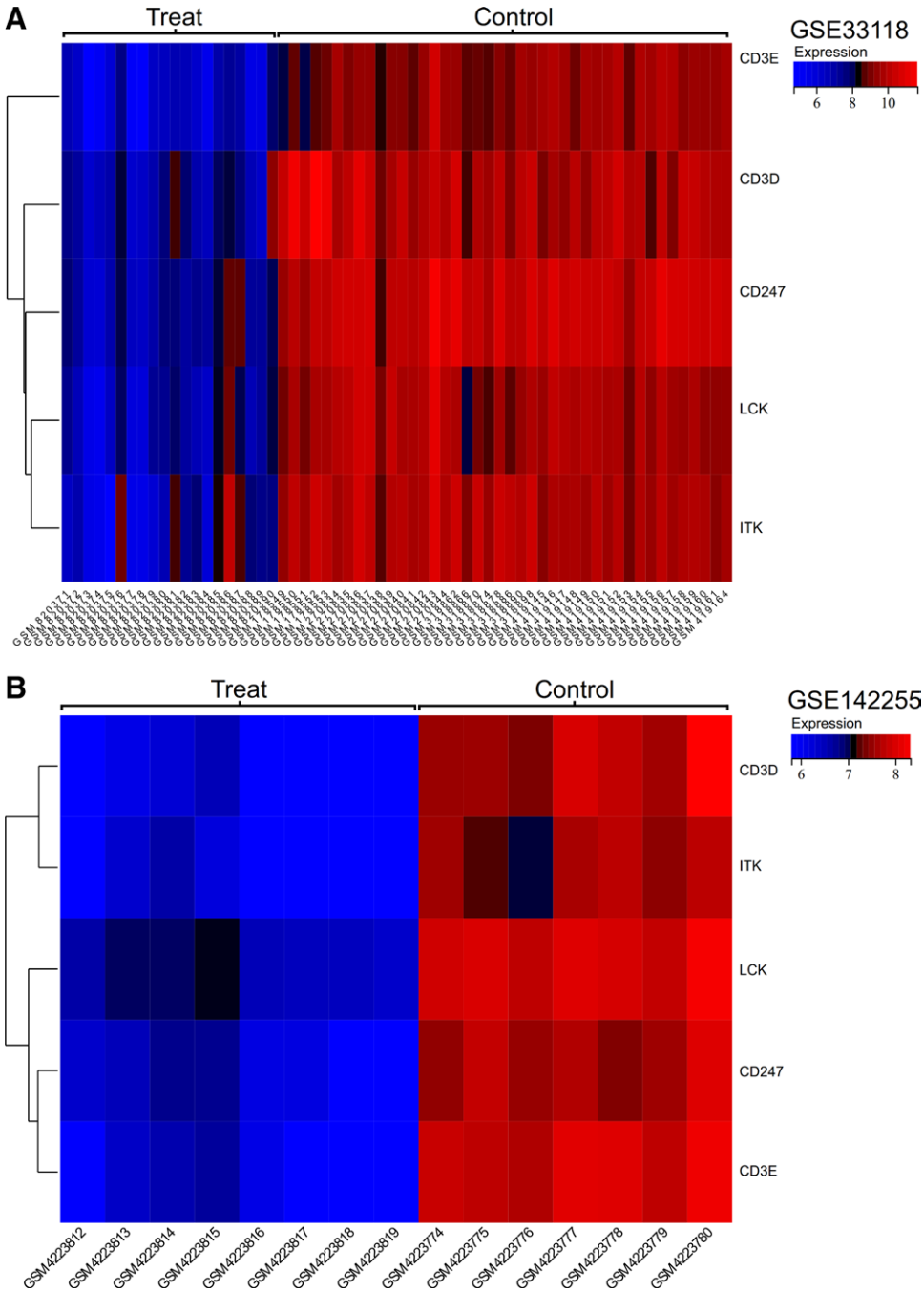


**Figure 10.** Gene expression heatmap. (A) Visualized and made heat maps to visualize the expression of the core genes in the sepsis shock datasets GSE33118. (B) Visualized and made heat maps to visualize the expression of the core genes in the sepsis shock datasets GSE142255.

inflammatory response of immune cells and attenuate the release of inflammatory mediators and organ function damage.[20,21] Immunomodulators such as mitomycin and IL-10 can modulate the function of the immune system and inhibit the activity of immune cells and inflammatory responses.[22] Antioxidants such as vitamin C, vitamin E, and glutathione can alleviate the damage of oxygen free radicals to cells and tissues and protect cells from oxidative stress.[23] Vasoactive drugs such as nitrates and dihydroergotamine can modulate vascular tone, improve circulation and tissue perfusion, and attenuate organ dysfunction and tissue damage.[24] β-Antimicrobials such as lactams and macrolides can directly kill bacteria, prevent further spread and exacerbation of infection, and protect the organism from damage due to infection.[25] The main results of this study were that cd3d, CD247 were abnormally expressed in sepsis shock, which may affect the development and progression of sepsis shock.

Cd3d is a human membrane-bound protein that is part of the TCE complex, and its encoding gene is located on human chromosome 11. Cd3d is a subunit in the CD3 complex, a complex composed of the TCR and CD3 molecules, which includes CD3 δ, CD3 ε, CD3 γ, and CD3 ζ. The 4 subunits as well as the TCR α and the TCR β both strands. Cd3d, along with other subunits, is involved in processes such as signal transduction, T cell activation and proliferation.[26] CD247, also known as cd3z, is a human membrane-bound protein that is part of the TCR complex. Its coding gene is located in the human chromosome 1q21.3 region. CD247 is a subunit of the CD3 complex, a complex composed of the TCR and CD3 molecules, which includes

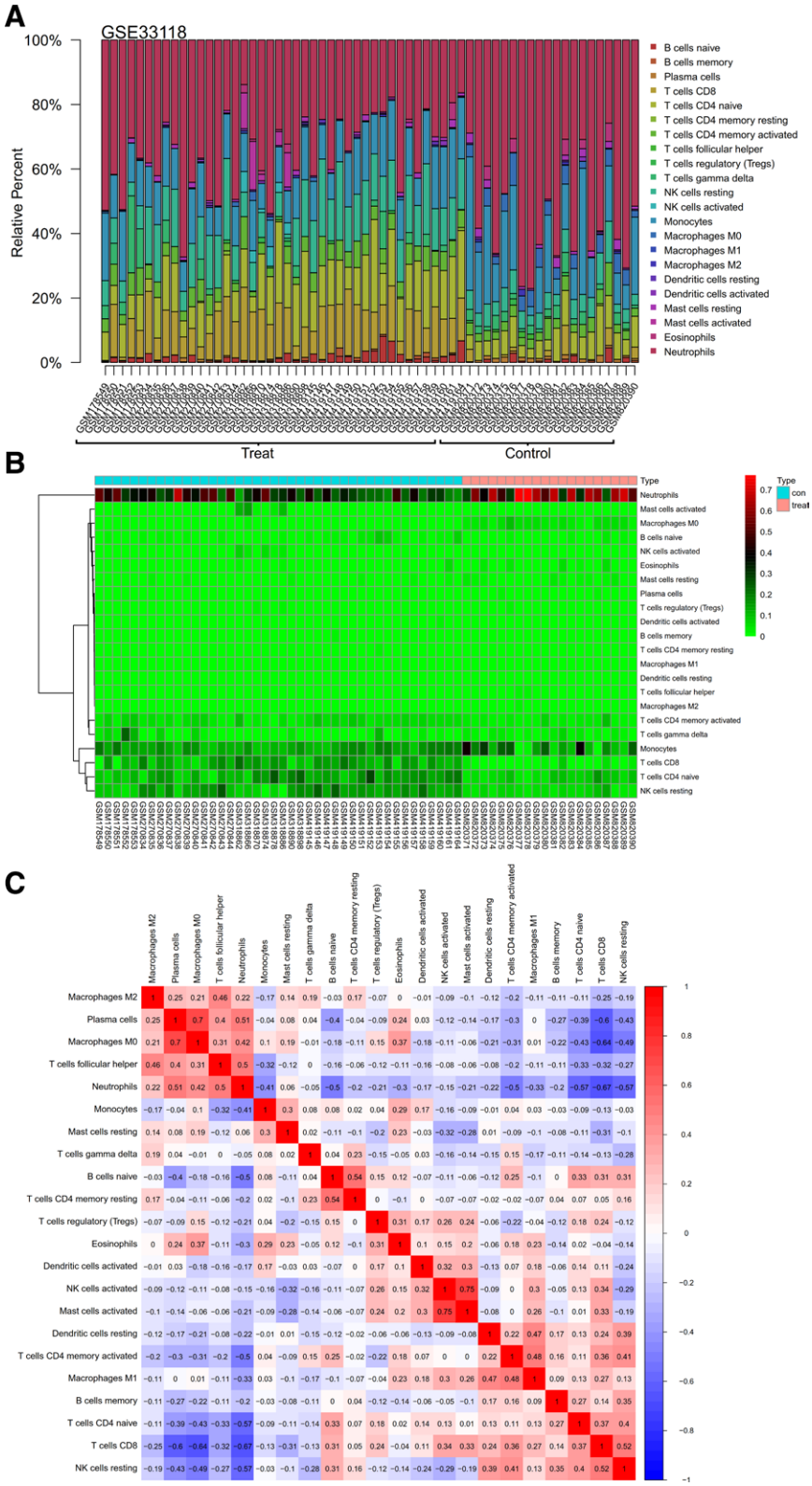


**Figure 11.** Immune infiltration analysis. Used the cibersort package to analyze the gene expression matrix of GSE33118. (A) The proportion results of immune cells of the full gene expression matrix. (B) The immune cell expression heatmap in the dataset. (C) The correlation analysis was also performed on infiltrating immune cells, resulting in a plot of co expression patterns among immune cell components.

CD3 δ, CD3 ε, CD3 γ, and CD3 ζ. The 4 subunits as well as the TCR α and the TCR β both strands. CD247 is the only molecule of the CD3 complex with a cytoplasmic domain that contains multiple tyrosine phosphorylation sites and is involved in regulating the binding of the CD3 complex to intracellular signaling molecules, thereby modulating T cell activation and function.[27] Researchers have suggested that the decrease in CD3 expression may be related to the failure of antigen presentation

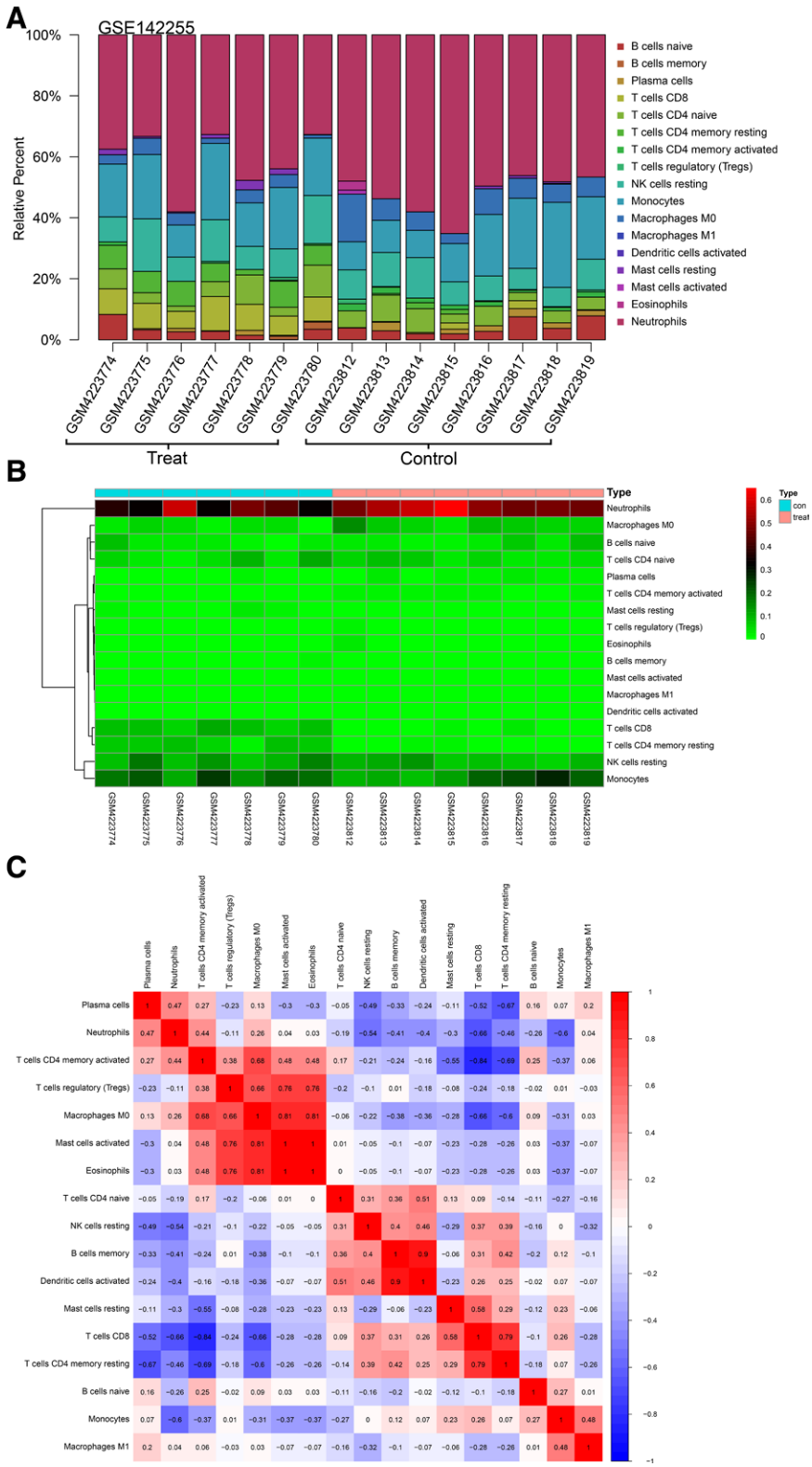


**Figure 12.** Immune infiltration analysis. Used the cibersort package to analyze the gene expression matrix of GSE142255. (A) The proportion results of immune cells of the full gene expression matrix. (B) The immune cell expression heatmap in the dataset. (C) The correlation analysis was also performed on infiltrating immune cells, resulting in a plot of co expression patterns among immune cell components.

after septic shock.[28] It has also been shown that the CD247 gene is mainly associated with upregulated inflammatory and metabolic responses and downregulated immune responses.[29] Another researchers indicated that Fyn and CD247 were at the center of a protein-protein interaction network, and survival analysis found that they positively correlated with sepsis survival.[30] Therefore, we speculate that cd3d, CD247 may play an important role in the development of septic shock.

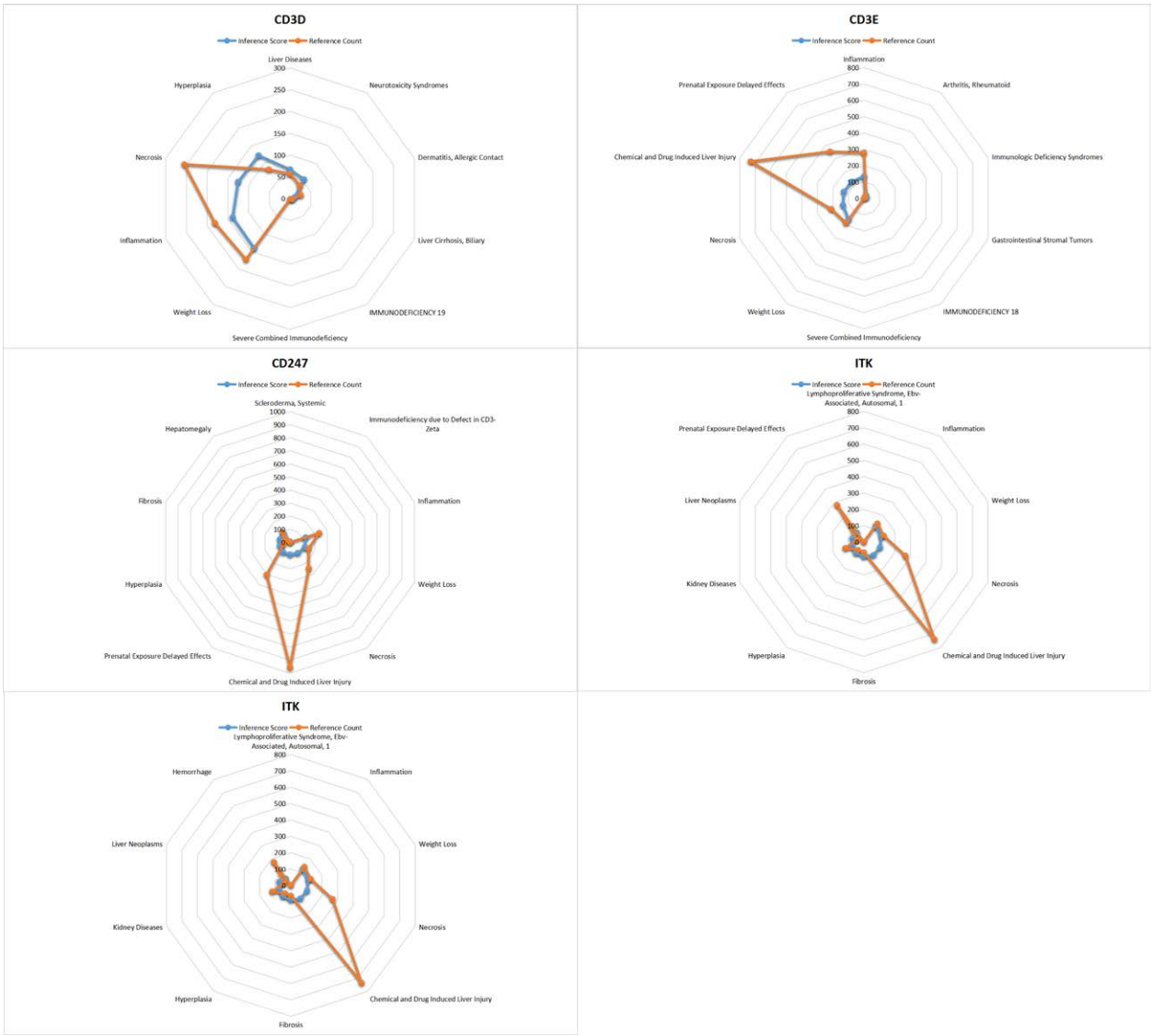


**Figure 13.** CTD analysis. Five core genes (CD247, Lck, cd3e, cd3d, ITK) were found to be associated with hemorrhage and necrosis. CTD = comparative toxicogenomics database.

**Table 1**

**A summary of miRNAs that regulate hub genes.**

| | Gene | MIRNA | | |
|---|---|---|---|---|
| 1 | CD247 | hsa-miR-330-5p | hsa-miR-326 | |
| 2 | LCK | hsa-miR-325-3p | | |
| 3 | CD3D | hsa-miR-7153-5p | hsa-miR-146a-5p | hsa-miR-146b-5p |
| 4 | ITK | hsa-miR-155-5p | | |
| 5 | CD3E | none | | |

The above literature review is consistent with our results that cd3d, CD247 was low expressed in septic shock, and the lower the level of cd3d, CD247, the worse the prognosis.

Despite the rigorous bioinformatics analysis in this paper, there are still some deficiencies. No animal experiments of gene overexpression or knockout were performed in this study to further verify its function. Therefore in future studies, we should conduct an in-depth exploration in this regard.

In conclusion, low expression of cd3d, CD247 was observed in septic shock, and the lower the level of cd3d, CD247, the worse the prognosis.

## Author contributions

**Conceptualization:** Qian Yang.
**Data curation:** Zhijuan Feng, Chunbo Kang.
**Formal analysis:** Qian Yang, Chunbo Kang.
**Methodology:** Zhijuan Feng, Danyang Ding, Chunbo Kang.
**Software:** Zhijuan Feng, Danyang Ding.
**Validation:** Qian Yang.
**Visualization:** Qian Yang.
**Writing – review & editing:** Qian Yang.
**Writing – original draft:** Danyang Ding, Chunbo Kang.

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