Identification and Exploration of Pharmacological pyroptosis-related Biomarkers of Ulcerative Colitis

Kaiwei Chen†, Shipeng Shang†, Shengnan Yu, Luwen Cui, Shangyong Li‡, Ningning He‡

School of Basic Medicine, Qingdao Medical College, Qingdao University, Qingdao 266003, China.

† Those authors contributed equally.

‡Corresponding author: Prof. Shangyong Li; Email: lisy@qdu.edu.cn

Prof. Ningning He; Email: heningning@qdu.edu.cn

Abstract

Ulcerative colitis (UC) is a ~~a type of~~ chronic inflammatory bowel disease (IBD). Its etiology is unclear. Much evidence suggests that the ~~abnormal intestinal epithelial cells (IECs) death~~death of abnormal intestinal epithelial cells (IECs) leads to intestinal barrier disruption, and the subsequent inflammatory response plays a~~n important~~ vital role in UC. Pyroptosis is a form of programmed inflammatory cell death, and the role of pyroptosis in UC etiology remains to be explored. ~~In this study, 10 hub genes in pyroptosis were identified~~This study identified 10 hub genes in pyroptosis by gene expression profiles obtained from the GSE87466 dataset. Meanwhile, the biomarkers were screened ~~according~~ based on~~to~~ gene significance (GS) ~~&~~ and module membership (MM) ~~in~~ through the Weighted Gene Co-Expression Network Analysis (WGCNA) analysis. The following analysis indicated those hub genes were ~~were~~ closely ~~related~~ associated with~~to~~ the UC progression and therapeutic drug~~s~~ response. The single-cell RNA (scRNA) sequencing data from UC patients ~~in~~ within the GSE162335 dataset indicated that macrophages were most related to ~~the occurrence of~~ pyroptosis. Finally, the expression of hub genes and response to the therapeutic drug (5-aminosalicylic acid, 5-ASA) were verified in dextran ~~sulphate~~ sulfate sodium (DSS) induced colitis mice. Our study identified *IL1B* ~~is~~ as the ~~key~~ critical pyroptosis-related biomarker in UC~~, and t~~. The crosstalk between macrophages pyroptosis and IECs pyroptosis may play an ~~important~~ essential role in UC, ~~which~~ deserv~~es~~ing further exploration.

Keywords: Ulcerative colitis; pyroptosis; Transcriptome; *IL1B*; DSS-induced colitis

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) of unclear etiology, ~~that usually~~ beginsning in the rectum and subsequently ~~spreads~~ spreading to the colonic mucosa (1). The incidence rate of UC is 9 to 20 cases per 100,000 person-years, and the prevalence rate is 156 to 291 cases per 100,000 people, ~~which has become~~constituting a global burden (2). The ~~life~~ quality of life of UC patients is commonly impaired ~~by~~ due to diarrhea, abdominal pain, bloody stools, and the ~~increased~~ elevated risks of colon and rectal cancer (3). ~~A variety of~~Various factors have been implicated in ~~the~~ developing~~ment of~~ UC, such as dysregulated immune response, gut microbial dysbiosis, genetic susceptibility, and environmental influences (4). UC patients ~~cannot~~ cannot be ~~completely~~ wholly cured and often take long-term medication (5). ~~Currently, t~~There is a widely accepted theory that a vicious cycle of intestinal barrier disruption, cell death, and subsequent inflammatory response ~~lies~~ rests at the heart of chronic inflammation (6).

The continuous monolayer of IECs ~~is~~, the first barrier against microbial and environmental pressure, ~~performs~~implements a critical innate immune function (7). Remarkably, the abnormal death of large numbers of IECs is ~~commonly~~ observed in preclinical models of UC patients ~~with UC~~ (8). Moreover, intestinal barrier dysfunction ~~can be~~is detected by confocal endoscopy before the occurrence of intestinal lesions, ~~which can~~thereby predicting the recurrence of IBD (9). Pyroptosis, also ~~known as~~called inflammatory cell necrosis, is a new form of programmed inflammatory cell death (10). The canonical pyroptosis pathway ~~relies~~ depends on caspase 1 for the cleavage of gasdermin D (GSDMD). In contrast,~~, whereas~~ the non-canonical pathway relies on caspase 4/5/11. After cleavage, the N-terminal end of GSDMD ~~forms~~ develops a transmembrane pore, ~~which~~ releasinges inflammatory cytokines such as IL-1β and IL-18, and interferes with ion and water regulation, ultimately ~~leading to~~causing intense inflammation and cell death (11). This pattern of cell death accompanied by a ~~strong~~ robust inflammatory response ~~is~~ becomes a double-edged sword for the host (10). An over-activated inflammatory response helps

the host ~~defend~~ defend against pathogenic infections~~,~~ but ~~may~~ could also cause various inflammatory diseases, including ~~such as~~ sepsis (12) and gout (13).

During the active phase of UC, ~~many~~ several different forms of cell death, including pyroptosis, are ~~widely~~ activated (14). The development of UC ~~may~~ could be related to pyroptosis of IECs and the release of inflammatory factors~~, t~~. The relationship between the two has attracted ~~extensive~~ significant interest (15). For ~~example~~instance, ~~it has been reported that~~ IL-36β, a member of the IL-36 subfamily of the IL-1 family, ~~enhances~~ increases the pathology of DSS-induced colitis in mice by ~~promoting~~ enhancing Th2 responses in LPL while decreasing Foxp3+ Treg responses (16). However, current molecular mechanisms ~~related~~ associated with~~to~~ pyroptosis in UC are ~~still~~ lacking in research. Therefore, explor~~ation of its regulatory mechanisms and gene expression characteristics will help to reveal the etiology of UC and provide~~ing its regulatory mechanisms and gene expression characteristics will help understand the etiology of UC and provide a new perspective ~~for~~ on UC treatment.

In this study, ~~using GSE87466 dataset as the base data,~~ hub genes associated with pyroptosis in UC were identified by Weighted Gene Co-Expression Network Analysis (WGCNA) combined gene expression matrix using the GSE87466 dataset as the base data. After validation by GSE92415, GSE107499, GSE59071, GSE73661, and GSE46451 datasets, the expression change of hub genes ~~after different drug treatments~~ w~~ere~~as ~~further~~ observed after different drug treatments to ~~reveal~~ demonstrate the role of pyroptosis in UC. Subsequently, ~~by single-cell RNA analysis of the GSE162335 dataset,~~ we explored the expression patterns of hub genes in the macrophage clusters ~~most~~ associated with pyroptosis of immune cells by single-cell RNA analysis of the GSE162335 dataset~~, and~~. We identified macrophage clusters ~~with~~ having different roles in UC pyroptosis ~~as a way~~ to ~~further~~ reveal the mechanisms ~~associated with~~related to pyroptosis in UC.

MATERIALS AND METHODS

Datasets and Preprocessing

Gene expression profiles and corresponding clinical data of UC were ~~obtained~~

retrieved from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo). Information ~~of~~ on all the datasets in this study ~~was~~ is ~~shown~~ described in **Table 1**. ~~Descriptions of~~The patient demographic characteristics for all the datasets are available in **Supplementary Material 1**. Pyroptosis-related genes (PRGs) were ~~obtained~~ procured from the Uniprot database (https://www.uniprot.org/), MSigDB database (https://www.gsea-msigdb.org/), and previous studies in the literature (17, 18) ~~, see (~~**Supplementary Table S1**~~) for more details~~.

Table 1 Information for all the datasets in this study

Dataset	Platform	Title
GSE87466	GPL13158	[HT_HG-U133_Plus_PM] Affymetrix HT HG-U133+ PM Array Plate
GSE92415	GPL13158	[HT_HG-U133_Plus_PM] Affymetrix HT HG-U133+ PM Array Plate
GSE107499	GPL15207	[PrimeView] Affymetrix Human Gene Expression Array
GSE59071	GPL6244	[HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array
GSE73661	GPL6244	[HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array
GSE46451	GPL10558	Illumina HumanHT-12 V4.0 expression beadchip
GSE162335	GPL20301	Illumina HiSeq 4000

Differential Expression Analysis

The dataset GSE87466 containing 87 UC patients and 21 healthy individuals, was ~~used~~ utilized to identify the differentially expressed genes (DEGs) between the UC and healthy controls (19). The DEGs were screened ~~with~~ having a threshold of *P*-value<0.05 ~~&~~ and |log2FC|>1 by using the "limma" package. ~~Visualization of DEGs was perform~~DEGs were visualized using the R packages "ggplot2" and "pheatmap~~"~~."

Construction of Co-Expression Network and Identification of Modules

~~In order to reduce the computational effort of the whole network and maintain the characteristics of scale-free topological network structure, t~~The 3,945 variant genes were screened (**Supplementary Table S2**) with a coefficient of variation > 0.08 to

reduce the computational effort of the whole network and maintain the characteristics of a scale-free topological network structure. A weighted gene co-expression network was constructed ~~using~~ with the R package "WGCNA" for the ~~screened~~ 3,945 variant genes. First, the sample data with abnormal gene expression values were filtered ~~by~~ using hierarchical clustering, and the "pickSoftThreshold" function was ~~used~~ utilized to ~~evaluate~~ assess the appropriate soft threshold β. Subsequently, the Pearson correlation coefficients of the gene ~~was~~ were ~~calculated~~determined, the weighted adjacency matrix was constructed ~~with~~ using a soft threshold β of 13, and the adjacency matrix was converted into ~~a~~ the topological overlap matrix (TOM). The minimum module size cutoff was set to 30, and the same modules were merged ~~with~~ using a threshold value of 0.2. Genes with similar expression patterns were grouped ~~into~~ within the same module. Module eigengene (ME) was calculated to represent the overall ~~level of gene expression~~gene expression level ~~within~~ inside the module and used to identify modules ~~highly~~ significantly correlated ~~to~~ with the disease.

Identification of Pyroptosis-Related Hub Genes and Biomarkers

The hub gene was ~~obtained~~ retrieved by taking the overlapping ~~of~~ PRGs, DEGs, and genes from the module ~~with~~ having the highest relevance to UC in WGCNA analysis. Biomarkers were screened from hub genes ~~based~~ depending on the criteria of gene significance (GS) > 0.2 & module membership (MM) > 0.8 in the WGCNA analysis (20). The Receiver Operating Characteristic (ROC) analysis of the hub genes ~~using~~ was performed with the R package "pROC~~".~~."

Immune Cell Infiltration Estimation

The relative abundance of immune cells ~~in~~ within the colonic mucosal tissue of the UC and healthy controls were ~~evaluated~~ assessed using the CIBERSORT algorithm (https://cibersort.stanford.edu/) (21). The difference~~s~~ between the two groups of immune cells ~~were~~ was compared ~~using~~ with Student's t-test and visualized by a "ggboxplot~~". .~~." The correlation between each infiltrating immune cell~~s~~ and the

relationship between hub genes and immune cells was visualized ~~by~~ using the "corrplot" package.

Single-Cell Analysis

The dataset GSE162335 ~~was~~ contained single-cell sequencing data of immune cells from the CD45+ colonic lamina propria of the 11 UC patients (22). The "Seurat" package was ~~used~~ utilized for subsequent data processing (23). The low-expressing cells and genes were filtered~~,~~ while ensuring that the percentage of mitochondria per cell was below 5% ~~&~~ and the features of genes ~~was~~ were below 6,000 and above 500. Finally, a total of 18,375 cells were ~~obtained~~identified. After normalization using the "LogNormalize" method, 3,000 highly variable genes (HVG) were identified ~~using~~ with the "vst" method. PCA was applied to identify significant principal components based on the expression of HVG. Thus, ~~and~~ 25 PCs were selected for t-SNE analysis ~~with~~ having a resolution of ~~"~~"2"~~"~~ to identify the different clusters. "FindAllMarkers" function (logfc.threshold = 0.25) was ~~applied~~ incorporated to identify DEGs in each cluster and marker gene for each cluster ~~with~~ using avg_log2FC>1.

Using the "BlueprintEncodeData" dataset from the R package "celldex" as a ~~a~~ reference, each cluster was initially annotated ~~by~~ with "SingleR~~".~~." The R package "scHCL" was used to ~~further~~ annotate the cluster of interest (24)~~,~~ ~~with~~ using the Human Cell Landscape (http://bis.zju.edu.cn/HCL/) & the scRNASeqDB (https://bioinfo.uth.edu/scrnaseqdb/) databases as ~~a~~ the secondary reference.

The R package "AUCell" was used to ~~assess~~ evaluate the response of single cells to PRGs (25), and the "Aucell_explorethreshold" function was used to determine the threshold for identifying gene set active cells. Then, the AUC score of each cell ~~were~~ was mapped to the t-SNE embedding ~~using~~ with ~~"~~"ggplot2"~~"~~ for visualization. The "Monocle" package was ~~used~~ utilized for cell pseudotime analysis (26).

Functional Annotation and Pathway Enrichment Analysis

GO annotations of genes from the R package ~~"~~"org.Hs.eg.db"~~"~~ (version 3.1.0) was

used utilized as background. The selected genes were mapped to the background set, and performed the enrichment analysis was performed using the R package ""clusterProfiler"" (version 3.14.3). $P < 0.05$ & FDR < 0.25 were considered statistically significant.

Animal experiment

The experimental mice and standard rodent chow food were purchased obtained from Jinan Pengyue Laboratory Animal Breeding Company (Jinan, China). The Eexperimental procedures were reviewed and approved by the Ethics Committee of the Medical College of Qingdao University (QDU-AEC-2022314). C57BL/6J mice (18-20 g) were randomized into 3 three groups (n=6) after 1 one week of acclimation. NC group: no extra treatment for 7seven days; DSS group: free drinking water containing 2.5% DSS during 7seven days; 5-aminosalicylic acid (5-ASA) group: the free drinking water containing with 2.5% DSS for 7seven days while 5-ASA (400 mg/kg/day) were was administered by gavage. At the end of the experiment, the entire colon was excised, and the length of coloncolon length was measureddetermined. The collected colon tissue was stored at -80°C for further analysis.

Histopathological analysis

The collected colonic tissues were fixed in 10% formalin overnight,. The fixed tissues were embedded in paraffin and sliced after dehydration in gradient concentrations of alcohol after dehydration at a thickness of 5 μm. Stained with hematoxylin and eosin (H&E) were observed using with a 200x magnification (E100, Nikon, Tokyo, Japan) and an imaging system (DS-U3, Nikon, Tokyo, Japan). Histological As previously described, the histological score for H&E staining was assessed in a blinded fashionscore for H&E staining was assessed in a blinded fashion as previously described (27).

RNA extraction and quantitative ~~real~~ real-time PCR (RT-qPCR) analysis

The extraction of total RNA and reverse transcription were performed ~~according~~ based on~~to~~ the kit instructions of SparkJade (Jinan, China). The RT-qPCR primers are depicted ~~were shown~~ in **Supplementary Table S3**. The gene expression levels were normalized ~~using~~ with GAPDH, and the relative quantification of gene expression was ~~calculated~~ determined using~~through~~ the $2^{-\Delta\Delta Ct}$ method.

Enzyme-linked immunosorbent assay (ELISA)

~~In~~ At the end of the experiment, mice were anesthetized ~~by~~ with chloral hydrate and separated from the serum by centrifugation (3500 rpm, 4°C, 30 min) after picking out the eyeball. The ELISA kit was purchased from ABclonal (#RK00027, Wuhan, China). The serum levels ~~of in the serum was~~ were measured ~~according~~ based on~~to~~ the instruction of the ELISA kit.

Statistical Analysis

All the statistical ~~analysis~~ analyses ~~was~~ were performed ~~by~~ using the R software (version 4.1.1). All the data are expressed as mean ± SE. Unpaired ~~Student's~~ Student's t-test was used ~~for~~ to compare two ~~comparison~~ groups, and ~~.~~ One-way ANOVA was used for three and more group comparison~~s groups.~~, ~~and~~ Tukey HSD was used for inter-comparison between multiple groups. This ~~e above~~ statistical analysis was performed ~~with~~ using the R package "ggpubr" and "stats~~".~~." P <0.05 was considered ~~as~~ statistically ~~different~~significant.

Result

Research Design Summary

The flow chart ~~was~~ is shown in **Figure 1**. Firstly, ~~DEGs were screened between the UC patient and the healthy controls from the GEO database~~the GEO database screened DEGs between the UC patient and the healthy controls. Subsequently, pyroptosis-related hub genes were identified ~~using~~ through DEGs combined with genes of the key module in WGCNA analysis and PRGs. ~~Six genes were screened as biomarkers among hub genes based on GS and MM valu~~Based on GS and MM values, six genes were screened as biomarkers from the hub genes.~~,~~ ~~and~~ ROC analysis was used to ~~assess~~ determine their diagnostic values. The obtained hub genes were validated from two aspects. Next, the differences ~~of~~ in hub genes between UC/healthy controls, mucosal lesional/nonlesional group, and active/inactive/healthy controls were validated ~~based~~ depending on three datasets obtained from the GEO database~~, respectively~~. The pattern of changes in the hub gene after treatment with 5-ASA, IFX (infliximab), and VDZ (vedolizumab) was ~~evaluated~~assessed. Infiltrating immune cells in UC patients ~~was~~ were analyzed ~~using~~ with CIBERSORT. Finally, the expression pattern of hub genes was ~~evaluated~~ evaluated in immune cells from the colonic lamina propria of UC patients at the single-cell level~~ and~~. ~~Verification of~~The hub gene expression was verified using~~by~~ animal experiments.

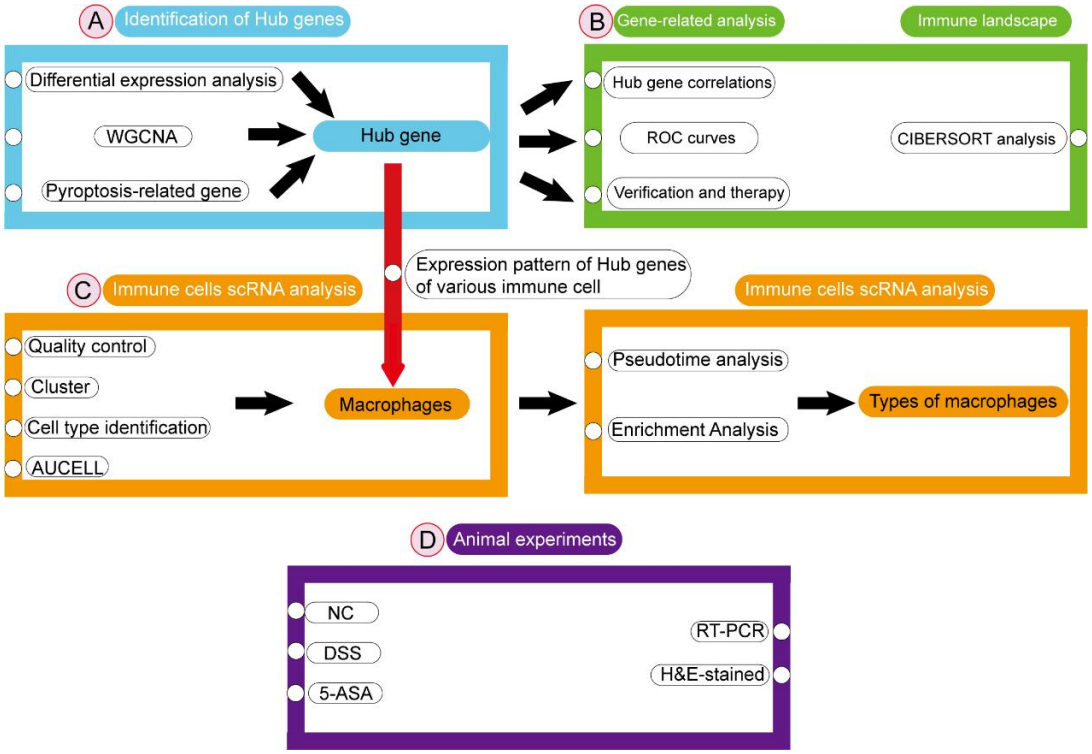


Figure 1. Flowchart of bioinformatics ~~analysis~~ analyses in this study.

Identification of DEGs and Functional Annotation and Pathway Enrichment of DEGs

Correlation analysis of the GSE87466 dataset ~~showed~~ revealed stronger intra-group correlations for the UC group (**Figure S1A-B**). ~~Using *P*-value<0.05 & |log2FC|>1 as the threshold,~~ ~~w~~We identified 1,247 DEGs, including 843 upregulated ~~genes~~ and 404 downregulated genes, using *P*-value<0.05 & |log2FC|>1 as the threshold (**Supplementary Table S4**). The up and ~~down~~ down-regulation distributions of DEGs were shown in the volcano plot (**Figure 2A**). Heatmap showed the expression patterns of DEGs and relative consistency within groups. Upregulated DEGs showed a positive correlation with the UC group and a negative correlation with healthy controls, while downregulated DEGs ~~showed~~ revealed the opposite (**Figure 2B**).

~~To better understand the functions of DEGs,~~ GO and KEGG enrichment analysis was performed to understand the functions of DEGs better. GO terms of biological process (BP) ~~showed~~ described that DEGs were ~~mainly~~ primarily enriched in immunomodulation and immune response, such as response to chemical~~,~~ and immune system processes ~~, immune response~~ (**Figure 2C**). Moreover, the enriched KEGG

pathway showed that a series of pathways ~~related~~ associated with~~to~~ the inflammatory response ~~were~~ was activated, ~~including~~ such as Cytokine-cytokine receptor interaction, B cell receptor signaling pathway, Chemokine signaling pathway, and the IL-17 signaling pathway (**Figure 2D)**. ~~More n~~Notably, DEGs were also enriched by pyroptosis-related inflammatory pathways, ~~such~~ including~~as~~ the NOD-like receptor signaling pathway, NF-kappa B signaling pathway, Toll-like receptor signaling pathway, and TNF signaling pathway. The enrichment results of these DEGs ~~showed~~ revealed that various external stimuli induced a series of immune responses, ~~including~~ such as ~~the activation of some inflammatory pathways associated with pyroptosis, which indicated~~activating some inflammatory pathways associated with pyroptosis, indicating that pyroptosis is widely activated in UC. ~~And~~ Moreover, the enrichment results of these DEGs also predicted a damaged intestinal barrier ~~in~~ among UC patients. Intestinal barrier function ~~consists~~ has~~of~~ three main components: mechanical~~barrier, ecological barrier and immune barrier~~, ecological, and immune. The disruption of the immune barrier often ~~causes~~ leads to functional impairment of the other two. As ~~we~~ mentioned above, these terms focusing on immune responses and inflammatory pathways indicate that the immune barrier is severely impaired ~~in~~ among UC patients~~ and t~~. This damage further ~~causes disruption of~~disrupts the mechanical barrier, ~~such as~~including the abnormal death of IECs.

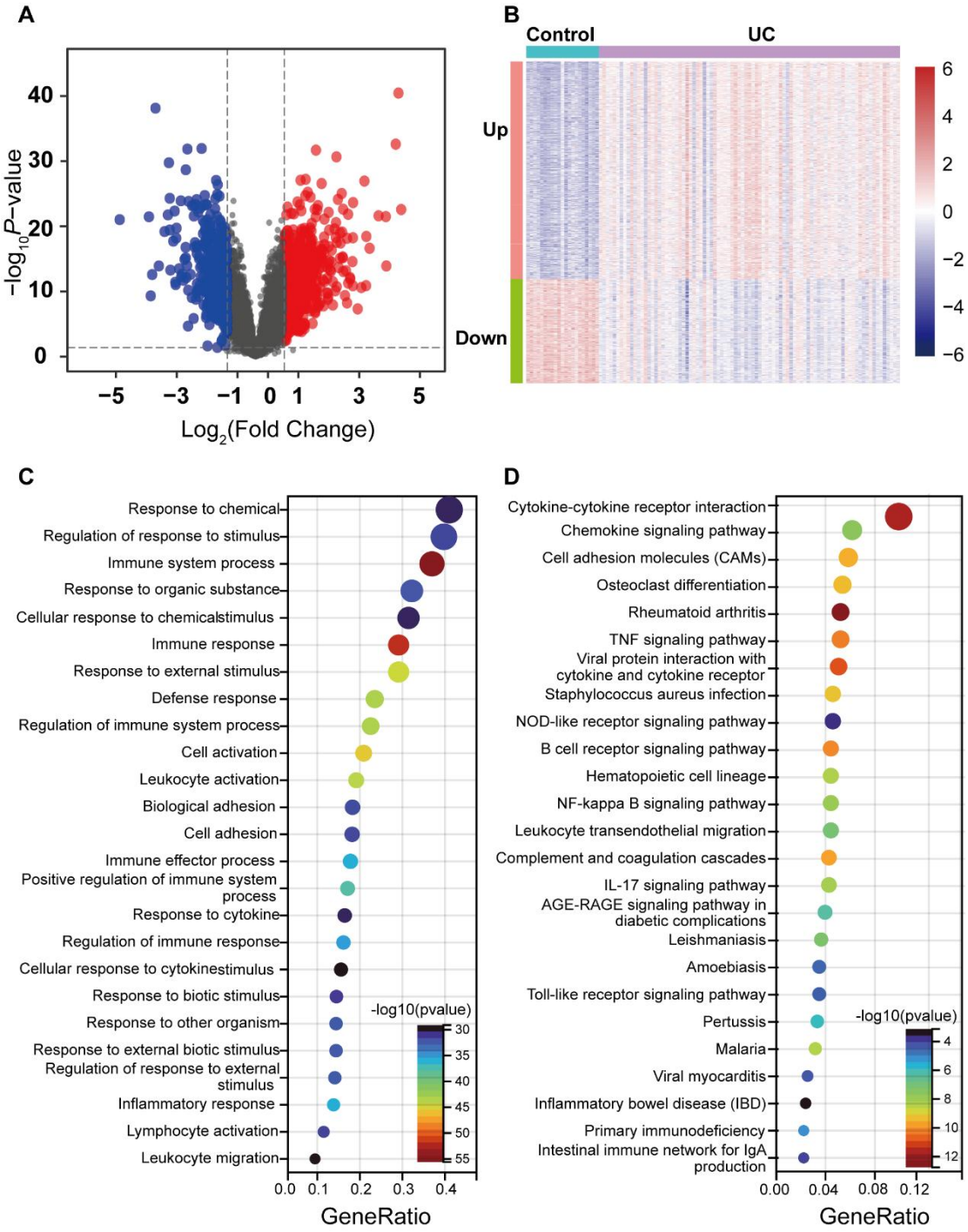


Figure 2. Identification of DEGs between the UC ~~group~~ and control groups in the dataset and enrichment analysis. (A) Volcano maps of DEGs between UC and control, where red dots represent the upregulated genes, blue dots represent the downregulated genes, and gray dots represent no differential gene. (B) Heatmap of DEGs in UC and control. The red indicates high expression, ~~,~~ and the green indicates low expression. (C) GO enrichment analysis of DEGs. (D) KEGG pathway enrichment analysis of DEGs.

WGCNA Construction and Key Modules Identification

The co-expression network was ~~constructed~~ developed to identify the most relevant modules for UC based on the expression of 3,945 CV genes. The 108 samples were clustered, and the two outlier data were removed (**Figure S1C**). A scale-independent topological network (soft threshold 13 scale-free R^2 0.87) and the mean connectivity network were established (**Figure 3A**). We obtained 11 gene modules ~~by~~ through hierarchical clustering and module merging (**Figure 3B**). Among these~~m~~, black (r = 0.52, *P* = 1e-8), blue (r = 0.53, *P* = 4e -9), and turquoise (r = 0.75, *P* = 3e-20) modules were ~~highly~~ significantly correlated with UC (**Figure 3C**). The turquoise module, ~~which containing a total of~~containing 1,186 genes, was selected as the feature module for UC d~~based~~ epending on the correlation coefficient and *P*-value~~,~~ (**Supplementary Table S5**). Thus, 354 genes were screened as module hub genes from the turquoise module ~~based~~ depending on GS > 0.2 & MM > 0.8 (**Figure 3D**).

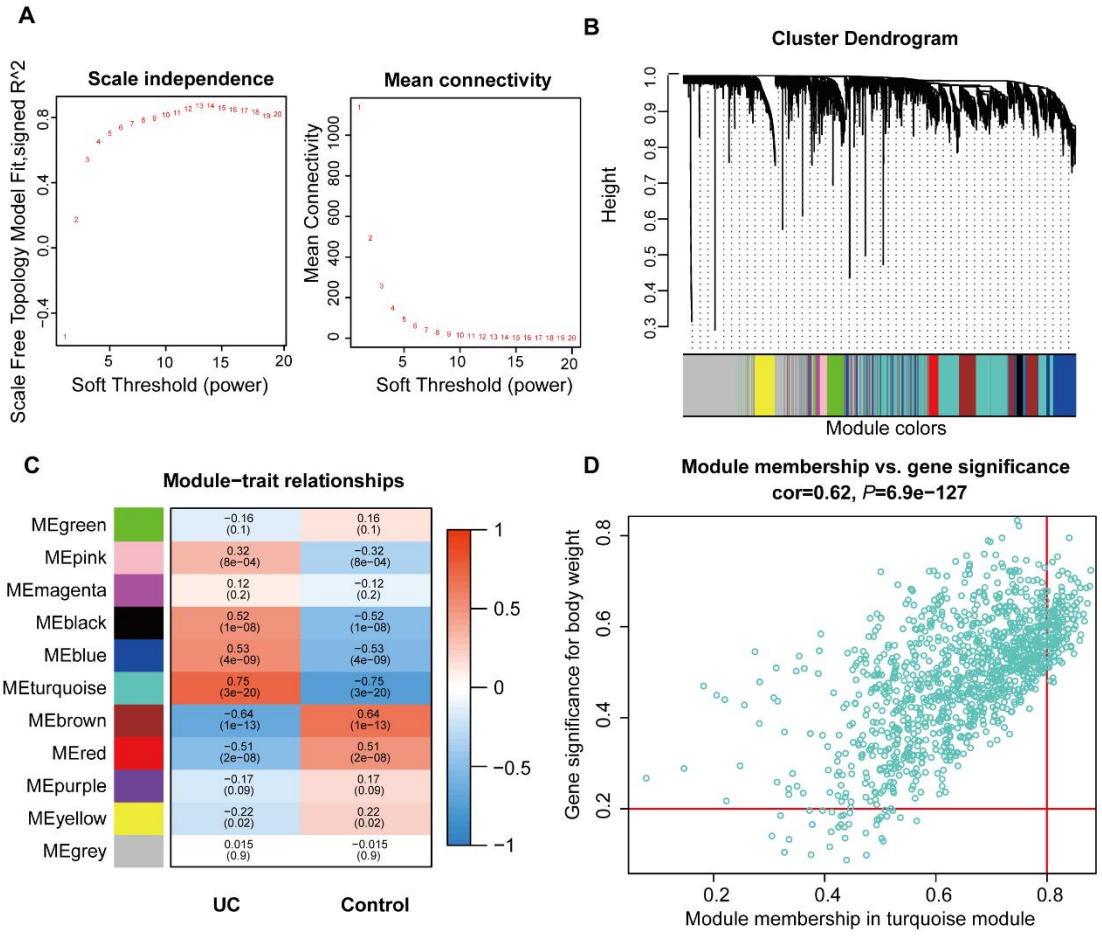


Figure 3. Identification of the key modules and genes ~~that relate to~~associated with UC

by WGCNA. (A) Estimati~~on of~~ng the scale independence index of the 1–20 soft threshold power and determin~~ingation of~~ the mean connectivity of the 1–20 soft threshold power. (B) Module clustering dendrogram derived from the 1-tom matrix. The different color bands represent different modules. (C) Correlations between the ~~different~~ various modules and traits. The number of each module ~~represents~~ depicts the correlation coefficient with the trait, and the color of the module ranges from red to green, ~~representing~~ showing ~~from~~ high to low correlation. (D) Scatter plot of the turquoise module genes. The vertical coordinate represents the GS score ~~for each gene,~~ and the horizontal coordinate represents the MM score for each gene.

Identification of Pyroptosis -Related Hub Genes and Biomarkers

We performed GO enrichment analysis to analyze 74 PRGs and ~~discovered~~ observed that these genes were enriched with interleukin-1-related biological processes ~~in addition to~~along with pyroptosis (**Figure S2**). This ~~suggested~~ indicated that pyroptosis ~~may~~ could be closely ~~related~~ associated with~~to~~ interleukin 1. The 10 hub genes (*AIM2*, *IL6*, *IL1B*, *NLRP7*, *TNF*, *IL1A*, *IL18R1*, *ZBP1*, *GZMB*, *TREM1*) were obtained from the overlap of DEGs, PRGs, and turquoise module genes (**Figure 4A**). *NLRP7* and *ZBP1* were ~~weakly~~ poorly interrelated with other hub genes, and *IL1B* had the highest correlation with *IL6*, *IL1A*, and *TREM1*, ~~based~~ depending on the expression patterns of the~~se~~ hub genes (**Figure 4B**).

~~In order t~~To identify new UC biomarkers related to pyroptosis, the overlapping parts of turquoise module hub genes, and DEGs, PRGs were taken as new biomarkers (**Figure 4C**). A total of ~~6~~ six genes (*IL1B*, *IL18R1*, *ZBP1*, *AIM2*, *GZMB*, *TREM1*) were obtained~~, respectively~~. ~~To verify the diagnostic significance of the six biomarkers,~~ ROC analysis was performed to verify the diagnostic significance of the six biomarkers (**Figure 4D-I**). The AUC values for all the genes were ~~greater~~ more significant than 0.75, with *IL1B* (AUC 0.97, 95%CI) ~~having~~ with the largest AUC value and *IL18R1* (AUC 0.91, 95%CI) ~~having~~ showing the smallest AUC value. This indicated that *IL1B*, *IL18R1*, *ZBP1*, *AIM2*, *GZMB*, ~~TREM1 may as~~and *TREM1* could

be new UC biomarkers ~~related~~ associated with~~to~~ pyroptosis.

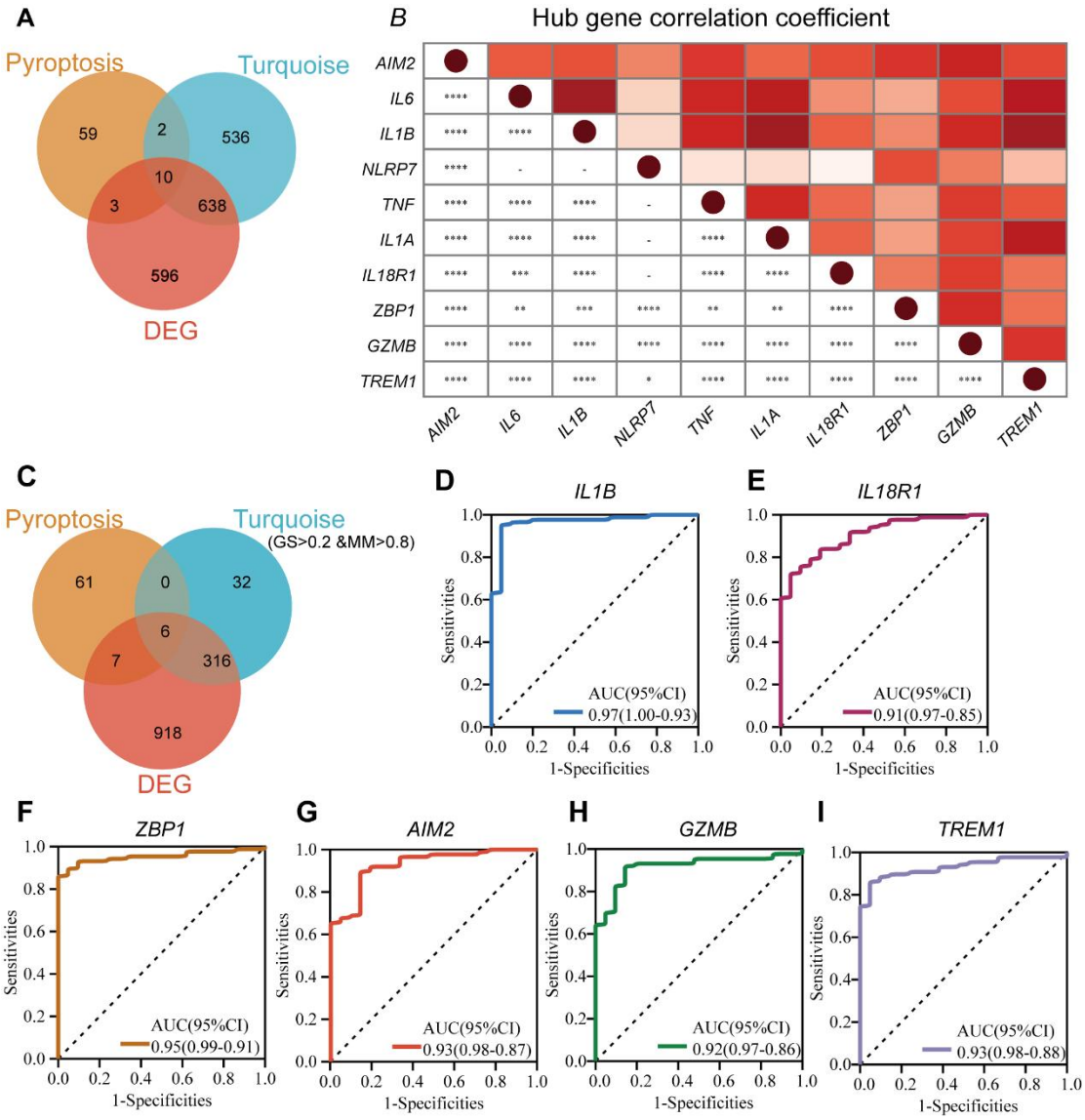


Figure 4. Identification of pyroptosis-related hub genes and biomarkers in UC. (A) ~~Overlapping genes generated by the intersection of turquoise module genes, DEGs and PRGs showed by venn diagram~~The Venn diagram shows the overlapping genes generated by the intersection of turquoise module genes, DEGs, and PRGs. (B) Expression correlation matrix of each PRG~~s~~ hub gene in UC. (C) ~~Overlapping genes generated by the intersection of turquoise module hub genes, DEGs and PRGs showed by venn diagram~~The Venn diagram shows the overlapping genes generated by the intersection of turquoise module hub genes, DEGs, and PRGs. ~~ROC (r~~Receiver operating characteristic (ROC) curves for *IL1B*(D), *IL18R1*(E), *ZBP1*(F), *AIM2*(G),

GZMB(H), and*TREM1*(I). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$

Verification of Pyroptosis-Related Hub Genes

The 10 hub genes were ~~further~~ validated ~~by~~ using the GSE92415 dataset (27). ~~It showed that a~~All the hub genes were ~~statistically~~ significantly different in UC and healthy controls ($P < 0.001$, **Figure 5A**). ~~It was verified that~~ Moreover, all the hub genes were ~~statistically~~ significantly different between lesional and nonlesional groups from the GSE107499 dataset (**Figure 5B**).

In addition, we compared the differences in hub genes between activated UC groups, inactivated UC groups, and healthy controls by the GSE59071 dataset (28). ~~Interestingly, only *IL1B* showed a statistical difference among the activate~~Only IL1B showed a statistical difference among the activated UC groups, inactivated UC groups, and healthy controls (**Figure 5C**). All the hub genes except *IL1B* were not ~~statistically~~ significantly different in healthy controls and inactivated UC groups included~~s~~ *IL6* and *TNF*, common pro-inflammatory factors. Subsequently, ~~we explored~~ four other pro-inflammatory factors, including *IL8*, *IL17A*, *IL18*, and *IL33*, were explored,~~all of~~ which ~~are~~ were thought to be closely associated with the development of UC disease~~, although~~. However, their role in pyroptosis remains unexplored. ~~Interestingly, t~~The expression pattern of *IL33* was similar to *IL1B*, with ~~statistically~~ significant differences between all three groups (**Figure S3**). The relationship between *IL33* and UC ~~may~~ could also ~~deserve to~~ be focused on ~~on~~ in the future (29). ~~In conclusion~~Therefore, *IL1B* was ~~probably~~ the more vital of the hub genes and ~~and it showed strong correlation~~trongly correlated with other hub genes.

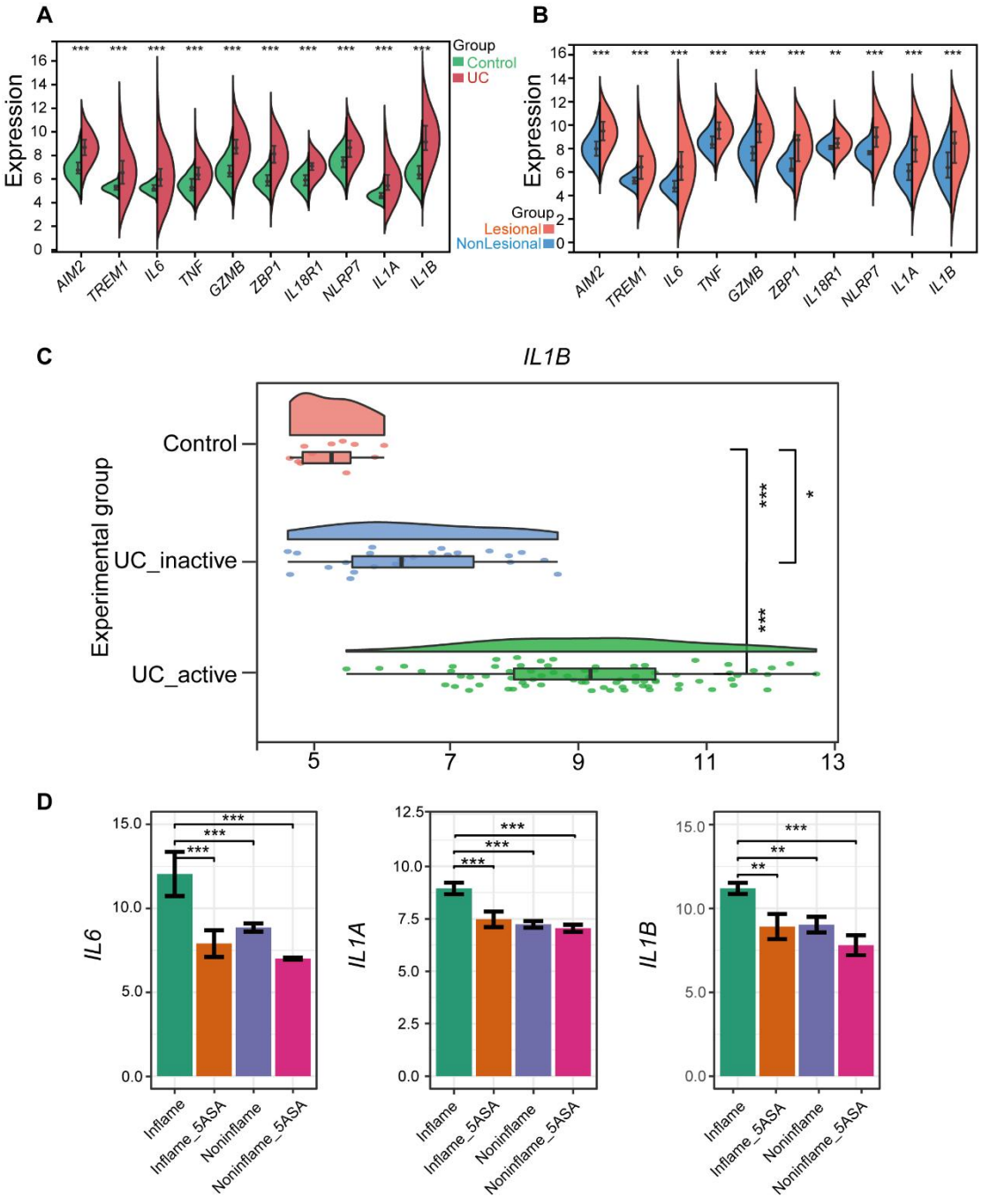


Figure 5. Verification of PRGs hub genes in UC and Evaluation of 5-ASA for ~~the treatment of~~reating UC pyroptosis. (A) ~~Expression~~ The violin plot showed the expression of 10 hub genes in the colonic mucosa of the UC group and the control group in GSE92415~~of 10 hub genes in the colonic mucosa of UC group and control group in GSE92415 showed by violin plot~~. (B) ~~Expression of 10 hub genes in the lesional and nonlesional colonic mucosa of UC patients in GSE107499 showed by violin plot~~Comp violin plot showed the expression of 10 hub genes in the lesional and nonlesional colonic mucosa of UC patients in GSE107499. (C) The ~~expression of~~

~~*IL1B* in the colonic mucosa of control, inactive and active UC patients in GSE59071 showed by raincloud plot~~raincloud plot showed the expression of IL1B in the colonic mucosa of control, inactive and active UC patients in GSE59071. (D) 5-ASA alleviates rectal mucosal damage in UC patients by ~~regulating~~ controlling *IL6*, *IL1A*, and *IL1B* in the RPGs hub genes. The relative expression levels of *IL6*, *IL1A*, and *IL1B* within the rectal mucosa of inflame (inflammation but no drug), inflame_5-ASA (inflammation and treated with 5-ASA), non-inflame (non- inflammation and no drug), and non-inflame_5-ASA (non- inflammation and treated with 5-ASA). $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

Drugs Improve Rectal and Colonic Mucosal Damage in UC Patients by Reducing Pyroptosis-Related Hub Genes

Subsequently, ~~we explored~~ the influence of drugs on changes in the expression of pyroptosis-related hub genes was explored using GSE46451 and GSE73661 (30). 5-ASA was the drug of choice for mild-to-moderate UC (31). IFX, adalimumab, and golimumab that target ~~to *TNF*-α, VDZ that target to α4β7 integrin, and ustekinumab that target to~~TNF-α, VDZ that target α4β7 integrin, and ustekinumab that target IL-12 and IL-23 ~~were~~ are the five most common biologics approved for ~~the~~ treat~~ment of~~ing UC, and they were recommended as the first-line treatment for moderate-to-severe UC (32). It ~~showed~~ revealed ~~that there was~~ no difference in the expression of *AIM2*, *TREM1*, *TNF*, *GZMB*, *ZBP1*, *IL18R1*, and *NLRP7* before and after 5-ASA effect on inflamed or non-inflamed rectal mucosa ~~*in vitro*~~ of UC patients *in vitro*, ~~suggesting~~ indicating that 5-ASA may ~~have no effect on~~not affect these ~~7~~ seven RPGs. (**Figure S4**). However, the expression levels of *IL6*, *IL1A*, and *IL1B* were significantly ~~reduced~~ decreased in the inflamed rectal mucosa after receiving 5-ASA~~, n~~. T~~ot only that, t~~hese three genes also ~~showed~~ depicted significant differences between inflamed ~~rectal mucosa~~ and non-inflamed rectal mucosa (**Figure 5D**). Meanwhile, the expression of hub genes in the colonic mucosa of patients ~~with~~ with active UC~~active UC was~~ significantly decreased after IFX treatment. Moreover, ~~we found~~ no difference –

between pre-IFX treatment and non-responders~~,~~ but a significant difference was observed between after-IFX treatment and non-responders. Most importantly, ~~except for *IL1A*,~~ the expression levels of the other ~~9~~ nine hub genes were restored to the levels of healthy controls, except for *IL1A* (**Figure 6A**). The ~~E~~expression patterns of pyroptosis-related hub genes before and after VDZ treatment of patients with active UC were ~~almost identical~~similar to IFX (**Figure 6B**). Therefore, we speculated that IFX and VDZ ~~probably~~ could have reduced the occurrence of pyroptosis while treating UC~~,~~. 5-ASA has been used to treat mild UC for nearly three decades, while biologics such as IFX~~,~~ and VDZ were often used to treat patients with moderate and severe UC. Based on the ~~above-mentioned chang~~abovementioned~~e~~ pattern of hub genes, we speculated that the progression of the UC condition ~~might~~ could be associated with pyroptosis.

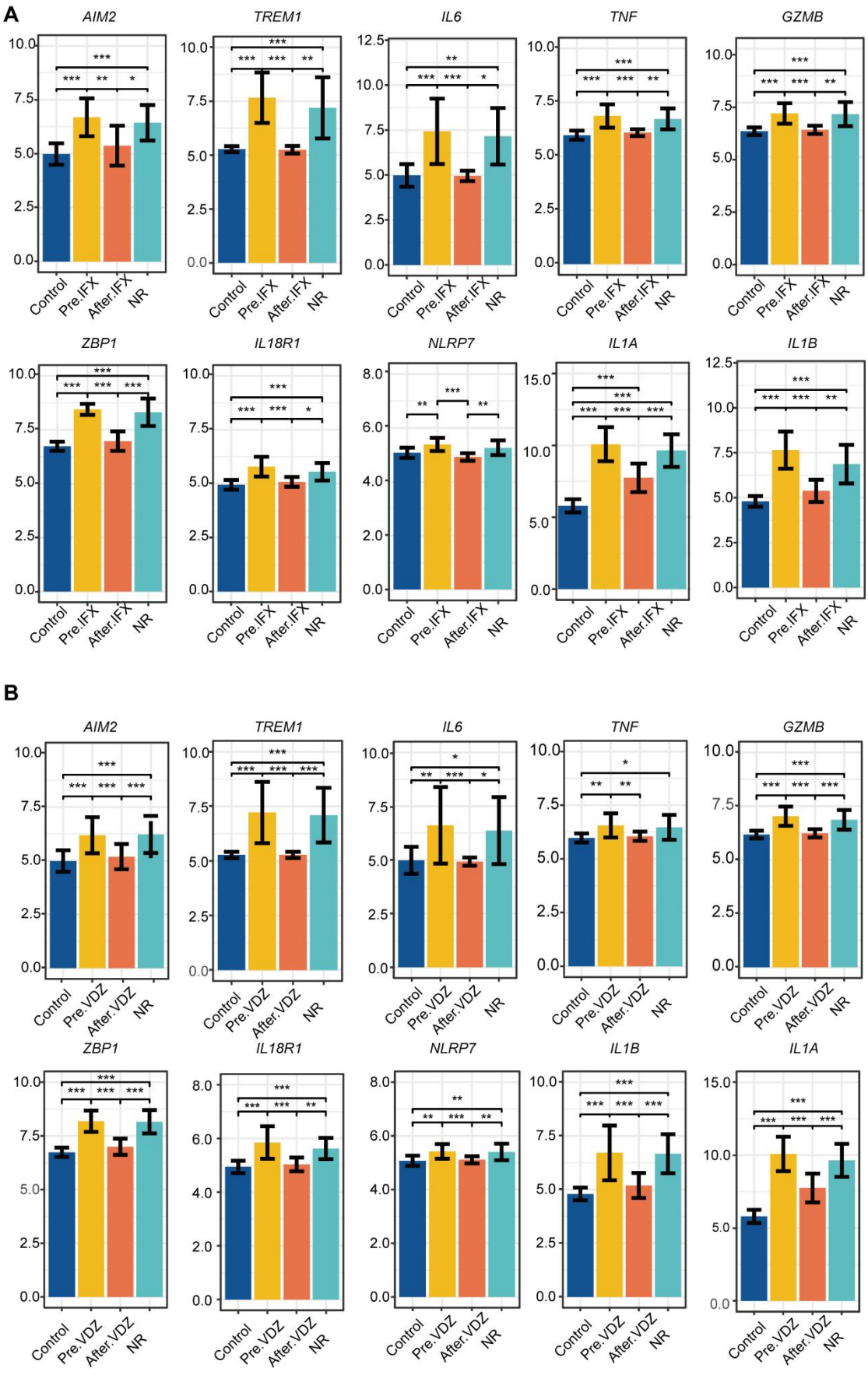


Figure 6. IFX and VDZ reduces impaired colonic mucosa of UC patients by

regulating the PRGs hub gene. (A, B) The relative expression levels of *AIM2*, *TREM1*, *IL6*, *TNF*, *GZMB*, *ZBP1*, *IL18R1*, *NLRP7*, *IL1A*, and *IL1B* in the colonic mucosa of control, Pre.IFX (UC patients before IFX therapy), After.IFX (UC patients in remission after IFX therapy) and NR (UC patients not ~~n~~responding to IFX therapy). **P* <0.05, ***P* < 0.01 and ****P* < 0.001.

Immune Infiltration Analysis

~~Infiltration immune cell of UC calculated by CIBERSORT algorithm~~The CIBERSORT algorithm determined the infiltration of immune cells of UC. The ~~was used to evaluated the~~ differences in immune cell abundance between the UC group and healthy controls were evaluated, and samples with $P > 0.05$ were filtered. The correlation between the 22 immune cells ~~was~~ is shown in **Figure 7A.** Mast cells activated and NK cells resting had the highest positive correlation (r = 0.58~~),~~). T cells follicular helper and B cells naive also ~~showed~~ revealed a positive correlation (r = 0.51). Meanwhile, there ~~is~~ was a negative correlation between T cells follicular helper and macrophages M2, mast cells resting, and mast cells activated (r = -0.52). **Figure 7B** ~~showed~~ demonstrates the difference in the abundance of immune cell infiltration between the UC ~~group~~ and the healthy control groups. The levels of T cells CD4 memory activated ($P < 0.001$), T cells follicular helper ($P < 0.01$), macrophages M0 ($P < 0.001$), macrophages M1 ($P < 0.01$), dendritic cells activated ($P < 0.001$), mast cells activated ($P < 0.01$), eosinophils ($P < 0.05$), and neutrophils ($P < 0.01$) ~~levels~~ were significantly higher in the UC group than healthy controls. On the other hand, healthy controls ~~had~~ showed higher levels of NK cells activated ($P < 0.001$), macrophages M2 ($P < 0.001$), and mast cells resting ($P < 0.001$). Moreover, T cells CD4 memory activated, T cells follicular helper, macrophages M1, and neutrophils ~~showed~~ positively correlated~~positive correlation~~ with all the hub genes~~, a~~. Among them, neutrophils ~~showed~~ had the highest correlation (**Figure 7C**). Macrophage~~s~~ M2 ~~showed negative correlation~~negatively correlated with all the hub genes ($P < 0.05$). *AIM2* ~~had~~ showed the highest negative correlation with macrophage M2, *IL1B*, and

TREM1 ~~had~~ depicted the highest positive correlation with Neutrophils. These results indicated that a severe immune imbalance occurred in the colon of UC patients, and the relationship between the different types of macrophages and UC was ~~more~~ notable.

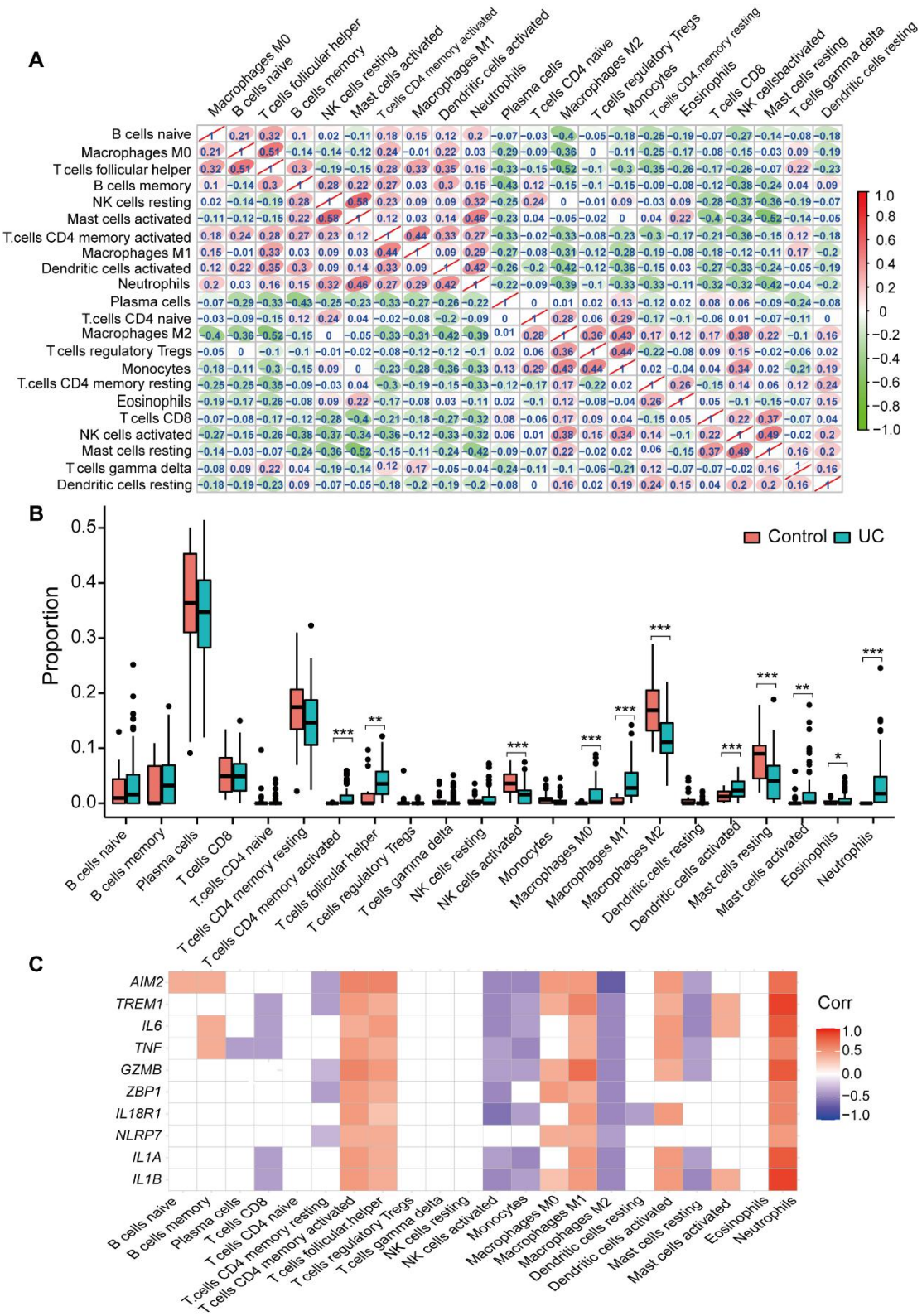
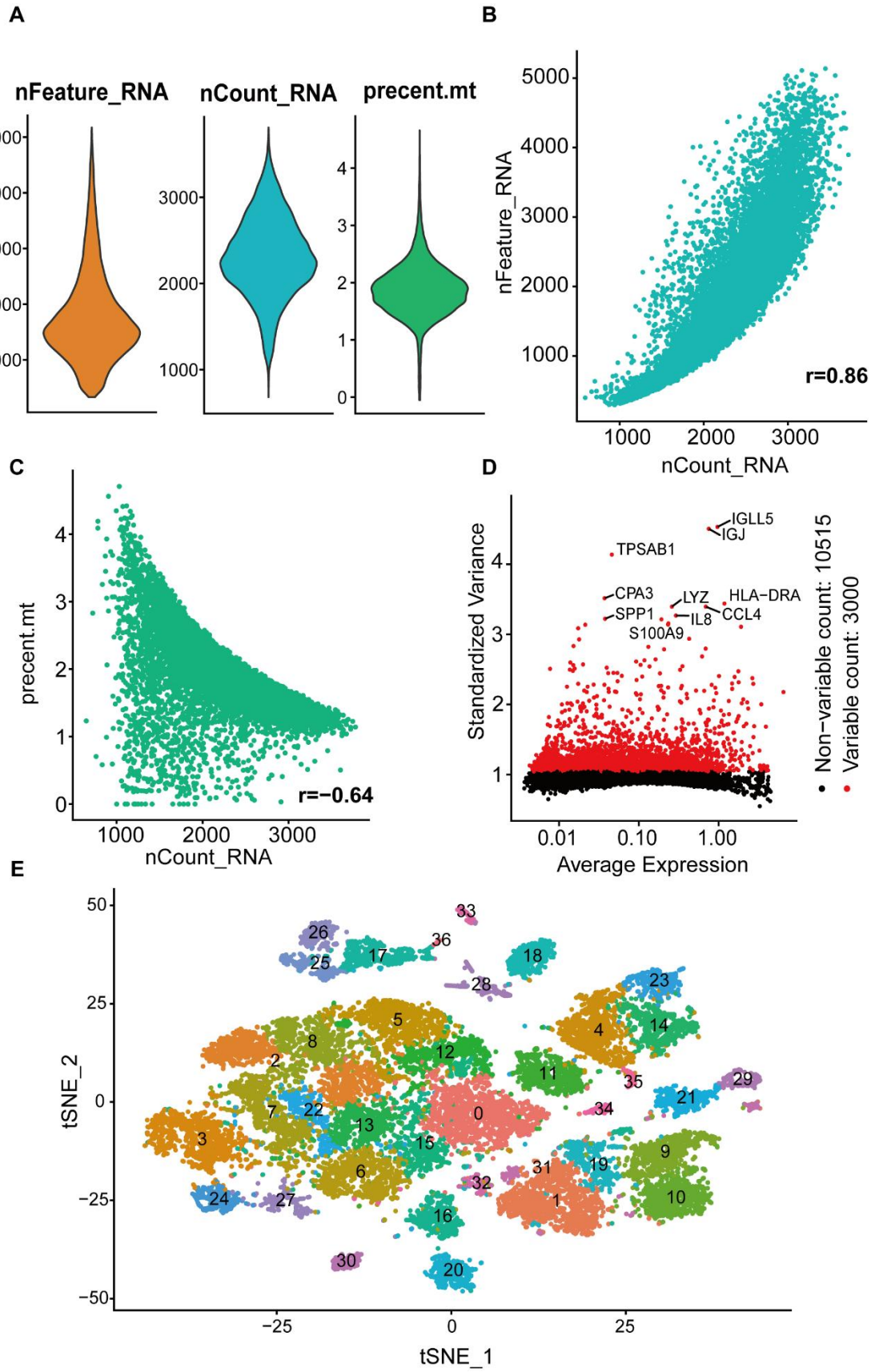


Figure 7. CIBERSORT probes the immune-infiltration landscape of UC. (A) ~~Interrelationship~~ The interrelationship between different infiltrating immune cells. The red to green indicates the change from high to low correlation. (B) Boxplot shows the ~~difference in colonic immune cell infiltration~~colonic immune cell infiltration difference between the control and UC groups. Blue represents the UC group; red represents the Control group. (C) The correlation between the hub gene and the immune cell. Blank area means the ~~S~~significant level is higher than 0.05 $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

Immune Cell scRNA Analysis of Colonic Lamina Propria in Inflamed UC

The GSE162335 dataset was ~~used~~ utilized for ~~single~~ single-cell analysis. After filtering, 18,375 immune cells from the colonic lamina propria of inflamed UC patients were ~~obtained~~collected. ~~Expression~~ The expression characteristics of the sample ~~was~~ are shown in **Figure 8**. We normalized the data and identified 3000 highly variable genes (HVG) using "VST~~",~~ ," of which the top 10 HVG were shown in **Figure 8D.** Principal component analysis ~~was performed~~ and heatmap of the top 10 PCs with signature genes ~~were~~ are shown in ~~the~~ **Figure S5.** ~~37~~ Thirty-seven ~~different was identified using the t-distributed stochastic neighbor embedding (t-SNE) method (**Figure 8E**), and the marker gene for each cell cluster were~~were identified using the t-distributed stochastic neighbor embedding (t-SNE) method (Figure 8E), and the marker gene for each cell cluster is available ~~from~~ in **Supplementary Table S6.**

Seven immune cells were identified ~~by~~ using different annotation methods, including B-cells, CD4+ T-cells, CD8+ T-cells, Fibroblasts, Hematopoietic Stem Cells (HSC), Macrophages, and NK cells (**Figure 9A**). B cells and CD4+ T-cells were the most numerous, while macrophages ~~could b~~were significantly divided into two clusters.



485

486 **Figure 8.** scRNA analysis of Colonic lamina propria ~~in~~ within inflamed UC. (A) The

487 genes (features), counts, and mitochondrial gene percentages of the sample. (B)

Correlation between genes and counts in the sample. (C) Correlation between genes and mitochondrial gene percentage in the sample. (D) The gene scatter plot with the top10 highly variable genes. The red dots represent the highly variable genes, and the black dots represent other genes. (E) t-SNE projection of 18,375 Immune cells~~. 18,375 Immune cells were~~ , clustered into 37 classes.

Macrophages Highly Correlated with Pyroptosis

First, the relationship ~~of~~ between immune cells and PRGs was explored, and we found that NK cells and macrophages had high AUC values (**Figure 9B-C**). Combining with the ~~results of the previous immune infiltration analysis~~previous immune infiltration analysis results, we hypothesized that macrophages could be more closely related to pyroptosis. Therefore, we performed a KEGG analysis of DEGs in macrophages. The results showed ~~DEGs were mainly enriched in inflammatory diseases and inflammatory signaling pathways, also~~that DEGs were mainly enriched in inflammatory diseases and signaling pathways and involved phagosome and lysosome (**Figure S6**).

We also analyzed the expression distribution of 10 hub genes ~~in~~ within various immune cells (**Figure 10A**). The results ~~showed~~ revealed that *TNF* was more widely distributed, and *NLRP7* was less distributed among the seven immune cells. ~~More interestingly, a~~Almost all hub genes were expressed in macrophages, especially *IL1B*, ~~as the more critical hub gene, was~~the more critical hub gene, strongly expressed (**Figure 10B-C**). This indicated that macrophages ~~may~~ could have a vital role in pyroptosis. Similarly, the high expression of *GZMB* in NK cells was also ~~noteworthy~~significant.

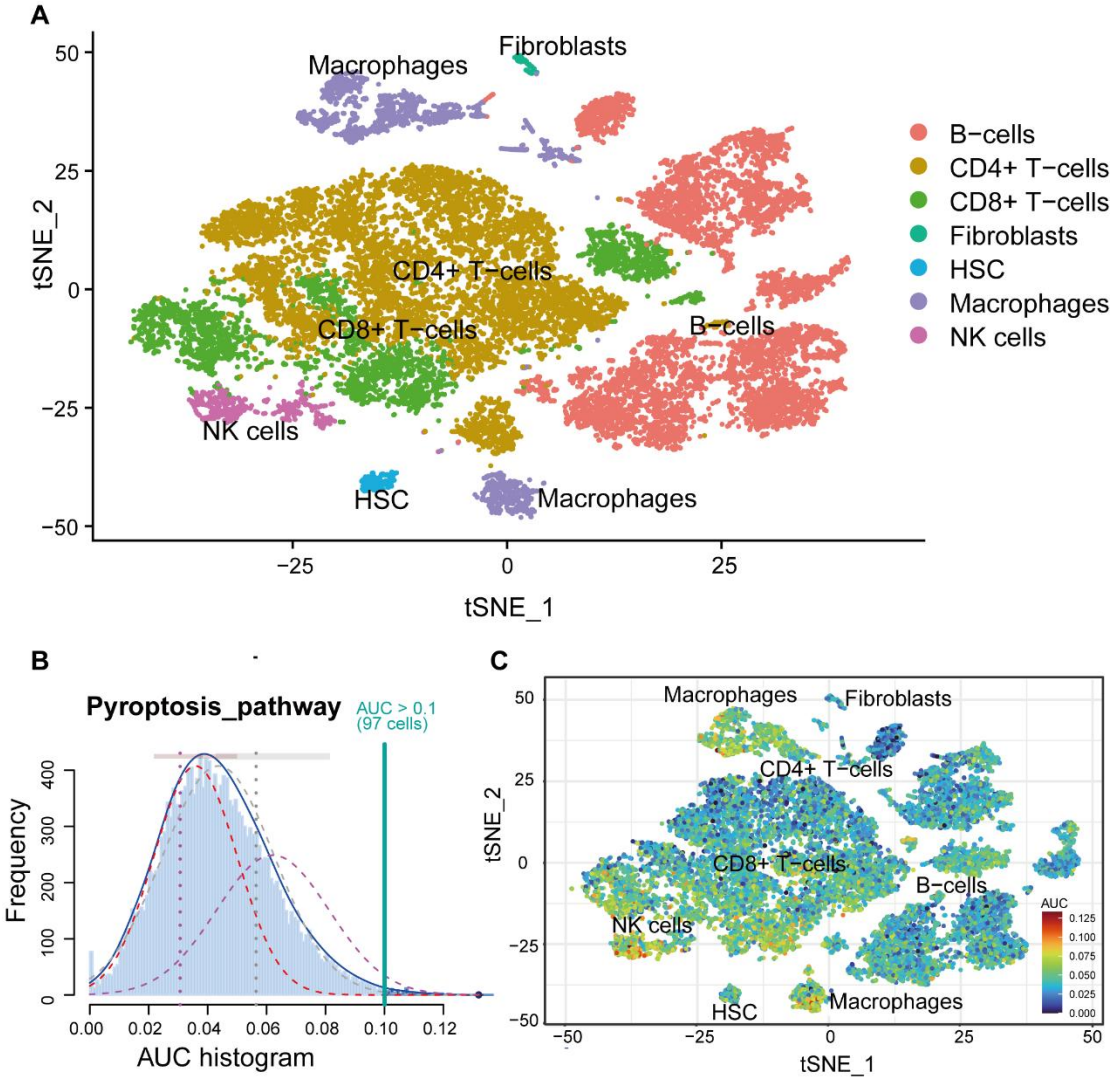


Figure 9. The ~~C~~cell annotation results and PRGs scores of colonic laminas propria immune cell types in inflamed UC. (A) The results of cell annotation with ~~.~~ ~~D~~different ~~Immune~~ immune cell types are ~~were colored with~~ distinctively colorsed. (B) ~~Score~~ A score of 74 screened PRGs. The threshold was chosen as 0.10. (C) t-SNE plots of RPGs score in all the cell types. Macrophages and NK cells express more genes and exhibit higher AUC values.

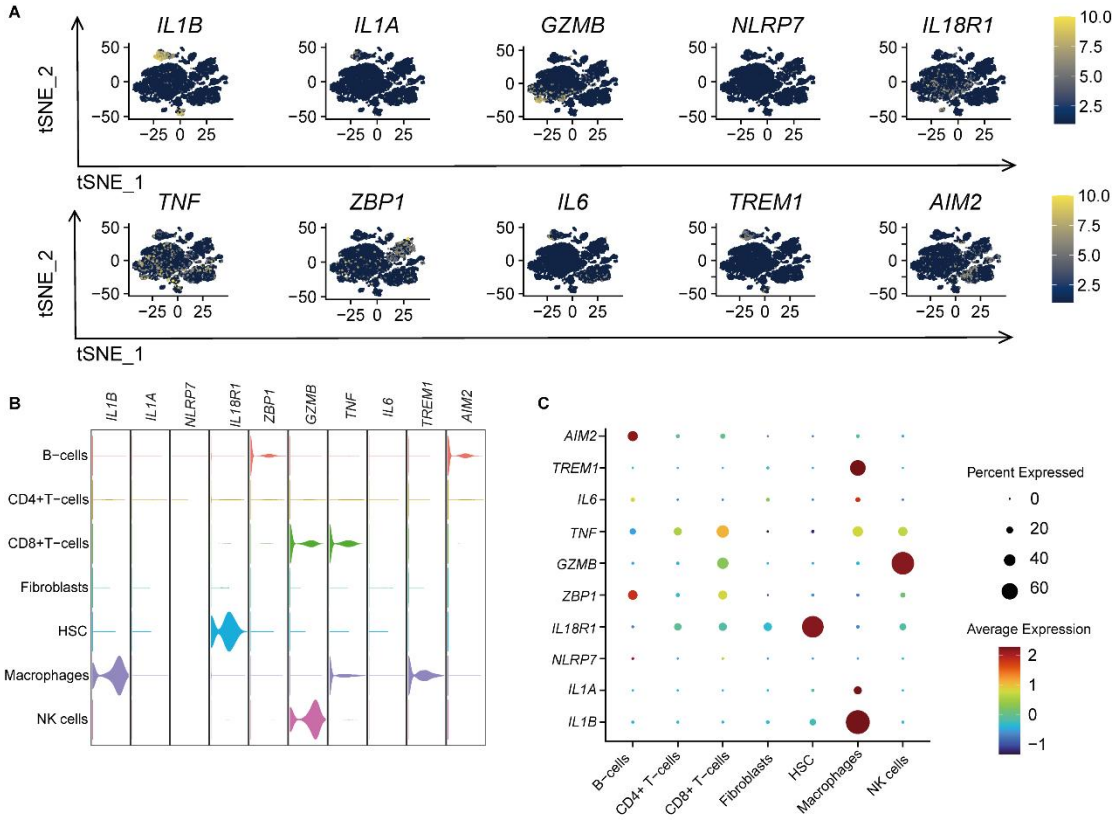


Figure 10. ~~10~~ Ten RPGs hub gene expression patterns in different immune cells. (A) The t-SNE plot shows the distribution of hub genes. (B) The Violin plot showed the expression levels of hub genes. (C) ~~Dot~~ The dot plot ~~showed~~ depicted the expression patterns of hub genes.

Pseudotime Analysis and Enrichment Analysis Identification of Pyroptosis Related-Macrophages Clusters in UC

~~To further reveal the relationship between macrophages and pyroptosis, w~~We performed a pseudotime analysis of the five macrophage clusters, including cluster17, 20, 25, 26, and 28, to reveal the relationship between macrophages and pyroptosis. The ~~analysis~~ research showed that almost all cells were projected onto two branches of a trunk, where clusters 17, 20, and 28 were located at one pole each, and clusters 25~~,~~ and 26 were on the evolutionary line (**Figure 11A, B**). Based on the expression of pyroptosis-related hub genes, cluster17 and ~~,~~ 20 were defined as *IL1B*$^{+}$*IL1A*$^{-}$*IL6*$^{-}$, cluster 25 ~~were~~ was ~~defined~~ described as *IL1B*$^{+}$*IL1A*$^{+}$*IL6*$^{+}$, cluster 26 ~~were~~ was ~~defined~~ designated as *IL1B*$^{+}$*IL1A*$^{+}$*IL6*$^{-}$, and cluster 28 ~~were~~ was defined as

IL1B-*IL1A*-*IL6*- cell group (**Figure 11C**). The expression of 74 RPGs in five cellular clusters was ~~evaluated~~ assessed (**Figure 11D**). Almost all the RPGs were not expressed in cluster 28, which also predicted that cluster 28 was ~~probably~~ not related to pyroptosis. Clusters 17~~,~~ and 20 had similar hub gene expression patterns, but cluster 17 expressed more RPGs than cluster 20.

~~To determine the molecular characteristics of the macrophages related with pyroptosis,~~ GO enrichment analysis of unique DEGs from each cluster ~~were~~ was ~~preformed~~ performed to determine the molecular characteristics of the macrophages related to pyroptosis (**Supplementary Table S7**). Clusters 17, 20, 25, and 26 identified 33, 120, 96, and 99 DEGs (**Figure 11E**). GO terms of BP ~~were~~ are shown in **Figure S7**. Enrichment analysis of cluster17 indicated this cluster was involved in ~~a number of~~several regulatory processes, including the positive regulation of immune response, ~~regulation of cell adhesion, positive regulation of~~cell adhesion regulation, and the immune system process (**Figure S7A**). Therefore, we named cluster17 as ~~“~~"Immunomodulatory macrophages~~”.~~." The GO terms in cluster 20 focused on the negative regulatory processes of cells, and cluster 20 was ~~named~~ termed ~~“~~"negative regulation macrophages~~”~~" (**Figure S7B**). The trajectory analysis of cluster25 ~~showed~~ revealed that it was located between cluster17~~,~~ and 20, and enrichment analysis showed that it was mainly focused on the response process to external stimuli and immunity~~, ; so~~ thus, it was named ~~as “~~"response macrophages~~”~~" (**Figure S7C**). Finally, cluster26 was named ~~as~~ a ~~“~~"transporter and secretory macrophages~~”~~" (**Figure S7D**). These results ~~suggested~~ indicated that macrophages related to pyroptosis ~~may~~ could be classified into four types and perform different functions in the colonic lamina propria of inflamed UC patients. Therefore, ~~E~~exploring the relationship between macrophages and pyroptosis may ~~contribute~~ facilitate~~to~~ a better understanding of ~~the occurrence of~~ pyroptosis occurrence in UC.

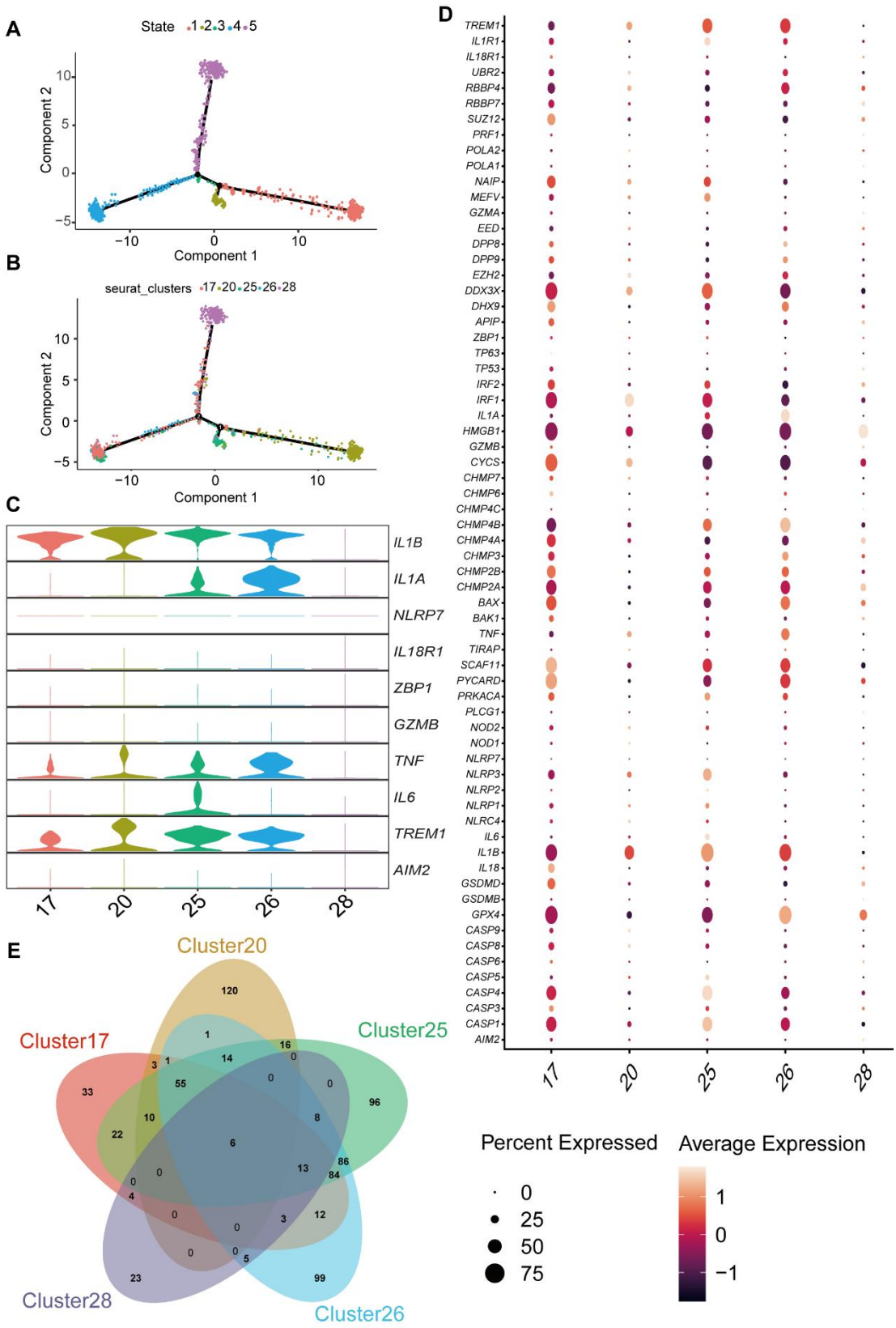


Figure 11. Pseudotime analysis of the clusters and expression patterns of PRGs hub genes ~~in~~ among different macrophage clusters. (A, B) Monocle pseudotime trajectory shows the progression of macrophages. (C) The Violin plot demonstrates the

expression levels of hub genes in five cluster macrophages. (D) ~~Dot~~ The dot plot shows the expression pattern of 74 RPGs in five cluster macrophages. (E) The ~~venn~~ Venn diagram ~~shows~~ depicts the DEGs ~~of~~ within each cluster macrophage.

Animal experiments to verify Hub gene expression levels

The DSS-induced acute colitis was ~~used~~ utilized to verify the gene expression of hub genes (**Figure 12A)**. The results ~~show~~ indicate that DSS-induced colitis in mice with bloody stools~~, which~~ was relieved ~~by~~ using 5-ASA treatment (**Figure 12B)**. DSS-induced colitis caused weight loss and shorter colonic length in mice, and 5-ASA could ~~slow~~ suppress ~~down~~ the weight loss and colon shortening (**Figure 12C-D)**. The symptoms ~~that~~ commonly occurringred during UC, ~~such as~~including bloody stools and shortening of the colon, are evidence of ~~increased~~ enhanced abnormal death of IECs. ~~To further observe the damage of intestinal mucosa during UC,~~ ~~w~~We performed histological staining and examination of colon sections of mice to observe the damage of intestinal mucosa during UC.~~,~~ ~~and w~~Compared with the NC group, we found a series of features such as disorganized mucosal structure, inflammatory cell infiltration, crypt detachment, and IECs death in colon sections of mice in the DSS~~e observed a series of features such as disorganized mucosal structure, inflammatory cell infiltration, crypt detachment and IECs death in colon sections of mice in the DSS group compared with the NC~~ group. In contrast, 5-ASA treatment showed a protective effect on colonic mucosal structures **(Figure 12E)**. ~~All of t~~There~~is~~ is strong evidence that UC is highly correlated with the death of IECs, ~~disruption of the intestinal barrier, and that~~intestinal barrier disruption, and therapeutic drugs can alleviate these symptoms. ~~Consistently~~Therefore, inflammatory factor (TNF-α) in serum of ~~DSS~~ DSS-induced mice ~~were~~ was increased, while 5-ASA intervention ~~reduced~~ decreased the elevation of TNF-α (**Figure 12F**). ~~As shown in~~ **Figure 12G-O** shows~~,~~ that the relative expression levels of hub genes in DSS-induced colitis mice ~~were~~ are consistent with bioinformatics analysis. The expression of most genes was elevated in the DSS group. Genes with inconsistent expression ~~may~~could

be due to the species differences between mice and humans; *NLRP7* ~~has been shown to be~~is associated with intestinal diseases such as colon cancer (33), but only a human-specific gene is ~~that~~ not found in rodents (34). *ZBP1*, a ~~key~~ critical innate sensor that recognizes and binds Z-RNA structures produced ~~mainly~~ by various viruses, triggers different forms of cell death (35)~~, yet~~. However, microorganisms with such structures ~~may~~ could be lacking in the pathogenic factors of DSS-induced colitis mice (36). A further ~~finding~~outcome was that 5-ASA significantly downregulated only one gene, *IL1B*, ~~and had no~~without any significant effect on other genes~~,~~. It ~~suggesting~~ suggests that there may be no relationship between the relief of colitis by 5-ASA and the regulation of pyroptosis, consistent with our previous ~~prediction~~findings.

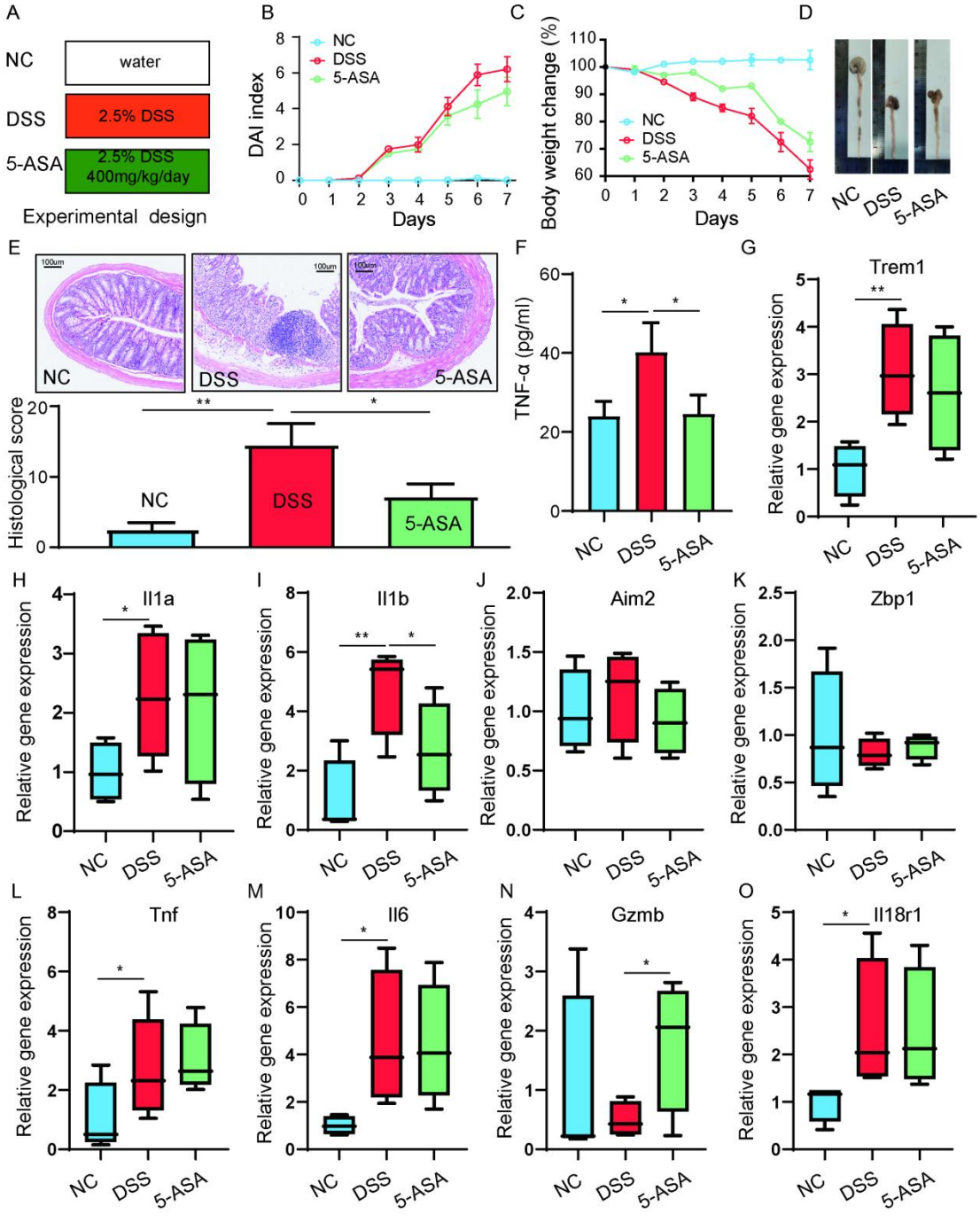


Figure 12. Animal experiments to verify the Hub gene expression levels. (A) Experimental design. (B) DAI index. (C) Body weight change. (D) Length of colons. (E) H&E-stained colon tissue. (F) Serum TNF-α was determined ~~using~~ with ELISA. The relative gene expression levels of *Trem1* (G), *Il1a* (H), *Il1b* (I), *Aim2* (J), *Zbp1* (K), *Tnf* (L), *Il6* (M), *Gzmb* (N), and *Il18r1* (O) were determined ~~using~~ with RT-PCR. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

DISCUSSION

UC is a common chronic inflammatory intestinal disease ~~of the intestine~~. The life quality of UC patients is ~~seriously~~ severely impaired due to its recurrent and incurable characteristics (37). A~~t present, although some progress has been made in the treatment of~~lthough some progress has been made in treating UC, further mitigation of recurrence and eradication remains a global challenge. Pyroptosis, ~~as~~ a new form of cell death discovered ~~in recent years~~recently, has shown great promise in UC (38). For example, ~~One has been~~a ~~reported~~ study claims that DSS potentiates NLRP3 inflammasome activation by ~~modulating~~ regulating the KCa3.1 potassium channel in a mouse model of colitis~~,~~. This provides direct evidence for the role of pyroptosis in UC (39). In another study, the authors ~~discovered~~ observed that Trans-10-Hydroxy-2-~~Decenoic~~ Decanoic ~~A~~acid could treat UC by inhibiting pyroptosis~~,~~ while also enhancing the barrier function of the colon (40). However, there is still a lack of reports ~~on~~ concerning the detailed mechanism of Pyroptosis in UC. Our study ~~attempts to explore the role of pyroptosis in UC by identification of~~explores the role of pyroptosis in UC by identifying the relevant hub genes and immune cells~~, t~~. This will provide a new perspective ~~for~~ on the pathogenesis and treatment of UC.

A total of 1,247 DEGs and 1,186 module genes were obtained ~~by~~ using differential ~~analysis~~ and WGCNA ~~analysis~~analyses. The ~~results of the enrichment analysis~~enrichment analysis ~~s showed~~revealed that these genes were ~~mainly~~ primarily involved in ~~inflammatory pathways related to pyroptosis~~pyroptosis-related inflammatory pathways, including NF-kappa B signaling pathway, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, and TNF signaling pathway. This suggested that there is a close relationship between UC and pyroptosis. Subsequently, ~~we identified~~ 10 pyroptosis-related hub genes (*AIM2*, *IL6*, *IL1B*, *NLRP7*, *TNF*, *IL1A*, *IL18R1*, *ZBP1*, *GZMB*, *TREM1*) were identified. *AIM2* is a

cytoplasmic sensor of double-stranded DNA from pathogens or damaged organelles. It recruits ASC and caspase-1 to ~~form~~ develop the *AIM2* inflammasome, ~~which~~ activate~~s~~ing caspase-1 and ~~the processing of~~processing IL-1β and IL-18, ~~causing~~ leading to pyroptosis (41). Innate immune sensor Z-DNA binding protein 1 (*ZBP1*) ~~as~~ is the apical sensor of fungal infection and ~~regulates~~ controls the NLRP3 inflammasome to participate in pyroptosis (42). ~~Notably, ~~ ~~i~~It has been reported that *AIM2* in complex with pyrin and *ZBP1* can ~~be engaged in pyroptosis, apoptosis, and necroptosis simultaneously~~simultaneously engage in pyroptosis, apoptosis, and necroptosis, ~~this~~ which is probably a ~~new~~ novel research direction (43). *IL1B*, *IL1A*, *IL6*, and *TNF* are important pro-inflammatory cytokines in pyroptosis (44). *IL18R1* is the receptor for pro-inflammatory cytokine IL18 and is responsible for the binding of IL18 (45). The interleukin-1 cytokine family play~~s~~ a ~~key~~ crucial role in maintaining intestinal barrier integrity and inflammatory responses, but their specific function~~s~~ ~~remains~~ are controversial. ~~More and more~~Various studies ~~suggest~~ indicate ~~that they play~~their ~~a~~ dual role in inflammation and homeostasis *in vivo* (46). *GZMB* catalyzes the cleavage of gasdermin-E (GSDME) and releases the pore-forming fraction of GSDME, thereby triggering pyroptosis (47). *TREM1* amplifies inflammatory signals involving Toll-like ~~receptors~~ and NOD-like receptors, ~~and in this way~~which mediates the exacerbation of pyroptosis (48). *NLRP7* is ~~related~~ associated with~~to~~ innate immune signaling and affects macrophage polarization, but its exact role ~~remains~~ is unclear~~somewhat controversial~~. ~~With~~ In recent ~~the~~ research ~~in recent years~~, different pathways of pyroptosis have been identified (49). However, the specific mechanism of pyroptosis in UC remains poorly ~~deciphered~~understood. Based on the 10 hub genes identified, we hypothesized that there ~~may~~ could be two main pyroptosis pathways in UC.~~,~~ ~~f~~Firstly the *AIM2* inflammasome-mediated caspase-1-dependent classical pyroptosis pathway was ~~formed~~ developed after dsDNA recognition by *AIM2* (50), and the caspase-independent pyroptosis pathway was ~~mediated~~ controlled by *GZMB* (51). *AIM2*, *TREM1*, LI1B, *GZMB*, *IL18R1*, ~~*ZBP1* were identified as~~*and ZBP1* were diagnostic biomarkers. The results of ROC analysis indicated that *IL1B* has the ~~greatest~~ most significant potential as a biomarker.

We observed the expression patterns of hub genes ~~in~~ within the lesional/nonlesional, active/inactive/control groups by different datasets~~,~~. ~~and a~~All the results showed significant differences except for inactive/control groups~~,~~. ~~A~~and among the inactive/control groups, *IL1B* became the only hub gene with significant differences. IL-1β is a ~~powerful~~ potent pro-inflammatory factor that plays a ~~key~~ crucial role in pyroptosis. The synthesis of pro-IL-1β is stimulated in response to an inflammatory signal~~, and t~~. Then ~~when a large number of~~, when many inflammatory vesicles activate caspase 1, pro-IL-1β is cleaved to ~~form~~ synthesize IL-1β, and subsequently, caspase-1 cleaves the GSDMD to ~~form~~ create the 22 kDa C-terminus (C-GSDMD) and 31 kDa N-terminal (N-GSDMD). N-GSDMD completes by ~~forming~~ forming pores in the plasma membrane and mitochondria to release IL-1β and IL-18~~, t~~. Thus, *IL1B* is a critical part of pyroptosis. ~~There have been many reports~~Many reports have revealed that targeting *IL1B* can effectively alleviate UC (52). Combined with the previous ROC ~~analysis~~analyses, we speculate that *IL1B* is the most crucial gene in the pyroptosis of UC.

A characteristic feature of UC is that 80-90% of patients have alternated between active and inactive intervals (53). The significant difference between the active and inactive phases of the hub gene ~~may~~ could indicate~~d~~ the ~~increased~~ elevated expression of the hub gene in patients, ~~resulting~~ causing~~in~~ damage to the intestinal mucosa triggered by pyroptosis in the IECs and an increase in pro-inflammatory factors, ~~causing~~ leading to recurrent disease (54). ~~And~~ Thus, focusing on ~~the development of~~ pyroptosis development is probably a new ~~idea~~ way of ~~thinking to~~ avoiding the recurrence of UC and a~~n important~~ crucial ~~direction for~~ future treatment direction. Moreover, we also explored the effects of some common UC drugs on hub genes to ~~better reveal the significance of pyroptosis in UC~~reveal the significance of pyroptosis in UC better. 5-ASA and biologics are common UC drugs but ~~showed~~ showed different effects on the hub gene. 5-ASA reduced the expression levels of *IL1B*, *IL1A*, and *IL6*~~,~~ but ~~had no effect on~~did not affect other hub genes. Biologics such as IFX~~,~~ and VDZ significantly ~~reduced~~ decreased all hub genes. For 30 years, 5-ASA has

been the treatment of choice ~~for~~ against mild UC (55). However, a recent study ~~has~~ shown~~ed~~ that compared to biologic agents such as IFX and VDZ, 5-ASA ~~was not helpful in the treatment of~~did not help treat patients with moderate to severe UC, especially in controlling inflammation (56). Therefore, we speculated that the progression of UC disease ~~may~~ could be ~~related~~ associated with~~to~~ the degree of pyroptosis, and patients with severe UC may be subjected to a higher degree of intestinal mucosa invasion ~~by~~ due to pyroptosis. ~~, and t~~Therefore, 5-ASA is not effective in treating patients ~~with~~ having severe UC. In conclusion, we verified that both UC drugs had a different effect on the pyroptosis-related hub gene and ~~reduced~~ decreased its expression while alleviating UC. The link between pyroptosis and the progression of UC deserves ~~more~~ in-depth exploration~~, especially, the expression of relevant genes/proteins need~~. The expression of relevant genes/proteins needs to be verified by using the tissue ~~of~~ from the patients. ~~By r~~Reviewing the research literature ~~using~~ with UC clinical samples, ~~we found that~~ many previous studies reported the test results of the hub genes in patient tissues. For ~~example~~instance, IL-1β in serum and tissues of UC patients was measured by ELISA, and the results ~~showed~~ depicted that the expression of IL-1β in UC patients was significantly higher than ~~that~~ in healthy controls (57, 58). In addition, the flow cytometry analysis ~~showed~~ revealed that the number of cells expressing GZMB and TREM1 in the intestinal mucosa of UC patients significantly increased ~~significantly~~(59, 60). Using western blot analysis, the expression of AIM2 in active UC is higher than ~~that~~ in inactive UC(28). These are consistent with the results ~~of~~ from our bioinformatics ~~analysis~~analyses.

~~To further explore the dysregulation of inflammatory cells in UC, an immune Infiltration analysis was performed~~An immune Infiltration analysis was performed to explore the dysregulation of inflammatory cells in UC. The results showed that~~,~~ immune cells were severely dysregulated ~~in~~ within the colonic tissue of UC patients. Neutrophils, Macrophages M0, M1, Dendritic cells activated, T cells CD4 memory resting, and T cells follicular helper were positively ~~correlated~~ associated with hub genes. ~~The results of s~~Single-cell analysis showed ~~that 9~~ nine hub genes were distributed in macrophages except for *NLRP7*, and *IL1B* was strongly expressed. In

addition, macrophages were identified by AUCell evaluation to ~~show~~ deliver the highest degree of response to the pyroptosis gene set. By ~~increasing~~ enhancing the resolution, we identified ~~5~~ five clusters of macrophages. ~~Among them, f~~Four clusters ~~are~~ were closely ~~related~~ associated with~~to~~ pyroptosis and may perform different biological functions. The ~~P~~pro-inflammatory and anti-inflammatory functions of macrophages ~~play~~ have a ~~key~~ crucial role in the development of UC, and many drugs alleviate UC by targeting the macrophages (61, 62). However, the interaction between different immune cell types ~~still needs~~requires further exploration. More importantly, the alleviation of UC by inhibiting macrophage pyroptosis has been ~~reported~~ described in recent years (15). It was also shown that the Lactic Acid-Producing Probiotic *Saccharomyces cerevisiae* inhibits the hyperactivation of the NLRP3 inflammasome and downstream caspase-1 pathway ~~in~~ among macrophages, thereby inhibiting ~~the process of~~ pyroptosis ~~in macrophages~~. When macrophage pyroptosis was inhibited, the colonic mucosal barrier was strengthened, the immune response in the intestine ~~was decreased, while~~decreased, and histological damage was ~~also~~ restored.

Abnormal death of IECs ~~leads to~~causes damage ~~of~~ to the intestinal mucosa, and this structural weakening of the intestinal mucosal barrier is an early event in ~~the~~ UC pathogenesis ~~of UC~~ (54). Pyroptosis is a~~n important~~ necessary form~~s~~ of death for IECs~~, and a~~. A series of pro-inflammatory factors secreted during pyroptosis, ~~such as~~including IL-18, and IL-1β, can disrupt the integrity of the intestinal mucosal barrier (63). Therefore, excessive death of IECs and the secretion of pro-inflammatory factors ~~caused~~ due to~~by~~ excessive activation of pyroptosis are ~~important~~ essential factors in disrupting the intestinal barrier, which ~~may~~ could be a critical mechanism in ~~the~~ UC pathogenesis ~~of UC~~. After ~~the disruption of the intestinal barrier, microorganisms in the gut invade into the intestine causing a series of immune responses while macrophages themselv~~disrupting the intestinal barrier, microorganisms in the gut invade the intestine, causing a series of immune responses while macrophag~~es undergo pyroptosis. The pro-inflammatory factors released in pyroptosis ~~may~~ could worsen the immune response in the intestine and lead to more

death of IECs, ~~which further disrupts the intestinal barrier and t~~further disrupting the intestinal barrier. This may be one of the reasons why UC is incurable and recurrent.

CONCLUSION

Ten ~~pyroptosis~~ pyroptosis-related hub genes in UC were identified, and the expression pattern of hub genes was validated. The effect of the existing UC treatment drugs on the gene expression of hub genes ~~were~~ was explored, and *IL1B* was identified as ~~a~~ the predictor for drug response and marker for the active state of UC. Combining single cell analysis and immune infiltration, we ~~determined~~ identified ~~that~~ macrophages as the most relevant immune cell type in ~~the~~ UC progression ~~of UC~~. Our study explored the molecular mechanisms of the pyroptosis process in UC ~~and~~. We proposed that the crosstalk ~~regarding pyroptosis~~ between macrophages and IECs regarding pyroptosis ~~is probably~~could be responsible for the incurability and recurrence of UC. The *IL1B*-macrophage-pyroptosis relationship chain provides a new perspective ~~for~~ on the pathogenesis and treatment of UC.

Supplementary data

Supplementary data are available online.

Acknowledgement

This study was supported by National Natural Science Foundation of China (NSFC) [grant number 31900031] and Shandong Provincial Natural Science Foundation [grant number ZR2019BD027]. We sincerely thank the Professor Shumei Zhang from College of Information and Computer Engineering of Northeast Forestry University for reviewing the statistical methods in this study.

Author contributions

N.H and S.L conceived the study and performed data analysis. K.C. and S.S. collected data and performed data analysis. S.Y and L.C performed animal experiments and related tests. N.H and S.L supervised the project. K.C., S.S., S.L and N.H. wrote the manuscript. All authors approved the final manuscript.

Data Availability Statement

The datasets presented in this study can be found in online repositories. These datasets were freely downloaded from GEO database.

Code Availability Statements

The code for data analysis have been uploaded to Github (https://github.com/illusion621/Identification-and-Exploration-of-Pharmacological-pyroptosis-related-Biomarkers-of-Ulcerative-Coliti

References

1. Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative Colitis. Lancet (London, England) (2012) 380(9853):1606-19. Epub 2012/08/24. doi: 10.1016/s0140-6736(12)60150-0.
2. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative Colitis. Lancet (London, England) (2017) 389(10080):1756-70. Epub 2016/12/05. doi: 10.1016/s0140-6736(16)32126-2.
3. Ozaki R, Kobayashi T, Okabayashi S, Nakano M, Morinaga S, Hara A, et al. Histological Risk Factors to Predict Clinical Relapse in Ulcerative Colitis with Endoscopically Normal Mucosa. Journal of Crohn's & colitis (2018) 12(11):1288-94. Epub 2018/06/26. doi: 10.1093/ecco-jcc/jjy092.
4. Xavier RJ, Podolsky DK. Unravelling the Pathogenesis of Inflammatory Bowel Disease. Nature (2007) 448(7152):427-34. Epub 2007/07/27. doi: 10.1038/nature06005.
5. Rozich JJ, Dulai PS, Fumery M, Sandborn WJ, Singh S. Progression of Elderly Onset Inflammatory Bowel Diseases: A Systematic Review and Meta-Analysis of Population-Based Cohort Studies. Clin Gastroenterol Hepatol (2020) 18(11):2437-47.e6. Epub 2020/03/07. doi: 10.1016/j.cgh.2020.02.048.
6. Patankar JV, Becker C. Cell Death in the Gut Epithelium and Implications for Chronic Inflammation. Nature reviews Gastroenterology & hepatology (2020) 17(9):543-56. Epub 2020/07/12. doi: 10.1038/s41575-020-0326-4.

7. Okumura R, Takeda K. Roles of Intestinal Epithelial Cells in the Maintenance of Gut Homeostasis. Experimental & molecular medicine (2017) 49(5):e338. Epub 2017/05/27. doi: 10.1038/emm.2017.20.

8. Iwamoto M, Koji T, Makiyama K, Kobayashi N, Nakane PK. Apoptosis of Crypt Epithelial Cells in Ulcerative Colitis. The Journal of pathology (1996) 180(2):152-9. Epub 1996/10/01. doi: 10.1002/(sici)1096-9896(199610)180:2<152::Aid-path649>3.0.Co;2-y.

9. Baxt LA, Xavier RJ. Role of Autophagy in the Maintenance of Intestinal Homeostasis. Gastroenterology (2015) 149(3):553-62. Epub 2015/07/15. doi: 10.1053/j.gastro.2015.06.046.

10. Kovacs SB, Miao EA. Gasdermins: Effectors of Pyroptosis. Trends in cell biology (2017) 27(9):673-84. Epub 2017/06/18. doi: 10.1016/j.tcb.2017.05.005.

11. Kayagaki N, Stowe IB, Lee BL, O'Rourke K, Anderson K, Warming S, et al. Caspase-11 Cleaves Gasdermin D for Non-Canonical Inflammasome Signalling. Nature (2015) 526(7575):666-71. Epub 2015/09/17. doi: 10.1038/nature15541.

12. Wu C, Lu W, Zhang Y, Zhang G, Shi X, Hisada Y, et al. Inflammasome Activation Triggers Blood Clotting and Host Death through Pyroptosis. Immunity (2019) 50(6):1401-11.e4. Epub 2019/05/12. doi: 10.1016/j.immuni.2019.04.003.

13. Szekanecz Z, Szamosi S, Kovács GE, Kocsis E, Benkő S. The Nlrp3 Inflammasome - Interleukin 1 Pathway as a Therapeutic Target in Gout. Archives of biochemistry and biophysics (2019) 670:82-93. Epub 2019/02/03. doi: 10.1016/j.abb.2019.01.031.

14. Marchiando AM, Shen L, Graham WV, Edelblum KL, Duckworth CA, Guan Y, et al. The Epithelial Barrier Is Maintained by in Vivo Tight Junction Expansion During Pathologic Intestinal Epithelial Shedding. Gastroenterology (2011) 140(4):1208-18.e1-2. Epub 2011/01/18. doi: 10.1053/j.gastro.2011.01.004.

15. Sun S, Xu X, Liang L, Wang X, Bai X, Zhu L, et al. Lactic Acid-Producing Probiotic Saccharomyces Cerevisiae Attenuates Ulcerative Colitis Via Suppressing Macrophage Pyroptosis and Modulating Gut Microbiota. Front Immunol (2021) 12:777665. Epub 2021/12/14. doi: 10.3389/fimmu.2021.777665.

16. Zhu J, Xu Y, Li Z, Liu S, Fu W, Wei Y. Interleukin-36β Exacerbates Dss-Induce Acute Colitis Via Inhibiting Foxp3(+) Regulatory T Cell Response and Increasing Th2 Cell Response. International immunopharmacology (2022) 108:108762. Epub 2022/04/19. doi: 10.1016/j.intimp.2022.108762.

17. Ye Y, Dai Q, Qi H. A Novel Defined Pyroptosis-Related Gene Signature for Predicting the Prognosis of Ovarian Cancer. Cell death discovery (2021) 7(1):71. Epub 2021/04/09. doi: 10.1038/s41420-021-00451-x.

18. Wei R, Li S, Yu G, Guan X, Liu H, Quan J, et al. Deciphering the Pyroptosis-Related Prognostic Signature and Immune Cell Infiltration Characteristics of Colon Cancer. Front Genet (2021) 12:755384. Epub 2021/10/30. doi: 10.3389/fgene.2021.755384.

19. Li K, Strauss R, Ouahed J, Chan D, Telesco SE, Shouval DS, et al. Molecular Comparison of Adult and Pediatric Ulcerative Colitis Indicates Broad Similarity of Molecular Pathways in Disease Tissue. Journal of pediatric gastroenterology and nutrition (2018) 67(1):45-52. Epub 2018/02/06. doi: 10.1097/mpg.0000000000001898.

20. Tang J, Kong D, Cui Q, Wang K, Zhang D, Gong Y, et al. Prognostic Genes of Breast Cancer Identified by Gene Co-Expression Network Analysis. Front Oncol (2018) 8:374. Epub 2018/09/27. doi: 10.3389/fonc.2018.00374.

21. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust Enumeration of Cell Subsets from Tissue Expression Profiles. Nature methods (2015) 12(5):453-7. Epub 2015/03/31. doi: 10.1038/nmeth.3337.

22. Devlin JC, Axelrad J, Hine AM, Chang S, Sarkar S, Lin JD, et al. Single-Cell Transcriptional Survey of Ileal-Anal Pouch Immune Cells from Ulcerative Colitis Patients. Gastroenterology (2021) 160(5):1679-93. Epub 2020/12/29. doi: 10.1053/j.gastro.2020.12.030.

23. Hao Y, Hao S, Andersen-Nissen E, Mauck WM, 3rd, Zheng S, Butler A, et al. Integrated Analysis of Multimodal Single-Cell Data. Cell (2021) 184(13):3573-87.e29. Epub 2021/06/02. doi: 10.1016/j.cell.2021.04.048.

24. Han X, Zhou Z, Fei L, Sun H, Wang R, Chen Y, et al. Construction of a Human

880 Cell Landscape at Single-Cell Level. Nature (2020) 581(7808):303-9. Epub
881 2020/03/28. doi: 10.1038/s41586-020-2157-4.
882 25. Lu Y, Li K, Hu Y, Wang X. Expression of Immune Related Genes and Possible
883 Regulatory Mechanisms in Alzheimer's Disease. Front Immunol (2021) 12:768966.
884 Epub 2021/11/23. doi: 10.3389/fimmu.2021.768966.
885 26. Wang L, He T, Liu J, Tai J, Wang B, Zhang L, et al. Revealing the Immune
886 Infiltration Landscape and Identifying Diagnostic Biomarkers for Lumbar Disc
887 Herniation. Front Immunol (2021) 12:666355. Epub 2021/06/15. doi:
888 10.3389/fimmu.2021.666355.
889 27. Sandborn WJ, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, et al.
890 Subcutaneous Golimumab Induces Clinical Response and Remission in Patients with
891 Moderate-to-Severe Ulcerative Colitis. Gastroenterology (2014) 146(1):85-95; quiz
892 e14-5. Epub 2013/06/06. doi: 10.1053/j.gastro.2013.05.048.
893 28. Vanhove W, Peeters PM, Staelens D, Schraenen A, Van der Goten J, Cleynen I, et
894 al. Strong Upregulation of Aim2 and Ifi16 Inflammasomes in the Mucosa of Patients
895 with Active Inflammatory Bowel Disease. Inflammatory bowel diseases (2015)
896 21(11):2673-82. Epub 2015/08/28. doi: 10.1097/mib.0000000000000535.
897 29. Yuan C. Il-33 in Autoimmunity; Possible Therapeutic Target. International
898 immunopharmacology (2022) 108:108887. Epub 2022/06/23. doi:
899 10.1016/j.intimp.2022.108887.
900 30. Arijs I, De Hertogh G, Lemmens B, Van Lommel L, de Bruyn M, Vanhove W, et
901 al. Effect of Vedolizumab (Anti-A4β7-Integrin) Therapy on Histological Healing and
902 Mucosal Gene Expression in Patients with Uc. Gut (2018) 67(1):43-52. Epub
903 2016/11/02. doi: 10.1136/gutjnl-2016-312293.
904 31. Le Berre C, Roda G, Nedeljkovic Protic M, Danese S, Peyrin-Biroulet L. Modern
905 Use of 5-Aminosalicylic Acid Compounds for Ulcerative Colitis. Expert opinion on
906 biological therapy (2020) 20(4):363-78. Epub 2019/09/10. doi:
907 10.1080/14712598.2019.1666101.
908 32. Bhattacharya A, Osterman MT. Biologic Therapy for Ulcerative Colitis.
909 Gastroenterology clinics of North America (2020) 49(4):717-29. Epub 2020/10/31.

910 doi: 10.1016/j.gtc.2020.08.002.
911 33. Li B, Qi ZP, He DL, Chen ZH, Liu JY, Wong MW, et al. Nlrp7 Deubiquitination
912 by Usp10 Promotes Tumor Progression and Tumor-Associated Macrophage
913 Polarization in Colorectal Cancer. J Exp Clin Cancer Res (2021) 40(1):126. Epub
914 2021/04/12. doi: 10.1186/s13046-021-01920-y.
915 34. Amoushahi M, Sunde L, Lykke-Hartmann K. The Pivotal Roles of the Nod-Like
916 Receptors with a Pyd Domain, Nlrps, in Oocytes and Early Embryo Development†.
917 Biology of reproduction (2019) 101(2):284-96. Epub 2019/06/16. doi:
918 10.1093/biolre/ioz098.
919 35. Yang D, Liang Y, Zhao S, Ding Y, Zhuang Q, Shi Q, et al. Zbp1 Mediates
920 Interferon-Induced Necroptosis. Cellular & molecular immunology (2020)
921 17(4):356-68. Epub 2019/05/12. doi: 10.1038/s41423-019-0237-x.
922 36. Balachandran S, Mocarski ES. Viral Z-Rna Triggers Zbp1-Dependent Cell Death.
923 Current opinion in virology (2021) 51:134-40. Epub 2021/10/25. doi:
924 10.1016/j.coviro.2021.10.004.
925 37. Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and Natural
926 History of Inflammatory Bowel Diseases. Gastroenterology (2011) 140(6):1785-94.
927 Epub 2011/05/03. doi: 10.1053/j.gastro.2011.01.055.
928 38. Wu G, Zhang D, Yang L, Wu Q, Yuan L. Microrna-200c-5p Targets Nima Related
929 Kinase 7 (Nek7) to Inhibit Nod-Like Receptor 3 (Nlrp3) Inflammasome Activation,
930 Mode-K Cell Pyroptosis, and Inflammatory Bowel Disease in Mice. Molecular
931 immunology (2022) 146:57-68. Epub 2022/04/22. doi:
932 10.1016/j.molimm.2022.03.121.
933 39. Zeng B, Huang Y, Chen S, Xu R, Xu L, Qiu J, et al. Dextran Sodium Sulfate
934 Potentiates Nlrp3 Inflammasome Activation by Modulating the Kca3.1 Potassium
935 Channel in a Mouse Model of Colitis. Cellular & molecular immunology (2022)
936 19(8):925-43. Epub 2022/07/08. doi: 10.1038/s41423-022-00891-0.
937 40. Huang S, Tao R, Zhou J, Qian L, Wu J. Trans-10-Hydroxy-2-Decenoic Acid
938 Alleviates Dextran Sulfate Sodium-Induced Colitis in Mice Via Regulating the
939 Inflammasome-Mediated Pyroptotic Pathway and Enhancing Colonic Barrier

940 Function. Molecular nutrition & food research (2022) 66(12):e2100821. Epub
941 2022/04/05. doi: 10.1002/mnfr.202100821.
942 41. Wang B, Tian Y, Yin Q. Aim2 Inflammasome Assembly and Signaling. Adv Exp
943 Med Biol (2019) 1172:143-55. Epub 2019/10/20. doi: 10.1007/978-981-13-9367-9_7.
944 42. Kuriakose T, Man SM, Malireddi RK, Karki R, Kesavardhana S, Place DE, et al.
945 Zbp1/Dai Is an Innate Sensor of Influenza Virus Triggering the Nlrp3 Inflammasome
946 and Programmed Cell Death Pathways. Science immunology (2016) 1(2). Epub
947 2016/12/06. doi: 10.1126/sciimmunol.aag2045.
948 43. Lee S, Karki R, Wang Y, Nguyen LN, Kalathur RC, Kanneganti TD. Aim2 Forms
949 a Complex with Pyrin and Zbp1 to Drive Panoptosis and Host Defence. Nature (2021)
950 597(7876):415-9. Epub 2021/09/03. doi: 10.1038/s41586-021-03875-8.
951 44. Li LL, Dai B, Sun YH, Zhang TT. The Activation of Il-17 Signaling Pathway
952 Promotes Pyroptosis in Pneumonia-Induced Sepsis. Ann Transl Med (2020) 8(11):674.
953 Epub 2020/07/04. doi: 10.21037/atm-19-1739.
954 45. Sergi B, Penttila I. Interleukin 18 Receptor. J Biol Regul Homeost Agents (2004)
955 18(1):55-61. Epub 2004/08/25.
956 46. Kanai T, Kamada N, Hisamatsu T. Clinical Strategies for the Blockade of Il-18 in
957 Inflammatory Bowel Diseases. Current drug targets (2013) 14(12):1392-9. Epub
958 2013/05/09. doi: 10.2174/13894501113149990006.
959 47. Liu Y, Fang Y, Chen X, Wang Z, Liang X, Zhang T, et al. Gasdermin E-Mediated
960 Target Cell Pyroptosis by Car T Cells Triggers Cytokine Release Syndrome. Science
961 immunology (2020) 5(43). Epub 2020/01/19. doi: 10.1126/sciimmunol.aax7969.
962 48. Bouchon A, Facchetti F, Weigand MA, Colonna M. Trem-1 Amplifies
963 Inflammation and Is a Crucial Mediator of Septic Shock. Nature (2001)
964 410(6832):1103-7. Epub 2001/04/27. doi: 10.1038/35074114.
965 49. Zhai Z, Yang F, Xu W, Han J, Luo G, Li Y, et al. Attenuation of Rheumatoid
966 Arthritis through the Inhibition of Tumor Necrosis Factor-Induced Caspase
967 3/Gasdermin E-Mediated Pyroptosis. Arthritis & rheumatology (Hoboken, NJ) (2022)
968 74(3):427-40. Epub 2021/09/05. doi: 10.1002/art.41963.
969 50. Sharma BR, Karki R, Kanneganti TD. Role of Aim2 Inflammasome in

970 Inflammatory Diseases, Cancer and Infection. European journal of immunology (2019)
971 49(11):1998-2011. Epub 2019/08/03. doi: 10.1002/eji.201848070.
972 51. Zhang Z, Zhang Y, Xia S, Kong Q, Li S, Liu X, et al. Gasdermin E Suppresses
973 Tumour Growth by Activating Anti-Tumour Immunity. Nature (2020)
974 579(7799):415-20. Epub 2020/03/20. doi: 10.1038/s41586-020-2071-9.
975 52. Yin Q, Pi X, Jiang Y, Ren G, Liu Z, Liu H, et al. An Immuno-Blocking Agent
976 Targeting Il-1β and Il-17a Reduces the Lesion of Dss-Induced Ulcerative Colitis in
977 Mice. Inflammation (2021) 44(5):1724-36. Epub 2021/04/21. doi:
978 10.1007/s10753-021-01449-4.
979 53. Blonski W, Buchner AM, Lichtenstein GR. Treatment of Ulcerative Colitis.
980 Current opinion in gastroenterology (2014) 30(1):84-96. Epub 2013/11/29. doi:
981 10.1097/mog.0000000000000031.
982 54. van der Post S, Jabbar KS, Birchenough G, Arike L, Akhtar N, Sjovall H, et al.
983 Structural Weakening of the Colonic Mucus Barrier Is an Early Event in Ulcerative
984 Colitis Pathogenesis. Gut (2019) 68(12):2142-51. Epub 2019/03/28. doi:
985 10.1136/gutjnl-2018-317571.
986 55. Cottone M, Renna S, Modesto I, Orlando A. Is 5-Asa Still the Treatment of
987 Choice for Ulcerative Colitis? Current drug targets (2011) 12(10):1396-405. Epub
988 2011/04/07. doi: 10.2174/138945011796818126.
989 56. Balram B, Joshi H, Wong K, Kroeker KI, Dieleman LA, Halloran BP, et al.
990 Concomitant 5-Aminosalicylate Therapy in Moderate-to-Severe Ulcerative Colitis
991 Patients Escalated to Infliximab Is Not Beneficial. Digestive diseases and sciences
992 (2021) 66(11):3985-92. Epub 2020/11/14. doi: 10.1007/s10620-020-06704-6.
993 57. Feng J, Zhu Y, Chen L, Wang M. Clinical Significance of Microrna-146a in
994 Patients with Ulcerative Colitis. Annals of clinical and laboratory science (2020)
995 50(4):463-7. Epub 2020/08/23.
996 58. Carter MJ, Jones S, Camp NJ, Cox A, Mee J, Warren B, et al. Functional
997 Correlates of the Interleukin-1 Receptor Antagonist Gene Polymorphism in the
998 Colonic Mucosa in Ulcerative Colitis. Genes and immunity (2004) 5(1):8-15. Epub
999 2004/01/22. doi: 10.1038/sj.gene.6364032.

59. Cupi ML, Sarra M, Marafini I, Monteleone I, Franzè E, Ortenzi A, et al. Plasma Cells in the Mucosa of Patients with Inflammatory Bowel Disease Produce Granzyme B and Possess Cytotoxic Activities. Journal of immunology (Baltimore, Md : 1950) (2014) 192(12):6083-91. Epub 2014/05/20. doi: 10.4049/jimmunol.1302238.

60. Brynjolfsson SF, Magnusson MK, Kong PL, Jensen T, Kuijper JL, Håkansson K, et al. An Antibody against Triggering Receptor Expressed on Myeloid Cells 1 (Trem-1) Dampens Proinflammatory Cytokine Secretion by Lamina Propria Cells from Patients with Ibd. Inflammatory bowel diseases (2016) 22(8):1803-11. Epub 2016/06/01. doi: 10.1097/mib.0000000000000822.

61. Wu MM, Wang QM, Huang BY, Mai CT, Wang CL, Wang TT, et al. Dioscin Ameliorates Murine Ulcerative Colitis by Regulating Macrophage Polarization. Pharmacological research (2021) 172:105796. Epub 2021/08/04. doi: 10.1016/j.phrs.2021.105796.

62. Wei YY, Fan YM, Ga Y, Zhang YN, Han JC, Hao ZH. Shaoyao Decoction Attenuates Dss-Induced Ulcerative Colitis, Macrophage and Nlrp3 Inflammasome Activation through the Mkp1/Nf-Kb Pathway. Phytomedicine (2021) 92:153743. Epub 2021/09/29. doi: 10.1016/j.phymed.2021.153743.

63. Bergmann S, von Buenau B, Vidal YSS, Haftek M, Wladykowski E, Houdek P, et al. Claudin-1 Decrease Impacts Epidermal Barrier Function in Atopic Dermatitis Lesions Dose-Dependently. Sci Rep (2020) 10(1):2024. Epub 2020/02/08. doi: 10.1038/s41598-020-58718-9.