

Identification of Biomarkers Modulating Osteogenic Differentiation With Immune and Inflammation Landscapes in Mesenchymal Stem Cells: A Bioinformatics-based Comprehensive Study

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Abstract

Background: Inducing osteogenic mesenchymal stem cell (MSC) differentiation brings a huge potential for clinical bone defect repair. This present study is aimed to identify novel key biomarkers for osteogenesis of MSCs, and analyze their possible regulation roles on immune and inflammation during this process.

Results: Seven datasets (GSE159137, GSE159138, GSE114117, GSE88865, GSE153829, GSE63754, GSE73087) were obtained from the gene expression omnibus database, and 200 differentially expressed genes (DEGs) were identified from the train datasets. Biomarkers were screened through Logical regression of the selection operator algorithm, whose expressions and diagnostic performances were verified in the test datasets. FKBP prolyl isomerase 5 (FKBP5), insulin like growth factor binding protein 2, prostaglandin E receptor 2, SAM and HD domain containing deoxynucleoside triphosphate triphosphohydrolase 1 (SAMHD1) and transmembrane O-mannosyltransferase targeting cadherins 1 were established to be osteogenesis-related biomarkers. Finally, the DEGs of immune and inflammation in osteogenesis were detected, and their correlation with the hub biomarkers were analyzed. The five selected biomarkers are strongly associated with immune and inflammation related genes.

Conclusion: Taken together, our manuscript identified five biomarkers related to stem cells osteogenesis and discussed their potential perspectives in immunoregulation, inflammation and tissue engineering during the process.

Background

Though omnipresent challenges exist in face of the clinical and surgical repairing of large-volume skeletal abnormalities, recent stem cell-based therapy (SCBT) has shown great potential for the treatment of severe bone defects(1, 2). In biological conditions, healthy bone tissues serve limited capabilities in self-renewal and regeneration which is majorly controlled by the activities of osteoclasts and osteoblasts. Nevertheless, such properties can be significantly suppressed, even halted in case of severe skeletal trauma or defects, resulting in great setbacks when taken into clinical consideration. Mesenchymal stem cells (MSCs), also known as mesenchymal stromal cells, including adipose-derived stem cells (ASCs) and bone-marrow stromal cells (BMSCs), plays a major role in regenerative medicine due to their fundamental properties in cell-regeneration and differentiation. They have high osteoinductive and osteogenic potential, which is theoretically ideal for bone regenerations and shows a promising alternative(3). Therefore, the search for and identification of important biochemical molecules and genes that may initiate or control seed osteogenic differentiation is vital for the establishment of relevant defect treatment strategies.

Depending on specific microenvironment, MSCs which have various properties in regulating immune and inflammation play crucial biological roles in ways of direct immune cell contact and the production of inflammatory regulatory molecules, namely, the paracrine effects(4, 5). Though the effect of “sensor and

switcher of the immune system”, MSCs have the capabilities of both up- and down- regulating inflammatory processes, mainly by responding to danger indicators and releasing anti-inflammatory mediators through activated Toll-like receptors (TLRs) ligands, such as TLR9 TLR7 and TLR2, and expressing the immune suppressors PD-L1 as well as PD-L2 under the stimulation of interferon gamma (IFN- γ), respectively(6). MSCs also has various effects in coordinating the migration, proliferation, and activation of immune cells according to different stages of osteogenesis and different categories of immune cells (7). And consistent with changes in proinflammatory and anti-inflammatory cytokines presenting in the micro-environment, MSCs secrete cytokines including prostaglandin E2 (PGE2), transforming growth factor beta (TGF- β), histocompatibility locus antigen-G (HLA-G) that induce the formation of regulatory T cells, suppress neutrophil migration and more importantly, serve the role in immune-inhibition by monocyte/macrophage regulation(8–10). Therefore, exploring the potential roles of immunomodulation and inflammatory regulation which involved MSC osteogenic differentiation is crucial in discovering novel therapeutic strategies and improving currently inefficient bone remodeling.

In this study, we performed a comprehensive strategy to determine biomarkers related to osteogenic differentiation of MSCs, and further investigated the relationship between those biomarkers and genes involving immunoregulation and inflammation participating in this process. The objective of our research is to form a basis for further explorations of significant genes, biochemical pathways as well as vital bioactive molecules affecting the osteogenic role of mesenchymal stem cells.

Methods

Data Collection and Processing

The study flowchart is presented in Figure 1. Three datasets which expression profiling by high throughput sequencing (GSE159137, GSE159138, GSE114117) and four datasets which expression profiling by array (GSE88865, GSE153829, GSE63754, GSE73087) were retrieved from the GEO database (<http://www.ncbi.nlm.nih.gov/geo>), and the characteristics of these included datasets were presented in Table 1. The three high throughput sequencing were selected as the train group containing a total of 7 uninduced MSCs samples and 12 osteogenic induced MSCs samples. And four datasets which expression profiling by array, with a total of 10 uninduced MSCs samples and 18 osteogenic MSCs induced samples, were utilized for test validation. The RNA algorithm was applied to background correction and data normalization(39). Then, probes were transmuted into their corresponding gene symbols based on platform annotation information.

Identification of Differentially Expressed Genes

The analysis of differentially expressed genes (DEGs) was performed by using the “limma” R package (<http://www.bioconductor.org/packages/release/bioc/html/limma.html>). Using false discovery rate (FDR) q-value < 0.05 and log2 fold change (FC) ≥ 2 as the filter condition. “Pheatmap” R package (<https://cran.r-project.org/web/packages/pheatmap/index.html>) , “ggplot2”

(<https://www.rdocumentation.org/packages/ggplot2/versions/2.1.0>) R package and “ggrepel” R package (<https://cloud.r-project.org/package=ggrepel>) were utilized to visualize DEGs.

Enrichment Analyses of DEGs

Gene Ontology (GO) (<http://geneontology.org/>) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) (<https://www.kegg.jp/>) were used for functional and pathway enrichment analyses, respectively. Disease Ontology (DO) (<http://disease-ontology.org>) comprises 8043 inherited, acquired, and developmental human diseases. Based on molecular signature database, Gene Set Enrichment Analysis (GSEA) (<http://software.broadinstitute.org/gsea/index.jsp>) is a statistical tool for interpreting gene expression data. GSEA 4.1.0 was utilized to predict the potential functions and downstream access of the two clusters. Prior to enrichment analyses, “org.Hs.eg.db” R package (<http://www.bioconductor.org/packages/release/data/annotation/html/org.Hs.eg.db.html>), a human genome annotation database was used for conversion of gene symbol codes into Entrez ID. FDR q-value < 0.05 were considered markedly enriched for GO terms, DO and GSEA analyses, while p-value < 0.05 was considered significant for KEGG analyses.

Screening for MSCs osteogenesis related biomarkers

LASSO was used to investigate osteogenesis-related biomarkers via R package “glmnet” R package (<https://mirrors.sjtug.sjtu.edu.cn/cran/web/packages/glmnetSE/index.html>) in train group. while “pROC” R package (<https://cran.rstudio.com/web/packages/pROC/index.html>) was used for computing the area under the curve (AUC) in train and test group. Accuracy, sensitivity, and specificity of a receiver operating characteristic (ROC) curve to assess the diagnostic ability of the markers. $p < 0.05$ was the cut-off for significance.

Selection of immune and inflammation related genes

To make the research more comprehensive, we selected immune and inflammation related genes, and the differential expressed genes in osteogenesis and non-osteogenesis groups was visualized by using the methods mentioned above. PPI networks were established to assess the associations among genes in selected modules using the Search Tool for the Retrieval of Interacting Genes version 11 (STRING V11, <https://string-preview.org/>) with > 0.4 as the confidence level. Cytoscape version 3.8.2 was used for network visualization. Hub genes, which are presented by highly interconnected nodes, may play vital roles in the PPI network.

Interaction analysis of selected biomarkers

Co-expression analysis of selected biomarkers with immune-related genes and inflammation-related genes was performed using “limma” in R (<http://www.bioconductor.org/packages/release/bioc/html/limma.html>), respectively. We then performed co-expression analysis, and the following parameters were used as filter conditions to select related genes: “correlation coefficient = 0.4” and “pvalueFilter = 0.05”.

Results

Identification of DEGs

Differential expression analysis was conducted based on the train datasets to screen for DEGs. There were clear differences between osteogenesis MSCs and the control ones. From our heatmap (Figure 2A) and volcano plots (Figure 2B), we observed there were 200 DEGs. Among them, 156 genes were upregulated while 44 genes were downregulated.

Functional enrichment analyses of DEGs

With regards to biological processes, GO analysis showed that robust DEGs were mainly enriched in cellular divalent inorganic cation homeostasis, epithelial cell proliferation, and cellular calcium ion homeostasis in the biological process (BP) part. The cellular component (CC) part, the DEGs mainly concentrated in collagen-containing extracellular matrix, postsynaptic density, and asymmetric synapse. In Molecular functions included peptide binding and amide binding among other important functions (Figure 3A). The KEGG analysis demonstrated that neuroactive ligand-receptor interaction, complement and coagulation cascades, as well as cytokine-cytokine receptor interaction were the most significant processes in MSCs osteogenic differentiation process (Figure 3B). DO analysis showed that DEGs were enriched in kidney disease and urinary system disease (Figure 4A).

Moreover, the GSEA analysis result indicated active pathways that varied between uninduced and osteogenic induced groups (Figure 4B), including cellular ion homeostasis, divalent inorganic cation homeostasis, inflammatory response, and metal ion homeostasis were established to be active in the osteogenic induced group. While the active pathways of uninduced group (Figure 4C) were chromosome segregation, polymeric cytoskeletal fiber, postsynapse, postsynaptic membrane, and synaptic membrane.

Verification of the hub biomarkers

Through LASSO logistic regression, a total of 7 genes were identified from robust DEGs as potential vitiligo-associated markers (Figure 5A). These 7 biomarkers included adrenoceptor alpha 1B (ADRA1B), FKBP prolyl isomerase 5 (FKBP5), insulin like growth factor binding protein 2 (IGFBP2), monoamine oxidase A (MAOA), prostaglandin E receptor 2 (PTGER2), SAM and HD domain containing deoxynucleoside triphosphate triphosphohydrolase 1 (SAMHD1) and transmembrane O-mannosyltransferase targeting cadherins 1 (TMTC1).

To assess the accuracy for the biomarkers, we used 4 datasets (GSE88865, GSE153829, GSE63754, GSE73087) as verification sets to validate the efficacies of candidate biomarkers. Differential expressions of these five genes, including FKBP5, IGFBP2, PTGER2, SAMHD1 and TMTC1, were verified in test group, and their expressions were all significantly higher in the osteogenic induced groups (Figure 5B-H). The five biomarkers were all with an AUC more than 0.7 in the test datasets, and SAMHD1 presented the highest AUC of 0.861, indicating the capabilities of the five biomarkers to diagnose MSCs osteogenic differentiation with excellent specificity and sensitivity (Figure 6).

Identification of DEGs related to immune and inflammation

Heatmaps revealed 38 differentially expressing immune-related regulators between osteogenic induced and control groups with FDR q-value < 0.05 (Figure 7A), with 31 upregulated and 7 downregulated. The interrelation of immune-related regulators was constructed using the STRING database, the protein-protein interaction (PPI) network analysis (Figure 7B) indicated that IL6, LEP and ANGPT1 had the most interactions of the studied genes. GO and KEGG analyses were performed to the potential functions and pathways of the differentially expressing immune-related genes (Figure 8). The GO results revealed that the differentially expressing immune-related regulators were majorly concentrated in epithelial cell proliferation in BP, basolateral plasma membrane, receptor ligand activity in CC, receptor ligand activity and signaling receptor activator activity in MF. In KEGG, it concentrated in neuroactive ligand-receptor interaction, as well as cytokine-cytokine receptor interaction.

We screened differentially expressing inflammation-related regulators between osteogenic induced and control groups through p-value < 0.05 and 32 upregulated and 18 downregulated genes were presented in Heatmaps (Figure 9A). The interaction as well as the expression number of inflammation-related regulators were shown in Figures 9B, CXCL8 and IL6 were treated as the central gene. GO and KEGG enrichment analyses found that response to molecule of bacterial origin and response to lipopolysaccharides in BP, secretory granule membrane and plasma membrane signaling receptor complex in CC, receptor ligand activity and signaling receptor activator activity in MF, while cytokine-cytokine receptor interaction were the most important functions and pathways of the differentially expressing inflammation-related regulators according to the enrichment scores in KEGG (Figure 10).

Potential roles of hub biomarkers in immune and inflammation

We analyzed RNA-seq transcriptome to explore the association between differentially expressed immune-related genes and selected biomarkers (Figure 11A) as well as the relationship between differentially expressed inflammation-related genes and the five hub biomarkers (Figure 11B). There seemed to be a regulating network of FKBP5, IGFBP2, PTGER2, SAMHD1, and TMTC1 with immune-related and inflammation-related genes. The correlation coefficients and positive and negative regulatory relationships between the genes we predicted are shown in the Table 2 and Table 3. In the process of stem cell osteogenesis, the biomarkers we screened showed a strong correlation with immunity and inflammation, which laid a foundation for us to further study the mechanism of stem cell osteogenesis.

Discussion

Although the significance of MSCs in bone tissue engineering have been evaluated, their efficacy in osteogenic differentiation is limited without extra interventions in vivo and in vitro(11, 12). How to improve the efficiency of regenerative abilities of MSCs in damage restoration is still urgent. The search of novel key biomarkers in stem cell osteogenesis will be helpful in clarifying osteogenic potentials and mechanisms of stem cells, thus designing more appropriate bone filling materials to induce bone regeneration with increased proficiency and biocompatibility.

In this study, we identified hub biomarkers of stem cells osteogenesis with a comprehensive strategy of LASSO logistic regression and ROC curve analysis. The five hub biomarkers we identified are FKBP5, IGFBP2, PTGER2, SAMHD1 and TMTC1. Although there have been studies focusing on exploring stem-cell-related osteogenic biomarkers via bioinformatics analyses(13, 14), the hub markers SAMHD1 and FKBP5 we determined in this study have an average AUC over 0.8 in test datasets, indicating their reliable predictive abilities.

Among these 5 hub biomarkers, the effects of IGFBP2 and PTGER2 on osteogenesis have been reported, but remain controversial. IGFBP2, an important member of the insulin-like growth factor family, plays crucial roles in growth, development, and metabolism of human body(15, 16). Hamidouchel et al. demonstrated that IGFBP2 has the property of promoting the osteogenic differentiation of MSCs by enhancing the osteoblast phenotype genes, including RUNX2, ALP and COI1A1B(17). And consistent with our results, the expression of IGFBP2 increased significantly when inducing osteogenesis(18). Moreover, a recent study revealed that IGFBP2 is also capable of activating the JNK/Akt pathway and inducing the adipogenic differentiation of MSCs(19). Further researches are needed to elucidate the mechanisms and specific roles played by IGFBP2 in promoting osteogenesis or adipogenesis in MSCs. PTGER2 acts as a main receptor of prostaglandin E2, which has been shown to encourage osteogenic differentiation of MSCs, and stimulate the resorption and formation of bones(20-22). It is suggested in several current studies that local application of PTGER2 agonists to fractured rats in vitro can effectively stimulate the formation of bone marrow and periosteum, thus promoting fracture repairing(23-25). However, the direct effect of PTGER2 on the osteogenic differentiation of MSCs needs further research to confirm.

TMTC1, a glycosyltransferase, promotes O-mannosylation of cadherin and maintains intracellular calcium homeostasis(26-28). Glycosylation is a key posttranslational modification process that participates in controlling extracellular matrix formation during osteogenesis(29, 30). GO analysis revealed that DEGs were mainly concentrated in extracellular matrix organization, and collagen-containing extracellular matrix. Previous studies have revealed that extracellular matrix modulates osteogenic effects by adhering to cytoskeletal proteins in MSCs(31, 32). Together with our results, TMTC1 and glycosylation may play crucial roles in the extracellular matrix formation during osteogenic differentiation.

The strong relationship between stem cells osteogenesis and immunity has been well reported within the osteoimmunology scope, and a controlled inflammatory response is vital for favoring osteogenesis(33-35). In this study, we identified differentially expressing immune and inflammatory related regulators between osteogenic induced and uninduced groups, and numerous DEGs between groups were found, demonstrating a significant difference of immune and inflammation modulation between osteogenic and nonosteogenic status. And we further analyzed their association with hub biomarkers. Among the five biomarkers, SAMHD1 and FKBP5 represented to be most correlated with immune-related and inflammation-related genes in osteogenic differentiation. SAMHD1 is a deoxynucleoside triphosphohydrolase that can suppress innate immune responses to viral infection through interacting with various key proteins in immune signaling pathways(36, 37). FKBP5 is a stress-responsive molecule

which also possesses the property of modulating immune function. Earlier researches have demonstrated the effects of SAMHD1 and FKBP5 on immune response and inflammation, but whether these effects present during the process of osteogenesis have not been fully elucidated(38). Our present results suggest that the potential mechanisms of SAMHD1 and FKBP5 exerting in osteogenesis might be greatly linked to immune and inflammation regulation.

In summary, we combined bioinformatic analysis and ROC analysis to screen the biomarkers associated with osteogenesis of stem cells, and analyzed their potential roles with immune and inflammation during this process. Although these hub genes are highly associated with osteogenesis of stem cells based on this study and other previous studies mentioned above, their biological mechanisms in osteogenesis remain unclear. Therefore, due further researches can be focused on specifying the precise mechanisms as well as discovering novel, related signaling pathways and bioactive molecules.

Conclusion

In conclusion, by combining bioinformatic and analysis, the current study has screened several hub biomarkers which are related to the osteogenic differentiation of mesenchymal stem cells, and analyzed their potential roles in immunomodulation and inflammatory responses during this process. It is suggested, by our results, the possible properties of these markers act through signaling pathways that are related to their effects in immune and inflammation regulation, also lay a foundation for us to further study the mechanism of mesenchymal stem cell osteogenesis.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors have provided their consent for publication.

Availability of data and materials

The datasets generated and/or analyzed included in the manuscript are available in the GEO repository, <http://www.ncbi.nlm.nih.gov/geo>.

Competing interests

Not applicable

Funding

Not applicable

Authors' contributions

SG and ZF conceived and designed the study. ZF and XS performed data acquisition and interpretation. ZF and TW wrote the paper. All authors approved the final manuscript.

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Tables

Table 1. Gene expression data from Gene Expression Omnibus (GEO) database.

Dataset ID	Total samples	Control	Treat	Data type	Tissue type	Country
GSE159137	6	6	0	RNA-seq	AMSCs	China
GSE159138	6	0	6	RNA-seq	AMSCs	China
GSE114117	7	1	6	RNA-seq	hMSCs	China
GSE88865	6	3	3	Microarray	PDMSCs	China
GSE153829	6	0	6	Microarray	MSCs	China
GSE63754	6	3	3	Microarray	ADSCs	Lithuania
GSE73087	7	1	6	Microarray	MSCs	China

Table 2. Co-expression of biomarkers and immune related genes.

Immune related gene	Biomarker	cor	p value	Regulation
SAMHD1	FKBP5	0.878497	0.000169	positive
APOD	FKBP5	0.841998	0.000591	positive
SAA1	FKBP5	0.657604	0.020119	positive
TSC22D3	FKBP5	0.632426	0.027332	positive
ANGPT1	FKBP5	0.698622	0.011488	positive
PTK2B	FKBP5	0.784954	0.002492	positive
ANOS1	FKBP5	-0.71911	0.008395	negative
NRG1	FKBP5	-0.61721	0.032506	negative
AGTR1	FKBP5	0.791893	0.002142	positive
EDNRB	FKBP5	0.612917	0.034084	positive
PDGFD	FKBP5	0.728478	0.00721	positive
SDC1	FKBP5	-0.60023	0.039067	negative
C7	FKBP5	0.679423	0.015088	positive
C5AR2	FKBP5	0.645377	0.023422	positive
GDF7	FKBP5	0.65612	0.0205	positive
EPGN	FKBP5	-0.65542	0.020681	negative
A2M	FKBP5	0.645669	0.023339	positive
UCN2	FKBP5	0.678846	0.015208	positive
PTH1R	FKBP5	0.577018	0.049494	positive
NGFR	FKBP5	0.746981	0.005242	positive
SAMHD1	IGFBP2	0.638202	0.025534	positive
APOD	IGFBP2	0.812225	0.001329	positive
LGR4	IGFBP2	-0.77284	0.003206	negative
ANGPTL4	IGFBP2	0.733831	0.006592	positive
PTK2B	IGFBP2	0.645715	0.023325	positive
NRG1	IGFBP2	-0.65704	0.020262	negative
AGTR1	IGFBP2	0.781886	0.00266	positive
PDGFD	IGFBP2	0.611956	0.034444	positive

LEP	IGFBP2	0.740169	0.005912	positive
SDC1	IGFBP2	-0.77122	0.003313	negative
NR2F1	IGFBP2	0.718353	0.008497	positive
C7	IGFBP2	0.814873	0.001244	positive
EPGN	IGFBP2	-0.87914	0.000165	negative
STC1	IGFBP2	0.635467	0.026375	positive
A2M	IGFBP2	0.703206	0.010733	positive
CCL13	IGFBP2	0.701208	0.011058	positive
UCN2	IGFBP2	0.816073	0.001206	positive
PTH1R	IGFBP2	0.645509	0.023384	positive
NGFR	IGFBP2	0.703752	0.010645	positive
SAMHD1	SAMHD1	1	1.03E-70	positive
APOD	SAMHD1	0.813539	0.001286	positive
PTGER2	SAMHD1	0.586136	0.04519	positive
SAA1	SAMHD1	0.698088	0.011579	positive
MMP7	SAMHD1	0.597863	0.040052	positive
TSC22D3	SAMHD1	0.652469	0.021461	positive
ANGPT1	SAMHD1	0.626446	0.029289	positive
PTK2B	SAMHD1	0.653912	0.021078	positive
ANOS1	SAMHD1	-0.7379	0.006149	negative
AGTR1	SAMHD1	0.706966	0.010141	positive
GDF7	SAMHD1	0.661101	0.01924	positive
EPGN	SAMHD1	-0.65035	0.022035	negative
SLC40A1	SAMHD1	0.679409	0.015091	positive
A2M	SAMHD1	0.616625	0.032717	positive
NGFR	SAMHD1	0.686714	0.013635	positive
IL6	SAMHD1	-0.57936	0.04836	negative
SAMHD1	TMTC1	0.82189	0.001038	positive
PTGER2	TMTC1	0.822676	0.001017	positive

LGR4	TMTC1	-0.65592	0.020552	negative
ANGPT1	TMTC1	0.700543	0.011167	positive
LEPR	TMTC1	0.582698	0.046781	positive
ANOS1	TMTC1	-0.79171	0.002151	negative
NRG1	TMTC1	-0.63645	0.026069	negative
EDNRB	TMTC1	0.617272	0.032483	positive
C5AR1	TMTC1	0.71421	0.00907	positive
C5AR2	TMTC1	0.639859	0.025034	positive
IL1RL1	TMTC1	0.650512	0.02199	positive
GDF7	TMTC1	0.840359	0.000621	positive
SLC40A1	TMTC1	0.687872	0.013415	positive
PPARGC1A	TMTC1	0.665063	0.018279	positive
AREG	TMTC1	0.6712	0.01686	positive
CRABP2	TMTC1	-0.59813	0.039942	negative
SAMHD1	PTGER2	0.586136	0.04519	positive
PTGER2	PTGER2	1	7.94E-69	positive
ANOS1	PTGER2	-0.70111	0.011074	negative
SLC40A1	PTGER2	0.630415	0.027979	positive
PPARGC1A	PTGER2	0.80576	0.001556	positive
CRABP2	PTGER2	-0.80215	0.001694	negative
IL6	PTGER2	-0.71855	0.00847	negative

Table 3. Co-expression of biomarkers and inflammation related genes.

Inflammation related gene	Biomarker	cor	p value	Regulation
BDKRB1	FKBP5	-0.65123958	0.021792337	negative
CD82	FKBP5	0.863575418	0.00029466	positive
CDKN1A	FKBP5	-0.782368639	0.002633147	negative
CHST2	FKBP5	0.659470315	0.019646225	positive
CXCR6	FKBP5	0.673761791	0.016292707	positive
INHBA	FKBP5	-0.721062794	0.00813693	negative
LPAR1	FKBP5	0.649708006	0.022209777	positive
MXD1	FKBP5	-0.680800725	0.014805463	negative
PTGER4	FKBP5	0.70652552	0.010209257	positive
RIPK2	FKBP5	-0.632218742	0.027398517	negative
SLC4A4	FKBP5	-0.623010993	0.030456877	negative
TNFSF9	FKBP5	-0.58604887	0.045229839	negative
ADM	IGFBP2	0.619614804	0.03164453	positive
CD40	IGFBP2	0.637875106	0.025633574	positive
CD82	IGFBP2	0.905790243	0.0000498	positive
CDKN1A	IGFBP2	-0.646818915	0.023013017	negative
CHST2	IGFBP2	0.872735878	0.000211527	positive
IFITM1	IGFBP2	0.757047546	0.00435922	positive
INHBA	IGFBP2	-0.749747119	0.004987399	negative
LPAR1	IGFBP2	0.593798189	0.041783501	positive
MXD1	IGFBP2	-0.794320094	0.002028742	negative
PTGER4	IGFBP2	0.788471206	0.002309513	positive
RIPK2	IGFBP2	-0.719995108	0.008277386	negative
SLC4A4	IGFBP2	-0.745148832	0.005416701	negative
TLR2	IGFBP2	0.604987195	0.037142038	positive
TNFRSF1B	IGFBP2	0.728583385	0.007197474	positive
BDKRB1	SAMHD1	-0.722948357	0.007893244	negative
CD82	SAMHD1	0.752408175	0.004750999	positive

CDKN1A	SAMHD1	-0.604274036	0.037426353	negative
CXCR6	SAMHD1	0.639525823	0.02513441	positive
IL1R1	SAMHD1	0.587393356	0.044618005	positive
IL6	SAMHD1	-0.579363545	0.048360329	negative
PTGER2	SAMHD1	0.586135805	0.045190099	positive
RIPK2	SAMHD1	-0.655653831	0.020621179	negative
SCN1B	SAMHD1	-0.636717191	0.025987976	negative
SLC4A4	SAMHD1	-0.582409884	0.046915516	negative
C5AR1	TMTC1	0.714209971	0.009070195	positive
CD82	TMTC1	0.721169657	0.00812297	positive
CXCR6	TMTC1	0.833635546	0.000753658	positive
LPAR1	TMTC1	0.608111384	0.035914653	positive
MXD1	TMTC1	-0.63942472	0.025164779	negative
PTGER2	TMTC1	0.822676097	0.001016776	positive
TNFAIP6	TMTC1	-0.582544685	0.046852295	negative
TNFSF9	TMTC1	-0.677741891	0.015438898	negative
CXCR6	PTGER2	0.69943985	0.011350871	positive
FZD5	PTGER2	0.603262432	0.037832306	positive
IL6	PTGER2	-0.718552304	0.00847006	negative
PTGER2	PTGER2	1	5.71E-66	positive
RAF1	PTGER2	0.656214723	0.020475727	positive
TNFAIP6	PTGER2	-0.586483484	0.045031415	negative
TNFSF9	PTGER2	-0.63902551	0.02528495	negative

Figures

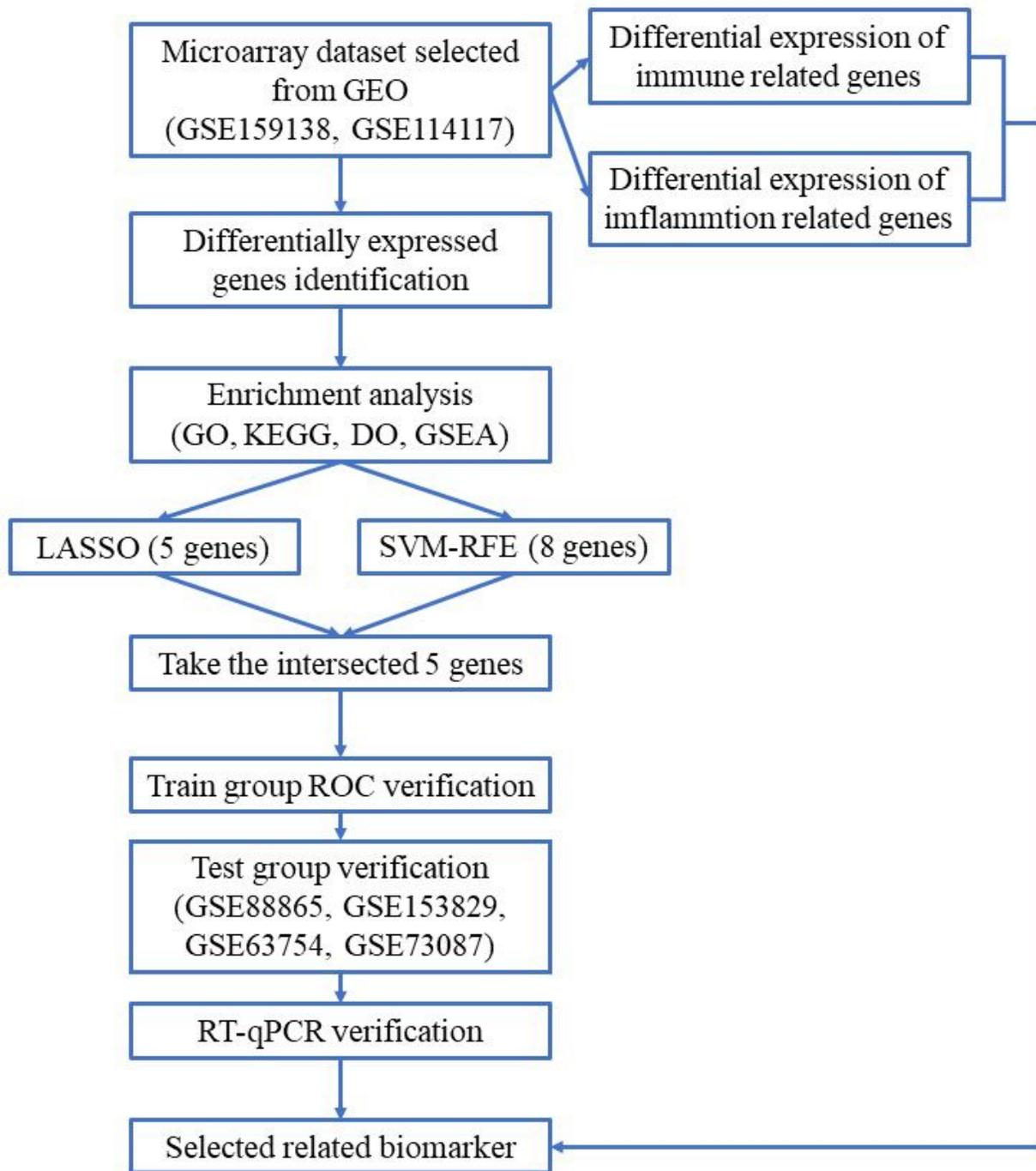


Figure 1 | Workflow chart of data generation and analysis.

Figure 1

See image above for figure legend.

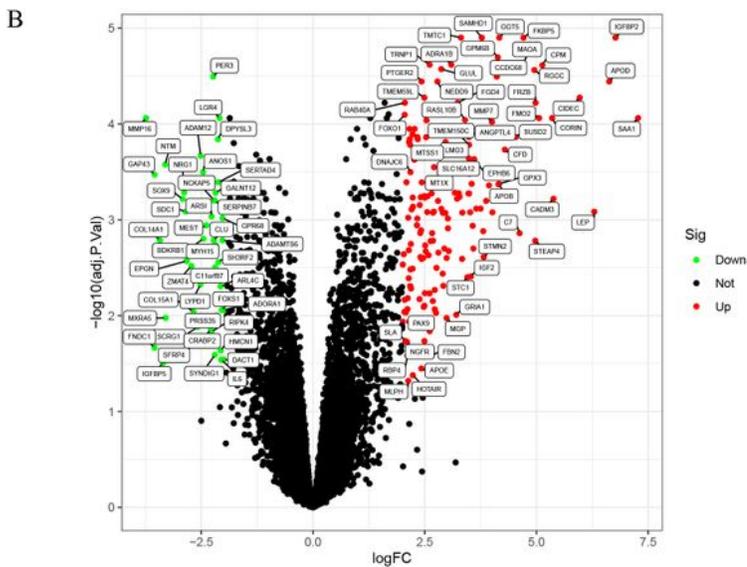
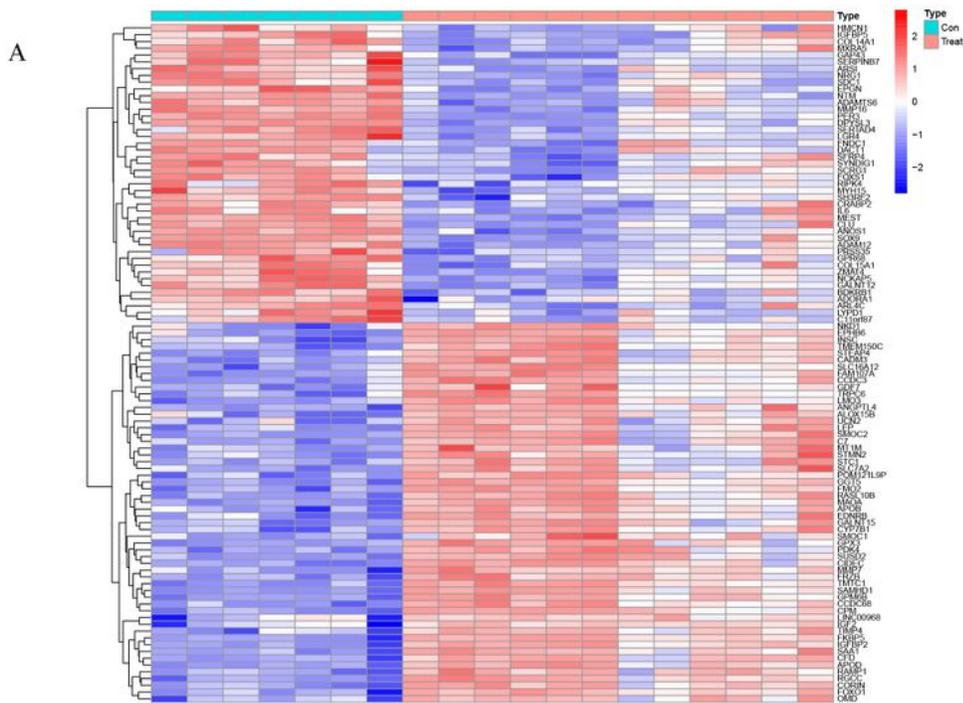


Figure 2 | The expression characteristics of genes granulosa cells in osteogenic mesenchymal stem cells (treat group) and uninduced mesenchymal stem cells (control group). (A) Heatmap presented the overall expression $FDR < 0.05$ in treat and control group. (B) Volcano plot visualized the upregulated (red) and downregulated (blue) genes in treat and control group.

Figure 2

See image above for figure legend.

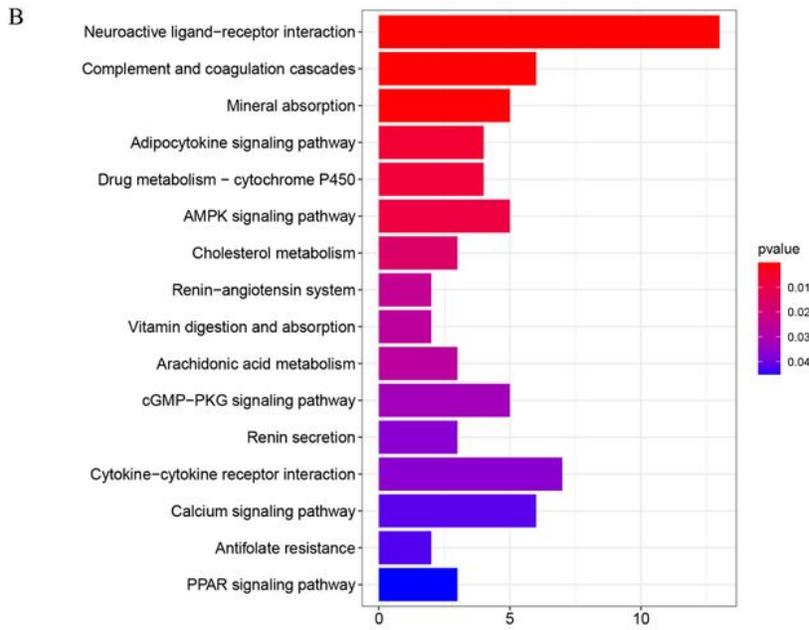
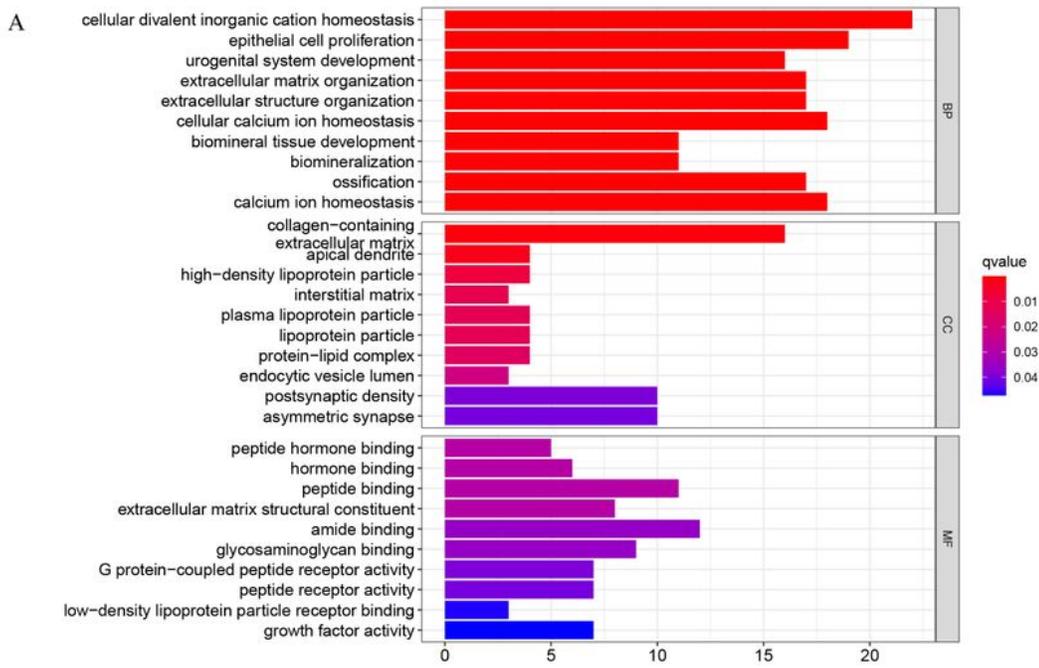


Figure 3 | (A) Gene Ontology (GO) analyses were conducted to predict the potential function of the differentially expressing genes between the treat and the control group. cellular component (CC), molecular function (MF), biological process (BP), respectively. (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) potential pathways regarding the differentially genes between the treat and the control group.

Figure 3

See image above for figure legend.

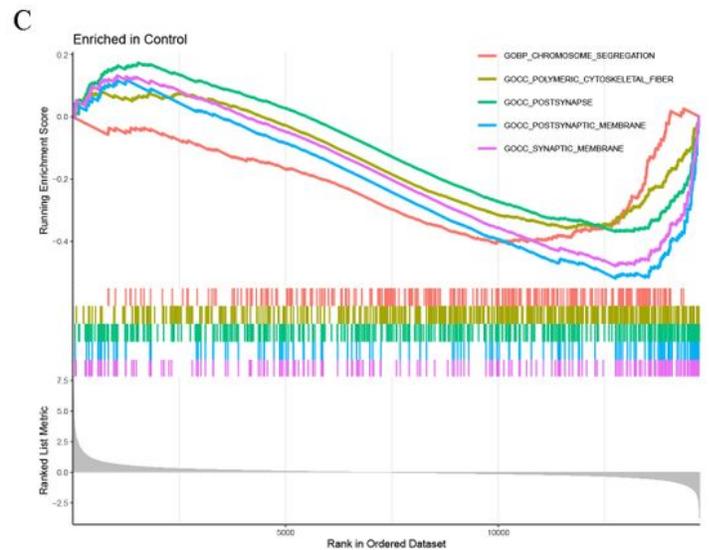
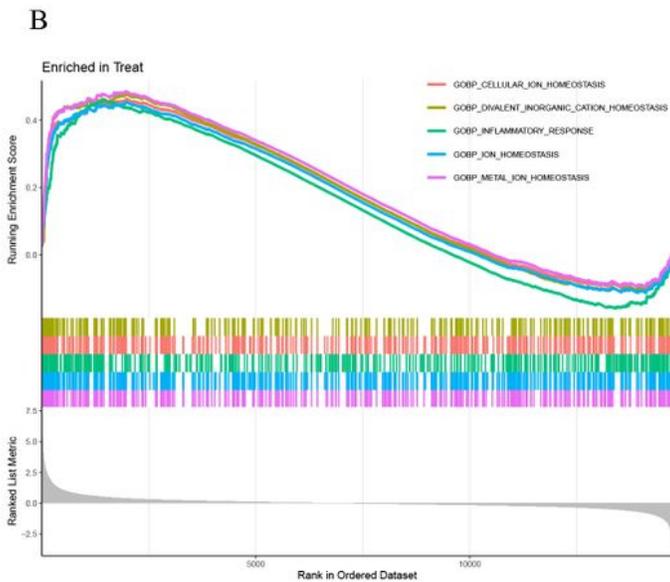
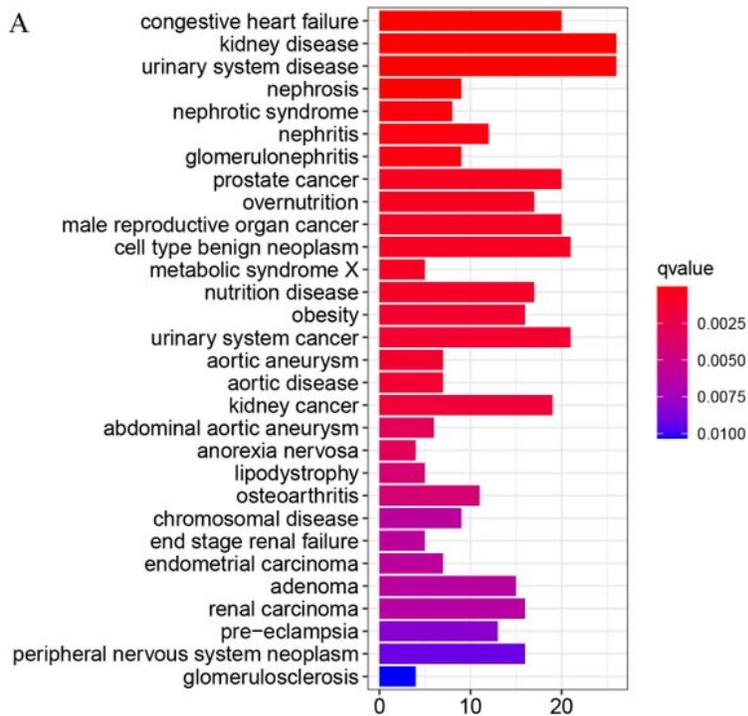


Figure 4 | (A) Disease ontology (DO) analyses were conducted to predict the potential related diseases of the differentially expressing genes between the treat and the control group. (B-C) GSEA showed that the top 5 signal pathways were most related to treat(B) and control group(C).

Figure 4

See image above for figure legend.

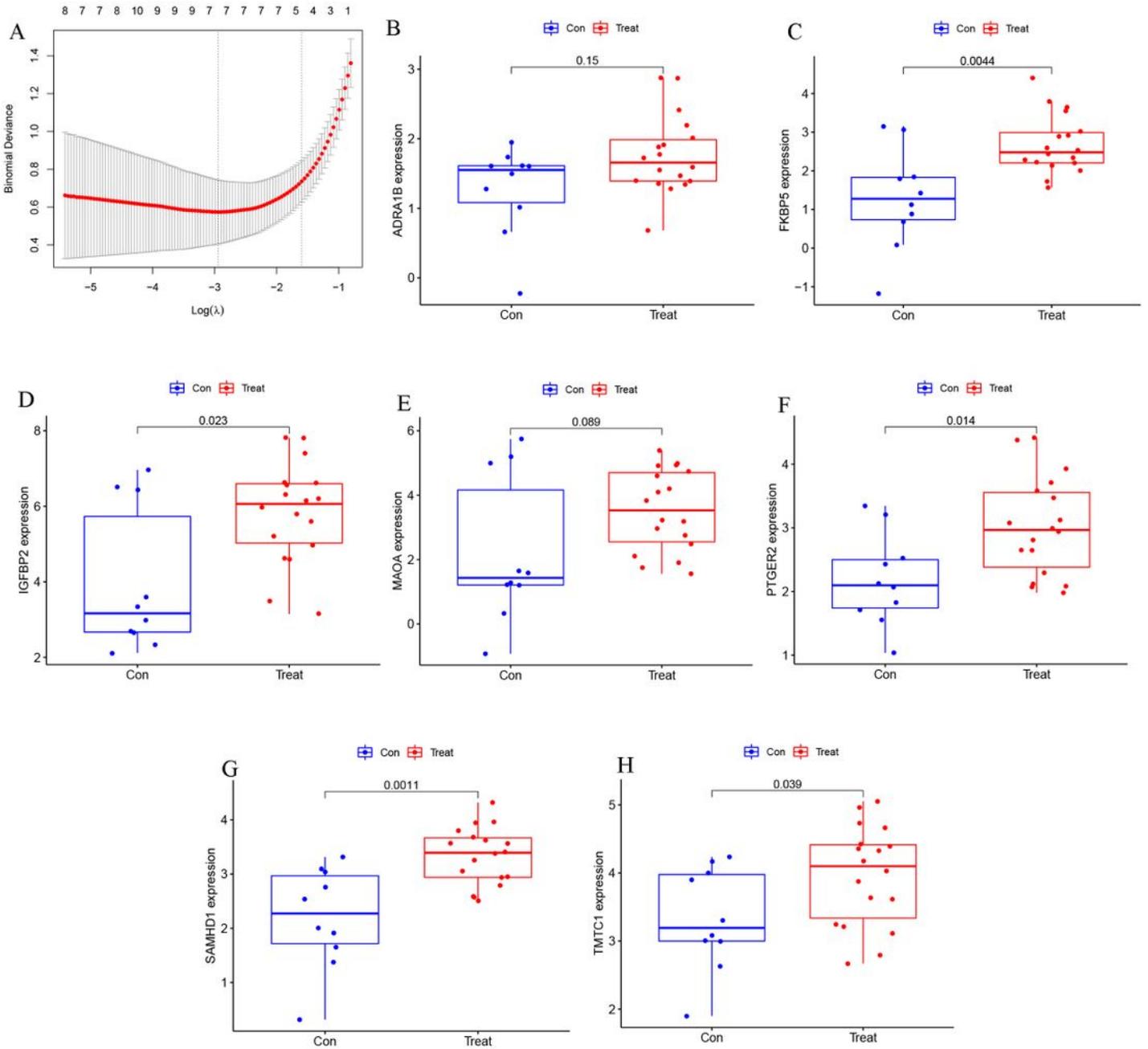


Figure 5 | (A) The least absolute shrinkage and selection operator (LASSO) logistic regression algorithm is used to retain the most predictive features. (B-H) The differential expression of ADRA1B (B), FKBP5(C), IGFBP2(D), MAOA(E), PTGER2 (F), SAMHD1(G) and TMTC1(H) in test group.

Figure 5

See image above for figure legend.

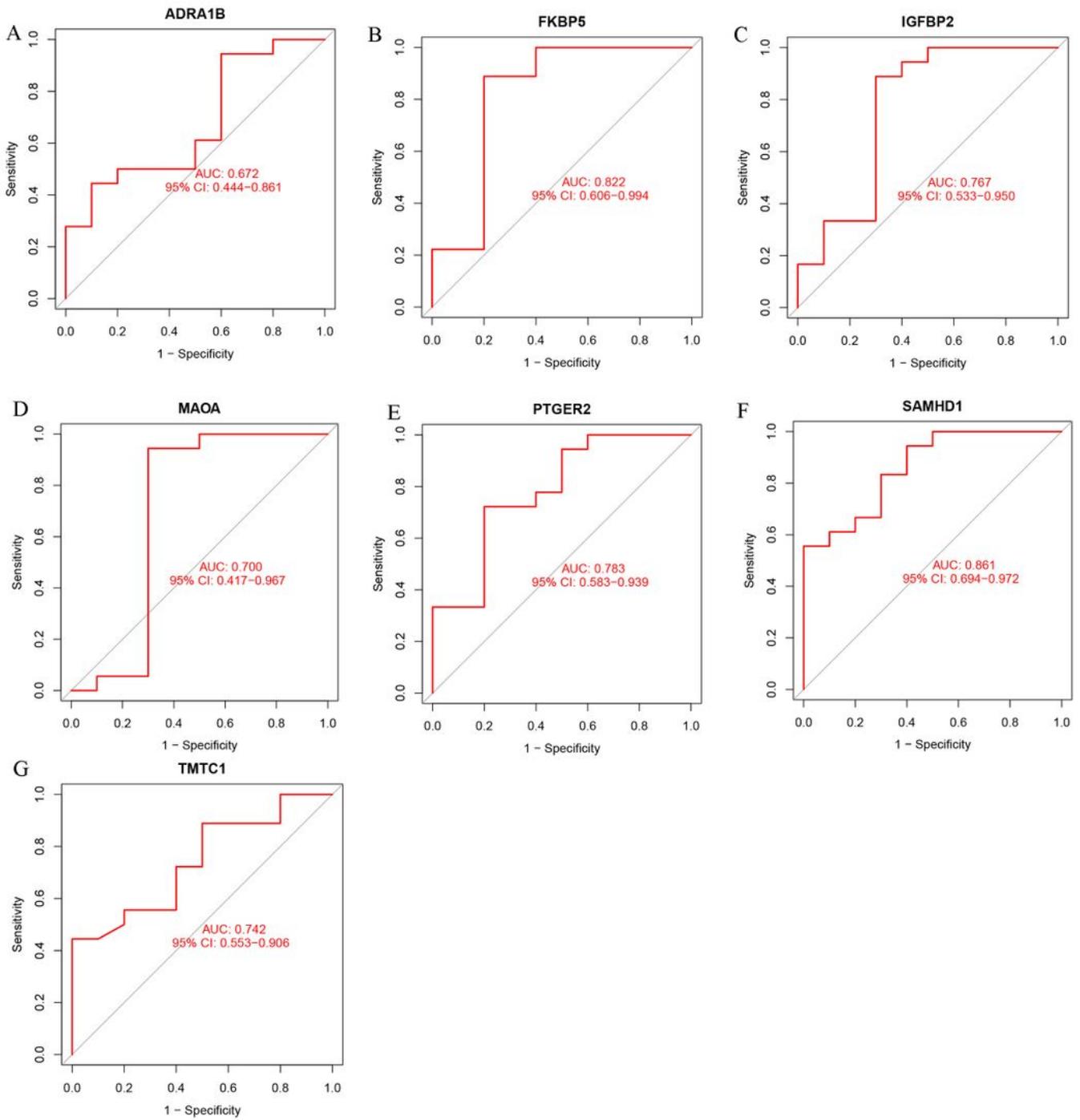


Figure 6 | (A-G) Receiver operating characteristic (ROC) curves of ADRA1B(A), FKBP5(B), IGFBP2(C), MAOA(D), PTGER2(E), SAMHD1(F) and TMTC1(G) in test group.

Figure 6

See image above for figure legend.

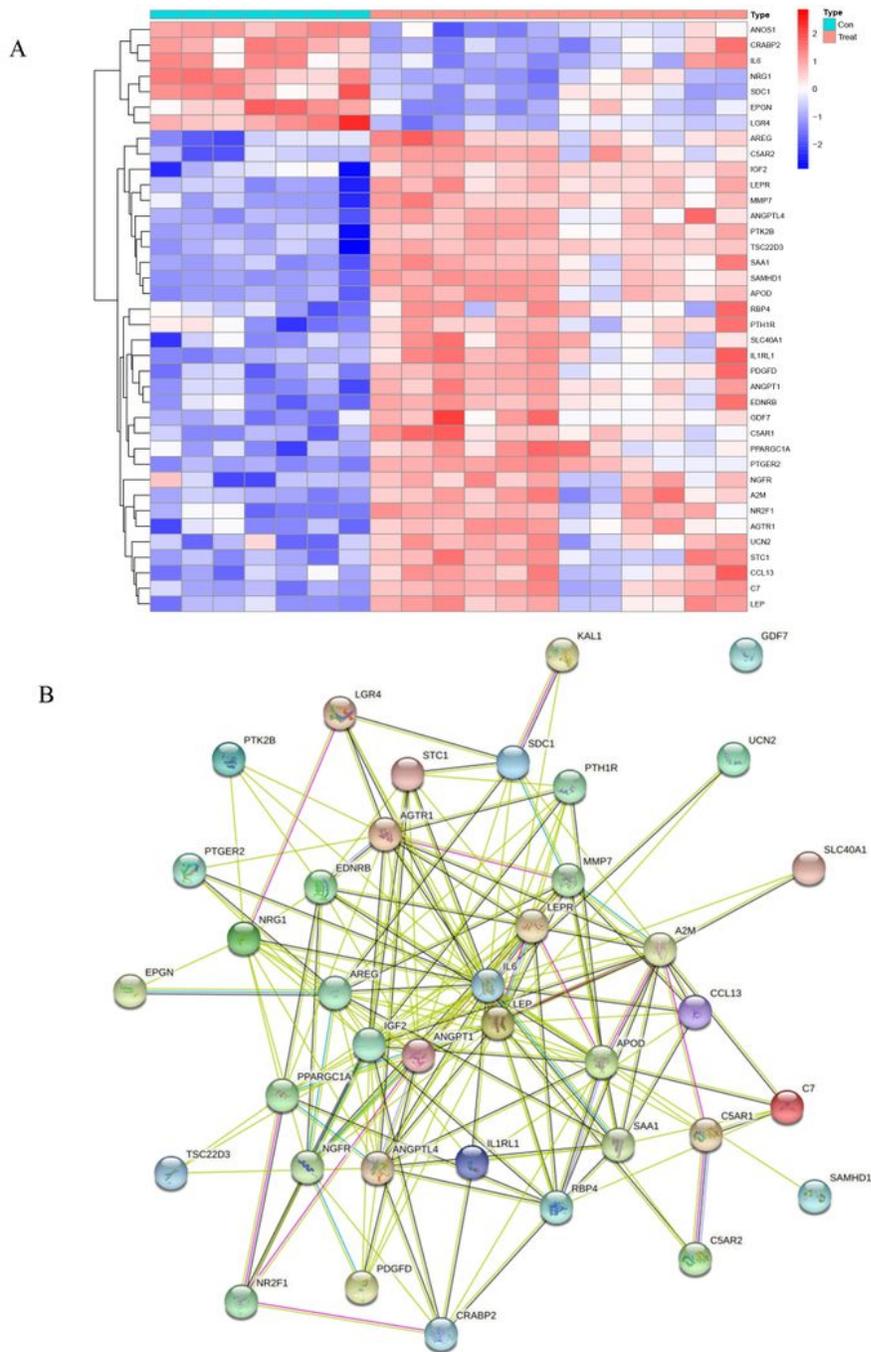


Figure 7 | The expression characteristics of immune related regulators. (A) Heatmaps presented the expression of inflammation related regulators in treat and control group. (B) The STRING Protein-Protein Interaction Networks of immune related regulators.

Figure 7

See image above for figure legend.

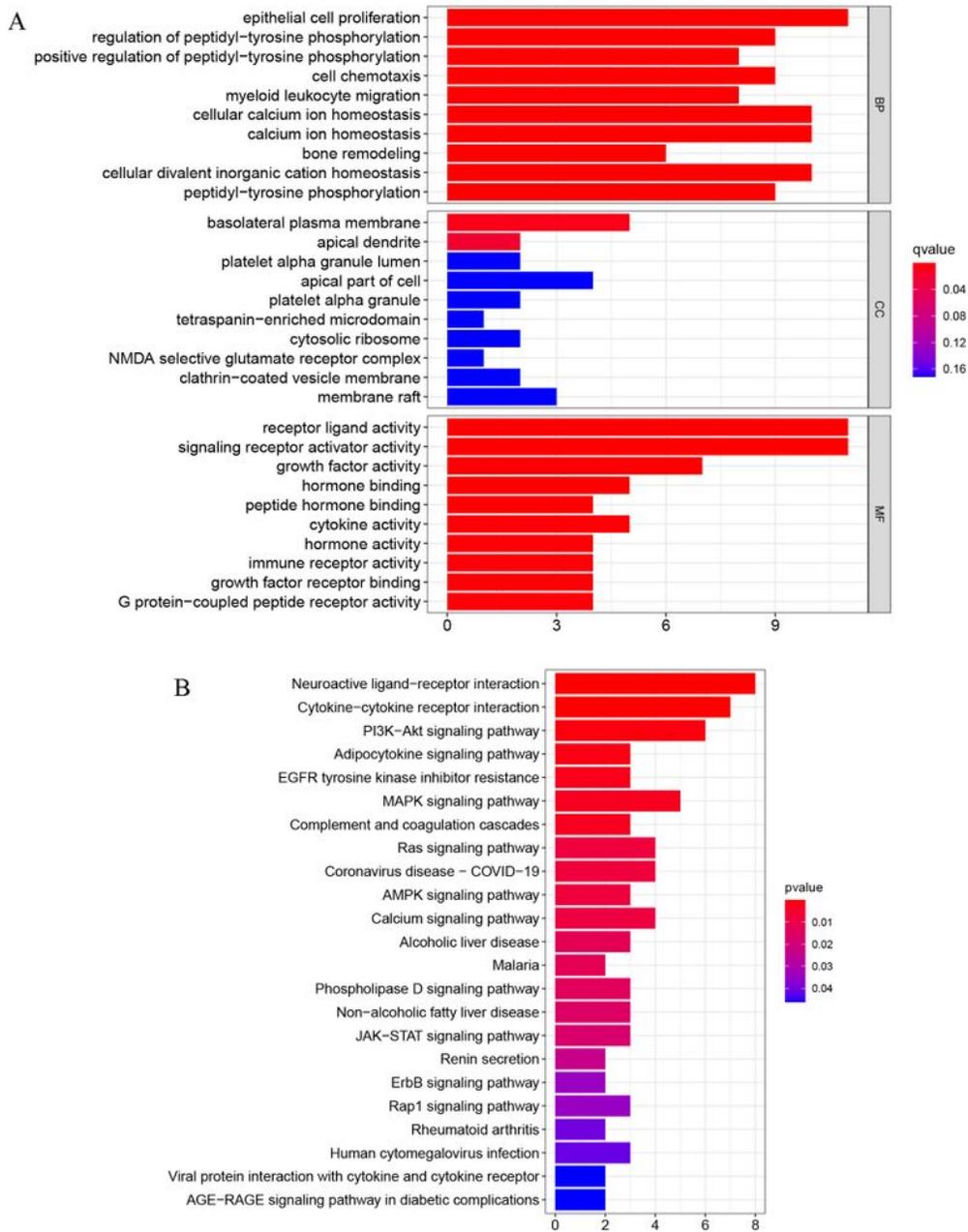


Figure 8 | (A) Gene Ontology (GO) analyses were conducted to predict the potential function of the differentially expressing immune related genes between the treat and the control group. cellular component (CC), molecular function (MF), biological process (BP), respectively. (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) potential pathways regarding the differentially immune related genes between the treat and the control group.

Figure 8

See image above for figure legend.

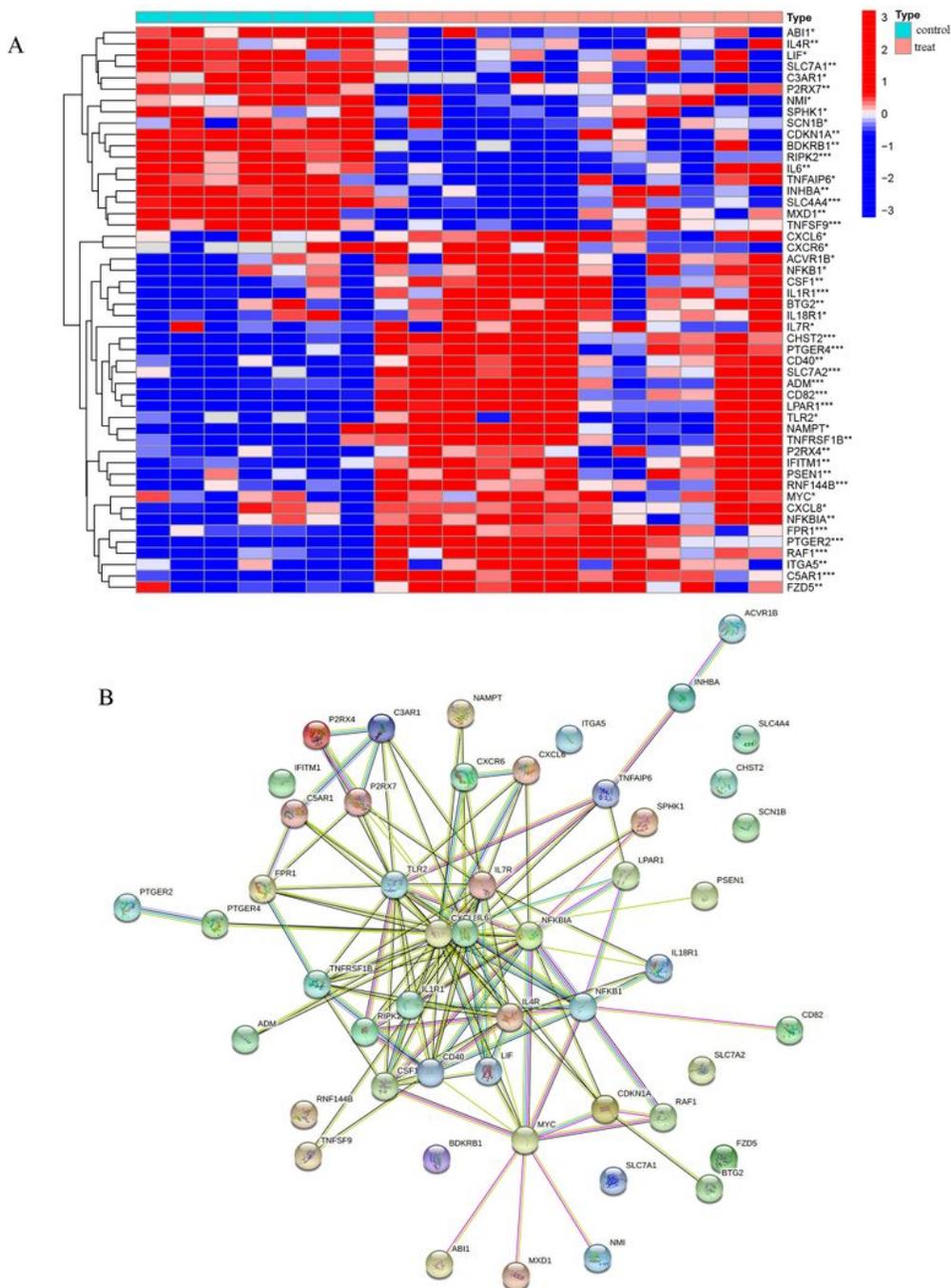


Figure 9 | The expression characteristics of inflammation related regulators. (A) Heatmaps presented the expression of inflammation related regulators in treat and control group. $P < 0.05$ (“*”), $P < 0.01$ (“**”) and $P < 0.01$ (“***”). (B) The STRING Protein-Protein Interaction Networks of inflammation related regulators.

Figure 9

See image above for figure legend.

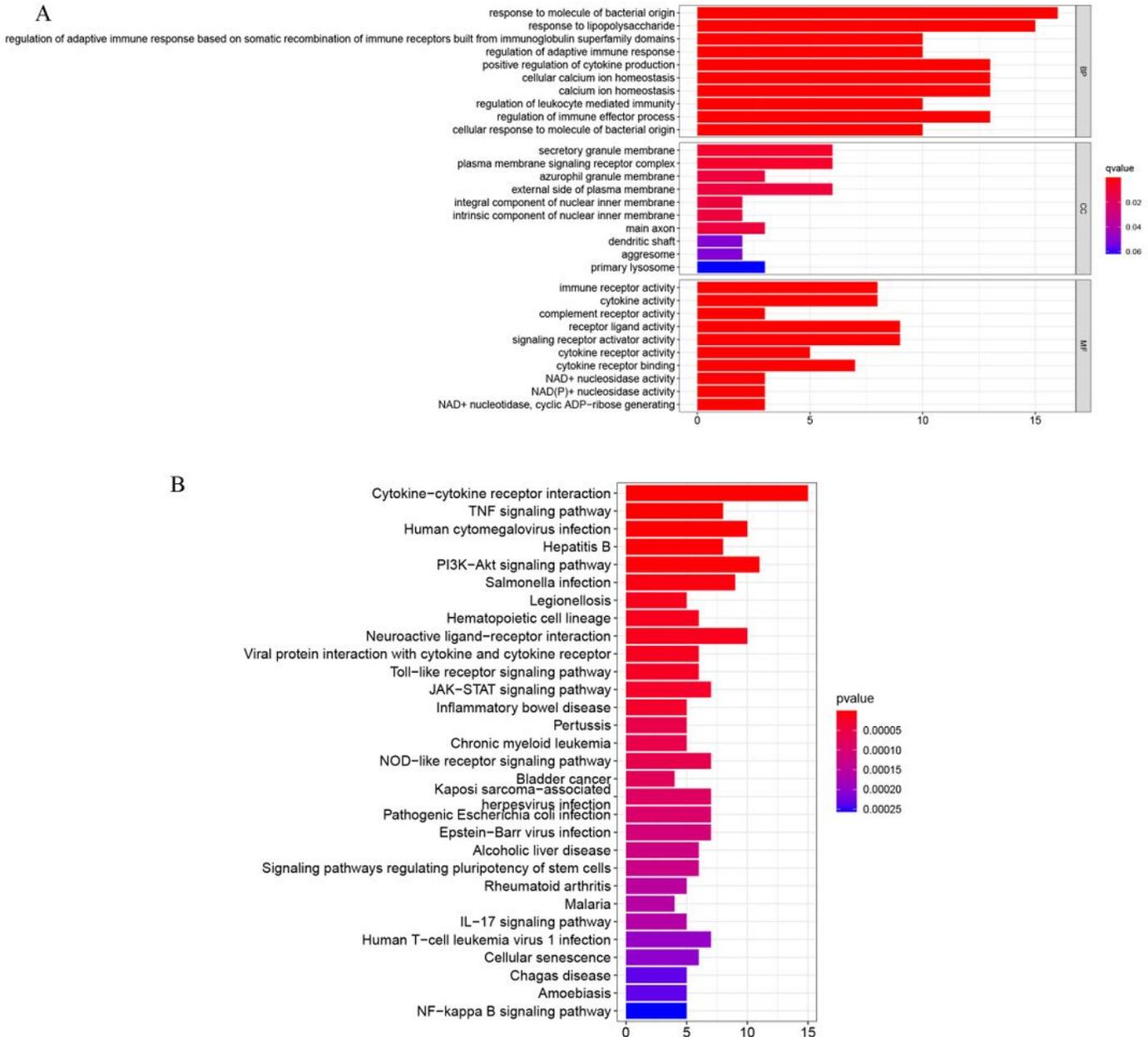


Figure 10 | (A) Gene Ontology (GO) analyses were conducted to predict the potential function of the differentially expressing inflammation related genes between the treat and the control group. cellular component (CC), molecular function (MF), biological process (BP), respectively. (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) potential pathways regarding the differentially inflammation related genes between the treat and the control group.

Figure 10

See image above for figure legend.

