Comparative Transcriptomic Analysis Reveals the Immunosuppressive Targets of Mesalazine in Dextran Sulfate Sodium-Induced Ulcerative Colitis

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Ulcerative colitis (UC) is a complex inflammatory bowel disorder that can induce colonic and rectal dysfunction. Mesalazine, a first-line medicine, is routinely prescribed for UC treatment. However, the pharmacological targets of mesalazine against UC are not detailed in current publications. In the current study, a transcriptomics strategy was applied to reveal the therapeutic targets and molecular mechanisms of mesalazine for treating dextran sulfate sodium (DSS)-induced UC in mice. Compared with the UC group, a total of 1,663 differentially expressed genes were identified in mesalazine-treated mice, of which 262 were upregulated and 1,401 were downregulated. GO and KEGG enrichment analyses indicated that the protective actions of mesalazine for treating UC were related to the functional regulation of immune inflammatory response, such as the regulation of T cells, white blood cells, and cytokine receptor pathways. In addition, ingenuity pathway analysis of the gene network further revealed the inhibitory action of mesalazine on C–C motif chemokine ligands (CCL11 and CCL21) and C–X–C motif chemokine ligands (CXCL3 and CXCR2). Taken together, the current transcriptomic findings revealed anti-UC pharmacological targets, including the newly discovered biotargets CCL11, CCL21, CXCL3, and CXCR2, of mesalazine against DSS-induced intestinal inflammation.

Keywords: ulcerative colitis, mesalazine, transcriptomics, differentially expressed genes, pharmacological targets

INTRODUCTION

Ulcerative colitis (UC), a refractory enteritis disease, is characterized by mucosal inflammation and enterocyte lesions present in the gastrointestinal tract (Guan, 2019). It is reported that the prevalence and burden of UC are increasing worldwide, including in China (Ordás et al., 2012). Genetic factors, environmental factors, and dietetic alterations may be crucial risk indicators...
for UC (Eisenstein, 2018). If left untreated, chronic UC in Asian patients may result in an elevated risk of developing colorectal cancer when compared with that of the general population (Bopanna et al., 2017). The pathological etiology of UC is multifaceted, including impaired barrier function in the mucus layer, lamina propria lesion, gut microbiota imbalance, and neutrophilic immune response (Kobayashi et al., 2020). Early and precise diagnosis of UC using endoscopy, CT scan, blood biomarker determination, fecal calprotectin testing, and bowel ultrasonography is critical to efficacious therapy (Conrad et al., 2014). Treat-to-target, a countermeasure used for treating chronic disorders, may be achieved by determining pathological targets and mitigating UC (Ungaro et al., 2019).

Mesalazine, a precursor of 5-aminosalicylic acid, can be used clinically for mild to moderate UC, and is characterized by a good safety profile and low tolerance (Sehgal et al., 2018). Mesalazine is clinically used as the first-line treatment for patients with UC based on its high effectiveness and safety (Kato et al., 2018). However, new mesalazine anti-UC targets remain minimally reported in preclinical studies. Transcriptomics, a next-generation sequencing technology, is an emerging and rapid method that can identify genome-wide annotation of DNA functions and perform detailed comparative genomic studies in humans and mice (Breschi et al., 2017). Interestingly, transcriptomic strategies have been used to unravel the detailed targets of pathological liver cancer (Lai et al., 2020),

![Diagram of gene expression](image-url)

**FIGURE 1** | Dextran sulfate sodium (DSS) induced colitis through the induction of inflammatory response. The volcano plot shows the differential expression of genes in the gut of DSS-induced colitis mice. The genes with |log2 fold change: DSS/Ctrl| > 1 and -log B and H corrected p-value > 1.3 were considered differentially expressed genes (DEGs). The red dot represents upregulated genes, the green dot represents downregulated genes, and the gray dot represents genes with no significant change. (B) The rich factor plot shows the alteration of biological processes related to immunity in the gut of DSS-induced colitis mice. The size of the dot represents the number of DEGs. The color intensity of the dots represents the significance of the biology processes. (C) The rich factor plot showed the alteration of cell signaling pathways related to immunity in the gut of DSS-induced colitis mice. The size of the dot represents the number of DEGs. The color intensity of the dots represents the significance of the signaling pathways.
# TABLE 1 | Inflammatory responses in gut caused by DSS.

| GO ID          | Description                                                        | Adjusted p-value | Number of gene | Gene ID                                                                 |
|----------------|--------------------------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:0005727     | regulation of inflammatory response                                 | 5.65E-32         | 90             | S100a8/S100a9/Il1f/Il6/Mmp8/Serpine1/Il1b/Alg2a/Ptg52/Il1f/Adop/Il1f/   |
|                |                                                                    |                  |                | Sema7a/C3/Fcgr3/Per1/Orm/Fcgr2b/Cdhs/Tnfssf/Il1f/Il2f/Adop/Il1f/Tnf/   |
|                |                                                                    |                  |                | Ednra/Il6/Tnn1/Tnf2/Il6a/Sipa/Adop/Tnf/Tox5/Il6a/Sipa/Cd15f/Il5ra/    |
| GO:0002526     | acute inflammatory response                                         | 4.54E-19         | 42             | Il6/Serpin3n/Alg2a/Il1b/Il6/Mmp8/Serpine1/C3/Fcgr3/Fcgr2b/Il1a/Tnf   |
|                |                                                                    |                  |                | Il6/Tnf/sf1/Fcgr1/Tnf/Tnf/Aloxpap/Vh1/Fcgr1/Serping1/Cd16f/Adop/Fn1/Tf |
| GO:0005729     | positive regulation of inflammatory response                       | 6.22E-14         | 37             | S100a8/S100a9/Il1f/Il6/Mmp8/Serpine1/Il1b/Ptg52/C3/Fcgr3/Orm/Tnfssf/  |
| GO:0002673     | regulation of acute inflammatory response                          | 6.08E-13         | 25             | Il6/Il1b/Ptg52/C3/Fcgr3/Fcgr2b/Tnfssf/Fcgr1/Tnf/Aloxpap/Fcgr1/Cd3f    |
| GO:0005728     | negative regulation of inflammatory response                       | 1.11E-12         | 37             | C3/Fcgr3/Per1/Orm/Fcgr2b/Il6a/Sipa/Adop/Tnf/Tox5/Tnf/Tox2/Il6a/Sipa   |
| GO:0002675     | positive regulation of acute inflammatory response                 | 1.15E-08         | 15             | Il6/Il1b/Ptg52/C3/Fcgr3/Tnfssf/Tnf/Aloxpap/Fcgr1/Cd3f/Adop/Orm/Fn1/Tf |
| GO:0002523     | leukocyte migration involved in inflammatory response              | 1.92E-07         | 10             | S100a8/S100a9/Cd12f/Tg6/Tg6/Tg6/Fcgr1/Tnf6/Sipa/Adop/Tnf/Tox5/Tnf    |
| GO:0002532     | production of molecular mediator involved in inflammatory response | 5.11E-07         | 19             | Serping1/Il1f/Adop/Tnf/Adop/Tnf/Adop/Tnf/Adop/Tnf/Adop/Tnf/Adop/Tnf  |
| GO:0002437     | inflammatory response to antigenic stimulus                        | 7.40E-07         | 17             | C3/Fcgr3/Fcgr2b/Fcgr1/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf     |
| GO:0002438     | acute inflammatory response to antigenic stimulus                  | 8.96E-07         | 12             | C3/Fcgr3/Fcgr2b/Fcgr1/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf     |
| GO:00090594    | inflammatory response to wounding                                  | 1.01E-05         | 7              | Timp1/Timp1/Timp1/Timp1/Timp1/Timp1/Timp1/Timp1/Timp1/Timp1/Timp1/T  |
| GO:0002561     | regulation of inflammatory response to antigenic stimulus         | 3.62E-05         | 11             | C3/Fcgr3/Fcgr2b/Fcgr1/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf     |
| GO:00150076    | neuroinflammatory response                                         | 0.000105408      | 11             | Mmp8/Ffz2/Il3f/Cd200/Ager/Tr2/Tr2/Tr2/Tr2/Tr2/Tr2/Tr2/Tr2/Tr2/Tr2/Tr |
| GO:0002864     | regulation of acute inflammatory response to antigenic stimulus    | 0.000161112      | 8              | C3/Fcgr3/Fcgr2b/Fcgr1/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf     |
| GO:0002863     | positive regulation of inflammatory response to antigenic stimulus | 0.000577499      | 7              | C3/Fcgr3/Fcgr1/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf    |
| GO:1900015     | regulation of cytokine production involved in inflammatory response| 0.000633959      | 10             | Il1f/Adop/Per1/Tam1/Adop/Sipa/Tam1/Adop/Sipa/Tam1/Adop/Sipa/Tam1/Adop |
| GO:0002866     | positive regulation of acute inflammatory response                 | 0.00098818       | 6              | C3/Fcgr3/Fcgr1/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf    |
| GO:0002534     | cytokine production involved in inflammatory response             | 0.000106648      | 10             | C3/Fcgr3/Fcgr2b/Fcgr1/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf     |
| GO:0002544     | chronic inflammatory response                                       | 0.0001405663     | 6              | C3/Fcgr3/Fcgr2b/Fcgr1/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf     |
| GO:0002699     | leukocyte activation involved in inflammatory response             | 0.0001869998     | 8              | C3/Fcgr3/Fcgr2b/Fcgr1/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf/Tnf     |
| GO:1900016     | negative regulation of cytokine production involved in inflammatory response | 0.0006573282  | 5              | Il1f/Adop/Sipa/Tam1/Adop/Sipa/Tam1/Adop/Sipa/Tam1/Adop/Sipa/Tam1/Adop |
| GO:0006925     | inflammatory cell apoptotic process                                | 0.01251732       | 6              | Il6/Cxcr2/Fcgr2b/Cdhs/Il6a/Sipa/Il6a/Sipa/Il6a/Sipa/Il6a/Sipa/Il6a/Sipa |
| GO:0002674     | negative regulation of acute inflammatory response                | 0.002079612      | 4              | C3/Fcgr3/Fcgr2b/Cdhs/Il6a/Sipa/Il6a/Sipa/Il6a/Sipa/Il6a/Sipa/Il6a/Sipa |
| GO:0002862     | negative regulation of inflammatory response to antigenic stimulus | 0.003274163      | 4              | C3/Fcgr3/Fcgr2b/Cdhs/Il6a/Sipa/Il6a/Sipa/Il6a/Sipa/Il6a/Sipa/Il6a/Sipa |
| KEGG ID       | Description                                                | Adjusted p-value | Number of gene | Gene ID                                                                                                                                 |
|---------------|-------------------------------------------------------------|-------------------|----------------|---------------------------------------------------------------------------------------------------------------------------------------|
| mmu04060      | Cytokine-cytokine receptor interaction                       | 1.03E-18          | 76             | Cxcl5/Il1r1/Ilb/Cxcl2/Csf3/Cxcr2/Inhibb/Ii11/Ii1b/Ii19/Cxcl1/Ii12/OsMr/Ii1r/Ii1c/Cxcl3/                                   |
|               |                                                             |                   |                | Csf3r/Cxcl/OsMr/Tnfrsf9/Il1a/Tnfrsf11/Ii33/Ppap/Cxcl13/Bmp3/Bmp7/Inhiba/Tnf/Cc1r1/Cc1l15ra/Cc3/Csf1r2/Csf2r/Pf4/Cxcl8/Ii18rap/Cxcl12/Bmp2/Ackr3/Il6st/Tgfbi1/Cxcl16/ |
|               |                                                             |                   |                | Tgfbi1/Il4ra/Cd4/Ii4ra2/Ii34/Cc1r1/Csf1r/Il10ra/I10ra/Tnfrsf11b/Ii12ra/Ii13ra2/Cc7/Cc2r/Cc5r/Bmp3/Tnfrsf11a/Gm10591/Cxcr4/Cnfr/Cf1/l1r18r1/Bmp8bb/Ret/ |
|               |                                                             |                   |                | II2/Tnfrsf9/Ii2rb/Tnfrsf8/Ii20rb/Kk1b4/Cc21a                                                                                   |
| mmu04061      | Viral protein interaction with cytokine and cytokine receptor | 4.83E-14          | 36             | Cxcl5/I6/Cxcl2/Cxcl1/Ii19/Cxcl3/Csf2/Ppbp/Cxcl13/Tnfr/Cc1r1/Cc3/I3/Pf4                                                             |
|               |                                                             |                   |                | Csf8/Ii18rap/Cxcl12/Ackr3/Il6st/I34/Cc1r1/Csf1r/Il10ra/I10ra/Ii2ra/Cc7/Cc2r/Cc5r/Gm10591/Cxcr4/Gm13304/Cf1/l1r18r1/Bmp8bb/Ret/ |
| mmu04610      | Complement and coagulation cascades                          | 7.62E-13          | 34             | Serpin1/Plaur/C3/Brkbl/Plaur/Pllat/Fgla/F10/C1q2/fgam/Fgci/C1s1/C4b/F13a1                                                    |
|               |                                                             |                   |                | C1qbi/Vw/Serping1/Cth/C1q2/C1r1/A2m/Pros1/Igtgb2/C7/Cd55/Bdkrbl/F2r/F2rl/Cr1b/tfag/Fhbd/F3/Cb3/C3r1                      |
| mmu05144      | Malaria                                                      | 1.21E-09          | 23             | Il6/Csf3/Hbb-b3/Thbs1/Ii1b-a2/Hba-a1/I1b1/Hbb-                                                                                 |
| mmu04151      | PI3K-Akt signaling pathway                                   | 4.99E-09          | 66             | Itgam/OsMr/Col4a1/Tnc/Col4a2/Lamb1/OsMr/Lamb1/Lama1                                                                  |
|               |                                                             |                   |                | /Pdgfra5/Pdgfr/Creb3/Lama4/Nos3/I1r/N4a1/Spp1/Fgf7/Wf/tfgb8/Fn1/lgf1/Anq1/                                                 |
|               |                                                             |                   |                | Tgfr5/Col1a2/Emab5/Phlp2/Ii4ra/Col4a4/Pdgfrb/Pdgcdf/Ifrar1/Col6a1/Dd14/Anq2p2/                                         |
|               |                                                             |                   |                | /Csf1r/Nras/I2ra/Gng2/Kdr/Col1a2/Lama5/Myb/F2r/PP2r2b/Pdgfb/Col1a1/Fgcl3/Tr12/   |
| mmu05146      | Amoebiasis                                                   | 2.62E-08          | 30             | Gsiga/Fthbd/F3/Cb3/C3r1                                                                                                        |
| mmu04933      | AGE-RAGE signaling pathway in diabetic complications          | 2.62E-08          | 29             | Il6/Serpin1/I1b1/Col4a1/Cc2/Col4a2/Sele/I1a/Vcam1/Mmp2/Tnf/Nos3/Nol1/Fn1/                          |
|               |                                                             |                   |                | /Ager/Egr/Tgfbi1/Tgfbr1/Nras/Icam1/Col1a2/Col3a1/Col1a1/Cyb2/Thbd/Col4a4/         |
| mmu04512      | ECM-receptor interaction                                     | 8.92E-08          | 26             | Thbs1/Col1a4/Tnc/Col4a2/Lamb1/Lama1/Lama1/Frem1/Lama4/Spl1/Vw/tfgb8/Fn1/Cd36/Il6tgs5/Col4a2/Col6a1/Col6a2/Lama5/    |
| mmu04514      | Cell adhesion molecules                                      | 2.63E-07          | 38             | Selp/Sele/Cd55/Vcam1/Igtgam/Pcam1/Itla4/Esm2/Vecm/Vgab/Fgbi8/Alv/Madcam1/                     |
|               |                                                             |                   |                | Col4a4/Il1a/Col4a4/Il12/Col4a2/Col1a4/Lama1/Lama1/Frem1/Lama4/Spl1/Vw/tfgb8/Fn1/Cd36/Il6tgs5/Col4a2/Col6a1/Col6a2/Lama5/ |
| mmu05618      | Fluid shear stress and atherosclerosis                       | 3.14E-07          | 34             | Il1b/I1f1/I1r1/Mmp9/Cc2/Dup1/Sele/Plat/Hmox1/Ii1a/Cdh5/                                                     |
|               |                                                             |                   |                | Vcam1/Mmp2/Pcam1/Gstms3/Tnfr/Nos3/Nol1/Kl/2/Inhiba/Tnfrsf11a/Gama1/Kdr/Cav1uent                                      |
| mmu05323      | Rheumatoid arthritis                                         | 1.16E-06          | 24             | Cxcl5/Il6/Mmp3/Cxcl2/Ii11/Ii1b/Cxcl1/Cxcr3/Ii2r/Tnfrsf11f/Tnf/Ctla4/F1t1/Cd33/ |
| mmu04380      | Osteoclast differentiation                                   | 1.38E-06          | 30             | Il1b/I1f1/Fgcr3/Fgcr2b/Ii1f1/Tnfrsf11b/Iib4a/Tfgbr1/Tnf1/Tnfrb/Fgr1/Tnfr/Tnfrb/Ctka/Nox1/                         |
|               |                                                             |                   |                | /Socs3/Tgfbi1/Tgfbr1/Spl1/Infrar2/Csf1r/Tnfrsf11b/Sirpa/tfgb3/Lcp2/Sirpb1b/Fyn/Fos1/                                   |

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| KEGG ID | Description                                      | Adjusted p-value | Number of gene | Gene ID                                                                 |
|---------|--------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| mmu04974 | Protein digestion and absorption                 | 1.40E-06         | 27             | Col18a1/Copa3/Col4a1/Col4a2/Gm2663/Kcne3/Col15a1/Col5a2/Atp1b2/          |
|          |                                                  |                  |                | Xpnpep2/Col13a1/Col6a2/ELr/Col5a1/Col5a4/Col5a5/Col12a1/Slc36a2/Col23a1/ |
|          |                                                  |                  |                | Col1a2/Col2a/Col3a/Col36a4/Col4a/Col4a4/Col16a1                      |
| mmu04640 | Hematopoietic cell lineage                       | 1.82E-06         | 23             | Il6/Cst5/Ilt1/Ilt1b/Ilt1r1l/Ilt3r/Ilta1/Tgamt/Tnf/Cd33/Fgfr1/Ilt5a/Cd36/Iltg5/Ilt4a/ |
|          |                                                  |                  |                | Col4/Col4/Ilt2a/Col55/fgfr3/Ilt7/Ilt7b/Cd34                          |
| mmu05133 | Pertussis                                        | 3.16E-05         | 20             | Cxcl5/Ilt6/Iltb/CIa1/Ctqa/Tgamt/Nlrp3/Tnf/C1s1/C4b/C1q/C1r/Sepr1g1/ |
|          |                                                  |                  |                | Itlg5a/Ilt7c/Nos2a/C1ra/fgfr2/Ilt10/C1r1                             |
| mmu04062 | Chemokine signaling pathway                      | 5.47E-06         | 35             | Cxcl5/Cxcl2/Cxcl1/Cxcl3/Col2/Phd2bpep/Cxcl13/Cor1/Col1/Col3/Qrk3/P44/ |
|          |                                                  |                  |                | Cxcl12/Prext/Arnb2/Cxcl16/Col4/Adcy4/Nras/Gng2/Cg2/Ccr7/Ccr5/Pckb6/ |
|          |                                                  |                  |                | Gm10591/Cxcl4/Pkdr5/Gmp13304/Gen4/NCf1/Fgr/Cxcl21a                   |
| mmu05150 | Staphylococcus aureus infection                  | 6.93E-06         | 26             | F2r2/F2r1/Selp/C3/Fgcr2/Fgcr2b/Ilt7a/Tgamt/Fgff/Fgcr1/C1s1/C4b/      |
|          |                                                  |                  |                | C1qbp/CIh1/C1qbc/Psat/C1ra/Ilt7b/Ilt10/Itlam1/Itlam2/C1r1/Selpqg/Krt14/Krt23/C3ar1 |
| mmu05143 | African trypanosomiasis                          | 8.69E-05         | 13             | Il6/Iltb-bb/Hba-a2/Hba-a1/Ilt1b/Iltb-bb/Sele/Vcam1/Tnf/Lamn4/Ilt10/Icam1/Apoa1 |
| mmu05140 | Leishmaniasis                                    | 9.47E-06         | 18             | Il1b/Ptg3a/Marcok1/C3/Cfgr3/Ilt1a/Tgamt/Fgcr4/Fn1/Fgtb1/Ilt10/      |
|          |                                                  |                  |                | Tr2/Cybb/Ncf1/Ncf1                                                   |
| mmu05321 | Inflammatory bowel disease                      | 0.000275632      | 16             | Il6/Ilt1b/Ilt1r/Ilt8rap/Tgfb1/Ilt4a/Fopx3/Ilt10/Rora/Tk2/2a3a/Stat4/Maf/Ilt1r1/Ilt22 |
| mmu04510 | Focal adhesion                                   | 0.000302024      | 34             | Tbs1/Col4a1/Trn/Col4a2/Lamb1/Lanc1/Lama1/Pdgfbraf/Plgf/Lama4/It1/Spp1/ |
|          |                                                  |                  |                | Vw/Vw/Itbgb6/F1/Itgb6/Itgb5/Cfgr5/Cfgr5a/Col6a2/Col6a4/Pdgbf/Pdgbf2/ |
|          |                                                  |                  |                | Col8a1/Lama5/Pdgfb/Parvb/Col1a1/Rac2/Htgfb3/Col4a4/Fyn                |
| mmu04657 | IL-17 signaling pathway                          | 0.000324392      | 20             | S100a5/S100a9/Cxcl5/Iltb6/Mmp3/Cxcl2/Tcfr5/Lcn2/Itlt4/Iltg2s2/      |
|          |                                                  |                  |                | Mmp9/Cxcl3/Cc2f/Tnf/Col11/Cebpp/Fos1/Map15                         |
| mmu04630 | JAK-STAT signaling pathway                       | 0.000738152      | 29             | Il6/Cst5/Iltf/Ilt19/Osmr/Cst22r/Osm/Pdgrf/gala/Ilt5a/Col6s2/Cf2b2/Cf2b2/Socs3/Itlb3/Ilt4a/ |
|          |                                                  |                  |                | Pdgfb/Sfz2/Stat4/Cnfrn/Ilt7/22/Ilt2b/Ilt2b/Itlb/Xox1               |
| mmu04010 | MAPK signaling pathway                           | 0.000904161      | 43             | Ereg/Ilt1b/Ilt1r/Dusp1/Ilt1a/Pdgfbraf/Plg/Itph2s2/Tnf/Flt1/Itphap1a/Nl4a1/Fgtj7/Flg1/  |
|          |                                                  |                  |                | Anogt1/Fgr1/Hspb1/Itphap1b/Elfnas/Arnb2/Tgfb1/Itmn1/Tgfrb1/Pdgfbf/Pdgfbf2/Anogtp2/ |
|          |                                                  |                  |                | Csf1r/Nras/Casca2n1/Itk/Pttn5/Pdgfb/Dusp2/Fgfr3/Rac3/Mp3k8/Hgf/Cagng7/  |
|          |                                                  |                  |                | Cacna1e/Lmp3k8/Pla2gc4/Rps6ka6/Kkb14                               |
| mmu04611 | Platelet activation                              | 0.001249729      | 23             | Fgr3/Fgcr1/Fggr/Nos3/VwF/Itptgs1/Adcy4/Gucy1b/C1la2/Pfg2/F2r3/Flr2/   |
|          |                                                  |                  |                | Col3a1/Col1a2/Pfes/Pfg2r/12TGf3b/Lcp2/Pfkg3/Fyn/Fermel/P2x1/Pla2g4c  |
| mmu04670 | Leukocyte transendothelial migration             | 0.001551043      | 22             | Mmp9/Cdtn5/Cam1/Tgamt/Mmp2/Pecam1/Msn/Esam/Cxcl12/Cldn4/Itgb2/Itcam1/ |
|          |                                                  |                  |                | Rac2/Cxcl4/Itbg/Cldn5/Itam2/Jam2/Jam1/Rass5f/Jam3/Itf/Ncf1           |
| mmu04015 | Rap1 signaling pathway                           | 0.001969048      | 33             | Tbs1/Flr1/Tgamt/Pdgfbraf/Pgt1/Flt1/Flg7/Ing1/Fgr1/Elfnas/Pdgbf/Pdgbf/ |
|          |                                                  |                  |                | Angt2/Adcy4/Itgb2/Itfr1/Nras/Kfr2/Flr2/F2r/Pdgfbf/Pdgfbf3/Rac2/Htgfb3/Lcp2/Adora2a/  |
|          |                                                  |                  |                | Arap3/Cntn1/Rass5f/Ph2/Kk1b4                                       |
| mmu04064 | NF-kappa B signaling pathway                     | 0.0020404       | 20             | Cxcl2/I1b/Ptg3s2/Iltcl1/Ilt1r/Cxcl3/Plau/Tnfls11/Itcam1/Tnf/Cxcl12/Bcl2a1b/ |
|          |                                                  |                  |                | Cxcl4/Bcl2a1a/Bcl2a1d/Cxam1/Gm10591/Lbp/Gm13304/Cc21a               |
| mmu04668 | TNF signaling pathway                            | 0.0020404       | 21             | Cxcl5/Ilt6/Mmp3/Cxcl2/I1b/Ptg3s2/Ilt11m/Pmp9/Cxcl2/Ct2/Sel/Itcam1/Creb33/  |
|          |                                                  |                  |                | Tnf/Cebpbb/Socs3/Mmp14/Itcam1/Map3k8/Csreb1/Itlb1                 |
| mmu05134 | Legionellosis                                    | 0.002307615      | 14             | Il6/Ilt2b/Iltb1/Cxcl1/Ilt3/C3Tgamt/Hipa2/Tnfl/Hipab1/Hnip3/Itgb2/T2r  |
| mmu04145 | Phagosome                                        | 0.00230738       | 29             | Tbs1/Ilt1/Itm0/Cc3/Cfcr3/Fgcr3/Fgr1b/Mfex/Itgamt/Gm7a/Cfcr4/Coleic12/Mr1c/Fcr1/  |
|          |                                                  |                  |                | /Cd6a/Iltg5/Mrc2/C1ra/tgb2/Tubb3/Nos1/C1r1b/H2-Q10/T1r2/tgb3/       |
|          |                                                  |                  |                | Cybb/Pis2r1/Coro1a/Itg4/Ncf1                                       |

(Continued)
| KEGG ID       | Description                              | Adjusted p-value | Number of gene | Gene ID                                                                 |
|--------------|------------------------------------------|------------------|----------------|------------------------------------------------------------------------|
| mmu05152     | Tuberculosis                             | 0.004243105      | 28             | Il6/I1b/Sphk1/C3/FcgR3/Clec4a/Fcgr2b/I1a/Fcer1g/Igcam/Clec7a/Fcgr4/Tnf/Mrc1/Fcgr1/Cebpb/Mrc2/Nos2/Tgfbr1/I10Ir1a/110/CarDi9/Igcam/Tlr2/Lbp/Pia2r1/Coro1a |
| mmu04926     | Relaxin signaling pathway                | 0.004501477      | 22             | Mmp13/Mmp9/Col4a1/Col4a2/Mmp2/Creb3/3/Nos3/Nos2/Arbb/Tgfbr1/Ednrbr/Tgfbr1/Adcy4/Nras/Gng2/Nos1/Col1a2/Col3a1/Col4a4/Creb5/Gng4 |
| mmu05202     | Transcriptional misregulation in cancer  | 0.006992589      | 32             | Il6/Mmp3/I1r2/Mmp9/Etv4/Plaau/Igcam/Flt1/FcgR1/Cebpb/Igfbp3/Igf1/Bcl2a1b/Erg/Sp1/Irun1/Bcl2a1a/Bcl2a1d/Csf1r/Nup1r/Rel/Zbta16/Cdb86/F11/Zeb1/Prom1/Ccna2/Mat/Runx2/Nr4a3/I1r2b |
| mmu05205     | Proteoglycans in cancer                  | 0.007038418      | 30             | Thbs1/Mmp9/Plaau/Plaau/Mmp2/Msrn/Dcn/Tnf/Twist2/Timp3/Fzd1/Fnl/Ilf5/Igf5/Fgt1/Tgfbr1/Fzd4/Nras/Wnts5a/Kdrl/Hcls1/Cav1/Col1a2/Lum/Col1a1/Tlr2/Twist1/Hgf/Ilg30/Hnt11 |
| mmu04350     | TGF-beta signaling pathway               | 0.009765721      | 17             | Inhba/Thbs1/Grem1/Nbl1/Fet/Dcn/Bmp7/Inhba/Tnf/Ltb1/Bmpr2/Chrd/Tgfbr1/Bmp5/Fbn1/Bmp6b |
| mmu04020     | Calcium signaling pathway                | 0.011240288      | 33             | Sphk1/Bdkrb1/Tac1/Polgfr/Ednra/Nos3/F1t/Fgf7/Fgf1/Platr/Ret/Pde1a/Nos2/Ednrb/Pdgfrb/Pdgfc/Adcy4/Htr4/Kdrl/Bdkrb2/Nos1/F2r/11r7/Pdgfrb/Fgfr3/Hgf/Ccna4/Adora2a/P2x1/Cacna1e/Avp1a/Pkd3/Klk1b4 |
| mmu05145     | Toxoplasmosis                            | 0.019183988      | 18             | Lamb1/Lama1/Hspa2/Tnf/Lama4/Hspalb/Il6/I1b/I10/I10a/I10/Lama5/Tlr2/Ccr5/Pkrd6/Pkrd5/Atp3 |
| mmu05142     | Chagas disease                           | 0.022038178      | 17             | Il6/Serpin1/I1b/C3/C3a/Tnf/C1qa/Tf/Clq/C3a/C1q/Nos2/Tgfbr1/Tgfbr1/I10/11r2/Bdkrb2/Pop2r1b/Tlr2 |
| mmu04072     | Phospholipase D signaling pathway        | 0.025003398      | 22             | Cxcr2/Sphk1/Fcer1g/Pdgfrb/Pp3c/Cyth3/Pdghbe/Pdghc/Adcy4/Nras/F2r/Pdghbe/Dnm1/Pkrd6/Pkrd5/Gmr7/Cyth4/Fyn/Agtr1a/Pla2g4c/Dnm3 |
| mmu05215     | Prostate cancer                          | 0.033625213      | 16             | Mmp3/I1r2/Mmp9/Plaau/Plag/Pdghbe/Creb3/3/Igf1/Fgt1/Erg/Pdgfrb/Pdgfc/Nras/Pdgfrb/Zeb1/Creb5 |
| mmu04620     | Toll-like receptor signaling pathway     | 0.036246562      | 16             | Il6/I1b/Tnf/Igcam/Col4/Cd80/Cd86/Tlr2/Tlr7/Mmp3/I1r2/Lbp/Irf5 |
| mmu04672     | Intestinal immune network for IgA production | 0.037643516   | 9              | Il6/Icos/Cxcl12/Tgfbr1/Madcam1/Cd80/I10/Cd86/Cxcr4 |
Li et al. Detailed Anti-UC Biotargets of Mesalazine

**FIGURE 2** Immunosuppressive effects of mesalazine on DSS-induced colitis. The volcano plot shows the differential expression of genes in the mesalazine-treated colitis mice. The genes with $|\log_2 (\text{fold change: mesalazine + DSS/DSS})| > 1$ and -log B and H corrected p-value $> 1.3$ were considered DEGs. The red dot represents upregulated genes, the green dot represents downregulated genes, and the gray dot represents genes with no significant change. (B) The rich factor plot shows the alteration of biological processes related to immunity in the gut of mesalazine-treated mice with colitis. The size of the dot represents the number of DEGs. The color intensity of the dots represents the significance of the biology processes. (C) The rich factor plot showed the alteration of cell signaling pathways related to immunity in the gut of mesalazine-treated colitis mice. The size of the dots represents the number of DEGs. The color intensity of the dots represents the significance of the signaling pathways.

perfluorooctanesulfonate-induced immunotoxicity (Li et al., 2020a), glutathione against cleft lip (Li et al., 2021b), and vitamin C against hepatotoxicity (Li et al., 2021a). Therefore, in the present study, we utilized transcriptomic analysis to reveal detailed and novel biotargets of mesalazine against UC in vivo.

**MATERIALS AND METHODS**

**Animal Maintenance and Treatment**

Mature male C57BL/6J mice, aged around 7 weeks, were purchased from STA Lab Animal Co., Ltd. (Changsha, China). All mice were adaptively maintained for approximately 7 days,
### TABLE 3 | Effects of Mesalazine on the DSS-induced biological inflammatory responses.

| GO ID   | Description                          | Adjusted p-value | Number of gene | Gene ID                                                                 |
|---------|--------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:0050900 | leukocyte migration                  | 7.42E-40         | 101            | S100a8/S100a9/Retigic/Cxcl5/Cxcl2/Cxcr2/Serpine1/Thbs1/Il1b/Fpr2/Cxcl1/Apod/Grem1/
|          |                                      |                  |                | Ilr1/Mmp9/Lgmn/Cxcl3/Cxcl5/Ptgs2/Cxcl2/Trem1/Dusp1/Selp/Bdkbr1/Fgcr3/Sele/
|          |                                      |                  |                | Cyp7b1/Tacr1/Il1a/Tnfsf11/Nbl1/Fcer1g/Vcam1/I33/tgcam/Pvap/Ppbb/Cxcl13/S1pr1/
|          |                                      |                  |                | /Ednna/Pecam1/Msp/Pgf/Jam1/Tnf/Flt1/Ccr1/Spp1/Cxcl1/Cxcl3/Ptgs2/Ch25tv/P4/Adam8/
|          |                                      |                  |                | Cxcl8/Cxcl12/Ptfr/Tmpr/Ex1/Em1lin1/Cd300a/Tgb1/Cxcl16/Ednrb/Snp2/Madcam1/
|          |                                      |                  |                | /Cmxkr1/Cmx/Mmp14/tgfb2/Wnt5a/Icam1/Sirpa/Ppt22/Ccr7/Rarres2/Pdgbf/Sell/
|          |                                      |                  |                | Ccr2/Tnpv4/Bst1/Tlr2/Rac2/Selepig/Adorat1/Gata3/P2r12/Lbp/tgb3/Gpms3/Dysf/
|          |                                      |                  |                | Podbl/Art1/Coro1a/Slimf8/Nckap11/Nkoc-3/Jam3/Cd34/Cd21a/C3ar1 |
| GO:0097529 | myeloid leukocyte migration            | 1.01E-29         | 68             | S100a8/S100a9/Retigic/Cxcl5/Cxcl2/Cxcr2/Serpine1/Thbs1/Il1b/Fpr2/Cxcl1/Grem1/
|          |                                      |                  |                | Ilr1/Lgmn/Cxcl3/Cxcl5/Ptgs2/Cxcl2/Trem1/Dusp1/Fgcr3/S1pr11/Nbl1/Fcer1g/
|          |                                      |                  |                | Cxcl5/Cxcl2/Selp/Ednna/Pecam1/Pgf/Jam1/Tnf/Flt1/Ccr1/Spp1/Cxcl1/Cxcl3/Ptgs2/
|          |                                      |                  |                | P4/Adam8/Cxcl8/Cxcl12/Px1/Em1lin1/Cd300a/Ednrb/Cmxkr1/Cmx/Mmp14/tgfb2/Sirp/
|          |                                      |                  |                | Ccr7/Rarres2/Pdgbf/Sell/Ccr2/Tnpv4/Bst1/Tlr2/P2r12/Lbp/Dysf/Art1/Slimf8/
|          |                                      |                  |                | Nckap11/Jam3/Cd21a/C3ar1 |
| GO:0030595 | leukocyte chemotaxis                | 3.63E-28         | 68             | S100a8/S100a9/Retigic/Cxcl5/Cxcl2/Cxcr2/Serpine1/Thbs1/Il1b/Fpr2/Cxcl1/Grem1/
|          |                                      |                  |                | Lgmn/Cxcl3/Cxcl5/Ptgs2/Cxcl2/Trem1/Dusp1/Fgcr3/S1pr11/Nbl1/Fcer1g/
|          |                                      |                  |                | Cxcl5/Cxcl2/Selp/Ednna/Pecam1/Pgf/Jam1/Tnf/Flt1/Ccr1/Spp1/Cxcl1/Cxcl3/Ptgs2/
|          |                                      |                  |                | P4/Adam8/Cxcl8/Cxcl12/Px1/Em1lin1/Cd300a/Ednrb/Cmxkr1/Cmx/Mmp14/tgfb2/Wnt5a/
|          |                                      |                  |                | Ccr7/Rarres2/Pdgbf/Sell/Ccr2/Tnpv4/Bst1/Tlr2/Lbp/Dysf/Art1/Slimf8/
|          |                                      |                  |                | Nckap11/Jam3/Cd21a/C3ar1 |
| GO:0071599 | leukocyte cell-cell adhesion        | 4.18E-25         | 79             | S100a8/S100a9/I6/l6r1/11b/Cd2/Selp/Sele/Tnfsf11/Icam1/I6r3/Nlrp3/Lrc32/
|          |                                      |                  |                | Pecam1/Msp/Bmp7/Tarn1/Tnf/Cfla4/Arg2/Vn1/Arg1/Icos/Gpnnb/Cebpb/Adam8/Cxcl12/
|          |                                      |                  |                | Cyp7b1/Zc3h12a/Eds1/Tgbf1/Ptfr/Hsp11/Retgic1/Cxcl13/Ptgs2/Cxcl2/Trem1/Dusp1/
|          |                                      |                  |                | Fcgr3/S1pr1/Cxcl1/Cxcl3/Ptgs2/Ch25tv/P4/Adam8/Cxcl8/Cxcl12/Px1/Em1lin1/Cd300a/
|          |                                      |                  |                | Ednrb/Cmxkr1/Cmx/Mmp14/tgfb2/Wnt5a/Icam1/Sirpa/Zbtb16/Cdx44a/
|          |                                      |                  |                | Ppt22/Cav1/Ccr7/Myc/Cd86/Sell/Ccr2/Selepig/Pk3rb6/Gata3/Tig3/Lag3/J3r/Adora2a/
|          |                                      |                  |                | /Tnfsf82/Fermt3/Art1/Coro1a/Nn4a3/Cd6/Tnfsf9/Nckap11/Rumx3/J20rb/Vtcn1 |
| GO:0026265 | regulation of leukocyte migration    | 2.03E-24         | 60             | Cxcl2/Serpine1/Thbs1/Il1b/Fpr2/Apod/Grem1/I1r1/Mmp9/Lgmn/Ptgs2/Cxcl2/Dusp1/Selp/
|          |                                      |                  |                | Bdkbr1/Sele/Tacr1/Il1a/Nbl1/I33/tgcam/Pvap/Cxcl13/Ednna/Pecam1/Msp/Pgf/Ccr7/
|          |                                      |                  |                | Adorat1/Gata3/P2r12/Lbp/tgb3/Gpms3/Dysf/Art1/Slimf8/Nckap11/
|          |                                      |                  |                | Jam3/Cd21a/C3ar1 |
| GO:0018919 | positive regulation of cytokine production | 2.74E-23         | 91             | Ilr1/I6b/Mmp8/Serpine1/Thbs1/Ereg/Il1b/Pgts2/I1r1/Sema7a/Mmp12/C2/C3/Fgcr3/Clec4e/
|          |                                      |                  |                | Cnsm/I1a/Fcgr3/I33/tgcam4/Nlrp3/Tarn1/Cd200/Hilpida/Tnf/Cebpb/Slc11a/Cxcl3/Ptgs2/
|          |                                      |                  |                | Dusp1/Selp/Bdkbr1/Fgcr3/Sele/
|          |                                      |                  |                | Cyp7b1/Tacr1/Il1a/Tnfsf11/Nbl1/Fcer1g/Vcam1/I33/tgcam/Pvap/Ppbb/Cxcl13/S1pr1/
|          |                                      |                  |                | /Ednna/Pecam1/Msp/Pgf/Jam1/Tnf/Flt1/Ccr1/Spp1/Cxcl1/Cxcl3/Ptgs2/Ch25tv/P4/Adam8/
|          |                                      |                  |                | Cxcl8/Cxcl12/Ptfr/Tmpr/Ex1/Em1lin1/Cd300a/Tgb1/Cxcl16/Ednrb/Snp2/Madcam1/
|          |                                      |                  |                | /Cmxkr1/Cmx/Mmp14/tgfb2/Art1/Coro1a/Slimf8/Nckap11/Rumx3/J20rb/Vtcn1 |
| GO:0018118 | negative regulation of cytokine production | 9.33E-23         | 66             | Ilr1/I6b/Mmp8/Inhbb/Thbs1/I1r1/Apod/Srgn/Tnfsf9/Fgcr2b/Hmxon1/I33/tgcam3/Lnc32/Tnf/Ar2/Twist2/
|          |                                      |                  |                | Arg1/I33/tgcam4/S1pr1/Adorat1/Kdf2/Fn1/Zc3h12a/Arg1/1g/Fcgr3/S1pr1/Cxcl2/
|          |                                      |                  |                | /Axl/Havcr2/Cmxkr1/Pgpg4/Cd34/Muc16/Foxp3/Ret/I10/Sirpa/Ppt22/Clec4a/Adora2a/
|          |                                      |                  |                | Art1/Fcgr3/Tacr1/Il1a/Tnfsf11/Nbl1/Fcer1g/Vcam1/I33/tgcam/Pvap/Ppbb/Cxcl13/S1pr1/
|          |                                      |                  |                | /Ednna/Pecam1/Msp/Pgf/Jam1/Tnf/Flt1/Ccr1/Spp1/Cxcl1/Cxcl3/Ptgs2/Ch25tv/P4/Adam8/
|          |                                      |                  |                | Cxcl8/Cxcl12/Ptfr/Tmpr/Ex1/Em1lin1/Cd300a/Tgb1/Cxcl16/Ednrb/Snp2/Madcam1/
|          |                                      |                  |                | /Cmxkr1/Cmx/Mmp14/tgfb2/Wnt5a/Icam1/Sirpa/Ppt22/Ccr7/Rarres2/Pdgbf/Sell/
|          |                                      |                  |                | Ccr2/Tnpv4/Bst1/Tlr2/Rac2/Selepig/Adorat1/Gata3/P2r12/Lbp/tgb3/Gpms3/Dysf/
|          |                                      |                  |                | Podbl/Art1/Coro1a/Slimf8/Nckap11/Nkoc-3/Jam3/Cd34/Cd21a/C3ar1 |

(Continued)
| GO ID         | Description                                     | Adjusted p-value | Number of gene | Gene ID                                                                 |
|---------------|-------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:0002683    | negative regulation of immune system process    | 2.77E-21         | 91             | Il1rl1/Cxcr2/Thbs1/ApoC1/Grem1/Mmp12/Dusp1/Fgcr2b/Hmox1/Nbl1/Fcjr1g/Ill33/Cd300f0/Srpf1/Fox1a/Lnc32/Tmem17a/Tam1/Cd200/Tnf/Ctca4/Sry2/2/Arg1/Gcnmb/Cebpba/Accod1/Serp1/S1c4/Cxcr3/Lgmn1/Pla2g7/Cd2/2/Selp/2/Sele/Tacr1/Ill1a/Pvap/Cxcr13/Ednra/Prcam1/Pgf/Ccr1/Ald8/Cxcl12/Plafr/Tgf1b/Madcam1/Cmk1/C1c8/Mmp14/Wnt5a/Cacm1/Ccr7/Frraes2/Sell/Ccr2/Tpipv4/Tpk/2/Rac2/Pory2/12/Lbp/Tgfb1/Gomp3/Dysf/Ref1/Nckap1/Cd21a/C3ar1 |
| GO:0002687    | positive regulation of leukocyte migration       | 1.59E-20         | 47             | Cxcr2/Serp1/Cxcr13/Ttnspp1/Cxcr2/Tnfspp1/Fgcr2b/Hmox1/Ill33/Cd300f0/Fox1a/Lnc32/Tmem17a/Tam1/Cd200/Tnf/Ctca4/Sry2/2/Arg1/Gcnmb/Cebpba/Accod1/Serp1/S1c4/Cxcr3/Lgmn1/Pla2g7/Cd2/2/Selp/2/Sele/Tacr1/Ill1a/Pvap/Cxcr13/Ednra/Prcam1/Pgf/Ccr1/Ald8/Cxcl12/Plafr/Tgf1b/Madcam1/Cmk1/C1c8/Mmp14/Wnt5a/Cacm1/Ccr7/Frraes2/Sell/Ccr2/Tpipv4/Tpk/2/Rac2/Pory2/12/Lbp/Tgfb1/Gomp3/Dysf/Ref1/Nckap1/Cd21a/C3ar1 |
| GO:0002274    | cytokine secretion                               | 2.09E-20         | 56             | Cxcl5/Ill1/Ill33/Cd300f0/Fox1a/Lnc32/Tmem17a/Tam1/Cd200/Tnf/Ctca4/Sry2/2/Arg1/Gcnmb/Cebpba/Accod1/Serp1/S1c4/Cxcr3/Lgmn1/Pla2g7/Cd2/2/Selp/2/Sele/Tacr1/Ill1a/Pvap/Cxcr13/Ednra/Prcam1/Pgf/Ccr1/Ald8/Cxcl12/Plafr/Tgf1b/Madcam1/Cmk1/C1c8/Mmp14/Wnt5a/Cacm1/Ccr7/Frraes2/Sell/Ccr2/Tpipv4/Tpk/2/Rac2/Pory2/12/Lbp/Tgfb1/Gomp3/Dysf/Ref1/Nckap1/Cd21a/C3ar1 |
| GO:0005663    | cytokine secretion                               | 7.30E-20         | 59             | Il1rl1/Ill33/Ill1a/Cd300f0/Fox1a/Lnc32/Tmem17a/Tam1/Cd200/Tnf/Ctca4/Sry2/2/Arg1/Gcnmb/Cebpba/Accod1/Serp1/S1c4/Cxcr3/Lgmn1/Pla2g7/Cd2/2/Selp/2/Sele/Tacr1/Ill1a/Pvap/Cxcr13/Ednra/Prcam1/Pgf/Ccr1/Ald8/Cxcl12/Plafr/Tgf1b/Madcam1/Cmk1/C1c8/Mmp14/Wnt5a/Cacm1/Ccr7/Frraes2/Sell/Ccr2/Tpipv4/Tpk/2/Rac2/Pory2/12/Lbp/Tgfb1/Gomp3/Dysf/Ref1/Nckap1/Cd21a/C3ar1 |
| GO:0042110    | T cell activation                                | 1.10E-18         | 89             | Il6/Ill3/Ill1a/Ill33/Cd300f0/Fox1a/Lnc32/Tmem17a/Tam1/Cd200/Tnf/Ctca4/Sry2/2/Arg1/Gcnmb/Cebpba/Accod1/Serp1/S1c4/Cxcr3/Lgmn1/Pla2g7/Cd2/2/Selp/2/Sele/Tacr1/Ill1a/Pvap/Cxcr13/Ednra/Prcam1/Pgf/Ccr1/Ald8/Cxcl12/Plafr/Tgf1b/Madcam1/Cmk1/C1c8/Mmp14/Wnt5a/Cacm1/Ccr7/Frraes2/Sell/Ccr2/Tpipv4/Tpk/2/Rac2/Pory2/12/Lbp/Tgfb1/Gomp3/Dysf/Ref1/Nckap1/Cd21a/C3ar1 |
| GO:0005070    | regulation of cytokine secretion                 | 1.97E-18         | 53             | Il1rl1/Ill33/Ill1a/Cd300f0/Fox1a/Lnc32/Tmem17a/Tam1/Cd200/Tnf/Ctca4/Sry2/2/Arg1/Gcnmb/Cebpba/Accod1/Serp1/S1c4/Cxcr3/Lgmn1/Pla2g7/Cd2/2/Selp/2/Sele/Tacr1/Ill1a/Pvap/Cxcr13/Ednra/Prcam1/Pgf/Ccr1/Ald8/Cxcl12/Plafr/Tgf1b/Madcam1/Cmk1/C1c8/Mmp14/Wnt5a/Cacm1/Ccr7/Frraes2/Sell/Ccr2/Tpipv4/Tpk/2/Rac2/Pory2/12/Lbp/Tgfb1/Gomp3/Dysf/Ref1/Nckap1/Cd21a/C3ar1 |
| GO:00019555   | cytokine binding                                 | 5.93E-18         | 43             | Il1rl1/Ill33/Ill1a/Cd300f0/Fox1a/Lnc32/Tmem17a/Tam1/Cd200/Tnf/Ctca4/Sry2/2/Arg1/Gcnmb/Cebpba/Accod1/Serp1/S1c4/Cxcr3/Lgmn1/Pla2g7/Cd2/2/Selp/2/Sele/Tacr1/Ill1a/Pvap/Cxcr13/Ednra/Prcam1/Pgf/Ccr1/Ald8/Cxcl12/Plafr/Tgf1b/Madcam1/Cmk1/C1c8/Mmp14/Wnt5a/Cacm1/Ccr7/Frraes2/Sell/Ccr2/Tpipv4/Tpk/2/Rac2/Pory2/12/Lbp/Tgfb1/Gomp3/Dysf/Ref1/Nckap1/Cd21a/C3ar1 |
| GO:00026335   | interleukin-6 production                         | 5.70E-18         | 45             | Il6/Ill3/Ill1a/Ill33/Ill1a/Cd300f0/Fox1a/Lnc32/Tmem17a/Tam1/Cd200/Tnf/Ctca4/Sry2/2/Arg1/Gcnmb/Cebpba/Accod1/Serp1/S1c4/Cxcr3/Lgmn1/Pla2g7/Cd2/2/Selp/2/Sele/Tacr1/Ill1a/Pvap/Cxcr13/Ednra/Prcam1/Pgf/Ccr1/Ald8/Cxcl12/Plafr/Tgf1b/Madcam1/Cmk1/C1c8/Mmp14/Wnt5a/Cacm1/Ccr7/Frraes2/Sell/Ccr2/Tpipv4/Tpk/2/Rac2/Pory2/12/Lbp/Tgfb1/Gomp3/Dysf/Ref1/Nckap1/Cd21a/C3ar1 |
| GO:1903037    | regulation of leukocyte cell-cell adhesion       | 1.07E-17         | 63             | Il1rl1/Ill33/Ill1a/Ill33/Ill1a/Cd300f0/Fox1a/Lnc32/Tmem17a/Tam1/Cd200/Tnf/Ctca4/Sry2/2/Arg1/Gcnmb/Cebpba/Accod1/Serp1/S1c4/Cxcr3/Lgmn1/Pla2g7/Cd2/2/Selp/2/Sele/Tacr1/Ill1a/Pvap/Cxcr13/Ednra/Prcam1/Pgf/Ccr1/Ald8/Cxcl12/Plafr/Tgf1b/Madcam1/Cmk1/C1c8/Mmp14/Wnt5a/Cacm1/Ccr7/Frraes2/Sell/Ccr2/Tpipv4/Tpk/2/Rac2/Pory2/12/Lbp/Tgfb1/Gomp3/Dysf/Ref1/Nckap1/Cd21a/C3ar1 |
| GO ID               | Description                                                                 | Adjusted p-value | Number of gene | Gene ID                                                                 |
|--------------------|------------------------------------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:1903555         | regulation of tumor necrosis factor superfamily cytokine production          | 7.43E-17         | 44             | Mmp8/Thbs1/Coc2/Fgcr3/Fcerg1g/Ltf/Arg2/Twist2/Gpnbmb/Cocl3/P4/Adam8/Cocl3/Zc3h12a/Angpt1/Ptfh1/Vsir/Arnb2/Ax/Vhavcr2/Arid5a/Cocl4/Nfatc4/Cocl4/Foxd3/Wnt5a/Ilt10/Cd300d/Sirpa/Ptpn22/Ccr7/Clec4a2/Cocl2/Crdfl/Tfr2/Cor5/Twist1/Inak3/Lbp/Cybbl/Spon2/Sytl1/Cd34 |
| GO:0032675         | regulation of interleukin-6 production                                        | 1.66E-16         | 42             | Il6/Mmp8/Ereg/Il11b/Fcerg1g/I33/Tn/Cebpb/Kf2/Cd3b/Zc3h12a/Ptfh1/Arnb2/Havcr2/Arid5a/Prg4/Foxd4/Muc16/Foxp3/Wnt5a/Atap2/Ilt10/Cd300d/Sirpa/Ptpn22/F2r/Ftrp4/Crdfl/Tfr2/Ccr5/Twist1/Trf8/Trf7/Hf/Hfak/I33/Lbp/Spon2/Sytl1/Tnfsf9/Nckap1/Inpp5d |
| GO:0001706         | tumor necrosis factor superfamily cytokine production                         | 1.66E-16         | 44             | Mmp8/Thbs1/Coc2/Fgcr3/Fcerg1g/Ltf/Arg2/Twist2/Gpnbmb/Cocl3/P4/Adam8/Cocl3/Zc3h12a/Angpt1/Ptfh1/Vsir/Arnb2/Ax/Vhavcr2/Arid5a/Cocl4/Nfatc4/Cocl4/Foxd3/Wnt5a/Ilt10/Cd300d/Sirpa/Ptpn22/Ccr7/Clec4a2/Cocl2/Crdfl/Tfr2/Cor5/Twist1/Inak3/Lbp/Cybbl/Spon2/Sytl1/Cd34 |
| GO:0060863         | regulation of T cell activation                                               | 1.09E-15         | 62             | Il6/Il1b/Clu/a/Cocl2/Tnfsf11/Trf/Caml1/Il33/Lm33/Tl1a/Clu/Ag2/Vtrn1/Ag1/Lcox/Gpnbmb/Cocl8b/Adam8/gf1/Zc3h12a/Ib/Ag1/Havsh1/Ilt6/Ist/Vsir/Cd300a/Tgfb1/Hav3/H4/Fra/Cocl4/Runk1/Cd80/Foxp3/Cocl3b88b/Ilt2a/Sirpa/Zfb16/Cd124a/Ftrp22/Cav1/Cor7/Myb/Cd38/Cocl2/ Rac2/Zebl/Pik4b6/Gata3/Tigl/Lag3/Tfr7/Adora2a/Pdml/Tnfsf82/Fancl/Cor1a/Cd6/Tnfssf9/Nckap1/Runkx4l20b/Vtcmn |
| GO:0019221         | cytokine-mediated signaling pathway                                           | 1.19E-15         | 66             | Cxcl5/I6/Cocl2/Ccoc2/Ifitm1/Ereg/Iltb/Ccl11/Ilt12/Osrm/Ilt11/Mmp12/Cocl3/Ifitm6b/Sphk1/Cocl2/Osm/Ilt11/Tnfsf11/Ifcerg1/Pvap/Slt3/Cd300f/Ppob/Cocl13/Tfng/Iltg/Irtb/Ccr1/Trem2/Cc11/Osm/Cc12b2b/Csfrd2p/P4/Cocl13/Ifitm12/Angt1/Ack3/I6ast/Egr1/Axld4/finar2/Cocl4/Csfr1/Wnt5a/Ilt10a/Cnmlb/Pdgb/Ccr2/Apoa1/Irk4/Cr3/Stat2/Cnfr4/Tf3/Prtn11/Ifr5/Irl2b/Irl20b/Cocl2a |
| GO:0026997         | regulation of immune effector process                                        | 2.58E-15         | 74             | Cxcl5/I6/Iltb/Cocl11/Ilt1/Eitg/Ilt11/Sema7a/Iltm12/Cocl2/Cocl3/C3/Fgcr3/Fgcr2/bsmmox1/Ifcerg1/Ilt33/gtam/Snp3/Fodt1/Tfng1/Fgcr1/Serpip1/Ilt18rap/Cd3b/Zc312a/Gpnc6b/Angip1/Cf/Hf/Ptug/Ptug/Axldr/Cd300a/Arnb2/Tfgh1/Havcr2/Ilt4a/Adm2/Ilt5a/Cocl4/Csfr1/Wnt5a/Ilt10a/Cnmlb/Pdgb/Ccr2/Apoa1/Irk4/Cric3/Stat2/Cnfr4/Tof7/Prtn11/Ifr5/Irl2b/Irl20b/Cocl2a |
| GO:0026995         | negative regulation of leukocyte activation                                   | 4.08E-15         | 45             | Fgcr2b/Hmox1/Cocl300s/Ifsp1/Fod1/Lrc32/Tarm1/Cocl200/Cocl4/Arg1/Apgmb/Cpecppb/Zc3h12a/Hlx/Sams1/Visr/Cd300a/Tgfb1/Adlx/Havcr2/It4a/Runx1/Cd80/Foxp3/Cd80/Ilt10a/Ilt12a3b/Ptnp22/Cocl6/Cocl2/Mir1/Tigl/Lag3/Adora2a/Pdml/Cnml1/Tnfsf82/Agdfios/Galo/Runx3/Ilt2b/Irl20b/Inpp5d/Vtcmn1 |
| GO:1903039         | positive regulation of leukocyte cell-cell adhesion                           | 4.31E-15         | 48             | Il6/Iltb/Cocl2/Tnfsf11/Vcam1/Iltmp3/Trf/Vitn1/Cocl8a/Adm8/gf1/Ets1/Iltb/Ptg1/Tfng1/Usp1/Havsh1/Ilt6/Ist/Vtsr/Tgfb1/Havcr2/Cocl4/Runx1/Cocl80/Foxp3/Cocl88b/Icam1/Ilt2a/Sirpa/Zfb16/Cd124a/Ptnp22/Cav1/Cor7/Myb/Cocl6b/Cocl2/Pik3b6/Gata3/Ilt7/Atf1/Cor1a/Ilt4a3/Cocl6/Tnfsf11/Nckap1/Runkx3/Vtcmn1 |
| GO:0067777         | negative regulation of immune response                                       | 6.11E-15         | 41             | Il111/Mmp12/FGcr2b/Hmox1/Ilt33/Fod1/Tnct4/Arg2/Arg1/Cocr1/Accl1/Serpip1/Zc3h12a/Angp1/Hlx/Sams1/Visr/Cd300a/Arnb2/Tfgh1/Aaxy15/Havcr2/Ilt4a/Adm2/Cocl4/Foxp3/Ilt10a/Ilt12a3b/Oqpx2/Cocr2/Cocl3a/Apoa1/Inr4/Ilt7/Sh2d1b1/Iltamf/Ilt2b/Irl20b/Krlb1b/Inpp5d |
| GO:0002688         | regulation of leukocyte chemotaxis                                            | 8.45E-14         | 34             | Ccr2/Serpin1/Tbhs1/Il11b/Fpr2/Grem1/Lgrmn/Plaz71/Cocl2/Dusp5/Nbp1/Cocl13/Edma/Pgfl/Cocr1/Cocl12/Cxkn1/Cocl4/Wnt5a/Cocr7/Rarres2/Sell/Cocr2/Trp4/Brst1/Rac2/Lbp/Gpsm3/Dysf/Afl1/Slmtf8/Nckap1/Jam3/Cstar1 |

(Continued)
| GO ID     | Description                                               | Adjusted p-value | Number of gene | Gene ID                                                                 |
|----------|-----------------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:0050870 | positive regulation of T cell activation                  | 7.75E-13         | 43             | Il6/Il1b/Cd22/Tnfsf11/Vcam1/Nlrp3/Vn1/Il6st/Tnfrsf9/Nckap11/Rbp1/Ccr7   |
| GO:0002699 | positive regulation of immune effector process             | 8.87E-13         | 52             | Il6/Il1b/Cxcl1/Il1f/Il1r1/Cdc2/Tnf1/Cd86/Cd34/Il6st/Vn1/Fcgr2            |
| GO:0050715 | positive regulation of cytokine secretion                 | 1.17E-12         | 37             | Il1rl1/Cxcl1/Il6/Il1b/Cxcl1/Il6b/Irp2/Ctla4a/Ctla4b/Ctla4c              |
| GO:0070661 | leukocyte proliferation                                   | 1.27E-12         | 59             | Il6/Il1b/Grem1/Tacr1/Fcgr2b/Itgam/Nlrp3/Tnf/Cd86/Cd34/Il6st/Vn1/Ctla4a |
| GO:002696  | positive regulation of leukocyte activation               | 1.74E-12         | 72             | Il1rl1/Mmp8/Il6b/Cd22/Tacr1/Tnf1/Itgam/Vcam1/Tnf/Cd86/Cd34/Il6st/Vn1  |
| GO:192105  | regulation of leukocyte differentiation                   | 1.94E-12         | 55             | Il6/Il1b/Ctxa2/Tnf1/Tnfrsf9/Il6b/Tnfrsf9/Nckap11/Rbp1/Ccr7             |
| GO:002690  | positive regulation of leukocyte chemotaxis                | 2.80E-12         | 28             | Cxcr2/Cxcr3/Cxcr4/Il1b/Cxcl1/Il6/Il1b/Irp2/Ctla4a/Ctla4b/Ctla4c         |
| GO:192107  | positive regulation of leukocyte differentiation           | 5.66E-12         | 39             | Il6/Il1b/Tnf1/Il6b/Tnfrsf9/Il6b/Cd86/Cd34/Il6b/Tnfrsf9/Nckap11/Rbp1   |
| GO:0042098 | T cell proliferation                                       | 5.80E-12         | 44             | Il6/Il1b/Ctla4b/Cxcr2/Cxcl1/Il6/Il1b/Irp2/Ctla4a/Ctla4b/Ctla4c/Irp2   |
| GO:0050710 | negative regulation of cytokine secretion                 | 6.64E-12         | 23             | Il6/Il1b/Tnf1/Il6b/Tnfrsf9/Nckap11/Rbp1/Ctla4a/Ctla4b/Ctla4c/Irp2   |

(Continued)
| GO ID       | Description                                           | Adjusted p-value | Number of gene | Gene ID                                                                 |
|------------|-------------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:0002443 | leukocyte mediated immunity                           | 9.26E-12         | 76             | Cxcl5/Il6/Ilb/Cxcl1/Il1r/Trem1/C3/Fgro/FCgr2b/Hmox1/C1qa/Fcer1g/Tgarn/Nlrp3/Fox1/Tnf/C1s1/Arg1/Prb/Fgro/C4/Cq/C1qa/Cl/Cq/C1/a/Serping1/If1rap/C1p/Ptf/Agcr/Visr/Ig/hv1-72/Cd300a/Arbit/2gfb/Tgfb/Havcr/Il4a/C1ra/Ah1a/Cxcl1/BattPIfg2/Cd84/If1g2b/Fgro3/Icam1/Rtn1/Cd35/If13a2/Cd300b/lghv2/c/C1tb/H1-2/Q10/Ccr3/Tr2/Rac2/Trdc/Mir1/Pkr3b/Gata3/Cadm1/1Gag3/1TrHg/h2/Sno2/Pgat2/Gata2/Clcf1/18r1/Cor1a/Nr4a3/lghv1-55/1I20b/Krblb1/lpp5d/Exo1/Nr4f/Myol1 |
| GO:0002444 | myeloid leukocyte mediated immunity                   | 1.05E-11         | 29             | Cxcl5/Cxcl1/Trem1/C3/Fgro/Hmox1/Fcer1g/Tgarn/Fox1/Arg1/Fgro/Ftfn/Cd13a/Il4ra/Sbx11/Psa2g/Cd84/lhfgb2/I13ra2/Cd300b/Ccr2/Rac2/Mir1/Psporn/Gata2/Nr4a3/Nr4f/Myol1 |
| GO:0070663 | regulation of leukocyte proliferation                | 1.75E-11         | 47             | I1b/I1b/Grem1/Tac1/Fgcr2b/Cam1/Lrc32/Tarm1/Ctla4/Arg2/Arg1/Gtnmb/Cebpb/Arg1/Agcr/Ilis/Visr/Cd300a/Tgb1/Havcr/Cd4/Cac1/Cd80/Faxon3/Ccd38bb/I1/I2ra/Cd244a/Ptnp22/Ccr7/Cd86/Ccr2/Bat2/Trpol1/Costampl/Afl1/Clcf1/Gal/Cor1a/Cd6/Tnsf9/1Nckap11/Tnfsf4/I120b/1pp5d/vCtn1 |
| GO:0002718 | regulation of cytokine production involved in immune response | 3.32E-11         | 25             | I1b/I1b/I1r1/Sema7a/Hmox1/Fcer1g/Nlrp3/Tfn/Arg1/Cd36/Gpocr5b/Argol/Visr/Tgbf/Ar1d5a/Flpox/Cdact5a/Il10/Apoa1/Trf2/Gata3/Irak3/Spon2/I1bI1r1/Nr4a3 |
| GO:0002367 | cytokine production involved in immune response      | 3.57E-11         | 28             | I1b/I1b/I1r1/Sema7a/Trem1/Hmox1/Fcer1g/Nlrp3/Tfn/Arg1/Cd13a/Il4ra/Sbx11/Psa2g/Cd84/lhfgb2/I13ra2/Cd300b/Ccr2/Rac2/Mir1/Psporn/Gata2/Nr4a3/Nr4f/Myol1 |
| GO:0005125 | cytokine activity                                     | 5.13E-11         | 45             | I1b/I1b/I1r1/Sema7a/Hmox1/Fcer1g/Nlrp3/Tfn/Arg1/Cd36/Gpocr5b/Argol/Visr/Tgbf/Ar1d5a/Flpox/Cdact5a/Il10/Apoa1/Trf2/Gata3/Irak3/Spon2/I1bI1r1/Nr4a3 |
| GO:0042035 | regulation of cytokine biosynthetic process           | 3.97E-11         | 29             | I1b/I1b/I1b/I1r1/Sema7a/Hmox1/Fcer1g/Nlrp3/Tfn/Arg1/Cd36/Gpocr5b/Argol/Visr/Tgbf/Ar1d5a/Flpox/Cdact5a/Il10/Apoa1/Trf2/Gata3/Irak3/Spon2/I1bI1r1/Nr4a3 |
| GO:0042107 | cytokine metabolic process                            | 6.78E-11         | 31             | I1b/I1b/I1b/I1r1/Sema7a/Hmox1/Fcer1g/Nlrp3/Tfn/Arg1/Cd36/Gpocr5b/Argol/Visr/Tgbf/Ar1d5a/Flpox/Cdact5a/Il10/Apoa1/Trf2/Gata3/Irak3/Spon2/I1bI1r1/Nr4a3 |
| GO:0002703 | regulation of leukocyte mediated immunity             | 1.10E-10         | 47             | I1b/I1b/I1b/I1r1/Sema7a/Hmox1/Fcer1g/Nlrp3/Tfn/Arg1/Cd36/Gpocr5b/Argol/Visr/Tgbf/Ar1d5a/Flpox/Cdact5a/Il10/Apoa1/Trf2/Gata3/Irak3/Spon2/I1bI1r1/Nr4a3 |
| GO:0002089 | cytokine biosynthetic process                         | 1.32E-10         | 30             | I1b/I1b/I1b/I1r1/Sema7a/Hmox1/Fcer1g/Nlrp3/Tfn/Arg1/Cd36/Gpocr5b/Argol/Visr/Tgbf/Ar1d5a/Flpox/Cdact5a/Il10/Apoa1/Trf2/Gata3/Irak3/Spon2/I1bI1r1/Nr4a3 |
| GO:0004896 | cytokine receptor activity                            | 2.43E-10         | 27             | I1f1/Cxcr2/I1r2/11r2/Osmr/11r1/Csfr3/Ccr1/I1ra/Cdr2b2/Cdr2b2/I1rap/Acrk3/I16st/I1ra/Cd4/Cd11r2/I1ra/Cdr2/Ccr2/Ccr1/Cdr2/Ccr1/Crtr1/I1r1/I1r2b |
| GO:1903557 | positive regulation of tumor necrosis factor superfamily cytokine production | 2.90E-10         | 26             | Mmpb/Thbs1/Cd2/Flcro3/Fcer1g/Cd3/Fp4/Adam8/Cd36/Ftfn/Psporn/Havcr/Cdr2/Ar5da/Cd4/Cd4/Cd3/Cd4/Cd11r2/Crtr1/I1r1/I1r2b |
| GO:0002700 | regulation of production of molecular mediator of immune response | 4.64E-10         | 33             | I1b/I1b/I1b/I1r1/Sema7a/Fcgr2b/Hmox1/Fcer1g/I1ra/I1tr3/IPfn/Arg1/Cd36/Gpocr5b/Angol/Visr/Tgbf/Ar1d5a/Flpox/Cdact5a/Il10/Apoa1/Trf2/Gata3/Irak3/Spon2/I1bI1r1/Nr4a3/Tnsf9/1

(Continued)
TABLE 3 | Continued

| GO ID     | Description                                                                 | Adjusted p-value | Number of gene | Gene ID                                                                 |
|-----------|-----------------------------------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:0002366 | leukocyte activation involved in immune response                            | 4.79E-10         | 47             | Il6/Clec4e/Hmox1/Fcer1g/I33/Tgam/Nr3p3/Fox1/1/Clec4d/Tyrobpb/Slt1a1/12/Zc3h12a/Hlx/Ptafr/ Cd300a/Tgfb1/Havcr2/I4ra/Stx11/Batt/Psa2g3/Cd84/Itgb2/Foxp3/Icam1/lm2a/Cd244a/I13ra2 /Ccr7/Mmb/Ccr2/Rora/Rac2/Mir1/Gata3/Lbp/Dysf/Sh2d1b1/Gata2/Cclfl/118r1/Coro1a/Nr4a3 /Nr2c-3/Exo1/Fgr/Myo1f |
| GO:0002263 | cell activation involved in immune response                                 | 8.10E-10         | 47             | Il6/Clec4e/Hmox1/Fcer1g/I33/Tgam/Nr3p3/Fox1/1/Clec4d/Tyrobpb/Slt1a1/12/Zc3h12a/Hlx/Ptafr/ Cd300a/Tgfb1/Havcr2/I4ra/Stx11/Batt/Psa2g3/Cd84/Itgb2/Foxp3/Icam1/lm2a/Cd244a/ I13ra2/Ccr7/Mmb/Ccr2/Rora/Rac2/Mir1/Gata3/Lbp/Dysf/Sh2d1b1/Gata2/Cclfl/118r1/Coro1a /Nr4a3/Nr2c-3/Exo1/Fgr/Myo1f |
| GO:0002275 | myeloid cell activation involved in immune response                         | 1.15E-09         | 24             | Hmox1/Fcer1g/I33/Tgam/Fox1/1/Tyrobpb/Ptafr/Cd300a/Havcr2/I4ra/Stx11/Psa2g3/Cd84/Itgb2/ I13ra2/Ccr2/Rac2/Mir1/Lbp/Dysf/Gata2/Nr4a3/Fgr/Myo1f |
| GO:0002698 | negative regulation of immune effector process                              | 2.05E-09         | 30             | Fcgr2b/Hmox1/I33/Fox1/Tnf/Arg1/Serping1/1/Zc3h12a/Angpt1/1/Hlx/Vair/Cd300a/Arnb2/Tgfb1 /Havcr2/I4ra/A2m/Cd84/Foxp3/ll10/I2ra/I13ra2/Ccr2/Apoa1/Irak3/I7r/Sh2d1b1/Slam8b/I206b/Kfrn1b |
| GO:0002573 | myeloid leukocyte differentiation                                           | 2.65E-09         | 40             | Csf3/Tnfsf11/Fcer1g/I33/Cd300f/Sirp1/Rbp1/Tnf/Prl/Ccr1/Tem2/Tyrobpb/Cebpb/Cc3/P4/ Adam8/1/1/C1qg/Tgfb1/Spl1/Cd4/I4ra/Lrunx1/Batt/Csfr1/Hlsl1/Ccr1/Fam20c/Fgtf3/Tir1/Gata3 /Itgb3/3/Zip38/f1/Gata2/Costamp/Fbr1/Tnfsf9/Nxox2-3/Tal1/Inpp5d/Niros |
| GO:0046631 | alpha-beta T cell activation                                               | 3.93E-09         | 26             | Il6/Nr3p3/Tam1/Arg2/Cebpb/Zc3h12a/Hlx/Ager/Hsp1/Visr/Cd300a/Tgfb1/I4ra/Runx1/Batt/ Cd80/Foxp3/Zbbrf1/Cd244a/Btnprn/22/Ccr7/Mmb/Ccr2/Gata2/Adora2a/Prdm1/Nkack1/i/Runx3 |
| GO:0046634 | regulation of alpha-beta T cell activation                                 | 6.42E-09         | 20             | Cxcl5/Cxcl1/C3/Fgr3/Hmox1/Fcer1g/I33/Fox1/1/Fgcr1/1/Tgam/Fox1/1/Gat2a/Fgr |
| GO:0002886 | regulation of myeloid leukocyte mediated immunity                           | 9.27E-09         | 20             | Lrrc32/Tam1/Ctla4/Arg2/Arg1/Gpnmb/Cebpb/Igfl/Ager/Hsp1/Visr/Tc3h12a/1/Hlx/Vair/Cd300a/ Tgfb1/Havcr2/Cd80/Foxp3/Cdcd88b/I2ra/Cd244a/Prtn22/Ccr7/Gata2/Adora2a/Ardc2/Arfl /Coro1a/Cd6/Tnfsf3/Nkack1/i/I206b/Vtcn1 |
| GO:0042129 | regulation of T cell proliferation                                          | 1.15E-08         | 34             | Lrc32/Tam1/Ctla4/Arg2/Arg1/Gpnmb/Cebpb/ADAM8/Cxcl12/Zc3h12a/Hlx/Vair/Cd300a/Tgfb1/Havcr2/ Cd80/Foxp3/Cdcd88b/I2ra/Cd244a/Prtn22/Ccr7/Gata2/Adora2a/Prdm1/Nkapp1/i/Runx3 |
| GO:1903038 | negative regulation of leukocyte cell-cell adhesion                        | 1.24E-08         | 29             | Lrrc32/Tam1/Ctla4/Arg2/Arg1/Gpnmb/Cebpb/ADAM8/Cxcl12/Zc3h12a/Hlx/Vair/Cd300a/Tgfb1/ Havcr2/Cd80/Foxp3/Cdcd88b/I2ra/Cd244a/Prtn22/Ccr7/Gata2/Adora2a/Prdm1/Nkapp1/i/Runx3 |
| GO:0032715 | negative regulation of interleukin-6 production                             | 1.65E-08         | 17             | Tnf/Kfl2/Zc3h12a/Arnb2/Havcr2/Cd84/Muc16/Foxp3/Il10/Sirpa/Prtn22/Hgfl/Irak3/Syt1l1/Nkack1/i/Inpp5d |
| GO:0050868 | negative regulation of T cell activation                                    | 2.30E-08         | 27             | Lrrc32/Tam1/Ctla4/Arg2/Arg1/Gpnmb/Cebpb/Zc3h12a/Hlx/Vair/Cd300a/Tgfb1/Havcr2/I4ra /Runx1/Cd80/Foxp3/I2ra/Prtn22/Cdr8b/Tgfl/Lag3/Adora2a/Tnfla8b2/Runx3/I206b/Vtcn1 |
| GO:0070664 | negative regulation of leukocyte proliferation                             | 2.51E-08         | 23             | Grem1/Fgr2b/Lrc32/Tam1/Ctla4/Arg2/Arg1/Gpnmb/Cebpb/Vair/Cd300a/Tgfb1/Havcr2/Cd80/Foxp3/Il10/I2ra/Cd86/Prdm1/Gal/I206b/Inpp5d/Vtcn1 |
| GO:0043299 | leukocyte degranulation                                                    | 2.73E-08         | 20             | Hmox1/Fcer1g/I33/Tgam/Fox1/1/Ptafr/Cd300a/I4ra/Stx11/Psa2g3/Cd84/Itgb2/I13ra2/Ccr2/ Rac2/Mir1/Gata2/Coro1a/Nr4a3/Fgr/Myo1f |
| GO ID       | Description                                                                 | Adjusted p-value | Number of gene | Gene ID                                                                 |
|------------|------------------------------------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:0071347 | cellular response to interleukin-1                                           | 2.97E-08         | 21             | Il6/Saa3/Serpin1/Ilt2/Ilt1/Ccl2/Ilt1a/Ccl11/Cebpb/Ccl3/Acocd1/Ccl8/Adams7/Fn1/ \
|            |                                                                               |                  |                | /Zc3h12a/Egr1/Ilt4/Rora/Ilt3/Adams12/Ccl21a                              |
| GO:0032611 | interleukin-1 beta production                                                | 3.50E-08         | 20             | Iltb1/Abca1/Nlrp3/Arg2/Ccl3/Cd36/Zc3h12a/Mefv/Hspb1/Egr1/Arb2/Wnt5a/ \
|            |                                                                               |                  |                | Ccr7/F2r/Apoa1/Ilt2/Ccr5/Tlr/Tlr8/S1pr3                                  |
| GO:0035710 | CD4-positive, alpha-beta T cell activation                                   | 3.65E-08         | 23             | Ilt6/Nlrp3/Tarm1/Arg2/Cebpb/Zc3h12a/Hbx/Ager/Vsir/Tgfbl/Ilt4a/Runx1/Batt/Foxp3/ \
|            |                                                                               |                  |                | Ccr7/Satb1/Ccyr2/Rora/Gata3/Ilt18r1/Nckap1/Runx3/Nkx2-3                 |
| GO:0032755 | positive regulation of interleukin-6 production                             | 4.77E-08         | 23             | Mmp8/Egr2/Ilt2/Ilt1a/Fcer1g/Il33/Tnf/Cd36/Pfmr/Ardia5/Wnt5a/F2r/Tprv4/Card9/ \
|            |                                                                               |                  |                | Tlr2/Ccr5/Tlrf/Tlrf/Lbp/Spon2/Tnfsf9                                   |
| GO:1903556 | negative regulation of tumor necrosis factor superfamily cytokine production | 5.69E-08         | 18             | Arg2/Twist2/Gpmmb/Zc3h12a/Vsir/Arb2/Arx/Havcr2/Foxp3/Ilt10/Sirpa/Pttn22/Clec4a2/ \
|            |                                                                               |                  |                | /Tlrf/Iltak3/Lbpl/Sly11/Cdd4                                        |
| GO:0030217 | T cell differentiation                                                       | 7.12E-08         | 43             | Ilt6/Iltb/Ctla2/Clec4e/Fcer1g/Nlrp3/Clt4a/Clec-cd/Vhnn1/Adam8/Zc3h12a/Hbx/Prex1/Vsir/ \
|            |                                                                               |                  |                | Egr1/Tgfbl/Ilt4a/Cd4/Runx1/Batt/Bcl2a1d/Foxp3/Ilt2a/Zbtl16/Pttn22/Ccr7/Satb1/ \
|            |                                                                               |                  |                | Myb/Ccr2/Rora/Zeb1/Plkdr6/Gata3/Ztf4/Sf6/Cd37/Ilt/Ptdm1/Fanca/Runx2/Ilt8r1/Tnfsf9/ \
|            |                                                                               |                  |                | Nckap1/Runx3/Nkx2-3                                                  |
| GO:0032612 | interleukin-1 production                                                     | 9.20E-08         | 22             | Iltb1/Ilt2/Sphk1/Abca1/Nlrp3/Arg2/Ccl3/Cd36/Zc3h12a/Mefv/Hspb1/Egr1/Arb2/ \
|            |                                                                               |                  |                | Havcr2/Wnt5a/Ccr7/F2r/Apoa1/Ilt2/Ccr5/S1pr3                             |
| GO:0005126 | cytokine receptor binding                                                    | 1.39E-07         | 50             | Cxcl5/Ilt6/Cxcl2/Csf3/Inhbb/Ilt1/Iltb/Cxcl1/Grem1/Cxcl3/Cd2/Lrg1/Osm/Ilt1a/Tgfbr3/Tnfsf1/ \
|            |                                                                               |                  |                | /Eng/Cd33000/Ppob/Cxcl13/New/3/Mmp8/Mmp7/Inhba/Pfgr/Tlrf/Cd3/Cd3/Pt2a/4/Pfcr/Cd4/Cxcl12/ \
|            |                                                                               |                  |                | Ang1/Elfa5/Ilt8t/Tgfb1/Iltx16/Tgfb1/Ilt34/Ccyr4/Angd2/Ilt10/Mmp5/Gata3/Igfb3/Cnfr/ \
|            |                                                                               |                  |                | /Cnfr/Cxcl8/Trnsf9/Cd21a/Ras11b                                      |
| GO:0002523 | leukocyte migration involved in inflammatory response                        | 1.06E-07         | 10             | S100a8/S100a9/Ccl2/Iltgam/Ppob/Adam8/Igt2b/Lbp/Slam8/Jam3                 |
| GO:0070665 | positive regulation of leukocyte proliferation                              | 1.58E-07         | 29             | Ilt6/Iltb/Tcr1/Vcam1/Igfl1/Ager/Ilt6t/Havcr2/Cd4/Tac1/Cd80/Foxp3/Cdcd88b/Ilt2a/Cd244a/ \
|            |                                                                               |                  |                | /Ptn22/Ccr/Cd86/Ccrr2/Bst1/Ocstamp/Afl1/Cclf1/Coro1a/Cd6/Tnfsf9/Nckap1/ \
|            |                                                                               |                  |                | Tnfsf4/Vtnc1                                                          |
| GO:0070555 | response to interleukin-1                                                   | 1.68E-07         | 22             | Ilt6/Saa3/Serpin1/Ilt2/Ilt1/Ccl2/Sele/Ilt1a/Ccyr2/Cebpb/Ccl3/Acocd1/Ccl8/Adams7/Fn1/ \
|            |                                                                               |                  |                | /Zc3h12a/Egr1/Ilt4/Rora/Ilt3/Adams3/Adams12/Ccl21a                       |
| GO:0042102 | positive regulation of T cell proliferation                                  | 1.85E-07         | 23             | Ilt6/Iltb/Vcam1/Igfl1/Ager/Ilt6t/Havcr2/Cd4/Cd80/Foxp3/Cdcd88b/Ilt2a/ \
|            |                                                                               |                  |                | Cd244a/Ptnp22/Ccr/Cd86/Ccrr2/Afl1/Coro1a/Cd6/Tnfsf9/Nckap1/1/Vtnc1     |
| GO:0032695 | negative regulation of interleukin-12 production                            | 2.04E-07         | 10             | Thbs1/Tnfsf9/Mefv/Arb2/Cmklr1/Ilt2/Tlrf/Tlrf/Tgitr/Ilt3                     |
| GO:2000514 | regulation of CD4-positive, alpha-beta T cell activation                     | 2.09E-07         | 18             | Ilt6/Nlrp3/Tarm1/Arg2/Zc3h12a/Hbx/Ager/Vsir/Tgfbl/Ilt4a/Runx1/Foxp3/Ccr7/Myb/ \
|            |                                                                               |                  |                | /Ccr2/Gata3/Nckap1/Runx3                                               |
| GO:00028119| regulation of adaptive immune response                                       | 2.98E-07         | 35             | Ilt1/Ilt2/Ilt3/Cfcr2/Fcgr2b/Sc11a/Nlrp3/Tnf/Arg1/Fcgr1/Sf1/11a/Zc3h12a/ \
|            |                                                                               |                  |                | Hbx/Ager/Samsn1/Vsir/Tgfbl1/Alex15/Havcr2/Ilt4a/Cd4/Anida5a/Foxp3/Cd55/Ccr7/H2- \
|            |                                                                               |                  |                | Q10/Ccr2/Gata3/Ilt2/Ilt8r1/Ilt20rb                                    |
| GO:0032651 | regulation of interleukin-1 beta production                                  | 3.15E-07         | 17             | Sphk1/Nlrp3/Arg2/Cc5/Zc3h12a/Mefv/Hspb1/Egr1/Arb2/Wnt5a/Ccr7/F2r/Apoa1/Ilt2/Ccr5/Tlrf/S1pr3 |
| GO ID          | Description                                                                 | Adjusted p-value | Number of gene | Gene ID                                                                 |
|---------------|------------------------------------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:0001776    | leukocyte homeostasis                                                        | 3.31E-07         | 24             | Cxcl5/Il6/Cxcr2/Ccl2/Fcgr2b/Fcer1g/Pirb/Pde4b/Tgfβ1/Axl/Spln2/B2c2a1a/Foxp3/Ccnb2 /Il2a/Cc2r/Hac2r/Sh2b2/Slc7a11/Cc2r1a/Nckap1/Nkx2-3/Tnfrsf4/Jam3 |
| GO:0045582    | positive regulation of T cell differentiation                               | 4.34E-07         | 22             | Il6/Il1b/Nlrp3/Vn1/Adam8/Hlx/Vsr/Tgfβ1/Ila4a/Runx1/Foxp3/Ila2a/2btxb16/Ccr7/Myb /Cc2r/Pk3rb/Gata3/Ilf7r/Tnfrsf9/Nckap1/Runc3 |
| GO:0002781    | negative regulation of production of molecular mediator of immune response  | 4.37E-07         | 13             | Fcgr2b/Hmox1/Ila3/Tnf/Arg1/Angpt1/Vsr/Tgfβ1/Foxp3/Ila0/Ila3ra2/Apoa1/Irk3  |
| GO:0061756    | leukocyte adhesion to vascular endothelial cell                              | 4.95E-07         | 12             | Selp/Sele/Vcam1/Tnf/Cxcl12/Ets1/Ptar/Madcam1/Icam1/Iell/Cc2r/Selpig      |
| GO:0002822    | regulation of adaptive immune response based on somatic recombination of     | 4.95E-07         | 33             | Il1r1/Ila6/Ila1b/Ila1r1/C3/Fcgr3/Fcgr2b/Fcer1g/Ila3/Ila3ra2/Tnf/Arg1/Cc2r/Csl1a1/Zc3h12a/ Hlx/Ager/Vsr/Tgfβ1/Hacv2/Ila4a/Cd4/Arid5a/Foxp3/Cd55/Cc2r7/H-2- Q10/Cc2r/Gata3/Ilf7r/Ccfl/Ila8r1/Ila20b |
| GO:0002460    | adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains | 5.73E-07         | 57             | Il1r1/Ila6/Ila1b/Ila1r1/C3/Fcgr3/Fcgr2b/C1qa/Fcer1g/Ila3/Ila3ra2/Tnf/Arg1/Pirb/ Fcgr1/Cd4b/C1qcb/Scl1a1/Sepeling1/Ila8rap/Cd3h12a/C1qcb/Hlx/Ager/Vsr/Ighv1- 72/Tgfβ1/Hacv2/Ila4a/Cd4/Arid5a/Stix11/Batf/Foxp3/Niras/Icam1/Rtnf1/Cd55/Ila3ra2 /Cc2r7/Ighv2/Ccl1b/H-2-01/Cc2r/For2/Trdc/Gata3/Ilf7r/Ccfl/Ila8r1/Ighv1- 55/Ila20b/lnnp5d/Exc1/C3a1 |
| GO:00033004   | negative regulation of mast cell activation                                  | 6.70E-07         | 8              | Hmox1/Cd3000f/Fox1/Cd300a/Cd84/Ila3ra2/Mir1/Cnr1                         |
| GO:0032652    | regulation of interleukin-1 production                                        | 6.80E-07         | 19             | Il1r2/Sphk1/Nlrp3/Arg2/Cd3/Cz3h12a/Mefv/Hsqb1/Egr1/Arbb2/Hacv2/Wnt5a/Cc2r7/F2r /Apopa1/Tir2/Ccfl/Tirb/S1pr3 |
| GO:0002825    | regulation of T-helper 1 type immune response                                 | 7.44E-07         | 12             | Il1r1/Ila6b/Ila1r1/C3/Ila3a1a1/Hlx/Hacv2/Ila4ra/Arid5a/Cc2r7/Cc2r1/Ila8r1 |
| GO:0045580    | regulation of T cell differentiation                                          | 7.87E-07         | 28             | Il6/Ila1b/Ctla2a/Nlrp3/Ctla4/Vn1/Adam8/Zc3h12a/Hlx/Vsr/Tgfβ1/Ila4a/Runx1/Foxp3/Ila2a /Zb1b16/Cc2r7/Myv/Cc2r2/Zeb1/Pik3gb/Gata3/Ilf7r/Pdmd1/Fanca/Tnfrsf9/Nckap1/Runc3 |
| GO:0002704    | negative regulation of leukocyte mediated immunity                           | 7.88E-07         | 16             | Fcgr2b/Hmox1/Fox1/Arg1/Vsr/Cd300a/Arb2/Hacv2/Cd84/ Fpox3/Ila3ra2/Cc2r7/Ilf7r/Sh2d1b1/Ila20b/Kirb1b |
| GO:0002761    | regulation of myeloid leukocyte differentiation                               | 8.51E-07         | 25             | Tnfrsf11/Sfr1/Frbp1/Tnf/Cc2r1/Tyrobp/Cebpbb/Cc3f/P44/Adam8/C1qcb.Tgfβ1/Cd4/Ila3/ Ranx1/Csf1r/Hc1s/Fgfr3/Ila3b3/Ip3b61/Gata2/Costmap/Fbn1/Tal1/Innp5d |
| GO:0045576    | mast cell activation                                                          | 8.62E-07         | 18             | Fgfr3/Hmox1/Fcer1g/Cd300tf/Fox1/Cd300a/Ila4a/Ila2g3/Cd84/Ila3ra2/Cd300b/ Rac2/Mir1/Lcp2/Ch1r/Gata2/Nra3/Fgr |
| GO:0002720    | positive regulation of cytokine production involved in immune response        | 8.74E-07         | 15             | Il6/Ila1b/Ila1r1/Sema7a/Fcer1g/Ila3/Ila3/Ila3ra2/Cd36/Gprc5b/Arid5a/Wnt5a /Tr2/Gata3/Sponder2/Ila8r1/Nra3 |
| GO:0002702    | positive regulation of production of molecular mediator of immune response    | 8.74E-07         | 22             | Il6/Ila1b/Ila1r1/Sema7a/Fcer1g/Ila3/Ila3ra2/Cd36/Gprc5b/Tgfβ1/Ila4a/Arid5a/Wnt5a/Cd244a /Ptprc22/Tir2/Gata3/Sponder2/Ccfl/Ila8r1/Nra3/Tnfrsf4 |

(Continued)
| GO ID         | Description                                      | Adjusted p-value | Number of gene | Gene ID                                                                 |
|--------------|--------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:0033003   | regulation of mast cell activation               | 1.15E-06         | 15             | Hmox1/Fcer1g/Cd300f/Fox1/Cd300a/I14ra/Cd84/I13ra2/Cd300b/Rac2/Mir1/Cnr1/Gata2/Nr4a3/Fgr |
| GO:0010935   | regulation of macrophage cytokine production      | 1.49E-06         | 8              | Sema7a/Cd36/Gprc5b/Tgb1/Wnt5a/Tlr2/Irak3/Spon2                           |
| GO:0045088   | regulation of innate immune response             | 1.50E-06         | 39             | Ereg/Mmp12/Mmp2/Cd300f/Ltf/Tlr13/Tnf/Colec12/Arg1/Ccr1/Ac01/Serping1/Adam8/I11rap/Cd36/Cd300a/Armb2/Hacv2/A2rM/wnt5a/Ittn1/Ptnp22/Cav1/Cd9/Cr9/Tlr2/Tlr7/Ptkb/Irak3/Cadm1/Lbp/Lag3/Sh2d1b1/Slamf8/Ptkk3ap1/Kibrb1b/Fgr/Myo1f |
| GO:0042108   | positive regulation of cytokine biosynthetic process | 2.12E-06         | 17             | Thbs1/Ereg/I11b/Fgr3/Cri/I1f/Tnfr/Phgp1/Egr1/Wnt5a/Rel/Ccr2/Tlr2/Tlr7/Lbp/Cybb |
| GO:0043367   | CD4-positive, alpha-beta T cell differentiation   | 2.25E-06         | 19             | It6/Nlrp3/Zc3h12a/Hlx/Tgbf1/I4ra/Ruxn1/Baf7/Foxp3/Cr7/Satb1/Myb/Ccr2/Rora/Gata3/I1lb/I1rb/Nkcap1/Runx3/Nkcap2-3 |
| GO:0032655   | regulation of interleukin-12 production           | 2.56E-06         | 15             | Thbs1/Tnfrs8/Cd36/Mefv/Ager/Armb2/Cmk1/F/Itl/I10/Ccr2/Tlr2/Tigf/Irak3/Tnfsf9 |
| GO:0061082   | myeloid leukocyte cytokine production             | 2.75E-06         | 11             | Sema7a/Hmox1/Fcer1g/Cd36/Gprc5b/Tgbf1/Wnt5a/Tlr2/Irak3/Spon2/Nr4a3       |
| GO:0034446   | substrate adhesion-dependent cell spreading       | 2.83E-06         | 20             | Lamb1/Lam1/Tdpn/Fpr1/Ephb3/Amnxt1/Fn1/Dab2/Prex1/Etna5/Ab2/Fzd4/Fndc3b/Lama5/Parvb/Apoa1/I1gb/Dbn1/Fermt3/Fbn1 |
| GO:0002719   | negative regulation of cytokine production       | 2.97E-06         | 10             | Hmox1/Tnfr/Arg1/Angpt1/Vsg/Tgbf1/Foxp3/I10/Apoa1/Irak3                  |
| GO:0070486   | leukocyte aggregation                            | 3.04E-06         | 8              | S100a8/S100a9/I11b/Msn/Bmp7/Adam8/Rac2/Nr4a3                            |
| GO:0072678   | T cell migration                                 | 3.23E-06         | 15             | Apo6/Cc2/Ocx13/S1pr1/Msn/Adamb8/Ocx12/Ocx16/Wnt5a/Icam1/Ccr7/Ccr2/I1gb3/Afi1/Cd21a |
| GO:0043300   | regulation of leukocyte degranulation             | 3.82E-06         | 14             | Hmox1/Fcer1g/I1tgam/Fox1/Ptnaf/Cd300a/I4ra/Cd84/I17ra2/Ccr2/Rac2/Gata2/Fgr |
| GO:0042088   | T-helper 1 type immune response                  | 3.82E-06         | 14             | I1f1/I1b/I1f3/I133/Si1a1/I11rap/Hlx/Hacv2/I4ra/Ariliba/Nrars/Ccr7/Ccr2/I1rb1 |
| GO:0002253   | activation of immune response                    | 4.30E-06         | 60             | Fpr2/Fpr1/Fcr3/Fgcr2b/C1qa/Fcrg1g/Cd300f/Ltf/Tlr13/Tnfr/Colec12/C1s1/C4b/C1qgb/Pde4b/Ac01/Serping1/Cd36/Zc3h12a/Cct/C1qc/Ighv1/I7-2/Cd300a/Armb2/Hacv2/C1raa/Cntmt3/Cmk1/A2m/Bc2a1d/Fox3/Nras/Ittn1/Cd55/Ptnp22/Cav1/Cfpra/C7r7/Ighg2/Cd86/I1rb/Fnra/Tfr/Tdrd/Tlr7/Tlr7/Gata3/Irk3/Snh2b2/Lbp/Lpcp2/Fyn/Nr4a3/Ighv1-55/Nkcap1/Ptkapb/I1/C6/Vctn/C3ar1 |
| GO:0002292   | T cell differentiation involved in immune response| 4.79E-06         | 17             | I1b/Clec4c/Fcer1g/Nr3p3/Clec4d/Zc3h12a/Hlx/Tgbf1/I4ra/Baf7/Foxp3/Cr7/Myb/Ccr2/Rora/Gata3/I18r1             |
| GO:0002448   | mast cell mediated immunity                      | 4.89E-06         | 14             | Hmox1/Fcer1g/Fox1/Cd300a/I4ra/Pia2g3/Cd84/I13ra2/Ccr2/Mir1/Spon2/Gata2/Nr4a3/Fgr |
| GO:0032615   | interleukin-12 production                        | 5.17E-06         | 15             | Thbs1/Tnfrs8/Cd36/Mefv/Ager/Armb2/Cmk1/F/Itl/I10/Ccr2/Tlr2/Tigf/Irak3/Tnfsf9 |
| GO:0002820   | negative regulation of adaptive immune response  | 5.59E-06         | 13             | I1f1/Fcrg2b/I33/Ang1/Zc3h12a/Samsn1/Vsir/Alox15/Hacv2/I4ra/Foxp3/I7r/I20r  |
| GO:0010934   | macrophage cytokine production                   | 5.67E-06         | 8              | Sema7a/Cd36/Gprc5b/Tgbf1/Wnt5a/Tlr2/Irak3/Spon2                         |
| GO:0043370   | regulation of CD4-positive, alpha-beta T cell differentiation | 8.07E-06         | 14             | I1b/Nr3p/Zc3h12a/Hlx/Tgbf1/I4ra/Ruxn1/Foxp3/Cr7/Myb/Ccr2/Gata3/Nkcap1/Runx3 |
| GO:0002369   | T cell cytokine production                       | 8.07E-06         | 12             | I1b/I1b/I1f3/Nr3p3/Arg1/S11a1/I11rap/Vsir/Ariliba/Foxp3/Gata3/I18r1     |
| GO:0059091   | leukocyte tethering or rolling                    | 1.01E-05         | 10             | Selp/Sele/Vcam1/Tnfr/Ocx12/Ptnaf/Madcam1/Sell/Ccr2/Selpgl             |
| GO ID         | Description                                                                 | Adjusted p-value | Number of gene | Gene ID                                                                 |
|--------------|------------------------------------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:0032653   | regulation of interleukin-10 production                                      | 1.02E-05         | 14             | Mmp8/Tnfrsf9/Fcgr2b/Fcer1g/Ager/Vsir/Cd84/Foxp3/Tlr2/Tig/t/Hgf/Trib2/Tld/Cd34 |
| GO:0002440   | production of molecular mediator of immune response                          | 1.08E-05         | 44             | Il6/I1b/I1r/Sema7a/Trem1/Fcgr2b/Hmxox1/Fcer1g/Il33/Nirp3/Tnf/Arg1/Slic11a1/gkv3-2/I1r/ap/Fc8d6/Gprc5b/Angpt1/Saur/Tgb1/I4ra/Arid5a/Batt/Foxp3/Wnt5a/I10/Itm2a/Cd244a/I13ra2/Pptn22/Apoa1/Tlr2/Gata3/Irak3/I1r/gkv12-89/Spon2/Cicf1/I18r1/Nir4a3/Poqlj/gkv10-96/Tnfrs4/Exo1 |
| GO:004226    | interleukin-6 biosynthetic process                                           | 1.11E-05         | 9              | Ereg/I1b/I1r/Cebpb/Pthar/Fgh4/Muc16/Cardi/Innp5d                        |
| GO:0032623   | interleukin-2 production                                                      | 1.14E-05         | 16             | I1b/I1r/Fcer1g/Slic11a1/Pde4b/Hvacr2/Runc1/Foxp3/Ccr2/Card9/Gata3/Lag3/Nav3/I20v/Ito/c1/Cd34 |
| GO:0046635   | positive regulation of alpha-beta T cell activation                          | 1.40E-05         | 16             | I1b/I6/Iru/Nirp3/Ihps1/Hra/Runc1/Devp3/Foxp3/Zbbr16/Cd244a/Pptn22/Ccr7/Myb/Ccr2/Nkcap1/Irun3 |
| GO:0002724   | regulation of T cell cytokine production                                       | 1.45E-05         | 10             | I1b/I1r/I1/Ir/Nirp3/Arg1/Vsir/Arid5a/Foxp3/Gata3/I1r8r1                |
| GO:0046637   | regulation of alpha-beta T cell differentiation                               | 1.68E-05         | 16             | I1b/I6/Iru/Nirp3/Zc3h12a/Hvks/Tgb1/I4ra/Runc1/Foxp3/Zbbr16/Ccr7/Myb/Ccr2/Gata3/Prdm1/I0kcap1/Irun3 |
| GO:0061081   | positive regulation of myeloid leukocyte cytokine production in immune response | 1.69E-05         | 8              | Sema7a/Fcer1g/Cd3b/Gpfc5b/Wnt5a/I1r2/Spon2/Nir4a3                       |
| GO:0035743   | CD4-positive, alpha-beta T cell cytokine production                          | 1.70E-05         | 9              | I1b/I1r/I1r/Nirp3/Arg1/I1r8ap/Arid5a/Gata3/I1r8r1                      |
| GO:2000106   | regulation of leukocyte apoptotic process                                      | 1.71E-06         | 21             | Ccr2/Fcgr2b/Fcer1g/Arg2/Adm6/Cxcl12/Ax/Be2a1a/Wnt5a/I10/Hcls1/Ccr7/Ccr5/Hcra2/Pdcd11r7/Auark/Slic7a11/Nir4a3/Siglec1/Tnfrs4 |
| GO:0002286   | T cell activation involved in immune response                                 | 1.71E-06         | 21             | I1b/I6/Ne4e/Fcer1g/Inirp3/Ne4e4d/Slic11a1/Zc3h12a/Hvks/Tgb1/Havorr2/I4ra/Strx11/Batf/Foxp3/Imac1/Ccr7/Myb/Ccr2/Rora/Gata3/I1r8r1 |
| GO:0032613   | interleukin-10 production                                                     | 1.98E-05         | 14             | Mmp8/Tnfrsf9/Fcgr2b/Fcer1g/Ager/Vsir/Cd84/Foxp3/Tlr2/Tig/t/Hgf/Trib2/Tld/Cd34 |
| GO:0043303   | mast cell degranulation                                                       | 1.98E-05         | 13             | Hmxox1/Fcer1g/Fox1/Cd30a/I4ra/Pls2g3/Cd8d/I13ra2/Rac2/Mir1/Gata3/Nir4a3/Gatr |
| GO:0002920   | regulation of humoral immune response                                         | 2.00E-05         | 11             | C3/Fcgr2b/Tnl/Acod1/Serping1/Ofly/Spons2/A2m/Cd55/Ccr7/Ccr6 |
| GO:0046632   | alpha-beta T cell differentiation                                             | 2.21E-05         | 21             | I1b/I6/Iru/Nirp3/Zc3h12a/Hvks/Tgb1/I4ra/Runc1/I1b/Foxp3/Zbbr16/Ccr7/Satb1/Myb/Ccr2/Rora/Gata3/Prdm1/I1r8r1/Nkcap1/Irun3/Nkox2-3 |
| GO:0002705   | positive regulation of leukocyte mediated immunity                            | 2.26E-06         | 28             | I1b/I1b/Cxcl11r/1r3/C5/Fcgr3/Fcer1g/Tggam/Nirp3/Tnf/Arg1/Fcgr1/I1r8ap/Pthar/Tgb1/I4ra/Arid5a/Tgf2/Foxp3/H2-Q10/Gata3/Cadm11/Lag/3h2c1b1/Gata3/Cfic1/I1r8r1/Fgrp |
| GO:0050702   | interleukin-1 beta secretion                                                  | 2.36E-06         | 12             | Aboa1/Nirp3/Arg2/Cc3d/Cd3b/Zc3h12a/Wnt5a/Ccr7/Apoa1/Tlr2/Ccr5/I18 |
| GO:0042130   | negative regulation of T cell proliferation                                   | 2.37E-06         | 16             | Lmc32/Tam1/Clda4/Arg2/Arg1/Opmb/Cebpb/Vsir/Tgb1/Havorr2/Cd8d/I3ra2/Cr3a2/Cd34/I20v/Bytct1/Gata3/Prdm1/I1r8r1/Nkcap1/Irun3/Nkox2-3 |
| GO:0002279   | mast cell activation involved in immune response                              | 2.44E-06         | 13             | Hmxox1/Fcer1g/Fox1/Cd30a/I4ra/Pls2g3/Cd8d/I13ra2/Rac2/Mir1/Gata2/Nir4a3/Gatr |
| GO:0071887   | leukocyte apoptotic process                                                   | 2.85E-06         | 23             | I6/Cc6r2/Fcgr2b/Fcer1g/Arg2/Adm6/Cxcl12/Ax/Be2a1a/Wnt5a/I10/I2ra/Hcls1/Ccr7/Ccr5/Hcra2/Pdcd11r7/Auark/Slic7a11/Nir4a3/Siglec1/Tnfrs4 |
| GO:0050701   | interleukin-1 secretion                                                       | 3.05E-06         | 13             | I1r2/Aboca1/Nirp3/Arg2/Cc3d/Cd3b/Zc3h12a/Wnt5a/Ccr7/Apoa1/Tlr2/Ccr5/I18 |
| GO:0042092   | type 2 immune response                                                        | 3.48E-06         | 11             | I6/Il33/Nirp3/Arg2/Arg1/Hvks/I4ra/Batf/Ccr2/Gata3/Cr3a2/Cfic1           |
| GO:0002828   | regulation of type 2 immune response                                          | 3.75E-06         | 10             | I6/I6/I33/Nirp3/Arg2/Arg1/Hvks/I4ra/Ccr2/Gata3/Cr3a2/Cfic1 |

(Continued)
### TABLE 3 | Continued

| GO ID       | Description                                                                 | Adjusted p-value | Number of gene | Gene ID                                                                 |
|-------------|------------------------------------------------------------------------------|-------------------|----------------|------------------------------------------------------------------------|
| GO:0002888  | positive regulation of myeloid leukocyte mediated immunity                  | 3.79E-05          | 12             | Cxcl1/C3/Fogr3/Fcer1g/Ftgam/Arg1/Fogr1/Ptafr/I4ra/Itgb2/Gata2/Fgr     |
| GO:0032663  | regulation of interleukin-2 production                                        | 4.33E-05          | 14             | If1b/I1a/Pdeo4b/Havcr2/Runx1/Foxp3/Ccr2/Card1f/Gata3/Lag3/Nav3/I20rb/  |
|             |                                                                               |                   |                | Vtcn1/Cd/Card50a/Cd98/If3ra2/Ccr2                                      |
| GO:0043301  | negative regulation of leukocyte degranulation                                | 4.39E-05          | 6              | Hmox1/Fox1/Cd300a/Cd84/If3ra2/Ccr2                                    |
| GO:0072604  | interleukin-6 secretion                                                      | 4.52E-05          | 11             | If1b/Zc3h12a/Nos2/Arid5a/Cd84/Ptpn22/F2r/Tpv4/Twist1/Tlr8/Sytl1     |
| GO:0032637  | interleukin-8 production                                                     | 5.48E-05          | 15             | Serpin1/I1b/Chil1/Tnf/Nos2/Runx5/Il24A/Ptpn22/F2r/Tlr2/Tlr8/Tlr7/Scc |
| GO:0032693  | negative regulation of interleukin-10 production                             | 6.12E-05          | 8              | Mmp8/Tnfr5/Fogr2b/Ager/Visr/Cd84/Foxp3/Tibh2                        |
| GO:0045408  | regulation of interleukin-6 biosynthetic process                            | 6.12E-05          | 8              | Ereg/I1b/Cebob/Ptafr/Prg4/Muc1b/Card1f/Npp5d                        |
| GO:0002374  | cytokine secretion involved in immune response                               | 6.12E-05          | 8              | Trem1/Nlrp3/Tnf/Angpt1/Wnt5a/I10/Apoa1/Tlr2                         |
| GO:0002763  | positive regulation of myeloid leukocyte differentiation                     | 6.24E-05          | 14             | Tnfr511/Tnf/Ccr1/Cd3/P45/Tgb1/Cd4/Il34/Runx1/Csf1fr/Hcts1/Igb3/Zlf3 |
|             |                                                                               |                   |                | Slf1/Cadm1/Visr                                                      |
| GO:0002823  | negative regulation of adaptive immune response based on somatic            | 7.33E-05          | 11             | If1r1/Fogr2b/I3/I3/Arg1/Zc3h12a/Visr/Havcr2/I4ra/Foxp3/I17/I120rb   |
|             | recombination of immune receptors built from immunoglobulin superfamily     |                   |                | domains                                                              |
| GO:0022283  | neutrophil activation involved in immune response                             | 0.000100258       | 7              | Fcer1g/Ftgam/Tyrobp/Ptafr/Stx11/Igb2/Myoif                         |
| GO:0045089  | positive regulation of innate immune response                                | 0.000100884       | 30             | Ereg/Mmp12/Mmp2/Cd300f/Tlt/Tlr3/Tnf/Clec12/Acad1/Adam8/I18rapt/CD36/ |
|             |                                                                               |                   |                | Cd300a/Armb2/Havcr2/Wnt5a/Rtnr1/Ptpn22/Cav1/Cd86/Card5/Tlr3/Tlr8/Tlr7/ |
|             |                                                                               |                   |                | Ira3/Cadm1/Lbpc/Lag3/Sh2d1b1/Pk3ap1                                 |
| GO:0002294  | CD4-positive, alpha-beta T cell differentiation involved in immune response  | 0.000105367       | 14             | I16/Nlrp3/Zc3h12a/Hx/Tgb1/I4ra/Batl/Foxp3/Ccr7/Myb/Ccr2/Rora/Gata3/I18r1 |
| GO:0043304  | regulation of mast cell degranulation                                         | 0.000112252       | 10             | Hmox1/Fcer1g/Fox1/Cd300a/I4ra/Cd84/If3ra2/Rac2/Gata2/Fgr           |
| GO:0002293  | alpha-beta T cell differentiation involved in immune response               | 0.000124943       | 14             | I16/Nlrp3/Zc3h12a/Hx/Tgb1/I4ra/Batl/Foxp3/Ccr7/Myb/Ccr2/Rora/Gata3/I18r1 |
| GO:0046638  | positive regulation of alpha-beta T cell differentiation                     | 0.00013566        | 12             | I16/Nlrp3/I4ra/Runx1/Foxp3/Ztbb1/Ccr7/Myb/Ccr2/Nckap1/Rnapx3     |
| GO:0002285  | lymphocyte activation involved in immune response                            | 0.000140936       | 27             | I16/Clec4e/Fcer1g/Nlrp3/Clec4d/Stc11a/Zc3h12a/Hx/Tgb1/Havcr2/I4ra/Stx11/Batl/Foxp3/Ccam1/Ttm2a/Cd244a/Ccr7/Myb/Ccr2/Rora/Gata3/Clec1/I18r1/Coro1a/Nko2-3/Exo1 |
| GO:0050706  | regulation of interleukin-1 beta secretion                                    | 0.000143647       | 10             | Nlrp3/Arg2/Cd3/Zc3h12a/Wnt5a/Ccr7/Apoa1/Tlr2/Ccr5/Tlr8             |
| GO:0033006  | regulation of mast cell activation involved in immune response               | 0.000143647       | 10             | Hmox1/Fcer1g/Fox1/Cd300a/I4ra/Cd84/I13ra2/Rac2/Gata2/Fgr           |
| GO:0050704  | regulation of interleukin-1 secretion                                         | 0.000144385       | 11             | I1r2/Nlrp3/Arg2/Cd3/Zc3h12a/Wnt5a/Ccr7/Apoa1/Tlr2/Ccr5/Tlr8       |

(Continued)
### TABLE 3 | Continued

| GO ID          | Description                                                                 | Adjusted p-value | Number of gene | Gene ID                                                                 |
|----------------|------------------------------------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:0046636     | negative regulation of alpha-beta T cell activation                          | 0.000144365      | 11             | Tarm1/Arg2/Zc3h12a/Hlx/Vsir/Cd300a/Il4ra/Runx1/Foxp3/Adora2a/Runx3     |
| GO:0002287     | alpha-beta T cell activation involved in immune response                     | 0.000145765      | 14             | Il6/Nlrp3/Zc3h12a/Hlx/Tgfbl/Il4ra/Batt/Foxp3/Ccr7/Myb/Ccr2/Flora/Gata3/Ii19r1 |
| GO:1904994     | regulation of leukocyte adhesion to vascular endothelial cell                | 0.000145956      | 6              | Tnf/Cxcl12/Ets1/Pfmr1/Ccam1/Ccr2                                      |
| GO:0042036     | negative regulation of cytokine biosynthetic process                         | 0.000165715      | 9              | Il6/Inhbb/Pgrh/Muc16/Foxp3/Ii10/Lag3/Trib2/Fnpp5d                      |
| GO:0002827     | positive regulation of T-helper 1 type immune response                       | 0.000175032      | 8              | Il1b/Ii1r1/Slc11a1/Hlx/Arid5a/Ccr7/Ccr2/Ii19r1                         |
| GO:0043372     | positive regulation of CD4-positive, alpha-beta T cell differentiation        | 0.000218687      | 9              | Il6/Nlrp3/Hlx/Ii4ra/Foxp3/Ccr7/Myb/Ccr2/Nckap11                       |
| GO:0032757     | positive regulation of interleukin-8 production                              | 0.000219443      | 11             | Serpine1/Ii1b/Tnf/Wnt5a/Atpa2/C2d44aA/F2r/Tlr2/Tlr8/Tlr7/Lbp          |
| GO:0002726     | positive regulation of T cell cytokine production                             | 0.000224164      | 7              | Il6/Ii1b/Ii1r1/Nlrp3/Arid5a/Gata3/Ii19r1                              |
| GO:0032753     | positive regulation of interleukin-4 production                              | 0.000237696      | 8              | Fcer1g/Ii33/Nlrp3/Cebpb/Havcr2/Foxp3/Gata3/Ii20rb                    |
| GO:2000515     | negative regulation of CD4-positive, alpha-beta T cell activation            | 0.00027983       | 9              | Tarm1/Arg2/Zc3h12a/Hlx/Vsir/Ii4ra/Runx1/Foxp3/Gata3/Ii20rb           |
| GO:0032677     | regulation of interleukin-8 production                                        | 0.000281381      | 13             | Serpine1/Ii1b/Tnf/Wnt5a/Atpa2/C2d44aA/Ptnp22/F2r/Tlr2/Tlr7/Scc5d/Lbp |
| GO:0006959     | humoral immune response                                                      | 0.000281381      | 41             | S100a9/Cxcl5/Cxcl2/Cxcl1/Cxcl3/Cd2/C3/Fcgr2b/Gfa/C1qa/Pbp/Cxcl13/Ltf/Tnf/ C1s1/C4b1/Cq6/Cmklr1/Mefv/Nos2/Ii1r2/Per1/Tarm1/Flora/Gata3/Ii19r1 |
| GO:0045824     | negative regulation of innate immune response                                | 0.000281381      | 12             | Mmp12/Arg1/Ccr1/Acc1/Havcr2/A2m/Irak3/Sh2d1b1/Slamf8/Kbrt1b           |
| GO:0002686     | negative regulation of leukocyte migration                                   | 0.000282957      | 10             | Apod/Grem1/Dusp1/Nbl1/Ii33/Cxcl12/Emilin1/Cd300a/Adora1/Slamf8       |
| GO:0002764     | immune response-regulating signaling pathway                                 | 0.000291533      | 49             | Fpr2/Fpr1/Clec4e/Fcgr2b/Fcgr1g/Cd300f/Ltf/Tlr13/Tnfl/Citna/Colec12/Clec4d/Pd4e4b/ Accod1/Cd36/Zc3h12a/Ighv1-72/Cd300a/Arb2/Havcr2/Cmklr1/Bcl2a1d/Foxp3/Flora/Rfh1/Ptnp22/Cav1/Ccr7 /Iighv1-2/Cd86/Tlr2/Tdrd/Tlr8/Tlr7/Gata3/Irak3/Sh2d2b/Lbp/Lcp2/Fyn/Sh2d1b1/Nra3/Ighv1-55/Nckap1/Plk3ap1/Ii20rb/Vtnc1/C3ar1 |
| GO:0072606     | interleukin-8 secretion                                                      | 0.000318798      | 8              | Chit1/Nos2/Wnt5a/Cd244aA/Ptnp22/F2r/Tlr2/Scc5d                       |
| GO:0050798     | activated T cell proliferation                                               | 0.000320711      | 11             | Itgam/Lrc32/Arg1/Igh1/Ager/lggb2/Ii2ra/Satb1/Cd86/Fyn/Tnsf9           |
| GO:1900015     | regulation of cytokine production involved in inflammatory response          | 0.000349998      | 10             | Il1r2/Adop/Pern1/Tarm1/Zc3h12a/Mefv/Nos2/Sirpa/Gpsm3/Cd6             |
| GO:0050869     | negative regulation of B cell activation                                     | 0.00035374       | 9              | Fcgr2b/Sfpr1/Citna/Sams5/Cd300a/Foxp3/Ii10/Pdram1/Inpp5d            |

(Continued)
| GO ID       | Description                                                                 | Adjusted p-value | Number of gene | Gene ID                                                                 |
|-------------|------------------------------------------------------------------------------|-------------------|----------------|-------------------------------------------------------------------------|
| GO:0002758  | innate immune response-activating signal transduction                        | 0.000423651       | 20             | Cx300fl/Tlr13/Tnf/Colec12/Accod1/Cx56/Cx300a/Arnb2/Havcr2/Tlrn1/Ptpn22/Cav1/Car86/Tlr2/Tlr7/Ira3/Lbp/Pk3ap1 |
| GO:0001909  | leukocyte mediated cytotoxicity                                               | 0.00050804        | 21             | Cxcl5/Cxcl1/Cxcl2/Trem1/Fgfr3/Arg1/Fgfr1/Il1rap/Arg1/Arnb2/Havcr2/Stxb11/H2Q10/Pk3r6/Cadm1/Lag3/L7rr/Sh2d1b1/Coro1a/Ilr1/Ilr2 |
| GO:2000404  | regulation of T cell migration                                                | 0.00051051        | 10             | Apor/Cxcl13/Adam8/Cxcl12/Wnt5a/Ccr7/Ccr2/Il2g/Alf1/Cc21a               |
| GO:0014033  | neural crest differentiation                                                  | 0.00052764        | 16             | Sema7a/Stxb1/Ednra/Bmp7/Rdh10/Fn1/Ret/Sema3f/Ednrb/Lama5/Sema6b/Twist1/Hand2/Sna2/Pkox2b/Sema3g |
| GO:0036037  | CD8-positive, alpha-beta T cell activation                                    | 0.000530507       | 8              | Vair/Runx1/Cd244a/Ptpn22/Satb1/Clec4a2/Nckap1/Runx3                     |
| GO:2000406  | positive regulation of T cell migration                                       | 0.000542479       | 9              | Cxcl13/Adam8/Cxcl12/Wnt5a/Ccr7/Ccr2/Il2g/Alf1/Cc21a                   |
| GO:2000516  | positive regulation of CD4-positive, alpha-beta T cell activation             | 0.000542479       | 9              | Il6/Nrp3/Il4a/Foxp3/Ccr7/Myb/Ccr2/Nckap1                               |
| GO:0002830  | positive regulation of type 2 immune response                                 | 0.000545532       | 6              | Il6/Il33/Nrp3/Il4a/Gata3/Clec1f                                      |
| GO:0002824  | positive regulation of adaptive immune response based on somatic             | 0.000549094       | 21             | Il6/Il12/Il11r/Cc3/Fggr3/Fcerrg3/Il6/Nlrp3/Tnf/Fgfr1/Scl11a1/Hx/Tgb1/Cd4/Arid5a/Foxp3/Ccr7/H2Q10/Ccr2/Gata3/Clec1f/I218r1 |
| GO:0035744  | T-helper 1 cell cytokine production                                           | 0.000552023       | 5              | Il1b/Il1r1/Il1rap/Arid5a/Il8r1                                        |
| GO:0002534  | cytokine production involved in inflammatory response                         | 0.000604767       | 10             | Il12/Adpo/Peri1/Tamm1/Zc3h12a/Mefv/Nos2/Sirpa/Gpsm3/Cd6                 |
| GO:00965929 | substrate-dependent cell migration                                           | 0.000650887       | 8              | Adam8/Fn1/Fgfr1/Pdgb/Tfnsf12a/Sna2/P2ry12/Fb1n1                         |
| GO:0014032  | neural crest cell development                                                 | 0.000668887       | 15             | Sema7a/Ednra/Bmp7/Rdh10/Fn1/Ret/Sema3f/Ednrb/Lama5/Sema6b/Twist1/Hand2/Sna2/Pkox2b/Sema3g |
| GO:0001755  | neural crest cell migration                                                    | 0.000706219       | 12             | Sema7a/Bmp7/Fn1/Ret/Sema3f/Ednrb/Lama5/Sema6b/Twist1/Hand2/Pkox2b/Sema3g |
| GO:0051852  | disruption by host of symbiont cells                                          | 0.000777607       | 6              | Cxcl5/Cxcl1/Trem1/Arg1/P4/Nckf1                                       |
| GO:0051783  | killing by host of symbiont cells                                            | 0.000777607       | 6              | Cxcl5/Cxcl1/Trem1/Arg1/P4/Nckf1                                       |
| GO:2000107  | negative regulation of leukocyte apoptotic process                            | 0.000849747       | 13             | Cxcr2/Fggr2b/Fcgr1g/Arg2/Cxcl12/Ax/Bo2a1a/Hcls1/Ccr7/Ccr5/I7rr/Aurkb/Tfnsf4 |
| GO:0070943  | neutrophil mediated killing of symbiont cell                                 | 0.000873833       | 5              | Cxcl5/Cxcl1/Trem1/Arg1/Nckf1                                          |
| GO:0032620  | interleukin-17 production                                                     | 0.000977077       | 9              | Osrm/Arg2/Ilr13/Tnf1/Arnb2/Havcr2/Tlrn1/Ptpn22/Cav1/Car86/Tlr2/Tlr7/Ira3/Lbp/Pk3ap1 |
| GO:0032633  | interleukin-4 production                                                      | 0.000977077       | 9              | Fcgr1/Il13/Nlrp3/Ilr3/Il1r3/Cepbb/Havcr2/Foxp3/Gata3/I200b/Vhcn1       |
| GO:0032731  | positive regulation of interleukin-1 beta production                          | 0.000977077       | 9              | Nlrp3/Cctl3/Hspb1/Egr1/Wnt5a/Ccr7/Tlr2/Ccr5/Tlr8                      |
| GO:0002821  | positive regulation of adaptive immune response                              | 0.000978168       | 21             | Il6/Il1b/Il1r1/Cc3/Fggr3/Fcerrg3/Il6/Nlrp3/Tnf/Fgfr1/Scl11a1/Hx/Tgb1/Cd4/Arid5a/Foxp3/Ccr7/H2Q10/Ccr2/Gata3/Clec1f/I218r1 |
| GO:0002732  | positive regulation of interleukin-1 production                              | 0.001010452       | 10             | Nlrp3/Cctl3/Hspb1/Egr1/Havcr2/Wnt5a/Ccr7/Tlr2/Ccr5/Tlr8              |
| GO ID       | Description                                           | Adjusted p-value | Number of gene | Gene ID                                                                 |
|-------------|-------------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:0002269 | leukocyte activation involved in inflammatory response | 0.001029033      | 8              | Mrp8/Fpr2/I33/Ager/Tlr2/Tlr8/Tlr7/Aif1                                  |
| GO:0032660 | regulation of interleukin-17 production               | 0.001029033      | 8              | Osrm/Arg2/Vsir/Tgfb1/Arid5a/Foxp3/Tlr2/Nckap1                           |
| GO:0032673 | regulation of interleukin-4 production                | 0.001029033      | 8              | Fcer1g/I33/Nlrp3/Cebpb/Havr2/Foxp3/Gata3/I20orb                         |
| GO:0032700 | negative regulation of interleukin-17 production      | 0.001060545      | 6              | Arg2/Vsir/Tgfb1/Foxp3/Tlr2/Nckap1                                     |
| GO:2001185 | regulation of CD8-positive, alpha-beta T cell activation | 0.001060545     | 6              | Vair/Ruin1/Cd244a/Ptnr22/Nckap1/Ruin3                                  |
| GO:0001959 | regulation of cytokine-mediated signaling pathway      | 0.001076097      | 16             | Itl6/I1r2/I1r1/Mrp12/Spkh1/Slk3/Cd300a/Arg1/Trem2/Angot1/I4ist/Axl/Wnt5a/Cav1/Apoa1/I33 |
| GO:0060759 | regulation of response to cytokine stimulus            | 0.001213528      | 17             | Itl6/I1r2/I1r1/Mrp12/Spkh1/Slk3/Cd300a/Arg1/Trem2/Angot1/I4ist/Axl/Wnt5a/Cav1/Apoa1/I33 |
| GO:0072538 | T-helper 17 type immune response                       | 0.00127248       | 8              | Itl6/Nlrp3/Zc3h12a/Tgfb1/Arid5a/Batt/Foxp3/Rora                        |
| GO:0002757 | immune response-activating signal transduction         | 0.001343103      | 45             | Fpr2/Fpr1/Fcer1g/Cd300a/Ilt13/Tnf/Cd300a/Arg1/Trem2/Angot1/I4ist/Axl/Wnt5a/Cav1/Apoa1/I33 |
| GO:0002456 | T cell mediated immunity                               | 0.001418302      | 19             | Itl6/I1b/I1r1/Nlrp3/I33/Slk11a/Ill8rap/Ager/Vair/Arid5a/Sto1/Foxp3/Im/cam1/Rfin1/H2-Q10/Gata3/I18r1/I20rb |
| GO:0032674 | regulation of interleukin-5 production                | 0.001433829      | 6              | I1r1/I33/Nlrp3/Ill5a/Foxp3/Gata3                                      |
| GO:0030889 | negative regulation of B cell proliferation            | 0.001433829      | 6              | Fcer1g/C4l300a/I10/Frdm1/I33                                           |
| GO:0032703 | negative regulation of interleukin-2 production        | 0.001607694      | 7              | Havr2/Foxp3/Gata3/Lag3/Nav3/I20rb/Cd34                                 |
| GO:0002218 | activation of innate immune response                   | 0.001884179      | 20             | Cd300a/Ilt13/Tnf/Cd300a/Arg1/Trem2/Angot1/I4ist/Axl/Wnt5a/Cav1/I33/I18r1/I20rb |
| GO:0032634 | interleukin-5 production                               | 0.001912936      | 6              | I1r1/I33/Nlrp3/Ill5a/Foxp3/Gata3                                      |
| GO:0002517 | T cell tolerance induction                             | 0.001916359      | 5              | Icos/Ruin1/Foxp3/I2ra/Cd86                                            |
| GO:0002281 | macrophage activation involved in immune response      | 0.001916359      | 5              | I33/Tyrobp/Havr2/Lbp/Disf                                             |
| GO:0070498 | interleukin-1-mediated signaling pathway               | 0.002486676      | 6              | I1/l1r1/I1r1/I1a/Egr1/I33                                             |
| GO:0006691 | leukotriene metabolic process                          | 0.003147625      | 6              | Gqgt5/Gqgt1/Aloxasap/Cyp4f118/Tlr2/Ncf1                                  |
| GO:2000482 | regulation of interleukin-8 secretion                 | 0.003147625      | 6              | Wnt5a/Cd244a/Ptnr22/F2r/Tlr2/Sec5d                                     |
| GO:0002709 | regulation of T cell mediated immunity                 | 0.003296751      | 14             | I1b/I1r1/I1r1/Nlrp3/Arg1/Ager/Vair/Arid5a/Foxp3/H2-Q10/Gata3/I18r1/I20rb |
| GO:0033089 | positive regulation of T cell differentiation in thymus | 0.003628826      | 5              | I1b/Vnin1/Adam8/Foxp3/I17r                                              |
| GO:0002739 | regulation of cytokine secretion involved in immune response | 0.003628826 | 5 | Trnf/Angot1/Wnt5a/I10/Apoa1 | (Continued)
| GO ID | Description                                                                 | Adjusted p-value | Number of gene | Gene ID                                                                                           |
|-------|----------------------------------------------------------------------------|------------------|----------------|--------------------------------------------------------------------------------------------------|
| GO:1900016 | negative regulation of cytokine production involved in inflammatory response | 0.003628826      | 5              | Il1r2/Adop/Zc3h12a/Mefv/Sirpa                                                                      |
| GO:0043302 | positive regulation of leukocyte degranulation                             | 0.003637478      | 7              | Fcer1g/1tgam/Ptraf/I4ra/Igzb2/Gata2/Fgr                                                           |
| GO:0050718 | positive regulation of interleukin-1 beta secretion                        | 0.003637478      | 7              | Nlrp3/Ccd3/Wnt5a/Ccr7/Tlr2/Ccr5/Tlr8                                                             |
| GO:0001960 | negative regulation of cytokine-mediated signaling pathway                 | 0.004611661      | 9              | Il6/Il1r2/Mmp12/Slt3/Arg1/Il6st/Cav1/Apoa1/Ira3                                                   |
| GO:0032621 | interleukin-18 production                                                  | 0.004750601      | 4              | Nlrp3/Tnf/Cd84/Tlr2                                                                               |
| GO:0050711 | negative regulation of interleukin-1 secretion                             | 0.004750601      | 4              | Il1r2/Nlrp3/Zc3h12a/Apoa1                                                                        |
| GO:0070673 | response to interleukin-18                                                | 0.004750601      | 4              | Il18rap/Pdgfbl/Cltn1/Il18r1                                                                     |
| GO:0043371 | negative regulation of CD4-positive, alpha-beta T cell differentiation     | 0.004760181      | 6              | Zc3h12a/Hlx/I4ra/Runx1/Runx3                                                                      |
| GO:0019724 | B cell mediated immunity                                                  | 0.005107466      | 28             | C3/Fgcr3/Flg2/Fc1g1a/Fc1g1/Tnf/Cd84/Fgcr1/C4b/Cq14/Cd55/Igh1-72/Tgfbl/I4ra/I4a/Baff/Flg3p3/Cd55/I13ra2/Igh2c/C1br/Tnc/Clcf1/Igh1-55/Innp5d/Exo1 |
| GO:0001910 | regulation of leukocyte mediated cytotoxicity                             | 0.005759764      | 15             | Ccl5/Ccl2/Arg1/Il18rap/Ager2/Arb2/Havcr2/H2/Q10/Pkt3p/Clf1p3/It7r/Sl2d1d1/Bkb1                   |
| GO:0050716 | positive regulation of interleukin-1 secretion                            | 0.005990978      | 7              | Nlrp3/Cc3/Wnt5a/Ccr7/Tlr2/Ccr5/Tlr8                                                              |
| GO:0032691 | negative regulation of interleukin-1 beta production                      | 0.005990978      | 5              | Nlrp3/Zc3h12a/Mefv/Arb2/Apoa1                                                                    |
| GO:0002922 | positive regulation of humoral immune response                            | 0.005990978      | 5              | C3/Tnf/Acc1p/Ccr7/C6                                                                             |
| GO:1902106 | negative regulation of leukocyte differentiation                           | 0.006243457      | 14             | Stf1/Tmem176a/Ctta4/Zc3h12a/C1qc/Hlx/I4ra/Runx1/Runx3/Cd55/I13ra2/Igh2c/C1br/Tnc/Clcf1/Igh1-55/Innp5d/Exo1 |
| GO:0002664 | regulation of T cell tolerance induction                                   | 0.006620994      | 4              | Runx1/Flg3p3/I4ra/Cd84                                                                            |
| GO:1901163 | regulation of trophoblast cell migration                                   | 0.006620994      | 4              | Timp1/Mmp12/Syde1/Arhgdib                                                                       |
| GO:0032692 | negative regulation of interleukin-1 production                           | 0.006940312      | 6              | Il1r2/Nlrp3/Zc3h12a/Mefv/Arb2/Apoa1                                                               |
| GO:0003761 | negative regulation of response to cytokine stimulus                      | 0.007342352      | 9              | Il6/Il1r2/Mmp12/Slt3/Arg1/Il6st/Cav1/Apoa1/Ira3                                                   |
| GO ID       | Description                                                                 | Adjusted p-value | Number of gene | Gene ID                                                                 |
|------------|------------------------------------------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| GO:0002710 | negative regulation of T cell mediated immunity                              | 0.007463658      | 5              | Arg1/Vsir/Foxp3/Ilf/I20rb                                              |
| GO:2000108 | positive regulation of leukocyte apoptotic process                           | 0.008044325      | 7              | Adam8/Wnt5a/I10/Hcar2/Pdcd1/Nr4a3/Siglec1                              |
| GO:0016064 | immunoglobulin mediated immune response                                      | 0.008070417      | 27             | C3/Fcg3r3/Fcg2b/C1qa/Fcer1g/Tnf/C1s1/Fcg1r1/C4b/C1qb/Serping1/C1qc/Ighv1-72/Tgf1b/I4ra/C1ra/Baff/Fopx3/Cd55/I13na2/Igh2c/C1rb/Tdcl/Clicf1/Ighv1-55/Inpp5d/Exo1 |
| GO:2000778 | positive regulation of interleukin-6 secretion                               | 0.008255899      | 6              | Iibt/Arid5a/F2r/Trpv4/Twist1/Tlr8                                     |
| GO:0045589 | regulation of regulatory T cell differentiation                              | 0.008255899      | 6              | Ctxa2a/Ctla4/Vsir/Tgf1b/Foxp3/Fanca                                    |
| GO:0032754 | positive regulation of interleukin-5 production                              | 0.008896526      | 4              | Iibt/I33/Nr3p3/Gata3                                                  |
| GO:0061450 | trophoblast cell migration                                                    | 0.008896526      | 4              | Timp1/Mmp12/Syde1/Arhgdib                                             |
| GO:0046718 | viral entry into host cell                                                    | 0.009178108      | 9              | Ifttm1/Ifttm6/Pbx3/As1/Cd4/Cav1/Igfb3/Trim10/Trim21                  |
| GO:0045076 | regulation of interleukin-2 biosynthetic process                             | 0.009244491      | 5              | Iibt/I1a/Foxp3/Card9/Lag3                                             |
| GO:0010818 | T cell chemotaxis                                                            | 0.009244491      | 5              | Cxcl13/Cxcl16/Wnt5a/Ccr7/Ccr2                                         |
| GO:0002712 | regulation of B cell mediated immunity                                        | 0.009482699      | 10             | C3/Fcg3r3/Fcg2b/Fcer1g/Tnf/Fcg1r1/Tgf1b/Foxp3/Cd55/Clic1              |
| GO:0002889 | regulation of immunoglobulin mediated immune response                        | 0.009482699      | 10             | C3/Fcg3r3/Fcg2b/Fcer1g/Tnf/Fcg1r1/Tgf1b/Foxp3/Cd55/Clic1              |
| GO:0046639 | negative regulation of alpha-beta T cell differentiation                     | 0.009725406      | 6              | Zc3h12a/Ihxs/I4ra/Runc1/Foxp3/Runc3                                   |
| GO:0046640 | regulation of alpha-beta T cell proliferation                                | 0.010641684      | 7              | Tarm1/Arg2/Vsir/Cd80/Cd244a/Ptprn22/Ccr2                               |
| GO:0042267 | natural killer cell mediated cytotoxicity                                     | 0.011450593      | 10             | Ilt18rap/Arnb2/Havcr2/Sbx11/Pik3rb/Cadm1/Lag3/Sh2d1b1/Coro1a/Krb1b    |
| GO:2000484 | positive regulation of interleukin-8 secretion                               | 0.01148009       | 4              | Wnt5a/Cd244a/F2r/Tlr2                                                 |
| GO:0035745 | T-helper 2 cell cytokine production                                           | 0.01148009       | 4              | I6b/Nr3p3/Arg1/Gata3                                                  |
| GO:0030260 | entry into host cell                                                         | 0.012367149      | 9              | Ifttm1/Ifttm6/Pbx3/As1/Cd4/Cav1/Itgfb3/Trim10/Trim21                 |
| GO:0071353 | cellular response to interleukin-4                                            | 0.01321804       | 6              | Cd2000l/Nr3/Mrc1/Gata3/Mcm2/Coro1a                                    |
| GO:0033005 | positive regulation of mast cell activation                                   | 0.01321804       | 6              | Fcer1g/I4ra/Cd300b/Gata2/Nr4a3/Fgr                                    |
| GO:0045066 | regulatory T cell differentiation                                             | 0.01321804       | 6              | Ctxa2a/Ctla4/Vsir/Tgf1b/Foxp3/Fanca                                    |
| GO:0042094 | interleukin-2 biosynthetic process                                           | 0.01342999       | 5              | Iibt/I1a/Foxp3/Card9/Lag3                                             |
| GO:0046606 | regulation of activated T cell proliferation                                 | 0.013571162      | 7              | Lrc32/Arg1/Igf1/Ager/I2ra/Cd86/Tnfsf9                                  |
| GO:0002228 | natural killer cell mediated immunity                                         | 0.013572012      | 10             | Ilt18rap/Arnb2/Havcr2/Sbx11/Pik3rb/Cadm1/Lag3/Sh2d1b1/Coro1a/Krb1b    |
| GO ID   | Description                                                                 | Adjusted p-value | Number of gene | Gene ID                                                                 |
|---------|-----------------------------------------------------------------------------|------------------|----------------|--------------------------------------------------------------------------|
| GO:0002768 | immune response-regulating cell surface receptor signaling pathway         | 0.01370038       | 32             | Fpr2/Fpr1/Clec4e/Fcgr2b/Clec4d/Pde4b/Zc3h12a/Ighv1-72/Cd300a/Cmntm3/Cmkrd1/Bcl2a1d/Foxp3/Nras/Rtn1/Ppbn22/Ccr7/Ighg2c/Tlr2/Trdc/Gata3/Sh2b2b/Lcp2/Fyn/Sh2d1b1/Nr4a3/Ighv1-55/Nckap11/Vtn1/C3ar1 |
| GO:0010820 | positive regulation of T cell chemotaxis                                    | 0.014564028      | 4              | Cxcl13/Wnt5a/Ccr7/Ccr2                                                   |
| GO:0002829 | negative regulation of type 2 immune response                               | 0.014564028      | 4              | Arg2/Arg1/Hlx/Ccr2                                                      |
| GO:0030888 | regulation of B cell proliferation                                          | 0.014756231      | 10             | Fcgr2b/Cd40a/I10/Bstl/Pdcd1/Tnfrsf4/Fn1p5d                              |
| GO:0046633 | alpha-beta T cell proliferation                                             | 0.015272178      | 7              | Tar1/Arg2/Vsr/Cd80/Cd244a/Ppbn22/Ccr2                                  |
| GO:0001911 | negative regulation of leukocyte mediated cytotoxicity                      | 0.015904422      | 5              | Arrb2/Havcr2/Il7r/Sh2d1b1/Xr1b1                                           |
| GO:0070670 | response to interleukin-4                                                    | 0.017380764      | 6              | Cd300f/Ilf3/Mrc1/Cd13/St18/Mcm2/Coro1a                                  |
| GO:0033081 | regulation of T cell differentiation in thymus                               | 0.017380764      | 6              | Itf1b/Vn1/Adm8/Foxp3/Zebl/I7r                                            |
| GO:1900024 | regulation of substrate adhesion-dependent cell spreading                   | 0.018025241      | 8              | Pdpn/Dab2/Pres1/Ena5/Apoa1/Itgb3/Dbn1/Fbn1                               |
| GO:0010819 | regulation of T cell chemotaxis                                             | 0.018074986      | 4              | Cxcl13/Wnt5a/Ccr7/Ccr2                                                   |
| GO:0070234 | positive regulation of T cell apoptotic process                             | 0.018074986      | 4              | Adam8/Wnt5a/Pdcd1/Sglec1                                                |
| GO:0032733 | positive regulation of interleukin-10 production                            | 0.019719759      | 6              | Fcgr1g/Tlr2/Tgbl/Hgf/It2Orb/Cd34                                        |
| GO:0032743 | positive regulation of interleukin-2 production                             | 0.019719759      | 6              | Itf1b/I1a/Pdcd4/Rn1/Xcr2/Vtn1                                            |
| GO:0045577 | regulation of B cell differentiation                                        | 0.019719759      | 6              | Strp1/Mmp14/Zf3/S61/Prdm1/Nckap11/Itgb3                                  |
| GO:0043374 | CD8-positive, alpha-beta T cell differentiation                             | 0.022221339      | 4              | Runx1/Stat1/Nckap11/Runx3                                               |
| GO:0046566 | regulation of viral entry into host cell                                    | 0.022322781      | 6              | Ifttm1/Ifttm6/Ptx3/Cd4/Trm10/Trm21                                      |
| GO:0070232 | regulation of T cell apoptotic process                                       | 0.0223818542     | 7              | Arg2/Adm8/Wnt5a/Pdcd1/I7r/Sglec1/Tnfrsf4                                |
| GO:0042269 | regulation of natural killer cell mediated cytotoxicity                      | 0.023869632      | 8              | It18rap/Arbb2/Havcr2/Pk3r6/Cadm1/Lag3/Sh2d1b1/Krib1b                    |
| GO:0032735 | positive regulation of interleukin-12 production                            | 0.025322878      | 6              | Cd36/Ager/Rel/Ccr7/Tlr2/Tnfrsf9                                         |
| GO:0002715 | regulation of natural killer cell mediated immunity                          | 0.026123245      | 8              | It18rap/Arbb2/Havcr2/Pk3r6/Cadm1/Lag3/Sh2d1b1/Krib1b                    |
| GO:0045581 | negative regulation of T cell differentiation                               | 0.026290355      | 7              | Cta4/Zc3h12a/Hlx/I4ra/Runx1/Foxp3/Runx3                                 |
| GO:0002689 | negative regulation of leukocyte chemotaxis                                 | 0.026549353      | 4              | Grem1/Dbp1/Nbl1/Samat8                                                  |
| GO:0045579 | positive regulation of B cell differentiation                               | 0.026549353      | 4              | Mmp14/Prdm1/Nckap11/Itgb3                                                |
| GO:0043373 | CD4-positive, alpha-beta T cell lineage commitment                           | 0.026549353      | 4              | It6/Tgbi1/Baf1/Foxp3                                                   |
and then 3% DSS solution was provided freely in drinking water for UC induction. Meanwhile, DSS-exposed mice were treated with 0.4 g/kg mesalazine for 10 days. In addition, mice treated with DSS-free solution were used as the control group. At the end of the experiment, all mice were euthanized via cervical dislocation, and colorectal tissue samples were immediately isolated and snap-frozen with liquid nitrogen before being used for transcriptomics analysis (Su et al., 2019).

**Transcriptome Sequencing**
Total RNA (n = 5 from each treatment) was extracted using TRIzol reagent, following the manufacturer's instructions. RNA samples with a RIN number > 8.0, were used for library construction. Briefly, poly(A)-mRNA was enriched from ten-micrograms of total RNA using poly T oligo-attached magnetic beads. After purification, the mRNA was fragmented and subjected to cDNA library construction using an Illumina mRNA Seq sample preparation kit. Paired-end sequencing (150 bp each) was performed on an Illumina HiSeq 4000, following the manufacturer’s instructions.

**Bioinformatic Analysis of Transcriptome**
The adaptor sequences and sequencing primers were trimmed. Low-quality reads with q quality scores lower than 20 were removed. The clean sequence reads were mapped to the UCSC reference genome using the HISAT package. StringTie and edgeR were used to determine the expression levels of all the transcripts. Genes with a \( \log_2 \) (fold change) > 1 and with statistical significance (q-value < 0.05) were defined as differentially expressed genes (DEGs). The DEGs were subjected to Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, and ingenuity pathway analysis (IPA) to examine the effects of mesalazine on DSS-induced colitis.

**Enzyme-Linked Immunosorbent Assay**
The colon samples were homogenized, and the supernatants were used for the measurement of inflammatory cytokines using ELISA kits (Elisa Biotech, Shanghai, China). The experimental procedures were performed as described previously (Xu et al., 2019).

**Immunostaining**
The colon samples from different groups were fixed with 4% paraformaldehyde prior to being cut into 4–5 µm thick sections using a microtome. Thereafter, the sections were dewaxed using xylene and were stained with hematoxylin and eosin dyes for histopathological examination (Wu et al., 2019; Li et al., 2020b).

**DISCUSSION**
In this study, we performed comparative transcriptomic analysis to understand the molecular basis of DSS-induced colitis and to delineate the molecular mechanism underlying the immunosuppressive effect of mesalazine. In the first part of the study, we attempted to understand the detailed mechanism underlying DSS-induced colitis in a mouse model. It has been well reported that the major cause of UC results from abnormal immune responses to antigens derived from the intestinal microbiota (Podolsky, 2002; Macdonald and Monteoleone, 2005). Our results showed that DSS could lead to a large number of differentially expressed genes. The results of GO and pathway analyses further highlighted that the induction of inflammatory and immune responses is mainly through alterations of cytokine-cytokine receptor interaction. IL-17 signaling pathway, leukocyte transendothelial migration, Toll-like receptor signaling pathway, and intestinal immune network for IgA production. In addition, IBD was highlighted in the analysis, suggesting that the model was valid. Cytokine-cytokine receptor interactions play an important role in immune regulation (Wu et al., 2005). It is involved in the T-cell receptor signaling pathway (Qian et al., 2019) and has been reported to be associated with autoimmune disorders (Liu et al., 2013; Xing et al., 2020). Many autoimmune and inflammatory diseases can result from excessive cytokine production or responsiveness. The IL-17 signaling pathway is the founding member of a novel family of inflammatory cytokines (Amaty et al., 2017). IL-17, the hallmark cytokine of the newly defined T helper 17 [T(H)17] cell subset, plays a crucial role in the inflammatory pathology of autoimmune diseases (Gaffen, 2009). The role of the IL-17 family in most common autoimmune diseases, such as psoriasis, IBD, and rheumatoid arthritis, has been extensively characterized (Zhang et al., 2015). Our funding is concordant with the previous IL-17R knockout mouse study that IL-17 signaling plays a critical role in the development of TNBS-induced colitis and may represent a target for therapeutic intervention for IBD (Zhang et al., 2006). The toll-like receptor signaling pathway has been recognized as a key repressor of inflammatory signaling and plays an essential role in the pathogenesis of autoimmune diseases (Chen et al., 2016; Shamilov and Aneskievich, 2018). It is a primary sensor of both innate and adaptive immune systems through its modulation of numerous genes that function in host defense, including inflammatory cytokines, chemokines, and antigen-presenting molecules (Liu et al., 2006). It has been reported that Toll-like receptor (TLR) signaling-related protein (TLR4) was detected in the colon tissue of DSS model mice (Bai et al., 2021). Furthermore, macrophages in the intestinal mucosa can rapidly induce TLR-mediated inflammatory responses (Wang et al., 2021). Our results provide a better understanding of the immunopathogenesis of UC and may help to identify novel targets for more potent interventions.

**RESULTS**

**DSS-Induced Colitis Through the Induction of Inflammatory Response**
To understand the molecular mechanism underlying DSS-induced colitis and the immunosuppressive effects of mesalazine...
## Effects of Mesalazine on the DSS-altered cell signaling pathways.

| KEGG ID | Description | Adjusted p-value | Number of Gene ID gene |
|---------|-------------|------------------|------------------------|
| mmu04512 | ECM-receptor interaction | 1.60E-10 | 30 |
| mmu04510 | Focal adhesion | 4.00E-08 | 43 |
| mmu04060 | Cytokine-cytokine receptor interaction | 4.67E-08 | 54 |
| mmu04974 | Protein digestion and absorption | 6.55E-08 | 29 |
| mmu04514 | Cell adhesion molecules | 9.44E-08 | 38 |
| mmu04061 | Viral protein interaction with cytokine and cytokine receptor | 8.66E-07 | 25 |
| mmu04151 | PI3K-Akt signaling pathway | 8.66E-07 | 58 |
| mmu05323 | Rheumatoid arthritis | 2.39E-06 | 23 |
| mmu05144 | Malaria | 2.89E-06 | 18 |
| mmu04657 | IL-17 signaling pathway | 1.97E-05 | 22 |
| mmu04020 | Calcium signaling pathway | 3.85E-05 | 40 |
| mmu04726 | Serotonergic synapse | 9.49E-05 | 26 |
| mmu04080 | Neuroactive ligand-receptor interaction | 0.000330284 | 50 |

(Continued)
| KEGG ID   | Description                                   | Adjusted p-value | Number of gene | Gene ID                                                                 |
|----------|-----------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| mmu04010 | MAPK signaling pathway                        | 0.000387987      | 43             | Il1r1/Ereg/Cacna2d1/Dusp5/Fgfr1/Cacna1h/Hspa2/Igf2/Casp3/Flna/Mapk8p2/  |
|          |                                               |                  |                | Cacnb2/Pdgfra/Mapt8p1/Anpgr2/Anpgr1/Pgf/Nr4a1/Ptpn5/Pdgfrb/Rasgrf2/Rasgrf1/  |
|          |                                               |                  |                | /Tfii/Tgfß3/Etna5/Map3k8/Ptpn7/Pdgfrb/Ngfr/Cacna1c/His1p1/Fasl/Mapk10/Tgfß2/  |
|          |                                               |                  |                | Kdr/Pdgfic/Ntrk2/Tfii10/Cacng7/Tfii4/Hgfr/Igf1                          |
| mmu04658 | Th1 and Th2 cell differentiation              | 0.000415763      | 19             | H2-DMb1/H2-DMa/H2-Asfng/H2-Ab1/H2-Eb1/Tbx21/Ii12rb2/Cd3g/Lck/Ii12rb1/Cd247/Lat/Cd3d/Matf  |
| mmu04659 | Th17 cell differentiation                     | 0.000437294      | 21             | H2-DMb1/H2-DMa/H2-Asfng/H2-Ab1/Ii11r1/H2-Eb1/Tbx21/Cd3g/Lck/Ii12rb1/Cd247/Lat/Cd3d/Ii12r2/Cd3e/Mapk10/Ii17d |
| mmu04713 | Circadian entrainment                         | 0.000510163      | 20             | Adcy5/Nox1/Cacna1h/Ryr2/Guyc1b1/Prkga1/Gnq4/Mtnr1a/Guyc1a1/Gnq1/Gnq3/Gna1/Cacna1c/Gna1/Gria/Kcnj5/Adcyap1r1/Prkga2/Kcnj3 |
| mmu04360 | Axon guidance                                 | 0.000510163      | 30             | Myo9/Slit3/Nfasc4/Omp7/Ntn4/Trpc4/Sema3a/Ntng1/Pgma/Pard6g/Etna5/Pak5/Epha5/  |
| mmu05144 | Dilated cardiomyopathy                        | 0.0008335        | 19             | Adcy5/Cacna2d1/Iggb8/Ry2/Igga7/Cacnb2/Iggb8/Iggb7/Iggb3/Cacna1c/Slc8a2/  |
|          |                                               |                  |                | /Igga9/Iggb7/Pfn/Cacng7/Agf/Igga8/Igf1                                     |
| mmu05145 | Toxoplasmosis                                 | 0.0008335        | 21             | H2-DMb1/H2-DMa/H2-Asfng/Citga/H2-Ab1/H2-Eb1/Lamc1/Lamb1/Hspa2/Casp3/  |
| mmu05146 | Amoebiasis                                    | 0.001461118      | 20             | Ifng/Col4a1/Ii1r1/Cctc1/Col4a2/Lamc1/Lamb1/Casp3/Lam4/Socs1/Ifng/Tgfß3/Alox5/Gna1/Gna1/Irgm2/Mapk10/Tgfß2 |
| mmu05032 | Morphine addiction                            | 0.001461118      | 18             | Adcy5/Pde1c/Gnq4/Gabrg3/Gabrbr3/Adora1/Gnkr3/Pde1a/Gnkr11/Gnq3/Gna1/Gna1  |
| mmu05410 | Hypertrophic cardiomyopathy                   | 0.001461118      | 18             | Cacna2d1/Iggb8/Ry2/Igga7/Cacnb2/Iggb8/Iggb7/Iggb3/Cacna1c/Slc8a2/Igga9/  |
| mmu04014 | Ras signaling pathway                         | 0.001541131      | 34             | Fgfr7/Fgfr1/Iggb7/Pla2g3/Pdgfra/Gnq4/Angpt2/Lat/Angpt1/Pgf/Pdgfrb/Rasgrf2/Rasgrf1/  |
| mmu05321 | Inflammatory bowel disease                   | 0.001645414      | 14             | H2-DMb1/H2-DMa/H2-Asfng/H2-Ab1/Ih2-Eb1/Tbx21/Ii12rb2/Ii12rb1/Iat/Tgfß3/  |
| mmu05142 | Arrhythmogenic right ventricular cardiomyopathy| 0.001645414     | 16             | Gq/a1/Cacna2d1/Iggb8/Ry2/Igga7/Cacnb2/Iggb7/Iggb1/Calma2/Iggb3/Cdfh2/Cacna1c/  |
| mmu04933 | AGE-RAGE signaling pathway in diabetic complications | 0.001648002 | 19             | Col4a1/Map2/Serpine1/Col4a2/Casp3/Col3a1/Nox1/Tgfß3/Agtr1a/Thbxd/Egr1/Mapk10/  |
| mmu04940 | Type 1 diabetes mellitus                      | 0.001668886      | 15             | H2-DMb1/H2-DMa/H2-Asfng/Gzmib/Cpe/H2-Ab1/Prf1/H2-Eb1/H2-Q7/Ptpn1/H2-  |
| mmu05332 | Graft-versus-host disease                     | 0.001668886      | 14             | H2-DMb1/H2-DMa/H2-Asfng/Gzmib/H2-Ab1/Prf1/H2-Eb1/H2-Q7/H2-Q6/Kfcr/  |
| mmu05990 | Arachidonic acid metabolism                   | 0.001668886      | 17             | Ptgls1/Pla2g3/Gf1/Cyp2c66/Alox5/Ptgls2/Cyp2c68/Cyp2e1/Ltc4s/Gpx3/Gpx3/  |

(Continued)
| KEGG ID   | Description                                | Adjusted p-value | Number of gene | Gene ID                                                                 |
|-----------|--------------------------------------------|------------------|----------------|-------------------------------------------------------------------------|
| mmu04640  | Hematopoietic cell lineage                 | 0.001666886      | 18             | H2-DMb1/H2-DMa/H2-Aa/H2-Ab1/1f11r1/Csf5/H2-EB1/Cd8a/Cd8b1/Cd3g/Tgα5/Il1rα/Cd3d1/Il1/tgb3/Cd3e/Il6/Cd2 |
| mmu04022  | cGMP-PKG signaling pathway                 | 0.001928048      | 27             | Rgs2/Kcnj8/Adcy5/Myl9/Gucy1b1/Prkg1/Ednra/Atptb4/Ntac4/Atpt1b2/βdkrb2/ |
| mmu04062  | Chemokine signaling pathway                | 0.001994739      | 29             | Cxcl9/Adcy5/Cxcl1/Cxcr6/Cxcl11/Cxcl2/Cxcl14/Gng3/Ppbd/Cxcl5/Cxcl3/Grk3/ |
| mmu04270  | Vascular smooth muscle contraction         | 0.002731842      | 23             | Adcy5/Myl9/Gucy1b1/Prkg1/Myh11/Ednra/Atptb2/Calcβ1/Mrf1/Ppp2r12b/Ramp3/ |
| mmu05143  | African trypanosomiasis                    | 0.003357978      | 10             | Hba-a2/Hbb-bt/Hbb-bs/Hba-a1/Ifng/Lama4/Il6/Itgb3/Atr1a/Gnai1/ |
| mmu04924  | Renin secretion                            | 0.003357978      | 15             | Adcy5/Atpq1/Gucy1b1/Pde1c/Ednra/C1ca1/Gucy1a/Ad/ora1/Pde1a/Agtr1a/ |
| mmu05142  | Chagas disease                             | 0.004334336      | 18             | Ifng/Serpin1/Cd3g/βdkrb2/Ppdr2b2/Cd2747/Cd3d7/Gna1/Gnai1/Fasl/   |
| mmu05165  | Human papillomavirus infection             | 0.004334336      | 45             | Thbs1/C1Ra1/Ctn/H2-Q7/Tmbx/Cd4a2/Itgb8/Lamc1/Lamb1/Tgα7/Casp3/Lama4/H2- |
| mmu04390  | Hippo signaling pathway                    | 0.00461878       | 24             | Serpin1/Nkd1/Wnt1/Rmdd/Impr7/Ppdr2b2/Itgb2/Lats2/Nkod2/Fzd1/D4/Pdand6/ |
| mmu04015  | Rap1 signaling pathway                     | 0.00476026       | 30             | Thbs1/Adcy5/Grf1/Fgfr1/Ptp2/Atp2r1b/Itgb2/Itgb2/Itgb2/Itgb2/Itgb2/ |
| mmu05205  | Proteoglycans in cancer                    | 0.004814656      | 29             | Thbs1/Mmp2/Fgfr1/Dbp3/Casp3/Gpc3/Mmp9/Fna1/Tgα5/Ppdr12b/   |
| mmu04612  | Antigen processing and presentation        | 0.006298916      | 16             | Cd74/H2-DMb1/H2-DMa/H2-Aa/Ifng/Ciita/H2-Ab1/H2-EB1/H2-Q7/Cd8a/Lgmm/Cd8b1/Hspa2/Tap1/H2-Q6/Knc1 |
| mmu04926  | Relaxin signaling pathway                  | 0.009364211      | 20             | Col4a1/Adcy5/Mmp2/Nos1/Col4a2/Mmp9/Mmp13/Gng4/Ins3/Gnai1/Col4a1/Edrnr/ |
| mmu04725  | Cholinergic synapse                        | 0.010129103      | 18             | Adcy5/Slc18a3/Chnma3/Grkn4/Grkn3/Knc12/Chmb4/Gng4/Chat/Gnai1/Grkn3/ |
| mmu04350  | TGF-beta signaling pathway                 | 0.010577012      | 16             | Ifng/Thbs1/Nb1b/Itnbbb/Dcrn/Fst/Itbp1/Grnm2/Bmpl/Grma/Fbn1/Tgb3/   |
| mmu04724  | Glutamatergic synapse                      | 0.010726962      | 18             | Adcy5/Gnrr3a/Gnkn3/Gng4/Grkn3/Gnai1/Shc4/Mapk10/Itgb2/Col4a5/   |
| mmu05330  | Allograft rejection                        | 0.012447412      | 12             | H2-DMb1/H2-DMa/H2-Aa/Ifng/Gzmmb/H2-Ab1/Ppp1r1b1/H2-Q7/H2-Q6/Fasl/Cd28 |
| mmu04261  | Adrenergic signaling in cardiomyocytes     | 0.012463153      | 22             | Adcy5/Cnca2d1/Ryr2/Sncn7a/Atptb4/Cacnb2/Atptb2/Ppdr2b2/Atptb3/Csn5a/ |
| mmu00591  | Linoelic acid metabolism                   | 0.018463113      | 10             | Cyp3a44/Cyp3a25/Cyp2a3/Cyp2c66/Cyp2c68/Cyp2c81/Alox15/Cyp2c55/Cyp2c65/ |
| mmu04610  | Complement and coagulation cascades        | 0.019839992      | 15             | Serpin1/βdkrb1/Fga/βdkrb2/tgb2/Pro1/Thbd1/Tpx/Cb/F10/C7/Pat/C2/Fggr1F13a1 |

(Continued)
on DSS-induced colitis, a comparative transcriptomic analysis was conducted, followed by bioinformatic analysis. Deep sequencing of RNA libraries derived from control and treatment groups each generated at least 43 million quality-trimmed clean reads (Supplementary Table 1); the read utilization ratio was over 97%. A total of 110 Gb quality-trimmed bases were obtained from transcriptome sequencing (Supplementary Table 1). Over 90% of the sequencing reads could be mapped to the exonic regions of the mouse reference genome (Supplementary Table 2). In the comparative transcriptomic analysis, a total of 1,627 differentially expressed genes (DEGs), including 1,326 upregulated and 301 downregulated genes, were identified in the DSS-induced colitis mice as compared to the control group mice (Figure 1A and Supplementary Table 3). The DEGs were then subjected to GO and KEGG enrichment analyses to understand the alteration of biological functions and signaling pathways in the DSS-induced colitis model. GO results suggested that numerous biological processes, cellular components, and molecular functions were altered in the DSS-induced colitis model. In the analysis of biological processes, we found the activation of numerous inflammatory processes (Figure 1B and Table 1). The KEGG pathway analysis further highlighted the alteration of pathways related to immune and inflammatory responses, including cytokine-cytokine receptor interaction, inflammatory bowel disease (IBD), IL-17 signaling pathway, leukocyte transendothelial migration, Toll-like receptor signaling pathway, and intestinal immune network for IgA production (Figure 1C and Table 2).

### Mesalazine Triggered Immunosuppressive Response in DSS-Induced Colitis

A similar approach was used to determine the immunosuppressive effect of mesalazine on DSS-induced colitis. When we compared the DSS group and DSS + mesalazine group, we identified 1,663 DEGs, of which 262 were upregulated and 1,401 were downregulated (Figure 2A and Supplementary Table 4). In the bioinformatic analysis, we primarily focused on the GO terms and pathways related to immune responses. The results of GO analysis showed that low-dose mesalazine treatment altered different immune processes. This could be due to the regulation of different immune cell types, such as T cells, B cells, natural killer cells, and leukocytes (Figure 2B and Table 3). This results in the regulation of cytokine functions such as cytokine biosynthetic process, cytokine production, cytokine secretion, and cytokine binding (Figure 2B and Table 3), leading to alterations in the immune response of the gut system (Figure 2B and Table 3). The results of KEGG pathway analysis further highlighted the immune-responsive pathways such as ECM-receptor interaction, T-cell receptor signaling pathway, TNF signaling pathway, cytokine-cytokine receptor interaction, chemokine signaling pathway, IL-17 signaling pathway, Th1, Th2, and Th17 cell differentiation, IBD, Hippo signaling

| Table 4 | Continued |
|---|---|
| Gene ID | Number of gene |
| adjusted | p-value |
| mmu04100 | T-cell receptor signaling pathway | 16 |
| mmu0430 | Regulation of actin cytoskeleton | 28 |
| mmu0430 | NF-kB signaling pathway | 17 |
| mmu0430 | Gap junction | 14 |
| mmu0430 | Glycogen metabolism | 5 |
| mmu0430 | Cytokine-cytokine receptor interaction | 21 |
| mmu0430 | Cytokine production | 19 |
| mmu0430 | Cytokine secretion | 22 |
| mmu0430 | Cytokine receptor binding | 6 |

**TABLE 4** KEGG ID Description

- mmu04100: T-cell receptor signaling pathway
- mmu0430: Regulation of actin cytoskeleton
- mmu0430: NF-kB signaling pathway
- mmu0430: Gap junction
- mmu0430: Glycogen metabolism
- mmu0430: Cytokine-cytokine receptor interaction
- mmu0430: Cytokine production
- mmu0430: Cytokine secretion
- mmu0430: Cytokine receptor binding

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**TABLE 1**

| KEGG ID | Description |
|---|---|
| mmu04100 | T-cell receptor signaling pathway |
| mmu0430 | Regulation of actin cytoskeleton |
| mmu0430 | NF-kB signaling pathway |
| mmu0430 | Gap junction |
| mmu0430 | Glycogen metabolism |
| mmu0430 | Cytokine-cytokine receptor interaction |
| mmu0430 | Cytokine production |
| mmu0430 | Cytokine secretion |
| mmu0430 | Cytokine receptor binding |

---

**FIGURE 1A**

**FIGURE 1B**

**FIGURE 1C**

**FIGURE 2A**

**FIGURE 2B**

**FIGURE 2C**

---

**TABLE 2**

| KEGG ID | Description |
|---|---|
| mmu04100 | T-cell receptor signaling pathway |
| mmu0430 | Regulation of actin cytoskeleton |
| mmu0430 | NF-kB signaling pathway |
| mmu0430 | Gap junction |
| mmu0430 | Glycogen metabolism |
| mmu0430 | Cytokine-cytokine receptor interaction |
| mmu0430 | Cytokine production |
| mmu0430 | Cytokine secretion |
| mmu0430 | Cytokine receptor binding |

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| KEGG ID | Description |
|---|---|
| mmu04100 | T-cell receptor signaling pathway |
| mmu0430 | Regulation of actin cytoskeleton |
| mmu0430 | NF-kB signaling pathway |
| mmu0430 | Gap junction |
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| mmu0430 | Glycogen metabolism |
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| mmu0430 | Cytokine production |
| mmu0430 | Cytokine secretion |
| mmu0430 | Cytokine receptor binding |

---

**FIGURE 1A**

**FIGURE 1B**

**FIGURE 1C**

**FIGURE 2A**

**FIGURE 2B**

**FIGURE 2C**

---

**TABLE 2**

| KEGG ID | Description |
|---|---|
| mmu04100 | T-cell receptor signaling pathway |
| mmu0430 | Regulation of actin cytoskeleton |
| mmu0430 | NF-kB signaling pathway |
| mmu0430 | Gap junction |
| mmu0430 | Glycogen metabolism |
| mmu0430 | Cytokine-cytokine receptor interaction |
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| mmu0430 | Cytokine receptor binding |

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**TABLE 3**

| KEGG ID | Description |
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| mmu04100 | T-cell receptor signaling pathway |
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**TABLE 4**

| KEGG ID | Description |
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| mmu0430 | Regulation of actin cytoskeleton |
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| mmu0430 | Cytokine-cytokine receptor interaction |
| mmu0430 | Cytokine production |
| mmu0430 | Cytokine secretion |
| mmu0430 | Cytokine receptor binding |

---

**FIGURE 1A**

**FIGURE 1B**

**FIGURE 1C**

**FIGURE 2A**

**FIGURE 2B**

**FIGURE 2C**

---

**TABLE 2**

| KEGG ID | Description |
|---|---|
| mmu04100 | T-cell receptor signaling pathway |
| mmu0430 | Regulation of actin cytoskeleton |
| mmu0430 | NF-kB signaling pathway |
| mmu0430 | Gap junction |
| mmu0430 | Glycogen metabolism |
| mmu0430 | Cytokine-cytokine receptor interaction |
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| mmu0430 | Cytokine secretion |
| mmu0430 | Cytokine receptor binding |

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**TABLE 3**

| KEGG ID | Description |
|---|---|
| mmu04100 | T-cell receptor signaling pathway |
| mmu0430 | Regulation of actin cytoskeleton |
| mmu0430 | NF-kB signaling pathway |
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| mmu0430 | Cytokine receptor binding |

---

**TABLE 4**

| KEGG ID | Description |
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| mmu0430 | Regulation of actin cytoskeleton |
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| mmu0430 | Glycogen metabolism |
| mmu0430 | Cytokine-cytokine receptor interaction |
| mmu0430 | Cytokine production |
| mmu0430 | Cytokine secretion |
| mmu0430 | Cytokine receptor binding |
| Ingenuity Canonical Pathways | Adjusted p-value | Molecules |
|-----------------------------|-----------------|-----------|
| Agranulocyte Adhesion and Diapedesis | 4.46684E-09 | AOC3,CCL11,Cc2l2,CCL21,CCL22,CC15,Cc7,CDH5,Ccxl11, Ccxl2,Ccxl3,Ccxl6,Ccxl9,Ccpr2,FN1,GNAI1,IL1R1, IL33,ITG5A,ITGB2,ITGB7,JAM3,MMP10,MMP13,MMP19, MMP2,MMP3,MMP7,MMP9,MYH10,MYH11,MYI9,PF4, PODXL2,Prppb,SEP1 |
| Th1 and Th2 Activation Pathway | 7.07946E-09 | CD247,CD28,CD3D,CD3E,CD3G,CD68,CXCR3,CXCR6,HLA-A, HLA-DMA,HLA-DMB,HLA-DQA1,HLA-DQB1,HLA-DRB5,FNG,IKF2F1, IL12B1,IL12B2,IL1R1,IL2RB,IL3L3,IL6,IRF1,ITGB2,ITG2, KL2,RC1,MAF,NFATC4,S1PR1,SOCS1,SOCS3,TBX21,TGFBR3,TNFSF11 |
| Th1 Pathway | 3.16228E-07 | CD247,CD28,CD3D,CD3E,CD3G,CD68,CXCR3,CXCR6,HLA-A,HLA-DMA,HLA-DMB,HLA-DQA1,HLA-DQB1,HLA-DRB5,FNG, IL12B1,IL12B2,IL1R1,IL2RB,IL3L3,ITGB2,ITG2,JAM3, KL2,RC1,MAF,NFATC4,S1PR1,SOCS1,SOCS3,TBX21,TGFBR3,TNFSF11 |
| Th2 Pathway | 8.91251E-07 | CD247,CD28,CD3D,CD3E,CD3G,CXCR6,HLA-A,HLA-DMA,HLA-DMB,HLA-DQA1,HLA-DQB1,HLA-DRB5,FNG,IKF2F1,IL12B1,IL12B2,IL1R1,IL2RB,IL3L3,ITGB2,ITG2,JAG2,MAF, S1PR1,SOCS3,TBX21,TGFBR3 |
| Antigen Presentation Pathway | 5.12861E-06 | CD74,CIITA,HLA-A,HLA-DMA,HLA-DMB,HLA-DQA1,HLA-DQB1, HLA-DRB5,FNG,FSMB8,PSMB9,TAP1 |
| T Helper Cell Differentiation | 7.58578E-05 | CD28,HLA-A,HLA-DMA,HLA-DMA-DMB,HLA-DQA1,HLA-DQB1, HLA-DRB5,FNG,IL12B1,IL12B2,IL6,IL6ST,NFGR,TBX21,TNFSF11 |
| Neuroinflammation Signaling Pathway | 0.0001 | CALB2,CASP3,CD200,FASLG,FZD1,GABRA3,GABRB3, GABRG3,GAD2,GNF,GRIA1,GRIN3A,HLA-A,HLA-DMA,HLA-DMB, HLA-DQA1,HLA-DQB1,HLA-DRB5,ITGB2,JAG2,KL2,MAF, NFATC4,S1PR1,SOCS1,SOCS3,TBX21,TGFBR3,TNFSF11 |
| Cardiac ß-adrenergic Signaling | 0.000169824 | ADCYS,AKAP12,AKAP6,CANCA1,CNG11,GNG33,GNG34,GNG3, PDE1A,PDE1C,PDE1B,PDE8A,PKB1,PKB3,PKB2,PKB2B, PPK2F2,PPE2,FMPK3,PKR2,SLC9A2,SMPDL3B |
| IL-15 Production | 0.000389045 | AATK,AXL,DDR2,EPH5A,FGR,FRT1,FLT4,JUL,FRI,TK,KD,KD, LCK,NTK2,NTK3,PDGFR,PGDFB,RET,ROR2,TIE1 |
| CCR5 Signaling in Macrophages | 0.000436516 | CACNA1C,CACNA1H,CACNB2D,CACNG7,CCL5,CD247, CD3D,CD3E,CD3G,CD68,FASLG,GNAI1,GNG31,GNG33,GNG4,MAPK10 |
| IL-17 Signaling | 0.000660693 | CCL11,CCL22,CHEPP,CSF3,CR3,C3,Eda,FASLG,FNG,IL1,IL17D, IL33,IL8,LCN2,MAPK10,MM13,MP13,MP2,MP3,MP9,PGDFC, PFG,TGFB2,TGFBR2,TGFBR3,TNFRSF11B,TNFSF11 |
| iCOS-iCOSL Signaling in T Helper Cells | 0.000123293 | CD247,CD28,CD3D,CD3E,CD3G,HLA-A,HLA-DMA,HLA-DMB, HLA-DQA1,HLA-DQB1,HLA-DRB5,ITGB2,ITK,LAT,LCK, NFATC4,PLEKHA4 |
| Leukocyte Extravasation Signaling | 0.001047129 | APBGAP6,ARHAP8,PRPS-AEP-HAP8,CD45,CTNNA2,DLCL1,EDIL3, GNA11,IT2K,ITKJAM2,JAM3,MAPK10,MM10,MP13,MM19, MMP2,MMP3,MMP7,MMP9,NOX1,RAPGEF4,RASGRP1, SPN,TIMP1,TIMP2 |
| Calcium-induced T Lymphocyte Apoptosis | 0.001230269 | CD247,CD3D,CD3E,CD3G,HLA-A,HLA-DMA,HLA-DMB, HLA-DQA1,HLA-DQB1,HLA-DRB5,ITGB2,ITK,LAT,LCK, NFATC4,PLEKHA4 |
| Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells | 0.001412538 | CASP3,CD247,CD3D,CD3E,CD3G,FASLG,HLA-A,PRF1 |
| ILK Signaling | 0.001819701 | CASP3,FERMT2,FLNA,FN1,ITGB2,ITGB3,ITGB7,ITGB8,ITGMS2, MAPK10,MM9,PXY10,MYH10,MYH11,MY3,PGDFC,PGF,PPR2P2B, PPR2P2P,PPR2R3,PTG522,RHOC,RND2,SNA11,TGFBR11 |
| Acute Phase Response Signaling | 0.001949845 | AG1,A2C2,CEBPA,CPA,FGB,FNG,FN1,HPIL1,IL13L,IL6ST,NFGR, RBP1,SA11,SA3,SEMP3A,SEMP3E1,SOCS1,SOCS2,SOCS3, TF,TNFRSF11B |
| Interferon Signaling | 0.002089026 | IFIT1,IFIT3,IFITM2,FNG,IRF1,PSMB8,SOCS1,TAP1 |
| PKCε Signaling in T Lymphocytes | 0.003311311 | CACNA1C,CACNA1H,CACNB2D,CACNG7,CD247,CD28, CD3D,CD3E,CD3G,HLA-A,HLA-DMA,HLA-DMB,HLA-DQA1, HLA-DQB1,HLA-DRB5,LAT,LCK,MAPKG9,NFATC4 |
| Autoimmune Thyroid Disease Signaling | 0.004468836 | CD28,FASLG,HLA-A,HLA-DMA,HLA-DMB,HLA-DQA1,HLA-DQB1, HLA-DRB5,PRF1 |

(Continued)
TABLE 5 | Continued

| Ingenuity Canonical Pathways | Adjusted p-value | Molecules |
|-----------------------------|------------------|-----------|
| CTLA4 Signaling in Cytotoxic T Lymphocytes | 0.005754399 | CD247, CD28, CD3D, CD3E, CD3G, CD8A, CD8B, HLA-A, LAT, LCK, PPP2R2B, PPP2R2C, PPP2R3A |
| CD28 Signaling in T Helper Cells | 0.006309573 | CD247, CD28, CD3D, CD3E, CD3G, HLA-A, HLA-DMB, HLA-DQA1, HLA-DRB1, HLA-DRB5, ITK, LAT, LCK, MAPK10, NFATC4 |
| Altered T Cell and B Cell Signaling in Rheumatoid Arthritis | 0.006309573 | CCL2, CD28, FASLG, HLA-A, HLA-DMB, HLA-DQA1, HLA-DRB1, HLA-DRB5, IFNG, IL33, IL6, TNSF11 |
| B Cell Development | 0.00851138 | HLA-A, HLA-DMB, HLA-DQA1, HLA-DRB1, HLA-DRB5, SPN |
| Role of IL-17A in Psoriasis | 0.011220185 | CXCL3, CXCL6, S100A8, S100A9 |
| Leukotriene Biosynthesis | 0.011220185 | ALOX5, GGT1, GGT5, LTC4S |
| Antiproliferative Role of TOB in T Cell Signaling | 0.013182567 | CD247, CD28, CD3D, CD3E, CD3G, TGFB2, TGFB3 |
| IL-8 Signaling | 0.013803843 | ANGPT1, ANGPT2, CXCXR2, FLT1, FLT4, GNA1, GNA3, GNA11, GNG3, GNG4, ITGB2, ITGB3, KDR, MAPK10, MPP2, MPP9, MYL9, NOX1, PDGFC, PGF, PTPG2S2, RHOJ, RND2 |
| Role of NFAT in Regulation of the Immune Response | 0.017378008 | CD247, CD28, CD3D, CD3E, CD3G, GNA1, GNA10, GNA11, GNG3, GNG4, HLA-A, HLA-DMB, HLA-DQA1, HLA-DRB5, ITK, LAT, LCK, NFATC4 |
| Role of JAK family kinases in IL-6-type Cytokine Signaling | 0.022387211 | IL6, IL6ST, MAPK10, SOCS1, SOCS3 |
| T Cell Receptor Signaling | 0.023442288 | CD247, CD28, CD3D, CD3E, CD3G, CD8A, CD8B, ITK, LAT, LCK, NFATC4, PTPN7, RASGRF1 |
| T Cell Exhaustion Signaling Pathway | 0.023442288 | CD28, HLA-A, HLA-DMB, HLA-DQA1, HLA-DRB1, HLA-DRB5, IFNG, IL12RB1, IL12RB2, IL6, KDR, MAPK10, NFATC4, PPP2R2B, PPP2R2C, PPP2R3A, TXB2, TGFBR3 |
| Nur77 Signaling in T Lymphocytes | 0.035481339 | CASP3, CD247, CD28, CD3D, CD3E, CD3G, FASLG, HDAC9, NR4A1 |
| IL-6 Signaling | 0.039810717 | CEBPB, HSPPB1, IL1R1, IL1R1, IL1RL1, IL6, IL6ST, MAPK10, NGFR, SOCS1, SOCS3, TFAP2E, TNSF11B |

FIGURE 3 | The gene network involved in the immunosuppressive effect of mesalazine on DSS-induced colitis. Ingenuity pathway analysis shows the contribution of different molecules in the immunosuppressive effect of mesalazine. The red symbol represents upregulated genes; the green shape represents downregulated genes.
Immunosuppressive Effect of Mesalazine via the Regulation of Canonical Pathway

The DEGs were then subjected to IPA to determine the immunosuppressive effect of mesalazine on the treatment of UC. The results of canonical pathway analysis highlighted the substantial alteration of a large number of cell signaling pathways related to immune and inflammatory responses ($p < 0.05$) (Table 5). They included the regulation of interleukin signaling, such as IL-6, IL-8, IL-15, and IL-17 signaling pathways (Table 5). Furthermore, the results highlighted T cell differentiation, including Th1, Th2, and T helper cells (Table 5). In addition to the T cells, we also observed the regulation of B cells by mesalazine treatment (Table 5). Finally, we conducted IPA gene network analysis to delineate the detailed molecular mechanisms underlying the immunosuppressive responses caused by mesalazine. Our results showed that mesalazine regulated a large number of C–C motif chemokine ligands (CCL5, CCL7, CCL11, and CCL21) and C–X–C motif chemokine ligands (CXCL3, CXCL6, CXCL9, and CXCL11) in the extracellular space (Figure 3). This led to the modulation of a group of transmembrane receptors, including interleukin receptors (IL2RB, IL12RB1, and IL12RB2), T-cell surface glycoprotein (CD3E, CD3G, CD28, and CD247), and chemokine receptors (CXCR2 and CXCR3) (Figure 3). The regulation of these receptors has been reported to regulate different enzymes (PDE1A, PDE1B, PDE1C, and MYH10) and kinases (ITK, LCK, NOX1, and MAP3K8) involved in immune responses (Figure 3). More importantly, mesalazine treatment reversed the suppressive effect of immune responsive genes, including chemokine (C–X–C motif) ligand (CXCL3), chemokine (C–C motif) ligand (CCL11 and CCL21), chemokine receptor (CXCR2), and colony-stimulating factor (CSF3) in the DSS-induced colitis model (Table 6). Taken together, our results suggest that mesalazine treatment could reverse the DSS-induced immune response through its immunosuppressive effect.

In the later part of this study, we aimed to determine the molecular pathways underlying the immunosuppressive effect of mesalazine on DSS-induced colitis. Therefore, we focused on immune-related biological processes and pathways in bioinformatic analysis. Our results show that mesalazine treatment can alter different immune cells, including T cells, B cells, and natural killer cells, leading to the control of autoimmune responses (Zitti and Bryceson, 2018; Meffre and O’Connor, 2019; Wing et al., 2019). T cells are reported to contribute to the pathogenesis of chronic intestinal inflammation and are considered targets for UC treatment due to their role in the autoimmune system (Eri et al., 2012). For example, a study using a T cell transfer colitis model demonstrated that CD4+ T helper cells strongly contributed to the pathogenesis of IBD (Maschmeyer et al., 2021). In addition, imbalanced CD4+ T cells have been reported as predisposing factors for colitis (Pandit et al., 2021). Furthermore, mesalazine treatment mediated cytokine biosynthesis and secretion. As mentioned before, cytokines play an important role in the autoimmune response. More importantly, our gene network analysis further highlighted the suppression of C–C motif chemokine ligands (CCL11 and CCL21) and C–X–C motif chemokine ligands (CXCL3 and CXCR2) by mesalazine treatment. All of these ligands were increased in the DSS-induced model. CCL11, a prototypical Th2 chemokine, is associated with enrichment in Th2 CD4+ T cells (Chao et al., 2014) and is involved in eosinophil recruitment (Hornig et al., 2016). CCL11 expression levels correlated with the expression levels of different ILs. Reduced expression of CCL11 has been reported to alleviate allergic symptoms and allergic inflammatory responses by reducing serum cytokine levels (Lei et al., 2020). It has been reported that CD4+ CD25+ T cells inhibit the development of colitis induced by both Th1 and Th2 cells in a mouse model (Xu et al., 2003). The Th1/Th2 balance of peripheral CD4-positive T cells is considered a biomarker for patients with refractory ulcerative colitis (Nakase et al., 2009). CCL21, a ligand of the receptor CCR7, contributes to the balance of immunity and tolerance through thymocyte development, secondary lymphoid organogenesis, high affinity antibody responses, and regulatory and memory T-cell function ( Förster et al., 2008; Comerford et al., 2013). It has been shown that the loss of CCL21 responsiveness in the normal development of the memory T-cell effector function does not hold for autoimmune diseases (Christopherson et al., 2003). A mouse colitis study demonstrated that CCL21 suppression could decrease damage induced by ulcerative colitis (Zhang et al., 2014), suggesting that CCL21 might be a therapeutic target for UC treatment.

Anti-colitis Actions of Mesalazine in vivo

To determine the pharmacological effects of mesalazine against colitis, the structure of the colon tissues was studied. Our results showed that the length of the colon was shortened and colon tissues became fragile in the colitis study using a T cell transfer colitis model demonstrated that CCL21 suppression could contribute to the pathogenesis of chronic intestinal inflammation and are considered targets for UC treatment due to their role in the autoimmune system (Eri et al., 2012). For example, a study using a T cell transfer colitis model demonstrated that CD4+ T helper cells strongly contributed to the pathogenesis of IBD (Maschmeyer et al., 2021). In addition, imbalanced CD4+ T cells have been reported as predisposing factors for colitis (Pandit et al., 2021). Furthermore, mesalazine treatment mediated cytokine biosynthesis and secretion. As mentioned before, cytokines play an important role in the autoimmune response. More importantly, our gene network analysis further highlighted the suppression of C–C motif chemokine ligands (CCL11 and CCL21) and C–X–C motif chemokine ligands (CXCL3 and CXCR2) by mesalazine treatment. All of these ligands were increased in the DSS-induced model. CCL11, a prototypical Th2 chemokine, is associated with enrichment in Th2 CD4+ T cells (Chao et al., 2014) and is involved in eosinophil recruitment (Hornig et al., 2016). CCL11 expression levels correlated with the expression levels of different ILs. Reduced expression of CCL11 has been reported to alleviate allergic symptoms and allergic inflammatory responses by reducing serum cytokine levels (Lei et al., 2020). It has been reported that CD4+ CD25+ T cells inhibit the development of colitis induced by both Th1 and Th2 cells in a mouse model (Xu et al., 2003). The Th1/Th2 balance of peripheral CD4-positive T cells is considered a biomarker for patients with refractory ulcerative colitis (Nakase et al., 2009). CCL21, a ligand of the receptor CCR7, contributes to the balance of immunity and tolerance through thymocyte development, secondary lymphoid organogenesis, high affinity antibody responses, and regulatory and memory T-cell function ( Förster et al., 2008; Comerford et al., 2013). It has been shown that the loss of CCL21 responsiveness in the normal development of the memory T-cell effector function does not hold for autoimmune diseases (Christopherson et al., 2003). A mouse colitis study demonstrated that CCL21 suppression could decrease damage induced by ulcerative colitis (Zhang et al., 2014), suggesting that CCL21 might be a therapeutic target for UC treatment.
FIGURE 4 | In vivo effects of mesalazine treatment on DSS-induced colitis. (A) Hematoxylin and eosin staining show the structural changes including visible crypt abscess, inflammatory infiltration, and cytoclastic in DSS-induced colitis. These alterations were reversed by mesalazine treatment. (B) Mesalazine treatment relieved DSS-induced inflammatory cytokines including TNF-α, IL-1α, IL-6, and IL-8 in the colon samples via its anti-inflammation action.

mouse model, and ulcers were formed, including mucosal abscess and congestion. Mesalazine intervention improved pathological changes in mice with colitis. Histomorphological observations using H&E staining showed that the colon tissue of the colitis mice changed in morphology and showed alterations, such as crypt abscess, decreased density in the colon, and inflammatory infiltration. Mesalazine treatment mitigated mucosal structural changes and reduced inflammatory infiltration (Figure 4A). ELISA data showed that the expression levels of TNF-α, IL-1α, IL-6, and IL-8 in colitis mice were significantly reduced compared with those in control samples. Interestingly, the elevation of inflammatory cytokines in mesalazine-treated colitis mice was decreased (Figure 4B).

CXCL3, a small cytokine, controls the migration and adhesion of monocytes through its interaction with the cell surface chemokine receptor CXCR2 (Smith et al., 2005). CXCR2 is a functional receptor for GRO-family chemokines involved in monocyte recruitment and inhibits inflammation. CXCR2 has been found to be significantly increased in colonic mucosal tissues of patients with active UC (Zhu et al., 2020). It has been reported that CXCL3 plays a role in controlling intestinal inflammation and gut mucosal healing, and is considered a key prognostic parameter in the management of IBD (Stronati et al., 2019). Therefore, the suppressive effect of mesalazine on these C–C motif chemokine ligands and C–X–C motif chemokine ligands suggests a possible strategy for the treatment of UC, and a better understanding of the molecular mechanism underlying the immunosuppressive effect of mesalazine has important implications for developing novel anti-UC drugs directed against the CXC chemokine signaling system. However, the newly identified markers need to be further confirmed and investigated using a human model.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the BioProject repository, accession number PRJNA732984.

ETHICS STATEMENT

The animal study was reviewed and approved by West China School of Basic Medical Sciences and Forensic Medicine of Sichuan University.

AUTHOR CONTRIBUTIONS

RL and LZ conceived and designed the experiments and wrote the manuscript. RL, LC, and QW performed the experiments. RL analysed the data. All authors read and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2021.698983/full#supplementary-material
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