Immunogenic Cell Death involves in SARS-CoV-2 and Pulmonary arterial hypertension Crosstalk

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Abstract

Background: Pulmonary arterial hypertension (PAH) is a complication of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and that individuals with PAH are at substantial risk for rapid deterioration once infected. However, the underlying mechanism and genetic signatures of PAH and SARTS-CoV-2 remains unclear.

Methods: The PAH and SARS-CoV-2 datasets were downloaded from the Gene Expression Omnibus (GEO) database, immunogenic cell death (ICD) genes were extracted, and differential expression analysis was performed to obtain shared differentially expressed genes (DEGs). Meta-analysis, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, and protein-protein interaction (PPI) network analysis were performed on DEGs to observe functional enrichment of these genes and associations between genes. The disease prediction model was constructed by artificial neural network (ANN), and its accuracy was further evaluated by receiver operating characteristic (ROC) curve. Using Single-sample GSEA (ssGSEA) to evaluate the correlation between disease signature genes and immune cell abundance in the PAH dataset and the SARS-CoV-2 dataset. Finally, the screened drugs were predicted by DEGs, and were molecularly docked with DEGs by AutoDock Vina.

Findings: TLR4, MYD88, IL1B, and HMGB1 four shared genes were identified by ICD differential gene expression analysis. NF-kappa B signaling pathway, Toll-like receptor signaling pathway and NOD-like receptor signaling pathway closely related to inflammation were observed by the enrichment analysis of DEGs. The result of immune infiltration showed that these DEGs were involved in the changes of PAH and the immune microenvironment of SARS-CoV-2, and the same regulatory process existed in Macrophage, Monocyte, Central memory CD8 T cell, and Central memory CD4 T cell. Nitric oxide, Resveratrol, and Curcumin may promise drugs for the treatment of SARS-CoV-2 and PAH. Further verification of Resveratrol and Curcumin by molecular docking showed that Resveratrol and Curcumin have good docking activity with their target proteins.

Interpretation: Analogous molecular mechanism between PAH and SARS-CoV-2 from the perspective of ICD. The key genes IL1B, MYD88, TLR4 and HMGB1 involve in the immune cell response process, thus affecting the progression of these two diseases. These findings may provide novel directions for treatment of PAH and SARS-CoV-2.

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Keywords: PAH, SARS-CoV-2, immunogenic cell death, Artificial neural networks, immune cell infiltration.

1. Introduction

Coronavirus disease (COVID-19) is a persistent global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, which has brought a huge threat to human health and society significant economic losses (1-3). Most patients with SARS-CoV-2 have symptoms such as fever, cough, shortness of breath, and pneumonia (4, 5). SARS-CoV-2 has been reported to cause pneumonia, acute respiratory distress, and cardiovascular complications (6-8). Approximately 2.5% to 16% of SARS-CoV-2 patients suffer from cardiovascular diseases such as myocarditis, pulmonary embolism, etc. (9-11). This highlights the importance of this virus's effect on blood vessels as a risk factor for cardiovascular disease (12). Severe pulmonary hypertension(PH) has been found to be an important sequela of SARS-CoV-2 after severe acute illness (13).However, the mechanisms underlying the effects of SARS-CoV-2 infection in patients with cardiovascular comorbidities remain unclear, including PH (14-16).

Pulmonary arterial hypertension (PAH) is considered a progressive disease that is estimated to affect up to 100 million people worldwide (17). The main pathological features of PAH are endothelial dysfunction and pulmonary vascular remodeling leading to increased pulmonary vascular resistance and pulmonary pressure, clinically manifested as decreased cardiac output, right heart failure, and death (18). Immune cells play a key role in the development of PAH, which can promote the release of inflammatory factors during pulmonary vascular remodeling, leading to perivascular inflammation in the lung (19-22). T cells and B cells in lymphocytes migrate to the lungs during SARS-CoV-2 infection (23). Interestingly, the lungs from SARS-CoV-2 patients have a morphological pattern of perivascular lymphocytic infiltration (24), but peripheral blood lymphopenia (25). In addition, immune responses are associated with the severity of SARS-CoV2, and serum levels of pro-inflammatory cytokines also rise as disease severity progresses (26). Meanwhile, morphometric analysis shows that the thick of pulmonary artery walls from SARS-CoV-2 infected patients were twice thicker than those of H1N1 influenza patients (27). The rats infected with SARS-CoV-2 may develop features of PAH and pulmonary vascular remodeling or enhanced sensitivity to PAH triggers (27-29). As structural remodeling of pulmonary arterioles is a hallmark of PAH (30). Progression of PAH might be associated with SARS-CoV-2 infection.

Recently, the Committee on Cell Death Nomenclature defined immunogenic cell death (ICD) as "a form of regulated cell death (RCD) sufficient to activate an adaptive immune response in an immunocompetent isogenic host" (31). ICD can trigger innate and adaptive immune responses (32-34). They are accompanied by the exposure and release of numerous damage-associated molecular patterns (DAMPs), which positively influence the recruitment and activation of antigen-presenting cells (35-37). ICDs are major initiators of adaptive immunity in infectious and malignant diseases that lead to increased antigenicity and adjuvants (38). At present, ICD research involves oncology, including application in colon cancer prevention and treatment (39), research on the treatment of non-small cell lung cancer (40) and predicting the prognosis of head and neck squamous cell carcinoma and immunotherapy response, etc. (41). There are very few studies of ICD in non-neoplastic diseases, including SARS-CoV-2 and PAH. Key DAMPs of ICD include surface exposed calretinin (CRT) and heat shock protein (HSP) 70/90, secreted adenosine triphosphate (ATP) and passively released high mobility group box 1 (HMGB1) protein (42). In recent years, DAMPs have emerged as important inflammatory mediators (43).Among them, HMGB1 acts as a potent pro-inflammatory factor (44-46). Expression levels are elevated in both SARS-CoV-2 patients and male PAH (47-50). These may suggest that HMGB1 plays the similar role in SARS-CoV-2 and PAH pathology. The molecular chaperone HSP90 is directly involved in malignant growth and proliferation under stressful conditions and is involved in many physiological and pathological processes (51). Studies have found that

inhibition of HSP90 may improve pulmonary arterial remodeling in PH (52, 53). ATP is known to accumulate at sites of tissue injury or inflammation (54). Compared with healthy children, children with SARS-CoV-2 have higher plasma ATP concentrations (55), a pro-inflammatory signal (56). SARS-CoV-2 promotes the release of ATP, an important mediator of inflammation that promotes immune cell proliferation and T cell activation (57), worsening immune responses and impairing cardiorespiratory function.

In summary, there are plausible links between SARS-CoV-2 and PAH in terms of pathological features. Therefore, we hypothesize that ICD may be an important mechanism for the link of SARS-CoV-2 and PAH. This study aims to explore common molecular mechanisms between PAH and SARS-CoV-2 from the perspective of ICD by means of bioinformatics technology and machine learning. Ultimately, we seek to explore novel targets for the treatment of PAH and SARS-CoV-2.

2. Methods

2.1. Workflow overview

First, we downloaded the PAH (GSE703 and GSE131793) dataset (58) (59) and the SARS-CoV-2 (GSE157103) dataset (60)from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database. The dataset was batch corrected by the limma package to extract ICD genes. Then differential analysis was performed on ICD genes, and intersection was taken to obtain ICD shared differential genes. Meta and protein-protein interaction (PPI) network analysis was performed on the shared differential genes to observe the functional enrichment of these genes and the association between genes. To predict the likelihood of PAH and SARS-CoV-2, the respective corresponding weights of these DEGs in PAH and SARS-CoV-2 were further calculated by building an ANN model, which was further evaluated using ROC. Furthermore, we used the Single-sample GSEA (ssGSEA) algorithm to quantify the extent of immune cell infiltration in PAH and SARS-CoV-2. Finally, the proposed drugs were screened by DEGs genes, and the screened drugs were further verified by molecular docking. See the GRAPHICAL ABSTRACT.

2.2. Data download and preprocessing

The details of each GEO dataset are shown in Table.1. The GSE131793, GSE703 data were batch corrected and merged using the ComBat method in the sva package for R software (version 4.2.1) to reduce sample bias between different batches. ICD-related genes were obtained from the paper published by Garg et al. (61).

2.3. Identification of differentially regulated genes

After batch correction, we performed the extraction and analysis of ICD-related genes on the PAH and SARS-CoV-2 datasets using the "Limma" software package (62) to screen out the ICDs shared by PAH and SARS-CoV-2 differential genes.

2.4. Protein Interaction Network Analysis

String (vision 11.5) (http://string-db.org/cgi/input.pl) database is integrated known and predicted protein interactions (63).We used this database to perform protein interaction networks on differential genes and set the confidence level to 0.4 to obtain their interactions.

2.5. Gene functional enrichment analysis

We imported the screened differential genes into the Metascape database (64) for functional enrichment analysis. Terms with a P-value (P) < 0.01, a minimum count of 3, and an enrichment factor > 1.5 are collected and grouped into clusters based on their membership similarities. Meanwhile, Go and KEGG analyses were performed on the screened DEGs using the "cluster Profiler" R package (65). Set the screening threshold to p<0.05.

2.6. Construction of the artificial neural network model

Artificial neural network (ANN) is an arithmetic model that can process and mine the internal structure of complex data and has a reliable performance in clinical diagnosis (66). We use the "neuralnet" R package (67) to build artificial neural network models. We set 3 hidden layers as model parameters. The area under the ROC curve was calculated by the pROC software package (68) to evaluate the model diagnostic performance.

2.7. Immune Analysis

We calculated the relative abundance of 28 immune cells in each sample using ssGSEA analysis in R software. ssGSEA was performed using the "GSVA" package in R version 4.2.1. The selection of immune cell types was obtained from the paper published by Charoentong et al(69).

2.8. Drug prediction

Drug molecular identification is one of the important contents of this research. The Drug Signatures database (DSigDB) database was used to explore potential drug molecules that exhibited significant interactions with genes. The Enrichr (https://maayanlab.cloud/Enrichr/). Website provides a link to DsigDB.

2.9. Molecular docking

Molecular docking of the predicted Resveratrol and Curcumin binding the target protein. Small molecule ligands (Resveratrol, Curcumin) in SDF format were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). We used Chem3D software to convert SDF format to mol2 format file. The 3D structure of the target protein was downloaded from the PDB database (https://www.rcsb.org), and the water molecules and original ligands in the structure were removed by PyMOL software. Then, we imported the small molecule ligand and target protein into AutoDock Tools 1.5.7 to determine the active pocket and convert it to pdbqt format, and run AutoDock Vina through the CMD command character for molecular docking (70).Finally, the molecular docking results were visualized and analyzed using PyMOL software.

2.10. Statistical Analysis

All data processing was done in R 4.2.1 software. Depending on the data type, t-test and Wilcox Test were used. For each analysis, statistical significance was set at P < 0.05.

3. Results

3.1. Data preprocessing

To reduce the difference between batches, we use the limma package to eliminate batch effects. Figure.1A is the result of combining the PAH datasets GSE703 and GSE131793 without normalization. As shown in the figure, the expression values between the data are not uniform, and there are obvious

differences. Figure.1B is the result of normalization after combining the PAH datasets GSE703 and GSE131793. The results shows that the expression values between the data are normalized. Figure.1C is the normalized result of the SARS-CoV-2 dataset GSE157103. The results shows that there were significant differences in the expression values. Figure.1D is the normalized result of SARS-CoV-2 dataset GSE157103. The results shows that the expression values have been relatively uniform. The above results suggest that after the data is batch corrected, the differences between the data sets are significantly reduced, which can be used for subsequent analysis.

3.2. Identification of common differentially expressed genes

To observe the differential expression of ICD genes in PAH and SARS-CoV-2, we used the limma R software package to perform differential analysis. It was found that ICD genes showed significant differences between PAH samples and control samples, with red representing positive correlation and blue representing negative correlation (Figure.2A). Among them, compared with the control group, the expressions of HMGB1 and PRF1 were down-regulated in the PAH group, and the expressions of IL10, IL1B, ENTPD1, myeloid differentiation primary response 88 (MYD88), IFNGR1, and Toll-like receptor4 (TLR4) were up-regulated in the PAH group (Figure.2C). Likewise, as shown in Figure.2B, there is a clear difference between the SARS-CoV-2 samples and the normal samples. Among them, compared with the control group, BAX, CD4, FOXP3, IL1B, and TNF were down-regulated in SARS-CoV-2, while IL1R1, MYD88, IL17RA, PIL3CA, TLR4, HSP90AA1, EIF2AK3, HMGB1, ATG5 and CASP1 were up-regulated in SARS-CoV-2 (Figure.2D). Then we examined the intersection between datasets and obtained 4 intersection genes that were shared by PAH and SARS-CoV-2 patients (MYD88, TLR4, IL1B and HMGB1) (Figure.2E). The above analysis results shows that ICD plays a crucial role in the occurrence and development of PAH and SARS-CoV-2.

3.3. Gene functional enrichment analysis

The string (vision 11.5) database was used to analyze the PPI protein interaction network of the seven ICD differential genes. The results shows that the ICD differential genes formed an interactive regulatory network (Figure.3A). As shown in Figure 3B, this biological process is highly correlated with positive regulation of interleukin-8 production, MYD88 deficiency (TLR2/4), LTF danger signal response pathway, Interleukin-1 signaling, and regulation of interferon-gamma production. GO functional enrichment analysis (Figure.3C) indicated that DEGs were enriched in positive regulation of interleukin-8 production, positive regulation of interleukin-6 production chemokine production, interleukin-1 receptor binding. EGG pathway analysis (Figure.3D) shows that DEGs were concentrated in NF-kappa B signaling pathway, Toll-like receptor signaling pathway and NOD-like receptor signaling pathway. These results suggest that shared ICD differential genes play a significant role in immune responses.

3.4. Screening and identification of hub gene

To further construct a DEGs-specific scoring model, we constructed an artificial neural network model for the screened IL1B, MYD88, TLR4, and HMGB1 genes. The model has three layers, the input layer accepts input information of five variables, the hidden layer has three nodes, and the output layer has two nodes. Lines between nodes represent weights. As shown in Figure.4A, these genes were best differentiated between PAH, SARS-CoV-2, and controls. Figure.4B shows the weight prediction results of each gene in PAH and SARS-CoV-2, respectively. To evaluate the results of the neural network model more effectively, the area under the ROC curve was used to evaluate the model

diagnostic performance. As shown in Figure.4C, in both PAH and SARS-CoV-2 diseases, the area under the ROC curve is greater than 0.5, indicating that the model has good diagnostic performance.

3.5. Association of disease signature genes with immune infiltration

To further explore the immune differences between PAH patients, SARS-CoV-2 patients, and control patients, we used the ssGSEA algorithm to analyze the abundance of immune cells in different samples. Figures.5A and 5B reflected the heat map of immune cell infiltration in PAH and SARS-CoV-2. We found that PAH patients, SARS-CoV-2 patients and control patients had significant differences in 28 immune cell subsets ($P < 0.05$, Red means positive correlation, blue means negative correlation). Activated CD8 T cell, Activated dendritic cell, Immature B cell, Mast cell, Natural killer T cell, Effector memory CD4 T cell, Central memory CD4 T cell, Central memory CD8 T cell, Effector memory CD8 T cell have statistical difference in PAH diseases(Figue.5C).Activated CD4 T cell,CD56dim natural killer cell, Immature dendritic cell, MDSC, Macrophage, Mast cell, Monocyte, Natural killer cell, Regulatory T cell, T follicular helper cell, type 2 helper cell and central memory CD8 helper cell have statistical differences in SARS-CoV-2 (Figure.5D).The above results suggest that these differential immune cells may be involved in the immunoregulatory process of PAH and the pathogenesis of SARS-CoV-2.

In addition, the correlation between disease-characterized genes and the type of different immune cells was also explored. As shown in Figure.5E,in PAH, HMGB1 was negatively correlated in Macrophage and Monocyte.IL1B was negatively correlated with Effector memory CD8 T cell and positively correlated with Plasmacytoid Dendritic cell;MYD88 was negatively correlated with Activated CD8 T cell, Central memory CD8 T cell, Central memory CD4 T cell and Type 1 T helper cell, and was positive correlated with Macrophage, Neutrophil and Natural killer T cell.TLR4 was negatively correlated with Activated CD8 T cell, Effector memory CD4 T cell, T follicular helper cell, and positively correlated with Activated dendritic cell, Immature dendritic cell, Macrophage, Natural killer T cell, Natural killer cell related. As shown in Figure.5F, in SARS-CoV-2, it was also observed that HMGB1 was negatively correlated with Macrophage and Monocyte; MYD88 was negatively correlated with Activated CD8 T cell, Central memory CD8 T cell, Central memory CD4 T cell and Type 1 T helper cell and were positively correlated with Macrophage and Neutrophil. TLR4 was negatively correlated with T follicular helper cells and positively correlated with Natural killer cells. In conclusion, these results suggest that ICD genes have similar pathological processes in the immune microenvironment of PAH and SARS-CoV-2.

3.6. Drug screening

We screened drugs based on key genes (MYD88, TLR4, IL1B and HMGB1) using the DSigDB database on the Enrichr website and screened the top fifteen drug compounds based on P-values. Table 2 shows the effective drugs predicted by the disease signature gene screening in the DSigDB database. Among them, Nitric oxide BOSS, Resveratrol BOSS and Curcumin BOSS are the drugs worthy of our attention. Nitric oxide (NO),a potent vasodilator, is an established mediator in approaches for pharmacological treatment of PAH (71).Meanwhile, there is evidence that NO is effective in reducing SARS-CoV-2 replication and hypoxia in patients with severe acute respiratory syndrome (72).In addition, studies have shown that Resveratrol, Curcumin have anti-inflammatory effects in both PAH and SARS-CoV-2, and can improve pulmonary vascular remodeling (73-76).These provide a potential drug therapy target for the treatment of PAH and SARS-CoV-2.

3.7 Molecular docking

For further validation of the screened potential drugs, we used Auto Dock Vina software to evaluate molecular docking of Resveratrol and Curcumin with DEGs proteins (HMGB1, TLR4, IL1B, MYD88). Among them, HMGB1 PDB ID is 2YRQ; TLR4 PDB ID is 5NAO; IL1B PDB ID is 9ILB; MYD88 PDB ID is 7BER. Figure.6A shows the Resveratrol docking results, resveratrol binds to HMGB1 through a binding pocket consisting of SAP-98 (2.2 Å), ALA-101 (2.3 Å), and ARG-170 (2.3 Å). Resveratrol binds to IL1B through a binding pocket composed of MET-20 (2.5 Å) and LYS-63 (5.7 Å).Resveratrol binds to MYD88 through a binding pocket composed of SAP-234 (2.4 Å), ILE-267 (2.2 Å),and SER-266 (2.3 Å).Resveratrol binds to TLR4 through a binding pocket consisting of LYS-31 (2.6 Å).The above shows that: Resveratrol can bind to the docking pocket and has good docking activity between target proteins.Figure.6B shows that Curcumin binds to HMGB1 through a binding pocket consisting of ARG-104 (2.1 Å), LYS-95 (2.3 Å) and LYS-93 (2.1 Å). Curcumin binds to IL1B, and its binding pocket consists of LEU-80 (1.7 Å), LEU-134 (2.1 Å), and SER-125 (2.8 Å). Curcumin binds to MYD88 through a binding pocket composed of LYS-262 (2.1 Å). Curcumin binds to TLR4 through a binding pocket composed of TYR-33 (2.8 Å). Figure.6C shows the binding energy of Resveratrol and Curcumin binding to the target protein, Kcal*mol-1<-1.2, indicating a good docking result. (The redder the color, the lower the binding energy, which proves the easier the combination between them.) These indicate that: Curcumin can bind into the docking pocket and has good docking activity above four target proteins. In summary, resveratrol and curcumin are expected to play a potential role in the treatment of PAH and SARS-CoV-2.

4. Discussion

This study explores the connection between PAH, SARS-CoV-2, and ICD by means of bioinformatics and machine learning. We show that IL1B, MYD88, TLR4, and HMGB1 expression were different in both PAH and SARS-CoV-2 compared with the control groups. These key genes are closely related to immune cell activation. which may contribute to the progression of the PAH and SARS-CoV-2 infection.

As two important diseases affecting cardiorespiratory function, PAH and SARS-CoV-2 are intricately linked. There is growing evidence that the two diseases have multiple common risk factors and may interact with each other (77, 78). A more unique feature of SARS-CoV-2 than SARS-CoV-1 and H1N1 influenza viruses is its association with pulmonary vascular remodeling (27, 79-81), and these results indicate that SARS-CoV-2 may contribute to the development of pulmonary vascular diseases.

To illustrate the mechanistic relationship between SARS-CoV-2 and PAH, we performed enrichment analysis of DEGs in these conditions. We found that the functions of gene enrichment in SARS-CoV-2 and PAH were highly correlated with immune response, including positive regulation of interleukin-8 production, positive regulation of interleukin-6 production, positive regulation of chemokine production, chemokine production, interleukin-1 receptor binding, regulation of chemokine production. The main cause of death from SARS-CoV-2 is the cytokine storm caused by the release of pro-inflammatory cytokines and chemokines by immune effector cells (82, 83), which triggers a series of serious complications (84). The immune pattern of SARS-CoV-2 includes lymphopenia, lymphocyte activation and dysfunction, granulocyte and monocyte abnormalities, increased cytokine production, and increased antibody production (85, 86).This is similar to the immune pattern of PAH (22, 87). At the same time, we also observed that there are multiple common important inflammatory signaling pathways between SARS-CoV-2 and PAH, Including NF-kappa B signaling pathway, Toll-like receptor signaling pathway, NOD-like receptor signaling pathway. NF-κB signaling has important functions in maintaining physiological homeostasis and preventing inflammatory diseases in tissues

(88, 89). The TLR signaling pathway induces various genes that play a role in host defense, including inflammatory cytokines, chemokines (90-92). The NODLR signaling pathway mediates the host innate immune system and is involved in inflammasome activation (93-95). Therefore, we have reason to believe that the expression imbalance of ICD plays a crucial role in the occurrence and development of PAH and SARS-CoV-2.

In this study, four key genes were identified: IL1B, MYD88, TLR4, and HMGB1. Among these key genes, IL1B is a key biomarker for PAH (96). IL-1B levels were significantly elevated in PAH patients compared to healthy controls (97). Furthermore,IL-1B as an inflammasome gene (98), may contribute to the formation of the cytokine storm in SARS-CoV-2 (99, 100). Plasma levels of IL1B are significantly elevated in SARS-CoV-2 patients (101). IL1B can be used as a biomarker of SARS-CoV-2 infection (102). Studies have found that IL-1B may be a potential target for the treatment of SARS-CoV-2 (103).

In addition, we found that there is a certain regulatory relationship between the other three key genes HMGB1, MYD88, and TLR4 by consulting relevant literature(104, 105). Therefore, we propose the hypothesis that the HMGB1/TLR4/MYD88 axis may be involved in the progression of patients with simultaneous SARS-CoV-2 infection and PAH.

HMGB1 is secreted by activated monocytes and macrophages and passively released by necrotic or damaged cells (45). The present study found that HMGB1 expression was significantly different in both PAH and SARS-CoV-2 monocytes and macrophages, suggesting that HMGB1 has a key role in PAH and SARS-CoV-2 progression. When cells undergo ICD, HMGB1 is released extracellularly (106).However, the molecular mechanism of HMGB1 in the context of ICD remains to be elucidated (38). In the extracellular environment, HMGB1 is a potent pro-inflammatory factor (44-46). Studies have found that extracellular HMGB1 may be a therapeutic target for SARS-CoV-2 (107). Increased levels of HMGB1 in patients can be used as a criterion for clinical symptoms and prognosis (47-49). Interestingly, male PAH patients shows higher levels of circulating HMGB1 (50). This seems to suggest that HMGB1 plays a similar role in the progression of SARS-CoV-2 and PAH.

TLR4 is an innate immune receptor on the cell surface that interacts with DAMPs (108). DAMPs of TLR4 further reinforce that TLR4-mediated hyperinflammation is a major contributor to the molecular pathogenesis of SARS-CoV-2 (109). The TLR4 pathway can produce IL6, a key cytokine associated with cytokine storm, suggesting that TLR4 is involved in SARS-CoV-2 invasion (110-112). Studies have identified TLR4 as a therapeutic target for respiratory complications of SARS-CoV-2 (113). This study found that TLR4 was negatively correlated with T follicular helper cells and positively correlated with Natural killer cells. Type I interferon signaling by dendritic cells selectively stimulates Tfh cell development in response to antigen binding to TLR4 agonists (114).Intracellular expression of TLR4 in human NK cells, which may play a deleterious role during systemic inflammation, is upregulated in NK cells in patients with sepsis (115).

MYD88 is a key adaptor protein involved in IL-1 receptor family signaling controlling innate immune responses and inflammation (116). Studies have shown that MYD88 is a central node in the inflammatory pathway (117), which aggregates macrophages in cardiovascular disease, leading to vascular remodeling and triggering chronic inflammation, such as hypertension, chronic heart failure, and Kawasaki disease (118). In addition, MYD88 is significantly overexpressed in the pulmonary vessels of idiopathic PAH patients (119). MYD88 expression is positively correlated with the pathogenesis of SARS-CoV-2 (120).

Extracellular HMGB1 may be involved in glomerular endothelial cell injury by activating the TLR4/MYD88 signaling pathway in lupus nephritis (104). Upregulation of the HMGB1-TLR4/MYD88 pathway in type II focal cortical dysplasia leads to increased release of pro-inflammatory cytokines (105). Animal experiments revealed that HMGB1 mediates cigarette smoke-induced lung inflammation in mice through a TLR4/MYD88-dependent signaling pathway (121). In a hypoxia-induced pulmonary hypertension rat model, the expression of HMGB1 and TLR4 was significantly up-regulated in pulmonary arteries (122). HMGB1-mediated activation of TLR4 shows to promote experimental PH in a mouse model of chronic hypoxia-induced PH (123). Based on the above studies, we are more convinced that the HMGB1-TLR4/MYD88 pathway may play a role in promoting SARS-CoV-2 infection and PAH progression.

Nitric oxide, Resveratrol and Curcumin are the potential drugs we screened. Modulation of Nitric oxide (NO) signaling, is an effective approaches for pharmacological treatment of PAH (71). At the same time, nitric oxide is also an antibacterial and anti-inflammatory molecule, and exogenous NO is effective for new coronary patients with mild symptoms or severe acute respiratory syndrome (72, 124, 125). In addition, studies have found that resveratrol exerts anti-inflammatory effects by inhibiting the expression of HMGB1-mediated signaling pathways, such as TLR4, MYD88 during acute inflammatory diseases (112). Therefore, resveratrol may treat SARS-CoV-2 patients by inhibiting SARS-CoV-2-induced cytokine storm (126). Due to its anti-inflammatory, antioxidant, and anti-apoptotic properties, curcumin may have a positive role in suppressing inflammation in SARS-CoV-2 (75, 127). Recently, it has been suggested that resveratrol can effectively inhibit the proliferation of pulmonary artery smooth muscle cells and the remodeling of the right ventricle, which may be a potential drug for the treatment of PH (73). In a rat model of pulmonary hypertension, both resveratrol and curcumin were effective in improving pulmonary vascular remodeling (74, 128, 129). This provides innovative ideas for the treatment and prevention of PAH and SARS-CoV-2.

Taken together, based on the current findings, we can hypothesize that dysregulation of immunogenic cellular genes is a common factor in PAH and SARS-CoV-2 exacerbation, and their dysregulation can cause immune system dysfunction. Interestingly, among these several key genes, we found that HMGB1-TLR4/MYD88 can act as a signaling pathway axis, and jointly participate in the disease development of PAH and SARS-CoV-2. However, these are still speculations, and the specific mechanism needs to be explored further.

5. Conclusion

This study revealed common mechanisms between PAH and SARS-CoV-2 from the perspective of ICD gene expression and regulation. The key genes IL1B, MYD88, TLR4 and HMGB1 are involved in the immune cell response process, thereby affecting the progression of these two diseases. Our findings may provide new insights into the pathogenesis of PAH and SARS-CoV-2 and provide potential targets for future treatments.

6. Contributors

D.S.W, Y.F.M, and Y.B. conceived the original scope of this manuscript. D.S.W. and Y.F.M. designed the experiments and manuscript, and figures. M.T.G., R.F.M., Y.B. critically reviewed the final manuscript. All authors have read and agreed to the published version of the manuscript.

7. Conflict of Interest

The authors declare no competing financial interests

8. Acknowledgments

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9. Data sharing statement

All the data pertaining to the study are available in the manuscript or in the supplementary materials.

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GRAPHICAL ABSTRACT

Figure.1 Data preprocessing. A: Unstandardized analysis result of merged PAH dataset. B: The standardized analysis results of the combined PAH dataset. C: Unstandardized analysis result of SARS-CoV-2 dataset. D: SARS-CoV-2 standardized analysis results.

Figure.2 Identification of common differential genes. A: Difference analysis of ICD gene between PAH samples and normal samples. B: Difference analysis of ICD gene between SARS-CoV-2 samples and normal samples. C: Boxplot of difference analysis between ICD gene PAH samples and normal samples. D: Boxplot of differential analysis of ICD gene in SARS-CoV-2 samples and normal samples E: Intersection of PAH and SARS-COV-2. *P< 0.05, **P< 0.01, and ***P< 0.001.

Figure.3 Gene function enrichment analysis. A: PPI protein interaction network analysis. B: Meta analysis gene function enrichment histogram. C: GO functional enrichment analysis. D: KEGG functional enrichment analysis.

Figure.4 Construction of the artificial neural network model. A: Model diagram of artificial neural network in PAH and SARS-CoV-2. 0 represents the normal control group, 1 represents the disease group. B: Results of weights in PAH and SARS-CoV-2 disease. C: ROC curves of disease signature genes in PAH and SARS-CoV-2.

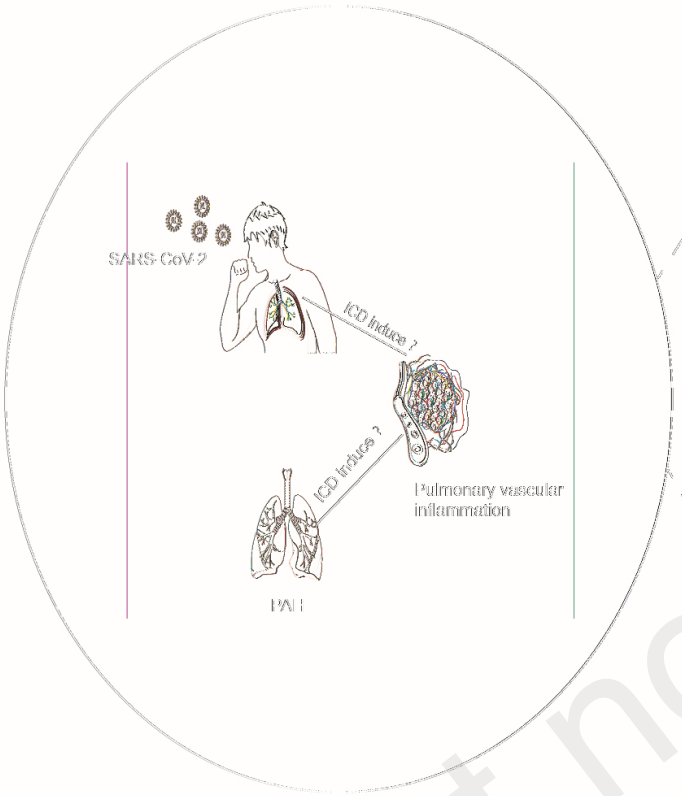
Figure.5 Immune infiltration analysis of shared disease signature genes. A: Heat map for differential analysis of immune cells in PAH. B: Heat map for differential analysis of immune cells in SARS-CoV-2. C: The violin plot of the proportions of 28 immune cells in PAH. D: The violin diagram of the proportion of 28 immune cells in SARS-CoV-2. E: Correlation analysis of five disease characteristic genes with immune cells in PAH. F: Correlation analysis of five disease characteristic genes and immune cells in SARS-CoV-2.

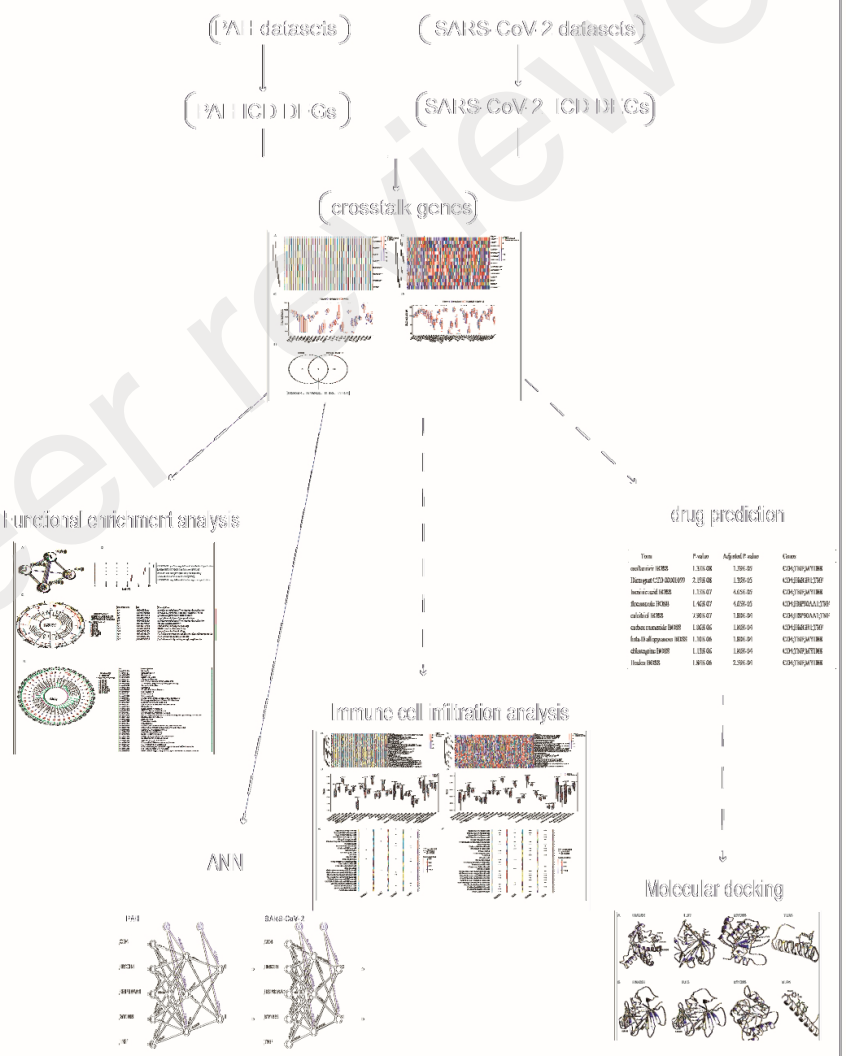
Figure.6 Molecular models of resveratrol, curcumin, and target proteins. A: HMGB1, IL1B, MYD88 and TLR4 interact with resveratrol. B: HMGB1, IL1B, MYD88 and TLR4 interact with curcumin. Dark blue represents amino acid residues in the binding site to resveratrol and Resveratrol, red dashed lines represent hydrogen bonds. C: Binding energy heat map.

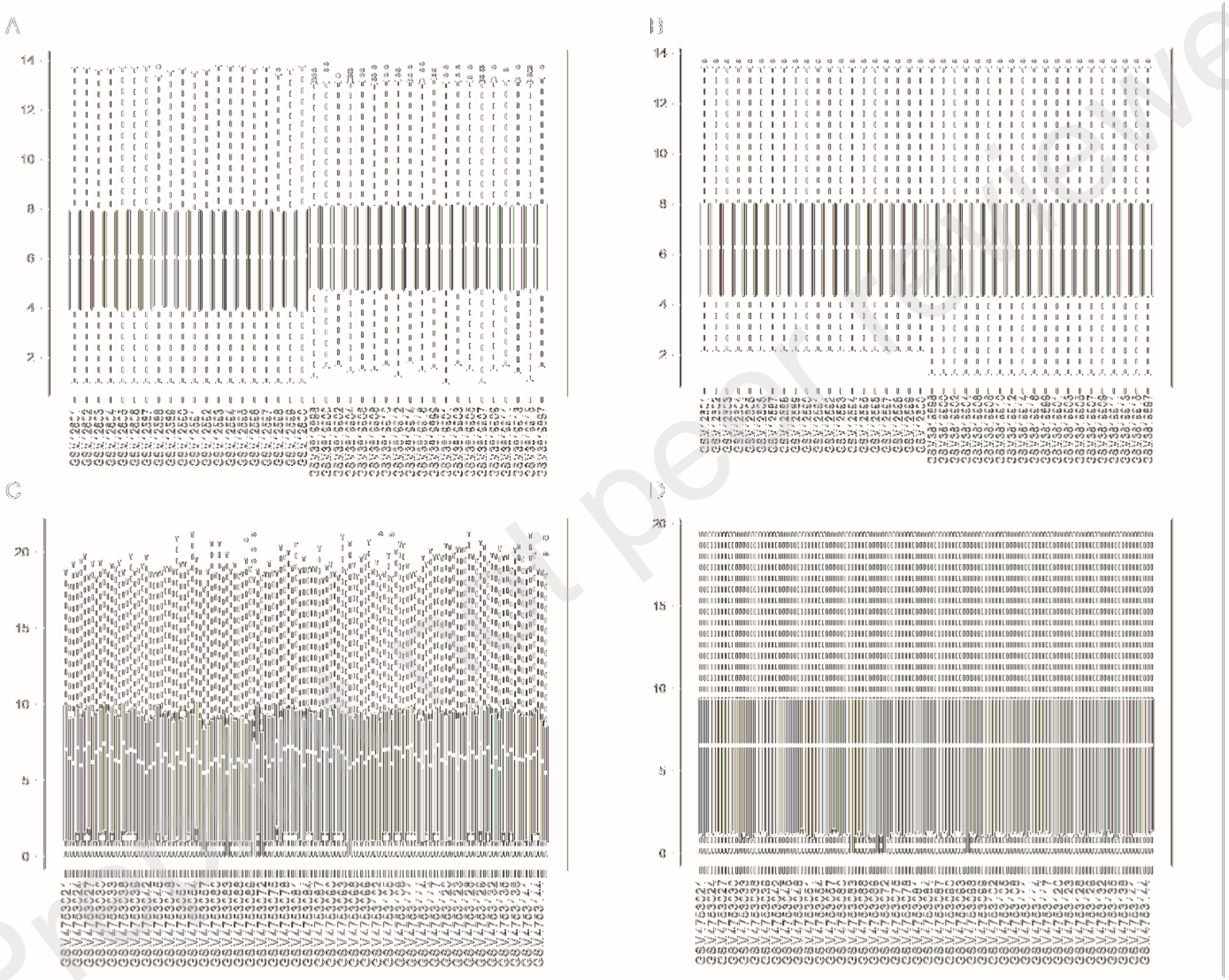
Table.1 Related dataset information

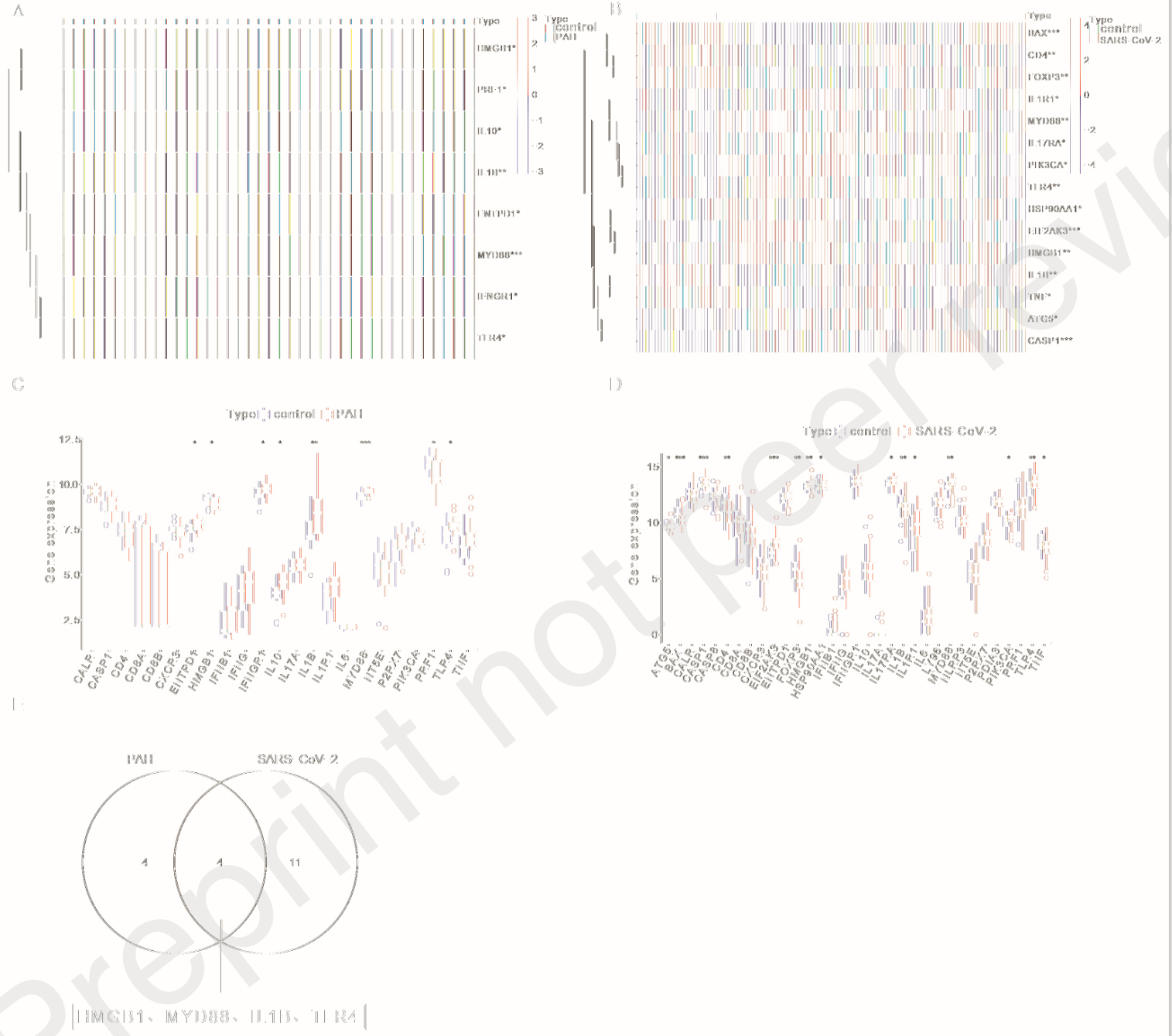
Table.2 Drug prediction

GRAPHICAL ABSTRACT

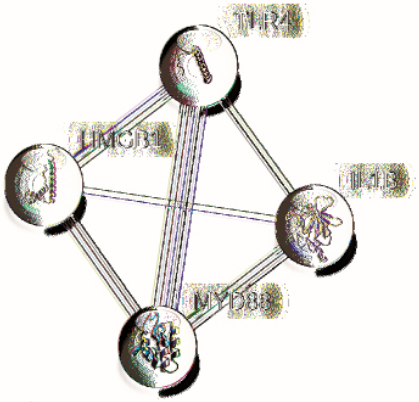




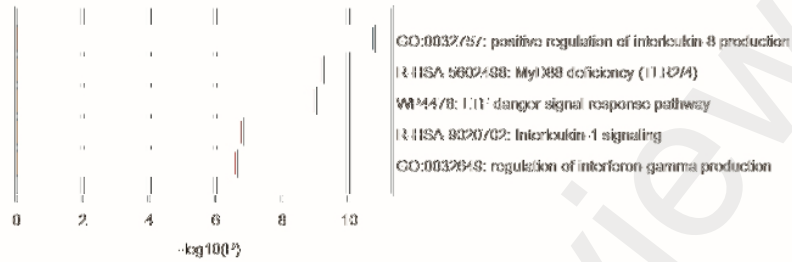




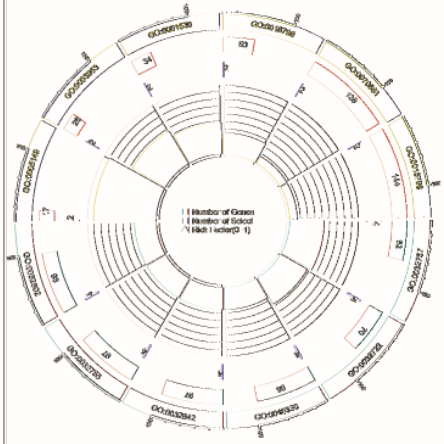
A



B

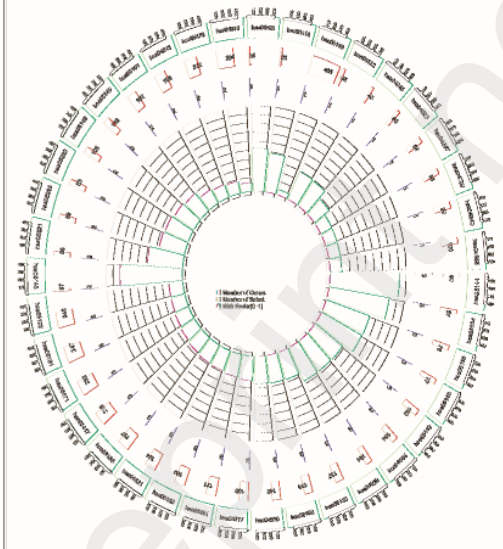


C

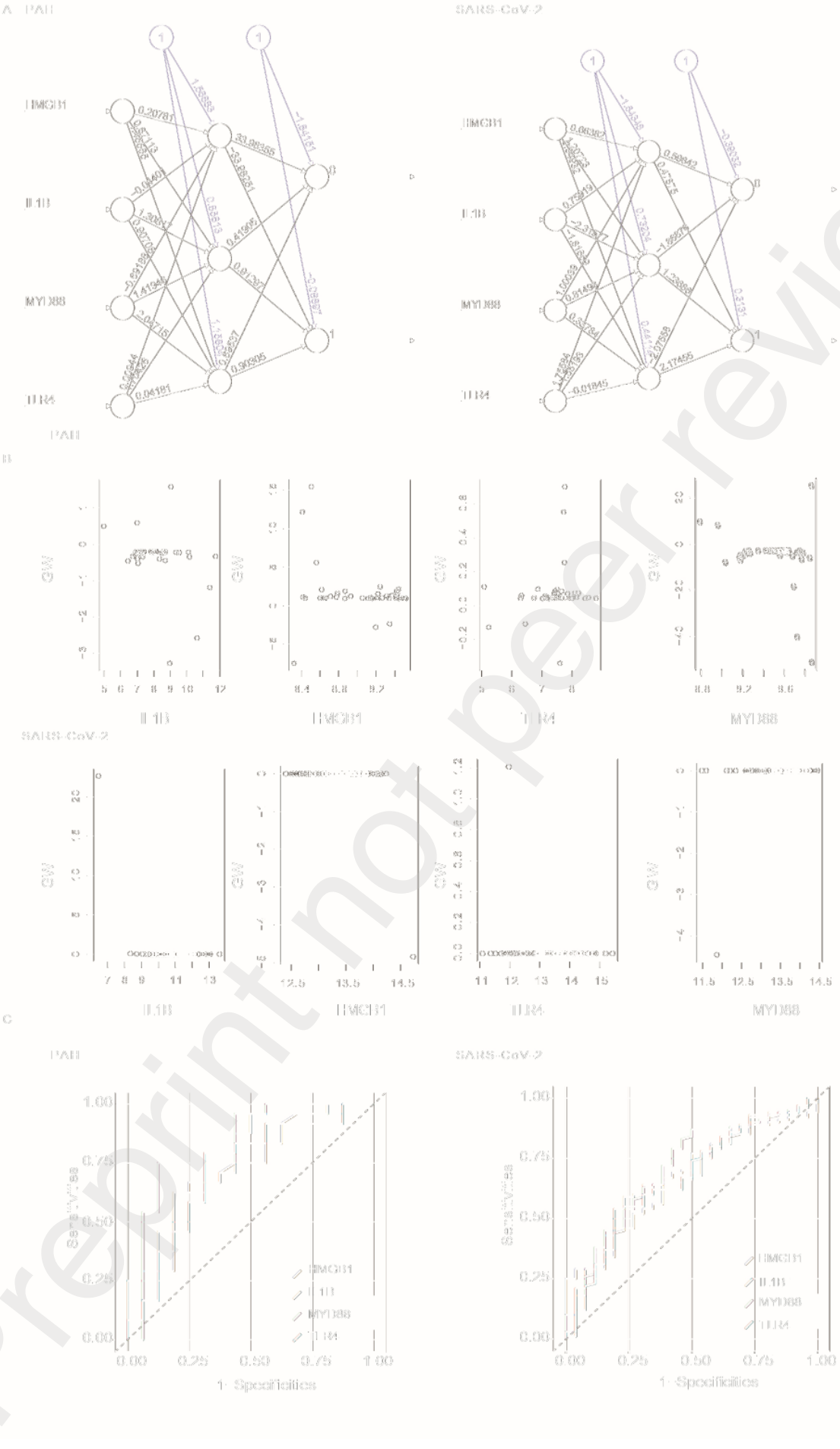


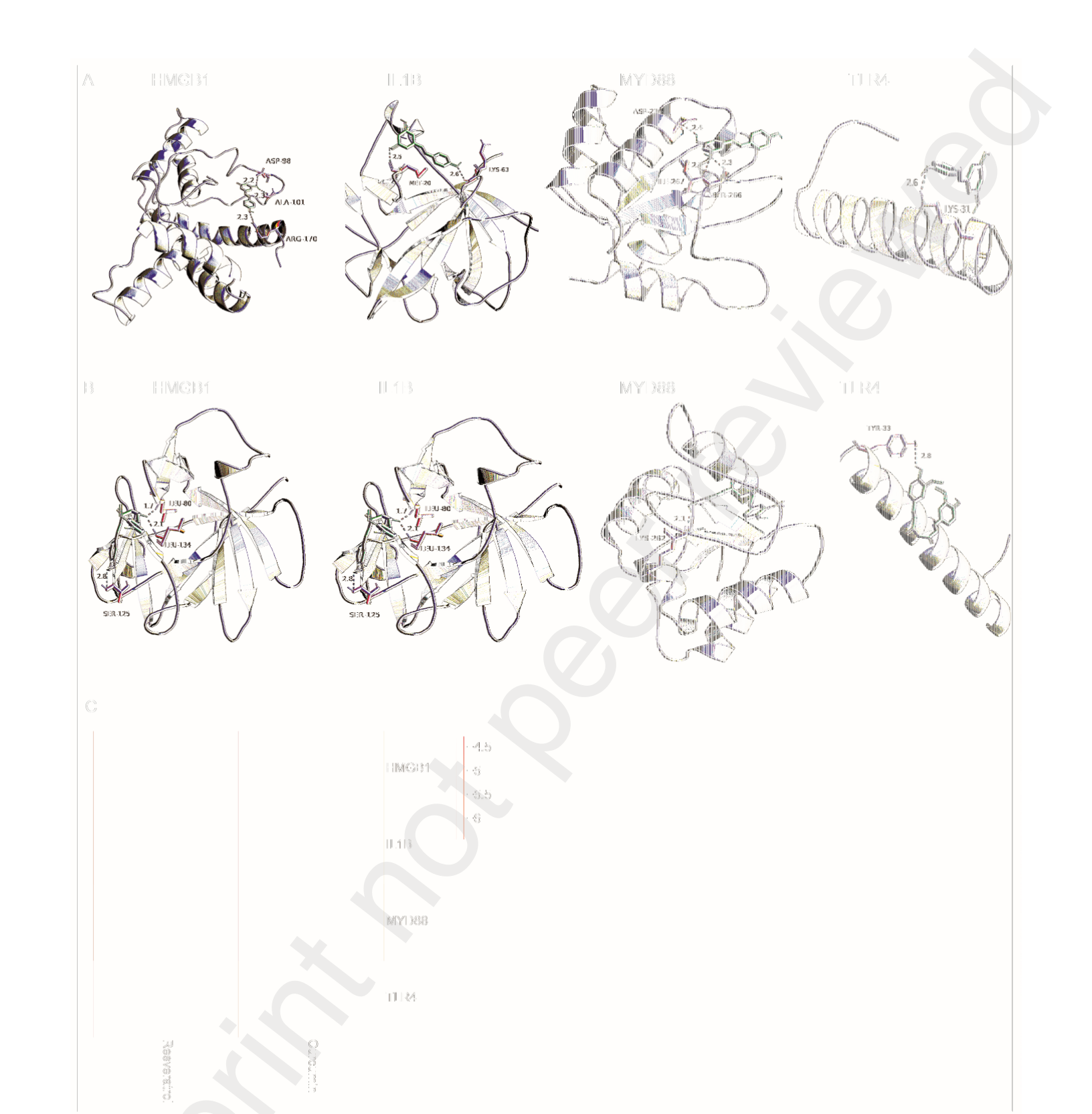
ONTOLOGY	ID	Description
BP	GO:0032757	positive regulation of interleukin-8 production
BP	GO:0032722	positive regulation of chemokine production
BP	GO:0046330	positive regulation of JNK cascade
BP	GO:0032642	regulation of chemokine production
BP	GO:0032755	positive regulation of interleukin-6 production
BP	GO:0032602	chemokine production
MF	GO:0005149	interleukin-1 receptor binding
MF	GO:0003953	NAD+ nucleosidase activity
MF	GO:0001530	lipopolysaccharide binding
MF	GO:0016799	hydrolase activity, hydrolyzing N-glycosyl compounds
MF	GO:0070851	growth factor receptor binding
MF	GO:0016798	hydrolase activity, acting on glycosyl bonds

D



ID	Description
hsa05144	Malaria
hsa05134	Legionellosis
hsa05133	Pertussis
hsa05140	Leishmaniasis
hsa05142	Chagas disease
hsa04064	NF-kappa B signaling pathway
hsa04620	Toll-like receptor signaling pathway
hsa05135	Yersinia infection
hsa05162	Measles
hsa04936	Alcoholic liver disease
hsa04217	Necroptosis
hsa05164	Influenza A
hsa05152	Tuberculosis
hsa04621	NOD-like receptor signaling pathway
hsa05130	Pathogenic Escherichia coli infection
hsa05417	Lipid and atherosclerosis
hsa05171	Coronavirus disease - COVID-19
hsa05131	Shigellosis
hsa05132	Salmonella infection
hsa05143	African trypanosomiasis
hsa05321	Inflammatory bowel disease
hsa05235	PD-L1 expression and PD-1 checkpoint pathway in cancer
hsa05323	Rheumatoid arthritis
hsa05146	Amoebiasis
hsa05145	Toxoplasmosis
hsa05161	Hepatitis B
hsa04613	Neutrophil extracellular trap formation
hsa05170	Human immunodeficiency virus 1 infection
hsa04010	MAPK signaling pathway
hsa01523	Antifolate resistance
hsa03410	Base excision repair
hsa05168	Herpes simplex virus 1 infection
hsa05332	Graft-versus-host disease
hsa04940	Type I diabetes mellitus
hsa04623	Cytosolic DNA-sensing pathway
hsa04657	IL-17 signaling pathway
hsa04750	Inflammatory mediator regulation of TRP channels
hsa04640	Hematopoietic cell lineage
hsa04933	AGE-RAGE signaling pathway in diabetic complications





Disease	GEO ID	Platform	Sample numbers	Organization type
PAH	GSE131793	GPL6244	20 (Normal: 10; Disease: 10)	peripheral blood
PAH	GSE703	GPL80	20 (Normal: 6; Disease: 14)	peripheral blood
SARS-CoV-2	GSE157103	GPL24676	126 (Normal: 26; Disease: 100)	plasma

Term	P-value	Adjusted P-value	Genes
Day 11-7082 CTD 00003959	2.75E-07	1.78E-04	IL1B; HMGB1; TLR4
Carbon monoxide BOSS	4.27E-07	1.78E-04	IL1B; HMGB1; TLR4
Ecalon BOSS	7.38E-07	2.05E-04	IL1B; TLR4; MYD88
Adenosine triphosphate BOSS	1.17E-06	2.43E-04	IL1B; HMGB1; TLR4
Superoxide BOSS	3.30E-06	2.78E-04	IL1B; HMGB1; TLR4
AGN-PC-0JHFVD BOSS	3.41E-06	2.78E-04	IL1B; TLR4; MYD88
Nitric oxide BOSS	3.41E-06	2.78E-04	IL1B; TLR4; MYD88
Trimethoprim BOSS	3.41E-06	2.78E-04	IL1B; TLR4; MYD88
Isoguanine BOSS	3.46E-06	2.78E-04	IL1B; TLR4; MYD88
Dinoprostone BOSS	3.68E-06	2.78E-04	IL1B; TLR4; MYD88
Water BOSS	3.68E-06	2.78E-04	IL1B; TLR4; MYD88
Resveratrol BOSS	5.57E-06	3.86E-04	IL1B; TLR4; MYD88
Muramyl Dipeptide CTD 00005307	6.92E-06	4.43E-04	IL1B; TLR4
Curcumin BOSS	8.31E-06	4.94E-04	IL1B; HMGB1; TLR4
Naloxone CTD 00006373	8.99E-06	4.98E-04	IL1B; TLR4