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Research Article

Keywords: higher-order interactions, self-organization, complex networks, major depression, viral pathogenesis, immune activation

Posted Date: October 9th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11109337/v1>

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Additional Declarations: No competing interests reported.

Self-Organizing Immune-Inflammatory Architectures in Severe Major Depression: Higher-Order Interactivity and Viral Pathogenesis

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Abstract

Background: Major depressive disorder (MDD) and its most severe phenotype, major dysmood disorder (MDMD), are distinguished by the activation of the immune-inflammatory response system, T cell activation, and a relative T regulatory cell suppression. While traditional models rely on dyadic protein associations, multi-protein coordination and emergent system dynamics remain largely uncharacterized in MDMD immune architectures.

Objectives: To investigate the self-organizing properties and higher-order structural organization of the MDMD immune interactome, identifying central coordinating nodes, functional bottlenecks, and enriched emergent biological processes.

Results: Network topological evaluation identified a core backbone of primary coordinating hubs: tumor necrosis factor- α (TNF), interleukin (IL)6, CXCL12, CXCL10, CCL5, CD4, CD8A, HLA-DR, and FOXP3. Functional overrepresentation revealed that the network self-organizes around systemic defense responses and viral-mediated processes involving viral protein interactions with cytokines and their receptors. Chemokine signaling cascades and TNF/NF- κ B pathways constituted primary feedback circuits. Molecular complex detection extracted a tightly coordinated higher-order module centered on RELA-regulated viral protein–cytokine interactions, reflecting group-level functional interdependence rather than isolated dyadic interactions.

Conclusions: Acute immune-inflammatory activation in MDMD behaves as an emergent, self-organized state driven by higher-order viral-associated pathway modules. These findings indicate that viral-immune interactions may serve as critical structural drivers that organize systemic T cell activation and chronic inflammatory recurrence, providing a systems-level framework for MDD pathogenesis.

Key words: higher-order interactions; self-organization; complex networks; major depression; viral pathogenesis; immune activation

Introduction

Evidence now indicates that the acute phase of severe major depressive disorder (MDD) is a neuro-immune condition (Almulla et al., 2024; Maes, 1995; Réus et al., 2023). MDD consists of two primary subcategories: major dysmood disorder (MDMD) and simple dysmood disorder (SDMD) (Maes, 2022; M. Maes et al., 2023; Maes, Zhou, Jirakran, et al., 2024). There exist clinical and biomarker-pathway distinctions between the two groups. MDMD, in contrast to SDMD, represents a more severe phenotype characterized by heightened levels of depression, anxiety, fatigue, and physiosomatic symptoms (Maes, 2022; Maes, Almulla, et al., 2024; M. Maes et al., 2023). Moreover, a greater recurrence of illness, characterized by escalating episode frequencies and suicidal tendencies, distinguishes MDMD from SDMD. Nonetheless, a first episode of MDD can exhibit the MDMD phenotype if the severity of the illness is sufficient (Almulla et al., 2024). Secondly, significant disparities exist in biomarkers and pathways between the two groups (M. Maes et al., 2023). These disparities include elevated cytokine networks, enhanced T cell activation, diminished neurotrophic growth factors, heightened atherogenicity, and reduced antioxidant defenses (Maes et al., 2022; Michael Maes, Muanpetch Rachayon, Ketsupar Jirakran, Pimpayao Sodsai, et al., 2023; Maes, Zhou, Jirakran, et al., 2024).

Investigations conducted on inpatients with severe MDMD revealed that this illness is marked by the activation of distinct cytokine profiles within the immune-inflammatory response system (IRS) and the compensatory immunoregulatory system (CIRS). The IRS indicates the activation of immunological and inflammatory networks, while the CIRS downregulates the IRS, thus averting an excessive IRS response and chronic inflammation, and facilitates a return to immune homeostasis or tolerance (Maes & Carvalho, 2018). The MDMD inpatients exhibited markedly activated M1 macrophages, M2 macrophages, T helper (Th)-1 cells, Th-1 polarization,

IRS, and CIRS profiles (Almulla et al., 2024). The key cytokines, chemokines, and growth factors distinguishing MDMD from controls or predicting depression severity were interleukin (IL)-16, TRAIL, IL-2 receptor (IL-2R), tumor necrosis factor (TNF)- β , IL-6, and platelet-derived growth factor (PDGF). Furthermore, MDMD, as opposed to SDMD, is distinguished by particular alterations in immune cell CD markers, signifying T cell activation and the suppression of T regulatory (Treg) cells (Maes, Zhou, Rachayon, et al., 2024). MDMD or its characteristics were markedly correlated with elevated numbers (or expression) of cells including CD4⁺ and CD8⁺, as well as CD140⁺, CD71⁺, and HLA-DR⁺, in addition to cells expressing CD20⁺ and CB2 (cannabinoid receptors) (Michael Maes, Muanpetch Rachayon, Ketsupar Jirakran, Atapol Sughondhabirorn, et al., 2023). Conversely, there was a notable reduction in Treg cells, specifically CD4⁺CD25⁺FOXP3⁺CD152⁺, and CD4⁺CD25⁺FOXP3⁺GARP⁺ Treg cells (Maes, Zhou, Rachayon, et al., 2024). These findings further suggest that MDMD is associated with an imbalance between IRS (T cell activation) and CIRS (Treg cell) activities (Maes & Carvalho, 2018). It is important to emphasize that different phenotypes of depression, such as MDD in outpatients (Maes, Jirakran, et al., 2024) or very mild SDMD (Michael Maes, Asara Vasupanrajit, et al., 2023), exhibit significantly distinct immunological profiles.

However, no research has examined the molecular processes or pathways that underlie the alterations in IRS (M1, Th-1, Th-1 polarization), CIRS (M2), and alteration in immune cells in MDMD. This undertaking can be executed using protein-protein interaction (PPI) or gene-gene interaction analysis, followed by annotation and enrichment analysis (Gevezova et al., 2023; Maes, Plaimas, et al., 2021). Network analysis will reveal the hubs and bottleneck genes by calculating degree and betweenness centrality. This method may identify the most important drug targets for network modification and disorder treatment. Additionally, annotation and enrichment analysis

will reveal potential underlying molecular mechanisms or intracellular networks that could function as novel therapeutic targets for the development of new treatments for the disorder (Maes, Plaimas, et al., 2021).

Accordingly, this study was designed to characterize the higher-order structural organization and self-organizing properties of the immune-inflammatory network in MDMD, examining coordinated multi-protein assemblies rather than isolated pairwise interactions. To achieve this, we synthesized the empirical immune profiles reported by Almulla et al. (2024)—(Almulla et al., 2024) including the expression values and associated weights of multiplex-quantified cytokines, chemokines, and growth factors—alongside the differentiated T cell markers documented by (Maes, Zhou, Rachayon, et al., 2024).

Our primary objective was to map the shared network architecture, identifying critical hubs, bottlenecks, and densely coordinated molecular modules that govern systemic immune activation in severe affective illness. Furthermore, by implementing the weighted functional enrichment framework established by (Fan & Cui, 2021) across these differentially expressed mediators, we sought to isolate the emergent biological pathways and intracellular patterns—particularly viral-associated processes—that underpin the acute inflammatory state in MDMD.

Participants

We enlisted 71 FE-MDMD patients from the psychiatric unit at Alhakeim Hospital in Najaf, Iraq, for a case-control study investigating cytokines, chemokines, and growth factors. Individuals with FE-MDMD exhibited an average disease duration of 2.5 months. Furthermore, the senior psychiatrist enlisted 40 healthy control participants who were acquaintances of patients, medical personnel, or associates of medical personnel from the same catchment area. The principal data

are disseminated by (Almulla et al., 2024). The flow cytometry study (Maes, Zhou, Rachayon, et al., 2024) involved 30 outpatients with MDD (19 with MDMD and 11 with SDMD) at the psychiatric department of Chulalongkorn Hospital in Bangkok, Thailand, to examine T cell activation in MDMD. Posters and word of mouth were employed to recruit healthy volunteers from the same vicinity (Bangkok, Thailand) as the patients. This study incorporated 20 healthy controls.

Both studies employed identical diagnostic techniques and clinical inclusion and exclusion criteria. All participants were aged 18 to 65 years and received a DSM-5 diagnosis of MDD from a qualified psychiatrist, scoring a minimum of 17 on the Hamilton Depression Rating Scale (HAM-D) (Çakici et al., 2020). Almulla et al. (2024) exclusively recruited patients with MDMD (Almulla et al., 2024). Maes et al. (2024) recruited individuals with MDD, categorized into MDMD and SDMD groups (Maes, Zhou, Rachayon, et al., 2024). These diagnoses can be established via machine learning models developed from the Hamilton Depression Rating Scale (HAMD), the Hamilton Anxiety Rating Scale (HAMA), the recurrence of illness (ROI) index, or when the HAMD score is ≥ 22 and the HAMA score is ≥ 22 . All patients included here were in the acute phase of MDMD, not in remission or partial remission.

The exclusion criteria were identical in both centers. We excluded participants who fulfilled the criteria for the following Axis I DSM-IV-TR disorders: psycho-organic disorders, dysthymia (unless part of double depression), bipolar disorder, schizoaffective disorder, schizophrenia, autism spectrum disorder, obsessive-compulsive disorder, generalized anxiety disorder, post-traumatic stress disorder, and substance use disorder (excluding nicotine dependence). The identical criteria were utilized for normal controls, who must also be devoid of a lifetime diagnosis of major depression and dysthymia, as well as a familial history of depression,

mania, psychosis, substance use disorder, or suicide. Patients and controls with chronic liver or kidney disease, as well as pregnant and breastfeeding women, were excluded. Furthermore, we eliminated subjects with neurodegenerative, neuroinflammatory, or neurological disorders, including Alzheimer's disease, Parkinson's disease, stroke, and multiple sclerosis. Additionally, we excluded participants with (auto)immune diseases, including inflammatory bowel disease, rheumatoid arthritis, type 1 diabetes, cancer, psoriasis, scleroderma, systemic lupus erythematosus, chronic obstructive pulmonary disease, acute COVID-19 infection, severe or critical COVID-19 illness, long COVID, other infectious diseases such as influenza and hepatitis, asthma, cardiovascular disorders, and a history of allergic or inflammatory responses within the preceding three months. Additionally, we excluded participants who received immunosuppressive or immunomodulatory therapies, including glucocorticoids, those who had recently (within the last three months) consumed nonsteroidal anti-inflammatory drugs (NSAIDs) or other analgesics, and individuals on therapeutic doses of antioxidants or omega-3 supplements.

Ethics statement

This work constitutes a secondary data analysis utilizing open, deidentified, and non-coded data sets; hence, it qualifies as non-human subjects research exempt from IRB approval. In our prior case-control research (Almulla et al., 2024; Maes, Zhou, Rachayon, et al., 2024), we detected significantly differentially expressed proteins (DEPs) in peripheral blood immune cells and in cytokines/chemokines/growth factors/CD markers compared to normal controls. Both case-control studies obtained permission from our local Institutional Review Boards, and all participants provided written informed consent prior to their involvement in the study. The Institutional Review Board of the College of Medical Technology of the Islamic University of Najaf, Iraq, sanctioned

the study under Document No. 18/2021. The study protocol of Maes et al. (2024) received approval from the Institutional Review Board of Chulalongkorn University, Faculty of Medicine, Bangkok, Thailand (#528/63). The study was executed in accordance with Iraqi, Thai, and international ethical and privacy standards, including, but not limited to, the World Medical Association's Declaration of Helsinki, the Belmont Report, the CIOMS Guidelines, and the International Conference on Harmonization of Good Clinical Practice. Furthermore, our institutional review board rigorously complies with the International Council for Harmonisation Good Clinical Practice (ICH-GCP) guidelines for human research safety.

Biochemical assays

In both investigations, blood samples were collected between 8:00 and 11:00 a.m. with a disposable syringe and serum tubes. Following centrifugation of the blood at 3,500 rpm, serum was isolated and stored in Eppendorf tubes at -80 °C in tiny aliquots until thawed for biomarker testing. The interested reader is directed at our publications detailing the analytical methodologies (Almulla et al., 2024; Maes, Zhou, Rachayon, et al., 2024).

WEAT analysis.

Weat analysis performed using cytokine/chemokine/growth factors in MDMD.

Using the method of (Fan & Cui, 2021) (<https://www.cuilab.cn/weat/>), we performed functional enrichment analysis using weighted scores obtained from all variables in the study by (Almulla et al., 2024). The gene identification of the cytokines/chemokines/growth factors that were analyzed in the initial phase of the study is depicted in **Table 1**. This table also displays the average expression values of the measurements in MDMD patients and controls. Using the formula

average_MDMD – average_controls, we determined the weight (or essentiality score) of each gene based on these discrepancies, with higher scores indicating greater essentiality or importance for MDMD. As a result, we employed the weights specified in Table 1 to conduct Weighted Enrichment Analysis Tools (WEAT) (available at <https://www.cuilab.cn/weat/>). Our results section (refer to the below) will display the results of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses and Gene Ontology (GO) enrichment (<https://genome.jp/kegg/>).

Selection of seed proteins in our network analysis.

STRING 11.9 (<https://string-db.org>) and Cytoscape (<https://cytoscape.org>) modules, including NetworkAnalyzer (Cytoscape App Store - NetworkAnalyzer), were employed to conduct the network analysis. The minimum required interaction score was 0.400, and the set organism was homo sapiens. We generated PPI networks with zero order, which consisted solely of the seed proteins. The latter was composed of the DEPs with respect to cytokines/chemokines/growth factors and CD markers as well. The DEPs utilized in this analysis are listed in **Table 1**. As a result, we analyzed the network characteristics of the zero network, which included the number of nodes and edges, network diameter and radius, clustering coefficient, and network density. Hub nodes were authenticated as the top nodes with the highest degree value, while top non-hub bottlenecks were identified as nodes with the highest betweenness centrality. The network's backbone is formed by both the DEPs with the highest degree and the non-hub bottlenecks.

Annotation and Enrichment analysis.

In addition to WEAT analysis performed on the weights of all cytokines/chemokines/growth factors, we also analyzed the DEPs from the combined (Almulla et al., 2024; Maes, Zhou, Rachayon, et al., 2024). The combined DEPs, which include cytokines/chemokines/growth factors/CD markers, were subjected to annotation-enrichment analyses. Therefore, in those analyses, we integrate the significant M1, Th1, Th2, IRS, and CIRS cytokines/chemokines and growth factors with CD markers of T cell activation, B cells bearing CB receptors, and T reg cells. We used False Discovery Rate (FDR) p correction to adjust for multiple associations.

The top 100 most relevant genes were included in an extended network, and the DEPs were applied to Enrichr (<https://maayanlab.cloud/Enrichr/>). The DEPs were searched against KEGG 2021 Human pathways, and the Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining (TRRUST) factors. The results were presented in the form of bar graphs that were generated using Appyters to display the top 10 performing enriched terms. In these bar graphs, the statistical significance is denoted by the asterisks located adjacent to the p-values.

Furthermore, the DEPs network was subjected to enrichment analysis of KEGG pathways and disease-gene associations using STRING. The latter method included the top 10 processes that were displayed using functional enrichment visualization sorted by $-\log$ (FDR) and grouped by >0.8 similarity.

The list of seed genes was supplemented with known protein interactions from Metascape (<http://metascape.org>). The combined GO molecular processes, immunologic signatures, and KEGG and Reactome (<https://reactome.org>) pathways were used to conduct enrichment analysis (min network size = 3, maximal network size = 5, beta testing, min enrichment = 1.5, set species = homo sapiens). Based on Kappa-statistical similarities among their gene memberships,

Metascape automatically clusters the significant terms into a tree in a hierarchical fashion. This is advantageous due to the substantial overlap between the terms employed, which may otherwise result in a significant amount of redundancy in the output terms. The molecular complex detection (MCODE) method was employed as the primary analysis to identify small components of densely connected nodes, which are indicative of molecular complexes (Bader & Hogue, 2003). Biological meanings were extracted from the network components and GO enrichment was applied. The top 3 best p-value terms are listed.

Using the module explorer in OmicsNet (<https://www.omicsnet.ca>), we constructed a Network with MultiOmics Enrichment Analysis. The inventory of seed DEPs was expanded to include known protein interactions from STRING. The OmicsNet function regulation explorer was employed to establish Panther biological processes (www.pantherdb.org) and KEGG gene-protein processes. The module explorer was used to delineate modules in the enlarged network. We created a 3-dimensional network graph that also displays the network's backbone.

Results.

The PPI network topography of FE-MDMD.

To represent the protein interactions in FE-MDMD, an undirected network was constructed by utilizing all seed genes (cytokine/chemokine/growth factor/CD marker DEPs). The zero-order network was composed of 34 nodes, as illustrated in Table 1. However, there were three singletons that were not part of the PPI network that had been established. Subsequently, these were eliminated: CNR1, CNR2, and CNGB1. The zero-order protein network, depicted in **Figure 1**, contained 31 nodes. The number of edges ($n=348$) exceeded the anticipated number of edges ($n=55$) with a p-enrichment value of $1.0e-16$. The average local clustering coefficient was 0.86, and the

average node degree was 22.5. The characteristic path length was 1.252, the network diameter was 2, the network radius was 1, and the network density was 0.748. The main hubs were TNF (degree=31), IL-6 (29), CD4 (29), CD8A (28), CCL5 (28), CXCL10 (28), and CXCL12 (27) according to the network degree analysis. No other non-hub bottlenecks were included in the top 5 bottleneck nodes. Consequently, TNF, IL-6, CD4, CD8A, CCL5, CXCL10, and CXCL12 constitute the zero-order network's backbone.

Consequently, the list of 31 seed genes was expanded (first shell only) to include known protein interactions from STRING. The extended network consists of 217 nodes and 5907 edges, whereas the anticipated number of edges was 1428 (PPI enrichment p value of 1.0×10^{-16}). The average local clustering coefficient was 0.678, and the average node degree was 54.4. The module explorer of OmicsNET revealed the presence of 5 significant modules. The first module ($n=54$, 1.81×10^{-17}) was centered around chemokines, particularly CXCL12 and CCL5, the second module ($n=42$, 9.28×10^{-18}) was centered around TNF, the third module ($n=22$, 1.25×10^{-5}) was centered around CD4, the fourth module centered around HLA-DR ($n=21$, $p=3.58 \times 10^{-5}$), and the fifth module was centered around FOXP3 ($n=14$, $p=0.00463$). The degree analysis indicated the top hubs as TNF (53), CD4 (47), CXCL12 (38), CCL5 (37), CCL11 (25) and HLA-DR (22). FOXP3 was the third most significant bottleneck, following CD4 and TNF, and preceding CCL5 and CXCL12.

Weat enrichment

The results of the WEAT enrichment analysis conducted on the weights of all cytokines/chemokines/growth factors obtained in the (Almulla et al., 2024) study is presented in **Table 2**. We conducted a search of the network against the KEGG pathways. The cytokine-

cytokine receptor interactions, viral protein interaction with cytokine and cytokine receptor, TNF signaling pathway, and chemokine signaling pathway were the top pathways (FDR all $< e^{-9}$).

STRING enrichment

Additionally, we conducted a search of the zero-order network using the 31 DEPs shown in Figure 1 against the KEGG pathways and disease-gene associations using STRING. The results of the KEGG enrichment are illustrated in **Figure 2**. The KEGG pathways that were most enriched in the zero-network were the viral protein interaction with cytokine and cytokine receptor, as well as the cytokine-cytokine receptor interactions. The chemokine signaling pathway followed at a distance. The nuclear factor- κ B and TNF signaling pathways were the fourth and fifth most significant pathways.

In Figure 1, we have projected a selection of GO functions, including the response to a stimulus (31/6357, $2.58e^{-10}$), regulation of T cell activation (11/376, $2.63e^{-9}$), defense response (20/1394, $5.35e^{-13}$), cellular response to a stimulus (30/6357, $2.58e^{-11}$), and one KEGG pathway, namely viral protein interaction with cytokine and cytokine receptor (15/96, $3.14e^{-24}$). The enrichment of disease-gene associations is illustrated in **Figure 3**. This analysis suggests that the zero-order network is substantially enriched in autoimmune disease, primary immunodeficiency disease, and immune system disease. A variety of viral infection diseases are included, in addition to inflammatory disorders.

Enrichr enrichment

The results of the Enrichr enrichment analysis (extended networks) search against KEGG pathways and TTRUST transcription factors are depicted in **Figure 4** and **Figure 5**, respectively,

as bar graphs. The KEGG pathway analysis yielded comparable results to the KEGG analysis conducted on the zero-order network and the WEAT analysis and additionally indicated that the Toll-Like Receptor (TLR) pathway was significantly enriched in the network. NFKB1 (nuclear factor (NF)- κ B), REL (REL Proto-Oncogene, NF- κ B Subunit), and RELA were the most important transcriptional factors associated with the Enrichr extended network (Figure 5).

Metascape enrichment

MCODE analysis performed using an extended network constructed with Metascape showed that one molecular complex could be extracted from the data, namely cytokine-cytokine receptor interaction, viral protein interaction with cytokine and cytokine receptor, and regulated by RELA (see **Table 3**).

OmicsNET enrichment

ESF, Figure 1 illustrates the enrichment visualization of the extended network based on the critical cytokine/chemokine/growth factor/CD marker DEPs, as described in the "The PPI network topography of FE-MDMD" section. Using perspective architecture, this figure illustrates the nodes of the five significant modules that were constructed using the OmicsNET module explorer. The backbone nodes of this extended network are also depicted in this graph, and they simultaneously represent various modules. We have investigated the enrichment of Panther biological processes using OmicsNET (refer to **Table 4**).

This table demonstrates that immune responses was the most significant process underpinning the extended network, followed by a viral process and, at a distance, antigen processing and presentation. The defense response to a bacterium was only marginally significant.

The KEGG gene/protein processes that underpin the extended network were analyzed in **Table 5**. The phosphatidylinositol signaling system exhibited the most significant enrichment in the network, followed by FoxO signaling, the sphingolipid signaling pathway, and the phospholipase D signaling pathway.

Discussion

Topology of the neuro-immune network in MDMD

The first major discovery of this study is that the zero and extended networks of the cytokine, chemokine, growth factor, and CD marker DEPs were primarily composed of TNF, IL-6, CXCL12, CXCL10, CCL5, CD4, CD8A, HLA-DR, and FOXP3. Consequently, chemokines and CD markers are crucial components of the immunological networks involved in MDMD. Furthermore, the findings indicate that T cell markers, such as CD4⁺ (T helper cells) and CD8⁺ (cytotoxic cells), along with the T cell activation marker HLA-DR⁺, are significant hotspots within the interconnected cohesive network.

The hubs of a PPI-network are of paramount importance, as they are fundamental to the network and have a significant impact on numerous nodes. Consequently, these influential nodes may be regarded as hotspots and, consequently, as legitimate drug targets. On the other hand, the information flow within the network is regulated by the bottlenecks, as they are the nodes where the network's traffic is regulated. Consequently, both the hotspots and bottlenecks are part of the backbone of the network that, when altered, will have a modulating effect on the entire network (Maes, Plaimas, et al., 2021).

Prior studies primarily examined pivotal M1 cytokines, including IL-1 β , IL-6, TNF- α , and IL-8, as evidenced by several studies and meta-analyses (Çakici et al., 2020; Cosma et al., 2021; Maes et al., 1995; Ng et al., 2018). Consequently, the findings of the current suggest that the

chemokine module is at least as significant as the M1 cytokines that were introduced three decades ago (Maes et al., 1991). Three meta-analyses that were conducted in MDD showed that CCL2 (Köhler et al., 2017), CCL2 and MCP-1 (Eyre et al., 2016), and CCL2, CCL3, CCL11, CXCL7 and CXCL8 (Leighton et al., 2018) were increased in MDD as compared with controls. Nevertheless, the results of our study show that chemokines such as CXCL12, CXCL10, and CCL5 emerge as hotspots within the network and thus as highly significant drug target candidates for the treatment of MDMD. It is important to note that the cytokine exhibiting the greatest effect size in MDMD, specifically IL-16, was neither a hub nor a bottleneck, whereas IL-6, a less significant cytokine in MDD, including in the study by (Almulla et al., 2024), was a significant hub.

Furthermore, our findings indicate that growth factors or colony-stimulating factors, which are highly increased in MDMD (such as PDGF and M-CSF), do not function as a hub or bottleneck. Therefore, IL-16, CXCL27, M-CSF, and PDGF are not the primary drug targets, despite the fact that they are more significantly increased and have a larger effect size in MDMD than for example IL-6. This is all important because it indicates that prior research concentrated excessively on the effects of the M1 cytokines such, as TNF- α , IL-6, and IL-1, and did partly neglect the most important chemokines and CD makers (Çakici et al., 2020; Islam et al., 2023; Jayakumar et al., 2023; Köhler et al., 2017; Maes et al., 1995; Ng et al., 2018; Osimo et al., 2020; Zhang et al., 2023). Consequently, the primary drug targets to treat MDMD are TNF- α , IL-6, CXCL12, CXCL10, CCL5, CD4, CD8A, HLA-DR, and FOXP3.

We have previously reviewed the mechanisms by which the activation of this cytokine/chemokine/growth factor network may induce depressive symptoms through neurotoxic effects on affective circuits in the brain, including diminished neuroplasticity and neurogenesis,

axonal dysfunctions, and structural deficits in neurons (Cui et al., 2024; Kubera et al., 2011; Maes & Carvalho, 2018).

Involvement of the PPI network in IRS responses

The most significant biological pathways enriched in the PPI network are an IRS response, cytokine-cytokine receptor interactions, a cellular defense response, and, in particular, a viral process that involves viral protein interaction with cytokine and cytokine receptors. This was illustrated by our different enrichment and annotation analyses. In the same vein, the network's backbone identified hub and bottleneck nodes, which are essential components of an IRS response and a defense response against viral infections.

As a result, TNF is a most critical hotspot, and TNF signaling is a critical pathway that supports the network. During an immune response, monocytes and macrophages (as well as other immune cells) produce both TNF- α and IL-6, which are pleiotropic cytokines (Idriss & Naismith, 2000; M. Maes et al., 2014). TNF- α is a critical component of the host's response to infection and is produced in response to viral infections (Winthrop et al., 2013). Its production triggers an IRS response, which involves the recruitment of immune cells to the infectious site and the activation of macrophages (and other cells) (Kim & Solomon, 2010). IL-6 is a critical factor in the acute phase response and numerous immune processes associated with MDD (Michael Maes et al., 2014). Additionally, the upregulation of IL-6 during infection may facilitate the survival of the virus and exacerbations (Velazquez-Salinas et al., 2019). The chemokine network is an additional mediator of the IRS response, and chemokines are essential for the defense against viral infections (Melchjorsen et al., 2003). Chemokines attract white blood cells to the infectious site and stimulate them to produce pro-inflammatory cytokines (Melchjorsen et al., 2003). Viral infections are

accompanied by elevated levels of CCL5 and CXCL10 including in the cerebrospinal fluid. CXCL12, a pre-B cell growth factor, is essential for viral infections and serves important homeostatic functions (Janssens et al., 2018).

The activation and proliferation of CD4⁺ and CD8⁺ T cells are another prominent response to viral infection (Harty et al., 2000). CD4⁺ T cells facilitate the generation of virus-specific memory CD8⁺ T cells and maximize CD8⁺ T cell populations during an IRS response (Swain et al., 2012). CD8⁺ T cells maintain the antiviral response by producing chemokines and they have direct effector functions through cytotoxic T lymphocyte-mediated lysis (Swain et al., 2012). In MDMD, activated T cells (as indicated by increased HLA-DR⁺ T cell numbers and other activation markers) trigger the production of the chemokine network and consequently white blood cell recruitment to viral replication sites (Swain et al., 2012). Other research indicates that HLA-DR may be a valuable marker for the identification of effector T cells and the surveillance of immune responses in the context of infections (Tippalagama et al., 2021).

The defense response to an invading pathogen comprises an IRS response that eliminates or manages the infection, and a CIRS response that mitigates collateral harm from an excessive IRS reaction (Belkaid, 2008; Maes & Carvalho, 2018). FOXP3⁺ T cells are crucial to these regulatory processes, hence averting tissue injury (Belkaid, 2008). Nonetheless, when Tregs effectively maintain host homeostasis by regulating excessive immune responses, a resultant effect of this regulation is increased pathogen survival and even pathogen resistance (Scott-Browne et al., 2007). Moreover, Treg cells may facilitate viral replication (Belkaid, 2008; Ciurkiewicz et al., 2020). Conversely, patients exhibiting diminished Treg cell counts and chronic viral infections (e.g., HCV) may manifest autoimmune processes. This is significant because MDMD is characterized by a relatively reduced level of Treg cells, which is linked to the severity of

depression (Maes, Zhou, Rachayon, et al., 2024). Furthermore, whereas Treg cells may decrease during the acute phase of severe depression, our findings indicate that in the (partially) remitted phase, the quantity of Treg cells is elevated (Maes, Nani, et al., 2021). Consequently, FOXP3+ cells may play a role in viral infections or (auto)immune responses during both the acute phase and the (partially) remitted phase of MDD.

It is imperative to note that viral processes, including EBV and CMV infections, are highly significant processes that underpin the PPI network constructed in this study. It is important to emphasize that the immune-risk phenotype of bipolar depression may be influenced by human cytomegalovirus (HCMV)-specific seropositivity (Maes, Nani, et al., 2021). In addition, depression due to multiple sclerosis and depression following Long COVID are two conditions that are distinguished by elevated IgG/IgA/IgM levels against deoxyuridine 5'-triphosphate nucleotidohydrolase (dUTPases) of EBV and HHV-6 (Maes, Almulla, Tang, et al., 2024; Maes, Almulla, Vojdani, et al., 2024). The latter findings indicate the presence of HHV-6 and/or EBV replication and reactivation in association with depressive symptoms.

Other relevant pathways

Other pathways that are enriched in the PPI network constructed around the DEPs of MDMD are the NF- κ B pathway, the TLR pathway, the phosphatidylinositol signaling system, the sphingolipid signaling pathway, the phospholipase D signaling pathway, and the FOXO signaling pathway. MCODE analysis discovered one molecular complex comprising activation of the cytokine network and viral protein interactions combined with regulation by RELA.

Previously, we used annotation and enrichment analyses on a DEP (cytokine) network in severe MDD (Maes et al., 2022). Apart from increased TNF signaling, hyper-responsive NF- κ B

and TLR pathways were major molecular pathway underpinning MDMD (Maes et al., 2022). Recent papers show that NF- κ B RNA and TLR-4 RNA and protein levels are significantly increased in peripheral blood mononuclear cells of patients with MDD (Kéri et al., 2014). The prefrontal cortex of individuals with MDD shows increased mRNA expression of TLR-4 and TLR-3 (Pandey et al., 2014). A recent review demonstrated an increased expression of TRIF (TIR domain-containing adaptor molecule 1), MYD88 (an innate immune signal transduction adaptor), TLR-3/4, and NF- κ B in individuals diagnosed with MDD (Mariani et al., 2021). TRIF and MYD88 are both key elements leading to NF- κ B pathway activation. Similar findings were reported by Elovainio et al. (2015) who discovered that individuals with severe depression exhibit gene-set pathways linked to the TLR pathway and the NF- κ B pathway (Elovainio et al., 2015).

Upon the engagement of a ligand to certain TLR complexes, NF- κ B can be transported to the nucleus, resulting in the activation of transcription for pro-inflammatory cytokines and other actors leading to a primed inflammasome. Viral pathogen-associated molecular patterns (PAMPs), which include viral proteins and nucleic acids, are sensed by TLRs, including TLR2, TLR3, TLR4, TLR7, TLR8, and TLR9 (Lester & Li, 2014). Consequently, the stimulation of these TLRs initiates distinct signaling pathways, which ultimately result in an antiviral response that is orchestrated by the increased production of interferons (IFNs), pro-inflammatory cytokines and chemokines (Lester & Li, 2014). These antiviral responses are influenced by two significant signal transduction pathways: MYD88 and TRIF, which both result in the activation of NF- κ B. It is imperative to emphasize that the NF- κ B complex (which includes RELA and REL) was the most significant transcription factor associated with our PPI network, as demonstrated by our TRRUST transcription factor enrichment analysis. The NF- κ B pathway induces proinflammatory cytokines and contributes to an increase in IFN production (Lester & Li, 2014). The NF- κ B subunit RELA

exhibits a dual function in viral infections. Firstly, RELA traverses the nucleus to maintain baseline and autocrine IFN- β signaling in uninfected cells, a process crucial to immune responses against viral infections. Secondly, RELA amplifies inflammatory and adaptive immune responses following infection and may facilitate the survival of infected cells throughout this phase (Thapa et al., 2011).

Cell signaling, cytoskeleton regulation, organelle identity, and membrane dynamics are all significantly influenced by phosphatidylinositol phosphate kinases. Numerous viral infections target this pathway (Beziau et al., 2020). Sphingolipids, which are crucial components of cell membranes, are involved in cell signaling, and their metabolites are essential for the host's defense against viral infections (Thomas et al., 2023). The IRS response to viral infections is significantly influenced by perturbations in sphingolipid metabolism (Janneh et al., 2021). Ceramides, a type of sphingolipid, may also function as a receptor for viral entry into host cells, thereby facilitating viral replication (Thomas et al., 2023). Also, phospholipase D may facilitate the entry of certain viruses into host cells and contribute to viral infections (Oguin et al., 2014). The FOXO signaling pathway is responsible for the regulation of IRS responses, oxidative stress, Th1 activation, and the enhancement of FOXP3 and Treg cell formation and, consequently, the maintenance of cellular quality (e.g. redox and inflammatory homeostasis) (Cabrera-Ortega et al., 2017). The FOXO regulatory pathway is crucial in viral infections, including influenza, as it mediates anti-inflammatory and anti-apoptotic mechanisms (Wu et al., 2019).

Conclusions

The primary organizational backbone nodes and key therapeutic targets for MDMD identified through our network analysis are TNF, IL6, CXCL12, CXCL10, CCL5, CD4, CD8A,

HLA-DR, and FOXP3. Functional enrichment revealed that the system spontaneously organizes around cellular defense mechanisms, IRS activation, and cytokine–receptor cascades, with marked overrepresentation of viral processes involving viral protein interactions with cytokine receptors. MCODE analysis resolved a tightly coordinated, higher-order molecular component integrating viral–immune pathways regulated by the NF- κ B subunit RELA. These findings suggest that viral mechanisms may serve as higher-order drivers orchestrating the self-organized activation of T cells, cytokines, and chemokine circuits in MDMD.

Future research into the immunological architecture of MDMD and MDD should focus on these coordinated multidimensional circuits, particularly understudied chemokine mediators, flow-cytometry-quantified CD and intracellular markers, and the RELA-regulated NF- κ B pathway. Longitudinal studies are needed to determine whether specific viral triggers or reactivations—such as HCMV, EBV, and HHV-6—act as critical tipping points for illness onset and recurrence. Translating these findings into clinical practice will require interventions capable of restoring systemic network equilibrium, targeting the dysregulated chemokine network (notably CCL5, CXCL10, and CXCL12), aberrant T cell activation (CD4, CD8, and HLA-DR), and attenuated FOXP3 expression.

Acknowledgments

The authors express gratitude to the personnel of Al-Hakeem General Hospital for their contributions to data collecting. This manuscript has been previously posted as a preprint on medRxiv and Research Square (doi<https://doi.org/10.1101/2024.11.05.24316765>), (<https://doi.org/10.21203/rs.3.rs-5396280/v1>) (Maes, Zhang, et al., 2024).

Ethical approval and consent to participate.

This study involves a secondary data analysis using open, deidentified, and non-coded data sets, so exempting it from IRB approval as non-human subjects' research. The utilized data are derived from two case-control investigations (Almulla et al., 2024; Maes, Zhou, Rachayon, et al., 2024). Both case-control studies received approval from the local Institutional Review Boards, and all participants furnished signed informed consent before their participation in the study. The Institutional Review Board of the College of Medical Technology of the Islamic University of Najaf, Iraq, approved the study under Document No. 18/2021. The research procedure by Maes et al. (2024) was sanctioned by the Institutional Review Board of Chulalongkorn University, Faculty of Medicine, Bangkok, Thailand (#528/63).

Declaration of interest

The authors assert the absence of any conflicts of interest. Michael Maes, Editor-in-Chief of the journal *Medicine Bulletin*, was not involved in the editorial handling, peer review, or decision-making process for this manuscript.

Funding

The C2F program at Chulalongkorn University in Thailand, grant number 64.310/436/2565 to AFA, the Thailand Science Research, and Innovation Fund at Chulalongkorn University (HEA663000016), and a Sompoch Endowment Fund (Faculty of Medicine) MDCU (RA66/016) to MM all provided funding for the project.

Author's contributions

MM and AFA designed the study. MM, KP and AS conducted the study's statistical analysis. MM. wrote the first draft of the paper AFA, KP, AS, YZ, and JL further edited the paper. All authors have read and approved the final manuscript. AS was funded by National Science, Research and Innovation Fund (NSRF), and King Mongkut's University of Technology North Bangkok (Project no. KMUTNB-FF-67-B-24).

Availability of data

All data used in the study are made available in the Tables and Figures.

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Table 1. Average (Avg) expression of the serum cytokines, chemokines and growth factors measured in patients with major dysmood disorder (MDMD) and healthy controls (HC) and their computed weights, which are used in enrichment analysis.

Name	Gene ID	Avg in HC	Avg in MDMD	Weight
C-C motif chemokine ligand 27	CCL27	-0.993200333	0.387590374	1.380791
C-C motif chemokine ligand 11	CCL11	-0.498178032	0.19441094	0.692589
Colony stimulating factor 3	CSF3	0.051415714	-0.020064669	-0.07148
Colony stimulating factor 2	CSF2	-0.340418184	0.132846121	0.473264
C-X-C motif ligand 1	CXCL1	-0.522932743	0.204071314	0.727004
Hepatocyte growth factor	HGF	-0.968750474	0.378048965	1.346799
Interferon gamma	IFNG	-0.222619502	0.086875903	0.309495
Interleukin 1-alpha	IL1A	0.081109168	-0.031652358	-0.11276
Interleukin 1-beta	IL1B	0.166606711	-0.065017253	-0.23162
Interleukin 1 receptor antagonist	IL1R1	-0.670766609	0.261762579	0.932529
Interleukin 2	IL2	0.065999419	-0.025755871	-0.09176
Interleukin 2 receptor	IL2RA	-0.827629261	0.322977273	1.150607
Interleukin 4	IL4	-0.276729039	0.10799182	0.384721
Interleukin 5	IL5	0.212457109	-0.082910091	-0.29537
Interleukin 6	IL6	-0.505301334	0.197190765	0.702492
C-X-C motif ligand 8	CXCL8	-0.108893613	0.042495068	0.151389
Interleukin 9	IL9	-0.702492752	0.274143513	0.976636
Interleukin 10	IL10	-0.059922232	0.023384286	0.083307
Interleukin 12	IL12P70	-0.260999911	0.101853624	0.362854
Interleukin 13	IL13	-0.26312619	0.102683391	0.36581
Interleukin 15	IL15	-0.275978897	0.107699082	0.383678
Interleukin 16	IL16	-1.17127045	0.457081151	1.628352
Interleukin 17	IL17A	-0.291181273	0.113631716	0.404813
Interleukin 18	IL18	-0.679701455	0.265249348	0.944951
C-X-C motif ligand 10	IP10	-0.548759209	0.214149935	0.762909
Leukemia inhibitory factor	LIF	-0.2147785	0.083816	0.298595
C-C Motif Chemokine Ligand 2	CCL2	-0.520846587	0.203257205	0.724104

C-C Motif Chemokine Ligand 7	CCL7	-0.246266484	0.096103994	0.34237
Colony stimulating factor 1	CSF1	-0.992314209	0.387244569	1.379559
Macrophage migration inhibitory factor	MIF	-0.191819097	0.074856233	0.266675
C-X-C motif ligand 9	CXCL9	-0.430871698	0.168145053	0.599017
C-C Motif Chemokine Ligand 3	CCL3	0.192464412	-0.075108063	-0.26757
C-C Motif Chemokine Ligand 4	CCL4	-0.711276431	0.27757129	0.988848
Nerve Growth Factor-beta	BNGF	-0.394633987	0.154003507	0.548637
Platelet-derived growth factor	PDGFBB	-0.999472898	0.390038204	1.389511
C-C Motif Chemokine Ligand 5	CCL5	-0.840278477	0.327913552	1.168192
Stem cell factor	KITLG	-0.679492209	0.265167691	0.94466
Stem cell growth factor	SCGFB	-1.1196397	0.436932566	1.556572
C-X-C motif ligand 12	CXCL12	-0.735480778	0.287016889	1.022498
Tumor necrosis factor-alpha	TNF	-0.506191239	0.197538045	0.703729
Lymphotoxin alpha	LTA	-0.721703817	0.281640514	1.003344
TNF Superfamily Member 10	TNFSF10	-1.04690335	0.408547649	1.455451
Vascular endothelial growth factor	VFGFA	0.144462726	-0.056375698	-0.20084

Table 2. Results of Weighted Enrichment Analysis Tools (WEAT) with the KEGG pathways associated with major dysmood disorder

Term	Counts	OR	Scores	W OR	W P-value	Bonferroni	FDR
Cytokine-cytokine receptor interaction	34/295	341.17	40.27/301.27	254.25	3.20e-53	1.12e-50	1.12e-50
Viral protein interaction with cytokine and cytokine receptor	20/100	118.21	27.98/107.98	164.79	6.90e-41	2.41e-38	1.21e-38
Rheumatoid arthritis	16/93	79.56	16.62/93.62	61.07	9.97e-21	3.49e-18	1.16e-18
TNF signaling pathway	11/112	33.58	13.43/114.43	33.72	1.77e-14	6.18e-12	1.54e-12
Hematopoietic cell lineage	12/99	44.31	12.55/99.55	35.64	9.86e-14	3.45e-11	6.90e-12
Malaria	10/50	74.8	9.52/49.52	54.1	3.32e-12	1.16e-09	1.94e-10
Chemokine signaling pathway	11/192	18.55	13.99/194.99	19.75	1.85e-11	6.47e-09	9.25e-10
IL-17 signaling pathway	15/94	69.38	10.82/89.82	32.17	3.17e-11	1.11e-08	1.39e-09
Lipid and atherosclerosis	10/215	14.3	12.08/217.08	14.15	1.11e-09	3.88e-07	4.31e-08
Influenza A	10/171	18.3	11.58/172.58	17.1	1.34e-09	4.69e-07	4.69e-08
NF-kappa B signaling pathway	8/104	23.05	9.70/105.70	22.94	3.86e-09	1.35e-06	1.23e-07
Human cytomegalovirus infection	10/225	13.61	11.39/226.39	12.44	2.35e-08	8.24e-06	6.47e-07
Asthma	7/31	78.69	5.79/29.79	49.71	2.40e-08	8.41e-06	6.47e-07
Inflammatory bowel disease	12/65	73.04	7.69/60.69	31.35	2.79e-08	9.78e-06	6.99e-07
JAK-STAT signaling pathway	13/162	28.97	9.90/158.90	15.07	1.40e-07	4.91e-05	3.28e-06
Chagas disease	10/102	32.31	7.56/99.56	17.61	9.00e-07	3.15e-04	1.96e-05
Amoebiasis	9/102	27.74	7.40/100.40	16.96	9.53e-07	3.33e-04	1.96e-05
Cytosolic DNA-sensing pathway	5/63	21.71	6.74/64.74	24.44	1.11e-06	3.87e-04	2.15e-05
Toll-like receptor signaling pathway	8/104	23.05	7.84/103.84	17.6	1.20e-06	4.20e-04	2.20e-05

W: weighted; FDR: false discovery rate.

Table 3. Results of Molecular Complex Detection (MCODE) analysis performed on the differently expressed genes of major dysmood disorder.

Networks	Pathway	GO	Annotation (Log10P)
MCODE_All	KEGG	hsa04060	Cytokine-cytokine receptor interaction (-34.9)
	KEGG	hsa04061	Viral protein interaction with cytokine and cytokine receptor (-29.2)
	TRRUST	TRR01158	Regulated by RELA (-21.1)
SUB MCODE_1	KEGG	hsa04060	Cytokine-cytokine receptor interaction (-34.9)
	KEGG	hsa04061	Viral protein interaction with cytokine and cytokine receptor (-29.2)
	TTRUST	TRR01158	Regulated by RELA (-21.1)

Table 4. Panther biological processes associated with major dysmood disorder.

Pathway	Total	Expected	Hits	p value	FDR
Immune response	387	6.59	106	3.99E-106	7.74E-104
Viral process	448	7.63	48	2.06E-25	1.99E-23
Immune system process	515	8.77	44	2.40E-19	1.55E-17
Cell-cell signaling	232	3.95	30	2.66E-18	1.29E-16
Antigen processing and presentation	43	0.732	15	1.75E-16	6.81E-15
Antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	16	0.272	9	1.04E-12	3.37E-11
Apoptotic process	699	11.9	40	8.46E-12	2.34E-10
Negative regulation of apoptotic process	577	9.82	32	3.00E-09	7.28E-08
Cellular defense response	59	1	11	3.66E-09	7.89E-08
Cellular calcium ion homeostasis	102	1.74	11	1.23E-06	2.39E-05
Protein phosphorylation	627	10.7	24	0.000169	0.00298
Cytokine production	31	0.528	4	0.00179	0.029
Defense response to bacterium	164	2.79	9	0.00197	0.0294
Cell adhesion	629	10.7	21	0.00244	0.0338

FDR: false discovery rate p correction

Table 5. KEGG gene/protein processes associated with major dysmood disorder.

Pathway	Total	Expected	Hits	P.Value	FDR
Phosphatidylinositol signaling system	100	2.61	70	6.43E-92	2.16E-89
FoxO signaling pathway	294	7.68	84	2.05E-68	3.44E-66
Sphingolipid signaling pathway	189	4.94	63	6.63E-55	7.43E-53
Phospholipase D signaling pathway	102	2.66	40	9.53E-38	8.01E-36
Thermogenesis	112	2.93	40	8.15E-36	5.48E-34
Chemical carcinogenesis	201	5.25	47	1.38E-32	7.71E-31
Dilated cardiomyopathy (DCM)	38	0.993	25	2.47E-31	1.18E-29
TNF signaling pathway	107	2.79	36	3.71E-31	1.56E-29
Fc epsilon RI signaling pathway	94	2.46	33	2.73E-29	1.02E-27
Viral myocarditis	41	1.07	24	2.72E-28	9.14E-27
Proximal tubule bicarbonate reclamation	43	1.12	24	1.37E-27	4.20E-26
Epstein-Barr virus infection	170	4.44	38	1.53E-25	4.30E-24
Hepatitis B	112	2.93	32	3.43E-25	8.86E-24
Transcriptional misregulation in cancer	186	4.86	39	4.25E-25	1.02E-23
JAK-STAT signaling pathway	104	2.72	31	4.75E-25	1.06E-23

FDR: false discovery rate p correction

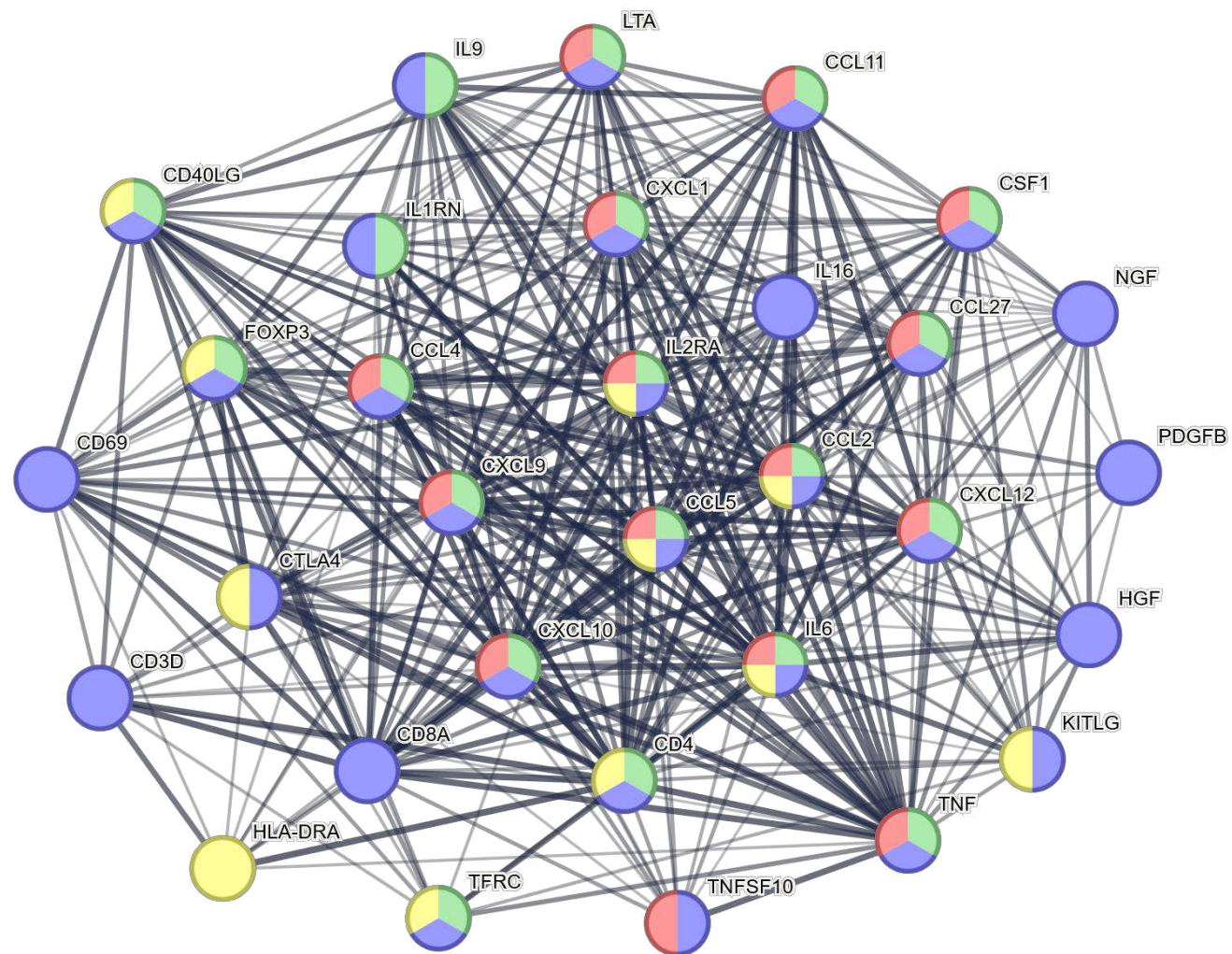


Figure 1. Zero-order protein-protein interaction network of the differently expressed cytokine / chemokine/ growth factor/CD marker genes of major dysmood disorder. Yellow: regulation of T cell activation, Red: Viral protein interaction with cytokine an cytokine receptor, Green: Defense response, Pink: cellular response to a stimulus, Al nodes: response to a stimulus.

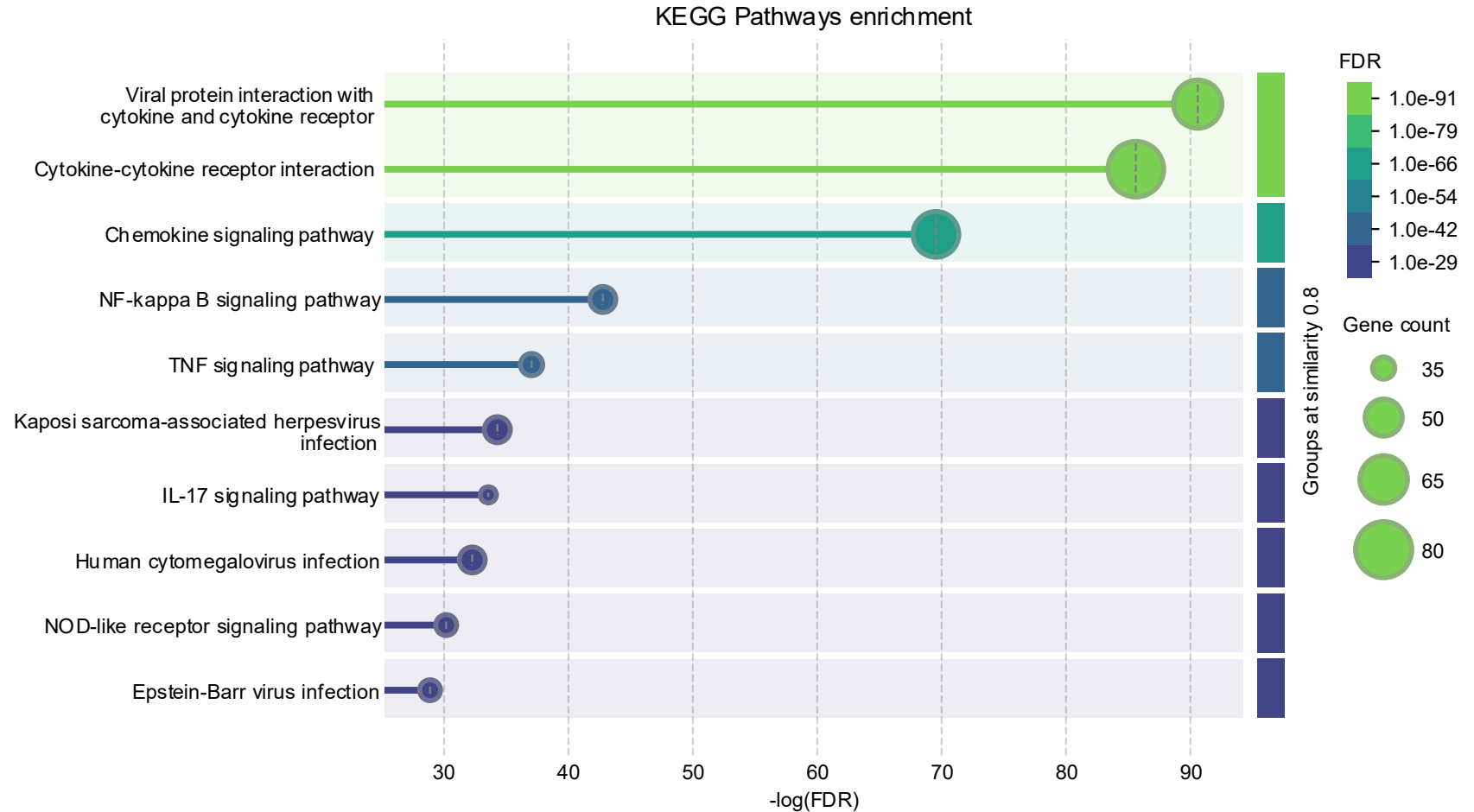


Figure 2. Functional enrichment visualization using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway.

Used are the differently expressed cytokine / chemokine/ growth factor/ CD marker genes of major dysmood disorder.

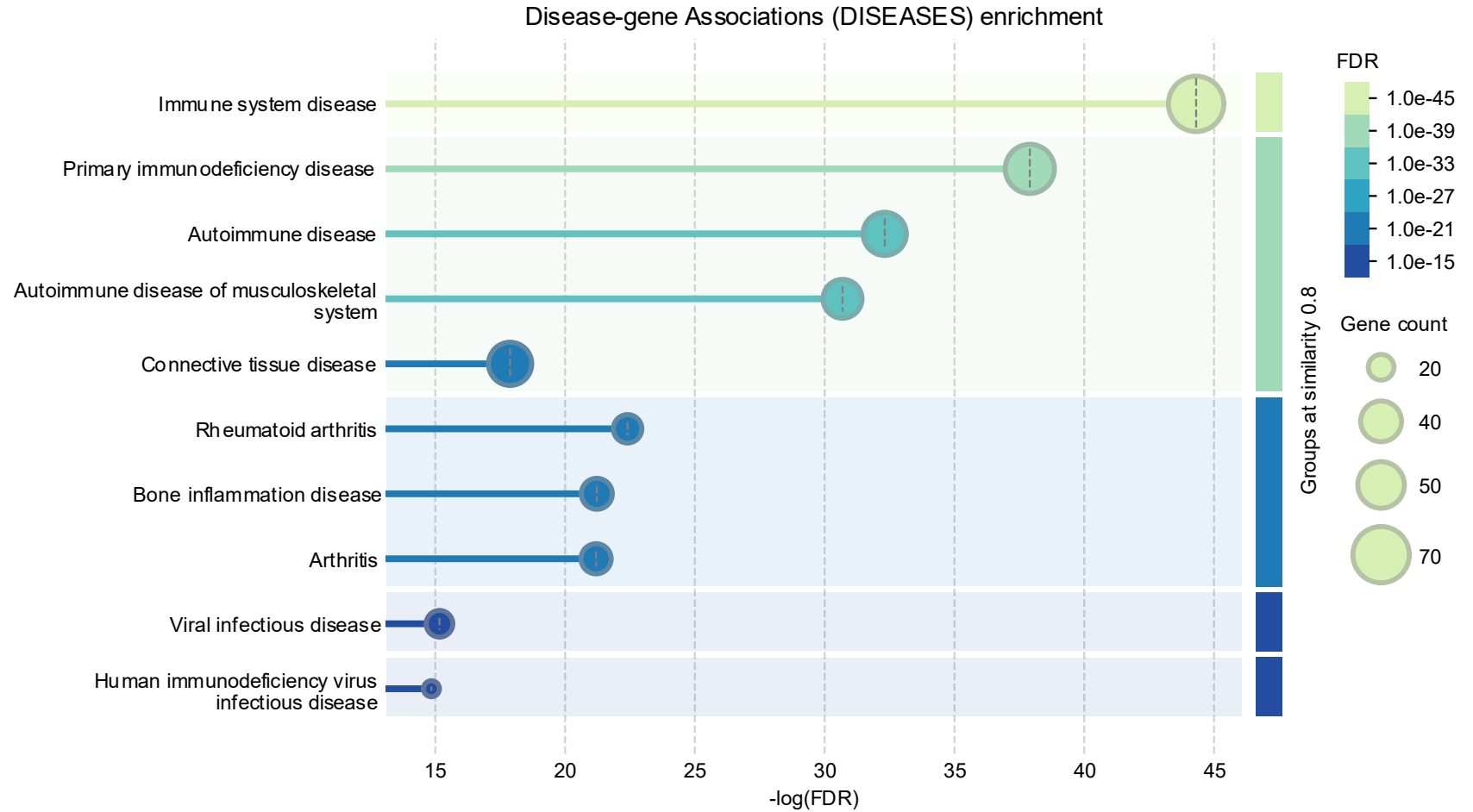


Figure 3. Functional enrichment visualization of Disease-Gene Associations. Used are the differently expressed cytokine / chemokine/ growth factor/ CD marker genes of major dysmood disorder.

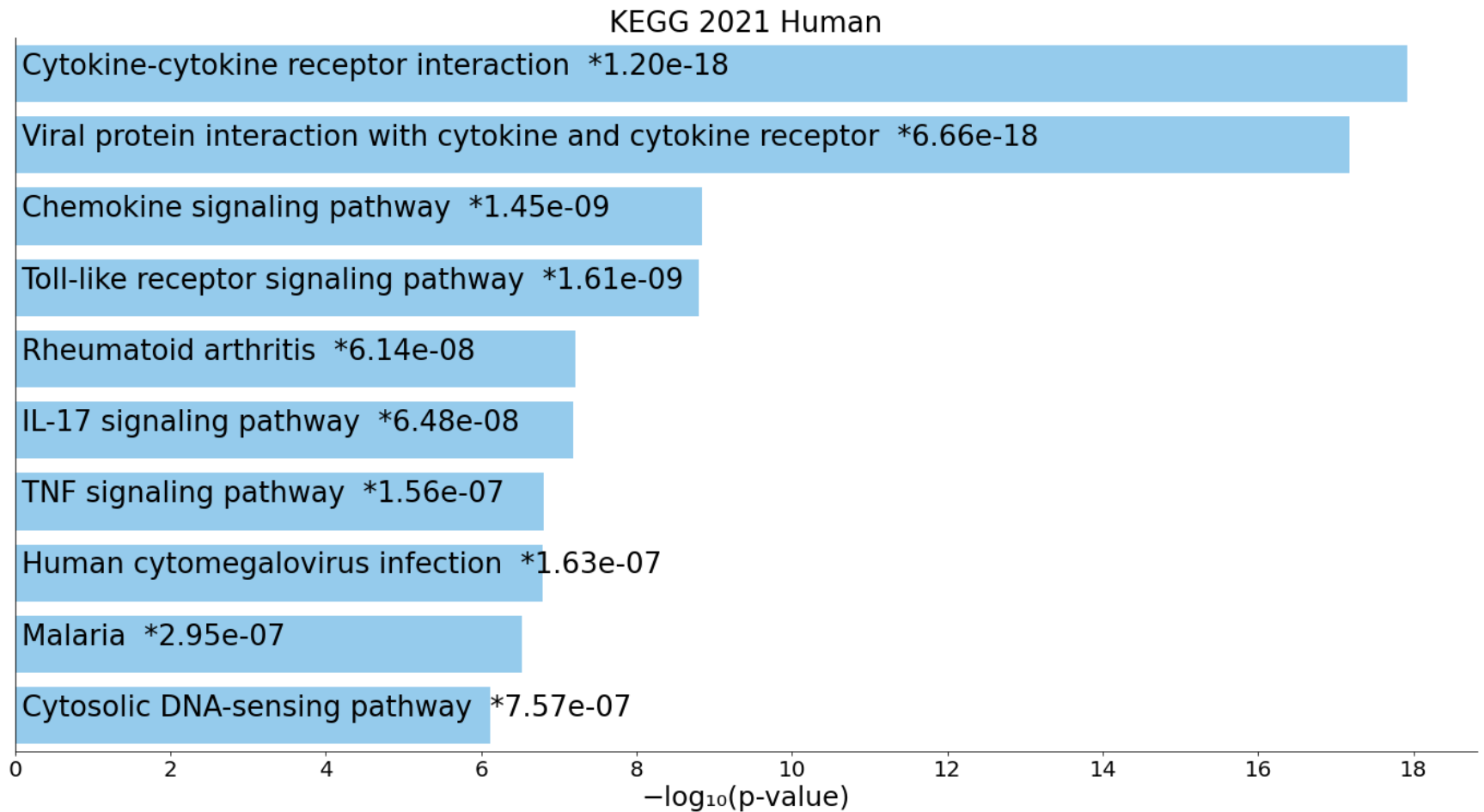


Figure 4. Heatmap (top-10) of the KEGG pathways accumulated in the differently expressed proteins of the zero-order network of major dysmood disorder.

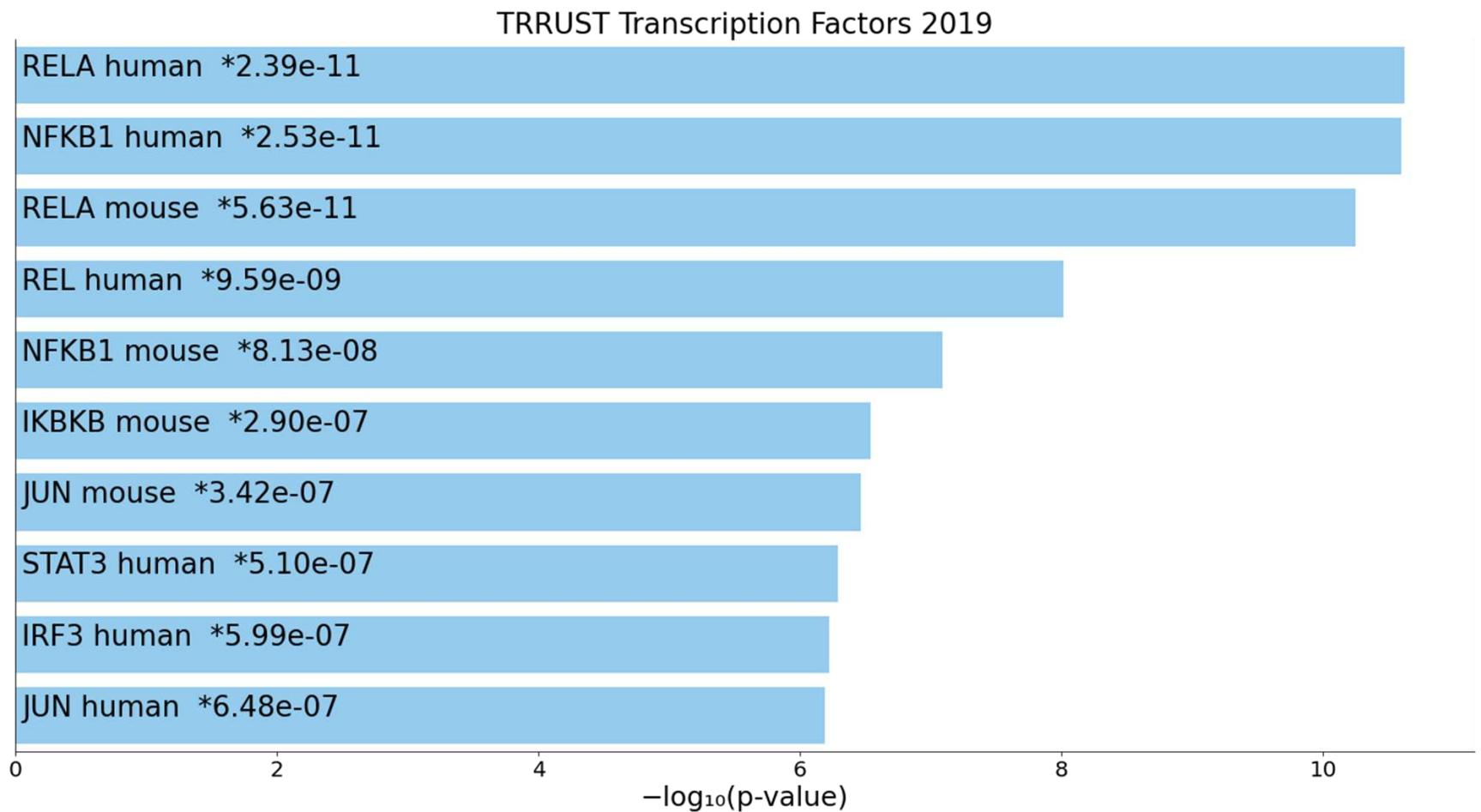


Figure 5. Heatmap (top-10) of the Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining (TRRUST) transcription factors accumulated in the differently expressed genes of the zero-order network of major dysmood disorder.

Supplementary Files

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