Pathways in the brain, heart and lung influenced by SARS-CoV-2 NSP6 and SARS-CoV-2 regulated miRNAs: an *in silico* study hinting cancer incidence

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Abstract

The influence of SARS-CoV-2 non-structural protein in the host's tissue-specific complexities remains a mystery and needs more in-depth attention because of COVID-19 recurrence and long COVID. Here we investigated the influence of SARS-CoV-2 transmembrane protein NSP6 (Non-structural protein 6) in three major organs - the brain, heart, and lung in silico. To elucidate the interplay between NSP6 and host proteins, we analyzed the protein-protein interaction network of proteins interacting with NSP6 interacting proteins. Reported host interacting partners of NSP6 were ATP5MG, ATP6AP1, ATP13A3, and SIGMAR1. Pathway enrichment analyses provided global insights into biological pathways governed by differentially regulated genes in the three tissues after COVID-19 infection. Hub genes of tissue-specific protein interactome were analysed for drug targets and many were found. miRNA-gene network for the tissue-specific regulated proteins was sought. Comparing this list with the gene list targetted by SARS-CoV-2 regulated miRNAs, we found three and two common genes in the brain and lung respectively. Among the five common proteins revealed as potential therapeutic targets across the three tissues, four non-approved drugs and one approved drug could target Galectin 3 (LGALS3) and AIFM1 respectively. Increased expression of LGALS3 (that was upregulated in the heart after COVID-19 infection) is observed in multiple cancers and acts as a modulator for tumor progression. COVID-19 infection also causes myocardial inflammation and heart failure (HF). HF is observed to be increasing cancer incidence. The present scenario of long COVID-19 and recurrent COVID-19 infections warrants in-depth studies to probe the effect of COVID-19 infection on increased cancer incidence.

Keywords: SARS-CoV-2 NSP6, tissue-specific, hub genes, pathways, drug targets, cancer incidence, heart failure

1. Introduction

After the disastrous COVID19 pandemic we are forced to live along with COVID-19 as a new normal. Unavailability of specific drugs that directly target COVID-19, ineffectiveness of vaccines against emerging variants and long COVID are some of the prominent responsible factors. In addition, reports of various animals as hosts,[1–5] emergence of variants and detection of these clades in humans coming in contact with them,[6] also increase the risks of emergence of zoonotic variants. Change in the symptoms of COVID-19 and decreased routine testing add to the present troubles in the identification of infected individuals and there by COVID-19 spread. Multiple organ systems get impacted due to COVID-19[7] in acute phase of the disease and several symptoms can persist from several weeks to two years,[8] now termed as "Post-acute sequelae of COVID-19" (PASC).[9] Increased risk of PASC including stroke, various cardiac disorders, after post COVID-19 as well as it's reinfection are well documented.[8–17] In addition other risks include chronic kidney diseases, fatigue, muscle weakness, acute coronary disease, memory problems, acute kidney injury, thromboembolism, constipation, GERD, etc.[8]

Various COVID19 proteins are shown to be interacting with different host proteins.[18,19] Among the multiple proteins coded in the SARS-CoV-2 genome can be mainly divided into structural proteins, nonstructural proteins (NSPs) and accessory proteins.[20–22] 16 NSPs are encoded in ORF1a and ORF1b that have multiple enzymatic functions.[22] Functions include regulation of viral RNA replication, transcription and genome replication.[22] One of the NSP proteins NSP6 formed by the cleavage of ORF1a polyprotein by NSP5 with chymotrypsin-like cysteine protease (3CLpro) activity contains 290 aa.[22,23] Predicted structure using Alphafold proposed "one C-terminal, two anti-parallel beta-sheets, sixteen turns and fourteen α helices".[24] Regarding the transmembrane domains, different reports suggest transmembrane helices as six,[23,25] seven,[26] and eight.[24] NSP6 is reported to be involved in SARS-CoV-2 replication by restricting ER luminal proteins accessing double membrane vesicles (DMVs) but allowing lipid flow, positioning and organising DMV clusters, and facilitating lipid droplets (LDs) through LD-tethering complex DFCP1-RAB18.[23] Observation of emerging deletion mutation of NSP6 (Δ106–108) in different variants[27,23] and its gain of function as reported by Ricciardi et al.,[23] increases its significance to be studied further. Two proteins ATP6AP1 and Sigma–1–receptor were reported to be interacting with NSP6 and are also drug targets.[18] Sigma–1–receptor ligands were observed to be inhibiting SARS-CoV-2 infection in Vero E6 cell line.[18] Overexpression of NSP6, NSP8 and M in stem cell differentiated cardiomyocytes showed altered transcriptome and proteome, and induced apoptosis.[28]

In the present *in silico* work, we analyzed to look for pathways influenced by NSP6 in three tissues, the brain, heart and lung. Proteins interacting with SARS-CoV-2 NSP6 interacting proteins expressed in these tissues and influenced by SARS-CoV-2 (18, 45 and 23 interacting proteins in the brain, heart and lung respectively) were analysed for finding out pathways and biological processes involved by these proteins. In the brain, heart and lung there were 25, 147 and 168 pathways respectively. There were two common pathways in the brain-lung, 52 in lung-heart and five in heart-brain pairs. 14 pathways were present in all three tissues. Unique pathways from Gene Ontology (GO) enrichment analysis revealed 4, 69 and 100 in the brain, heart and lung respectively. To find out the prominent players, hub genes were found and drug targets among these were searched for in the Drug-Gene Interaction Database (DGIdb). In the brain, five hub genes were targetted by 30 approved and 89 non-approved drugs. Four hub genes in the heart, had 44 approved and 56 non-approved drugs targetted, whereas in the lungs 34 approved and 31 non-approved drugs were targetting four hub genes. Finding out miRNA influence in these proteins, putative miRNA players were searched that could influence the proteins of our interest. Out of 82 miRNAs in the brain and 43 miRNAs in the lung number of miRNAs common were 16. In addition, reported

SARS-CoV-2 influenced miRNAs were probed for their targets in the interacting proteins of SARS-CoV-2 NSP6 interacting protein list influenced by SARS-CoV-2 infection in the brain and lung. We were able to find three genes targetted by SARS-CoV-2 influenced miRNAs in the brain and in the lung two genes were targetted.

2. Materials and methods

2.1. SARS-CoV-2 NSP6 interacting proteins

To find out SARS-CoV-2 NSP6 influenced pathways in the brain, heart and lungs, we took list of proteins interacting with SARS-CoV-2 NSP6.[18] Four interacting proteins reported were considered for further analyses.

2.2. Tissue specific genes interacting with NSP6 interacting proteins

For finding the interacting proteins of SARS-CoV-2 NSP6 interacting proteins in a tissue specific manner, IID (Integrated Interactions Database) (http://ophid.utoronto.ca/iid) was used based on two or more experimental pieces of evidence (studies or bioassays).[29] Common genes present in the gene list obtained from IID and genes regulated after SARS-CoV-2 infection in the brain choroid plexus epithelium,[30] heart[31] and lung[32] were used for further analyses. We considered genes having the p-value of ≤ 0.05 for our further analyses. To find out the common and unique genes from different tissues, we used jvenn.[33]

2.3. Construction of Tissue specific protein-protein-interaction network of SARS-CoV-2 NSP6 influenced proteins

Tissue specific and SARS-CoV-2 NSP6 inluenced proteins were used to make protein-protein-interaction network using IID. Common genes present in the gene list obtained from IID and genes regulated after SARS-CoV-2 infection in the brain choroid plexus epithelium,[30] heart[31] and lung[32] were used. In these common genes, those with p-value ≤ 0.05 were selected. Further we discerned up– and downregulated genes, that had a cut off value of $\log_2$ fold change $\pm$ 2.0. These categorized gene lists were poised for subsequent in-depth investigation and analyses.

2.4. Identification of central hub genes in tissue-specific protein-protein interactome

The tissue-specific genes were the input for the construction of protein–protein interaction network in STRING version 12.0[34] with default settings (Network Type: full STRING network, Required score: medium confidence (0.400), FDR stringency: medium (5 percent)). Network was further grown to obtain more tissue specific interactions and these were uploaded in Cytoscape version 3.10.1.[35] Hub genes for tissue-specific network were found using cytoHubba plugin.[36] To initiate the hub gene identification process, a Node Score was calculated, employing the Maximum Clique Centrality (MCC) metric. The MCC metric, the most accurate among the topological analysis methods,[36] focuses on identifying cliques which is a subset of nodes within a network. Based on the node's centrality the MCC calculates the size of the maximum clique by providing rank for a specific node. Subsequently, an analysis was executed, employing the criteria of the top 10 hub genes within the designated PPI network. Further refinement was carried out by selecting parameters pertaining to the first stage node, shortest path, and expanded network analysis. These steps collectively led to the delineation of the hub gene network.

2.5. Gene ontology and KEGG pathway analysis of central hub genes in tissue-specific protein-protein interactome

Subsequent to ranking the top 10 hub genes using the MCC method, we proceeded with a comprehensive pathway analysis. For this purpose, we employed ClueGO, an additional plugin available in the Cytoscape software suite.[37] Using the functional analysis mode, we conducted

targeted searches, with default medium network specificity settings and a pathway significance threshold set at ≤ 0.05 for p-values. With the help of ClueGO, GO terms of the identified hub genes network were based on Gene Ontology (GO) Biological Process, GO Cellular Component, GO Molecular Function, GO Immune System Processes, Reactome pathways, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Kappa score threshold was taken as 0.4 and for statistical analysis, we used Enrichment/Depletion (two-sided hyper geometric test). Kappa score also known as Cohen's coefficient, provides inter-relatability between two functional groups based on the genes shared by them.

2.6. Drug-gene interaction analysis of hub genes in DGIdb

To find out possibility of detected hub genes as probable drug targets, we searched for drugs targeting these genes using the Drug-Gene Interaction database and further identified the gene categories for the probable prognostic hub genes (DGIdb v.5.0.1, https://www.dgidb.org/).[38,39] Interactions of drug-gene network and gene categories were uploaded in Cytoscape version 3.10.1.[35] to construct network.

2.7. Tissue specific pathway enrichment analysis

To obtain the Gene Ontology (GO) IDs for particular tissues, we utilized the R package ClusterProfiler.[40] We executed this process individually for the genes associated with the brain, heart, and lung tissues. This enabled us to extract pathways specific to each tissue. For a more comprehensive analysis, we further categorized the genes into upregulated and downregulated groups. We performed separate analyses on the genes exhibiting upregulation and downregulation to find the biological processes and pathways associated with them. Subsequently, we employed a significance threshold by setting a q-value cutoff of 0.05 and only these significant pathways were considered for dot-plot construction by using ggplot2 package in R.[41]

2.8. Tissue-Specific miRNA Network Construction

NSP6 may regulate the activities of a diverse array of miRNAs. miRNAs are well-known influencers of gene expression. Using miRNet (https://www.mirnet.ca),[42] we have identified and constructed a tissue-specific interaction network of SARS-CoV-2 influenced interacting partners of SARS-CoV-2 NSP6 interacting 4 proteins with miRNAs. Based on the availability of data in miRNet, we only constructed miRNA networks for the brain and lung. The degree of a node has been calculated by the number of other nodes it connected, and betweenness centrality signifies the bridging role of a node in a network. We have used the GO-BP and KEGG database for functional enrichment analysis of the miRNA network and identified significant ($p \leq 0.05$) biological processes associated with the miRNA networks. Enrichment analyses were based on the hypergeometric tests after adjustment for FDR.

2.9. Tissue-Specific miRNA Network Construction for heart

Tissue filter was absent for heart in miRNet. We tried to find possible regulated pathways in a different way using miRNet. Circulating miRNAs regulated by SARS-CoV-2 infection were chosen to find out the target genes. Among these genes, only those were chosen that were regulated by SARS-CoV-2 infection in heart. miRNA-mRNA interactome for these genes and their respective miRNA were deduced.

2.10. Comparison of SARS-CoV-2 influenced circulatory miRNAs and tissue specific miRNAs targeting interacting proteins of SARS-CoV-2 NSP6 interacting proteins

As SARS-CoV2 influences miRNAs, we wanted to study the regulated circulatory miRNAs and miRNAs that target SARS-CoV2-regulated proteins interacting with SARS-CoV2 NSP6 binding proteins in the brain and lung. Common miRNAs in the reported list of SARS-CoV2-influenced circulating miRNAs[43,44] and tissue specific miRNAs influencing interacting proteins of SARS-CoV2 NSP6 binding proteins were probed to find out regulated miRNAs and their respective target proteins.

3. Results

3.1. Interacting partners of NSP6 interacting proteins varies in different tissues

SARS-CoV-2 NSP6 is reported to interact with four host proteins, namely ATP synthase membrane subunit g (ATP5MG), ATPase H+ transporting accessory protein-1 (ATP6AP1), ATPase 13A3 (ATP13A3), Sigma-1 receptor (SIGMAR1).[18] To know the tissue specific effect in the brain, heart and lung, interactome analyses were done to identify interacting partners of these four NSP6 interacting proteins in each of the tissues. Proteins were selected that were regulated after COVID infection[30–32] as well as that were expressed in these tissues. Tissue specific experiment-based interaction network from IID data base analyses revealed 18, 45 and 23 interacting proteins in the brain, heart and lung respectively (Supporting Information Data 1). By using Venn diagram analysis (Figure 1a), we analyzed common and unique proteins among the brain, heart and lung. Results showed 5 of these interacting proteins were common in these three tissues and they were apoptosis inducing factor mitochondria associated 1 (AIFM1), ATPase H+ transporting V0 subunit d1 (ATP6V0D1), tectonic family member 2 (TCTN2), galectin 3 (LGALS3), and pleckstrin homology like domain family A member 3 (PHLDA3). Regulation of these genes in different tissues are shown in figure 1b. Apart from this we further observed that ten interacting proteins interferon-induced transmembrane protein 3 (IFITM3), galectin-9 (LGALS9), P2Y receptor family member 8 (P2RY8), leucine-rich repeat kinase 2 (LRRK2), transforming growth factor beta receptor 2 (TGFBR2), amyloid beta precursor protein binding family B member (APBB1), Erb-b2 receptor tyrosine kinase 2(ERBB2), Protein interacting with PRKCA 1 (PICK1), Synoviolin 1 (SYVN1), and PDZ domain containing 11 (PDZD11) were common between the heart and lung. Two interacting proteins, transmembrane and coiled-coil domains 3 (TMCO3) and ATPase H+ transporting V0 subunit a2 (ATP6V0A2) were common in the brain and lung, while in the brain and heart, eight shared proteins were observed, namely ATPase H+ transporting accessory protein 1 (ATP6AP1), tectonic family member 3 (TCTN3), ATPase 13A3 (ATP13A3), calnexin (CANX), caveolin 2 (CAV2), mitogen-activated protein kinase 6 (MAPK6), stomatin (STOM), and tubulin alpha 1c (TUBA1C).

Considering tissue specific regulated unique proteins, only three were present in the brain namely golgi transport 1B (GOLT1B), reticulon 1 (RTN1), cyclin-dependent kinase 2 (CDK2). 22 proteins were unique in the heart and they were Yip1 domain family member 3 (YIPF3), mitochondrial ribosomal protein L58 (MRPL58), amyloid beta precursor protein (APP), immunoglobulin superfamily containing leucine-rich repeat (ISLR), serpin family H member 1 (SERPINH1), lysosomal-associated membrane protein 2 (LAMP2), transmembrane protein 216 (TMEM216), adhesion G protein-coupled receptor G5 (ADGRG5), ATP synthase membrane subunit g (ATP5MG), trans-golgi network protein 2 (TGOLN2), HscB mitochondrial iron-sulfur cluster co-chaperone (HSCB), transmembrane protein 67 (TMEM67), myelin expression factor 2 (MYEF2), RAB7A, member RAS oncogene family (RAB7A), pyrimindinergic receptor P2Y6 (P2RY6), solute carrier family 15 member 3 (SLC15A3), sigma non-opioid intracellular receptor 1 (SIGMAR1), transmembrane protein adipocyte associated 1 (TPRA1), SH3 domain-containing GRB2-like (SH3GLB1), estrogen receptor 1 (ESR1), galectin-8 (LGALS8), sideroflexin 1 (SFXN1). In the lungs, six unique proteins observed were angiogenin (ANG), drebrin 1 (DBN1), battenin(CLN3), POC5 centriolar protein (POC5), acyl-CoA dehydrogenase family member 9 (ACAD9), and pericentriolar material 1 (PCM1).

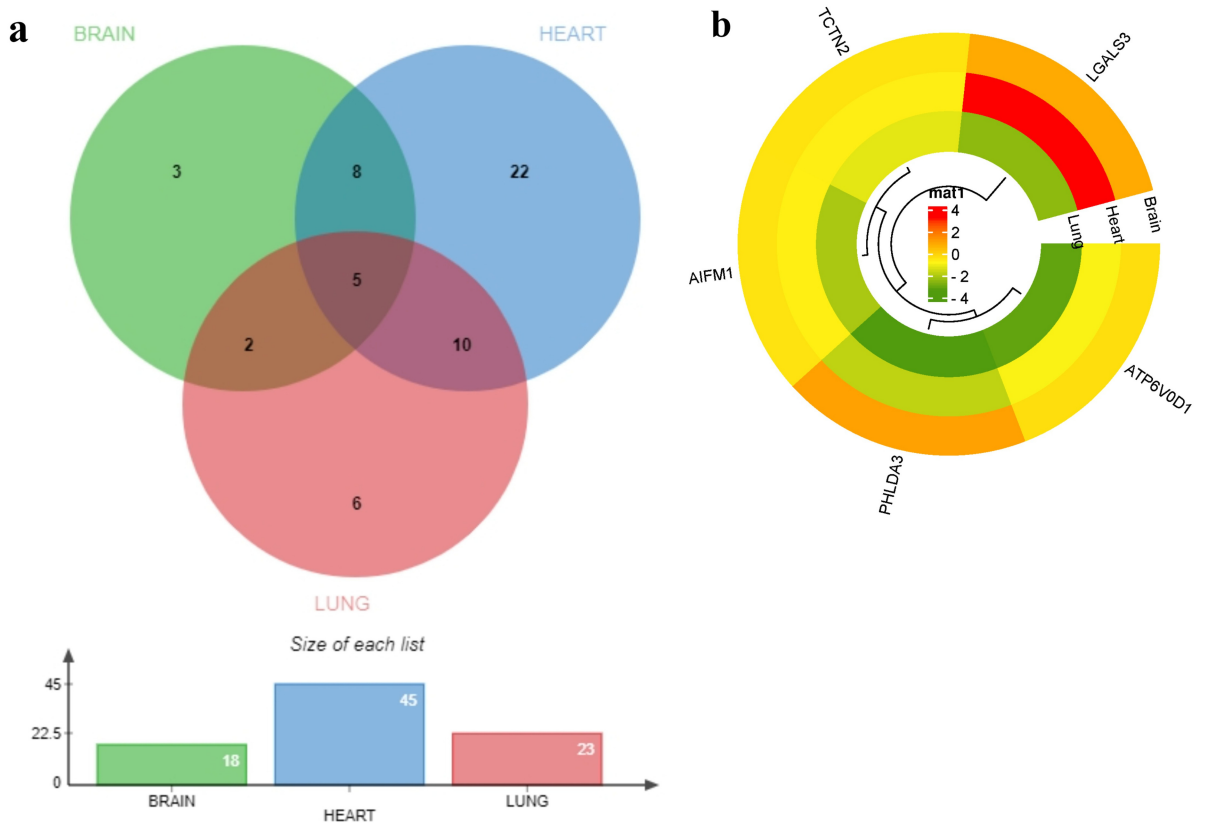


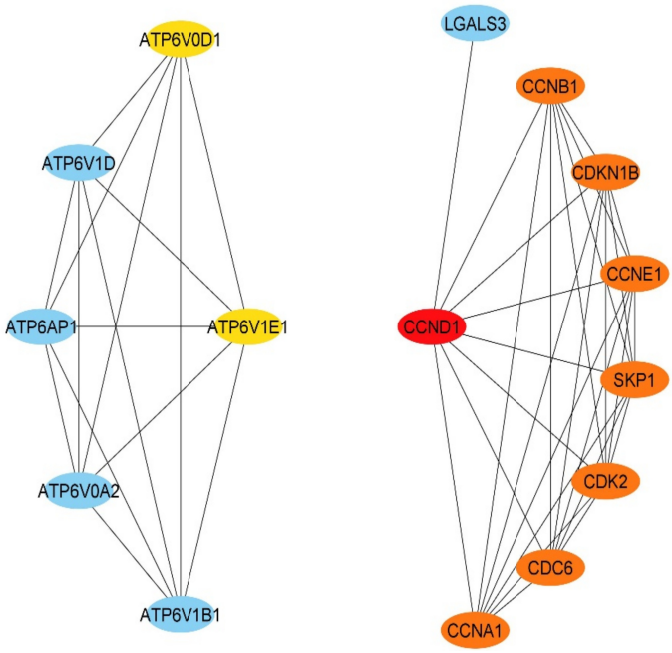
Figure 1. a) Venn diagram showing the number of proteins interacting with SARS-CoV-2 NSP6 interacting proteins in different tissues influenced by SARS-CoV-2 infection. Number of proteins in the brain, heart and lung were 18, 45 and 23 respectively. Among these, 5 genes were common in brain, lung and heart. Common genes between the brain and heart were 8, heart and lung were 10, and lung and brain were 2. **b)** Circular cluster heatmap showing expression in fold change of the 5 common genes in the brain heart and lung.

3.2. Hub genes network and its associated pathways

3.2.1. Hub genes network

After finding out various proteins interacting with SARS-CoV-2 NSP6 in a tissue specific manner, we wanted to find out networks and the prominent players. For this STRING database was used. In the STRING database, the compilation of tissue specific regulated genes pertaining to the brain, heart and lung is employed to construct a Protein-Protein (PP) Interactome network, consisting of the query proteins and their primary interactors of the first shell. Within the context of the brain tissue, this interactome yielded a network characterized by the following features: a total count of 28 nodes, accompanied by 46 edges, thereby resulting in an average node degree of 3.29. Similarly in the heart, the PP interactome revealed 50 nodes corresponding to 70 edges along with an average node degree of 2.8. Where as in the lung, the PP interactome consisted of 33 nodes and 38 edges, having an average node degree of 2.3.

For the key genes in the PP interactome network of the brain, lung and heart, we identified top 10 hub genes in cytoHubba (Supporting Information Data 2). In brain, these hub genes were cyclin D1 (CCND1), cyclin-dependent kinase 2 (CDK2), cyclin A1 (CCNA1), cell division cycle 6 (CDC6), cyclin-dependent kinase inhibitor 1B (CDKN1B), S-phase kinase-associated protein 1 (SKP1), cyclin B1 (CCNB1), cyclin E1 (CCNE1), ATPase H+ transporting V1 subunit B1 (ATP6V1B1), and ATPase H+ transporting V1 subunit E1 (ATP6V1E1) and among them CCND1 was the most significant one (Figure 2a). Similarly in the heart, the ras-related protein Rab-7a (RAB7A), ATPase H+ transporting V0 subunit d1 (ATP6V0D1), lysosomal-associated membrane protein 2 (LAMP2), transforming growth factor beta 1 (TGFB1), calnexin (CANX), galectin-3 (LGALS3), ATPase H+ transporting V1 subunit subunit B1 (ATP6V1B1), ATPase H+ transporting V1 subunit D (ATP6V1D), ATPase H+-transporting accessory protein 1 (ATP6AP1), and Amyloid beta precursor protein (APP) were the hub genes where RAB7A, ATP6V0D1 and LAMP2 were more significant than other hub genes (Figure 2b). In case of the lungs, ATPase H+ transporting V0 subunit a1 (ATP6V0A1), ATPase H+ transporting V0 subunit a2 (ATP6V0A2), ATP6V0D1, ATP6V1D, ATPase H+ transporting V1 subunit E1 (ATP6V1E1), Transforming growth factor beta 1 (TGFB1), Epidermal growth factor receptor-binding protein 1 (EGFB1), Epidermal growth factor (EGF), Transforming growth factor beta receptor 2 (TGFBR2), and Galectin-3 (LGALS3) were identified as hub genes (Figure 2c).



Rank	Node
1	CCND1
2	CDK2
2	CCNA1
2	CDC6
2	CDKN1B
2	SKP1
2	CCNB1
2	CCNE1
9	ATP6V1B1
9	ATP6V1E1

Figure 2a. Top 10 hub genes of the NSP6 interactome proteins from STRINGdb analyzed by cytoHubba in the brain. Node color from red to yellow represents higher to lower significance. Analysis showed CCND1 to be the highly significant major hub gene.

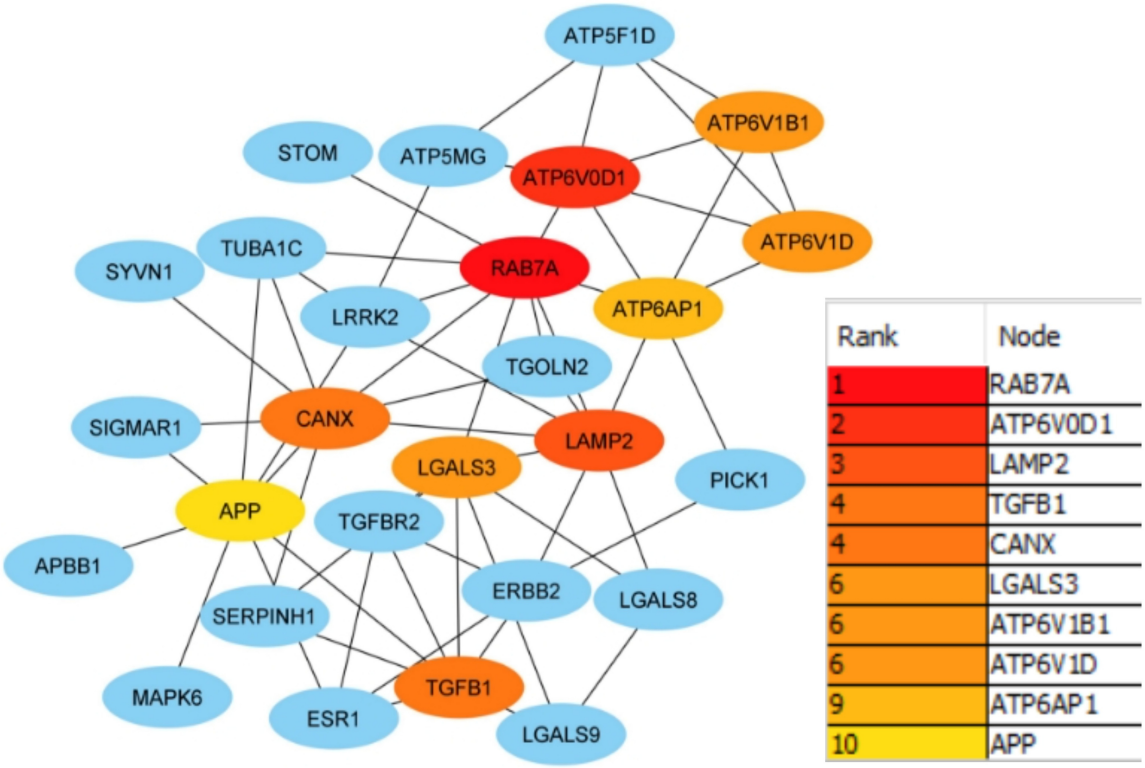
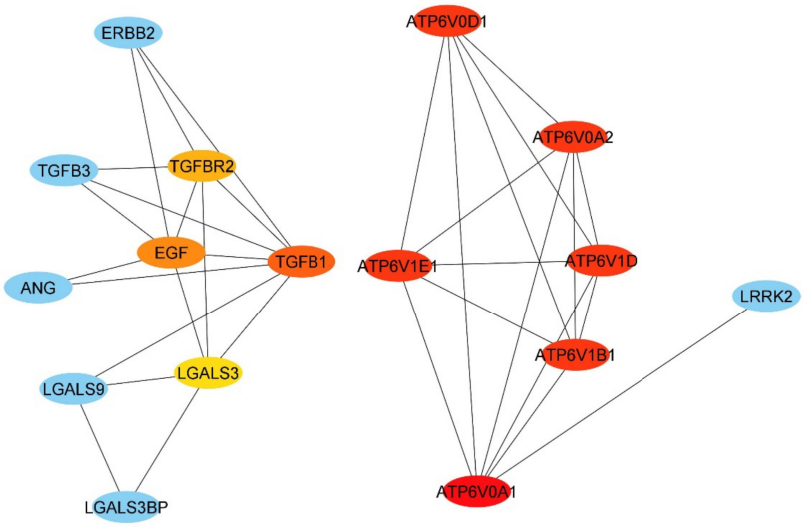


Figure 2b. Top 10 hub genes of the NSP6 interactome proteins from STRINGdb analyzed by cytoHubba in the heart. Node color from red to yellow represents higher to lower significance. Analysis showed RAB7A, ATP6V0D1 and LAMP2 to be the highly significant major hub genes.



Rank	Node
1	ATP6V0A1
2	ATP6V0A2
2	ATP6V0D1
2	ATP6V1D
2	ATP6V1B1
2	ATP6V1E1
7	TGFB1
8	EGF
9	TGFBR2
10	LGALS3

Figure 2c. Top 10 hub genes of the NSP6 interactome proteins from STRINGdb analyzed by cytoHubba in the lung. Node color from red to yellow represents higher to lower significance. Analysis showed ATP6V0A1, ATP6V0A2, ATP6V0D1, ATP6V1D, ATP6V1B1 and ATP6V1E1 to be the highly significant major hub genes.

3.2.2. Hub genes associated pathways

Pathway enrichment analyses associated with the top 10 hub genes from the brain, lung and heart were performed to identify the regulated biological and cellular processes. In case of the brain, iron uptake and transport and G1/S transition were the two main key biological ontologies found. In addition, 97% of biological pathways were associated with G1/S transition showing interaction with 8 of the total hub genes. Whereas two pathways, namely small cell lung cancer (CDK2, CDKN1B, CCNE1, CCND1) and Prostate cancer (CDK2, CDKN1B, CCNE1, CCND1) were also identified. Activation of PTK6 and regulation of cell cycle associated pathways and p53 signalling pathways are also found in the analysis (Figure 3a).

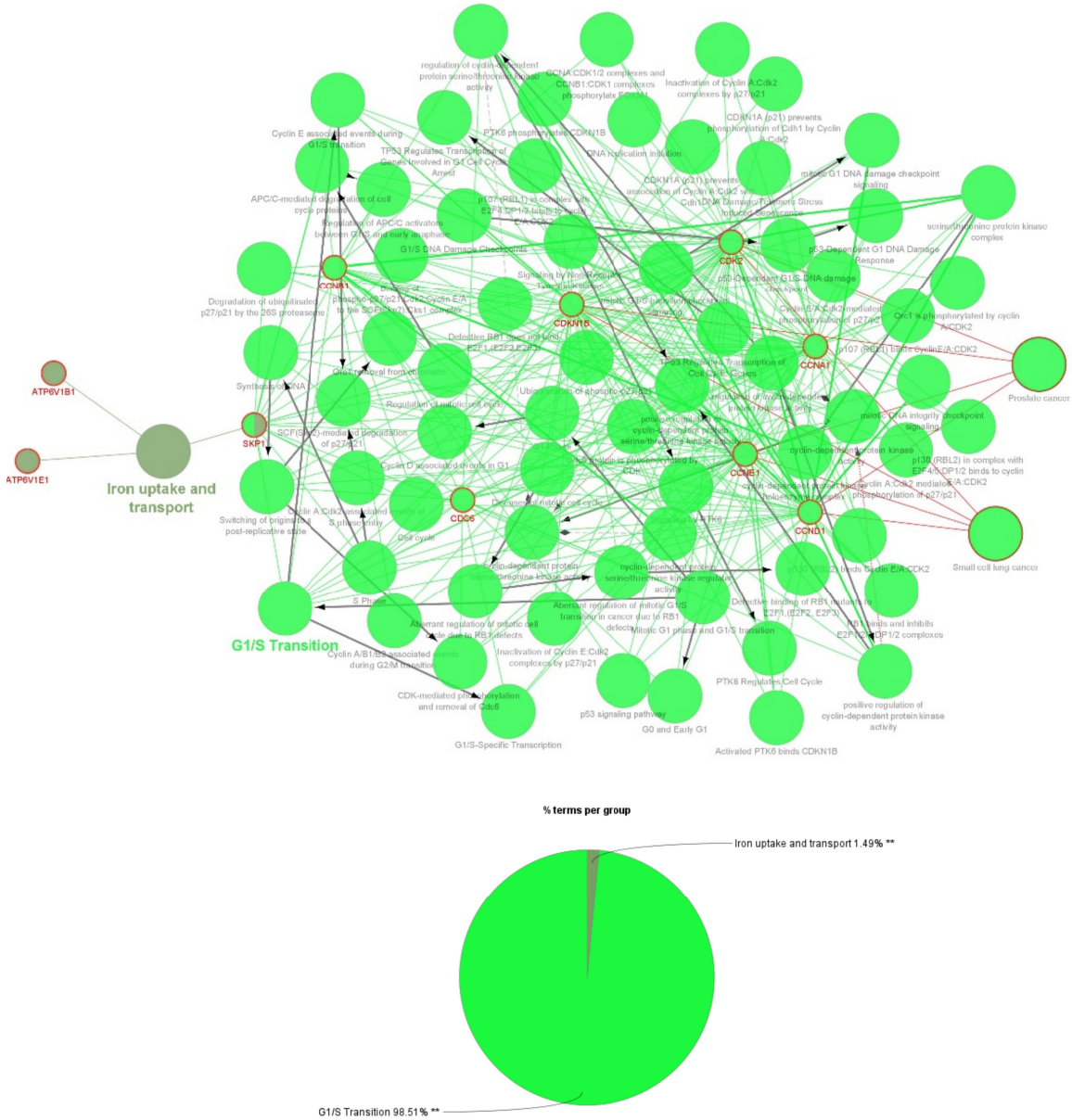


Figure 3a. Gene ontology processes, KEGG pathway and reactome pathway analyses of top 10 hub genes in the brain by CluGO analysis.

In the context of lung biology, two prominent biological pathways had been identified as crucial and they were the differentiation of dendritic cells and the reduction of intraphagosomal pH to a level of 5, primarily facilitated by V-ATPase. Furthermore, it has been observed that 97.22% of biological

pathways were linked to the reduction of intraphagosomal pH, indicating significant interaction with six of the total hub genes. Notably, our analyses also revealed associations with other processes, including rheumatoid arthritis, insulin receptor cycling, V*ibrio cholerae* infection, and epithelial cell signaling in *Helicobacter pylori* infection. Among these hub genes, LGALS3, TGFBR2, and TGFB1 were specifically associated with the dendritic cell differentiation pathway (Figure 3b).

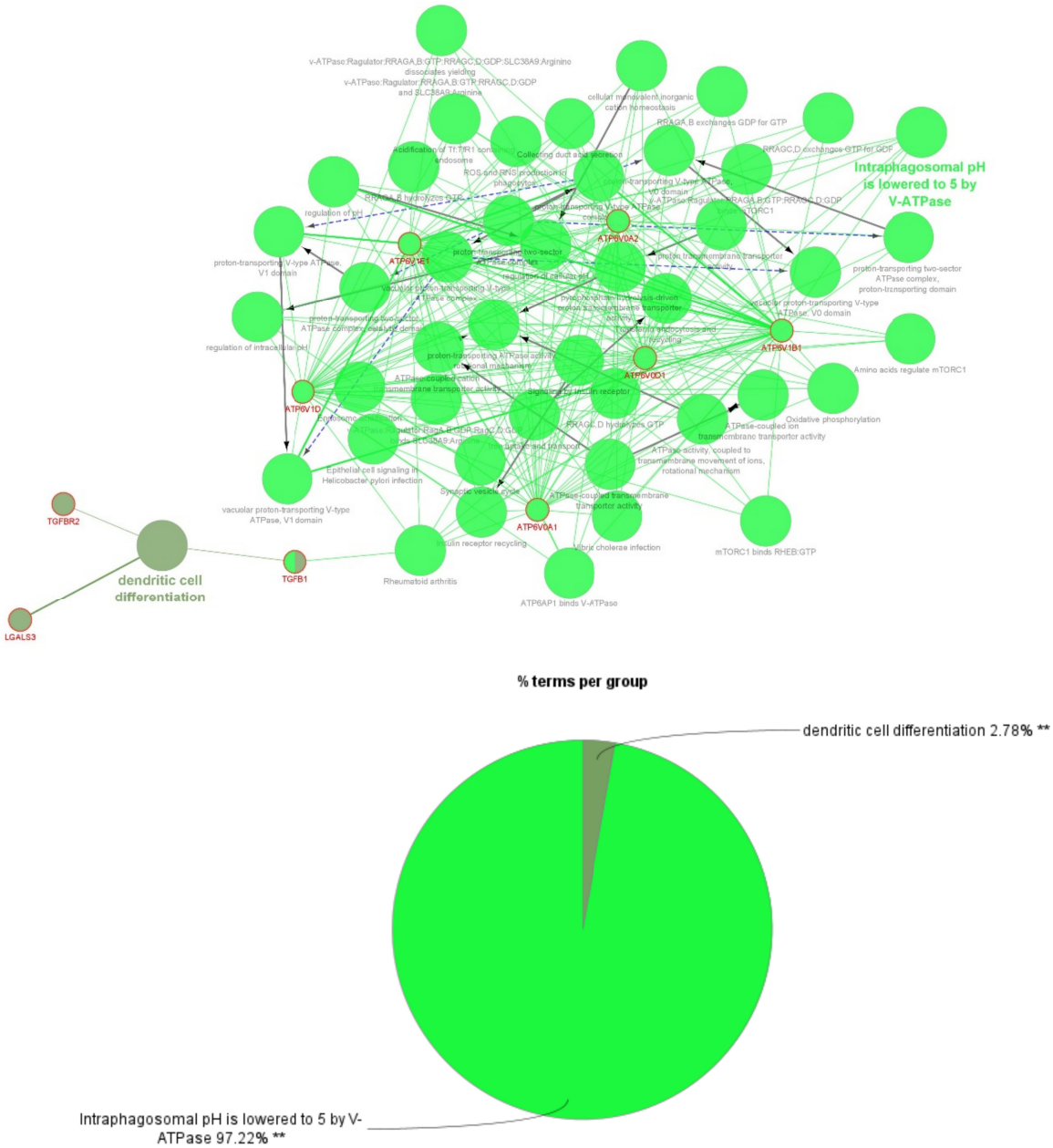


Figure 3b. Gene ontology processes, KEGG pathway and reactome pathway analyses of top 10 hub genes in the lung by CluGO analysis.

For the heart ClueGO analysis revealed, phagosome associated pathways (2.86% terms per group) and regulation of intracellular pH (97.14% terms per group) as the leading term of the network. Proteins associated with phagosome associated pathways had close interaction with all the hub

genes (ATP6V1B1, ATP6V1D, ATP6AP1, ATP6V0D1, RAB7A, CANX and LAMP2) except TGFB1. Proteins associated with regulation of intracellular pH were ATP6V1B1, ATP6V1D, ATP6AP1, ATP6V0D1, and RAB7A. Some of the functions influenced by the genes of the network were vascular proton-transporting V-type ATPase complex (ATP6V1B1, ATP6V1D, ATP6V0D1), rheumatoid arthritis (TGFB1, ATP6V1B1, ATP6V1D, ATP6AP1, ATP6V0D1) and additional pathways like mTORC1 pathways, iron uptake and transport, signalling by insulin receptor, ATPase-coupled cation transmembrane transporter activity etc. (Figure 3c).

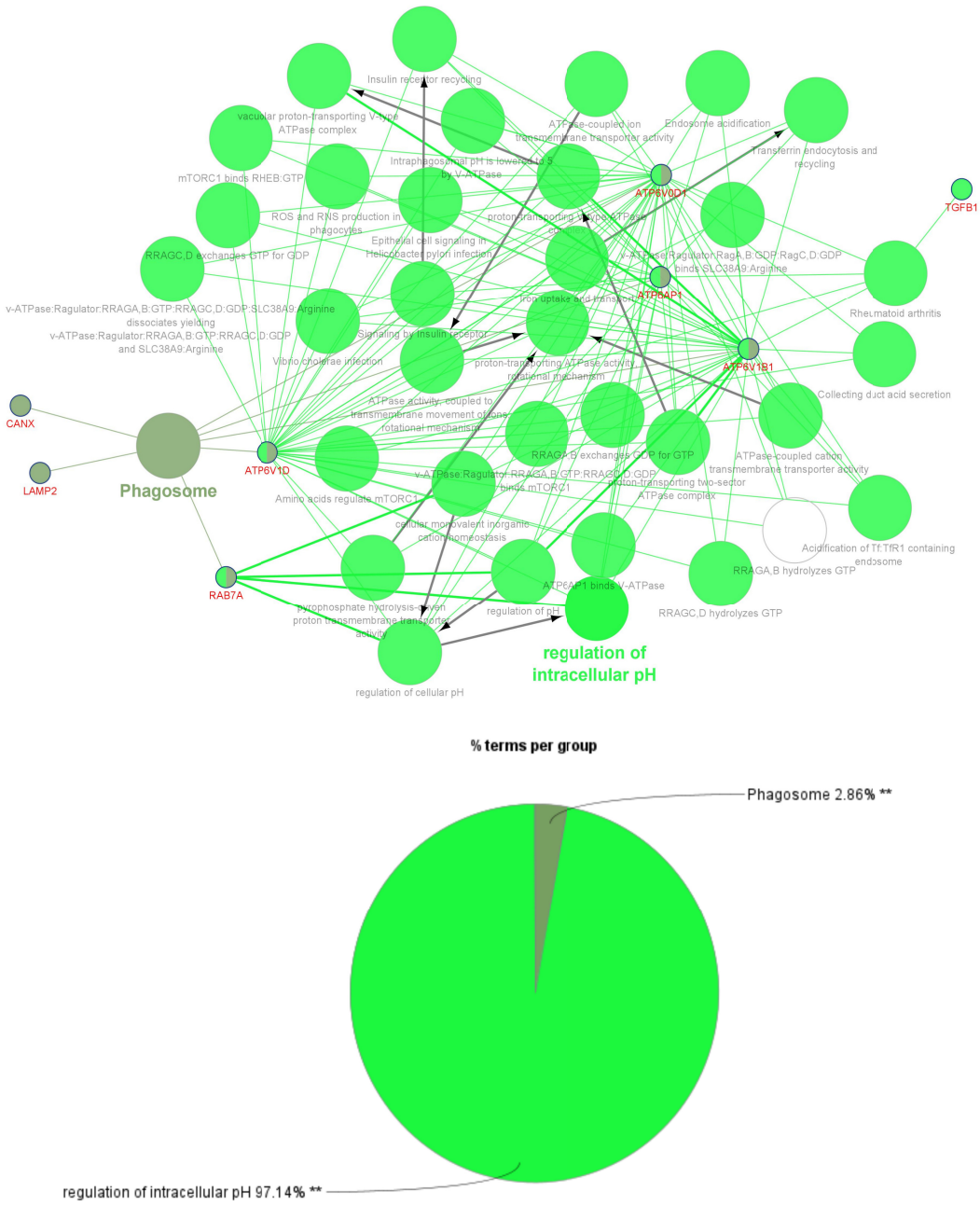


Figure 3c. Gene ontology processes, KEGG pathway and reactome pathway analyses of top 10 hub genes in the heart by CluGO analysis.

3.3. Drug-gene interaction analysis of hub genes in DGIdb

After deducing pathways where the hub genes were involved, we employed DGIdb to assess potential drug interactions with the hub genes in the brain, heart and lung. In the brain among the top ten hub genes, five genes (CDKN1B, CCND1, CDK2, CCNB1, and CCNE1) were drug targets. Few drugs were targetting multiple proteins. There were 30 approved drugs and 89 non-approved drugs (Figure 4a) (Supporting Information Data 3). In the heart (TGFB1, CANX, APP, and LGALS3) and in the lungs (TGFB1, LGALS3, TGFBR2, and EGF), four genes each were drug targets (figure 4b and 4c respectively and Supporting Information Data 3). Approved and non-approved drugs in the heart were 44 and 56 respectively and in the lung the counts were 34 and 31 respectively.

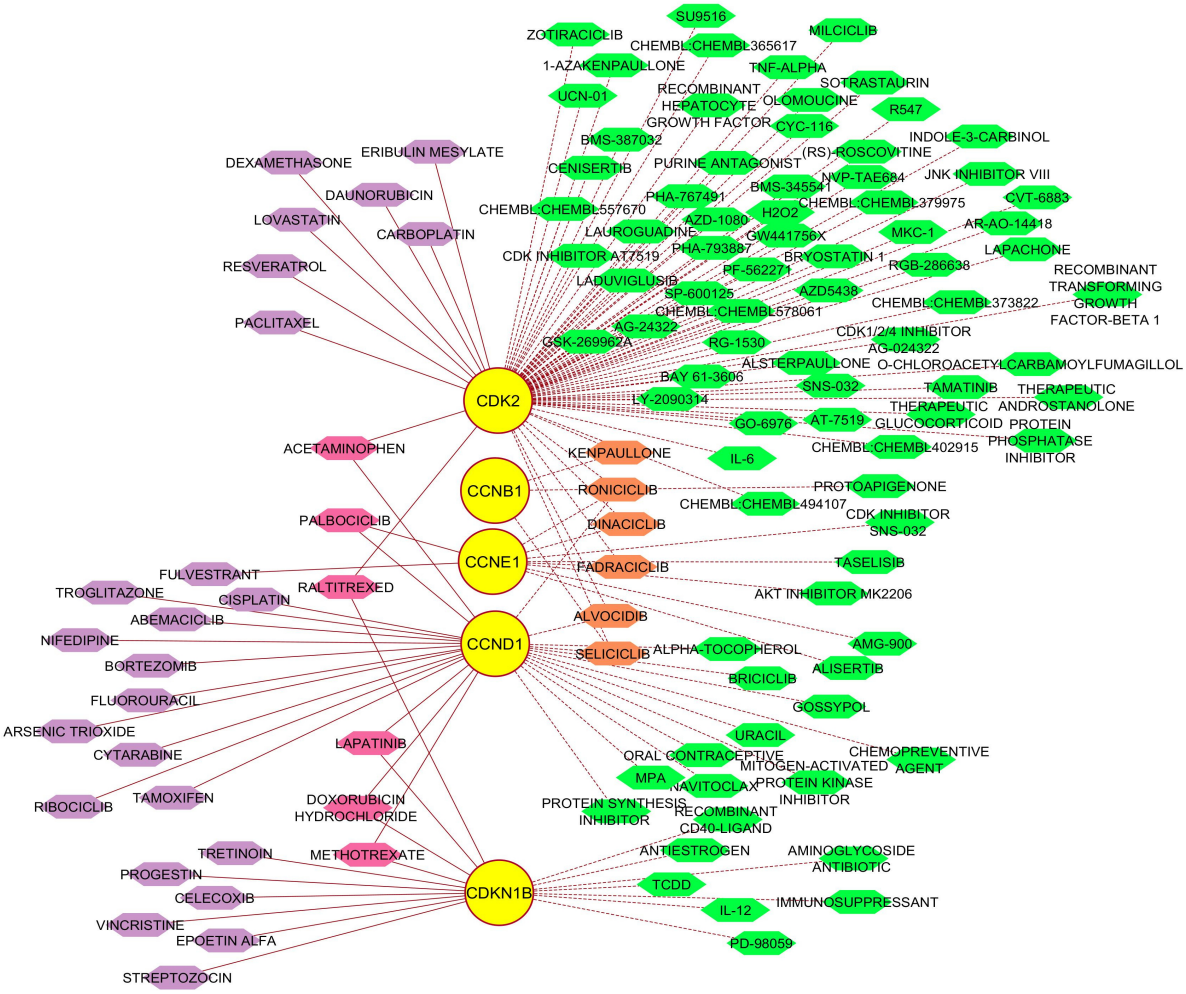


Figure 4a. Drug targets among hub genes in the brain. Circular yellow nodes are hub genes and hexagonal nodes denote drugs. Violet hexagonal nodes are approved drugs and pink nodes denote approved drugs targeting more than one hub genes. Green colored hexagonal nodes with dotted lines are non-approved drugs. Solid edges are approved drugs and dotted edges are for non-approved drugs. Orange colored hexagonal nodes denote non-approved drugs targeting more than one hub genes. 30 approved and 89 non-approved drugs were found for the five among 10 top hub genes.

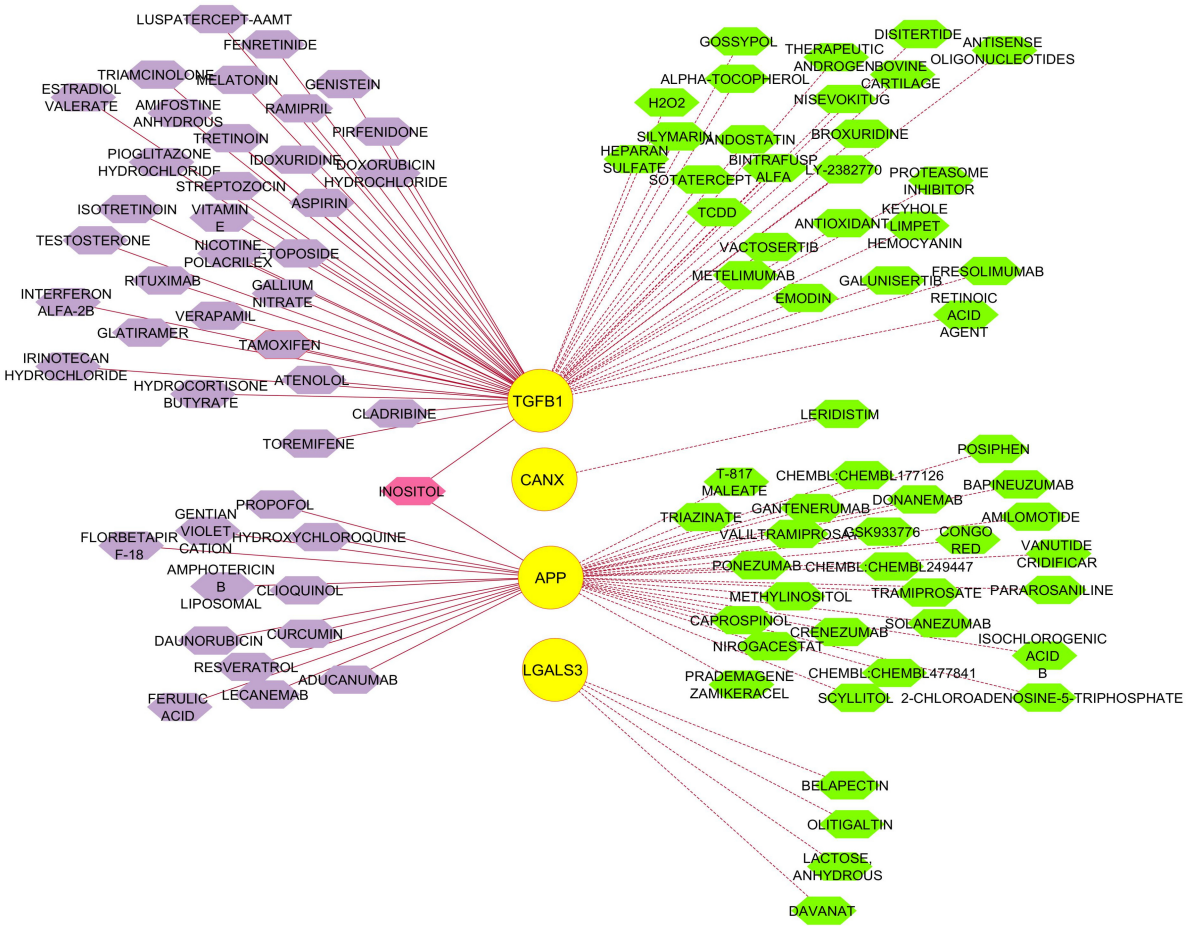


Figure 4b. Drug targets among hub genes in the heart. Circular yellow nodes are hub genes and hexagonal nodes denote drugs. Violet hexagonal nodes are approved drugs and pink nodes denote approved drugs targeting more than one hub genes. Solid edges are approved drugs and dotted edges are for non-approved drugs. Green colored hexagonal nodes are non-approved drugs. 44 approved and 56 non-approved drugs were found for the four among 10 top hub genes.

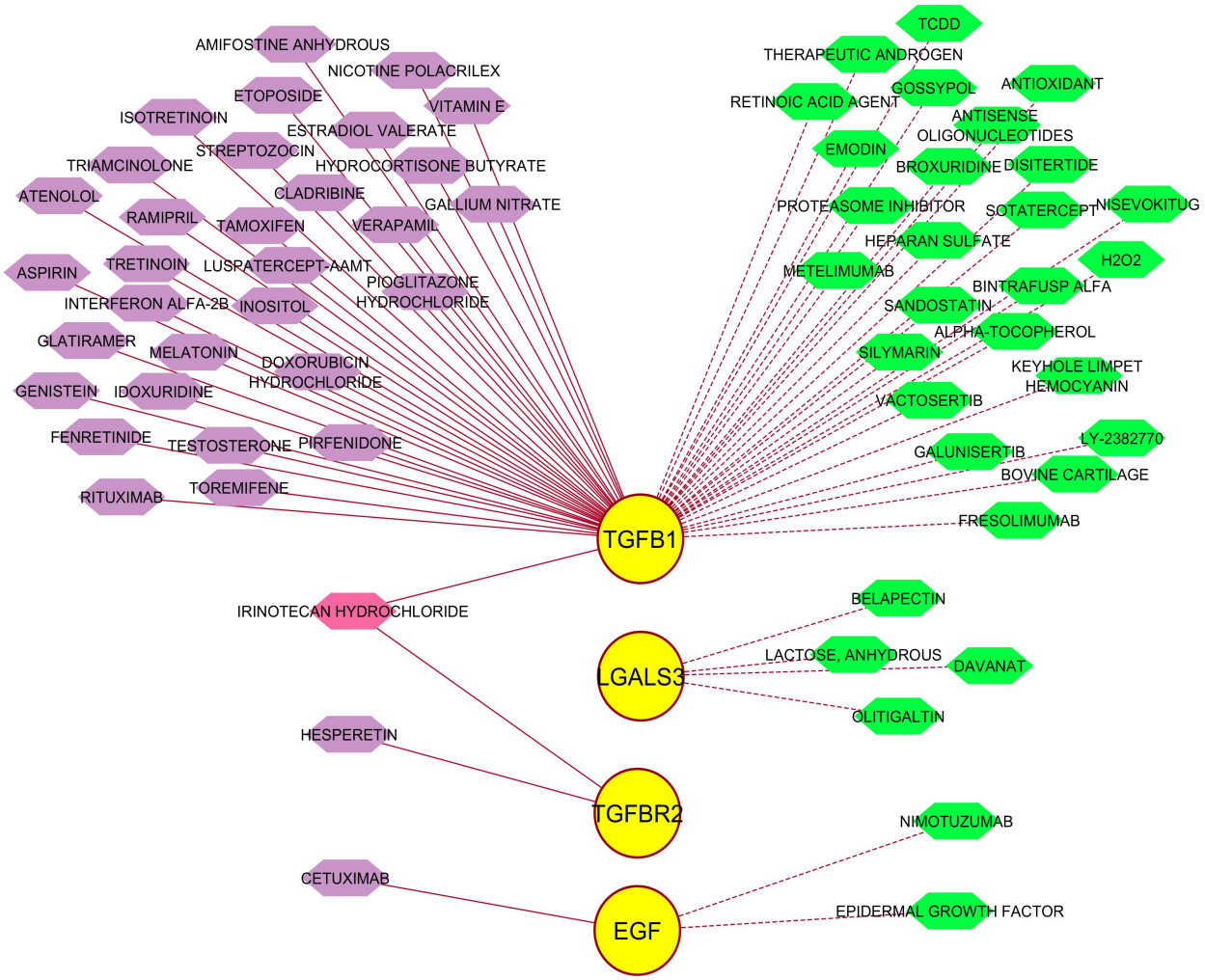


Figure 4c. Drug targets among hub genes in the lung. Circular yellow nodes are hub genes and hexagonal nodes denote drugs. Violet hexagonal nodes are approved drugs and pink nodes denote approved drugs targeting more than one hub genes. Solid edges are approved drugs and dotted edges are for non-approved drugs. Green colored hexagonal nodes are non-approved drugs. 34 approved and 31 non-approved drugs were found for the four among 10 top hub genes.

3.4. NSP6 interacting proteins can influence general and unique tissue specific biological processes

Here, tissue specific interacting-interacting protein partners had been used to bring out the related pathways those are the most susceptible and probably influenced due to COVID-19 infections in the brain, heart and lung. Pathways in the brain, heart, and lung were 25, 147 and 168 respectively. There are 2, 52 and 5 common GO pathway between brain-lung, lung-heart and heart-brain pairs respectively and 14 common GO pathways were present in all supplementary. This aids in identifying pathways of notable significance across all of these tissue types. Gene Ontology (GO) enrichment analysis revealed 4, 69 and 100 unique pathways are there in brain, heart and lung respectively (Figure 5 and Supporting Information Data 4). Common pathways in the brain, heart and lung is shown in figure 6 and supporting information data 4.

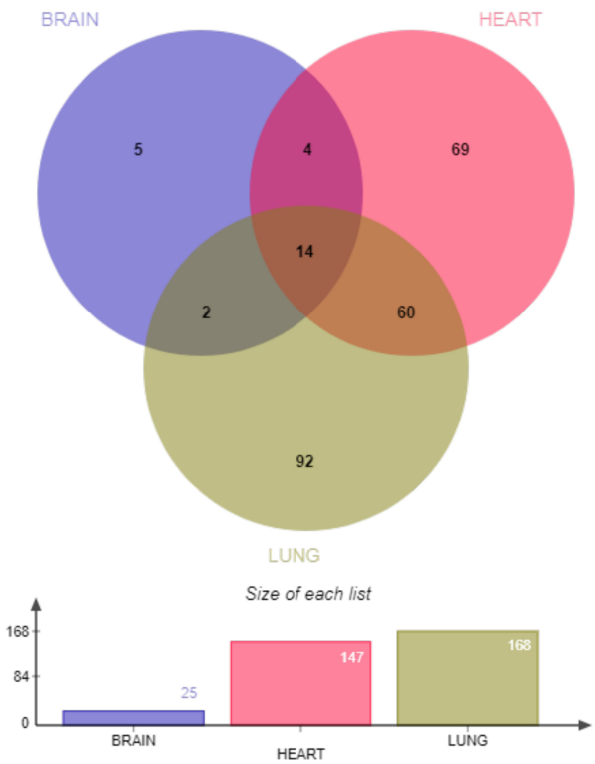


Figure 5. Venn diagram representing common and unique pathways influenced by proteins interacting with SARS-CoV-2 NSP6 interacting proteins in the brain, heart and lungs. 14 pathways were common in all the three tissues, whereas unique pathways present in the brain, heart and lungs were 4, 69 and 100 respectively.

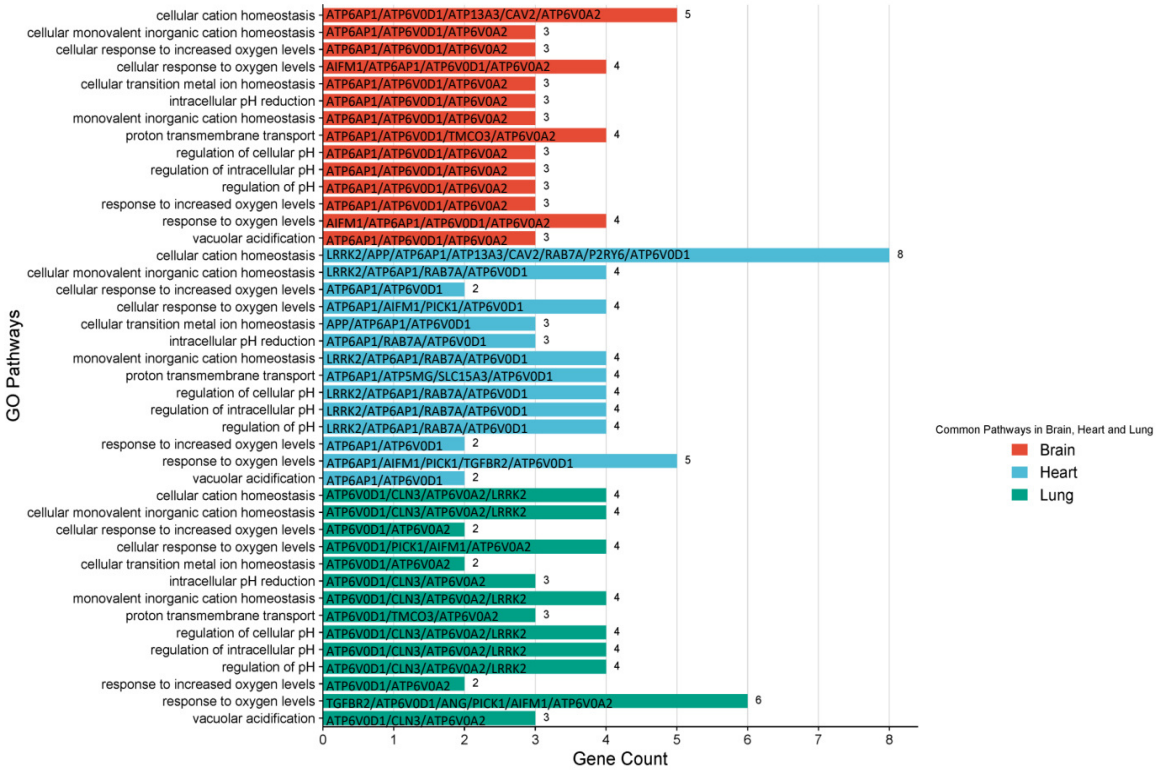


Figure 6. Bar diagram showing common pathways influenced by proteins interacting with SARS-CoV-2 NSP6 interacting proteins in the brain, heart and lungs. Length of the bars represent the number of genes involved in the pathways. X-axis represents gene count and Y-axis represents GO terms.

Generally, SARS-CoV-2 NSP6 triggers autophagy activity via the omegasome pathway,[45] inducing the ER zippering activity,[23] inducing inflammasome activation in lung epithelial cells.[46] In addition, among these three major organs (brain, heart and lung), NSP6 influenced pathways were noticed to be more in heart and lung than in brain. In brain, unique pathways included response to nitric oxide, golgi lumen acidification, cellular response to reactive nitrogen species, cellular response to nitric oxide and modulation by host of symbiont process (Figure 7a and Supporting Information Data 4).

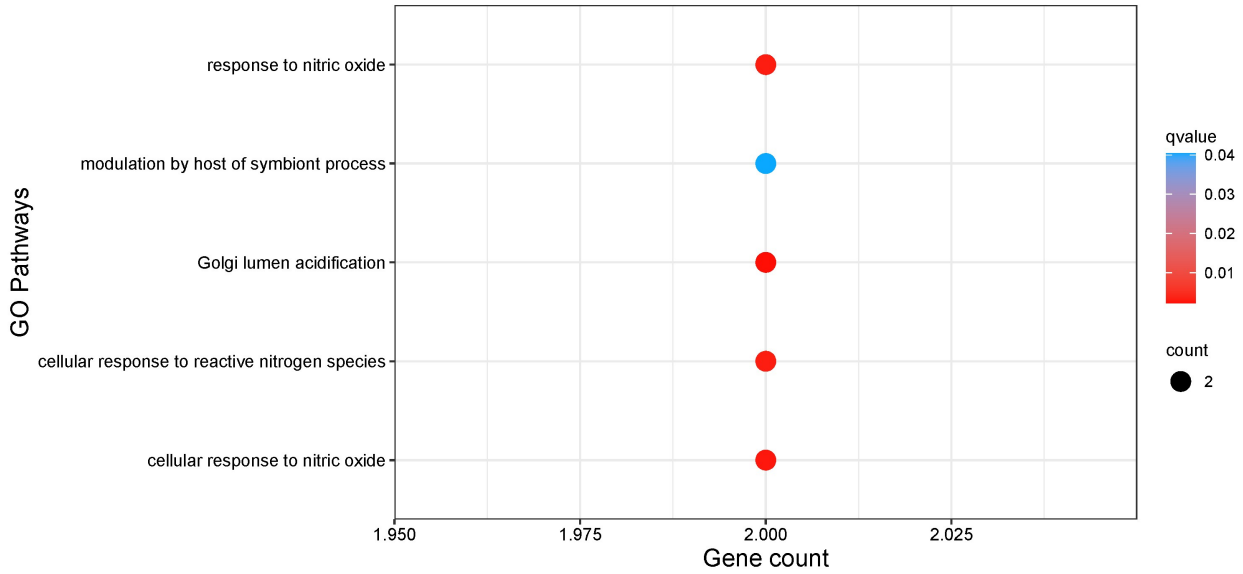


Figure 7a. Dot plot representing unique pathways influenced by proteins interacting with SARS-CoV-2 NSP6 interacting proteins in the brain. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

In heart, NSP6 can also influenced some unique biological pathways including viral process, regulation of viral process, viral life cycle, biological process involved in symbiotic interaction, cellular response to starvation, regulation of dopamine receptor signaling pathway, G protein-coupled purinergic nucleotide receptor signaling pathway, endosome to plasma membrane protein transport, chondrocyte differentiation involved in endochondral bone morphogenesis, cellular response to manganese ion, etc., (Figure 7b and Supporting Information Data 4).

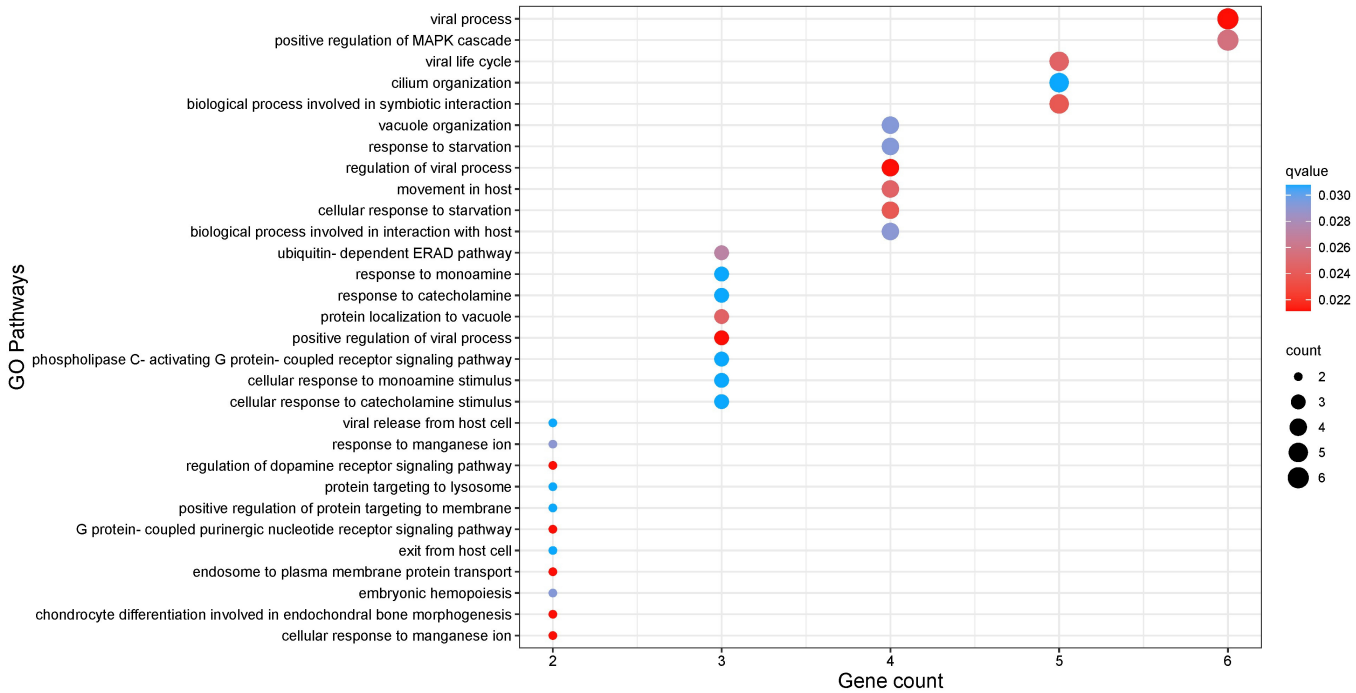


Figure 7b. Dot plot representing top 30 unique pathways influenced by proteins interacting with SARS-CoV-2 NSP6 interacting proteins in the heart. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

In case of lung, synapse organisation, regulation of endocytosis, dendritic spine organization, response to decreased oxygen levels, regulation of T-cell activation and apoptotic process, regulation of leukocyte proliferation and leukocyte cell-cell adhesion, lymphocyte proliferation, intrinsic apoptotic cell signalling are mostly prominent (Figure 7c and Supporting Information Data 4).

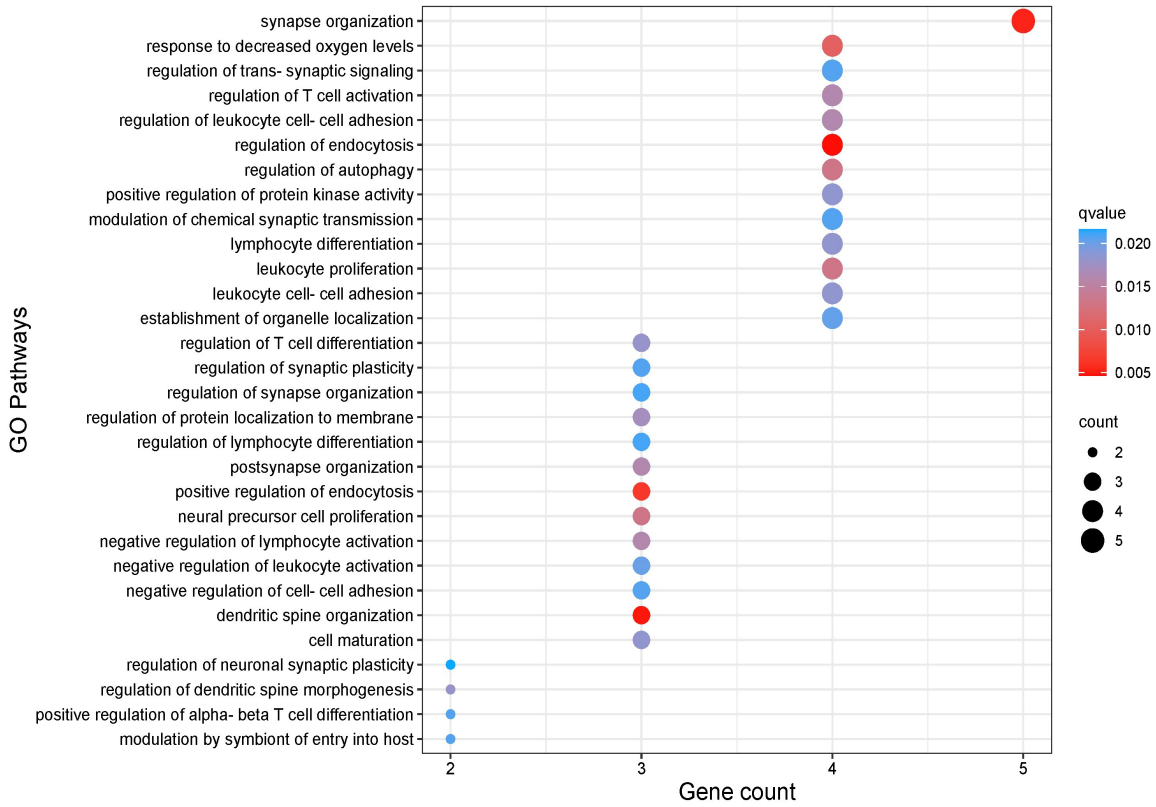


Figure 7c. Dot plot representing top 30 unique pathways influenced by proteins interacting with SARS-CoV-2 NSP6 interacting proteins in the lungs. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

In the subsequent phase of our study, we aimed to conduct an analysis of both upregulated and downregulated pathways, taking into account of the tissue-specific context, using differentially expressed genes influenced by SARS-CoV-2. We delineated both up and down regulated genes to identify pathways they influence. In brain, among the upregulated genes impacted pathways prominent ones were positive regulation and modulation of host viral process (Figure 8a and Supporting Information Data 5).

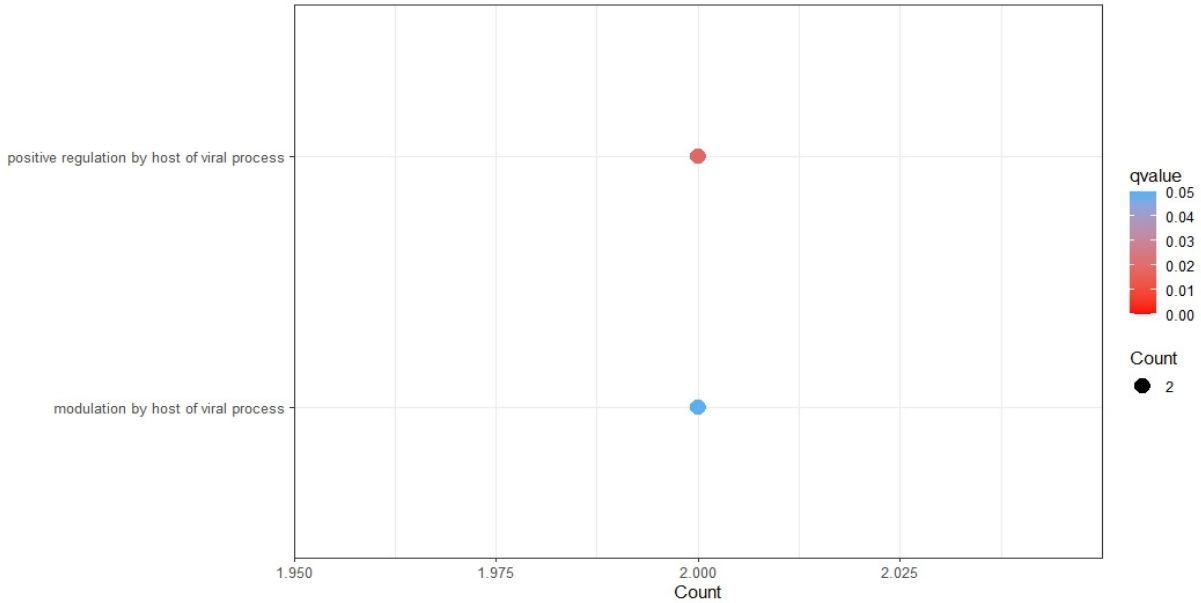


Figure 8a. Dot plot representing pathways in the brain influenced by upregulated proteins interacting with SARS-CoV-2 NSP6 interacting proteins. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the *q*-value using the Benjamini-Hochberg method.

Whereas, downregulated genes were observed to be associated with negative regulation of cellular and intracellular pH, vascular acidification cellular response to increased oxygen level, transmembrane transport of proton, cellular iron homeostasis are the most relevant (Figure 8b and Supporting Information Data 5).

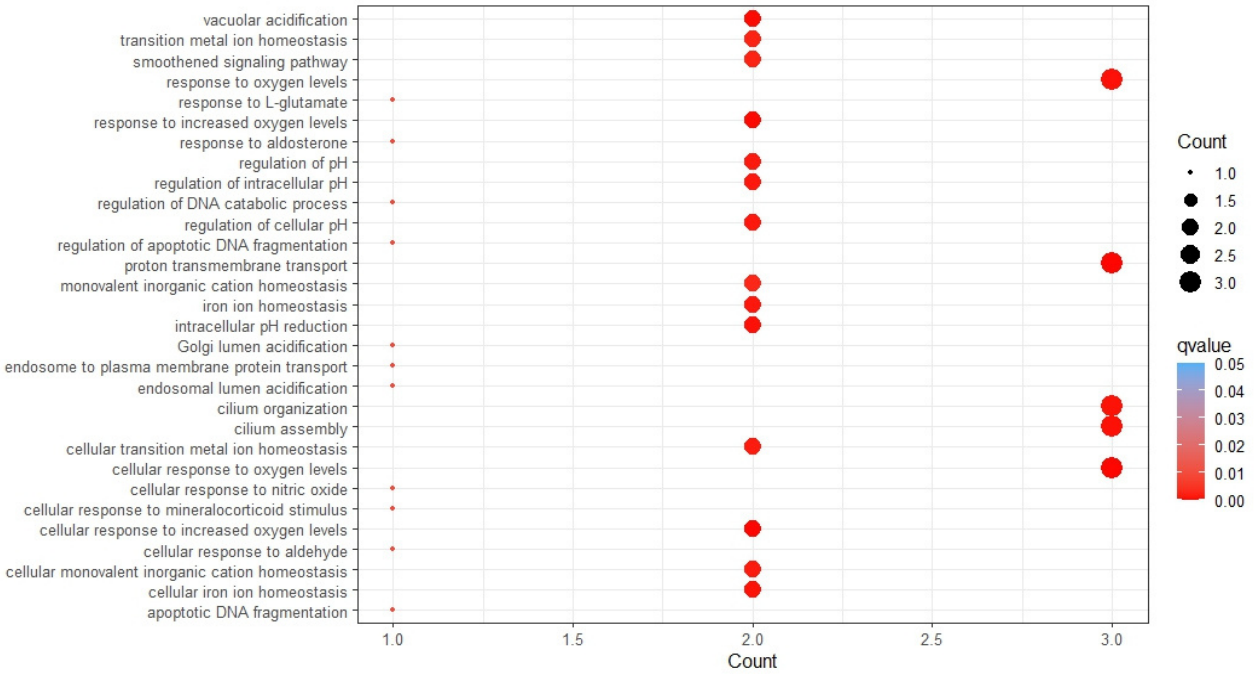


Figure 8b. Dot plot representing top 30 pathways in the brain influenced by downregulated proteins interacting with SARS-CoV-2 NSP6 interacting proteins. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the *q*-value using the Benjamini-Hochberg method.

In case of heart, the influenced pathways by upregulated genes are protein targeting and regulation of protein containing-complex assembly, regulation of viral process, positive regulation of tumor necrosis factor superfamily cytokine production, regulation of mononuclear and leukocyte cell migration, Phospholipase C activating G protein coupled receptor signalling pathway, neuron maturation and projection organisation, cellular response to starvation etc. (Figure 9a and Supporting Information Data 5).

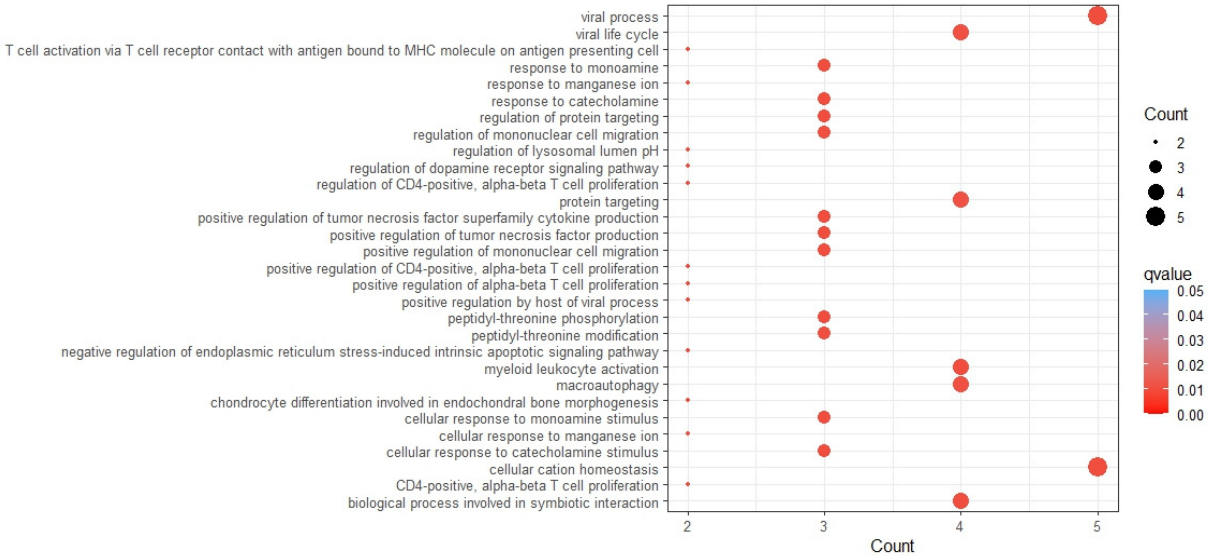


Figure 9a. Dot plot representing top 30 pathways in the heart influenced by upregulated proteins interacting with SARS-CoV-2 NSP6 interacting proteins. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

Whereas, down regulated genes are seen to act on cilium assembly and organisation, cellular response to increased oxygen level, selective and macroautophagy, intracellular pH reduction etc (Figure 9b and Supporting Information Data 5). None of the pathways had a significant q-value.

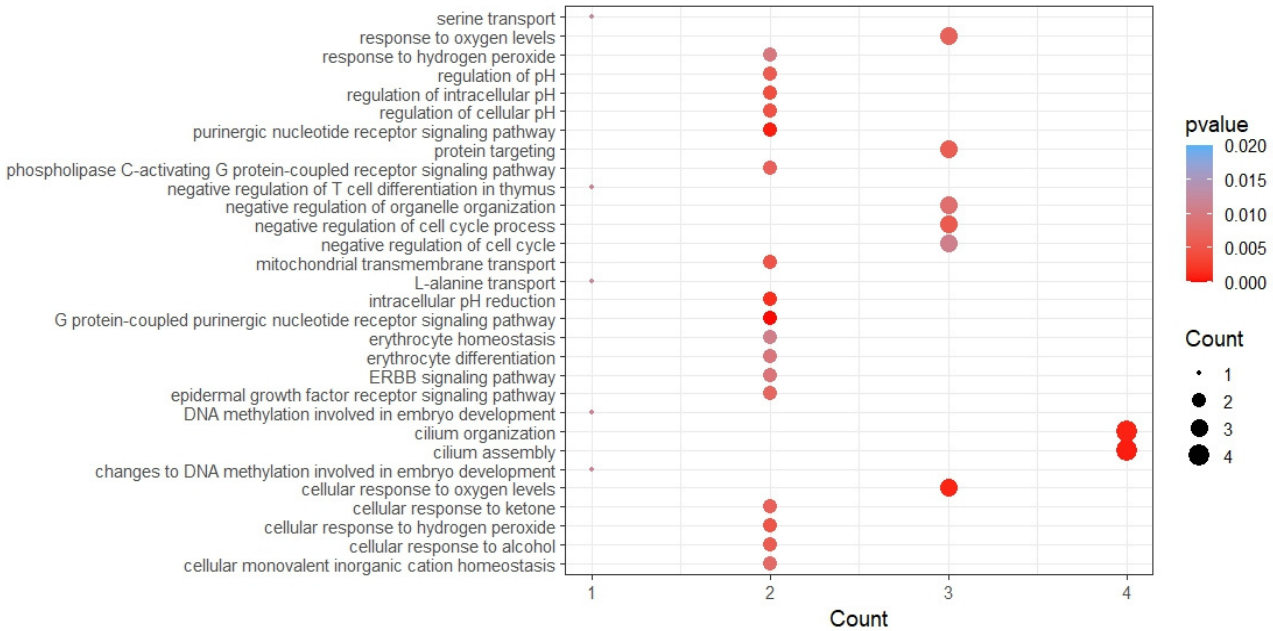


Figure 9b. Dot plot representing top 30 pathways in the heart influenced by downregulated proteins interacting with SARS-CoV-2 NSP6 interacting proteins. Dot size represent the gene ratio and dot color depicts the p-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

Within lung, the upregulated genes have been noticed to influence some pathways, like immune system related biological processes there are T-cell activation via T-cell receptor contact with antigen bound to MHC molecule, response to interferon gamma, T-cell differentiation, some viral entry related pathways like modulation by symbiont of entry into host cell, in cell signalling processes, stress-activated protein kinase signalling cascade, stress activated MAPK cascade, movement and modulation by symbiont of entry into host, neurotransmitter secretion, synapse signalling (Figure 10a and Supporting Information Data 5).

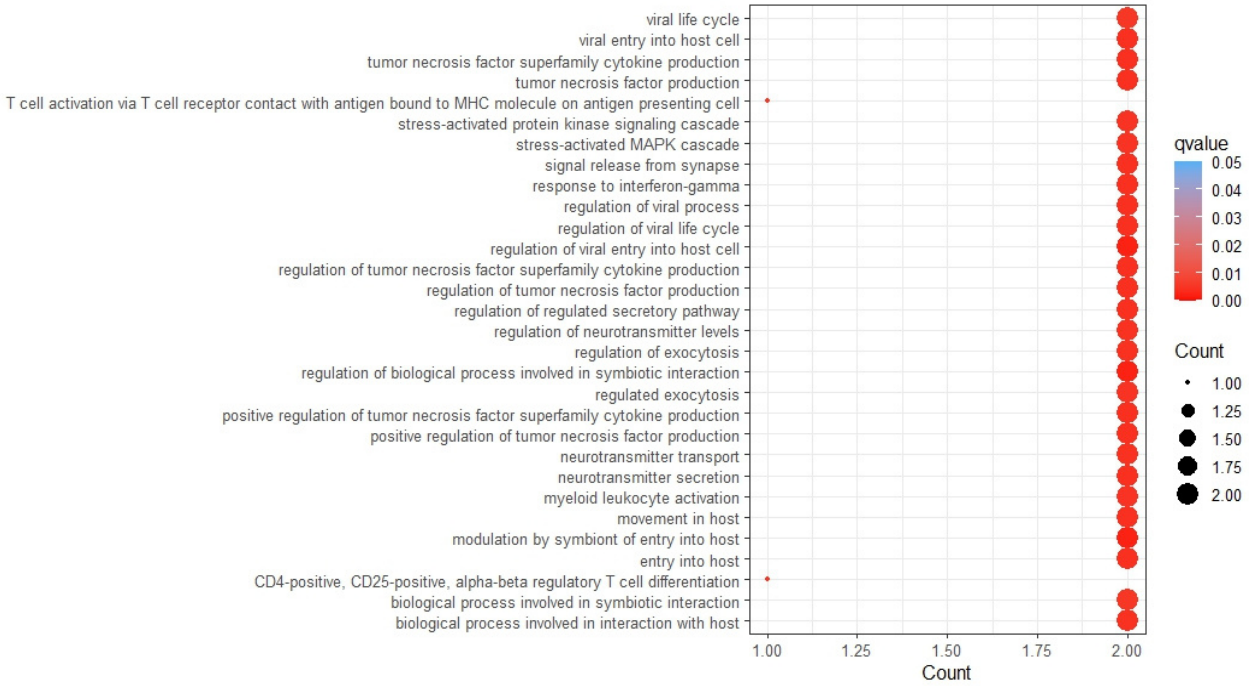


Figure 10a. Dot plot representing top 30 pathways in the lung influenced by upregulated proteins interacting with SARS-CoV-2 NSP6 interacting proteins. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

In case of down regulated pathway in lung, we found positive regulation of protein regulation and transport, response to increased and decreased oxygen level, mononuclear T-cell proliferation, proton trans membrane transport, intercellular pH reduction and vascular acidification, regulation of neural synaptic plasticity (Figure 10b and Supporting Information Data 5).

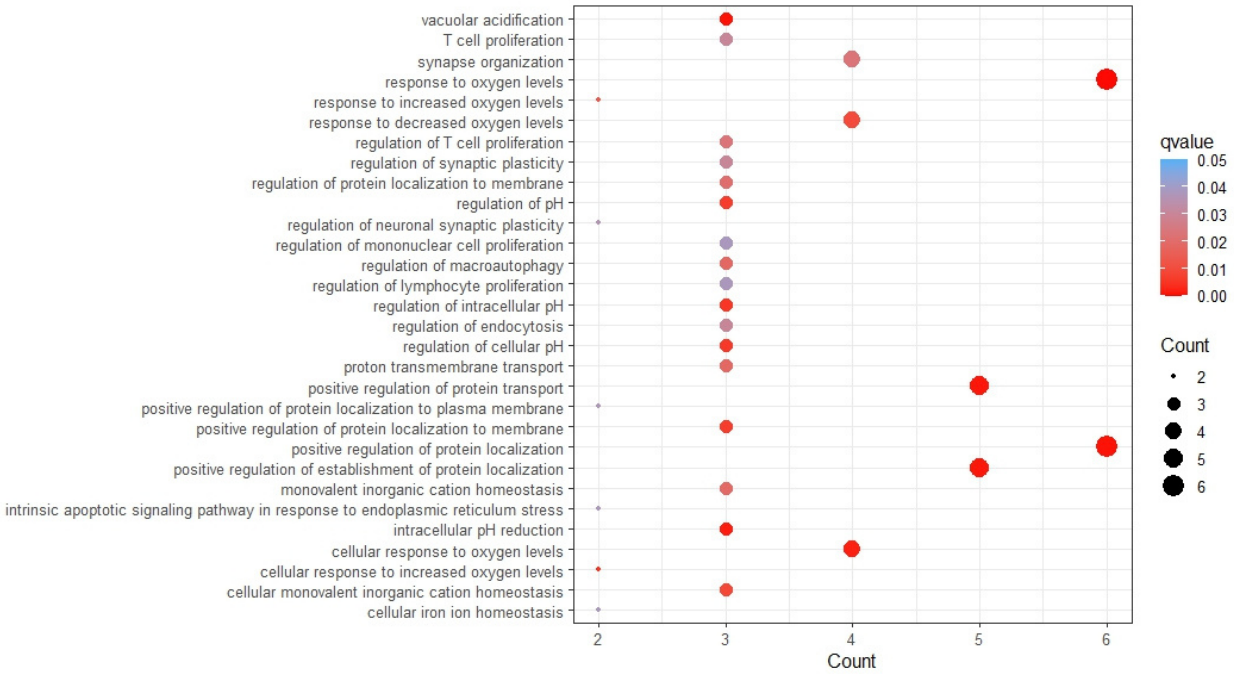


Figure 10b. Dot plot representing top 30 pathways in the lung influenced by downregulated proteins interacting with SARS-CoV-2 NSP6 interacting proteins. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

3.7. SARS-CoV2 influenced miRNA population is different in the brain and lung

Protein regulation by miRNAs could affect protein abundance in cells and influence pathways where these proteins are involved. After finding out the different pathways in the brain, heart and lung we looked for miRNAs expressed in these tissues that can target interacting partners of SARS-CoV-2 NSP6 interacting proteins regulated by SARS-CoV-2 infection using miRNet. We found 82 miRNAs in the brain and 43 miRNAs in the lung. Among these 16 were common in the brain and lung. 66 miRNAs were unique for brain whereas for lung it was 27 (Figure 11 and Supporting Information Data 6). In miRNet, miRNAs expressed in the heart was not available.

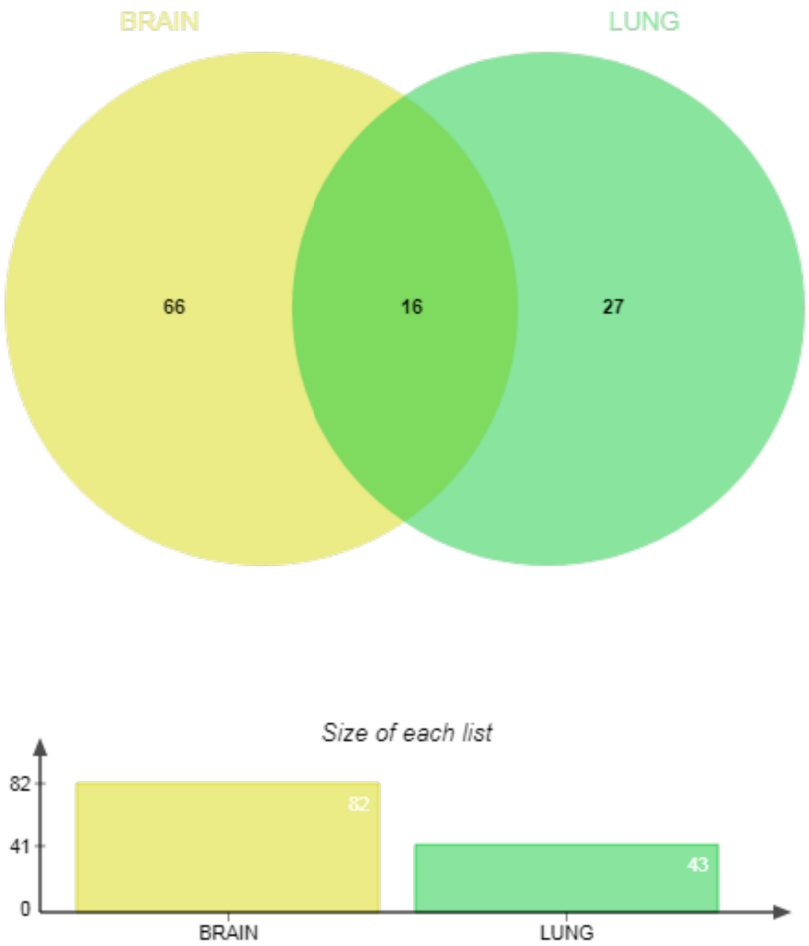


Figure 11. Common miRNAs in the brain and lung. Proteins influenced by SARS-CoV-2 infection that interact with SARS-CoV-2 NSP6 in the brain and lung was taken to find out miRNAs that can target these protein mRNAs. Among the total of 82 miRNAs in brain and the total of 43 miRNAs in the lung, 16 miRNAs were common where as 66 and 27 miRNAs were unique in the brain and lung respectively.

3.8. miRNA-mRNA interaction network involving the brain and lung expressed miRNAs targeting SARS-CoV2 influenced interacting partners of SARS-CoV-2 NSP6 interacting proteins and their corresponding pathways

From gene-microRNA interaction network analysis of the brain, the gene-microRNA network consists of 113 nodes, (82 miRNAs, 18 genes and 13 influencing transcription factors (TF)) and 297 edges. In the miRNA interaction network, we have identified five miRNAs with comparatively high degree (≥10) and betweenness values (hsa-mir-124-3p with degree 14 and betweenness 484.1903, hsa-mir-1-3p with degree 13 and betweenness 431.2286, hsa-mir-16-5p with degree 12 and betweenness 379.2163, hsa-let-7b-5p with degree 11 and betweenness 239.3807 and hsa-mir-155-5p with degree 10 and betweenness 243.6051) (Figure 12). Pathways influenced by these miRNAs are given in supporting information data 7 that was obtained from GO-BP and KEGG pathways. These pathways included transcription related pathways, pathways regulating cell proliferation, pathways regulating nucleotide metabolism, apoptotic signalling pathways, regulation of myeloid cell and leukocyte differentiation, etc., in the GO-BP list. KEGG list consisted of pathways ($p \leq 0.05$) in viral infection (Epstein-Barr virus, HTLV-I, Hepatitis C, and Herpes simplex) various cancer pathways (chronic myeloid leukemia, acute myeloid leukemia, colorectal cancer, and prostate cancer), different signalling pathways (MAPK signalling, Wnt signalling, p53 signalling, and ErbB signalling), and pathways in bacterial infection (pertussis, and tuberculosis) (Supporting Information Data 8).

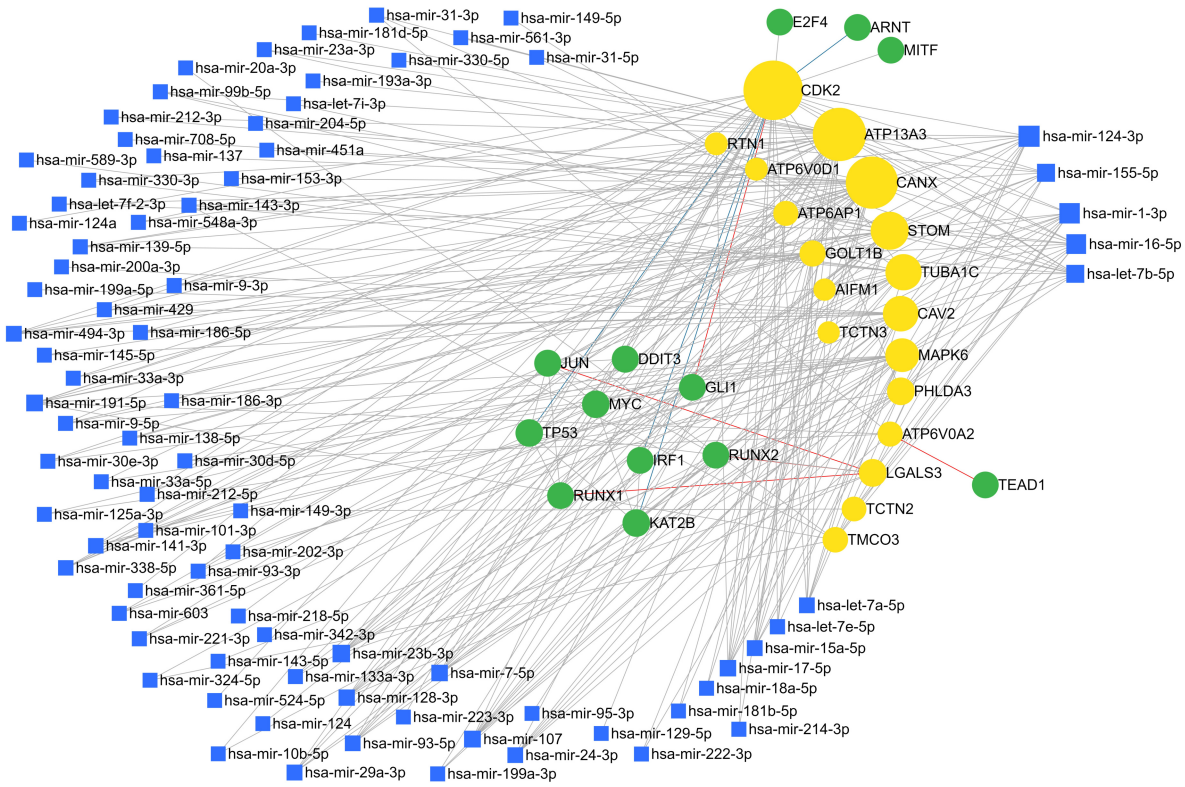


Figure 12. miRNA interaction network with interacting proteins of SARS-CoV2 NSP6 interacting proteins in the brain. Yellow colored nodes denote proteins and green colored nodes are transcription factors influencing proteins and miRNAs in the network. Blue squares represent miRNA nodes. The Increased size of node represent a higher degree.

In the lung tissue 108 nodes consisting 43 miRNAs, 22 genes and 43 TFs exhibited 337 edges. In the lung specific miRNA interaction network, we identified two miRNAs with comparatively high degree (≥10) and betweenness values (hsa-mir-1-3p with degree 14 and betweenness 403.7072 and hsa-mir-34a-5p with degree10 and betweenness 569.9464) (Figure 13 and Supporting Information Data 9). This indicates the significance of these miRNAs as hubs (for their high degree) and their important role in the interaction network (for their high betweenness). Influenced pathways by these miRNAs are given in supporting information data 10 obtained from GO-BP and KEGG. GO-BP pathway list consisted of regulation of DNA dependent transcription, regulation of RNA metabolic processes, positive regulation of transcription from RNA polymerase II promoter, regulation of cell differentiation, cell proliferation, organ development, embryo development, nervous system development, etc. Pathways with p-value≤ 0.05 in KEGG pathway list were HTLV-1 infection, cancer pathways (prostate, renal cell, pancreas, colorectal, and chronic myeloid leukemia), osteoclast differentiation, prion diseases, signalling pathways (TGF-beta, ErbB, Notch, Wnt, and MAPK), tuberculosis, addiction (cocaine, amphetamine, and alcoholism), Leishmaniasis, mineral absorption, and adherens junction.

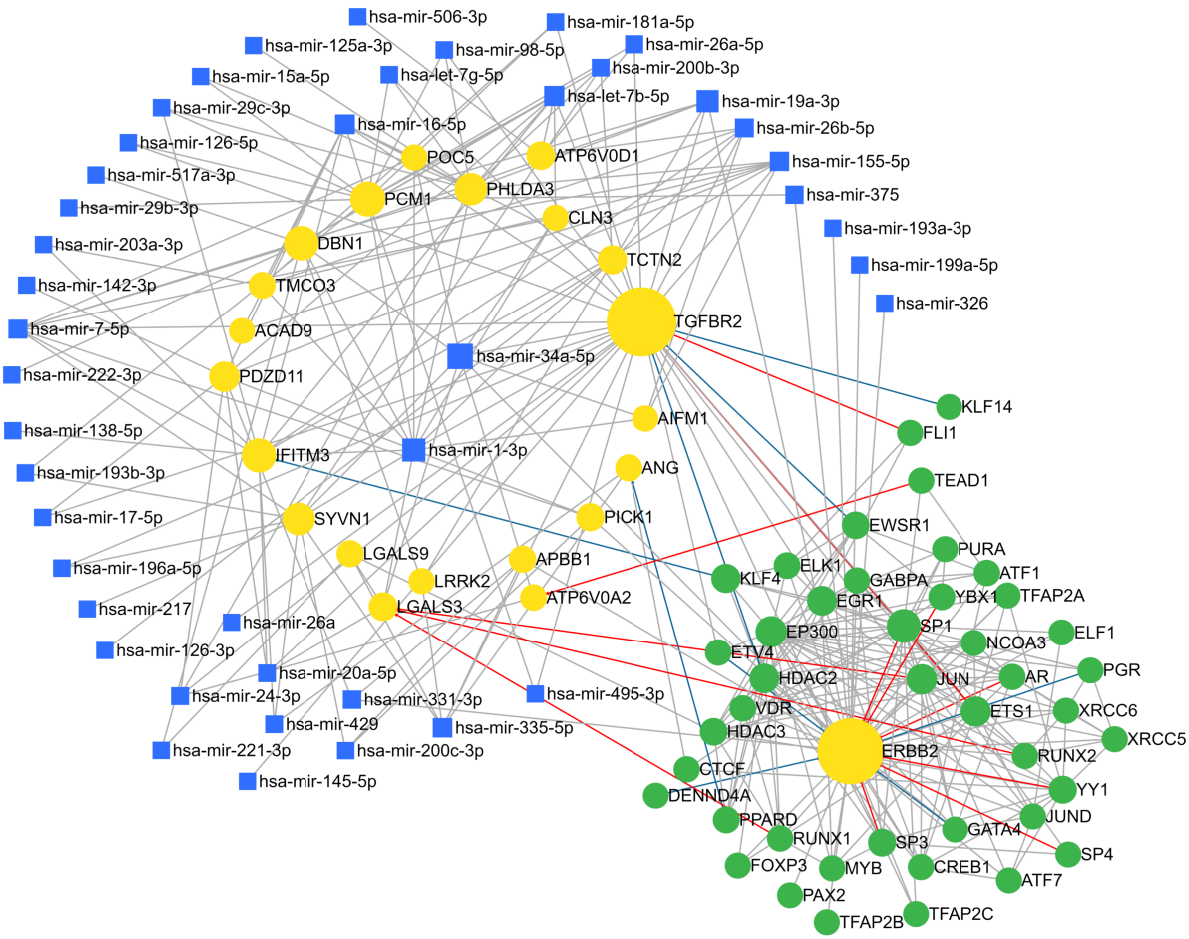


Figure 13. miRNA interaction network with interacting proteins of SARS-CoV2 NSP6 interacting proteins in the lung. Yellow colored nodes denote proteins and green colored nodes are transcription factors influencing proteins and miRNAs in the network. Blue squares represent miRNA nodes. The Increased size of node represent a higher degree.

3.8. Possible pathways in heart influenced by SARS-CoV2 regulated circulating miRNAs in the heart

In miRNet heart specific miRNA database is absent. To construct probable miRNA-RNA interaction network of heart that is influenced by SARS-CoV-2 NSP6, we took miRNAs and genes that satisfied two conditions – i) SARS-CoV2 regulated circulating miRNAs[47,44] and (ii) among this list, miRNAs targetting SARS-CoV2 regulated proteins in the heart interacting with SARS-CoV-2 NSP6 interacting proteins. Resulted miRNA-RNA interaction network is shown in figure 14 and supporting information data 11.

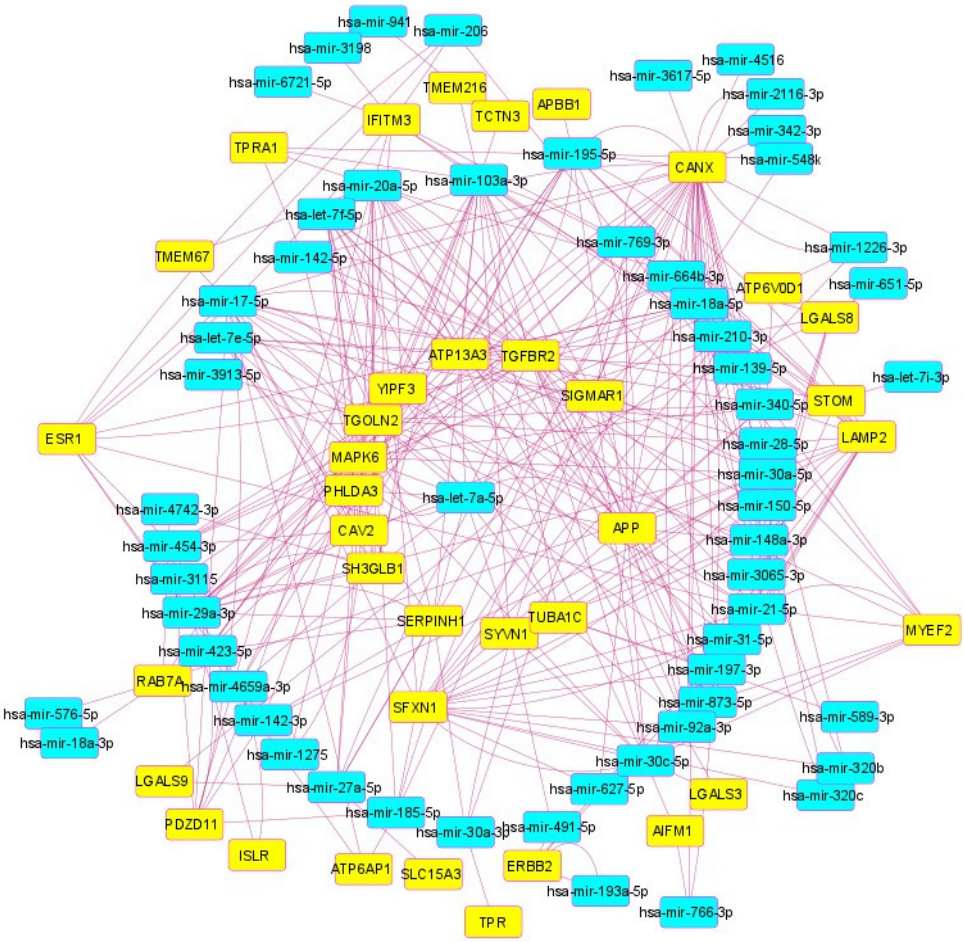


Figure 14. miRNA interaction network with interacting proteins of SARS-CoV2 NSP6 interacting proteins in the heart. Yellow colored nodes denote proteins and blue colored nodes are miRNAs in the network.

3.9. Regualted SARS-CoV2 influenced circulatory miRNAs were found to be targeting tissue specific interacting proteins of SARS-CoV-2 NSP6 interacting proteins

As SARS-CoV2 is reported to be influencing miRNAs[44,47], we wanted to study the regulated circulatory miRNAs and miRNAs that target SARS-CoV2-regulated proteins interacting with SARS-CoV2 NSP6 binding proteins in the brain and lung. Common miRNAs in the reported list of SARS-CoV2-influenced circulating miRNAs[44,47] and tissue specific miRNAs influencing interacting proteins of SARS-CoV2 NSP6 binding proteins were probed to find out regulated miRNAs and their respective target proteins. Among the common miRNAs in the lung we observed three miRNAs (hsa-mir-17-5p, hsa-mir-20a-5p, and hsa-mir-142-3p) whereas, in the brain it was ten miRNAs (hsa-let-7a-5p, hsa-let-7e-5p, hsa-mir-17-5p, hsa-mir-18a-5p, hsa-mir-29a-3p, hsa-mir-31-5p, hsa-mir-139-5p, hsa-mir-342-3p, hsa-mir-589-3p, and hsa-let-7i-3p) (Supporting Information Data 12). With the help of miRNET, we found out genes targetted by these miRNAs in the brain and lung. In the lung 1616 genes were targetted by the three observed miRNAs (Figure 15 and supporting information data 13) and 2782 genes were observed to be targetted by the 10 miRNAs in the brain (Figure 16) (Supporting Information Data 14). We wanted to see the common proteins that were targetted by the regulated miRNAs (both in the brain and lung). We got 3 genes common in the brain and 2 genes common in the lung. Checking for the pathways that were involving these genes, we were able to see pathways like induction of tolerance to self antigen (B cell, T cell, NK T cell), protein amino acid phosphorylation, protein metabolic process, phosphorous metabolism, regulation of mitosis, response to cholesterol, myeloid dendritic cell differentiation, etc., (Figure 17) (Supporting Information Data 15).

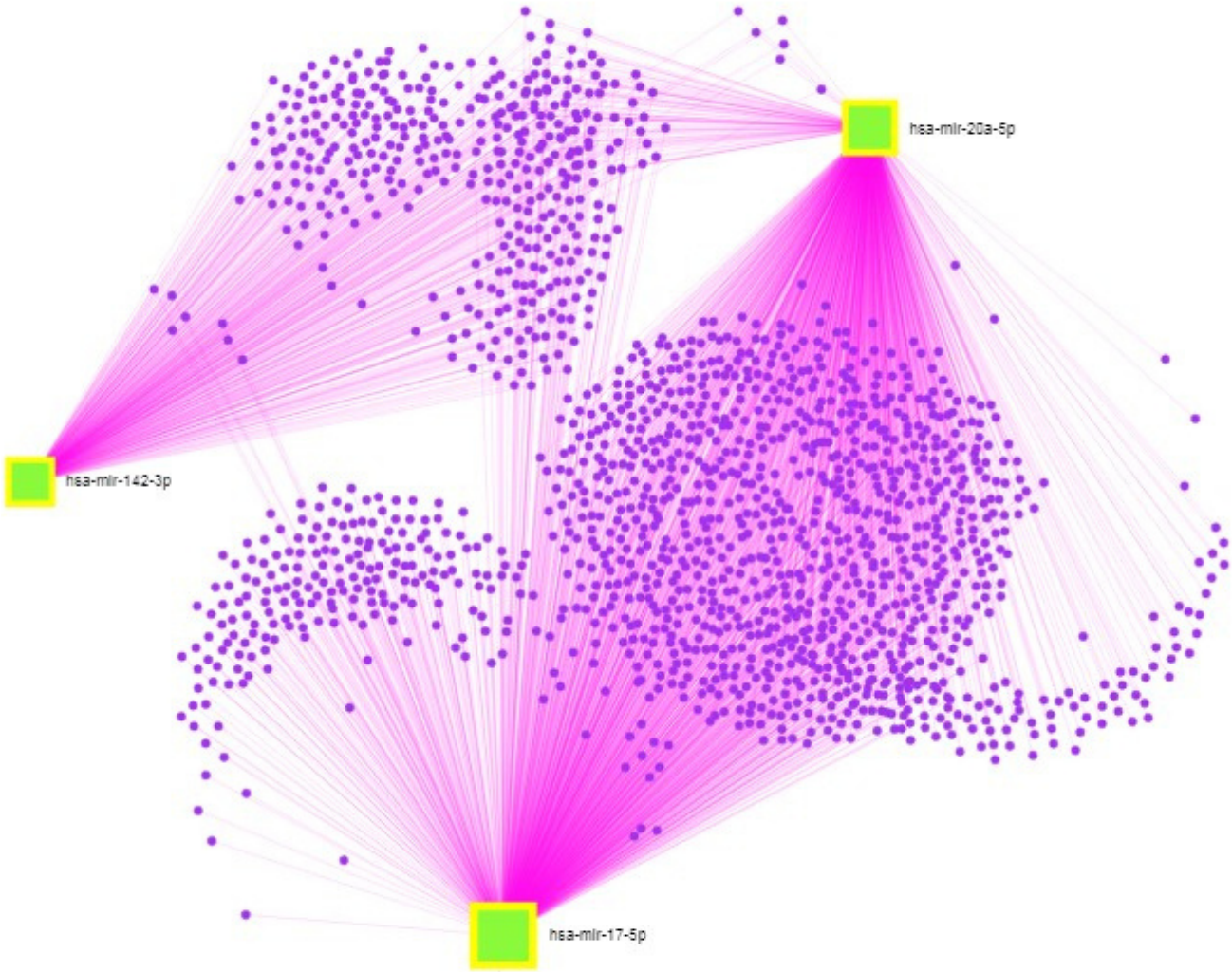


Figure 15. Common among SARS-CoV2 influenced circulating miRNAs and lung miRNAs. Square nodes denotes miRNAs and circular nodes represent targetted mRNAs. Three miRNAs were targeting 1616 mRNAs.

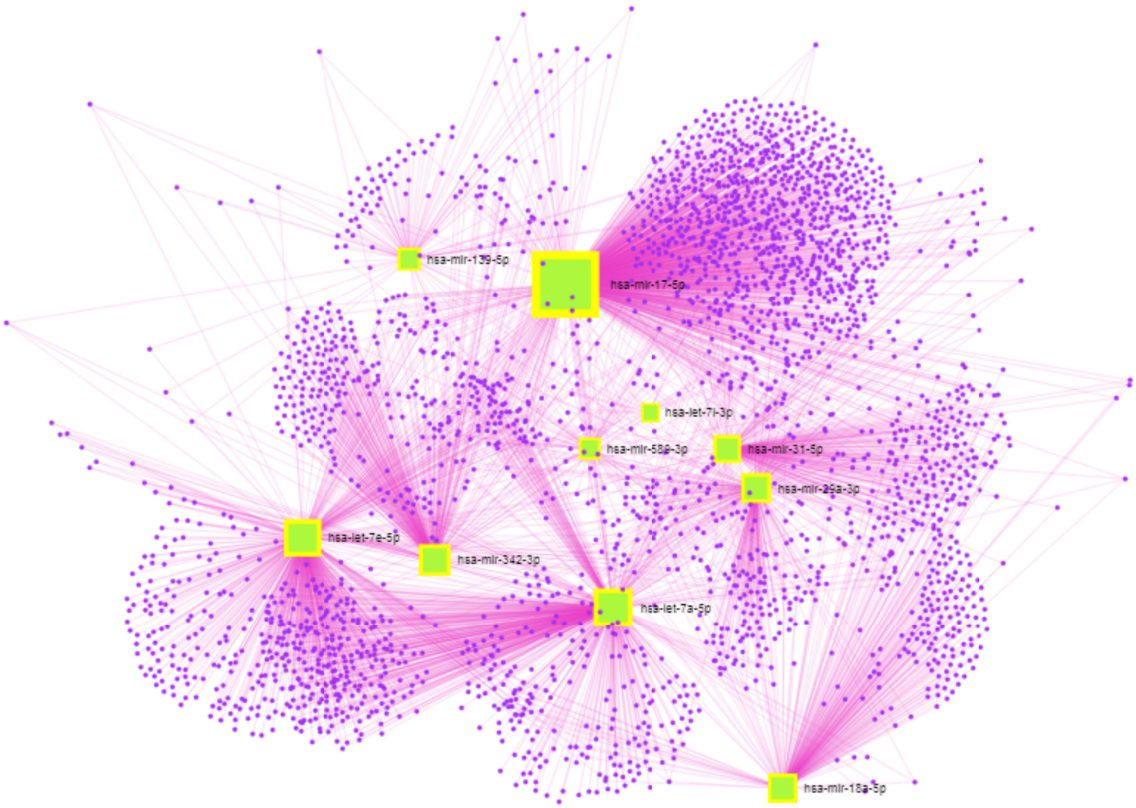


Figure 16. Common among SARS-CoV2 influenced circulating miRNAs and brain miRNAs. Square nodes denotes miRNAs and circular nodes represent targetted mRNAs. 10 miRNAs were targeting 2782 genes.

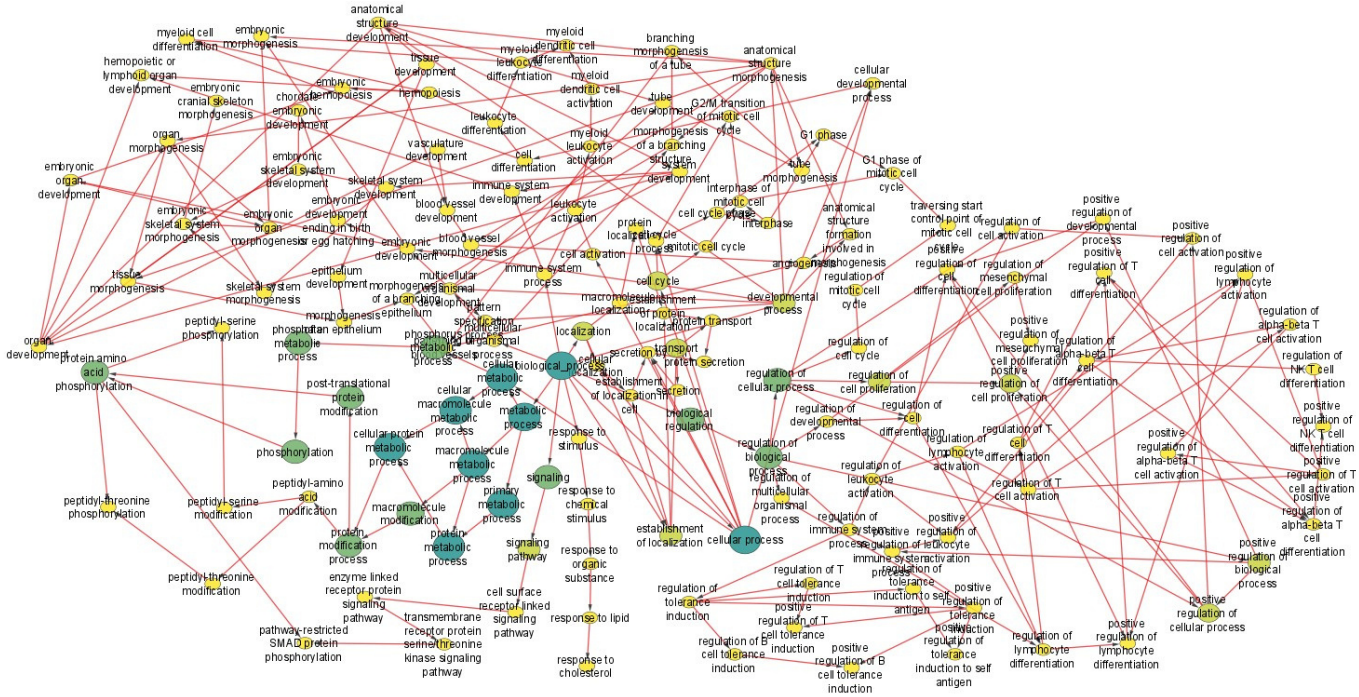


Figure 17. Pathways involving common 5 genes observed from the brain and lung. Circular nodes represent pathways associated with 5 genes. Continuous color mapping from green to yellow represent maximum to minimum significance.

4. Conclusions and Discussion

In various analyses, tissue specific complications due to SARS-CoV-2 infection has been delineated, but in case of individual influence of SARS-CoV-2 accessory proteins are yet to be delineated in detail. Here we have taken SARS-CoV-2 NSP6 protein to find its tissue specific host response. Proteins influenced after SARS-CoV-2 infection in the brain, heart and lung that are interacting with SARS-CoV-2 NSP6 interacting proteins were taken for finding pathways influenced by these proteins as well as the hub genes that could be important players in host tissue specific pathogenesis. Clue GO analysis provides a broad picture of probable pathways and biological processes undergoing perturbations related to these hub genes in brain, heart and lung of COVID infected individuals. In addition, reported circulatory miRNAs regulated after SARS-CoV-2 infection were also taken for miRNAs expressed in the brain, and lung to find out miRNA-mRNA network and miRNA influenced pathways in the brain and lung. Finding out possible miRNA influenced pathways in the heart, SARS-CoV-2 regulated genes targetted by reported circulatory miRNAs regulated after SARS-CoV-2 infection were used.

Among the common proteins in the brain, heart and lung, that were influenced by SARS-CoV-2 AIFM1 reported to be involved in apoptosis[48] and in COVID19, anti-neuronal autoantibody against this protein is formed[49]. This protein is shown to be involved in central respiratory drive and reflex responses in impaired lung function[49]. ATP6V0D1 was observed to be upregulated in A549 cells transfected with SARS-CoV-2 S plasmid and intracellular/lysosomal pH was observed to be decreased.[50] TCTN2, another regulated protein is shown to be associated with osteoarthritis.[51] PHLDA3 knock down is observed to be negatively affecting colony forming efficiency of MM468 cells expressing myr-Akt and high frequency of chromosome loss at PHLDA3 locus is also reported in large cell neuroendocrine carcinoma (LCNEC).[52]

Our study of hub genes influenced pathways in brain included pathways observed in cancer like small cell lung cancer (CDK2, CDKN1B, CCNE1, CCND1), and prostate cancer (CDK2, CDKN1B, CCNE1, CCND1) and these genes identified could be indirectly linked to genes regulating dysregulation of cell cycle, it's repair and checkpoints. With the hub genes regulated by SARS-CoV-2 infection affecting these pathways could have an effect in these cancers. It is reported that NSP6 and ORF7a through transforming growth factor *β*-activated kinase 1 (TAK1) activates NF-κB which in turn promotes proinflammatory cytokine levels.[53] In lung cancer CCND1 overexpression by NF-κB translocation was observed and was shown to be promoting cancer through PI3K/AKT signaling pathway.[54,55] Protein-protein interactome with NSP6 interacting partners in brain exhibits CCND1, CDK2, and CCNA1 as crucial players mainly altering cell cycle processes. Majority of the of the pathway terms were G1/S transition and the next highest group consisted of iron uptake and transport terms. Activation of PTK6 and regulation of cell cycle associated pathways and p53 signalling pathways are also found in the analysis. This instigates for further exploration and elucidation of impact of coronavirus NSP6 on cancer. For the lung hub genes influenced pathways, ATP6V0A1, ATP6V0A2, ATP6V0D1, ATP6V1D, ATP6V1B1, and ATP6V1E1 were involved in intraphagosomal pH lowering by the V-ATPase. SARS-CoV-2 were shown to be increasing the expression of V-ATPase.[50] Pathway involving other hub genes TGFB1, TGFBR2, and LGALS3 was dendritic cell differentiation. Further analysis of pathways involving heart hub genes RAB7A, ATPV0D1, and LAMP2 were associated with phagosome related processes. Another hub gene TGFB1 showed association with rheumatoid arthritis that is also one of the reported disease in postacute sequelae of COVID-19.[56]

For the hub genes in the brain CDK2, CCNB1, CCNE1, CCND1, and CDKN1B were drug targets for both approved and non-approved drugs with some of them having more than one target protein.

Some approved drugs were targeting multiple proteins in our analyses. Acetaminophen targetted CDK2 and CCND1, palbociclib targetted CCND1 and CCNE1, raltitrexed targetted CDK2 and CDKN1B, lapatinib targetted CCND1 and CDKN1B, doxorubicin hydrochloride and methotrexate targetted CCND1 and CDKN1B. Considering the hub genes in heart, TGFB1, CANX, APP, and LGALS3 were the drug targets and one of the drug inositol targetted TGFB1 and APP. In the lung irinotecan hydrochloride targetted TGFB1 and TGFBR2 among the drug targets TGFB1, LGALS3, TGFBR2, and EGF. Drugs targeting hub genes were anti-cancer drugs like palbociclib, lapatinib, irinotecan, etc., anti-rheumatic like methotrexate, anti-pyretic like acetaminophen, etc.

Deleterious effect of NSP6 in heart or cardiomyocytes are reported. Expression of NSP6 in *Drosophila* heart caused cardiac actin filaments getting disorganized and significantly reducing fiber density affecting its function with reduced diastolic diameter of the heart tube.[58] SARS-CoV-2 NSP6, NSP8 and M overexpression caused transcriptome and proteome change in human pluripotent stem cell (hPSC) derived cardiomyocytes as well as undergo apoptosis.[28] One of the binding partners of SARS-CoV-2 NSP6 interacting proteins regulated in COVID-19 infection was galectin 3. It was observed to be upregulated in heart after SARS-CoV-2 infection. Myocardial galectin 3 expression was reported to be significantly correlated with inflammatory cell count on endomyocardial biopsy in inflammatory cardiomyopathy.[59] Galectins act as modulators of tumour progression.[60] SARS-CoV-2 infection lead to many cardiovascular complications and postacute sequelae,[8,9,11,56,61,62] including myocardial inflammation.[63–66] Many reports point out link between heart failure (HF) and cancer incidence.[62,67,68,68–74] Systemic inflammatory signals were shown to be promoting cancer.[69] Cancer incidence post COVID is still unclear and our study hints in this direction. In our analyses, increased expression of galactin 3 that has a positive influence in the cancer incidence, prompts a study in the incidence of cancer after SARS-CoV-2 infection. This could help in the inclusion of early cancer detection protocols in the health care system after SARS-CoV-2 infection for better prognosis in the event of cancer incidence.

Supporting information Data

Data 1: Supporting Information Data 1_All interacting proteins
Data 2: Supporting Information Data 2_Hub genes
Data 3: Supporting Information Data 3_Hub gene drug targets
Data 4: Supporting Information Data 4_Common and unique pathways
Data 5: Supporting Information Data 5_DEG biological pathways
Data 6: Supporting Information Data 6_Common and unique miRNA
Data 7: Supporting Information Data 7_Brain miRNET miR gene
Data 8: Supporting Information Data 8_Brain miRNA pathways
Data 9: Supporting Information Data 9_Lung miRNET miR gene
Data 10: Supporting Information Data 10_Lung miRNA pathways
Data 11: Supporting Information Data 11_miRNET heart genes
Data 12: Supporting Information Data 12_miRNAs_common_circulatory_brain_lung
Data 13: Supporting Information Data 13_Common miRNA_Circulatory_lung genes
Data 14: Supporting Information Data 14_Common miRNA_Circulatory_brain genes
Data 15: Supporting Information Data 15_Pathway list of 5 genes common in lung and brain

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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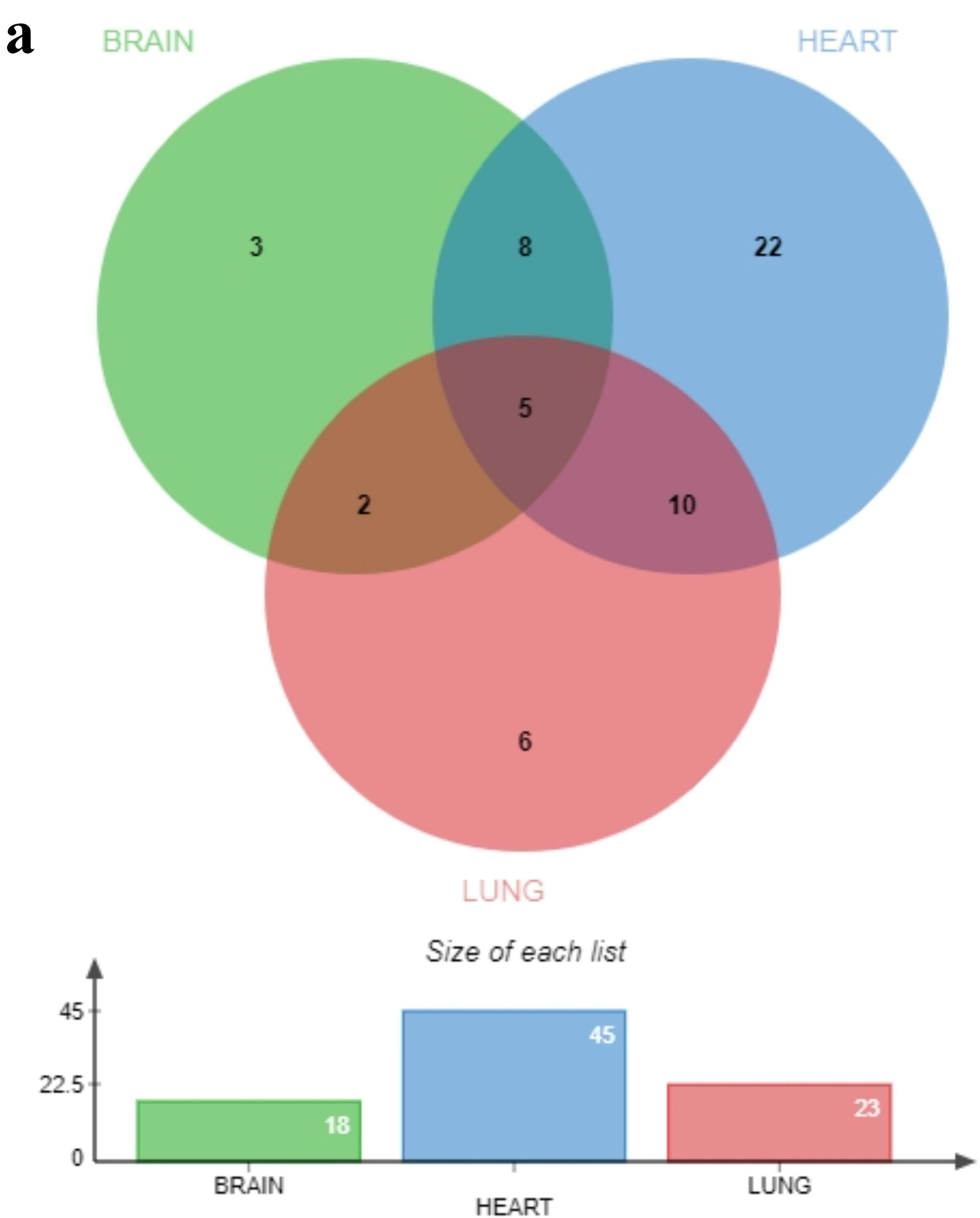
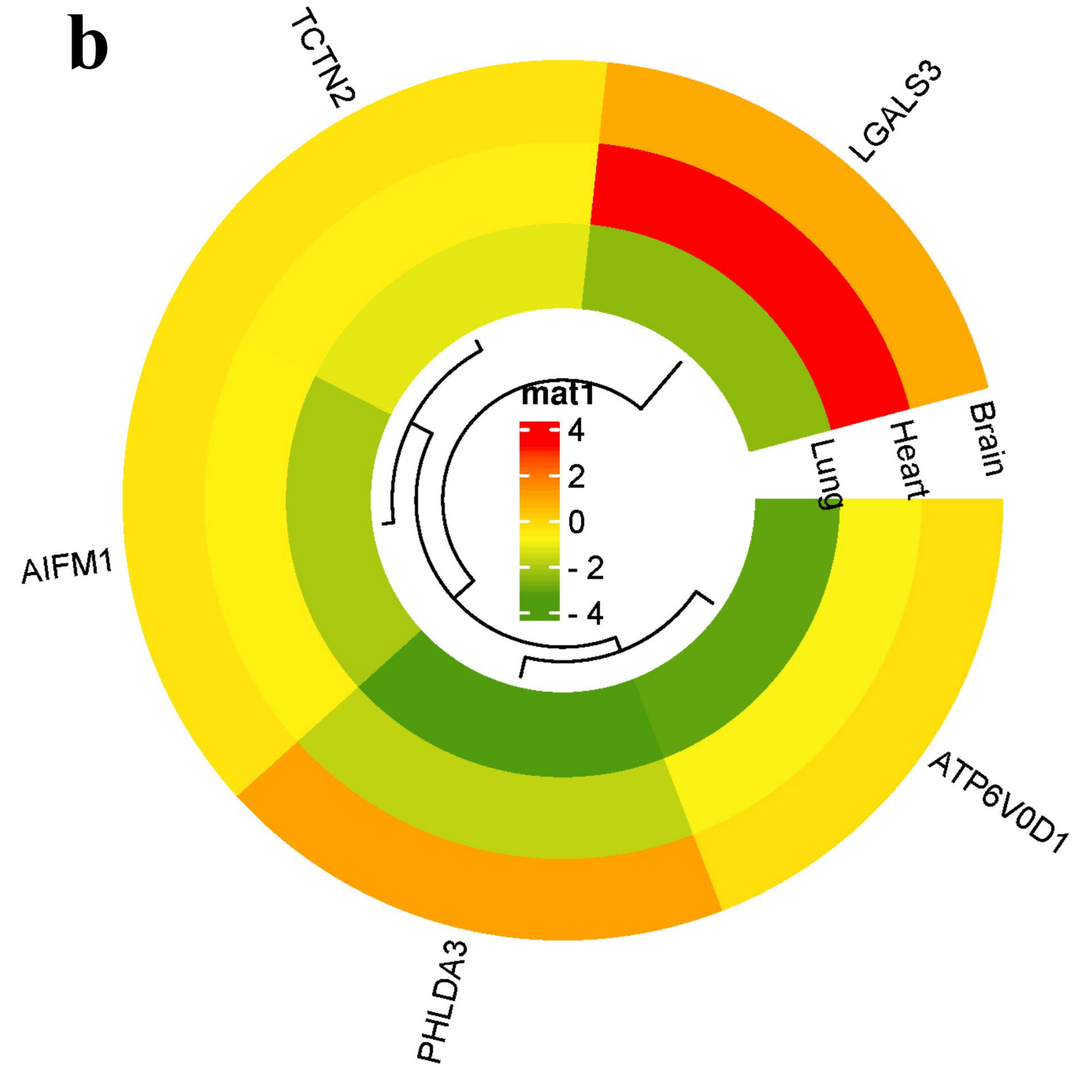
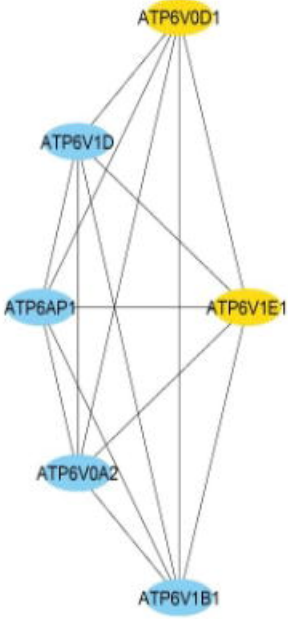
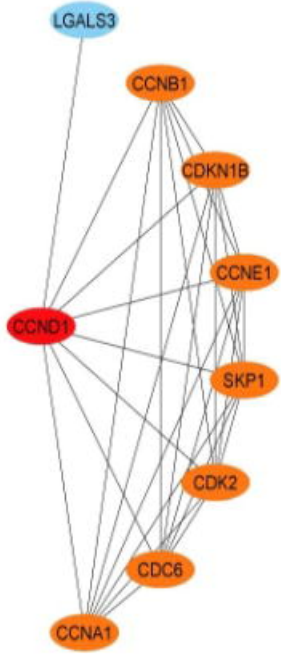
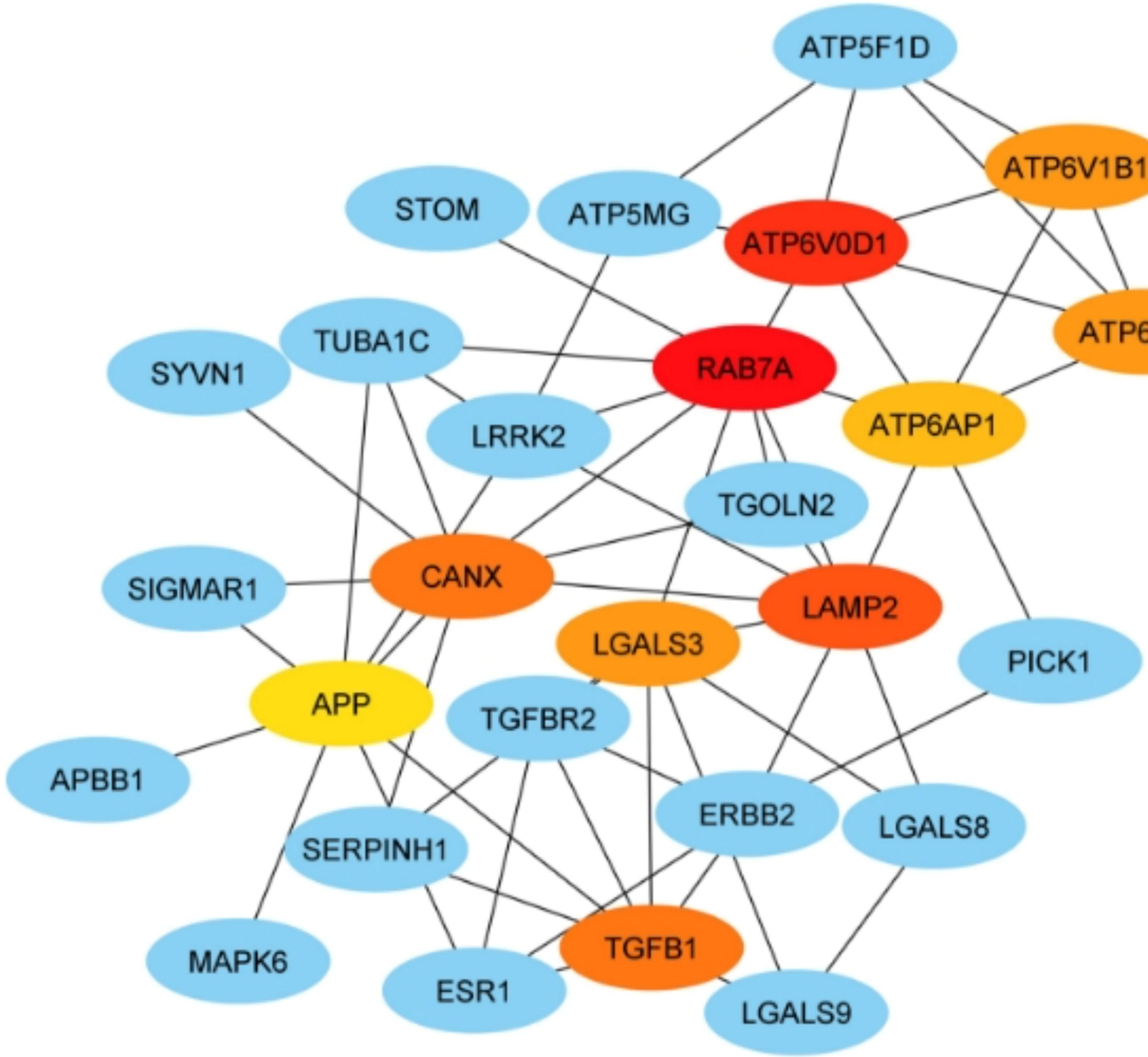

a
BRAIN
HEART
LUNG
3
8
22
5
2
10
6
Size of each list
45
22.5
0
18
45
23
BRAIN
HEART
LUNG


b
TCTN2
LGALS3
Brain
Heart
Lung
ATP6V0D1
PHLDA3
AIFM1
mat1
4
2
0
- 2
- 4

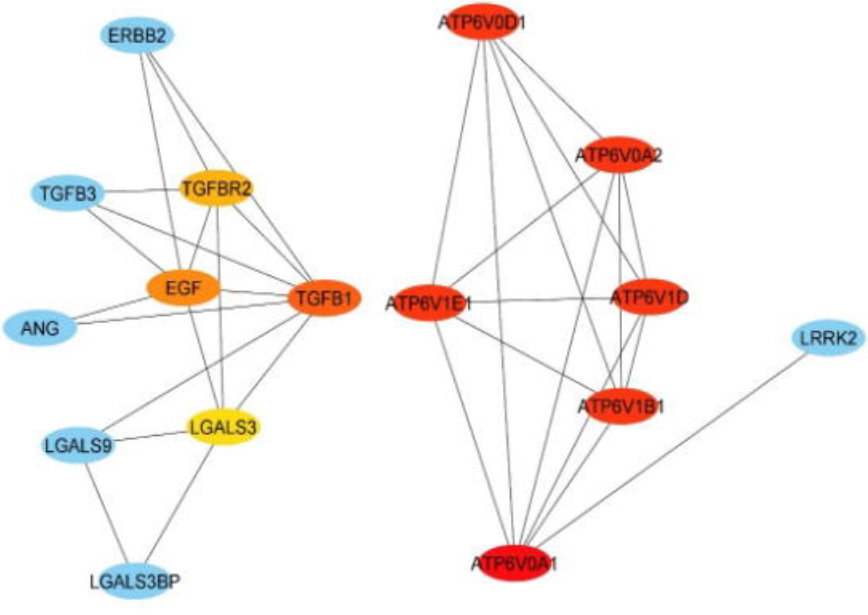




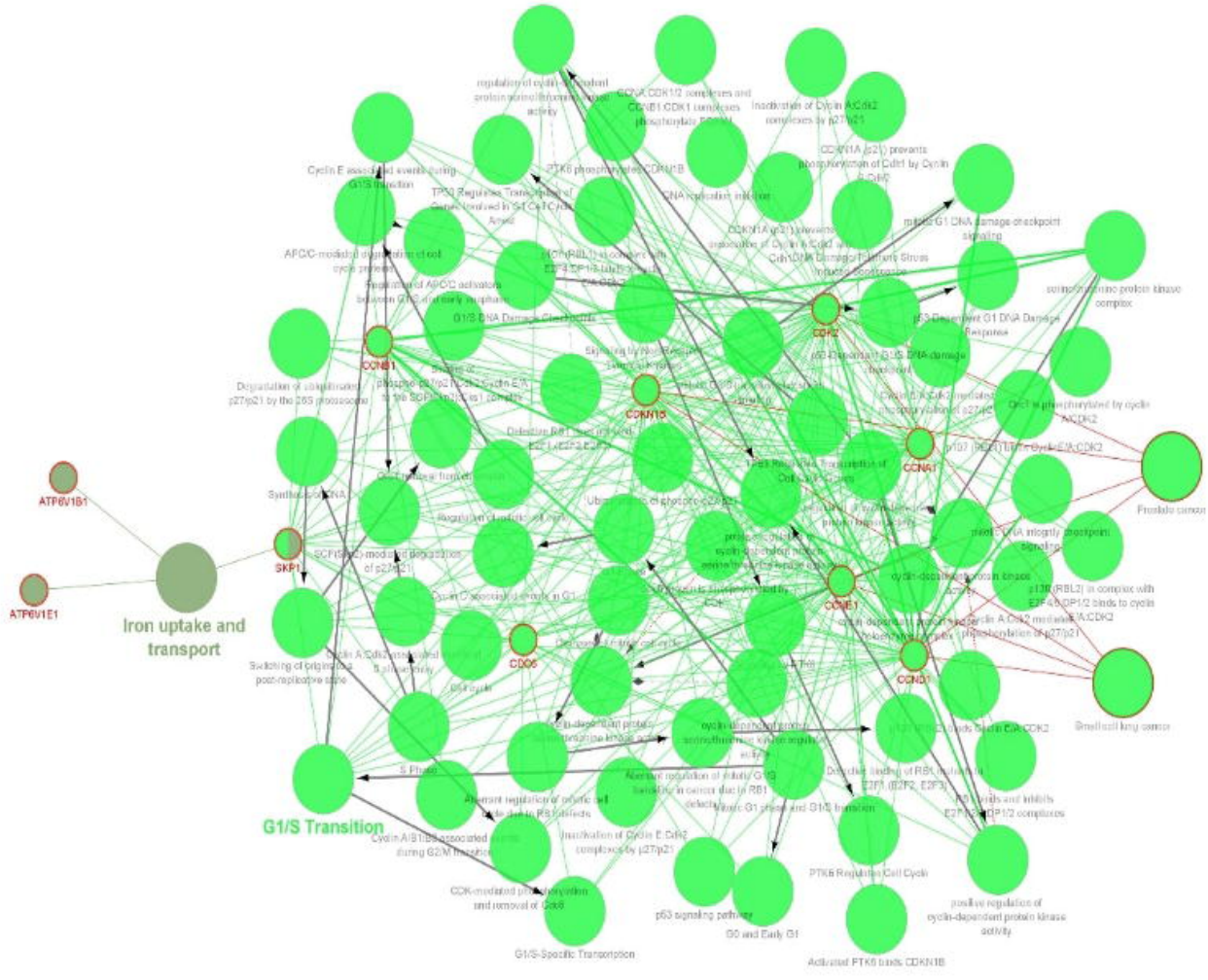
Rank	Node
1	CCND1
2	CDK2
2	CCNA1
2	CDC6
2	CDKN1B
2	SKP1
2	CCNB1
2	CCNE1
9	ATP6V1B1
9	ATP6V1E1



Rank	Node
1	RAB7A
2	ATP6V0D1
3	LAMP2
4	TGFB1
4	CANX
6	LGALS3
6	ATP6V1B1
6	ATP6V1D
9	ATP6AP1
10	APP



Rank	Node
1	ATP6V0A1
2	ATP6V0A2
2	ATP6V0D1
2	ATP6V1D
2	ATP6V1B1
2	ATP6V1E1
7	TGFB1
8	EGF
9	TGFBR2
10	LGALS3


ATP6V1B1
ATP6V1E1
Iron uptake and transport
SKP1
CCNB1
CDK2
CDKN1B
CCNA1
CCNE1
CDC6
CCND1
G1/S Transition

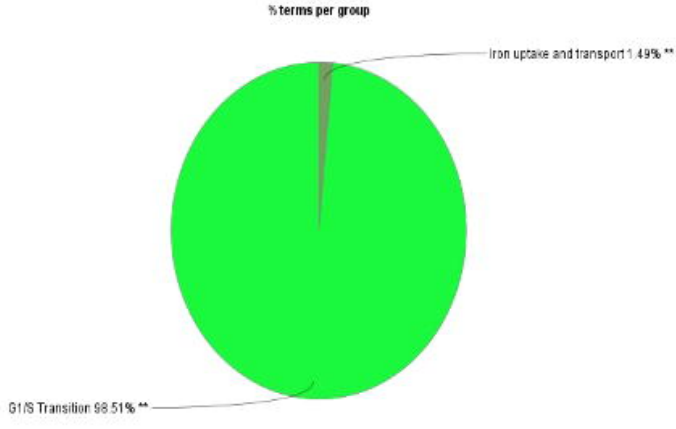
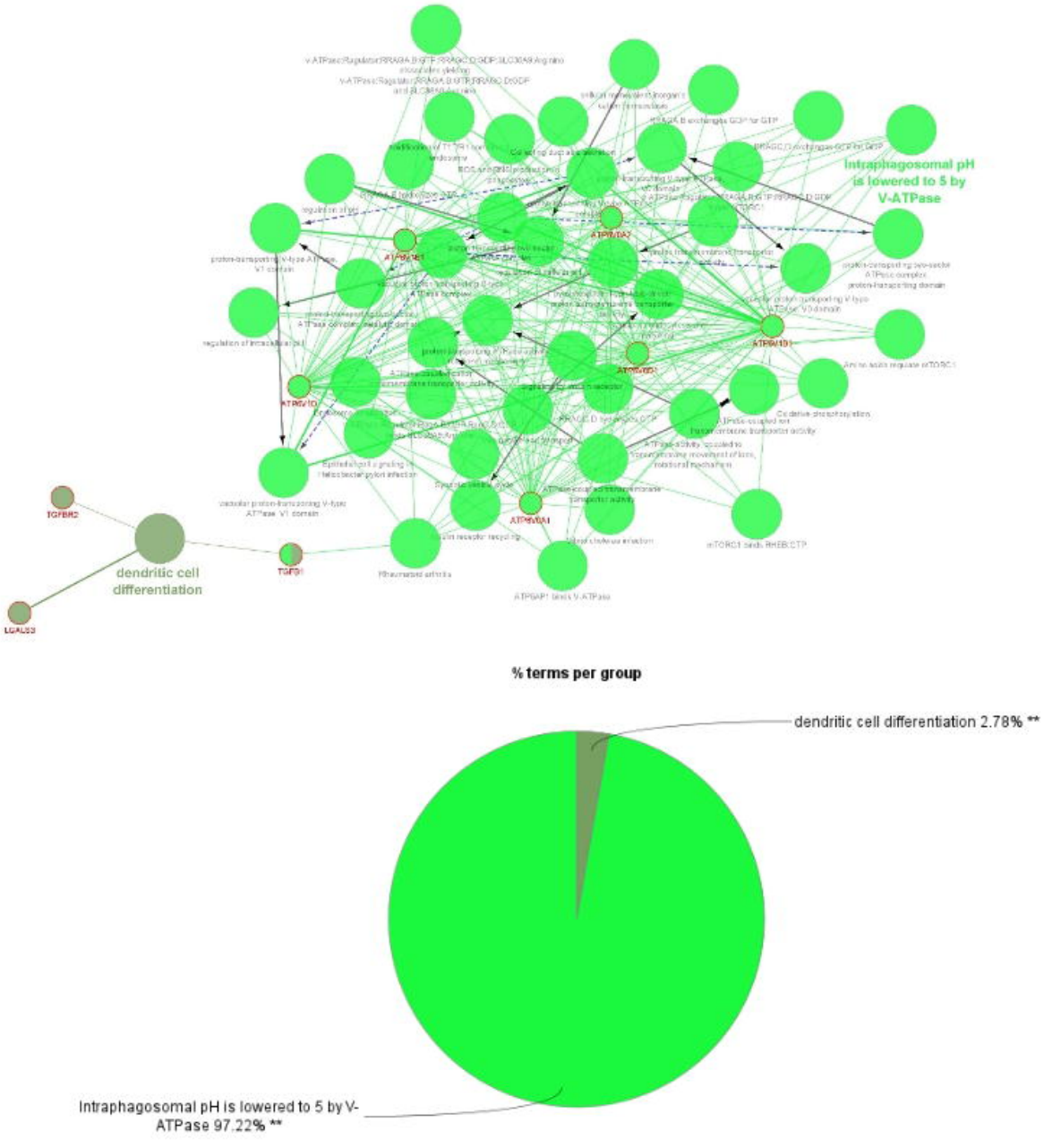
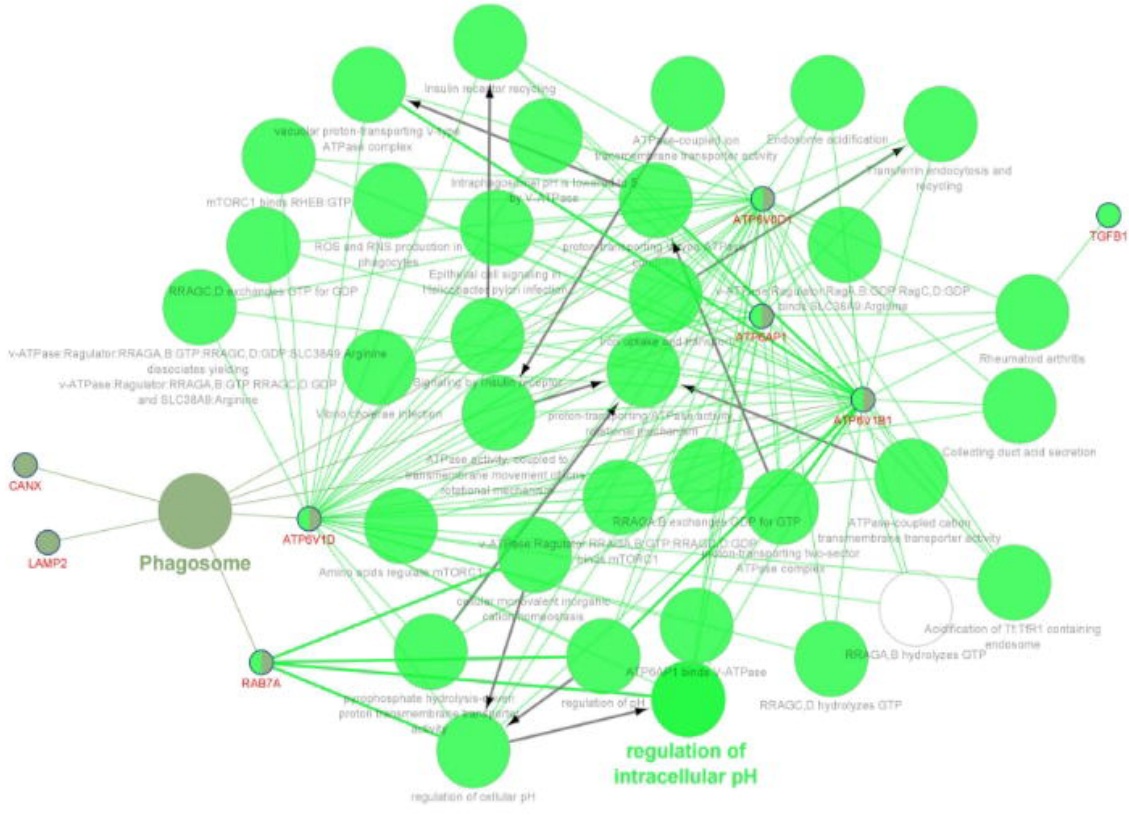
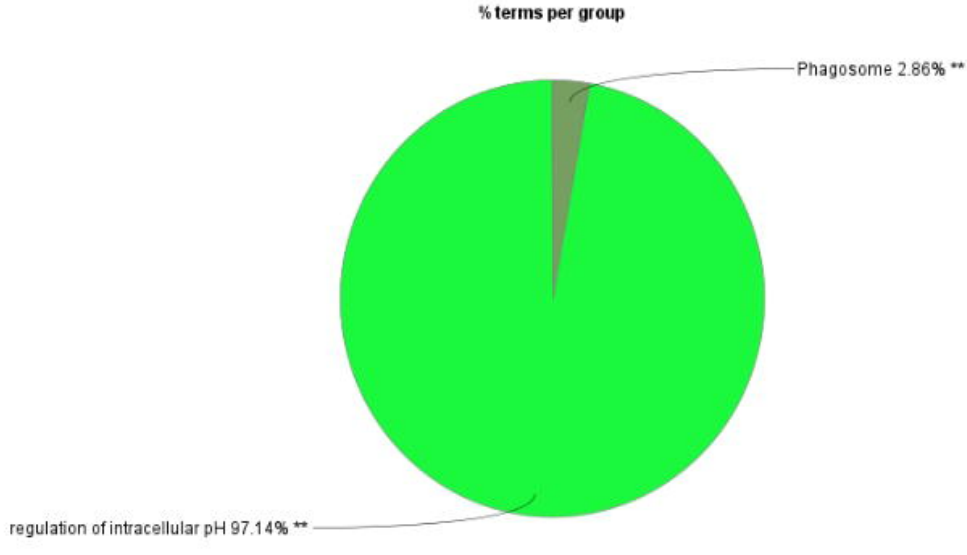

% terms per group
Iron uptake and transport 1.49% **
G1/S Transition 98.51% **

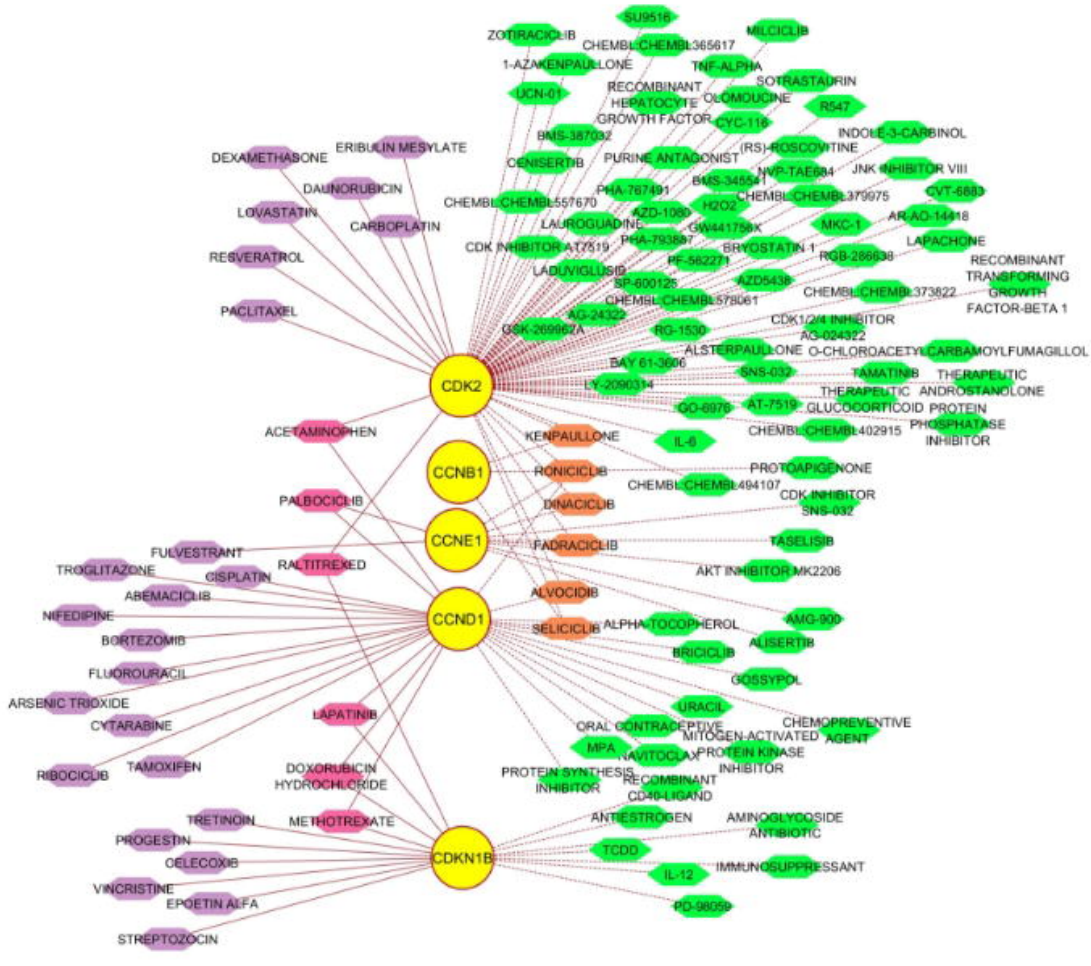
Figure 3b. Gene ontology processes, KEGG pathway and reactome pathway analyses of top 10 hub genes in the lung by CluGO analysis



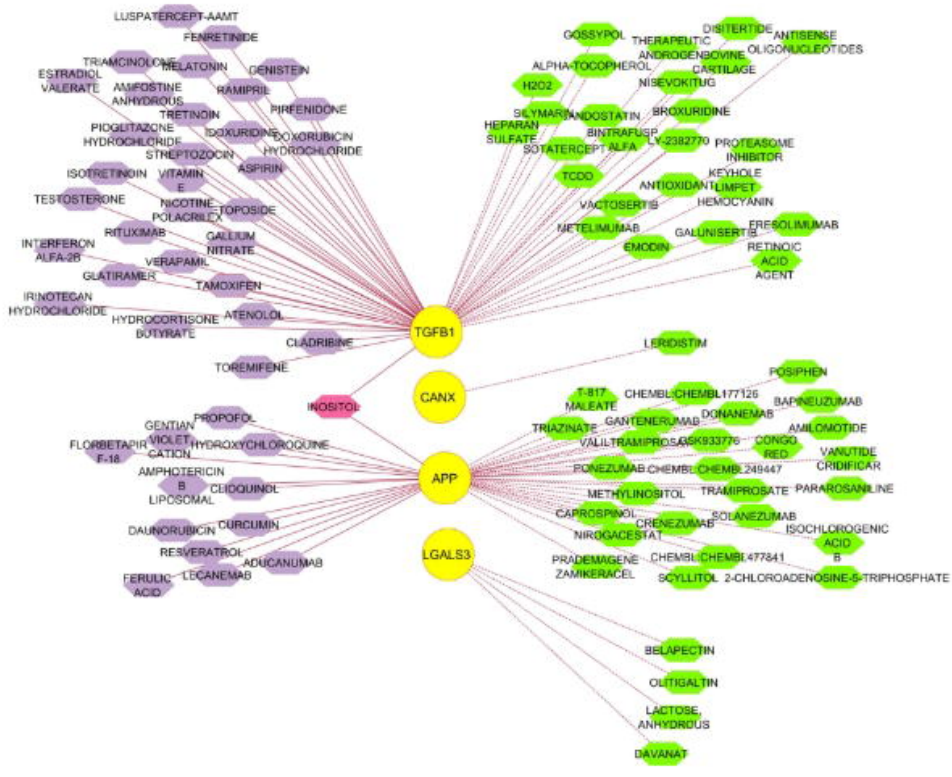

Phagosome
CANX
LAMP2
ATP6V1D
RAB7A
ATP6V0D1
ATP6AP1
ATP6V1B1
TGFB1
regulation of intracellular pH
regulation of cellular pH
Rheumatoid arthritis
Collecting duct acid secretion
Endosome acidification
Transferrin endocytosis and recycling
Insulin receptor recycling
RRAGC,D exchanges GTP for GDP
RRAGC,D hydrolyzes GTP
RRAGA,B hydrolyzes GTP
Acidification of Tf:TfR1 containing endosome


% terms per group
Phagosome 2.86% **
regulation of intracellular pH 97.14% **

Brain


CDK2
CCNB1
CCNE1
CCND1
CDKN1B
SU9516
ZOTIRACICLIB
MILCICLIB
CHEMBL:CHEMBL365617
1-AZAKENPAULLONE
TNF-ALPHA
UCN-01
RECOMBINANT HEPATOCYTE GROWTH FACTOR
SOTRASTAURIN
OLOMOUCINE
R547
CYC-116
BMS-387032
INDOLE-3-CARBINOL
(RS)-ROSCOVITINE
DEXAMETHASONE
ERIBULIN MESYLATE
CENISERTIB
PURINE ANTAGONIST
NVP-TAE684
JNK INHIBITOR VIII
DAUNORUBICIN
PHA-767491
BMS-345541
CVT-6883
LOVASTATIN
CHEMBL:CHEMBL557670
CHEMBL:CHEMBL379975
AZD-1080
H2O2
CARBOPLATIN
LAUROGUADINE
GW441756X
MKC-1
AR-AO-14418
CDK INHIBITOR AT7519
PHA-793887
BRYOSTATIN 1
LAPACHONE
RESVERATROL
PF-562271
RGB-286638
LADUVIGLUSIB
RECOMBINANT TRANSFORMING GROWTH FACTOR-BETA 1
SP-600125
AZD5438
CHEMBL:CHEMBL578061
CHEMBL:CHEMBL373822
PACLITAXEL
AG-24322
CDK1/2/4 INHIBITOR
GSK-269962A
RG-1530
AG-024322
ALSTERPAULLONE
O-CHLOROACETYL CARBAMOYLFUMAGILLOL
BAY 61-3606
SNS-032
TAMATINIB
THERAPEUTIC ANDROSTANOLONE
LY-2090314
THERAPEUTIC GLUCOCORTICOID PROTEIN
GO-6976
AT-7519
PHOSPHATASE INHIBITOR
CHEMBL:CHEMBL402915
ACETAMINOPHEN
KENPAULLONE
IL-6
RONICICLIB
PROTOAPIGENONE
CHEMBL:CHEMBL494107
CDK INHIBITOR SNS-032
PALBOCICLIB
DINACICLIB
FULVESTRANT
FADRACICLIB
TASELISIB
TROGLITAZONE
CISPLATIN
RALTITREXED
AKT INHIBITOR MK2206
ABEMACICLIB
ALVOCIDIB
NIFEDIPINE
SELICICLIB
ALPHA TOCOPHEROL
AMG-900
BORTEZOMIB
BRICICLIB
ALISERTIB
FLUOROURACIL
GOSSYPOL
ARSENIC TRIOXIDE
URACIL
LAPATINIB
CYTARABINE
ORAL CONTRACEPTIVE
CHEMOPREVENTIVE AGENT
MITOGEN-ACTIVATED PROTEIN KINASE INHIBITOR
MPA
NAVITOCLAX
RIBOCICLIB
TAMOXIFEN
DOXORUBICIN HYDROCHLORIDE
PROTEIN SYNTHESIS INHIBITOR
RECOMBINANT CD40-LIGAND
TRETINOIN
METHOTREXATE
ANTIESTROGEN
AMINOGLYCOSIDE ANTIBIOTIC
PROGESTIN
TCDD
CELECOXIB
IL-12
IMMUNOSUPPRESSANT
VINCRISTINE
EPOETIN ALFA
PD-98059
STREPTOZOCIN

Heart


LUSPATERCEPT-AAMT
FENRETINIDE
TRIAMCINOLONE
MELATONIN
GENISTEIN
ESTRADIOL VALERATE
AMIFOSTINE ANHYDROUS
RAMIPRIL
TRETINOIN
PIRFENIDONE
PIOGLITAZONE HYDROCHLORIDE
IDOXURIDINE
DOXORUBICIN HYDROCHLORIDE
STREPTOZOCIN
ASPIRIN
ISOTRETINOIN
VITAMIN E
TESTOSTERONE
NICOTINE POLACRILEX
ETOPOSIDE
RITUXIMAB
GALLIUM NITRATE
INTERFERON ALFA-2B
VERAPAMIL
GLATIRAMER
TAMOXIFEN
IRINOTECAN HYDROCHLORIDE
HYDROCORTISONE BUTYRATE
ATENOLOL
CLADRIBINE
TOREMIFENE
GOSSYPOL
THERAPEUTIC
DISITERTIDE
ANTISENSE OLIGONUCLEOTIDES
ANDROGENBOVINE CARTILAGE
ALPHA-TOCOPHEROL
NISEVOKITUG
H2O2
SILYMARIN
INDOSTATIN
BROXURIDINE
HEPARAN SULFATE
BINTRAFUSP ALFA
LY-2382770
PROTEASOME INHIBITOR
SOTATERCEPT
TCDD
KEYHOLE LIMPET HEMOCYANIN
ANTIOXIDANT
VACTOSERTIB
METELIMUMAB
GALUNISERTIB
FRESOLIMUMAB
EMODIN
RETINOIC ACID AGENT
TGFB1
LERIDISTIM
POSIPHEN
CANX
T-817 MALEATE
CHEMBL:CHEMBL177126
BAPINEUZUMAB
INOSITOL
DONANEMAB
GANTENERUMAB
TRIAZINATE
AMILOMOTIDE
VALILTRAMIPROSATE
GSK933776
CONGO RED
VANUTIDE CRIDIFICAR
PROPOFOL
GENTIAN VIOLET CATION
FLORBETAPIR F-18
HYDROXYCHLOROQUINE
PONEZUMAB
CHEMBL:CHEMBL249447
APP
AMPHOTERICIN B LIPOSOMAL
CEIDQUINOL
METHYLINOSITOL
TRAMIPROSATE
PARAROSANILINE
CAPROSPINOL
SOLANEZUMAB
CRENEZUMAB
ISOCHLOROGENIC ACID B
DAUNORUBICIN
CURCUMIN
NIROGACESTAT
RESVERATROL
ADUCANUMAB
LGALS3
PRADEMAGENE ZAMIKERACEL
CHEMBL:CHEMBL477841
FERULIC ACID
LECANEMAB
SCYLLITOL
2-CHLOROADENOSINE-5-TRIPHOSPHATE
BELAPECTIN
OLITIGALTIN
LACTOSE, ANHYDROUS
DAVANAT

Lung

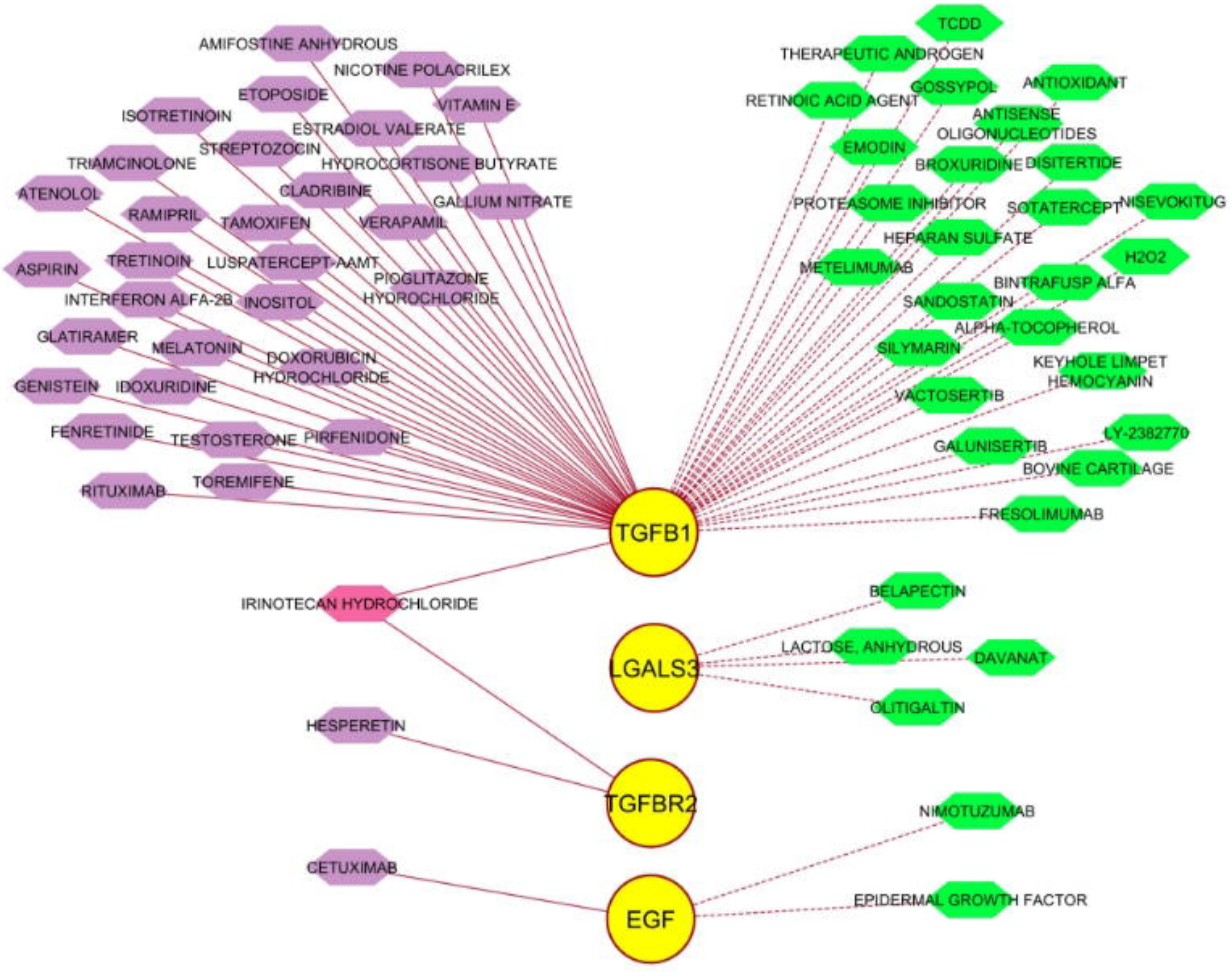

AMIFOSTINE ANHYDROUS
NICOTINE POLACRILEX
ETOPOSIDE
VITAMIN E
ISOTRETINOIN
ESTRADIOL VALERATE
STREPTOZOCIN
TRIAMCINOLONE
HYDROCORTISONE BUTYRATE
ATENOLOL
CLADRIBINE
GALLIUM NITRATE
RAMIPRIL
TAMOXIFEN
VERAPAMIL
ASPIRIN
TRETINOIN
LUSPATERCEPT-AAMT
PIOGLITAZONE HYDROCHLORIDE
INTERFERON ALFA-2B
INOSITOL
GLATIRAMER
MELATONIN
DOXORUBICIN HYDROCHLORIDE
GENISTEIN
IDOXURIDINE
FENRETINIDE
TESTOSTERONE
PIRFENIDONE
RITUXIMAB
TOREMIFENE
TCDD
THERAPEUTIC ANDROGEN
GOSSYPOL
ANTIOXIDANT
RETINOIC ACID AGENT
ANTISENSE OLIGONUCLEOTIDES
EMODIN
BROXURIDINE
DISITERTIDE
PROTEASOME INHIBITOR
SOTATERCEPT
NISEVOKITUG
HEPARAN SULFATE
H2O2
METELIMUMAB
BINTRAFUSP ALFA
SANDOSTATIN
ALPHA-TOCOPHEROL
SILYMARIN
KEYHOLE LIMPET HEMOCYANIN
VACTOSERTIB
LY-2382770
GALUNISERTIB
BOVINE CARTILAGE
FRESOLIMUMAB
TGFB1
IRINOTECAN HYDROCHLORIDE
BELAPECTIN
LGALS3
LACTOSE, ANHYDROUS
DAVANAT
OLITIGALTIN
HESPERETIN
TGFBR2
NIMOTUZUMAB
CETUXIMAB
EGF
EPIDERMAL GROWTH FACTOR

Figure8

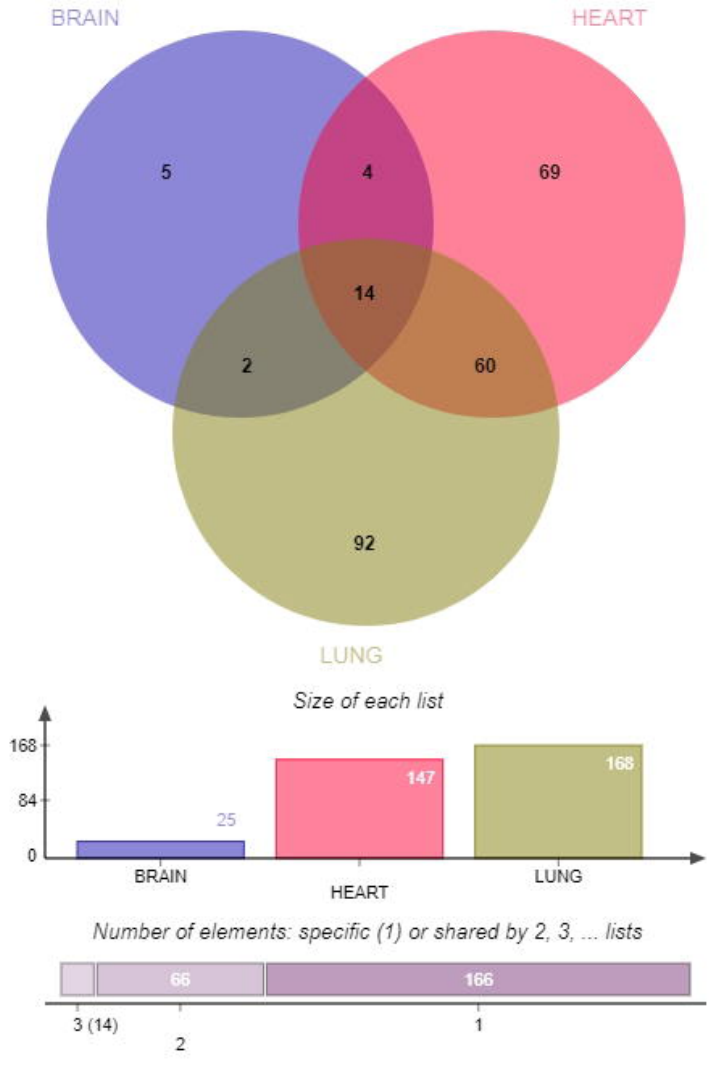

BRAIN
HEART
LUNG
5
4
69
14
2
60
92
Size of each list
168
84
0
25
147
168
BRAIN
HEART
LUNG
Number of elements: specific (1) or shared by 2, 3, ... lists
66
166
3 (14)
2
1

Figure 6

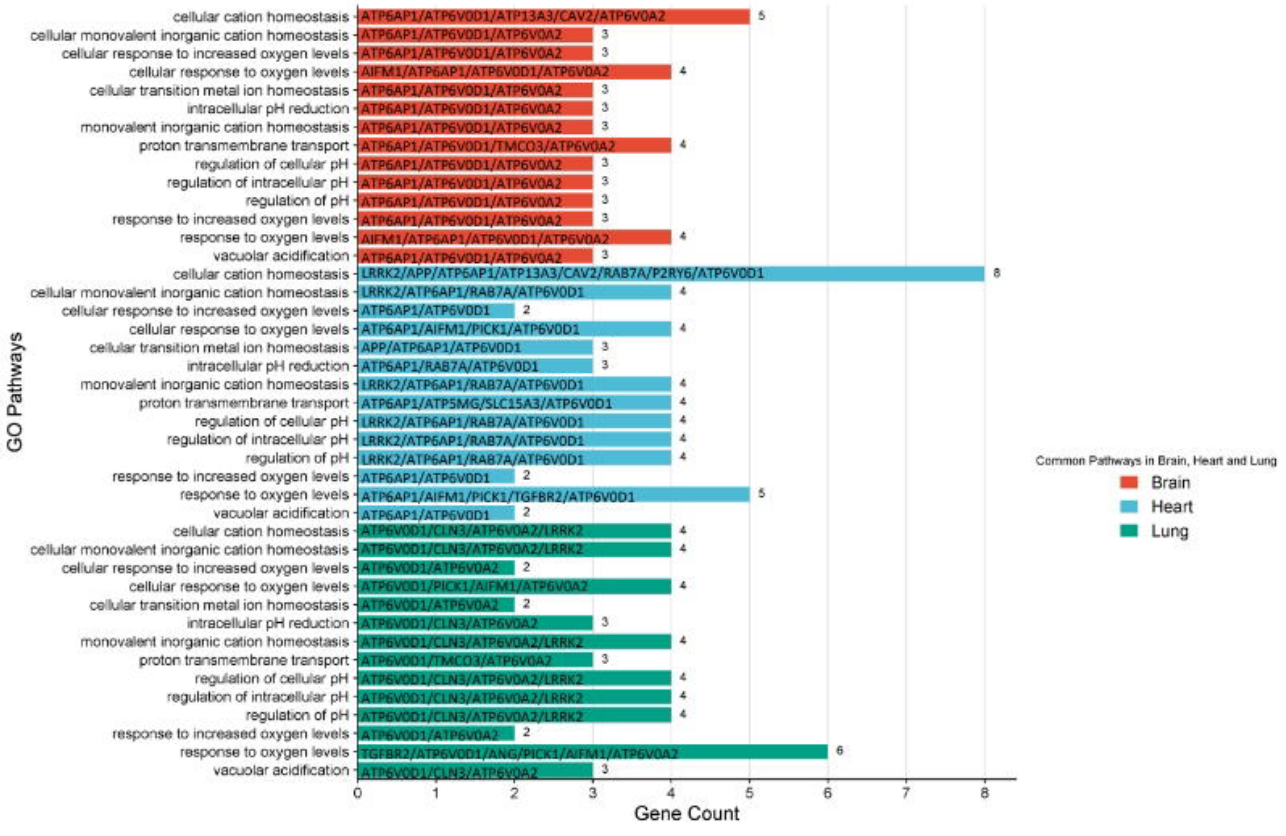

GO Pathways
cellular cation homeostasis
cellular monovalent inorganic cation homeostasis
cellular response to increased oxygen levels
cellular response to oxygen levels
cellular transition metal ion homeostasis
intracellular pH reduction
monovalent inorganic cation homeostasis
proton transmembrane transport
regulation of cellular pH
regulation of intracellular pH
regulation of pH
response to increased oxygen levels
response to oxygen levels
vacuolar acidification
cellular cation homeostasis
cellular monovalent inorganic cation homeostasis
cellular response to increased oxygen levels
cellular response to oxygen levels
cellular transition metal ion homeostasis
intracellular pH reduction
monovalent inorganic cation homeostasis
proton transmembrane transport
regulation of cellular pH
regulation of intracellular pH
regulation of pH
response to increased oxygen levels
response to oxygen levels
vacuolar acidification
cellular cation homeostasis
cellular monovalent inorganic cation homeostasis
cellular response to increased oxygen levels
cellular response to oxygen levels
cellular transition metal ion homeostasis
intracellular pH reduction
monovalent inorganic cation homeostasis
proton transmembrane transport
regulation of cellular pH
regulation of intracellular pH
regulation of pH
response to increased oxygen levels
response to oxygen levels
vacuolar acidification
ATP6AP1/ATP6V0D1/ATP13A3/CAV2/ATP6V0A2
ATP6AP1/ATP6V0D1/ATP6V0A2
AIFM1/ATP6AP1/ATP6V0D1/ATP6V0A2
ATP6AP1/ATP6V0D1/TMCO3/ATP6V0A2
LRRK2/APP/ATP6AP1/ATP13A3/CAV2/RAB7A/P2RY6/ATP6V0D1
LRRK2/ATP6AP1/RAB7A/ATP6V0D1
ATP6AP1/ATP6V0D1
ATP6AP1/AIFM1/PICK1/ATP6V0D1
APP/ATP6AP1/ATP6V0D1
ATP6AP1/RAB7A/ATP6V0D1
ATP5MG/SLC15A3/ATP6V0D1
ATP6AP1/AIFM1/PICK1/TGFBR2/ATP6V0D1
ATP6V0D1/CLN3/ATP6V0A2/LRRK2
ATP6V0D1/ATP6V0A2
ATP6V0D1/PICK1/AIFM1/ATP6V0A2
ATP6V0D1/CLN3/ATP6V0A2
ATP6V0D1/TMCO3/ATP6V0A2
TGFBR2/ATP6V0D1/ANG/PICK1/AIFM1/ATP6V0A2
0
1
2
3
4
5
6
7
8
Gene Count
Common Pathways in Brain, Heart and Lung
Brain
Heart
Lung

Figure 7

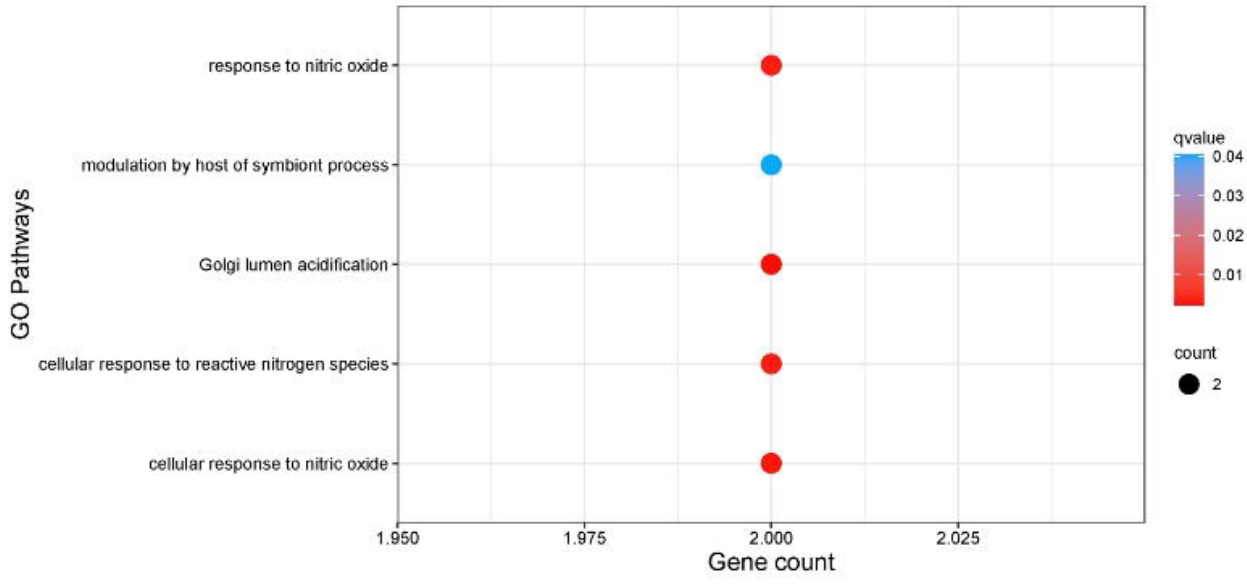

response to nitric oxide
modulation by host of symbiont process
Golgi lumen acidification
cellular response to reactive nitrogen species
cellular response to nitric oxide
GO Pathways
1.950
1.975
2.000
2.025
Gene count
qvalue
0.04
0.03
0.02
0.01
count
2

Figure 7b

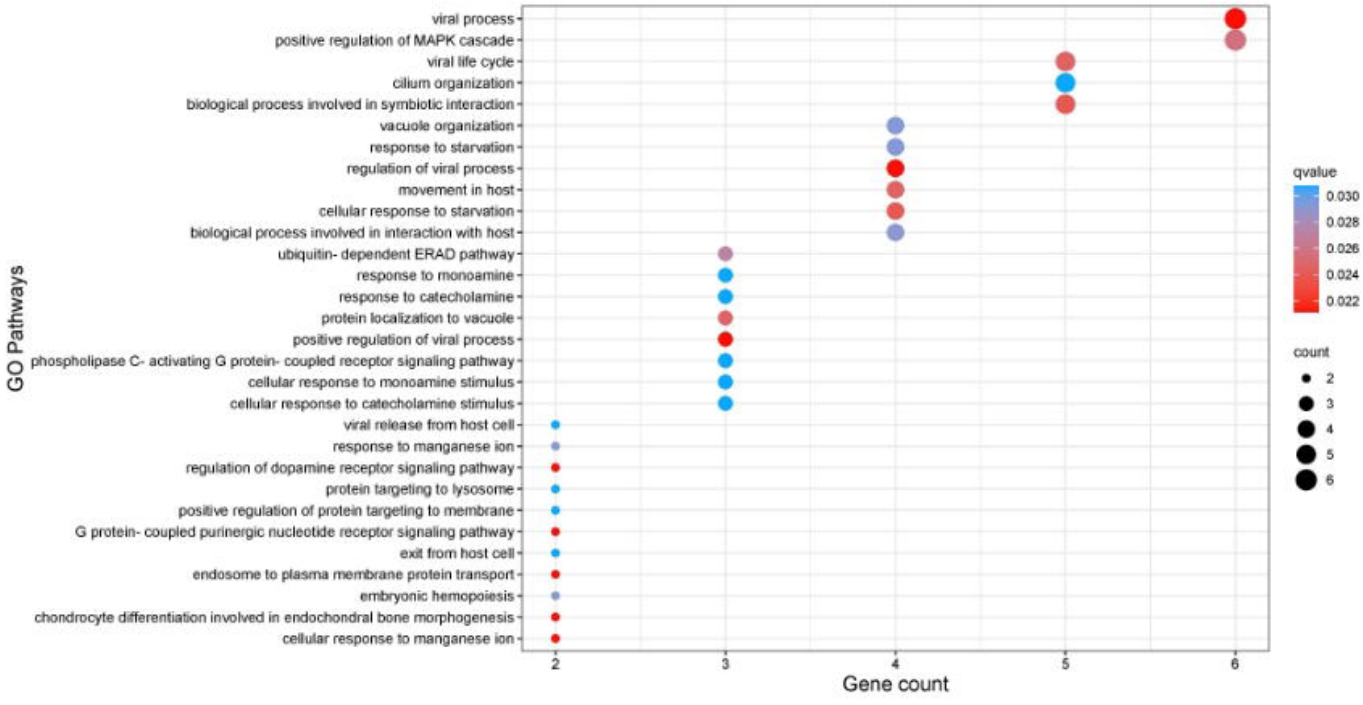

viral process
positive regulation of MAPK cascade
viral life cycle
cilium organization
biological process involved in symbiotic interaction
vacuole organization
response to starvation
regulation of viral process
movement in host
cellular response to starvation
biological process involved in interaction with host
ubiquitin- dependent ERAD pathway
response to monoamine
response to catecholamine
protein localization to vacuole
positive regulation of viral process
phospholipase C- activating G protein- coupled receptor signaling pathway
cellular response to monoamine stimulus
cellular response to catecholamine stimulus
viral release from host cell
response to manganese ion
regulation of dopamine receptor signaling pathway
protein targeting to lysosome
positive regulation of protein targeting to membrane
G protein- coupled purinergic nucleotide receptor signaling pathway
exit from host cell
endosome to plasma membrane protein transport
embryonic hemopoiesis
chondrocyte differentiation involved in endochondral bone morphogenesis
cellular response to manganese ion
GO Pathways
2
3
4
5
6
Gene count
qvalue
0.030
0.028
0.026
0.024
0.022
count
2
3
4
5
6

Figure 7c

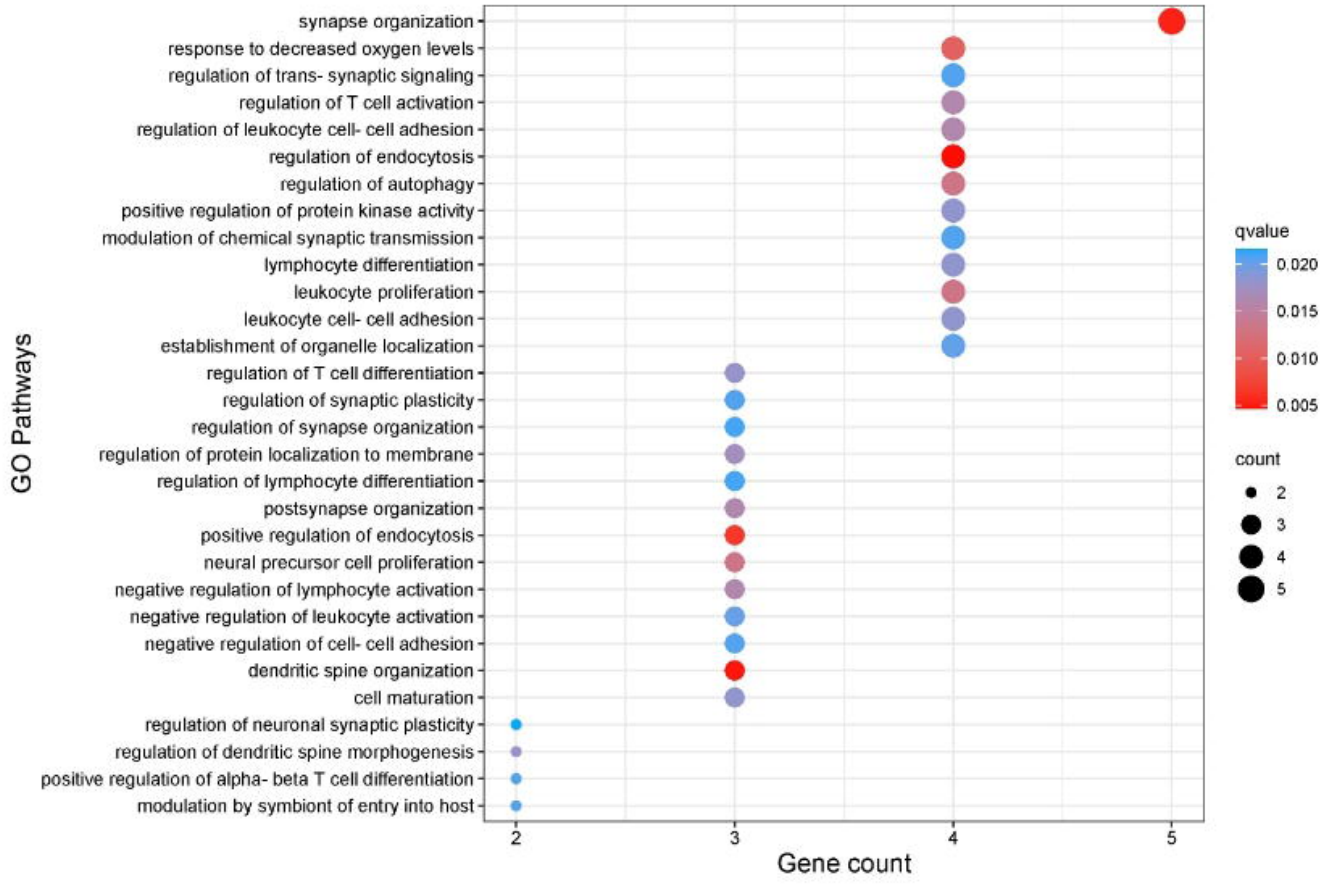

synapse organization
response to decreased oxygen levels
regulation of trans- synaptic signaling
regulation of T cell activation
regulation of leukocyte cell- cell adhesion
regulation of endocytosis
regulation of autophagy
positive regulation of protein kinase activity
modulation of chemical synaptic transmission
lymphocyte differentiation
leukocyte proliferation
leukocyte cell- cell adhesion
establishment of organelle localization
regulation of T cell differentiation
regulation of synaptic plasticity
regulation of synapse organization
regulation of protein localization to membrane
regulation of lymphocyte differentiation
postsynapse organization
positive regulation of endocytosis
neural precursor cell proliferation
negative regulation of lymphocyte activation
negative regulation of leukocyte activation
negative regulation of cell- cell adhesion
dendritic spine organization
cell maturation
regulation of neuronal synaptic plasticity
regulation of dendritic spine morphogenesis
positive regulation of alpha- beta T cell differentiation
modulation by symbiont of entry into host
GO Pathways
2
3
4
5
Gene count
qvalue
0.020
0.015
0.010
0.005
count
2
3
4
5

Figure 8 a

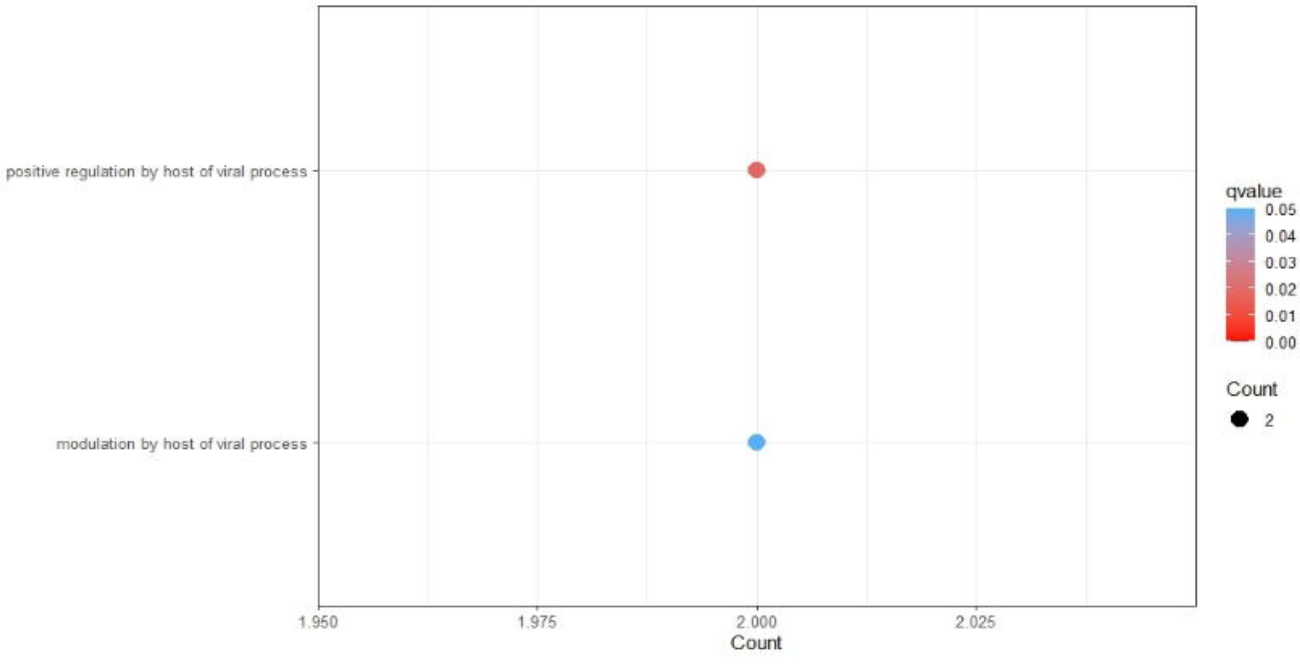

positive regulation by host of viral process
modulation by host of viral process
1.950
1.975
2.000
2.025
Count
qvalue
0.05
0.04
0.03
0.02
0.01
0.00
Count
2

Figure 8b

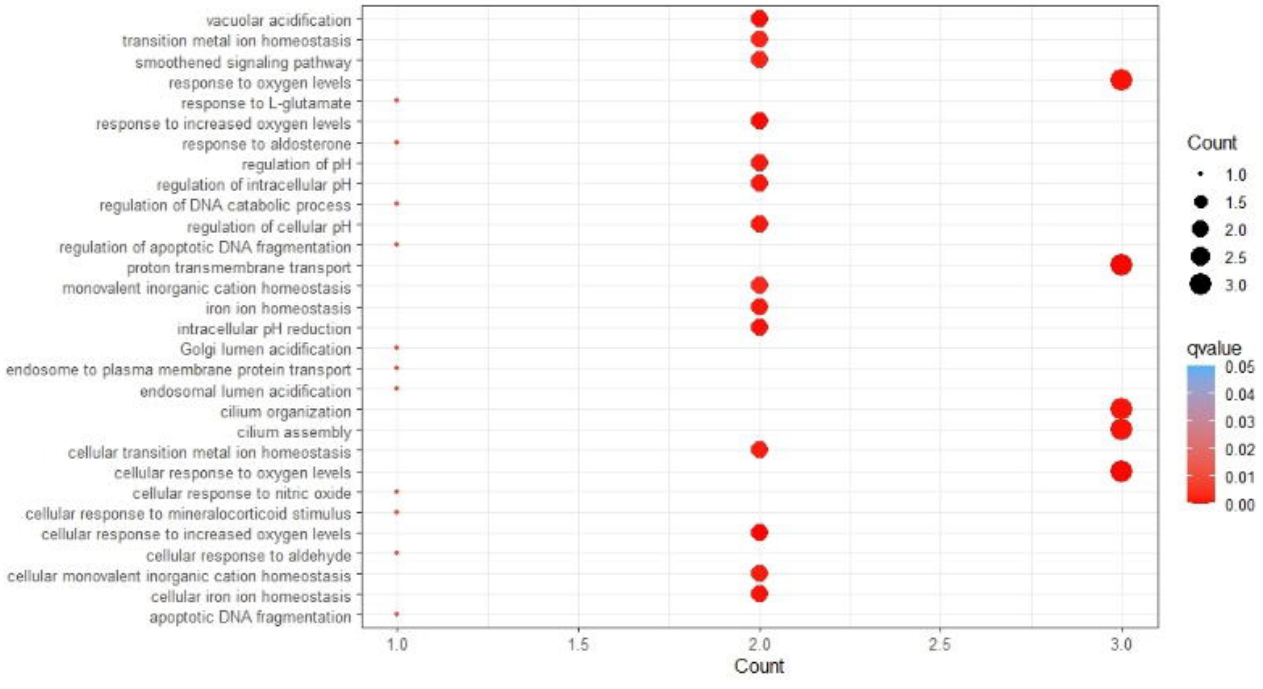

vacuolar acidification
transition metal ion homeostasis
smoothened signaling pathway
response to oxygen levels
response to L-glutamate
response to increased oxygen levels
response to aldosterone
regulation of pH
regulation of intracellular pH
regulation of DNA catabolic process
regulation of cellular pH
regulation of apoptotic DNA fragmentation
proton transmembrane transport
monovalent inorganic cation homeostasis
iron ion homeostasis
intracellular pH reduction
Golgi lumen acidification
endosome to plasma membrane protein transport
endosomal lumen acidification
cilium organization
cilium assembly
cellular transition metal ion homeostasis
cellular response to oxygen levels
cellular response to nitric oxide
cellular response to mineralocorticoid stimulus
cellular response to increased oxygen levels
cellular response to aldehyde
cellular monovalent inorganic cation homeostasis
cellular iron ion homeostasis
apoptotic DNA fragmentation
1.0
1.5
2.0
2.5
3.0
Count
Count
1.0
1.5
2.0
2.5
3.0
qvalue
0.05
0.04
0.03
0.02
0.01
0.00

Figure 9a

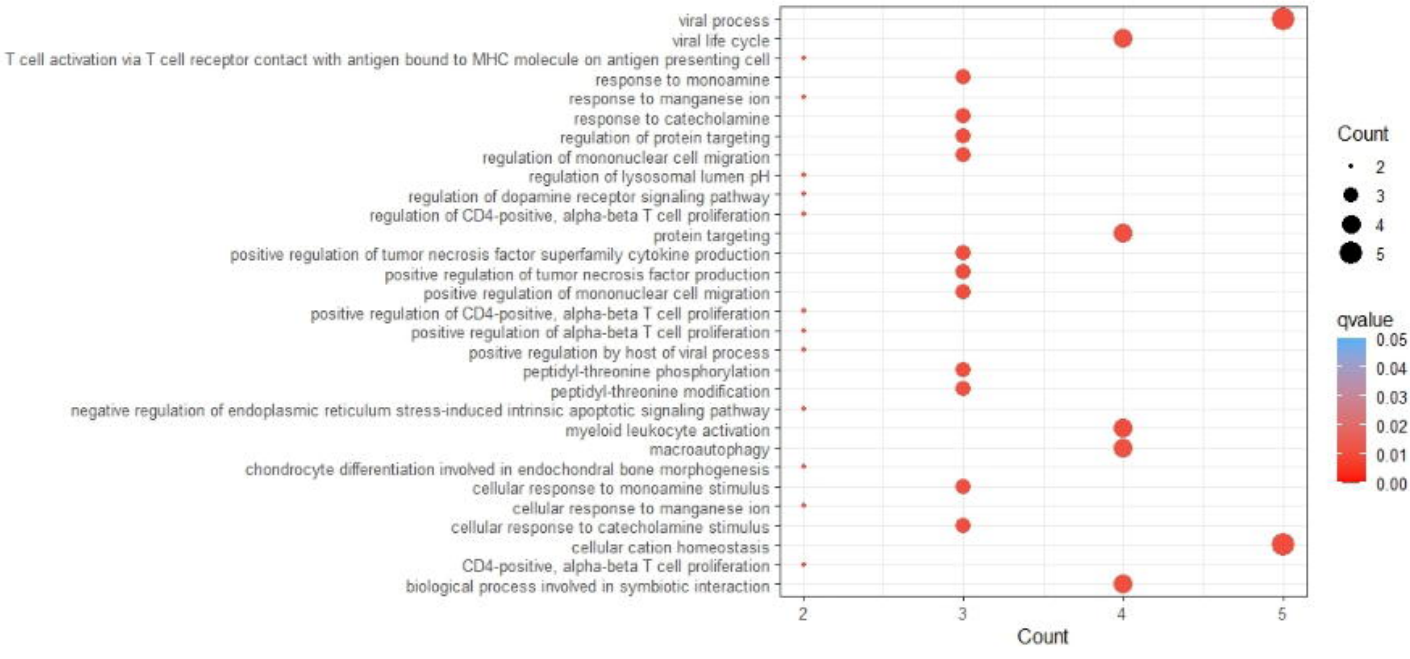

viral process
viral life cycle
T cell activation via T cell receptor contact with antigen bound to MHC molecule on antigen presenting cell
response to monoamine
response to manganese ion
response to catecholamine
regulation of protein targeting
regulation of mononuclear cell migration
regulation of lysosomal lumen pH
regulation of dopamine receptor signaling pathway
regulation of CD4-positive, alpha-beta T cell proliferation
protein targeting
positive regulation of tumor necrosis factor superfamily cytokine production
positive regulation of tumor necrosis factor production
positive regulation of mononuclear cell migration
positive regulation of CD4-positive, alpha-beta T cell proliferation
positive regulation of alpha-beta T cell proliferation
positive regulation by host of viral process
peptidyl-threonine phosphorylation
peptidyl-threonine modification
negative regulation of endoplasmic reticulum stress-induced intrinsic apoptotic signaling pathway
myeloid leukocyte activation
macroautophagy
chondrocyte differentiation involved in endochondral bone morphogenesis
cellular response to monoamine stimulus
cellular response to manganese ion
cellular response to catecholamine stimulus
cellular cation homeostasis
CD4-positive, alpha-beta T cell proliferation
biological process involved in symbiotic interaction
2
3
4
5
Count
Count
2
3
4
5
qvalue
0.05
0.04
0.03
0.02
0.01
0.00

Figure 9b

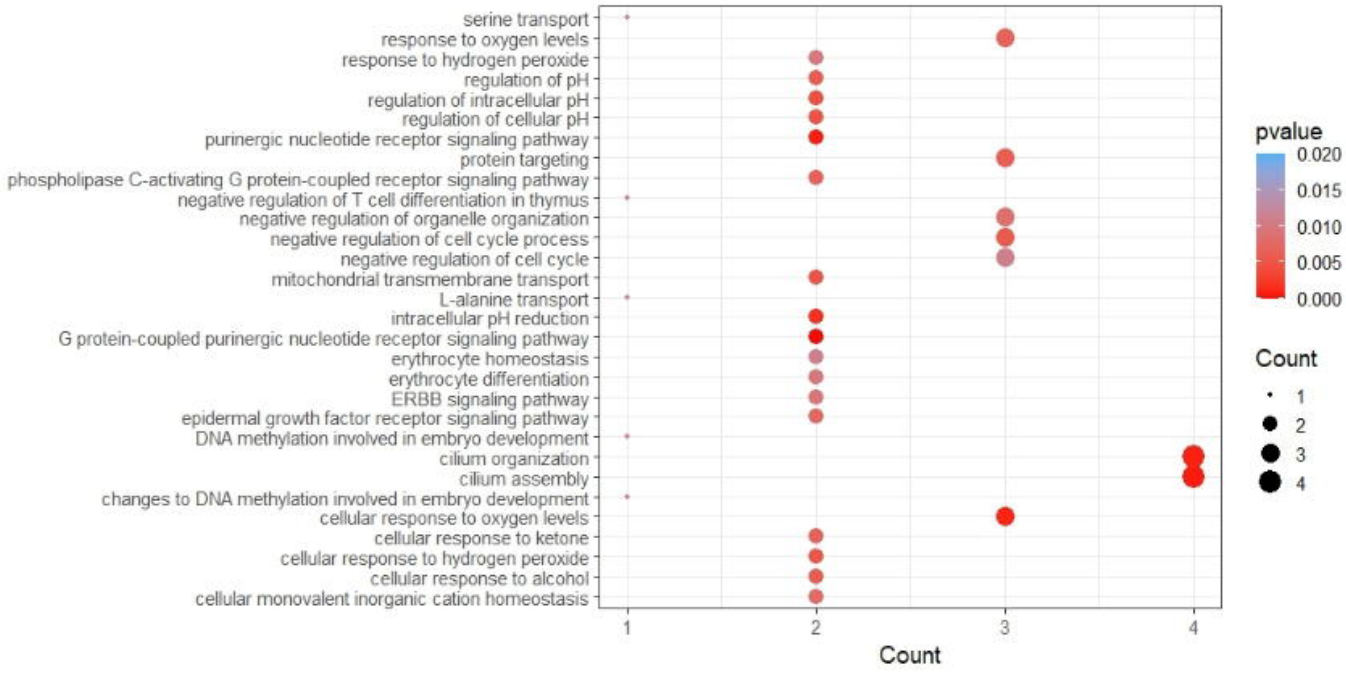

serine transport
response to oxygen levels
response to hydrogen peroxide
regulation of pH
regulation of intracellular pH
regulation of cellular pH
purinergic nucleotide receptor signaling pathway
protein targeting
phospholipase C-activating G protein-coupled receptor signaling pathway
negative regulation of T cell differentiation in thymus
negative regulation of organelle organization
negative regulation of cell cycle process
negative regulation of cell cycle
mitochondrial transmembrane transport
L-alanine transport
intracellular pH reduction
G protein-coupled purinergic nucleotide receptor signaling pathway
erythrocyte homeostasis
erythrocyte differentiation
ERBB signaling pathway
epidermal growth factor receptor signaling pathway
DNA methylation involved in embryo development
cilium organization
cilium assembly
changes to DNA methylation involved in embryo development
cellular response to oxygen levels
cellular response to ketone
cellular response to hydrogen peroxide
cellular response to alcohol
cellular monovalent inorganic cation homeostasis
1
2
3
4
Count
pvalue
0.020
0.015
0.010
0.005
0.000
Count
1
2
3
4

Figure 10a

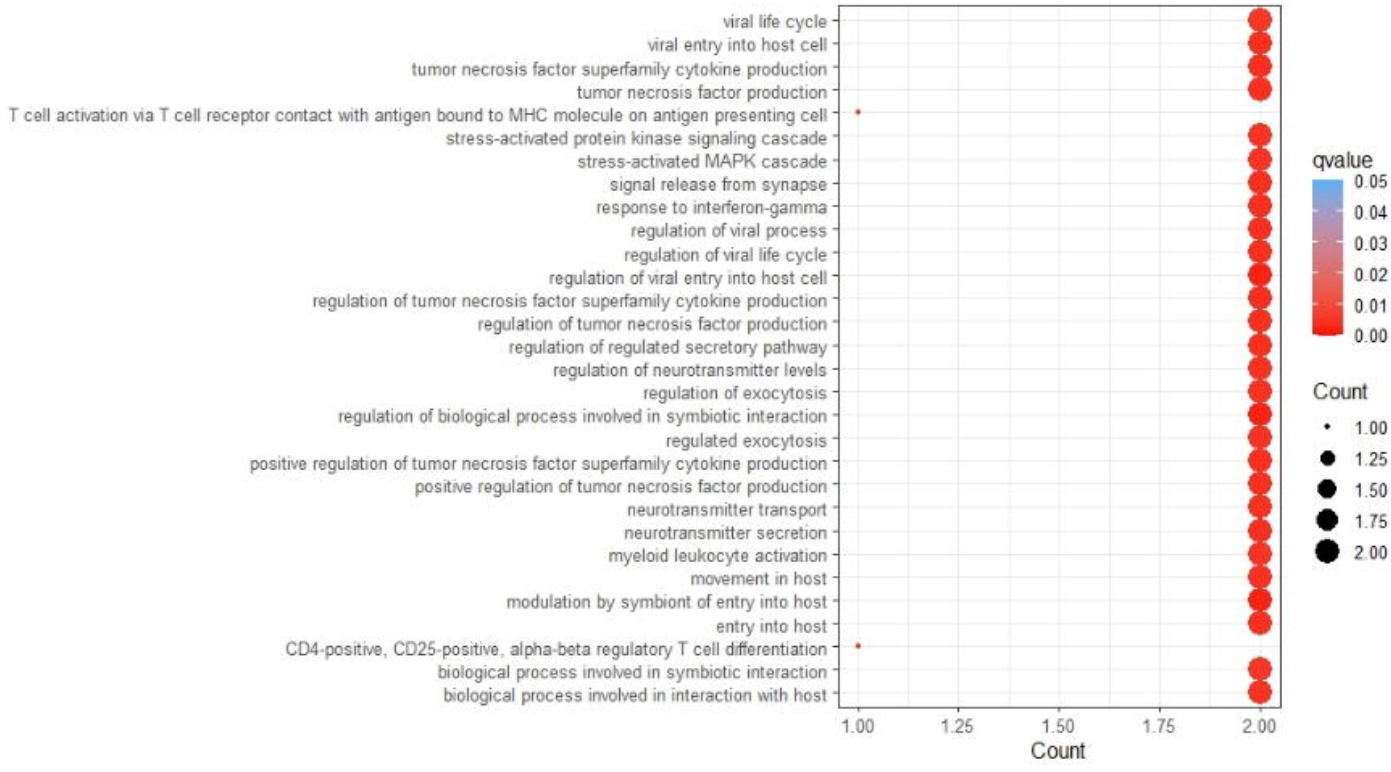

viral life cycle
viral entry into host cell
tumor necrosis factor superfamily cytokine production
tumor necrosis factor production
T cell activation via T cell receptor contact with antigen bound to MHC molecule on antigen presenting cell
stress-activated protein kinase signaling cascade
stress-activated MAPK cascade
signal release from synapse
response to interferon-gamma
regulation of viral process
regulation of viral life cycle
regulation of viral entry into host cell
regulation of tumor necrosis factor superfamily cytokine production
regulation of tumor necrosis factor production
regulation of regulated secretory pathway
regulation of neurotransmitter levels
regulation of exocytosis
regulation of biological process involved in symbiotic interaction
regulated exocytosis
positive regulation of tumor necrosis factor superfamily cytokine production
positive regulation of tumor necrosis factor production
neurotransmitter transport
neurotransmitter secretion
myeloid leukocyte activation
movement in host
modulation by symbiont of entry into host
entry into host
CD4-positive, CD25-positive, alpha-beta regulatory T cell differentiation
biological process involved in symbiotic interaction
biological process involved in interaction with host
1.00
1.25
1.50
1.75
2.00
Count
qvalue
0.05
0.04
0.03
0.02
0.01
0.00
Count
1.00
1.25
1.50
1.75
2.00

Figure 10b

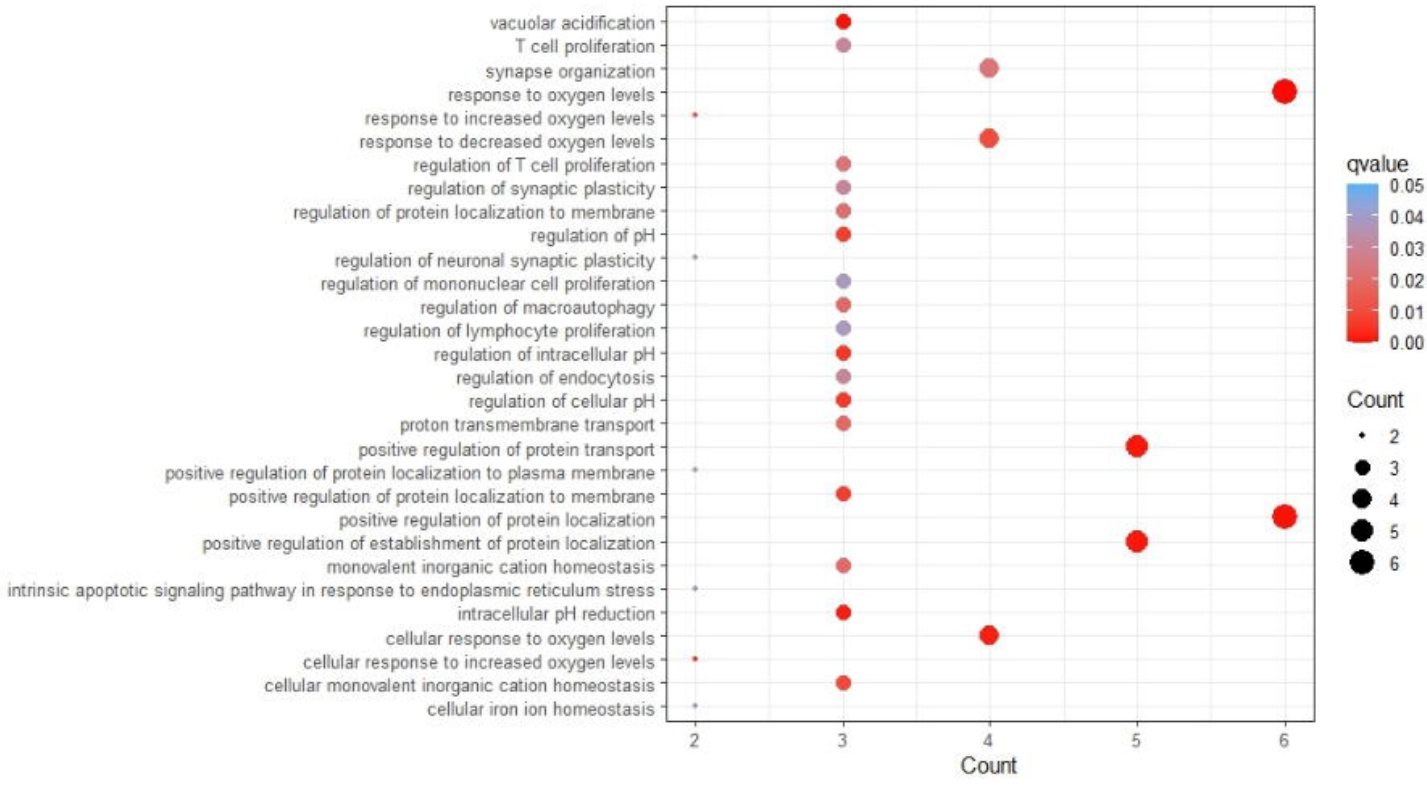

vacuolar acidification
T cell proliferation
synapse organization
response to oxygen levels
response to increased oxygen levels
response to decreased oxygen levels
regulation of T cell proliferation
regulation of synaptic plasticity
regulation of protein localization to membrane
regulation of pH
regulation of neuronal synaptic plasticity
regulation of mononuclear cell proliferation
regulation of macroautophagy
regulation of lymphocyte proliferation
regulation of intracellular pH
regulation of endocytosis
regulation of cellular pH
proton transmembrane transport
positive regulation of protein transport
positive regulation of protein localization to plasma membrane
positive regulation of protein localization to membrane
positive regulation of protein localization
positive regulation of establishment of protein localization
monovalent inorganic cation homeostasis
intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress
intracellular pH reduction
cellular response to oxygen levels
cellular response to increased oxygen levels
cellular monovalent inorganic cation homeostasis
cellular iron ion homeostasis
2
3
4
5
6
Count
qvalue
0.05
0.04
0.03
0.02
0.01
0.00
Count
2
3
4
5
6

Figure 11 miRNA common in brain and Lung

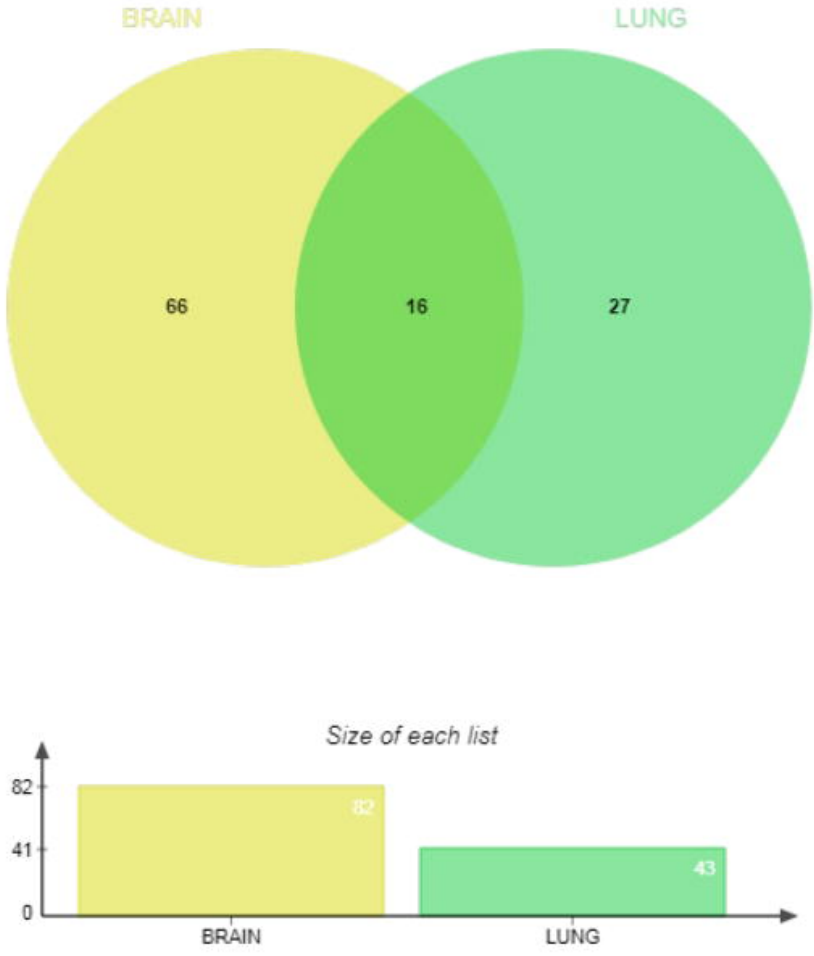


Figure 12 miRNA interaction network Brain

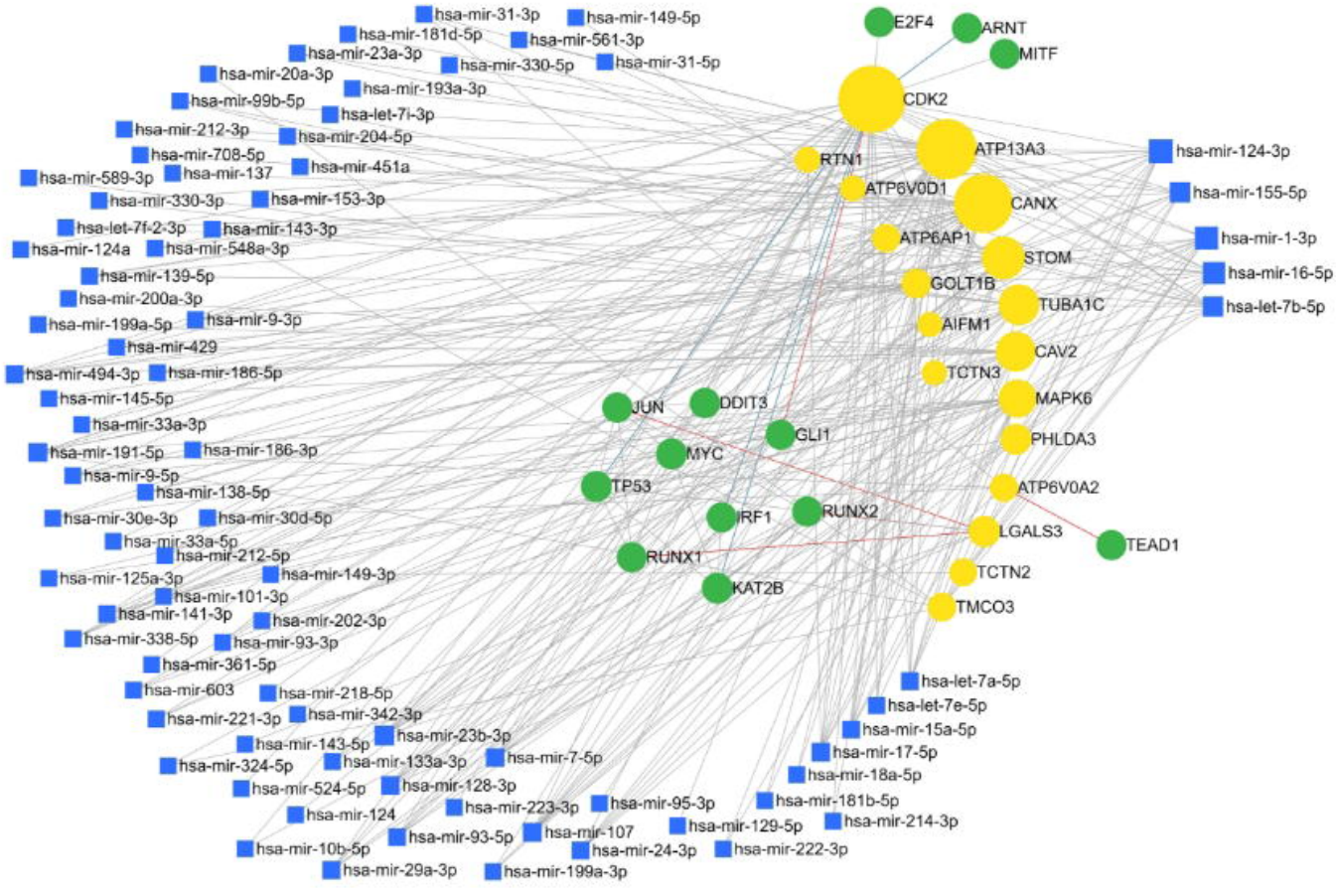


Figure 13 miRNA Lung

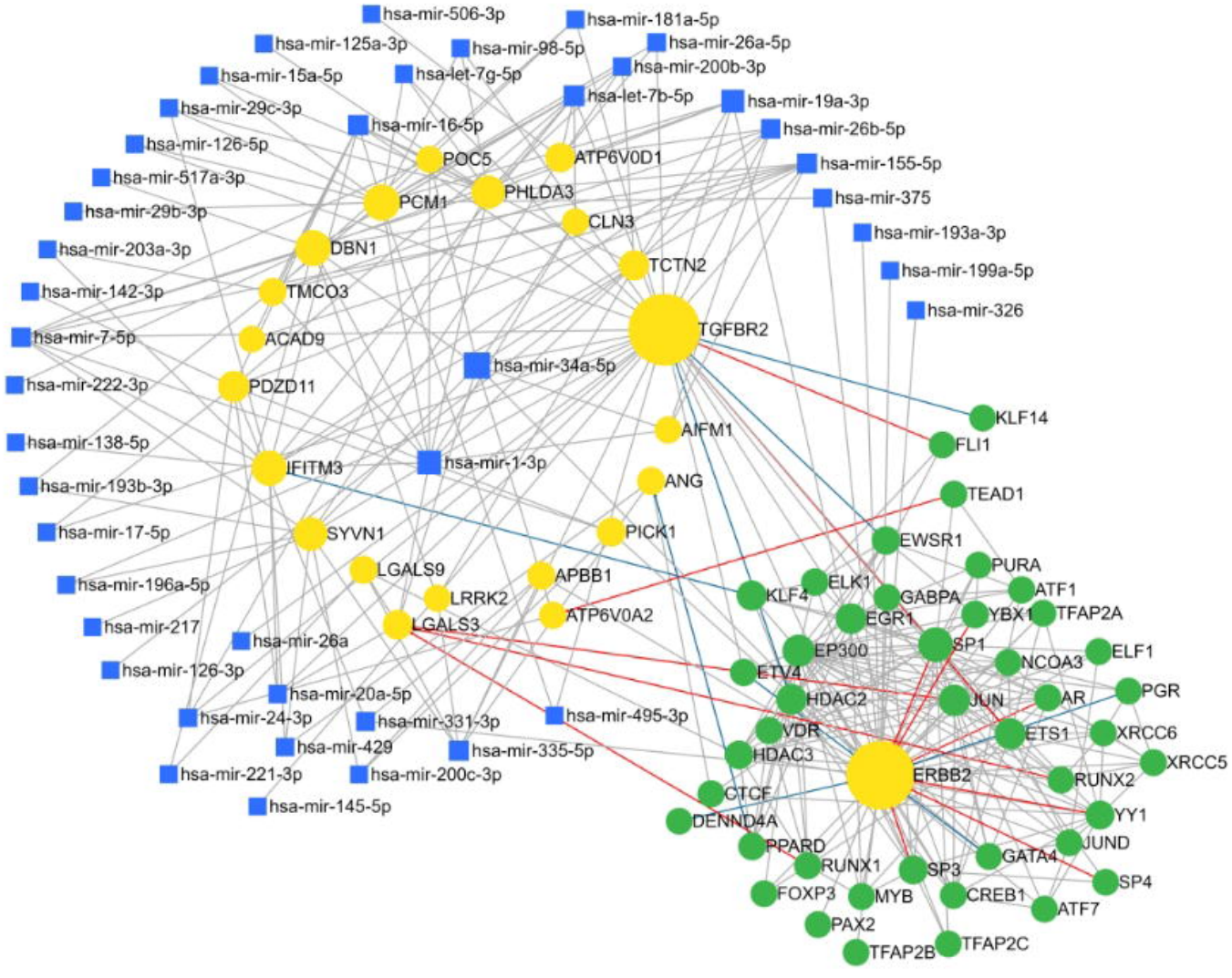


Figure 14 miRNA-RNA interaction network in the heart

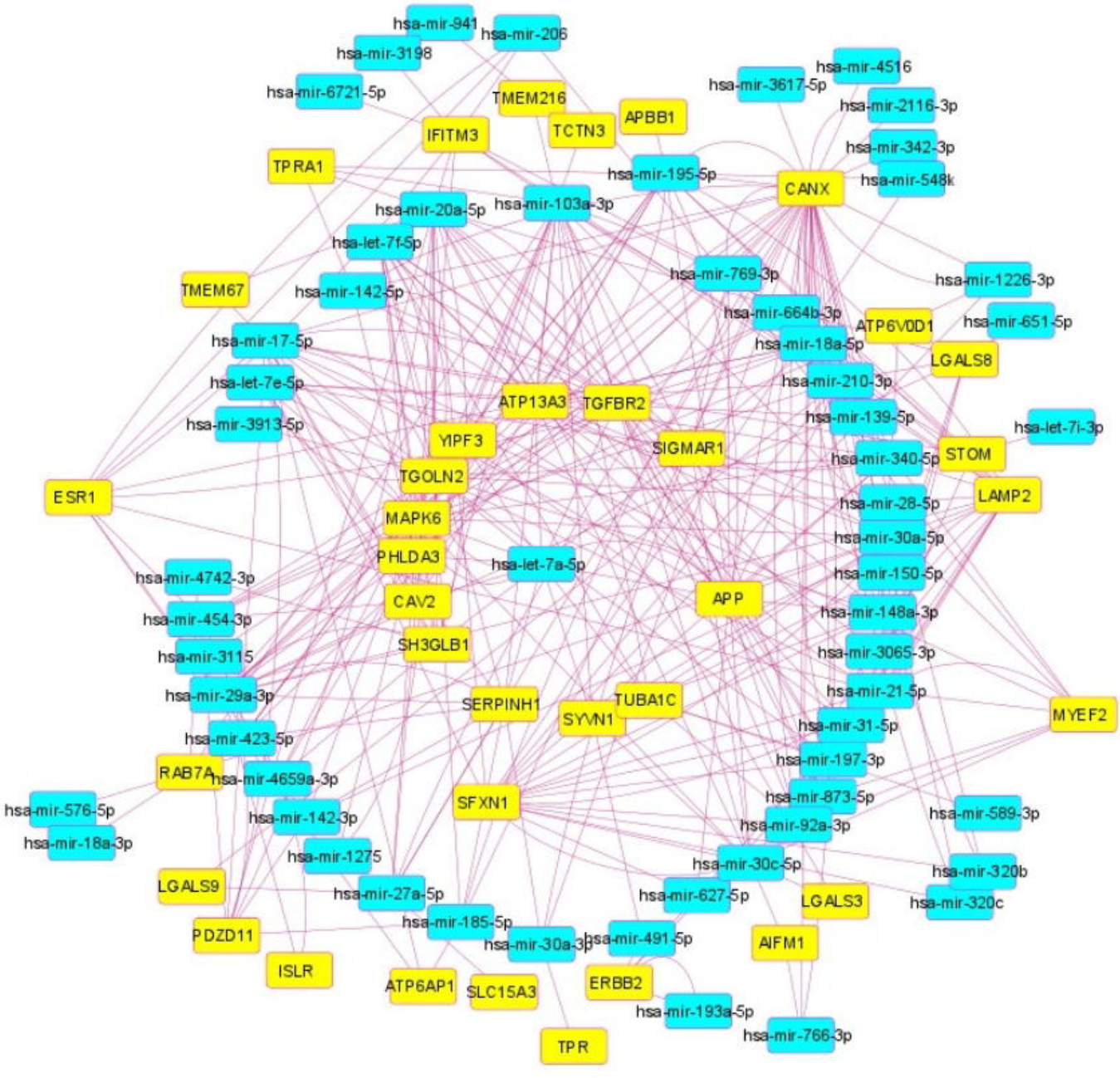


Figure 15_NSP6_cimiRNA_lung

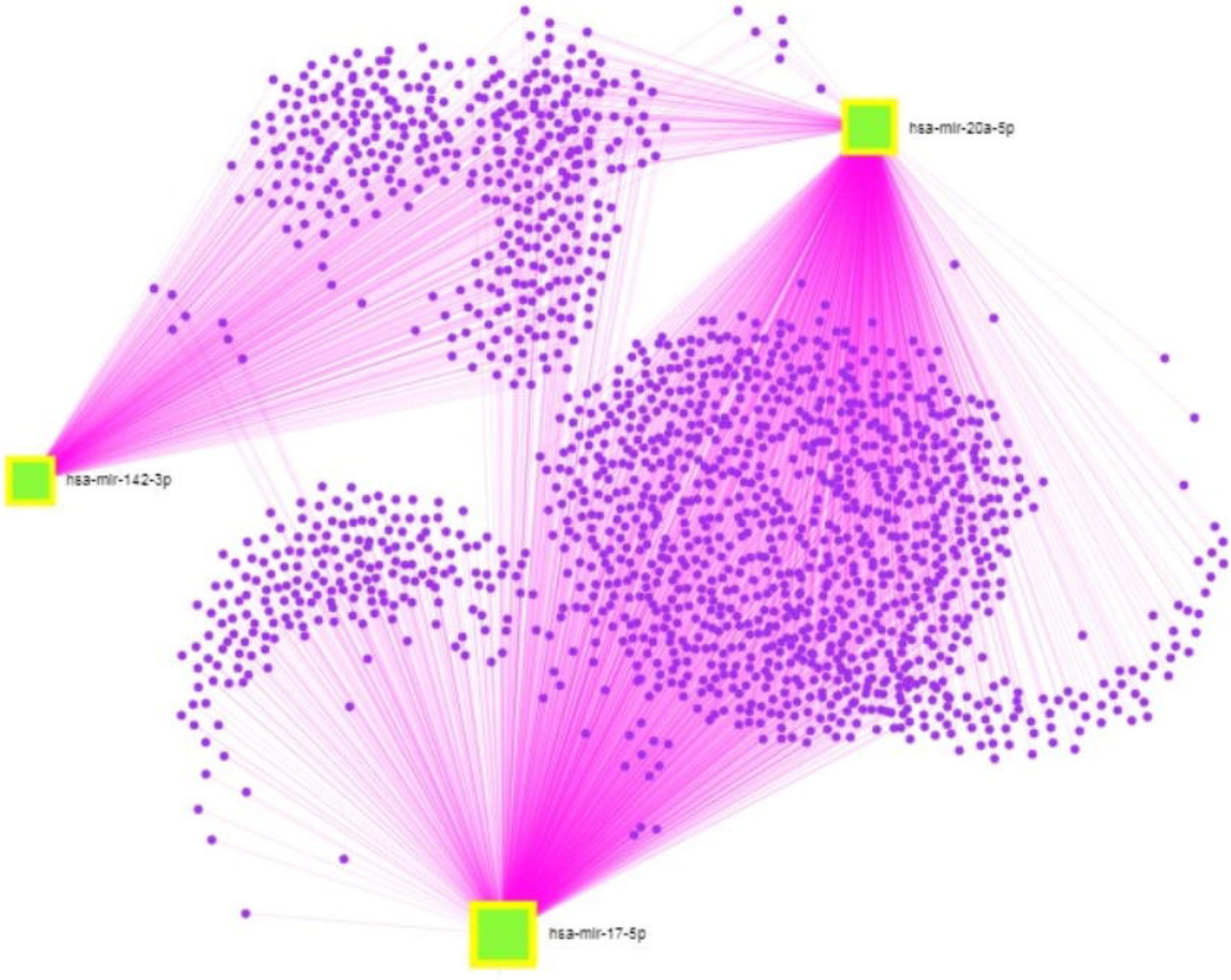


Figure 16_NSP6_cimiRNA _brain

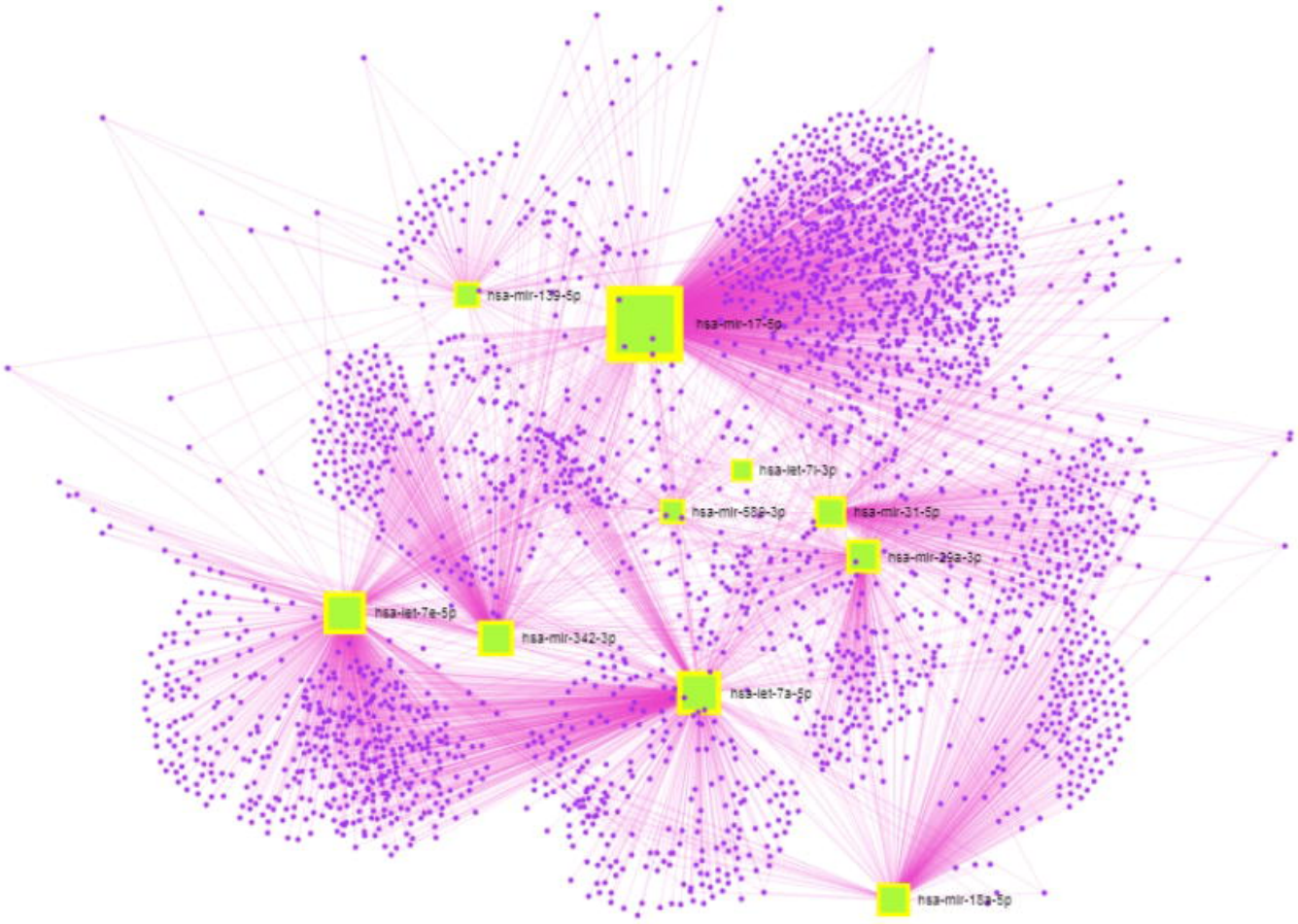
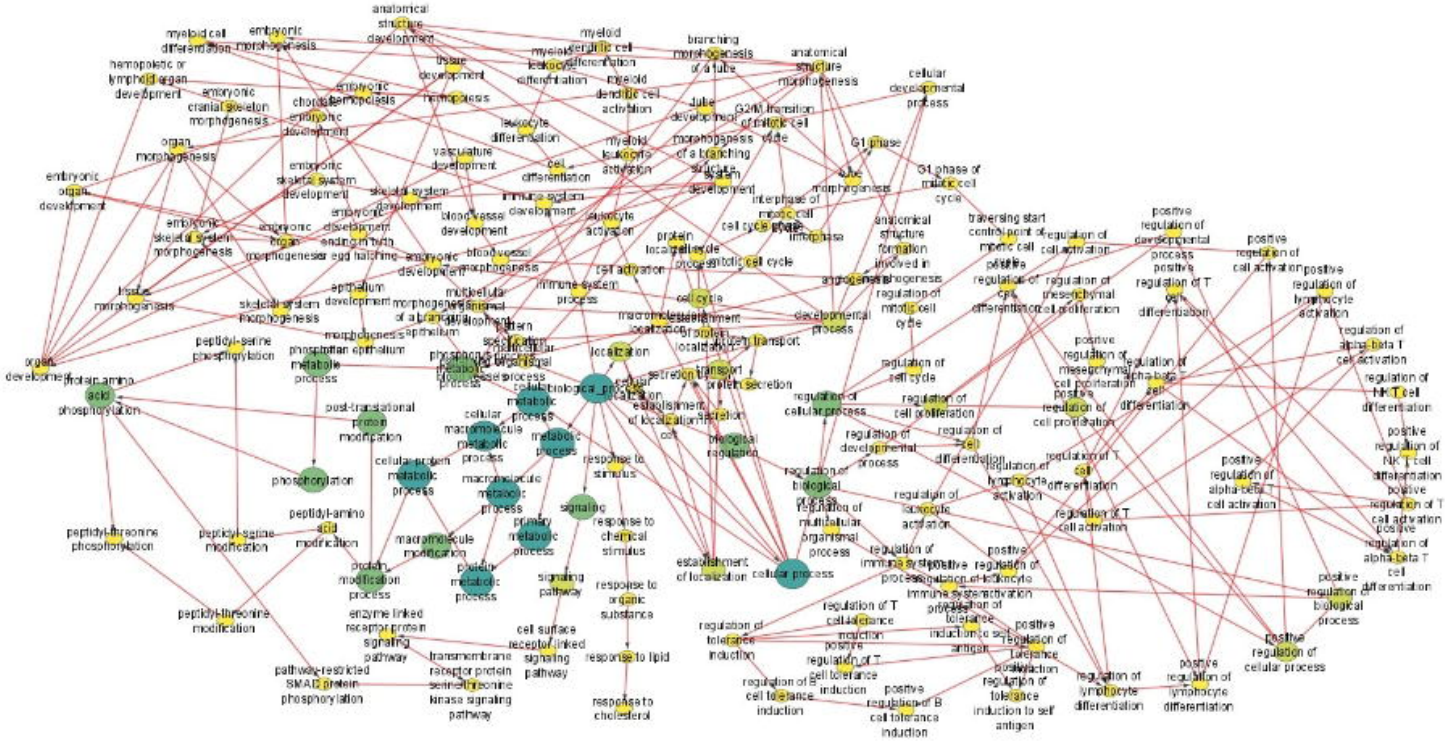


Figure 17 NSP6_common miRNA _brain _lung pathways



Pathways in the brain, heart and lung influenced by SARS-CoV-2 NSP6 and SARS-CoV-2 regulated miRNAs: an *in silico* study hinting cancer incidence

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[3] Authors contributed equally
[4] Corresponding author

Figure 1. a) Venn diagram showing the number of proteins interacting with SARS-CoV-2 NSP6 interacting proteins in different tissues influenced by SARS-CoV-2 infection. Number of proteins in the brain, heart and lung were 18, 45 and 23 respectively. Among these, 5 genes were common in brain, lung and heart. Common genes between the brain and heart were 8, heart and lung were 10, and lung and brain were 2. **b)** Circular cluster heatmap showing expression in fold change of the 5 common genes in the brain heart and lung.

Figure 2a. Top 10 hub genes of the NSP6 interactome proteins from STRINGdb analyzed by cytoHubba in the brain. Node color from red to yellow represents higher to lower significance. Analysis showed CCND1 to be the highly significant major hub gene.

Figure 2b. Top 10 hub genes of the NSP6 interactome proteins from STRINGdb analyzed by cytoHubba in the heart. Node color from red to yellow represents higher to lower significance. Analysis showed RAB7A, ATP6V0D1 and LAMP2 to be the highly significant major hub genes.

Figure 2c. Top 10 hub genes of the NSP6 interactome proteins from STRINGdb analyzed by cytoHubba in the lung. Node color from red to yellow represents higher to lower significance. Analysis showed ATP6V0A1, ATP6V0A2, ATP6V0D1, ATP6V1D, ATP6V1B1 and ATP6V1E1 to be the highly significant major hub genes.

Figure 3a. Gene ontology processes, KEGG pathway and reactome pathway analyses of top 10 hub genes in the brain by CluGO analysis.

Figure 3b. Gene ontology processes, KEGG pathway and reactome pathway analyses of top 10 hub genes in the lung by CluGO analysis.

Figure 3c. Gene ontology processes, KEGG pathway and reactome pathway analyses of top 10 hub genes in the heart by CluGO analysis.

Figure 4a. Drug targets among hub genes in the brain. Circular yellow nodes are hub genes and hexagonal nodes denote drugs. Violet hexagonal nodes are approved drugs and pink nodes denote approved drugs targeting more than one hub genes. Green colored hexagonal nodes with dotted lines are non-approved drugs. Solid edges are approved drugs and dotted edges are for non-approved drugs. Orange colored hexagonal nodes denote non-approved drugs targeting more than one hub genes. 30 approved and 89 non-approved drugs were found for the five among 10 top hub genes.

Figure 4b. Drug targets among hub genes in the heart. Circular yellow nodes are hub genes and hexagonal nodes denote drugs. Violet hexagonal nodes are approved drugs and pink nodes denote approved drugs targeting more than one hub genes. Solid edges are approved drugs and dotted edges are for non-approved drugs. Green colored hexagonal nodes are non-approved drugs. 44 approved and 56 non-approved drugs were found for the four among 10 top hub genes.

Figure 4c. Drug targets among hub genes in the lung. Circular yellow nodes are hub genes and hexagonal nodes denote drugs. Violet hexagonal nodes are approved drugs and pink nodes denote approved drugs targeting more than one hub genes. Solid edges are approved drugs and dotted edges are for non-approved drugs. Green colored hexagonal nodes are non-approved drugs. 34 approved and 31 non-approved drugs were found for the four among 10 top hub genes.

Figure 5. Venn diagram representing common and unique pathways influenced by proteins interacting with SARS-CoV-2 NSP6 interacting proteins in the brain, heart and lungs. 14 pathways were common in all the three tissues, whereas unique pathways present in the brain, heart and lungs were 4, 69 and 100 respectively.

Figure 6. Bar diagram showing common pathways influenced by proteins interacting with SARS-CoV-2 NSP6 interacting proteins in the brain, heart and lungs. Length of the bars represent the number of genes involved in the pathways. X-axis represents gene count and Y-axis represents GO terms.

Figure 7. Dot plot representing unique pathways influenced by proteins interacting with SARS-CoV-2 NSP6 interacting proteins in the brain, heart and lungs. **a)** Unique pathways in the brain. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

Figure 7. Dot plot representing top 30 unique pathways influenced by proteins interacting with SARS-CoV-2 NSP6 interacting proteins in the brain, heart and lungs. **b)** Unique pathways in the heart. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

Figure 7. Dot plot representing top 30 unique pathways influenced by proteins interacting with SARS-CoV-2 NSP6 interacting proteins in the brain, heart and lungs. **c)** Unique pathways in the lungs. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

Figure 8. a) Dot plot representing pathways in the brain influenced by upregulated proteins interacting with SARS-CoV-2 NSP6 interacting proteins. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the *q*-value using the Benjamini-Hochberg method.

Figure 8. b) Dot plot representing top 30 pathways in the brain influenced by downregulated proteins interacting with SARS-CoV-2 NSP6 interacting proteins. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the *q*-value using the Benjamini-Hochberg method.

Figure 9. a) Dot plot representing top 30 pathways in the heart influenced by upregulated proteins interacting with SARS-CoV-2 NSP6 interacting proteins. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

Figure 9. b) Dot plot representing top 30 pathways in the heart influenced by downregulated proteins interacting with SARS-CoV-2 NSP6 interacting proteins. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

Figure 10. a) Dot plot representing top 30 pathways in the lung influenced by upregulated proteins interacting with SARS-CoV-2 NSP6 interacting proteins. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

Figure 10. b) Dot plot representing top 30 pathways in the lung influenced by downregulated proteins interacting with SARS-CoV-2 NSP6 interacting proteins. Dot size represent the gene ratio and dot color depicts the q-value as in the heat map. X-axis shows the count of genes per GO term and Y-axis indicates the GO terms. The color gradient indicates the q-value using the Benjamini-Hochberg method.

Figure 11. Common miRNAs in the brain and lung. Proteins influenced by SARS-CoV-2 infection that interact with SARS-CoV-2 NSP6 in the brain and lung was taken to find out miRNAs that can target these protein mRNAs. Among the total of 82 miRNAs in brain and the total of 43 miRNAs in the lung, 16 miRNAs were common where as 66 and 27 miRNAs were unique in the brain and lung respectively.

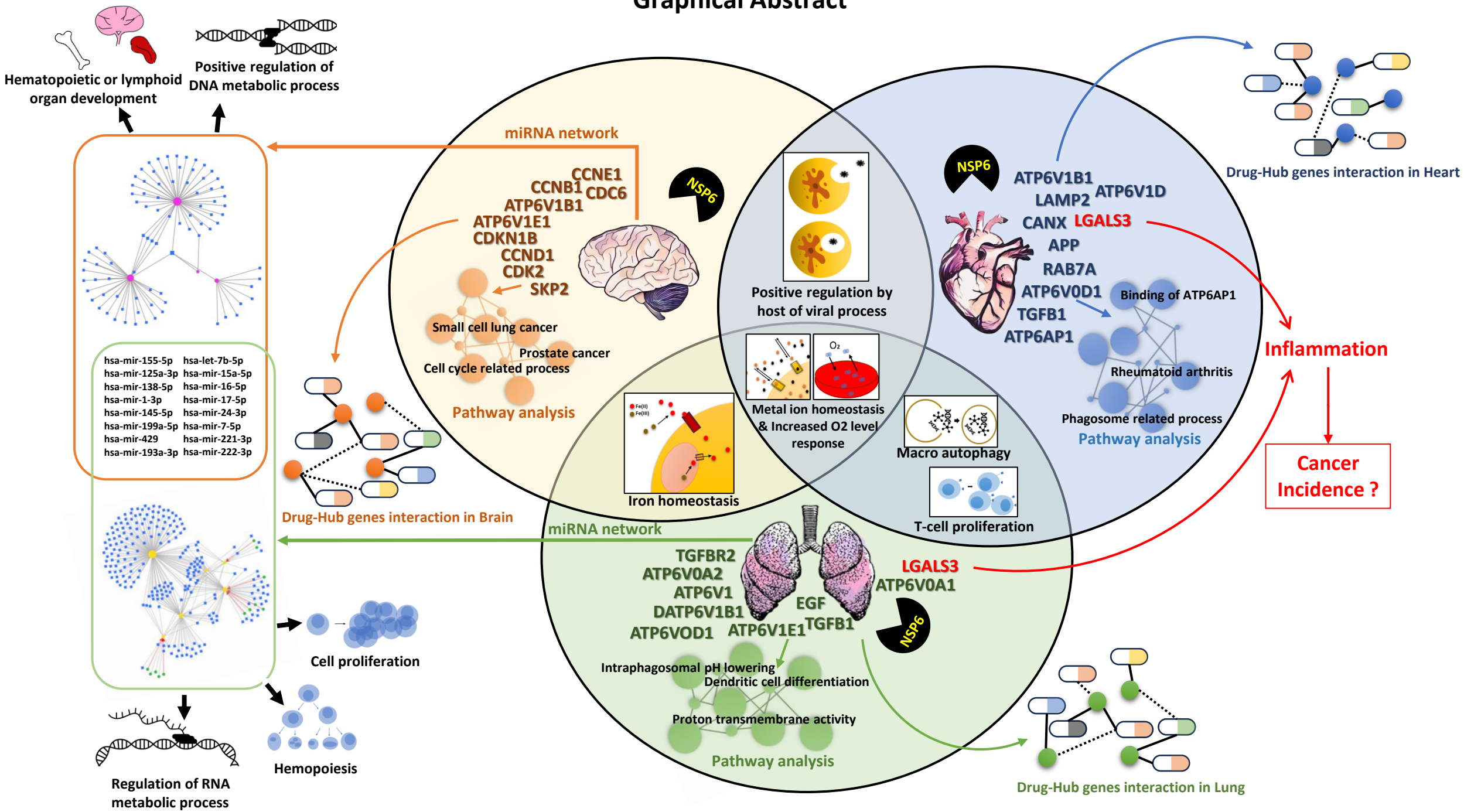
Figure 12. miRNA interaction network with interacting proteins of SARS-CoV2 NSP6 interacting proteins in the brain. Yellow colored nodes denote proteins and green colored nodes are transcription factors influencing proteins and miRNAs in the network. Blue squares represent miRNA nodes. The Increased size of node represent a higher degree.

Figure 13. miRNA interaction network with interacting proteins of SARS-CoV2 NSP6 interacting proteins in the lung. Yellow colored nodes denote proteins and green colored nodes are transcription factors influencing proteins and miRNAs in the network. Blue squares represent miRNA nodes. The Increased size of node represent a higher degree.

Figure 14. miRNA interaction network with interacting proteins of SARS-CoV2 NSP6 interacting proteins in the heart. Yellow colored nodes denote proteins and blue colored nodes are miRNAs in the network.

Figure 15. Common among SARS-CoV2 influenced circulating miRNAs and lung miRNAs. Square nodes denotes miRNAs and circular nodes represent targetted mRNAs. Three miRNAs were targeting 1616 mRNAs.

Figure 16. Common among SARS-CoV2 influenced circulating miRNAs and brain miRNAs. Square nodes denotes miRNAs and circular nodes represent targetted mRNAs. 10 miRNAs were targeting 2782 genes.

Figure 17. Pathways involving common 5 genes observed from the brain and lung. Circular nodes represent pathways associated with 5 genes. Continuous color mapping from green to yellow represent maximum to minimum significance.

Graphical Abstract