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# Mapping molecular gene signatures mediated by SARS-COV-2 and large-scale and genome-wide transcriptomics comparative analysis among respiratory viruses of medical importance

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## ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an emerging RNA virus causing COVID-19 disease, across the globe. SARS-CoV-2 infected patients may exhibit acute respiratory distress syndrome which can be compounded by endemic respiratory viruses and thus highlighting the need to understand the genetic bases of clinical outcome under multiple respiratory infections. In this study, 42 individual datasets and a multi-parametric based selected list of over 12,000 genes against five medically important respiratory viruses (SARS-CoV-2, SARS-CoV-1, influenza A, respiratory syncytial virus (RSV) and rhinovirus) were collected and analysed in an attempt to understand differentially regulated gene patterns and to cast genetic markers of individual and multiple co-infections. While a certain cohort of virus-specific genes were regulated (negatively and positively), notably results revealed a greatest correlation among genes regulation by SARS-CoV-2 and RSV. Furthermore, out of analysed genes, the MAP2K5 and NFKBIL1 were specifically and highly upregulated in SARS-CoV-2 infection both in vivo or in vitro. The most conserved genetic signature was JAK2 gene as well as the constitutively downregulated ZNF219 gene. In contrast, several genes including GPBAR1 and SC5DL were specifically downregulated in SARS-CoV-2 datasets. Finally, we catalogued a set of genes that were conserved or differentially regulated across studied respiratory viruses. These findings provide foundational and genome-wide data to gauge the markers of respiratory viral infections individually and under co-infection. This work compares the virogenomic signatures among human respiratory viruses and provides valid targets for potential antiviral therapy.

## 1. Background

Since its first appearance in Wuhan, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread across the world in a way unlike any other respiratory viruses. Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, is considered the third highly pathogenic coronavirus following SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV) [1]. The most striking feature of the incidences and epidemiology of SARS-CoV-2 is its high ability for transmission among people [2]. The clinical outcome and incidence vary that most COVID-19 patients show mild and moderate symptoms, and the elderly show serious symptoms [3]. Additionally, severely affected patients had shown respiratory complications such as moderate to severe pneumonia, acute respiratory distress syndrome (ARDS), sepsis, acute lung injury (ALI), and multiple organ dysfunction (MOD) [4].

ARDS in COVID-19 patients is thought to be the main cause of death because of the cytokine storm caused by an over-activation of the human innate immune response [5]. However, there are multiple immune regulators and host genetic and epigenetic factors that are capable of significant contributions to the disease manifestation [5]. Host-pathogen interactions can act as a double-edged sword in different coronavirus infections as they might be useful not just for hosts, but also for viruses [6]. Similar tug-of-war host-viruses can also be present in COVID-19, which could lead to overcomplicated outcomes of the disease [7].

Although recent studies have shown the transcriptomic analysis of host responses to SARS-CoV-2 infection at different time points within multiple cell lines [8,9], the transcriptional dynamics of host response to multiple virus infection remained largely unknown. In general, the host innate immune responses play an essential role in suppressing the

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**Table 1**

Inclusion and Exclusion criteria used to select datasets to be included within this study.

Inclusion Criteria	Exclusion Criteria
Transcriptomic Study	Sample is taken from another species other than human
Sample has been collected from infected humans with either SARS-CoV-2, Influenza A, SARS-CoV-1, RSV or Rhinovirus	Studies that include less than 10 up or down regulated genes
Sample is taken from humans In vivo or in vitro study	Studies that have modified genes Studies where subjects had been given a preventative drug or treatment

**Table 2**

Complete list of all datasets selected to be used within this study. The virus, type of sample and analysis method are also shown.

Virus	Sample Type	Dataset ID	Analysis Method
SARS-CoV-2	Nasopharyngeal swabs	GSE152075	RNA sequencing
SARS-CoV-2	PBMCs	GSE150728	RNA sequencing
SARS-CoV-2	Intestinal Organoids	GSE149312	RNA sequencing
SARS-CoV-2	NHBE	GSE147507	RNA sequencing
SARS-CoV-2	Autopsy samples	GSE150316	RNA sequencing
SARS-CoV-2	PBMCs and BALF	CRA002390	RNA sequencing
SARS-CoV-2	BALF	HRA000143	RNA sequencing
SARS-CoV-2	Upper Airway Samples	GSE156063	RNA sequencing
SARS-CoV-2	PBMCs	GSE152418	RNA sequencing
SARS-CoV-2	pHAE	GSE153970	RNA sequencing
SARS-CoV-2	Nasopharyngeal swabs	GSE154770	RNA sequencing
SARS-CoV-2	FFPE samples	GSE159787	RNA sequencing
SARS-CoV-2	Human Lung-only mice	GSE155286	RNA sequencing
SARS-CoV-2	nHTBE	GSE157526	RNA sequencing
Influenza	PBMCs	GSE34205	Microarray
Influenza	PBMCs	GSE6269	Microarray
Influenza	Nasal Wash Samples	GSE68310	Microarray
Influenza	Whole Blood	GSE38900	Microarray
Influenza	Whole Blood	GSE90732	Microarray
Influenza	Whole Blood	GSE61754	Microarray
Influenza	NHBE	GSE147507	RNA Sequencing
Influenza	Whole Blood	GSE21802	Microarray
SARS-CoV-1	PBMCs	GSE1739	Microarray
SARS-CoV-1	Intestinal Organoids	GSE149312	RNA Sequencing
SARS-CoV-1	NHBE	GSE147507	RNA Sequencing
RSV	Whole Blood	GSE38900	Microarray
RSV	PBMCs	GSE34205	Microarray
RSV	NHBE	GSE147507	RNA Sequencing
Rhinovirus	PBMCs	GSE53543	Microarray
Rhinovirus	Whole Blood	GSE38900	Microarray

replication of the virus once the virus enters the host, such as antiviral-mediated interferons and cytokines, which could lead to the virus pathogenesis. Increased cytokine levels are also observed in patients hospitalised with COVID-19 in the same way as both SARS-CoV-1 and MERS-CoV, which induce high levels of cytokine [10,11]. Accordingly, understanding the magnitude and dynamics of human transcriptome in response to medically important respiratory viruses will help in understanding their pathogenesis, molecular genetic markers and in repurposing existing antivirals to combat respiratory viral infections.

The current study aims to compare a large cohort of transcriptomic dataset map the gene regulation (up or down regulated) by SARS-CoV-2 infection and the compounding impact of other respiratory viruses such as influenza, SARS-CoV-1, respiratory syncytial virus (RSV) and rhinovirus. This parallel comparison showcases common and unique genetic signatures of respiratory viruses under individual and co-infection scenarios to guide future investigational studies and designing therapies.

## 2. Materials and methods

### 2.1. Data collection, inclusion and exclusion criteria

Gene Expression Omnibus (GEO) and PubMed datasets were used to search for literature that contained data relating to upregulated and downregulated genes in response to infection with respiratory viruses (SARS-CoV-2, influenza, SARS-CoV-1, RSV and rhinovirus). The collection began with searching for datasets for the more recent COVID-19 pandemic. On GEO, the terms “(“*Severe acute respiratory syndrome coronavirus 2*”[Organism] OR SARS-CoV-2[All Fields]) AND “*Homo sapiens*”” were used whereas when searching on PubMed, the terms “(SARS-CoV-2) AND (Transcriptome)” were used. Once datasets were identified, inclusion and exclusion criteria were carried out as outlined in Table 1 to ensure parallel comparison of gene signatures.

### 2.2. Included datasets and data synchronisation

The collected datasets from various sources were compiled into one set of data using Microsoft Excel program. The studied viruses and their respective analysed datasets are provided in a spreadsheet (Table 2). An overview of each dataset is provided in the **Supplementary dataset 1**. Each dataset carried genes found in a specific study mentioned in the category, and the corresponding level of gene expression is displayed next units originally used by the datasets. To ensure that all the included datasets for each virus could be compared, these were converted to the same units. The raw data was often listed in three units; Fold Change, Log Fold Change and Log 2-Fold Change, and all the data was converted into the Log 2-Fold Change format. Log 2-Fold Change was used as it allows easier visualisation of the data, as the range of the values of the data becomes narrower, allowing for easier comparison of the up/down regulated genes (**Supplementary dataset 2**).

### 2.3. Ranking system

Owing to large diversity among datasets in areas such as cell types and media in which the experiments were carried out, it could introduce biasness to compare genes specifically by their Log 2-Fold Change values, which is calculated to the baseline gene expression. To introduce a novel method of comparing each gene up or down regulated in a dataset compared to datasets from another virus or different cell types, a ranking system next to each Log 2-Fold Change column was proposed. This system ranked the genes based on which percentage group they were in, depending on whether they were up or down-regulated. Then, a mean score was taken across datasets within the same studied viruses and these means were used to compare between the viruses. For avoidance of confusion, this system synchronizes the dataset such that at the top 10% of upregulated genes for one virus while only at the top 80% of genes for another virus.

Using the GraphPad Prism 9.0.0 software, a scatter bar graph was generated using the overall ranking score for each gene of each virus. Two versions were created; first had the uncut data taken directly from the ranking system, containing roughly 24,000 genes, and secondly a cut down version of the data where non-significant genes were removed. Additionally, the non-coding gene loci and non-annotated genes were removed, as these often yielded zero values for up or down regulated genes reducing 6200 genes. Furthermore, other genes were removed which contained more than three or more zero values for up or down-regulation across the five viruses removing a further 200 genes. Finally, using influenza virus as a model virus, all genes were removed that lied within the ranking scores of +20 (bottom 20% upregulated) and -20 (bottom 20% downregulated genes), unless a gene had a ranking score of above +50 or below -50 in any other of the viruses. This removed a further 5005 genes leaving a total of just over 12,000 genes in the cut down version, which removed the large proportion of genes containing zero values for clearer view for the spread of gene ranking scores.

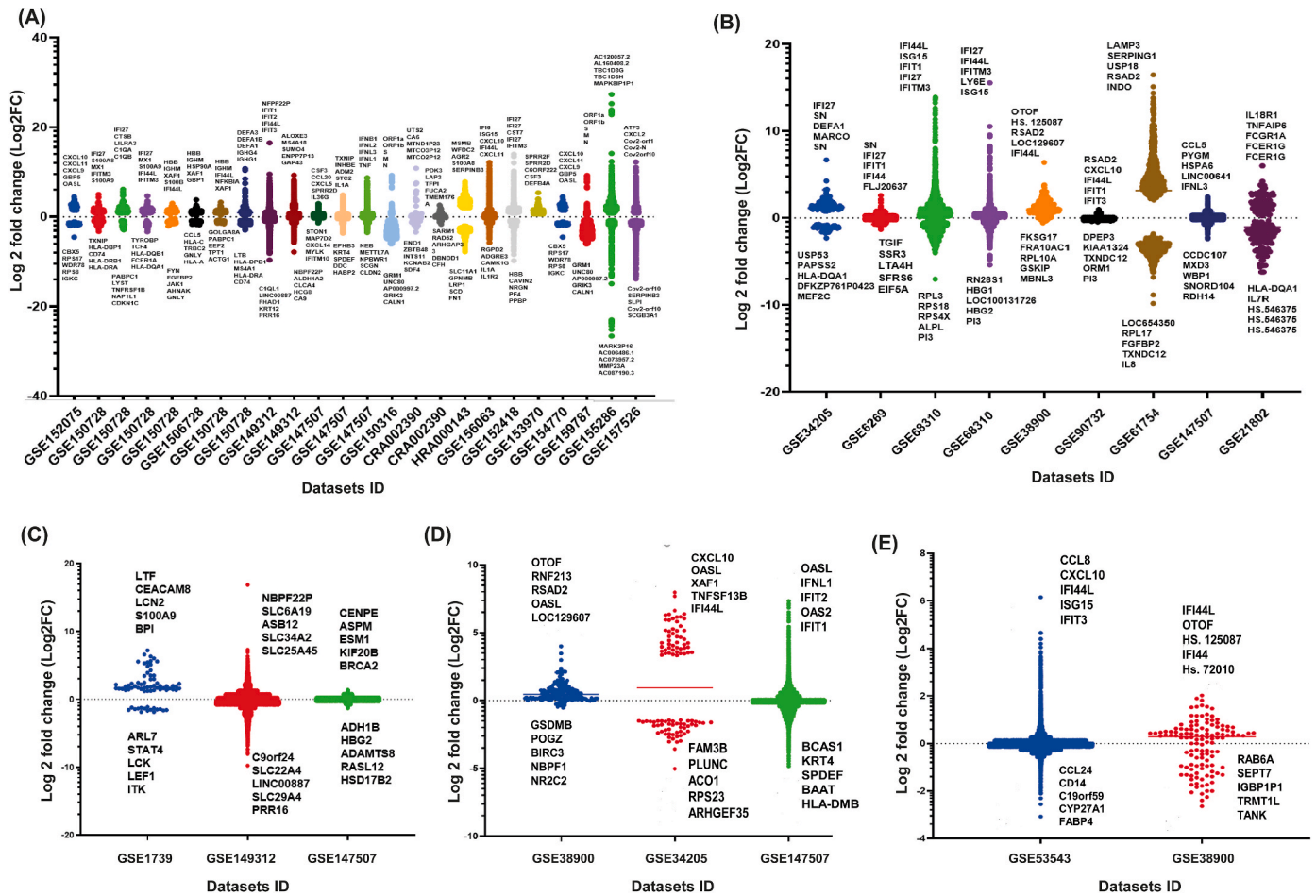


Fig. 1. Scatter bar graphs of the Log<sub>2</sub>-Fold Change of each gene for each dataset for A) SARS-CoV-2, B) Influenza, C) SARS-CoV-1, D) RSV and E) Rhinovirus. A horizontal line is also shown on each bar, which marks the average Log<sub>2</sub> fold change (Log<sub>2</sub>-FC) of the selected genes.

Table 3

Top five up regulated genes shared across SARS-CoV-2, Influenza, SARS-CoV-1, RSV and Rhinovirus.

Gene	Function	Location	Virus				
			SARS-CoV-2	Influenza	SARS-CoV-1	RSV	Rhinovirus
DDX60L	This gene encodes a member of the DExD/H-box helicase family of proteins, a subset of the super family 2 helicases. In addition to functions in RNA metabolism, members of this family are involved in anti-viral immunity and act as cytosolic sensors of viral nucleic acids	chr4	76.89471	81.07511	88.881	98.23783	98.44945
JAK2	This gene encodes a non-receptor tyrosine kinase that plays a central role in cytokine and growth factor signalling. This gene and the IL6/JAK2/STAT3 signalling pathway is a therapeutic target for the treatment of excessive inflammatory responses to viral infections.	chr9	65.71339	81.63137	94.10571	87.08943	97.70603
IFI44	Predicted to be involved in immune response.	chr1	75.19402	88.06365	68.81404	96.18025	97.14861
FOXP2	This gene encodes a forkhead domain binding protein and may function in the transcriptional regulation of the human T-cell leukemia virus long terminal repeat.	chr2	66.57874	89.72695	99.19854	82.8818	85.6627
DDX60	DX60 (DExD/H-Box Helicase 60) is a Protein Coding gene that functions as an antiviral factor and promotes RIG-I-like receptor-mediated signaling.	chr4	60.44368	69.17354	92.75642	99.07696	99.12914

Means of the total ranking score of every gene were used to calculate the top five up regulated genes.

2.4. Log<sub>2</sub> fold change

The selected dataset was converted into Log<sub>2</sub> Fold Change for the gene expression. The datasets that were unconvertible into Log<sub>2</sub> were removed for Log<sub>2</sub> Fold Change analysis. In addition, only datasets that compared infected and non-infected patients were used while high vs low viral load datasets were removed. Finally, Log<sub>2</sub> Fold Change values for each gene and for each dataset were inputted into the software and the graphing tool was used to generate scatter bar charts for each virus-

specific dataset. These include the top five upregulated and/or down-regulated genes for each dataset taken from the original data.

2.5. iDEP.91 software

Once all the data had been converted into the ranking score format, it was exported into a separate Excel File to be compiled into one concise table (Supplementary dataset 3), then saved as a text document and uploaded to the iDEP web application for expression and pathway

**Table 4**

Top five down regulated genes shared across all SARS-CoV-2, Influenza, SARS-CoV-1, RSV and Rhinovirus.

Gene	Function	Location	SARS-CoV-2	Influenza	SARS-CoV-1	RSV	Rhinovirus
FCGRT	This gene encodes a receptor that binds the Fc region of monomeric immunoglobulin G. This protein also binds immunoglobulin G to protect the antibody from degradation. Alternative splicing results in multiple transcript variants.	chr19	-39.6042	-82.1815	-90.1054	-93.0122	-99.3856
MFSD3	MFSD3 (Major Facilitator Superfamily Domain Containing 3) is a Protein Coding gene. Predicted to be involved in proton transmembrane transport. Predicted to be integral component of membrane.	chr8	-42.4523	-76.7588	-93.4394	-97.8397	-94.4382
IMPA2	IMPA2 (Inositol Monophosphatase 2) is a Protein Coding gene. The encoded protein catalyzes the dephosphorylation of inositol monophosphate and plays an important role in phosphatidylinositol signaling.	chr18	-42.7931	-77.4724	-97.8705	-92.2407	-97.9466
CCDC106	CCDC106 (Coiled-Coil Domain Containing 106) is a Protein Coding gene. Promotes the degradation of p53/TP53 protein and inhibits its transactivity.	chr19	-39.2854	-89.8161	-88.2233	-98.4019	-93.7914
ZNF219	ZNF219 (Zinc Finger Protein 219) is a Protein Coding gene. The encoded protein functions as a transcriptional repressor of the high mobility group nucleosome binding domain 1 protein, which is associated with transcriptionally active chromatin.	chr14	-75.3585	-85.8418	-83.4911	-72.589	-98.0437

Means of the total ranking score of every gene were used to calculate the top five down regulated genes.

analysis as described earlier [12].

### 3. Results

#### 3.1. Overview of the differences in the Log-2 fold change values and ranking scores across multiple respiratory viruses

The scatter bar graphs for each of the individual datasets within each of the five viruses were drawn to provide an overview of the differences in the Log-2 Fold Change values obtained from each dataset (Fig. 1). The scatter bar graph for the datasets collected for the SARS-CoV-2 uses the original Fold change values given by each study where each bar represents a separate dataset that showed the up and down regulated genes in response to viral infection (Fig. 1A). A vast majority of top five upregulated genes were summarized (Table 3) while the top five down regulated genes involved in the innate immune response to SARS-CoV-2, SARS-CoV-1, influenza, RSV and rhinovirus infection were concluded (Table 4). Interestingly, each dataset shown was distinctive showing a varying pattern where host genes are mildly up or down regulated and only a few that are highly differentially up or down regulated. This highlights selective genes of the innate immune response are affected in response to a specific virus infection. Collectively, dataset GSE155286 has the widest spread of data while dataset GSE147507 has the lowest (Table 2). In addition, all the datasets carried both downregulated and upregulated genes except GSE153790, which has only upregulated genes. Amongst the top five up-regulated genes, five genes including IFI27 and C-X-C motif chemokine ligand (CXCL) group of cytokine-producing genes, specifically CXCL10 showed a virus-specific trend.

Using the same approach, we used the data collected for innate immune genes in response to influenza virus infection that contains nine datasets. The data was presented for better visualising to gauge the innate immune genes play critical roles in the virus infection. Consistently, amongst all datasets, the up regulated genes for the influenza virus were interferon alpha-inducible protein 27 (IFI27) and interferon induced protein 44 producing gene IFI44/IFI44L, which involves in type-1 interferon signalling process leading to apoptosis and the formation of tubular structures, respectively.

The scatter bar graphs for SARS-CoV-1, RSV and rhinovirus indicate a unique set of genes up or down regulated during infection (Fig. 1C, D and 1E), respectively. While limited datasets were available against some viruses, minimum eight datasets provided approximately 12,000 different genes. Datasets that have gaps around the zero value for Log-2 fold change are the datasets that only include genes that were significantly up or down regulated. All datasets shown in Fig. 1C, D and 1E show a clear abundance of genes that are mildly differentially regulated with significantly less genes at the high fold change values, highlighted

by the shape of the GSE53543 dataset. Interestingly, there was marked variation between the highest and lowest values obtained for log-2 fold change for different datasets within SARS-CoV-1. In addition, most of innate immune genes fall within +10 or -10 log-2 fold change for these viruses. However, SARS-CoV-1 appears to have a unique set of top five up regulated genes compared to the other viruses whereas both RSV and rhinovirus datasets showed IFI44 gene and the CXCL family. OASL remained a consistently upregulated gene in RSV datasets.

The log-2 fold change values of each gene for each dataset was changed into a ranking score due to the high variation of experimental method used to collect data for each dataset, which meant that log-2 fold change values were rarely consistent between datasets for differential gene regulation of patients/cells infected with the same respiratory virus. Thus, the ranking score removed this issue by assigning each gene a value based on its position among other differentially regulated genes within the same dataset (i.e., a gene placing as the 5th highest upregulated gene in a list of 100 genes would receive a score of 95). These synchronized values were averaged across all datasets within each virus that enabled the data collected from different experimental approaches to be compared more effectively between datasets within the same virus and a combination of datasets to be compared between different viruses (Tables 5 and 6).

#### 3.2. iDEP.91 statistical analysis

The application of ranking scores facilitated the generation of a dataset consisting of 12,000 genes across all viruses by removing many non-significant low expressed genes (Fig. 2A). This newly and reduced set of genes and the data provided a higher resolution of genes distribution across multiple respiratory viruses (Fig. 2B). Thereafter, all analyses were conducted using dataset generated through ranking system. The iDEP (an integrated web application for differential expression and pathway analysis), helped to remove low expressed genes, convert gene IDs, fold change calculation and gene clustering.

The scatter plots generated based on 12,000 genes highlighted the distribution patterns of genes for SARS-CoV-2 and other respiratory viruses (Fig. 2C to F). The relationship between SARS-CoV-2 and influenza virus gene regulation revealed a uniform scatter data (Fig. 2C), while the relationship between SARS-CoV-2 and SARS-CoV-1 gene regulation contains more spread of data points except towards the centre of the graph due to the removal of less important data towards zero values (Fig. 2D). A slightly different patterns was observed where a linear relationship between SARS-CoV-2 and RSV (Fig. 2E) was noticed. An overall less uniform spread of data points with a skew to the right towards the top of the graph, and additional upregulated genes were observed in SARS-CoV-2 and rhinovirus comparison (Fig. 2F).

**Table 5**

Ranking scores of up regulated genes related to SARS-CoV-2 compared to down regulated genes correlated with other respiratory viruses (Influenza, SARS-CoV-1, RSV and Rhinovirus).

Gene	Fuction	Location	Virus				
			SARS-CoV-2	Influenza	SARS-CoV-1	RSV	Rhinovirus
RGS11	RGS11 (Regulator Of G Protein Signaling 11) is a Protein Coding gene. This protein inhibits signal transduction by increasing the GTPase activity of G protein alpha subunits, thereby driving them into their inactive GDP-bound form.	chr16	65.1003246	-96.6809	-93.256614	-94.2356	0
MAP2K5	MAP2K5 (Mitogen-Activated Protein Kinase Kinase 5) is a Protein Coding gene. The protein encoded by this gene is a dual specificity protein kinase that belongs to the MAP kinase kinase family. This kinase specifically interacts with and activates MAPK7/ERK5. This kinase itself can be phosphorylated and activated by MAP3K3/MEKK3, as well as by atypical protein kinase C isoforms (aPKCs). The signal cascade mediated by this kinase is involved in growth factor stimulated cell proliferation and muscle cell differentiation.	chr15	47.014237	-58.371	-65.336632	-55.5825	-78.04365
GPR162	GPR162 (G Protein-Coupled Receptor 162) is a Protein Coding gene. This gene was identified upon genomic analysis of a gene-dense region at human chromosome 12p13. It appears to be mainly expressed in the brain; however, its function is not known.	chr12	17.8299687	-96.1071	-82.211228	-99.2836	-97.15441
MSC	MSC (Musculin) is a Protein Coding gene. This protein is capable of inhibiting the transactivation capability of E47, an E2A protein, in mammalian cells. This gene is a downstream target of the B-cell receptor signal transduction pathway.	chr8	47.471529	-72.2297	-91.707894	-20.3681	-93.69442
ZNF581	ZNF581 (Zinc Finger Protein 581) is a Protein Coding gene. Predicted to enable DNA-binding transcription factor activity and RNA polymerase II cis-regulatory region sequence-specific DNA binding activity. Predicted to be involved in regulation of transcription by RNA polymerase II.	chr19	58.2545732	-44.4351	-86.534739	-68.4118	-73.32255
RINL	RINL (Ras And Rab Interactor Like) is a Protein Coding gene. Predicted to enable guanyl-nucleotide exchange factor activity. Predicted to be involved in endocytosis.	chr19	42.9596735	-57.4864	-21.907937	-96.4069	-75.6831
CENPBD1	Predicted to enable DNA binding activity.	chr16	55.3289963	-67.2357	-42.449989	-55.814	-93.1447
THBS3	THBS3 (Thrombospondin 3) is a Protein Coding gene. The protein encoded by this gene belongs to the thrombospondin family. Thrombospondin family members are adhesive glycoproteins that mediate cell-to-cell and cell-to-matrix interactions. This protein forms a pentameric molecule linked by a single disulfide bond. This gene shares a common promoter with metaxin 1.	chr1	62.2955312	-84.3552	-29.791353	-78.6179	-56.05497
CDA	CDA (Cytidine Deaminase) is a Protein Coding gene. This gene encodes an enzyme involved in pyrimidine salvaging. The encoded protein forms a homotetramer that catalyzes the irreversible hydrolytic deamination of cytidine and deoxycytidine to uridine and deoxyuridine, respectively. It is one of several deaminases responsible for maintaining the cellular pyrimidine pool.	chr1	45.317302	-79.2657	-51.989675	-8.71817	-99.35327
LIMS2	LIMS2 (LIM Zinc Finger Domain Containing 2) is a Protein Coding gene. This gene encodes a member of a small family of focal adhesion proteins which interacts with ILK (integrin-linked kinase), a protein which effects protein-protein interactions with the extracellular matrix. The encoded protein has five LIM domains, each domain forming two zinc fingers, which permit interactions which regulate cell shape and migration.	chr2	46.6092978	-76.5029	-81.910088	-75.113	0
NFKBIL1	NFKBIL1 (NFKB Inhibitor Like 1) is a Protein Coding gene. Involved in the regulation of innate immune response. Acts as negative regulator of Toll-like receptor and interferon-regulatory factor (IRF) signaling pathways. Contributes to the negative regulation of transcriptional activation of NF-kappa-B target genes in response to endogenous proinflammatory stimuli.	chr6	53.4792011	-80.9981	-78.780383	-80.205	0
DTX3	DTX3 (Deltex E3 Ubiquitin Ligase 3) is a Protein Coding gene. Regulator of Notch signaling, a signaling pathway involved in cell-cell communications that regulates a broad spectrum of cell-fate determinations.	chr12	58.4436487	-79.989	-64.132071	-46.0818	-43.75101

### 3.3. Heatmap analyses and gene differences between respiratory viruses

The heatmap were generated to provide an insight into pathways that are differently regulated by each of the five studied respiratory viruses (Fig. 3). SARS-CoV-2 appeared unique in eliciting a separate viral response compared to the other respiratory viruses. Notably, there was a region at the bottom of the heatmap between genes DDX21 and GBP3 where other viruses have no effect or a slight upregulation of the genes, however, SARS-CoV-2 causes a downregulation (Fig. 3). Perhaps the most unique out of all the respiratory viruses is SARS-CoV-1 which showed large areas of each heatmap where it is causing a downregulation of genes where all other respiratory viruses were eliciting upregulation.

The heatmap results highlighted the differences between each of the respiratory viruses, even though they are in the same group based on

their target within the host; the genes that being affected are substantially different. Each virus shown in the heatmap carried different and distinct green and red areas, with very few coloured areas shared between more than two viruses. The most substantial difference was noticed between SARS-CoV-2 and SARS-CoV-1, whereas almost no colours in common. However, SARS-CoV-1 appeared to be the only virus that has both up and down regulated genes in two specific groups.

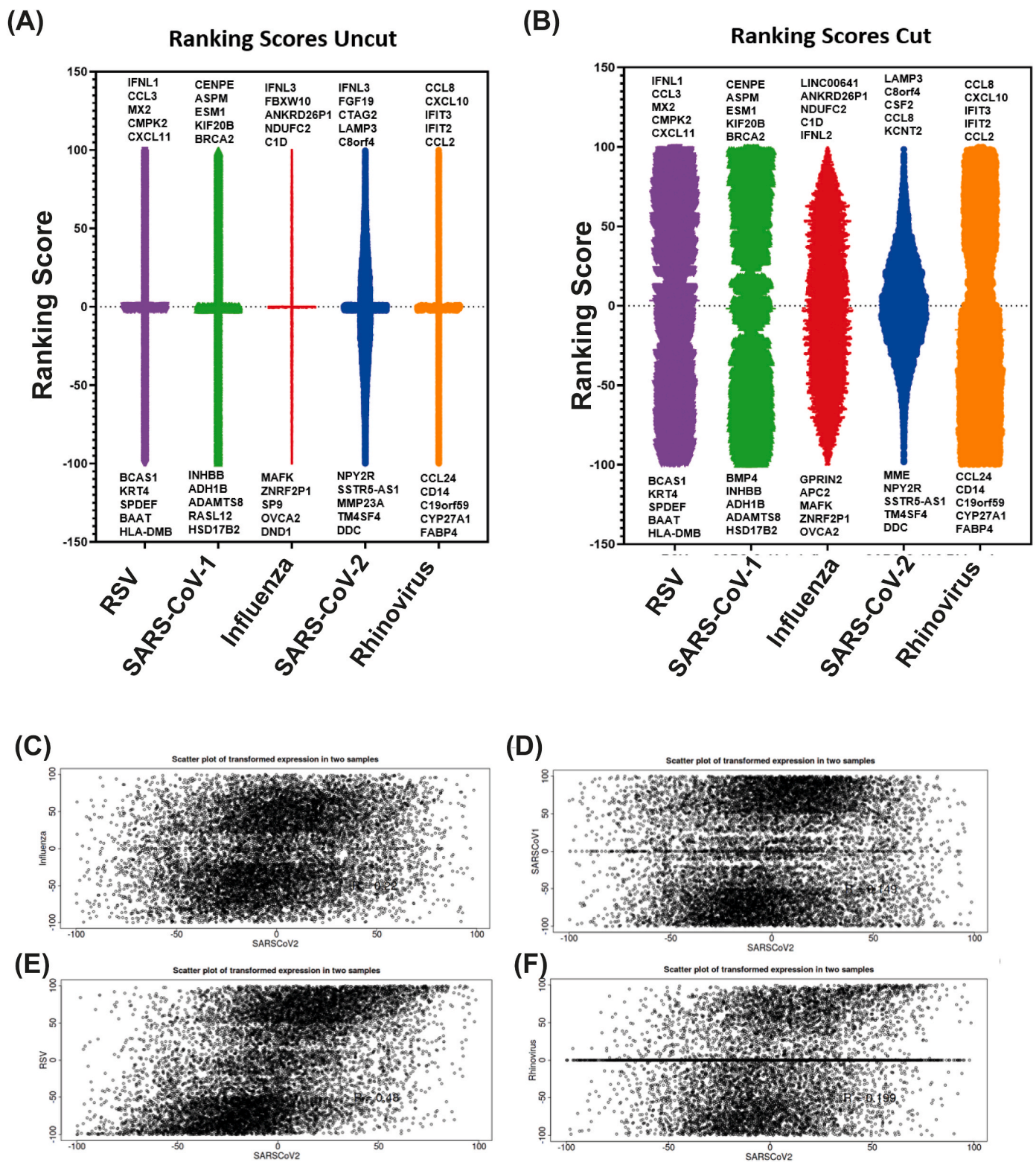
### 3.4. Standard deviation calculation and T-SNE plot analyses

The SD graph highlights the extremely high standard deviation across all the regulated genes in response to different viruses (Fig. 4A). A standard deviation above 1 was considered high unless the standard deviation in this case was between 25 and 75 indicating that there are high differences in the differentially regulated genes in response to each

**Table 6**

Ranking scores of down regulated genes related to SARS-CoV-2 compared to up regulated genes correlated with other respiratory viruses (Influenza, SARS-CoV-1, RSV and Rhinovirus).

Gene	Function	Location					
			SARS-CoV-2	Influenza	SARS-CoV-1	RSV	Rhinovirus
GPBAR1	GPBAR1 (G Protein-Coupled Bile Acid Receptor 1) is a Protein Coding gene. This gene encodes a member of the G protein-coupled receptor (GPCR) superfamily. This enzyme functions as a cell surface receptor for bile acids. Treatment of cells expressing this GPCR with bile acids induces the production of intracellular cAMP, activation of a MAP kinase signaling pathway, and internalization of the receptor. The receptor is implicated in the suppression of macrophage functions and regulation of energy homeostasis by bile acids.	chr2	-44.515674	82.44926	41.1585675	83.27739	98.47069
IDI1	IDI1 encodes a peroxisomally-localized enzyme that catalyzes the interconversion of isopentenyl diphosphate (IPP) to its highly electrophilic isomer, dimethylallyl diphosphate (DMAPP), which are the substrates for the successive reaction that results in the synthesis of farnesyl diphosphate and, ultimately, cholesterol.	chr10	-40.808037	93.10592	60.5356599	76.79214	63.57264
SC5DL	This gene encodes an enzyme of cholesterol biosynthesis. The encoded protein catalyzes the conversion of lathosterol into 7-dehydrocholesterol.	chr11	-71.53255	60.62026	74.15035	88.31216	77.25149
FAM49B	Enables small GTPase binding activity. Involved in several processes, including cellular response to molecule of bacterial origin; negative regulation of small GTPase mediated signal transduction; and regulation of organelle organization.	chr8	-44.603995	74.26559	59.9066653	58.04363	63.74257
MYH15	MYH15 (Myosin Heavy Chain 15) is a Protein Coding gene. Predicted to enable several functions, including ATP binding activity; actin filament binding activity; and calmodulin binding activity. Predicted to be involved in extraocular skeletal muscle development.	chr3	-60.985801	96.50129	79.5576747	86.08247	0
INA	INA (Internexin Neuronal Intermediate Filament Protein Alpha) is a Protein Coding gene. They may also play a role in intracellular transport to axons and dendrites. This gene is a member of the intermediate filament family and is involved in the morphogenesis of neurons.	chr10	-62.020737	95.6463	74.2010754	77.4994	0
KIAA1009	CEP162 (Centrosomal Protein 162) is a Protein Coding gene. Involved in cilium assembly.	chr6	-50.576886	63.53921	96.7637212	13.33014	58.41121
SGOL2	SGO2 (Shugoshin 2) is a Protein Coding gene. Predicted to be involved in homologous chromosome segregation; meiotic sister chromatid cohesion; and mitotic sister chromatid segregation. Predicted to act upstream of or within meiotic nuclear division; positive regulation of maintenance of meiotic sister chromatid cohesion, centromeric; and protein localization. Located in chromosome, centromeric region and nuclear body.	chr2	-59.393092	88.30258	99.9289845	-4.27642	52.46389
BCAS2	BCAS2 (BCAS2 Pre-mRNA Processing Factor) is a Protein Coding gene. Involved in mRNA splicing, via spliceosome. Located in centrosome and nuclear speck. Part of U2-type catalytic step 2 spliceosome. Colocalizes with DNA replication factor A complex. Implicated in breast cancer.	chr1	-42.301185	44.86425	69.5343411	42.43587	35.70518
PKD2	PKD2 (Polycystin 2, Transient Receptor Potential Cation Channel) is a Protein Coding gene. The encoded protein is a multi-pass membrane protein that functions as a calcium permeable cation channel, and is involved in calcium transport and calcium signaling in renal epithelial cells. This protein interacts with polycystin 1, and they may be partners in a common signaling cascade involved in tubular morphogenesis.	chr4	-45.37472	46.51688	62.7675763	29.76504	53.48343



**Fig. 2.** Uncut (A) and Cut (B) ranking scores for each gene combining all datasets for each respiratory virus. Also, in this figure are scatter plots of ranking scores of all genes collected for each respiratory virus, using SARS-CoV-2 as the comparison. (C) Shows a comparison of Influenza and SARS-CoV-2, (D) between SARS-CoV-1 and SARS-CoV-2, (E) between RSV and SARS-CoV-2 and (F) between Rhinovirus and SARS-CoV-2.



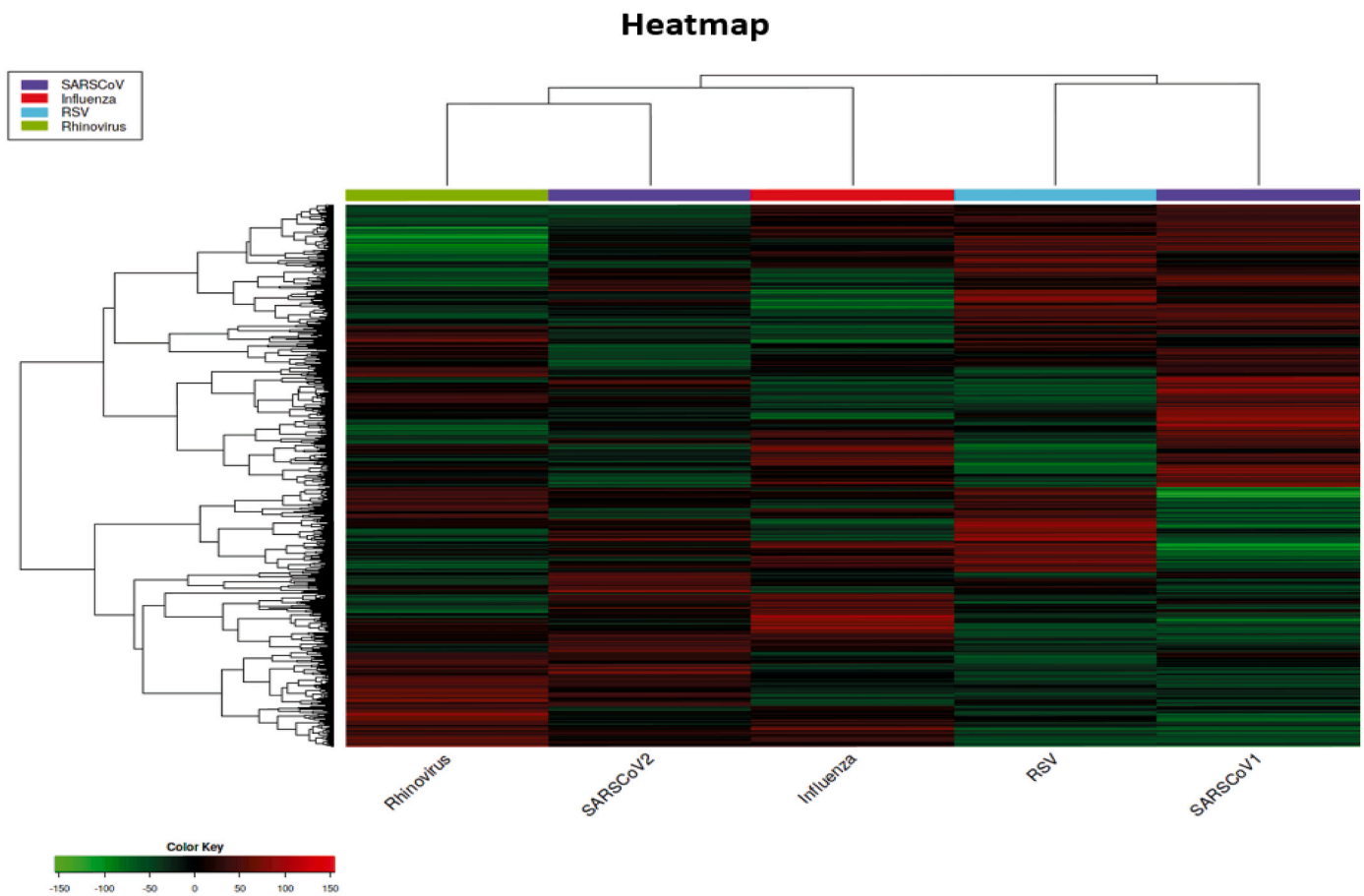


Fig. 3. Heatmap of DEGs for all respiratory viruses studied in this analysis.

virus. On the other hand, a correlation matrix that shows the correlation between each of the viruses revealed that the most similar virus to SARS-CoV-2 was RSV with a Pearson's correlation coefficient of 0.48 (Fig. 4B) while the least similar one was SARS-CoV-1 with a Pearson's correlation coefficient of 0.15 (Fig. 4B). A correlation value of 1 implies that there is a perfectly linear distribution of data between the two variables and a value of 0.48 generated for RSV compared to SARS-CoV-2 is relatively high that highlight how close the two viruses are in comparison to other viruses.

Differentially regulated genes were classified into 20 clusters based on their K means (Fig. 4C) where we used them to break down for better understanding whereabouts the differences between these emerged viruses. Each cluster contains genes involved in specific pathways that allows for the comparison of gene regulation in a variety of pathways depending on the virus (Supplementary dataset 4). After K-means clustering, cluster O appeared to contain the most pathways involved in the innate immune response, such as the JAK-STAT signalling pathway, TNF signalling pathway and IL-17 signalling pathway indicating that cluster O could be used as a sign of a virus's regulation for the overall innate immune response signalling. Both influenza and SARS-CoV-2 showed both up and down regulated genes within the cluster with specific areas either being highly up- or down-regulated, suggesting that these viruses target specific areas within this cluster. While SARS-CoV-1 and RSV upregulated and down regulated this region, respectively.

The T-SNE plot analyses for all the data was coloured based on their belonging cluster. The T-SNE allowed multi-dimensional data to visualise in a low dimension space such as the 2D graph (Fig. 4D). The distance between each of the points reflected the similarity of each data point. Whilst T-SNE should not always be used for gene expression data analysis, due to its high intrinsic dimensionality. Therefore, it has been

used to highlight that even though there are a high number of clusters present, they are still very much distinguishable, despite there being some clusters that exhibit more separation of data points compared to others. In addition, there was a slight problem with crowding towards the centre of the dataset; however, this was observed in most SNE forms.

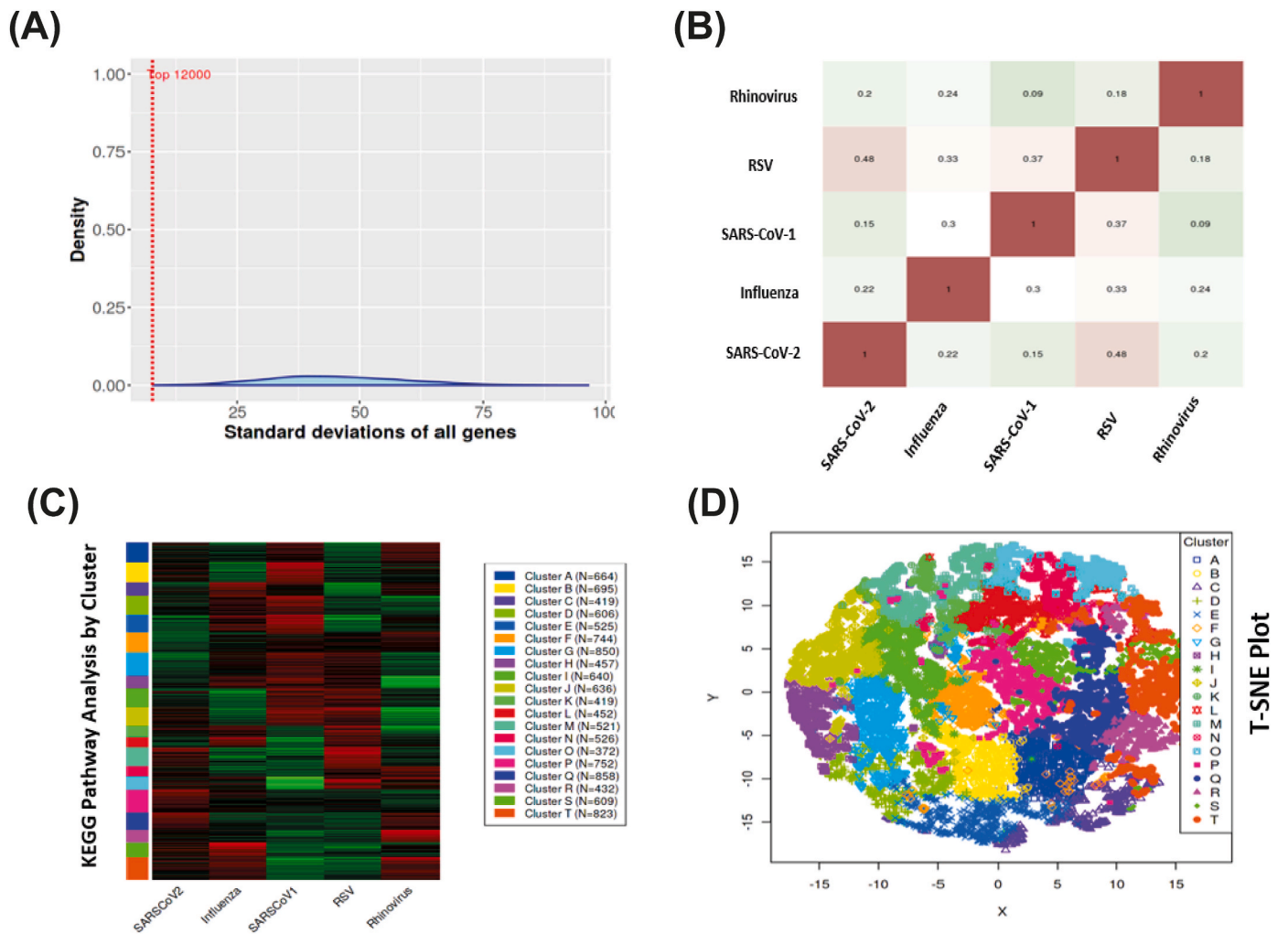
### 3.5. Comparison between differentially regulated genes among multiple respiratory viruses

Generally, the number of upregulated genes is relatively even with the number of downregulated genes, however, there are more downregulated genes than upregulated genes for each of the five tested viruses. The standouts are substantially downregulated than upregulated genes in case of rhinovirus infection in (Fig. 5A). Moreover, rhinovirus showed less differentially regulated genes in total compared to the other respiratory viruses.

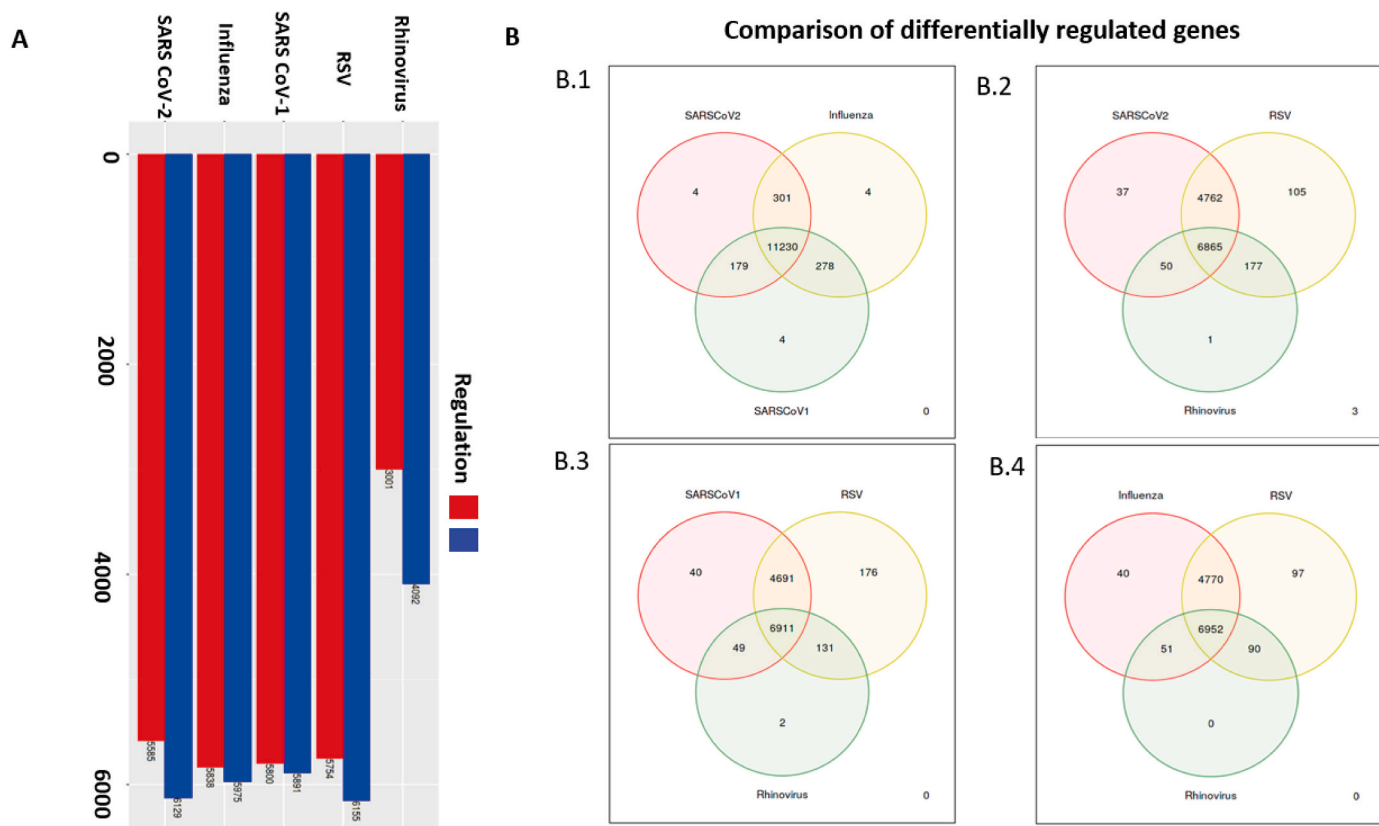
The Venn diagrams showed a comparison between each of the viruses by how many differentially regulated genes they have in common, regardless of whether they are up or down regulated. This highlighted genes that are differently regulated within only one virus compared to others within the same diagram (Fig. 5B). Vast majority of genes are found to be differentially expressed across all viruses; however, there were some exceptions mainly found within RSV that has the highest number of genes unique to itself while rhinovirus rarely had any uniquely expressed genes.

### 3.6. Impact of SARS-CoV-2 on cellular DNA replication

A visual representation for the impact of SARS-CoV-2 on the DNA replication within infected cells was outlined (Fig. 6). The upregulated



**Fig. 4.** (A) Standard deviation of all genes across all viruses. (B) Correlation matrix using data taken from the top 75% of genes. (C) KEGG pathway analysis by cluster. (D) T-SNE plot of all 12,000 genes.



**Fig. 5.** Nature of differentially regulated genes. (A) Total number of upregulated and down regulated genes for each virus. (B) Venn diagrams representing the differentially regulated genes that are in common between each of the respiratory viruses.

genes (2/32) were shown in red while the downregulated (27/32) were shown in green (Fig. 6). Genes responsible to produce DNA ligase and helicase were notably down regulated, which are important in the DNA replication and being used by the virus as a means of slowing down the cell cycle to enhance viral replication.

### 3.7. Regulation of JAK-STAT immune signalling pathway in response to SARS CoV-2 infection

There are more upregulated genes in JAK-STAT immune signalling (Fig. 7A) and the cytokine-cytokine receptor interaction pathways (Fig. 7B) than downregulation, highlighted by the prominence of the red colouring over the green colouring. While there were several upregulated genes as GFAP and Ras, which are involved in cell differentiation and MAPK signalling pathway, respectively.

The genes that are up or down regulated in relation to the immune signalling pathways and are affected in response to SARS-CoV-2 infection were analysed using KEGG pathway database (Fig. 7A and B and Supplementary Fig. 1). These results revealed that SARS-CoV-2 does not affect every pathway in a simple manner by either upregulating or downregulating all genes involved in that pathway, but instead having multiple effects.

Using the ranking scores, C8orf4 was the second most highly upregulated gene in cells/patients infected with SARS-CoV-2. The C8orf4 (also known as TC1M) is responsible for producing the c8orf4 protein (also known as TC1) which is involved in the enhancement of NF-kappaB activity and leading to up-regulating several cytokines involved in the process of inflammation [13]. This is the main factor attributed to the cytokine storm exhibited in patients following SARS-CoV-2 infection. In addition, our analyses show that each virus has a different effect on the regulation of C8orf4 and its regulation could therefore be used as a biomarker to differentiate between aetiology of

infection, with extremely high levels of TC1 protein pointing towards a SARS-CoV-2 infection (Fig. 7C). Of course, many other genes could be used as markers for SARS-CoV-2 infection but also genes that are conserved between all viruses. Previous study, based on transcriptome overlapping analysis induced on bronchial epithelium cells infected with SARS-CoV-2, SARS-CoV, MERS-CoV, and H1N1, has revealed that c8orf4 gene was commonly regulated in NHBE and HAE under the infection of the four different viruses [14]. In addition, c8orf4 gene enhances the proliferation of follicular dendritic cells [15].

After individual identification of the upregulated or downregulated genes and their respective pathways, we aim to visualise where those genes are located within the human chromosome (Fig. 7D). Human genome map analyses show each chromosome with its own line with genes where the upregulated genes appear above the line in red colour while genes that are downregulated appear below the line in blue colour (Fig. 7D). This genomic map shows the regulation in response to SARS-CoV-2 infection and revealed that every chromosome in the human genome has been affected whereas the mostly affected chromosome was chromosome 19. However, the least affected chromosomes were X and Y sex chromosomes. In addition, chromosome 17 also shows a notable pattern. There are many areas across many chromosomes that showed notable gaps where SARS-CoV-2 appears to have no effect on gene regulation (Fig. 7D).

There is a large amount of consistency between all the genome maps within the most affected chromosome, in all cases, being chromosome 19. In case of rhinovirus, there is a lack of altered genes regulation on the X and Y chromosomes. Furthermore, a much blander overall picture on fewer data points (Supplementary Fig. 2D) because there were less genes recorded to have been up or downregulated in the rhinovirus dataset.

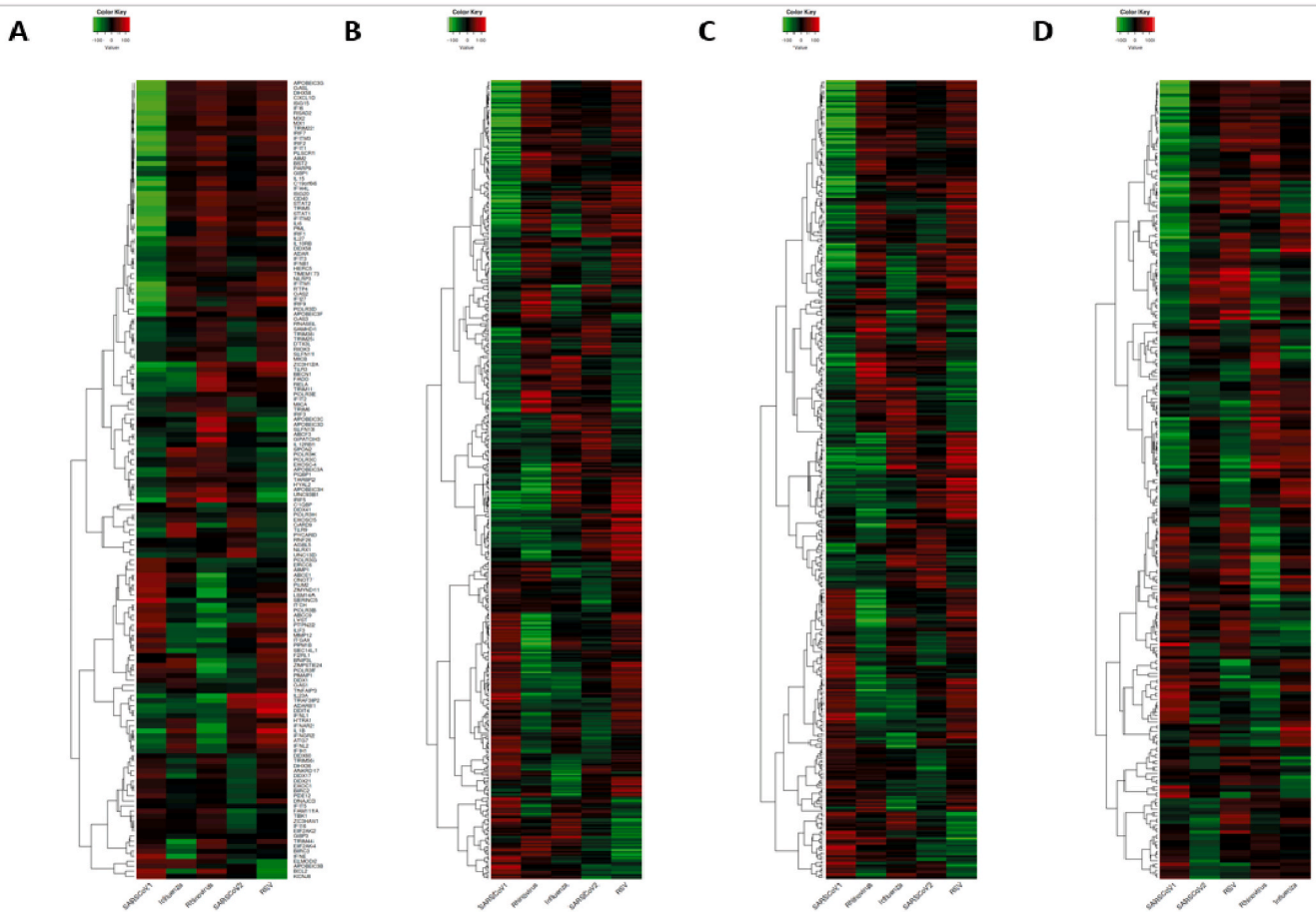


Fig. 6. Heatmaps specific to different pathways compiled by GAGE pathway analysis. (A) For Defence response to virus, (B) for cytokine response, (C) for regulation of cytokine production and (D) for positive regulation of innate immune response.

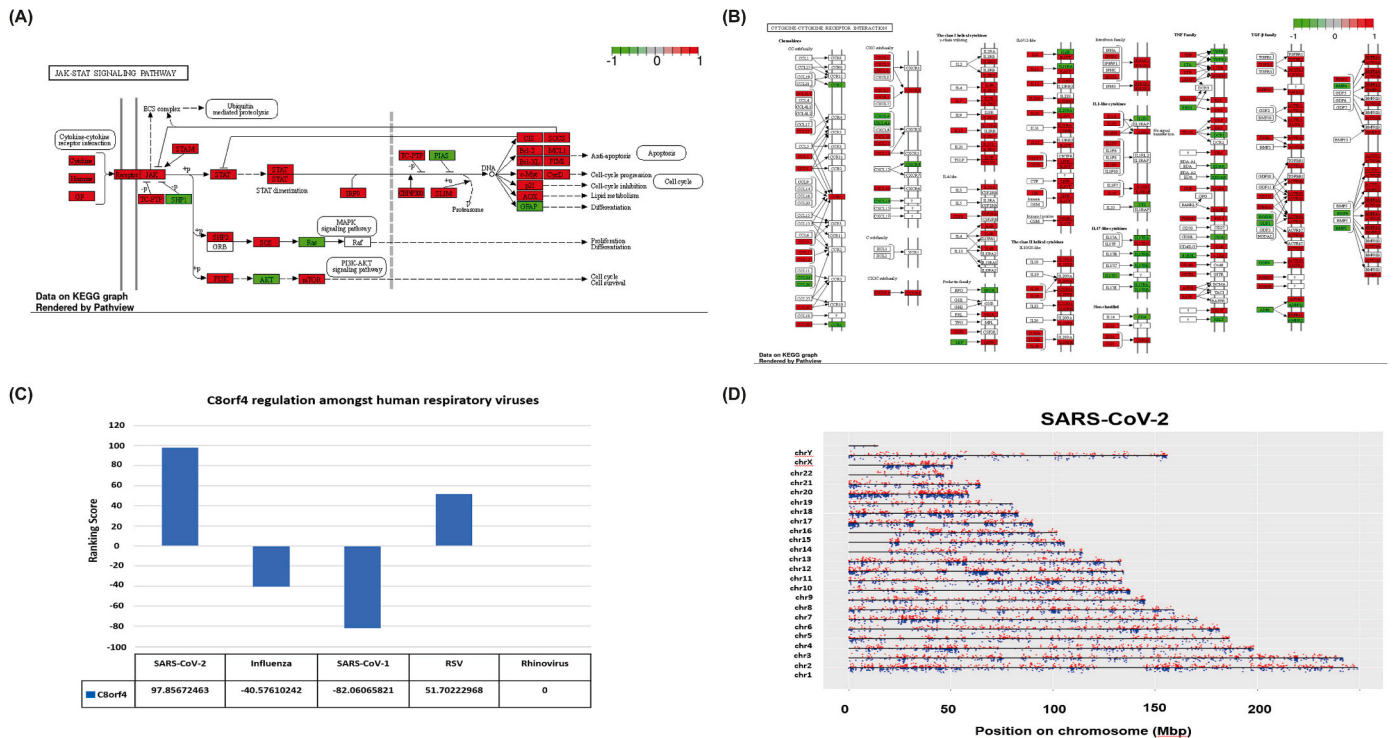


Fig. 7. Regulation of different pathways by studied respiratory viruses. (A) Regulation of genes associated with the JAK-STAT signalling pathway. (B) Regulation of genes associated with cytokine-cytokine receptor interaction. (C) Ranking scores of the C8orf4 gene for each respiratory virus. (D) Genome map showing SARS-CoV-2 upregulated genes in red and downregulated genes in blue.

#### 4. Discussion

Despite majority of the human respiratory viruses show similar pathology by infecting the same respiratory system, they all showed clear and substantial differences, which have highlighted unique markers related to differential gene regulation. The scatter plots showed the correlation between the effects of each virus on human gene expression, and a specific removal of genes was evident in this analysis which are less dramatically differentially regulated and therefore of less importance to this study. These results indicated that SARS-CoV-2 is like RSV compared to other respiratory viruses because of the high correlation between the data points within the scatter graphs showing a rising diagonal line suggesting a positive correlation between the upregulated and downregulated genes. These results are supported by the correlation matrix, where the Pearson's correlation coefficient between SARS-CoV-2 and RSV was 0.48, much higher than the 0.22, 0.2 and 0.15 for influenza, rhinovirus, and SARS-CoV-1, respectively. The SARS-CoV-2 and RSV showed high similarity in differentially regulated genes. This aligns with the fact that affected patients exhibit similar symptoms when infected with any of SARS-CoV-2 or RSV, mainly upper respiratory tract symptoms and often lower respiratory tract symptoms such as a dry cough [16]. Interestingly, both viruses appear to cause damage to the respiratory tract that result in persistent problems long after infection such as persistent airway obstruction as well as hyper-responsiveness can be seen in patients 30 years after infection with RSV [17]. These symptoms are like the long-term lung dysfunction reported after SARS-CoV-2 infection [18]. However, the main difference between these two viruses is the age of the patients that are more susceptible for infection, with RSV commonly causing respiratory tract infection in young infants and children [16], whereas SARS-CoV-2 is known for more severe cases being present in the elderly albeit infection potential among all ages. Further research in this area could be useful to compare influenza, RSV, SARS-CoV-1 and rhinovirus against SARS-CoV-2 but specifically for each pathway/area such as the innate immune response or the cytokine activation pathway.

Insights into the human chromosomes in response to SARS-CoV-2 infection revealed that the mostly affected chromosome was chromosome 19 suggesting a high number of genes involved in the immune response to viral infection could be present within chromosome 19 and severe cases of infection could be attributed to the genetic mutations within this chromosome. Another interesting point is the presence of differential gene expression on the X chromosome for patients suffering from COVID-19. Altered genes on the X-chromosome could lead to a difference in the clinical outcome between men and women infected with SARS-CoV-2. Previous studies reported that the immune regulatory genes encoded by the X chromosome in women could cause lower viral load levels resulting in a reduction in the inflammatory response compared to men [19].

The top and bottom five consistently up and down-regulated genes across all five viruses could potentially be used as markers for specific respiratory viral infection. JAK2 is one of the genes, which is consistently, and highly upregulated among all the studied viruses and it encodes for the Janus Kinase 2 protein (JAK2). JAK2 plays a crucial role in the cytokine signalling where it associates with type II cytokine receptors, hormone-like cytokine receptors and being activated by IFN-gamma [20]. Additional four-upregulated genes were DDX60L, IFI44, FOXN2 and DDX60, which may be a target for drugs.

The upregulated genes in response to SARS-CoV-2 infection have been identified while those were downregulated in the other respiratory viruses. These genes could be used as markers for a SARS-CoV-2 infection and to distinguish SARS-CoV-2 from other respiratory viruses. The most important gene was the NFKBIL1 gene that encodes for the NF-kappa-B inhibitor-like protein 1 and it is involved in the NF-kappa-B signalling, which plays a major role in the inflammatory response by increasing the cytokine expression [21]. On the other hand, the down-regulated genes in response to SARS-CoV-2 could possibly be used as a

marker to distinguish SARS-CoV-2 infection in case of suspicion with a respiratory virus infection associated with respiratory symptoms. One of these genes is GPBAR1, which encodes for the G-protein acid receptor 1. Previously, it has been reported that GPBAR1 was able to regulate and increase the expression of IL-10 [22] suggesting that levels of IL-10 in patients suffering with COVID-19 would be lower, however, recent studies contradict that as IL-10 levels are found to be unexpectedly increased in severe cases [23].

#### 5. Conclusions and limitations

The aim of this study was to determine the influence of SARS-COV-2 on the immune regulation and gene induction in comparison to other respiratory viruses. It appeared that SARS-CoV-2 was unique in its impact on gene regulation and matches none of the other respiratory viruses except RSV. Genes such as MAP2K5 and NFKBIL1 have been found to be greatly upregulated in SARS-CoV-2 whilst being down-regulated in the compared viruses. MAP2K5 is a dual specificity protein kinase that belongs to the MAP kinase family that specifically interacts with and activates MAPK7/ERK5. The signal cascade mediated by this kinase is involved in growth factor stimulated cell proliferation and muscle cell differentiation. The expression of these kinases inhibited the virus at post-entry stages. Specifically, it can inhibit the viral RNA replication. NFKBIL1 gene lies within the major histocompatibility complex (MHC) class I region on chromosome 6 that involved in the regulation of innate immune response by acting as negative regulator of Toll-like receptor and interferon-regulatory factor (IRF) signalling pathways.

Whereas genes such as GPBAR1 and SC5DL were contrastingly found to be significantly downregulated in SARS-CoV-2 but upregulated in influenza, SARS-CoV-1, RSV and rhinovirus. The GPBAR1 gene encodes a member of the G protein-coupled receptor (GPCR) superfamily. This enzyme functions as a cell surface receptor for bile acids, which is implicated in the suppression of macrophage functions and regulation of energy homeostasis by bile acids. SC5DL gene encodes an enzyme of cholesterol biosynthesis pathway and it catalyzes the conversion of lathosterol into 7-dehydrocholesterol. Despite all the reported differences, the most conserved genetic signature was JAK2 gene as well as the constitutively downregulated ZNF219 gene. While the resolution of analysis provides foundational finding, further research is warranted to validate the impact of these molecular signature against individual or multiple infections. This study might open the way for further investigations aimed at elucidating the molecular mechanisms that underlay these observations. This study also suggests that it may be possible to identify a signature, which could be useful to identify early patients at risk of adverse outcome. Our analysis identified several key aspects of the host response among human respiratory viruses' infection where essential immunity genes and biological pathways could be used for understanding the pathogenesis of SARS-CoV-2 infection.

We observed a limitation of the study that the gene regulation may be affected by the experimental characteristics such as time length post infection, the culturing conditions, phenotypes of the cells, and the nature of the virus stimulation (in vivo or in vitro studies). Finally, different cell types (A549, BALF or PBMC cells) were used for virus infection, which may respond differently to different viral infections. Nevertheless, the provided analysis provides a foundation for the impact of respiratory viruses on the gene regulation.

In addition, like other transcriptomic studies, this work has several limitations. The number of patients included in the different groups was limited, a factor that may have restricted the number of DEG reported. Samples were taken from different organs, whole blood or saliva do not necessarily reflect the gene expression patterns in clinically affected organs and/or individual cells and depict sample heterogeneity. The sequencing depth may have restricted differential detection of less abundantly expressed genes. Finally, the samples were issued from a single cohort of patients, and thus validation from other cohorts would

be useful.

### Ethics statement

No experimental procedures were carried out in this project and all data was collected from a range of previous research papers; therefore, no steps of this study were required to seek ethical approval.

### Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mcp.2022.101820>.

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