SARS-CoV-2 contributes to altering the post-transcriptional regulatory networks across human tissues by sponging RNA binding proteins and micro-RNAs

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Abstract

The outbreak of a novel coronavirus SARS-CoV2 responsible for COVID-19 pandemic has caused worldwide public health emergency. Due to the constantly evolving nature of the coronaviruses, SARS-CoV-2 mediated alteration on post-transcriptional gene regulation across human tissues remains elusive. In this study, we systematically dissected the crosstalk and dysregulation of human post-transcriptional regulatory networks governed by RNA binding proteins (RBPs) and micro-RNAs (miRs), due to SARS-CoV-2 infection. We uncovered that 13 out of 29 SARS-CoV-2 encoded proteins directly interact with 51 human RBPs of which majority of them were abundantly expressed in gonadal tissues and immune cells. We further performed functional analysis of differentially expressed genes in mock treated versus SARS-CoV-2 infected lung cells that revealed an enrichment for immune response, cytokine mediated signaling, and metabolism associated genes. This study also characterized the alternative splicing events in SARS-CoV-2 infected cells compared to control demonstrating that skipped exons and mutually exclusive exons were the most abundant events that potentially contributed to differential outcomes in response to viral infection. Motif enrichment analysis on the RNA genomic sequence of SARS-CoV-2 clearly revealed an enrichment for RBPs such as SRSFs, PCBPs, ELAVs and HNRNPs illustrating the sponging of RBPs by SARS-CoV-2 genome. Similar analysis to study the interactions of miRs with SARS-CoV-2 revealed the potential for several miRs to be sponged, suggesting that these interactions may contribute to altered pos-transcriptional regulation across human tissues. Given the need to understand the interactions of SARS-CoV-2 with key pos-transcriptional regulators in the human genome, this study provides a systematic analysis to dissect the role of dysregulated post-transcriptional regulatory networks controlled by RBPs and miRs, across tissues types during SARS-CoV2 infection.

Introduction

An outbreak of coronavirus disease (COVID-19) caused by the newly discovered severe acute respiratory syndrome coronavirus (SARS-CoV-2), started in December 2019, in the city of Wuhan, Hubei province, China. As of July 6, 2020, COVID-19 has expanded globally with more than 11.6 million confirmed cases with over 538,000 deaths worldwide, imposing an unprecedented threat to public health (https://www.worldometers.info/coronavirus/). In the past two decades, coronavirus outbreak has resulted in viral epidemics including severe acute respiratory syndrome (SARS-CoV) in 2002 with fatality of 10% and middle east respiratory syndrome (MERS-CoV) in 2012 with fatality of 36% [1-4]. Both SARS-CoV and MERS-CoV were zoonotic viruses originating in bat and camels respectively [5, 6]. However, the recurring emergence of highly pathogenic SARS-CoV, MERS-CoV and now SARS-CoV2 have indicated the potential for cross-species transmission of these viruses thus raising a serious public health concern [7, 8]. SARS CoV-2 shares a sequence similarity of 80% and 50% with previously identified SARS-CoV and MERS-CoV respectively [9-12]. Since its emergence, rapid efforts have illustrated the molecular features of SARS-CoV-2 that enables it to hijack the host cellular machinery and facilitates its genomic replication and assembly into new virions during the infection process [13-16].

Coronaviruses carry the largest genome among all RNA viruses, ranging from 26 to 32 kilobases in length [12]. These viruses have a characteristic "crown" like appearance under two-dimensional transmission electron microscopy. SARS-CoV-2 is an enveloped positive -sense, single-stranded ribonucleic acid (RNA) viruses that belongs to the genus beta-coronavirus. Upon entry in the cell, SARS-CoV-2 RNA is translated into non-structural proteins (nsps) from two open reading frames (ORFs), ORF1a, and ORF1b [17, 18]. The ORF1a produces polypeptide 1a that is cleaved further into 11 nsps, while ORF1b, yields polypeptide 1ab, that is cleaved into 16 nsps [17, 18]. Since, SARS-CoV-2 utilizes human machinery to translate its RNA after the entry into the cell, it could possibly impact several RNA-binding proteins from the host to bind the viral genome resulting in altered post-transcriptional regulation. Next, the viral genome is used as the template for replication and transcription, mediated by non-structural protein, RNA-dependent RNA polymerase (RdRP) [18, 19]. SARS-CoV-2 encodes four main structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N) that are conserved and several other accessory proteins (3a, 6, 7a, 7b, 8, and 10) according to the current annotation (GenBank: NC_045512.2) [17, 20]. The spike protein, that has evolved the most during the COVID-19 outbreak, enables the virus to bind to angiotensin-converting enzyme 2 (ACE2) on the host cell membrane, following which it undergoes structural changes and subsequently allows the viral genome to make its way inside the host cell [21]. Infections caused by these viruses result in severe pneumonia, fever and breathing difficulty [22].

Protein-protein interaction map between SARS-CoV-2 and human proteins published recently has revealed several important targets for drug repurposing [23]. Given the evolving nature of coronaviruses that results in frequent genetic diversity in their genome, it is crucial to identify the regulators in humans that interacts with the viral genome and their cross talk that results in altered regulatory mechanisms in the host during the infection process. Therefore, it is imperative to

investigate the interacting post-transcriptional regulators that asset these viral proteins in different tissues.

RNA-binding proteins (RBPs) and miRNAs (micro-RNAs) have been widely recognized in regulating the post-transcriptional gene regulatory network in humans [24-26]. Dysregulated RBPs and miRNAs have been shown to contribute significantly to altered regulatory network in a plethora of diseases such as cancer, genetic diseases and viral infections [27-33]. Till date, the potential RBPs and miRNAs that either interact directly with the virus or cross talk with each other to facilitate SARS-CoV-2 infection has not been investigated and hence require a systematic investigation of their role in SARS-SoV-2 infection.

Currently, there are no proven vaccines or anti-viral therapies that are effective against the novel coronavirus. Although, analysis of therapeutic targets for SARS-CoV-2 has been conducted to identify potential drugs by computational methods [34], the targets have not clinically approved for vaccine development. Alternative therapeutics like angiotensin receptor blockers have been identified as tentative vaccine candidates but have shown concerns associated with disrupted angiotensin functions crucial for cell [35]. Therefore, to devise effective therapeutics, there is a need to determine the cellular targets in humans that interact with the virus and result in altered functional outcomes. In this study, we uncovered that several human RBPs and miRNAs harbor abundant binding sites across the SARS-CoV-2 genome, illustrating the titration of post-transcriptional regulators. Interestingly, we show that most of these regulators were predominantly expressed in gonadal tissues, adrenal, pancreas, and blood cells. Overall, this study will bridge the gap in our understanding of the impact of SARS-CoV-2 infection on post-transcriptional regulatory networks.

RESULT AND DISCUSSION

Protein-protein interaction network analysis suggest an immediate interaction of functionally important human RBPs with SARS-CoV-2 viral proteins

We obtained the affinity purification-mass spectrometry (AP-MS) based SARS-CoV-2 and human proteins interaction network established in HEK293 cells [23] and investigated the human RBPs that directly interact with viral proteins. Our analysis revealed that SARS-CoV-2 encoded proteins interact directly with 51 human RBPs (Fig.1A). We observed that these primary interacting RBPs were proven to serve several vital functions in the cells including RNA processing enzymes such as polyadenylate binding protein 4 (PABP4) and Dead-box helicases (DDX21 and DDX10), enzymes involved in translation machinery such as eukaryotic translation initiation factor 4H (EIF4H) and ribosomal protein L36 (RPL36) (Fig. 1A). Among these immediate interactions, we also found human RBPs such as signal recognition particle 19 (SRP19 and SRP54) and Golgin subfamily B member 1 (GOLGB1) that have been well recognized for co-translational protein targeting to membrane and ER to Golgi vesicle-mediated transport [36, 37] (Fig. 1A). These results suggest that several human RBPs that come in direct contact with SARS-CoV-2 proteins could contribute to virus assembly and export. However, such findings require in-depth experimental validation to support the functional involvement of the identified RBPs in response to SARS-CoV-2 infection.

Further, we observed that among the SARS-CoV-2 encoded proteins, majority of direct interactions occurred with a range of non-structural proteins (nsp 2,5,8,9,11,13) that contribute to viral replication and transcription along with structural proteins (E,N and M) (Fig. 1A). It is likely that these identified RBPs assist in the viral transcription in the host cell. Furthermore, we also observed ~65% of annotated human RBPs [38] were in immediate neighborhood (obtained from BioGRID, shown at the center in Fig. 1A) of virus-protein interacting RBPs and could indirectly regulate the SARS-CoV-2 proteins, representing a comprehensive network of human RBPs and SARS-CoV-2 proteins likely to rewire the post-transcriptional gene regulatory mechanisms in human cells.

Next, we examined the abundance of SARS-CoV-2 interacting RBPs across human tissues using the protein expression data from human proteome map[39]. Our results suggest that majority of the human RBPs that have direct interaction with SARS-CoV-2 proteins were predominantly expressed in gonadal tissues (testis and ovary) (Fig. 1B). These findings agreed with a recent study showing that male reproductive systems are vulnerable to SARS-CoV-2 infection, that was evident by dramatic changes in sex hormones of the infected patients suggesting gonadal function impairment [40, 41]. Additionally, we also found that, these SARS-CoV-2 interacting human RBPs were showing relatively higher expression in immune cell types such as T cells (CD4+ and CD8+) and NK cells that are a part of innate anti-viral immune response (Fig. 1B). Our observations are supported by recently published studies suggesting that T cells, CD4+ T cells, and CD8+ T cells play a significant antiviral role during SARS-CoV-2 infection [20, 42-45]. Overall, the results from this analysis provide a systematic dissection of potential RBPs in humans interacting with SARS-CoV-2 proteins across tissues.

SARS-CoV-2 infected lung epithelial cells reveal the enrichment of several immunological and metabolic pathways.

Due to rapidly evolving nature of SARS-CoV-2, the transcriptomic alterations contributed by the virus in humans remains unclear. To gain insight into the effect of SARS-CoV-2 infection on host gene expression, we obtained the raw RNA sequencing data in normal versus SARS-CoV-2 infected human bronchial epithelial (NHBE) cells [46] deposited in Gene Expression Omnibus (GEO) [47]. We investigated the differentially expressed genes between the mock treated versus SARS-CoV-2 infected cells and assessed the functional pathways associated with these genes (Fig. 2A, Fig. S1). Functional analysis of differentially expressed genes (at 5% fdr) using ClueGo [48] revealed an enrichment for immune response, cytokine mediated signaling, inflammatory response and metabolism associated genes (Fig. 2A, Table S1).

We observed a significant enrichment for the IL-17 pathway associated genes during SARS-CoV-2 infection (Fig. 2A). Our observation of overrepresented IL-17 pathway was in accordance with recent studies that show overactivation of IL-17 producing Th17 cells during severe immune injury in SARS-CoV-2 patients [49-51]. In addition to this, a recent review has summarized that targeting IL-17 is immunologically plausible as a therapeutic strategy to prevent acute respiratory distress syndrome (ARDS) during SARS-CoV-2 infection, based on previous evidence that mice lacking functional IL-17 receptor (Il17ra−/−) signaling were shown to be more susceptible than wild-type mice to secondary pneumonia following infection with influenza A [52, 53]. Thus, our analysis shows several enriched pathways including IL-17 cytokine response in SARS-CoV-2 infected cells.

We also provide parallel support for the dysregulation of multiple RNA binding proteins (FLNB, HDGF, ASS1, ZC3H12A, HK2, BST2, PPARGC1A) involved in immune response, cytokine mediated signaling, and metabolism by employing mock treated versus SARS-CoV2 infected lung cells (Fig. 2B). We also observed that six of these differentially expressed genes that encode for RBPs were involved in at least 30% of the overrepresented pathways. Overall, our results imply that differentially expressed genes in SARS-CoV-2 infected cells may contribute to alterations in the post-transcriptional regulatory networks governed by the RBPs encoded by them.

Alternative splicing analysis revealed the abundance of skipped and mutually exclusive exons during SARS-CoV-2 infection in lung epithelial cells.

Alternative splicing is a principal mechanism that contributes to protein diversity in eukaryotes, while regulating physiologically important immune responses during bacterial and viral infections [54]. Viral infections have been shown to cause global changes in the alternative splicing signatures in the infected cells that may arise due to intrinsic factors like polymorphism at the splice sites or due to direct intervention by virulence factors [55-57]. A previous study on virus-host interactions has demonstrated that human coronavirus targets various signaling pathways of

ER stress resulting in differential splicing outcomes [58]. Another study has shown that deletion of E protein in recombinant SARS-CoV resulted in significant XBP1 gene splicing and higher rate of apoptosis, suggesting that coronavirus infected cells are susceptible to differential splicing events [59]. Therefore, we next investigated the alternative splicing events in mock vs SARS-CoV-2 treated NHBE cells using rMATS (replicate Multivariate Analysis of Transcript Splicing) [60].

Our analysis revealed an abundance for skipped and mutually exclusive exonic events in the genes exhibiting alternative splicing events during SARS-CoV-2 infection at 5% fdr (Fig. 3A). We also observed that 81 of the alternatively spliced genes encoded for RBPs (indicated in blue, Fig. 3A) and hence could result in altering the downstream post-transcriptional regulatory networks in SARS-CoV-2 infected cells (Fig. 3A). These findings enhance our mechanistic understanding of the SARS-CoV-2 induced alternative splicing dyregulation in human cells and could be critical for developing novel therapeutic strategies. Next, we identified the functional annotation of the enriched GO-terms related to the genes exhibiting alternative splicing using ClueGO [48]. Our data revealed that majority of these genes were enriched for vital biological processes including cellular protein localization, protein metabolism, organelle organization, cellular biosynthetic process, cellular component assembly and cytochrome c-mediated apoptotic response (Fig. 3B). These findings suggest that SARS-CoV-2 infection could dysregulate the functionally important biological catalogue via alternatively spliced genes predominantly through skipped exons and mutually exclusive exons. In summary, this analysis provides a clustered network of enriched biological functions that could be significantly dysregulated in SARS-CoV-2 infected cells.

Motif enrichment analysis reveals potential human RBPs titrated by SARS-CoV-2 viral genome.

SARS-CoV-2 genome is the largest among the coronavirus family (~30kb) and has been attributed to enhanced virus pathogenicity in the newly evolved strains of COVID-19 pandemic [18, 61]. Among the host derived cellular factors, RBPs have been recognized as active participants in all steps of viral infection [29, 62, 63]. A recent review has shown linkage of 472 human proteins with viruses through unconventional RNA binding domains [63]. In the present research, we conducted a systematic and comprehensive bioinformatic study to investigate the RBPs that could potentially bind on RNA genome of SARS-CoV-2 by motif enrichment analysis of human RBPs using FIMO [64]. Motif analysis for RBPs with established position specific weight matrices (PWMs) revealed significant number of binding sites spread across the SARS-CoV-2 genome illustrating the possible titration of post-transcriptional regulators by viral genome (Fig. 4A, Fig. S2). Importantly, the binding pattern of the RBP motifs across the entire normalized length of the virus suggesting that, several of human RBPs could be titrated randomly across the viral genome (Fig. 4A). Our results showed an enrichment for RBPs such as SRSFs, PCBPs, ELAVs and HNRNPs being most likely to get sponged on the viral genome (Fig. 4B). Specifically, our observation of RBPs well known for their role in splicing such as SRSF7, HNRNPA1 and TRA2A was in accordance with a recently published study that predicted binding sites of these RBPs on SARS-CoV-2 RNA [65]. In summary, these findings suggest that SARS-CoV-2 could sponge human RBPs on its genome resulting in altered post-transcriptional gene regulatory network in the host cells. Targeting host proteins has been appreciated as an effective strategy to combat a wide

range of viral infections and therefore an understanding of the potential RBPs that are likely sponged on the viral genome is crucial to develop novel therapeutics [30].

SARS-CoV-2 genome titrates the abundance of functionally important miRs in human tissue

Micro RNAs (miRs) are small non-coding RNA molecules that function as central regulators of post-transcriptional gene regulation. Human miRs have been associated with a variety of pathophysiological pathways and demonstrate differential expression during viral infections [66, 67]. Recently, a machine learning based study predicted that miRs could impact SARS-CoV-2 infection through several mechanisms such as interfering with replication, translation and even modulating the host gene expression [68]. In this study, we used computational approach to investigate the potential binding sites of human miRs in SARS-CoV-2 genome using FIMO [64]. We identified 22 miRNAs that could potentially bind throughout the length of the SARS-CoV2 viral genome (Fig.5A, Table S5). These results suggest that several important miRs are likely being titrated by SARS-CoV-2 genome that could result in dysregulation of post-transcriptional networks in the infected cells.

Majority of the identified miRs were highly expressed in immune cells including CD8+T cells, CD4+T, NK cells, CD14 cells and mast cells, suggesting that these miRs might contribute to altered post-transcriptional regulation in specialized immune cells and could contribute to the progression of the viral infection and host immune response across other human tissues (Fig.5B). Our results also indicate that the high confident genes targeted by these sponged miRs were enriched for functional themes including 'regulation of metabolic processes', 'post transcriptional gene regulation' and 'cell to cell communication' suggestive of a large-scale dysregulation across tissues (Fig.5C). Overall, our results present a comprehensive analysis of the miRs being potentially titrated on the viral genome resulting in altered post-transcriptional gene regulation. These findings provide important clues that enhances our understanding of miR associated mechanism in viral pathogenesis.

CONCLUSION

Our analysis integrates a comprehensive interaction network to map the immediate interactions between SARS-CoV-2 genome and proteome with human post-transcriptional regulators such as RBPs and miRNAs along with their tissue specific expression and functional annotations. Given the importance of developing effective therapeutic strategies in the current pandemic, understanding the effect of SARS-CoV-2 infection on human transcriptional and post-transcriptional regulatory networks is crucial for identifying effective drug targets. To delineate the impact of SARS-CoV-2 on host cells post-transcriptional gene regulatory network, we integrated the immediate interactions between SARS-CoV-2 encoded proteins with human RNA binding proteins. Our findings indicate 51 human RBPs interact directly with the viral structural and non-structural proteins, that in turn interact indirectly with ~65% other secondary neighbor RBPs. We show that the expression profiles of majority of the directly interacting RBPs were associated with gonadal tissues and immune cell types. Our study highlights that several of the differentially expressed genes in SARS-CoV-2 infected cells were enriched for biological

pathways such as immune response, cytokine mediated signaling, inflammatory response and metabolism associated genes that are indispensable for cell survival. Importantly, we found that among the differentially expressed genes, six genes encoded for RBPs implying a potential impact of SARS-CoV-2 infection on post-transcriptional regulation. Further, our analysis demonstrates the abundance of skipped exonic and mutually exclusive exonic events in the SARS-CoV-2 infected cells, suggesting these alternative splicing events as a plausible cause for altered post-transcriptional regulation in human cells. Using motif enrichment analysis, we show that two key post-transcriptional regulators, RBPs and miRs are likely to be titrated by SARS-CoV-2 genome that could result in dysregulated transcriptional regulation in the infected cells. Currently, there are no anti-viral therapies or vaccines available for COVID-19. Therefore, our analyses provide a roadmap to enhance our understanding of the interactions of SARS-CoV-2 with key pos-transcriptional regulators in the human genome.

MATERIALS AND METHODS

Dissection of SARS-CoV-2 proteins interacting with human RBPs

We obtained the high confidence mass spectrometry based SARS-CoV-2 viral protein to human protein interaction network established by Gordan et al, 2020 [23] in HEK293 cells. We dissected the human RBPs directly interacting with viral proteins and integrated with 1st neighbor interacting RBPs (obtained from BioGRID). We also extracted the protein abundance of these SARS-CoV2 interacting RBPs across human tissues from Human Proteome Map [39]. The abundance of these proteins were hierarchically clustered and row normalized and represented as heatmap generated from Morpheus (https://software.broadinstitute.org/morpheus/).

Differential expression analysis of mock treated versus SARS-CoV-2 infected primary human lung epithelial cells.

We downloaded the raw RNA sequencing data deposited in Gene Expression Omnibus (GEO)[47]. Specifically, we downloaded the paired end raw sequencing (FASTQ) files of mock treated and SARS-CoV-2 (USA-WA1/2020) infected primary human lung epithelial cells (in biological triplicates) using the Sequence Read Archive (SRA) Toolkit (fastq-dump command), from the GEO cohort GSE147507 [46]. The quality of the sequence reads were ensured using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) with a minimum of Phred quality score 20 for each sample. We processed the raw sequencing reads using the in-house NGS data processing pipeline as described previously[69, 70]. Briefly, we used Hierarchical Indexing for Spliced Alignment of Transcripts (HISAT) [71] for aligning the short reads from RNA-seq experiments onto human reference genome (hg38). SAM (Sequence Alignment/Map) files obtained from HISAT were post-processed using SAMtools (version 0.1.19) [72, 73] for converting SAM to BAM (Binary Alignment/Map) followed by sorting and indexing the output BAMs. The sorted BAM files were parsed using the python script provided by StringTie (version 1.2.1) [74] to obtain the count matrix of for gene levels across the samples. This count matrix was used to perform differential expression analysis between mock vs SARS-CoV-2 infected NHBE cells using DE-seq2 [75]. Statistically significant (at 5% fdr) differentially expressed genes were collected for

downstream data analysis. Functional enrichment analysis of these genes waere performed with p-value threshold < 10^{-10} using ClueGO [48] (a Cytoscape [76] plugin) and were represented as bar-plot illustrating the significant pathways obtained from GO-term based functional grouping of differentially expressed genes.

Identification of alternative splicing events during SARS-CoV2 infection.

We used rMATS (replicate Multivariate Analysis of Transcript Splicing) [60] to identify differential alternative splicing (AS) events between the mock vs SARS-CoV-2 treated NHBE cells. rMATS used sorted BAM (Binary Alignment/Map) files, obtained from aligning the fastq files against hg38 reference genome using HISAT (as discussed above). It also uses a GTF file (gene transfer file format), downloaded from Ensembl (version 97) [77] for existing annotation of exons. Briefly, rMATS enabled the analysis of the inclusion/exclusion of target exons/introns contributing to different types of alternative splicing events, namely skipped exon (SE), alternative 5' splice site (A5SS), alternative 3' splice site (A3SS), mutually exclusive exons (MXE) and retained intron (RI), between pair of conditions and provides the difference in level of inclusion of an exon denoted by Percentage Splicing Index ($\psi\ score$) (as described previously [69]). Genes exhibiting alternatively spliced events detected below 5% FDR threshold were documented in Table S3. Functional enrichment analysis of these genes was performed using ClueGO [48].

Identification of potential binding blocks of RBPs in SARS-CoV-2 viral genome using motif enrichment analysis.

We obtained the RBP-motifs from Attract[78] and scanned across the SARS-CoV-2 viral genome using FIMO[64, 79] with default parameters. Resulting genomic locations for each RBP motif were documented in Table S4. For each binding motif, the scanned genomic location was normalized by considering the mid-point of genomic coordinate divided with the SARS-CoV-2 genome length. Also, the occurrence of each RBP motif binding across the viral genome was computed. Statistically significant (p-value < e-05) preferential binding profile of RBP motifs (sorted by frequency of binding and greater than 10 sites) across the SARS-CoV-2 viral genome (length normalized) identified using FIMO was visualized in violin plot. Also, the protein abundance of corresponding RBPs were extracted from human proteome map[39] and represented as hierarchically clustered heatmap across the tissues.

Identification of potential binding blocks of microRNAs in SARS-CoV-2 viral genome using motif enrichment analysis.

We obtained the miR-motifs from MEME[79] and scanned across the SARS-CoV-2 viral genome using FIMO[64, 79] with default parameters. Resulting genomic locations for each miR-motif were documented in Table S6. For each miR motif, the scanned genomic location was normalized by considering the mid-point of genomic coordinate divided with the SARS-CoV-2 genome length. Also, the occurrence of each miR-motif binding across the viral genome was computed. Statistically significant (p-value < e-05) preferential binding profile of miR-motifs (sorted by frequency of binding and greater than 15 sites) across the SARS-CoV-2 viral genome (length normalized) identified using FIMO was visualized in violin plot. Also, the expression profile of corresponding miRs were extracted from FANTOM5 project[80] and represented as hierarchically

clustered heatmap across the tissues. To understand the generic biological function of these miRs, that could be altered by being titrated by SARS-CoV-2 genome in host cells, we downloaded the high confidence miR target genes (obtained from miRNet [81, 82]) and performed function annotation analysis. Resulting significant biological processes, obtained from gene ontology enrichment based functional grouping of these miR target genes were illustrated in barplot. Significant clustering (adj. $p < 1e\text{-}10$) of genes enriched in GO-biological processes were generated by ClueGO [48] analysis (a cytoscape [76] plugin).

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Authors' contribution

RS, MS and SCJ conceived and designed the study. RS processed the publicly available dataset and implemented the bioinformatic tools. RS and SVD integrated the supplementary dataset required for downstream data analysis. RS, MS and SCJ interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

Competing financial interest

The authors report no financial or other conflict of interest relevant to the subject of this article.

Figure Legends:

Figure 1. Protein-protein interaction network analysis suggest an immediate interaction of human RBPs with SARS-CoV2 viral proteins (A) An integrated SARS-CoV-2 – human RBP interaction network. We obtained the MS-based SARS-CoV-2 viral protein to human protein interaction network established in HEK293 cells and integrated with 1st neighbor interacting RBPs (obtained from BioGRID). (B) Protein abundance of SARS-CoV2 interacting RBPs across human tissues. Expression data were obtained from human protein map and row normalized. SARS-CoV-2 proteins were color coded and highlighted in the network.

Figure 2. Differential expression analysis of mock treated versus SARS-CoV-2 infected primary human lung epithelial cells. (A) Bar-plot illustrating the significant pathways obtained from GO-term based functional grouping of differentially expressed genes (at 5% fdr) using ClueGO analysis (Cytoscape plugin) (B) Row normalized expression profile of differentially expressed RBPs.

Figure 3. Alternative splicing events during SARS-CoV2 infection. (A) Bar plot showing the genes (RBP encoding genes in blue) exhibiting alternative splicing during SARS-CoV-2 infection in primary human lung epithelial cells (at 5% fdr). (B) Clustered GO-term network obtained from function annotation analysis and grouping of the GO-term for the genes exhibiting alternative splicing using ClueGo (cytoscape plugin). Significant clustering (adj. $p < 1e\text{-}05$) of functional groups were color coded by functional annotation of the enriched GO-biological processes, with size of the nodes indicating the level of significant association of genes per GO-term, were shown.

Figure 4. Motif enrichment analysis reveals potential human RBPs titrated by SARS-CoV-2 viral genome. (A) Violin plot shows the statistically significant (p-value < e-05) preferential binding profile of RBP motifs (sorted by frequency of binding and greater than 10 sites) across the SARS-CoV-2 viral genome (length normalized) identified using FIMO. (B) Hierarchically clustered heatmap showing the protein abundance (row normalized) of RBPs across tissues.

Figure 5. SARS-CoV-2 genome titrates the abundance of functionally important miRs in human tissue (A) Violin plot shows the statistically significant (p-value < e-05) preferential binding profile of miR- motifs (sorted by frequency of binding >15 sites) across the SARS-CoV-2 viral genome (length normalized) identified using FIMO. (B) Hierarchically clustered heatmap showing the $\log_{10}$ expression (CPM, row normalized) of miRs across tissues. (C) Bar-plot illustrating the significant biological processes, obtained from gene ontology enrichment based functional grouping of miR target genes (obtained from miRNet). Significant clustering (adj. $p < 1e\text{-}10$) of genes enriched in GO-biological processes generated by ClueGO analysis (cytoscape plugin).

REFERENCES

1. de Wit, E.; van Doremalen, N.; Falzarano, D.; Munster, V. J., SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol* **2016,** 14, (8), 523-34.
2. Meo, S. A.; Alhowikan, A. M.; Al-Khlaiwi, T.; Meo, I. M.; Halepoto, D. M.; Iqbal, M.; Usmani, A. M.; Hajjar, W.; Ahmed, N., Novel coronavirus 2019-nCoV: prevalence, biological and clinical characteristics comparison with SARS-CoV and MERS-CoV. *Eur Rev Med Pharmacol Sci* **2020,** 24, (4), 2012-2019.
3. Zaki, A. M.; van Boheemen, S.; Bestebroer, T. M.; Osterhaus, A. D.; Fouchier, R. A., Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* **2012,** 367, (19), 1814-20.
4. Cheng, V. C.; Lau, S. K.; Woo, P. C.; Yuen, K. Y., Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. *Clin Microbiol Rev* **2007,** 20, (4), 660-94.
5. Hajjar, S. A.; Memish, Z. A.; McIntosh, K., Middle East Respiratory Syndrome Coronavirus (MERS-CoV): a perpetual challenge. *Ann Saudi Med* **2013,** 33, (5), 427-36.
6. Bolles, M.; Donaldson, E.; Baric, R., SARS-CoV and emergent coronaviruses: viral determinants of interspecies transmission. *Curr Opin Virol* **2011,** 1, (6), 624-34.
7. Su, S.; Wong, G.; Shi, W.; Liu, J.; Lai, A. C. K.; Zhou, J.; Liu, W.; Bi, Y.; Gao, G. F., Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. *Trends Microbiol* **2016,** 24, (6), 490-502.
8. Menachery, V. D.; Graham, R. L.; Baric, R. S., Jumping species-a mechanism for coronavirus persistence and survival. *Curr Opin Virol* **2017,** 23, 1-7.
9. Zheng, J., SARS-CoV-2: an Emerging Coronavirus that Causes a Global Threat. *Int J Biol Sci* **2020,** 16, (10), 1678-1685.
10. Xu, J.; Zhao, S.; Teng, T.; Abdalla, A. E.; Zhu, W.; Xie, L.; Wang, Y.; Guo, X., Systematic Comparison of Two Animal-to-Human Transmitted Human Coronaviruses: SARS-CoV-2 and SARS-CoV. *Viruses* **2020,** 12, (2).
11. Gralinski, L. E.; Menachery, V. D., Return of the Coronavirus: 2019-nCoV. *Viruses* **2020,** 12, (2).
12. Wang, H.; Li, X.; Li, T.; Zhang, S.; Wang, L.; Wu, X.; Liu, J., The genetic sequence, origin, and diagnosis of SARS-CoV-2. *Eur J Clin Microbiol Infect Dis* **2020**.
13. He, J.; Tao, H.; Yan, Y.; Huang, S. Y.; Xiao, Y., Molecular Mechanism of Evolution and Human Infection with SARS-CoV-2. *Viruses* **2020,** 12, (4).
14. Trubsbach, A.; Dokert, B.; Schentke, K. U.; Jaross, W., [Lipid analysis of bile for determination of lithogeneity of bile and reproducibility of results with duodenal B bile]. *Z Med Lab Diagn* **1982,** 23, (1), 30-4.
15. Kang, S.; Peng, W.; Zhu, Y.; Lu, S.; Zhou, M.; Lin, W.; Wu, W.; Huang, S.; Jiang, L.; Luo, X.; Deng, M., Recent progress in understanding 2019 novel coronavirus (SARS-CoV-2) associated with human respiratory disease: detection, mechanisms and treatment. *Int J Antimicrob Agents* **2020**, 105950.
16. Malle, L., A map of SARS-CoV-2 and host cell interactions. *Nat Rev Immunol* **2020**.
17. Kim, D.; Lee, J. Y.; Yang, J. S.; Kim, J. W.; Kim, V. N.; Chang, H., The Architecture of SARS-CoV-2 Transcriptome. *Cell* **2020**.
18. Khailany, R. A.; Safdar, M.; Ozaslan, M., Genomic characterization of a novel SARS-CoV-2. *Gene Rep* **2020**, 100682.
19. Elfiky, A. A., SARS-CoV-2 RNA dependent RNA polymerase (RdRp) targeting: an in silico perspective. *J Biomol Struct Dyn* **2020**, 1-9.
20. Astuti, I.; Ysrafil, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. *Diabetes Metab Syndr* **2020,** 14, (4), 407-412.

21. Guzzi, P. H.; Mercatelli, D.; Ceraolo, C.; Giorgi, F. M., Master Regulator Analysis of the SARS-CoV-2/Human Interactome. *J Clin Med* **2020,** 9, (4).
22. Shereen, M. A.; Khan, S.; Kazmi, A.; Bashir, N.; Siddique, R., COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. *J Adv Res* **2020,** 24, 91-98.
23. Gordon, D. E.; Jang, G. M.; Bouhaddou, M.; Xu, J.; Obernier, K.; White, K. M.; O'Meara, M. J.; Rezelj, V. V.; Guo, J. Z.; Swaney, D. L.; Tummino, T. A.; Huettenhain, R.; Kaake, R. M.; Richards, A. L.; Tutuncuoglu, B.; Foussard, H.; Batra, J.; Haas, K.; Modak, M.; Kim, M.; Haas, P.; Polacco, B. J.; Braberg, H.; Fabius, J. M.; Eckhardt, M.; Soucheray, M.; Bennett, M. J.; Cakir, M.; McGregor, M. J.; Li, Q.; Meyer, B.; Roesch, F.; Vallet, T.; Mac Kain, A.; Miorin, L.; Moreno, E.; Naing, Z. Z. C.; Zhou, Y.; Peng, S.; Shi, Y.; Zhang, Z.; Shen, W.; Kirby, I. T.; Melnyk, J. E.; Chorba, J. S.; Lou, K.; Dai, S. A.; Barrio-Hernandez, I.; Memon, D.; Hernandez-Armenta, C.; Lyu, J.; Mathy, C. J. P.; Perica, T.; Pilla, K. B.; Ganesan, S. J.; Saltzberg, D. J.; Rakesh, R.; Liu, X.; Rosenthal, S. B.; Calviello, L.; Venkataramanan, S.; Liboy-Lugo, J.; Lin, Y.; Huang, X. P.; Liu, Y.; Wankowicz, S. A.; Bohn, M.; Safari, M.; Ugur, F. S.; Koh, C.; Savar, N. S.; Tran, Q. D.; Shengjuler, D.; Fletcher, S. J.; O'Neal, M. C.; Cai, Y.; Chang, J. C. J.; Broadhurst, D. J.; Klippsten, S.; Sharp, P. P.; Wenzell, N. A.; Kuzuoglu, D.; Wang, H. Y.; Trenker, R.; Young, J. M.; Cavero, D. A.; Hiatt, J.; Roth, T. L.; Rathore, U.; Subramanian, A.; Noack, J.; Hubert, M.; Stroud, R. M.; Frankel, A. D.; Rosenberg, O. S.; Verba, K. A.; Agard, D. A.; Ott, M.; Emerman, M.; Jura, N.; von Zastrow, M.; Verdin, E.; Ashworth, A.; Schwartz, O.; d'Enfert, C.; Mukherjee, S.; Jacobson, M.; Malik, H. S.; Fujimori, D. G.; Ideker, T.; Craik, C. S.; Floor, S. N.; Fraser, J. S.; Gross, J. D.; Sali, A.; Roth, B. L.; Ruggero, D.; Taunton, J.; Kortemme, T.; Beltrao, P.; Vignuzzi, M.; Garcia-Sastre, A.; Shokat, K. M.; Shoichet, B. K.; Krogan, N. J., A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature* **2020**.
24. Glisovic, T.; Bachorik, J. L.; Yong, J.; Dreyfuss, G., RNA-binding proteins and post-transcriptional gene regulation. *FEBS Lett* **2008,** 582, (14), 1977-86.
25. Zanzoni, A.; Spinelli, L.; Ribeiro, D. M.; Tartaglia, G. G.; Brun, C., Post-transcriptional regulatory patterns revealed by protein-RNA interactions. *Sci Rep* **2019,** 9, (1), 4302.
26. Chen, P. Y.; Meister, G., microRNA-guided posttranscriptional gene regulation. *Biol Chem* **2005,** 386, (12), 1205-18.
27. Brinegar, A. E.; Cooper, T. A., Roles for RNA-binding proteins in development and disease. *Brain Res* **2016,** 1647, 1-8.
28. Lukong, K. E.; Chang, K. W.; Khandjian, E. W.; Richard, S., RNA-binding proteins in human genetic disease. *Trends Genet* **2008,** 24, (8), 416-25.
29. Li, Z.; Nagy, P. D., Diverse roles of host RNA binding proteins in RNA virus replication. *RNA Biol* **2011,** 8, (2), 305-15.
30. Garcia-Moreno, M.; Noerenberg, M.; Ni, S.; Jarvelin, A. I.; Gonzalez-Almela, E.; Lenz, C. E.; Bach-Pages, M.; Cox, V.; Avolio, R.; Davis, T.; Hester, S.; Sohier, T. J. M.; Li, B.; Heikel, G.; Michlewski, G.; Sanz, M. A.; Carrasco, L.; Ricci, E. P.; Pelechano, V.; Davis, I.; Fischer, B.; Mohammed, S.; Castello, A., System-wide Profiling of RNA-Binding Proteins Uncovers Key Regulators of Virus Infection. *Mol Cell* **2019,** 74, (1), 196-211 e11.
31. Ardekani, A. M.; Naeini, M. M., The Role of MicroRNAs in Human Diseases. *Avicenna J Med Biotechnol* **2010,** 2, (4), 161-79.
32. Drury, R. E.; O'Connor, D.; Pollard, A. J., The Clinical Application of MicroRNAs in Infectious Disease. *Front Immunol* **2017,** 8, 1182.
33. Girardi, E.; Lopez, P.; Pfeffer, S., On the Importance of Host MicroRNAs During Viral Infection. *Front Genet* **2018,** 9, 439.
34. Wu, C.; Liu, Y.; Yang, Y.; Zhang, P.; Zhong, W.; Wang, Y.; Wang, Q.; Xu, Y.; Li, M.; Li, X.; Zheng, M.; Chen, L.; Li, H., Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharm Sin B* **2020**.

35. Gurwitz, D., Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics. *Drug Dev Res* **2020**.
36. Nyathi, Y.; Wilkinson, B. M.; Pool, M. R., Co-translational targeting and translocation of proteins to the endoplasmic reticulum. *Biochimica et biophysica acta* **2013,** 1833, (11), 2392-402.
37. Saraogi, I.; Shan, S. O., Molecular mechanism of co-translational protein targeting by the signal recognition particle. *Traffic* **2011,** 12, (5), 535-42.
38. Hashemikhabir, S.; Neelamraju, Y.; Janga, S. C., Database of RNA binding protein expression and disease dynamics (READ DB). *Database : the journal of biological databases and curation* **2015,** 2015, bav072.
39. Kim, M. S.; Pinto, S. M.; Getnet, D.; Nirujogi, R. S.; Manda, S. S.; Chaerkady, R.; Madugundu, A. K.; Kelkar, D. S.; Isserlin, R.; Jain, S.; Thomas, J. K.; Muthusamy, B.; Leal-Rojas, P.; Kumar, P.; Sahasrabuddhe, N. A.; Balakrishnan, L.; Advani, J.; George, B.; Renuse, S.; Selvan, L. D.; Patil, A. H.; Nanjappa, V.; Radhakrishnan, A.; Prasad, S.; Subbannayya, T.; Raju, R.; Kumar, M.; Sreenivasamurthy, S. K.; Marimuthu, A.; Sathe, G. J.; Chavan, S.; Datta, K. K.; Subbannayya, Y.; Sahu, A.; Yelamanchi, S. D.; Jayaram, S.; Rajagopalan, P.; Sharma, J.; Murthy, K. R.; Syed, N.; Goel, R.; Khan, A. A.; Ahmad, S.; Dey, G.; Mudgal, K.; Chatterjee, A.; Huang, T. C.; Zhong, J.; Wu, X.; Shaw, P. G.; Freed, D.; Zahari, M. S.; Mukherjee, K. K.; Shankar, S.; Mahadevan, A.; Lam, H.; Mitchell, C. J.; Shankar, S. K.; Satishchandra, P.; Schroeder, J. T.; Sirdeshmukh, R.; Maitra, A.; Leach, S. D.; Drake, C. G.; Halushka, M. K.; Prasad, T. S.; Hruban, R. H.; Kerr, C. L.; Bader, G. D.; Iacobuzio-Donahue, C. A.; Gowda, H.; Pandey, A., A draft map of the human proteome. *Nature* **2014,** 509, (7502), 575-81.
40. Wang, S.; Zhou, X.; Zhang, T.; Wang, Z., The need for urogenital tract monitoring in COVID-19. *Nat Rev Urol* **2020**.
41. Ling Ma, W. X., Danyang Li, Lei Shi, Yanhong Mao, Yao Xiong, Yuanzhen Zhang, Ming Zhang, Effect of SARS-CoV-2 infection upon male gonadal function: A single center-based study. *medRxiv* **2020**.
42. Li, G.; Fan, Y.; Lai, Y.; Han, T.; Li, Z.; Zhou, P.; Pan, P.; Wang, W.; Hu, D.; Liu, X.; Zhang, Q.; Wu, J., Coronavirus infections and immune responses. *J Med Virol* **2020,** 92, (4), 424-432.
43. Julian Braun, L. L., Marco Frentsch, Daniel Wendisch, Philipp Georg, Florian Kurth, Stefan Hippenstiel, Manuela Dingeldey, Beate Kruse, Florent Fauchere, Emre Baysal, Maike Mangold, Larissa Henze, Roland Lauster, Marcus Mall, Kirsten Beyer, Jobst Roehmel, Juergen Schmitz, Stefan Miltenyi, Marcel A Mueller, Martin Witzenrath, Norbert Suttorp, Florian Kern, Ulf Reimer, Holger Wenschuh, Christian Drosten, Victor M Corman, Claudia Giesecke-Thiel, Leif-Erik Sander, Andreas Thiel, Presence of SARS-CoV-2 reactive T cells in COVID-19 patients and healthy donors. *medRxiv* **2020**.
44. Wang, X.; Xu, W.; Hu, G.; Xia, S.; Sun, Z.; Liu, Z.; Xie, Y.; Zhang, R.; Jiang, S.; Lu, L., SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion. *Cell Mol Immunol* **2020**.
45. Zheng, M.; Gao, Y.; Wang, G.; Song, G.; Liu, S.; Sun, D.; Xu, Y.; Tian, Z., Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell Mol Immunol* **2020,** 17, (5), 533-535.
46. Daniel Blanco-Melo, B. E. N.-P., Wen-Chun Liu, Rasmus Møller, Maryline Panis, David Sachs, Randy A. Albrecht, Benjamin R. tenOever, SARS-CoV-2 launches a unique transcriptional signature from in vitro, ex vivo, and in vivo systems. *bioRxiv* **2020**.
47. Barrett, T.; Wilhite, S. E.; Ledoux, P.; Evangelista, C.; Kim, I. F.; Tomashevsky, M.; Marshall, K. A.; Phillippy, K. H.; Sherman, P. M.; Holko, M.; Yefanov, A.; Lee, H.; Zhang, N.; Robertson, C. L.; Serova, N.; Davis, S.; Soboleva, A., NCBI GEO: archive for functional genomics data sets--update. *Nucleic Acids Res* **2013,** 41, (Database issue), D991-5.

48. Bindea, G.; Mlecnik, B.; Hackl, H.; Charoentong, P.; Tosolini, M.; Kirilovsky, A.; Fridman, W. H.; Pages, F.; Trajanoski, Z.; Galon, J., ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* **2009,** 25, (8), 1091-3.
49. Xu, Z.; Shi, L.; Wang, Y.; Zhang, J.; Huang, L.; Zhang, C.; Liu, S.; Zhao, P.; Liu, H.; Zhu, L.; Tai, Y.; Bai, C.; Gao, T.; Song, J.; Xia, P.; Dong, J.; Zhao, J.; Wang, F. S., Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* **2020,** 8, (4), 420-422.
50. Zhang, C.; Wu, Z.; Li, J. W.; Zhao, H.; Wang, G. Q., The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. *Int J Antimicrob Agents* **2020**, 105954.
51. Wu, D.; Yang, X. O., TH17 responses in cytokine storm of COVID-19: An emerging target of JAK2 inhibitor Fedratinib. *J Microbiol Immunol Infect* **2020**.
52. Kudva, A.; Scheller, E. V.; Robinson, K. M.; Crowe, C. R.; Choi, S. M.; Slight, S. R.; Khader, S. A.; Dubin, P. J.; Enelow, R. I.; Kolls, J. K.; Alcorn, J. F., Influenza A inhibits Th17-mediated host defense against bacterial pneumonia in mice. *J Immunol* **2011,** 186, (3), 1666-1674.
53. Pacha, O.; Sallman, M. A.; Evans, S. E., COVID-19: a case for inhibiting IL-17? *Nat Rev Immunol* **2020**.
54. Chauhan, K.; Kalam, H.; Dutt, R.; Kumar, D., RNA Splicing: A New Paradigm in Host-Pathogen Interactions. *Journal of molecular biology* **2019,** 431, (8), 1565-1575.
55. Ashraf, U.; Benoit-Pilven, C.; Lacroix, V.; Navratil, V.; Naffakh, N., Advances in Analyzing Virus-Induced Alterations of Host Cell Splicing. *Trends Microbiol* **2019,** 27, (3), 268-281.
56. Boudreault, S.; Roy, P.; Lemay, G.; Bisaillon, M., Viral modulation of cellular RNA alternative splicing: A new key player in virus-host interactions? *Wiley Interdiscip Rev RNA* **2019,** 10, (5), e1543.
57. Denisa Bojkova, K. K., Benjamin Koch, Marek Widera, David Krause, Sandra Ciesek, Jindrich Cinatl, Christian Münch, SARS-CoV-2 infected host cell proteomics reveal potential therapy targets. **2020**.
58. Lim, Y. X.; Ng, Y. L.; Tam, J. P.; Liu, D. X., Human Coronaviruses: A Review of Virus-Host Interactions. *Diseases* **2016,** 4, (3).
59. DeDiego, M. L.; Nieto-Torres, J. L.; Jimenez-Guardeno, J. M.; Regla-Nava, J. A.; Alvarez, E.; Oliveros, J. C.; Zhao, J.; Fett, C.; Perlman, S.; Enjuanes, L., Severe acute respiratory syndrome coronavirus envelope protein regulates cell stress response and apoptosis. *PLoS Pathog* **2011,** 7, (10), e1002315.
60. Shen, S.; Park, J. W.; Lu, Z. X.; Lin, L.; Henry, M. D.; Wu, Y. N.; Zhou, Q.; Xing, Y., rMATS: robust and flexible detection of differential alternative splicing from replicate RNA-Seq data. *Proceedings of the National Academy of Sciences of the United States of America* **2014,** 111, (51), E5593-601.
61. Forster, P.; Forster, L.; Renfrew, C.; Forster, M., Phylogenetic network analysis of SARS-CoV-2 genomes. *Proceedings of the National Academy of Sciences of the United States of America* **2020,** 117, (17), 9241-9243.
62. Zhu, J.; Gopinath, K.; Murali, A.; Yi, G.; Hayward, S. D.; Zhu, H.; Kao, C., RNA-binding proteins that inhibit RNA virus infection. *Proceedings of the National Academy of Sciences of the United States of America* **2007,** 104, (9), 3129-34.
63. Garcia-Moreno, M.; Jarvelin, A. I.; Castello, A., Unconventional RNA-binding proteins step into the virus-host battlefront. *Wiley Interdiscip Rev RNA* **2018,** 9, (6), e1498.
64. Grant, C. E.; Bailey, T. L.; Noble, W. S., FIMO: scanning for occurrences of a given motif. *Bioinformatics* **2011,** 27, (7), 1017-8.
65. Bartas, M.; Brázda, V.; Bohálová, N.; Cantara, A.; Volná, A.; Stachurová, T.; Malachová, K.; Jagelská, E. B.; Porubiaková, O.; Červeň, J.; Pečinka, P., In-Depth Bioinformatic Analyses of

Nidovirales Including Human SARS-CoV-2, SARS-CoV, MERS-CoV Viruses Suggest Important Roles of Non-canonical Nucleic Acid Structures in Their Lifecycles. *Frontiers in Microbiology* **2020,** 11, (1583).
66. Bruscella, P.; Bottini, S.; Baudesson, C.; Pawlotsky, J. M.; Feray, C.; Trabucchi, M., Viruses and miRNAs: More Friends than Foes. *Front Microbiol* **2017,** 8, 824.
67. Roberts, A. P.; Lewis, A. P.; Jopling, C. L., The role of microRNAs in viral infection. *Prog Mol Biol Transl Sci* **2011,** 102, 101-39.
68. Müşerref Duygu Saçar Demirci, A. A., Computational analysis of microRNA-mediated interactions in SARS-CoV-2 infection. *medRxiv* **2020**.
69. Srivastava, R.; Budak, G.; Dash, S.; Lachke, S. A.; Janga, S. C., Transcriptome analysis of developing lens reveals abundance of novel transcripts and extensive splicing alterations. *Sci Rep* **2017,** 7, (1), 11572.
70. Budak, G.; Dash, S.; Srivastava, R.; Lachke, S. A.; Janga, S. C., Express: A database of transcriptome profiles encompassing known and novel transcripts across multiple development stages in eye tissues. *Experimental eye research* **2018,** 168, 57-68.
71. Kim, D.; Langmead, B.; Salzberg, S. L., HISAT: a fast spliced aligner with low memory requirements. *Nature methods* **2015,** 12, (4), 357-60.
72. Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R.; Genome Project Data Processing, S., The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **2009,** 25, (16), 2078-9.
73. Wheeler, D. L.; Barrett, T.; Benson, D. A.; Bryant, S. H.; Canese, K.; Chetvernin, V.; Church, D. M.; Dicuccio, M.; Edgar, R.; Federhen, S.; Feolo, M.; Geer, L. Y.; Helmberg, W.; Kapustin, Y.; Khovayko, O.; Landsman, D.; Lipman, D. J.; Madden, T. L.; Maglott, D. R.; Miller, V.; Ostell, J.; Pruitt, K. D.; Schuler, G. D.; Shumway, M.; Sequeira, E.; Sherry, S. T.; Sirotkin, K.; Souvorov, A.; Starchenko, G.; Tatusov, R. L.; Tatusova, T. A.; Wagner, L.; Yaschenko, E., Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* **2008,** 36, (Database issue), D13-21.
74. Pertea, M.; Pertea, G. M.; Antonescu, C. M.; Chang, T. C.; Mendell, J. T.; Salzberg, S. L., StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol* **2015,** 33, (3), 290-5.
75. Love, M. I.; Huber, W.; Anders, S., Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology* **2014,** 15, (12), 550.
76. Smoot, M. E.; Ono, K.; Ruscheinski, J.; Wang, P. L.; Ideker, T., Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics* **2011,** 27, (3), 431-2.
77. Cunningham, F.; Amode, M. R.; Barrell, D.; Beal, K.; Billis, K.; Brent, S.; Carvalho-Silva, D.; Clapham, P.; Coates, G.; Fitzgerald, S.; Gil, L.; Giron, C. G.; Gordon, L.; Hourlier, T.; Hunt, S. E.; Janacek, S. H.; Johnson, N.; Juettemann, T.; Kahari, A. K.; Keenan, S.; Martin, F. J.; Maurel, T.; McLaren, W.; Murphy, D. N.; Nag, R.; Overduin, B.; Parker, A.; Patricio, M.; Perry, E.; Pignatelli, M.; Riat, H. S.; Sheppard, D.; Taylor, K.; Thormann, A.; Vullo, A.; Wilder, S. P.; Zadissa, A.; Aken, B. L.; Birney, E.; Harrow, J.; Kinsella, R.; Muffato, M.; Ruffier, M.; Searle, S. M.; Spudich, G.; Trevanion, S. J.; Yates, A.; Zerbino, D. R.; Flicek, P., Ensembl 2015. *Nucleic Acids Res* **2015,** 43, (Database issue), D662-9.
78. Giudice, G.; Sanchez-Cabo, F.; Torroja, C.; Lara-Pezzi, E., ATtRACT-a database of RNA-binding proteins and associated motifs. *Database : the journal of biological databases and curation* **2016,** 2016.
79. Bailey, T. L.; Johnson, J.; Grant, C. E.; Noble, W. S., The MEME Suite. *Nucleic Acids Res* **2015,** 43, (W1), W39-49.

80. de Rie, D.; Abugessaisa, I.; Alam, T.; Arner, E.; Arner, P.; Ashoor, H.; Astrom, G.; Babina, M.; Bertin, N.; Burroughs, A. M.; Carlisle, A. J.; Daub, C. O.; Detmar, M.; Deviatiiarov, R.; Fort, A.; Gebhard, C.; Goldowitz, D.; Guhl, S.; Ha, T. J.; Harshbarger, J.; Hasegawa, A.; Hashimoto, K.; Herlyn, M.; Heutink, P.; Hitchens, K. J.; Hon, C. C.; Huang, E.; Ishizu, Y.; Kai, C.; Kasukawa, T.; Klinken, P.; Lassmann, T.; Lecellier, C. H.; Lee, W.; Lizio, M.; Makeev, V.; Mathelier, A.; Medvedeva, Y. A.; Mejhert, N.; Mungall, C. J.; Noma, S.; Ohshima, M.; Okada-Hatakeyama, M.; Persson, H.; Rizzu, P.; Roudnicky, F.; Saetrom, P.; Sato, H.; Severin, J.; Shin, J. W.; Swoboda, R. K.; Tarui, H.; Toyoda, H.; Vitting-Seerup, K.; Winteringham, L.; Yamaguchi, Y.; Yasuzawa, K.; Yoneda, M.; Yumoto, N.; Zabierowski, S.; Zhang, P. G.; Wells, C. A.; Summers, K. M.; Kawaji, H.; Sandelin, A.; Rehli, M.; Consortium, F.; Hayashizaki, Y.; Carninci, P.; Forrest, A. R. R.; de Hoon, M. J. L., An integrated expression atlas of miRNAs and their promoters in human and mouse. *Nat Biotechnol* **2017,** 35, (9), 872-878.
81. Fan, Y.; Siklenka, K.; Arora, S. K.; Ribeiro, P.; Kimmins, S.; Xia, J., miRNet - dissecting miRNA-target interactions and functional associations through network-based visual analysis. *Nucleic Acids Res* **2016,** 44, (W1), W135-41.
82. Fan, Y.; Xia, J., miRNet-Functional Analysis and Visual Exploration of miRNA-Target Interactions in a Network Context. *Methods Mol Biol* **2018,** 1819, 215-233.

SARS-CoV-2 contributes to altering the post-transcriptional regulatory networks across human tissues by sponging RNA binding proteins and micro-RNAs

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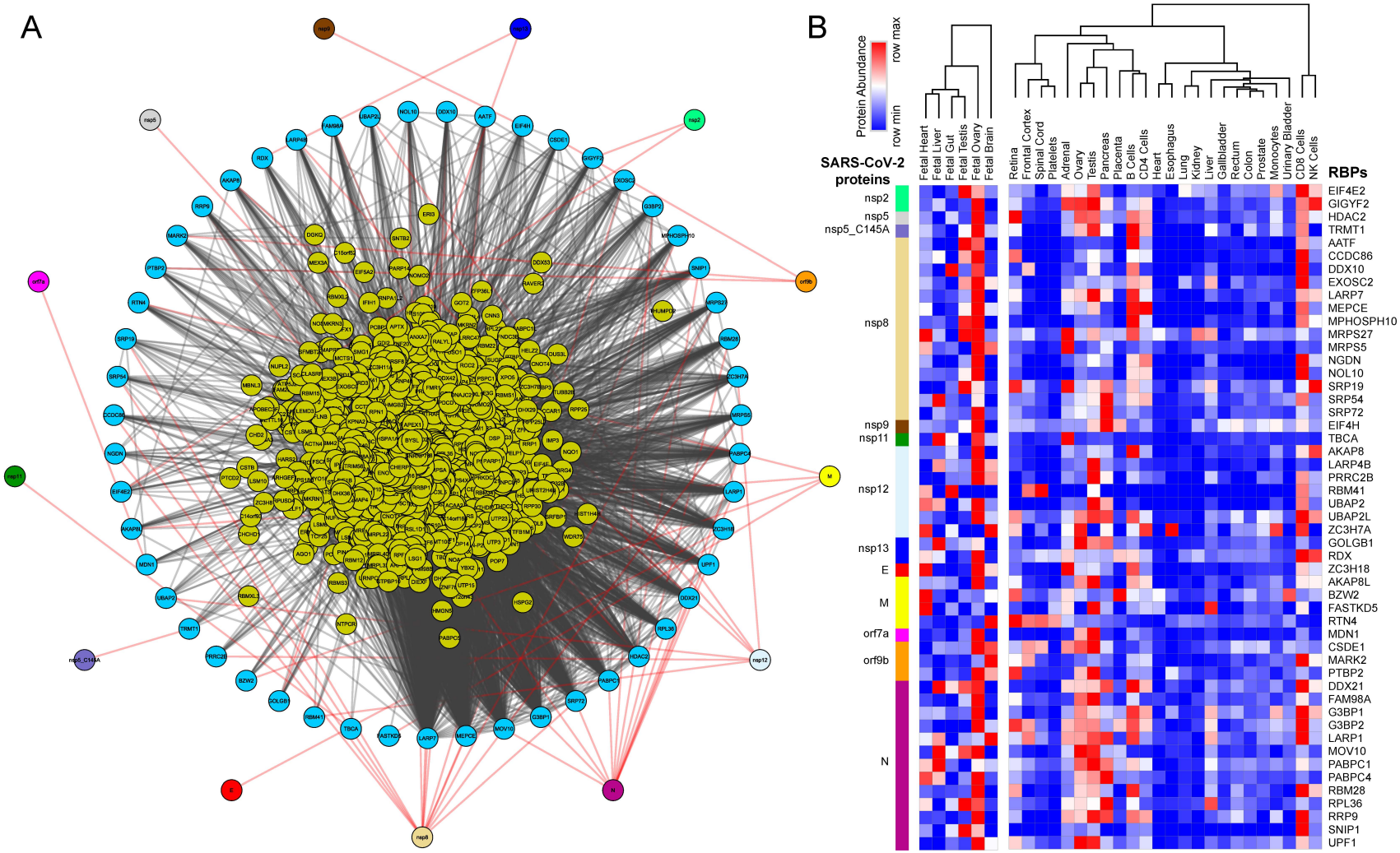

Figure 1. Protein-protein interaction network analysis suggest an immediate interaction of human RBPs with SARS-CoV2 viral proteins (A) An integrated SARS-CoV-2 – human RBP interaction network. We obtained the MS-based SARS-CoV-2 viral protein to human protein interaction network established in HEK293 cells and integrated with 1st neighbor interacting RBPs (obtained from BioGRID). (B) Protein abundance of SARS-CoV2 interacting RBPs across human tissues. Expression data were obtained from human protein map and row normalized. SARS-CoV-2 proteins were color coded and highlighted in the network.

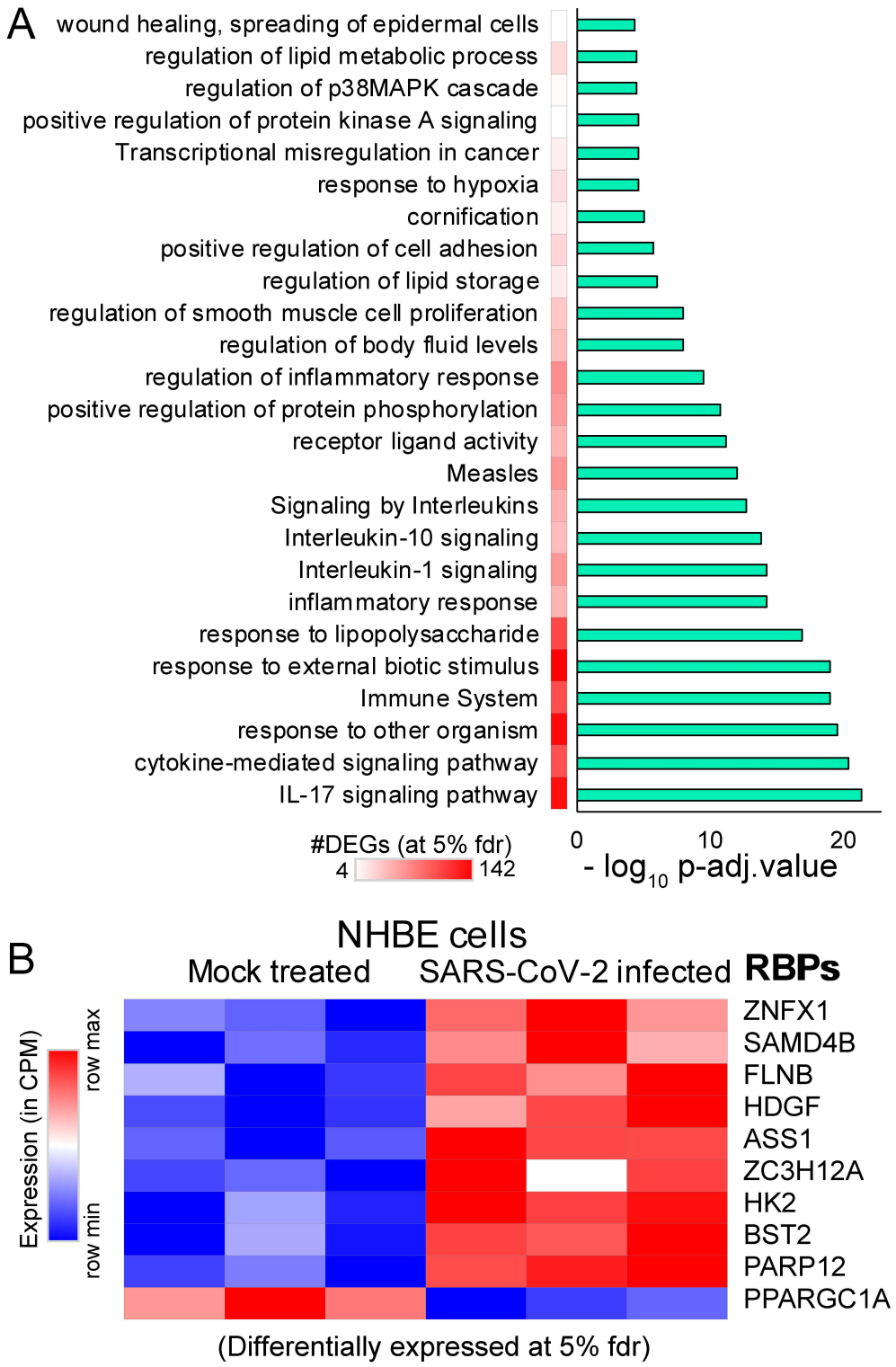

Figure 2. Differential expression analysis of mock treated versus SARS-CoV-2 infected primary human lung epithelial cells. (A) Bar-plot illustrating the significant pathways obtained from GO-term based functional grouping of differentially expressed genes (at 5% fdr) using ClueGO analysis (Cytoscape plugin) (B) Row normalized expression profile of differentially expressed RBPs.

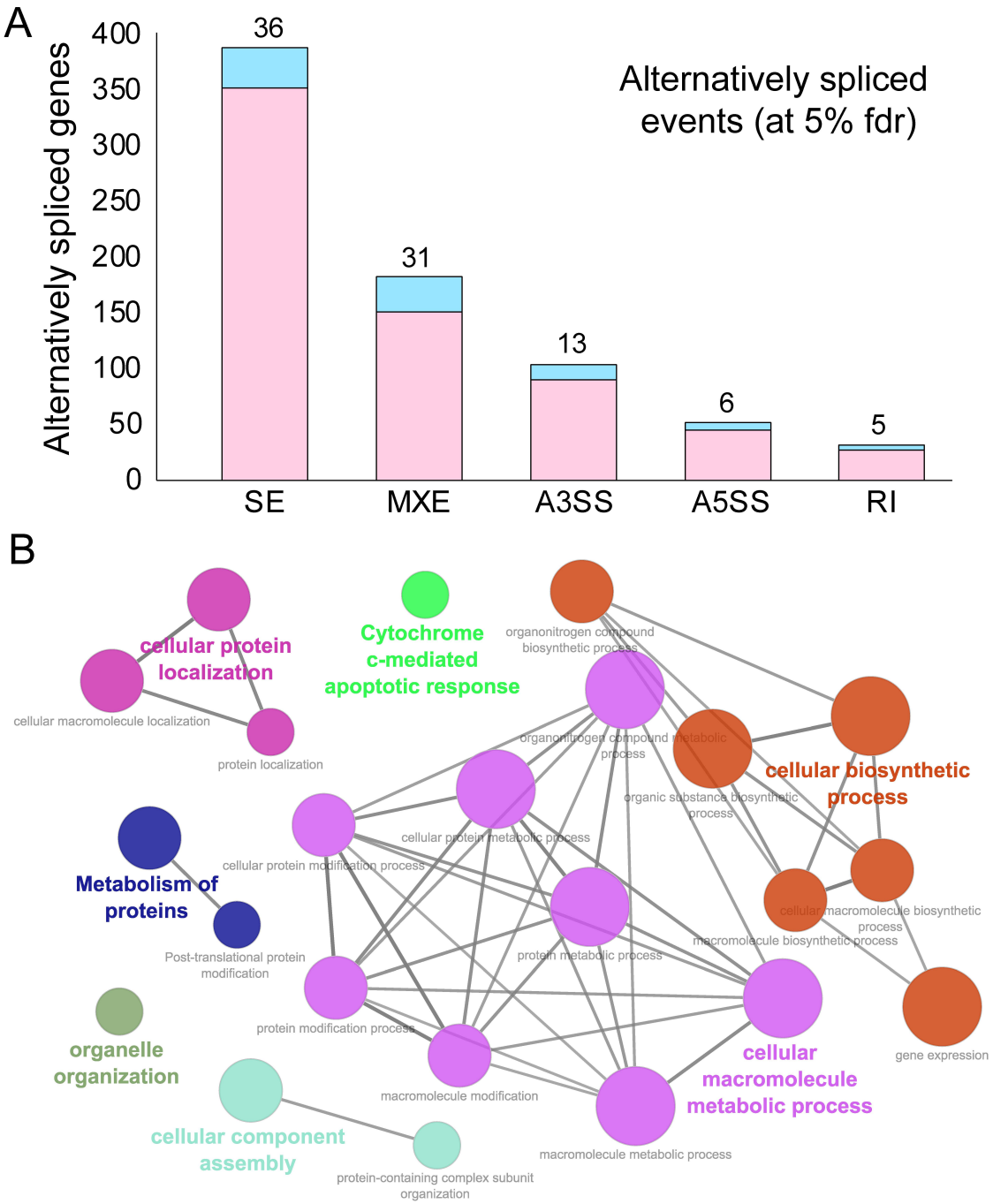

Figure 3. Alternative splicing events during SARS-CoV2 infection. (A) Bar plot showing the genes (RBP encoding genes in blue) exhibiting alternative splicing during SARS-CoV-2 infection in primary human lung epithelial cells (at 5% fdr). (B) Clustered GO-term network obtained from function annotation analysis and grouping of the GO-term for the genes exhibiting alternative splicing using ClueGo (cytoscape plugin). Significant clustering (adj. $p < 1e\text{-}05$) of functional groups were color coded by functional annotation of the enriched GO-biological processes, with size of the nodes indicating the level of significant association of genes per GO-term, were shown.

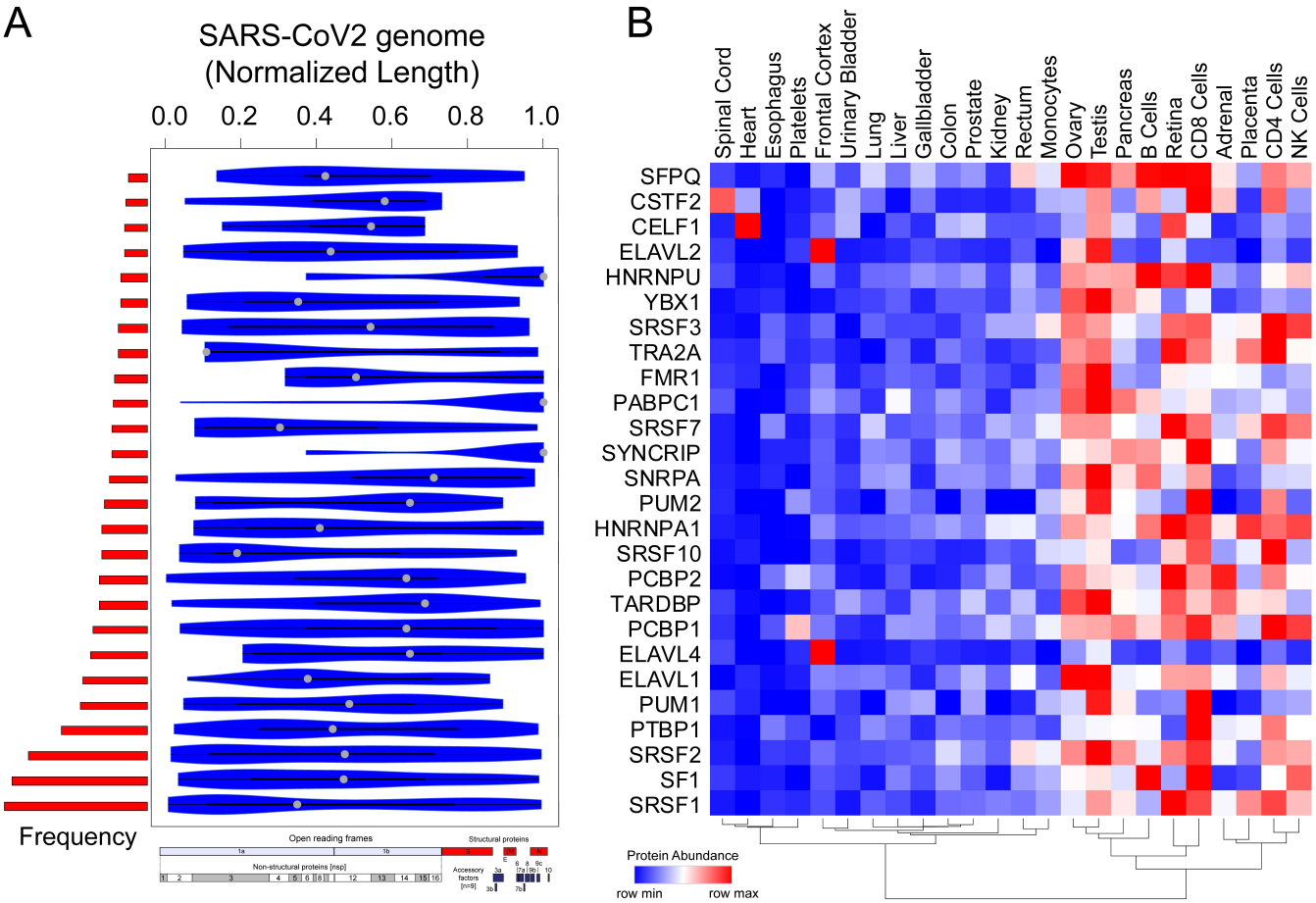

Figure 4. Motif enrichment analysis reveals potential human RBPs titrated by SARS-CoV-2 viral genome. (A) Violin plot shows the statistically significant (p-value < e-05) preferential binding profile of RBP motifs (sorted by frequency of binding and greater than 10 sites) across the SARS-CoV-2 viral genome (length normalized) identified using FIMO. (B) Hierarchically clustered heatmap showing the protein abundance (row normalized) of RBPs across tissues.

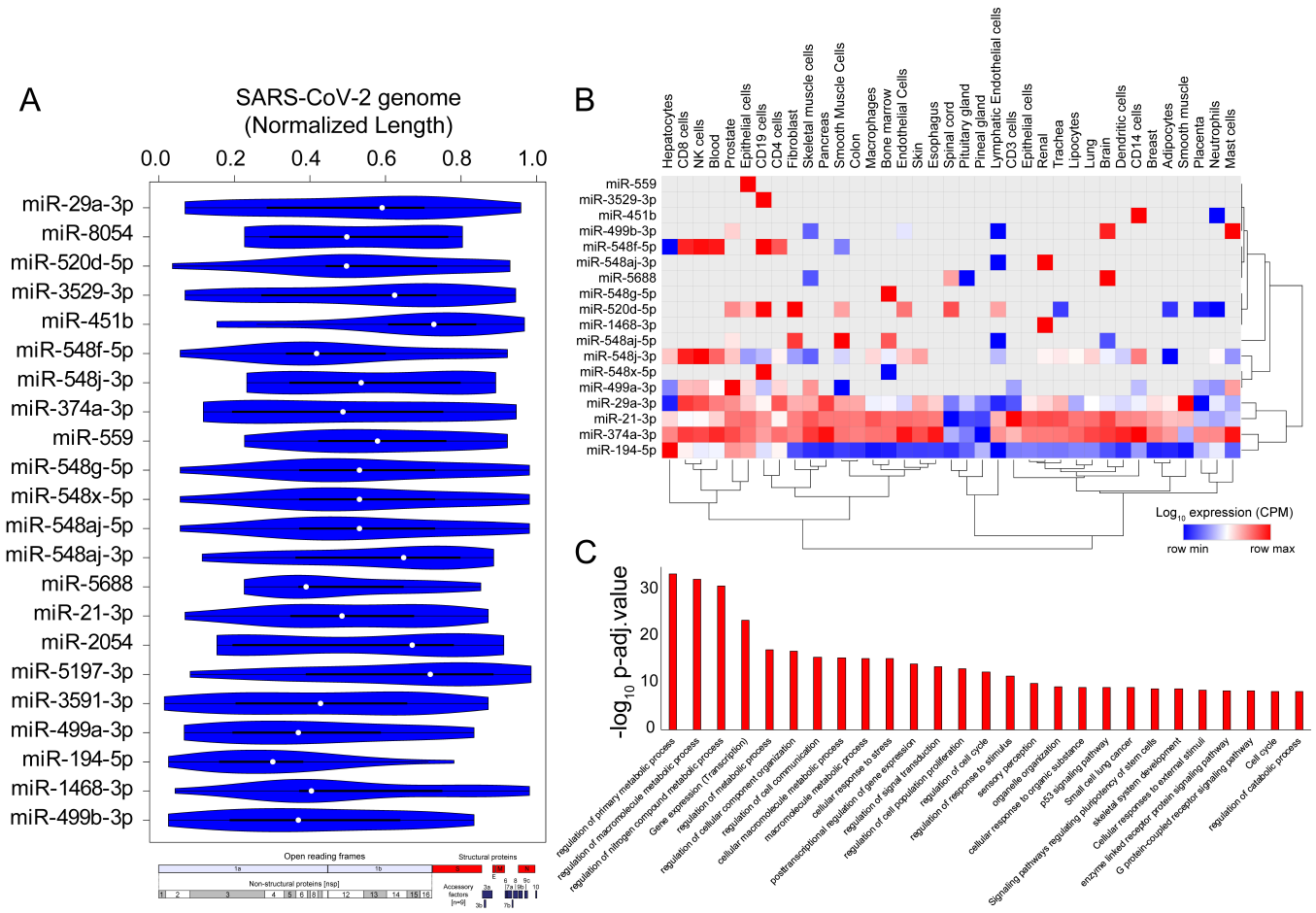

Figure 5. SARS-CoV-2 genome titrates the abundance of functionally important miRs in human tissue (A) Violin plot shows the statistically significant (p-value < e-05) preferential binding profile of miR-motifs (sorted by frequency of binding >15 sites) across the SARS-CoV-2 viral genome (length normalized) identified using FIMO. (B) Hierarchically clustered heatmap showing the log10 expression (CPM, row normalized) of miRs across tissues. (C) Bar-plot illustrating the significant biological processes, obtained from gene ontology enrichment based functional grouping of miR target genes (obtained from miRNet). Significant clustering (adj. $p < 1e\text{-}10$) of genes enriched in GO-biological processes generated by ClueGO analysis (Cytoscape plugin).

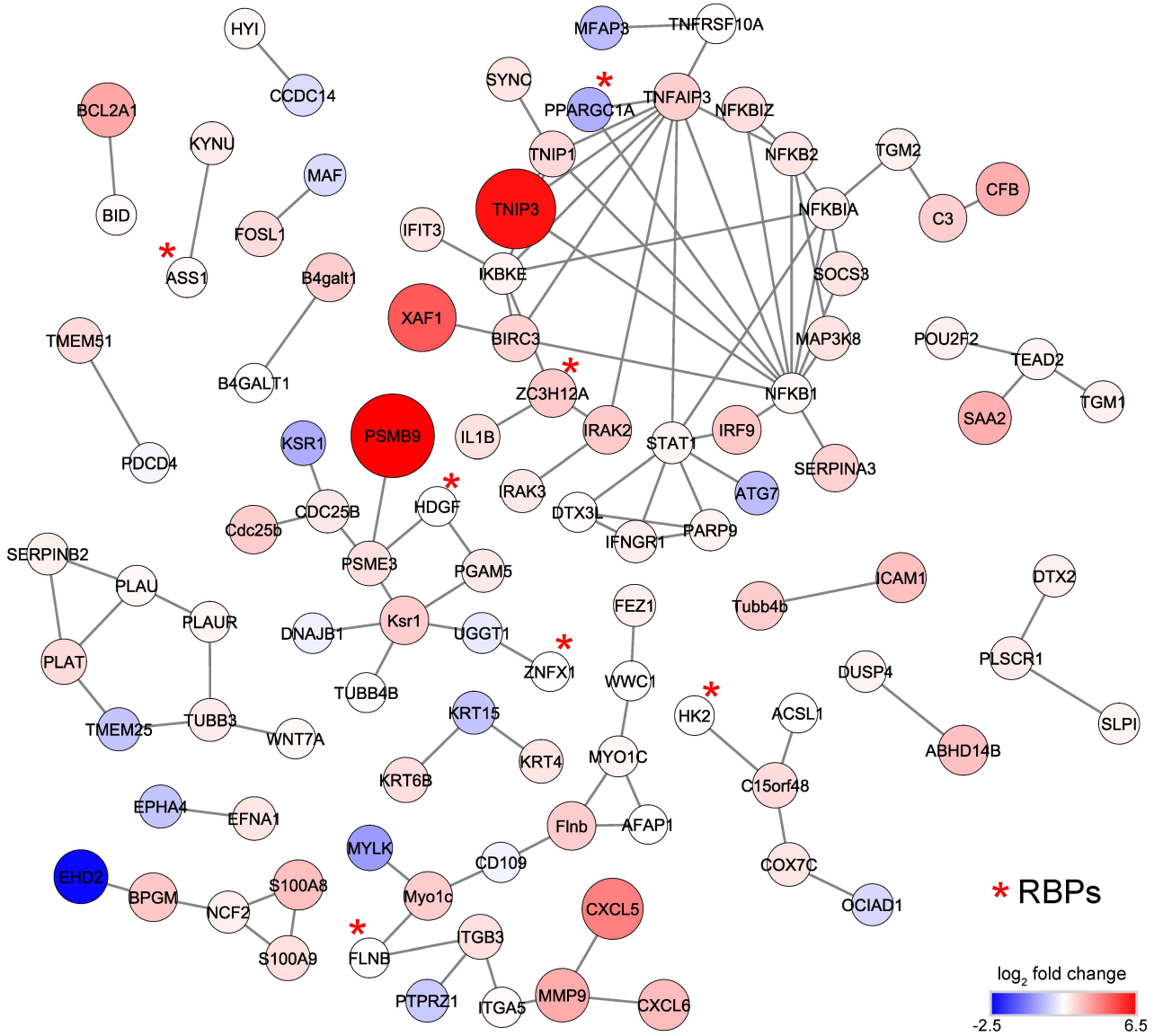

Figure S1. Protein-protein interaction network of differentially expressed genes in mock treated versus SARS-CoV-2 infected primary human lung epithelium (NHBE cells) where node size and color show the absolute and relative log2 fold changes, respectively.

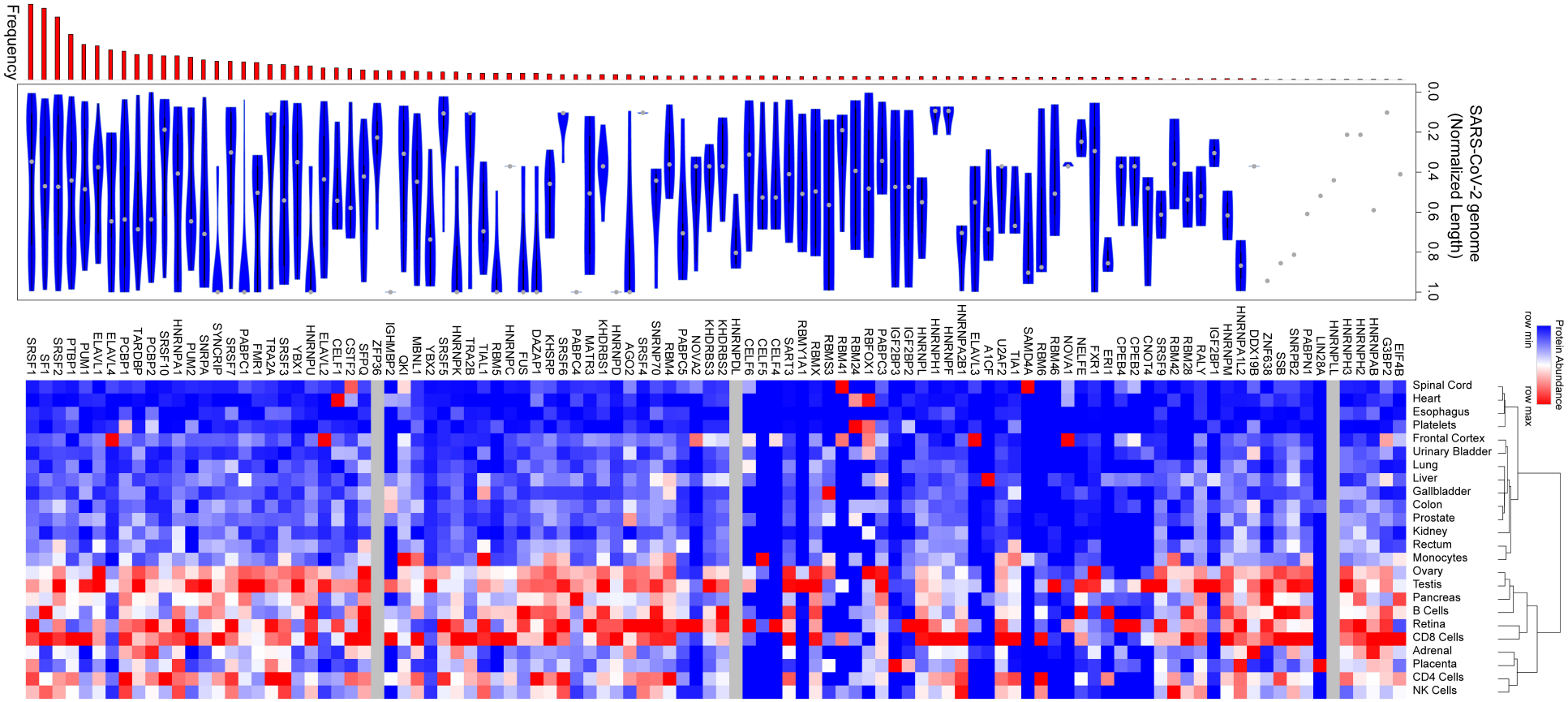

Figure S2. (A) Violin plot shows the statistically significant (p-value < e-05) preferential binding profile of RBP motifs (sorted by frequency of binding) across the SARS-CoV2 viral genome (length normalized), identified using FIMO (B) Hierarchical clustered heatmap showing the protein abundance (row normalized) of RBPs across tissues.

Supplementary Table Legends:

Table S1. Significant biological pathways obtained from functional annotation analysis of differentially expressed genes (at 5% fdr) in mock treated versus SARS-CoV-2 infected primary human lung epithelium (NHBE cells) using ClueGO (a cytoscape plugin).

Table S2. Identification of alternative splicing events using rMATS (replicate Multivariate Analysis of Transcript Splicing) in mock treated versus SARS-CoV-2 infected primary human lung epithelium (NHBE cells).

Table S2. Significant biological pathways obtained from functional annotation analysis of alternatively spliced genes (at 5% fdr) in mock treated versus SARS-CoV-2 infected primary human lung epithelium (NHBE cells) using ClueGO (a cytoscape plugin).

Table S4. Preferential binding location of RBP motifs identified by FIMO across the SARS-CoV2 viral genome.

Table S5. Preferential binding location of miR-motifs identified by FIMO across the SARS-CoV2 viral genome.

Table S6. Significant biological pathways obtained from functional annotation analysis of mir-targeted genes (from mirNet) using ClueGO (a cytoscape plugin).