

# MicroRNAs and long non-coding RNAs as potential candidates to target specific motifs of SARS-CoV-2

Lucia Natarelli (✉ [lucia.natarelli@med.uni-muenchen.de](mailto:lucia.natarelli@med.uni-muenchen.de))

Institute for cardiovascular prevention (IPEK)

Luca Parca

IRCCS Casa Sollievo della Sofferenza

Fabio Virgili

Council for Agricultural Research and Economics

Tommaso Mazza

IRCCS Casa Sollievo della Sofferenza

Christian Weber

Institute for Cardiovascular Prevention (IPEK)

Deborah Fratantonio

University of Bari Aldo Moro

---

## Research Article

**Keywords:** oligosequences, SARS-CoV-2, target therapy, non-coding RNAs

**Posted Date:** September 25th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-80442/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published on February 18th, 2021. See the published version at <https://doi.org/10.3390/ncrna7010014>.

# MicroRNAs and long non-coding RNAs as potential candidates to target specific motifs of SARS-CoV-2

Lucia Natarelli<sup>1\*</sup>, Luca Parca<sup>2</sup>, Fabio Virgili<sup>3</sup>, Tommaso Mazza<sup>2</sup>, Christian Weber<sup>1</sup>, and Deborah Fratantonio<sup>4\*</sup>

<sup>1</sup> Ludwig-Maximilians-Universität (LMU) München, Institute for Cardiovascular Prevention (IPEK), 800336, Munich, Germany

<sup>2</sup> IRCCS Casa Sollievo della Sofferenza, Laboratory of Bioinformatics, San Giovanni Rotondo (FG), Italy

<sup>3</sup> Council for Agricultural Research and Economics, Research Center for Food and Nutrition, 00178, Rome, Italy

<sup>4</sup> University of Bari Aldo Moro, Department of Biosciences Biotechnology and Biopharmaceutics, 70125, Bari, Italy

\*Correspondence to: lnatarel@med.lmu.de

\*Correspondence may also be addressed to: deborah.fratantonio@uniba.it

## Abstract

The novel Coronavirus, SARS-CoV-2 disease (COVID-19) was defined as a global pandemic and induced a severe public health crisis in 2020. Covid-19 viral infection targets the human respiratory system and, at present, no specific treatment has been identified even though certain drugs have been studied and considered apparently effective in viral progression by reducing the complications in the lung epithelium. Researchers and clinicians are still struggling to find a vaccine or a specific innovative therapeutic strategy to counter COVID-19 infection.

Here we describe our study indicating that SARS-CoV-2 genome contains motif sequences in the 5'UTR leader sequence that can be selectively recognized by specific human non-coding RNAs (ncRNAs), such as micro and long non-coding RNAs (miRNAs and lncRNA). Notably, some of these ncRNAs have been already utilized as oligo-based drugs in pulmonary and virus-associated diseases. We identified three selective motifs at the 5'UTR leader sequence of SARS-CoV-2 that allow viral recognition and binding of a specific group of miRNAs, some of them characterized by "GU" seed alignments. Additionally, one seed motif within miRNAs has been found to be able to bind the 5'UTR leader sequence. Among miRNAs having thermodynamically stable binding site against leader sequence and that are able interacted with Spike transcript some are involved in pulmonary arterial hypertension and anti-viral response, i.e. miR-204, miR-3661, and miR-1343. Moreover, several miRNA candidates have been already validated in vivo and specific oligo sequence are indeed available for their inhibition or overexpression.

Four lncRNAs (H19, Hotair, Fendrr, and LINC05) directly interact with spike transcript (mRNA) and viral genome.

In conclusion, we suggest that specific miRNAs and lncRNAs can be potential candidates to design oligonucleotide-drugs to treat COVID-19 and that our study can provide candidate hypothesis to be eventually tested in further experimental studies.

**Keywords:** oligosequences, SARS-CoV-2, target therapy, non-coding RNAs

## **Introduction**

The virus causing COVID-19 disease(1), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a subgroup member of coronaviruses family that together with Middle East respiratory syndrome coronavirus (MERS-CoV), H1N1, and H5N1 influenza A, causes acute respiratory distress syndrome (ARDS) and acute lung injury (ALI), and in chronic stages pulmonary failure and fatality. Compared to World Health Organization estimated fatality rate for SARS-CoV patients at 14-15% in 2003 (update 49, 7 May 2003, WHO), SARS-CoV-2 showed a pandemic transmission rate, all over the globe, resulting in a pandemic global lock-down(2). One of the reasons proposed for such increased transmission rate resides in the high mutation rate in glycoprotein Spike, which made SARS-CoV-2 viral infection more rapidly(3)<sup>1</sup>. Coronavirus entry mechanism requires the interaction of Spike with the cellular receptor Angiotensin Converting Enzyme 2 (ACE2), which promotes Spike conformational changes and facilitates envelope fusion with the cell membrane and virus endosomal entrance. Interim trial data on vaccination are still ongoing and only preliminary data have been released. Moreover, there is no clinically approved nor effective vaccine available against SARS-CoV-2. Current therapeutic interventions have been reported in patients treated with chloroquine and mostly relevant with its derivative, hydroxychloroquine, a malaria drug that may alter the SARS-CoV-2 cellular uptake(4). However, recent evidences indicate that hydroxychloroquine was used more for symptoms prevention or progression of Covid-19, rather than prevention of SARS-CoV-2 infection(5). Overall, the potential prevention benefits of hydroxychloroquine remain to be determined, since long-term usage is ineffective and imply severe side effects, i.e. eye damage(6). Therefore, it is important to discover new potential therapeutic molecules with a low cost-effective ratio and with low side-effects, potentially already clinically approved for other indications. Nevertheless, target-selectivity is crucial to avoid side effects.

RNA-based drugs, i.e. small interference RNAs (siRNAs) and long RNA antisense locked nucleic acid oligos (LNAs), are catching the interest of the scientific community due to their low side-effects and high efficiency ratio, partly due to their mechanism of action that mimics an endogenous and physiological mechanism of defense proper of many organisms, which indeed include small RNAs. Notably, microRNAs (miRNAs) have been already proposed as potential non-coding RNA (ncRNA) candidates to counter cardiovascular and inflammatory diseases(7), and COVID-19(8), using the MirTarget program to predict putative miRNA candidates<sup>2</sup>. In this manuscript, we aimed to achieve two different goals. First, we wanted to dissect the existence of endogenous ncRNAs, like miRNAs and long non-coding RNAs, that could potentially be used to generate oligosequence-like based anti-viral therapeutic drugs or for which therapeutic trials have been already developed or are in advanced clinical trials. Second, we wanted to determine the existence of COVID-19-selective motifs that might be recognized from specific ncRNA candidates and that therefore could be selective for exogenous RNA genomes, promoting high selectivity for oligo-based antiviral drugs.

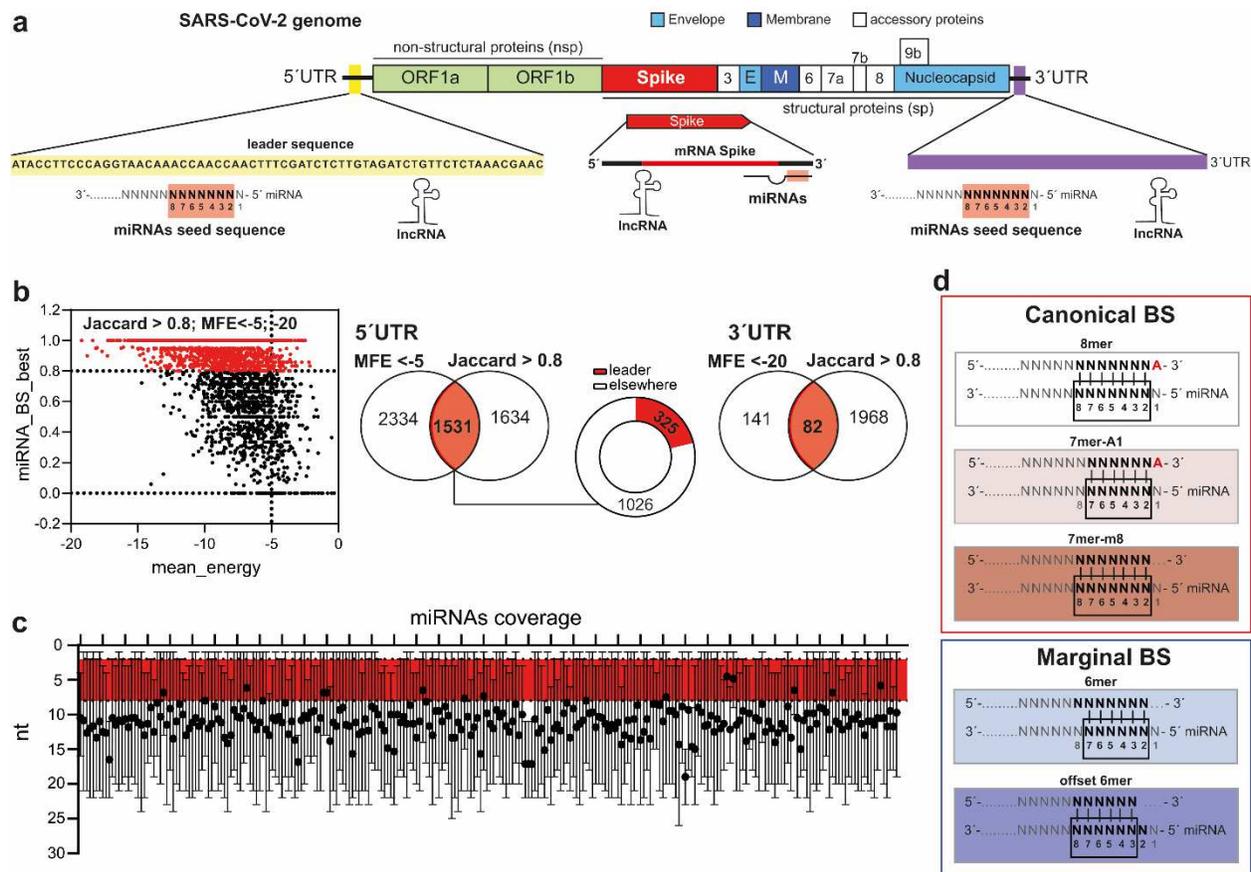
## Results

### **MiRNAs bind the 5'UTR-leader sequence, the 3'UTR of SARS-CoV-2 and Spike mRNA through noncanonical bindings**

MiRNAs act as translational repressors by targeting mRNA transcripts in the RNA-induced silencing complex (20,21), by recognizing mRNA 3'-UTR with 7-8 nucleotides at their 5' end, termed miRNA seed. Animal but not plant-derived miRNAs can recognize also the mRNA 5'UTR to promote or inhibit mRNA-to-protein translation(22-24). This mechanism has been modulated through artificially designed miRNA enhancers, termed miRancers(25).

To identify miRNAs as potential therapeutic candidates against SARS-CoV-2 infection, we depicted putative miRNA binding sites (BS) on SARS-CoV-2 genome, focusing on its 3'UTR and 5'UTR. We also depicted miRNA BS against the mRNA of the glycoprotein Spike (**Fig. 1a**), which facilitates SARS-CoV-2 envelope fusion with the cell membrane and virus endosomal entrance by interacting with the cellular receptor Angiotensin Converting Enzyme 2 (ACE2). Although ACE2 has been proposed as target for anti-viral treatments<sup>(26)</sup>, its fundamental protective role against acute lung failure(27) led us to hypothesize that identification of putative miRNA and lncRNA BS on Spike mRNA may represent a more effective mechanism, with less side-effects, to prevent SARS-CoV-2 transcription.

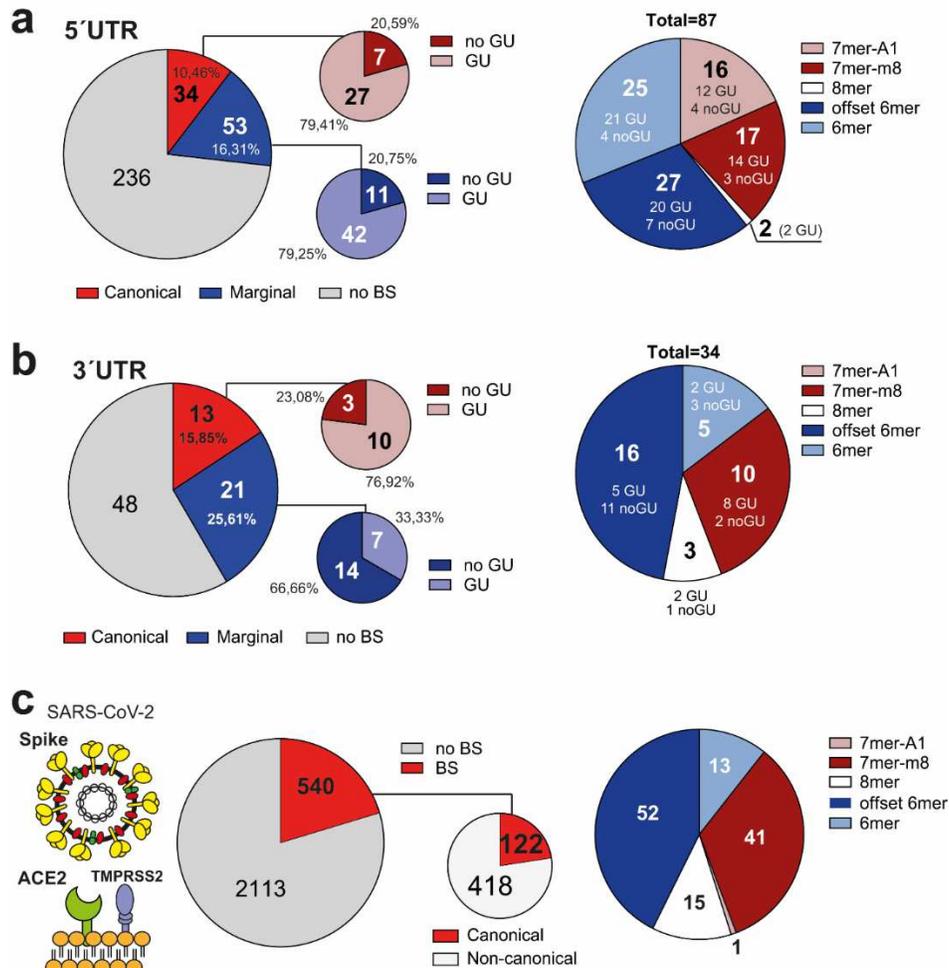
We initially screened 2.656 human mature miRNA sequences using three RNA-RNA binding site prediction methods(11,13). Although miRNAs interact with the 3'UTR of mRNA transcripts, the 5'UTR of SARS-CoV-2 contain a highly conserved sequence of 90 nucleotides, termed "leader sequence", that is pivotal for viral transcription and that is used for the identification of all viral subgenomic mRNAs (**Fig. 1a**). Therefore, we also considered miRNA noncanonical bindings against viral 5'UTR. Overall, we identified BS for 1.531 miRNAs against the 5'UTR and 82 miRNAs BS against the 3'UTR of SARS-CoV-2 genome (**Fig. 1b** and **Suppl. Table 1-4**).



**Figure 1. Canonical and noncanonical microRNAs binding sites identified within the 5'UTR and 3'UTR of SARS-CoV-2 genome.** (a) structural representation of SARS-CoV-2 genome with underlined leader sequence, 3'UTR, and spike transcript against which we identified (b) miRNA binding sites (BS) using 3 RNA:RNA prediction algorithms. Only binding sites with a Jaccard index  $\geq 0.8$  and a minimum free energy (MFE)  $< -5$  (for 5'UTR), or  $< -20$  (for 3'UTR of viral-RNA and Spike transcript) were considered. (c) Only those miRNA BS involving the seed sequence (nt 2-8) were considered and (d) screened in canonical and marginal according to Bartel classification.

According to Bartel classification(20,21), 325 out of 1,531 5'UTR-identified miRNA BS were located in the leader sequence, 53 classified as marginal BS and 34 as canonical BS (Fig. 1b-d and Fig. 2a). 21 out of 82 3'UTR-identified miRNA BS were marginal and 13 were canonical (Fig. 1d and Fig. 2b). Identified miRNAs bound the leader sequence and the 3'UTR of viral genome through a more “noncanonical” binding, which devoid of GU-alignments (Fig. 2a,b and Suppl. Table 1-2). Conversely, 540 potential miRNA BS identified against Spike mRNA (3'UTR) lack of GU annealing (Fig. 2c, and Suppl. Table 3). Notably, miRNAs can bind “nonclassical targets”, like lncRNAs, through a more noncanonical and GU-enriched binding(28). Irrespective from the genomic region considered, miRNAs bound the leader sequence, the genomic 3'UTR, and Spike transcript preferentially through an offset-6mer binding (Fig. 2a-c). Moreover, although the number of 8mer and 7mer-m8 miRNA BS identified against Spike transcript was increased, according to the increased chance to predict 3'UTR-like interactions, the offset-6mer site was still

the most enriched BS (**Fig. 2c** and **Suppl. Table 3**). Taken together, these data indicate the presence of a conserved and/or preferred seed sequence within the viral genome.



**Figure 2. Identified miRNA BS on SARS-CoV-2 leader sequence, genomic 3'UTR, and Spike 3'UTR transcript.** Binding sites (BS) identified and classified according to Bartel classification against (a) viral-leader sequence, (b) the viral genomic 3'UTR, and (c) against the 3'UTR of glycoprotein Spike transcript, which interact with cell receptors ACE2 and TMPRSS2 of the target cell.

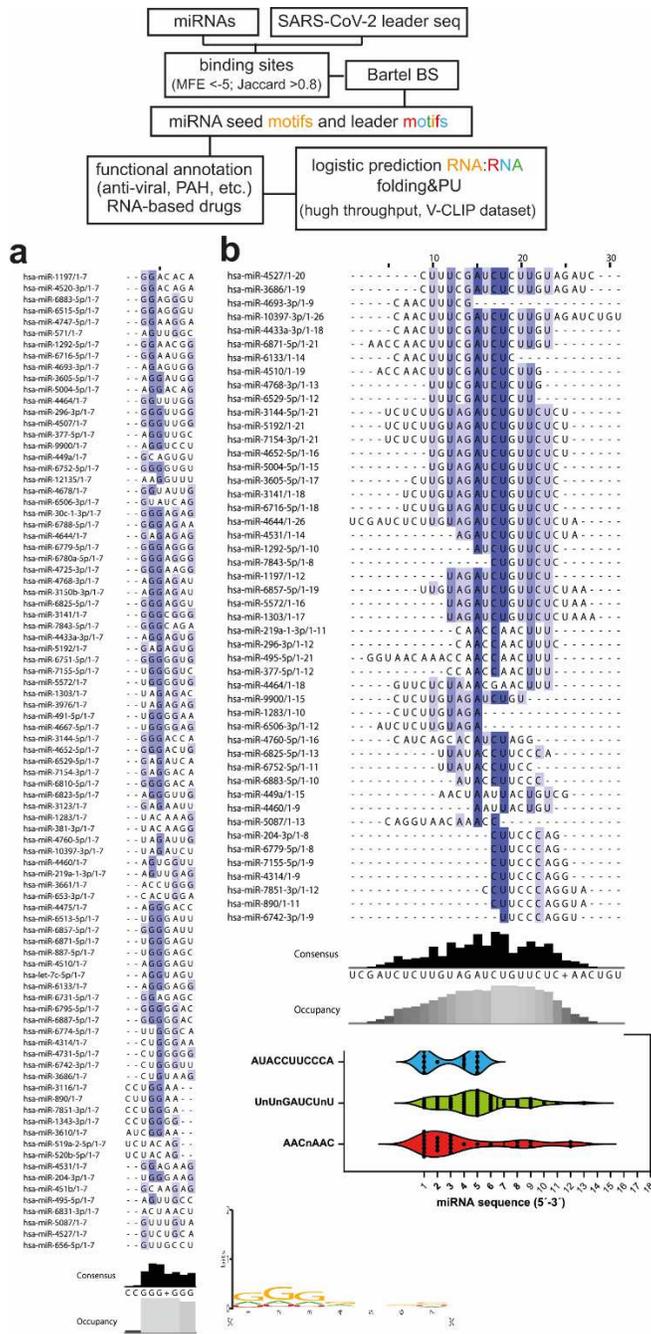
### MiRNAs bind the leader sequence of SARS-CoV-2 through a seed-enriched triplet motif

We identified a nucleotide motif enriched in the seed sequence of miRNAs targeting the leader sequence through a noncanonical binding (GU enriched) that we define as “miRNA-leader triplet motif”. The motif consists of a “GGG” nucleotide triplet within the miRNA seed (nt 2,3,4) (**Fig. 3a** and **Suppl. Table 4**). The consensus motif bits rate was significantly high and independent from the type of BS classification. Moreover, the “GGG” motif was absent in miRNA seeds binding the genomic 3'UTR or the Spike transcript. Notably, we did not identify conserved motifs among all 2,656 human miRNAs, nor references indicating conserved motifs in miRNAs seed sequences that can be used to increase miRNA selectivity for their mRNA targets. These data suggest the existence of specific miRNAs that can recognize the SARS-CoV-2 leader sequence

through a triplet motif. The triplet may stabilize noncanonical bindings of identified miRNAs with exogenous genomes.

**SARS-CoV-2 leader sequence contains three motifs matching GGG-seed enriched miRNAs**

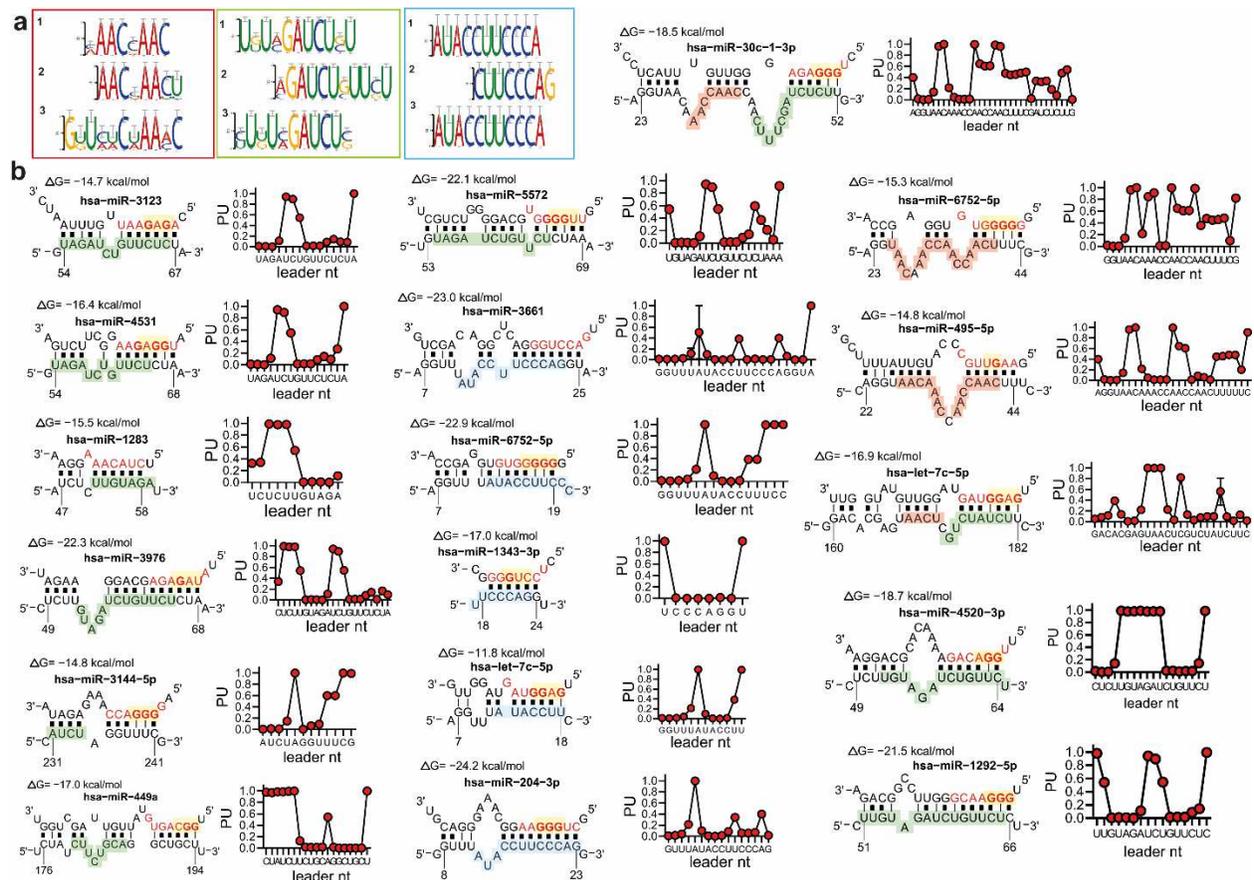
During the analysis of miRNA BS identified against the 5'UTR of SARS-CoV-2 we observed that almost all miRNAs recognize recurrent nucleotide motifs located in the leader sequence of viral-RNA. We hypothesized that these motifs may increase and stabilize miRNAs-viral RNA interaction, especially that of miRNAs containing noncanonical BS. MEME suite analysis of enriched motifs in the leader sequence of SARS-CoV-2 identified three consensus motifs (**Fig. 3b** and **Suppl. Table 4**). In line with our findings, the motifs completely bounded the seed of miRNAs with BS against the leader sequence, including those containing the “GGG” motif and comprising GU alignments (**Fig. 3b** and **Suppl. Table 4**).



**Figure 3.** GGG and leader sequence motifs identified using RNA:RNA algorithms against all miRNA candidates. Schematic representation and relative GGG and (b) leader motifs identified on selective miRNAs, with an RNA:RNA thermodynamic predisposition to interact. The violin graph indicate the motif conservation and the corresponding miRNA region involved in the interaction.

In detail, we identified an “AACnAAC”, “AUACCUUCCA”, and “nUnGAUCUnU” motif recognized within the miRNA sequence, and within the nt 3-8 of miRNAs seed (Fig. 4a-b, Suppl. Fig., and Suppl. Table 4). Several GGG-enriched miRNA candidates recognized more than one motif (Fig. 4b, Suppl. Fig., and Suppl. Table 4). Next, using StarMir algorithm(17,18), we

performed a logistic prediction of miRNA:leader-sequence folding and pairing using available high throughput and V-CLIP datasets. In particular, miRNA:leader bindings were analyzed considering thermodynamic, structural, and sequence features. This prediction returns an output value termed “probability of unpaired” (PU), which is the probability for each nt of the leader sequence to bind to a miRNA nt, therefore to be unpaired according to the miRNA:leader-sequence thermodynamic features. Accordingly, the logistic prediction confirmed the propensity of GGG-enriched miRNAs to selectively interact with identified leader-enriched motifs, especially the “nUnGAUCUnU” motif (**Fig. 4b, Suppl. Fig., and Suppl. Excel File**). The logistic prediction identified thermodynamically stable miRNA:leader-sequence BS, i.e. those for miR-4531 and miR-449a, and revealed those thermodynamically unstable and therefore improbable, i.e. those for miR-6752 and miR-5572 (**Fig. 4b and Suppl. Fig.**). Taken together, these data indicated that leader-enriched motifs are recognized by GGG-enriched miRNAs that interact with viral genome through noncanonical BS. The motifs may contribute to miRNA final recognition of viral genome and increase binding stability of those miRNAs that use a noncanonical binding to target SARS-CoV-2.



**Figure 4. Leader enriched motifs and localization in miRNA BS GGG enriched.** (a) Three leader motifs identified with RNAup (1), IntaRNA (2), and RNAplex (3). (b) MiRNA BS containing in yellow the GGG motif and localization of leader-enriched AACnAAC (red), UnUnGAUCUnU (green), and AUACCUUCCCA motifs (light blue). The graphics represent the probability of each nucleotide to be unpaired (PU), considering thermodynamic and structural features analyzed with StarMir algorithms



sites for Spike were identified for cytoplasmic **(b)** lncRNAs H19 (and for 5'UTR), **(c)** Fendrr, and **(d)** LINC05. Nuclear lncRNAs were used as negative control. Rectangles represent the zoomed view of lncRNA interaction loops. lncRNA minimum free energy secondary structures were predicted using RNAfold web tool. Colors represent base-pair probabilities. \* $p < 0.05$ ; \*\* $p < 0.01$ ;  $p < 0.001$

## Discussion

COVID-19-related global pandemic and severe public health crisis have underlined the importance to find strategies able to avoid the risk of future re-emergence events. Waiting for a capillary utilization of an efficient vaccine and considering the severe respiratory failure at which patients affected by COVID-19 are subjected, priority is to be given to prevent or at least to reduce viral-related complications.

ncRNAs can represent a class of molecules offering an evolutionary and adaptive advantage in humans. miRNAs and lncRNAs are epigenetic modulators of various biological processes and comprise almost 90% of the genome, underlying their critical role as fine-tuning regulators of cell adaptive processes, including viral infections. Indeed, RNA-based drugs, such as siRNAs, Gapmers, and LNA-flanked oligos, have been already extensively used *in vitro* and *in vivo* to treat inflammatory and viral-related diseases, show high cell-type specificity and lack of side effects(7,30,31). Therefore, modulation of an endogenous anti-viral and anti-inflammatory mechanism can represent a more attractive and promising strategy against COVID-19. However, viral specificity of ncRNA-based drugs may represent an obstacle when modulating endogenous ncRNAs. Here, we described the existence of selective motifs located in the SARS-CoV-2 genome and in the seed of certain miRNAs that may represent a way to generate viral-selective RNA-based drugs with unwanted side effects.

We performed an *in silico* analysis to identify miRNA BS against the SARS-CoV-2 leader sequence, 3'UTR, and against the Spike transcript. We identified a triplet motif enriched in the seed of those miRNAs binding the SARS-CoV-2 leader sequence, and three motifs within the leader sequence that were recognized by GGG-enriched miRNAs. All together, these motifs may consent a more thermodynamically stable interaction of those miRNAs targeting the leader sequence through a noncanonical binding. Nevertheless, these motifs may direct the interaction of certain miRNAs against viral rather than endogenous RNAs. These motifs are absent in the seed of miRNAs binding the 3'UTR of SARS-CoV-2 genome or the Spike transcript. Therefore, they represent a promising site to design RNA-based anti-viral drugs.

To accelerate the chance to generate effective RNA-based anti-viral drugs, we screened currently available literature to depict miRNAs involved in viral response, pulmonary and cardiovascular diseases, and inflammatory processes. We identified 35, 16, and 44 miRNA candidates binding the leader sequence, the viral 3'UTR, and the Spike transcript, respectively (**Table 1** and **Suppl. Table 5**). Fifteen GGG-enriched miRNAs binding the leader sequence are known modulators of viral replication upon infection in humans. In example, miR-3661 has been reported to be directly involved in SARS-CoV-2 proteins formation in lung(32). MiR-3145-5p and let-7c-5p inhibit viral H1N1-deriving protein synthesis in patients with chronic obstructive

pulmonary disease (COPD)(33,34). Seven miRNAs were involved in arthritis or osteonecrosis(35,36). Several miRNA candidates, e.g. Spike-binding miR-648(37), miR-19a-3p(38), miR-644a(39), and miR-320d(40), and leader-binding miR-449a(41), miR-4531(42), and miR-204(43) were involved in PAH, asthma, COPD and pulmonary fibrosis. Leader-binding miR-6752 is highly enriched in airway epithelial cells upon mucin overproduction(44), miR-4531 is upregulated in children with asthma, and miR-4520-3p is associated with familial Mediterranean fever(45). Hence, miRNAs targeting the leader sequence of SARS-CoV-2 might partly explain increased susceptibility of certain patients with familial or previous complications. Additionally, other leader-binding and Spike-binding miRNAs, and lncRNA H19(29), are involved in cardiovascular diseases, diabetic retinopathy, and other inflammatory and metabolic diseases. Most of miRNAs indicated in **Table 1** have been inhibited or overexpressed using RNA-based oligos, tested *in vitro* and *in vivo*, or used as disease-associated markers, and can therefore be potentially applied as anti-viral drugs.

## Methods

### Dataset

Covid genome has been downloaded from ENA (MN908947) (<http://www.ebi.ac.uk/ena/data/view/<accession>>), 3'-5'-UTR and Spike portion have been collected from the same entry. 2656 human miRNA sequences have been downloaded from miRBase (May 2020) (9). The sequences for the following lncRNA have been downloaded from RNAcentral(10): Fender, Ftx, H19, Hotair, Malat1, Meg3, Mhrt, Miat, Nron, Sencr, lncWDR59, LINC05, APOA1-AS.

### miRNA and lncRNA interaction with SARS-CoV-2 genome

3 RNA-RNA binding site prediction methods have been considered: IntaRNA(11), RNAplex (12) and RNAup (12). The choice is based on two recent comparative studies which highlighted them as the best overall methods (13) (14). These programs have been run with default parameters, exceptions made for the maximum matching interaction length which has been set to the miRNA length and to 100 for lncRNA-RNA matches. Average pair probabilities for locally stable secondary structures, necessary for RNAplex, have been calculated with RNAplfold (12). Examples of the running commands are:

#### IntaRNA

```
IntaRNA -t <input_file_query> -q <input_file_target> > <output_file>
```

#### RNAplex

```
RNAplfold -W <mirna_length> -u <mirna_length> -O --plex_output < <input_file>
```

```
RNAplex -l <mirna_length> -q <input_file_query> -t <input_file_target> -a ./
```

### **RNAup**

```
RNAup -w <mirna_length> -b -o -3 -5 --interaction_first < <input_file>
```

### **RNA-RNA interaction analysis**

Results of the 3 methods have been analyzed and merged, giving high priority to consensus matches. Match ranges, the overlap of which has been expressed through the Jaccard indexes of the matching residues in both RNAs, and interaction energy (minimum free energy, MFE, expressed in Kcal/moll) are then considered (**Supplementary Tables 1-3**). We considered only match ranges with Jaccard index threshold of  $\geq 0.8$ . For miRNA BS predicted at the viral 3'UTR or at the Spike mRNA transcript (3'UTR) we considered a MFE threshold of  $< -20$ . For miRNA BS predicted at the viral 5'UTR of SARS-CoV-2 genome we considered a MFE threshold of  $< -5$ , since it is considered a noncanonical site of interaction and datasets refers mainly at 3'UTR bindings. An interaction propensity threshold of  $< -15$  was arbitrary set-up for lncRNA:RNA interactions.

### **MiRNAs and SARS-CoV-2 leader sequence motifs analysis**

Enriched motifs have been searched with the MEME suite(15) with default parameters. A logo of the miRNA seed motifs and leader motifs has been generated using WebLogo(12,16) with default parameters.

### **Logistic prediction of miRNA-target sites using high throughput and V-CLIP studies**

For further analysed each miRNA candidate showing BS in the leader sequence and with a functional annotation using STarMir Tool (17,18) to implement the logistic prediction, crosslinking high throughput miRNA binding data with immune-precipitation (CLIP) studies. The advantage of this additional prediction is that we can incorporate comprehensive thermodynamic, structural and sequence features. First, for each miRNA candidate interacting with the leader sequence according to RNAup, RNAplex, and IntaRNA analysis, we further analysed the interaction propensity using the available “5'UTR” filter to screen the high throughput and V-CLIP datasets. All interactions were confirmed and followed Bartel classification. Next, we evaluated the target site probability score, ranking the target sites based on their logistic probability, site and seed access score. The Site Access is the measure of structural accessibility as computed by the average probability of a nucleotide being single strand (i.e., unpaired) for the nucleotides in the predicted binding site. The Seed access is the measure of structural accessibility as computed by the average of single-strand probabilities of nucleotides in the target sub-region complementary to the miRNA seed. The potential of nucleation ( $\Delta G_{\text{nucl}}$ ) and stability ( $\Delta G_{\text{hybrid}}$ ) of the target site annealing were used to measure the total energy change of the hybridization ( $\Delta G_{\text{total}}$ ) (see Supplementary Excel file for additional details).

We further analysed the miRNA:leader sequence interaction. Considering the thermodynamic of predicted BS, we calculated the probability of each nucleotide within the leader sequence to be involved in a binding with each miRNA. A probability unpaired value (PU) for each nt below 0.05 corresponded to a significantly high propensity of interaction. In contrast, a high PU value corresponds to miRNA:RNA loops.

### **LncRNA secondary structures**

MFE secondary structures of lncRNAs interacting with Spike transcript, SARS-CoV-2 5' or 3'UTR were predicted using RNAfold web tool(19). Colors represent base-pair probabilities.

### **Conflict of interest**

Authors declare to do not have conflicts of interest

### **Main References**

1. Cui, J., Li, F. and Shi, Z.-L. (2019) Origin and evolution of pathogenic coronaviruses. *Nature Reviews Microbiology*, **17**, 181-192.
2. Lai, C.-C., Shih, T.-P., Ko, W.-C., Tang, H.-J. and Hsueh, P.-R. (2020) Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *International Journal of Antimicrobial Agents*, **55**, 105924.
3. Shereen, M.A., Khan, S., Kazmi, A., Bashir, N. and Siddique, R. (2020) COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. *Journal of Advanced Research*, **24**, 91-98.
4. Geleris, J., Sun, Y., Platt, J., Zucker, J., Baldwin, M., Hripcsak, G., Labella, A., Manson, D.K., Kubin, C., Barr, R.G. *et al.* (2020) Observational Study of Hydroxychloroquine in Hospitalized Patients with Covid-19. *New England Journal of Medicine*, **382**, 2411-2418.
5. Cohen, M.S. (2020) Hydroxychloroquine for the Prevention of Covid-19 — Searching for Evidence. *New England Journal of Medicine*.
6. Abdulaziz, N., Shah, A.R. and McCune, W.J. (2018) Hydroxychloroquine: balancing the need to maintain therapeutic levels with ocular safety: an update. *Current opinion in rheumatology*, **30**, 249-255.
7. Natarelli, L. and Weber, C. (2019) Next-Generation Therapeutic Concepts for Atherosclerosis: Focus on Cell Specificity and Noncoding RNAs. *Thrombosis and haemostasis*, **119**, 1199-1201.
8. Anatoliy Ivashchenko, A.R., Dana Aisina. (2020) How miRNAs can protect humans from coronaviruses COVID-19, SARS-CoV, and MERS-CoV. *Research Square*.
9. Kozomara, A. and Griffiths-Jones, S. (2010) miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res*, **39**, D152-D157.
10. The RNACentral Consortium. (2018) RNACentral: a hub of information for non-coding RNA sequences. *Nucleic Acids Res*, **47**, D221-D229.

11. Mann, M., Wright, P.R. and Backofen, R. (2017) IntaRNA 2.0: enhanced and customizable prediction of RNA-RNA interactions. *Nucleic Acids Res*, **45**, W435-W439.
12. Lorenz, R., Bernhart, S.H., Höner zu Siederdisen, C., Tafer, H., Flamm, C., Stadler, P.F. and Hofacker, I.L. (2011) ViennaRNA Package 2.0. *Algorithms for Molecular Biology*, **6**, 26.
13. Umu, S.U. and Gardner, P.P. (2017) A comprehensive benchmark of RNA-RNA interaction prediction tools for all domains of life. *Bioinformatics (Oxford, England)*, **33**, 988-996.
14. Lai, D. and Meyer, I.M. (2016) A comprehensive comparison of general RNA-RNA interaction prediction methods. *Nucleic Acids Res*, **44**, e61.
15. Bailey, T.L., Boden, M., Buske, F.A., Frith, M., Grant, C.E., Clementi, L., Ren, J., Li, W.W. and Noble, W.S. (2009) MEME Suite: tools for motif discovery and searching. *Nucleic Acids Res*, **37**, W202-W208.
16. Crooks, G.E., Hon, G., Chandonia, J.M. and Brenner, S.E. (2004) WebLogo: a sequence logo generator. *Genome Res*, **14**, 1188-1190.
17. Kanoria, S., Rennie, W., Liu, C., Carmack, C.S., Lu, J. and Ding, Y. (2016) STarMir Tools for Prediction of microRNA Binding Sites. *Methods Mol Biol*, **1490**, 73-82.
18. Rennie, W., Liu, C., Carmack, C.S., Wolenc, A., Kanoria, S., Lu, J., Long, D. and Ding, Y. (2014) STarMir: a web server for prediction of microRNA binding sites. *Nucleic Acids Res*, **42**, W114-118.
19. Gruber, A.R., Lorenz, R., Bernhart, S.H., Neuböck, R. and Hofacker, I.L. (2008) The Vienna RNA websuite. *Nucleic Acids Res*, **36**, W70-W74.
20. Bartel, D.P. (2009) MicroRNAs: Target Recognition and Regulatory Functions. *Cell*, **136**, 215-233.
21. Agarwal, V., Bell, G.W., Nam, J.W. and Bartel, D.P. (2015) Predicting effective microRNA target sites in mammalian mRNAs. *eLife*, **4**.
22. Gu, W., Xu, Y., Xie, X., Wang, T., Ko, J.-H. and Zhou, T. (2014) The role of RNA structure at 5' untranslated region in microRNA-mediated gene regulation. *RNA*, **20**, 1369-1375.
23. Lee, I., Ajay, S.S., Yook, J.I., Kim, H.S., Hong, S.H., Kim, N.H., Dhanasekaran, S.M., Chinnaiyan, A.M. and Athey, B.D. (2009) New class of microRNA targets containing simultaneous 5'-UTR and 3'-UTR interaction sites. *Genome Res*, **19**, 1175-1183.
24. Ørom, U.A., Nielsen, F.C. and Lund, A.H. (2008) MicroRNA-10a Binds the 5'UTR of Ribosomal Protein mRNAs and Enhances Their Translation. *Molecular Cell*, **30**, 460-471.
25. Li, G., Wu, X., Qian, W., Cai, H., Sun, X., Zhang, W., Tan, S., Wu, Z., Qian, P., Ding, K. *et al.* (2016) CCAR1 5' UTR as a natural miRancer of miR-1254 overrides tamoxifen resistance. *Cell research*, **26**, 655-673.
26. Monteil, V., Kwon, H., Prado, P., Hagelkrüys, A., Wimmer, R.A., Stahl, M., Leopoldi, A., Garreta, E., Hurtado del Pozo, C., Prosper, F. *et al.* (2020) Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2. *Cell*, **181**, 905-913.e907.
27. Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B., Yang, P., Sarao, R., Wada, T., Leong-Poi, H. *et al.* (2005) Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*, **436**, 112-116.
28. Natarelli, L., Geißler, C., Csaba, G., Wei, Y., Zhu, M., di Francesco, A., Hartmann, P., Zimmer, R. and Schober, A. (2018) miR-103 promotes endothelial maladaptation by targeting lncWDR59. *Nature communications*, **9**, 2645.
29. Su, H., Xu, X., Yan, C., Shi, Y., Hu, Y., Dong, L., Ying, S., Ying, K. and Zhang, R. (2018) LncRNA H19 promotes the proliferation of pulmonary artery smooth muscle cells through AT(1)R via sponging let-7b in monocrotaline-induced pulmonary arterial hypertension. *Respiratory research*, **19**, 254.

30. Bajan, S. and Hutvagner, G. (2020) RNA-Based Therapeutics: From Antisense Oligonucleotides to miRNAs. *Cells*, **9**.
31. Lundstrom, K. (2015) RNA-based drugs and vaccines. *Expert review of vaccines*, **14**, 253-263.
32. Islam, A.B.M.M.K. and Khan, M.A.-A.-K. (2020) Lung biopsy cells transcriptional landscape from COVID-19 patient stratified lung injury in SARS-CoV-2 infection through impaired pulmonary surfactant metabolism. *bioRxiv*, 2020.2005.2007.082297.
33. Khongnomnan, K., Makkoch, J., Poomipak, W., Poovorawan, Y. and Payungporn, S. (2015) Human miR-3145 inhibits influenza A viruses replication by targeting and silencing viral PB1 gene. *Exp Biol Med (Maywood)*, **240**, 1630-1639.
34. Ma, Y.J., Yang, J., Fan, X.L., Zhao, H.B., Hu, W., Li, Z.P., Yu, G.C., Ding, X.R., Wang, J.Z., Bo, X.C. *et al.* (2012) Cellular microRNA let-7c inhibits M1 protein expression of the H1N1 influenza A virus in infected human lung epithelial cells. *Journal of cellular and molecular medicine*, **16**, 2539-2546.
35. Aquino-Martinez, R., Farr, J.N., Weivoda, M.M., Negley, B.A., Onken, J.L., Thicke, B.S., Fulcer, M.M., Fraser, D.G., van Wijnen, A.J., Khosla, S. *et al.* (2019) miR-219a-5p Regulates Ror $\beta$  During Osteoblast Differentiation and in Age-related Bone Loss. *J Bone Miner Res*, **34**, 135-144.
36. Li, P., Zhai, P., Ye, Z., Deng, P., Fan, Y., Zeng, Y., Pang, Z., Zeng, J., Li, J. and Feng, W. (2017) Differential expression of miR-195-5p in collapse of steroid-induced osteonecrosis of the femoral head. *Oncotarget*, **8**, 42638-42647.
37. Li, C., Gonsalves, C.S., Eiyomo Mwa Mpollo, M.-S., Malik, P., Tahara, S.M. and Kalra, V.K. (2015) MicroRNA 648 Targets ET-1 mRNA and Is Cotranscriptionally Regulated with *MICAL3* by PAX5. *Molecular and Cellular Biology*, **35**, 514-528.
38. Chen, W. and Li, S. (2017) Circulating microRNA as a Novel Biomarker for Pulmonary Arterial Hypertension Due to Congenital Heart Disease. *Pediatric Cardiology*, **38**, 86-94.
39. Zhong, S., Chen, C., Liu, N., Yang, L., Hu, Z., Duan, P., Shuai, D., Zhang, Q. and Wang, Y. (2019) Overexpression Of hsa-miR-664a-3p Is Associated With Cigarette Smoke-Induced Chronic Obstructive Pulmonary Disease Via Targeting FHL1. *Int J Chron Obstruct Pulmon Dis*, **14**, 2319-2329.
40. Faiz, A., Steiling, K., Roffel, M.P., Postma, D.S., Spira, A., Lenburg, M.E., Borggrewe, M., Eijgenraam, T.R., Jonker, M.R., Koppelman, G.H. *et al.* (2019) Effect of long-term corticosteroid treatment on microRNA and gene-expression profiles in Chronic Obstructive Pulmonary Disease. *European Respiratory Journal*, 1801202.
41. Han, R., Ji, X., Rong, R., Li, Y., Yao, W., Yuan, J., Wu, Q., Yang, J., Yan, W., Han, L. *et al.* (2016) MiR-449a regulates autophagy to inhibit silica-induced pulmonary fibrosis through targeting Bcl2. *Journal of molecular medicine (Berlin, Germany)*, **94**, 1267-1279.
42. Zhang, X., Zhao, X., Sun, H., Yan, Y., Huang, L., Gu, W., Jiang, W., Wang, Y., Zhu, C., Ji, W. *et al.* (2018) The role of miR-29c/B7-H3 axis in children with allergic asthma. *Journal of Translational Medicine*, **16**, 218.
43. Courboulin, A., Paulin, R., Giguère, N.J., Saksouk, N., Perreault, T., Meloche, J., Paquet, E.R., Biardel, S., Provencher, S., Côté, J. *et al.* (2011) Role for miR-204 in human pulmonary arterial hypertension. *Journal of Experimental Medicine*, **208**, 535-548.
44. Gupta, R., Radicioni, G., Abdelwahab, S., Dang, H., Carpenter, J., Chua, M., Mieczkowski, P.A., Sheridan, J.T., Randell, S.H. and Kesimer, M. (2019) Intercellular Communication between Airway Epithelial Cells Is Mediated by Exosome-Like Vesicles. *American journal of respiratory cell and molecular biology*, **60**, 209-220.
45. Latsoudis, H., Mashreghi, M.F., Grün, J., Chang, H.-D., Stuhlmüller, B., Repa, A., Gergiannaki, I., Kabouraki, E., Häupl, T., Radbruch, A. *et al.* (2016) Differential Expression of miR-4520a Associated with Pypin Mutations Suggesting a Role of Autophagy in Familial Mediterranean Fever (FMF). *Journal of Cellular Physiology*, **232**.

46. He, L., Yuan, J., Xu, Q., Chen, R., Chen, L. and Fang, M. (2016) miRNA-1283 Regulates the PERK/ATF4 Pathway in Vascular Injury by Targeting ATF4. *PLoS One*, **11**, e0159171-e0159171.
47. Fu, J., Bai, P., Chen, Y., Yu, T. and Li, F. (2019) Inhibition of miR-495 Improves Both Vascular Remodeling and Angiogenesis in Pulmonary Hypertension. *Journal of Vascular Research*, **56**, 97-106.
48. Au, K.Y., Pong, J.C.H., Ling, W.L. and Li, J.C.B. (2016) MiR-1303 Regulates Mycobacteria Induced Autophagy by Targeting Atg2B. *PLoS One*, **11**, e0146770.
49. Ioannidis, J. and Donadeu, F.X. (2018) Comprehensive analysis of blood cells and plasma identifies tissue-specific miRNAs as potential novel circulating biomarkers in cattle. *BMC Genomics*, **19**, 243.
50. Stolzenburg, L.R., Wachtel, S., Dang, H. and Harris, A. (2016) miR-1343 attenuates pathways of fibrosis by targeting the TGF- $\beta$  receptors. *Biochem J*, **473**, 245-256.
51. Stolzenburg, L.R. and Harris, A. (2018) Microvesicle-mediated delivery of miR-1343: impact on markers of fibrosis. *Cell and tissue research*, **371**, 325-338.
52. Rothschild, S.I., Tschan, M.P., Jaggi, R., Fey, M.F., Gugger, M. and Gautschi, O. (2012) MicroRNA-381 represses ID1 and is deregulated in lung adenocarcinoma. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer*, **7**, 1069-1077.
53. Wang, C., Liu, L., Zhu, H., Zhang, L., Wang, R., Zhang, Z., Huang, J., Zhang, S., Jian, F., Ning, C. *et al.* (2019) MicroRNA expression profile of HCT-8 cells in the early phase of *Cryptosporidium parvum* infection. *BMC Genomics*, **20**, 37.
54. Zhang, L. and Yu, S. (2018) Role of miR-520b in non-small cell lung cancer. *Experimental and therapeutic medicine*, **16**, 3987-3995.
55. Lin, L., Cai, Q., Zhang, X., Zhang, H., Xu, C. and Li, Y. (2015) Two less common human microRNAs miR-875 and miR-3144 target a conserved site of E6 oncogene in most high-risk human papillomavirus subtypes. *Protein & cell*, **6**.
56. Li, R., Yang, Y.E., Jin, J., Zhang, M.Y., Liu, X., Liu, X.X., Yin, Y.H. and Qu, Y.Q. (2019) Identification of lncRNA biomarkers in lung squamous cell carcinoma using comprehensive analysis of lncRNA mediated ceRNA network. *Artificial cells, nanomedicine, and biotechnology*, **47**, 3246-3258.
57. Biju, B., Chhabra, S. and Sarkar, C. (2020) *Elucidation of novel miRNA candidates and their role in unraveling the pathology of Non-Alcoholic Fatty Liver Disease.*
58. Wang, Q., Wang, Y., Minto, A.W., Wang, J., Shi, Q., Li, X. and Quigg, R.J. (2008) MicroRNA-377 is up-regulated and can lead to increased fibronectin production in diabetic nephropathy. *FASEB J*, **22**, 4126-4135.
59. Wu, H., Liu, H.Y., Liu, W.J., Shi, Y.L. and Bao, D. (2018) miR-377-5p inhibits lung cancer cell proliferation, invasion, and cell cycle progression by targeting AKT1 signaling. *Journal of cellular biochemistry*.
60. Fan, J., An, X., Yang, Y., Xu, H., Fan, L., Deng, L., Li, T., Weng, X., Zhang, J. and Chunhua Zhao, R. (2018) MiR-1292 Targets FZD4 to Regulate Senescence and Osteogenic Differentiation of Stem Cells in TE/SJ/Mesenchymal Tissue System via the Wnt/ $\beta$ -catenin Pathway. *Aging Dis*, **9**, 1103-1121.
61. Mao, L., Liu, S., Hu, L., Jia, L., Wang, H., Guo, M., Chen, C., Liu, Y. and Xu, L. (2018) miR-30 Family: A Promising Regulator in Development and Disease. *BioMed Research International*, **2018**, 9623412.
62. Zhang, Q., Huang, C., Yang, Q., Gao, L., Liu, H.-C., Tang, J. and Feng, W.-h. (2016) MicroRNA-30c Modulates Type I IFN Responses To Facilitate Porcine Reproductive and Respiratory Syndrome Virus Infection by Targeting JAK1. *The Journal of Immunology*, **196**, 2272-2282.

63. Miao, R., Wang, Y., Wan, J., Leng, D., Gong, J., Li, J., Liang, Y., Zhai, Z. and Yang, Y. (2017) Microarray expression profile of circular RNAs in chronic thromboembolic pulmonary hypertension. *Medicine (Baltimore)*, **96**, e7354-e7354.
64. Keller, A., Ludwig, N., Fehlmann, T., Kahraman, M., Backes, C., Kern, F., Vogelmeier, C.F., Diener, C., Fischer, U., Biertz, F. *et al.* (2019) Low miR-150-5p and miR-320b Expression Predicts Reduced Survival of COPD Patients. *Cells*, **8**, 1162.
65. Zhang, Y., Wang, J. and Su, H. (2020) MiR-3150b inhibits hepatocellular carcinoma cell proliferation, migration and invasion by targeting GOLPH3. *Journal of Investigative Medicine*, **68**, 770-775.
66. Wang, S.-N., Luo, S., Liu, C., Piao, Z., Gou, W., Wang, Y., Guan, W., Li, Q., Zou, H., Yang, Z.-Z. *et al.* (2017) miR-491 Inhibits Osteosarcoma Lung Metastasis and Chemoresistance by Targeting  $\alpha$ B-crystallin. *Mol Ther*, **25**, 2140-2149.
67. Xu, Y., Lin, J., Jin, Y., Chen, M., Zheng, H. and Feng, J. (2019) The miRNA hsa-miR-6515-3p potentially contributes to lncRNA H19-mediated-lung cancer metastasis. *Journal of cellular biochemistry*, **120**, 17413-17421.
68. Yu, J.H., Long, L., Luo, Z.X., Li, L.M. and You, J.R. (2016) Anti-inflammatory role of microRNA let-7c in LPS treated alveolar macrophages by targeting STAT3. *Asian Pacific journal of tropical medicine*, **9**, 72-75.
69. Higaki, M., Shintani, T., Hamada, A., Rosli, S.N.Z. and Okamoto, T. (2020) Eldecalcitol (ED-71)-induced exosomal miR-6887-5p suppresses squamous cell carcinoma cell growth by targeting heparin-binding protein 17/fibroblast growth factor-binding protein-1 (HBp17/FGFBP-1). *In Vitro Cellular & Developmental Biology - Animal*, **56**, 222-233.

## Contributions

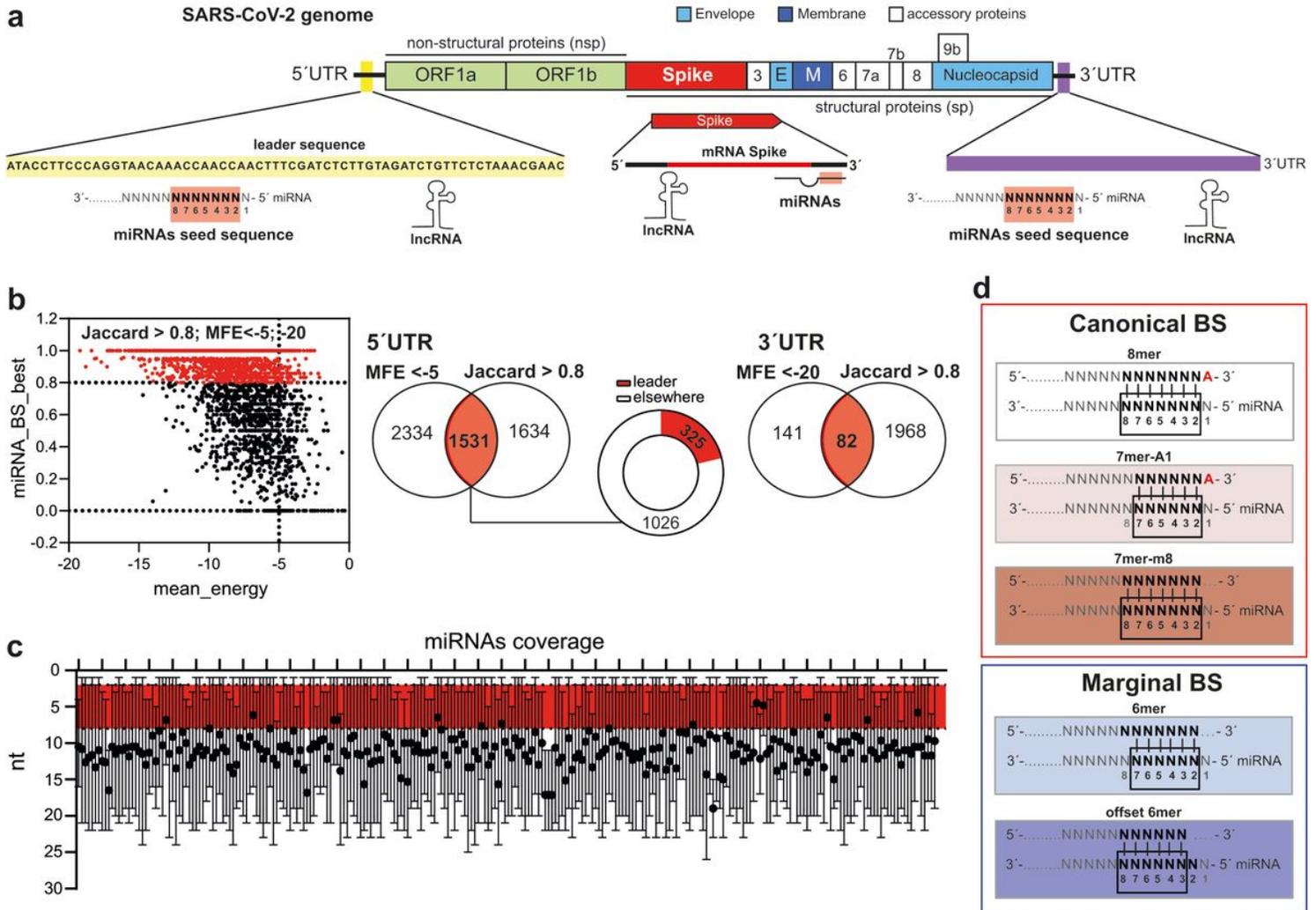
LN, DF, and LP performed conceptualization and data curation; LP data generation; LN performed formal analysis and drafted the manuscript; LN and DF curated final analysis, drafted the manuscript, conceptualization, review and editing of the final version of the manuscript; DF project administration, supervision; FV, TM, and CW conceptualization, review and editing the final version of the manuscript.

**Table 1. Role of main miRNA candidates binding the leader sequence of SARS-CoV-2**

miRNAs	Reported function	ref
hsa-miR-1283	Endothelial vascular injury	(46)
hsa-miR-495-5p	Inhibits vascular remodeling and angiogenesis in PAH	(47)
hsa-miR-1303	Regulate the autophagy process in mycobacteria infection	(48)
hsa-miR-204-3p	Promoter of PAH	(43)
hsa-miR-6529-5p	Novel potential tissue specific biomarker in cattle	(49)
hsa-miR-1343-3p	Attenuate fibrosis in fibrotic lung disease/microvesicle	(50,51)
hsa-miR-3661	Direct involvement with SARS-CoV-2 proteins from lung biopsy	(32)
hsa-miR-381-3p	Deregulated in lung adenocarcinoma	(52)
hsa-miR-3976	Regulates apoptosis in hosts after microbial infection	(53)
hsa-miR-520b-5p	Inhibits NSCLC	(54)
hsa-miR-3144-5p	Interact with viral proteins	(55)
hsa-miR-4652-5p	Lung cancer expressed miRNA	(56)
hsa-miR-6857-5p	Prognostic of viral-related cervical cancer/ marker of NSCLC	(57)

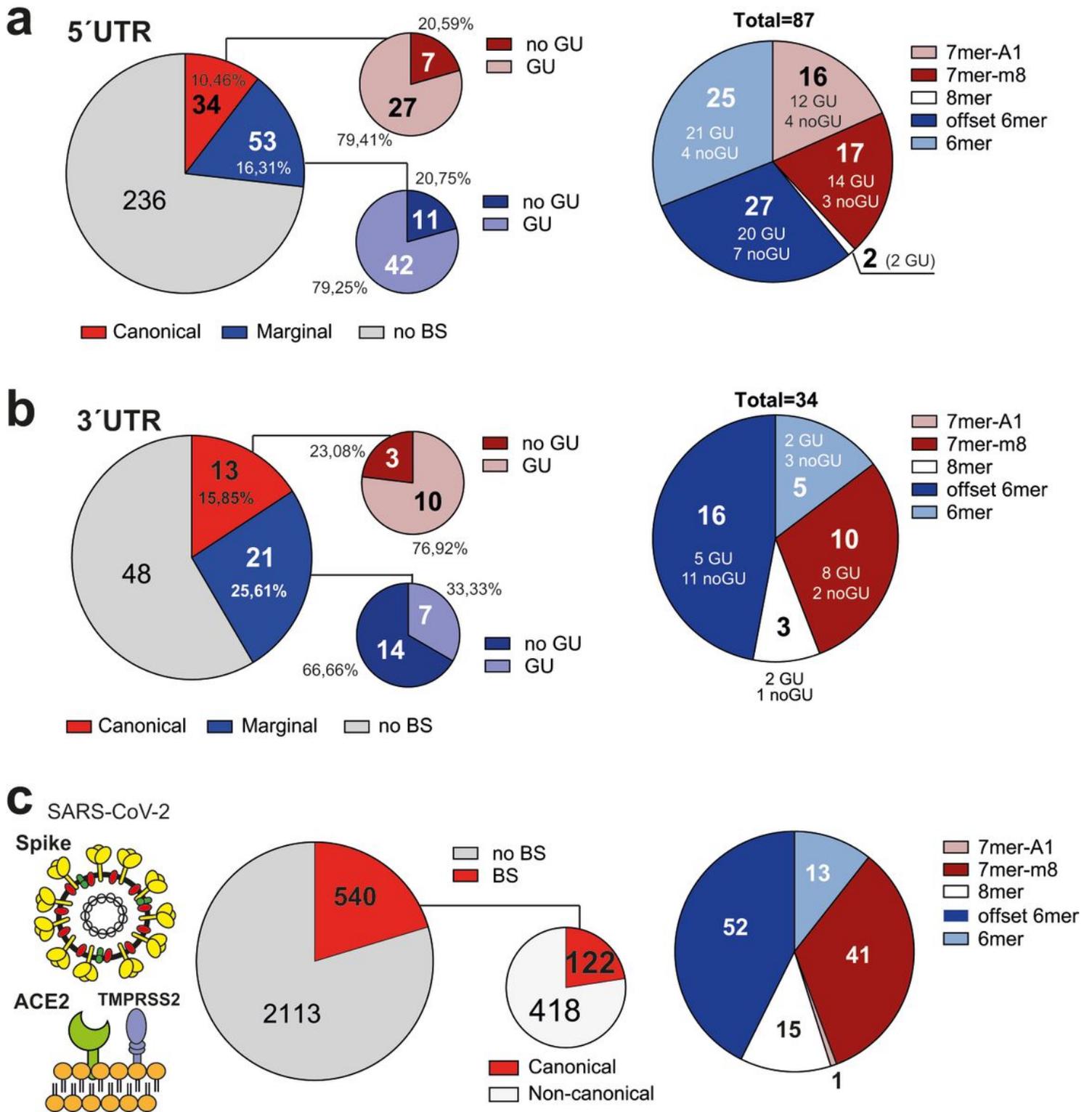
hsa-miR-377-5p	Promote fibronectin production/inhibits lung cell proliferation	(58,59)
hsa-miR-1292-5p	Inhibitor of osteogenic differentiation, promotes osteoporosis	(60)
hsa-miR-219a	Arthritis/NSCLC	(35)
hsa-miR-30c-1-3p	Positive bone development/promotes viral infection	(61,62)
hsa-miR-449a	Inhibits pulmonary fibrosis	(41)
hsa-miR-5572	Upregulated in osteonecrosis femoral head	(36)
hsa-miR-6752-5p	Highly expressed in airway epithelial cells/mucin overproduction	(44)
hsa-miR-4531	Upregulated in children with asthma	(42)
hsa-miR-6831-3p	Anti-atherogenic/PAH	(44,63)
hsa-miR-377-5p	Inhibits lung cancer cell proliferation	(59)
hsa-miR-3123	Negative correlation with survival of COPD patients	(64)
hsa-miR-3150b-3p	Inhibits cell proliferation in NSCLC patients	(65)
hsa-miR-451b	Inhibits osteosarcoma lung metastasis	(66)
hsa-miR-4520-3p	Associated with FMF-related mutations	(45)
hsa-miR-491-5p	Inhibits osteosarcoma lung metastasis	(66)
hsa-miR-6515	Contributes to lncRNA H19-mediated lung cancer metastasis	(67)
hsa-let-7c-5p	Inhibits H1N1 protein synthesis/anti-inflammatory role in COPD	(34,68)
hsa-miR-6887-5p	Inhibits squamous cell carcinoma cell growth	(69)
PAH: Pulmonary arterial hypertension; NSCLC: Non-Small Cell Lung Cancer; COPD: Chronic obstructive pulmonary disease; FMF: familial Mediterranean fever		

# Figures



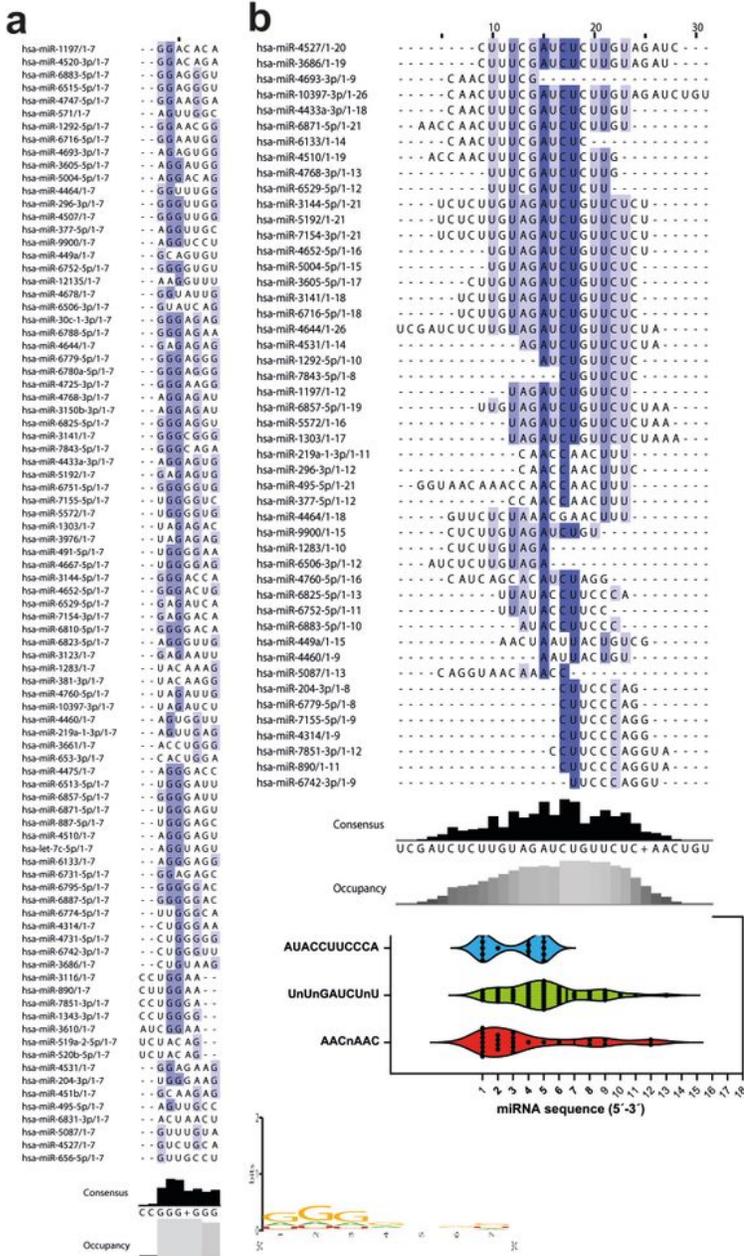
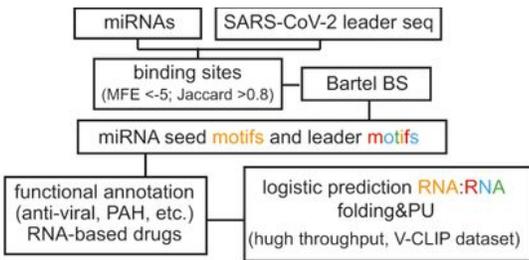
**Figure 1**

Canonical and noncanonical microRNAs binding sites identified within the 5'UTR and 3'UTR of SARS-CoV-2 genome. (a) structural representation of SARS-CoV-2 genome with underlined leader sequence, 3'UTR, and spike transcript against which we identified (b) miRNA binding sites (BS) using 3 RNA:RNA prediction algorithms. Only binding sites with a Jaccard index  $\geq 0.8$  and a minimum free energy (MFE)  $< -5$  (for 5'UTR), or  $< -20$  (for 3'UTR of viral-RNA and Spike transcript) were considered. (c) Only those miRNA BS involving the seed sequence (nt 2-8) were considered and (d) screened in canonical and marginal according to Bartel classification.



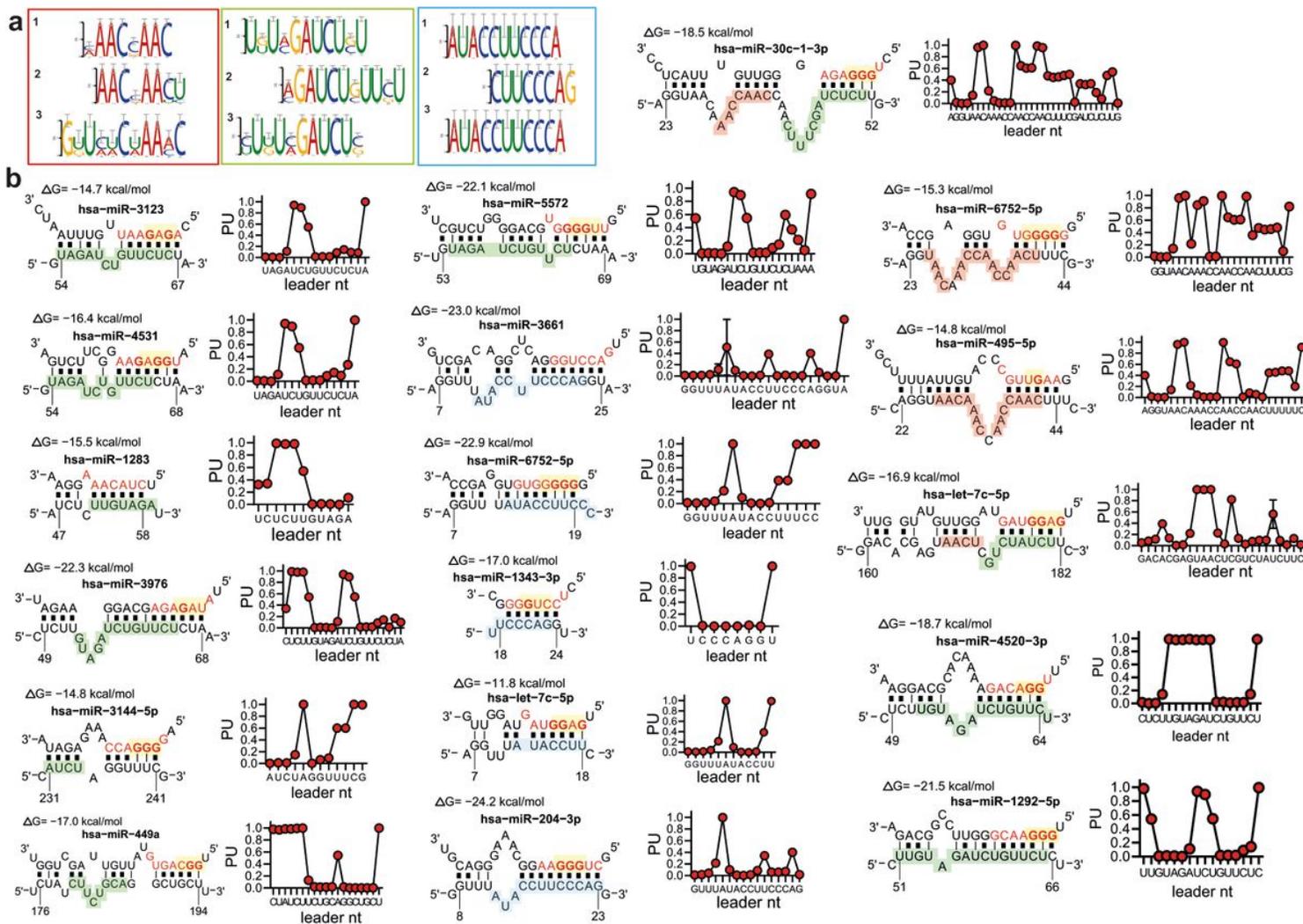
**Figure 2**

Identified miRNA BS on SARS-CoV-2 leader sequence, genomic 3'UTR, and Spike 3'UTR transcript. Binding sites (BS) identified and classified according to Bartel classification against (a) viral-leader sequence, (b) the viral genomic 3'UTR, and (c) against the 3'UTR of glycoprotein Spike transcript, which interact with cell receptors ACE2 and TMPRSS2 of the target cell.



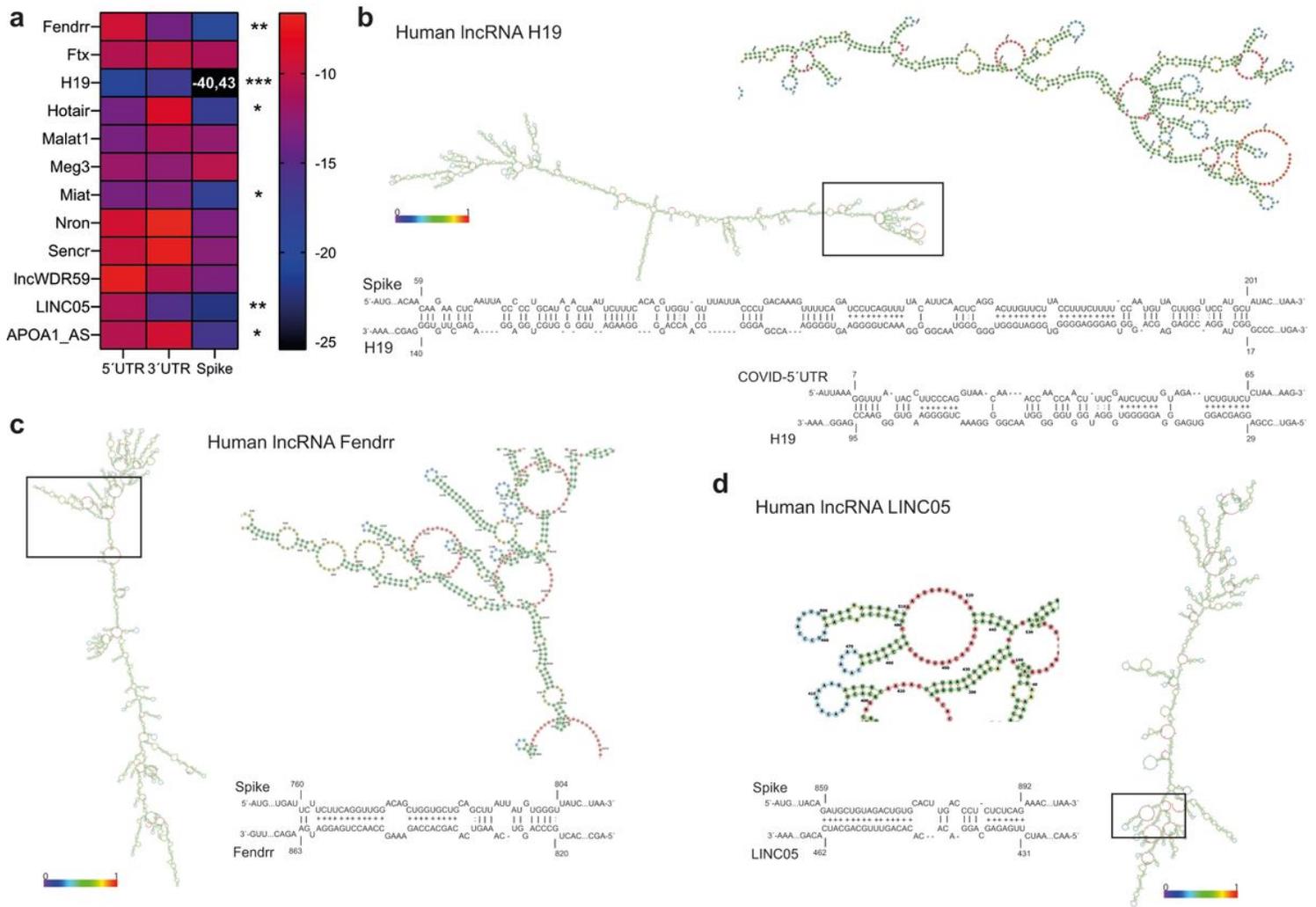
**Figure 3**

GGG and leader sequence motifs identified using RNA:RNA algorithms against all miRNA candidates. Schematic representation and relative (a) GGG and (b) leader motifs identified on selective miRNAs, with an RNA:RNA thermodynamic predisposition to interact. The violin graph indicate the motif conservation and the corresponding miRNA region involved in the interaction.



**Figure 4**

Leader enriched motifs and localization in miRNA BS GGG enriched. (a) Three leader motifs identified with RNAup (1), IntaRNA (2), and RNAPlex (3). (b) MiRNA BS containing in yellow the GGG motif and localization of leader-enriched AACnAAC (red), UnUnGAUCUnU (green), and AUACCUUCCCA motifs (light blue). The graphics represent the probability of each nucleotide to be unpaired (PU), considering thermodynamic and structural features analyzed with StarMir algorithms



**Figure 5**

Interaction propensity of lncRNAs involved in pulmonary arterial hypertension, anti-viral response, and inflammatory diseases. (a) Heat map of lncRNA interaction propensity with SARS-CoV-2 5'UTR, 3'UTR, and with Spike mRNA using IntaRNA, RNAup, and RNAplex. Significantly binding sites for Spike were identified for cytoplasmic (b) lncRNAs H19 (and for 5'UTR), (c) Fendrr, and (d) LINC05. Nuclear lncRNAs were used as negative control. Rectangles represent the zoomed view of lncRNA interaction loops. lncRNA minimum free energy secondary structures were predicted using RNAfold web tool. Colors represent base-pair probabilities. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplement1.pdf](#)
- [supplement2.xlsx](#)