Supplementary Information:

miR-24-3p Is Antiviral Against SARS-CoV-2 by Downregulating Critical Host Entry Factors

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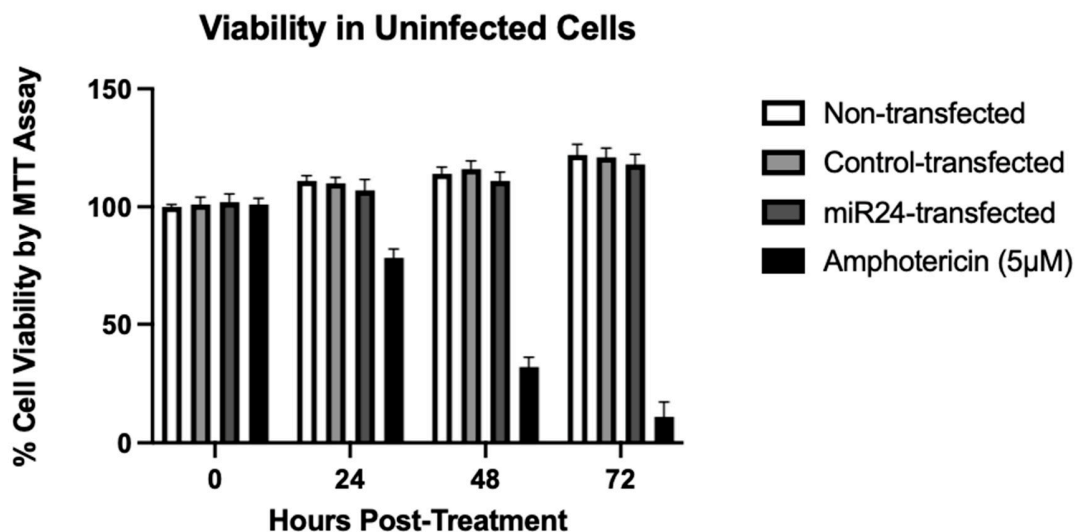[†] These authors contributed equally to this work.

Figure S1. MTT Calu-3 cell viability assay during transfection with miR-24. Calu-3 cells were treated with 100 nM of miR-24 or con-miR for 24-72h before incubation with 2.5 mg/mL formazan and quantifying by absorbance at 570 nm. Amphotericin was included as a positive control for cytotoxicity.
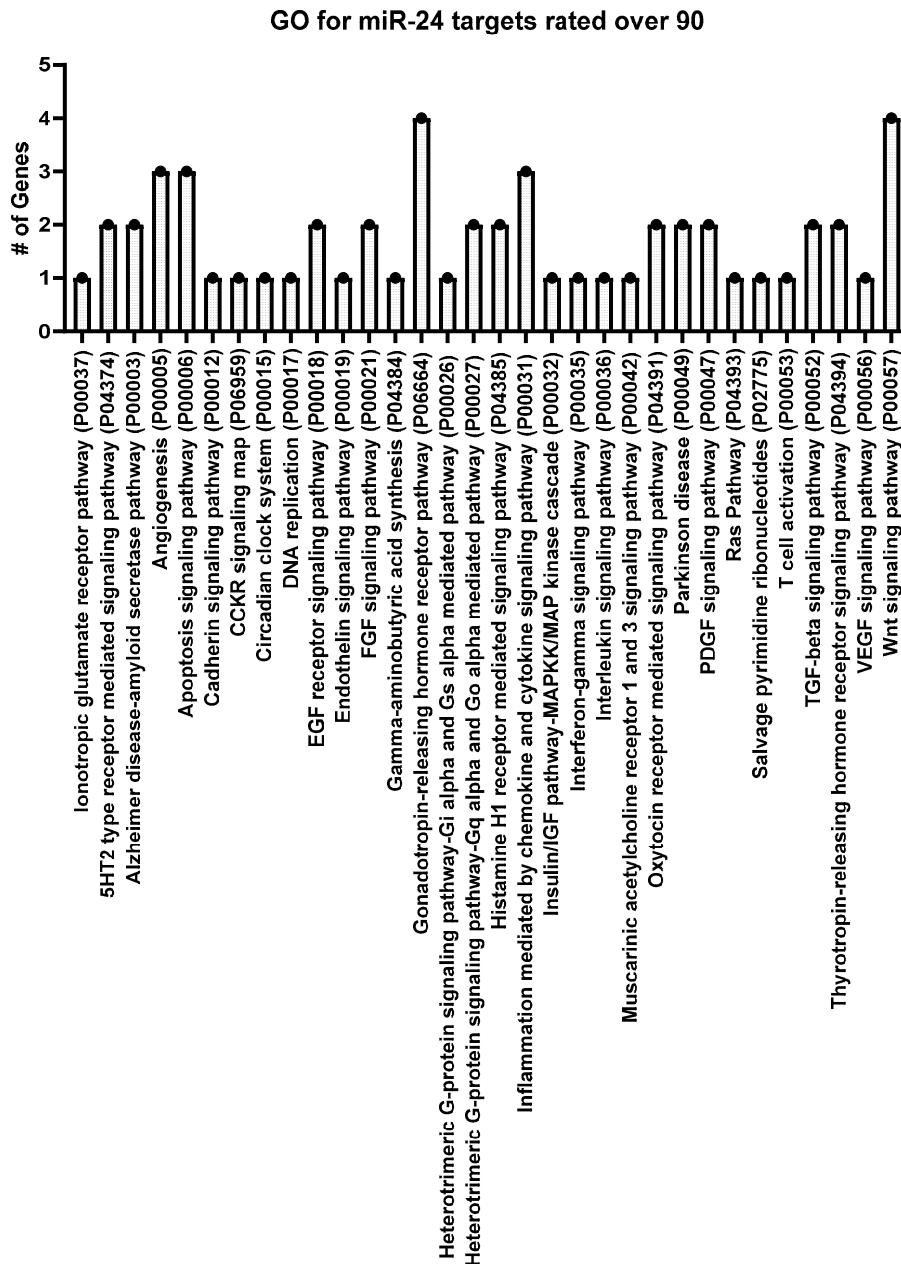
GO for miR-24 targets rated over 90

Figure S2. Gene Ontology for predicted miR-24 targets with a score of 90 of greater. Panther GO was performed on all miRDB predicted targets regardless of target expression. Most genes belonged to unclassified categories; however, these were removed for clarity. Of the 959 targets, the targets with a miRDB score of 90 or over (n = 130) were included for generating the following GO analysis.

Example Gene, 3'UTR nucleotide position: ABC-XYZ 5' ...XXXXXXXXXXXXXXXXXXXXXXXXXXX... Calculated ΔG Conservation

hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG

NRP1 3'UTR nucleotide position : 110-117 5' ...GUGUGUUGAUGACCACUGAGCCA... Calculated ΔG= -16.23 kcal/mol Highly conserved
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                          :       |||||||
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hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG= -41.48 kcal/mol

NRP2 3'UTR nucleotide position: 2935-2942 5' ...UCGACCAUUCACUGGCUGAGCCU... Calculated ΔG= -12.93 kcal/mol Highly conserved
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                     :      :     |||||||
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hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG= -45.71 kcal/mol

NRP2 3'UTR nucleotide position: 2346-2352 5' ...UGCUGUCAUGCUCAG – UGAGCCAU... Calculated ΔG= -13.29 kcal/mol Poorly conserved
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                        ::::      |||||||
```
hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG= -42.91 kcal/mol

Furin 3' UTR nucleotide position: 724-730 5' ...CCAAGUCCCUGUUU—CUGAGCCU... Calculated ΔG= -19.77kcal/mol Highly conserved
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                       ::::       |||||||
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hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG= -42.51 kcal/mol

Furin 3' UTR nucleotide position: 1211-1217 5' ...GGGCAGGUGGUGGCACUGAGCCC... Calculated ΔG= -14.28 kcal/mol Poorly conserved
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                       :   :      |||||||
```
hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG= -51.07 kcal/mol

SR-B1 3' UTR nucleotide position: 117-123 5' ...CCCCAGAAAACAGCCUGAGCCU... Calculated ΔG= -12.93 kcal/mol Highly conserved
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                         :        |||||||
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hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG= -45.8 kcal/mol

SR-B1 3' UTR nucleotide position: 715-721 5' ...ACAUCAUCCUAUGGA—CUGAGCCG... Calculated ΔG= -14.28 kcal/mol Highly conserved
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                        ::::      |||||||
```
hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG= -42.94 kcal/mol

SR-B1 3' UTR nucleotide position: 17-23 5' ...GUCCUGAGGACACCGUGAGCCAG... Calculated ΔG= -13.29 kcal/mol Poorly conserved
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                       :: ::      |||||||
```
hsa-miR-24-3p 3' GACAAGGACGACUUG --- ACUCGGU Calculated Maximum ΔG= -44.9 kcal/mol

SREBP2 3' UTR nucleotide position 4123-4146 5' ...TCCCTCCCCTGGGCCUGACUGAGCCU... Calculated ΔG= -14.28 kcal/mol Poorly conserved
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                           ::     |||||||
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hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG= -57.0 kcal/mol

PTGER4 3' UTR nucleotide position: 1274-1280 5' ...GGUUGUAAUUAAAUUCUGAGCCU... Calculated ΔG= -12.93 kcal/mol Highly Conserved
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hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG= -41.48 kcal/mol

CDH7 3' UTR nucleotide position: 1020-1027 5' ...AUAUAAAUUGUUCUUCUGAGCCU... Calculated ΔG= -12.93 kcal/mol Highly conserved
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                       :: :       |||||||
```
hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG = -41.48 kcal/mol

CDH7 3' UTR nucleotide position: 4752-4759 5' ...CUGGGAUUACAGGCGUGAGCCAC... Calculated ΔG= -13.29 kcal/mol Poorly conserved
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                       :   : :    |||||||
```
hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG = -46.33 kcal/mol

TOP1 3' UTR nucleotide position 163-170 5' ...AUUUAUAAGGGAGAGCUGAGCCU... Calculated ΔG= -12.93 kcal/mol Highly conserved
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                       :   :      |||||||
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hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG= -42.46 kcal/mol

TOP1 3' UTR nucleotide position: 62-68 5' ...GGAAAGAUGGAUAAACUGAGCCU... Calculated ΔG= -16.22 kcal/mol Poorly conserved
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                       :   :      |||||||
```
hsa-miR-24-3p 3' GACAAGGACGACUUGACUCGGU Calculated Maximum ΔG= -41.98 kcal/mol

Figure S3. All 3'-UTR binding interactions and associated binding free energy for selected predicted targets. Target seed sites were predicted using miRDB [29] and additional interactions outside the seed, in addition to binding energies, were calculated/determined using Oligo Analyzer. Watson-crick pairs within the seed site are indicated by solid black lines (**-**) while Watson-Crick pairs and non-Watson-Crick pairs outside the seed site are indicated by a colon (:). The degree of evolutionary conservation is not part of the miRDB parameters for prediction [29]; however, the degree of conservation has been determined using Targetscan [60] and included for each target.
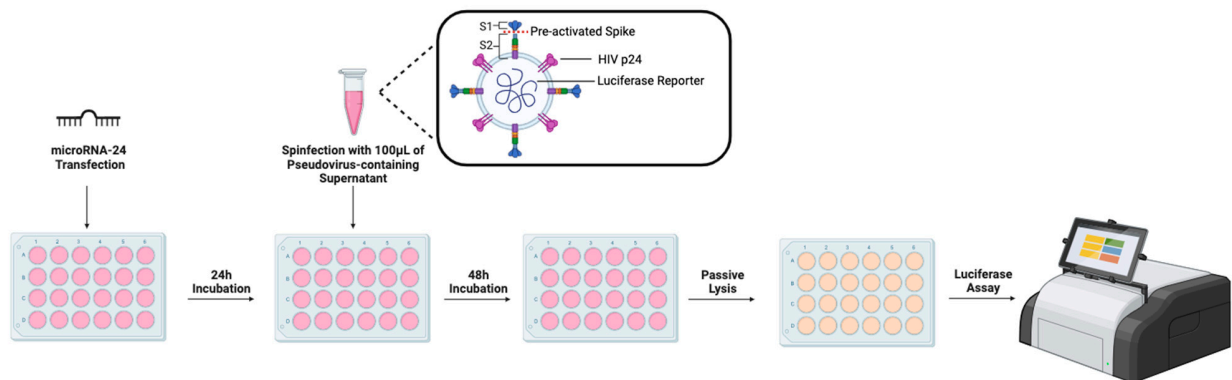
Figure S4. Scheme depicting the quantification of SARS-CoV-2 S pseudovirus entry assay.
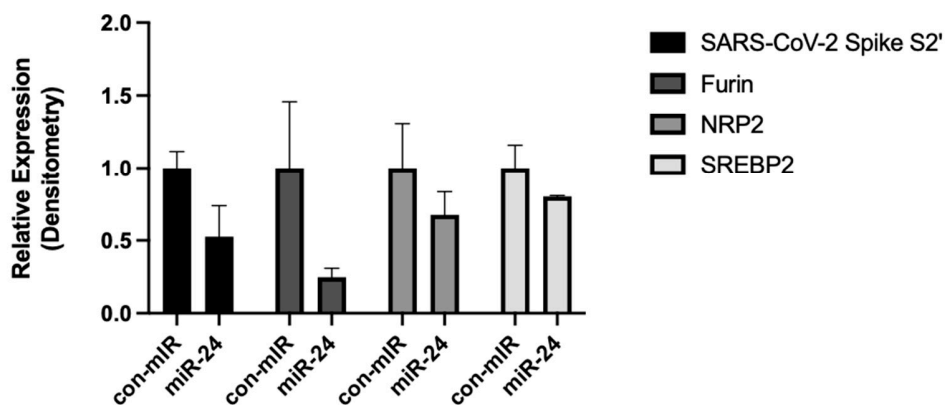

Figure S5. Densitometric analysis of western blots of miR-24 pre-treated cell lysates. HEK293T cells were pre-treated with miR-24 or con-miR 24h before transfection with the plasmids to produce pseudovirions. After 48h, the pseudovirus was collected and the pseudovirus pro-duced during miR-24 treatment or con-miR treatment were then used to infect healthy untreated Huh7 cells. A luciferase assay performed on these Huh7 cells to quantify amount of pseudovirus produced**.** The densitometry is performed on the blot shown in Figure 6B & 6C. n = 2; error bares represent SEM.
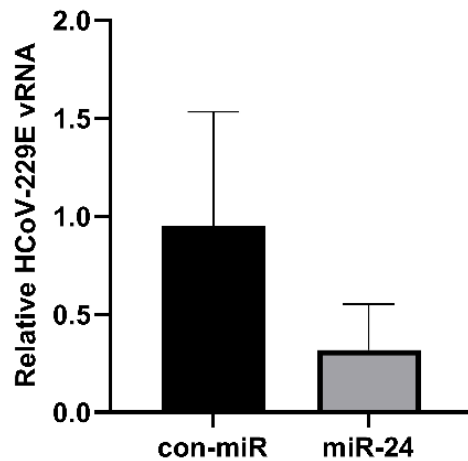
Figure S6. miR-24-3p shows a trending inhibition against Human coronavirus 229E. A549 cells were reverse transfected with miR-24-3p or a control miRNA for 24h before infecting with HCoV-229E, MOI of 0.05, for 48h. Following infection, cells were lysed and RT-qPCR was performed (technical duplicate) for vRNA of HCoV-229E normalized to GAPDH mRNA. n = 2. Error bars represent SEM. p = 0.2907.
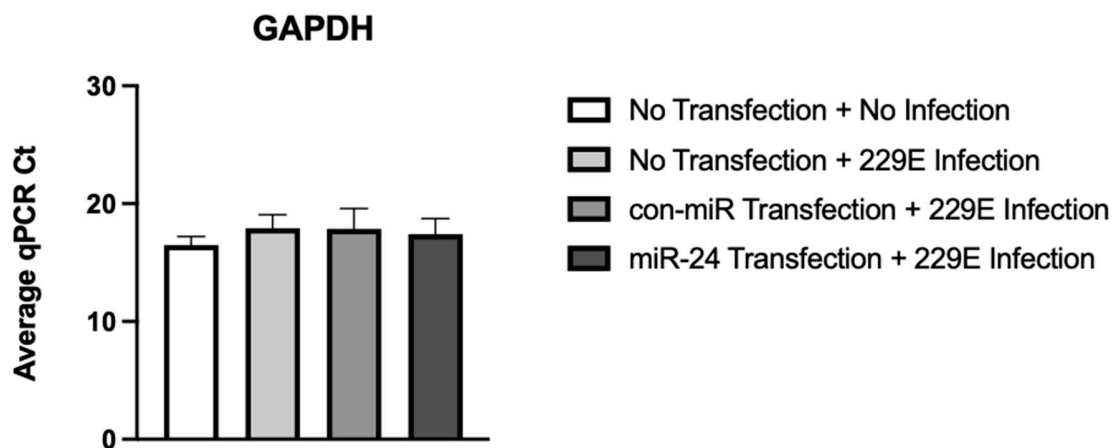

Figure S7. GAPDH is not affected by transfection of miR-24 followed by HCoV-229E infection. A549 cells were reverse transfected with miR-24 or a control miRNA for 24h before infecting with HCoV-229E, MOI of 0.05, for 48h. Following infection, cells were lysed and RT-qPCR was performed in technical duplicate. n = 2. Error bars represent SEM.