Supplementary Materials

Clinically Approved Anti-Viral Drug in an Orally Administrable Nanoparticle for COVID-19

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Materials: All chemicals were used as received without further purification unless otherwise mentioned. Ivermectin (IVM) was purchased from Sigma-Aldrich. Regenerative cellulose membrane Amicon Ultra centrifugal 100 kDa filters were purchased from Millipore Sigma Ltd. Phosphate buffered saline (1X PBS) was purchased from Gibco (catalog number 10010-023). Goat serum was obtained from Sigma Aldrich (catalog number G9023). Glutamine, penicillin/streptomycin trypsin-EDTA solution, HEPES buffer (1 M in water), and sodium pyruvate were procured from Sigma Life Sciences. Dulbecco's Modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco Life Technologies. Mouse monoclonal IgG, Fc-Rn (A-6) (Catalog number SC-393064) was purchased from Santa Cruz Biotechnology. SARS-CoV-2 spike protein plasmid, pcDNA3-SARS-CoV-2-S-RBD-Fc was a gift from Erik Procko (Addgene plasmid # 141183; http://n2t.net/addgene:141183; RRID:Addgene-141183) (Catalog number 141183) was procured from Addgene.[1] MERS-CoV spike protein plasmid, pCMV3-Flag-Spike (beta coronavirus 2c EMC 2012) was purchased from Sino Biological. HA-tag (1:1000, C29F4) and Flag-taf (1:1000, #14793) was purchased from Cell signaling. ACE2 (1:1000, ab15348) and β-actin (1:1000, ab205718) antibodies were purchased from Abcam. Imp α (I1784) was purchased from Millipore Sigma and Imp β1 (51186) was purchased from cell signaling. Native human IgG Fc fragment protein (catalog number Ab90285) was procured from Abcam. Alexa Fluor 488 goat anti-rabbit IgG secondary antibody (Ab11008) and HRP conjugated mouse (Ab150113) and rabbit (Ab6727) secondary antibodies were purchased from Abcam. Ammonium persulfate (Catalog number 161-0180), tris/glycine/SDS buffer (Catalog number 161-0732), Criterion™ TGX Stain-Free™ Gels (Catalog number 5671094), and Clarity™ western ECL substrate

(Catalog number 170-5060) were purchased from Bio-Rad Inc. Nitrocellulose membrane (catalog number 88018), and tween-20 was purchased from Fisher Bioreagents. The Angiotensin Converting Enzyme 2 (ACE2)-Red reporter kit (Catalog number #C1100R) and Pseudo SARS-CoV-2 Green Reporter (Catalog number #C1110G) were purchased from Montana Molecular. Importazole (SML0341) was purchased from Sigma Aldrich. Remdesivir (#329511) was purchased from MedKoo Biosciences. Mitochondrial Complex IV (#700990) and complex V (#701000) activity kits were purchased from Cayman Chemicals. PLGA-*b*-PEG-OH and PLGA-*b*-PEG-MAL polymers were prepared using a previously published method.[2]

Instruments: Dynamic light scattering (DLS) measurements were carried out using a Malvern Zetasizer Nano ZS system. Distilled water was purified by passage through a Millipore Milli-Q Biocel water purification system (18.2 MΩ) containing a 0.22 μm filter. Absorbance analyses were performed on a Bio-Tek Synergy HT microplate reader. High-performance liquid chromatography (HPLC) analyses were made on an Agilent 1200 series instrument equipped with a multi-wavelength UV-visible and a fluorescence detector. Cells were counted using Countess® Automated Cell Counter procured from Invitrogen. Mitochondrial bioenergetics assays were performed on XF^e96 Extracellular Flux Analyzer (Agilent Seahorse Biosciences). CFX Connect System from BIO-RAD used for Real-Time PCR studies. Confocal microscopy images were obtained using an Olympus FluoView FV3000.

Methods:

1. Cell Culture Methods: A transfectable derivative of human embryonic kidney 293 (HEK293T) cells and HeLa malignant cervical epithelial cells were procured from ATCC. These cells were grown in Dulbecco's Modified Eagle Medium (DMEM) along with 10% fetal bovine serum. Cell cultures were maintained in a humidified cell culture incubator at 37 °C and with 5% CO_2. A549 adenocarcinomic alveolar basal epithelial cells were procured from ATCC. These cells were grown in Ham's F-12K (Kaighn's) Medium along with 10% fetal bovine serum. Cell cultures were maintained in a humidified cell culture incubator at 37 °C and with 5% CO_2. Human small airway epithelial cells (HSAEC) were procured from ATCC and grown in Airway Cell Basal Medium (ACBM) with supplements (HSA 500 μg/mL, linoleic acid 0.6 μM, lecithin 0.6 μg/mL, epinephrine 1.0 μM, transferrin 5 μg/mL, T3 10 nM, hydrocortisone 1 μg/mL, rh EGF 5 ng/mL, rh insulin 5 μg/mL), 0.4 %extract P, 1% pen/strep and 6 mM L-glutamine. All the cultures were maintained in a humidified cell culture incubator at 37 °C and with 5% CO_2.

2. Synthesis of Nanoparticle: Nanoparticles were prepared using a previously published method, which is summarized here.[2] A solution of the polymer (10 mg mL^{-1}) and ivermectin (2 mg mL^{-1}) was made in 1 mL of DMF. This solution was added dropwise to 10 mL of nanopure water with constant stirring (900 RPM) at room temperature for 2 h in a fume hood. The NPs were washed 3 times with nanopure water with amicon ultracentrifugation filtration membranes with a molecular weight cutoff of 100 kDa (2800 rpm, 4 °C). The formed NPs were suspended in water and stored at 4 ^{o}C. The NP size and surface charge were obtained from three independent measurements.

The T-Fc-IVM-NPs were created through covalent attachment of the polyglonal IgG Fc fragments to the PLGA-*b*-PEG-Mal nanoparticles using thiolene chemistry. 2-Iminothiolane (4.5 μL from a 5 mg mL^{-1} solution in 5 mM EDTA) was mixed with 35 μL of Fc fragment (2.48 mg mL^{-1}) at 37 °C for 1 h. This mixture was added to the NP solution (3 mg mL^{-1}) and allowed to react at 4 °C for 1 h. The NPs were purified in a similar fashion as mentioned before and the change in diameter and zeta potential of the nanoparticles was studied using DLS. The amount of Fc was quantified using BCA assay.

3. Cell Viability Assay: The cytotoxicity of ivermectin, NT-IVM-NP, and T-Fc-IVM-NP was tested in HEK293T cells using the MTT assay. HEK293T cells were plated in a 96-well plate with a density of 3,000 cells/well and allowed to grow overnight. The articles were added in varying concentrations from 0 to 100 μM with respect to ivermectin. After 24 h, the media was replenished with fresh media and kept the cells for additional 48 h. After the given incubation time, 20 μL/well MTT was added (5 mg/mL stock in PBS) and incubated for 5 h in order for MTT to be reduced to purple formazan. The media was removed and the cells were lysed with 100 μL of DMSO. In order to homogenize the formazan solution, the plates were subjected to 1 min of gentle shaking and the absorbance was read at 550 nm with a background reading at 800 nm with a plate reader. Control values were set to 100% of cell viability. Cytotoxicity data was fitted to a sigmoidal curve and a three-parameter logistic model was used to calculate the IC_{50}, which is the concentration of articles causing 50% inhibition in comparison to untreated controls. The mean IC_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions and is the average from at least three independent

measurements that were reproducible and statistically significant. These analyses were performed with GraphPad Prism.

4. Cellular Uptake of the Articles: HEK293T cells were plated (50,000 cells/well) in a 6-well plate and allowed to grow overnight. The media was changed and the articles were given for time points of 2, 4, 6, and 24 h at a concentration of 10 μM with respect to IVM. After each time point was complete, media was removed and fresh media was kept in the well up to 24 h, so that all wells received articles and fresh media afterward for a total time of 24 h. After the given incubation time, media was removed and cells were washed three times with PBS. Then, cells were collected in 1 mL methanol and run through a Strata C18-T column (previously activated by passing 1 mL of methanol and water through the filter in sequence). The cell suspension was passed through the activated column in order to get rid of remaining debris and impurities. The column was then washed with 1-2 mL of 5% methanol in order to remove the impurities. The ivermectin from the column was then collected in 1 mL of methanol and quantified using HPLC. A Zorbax C18 column was used as a solid phase and a 10:70:20 water:acetonitrile:methanol solution served as the mobile phase. The wavelength of detection used for these experiments was 243 nm and the elution time IVM was at 20.03 min.

5. SARS-CoV-2 Spike Protein Plasmid Purification. Bacterial agar plates (1.5%) containing 100 μg/mL ampicillin were prepared and streaked with SARS-CoV-2 spike protein plasmid and incubated at 37 °C for 16 h. A single colony from the plate was inoculated in 5 mL of LB broth media containing 100 μg/mL ampicillin for primary culture. The culture was incubated at 37 °C in an incubator shaker for 16 h. A 1:100 dilution of

primary culture was used for secondary culture. Spike protein plasmid was isolated using Qiagen Midiprep plasmid isolation kit. Bacterial culture grown overnight was harvested by centrifuging at 1900g for 20 min at 4 °C. The supernatant was discarded and the bacterial pellet was dissolved in 4 mL of buffer P1 and mixed properly. To this, 4 mL of buffer P2 was mixed thoroughly by vigorously inverting the tube four to six times and incubated at room temperature for 5 min. Finally, 4 mL of buffer P3 was mixed thoroughly by vigorously inverting the tube four to six times and incubated on ice for 15 min. The tube was centrifuged at 1900g for 30 min at 4 °C. The Qiagen midi column was equilibrated with 4 mL of buffer QBT and bacterial supernatant was loaded onto the column, and the column was allowed to empty by gravity flow. The plasmid was eluted from the column by using QF buffer. Isopropanol was used to precipitate the plasmid. Finally, plasmid was dissolved in autoclaved water. The purity and quantification of plasmid were checked using Nanodrop by measuring the absorbance ratio at 260/230 nm.

6. ACE2 and Spike Protein Expression by Western Blot: HEK293T cells, HeLa cells, and A549 cells (1×10^5) were each seeded in 6-well plates in 10% FBS-containing DMEM medium and were incubated overnight. Cells were transfected with 2 μg of SARS-CoV-2 spike protein plasmid using the transfection reagent turbofectin (turbofectin: DNA-3:1) in Opti-MEM medium. After 6 h post-transfection, media was removed and replaced with fresh media and incubated for additional 6 h. Cells were treated with 10 μM concentration of IVM, NT-IVM-NP, and T-Fc-IVM-NP for 24 h. In a separate experiment to test the time-dependent effects of free IVM and IVM-NPs, the articles in the same concentration were added to cells for 2, 4, and 6 h, followed by incubation of cells in normal media up to 24 h total. In separate experiment, to study the efficacy of articles against MERS-CoV spike

protein, the HEK293T cells were transfected with 1 μg of MERS-CoV spike protein plasmid using the transfection reagent turbofectin (turbofectin: DNA-3:1) in Opti-MEM medium. After 6 h post-transfection, media was removed and replaced with fresh media and incubated for additional 6 h. For experiments studying the viral spike protein expression for both SARS-CoV-2 and MERS-CoV, in which remdesivir was used, the remdesivir treatment was conducted for 4 h at a concentration of 10 μM, followed by incubation of cells in normal media up to 24 h total. Cells were lysed with RIPA buffer and the total cell lysate (30 μg) was resolved on a 4−20% gradient gel. Proteins were transferred to PVDF membrane and probed with HA-tag (1:1000), ACE2 (1:1000), and β-actin (1:1000) antibodies overnight at 4 °C. After 3 TBST washes, the membrane was probed with HRP-conjugated secondary antibody (1:2000) for 1 h at room temperature. The membrane was washed 5 times with TBST and developed using Super Signal west pico-chemiluminescence substrate (Thermo Scientific). Densitometric analysis of western blots was performed using ImageJ software.

7. Importin Proteins expression by Western Blot: HEK293T cells (1×10^5) were each seeded in 6-well plates in 10% FBS-containing DMEM medium and were incubated overnight. Cells were transfected with 2 μg of SARS-CoV-2 spike protein plasmid using the transfection reagent turbofectin (turbofectin: DNA-3:1) in Opti-MEM medium. After 6 h post-transfection, media was removed and replaced with fresh media and incubated for additional 6 h. IVM and its nanoformulations were added to cells at 10 μM concentrations with respect to IVM to cells for 2, 4, and 6 h, followed by incubation of cells in normal media up to 24 h total. In experiments where remdesivir was used, remdesivir treatment was conducted for 4 h at a concentration of 10 μM, followed by incubation of cells in

normal media up to 24 h total. Cells were lysed with RIPA buffer and the total cell lysate (30 μg) was resolved on a 4−20% gradient gel. Proteins were transferred to PVDF membrane and probed with IMPα (1:1000), IMPβ1 (1:1000), and β-actin (1:1000) antibodies overnight at 4 °C. After 3 TBST washes, the membrane was probed with HRP-conjugated secondary antibody (1:2000) for 1 h at room temperature. The membrane was washed 5 times with TBST and developed using Super Signal west pico-chemiluminescence substrate (Thermo Scientific). Densitometric analysis of western blots was performed using ImageJ software.

8. ACE2 and Spike Protein Expression by Immunofluorescence: HEK293T cells were each plated on coverslips in separate 12-well plates with density of 20,000 cells/well in 1 mL of DMEM (with 10% FBS) media. Cells were transfected with 2 μg of SARS-CoV-2 spike protein plasmid using transfection reagent turbofectin (turbofectin: DNA-3:1) in Opti-MEM medium. After 6 h post transfection, medium was removed and replaced with fresh medium and incubated for additional 6 h. Cells were treated with IVM, NT-IVM-NP, or T-Fc-IVM-NP at a concentration of 10 μM with respect to IVM for 4 h, after which media was replaced for an additional 20 h. The cells were washed with PBS (1X) 3 times and fixed with 4% paraformaldehyde for 1 h at 37 °C. After performing 3 washes, the cells were permeabilized using 0.1% Triton-X100 for 10 min at 37 °C. The cells were washed with 1X PBS 3 times and blocked with 1% goat serum in 1X PBS for 12 h. Cells were treated with the respective primary antibody in 1% goat serum containing 1X PBS) for 12 h at 4 °C in humidified chamber. After washing the cells three more times with 1% goat serum containing 1X PBS, the respective secondary antibody (Alexa 488 conjugated antibody) solution in 1% goat serum containing 1X PBS was added along with DAPI and incubated

for 1 h at room temperature. Cells were finally washed three more times with 1% goat serum containing 1X PBS. The membrane was gently removed and kept on glass slides and covered with coverslips using mounting solution (n-propyl gallate, Tris and glycerol in nanopure water, pH = 8.0). Confocal images were recorded using an Olympus FluoView FV3000 confocal microscope using 405/460 nm for DAPI and 488/510 nm for Alexa488. Sampling speed was kept as 8.0 us/pixel (0.56 min per image). In a similar way, the ACE2 expression in HeLa and A549 cells was studied.

9. RT-PCR: HEK293T cells were seeded in 6-well plate and transfected with 2 μg of plasmid using turbofectin. Cell were treated with ivermectin, NT-IVM-NP and T-Fc-IVM-NP at a concentration of 10 μM with respect to IVM and remdesivir for 4 h, followed by incubation of cells in normal media up to 24 h total. In experiments where remdesivir was used, remdesivir treatment was conducted for 4 h at a concentration of 10 μM, followed by incubation of cells in normal media up to 24 h total. RNA was extracted using a kit from Qiagen. Briefly, cells were harvested with trypsin and lysed with buffer RLT. 1 volume of 70% ethanol was added to the cell lysate and mixed well. Lysate were transferred to RNeasy mini spin column and centrifuged for 1 minute at 8000 rpm. Flow-through was discarded. 700 μL of buffer RW1 was added to the mini spin column and centrifuged for 1 for minute at 8000 rpm. Flow-through was discarded. 500 μL of buffer RPE was added to the mini spin column and centrifuged for 1 minute at 8000 rpm. RNA was recovered from mini spin column using RNase-free water. Purity and concentration of RNA was checked using Nanodrop. Reverse transcription from each sample was carried out using 1 μg of RNA from each sample in a 20 μL reaction volume using iScript Reverse Transcription Supermix for RT-qPCR kit from Bio-Rad. Real time PCR reaction was

carried out using SsoAdvanced Universal SYBR® Green Supermix in 20 μL reaction using Bio-Rad machine. β-actin was used as an internal control. Data were analyzed according to the comparative Ct value and expressed as fold change $2^{-\Delta\Delta CT}$. The forward and reverse primer sequence was 5'GCAGTACGCCATGTAACGGA3' and 5'CGTGGAGGAGCTCAAAGGAC 3', respectively, for the spike protein gene. The primer for human ACE2 gene was purchased from Sino Biological Inc (catalogue number: HP100185). The primer sequence for β-actin gene was: forward 5' GCATCCTCACCCTGAAGTAC 3' and reverse 5' GATAGCACAGCCTGGATAGC 3'. The primers for MERS-CoV spike gene were purchased from Sigma-Aldrich (pCMV3-F, 5' CAGGTGTCCACTCCCAGGTCCAAG 3'; pcDNA3-R, 5' GGCAACTAGAAGGCACAGTCGAGG 3').

10. Enzyme-linked immunosorbent assay (ELISA) to determine immunogenic effect: The levels of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α in the supernatant of treated cells was determined using ELISA kits for the respective cytokines following the manufacturer's protocol. Briefly, 100 μL/well of coating buffer with capture antibody was added to the 96-well ELISA plate. After that, the plate was sealed and kept overnight at 4 °C. The buffer was aspirated from the wells and the cells were washed 3 times with 300 μL/well of wash buffer. After the last wash, the plate was inverted and blotted on absorbent paper to remove any residual buffer. Wells were blocked using 200 μL/well of assay diluent and incubated at room temperature for 1 h. The diluent was aspirated from the wells and the wells were washed as mentioned above. Then, 100 μL of each standard in assay diluent, as well as the samples (50 μL of plasma sample), were added to the appropriate wells. The plate was then sealed and incubated for 2 h at room temperature.

The solutions were then aspirated from the wells and they were washed five times as mentioned above. 100 μL of detection antibody (IL-1β, IL-6, and TNF-α) diluted in assay diluent was added to each well, then the plate was sealed and incubated for 1 h at room temperature. Similar washing steps were carried out five times. Then, 100 μL of enzyme reagent diluted in assay diluent was added to each well. The plate was sealed and incubated at room temperature for 30 min. After the reagent was aspirated from wells, similar washing steps were carried out seven times. Substrate solution (100 μL) was added to each well, and the plate was sealed and incubated for 30 minutes at room temperature in the dark. After this, 50 μL of stop solution was added to each well, and the absorbance was recorded at 450 nm.

11. Pseudo SARS-CoV-2 Reporter Assay and Virus Uptake: The Angiotensin Converting Enzyme 2 (ACE2)-Red reporter assay and Pseudo SARS-CoV-2 Green Reporter assay were performed with the goal of increasing ACE2 expression in HEK293T cells and A549 cells and observing the effects of IVM-loaded nanoparticle treatment on ACE2 expression and SARS-CoV-2 virus cell entry. These effects were measured through fluorescence imaging. Through these tests, we aimed examine two different methods of treatment: a preventive method, in which IVM and IVM nanoformulation treatment preceded pseudovirus infection in the cells, and a therapeutic method, in which IVM and IVM nanoformulation treatment followed pseudovirus infection in the cells. The preventive method's aim was to decrease ACE2 levels initially through IVM treatment so that the rate at which pseudoviruses entered cells would decrease. In contrast, the therapeutic method aimed to interrupt ACE2 and pseudovirus binding and show a lowering in pseudovirus infection presence after the treatment.

a. Pseudo SARS-CoV-2 Uptake by Plate Reader

HEK293T cells were detached using a standard trypsinization protocol and then counted. Cells were prepared for plating in a 96-well plate at 50,000 cells per well, using 100 μL of media for each well. These cells were maintained in a single test tube at 500,000 cells per mL, ready to be mixed with the viral transduction reaction. For each well with a transduction reaction, using the Red ACE2 Reporter kit (C1100R), 5 μL of the fluorescent ACE2 BacMam was mixed with 0.6 μL of the 500 mM sodium butyrate (SB) solution and 44.4 μL of the complete culture media for the HEK293T cells, for a total volume of 50 μL. Next, the tube containing cells and tube containing the transduction reaction mix were mixed and kept for 5 minutes at room temperature. 150 μL of this mixture was added to each well of the 96-well plate. Cells were then incubated for 48 h under standard cell growth conditions. Then cells were treated with IVM, NT-IVM-NPs, and T-Fc-IVM-NPs at a concentration of 10 μM with respect to IVM. The cells were treated with articles for 4 h, the media was replenished and kept for an additional 20 h of incubation. Then, 100 μL of media containing 8 x 10^7 viral genes of pseudo-SARS-CoV-2 were added to each well from the stock solution of 2 x 10^{10} viral genes per mL (C1110G). The cells were then incubated for 12 h in the dark, under normal cell growth conditions. Prior to fluorescence reading, the cells were washed (2 times, 1X PBS) gently. Finally, 100 μL of PBS was added and the florescence levels were measured using plate reader measurements at 506/517 (Ex/Em) and 558/603 (Ex/Em). This described procedures details the preventative method, but the therapeutic option was also explored, in which after the pseudovirus addition, IVM and IVM-loaded nanoparticle treatments was carried out. Besides this change in order, the rest of the protocol was kept the same.

b. Pseudo SARS-CoV-2 Uptake by Confocal Microscopy

Pseudovirus uptake was also observed using confocal microscopy in HEK293T and HSAEC cells. The cells were detached using a standard trypsinization protocol and then counted. Cells were prepared for plating in an 8-well live cell imaging chamber at 20,000 cells per well, using 100 μL of media for each well. These cells were maintained in a single test tube at 500,000 cells per mL, ready to be mixed with the viral transduction reaction. For each well with a transduction reaction, using the Red ACE2 Reporter kit (C1100R), 5 μL of the fluorescent ACE2 BacMam was mixed with 0.6 μL of the 500 mM sodium butyrate (SB) solution and 44.4 μL of the complete culture media for the HEK293T cells, for a total volume of 50 μL. Next, 150 μL of this mixture was added to each well. Cells were then incubated for 48 hours under standard cell growth conditions. Then cells were treated with IVM, NT-IVM-NPs, and T-Fc-IVM-NPs at a concentration of 10 μM with respect to IVM. The cells were treated with articles for 4 h, the media was replenished and kept for an additional 20 h of incubation. Then, 100 μL of media containing 8 x 10^7 viral genes of pseudo-SARS-CoV-2 were added to each well from the stock solution of 2 x 10^{10} viral genes per mL (C1110G). The cells were then incubated for 12 h in the dark, under normal cell growth conditions. Prior to imaging, the cells were washed (2 times, 1X PBS) gently. Finally, 100 μL of phenol red free media was added and images were obtained using an Olympus FluoView FV3000 confocal microscope. The images were visualized for Red at Ex/Em = 561/593 and for green at Ex/Em = 516/561. Sampling speed was kept as 8.0 us/pixel (0.56 min per image).

12. Pseudovirus Assay for Effect of Importazole

HEK293T cells were detached using a standard trypsinization protocol and then counted. Cells were prepared for plating in a 96-well plate at 50,000 cells per well, using 100 μL of media for each well. These cells were maintained in a single test tube at 500,000 cells per mL, ready to be mixed with the viral transduction reaction. For each well with a transduction reaction, using the Red ACE2 Reporter kit (C1100R), 5 μL of the fluorescent ACE2 BacMam was mixed with 0.6 μL of the 500 mM sodium butyrate (SB) solution and 44.4 μL of the complete culture media for the HEK293T cells, for a total volume of 50 μL. Next, the tube containing cells and tube containing the transduction reaction mix were mixed and kept for 5 min at room temperature. 150 μL of this mixture was added to each well of the 96-well plate. This plate was covered to protect from light, and kept for 30 minutes at room temperature. Cells were then treated with 20 μM importazole and incubated for 24 h under standard cell growth conditions. Then cells were treated with IVM, NT-IVM-NPs, and T-Fc-IVM-NPs at a concentration of 10 μM with respect to IVM. These treatments were kept for 4 h, after which media was removed and replaced with fresh media for an additional 20 h. Then, 100 μL of media containing 8×10^7 viral genes of pseudo-SARS-CoV-2 were added to each well from the stock solution of 2×10^{10} viral genes per mL (C1110G). Control cells were treated with a bald control, a spike-less pseudovirus. Cells were incubated for 24 h at normal incubation conditions, after which media was changed to remove the pseudovirus and replaced with fresh media containing the 2 mM sodium butyrate. Prior to fluorescence reading, the cells were washed (2 times, PBS) gently. Finally, 100 μL of PBS was added and the florescence levels were measured using plate reader measurements at 506/517 (Ex/Em) and 558/603 (Ex/Em).

13. Mitostress Assay: Different parameters of mitochondrial respiration such as basal respiration, maximal respiration, and ATP production were investigated using Seahorse XF^e96 Analyzer. One day prior to the assay, XF sensor cartridges were hydrated using 200 μL of XF calibrant buffer and kept at 37 °C incubator without CO_2 overnight. HEK293T cells were plated at a density of 20,000 cells per well in 80 μL DMEM media (with 10% FBS) and the plate was kept 1 h at room temperature followed by incubation at 37°C with 5% CO_2 for 3 h. Finally, 130 μL of fresh media was added to have total 180 μL per well and incubated for 16 h. These cells were transfected with SARS-CoV-2 spike protein plasmid as previously described and later on, media was aspirated and various compounds (10 μM with respect to IVM) were added so that final volume in each well was 180 μL. They were incubated for additional 24 h. Before conducting the Mitostress assay, Seahorse media (XF Assay Medium Modified DMEM) was reconstituted with glucose (1.8 mg/mL), sodium pyruvate (1%) and L-glutamine (1%) and adjusted for pH 7.4 by using 0.1 N NaOH. The cells were washed thrice with freshly prepared seahorse medium and incubated at 37 °C in non-CO_2 incubator for 1 h. Meanwhile, cartridge ports were added with various inhibitors. The stocks of oligomycin (10 μM), FCCP (10 μM) and antimycin-A/rotenone mixture (10 μM each) were made in seahorse media. The port A was filled with 20 μL of oligomycin, port B with 22 μL of FCCP and port C with 25 μL of antimycin A/rotenone to have a final concentration of 1 μM in each well. The cartridge was calibrated for pH and O_2. After calibration, the experiment plate was run where 3 measurements were recorded for basal OCR and after addition of each reagent. The media was aspirated and 20 μL of RIPA buffer was added to each well and incubated for 10 mins at 37 ^oC. Further BCA assay was performed to obtain protein normalized OCR values.

14. Mitochondrial Complex Assays

a. Mitochondrial Complex IV activity assay: HEK293T cells were seeded in 6-well plates in with the density of 1 × 10^5 cells per well and were incubated overnight. Cells were transfected with 2 μg of SARS-CoV-2 spike protein plasmid using the transfection reagent turbofectin (turbofectin: DNA-3:1) in Opti-MEM medium. After 6 h of transfection, media was removed and replaced with fresh media and incubated for additional 6 h. IVM and its nanoformulations were added to cells at 10 μM concentrations with respect to IVM for 4 h followed by incubation of cells in normal media up to 24 h total. The cells were washed with 1X PBS two times. The cells were lysed using cells lysis buffer, provided in the kit. The assay was conducted in accordance with the manufacturer's protocols. Briefly, reagent A was prepared using complex IV assay buffer and cell lysate. 50 μL of this solution was added to each well. The cell lysate was incubated for 5 minutes while the plate reader was brought to 37 ºC. Immediately before the absorbance was read, reagent B was added to each well. Reagent B was comprised of complex IV assay buffer and reduced cytochrome c assay reagent. The absorbance was then read on kinetic mode for 30 minutes immediately at 550 nm. BCA was carried out on cell lysate in order to normalize the results with mg per protein.

b. Mitochondrial Complex V Activity Assay: HEK293T cells were seeded in 6-well plates in with the density of 1 × 10^5 cells per well and were incubated overnight. Cells were transfected with 2 μg of SARS-CoV-2 spike protein plasmid using the transfection reagent turbofectin (turbofectin: DNA-3:1) in Opti-MEM medium. After 6 h of transfection, media was removed and replaced with fresh media and incubated for additional 6 h. IVM and its nanoformulations were added to cells at 10 μM concentrations with respect to IVM

for 4 h followed by incubation of cells in normal media up to 24 h total. The cells were washed with 1X PBS two times. The cells were lysed using cells lysis buffer, provided in the kit. The assay was conducted in accordance with the manufacturer's protocols. Briefly, reagent A was prepared using complex V assay buffer, rotenone and cell lysate. 50 μL of this solution was added to each well or positive control. Here, 1 μM of antimycin A was used as positive control. The reaction mixture was incubated for 5 minutes while the plate reader was brought to 25 ºC. Immediately before the absorbance was read, reagent B was added to each well. Reagent B was comprised of complex V assay enzyme mix, complex V ATP reagent, and complex V NADH reagent. The absorbance was then read on kinetic mode for 30 minutes at 340 nm. BCA was carried out on cell lysate in order to normalize the results with mg per protein.

Table S1. Completed Clinical Studies for COVID-19 Therapy					
Sponsor	**Title**	**ClinicalTrials.gov Identifier**	**Therapeutic(s)**	**Type of trial**	**Results**
Guangzhou Eighth People's Hospital Guangzhou, Guangdong, China	The Efficacy of Lopinavir Plus Ritonavir and Arbidol Against Novel Coronavirus Infection	NCT04252885	Lopinavir, ritonavir, arbidol	Phase 4	Completed, but no results posted
Shaare-Zedek Medical Center Jerusalem, Israel	Compassionate Use of Opaganib in Patients with Coronavirus Disease 2019 (COVID-19)	NCT04435106	Opaganib	Retrospective Observational Study	Completed, but no results posted
The University of Hong Kong	Lopinavir/ Ritonavir, Ribavirin and IFN-beta Combination for nCoV Treatment	NCT04276688	Lopinavir/ritonavir, ribavirin, IFN β-1B	Phase 2	Completed, but no results posted
National Institute of Allergy and Infectious Diseases (NIAID)	A Safety, Tolerability, Pharmacokinetics and Immunogenicity Trial of Co-administered MERS-CoV Antibodies REGN3048 and REGN3051	NCT03301090	MERS-CoV Antibodies REGN3048, REGN3051	Phase 1	Completed, but no results posted
Fundação de Medicina Tropical Dr. Heitor Vieira Dourado	Chloroquine Diphosphate for the Treatment of Severe Acute Respiratory Syndrome Secondary to SARS-CoV2 (CloroCOVID19)	NCT04323527	Chloroquine diphosphate	Phase 2	Completed, but no results posted
Marmara University	Ozone Therapy in the Prevention of COVID-19 Infection	NCT04400006	Ozone	Observational Trial	Completed, but no results posted
University of Trieste	Methylprednisolone for Patients With COVID-19 Severe Acute Respiratory Syndrome (MP-C19)	NCT04323592	Methylprednisolone	Observational Trial	Patients treated with methylprednisolone showed lower admission to ICU, need for invasive mechanical ventilation (intubation), and all-cause death by day 28
University of Minnesota	Post-exposure Prophylaxis / Preemptive Therapy for	NCT04308668	Hydroxychloroquine	Phase 3	Completed, but no results posted

	SARS-Coronavirus-2 (COVID-19 PEP)				
Shanghai Public Health Clinical Center	Efficacy and Safety of Hydroxychloroquine for Treatment of COVID-19	NCT04261517		Phase 3	Completed, but no results posted
Ain Shams University	Efficacy and Safety of Favipiravir in Management of COVID-19 (FAV-001)	NCT04349241	Favipiravir	Phase 3	Completed, but no results posted
Hospital Israelita Albert Einstein	Safety and Efficacy of Hydroxychloroquine Associated with Azithromycin in SARS-CoV2 Virus (Coalition Covid-19 Brasil II)	NCT04321278	Hydroxychloroquine + azithromycin	Phase 3	Completed, but no results posted
A.O. Ospedale Papa Giovanni XXIII	An Observational Study of the Use of Siltuximab (SYLVANT) in Patients Diagnosed With COVID-19 Infection Who Have Developed Serious Respiratory Complications (SISCO)	NCT04322188	Siltuximab (SYLVANT)	Retrospective Observational Trial	Completed, but no results posted
University of Milan	Enhanced Platelet Inhibition in Critically Ill Patients With COVID-19 (PIC-19)	NCT04368377	Tirofiban Injection, Clopidogrel, Acetylsalicylic acid, Fondaparinux	Phase 2	Completed, but no results posted
Shahid Beheshti University of Medical Sciences	An Investigation into Beneficial Effects of Interferon Beta 1a, Compared to Interferon Beta 1b And the Base Therapeutic Regiment in Moderate to Severe COVID-19: A Randomized Clinical Trial (COVIFERON)	NCT04343768	Hydroxychloroquine, Lopinavir / Ritonavir, Interferon Beta-1A, Interferon Beta-1B	Phase 2	Completed, but no results posted

Table S2. Ongoing Clinical Studies for COVID-19 Therapy				
Sponsor	**Title**	**ClinicalTrials.gov Identifier**	**Therapeutic(s)**	**Type of trial**
Eurnekian Public Hospital	IVERMECTIN Aspirin Dexametasone and Enoxaparin as Treatment of Covid 19	NCT04425863	Ivermectin, aspirin, dexamethasone, and enoxaparin	Prospective Observational Trial
Washington University School of Medicine	Duvelisib to Combat COVID-19	NCT04372602	Duvelisib	Phase 2
Tufts Medical Center	A Phase 2 Trial of Infliximab in Coronavirus Disease 2019 (COVID-19).	NCT04425538	Infliximab	Phase 2
Tanta University	Safety and Efficacy of Tramadol in COVID-19 Egyptian Patients	NCT04454307	Tramadol	Phase 1 / Phase 2
Vicore Pharma AB	Safety and Efficacy of C21 in Subjects With COVID-19	NCT04452435	C21 (Compound 21)	Phase 2
Kermanshah University of Medical Sciences	Application of Desferal to Treat COVID-19	NCT04333550	Deferoxamine	Phase 1 / Phase 2
Acibadem University	Dornase Alpha for the Treatment of COVID-19	NCT04432987	Pulmozyme	Phase 2
Guiliano Rizzardini	Clinical Study to Evaluate the Performance And Safety Of Favipiravir in COVID-19	NCT04336904	Favipiravir	Phase 3
Fundacion SEIMC-GESIDA	Clinical Trial to Evaluate the Effectiveness and Safety of Tocilizumab for Treating Patients With COVID-19 Pneumonia	NCT04445272	Tocilizumab	Phase 2
Tanta University	Isotretinoin in Treatment of COVID-19 (Randomized)	NCT04361422	Isotretinoin (oral)	Phase 3
Cairo University	Utility of Lactoferrin as an Adjunct Therapeutic Agent for COVID-19	NCT04421534	Lactoferrin	Phase 2 / Phase 3
Bukwang Pharmaceutical	The Phase 2 Study to Evaluate the Safety and Efficacy of Clevudine in Patients with Moderate COVID-19	NCT04347915	Clevudine	Phase 2
Abivax S.A.	ABX464 in Treating Inflammation and	NCT04393038	ABX464	Phase 2 / Phase 3

	Preventing Acute Respiratory Failure in Patients With COVID-19 (Mir-Age)			
Masonic Cancer Center	Study of FT516 for the Treatment of COVID-19 in Hospitalized Patients with Hypoxia	NCT04363346	FT516	Phase 1
Kermanshah University of Medical Sciences	Colchicine Plus Phenolic Monoterpenes to Treat COVID-19	NCT04392141	Colchicine, Herbal Phenolic Monoterpene Fractions	Phase 1 / Phase 2
InflaRx GmbH	Open-label, Randomized Study of IFX-1 in Patients with Severe COVID-19 Pneumonia (PANAMO)	NCT04333420	IFX-1	Phase 2 / Phase 3

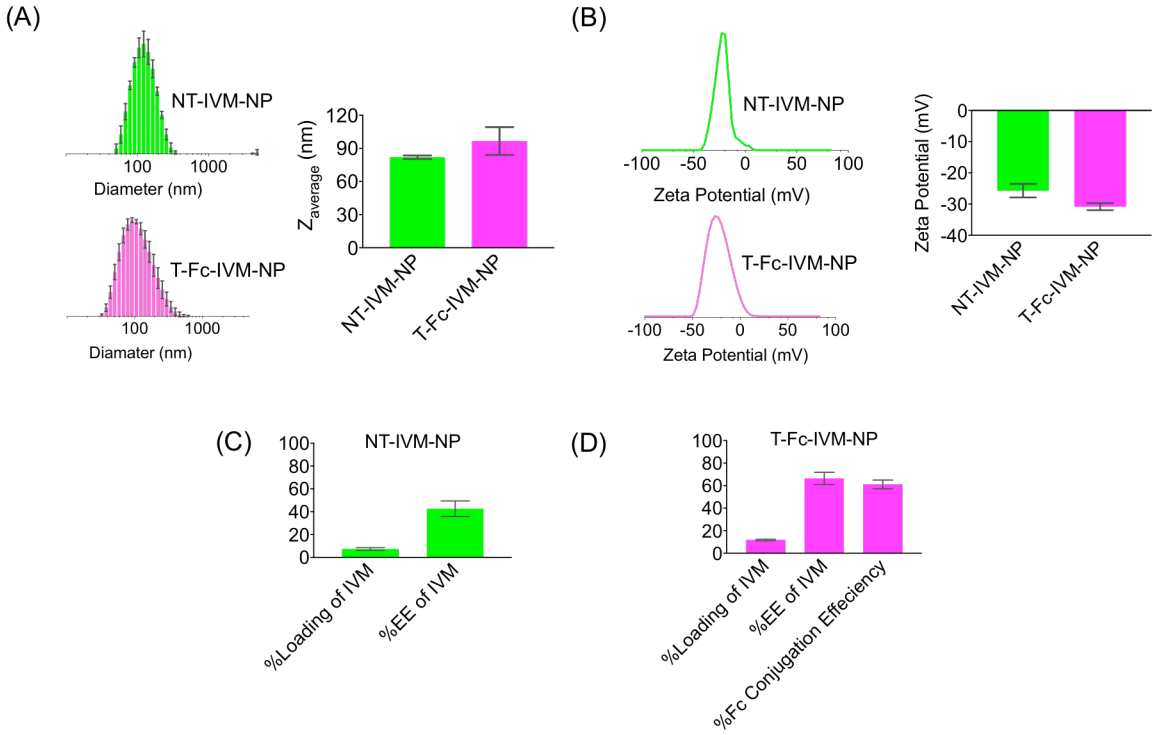

Figure S1. (A) Diameter and (B) zeta potential of NT-IVM-NP and T-Fc-IVM-NP. (C) %loading and %EE of IVM in NT-NPs, and (D) %loading, %EE of IVM and %Fc conjugation in T-Fc-IVM-NPs.

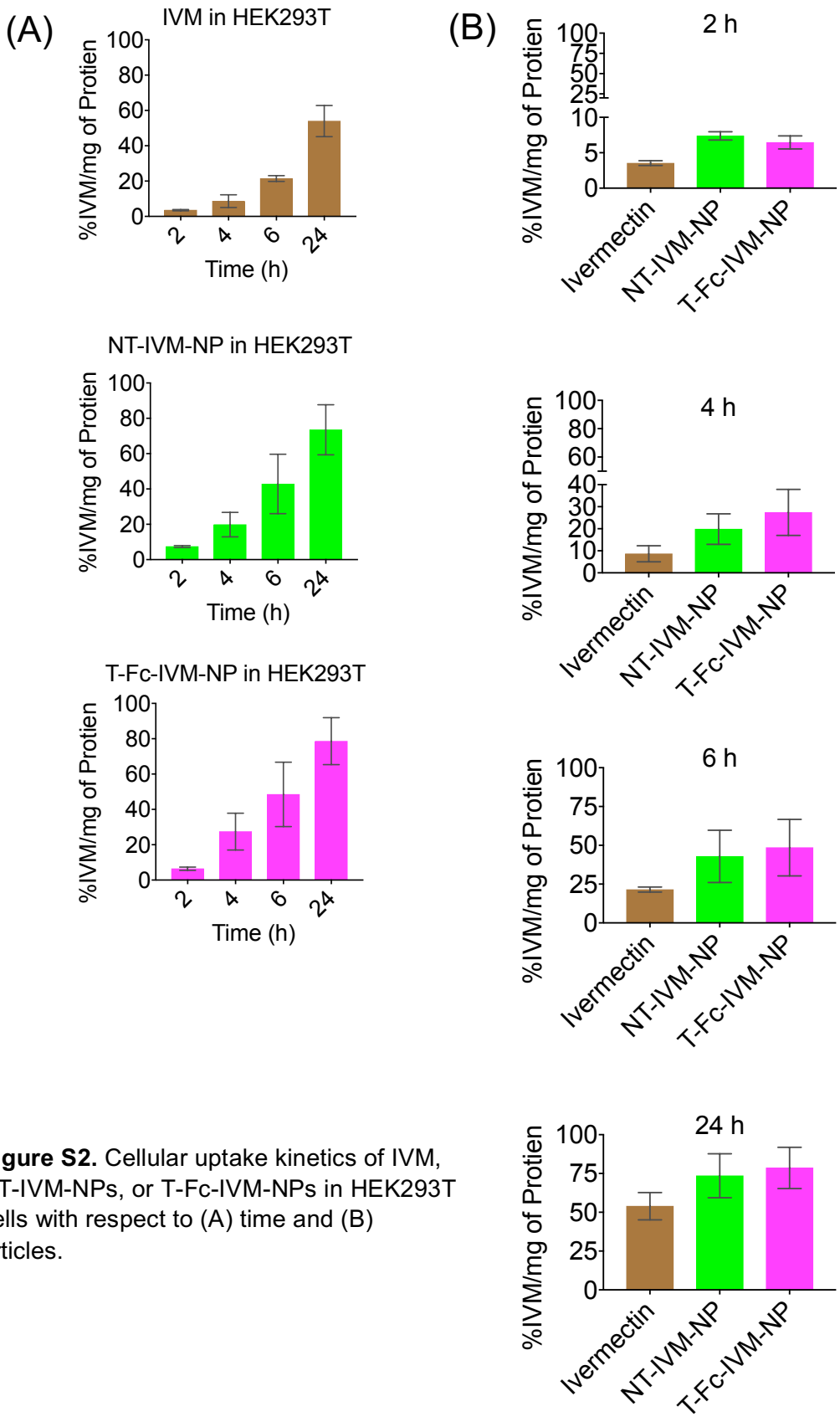

Figure S2. Cellular uptake kinetics of IVM, NT-IVM-NPs, or T-Fc-IVM-NPs in HEK293T cells with respect to (A) time and (B) articles.

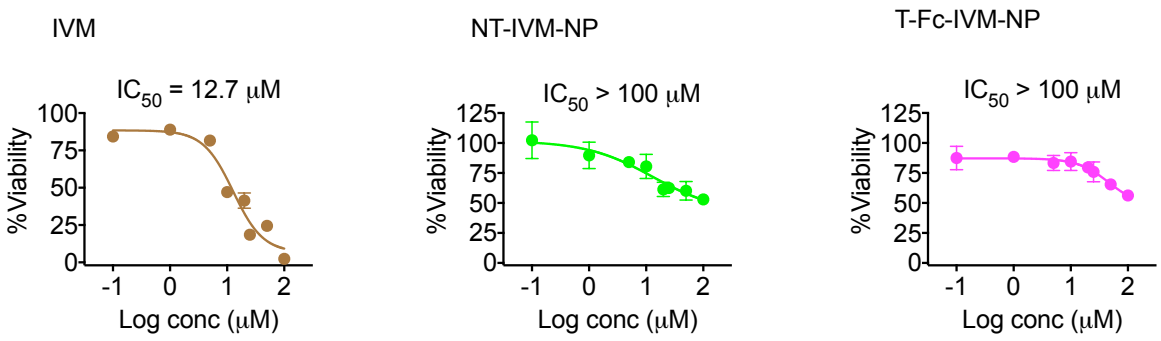

Figure S3. Cytotoxicity of IVM, NT-IVM-NPs, and T-Fc-IVM-NPs in HEK293T cells as determined by the MTT assay. The cells were treated with articles for 24 h, after which media was replenished and followed by an additional 48 h of incubation.

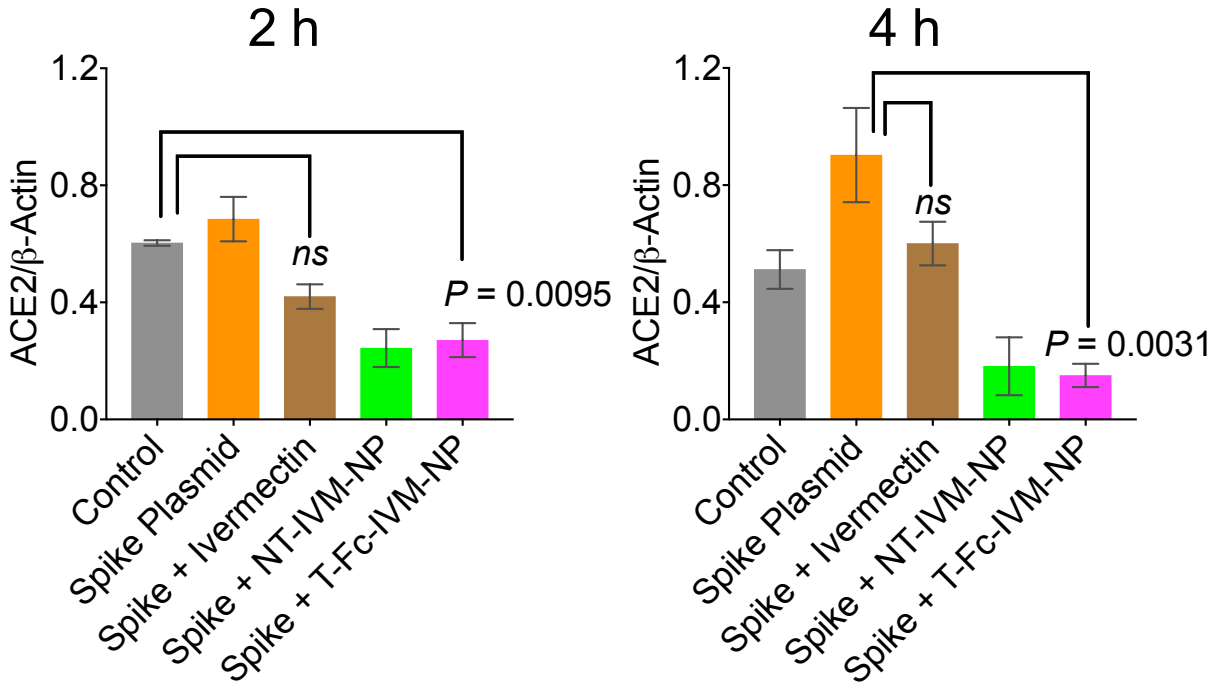

Figure S4. Densitometric analyses of western blots showing expression of ACE2 protein in HEK293T cells transfected with plasmid expressing spike protein with a treatment of IVM, NT-IVM-NPs, or T-Fc-IVM-NPs. Cells were treated with the articles for 2 h or 4 h at a concentration of 10 μM with respect to IVM, the media was changed, and further incubated up to a total of 24 h. Analyses were performed by ImageJ software.

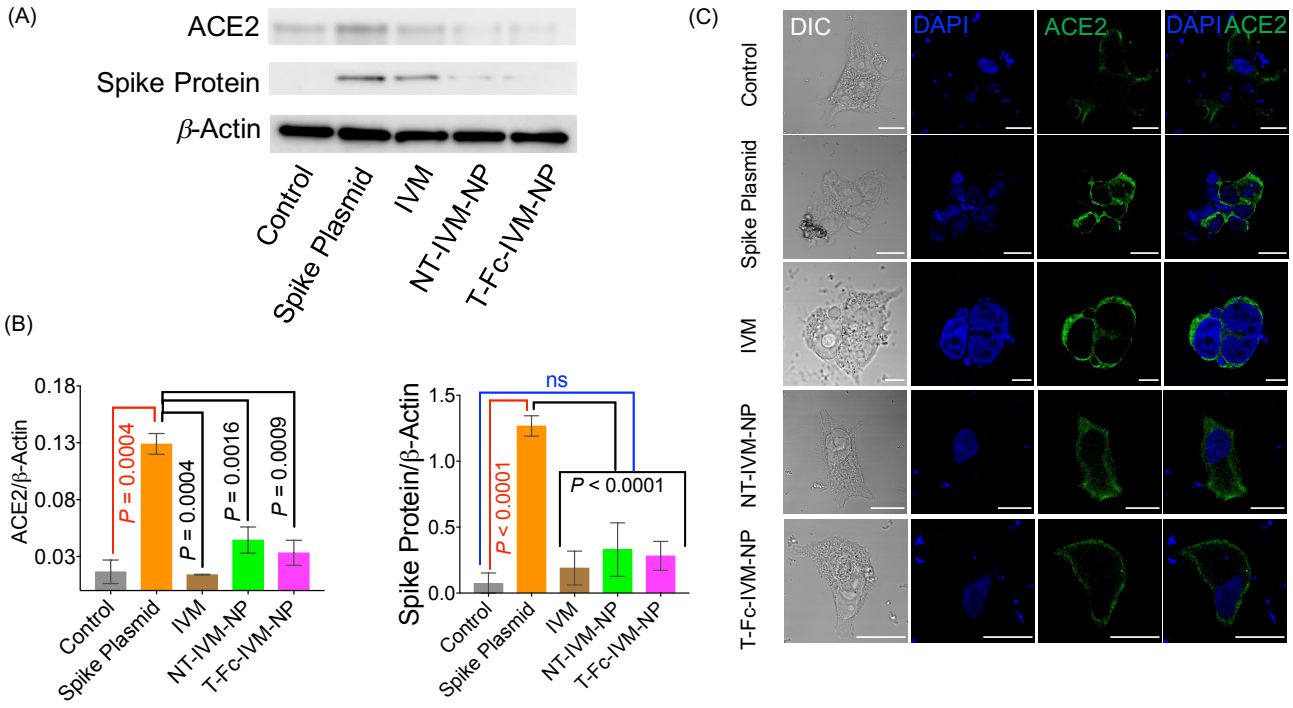

Figure S5. (A) Western blot analysis showing expression of ACE2 and spike protein in HEK293T cells transfected with a plasmid expressing spike protein, with treatment of IVM, NT-IVM-NPs, or T-Fc-IVM-NPs. (B) Densitometric analyses of the western blots showing expression of ACE2 and spike protein. (C) Immunofluorescence staining showing expression of ACE2 in HEK293T cells transfected with plasmid expressing spike protein with treatment of IVM, NT-IVM-NPs, or T-Fc-IVM-NPs. Cells were treated with the articles for 24 h at a concentration of 10 μM with respect to IVM.

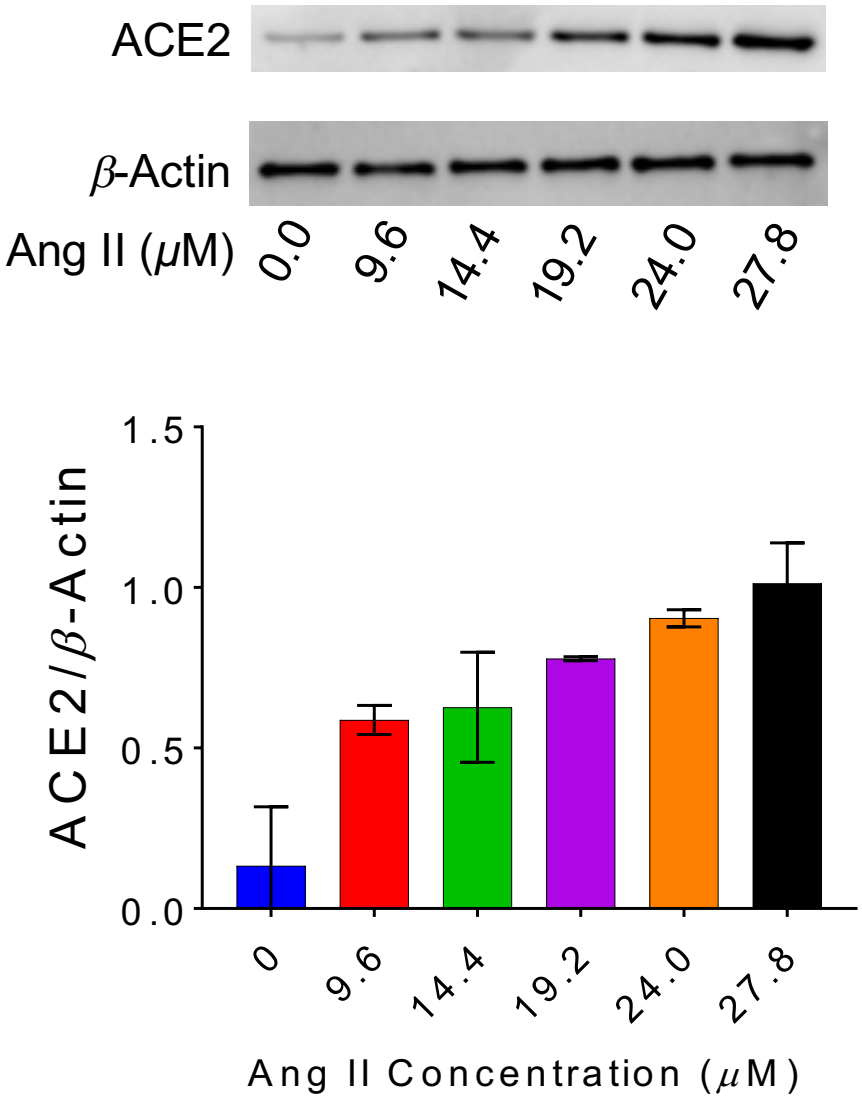

Figure S6. Western blot and densitometric quantification showing increased expression of ACE2 in HEK293T cells upon treatment with Ang II at various concentrations.

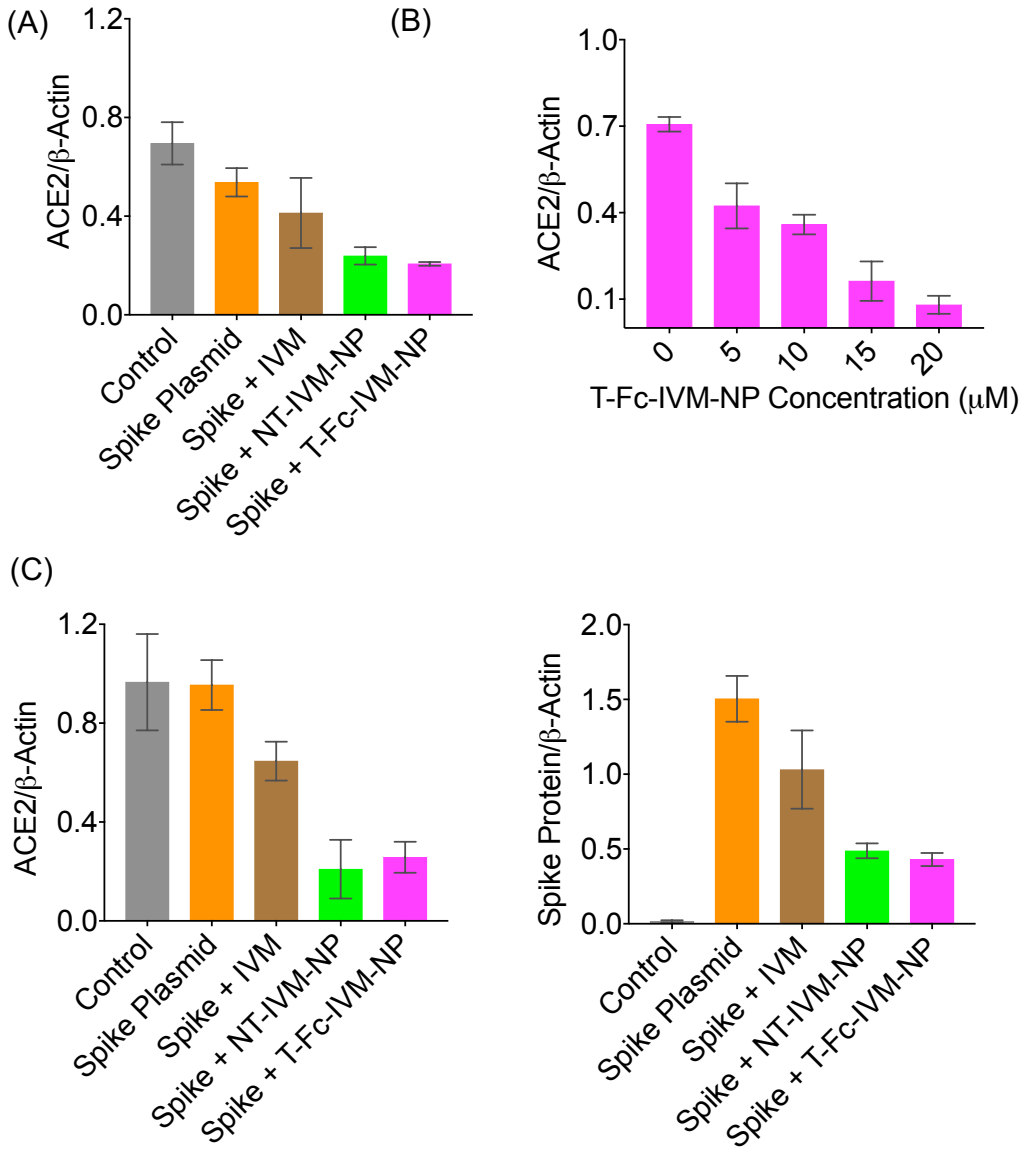

Figure S7. (A) Densitometric analyses of western blots showing expression of ACE2 in A549 adenocarcinomic alveolar basal epithelial cells transfected with a plasmid expressing spike protein, with treatment of IVM, NT-IVM-NPs, or T-Fc-IVM-NPs. Cells were treated with the articles for 4 h at a concentration of 10 μM with respect to IVM. (B) Densitometric analyses of western blots showing dose-dependent decrease in ACE2 expression after treatment with varying concentrations of T-Fc-IVM-NPs with respect to IVM in A549 cells. (C) Densitometric analyses of western blot showing expression of ACE2 and spike in HeLa malignant epithelial cells transfected with a plasmid expressing spike protein, with treatment of IVM, NT-IVM-NPs, or T-Fc-IVM-NPs. Cells were treated with the articles for 4 h at a concentration of 10 μM with respect to IVM.

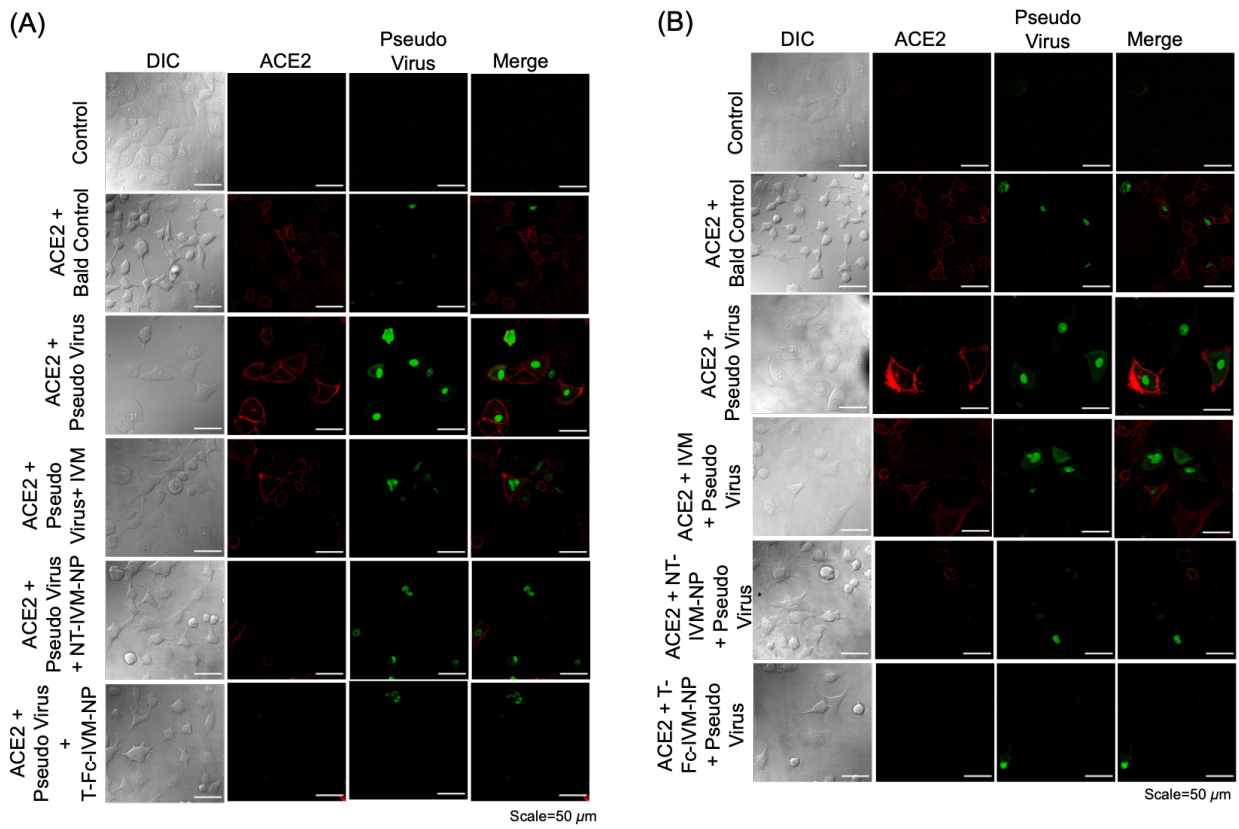

Figure S8. Accumulation of mNeonGreen pseudovirus reporter protein in human small airway epithelial cells (HSAEC) and the efficacy of articles showing inhibition of both ACE2 and pseudovirus uptake under (A) therapeutic and (B) preventative treatment methods as evaluated by confocal microscopy. The article concentration was kept at 10 μM with respect to IVM for 4 h followed by additional 20 h of incubation.

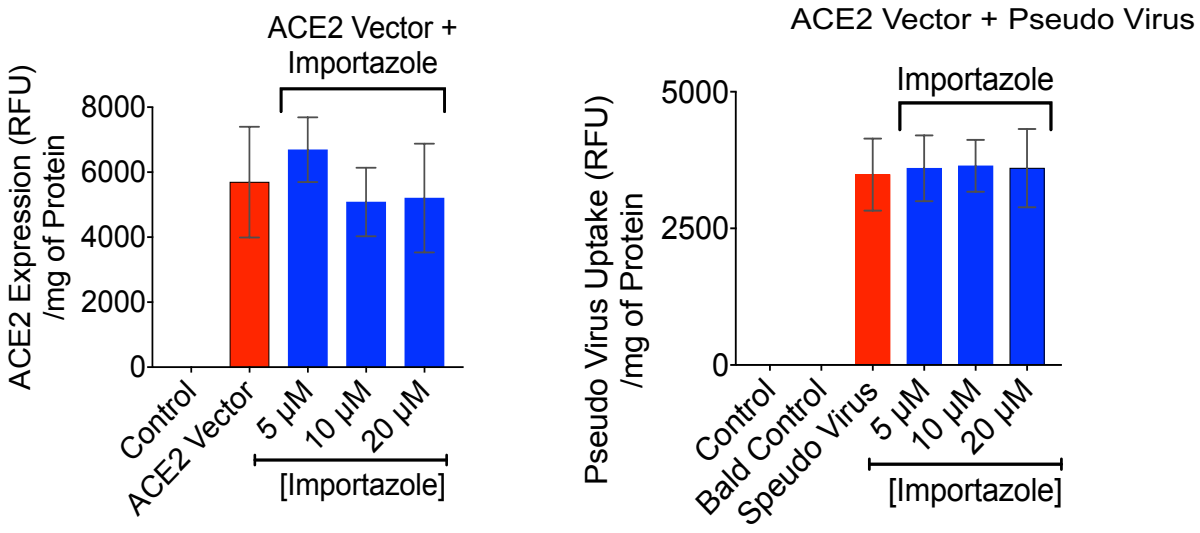

Figure S9. Effects of importazole at varying concentrations on both ACE2 expression and mNeonGreen pseudovirus uptake as measured by the microplate reader.

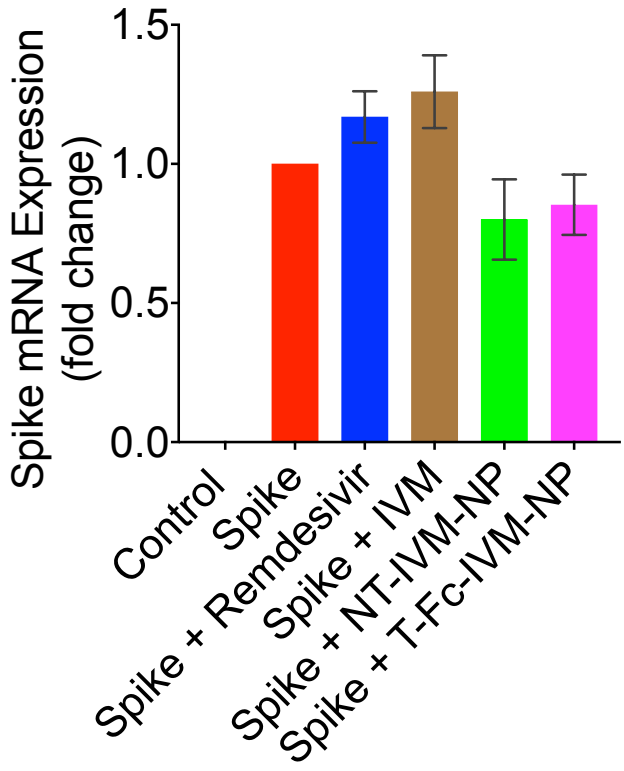

Figure S10. RT-PCR data showing the SARS-CoV-2 spike mRNA expression in HEK293T cells. Cells were treated with the articles for 4 h at a concentration of 10 μM with respect to IVM or remdesivir, followed by incubation in media for an additional 20 h.

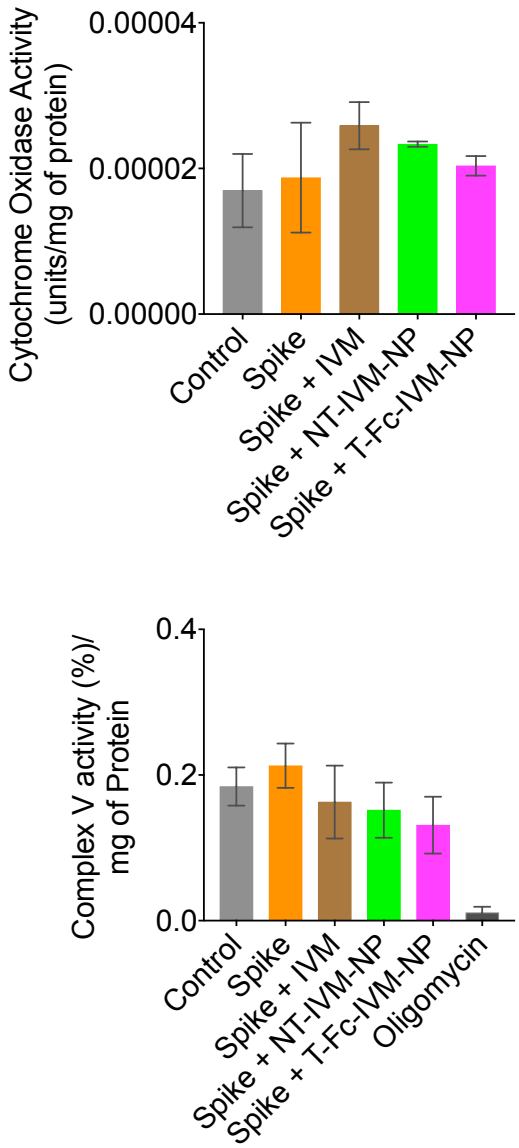

Figure S11. Effects of IVM and IVM-loaded NPs on mitochondrial complex IV and complex V activity in spike protein-expressing HEK293T cells.

References

1. Procko, E., The sequence of human ACE2 is suboptimal for binding the S spike protein of SARS coronavirus 2. *bioRxiv* **2020**.
2. Surnar, B.; Kamran, M. Z.; Shah, A. S.; Basu, U.; Kolishetti, N.; Deo, S.; Jayaweera, D. T.; Daunert, S.; Dhar, S., Orally Administrable Therapeutic Synthetic Nanoparticle for Zika Virus. *ACS Nano* **2019,** *13*, 11034-11048.