

1 **Metformin Suppresses SARS-CoV-2 in Cell Culture**

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10 **Keywords**

11 SARS-CoV-2, COVID-19, anti-viral activity, Metformin, AMPK, Diabetes

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15 **ABSTRACT**

16 People with diabetes are reported to have a higher risk of experiencing severe
17 COVID-19 complications. Metformin, a first-line medication for type 2 diabetes, has
18 antiviral properties. Some studies have indicated its prognostic potential in COVID-
19 19. Here, we report that metformin significantly inhibits SARS-CoV-2 growth in cell
20 culture models. SARS-CoV-2 infection of gut epithelial cell line, Caco2, resulted in
21 higher phosphorylation of AMPK. Metformin reduced viral titers in the infected cells
22 by nearly 99%, and by about 90% when cells were treated prior to infection.
23 Metformin pre-treatment resulted in further phosphorylation of AMPK and caused a
24 ten-fold reduction of viral titers indicating its potential in preventing naïve infections.
25 Confirming the positive impact of AMPK activation, another AMPK activator AICAR
26 substantially inhibited of viral titers and, AMPK inhibitor Compound C, augmented it
27 considerably. Metformin treatment post-SARS-CoV-2 infection resulted in nearly
28 hundred-fold reduction of viral titers, indicating that the antiviral potency of the drug
29 is far higher in infected cells, while still being able to reduce fresh infection.
30 Metformin displayed SARS-CoV-2 TCID₅₀ and TCID₉₀ at 3.5 and 8.9 mM,
31 respectively. In conclusion, our study demonstrates that metformin is very effective in
32 limiting the replication of SARS-CoV-2 in cell culture and thus possibly could offer
33 double benefits to diabetic COVID-19 patients by lowering both blood glucose levels
34 and viral load.

35 **INTRODUCTION**

36 As the COVID-19 pandemic still rages on in most parts of the world, countries are
37 racing to get their citizens fully vaccinated. While the vaccines are effective in most
38 cases, emergence of newer variants of the causative agent, SARS-CoV-2, is a

39 cause for concern (Bernal et al., 2021). Particularly vulnerable to COVID-19 are
40 patients with comorbidities such as cancer, auto-immune diseases, cardiovascular
41 conditions, and diabetes. The hospitalization rate for patients with comorbidities who
42 contracted COVID-19 was significantly higher during the first wave of the pandemic,
43 associated with poor prognosis (Sanyaolu et al., 2020). Type 2 diabetes, one of the
44 most common metabolic disorders, is universally treated using insulin and a number
45 of other drugs, chiefly metformin (Davidson & Peters, 1997). Metformin is a
46 biguanide compound, used as first-line antidiabetic medication worldwide. It acts
47 primarily by increasing glucose intake and limiting gluconeogenesis in the liver, and
48 its action is mediated in part by the energy-sensing kinase, 5'-AMP-activated protein
49 kinase (AMPK) (Zhou et al., 2001). Metformin inhibits Complex I of the electron
50 transport chain and suppresses ATP synthesis, which triggers AMPK activation. This
51 results in a cascade of events that decreases anabolic processes and initiates
52 macromolecular breakdown to reinstate homeostasis.

53 In the past year, a number of reports have debated the clinical use of metformin in
54 COVID-19 (Bramante et al., 2021; Dardano & del Prato, 2021; Ibrahim et al., 2021;
55 Zangiabadian et al., 2021). Many case studies on metformin treatment report a
56 decrease in hospital mortality rates for patients that were on metformin prior to
57 admission (Dardano & del Prato, 2021; L et al., 2020; Marmor et al., 2021). Reports
58 suggest that high glucose levels are associated with poorer prognosis and well-
59 controlled glucose levels were indicative of lesser complications (L et al., 2020).
60 Metformin has also been reported to show anti-viral activity against other viruses (X.
61 Chen et al., 2020). In this study, we aimed to investigate the effect of metformin on
62 SARS-CoV-2, and identify the effects of AMPK perturbation on infection. Pre-
63 treatment of cells with metformin prior to infection substantially lowered the viral titer.

64 Pharmacological activation of AMPK suppressed viral infection while its inhibition
65 promoted it. Treatment of SARS-CoV-2 infected cells with metformin resulted in
66 stronger restriction of the virus in a dose-dependent manner, as compared to the
67 pre-treatment. Our results support the promising use of metformin as a therapeutic
68 drug in COVID-19.

69 **MATERIALS AND METHODS**

70 **Cell culture and reagents**

71 Caco2, Huh7, and Vero cells were grown in DMEM supplemented with FBS, and
72 Pen Strep, at 37°C and 5% CO₂. Anti-AMPK α antibody was procured from CST.
73 GAPDH, β -tubulin, and anti-Nucleocapsid antibodies were from ThermoFisher
74 Scientific. HRP-conjugated secondary antibodies were purchased from Jackson
75 ImmunoResearch. Metformin, AICAR, and Compound C were procured from Merck
76 Millipore.

77 **Infections and treatments**

78 All experiments involving virus culture were carried out in the biosafety level-3
79 laboratory at the Centre for Cellular and Molecular Biology (CCMB). SARS-CoV-2
80 strain B.1.1.8 (TG-CCMB-L1021/2020 isolate) was used for all experiments (Gupta
81 et al., 2021) at 1 MOI. Cells were grown to 80% confluency and treated as
82 described. For pre-treatments, cells were subjected to 10 mM metformin or 1 mM
83 AICAR for 24 h, followed by infection with SARS-CoV-2 in serum-free medium (SFM)
84 for 3 h in the presence of the respective compound. The inoculum was subsequently
85 replaced with complete medium containing the compound and the cells were
86 harvested at 24 h post-infection (hpi). In post-infection mode, the cells were first
87 infected for 3 h after which the inoculum was replaced by media containing
88 metformin and further incubated until 24 hpi. Dose-dependent effect of metformin

89 was studied by subjecting cells to varying doses of metformin (5, 10, 20, and 40 mM)
90 similar to the post-treatment regimen mentioned above. Compound C treatment was
91 carried out by infecting cells for 3 h at 1 MOI, complete media for 21 h, and then an
92 additional 24 h with 10 μ M Compound C. For all treatments, media supernatant was
93 collected to measure extracellular viral RNA as well as infectious viral titres, and
94 cells were processed for immunoblotting.

95 **Virus quantification and titration**

96 RNA from viral supernatants was isolated using Nucleospin Viral RNA isolation kit
97 (Macherey-Nagel GmbH & Co. KG). qRT-PCR was carried out using nCOV-19 RT-
98 PCR detection kit from Q-line Molecular to quantify SARS-CoV-2 RNA following
99 manufacturer's protocol on Roche LightCycler 480.

100 Infectious titres of the supernatants were calculated using plaque forming assay
101 (PFU/mL) as mentioned previously (Gupta et al., 2021). Briefly, the supernatant was
102 serially diluted from 10^{-1} to 10^{-7} in SFM and added to a confluent monolayer of Vero
103 cells for infection for 3 h. The medium was then replaced with a 1:1 mixture of
104 agarose: 2 \times DMEM (1% low-melting agarose (LMA) containing a final concentration
105 of 5% FBS and 1 \times Pen-Strep). Six days post-infection, cells were fixed in 4%
106 formaldehyde prepared in 1 \times PBS and subsequently washed and stained with 0.1%
107 crystal violet to count the plaques.

108 **Immunoblotting**

109 Protein pellets were lysed in an NP-40 lysis buffer as described earlier (Gupta et al.,
110 2021). Protein quantification was done using BCA method (G Biosciences). Lysates
111 were then mixed with 6 \times Laemmli buffer, and equal amounts of protein were run on
112 SDS-PAGE, followed by transfer onto PVDF membrane. Blots were blocked in 5%

113 BSA and incubated with specific primary antibodies at 4°C overnight. Incubation with
114 HRP-conjugated secondary antibodies was done for 1 hour and the blots were
115 developed on a BioRad Chemidoc MP system using ECL reagents (ThermoFisher
116 and G Biosciences). Quantification was performed using ImageJ (Schneider et al.,
117 2012).

118 **Cell viability assay**

119 Effect of different doses of metformin on viability of mock and infected Caco2 cells
120 was measured using MTT assay. Cells were subjected to varied doses of metformin
121 as mentioned in the previous section. After incubation, media containing 0.5 mg/mL
122 MTT was added to cells and incubated at 37°C for 3.5 h. Formazan crystals were
123 dissolved in 100 µL DMSO and incubated for 30 min with mild agitation. Viability was
124 read as absorbance measured at 570 nm, with a reference reading at 620 nm.

125 **Statistical analysis**

126 All experiments were performed in triplicate to calculate mean \pm SEM. Statistical
127 significance was calculated using two-tailed, unpaired Student's *t*-test and *p* values
128 are represented as *, **, ***, indicating $p \leq 0.05$, 0.005, and 0.0005, respectively.
129 IC50 and IC90 were calculated from qRT-PCR data, while TCID50 and TCID90 were
130 calculated from PFU data from metformin titration experiments.

131 **RESULTS**

132 **SARS-CoV-2 infection causes long-term phosphorylation of AMPK**

133 Caco2 cells were infected with 1 MOI of SARS-CoV-2 for several time points and the
134 samples were analyzed for AMPK phosphorylation. Though no significant change in
135 the AMPK phosphorylation was detected until 48 h post-infection (hpi) (Figure 1 A
136 and B), marked increase in phosphorylation was evident from 48 hpi that further

137 strengthened until 96 hpi, despite a drop in the abundance of the protein. These
138 results indicated a major metabolic reprogramming, resulting in AMPK
139 phosphorylation occurring after 24 hpi. Interestingly, AMPK phosphorylation
140 coincided with the accumulation of viral proteins (Figure 1A).

141 **Metformin protects cells from SARS-CoV-2 infection**

142 We investigated the role of AMPK activation during SARS-CoV-2 infection as
143 previous reports have clearly established the roles played by this molecule on the
144 outcome of viral infections (Bhutta et al., 2021). Caco2 cells were pre-treated with
145 10mM concentration of metformin for 24 h after which they were infected with 1 MOI
146 of SARS-CoV-2 for 3 h in presence of metformin. Subsequently, the viral media was
147 replaced with growth medium containing metformin and incubated until 24 hpi
148 (Figure 1C). Increased phosphorylation of AMPK in the drug-treated cells confirmed
149 the effect of metformin (Figures 1 D and E). Metformin treatment resulted in nearly
150 50% drop in the viral RNA (Figure 1F), and nearly one-log drop in the infectious viral
151 titers (Figure 1G) indicating that metformin treatment is protective against SARS-
152 CoV-2 infection. However, no prominent change in the viral protein N was observed
153 (Figures 1 D and H), suggesting that viral translation or its stability is not negatively
154 impacted by metformin. Metformin treatment in Huh7 cells also brought about
155 significant reduction in the infectious viral titer of SARS-CoV-2 (Supplementary
156 Figures S1 A-D), albeit, less pronounced than in Caco2 cells, confirming that
157 metformin has strong protective effects against SARS-CoV-2 infection.

158 **AMPK activation restricts SARS-CoV-2**

159 To further verify if the protective effect of metformin involves AMPK, we used AICAR,
160 another activator of AMPK. Cells were pre-treated with 1mM of AICAR for 24 h as in

161 the case of metformin (Figure 2A). AICAR treatment (Figure 2B) significantly lowered
162 the infectious viral titer of SARS-CoV-2 (Figure 2C) by almost one-log, as did
163 metformin. These results demonstrate that AMPK activation is certainly beneficial to
164 the host cells by significantly limiting the viral titers. We further confirmed this effect
165 by inhibiting AMPK by using Compound C (CC) during SARS-CoV-2 infection. Since
166 AMPK phosphorylation peaked beyond 24 h, cells were first infected at 1 MOI for 3 h
167 followed by supplementation with growth medium. 24 hpi, media containing 10 μ M
168 CC was added and incubated for an additional 24 h (Figure 2D). Though CC
169 treatment caused a visible drop in AMPK phosphorylation in mock cells, there was
170 no apparent decrease observed in infected cells, indicating that the virus-induced
171 AMPK activation overrides CC inhibition (Figure 2 E and F). As anticipated, CC
172 treatment resulted in over four-fold higher viral titers in the supernatants as against
173 the control sample (Figure 2G), accompanied by a modest drop in N levels (Figure
174 2H). CC treatment of infected Huh7 cells also resulted in substantial increase in the
175 infectious viral titres (Supplementary Figures S2 A, B, and D), again accompanied by
176 considerable drop in N levels (Supplementary Figure S2C). These results confirm
177 that AMPK coordinates strong antiviral measures in SARS-CoV-2 infected cells.
178 Thus, activation of AMPK during the infection is protective against SARS-CoV-2
179 infection.

180 **Metformin treatment post-infection causes more profound restriction of SARS-** 181 **CoV-2**

182 We next tested the effect of metformin in Caco2 cells previously infected with SARS-
183 CoV-2 to extrapolate its impact on the infected patients. Cells infected with 1 MOI of
184 SARS-CoV-2 for 3 h were subsequently treated with 10 mM metformin until
185 harvested at 24 hpi (Figure 3 A). Metformin caused higher AMPK phosphorylation

186 (Figures 3 B and C). In comparison with the pre-treatment regimen, the post-
187 infection regimen caused a more profound drop in the viral RNA and nearly two log
188 decrease of infectious titer in the supernatant (Figures 3 D and E, respectively).
189 Interestingly, considerable drop in the levels of N was observed in the metformin
190 treated samples (Figure 3F), further confirming the profound restrictive effect of the
191 drug on SARS-CoV-2. In comparison, N levels were relatively unchanged in the
192 samples pre- and co-treated with metformin and AICAR (Figures 1 D, H and 2B
193 respectively). A higher drop in the viral titers were observed in metformin treated
194 Huh7 cells as well (Supplementary Figures S3 A and B). These results indicate that
195 the protection offered by metformin from SARS-CoV-2 infection is more profound in
196 cells previously infected with SARS-CoV-2 than the uninfected cells.

197 Since post-infection regimen had a higher impact on SARS-CoV-2, we performed a
198 dose-dependence analysis. A dose-dependent increase in AMPK phosphorylation
199 was evident in SARS-CoV-2 infected cells from 5-40 mM metformin concentrations
200 (Figures 4 A and B). A gradual and dose-dependent decrease in N levels was
201 evident (Figures 4 A and C). The drop in viral RNA levels was more dramatic with a
202 very significant drop detected even at 5 mM concentration and was further stabilized
203 at 20 mM concentration (Figure 4D). 5 mM concentration of metformin inhibited
204 infectious viral titers by 70% while the highest inhibition of nearly 2 logs was
205 observed at 20 mM concentration where once again, the inhibition was stabilized
206 (Figure 4E). The calculated IC₅₀ and IC₉₀ for viral RNA were measured to be 2.9
207 mM and 8.8 mM respectively (Figure 4F). We also measured the TCID₅₀ and
208 TCID₉₀ values at 3.5 mM and 8.9 mM respectively (Figure 4G). MTT experiments
209 carried out with the different doses indicate a steady decrease in viability with

210 increasing metformin (Figure 4H). Together, these results unambiguously
211 demonstrate a potent anti-SARS-CoV-2 effect of metformin.

212 **DISCUSSION**

213 The anti-diabetic drug, metformin, has been projected to influence the prognosis of
214 COVID-19 patients. As patients with comorbidities fared worse when infected with
215 SARS-CoV-2, management of an ongoing illness alongside COVID-19 treatment
216 became paramount. Some studies early during the pandemic identified a positive
217 correlation between improved glucose levels in diabetic patients on metformin and
218 better clinical outcome (Y. Chen et al., 2020; Crouse et al., 2020; L et al., 2020; P et
219 al., 2020). A number of reports proposed metformin as a possible “miracle or
220 menace” in COVID-afflicted patients, based on retrospective data from
221 hospitalisations, (Lui & Tan, 2021; Marmor et al., 2021) comparing the length of
222 hospitalisation, severity of symptoms, or mortality. In this study, we demonstrated
223 that metformin profoundly lowers SARS-CoV-2 infectivity. While extrapolating these
224 results to a clinical set up may not be appropriate, our data indicate that metformin
225 can be effective not only as a treatment option, but as a prophylactic agent as well.
226 With this data, we suggest that treatment of patients with metformin prior to infection
227 with SARS-CoV-2 may have assisted in decreasing their symptoms of COVID-19.
228 Our results also suggest that metformin could be beneficial in non-diabetic, COVID-
229 19 patients and expand the scope of its coverage. In summary, this data lies in
230 agreement with the numerous case studies published during the pandemic that
231 suggested an antiviral role for this known anti-diabetic drug.

232 Metformin plays a major role in modulating lipid metabolism, but the mechanisms are
233 multi-dimensional. Metformin is a soluble compound that interferes with Complex I

234 of the electron transport chain, and the decrease in ATP production causes AMPK
235 activation. It also decreases hepatic lipids, increases skeletal muscle uptake of
236 glucose, and in parallel helps in decreasing circulating lipids that can eventually
237 increase cardiovascular risk especially in diabetic adults who are obese (Pernicova &
238 Korbonits, 2014; Rena et al., 2017). RNA viruses have an intimate relationship with
239 the cytoplasmic membrane network and modulate lipogenesis to steer cells to
240 produce more vesicles to aid replication, as well as packaging and release (Herker &
241 Ott, 2012; Pereira-Dutra et al., 2019). The impairment of such activities leading to
242 loss in infectivity has been reported for several viruses (Abu-Farha et al., 2020; X.
243 Chen et al., 2020; CN et al., 2021). A recent report on SARS-CoV-2 highlighted the
244 possible role that lipid droplets play in its infection (Dias et al., 2020). Not only did
245 they observe higher colocalization of viral RNA with the lipid droplets, they also
246 demonstrated that inhibition of its formation decreased viral load as well as pro-
247 inflammatory cytokines and apoptosis markers. These results in conjunction with
248 ours indicates that the anti-viral effect of metformin is probably a resultant of altered
249 lipid metabolism.

250 We speculate that the loss in infectivity of SARS-CoV-2 by metformin could also be,
251 an outcome of altered lipid metabolism mediated by AMPK. AMPK affects cellular
252 lipid levels through a number of its substrates, such as ACC and SREBP1. AMPK
253 also regulates macromolecular metabolism, mitochondrial homeostasis, autophagy
254 as well as apoptosis. As its role is multifaceted and vital for maintaining energy
255 levels, it has been reported to play key roles in many virus infections. Multiple reports
256 shows that AMPK activation can be either detrimental or beneficial for virus survival
257 and propagation (Bhutta et al., 2021). Our results using AICAR and CC in SARS-
258 CoV-2 infection implies an unfavorable/antiviral environment for the virus when

259 AMPK is activated. In this context, it is interesting to note that N protein was detected
260 at modestly higher abundance during pharmacological activation of AMPK unlike the
261 viral RNA and infectious titer, indicating that viral protein translation is not inhibited
262 during the treatments. However, a significant drop in N levels during the post-
263 infection treatment suggested an overall inhibition of viral life-stages concurrent with
264 an overall drop of cellular activities indicated by MTT results. Inhibition of metabolic
265 activities particularly in the infected cells upon post-infection metformin treatment
266 indicated that metformin treatment specifically targeted the infected cells for
267 destruction. This could be viewed as beneficial to the system fighting to clear the
268 virus from it.

269 Although multiple reports show AMPK as the major effector of metformin action, it is
270 now well established that metformin exerts its affects through other pathways such
271 as PKA and FBPase-1 mediated regulation as well (Pernicova & Korbonits, 2014).
272 Further study into the mechanism SARS-CoV-2 inhibition by metformin could pave
273 the way for it to be a possible therapeutic target for COVID-patients. From a clinical
274 perspective, our study provides some answers to the favorable prognosis of
275 metformin-treated diabetic patients who contracted COVID-19.

276 **Institutional biosafety**

277 Institutional biosafety clearance was obtained for the experiments pertaining to
278 SARS-CoV-2.

279 **Author contributions**

280 H.P. performed treatments, infections, quantification and immunoblotting. D.N.
281 performed qRT-PCR experiments. H.P. and K.H.H. conceptualized the study and
282 wrote the manuscript.

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380 LEGENDS

381 **Figure 1. SARS-COV-2 infection induces AMPK phosphorylation and**
382 **metformin treatment inhibits SARS-CoV-2. (A)** Immunoblots analyzing the
383 phosphorylation of AMPK in SARS-CoV-2 infected Caco2 cells for early (1, 2, 6, and
384 12 h) and late (24, 48, 72, and 96 h) time points. Cells infected with 1 MOI of SARS-
385 CoV-2 were harvested at various time intervals post-infection and analyzed by
386 immunoblotting. **(B)** Quantitative representation of AMPK phosphorylation from three
387 independent replicates. Densitometric values of p-AMPK bands were normalized
388 against those of T-AMPK and GAPDH belonging to the corresponding samples and
389 the values were plotted graphically. **(C-H)** Pre-treatment with metformin suppresses

390 SARS-CoV-2 infection in Caco2 cells. **(C)** Schematic of the experimental set up for
391 pre-treatment. Cells were infected with SARS-CoV-2 24 h after metformin treatment.
392 The vehicle control cells were also infected with the virus in parallel. **(D)**
393 Immunoblots confirming AMPK phosphorylation by metformin or vehicle treatment
394 and SARS-CoV-2 infection. **(E)** Densitometric quantification of AMPK
395 phosphorylation in infected cells. **(F)** SARS-CoV-2 RNA levels in the supernatant of
396 metformin treated samples, measured by qRT-PCR of E gene. Relative fold change
397 in the E levels between metformin and vehicle treated samples is depicted. **(G)**
398 Relative fold change in the infectious viral titers of SARS-CoV-2 in metformin treated
399 samples compared against that treated with vehicle, represented as fold change in
400 PFU/mL. **(H)** Densitometric analysis of N expression during metformin treatment.

401 **Figure 2. AMPK activation is beneficial to the host against SARS-CoV-2. (A)**
402 Schematic of the treatment of Caco2 cells with AICAR and infection by SARS-CoV-
403 2. **(B)** Immunoblot confirming the infection. **(C)** Relative infectious titers of SARS-
404 CoV-2 in samples that underwent pre-treatment with AICAR, as against the vehicle.
405 **(D)** Schematic of the treatment of SARS-CoV-2 infected Caco2 cells with CC. **(E)**
406 Immunoblot confirmation of the infection and inhibition of AMPK activity. **(F)**
407 Quantification of AMPK phosphorylation in CC treated, infected samples. **(G)**
408 Relative infectious titers of SARS-CoV-2 in samples that underwent CC-treatment,
409 as against the vehicle, DMSO. **(H)** Relative expression of N in SARS-CoV-2 infected
410 cells treated with CC or DMSO, quantified from densitometric values.

411 **Figure 3. Metformin suppresses SARS-CoV-2 replication in the infected cells.**
412 **(A)** Schematic of the post-infection treatment by metformin. **(B)** Confirmation of
413 SARS-CoV-2 infection and AMPK phosphorylation by immunoblotting. **(C)**
414 Densitometric analysis of AMPK phosphorylation in the treated, infected cells. **(D)**

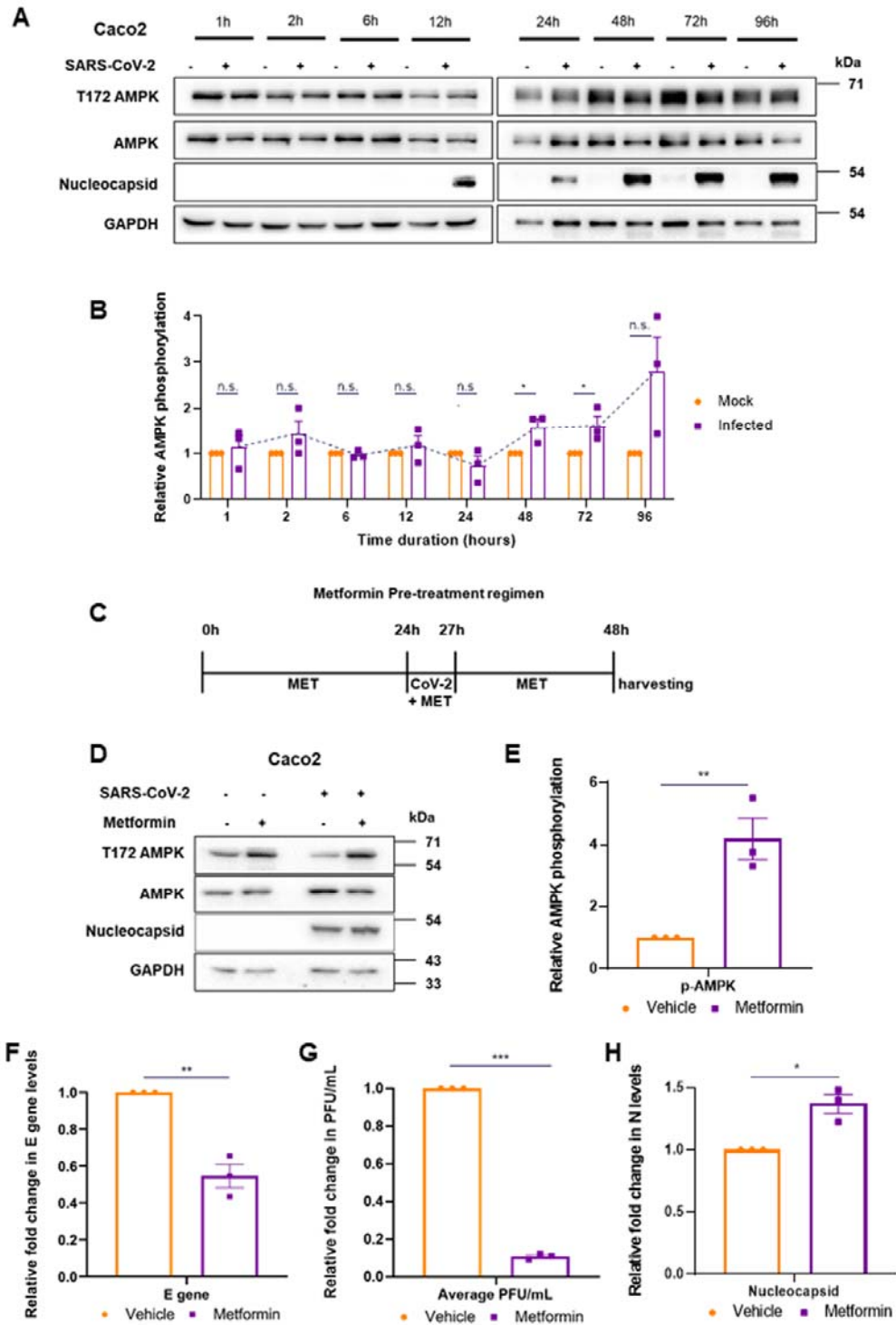
415 Relative fold change in SARS-CoV-2 E gene measured by qRT-PCR in the
416 supernatants of metformin treated cells compared with the those treated with vehicle.
417 **(E)** Relative SARS-CoV-2 infectious titers of the supernatant from samples treated
418 with metformin represented as fold change in PFU/mL. **(F)** Relative abundance of N
419 levels in the metformin treated samples against the control.

420 **Figure 4. Metformin treatment inhibits SARS-CoV-2 replication in a dose-**
421 **dependent manner. (A)** Immunoblots confirming the infection and AMPK
422 phosphorylation following the treatment of SARS-CoV-2 infected cells with metformin
423 at the doses described above the panel. **(B)** Relative AMPK phosphorylation in the
424 samples treated with metformin. The graph was generated from the densitometric
425 analysis of the immunoblots. **(C)** Relative fold change in SARS-CoV-2 E gene
426 measured by qRT-PCR in the supernatants of metformin treated cells compared with
427 the those treated with vehicle. **(D)** Relative SARS-CoV-2 infectious titers of the
428 supernatant from samples treated with metformin represented as fold change in
429 PFU/mL. **(E)** Relative abundance of N quantified from the immunoblots from the
430 panel (A). **(F)** Calculation of IC₅₀ and IC₉₀ for metformin on SARS-CoV-2 replication
431 measured by plotting E gene levels present in the supernatants of the samples
432 treated with the respective concentrations of metformin. **(G)** Measurement of TCID₅₀
433 and TCID₉₀ for metformin on SARS-CoV-2 infectious virus particle production.
434 PFU/mL data for the individual samples treated with the specific concentrations were
435 plotted in the graph to calculate the respective values. **(H)** Analysis of cell viability in
436 the metformin-treated or the control cells by MTT assay. % viability of cells from
437 absorbance measurements at 570 and 620 nm relative to the respective vehicle
438 control are plotted graphically.

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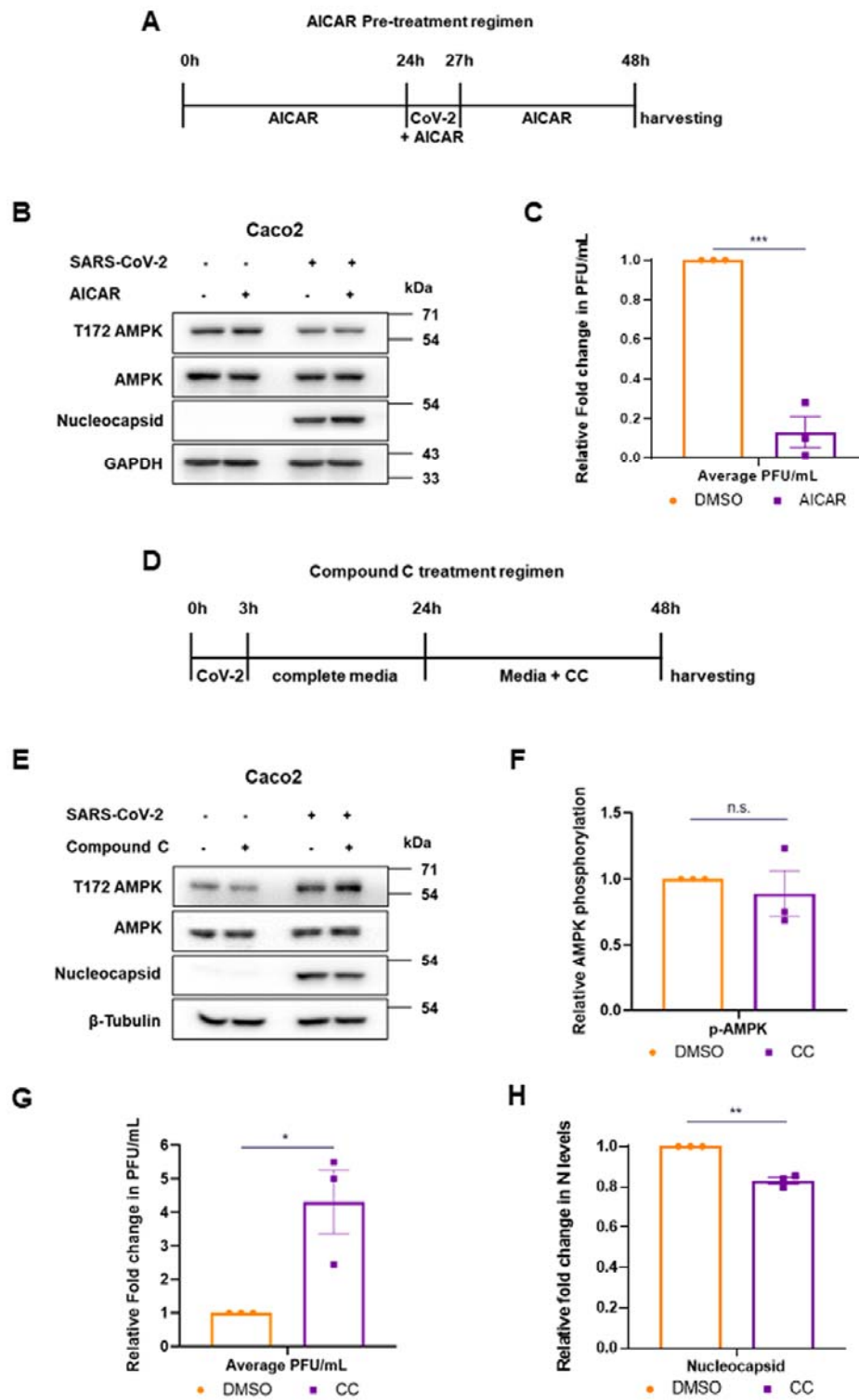
440 FIGURES

441 Figure 1



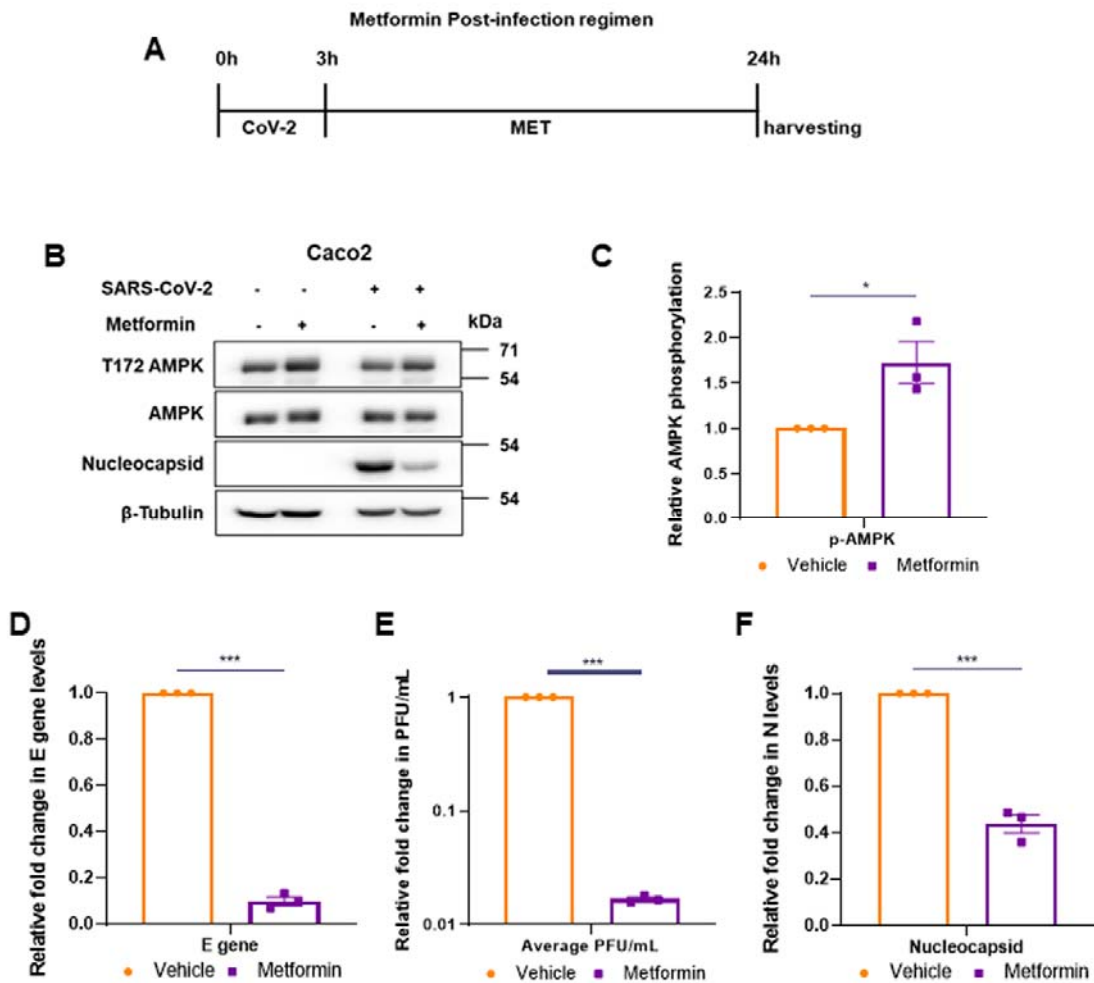
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443 **Figure 2**



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445 **Figure 3**



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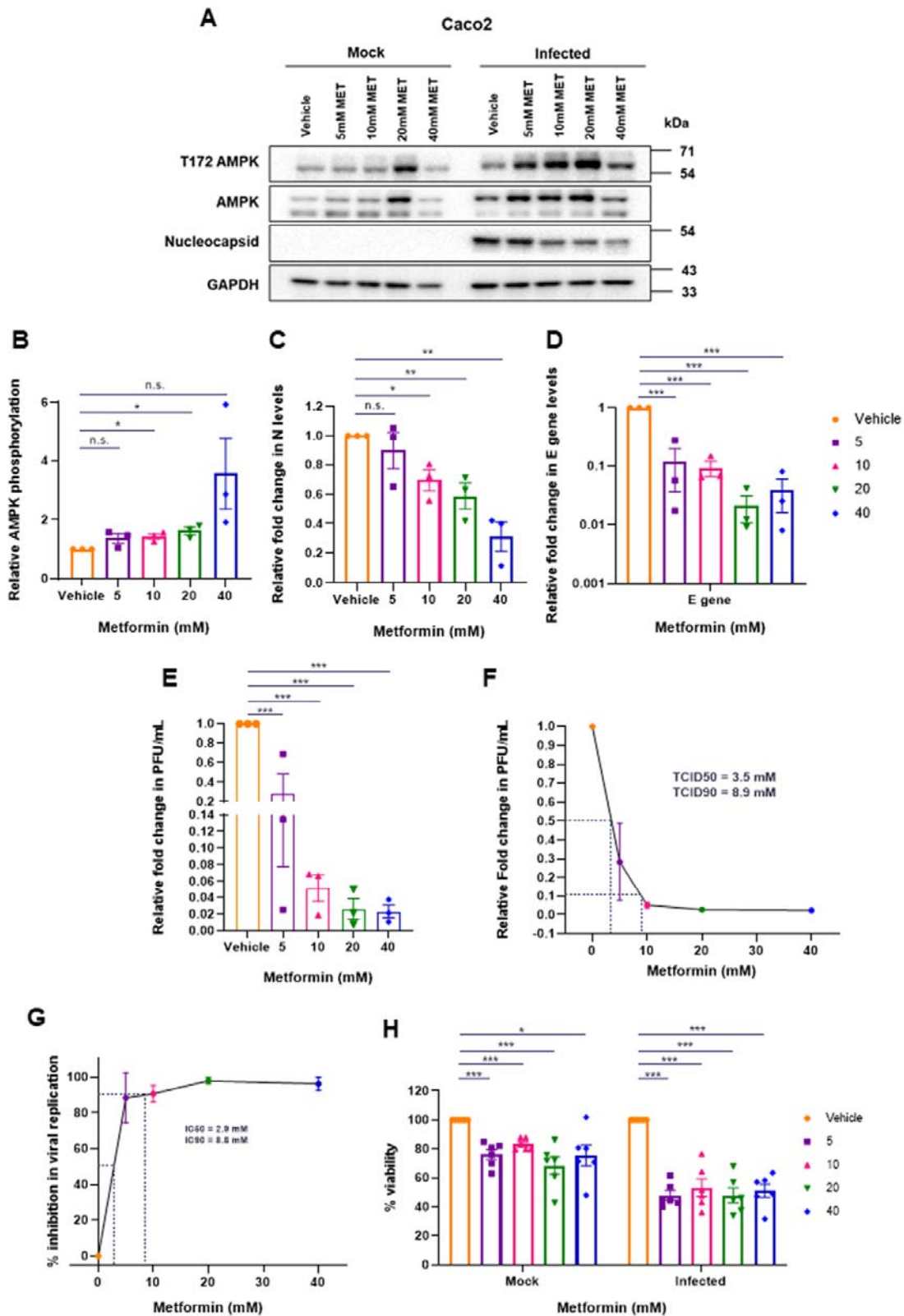
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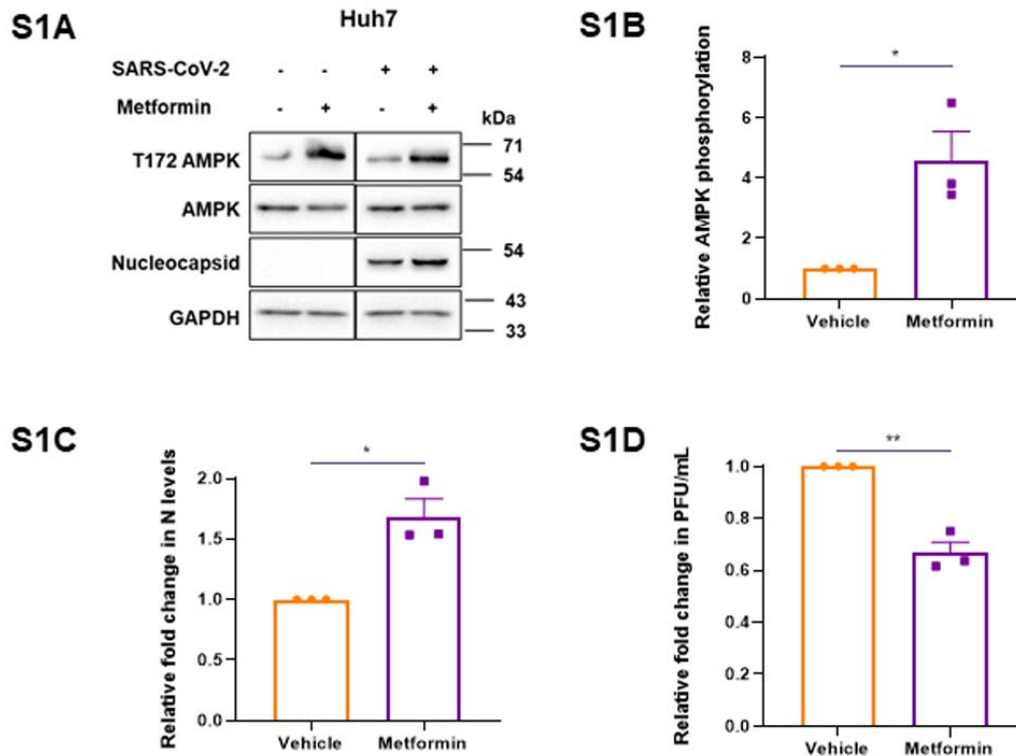
452 **Figure 4**



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454 **Supplementary figures**

455 **Figure S1**

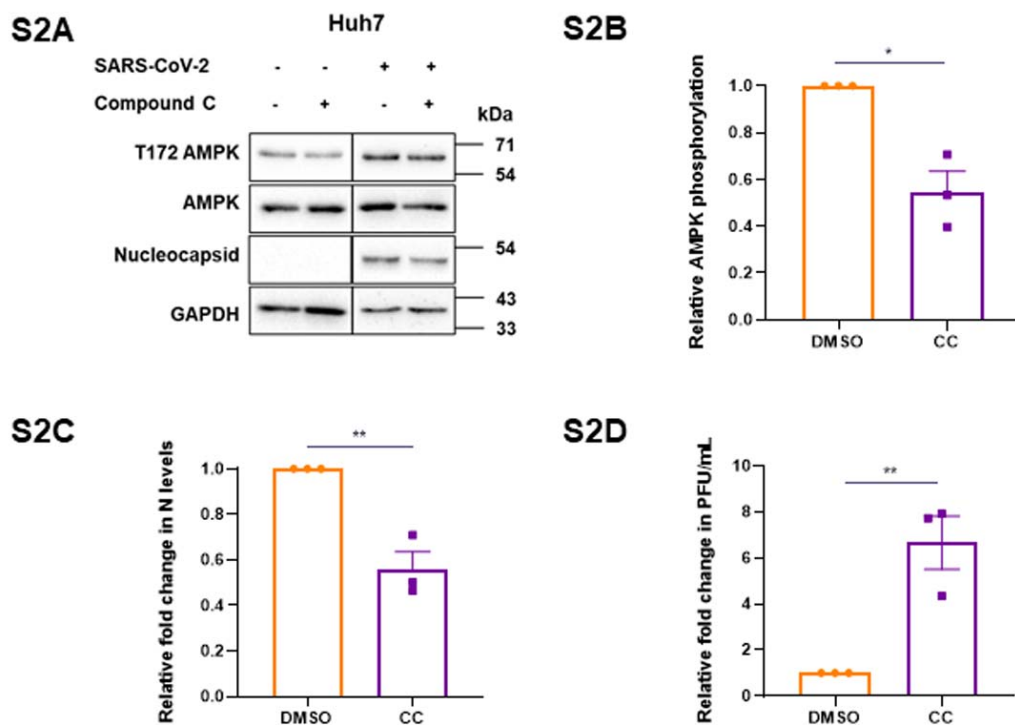


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458 **Figure S1.** Metformin treatment reduces SARS-CoV-2 infection in Huh7 cells. **(A)**
459 Immunoblotting of the metformin treated or control samples to confirm SARS-CoV-2
460 infection and AMPK phosphorylation. Huh7 cells were treated with metformin and
461 infected with SARS-CoV-2 as demonstrated in Figure 1D and the samples
462 processed as in Figure 1E. **(B)** Densitometric quantification of AMPK
463 phosphorylation and **(C)** that of N abundance in metformin treated or control
464 samples. **(D)** Relative infectious titer of SARS-CoV-2 in the supernatants of
465 metformin treated samples or the control samples.

466 **Figure S2**



467

468 **Figure S2.** AMPK inhibition promotes SARS-CoV-2 titers. **(A)** Confirmation of
469 SARS-CoV-2 infection in Huh7 cells by immunoblotting. **(B)** Relative AMPK
470 phosphorylation levels in samples treated with CC or the control samples. **(C)**
471 Relative abundance of N in the control samples or those treated with CC. **(D)**
472 Relative infectious titers in the control or CC-treated samples represented as relative
473 PFU/mL.

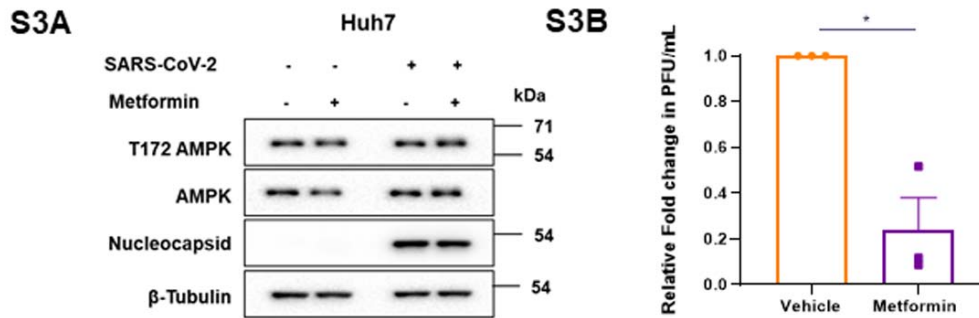
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478 **Figure S3**



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480 **Figure S3.** Metformin inhibits SARS-Cov-2 replication in the infected Huh7 cells. **(A)**

481 Immunoblotting of SARS-Cov-2 infected Huh7 cultures treated with metformin

482 compared with the control samples. The cells were first infected with the virus and

483 followed by metformin treatment as described in Figure 3A. **(B)** Relative infectious

484 titer of SARS-Cov-2 from the control samples or those treated with metformin.