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**SARS-CoV-2 aberrantly elevates mitochondrial bioenergetics to induce robust
virus propagation**

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aberrant mitochondrial bioenergetics; Epidermal growth factor receptor; EGFR inhibitors

19 **Abstract**

20 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a respiratory pathogen leading
21 to serious multi-organ damage. However, little is known about SARS-CoV-2-induced cellular
22 alterations for understanding robust virus propagation yet. Here we report that SARS-CoV-2 aberrantly
23 elevates mitochondrial bioenergetics and activates epidermal growth factor receptor (EGFR)-mediated
24 cell survival signal cascade for sustaining persistence of SARS-CoV-2. We found that SARS-CoV-2
25 causes increase in mitochondrial transmembrane potential by SARS-CoV-2 RNA-nucleocapsid cluster,
26 thereby abnormally promoting mitochondrial biogenesis and oxidative phosphorylation (OXPHOS)
27 process followed by abundant ATP production. SARS-CoV-2 also activated EGFR signal cascade and
28 subsequent mitochondrial EGFR accumulation which contributes to the maintenance of abnormal
29 OXPHOS and viral propagation. Therapeutic options for the treatment of COVID-19 are still
30 inadequate. The FDA-approved EGFR inhibitors caused a remarkable reduction in SARS-CoV-2
31 propagation. Among EGFR inhibitors, vandetanib showing the highest anti-SARS-CoV-2 efficacy
32 exhibited the potent antiviral activity against various SARS-CoV-2 variants including B.1.1.7 (UK
33 variant) and B.1.351 (SA variant) lineages in both *in vitro* cell culture and *in vivo* animal experiments
34 using wild-type aged mouse susceptible to SARS-CoV-2 infection, suggesting that EGFR is an
35 attractive host target for combatting COVID-19. Overall, our results suggest that SARS-CoV-2 induces
36 aberrant mitochondrial dynamics and bioenergetics, which significantly contributes to robust SARS-
37 CoV-2 propagation.

38

39 **Introduction**

40 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a global threat leading to
41 serious respiratory illness and death since the first case was reported as a fatal pathogen in 2019^{1,2}.
42 SARS-CoV-2 phylogenetically closely related to SARS-CoV causing an epidemic in 2003^{3,4} is an
43 enveloped, single-stranded positive-sense RNA virus belonging to the family *Coronaviridae*⁵. SARS-
44 CoV-2 enters into the host cells via host factors angiotensin-converting enzyme carboxypeptidase
45 (ACE2) and transmembrane serine protease 2 (TMPRSS2) and utilizes own RNA-dependent RNA
46 polymerase (RdRp) for the replication of viral genome in the cytoplasm⁶⁻⁸. In particular, when SARS-
47 CoV-2 infects cells by ACE2-mediated endocytosis, SARS-CoV-2 nucleocapsid protein binding to the
48 packaging signals in the virus RNA (SARS-CoV-2 RNA-nucleocapsid cluster) enters into the cells^{9,10}
49 unlike other RNA viruses such as hepatitis C virus (HCV) and Zika virus (ZIKV) and so on^{29,30}.

50 Viruses manipulate various intracellular events such as endoplasmic reticulum (ER) stress,
51 oxidative stress, and Ca^{2+} flux between ER and mitochondria that affects viral propagation and
52 inflammation¹¹⁻¹⁴. Particularly, virus infection induces mitochondrial Ca^{2+} overload thereby resulting
53 in collapse of mitochondria membrane potential ($\Delta\Psi_m$) which is the key step in modulating
54 mitochondrial dynamics for the maintenance of cellular homeostasis^{15,16}. Recent evidences showed
55 that virus infection induces abnormal mitochondrial dynamics and perturbs mitochondrial
56 bioenergetics that are critical for viral propagation and antiviral innate immune response^{17,18}.

57 The regulation of homeostasis in host cells is important for the replication of coronaviruses¹⁹.
58 The pro-survival function of epidermal growth factor receptors (EGFR) is involved in many viral
59 infections. The activation of EGFR is not only required for viral entry to the plasma membrane but
60 also for mitochondrial biogenesis and bioenergetics for viral replication²⁰⁻²⁵. Recent reports showed
61 that beyond a significant contribution of EGFR signaling required for sustaining cell survival and
62 proliferation, EGFR migration to subcellular organelles such as ER and mitochondria following EGFR

63 activation has been reported³⁶⁻³⁸.

64 In this study, we investigated the SARS-CoV-2-induced mitochondrial alteration which affects
65 robust virus propagation. Our results demonstrate that SARS-CoV-2 elevates mitochondrial
66 bioenergetics efficiency to facilitate SARS-CoV-2 propagation. Unlike other RNA viruses such as
67 HCV and ZIKV, SARS-CoV-2 infection caused increase in $\Delta\Psi_m$ by viral RNA-nucleocapsid cluster
68 leading to the elongated mitochondria. SARS-CoV-2 infection also increased mitochondrial biogenesis
69 and promoted oxidative phosphorylation (OXPHOS) process followed by abundant ATP production.
70 Further, SARS-CoV-2 activated EGFR/Akt-mediated cell survival signaling and subsequently
71 triggered EGFR translocation to the outer membrane of mitochondria (OMM) and its accumulation,
72 which was convincingly proved by confocal microscopy and Western blot analysis using mitochondrial
73 fractions isolated from SARS-CoV2-infected cells and lung tissue of SARS-CoV-2-infected mouse.
74 SARS-CoV-2-induced activation of EGFR signaling and subsequent EGFR accumulation on the OMM
75 was crucial for sustaining SARS-CoV-2-induced abnormal increase in mitochondrial OXPHOS
76 process and SARS-CoV-2 propagation. Treatment of SARS-CoV-2-infected cells with FDA-approved
77 EGFR inhibitors resulted in drastic reduction in SARS-CoV-2 replication and egress. Furthermore,
78 SARS-CoV-2 viral load in the lung tissue of SARS-CoV-2-infected mouse was dramatically
79 suppressed by oral administration of EGFR inhibitor vandetanib, suggesting that EGFR is a promising
80 host target for combatting SARS-CoV-2 propagation. Overall, our results implicate that SARS-CoV-
81 2-induced mitochondrial alterations are physiologically relevant in maintenance of persistence of
82 SARS-CoV-2 and pathogenesis. In addition, our results also provide novel insights into the antiviral
83 potential of EGFR inhibitors as promising therapeutic agents in the current lack of effective therapies
84 for the treatment of coronavirus disease 19 (COVID-19).

85

86

87 **Results**

88 **SARS-CoV-2 aberrantly elevates mitochondrial bioenergetics**

89 Recently, we and others have shown that SARS-CoV-2 infects the human cells through host factors
90 ACE2 and TMPRSS2 and replicates in various human epithelial cells^{7,8}. In order to investigate SARS-
91 CoV-2-induced alteration in intracellular events which affect a rapid and sustained viral propagation,
92 we first analysed the aspects of SARS-CoV-2 infectivity and replication in several human cells
93 including human normal primary bronchial cells as evidenced by confocal microscopy using anti-
94 SARS-CoV-2 nucleocapsid antibody, real-time qRT-PCR using primer sets specific to SARS-CoV-2
95 nucleocapsid gene, and Western blot analysis with antibodies specific to SARS-CoV-2 spike and
96 nucleocapsid antigens (Extended Data Fig. 1, Extended Data Fig. 2a-c, Extended Data Fig. 3b-c).

97 Interestingly, we observed a prominent elevation in intracellular ATP level paralleled by a rapid
98 increase in viral replication after being infected with SARS-CoV-2 compared to uninfected cells (Fig.
99 1a, Extended Data Fig. 1b, Extended Data Fig. 2b, d). To examine whether SARS-CoV-2 promotes
100 increase in intracellular ATP synthesis during viral replication, we measured the mitochondrial
101 membrane potential ($\Delta\Psi_m$) in SARS-CoV-2-infected cells, as mitochondria are dynamic organelles
102 known as central hub for ATP generation by oxidative phosphorylation^{26,27} and the $\Delta\Psi_m$ is a
103 bioenergetics parameter modulating mitochondria respiration and critical for mitochondrial
104 homeostasis²⁸. As a result, unlike other RNA viruses such as HCV and ZIKV^{29,30}, SARS-CoV-2
105 infection unusually caused increase in $\Delta\Psi_m$ (Fig. 1b). At this point, we wondered how SARS-CoV-2
106 induces $\Delta\Psi_m$ increase. To substantiate SARS-CoV-2-induced increase in $\Delta\Psi_m$, we focused on the
107 early stage of SARS-CoV-2 infection, as most viruses trigger endoplasmic reticulum (ER) stress at the
108 early stage of infection, leading to the release of Ca^{2+} from the ER lumen and subsequent Ca^{2+}
109 accumulation in the mitochondrial matrix, thereby resulting in abnormal $\Delta\Psi_m$ and mitochondrial
110 function^{14,31}. Unlike other RNA viruses, SARS-CoV-2 ssRNA and nucleocapsid protein together enters

111 into the cells when SARS-CoV-2 infects host cells by ACE2-mediated endocytosis^{9,10}. Hence, we
112 sought to analyse $\Delta\Psi_m$ in the presence of SARS-CoV-2 RNA and nucleocapsid protein. As shown in
113 Fig. 1c, we first analysed that the expression of SARS-CoV-2 nucleocapsid alone does not significantly
114 affect $\Delta\Psi_m$ (Fig. 1c, left graph). Next, we found that the transfection of SARS-CoV-2 RNA in cells
115 causes loss in $\Delta\Psi_m$ (right graph), whereas the transfection of SARS-CoV-2 RNA in cells expressing
116 nucleocapsid protein induces dramatic increases in $\Delta\Psi_m$ (Fig. 1c, right graph). poly(I:C) was used for
117 the control for $\Delta\Psi_m$ loss³². poly(I:C) did not induce $\Delta\Psi_m$ increase in the presence and absence of
118 SARS-CoV-2 nucleocapsid protein, suggesting that the elaborate structure or formation of SARS-CoV-
119 2 ssRNA and nucleocapsid protein might affect $\Delta\Psi_m$ increase. The accompanying schematic diagram
120 represents that the expression of SARS-CoV-2 RNA and nucleocapsid together causes $\Delta\Psi_m$ increase
121 (Fig. 1c).

122 Next, we examined a mitochondrial shape on confocal microscope, as change in $\Delta\Psi_m$ usually
123 causes alteration in mitochondrial morphology and induces abnormal mitochondrial function. As
124 shown in Fig. 1d, we observed that aberrant elongated and swollen mitochondrial shape is sustained
125 in SARS-CoV-2-infected cells. The accompanying graph presents the quantification of mitochondrial
126 length in SARS-CoV-2-infected cells and uninfected cells shown in confocal microscope. Lack of
127 mitofusin (MFN) creates mitochondrial fusion deficiency and causes severe mitochondrial
128 fragmentation³³. Hence, we examined the stability of MFN in SARS-CoV-2-infected cells. As shown
129 in Fig. 1e, we found that MFN1/2 is increased and sustained in SARS-CoV-2-infected cells,
130 demonstrating that MFN acts as a modulator for sustaining abnormal mitochondrial elongation in
131 SARS-CoV-2-infected cells. We also examined mitochondrial biogenesis through analysis of
132 mitochondrial DNA (mtDNA) level in SARS-CoV-2-infected cells. Analysis of mitochondrial NADH
133 dehydrogenase 2 (ND2) and cytochrome c oxidase 2 (COX2) reveals that the abnormal increase in
134 mitochondrial mass is maintained during SARS-CoV-2 replication (Fig. 1f).

135 Most of the ATP is mainly generated in mitochondria by the oxidative phosphorylation (OXPHOS)

136 process³⁴. Thus, we subsequently examined a change in the expression levels of mitochondrial
137 OXPHOS complex that might be associated with abundant ATP production on SARS-CoV-2-induced
138 abnormal mitochondrial fusion. As a result, we found that the expression levels of mitochondrial
139 OXPHOS complex I, II, III, IV, and V enzymes are entirely increased in SARS-CoV-2-infected cells
140 (Fig. 1g). Further study by RNAseq and subsequent analysis demonstrated that most of the genes
141 involved in OXPHOS process were upregulated in SARS-CoV-2 (Fig. 1h). Taken together, these
142 results indicate that SARS-CoV-2 induces increase in $\Delta\Psi_m$, mitochondrial biogenesis, and OXPHOS
143 process, thereby abnormally promoting ATP production.

144

145 **SARS-CoV-2 activates EGFR signal cascade and subsequently induces mitochondrial EGFR** 146 **translocation**

147 Next, we observed the prolonged survival and proliferation of human cells infected with SARS-
148 CoV-2 without a distinct viral cytopathogenic effect while we analyse SARS-CoV-2 infectivity in
149 various human cells (Fig. 2a-b, Extended Data Fig. 2e-f, Extended Data Fig.3). Thus, we sought to
150 analyse SARS-CoV-2-induced activation in EGFR signal cascade, as contribution of EGFR signaling
151 is critical for sustaining cell survival and increased cell proliferation^{35,36}. As shown in Fig. 2c, SARS-
152 CoV-2 promoted EGFR gene expression at the transcriptional levels at an early time point of SARS-
153 CoV-2 infection. Subsequent Western blot analysis also revealed that SARS-CoV-2 results in activation
154 of EGFR-mediated signal cascade as evidenced by increase in the expression levels of phosphorylated
155 EGFR and phosphorylated Akt (Fig. 2d). Together, these results indicate that SARS-CoV-2 promotes
156 EGFR-mediated cell survival and proliferation of infected cells.

157 Beyond a significant contribution of EGFR signal cascade required for sustaining cell survival and
158 proliferation, EGFR migration to subcellular organelles following EGFR activation has been
159 reported³⁶⁻³⁸. Particularly, mitochondrially localized EGFR contributes to cell survival and regulation

160 of mitochondrial function³⁶. Hence, we further examined if SARS-CoV-2 induces EGFR translocation
161 to mitochondria. We isolated the mitochondrial fraction from SARS-CoV-2-infected cells and analysed
162 SARS-CoV-2-induced mitochondrial EGFR translocation by Western blotting with antibodies specific
163 to phospho-EGFR and EGFR, respectively. As shown in Fig. 2e, SARS-CoV-2 elevated mitochondrial
164 translocation of EGFR and its accumulation compared to those in uninfected cells. A quantitative
165 analysis reveals the difference in relative EGFR accumulation on mitochondrial fraction of SARS-
166 CoV-2-infected cells (Fig. 2e). Further analysis by confocal microscopy also identified EGFR
167 accumulation on mitochondria of SARS-CoV-2-infected cells (Fig. 2f). Here, we note that SARS-CoV-
168 2 nucleocapsid is localized on mitochondria as evidenced by Western blot analysis and confocal
169 microscopy using antibody specific to SARS-CoV-2 nucleocapsid antigen (Fig. 2e-f).

170 To strengthen this observation in vivo animal model, we first investigated SARS-CoV-2 infectivity
171 using various age groups of wild-type C57BL/6 mouse to establish wild-type mouse model that is
172 proper for studying SARS-CoV-2 pathogenesis, as we considered that wild-type mouse model is more
173 appropriate for studying SARS-CoV-2 pathogenesis than hACE2 transgenic mouse model which
174 induces an extreme infection of SARS-CoV-2 via overexpression of hACE2 gene. As a result, we
175 found that 15-month aged group of wild-type C57BL/6 mouse is susceptible to SARS-CoV-2 infection
176 (Extended Fig. 4). SARS-CoV-2-infected wild-type 15-month aged mice revealed changes in body
177 weight, survival rate, and viral infectivity in the lung tissue which is very similar with those shown in
178 Golden hamster and Syrian hamster (Extended Fig. 4)^{39,40}. Subsequently, we isolated mitochondrial
179 fraction from the lung tissue of wild-type 15-month aged mouse infected with SARS-CoV-2 and
180 analysed mitochondrial EGFR accumulation by Western blotting with anti-EGFR antibody. As shown
181 in Fig. 2g, SARS-CoV-2 infection resulted in enhanced mitochondrial EGFR accumulation in the lung
182 tissue compared to those of uninfected wild-type 15-month aged mouse. A quantitative analysis shows
183 the difference in relative EGFR accumulation on mitochondrial fraction of lung tissue of SARS-CoV-
184 2-infected mouse (Fig. 2e). Taken together, these results indicate that SARS-CoV-2 activates EGFR

185 signal cascade followed by mitochondrial EGFR accumulation during viral infection.

186

187 **EGFR inhibitors reveals the potent antiviral activity against SARS-CoV-2**

188 Next, we wondered if robust SARS-CoV-2 propagation is suppressed by inhibiting of SARS-CoV-
189 2-activated EGFR signal cascade. Hence, we evaluated antiviral activity of FDA-approved 12 EGFR
190 inhibitors including gefitinib, olmutinib, erlotinib, lapatinib, bosutinib, cabozantinib, icotinib,
191 vandetanib, brigatinib, afatinib, dacomitinib, and neratinib in SARS-CoV-2-infected cells (Fig. 3a).
192 As shown in Fig. 3b, treatment of SARS-CoV-2-infected cells with EGFR inhibitors caused drastic
193 reduction in the level of extracellular SARS-CoV-2 RNA. Western blot analysis also exhibited that
194 EGFR inhibitors result in the reduction in the expression level of SARS-CoV-2 nucleocapsid protein
195 (Fig. 3c). Among EGFR inhibitors we tested, afatinib, dacomitinib, and neratinib induced a striking
196 cytotoxicity in SARS-CoV-2-infected cells (Table 2). Together, these results suggest that FDA-
197 approved EGFR inhibitors can be utilized as an antiviral therapeutic agent against SARS-CoV-2
198 infection.

199

200 **EGFR inhibitor restores SARS-CoV-2-induced abnormal mitochondrial bioenergetics**

201 To substantiate the action of EGFR inhibitors on inhibition of SARS-CoV-2 propagation, we
202 further examined the effect of EGFR inhibitors in SARS-CoV-2-induced mitochondrial alteration. The
203 FDA-approved vandetanib revealed the highest antiviral efficacy against SARS-CoV-2 infection
204 among EGFR inhibitors we tested (Fig. 3b, c). Hence, we examined if vandetanib is capable of
205 restoring SARS-CoV-2-induced abnormal OXPHOS process and subsequent increase in ATP synthesis.
206 We first analysed that vandetanib reduces mitochondrial EGFR accumulation in SARS-CoV-2-infected
207 cells (Fig. 3d). Further, treatment of SARS-CoV-2-infected cells with vandetanib dramatically restored

208 SARS-CoV-2-induced increase in intracellular ATP level and the expression levels of mitochondrial
209 OXPHOS complex I, II, III, IV, and V enzymes (Fig. 3e, f). In addition, RNAseq and subsequent
210 analysis demonstrated that vandetanib restores SARS-CoV-2-induced abnormal upregulation in most
211 of genes involved in OXPHOS process (Fig. 3g). Taken together, these results indicate that SARS-
212 CoV-2-induced activation of EGFR signal cascade followed by mitochondrial EGFR translocation
213 contributes to SARS-CoV-2-induced abnormal mitochondrial dynamics and bioenergetics supporting
214 robust SARS-CoV-2 propagation.

215

216 **EGFR inhibitor vandetanib exhibits the potent anti-SARS-CoV-2 activity**

217 To substantiate the antiviral effect of vandetanib on SARS-CoV-2 propagation, we first analysed
218 cell cytotoxicity of vandetanib. As shown in Fig. 4a, vandetanib did not significantly affect viability
219 of both uninfected and SARS-CoV-2-infected cells. Vandetanib also led to dose-dependent reduction
220 in SARS-CoV-2 nucleocapsid expression as assessed by Western blot analysis (Fig. 4b). To further
221 understand the action of anti-SARS-CoV-2 effect of vandetanib, we analysed the expression level of
222 intracellular SARS-CoV-2 RNA in infected cells under post-treatment or pre-treatment of vandetanib.
223 As shown in Fig. 4c and d, intracellular SARS-CoV-2 RNA level was drastically declined by post-
224 treatment of vandetanib, but not pre-treatment as evidenced by real-time qRT-PCR data, suggesting
225 that vandetanib affects an intracellular replication process during SARS-CoV-2 propagation.

226 We further analysed the in vivo anti-SARS-CoV-2 efficacy of vandetanib using wild-type 15-
227 month aged mice. Vandetanib is initially developed as an orally available agent⁴¹. Hence, we orally
228 administrated vandetanib to SARS-CoV-2-infected mice at dose of 25 mg/kg daily and terminated
229 SARS-CoV-2-infected mice at 4 days post-infection to analyse intracellular SARS-CoV-2 RNA level
230 in lung tissue of mice (Fig. 4e). As a results, we analysed that vandetanib strongly suppresses SARS-
231 CoV-2 propagation as assessed by qRT-PCR analysis (Fig. 4f). The intramuscular administration of

232 vandetanib in SARS-CoV-2-infected mice did not reveal anti-SARS-CoV-2 efficacy (Fig. 4f).

233 A combination regimen targeting both virus and host might be more effective for curing COVID-
234 19. Thus, we additionally tested the anti-SARS-CoV-2 effect of vandetanib in combination with
235 remdesivir at a 100-fold lower concentration than the IC₅₀. The advantage of this regimen with EGFR
236 inhibitors in combination with remdesivir is the drastic reduction in toxicity caused by the continuous
237 administration of remdesivir to patients with COVID-19^{42,43}. As shown in Fig. 4g, treatment of
238 remdesivir in combination with vandetanib caused an additive reduction in intracellular SARS-CoV-2
239 RNA level, suggesting that vandetanib can be utilized in combination with remdesivir without toxicity.

240 Recently, SARS-CoV-2 variants has been constantly spreading worldwide. Thus, we examined if
241 vandetanib reveals antiviral activity against various SARS-CoV-2 (S, V, G, GH, and GR clades) and
242 its variants including B.1.1.7 (United Kingdom variant) and B.1.351 (South Africa variant) lineages.
243 The anti-SARS-CoV-2 activity of vandetanib was evaluated by qRT-PCR analysis and SARS-CoV-2
244 foci reduction assay. As shown in Fig. 5a, treatment of SARS-CoV-2 variants-infected cells with
245 vandetanib caused drastic reduction in the expression levels of extracellular SARS-CoV-2 RNA.
246 Furthermore, vandetanib potently inhibited the infectivity of various SARS-CoV-2 strains in a dose-
247 dependent manner (Fig. 5b). SARS-CoV-2 strains-induced foci formation was completely inhibited at
248 10 μ M of vandetanib (Fig. 5b). Taken together, these results suggested that vandetanib is a very
249 efficient inhibitor against various SARS-CoV-2 strains.

250

251 Discussion

252 Although the current COVID-19 pandemic caused by SARS-CoV-2 infection is a severe threat
253 globally, our understanding of persistent SARS-CoV-2 infection and the pathogenesis of COVID-19
254 is still insufficient. In this study, we show that SARS-CoV-2 alters intracellular events and abnormally
255 modulates mitochondrial bioenergetics for robust virus propagation. We found that SARS-CoV-2

256 increases $\Delta\Psi_m$ which leads to the mitochondrial elongation and swelling. SARS-CoV-2 RNA and
257 nucleocapsid complex promoted $\Delta\Psi_m$ increase. SARS-CoV-2 infection promoted mitochondrial
258 biogenesis and activated mitochondrial OXPHOS process to generate abundant ATP. We also found
259 that SARS-CoV-2 induces EGFR-mediated cell survival signal cascade and subsequently promotes
260 mitochondrial EGFR accumulation which contributes to maintenance of abnormal mitochondrial
261 bioenergetics. Overall, our results suggest that the SARS-CoV-2-modulated abnormal mitochondrial
262 bioenergetics is physiologically relevant to the maintenance of cellular homeostasis of SARS-CoV-
263 2-infected cells and robust SARS-CoV-2 replication and propagation (Fig. 6).

264 The involvement of EGFR internalization and transport to cellular organelles provides a favourable
265 environment for virus replication^{20-24,44}. We showed that SARS-CoV-2 stimulates EGFR-mediated cell
266 survival signal and mitochondrial EGFR accumulation for the maintenance of cell proliferation and
267 homeostasis. SARS-CoV-2 infection dramatically triggered the translocation of EGFR to mitochondria
268 as demonstrated by analyses of subcellular fractions and confocal microscope. Subsequently, SARS-
269 CoV-2 maintained the abnormally elevated mitochondrial bioenergetics via EGFR internalization and
270 accumulation on mitochondria, which affects viral replication and ATP production. In hepatitis C virus
271 (HCV), EGFR acts as a co-factor for entry and upregulates its internalization and transport to the
272 cellular organelles, and similar processes have been also reported for hepatitis B virus (HBV) and
273 gastroenteritis virus^{20-25,45}. SARS-CoV infection also involved the overactivation of EGFR, which
274 leads to increased pulmonary fibrosis⁴⁶. Of note, many respiratory viruses induce EGFR activation in
275 the airway epithelium^{24,47}. Moreover, the aberrant expression and activation of EGFR have been
276 implicated in multiple diseases including cancer, diabetes, pulmonary fibrosis, and various lung, heart,
277 and renal diseases^{35,48-51}.

278 Notably, vandetanib caused a remarkable inhibition of SARS-CoV-2 propagation in our study
279 against all clades of SARS-CoV-2. Vandetanib, an FDA-approved EGFR inhibitor, is an inexpensive
280 drug with low toxicity that has been used for the treatment of certain tumours of the thyroid gland^{52,53}.

281 Several EGFR inhibitors have already been shown to block multiple steps of replication in influenza
282 virus⁵⁴ and dengue virus⁵⁵. Therapeutic options in response to the various SARS-CoV2 strains are still
283 limited. In the context of the current COVID-19 pandemic, our study suggests that EGFR inhibitors
284 are a druggable regulator to reduce viral replication by modulation of mitochondrial bioenergetics and
285 EGFR signal cascade against SARS-CoV-2 variants.

286

287 **Materials and Methods**

288 **Cell culture**

289 Human embryonic kidney 293 cells (ATCC), human hepatoma Huh7 cells (JCRB), and human
290 lung epithelial A549 cells (ATCC) were grown in high-glucose DMEM (Gibco) containing 1%
291 nonessential amino acids (Gibco), 1% sodium pyruvate (Gibco), and 10% foetal bovine serum
292 (Hyclone). Human normal primary bronchial cells (ATCC) were grown in bronchial epithelial basal
293 medium (LONZA). All cell lines were incubated in a 5% CO₂ humidified atmosphere at 37°C.

294

295 **Viruses**

296 SARS-CoV-2 variants were kindly provided by the Korean Centers for Disease Control and
297 Prevention. SARS-CoV-2 variants used in this study were listed in Table 1⁵⁶. SARS-CoV-2 was
298 propagated and prepared in African green monkey kidney Vero E6 cells (ATCC). SARS-CoV-2
299 infections was performed at a multiplicity of infection (MOI) of 1 in biosafety level 3 (BSL3) facility.
300 All experiments using SARS-CoV-2 was handled in the enhanced BSL3 containment laboratory as
301 approved by the Korean Center for Disease Control and Prevention.

302

303 **Reagents and antibodies**

304 The US Food and Drug Administration (FDA)-approved EGFR inhibitors including gefitinib,
305 olmutinib, erlotinib, lapatinib, bosutinib, cabozantinib, icotinib, vandetanib, brigatinib, afatinib,
306 dacomitinib, and neratinib as well as remdesivir were provided by the Korean Chemical Bank at the
307 Korean Research Institute of Chemical Technology (KRICT, Daejeon, South Korea). Primary
308 antibodies used in this study include the following: rabbit monoclonal anti-phospho-EGFR (Y1068,
309 Y1092) (Cell Signaling), rabbit polyclonal anti-EGFR (Millipore), rabbit monoclonal anti-phospho-

310 Akt (T308) (Cell Signaling), rabbit monoclonal anti-Akt (Cell Signaling), mouse monoclonal anti-
311 MFN1/2 (Abcam), goat polyclonal anti-VDAC (Abcam), mouse monoclonal anti- β -actin (Cell
312 Signaling), mouse monoclonal anti-TOM20 (BD), rabbit monoclonal anti-GAPDH (Cell Signaling),
313 mouse monoclonal anti-Hu total OxPhos complex (Thermo Fisher Scientific), rabbit monoclonal anti-
314 SARS-CoV-2 nucleoprotein (Sino Biological), and rabbit polyclonal anti-SARS-CoV-2 spike (Sino
315 Biological). The secondary antibodies used for immunofluorescence were Alexa Fluor 633 goat anti-
316 rabbit antibody. The secondary antibodies used for western blot analyses were HRP-conjugated anti-
317 mouse IgG (Invitrogen, Waltham, MA, USA) and HRP-conjugated anti-rabbit IgG (Invitrogen).

318

319 **Western blot analysis**

320 For the immunoblotting assay, denatured protein samples were prepared by boiling in SDS loading
321 buffer (50 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 0.1 M DTT, 0.05% bromophenol blue). Proteins
322 were separated via SDS-PAGE and transferred onto PVDF membranes (Millipore). The membranes
323 were blocked with 5% skim milk in PBS containing 0.1% Tween-20 (PBS-T) and stained with
324 antibodies against the indicated proteins. The membranes were washed three times with PBS-T and
325 then developed using the western ECL Femto Kit (LPS Solution). Images were captured using
326 ImageQuant LAS 4000 (GE Healthcare). The intensity of protein expression was quantified using
327 ImageJ (Bethesda).

328

329 **TMRE (tetramethylrhodamine, ethyl ester) assay**

330 A Mitochondrial Membrane Potential Assay kit (BioVision) was used according to the
331 manufacturer's instructions. A total cells were incubated with the fluorescent TMRE (200nM) dye for
332 20 min at 37°C, 5% CO₂. For a negative control, FCCP (carbonyl cyanide 4-(trifluoromethoxy)
333 phenylhydrazon, 400nM) was added into one well and incubated at 37°C for 10 min prior to TMRE

addition. Following incubation, the stained cells were harvested and gently washed with assay buffer. The fluorescence signal was measured at 549/575 nm of excitation/emission wavelengths using a Synergy H1 multi-mode microplate reader (BioTek). Two technical and biological replicates were performed for each sample.

338

Real-time qRT-PCR and qPCR

To analyse the expression levels of EGFR and SARS-CoV-2 viral genes, total RNA was extracted from cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Extracellular viral RNA from supernatants was isolated using a QIAamp Viral RNA Mini Kit (Qiagen). The cellular RNA level of *EGFR* was quantified by real-time qRT-PCR using the One-Step SyBr Green RT-PCR Kit II (Takara). The intracellular and extracellular SARS-CoV-2 RNA copy numbers were determined by real-time qRT-PCR using the One Step PrimeScript III RT-PCR Kit (Takara). The following primer sets were used for RT-PCR: *EGFR* forward, 5'-AGGCAGGAGTAACAAGCTCAC; *EGFR* reverse, 5'-ATGAGGACATAACAAGCCACC; SARS-CoV-2-N forward, 5'-TTACAAACATTG GCCGCAA; SARS-CoV-2-N reverse 5'-GCGCGACATTCCGAAGAA; SARS-CoV-2-N probe 5'-FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQ1; β -actin forward, 5'-ACAGAGCCTCGCCTTTG; β -actin reverse, 5'-CCTTGCACATGCCGGAG, β -actin probe; 5'-/56-FAM/TCATCCATG/ZEN/GTGAGCTGGCGG/3IABkFQ.

To analyze the expression levels of mitochondrial DNA, total cellular DNA was extracted from the cells using AllPrep DNA kit (Qiagen) and subsequently quantified by qPCR using DyNAmo HS SYBR Green qPCR kit according to the manufacturer's instructions. The following primer sets were used for qPCR: ND-2 forward, 5'-TAGCCCCCTTTCACCTTCTGA; ND-2 reverse, 5'-GCGTAGCTGGGTTTGGTTTA; COX-2 forward, 5'-GGCCACCAATGGTACTGAAC; COX-2

reverse, 5'-CGGGAATTGCATCTGTTTTT. Real-time qPCR was conducted using the QuantStudio3 Real-Time PCR System (Applied Biosystems).

Immunofluorescence

For immunofluorescence, cells grown on glass cover slips were infected with SARS-CoV-2 at an MOI of 1. At 2 days post-infection, cells were fixed with 4% paraformaldehyde and permeabilized with 0.2% Triton X-100 in phosphate-buffered saline. Cells were blocked in blocking buffer (2% BSA, PBS) and immunostained with the indicated antibodies. The coverslips were mounted in antifade medium containing DAPI (Vector Laboratories). Images were captured using a Zeiss LMS700 confocal laser-scanning microscope and evaluated using ZEM 2012 SP5.

Cell viability assay

Cell viability and ATP levels were measured using a Synergy H1 Microplate Reader (BioTek) using the CellTiter-Glo assay (Promega) according to the manufacturer's instructions. Total cell number and cell viability were quantified using a haemocytometer and the Trypan Blue exclusion method.

Subcellular fractionation

Mitochondria were isolated from SARS-CoV-2-infected cells or lung tissue of SARS-CoV-2-infected mice using a Mitochondria Isolation Kit (Thermo Scientific) according to the manufacturer's instructions. Protein extracted from each cellular fraction was analysed by western blotting with the indicated antibodies.

Animal experiment

380 Mouse experiments were approved by the Institutional Animal Care and Use Committee (IACUC)
381 of the Korea Research Institute of Chemical Technology (2020-8B-07-01). Nine wild-type C57BL/6
382 aged mice (15 month old) were evenly divided into three groups and housed in the ABSL-3 facility.
383 Mice from each group were lightly euthanized with isoflurane and injected with SCoV2 (1×10^5
384 pfu/head, clade S) into each mouse via the intranasal route. Mice were orally administered vehicle or
385 vandetanib (25 mg/kg per mouse) once daily. All mice was terminated for further analysis at 4 day
386 post-infection. Mouse lung samples were washed with PBS and harvested to test the inhibition of
387 SCoV2. Tissue samples from the lung were homogenized in cold phosphate-buffered saline (PBS)
388 solution with a FastPrep-24 homogenizer (MP Biomedicals) for 5 cycles (20 s on/ 20 s off). Three
389 additional freeze-thaw cycles were performed at -80°C , and cell debris was removed by centrifugation
390 at 13000 rpm for 1 min to measure the viral RNA and proteins in the lung.

391

392 **FFU (Focus forming unit) assay**

393 The titer of infectious virus in the culture medium was determined by FFU assay. Vero E6 cells
394 were seeded in a 96 well plate. The infectious cell culture medium was used to infect the cells and, the
395 plates were incubated at 37°C . After 8 h, cells were washed with PBS and fixed with 4%
396 paraformaldehyde and permeabilized with methanol. SCoV2 foci were developed using anti-SCoV2
397 N antibody followed by addition of horseradish peroxidase-conjugated goat anti-rabbit IgG secondary
398 antibody and TMB substrate (Promega). Plates were incubated in dark condition at room temperature
399 for 30 min. The plates were washed and dried for observation under a light microscope. The number
400 of SCoV2 positive foci was scanned and counted in each well using an ImmunoSpot reader (CTL,
401 Shaker Height, OH).

402

403 **RNAseq analysis and data processing**

Raw reads were mapped to the human genome assembly hg38 using the aligner STAR v.2.4.0b⁵⁷. To measure gene expression level, the gene annotation database of the species was used with Cufflinks v2.1.1⁵⁸. For differential expressed gene (DEG) analysis, gene level count data were generated by using HTSeq-count v0.6.1p1⁵⁹. The R package called TCC⁶⁰ was used to identify DEGs. For comparing tag count data, this package applies robust normalization strategies. Normalization factors were measured by using the iterative DEGES/edgeR method. For correcting errors caused by multiple-testing, the DEGs were identified based on the qvalue threshold less than 0.05⁶¹.

Statistical analysis

Unpaired Student's *t*-tests and one-way ANOVA were performed using GraphPad Prism 8 (Graph Pad, La Jolla, CA, USA). A value of $P < 0.05$ was considered significant.

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Author contributions

H.J.S. and S.-J.K. conceptualised and designed the study. H.J.S., K.B.K., G.Y.Y., H.-W.M., and

427 C.K. conducted the experiments. H.J.S., B.-T.K., J.-W.O., A.S., and S.-J.K. analysed the data. H.J.S.
428 and S.-J.K. wrote the paper.

429

430 **Competing interests**

431 The authors declare no competing interests.

432

433 **References**

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600 **Figure legends**

601 **Fig. 1 | SCoV2 induces aberrant mitochondrial elongation and alterations in mitochondrial**
602 **bioenergetics. a**, Quantification of the elevated level of intracellular ATP in SCoV2-infected HEK293
603 cells at the indicated time points. Data, average of two independent experiments (mean $\pm$ SD; n=3;
604 *p<0.001). **b**, Quantification of $\Delta\Psi_m$ increase in HEK293 cells infected with SCoV2 at an MOI of 1.
605 $\Delta\Psi_m$ in SCoV2-infected cells were monitored by TMRE assay at 1 day post-infection (mean $\pm$ SD;
606 n=3; **p<0.005). $\Delta\Psi_m$, mitochondrial membrane potential; FCCP, synthetic mitochondrial uncoupler,
607 a control for $\Delta\Psi_m$ loss; accompanying diagram represents SCoV2-induced increase in $\Delta\Psi_m$. **c**,
608 Quantification of $\Delta\Psi_m$ increase by SCoV2 RNA-N cluster. HEK293 cells transiently expressing
609 SCoV2 N gene were transfected with SCoV2 RNA (1 μ g/ml). $\Delta\Psi_m$ in cells were monitored by TMRE
610 assay at 4 h post-transfection (mean $\pm$ SD; n=3; *p<0.001, ***p<0.01). poly(I:C), synthetic analog of
611 double-stranded RNA, a control for $\Delta\Psi_m$ loss; accompanying diagram represents SCoV2 RNA-N
612 cluster-induced increase in $\Delta\Psi_m$. **d**, Confocal microscopy showing the elongated and swollen shape of
613 mitochondria in SCoV2-infected cells. SCoV2-infected HEK293 cells were stained with SCoV2-S
614 (red) and TOM20 (green) antibodies. Nuclei, DAPI (blue); Infection, SCoV2 spike (S) antigen;
615 accompanying graph represents the quantification of mitochondrial length by MBF ImageJ. **e**, Western
616 blot analysis of MFN1 expression in SCoV2-infected cells. At 1 days post-infection, mitochondrial
617 fraction (Mito) isolated from SCoV2-infected HEK293 cells was analyzed by immunoblotting with
618 anti-MFN1 antibody. **f**, qPCR data showing the increase of mitochondrial DNA in SCoV2-infected
619 cells. At 1 day post-infection, the expression level of mitochondrial ND2 and COX2 DNA was
620 analyzed by real-time qPCR. GAPDH was used to normalized changes in ND2 and COX2 expression
621 (mean $\pm$ SD; n=3; *p<0.001). **g**, Western blot analysis of mitochondrial respiratory chain complex
622 enzyme expression in SCoV2-infected cells. At a day post-infection, the expression level of complex
623 I, II, III, IV, and V enzymes was analyzed by immunoblotting with anti-Hu total OXPHOS complex
624 antibody. β -actin, an internal loading control; accompanying graph represents the quantification of

625 increase in complex I, II, III, IV, and V expression in SCoV2-infected cells by ImageJ. P value was
626 calculated by using an unpaired Student's t-test. **h**, Heat maps of relative mRNA and protein expression
627 of the indicated mitochondrial OXPHOS genes isolated from uninfected and SARS-CoV-2-infected
628 cells. Each box indicates an average of three independent experiments. Colour indicates log1 fold-
629 change for uninfected vs SARS-CoV-2-infected cells.

630

631 **Fig. 2 | SCoV2 promotes EGFR-mediated cell survival signal cascade and mitochondrial EGFR**
632 **translocation. a**, Quantification of SCoV2-infected HEK293 cell viability. Total number of viable
633 HEK293 cells infected with SCoV2 at an MOI of 1 was measured at the indicated time points. **b**, Light
634 microscopic analysis of SCoV2-infected HEK293 cells at 5 days post-infection. **c**, Quantitative
635 analysis of EGFR gene expression in SCoV2-infected cells. At 1 day post-infection, the expression
636 level of EGFR mRNA in SCoV2-infected cells were analysed by real-time qRT-PCR (mean $\pm$ SD; n=3;
637 *p<0.001). **d**, Western blot analysis of EGFR-mediated signal cascade in SCoV2-infected cells. At 1
638 days post-infection, whole cell lysates of SCoV2-infected HEK293 cells were analysed by
639 immunoblotting with antibodies specific to p-EGFR, EGFR, p-Akt (Thr308), and Akt. β -actin, an
640 internal loading control. **e**, SCoV2-induced mitochondrial accumulation of EGFR. Cytosolic and
641 mitochondrial fractions isolated from uninfected and SCoV2-infected HEK293 cells were evaluated
642 by immunoblotting with antibodies specific to p-EGFR and EGFR. Fractions: purified cytoplasm, Cyto;
643 purified mitochondria, Mito. Organelle marker: TOM20, mitochondria. Infection marker: SCoV2
644 nucleocapsid (N) antigen. The relative intensity of EGFR normalized to TOM20 and p-EGFR
645 normalized to EGFR respectively was analysed by ImageJ. **f**, Confocal microscopy showing the
646 mitochondrial accumulation of EGFR in SCoV2-infected cells. SCoV2-infected HEK293 cells were
647 stained with EGFR (green), VDAC (red), and SCoV2-N (white on upper panel, blue on lower panel)
648 antibodies. Infection, SCoV2 nucleocapsid (N) antigen; Arrow (white), colocalization of VDAC and
649 SCoV2 N antigen. **g**, Western blot analysis of mitochondrial EGFR accumulation in lung tissue of

650 wild-type aged mouse infected with SCoV2. Mitochondrial fraction (Mito) isolated from lung tissue
651 of uninfected and SCoV2-infected wild-type aged mouse were analysed by immunoblotting with
652 EGFR antibody. Organelle marker: TOM20, mitochondria. Infection marker: SCoV2 nucleocapsid (N)
653 antigen. The relative intensity of EGFR expression level normalized to TOM20 was analysed by
654 ImageJ.

655

656 **Fig 3 | Tyrosine kinase inhibitors targeting EGFR signaling pathway suppresses SCoV2**
657 **propagation. a**, A scheme for screening antiviral effect of EGFR inhibitors against SCoV2
658 propagation. At 4 h post-infection, HEK293 cells infected with SCoV2 at an MOI of 1 were washed
659 with fresh cell culture media 5 times and subsequently treated with the indicated EGFR inhibitors (10
660 μ M) for 44 h. Cell culture supernatant and pellet were used for further analysis. **b**, Extracellular RNA
661 isolated from culture supernatant of SCoV2-infected cells was used for the analysis of SCoV2 RNA
662 level by qRT-PCR using PCR primers set specific to the SCoV2 N gene. **c**, Whole cell lysates of
663 SCoV2-infected cells were analysed with SCoV2 N antibody. Accompanying graph, ImageJ
664 quantification of SCoV2 N expression level. **d**, Rescue of SCoV2-induced abnormal mitochondrial
665 accumulation of EGFR. Cytosolic and mitochondrial fractions isolated from uninfected and SCoV2-
666 infected cells treated with vandetanib were analysed by immunoblotting with EGFR antibody.
667 Fractions: purified cytoplasm, Cyto; purified mitochondria, Mito. Organelle marker: TOM20,
668 mitochondria; GAPDH, cytoplasm. SCoV2 infection marker: SCoV2 nucleocapsid (N). The relative
669 intensity of EGFR normalized to TOM20 was analysed by ImageJ. **e**, Rescue of SCoV2-induced
670 elevation in intracellular ATP level by vandetanib treatment. At 1 day post-treatment of vandetanib,
671 intracellular ATP level in SCoV2-infected HEK293 cells was analysed as described in the Materials
672 and Methods. Data, average of two independent experiments (mean $\pm$ SD; n=3; *p<0.001). **f**, Western
673 blot analysis of mitochondrial respiratory chain complex enzyme expression. At 1 day post-treatment
674 of vandetanib, the expression level of complex I, II, III, IV, and V enzymes was analysed by

immunoblotting with anti-Hu total OxPhos complex antibody. β -actin, an internal loading control; SCoV2 N, infection marker. **g**, Heat maps of relative mRNA and protein expression of the indicated mitochondrial OXPHOS genes isolated from SARS-CoV-2-infected and vandetanib-treated SARS-CoV-2-infected cells. Each box indicates an average of three independent experiments. Colour indicates log₁ fold-change for SARS-CoV-2-infected vs vandetanib-treated SARS-CoV-2-infected cells.

681

Fig 4 | Vandetanib is a potent antiviral agent for SCoV2 propagation. **a**, Viability of SCoV2-infected cells treated with vandetanib for 2 days. Cell viability was analysed as described in the Materials and Methods. **b**, Dose dependent antiviral effect of vandetanib against SCoV2 infection. At 2 days post-treatment, whole cell lysates of SCoV2-infected cells treated with vandetanib at the indicated concentrations were analysed by immunoblotting with SCoV2 N antibody. The relative intensity of SCoV2 N expression level normalized to β -actin was analysed by ImageJ. **c**, Anti-SCoV2 effect by post-treatment of vandetanib. HEK293 cells infected with SCoV2 at an MOI of 1 for 4 h were washed with fresh cell culture media and then treated with vandetanib (0.1, 1, or 10 μ M) for 44 h. **d**, Anti-SCoV2 effect by pre-treatment of vandetanib. HEK293 cells treated with vandetanib (0.1, 1, or 10 μ M) for 4 h were infected with SCoV2 at an MOI of 1 for 44 h. Intracellular SCoV2 RNA level in SCoV2-infected cells was analysed by qRT-PCR using PCR primers set specific to the SCoV2 N gene (c-d). **e**, In vivo anti-SCoV2 efficacy of vandetanib in mouse model susceptible to SCoV2 infection. Wild-type aged mice (15 months old) intranasally inoculated with SCoV2 (1×10^5 pfu/head, clade S) were orally administrated with vandetanib daily. At 4 days post-infection, all mice was terminated for further analysis. **f**, Intracellular SCoV2 RNA level in lung tissue isolated from SCoV2-infected mice was analysed by qRT-PCR using PCR primers set specific to the SCoV2 N gene. **g**, Additive anti-SCoV2 effect of vandetanib in combination with remdesivir. HEK293 cells infected with SCoV2 at an MOI of 1 were treated with remdesivir (0.1 nM, about 10,000-fold lower concentration

700 than the IC₅₀ for SCoV2) in the presence or absence of vandetanib (10 µM) for 48 h. Intracellular
701 SCoV2 RNA level was analysed by qRT-PCR using PCR primers set specific to the SCoV2 N gene. P
702 values were calculated by one-way ANOVA (mean ± SD; n =3; *P ≤ 0.0001).

703

704 **Fig 5 | Vandetanib is a very effective against SCoV2 variants. a-b,** Antiviral effect of vandetanib
705 against SCoV2 variants. HEK293 cells infected with SARS-CoV-2 (S, V, G, GH, and GR clades) and
706 its variants including United Kingdom variant (B.1.1.7) and South Africa variant (B.1.351) at an MOI
707 of 1, respectively, were treated with vandetanib for 48 h. **a,** Total RNA isolated from cell culture media
708 of SCoV2-infected cells was used for analysis of extracellular SCoV2 RNA level by qRT-PCR using
709 PCR primers set specific to the SCoV2 N gene. **b,** Suppression of SCoV2 infectivity by vandetanib
710 treatment was analysed by focus forming unit assay as described in the Materials and Methods.

711

712 **Fig 6 | A schematic diagram showing a significant contribution of SCoV2-induced altered**
713 **mitochondrial dynamics and mitochondrial EGFR accumulation in sustaining viral propagation.**
714 First, SCoV2 RNA and nucleocapsid complex increases ΔΨ_m during SCoV2 infection. This alteration
715 subsequently promotes mitochondrial elongation and swelling. SCoV2 also induces mitochondrial
716 biogenesis and activates mitochondrial OXPHOS process, thereby promoting abundant ATP
717 production. Second, SCoV2 activates EGFR-mediated cell survival signaling and subsequently
718 promotes mitochondrial EGFR internalization which contributes to maintenance of abnormal
719 mitochondrial bioenergetics. These alterations are physiologically relevant to the maintenance of
720 homoeostasis of SCoV2-infected cells and robust SCoV2 propagation

721

722 **Extended Data Fig. 1 | SCoV2 infectivity and replication in human embryonic kidney cells. a,**

723 Immunofluorescence microscopic analysis of SCoV2 infectivity in HEK293 cells. SCoV2-infected
724 cells were stained with SCoV2-N antibody (green) at 2 days post-infection. Nuclei, DAPI (blue);
725 Infection, SCoV2 nucleocapsid (N) antigen (green); accompanying graph, quantification of SCoV2-
726 N-positive cells. **b**, Real-time qRT-PCR data showing a rapid increase in intracellular SCoV2 RNA
727 level in HEK293 cells. Data, average of two independent experiments. **c**, Western blot data showing
728 the expression of spike and N antigens in SARS-CoV-2-infected HEK293 cells. Whole cell lysates of
729 SCoV2-infected cells were analysed by immunoblotting with antibodies specific to SCoV2 spike and
730 N antigens. N, SCoV2 nucleocapsid; β -actin, an internal loading control.

731

732 **Extended Data Fig. 2 | SCoV2 infection leads to the elevation in intracellular ATP synthesis and**
733 **prolonged survival of normal human primary bronchial cells. a**, Confocal microscopy showing
734 SCoV2 infectivity in normal human primary bronchial cells. At 3 days post-infection, SCoV2-infected
735 cells were stained with SCoV2 spike antibody (red). Nuclei, DAPI (blue). **b**, Real-time qRT-PCR data
736 showing a rapid increase in intracellular SCoV2 RNA level in normal human primary bronchial cells.
737 Data, average of two independent experiments. **c**, Western blot data showing the expression of spike
738 and N antigens in SARS-CoV-2-infected normal human primary bronchial cells. Whole cell lysates of
739 SCoV2-infected cells were analysed by immunoblotting with antibodies specific to SCoV2 spike and
740 N antigens. N, SCoV2 nucleocapsid; β -actin, an internal loading control. **d**, Quantification of increased
741 intracellular ATP level in normal human primary bronchial cells infected with SCoV2 at an MOI of 1
742 at the indicated time points. Data, average of two independent experiments. **e**, Viability of SCoV2-
743 infected normal human primary bronchial cells. At 3 days post-infection, total number of normal
744 human primary bronchial cells infected with SCoV2 was measured using a hemocytometer and trypan
745 blue. **f**, Microscopic analysis of SCoV2-infected normal human primary bronchial cells at 5 days post-
746 infection.

747

748 **Extended Data Fig. 3 | SCoV2 does not induce cytopathic effect during robust virus propagation.**

749 **a**, Light microscopic analysis of SCoV2-infected human-derived liver hepatoma Huh7 cells and lung
750 carcinoma epithelial A549 cells. At 3 days post-infection, cells were observed under a light microscope
751 at the BSL3 facility. **b-c**, Quantification of intracellular and extracellular SCoV2 RNA levels in Huh7
752 and A549 cells. Cells were infected with SCoV2 at an MOI of 1 for 3 days. Total RNAs purified from
753 SCoV2-infected cells or culture supernatants were used for analyses of intracellular and extracellular
754 SCoV2 RNA levels by real-time qRT-PCR using PCR primers set specific to the SCoV2 N gene. Data,
755 average of two independent experiments.

756

757 **Extended Data Fig. 4 | Wild-type aged mouse susceptible to SCoV2 infection. a**, Wild-type

758 C57BL/6 aged mice (15 months old) were intranasally inoculated with SCoV2 (1×10^5 pfu/head, clade
759 S). **b-c**, Body weight and survival rate of SCoV2-infected mice were monitored daily. **d**, At 1, 3, and
760 7 days post-infection, uninfected and SCoV-2-infected mice was terminated for further analysis.
761 Intracellular SCoV2 RNA level in lung tissue isolated from uninfected and SCoV2-infected mice was
762 analysed by qRT-PCR using PCR primers set specific to the SCoV2 N gene.

763

764 **Table 1. SARS-CoV-2 viruses used in this study**

Virus	Clade		NCCP* number	Cell line for virus propagation	Virus stock concentration**
SARS-CoV-2	S		43326	Vero E6	1x10 ⁶ pfu/ml
	V		43342		5x10 ⁵ pfu/ml
	G		43343		1x10 ⁶ pfu/ml
	GR		43344		1x10 ⁶ pfu/ml
	GH		43345		1x10 ⁶ pfu/ml
	GR	UK variant (B.1.1.7)	43381		1x10 ⁷ pfu/ml
	GH	SA Variant (B.1.351)	43382		1x10 ⁷ pfu/ml

765

766 *NCCP: National Culture Collection for Pathogens, National Institute of Health, South Korea

767 ** After virus propagation using Vero cell, virus concentration was titrated by plaque assay

768

Table 2. Summary of FDA-approved EGFR inhibitors showing anti-SARS-CoV-2 efficacy

EGFR inhibitors (TKIs)	Structure	Final concentration for primary screening	Cytotoxicity*	Anti-SARS-CoV-2 efficacy**
Gefitinib		10 μ M	– (negative)	$\geq 4.1 \times 10^1$ RNA***
Olmутinib		10 μ M	– (negative)	$\geq 7.6 \times 10^3$ RNA***
Erlotinib		10 μ M	– (negative)	$\geq 1.6 \times 10^3$ RNA***
Lapatinib		10 μ M	– (negative)	$\geq 2.4 \times 10^3$ RNA***
Bosutinib		10 μ M	– (negative)	$\geq 1.4 \times 10^3$ RNA***
Cabozantinib		10 μ M	– (negative)	$\geq 1.4 \times 10^4$ RNA***
Icotinib		10 μ M	– (negative)	$\geq 4.0 \times 10^3$ RNA***
Vandetanib		10 μ M	– (negative)	$\geq 1.9 \times 10^4$ RNA***

Brigatinib		10 μ M	– (negative)	$\geq 4.1 \times 10^2$ RNA ^{***}
Afatinib		10 μ M	+ (positive)	N.D. ^{****}
Dacomitinib		10 μ M	+ (positive)	N.D. ^{****}
Neratinib		10 μ M	+ (positive)	N.D. ^{****}

770

771 *In the presence of EGFR inhibitors for 48 h, cytotoxicity of SARS-CoV-2-infected HEK293 cells was
772 observed by light microscope in biosafety laboratory 3 facility.

773 **Anti-SARS-CoV-2 efficacy of EGFR inhibitors was determined by extracellular SARS-CoV-2 RNA
774 level analysed by real-time quantitative RT-PCR with primer set specific to SARS-CoV-2 N gene.

775 ***Unit: copies/ μ l

776 ****N.D.: Not determined

Fig. 1

Fig. 2

Fig. 3

Fig. 4

b

Fig. 6

Extended Data Fig. 1

Extended Data Fig. 2

Extended Data Fig. 3

a

	Uninfected	SCoV2 (3 dpi)	Cell lines	CPE observation
Huh7			Human-derived liver hepatoma cells	No CPE
A549			Human-derived lung carcinoma epithelial cells	Very marginal CPE

b

c

Extended Data Fig. 4