SRSF protein kinases 1 and 2 are essential host factors for human coronaviruses including SARS-CoV-2

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ABSTRACT

Antiviral therapeutics against SARS-CoV-2 are needed to treat the pandemic disease COVID-19. Pharmacological targeting of a host factor required for viral replication can suppress viral spread with a low probability of viral mutation leading to resistance. Here, we used a genome-wide loss of function CRISPR/Cas9 screen in human lung epithelial cells to identify potential host therapeutic targets. Validation of our screening hits revealed that the kinase SRPK1, together with the closely related SRPK2, were jointly essential for SARS-CoV-2 replication; inhibition of SRPK1/2 with small molecules led to a dramatic decrease (more than 100,000-fold) in SARS-CoV-2 virus production in immortalized and primary human lung cells. Subsequent biochemical studies revealed that SPRK1/2 phosphorylate the viral nucleocapsid (N) protein at sites highly conserved across human coronaviruses and, due to this conservation, even a distantly related coronavirus was highly sensitive to an SPRK1/2 inhibitor. Together, these data suggest that SRPK1/2-targeted therapies may be an efficacious strategy to prevent or treat COVID-19 and other coronavirus-mediated diseases.

INTRODUCTION

In December 2019, a novel human coronavirus, now known as SARS-CoV-2, emerged and began causing a human disease termed COVID-19[1,2]. Since then, a global pandemic has infected millions and caused hundreds of thousands of deaths to date. Due to the prevalence and severity of this disease, the development of therapeutic interventions is of the highest importance. Much attention has been focused on targeting viral proteins and their associated enzymatic activities. In particular, the virally encoded RNA-

67 dependent RNA polymerase (RdRp) and the viral proteases, are attractive potential
68 targets. Remdesivir, the only FDA-approved antiviral for SARS-CoV-2, is a nucleoside
69 analogue which targets the viral RdRp and causes premature termination of
70 transcription[3]. Efficacy of this treatment however, unfortunately, appears limited[4].
71
72 In addition to targeting viral proteins directly, other antiviral development strategies
73 attempt to target host factors that the virus requires to complete its lifecycle. Relative to
74 their hosts, viruses have dramatically less coding space in their genomes and therefore
75 utilize host proteins to supplement the activities of their own proteins. The major
76 advantages of inhibiting a virus indirectly via an essential host factor are two-fold: (1)
77 many viruses may utilize the same host protein, therefore host-directed therapeutics have
78 the potential to be broadly acting and (2) while direct targeting of the virus can rapidly
79 select for resistant viral mutants, it is thought to be much more difficult for a viral mutation
80 to overcome inhibition of a co-opted host protein.
81
82 The development of host-directed COVID-19 therapeutics has been hindered however,
83 due to an incomplete understanding of the host factors required for SARS-CoV-2
84 replication; this is in no small part due to its recent emergence into the human population.
85 Although a genetic screen for the host factors utilized by SARS-CoV-2 in African green
86 monkey cells (*Chlorocebus sp.*) was recently reported[5], we wanted to focus on the human
87 proteins essential for SARS-CoV-2 replication. We therefore generated a human lung
88 epithelial cell line (A549) modified to over-express the SARS-CoV-2 receptor ACE2 and
89 performed a genome wide CRISPR/Cas9 screen in BSL3 containment using the

90 prototype USA-WA1/2020 SARS-CoV-2 isolate. Pharmacological targeting of our top hit,
91 SRSF Protein Kinase 1 (SPRK1) and the highly related SRPK2, strongly inhibited the
92 replication of both human alpha- and beta-coronaviruses, likely due to altered
93 phosphorylation of the respective viral nucleocapsid proteins. Thus, not only are the
94 enzymatic activities of SRPK1/2 essential for the replication of multiple human
95 coronaviruses, but targeting these kinases may represent an effective therapeutic
96 approach for coronavirus mediated diseases, including COVID-19.
97

98 **RESULTS**

99 While CRISPR/Cas technology allows most known genes in the host genome to be
100 rapidly screened for effects on viral replication, efficient viral killing of the cells with
101 irrelevant genetic modifications is required. Unfortunately, SARS-CoV-2 cytopathic
102 effects have been reported to be mostly restricted to cell lines derived from non-human
103 primates, which precludes efficient screening in human cell lines[6]. In order to overcome
104 this limitation (and also avoid enriching for well characterized viral entry factors), we
105 generated lentiviruses harboring the SARS-CoV-2 receptor ACE2, and stably transduced
106 a human A549 lung epithelial cell clone that had already been engineered to express
107 Cas9 from *Streptococcus pyogenes*. This cell line expressed ~100x more ACE2 than the
108 parental line, and was dramatically more permissive to SARS-CoV-2 infection.
109 Importantly for our screening goals, the A549-ACE2 line was efficiently killed by SARS-
110 CoV-2 infection as revealed by crystal violet staining (**Supplemental Figure 1**).
111

112 To perform the screen, $2x10^7$ A549-Cas9-Ace2 cells (in two biological replicates) were
113 transduced at a multiplicity of infection (MOI) of 0.8 with lentiviruses harboring the GeCKO
114 V2 sgRNA library. This library contains three individual sgRNAs against most annotated
115 genes in the human genome[7]. Five days post-transduction, a timepoint after which
116 genome editing and protein turnover has occurred, half of the cells ($6x10^7$ cells/replicate)
117 were infected with SARS-CoV-2 at an MOI of 0.05 followed by multicycle viral growth for
118 72 hours. The remaining half of the cells were collected and used as an unselected
119 comparison. Most of the cells were killed by viral infection, which was expected since
120 most host proteins are not essential for viral replication. Those cells whose genomic
121 modifications rendered them refractory to viral killing however, were collected and the
122 sgRNAs that they harbored were sequenced via Illumina sequencing (**Figure 1A,**
123 **Supplemental Dataset 1**).
124
125 Statistical analysis of the sgRNA sequencing data, which considered sgRNA abundance,
126 reproducibility, and enrichment of multiple independent sgRNAs[8], indicated that the host
127 gene with the highest probability of being required for SARS-CoV-2 infection was the
128 serine/arginine-rich protein kinase, SRPK1 (**Figure 1B**). As expected from the design of
129 our screen, we did not enrich for ACE2, however a number of additional human genes
130 were also significantly enriched **(Supplemental Dataset 2)**. Rather than broadly
131 validating predicted screen hits, we instead chose to focus on our top hit as kinases are
132 generally thought to be good drug targets[9], and identifying drug targets was the initial goal
133 of our screen. Since a number of SARS-CoV-2 proteins have been reported to be
134 phosphorylated, we took a bioinformatic approach to predict potential SRPK1

135 phosphorylation sites[10] across the entire SARS-CoV-2 genome (**Figure 1C,**
136 **Supplemental Table 1**). The viral protein with by far the highest confidence predicted
137 SRPK1 sites was the viral nucleoprotein (N). Phosphoproteomic studies of SARS-CoV-
138 2 proteins have reported that some of the predicted SRPK1 phosphorylation sites in the
139 N protein are indeed phosphorylated[11-13] (**Fig 1C**). We therefore hypothesized that
140 SRPK1 was likely responsible for the phosphorylation of at least some sites in the N
141 protein.
142
143 We next wanted to define the degree to which inhibition of SRPK1 mediated N
144 phosphorylation would affect viral replication, especially since other non-SRPK1 kinases
145 have been predicted to be responsible for SARS-CoV-2 protein phosphorylation[12,13]. We
146 selected the SRPK1 inhibitor SPRIN340 (**Figure 1D**) as it has been reported to inhibit
147 SRPK1 (and SRPK2 with an ~10x higher IC50) without affecting other kinases including
148 the related serine/arginine-rich protein kinase family members (SRPKs)[14]. Treatment of
149 A549-ACE2 cells with increasing concentrations of SRPIN340 revealed significant toxicity
150 at a concentration of 160μM; lower concentrations however, were well tolerated (**Figure**
151 **1E**). We next infected cells treated with non-toxic concentrations of SRPIN340 with
152 SARS-CoV-2 and allowed multicycle viral growth for 24 hours. We observed a dramatic
153 SRPIN340-mediated decrease in viral RNA and infectious viral titer (up to 100,000-fold)
154 at the higher drug concentrations (**Figure 1F,G**).
155
156 The stronger viral inhibitory phenotype at higher concentrations of SRPIN340 suggested
157 that SRPK1 and SRPK2 (which are known to have similar substrate specificities[14,15]) likely

158 need to be inhibited simultaneously for full suppression of viral replication. To further test
159 that hypothesis, we made use of another small molecule (SPHINX31) that targets SRPK1
160 without appreciably affecting SRPK2[16]. Consistent with our previous data, while we could
161 significantly inhibit SARS-CoV-2 replication by targeting SRPK1 alone, the magnitude of
162 the effect was smaller than with SRPIN340 (**Supplemental Figure 2**). Finally, in order
163 to provide direct evidence that both SRPK1 and SRPK2 could indeed phosphorylate the
164 SARS-CoV-2 N protein, we performed *in vitro* phosphorylation assays with purified N,
165 SRPK1 or SRPK2, and γ32-P ATP. In a dose dependent manner, increasing
166 concentrations of the kinases led to increased N protein phosphorylation (**Figure 1H,I**).
167 Together, these data indicate that while SRPK1 is the dominant kinase (and thus most
168 enriched in the screen), SRPK2 likely provides at least partial redundancy for N
169 phosphorylation.
170
171 Since all of our experiments had been performed in our artificially generated A549-ACE2
172 cell lines, we next repeated the SRPIN340 inhibitor experiments in Calu-3 cells. Calu-3
173 is a human lung adenocarcinoma line that is naturally permissive to SARS-CoV-2
174 infection (**Figure 2A**). Toxicity of SRPIN340 in Calu-3 cells was similar to A549-ACE2
175 cells, and as expected, SARS-CoV-2 was significantly inhibited at the higher
176 concentrations of SRPIN340 (**Figure 2B-E**). After SRPIN340 treatment, the viral
177 replication intermediate double stranded RNA (dsRNA) became un-detectable,
178 expression of the viral S protein was significantly reduced, and viral RNA and cell free
179 infectious viral particles were reduced by more than three orders of magnitude (**Figure
180 2C-E, Supplemental Figure 3**).

181

182 N proteins of diverse human coronaviruses have highly conserved domains[17,18], including
183 the predicted SRPK1 phosphorylation sites (**Figure 2F, Supplemental Table 2**). We
184 therefore wanted to determine if the viral requirement for SRPK1/2 activity might be
185 broadly conserved as well. SRPIN340 treatment of cells infected with the
186 alphacoronavirus 229E (which is only distantly related to betacoronavirus SARS-CoV-2)
187 inhibited the virus by more than 1,000-fold at non-toxic concentrations of the drug (**Figure**
188 **2G-H**). These data indicate that the requirement for SRPK1/2 activity is not restricted to
189 SARS-CoV-2.

190

191 Finally, we wanted to understand the potential for targeting SARS-CoV-2 via inhibition of
192 SRPK1/2 in primary human lung cells. Type-2 pneumocytes isolated from four
193 independent healthy human donor lungs were first expanded in organoids embedded in
194 Matrigel (**Figure 2I**). For viral infection studies, pneumocytes were then cultured on
195 transwell membranes as monolayers. These distal alveolar cells were then treated with
196 drug or vehicle followed by infection with SARS-CoV-2. Viral replication (as measured by
197 viral protein staining) was strongly inhibited and reduced almost to the level of detection
198 (**Figure 2J,K**). Thus, the viral requirement for SRPK1/2 is not restricted to immortalized
199 cell lines and their kinase activities are essential for viral replication in some of the most
200 vulnerable populations of lung cells affected during severe COVID-19 disease[19].

201

202 **DISCUSSION**

203 Our screen for human host targets that could be pharmacologically inhibited to treat
204 COVID-19 disease identified SRPK1 and SRPK2 as kinases that are critical for the
205 replication of coronaviruses as divergent as 229E and SARS-CoV-2. SRPK1 and 2 are
206 expressed in most human tissues and have been implicated in a number of basal
207 processes including the regulation of transcript splicing and cellular stress responses[20,21].
208 Further, SRPK1 is upregulated in multiple cancers including prostate, breast, lung, and
209 glioma[22]. In particular, it is thought that its regulation of vascular endothelial growth factor
210 (VEGF) is central to its importance for a number of different diseases[23]. As such, a
211 number of different inhibitors of SRPK1 have been developed and used in various pre-
212 clinical disease models, however none of these compounds have yet been FDA
213 approved. In addition to its normal physiological roles and potential roles in cancer,
214 SRPK1/2 kinase activity has been previously reported as important for the replication of
215 a number of different viruses. These viruses include: hepatitis B virus, human
216 papillomavirus, hepatitis C virus, SARS-CoV, Ebola virus, human cytomegalovirus, and
217 herpes simplex virus-1[24-32]. While the mechanisms underlying how SRPK1/2 contribute
218 to the replication of these viruses differ, it is clear that many viruses have evolved to take
219 advantage of these host kinases.
220
221 One major question raised by our work is the relative contributions of different kinases to
222 SARS-CoV-2 viral protein phosphorylation. At least two recent reports have implicated
223 different kinases including: growth factor receptor (GFR) activated kinases, casein kinase
224 II (CK2), cyclin-dependent kinase (CDK), and protein kinase C (PKC) as generally
225 important, but not necessarily directly linked to N protein phosphorylation[12,13]. Previous

226 work with the related betacoronavirus SARS-CoV has shown that, at least in vitro, CDK,
227 glycogen synthase kinase (GSK), mitogen-activated protein kinase, SRPK1, and CK2 can
228 all phosphorylate the SARS-CoV N protein[32,33]. SRPK1 phosphorylation was reported to
229 affect ability of the SARS-CoV N protein to multimerize and inhibits host translation,
230 however any requirement for SRPK2, as well as effects on viral growth were not
231 reported[32]. Additionally, the growth of both SARS-CoV and mouse hepatitis virus (MHV)
232 have been reported to suppressed after treatment with GSK-3 inhibitors, presumably by
233 affecting N protein phosphorylation[34]. Further studies will be required to understand how
234 viral phosphorylation of SARS-CoV-2 protein affects their functions, as well as
235 experimentally define which kinases phosphorylate which viral proteins at which specific
236 locations. It will also be important to define potential kinase redundancy and the effects
237 on viral replication. At least for SARS-CoV-2, it appears that SRPK1/2 are central to the
238 regulation of viral infection, but it is worth noting that although our study has focused on
239 the phosphorylation of the N protein, we cannot rule out that the viral inhibition phenotype
240 observed is also or instead due to altered phosphorylation of other viral, or indeed host,
241 proteins.
242
243 Additionally, while we have provided data that SRPK1/2 inhibitors are effective in both
244 immortalized and primary cells, it remains unclear if targeting SRPK1/2 *in vivo* will have
245 a similar magnitude of effect. In support of potentially using SRPK1/2 inhibitors to treat
246 COVID-19 disease, several reports have utilized inhibitors of SRPK1 in animal models
247 and shown efficacy in suppressing phenotypes such as neovascular eye disease and
248 tumor growth, without apparent toxicity[16,35]. In conclusion, the development of safe and

249 efficacious anti-COVID-19 therapeutics is of the highest importance due to the ongoing
250 pandemic. Targeting of host factors essential for viral replication represents an approach
251 which may help to limit the emergence of viral resistance. Our study identified two host
252 kinases, against which inhibitors have already been developed and for which the
253 magnitude of inhibition of viral replication is on par with direct targeting of the viral
254 polymerase[36]. Continued development, optimization, and use of these kinase inhibitors
255 may lead to a class of host-directed antiviral therapeutics that could help combat the
256 current, and potentially future, coronavirus pandemics.
257

258 **MATERIALS AND METHODS**

259 Cell culture. All cells were obtained from ATCC and grown at 37°C in 5% CO_2. 293T and
260 A549 and Huh7 cells were grown in DMEM with 10% FBS, Glutamax, and
261 Penicillin/Streptomycin. VeroE6 cells were grown in MEM media supplemented with
262 Penicillin/Streptomycin, 10% FBS, 1 mM Pyruvate, and 1X MEM NEAA. Calu-3 cells
263 were grown in EMEM with 10% FBS and Penicillin/Streptomycin. Cells were selected in
264 0.5μg/ml Puromycin and 1μg/ml Blasticidin.
265

266 Plasmids. The ACE2-pLEX overexpression plasmid was generated by cloning a
267 synthesized gBlock of the ACE2 ORF (Ref. seq. NM_001371415.1) into the pLEX plasmid
268 via HiFi DNA Assembly (NEB Cat# M5520AA) and subsequent bacterial transformation.
269 Bacterial colonies were then selected and plasmid DNA isolated using the Genejet
270 Plasmid Miniprep kit (Thermo Fisher Cat# K0503). Plasmids were then sequenced via
271 Sanger sequencing to confirm successful cloning of the ACE2 ORF. Lentiviruses were

272 packaged as per standard protocols with a VSV-G envelope protein, A549-Cas9 cells
273 were transduced and selected with puromycin at 2μg/mL.
274
275 SARS-CoV-2 infections and titering
276 A stock of BEI isolate SARS-CoV-2 USA-WA1/2020 (kind gift of Greg Sempowski) was
277 grown on VeroE6 cells in viral growth media (MEM supplemented with 1% Pen/Strep, 2
278 % FBS, 1 mM Sodium Pyruvate, and 1x MEM NEAA). Infection was incubated for 1 hr
279 at 37℃. After infection total volume of media was brought to 30 mL. Virus was harvested
280 after 72 hrs of infection. To determine viral titer of stock and after drug treatment, a
281 monolayer of VeroE6 cells was infected with serially diluted virus for 1 hr. Virus was
282 removed and an agar overlay was added to each well (MEM, Penicillin/Streptomycin, 2%
283 FBS, 1 mM Pyruvate, 1x MEM NEAA, 0.3% Sodium Bicarbonate, Glutamax, 0.7% oxoid
284 agar). Plaque assays were incubated for 72 hrs then stained with either 0.1% Crystal
285 Violet in 10% Neutral Buffered Formalin or 0.05% Neutral Red. All viral infections took
286 place in cell specific media with only 2% FBS.
287
288 HCoV-229E infections and titering
289 A stock of isolate HCoV-229E VR-740 (ATCC) was grown on Huh7 cells in complete
290 media (DMEM supplemented with 10% FBS, 1% Pen/Strep, Glutamax). Infection was
291 incubated for 1hr at 37°C. After infection total volume of media was brought to 20 ml.
292 Virus was harvested after 36 hrs of infection. Viral titer of stock was determined via plaque
293 assay on Huh7 cells. A confluent monolayer of cells was infected with serial dilutions of
294 virus in complete media for 1 hr at 37°C. Virus was removed and an agar overlay was

295 added to each well (DMEM, 10% FBS, 1% Pen/Strep, Glutamax, 0.5% oxoid agar).
296 Plaque assays were incubated for 72 hrs then stained with 0.1% Crystal Violet in PBS.
297 Viral infection assays took place in complete media (DMEM, 10% FBS, 1% Pen/Strep,
298 Glutamax).
299
300 Quantitative Reverse Transcription PCR. Cells were resuspended in TRIzol Reagent
301 (Thermo Fisher Cat #15596018) and total RNA was isolated by either phase separation
302 with chloroform and isopropanol or using the Zymo Direct-zol RNA Miniprep kit (Zymo
303 Cat #R2050). One-step qRT-PCR was performed using the Invitrogen EXPRESS One-
304 Step Superscript qRT-PCR kit (Thermo Fisher Cat #11781200) and the commercial
305 Taqman probe ACE2 (Hs01085333_m1). The SARS-CoV2 primer/probe set were
306 synthesized using IDT DNA based on the sequences provided by the CDC "Research
307 Use Only 2019-Novel Coronavirus (2019-nCov) Real-time RT-PCR Primers and Probes"
308 set N1. For RNA extractions of 229E infected cells, RNA was extracted using NEB
309 Monarch total RNA miniprep kit (T2010S). To quantify viral RNA we used commercial
310 taqman probe CoV_229E (Vi06439671_s1). Reactions were cycled on the Applied
311 Biosystems QuantStudio 3 Real-Time PCR System and analyzed using QuantStudio
312 software version v1.4.1. RNA was normalized to the endogenous 18S primer-probe set
313 (Thermo Fisher Cat #4319413E).
314
315 Crystal violet staining
316 To visualize CPE of A549 cells expressing Ace2 or not, cells were infected with a MOI of
317 0.1 for 1 hr at 37℃. After 1 hr incubation 0.5 ml DMEM with 2% FBS was added to each

318 well and infection was allowed to continue for 72 hrs. After infection, the monolayer was
319 stained with 0.1% Crystal violet in 10% Neutral Buffered Formalin for 15 minutes. Stain
320 was then removed and cells were rinsed with ultra-pure water and then imaged.
321
322 CRISPR Screening and analysis
323 For the CRISPR screen, $2x10^7$ cells per replicate were transduced with CRISPR GeCKO
324 Library A lenti virus at a MOI of 0.8 in 7.5 ml DMEM with DEAE dextran. After 2 days,
325 cells were expanded and then seeded on day 4 post-transduction in 8 T75 plates per
326 replicate. Cells were infected at a MOI of 0.05 for 1 hr at 37℃. 10 ml of 2% DMEM was
327 added to the infection and cells were placed back in the incubator for 72 hrs. After
328 infection, cells were trypsinized, and centrifuged for 5 minutes at 500xg. Cells were
329 resuspended in DMEM with 10% FBS and replated in 4 T75 flasks. Cells grew for 48hrs
330 and then were expanded to 8 T75 flasks. The next day, 4 flasks of cells were collected
331 and the pellet was resuspended in DNA/RNA shield (Zymo Research). Genomic DNA
332 was purified using QiaAmp Blood Maxi (Qiagen). PCR was performed using Ex Taq DNA
333 Polymerase (Takara Cat #RR001) on 18.5 μg of genomic DNA. PCR bands were gel
334 purified using the GeneJet gel extraction kit (Thermo Scientific Cat #K0832). PCR
335 amplicons were quantified using an Agilent Bioanalyzer and samples were then
336 sequenced on an Illumina MiSeq. Raw reads were extracted and analyzed using the
337 MAGeCK pipeline[8]. Genes were ranked by the p-value assigned by MAGeCK.
338
339 SRPK inhibitor treatment assays

340 Two different inhibitors of SRPK1 and SRPK2 were used to assay effects on viral
341 replication, SRPIN340 and SPHINX31 (MedChem Express). Both drugs were suspended
342 in DMSO at 1000X the final concentration indicated. They were then diluted to the
343 indicated concentrations in cell specific media. Cells were treated with drug 12 hours
344 before infection. Cells were then infected for 1 hour as described above. After infection,
345 media with 2% FBS + drug or DMSO control was added to each well (qRT-PCR and Calu-
346 3 microscopy) or inoculum was removed and media with 2% FBS + drug or DMSO control
347 was added to each well (infectious titer quantification).
348
349 Cytotoxicity Assays. A549-Cas9 ACE2, Calu-3, or Huh7 cells were treated with the
350 indicated concentrations of SRPIN-340 or SPHINX-31 in a consistent volume of vehicle
351 (DMSO). 48 hours after treatment, samples were processed according to the CellTiter-
352 Glo (Promega) protocol and luminescence was determined using a luminometer.
353
354 Calu-3 Infection and immunofluorescence assays. For infections of Calu-3 cells for
355 immunofluorescence assays, cells were treated with DMSO or 53 μM SRPIN340 12 hrs
356 before infection. To infect Calu-3 cells media was removed and cells were washed with
357 0.5 mL PBS. Virus was added at MOI 2.5 and cells were infected at 37°C for 1 hr. After
358 infection, 200 μL media + 2x SRPIN340 or DMSO was added to cells and incubated at
359 37°C for 24 hrs. To fix cells, the culture plate was submerged in 10% neutral buffered
360 formalin for 2 hrs. NBF was removed and cells were placed in PBS. For immunostaining
361 cells were permeabilized with 0.1% Triton X-100 and blocked in PBS+ 5% BSA + 0.1%
362 Tween-20. Cells were stained with either an anti-dsRNA antibody (J2, Sigma

363 MABE1134) at a 1:125 dilution or an anti-SARS-CoV-2 Spike RBD protein (ProSci #9087)
364 at a 1:150 dilution. Alexa fluor 488 or 594 conjugated secondary antibodies were used at
365 a 1:1000 dilution. DNA was stained with Hoechst 33342 dye at a 1:10,000 dilution in
366 PBS. Images were taken on Bio-Rad Zoe fluorescent cell imager.
367
368 In vitro SRPK1/2 phosphorylation assays. To produce SARS-CoV-2 N protein, a 10 cm
369 plate of 293T cells were transfected with 15 μg of the pLVX-EF1alpha-nCOV2019-N-
370 2xStrep-IRES-Puro plasmid[12] using 45 μl Trans-IT LT1. 48 hrs post-transfection, cells
371 were lysed with 2 ml lysis buffer (50mM Tris pH 7.4, 150mM NaCl, 1% Triton X-100, 10%
372 Glycerol, cOmplete, EDTA-free protease inhibitor cocktail (Roche), PhosSTOP (Roche)
373 phosphatase inhibitor (1 tab per 10ml buffer)). Cellular debris was removed by
374 centrifugation 16,000xg for 5 minutes, and lysate was transferred to 2x 2ml tubes each
375 with 100 μl of Dynabeads Streptavidin M-280 (ThermoFisher) that were washed 1 time
376 with lysis buffer. Lysates rotated for 4 hrs at 4°C then beads were collected with a
377 magnet. The lysate was removed, and the beads were washed 3 times with 500 μl TBS
378 (50mM Tris pH 7.4, 150mM NaCl, EDTA-free protease inhibitor cocktail (Roche)). The 2
379 tubes of beads were combined and resuspended in 300μl TBS.
380
381 For kinase assays, N-bound beads were collected, and buffer was removed. The beads
382 were resuspended in 300 μl Kinase buffer (50 mM Tris pH7.4, 50 mM KCl, 5 mM $MgCl_2$,
383 1mM DTT) and 10 μl of beads+N or beads only were placed in 1.5 ml screw cap tubes.
384 1 ng, 10 ng, or 100 ng of SRPK1 or SRPK2 (ThermoFisher) were added to reactions as
385 well as Kinase buffer to bring total volume to 95 μl. Finally, ATP, [γ-32P]- 6000Ci/mmol

386 10mCi/ml was diluted 1:250 in Kinase buffer and 5μl of the diluted hot ATP (0.2 μCi) was
387 added to each reaction. Each reaction was done in triplicate and the reactions were
388 incubated at 30 degrees for 60 minutes with shaking. The samples were washed 3 x with
389 1 ml PBS (Gibco) and radioactivity incorporated was counted on a Beckman LS 6500
390 scintillation counter. The samples were then resuspended in loading dye and run on
391 BioRad Mini-PROTEAN TGX 4-20% stain-free gels. The gels were dried and radioactivity
392 visualized using Carestream BIOMAX MS film. A second gel was stained with
393 Coomassie Brilliant Blue to visualize bead bound proteins.
394
395 Bioinformatic prediction of SRPK1 phosphorylation sites. Sites predicted to be
396 phosphorylated by SRPK1 were identified using Group-based Prediction System v5.0[10]
397 and the following filter: Phosphorylation/Serine/threonine kinase/CMGC/SRPK/SRPK1.
398 Amino acid sequences from annotated ORFs in the following genbank accession
399 numbers (MN985325.1, NC_004718.3, JX869059.2, KF686340.1, MN306036.1,
400 KF530114.1, KF514433.1) were entered individually and a threshold of "high" was
401 applied. Only phosphorylation sites with a prediction score greater than the threshold of
402 71.312 were included in further analyses.
403
404 Human lung dissociation, primary type two pneumocytes purification, culturing, and
405 immunostaining. Human lung tissues were washed with PBS containing 1% Antibiotic-
406 Antimycotic and cut into small pieces. Then samples were digested with enzyme mixture
407 (Collagenase type I: 1.68 mg/ml, Dispase: 5U/ml, DNase: 10U/ml) at 37°C for 1h with
408 rotation. The cells were filtered through a 100 μm strainer, rinsed with 15 ml

409 DMEM/F12+10% FBS medium and centrifuged at 450x g for 10 min. The supernatant
410 was removed and the cell pellet was resuspended in red blood cell lysis buffer for 10min,
411 washed with DMEM/F12 containing 10% FBS and filtered through a 40 µm strainer. Total
412 cells were centrifuged at 450xg for 5 min at 4ºC and the cell pellet was processed for AT2
413 purification.
414
415 For human type two pneumocytes purification, approximately 2-10 million total human
416 lung cells were resuspended in MACS buffer and blocked with Human TruStain FcX
417 (Biolegend cat# 422032) for 15min at 4ºC followed by HTII-280 antibody (Terrace Biotech,
418 TB-27AHT2-280) staining for 1h at 4ºC. The cells were washed twice with MACS buffer
419 followed by incubation with anti-mouse IgM microbeads for 15 min at 4ºC. The cells were
420 loaded into the LS column and collected magnetically. AT2 cells ($3x10^3$) were
421 resuspended in serum free medium, mixed with an equal amount of Matrigel (Corning
422 Cat# 354230) and plated as drops on 6 well plates. The medium was changed every three
423 days. Alveolospheres were passaged every 14 days. For virus infection experiments
424 human AT2 cells were seeded at 10×10^4 cells per insert on 5% matrigel coated Transwell
425 with 0.4-µm pore-sized inserts (Corning). Cells were treated with DMSO or 53 µM
426 SRPIN340 12 hrs before infection with media on the top and bottom of transwell. To
427 infect cells, media was removed from top of transwell and cells were washed with 200 µl
428 PBS. Cells were then infected at a MOI of 1 for 1 hr. Virus was then removed, and cells
429 were cultured for 24 hrs. To fix cells and remove from BSL3, plates and wells were
430 submerged in 10% Neutral buffered formalin for 2 hrs. NBF was removed and cells were
431 placed in PBS until staining.

432

433 For immunostaining of the cultures, the membrane from the transwell insert was cut
434 out with a scalpel and washed with PBS, permeabilized in PBST (0.1% Triton X-100 in
435 PBS), and incubated with blocking buffer (1% BSA in PBST) for 1h at room temperature.
436 Samples were incubated with primary antibodies: Prosurfactant protein C (1:500,
437 Milipore, cat# AB3786) and SARS-CoV-2 (1:500, Genetex cat# GTX632604) in blocking
438 buffer at 4°C overnight. Membranes were then washed 3 times in PBST, incubated with
439 secondary antibodies in blocking buffer for 1h at room temperature followed be three
440 washes with PBST and mounted using Fluor G reagent with DAPI. All confocal images
441 were collected using Olympus Confocal Microscope FV3000 using a 40X objectives.

442

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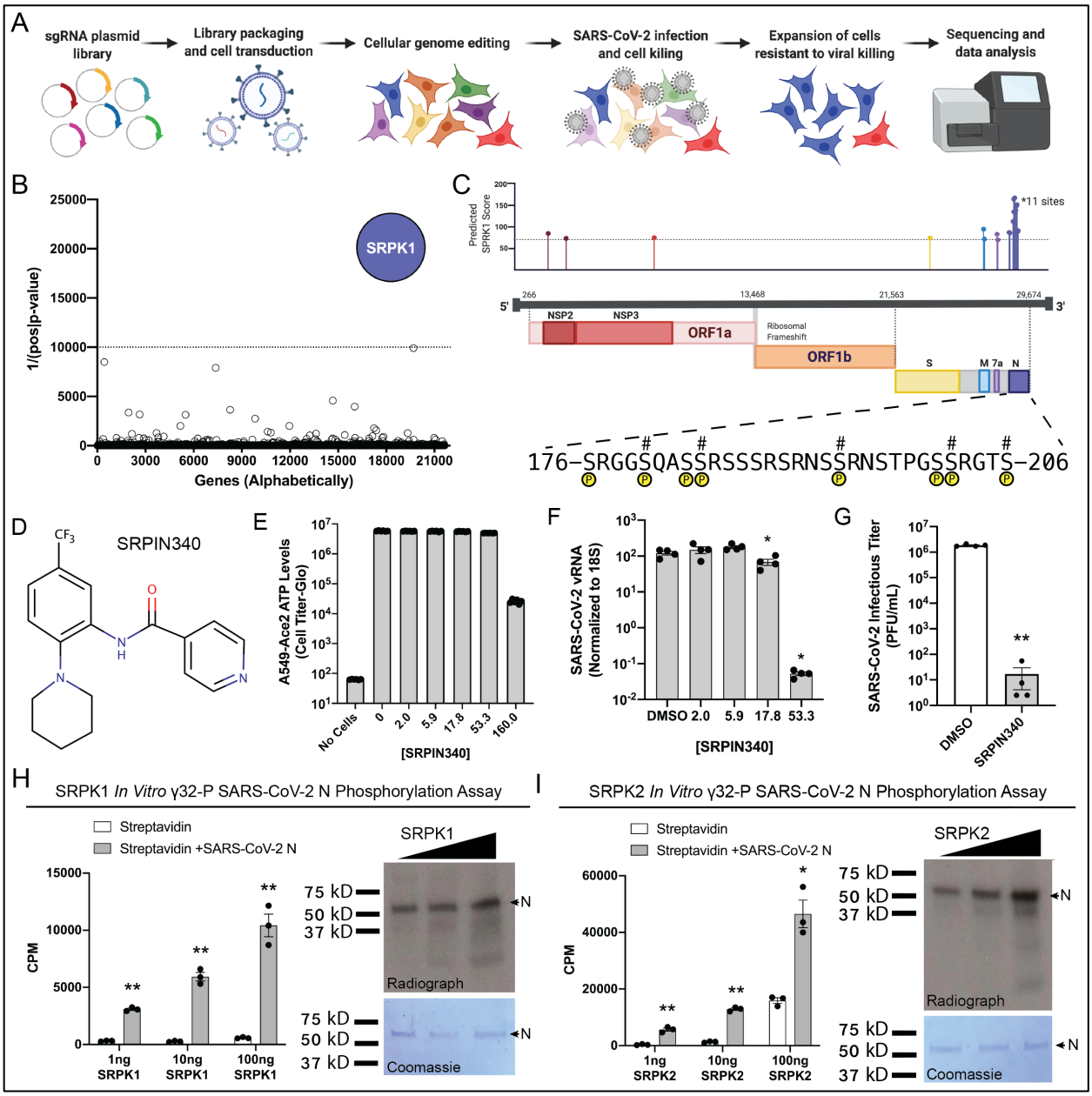
572 **MAIN FIGURES AND LEGENDS**



573

574 **Figure 1. CRISPR screening identifies SRPK1/2 as kinases that mediate**
575 **phosphorylation of the SARS-CoV-2 N protein.** (a) Diagram of CRISPR GeCKO
576 screen in A549-Cas9-Ace2 cells with SARS-CoV-2. (b) Representation of predicted hits
577 from the CRISPR screen. Genes are arranged on x-axis alphabetically and y-axis score
578 is inverse of the p-value for enriched genes. Genes with p-values of less than 10^{-4} were

579 represented with larger dots and the gene name. (c) Map of the SARS-CoV-2 genome
580 with potential SRPK1 phosphorylation sites indicated in graph on the top. Zoom-in of
581 amino acids 176-204 of the N protein that contain multiple potential SRPK1
582 phosphorylation sites. # above the amino acids indicate predicted SRPK1
583 phosphorylation sites while the yellow circles bellow the amino acids indicate the serines
584 reported to be phosphorylated by both Davidson et al. and Bouhaddou et al.[11,12]. (d)
585 Chemical structure of SRPIN340. (e) CellTiter-GLO assay in A549-Cas9-Ace2 cells (n=6)
586 determining the toxicity of SRPIN340 at multiple concentrations of drug. (f) After
587 treatment of A549-Cas9-Ace2 cells with different concentrations of SRPIN 340, SARS-
588 CoV-2 mRNA (n=4) was quantified by qRT-PCR. (g) Infectious virus was quantified (n=4)
589 by plaque assay 48 hrs after infection of A549-Cas9-Ace2 cells that were treated with
590 DMSO or 53 μM SRPIN340. (h) *In vitro* phosphorylation assay using purified SRPK1
591 protein and SARS-CoV-2 N protein on streptavidin beads or streptavidin beads only as
592 substrate. On the left CPM were quantified after phosphorylation reaction, n=3. On the
593 right top panel radiograph of phosphorylated N protein, on the bottom panel Coomassie
594 stained gel showing equal loading of N protein. (i) *In vitro* phosphorylation assay using
595 purified SRPK2 protein and SARS-CoV-2 N protein on streptavidin beads or streptavidin
596 beads only as substrate. On the left CPM were quantified after phosphorylation reaction,
597 n=3. On the right top panel radiograph of phosphorylated N protein, on the bottom panel
598 Coomassie stained gel showing equal loading of N protein. All experiments are
599 representative of at least two independent biological replicates. In all panels, error bars
600 represent SEM, *$p<0.05$ **$p<0.001$ by an unpaired, student's t-test
601

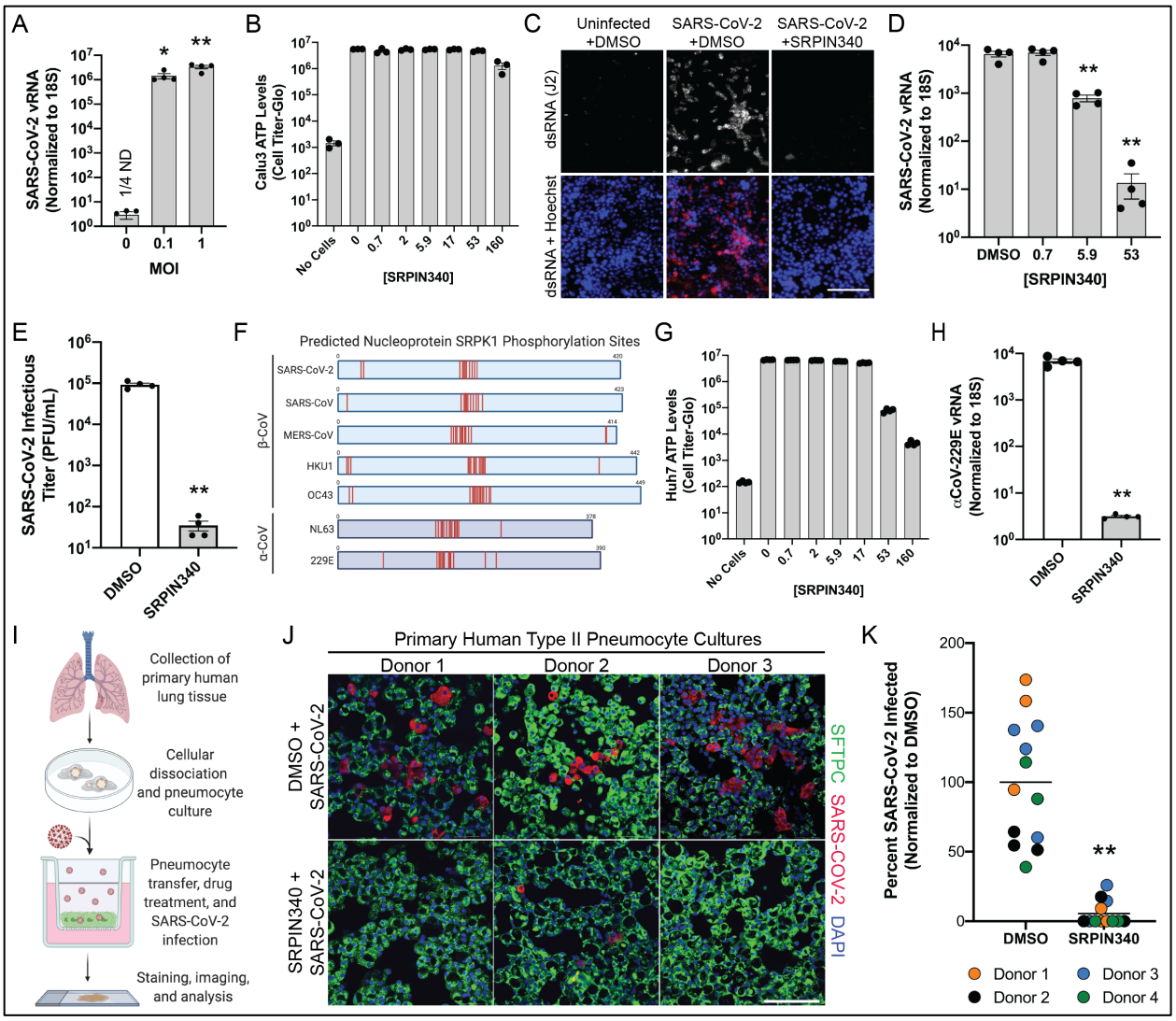


Figure 2. Pharmacological inhibition of SRPK1/2 compromises the replication of multiple human coronaviruses. (a) Quantification of SARS-CoV-2 mRNA by qRT-PCR after infection of Calu-3 cells at different MOIs n=4. (b) CellTiter-Glo assay in Calu-3 cells determining the toxicity of SRPIN340 at multiple concentrations of drug n=3. (c) Calu-3 cells were uninfected or infected with SARS-CoV-2, MOI=2.5 after treatment with DMSO control or 53 μM SRPIN340. DNA was stained with Hoechst (blue) and dsRNA stained with J2 antibody (red). Scale bar represents 150 μm. (d) Calu-3 cells were treated with DMSO control or different concentrations of SRPIN340 then infected with MOI 0.005.

611 SARS-CoV-2 mRNA was quantified by qRT-PCR n=4. (e) Calu-3 cells were treated with
612 DMSO control or 53 μM SRPIN340 then infected with MOI 0.01 for 48 hrs. Infectious
613 virus was quantified by plaque assay n=4. (f) Diagram of the N protein of multiple
614 coronaviruses. Red lines within protein indicate predicted SRPK1 phosphorylation sites.
615 (g) Huh7 cells were treated with different concentrations of SRPIN340 and cytotoxicity
616 was assayed with the CellTiter-Glo assay n=4. (h) Huh7 cells were treated with DMSO
617 or 17 μM SRPIN340 then infected with 229E MOI 0.01. Viral RNA was quantified with
618 qRT-PCR n=4. (i) Diagram of primary type II pneumocyte cell culturing and infections.
619 (j) Primary type II pneumocytes were isolated from different donors, cultured on transwell
620 membranes, treated with DMSO or 53 μM SRPIN340 and then infected at MOI=1. After
621 24 hrs cells were fixed and stained for type II marker SFTPC (green), DAPI (blue) or
622 SARS-CoV-2 (red). Scale bar= 100 μm. (k) Microscopy images from (j) were quantified
623 and % positive cells from four independent donors were plotted, $n \geq 3$. For all panels, error
624 bars represent SEM and *$p<0.05$ **$p<0.001$ by an unpaired, student's t-test.
625