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Virus-induced senescence is driver and therapeutic target in COVID-19

Soyoung Lee[1-3]*, Yong Yu[4]*, Jakob Trimpert[5], Fahad Benthani[4], Mario Mairhofer[4], Paulina Richter-Pechanska[1], Emanuel Wyler[2], Dimitri Belenki[1,2], Sabine Kaltenbrunner[4], Maria Pammer[4], Lea Kausche[1,6], Theresa C. Firsching[7], Kristina Dietert[7,8], Michael Schotsaert[9,10], Carles Martínez-Romero[9,10], Gagandeep Singh[9,10], Séverine Kunz[2], Daniela Niemeyer[11], Riad Ghanem[6], Helmut J. F. Salzer[12], Christian Paar[13], Michael Mülleder[14], Melissa Uccellini[9,10]†, Edward G. Michaelis[15], Amjad Khan[16], Andrea Lau[1], Martin Schönlein[1,17], Anna Habringer[13], Josef Tomasits[13], Julia M. Adler[5], Susanne Kimeswenger[4], Achim D. Gruber[7], Wolfram Hoetzenecker[4,18], Herta Steinkellner[19], Bettina Purfürst[2], Reinhard Motz[20], Francesco Di Pierro[21,22], Bernd Lamprecht[4,12], Nikolaus Osterrieder[5,23], Markus Landthaler[2], Christian Drosten[11], Adolfo García-Sastre[9,10,24,25], Rupert Langer[4,20], Markus Ralser[13,26], Roland Eils[27], Maurice Reimann[1], Dorothy N. Y. Fan[1,3], and Clemens A. Schmitt[1-4,6]

[1]Charité - Universitätsmedizin and Berlin Institute of Health (BIH), Medical Department of Hematology, Oncology and Tumor Immunology, and Molekulares Krebsforschungszentrum – MKFZ, Campus Virchow Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany

[2]Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association, Robert-Rössle-Straße 10, 13125 Berlin, Germany

[3]Deutsches Konsortium für Translationale Krebsforschung (German Cancer Consortium), Partner site Berlin, Germany

22 [4]Johannes Kepler University, Altenbergerstraße 69, 4040 Linz, Austria

23 [5]Institute of Virology, Freie Universität Berlin, Robert von Ostertag-Straße 7-13, 14163 Berlin,
24 Germany

25 [6]Kepler University Hospital, Department of Hematology and Oncology, Krankenhausstraße 9,
26 4021 Linz, Austria

27 [7]Institute of Veterinary Pathology, Freie Universität Berlin, Robert-von-Ostertag-Straße 15,
28 14163 Berlin, Germany

29 [8]Veterinary Centre for Resistance Research, Freie Universität Berlin, Robert-von-Ostertag-
30 Straße 8, 14163 Berlin, Germany

31 [9]Department of Microbiology, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy
32 Place, New York, NY 10029, USA

33 [10]Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai,
34 1 Gustave L. Levy Place, New York, NY 10029, USA

35 [11]Charité - Universitätsmedizin, Institute of Virology; German Center for Infection Research
36 (DZIF), Charitéplatz 1, 10117 Berlin, Germany

37 [12]Kepler University Hospital, Department of Pulmonology, Krankenhausstraße 9, 4021 Linz,
38 Austria

39 [13]Kepler University Hospital, Laboratory Medicine, Krankenhausstraße 9, 4021 Linz, Austria

40 [14]Charité - Universitätsmedizin, Department of Biochemistry, Charitéplatz 1, 10117 Berlin,
41 Germany

42   [15]Charité - Universitätsmedizin, Institute of Pathology, Charitéplatz 1, 10117 Berlin, Germany

43   [16]Department of Chemistry, University of Oxford, Mansfield Road, OX1 3TA, Oxford, UK

44   [17]Department of Oncology, Hematology and Bone Marrow Transplantation with Section of

45   Pneumology, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20251

46   Hamburg, Germany

47   [18]Kepler University Hospital, Department of Dermatology, Krankenhausstraße 9, 4021 Linz,

48   Austria

49   [19]Department of Applied Genetics and Cell Biology, University of Natural Resources and Life

50   Sciences, Muthgasse 18, 1190 Vienna, Austria

51   [20]Kepler University Hospital, Institute of Pathology, Krankenhausstraße 9, 4021 Linz, Austria

52   [21]Scientific & Research Department, Velleja Research, Viale Lunigiana, 23, 20125 Milan, Italy

53   [22]Digestive Endoscopy, Fondazione Poliambulanza, Via Leonida Bissolati 57, 25124 Brescia,

54   Italy

55   [23]Department of Infectious Diseases and Public Health, City University of Hong Kong,

56   Kowloon Tong, Hong Kong

57   [24]Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount

58   Sinai, 1 Gustave L. Levy Place, New York, NY 10029, USA

59   [25]The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy

60   Place, New York, NY 10029, USA

61 [26]The Molecular Biology of Metabolism Laboratory, The Francis Crick Institute, London NW1

62 1AT, UK

63 [27]Center for Digital Health, Berlin Institute of Health (BIH) and Charité - Universitätsmedizin

64 Berlin, Anna-Louisa-Karsch-Straße 2, 10178 Berlin, Germany

65

66 *) These authors contributed equally

67 †) Current address: Regeneron Pharmaceuticals Inc., Tarrytown, New York, NY 10591, USA.

68

Derailed cytokine and immune cell networks account for organ damage and clinical severity of COVID-19[1-4]. Here we show that SARS-CoV-2, like other viruses, evokes cellular senescence as a primary stress response in infected cells. Virus-induced senescence (VIS) is indistinguishable from other forms of cellular senescence and accompanied by a senescence-associated secretory phenotype (SASP), composed of pro-inflammatory cytokines, extracellular matrix-active factors and pro-coagulatory mediators[5-7]. COVID-19 patients displayed markers of senescence in their airway mucosa *in situ* and elevated serum levels of SASP factors. Mirroring COVID-19 hallmark features such as macrophage and neutrophil infiltration, endothelial damage and widespread thrombosis in affected lung tissue[1,8,9], *in vitro*-assays demonstrated macrophage activation with SASP-reminiscent secretion, complement lysis and SASP-amplifying secondary senescence of endothelial cells, neutrophil extracellular trap (NET) formation as well as activation of platelets and the clotting cascade in response to supernatant of VIS cells, including SARS-CoV-2-induced senescence. Senolytics such as Navitoclax and Dasatinib/Quercetin selectively eliminated VIS cells, mitigated COVID-19-reminiscent lung disease and reduced inflammation in SARS-CoV-2-driven hamster and mouse models. Our findings mark VIS as pathogenic trigger of COVID-19-related cytokine escalation and organ damage, and suggest senolytic targeting of virus-infected cells as a novel treatment option against SARS-CoV-2 and perhaps other viral infections.

The pandemic human pathogenic SARS-CoV-2 coronavirus causes upper respiratory infections and subsequently COVID-19 lung disease that may get further complicated by septic multi-organ failure and comes with significant mortality[10,11]. Escalating immune activation with massive cytokine release seems to drive severe COVID-19[1-3], possibly more than the virus

93   infection itself. Mechanisms of viral entry – primarily *via* interaction of CoV-2 spike protein S

94   with the host cell receptor ACE2 (angiotensin-converting enzyme 2) – and consecutive steps of

95   RNA replication, virus assembly and budding represent therapeutic targets[12,13]. While anti-

96   inflammatory drugs are clinically being used or currently tested with mixed results[14,15], more

97   comprehensive and causal measurements to control the cytokine storm are needed.

98   Viral entry reflects a major cellular insult, and evokes profound biological changes in infected

99   host cells – potentially leading to cellular senescence as a virus-triggered state switch[16,17], with

100   controversial impact on virus propagation[18,19]. Senescence is also acutely stress-induced by

101   activated oncogenes (OIS) or DNA-damaging cancer therapy, and physiologically occurs in

102   embryonic development, wound healing and ageing[6]. Little is known about systemic

103   ramifications of cellular senescence as an anti-viral host defense. We hypothesize here that

104   cellular senescence with its massive SASP, largely composed of pro-inflammatory cytokines,

105   matrix-degrading proteases and pro-coagulatory factors[5-7], underlies the cytokine storm,

106   macrophage activation, NET formation, endothelialitis and widespread pulmonary thrombosis

107   frequently observed in COVID-19 patients[1,8,9].

108

109   **Properties of virus-induced senescence**

110   We first tested signs of senescence in human diploid fibroblast (HDF) models exposed to high-

111   titer retrovirus. At day 5 post infection (p.i.), most of the cells presented with a senescence-

112   typical flattened, enlarged morphology, stained positive in the senescence-associated β-

113   galactosidase (SA-β-gal) assay[20], exhibited DAPI (4′,6-diamidin-2-phenylindol)-dense

114   chromatin reminiscent of senescence-associated heterochromatin foci (SAHF)[21], and

115   overexpressed p16[INK4a], an inhibitor of cyclin-dependent kinases (CDK) 4 and 6, in the nucleus

116   (Fig. 1a, Extended Data Fig. 1a). Gene set enrichment analyses (GSEA) of HDF RNA

117   sequencing (RNA-seq) datasets found senescence- and SASP-related gene ontology (GO) terms

118   skewed towards the VIS state, and to overlap with the OIS state of the very same cells in Ras-

119   induced senescence (Fig. 1b). Further underlining the context-overarching role of a senescent

120   stress response, GSEA retrieved host responses to viral infection in OIS (Extended Data

121   Fig. 1b). Specific analyses of VIS-associated expression changes by quantitative RT-PCR

122   (qRT-PCR) found senescence-typical transcriptional changes[21,22] in VIS (Fig. 1c, Extended

123   Data Fig. 1c). Notably, lower virus titers did not evoke a senescent cell-cycle arrest, as aimed

124   for in retro- or lentivirus-assisted gene transfer experiments (Extended Data Fig. 1d). Multiplex

125   protein analysis found VIS cells to secrete a plethora of SASP factors, many of them *bona fide*

126   NF-κB targets[23,24] (Fig. 1d). Importantly, these responses depended on senescence-essential

127   gene activities: HDF expressing the H3K9me3-active demethylase JMJD2C or engineered with

128   a small hairpin stably knocking down p53 (*shp53*) to genetically disable OIS[22,25], as well as

129   mouse embryo fibroblasts (MEF) without intact *p53* alleles failed to enter VIS, and lacked

130   senescence-typical changes by qRT-PCR and multiplex secretome analyses (Fig. 1e, Extended

131   Data Fig. 1e-g). Upon viral infection, only VIS-capable cells presented with cGAS/STING

132   activation (Extended Data Fig. 1h), a SASP-underlying interferon response-driving

133   cytoplasmic DNA sensor[26]. Importantly, blocking retroviral replication *via* the reverse

134   transcriptase inhibitor Azidothymidine prevented VIS, and blunted cGAS/STING activation

135   (Extended Data Fig. 1i, j). cGAS/STING-mediated phospho-activation of the interferon

136   regulatory factor IRF3[27], the NF-κB transcription factor p65[28], and other nuclear senescence

137   markers such as p21^{CIP1} and heterochromatin-associated H3K9me3 were strongly increased, or,

138   regarding non-G1-phase-hyperphosphorylated retinoblastoma (Rb) protein, markedly

139   decreased in VIS (Fig. 1f). Hence, viral infection evokes a host cell response reminiscent of

140   and genetically dependent on key features of other well-established types of cellular senescence

141   and their associated secretome.

142   Notably, VIS became detectable in response to a broad spectrum of viruses, including

143   lentivirus, adeno-associated virus (AAV), vesicular stomatitis virus (VSV) and the low-

144   pathogenic human alpha-coronavirus NL63 (HCoV-NL63) in human lung carcinoma and non-

145   malignant epithelial cells (Fig. 1g, Extended Data Fig. 2a; see human primary bronchial

146   HBEpC and nasal HNEpC epithelial cells, and multiplicity-of-infection [MOI]/SA-β-gal

147   relationships in Extended Data Fig. 2b-d). To more closely mimic SARS-CoV-2 infection, we

148   exposed ACE2-expressing HNEpC to SARS-CoV spike protein-pseudotyped VSV

149   glycoprotein G-deletion mutants (VSV-ΔG*/CoV-S and VSV-ΔG*/CoV-2-S), which all

150   induced senescence in these cells in a spike protein-dependent manner, since ACE2-negative

151   NIH3T3 cells[29] failed to senesce (Fig. 1h, Extended Data Fig. 2e). Retrovirus- as well as VSV-

152   induced senescence exhibited enhanced reactive oxygen species (ROS) and γ-H2AX DNA

153   damage foci (Extended Data Fig. 2f). Conversely, the ROS scavenger N-acetylcysteine (NAC)

154   reduced γ-H2AX foci and abrogated VIS. Similarly affecting γ-H2AX foci, GS-441524, the

155   active metabolite of the viral RNA polymerase inhibitor Remdesivir, blunted cGAS/STING

156   activation in VSV-infected cells (Extended Data Fig. 2g, h).

157   Importantly, HNEpC infected with the authentic SARS-CoV-2 virus also displayed SASP-

158   positive senescence that was preventable by *shp53*-mediated inability to senesce or by GS-

159   441524 treatment (Fig. 1i, j, Extended Data Fig. 2i, j). Moreover, RNA-seq analysis of SARS-

160   CoV-2-infected alveolar epithelial cells (AEC) as well as publicly available RNA-seq datasets

161   of normal human bronchus epithelial (NHBE) cells and lung cancer cell lines[4] corroborated

162   robust signs of senescence induction (Fig. 1k, Extended Data Fig. 2k). Thus, virus infection in

163   general and SARS-CoV-2 infection of respiratory epithelial cells in particular evoke full-

164 featured cellular senescence, underscoring our hypothesis that VIS might play a critical

165 pathogenic role in COVID-19 lung disease.

166

167 **Cellular senescence in COVID-19 patients**

168 Next, we asked whether VIS would be a typical feature detectable in SARS-CoV-2-infected

169 patients and specifically analyzed nasopharyngeal and lung tissue specimens from patients with

170 a fatal course of PCR-proven COVID-19. Strikingly, immunohistochemical analyses (IHA) of

171 the senescence markers lipofuscin, p16^{INK4a}, p21^{CIP1}, H3K9me3 and IL-8 as a SASP

172 representative unveiled robust reactivity in samples from infected individuals with or without

173 remaining SARS-CoV-2 RNA *in situ* but not in non-COVID-19 controls (Fig. 2a, Extended

174 Data Fig. 3a). Single-cell (sc)RNA-seq analyses of upper airway mucosa samples provided

175 further evidence of a senescence switch in COVID-19 patients[30], showing induced transcript

176 levels of p16^{INK4a} (CDKN2A) and multiple SASP factors not only in SARS-CoV-2-permissive

177 ciliated respiratory epithelial cells but also in macrophages, implying paracrine senescence

178 spreading to SASP-attracted macrophages as well as to other less virus-susceptible p16^{INK4a}-

179 elevated mucosal epithelial cell types in the nasopharyngeal specimens (Fig. 2b). By gene

180 expression profiling (GEP) of upper and lower airway specimens, we found SASP-reminiscent

181 cytokines in COVID-19 specimens induced at the transcript level (Fig. 2c). scRNA-seq data

182 further underscored that predominantly ciliated epithelial cells and macrophages scored

183 positive for p16^{INK4a}/CDKN2A transcripts (Extended Data Fig. 3b).

184 Key components of severe COVID-19 are a cytokine storm and macrophage activation

185 syndrome[3]. We reasoned that the SASP of senescent upper airway epithelial cells may attract

186 and activate macrophages through paracrine senescence induction[31], thereby contributing to

187   MAS escalation, a condition in which macrophages subsequently infiltrate the lungs. Notably,

188   COVID-19 lungs showed infiltration by pro-inflammatory CD86$^+$ macrophages and an M1-

189   macrophage polarization profile[32,33] (Fig. 2d, Extended Data Fig. 3c). In serum samples of

190   COVID-19 patients, we found SASP peptides such as IL-8, PAI-1/Serpin E1, CCL2/MCP-1,

191   MMP1, MMP9, and TIMP-1 among the most strongly upregulated factors (Fig. 2e). Selective

192   scanning for clotting-relevant factors by ultra-high-throughput mass spectrometry-based

193   proteomics[34] also unveiled a massive induction of those factors in COVID-19 serum probes

194   (Fig. 2f), in line with the transcriptional skewing of GO terms "coagulation" and "complement"

195   in SARS-CoV-2-infected NHBE cells, retrovirally senescent and even OIS or irradiation-

196   senescent HDF cells (Extended Data Fig. 1b, 2k)[7]. Further underscoring the pro-thrombogenic

197   propensity in COVID-19 patients, we detected elevated CD62$^+$-marked platelet activation in

198   their blood samples (Extended Data Fig. 3d). Accordingly, clinically more severe cases of

199   COVID-19 exhibited a tight association of higher SASP serum levels, clotting-relevant factors

200   and activated coagulation[35] (Fig. 2g, Extended Data Fig. 3e, f), and a close correlation between

201   senescence marker transcripts and microthrombosis in COVID-19 lungs *in situ* (Extended Data

202   Fig. 3g, h). Thus, findings in COVID-19 patient material unveiled alterations consistent with

203   VIS and its SASP in the upper airway epithelia, and suggest SASP-mediated effects as critical

204   contributors to the respective lung disease and associated pro-inflammatory, tissue-damaging

205   and pro-thrombogenic features.

206

207   **Linking COVID-19 features to VIS**

208   Next, we mechanistically interrogated whether supernatant (SN) from VIS cells (SN$_{VIS}$) might

209   evoke COVID-19-typical functionalities in critical target cell types. Indeed, human THP-1

210   macrophages turned to a CD86$^+$ or CD13$^+$ M1-like state[32] when incubated with SN$_{VIS}$, but not

211   SN of non-infected proliferating cells ($SN_{prol.}$) or genetically senescence-incapable virus-

212   infected HDF (Fig. 3a, Extended Data Fig. 4a). They themselves exhibited a strong SASP-

213   reminiscent secretory profile, indicative of paracrine senescence, since only replication-

214   incompetent viruses were used here (Fig. 3b, Extended Data Fig. 4b). Notably, transcripts

215   reminiscent of SN_{VIS}-induced CD86^{+}-associated THP1 were similarly detected in COVID-19

216   lungs (Fig. 3c). Indeed, SN_{VIS} exposure enforced paracrine, SA-β-gal-positive senescence in

217   macrophages (Fig. 3d), all in line with their contribution as a VIS-triggered SASP-amplifying

218   cellular messenger to COVID-19 disease. Underscoring the critical role of an altered

219   endothelium as a cytokine-releasing relay[36], SN_{VIS} also evoked paracrine senescence in

220   HUVEC human endothelial cells, which, in turn, launched a pro-inflammatory and pro-

221   thrombogenic SASP (Fig. 3e, Extended Data Fig. 4c). Consistent with microangiopathic

222   thrombosis observed in severely affected COVID-19 lungs, transfer of SN_{VIS}, but not $SN_{prol.}$ or

223   SN of virus-infected senescence-incapable HDF, prompted platelet activation[37] (Fig. 3f),

224   accelerated clotting time (Fig. 3g), and provoked NET formation *in vitro*[9] (Fig. 3h). Moreover,

225   mirroring the strongly elevated complement-activating factors in COVID-19 patient samples,

226   we observed enhanced formation of the C5b-C9 membrane attack complex and its ultimate

227   cytotoxic action on HUVEC cells in response to SN_{VIS}-activated human serum (Fig. 3i, j). In

228   essence, the pathogenesis of COVID-19 lung disease appears to be closely linked to

229   senescence-governed immune escalation and pro-thrombotic effects.

230

231   **Eliminating VIS cells by senolytic drugs**

232   Based on these findings we considered VIS cells a central therapeutic target in SARS-CoV-2

233   infection whose early elimination might mitigate the course of disease. Previously, we and

234   others presented evidence that senolysis, *i.e.* the selective depletion of senescent cells by drugs

235   such as Bafilomycin, the BCL-2 family inhibitor ABT-263 (a.k.a. Navitoclax) or the multiple

236   kinases-inhibiting flavonoids Fisetin and Quercetin, the latter typically in combination with the

237   tyrosine kinase inhibitor Dasatinib (D/Q), reduced undesirable local and system-wide effects

238   that persistent senescent cells might otherwise exert[38-42]. We first profiled transcript and protein

239   expression of anti-apoptotic BCL-2 family members in VIS cells, and found BCL-xL

240   (BCL2L1), BCL-w (BCL2L2), and MCL-1 (BCL2L3) induced (Fig. 4a, Extended Data Fig. 5a;

241   see also Fig. 2a, Extended Data Fig. 3a, b for elevated BCL2L1 and BCL2L2 expression in

242   COVID-19 patients), thereby providing a rationale for Navitoclax to selectively kill VIS cells.

243   We also found the PI3K/AKT/p70S6K axis, p38MAPK and the tyrosine kinase SRC to be

244   deregulated in VIS, and, hence, to serve as potential targets especially for Fisetin and D/Q

245   according to predicted protein-chemical interactions[43-45] (Fig. 4a, Extended Data Fig. 5b).

246   Indeed, Navitoclax, unlike the BCL-2-specific Venetoclax (a.k.a. ABT-199), as well as Fisetin

247   and D/Q exerted cytotoxic potential selectively against retrovirus- or VSV-driven VIS cells but

248   not equally infected senescence-incapable HDF *in vitro* (Fig. 4b, Extended Data Fig. 5c-e).

249   Considering release of infectious virions as a potential concern, we found no enhanced

250   infectivity upon lysis of VIS cells (Extended Data Fig. 5f, g).

251   Importantly, infection by the authentic SARS-CoV-2 virus rendered HNEpC susceptible to

252   Navitoclax, Fisetin or D/Q (Fig. 4c). Moreover, as seen before with other viruses, SN transfer

253   from SARS-CoV-2-infected cells expanded CD86$^+$ THP-1 macrophages, and led to paracrine

254   senescence or complement-mediated lysis of HUVEC cells, if not carried out in *shp53-* or GS-

255   441524-pretreated epithelial cells or under exposure to any of the three senolytic treatment

256   options (Fig. 4d-f, Extended Data Fig. 5h, and i-l regarding VIS, SASP and senolytic

257   susceptibility in the context of the spike protein-affecting SARS-CoV-2 mutations alpha and

258   beta).

259

260   **Senolytic targeting of COVID-19 *in vivo***

261   To determine the impact of senolytic treatment on clinical severity of COVID-19-like lung

262   disease, we first tested early intervention with Navitoclax in the well-established Syrian golden

263   hamster model of SARS-CoV-2-driven lung disease [46,47]. After intranasal infection with SARS-

264   CoV-2 virus, animals developed COVID19-like pneumonia within the first three to five days.

265   scRNA-seq profiling of SARS-CoV-2-infected *vs.* mock-infected hamster lungs unveiled

266   upregulated p16^{INK4a}/CDKN2A, core senescence and SASP factor as well as SRC transcript

267   expression in alveolar epithelial cells, macrophages and neutrophils due to primary virus

268   infection or paracrine senescence induction during days 3 to 5 p.i. (Extended Data Fig. 6a). This

269   analysis also demonstrated induction of *BCL-w* transcripts in these cell types at day 5 p.i.

270   (Extended Data Fig. 6b), in support of Navitoclax as a suitable senolytic. Animals on the

271   Navitoclax protocol exhibited moderate adverse effects (Extended Data Fig. 6c, d), presented

272   with profoundly reduced senescent cells and improvement of some histopathological features

273   when compared to a solvent-only-treated but equally SARS-CoV-2-infected cohort on day 7

274   p.i. (Fig. 5a, Extended Data Fig. 6e-g, Extended Data Table 1). Strikingly, we detected much

275   lower concentrations of key pro-inflammatory SASP factors in the serum of Navitoclax-

276   exposed hamsters on day 7 p.i., virtually indistinguishable from uninfected controls (Extended

277   Data Fig. 6h).

278   To evaluate the potential of senolytic intervention in model systems with a more severe

279   COVID-19-like clinical course, we next considered the Roborovski dwarf hamster model[48]. On

280   day 4 p.i. and treatment with either Navitoclax, D/Q or solvent-only, animals with senolytic

281   intervention presented with a substantial reduction of H3K9me3- or lipofuscin-marked

282   senescent cells in their respiratory epithelium, and a profound reduction in lung disease features,

283   especially in the D/Q arm (Fig. 5b-d). Both senolytic regimens also produced in this model a

284   dramatic reduction in SASP-reminiscent serum cytokines on day 4 p.i., close to healthy controls

285   (Fig. 5e). Effects in the D/Q-treated group were particularly meaningful; 5/5 animals survived,

286   displayed no apparent signs of disease and no or only moderate weight loss (Extended Data

287   Fig. 7a). By contrast, 4/5 animals in the Navitoclax- and 3/5 animals in the placebo-treated

288   group had to be terminated due to weight loss and clinical impairment by day 4 p.i. Lastly, we

289   investigated the *K18-hACE2*-transgenic mouse model, which also supports a more severe and

290   typically lethal SARS-CoV-2 infection[49,50]. Mice were treated comparably to the dwarf hamster

291   model with oral administrations of Navitoclax, D/Q and here also Fisetin, and scheduled for

292   lung analysis on day 6 p.i.. While 2/5 solvent-only-treated and 1/5 Navitoclax-treated mice died

293   on or before day 6, and another Navitoclax-treated mouse just slipped underneath the

294   acceptable weight limit on day 6, all D/Q- and Fisetin-treated mice were alive, had limited

295   weight loss and presented clinically well on day 6 (Extended Data Fig. 7a, b). Although overall

296   histopathological analyses could not unveil overt differences regarding COVID-19-like

297   parameters (Extended Data Fig. 7c, and d regarding virus loads in this and the hamster models),

298   markers of senescent cells were found to significantly lesser extents in the lungs of senolytics-

299   treated mice across all regimens applied (Extended Data Fig. 7e). Moreover, Quercetin was

300   recently tested as an oral formulation with sunflower lecithin against standard care (SC) in two

301   randomized clinical trials (NCT04578158 and NCT04861298) for patients with confirmed

302   SARS-CoV-2 infection and mild COVID-19-associated symptoms. Both trials, based on 152

303   and 42 patients, respectively, demonstrated clinical improvement by the senolytic

304   intervention[51,52]. Agglomerative hierarchical clustering of the second trial across numerous

305   parameters separated the baseline-indistinguishable patient population by Quercetin *vs.* SC at

306   day 7 (Fig. 5f, Extended Data Table 2). Moreover, collective analysis of all 194 patients

307   concluded significant risk reductions regarding the needs of hospitalization and oxygen therapy

308 for the Quercetin group, which was also superior to SC in terms of length of hospitalization,

309 referral to the intensive care unit and number of deaths (Extended Data Fig. 8a, b). Taken together,

310 early senolytic intervention during SARS-CoV-2 infection significantly attenuates COVID-19-

311 like lung disease and systemic inflammation.

312 Our investigation marks VIS as a central pathogenic principle and valid therapeutic target in

313 SARS-CoV-2 infection to prevent severe COVID-19 lung disease. The primary virus/host cell

314 encounter evokes a senescence response associated with massive pro-inflammatory cytokine

315 secretion that complements virus spreading to the lower airway tract. Our data pinpoint

316 macrophages as pivotal cellular messengers in this process. Attracted to and primed by upper

317 airway VIS cells, macrophages undergo a secondary, SASP-amplifying senescent state switch.

318 Subsequently deployed in the lungs, they further induce senescence, particularly in endothelial

319 cells. System-wide and locally active pro-coagulatory and complement-activating SASP factors

320 contribute to hallmark histopathological features and clinical severity of COVID-19. Hence,

321 early senolytic intervention is an appealing strategy to interrupt such escalating immune

322 cascade at its beginnings. The D/Q-, Fisetin-, and Navitoclax-mediated effects we observed

323 here are encouraging, but optimized dose-scheduling to maximize their clinical potential and

324 limit potential toxicities is needed. Due to their excellent safety profiles, compounds such as

325 the flavonoids Quercetin and Fisetin are particularly attractive candidates as VIS-targeting

326 senolytics. As already demonstrated for Quercetin[51,52], Fisetin is currently being investigated in

327 COVID-19 trials. Robbins and colleagues recently reported enhanced SASP production of pre-

328 existing senescent cells in old mice by pathogen-associated molecular pattern factors such as

329 lipopolysaccharide or the SARS-CoV-2 spike protein[53]. In turn, pharmacological removal of

330 senescent cells by Fisetin or D/Q in mouse β-coronavirus-infected animals was clinically

331 effective – thereby adding to the rationale of senolytic VIS cell elimination as proposed here

332 for SARS-CoV-2-evoked senescence. Moreover, it will be interesting to explore whether early

333 senolytic intervention may also mitigate chronic post-COVID-19 impairment known as long

334 COVID[54]. Finally, we highlight that although effective vaccinations are available worldwide

335 and will hopefully make COVID-19 history soon, our approach remains conceptually valid in

336 the context of other viral epidemics or pandemics in the future.

337

338 **Online Content** Methods, along with any additional Extended Data display items and Source

339 Data are available in the online version of the paper; references unique to these sections appear

340 only in the online paper.

341

342 **Supplementary Information** is linked to the online version of the paper at

343 www.nature.com/nature.

344

376

401 Manufacturing, Avimex, Johnson & Johnson, Dynavax, 7Hills Pharma, Pharmamar,

402 ImmunityBio, Accurius, Nanocomposix and Merck. A.G.-S. has consulting agreements for the

403 following companies involving cash and/or stock: Vivaldi Biosciences, Contrafect, 7Hills

404 Pharma, Avimex, Vaxalto, Pagoda, Accurius, Esperovax, Farmak and Pfizer. A.G.-S. is

405 inventor on patents and patent applications on the use of antivirals and vaccines for the

406 treatment and prevention of virus infections, owned by the Icahn School of Medicine at Mount

407 Sinai, New York.

408

413

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548

549

550   **FIGURE LEGENDS**

551   **Figure 1 | Senescence is a universal host cell response to viral stress. a**, SA-β-gal, SAHF

552   formation by DAPI, and p16^{INK4a} staining of IMR90 five days after retroviral infection or mock

553   control. **b**, GSEA probing senescence-associated genes in WI38 HDF in VIS and OIS. Positive

554   Normalized Enrichment Scores (NES) indicate enrichment compared to proliferating

555   counterparts. Presented NES are statistically significant (FDR *q*-value < 0.05, Supplementary

556   Information for individual values). **c**, Relative expression of core senescence and SASP

557   transcripts in cells as in *a* by qRT-PCR compared to mock control. **d**, Multiplex bead-based

558   protein analysis in the SN of HDF IMR90 (as in *a*), WI38 and Tig3. **e**, SA-β-gal staining of

559   IMR90 cells expressing JMJD2C, *shp53*, or control vector. **f**, Immunoblot analysis of nuclear

560   senescence markers in samples as in *d*. α-Tubulin as a loading control. **g**, Quantification of SA-

561   β-gal positivity in human cell lines (RPE1, A549) infected with AAV, lentivirus, HCoV-NL63,

562   and VSV. **h**, Quantification of SA-β-gal positivity in human cell lines and primary HNEpC

563   infected with VSV-ΔG*-CoV-S, VSV-ΔG*-CoV-2-S, or VSV-ΔG*/empty vector (VSV-ΔG*-

564   emp). NIH3T3 as ACE2-negative, infection-resistant control. **i**, SA-β-gal staining of stably

565   hACE2-engineered primary nasal epithelial cells (HNEpC-hACE2) infected with SARS-CoV-

566   2 and treated as indicated at 48 hours p.i.. **j,** Relative expression of the indicated transcripts by

567   qRT-PCR of cells as in *i*. **k**. GSEA of RNA-seq-based GEP from AEC infected with SARS-

568   CoV-2 *vs.* uninfected controls (*n* = 4). Preselected senescence-associated terms with FDR *q*-

569   value ≤ 0.01 are shown (see Supplementary Information for individual values). All scale bars

570   in this figure represent 100 μm, except 5 μm for SAHF in *a*. All quantifications in this figure

571   refer to mean ± s.d. of *n* = 3 independent experiments (except *k*) with individual values as dots

572   in bar plots or representative photomicrographs shown.

573

574 **Figure 2 | COVID-19 patients exhibit features of VIS. a**, Immunohistochemical analysis

575 (IHA) of senescence markers, BCL-w, and *in situ* hybridization of SARS-CoV-2 RNA in the

576 respiratory mucosa of nasopharyngeal specimens from COVID-19 patients ($n = 6$ for $p16^{INK4a}$

577 and IL-8, otherwise $n = 5$) *vs.* non-COVID-19 individuals ($n = 2$). Quantification of affected

578 areas as mean percentages of positive cells ± s.d. or mean percentage of positive area ± s.d

579 (lipofuscin only). **b**, scRNA-seq-based GEP of upper airway tissue from COVID-19 patients

580 ($n = 19$) and normal controls ($n = 5$). Color-coded average-scaled (z-scores) expression levels

581 of the respective transcripts and cell types. (n)rMa = (non-)residential macrophage. **c**, GEP of

582 SASP-reminiscent cytokines in upper (nasopharynx) and lower airway (lung) tissue samples

583 from COVID-19 *vs.* non-COVID-19 autopsies ($n = 3$ each) by oligonucleotide-based transcript

584 hybridization. **d**, CD86-IHA of macrophages in lung specimens from COVID-19 ($n = 13$) *vs.*

585 non-COVID-19 patients ($n = 6$). Mean number of positive non-epithelial cells in 0.1 mm^2 ±

586 s.d.. $p = 0.0005$. **e**, Multiplex bead-based protein analysis of serum samples from COVID-19

587 patients ($n = 32$) and healthy donors ($n = 8$). **f**, Mass spectrometry-based proteome analysis of

588 serum from COVID-19 patients ($n = 26$) and healthy donors (serum $n = 3$ and plasma $n = 11$).

589 Heatmaps in **e** and **f** present scaled (z-scores) protein intensities. **g**, 3D-plot showing serum

590 levels of D-Dimer (clotting activity), SERPINA3 (coagulation-related SASP factor), and IL6

591 (pro-inflammatory SASP factor) correlated to WHO-graded clinical COVID-19 severity.

592 $n = 133$ patients; size of the sphere is proportional to the population size. All photomicrographs

593 are representative and scale bars mark 50 μm in this figure.

594

595 **Figure 3 | The VIS-associated secretome drives key immune-thrombotic components of**

596 **COVID-19. a**, Percentage of CD86$^+$ cells in the THP-1 macrophage population exposed to SN

597 from retroviral VIS *vs.* proliferating (left) or senescence-incapable (right) IMR90 cells. **b**,

598   Multiplex bead-based protein analysis of the SN of THP-1 exposed to SN as in *a* (SN$_{prol.}$ = SN

599   of proliferating cells, SN$_{VIS}$ = SN of VIS cells), SN$_{VIS}$ without THP-1 cells shown on the right.

600   **c**, GEP of M1-macrophage polarization-related transcripts by RNA-seq of THP-1 cells exposed

601   to SN$_{prol.}$ or SN$_{VIS}$ and sorted for CD86-positivity (*n* = 2 each, left) and by oligonucleotide-

602   based transcript hybridization of lung tissue from COVID-19 *vs.* non-COVID-19 autopsies

603   (*n* = 3 each, right). **d**, SA-β-gal and the CD86$^+$ fraction of THP-1 exposed to SN as indicated.

604   **e**, SA-β-gal and transcript expression by qRT-PCR of HUVEC endothelial cells incubated with

605   the SN as indicated. **f**, Platelet activation measured by ADP release from healthy donor platelets

606   incubated with the SN of IMR90 as in *a*. *n* = 5 donors. **g**, Clotting time in whole blood samples

607   (*n* = 4 healthy donors) mixed with indicated SN volumes (WI38, as in *b*). **h**, NET formation

608   after exposing neutrophils to SN from HDF as in *b*. *n* = 4 healthy donors, two measurements

609   each. **i**, Quantification of the cytolytic complement complex C5b-9 on HUVEC incubated with

610   human serum and SN as indicated. **j**, Cytotoxicity assay of HUVEC as in *i*. All quantifications

611   (mean ± s.d. with individual values as dots) and representative photomicrographs are from *n* = 3

612   independent experiments except otherwise noted. '*' indicates *p* < 0.05 by unpaired-t-test (two-

613   tailed; *a*, *f*, *h*, *i*, *j*), unpaired-t-test (one-tailed; *d*), paired-t-test (two-tailed; *g*). All scale bars in

614   this figure represent 100 μm.

615

616   **Figure 4 | VIS cells are sensitive to senolytic targeting. a,** Immunoblot of BCL2 family

617   members (left) and kinases (right) in HDF lysates as indicated. α-Tubulin as a loading control.

618   **b**, Relative viability of IMR90 cells as in Fig. 1e, 48 hours after treatment with senolytics as

619   indicated or solvent-only (DMSO). **c,** Viability of HNEpC-hACE2 infected with SARS-CoV-

620   2 or mock, and treated with senolytics as indicated for *c-f*. **d,** Relative change of the CD86$^+$

621   fraction in the THP-1 cell population after receiving SN of HNEpC-hACE2 as in *c*. Of note,

622   SN transfer experiments were carried out in the presence of SARS-CoV-2 neutralizing anti-

623   spike antibodies, preventing transmission of infection (as evidenced by negative target cell

624   SARS-CoV-2 PCR, data not shown). **e,** SA-β-gal staining of HUVEC endothelial cells after

625   receiving SN of HNEpC-hACE2 as in *d*. **f,** Quantification of C5b-9 on HUVEC cells exposed

626   to human serum with SN of HNEpC-hACE2 as in *d*. All bar plots in this figure show mean

627   results ± s.d. of $n = 3$ independent experiments with individual values as dots. All '*' in this

628   figure indicate $p < 0.05$ by unpaired t-test (two-tailed).

629

630   **Figure 5 | Senolytic targeting mitigates disease features of COVID-19 in animal models**

631   **and patients. a,** H3K9me3 IHA in nasopharyngeal (top) and lung (middle) specimens, as well

632   as lipofuscin-based detection (bottom) of senescent cells in the lung of golden hamsters infected

633   with SARS-CoV-2 and treated with Navitoclax or solvent-only ($n = 5$ animals each), or mock-

634   infected and untreated ($n = 3$ animals) at day 7 p.i.. **b,** H3K9me3 IHA and lipofuscin staining

635   in nasopharyngeal sections of Roborovski dwarf hamsters infected with SARS-CoV-2 or mock,

636   at day 4 p.i. after treatment as indicated. **c** Hematoxylin-eosin (H/E)-stained lung tissue of dwarf

637   hamsters as in *b* on day 4 p.i.; uninfected healthy hamsters (mock) for comparison (xiv–xvii).

638   Lung sections for overview (i, v, x) with signs of COVID-19-like pneumonia and hemorrhage:

639   hyperplasia of bronchial epithelium (double-headed arrow), hemorrhage (asterisks) and

640   necrosis (black arrows) of alveolar epithelial cells with neutrophil infiltration (white arrows),

641   and endothelialitis (lined black arrows). '*' indicates $p < 0.05$ by unpaired-t-test (one-tailed).

642   **d,** Quantification of the findings in *c*. **e,** Multiplex bead-based protein analysis of dwarf hamster

643   serum samples on day 4 p.i. (as in *b-d*). $n = 5$ each (*b-d*; $n = 4$ each in *e*) for SARS-CoV-2-

644   infected and treated animals. $n = 2$ for healthy (mock) control. **f,** Constellation plots of COVID-

645   19 patients[52] before (day 0) and after 7 days of standard care (SC) only, or Quercetin plus SC.

646   Variables (Extended Data Table 2) were simultaneously analyzed by agglomerative

647   hierarchical clustering to visualize similarity (distance) between patients and separation of the

648   cohorts after treatment. $n = 21$ for each group. All quantifications are mean $\pm$ s.d. All

649   photomicrographs in this figure are representative with scale bars indicating 50 μm (except as

650   indicated in *c*).

651

652   **METHODS**

653   **Cell culture and virus production**

654   Cell lines were maintained according to the guideline of suppliers. Human diploid fibroblast

655   (HDF) IMR90, WI38, Tig3 were described previously[22]. Human cell lines RPE1 (hTERT

656   RPE1), A549 and HUVEC were purchased from ATCC. Primary human nasal or bronchial

657   epithelial cells (HNEpC and HBEpC) were purchased from PromoCell. HNEpC cells with

658   exogenous ACE2 expression (HNEpC-hACE2) were generated by lentiviral infection (hACE2

659   lentivirus, BPS Bioscience). THP-1 cells were kindly provided by Anna Walter (Charité).

660   Senescence-incapable cell lines were generated by transduction with lentiviruses encoding

661   JMJD2C or *shp53* as previously described[22,55].

662   All viruses used in this work are tagged with GFP, with the exception of HCoV-NL63 and

663   SARS-CoV-2, and infection efficiency was monitored by detecting fluorescence in infected

664   cells. Retrovirus was produced by transfecting Phoenix packaging cells with a murine stem cell

665   virus (MSCV) GFP plasmid[56]. Lentivirus was produced by transfecting 293T cells with

666   psPAX2, pMD2.G (Addgene nr. 12260 and 12259) and pCDH-CMV-MCS-EF1-GFP

667   plasmids[22]. AAV was obtained from the Charité Viral Core Facility. Caco-2 cell-adapted wild-

668   type HCoV-NL63 virus at a titer of 3 x 10^6 pfu/ml was provided by the M.L. lab. VSV-GFP

669   was a kind gift from W. Brune (Heinrich Pette Institute, Hamburg, Germany). VSV-ΔG* GFP

670   (Kerafast) and plasmids encoding SARS-CoV spike (pKS SARS St19; kindly provided by S.

671   Fukushi, National Institute of Infectious Disease, Tokyo, Japan), SARS-CoV-2 spike (Wuhan

672   SARS-CoV-2 spike deltaCT28; kindly provided by C. Ratswohl and K. de la Rosa, MDC),

673   SARS-CoV-2 spike variants Alpha and Beta (pCDH-CMV-spike-B117-EF1-puro and pCDH-

674   CMV-spike-B1351-EF1-puro synthesized by GENEWIZ, China) proteins were used to

675   generate   mutant   pseudotypes.   SARS-CoV-2   (hCoV-19/Germany/BY-ChVir-984/2020,

676   sequence reference EPI_ISL_406862) was propagated for *in vitro* applications in Vero E6 cells

677   (ATCC CRL-1586) and genome-sequenced to exclude stocks with mutations from further

678   experiments. Infection was monitored by PCR, detecting viral genomic RNA in the supernatant

679   48 or 72 h p.i., as previously described[57]. Experiments were conducted under appropriate

680   biosafety conditions with enhanced respiratory personal protection equipment in the BSL-3

681   facility at the Institute for Virology, Charité, Berlin. Self-made virus stocks were concentrated

682   10-times using virus concentrations kit (TaKaRa Bio and Abcam). High-titer virus infection to

683   induce VIS reflect MOI of 50 for replication-incompetent retrovirus and lentivirus, 10 for

684   replication-incompetent AAV and VSV-ΔG*, 10 as well for replication-competent HCoV-

685   NL63, 1 for replication-competent VSV, and 0.1 for replication-competent SARS-CoV-2. VIS

686   features were assessed on day 5 (retrovirus, lentivirus), day 3 (HCoV-NL63, VSV-ΔG*, SARS-

687   CoV-2) or day 2 (VSV) p.i.

688

689   **COVID-19 patients, non-COVID-19 patients and healthy donor material**

690   Upper (nasopharyngeal) and lower airway (lung) tissue FFPE samples were derived from

691   deceased SARS-CoV-2-infected COVID-19 patients as well as archive material from patients

692   who passed away without significant clinical signs of a respiratory infection prior to October

693   2019 (*i.e.* non-COVID-19 patients), blood samples of SARS-CoV-2-infected and hospitalized

694   patients in the course of their COVID-19 disease, or blood samples from healthy volunteers

695   were used based on approval by the local ethics commission of the Johannes Kepler University

696   (reference number 1070/2020). All COVID-19 patients were PCR-proven SARS-CoV-2-

697   positive, however, SARS-CoV-2 RNA was detectable in 4/5 COVID-19 cases shown in Fig.

698   2a. All participants gave informed consent. In addition, we conducted re-analyses of additional

699   patient and control materials that were obtained before and published elsewhere[30,34,51,52].

700

701   **Flow cytometry, immunoblotting, immunofluorescence, and immunohistochemical**

702   **analysis**

703   Flow cytometry, immunoblotting (IB), immunofluorescence (IF), and immunohistochemical

704   analysis (IHA) were performed as described previously[24,58]. Surface antigen detection by flow

705   cytometry was carried out with fluorescence-conjugated antibodies against human CD86-PE-

706   Cy7 (BioLegend, #374209, 1:200), CD13-APC-Cy7 (BioLegend, #301709, 1:200), CD62P-PE

707   (BioLegend, #304905, 1:200). Antigen detection by IB was carried out with antibodies against

708   BCL2 (Cell Signaling Technology [CST], #15071, 1:1000), BCLXL (CST, #2764, 1:1000),

709   BCLW (CST, #2724, 1:1000), MCL1 (CST, #39224, 1:1000), phospho-p65-Ser536 (CST,

710   #3033, 1:1000), phospho-IRF3-Ser386 (CST, #37829, 1:1000), phospho-Rb-Ser807/811 (CST,

711   #8516, 1:1000), p21^{CIP1} (Santa Cruz Biotechnology [SCBT], #sc-6246, 1:200), p16^{INK4a}

712   (SCBT, #sc-1661, 1:200), H3K9me3 (Abcam, #ab8898, 1:2000), phospho-Akt (Thr308) (CST,

713   #13038T, 1:2000), phospho-p38 MAPK (Thr180/Tyr182) (CST, #4511T, 1:1000), phospho-

714   p70 S6 Kinase (Thr389) (CST, #9234T, 1:1000), phospho-Src (Tyr416) (CST, #6943T, 1:1000)

715   and α-Tubulin (Sigma, #T5168, 1:2000). Anti-mouse or anti-rabbit horseradish peroxidase-

716   conjugated antibodies were used as secondary antibodies (GE Healthcare, #RPN4301 and

717   #NA931V, respectively). Antigen detection by IF was performed with antibodies against

718   phospho-H2A.X (Cell Signaling, #9718, 1:400), p16^{INK4a} (Abcam, #ab211542, 1:250), C5b-9

719   (SCBT, #sc-58935, 1:250), anti-mouse-Cy3 secondary antibody (Thermo Fisher, #A10521,

720   1:1000), anti-rabbit-594 secondary antibody (Thermo Fisher, #A11012, 1:1000). Antigen

721   detection by IHA was performed using the HiDef Detection HRP Polymer System (Cell

722 Marque, #954D-30) with antibodies against p21^{CIP1} (SCBT, #sc-6246, 1:200), IL-8 (Abcam,

723 #ab18672, 1:200), H3K9me3 (Abcam, #ab8898, 1:2000), BCL2L2 (Abcam, #ab190952,

724 1:1000), CD86 (Antibodies-online, ABIN736701, 1:100).

725 Original scans of the immunoblot information presented in Fig. 1f and 4a, with molecular

726 weight markers and crop area indicated, are shown in Supplementary Information.

727

728 *In situ* **hybridization (ISH)**

729 ISH was performed as previously described[46] using the ViewRNA™ Tissue Assay Core Kit

730 (Invitrogen, #19931) and the ViewRNA™ Tissue Assay Blue Module (Invitrogen, #19932)

731 according to the manufacturer's instructions. For the detection of SARS-CoV-2, ViewRNA™

732 Type 1 Probe Set COVID19 polyprotein (Invitrogen, #CVX-06, Assay ID VPNKRHH) and

733 ViewRNA™ Type 6 Probe Set Human ACTB (Invitrogen, #VX-06, Assay ID VA6-10506)

734 were used. Following hybridization, sections were counterstained with hematoxylin and

735 mounted with ProLong™ Glass Antifade Mountant (Invitrogen, #P36982). Images were taken

736 using the Olympus Slide Scanner VS200.

737

738 **Quantification of gene transcription**

739 *Quantitative reverse transcriptase PCR (qRT-PCR)*: RNA isolated from cell pellets using

740 Trizol (Invitrogen) was transcribed to cDNA using SuperScript II reverse transcriptase

741 (Invitrogen). Individual gene expression was analyzed by qRT-PCR using Taqman assays from

742 Applied Biosystems. A list of Taqman assays used here is available in Supplementary

743 Information.

744 *RNA sequencing*: Total RNA was isolated using the RNAeasy Mini kit (Qiagen) and sequenced

745 at the BGI Genomics. Single-cell RNA sequencing (scRNA-seq) was conducted as described[59].

746 Further processing of the raw sequencing data is described in 'Statistics and data analysis'.

747 *Oligonucleotide hybridization-based transcriptome profiling:* RNA was extracted from FFPE

748 tissue sections with the PureLink™ FFPE RNA Isolation Kit (Thermo Fisher Scientific, Cat.

749 No.: K156002), hybridized with the probe set of the NanoString nCounter® Human Tumor

750 Signaling 360 Panel (Cat. No.: XT-CSPS-H-T360-12), and analyzed on the NanoString

751 nCounter® SPRINT.

752

753 **Quantification of protein expression**

754 *Multiplex bead-based protein detection (MAGPIX)*: MAGPIX assays were conducted

755 according to the manufacturer's manual with following bead panels: Custom Procartaplex 19-

756 plex (Thermo Fisher Scientific, PPX-19, Assay ID MXAACDR), Custom Procartaplex 4-plex

757 (Thermo Fisher Scientific, PPX-04, Assay ID MXCE4XN), Bio-Plex Pro™ Rat Cytokine 23-

758 Plex Assay (Bio-Rad, 12005641). The assay was measured with the BioPlex® Magpix™

759 Multiplex Reader (Bio-Rad) and analyzed with a 5PL algorithm provided by the Bio-Plex

760 Manager™ software (Version 6.1). Detailed procedures for sample preparation and

761 measurement are described in Supplementary Information. Of note, due to a different

762 normalization procedure, values cannot be cross-read between different panels, *e.g.* Fig. 1d *vs.*

763 Fig. 3b.

764 *Mass spectrometry-based proteome***:** Detailed protocol of sample preparation, mass

765 spectrometry and data processing were previously described[34], for further details see also

766 Supplementary Information. Briefly, serum samples were reduced, alkylated, digested and

767   conditioned on a Biomek i7 workstation, and approximately 5 µg of peptides per injection were

768   used for the gradient LC-MS/MS analysis. The raw data were processed using DIA-NN 1.7.12.

769

770   **VIS assays *in vitro***

771   *Growth curve analysis*: HDF IMR90, WI38 or Tig3 cells were infected with MSCV retrovirus

772   at day 0 and day 3, and SA-β-gal activity were measured at day 5. For growth curve analyses,

773   2×10^4 cells were seeded into 12-well plates and cell numbers were counted at day 3, 6 and 9.

774   *2'3'-cGAMP ELISA* assay: Cell cytoplasmic 2'3'-cGAMP concentration was measured using

775   the Caymanchem ELISA kit. Briefly, cells were lysed in M-PER extraction reagent (Thermo

776   Fisher) and 10 µg protein lysate was used to determine 2'3'-cGAMP content.

777   *Analysis of cell viability and senescence*: Viability and cell numbers were analyzed by trypan

778   blue dye exclusion or Guava Viacount (Millipore). SA-β-gal activity at pH 6.0 (for human cells)

779   or pH 5.5 (for rodent cells) as a senescence marker in fresh or cryo-preserved cells was assessed

780   as described[60]. *In situ* detection of lipofuscin as a senescence marker in FFPE samples was

781   carried out with the SenTraGor[TM] reagent (Lab Supplies Scientific; kindly provided by Vassilis

782   G. Gorgoulis, University of Athens) according to the manufacturer's instructions.

783   *ROS detection*: 20 µM Dihydroethidium (DHE, MedChemExpress) was directly added to cells

784   and incubated at 37°C for 20 minutes. DHE was then washed away with PBS. Cells were fixed

785   in 4% paraformaldehyde and staining intensity was analyzed with Nikon fluorescent

786   microscope.

787

788   **VIS supernatant transfer and secondary, paracrine senescence**

789   Conditioned medium was then collected from proliferating and VIS cells after 24 hours of

790   incubation and centrifuged at 5,000 rpm for 5 minutes, and the SN ($SN_{prol.}$ or SN_{VIS},

791   respectively) was transferred to HUVEC or THP-1 cells for further analysis. For the SN transfer

792   from SARS-CoV-2-infected HNEpC, self-made neutralizing antibodies against SARS-CoV-2

793   (provided by the H.S. lab) were mixed in to block secondary infection.

794

795   **NET formation assay**

796   NET formation was measured by the concentration of NET-DNA bound to myeloperoxidase

797   (MPO). Neutrophils were isolated from whole blood of four healthy donors. NET were

798   quantified as previously described[61]. Briefly, 10 µg/ml myeloperoxidase (MPO) antibody

799   (Abcam; #ab25989) was immobilized on the surface of black MaxiSorp-treated plates (Thermo

800   Scientific). The plate was subsequently washed with PBS and blocked with 5% non-fat milk

801   for 2 hours at RT. Neutrophils incubated for 4 hours at 37°C in RPMI medium from

802   proliferating or VIS WI-38 or IMR-90 cells were pipetted onto the MPO-coated plate and

803   incubated for 1h at RT. NET quantification was performed using the MPO-DNA PicoGreen

804   assay according to the manufacturer's instructions (Quant-iT PicoGreen dsDNA Assay Kit,

805   Invitrogen). Sample fluorescence was measured using the GloMax Discover microplate reader

806   (Promega).

807

808   **Rotational thromboelastometry**

809   To measure clotting time, a rotational thromboelastometry assay was performed on a

810   ROTEM® delta (Werfen) device according to manufacturer's instructions. INTEM captures

811   intrinsic, platelet-dependent and EXTEM extrinsic, non-platelet-dependent clotting. Whole

812   blood samples of healthy donors were spiked with the SN of WI38 in proliferation or VIS.

813   Exclusion criteria for the healthy donors were coagulation disorders and clotting-affecting

814   medication. Gender distribution was equal among the donors.

815

816   **Platelet activation and ADP release measurement by VIS supernatant**

817   Platelet activation was measured by flow cytometry as percentage of CD62/P-selectin-positive

818   cells in whole blood. To measure VIS-evoked ADP release, 2×10^6 platelets from healthy

819   donors were resuspended in 200 μl conditioned medium and incubated at 37 ºC for 1 hour.

820   Supernatant was collected by centrifugation at 2,000 × g for 5 minutes, and ADP concentration

821   was measured using the ADP Colorimetric Assay Kit II (BioVision) according to the

822   manufacturer's instruction.

823

824   **Complement-mediated cytotoxicity in HUVEC endothelial cells**

825   500 μl normal human serum of healthy donors was incubated with proliferating or VIS HDF

826   (Fig. 3i, j) or HNEpC (Fig. 4f, Extended Data Fig. 5h) in 5 ml medium at 37ºC for 30 minutes.

827   Heat-inactivated (56ºC for 30 minutes) serum was used as a control. Supernatant was collected

828   and incubated with HUVEC endothelial cells at 37ºC. 2 hours after incubation, HUVEC cells

829   were fixed in 4% paraformaldehyde, and C5b-9 immunofluorescent staining was carried out.

830   Alternatively, 24 hours after incubation, a cytotoxicity assay was performed using the

831   CyQUANT LDH Cytotoxicity commercial kit (Invitrogen).

832

833   **Senolytic treatment *in vitro* and secondary viral infection**

834   VIS cells were treated with Venetoclax (1 µM), Navitoclax (1 µM), Fisetin (20 µM), D/Q

835   (20 µM, reflecting 2 µM Dasatinib + 20 µM Quercetin) or AT-101 (1 µM) for 48 hours and

836   cell viability was determined by trypan blue staining using the Countess™ II FL Automated

837   Cell Counter (Thermo Fisher). To generate the SN used for secondary viral infection, wild-type

838   MEF were infected with VSV for 6 hours and treated with 1 µM Navitoclax for 24 hours. SN

839   was centrifuged and filtered through a 0.22 µm PVDF filter and added to THP-1 cells. After 24

840   hours, secondary infectivity was measured by counting GFP-positive THP-1 cells.

841

842   **SARS-CoV-2-driven COVID-19 animal models**

843   *In vitro* and *in vivo* work related to hamsters was conducted under appropriate biosafety

844   conditions in the BSL-3 facility at the Institute for Virology, Freie Universität Berlin, Germany.

845   All hamster experimentation was approved by the relevant state authority (Landesamt für

846   Gesundheit und Soziales Berlin, Germany [permit number 0086/20]), and performed in

847   compliance with national and international guidelines for care and humane use of animals.

848   Preparation of SARS-CoV-2 virus stock and intranasal infection of Syrian hamsters

849   (*Mesocricetus auratus*) as a faithful in vivo-model for COVID-19 lung disease were previously

850   described[46]. Briefly, a SARS-CoV-2 wild-type isolate (BetaCoV/Munich/BavPat2-ChVir984-

851   ChVir1017/2020) was grown in Vero E6 cells. To ensure genetic integrity, passage 3 stocks

852   were genome-sequenced, results showed conformity with the published sequence (GenBank

853   MT270112.1) and confirmed the presence of the furin cleavage site essential for *in vivo*

854   pathogenesis. Anaesthetized male and female hamsters at 6-10 weeks of age were inoculated

855   with 1 x 10^5 pfu SARS-CoV-2 in a total volume of 60 µl cell culture medium. Control animals

856   were mock infected with the same volume of cell culture medium. One day after infection,

857   animals were randomized and half of the animals ($n = 5$) received a daily single 85 mg/kg body

858   weight dose of Navitoclax in solvent (60% Phosal PG, 30% PEG 300, 20% ethanol)

859   intraperitoneally for six consecutive days. The other half of infected animals ($n = 5$) received

860   the same volume of solvent only. The control group ($n = 3$) did neither receive virus nor

861   treatment. On day 7 p.i., one day after the last treatment, all hamsters were euthanized, and

862   tissue and peripheral blood samples were prepared for further analyses.

863   The Roborovski dwarf hamster (*Phodopus roborovskii*), a species highly susceptible to a severe

864   course of SARS-CoV-2 infection, was used under the permit mentioned above. Infections were

865   performed as previously described[48]. Female Roborovski dwarf hamsters at 5-7 weeks of age

866   underwent the procedure described above for Syrian hamsters with the exception that the total

867   volume of the inoculum was reduced to 20 µl. The same virus stock was used. Animals were

868   randomly distributed into three treatment groups ($n = 5$) and one non-infected, non-treated

869   group ($n = 2$). From 6 h hours p.i. on, animals were treated once daily with oral application of

870   Navitoclax at 85 mg/kg, D/Q at 5/50 mg/kg dissolved in 60% Phosal PG, 30% PEG 300, 20%

871   ethanol and mixed 1:1 with strawberry syrup, the placebo-treatment group received the same

872   solvent/syrup combination without drug. On Day 4 p.i., animals were sacrificed and tissue as

873   well as peripheral blood specimens were collected for downstream analyses.

874   Infection of *K18-hACE2* mice (Jax strain 034860) with SARS-CoV-2 was performed according

875   to the approval by the Institutional Animal Care and Use Committee (IACUC) of Icahn School

876   of Medicine at Mount Sinai (ISMMS) as previously reported[62]. Briefly, 6-week old female mice

877   were infected with 1 x 10^4 pfu SARS-CoV-2 (USA-WA1/2020, BEI resources NR-52281)

878   grown in Vero E6 cells and validated by genome sequencing, and randomly distributed into

879   three treatment groups (*n* = 5 each) and one solvent control group (*n* = 5). From one day after

880   infection, randomly chosen animals were treated with oral application of Navitoclax at

881   85 mg/kg, D/Q at 5/50 mg/kg, and Fisetin at 100 mg/kg dissolved in 60% Phosal PG, 30%

882   PEG 300, 20% ethanol every other day. On Day 6 p.i. (one day after the last treatment), animals

883   were sacrificed and tissue specimens were collected for histopathological analyses.

884   Hamster and mouse lung tissues were processed for histopathology as described[47] and analyzed

885   by board-certified veterinary pathologists following suggested standard criteria[63]. Transmission

886   electron microscopy was performed with trachea tissue from infected and treated Syrian golden

887   hamsters. Fixation and further processing of the samples, as well as image acquisition

888   procedures are described in Supplementary Information.

889

890   **Statistics and data analysis**

891   Based on previous experience[22,24,41,47,50,53,54,57], sample sizes typically reflect three to five, in

892   some experiments also much higher numbers of individual primary material as biological

893   replicates. All quantifications from staining reactions were carried out by an independent and

894   blinded second examiner and reflect at least three samples with at least 100 events counted

895   (typically in three different areas) each. For patients and animal models, IHA quantification

896   was carried out in SARS-CoV-2-affected areas. Regarding Fig. 5, Extended Data Fig. 6 and 7,

897   quantification of the indicated features was based on the following numbers of individual

898   photomicrographs from animals as indicated in legend and text: *Fig. 5a* – nasopharynx

899   H3K9me3 (*n* = 3 for mock, *n* = 6 for solvent and Navitoclax), lung H3K9me3 and lipofuscin

900   ($n = 3$ for mock, $n = 5$ for solvent and Navitoclax); *Fig. 5b* – H3K9me3 ($n = 5$ for solvent and

901   Navitoclax, $n = 6$ for D/Q), lipofuscin ($n = 5$ for all three groups); *Extended Data Fig. 7e* –

902   p16[INK4a] ($n = 5$ for solvent, Navitoclax and D/Q, $n = 4$ for Fisetin), H3K9me3 ($n = 4$ for solvent

903   and Navitoclax, $n = 5$ for Fisetin and D/Q), lipofuscin ($n = 5$ for solvent and Navitoclax, $n = 4$

904   for Fisetin, $n = 7$ for D/Q).

905   Data analysis was performed with GraphPad Prism 9. For statistical comparisons, a *t*-test was

906   applied. Unless otherwise stated, a *p*-value < 0.05 was considered statistically significant. For

907   multiple testing corrections the method by Benjamini & Hochberg (BH) to control for false

908   discovery rate was applied[64]. Bioinformatics Analysis was performed in R, version 4.0.3, and

909   Bioconductor 3.12 using various R packages as described in the Supplementary Information in

910   greater detail. High-performance computation has been performed on the Research/Clinic

911   cluster of the Berlin Institute of Health. For further analyses of COVID-19 patient clinical

912   data[51,52] as presented in Fig. 5f and Extended Data Fig. 8a, b, JMP Pro 14 (SAS Institute) was

913   used for agglomerative hierarchical clustering, GraphPad Prism9 was used for Fisher's exact

914   test for *p*-values, and a Haldane-Anscombe correction was applied to calculate relative risks.

915   Lipofuscin (SenTraGor®) staining was quantified with the pixel counting function of the ImageJ

916   program (bundled with Java 1.8.0_172, NIH)[65].

917

918   **Data availability**

919   The WI38 VIS- and OIS-derived raw RNA-seq data (Fig. 1b and Extended Data Fig. 1b) were

920   deposited at the Gene Expression Omnibus (GEO) repository of the National Center for

921   Biotechnology Information (NCBI) under accession number GSE165532

922   (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE165532). Gene expression data of

923   human AEC cells infected with SARS-CoV-2 (Fig. 1k) are publicly available from at the NCBI

924   GEO under the accession number GSE148729

925   (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE148729). Raw sequencing data of

926   NHBE, Calu-3 and A549 cells infected with SARS-CoV-2 (Extended Data Fig. 2k) are publicly

927   available at the NCBI GEO under the accession number

928   GSE147507[4](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE147507). Seurat

929   objects comprising scRNA-seq datasets of nasopharyngeal, protected specimen brush, and

930   bronchial lavage samples of 19 COVID-19 patients and five healthy controls[30], comprising a

931   total of 36 samples that were analyzed in this study (Fig. 2b), are available at FigShare

932   (https://doi.org/10.6084/m9.figshare.12436517; covid_nbt_main.rds, covid_nct_loc.rds). The

933   mass spectrometry-based proteome data of serum from COVID-19 patients and healthy donors

934   in Fig. 2f are described[34]. Patient proteomics data (Fig. 2g, Extended Data Fig. 3e) are publicly

935   available at https://www.who.int/teams/blueprint/covid-19, in which disease severity was

936   defined: g3 = hospitalized, no oxygen therapy; g4 = oxygen by mask or nasal prongs; g5 = non-

937   invasive ventilation or high-flow oxygen; g6 = intubation and mechanical ventilation; g7 =

938   ventilation and additional organ support. scRNA-seq data of hamsters infected with SARS-

939   CoV-2 (Extended Data Fig. 6a, b) are available (GSE162208,

940   https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE162208)[59]. All information related

941   to the two randomized trials on early Quercetin intervention in COVID-19 patients

942   (NCT04578158 and NCT04861298) can be found in the respective publications[51,52] and in

943   Extended Data Table 2.

944

945

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984 **EXTENDED DATA FIGURE LEGENDS**

985 **Extended Data Figure 1 | Additional biological properties of retroviral VIS. a**, SA-β-gal

986 staining, SAHF formation by DAPI, and p16^{INK4a} staining of HDFs (Tig3 and WI38) five days

987 after retroviral infection or mock control. Representative photomicrographs of $n = 3$

988 independent experiments. **b**, Gene set enrichment analysis (GSEA) of virus infection-relevant

989 GO terms probing RNA-seq datasets of WI38 HDF in VIS and OIS. Positive normalized

990 enrichment scores (NES) indicate enrichment in VIS (dark grey bars) or OIS (light grey bars)

991 compared to proliferating counterparts (mock infection for VIS, or empty vector control for

992 OIS). NES with FDR q-value ≤ 0.1 are considered statistically significant and presented (for

993 individual q-values, see Supplementary Information); $n = 3$ biological replicates each. **c**, Gene

994 expression analysis for core senescence and SASP genes by quantitative RT-PCR in WI38 and

995 Tig3 as in *a*. Mean relative transcript level compared to mock control + s.d. of $n = 3$ independent

996 experiments are shown. **d**, Growth curve analyses of HDF infected with retrovirus at different

997 MOI as indicated, showing that high-titer virus induced VIS, reflected by stable cell numbers

998 over time, while lower virus titer remained compatible with exponential cell growth. $n = 3$

999 independent experiments are presented as mean cell numbers ± s.d.. **e**, SA-β-gal staining (left)

1000 and gene expression analysis of the indicated transcripts by qRT-PCR (right) in wild-type (WT)

1001 or senescence-defective $p53^{-/-}$ MEF, five days after infection with high-titer retrovirus or mock

1002 as a control. Representative photomicrographs with fractions of SA-β-gal-positive cells, and

1003 mean relative transcript levels normalized to mock control ± s.d. of $n = 4$ independent

1004 experiments are shown. **f**, Gene expression analysis of the indicated transcripts by qRT-PCR in

1005 IMR90 cells expressing JMJD2C, *shp53*, or control vector as in Fig. 1e. **g**, Multiplex bead-

1006 based protein analysis of SN of senescence-incapable IMR90 as in Fig. 1e. SN$_{mock}$ = SN of

1007 mock-infected cells; SN$_{virus}$ = SN of retrovirus-infected cells. Mean expression levels of $n = 3$

1008 biological replicates are shown. **h**, cGAS/STING activation upon viral infection, as evidenced

1009   by a higher induction of cyclic guanosine monophosphate-adenosine monophosphate (2'3'-

1010   cGAMP) by ELISA analysis in matched pairs of HDF after either mock or retrovirus infection

1011   (mean of $n = 4$ independent experiments for each cell line, upper panel). Mean cGAMP levels

1012   + s.d. for senescence-incapable IMR90 as in Fig. 1e ($n = 4$ independent experiments, lower

1013   panel). **i**, SA-β-gal staining of IMR90 cells, treated with reverse transcriptase inhibitor

1014   azidothymidine Zidovudine (50 μM), cGAS inhibitor G150 (5 μM), STING inhibitor H-151

1015   (1 μM), or DMSO. Mock infection and DMSO solvent treatment as negative controls.

1016   Representative photomicrographs and quantification of positively stained cells as mean ± s.d.

1017   of $n = 3$ independent experiments are shown. **j**, 2'3'-cGAMP ELISA analysis of VIS IMR90

1018   cells as in *i*.

1019

1020   **Extended Data Figure 2 | Additional biological properties of VIS exerted by a variety of**

1021   **viruses. a**, SA-β-gal staining in human cell lines (RPE1, A549) infected with AAV, lentivirus,

1022   HCoV-NL63, and VSV as in Fig. 1g. **b**, SA-β-gal staining of human primary bronchial or nasal

1023   epithelial cells (HBEpC and HNEpC, respectively) infected with HCoV-NL63 and VSV. Mock

1024   infected cells as negative control. Quantification of positive cells for $n = 3$ independent

1025   experiments is shown as mean ± s.d.; scale bar = 100 μm. **c**, Quantification result of SA-β-gal-

1026   positive cells (RPE1, A549) after lentiviral infection at MOI as indicated are shown as mean

1027   percentage ± s.d. for $n = 3$ independent experiments. Note that MOI 50 was chosen for VIS

1028   induction. **d**, Quantification result of SA-β-gal-positive RPE1 cells after infection with VSV-

1029   ΔG*-CoV-S or VSV-ΔG*-CoV-2-S at MOI as indicated are shown as mean percentage ± s.d.

1030   for $n = 3$ independent experiments. Note that MOI 10 was chosen for VIS induction. **e**, SA-β-

1031   gal staining in human cell lines (RPE1, A549) and primary HNEpC infected with VSV-ΔG*-

1032   CoV-S, VSV-ΔG*-CoV-2-S, or VSV-ΔG*/empty vector (VSV-ΔG*-emp) as in Fig. 1g.

1033   NIH3T3 as ACE2-negative, infection-resistant control.**f,** Fluorescence detection of ROS in

1034   IMR90 after infection with retrovirus or VSV and treatment with NAC as indicated (upper

1035   panels). SA-β-gal staining and quantification of corresponding samples (lower panels). Mock

1036   infection or solvent treatment controls (UT) are shown. Representative photomicrographs and

1037   quantification are shown as mean percentages ± s.d. for $n = 3$ independent experiments. Scale

1038   bar for ROS and SA-β-gal = 100 μm; for γH2A.X (insets; pink dots reflect foci) 5 μm. **g,**

1039   Quantification of γH2A.X-positive IMR90 cells as in *f*. $n = 3$ independent experiments is shown

1040   as mean ± s.d. **h,** 2'3'-cGAMP ELISA analysis of IMR90 infected and treated with GS-441524

1041   as in *g*. $n = 8$ independent experiments is shown as mean ± s.d. **i,** Viral RNA detection in the

1042   supernatant of primary nasal epithelial cells with exogenous hACE2 expression (HNEpC-

1043   hACE2) infected with SARS-CoV-2, at the indicated time-points. *shp53* renders cells

1044   senescence–incapable, but, unlike treatment with 10 μM GS-441524 (GS), does not block viral

1045   replication. Data are shown as mean values + s.d. for $n = 3$ independent experiments. **j,** Relative

1046   viability of the indicated conditions, each compared to the corresponding untreated (UT) control

1047   of SARS-CoV-2-infected or mock-infected cells as in *i*, 72 hours p.i. Data are shown as mean

1048   values + s.d. for $n = 3$ independent experiments. **k,** GSEA probing selected senescence-related

1049   gene sets[66] by RNA-seq (GSE147507) analysis of NHBE, Calu-3, and A549 cells infected with

1050   SARS-CoV-2, compared to corresponding mock-infected controls. Positive NES indicate

1051   enrichment in virus-infected cells (dark grey bars), negative NES indicate downregulation in

1052   virus-infected cells (light grey bars). NES of FDR *q*-value < 0.05 are considered statistically

1053   significant and presented (for individual *q*-values, see Supplementary Information). Biological

1054   replicates comprise $n = 7$ control and $n = 3$ infected regarding NHBE, $n = 3$ for each condition

1055   regarding Calu-3, and $n = 5$ control and $n = 3$ infected regarding A549.

1056

1057   **Extended Data Figure 3 | Vignettes of VIS in human COVID-19 lung specimens. a**, IHA

1058   of senescence markers in autopsy lung specimens from non-COVID-19 individuals (with no

1059   obvious airway pathology) *vs.* SARS-CoV-2-PCR-proven COVID-19 patients. Representative

1060   photomicrographs and quantification of affected area as mean percentages of positive cells ±

1061   s.d. or mean percentage of positive area ± s.d (only for lipofuscin) of COVID-19 patients

1062   (lipofuscin, p21$^{\text{CIP1}}$, H3K9me3, BCL2L2, *n* = 5; IL-8, *n* = 9; SARS-CoV-2 RNA, *n* = 4) and

1063   Non-COVID-19 individuals (*n* = 2). Note that *in situ* hybridization of SARS-CoV-2 RNA was

1064   occasionally, but not consistently positive in COVID-19 samples at autopsy (2/4). Scale bars

1065   represent 50 μm. **b**, scRNA-sequencing-based expression analysis of *CDKN2A* and targets of

1066   Navitoclax (*BCL2L1* and *BCL2L2*) in bronchiolar protected specimen brushes (PS), and

1067   bronchoalveolar lavages (BL) of COVID-19 patients with critical course of the disease (*n* = 4).

1068   UMAPs of cell types (top). Orange color code marks cells expressing *CDKN2A*, purple *BCL2L1*

1069   (lower left) or *BCL2L2* (lower right), and red for both. **c,** Gene expression profile (GEP)

1070   indicating elevated transcript expression of cytokines and markers related to M1 macrophage

1071   polarization and activation[32,33] by oligonucleotide-based transcript hybridization of lung

1072   specimens as in *a* (*n* = 3 each). **d**, CD62/P-selectin-marked platelet activation by flow

1073   cytometry in whole blood samples of healthy donors (*n* = 7) or COVID-19 patients (*n* = 10).

1074   Mean ± s.d. with individual values as dots. *p = 0.0368 by unpaired t-test (two-tailed). **e,**

1075   Volcano plot of proteins differentially (*p* < 0.05) expressed in COVID-19 patient serum; SASP

1076   factor SERPINA3[67] highlighted in red as presented in Fig. 2g. Combined clinical COVID-19

1077   severity WHO grade 3 and grade 4 (*n* = 86 patients) *vs.* grade 7 (*n* = 52 patients) are compared.

1078   Log2-fold changes are plotted against log10 adjusted (Benjamini-Hochberg) *p*-values. Vertical

1079   red lines indicate log-fold change cutoffs -0.5/0.5, horizontal red line indicates a significance

1080   cutoff *p* = 0.05. Analysis was performed using the DEqMS software package. **f,** Scatter plot

1081   showing average level of 16 SASP factors measured as in Fig. 2e and fibrinogen concentration

1082   in the serum (y-axis) of the same individual patients. $n = 3$ severe, $n = 5$ moderate, and $n = 18$

1083   mild COVID-19 patients and healthy control ($n = 6$) are shown as color-coded dots. Correlation

1084   coefficiency (Pearson) $r = 0.4139$ with $p = 0.0103$. **g**, Expression levels of senescence-related

1085   transcripts (gene sets: Reactome_SASP and SASP_Schleich[68]) by oligonucleotide-based

1086   transcript hybridization of nasopharyngeal and lung specimens of COVID-19 patients ($n = 3$)

1087   separated by their histopathological microthrombosis status. Shown are genes with fold-

1088   change > 1.5. **h**, Percentages of IL-8-positive cells in COVID-19 lung specimens as in *a*, now

1089   stratified by their microthrombosis status (positive, $n = 4$; negative $n = 5$), are shown as mean

1090   values + s.d.. $p = 0.0397$ by unpaired t-test (two-tailed).

1091

1092   **Extended Data Figure 4 | Additional COVID-19-relevant biological features evoked by**

1093   **the VIS-associated secretome. a**, Percentage of CD13$^+$ THP-1 macrophages exposed to SN

1094   from retroviral VIS *vs.* proliferating HDF as indicated and shown in Fig. 3a. $n = 3$ ($n = 4$ for

1095   medium control) independent experiments; shown are mean percentages + s.d.. '*' indicates p

1096 $= 0.0017$ for IMR90 and 0.0029 for WI38 by unpaired t-test (two-tailed). **b**, GSEA of SN$_{VIS}$-

1097   primed macrophages unveiled vignettes of senescence and inflammation in the CD86$^+$

1098   population by probing of selected senescence- or macrophage activation-related GO terms and

1099   gene sets[66,68] in RNA-seq analyses of THP-1 macrophages incubated with IMR90 SN$_{VIS}$ and

1100   sorted by CD86 expression status (as in Fig. 3c, left). $n = 2$ for each condition. Gene sets with

1101   FDR q-value ≤ 0.05 are shown. **c**, SA-β-gal staining (left) and gene expression analysis by qRT-

1102   PCR (right) of HUVEC endothelial cells that entered secondary, paracrine senescence upon

1103   exposure to SN$_{VIS}$ of WI38 as indicated. Representative photomicrographs and quantification

1104   of $n = 3$ independent experiments; shown are mean percentages $\pm$ s.d.. Relative expression

1105 levels of core senescence-related transcripts (left panel) and SASP transcripts (right panel) are

1106 shown as mean values + s.d.

1107

1108 **Extended Data Figure 5 | Targets of senolytic drugs are induced after viral infection and**

1109 **render VIS cells sensitive to senolytics. a,** Gene expression analysis of BCL2 family

1110 transcripts by qRT-PCR in VIS HDF, with mock infection as a control, as indicated. $n = 3$

1111 independent experiments for each cell line and infection. **b**, STITCH plot showing interactions

1112 between senolytic drugs (Navitoclax, Fisetin, Dasatinib, Quercetin) and their potential targets.

1113 Protein-protein interactions are shown in grey, chemical-protein interactions in green and

1114 interactions between chemicals in red. Modified from http://stitch.embl.de. Note that all

1115 senolytics shown are working in close proximity and have overlapping targets at the level of

1116 kinase networks and BCL2-family members. **c**, Relative viability of proliferating or VIS

1117 (retrovirus) WI38 and Tig3 HDF as in Extended Data Fig. 1a, 24 hours after treatment with

1118 1 µM Venetoclax, 1 µM Navitoclax, or solvent-only (DMSO). $n = 3$ independent experiments.

1119 **d**, Relative viability of senescence-incapable IMR90 (as in Fig. 1e and 4b) infected with VSV,

1120 and 24 hours after treatment with 1 µM Venetoclax, 1 µM Navitoclax, 20 µM Fisetin, 20 µM

1121 D/Q or solvent-only (DMSO). $n = 3$ independent experiments. **e**, Relative viability of

1122 proliferating or VIS IMR90 HDF compared to solvent-only (DMSO) control, two days after

1123 treatments with 1 µM Navitoclax, 1 µM MCL1 inhibitor AT101, or both. Results shown as

1124 mean relative viability + s.d. for $n = 3$ independent experiments. Of note, strongly Mcl-1-

1125 expressing IMR90 VIS cells (see Fig. 4a) were not more susceptible to combined treatment

1126 with Navitoclax plus an Mcl-1 inhibitor as compared to Navitoclax alone. **f**, Secondary viral

1127 infectivity by SN transfer. Wild-type MEF that entered VIS in response to replication-

1128 competent VSV/GFP (or mock) were treated with 1 µM Navitoclax before transferring the SN

1129   to uninfected THP-1 macrophages, here used as an infectivity reporter line. SA-β-gal staining

1130   to illustrate VIS, and GFP detection to visualize VSV infection of MEF. Secondary infectivity

1131   was measured as the GFP-positive fraction of THP-1 cells exposed to SN from MEF after

1132   Navitoclax or solvent-only treatment (bar plot). Senescence-dependent killing of VIS MEF did

1133   not increase GFP-positive THP-1 cells, thereby demonstrating that non-assembled cytoplasmic

1134   virus material potentially released upon senolysis does not add to infectivity. $n = 3$ independent

1135   experiments. **g**, Secondary viral infectivity (% GFP) and relative viability (Rel. viability) of

1136   THP-1 cells after SN transfer from VSV (or mock)-infected MEFs. Dilution of VIS SN as

1137   indicated below decreases secondary infection and increases viability. $n = 3$ independent

1138   experiments. **h,** Viability of HUVEC endothelial cells after exposure to human serum and SN

1139   of proliferating ($SN_{prol.}$) or VIS (SN_{VIS}) primary nasal epithelial cells (HNEpC-ACE2) as in

1140   Fig. 4f. $n = 3$ independent experiments. **i – l**, SARS-CoV-2 spike protein-pseudotyped VSV-

1141   ΔG*/CoV-2-S system to probe VIS in response to spike protein-affecting mutations. **i,** SA-β-

1142   gal staining of human cell line RPE1 infected with VSV-ΔG*-CoV-2-S (WT), VSV-ΔG*-CoV-

1143   2-S variant B.1.1.7 (Alpha), VSV-ΔG*-CoV-2-S variant B.1.351 (Beta), or mock infection as

1144   control. No obvious difference in the ability to drive VIS was detected. Representative

1145   photomicrographs and quantifications are shown as mean percentages ± s.d. for $n = 3$

1146   independent experiments. Scale bar represents 100 μm. **j,** CD86-marked activation of THP-1

1147   cells exposed to SN of RPE1 cells in VIS after infection with VSV-ΔG* pseudotypes as

1148   indicated, or mock infection, as in *i*. Fractions of CD86$^+$ cells relative to mock are shown. SN

1149   of cells infected with either mutant- or wild-type-pseudotyped virus equally enhance the

1150   fraction of CD86$^+$ THP-1 cells. $n = 3$ independent experiments. **k**, Viability of cells as in *i*, after

1151   senolytic treatments as indicated. $n = 3$ independent experiments. All senolytic regimens,

1152   Navitoclax, Fisetin and D/Q proved equally effective in selectively eliminating VIS cells

1153   induced by the wild-type or the alpha or beta mutants. **l**, Gene expression analysis of

1154   senescence-related genes by qRT-PCR in RPE1 cells as in *i*. While transcript profiles of cell-

1155   autonomous senescence markers and pro-coagulatory SASP factors were similar between wild-

1156   type and mutants, pro-inflammatory cytokines and extracellular matrix-active factors appeared

1157   to be significantly higher expressed in the two mutant scenarios compared to wild-type. $n = 3$

1158   independent experiments. All bar plots in this figure show mean results + s.d. with individual

1159   values as dots. '*' indicates $p < 0.05$ by unpaired t-test (two-tailed) in all panels of this figure.

1160

1161   **Extended Data Figure 6 | Senolysis as a novel treatment concept in the COVID-19 golden**

1162   **hamster model. a**, scRNA-seq-based expression analysis of viral RNA and senescence-

1163   associated genes in SARS-CoV-2-infected Syrian golden hamster lungs ($n = 3$ per time-point).

1164   UMAPs of cell types (top left, cell types of special interest in red), core senescence genes (top

1165   right), *CDKN2A* (bottom left), SASP genes (bottom second from left), and *SRC* (bottom right)

1166   on the indicated day p.i.; orange color code marks cells expressing senescence-associated genes,

1167   purple viral RNA, and red both. **b**, scRNA-seq-based expression analysis of *BCL-w, BCL2* and

1168   *MCL1* transcripts in the indicated cell types in SARS-CoV-2-infected Syrian golden hamster

1169   lungs ($n = 3$ per time-point) at the indicated days p.i.; average-scaled (z-scores) expression

1170   levels are shown in color code. Note that *BCL-2* and *MCL-1* transcript levels followed no clear

1171   trend, while *BCL-w* transcripts were induced at day 5 p.i. in all cell types presented. **c**, Daily

1172   body weight assessment of Syrian golden hamsters infected with SARS-CoV-2 (or uninfected

1173   healthy controls), and subsequently treated with Navitoclax or solvent-only as in Fig. 5a. $n = 5$

1174   each for SARS-CoV-2-infected solvent-only- and Navitoclax-treated animals; $n = 3$ for the

1175   healthy control group. **d**, Number of platelets per 500 erythrocytes in blood smears of golden

1176   hamsters infected with SARS-CoV-2 and treated as in *c*, on day 7 p.i.. Mean counts ± s.d. of

1177 $n = 5$ hamsters per treatment group. *$p = 0.0127$ by unpaired t-test (two-tailed). Note for *c* and

1178   *d*: Although the Navitoclax group presented with relative weight loss of around nine percent

1179   and a reduction of nearly 40% of the initial thrombocyte concentration compared to the

1180   infection-only group at the end of therapy, a prominent adverse effect of Navitoclax, no obvious

1181   external or internal signs of enhanced hemorrhage was observed. **e**, Transmission electron

1182   microscopy analysis of trachea epithelial cells from hamsters as in *b* and *c*. In infected and

1183   solvent treated animals (with the enlarged photomicrograph of a nucleus on the right), ciliated

1184   epithelial cells show senescent features as dilated endoplasmic reticulum (*), disrupted and

1185   dilated nuclear envelope (N = nucleus), vacuolization and altered mitochondria. Scale bar =

1186   1µm. *n* = 4 for SARS-CoV-2 infected groups and *n* = 3 for the mock infection group. **f**,

1187   Representative photomicrographs of H/E-stained lung tissue of Syrian golden hamsters as in

1188   Fig. 5a and *c-e* at day 7 p.i.. Lung sections for overview (i, v, ix) with active inflammation

1189   (arrow), signs of necrosis (inset, arrowhead), and hyperplasia (arrowheads) of alveolar

1190   epithelial cells (AEC) II (ii, vi). Vascular endothelialitis (iii, vii) with sloughing of endothelial

1191   cells (arrow), and subendothelial infiltration by lymphocytes (arrowhead). Regeneration of

1192   bronchial epithelium (iv, viii; double-headed arrow). Lung of mock-infected, untreated animals

1193   as control (ix, x, xi, xii). General signs of inflammation and AEC II hyperplasia in the

1194   Navitoclax group were rather similar to untreated animals. while the Navitoclax group

1195   presented with a less extensive patchy pneumonia pattern when compared to the solvent-only

1196   group (compare f-v to f-i). *n* = 5 each for SARS-CoV-2-infected solvent-only- and Navitoclax-

1197   treated animals. *n* = 3 for the healthy control group. Scale bars in i, v, ix = 1 mm, ii-iv, vi-viii,

1198   x-xii = 50 µm, insets ii, vi, x = 20 µm. **g**, Quantification of the findings shown in *f*. *p = 0.0042

1199   by unpaired t-test (two-tailed). **h**, Multiplex bead-based protein analysis of golden hamster

1200   serum samples at day 7 p.i.; hamsters as in *c–g*. All bar plots in this figure show mean results +

1201   s.d. with individual values as dots.

1202

1203   **Extended Data Figure 7 | Senolysis as a novel treatment concept in COVID-19 in**

1204   **additional animal models. a**, Daily body weight assessment of dwarf hamsters and *K18-*

1205   *hACE2* mice infected with SARS-CoV-2 (or uninfected healthy controls), and subsequently

1206   treated with senolytics as in *b-e* and Fig. 5b-e. Dwarf hamsters: *n* = 5 each for SARS-CoV-2-

1207   infected solvent-only-, Navitoclax- or D/Q-treated; *n* = 2 for the healthy control group. *K18-*

1208   *hACE2*-transgenic mice: *n* = 5 for solvent, Navitoclax, and D/Q; *n* = 4 for Fisetin. **b**, Survival

1209   rate of *K18-hACE2*-transgenic mice on day 6d p.i. with SARS-CoV-2 and subsequent senolytic

1210   treatment as in *a*. **c**, Histological scoring of H/E-stained lung tissue of K18-hACE2 mice as in

1211   *a*, *b*. **d**, Viral load in hamster and mouse tissues on day 6 (mouse, as in *a-c*), day 7 (golden

1212   hamster, as in Fig. 5a and Extended data 6), and day 4 (dwarf hamster, as in Fig. 5b-e) p.i.. **e**,

1213   IHA of senescence markers (p16^{INK4a}, H3K9me3) and lipofuscin-staining in lung specimens of

1214   *K18-hACE2*-transgenic mice as in *a-d*. All bar plots in this figure show mean results + s.d. with

1215   individual values as dots.

1216

1217   **Extended Data Figure 8 | Senolysis as a novel treatment concept in COVID-19 patients. a**,

1218   Frequency (bars) and risk (odds ratio) of COVID-19 patients to be hospitalized or in need of

1219   oxygen support after either standard care (SC) or Quercetin plus standard care (Quercetin).

1220   *n* = 97 for each group, based on two randomized trials combined and re-analyzed here[51,52]. **b**,

1221   Days of hospitalization (left) of COVID-19 patients (left; *p < 0.0001 by unpaired t-test, two-

1222   tailed*), the fraction of patients admitted to the intensive care unit (ICU; middle) or deceased in

1223   the course of the disease (right). Note that there was no patient in ICU or deceased in the

1224   Quercetin group. Patients as in *n*; *n* = 97 for each group.

1225

1226 **Extended Data Table 1 | Histological scoring of COVID-19 features in the SARS-CoV-2-**

1227 **infected hamsters.** Histopathological scoring of FFPE lung tissue from hamsters infected and

1228 treated as indicated.

1229 * % affected score is scaled as (0) not present, (1) present but $\leq$ 25%, (2) > 25 but $\leq$ 50%, (3)

1230 > 50 but $\leq$ 75%, (4) > 75 %;

1231 ** degree of inflammation is scaled as (1) minimal, (2) mild, (3) moderate or (4) severe.

1232 For all other parameters, rating refers to occurrence rate of (1) sporadic, (2) mild, (3) moderate,

1233 (4) severe.

1234 [1]Pneumonia score is the average of five pneumonia criteria (% affected score, degree of

1235 inflammation, occurrence rates of lymphocytes, macrophages, neutrophils).

1236 [2]Alveolar damage is the average of alveolar epithelial necrosis and alveolar edema.

1237 [3]Endothelial damage is the average of endothelialitis and endothelial activation

1238

1239 **Extended Data Table 2 | Input data for Figure 5f.** Treatment, sex, age, symptoms, CRP,

1240 LDH, RT-PCR were collectively analyzed in a multi-variant analysis by agglomerative

1241 hierarchical clustering underlying the constellation plots.

1242

a

	lipofuscin	p16^{INK4a}	p21^{CIP1}	H3K9me3	IL-8	BCL2L2 (BCL-w)	SARS-CoV-2 RNA
Non-COVID-19	5.8% ± 2.7	9.1% ± 2.8	0.3% ± 0.4	21.2% ± 0.5	22.6% ± 2.2	1.0% ± 0.7	
COVID-19	60.8% ± 14.7	48.3% ± 11.7	44.8% ± 15.2	83.7% ± 5.1	80.8% ± 13.8	97.2% ± 3.2	

b

c

d

e healthy COVID-19

EGF, IL-8, MMP-1, uPAR, HGF, ICAM-1, TIMP-1, IL-6, MIP-1α, PAI-1(SERPIN), MMP-9, IL-1α, IL-10, CCL4, MMP-3, FGF-2, IFN γ, IL-1β, MCP-1, G-CSF1, IFNα, GM-CSF, TNFα

row min — row max

f healthy COVID-19

ORM1, SERPINA1, SAA1, SERPINA3, LRG1, C9, C1QC, SERPING1, PF4, THBS1

rel. intensity

g

a

% CD86+ cells

med. prol. VIS | prol. VIS
IMR90 | WI38

med. | vec. | JMJD2C | shp53
virus - + - + - +

b

medium
WI38 SNprol.
IMR90 SNprol.
WI38 SNVIS
IMR90 SNVIS
WI38 SNVIS
IMR90 SNVIS

- - + + + + - - THP-1

GM-CSF
G-CSF1
IL-6
IL-1 b
FGF-2
TNF a
IL-1 a
IL-8
MCP-1
IL-10
MMP-3
MMP-9
HGF
TIMP-1
ICAM-1
IFN a
MIP-1 b
EGF
IFN g
MIP-1a
MMP-1

min — max

c

THP-1 · CD86+ · unsorted

WI38 SNprol.
IMR90 SNprol.
WI38 SNVIS
IMR90 SNVIS
Non-COVID-19
COVID-19

CCR2
NCF1
CSF2RB
MMP9
TNFSF14
CCR1
CCL2
HLA-B
TLR4
CD163
CD14
RRAGC
CCL4
MYD88
S100A12
IL12RB1
HLA-A
IL27RA
CCL5
CD33
NFKB2
HLA-DRB1
TNFRSF9
TNFSF13B
B2M
IRF1
CCR5
IL10RA
CD40
HIF1A

-4 -2 0 2 4

d

Supernatant of RPE1 infected VSV-ΔG*

mock | emp | CoV S | CoV-2 S
16.9% ± 1.4 | 22.5% ± 6.2 | 62.3% ± 8.9 | 72.4% ± 5.9

SA-β-gal   100 μm

CD86+ (rel. to mock)

e

Supernatant of IMR90

proliferating | VIS
1.2% ± 0.6 | 95.3% ± 1.6

HUVEC   100 μm

Rel. transcription

CDKN1A LMNB1 MKI67 DHFR | IL1A IL1B IL6 IL8 SERPINE1 MMP1 THBS1

SNprol. SNVIS

f

ADP (μM)

virus - + - + - +
vector | JMJD2C | shp53

g

clotting time (sec)

SNprol. SNVIS

INTEM 15 μl | 25 μl   EXTEM 15 μl | 25 μl

h

MPO-DNA (ng/ml)

prol. VIS | prol. VIS
IMR90 | WI38

i

%C5b-9-pos.

serum ina. + + | ina. + +
SN - prol. VIS | - prol. VIS
IMR90 | WI38

j

% Cytotoxicity

serum ina. + +
SN - prol. VIS

a

	IMR90	Tig3	Wi38		IMR90	Wi38	Tig3
virus	- +	- +	- +	virus	- +	- +	- +
BCL2				p-SRC			
BCL2L1 (BCL-XL)				p-AKT			
BCL2L2 (BCL-W)				p-p70S6K			
MCL1				p-p38			
α-Tubulin				α-Tubulin			

b — DMSO, Venetoclax, Navitoclax, Fisetin, D/Q

Relative viability; virus − / +; vector, *shp53*, JMJD2C

Legend for c - f — UT, *shp53*, GS, Navitoclax, Fisetin, D/Q

c — Relative viability; mock, SARS-CoV-2

d — CD86-pos. THP-1 cells (relative to mock UT); SN_{mock}, $SN_{SARS-CoV-2}$

e — % SA-β-gal; SN_{mock}, $SN_{SARS-CoV-2}$

f — % C5b-9-positive; SN_{mock}, $SN_{SARS-CoV-2}$

a golden hamster

H3K9me3 | mock 26.1% ± 8.7 | SARS-CoV-2 + sol. 57.2% ± 17.4 | SARS-CoV-2 + Navito. 13.8% ± 8.4

H3K9me3 | 17.6% ± 1.8 | 30.8% ± 11.4 | 14.2% ± 10.3

lipofuscin | 12.3% ± 2.7 | 36.8% ± 4.7 | 15.7% ± 4.0

50 μm

b dwarf hamster + SARS-CoV-2

H3K9me3 | solvent 50.8% ± 25.9 | Navitoclax 32.4% ± 22.7 | D/Q 15.3% ± 5.3

lipofuscin | 31.1% ± 11.3 | 7.9% ± 6.4 | 8.5% ± 5.5

50 μm

c dwarf hamster

SARS-CoV-2

solvent: i, ii (bronchiolitis), iii (pneumonia), iv (endothelialitis)

Navitoclax: v, vi, vii/viii, ix

D/Q: x, xi, xii, xiii

mock: xiv, xv, xvi, xvii

1 mm | 20 μm | 20 μm | 50 μm

d dwarf hamster
- SARS-CoV-2 + solvent
- SARS-CoV-2 + Navitoclax
- SARS-CoV-2 + D/Q
- healthy

pneumonia*, bronchial epithelial hyperplasia*, alveolar damage*, perivasc. lymphocyte cuffs, perivasc. edema, endothelialitis

e healthy | SARS-CoV-2 sol. Navito. D/Q

G-CSF, GM-CSF, GRO/KC, INF-g, IL-1a, IL-1b, MCP-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12p70, IL-13, IL-17A, IL-18, M-CSF, MIP-1a, MIP-3a, RANTES, TNF-a, VEGF

row min — row max

f Day 0 / Day 7 (assigned)
- SC
- Quercetin

Y: 30, 20, 10, 0, -10, -20, -30
X: -40 -30 -20 -10 0 10 20 30

a

Tig3 — mock: SA-β-gal 5.3% ± 2.0, retrovirus 88.8% ± 5.5; 100 µm
WI38 — mock 3.0% ± 1.8, retrovirus 89.2% ± 4.3
SAHF — 5 µm
p16INK4A — 100 µm

b

HOSTPATHOGEN_INTERACTION_OF_HUMAN_CORONA_VIRUSES_MAPK_SIGNALING
HOSTPATHOGEN_INTERACTION_OF_HUMAN_CORONA_VIRUSES_INTERFERON_INDUCTION
COMPLEMENT
TYPE_I_INTERFERON_INDUCTION_AND_SIGNALING_DURING_SARSCOV2_INFECTION
SARS_CORONAVIRUS_AND_INNATE_IMMUNITY
COAGULATION
HOSTPATHOGEN_INTERACTION_OF_HUMAN_CORONA_VIRUSES_APOPTOSIS

VIS / OIS

Normalized Enrichment Score

c

Relative transcription: CDKN1A, LMNB1, MKI67, DHFR, IL1A, IL6, IL8, CCL2, CXCL10, MMP3, MMP9, SERPINE1, THBS1, TIMP1

WI38 mock / WI38 virus / Tig3 mock / Tig3 virus

d

Cell number (10^4) vs days

Tig3 mock, Wi38 mock, IMR90 mock
Tig3 1* retro, Wi38 1* retro, IMR90 1* retro
Tig3 10* retro, Wi38 10* retro, IMR90 10* retro

e

WT — mock 7.8% ± 1.5, retrovirus 92.8% ± 2.4; 100 µm
$p53^{-/-}$ — mock 5.0% ± 1.0, retrovirus 12.3% ± 2.3

Relative transcription: CDKN1A, CDKN2A, MKi67, CDK2, IL6

WT mock / WT virus / $p53^{-/-}$ mock / $p53^{-/-}$ virus

f

Relative transcription: CDKN1A, LMNB1, MKI67, IL6, IL8, MMP1, SERPINE1, THBS1, TIMP1

vector mock / vector virus / JMJD2C mock / JMJD2C virus / shp53 mock / shp53 virus

g

empty, SN_mock; empty, SN_virus; JMJD2C, SN_mock; JMJD2C, SN_virus; shp53, SN_mock; shp53, SN_virus

EGF, FGF-2, G-CSF (CSF-3), GM-CSF, HGF, INF alpha, IFN gamma, IL-1 alpha, IL-1 beta, IL-10, IL-6 (, IL-8 (CXCL8), MCP-1 (CCL2), MIP-1 alpha (CCL3), MIP-1 beta (CCL4), MMP-1, PAI-1 (Serpin), TNF alpha, uPAR, ICAM-1, MMP-3, MMP-9, TIMP-1

row min — max

h

2'3'-cGAMP (pg/mL): mock vs virus — Wi38, IMR90, Tig3

2'3'-cGAMP (pg/mL) vs virus (– / +): vector, JMJD2C, shp53

i

retrovirus: – / + / + / + / +
treatment: – / – / Zidovudine / G150 / H151

3.8% ± 1.3, 86.0% ± 1.3, 4.5% ± 1.3, 77.3% ± 6.5, 57.8% ± 5.6; 100 µm

j

cGAMP (pg/ml)
virus: – / + / + / + / +
treatment: – / – / Z / G150 / H

a

	mock	AAV	lentivirus	HCoV-NL63	VSV
RPE1					
A549					

100 µm

b

	mock	HCoV-NL63	VSV
HBEpC	25.5% ± 7.0	56.7% ± 3.7	n.d.
HNEpC	7.5% ± 2.5	34.0% ± 4.0	63.1% ± 1.7

100 µm

c

% SA-β-gal positive vs MOI (log2)
● RPE1
■ A549

d

% SA-β-gal positive vs MOI (log2)
● CoV S
■ CoV-2 S

e

	VSV-ΔG* emp	VSV-ΔG*-CoV S	VSV-ΔG*-CoV-2 S
RPE1			
A549			
HNEpC			
NIH3T3			

100 µm

f

	mock	retrovirus	VSV
ROS (inset γH2A.X) UT			
ROS (inset γH2A.X) NAC			
SA-β-gal UT	6.6% ± 3.3	93.0% ± 1.8	95.3% ± 1.5
SA-β-gal NAC	3.8% ± 1.3	32.5% ± 4.4	50.7% ± 8.2

100 µm

g

% γH2AX positive

virus	-	-	retro	retro	VSV	VSV	VSV
treatment	-	NAC	-	NAC	-	NAC	GS

h

relative cGAMP level
mock / VSV / VSV+GS

i

viral RNA in SN (GE/ml)
□ UT
▨ shp53
▨ GS

hrs p.i. 0 72 72 72

j

Relative viability (to UT)
□ UT
▨ shp53
▨ GS

mock / SARS-CoV-2

k

Normalized Enrichment Score

NHBE
- SASP
- CLASSICAL SASP FACTORS
- COMPLEMENT
- COAGULATION

Calu-3
- FRIDMAN_SENESCENCE_UP
- CLASSICAL SASP FACTORS
- REACTOME_CELLULAR_SENESCENCE
- REACTOME_DNA_DAMAGE_TELOMERE_STRESS_INDUCED_SENESCENCE
- FRIDMAN_SENESCENCE_DN
- HALLMARK_E2F_TARGETS

A549
- KEGG_CELLULAR_SENESCENCE
- SASP
- CLASSICAL SASP FACTORS

a

	lipofuscin	p21^{CIP1}	H3K9me3	IL-8	BCL2L2 (BCL-w)	SARS-CoV-2 RNA
Non-COVID-19	5.1% ± 1.4	15.8% ± 12.0	14.2% ± 13.1	31.9% ± 5.9	31.5% ± 6.6	
COVID-19	32.0% ± 18.7	44.5% ± 15.9	65.0% ± 8.1	82.7% ± 13.2	86.4% ± 10.0	

b cell type

c

d

e

f

g

h

a

% CD13^high

med. | prol. | VIS | prol. | VIS
IMR90 | | | WI38

b

SASP_SCHLEICH
FRIDMAN_SENESCENCE_UP
CLASSICAL_SASP
HALLMARK_P53_PATHWAY
GO_MACROPHAGE_ACTIVATION
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_INFLAMMATORY_RESPONSE

Normalized Enrichment Score

c

Supernatant of WI38

proliferating | VIS

HUVEC

3.5% ± 1.3 | 96.5% ± 1.8

100 μm

Relative transcription

CDKN1A LMNB1 MKI67 DHFR

IL1A IL1B IL6 IL8 SERPINE1 MMP1 THBS1

Wi38-SN_prol.
Wi38-SN_VIS

a

cell type

Core senescence genes   SARS-CoV-2   Both

3 days p.i.   5 days p.i.

Cdkn2a   SARS-CoV-2   Both

SASP   SARS-CoV-2   Both

3 days p.i.   5 days p.i.

Src   SARS-CoV-2   Both

3 days p.i.   5 days p.i.

b

BCL2L2 (BCL-w)

BCL2

MCL1

Average expression

c

golden hamster

- SARS-CoV-2 + solvent
- SARS-CoV-2 + Navitoclax
- healthy control

d

Navitoclax   −   +

e

SARS-CoV-2 + sol.

SARS-CoV-2 + Navito.

mock

f

golden hamster

pneumonia   endothelialitis   bronchi. epithel. hyperpl.

SARS-CoV-2 + sol.   i   ii   iii   iv

SARS-CoV-2 + Navito.   v   vi   vii   viii

mock   ix   x   xi   xii

g

golden hamster

- SARS-CoV-2 + solvent
- SARS-CoV-2 + Navitoclax
- healthy

p = 0.066

pneumonia   bronchial epithelial hyperplasia   alveolar damage   perivasc. lymphocyte cuffs   perivasc. edema   endothelial damage   mesothelial activation

h

golden hamster

healthy   SARS-CoV-2 + solvent   SARS-CoV-2 + Navitoclax

IL-7
GM-CSF
GRO/KC
IFN-g
IL-18
TNF-a
IL-6
IL-13
MIP-3a
MIP-1a
M-CSF
IL-17
VEGF
MCP-1
IL-2
IL-12(p70)
IL-10
G-CSF
IL-1a
IL-5
RANTES
IL-4
IL-1b

row min   row max

a

dwarf hamster

Body weight (g)

- SARS-CoV-2 + solvent
- SARS-CoV-2 + Navitoclax
- SARS-CoV-2 + D/Q
- healthy control

days p.i.

K18-hACE2 mouse

Body weight (g)

- SARS-CoV-2 + solvent
- SARS-CoV-2 + Navitoclax
- SARS-CoV-2 + Fisetin
- SARS-CoV-2 + D/Q

days p.i.

b

K18-hACE2 mouse
+ SARS-CoV-2

- + solvent
- + Navitoclax
- + Fisetin
- + D/Q

% survival on d6

c

K18-hACE2 mouse

pneumonia · bronchial epithelial hyperplasia · alveolar damage · perivasc. lymphocyte cuffs · perivasc. edema · endotheliitis

d

K18-hACE2 mouse

SARS-CoV-2 (PFU/ml)

Mock · Navitoclax · Fisetin · D/Q

e

golden hamster

viral RNA (e+4 GE/ml)

nasal · lung

dwarf hamster

viral RNA (e+6 GE/ml)

nasal · lung

- SARS-CoV-2 + solvent
- SARS-CoV-2 + Navitoclax
- SARS-CoV-2 + D/Q
- healthy

K18-hACE2 mouse + SARS-CoV-2

solvent | Navitoclax | Fisetin | D/Q

p16INK4a

59.8% ± 17.4 | 19.2% ± 4.8 | 4.6% ± 4.2 | 11.2% ± 5.7

H3K9me3

60.7% ± 5.5 | 24.1% ± 10.4 | 26.6% ± 3.8 | 19.8% ± 4.4

lipofuscin

29.3% ± 4.3 | 9.2% ± 3.7 | 13.4% ± 2.4 | 12.2% ± 4.2

50 µm

a

Frequency: Hospitalization		
N		Y
Quercetin		
SC		
p = 0.0024 Odds Ratio (SC/Q)	Lower 95%	Upper 95%
3.996	1.624	9.831

Frequency: Need of oxygen		
N		Y
Quercetin		
SC		
p = 0.0002 Odds Ratio (SC/Q)	Lower 95%	Upper 95%
18.963	2.461	146.101

b

standard care
Quercetin

Roborovski dwarf hamster Animal Nr.	Infection	Treatment	Time (dpi)	Pneumonia					Pneumonia score[1]	Bronchial epithelial necrosis	Bronchial inflammation	Bronchial epithelial hyperplasia	Alveolar epithelial necrosis	Alveolar edema	Alveolar damage[2]	Perivascular edema	Perivascular lymphocytic cuffing	Hyperplasia ATII	Endotheliailitis
				% affected score*	Degree of inflammation**	Lymphocytes	Macrophages	Neutrophils											
ND/Q_1	SARS-CoV-2	solvent	4	3	2-3	2	3	2	2.5	1-2	2	2	2-3	2-3	2.5	1	1	1-2	1
ND/Q_2	SARS-CoV-2	solvent	4	4	3	1	2-3	3	2.7	2-3	2	2-3	3	3	3.0	2	0	1	2
ND/Q_3	SARS-CoV-2	solvent	4	0	1	1	1	1	1.0	0	0	0	0	0	0.0	0	1	0	0
ND/Q_4	SARS-CoV-2	solvent	2	3	2	1-2	2	1-2	2.0	2	2	1	1	1	1.0	0	1	1	1
ND/Q_5	SARS-CoV-2	solvent	3	3	2-3	1	2	2-3	2.2	1-2	2	3	2-3	2	2.3	1-2	0	0	1
ND/Q_6	SARS-CoV-2	navitoclax	4	1	1	1	2	1	1.2	1	1	1	0	0	0,0	1	0	0	1
ND/Q_7	SARS-CoV-2	navitoclax	3	4	3	1	2-3	2	2.5	2	1	2-3	3	3	3.0	2	0	1	1
ND/Q_8	SARS-CoV-2	navitoclax	3	2	2	1	2	2	2.0	3	1	1	2	2	2.0	2	0	1	1
ND/Q_9	SARS-CoV-2	navitoclax	2	2	2-3	1	2	2	1.9	3	1-2	1	2	2	2.0	1	0	1	1-2
ND/Q_10	SARS-CoV-2	navitoclax	3	3	2-3	1	2-3	2	2.2	3	1	1	2	2-3	2.3	1	0	1	1
ND/Q_11	SARS-CoV-2	D/Q	4	0	1	1	2	1	1.2	0	0	0	0	1	0.5	0	0	0	1
ND/Q_12	SARS-CoV-2	D/Q	4	1	1	1	1-2	1	1.1	0	1	1	0	1	0.5	0	0	0	0
ND/Q_13	SARS-CoV-2	D/Q	4	2	1	1	1-2	1-2	1.4	1	1	1	1	1-2	1.3	1	0	0	1
ND/Q_14	SARS-CoV-2	D/Q	4	2	1	1	2	1	1.4	1	1	1	1	1	1.0	0	0	0	0
ND/Q_15	SARS-CoV-2	D/Q	4	2	2	1	2	2	1.8	2	1	1-2	2	2	2.0	1-2	1	1	1-2
ND/Q_16	mock	mock	4	0	1	1	2	1	1.2	0	0	0	0	1	0.5	0	0	0	0
ND/Q_17	mock	mock	4	0	1	1-2	2	1	1.3	0	0	0	0	1	0.5	0	1	0	0

Syrian Golden hamster Animal Nr.	Infection	Treatment	Time (dpi)	Pneumonia					Pneumonia score[1]	Bronchial epithelial necrosis	Bronchial inflammation	Bronchial epithelial hyperplasia	Alveolar epithelial necrosis	Alveolar edema	Alveolar damage[2]	Perivascular edema	Perivascular lymphocytic cuffing	Hyperplasia ATII	Endothe-litis	Endothelial activation	Endothelaial damage[3]	Mesothelial activation
				% affected score*	Degree of inflammation**	Lymphocytes	Macrophages	Neutrophils														
NX1	SARS-CoV2	Navitoclax	7	2	2-3	3	2-3	2	2.4	0	1	2	2-3	0	1.3	2	2	3	0	2	1	3
NX2	SARS-CoV2	Navitoclax	7	3	2-3	3	2-3	2	2.6	0	0	3-4	3.5	2	2.8	1-2	1	4	0	3	1.5	4
NX3	SARS-CoV2	Navitoclax	7	2	2-3	2	2-3	3	2.4	0	2	2-3	2-3	0	1.3	0	1	2-3	0	2	1	1
NX4	SARS-CoV2	Navitoclax	7	2	3	2-3	3	3	2.7	0	1	2	3	2	2.5	2	2	3-4	0	3	1.5	0
NX5	SARS-CoV2	Navitoclax	7	3	4	3	3-4	3	3.3	0	0	0	4	2-3	3.3	2-3	2	4	0	2-3	1.25	3
NX6	SARS-CoV2	solvent	7	2	3	3	3	3	2.8	0	1	3	3	2-3	2.8	2-3	2	3	1-2	3	2.25	2-3
NX7	SARS-CoV2	solvent	7	2	2-3	3	2-3	2-3	2.5	0	0	3	2	0	1.0	2-3	2-3	3	0	3	1.5	3
NX8	SARS-CoV2	solvent	7	2	3	3	3	3	2.8	0	0	2	2	2	2.0	3	1	3-4	1-2	3	2.25	2
NX9	SARS-CoV2	solvent	7	3	4	4	3-4	2-3	3.4	0	0	3-4	2	2-3	2.3	3	3	4	2	3	2.5	4
NX10	SARS-CoV2	solvent	7	1	2-3	3	2-3	3	2.4	0	0	3	2-3	0	1.3	2	2-3	3	1	3	2	2
NX11	mock	mock	7	0	0	0	0	0	0.0	0	0	0	0	0	0.0	0	0	0	0	0	0	0
NX12	mock	mock	7	0	0	0	0	0	0.0	0	0	0	0	0	0.0	0	1	0	0	0	0	0
NX13	mock	mock	7	0	0	0	0	0	0.0	0	0	0	0	0	0.0	0	1	0	0	0	0	0

Patient Nr.	Treatment	Sex	Gender code	Age (years)	CRP d1	CRP d7	CPR d7 - d1	LDH d1	LDH d7	LDH d7 - d1	Symptoms d7 - d1	RT-PCR d1	RT-PCR d7
1	Q	F	0	45	4,05	0,43	-3,62	240	190	-50	-3	Positive	Negative
2	Q	F	0	20	6,7	0,99	-5,71	341	230	-111	-2	Positive	Negative
3	Q	M	1	68	3,54	0,34	-3,2	298	240	-58	-2	Positive	Negative
4	Q	F	0	40	3	0,5	-2,5	220	140	-80	0	Positive	Negative
5	Q	M	1	29	10,2	1,5	-8,7	560	331	-229	-1	Positive	Negative
6	Q	F	0	32	19	0,75	-18,25	605	256	-349	-2	Positive	Negative
7	Q	M	1	38	0,42	0,41	-0,01	175	127	-48	-3	Positive	Negative
8	Q	M	1	30	1	1	0	300	202	-98	-2	Positive	Negative
9	Q	F	0	24	2,1	0,5	-1,6	402	264	-138	-2	Positive	Negative
10	Q	M	1	56	38	55	17	709	678	-31	-3	Positive	Positive
11	Q	F	0	53	5	1	-4	340	220	-120	-3	Positive	Negative
12	Q	M	1	70	37	46	9	300	309	9	-2	Positive	Positive
13	Q	F	0	66	15	3	-12	267	220	-47	-2	Positive	Negative
14	Q	M	1	30	24	5	-19	176	160	-16	-4	Positive	Negative
15	Q	F	0	32	16	21	5	250	274	24	-1	Positive	Positive
16	Q	F	0	34	67	3,1	-63,9	345	213	-132	-4	Positive	Negative
17	Q	M	1	43	45	10	-35	678	248	-430	-3	Positive	Negative
18	Q	M	1	38	60	25	-35	770	279	-491	-2	Positive	Negative
19	Q	F	0	45	80	27	-53	670	370	-300	-3	Positive	Negative
20	Q	F	0	47	75	30	-45	535	270	-265	-3	Positive	Positive
21	Q	M	1	52	60	25	-35	610	450	-160	-2	Positive	Positive
22	Standard	F	0	54	62	40	-22	352	320	-32	-2	Positive	Positive
23	Standard	F	0	56	80	77	-3	512	459	-53	-3	Positive	Positive
24	Standard	M	1	62	55	43	-12	323	310	-13	-3	Positive	Positive
25	Standard	F	0	45	66	48	-18	402	380	-22	-2	Positive	Positive
26	Standard	F	0	59	78	66	-12	612	551	-61	-3	Positive	Positive
27	Standard	M	1	95	17,14	12,2	-4,94	320	280	-40	-2	Positive	Positive
28	Standard	M	1	37	12,5	1,6	-10,9	262	257	-5	-3	Positive	Positive
29	Standard	F	0	33	7,3	2,3	-5	203	285	82	-1	Positive	Negative
30	Standard	F	0	73	16	3,3	-12,7	615	359	-256	-1	Positive	Positive
31	Standard	F	0	57	17,5	5	-12,5	267	231	-36	-2	Positive	Positive
32	Standard	M	1	72	73	5	-68	256	231	-25	-1	Positive	Positive
33	Standard	F	0	57	12	24	12	232	250	18	-2	Positive	Positive
34	Standard	M	1	60	34	11,4	-22,6	640	250	-390	-1	Positive	Positive
35	Standard	M	1	51	48,7	12	-36,7	305	259	-46	-2	Positive	Positive
36	Standard	M	1	73	15	6	-9	347	243	-104	-1	Positive	Positive
37	Standard	M	1	68	4,78	1	-3,78	573	433	-140	-2	Positive	Positive
38	Standard	M	1	50	0,9	0,5	-0,4	344	250	-94	-2	Positive	Positive
39	Standard	M	1	42	0,5	0,5	0	250	253	3	-3	Positive	Negative
40	Standard	F	1	69	5,51	6,2	0,69	358	745	387	-2	Positive	Positive
41	Standard	F	0	42	1	0,5	-0,5	240	320	80	-1	Positive	Positive
42	Standard	F	0	25	33,9	15,2	-18,7	250	214	-36	-2	Positive	Positive