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2 **Solar simulated ultraviolet radiation inactivates HCoV-NL63**

3 **and SARS-CoV-2 coronaviruses at environmentally relevant doses**

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21 **Keywords:** Coronavirus; HCoV-NL63; SARS-CoV-2; Solar simulated ultraviolet radiation; Viral  
22 inactivation

23 **ABSTRACT**

24 The germicidal properties of short wavelength ultraviolet C (UVC) light are well established and  
25 used to inactivate many viruses and other microbes. However, much less is known about  
26 germicidal effects of terrestrial solar UV light, confined exclusively to wavelengths in the UVA  
27 and UVB regions. Here, we have explored the sensitivity of the human coronaviruses HCoV-  
28 NL63 and SARS-CoV-2 to solar-simulated full spectrum ultraviolet light (sUV) delivered at  
29 environmentally relevant doses. First, HCoV-NL63 coronavirus inactivation by sUV-exposure  
30 was confirmed employing (*i*) viral plaque assays, (*ii*) RT-qPCR detection of viral genome  
31 replication, and (*iii*) infection-induced stress response gene expression array analysis. Next, a  
32 detailed dose-response relationship of SARS-CoV-2 coronavirus inactivation by sUV was  
33 elucidated, suggesting a half maximal suppression of viral infectivity at low sUV doses. Likewise,  
34 extended sUV exposure of SARS-CoV-2 blocked cellular infection as revealed by plaque assay  
35 and stress response gene expression array analysis. Moreover, comparative (HCoV-NL63 versus  
36 SARS-CoV-2) single gene expression analysis by RT-qPCR confirmed that sUV exposure blocks  
37 coronavirus-induced redox, inflammatory, and proteotoxic stress responses. Based on our findings,  
38 we estimate that solar ground level full spectrum UV light impairs coronavirus infectivity at  
39 environmentally relevant doses. Given the urgency and global scale of the unfolding SARS-CoV-  
40 2 pandemic, these prototype data suggest feasibility of solar UV-induced viral inactivation, an  
41 observation deserving further molecular exploration in more relevant exposure models.

42

43 Abbreviations: MOI, multiplicity of infection; sUV, solar simulated ultraviolet light; UV,  
44 ultraviolet.

45

46 **1. Introduction**

47 The germicidal properties of short wavelength ultraviolet C (UVC) light are well established  
48 and widely used to inactivate many viruses and other microbes, and virucidal activity of solar UVC  
49 targeting pathogenic coronaviruses has been explored in much detail before [1-3]. Given the  
50 urgency and global scale of the unfolding SARS-CoV-2-caused COVID-19 pandemic, UV-  
51 induced inactivation of coronaviruses including SARS-CoV-2 has reemerged as a matter of much  
52 contemporary research interest [2-8]. Indeed, recently, rapid and complete inactivation of SARS-  
53 CoV-2 by UVC has been substantiated experimentally, and virucidal UVC light sources (254 nm  
54 emission) are used for surface disinfection and decontamination [5,8]. Moreover, far UVC (222  
55 nm) has attracted considerable attention due to its potent virucidal activity [2]. However, much  
56 less is known about germicidal (and coronavirus-directed) effects of terrestrial (ground level) solar  
57 UV light, a matter of much interest given the airborne spread of coronaviruses including SARS-  
58 CoV-2 [2,6]. UVC (< 290 nm) is not present in the solar spectrum reaching the Earth's surface,  
59 and most of solar UV energy incident on the skin is from the UVA region (>95%; from 320–400  
60 nm). Remarkably, the UVB (290–320 nm) proportion of total solar UV-flux received by skin can  
61 be well below 2% depending on the solar angle, which determines the atmospheric light path length  
62 and thereby the degree of ozone-filtering and preferential Rayleigh scattering of short wavelength  
63 UV light [9].

64 Recently, the role of ground level (environmentally relevant) solar UV has been explored in the  
65 context of SARS-CoV-2 disinfection, and a role of solar UVB in human coronavirus inactivation  
66 has been substantiated based on atmospheric and geophysical simulations [2,6,10,11].  
67 Specifically, inactivation times of SARS coronaviruses exposed to environmental photons with  
68 wavelengths between 290-315 nm have been calculated using OMI (ozone monitoring instrument)

69 satellite data for the sunlit earth [10]. Moreover, recent research has demonstrated that simulated  
70 sunlight rapidly inactivates SARS-CoV-2 on surfaces including human saliva when exposed to  
71 simulated sunlight representative of the summer solstice at 40 °N latitude at sea level on a clear  
72 day [10]. Also, indirect effects of solar UVB exposure in reducing COVID-19 deaths have been  
73 substantiated, potentially mediated by UVB-driven cutaneous vitamin D synthesis, among other  
74 factors [12-14]. In addition, a role of solar UVA photons in the inactivation of coronaviruses has  
75 been proposed [7].

76 Given the complexity of virucidal activity as a function of spectral composition from ultraviolet  
77 to infrared, a topic recently reviewed by various authors, a more detailed knowledge and direct  
78 evidence of solar UV-induced coronavirus inactivation (achievable at ground level and  
79 environmentally relevant doses) would offer improved options that inform decisions at the basic  
80 research, clinical care, and public health levels [2,6,8]. Here, for the first time, we have explored  
81 the sensitivity of the human coronaviruses HCoV-NL63 and SARS-CoV-2 to solar simulated  
82 ultraviolet light (sUV). Our findings suggest that solar UV delivered at environmentally relevant  
83 dose levels inactivates HCoV-NL63 and SARS-CoV-2 coronaviruses with pronounced blockade  
84 of infectivity protecting mammalian host cells.

85

## 86 **2. Materials and Methods**

### 87 ***2.1. Chemicals***

88 All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA).

89

### 90 ***2.2. Mammalian cell culture, viral propagation, and target cell infection***

91 As established viral target cells infected by HCoV-NL63 and SARS-CoV-2, Calu-3 human  
92 metastatic lung epithelial adenocarcinoma (HTB-55), Caco-2 human colorectal epithelial

93 adenocarcinoma (HTB-37) and Vero normal epithelial monkey kidney (CCL-81) cells (all from  
94 ATCC, Manassas, VA, USA) were maintained according to published standard procedures [15-  
95 18]. In brief, all cells (Calu-3, Caco-2 and Vero) were cultured in Eagle's Minimum Essential  
96 Medium (MEM) medium (Corning, Manassas, VA) supplemented with 10% bovine calf serum  
97 (BCS, HyClone<sup>TM</sup> Laboratories, Logan, UT). Coronavirus HCoV-NL63 (NR-470) and its  
98 genomic RNA (NR-44105) were obtained from BEI Resources (NIAID, NIH). SARS-CoV-2  
99 strain WA1 (NR-52281; BEI Resources) was propagated in Vero cells unless specified otherwise  
100 [6]. For viral stocks, cells were infected at a multiplicity of infection (MOI) of 0.01 and cultured  
101 for 48 h. At that point, cells were harvested, homogenized, subjected to a single freeze-thaw cycle,  
102 and then combined with the culture supernatant followed by centrifugation (3000 rpm, 10 min).  
103 The viral titers of the final supernatant (after serial dilution) was determined by plaque forming  
104 assay. All work with SARS-CoV-2 was performed under BSL3 conditions in a facility with  
105 negative pressure and PPE that included Tyvek suits and N95 masks for respiratory protection.

106

### 107 **2.3. Viral irradiation with solar simulated UV light (sUV)**

108 A KW large area light source solar simulator, model 91293, from Oriel Corp. (Stratford, CT)  
109 was used, equipped with a 1000W xenon arc lamp power supply, model 68920, and a VIS-IR band  
110 pass blocking filter plus either an atmospheric attenuation filter (output 290–400 nm plus residual  
111 650–800 nm for solar simulated light) [19,20]. For viral irradiation, viral stocks were diluted  
112 >1:100 in PBS and irradiated in a sealed UV-transparent cuvette [BrandTech<sup>TM</sup> BRAND<sup>TM</sup> UV-  
113 Cuvets, providing transparency from 230 to 900 nm, widely used for DNA, RNA and protein  
114 analysis (BrandTech<sup>TM</sup> 759170, Fisher Scientific)]. The cuvette was inserted into a fully UV-  
115 transparent scintillation counter vial (Wheaton '180' low-potassium glass, SigmaAldrich

116 Z253081). The UV output was quantified using a dosimeter from International Light Inc.  
117 (Newburyport, MA), model IL1700, with an SED240 detector for UVB (range 265–310 nm, peak  
118 at 285 nm) or a SED033 detector for UVA (range 315–390 nm peak 365 nm) at a distance of 365  
119 mm from the source, which was used for all experiments. In order to avoid artifactual thermal  
120 effects of photon exposure on viral activity, cuvettes were placed on ice during irradiation. At 365  
121 mm from the source, total solar UV intensity was 5.34 mJ/cm<sup>2</sup> s (UVA) and 0.28 mJ/cm<sup>2</sup> s (UVB).

122

123 **2.4. HCoV-NL63 plaque forming assay and viral RNA quantification**

124 A published standard procedure was followed [15,21]. For HCoV-NL63, target cells (CaCo-2  
125 or Calu-3) were seeded in 6-well plates at approximately  $4 \times 10^5$  cells per well and incubated until  
126 the monolayer was 80–90% confluent. Prior to infection, cells were washed with phosphate  
127 buffered saline (PBS). Virus inoculum (MOI=0.01) in 500  $\mu$ L of growth media supplemented with  
128 2% horse serum (with standard penicillin/streptomycin and L-glutamine supplementation) was  
129 added to each well. Viral entry was performed by incubation at 4°C for 30-60 min with gentle  
130 agitation followed by 1 h incubation in 33°C, 5% CO<sub>2</sub>. Then, inoculum was removed and cells  
131 were washed twice with PBS and replaced by 2 mL of normal growing media. After infection,  
132 cells were washed twice with PBS and placed in the incubator and cultured in normal growth  
133 media. Once plaques appeared (~5-7 d post infection), cells were fixed with 10% neutral buffered  
134 formalin for 30 min at room temperature and stained with 1% crystal violet in 20% methanol for  
135 20 min. Then, cells were washed several times with water, and plaques were counted and  
136 representative pictures taken at 10x magnification using an inverted microscope (Nikon  
137 Instruments, Melville, NY). In addition, viral RNA was extracted from cells and the respective  
138 culture supernatant with the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). One step

139 RT-qPCR for HCoV-NL63 with absolute virus RNA quantification was performed using the  
140 following primer/probe set as published before [22]:

141 forward primer – 5'-ACGTACTTCTATTATGAAGCATGATATTAA-3'

142 reverse primer – 5'-AGCAGATCTAATGTTACTTAAACTACG-3'

143 probe – FAM-5'- ATTGCCAAGGCTCCTAACGTACAGGTGTT -3'-NFQ-MGB

144 Briefly, RT-qPCR was carried out in a 20  $\mu$ L reaction mixture with extracted RNA and One  
145 step RT-qPCR 2x Master Mix containing ROX as a passive reference dye (Gold Biotechnology,  
146 St. Louis, MO) and 300 nM forward and reverse primers and 200 nM MGB probe. Amplification  
147 and detection were performed in ABI 7500 system (Applied Biosystems, Foster city, CA) under  
148 the following conditions: first strand cDNA synthesis at 42°C for 30 min; initial denaturation/RT  
149 inactivation at 95°C for 3 min; denaturation at 95°C for 10 sec and annealing/extension at 55°C  
150 for 30 sec followed by 45 sec for data acquisition at 72°C. During amplification, the ABI PRISM  
151 7500 sequence detector monitored real-time PCR amplification by quantitative analysis of the  
152 fluorescence emissions. The reporter dye (FAM) signal was measured against the internal  
153 reference dye (ROX) signal to normalize the signals for non-PCR-related fluorescence fluctuations  
154 that occur from well to well. The cycle threshold (Ct) represented the refraction cycle number at  
155 which a positive amplification was measured and was set at ten times the standard deviation of the  
156 mean baseline emission calculated for PCR cycles 3 to 15. Genomic RNA from HCoV-NL63 was  
157 used as a positive control.

158

### 159 **2.5. SARS-CoV-2 plaque forming assay and viral RNA quantification**

160 The quantification of infectious SARS-CoV-2 has been published before [18]. Target cells  
161 (Vero or Calu-3) were infected in triplicates at an MOI of 0.005 (high titer) or 0.001 (low titer).

162 Briefly, cells were incubated with SARS-CoV-2 for 2 h and subsequently overlaid with 1%  
163 methylcellulose in culture medium. After 3-4 days, the cells were fixed in 10% neutral buffered  
164 formalin for 30 min, washed under tap water, and stained with 1% crystal violet. The number of  
165 plaques was counted on a light table. Alternatively, infection of cells was determined by measuring  
166 the amount of viral RNA. Cells were lysed in Trizol followed by RNA extraction with the  
167 RNAeasy kit (Qiagen). After reverse transcription, cDNA corresponding to the gene encoding the  
168 SARS-CoV-2 spike protein was quantified by qPCR with the Perfecta FastMix (QuantaBio) using:  
169 forward primer (SARS-CoV-2) 5'-GCTGGTGCTGCAGCTTATTA-3'  
170 reverse primer (SARS-CoV-2) 5'-AGGGTCAAGTGCACAGTCTA-3'  
171 at an annealing temperature of 60 °C. For normalization, *GAPDH* expression was measured using  
172 the following primers:  
173 forward primer (*GAPDH*) 5'-TGGTGAAGGTCGGTGTGAAC-3'  
174 reverse primer (*GAPDH*) 5'-CCATGTAGTTGAGGTCAATGAAGG-3' .  
175

176 **2.6. Human Stress & Toxicity PathwayFinder RT<sup>2</sup> Profiler<sup>TM</sup> gene expression array analysis of  
177 infected host cells**

178 Seven days post infection of Calu-3 host cells with either HCoV-NL63 (MOI=0.01) or HCoV-  
179 NL63 exposed to sUV (UVB portion: 706 mJ/cm<sup>2</sup>), total mRNA from host cells was isolated using  
180 the RNeasy Mini kit (Qiagen, Valencia, CA) following our published standard procedures. Reverse  
181 transcription was then performed using the RT<sup>2</sup> First Strand kit (Qiagen) from 500 ng total RNA.  
182 For gene expression array analysis, the human Stress & Toxicity PathwayFinder RT<sup>2</sup> Profiler<sup>TM</sup>  
183 technology (Qiagen), assessing expression of 84 stress response-related genes, was used as  
184 published before [23,24]. Quantitative PCR was run using the following conditions: 95 °C (10

185 min), followed by 40 cycles at 95 °C (15 s) alternating with 60 °C (1 min) (Applied Biosystems,  
186 Carlsbad, CA). Gene-specific products were normalized to a group of 5 housekeeping genes  
187 (*ACTB*, *B2M*, *GAPDH*, *HPRT1*, *RPLP0*) and quantified using the comparative  $\Delta\Delta Ct$  method (ABI  
188 PRISM 7500 sequence detection system user guide). Expression values were averaged across at  
189 least three independent array experiments, and standard deviation was calculated for graphing and  
190 statistical analysis as published before.

191

192 **2.7. Individual RT-qPCR analysis**

193 Total cellular mRNA was isolated using the Qiagen RNeasy Mini Kit (Qiagen, Gaithersburg,  
194 MD) according to the manufacturer's protocol as published by us before [24]. Human primer  
195 probes [*CCL3* (Hs\_00234142\_m1), *CSF2* (Hs\_00929873\_m1), *HSPA6* (Hs\_00275682\_s1), *IL1B*  
196 (Hs\_00174097\_m1), *IL6* (Hs\_00985639\_m1), *SOD2* (Hs\_00167309\_m1), *TNF*  
197 (Hs\_00174128\_s1), and *RSP18* (housekeeping gene; Hs\_01375212\_g1)], were obtained  
198 from ThermoFisher Scientific (Waltham, MA). After cDNA synthesis, quantitative PCR reactions  
199 were performed as follows: 10 min (95 °C) followed by 15 sec (95 °C), 1 min (60 °C), 40 cycles,  
200 using the ABI7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Amplification  
201 plots were generated, and Ct values were recorded as published before [24].

202

203 **2.8. Statistical analysis**

204 Unless stated differently, data sets were analyzed employing analysis of variance (ANOVA)  
205 with Tukey's posthoc test using the GraphPad Prism 9.1.0 software (Prism Software Corp., Irvine,  
206 CA); in respective bar graphs (analyzing more than two groups), means without a common letter  
207 differ ( $p < 0.05$ ) as published before [24]. For bar graphs comparing two groups only, statistical

208 significance was calculated employing the Student's two-tailed t-test, utilizing Excel (Microsoft,  
209 Redmond, WA). Experiments were performed in sets of at least three independent repeats. The  
210 level of statistical significance was marked as follows: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001.

211

212 **3. Results**

213 ***3.1. Solar simulated UV exposure of HCoV-NL63 blocks subsequent viral infection and***  
214 ***replication in Calu-3 human epithelial lung cells***

215 First, we examined the feasibility of UV-inactivation of a pathologically relevant coronavirus  
216 by employing a single dose of solar simulated UV light using a commercial xenon light source  
217 with quantified spectral power distribution (Fig. 1A). To this end, we exposed human coronoavirus  
218 NL63 (HCoV-NL63) in PBS to a high dose of sUV [equivalent to approximately 6 minimal  
219 erythema doses (MEDs; UVA: 13.46 J/cm<sup>2</sup>; UVB: 706 mJ/cm<sup>2</sup>)] and subsequently used it to infect  
220 Calu-3 target cells for 7 days [2, 6, 8]. We used unexposed virus as controls. Strikingly, sUV pre-  
221 exposure strongly suppressed viral infectivity of target cells as demonstrated by quantitative  
222 plaque assay analysis, indicating that sUV exposure caused a more than 8-fold decrease in viral  
223 infectivity (Fig. 1B).

224 Next, we examined the dose-response relationship characterizing the inhibition of HCoV-NL63  
225 viral replication (induced by sUV pre-exposure) by one step RT-qPCR analysis of the genomic  
226 RNA copy number. We detected a significant inhibition at low sUV doses [UVA: 0.25 J/cm<sup>2</sup>; 13  
227 mJ/cm<sup>2</sup> UVB]. Viral inactivation of more than 98 % occurred at doses equal and above 480 mJ/cm<sup>2</sup>  
228 UVB (UVA: 9.04 J/cm<sup>2</sup>; Fig. 1C).

229

230 >Figure 1<

231 **3.2. Solar simulated UV exposure of HCoV-NL63 blocks subsequent infection of Caco-2 human**  
232 **epithelial colorectal cells**

233 In order to explore sUV effects on HCoV-NL63 infectivity in another human target cell, we  
234 exposed the virus (in PBS) to a high dose of sUV [equivalent to approximately 6 MEDs (UVA:  
235 13.46 J/cm<sup>2</sup>; UVB: 706 mJ/cm<sup>2</sup>)] and subsequently infected Caco-2 epithelial colon cells (Fig. 2).  
236 As observed before with Calu-3 cells (Fig. 1), our quantitative plaque assay analysis showed that  
237 the suppression of viral infectivity of Caco-2 target cells by sUV exposure caused a more than 4-  
238 fold decrease in plaque formation (Fig. 2A). Likewise, our dose response analysis by RT-qPCR of  
239 genomic RNA copy numbers indicated that sUV exposure caused a pronounced suppression of  
240 HCoV-NL63 viral replication at doses as low as 240 mJ/cm<sup>2</sup> UVB (UVA: 4.52 J/cm<sup>2</sup>; Fig. 2B).

241

242 >Figure 2<

243

244 **3.3. Stress response gene expression array analysis confirms solar UV-induced inhibition of**  
245 **HCoV-NL63 infectivity targeting Calu-3 human epithelial lung cells**

246 Next, the cellular stress response of Calu-3 human epithelial lung cells, elicited by infection  
247 with either mock-irradiated or sUV pre-exposed HCoV-NL63, was examined at the gene  
248 expression level using the RT<sup>2</sup> Human Stress and Toxicity PathwayFinder<sup>TM</sup> PCR Array  
249 technology. To this end, we infected Calu-3 target cells with sUV or mock-treated virus (doses as  
250 in Figs. 1, 2) and profiled the gene expression at the end of the experiment. We observed global  
251 HCoV-NL63-induced expression changes (antagonized by viral pre-exposure to sUV) as depicted  
252 by Volcano plot (Fig. 3). As expected, HCoV-NL63 viral infection caused a pronounced  
253 upregulation of stress response gene expression including genes encoding key regulators of

254 inflammatory signaling (such as *CSF2*, *TNF*, *IL1B*, *IL1A*, *CCL3*, *CXCL10*, *NFKBIA*, and *IL6*),  
255 oxidative stress defense (such as *SOD2*), and heat shock response (such as *HSPA6*; Fig. 3). In  
256 contrast, after viral sUV-exposure performed pre-infection, most of these infection-associated  
257 expression changes were either attenuated or completely obliterated, an observation consistent  
258 with pronounced suppression of HCoV-NL63 viral infectivity as a consequence of sUV-exposure.  
259 Likewise, HCoV-NL63 viral infection-induced expression changes causing downregulation of  
260 specific apoptotic modulators including *BCL2L1*, *EGR1*, *CASP8*, and *CASP1*, proliferation  
261 markers such as *PCNA*, and heat shock response factors such as *HSPA4*, *HSPH1*, and *HSP90AA2P*  
262 were completely absent in samples obtained from cells exposed to the pre-irradiated virus.  
263 Strikingly, expression of seven specific genes (*CDKN1A*, *CYP1A1*, *MDM2*, *HMOX1*, *RAD50*,  
264 *HSPA1L*, and *E2F1*) was modulated uniquely in response to exposure to sUV-preirradiated HCoV-  
265 NL63, a finding consistent with gene expression changes responsive to sUV-induced chemical  
266 damage to viral components (including ribonucleic acids, proteins, and lipids) [1-3].

267

268 >Figure 3<

269

270 **3.4. Dose-response relationship of solar simulated UV-induced inhibition of SARS-CoV-2**  
271 **infectivity targeting Vero and Calu-3 mammalian cells**

272 After demonstrating HCoV-NL63 coronavirus inactivation by sUV at an environmentally  
273 relevant dose level, we examined whether sUV-inactivation might also be applicable to SARS-  
274 CoV-2. To this end, we exposed the virus with a dose range of sUV, subsequently infected Vero  
275 monkey epithelial cells at two different multiplicities of infection (MOIs, high versus low titer),  
276 and measured the number of infectious virions three days later by plaque forming assay.

277 Strikingly, as observed with HCoV-NL63, sUV exposure caused a pronounced suppression of viral  
278 infectivity. This antiviral effect, observable over a broad range of sUV doses, followed an  
279 exponential decay curve with an effective ED<sub>50</sub> (sUV dose diminishing SARS-CoV-2 viral  
280 infectivity by 50%) approximating 55 mJ/cm<sup>2</sup> (low titer) and 62 mJ/cm<sup>2</sup> (high titer) (Fig. 4A).

281 Next, we tested feasibility of achieving complete inhibition of SARS-CoV-2 replication by high  
282 dose sUV [UVB portion: 1010 mJ/cm<sup>2</sup>, a maximum dose level similar to the one used in the HCoV-  
283 NL63-directed dose-response experiments (Fig. 1C)]. To this end, we pre-exposed SARS-CoV-2  
284 to sUV and measured the amount of viral RNA (corresponding to the region of the viral genome  
285 encoding the S protein) by RT-qPCR analysis. Indeed, complete inhibition was achieved at that  
286 dose (Fig. 4B). We obtained similar results for sUV-exposed SARS-CoV-2 infections of Calu-3  
287 human lung epithelial target cells with viral load in supernatants being monitored over three days  
288 by RT-qPCR (Fig. 4C). Taken together, we conclude that SARS-CoV-2 is sensitive to sUV  
289 suggesting viral inactivation at environmentally relevant exposure levels.

290

291 >Figure 4<

292

293 ***3.5. Solar simulated UV exposure of SARS-CoV-2 prevents stress response gene expression  
294 elicited by viral infection of Calu-3 human epithelial lung cells as detected by array analysis***

295 Next, to determine Calu-3 human epithelial lung cell stress response gene expression elicited  
296 by SARS-CoV-2 as a function of viral pre-exposure to sUV, we employed expression analysis  
297 using the Human Stress and Toxicity PathwayFinder™ PCR Array technology. To this end, we  
298 infected Calu-3 target cells with sUV or mock-treated virus as outlined before, followed by  
299 comparative gene expression profiling at the end of the experiment. We observed multiple SARS-

300 CoV-2-induced expression changes (antagonized by viral pre-exposure to sUV) as shown in the  
301 Volcano plot depiction [displaying statistical significance (P value) versus magnitude of change  
302 (fold change)] (Fig. 5). SARS-CoV-2 infection caused a pronounced upregulation of stress  
303 response gene expression including genes encoding key regulators of inflammatory signaling  
304 including *IL1A*, *IL1B*, *IL6*, *TNF*, *CCL3*, *CXCL10*, *CSF2*, and *NFKBIA*, oxidative stress defense  
305 such as *SOD2*, and heat shock response such as *HSPA6* (Fig. 5). In contrast, after infection with  
306 sUV-exposed virus, most of these infection-associated expression changes were either attenuated  
307 or completely obliterated, an observation consistent with pronounced suppression of SARS-CoV-  
308 2 infectivity as a consequence of sUV-exposure. Remarkably, these expression changes closely  
309 mirrored those observed in response to HCoV-NL63 infection that occurred with or without viral  
310 exposure to sUV (Fig. 3).

311

312 >Figure 5<

313

314 Likewise, we observed a striking similarity between the gene expression changes elicited by  
315 HCoV-NL63 and SARS-CoV-2 (and blocked by viral sUV pre-exposure), modulating redox,  
316 inflammatory, and proteotoxic stress responses in Calu-3 human epithelial lung cells (Fig. 6).  
317 Specifically, sUV-induced (UVB portion: 706 mJ/cm<sup>2</sup>) viral inactivation was apparent from  
318 independent RT-qPCR assessment of mRNA levels ('no sUV' versus 'sUV') interrogating genes  
319 encoding key regulators of redox (*SOD2*), inflammatory (*IL1B*, *TNF*, *CCL3*, *IL6*, *CSF2*), and  
320 proteotoxic ('heat shock'; *HSPA6*) stress responses in Calu-3 target cells as detailed above. Thus,  
321 our data suggest that similar to HCoV-NL63, sUV exposure of SARS-CoV-2 interrupts the viral

322 life cycle causing suppression of viral replication and virus-induced inflammatory and cellular  
323 stress responses in mammalian target cells.

324

325 >Figure 6<

326

327 **4. Discussion**

328 Identification and mechanistic exploration of environmental factors that might determine  
329 coronavirus infectivity are of significant interest with relevance to both basic molecular research  
330 and public health-related preventive and interventional investigations [2]. Here, we have explored  
331 for the first time the effects of full spectrum (UVA + UVB) solar ultraviolet radiation on  
332 coronavirus infectivity and demonstrate that sUV inactivates HCoV-NL63 and SARS-CoV-2  
333 coronaviruses at environmentally relevant doses. First, we observed that exposure of HCoV-NL63  
334 and SARS-CoV-2 to sUV (performed at acute dose levels relevant to human populations  
335 worldwide) blocks subsequent viral infection and replication in relevant primate target cells  
336 [human: Calu-3 lung epithelial, Caco-2 colorectal epithelial; monkey: Vero kidney epithelial (Figs.  
337 1, 2, 4)]. Blockade of viral infectivity in response to sUV pre-exposure was also confirmed using  
338 stress response gene expression profiling in array (Figs. 3, 5) and independent RT-qPCR format  
339 (Fig. 6) elicited in Calu-3 target cells by coronavirus infection (HCoV-NL63 and SARS-CoV-2).

340 Remarkably, dose levels used throughout this pilot study are representative of terrestrial ground  
341 level exposure suggesting environmental relevance, and significant coronavirus inactivation was  
342 detectable even at low exposure levels expected to be beneath the cutaneous sunburn-inducing  
343 threshold (Figs. 1, 2, 4) [2,6,8]. In this context, it is remarkable that recent research has already  
344 indicated that ground level solar UV displays significant virucidal effects targeting coronaviruses

345 including SARS-CoV-2 [2,6,11,13]. However, the complexity of human exposure levels to solar  
346 UV as a function of solar zenith angle, seasonality, spectral distribution, and latitude remain to be  
347 addressed before any firm conclusions relevant to human populations can be drawn. Specifically,  
348 the anti-viral activity of specific spectral components of sUV remains to be determined since the  
349 light source employed in our prototype studies emitted full spectrum simulated solar UV, and the  
350 action spectrum of virus inactivation by solar UV remains largely undefined. For example, it is  
351 possible that the UVA portion of ground level sUV significantly contributes to the coronavirus-  
352 directed effects described by us [7]. It therefore remains to be seen if indirect impairment of viral  
353 structure and infectivity occurs by alternative mechanisms, such as UVA-driven photosensitization  
354 and oxidative stress (mediated by formation of reactive oxygen species including singlet oxygen),  
355 that might be operative in addition to direct inactivation of viral genomic RNA through nucleic  
356 acid base photodamage. It will also be interesting to explore potential mechanistic synergisms  
357 underlying virucidal effects that occur upon combined UVA and UVB as compared to separate  
358 spectral exposure. Likewise, experimental conditions used throughout our studies (including viral  
359 irradiation in PBS and exposure performed in cell culture medium) might limit the applicability of  
360 our conclusions in the context of relevant coronavirus transmission situations that involve more  
361 complex determinants of infectivity including the role air-borne and aerosol transmission and  
362 intermediate surface retention [6].

363 Addressing urgency and global scale of the unfolding SARS-CoV-2 pandemic requires an  
364 improved understanding of environmental factors that modify viral infectivity [2,6,8]. Taken  
365 together, our data suggest feasibility of sUV-induced viral inactivation targeting HCoV-NL63 and  
366 SARS-CoV-2 coronaviruses, a finding to be substantiated by future mechanistic exploration  
367 performed in more relevant *in vivo* exposure models.

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372

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375

376 **Declaration of competing interest**

377 The authors state no conflict of interest.

378

379 **References**

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495 **Figure legends**

496 **Figure 1. Solar simulated UV pre-exposure antagonizes HCoV-NL63 viral infectivity**  
497 **targeting Calu-3 human epithelial lung cells.** Virus in PBS was exposed to sUV or left  
498 unexposed followed by Calu-3 target cell infection (0.01 MOI) and post infection culture over 7  
499 days followed by analysis. (A) Spectral power distribution (irradiance) of the solar simulator light  
500 source equipped with appropriate cut-off filter (sUV: UVB + UVA, solid black line). (B) Plaque  
501 assay after viral exposure to sUV (UVB portion: 706 mJ/cm<sup>2</sup>) as visualized by light microscopy  
502 (10 x magnification); bar graph summarizes numerical data. (C) RT-qPCR of viral genome  
503 replication in target cells [left panel: amplification curves as a function of sUV dose (UVB portion  
504 as indicated); right panel: bar graph summarizing numerical data].

505

506 **Figure 2. Solar simulated UV pre-exposure antagonizes HCoV-NL63 viral infectivity**  
507 **targeting Caco-2 human epithelial colorectal cells.** Virus in PBS was exposed to sUV or left  
508 unexposed followed by Caco-2 target cell infection (0.01 MOI) and post infection culture (7 days)  
509 followed by analysis. (A) Plaque assay after viral exposure to sUV (UVB portion: 706 mJ/cm<sup>2</sup>)  
510 as visualized by light microscopy (10 x magnification); bar graph summarizes numerical data. (B)  
511 RT-qPCR detection of viral genome replication in target cells; left panel: amplification curves (as  
512 a function of sUV-dose); right panel: bar graph summarized numerical data.

513

514 **Figure 3. Solar simulated UV pre-exposure of HCoV-NL63 prevents stress response gene**  
515 **expression elicited in Calu-3 human epithelial lung target cells.** Treatments were performed as  
516 detailed in Fig. 1. (A) Target cell stress response [control (HCoV-NL63) versus sUV (UVB  
517 portion: 706 mJ/cm<sup>2</sup>) pre-exposed virus] assessed by *RT<sup>2</sup> Profiler<sup>TM</sup> Stress and Toxicity Pathway*

518 gene expression array analysis [volcano plot depiction: p value over log2 (fold expression  
519 change)]. (B) Scatter plot depiction comparing expression changes elicited by untreated control  
520 virus (top panel) or sUV pre-exposed virus (bottom panel). (C) Venn diagram depicting expression  
521 changes induced by mock-irradiated virus (control) versus sUV pre-irradiated virus. (D) Tabular  
522 summary of numerical values specifying gene expression changes at the mRNA level (p<0.05).

523

524 **Figure 4. Solar simulated UV exposure of SARS-CoV-2 antagonizes subsequent viral**  
525 **infection and replication in African green monkey Vero and Calu-3 human epithelial lung**  
526 **cells.** (A) SARS-CoV-2 was sUV-irradiated (UVB portion: up to 480 mJ/cm<sup>2</sup>; or remained  
527 unirradiated) in PBS and subsequently used to infect Vero cells at two different MOIs (high versus  
528 low titer). Dose response of plaque formation as a function of sUV pre-exposure dose was  
529 assessed; a representative experiment (left panel, top and bottom rows) and quantification (right  
530 panels) are depicted. (B) Detection of viral genome replication in Vero cells with quantification  
531 of viral RNA after infection using mock or sUV pre-irradiated virus (UVB portion: 1010 mJ/cm<sup>2</sup>)  
532 as assessed by RT-qPCR after 24 h. (C) Infection of Calu-3 cells with SARS-CoV-2 [sUV pre-  
533 exposed (UVB portion: 706 mJ/cm<sup>2</sup>) versus unirradiated virus]. The presence of infectious virions  
534 in the supernatants was quantified over the course of three days post infection by RT-qPCR (nd:  
535 not detectable).

536

537 **Figure 5. Solar simulated UV pre-exposure of SARS-CoV-2 prevents stress response gene**  
538 **expression elicited in Calu-3 human epithelial lung target cells.** Treatment and analysis were  
539 performed as detailed in Fig. 3. (A) Target cell stress response [control (SARS-CoV-2) versus  
540 sUV (UVB portion: 706 mJ/cm<sup>2</sup>)-preirradiated virus] assessed by *RT<sup>2</sup> Profiler<sup>TM</sup> Stress and*

541 *Toxicity Pathway* gene expression array analysis [volcano plot depiction: p value over log2 (fold  
542 expression change)]. (B) Scatter plot depiction comparing expression changes elicited by untreated  
543 control virus (top panel) or sUV pre-exposed virus (bottom panel). (C) Venn diagram depicting  
544 expression changes induced by mock-irradiated virus (control) versus sUV pre-irradiated virus.  
545 (D) Tabular summary of numerical values of gene expression changes at the mRNA level (p<0.05).

546

547 **Figure 6. Comparative analysis of redox, inflammatory, and proteotoxic stress response gene**  
548 **expression in Calu-3 human epithelial lung cells elicited by HCoV-NL63 and SARS-CoV-2**  
549 **(with and without viral sUV pre-exposure).** Gene expression as assessed by single RT-qPCR  
550 quantification in virus-exposed target cells as a function of viral pre-exposure ['no sUV' versus  
551 'sUV' (UVB portion: 706 mJ/cm<sup>2</sup>)]. Bar graphs depict fold change ('sUV' versus 'no sUV')  
552 normalized to housekeeping gene expression (*RPS18*; gray bar: no sUV pretreatment; black bar:  
553 sUV-pretreatment).

554

Fig. 1

Calu-3

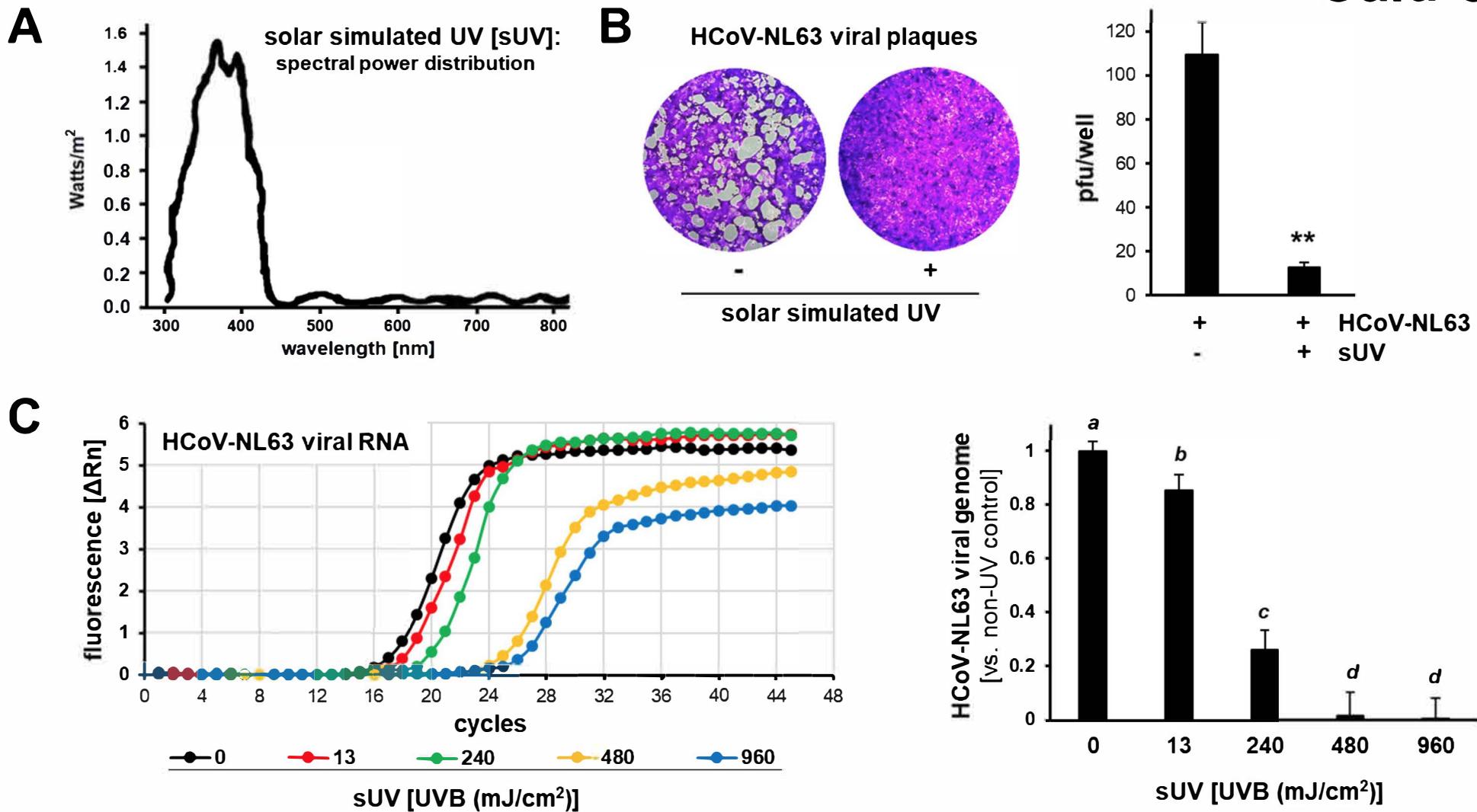
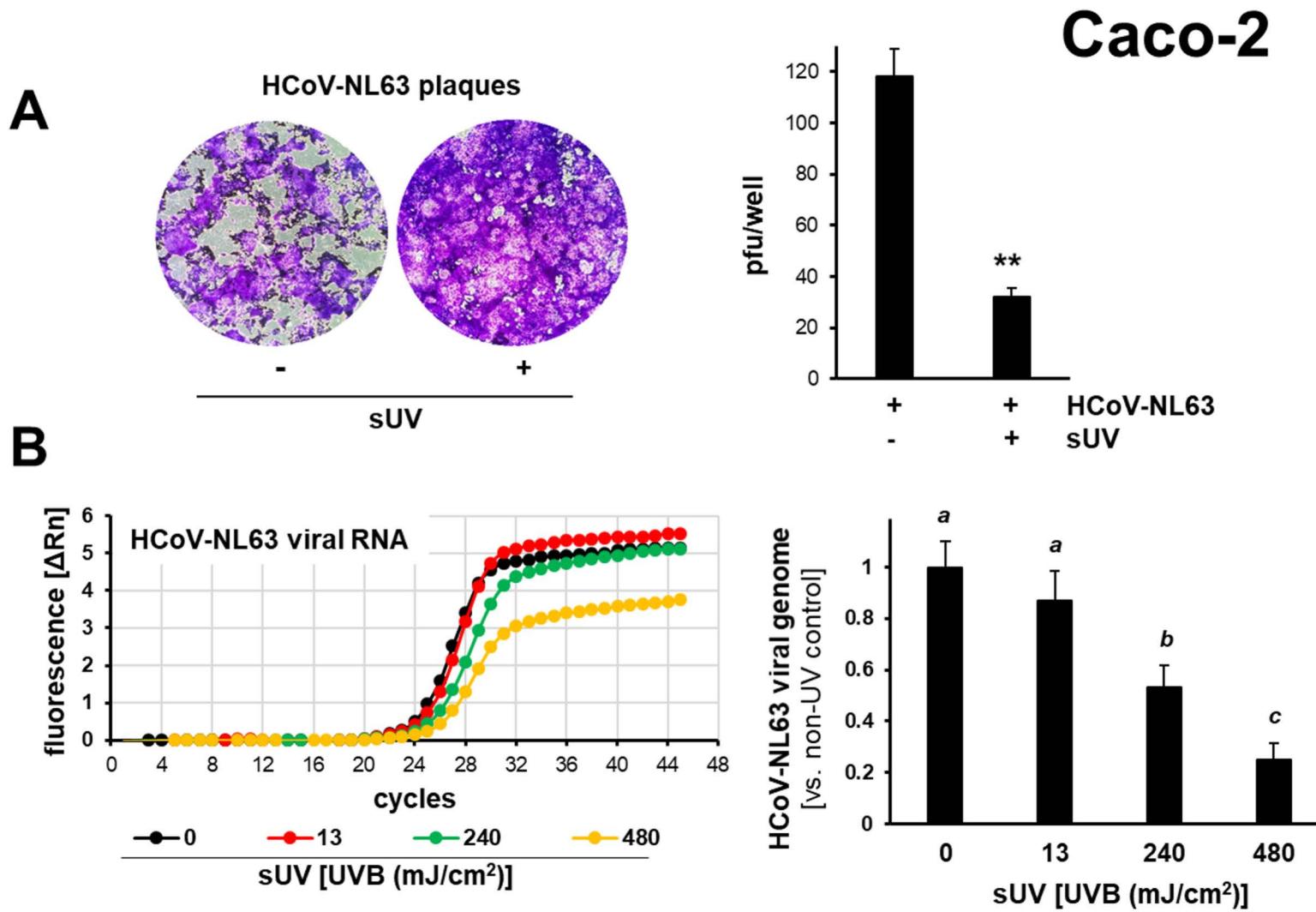
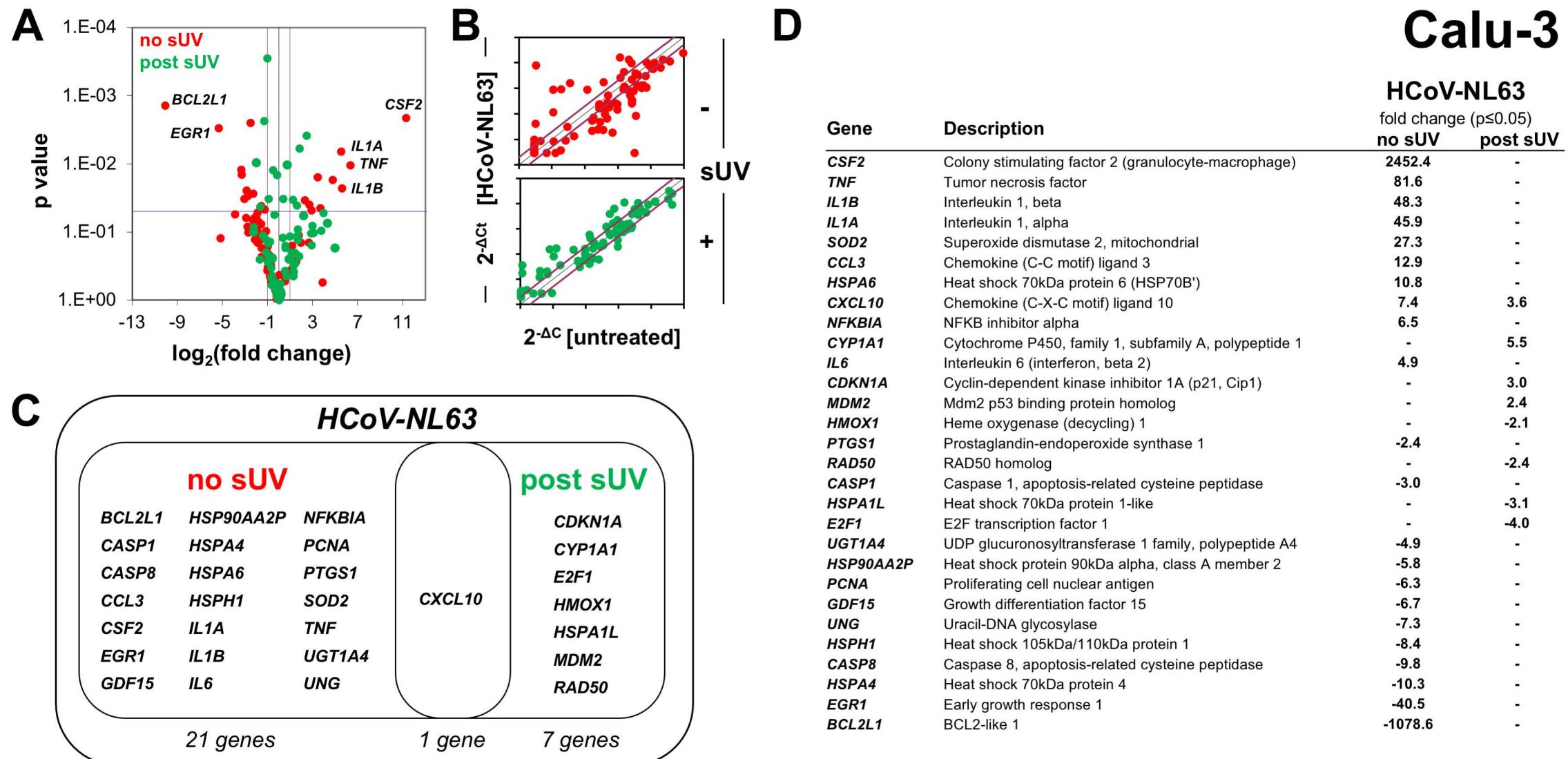


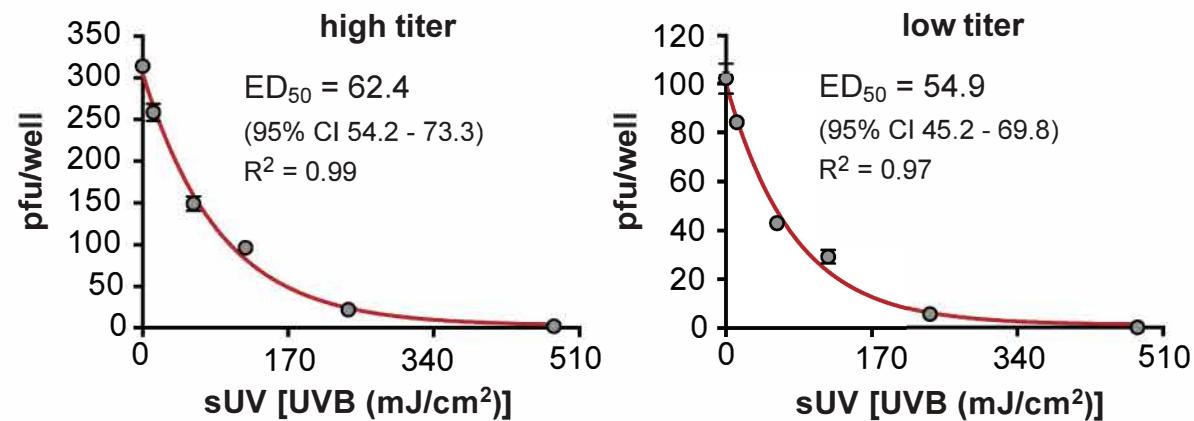
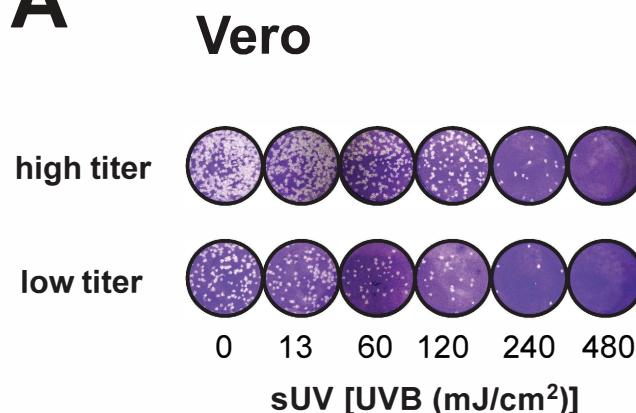
Fig. 2



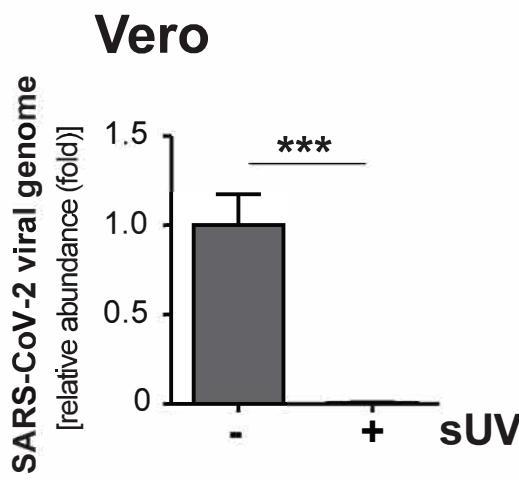
**Fig. 3**

**Fig. 4**

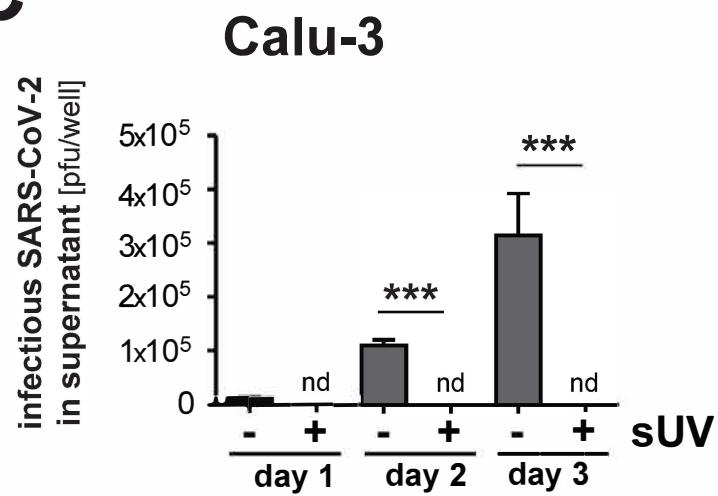
**A**



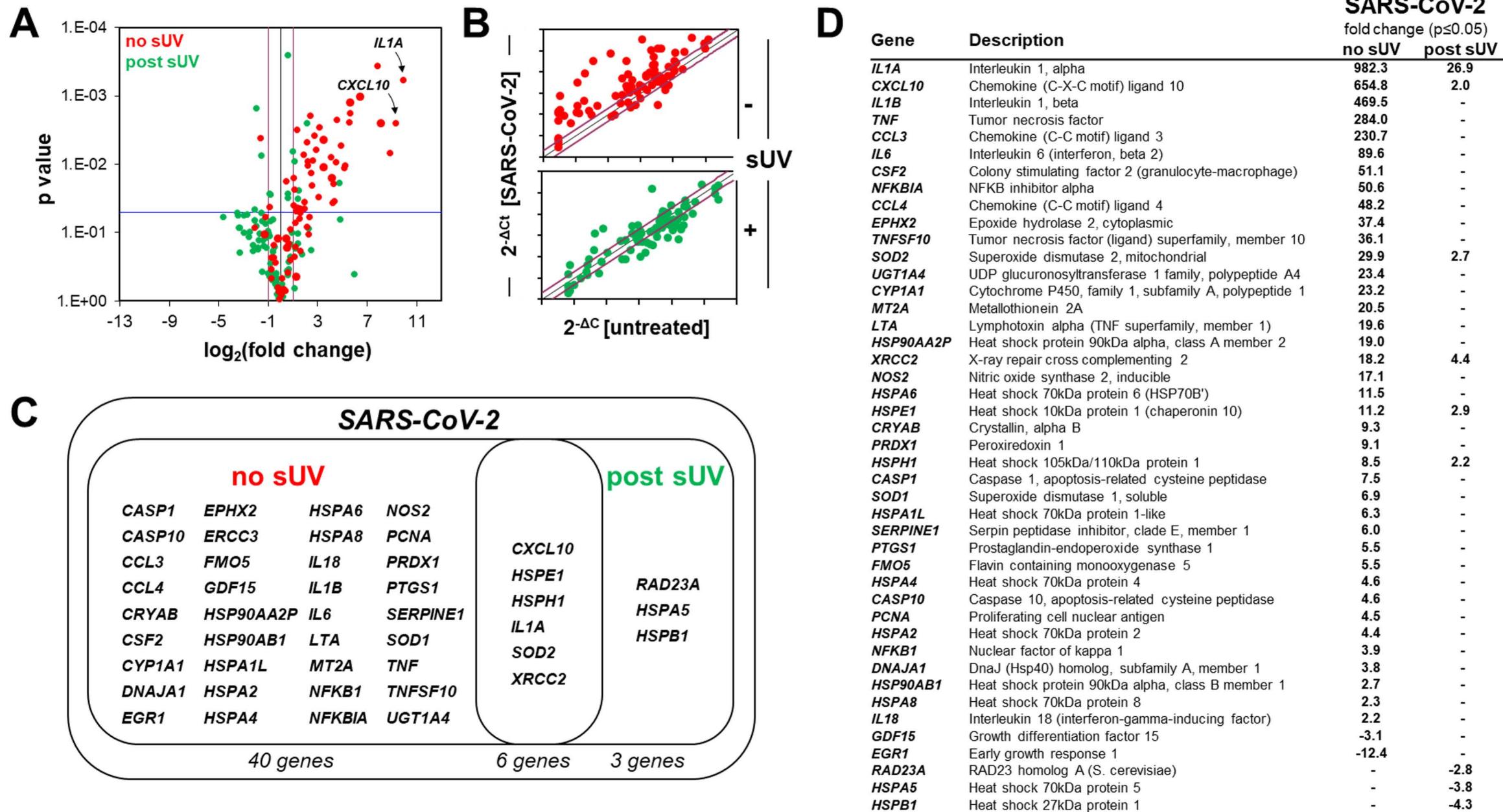
**B**



**C**



**Fig. 5**



**Fig. 6**