Supplementary Materials for

SARS-CoV-2-induced disruption of a vascular bed in a microphysiological system caused by type-I interferon from bronchial organoids

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Supplementary Discussion

Estimation of virus particle distribution.

Here, we assessed the effect of gravity and diffusion driven migration of virus particles. Firstly, the terminal settling velocity (v) of a virus particle is calculated using Stokes's law, considering low Reynolds number,

$$v = \frac{2\rho_p - \rho_w}{9 \quad \mu} gR^2, \qquad (1)$$

where ρ_p, ρ_w, μ, g, and R are density of particle, density of water, viscosity of water, gravitational acceleration, and virus particle radius, respectively. The particle radius were set as 50 nm based on a previous report.[1] Other parameters, as outlined in Table S1 yield a terminal velocity of 2.7 nm/s.

Diffusion constant D is described by Stokes-Einstein equation,

$$D = \frac{k_B T}{6\pi\mu R}, \qquad (2)$$

where k_B and T are Boltzmann constant and temperature, respectively. The value of D was 4.4×10^{-12} m^2/s. Regarding these values, Peclet number Pe, which represents the ratio of convection flow against diffusion is calculated

$$Pe = \frac{Lv}{D}, \qquad (3)$$

Where L is the representative length of the system. Here, we applied L = 500 μm which matches to the sum of the VB-culture channel height and the height of hole connecting the VB-culturechannel and the BO-culture well. Calculation gave us Pe = 0.31, indicating that diffusion is governing virus particle distribution.

Static distribution of virus particle under gravity is derived previously.[2] Using convection parameter c and diffusion constant D it is written as,

$$w(z) = \frac{c}{D} e^{-cz/D}. \qquad (4)$$

c/D is also expressed as

$$\frac{c}{D} = (1 - \frac{\rho_p}{\rho_w}))(\frac{mg}{k_B T}), \qquad (4)$$

where m represents a mass of single particle. When we set z = 500 μm, representing the distance from the bottom of the VB-culture channel to the bottom of the BO-culture well, the density is calculated as 94% of the value with z = 0 μm. This suggests that the effect of gravity on the distribution of the virus particle is limited.

1. Ke, Z. *et al.* Structures and distributions of SARS-CoV-2 spike proteins on intact virions. *Nature* **588**, 498–502 (2020).
2. Chandrasekhar, S. Stochastic Problems in Physics and Astronomy. *Rev. Mod. Phys.* **15**, 1–89 (1943).

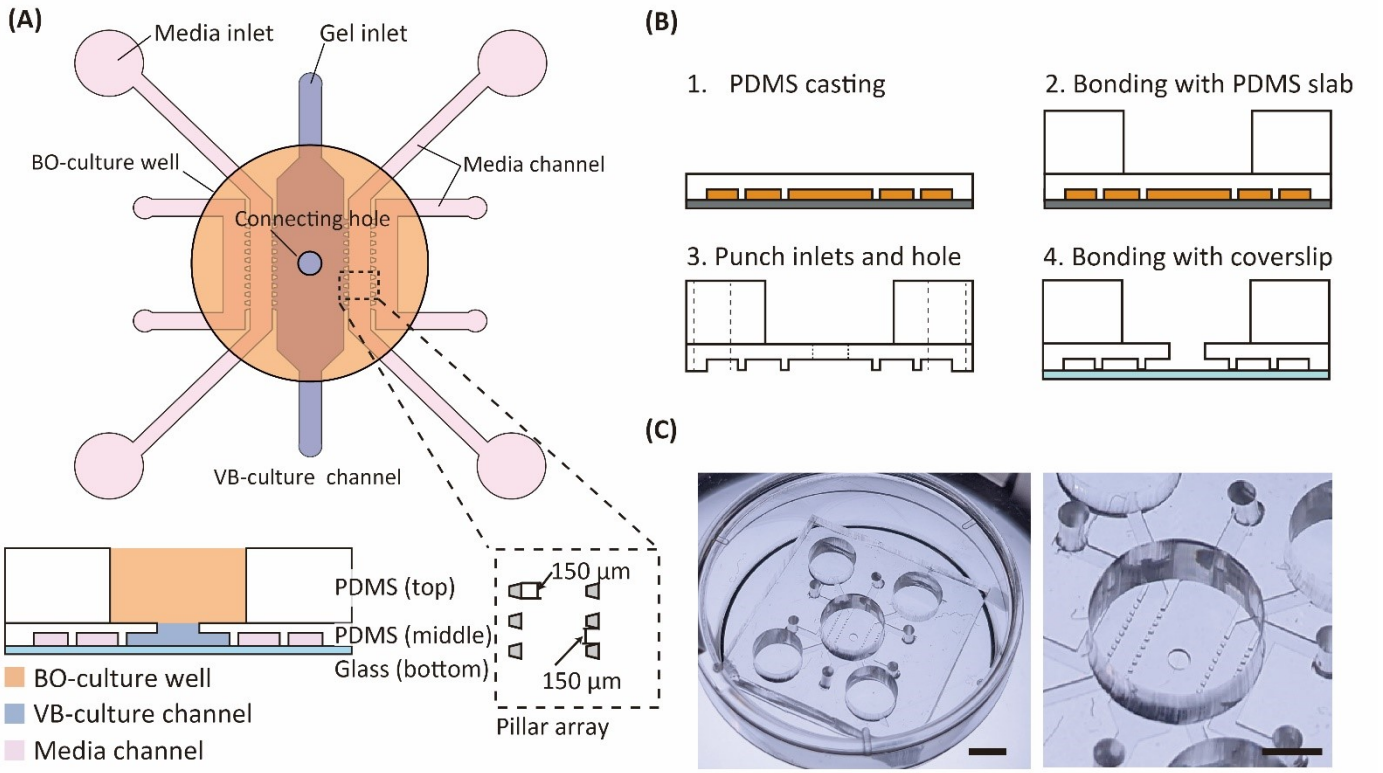

Fig. S1. Device design and fabrication. (A) Design and dimensions of the microfluidic device. The VB-culture channel (light blue) is separated from the media channels (pink) by an array of pillars. A connecting hole opens to the VB-culture channel and the BO-culture well (orange) at the center of the channel. (B) Fabrication process. (1) PDMS prepolymer was cast on the mold as the middle PDMS layer. (2) The top PDMS layer was bonded to the cured middle layer. (3) Gel inlets, media reservoirs, and connecting holes were punched after removing the top and middle layers from the mold. (4) Two layers were bonded with a coverslip to form closed channels. (C) The microfluidic device in a 35-mm dish (left) and magnified view (right). Scale bar: 1 mm.

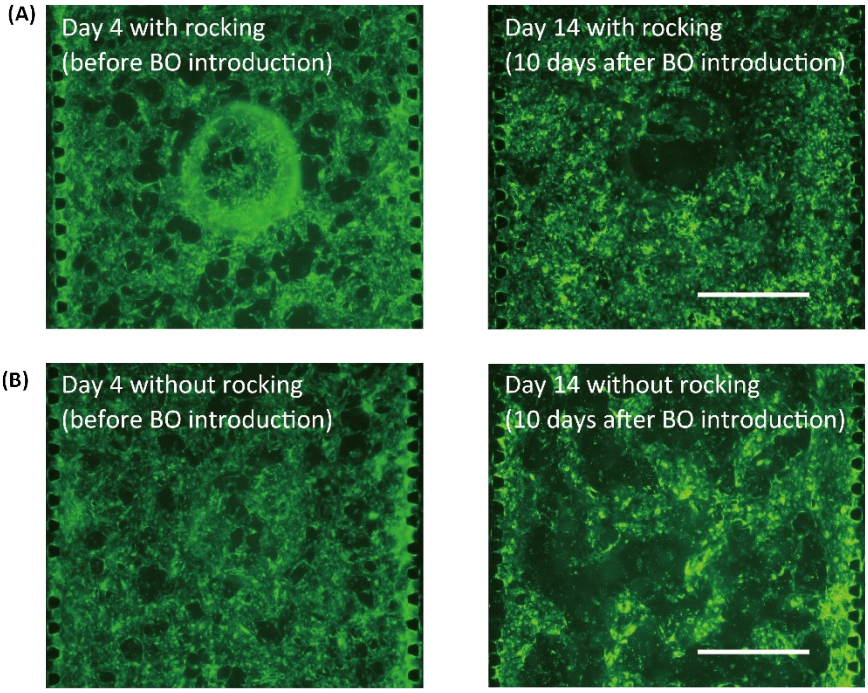

Fig. S2. VB before and after co-culture with the BOs. VB image before the BOs were introduced (day 4) and after 10 days of co-culturing with the BOs (day 14), GFP: Green. With rocking 1 cycle / min (A) or without rocking (B). Scale bar: 1 mm.

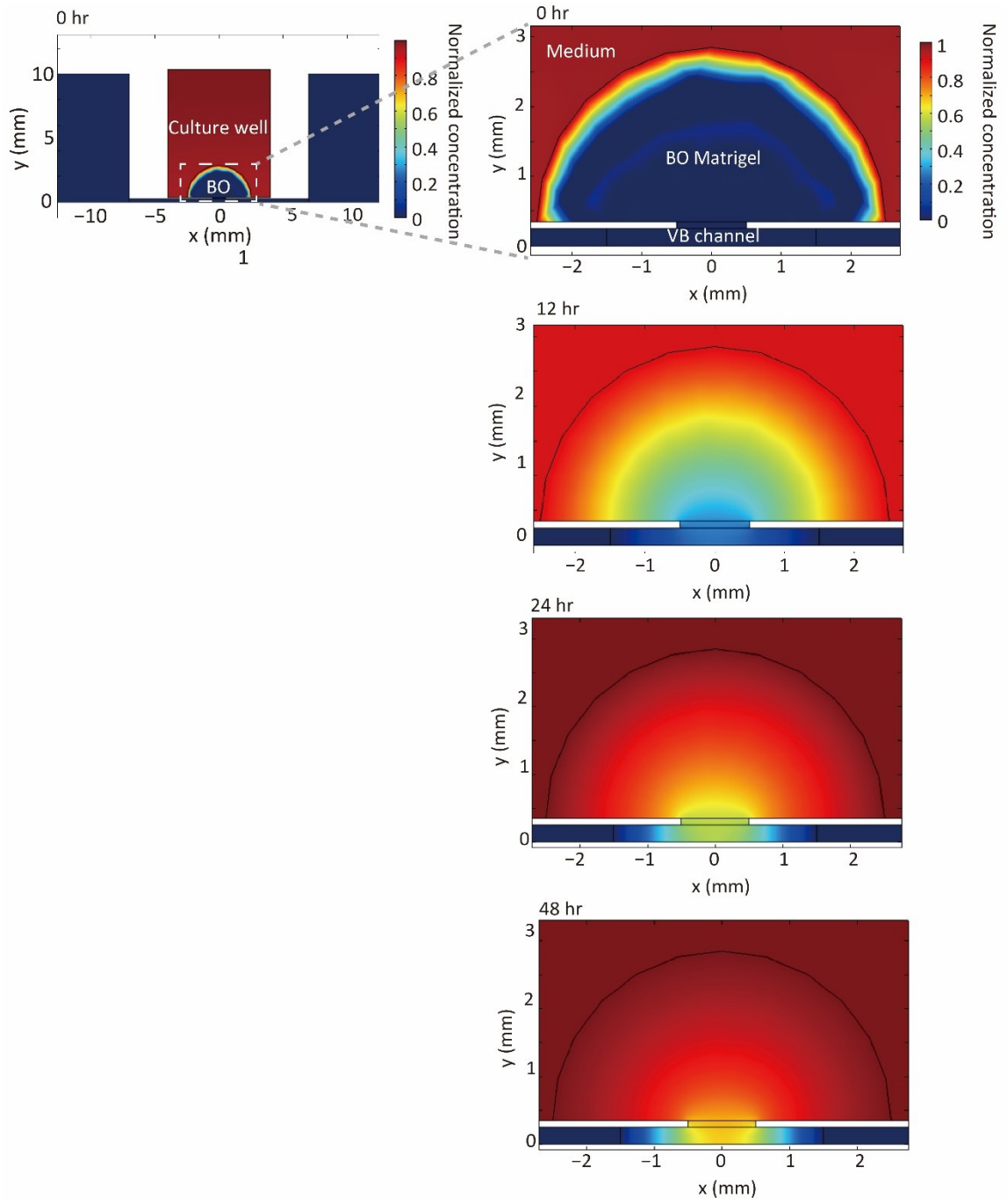

Fig. S3. Numerical simulation of virus particle diffusion in the ECM. Numerical simulation results of virus particle diffusion in the ECM. The diffusion of virus particles into Matrigel containing BOs and ECM gel in the VB-culture channel was numerically simulated and visualized for 48 hours.

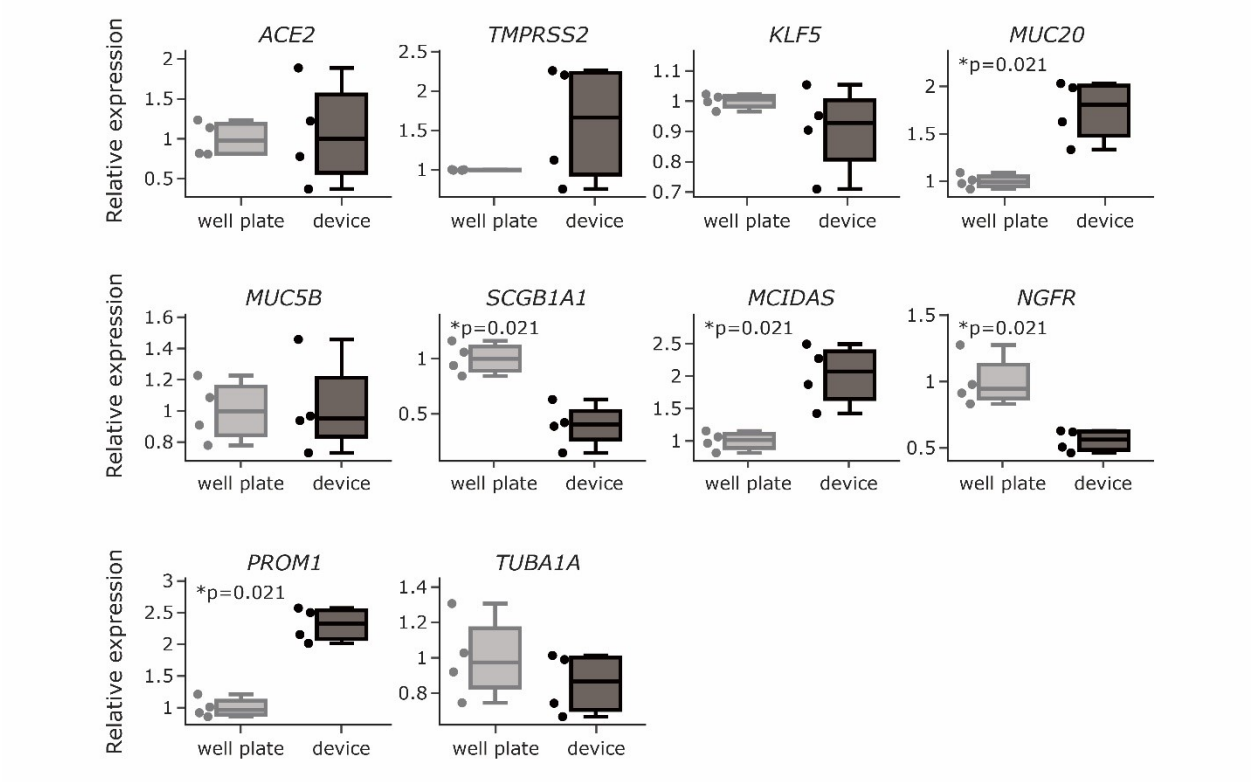

Fig. S4. Generation of BOs in a device and well plate. Expression of virus infection-related genes and bronchial markers was measured in BOs in well plates and devices. p-values were calculated using Wilcoxon's rank sum test. N = 5 replications.

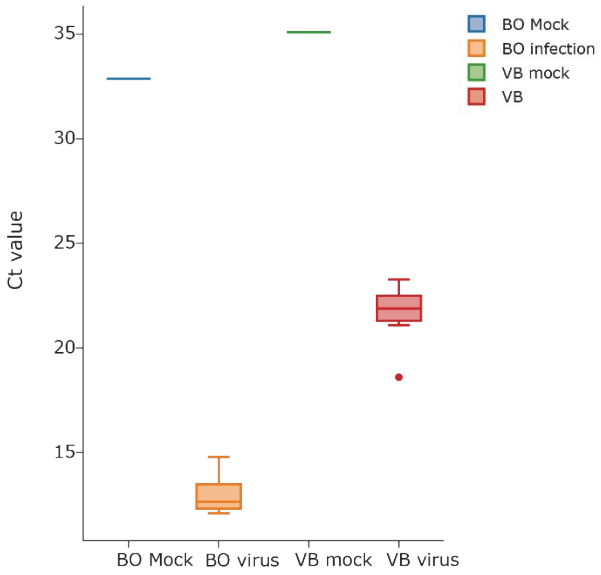

Fig. S5. Ct values for *SARS-CoV-2 N*. Distribution of Ct values obtained from qPCR test implemented with VB and BO samples without the virus (mock) and with the virus (infected).

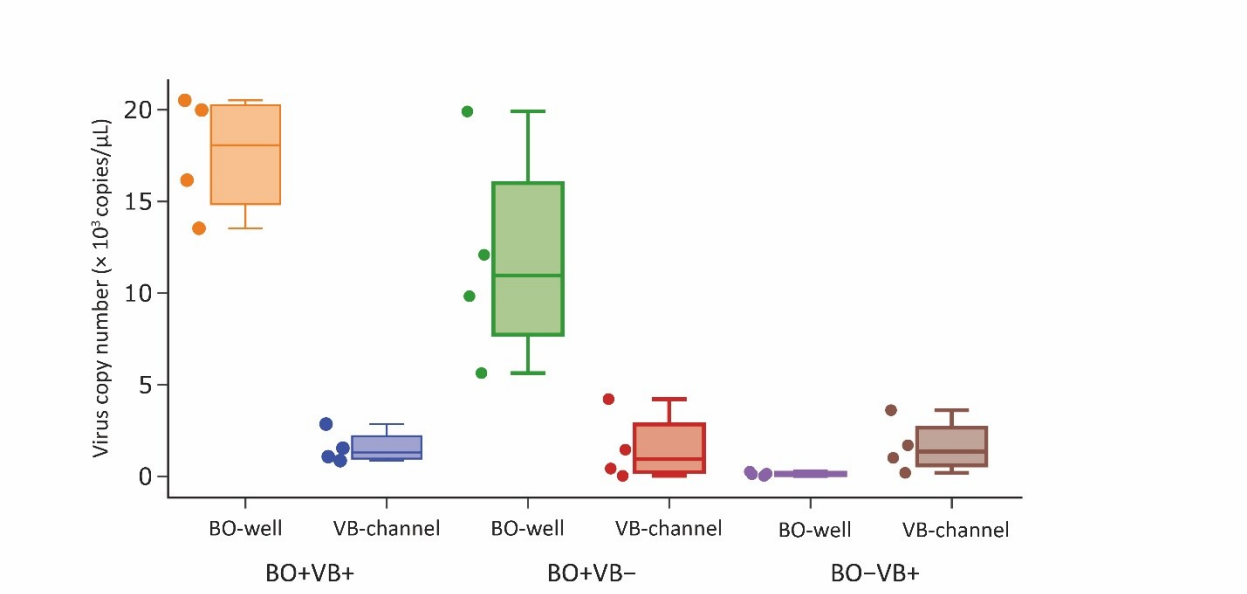

Fig. S6. Virus copy number in supernatants from different virus applications. Virus copy numbers in supernatants from the BO-culture well and the VB-culture channel were measured when the virus was applied to the BO-culture well and the VB-culture channel (BO+VB+), only the BO-culture well (BO+VB−), and only the VB-culture channel (BO−VB+).

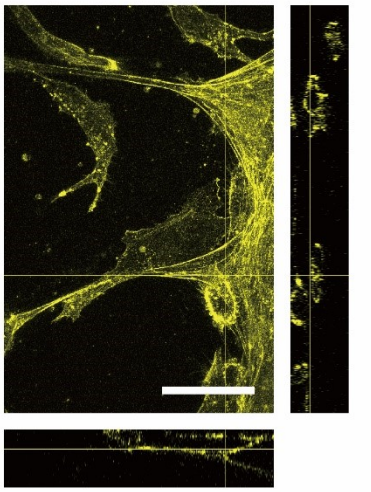

Fig. S7. Orthogonal view of the VB in the SARS-CoV-2-infected device. Fluorescent orthogonal images of actin filament (yellow) from three sides (top view and two orthogonal cross-sectional views) were taken from a damaged vascular structure in a SARS-CoV-2-infected device. Scale bar: 100 μm.

Table S1

Parameters in virus distribution considerations

Parameter	**Symbol**	**Value**
Boltzmann constant	k_B	1.38×10^{-23} m^2 kg s^{-2} K^{-1}
Temperature	T	300 K
Viscosity	μ	1×10^{-3} Pa s
Particle radius	R	5×10^{-8} m
Particle density	ρ_p	1.5×10^3 kg m^{-3}
Solution density	ρ_w	1.0×10^3 kg m^{-3}
Gravitational acceleration	g	9.81 m s^{-2}
Representative length	L	5×10^{-4} m

Table S2

Antibodies and labeling reagents

Reagent	**Item number**	**Company**
Alexa Fluor 647 Phalloidin	A30107	Thermo Fisher Scientific
DAPI	D3571	Thermo Fisher Scientific
Cytokeratin Antibody	Ab193895	Abcam
Acetylated tubulin Antibody	T7451	Sigma-Aldrich
CD31 Antibody	555444	Becton
Alexa fluor 647 labeled E-Cadherin Antibody	EP700Y	Abcam

Table S3

Sequences of primers used for qPCR

Target gene	Fwd primer	Rev primer
KRT5	CCAAGGTTGATGCACTGATGG	TGTCAGAGACATGCGTCTGC
MCIDAS	ATTCCCACCAAACGGAAGCAG	CCAGGGTAGGCGACATCATAG
NGFR	CCTACGGCTACTACCAGGATG	CACACGGTGTTCTGCTTGT
TP63	GGACCAGCAGATTCAGAACGG	AGGACACGTCGAAACTGTGC
TUBA1A	TCGATATTGAGCGTCCAACCT	CAAAGGCACGTTTGGCATACA
ACE2	ACAGTCCACACTTGCCCAAAT	TGAGAGCACTGAAGACCCATT
TMPRSS2	GTCCCCACTGTCTACGAGGT	CAGACGACGGGGTTGGAAG
IFNA1	GCAGATCACCCAGAAGATCG	GGCCCTTGTTATTCCTCACC
IFNB1	CCTTGCTGAAGTGTGGAGGA	CCAGGCGATAGGCAGAGA
MxA	CTTATCCGTTAGCCGTGGTG	CAAGGTGGAGCGATTCTGAG
ISG56	CCTTGCTGAAGTGTGGAGGA	CCAGGCGATAGGCAGAGA
ISG15	GCAGATCACCCAGAAGATCG	GGCCCTTGTTATTCCTCACC
ICAM1	ATGCCCAGACATCTGTGTCC	GGGGTCTCTATGCCCAACAA
VCAM1	GGGAAGATGGTCGTGATCCTT	TCTGGGGTGGTCTCGATTTTA
VE-CAD (CDH5)	TTGGAACCAGATGCACATTGAT	TCTTGCGACTCACGCTTGAC
ZO-1 (TJP1)	CTTACCACACTGTGCGTCCAT	AGGAGTCGGATGATTTTAGAGCA
IL-6	CCTGAACCTTCCAAAGATGGC	TTCACCAGGCAAGTCTCCTCA
SARS-CoV-2	AGCCTCTTCTCGTTCCTCATCAC	CCGCCATTGCCAGCCATTC