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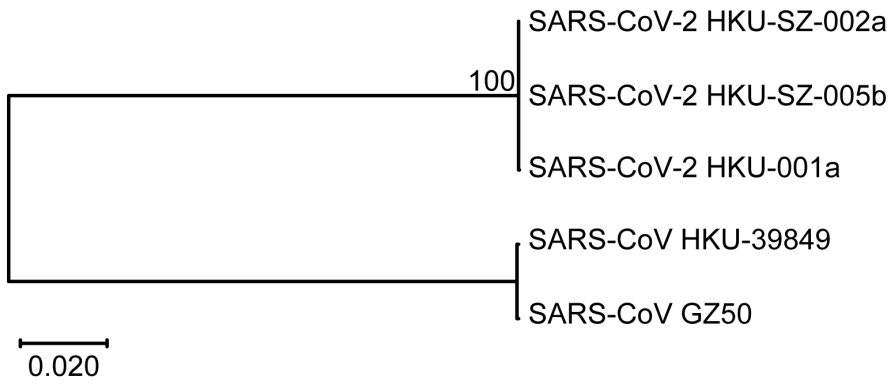
Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Chu H, Chan J F-W, Yuen T T-T, et al. Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study. *Lancet Microbe* 2020; published online April 21. https://doi.org/10.1016/S2666-5247(20)30004-.

Supplementary Figures and Figure Legends:

A

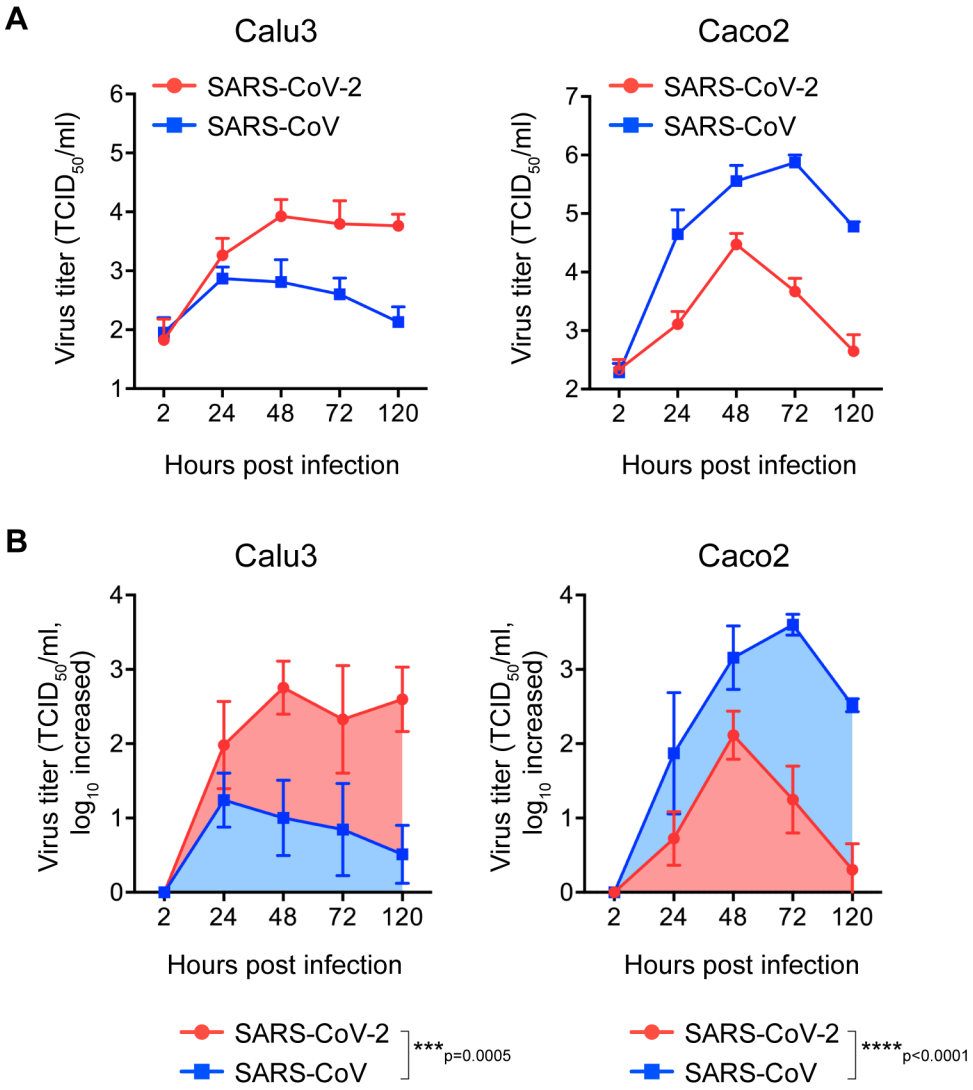


B

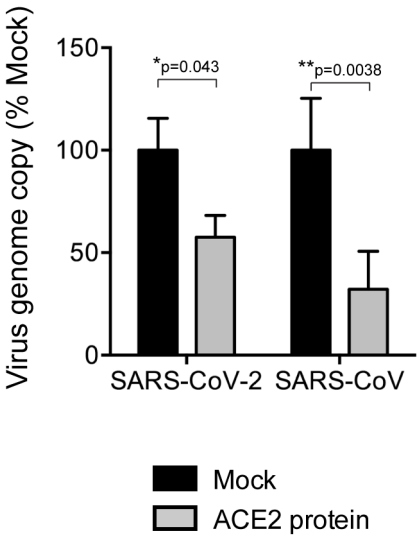
Genome	Position (nucleotide)		Gene	Amino acid
HKU-SZ-005b	1663	(GAC)	NSP2	D286
HKU-SZ-002a	1663	(GAC)	NSP2	D286
HKU-001a	1663	(GAT)	NSP2	D286
HKU-SZ-005b	9561	(TTA)	NSP4	L336
HKU-SZ-002a	9561	(TCA)	NSP4	S336
HKU-001a	9561	(TCA)	NSP4	S336
HKU-SZ-005b	15607	(CTA)	NSP12	L723
HKU-SZ-002a	15607	(TTA)	NSP12	L723
HKU-001a	15607	(TTA)	NSP12	L723
HKU-SZ-005b	22661	(GTC)	S	V367
HKU-SZ-002a	22661	(GTC)	S	V367
HKU-001a	22661	(TTC)	S	F367
HKU-SZ-005b	24034	(AAC)	S	N824
HKU-SZ-002a	24034	(AAC)	S	N824
HKU-001a	24034	(AAT)	S	N824
HKU-SZ-005b	26729	(GCT)	M	A69
HKU-SZ-002a	26729	(GCT)	M	A69
HKU-001a	26729	(GCG)	M	A69
HKU-SZ-005b	28077	(GTG)	orf8	V62
HKU-SZ-002a	28077	(GTG)	orf8	V62
HKU-001a	28077	(CTG)	orf8	L62
HKU-SZ-005b	29095	(TTT)	N	F274
HKU-SZ-002a	29095	(TTT)	N	F274
HKU-001a	29095	(TTC)	N	F274

Supplementary Figure 1. Phylogenetic analysis of SARS-CoV-2 HKU-001a. (A) The tree was constructed by Neighbor-Joining method using Tajima-Nei model with uniform rates. The bootstrap value (1000 replicates) was shown next to the branch. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The SARS-CoV-2 strains included in the analysis were HKU-SZ-005b (MN975262), HKU-SZ-002a (MN938384), and HKU-001a (MT230904). The SARS-

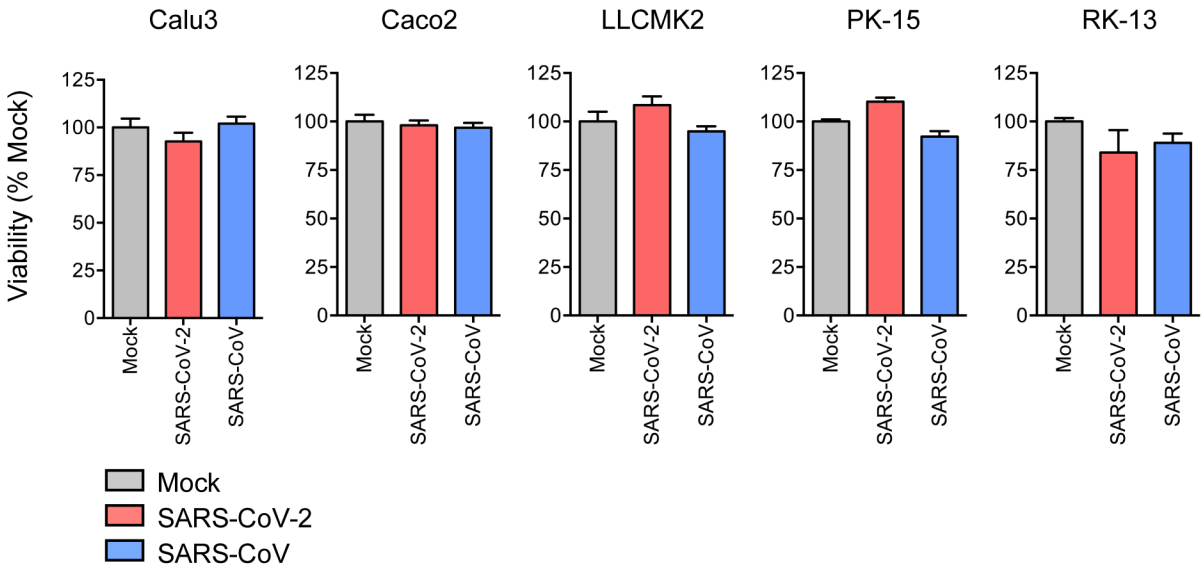
CoV strains included in the analysis were HKU-39849 (AY278491) and GZ50 (AY304495). (B) Nucleotide and amino acid substitutions. The nucleotide and amino acid sequences of two SARS-CoV-2 clinical strains (HKU-SZ-005b, HKU-SZ-002a) and the SARS-CoV-2 culture strain (HKU-001a) used in this study were compared. The amino acid substitutions of the culture isolate were labelled in red.



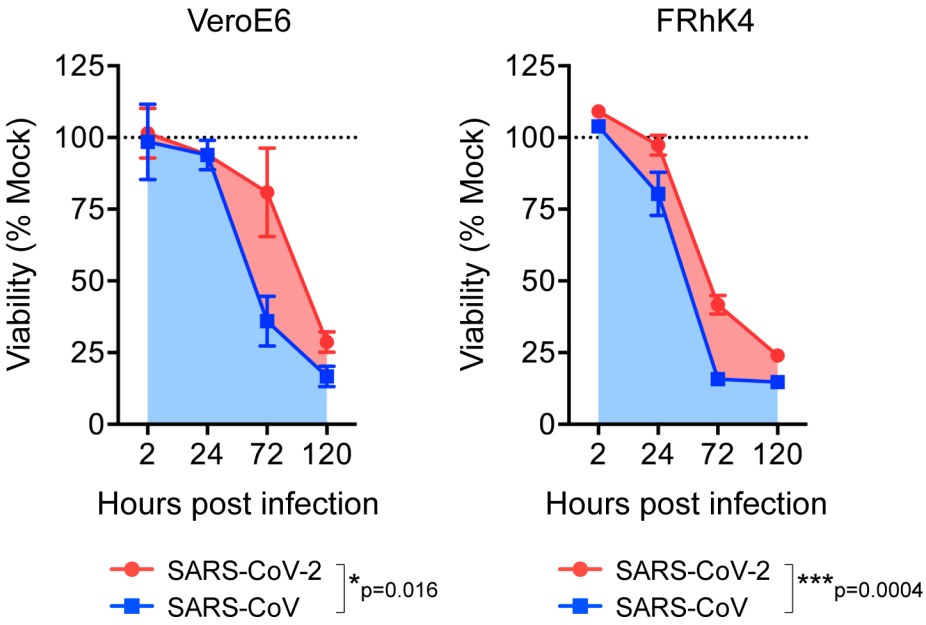
Supplementary Figure 2. $TCID_{50}$ assay for SARS-CoV-2- and SARS-CoV-infected Calu3 and Caco2 cells. (A) Calu3 and Caco2 cells were inoculated with SARS-CoV-2 or SARS-CoV at 0.1 MOI and the supernatants were harvested at 2, 24, 48, 72, and 120 hours post-inoculation. The number of infectious virus particles in the samples was titrated with $TCID_{50}$ assays. (B) AUC analyses of SARS-CoV-2- and SARS-CoV-infected Calu3 and Caco2 cells demonstrated the total amount of infectious virus particles increased over the 120-hours period. Statistical significance of the AUC analyses was determined with Student's t-test. *** indicated $P < 0.001$ and **** indicated $P < 0.0001$.



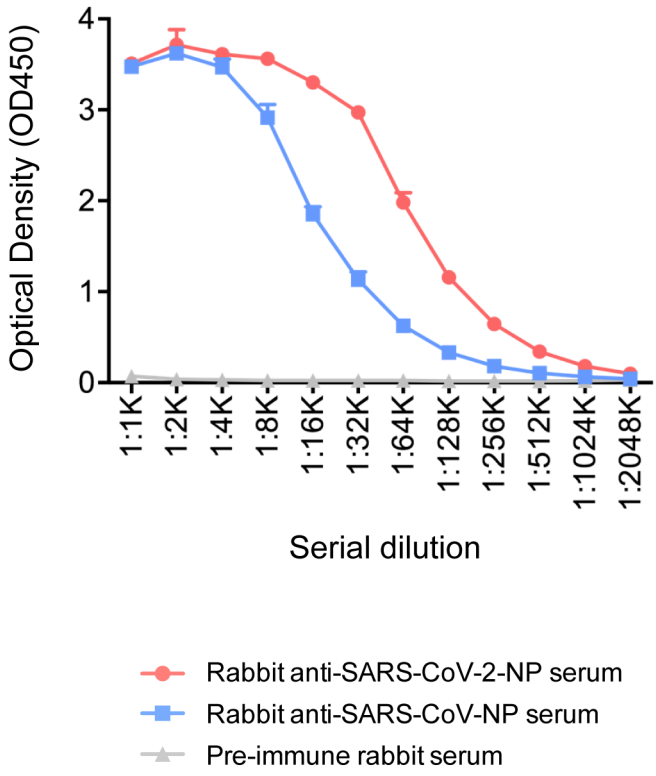
Supplementary Figure 3. Recmobinant human ACE2 (rhACE2) protein blocking assay for SARS-CoV-2- and SARS-CoV infection. SARS-CoV-2 or SARS-CoV was pre-incubated with 80μg/ml rhACE2 for 1 hour. The mixture was added to VeroE6 cells for 30 minutes. The cells were then washed and harvested at 4 hours post infection. Virus genome copies in the cell lysates were determined with qRT-PCR. The histograms represented mean and standard deviations. Statistical significance was determined with two-way ANOVA. * indicated $P < 0.05$ and ** indicated $P < 0.01$.



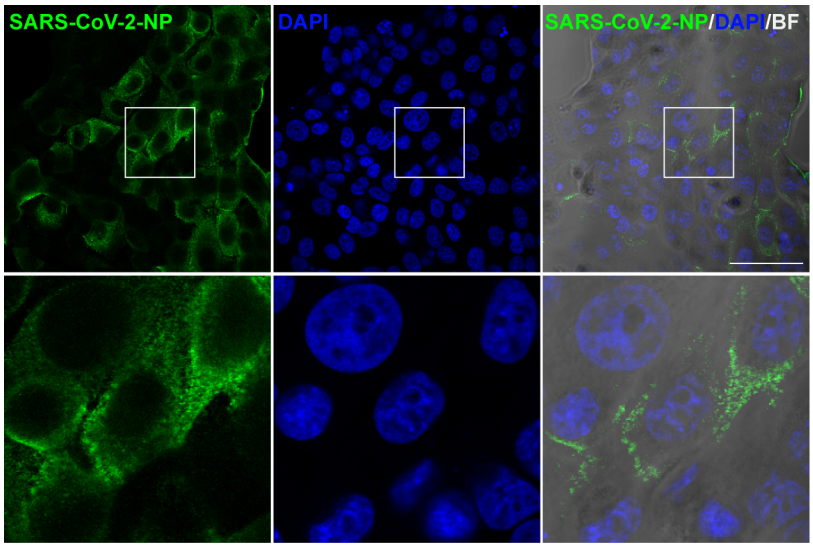
Supplementary Figure 4. Viability of SARS-CoV-2- or SARS-CoV-infected cells at day 7 post-infection. Calu3, Caco2, LLCMK2, PK-15, and RK-13 cells were infected with SARS-CoV-2 or SARS-CoV at 0.1 MOI and the cell viability was quantified at day 7 post-infection with the CellTiterGlo assay. The histograms represented mean and standard deviations. Statistical significance was determined with one-way ANOVA. No significant decrease in cell viability was detected.



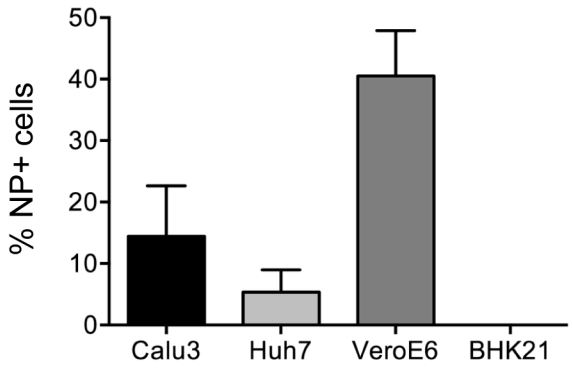
Supplementary Figure 5. Area under the curve analysis of the cell damages induced by SARS-CoV-2 or SARS-CoV in VeroE6 and FRhK4 cells. VeroE6 and FRhK4 cells were inoculated with SARS-CoV-2 or SARS-CoV at 0.1 MOI and the cell viability was quantified at 2, 24, 72, and 120 hours post-inoculation. The total amount of viable cells over the 120-hour period upon SARS-CoV-2 or SARS-CoV inoculation relative to mock-inoculated controls was compared by the area under the curve analysis. Statistical significance was determined with Student's t-test. * indicated $P < 0.05$ and *** indicated $P < 0.001$.



Supplementary Figure 6. Titration of SARS-CoV-2-NP and SARS-CoV-NP immune sera against SARS-CoV-2-NP. Serial diluted SARS-CoV-2-NP or SARS-CoV-NP immunized rabbit sera were added to SARS-CoV-2-NP-coated 96-well plates for 1 hour. Pre-immune rabbit serum was included as a control. Optical density (OD) was determined at 450 nm.



Supplementary Figure 7. Cellular localization of SARS-CoV-2-NP. VeroE6 cells were infected with SARS-CoV-2 at 0.01 MOI. At 16 hours post-inoculation, the cells were fixed in 4% paraformaldehyde and immunolabeled with the in-house rabbit anti-SARS-CoV-2-NP immune serum. The confocal images were acquired with a ZEISS LSM780 system. Bars represented 50 μm. BF = bright field.



Supplementary Figure 8. Quantification of SARS-CoV-2-infected cells. The number of SARS-CoV-2-infected Calu3, Huh7, VeroE6, and BHK21 was quantified. The number of antigen-positive cells were quantified from three randomly selected 768x768 pixel fields. The histograms represented mean and standard deviations.