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Received: 13 January 2022

Accepted: 27 January 2022

Accelerated Article Preview

Cite this article as: Hui, K. P. Y. et al. SARS-CoV-2 Omicron variant replication in human bronchus and lung ex vivo. *Nature* <https://doi.org/10.1038/s41586-022-04479-6> (2022).

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SARS-CoV-2 Omicron variant replication in human bronchus and lung ex vivo

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Emergence of SARS-CoV-2 variants of concern (VOC) with progressively increased transmissibility between humans is a threat to global public health. The omicron variant also evades immunity from natural infection or vaccines¹, but it is unclear whether its exceptional transmissibility is due to immune evasion or intrinsic virological properties. We compared the replication competence and cellular tropism of the wild-type (WT) virus, D614G, Alpha, Beta, Delta and Omicron variants in *ex vivo* explant cultures of human bronchus and lung. Dependence on TMPRSS2 for infection

was also evaluated. We show that Omicron replicated faster than all other SARS-CoV-2 in the bronchus but less efficiently in the lung parenchyma. All VOCs had similar cellular tropism as the WT. Omicron was more dependent on cathepsins than other VOC tested, suggesting that the omicron variant enters cells by a different route than other variants. The lower replication competence of Omicron in human lung may explain the reduced severity of Omicron that is now being reported in epidemiological studies although determinants of severity are multifactorial. These findings provide important biological correlates to observed epidemiological observations.

Since the emergence of COVID-19 in December 2019, the causative agent SARS-CoV-2 has continued to evolve in humans, generating variants of progressively increased transmissibility between humans. The World Health Organization (WHO) has classified several lineages as Variant of Concern (VOC) on the grounds of their high transmission rate, potential for immune evasion, unusual epidemiological properties or adverse impact on diagnostics and therapeutics². These VOCs include the B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), and B.1.1.529 (Omicron) virus lineages.

The first virus variant emerged in February 2020 carrying the D614G amino acid substitution in the S protein and this quickly became the dominant virus variant globally. It was shown to replicate more rapidly in primary airway epithelial cells and in the nasal cavity of experimentally infected hamsters, explaining its greater transmissibility in humans³. The B.1.1.7 lineage (Alpha variant), first reported in the UK in September 2020, had 13 mutations in the spike (S) protein and the N501Y, P681H mutations in the spike protein contributed to its increased transmissibility^{4,5}. The deletion at position 60-70 in the spike led to loss of detection of the S gene target in some diagnostic RT-PCR, termed “S-gene target failure”⁶.

The B.1.351 lineage (Beta variant) first detected in South Africa in August 2020, contained 10 mutations in the S protein and mutations N501Y and K417N enhanced viral transmission while E484K contributed to immune evasion^{7,8}. The B.1.617.2 lineage (Delta variant) was first identified in the state of Maharashtra, India in October 2020, had multiple amino acid substitutions in the S protein including L452R and E484Q which cause enhancement of ACE2 binding, transmission and immune evasion^{9,10}. The Omicron variant was identified in Botswana and South African in November 2021 and was designated as VOC by the WHO in the same month. It has 37 amino acid substitutions in the S protein, 15 of these being in the receptor binding domain. Thus, it was likely that the phenotype of the virus would be markedly affected for transmission, escape from prior immunity, or both. As of 14 December 2021, the Omicron variant has been detected in 76 countries globally¹¹. Differences in disease severity between variants has been more subtle. A modest increase in disease severity has been reported with Delta VOC with higher hospital admissions compared with the alpha VOC^{12,13}. The extensive amino acid substitutions in the spike protein of Omicron¹⁴ are likely to have major implications on transmissibility, disease severity and immune evasion from serum neutralizing antibodies from both infection-, vaccine-elicited and therapeutic monoclonal antibodies. However, there is limited information on the biological or virological characteristics of this variant. Omicron virus replication is ACE2 dependent and it is able to markedly evade neutralizing antibody elicited by past infection or vaccination¹.

Correlation of the phenotype of a SARS-CoV-2 variant *in vitro* with epidemiology is well illustrated by the experimental observations with the D614G mutation. An isogenic wild-type virus engineered to carry the spike D614G substitution had enhanced viral replication in human lung epithelial cells and primary human airway tissues by increasing the infectivity and stability of virions¹⁵. Hamsters infected with this virus produced higher infectious titres

in nasal washes and the trachea, but not in the lungs, supporting clinical evidence showing that the mutation enhances viral loads in the upper respiratory tract of COVID-19 patients and may increase transmission. But there are no studies using physiologically relevant experimental models investigating the phenotype of the Omicron variant in comparison with previous variants of SARS-CoV-2.

We have previously used *ex vivo* explant cultures of the human bronchus and lung parenchyma to study virus replication competence and cellular tropism for avian influenza viruses^{16,17}, MERS¹⁸⁻²⁰ and SARS-CoV-2²¹. This provided a suitable platform to rapidly compare the replication profile and tropism of the Omicron variant with others and to provide insight into the observed epidemiology of this variant. We initially compared the virus replication kinetics of wild type SARS-CoV-2 (WT) with the D614G variant and VOCs from B.1.1.7 (Alpha) and B.1.351 (Beta) lineages in *ex vivo* explant cultures of human bronchus and lung. In a subsequent set of experiments, we compared viral replication profiles of WT with B.1.617.2 (Delta) and B.1.1.529 (Omicron) lineages. We also investigated the cellular tropism of the virus using immunohistochemistry and compared the dependency of WT, Delta and Omicron variants on TMPRSS2 and cathepsins for their replication.

Higher replication of Omicron in bronchus

We first compared the replication kinetics of WT strain, D614G, Alpha and Beta virus strains isolated from returning travellers or the community in Hong Kong in *ex vivo* cultures of human bronchus and lung by titrating infectious virus using 50% tissue culture infectious dose (TCID₅₀) titrations. The only significant difference observed was a higher replication Beta variant in the human bronchus at 72 hpi; no significant difference being noted between

viruses at 24 and 48 hpi (Figure 1a). When WT, Delta and Omicron variants were compared, Omicron variant replicated to significantly higher titres than WT or Delta at 24 and 48 hpi; the difference being over 70-fold (Figure 1b). At 72 hpi, both Delta and Omicron viruses replicated significantly more than WT in human bronchus but there was no significant difference between Delta and Omicron viruses. When these experiments were done at 33°C, the viral titres were similar to those at 37°C for each virus (data not shown). In *ex vivo* cultures of human lung, the only significant difference observed was reduced replication of Omicron compared to WT at 24, 48 and 72 hpi (Figure 1c,d). These findings in bronchus and lung were confirmed in area-under-curve (AUC) analysis of aggregate virus titres at 24-72 hpi (Figure 1e,f). The individual donor data-sets of viral titers for the comparison of WT, Delta and Omicron were shown in Extended Data Figure 1. The same consistent trend was observed with each individual donor. Similar trends were observed between ORF-1b gene quantification and TCID₅₀ results, that a higher level of viral gene was detected in Omicron infected bronchus compared to WT and Delta while a higher number of viral gene copies was measured in WT infected lung tissues than Delta and Omicron infection (Extended Data Figure 2).

The tropism of each virus variant in the bronchus and lung was visualized with immunohistochemical staining for SARS-CoV-2 nucleoprotein (Figure 2). The virus variants did not appear to differ in cell tropism in the bronchus tissues infected *ex vivo*. Omicron had evidence of extensive virus infection with the ciliated epithelium, goblet cells and club cells showing infection in WT, Alpha, Beta, Delta and Omicron viruses by using immunofluorescent staining (Figure 2a and 3). Transmission electron micrograph also showed the presence of Omicron viral particles in membrane bound vesicles in the cytoplasm, as well as on the surface attached to microvilli of ciliated cells (Extended Data Figure 4). The

virus variants did not appear to differ in cell tropism in the lung (Figure 2b).

Immunohistochemical staining showed positive staining in the lung tissues with the spindle shaped antigen positive cells morphologically resembling type 1 pneumocytes. In lung tissues infected with the omicron variant there were decreased staining intensities of viral antigen identified (Extended Data Figure 3). On the contrary, higher numbers of cells with positive staining for viral antigen was observed in the bronchial epithelium infected with Omicron when compared to all the other variants and wild-type virus.

ACE2 and TMPRSS2 expression in respiratory tract

Angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) contribute to the entry of SARS-CoV-2 into cells. The latter cleaves the spike S2 domain (S2') allowing the virus to enter the cell via cell fusion at the outer cell membrane as distinct from virus entry via the endocytic pathway²². ACE2 expressed at cell surface exists as short and long forms, with the short form lacking the domain that binds to SARS-CoV-2 spike²³. We showed that there was significantly higher expression of both long and short form ACE2 in human bronchus than in the lung (Figure 4a). Immunohistochemical staining also showed clear evidence of more extensive ACE2 staining in bronchus than lung (Figure 4b and 4c). We next investigated the TMPRSS2 expression and showed significantly higher expression of TMPRSS2 in the bronchus than the lung by mRNA expression levels (Figure 4a).

Omicron preference on endocytic entry

We investigated WT, Delta and Omicron virus replication for their dependence on TMPRSS2. We compared the viral replication kinetics of wild-type, Delta and Omicron in Vero E6 and Vero E6-TMPRSS2 (E6/T2) cells. While virus titres at 24 hpi was higher in E6/T2 cells in

WT and Delta, the magnitude of the difference was 100-1000 fold in Delta, 3-100 fold for Omicron and around 10 fold for WT (Figure 4d). The AUC levels of the viral titres at 24-48 hours confirmed these findings (Figure 4e). Omicron replicated to lower titres than WT or Delta at 24 hpi in E6/T2 cells (Extended Data Figure 5), which was also shown in their AUC levels (Figure 4e). We next infected Vero E6/T2 cells with WT, Delta and Omicron in the presence of camostat mesylate (an inhibitor of serine proteases including TMPRSS2²²) or E64d (an inhibitor of cathepsins)²⁴. The viability of Vero E6/T2 was above 80% when treated with camostat mesylate at concentrations up to 300 μ M or E64d at concentrations up to 120 μ M (Extended Data Figure 6). While both WT and Omicron infection was partially reduced by camostat mesylate, Delta was the more sensitive to the inhibitor (Figure 4f). In contrast, while both WT and Delta infection was partially suppressed by E64d, Omicron infection was reduced more markedly to around 5% of control. The combined treatment of camostat mesylate and E64d completely blocked the infection of all three viruses. This suggests that in contrast to Delta, that Omicron may be more dependent on the endocytic pathway to enter cells.

Discussion

Preliminary epidemiological data from the United Kingdom suggests that the risk of household transmission with Omicron is 3.2 (95% confidence interval 2.0-5.0) higher than with Delta, which in turn was a highly transmissible virus compared to the wild-type virus. Omicron has a Reproduction number (R) of 3.7²⁵. It has been unclear whether this increased efficiency of transmission is driven by mutations that confer immune escape from antibody in the population elicited by prior infection or vaccination, by intrinsic viral factors or a combination of the two. It is increasingly clear that high neutralizing antibody titres elicited

by prior infection or immunization are markedly compromised by Omicron virus¹. Our results suggest that the Omicron variant has a substantial (over 70-fold increase) and significantly higher replication competence in the human bronchus compared to both wild-type and Delta viruses at 24 hpi. To what extent this faster replication in the bronchus might contribute to transmissibility is unclear, but higher infectious virus load in conducting airways might result in increased amounts of infectious virus released while breathing or speaking thus enhancing transmission by the airborne route. There are also recent reports suggesting increased viral replication of Omicron compared to Delta and WT virus in differentiated human nasal epithelial cultures in vitro²⁶. Infectious SARS-CoV-2 has been detected in fine aerosol particles in air exhaled by patients with COVID-19²⁷. The mechanistic reasons underlying the increased replication competence in bronchus remain to be elucidated. The Omicron variant has 37 amino acid substitutions in the spike protein, 15 of which are in the receptor binding domain¹⁴. Infection with Omicron is ACE2 dependent¹ and that the binding of the spike of Omicron spike to ACE2 is enhanced when compared to the wild-type virus²⁸. We showed that there is more extensive ACE2 expression in human bronchus than lung which may explain the enhanced replication of SARS-CoV-2 in the bronchus. Omicron also has amino acid substitutions in the nucleocapsid protein (R203K and G204R) that have been associated with enhanced virus replication²⁹.

Epidemiological studies suggested that Delta variant was substantially more transmissible than Alpha variant³⁰, which was itself was more transmissible than the earlier virus strains⁴. Thus, our data indicating that Delta variant has higher titres of infectious virus than wild-type virus in the bronchus is also compatible with the epidemiological observations. It has been shown that pseudoviruses expressing Delta spike are better able to infect ACE2^{low} human bronchial epithelial cells compared to previous variants³¹. Moreover, the spike of Delta is in a

predominantly cleaved state which may promote its replication efficiency in human airway³². P681R enhanced the cleavage of furin-cleavage sequences, which attributes to the enhanced fitness of Delta over Alpha in a competition assay in Calu-3 and human airway epithelium *in vitro* models^{33,34}.

Our data showing that the Omicron variant had lower viral replication competence in the lung, compared to the bronchus is of particular interest. This difference is also confirmed by the immunohistochemistry studies showing less virus infected cells in human lung explant cultures *ex vivo*. The biological determinants of this divergence in comparative replication competence of Omicron and Delta variants in the bronchus and lung remains to be explored. These observations may suggest that Omicron may have reduced clinical severity but such interpretations need to be qualified because the disease severity of COVID-19 is determined not only by virus replication but also by dysregulated innate immune responses. Recent epidemiological studies from South Africa and the United Kingdom suggest that Omicron has caused less hospitalization than Delta variant^{35,36}. Thus, the findings from the *ex vivo* cultures are in concordance with recent epidemiological assessments both in regard to transmission and disease severity.

Our finding that Omicron infection was less dependent on TMPRSS2 activities but more sensitive to a cathepsin inhibitor compared with Delta suggests that Omicron may enter cells primarily via the endocytic pathway while Delta preferentially enters cells via fusion at the cell surface. The adoption of a ubiquitous endocytic pathway potentially broadens the cellular spectrum for Omicron infection and enables Omicron to infect cells with ACE2 expression irrespective of the presence of TMPRSS2. Single cell sequencing results indicate that cells with co-expression of ACE2 and cathepsins are more abundant in the upper airway than cells that co-express ACE2 and TMPRSS2, which may explain the increased replication

competence of Omicron in the bronchus²⁶. Our findings that Omicron preferentially enters cells via the endosomal pathway while Delta is more reliant on the cell surface fusion has been in line with the reports on alveolar epithelial cells and nasal epithelial cells^{24,26}. The use of therapeutic inhibitors of TMPRSS2 may be of limited benefit in the management of clinical infections with the Omicron variant. Though there have been single-cell studies on the distribution of TMPRSS2 in cell cultures, the data on the extent of its distribution is hampered by sensitivity and specificity of antibodies in fixed tissues.

One of the limitations of this study is that only one virus strain from each lineage was tested. Furthermore, all six virus variants were not tested in parallel in the same experiment, but this is very difficult to carry out logistically because of limited tissue available to test six viruses with replicates.

In summary, our findings reveal faster and enhanced viral replication efficiency of Omicron in the human bronchus compared with the precedent lineages, suggesting an intrinsic capacity for enhanced transmission. The lower replication competence of Omicron in human lung is compatible with lower disease severity compared with Delta. Both of these observations are concordant with epidemiological data. Even if disease severity is modestly reduced, the very efficient transmissibility of Omicron will pose a major threat to global public health and health care systems. Investigations on preventing Omicron infection via vaccination booster and therapeutic options are urgently needed.

Online content Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at [<https://doi.org/10.1038/s41586-022-04479-6>].

Received 13 January 2022; accepted 27 January 2022

- 1 Cele, S. *et al.* SARS-CoV-2 Omicron has extensive but incomplete escape of Pfizer
BNT162b2 elicited neutralization and requires ACE2 for infection. *medRxiv : the*
2 *preprint server for health sciences*, doi:10.1101/2021.12.08.21267417 (2021).
- 3 WHO. *Tracking SARS-CoV-2 variants*,
<<https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/>> (2021).
- 4 Baric, R. S. Emergence of a Highly Fit SARS-CoV-2 Variant. *The New England*
journal of medicine **383**, 2684-2686, doi:10.1056/NEJMcibr2032888 (2020).
- 5 Davies, N. G. *et al.* Estimated transmissibility and impact of SARS-CoV-2 lineage
B.1.1.7 in England. *Science (New York, N.Y.)* **372**, doi:10.1126/science.abg3055
(2021).
- 6 Lubinski, B., Tang, T., Daniel, S., Jaimes, J. A. & Whittaker, G. R. Functional
evaluation of proteolytic activation for the SARS-CoV-2 variant B.1.1.7: role of the
P681H mutation. *bioRxiv : the preprint server for biology*,
doi:10.1101/2021.04.06.438731 (2021).
- 7 Srivastava, S., Banu, S., Singh, P., Sowpati, D. T. & Mishra, R. K. SARS-CoV-2
genomics: An Indian perspective on sequencing viral variants. *Journal of biosciences*
46, doi:10.1007/s12038-021-00145-7 (2021).
- 8 Zhou, D. *et al.* Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and
vaccine-induced sera. *Cell* **184**, 2348-2361.e2346, doi:10.1016/j.cell.2021.02.037
(2021).
- 9 Khan, A. *et al.* Higher infectivity of the SARS-CoV-2 new variants is associated with
K417N/T, E484K, and N501Y mutants: An insight from structural data. *Journal of*
cellular physiology **236**, 7045-7057, doi:10.1002/jcp.30367 (2021).
- 10 Ozono, S. *et al.* SARS-CoV-2 D614G spike mutation increases entry efficiency with
enhanced ACE2-binding affinity. *Nature communications* **12**, 848,
doi:10.1038/s41467-021-21118-2 (2021).
- 11 Augusto, G. *et al.* In vitro data suggest that Indian delta variant B.1.617 of
SARS-CoV-2 escapes neutralization by both receptor affinity and immune evasion.
Allergy, doi:10.1111/all.15065 (2021).
- 12 WHO. Weekly epidemiological update on COVID-19 - 14 December 2021. (2021).
- 13 Sheikh, A., McMenamin, J., Taylor, B. & Robertson, C. SARS-CoV-2 Delta VOC in
Scotland: demographics, risk of hospital admission, and vaccine effectiveness. *Lancet*
(*London, England*) **397**, 2461-2462, doi:10.1016/s0140-6736(21)01358-1 (2021).
- Twohig, K. A. *et al.* Hospital admission and emergency care attendance risk for
SARS-CoV-2 delta (B.1.617.2) compared with alpha (B.1.1.7) variants of concern: a

- cohort study. *The Lancet. Infectious diseases*, doi:10.1016/s1473-3099(21)00475-8 (2021).
- 14 Gu, H. *et al.* Probable Transmission of SARS-CoV-2 Omicron Variant in Quarantine Hotel, Hong Kong, China, November 2021. *Emerging infectious diseases* **28**, doi:10.3201/eid2802.212422 (2021).
 - 15 Plante, J. A. *et al.* Spike mutation D614G alters SARS-CoV-2 fitness. *Nature* **592**, 116-121, doi:10.1038/s41586-020-2895-3 (2021).
 - 16 Hui, K. P. *et al.* Tropism and innate host responses of influenza A/H5N6 virus: an analysis of ex vivo and in vitro cultures of the human respiratory tract. *The European respiratory journal* **49**, doi:10.1183/13993003.01710-2016 (2017).
 - 17 Chan, M. C. *et al.* Tropism and innate host responses of a novel avian influenza A H7N9 virus: an analysis of ex-vivo and in-vitro cultures of the human respiratory tract. *The Lancet. Respiratory medicine* **1**, 534-542, doi:10.1016/s2213-2600(13)70138-3 (2013).
 - 18 Chan, R. W. *et al.* Tropism and replication of Middle East respiratory syndrome coronavirus from dromedary camels in the human respiratory tract: an in-vitro and ex-vivo study. *The Lancet. Respiratory medicine* **2**, 813-822, doi:10.1016/s2213-2600(14)70158-4 (2014).
 - 19 Zhou, Z. *et al.* Phenotypic and genetic characterization of MERS coronaviruses from Africa to understand their zoonotic potential. *Proceedings of the National Academy of Sciences of the United States of America* **118**, doi:10.1073/pnas.2103984118 (2021).
 - 20 Chu, D. K. W. *et al.* MERS coronaviruses from camels in Africa exhibit region-dependent genetic diversity. *Proceedings of the National Academy of Sciences of the United States of America* **115**, 3144-3149, doi:10.1073/pnas.1718769115 (2018).
 - 21 Hui, K. P. Y. *et al.* Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures. *The Lancet. Respiratory medicine*, doi:10.1016/s2213-2600(20)30193-4 (2020).
 - 22 Hoffmann, M. *et al.* SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, doi:10.1016/j.cell.2020.02.052 (2020).
 - 23 Blume, C. *et al.* A novel ACE2 isoform is expressed in human respiratory epithelia and is upregulated in response to interferons and RNA respiratory virus infection. *Nature genetics* **53**, 205-214, doi:10.1038/s41588-020-00759-x (2021).

- 24 Willett, B. J. *et al.* The hyper-transmissible SARS-CoV-2 Omicron variant exhibits significant antigenic change, vaccine escape and a switch in cell entry mechanism. 2022.2001.2003.21268111, doi:10.1101/2022.01.03.21268111 %J medRxiv (2022).
- 25 Agency, U. H. S. SARS-CoV-2 variants of concern and variants under investigation (2021).
- 26 Peacock, T. P. *et al.* The SARS-CoV-2 variant, Omicron, shows rapid replication in human primary nasal epithelial cultures and efficiently uses the endosomal route of entry. 2021.2012.2031.474653, doi:10.1101/2021.12.31.474653 %J bioRxiv (2022).
- 27 Adenaiye, O. O. *et al.* Infectious SARS-CoV-2 in Exhaled Aerosols and Efficacy of Masks During Early Mild Infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, doi:10.1093/cid/ciab797 (2021).
- 28 Cameroni, E. *et al.* Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. 2021.2012.2012.472269, doi:10.1101/2021.12.12.472269 %J bioRxiv (2021).
- 29 Wu, H. *et al.* Nucleocapsid mutations R203K/G204R increase the infectivity, fitness, and virulence of SARS-CoV-2. *Cell host & microbe* **29**, 1788-1801.e1786, doi:10.1016/j.chom.2021.11.005 (2021).
- 30 Allen, H. *et al.* Household transmission of COVID-19 cases associated with SARS-CoV-2 delta variant (B.1.617.2): national case-control study. *The Lancet regional health. Europe* **12**, 100252, doi:10.1016/j.lanepe.2021.100252 (2022).
- 31 Li, H., Liu, T., Wang, L., Wang, M. & Wang, S. SARS-CoV-2 Delta variant infects ACE2(low) primary human bronchial epithelial cells more efficiently than other variants. *Journal of medical virology*, doi:10.1002/jmv.27372 (2021).
- 32 Mlcochova, P. *et al.* SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. *Nature* **599**, 114-119, doi:10.1038/s41586-021-03944-y (2021).
- 33 Liu, Y. *et al.* Delta spike P681R mutation enhances SARS-CoV-2 fitness over Alpha variant. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.08.12.456173 (2021).
- 34 Lubinski, B. *et al.* Spike protein cleavage-activation mediated by the SARS-CoV-2 P681R mutation: a case-study from its first appearance in variant of interest (VOI) A.23.1 identified in Uganda. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.06.30.450632 (2021).
- 35 Wolter, N. *et al.* Early assessment of the clinical severity of the SARS-CoV-2 Omicron variant in South Africa. 2021.2012.2021.21268116, doi:10.1101/2021.12.21.21268116 %J medRxiv (2021).

36 Agency, U. H. S. SARS-CoV-2 variants of concern and variants under investigation in Egnland. Technical Briefing: Updata on hospitization and vaccine effectiveness for Omicron VOC-21Nov-01 (B.1.1.529). (2021).

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Methods

SARS-CoV-2 isolation

Vero E6 cells were used for virus isolation and propagation of wild-type virus, D614G strain and Alpha variant, and Vero E6-TMPRSS2 overexpressed cells³⁷ were used for Beta, Delta and Omicron variant. Both cell-lines were cultured in DMEM with 10% FBS. The original clinical specimens were collected from SARS-CoV-2 confirmed patients in Hong Kong from January 2020 to November 2021 (Extended Data Table 1) and isolated as previously described³⁸. Viruses were isolated from clinical specimen of the nasopharyngeal and throat swab from patients infected with SARS-CoV-2 in virus transport medium. Vero E6-TMPRSS2 (E6/T2) cells seeded at 1×10^5 cells in 24-well plate were inoculated with 50uL of specimen and topped up with 2% FBS DMEM medium to 1mL for 1 hour at 37°C. Cells were washed once with PBS, replenished with fresh medium and observed daily for cytopathic effect (CPE). Culture supernatants were collected when the CPE reached around 50% and was defined as P1. The virus was further propagated in E6/T2 cells. The virus stock was aliquoted and stored frozen at -80°C. Aliquots were titrated to determine 50% tissue culture infection dose (TCID₅₀) in respective cell lines. The experiments were carried out in a Bio-safety level 3 (BSL-3) facility at the School of Public Health, LKS Faculty of Medicine, The University of Hong Kong. Informed consent was obtained from all subjects and approval

was granted by the Institutional Review Board (IRB) of the University of Hong Kong and the Hospital Authority (Hong Kong West) (IRB approval no: UW 20-862 and UW20-863).

***Ex vivo* cultures and infection of human respiratory tract**

Fresh non-tumour bronchus (n=12) and lung (n=12) tissues were obtained from patients aged 51-78 years undergoing elective surgery in Department of Surgery at Queen Mary Hospital (Pok Fu Lam, Hong Kong, China) from May to December 2021 and were removed as part of routine clinical care but surplus for routine diagnostic requirements as detailed previously.^{39,40} The donor information is listed in the Extended Data Table 2. The virus infection procedures were performed as previously described³⁸. Briefly, similar sized pieces of human bronchus and lung tissues were infected with each virus at 5×10^5 TCID₅₀/ml for 1 h at 37°C. Each tissue fragment was washed three times in culture medium to remove residual virus inoculum, topped up with fresh medium and incubated at 37°C as indicated. Mock-infected tissues served as negative controls. Aliquots of culture medium were removed at times indicated and stored at -80°C until titration. Infectious viral titres in culture supernatants were assayed by TCID₅₀ in Vero E6 or Vero E6-TMPRSS2 cells respectively, depending on the cells used for virus isolation and passage. Infected tissues were fixed in 10% formalin and processed for immuno-staining at 72 hours post infection. Bronchus tissues were also fixed in formalin and then processed for transmission electron microscopy as previously described⁴¹.

Viral titration by TCID₅₀ assay

A confluent 96-well tissue culture plates of Vero-E6 or Vero E6-TMPRSS2 cells was prepared one day before the virus titration (TCID₅₀) assay. Cells were washed once with PBS and replenished with DMEM (Gibco) with 2% foetal bovine serum (Gibco) supplemented with 100 units/ml penicillin and 100 µg/ml streptomycin (Gibco). Serial dilutions of virus supernatant, from 0.5 log to 7 log, were performed and each virus dilution added to the plates in quadruplicate. The plates were observed for cytopathic effect daily. The end-point of viral dilution leading to CPE in 50% of inoculated wells was estimated using the Karber method⁴². Area under the curve (AUC) was calculated from the viral titers from different time points indicated in the Y-axis.

Immunohistochemistry staining of paraffin-embedded tissues

Human respiratory tract tissues (lung and bronchus) were fixed with 10% formalin overnight at 4 °C and the fixed tissues were embedded in paraffin blocks. For immunohistochemistry, the 4µm sliced sections were microwaved for 15 min for antigen retrieval. Endogenous peroxidase activity was stopped by quenching the tissue sections with 3% H₂O₂ for 20 min. The slides were then blocked with 10% normal horse serum at room temperature (RT) and incubated with primary antibodies (SARS-CoV-2 Nucleoprotein (NP)(40143-T62, Sino Biological) or ACE2 (long form: ab108252; short form: ab15348, Abcam) antibodies for 90 min at RT followed by peroxidase (HRP)-conjugated anti-rabbit antibody (Vector Laboratory). The sections were developed using NovaRED Substrate Kit (Vector Laboratory). The cell nuclei were counterstained with Mayer's Hematoxylin.

To characterize the SARS-CoV-2-infected cells, double-antibody immunofluorescence staining of SARS-CoV-2 NP antibody with different cellular markers was performed. The tissue sections were first stained with SARS-CoV-2 NP antibody similarly as mentioned above, except after SARS-CoV-2 NP antibody incubation, the sections were incubated with alkaline phosphatase conjugated anti-rabbit or anti-mouse antibody (Vector Laboratory) and developed using Vector® Red (VR) Substrate Kit (Vector Laboratory). The sections were then microwaved, incubated with SCGB1A1/CC10 (Protein-tech), acetylated α Tubulin (Santa Cruz), MUC5AC (Thermo-Fisher), for 90 min at room temperature followed by goat anti rabbit-AF488 or goat anti mouse-AF488. The cell nuclei were counterstained with DAPI (blue). The sections were imaged using Nikon Eclipse Ti-S microscope.

Quantification of immunohistochemistry

Immunohistochemically stained specimens were analyzed by ImageJ Fiji software (version 2.1.0) as previously described^{43,44}. Briefly, each original IHC (RGB) image was split into single-colored images and Nova-red (SARS-CoV-2 NP) stained image was selected for the quantification of SARS-CoV-2 NP expression. The Nova-red (SARS-CoV-2 NP) stained signal was quantified after adjusting the threshold, which is set at the same value for all the images. The results are presented as frequency of expression and was calculated as percentage of positively stained area divided by the total image area of lung tissues or by the epithelium area of bronchial tissues.

Real-time PCR assay

The viral RNA in culture supernatants were extracted using QIAamp Viral RNA Mini Kit (Qiagen). RNA was reverse-transcribed by using random 6-mer primers with PrimeScript RT reagent Kit (Takara). mRNA expression of target genes was detected with the corresponding primers (Extended Data Table 3) using an ABI ViiA 7 real-time PCR system (Applied Biosystems). All procedures were performed according to the manufacturers' instructions. The viral gene and gene expression profiles of cell lysates were quantified and normalized with β -actin as previously described⁴⁵⁻⁴⁸.

Replication of SARS-CoV-2 on Vero-E6 and Vero-E6-TMPRSS2 and drug treatment

Vero-E6 and Vero-E6-TMPRSS2 cells in 48-well plates were infected with SARS-CoV-2 at a multiplicity of infection (MOI) of 0.01 for viral replication kinetics. Viral titers in culture supernatants (1-48 hpi) were determined using TCID₅₀ assay (see above). To assess effect of TMPRSS2 inhibitor and cathepsins inhibitor, the Vero-E6-TMPRSS2 cells were infected with SARS-CoV-2 at a MOI of 0.01. Camostat mesylate (Sigma), or E64d (Sigma) at 30 μ M, was added 1 h before, during and after infection. Vehicle was used as a negative control. Viral replication was evaluated by quantification of SARS-CoV-2 *ORF1b* copy number in the culture supernatants by using qPCR at 24 hpi⁴⁸.

Quantification of cell viability

Cell viability of Vero-E6-TMPRSS2 following the drug treatment was evaluated using Cell Counting Kit 8 (WST-8 / CCK8) (Abcam). In brief, cells grown to 70% confluency in 48-well plates were incubated for 24 h in the absence or presence of various concentrations (0.41-300 μ M) of camostat mesylate and (1.88-120 μ M) of E64d. Then, the culture medium

was aspirated and washed once with PBS, incubated with CCK8 working solution (CCK8: medium = 1:10) at 37 °C, 5% CO₂ for 2 h. Subsequently, OD values at 450 nm were measured by a multiplate reader (BMG FLUOstar OPTIMA). Cell viability was expressed as a percentage of that of the control cells.

Statistical analysis

Experiments with the human *ex vivo* cultures were performed independently with six different donors. Results shown in figures are geometric mean (+/-SD). Area-under-curve (AUC) was calculated by integrating infectious virus titres at 24-72 hpi in *ex vivo* bronchus, lung tissues or at 24-48 hpi in cell lines. The differences in log₁₀-transformed viral titres and quantitative viral RNA of *ORF1b* between viruses and over time were compared using two-way ANOVA followed by a Tukey's multiple-comparison test using GraphPad Prism version 9.1.2. Comparison of AUC and quantitative viral RNA of *ORF1b* between viruses were calculated using one-way ANOVA followed by a Tukey's multiple-comparison test. Differences were considered significant at a p value less than 0.05.

Reporting summary

Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

The sequences of all SARS-CoV-2 viruses used in this study are available in GISAID and the accession IDs are listed in Extended Data Table 1. The data generated during the current study are available in the source data file in this manuscript.

- 37 Matsuyama, S. *et al.* Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. *Proceedings of the National Academy of Sciences of the United States of America* **117**, 7001-7003, doi:10.1073/pnas.2002589117 (2020).
- 38 Hui, K. P. Y. *et al.* Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures. *The Lancet. Respiratory medicine* **8**, 687-695, doi:10.1016/s2213-2600(20)30193-4 (2020).
- 39 Chan, M. C. *et al.* Tropism and innate host responses of the 2009 pandemic H1N1 influenza virus in ex vivo and in vitro cultures of human conjunctiva and respiratory tract. *The American journal of pathology* **176**, 1828-1840, doi:10.2353/ajpath.2010.091087 (2010).
- 40 Hui, K. P. *et al.* Tropism and innate host responses of influenza A/H5N6 virus: an analysis of ex vivo and in vitro cultures of the human respiratory tract. *The European respiratory journal* **49**, doi:10.1183/13993003.01710-2016 (2017).
- 41 Yen, H. L. *et al.* Cellular tropism of SARS-CoV-2 in the respiratory tract of Syrian hamsters and B6.Cg-Tg(K18-ACE2)2Prln/J transgenic mice. *Veterinary pathology*, 3009858211043084, doi:10.1177/03009858211043084 (2021).
- 42 Kärber, G. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Naunyn-Schmiedeberg's Archiv für experimentelle Pathologie und Pharmakologie* **162**, 480-483, doi:10.1007/BF01863914 (1931).
- 43 Crowe, A. R. & Yue, W. Semi-quantitative Determination of Protein Expression Using Immunohistochemistry Staining and Analysis: An Integrated Protocol. *Bio-protocol* **9**, e3465, doi:10.21769/BioProtoc.3465 (2019).
- 44 Berglin, L. *et al.* In situ characterization of intrahepatic non-parenchymal cells in PSC reveals phenotypic patterns associated with disease severity. *PloS one* **9**, e105375, doi:10.1371/journal.pone.0105375 (2014).
- 45 Chan, R. W. *et al.* DAS181 inhibits H5N1 influenza virus infection of human lung tissues. *Antimicrob Agents Chemother* **53**, 3935-3941, doi:AAC.00389-09 [pii] 10.1128/AAC.00389-09 (2009).
- 46 Hui, K. P. *et al.* H5N1 influenza virus-induced mediators upregulate RIG-I in uninfected cells by paracrine effects contributing to amplified cytokine cascades. *The Journal of infectious diseases* **204**, 1866-1878, doi:10.1093/infdis/jir665 (2011).

- 47 Hui, K. P. *et al.* Induction of proinflammatory cytokines in primary human macrophages by influenza A virus (H5N1) is selectively regulated by IFN regulatory factor 3 and p38 MAPK. *J Immunol* **182**, 1088-1098, doi:10.4049/jimmunol.182.2.1088 (2009).
- 48 Bui, C. H. T. *et al.* Tropism of SARS-CoV-2, SARS-CoV, and Influenza Virus in Canine Tissue Explants. *J Infect Dis* **224**, 821-830, doi:10.1093/infdis/jiab002 (2021).

Acknowledgements Timmy Shack, Hin-Wo Yeung, Cassia Lin (School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China) and Kevin Fung (Department of Pathology, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China) provided technical support. We acknowledge research funding from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services (Contract No. 75N93021C00016) and the Theme-Based Research Scheme (Ref: T11-705/21N and T11-712/19-N) under University Grants Committee of Hong Kong Special Administrative Region. The funding sources had no roles in the writing of the manuscript or the decision to submit it for publication. The authors have not been paid to write this article by a pharmaceutical company or other agency. The authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. All data generated or analysed during this study are included in this published article. The funding source had no role in study design, data collection, analysis, or interpretation of the data, or in the writing of the report. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Author contributions KPYH: study design, coordination, analysis, interpretation of results, perform experiments and writing of the manuscript; JCWH, MCC, KCN, RHHC, KLL, TTK: perform experiments, analysis and interpretation of results; HG: perform analysis and interpretation of sequencing results; KYS, MKYH, TWKA: provide human lung and bronchus tissue and critical review of the manuscript; LLMP: analysis and interpretation of

sequencing results and critical review of the manuscript; MP: analysis and interpretation of results, writing and revision of the manuscript; JMN: analysis, co-ordination of sample collection, and interpretation of results from immunohistochemical staining and critical review of the manuscript; MCWC: study design, overall coordination, interpretation of results and writing of the manuscript.

Competing interests The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at

Correspondence and requests for materials should be addressed to Michael CW Chan

Peer review information *Nature* thanks Peter Openshaw and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Figure 1. Viral replication kinetics of SARS-CoV-2 variants in *ex vivo* cultures of human respiratory tract. Human *ex vivo* cultures of bronchus and lung were infected with 5×10^5 TCID₅₀/mL at 37°C. Virus released in the culture supernatants were measured over time by TCID₅₀ assay. a, c. Viral replication kinetics of wild-type (WT), D614G, Alpha and Beta in human *ex vivo* cultures of bronchus and lung. b, d. Viral replication kinetics of wild-type (WT), Delta and Omicron in human *ex vivo* cultures of bronchus and lung. The horizontal dotted line denotes the limit of detection in the TCID₅₀ assay. Data are presented as geometric mean values (n=6) (+/-SD). Statistics were performed using Two-way ANOVA followed by Tukey's test. Statistical significances were defined as $p < 0.05$ and exact p values are presented. e, f. Viral titers from a to d are depicted as area under the curve (AUC). Bar-charts show the geometric mean (n=6) (+/-SD). Statistical significances were defined as $p < 0.05$ and exact p values are presented. Statistics were performed using One-way ANOVA followed by Tukey's test.

Figure 2. Tissue tropism of SARS-CoV-2 variants in *ex vivo* cultures of human respiratory tract. *Ex vivo* cultures of a. human bronchus and b. human lung infected with wild-type (WT) and various variants of SARS-CoV-2 with 5×10^5 TCID₅₀/mL or mock at 37°C. The tissues were fixed with formalin at 72 hpi. Paraffin-embedded sections were subjected to immunohistochemical staining with a polyclonal antibody against the SARS-CoV-2 nucleoprotein. Positive cells are red-brown. Scale bar, 100 μm. The images are representative of two individual donors.

Figure 3. Cellular tropism of SARS-CoV-2 variants in *ex vivo* cultures of human bronchus. *Ex vivo* cultures of human bronchus were infected with SARS-CoV-2 wild-type (WT), Alpha, Beta, Delta, Omicron or mock. At 72 hpi the tissues were fixed with formalin, embedded in paraffin and stained for immunofluorescence (green) for indicated cell markers: acetyl- α -tubulin-positive for ciliated cells, mucin 5AC-positive for secretory goblet cells and club cell protein 10-positive for club cells; a monoclonal antibody against the SARS-CoV-2 nucleoprotein (red) and 4',6-diamidino-2-phenylindole-positive for nuclei (blue). The images are representative of two individual donors. Scale bar, 100 μ m.

Fig. 4 | See next page for caption.

Figure 4. ACE2 expression of respiratory tissues, TMPRSS2 and cathepsins dependence of SARS-CoV-2 variants. a. mRNA expression of both long and short, short, long form of ACE2 and TMPRSS2 was measured by real-time PCR. Fold-change of bronchus (n=6) and lung (n=8) was normalized with the average gene expression of lung tissues. Data are presented as mean (+/-SD). Statistics were performed using unpaired two-sided Student's t-test. b, c. Immunohistochemical staining on Paraffin-embedded sections with antibodies distinguishing non-specific and long form of ACE2 in bronchus and lung. Positive cells are red-brown. Scale bar, 100 μ m. The images are representative of two individual donors. d. Viral replication kinetics of Wild-type (WT), Delta and Omicron in Vero E6 (E6) and Vero E6-TMPRSS2 (T2) cells by TCID₅₀ assay. Cells were infected with SARS-CoV-2 viruses at MOI 0.01. Bar-charts show geometric mean virus titer (n=3) (+/-SD). The horizontal dotted line denotes the detection limit of TCID₅₀ assay. Statistics were performed using Two-way ANOVA followed by Tukey's test. e. Viral titers from d are depicted as area under the curve (AUC). Bar-charts show the mean (n=3) (+/-SD). Statistics were performed using One-way ANOVA followed by Tukey's test. f. Effects of viral replication by treatment with camostat mesylate (Cam) and E64d. T2 cells were pretreated with Cam, E64d or combined of two for 1 h. Infection of WT, Delta and Omicron was performed at MOI 0.01. After infection, the cells were replenished with fresh medium with the drug. *ORF1b* gene copies were measured in culture supernatants at 24 hpi by real-time PCR. Bar-charts show the mean percentages of *ORF1b* gene copies using vehicle treatment as reference. Data are mean (n=6) (+/-SD). Statistics were performed using One-way ANOVA followed by Tukey's test. Statistical significances were defined as p<0.05 and exact p values are presented.

a**Bronchus** **b****Lung**

Extended Data Figure 1. Viral replication kinetics of SARS-CoV-2 variants in *ex vivo* cultures of human respiratory tract from individual donors. Human *ex vivo* cultures of bronchus and lung were infected with 5×10^5 TCID₅₀/mL at 37°C. Virus released in the culture supernatants were measured over time by TCID₅₀ assay. Viral replication kinetics of wild-type (WT), Delta and Omicron in human *ex vivo* cultures of a. bronchus and b. lung from individual donors are shown. The horizontal dotted line denotes the limit of detection in the TCID₅₀ assay. Bar-charts show the geometric mean.

Extended Data Figure 2. Viral RNA of SARS-CoV-2 variants in *ex vivo* cultures of human respiratory tract. Human *ex vivo* cultures of bronchus and lung were infected with 5×10^5 TCID₅₀/mL at 37°C. Viral RNA of *ORF1b* gene released in the culture supernatants were measured over time by real time PCR. Viral *ORF1b* gene copies of wild-type (WT), Delta and Omicron in human *ex vivo* cultures of bronchus and lung are shown. Bar-charts show the mean (n=4) (+/-SD). Statistics were performed using Two-way ANOVA followed by a Tukey's multiple-comparison test. $p < 0.05$ was considered as statistically significant and exact p values are shown.

a Bronchus

b Lung

Extended Data Figure 3. Quantification of viral antigen staining of SARS-CoV-2 variants in *ex vivo* cultures of human bronchus and lung. *Ex vivo* cultures of human bronchus and human lung were infected with wild-type (WT) and various variants of SARS-CoV-2 with 5×10^5 TCID₅₀/mL or mock at 37°C and the tissues were fixed with formalin at 72 hpi. Paraffin-embedded sections were subjected to immunohistochemical staining with a polyclonal antibody against the SARS-CoV-2 nucleoprotein (SARS-2 NP). The expression of SARS-2 NP in a. bronchus for wild-type (WT) (n=4), D614G (n=3), Alpha (n=5), Beta, (n=2), Delta (n=2), Omicron (n=3) and mock (n=2) and b. lung for wild-type (WT) (n=5), D614G (n=5), Alpha (n=2), Beta, (n=2), Delta (n=3), Omicron (n=3) and mock (n=2) is presented. Bar-charts show the mean (+/-SD). Statistics were performed using One-way ANOVA followed by a Tukey's multiple-comparison test and samples with sample number < 3 were excluded in the test. $p < 0.05$ was considered as statistically significant and exact p values are shown.

Extended Data Figure 4. Omicron viral particles in human bronchial mucosa. Transmission electron micrograph of human bronchial mucosa infected with SARS-CoV-2 Omicron variant. Tissues were fixed after 72 hours in formalin, followed by processing into Epon, ultrathin sectioning and staining. Ciliated cells are present and there are virus particles present in membrane bound vesicles in the cytoplasm indicated by red boxes, as well as on the surface attached to microvilli indicated by blue boxes. Micrograph is a representative photo of three individual donors. Scale bar: 1 μ m.

Extended Data Figure 5. Viral replication kinetics of SARS-CoV-2 variants in Vero E6-TMPRSS2 cells.

Vero E6-TMPRSS2 cells were infected with SARS-CoV-2 wild-type (WT), Delta and Omicron at MOI 0.01. Virus released in culture supernatants were measured over time by TCID₅₀ assay. The horizontal dotted line denotes the limit of detection in the TCID₅₀ assay. Bar-charts show the geometric mean of three independent experiments (n=3) (+/-SD). Statistics were performed using Two-way ANOVA followed by a Tukey's multiple-comparison test. p<0.05 was considered as statistically significant and exact p values are shown.

Extended Data Figure 6. Viability of Vero E6-TMPRSS2 cells in the presence of camostat mesylate and E64d. Vero E6-TMPRSS2 cells were treated with a. camostat mesylate (Cam) from 0.41 to 300 μ M or b. E64d from 1.88 to 120 μ M for 48 h. Cell viability was measured using Cell Counting Kit 8. Untreated cells were used as 100 % viability. Results are mean of three independent experiments (n=3) (+/-SD). The half-maximal inhibitory concentration (IC₅₀) was calculated by using Prism.

Extended Data Table 1 | Information of virus isolates

Lineage (Variant)	Strain name	Genbank accession ID
A (WT)	hCoV-19/Hong Kong/WHV-HK61-P3/2020	OM403304
B.1.1.63 (D614G)	hCoV-19/Hong Kong/2161-P5/2020	OM403305
B.1.1.7 (Alpha)	hCoV-19/Hong Kong/3268-P2/2020	OM403306
B.1.351 (Beta)	hCoV-19/Hong Kong/4527-P2/2021	OM403307
B.1.617.2 (Delta)	hCoV-19/Hong Kong/VOC0013P2D5/2021	OM403308
B.1.1.529 (Omicron)	hCoV-19/Hong Kong/VOC0195-P2/2021	OM403309

Extended Data Table 2 | Information of tissue donor

PATIENT	TISSUE USED	VIRUSES USED	AGE	GENDER
1	Lung	WT, D614G, Alpha, Beta	66	M
2	Lung	WT, D614G, Alpha, Beta	74	F
3	Lung	WT, D614G, Alpha, Beta	71	F
4	Lung	WT, D614G, Alpha, Beta	67	F
5	Lung	WT, D614G, Alpha, Beta	70	M
6	Lung	WT, D614G, Alpha, Beta	62	F
7	Lung	WT, Delta, Omicron	73	M
8	Lung	WT, Delta, Omicron	57	F
9	Lung	WT, Delta, Omicron	59	F
10	Lung	WT, Delta, Omicron	78	F
11	Lung	WT, Delta, Omicron	63	M
12	Lung	WT, Delta, Omicron	51	F

Extended Data Table 3 | Primer sequences used for quantitative RT-PCR

Target gene	Primer sequences (5'- 3')
Actin, beta (ACTB)	Forward: TGGATCAGCAAGCAGGAGTATG Reverse: GCATTTGCGGTGGACGAT
SARS-CoV-2, open reading frame 1b (ORF1b)	Forward: TGGGGYTTTACRGGTAACCT Reverse: AACRCGCTTAACAAAGCACTC
Angiotensin converting enzyme 2 (ACE2, non-specific forms)	Forward: TGGGACTCTGCCATTTACTTAC Reverse: CCAACTATCTCTCGCTTCATC
ACE2 (short form)	Forward: GTGAGAGCCTTAGGTTGGATTC Reverse: TAAGGATCCTCCCTCCTTTGT
ACE2 (long form)	Forward: CAAGAGCAAACGGTTGAACAC Reverse: CCAGAGCCTCTCATTGTAGTCT
Transmembrane serine protease 2 (TMPRSS2)	Forward: CAGACCAGGAGTGTACGGGAAT Reverse: TCTGCCCTCATTTGTCGATAAA

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Data collection GraphPad Prism v9.1.2 were used to produce figures; ImageJ Fiji software (version 2.1.0) were used to measure intensities of IHC photos.

Data analysis GraphPad Prism v9.1.2 were used for statistical analyses; ImageJ Fiji software (version 2.1.0) were used to analyse intensities of IHC photos.

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Sample size	For all experiments were performed at least three times to provide $n =$ or >3 . The sample number has been indicated in the figure legends. The $n > 3$ was chosen so that statistical analysis can be performed to determine significances.
Data exclusions	There is no data exclusions.
Replication	All in vitro experiments were performed at least three times independently. Some in vitro experiments had biological replicates which were defined as cells culturing in different wells and samples collected from each well were processed and analyzed separately. Reproducible data are obtained.
Randomization	N/A, Recruitment of individual donor for ex vivo infection was random and based on accessibility. There is no randomization for in vitro experiments since the cell line exhibited a distinct genotype and phenotype.
Blinding	The experiments and sample processing were performed by different individuals. Sample identities were blinded during data analysis.

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Materials & experimental systems

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☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

- Immunocytochemistry/Immunohistochemistry (Paraffin) SARS-CoV/SARS-CoV-2 Nucleocapsid Antibody Polyclonal Rabbit 40143-T62 Sino Biological 1:2000
- Immunohistochemistry (Paraffin) Recombinant Anti-ACE2 antibody Monoclonal Rabbit [EPR4435(2)] ab108252 abcam 1:200
- Immunohistochemistry (Paraffin) Anti-ACE2 antibody Polyclonal Rabbit ab108252 abcam 1:200
- Immunocytochemistry SARS-CoV/SARS-CoV-2 Nucleocapsid Antibody Monoclonal Mouse 40143-MM05 [IgG1 Clone #05] Sino Biological 1:1000
- Immunocytochemistry Anti-acetylated α Tubulin Antibody (6-11B-1) Monoclonal Mouse [6-11B-1] sc-23950 Santa Cruz Biotechnology 1:100
- Immunocytochemistry MUC5AC Monoclonal Antibody (45M1) Monoclonal Mouse MA5-12178 [45M1] Thermo Fisher Scientific 1:50
- Immunocytochemistry Uteroglobin/CC10 Polyclonal antibody Polyclonal Rabbit 10490-1-AP proteintech 1:100
- Immunocytochemistry Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 Polyclonal Goat # A11001 Thermo Fisher Scientific 1:1000
- Immunocytochemistry Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 Polyclonal Goat # A11034 Thermo Fisher Scientific 1:1000
- Immunohistochemistry (Paraffin) ImmPress Horse Anti Rabbit IgG Kit MP-7401-50 Vector Laboratory
- Immunohistochemistry (Paraffin) ImmPress AP Anti-Rabbit Ig Kit MP-5401-15 Vector Laboratory
- Immunohistochemistry (Paraffin) ImmPress AP Anti-Mouse Ig Kit MP-5402-15 Vector Laboratory

Validation

All antibodies used in this work were commercially developed. Information about validation as well as references using same antibodies are available on the manufacturer's websites. The applications of antibodies are listed below:

1. SARS-CoV/SARS-CoV-2 Nucleocapsid Antibody Polyclonal Rabbit 40143-T62 Sino Biological 1:2000
 - Validated applications: ELISA, WB
2. Recombinant Anti-ACE2 antibody Monoclonal Rabbit [EPR4435(2)] ab108252 abcam 1:200
 - Validated applications: ELISA, WB, IP, IHC-P
3. Anti-ACE2 antibody Polyclonal Rabbit [EPR4435(2)] ab15348 abcam 1:200
 - Validated applications: ELISA, WB, ICC/IF, IHC (PFA fixed), IHC-Fr, IHC-P
4. SARS-CoV/SARS-CoV-2 Nucleocapsid Antibody Monoclonal Mouse 40143-MM05 [IgG1 Clone #05] Sino Biological 1:1000. Validated applications: ELISA, WB, IHC-P, Flow
5. Anti-acetylated α Tubulin Antibody (6-11B-1) Monoclonal Mouse [6-11B-1] sc-23950 Santa Cruz Biotechnology 1:100. Validated applications: WB, IP, IHC-P, IF
6. MUCSAC Monoclonal Antibody (45M1) Monoclonal Mouse MA5-12178 [45M1] Thermo Fisher Scientific 1:50
 - Validated applications: IHC-P, IF
7. Uteroglobin/CC10 Polyclonal antibody Polyclonal Rabbit 10490-1-AP proteintech 1:100
 - Validated applications: ELISA, IHC-P, IF, Flow
8. Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 Polyclonal Goat # A11001 Thermo Fisher Scientific 1:1000. Validated applications: ICC/IF, Flow
9. Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 Polyclonal Goat # A11034 Thermo Fisher Scientific 1:1000. Validated applications: IF, Flow
10. ImmPress Horse Anti Rabbit IgG Kit MP-7401-50 Vector Laboratory
 - Validated applications: ELISA, IHC, ICC
11. ImmPress AP Anti-Rabbit Ig Kit MP-5401-15 Vector Laboratory
 - Validated applications: ELISA, IHC, ICC
12. ImmPress AP Anti-Mouse Ig Kit MP-5402-15 Vector Laboratory
 - Validated applications: ELISA, IHC, ICC

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

Vero E6 cells from ATCC.
Vero E6-TMPRSS2 cells were developed and provided by Dr. Makoto Takeda (Matsuyama, S. et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. Proceedings of the National Academy of Sciences of the United States of America 117, 7001-7003, doi:10.1073/pnas.2002589117 (2020).

Authentication

None of the cell lines used were authenticated.

Mycoplasma contamination

All cell lines used were regularly tested for mycoplasma contamination and were mycoplasma free.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified lines were used in this study.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

The tissues came from those patients under surgery for tumor removal from the lung. The patient characteristics are provided in the Extended Data Table 2.

Recruitment

Recruitment of individual donor for ex vivo infection was random and based on accessibility. The tissues came from those patients under surgery for tumor removal from the lung.

Ethics oversight

Informed consent was obtained from all subjects and approval was granted by the Institutional Review Board (IRB) of the University of Hong Kong and the Hospital Authority (Hong Kong West) (IRB approval no: UW 20-862 and UW20-863).

Note that full information on the approval of the study protocol must also be provided in the manuscript.