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Attenuated replication and pathogenicity of SARS-CoV-2 B.1.1.529 Omicron

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SARS-CoV-2 Omicron emerged in November 2021 and is rapidly spreading among the human population¹. While recent reports reveal that the Omicron variant robustly escapes from vaccine and therapeutic neutralization antibodies²⁻¹⁰, the pathogenicity of the virus remains unknown. Here we show that the replication of the Omicron variant is dramatically attenuated in Calu3 and Caco2 cells. Further mechanistic investigations reveal that the Omicron variant is inefficient in transmembrane serine protease 2 (TMPRSS2) usage in comparison to that of WT and previous variants, which may explain

its reduced replication in Calu3 and Caco2 cells. Omicron replication is markedly attenuated in both the upper and lower respiratory tract of infected K18-hACE2 mice in comparison to that of WT and Delta variant, which results in its dramatically ameliorated lung pathology. When compared with SARS-CoV-2 WT, Alpha, Beta, and Delta variant, infection by the Omicron variant causes the least body weight loss and mortality rate. Overall, our study demonstrates that the Omicron variant is attenuated in virus replication and pathogenicity in mice in comparison with WT and previous variants.

Coronavirus Disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)^{11,12}. Since its emergence in 2019, SARS-CoV-2 continues to evolve by acquiring mutations that increase transmissibility, modulate pathogenicity, or confer resistance to neutralization antibodies. A new SARS-CoV-2 variant (PANGO lineage B.1.1.529) was reported on 24 November 2021 from Botswana and South Africa. Within 2 days, the Technical Advisory Group on Virus Evolution (TAG-VE) of the World Health Organization (WHO) classified B.1.1.529 as the fifth variant of concern (VOC), and designated it as the Omicron variant¹.

The Omicron variant is characterized by an unusually large number of mutations in the spike protein, including 30 amino acid substitutions, three short deletions, and one insertion, compared with the original SARS-CoV-2. Among these changes, 15 of the amino acid substitutions are located in the receptor-binding domain (RBD) with additional amino acid substitutions located at or near the furin-like cleavage site at the S₁/S₂ junction and S2 that may modulate host protease cleavage by furin and transmembrane serine proteases. The abundant mutations at these regions hinted that the Omicron variant may escape from neutralizing

antibody in convalescent or vaccinated sera, and modify its capacity in cell entry, replication, and pathogenesis.

Preliminary reports suggest that the Omicron variant is highly transmissible, even in fully vaccinated individuals and individuals that received a booster dose¹³⁻¹⁵. These results are congruous with recent findings that the Omicron variant is markedly resistant to neutralization by sera not only from convalescent patients, but also from individuals vaccinated with many widely used COVID-19 vaccines²⁻⁷. In addition, the neutralization capacity of monoclonal antibodies (mAbs) in clinical use against COVID-19 is abolished or severely impaired against the Omicron variant^{8,10,16}. In this regard, the Omicron variant represents a critical threat to the pandemic control and disease treatment of the COVID-19 pandemic.

The number of infections caused by the Omicron variant is growing rapidly with a propensity to replace the Delta variant as the predominant circulating SARS-CoV-2 variant. In contrast to its capacity of escaping antibody neutralization, whether or not the Omicron variant can cause milder or more severe disease remains unknown. In this study, we evaluated the virological features and pathogenesis of the Omicron variant in vitro and in vivo, and compared the results with that of SARS-CoV-2 wildtype (WT) and other VOCs. Our results demonstrate that the replication capacity of the Omicron variant is significantly attenuated both in vitro and in vivo compared with SARS-CoV-2 WT and VOCs, which is explained by its lowered efficiency of transmembrane serine protease 2 (TMPRSS2) usage. In the K18-hACE2 mouse model, the Omicron variant replicated significantly less efficiently than SARS-CoV-2 WT and Delta variant in both nasal turbinates and lungs, and induced substantially attenuated lung pathology. Finally, by comparing body weight loss and survival, our results suggest that the Omicron variant is

attenuated in pathogenicity when compared with SARS-CoV-2 WT and previous VOCs including Alpha, Beta, and Delta.

Attenuated replication of the Omicron variant

The Delta variant replaced all VOC and was the predominant circulating SARS-CoV-2 variant since mid-2021. Infection caused by the Omicron variant is increasing rapidly since late November 2021, which may become the next predominant circulating SARS-CoV-2 variant. We first compared the replication efficiency of the Omicron variant with that of SARS-CoV-2 WT, Alpha, Beta, and Delta variants. We included Omicron (R346K) in the comparison, which contains a R346K substitution in the spike protein that represents 8.5% (956/11242) of the total Omicron variant sequences deposited into GISAID database as of 19th December 2021. By measuring the subgenomic envelope (sgE) gene that represents replication intermediates, our results suggested that the replication of the Omicron variant was severely attenuated in the Calu3 human lung epithelial cells. Area under the curve (AUC) quantification of the sgE gene generated over a period of 48 hours suggested that Omicron and Omicron (R346K) replicated 3.4- and 4.2-fold less efficiently comparing to SARS-CoV-2 WT (**Fig. 1a**). In contrast, the Alpha, Beta, and Delta variants replicated to similar or higher levels in comparison to SARS-CoV-2 WT in Calu3 cells (**Fig. 1a**). We next measured the amount of infectious virus particles produced from SARS-CoV-2 WT- and variants-infected Calu3 cells. In keeping with the sgE gene results, we demonstrated that while Alpha, Beta, and Delta variants produced similar or higher levels of infectious virus particles between 8-48 hours post infection (hpi), the Omicron variant produced significantly less infectious virus particles than that of SARS-CoV-2 WT from Calu3 cells (**Fig. 1b**). At 48 hpi, the infectious virus titres of Omicron and Omicron (R346K)

were 501.4-fold ($p=0.0163$) and 114.7-fold ($p=0.0168$) lower than that of SARS-CoV-2 WT, respectively (**Fig. 1b**). In parallel, we evaluated the replication of the Omicron variant in Caco2 human intestinal epithelial cells. Similar to the results from Calu3 cells, the replication of the Omicron variant was dramatically lower in comparison with that of SARS-CoV-2 WT, Alpha, Beta, and Delta variants (**Extended Data Fig. 1a**). At 48 hpi, the infectious virus titres of Omicron and Omicron (R346K) were 407.2-fold ($p=0.0123$) and 238.1-fold ($p=0.0124$) lower than that of SARS-CoV-2 WT, respectively (**Fig. 1c**). Interestingly, in VeroE6 cells, the replication of the Omicron variant was reduced to a much lesser extent in comparison to that of SARS-CoV-2 WT, and was at a comparable level to the Delta variant (**Fig. 1d and Extended Data Fig. 1b**). Next, we evaluated the cytopathic effect of the Omicron variant in VeroE6-TMPRSS2 cells. Our results demonstrated that both Omicron and Omicron (R346K) were less cytopathic than SARS-CoV-2 WT as evidenced by the significantly higher cell viability between 12-36 hpi (**Fig. 1e**). Overall, these findings indicate that the omicron variant replicates less efficiently than SARS-CoV-2 WT, Alpha, Beta, and Delta variants, and induces a comparatively lower level of cell damage in the infected cells.

Mechanism of the attenuated replication of the Omicron variant

Our live virus infection assays demonstrate that the replication of Omicron variant is markedly attenuated in Calu3 and Caco2 cells in comparison to SARS-CoV-2 WT and other variants, while in VeroE6 cells, Omicron replication is only modestly lowered. Our results are in line with a recent preprint that showed a decrease in pseudovirus entry of the Omicron variant in Calu3 and Caco2 cells but not Vero, 293T, or A549-ACE2 cells¹⁷. Previous reports have demonstrated that SARS-CoV-2 enters Calu3 and Caco2 cells through the plasma membrane

entry pathway mediated by TMPRSS2, while the virus enters TMPRSS2-deficient cells (Vero, VeroE6, 293T, A549) through the endocytic pathway mediated by cathepsin L or other endosomal proteases¹⁸⁻²⁰. Combining previous knowledge and current evidence from us and others, we postulate that the spike mutations of the Omicron variant might result in a reduced capacity in TMPRSS2 usage, which severely impaired their entry and replication in Calu3 and Caco2 but not in VeroE6 cells. To evaluate this possibility, we assessed the entry of pseudovirus carrying the spike protein of SARS-CoV-2 WT, Alpha, Beta, Delta, or Omicron variant side-by-side in 293T cells transfected with ACE2 with or without additional TMPRSS2 overexpression. Our result demonstrated that TMPRSS2 overexpression increased SARS-CoV-2 WT, Alpha, Beta, and Delta pseudovirus entry in 293T-ACE2 cells by 11.3-, 12.6-, 11.7-, and 16-folds, respectively (**Fig. 2a**). In comparison, TMPRSS2 overexpression increased Omicron pseudovirus entry by only 4.3-folds, which was significantly lower than that of SARS-CoV-2 WT or other variants ($p<0.0001$) (**Fig. 2a**). To further validate this result, we compared pseudovirus entry in VeroE6 and VeroE6-TMPRSS2 cells side-by-side. Our results demonstrated that TMPRSS2 expression in the VeroE6-TMPRSS2 stable cell-line increased pseudovirus entry for SARS-CoV-2 WT, Alpha, Beta, and Delta variant by 16.1- ($p<0.0001$), 7.9- ($p=0.0005$), 32.1- ($p<0.0001$), and 48.7-folds ($p<0.0001$), respectively. In contrast, the Omicron pseudovirus was less sensitive to TMPRSS2 expression and its entry was marginally increased by only 1.3-fold ($p=ns$) in VeroE6-TMPRSS2 cells when compared to VeroE6 cells (**Fig. 2b**). Next, we evaluated pseudovirus entry on VeroE6-TMPRSS2 cells in the presence of camostat, a potent TMPRSS2 inhibitor. Our data showed that the inhibitory effect of camostat on Omicron pseudovirus entry was significantly reduced in comparison to that of WT, Alpha, Beta, or Delta pseudovirus (**Fig. 2c**). Camostat was similarly less potent in inhibiting the virus replication of Omicron and

Omicron (R346K) comparing to WT and other variants in VeroE6-TMPRSS2 cells (**Fig. 2d**). In line with these results, we detected a reduced spike cleavage of Omicron comparing to that of SARS-CoV-2 WT and Delta in the infected VeroE6-TMPRSS2 cells (**Extended Data Fig. 2**). Collectively, our results indicate that the reduced replication capacity of the Omicron variant in cells that the virus enters through the plasma membrane entry pathway, including Calu3, Caco2, and VeroE6-TMPRSS2, is caused by its reduced spike cleavage, leading to inefficient recognition by TMPRSS2.

Attenuated replication and pathogenesis of the Omicron variant in vivo

To explore whether the Omicron variant is also attenuated in vivo, we first compared virus propagation of the Omicron variant to SARS-CoV-2 WT and Delta variant in the K18-hACE2 transgenic mouse model. 6- to 8-week-old hACE2 transgenic mice were intranasally challenged with SARS-CoV-2 WT, Delta, or Omicron variant, and their tissues were collected for virological assessment. Our results demonstrated that the infection of WT SARS-CoV-2 was very robust along the respiratory tract of the hACE2 transgenic mice as evidenced by the high RdRp gene copy numbers and Delta variant propagated to a largely comparable level (**Fig. 3a**). In stark contrast, the viral RdRp gene copies in the nasal turbinate of mice challenged with the Omicron variant was markedly reduced by 1138- ($p=0.0324$) and 1821-folds ($p=0.001$) compared to those with WT and Delta variant at 2 dpi, respectively (**Fig. 3a**), despite using the same inoculation titres for virus challenge among the aforementioned variants. At 4 dpi, the viral gene copies in the Omicron-infected mouse nasal turbinate were reduced by approximately 10-fold compared to mice infected with WT or Delta variant. Similarly, the RdRp gene copies were

also significantly lower in the lung of the Omicron variant-infected mice at both time points in comparison with their counterparts challenged with WT or Delta variant (**Fig. 3a**).

To understand whether the reduction of viral gene production was due to attenuated virus replication, we quantified the sgRNA of the SARS-CoV-2 E gene. Our results showed that the subgenomic E gene in the nasal turbinate of the Omicron variant-infected mice was significantly lower than that of WT and Delta variant at both 2 and 4 dpi (**Fig. 3b**). In particular, at 2 dpi, the sgRNA level was diminished by 442- ($p=0.0129$) and 1450-folds ($p<0.0001$) compared to mice infected with WT and Delta variant, respectively (**Fig. 3b**). Consistently, sgRNA synthesis was also lower in the lungs of Omicron-infected mice when compared with those infected with WT or Delta variant, although to a lesser extent than in nasal turbinates (**Fig. 3b**). In keeping with the RdRp and subgenomic E gene results, the infectious virus titre in both nasal turbinate and lung samples of Omicron variant-infected mice was significantly lower than that of the WT-infected mice (**Fig. 3c**). Intriguingly, the infectious virus titre at the nasal turbinate of Omicron variant-infected mice was 48-fold ($p=0.0476$) lower than that of the Delta variant, suggesting less infectious virus shedding from the Omicron-infected nasopharynx than that by the Delta variant at 2 dpi (**Fig. 3c**). Consistently, viral gene copies in the nasal wash harvested from the Omicron-infected transgenic mice were also reduced compared to samples harvested from the other two groups (**Extended Data Fig. 3**). Since the Omicron variant also carries the N501Y mutation, which allows the infection of wildtype mice and rats as we and others previously reported^{21,22}, we also characterized the replication of the N501Y-carrying Alpha and Omicron variant in wildtype C57B6 mice side-by-side. Consistent with the findings in hACE2 transgenic mice, the replication of the Omicron variant in nasal turbinates and lungs of wildtype C57B6 mice was significantly attenuated when compared with that of the Alpha variant (**Extended Data Fig. 4**).

In parallel with the virological assessments, we quantified the gene expression of IP-10 and IFN γ , which are hallmark proinflammatory cytokines induced by SARS-CoV-2 infection^{23,24}. We found that the transcription level of IP-10 and IFN γ in both nasal turbinates and lungs of Omicron variant-infected mice were significantly down-regulated when compared to those challenged with WT and Delta variant, which represented an alleviated proinflammatory response during the infection by the Omicron variant (**Fig. 3d**).

Next, we investigated the infection outcome of the Omicron variant in comparison to SARS-CoV-2 WT, Alpha, Beta, and Delta variant in K18-hACE2 mice. Our results showed that when compared to WT and previously emerged variants, the body weight loss of mice infected by Omicron was significantly milder with onset time at a later stage during the course of infection (**Fig. 3e**). Importantly, we took advantage of the K18-hACE2 infection model and compared animal survival upon infection with SARS-CoV-2 WT and variants. Under the same inoculum conditions, mouse survival was lowest for the Alpha variant (0%), followed by WT (20%), Beta (33%), Delta (44%), and was highest for the Omicron variant (57%) (**Fig. 3f**). Statistically, mouse survival for Omicron was significantly higher than Alpha ($p=0.0001$) and WT ($p=0.0377$), but did not reach significance for Beta and Delta. Nevertheless, the trend of mouse survival was in keeping with results of body weight loss, which demonstrated a propensity of better survival in the order of Alpha, WT, Beta, Delta, and Omicron (**Fig. 3f**).

To evaluate the pathogenicity of Omicron infection in the respiratory tract, we carried out histopathological analysis of the lung tissue of infected hACE2 transgenic mice. In corroboration with findings in the virological assessment, multi-focal expression of the viral nucleocapsid (N) protein was frequently detected in both WT and Delta variant-infected mice, but not in those challenged with the Omicron variant at 2 dpi (**Fig. 4a and Extended Data Fig. 5**). At 4 dpi,

abundant N protein continued to accumulate in the alveoli of mice infected with WT and Delta variant. However, only a small amount of N protein expression was sparsely detected in the lung of mice infected with the Omicron variant (**Fig. 4a and Extended Data Fig. 5**). Histological examination of both WT- and Delta variant-infected mouse lungs revealed prominent pathological changes in the alveoli, including collapse of the alveoli wall, proteinaceous exudation in the alveoli cavity, and epithelial damage in the small bronchioles, while interstitial congestion was commonly observed, most likely contributed by infiltrations of inflammatory cells and mononuclear lymphocytes (**Fig. 4b and Extended Data Fig. 6**). In sharp contrast, histopathological changes in the lung of mice infected with the Omicron variant was not evident at 2 dpi. At 4 dpi, localized inflammatory infiltrations leading to thickening of the alveoli septa could be scarcely detected (**Fig. 4b and Extended Data Fig. 6**). Semi-quantitative histological scoring also indicated attenuated histopathological changes in the lungs of Omicron-infected mice compared to those infected with WT or Delta at both time points examined (**Extended Data Fig. 7**). Together, our results indicate that the Omicron variant replicates inefficiently in lung cells due to reduced spike cleavage and TMPRSS2 usage. The reduced replication capacity of the Omicron variant results in attenuated lung pathology, milder body weight loss, and improved animal survival *in vivo*.

Discussion

The exact change on the Omicron variant that results in the severely impaired replication in Calu3 human lung epithelial cells and the respiratory tract of mice is currently unknown. Interestingly, our live virus infection assays demonstrate that while the replication of Omicron variant is dramatically attenuated in Calu3 and Caco2 cells, it is less affected in VeroE6 cells.

Our results are in agreement with a recent preprint that suggested a decreased Omicron-pseudovirus entry in Calu3 and Caco2 cells but not in Vero, 293T, or A549-ACE2 cells¹⁷, as well as recent findings that demonstrated inefficient infection of human lung cells and organoids by Omicron²⁵⁻²⁷. Mechanistically, we further demonstrate that Omicron-pseudovirus does not utilize TMPRSS2 for entry as efficiently as SARS-CoV-2-WT-, Alpha-, Beta-, or Delta-pseudoviruses. At the same time, the inhibitory effect on Omicron entry and replication in VeroE6-TMPRSS2 cells by the TMPRSS2 inhibitor, camostat, is significantly weakened in comparison to that of WT or other variants. SARS-CoV-2 spike contains a PRRA insertion at the S₁/S₂ junction that creates a multi-basic furin-like cleavage site. This motif is absent from the spike of SARS-CoV-1, SARS-CoV-related coronaviruses (SARSr-CoVs), or the closely related bat coronavirus RaTG13²⁸. Recent reports suggest that this acquired furin-like cleavage site facilitates TMPRSS2 usage²⁹⁻³¹ and is critical for the highly efficient infection of human lung cells^{18,32,33}. Our Western blot analysis of virus-infected VeroE6-TMPRSS2 cells reveals a decreased S₁/S₂ cleavage of Omicron spike in comparison to that of SARS-CoV-2 WT and Delta. Since prior S₁/S₂ cleavage facilitates the exposure of the S₂' cleavage site upon receptor binding, which is required for TMPRSS2-mediated cleavage at S₂' and fusion³⁴. Together, these results suggest that the attenuated replication of the Omicron variant in lung cells is due to a reduced efficiency in host proteases cleavage by changes in the spike protein. Importantly, Omicron spike contains five substitutions at or near the furin-like cleavage site, including T547K, D614G, H655Y, N679K, and P681H, as well as six substitutions in S₂, including N764K, D796Y, N856K, Q954H, N969K, and L981F. Four of these substitutions (T547K, N764K, N856K, N969K) introduce electrostatic contacts formed between core S₂ subunit helices and the S₁ subunit³⁵. Thus, the enhanced interactions between the S₁ and S₂ subunits in

Omicron spike, along with substitutions at or near the furin-like cleavage site, may result in the altered S₁/S₂ cleavage. The exact role of these substitutions on S₁/S₂ cleavage should be further investigated.

A large body of timely reports has revealed that the Omicron variant can robustly escape from neutralization antibodies, allowing it to infect fully vaccinated or even booster vaccinated individuals^{2-8,10,13,14,16}. Our results in this study suggest that the virus replication fitness of the Omicron variant is severely compromised potentially as a trade-off of escaping from neutralization antibodies. SARS-CoV-2 variants emerged prior to Omicron were known to acquire mutations in spike that modulated their propagation in the human respiratory tract and the efficiency of virus transmission³⁶⁻³⁸. By comparing the pathogenicity of SARS-CoV-2 WT, Alpha, Beta, Delta, and Omicron variants side-by-side in the K18-hACE2 transgenic mouse model, we observed an attenuating trend for the emerging variants with the Omicron variant being the mildest. The lower pathogenicity in mouse lung infected with the Omicron variant hints that patients infected with the Omicron variant might develop milder respiratory symptoms in comparison to that of the previous variants.

Our study has limitations including the use of the K18-hACE2 mouse model for pathogenesis studies instead of the primate models that are more similar to humans. However, K18-hACE2 mouse model is a well-established model for SARS-CoV-2 study that allows survival measurement. Using this lethal model, we are able to compare the survival of mice upon infection by SARS-CoV-2 WT, Omicron, and other variants side-by-side in a quantitative manner, which is not possible by using other non-human primate, hamster, or ferret models.

Our study has a number of implications. First, we show that the Omicron variant is impaired in virus replication in human lung cells as well as in the lung of infected animals,

suggesting that Omicron infections in the human population may potentially result in milder lung symptoms in comparison to that of previous variants. However, the Calu3 cells used in the current study are carcinoma cells derived from the human lung and may not be accurate representations of the human lung. Similarly, the K18-hACE2 mice may not fully resemble the pathogenesis of Omicron infection in humans. In addition, data from our mouse models suggest that the difference in virus replication and pathogenicity between Omicron and Delta is smaller than the difference between Omicron and WT SARS-CoV-2. Thus, the pathogenicity of Omicron in humans should be further evaluated with human clinical data. Second, we show that the impaired replication of the Omicron variant results from its decreased spike cleavage and capacity of utilizing TMPRSS2. In this regard, our results suggest that therapeutics targeting TMPRSS2 may be less effective against the Omicron variant. Overall, our study reveals key features of the Omicron variant that provide critical information for the prevention, control, and treatment of the current COVID-19 pandemic.

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

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Methods

Viruses and safety

Wild type SARS-CoV-2 HKU-001a (GenBank: MT230904), B.1.1.7/Alpha (GenBank: OM212469), B.1.351/Beta (GenBank: OM212470), B.1.617.2/Delta (GenBank: OM212471), B.1.1.529/Omicron (GenBank: OM212472), and B.1.1.529/Omicron (R346K) (GenBank: OM212473) were isolated from laboratory-confirmed COVID-19 patients in Hong Kong^{4,39}. All variants of SARS-CoV-2 were cultured and titrated by plaque assays using VeroE6-TMPRSS2 cells. After obtaining the virus culture, sequences of all variants used in this study were confirmed with nanopore sequencing. In vivo and in vitro experiments with infectious SARS-CoV-2 were performed according to the approved standard operating procedures of the Biosafety Level 3 facility at Department of Microbiology, HKU.

Cell cultures

Caco2, 293T, and VeroE6 were obtained from ATCC and maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Amarillo, Texas, USA) according to supplier's instructions. Calu3 was obtained from ATCC and maintained in DMEM/F12 (Gibco). VeroE6-TMPRSS2 was obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank and cultured in DMEM. All cell lines used are routinely tested for mycoplasma and are maintained mycoplasma-free.

In vivo virus challenge in mice

The use of animals was approved by the Committee on the Use of Live Animals in Teaching and Research of The University of Hong Kong under CULATR #5440-20 and #5370-20.

Heterogenous K18-hACE2 C57BL/6J mice (2B6.Cg-Tg(K18-ACE2)2Prln/J) were obtained from The Jackson Laboratory. Animals were kept in cages with individual ventilation with 65% humidity and ambient temperature ranging between 21-23 °C with 12-hour-interval day/night cycle for housing and husbandry. For virus challenge in mice, 6- to 8-week-old female C57BL/6J mice or 6- to 8-week-old female and male K18-hACE2 transgenic mice were anaesthetized with ketamine and xylazine, followed by intranasal inoculation with 20 µl/mouse of Alpha, Beta, Delta or Omicron variants or WT SARS-CoV-2 at 2×10^3 PFU/mouse (for K18-hACE2 transgenic mice) or 1×10^5 PFU/mouse (for C57BL/6J mice) as we previously described^{21,40}. Mice were sacrificed at 2 and 4 dpi for harvesting nasal turbinate and lung tissues for virological assessment, proinflammatory cytokine quantification, or histological examination. Nasal wash was harvested by flushing with 200 µl PBS on a daily basis for four days post virus challenge for viral load detection. Survival and body weight of the infected animals were monitored for 14 days or until death of the animal.

Histology and immunohistochemistry staining

Animal tissues were harvested and fixed with 10% neutral-buffered formalin. Nasal turbinates were decalcified with 10% formic acid for 7 days before being processed with the TP1020 Leica semi-enclosed benchtop tissue processor. IHC was performed with the DAB (3,3'-diaminobenzidine) substrate kit (Vector Laboratories) as we previously described⁴⁰. To detect the viral antigen, in-house mouse monoclonal biotinylated anti-SARS-CoV-2 nucleocapsid protein antibody (1:4000) was used, followed by color development with the DAB substrate kit. The

nuclei were detected with haematoxylin before the tissue sections was mounted with the VectaMount permanent mounting medium (Vector Laboratories). For H&E staining, tissue sections were stained with Gill's haematoxylin and eosin-Y. Images were acquired with the Olympus BX53 light microscope. Three to four mice were sampled each group (as specified in the figure legends) and four to six sections from each animal were used for histology analysis. To obtain the semiquantitative histology scoring, tissue sections were graded in a blinded manner according to the pathological changes in bronchioles, alveoli, and blood vessels by an experienced pathologist as previously reported by us and others^{41,42}.

Infectious virus titration by plaque assays

Organs harvested from infected mice were homogenized in DMEM with Tissue Lyzer II (Qiagen, Germany) and clarified supernatants were 10-fold serially diluted inoculated to monolayered VeroE6-TMPSS2 cells for 2h at 37°C. After inoculation, the cells were washed with PBS three times, and covered with 1% low-melting agarose DMEM containing 1% FBS. The cells were fixed by 4% paraformaldehyde after 72h incubation. Fixed samples were stained with 0.5% crystal violet in 25% ethanol/distilled water for plaque visualization.

Infectious virus titration by TCID₅₀ assays

Supernatants from cells infected with Alpha, Beta, Delta, Omicron variant or WT SARS-CoV-2 were harvested and performed 10-fold serial dilution before inoculation into VeroE6-TMPRSS2 cells with 4 replicates per sample. Cytopathic effect (CPE) was observed at 72 hpi for the quantification of the median tissue culture infectious dose.

Cell viability assays

VeroE6-TMPRSS2 cells were infected with Alpha, Beta, Delta, Omicron variant or WT SARS-CoV-2 at 0.1 MOI. Cell viability was quantified by CellTiter-Glo luminescent cell viability assay

kit (Promega, USA), following manufacturer's manual with the EnSight Multimode Microplate Reader (Perkin Elmer, USA) at the designated time points.

Production of SARS-CoV-2-Spike-pseudoviruses and pseudovirus entry assays

All variants of SARS-CoV-2-spike pseudoviruses were packaged as described previously^{32,43}. In brief, 293T cells were transfected with different spikes with Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, USA). At 24 h post transfection, the cells were transduced with VSV-deltaG-firefly pseudotyped with VSV-G. At 2 h post transduction, the cells were washed 3 times with PBS and cultured in fresh media with anti-VSV-G (8G5F11) antibody (EB0010, kerafast, Boston, MA, USA) (1:1000). The pseudoviruses were then harvested 16 hours post transduction and titrated with TCID₅₀. For pseudovirus entry assays, target cells were inoculated with pseudoviruses for 2h and cultured in 1% FBS media for 24 h, before washed and lysed for detection of luciferase signal with a luciferase assay system (E1501, Promega, Madison, WI, USA).

RNA extraction and real-time reverse-transcription polymerase chain reaction

Viral RNA from infected cells was extracted using QIAasympyphony RNA Kit (931636, Qiagen, Germantown Road Germantown, MD, USA). Viral RNA from mice lung and nasal turbinate samples were extracted with the RNeasy Mini kit (74106, Qiagen). SARS-CoV-2 gene copies targeting the RNA-dependent RNA polymerase (RdRp) were quantified using the QuantiNova Probe RT-PCR Kit (208354, Qiagen). Viral subgenomic RNA was detected using primer targeting E gene. The expression of IP-10 and IFN-gamma were detected by qRT-PCR using the QuantiNova SYBR Green RT-PCR kit. The primer and probe sequences are available upon request.

Protease inhibitor treatment assay

The serine protease inhibitor, camostat, was purchased from MedChemExpress (Monmouth Junction, NJ, USA). VeroE6-TMPRSS2 cells were treated with DMSO or camostat at concentrations of 1, 25, and 50 μ M for 2 h before virus infection or pseudovirus transduction. At 24 hpi, the cell lysates were harvested for qRT-PCR quantification of virus replication or lysed for detection of luciferase signal.

Western blot analysis of spike cleavage from virus-infected VeroE6-TMPRSS2 cells.

VeroE6-TMPRSS2 cells were infected by SARS-CoV-2 WT, Delta, or Omicron at 0.01 MOI. Cell lysates were harvested at 48 hours post infection for Western blot analysis. Specific primary antibodies were incubated with the blocked membranes at 4°C overnight, followed by horseradish peroxidase (HRP) conjugated secondary antibodies (Thermo Fisher Scientific) for 1h at room temperature. The signal was developed by SuperSignal West Pico PLUS Chemiluminescent Substrate (34580, Thermo Scientific, USA) and detected using Alliance Imager apparatus (Uvitec, Cambridge, UK). Full-length spike and S2 was detected with a rabbit anti-SARS-CoV-2 spike S2 antibody (40590-T62, Sino Biological) (1:5000). Nucleocapid (N) was detected with an in-house rabbit anti-SARS-CoV-2 N immune serum (1:10000) and β -Actin was detected with a β -Actin antibody (clone AC-74, A5316, Sigma, USA) (1:5000).

Statistical analysis

Statistical comparison between two experiment groups were performed with unpaired two-tailed Student's *t*-test. Comparison among three or more experiment groups was performed with one-way or two-way ANOVA with Tukey's multiple comparison test. Area under the curve were calculated and analyzed with one-way ANOVA. Survival of animals were compared with Log-rank (Mantel-Cox) test. Differences were considered statistically significant when $p < 0.05$. Data analysis were performed with Graphpad prism 8.0.

Reporting summary

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

Data availability

The complete sequence of SARS-CoV-2 HKU-001a (GenBank: MT230904), B.1.1.7/Alpha (GenBank: OM212469), B.1.351/Beta (GenBank: OM212470), B.1.617.2/Delta (GenBank: OM212471), B.1.1.529/Omicron (GenBank: OM212472), and B.1.1.529/Omicron (R346K) (GenBank: OM212473) are available on GenBank. All source data is provided in the source data file.

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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 Hin Chu and Kwok-Yung Yuen.

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Fig. 1 Attenuated virus replication of SARS-CoV-2 Omicron. **a-d** Cells were challenged with WT SARS-CoV-2 or Alpha, Beta, Delta, Omicron, or Omicron (R346K) at 0.5 MOI (for Calu3) or 0.1 MOI (for Caco2 and VeroE6). **(a)** Cell lysates were harvested at the designated time points for quantification of the subgenomic RNA of the envelope (sgE) gene ($n = 7$). Robustness of sgE production was quantified with the area under the curve (AUC) analysis. **(b-d)** Infectious viral particles were titrated with TCID₅₀ assay ($n = 4$). **e** Cell viability of VeroE6-TMPRSS2 infected with WT SARS-CoV-2 and other variants at 0.1 MOI was quantified at the designated time points ($n = 6$). Data represents mean $\pm$ SD from the indicated number of biological repeats. Statistical significances were determined with one way-ANOVA in **(a)**, two-tailed Student's t -test in **(b-d)**, or two way-ANOVA in **(e)**. Multiple comparisons among different groups were adjusted with Tukey's multiple comparison tests. Data were obtained from three independent experiments. Each data point represents one biological repeat. * represented $p < 0.05$ and ** represented $p < 0.01$. *** represented $p < 0.001$, **** represented $p < 0.0001$. ns, not statistically significant; WT, wildtype SARS-CoV-2.

Fig. 2 SARS-CoV-2 Omicron is inefficient in TMPRSS2 usage. **a** 293T cells were transfected with ACE2 or co-transfected with ACE2 and TMPRSS2, followed by transduction with pseudoviruses expressing the spike of SARS-CoV-2 WT, Alpha, Beta, Delta, or Omicron variant at 24 hours post transfection. Pseudovirus entry was quantified by measuring the luciferase signal ($n = 9$ for Delta; $n = 10$ for WT, Alpha, Beta and Omicron). Fold changes in the luciferase signal was normalized to the mean luciferase readouts of cells with only ACE2 overexpression. **b** VeroE6 and VeroE6-TMPRSS2 cells were transduced with pseudoviruses expressing the spike of SARS-CoV-2 WT, Alpha, Beta, Delta, or Omicron variant. Pseudovirus entry was quantified by measuring the luciferase signal ($n = 6$). Fold changes in the luciferase signal was normalized to the mean luciferase readouts of VeroE6 cells. **c-d** VeroE6-TMPRSS2 cells were pre-treated with 1, 25, or 50 μM camostat or DMSO for 2 h followed by transduction with pseudoviruses expressing the spike of SARS-CoV-2 WT, Alpha, Beta, Delta, or Omicron variant (c) or challenged with the indicated authentic SARS-CoV-2 variants at 0.1 MOI (d). Pseudovirus entry was quantified by measuring the luciferase signal of the cell lysates at 24 hours post transduction ($n = 6$). The amount of viral subgenomic envelope RNA in harvested cell lysates at 24 hpi was determined by qRT-PCR [$n = 7$ for Delta, Omicron, and Omicron (R346K) at 1, 25, and 50 μM; $n = 6$ all other samples]. Data represents mean ± SD from the indicated number of biological repeats. Statistical significance was determined with two way-ANOVA (a-d). Multiple comparisons among different groups were adjusted with Tukey's multiple comparison tests. Data were obtained from three independent experiments. Each data point represents one biological repeat. * represented $p < 0.05$ and ** represented $p < 0.01$. *** represented $p < 0.001$, **** represented $p < 0.0001$. ns, not statistically significant. WT, wildtype SARS-CoV-2.

Fig. 4 Reduced in vivo pathology induced by Omicron infection. 6-to-8-week-old female and male K18-hACE2 transgenic mice were intranasally inoculated with 2×10^3 PFU Alpha, Beta, Delta, Omicron, or WT SARS-CoV-2. Lung of the infected mice were collected on 2 or 4 dpi for histological analysis. **a** Representative images of immunohistochemistry staining for the detection of nucleocapsid protein (brown, pointed by black arrows) of SARS-CoV-2 in lung of the infected mice ($n = 3$ for WT and Delta; $n = 4$ for Omicron). **b** Representative images of hematoxylin and eosin (H&E) staining for the detection of pathological tissue damage in the lung of the infected mice. Images in (a and b) are representative images from three to four mice ($n = 3$ for WT and Delta; $n = 4$ for Omicron). Data were obtained from two independent experiments. Four to six sections were taken from each mouse for histology and immunochemistry analysis. Scale bar represents 200 μm . WT, wildtype SARS-CoV-2.

Extended Data Figure 1

Extended Data Fig. 1 Virus replication of Omicron, WT SARS-CoV-2, and other VOCs in Caco2 and VeroE6 cells. **a** Caco2 and **b** VeroE6 cells were challenged by SARS-CoV-2 WT, Alpha, Beta, Delta, Omicron, or Omicron (R346K) at 0.1 MOI. Cell lysates were harvested at the designated time points for the quantification of subgenomic RNA of the envelope (sgE) gene. Robustness of sgE production was quantified with the area under the curve (AUC) analysis ($n = 7$). Data represents mean $\pm$ SD from the indicated number of biological repeats. Statistical significances were determined with one way-ANOVA with Tukey's multiple comparison tests. Each data point represents one biological repeat. * represented $p < 0.05$ and ** represented $p < 0.01$. *** represented $p < 0.001$, **** represented $p < 0.0001$. ns, not statistically significant; WT, wildtype SARS-CoV-2.

Extended Data Figure 2

Extended Data Fig. 2 Western blot analysis of SARS-CoV-2 spike from virus infected VeroE6-TM-PRSS2 cells. VeroE6-TMPRSS2 cells were infected by SARS-CoV-2 WT, Delta, or Omicron at 0.01 MOI. Cell lysates were harvested at 48 hours post infection for detection of SARS-CoV-2 spike cleavage using an anti-spike S2 antibody. Representative images of SARS-CoV-2 spike and nucleocapsid Western blots were shown with β -actin added as a sample processing control. Spike, nucleocapsid, and β -actin was run on three different gels and detected on three different membranes. The experiment was repeated three times independently with similar results.

Extended Data Fig. 3 Viral burden in nasal wash of K18-hACE2 transgenic mice infected with SARS-CoV-2 WT, Delta, or Omicron. Male and female 6- to 8-week old K18-hACE2 mice were infected with 2×10^3 pfu SARS-CoV-2 WT, Delta, or Omicron ($n = 4, 5$ and 7 for WT, Delta and Omicron, respectively). Nasal wash was collected on a daily basis for four days for determining RdRp gene copies with RT-qPCR. Statistical significances were determined with one way-ANOVA. Multiple comparisons among different groups were adjusted with Tukey's multiple comparison tests. Data were obtained from two independent experiments. Each data point represents one biological repeat. * represented $p < 0.05$. ns, not statistically significant; WT, wildtype SARS-CoV-2.

Extended Data Figure 4

Extended Data Fig. 4 Omicron infection in C57B6 wildtype mice. 6-to-8-week-old female C57B6 wild-type mice were intranasally inoculated with 1×10^5 PFU of Alpha or Omicron. Nasal turbinates and lungs of the infected mice were collected on 2 or 4 dpi for viral burden determination ($n = 8$). **a** Viral RNA-dependent RNA polymerase (RdRp) gene copies were quantified with probe-specific RT-qPCR. **b** Subgenomic envelope (E) gene were quantified with probe-specific RT-qPCR. **c** Infectious viral titres were quantified with plaque assay in VeroE6-TMPRSS2 cells. Data represents mean $\pm$ SD from the indicated number of biological repeats. Statistical differences were determined with two-tailed Student's *t*-test in (a-c). Data were obtained from two independent experiments. Each data point represents one biological repeat. * represented $P < 0.05$; ** represented $P < 0.01$; *** represented $P < 0.001$; **** represented $P < 0.0001$.

Extended Data Figure 5

Extended Data Fig. 5 Immunohistochemistry staining of lung tissue of K18-hACE2 transgenic mice infected with SARS-CoV-2 WT, Delta, or Omicron. 6-to-8-week-old female and male K18-hACE2 transgenic mice were intranasally inoculated with 2×10^3 PFU SARS-CoV-2 WT, Delta, or Omicron. Lung of the infected mice were collected on 2 or 4 dpi for histological analysis. Representative images of immunohistochemistry staining for the detection of nucleocapsid protein (brown, pointed by black arrows) of SARS-CoV-2 in lung of the infected mice ($n = 3$ for WT and Delta; $n = 4$ for Omicron). Images are the source images with lower magnification for Figure 4a. Dashed rectangles indicate the region enlarged in Figure 4a. Four to six sections were taken from each mouse for immunochemistry analysis. Scale bar represents 500 μm .

Extended Data Figure 6

Extended Data Fig. 6 H&E images of lung tissue of K18-hACE2 transgenic mice infected with SARS-CoV-2 WT, Delta, or Omicron. 6-to-8-week-old female and male K18-hACE2 transgenic mice were intranasally inoculated with 2×10^3 PFU SARS-CoV-2 WT, Delta, or Omicron. Lung of the infected mice were collected at 2 or 4 dpi for histological analysis. Representative images of hematoxylin and eosin (H&E) staining for the detection of pathological tissue damage in the nasal turbinate and lung of the infected mice ($n = 3$ for WT and Delta; $n = 4$ for Omicron). Images are the source images with lower magnification for Figure 4b. Dashed rectangles indicate the region enlarged in Figure 4b. Four to six sections were taken from each mouse for histology analysis. Scale bar represents 500 μm .

Extended Data Figure 7

Extended Data Fig. 7 Histological scoring of lung tissue of K18-hACE2 transgenic mice infected with SARS-CoV-2 WT, Delta, or Omicron. Semiquantitative histology scores were given to each H&E image of the lung tissue by grading the severity of damage in bronchioles, alveoli, and blood vessels and accumulating the total scores. For bronchioles: 0 = normal structure; 1 = mild peribronchiolar infiltration; 2 = peribronchiolar infiltration plus epithelial cell death; and 3 = score 2 plus intrabronchiolar wall infiltration and epithelium desquamation. For alveoli: 0 = normal structure; 1 = alveolar wall thickening and congestion; 2 = focal alveolar space infiltration or exudation; and 3 = diffuse alveolar space infiltration or exudation or haemorrhage. For blood vessels: 0 = normal structure; 1 = mild perivascular oedema or infiltration; 2 = vessel wall infiltration; and 3 = severe endothelium infiltration. Data are mean $\pm$ SD (n = 3 for WT and Delta; n = 4 for Omicron). Statistical differences were determined with one-way ANOVA. Multiple comparisons among different groups were adjusted with Tukey's multiple comparison tests. Images were obtained from two independent in vivo experiments. Each data point represents one biological repeat. * represented $P < 0.05$; ** represented $P < 0.01$; *** represented $P < 0.001$; **** represented $P < 0.0001$.

Reporting Summary

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Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Glomax Explorer 3.2.3 was used to collect luciferase data for pseudovirus entry assay.
cellSens Dimension 1.16 (Olympus) software was used to obtain H&E and IHC images.
The LightCycler 480 software release 1.5.1.62 SP3 was used to collect qPCR data.
Kaleido 1.2 was used to obtain luciferase data for the cell viability assay.
Image J (version 1.51) was used to analyze the ratio of S2/S full length of SARS-CoV-2 spike.

Data analysis

GraphPad Prism 8.0 was used for analysis and plotting the figures.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The complete sequence of SARS-CoV-2 HKU-001a (GenBank: MT230904), B.1.1.7/Alpha (GenBank: OM212469), B.1.351/Beta (GenBank: OM212470), B.1.617.2/Delta (GenBank: OM212471), B.1.1.529/Omicron (GenBank: OM212472), and B.1.1.529/Omicron (R346K) (GenBank: OM212473) are available on GenBank. All source data is provided in the source data file.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For the in vitro experiments, experiments were repeated for at least 3 times to give a sample size of 4 or above. A $n > 3$ is chosen so that statistical tests can be performed to determine statistical significance. For the in vivo experiments, group sizes were chosen based on statistical power analysis and our prior experience in examining viral titres in SARS-CoV-2 infected K18-hACE2 transgenic and C57B6 mice. Experiments were repeated to give a sample size of 6 or above.
Data exclusions	All data collected was included without exclusion of outliers.
Replication	For all in vitro experiments, all experiments were repeated at least on three separate occasions. For the mice experiments, all were performed in two separate occasions. Reproducible findings were obtained from all repeats.
Randomization	For all in vivo experiments, gender- and age- matched mice were randomized into different experimental groups. For the in vitro experiments, cells were randomized into different infection/treatment groups.
Blinding	For the measurement of quantitative values, such as viral genome copies, infectious viral titres, cytokine/chemokine gene copies, body weight and survival, data acquiring does not involve subjective judgments therefore no blinding procedures were applied to the experimentalists involved. For the in vitro and in vivo experiments, blinding was not possible because researchers were involved in infection/treatment procedures. For the histological examinations, qualified pathologists were blinded to group allocation to ensure the assessment was unbiased.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ 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	Rabbit anti-SARS-CoV-2 spike S2 antibody (40590-T62, Sino Biological) (1:5000). In-house rabbit anti-SARS-CoV-2-nucleocapsid (N) immune serum (1:10000). In-house biotinylated mouse anti-SARS-CoV-2 nucleocapsid (N) immune serum (1:4000). β -Actin antibody (Sigma, USA, A5316, clone AC-74) (1:5000). Goat anti-Rabbit IgG (H+L) HRP (Thermo Fisher Scientific, USA, 31460) (1:5000). Goat anti-Mouse IgG (H+L) HRP (Thermo Fisher Scientific, USA, 31430) (1:5000). Mouse anti-VSV-G Antibody (Kerafast, USA, # EB0010, clone 8G5FL11 (I1)) (1:1000).
Validation	Commercial primary antibodies were validated by the manufacturers and validation statements are available on the manufacturer's website. In-house biotinylated mouse anti-SARS-CoV-2 nucleocapsid (N) immune serum was validated with immunohistochemistry staining as we previously reported (doi:10.1016/j.ebiom.2021.103643). In-house rabbit anti-SARS-CoV-2-N immune serum was validated with immunofluorescence staining as we previously reported (doi:10.1016/S2666-5247(20)30004-5).

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Caco2, 293T, and VeroE6 were obtained from ATCC. VeroE6-TMPRSS2 was obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank.
Authentication	The cell lines were not authenticated.
Mycoplasma contamination	All cell lines have been recently tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	6- to 8-week-old female or male K18-hACE2 transgenic mice (2B6.Cg-Tg(K18-ACE2)2Prln/J) and same age female C57BL/6J mice were used. Animals were kept in cages with individual ventilation with 65% humidity and ambient temperature ranging between 21-23 °C with 12-hour-interval day/night cycle for housing and husbandry. Details were provided in the manuscript.
Wild animals	The current study does not involve wild animals.
Field-collected samples	The current study does not involve field-collected samples.
Ethics oversight	The use of animals was approved by the Committee on the Use of Live Animals in Teaching and Research of The University of Hong Kong under CULATR #5440-20.

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