

1 **SARS-CoV-2 Omicron BA.1 variant infection of human colon epithelial cells**

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22 All data, analytic methods, and study materials will be made available to other researchers upon request.

23

24 **Abstract:** Omicron B.1.1.529 became the predominant SARS-CoV-2 variant in early 2022, causing a new  
25 wave of public anxiety. Compared to the ancestral strain, Omicron has 50 mutations, with over 30  
26 mutations in the spike protein. These differences likely underlie the changes in Omicron biology noted in  
27 other studies, including an attenuation in the lung parenchyma, compared to the ancestral SARS-CoV-2  
28 strain and other variants, as well as a preference for endosomal entry, in place of the TMPRSS2-mediated  
29 membrane fusion pathway. This raises questions on Omicron tropism and infectivity in various target  
30 organ systems, including the gastrointestinal (GI) tract. Up to 70% of COVID-19 patients report GI  
31 symptoms, including nausea, vomiting, and diarrhea. Here, we show that in the context of donor intrinsic  
32 genetic heterogeneity, the SARS-CoV-2 Omicron variant infects human colonoids similarly, if not less  
33 effectively, than the ancestral WT (WA1) strain or the Delta variant. Additionally, we note a higher ratio  
34 of viral RNA to infectious virus titer, which may suggest that Omicron is potentially less infectious in the  
35 intestine. This study lays the foundation for further work defining mechanisms mediating intestinal  
36 infection and pathogenesis by Omicron.

37 Omicron B.1.1.529 (including BA.1, BA1.1, and BA.2 subvariants) became the predominant SARS-CoV-2  
38 variant in early 2022, causing a new wave of public anxiety. Omicron has 50 mutations compared to the  
39 ancestral strain, with over 30 mutations in the spike protein. These differences likely underlie the  
40 attenuated replication of Omicron in the lung parenchyma when compared to the ancestral SARS-CoV-2  
41 strain and other variants<sup>1</sup>. Omicron may also preferentially use endosomal entry over the TMPRSS2-  
42 mediated plasma membrane fusion pathway, raising further questions on Omicron tropism and  
43 infectivity<sup>2,3</sup>. It is crucial to understand the virulence and host immune responses of Omicron in various  
44 target organs, including the gastrointestinal (GI) tract. Up to 70% of COVID-19 patients experience GI  
45 symptoms, including nausea, vomiting, and diarrhea. We and others have previously shown that human  
46 small and large intestines express high levels of ACE2 and TMPRSS2/4, the host receptor and proteases,  
47 respectively, required for SARS-CoV-2 cell entry into host cells<sup>4</sup>. Here, we show that in the context of donor  
48 intrinsic genetic heterogeneity, the SARS-CoV-2 Omicron variant infects human colonoids similarly, if not  
49 less effectively, than the ancestral WT (WA1) strain or the Delta variant. This study lays the foundation for  
50 further work defining mechanisms mediating intestinal infection and pathogenesis by Omicron.

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52 All study procedures and reagents were approved by the Washington University IRB (#202011003).  
53 Primary colon epithelial cells (colonoids) were derived from healthy donor biopsies and cultured as  
54 previously described<sup>5</sup>. Each SARS-CoV-2 isolate and passage was confirmed by RNA sequencing  
55 (**Supplemental Table S1**). Supernatant from infected transwell colonoid monolayers was titrated by focus  
56 forming assay. Fixed monolayers were stained for SARS-CoV-2 nucleocapsid (N), actin, and DAPI prior to  
57 confocal imaging. Expression levels of SARS-CoV-2 N, GAPDH, interferon lambda (IFNL3), interferon beta  
58 (IFNB), and MX1 were quantified by RT-qPCR (primers and probes in **Supplemental Table S2**). HEK293-  
59 hACE2-TMPRSS2 cells were transfected with plasmids encoding variant spike proteins and plasmids  
60 encoding GFP and imaged at 24 hours post-transfection for syncytia formation. HEK293-hACE2 cells were  
61 transfected with plasmids encoding variant spike proteins and plasmids encoding empty vector control or  
62 V5-tagged host proteases TMPRSS2 or furin, and analyzed for spike cleavage by western blot at 24 hours  
63 post-transfection. Additional methodology is found in the **Supplementary Methods**.

64

65 Colonoids derived from four individual donors were seeded onto 2D transwell monolayers, differentiated,  
66 and infected apically with WT, Delta, or Omicron (MOI = 0.01 for 24 hours). Compared to WT and Delta,  
67 Omicron showed significantly increased replication as measured by intracellular viral RNA levels in 211A  
68 and 251A (**Fig. 1A**). Despite inter-individual differences in infectivity with each variant, a similar trend was  
69 observed in the colonoids of donor 262A (**Fig. 1A**). We conducted immunofluorescence to visualize  
70 intracellular SARS-CoV-2 N antigens in infected colonoids to confirm active replication (**Fig. 1B**). We  
71 additionally performed a focus forming assay to measure the amount of infectious SARS-CoV-2 progenies  
72 secreted into the apical colonoid supernatants, which demonstrated that Omicron produced comparable  
73 or numerically lower levels of infectious viruses than Delta and WT (**Fig. 1C**). This higher ratio of viral RNA  
74 to infectious virus titer suggests that Omicron is potentially less infectious in the intestine. Omicron also  
75 induced variable, but statistically similar, levels of type III IFN (IFNL3) expression, compared to the other  
76 SARS-CoV-2 strains (**Fig. 1D**). There was little induction of type I IFN (IFNB) and MX1, a canonical  
77 interferon-stimulated gene highly induced by type I and III IFNs, at 24 hours post-infection (**Fig. S1A**).

78 To understand the molecular basis of high viral RNA and low virus titer of Omicron in human intestinal  
79 epithelial cells, we tested whether Omicron has more effective cell-to-cell spread. We ectopically  
80 expressed SARS-CoV-2 variant spike proteins in HEK293 cells stably expressing human ACE2 and  
81 TMPRSS2<sup>6</sup>. Omicron spike induced the formation of fewer syncytia than either WT or Delta (**Fig. S1B**),  
82 consistent with a recent study<sup>2</sup>. Instead, we found inefficient Omicron spike cleavage by TMPRSS2 and  
83 furin proteases (**Fig. S1C**), suggesting possible attenuation of Omicron upon viral egress, when processed  
84 into mature infectious viruses.

85 In this study, we found that the SARS-CoV-2 Omicron BA.1 variant effectively infects healthy donor-  
86 derived colonoids, producing high levels of intracellular viral RNA in some donors, but comparably lower  
87 levels of infectious particles. We also found that Omicron induced weak IFN response after 24 hours,  
88 possibly due to reduced recognition by cytosolic sensors or viral antagonism of IFN responses. To date,  
89 only one study has compared infectivity of SARS-CoV-2 variants in human enteroids. Using spike-  
90 pseudotyped lentiviral viruses and a luciferase-based reporter assay to quantify infection, the authors  
91 observed a 2.5- and 5-fold higher infection of colonoids with the Omicron pseudotype spike compared to  
92 Delta and D614G spikes, respectively<sup>4</sup>. Our study's use of authentic SARS-CoV-2 virus and quantitation of  
93 both viral RNA and infectious particles may help explain this potential discrepancy.

94 GI symptoms in COVID-19 patients are strikingly frequent and have generated great interest for  
95 understanding how SARS-CoV-2 interacts with intestinal physiology. Disease states and commonly  
96 prescribed anti-inflammatory drugs can modulate intestinal ACE2 and protease expression, which  
97 potentially altered infectivity and disease severity in the initial waves<sup>5,7,8</sup>. Further, COVID-19 causes gut  
98 microbial dysbiosis and microbial diversity does not recover to pre-infection levels, even 6 months post-  
99 initial infection<sup>9</sup>. It is posited that such dysbiosis may be one potential contributor to long COVID-19. It is  
100 now of great interest to examine these possibilities in further studies in the context of Omicron and other  
101 SARS-CoV-2 variants. Due to the presence of viral RNA in stool and wastewater, there was concern for  
102 potential fecal-oral SARS-CoV-2 transmission<sup>10</sup>. Here, we show that at least in the colon, the Omicron  
103 variant does not produce more infectious particles, potentially reducing concern for GI virus shedding. In  
104 summary, our study establishes SARS-CoV-2 variant specific replication differences in human colonoids.  
105 As the pandemic evolves, there is already evidence for future variants and recombinants that can have  
106 unique features of transmission and pathology. As a potential viral reservoir, it is crucial to understand  
107 the molecular mechanisms of Omicron infection in the intestines, to better prepare for the return to  
108 normalcy.

109

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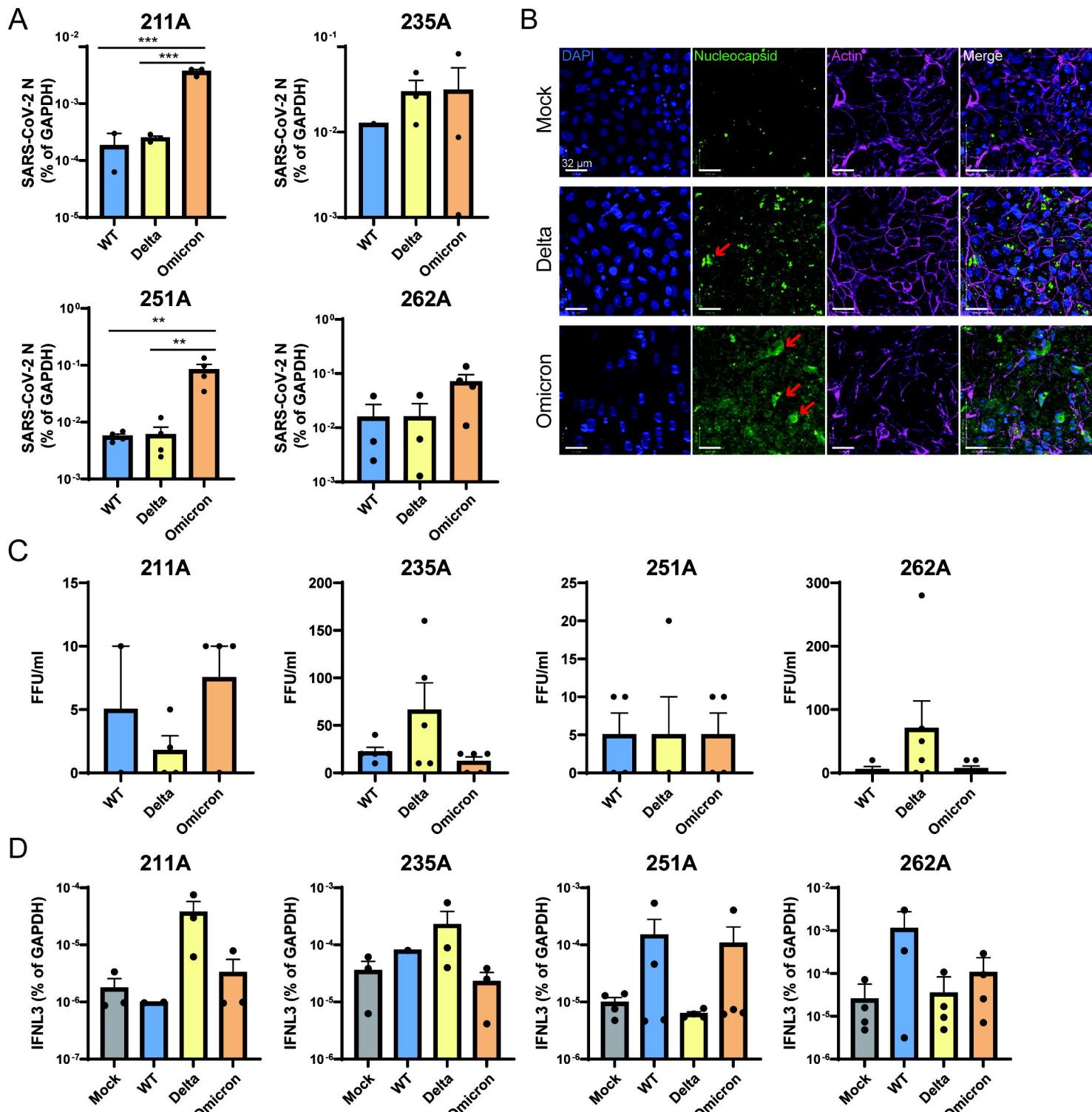
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**Figure 1.** SARS-CoV-2 infection of donor derived colonoids. **(A)** Colonoid lines in 2D transwell monolayers derived from four donors were infected with indicated SARS-CoV-2 variants at an MOI of 0.01. RNA was harvested at 24 hours post infection and SARS-CoV-2 N level was quantified by RT-qPCR and normalized to that GAPDH. (Mean with SEM, One-way ANOVA with Tukey's multiple comparisons test. \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ). **(B)** Colonoid 262A monolayers were infected by Delta or Omicron at an MOI of 0.01 and fixed at 24 hours post infection. Z-stacked confocal microscopic images were acquired and stained for SARS-CoV-2 N (green), actin (magenta), and DAPI (blue). Merge image demonstrates intracellular viral nucleocapsid staining. Red arrows indicate intracellular viral staining. Scale bar: 32 μm. **(C)** Quantification of infectious viral particles in the supernatants collected from the apical compartments of corresponding transwells at 24 hours post infection by a focus forming unit assay. **(D)** Same as (A) except that cellular IFNL3 mRNA level was measured instead (Mean with SEM).