Mutations accumulated in the spike of SARS-CoV-2 variants of concern allow for more efficient counteraction of the restriction factor BST2/Tetherin

Yuhang Shi, Sydney Simpson, Yuexuan Chen, Jared Benjamin and Ruth Serra-Moreno

Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY, USA

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ABSTRACT

BST2/Tetherin is a restriction factor with broad antiviral activity against enveloped viruses, including coronaviruses. Specifically, BST2 traps nascent particles to membrane compartments, preventing their release and spread. In turn, viruses have evolved multiple mechanisms to bypass BST2. Here, we examined the interactions between BST2 and SARS-CoV-2. Consistent with studies with other coronaviruses, BST2 reduces SARS-CoV-2 virion production. However, the virus uses the Spike (S) to downregulate BST2. This requires a physical interaction between S and BST2, which routes BST2 for lysosomal degradation. By surveying different SARS-CoV-2 variants of concern (Alpha-Omicron), we found that each variant is more efficient than the previously circulating strain at downregulating BST2 and facilitating virion production, and that mutations in S account for their enhanced BST2 antagonism. Therefore, as part of its adaptation to humans, SARS-CoV-2 is improving its capacity to counteract BST2, highlighting that BST2 antagonism is important for SARS-CoV-2 infectivity and transmission.

RESULTS

BST2 inhibits SARS-CoV-2 virus production and in turn SARS-CoV-2 down-regulates BST2 levels

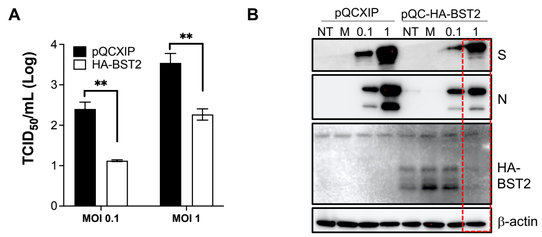


Figure 1. The antiviral impact of BST2 on SARS-CoV-2. (**A**) 293T-ACE2 stably expressing pQCXIP or pQCXIP-BST2 were infected with SARS-CoV-2 HK variant at MOI 0.1 and 1. Virion production was measured by ELISA (not shown) and TCID50 from the culture supernatants 24 hpi. (**B**) The level of BST2 was examined by western blot. *M: Mock infection. NT: non-treated cells **: p<0.01. Data correspond to the mean and SEM of 3 independent experiments.*

SARS-CoV-2 variants of concern have improved anti-BST2 activity

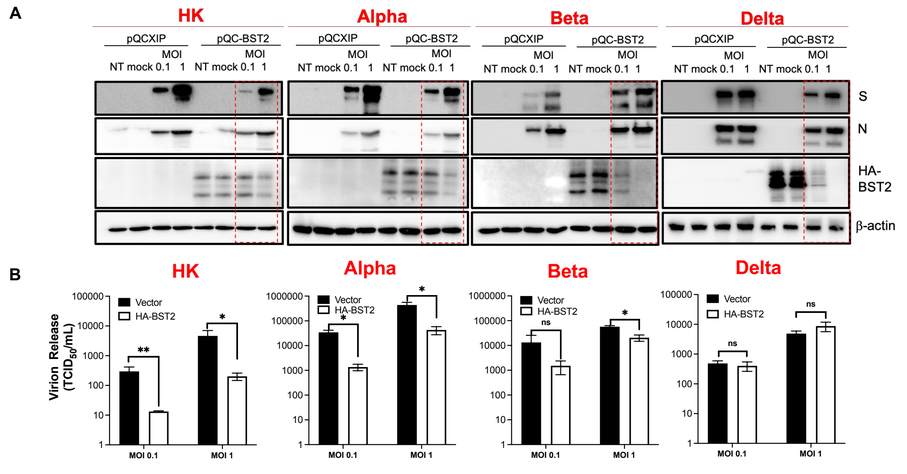


Figure 2. The new emerging variants have a better ability to antagonize BST2. (**A**) 293T-ACE2 cells stably expressing pQCXIP or pQCXIP-BST2 were infected with SARS-CoV-2 HK, Alpha, Beta, Delta variants at MOI 0.1 and 1. The level of BST2 and viral proteins were examined by western blot. (**B**) Virion production was examined by $TCID_{50}$, of the culture supernatants 24 hpi. **: p<0.05, **: p<0.01, ns: not significant. Data correspond to the mean and SEM of 3 independent experiments.*

SARS-CoV-2 uses the Spike protein to downregulate BST2

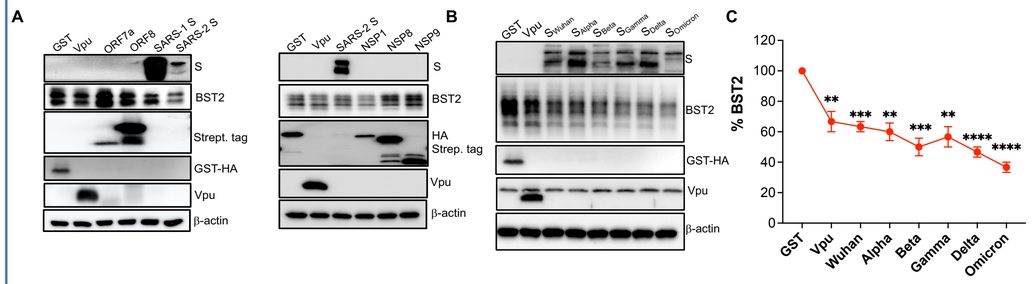


Figure 3. SARS-CoV-2 downregulates BST2 through Spike protein. (**A**) 293T-ACE2-BST2 stable cells were transfected with plasmids harboring each open reading frame of SARS-CoV-2 (panel A and not shown). Constructs encoding GST and HIV-1 Vpu were included as negative and positive control, respectively. The level of BST2 was measured level by western blot. (**B**) 293T-ACE2-BST2 stable cells were transfected with plasmids encoding the Spike gene from each emerging variants. BST2 levels were measured by western blot. (**C**) Relative BST2 expression was calculated by densitometry analyses from 3 biological replicates. ***: p<0.01, ***: p<0.001, ****: p<0.0001. Data correspond to the mean and SEM of 3 independent experiments.*

The Spike protein physically interacts with BST2, causing its lysosomal degradation

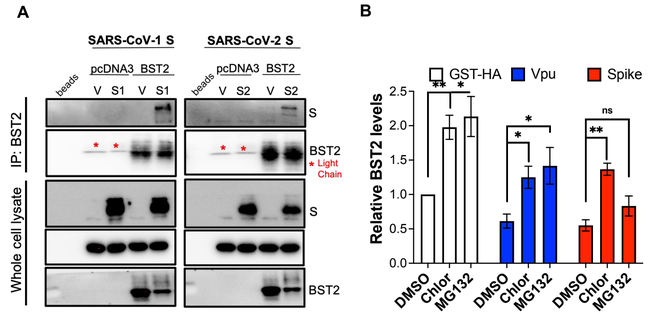


Figure 4. Fate of BST2 in presence of SARS-CoV-2 S. (**A**) The interaction of BST2 and SARS-CoV-2 S (S2) was evaluated by co-immunoprecipitation. Empty vector (V) and SARS-CoV-1 S (S1) were included as controls. (**B**) 293T-ACE2-BST2 stable cells were transfected with plasmids encoding GST, HIV-1 Vpu, and SARS-CoV-2 S. 24 h later, cells were treated with chloroquine and MG132. BST2 levels were measured 18 h later by western blot and expressed as relative to the vector control .**: p<0.05, **: p<0.01, ns: not significant. Data correspond to the mean and SEM of 3 independent experiments.*

MODEL

