

Cell Reports, Volume 33

Supplemental Information

An ACE2 Microbody

Containing a Single Immunoglobulin Fc Domain

Is a Potent Inhibitor of SARS-CoV-2

Takuya Tada, Chen Fan, Jennifer S. Chen, Ramanjit Kaur, Kenneth A. Stapleford, Harry Gristick, Belinda M. Dcosta, Craig B. Wilen, Crina M. Nimigean, and Nathaniel R. Landau

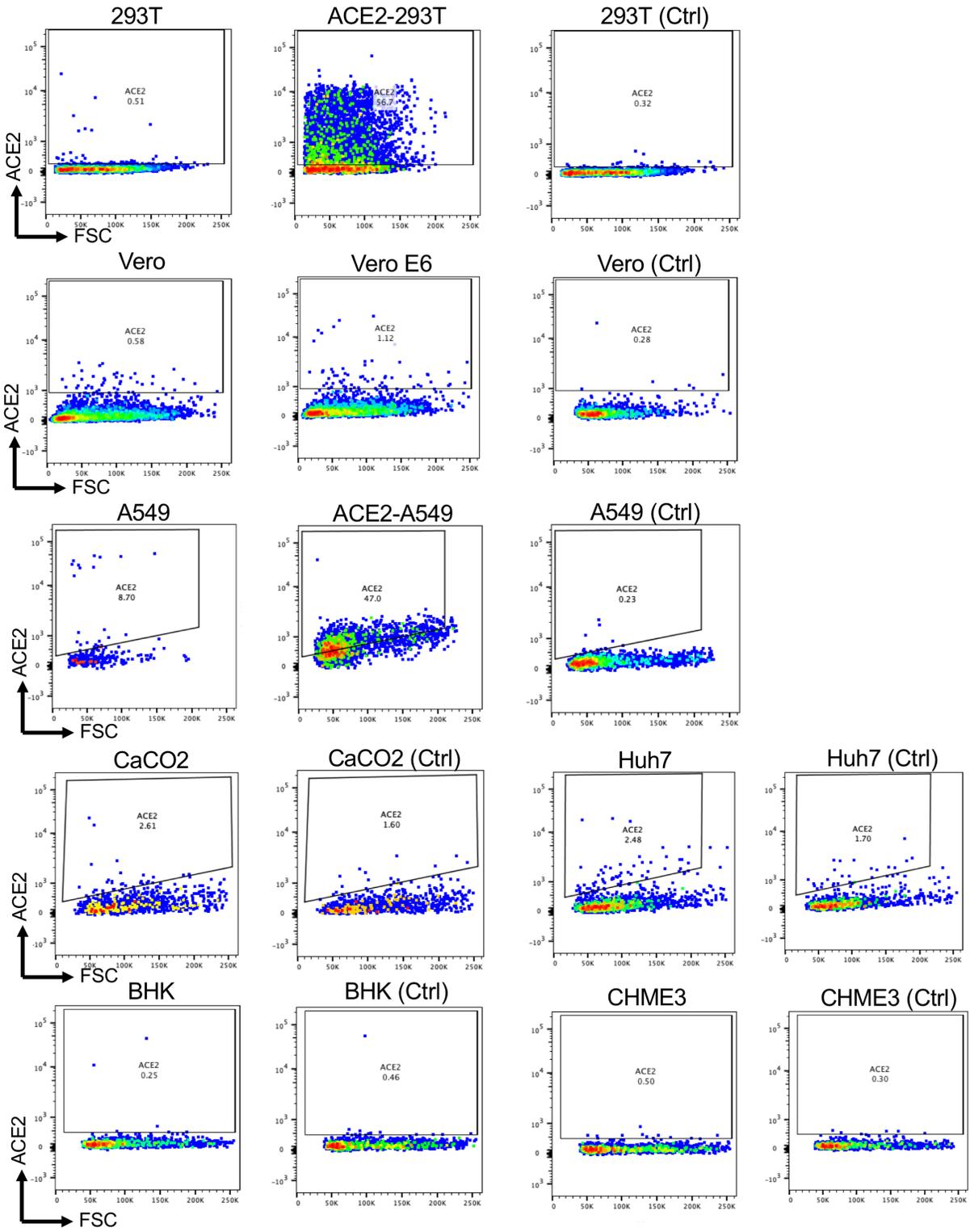


Figure S1. ACE2 expression levels in cell lines. Related to Figure 1. (A) The indicated cell lines were stained with anti-ACE2 antibody and Alexa fluor 594-conjugated anti-mouse IgG secondary antibody and analyzed by flow cytometry. As a negative control, cells were tained with Alexa fluor 594-conjugated anti-mouse IgG secondary antibody and analyzed by flow cytometry. The experiment was done twice with similar results.

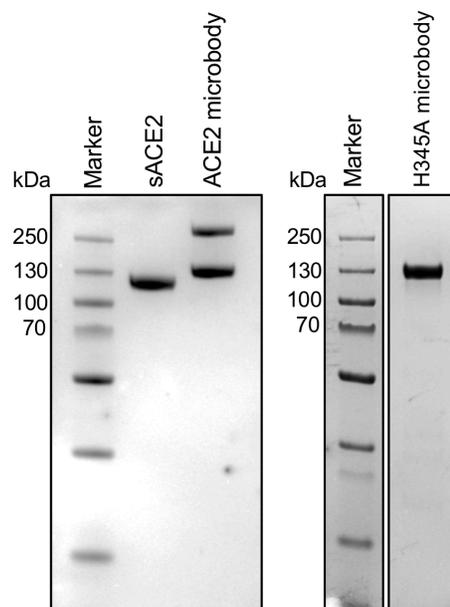


Figure S2. SDS-PAGE analysis of purified proteins. Related to Figure 2. 30 μ g of soluble ACE2, ACE2 microbody (left) and ACE2.H345A microbody (right) were analyzed by Coomassie blue stained SDS-PAGE under reducing conditions. Note, the ACE2 microbody dimer is partially resistant to reduction.

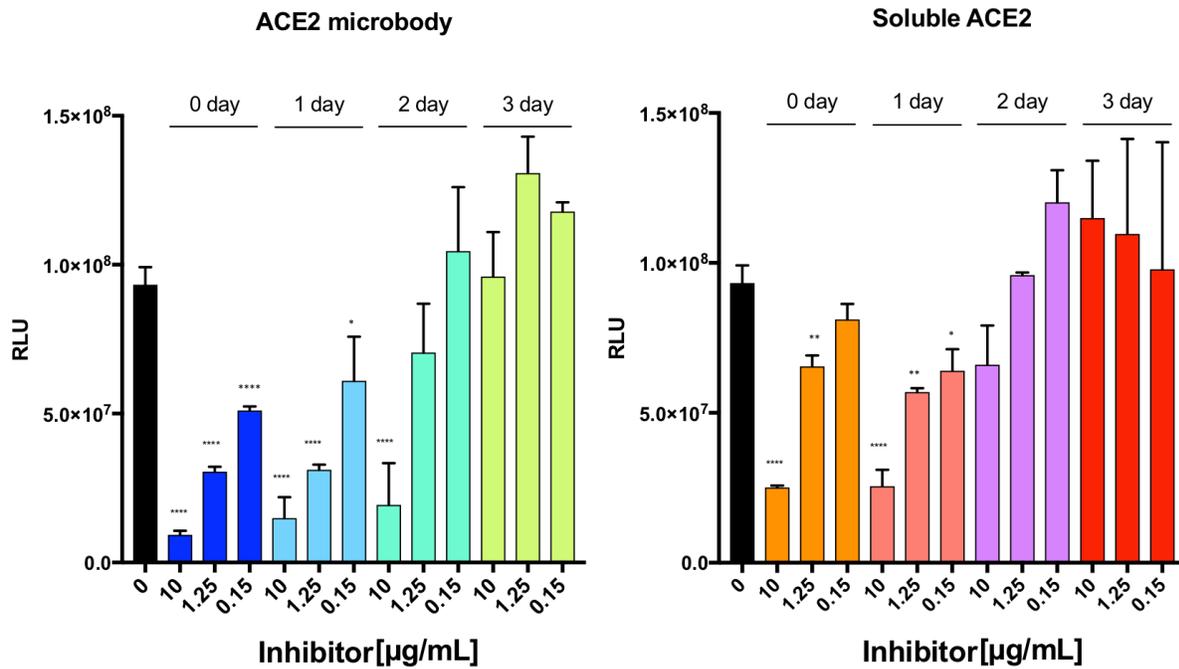
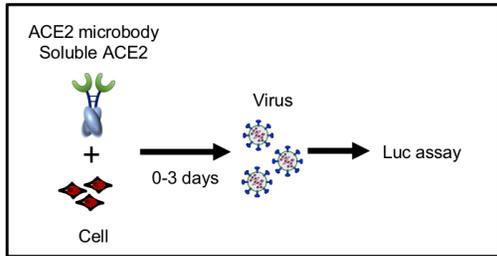


Figure S3. Increased stability of the ACE2 microbody. Related to Figure 5. Serially diluted sACE2 and ACE2 microbody proteins were added to ACE2.293T target cell cultures. The cells were infected either immediately with SARS-CoV-2 pseudotyped lentivirus or 1, 2 or 3 days later. Luciferase activity was measured 2 days post-infection. The data are presented as the mean of triplicates \pm SD. Statistical significance was calculated by the student-t test. The experiment was done twice with similar results.

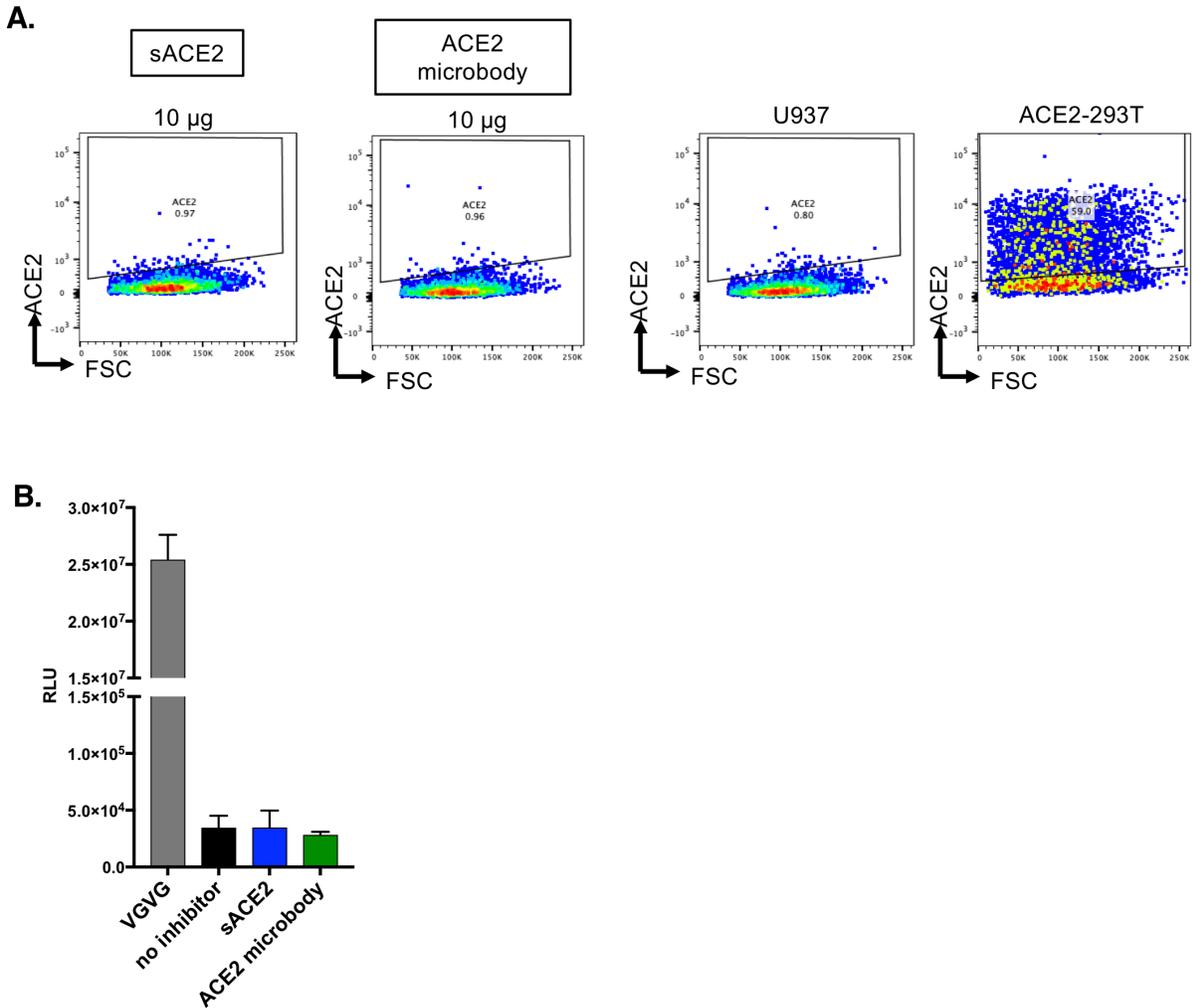
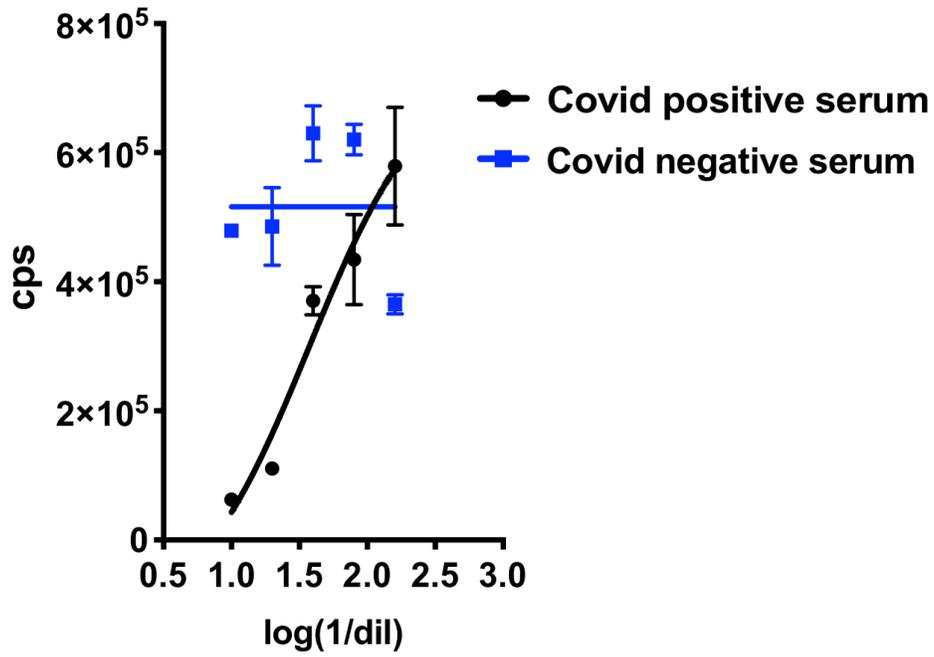


Figure S4. ACE2 microbody does not bind to Fc receptors. Related to Figure 5. (A) U937 cells were incubated for 30 minutes. with serially diluted soluble ACE2 or ACE2 microbody. Unbound soluble ACE2 proteins were removed and cell surface-bound proteins were detected by flow cytometry with anti-ACE2 antibody and Alexa fluor 594-conjugated anti-mouse IgG secondary antibody. ACE2.293T cells were analyzed as a positive control for ACE2 staining. The experiment was done twice with similar results.

A.



B.

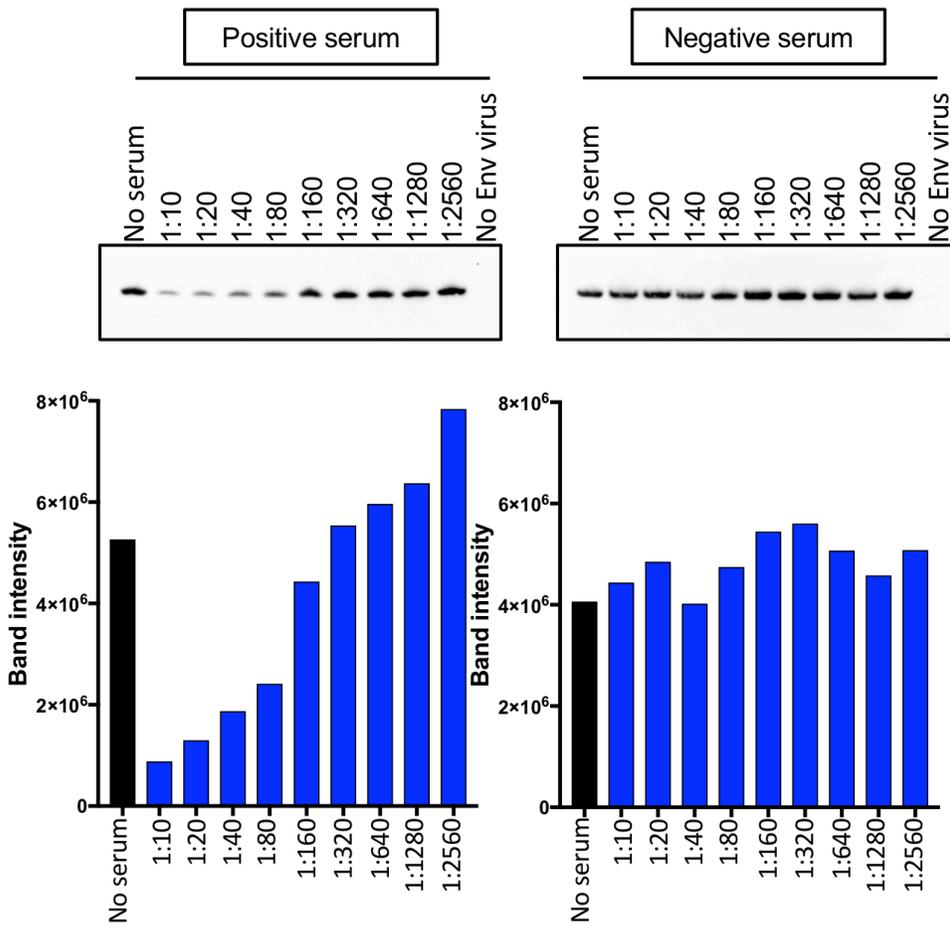


Figure S5. Titer of convalescent patient serum with SARS-CoV-2 lentiviral pseudotyped virus and effect of serum in virion binding assay. Related to Figure 5. (A) Serially diluted serum from a COVID-19 patient (black) and healthy donor (blue) was incubated for 30 minutes with pseudotyped virus and then added to Vero E6 cells. Two days post-infection, luciferase activity was measured. Nevirapine was added to one sample to confirm that signals were the result of bone fide infection. The data are displayed as the mean of triplicates \pm SD. Statistical significance was calculated by the student-t tests. (B) The ability of convalescent patient serum to block virus binding to ACE2 was tested. Ni-NTA agarose beads were coated with ACE2 microbody proteins. Serially diluted convalescent patient serum or healthy donor serum was incubated for 30 minutes with pseudotyped virions. The virions were then incubated for 1 hour with ACE2 microbody coated-beads. Free virions were removed and the bound protein was analyzed on an immunoblot probed with anti-p24 antibody. A histogram showing band intensities is shown below. The experiments were done three times with similar results.