Supplementary Figure 1

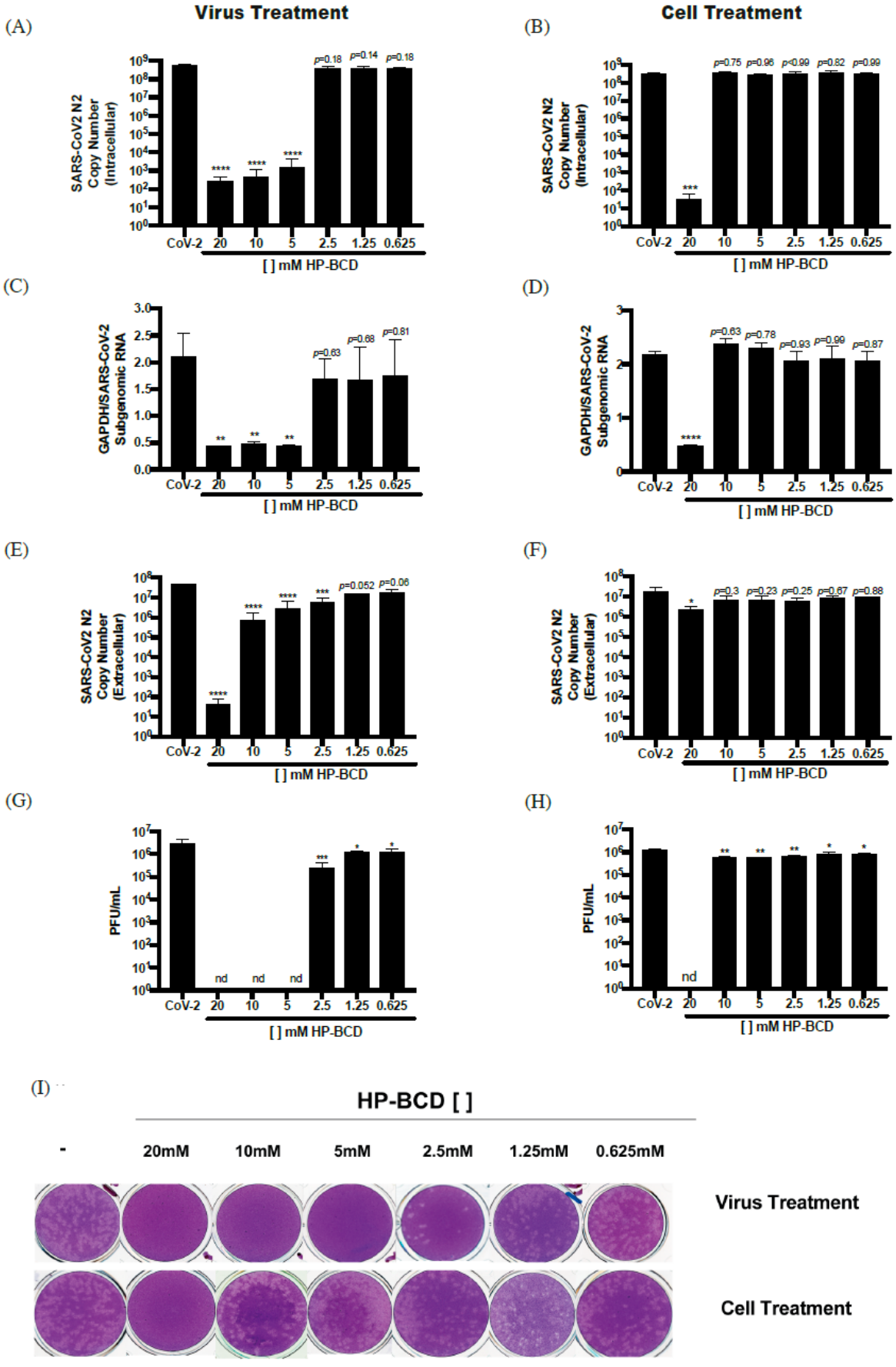

Figure S1: Inhibition of SARS-CoV-2 replication by HP-BCD. A-H) ***Virus treatment*** **(A, C, E, G):** SARS-CoV-2 stock samples were treated or not with the indicated concentrations of HP-BCD for 1 h, and then inoculated in Vero cells at a MOI of 0.1. After 1 h of virus adsorption, the cells were washed and cultured in HP-BCD-free culture medium for additional 72 h. ***Cell treatment*** **(B, D, F, H):** Vero cells were treated or not with the indicated concentrations of HP-BCD for 1h, the cells were washed, and the medium substituted by HP-BCD-free culture medium. Then, the cells were infected with SARS-CoV-2 (MOI of 0.1), and, after 72 h, the cell lysates and supernatants were harvested. The concentration (copy numbers) of intracellular genomic **(A, B)** and subgenomic virus RNA **(C, D)**, and of released genomic RNA **(E, F)** were measured by RT-qPCR; titration of released infectious virus particles was performed by plaque assay and represented as PFU/ml **(G, H)**. The bars indicate the average and SD of five independent experiments. Statistical analyses were performed by one-way anova followed by Dunnett's multiple comparison test; * represents $p<0.05$; ** $p<0.01$; *** $p<0.001$; ****$p< 0.0001$; nd-not detected. **I)** Representative experiment showing the plaques obtained at 72 hpi after cell or virus treatment with HP-BCD, as in **(G)** and **(H)**.

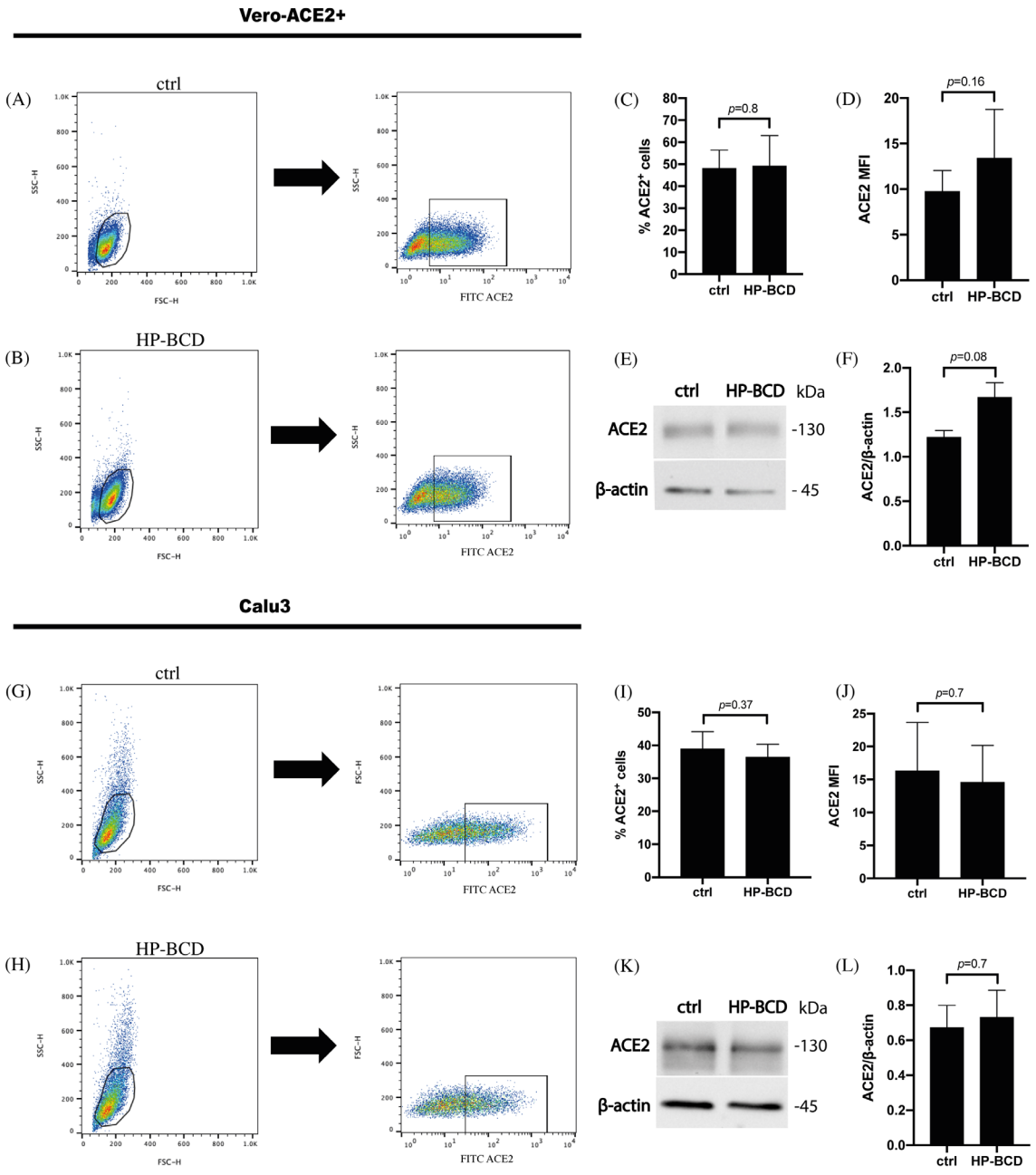

Figure S2: Evaluation of ACE2 expression in HP-BCD-treated cells using different monoclonal antibodies. ACE2-expressing Vero cells (Vero-ACE2+) or Calu-3 cells were treated with 20 mM HP-BCD for 1 h. The expression of ACE2 in intact nonpermeabilized cells was analyzed by flow cytometry using Ab272500 Abcam antibody, whereas total expression of ACE2 was evaluated in the cell lysates by western blotting, using Ab108252 Abcam antibody. The data obtained after the analyses of Vero-ACE2+ cells are demonstrated in **(A-F)**, whereas the data from Calu-3 cells are shown in **(G-L)**. **A, B, G, H)** Representative flow cytometry data showing the gate strategy and ACE2 staining of control and HP-BCD-treated cells. **C, D, I, J)** The bars

show the average and SD of the frequency of ACE2+ cells (% ACE2) and mean fluorescence intensity (MFI) from two independent experiments; statistical analyses were performed by paired t-test. **E, K, F, L)** Western blotting analysis, after staining the membranes with anti-ACE2, and anti-β-actin, as a loading control. Representative blots are demonstrated in **(E, K)**; and the expression level of ACE-2, in two independent experiments, were estimated using ImageJ software and normalized according to β-actin expression **(F, L)**. The bars show the average and SD from two independent experiments; statistical analyses were performed by paired t-test.