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Supplementary Information

Degradative Effect of Nattokinase on Spike Protein of SARS-CoV-2

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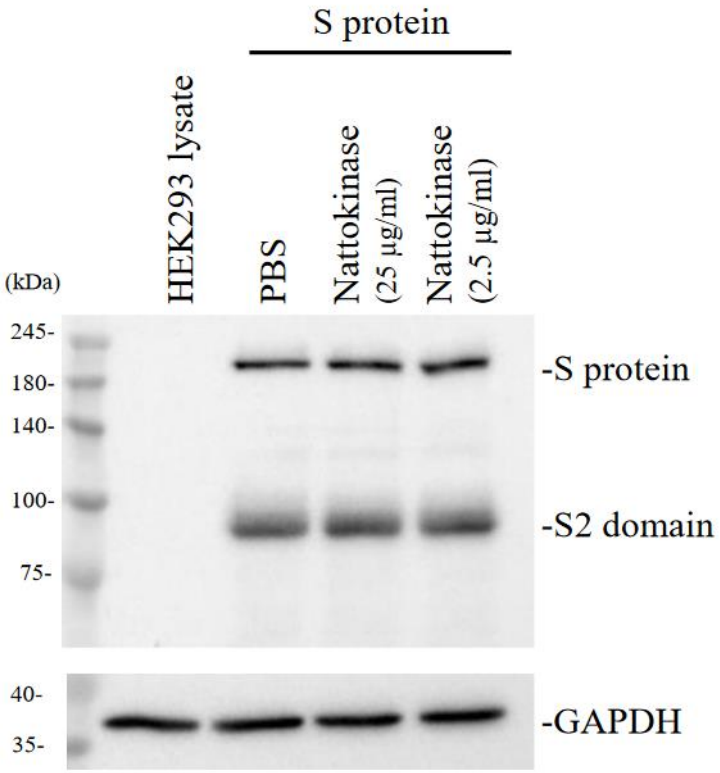

Figure S1. Effect of nattokinase addition to S protein expressing cell culture medium.was evaluated by westernblotting analysis. HEK293 cells were cultured at a density of3.5 × 105 cells/ml in DMEM supplemented with 10% FBS, L-glutamine, 100 U/mlpenicillin, and 100 μg/ml streptomycin. The cells were transfected with pcDNA3.1-SARS2-Spike and incubated for 9 h. After incubation, the cells were treated with nattokinase (25 and 2.5 μg/ml), incubated for 13 h. Western blotting was performed using anti-rhodopsin (C9) mouse monoclonal antibody (1D4) (Santa Cruz Biotechnology, TX, USA), anti-GAPDH mouse monoclonal antibody (FUJIFILM Wako)

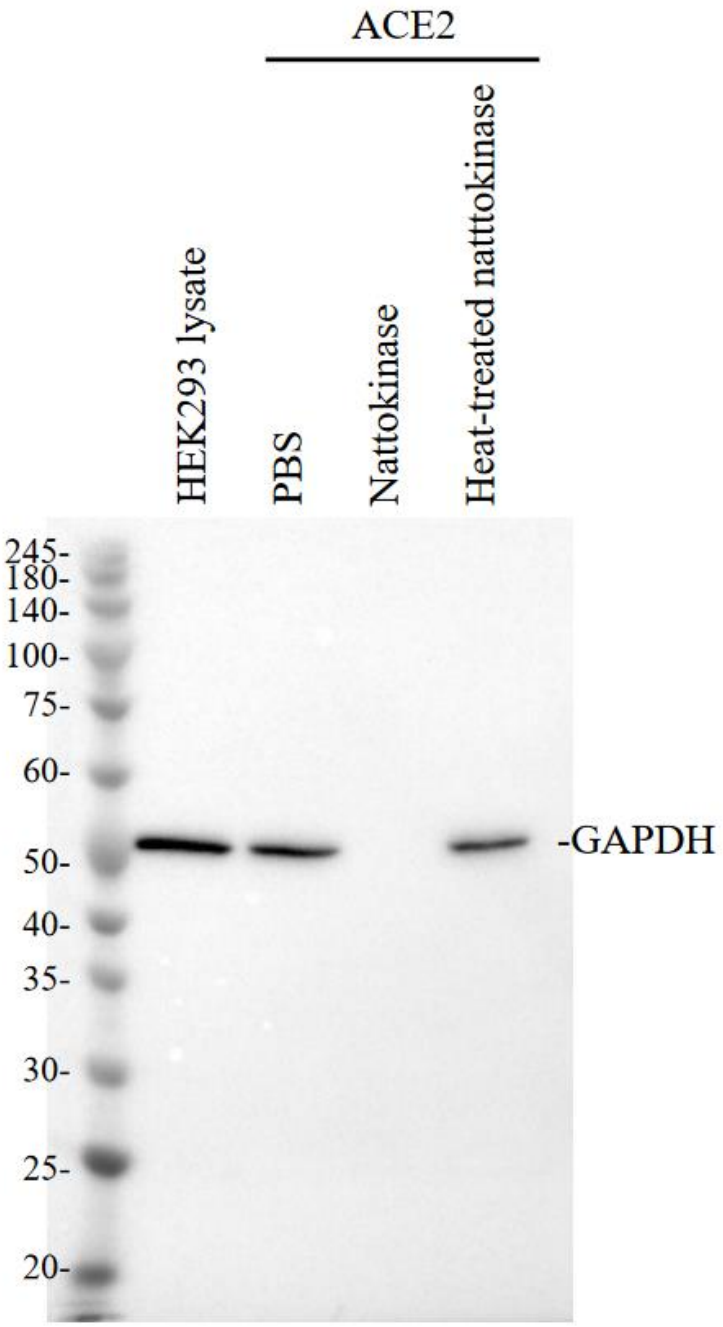

Figure S2. Degradative effects of GAPDH in vitro. The HEK293 cells were transfected with pcDNA3.1-hACE2 and incubated for 22 h. After incubation, the cultured cells were scraped and washed with ice-cold Dulbecco's phosphate-buffered saline (D-PBS). Cell lysates were incubated with nattokinase (7.5 μg/ml) and heat-treated nattokinase (7.5 μg/ml) and western blotting were performed. Equal volumes of the reaction mixture were loaded and western blotting was performed. The primary antibodies included anti-GAPDH mouse monoclonal antibody (FUJIFILM Wako) and secondaryantibodies include HRP-conjugated goat anti-mouse antibody (Proteintech).