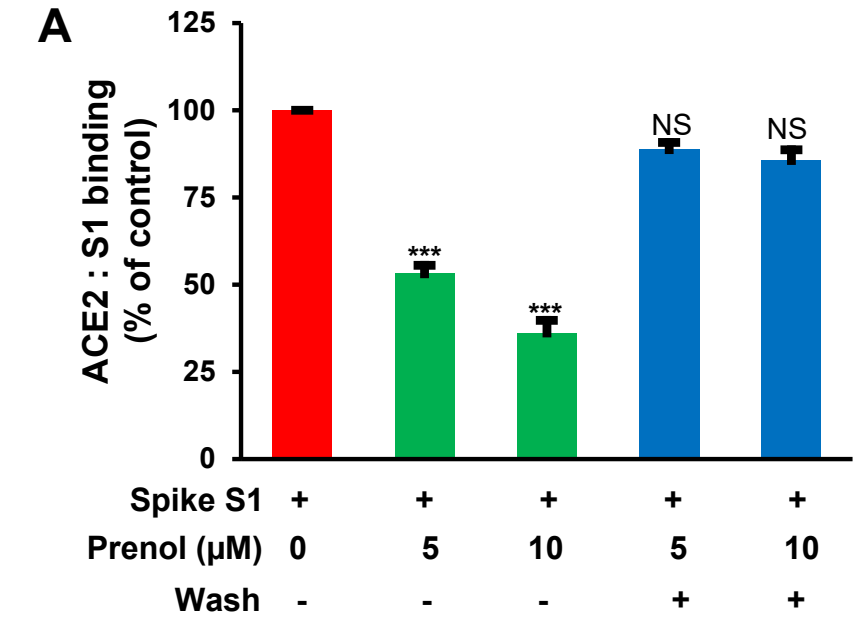

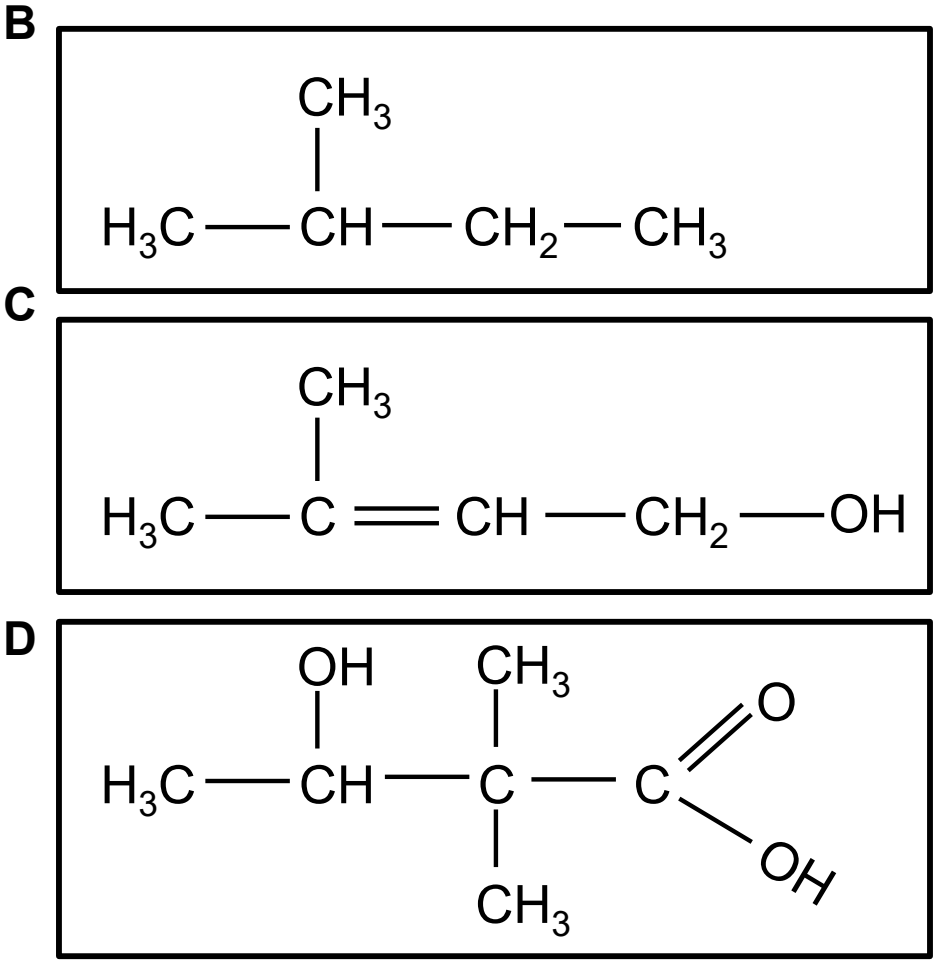

Figure S1. Prenol interferes with spike S1-to-ACE2 interaction through competitive binding. A) During the AlphaScreen)-based assay in which ACE2-coated 96-well plate was treated with different concentrations of prenol followed by addition of SARS-CoV-2 Spike (RBD)-Fc, in one set, prenol was washed out thrice after 30 min of incubation. Resultant chemiluminescence was monitored after treatment with anti-mouse Fc-HRP and an HRP substrate using a Perkin Elmer Victor X5 as mentioned under Materials and Methods. Results are mean ± SD of three independent experiments. $^{***}p < 0.001$; NS, not significant vs. spike S1 control without prenol.

Chemical structures of 2-methyl butane (B), prenol (C) and 3-hydroxy-2,2-dimethyl butyric acid or HDMB (D).

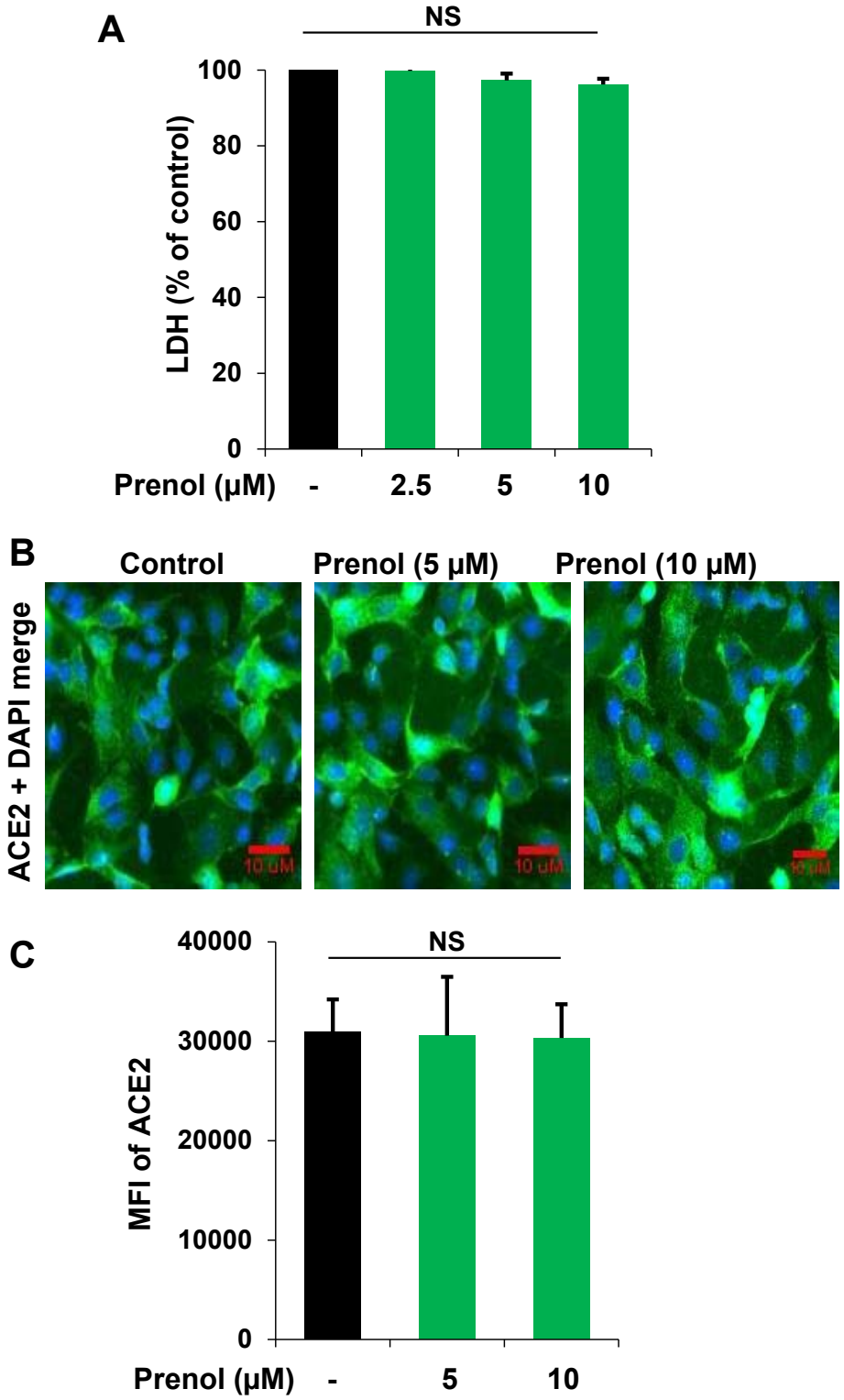

Figure S2. Effect of prenol on the survival and the level of ACE2 in A549 cells. A) After 5 h of stimulation with different concentrations of prenol, cell viability was examined by the release of LDH. Values obtained from the control group served as 100%. Data obtained in other groups were calculated as percent of control. Results are mean ± S.D. of three different experiments. NS, not significant. Cells were treated with 5 and 10 μM prenol for 6 h followed by immunofluorescence analysis with ACE2 antibody (B). DAPI was used to visualize nuclei. C) Mean fluorescence intensity (MFI) of ACE2 was calculated from a total of 6 images collected from three different experiments. Data are mean ± SEM. NS, not significant

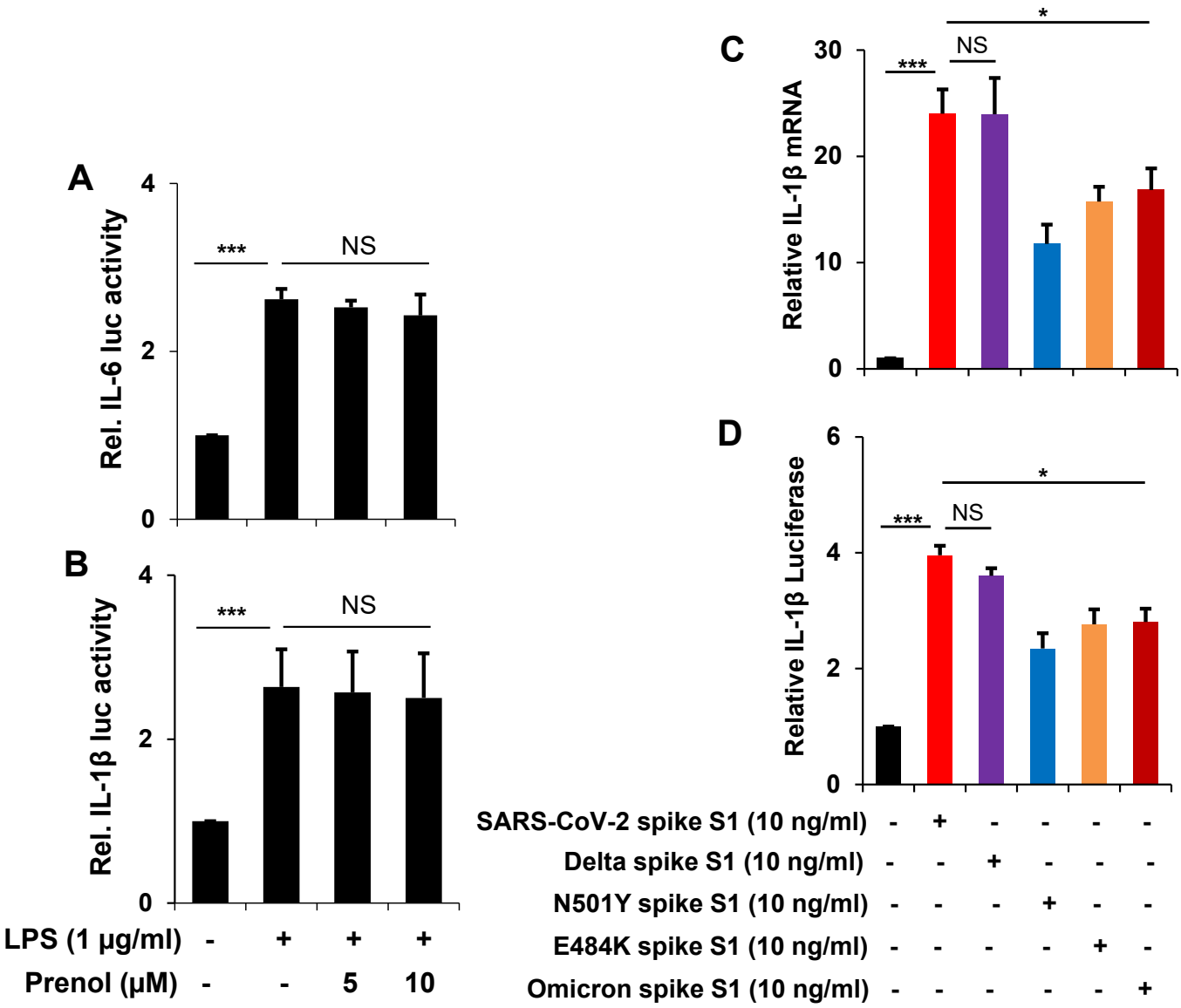

Figure S3. Effect of prenol on LPS-induced activation of IL-1β promoter and IL-6 promoter in A549 cells. Cells were transfected with either pIL-6-promoter-Luc (A) or pIL-1β-promoter-Luc (B). Twenty-four h after transfection, cells were treated with LPS (1 μg/ml) in the presence of different doses of prenol under serum-free condition. After 4 h, luciferase activities were measured in cell lysates. Results are mean ± SD of three different experiments. $^{***}p < 0.001$; NS, not significant.

Effect of different variants of SARS-CoV-2 on the mRNA expression of IL-1β and IL-1β promoter-driven luciferase activity in human A549 lung cell line. C) Cells were stimulated with 10 ng/ml SARS-CoV-2 spike S1, delta variant SARS-CoV-2 spike S1, alpha (N501Y) variant SARS-CoV-2 spike S1, beta (E484K) variant SARS-CoV-2 spike S1, and omicron variant SARS-CoV-2 spike S1, separately, under serum-free condition. After 4 h of stimulation, the mRNA expression of IL-1β was monitored by quantitative real-time PCR. D) Cells plated in 12-well plates at 60-70% confluence were transfected with 0.25 μg pIL-1b-promoter-Luc construct using Lipofectamine Plus. Twenty-four h after transfection, cells were stimulated with SARS-CoV-2 spike S1, delta variant SARS-CoV-2 spike S1, N501Y variant SARS-CoV-2 spike S1, E484K variant SARS-CoV-2 spike S1, and omicron variant SARS-CoV-2 spike S1, separately, under serum-free condition for 4 h followed by monitoring luciferase activity in cell extracts. Results are mean ± SD of three independent experiments. $^{*}p < 0.05$; $^{***}p < 0.001$; NS, not significant.

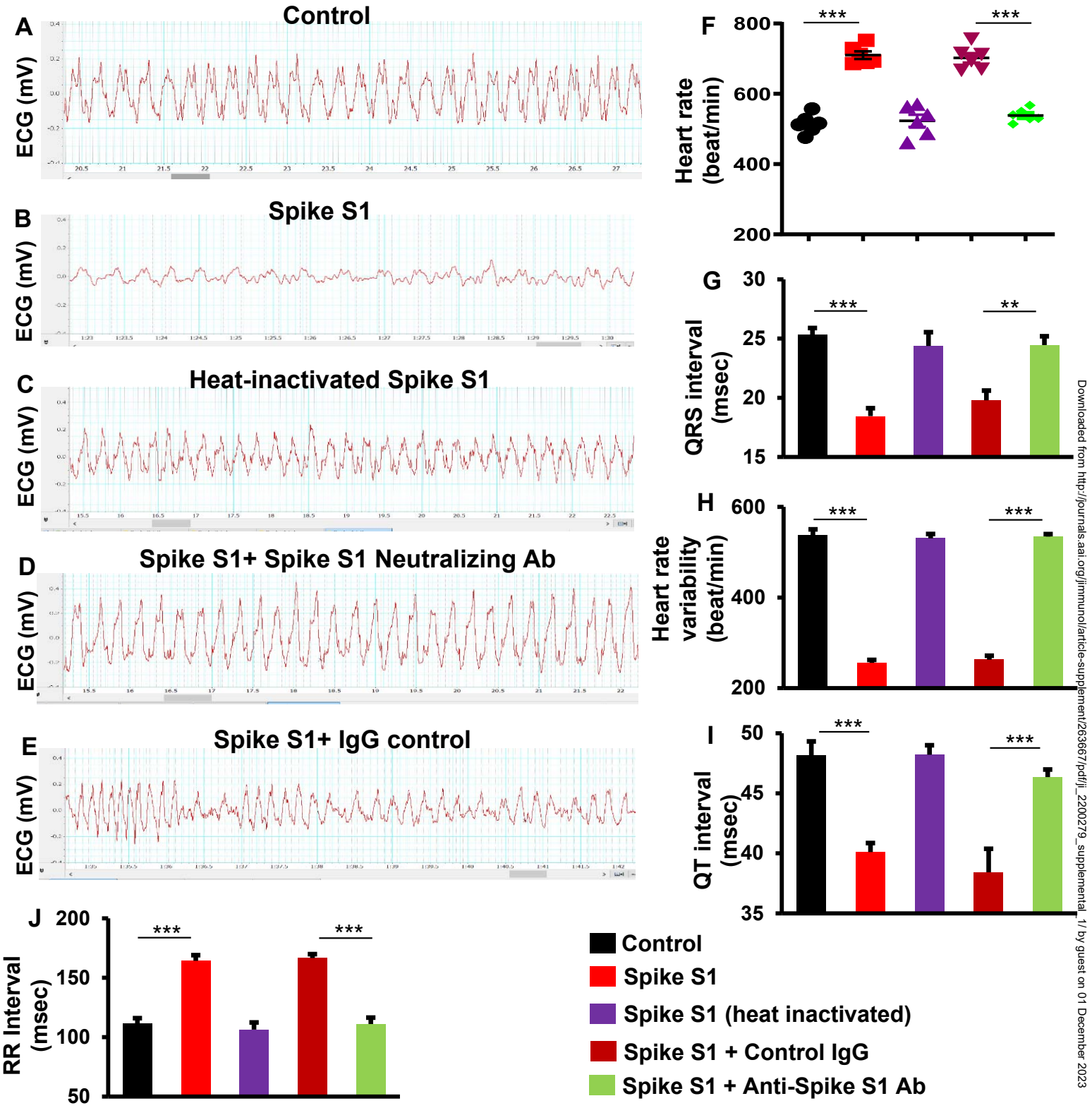

Figure S4. Specificity of SARS-CoV-2 spike S1- mediated arrhythmia in mice. Eight-to-ten week old C57/BL6 mice (n=6) of both sexes were intoxicated with recombinant SARS-CoV-2 spike S1 (50 ng/mouse/d) via intranasal route. To understand the specificity, one group of mice received same amount of boiled SARS-CoV-2 spike S1. Other groups of mice received the combination of SARS-CoV-2 spike S1 (50 ng) and 200 ng of either anti-spike S1 antibody or control IgG. After 12 d, heart functions were monitored by non-invasive electrocardiography (ECG) using the PowerLab (ADInstruments) [A, chromatogram of control mice; B, chromatogram of spike S1-intoxicated mice; C, chromatogram of heat-inactivated spike S1-intoxicated mice; D, chromatogram of (spike S1 + spike S1 neutralizing antibody)-intoxicated mice; E, chromatogram of (spike S1 + control IgG)-intoxicated mice; F, heart rate; G, QRS interval; H, heart rate variability; I, RR interval; J, QT interval]. Results are mean ± SEM of 6 mice per group. $^{**}p < 0.01$; $^{***}p < 0.001$.