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## Supplemental information

# A pathogen-like antigen-based vaccine confers immune protection against SARS-CoV-2 in non-human primates

**Chang Guo, Yanan Peng, Lin Lin, Xiaoyan Pan, Mengqi Fang, Yun Zhao, Keyan Bao, Runhan Li, Jianbao Han, Jiaorong Chen, Tian-Zhang Song, Xiao-Li Feng, Yahong Zhou, Gan Zhao, Leike Zhang, Yongtang Zheng, Ping Zhu, Haiying Hang, Linqi Zhang, Zhaolin Hua, Hongyu Deng, and Baidong Hou**

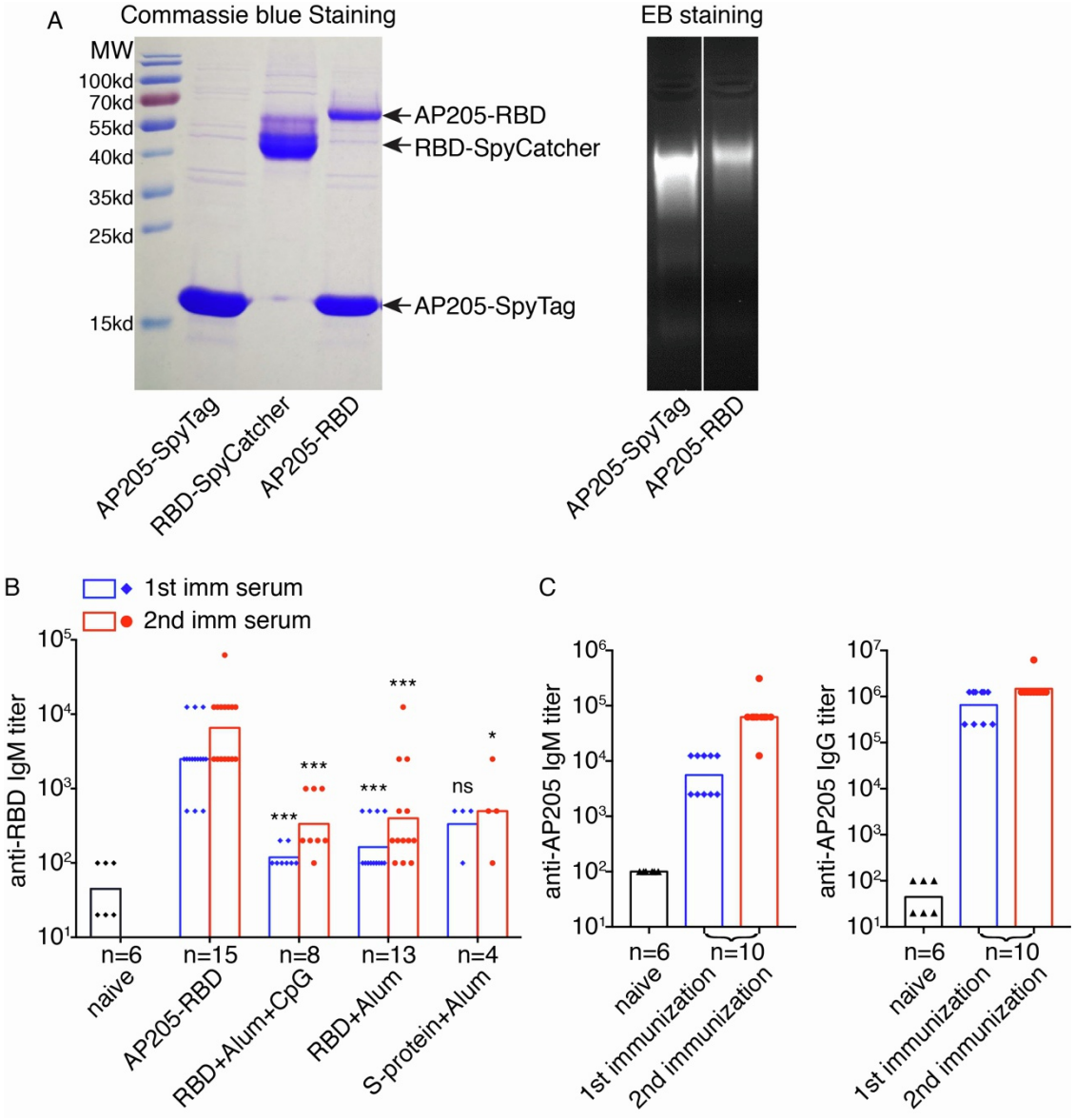


**Figure S1 AP205-RBD elicited anti-RBD and anti-AP205 antibody responses in mice, Related to Figure 1.**

(A) AP205-SpyTag, RBD-SpyCatcher and AP205-RBD were subjected to SDS-PAGE and Commassie Blue staining to examine the conjugation efficiency (left panel), or electrophoresis in 1% agarose gel and ethidium bromide staining to examine the nucleic acids (right panel). (B-C) Serum anti-RBD IgM (B) and anti-AP205 IgM or IgG (C) from mice immunized with AP205-RBD or the indicated antigens were measured by ELISA. Endpoint titers were presented. Symbols indicate data collected from individual mice. Bars indicate the geometric mean of each group. The number (n) of mice examined in each group was shown. For (B), Kruskal-Wallis test was used to compare the five immunized groups within either the '1st imm serum' or the '2nd imm serum'. There are significant differences among the five groups for both the '1st' and the '2nd imm serum'. Dunn's multiple comparisons test was then used to compare between the AP205-RBD immunized group and one of the other immunized groups, and the adjusted p value was used to determine the statistical significance and indicated in the graph. (* < 0.05, ** < 0.01, *** <0.001).

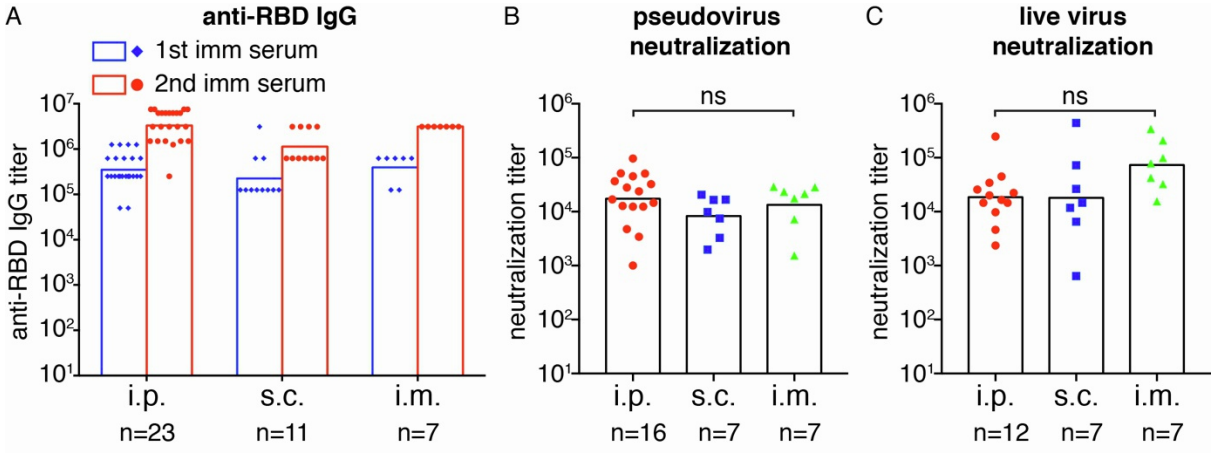


**Figure S2 AP205-RBD-elicited antibody response was not affected by immunization routes, Related to Figure 2.** AP205-RBD was administered through one of the indicated routes: i.p. (intraperitoneal), s.c. (subcutaneous), and i.m. (intramuscular). (A) Serum anti-RBD IgG from mice immunized after the first and the second immunization. (B-C) Neutralization titers (defined as half-maximal inhibitory concentrations) against pseudovirus (B) or live SARS-CoV-2 virus (C) in sera collected after the second immunization. Kruskal-Wallis test was used to compare among the groups. There is no significant difference among them. Symbols indicate data collected from individual mice. Bars indicate the geometric mean of each group. The number (n) of mice examined in each group was shown. The data of the i.p. group was also used in Figures 1D, 2A and 2B.

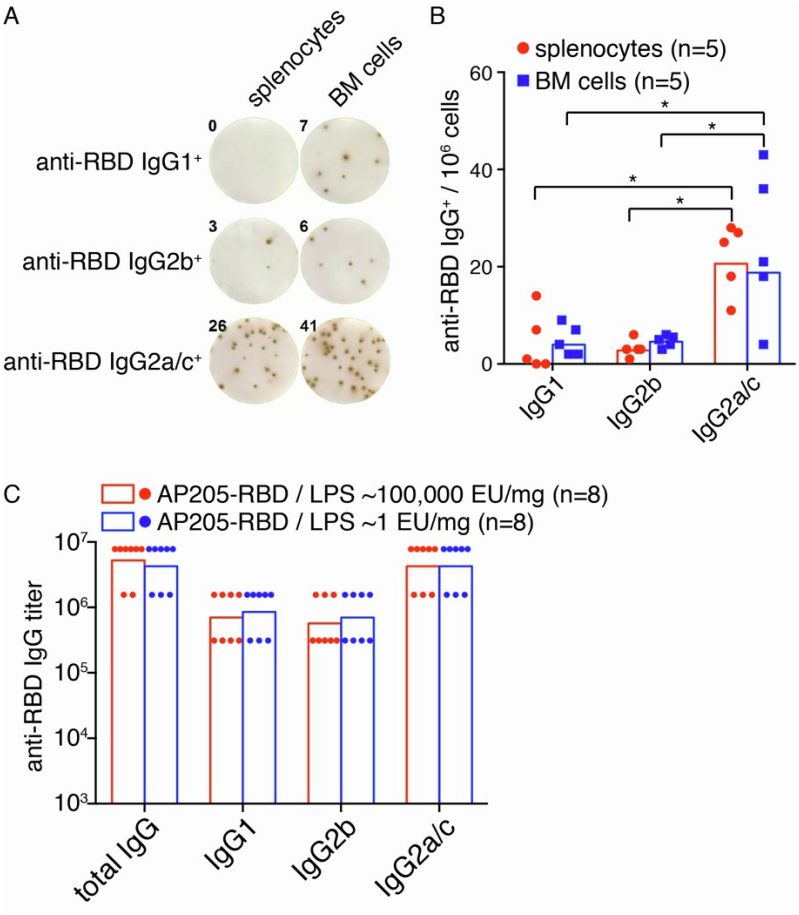


**Figure S3 AP205-RBD induced IgG2a/c$^+$ long-lived plasma cells, Related to Figure 3.**

(A-B) Splenocytes or bone marrow (BM) cells from mice immunized with AP205-RBD twice 3-4 months previously were examined for isotype-specific anti-RBD Ig secreting cells. Representative data is shown in (A) and quantification of data is shown in (B). Paired t test was used to compare between the IgG2a/c$^+$ and the IgG1$^+$ or the IgG2b$^+$ cell numbers (* < 0.05). (C) Mice were immunized i.p. with AP205-RBD containing different levels of LPS. Sera collected after the second immunization were examined for total anti-RBD IgG and IgG sub-isotypes. Symbols indicate data collected from individual mice. Bars indicate the geometric mean of each group. The number (n) of mice examined in each group was shown.

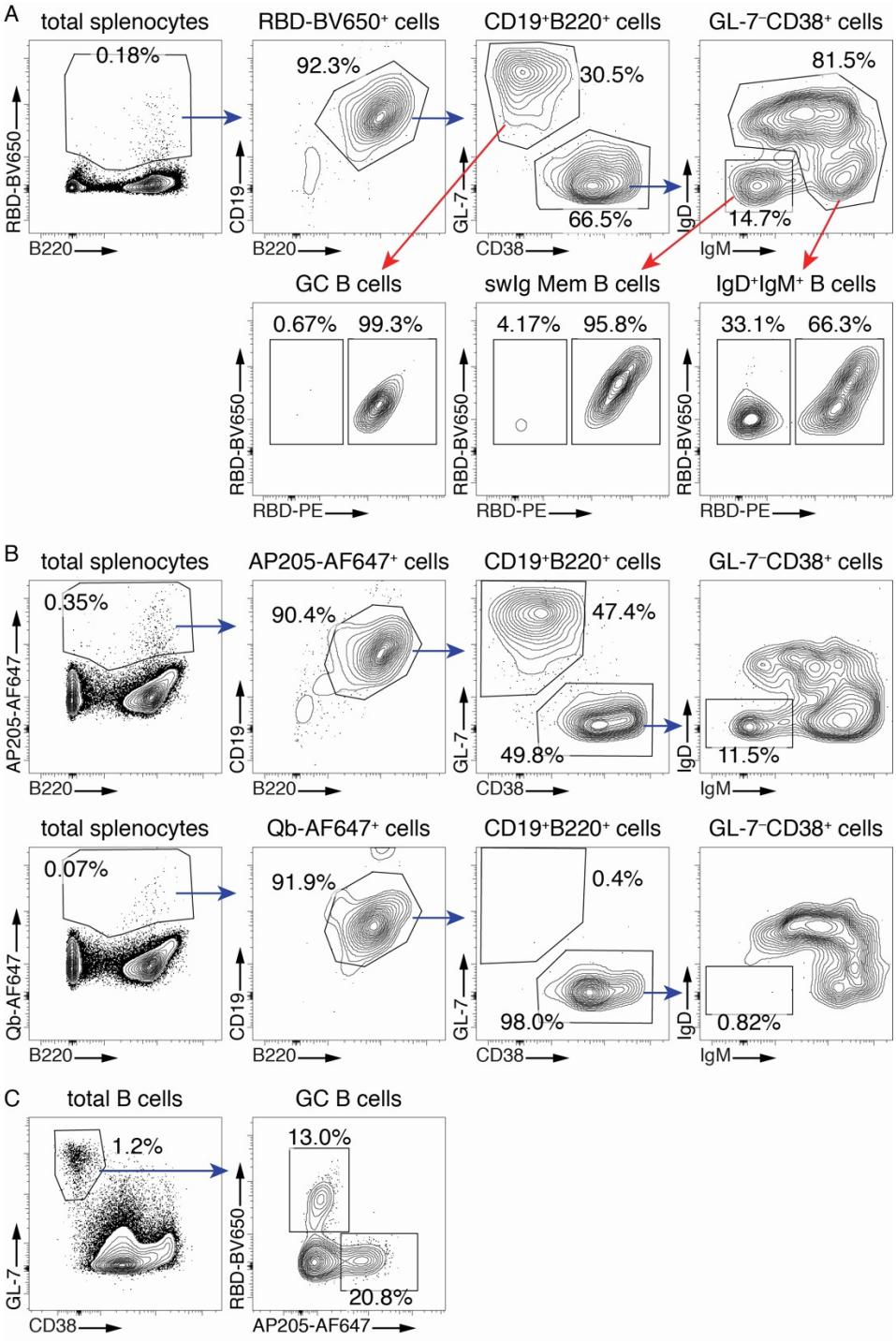


**Figure S4 Fluorophore-binding cells maintained naïve upon immunization, Related to Figure 4.**

(A-C) Splenocytes from mice immunized with AP205-RBD once 14 days previously were examined. (A) Cells were stained with both RBD-BV650 and RBD-PE. GC B cells and swIg Mem B cells are mostly RBD-BV650$^{+}$RBD-PE$^{+}$, whereas IgD$^{+}$IgM$^{+}$ B cells consist both RBD-BV650$^{+}$RBD-PE$^{+}$ and RBD-BV650$^{+}$RBD-PE$^{-}$ cells. (B) Cells were stained with AP205-AF647 and Qb-AF647, an AF647-conjugated irrelevant antigen. AP205-AF647$^{+}$ cells consist of both GC B cells and swIg Mem B cells whereas Qb-AF647$^{+}$ cells do not. (C) Cells were labeled with both RBD-BV650 and AP205-AF647. RBD-650$^{+}$ GC B cells and AP205-AF647$^{+}$ GC B cells are distinct from each other.

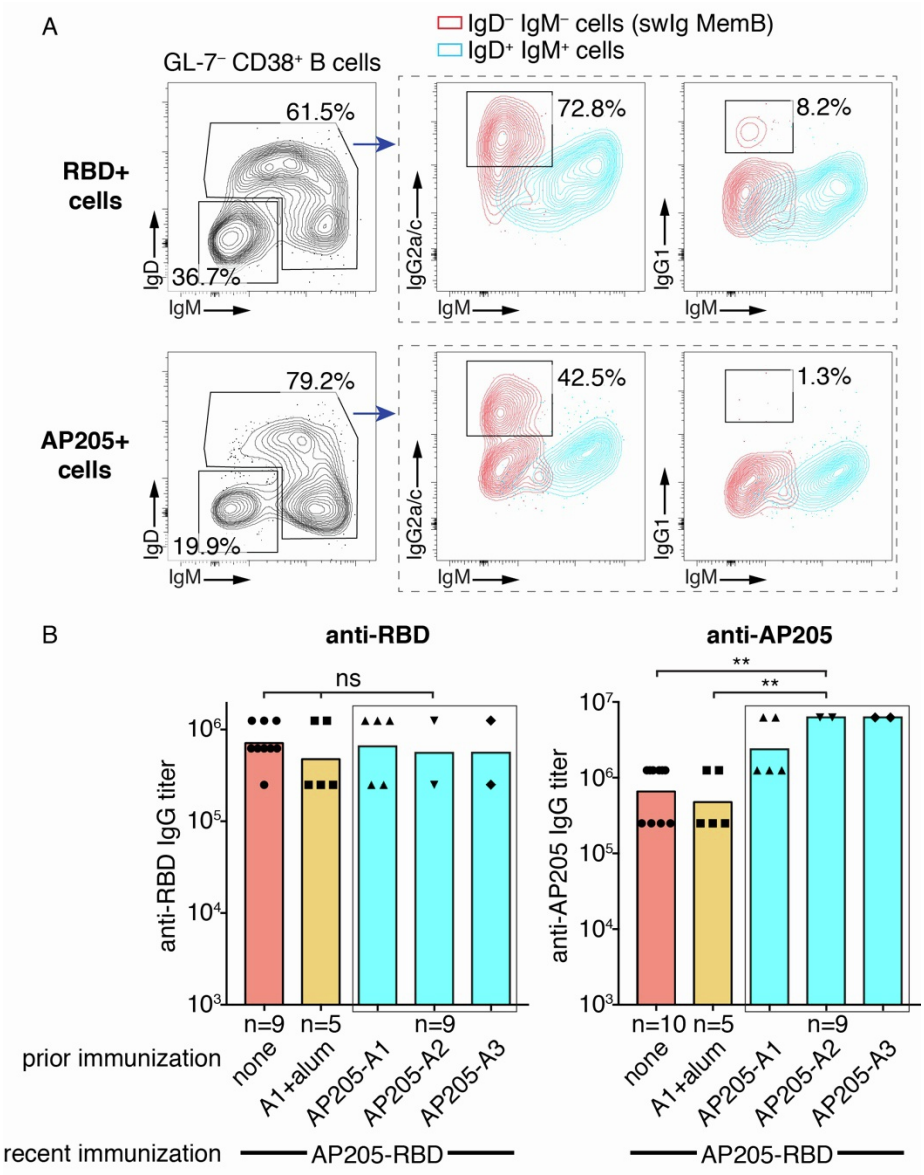


**Figure S5 AP205-RBD induced durable humoral memory, Related to Figure 5.**

(A) AP205-RBD induced IgG2a/c$^+$ memory B cells. Splenocytes from mice immunized with AP205-RBD twice previously were examined for antigen-specific B cells (RBD$^+$ or AP205$^+$). GL7$^-$CD38$^+$ non-GC B cells were further gated as IgD$^-$IgM$^-$ (swIg MemB) and IgD$^+$IgM$^+$ cells. The staining of IgG2a/c or IgG1 in the swIg MemB cells was overlaid with that in the IgD$^+$IgM$^+$ cells. Data is representative of at least three independent experiments. (B) AP205-RBD induced anti-RBD response was not affected by previous exposure to the AP205 carrier. Mice that have no prior immunization history (none) or that have been immunized with SARS-CoV-2 irrelevant antigens 4-6 months previously were immunized with AP205-RBD once and examined for serum anti-RBD and anti-AP205 IgG two weeks later. A1, A2 and A3 are three different antigens with no homology to SARS-CoV-2 S protein and were tagged with SpyCatcher and conjugated to AP205-SpyTag in the same manner as AP205-RBD was generated. In addition, mice immunized with A1 adjuvanted in alum (not conjugated to AP205) were also included for comparison. Kruskal-Wallis test was used for statistical analysis among groups. Mice immunized with AP205-A1, AP205-A2 and AP205-A3 were treated as one group. There is no significant difference of anti-RBD IgG but a statistically significant difference of anti-AP205 IgG among the compared groups. Dunn's multiple comparisons test was then used to compare between the AP205-antigen immunized group and one of the other two groups, and the adjusted p value was indicated in the graph (** < 0.01).

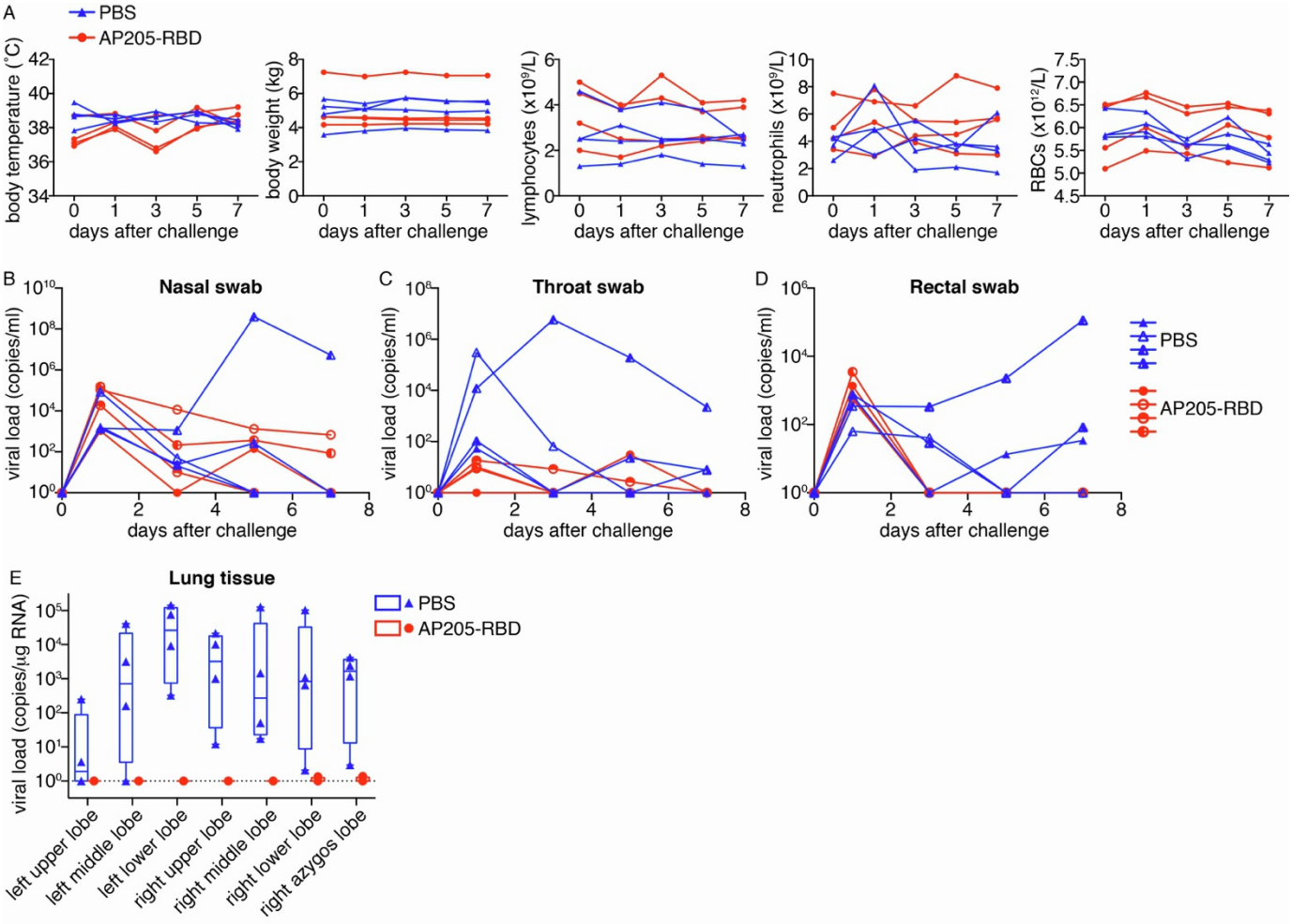


**Figure S6 Vaccination with AP205-RBD accelerated the viral clearance in the infected animals, Related to Figure 7.** (A) Changes of body temperature, weight, blood lymphocyte count, neutrophil count and red blood cell (RBC) count in the animals after viral challenge. (B-D) Swab samples were taken from nasal cavity, throat and rectum at the indicated time points after SARS-CoV-2 challenge and examined for viral loads. Data from the same animal were connected by lines. The same data are used to generate Figures 7A-C. (E) The lung tissue samples were taken at day 7 after viral challenge from each of the 7 lung lobes. The viral loads in each lung lobe were examined. Boxes indicate the quartiles of each group and symbols indicate the data from individual animals. The same data was presented in Figure 7E as the mean of the viral loads in 7 lung lobes from each individual animal.