

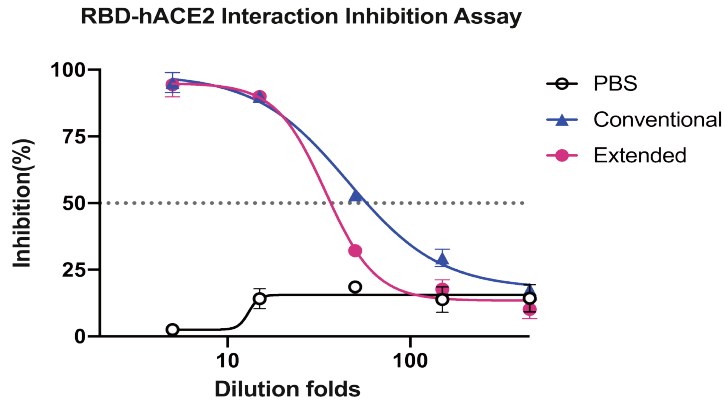
Supplemental information

**Extended SARS-CoV-2 RBD booster
vaccination induces humoral and cellular
immune tolerance in mice**

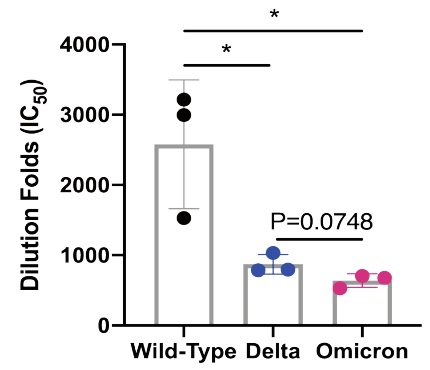
Feng-Xia Gao, Rui-Xin Wu, Mei-Ying Shen, Jing-Jing Huang, Ting-Ting Li, Chao Hu, Fei-Yang Luo, Shu-Yi Song, Song Mu, Ya-Nan Hao, Xiao-Jian Han, Ying-Ming Wang, Luo Li, Sheng-Long Li, Qian Chen, Wang Wang, and Ai-Shun Jin

Figure S1

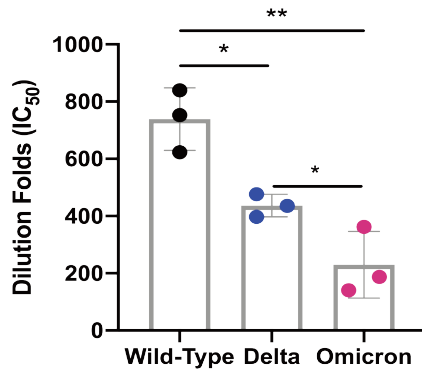
A.



B.



C.



D.

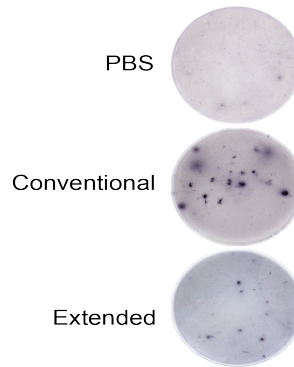
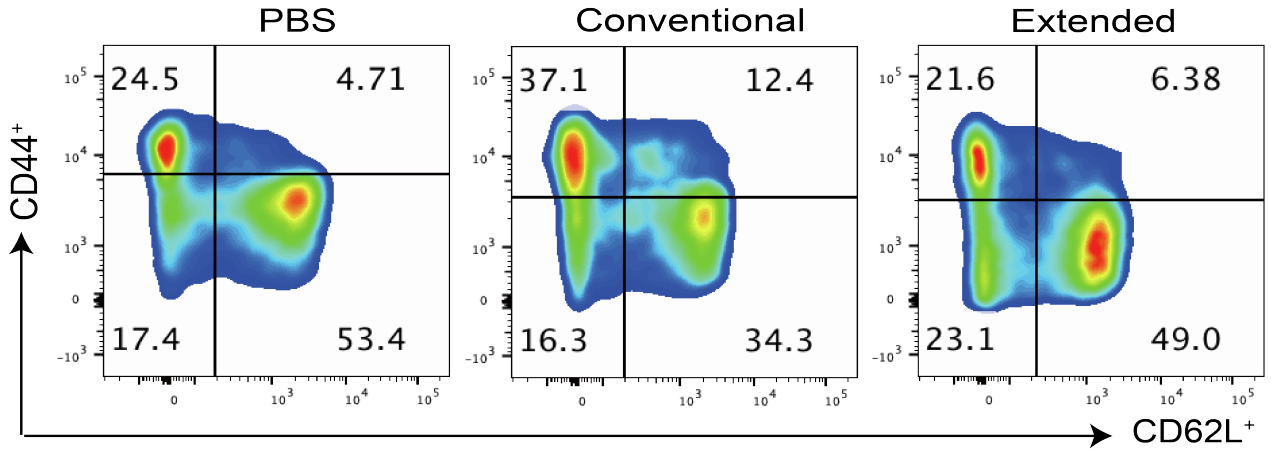


Figure S1. Extended immunization inefficiently induced humoral immunity in Balb/c mice. Related to Figure 2 and Figure 3.

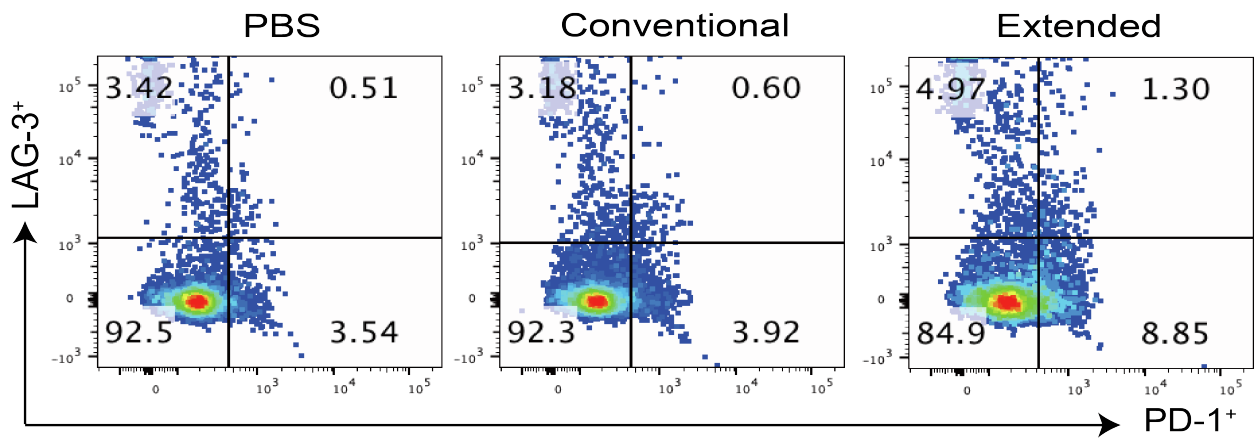
(A) The RBD blocking ability of mice serum was determined by RBD-hACE2 interaction inhibition assay. Pseudo-viruses neutralization curves for SARS-CoV-2 (wild-type), Delta and Omicron strains by mice sera taken 7 days following the last dose of the conventional vaccination group. Comparison of neutralization titers between SARS-CoV-2 (wild-type) and 2 variant strains for the conventional vaccination serum (B) or the extended immunization serum (C). Points represent individual mice in (B) and (C). (D) ELISPOT results of RBD-specific memory B cells in the spleen of mice after last immunization. Results were expressed as the numbers of RBD-specific IgG spots per 5×10^5 splenocytes of each mouse, subtracted those from the corresponding DMSO groups. Data were representative of two independent experiments. Data were presented as mean \pm (SEM). * $P < 0.05$, ** $P < 0.01$.

Figure S2

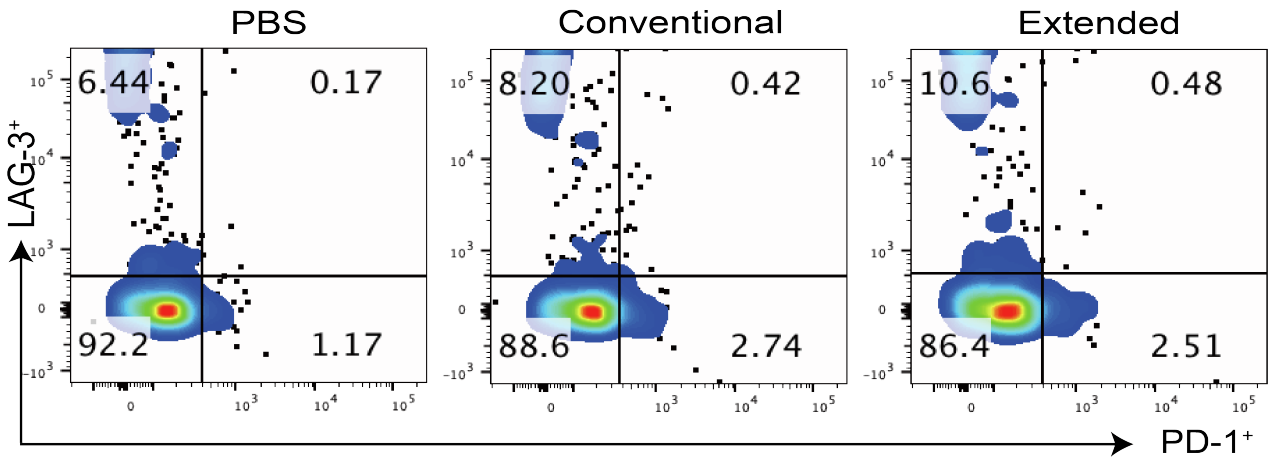
A.



B.



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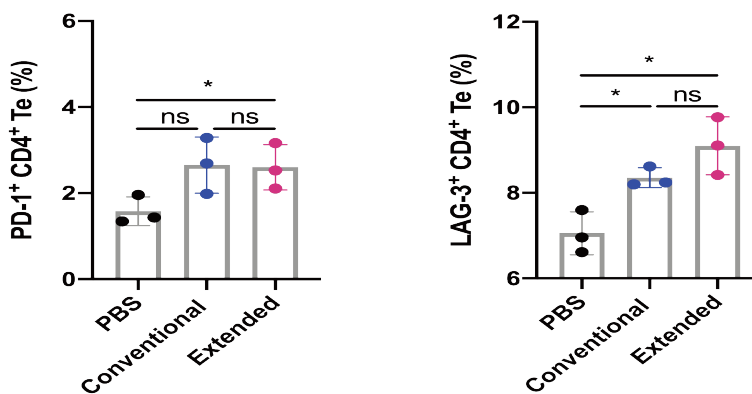


Figure S2. Extended immunization inhibited the activation of CD4⁺ T cell immune responses. Related to Figure 5.

(A) The ratio of Tn (CD62L⁺ CD44⁻), Te (CD62L⁻ CD44⁻), Tem (CD62L⁻ CD44⁺) and Tcm (CD62L⁺ CD44⁺) of CD4⁺ T cells on day 7 after the last immunization. (B) The expression of PD-1 and LAG-3 (gated on CD4⁺ T cells) were detected on day 7 after the last immunization. (C-D) The expression of PD-1 and LAG-3 (gated on Te (CD62L⁻ CD44⁻)) were detected. Points represent individual mice. Data were presented as mean \pm (SEM). * P < 0.05. ns represented non-significant.

Figure S3

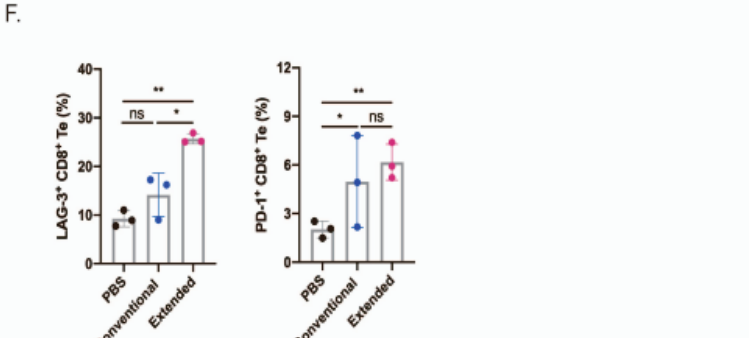
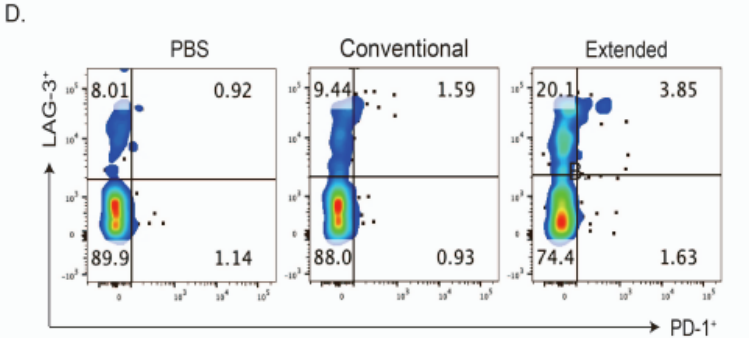
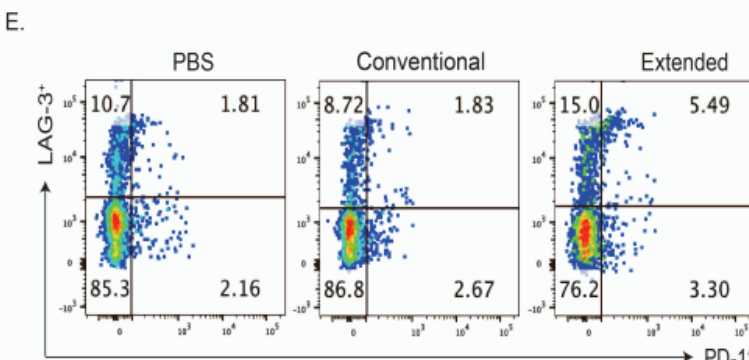
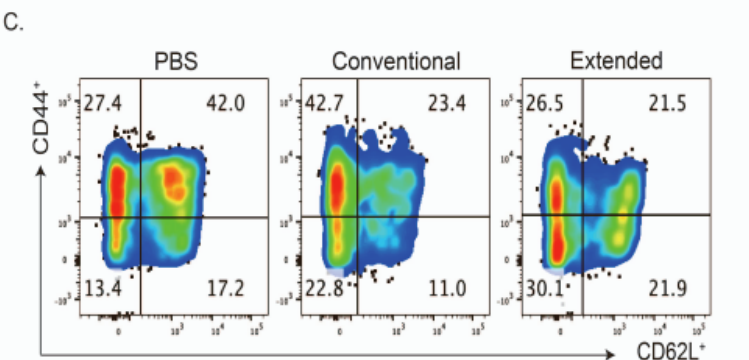
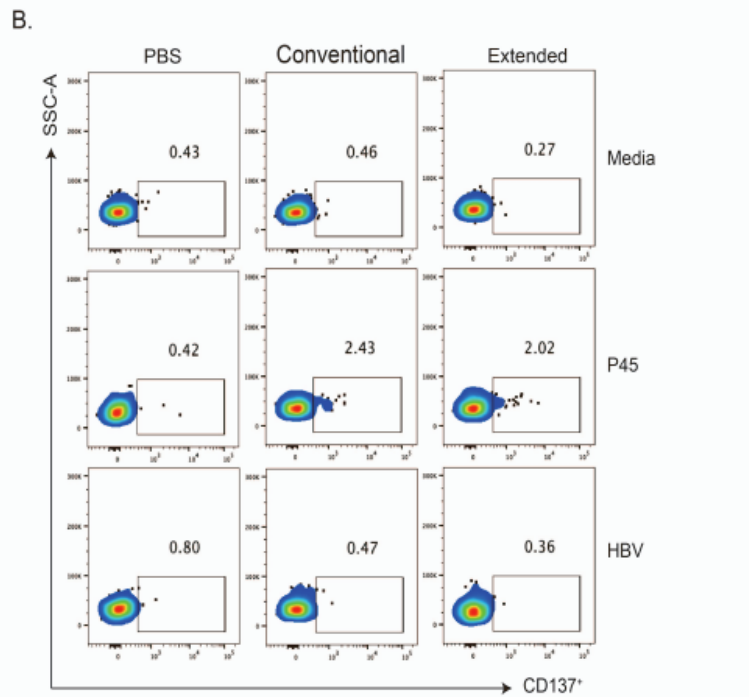
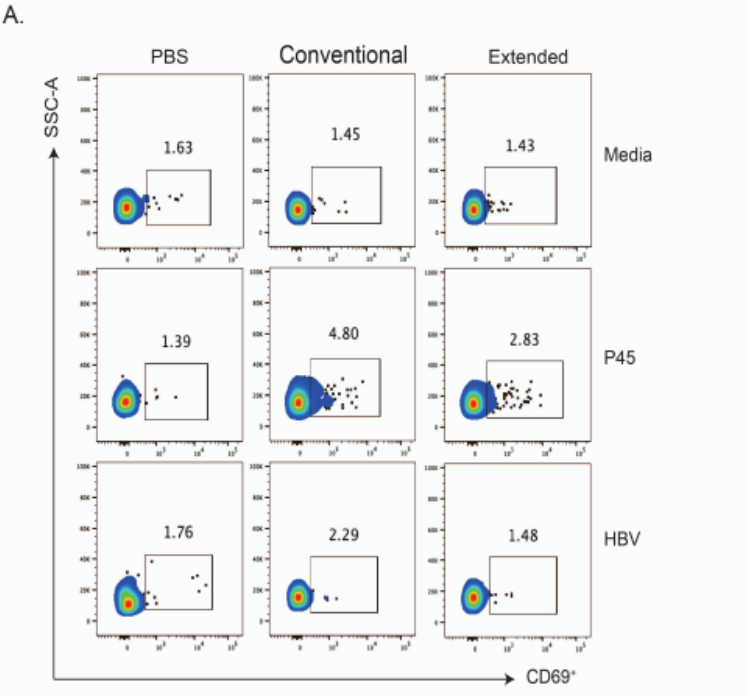


Figure S3. Extended immunization inhibited CD8+ T cell-mediated immune response. Related to Figure 6.

The expression of CD69 (A) and CD137 (B) in spleen (gated on CD8+ T cells) were detected by flow cytometry after 10 µg/mL RBD or HBV peptide stimulation for 24 hours, conventional media was used as negative control. ns represented non-significant. (C) The ratio of Tn (CD62L+ CD44-), Te (CD62L- CD44-), Tem (CD62L- CD44+) and Tcm (CD62L+ CD44+) of CD8+ T cells on day 7 after the last immunization. (D) The expression of PD-1 and LAG-3 (gated on CD8+ T cells) were detected on day 7 after the last immunization. (E-F) The expression of PD-1 and LAG-3 (gated on CD8+ T cells) were detected on day 7 after the last immunization. Data were presented as mean ± (SEM). * P < 0.05, ** P < 0.01. ns represented non-significant.