Foot-and-mouth disease virus-like particle mRNA vaccine induces strong humoral and cellular immunity

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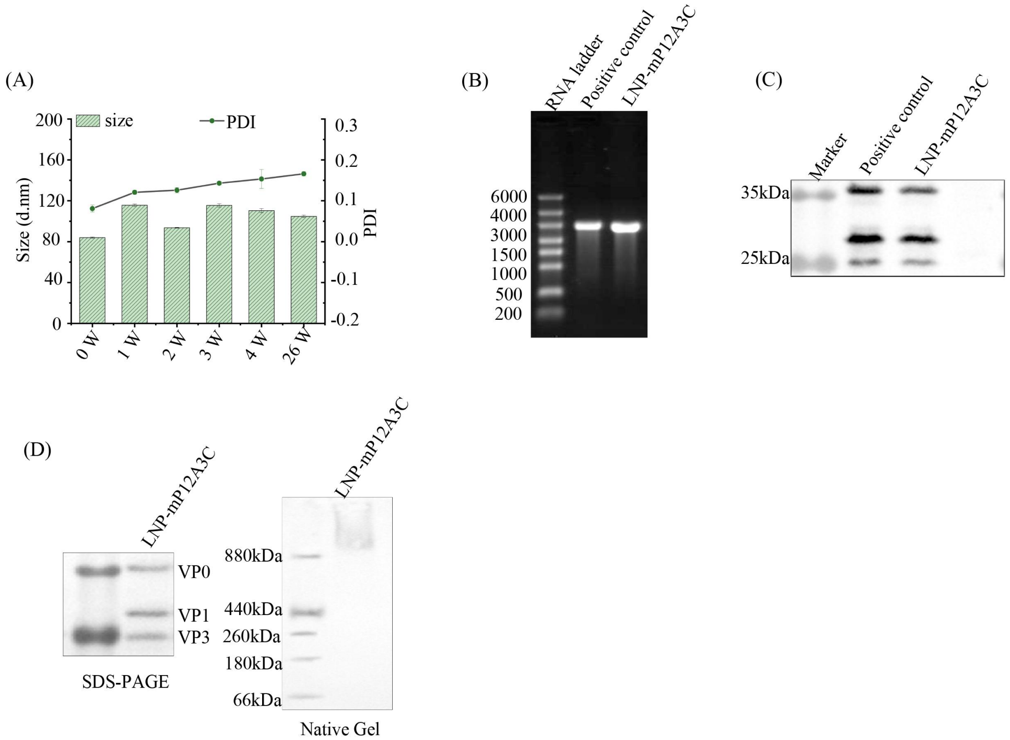


Fig. S1 Characterization of the FMDV VLP mRNA Vaccine. (A) The changes in particle size and polydispersity index (PDI) of LNP-mP12A3C during storage at 4 °C were monitored over time; (B) Denaturing agarose gel electrophoresis of LNP-mP12A3C stored at 4 °C for up to 26 weeks; (C) Western blot analysis of LNP-mP12A3C stored at 4 °C for up to 26 weeks; (D): Denaturing and non-denaturing western blot analyses confirmed that transfection with LNP-mP12A3C successfully induced the expression of foot-and-mouth disease virus (FMDV)-specific structural proteins (VP0, VP1, and VP3), as well as their corresponding large molecular aggregates.

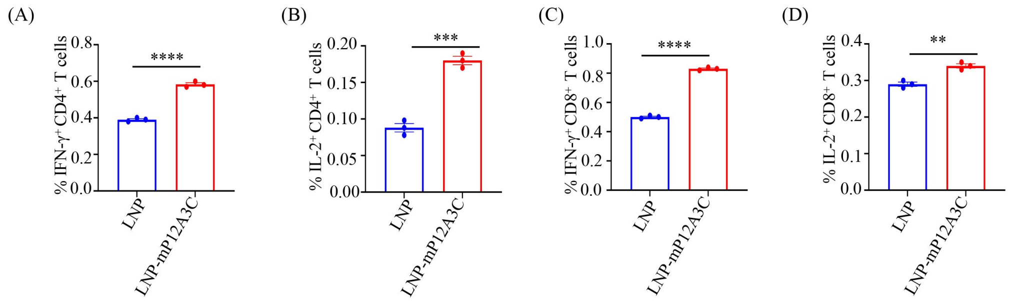


Fig. S2 Intracellular cytokine staining analysis of splenic lymphocytes from mice immunized with FMD VLP mRNA vaccine. (A-D) Flow cytometry analysis of IFN-γ-producing $CD4^+$ (A) and $CD8^+$ (C) T cells, and IL-2-producing $CD4^+$ (B) and $CD8^+$ (D) T cells.

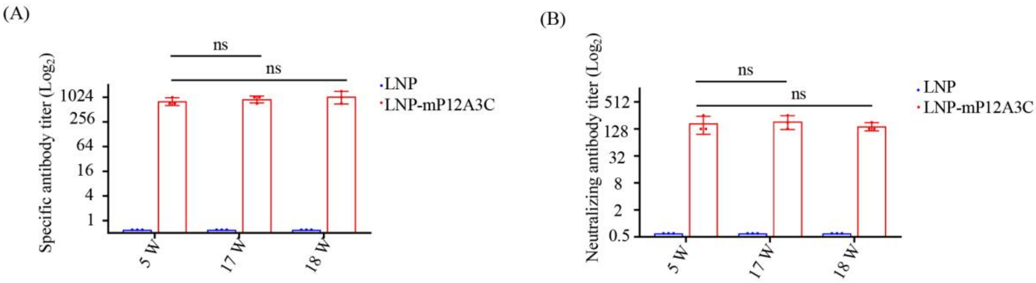


Fig. S3 The long-term antibody levels induced by FMDV VLP mRNA Vaccine. (A) Specific antibody; (B) Neutralizing antibody titers.

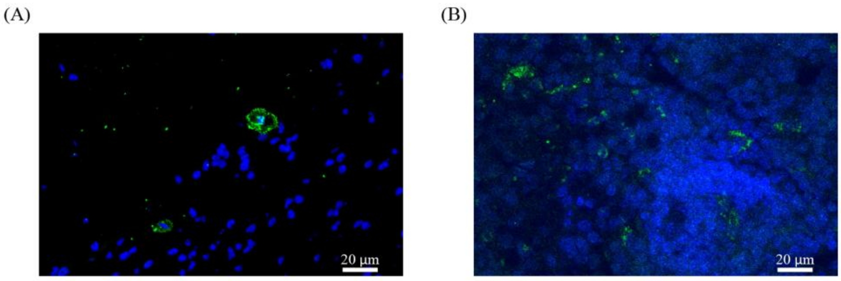
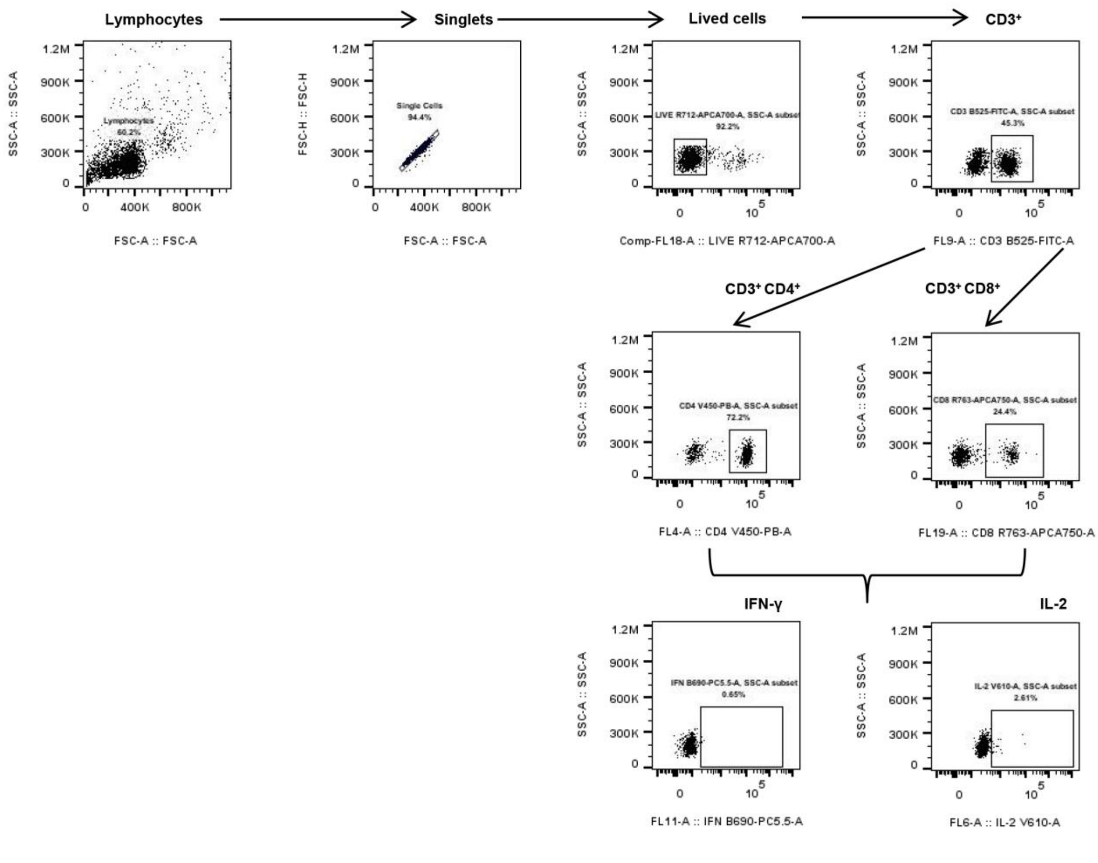
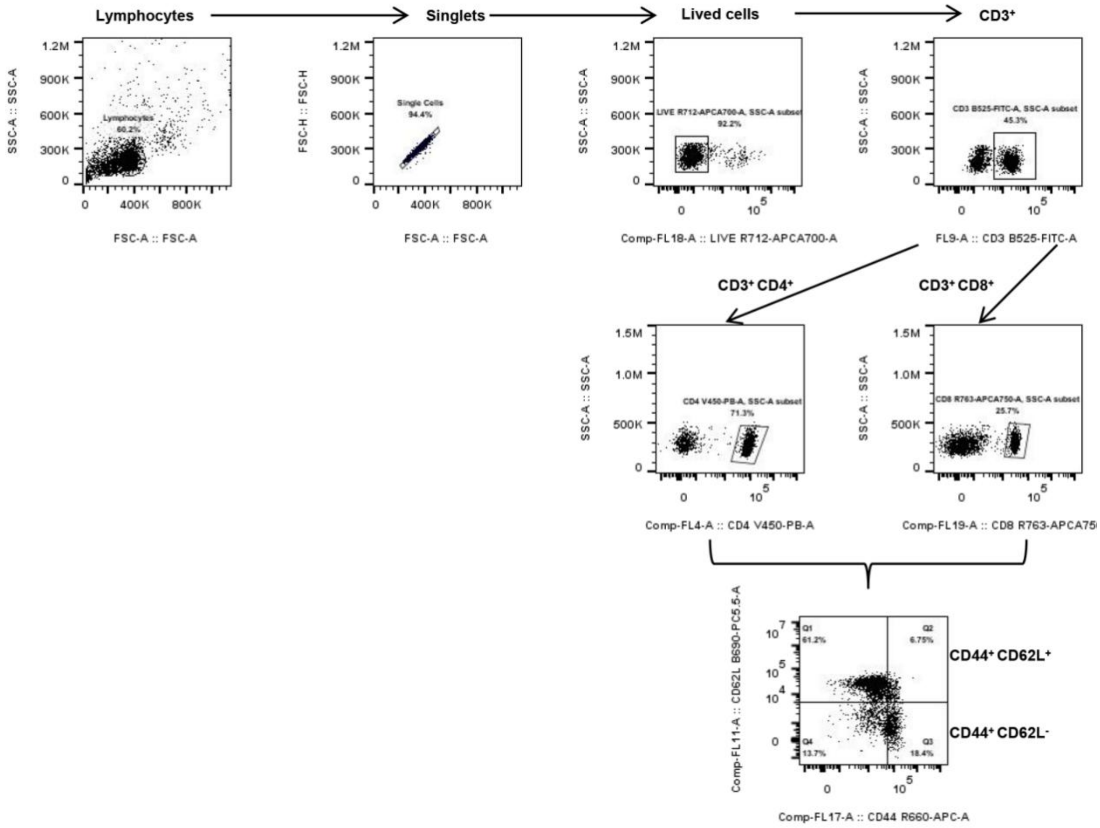


Fig. S4 Immunofluorescence analysis of muscle tissue at the injection site and inguinal lymph node tissue in mice following immunization with FMD VLP mRNA vaccine. (A) Muscle tissue; (B) Inguinal lymph node tissue.

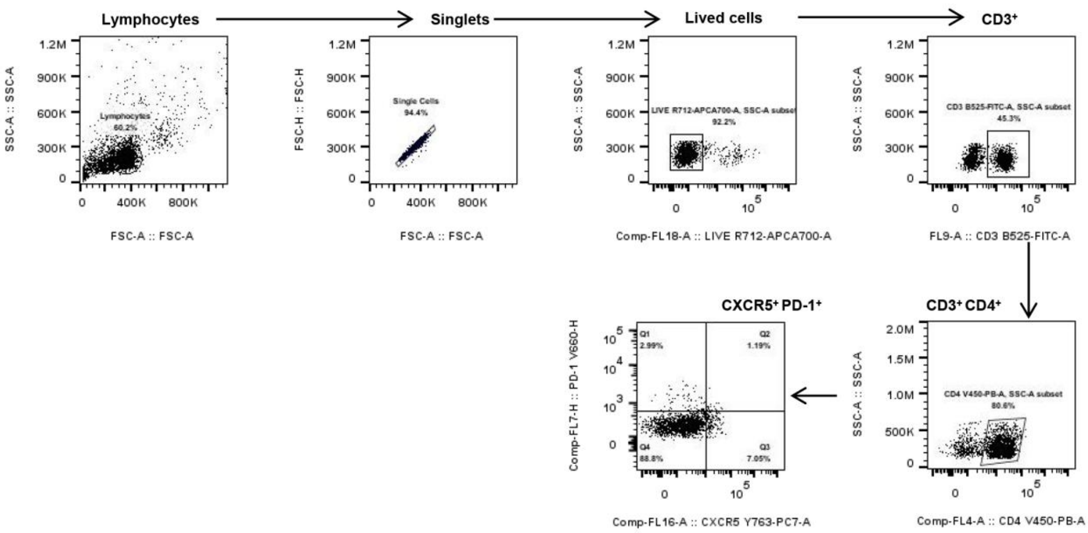
（A）



（B）


Lymphocytes
Singlets
Lived cells
CD3⁺
SSC-A :: SSC-A
FSC-H :: FSC-H
Lymphocytes
60.2%
Single Cells
94.4%
LIVE R712-APCA700-A, SSC-A subset
92.2%
CD3 B525-FITC-A, SSC-A subset
45.3%
FSC-A :: FSC-A
Comp-FL18-A :: LIVE R712-APCA700-A
FL9-A :: CD3 B525-FITC-A
CD3⁺ CD4⁺
CD3⁺ CD8⁺
CD4 V450-PB-A, SSC-A subset
71.3%
CD8 R763-APCA750-A, SSC-A subset
25.7%
Comp-FL4-A :: CD4 V450-PB-A
Comp-FL19-A :: CD8 R763-APCA75
Q1
61.2%
Q2
6.75%
Q3
18.4%
Q4
13.7%
CD44⁺ CD62L⁺
CD44⁺ CD62L⁻
Comp-FL11-A :: CD62L B690-PC5.5-A
Comp-FL17-A :: CD44 R660-APC-A

（C）


Lymphocytes
Singlets
Lived cells
CD3⁺
SSC-A :: SSC-A
FSC-H :: FSC-H
Lymphocytes
60.2%
Single Cells
94.4%
LIVE R712-APCA700-A, SSC-A subset
92.2%
CD3 B525-FITC-A, SSC-A subset
45.3%
FSC-A :: FSC-A
Comp-FL18-A :: LIVE R712-APCA700-A
FL9-A :: CD3 B525-FITC-A
CXCR5⁺ PD-1⁺
CD3⁺ CD4⁺
Q1
2.99%
Q2
1.19%
Q3
7.05%
Q4
88.8%
Comp-FL7-H :: PD-1 V660-H
Comp-FL16-A :: CXCR5 Y763-PC7-A
CD4 V450-PB-A, SSC-A subset
80.6%
Comp-FL4-A :: CD4 V450-PB-A

（D）

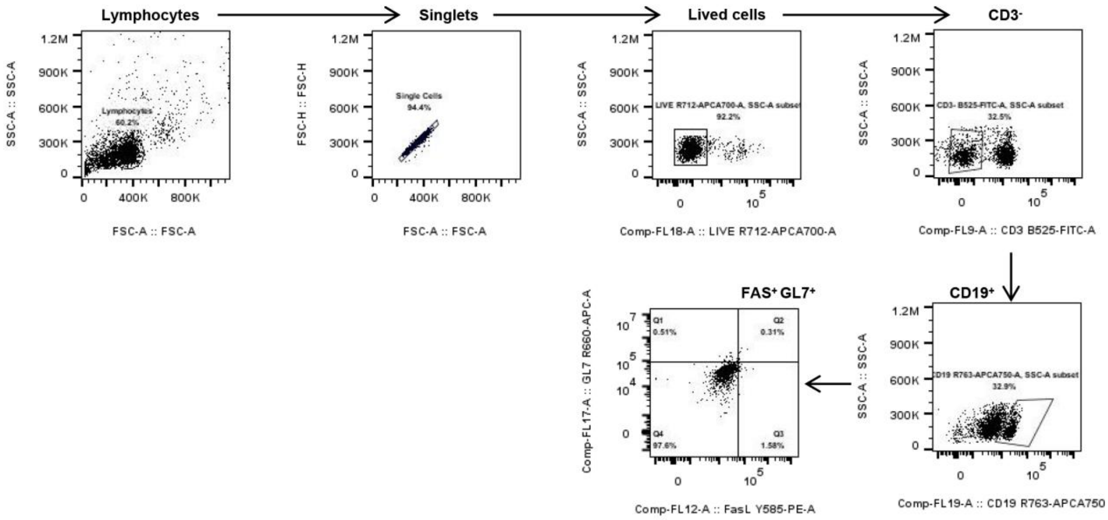


Fig. S5 Flow cytometry gating strategy. (A) Gating strategy for intracellular cytokine staining; (B) Gating strategy for TEM and TCM; (C) Gating strategy for Tfh; (D)Gating strategy for GCB.

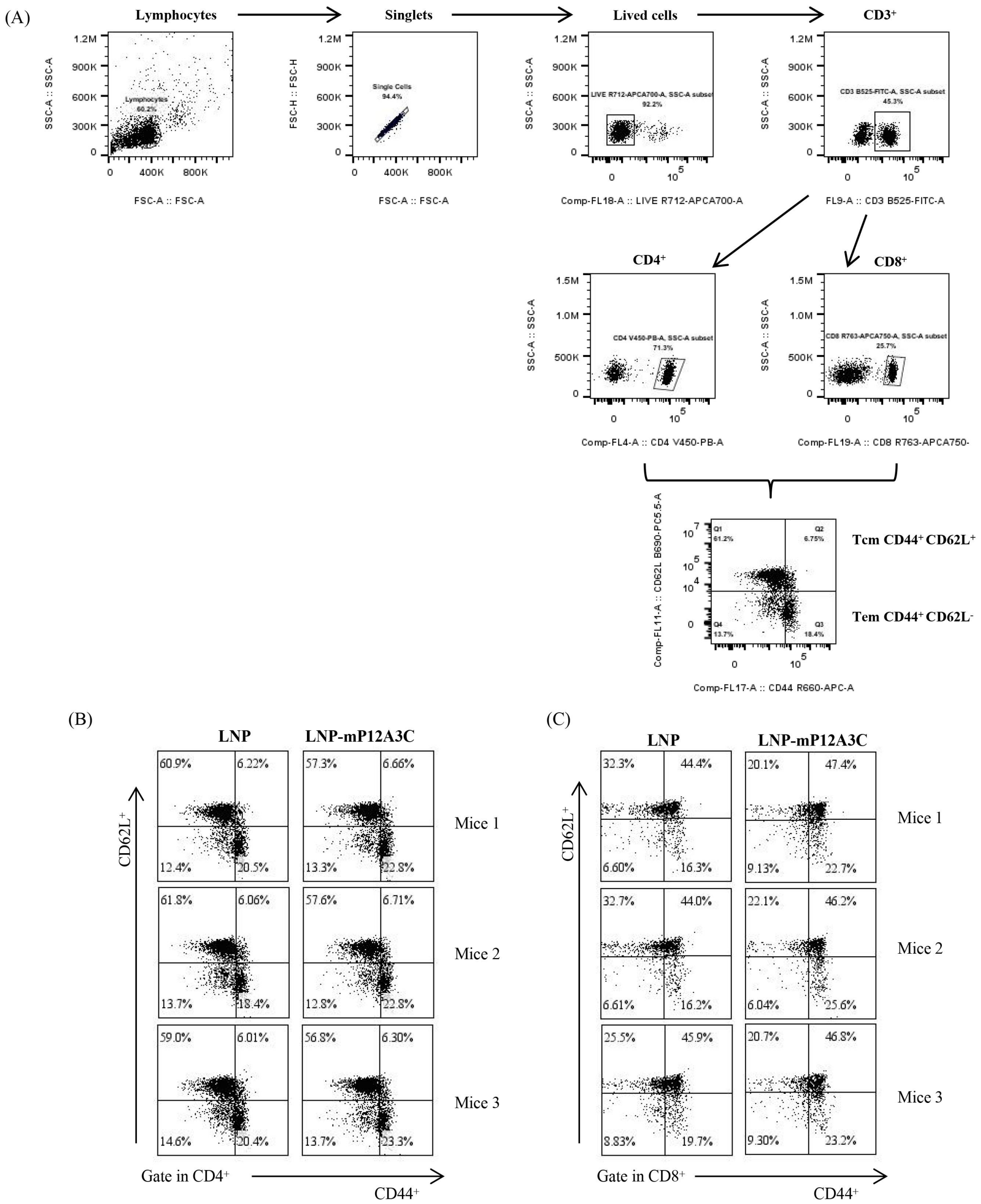


Fig. S6 Gating strategy for flow-cytometric analysis of memory T-cell subsets and raw dot plots from individual mice in all experimental groups.

(A) Sequential gating strategy used to identify central memory T (Tcm, CD44$^+$CD62L$^+$) and effector memory T (Tem, CD44$^+$CD62L$^-$) cell subsets; (B) Raw dot plots of CD44 versus CD62L within gated CD4$^+$ T cells from individual mice in the LNP and LNP-mP12A3C groups; (C) Raw dot plots of CD44 versus CD62L within gated CD8$^+$ T cells from individual mice in the LNP and LNP-mP12A3C groups. The percentages shown in the plots represent the frequencies of Tcm or Tem cells in each individual mouse and correspond directly to the animal-level data used for quantification in Figure 3.

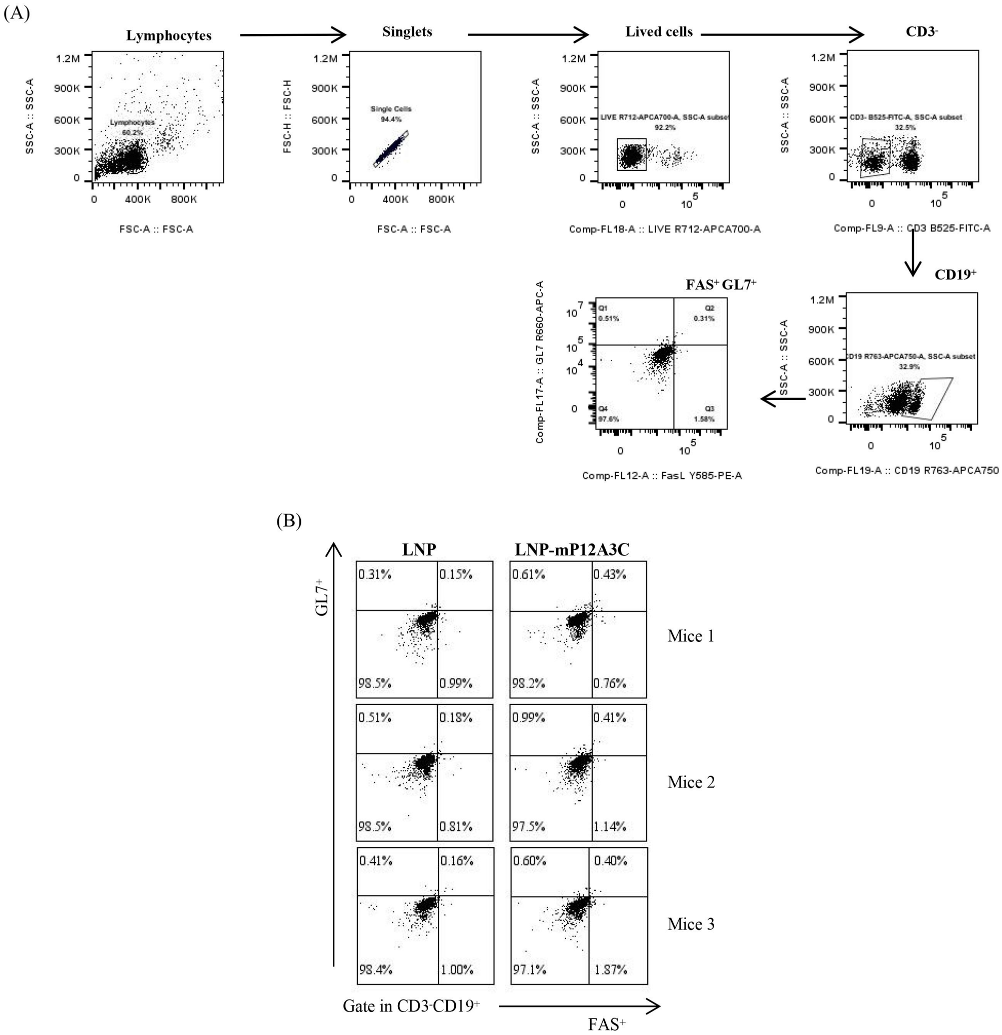


Fig. S7 Gating strategy for flow-cytometric analysis of germinal center B (GC B) cells and raw dot plots from individual mice in all experimental groups.

(A) Sequential gating strategy used to identify GC B cells (FAS$^+$GL7$^+$) within gated CD19$^+$ B cells; (B) Raw dot plots of FAS versus GL7 within gated CD3$^-$CD19$^+$ cells from individual mice in the LNP and LNP-mP12A3C groups. The percentages shown in the plots represent the frequencies of GC B cells in each individual mouse and correspond directly to the animal-level data used for quantification in Figure 3.

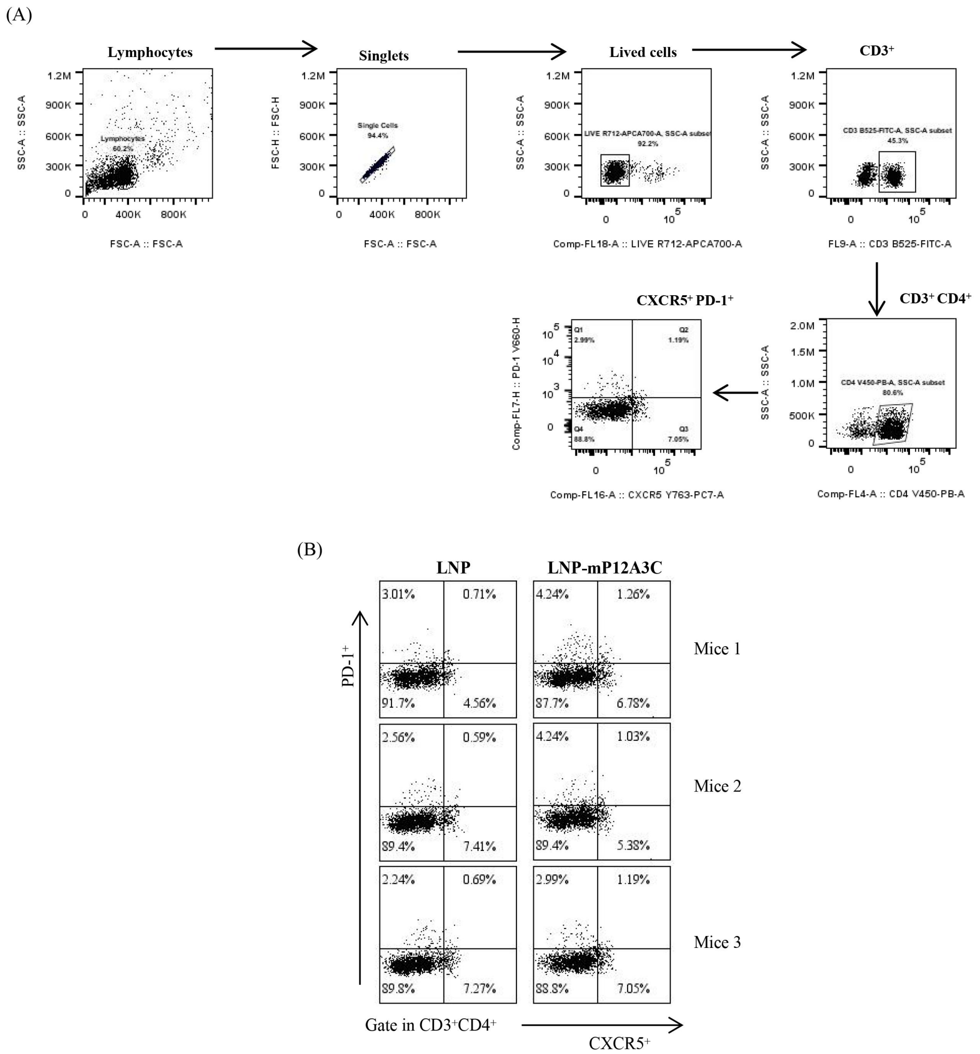


Fig. S8 Gating strategy for flow-cytometric analysis of T follicular helper (Tfh) cells and raw dot plots from individual mice in all experimental groups.

(A) Sequential gating strategy used to identify Tfh cells ($CXCR5^{+}PD\text{-}1^{+}$) within gated $CD3^{+}CD4^{+}$ T cells; (B) Raw dot plots of CXCR5 versus PD-1 within gated $CD3^{+}CD4^{+}$ cells from individual mice in the LNP and LNP-mP12A3C groups. The percentages shown in the plots represent the frequencies of Tfh cells in each individual mouse and correspond directly to the animal-level data used for quantification in Figure 3.

The mRNA sequence of P12A3C(L127P) consists of the following elements: 5′UTR–P12A3C(L127P)–3′UTR–poly(A) tail.

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CCTGGGTACCAAATTGATCGAGCAGGAGACTGACAAAGACACACTCATCTTGGATCTAGTG
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