Supporting information for

IL-7 promotes mRNA vaccine-induced long-term immunity

Lingli Wang[1,2,3,4,5], Jiawu Wan[1,2,3,4,5], Wenna He[1,2,3,4], Zongmei Wang[1,2,3,4], Qiong Wu[1,2,3,4], Ming Zhou[1,2,3,4], Zhen F. Fu[1,2,3,4], Ling Zhao[1,2,3,4,6,*]

[1]National Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China

[2]Hubei Hongshan Laboratory, Wuhan 430070, China

[3]Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan 430070, China

[4]Key Laboratory of Preventive Veterinary Medicine of Hubei Province, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China

[5]These authors contributed equally

[6]Lead contact

*Correspondence: Ling Zhao, zling604@outlook.com, lingzhao@mail.hzau.edu.cn

This file includes: Fig. S1 to S7

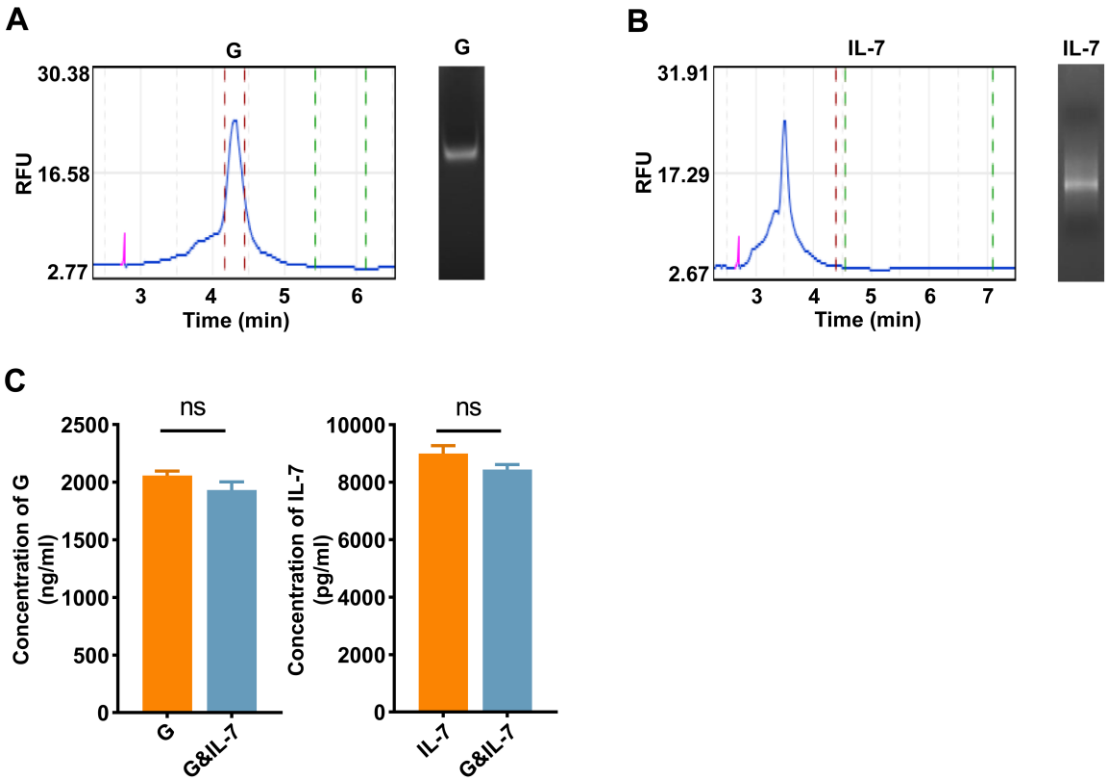

Figure S1. The quality and expression of the in vitro-transcribed mRNA. (A) The capillary electropherograms and agarose gel electropherograms of G mRNA. (B) The capillary electropherograms and agarose gel electropherograms of IL-7 mRNA. (C) HEK-293T cells were transfected with LNP-encapsulated G mRNA (1 μg), IL-7 mRNA (1 μg) or G&IL-7 mRNA (1 μg G mRNA + 1 μg IL-7 mRNA). After 24 hours, ELISA was performed to measure the expression levels of G and IL-7 in the cell lysates (n = 3).

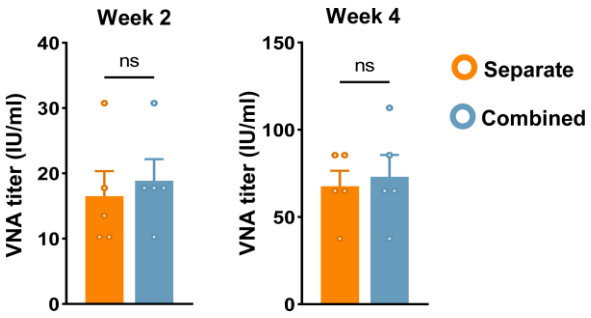

Figure S2. Different encapsulation strategies of IL-7 mRNA as an adjuvant. Mice were immunized using two different mRNA encapsulation methods: G mRNA and IL-7 mRNA were either co-encapsulated in the same lipid nanoparticle (LNP) for injection or separately encapsulated in individual LNPs and then mixed prior to injection. Virus-neutralizing antibody (VNA) titers were assessed using fluorescent antibody virus neutralization (FAVN) assays (n = 5).

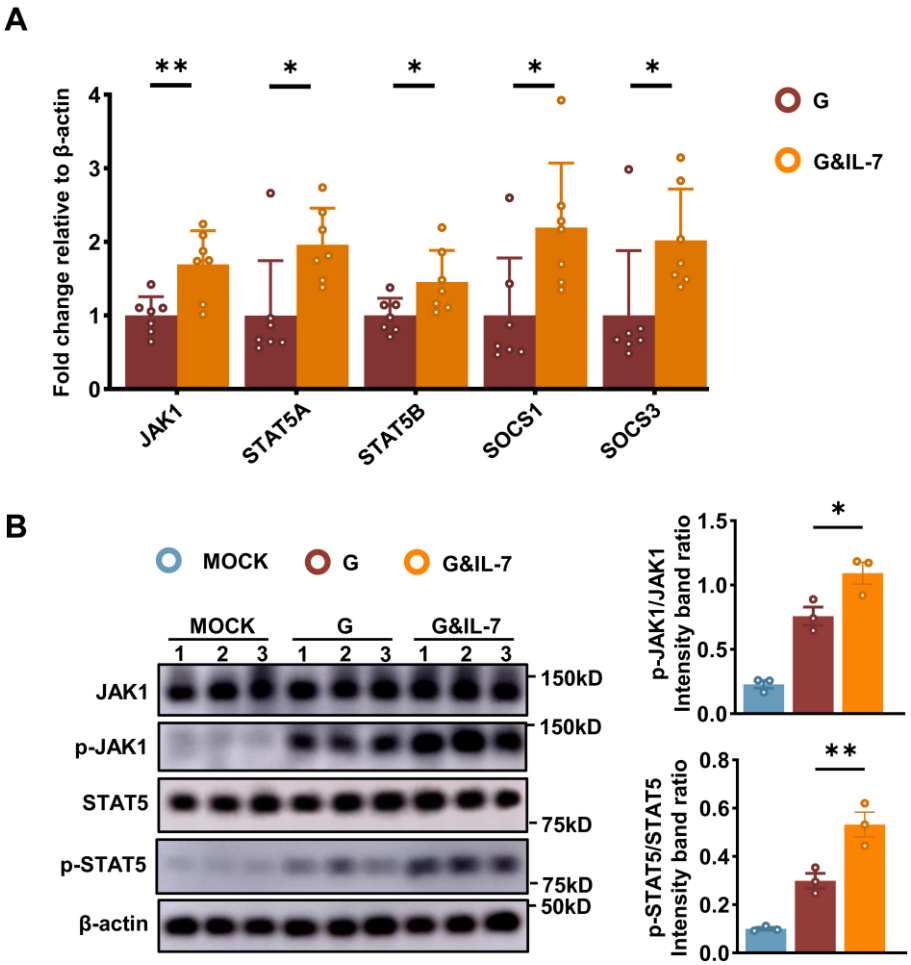

Figure S3. Expression levels of key genes and proteins in the JAK-STAT signaling pathway in mRNA vaccine-immunized and control mice. (A) The mRNA expression levels of JAK1, STAT5A, STAT5B, SOCS1, and SOCS3 in the inguinal lymph nodes (LNs) of mice (n = 7) inoculated with G mRNA and G&IL-7 mRNA. (B) The LNs from mice (n = 3) inoculated with G mRNA, G&IL-7 mRNA, and PBS were collected and homogenized. Western blotting was performed to assess the expression levels of JAK1, phosphorylated JAK1 (p-JAK1), STAT5, and phosphorylated STAT5 (p-STAT5).

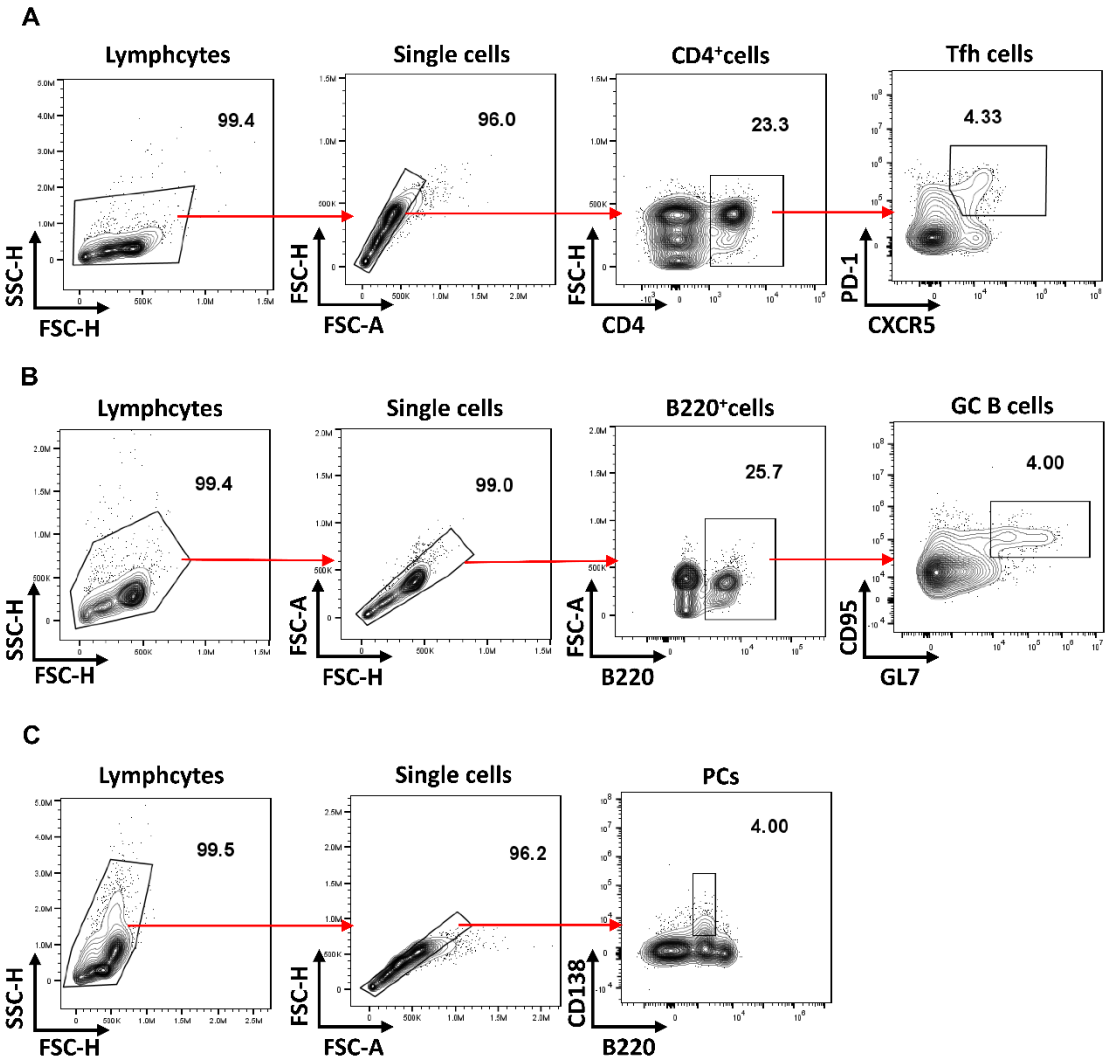

Figure S4. Flow cytometry gating strategy for the detection of Tfh cells, GC B cells and PCs. (A) Gating strategy to identify Tfh cells ($CD4^+CXCR5^+PD1^+$). (B) Gating strategy to identify GC B cells ($B220^+GL7^+CD95^+$). (C) Gating strategy to identify PCs ($B220^{low}CD138^+$).

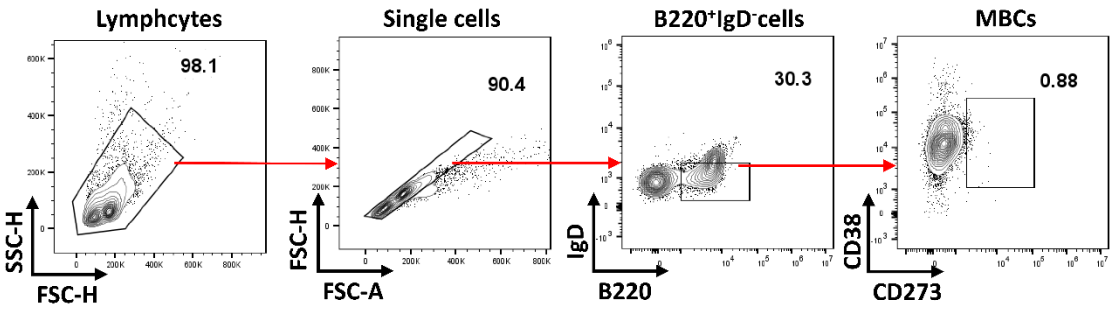

Figure S5. Flow cytometry gating strategy for the detection of MBCs. Gating strategy to identify MBCs ($B220^{+}IgD^{-}CD273^{+}CD38^{+}$).

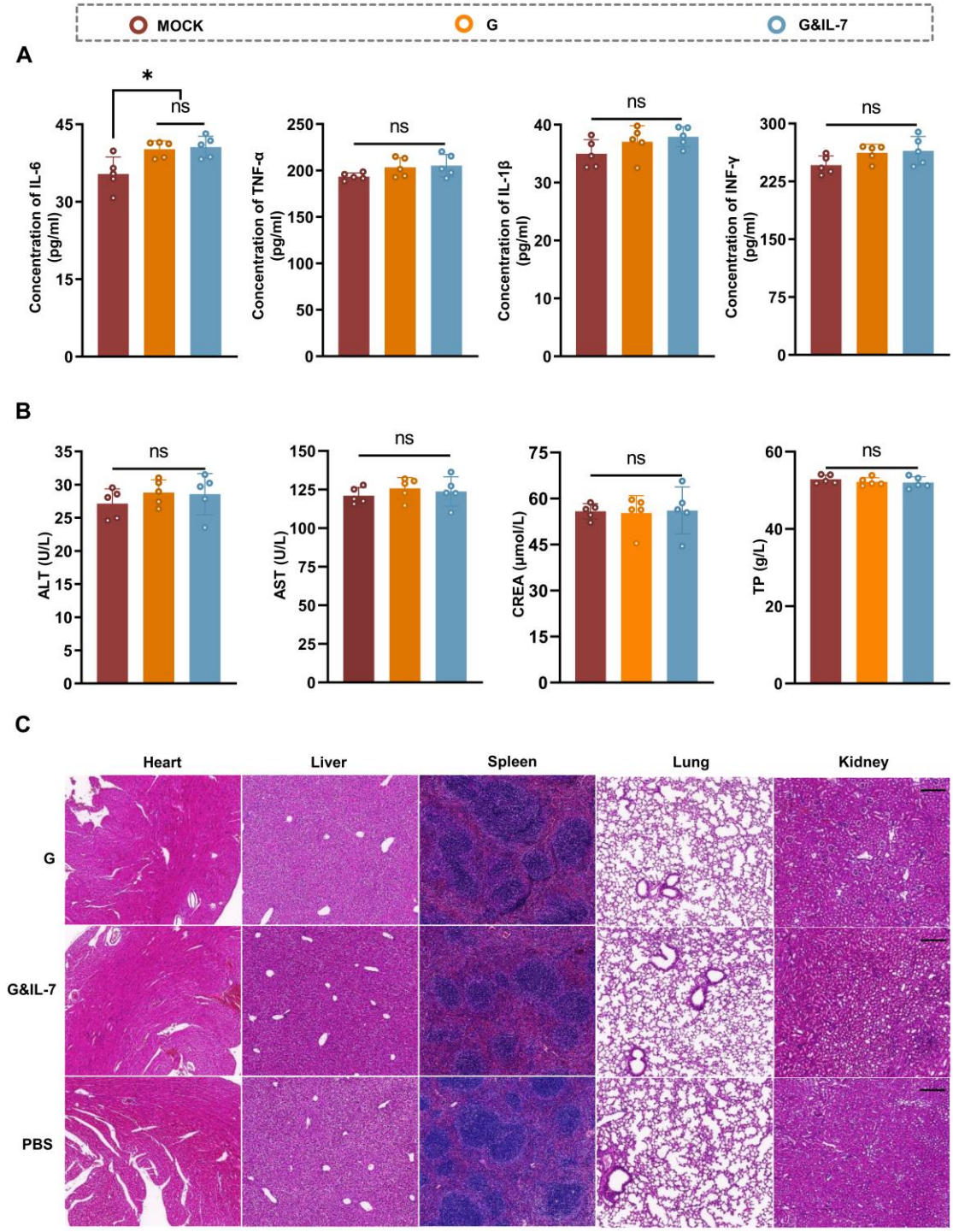

Figure S6. Safety evaluation of G&IL-7 mRNA. Vaccination of C57BL/6 mice via intramuscular injection with G mRNA, G&IL-7 mRNA or PBS, respectively. (A) Cytokine levels in the serum samples from mice were detected (n = 5). (B) The biochemical indicators of mice were analyzed using an automated hematological biochemical analyzer (n = 5). (C) Hematoxylin and eosin (H&E) staining was conducted on the major organs of mice on day 2. Scale bars represent 200 μm.

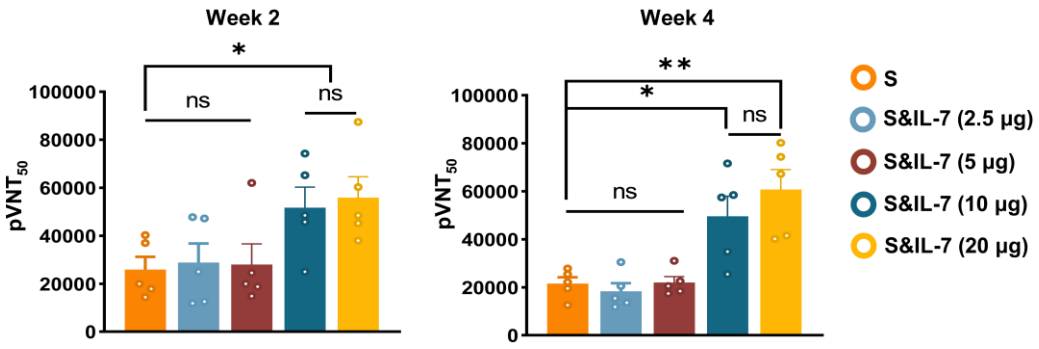

Figure S7. IL-7 mRNA concentration screening as an adjuvant for SARS-CoV-2 mRNA vaccine. Mice were immunized with S mRNA encapsulated alongside varying concentrations of IL-7 mRNA. Neutralizing antibodies against the original Wuhan strain were assessed using a pseudovirus neutralization assay (n = 5).