Supporting Information

An Optimized Intranasal LNP Formulation for Potent Immune Responses of mRNA-Based RSV Vaccines

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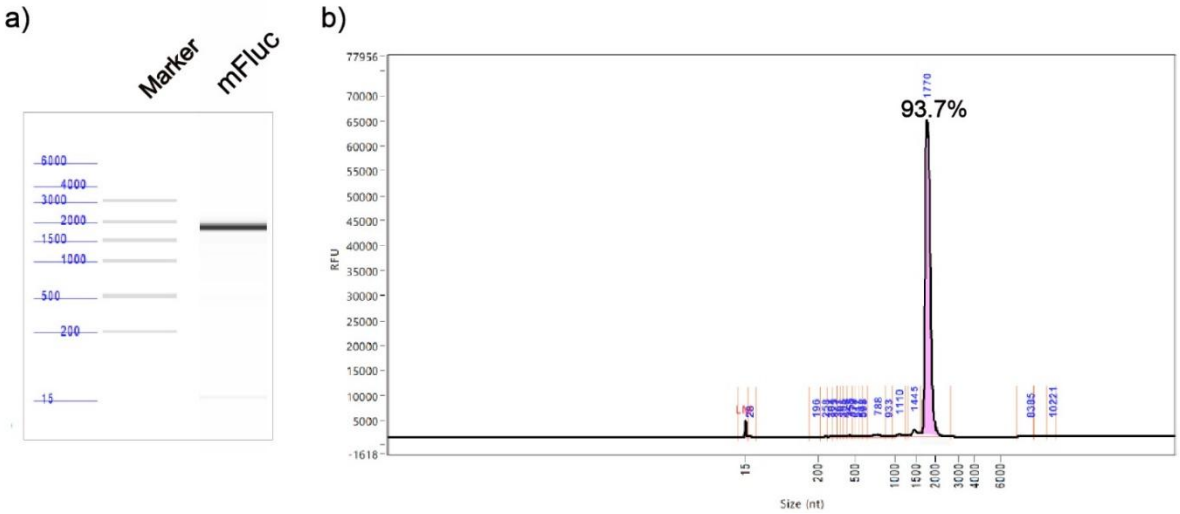

Figure S1. a) Microfluidic capillary electrophoresis analysis of mRNA encoding luciferase (mFluc) obtained by IVT. b) Purity assessment depending on the band intensity of the target molecule was calculated by software analysis.

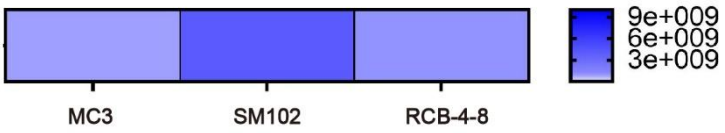

Figure S2. The total luminescence values for both the nasal and pulmonary regions of each mouse were calculated (n = 5).

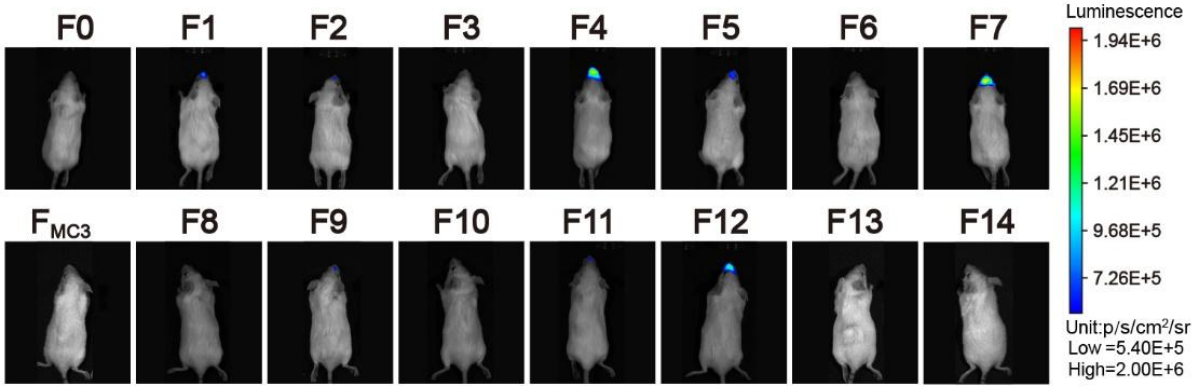

Figure S3. Bioluminescence imaging was conducted 24 h after the intranasal administration of mRNA-loaded LNPs to mice.

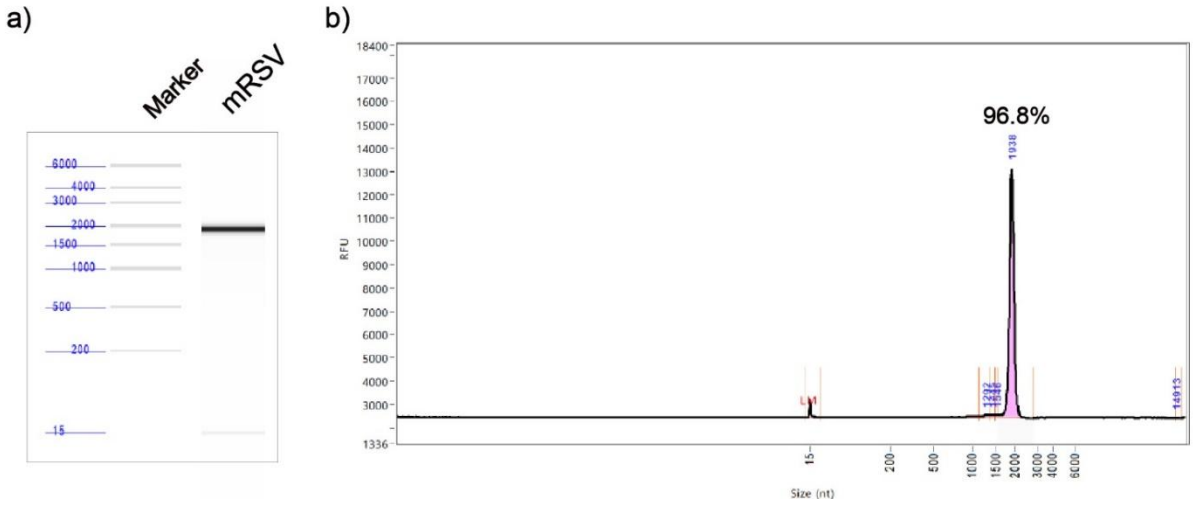

Figure S4. a) Microfluidic capillary electrophoresis analysis of mRNA encoding RSV antigenic proteins (mRSV) obtained by IVT. b) Purity assessment depending on the band intensity of the target molecule was calculated by software analysis.

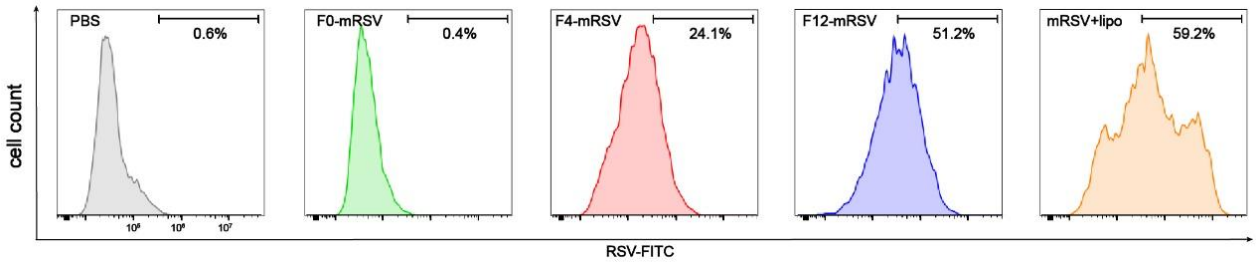

Figure S5. Expression efficiency of RSV proteins in A549 cells was assessed by flow cytometry using F488-FITC fluorescence. Cells were transfected with 2 μg/mL of RSV mRNA.

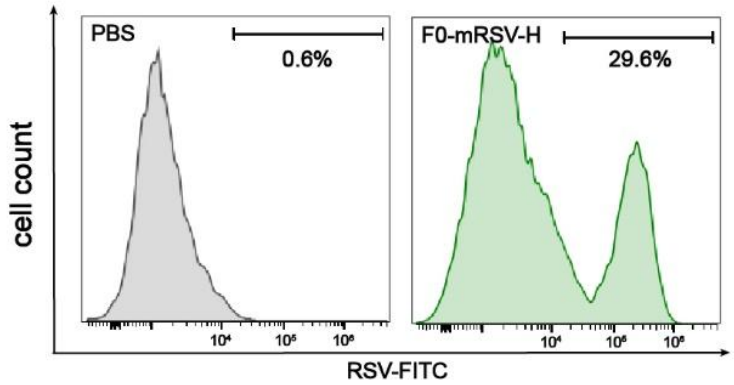

Figure S6. Expression efficiency of RSV proteins in A549 cells was assessed by flow cytometry using F488-FITC fluorescence. Cells were transfected with 4 μg/mL of RSV mRNA.

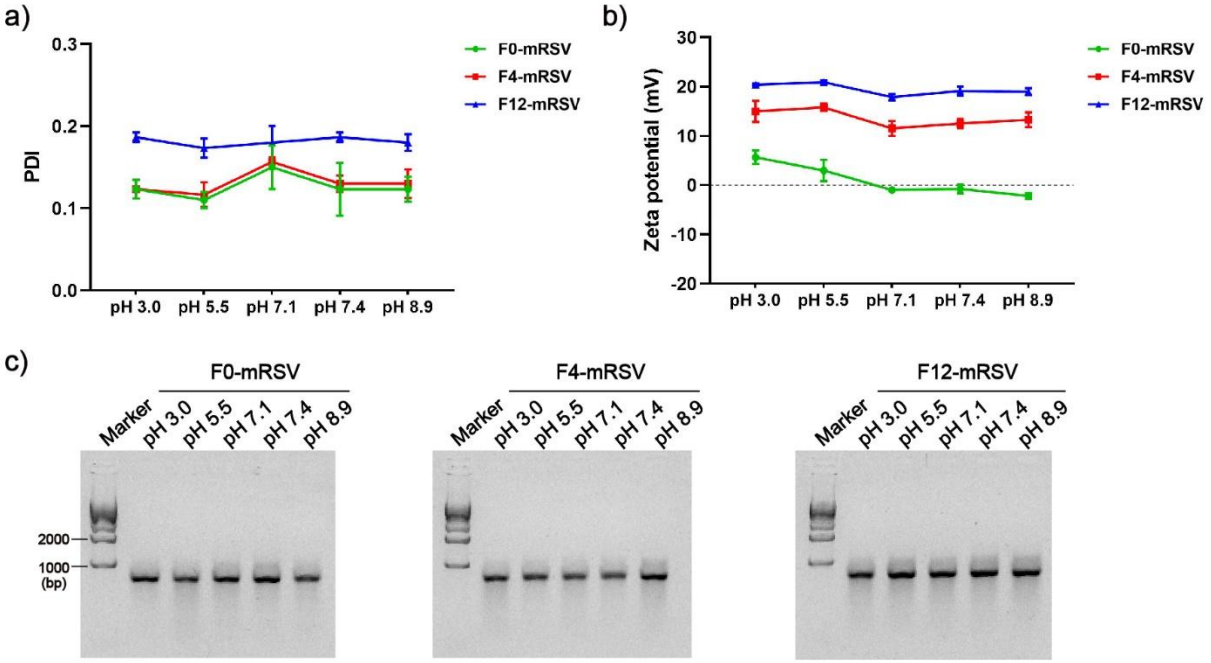

Figure S7. Stability of F0-mRSV, F4-mRSV, and F12-mRSV vaccines under simulated acidic and alkaline conditions, reflecting normal and disease states. After mixing with PBS buffers at pH levels of 3.0, 5.5, 7.1, 7.4, and 8.9, a) the polydispersity index (PDI) and b) zeta potential of the mRNA-loaded LNPs were measured. c) mRNA integrity assessment by 1% agarose gel electrophoresis.

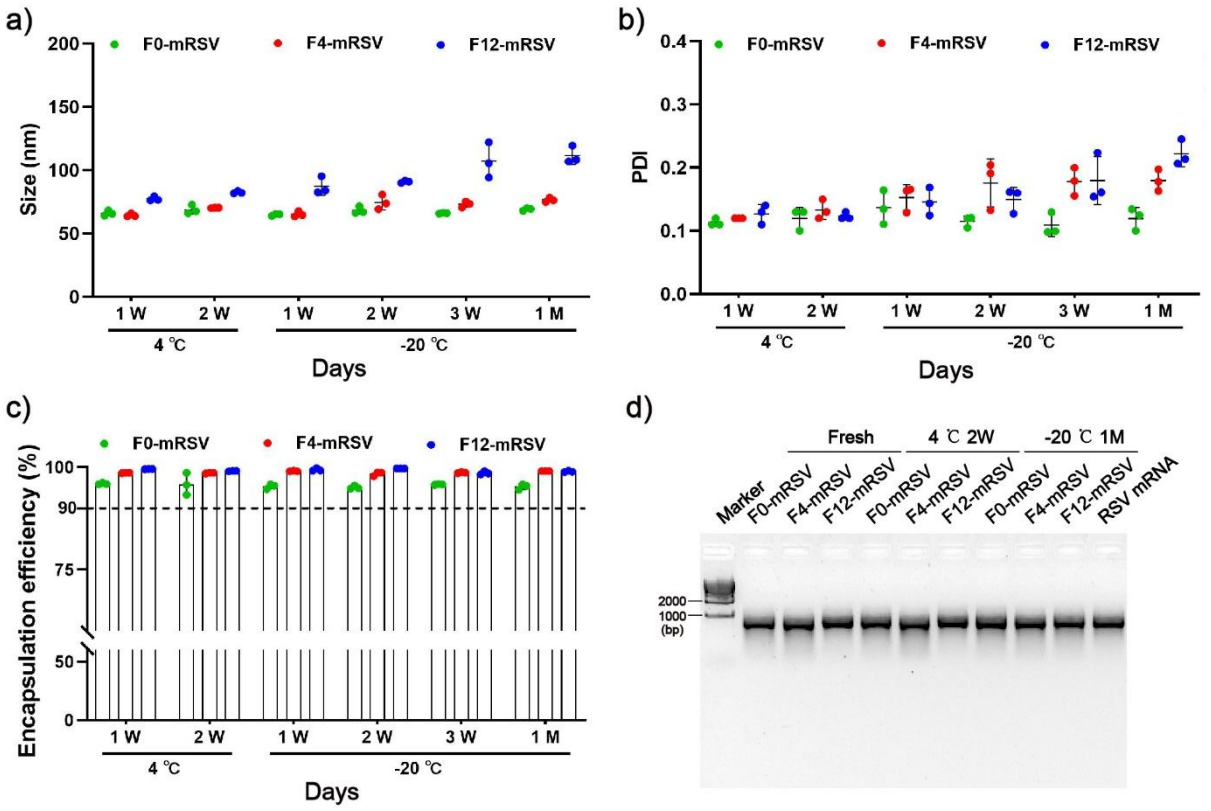

Figure S8. Storage stability of the F0-mRSV, F4-mRSV, and F12-mRSV vaccines at 4°C and –20°C. a) The particle size, b) PDI, c) encapsulation efficiency, and d) mRNA integrity were analyzed at different time points (W represents weeks, and M represents months). Compared with the same fresh formulation on day 0, there were no statistically significant differences at the detection time points.

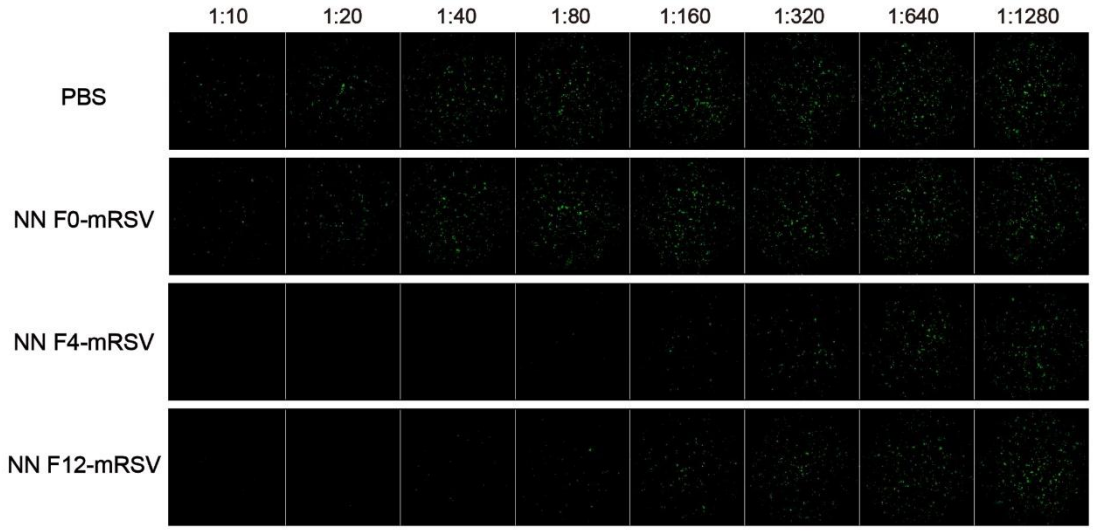

Figure S9. Representative fluorescence microscopy images of serum neutralizing antibody titers with FITC-labeled RSV-specific antibody. Serum samples were serially diluted from 1:10 to 1:1280 in 96-well plates.

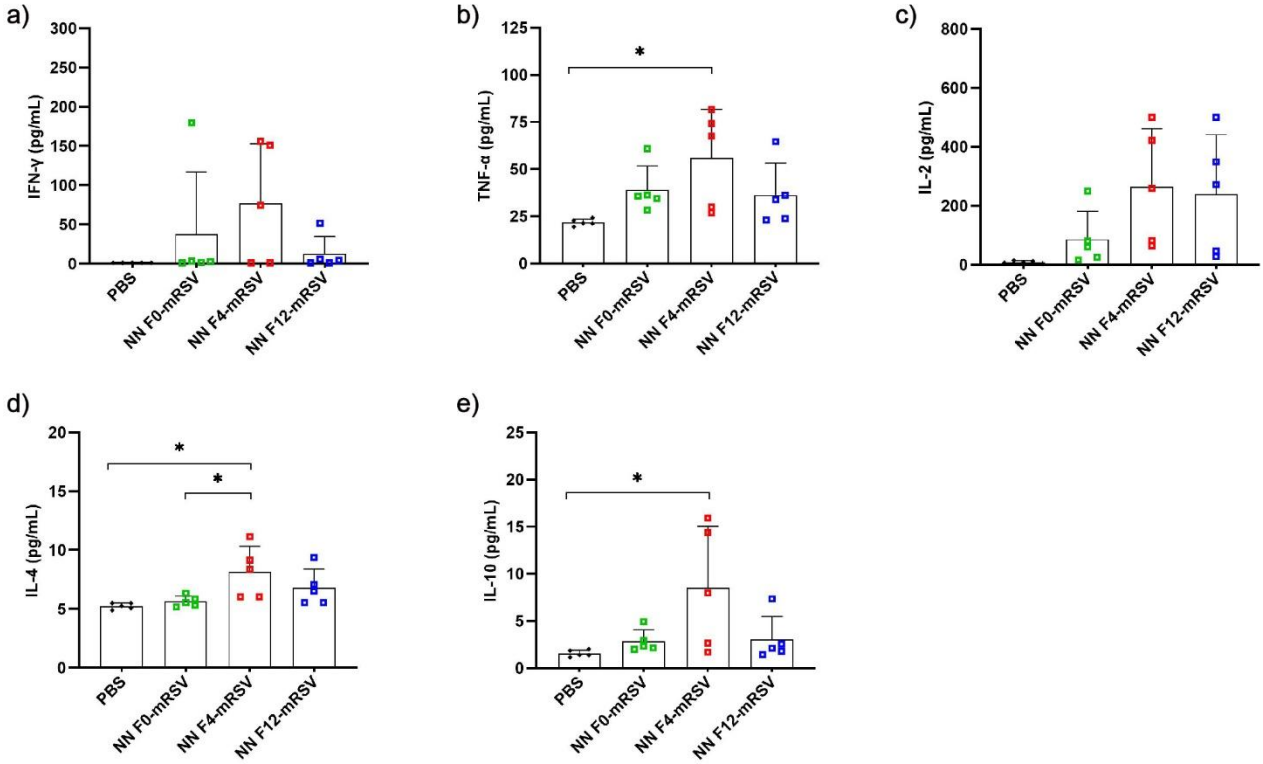

Figure S10. Cellular immune responses in the spleen of mice of mice following intranasal administration of RSV mRNA vaccines of F0-mRSV, F4-mRSV, and F12-mRSV. Cytokines from isolated spleen lymphocytes were detected using ELISA, including a and b) INF-γ and TNF-α, as markers of the Th1 response, c) IL-2, which promotes T cell proliferation and differentiation, and d and e) IL-4 and IL-10, as markers of the Th2 response; n = 5. *$P < 0.05$. **$P < 0.01$.

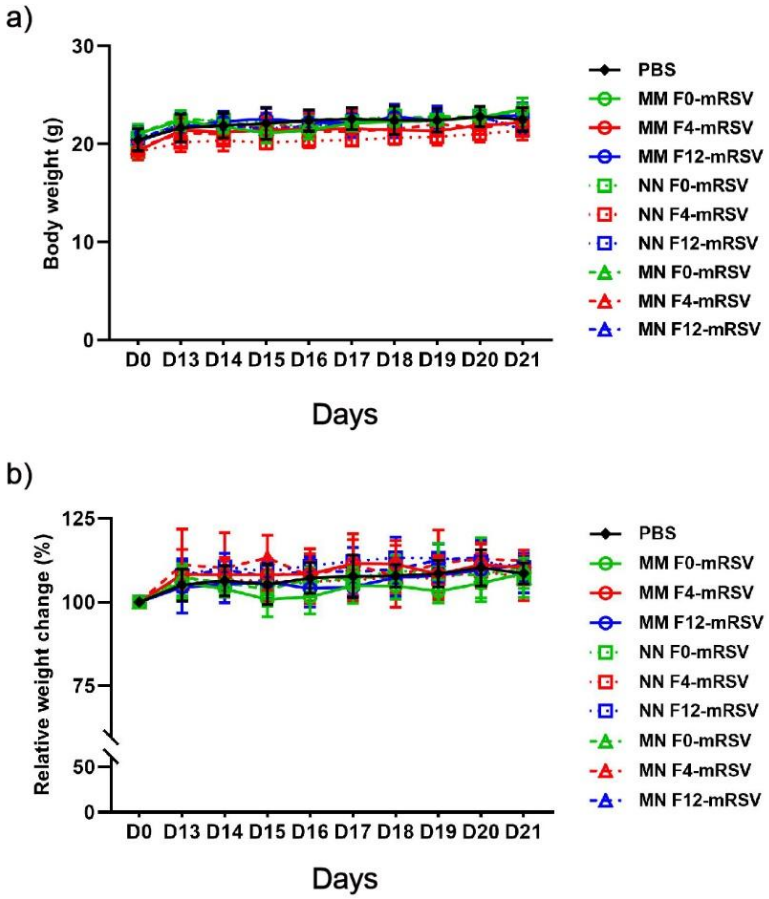

Figure S11. a) Body weight of the mice after administration of the second dose on day 14 (D14). b) Relative weight change compared with day 0 before drug administration.

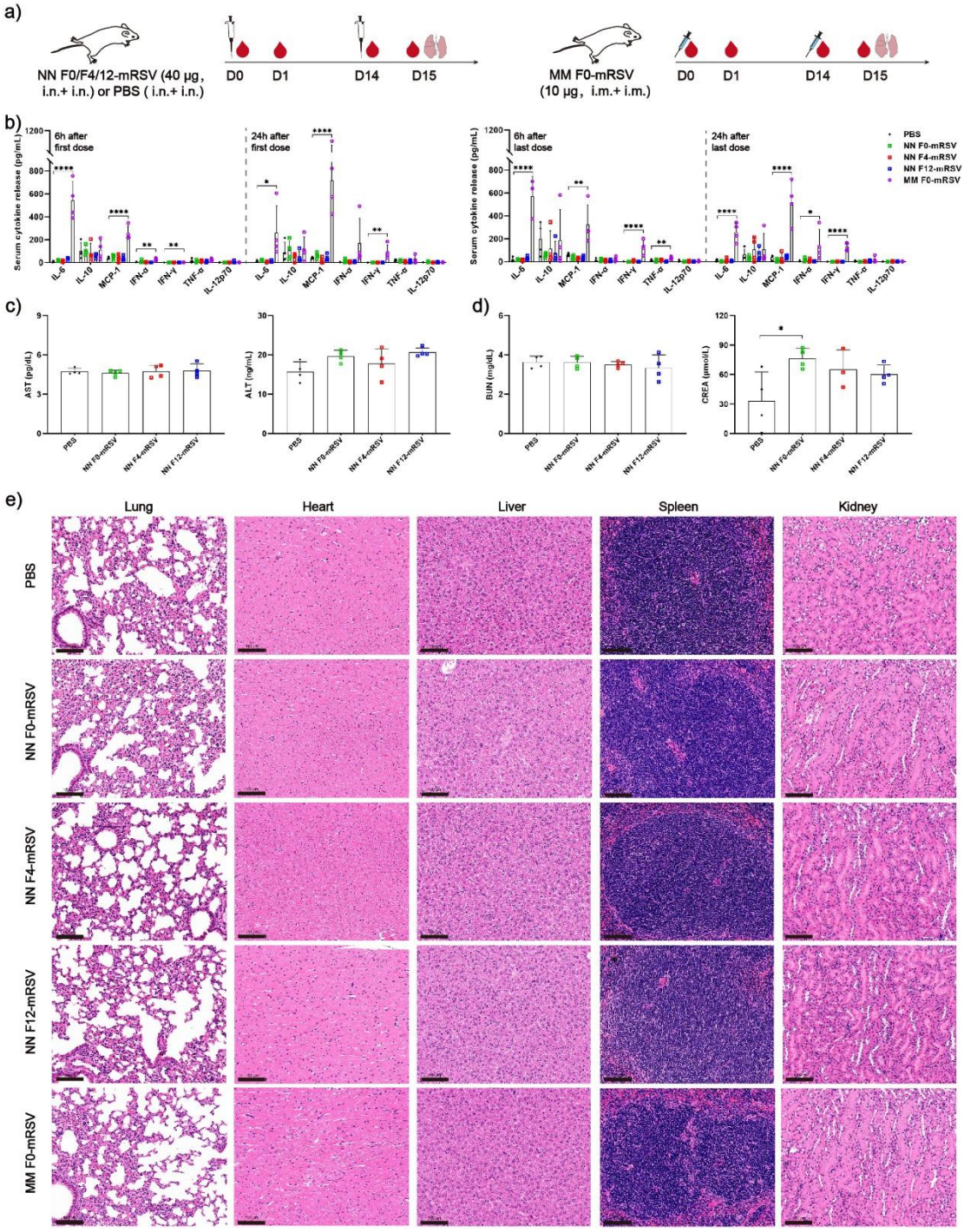

Figure S12. Systemic toxicity and biosafety evaluation of intranasal RSV mRNA vaccines a) Schematic representation of the immunization schedule for mice spanning days 0 to 14. Mice were immunized with three RSV mRNA vaccines via intranasal administration (NN F0, F4, F12-mRSV) or PBS (control group), or intramuscular injection (MM F0-mRSV). b) Serum cytokine levels measured at 6 h and 24 h post-administration (n = 3~4). Serum analysis of c) hepatic function and d) renal function markers at 24 h of the last administration (n = 3~4). e) Representative H&E-stained

tissue sections of lung, heart, liver, spleen, and kidney at 24 h of the last administration (Scale bar, 100 μm). *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$.