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Supplemental information

Self-amplifying mRNA SARS-CoV-2 vaccines raise cross-reactive immune response to variants and prevent infection in animal models

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SUPPLEMENTAL DATA

Table S1: Biophysical characterization of LNP attributes for sa-mRNA vaccines

	Sa-mRNA concentration (ug/mL)	Encapsulation efficiency (%)	Particle size (nM)	PDI	Zeta potential (mV)
sa-mRNA S	44.6	98.7	96	0.191	31.5
sa-mRNA S-N	53.9	97.7	98	0.103	32.7

Figure S1. Anti-N antibody responses raised by sa-mRNA S and sa-mRNA S-N vaccines in BALB/C and C57BL/*6J* mice. BALB/c (A) and C57BL/6J (B) mice (n = 10 each) were immunized twice, 3 weeks apart, with 1.0 µg or 0.01 µg of sa-mRNA S (blue) or sa-mRNA S-N (red). Serum samples were collected at Day 43. Sera were tested by ELISA for anti-N specific IgG. Each dot represents an individual serum sample and the column represents the geometric mean for the group. The dotted line in each panel represents the lower limit of quantitation for the assay. The dotted line in each panel represents the lower limit of quantitation for the assay. Statistical analysis by two-way ANOVA with Tukey's multiple comparison test was performed using GraphPad Prism 9.1.0. **P*<0.05. ***P*<0.01. ****P*<0.001. *****P*<0.0001. ns, not significant (*P*≥0.05).

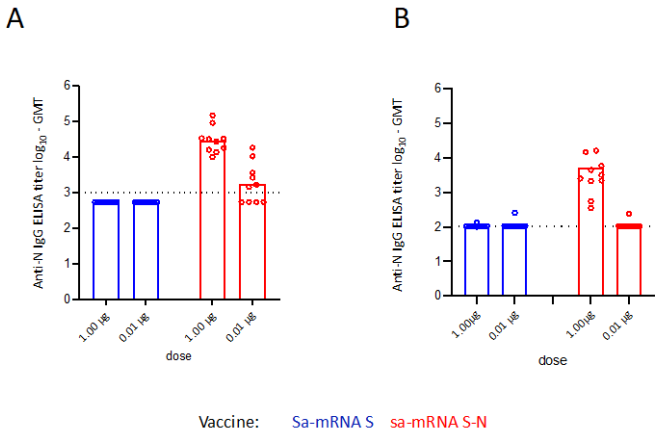

Figure S2. Anti-S IgG subclass antibody responses raised by sa-mRNA S and sa-mRNA S-N vaccines in BALB/C and C57BL/*6J* mice. Sera from BALB/C (A) and C57BL/6J (B) mice immunized with 1.0 µg of vaccines were tested by ELISA for S specific IgG1 and IgG2a subclasses. Each dot represents an individual serum sample, and the column represents the geometric mean for the group. The dotted line in each panel represents the lower limit of quantitation for the assay. (C) Correlation of MN titer with ELISA titers for S specific IgG1 and IgG2a subclasses.

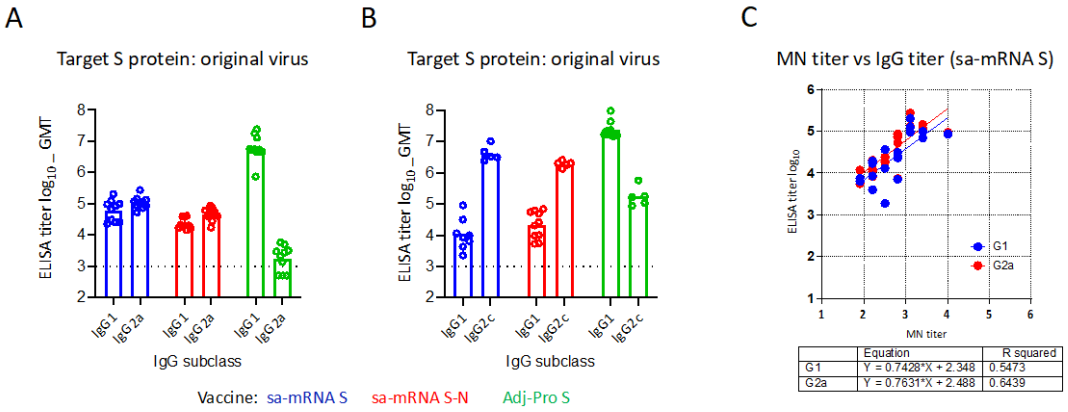

Figure S3. Antigen-specific CD4+ and CD8+ T cells raised by sa-mRNA S and sa-mRNA S-N vaccines or Adj-Pro S vaccines in C57BL/*6J* mice. C57BL/*6J* female mice (n = 10) were immunized twice, 3 weeks apart, with 1.0 µg or 0.01 µg of sa-mRNA S or sa-mRNA S-N vaccines or with 1.0 µg Adj-Pro S vaccine. Spleens were collected on day 43 and pooled (5 spleens/vaccine). Splenocytes were prepared, cultured in the presence or absence of antigen peptide mixtures, and analyzed by flow cytometry. The net (antigen-specific) percentage of cytokine-producing CD4+ and CD8+ T cells induced by each vaccine are shown for S1-specific CD4+ T cells (A), S1-specific CD8+ T cells (B), S2-specific CD4+ T cells (C), S2-specific CD8+ T cells (D), and N-specific CD4+ T cells (E). Contribution of various T helper subsets to the overall CD4 response was determined as follows: Th1, CD4+IFNγ+IL-5negIL-13neg; Th2, CD4+IFNγ−IL-5+/IL-13+; and Th0, CD4+IL-2+/TNFα+.

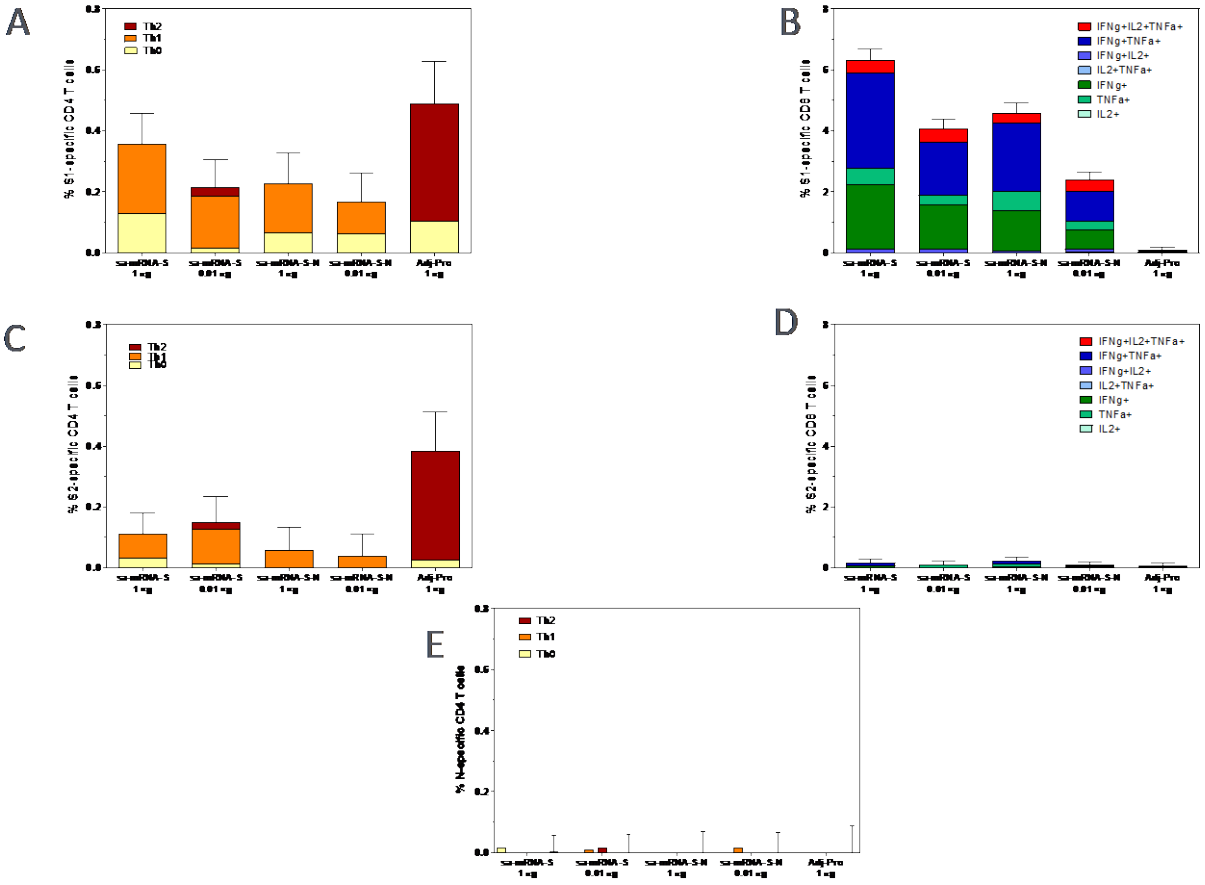

Figure S4. Body weight changes by challenge with SARS-CoV-2 virus for hamsters immunized with sa-mRNA S and sa-mRNA S-N or Adj-Pro S vaccine.
The body weight changes post the challenge with SARS-CoV-2 virus for hamsters immunized with sa-mRNA S (blue) and sa-mRNA S-N (red) vaccines or Adj-Pro S (green) vaccine.

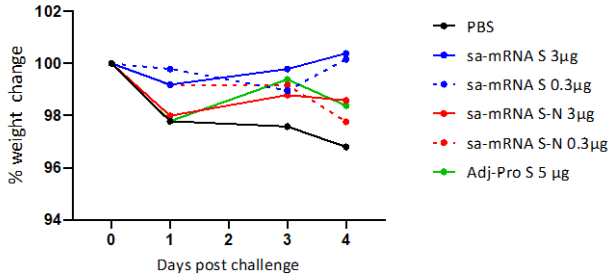

Figure S5. ACE2-binding inhibition raised by sa-mRNA S in BALB/c mice. Titers of antibodies inhibiting virus binding to ACE2 receptor in mice immunized with sa-mRNA S vaccines that encoded for either the wildtype sequence of the S protein (wildtype, blue), a mutated sequence that inhibits proteolytic cleavage between S1/S2 (uncleavable, red), or a mutated sequence that inhibits proteolytic cleavage between S1/S2 and a double proline mutation (uncleavable+PP, green). BALB/c female mice (n = 10) were immunized twice, three weeks apart, with 1.0 µg or 0.01 µg of sa-mRNA. Serum samples were collected at Day 43 and tested in an ACE2-binding inhibition assay. Each dot represents an individual serum sample, and the column represents the geometric mean for the group. The dotted line represents the lower limit of quantitation for the assay. Statistical analysis by two-way ANOVA with Tukey's multiple comparison test was performed using GraphPad Prism 9.1.0. **P*<0.05. ns, not significant (*P*≥0.05).