Supplemental Information

A Thermostable mRNA Vaccine against COVID-19

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Figure S1. Amino acid sequence alignment of full S protein of SARS-CoV-2 isolates used in this study. Related to Figures 1 and 3.

Invariant residues are shown as black dots. RBD sequences are shown in gray. Variant mutations are marked in light red.

Figure S2. Characterization of the expression of RDB encoding mRNA. Related to Figure 1.

(A) RBD expression in transfected HEK293F cells determined by ELISA.

(B) Immunofluorescence analysis of RBD expression (FITC, green) in HeLa cells. HeLa cells were transfected with RBD mRNA (2 μg/ml), and RBD expression was detected with a panel of SARS-CoV-2 specific monoclonal antibodies at 24 hours post transfection. Nuclei was stained using Hhechst (blue). Scale bar: 50 μm.

Figure S3. Flow sheet of mRNA-LNP manufacture. Related to Figure 1.

ARCoV is manufactured through rapid mixing of mRNA in aqueous solution and a mixture of lipids in ethanol. This process yields self-assembled LNPs with mRNA encapsulated inside. Tangential flow filtration was used to remove ethanol and to concentrate the solution. Following the Quality Control (QC) procedure, the final product was filtered into sterilized glass syringes or glass vials.

Figure S4. SARS-CoV-2 RBD expression profile in muscle tissue of ARCoV immunized mice. Related to Figure 2.

Intramuscular injection of ARCoV induced local RBD expression in intramuscular lymph nodes. Multiplex immunofluorescent staining of intramuscular injection sites showed SARS-CoV-2 RBD and CD11b-positive monocytes expression in the intramuscular lymph nodes of the ARCoV mRNA-LNP -inoculated mice. Scale bar: 500 μm. Magnifications of the areas boxed in white are shown on the right. Colored arrows indicate the double-stained cells that are magnified beside. Scale bar: 200 μm.

Figure S5. The immunogenicity and protection of a single dose of ARCoV in mice. Related to Figure 3.

BALB/c mice were intramuscularly immunized with 2 μg (n=7) or 30 μg (n=8) of the ARCoV vaccine or Placebo (n=5). Serum was collected at 14, 28 days post immunization and analyzed
by ELISA (A) and pseudovirus neutralization assay (B). Data are shown as mean ± SEM. Significance was calculated using a two-way ANOVA with multiple comparison tests (n.s., not significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001).

Six to eight weeks after immunization, all immunized mice were inoculated intranasally with the SARS-CoV-2 mouse-adapted strain MASCp6, and their lungs (C) and trachea (D) were collected for detection of viral RNA loads at 5 days post challenge. Data are shown as mean ± SEM. Significance was calculated using a one-way ANOVA with multiple comparison tests. (**, P < 0.01; ****, P < 0.0001).

**Figure S6.** Serum neutralization comparison between SARS-CoV-2 clinical isolate and the mouse adapted strain MASCp6. Related to Figure 5.

Standard PRNT assay were performed with sera from ARCoV immunized mice (n=15) using SARS-CoV-2 strains 131 and MASCp6, respectively. Data are analyzed by paired t-test. (n.s., not significant).

**Figure S7.** Neutralizing antibody response in male and female cynomolgus monkeys. Related to Figure 7.

Ten cynomolgus macaques were immunized intramuscularly with 100 μg or 1000 μg of ARCoV, respectively, and boosted with the same dose at a 14-day interval. The serum neutralizing antibody titers from male and female macaques were calculated respectively. Dotted lines indicate the limits of detection. Significance was calculated using a one-way ANOVA with multiple comparison tests. (**, P < 0.01; ****, P < 0.0001).

**Figure S8.** Thermostability of mRNA-LNP formulations under different temperatures. Related to Figure 2.

(A) BLI of FLuc expression in mice. The FLuc encoding mRNA-LNPs were stored at 4°C, 25°C or 37°C for 1, 4, and 7 days before being dosed to BALB/c mice. IVIS imaging was performed 6 hours post inoculation.

(B) Photon flux was quantified from ROI analysis. The data are representative of at least three independent experiments, and error bars indicate the SEM. Significance was calculated using two-way ANOVA with multiple comparison tests. (n.s., not significant; ***, P < 0.001; ****, P < 0.0001).
Figure S9. Comparison of neutralizing antibody titers in ARCoV-immunized cynomolgus monkeys and convalescent sera from COVID-19 patients. Related to Figure 6.

The serum neutralizing antibody titers were calculated from cynomolgus macaques immunized with 100 µg (n=10) and 1000 µg (n=10) ARCoV and COVID-19 patients’ convalescent sera (n=20), respectively. Dotted lines indicate the limits of detection. Significance was calculated using a one-way ANOVA with multiple comparison tests. (n.s., not significant; **, P < 0.01).
Table S1. Characterization of mRNA-LNPs in different batches (related to Figure 1)

|                      | Dₜ (nm) | PDI  | Encapsulation Efficiency |
|----------------------|---------|------|--------------------------|
| ARCoV mRNA-LNP       | 88.8    | 0.068| 96.80%                   |
| FLuc mRNA-LNP        | 98.7    | 0.05 | 98.60%                   |
| Empty LNP            | 55      | 0.164| N/A                      |
Table S2. SARS-CoV-2 strains used in this study

| Abbreviation in our study | Strain name                  | Accession Nos        | Patients originated from |
|---------------------------|------------------------------|----------------------|--------------------------|
| 131                       | BetaCoV/Beijing/IME-BJ01/2020 | GWHACAX01000000      | Beijing, China           |
| V34                       | BetaCoV/Beijing/IME-BJ05/2020 | GWHACBB01000000      | Wuhan, China             |
| 5N                        | BetaCoV/Beijing/IME-BJ08/2020 | GWHAMKA01000000      | Italy                    |
| MASCp6                    | BetaCoV/Beijing/IME-BJ05-P6/2020 | GWHACFH01000000      | N/A                      |
Table S3. Protein peptide pools used for ELISPOT assay (related to Figure 4)

| Sequence                      | Protein | Start-End |
|-------------------------------|---------|-----------|
| SASFSTFKCYGVSPTKL             | RBD     | 371-387   |
| KLPDDFTGCV                    | RBD     | 424-433   |
| NLDSKVGGNYN                   | RBD     | 440-457   |
| YLYRLFRKSNLK PFERDI           | RBD     | 451-468   |
| KPFERDISTEIYQ                 | RBD     | 462-474   |
Table S4. Characteristics of cynomolgus macaques used in this study (related to Figure 7)

| Group | Weight (Kg) | Sex | DOB* | IgG (Day 28) | NT<sub>50</sub> (Day 28) | IFN-γ (Day 19) |
|-------|-------------|-----|------|--------------|----------------|---------------|
| Placebo | 2.64 | M | 2017/1/6 | <50 | <30 | 20 |
| | 2.98 | M | 2017/1/12 | <50 | <30 | 210 |
| | 2.88 | M | 2017/1/2 | <50 | <30 | 220 |
| | 2.46 | M | 2017/1/5 | <50 | <30 | 20 |
| | 3.32 | M | 2016/1/31 | <50 | <30 | 90 |
| | 2.5 | F | 2017/1/7 | <50 | <30 | 110 |
| | 2.81 | F | 2017/1/5 | <50 | <30 | 120 |
| | 3.33 | F | 2016/5/31 | <50 | <30 | 190 |
| | 2.58 | F | 2017/1/9 | <50 | <30 | 190 |
| | 3.22 | F | 2016/12/3 | <50 | <30 | 390 |
| ARCoV 100 μg | 4.66 | M | 2015/7/29 | 12800 | 1866 | 780 |
| | 2.93 | M | 2016/8/19 | 50 | <30 | 520 |
| | 2.28 | M | 2017/1/12 | 50 | 70 | 650 |
| | 3.15 | M | 2017/1/12 | 3200 | 2138 | 180 |
| | 2.96 | M | 2017/1/8 | 3200 | 771 | 550 |
| | 2.43 | F | 2016/5/27 | 3200 | 385 | 530 |
| | 2.5 | F | 2017/1/13 | 800 | 103 | 220 |
| | 3 | F | 2017/1/8 | 3200 | 517 | 280 |
| | 2.41 | F | 2017/1/3 | 12800 | 272 | 870 |
| | 4.01 | F | 2015/2/17 | 12800 | 836 | 230 |
| ARCoV 1000 μg | 3.24 | M | 2017/1/11 | 51200 | 4390 | 960 |
| | 2.68 | M | 2017/1/14 | 12800 | 3197 | 570 |
| | 3.02 | M | 2017/1/7 | 12800 | 1807 | 790 |
| | 2.39 | M | 2017/1/10 | 51200 | 14189 | 1330 |
| | 2.78 | M | 2017/1/1 | 51200 | 8500 | 880 |
| | 2.85 | F | 2017/1/13 | 3200 | 89 | 970 |
| | 2.35 | F | 2017/1/14 | <50 | <30 | 620 |
| | 3.09 | F | 2015/3/19 | 12800 | 25185 | 720 |
| | 2.54 | F | 2016/9/7 | 12800 | 2595 | 660 |
| | 2.64 | F | 2017/1/10 | 12800 | 4840 | 1020 |

* Date of birth