

## Supplemental information

**Supplementary Table 1. Statistical comparisons of S-specific serum binding IgG antibody titers after vaccination**

Comparison	Estimated Fold-change (95% CI)	Adjusted P-value
<i>Dose 1 (Day 21)</i>		
25- $\mu$ g mRNA-LNP1 over 5- $\mu$ g mRNA-LNP1	68.53 (25.63-185.69)	<1e-6
25- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP1	67.68 (25.04-182.43)	<1e-6
5- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP1	50.61 (22.1-117.24)	<1e-6
25- $\mu$ g mRNA-LNP1 over 0.4- $\mu$ g IM	3.11 (0.86-11.06)	0.9619
25- $\mu$ g mRNA-LNP1 over 1- $\mu$ g IM	1.63 (0.61-4.38)	0.8426
5- $\mu$ g mRNA-LNP1 over 0.4- $\mu$ g IM	0.05 (0.02-0.14)	<1e-6
5- $\mu$ g mRNA-LNP1 over 1- $\mu$ g IM	0.02 (0.01-0.05)	<1e-6
25- $\mu$ g mRNA-LNP2 over 25- $\mu$ g mRNA-LNP1	0.99 (0.31-3.14)	0.4892
25- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP2	1.34 (0.5-3.71)	0.7183
25- $\mu$ g mRNA-LNP2 over 0.4- $\mu$ g IM	3.07 (0.9-11.02)	0.9635
25- $\mu$ g mRNA-LNP2 over 1- $\mu$ g IM	1.61 (0.6-4.4)	0.8312
5- $\mu$ g mRNA-LNP2 over 25- $\mu$ g mRNA-LNP1	0.74 (0.27-2.03)	0.2668
5- $\mu$ g mRNA-LNP2 over 0.4- $\mu$ g IM	2.3 (0.75-6.89)	0.9359
5- $\mu$ g mRNA-LNP2 over 1- $\mu$ g IM	1.2 (0.54-2.69)	0.6883
1- $\mu$ g IM over 0.4- $\mu$ g IM	1.91 (0.63-5.83)	0.8853
<i>Dose 2 (Day 41)</i>		
25- $\mu$ g mRNA-LNP1 over 5- $\mu$ g mRNA-LNP1	3.64 (1.54-8.55)	0.0024
25- $\mu$ g mRNA-LNP2 over 25- $\mu$ g mRNA-LNP1	3.68 (1.78-7.55)	6e-04
25- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP1	13.38 (5.58-31.97)	<1e-6
25- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP2	2.7 (1.1-6.61)	0.0156
5- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP1	4.95 (1.8-13.8)	0.0023
25- $\mu$ g mRNA-LNP1 over 0.4- $\mu$ g IM	0.61 (0.27-1.34)	0.1004
25- $\mu$ g mRNA-LNP1 over 1- $\mu$ g IM	0.37 (0.17-0.86)	0.0138
5- $\mu$ g mRNA-LNP1 over 0.4- $\mu$ g IM	0.17 (0.06-0.43)	2e-04
5- $\mu$ g mRNA-LNP1 over 1- $\mu$ g IM	0.1 (0.04-0.27)	2e-04

25- $\mu$ g mRNA-LNP2 over 0.4- $\mu$ g IM	2.24 (0.99-4.93)	0.9742
25- $\mu$ g mRNA-LNP2 over 1- $\mu$ g IM	1.38 (0.61-3.2)	0.7796
5- $\mu$ g mRNA-LNP2 over 25- $\mu$ g mRNA-LNP1	1.36 (0.56-3.32)	0.7592
5- $\mu$ g mRNA-LNP2 over 0.4- $\mu$ g IM	0.83 (0.32-2.27)	0.3406
5- $\mu$ g mRNA-LNP2 over 1- $\mu$ g IM	0.51 (0.19-1.38)	0.0894
1- $\mu$ g IM over 0.4- $\mu$ g IM	1.63 (0.63-3.98)	0.8621

---

CI, confidence interval; IgG, immunoglobulin G; IM, intramuscular; LNP, lipid nanoparticle; mRNA, messenger RNA.

**Supplementary Table 2. Statistical comparisons of S-specific serum binding IgA antibody titers after vaccination**

Comparison	Estimated Fold-change (95% CI)	Adjusted P-value
<i>Dose 1 (Day 21)</i>		
25- $\mu$ g mRNA-LNP2 over 25- $\mu$ g mRNA-LNP1	83.81 (38.04-224.25)	<1e-6
25- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP2	32.7 (3.92-716.22)	0.0035
25- $\mu$ g mRNA-LNP2 over 0.4- $\mu$ g IM	2.84 (1.18-6.73)	0.0104
25- $\mu$ g mRNA-LNP1 over 0.4- $\mu$ g IM	0.03 (0.01-0.09)	<1e-6
25- $\mu$ g mRNA-LNP1 over 1- $\mu$ g IM	0.02 (0.01-0.05)	<1e-6
25- $\mu$ g mRNA-LNP2 over 1- $\mu$ g IM	1.7 (0.72-3.93)	0.8993
5- $\mu$ g mRNA-LNP2 over 25- $\mu$ g mRNA-LNP1	2.56 (0.12-24.18)	0.8043
5- $\mu$ g mRNA-LNP2 over 0.4- $\mu$ g IM	0.09 (<1e-6-0.8)	0.0171
5- $\mu$ g mRNA-LNP2 over 1- $\mu$ g IM	0.05 (<1e-6-0.44)	0.0066
1- $\mu$ g IM over 0.4- $\mu$ g IM	1.67 (0.67-4.14)	0.8746
<i>Dose 2 (Day 41)</i>		
25- $\mu$ g mRNA-LNP1 over 5- $\mu$ g mRNA-LNP1	3.74 (1.37-10.19)	0.0051
25- $\mu$ g mRNA-LNP2 over 25- $\mu$ g mRNA-LNP1	5.77 (2.57-12.95)	<1e-6
25- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP1	21.57 (7.93-60.5)	<1e-6
25- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP2	4.05 (1.37-11.73)	0.0066
5- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP1	5.33 (1.55-18.18)	0.006
25- $\mu$ g mRNA-LNP1 over 0.4- $\mu$ g IM	0.21 (0.08-0.52)	0.0025
25- $\mu$ g mRNA-LNP1 over 1- $\mu$ g IM	0.13 (0.05-0.33)	<1e-6
5- $\mu$ g mRNA-LNP1 over 0.4- $\mu$ g IM	0.06 (0.02-0.18)	<1e-6
5- $\mu$ g mRNA-LNP1 over 1- $\mu$ g IM	0.03 (0.01-0.1)	<1e-6
25- $\mu$ g mRNA-LNP2 over 0.4- $\mu$ g IM	1.21 (0.47-3.09)	0.6658
25- $\mu$ g mRNA-LNP2 over 1- $\mu$ g IM	0.73 (0.28-1.88)	0.2411
5- $\mu$ g mRNA-LNP2 over 25- $\mu$ g mRNA-LNP1	1.43 (0.51-4.12)	0.7531
5- $\mu$ g mRNA-LNP2 over 0.4- $\mu$ g IM	0.3 (0.09-0.95)	0.0219
5- $\mu$ g mRNA-LNP2 over 1- $\mu$ g IM	0.18 (0.06-0.57)	0.0044
1- $\mu$ g IM over 0.4- $\mu$ g IM	1.67 (0.58-4.79)	0.8428

CI, confidence interval; IgA, immunoglobulin A; IM, intramuscular; LNP, lipid nanoparticle; mRNA, messenger RNA.

**Supplementary Table 3. Statistical comparisons of serum neutralizing antibody titers after vaccination**

Comparison	Estimated Fold-change (95% CI)	Adjusted P-value
<i>Dose 1 (Day 21)</i>		
25- $\mu$ g mRNA-LNP2 over 25- $\mu$ g mRNA-LNP1	38.84 (8.7-287.37)	<1e-6
25- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP2	17.46 (2.21-275.24)	0.0044
25- $\mu$ g mRNA-LNP2 over 0.4- $\mu$ g IM	8.09 (1.09-96.32)	0.0205
25- $\mu$ g mRNA-LNP1 over 0.4- $\mu$ g IM	0.21 (0.02-2.64)	0.0801
25- $\mu$ g mRNA-LNP1 over 1- $\mu$ g IM	0.06 (0.01-0.21)	<1e-6
25- $\mu$ g mRNA-LNP2 over 1- $\mu$ g IM	2.16 (0.63-7.5)	0.8996
5- $\mu$ g mRNA-LNP2 over 25- $\mu$ g mRNA-LNP1	2.22 (0.12-24.4)	0.7781
5- $\mu$ g mRNA-LNP2 over 0.4- $\mu$ g IM	0.46 (0.02-7.72)	0.275
5- $\mu$ g mRNA-LNP2 over 1- $\mu$ g IM	0.12 (0.01-0.77)	0.0149
1- $\mu$ g IM over 0.4- $\mu$ g IM	3.74 (0.6-39.29)	0.9309
<i>Dose 2 (Day 41)</i>		
25- $\mu$ g mRNA-LNP1 over 5- $\mu$ g mRNA-LNP1	5.07 (1.53-18.22)	0.0054
25- $\mu$ g mRNA-LNP2 over 25- $\mu$ g mRNA-LNP1	5.7 (2.28-14.07)	0.0008
25- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP1	28.89 (8.5-111.28)	<1e-6
25- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP2	5.08 (1.46-18.06)	0.0059
5- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP1	5.69 (1.32-27.13)	0.0119
25- $\mu$ g mRNA-LNP1 over 0.4- $\mu$ g IM	0.36 (0.14-0.91)	0.0173
25- $\mu$ g mRNA-LNP1 over 1- $\mu$ g IM	0.17 (0.06-0.45)	0.0004
5- $\mu$ g mRNA-LNP1 over 0.4- $\mu$ g IM	0.07 (0.02-.024)	0.0002
5- $\mu$ g mRNA-LNP1 over 1- $\mu$ g IM	0.03 (0.01-0.12)	<1e-6
25- $\mu$ g mRNA-LNP2 over 0.4- $\mu$ g IM	2.06 (0.83-5.26)	0.94
25- $\mu$ g mRNA-LNP2 over 1- $\mu$ g IM	0.96 (0.33-2.65)	0.4737
5- $\mu$ g mRNA-LNP2 over 25- $\mu$ g mRNA-LNP1	1.12 (0.32-3.86)	0.5776
5- $\mu$ g mRNA-LNP2 over 0.4- $\mu$ g IM	0.41 (0.12-1.43)	0.0758
5- $\mu$ g mRNA-LNP2 over 1- $\mu$ g IM	0.19 (0.05-0.69)	0.0065
1- $\mu$ g IM over 0.4- $\mu$ g IM	2.15 (0.77-6.24)	0.9333

CI, confidence interval, IM, intramuscular; LNP, lipid nanoparticle; mRNA, messenger RNA.

**Supplementary Table 4. Statistical comparisons of viral load by plaque assay<sup>a</sup> in the lungs of vaccinated hamsters at 3 days after SARS-CoV-2 challenge**

Comparison	Fold-change (standard error)	t-ratio	P-value
0.4- $\mu$ g IM over 1- $\mu$ g IM	2.32 (1.23)	1.88	0.783
0.4- $\mu$ g IM over 25- $\mu$ g mRNA-LNP1	1.15 (1.17)	0.98	1
0.4- $\mu$ g IM over 5- $\mu$ g mRNA-LNP1	-1.91 (1.11)	-1.72	0.881
0.4- $\mu$ g IM over 25- $\mu$ g mRNA-LNP2	2.77 (1.05)	2.64	0.245
0.4- $\mu$ g IM over 5- $\mu$ g mRNA-LNP2	0.99 (0.98)	1.01	1
0.4- $\mu$ g IM over mock	-2.60 (0.90)	-2.88	0.148
1- $\mu$ g IM over 25- $\mu$ g mRNA-LNP1	0.01 (1.23)	0.01	1
1- $\mu$ g IM over 5- $\mu$ g mRNA-LNP1	-3.05 (1.17)	-2.60	0.266
1- $\mu$ g IM over 25- $\mu$ g mRNA-LNP2	1.63 (1.11)	1.46	0.97
1- $\mu$ g IM over 5- $\mu$ g mRNA-LNP2	-0.15 (1.05)	-0.15	1
1- $\mu$ g IM over mock	-3.73 (0.98)	-3.82	0.014
25- $\mu$ g mRNA-LNP1 over 5- $\mu$ g mRNA-LNP1	-1.88 (1.23)	-1.53	0.955
25- $\mu$ g mRNA-LNP1 over 25- $\mu$ g mRNA-LNP2	2.79 (1.17)	2.39	0.401
25- $\mu$ g mRNA-LNP1 over 5- $\mu$ g mRNA-LNP2	1.02 (1.11)	0.91	1
25- $\mu$ g mRNA-LNP1 over mock	-2.57 (1.05)	-2.45	0.354
5- $\mu$ g mRNA-LNP1 over 25- $\mu$ g mRNA-LNP2	5.85 (1.23)	4.76	0.001
5- $\mu$ g mRNA-LNP1 over 5- $\mu$ g mRNA-LNP2	4.07 (1.17)	3.48	0.035
5- $\mu$ g mRNA-LNP1 over mock	0.49 (1.11)	0.44	1
25- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP2	-0.60 (1.23)	-0.49	1
25- $\mu$ g mRNA-LNP2 over mock	-4.18 (1.17)	-3.57	0.027
5- $\mu$ g mRNA-LNP2 over mock	-2.40 (1.23)	-1.96	0.731

<sup>a</sup>Viral loads ( $\log_{10}$  transformed) were assessed by ordinary linear regression; only modeled data at Day 3 after challenge was evaluated since viral loads on Day 14 were zero for all hamsters. A degree of freedom of 28 was used.

IM, intramuscular; LNP, lipid nanoparticle; mRNA, messenger RNA.

**Supplementary Table 5. Statistical comparisons of viral load by plaque assay in the nasal turbinates of vaccinated hamsters at 3 days after SARS-CoV-2 challenge**

Comparison	Fold-change (standard error)	<i>t</i> -ratio	P-value
0.4- $\mu$ g IM over 1- $\mu$ g IM	3.09 (0.87)	3.56	0.028
0.4- $\mu$ g IM over 25- $\mu$ g mRNA-LNP1	0.55 (0.83)	0.67	1
0.4- $\mu$ g IM over 5- $\mu$ g mRNA-LNP1	-0.97 (0.78)	-1.24	0.995
0.4- $\mu$ g IM over 25- $\mu$ g mRNA-LNP2	2.59 (0.74)	3.52	0.031
0.4- $\mu$ g IM over 5- $\mu$ g mRNA-LNP2	1.30 (0.69)	1.89	0.779
0.4- $\mu$ g IM over mock	-2.02 (0.64)	-3.18	0.073
1- $\mu$ g IM over 25- $\mu$ g mRNA-LNP1	-1.53 (0.87)	-1.76	0.86
1- $\mu$ g IM over 5- $\mu$ g mRNA-LNP1	-3.05 (0.83)	-3.69	0.02
1- $\mu$ g IM over 25- $\mu$ g mRNA-LNP2	0.52 (0.78)	0.66	1
1- $\mu$ g IM over 5- $\mu$ g mRNA-LNP2	-0.78 (0.74)	-1.05	0.999
1- $\mu$ g IM over mock	-4.09 (0.69)	-5.95	0
25- $\mu$ g mRNA-LNP1 over 5- $\mu$ g mRNA-LNP1	-0.51 (0.87)	-0.59	1
25- $\mu$ g mRNA-LNP1 over 25- $\mu$ g mRNA-LNP2	3.06 (0.83)	3.70	0.019
25- $\mu$ g mRNA-LNP1 over 5- $\mu$ g mRNA-LNP2	1.76 (0.78)	2.25	0.5
25- $\mu$ g mRNA-LNP1 over mock	-1.56 (0.74)	-2.11	0.607
5- $\mu$ g mRNA-LNP1 over 25- $\mu$ g mRNA-LNP2	4.58 (0.87)	5.29	0
5- $\mu$ g mRNA-LNP1 over 5- $\mu$ g mRNA-LNP2	3.28 (0.83)	3.98	0.009
5- $\mu$ g mRNA-LNP1 over mock	-0.03 (0.78)	-0.04	1
25- $\mu$ g mRNA-LNP2 over 5- $\mu$ g mRNA-LNP2	-0.28 (0.87)	-0.33	1
25- $\mu$ g mRNA-LNP2 over mock	-3.60 (0.83)	-4.36	0.003
5- $\mu$ g mRNA-LNP2 over mock	-2.31 (0.87)	-2.66	0.236

<sup>a</sup>Viral loads ( $\log_{10}$  transformed) were assessed by ordinary linear regression; only modeled data at Day 3 after challenge was evaluated since viral loads on Day 14 were zero for all hamsters. A degree of freedom of 28 was used.

IM, intramuscular; LNP, lipid nanoparticle; mRNA, messenger RNA.

**Supplementary Table 6. Major pulmonary histopathological findings and severity scores after SARS-CoV-2 challenge by vaccine group.**

<b>Day 3 (After Challenge)</b>							
	<b>Intranasal</b>				<b>Intramuscular</b>		
	Mock (n = 5)	mRNA- LNP1 5 µg (n = 5)	mRNA- LNP1 25 µg (n = 5)	mRNA- LNP2 5 µg (n = 5)	mRNA- LNP2 25 µg (n = 5)	0.4 µg (n = 5)	1 µg (n = 5)
<b>Interstitial inflammation, n</b>							
Group total	5	5	5	5	5	5	5
2 (mild)	2	4	2	2	1	5	2
3 (moderate)	3	1	3	3	4	0	3
<b>Bronchial/bronchiolar inflammation, n</b>							
Group total	4	5	1	3	1	3	4
1 (minimal)	2	1	1	1	0	2	3
2 (mild)	0	3	0	2	1	1	1
3 (moderate)	2	1	0	0	0	0	0
<b>Vascular inflammation, n</b>							
Group total	4	3	2	3	2	4	1
1 (minimal)	0	1	1	1	1	3	0
2 (mild)	2	2	1	1	0	1	1
3 (moderate)	2	0	0	1	1	0	0
<b>Day 14 (After Challenge)</b>							
	<b>Intranasal</b>				<b>Intramuscular</b>		
	Mock (n = 5)	mRNA- LNP1 5 µg (n = 5)	mRNA- LNP1 25 µg (n = 5)	mRNA- LNP2 5 µg (n = 4)	mRNA- LNP2 25 µg (n = 4)	0.4 µg (n = 5)	1 µg (n = 5)
<b>Interstitial inflammation, n</b>							
Group total	5	5	5	4	4	5	5
2 (mild)	0	5	4	0	4	4	3
3 (moderate)	5	0	1	4	0	1	2
<b>Type II pneumocyte hyperplasia, n</b>							
Group total	5	4	5	4	2	1	5
1 (minimal)	0	3	3	1	2	0	0
2 (mild)	4	1	1	2	0	0	3
3 (moderate)	1	0	1	1	0	1	2
<b>Bronchial/bronchiolar inflammation, n</b>							
Group total	4	3	2	3	1	1	3
1 (minimal)	2	3	2	1	1	1	3
2 (mild)	2	0	0	2	0	0	0
<b>Vascular inflammation, n</b>							
Group total	1	4	4	4	1	5	3
1 (minimal)	1	4	4	4	1	5	3

IM, intramuscular; LNP, lipid nanoparticle; mRNA, messenger RNA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

**Supplementary Table 7. Summary of hamsters positive for SARS-CoV-2 N-protein at 3 days after challenge**

<b>Vaccine Group</b>	<b>Administration Route</b>	<b>Ratio of N-protein Positive Hamsters to Total Population (n/N)</b>
Mock	IN	5/5
mRNA-LNP1 5 µg	IN	5/5
mRNA-LNP1 25 µg	IN	2/5
mRNA-LNP2 5 µg	IN	4/5
mRNA-LNP2 25 µg	IN	4/5
Intramuscular 0.4 µg	IM	3/5
Intramuscular 1 µg	IM	2/5

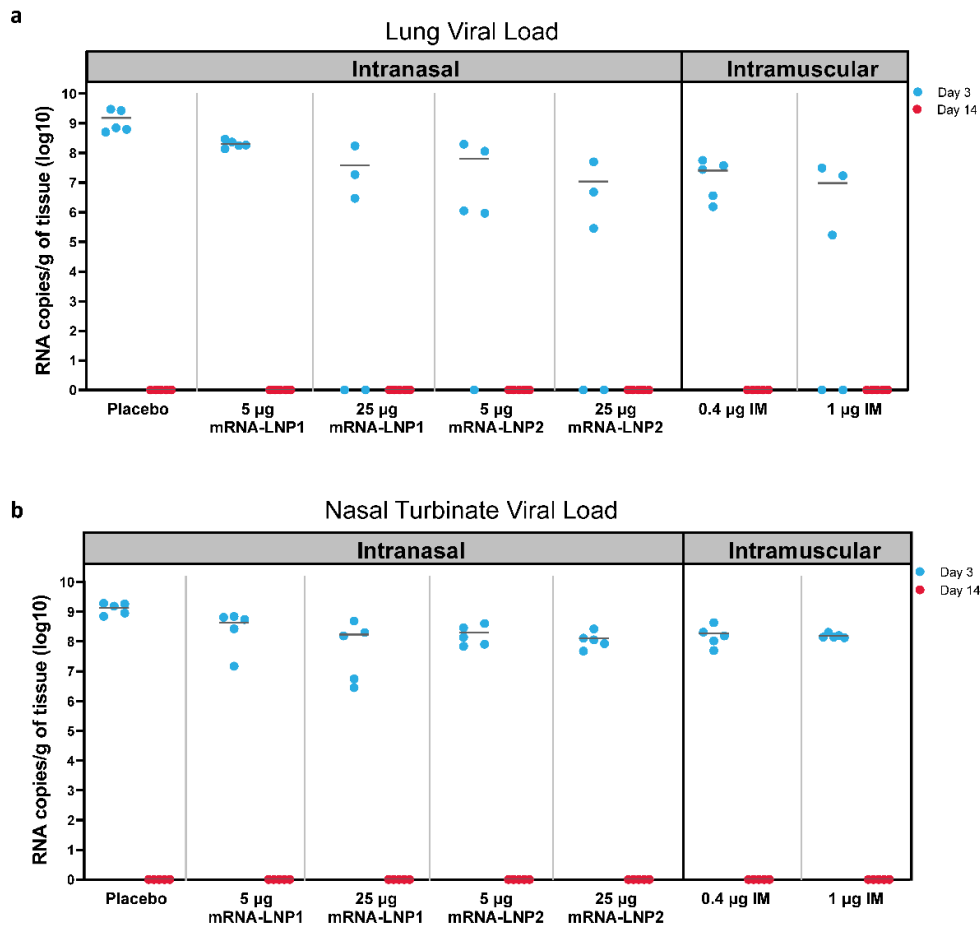
IN, intranasal; IM, intramuscular; LNP, lipid nanoparticle; mRNA, messenger RNA; N protein, nucleocapsid protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.



**Supplementary Figure 1. Viral load as determined via qRT-PCR through 14 days after SARS-CoV-2 challenge in vaccinated hamsters.**

Viral load (sgRNA copies per gram of tissue) at 3 days and 14 days after SARS-CoV-2 challenge in (a) lungs and (b) nasal turbinates of vaccinated hamsters. Animal-level data are shown as dots (n = 5 animals per group), with the grey lines representing the geometric mean of each group. LLOD = 10<sup>3</sup> copies/g of tissue.

IM, intramuscular; IN, intranasal; LNP, lipid nanoparticle; mRNA, messenger RNA; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sgRNA, subgenomic RNA.



**Supplementary Figure 2. Pulmonary pathology characteristics at 14 days after SARS-CoV-2 challenge in vaccinated hamsters.**

Lung sections from hamsters at 14 days after SARS-CoV-2 challenge were stained with H&E. Representative images of (a) interstitial inflammation, (b) type II pneumocyte hyperplasia (arrows), or (c) airways and blood vessels are shown for hamsters intranasally administered 2 doses of tris/sucrose buffer (mock-vaccinated), mRNA-LNP1 (25  $\mu$ g), mRNA-LNP2 (25  $\mu$ g), or were intramuscularly vaccinated with 2 doses of vaccine (1.0  $\mu$ g). Scale bars = 100  $\mu$ m.

H&E, hematoxylin and eosin; IN, intranasal; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

