

1 **SUPPLEMENTAL FIGURES, TABLES, AND FILES**

2 **Supplemental Figure 1. Evaluation of the immunogenicity of RBD+SpyCage determined**

3 **by serum IgG and neutralizing antibody titers.** Shown are the antibody titers from Trial 1 that

4 evaluated immunogenicity of the RBD+SpyCage vaccine in hamsters. Plotted are (A) IgG

5 antibody titers in serum samples collected on days 0, 26, and 55, and (B) neutralizing antibody

6 titers against SARS-CoV-2 on day 0 and 55. * significantly different from all other groups,

7 ($p < 0.001$) determined by Kruskal-Wallis test with Dunn's multiple comparison correction.

8

9 **Supplemental Figure 2. Weight loss and viral titers in the nasal turbinates and lungs after**

10 **SARS-CoV-2 challenge of vaccinated hamsters.** After viral challenge, hamsters in Trial 1

11 (immunogenicity study) hamsters were monitored for (A) weight loss, and viral titers were

12 evaluated in (B) lung tissues and (C) nasal turbinates on days 3 and 6 post-infection.

13

14 **Supplemental Figure 3. Weight loss in unvaccinated hamsters after challenge with 10^5 ,**

15 **10^4 , and 10^3 TCID₅₀ of SARS-CoV-2.** To determine if lower challenge doses of SARS-CoV-2

16 would induce weight loss, equal numbers of male and female hamsters ($n=3/\text{sex}$, $n=6/\text{group}$)

17 were intranasally inoculated with 10^5 , 10^4 , and 10^3 TCID₅₀ of SARS-CoV-2/USA/WA1/2020.

18 Weight loss was then monitored for 14 days until the animals recovered.

19

20 **Supplemental Figure 4. Lung histopathology scoring for multiple parameters in**

21 **vaccinated hamsters challenged with SARS-CoV-2.** On days 3, 5, and 7 post-infection, lung

22 tissues were processed for H&E staining and scored by a veterinary pathologist. Panels display

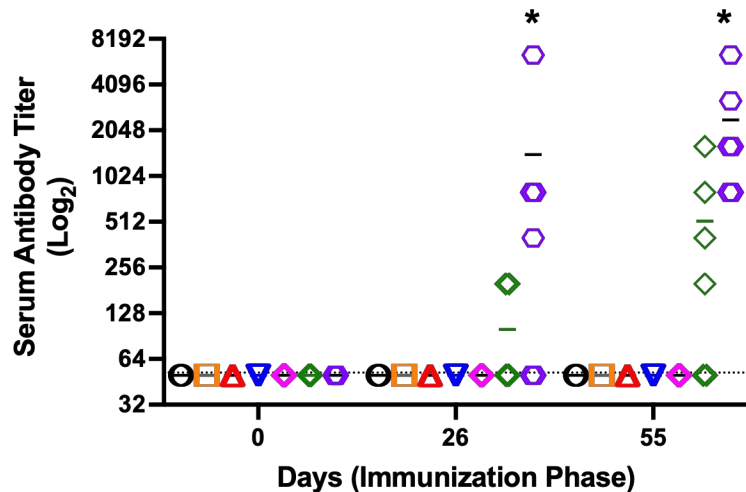
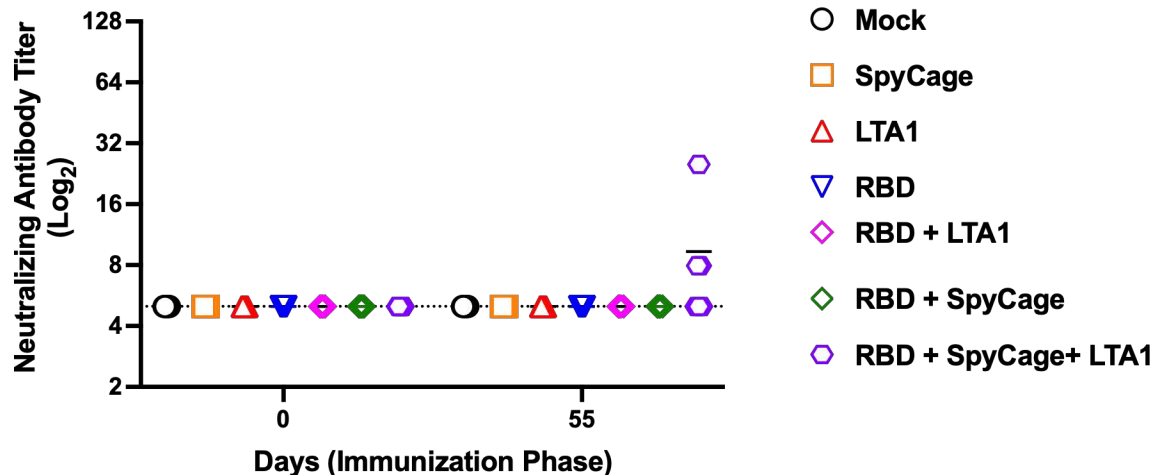
23 scoring for (A) Type II Pneumocyte Hyperplasia, (B) Alveoli Pathology, (C) Hemorrhage, (D)

24 Blood Vessels Pathology, and (E) Bronchi Pathology. Black horizontal lines indicate median

25 score with the distribution of scores displayed as violin plots.

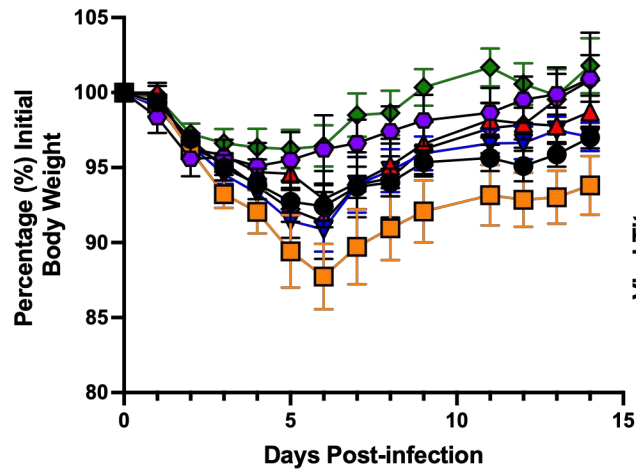
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- 27 **Supplementary Table 1: Cryo-EM data collection, processing, and model refinement**
28 **statistics.**
29
30 **Supplemental File 1. Sequences of plasmids used in this study.**

(A)**IgG Antibody Titers****(B)****Neutralizing Antibodies**

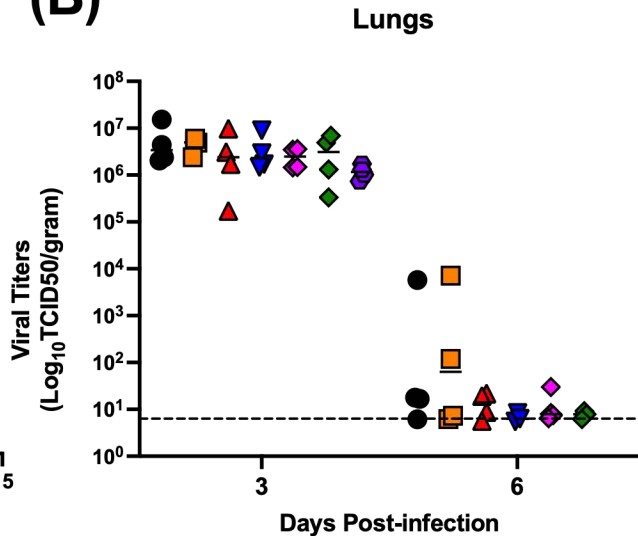
- Mock
- SpyCage
- △ LTA1
- ▽ RBD
- ◇ RBD + LTA1
- ◇ RBD + SpyCage
- ◇ RBD + SpyCage+ LTA1

Figure S1

(A)

● Mock

■ SpyCage

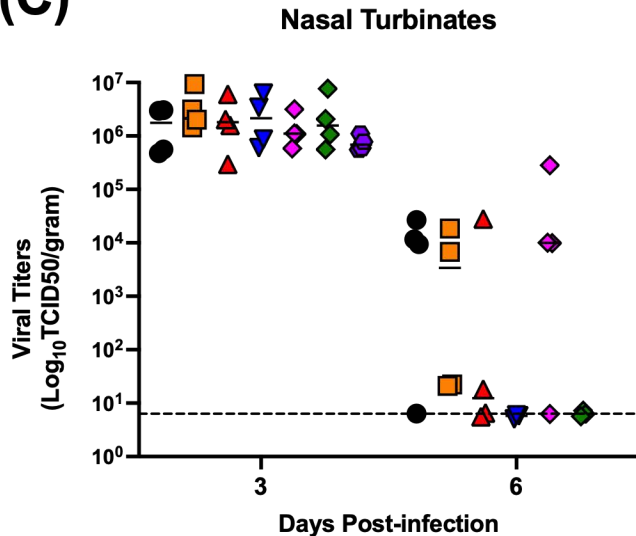
(B)

▼ RBD

▲ LTA1

◆ RBD + LTA1

◆ RBD + SpyCage

(C)

◆ RBD + SpyCage+ LTA1

Figure S2

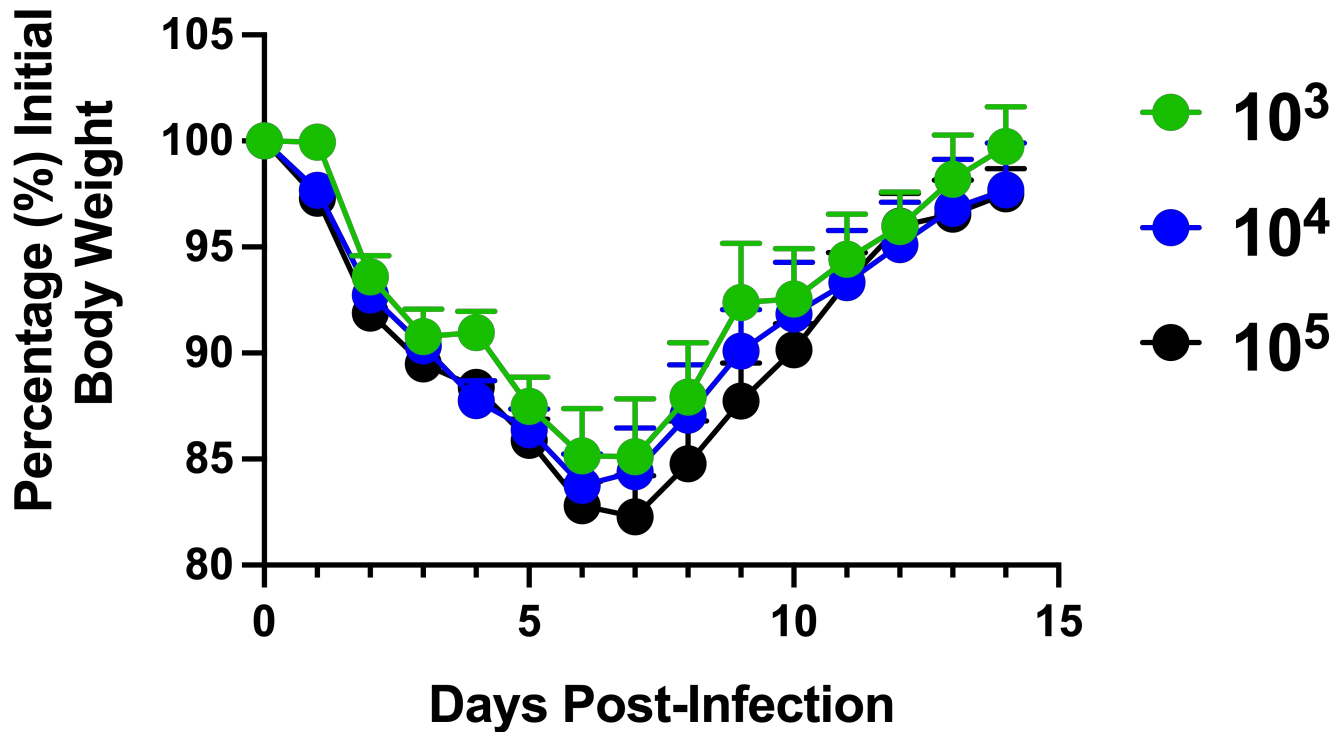


Figure S3

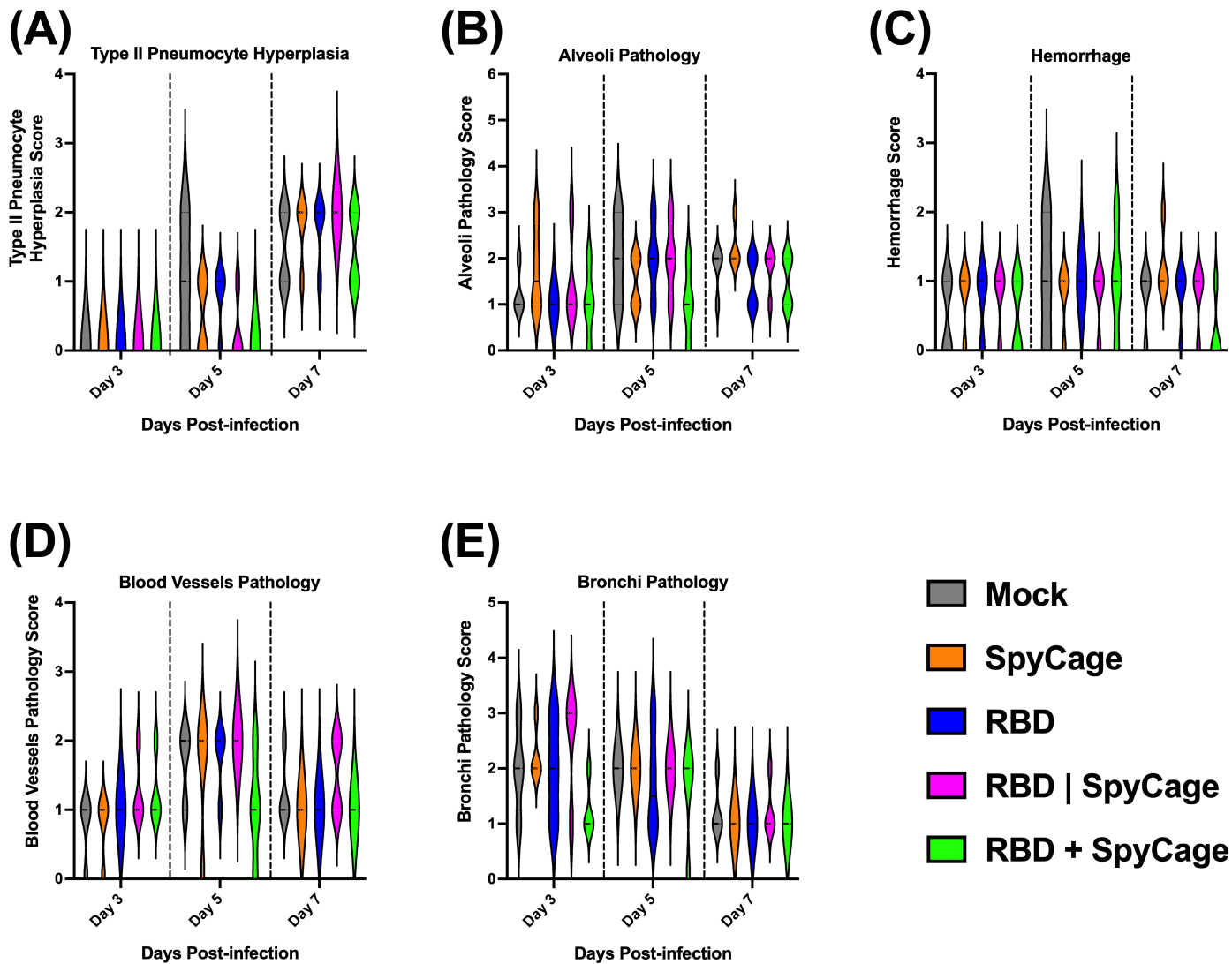


Figure S4

Supplementary Table 1: Cryo-EM data collection, processing, and model refinement statistics

Data collection and processing

| | |
|--|--------------|
| Microscope | Titan Krios |
| Detector | Falcon 3EC |
| Voltage (kV) | 300 |
| Recording mode | Counting |
| Nominal magnification | x59,000 |
| Electron exposure ($e^-/\text{\AA}^2$) | 45 |
| No. of frames | 39 |
| Defocus range (μm) | -1.2 to -3.0 |
| Pixel size (\AA) | 1.11 |
| Symmetry | Icosahedral |
| Initial particles (no.) | 129,792 |
| Final particles (no.) | 63,430 |
| Map resolution (\AA) | 3.4 |
| FSC Threshold | 0.143 |
| Map sharpening B-factor (\AA^2) | -196.1 |

Model refinement

| | |
|-------------------------------------|-------|
| Initial model used | 13-01 |
| FSC model vs. map at FSC=0.5 | 3.77 |
| CC model vs. map (masked) | 0.77 |
| Average B-factor (\AA^2) | 69.1 |
| Bond length rmsd (\AA) | 0.002 |
| Bond angle rmsd ($^\circ$) | 0.562 |

Model composition

| | |
|--------------------------|-----------------|
| No. of chains | 60 |
| Non-hydrogen atoms/chain | 1,519 |
| protein residues/chain | 201 (aa 22-222) |

Validation

| | |
|-------------------|------|
| Molprobity score | 1.5 |
| Clashscore | 9.4 |
| Poor rotamers (%) | none |

Ramachandran plot

| | |
|----------------|-------|
| Favored (%) | 97.99 |
| Allowed (%) | 2.01 |
| Disallowed (%) | none |

Supplemental File 1: Sequences of plasmids used in this study.

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ACCESSION
VERSION
SOURCE .
ORGANISM .
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//

>pSL1040_SpyCage-Flexible_Expression_Plasmid

LOCUS pSL1040 6259 bp DNA circular 12-MAY-2022

DEFINITION .

ACCESSION

VERSION

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ORGANISM .

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DEFINITION .

ACCESSION

VERSION

SOURCE .

ORGANISM .

COMMENT

COMMENT

COMMENT ApEinfo:methylated:1

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DEFINITION .
ACCESSION
VERSION
SOURCE .
ORGANISM .
COMMENT
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ORIGIN

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