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2 <sup>6</sup>These authors contributed equally to this work.

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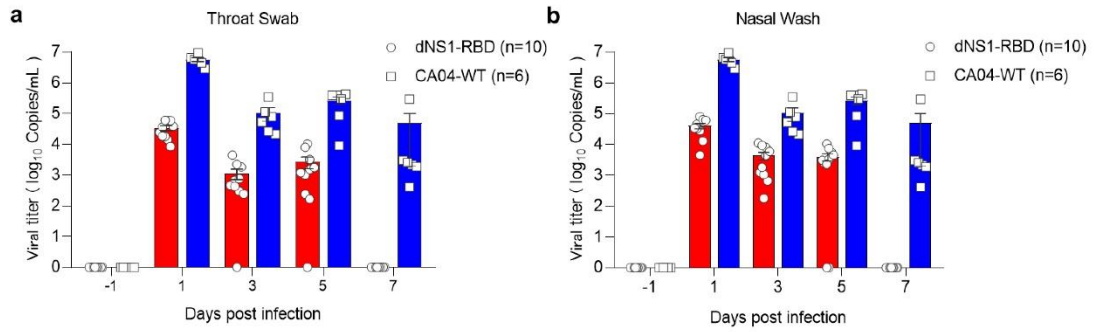
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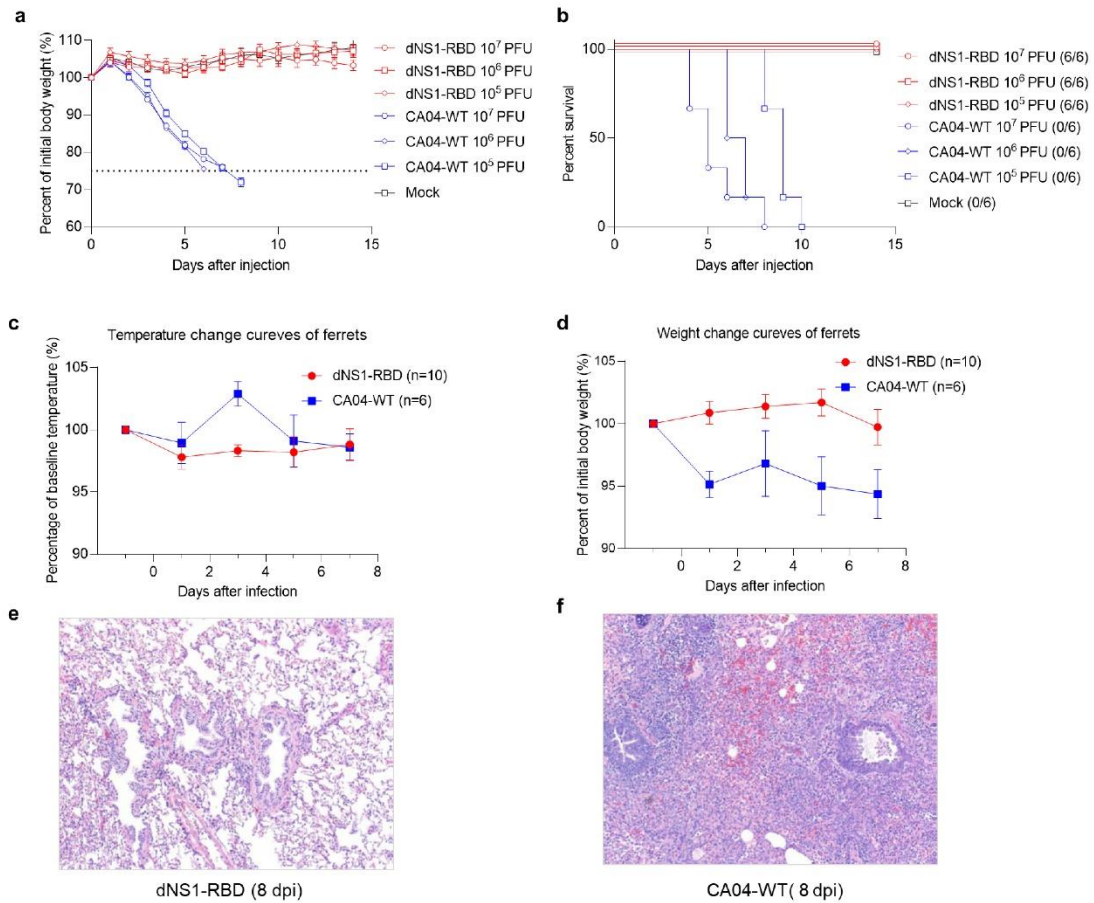
2 **Supplementary Fig. S1 Viral shedding of dNS1-RBD in ferrets. a-b,** Two groups of ferrets

3 were immunized with a single dose of the dNS1-RBD (red) and CA04-WT (blue) vaccines

4 through the intranasal route. Throat swabs (a) and nasal washes (b) of ferrets collected at days

5 -1, 1, 3, 5, and 7 were assayed.

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2 **Supplementary Fig. S2 Pathogenicity evaluation of dNS1-RBD in mice and ferrets.**

3 **a-b**, Body weight changes (**a**) and survival (**b**) of female BALB/c mice following intranasal  
 4 administration of different doses of dNS1-RBD (10<sup>5</sup>, 10<sup>6</sup> and 10<sup>7</sup> PFU) in comparison to those  
 5 following intranasal administration of different doses of CA04-WT (10<sup>5</sup>, 10<sup>6</sup> and 10<sup>7</sup> PFU). **c-**

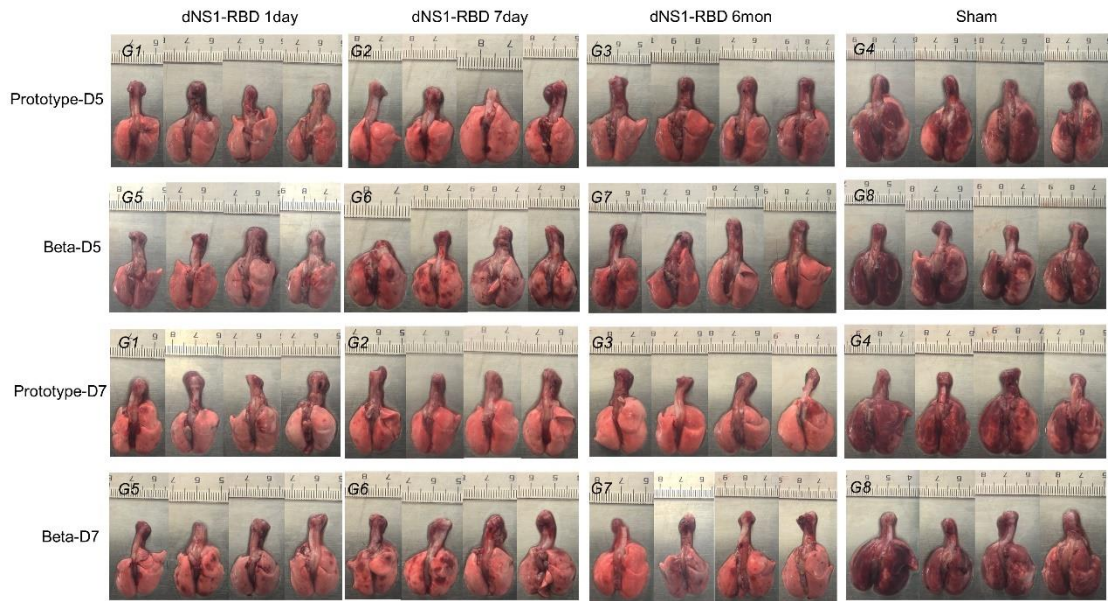
6 **d**, Two groups of ferrets intranasally administered a single-dose dNS1-RBD vaccine (n=10) or  
 7 CA04-WT (n=6) showed attenuation of dNS1-RBD infection. (**c**) Body weight and (**d**) body

8 temperature change of ferrets. **e-f**, Histopathological evaluations of the lungs from the two  
 9 groups of ferrets at day 8 post administration. Lung tissues were collected and stained with

10 hematoxylin and eosin. Inoculation with 10<sup>7</sup> PFU of CA04-WT resulted in obvious influenza-  
 11 like symptoms with fever, weight loss and pathological injury in lung tissues from ferrets.

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3 **Supplementary Fig. S3 Gross observations of lung tissues from hamsters in groups 1 to 8**

4 **as indicated in Figure 2 of the main manuscript.** The immunized hamsters were euthanized,

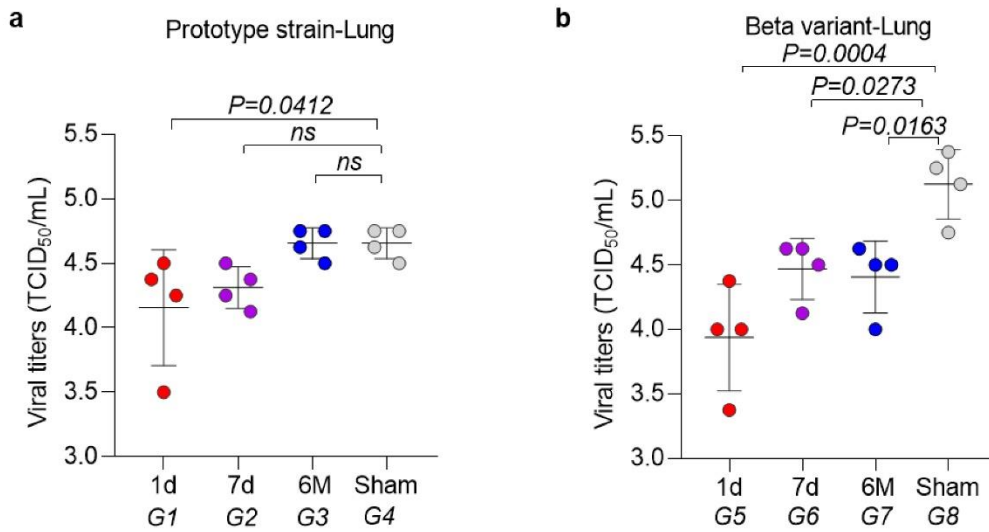
5 and the lungs were isolated at 5 dpi and 7 dpi. Severe lung lesions, including consolidation and

6 multifocal and diffuse hyperemia, were observed in G4 and G8 hamsters, while focal

7 histopathological changes in a few lobes of the lung were observed in the vaccinated hamsters.

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3 **Supplementary Fig. S4 Viral loads of lung tissue from challenged hamsters. a-b,** There

4 were eight total experimental groups each containing eight hamsters (males:females=1:1). **a,**

5 Groups 1, 2, 3 and 4 were challenged with the prototype SARS-CoV-2 strain; **b,** groups 5, 6, 7

6 and 8 were challenged with the beta variant. Groups 1 and 5 received a single dose of dNS1-

7 RBD 1 day before challenge; groups 2 and 6 received a single dose of dNS1-RBD 7 days before

8 challenge; groups 3 and 7 received two doses of dNS1-RBD at a 14-day interval 6 months

9 before challenge; and groups 4 and 8 served as sham controls and were not treated. Viral loads

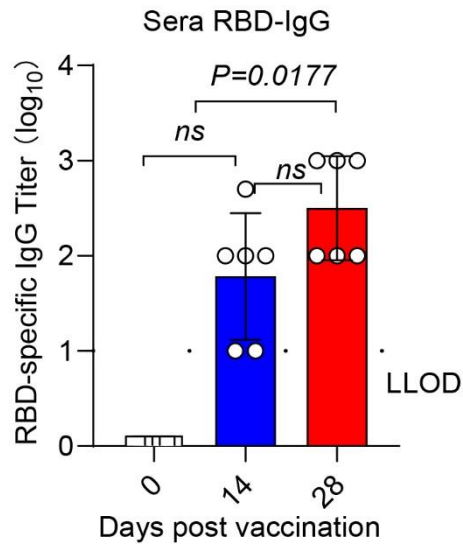
10 of lung tissue obtained at 5 dpi from hamsters challenged by the prototype (**a**) or beta strain (**b**)

11 were determined by TCID50 assay. Data are the mean  $\pm$  SD; ns, not significant ( $P > 0.05$ );

12 significance was determined by ordinary one-way ANOVA multiple comparison.

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3 **Supplementary Fig. S5 Quantification of humoral response levels in hamsters.** RBD-

4 specific IgG levels in serum were measured by ELISA for hamsters vaccinated twice, at day 0

5 and day 14. Data for the antibody analysis are presented as the geometric mean with the

6 geometric SD from four independent experiments. LLOD-lower limit of detection. Data are the

7 mean  $\pm$  SD; ns, not significant ( $P > 0.05$ ); significance was determined by ordinary one-way

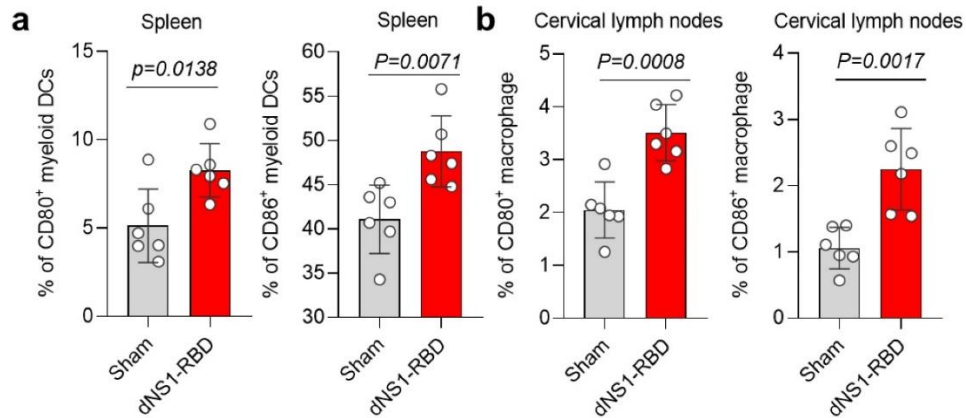
8 ANOVA multiple comparison.

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3 **Supplementary Fig. S7 Activation and differentiation of various innate immune cells. a-b,**

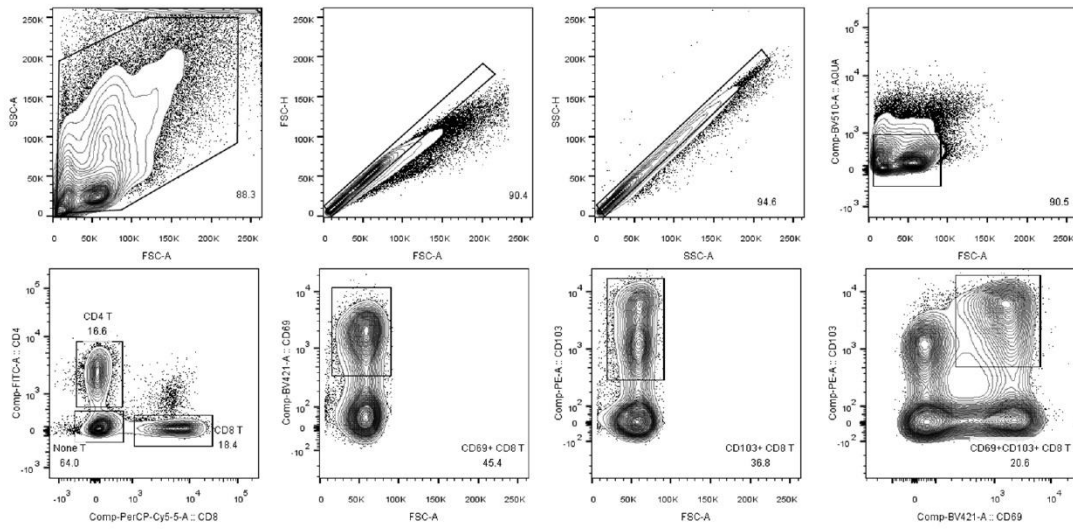
4 CD80 and CD86 expression on antigen-presenting cells from immunized C57BL/6 mouse

5 spleens (a) and cervical lymph nodes (b) at 14 days after vaccination. Data are the mean  $\pm$  SD;

6 significance was determined by two-tailed Student's t-test.

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3 **Supplementary Fig. S8 Gating strategy for identifying pulmonary T cells expressing**

4 **CD69<sup>+</sup> and CD103<sup>+</sup> in dNS1-RBD-infected mice.** Mice inoculated intranasally with dNS1-

5 RBD. Representative flow cytometry profiles are from mice harvested at different times.

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