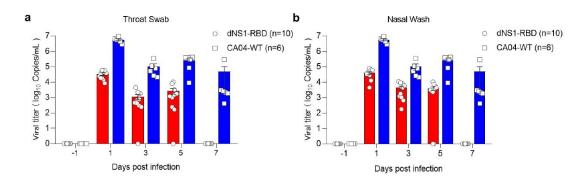
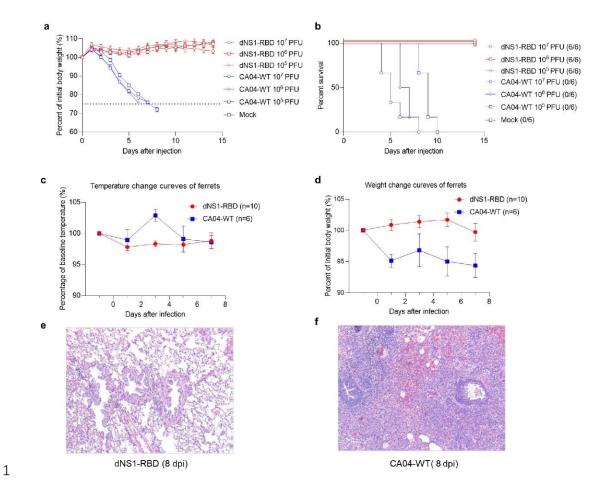
1	Supplementary information for
2	A live attenuated influenza virus-vectored intranasal COVID-19
3	vaccine provides rapid, prolonged, and broad protection against
4	SARS-CoV-2 infection
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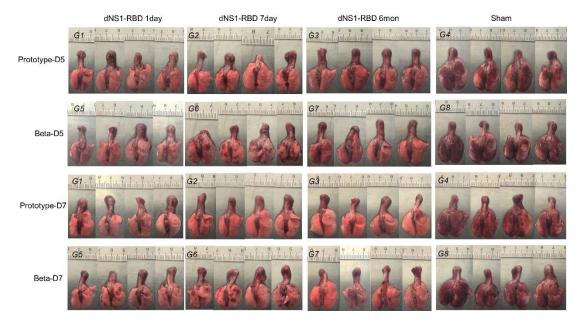


Supplementary Fig. S1 Viral shedding of dNS1-RBD in ferrets. a-b, Two groups of ferrets were immunized with a single dose of the dNS1-RBD (red) and CA04-WT (blue) vaccines through the intranasal route. Throat swabs (a) and nasal washes (b) of ferrets collected at days -1, 1, 3, 5, and 7 were assayed.

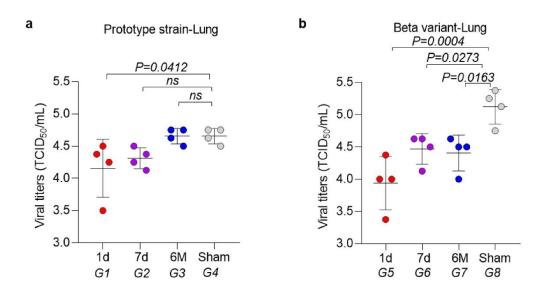


Supplementary Fig. S2 Pathogenicity evaluation of dNS1-RBD in mice and ferrets.

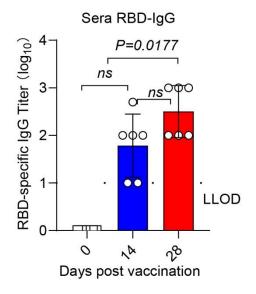
a-b, Body weight changes (**a**) and survival (**b**) of female BALB/c mice following intranasal administration of different doses of dNS1-RBD (10⁵, 10⁶ and 10⁷ PFU) in comparison to those following intranasal administration of different doses of CA04-WT (10⁵, 10⁶ and 10⁷ PFU). **c**-**d**, Two groups of ferrets intranasally administered a single-dose dNS1-RBD vaccine (n=10) or CA04-WT (n=6) showed attenuation of dNS1-RBD infection. (**c**) Body weight and (**d**) body temperature change of ferrets. **e-f**, Histopathological evaluations of the lungs from the two groups of ferrets at day 8 post administration. Lung tissues were collected and stained with hematoxylin and eosin. Inoculation with 10⁷ PFU of CA04-WT resulted in obvious influenzalike symptoms with fever, weight loss and pathological injury in lung tissues from ferrets.



Supplementary Fig. S3 Gross observations of lung tissues from hamsters in groups 1 to 8 as indicated in Figure 2 of the main manuscript. The immunized hamsters were euthanized, and the lungs were isolated at 5 dpi and 7 dpi. Severe lung lesions, including consolidation and multifocal and diffuse hyperemia, were observed in G4 and G8 hamsters, while focal histopathological changes in a few lobes of the lung were observed in the vaccinated hamsters.



Supplementary Fig. S4 Viral loads of lung tissue from challenged hamsters. a-b, There were eight total experimental groups each containing eight hamsters (males:females=1:1). a, Groups 1, 2, 3 and 4 were challenged with the prototype SARS-CoV-2 strain; b, groups 5, 6, 7 and 8 were challenged with the beta variant. Groups 1 and 5 received a single dose of dNS1-RBD 1 day before challenge; groups 2 and 6 received a single dose of dNS1-RBD 7 days before challenge; groups 3 and 7 received two doses of dNS1-RBD at a 14-day interval 6 months before challenge; and groups 4 and 8 served as sham controls and were not treated. Viral loads of lung tissue obtained at 5 dpi from hamsters challenged by the prototype (a) or beta strain (b) were determined by TCID50 assay. Data are the mean \pm SD; ns, not significant (P > 0.05); significance was determined by ordinary one-way ANOVA multiple comparison.



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

specific IgG levels in serum were measured by ELISA for hamsters vaccinated twice, at day 0 and day 14. Data for the antibody analysis are presented as the geometric mean with the geometric SD from four independent experiments. LLOD-lower limit of detection. Data are the mean \pm SD; ns, not significant (P > 0.05); significance was determined by ordinary one-way ANOVA multiple comparison.

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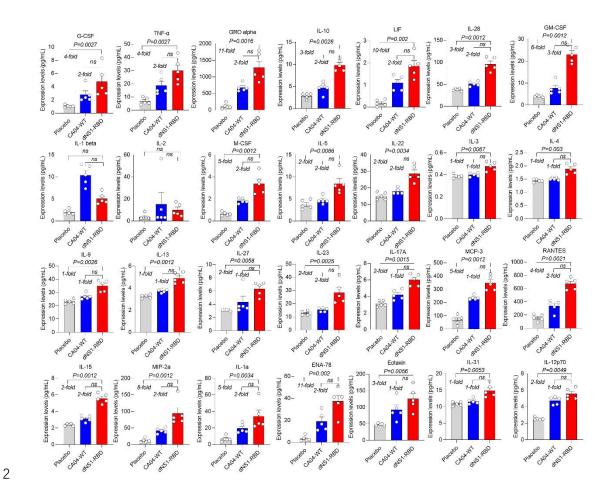
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homogenates from BALB/c mice vaccinated with dNS1-RBD one day prior to sacrifice were

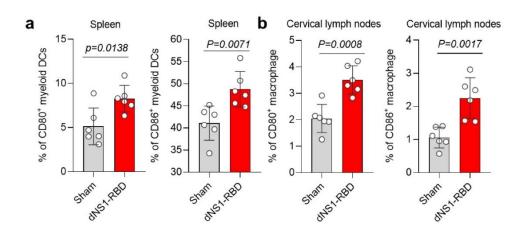
Supplementary Fig. S6 Quantification of cytokine and chemokine expression levels. Lung

collected for quantification of the other 28 cytokine and chemokine expression levels except

for IL-6, IL-18, IFN- γ , IFN- α , MCP-1, IP-10, MIP-1 α , and MIP-1 β by ProcartaPlex

immunoassays. The data are expressed as ng/mL. Data are the mean \pm SD; ns, not significant

(P > 0.05); significance was determined by ordinary one-way ANOVA multiple comparison.

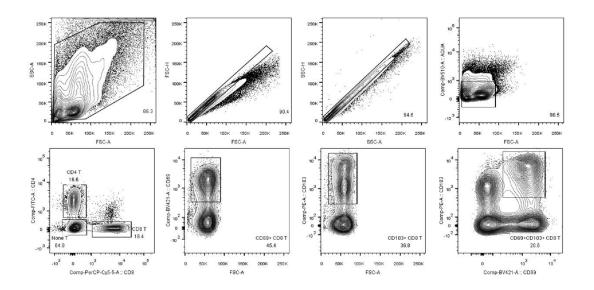


Supplementary Fig. S7 Activation and differentiation of various innate immune cells. a-b,

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

2





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

- CD69+ and CD103+ in dNS1-RBD-infected mice. Mice inoculated intranasally with dNS1-
- 5 RBD. Representative flow cytometry profiles are from mice harvested at different times.