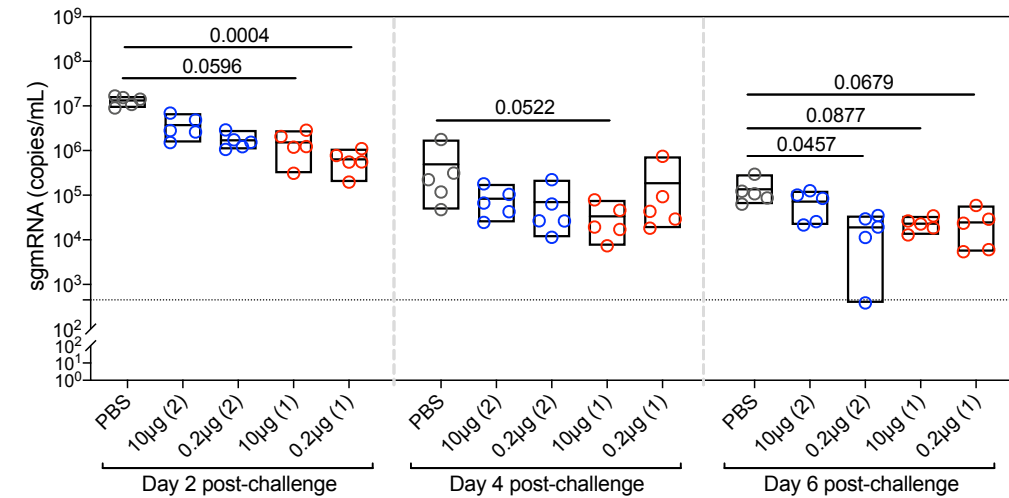
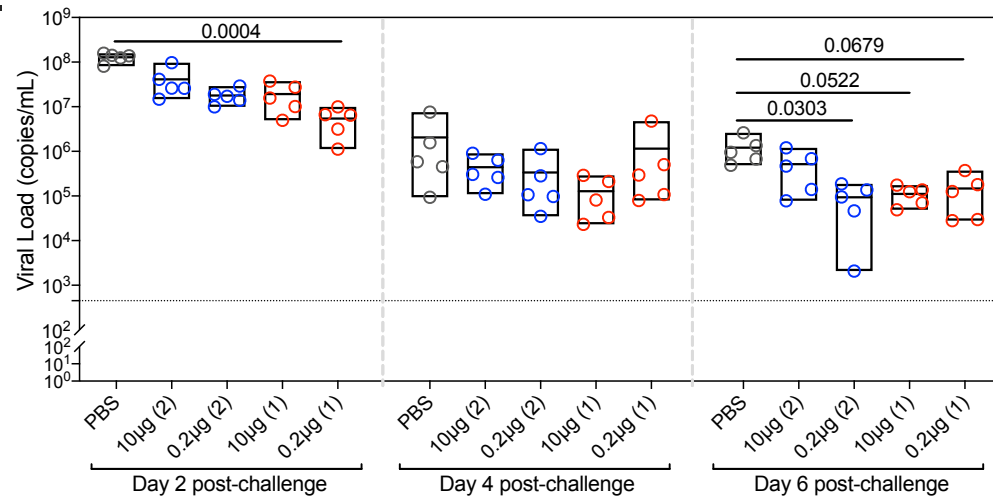
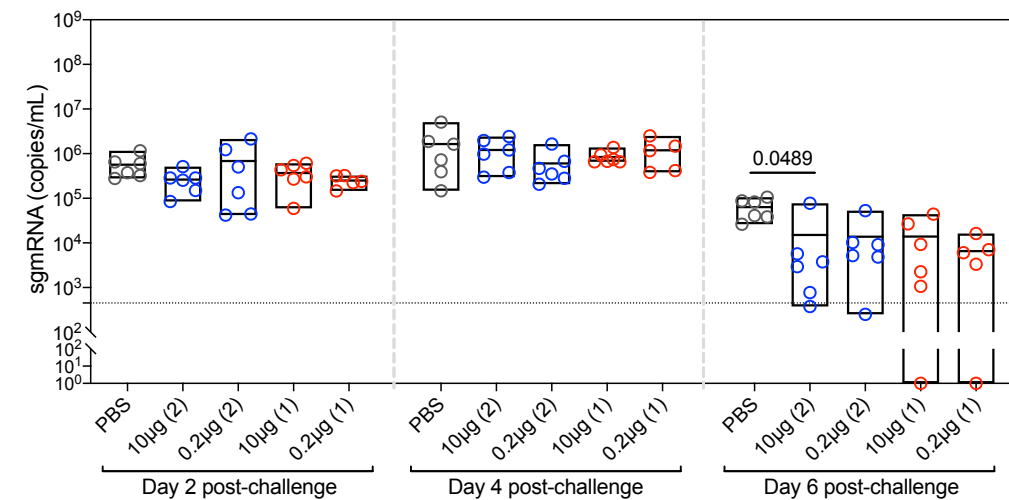
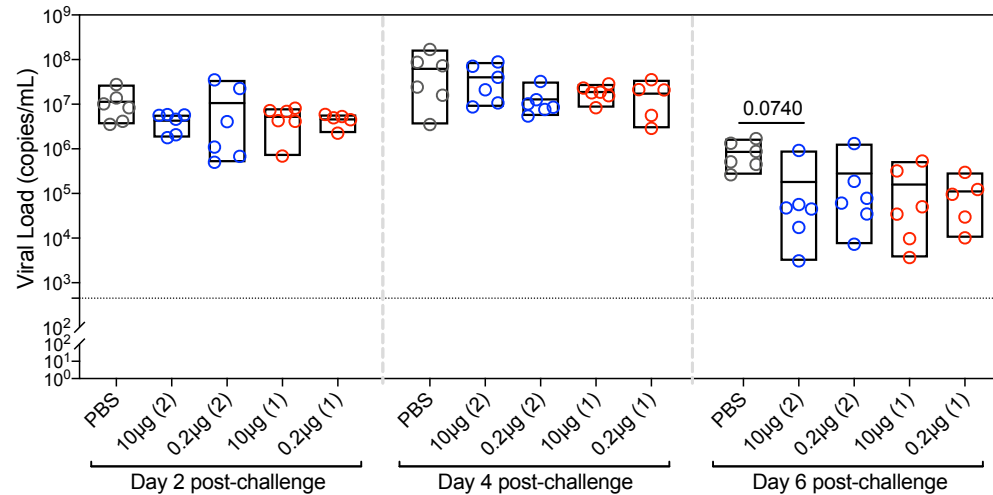


a. B.1.1.7 Oral Swab sgmRNA**b.** B.1.1.7 Oral Swab Viral Load**c.** B.1.351 Oral Swab sgmRNA**d.** B.1.351 Oral Swab Viral Load

Supplemental Table 1A

			IP Pathology (# observed/total n)				
Challenge Strain	Vaccine regimen	Vaccine dose	None	Minimal	Mild	Moderate	Marked
B.1.1.7	Prime-boost	PBS				3/5	2/5
		10 µg (2)	3/5	2/5			
		0.2 µg (2)	2/5	3/5			
	Single	10 µg (1)	2/5	1/5	1/5		1/5
		0.2 µg (1)	1/5	1/5		2/5	1/5
B.1.351	Prime-boost	PBS				4/6	2/6
		10 µg (2)	4/6	1/6	1/6		
		0.2 µg (2)	1/6	2/6	1/6	1/6	1/6
	Single	10 µg (1)	1/6	3/6	2/6		
		0.2 µg (1)*		3/5	1/5		1/5

*one animal lost to study due to injury unrelated to infection.

Supplemental Table 1B

			SARS-CoV-2 Viral Antigen per Section (# animals observed/total n)					
Challenge Strain	Vaccine regimen	Vaccine dose	None	Single cell	<5 cells	<20 cells	<40 cells	>500 cells
B.1.1.7	Prime-boost	PBS						5/5
		10 µg (2)	3/5	2/5				
		0.2 µg (2)	4/5	1/5				
	Single	10 µg (1)	3/5		1/5	1/5		
		0.2 µg (1)	1/5	1/5	1/5	2/5		
B.1.351	Prime-boost	PBS						6/6
		10 µg (2)	5/6			1/6		
		0.2 µg (2)	1/6	5/6				
	Single	10 µg (1)	4/6			2/6		
		0.2 µg (1)*		2/5	2/5		1/5	

*one animal lost to study due to injury unrelated to infection.

1 **Supplemental material**

2

3 **Supplemental Figure 1. Additional antibody responses following SpFN-ALFQ immunization. a,**

4 ELISA was performed using either WA1 derived Receptor Binding Domain (RBD) or S-2P Spike

5 proteins from sera taken at week 8. The vaccine regimens are indicated on the x-axis by

6 phosphate buffered saline (PBS) control or SpFN dose with the number of vaccinations in the

7 regimen given parenthetically and by color code (blue, 2- dose, red, 1- dose). Endpoint titers are

8 given on the y-axis as geometric mean titers with data displayed in box plots with the top and

9 bottom bars of the box the standard deviation and the middle bar as the median value. P-values

10 for SpFN-ALFQ vaccination groups compared with PBS control are given just above the boxes in

11 light grey while inter- and intra-regimen p-values are given above the boxes in black. ns, not

12 significant ($p > 0.05$). **b,** Octet Biolayer Interferometry (BLI) responses against the WA1, B.1.1.7,

13 and B.1.351 sequences of the RBD are given for the vaccination regimens as in **a** on the x-axis

14 from sera collected at week 8. BLI responses are given in nanometers (nm) on the y-axis.

15 Vaccination regimen color coding and statistical treatments are as in **a**. **c,** IgG opsonization as

16 measured by binding to SARS-CoV-2 Spike protein expressing expi293F cells subsequently stained

17 by fluorescently tagged goat anti-hamster IgG and detected by flow cytometry. Fluorescence is

18 given as mean fluorescence intensity (MFI) on the y-axis. Vaccination regiment color coding and

19 statistical treatments are as in **a**.

20

21 **Supplemental Fig 2. Quantitative SARS-CoV-2 total and subgenomic mRNA (sgmRNA) viral load**

22 **from oral swabs following challenge.** Oral fluid collection from were obtained by swabbing at

23 days 2, 4, and 6 post challenge in hamsters and submitted to quantitative SARS-CoV-2 total and

24 sgmRNA analysis. Viral load is given on y-axes at copies/mL of oral fluid plotted on a log₁₀ scale.
25 Vaccination regimen groups, stratified by day post-challenge, are given on the x-axes: Phosphate
26 buffered saline (PBS) control, gray circles; 10 µg and 0.2 µg 2- dose regimen groups, blue circles;
27 10 µg and 0.2 µg 1- dose regimen groups, red circles. Data are given in box plots where the
28 horizontal bar is the median value for each group. Where values between groups are statistically
29 meaningfully different, p-values are given above horizontal black bars showing the analyzed
30 groups. In all other cases, the p-values were > 0.05. **a**, sgmRNA viral load from B.1.1.7 challenged
31 hamsters; **(B)** total RNA viral load from B.1.1.7 challenged hamsters; **c**, sgmRNA viral load from
32 B.1.351 challenged hamsters; **d**, total RNA viral load from B.1. 351 challenged hamsters.

33

34 **Supplemental Table 1. Semi-quantitative histopathology and IHC scores**

35 Lung tissues were collected at necropsy on day 6 post-challenge, fixed with neutral buffered
36 formalin, and stained with hematoxylin and eosin (H&E) for standard microscopic examination
37 as well as submitted for immunohistochemical (IHC) staining for SARS-CoV-2 nucleocapsid (N)
38 protein. (A) H&E sections were semi-quantitatively scored on the histopathology present in all
39 lung sections examined (Methods). The overall severity of interstitial pneumonia (IP) present in
40 individual animals in the PBS control and vaccinated groups is given. (B) IHC sections were
41 examined at 400X magnification, and evaluated for the number of immunopositive cells per slide.
42 Distribution of the number of immunopositive cells per section is given per PBS control and 2-
43 dose and 1- dose vaccine group. The number of vaccinations per vaccine group are given
44 parenthetically.

45