

Supplementary Materials for
**An influenza mRNA vaccine protects ferrets from lethal infection with highly
pathogenic avian influenza A(H5N1) virus**

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Legend for data file s1

Other Supplementary Material for this manuscript includes the following:

Data file S1
MDAR Reproducibility checklist

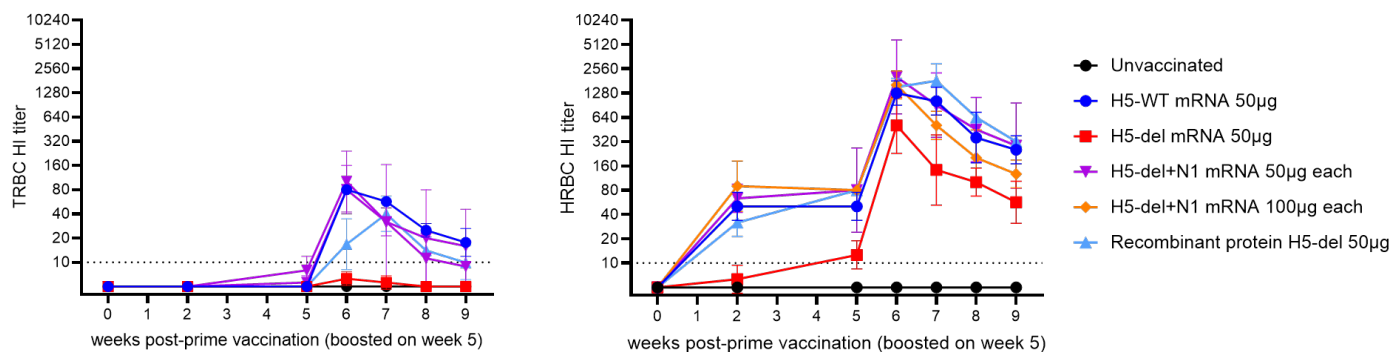


fig. S1. Comparison of hemagglutination inhibition (HI) titers of ferret serum samples with turkey or horse red blood cells.

HI assays were performed against the candidate vaccine virus (CVV) of A/American wigeon/South Carolina/22-000345-001/2021 (AW/SC/21) with 0.5% turkey red blood cells (TRBCs) or 1% horse red blood cells (HRBCs). The same week 0 to week 9 ferret serum samples as in Fig. 1 were assayed with both RBCs. Higher HI titers were detected against the AW/SC/21 CVV when HRBCs were used instead of TRBCs, indicating that the HI assay with HRBCs is more sensitive than that using TRBCs. Data are presented as geometric mean $\pm$ SD. Dotted horizontal lines indicate the limit of detection.

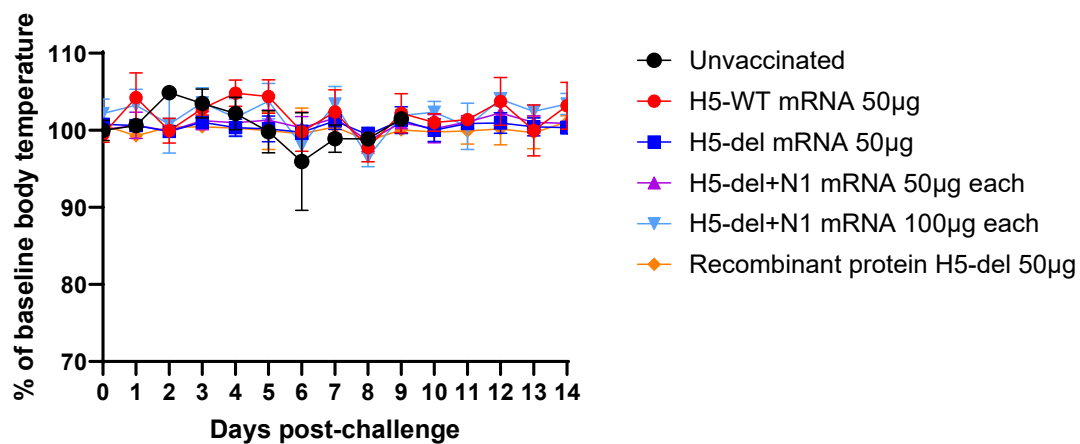


fig. S2. Body temperatures after challenge.

The body temperatures of the ferrets were monitored for 14 days after challenge for the experiment shown in Fig. 1. Data are presented as arithmetic mean $\pm$ SD.

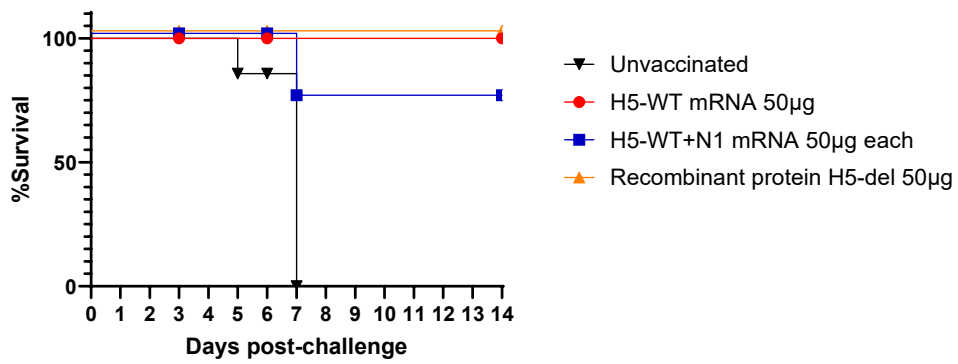


fig. S3. Survival rates after challenge.

Survival was monitored for 14 days after challenge described in Fig. 2. Because 3 ferrets were euthanized each on day 3 and 6 post-challenge for organ sampling, the survival rate were calculated with the ferrets remaining in each group at each time point.

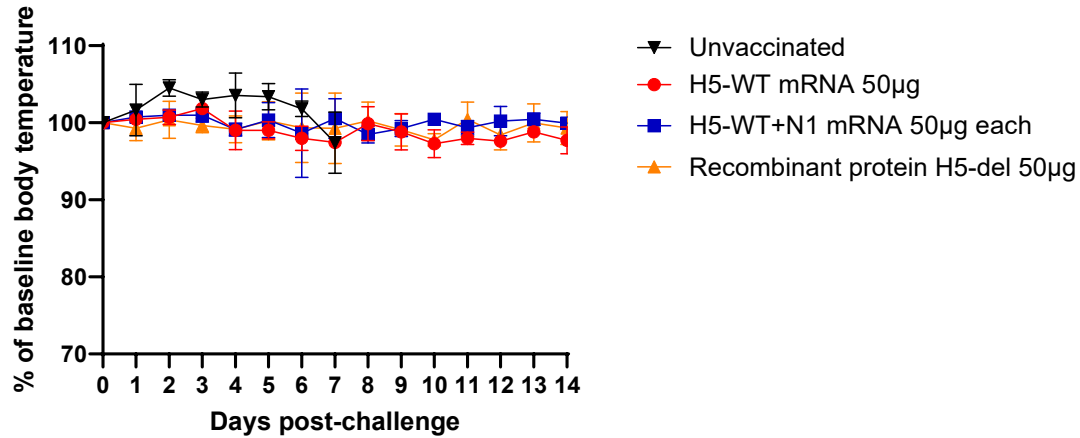


fig. S4. Body temperatures after challenge.

The body temperatures of the ferrets were monitored for 14 days after challenge for the experiment shown in Fig. 2. The averages of their body temperatures and their standard deviations were calculated only on the remaining ferrets (3 or 4 ferrets) in each group by excluding the ferrets used for organ sampling. Data are presented as arithmetic mean $\pm$ SD.

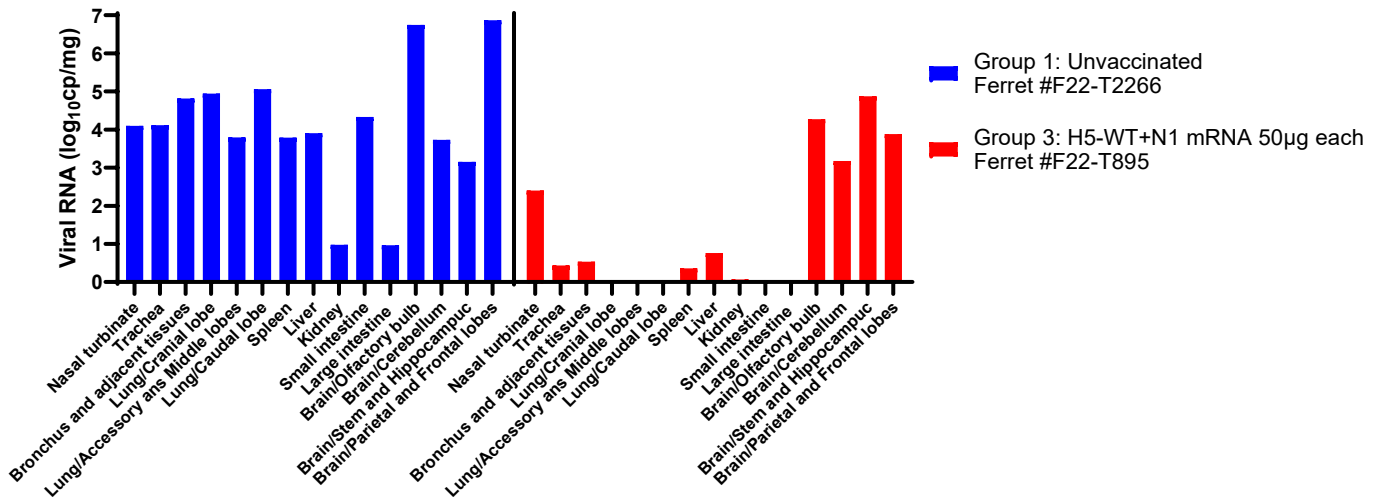


fig. S5. Viral RNAs detection in organ samples from the euthanized ferrets showing neurologic symptoms in the experiment shown in Fig. 2.

Organ samples were collected from the ferrets showing neurologic symptoms in Group 1 and Group 3 which were euthanized on day 5 and 7, respectively, and homogenized. RNA was extracted from the supernatants of their homogenates and the viral RNA copies per mg of tissue (cp/mg) were determined by digital polymerase chain reaction (dPCR).

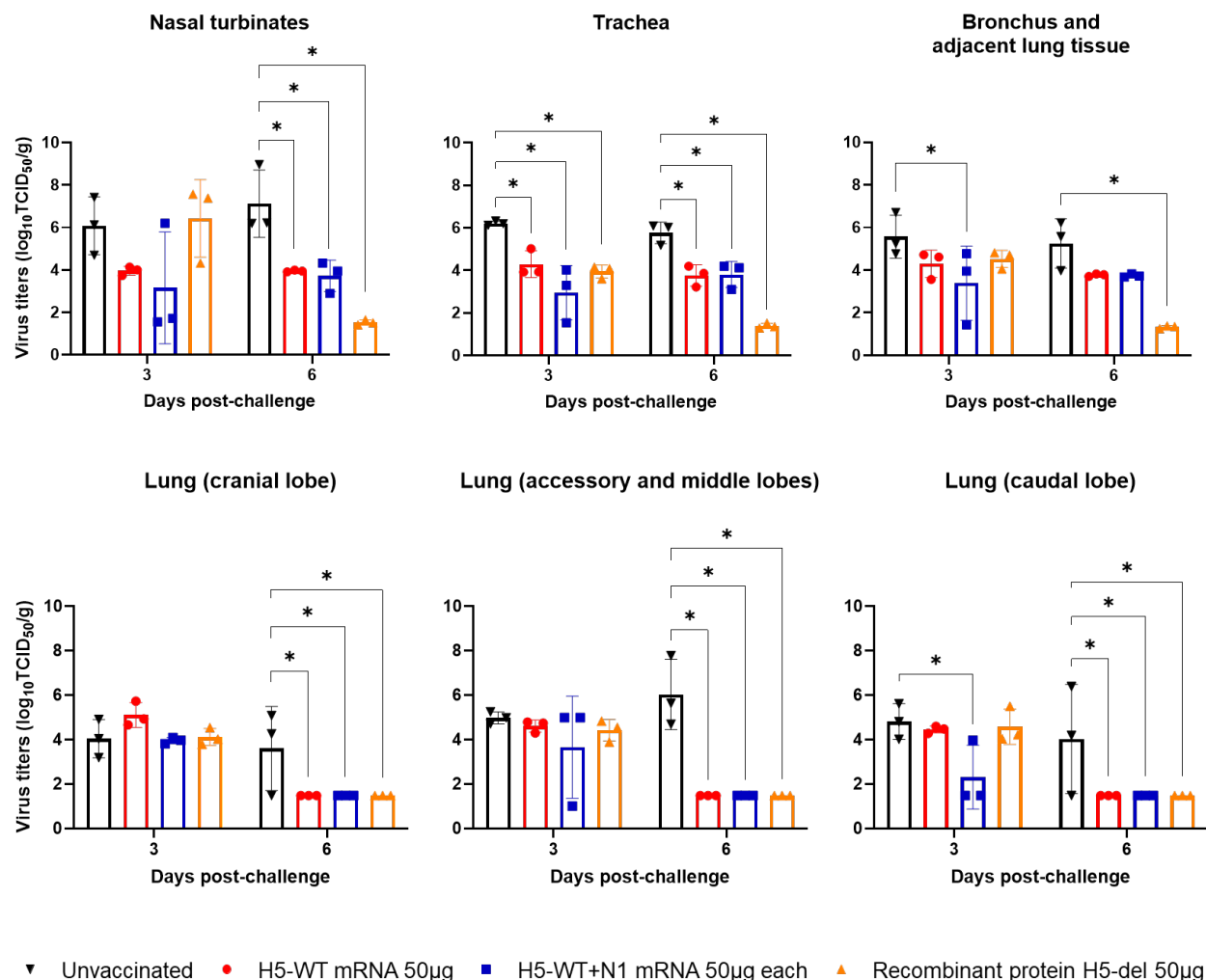


fig. S6. Virus titers in organ samples of infected ferrets in the experiment shown in Fig. 2.

Organ samples were collected from 3 ferrets in each group on days 3 and 6 post-challenge and homogenized. The viral titers in the supernatants of their homogenates were determined on Madin-Darby canine kidney (MDCK) cells by 50% Tissue Culture Infectious Dose (TCID₅₀) assay. The limit of detection is approximately 1.5 $\log_{10}$ TCID₅₀/g but it varies across samples depending on the sample weight used in the assay. Data are presented as geometric mean $\pm$ SD. Viral titers were analyzed by pairwise t-test with Tukey-Kramer's adjustment for multiple comparisons on the log titers. Significance values between vaccinated groups and the unvaccinated groups are indicated by * $P < 0.05$.

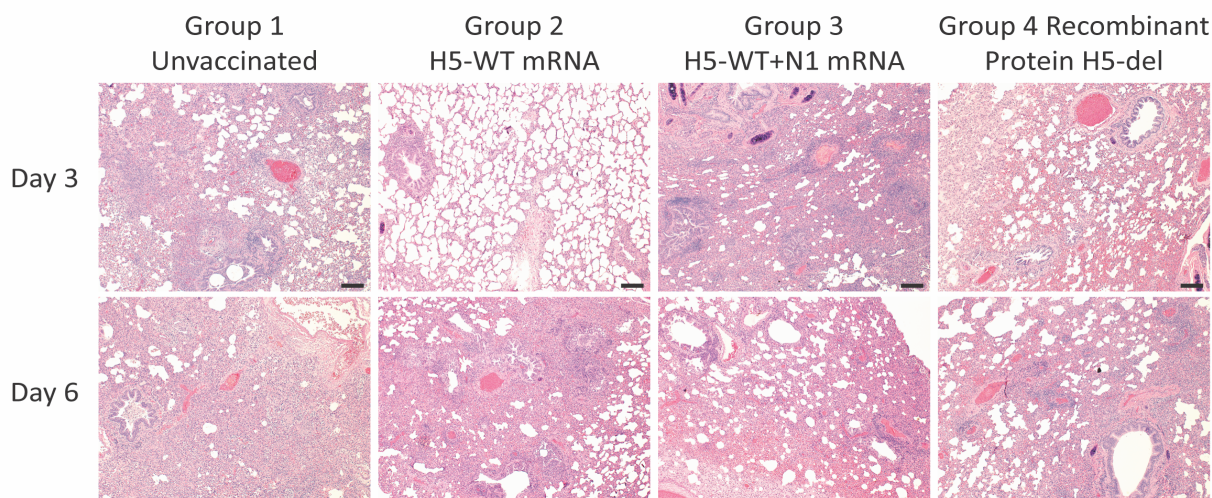


fig. S7. Representative lung histopathology from unvaccinated and vaccinated ferrets on days 3 and 6 post-challenge with A(H5N1).

Lung from an unvaccinated (Group 1) animal show necrotizing and suppurative bronchiolitis at day 3 post-challenge. Lungs from H5-WT (Group 2) and recombinant protein (Group 4) vaccinated animals had no appreciable changes on day 3 post-challenge, whereas lung from a H5-WT + N1 (Group 3) vaccinated animal showed perivascular inflammation. On day 6 post-challenge, lung from an unvaccinated animal (Group 1) showed bronchiointerstitial inflammation and reactive bronchiolar changes, whereas lungs from animals in vaccinated groups (Groups 2 to 4) had only minimal or focal inflammatory changes. Hematoxylin-eosin stain, Original magnifications: x50; scale bar = 200µm.

table S1. Virus titers in brain and intestines. Number of the virus-positive animals/total 3 animals (virus titers in positive animals, log₁₀TCID₅₀/gram) is shown in red.

	Group 1: Unvaccinated		Group 2: H5-WT mRNA 50µg		Group 3: H5-WT + N1 mRNA 50µg each		Group 4: Recombinant protein 50µg	
	Day 3	Day 6	Day 3	Day 6	Day 3	Day 6	Day 3	Day 6
Brain (cerebellum)	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Brain (stem and hippocampus)	0/3	1/3 (5.5)	0/3	0/3	0/3	0/3	0/3	0/3
Brain (parietal and frontal lobes)	0/3	1/3 (4.1)	0/3	0/3	0/3	0/3	0/3	0/3
Brain (olfactory bulb)	0/3	3/3 (>6.1, >6.2, >6.3)	0/3	0/3	1/3 (3.7)	0/3	0/3	1/3 (>6.1)
Small intestine	2/3 (2.9, 3.2)	2/3 (>5.8, >5.9)	0/3	0/3	0/3	0/3	0/3	0/3
Large intestine	1/3 (4.5)	3/3 (4.6, 4.8, >5.8)	0/3	0/3	0/3	0/3	0/3	0/3

Data file S1. Individual-level data.