



Figure S1. Intra-host Single-Nucleotide Variants (iSNV) in A/mink (H5N1) shed by infected donor and contact ferrets during transmission experiments. Shown are the iSNV identified and sequencing coverage across the full viral genome in nasal wash samples at the time of peak viral replication for individual animals. vRNA was isolated from nasal wash samples and subject to next-generation sequencing. Top panel displays iSNVs identified during contact transmission of wild-type A/mink (H5N1). Middle panels show iSNVs identified during airborne transmission study 1 and 2 with wild-type A/mink (H5N1), respectively. Bottom panel displays iSNV identified during airborne transmission of A/mink (H5N1) with PB2 A271T. Individual donor and contact ferrets are represented in each panel by different shades of blue and red, respectively. Non-synonymous mutations are denoted by diamond symbols, and synonymous mutations are denoted by circle symbols.

	Synonymous	PB2 HA	T947C C144T	PB2-315G (S) HA-48L (S)	G L				0.04 (L)			0.04 (G)	
Respiratory Transmission - PB2 A271T	Non- Synonymous	PB2 PB1 HA	G561A T127C A141T	PB2-E187K (NS) PB1-V42A (NS), PB1-F2-S11P (NS) HA-I47L (NS)	E V,S I			0.03 (L)				0.08 (K) 0.03 (A,P)	
	Synonymous	NP	C764T	NP-254D (S)	D					0.05 (D)			

Notes

Viral RNA was isolated from the nasal wash of each animal at the time of peak viral shedding.

Grey shading denotes no transmission to contact

Red font indicates mutations non-synonymous mutations present at a frequency greater than or equal to 5%

* denotes mutation also confers a synonymous change in another gene expressed from this segment.

** denotes mutation also confers a non-synonymous change in another gene expressed from this segment.

Table S2. Maximum change in ferret body temperature during dose de-escalation study.

Group	Ferret Number	Baseline Temp. (°C)	Maximum Temp. (°C)	Change in Temp. (°C) from Baseline	Day (p.i.) of Maximum Temp.
10⁰	1265*	37.9	38.9	1.0	1
	382	37.8	40.3	2.5	6
	1279	39.0	41.1	2.1	3
	1283	37.2	40.1	2.9	7
10¹	1267	37.6	39.7	2.1	4
	1268	38.7	41	2.3	5
	1282	39.0	40.7	1.7	6
	1285	38.6	40.8	2.2	5
10²	1264	38.7	40.7	2.0	6
	1266	38.6	39.8	1.2	3
	1277	39.4	40.6	1.2	4
	1278	38.4	40.6	2.2	3
10³	1256	38.3	40.5	2.2	7
	1258	37.2	39.5	2.3	6
	1270	38.2	41.2	3	5
	1276[#]	38.2	40.2	2	6

Notes: *Did not shed virus or display symptoms, #Shed virus, but did not display symptoms
 The only symptom that FT 1283 in group 10⁰ displayed was fever. The first two ferrets of each group were male and second two were female.

Table S3. Daily clinical scores of ferrets during dose de-escalation study

Group	Ferret Number	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6			Day 7					
		Activity	Respiratory	Other	Activity	Respiratory	Other	Activity	Respiratory	Other	Activity	Respiratory	Other	Activity	Respiratory	Other	Activity	Respiratory	Other	Activity	Respiratory	Other			
10^0	1265	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	382	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
	1279	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1
	1283	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^1	1267	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	2
	1268	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	1282	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1285	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
10^2	1264	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3
	1266	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	1277	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	2	0	0	2
	1278	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
10^3	1256	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	2	0	0
	1258	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	2	1	1	2	1	1	2
	1270	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
	1276	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Group	Ferret Number	Day 8			Day 9			Day 10			Day 11			Day 12			Day 13			Day 14					
		Activity	Respiratory	Other	Activity	Respiratory	Other	Activity	Respiratory	Other	Activity	Respiratory	Other	Activity	Respiratory	Other	Activity	Respiratory	Other	Activity	Respiratory	Other			
10^0	1265	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	382	0	1	2	1	0	2	1	0	2	3	1	0												
	1279	1	0	0	3	0	3																		
	1283	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^1	1267	2	1	2	3	3	3																		
	1268	2	0	3																					
	1282	0	0	0	3	3	3																		
	1285	2	0	0																					
10^2	1264																								
	1266	0	0	2	3	3	3																		
	1277	0	0	2	3	0	3																		
	1278	0	0	2	0	0	2																		
10^3	1256	2	1	0	2	3	2																		
	1258	2	1	2	2	2	3																		
	1270	2	0	0	2	2	3																		
	1276	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Notes: Grey box indicates no clinical score as animal had been euthanized. As described in the "Methods" section, 0 denotes no clinical score and 3 denotes most severe clinical score.

Table S4. Amino acid differences between select H5N1 strains isolated from mink.

Virus Strain	Viral Protein										Collection Date (year-month-day)
	PB2		PB1	PA		NA	NS1			NS2	
	138	598	480	349	716	395	73	155	C-terminus	74	
<i>A/mink/Spain/3691-8_22VIR10586-10/2022 (H5N1)</i>	R	T	K	E	K	A	S	A	-----	E	2022-10-18
<i>A/mink/Spain/22VIR12774-13_3869-2/2022 (H5N1)</i>	R	T	X*	E	<u>N</u>	A	<u>P</u>	A	<u>WRNEMVD</u>	<u>G</u>	2022-10-26
<i>A/mink/Spain/22VIR12774-14_3869-2/2022 (H5N1)</i>	Q	I	K	X*	K	<u>I</u>	S	<u>V</u>	-----	E	2022-10-26

Notes: Italic denotes virus strain used in this manuscript. Sequence alignment of proteins was performed using the 6 sequences published in GISAID for H5N1 viruses recovered during the mink outbreak. Sequence alignment and comparison of amino acid changes were performed using GISAID analyses tools. This table is modified from Maemura et al., 2023²⁶. X* published sequence is ambiguous; however, alignment of the 6 reported virus sequences indicates the consensus amino acids are PB1 480K and PA 349E. Underline indicates amino acids that differ from *A/mink/Spain/3691-8_22VIR10586-10/2022 (H5N1)*.