Additional information

Supplementary Table 1. Total reads, mean coverage, and name of fastq file stored in ENA repository (check data availability section) of all the samples sequenced.

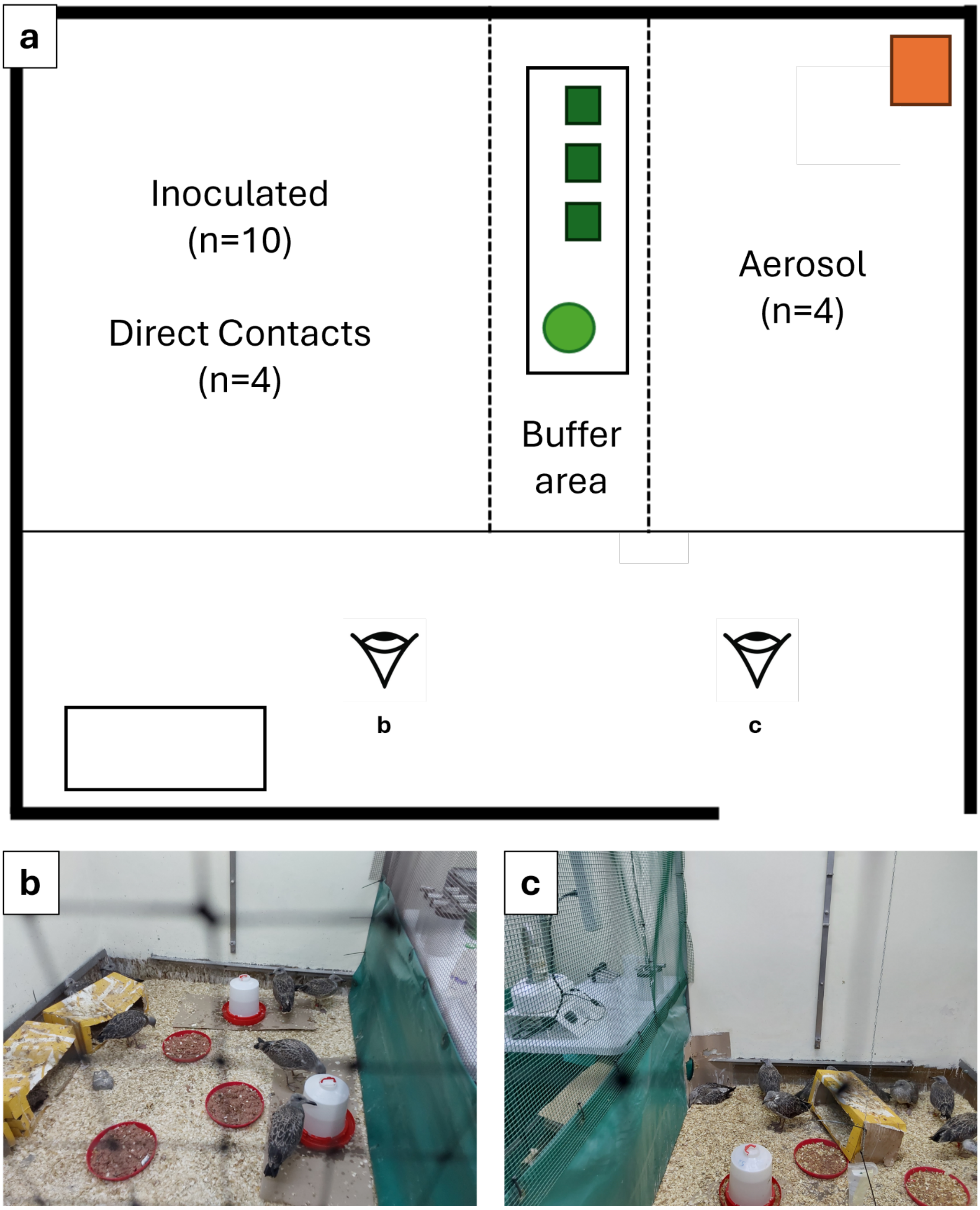
Sample	Total reads	Mean coverage	Fastq file (ENA)
inoculum H5N1/mink	1109994	17073	H5N1_mink_inoc.fastq
inoculum H5N1/gull	2293245	56777	H5N1_gull_inoc.fastq
mink #2 brain	1339928	945	mink2_brain.fastq
mink #4 brain	1460345	16864	mink4_brain.fastq
mink #2 OP	228346	1362	mink2_OPswab_7dpi.fastq
mink #4 OP	310817	3842	mink4_OPswab_7dpi.fastq
mink #19 OP	153002	2694	mink19_OPswab_4dpi.fastq
mink #20 OP	302248	4280	mink20_OPswab_4dpi.fastq
mink #23 OP	345232	984	mink23_OPswab_4dpi.fastq
gull #2B OP	115725	2644	gull2B_OPswab_10dpi.fastq
gull #3B OP	81852	1901	gull3B_OPswab_10dpi.fastq
gull #4B OP	203591	4989	gull4B_OPswab_10dpi.fastq
gull #1Y OP	245257	5622	gull1Y_OPswab_10dpi.fastq
gull #3Y OP	120946	2838	gull3Y_OPswab_10dpi.fastq
gull #4Y OP	102297	2255	gull4Y_OPswab_10dpi.fastq

Supplementary Table 2. Viral antigenic distribution in tissues of minks experimentally infected with H5N1/gull or H5N1/mink H5N1 HPAIVs. Results are expressed as the number of positive minks per total number of minks, and the average detection score in brackets including + (focal/oligofocal), ++ (multifocal), +++ (coalescing to diffuse) For each tissue and group, absence of brackets indicates absence of immunodetection (-). Neg (negative controls); Inoc3 = inoculated euthanised at 3 dpi; Inoc16 = inoculated euthanised at 16 dpi; InocCl = Inoculated and clinically affected minks euthanised at 5 dpi (n=2), 6 dpi (n=1), 7 dpi (n=1); DC: direct contacts; AC= aerosol contacts.

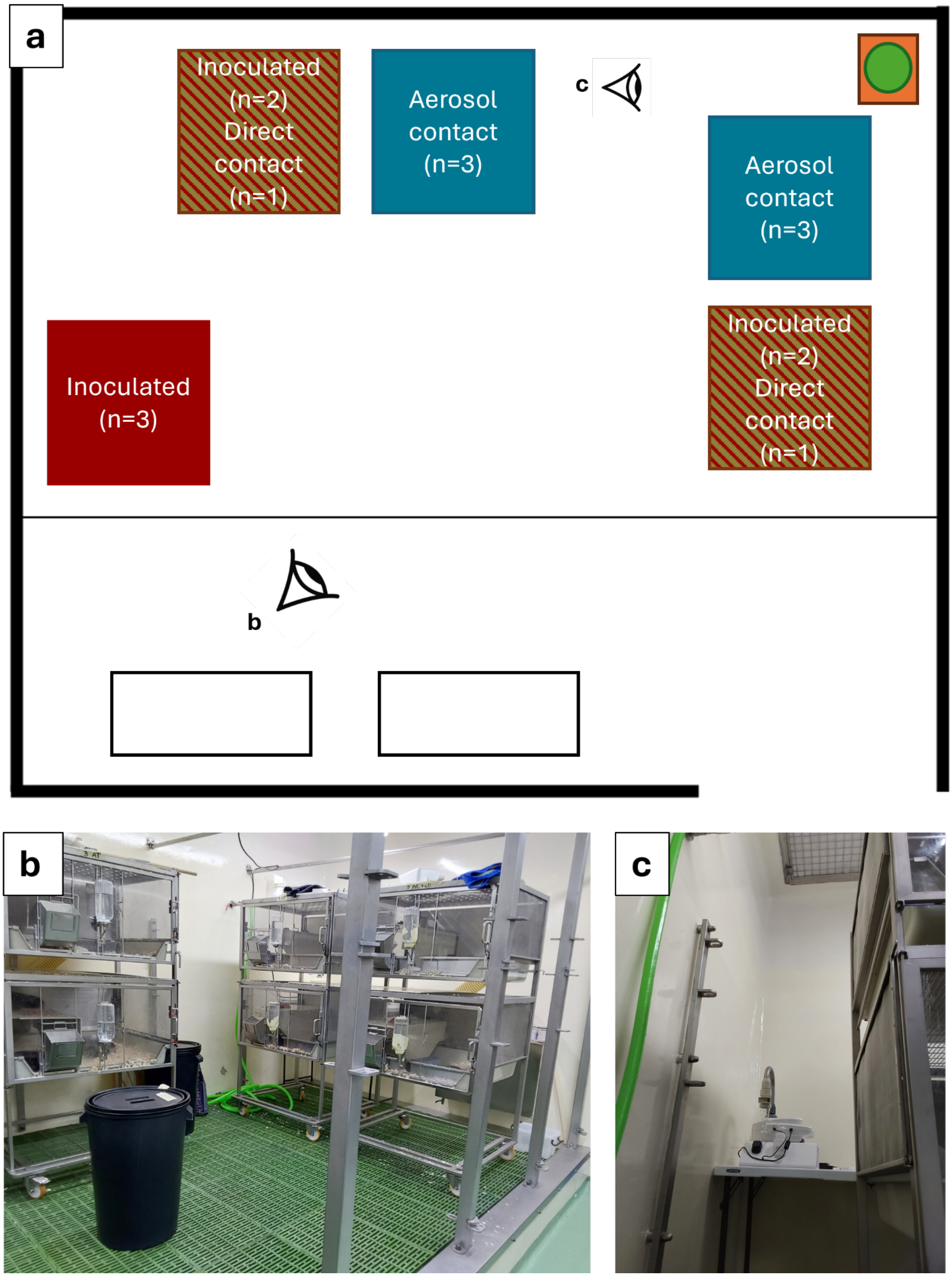
	H5N1/gull					H5N1/mink				
Tissue	Neg	Inoc3	Inoc16	DC16	AC16	Neg	Inoc3	InocCl	DC	AC
Nasal Cavity	0/3	2/3 (+)	0/4	0/2	0/6	0/2	1/3 (+)	4/4 (++)	0/2	0/6
Lung	0/3	0/3	0/4	0/2	0/6	0/2	1/3 (+)	4/4 (++)	0/2	0/6
Brain	0/3	0/3	1/4 (+)	0/2	0/6	0/2	0/3	4/4 (++)	0/2	0/6

Supplementary Table 3. Variants detected in the brain of mink #4 inoculated with the H5N1/gull virus. The table includes the viral genome segment, position (POS), reference (REF) and alternate (ALT) nucleotides, the variant frequency in mink brain (FREQ_mink), total sequencing depth (DEPTH_mink), number of reads supporting the variant (VARIANT_DEPTH_mink), and the corresponding amino acid substitution (PROTEIN_CHANGE_mink).

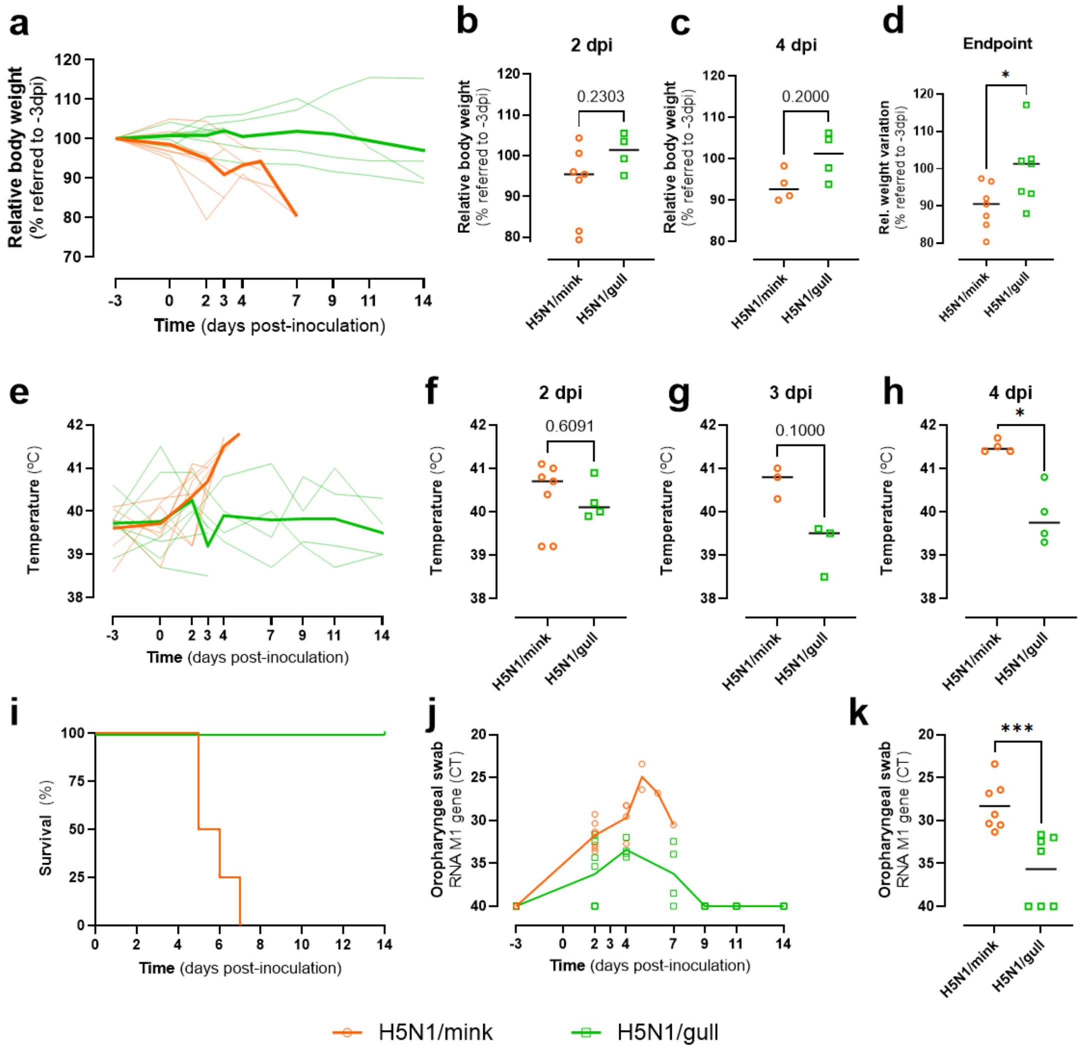
Segment	**POS**	**REF**	**ALT**	**FREQ_ mink**	**DEPTH_ mink**	**VARIANT_DEPTH _mink**	**PROTEIN_CHANGE_ mink**
M	610	G	A	0.9754	17208	16785	E204K
PB2	811	A	G	0.9637	634	611	T271A
HA	878	A	G	0.957	6442	6165	K293R



Supplementary Figure 1. Gull housing and distribution of the experimental room. **a,** Top view schematics. Each of the two experimental rooms (one per virus) had three areas: i) Inoculated and direct contacts (left), which housed 10 inoculated birds and 4 direct contacts; ii) Buffer area (middle), 1-m-wide, where environmental samplers were located (green); and iii) Aerosol contacts (right), which housed 4 birds. Areas were set favouring aerosol transmission, with aerosol contacts being closer to the exhaust (orange). **b,** Inoculated and direct contact area. **c,** Buffer area with the Coriolis µ active air sampler and the Modified Wilson and Cooke passive air samplers (left) and aerosol contacts below the exhaust (right).

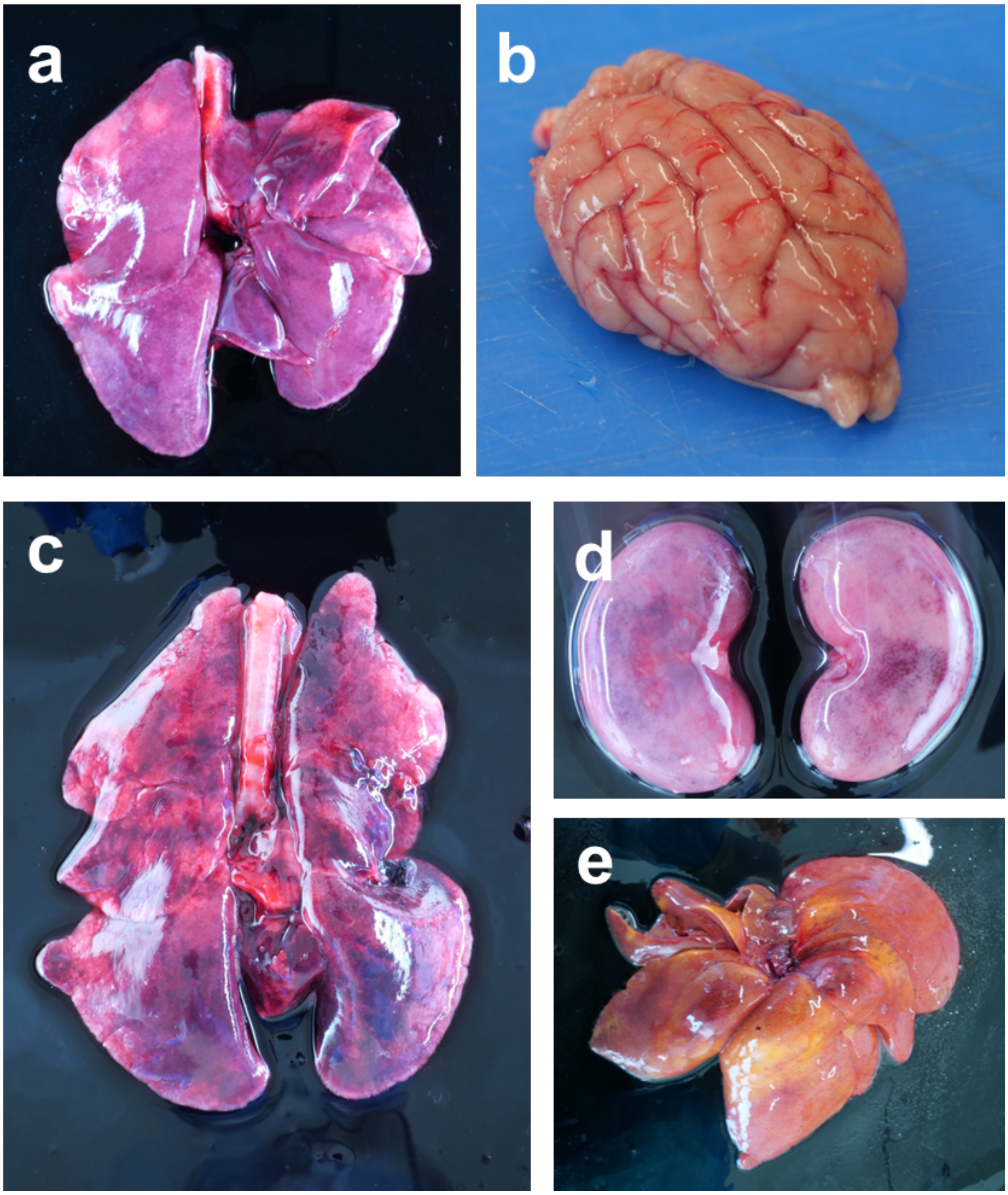


Supplementary Figure 2. Mink housing and distribution in the experimental room. **a,** Top view schematics. Each of the two experimental rooms (one per virus) had two transmission settings. Each transmission setting consisted of two cages: i) Inoculated (n=2) and direct contacts (n=1) (red and gold stripes); ii) Aerosol contacts (n=3) (blue). Cages were set favouring aerosol transmission, with aerosol contacts and the Coriolis µ active air sampler (green) being closer to the exhaust (orange). **b,** Transmission setting. **c,** Location of the Coriolis µ active air sampler below the exhaust.



Supplementary Figure 3. Comparison of mink experimentally inoculated with A/Larus ridibundus/Spain/CR4063/2023 (H5N1/gull, green) and A/Mink/Spain/3691-8_22VIR10586-10/2022 (H5N1/mink, orange). **a,** Follow-up of the body weight variation relative to -3 dpi. **b,** Relative body weight at 2 dpi. **c,** Relative body weight at 4 dpi. **d,** Relative body weight variation at endpoint (humane or experimental: 3-6 dpi for H5N1/mink and 3 or 16 dpi for H5N1/gull). Statistically significant differences were found between groups using a Mann-Whitney unpaired t test (*p<0.05). **e,** Follow-up of rectal temperature. **f,** Rectal temperature at 2 dpi. **g,** Rectal temperature at 3 dpi. **h,** Rectal temperature at 4 dpi.

Statistically significant differences were found between groups using a Mann-Whitney unpaired t test (*$p<0.05$). **i,** Kaplan-Meier survival curve with significant differences found between H5N1/mink and H5N1-gull inoculated animals using a log-rank Mantel-Cox test (*$p<0.05$). **j,** Follow-up of viral RNA load expressed as CT values of M1 gene. **k,** Lowest CT value achieved per animal (2-7 dpi). Statistically significant differences were found between groups using a Mann-Whitney unpaired t test (**$p<0.005$).



Supplementary Figure 4. Gross lesions observed in H5N1/mink-inoculated animals during necropsy. **a**, Lung, within normal limits (uninfected control). **b**, Brain, within normal limits (uninfected control). **c**, Lung, red necrotic foci scattered in parenchyma. **d**, Kidney, red necrotic foci scattered in parenchyma. **e**, Liver, icteric and friable.

Supplementary Data 1. Pairwise nucleotide comparison of original H5N1/gull and H5N1/mink viruses used in experimental infections.

Eight plain-text files (.txt), each corresponding to one genomic segment (HA, NA, PB2, PB1, PA, NP, M, NS). Each file includes alignment length, percentage nucleotide identity, and list of nucleotide differences between A/Larus ridibundus/Spain/CR4063/2023 (EPI_ISL_18983379) and A/Mink/Spain/3691-8_22VIR10586-10/2022 (EPI_ISL_15878539). Data available at Zenodo repository [https://zenodo.org/records/16813242].