nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	ELISA: GloMax Discover GM3000, software 4.0.0, firmware 4.92.0 (Promega) RT-qPCR: Design and Analysis Software v1.5.2 (Thermo Fisher Scientific) Virus neutralisation test: Manual recording of data by experimenter
Data analysis	GraphPad Prism 10 (version 10.1.2)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data are available without restriction in the main text, figures and supplementary files of this publication. The nucleotide sequences of genome segments 4 and 6 of the HPAI isolate A/Pelican/Bern/1/2022 (H5N1) have been deposited at GenBank under the accession numbers PX149233 and PX149234, respectively. Source data are available at Zenodo (https://doi.org/10.5281/zenodo.15753718).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	A power analysis using the online software of Columbia University Medical Center (www.biomath.info) was performed to calculate the appropriate group size for the SPF chicken experiments. For experimental immunisation of zoo birds the sample size was specified by the number of animals present at Basel Zoo and Bern Animal Park.
Data exclusions	One Greater flamingo at Basel Zoo showed extraordinary high virus-neutralizing antibody titers (Fig. 3e). The ND50 values of this outlier were excluded from the statistical analyses. All data collected from any other animal included in this study were taken into account.
Replication	Immunization and challenge experiments with SPF chickens were performed in groups of 8 animals. Virus-neutralization tests were performed with immune sera and virus loads determined in swab samples that were collected from all animals enrolled in this study. The vaccination trial in Swiss zoos was performed with 317 animals representing 23 species with animal group sizes ranging from n = 2 to n = 101. Pre-immune and immune sera were analyzed for all animals in all groups except for the Greater flamingos at Basel Zoo and Bern Animal park. Due to the large sizes of these two groups, only 50% of the vaccinated animals were serologically analyzed. All attempts at replication were successful.

Randomization	SPF chickens were randomly allocated to experimental groups. At Basel Zoo and Bern Animal Park, vaccine groups were specified by the existing bird populations at both sites. Non-vaccinated control groups were formed only for species with large populations such as Greater flamingos. Young animals were preferentially allocated to non-vaccinated control groups in order to allow exchange of these serologically negative animals with other zoos after the experiment has finished.
Blinding	Vaccination and infection of chicken: Investigators involved in clinical recording and RT-qPCR analysis of swab samples were blinded to group allocation. Investigators performing virus-neutralisation tests were not blinded to group allocation. Investigators analysing virus-neutralising antibody titers were blinded. Vaccination of zoo birds: The investigators who performed ELISA and virus-neutralization tests were blinded to the assignment of animals to vaccinated and unvaccinated groups.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Mouse monoclonal antibody directed to the VSV matrix protein (clone 23H12, KeraFast, Boston, MA, cat. no. EB0011; chicken polyclonal antiserum directed to low-pathogenic A/duck/Hokkaido/Vac-1/2004 (H5N1), Gert Zimmer, IVI Mittelhäusern, Switzerland; bovine immune serum from a dairy cow experimentally infected with A/cattle/Texas/063224-24-1/2024 (H5N1) (provided by Martin Beer, FLI, Riems-Greifswald, Germany). The following commercially available secondary antibodies were used: - Goat anti-mouse IgG conjugated with Alexa Fluor-488 (Life Technologies, Zug, Switzerland; cat. no. A28175), - IRDye 800CW donkey anti chicken IgY (LI-COR Biosciences, Bad Homburg, Germany; cat. no. 926-32218), - Goat anti-mouse IgG, cross-adsorbed, conjugated with Alexa Fluor-488 (Life Technologies; cat. no. A11001), - Rabbit anti-bovine IgG conjugated to Rhodamine Red-X (Jackson ImmunoResearch Laboratories; cat. no. 301-295-003).
Validation	The monoclonal antibody against the VSV matrix protein has been validated by indirect immunofluorescence and Western blot analysis (https://www.kerafast.com/productgroup/1011/anti-vsv-m-23h12-antibody). The polyclonal chicken anti-H5N1 serum has been validated by Western blot and virus-neutralisation test (Halbherr et al. 2013, PLoS ONE 8(6): e66059. doi:10.1371/journal.pone.0066059. The bovine anti-H5N1 immune serum has been characterized as described in Halwe et al. (2025) Nature 637, 903-912.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Madin-Darby canine kidney (MDCK, type II) (Georg Herrler, University of Veterinary Medicine, Hannover, Germany) ; Baby hamster kidney (BHK-21) cells (ACC 61, German cell culture collection, DSZM, Braunschweig, Germany); BHK-G43 cells (genetically modified BHK-21 cell clone; Gert Zimmer, IVI, Mittelhäusern, Switzerland); Vero E6 cells (ATCC CRL 1586, provided by Christian Drosten and Marcel Müller, Charite, Berlin, Germany).
Authentication	None of the cell lines used were authenticated by the authors themselves.
Mycoplasma contamination	All cell lines were tested negative for contamination by mycoplasma.
Commonly misidentified lines (See ICLAC register)	Commonly misidentified cell lines were not used in this study.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Chicken (Gallus gallus domesticus), White Leghorn breed, 5-6 weeks old (at the beginning of the vaccination experiment), obtained from the IVI breeding unit.
Wild animals	No wild birds were captured in the field. All birds included in the vaccine trial were captive birds living in the zoo of Basel and Bern Animal Park. Species name and age of the zoo birds are listed in Supplementary Table 2. None of the zoo birds were euthanized at the end of the study.
Reporting on sex	For immunisation and challenge infection of chickens, animals of mixed sex were randomly allocated to the vaccine groups. All zoo bird groups with at least two animals in the group were of mixed sex (see Supplementary Data 1). Data were not disaggregated for sex as impact of sex on vaccination efficacy was not addressed.
Field-collected samples	The study did not involve samples collected from animals in the field.
Ethics oversight	The committee on animal experiments of the canton of Bern, Switzerland, reviewed the experiments, and the cantonal veterinary authority (Amt für Landwirtschaft und Natur LANAT, Veterinärdienst VeD, Bern, Switzerland) approved the study under the authorization numbers BE24/2023 (zoo birds) and BE26/2023 (chickens).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*