

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 |
|--------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of all covariates tested |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> 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 Genetic sequences of influenza A viruses were collected from the NCBI Influenza Virus Database. No custom software or code was used for data collection.

Data analysis Data analysis was performed using MAFFT v7.520 for sequence alignment and IQ-TREE v2.3.6 for phylogenetic reconstruction. PAML package v4.10.9 was used for ancestral state reconstruction to trace the evolutionary trajectory of the NP mutation quartet. Statistical analysis was performed using GraphPad Prism 8. No custom code was used.

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](#)

Provide your data availability statement here.

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	Not applicable. This study did not involve human participants, human data, or human biological material.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable. This study did not involve human participants, human data, or human biological material.
Population characteristics	Not applicable. This study did not involve human participants, human data, or human biological material.
Recruitment	Not applicable. This study did not involve human participants, human data, or human biological material.
Ethics oversight	Not applicable. This study did not involve human participants, human data, or human biological material.

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](https://www.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	No statistical methods were used to predetermine sample size. The sample size (n=4) per group was chosen based on previous literature on H3N2 canine influenza virus infection studies and the 3Rs principle (Replacement, Reduction, and Refinement) to minimize the use of large animals while maintaining statistical relevance. Based on our experience, this sample size is sufficient to detect significant differences in viral shedding titers and clinical scores between the wild-type and mutant groups.
Data exclusions	No data were excluded from the analyses; all collected data points were included in the final statistical evaluations.
Replication	All attempts at replication were successful. Each in vitro experiment was performed with three independent biological replicates. For in vivo studies, four animals were used per group to ensure the consistency of the findings. All key experimental results were consistently reproduced across independent trials.
Randomization	Animals (beagle dogs and ducks) were randomly allocated into experimental groups.
Blinding	Investigators were blinded to group allocation during data collection and analysis.

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	Involvement in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	The following antibodies were used: NP (GTX125989, GeneTex), GAPDH (GB15004-100, Servicebio), DYKDDDDK tag (20543-1-AP, Proteintech), and Mouse IgG (A7028, Beyotime).
Validation	All primary antibodies were validated by the manufacturers for WB and IP. Their specificity was further confirmed in-house using protein-overexpressing cells, showing consistent and specific target detection.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	HEK 293T (CRL-3216) and MDCK (CCL-34) were purchased from ATCC. The ANP32A/B/E knockout 293T cell line was kindly provided by Prof. Xiaojun Wang from Harbin Veterinary Research Institute.
Authentication	The HEK 293T and MDCK cell lines were authenticated by the manufacturer (ATCC). The ANP32A/B/E knockout 293T cell line was authenticated in-house by Western blot analysis, which confirmed the successful knockout of the target proteins.
Mycoplasma contamination	All cell lines were tested for mycoplasma contamination and tested negative. Testing was performed using a PCR-based assay to ensure the absence of mycoplasma throughout the study.
Commonly misidentified lines (See ICLAC register)	None of the cell lines used in this study are listed in the ICLAC database of commonly misidentified cell lines.

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	Experimental animals included 10-week-old beagle dogs and 3-week-old domestic ducks.
Wild animals	This study did not involve wild animals. All animals were commercially bred and provided by professional animal supply companies, and transported to our facility following standard biosafety and animal welfare protocols.
Reporting on sex	Sex was considered during the study design, and appropriate methods were used to assign sex and ensure balance between groups. Our results confirmed that the host range of H3N2 CIV—specifically its ability to infect dogs but not ducks—was consistent across both sexes. No sex-linked differences were observed in viral susceptibility or shedding, indicating that the observed infection patterns are independent of host sex.
Field-collected samples	Animals were housed with ad libitum access to food and water under a controlled environment (temperature maintained at 26°C with appropriate humidity) and a natural light/dark cycle. At the conclusion of the study, all animals were humanely euthanized. Strict decontamination and sterilization procedures were followed to prevent any potential viral leakage.
Ethics oversight	All animal experiments were approved by the Experimental Animal Welfare Ethics Committee of South China Agricultural University (approval number: 2023c030).

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

Plants

Seed stocks	The study did not involve plants; therefore, no relevant information is provided.
Novel plant genotypes	The study did not involve plants; therefore, no relevant information is provided.
Authentication	The study did not involve plants; therefore, no relevant information is provided.