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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

For the three main figures: figure 1 calculated all sick pigs or used more than five samples in epidemiology, or used three animals in tissue distribution (meets the minimal statistical requirements). Figure 2 used most of the representative CoV genomes thus should be adequate. For all other tables or figures that sample size involved, we used more than three samples per group. For animal experiments, we used at least five animal per group.

2. Data exclusions

Describe any data exclusions.

No data exclusion.

3. Replication

Describe whether the experimental findings were reliably reproduced.

As epidemiology study, we presented all results including positive or negative here. The authors guarantee the findings are reliably reproducible. At least three independent experiments were performed, which was stated in the text.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Animals were randomly assigned to groups prior to any experimentation.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

SADS-CoV histology was performed in a blinded manner.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☒	☐	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☒	☐	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☒	☐	The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	BLAST+ v2.2.3, CLC Genomic Workbench v9.0, Clone Manager v8, MAFFT v7.221, MrBayes v3.2, DIAMOND v0.9.0, BaTS beta-build2, PopART v1.7, RAxML v8.2.11, TempEst v1.5, RDP v4.72.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	There is no restriction to material availability.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	1, rabbit anti-HKU2-NP polyclonal antibody, made by ourselves, validated by immunogen in a WB (titer 1: 10000); 2, anti-HIS tag monoclonal antibody (Proteintect Group), validated in a WB (titer 1: 1000); 3, anti-S tag monoclonal antibody, made by ourselves, validated in a WB (titer 1: 10000); 4, cyanin 3-labeled goat anti-mouse/rabbit IgG (Proteintech Group), validated in IFA (titer 1: 1000); 5, mouse anti-FLAG tag antibody (Thermo Fisher Scientific), validated in a WB (titer 1: 1000); 6, mouse anti-Cytokeratin 8+18+19 mAb (Abcam), validated in IHC (1:100); 7, FITC conjugated goat-anti-rabbit IgG (Proteintech), validated in IHC (1:100);

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	1, African green monkey origin, Vero from ATCC; 2, bat origin Rhinolophus sinicus (made by ourselves), Kidney primary RsKi9409, lung primary RsLu4323, lung immortalized RsLuT, brain immortalized RsBrT and heart immortalized RsHeT; all bats were made in house; 3, Swine cells: intestinal IPEC and SIEC, kidney PK15, LLC-PK1 and IBRS, testes cell ST; all swine cells were from ATCC; 4, human cells: Hela and HEK293T were from ATCC.
b. Describe the method of cell line authentication used.	All monkey and human cells were from ATCC with authentication. Swine cells (commercially available) were gifts of collaborators and were originally from ATCC with authentication. They were authentication by microscope observation during culture. Bat cells made by ourselves were from organ or cultured and immortalized. We guarantee they were from the organs described but there was no further authentication.
c. Report whether the cell lines were tested for mycoplasma contamination.	We confirm that all cells were tested as mycoplasma negative.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	None of the cell lines used are listed in the ICLAC database.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	Swine used in animal infection study aged between 2-4 days. The first experiment used healthy Chinese Bamaxiang SPF piglets that were cultured free of SADS-CoV or other known swine disease agents. The second experiment used healthy duroc-landrace-yokshire piglets (not SPF) that were not affected by SADS-CoV before. No gender preference when choose the animal. Piglets were from same breed and at same age and were randomly assigned into groups for the experiments.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	Pig farm workers were bleed for testing possible spillover of SADS-CoV. These workers are also adult male who had close contact with sick pigs. Non of them had clinical signs of diseases during sampling.