

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 MODELLER v. 10.1 (<https://salilab.org/modeller/>) and GROMACS v. 2020.4 (<http://www.gromacs.org>) were used for MD simulations.

Data analysis XDS (v. feb 5 2021), CCP4 (v 7.0), COOT (v. 0.8.6) and PHENIX (v. 1.19.2-4158) softwares were used for X-ray diffraction data processing, model building and refinement of BANAL 236 RBD-hACE2 complex. PyMOL (v 2.4.2) was used for structural image rendering.

PLUMED v. 2.7 (<https://www.plumed.org>); ROSETTA v. 3.11 (<https://www.rosettacommons.org/>); FoldX v. 4 (<http://foldxsuite.crg.eu/>); MDAnalysis v. 1.0.0 (<https://www.mdanalysis.org/>); MDTraj v. 1.9.5 (<https://www.mdtraj.org/1.9.5/index.html>); and in-house scripts available at <https://github.com/maxbonomi/bat-MD> were used for MD simulations.

hyphy 2.5.31; PhyML & MAFFT implemented through NGPphylogeny (<https://ngpphylogeny.fr/>); Simplot 3.5.1 were used for phylogenetic and recombination analyses.

MSSPE 1 was used for Betacoronavirus primer enrichment design.

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](#)

The sequences generated through the study were deposited into the GISAID database (<https://www.gisaid.org/>) under the accessions EPI_ISL_4302644 - 48. Raw reads used to assemble the genomes were deposited onto SRA database (BioProject PRJNA796968 : BioSamples SAMN24959173-77).

The crystal structure presented in this manuscript was deposited in the Protein Data Bank (PDB) with accession code 7PKI.

The GROMACS topology and input files as well as the analysis scripts used are freely available on PLUMED-NEST (www.plumed-nest.org) under accession ID plumID:21.037

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)

Ecological, evolutionary & environmental sciences study design

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

Study description	description of the fecal virome of bats from Laos, with a focus on sarbecoviruses
Research sample	A total of 645 bats belonging to six families and 46 species were captured . Two hundred and forty-seven blood samples, 608 saliva, 539 anal/feces, and 157 urine swabs were collected from the northern part of Laos
Sampling strategy	No calculation of sample size
Data collection	A total of 645 bats belonging to six families and 46 species were captured. Bats were collected using four-banks harp traps and mist nets. Captured bats were held individually in cloth bags and transport in polystyrene foam box to field station for ID and sampling. After samples were taken, bats were marked with wing bands for individual identification and released at the captured point within 12 hours. Those infected with a sarbecovirus were all from the Fueng district in Vientiane Province
Timing and spatial scale	First quarter 2021
Data exclusions	no
Reproducibility	without object
Randomization	without object
Blinding	without object
Did the study involve field work?	☒ Yes ☐ No

Field work, collection and transport

Field conditions	All samples were stored at -20°C in the field using portable mini freezer (-20°C). The samples were then transported with dry ice to IPL and stored at -80°C till laboratory analysis.
Location	The GPS coordinates of sampling are provided below 18° 32.879'N 101° 58.938'E 18° 32.914'N 101° 58.459'E 18° 34.018'N 101° 58.338'E 18° 31.743'N 101° 58.648'E 18° 30.495'N 101° 57.421'E 18° 54.426'N 101° 58.789'E 18° 54.898'N 101° 59.048'E 18° 53.717'N 101° 56.957'E

18° 54.787'N 101° 58.292'E
 18° 55.386'N 101° 57.723'E
 20° 43.071'N 101° 45.811'E
 20° 43.111'N 101° 46.577'E
 20° 41.647'N 101° 47.179'E
 20° 44.143'N 101° 46.770'E
 20° 52.396'N 101° 46.973'E
 20° 51.907'N 101° 46.926'E

Access & import/export	The bat study was approved by the wildlife authorities of the Department of Forest Resource Management (DFRM), and the Ministry of Agriculture and Forestry, Lao PDR, No. 2493/DFRM, issued on May 21, 2020; No. 0755/MAF issued on June 2, 2020. Importation in France was approved (import permit 2021-75-13530)
Disturbance	no disturbance

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
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	GnTI- Expi293™ cell lines for recombinant protein production were obtained from ThermoFisher Scientific. Calu-3 (ATCC HTB-55), Caco-2 (ATCC HTB-37), 293T (ATCC CRL-3216), VeroE6 (ATCC CRL-1586) were obtained from ATCC. 293T-ACE2 were developed at Institut Pasteur and described in PMID: 33259646
Authentication	Cells were authenticated by the provider
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	The study did not involve samples from laboratory animals.
Wild animals	A total of 645 bats belonging to six families and 46 species were captured. Bats were collected using four-banks harp traps and mist nets. Captured bats were held individually in cloth bags and transport in polystyrene foam box to field station for ID and sampling. After samples were taken, bats were marked with wing bands for individual identification and released at the captured point within 12 hours.
Field-collected samples	All samples were stored at -20°C in the field using portable mini freezer (-20°C). The samples were then transported with dry ice to IPL and stored at -80°C till laboratory analysis.
Ethics oversight	The bat study was approved by the wildlife authorities of the Department of Forest Resource Management (DFRM), and the Ministry of Agriculture and Forestry, Lao PDR, No. 2493/DFRM, issued on May 21, 2020; No. 0755/MAF issued on June 2, 2020.

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

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	Serum samples were previously tested by ELISA and seroneutralization The study protocol was approved by the Lao National Ethics Committee for Health Research (NECHR) (Ref #052/2020). Oral and written informed consent was obtained from people aged 15 years and older. Parental consent was taken for children aged between 5 and 12 years, and assent from children aged between 12 and 14 years in addition to parental consent, before the survey
Recruitment	Sera were already available annd selected based on their status regarding seroneutralization of SARS-CoV-2
Ethics oversight	Lao National Ethics Committee for Health Research (NECHR) (Ref #052/2020)

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