

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 checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested |
| <input checked="" type="checkbox"/> | <input 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 checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input checked="" type="checkbox"/> | <input 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

Data analysis

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](#)

New sequences have been deposited in GenBank under accession numbers OQ759652-OQ760076 listed in Supplementary Data 1. Input files used for the phylogenetic and mutational profile analyses are available at <https://doi.org/10.6084/m9.figshare.22335331.v2>.

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	Patients sex data (female or male) were collected from self-report forms filled by patients upon sample collection at local public laboratories. Research team was not able to access patients for consent collection, and such limitation was described in the study protocol reviewed and approved by Research Ethics Committee. 246 participants were reported as female, while 179 were reported as male. Descriptive statistics (mean, median, standard deviation) and Mann-Whitney U test on median years of age with a significance level $\alpha = 0.05$ were performed using participants sex data.
Reporting on race, ethnicity, or other socially relevant groupings	Socially constructed variables were not reported in this study since this information was not available.
Population characteristics	Patients' mean age upon sample collection was similar for both females and males (female= mean 39.10 SD \pm 18.83; male= mean 39.37 SD \pm 22.03), with 57.88% (n=246) of the participants identified as female. The clinical status of patients at the time of sample collection, and travel history data were not available for these samples.
Recruitment	There was no specific criteria for patient recruitment. Samples were obtained from patients that actively sought medical care from local clinics after presenting symptoms such as fever and myalgia.
Ethics oversight	Study protocol was reviewed and approved by Research Ethics Committee of the Universidade Federal de Minas Gerais with approval No. 32912820.6.1001.5149.

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	Nanopore sequencing was performed on 425 selected CHIKV-positive samples provided by Brazilian Central Public Health Laboratories (LACEN) from 12 states across 4 geographic regions (Northeast, Midwest, Southeast, and South) of Brazil during the years 2021-2022. No sample size calculation was performed because these samples are residual samples (serum or plasma) available during an epidemic and they were obtained from the epidemiological surveillance routine from local Laboratories (LACEN). This sample size is sufficient for this study as it corresponds to more than twice the amount of sequences available at NCBI from Brazil. Sample size-related study limitations that might apply have already been addressed in the manuscript.
Data exclusions	CHIKV RT-qPCR positive samples were selected for sequencing based on the cycle threshold value equal or greater than 30. Samples with Ct value less than 30 were not processed. Sequences with viral genome coverage less than 60% were excluded from the phylogenetic analyses and previously discussed by Thézé et al. 2018 (full citation in the manuscript). Samples 736.22_RED, FS0116, and FS0132 were discarded due to low genome coverage.
Replication	Bayesian analysis of molecular sequences using MCMC was performed in duplicate, with each run of 200 million states. The convergence of MCMC chains was checked using Tracer software.
Randomization	Access to participants was not possible since positive samples were obtained from the epidemiological surveillance routine of local public Laboratories. Allocation of participants/samples to experimental groups is not relevant to this study since this work aimed to characterize viral isolates from all positive samples with Ct value equal or greater than 30 and genome coverage greater than 60%.

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 checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input 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