

## 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

NA

Data analysis

Our M. tuberculosis variant identification pipeline is available at [https://github.com/ksw9/mtb\\_pipeline](https://github.com/ksw9/mtb_pipeline) (v1).

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](#)

Raw sequence data generated in this study have been deposited in the Sequence Read Archive under accession PRJNA870648 [<https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA870648>]. The H37Rv reference genome is available on NCBI under accession NC\_000962.3 [[https://www.ncbi.nlm.nih.gov/nucleotide/NC\\_000962.3](https://www.ncbi.nlm.nih.gov/nucleotide/NC_000962.3)]. The phylogenetic trees and results of ancestral state reconstruction and all other information displayed in figures are provided in the Source Data files.

## Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender	Sex was not considered in study design; it was determined through self-report. Of the 488 individual participants, 394 were men and 94 were women, reflecting that recruitment focused on prisons. In 2022, the incarcerated population in Paraguay was 95% male.
Population characteristics	Median age of study participants was 31 (IQR: 24-44). Of the samples passing filters, 158 were from individuals diagnosed with TB while in prison and 330 were from people diagnosed in the community. TB isolates were collected in Asunción (274/488) and in Ciudad del Este (214/488).
Recruitment	Sputum samples are routinely collected from all individuals presenting with symptoms of TB at primary health clinics and sent to the National Program for Tuberculosis Control (NPTC) of Paraguay reference laboratory for microbial diagnostics including culture and smear microscopy. We sequenced all available culture-positive isolates from these three departments. Study recruitment was done by study staff who visited patients at home and in prisons at the time patients began treatment (Directly Observed Therapy, DOT). At this time, the standard National TB Control Program questionnaire was conducted and patients who chose to enroll provided written consent for sequencing residual mycobacterial cultures for culture-positive samples. Study staff also collected additional demographic, clinical, residential, and epidemiological data, including information on history of prior or current incarceration with a structured questionnaire. No compensation was provided to study participants. It is possible that participants differed from non-participants though it is difficult to know the impact of this on reported results.
Ethics oversight	This research complies with all ethical regulations. The study was approved by the ethics committee of the Central Laboratory of Public Health of the Ministry of Health and Social Welfare of Paraguay (International Certification FWA N° FWA00020088) with code CEI-LCSP 91/010217. Informed consent was provided.

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://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	We did not conduct a sample size calculation. In this prospective genomic surveillance study, we included all M. tuberculosis genomes available and that met quality thresholds from cases diagnosed in the two study cities and in the study period. Our goal was to describe M. tuberculosis genomic diversity.
Data exclusions	We excluded M. tuberculosis isolates with evidence of mixed infections, as these can bias phylogenetic inferences. We excluded samples with less than 25X median coverage or with less than 80% of the M. tuberculosis genome covered by at least 10X coverage.
Replication	We did not conduct replication of sequencing. We obtained relatively high coverage of the M. tuberculosis genome.
Randomization	We did not conduct randomization. We did not conduct a clinical trial or test an intervention; our goal was to characterize M. tuberculosis diversity.
Blinding	We did not conduct blinding. We did not conduct a clinical trial or test an intervention; our goal was to characterize M. tuberculosis diversity.

## Behavioural & social sciences study design

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

Study description	Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).
Research sample	State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic

Research sample	<i>information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.</i>
Sampling strategy	<i>Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.</i>
Data collection	<i>Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.</i>
Timing	<i>Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.</i>
Data exclusions	<i>If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.</i>
Non-participation	<i>State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.</i>
Randomization	<i>If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.</i>

## Ecological, evolutionary & environmental sciences study design

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

Study description	<i>Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.</i>
Research sample	<i>Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i>, all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.</i>
Sampling strategy	<i>Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.</i>
Data collection	<i>Describe the data collection procedure, including who recorded the data and how.</i>
Timing and spatial scale	<i>Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken</i>
Data exclusions	<i>If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.</i>
Reproducibility	<i>Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.</i>
Randomization	<i>Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.</i>
Blinding	<i>Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.</i>

Did the study involve field work?  Yes  No

## Field work, collection and transport

Field conditions	<i>Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).</i>
Location	<i>State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).</i>
Access & import/export	<i>Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in</i>

Access & import/export	<i>compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).</i>
Disturbance	<i>Describe any disturbance caused by the study and how it was minimized.</i>

## 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
<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

### Methods

n/a	Involved 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	<i>Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.</i>
Validation	<i>Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.</i>

## Eukaryotic cell lines

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

Cell line source(s)	<i>State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.</i>
Authentication	<i>Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.</i>
Mycoplasma contamination	<i>Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.</i>
Commonly misidentified lines (See <a href="#">ICLAC</a> register)	<i>Name any commonly misidentified cell lines used in the study and provide a rationale for their use.</i>

## Palaeontology and Archaeology

Specimen provenance	<i>Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.</i>
Specimen deposition	<i>Indicate where the specimens have been deposited to permit free access by other researchers.</i>
Dating methods	<i>If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.</i>
<input type="checkbox"/>	Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.
Ethics oversight	<i>Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.</i>

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

## 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	<i>For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.</i>
Wild animals	<i>Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.</i>
Reporting on sex	<i>Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.</i>
Field-collected samples	<i>For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.</i>
Ethics oversight	<i>Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.</i>

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

## Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	<i>Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.</i>
Study protocol	<i>Note where the full trial protocol can be accessed OR if not available, explain why.</i>
Data collection	<i>Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.</i>
Outcomes	<i>Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.</i>

## Dual use research of concern

Policy information about [dual use research of concern](#)

### Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Public health
<input checked="" type="checkbox"/>	<input type="checkbox"/>	National security
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Crops and/or livestock
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Ecosystems
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Any other significant area

## Experiments of concern

Does the work involve any of these experiments of concern:

- | No                                  | Yes                      |   |
|-------------------------------------|--------------------------|---|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Demonstrate how to render a vaccine ineffective                             |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Confer resistance to therapeutically useful antibiotics or antiviral agents |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Enhance the virulence of a pathogen or render a nonpathogen virulent        |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Increase transmissibility of a pathogen                                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Alter the host range of a pathogen  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Enable evasion of diagnostic/detection modalities                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Enable the weaponization of a biological agent or toxin                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Any other potentially harmful combination of experiments and agents         |

## ChIP-seq

### Data deposition

- Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

*May remain private before publication.*

*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*

Files in database submission

*Provide a list of all files available in the database submission.*

Genome browser session

(e.g. [UCSC](#))

*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

### Methodology

Replicates

*Describe the experimental replicates, specifying number, type and replicate agreement.*

Sequencing depth

*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*

Antibodies

*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*

Peak calling parameters

*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*

Data quality

*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*

Software

*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

## Flow Cytometry

### Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

### Methodology

Sample preparation

*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*

Instrument

*Identify the instrument used for data collection, specifying make and model number.*

- Software *Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
- Cell population abundance *Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
- Gating strategy *Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*
- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

## Magnetic resonance imaging

### Experimental design

- Design type *Indicate task or resting state; event-related or block design.*
- Design specifications *Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
- Behavioral performance measures *State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

### Acquisition

- Imaging type(s) *Specify: functional, structural, diffusion, perfusion.*
- Field strength *Specify in Tesla*
- Sequence & imaging parameters *Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
- Area of acquisition *State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*
- Diffusion MRI  Used  Not used

### Preprocessing

- Preprocessing software *Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
- Normalization *If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
- Normalization template *Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*
- Noise and artifact removal *Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*
- Volume censoring *Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

### Statistical modeling & inference

- Model type and settings *Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*
- Effect(s) tested *Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*
- Specify type of analysis:  Whole brain  ROI-based  Both
- Statistic type for inference (See [Eklund et al. 2016](#)) *Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*
- Correction *Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

## Models & analysis

- n/a | Involved in the study
- Functional and/or effective connectivity
  - Graph analysis
  - Multivariate modeling or predictive analysis

Functional and/or effective connectivity

*Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*

Graph analysis

*Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*

Multivariate modeling and predictive analysis

*Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*