nature portfolio

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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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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	Cor	firmed
	\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\boxtimes	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	\boxtimes	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.
	\boxtimes	A description of all covariates tested
	\square	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	\boxtimes	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)
	\boxtimes	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.
\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
	\square	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 collectionABI 7500 Real-Time PCR systems software v2.3 was used for qPCR quantification data collection.
Bio-Plex Manager Software 6.1 was used for antibody data collection.
EM images were collected using a Tecnai Spirit BioTwin and a CMOS camera Phurona, Radius software, version 2.1, EMSIS.Data analysisMicrosoft Excel 16.0 was used for data collection (output data). Statistical analysis were done using the software R (v4.1.2) and the packages dplyr, rstatix, Rmisc and dunn.test. Visualization were done using the package ggplot2 in R and the comercially available software Adobe Illustrator and Adobe Photoshop CS6 (64 bit). For genome assembly, Trimmomatic, ClipAndMerge and Geneious Prime 2021.2.2 were used. For histological image analysis, ImageJ 1.52a 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 <u>data availability statement</u>. 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

Source data are provided with this paper. The main data supporting the results in this study are available within the main manuscript and the supplementary materials. The accession codes for the GenBank sequences used for the genomic analyses herein, are: MG572232.1, DQ217792.1. The myBaits Hybridization Capture for Targeted NGS protocol covers the following GenBank sequences: KC242801, KC242783, NC_004161, NC_014372, KC545395 and FJ750956. The resulting raw aligned bam files are available from the Genbank Sequence Read Archive (SRA) via the accession number PRJNA1001398. The BioSamples individual accession codes are: SAMN36810537, SAMN36810535, SAMN36810534, SAMN36810533, SAMN36810532, SAMN36810531, SAMN36810530, SAMN36810529, SAMN36810528, SAMN36810527, SAMN36810526 and SAMN36810525. The individual accession codes are also included in the main manuscript.

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Life sciences study design

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

Sample size

Since wild-caught animals with large individual variance and imprecise age determination must be used for this experiment, the number of animals per experimental group would have to be very high for a valid statistical evaluation. Therefore, we compromised sample size and included a minimum of 3 animals per variable (time points) within cohorts (6 animals per group). This was not possible for 2 groups. At least 2 technical replicates were included for downstream analyses. The comparison between the number of infected and non-infected animals was planned to be statistically analysed using Fisher's exact test, and the comparison of viral load in the organs was planned to use two way repeated measures ANOVA. In accordance with the planned statistical analyses, the initial sample size estimation per group was as following:

Ecological, evolutionary & environmental sciences

GPower Results of Power Analysis from SPSS F tests - ANOVA: Repeated measures, between factors Analysis: A priori: Compute required sample size Input: Effect size f=1.5 α err prob=0.05 Power (1- β err prob)=0.8 Number of groups=2 Number of measurements=3 Corr among rep measures=0.5 Output: Noncentrality parameter λ =20.2500000 Critical F=7.7086474 Numerator df=1.000000 Denominator df=4 .000000 Total sample size=6 Actual power=0.9119762

Data exclusions	No data was excluded in this study		
Replication	All attempts at replication were successful. All samples were run with technical duplicates, expect for the clinical biochemistry (insufficient sample) . A fraction of RNA extraction samples, randomly assigned from each virus cohort, were re-quantified by qPCR (also using technical duplicates).		
Randomization	Animals were randomly assigned to groups separately by sex, to allocate at least one female per cohort.		
Blinding	Investigators were not blinded for the following analyses (clinical observations, clinical measurements and collection of shedding samples). Blinding is not possible to follow up clinical progresion of individual animals and due to safety protocols within the BSL4. Analysis of samples was not blinded. This was deemed unnecesary for sample processing (e.g. qPCR, serology and titration) for the data collection and analyses.		

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 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.
Sampling strategy	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.
Data collection	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.
Timing	Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.
Data exclusions	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.
Non-participation	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.
Randomization	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.

Ecological, evolutionary & environmental sciences study design

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

Study description	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.
Research sample	Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi 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.
Sampling strategy	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.
Data collection	Describe the data collection procedure, including who recorded the data and how.
Timing and spatial scale	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
Data exclusions	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.

Reproducibility	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.
Randomization	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.
Blinding	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.

Did the study involve field work?

Yes No

Field work, collection and transport

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

Reporting for specific materials, systems and methods

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	n/a	Involved in the study
	Antibodies	\boxtimes	ChIP-seq
	Eukaryotic cell lines	\boxtimes	Flow cytometry
\boxtimes	Palaeontology and archaeology	\boxtimes	MRI-based neuroimaging
	Animals and other organisms		•
\boxtimes	Clinical data		
\boxtimes	Dual use research of concern		
\boxtimes	Plants		

Antibodies

Antibodies used	 EBOV VP40 polyclonal rabbit antibody (in-house) at a 1:2000 dilution Provided by Yoshihiro Kawaoka - University of Wisconsin-Madison, USA MARV NP polyclonal rabbit antibody at a 1:1000 dilution Provided by Ayato Takada - Hokkaido University, Japan A horse anti–rabbit IgG/polymer "ready-to-use" system from Vector Laboratories ImPress VR (catalog #MP-6401-15, lot number: ZH1216).
Validation	 PMID: 37386692, 28099857 PMID: 27976688, 30215737 Commercial vendor. We rely on the information provided in the Data Sheets provided by the manufacturer.

Eukaryotic cell lines

olicy information about <u>cell lines and Sex and Gender in Research</u>				
Cell line source(s)	Vero E6 cells (PMID: 21533129): provided by B. Rockx and R. Baric, University of North Carolina, Chapel Hill, North Carolina, USA MoKi cells (PMID: 31961874): Inmortilized cell lines within our lab, derived from kidney tissue of bats of the species Mops condylurus. Cells were immortalized by lentiviral transduction of SV40 large T antigen (G256-GVO-ABM, ABM).			
Authentication	These cells are routinely maintained in our lab. No other authentication at the lab level was performed.			
Mycoplasma contamination	All cells were tested and negative for Mycoplasma			

Palaeontology and Archaeology

Specimen provenance	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.	
Specimen deposition	Indicate where the specimens have been deposited to permit free access by other researchers.	
Dating methods	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.	
Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.		
Ethics oversight	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.	

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	The study did not involve laboratory animals.
Wild animals	Only adult Angolan-free tailed bats were included in the study. Adult females that were pregnant or lactating were excluded from the study and realease on-site.
Reporting on sex	Bats of both sex were included in the study. Due to challenges inheret to the animal collection, virus group had disproportionate sex composition. At least one female per virus group was allocated before random selection of the animals.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	Bat capture and work was performed following permission from the Laboratoire Central Vétérinair, Laboratoire National d'Appui au Développement Agricole (LANADA), Bingerville, Côte d'Ivoire (No. 05/virology/2016) and the Ministère des Eaux et Forêts (No. 0474/ MINEF/DGFF/FRC-aska). The animal care and use protocol adhered with the Ethics Committee of LANADA and the National Ethics Committee for the Research (CNER). Consent to capture bats was obtained by the residence owners in Bregbo Village (colony location). Animal transportation was conducted following appropriate IATA Live Animals Regulations. Permission for housing of bats and infection experiments in the BSL-4 facility at the Robert Koch-Institute, Berlin, Germany, was granted by the Regional Office for Health and Social Affairs Berlin (LAGeSo, No. ZH 180 and No. G 0256/18, respectively).

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

Clinical data

Policy information about <u>clinical studies</u> All manuscripts should comply with the ICMJE <u>guidelines for publication of clinical research</u> and a completed <u>CONSORT checklist</u> must be included with all submissions.

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

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:



Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
\boxtimes	Demonstrate how to render a vaccine ineffective
\boxtimes	Confer resistance to therapeutically useful antibiotics or antiviral agents
\boxtimes	Enhance the virulence of a pathogen or render a nonpathogen virulent
\boxtimes	Increase transmissibility of a pathogen
\boxtimes	Alter the host range of a pathogen
\boxtimes	Enable evasion of diagnostic/detection modalities
\boxtimes	Enable the weaponization of a biological agent or toxin
\boxtimes	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.
Authentication	Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene edition) were examined

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. <u>UCSC</u>)	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	laentijy 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).

Both

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

ANOVA or factorial designs were used.

Whole brain ROI-based

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Correction

Effect(s) tested

Specify type of analysis:

Statistic type for inference

(See Eklund et al. 2016)

n/a Involved in the study Functional and/or effective connectivity Graph analysis Multivariate modeling or predictive analys	is
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.