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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 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<br>*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<br>*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 | Custom scripts used for data analysis are available at GitHub (https://github.com/ariadnamorales/2023_Bat1Kimmunity). |
| Data analysis | Pacific Biosciences pipeline v.4.2.0<br>621 (https://github.com/PacificBiosciences/ccs), hifiasm v.0.13, purge_dups v.1.2.3, hifiasm v0.15.5-r352, hifiasm v0.15.4-r432 (see methods), HiCanu v.2.1, Canu v2.2, 10X Genomics longranger v2.2.2, Scaff10X v4.2, Break10X v3.1, Bionano Solve v1.6.1, g bwa-mem v0.7.17-r1188, Arima v2 (VGP) https://github.com/VGP/vgp-653, salsa2 v2.2, pbmm2 https://github.com/PacificBiosciences/pbmm2, DeepVariant v1.2.0, Merfin v1.1-development r197, bcftools consensus v1.12, RepeatClassifier https://github.com/davidaray/bioinfo_tools/blob/master/extract_align.py, RepeatAfterMe (RAM) https://zenodo.org/record/7076442, TE-Aid, USEARCH, RM2Bed.py, RepeatMasker v4.0.6, RNAfold v2.4.18, LASTZ v.1.04.15, axtChain v.1, ChainCleaner v.1, TOGA https://github.com/hillerlab/TOGA, commit v.c4bce48, MACSE v.2, HmmCleaner v.0.180750, ASTRAL v.5.5.9, IQTREE v.2.1.3, BOOSTER v.1, ModelFinder (as implemented in IQTREE), TreePL https://github.com/blackrim/treePL, gProfiler (database built on 2022-05-18 e106_eg53_p16_65fcd97), phyloFit v.1, brms v.2.20.4, AlphaFold2 Colabfold v.1.3.0 (release 13-45111), MMseqs2, Carma v.2.01 1, Partitioning Around Medoids algorithm implemented in Cluster v2.1.3, ggPlot2, FlowJo v10.0, Fiji IMageJ 2.9.0/1.53t, Graphpad Prism 9.4.1. |

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

Genome assemblies and sequencing data is available at NCBI for Aselliscus stoliczkanus (PRJNA949177), Doryrhina cyclops (PRJNA938463), Hipposideros larvatus (PRJNA938461), Rhinolophus affinis (PRJNA938462), Rhinolophus perniger lanosus (PRJNA955779), Rhinolophus yonghoiseni (PRJNA938455), Rhinolophus trifoliatus (PRJNA939732), Rhinopoma microphyllum (PRJNA971926), Megaderma spasma (PRJNA940731), and Mops condylurus (PRJNA949178). TOGA, transposable element and miRNA annotations of newly-sequenced bats and alignments of selected genes are available for download at http://genome.senckenberg.de/download/Bat1KImmune/. Accession codes and identifiers of genomic data are listed in Supplementary Tables 3 and 5. gRNAs, primers and geneblock sequences are listed in Supplementary Table 27.

Other databases used are: Dfam database (v3.5) (https://www.dfam.org/releases/Dfam_3.5/, Infernal (v1.1.2), GENCODE V38 (Ensembl 104), Gene Ontology http://geneontology.org/, KEGG https://www.genome.jp/kegg/, WikiPathways https://www.wikipathways.org/index.php/WikiPathways, miRTarBase http://mirtarbase.mbc.nctu.edu.tw, TRANSFAC http://genexplain.com/transfac/, Human Protein Atlas https://www.proteinatlas.org/, CORUM http://mips.helmholtz847muenchen.de/corum/, Human Phenotype Ontology https://hpo.jax.org/app/, uniref100

## Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

| | |
|---|---|
| Reporting on sex and gender | not applicable |
| Population characteristics | not applicable |
| Recruitment | not applicable |
| Ethics oversight | not applicable |

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

# Life sciences study design

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

| | |
|---|---|
| Sample size | For in vitro cellular work, where single cell quantification was required a minimum of 100 cells was utilized (microscopy) or 10,000 cells (FACS) based on minimal statistical requirements for each experiment (individual t test comparisons, one-way ANOVA etc). All other work was an average for each well of a plate as measured by the Plate Reader, counted for plaque assays or combined pool for qPCR/western blot and was performed in at least biological triplicate then replicated as below. |
| Data exclusions | No data was specifically excluded from the analysis. |
| Replication | All experiments were performed in triplicate and replicated (biologically) a minimum of 3 times. |
| Randomization | Randomization was generally not used due to blinding of genotypes and samples and internal normalization against controls (e.g. empty vector). Samples for genome extraction were randomly selected amongst those with high enough quality of HMW DNA. Randomization of genomes was performed for the phylogenetic downsampling by randomly selecting 10 species of the 20-species orders. |
| Blinding | Where possible- sample ID (genotype) was blinded and coded with a number. Viral infections, transfection experiments were coded/blinded without revealing the species. Quantification for microscopy and FACS was performed on blinded codes. WB samples were coded until after lysate collection then unblinded to ensure appropriate loading order was maintained. All data was unblinded at the time of generating graphs/visual representation. |

# 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 |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |

## Methods

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

## Antibodies

Antibodies used

Anti-CD13 Antibody Rabbit Polyclonal (Sinobiological, 10051-T60), Human coronavirus (HCoV-229E) Spike S1 Antibody, Rabbit PAb, Antigen Affinity Purified (Sinobiological, 40601-T62) , Ki67 Rabbit Monoclonal Antibody (Beyotime, AF1738), 20282 CF647 Goat Anti-Rabbit IgG (H+L), highly cross-adsorbed (biotium, 20282), 20281 CF647 Goat Anti-Mouse IgG (H+L), highly cross-adsorbed (biotium 20281), 20101 CF568 Goat Anti-Mouse IgG (H+L), highly cross-adsorbed (biotium 20181), Rabbit Anti-VSV-G tag antibody (Bioss, bs-2110R), ISG15 Antibody - middle region (avivasysbio, ARP59386_P050), Rabbit anti-IRF3 monoclonal antibody – Huabio (ET1612-14), Rabbit anti-Phospho IRF3 monoclonal antibody– Huabio (ET1608-22), MX1**抗体** [N2C2], Internal (Genetex, GTX110256), Mouse anti-MYC monoclonal antibody- Sino Biological (100029-MM08), Mouse anti-TNF alpha monoclonal antibody – Salarbio (K009343M), Mouse anti-IFIT1 monoclonal antibody – Sangon (D199761), GAPDH (14C10) Rabbit mAb (CST, #2118), ProteinFind ® Goat Anti-Mouse IgG (H+L), Anti-Rabbit Goat Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed Secondary Antibody, Alexa Flour 488/568/647, HRP Conjugate (transgen, HS201-01), ProteinFind ® Goat Anti-Rabbit IgG (H+L), HRP Conjugate (transgen, HS101-01), **辣根**过氧化物酶标记的驴抗羊IgG Donkey Anti-Goat IgG/HRP (SolarBio, K0038D-HRP-100ul).rabbit anti-UBE1L monoclonal antibody (Huabio, HA721228, dilution:1:500), rabbit polyclonal anti-UBE2L6 antibody (Abclonal, A13670), rabbit polyclonal anti-HERC5 antibody (Abclonal, A14889)

Validation

Commercial companies performed specific validation via peptide binding to antibodies as per each spec sheet, additional validation was performed in the laboratory with non-transfected or non-infected controls showing no staining. Rabbit anti-ISG15 polyclonal antibody - Aviva Systems Biology (ARP59386_P050)
This antibody alone was validated to use for detecting human and mouse and predicted to have a broad-spectrum reactivity in following species: cow, dog, goat, horse, pig, rabbit, sheep by the manufacturer.
Myc-tag inserted into ISG15 plasmids was tested with anti-Myc antibody as the surrogate for detecting ISG15 thereafter.
Remaining antibodies were validated by the providers, concentration used for western blot and FACS were further however, optimized to ensure the results are accurate and repeatable.Rabbit anti-CD13 polyclonal antibody (Sinobiological, 10051-T60) was validated by the manufacturer by using U937 and THP-1 cell lines which naturally express ANPEP/CD13. Rabbit polyclonal anti-human coronavirus (HCoV-229E) nucleocapsid antibody (Sinobiological, 40640-T62) was validated by the manufacturer by using human coronavirus (HCoV-229E) nucleoprotein for western blot. Rabbit anti-Ki67 monoclonal antibody (Beyotime, AF1378) was validated by the manufacturer using HepG2 cell lines for western blot, human tonsil tissue for IHC and Hela cells stained with Ki67 antibody for flow cytometry analysis. Rabbit Anti-VSV-G tag antibody (Bioss, bs-2110R) was validated by the manufacturer using overexpressed E.coli as positive control for western blot. Rabbit anti-IRF3 monoclonal antibody (Huabio, ET1612-14) was validated by the manufacturer using 5 different cell lines including Hela, Jurkat and THP-1, along with more than 10 types of human and mouse tissue as positive control for western blot. Rabbit anti-Phospho IRF3 monoclonal antibody (Huabio, ET1608-22) was validated by the manufacturer using NIH/3T3 cell lysate and NIH/3T3 cells treated with 100nM Calyculin as positive control for western blot. Polyclonal rabbit anti-MX1 antibody (GeneTex, GTX110256) was validated by the manufacturer using MX1-overexpressed 293T cell lysate as positive control for western blot. Mouse anti-TNF alpha monoclonal antibody (Solarbio, K009343M) was validated by the manufacturer using rat plasma and rat serum as positive control for western blot. Mouse anti-IFIT1 monoclonal antibody (Sangon, D199761) was validated by the manufacturer using IFIT1-overexpressed cell lysates for western blot, validated for predicting human, mouse, rat and monkey IFIT1, this antibody was only used on human cell line in our research. Rabbit monoclonal anti-GAPDH antibody (CST, #2118) was validated using HELA cells as positive control for western blot and IH, this antibody was also validated in bat cell lines in our lab. Rabbit anti-UBE1L monoclonal antibody (Huabio, HA721228) was validated by the manufacturer using THP-1 cell lysates, rat kidney tissue, human testis tissue, mouse cerebellum tissue as positive control for western blot, validated for human, mouse and rat Ube1L. Rabbit polyclonal anti-UBE2L6 antibody (Abclonal, A13670) was validated by the manufacturer using mouse lung and rat kidney tissues as positive control for western blot. Rabbit polyclonal anti-HERC5 antibody (Abclonal, A14889) was validated by the manufacturer using U-87MG, OVCAR3 and HT-1080 cell lysates and mouse kidney and rat testis tissues as positive control for western blot.

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Huh7/MRC-5 was supplied from Prof. Jincun Zhao (Guangzhou Medical University, original source ATCC), HEK293-subclone (original source: ATCC), A549 (CCL-185, ATCC), Vero-76 (CRL-1587, original source: ATCC), Vero-E6 cells sourced from Prof.

| | |
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| | Linrong Lu (Zhejiang University, original source: CTCCC repository). A549-ACE2 cells from Dr. Colpitt's laboratory (clone A549-ACE2 B9 as published). Hek293-CD13 cells and A549-ACE2-TMPRSS2 were generated for this project, as described. Hek293 subclone was sorted from parental Hek293 for high viral production. Cas9/crispr KO for ISG15 or scrambled guide were generated from this subclone. RsKT.01 (original cell line, Zhengli Shi, Wuhan Institute of Virology) |
| Authentication | No independent verification was performed by a commercial service however, Vero-E6 cells, RsKT, A549 and Hek293 cells transcriptome matches the appropriate species. ISGylation machinery was validated as shown in the supplemental figures. |
| Mycoplasma contamination | All cell lines were routinely tested for mycoplasma and found negative by pan-mycoplasma PCR. |
| Commonly misidentified lines (See ICLAC register) | None of the cell lines used belong to ICLAC |

## 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 | not applicable |
| Wild animals | Tissue samples were acquired from the Royal Ontario Museum mammal collection or field expeditions for:<br>Aselliscus stoliczkanus China – Shuipu Village, Yuping Town M 3/28/2007<br>Doryrhina cyclops Ivory Coast – Parc National De Tai, Institute D'ecologie Tropicale F 2/18/1992<br>Hipposideros larvatus China – Shuipu Village, Yuping Town M 04/12/2007<br>Rhinolophus affinis China – Shiwandashan National Reserve M 4/25/2005<br>Rhinolophus perniger lanosus China – Shuipu Village, Yuping Town M 39174<br>Rhinolophus yonghoiseni Malaysia – Nature Education and Research Centre, Endau Rompin National Park F 8/14/2001<br>Rhinolophus trifoliatus Malaysia – Nature Education and Research Centre, Endau Rompin National Park M 08/10/2001<br>Rhinopoma microphyllum Israel – Northern Israel M 1/13/2013<br>Megaderma spasma Vietnam – Dong Nai, Cat Tien National Park Headquarters M 05/05/1998<br>Mops condylurus Côte d'Ivoire – Bregbo Village M 11/20/2019<br>All animals were euthanized and tissues were used for genome sequencing (all bat samples were re-purposed samples for genome sequencing with the exception of Mops condylurus which was collected specifically for generation of a genome). |
| Reporting on sex | Where possible Males were utilized for presence of both X and Y chromosomes, cyclops and yonghoiseni were F due to limited sample availability. |
| Field-collected samples | No housing, instant collection. |
| Ethics oversight | Doryrhina cyclops (ROM-M100513) – Permit number 81 DPN from Direction de la Protection de la Nature, République de Côte d'Ivoire<br>Rhinolophus yonghoiseni (ROM-M113050), Rhinolophus trifoliatus (ROM-M113012) – Reference number PTN(J) 3/8 from Perbadanan Taman Negara (National Parks Corporation) Johor, Malaysia<br>Aselliscus stoliczkanus (ROM-M118506), Hipposideros larvatus (ROM-M118627), Rhinolophus affinis (ROM-M116429), Rhinolophus perniger lanosus (ROM-M118548) – Certificate numbers 2007/CN/ES133-137/KM from The Endangered Species Import and Export Management Office of the People's Republic of China<br>Megaderma spasma (ROM-M110751) – Number 138/STTN from Institute of Ecology and Biological Resources, National Center for Science and Technology, Vietnam<br>Mops condylurus (ID: 03#106) – Capture of bats and animal work were performed with<br>547 the permission of the Laboratoire Central Veterinaire, 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).<br>Rhinopoma microphyllum – National Parks Authority, permit 2013/04169. IACUC 04-20-551 019. NB: All Ethics and guidelines for each specific country were valid at the time of collection. |

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

## 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

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| Sample preparation | To generate ISG15 stable cell lines, lentiviral transduced Huh7, HEK293, A549 and Vero-E6 cells were sorted by fluorescence activated cell sorting (FACS) using the BD Influx System for mCherry-positive cells, normalized against autofluorescence in the |

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| | respective parental cell line. VSV-GFP load was measured directly via GFP fluorescent intensity. For HCoV-229E, CD13 stable HEK293 cells were stained with 229E N protein (Sino biological, 1:2000) and Ki67 (Beyotime, 1:500) for 30 minutes in FACS buffer containing 1x PBS (Gibco), 1% FBS and 1% P/S, after permeabilization with 0.05% TX-100 in TBS and blocking in 5% BSA in TBS-T. Cells were subsequently rinsed, stained with anti-mouse / rabbit CF®-488/568/647 secondary antibody for 15 minutes (Biotium, dilution 1:10000), rinsed thrice and run on the ACEA Novocyte flow system. No primary controls, untransfected controls or uninfected controls and cells only for autofluorescence were used to generate compensation matrices. |
| Instrument | Acea Novocyte; BD Influx |
| Software | Acea Novocyte capture software, FlowJo v10.0 |
| Cell population abundance | minimum of 10,000 events captured for the target population (transfected + infected). |
| Gating strategy | primary gates were used to separate live cells from dead based on SSC-H/FSC-H, then SS-H/SSC-A for single cells, then separate gates for mcherry positive or negative based on untransfected controls, followed by differential gates for infected or non-infected, gates drawn on uninfected control cells (stained with same antibodies). |

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.