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Life Sciences Reporting Summary

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.
For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

   Describe how sample size was determined.

   This study represents discovery of a new virus and not surveillance for a known pathogen, and thus sample size could not be determined

2. Data exclusions

   Describe any data exclusions.

   No data were excluded

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   Samples were retested to confirm they were positive, tested by multiple assays and sequenced for viral confirmation

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   This does not apply to our study, samples from all animals that were captured and sampled, were tested

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   These are not human samples or part of any clinical trials, thus blinding was not needed

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ 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 any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	ClustalW, BEAST, jModelTest, TreeAnnotator, FigTree, Recombination Detection Program (RDP version 4.87), PASC tool, Geneious, SLAC, FEL, MEME, and FUBAR algorithms executed in datamonkey, the M7 and M8 codon models in codeml (PAML package), CD-HIT, SKA program, PRINSEQ software (v 0.20.2), Bowtie2 mapper (v 2.0.6), MIRA assembler (v4.0)

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.	No restrictions

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Anti EBOV, TAFV, SUDV, BUDV antibodies were purchased from IBT Bioservices, eEnzyme, Sino Biological Inc., Alpha Diagnostic and R&D systems and validated by the company for use in ELISA platforms. Checkerboard dilutions were performed in our lab to determine the appropriate dilution to use for our ELISA assays and negative controls were included to determine if they cross reacted with non-ebolavirus antigens. Likewise, Rabbit antiserum specific for ebolavirus GP1 was purchased from Santa Cruz Biotechnology and used in immmunoblotting to detect BOMV GP1 VSV particles, used per manufacturer instructions. NPC1 domain C was detected by a horseradish-conjugated anti-Flag antibody (Sigma-Aldrich), using ultra-TMB substrate (Thermo Scientific). This is a standard antibody used to detect Flag tags and is well validated by the company. All appropriate negative controls were used to confirm specific binding. All commercial vendors provide physical quality control and data sheets for all antibodies.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	All commercially-available cell lines and primary cells are obtained from American Type Culture Collection (ATCC, subsidiary of BEI Resources) or Lonza Group, and are validated at their source. These include Vero African grivet kidney cells, U2OS human carcinoma cells and 293T human embryonic kidney fibroblast cells. This work also involves the generation of a cell line (U2OS-KO) genetically-modified by CRISPR/Cas9 engineering. All engineered cell lines are sequenced to genetically define each allele of the target gene. Further, functional knockout of the target gene and lack of major off-target effects are validated by genetic complementation, by transducing them with retroviruses expressing NPC1 gene variants.
b. Describe the method of cell line authentication used.	All commercial cell lines were authenticated at their source
c. Report whether the cell lines were tested for mycoplasma contamination.	We test all cell lines in culture for mycoplasma on a monthly basis, since contamination can occur sporadically. All incoming cell lines are routinely 'quarantined' until they have been confirmed to be mycoplasma-free.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	Not applicable

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.	Oral and rectal swabs, and whole blood when possible, were humanely collected (capture and release) from live captured animals under permits provided by The Ministry of Agriculture, Forestry and Food Security and under the Institutional Animal Care and Use Committee at the University of California, Davis (protocol number: 16048).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	Not applicable