

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

Immunofluorescence images were acquired using an EVOS M5000 Invitrogen Microscope.
Quantitative PCR data were collected using an Applied Biosystems StepOnePlus Real-Time PCR System.
Transmission Electron Microscopy Images were acquired using an JEM-JEOL2100+ Microscope.
Proteomic data was obtained through use of timsTOF Mass Spectrometry.
Viral titers were manually assessed through plaque assay and registered in Microsoft Excel.
Western blotting acquisitions were obtained using BioRad Chemi-Doc MP Imaging System.

Data analysis

GraphPad Prism 8 was used for the analysis of viral titers, viral forms and qPCR assays.
ImageJ2 2.16.0/1.54p (Fiji) was used for the analysis of immunofluorescence and transmission electron microscopy images.
Gene Ontology Analysis was performed using the websoftware ShinyGo 0.85.
Kinase Enrichment Analysis was performed with Kinase Enrichment Analysis 3 (KEA3) web software
Venn diagram analysis was performed with use of InteractiVenn software.
Figures were prepared using Microsoft Powerpoint.

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](#)

Source data are provided with this paper. Proteomic data has been deposited in the PRIDE Database and are available under accession codes PXD068865 (Reviewer code B1ASiuXsNJEy) and PXD075835 (Reviewer code 033d6lqyhtAQ).

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	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 ☐ 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	Sample sizes were chosen based on previous experiences to ensure adequate power. Sample sizes are indicated in figure legends and methods section.
Data exclusions	No data were excluded.
Replication	For in vitro viral growth experiments, WB and immunofluorescence a minimum of three independent replicates were performed. For Mass Spectrometry four independent biological replicates were used. For qPCR assay a minimum of five independent replicates were used. For TEM quantification, 15 distinct cell profiles of each condition were used for viral form quantification. All replications were successful.
Randomization	N/A
Blinding	Investigators were not blinded since prior knowledge of samples is required for data interpretation.

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
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

Beta-Actin (Cell Signalling #3700; 1:1000), Poli-VACV (Invitrogen Ref PA1-7258; 1:1000), DAPI (Sigma Aldrich, Ref D9542; 1:200), VACV E3 (Kindly Provided by Dr. Bertram Jacobs; 1:1000), VACV F13 (Kindly Provided by Dr. Rafael Blasco; 1:1000), VACV D8 (Kindly Provided by Dr. Mariano Esteban; 1:1000), VACV A26 (Kindly Provided by Dr. Wen Chang; 1:000), VACV A4 (Kindly Provided by Dr. Mariano Esteban; 1:1000), VACV Polyclonal (Kindly Provided by Dr. Mariano Esteban; 1:5000), Anti-rabbit IgG-peroxidase (Sigma-Aldrich, Cat. No. A0545; 1:10.000), Anti-mouse IgG-peroxidase (Sigma-Aldrich, Cat. No. A9044; 1:10.000), Anti-rabbit IgG Alexa Fluor 488 (Invitrogen, Cat. No. A-11012; 1:10.000)

Validation

Antibodies were validated through infection experiments in this study. In addition, commercial antibodies have been validated by manufacturers.

Eukaryotic cell lines

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

Cell line source(s)

Wild Type and ISG15 KO Mouse Embryonic Fibroblasts (MEFs) were kindly provided by Dr. Klaus Peter Knobloch. Wild Type and ISG15 KO HeLa cells were kindly provided and generated by Dr. Antje Beling. BSC-40 (ATCC CRL-2761) and 293T (ATCC-CRL-3216) cells were purchased from ATCC.

Authentication

MEFs and HeLa cell lines authenticated through genotyping and western blotting.

Mycoplasma contamination

Cell lines were routinely checked and were found negative for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell lines.

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A