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Reporting Summary

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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	ABI 7500 software, BD FACS DIVA Software
Data analysis	GraphPad Prism 8.4.3, MS Excel, Flowjo, Image J, ABI 7500 software

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

There are no restrictions on availability of data. The data that support the findings of this study are available from the corresponding author upon request.

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	The number of experiments and replicates was based on standard practices. In general, two or three independent experiments were conducted with three biological replicates or technical replicates where applicable. Details for each experiment are included in the respective figure legend.
Data exclusions	n/a
Replication	The experimental findings were reliably reproduced. See details in respective figure legends.
Randomization	n/a
Blinding	n/a

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	Details about all antibodies used in our study are described in the Methods section of the manuscript including vendor name, Cat# and clone.
Validation	All antibodies, except the anti-phospho-MDA5 (Ser88) and anti-MDA5 antibodies, were purchased from commercial vendors who have validated the antibodies for the use of Western blot, Co-immunoprecipitation, and/or FACS analysis. The anti-phospho-MDA5 (Ser88) antibody was previously validated by the Gack laboratory and has been published (Wies et al., Immunity 2013). The anti-MDA5 antibodies were provided by Dr. Jan Rehwinkel (Oxford University) and has been published (Hertzog et al., Eur J Immunol 2018).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	NHLF (Lonza), HEK293T (ATCC), Vero (ATCC), BHK-21 (ATCC), Aedes albopictus clone C6/36 (ATCC), HAP-1 WT and ISG15 KO cells (Horizon Discovery). Human PBMCs were isolated from unidentified healthy donor peripheral blood (HemaCare). The WT and Isg15 -/- MEF, WT and ISG15 KO HeLa, SVGA, MDA5 KO HEK293, RIG-I KO HEK293, HEK293T-hACE2, Vero-hACE2, and A549-hACE2 cells were obtained from other investigators (details are described in the Methods section of our manuscript).
Authentication	Cell lines from ATCC, Lonza, and Horizon Discovery were authenticated by the vendors and were not further authenticated in our laboratory. Cell lines that were obtained and validated by other groups were not further authenticated. All ISG15 KO or MDA5 KO cell lines were validated by confirming the absence of ISG15 or MDA5 protein expression by immunoblotting.
Mycoplasma contamination	Cell lines have been regularly tested for potential mycoplasma contamination by PCR and/or using the MycoAlert Kit (Lonza).
Commonly misidentified lines (See ICLAC register)	n/a

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	Cells were harvested and then pelleted by centrifugation at 300 x g for 5 min. Cells were washed with PBS and fixed with 4% (v/v) formaldehyde in PBS at room temperature for 30 min, followed by washing cells twice with PBS. For antibody staining, cells were incubated with 1× BD Perm/Wash buffer (BD Biosciences), followed by staining with primary (anti-flavivirus E [4G2] ; 1:100) and secondary (goat anti-mouse Alexa 488; 1:500) antibody at 4 degree in 1× BD Perm/Wash buffer for 30 min each.
Instrument	LSR Fortessa flow cytometer (BD Biosciences)
Software	FACS Diva (BD Biosciences), FlowJo (Tree Star)
Cell population abundance	n/a
Gating strategy	The main cell population was gated in the FCS/SSC blot and then analyzed in the Alexa 488 channel. Positive cells were above cutoff value, which is set to 98% of measure events of the negative control.

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