

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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ | 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | 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 Software used for data collection: Icy Software Version 2.1.3, Image J Version 1.52p, easyFRAP (a MatLab stand-alone application) with version 9.0.1 (R2016a) of MatLab, FlowJo Version 10, Living Image software Version 4.0(Caliper Life Sciences) and CLC Main Workbench 21.0.3.

Data analysis Data were analysed using Microsoft Office Excel 2019, GraphPad Prism 9.1 and Rstudio Version 1.2.5019.

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 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

The authors declare that all other data supporting the findings of this study are available within the article and its Supplementary information files, or are available from the authors 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](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	In vitro experiments were done at least twice, either in duplicate or triplicate. No calculation of sample size was done for animal experiments.
Data exclusions	One mouse was excluded from the uninfected control group data analysis (Fig. 3c, Histopathology). Mouse#2 in that group showed severe histopathology. We infer the mouse might have had some congenital health problems, and therefore excluded it from the data analysis.
Replication	For in vitro experiments, numerous cells/events were analyzed to ensure representativity and statistical significance. All data were consistent and data presented here reflect show the variability intra and inter experiment. No sample size calculation was done for animal experiments.
Randomization	Not applicable
Blinding	Histopathology scores were determined by three independent pathologists who were blinded as to the treatment that the animals received

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

Methods

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

Antibodies

Antibodies used	Rabbit polyclonal anti-N antibody mouse anti-RSV fusion protein monoclonal antibody (Abcam-ab94968) Purified mouse-anti-RSV phosphoprotein hybridoma-supernatant
Validation	Rabbit polyclonal anti-N antibody validated in reference doi:10.1038/s41467-017-00655-9 (2017) Purified mouse-anti-RSV phosphoprotein hybridoma-supernatant. doi: 10.1016/j.antiviral.2020.104774. (2017)

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HEp-2 cells : ATCC number CCL-23 BHK-21 cells (clone BSRT7/5) constitutively expressing the T7 RNA polymerase . Obtained from the Conzelmann group. Reference doi:10.1128/JVI.73.1.251-259.1999 (1999).
Authentication	None
Mycoplasma contamination	Negative for mycoplasma contamination

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

Yes, HEp-2 cells are known to result from a contamination by Hela cells. This is the cell line recommended by ATCC to grow RSV.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Female BALB/c mice of approximately 8 weeks of age were purchased from the Centre d'Élevage R. Janvier (Le Genest Saint-Isle, France)
Wild animals	None
Field-collected samples	None
Ethics oversight	All animal experiments except Fig. 3c were carried out at INRAE in accordance with INRAE guidelines and in compliance with European animal welfare regulations. The protocols were approved by the Animal Care and Use Committee at "Centre de Recherche de Jouy-en-Josas" (COMETHEA) under relevant institutional authorization ("Ministère de l'éducation nationale, de l'enseignement supérieur et de la recherche"), authorization number 2015100910396112v1 (APAFIS#1487). All experimental procedures were performed in a biosafety level 2 facility. Histopathology experiments (Fig. 3c) were carried out at WuxiAppTec. The animal care and use followed the Wuxi IACUC approved animal use protocol (IACUC #: ID01-QD029-2020v1.0). Female BALB/c mice were housed in the IVC in the BSL-2 labs of Wuxi AppTec after quarantine.

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

Sample preparation	HEp-2 cells were seeded at 2x10 ⁴ cells per well in 96-well plates the day before virus inoculation. Cells were infected at MOI=1 in presence of the respective compound. After 2 h the supernatant was removed and fresh medium containing the same concentration of the compound was added. At 24 h after inoculation, cells were washed once with PBS, and detached by trypsin-treatment. Cells were washed, centrifuged at 400xg, resuspended in fixation buffer (0.5% PFA and 1% FBS in PBS) and incubated at 4°C for at least 30 min or stored overnight. Subsequently, cells were permeabilized with 0.1% Saponin and 1% FCS in PBS on ice for 20 min.
Instrument	BD Accuri C6 Flow Cytometer
Software	FlowJo V10
Cell population abundance	The abundance of RSV infected and uninfected cells was quantified based on an RSV-P protein staining and a FACS analysis. The gating strategy for quantification of RSV-positive infected and uninfected cells were set as defined below.
Gating strategy	First, cells were gated based on forward (FSC) and sideward scatter (SSC) parameters with cells falling within a range of SSC between 5x10 ⁵ to 1x10 ⁷ and FSC between 3x10 ⁶ and 3x10 ⁷ selected for onward analysis. Roughly 70 to 80% of detected events fell into this range of SSC/FSC. Among these gated cells, RSV-positive cells were identified based on intracellular detection of RSV P protein expression, as detected by a P protein specific monoclonal antibody. The gate for RSV positive and negative cells was set by using the mock infected cell population stained with the P-protein specific antibody and a secondary antibody recognizing mouse IgG and coupled to Alexa 488. The gate was set using a scatter plot display with FSC signal of cells given on the X axis and the RSV P Alexa 488 staining on the Y axis. The quadrant directly above the signal of P-protein stained mock infected cells was used as gate to score RSV positive cells.

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