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Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work we publish. This form is published with all life science papers and is intended to promote consistency and transparency in reporting. All life sciences submissions use this form; while some list items might not apply to an individual manuscript, all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   Experiments presented in figures 4, 5a, 5b, 5c, 5d and supplementary figures 7 and 8 were independently repeated at least 3 times, based on standard practice. The exact number of independent repeats is the n number stated in the figure legends.

2. Data exclusions

   Describe any data exclusions.

   No data were excluded from the analysis of experiments presented in figures 4, 5a, 5b, 5c, 5d and supplementary figures 7 and 8.

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   All attempts at replication of experiments presented in figures 4, 5a, 5b, 5c, 5d and supplementary figures 7 and 8 were successful, with variations indicated by the standard error of the mean (SEM) represented as error bars in the graphs and by the 95% confidence interval of the resulting IC50s indicated in the results text.

4. Randomization

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

   This is not relevant to the study.

5. Blinding

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

   Data from experiments presented in figures 4a, 4b, 5b, 5c, 5d and supplementary figures 7b, 7c, 7d, 8b, 8c and 8d were blindly collected by machines. Virus titre data from figures 4c, 4d, 4e, 4f, 5a, and supplementary figures 7 and 8a were objectively but not blindly collected, as TCID50 read-outs are very obvious after 5 days and not prone to subjective interpretation.

Note: all studies involving animals and/or human research participants must disclose 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 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 (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *p* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

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.

Statistical analysis of the data in presented in figure 4 and supplementary figure 7 was performed using GraphPad Prism 7 software.

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The *Nature Methods* guidance for providing algorithms and software for publication may be useful for any submission.

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 for-profit company.

No restrictions; note that very limited amounts of synthetic NMT inhibitors are available, and are unlikely to be distributed for this reason.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The anti-2C antibody used in figure 5c and 8c has been custom-made for us by Covalab, France and has been used for Western blot of RV-A16 infected cells and validated for this application in Mousnier A. et al., J. Virol. 2014, 88(20):11671 (figure 11c). It recognizes two bands at the predicted molecular weight of 2C and 2BC, which specifically appear over time upon infection of cells with RV-A16 and are not detected in uninfected cells.
The anti-Lamin B1 antibody used in figure 5c and 8c is a rabbit polyclonal antibody from Proteintech Europe, catalogue number 12987-1-AP. It has been used and validated for Western blot on HeLa cell lysates (see manufacturer's website for images and publications: http://www.ptglab.com/Products/LMNB1-Antibody-12987-1-AP.htm#validation).
The antibody used in figure 3e is a mouse monoclonal antibody directed against purified RV-A16 (QED Biosciences catalogue number 18758, clone R16-7, lot number 080414), It recognizes RV-A16 VP2 and its precursor VP0. It has been used for Western blot of RV-A16 infected cells and validated for this application in Mosser AG et al., J Infect Dis, 2002, 185: 734 (figure 1). It recognizes two bands at the predicted molecular weight of VP2 and VP0, which specifically appear upon infection of cells with RV-A16 and are not detected in uninfected cells.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

HeLa H1 (ATCC CRL-1958) and HeLa Ohio (ECACC 84121901) cells were from ATCC and the European Collection of Authenticated Cell Cultures (ECACC), respectively. They are clones derived from HeLa cells that are permissive for RV-A16 replication.

b. Describe the method of cell line authentication used.

The HeLa H1 and HeLa Ohio cells used have been authenticated by the fact that they are permissive for RV-A16 replication, contrary to other clones of HeLa cells or other cell lines.

c. Report whether the cell lines were tested for mycoplasma contamination.

All cell lines tested negative.

d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No ICLAC registry lines used.

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 details on animals and/or animal-derived materials used in the study.

No animals were used.

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.

Did not involve human research participants.