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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	For sequence analysis, read mapping and GO analysis we used STAR aligner and R with the software packages cutadapt, DEseq2, bcftools, umi_tools and samtools. Exact details are provided in the methods section. No custom code was generated for the analysis.
Data analysis	For statistical analysis we used Graphpad Prism version 7. Exact details are provided in the methods section

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 upon request. 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

All sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under accession number SUB3758924

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The number of biologically independent repeats is indicated in each legend or figure.
Data exclusions	One ferret from the group of 1918 H1N1 influenza virus infections was excluded from analysis, because the previously-published viral titres in the ferret lung showed that this ferret had not been infected by the virus. We have fully disclosed this point in the manuscript.
Replication	All findings were reproducible by multiple scientists and in at least two labs. The finding that mutations in the template channel increase interferon induction was independently confirmed by Du et al Science 2018.
Randomization	Animal infections were previously performed and described in De Wit et al 2018 J Inf Dis and van den Brand et al 2012 PLOS One. We have repeated how the animals were assigned to groups in the methods.
Blinding	Blinding was not performed.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☐	☒ Unique materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☐	☒ Research animals
☒	☐ Human research participants

Unique materials

Obtaining unique materials	Plasmids are freely available from the authors

Antibodies

Antibodies used	All antibodies used are commercially available from e.g. GeneTex (RIG-I: GTX85488; NP: GTX125989; PB2: GTX125926; Myc: GTX115046)
Validation	Antibodies were validated by the manufacturer by Western blot.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK 293T cells and A549 cells were sourced from the ATCC
Authentication	RNA samples from cells were deep sequenced by RNAseq and confirmed to be human cells.
Mycoplasma contamination	All cells were routine mycoplasma tested.
Commonly misidentified lines (See ICLAC register)	N/A

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials	Ferret experiments have been described by De Wit et al 2018 J Inf Dis and van den Brand et al 2012 PLOS One. The information is also repeated in the methods.

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ Magnetic resonance imaging