nature research

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Last updated by author(s): Jul 17, 2020

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.

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	Image lab software 5.2.1
Data analysis	Image lab software 5.2.1 ,Prism5, MaxQuant 1.6.5, Perseus 1.6.5, Pymol (1.7.6.0), phenix (1.17.1-3660), ccp4 (7.0.078), coot (0.8.9.2), Modeller (9.24), Gromacs (2019.6)

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

The atomic coordinates of PLpro-ISG15 (murine) have been deposited in the PDB with accession code 6YVA in the Protein Data Bank. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium73 via the PRIDE partner repository74 with the dataset identifier PXD018983. The papain-like protease domain sequence is obtained from SARS-CoV-2 complete genome (NCBI genome databank, Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome; NC_045512). Protein sequence for CoV2 PLpro-Ubl domain (amino acids, 746-1060) of Nsp3 protein from SARS-CoV-2 (Nsp3; YP_009725299.1). Full gel images can be found in Supplementary Figure 1 and source data that support this study and can be found in Supplementary Information. Any other relevant data are available from the corresponding authors upon reasonable 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	No sample size calculation was done. Experiments were repeated three times with similar results and sample size was chosen based on the consistency and significance a of measured differences between groups. We have not mentioned any differences between groups if there the differences are not statistically significant.
Data exclusions	No data were excluded from analysis.
Replication	We have repeated each experiment in the manuscript at least three times to ensure consistent results.
Randomization	No randomization was necessary as various infection samples were recorded and analyzed by a computer software for extracting the significant differences.
Blinding	Blinding was not relevant for the experiments done as various infection samples were analyzed by a computer software for extracting the significant differences.

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	Ubiquitin (Cat# 3936S, Provider: Cell signaling Technology, 1:2000), ISG15 (Cat# HPA004627, Sigma Aldrich/Merck, 1:1000), GAPDH (Cat# 2118, Cell signaling Technology, 1:2000), GFP trap beads (Cat #: gta-100, Provider: ChromoTek), GFP (Cat# sc-9996, Santa Cruz Biotechnology, 1:2000), IRF3 (Cat# 4302, Cell signaling Technology, 1:2000), phospho-IRF3(Ser396) (Cat# 4947, Cell signaling Technology, 1:1000), IκBα (Cat# 4812, Cell signaling Technology, 1:2000), phospho-IκBα(Ser32/36) (Cat# 9246, Cell signaling Technology, 1:1000), TBK1 (Cat# 3013, Cell signaling Technology, 1:2000), pTBK1 (Cat # 3300-1 Epitomics, 1:1000), P65 (NFkB) (Cat# 8008, Santa Cruz Biotechnology, 1:2000), Lamin B1 (Cat# sc-373918, Santa Cruz Biotechnology, 1:2000).
Validation	Ubiquitin (Cat# 3936S, Provider: Cell signaling Technology) Validation statement from the manufacturer: Ubiquitin (P4D1) Mouse mAb detects ubiquitin, polyubiquitin and ubiquitinated proteins. This antibody may cross-react with recombinant NEDD8. Validation found at provider's website: https://www.cellsignal.com/products/primary-antibodies/ubiquitin-p4d1-mouse-mab/3936 ISG15 (Cat# HPA004627, Sigma Aldrich/Merck) Validation statement from the manufacturer: species reactivity-human, validation-recombinant expression, orthogonal RNA seq Validation found at provider's website: https://www.sigmaaldrich.com/catalog/product/sigma/hpa004627?lang=en&region=DE GAPDH (Cat# 2118, Cell signaling Technology) Validation statement from the manufacturer: GAPDH (14C10) Rabbit mAb detects endogenous levels of total GAPDH protein. Species Reactivity: Human, Mouse, Rat, Monkey, Bovine, Pig Validation found at provider's website: https://www.cellsignal.com/products/primary-antibodies/gapdh-14c10-rabbit-mab/2118

GFP trap beads (Cat #: gta-100, Provider: ChromoTek)
Validation statement from the manufacturer: GFP-Trap® Agarose is an affinity resin for immunoprecipitation of GFP-fusion proteins.
It consists of a GFP Nanobody/ VHH coupled to agarose beads.
Validation found at provider's website: https://www.chromotek.com/products/detail/product-detail/gfp-trap-agarose/

GFP (Cat# sc-9996, Santa Cruz Biotechnology)
Validation statement from the manufacturer: Anti-GFP Antibody (B-2) is a mouse monoclonal IgG2a (kappa light chain) GFP antibody provided at 200 µg/ml, raised against amino acids 1-238 representing full length GFP (green fluorescent protein) of Aequorea victoria origin
Validation found at provider's website: https://www.scbt.com/p/gfp-antibody-b-2?productCanUrl=gfp-antibody-b-2&_requestid=272661

IRF3 (Cat# 4302, Cell signaling Technology)
Validation statement from the manufacturer: IRF-3 (D83B9) Rabbit mAb detects endogenous levels of total IRF-3 protein. Species Reactivity:Human, Mouse, Rat, Monkey
Validation found at provider's website: https://www.cellsignal.com/products/primary-antibodies/irf-3-d83b9-rabbit-mab/4302

phospho-IRF3(Ser396) (Cat# 4947, Cell signaling Technology)
Validation statement from the manufacturer: phopho-IRF-3 (Ser396) (4D4G) Rabbit mAb detects endogenous levels of IRF-3 when phosphorylated at Ser396. Species Reactivity:Human, Mouse
Validation found at provider's website: https://www.cellsignal.com/products/primary-antibodies/phospho-irf-3-ser396-4d4g-rabbit-mab/4947

IκBα (Cat# 4812, Cell signaling Technology)
Validation statement from the manufacturer: IκBα (44D4) Rabbit mAb detects endogenous levels of total IκBα protein.Species Reactivity:Human, Mouse, Rat, Hamster, Monkey, Mink
Validation found at provider's website: https://www.cellsignal.com/products/primary-antibodies/ikba-44d4-rabbit-mab/4812

phospho-IκBα(Ser32/36) (Cat# 9246, Cell signaling Technology)
Validation statement from the manufacturer: Phospho-IκBα (Ser32/36) (5A5) Mouse mAb detects endogenous levels of IκBα only when phosphorylated at Ser32/36.Species Reactivity:
Human, Mouse, Rat, Monkey
Validation found at provider's website: https://www.cellsignal.com/products/primary-antibodies/phospho-ikba-ser32-36-5a5-mouse-mab/9246

TBK1 (Cat# 3013, Cell signaling Technology)
Validation statement from the manufacturer: TBK1 Antibody detects endogenous levels of total TBK1/NAK protein.Species Reactivity:Human, Mouse, Rat, Monkey
Validation found at provider's website: https://www.cellsignal.com/products/primary-antibodies/tbk1-nak-antibody/3013

pTBK1 (Cat # ab109272 abcam)
Validation statement from the manufacturer: This antibody only detects NAK/TBK1 phosphorylated at serine 172.Validation found at provider's website: https://www.abcam.com/naktbk1-phospho-s172-antibody-epr28672-ab109272.html

P65(NFkB) (Cat# 8008, Santa Cruz Biotechnology)
Validation statement from the manufacturer: Anti-NFκB p65 Antibody (F-6) is a mouse monoclonal IgG1 (kappa light chain) NFκB p65 antibody provided at 200 µg/ml, raised against amino acids 1-286 mapping at the N-terminus of NFκB p65 of human origin
Validation found at provider's website: https://www.scbt.com/p/nfkappab-p65-antibody-f-6?productCanUrl=nfkappab-p65-antibody-f-6&_requestid=285577

Lamin B1 (Cat# sc-373918, Santa Cruz Biotechnology)
Validation statement from the manufacturer: Lamin B1 Antibody (G-1) is a mouse monoclonal IgG3 (kappa light chain) provided at 200 µg/ml, specific for an epitope mapping between amino acids 559-586 at the C-terminus of Lamin B1 of mouse origin
Validation found at provider's website: https://www.scbt.com/p/lamin-b1-antibody-g-1?requestFrom=search

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	A549 cells (ATCC® CCL-185™), HeLa (ATCC® CCL-2™), CaCo-2 (DSMZ, ACC 169)
Authentication	Cell lines were authenticated using STR DNA profiling.
Mycoplasma contamination	All the cell lines used tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	The cell lines used in the study are not in the commonly misidentified lines list.