

Reporting Summary

Nature Portfolio 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 Portfolio policies, see our [Editorial Policies](#) 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 (<i>n</i>) 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> 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 <i>d</i> , Pearson's <i>r</i>), 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	-Western blot images were acquired with Image Quant LAS4000 (GE Healthcare). -RT-qPCR was performed using QuantStudio 6 Pro (Applied Biosystem). -Confocal microscopy images were acquired by using a Leica Stellaris 8 confocal microscope or a Leica SP8 system. -Luminescence detection in the GTPase assays was performed using an Orion II microplate Luminometer (Berthod Detection Systems). -Structural modeling was performed and illustrated by PyMol (Schrodinger, Inc.). -Mass spectrometry analysis was performed at the Harvard Taplin Biological Mass Spectrometry Facility, Boston. -IHC was performed by The University of Chicago's Human Tissue Resource Center Core Facility.
Data analysis	-The relative mRNA expression of the gene of interest for RT-qPCR data was calculated in Microsoft Excel (version 2501) by using the comparative threshold ($\Delta\Delta Ct$) method and expressed relative to the values for respective control cells. -ImageJ (version 1.51) was used for image analysis. -Data management and analysis were performed on Microsoft Excel (version 2501) and GraphPad prism (Version 10). -All statistical analysis was done by using GraphPad prism (Version 10).

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 Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All data are available in the article main text itself and its supplementary materials. This paper does not report original code.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

n/a

Reporting on race, ethnicity, or other socially relevant groupings

n/a

Population characteristics

n/a

Recruitment

n/a

Ethics oversight

n/a

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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

Sample sizes for the in vivo studies were determined by power analysis. For in vitro experiments, the number of replicates were chosen based on standard practices. In general, experiments were performed with three biological replicates unless indicated otherwise. Details for each experiment are included in the respective figure legends.

Data exclusions

No data were excluded from the analysis.

Replication

All major assays have been reproduced at least 2 independent times (as noted in the figure legends) with comparable results and reproducibility.

Randomization

Not applicable to in vitro experiments. For in vivo experiments, mice were randomized into vehicle and treatment groups.

Blinding

Not applicable to the study. No human participants were involved in the study.

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 and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

Antibodies used for immuno-precipitation and immunoblotting:

-Primary antibodies that were used for IB and their dilutions include: anti-FLAG (1:2,000, F1804; MilliporeSigma), anti-myc (1:2,000, 2276S; Cell Signaling Technology), anti-HA (1:2,000, H9658; MilliporeSigma), anti- β -actin (1:5,000, A5441; MilliporeSigma), anti-p-S39-TRIM23 (custom made, 1:500), anti-TBK1 (1:1,000, 3504S; Cell Signaling Technology), anti-p-S172-TBK1 (1:1,000, 5483S; Cell Signaling Technology), anti-LC3B (1:2,000, CAC-CTB-LC3-1-50; Cosmo Bio), anti-p62 (1:2,000, GP62-C; Progen), anti-p-S403-p62 (1:1,000, 39786; Cell Signaling Technology), anti-IFIT1 (1:1,000, 14769; Cell Signaling Technology), anti-viperin (1:1,000, 13996S; Cell Signaling Technology), anti-ISG15 (1:1000, sc-166755; Santa Cruz), anti-ubiquitin (1:1,000, sc-8017; Santa Cruz), anti-HSV-1 (1:1,000, B0114; Dako).

-Secondary (HRP-linked) antibodies that were used for IB as well as their dilutions are: anti-mouse IgG (1:4000, 7076; Cell Signaling Technology); anti-rabbit IgG (1:4000, 7074; Cell Signaling Technology); light chain specific anti-rabbit IgG (1:4000, 211-032-171; Jackson ImmunoResearch); light chain specific anti-mouse IgG (1:5000, 115-035-174; Jackson ImmunoResearch); anti-guinea pig IgG (1:4000; PA128597; Invitrogen).

Antibodies used for IHC:

-anti-p62 (1:100, GP62-C; Progen) and anti-HSV-1 (1:100, B0144; Dako)

Antibodies used for confocal microscopy:

-anti-FLAG (1:200, F1804; Millipore Sigma), anti-p62 (1:200, GP62-C; Progen), anti-HSV-1/2 ICP27 (1:300, sc-69806; Santa Cruz), anti-TRIM23 (1:150, sc-393923; Santa Cruz), anti-p-S172-TBK1 (1:500, 558397; BD Biosciences), and anti-STING (1:500, AF6516; R&D Systems), Alexa Fluor 488 (1:500, A21202; Invitrogen), Alexa Fluor 555 (1:500, A21435; Invitrogen), and Alexa Fluor 633 (1:500, A21050; Invitrogen)

Antibodies used for PLA:

-anti-p-S39-TRIM23 (1:200), anti-GM130 (1:100, sc55591; Santa Cruz), anti-ATG16 (1:200, sc-393274; Santa Cruz), and anti-p-S172-TBK1 (1:200, 558397; BD Bioscience)

Validation

The custom-made phospho(p)-site specific p-S39-TRIM23 antibody (generated in this study; see details in Methods section) was validated by testing its reactivity against the TRIM23 mutant protein in which S39 was mutated (S39A). All other antibodies used in this study were obtained from commercial sources (product details including catalogue numbers are described in the Methods section) and were validated by the respective vendors.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

The following cell lines were used in this study:

Human embryonic kidney cells (HEK293T, CRL3216; ATCC); hTERT immortalized human dermal fibroblasts (HDFs, a gift from P. Hearing, Stony Brook University); human adenocarcinoma cells (HeLa, CCL-2; ATCC); HeLa cells stably expressing GFP-LC3B (a gift from W.-X. Ding, University of Kansas Medical Center); African green monkey kidney epithelial cells (Vero, CCL-81; ATCC); STAT2-deficient (U6A) and parental human sarcoma cells (2fTGH, gifts from G. Stark, Cleveland Clinic Lerner Research Institute); human neuroblastoma (SH-SY5Y, CRL-2266; ATCC); mouse neuroblasts (Neuro-2a, CCL-131; ATCC); human alveolar epithelial cells (A549, CCL-185; ATCC); and mouse embryonic fibroblasts (MEFs) generated from WT and *lfnar1*^{-/-} mice (provided by M.S. Diamond, Washington University in St. Louis).

The immortalized fibroblasts from a human patient carrying the heterozygous TBK1 germline mutation TBK1 p.G159A/WT or from health control were isolated previously by J.-L. Casanova for his study published in Herman et al., J. Exp. Med. 2012 (PMID: 22851595) under his approved protocols. For the current study, pre-existing frozen cell stocks were used and provided to M.U. Gack's laboratory without the release of any patient information (i.e., de-identified) (determined as 'IRB EXEMPT—NOT HUMAN SUBJECTS RESEARCH' by WCG IRB).

The TBK1 KO HEK293T, TBK1 KO A549, and HDFs stably expressing FLAG-tagged human TRIM23 were generated in this study using the respective parental cell lines described above.

Authentication

Cell lines obtained from commercial sources were authenticated by the respective vendors. Cell lines obtained from other investigators have been validated and published by the providers. The TBK1 KO HEK293T, TBK1 KO A549, and HDFs stably

expressing FLAG-tagged human TRIM23 generated in this study were validated as described in the manuscript.

Mycoplasma contamination

All cell lines were periodically tested for potential mycoplasma contamination and were negative for mycoplasma.

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

No cell lines used in this study are among commonly misidentified lines.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

Trim23 knockout mice (Trim23^{-/-}) in C57BL/6J background were regenerated for the current study and validated by genotyping (see Methods section). Eight-week-old WT and Trim23^{-/-} mice of both sexes (i.e., male and female) were used in this study. Mice were co-housed in a 12:12 light/dark cycle with a room temperature of 68-79°F and humidity of 30-70% and ad libitum access to standard diet and water.

Wild animals

This study did not use wild animals.

Reporting on sex

Both male and female mice were used and evaluated in this study. No differences in phenotype were observed between female and male mice.

Field-collected samples

This study did not utilize field-collected samples.

Ethics oversight

Institutional Animal Care and Use Committee (IACUC) at The University of Chicago (IACUC protocol # 72454) and Cleveland Clinic's Florida Research and Innovation Center (IACUC protocol #2486).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a