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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<br>*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<br>*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<br>*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 | Cell death was recorded with Incucyte and Cell Titer Glo assays were run on the Mithras LB 940.<br><br>For RNA seq libraries to be multiplexed in the same run were pooled in equimolar quantities, calculated from Qubit and Bioanalyser fragment analysis. Samples were sequenced on the NextSeq 500 instrument (Illumina, San Diego, US) using a either a 43bp or 81bp paired end run.<br><br>LC-MS/MS was performed on a Q Exactive Orbitrap Plus interfaced to a NANOSPRAY FLEX ion source and coupled to an Easy-nLC 1000 (Thermo Scientific). Peptides were separated on a 24 cm fused silica emitter, 75 µm diameter, packed in-house with Reprosil-Pur 200 C18-AQ, 2.4 µm resin (Dr. Maisch) using a linear gradient from 5% to 30% Acetonitrile/ 0.1% Formic acid over 10min (for TBK1 AP-MS) or 30 min (for TNFR-SC AP-MS and kinase assay), at a flow rate of 250 nL/min. Precursor ions were measured in a data-dependent mode in the orbitrap analyser at a resolution of 70,000 and a target value of 3e6 ions. The ten most intense ions from each MS1 scan were isolated, fragmented in the HCD cell, and measured in the orbitrap at a resolution of 17,500. |
| Data analysis | Quantifications were performed with Excel, ImageJ and GraphPad and statistical analysis with GraphPad. Images and figures were processed with Adobe Photoshop and Illustrator CS6, respectively.<br><br>For RNAseq analysis data were demultiplexed and converted to fastq files using Illumina's bcl2fastq Conversion Software v2.19. Next, the expression of Illumina paired RNA-Seq transcript counts was quantified using kallisto software65 and a GRCh38 transcript model. The data was imported to the R statistical environment and summarised at the gene level (i.e transcript counts summed) using tximport. |

Statistical transformations and analysis of differential expression were performed with DESeq271.
Relevant transcripts were illustrated using BioVenn software.

For protein and phosphosite identification raw data were analysed with MaxQuant version 1.5.2.8 where they were searched against the human UniProt database (http://www.uniprot.org/, downloaded 22/10/2015 (for TNFR-SC AP-MS) and 05/10/2017 (for TBK1 AP-MS and kinase assay) using default settings. Label-free quantification in MaxQuant was used to quantify the AP-MS data.

For processing of the TNFR1-SC and TBK1 AP-MS data a protein-protein interaction network was generated in the STRING protein-protein interaction database using known interactions from curated databases and experimentally determined at default settings.

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

The RNA-Seq dataset generated during the current study are available in the SRA repository and can be accessed by using the following BioProject accession: PRJNA422567 or SRA accession: SRP126844 (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=SRP126844).

The proteomic data has been deposited on the ProteomeXchange Consortium via the PRIDE(ref) partner repository with the dataset identifier PXD008497 (TNFR1-SC analysis), PXD010777 (TBK1 analysis), and PXD008518 (RIPK1 kinase assay).

# 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 ☐ Ecological, evolutionary & environmental 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 | No statistical method was used to determine the correct sample size. Instead the sample sizes were determined based on our experiences from previous studies using similar methodologies. |
| Data exclusions | No data was excluded from the study. |
| Replication | All biological experiments were carried out under clearly defined and standard conditions and were repeated at least twice whenever possible. All replication attempts were successful. |
| Randomization | Mice were randomly allocated to experimental groups. |
| Blinding | In vivo experiment was done by a scientist blinded to the treatment schedule. The in vitro experiments were not carried out blinded but most of them were done in parallel by at least two researchers. |

# Reporting for specific materials, systems and methods

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Unique biological materials |
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology |
| ☐ | ☒ Animals and other organisms |
| ☒ | ☐ Human research participants |

## Methods

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

# Unique biological materials

Policy information about availability of materials

Obtaining unique materials: Knockout cell lines were generated using the CRISPR-Cas9 system and are readily available under request upon publication of the study.

# Antibodies

Antibodies used: p-TBK1 (Cell Signaling, 5483); TBK (Cell signaling, 3013); p-IKKe (Cell signaling, 8766); IKKe (Cell signaling, 2905); p-IKKa/b (Cell signaling, 2697); RIPK1 (Cell signaling, 3493); p-IkBa (Cell Signaling, 9246); IkBa (Cell signaling, 9242); p-ERK1/2 (Cell signaling, 4370); ERK1/2 (Cell signaling, 4695); p-JNK (Cell signaling, 4671); JNK (Cell signaling, 9258); p-p38 (Cell signaling, 9215); p-RIPK1 S166 (Cell signaling, 65746); murine p-RIPK1 S166 (Cell signaling, 31122); cleaved murine Caspase-8 (Cell signaling, 9429); HOIP (Ubiquigent, 68-0013-100); SHARPIN (Proteintech, 14626-1-AP); RIPK1 (BD, 610459); TNFR-1 (Santa Cruz, SC-8436); GAPDH (Abcam, ab8245); Actin (Sigma, A1978); M1-Ubiquitin (Merck Millipore, MABS199); p38 (Santa Cruz Biotech, sc-728); murine caspase-8 (Enzo Life Sciences, C15); p-MLKL (Abcam, ab187091); murine p-MLKL (Abcam, ab196436); murine RIPK3 (Enzo Life Sciences, ADI-905-242-100); FLAG (Sigma, M2); FADD (Enzo Life Sciences, 1F7); FADD (Santa Cruz, H-181); TANK (R&D Systems; AF4755); AZI2 / NAP1 (abcam, ab192253); Optineurin (abcam, ab151240); IKKγ (Santa Cruz,FL-419; sc-8330); SINTBAD (Cell Signaling, 8605); HOIL-1 (made in house); IKKa (Santa Cruz, B-8; sc-7606); TRAF2 (Enzo Life Sciences, ADI-AAP-422-D); FADD (Santa Cruz Biotech, sc-6036/M-19).
All antibodies used in this study were diluted 1:1000. Only p-IKKe (Cell signaling, 8766) was used 1:500 and Actin (Sigma, A1978) was diluted 1:10000.

Validation: p-TBK1 (Cell Signaling, 5483); validation https://www.cellsignal.com/products/primary-antibodies/phospho-tbk1-nak-ser172-d52c2-xp-rabbit-mab/5483
TBK (Cell signaling, 3013); validation: https://www.cellsignal.com/products/primary-antibodies/phospho-tbk1-nak-ser172-d52c2-xp-rabbit-mab/5483?site-search-type=Products&N=4294956287&Ntt=phospho-tbk1+%28s172%29+%28d52c2%29+xp%C3%82%C2%AE+&fromPage=plp
p-IKKe (Cell signaling, 8766); validation: https://www.cellsignal.com/products/primary-antibodies/phospho-ikke-ser172-d1b7-rabbit-mab/8766
IKKe (Cell signaling, 2905); validation: https://www.cellsignal.com/products/primary-antibodies/ikke-d20g4-rabbit-mab/2905
p-IKKa/b (Cell signaling, 2697); validation: https://www.cellsignal.com/products/primary-antibodies/phospho-ikka-bser176-180-16a6-rabbit-mab/2697
RIPK1 (Cell signaling, 3493); validation: https://www.cellsignal.com/products/primary-antibodies/rip-d94c12-xp-rabbit-mab/3493
p-IkBa (Cell Signaling, 9246); validation: https://www.cellsignal.com/products/primary-antibodies/phospho-ikba-ser32-36-5a5-mouse-mab/9246
IkBa (Cell signaling, 9242); validation: https://www.cellsignal.com/products/primary-antibodies/ikba-antibody/9242
p-ERK1/2 (Cell signaling, 4370); validation: https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapkerk1-2-thr202-tyr204-d13-14-4e-xp-rabbit-mab/4370
ERK1/2 (Cell signaling, 4695); validation: https://www.cellsignal.com/products/primary-antibodies/p44-42-mapk-erk1-2-137f5-rabbit-mab/4695
p-JNK (Cell signaling, 4671); validation: https://www.cellsignal.com/products/primary-antibodies/phospho-sapk-jnk-thr183-tyr185-98f2-rabbit-mab/4671
JNK (Cell signaling, 9258); validation: https://www.cellsignal.com/products/primary-antibodies/jnk2-56g8-rabbit-mab/9258
p-p38 (Cell signaling, 9215); validation: https://www.cellsignal.com/products/primary-antibodies/phospho-p38-mapk-thr180-tyr182-3d7-rabbit-mab/9215
p-RIPK1 S166 (Cell signaling, 65746); validation: https://www.cellsignal.com/products/primary-antibodies/phospho-rip-ser166-d1l3s-rabbit-mab/65746
murine p-RIPK1 S166 (Cell signaling, 31122); validation: https://www.cellsignal.com/products/primary-antibodies/phospho-ripser166-
antibody-rodent-specific/31122
cleaved murine Caspase-8 (Cell signaling, 9429) validation: https://www.cellsignal.com/products/primary-antibodies/cleavedcaspase-
8-asp387-antibody-mouse-specific/9429
HOIP (Ubiquigent, 68-0013-100); validated by using ctr versus HOIP KO cells
SHARPIN (Proteintech, 14626-1-AP); validated by using ctr versus SHARPIN KO cells
RIPK1 (BD, 610459); validation: http://www.bdbiosciences.com/us/applications/research/apoptosis/purified-antibodies/purifiedmouse-
anti-rip-38rip/p/610459
TNFR-1 (Santa Cruz, SC-8436); validation: https://www.scbt.com/scbt/product/tnf-r1-antibody-h-5

GAPDH (Abcam, ab8245); validation: https://www.abcam.com/gapdh-antibody-6c5-loading-control-ab8245.html
Actin (Sigma, A1978); validation: https://www.sigmaaldrich.com/catalog/product/sigma/a1978?lang=en&region=GB&gclid=EAIaIQobChMImI25oIuA3QIVDbTtCh2DJgebEAAYASAAEgJlm_D_BwE
M1-Ubiquitin (Merck Millipore, MABS199); validation: http://www.merckmillipore.com/GB/en/product/Anti-Linear-Ubiquitinclone-1E3-Antibody,MM_NF-MABS199
p38 (Santa Cruz Biotech, sc-728); validation: https://www.scbt.com/scbt/product/p38alpha-antibody-n-20
murine caspase-8 (Enzo Life Sciences, C15); validation by using Caspase-8 KO MEFs versus wt MEFs
p-MLKL (Abcam, ab187091); validation: https://www.abcam.com/mlkl-phospho-s358-antibody-epr9514-ab187091.html
murine p-MLKL (Abcam, ab196436); validation: https://www.abcam.com/mlkl-phospho-s345-antibody-epr95152-ab196436.html
murine RIPK3 (Enzo Life Sciences, ADI-905-242-100); validation: http://www.enzolifesciences.com/ADI-905-242/rip3-polyclonalantibody/
FLAG (Sigma, M2); validation: https://www.sigmaaldrich.com/catalog/product/sigma/f1804?lang=en&region=GB&gclid=EAIaIQobChMIgrjX1I2A3QIVBbXtCh1HrQLgEAAYASAAEgJhUfD_BwE
FADD (Enzo Life Sciences, 1F7); validation: http://www.enzolifesciences.com/ADI-AAM-212/fadd-monoclonal-antibody-1f7/
FADD (Santa Cruz, H-181); validation: https://www.scbt.com/scbt/product/fadd-antibody-h-181
TANK (R&D Systems; AF4755); validation: https://www.rndsystems.com/products/human-mouse-tank-antibody_af4755
AZI2 / NAP1 (abcam, Ab192253); validation: https://www.abcam.com/azi2-antibody-epr14698-c-terminal-ab192253.html
Optineurin (abcam, ab151240); validation: https://www.abcam.com/optineurin-antibody-ab151240.html
IKKγ (Santa Cruz,FL-419; sc-8330); validation: https://www.scbt.com/scbt/product/ikkgamma-antibody-fl-419
SINTBAD (Cell Signaling, 8605); validation: https://www.cellsignal.co.uk/products/primary-antibodies/sintbad-d1a5-rabbitmab/8605
HOIL-1 (Home made; validated by comparing lysates from HOIL-1 KO to control cells)
IKKa (Santa Cruz, B-8; sc-7606); validation: https://www.scbt.com/scbt/product/ikkalpha-antibody-b-8#
TRAF2 (Enzo Life Sciences, ADI-AAP-422-D); validation: http://www.enzolifesciences.com/ADI-AAP-422/traf2-polyclonal-antibody/
FADD (Santa Cruz Biotech, sc-6036/M-19); validation: https://www.scbt.com/scbt/product/fadd-antibody-m-19

Most of the listed antibodies have been used in our laboratory for many years and have been carefully validated using knockout MEFs or more recently via different techniques including siRNA kd, CrispR, TALEN or ZNF approach to achieve knockout cells. Many antibodies were also validated by the provider as stated on their website.

## Eukaryotic cell lines

Policy information about cell lines

| | |
|---|---|
| Cell line source(s) | A549 and HeLa cells were sourced from ATCC, IKKa/b KO MEFs were provided by Matthieu Bertrand, NEMO KO MEFs were provided from Manolis Pasparakis. HOIP KO MEFs were generated in our lab as well as TNF KO MEFs. All other knockout cells presented in this study were generated in our lab using the CRISPR-Cas9 system. |
| Authentication | Authentication was not performed as none of the cells used have been listed in the commonly misidentified lines. |
| Mycoplasma contamination | All cell lines were regularly tested for mycoplasma using the MycoAlert™ Mycoplasma Detection Kit (LONZA) |
| Commonly misidentified lines (See ICLAC register) | No misidentified lines were used in this study |

## Animals and other organisms

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

| | |
|---|---|
| Laboratory animals | Six- to eight-week old female C57BL/6N mice were used in this study |
| Wild animals | No wild animals were used in this study |
| Field-collected samples | No field-collected samples were used in this study |