

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	-Cell death: BioTek SYNERGY HI microplate reader,Gen5 3.08.01; -q-RT-PCR: QuantStudio 12K Flex software version 1.3 (Applied Biosystems) -Gel blots scan: EPSON scan version 3.9.3.6(EPSON) -Proteomics raw data: TimsTOF Pro mass spectrometer (Bruker, Bremen, Germany)
Data analysis	-Mass spectrometry: Fragpipe(v21.1) -Image analysis: HALO (v4.0.5107.318) -Statistical analysis: GraphPad Prism version 9.5 (v9.5.0)

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 supporting the findings of this study are available within the paper and its Supplementary Information. The mass spectrometry proteomics data generated in this study have been deposited in the ProteomeXchange Consortium via the iProX partner repository under accession code PXD056588 (<https://proteomecentral.proteomexchange.org>). The RNA-seq data generated in this study have been deposited at Gene Expression Omnibus (GEO) database under accession code GSE279034, GSE279700 and GSE279702 (<https://www.ncbi.nlm.nih.gov/geo>). The 16S rDNA seq data generated in this study are available in Supplementary Dataset file. Source data are provided as a Source Data file. Uncropped western blot scans are shown in the Source Data File. Source data are provided with this paper. Anyone who wishes to share, reuse, remix, or adapt the data must obtain permission from corresponding author.

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	Data for cell death measurement are presented as mean $\pm$ SEM from biological replicates. Mouse data are presented as mean $\pm$ SEM of the indicated <i>n</i> values. No statistical method was used to calculate sample size. Sample size was determined according to our experience as well as literature reporting in terms of specific experiments. Sample numbers were well described in the figure legends.
Data exclusions	No data were excluded from the analyses.
Replication	For in vitro experiment, each experiment was repeated at least 3 times with similar results. For animal experiment at least 3 mice were used in each group. All findings were replicated successfully.
Randomization	For animal experiments, age-matched mice with different genotypes were randomly divided into different experimental groups.
Blinding	For the in vivo experiments, the blinding was performed when the in vivo data were collected. Blinding during analysis in vivo experiments was performed between experimental and control group. For experiments other than the in vivo studies, the investigators were blinded during data collection and analysis where possible. For the in vitro study such as cell death assay, blinding is not performed as the results of cell viability were determined by an automated pipeline using a fluorescent plate reader in an unbiased manner.

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

For WB: TAK1 (Abcam, ab109526, 1:1,000), p-S166 RIPK1 (Biolynx, BX60008, 1:1,000), RIPK1 (homemade, 1:1,000), TRADD (homemade, 1:1,000), Cleaved Caspase-3 (CST, 9661, 1:1,000), Cleaved Caspase-8 (CST, 8592, 1:1,000), Caspase-3 (CST, 9662, 1:1,000), Caspase-8 (CST, 4790, 1:1,000), GSDMD (Abcam, ab209845, 1:1,000), Caspase-1 (p20) (AdipoGen, AG-20B-0042-C100, 1:1,000), p-ERK1/2 (CST, 4370, 1:1,000), p-p38 (CST, 4511, 1:1,000), p-JNK (CST, 4668, 1:1,000), p-NF-κB p65 (CST, 3033, 1:1,000), ERK1/2 (CST, 9102, 1:1,000), p38 (CST, 8690, 1:1,000), p-JNK (CST, 9252, 1:1,000), NF-κB p65 (CST, 8242, 1:1,000), FADD (Abcam, ab124812, 1:1,000), RIPK3 (ProSci, 2283, 1:1,000), MLKL (Abgent, AP14272b, 1:1,000), p-MLKL (Abcam, ab196436, 1:1,000), p-RIPK3 (Abcam, ab195117, 1:1,000), HA (CST, 3724, 1:1,000), β-Tubulin (MBL, PM054, 1:2000), β-actin (TransGen Biotech, HC201, 1:2000). For IHC: Cleaved Caspase-3 (CST, 9661, 1:200), Cleaved Caspase-8 (CST, 8592, 1:200), lysozyme-1 (Invitrogen, PA5-16668, 1:200) and Ly-6G (Biolegend, 127608, 1:200)

Validation

TAK1 (Abcam, ab109526, 1:1,000), <https://www.abcam.cn/products/primary-antibodies/tak1-antibody-epr5984-ab109526.html>

p-S166 RIPK1 (Biolynx, BX60008, 1:1000), <https://www.biolynxtec.com/products/antibody/p-ripk1s166.html>. Validated for WB by many previous publications (PMID: 33311474).

RIPK1 (homemade, 1:1,000). Validated for WB by many previous publications (PMID: 33311474)

TRADD (homemade, 1:1,000). Validated for WB in this paper.

Cleaved Caspase-3 (CST, 9661, 1:1,000 for WB, 1:200 for IHC), <https://www.cellsignal.cn/products/primary-antibodies/cleaved-caspase-3-asp175-antibody/9661>

Cleaved Caspase-8 (CST, 8592, 1:1,000 for WB, 1:200 for IHC), <https://www.cellsignal.cn/products/primary-antibodies/cleaved-caspase-8-asp387-d5b2-xp-rabbit-mab/8592>

Caspase-3 (CST, 9662, 1:1,000), <https://www.cellsignal.cn/products/primary-antibodies/caspase-3-antibody/9662>

Caspase-8 (CST, 4790, 1:1,000), <https://www.cellsignal.cn/products/primary-antibodies/caspase-8-d35g2-rabbit-mab/4790>

GSDMD (Abcam, ab209845, 1:1,000), <https://www.abcam.cn/products/primary-antibodies/gsdmd-antibody-epr19828-ab209845.html>

Caspase-1 (p20) (AdipoGen, AG-20B-0042-C100, 1:1,000), <https://adipogen.com/ag-20b-0042-anti-caspase-1-p20-mouse-mab-casper-1.html>

p-ERK1/2 (CST, 4370, 1:1,000), <https://www.cellsignal.cn/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-d13-14-4e-xp-rabbit-mab/4370>

p-p38 (CST, 4511, 1:1,000), <https://www.cellsignal.cn/products/primary-antibodies/phospho-p38-mapk-thr180-tyr182-d3f9-xp-rabbit-mab/4511>

p-JNK (CST, 4668, 1:1,000), <https://www.cellsignal.cn/products/primary-antibodies/phospho-sapk-jnk-thr183-tyr185-81e11-rabbit-mab/4668>

p-NF-κB p65 (CST, 3033, 1:1,000), <https://www.cellsignal.cn/products/primary-antibodies/phospho-nf-kb-p65-ser536-93h1-rabbit-mab/3033>

ERK1/2 (CST, 9102, 1:1,000), <https://www.cellsignal.cn/products/primary-antibodies/p44-42-mapk-erk1-2-antibody/9102>

p38 (CST, 8690, 1:1,000), <https://www.cellsignal.cn/products/primary-antibodies/p38-mapk-d13e1-xp-rabbit-mab/8690>

p-JNK (CST, 9252, 1:1,000), <https://www.cellsignal.cn/products/primary-antibodies/sapk-jnk-antibody/9252>

NF-κB p65 (CST, 8242, 1:1,000), <https://www.cellsignal.cn/products/primary-antibodies/nf-kb-p65-d14e12-xp-rabbit-mab/8242>

FADD (Abcam, ab124812, 1:1,000), <https://www.abcam.cn/products/primary-antibodies/fadd-antibody-epr5030-ab124812.html>

RIPK3 (ProSci, 2283, 1:1,000), <https://www.prosci-inc.com/rip3-antibody-2283.html>

MLKL (Abgent, AP14272b, 1:1,000), <https://www.abcepta.com/products/AP14272b-Mouse-Mlkl-Antibody-C-term>

p-MLKL (Abcam, ab196436, 1:1,000), <https://www.abcam.cn/products/primary-antibodies/mlkl-phospho-s345-antibody-epr95152-ab196436.html>

p-RIPK3 (Abcam, ab195117, 1:1,000), <https://www.abcam.cn/products/primary-antibodies/rip3-phospho-s232-antibody-epr9516n-25-ab195117.html>

HA (CST, 3724, 1:1,000), <https://www.cellsignal.cn/products/primary-antibodies/ha-tag-c29f4-rabbit-mab/3724>

α -Tubulin (MBL, PM054, 1:2000), <https://products.mblintl.com/products/pm054/>

β -actin (TransGen Biotech, HC201, 1:2000), https://www.transgenbiotech.com/loading_controls/proteinfind_anti_b_actin_mouse_monoclonal_antibody.html

lysozyme-1 (Invitrogen, PA5-16668, 1:200 for IHC), <https://www.thermofisher.cn/antibody/product/PA5-16668.html?CID=AFLCA-PA5-16668>

Ly-6G (Biolegend, 127608, 1:200 for IHC), <https://www.biolegend.com/en-us/products/pe-anti-mouse-ly-6g-antibody-4777>

Eukaryotic cell lines

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

Cell line source(s)	-HEK293T, ATCC, CRL3216 -MEFs were homemade from mice of indicated genotypes, this study -Primary BMDMs, this study
Authentication	No additional authentication was performed.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	There is no ICLAC line used in this study.

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	All mice were in the C57BL/6J background. Tak1 ^{fl/fl} mice were kindly provided by Shizuo Akira of Osaka University, Japan (PMID: 16186825). Villin-cre mice were purchased from SHANGHAI MODEL ORGANISMS company. Villin-creERT2 mice were gifts kindly provided by Dr. Wei Mo (PMID: 32296174). Ripk1D138N/D138N mice were generated via CRISPR/Cas9 system as described previously (PMID: 32513687). Tradd ^{fl/fl} mice were purchased from Cyagen Biosciences (strain no. S-CKO-15109). Fadd ^{-/-} Mlkl ^{-/-} mice were gifts kindly provided by Dr. Haibing Zhang (PMID: 27498868).
Wild animals	This study did not involve wild animals
Reporting on sex	Male and female Tak1 ^{IEC-KO} mice with or without Ripk1D138N/D138N, Tradd ^{IEC-KO} , Fadd ^{-/-} , Mlkl ^{-/-} ranging in age from P0 to P4 were analyzed. Only male mice of 7-8 weeks were used for Villin-creERT2 recombinase activity induction.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	Animal experiments were conducted according to the protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the Institute of Interdisciplinary Research Center on Biology and Chemistry and the experiments involving mice were performed according to the IACUC's guidelines (Approval no. ECSIOC2021-04).

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.