

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

qRT-PCR data were generated by ABI ViiA7 Real-Time PCR system (Applied Biosystems). Luciferase reporter was measured by a Lumat Luminometer (Berthold). Western blotting analysis was measured by a LAS4000mini or LAS3000 instrument (GE Healthcare). LDH release assay was measured by SpectraMax M5 (molecular device). DNA concentrations were measured by DU 800 (BECKMAN COULTER). Protein concentration was measured by SpectraMax M5 (molecular device) or DU 800 (BECKMAN COULTER). For Mass spectrometric analysis we used Nanoflow UHPLC, Easy nLC 1000 (Thermo Fisher Scientific), and Q Exactive instrument (Thermo Fisher Scientific). Cell survival data on iCelligence system were acquired by using RTCA iCelligence Software version 1.0 (ACEA Biosciences). H/E staining images were collected using cellSens standard Software version 1.14 (Olympus), and Immunofluorescence images were collected using BZ-II viewer application (Keyence), FV10-ASW ver1.6 (Olympus), Zeiss 710 Confocal Microscope (ZEISS) and Structured illumination microscopy (SIM) images were obtained using Nikon N-SIM system (Nikon). Flow cytometry data was collected by using FACS Canto II (BD Biosciences) and FACS Diva software ver 6.1.2 (Becton Dickinson).

Data analysis

GraphPad Prism8 version 8.4.0 was used to perform statistical analysis. Mass spectrometric data were analyzed using Mascot and SEQUEST in Proteome Discoverer 1.3 (Thermo Fisher Scientific). Image Gauge ver4.22 (FUJIFILM) was used for chemiluminescent images from LAS-4000 Image reader. ViiA7 RUO Software ver1.2.3 (Thermo Fisher Scientific) was used for RNA expression analysis. Recording data of cell survival using iCelligence system was analyzed by RTCA Data Analysis Software 1.0 (ACEA Biosciences). Immunofluorescence images were processed using the BZ-II image analysis application (Keyence) and SIM Image processing were carried out using the NIS-Element advanced research software version 5.1 (Nikon). Figures in the manuscript were arranged and converted into PDF files by using Illustrator version 24.0.3 (Adobe). Plots of skin scores (Figure.8d) were made using Excel for Mac version 16.34 (Microsoft). FlowJo software (Tomy Digital Biology, version 9.9.6) was used for all analyses of flow cytometry data.

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

We got protein sequences from uniprot and got DNA sequences from Genbank(NM_001083921, NM_031229, NM_017999.5, NM_194346).The authors declare that the other data supporting the findings of this study are available within the paper and its supplementary information files.

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	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. At least 3 animals of each genotype were analyzed.
Data exclusions	A few animals were excluded from skin scores due to fight wounds, dystocia or genotype-independent hypotrophy.
Replication	All findings were confirmed by at least two independent experiments including at least 3 animals of each genotypes or at least three independent in vitro assays.
Randomization	For in vivo experiments, animals were allocated to different experimental groups based on gender and age to equal distribution.
Blinding	In general, all animal experiments were under a blinded condition because mice were identified only by mice ID but not genotype during data collection.

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

All antibodies including dilution were described in Supplementary Table1.
 IκBα (# 4812, 44D4, Cell Signaling Technology), pIκBα (#9246, 5A5, Cell Signaling Technology), Caspase3 (#9662, Cell Signaling Technology), RIP1 (#3493, D94C12, Cell Signaling Technology), cleaved Caspase3 (#9661, D175, Cell Signaling Technology), cleaved Caspase8 (#9496, 18C8, Cell Signaling Technology), OTULIN (#14127S, Cell Signaling Technology), SHARPIN (#4444, Cell Signaling Technology), Anti-mouse IgG, HRP-linked Antibody (#7076, Cell Signaling Technology), Myc (#05-724, 4A6, Millipore), Linear ubiquitin chains (#MABS199, 1E3, Millipore), Lys-48 specific ubiquitin (#05-1307, Apu2, Millipore), Lys-63 specific ubiquitin (#05-1308, Apu3, Millipore), HA (#sc-805, Y11, Santa Cruz Biotechnology), IKKγ(sc-8330, FL-419, Santa Cruz Biotechnology), FADD (sc-6036, M-19, Santa Cruz Biotechnology), TRADD(sc-46653, A-5, Santa Cruz Biotechnology), Ubiquitin (sc-8017, P4D1, Santa Cruz Biotechnology), DDDDK (#M185-3S, FLA-1, MBL), multi-ubiquitin (#D058-3, FK2; MBL), IKKγ (#K0159-3, EA2-6, MBL), HA (#M180-3, Tana2, MBL), DDDDK-tag (PM020, MBL), His (#D291-3, OGHIS, MBL), FLAG (#F-3165, M2, Sigma-Aldrich), β-Actin (AC-74, A5316, Sigma-Aldrich), FADD (#ADI-AAM-212-E, 1F7, ENZO), Caspase8 (#ALX-804-447-C100, 1G12, ENZO), TNFR1

(ab19139, Abcam), tubulin (#CLT9002, CEDARLANE), RIP3 (# IMG-5523-2, IMGENEX), HRP conjugated anti-rabbit secondary antibody (NA934V, Amersham Pharmacia), AffiniPure Goat Anti-Mouse IgG light chain specific (115-005-174, Jackson Laboratory), Goat anti-Mouse IgG Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (A-21235, Thermo Fisher Scientific), Goat anti-Rabbit IgG Highly Cross-Adsorbed Secondary Antibody Alexa Fluor 488 (A-11034, Thermo Fisher Scientific), HOIL-1L (2E2, our laboratory), HOIL-1L N-terminal (our laboratory), SHARPIN (our laboratory), and HOIP (our laboratory), HOIP 1CB2 (our laboratory), Linear ubiquitin chains (LUB9, our laboratory), Myc (9E10, our laboratory), PE/Cy7 CD19 (#115520, 6D5, BioLegend), PE anti-CD8 α (#100708, 53-6.7, BioLegend), FITC-CD38 (#102705, 90, BioLegend), CD4 (#13-0041-85, GK1.5, eBioscience), FITC-CD3 ϵ (#100306, 145-2C11, BioLegend), PerCP Streptavidin (#405213, BioLegend), CD25, PE (#12-0251-82, PC61.5, BD Biosciences), PE-CD95 (FAS) (554258, BD Biosciences), PNA-biotin (#B-1075, Vector Laboratories), TCR beta monoclonal antibody FITC (H57-597 #11-5961-82 eBioscience)

Validation

All primary antibodies except for HOIL-1L, SHARPIN and HOIP are commercial and validated by manufactures. Antibodies for HOIL-1L, SHARPIN and HOIP were made in our laboratory and validated previously (Tokunaga et al., Nature Cell Biology, 2009)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

We reported previously generations of LUBAC TKO MEFs (Fujita et al., cell reports, 2018), cpdmMEFs (Tokunaga et al., Nature, 2011) and HEK293T cells in house.

Authentication

None of the cell lines used were not authenticated.

Mycoplasma contamination

We checked regularly mycoplasma contaminations for cell lines using Salmonella infectious experiments. For other experiments, we didn't test for mycoplasma contaminations.

Commonly misidentified lines (See ICLAC register)

No commonly misidentified cell line was used.

Animals and other organisms

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

Laboratory animals

Mice were bred and housed in a full barrier, specific pathogen free (SPF) animal facility at Kyoto University. SHARPINcpdm/cpdm mice were obtained from the Jackson Laboratory. CAG-Cre mice were obtained from RIKEN BioResource Center (RBRC No. RBRC01828). Information regarding animals is available in the Methods section and Figure legends. In general, age-matched male and female C57BL/6 adult mice were used.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight

All animal studies were approved by Animal Research Committee, Graduate School of Medicine, Kyoto University.

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

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Single-cell suspensions from spleens of mice were generated by macerating the tissues through a 70mm nylon mesh. For flow cytometry, 2×10^6 cells were incubated with antibodies described above.

Instrument

FACSCanto II (BD Biosciences) for flow cytometric analyses

Software

FACS Diva software ver 6.1.2 (Becton Dickinson) for data collection.
FlowJo version 9.9.6 for data analysis..

Cell population abundance

After antibody staining of single cells, FSC - SSC gating was used to determine pure live cell population to be analyzed.

Gating strategy

Gating strategies were clearly described in Supplementary Figure1. FSC-A and FSC-H was used to identify single cells. Basically, live cells were gated with FCS and SSC and subsequently gated with various markers as indicated in the figures.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.