nature portfolio

Corresponding author(s): Jaewhan Song

Last updated by author(s): Nov 5, 2024

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 (*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	For western blot images acquired by Vilber Fusion Imaging System. Images for immunofluorescence analysis were conducted with Zeiss Zen LSM980 confocal microscope
Data analysis	The data were analyzed using Microsoft Excel and statistical analysis was performed using GraphPad Prism.

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

The corresponding author can provide the datasets used in this study upon reasonable request. Source data are provided with this paper.

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	This study did not involve samples from human participants
Reporting on race, ethnicity, or other socially relevant groupings	This study did not involve samples from human participants
Population characteristics	This study did not involve samples from human participants
Recruitment	This study did not involve samples from human participants
Ethics oversight	This study did not involve samples from human participants

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Data from in vitro studies were obtained from at least three independent experiments. For mouse experiments, we aimed to have a minimum of 10 mice per each subtype to capture the heterogeneity present in each group. Specific sample numbers for mouse experiments are described in figure legends
Data exclusions	Outliers were excluded in some experiments.
Replication	The experiments were performed at least three times with similar results, the findings were reliably reproduced.
Randomization	Mice were randomly assigned to experimental groups
Blinding	Mice were treated blindly to the experiments.

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	RIPK1 (#610459; BD Biosciences), RIPK3 (#13526; Cell Signaling Technology), MLKL (#GTX107538; GeneTex), phospho-RIPK1 Ser166 (#65746; Cell Signaling), phospho-RIPK3 Ser227 (#ab209384; Abcam), phospho-MLKL Ser358 (#91689S; Cell Signaling), mouse RIPK3

(NBP1-77299; Novus Biologicals), mouse MLKL (AP14272b; Abgent), phospho-mouse MLKL Ser 345 (#37333; Cell Signaling), SMURF1 (#sc-100616; Santacruz), USP5 (sc-390943; Santacruz), IKKβ (#2370, Cell Signaling), IκBα (#4814, Cell Signaling), phospho-IκBα (Ser539) (#2859, Cell Signaling), TNFR1 (#AF225;R&D Systems), TRADD (#610572; BD transduction), Ubiqutin (P4D1) mouse mAB(HRP conjugate) (#14049, Cell Signaling), K63-linkage Specific Polyubiquitin (D7A11) Rabbit mAb (HRP Conjugate) (#12930, Cell Signaling), Actin (#A5316; Sigma-Aldrich), HA-Peroxidase (#12013819001; Sigma-Aldrich), FLAG-Peroxidase (#SAB4200119; Sigma-Aldrich), MYC (#sc-40: Santacruz) were utilized.

Validation

Antibodies were used according to the validated listed by the manufacturer's.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HT-29 (human colorectal carcinoma; HTB-38; ATCC, Manassas, VA, USA), TC-1 (mouse lung cancer cell line; CRL-2785; ATCC), and Molm-13 (acute myeloid leukemia; ATCC), NB-4 (acute myeloid leukemia; ATCC) cells were maintained in Roswell Park Memorial Institute (RPMI; HyClone, Chicago, IL, USA) in 5% CO2 at 37°C. HEK 293T (human embryonic kidney; CRL-3216; ATCC), HeLa (cervical cancer cell line, ATCC) were maintained in Dulbecco's modified Eagle's medium (DMEM; HyClone) in 5% CO2 at 37°C. All media were supplemented with 10% fetal bovine serum (HyClone) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA). All cells were tested mycoplasma contamination using e-Myco™ plus Mycoplasma PCR Detection Kit (#25237; Intron, Seongnam, Gyeonggi, South Korea).
Authentication	All cell lines used in this study were authenticated by STR profile report.
Mycoplasma contamination	All cell lines were protected from mycoplasma infection by treatment with Plasmocin TM (InvivoGen).
Commonly misidentified lines (See ICLAC register)	We did not use any misidentified cell 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	6-week-old female BALB/c nude mice. Mice were housed in a 12 h light-dark cycle environment, wherein the ambient temperature was maintained at 21-23 degrees celsius
Wild animals	No wild animals were used for this study
Reporting on sex	Only female mice were used in the experiments because male mice are typically more aggressive, potentially causing stress and variability in the results.
Field-collected samples	No field-collected samples were used.
Ethics oversight	All animal experiments involving the subcutaneous tumor xenograft model were approved by the Institutional Animal Care and Use Committee of the Laboratory Animal Research at Yonsei University (IACUC-A-202407-1881-01)

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

Plants

Seed stocks	This study did not involve samples from plants
Novel plant genotypes	This study did not involve samples from plants
Authentication	This study did not involve samples from plants

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	To determine cell death, HT-29 and TC-1 cells using Annexin V and 7-AAD double staining. The treated cells were collected and washed with PBS, then incubated with Annexin V-FITC (556547; BD Biosciences) and 7-AAD (00-6993-50; eBioscience) in Annexin V binding buffer (51-66121E; BD Biosciences) for 15 minutes. The HeLa-RIPK3 WT, K55R, K363R, K55/363R, Molm-13 and NB-4 cells were using propidium iodide (PI) single staining The treated cells were collected and washed with PBS, then incubated with PI (P4170; Sigma-Aldrich) for 15 minutes. Dead cells were identified as the PI-positive population. The stained cells were analyzed by flow cytometry (BD Accuri C6, BD Biosciences). Data analysis was performed using BD Accuri C6 Plus software (BD Biosciences). All cell viability assays were conducted in triplicate, with results presented as the mean±standard deviation.
Instrument	Cell fluorescence was measured using an Accuri 6 instrument (BD Biosciences).
Software	BD Accuri C6 software used in this study.
Cell population abundance	After checking FSC (Forward Scatter) and SSC (Side Scatter), gate the population of normal cells and analyze 10,000 cells.
Gating strategy	Based on FSC and SSC, gate 10,000 normal cells. Then, quantify cell death by calculating the percentage of PI-positive or Annexin V-7AAD-positive cells.

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