

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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>
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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)
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input checked="" type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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 data were acquired on an Incucyte 2024B instrument. RNA sequencing was carried out on the Illumina NovaSeq X+ platform. Confocal microscopy images were collected on a Leica SP8 instrument.
Data analysis	<p>All code used to analyze sequencing data in this study is publicly available at https://github.com/alnfedorov/Z-DoTT. Key dependencies are summarized below.</p> <p>* Human annotation was acquired from the GRCh38 to the CHM13v2 assembly using Liftoff (v1.6.3).</p> <p>* RNA-seq and RIP-seq experiments were preprocessed using a custom fork of the nf-core "rnaseq" pipeline (https://github.com/alnfedorov/rnaseq, commit 41e95d6a4a24d1f4fe4f2c50bb4d9a4744158c9b). During preprocessing, the following core tools were used: fastp (v0.23.4), STAR (v2.7.10a), samtools (v1.2), picard (v3.1.1), and salmon (v1.10.1).</p> <p>* Subsequent analysis involved using biobit (https://github.com/biomancy/biobit, v0.0.4), DESeq2 (v1.46.0), DEXSeq (v1.52.0), apeglm (v1.28.0), pyCircIzle (v1.7.1), ViennaRNA (v2.7.0), and Z-Hunt[rs] (https://github.com/biomancy/zhuntrs, v0.0.4).</p> <p>A complete Micromamba environment for conducting the customized data analysis is available in the repository under the setup/env directory.</p> <p>ImageJ, GraphPad Prism 10, Incucyte 2024B, Leica LAS X software package.</p>

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 data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002, PMID: 11752295) and are accessible using GEO Series accession number GSE308489 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE308489>).

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 methods were used to predetermine sample size. Sample size was determined based on our prior studies and on literature in the field (e.g., PMID: 35614224).
Data exclusions	No data were excluded.
Replication	In vitro and in cellulo experiments were performed with at least three independent biological replicates. All attempts at replication were successful.
Randomization	Cell samples with similar numbers were randomly allocated into each group. For immunostaining experiments, images were acquired from randomly selected regions of the sample chamber to avoid selection bias.
Blinding	Image acquiring and quantifications were performed by researchers blind to experiment group except positive and negative control group. Blinding was not technically applicable to other 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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

Z-RNA (clone Z22, Ab00783-23.0, Absolute Antibody), A-RNA (clone 9D5, 3361, Millipore), phosphorylated murine MLKL (Cat. 37333, Cell Signaling), FLAG (Cat.A00187, GenScript), FLAG (Cat.20543-1-AP, Proteintech), Emerin (ab40688, Abcam), phosphorylated murine MLKL (Ab196436, Abcam), total murine MLKL (MABC60, EMD Millipore), RIPK3 (#2283, ProSci), GAPDH (#60004-1-Ig, Prointech), Cleaved Caspase-3 (#9664, Cell Signaling Technology), Caspase-3 (#9662, Cell Signaling Technology), HSV-1/2 gB (ab6506, Abcam), HSV ICP5 (ab6508, Abcam), c-Myc (MA1-980, Invitrogen), FLAG (F1804, Sigma-Aldrich), ICP27 (SC-69806), ICP0 (sc-53070, Santa Cruz Biotechnology), ICP5 (sc-56989, Santa Cruz Biotechnology), NS1(sc-130568, Santa Cruz Biotechnology), NS1 (clone 1A7, gift from Adolfo García-Sastre), NP (GTX125989, GeneTex), human MLKL (#14993, Cell Signaling Technology), human RIPK3 (#13526, Cell Signaling Technology), murine ZBP1 (AG-20B-0010-C100, AdipoGen Life Sciences), human ZBP1 (PA5-20455,

Thermo Fisher Scientific), CPSF3 (ab72295, Abcam), V5 (R960-25, Thermo Fisher Scientific), FAM (A-889, Thermo Fisher Scientific), NP (MCA400, Biorad).

Validation

All antibodies have been previously validated by us (PMID: 32200799 and 35614224), and/or by the respective manufacturers.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

MEFs (produced in-house from timed matings), HS68 (ATCC, obtained from the Cell Culture Facility at Fox Chase), HT-29 cells (ATCC, obtained from the Cell Culture Facility at Fox Chase), Vero (ATCC, obtained from the Cell Culture Facility at Fox Chase).

Authentication

MEFs from genetically modified mice are routinely genotyped by qPCR (when mice are born) and by immunoblot analyses for protein expression. ATCC cell lines are routinely authenticated by the Cell Culture Facility at Fox Chase. The FCCC Cell Culture Facility genetically authenticates cell lines using short tandem repeat (STR) profiling.

Mycoplasma contamination

All cell lines used in this study are routinely tested for mycoplasma contamination, and were all negative for mycoplasma.

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

No commonly misidentified cell lines were used.