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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

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For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Sample size was determined empirically and was based on our previous work using HOIP-deficient. We aimed for a number of at least 3 animals per group to allow basic statistical analysis while using a justifiable number of mutant mice/embryos.

2. Data exclusions

Describe any data exclusions.

Exceptional embryos (either wildtype or knockout) found dead in utero were excluded from the analyses

3. Replication

Describe whether the experimental findings were reliably reproduced.

Preliminary data showed that the phenotype observed in the study was consistent and that every single mouse bearing a mutation carried the exact same phenotype. Thus, a sample size of n=3 was sufficient to reach statistical significance. More mice were however included and reliably reproduced

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

animal experiments were randomised since the genotypes of the mice where unknown at the time of experimentation. Genotyping was perform at the end of each experiment except when a pool of embryos was used for AGM FACS analysis.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

In all experiments with the exceptions mentioned below the genotypes of the mice where unknown at the time of experimentation and therefore the analyses were blinded. Quantifications in figure 4, TUNEL and branching points was performed by a scientist who was blinded to the identity of the samples.
RNA seq and AGM FACS analysis as well as ELISAs, Western blotting and in vitro experiments were not blinded.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Quantifications were performed with Excell, ImageJ and GraphPad and statistical analysis with GraphPad. For FACS analysis FlowJo 7.6.1 software (Treestar) was used. For RNA seq analysis samples were sequenced on the NextSeq 500 instrument (Illumina, San Diego, US) using a 43bp paired end run. Run data were de-multiplexed and converted to fastq files using Illumina's bcl2fastq Conversion Software v2.18 on BaseSpace. Fastq files were then aligned to a reference genome using STAR on the BaseSpace RNA-Seq alignment app v1.1.0. Reads per transcript were counted using HTSeq and differential expression was estimated using the BioConductor package DESeq2 (BaseSpace app v1.0.0). Functional analysis was performed using Venny 2.1 and STRING (string-db.org) softwares Microfocus CT scans were reconstructed using CTPro3D; Nikon Metrology and analysed with VG Studio MAX (Volume Graphics GmbH, Heidelberg, Germany). Images and figures were processed with Adobe Photoshop and Illustrator CS6, respectively.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	Mice generated in this study as well as unique reagents are available upon request. GSK'547A is property of GSK, RIPK3 KO mice are from Genentech, Caspase-8 KO mice are from Razq Hakem and RIPK1 KO from Philip Leder.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The following antibodies were used for immunofluorescence staining: PECAM-1 (BD Biosciences, 5533370 Clone MEC13.3, dilution 1/250 lot n 2251541, validation http://www.bdbiosciences.com/eu/applications/research/stem-cell-research/cancer-research/mouse/purified-rat-anti-mouse-cd31-mec-133/p/550274), cleaved caspase-3 (Cell Signaling, 9661, dilution 1/250, lot ns 43 and 12, validated by immunohistochemical analysis of paraffin-embedded mouse embryo, using Cleaved Caspase-3 (Asp175) Antibody preincubated with control peptide or Cleaved Caspase-3 (Asp175) Blocking Peptide #1050 in website). Alexa Fluor 594 Goat anti-Rat IgG (Invitrogen, A-11007, lot n 1241432), Alexa Fluor 488 Goat anti-Rabbit IgG (Invitrogen, A-11034, lot n 1408830). All secondary antibodies were used at a 1/500 dilution.
The following antibodies were used for Western blot analysis: HOIP (custom-made, Thermo Fisher Scientific, validated using HOIP deficient cells in Peltzer et al. 2014 Cell Reports), SHARPIN (ProteinTech, 14626-1-AP, lot n 00005729, validated using cpdm mice in Figure 2), HOIL-1 (home-made, validated using HOIL-1 deficient MEFs in Figure 2), TNFR1 (Abcam, ab19139, lot n GR3187980-1), Actin (Sigma, A1978, lot n 026M4780V), pIκBα (Cell Signaling, 9246, lot 19, validated in MEFs stimulated with TNF in this work), IκBα (Cell Signaling, 9242, lot n 10, validated in MEFs stimulated with TNF in this work), cleaved caspase-8 (Cell Signaling, 9429, validated in Caspase-8 deficient embryo homogenates in Extended Data Fig. 7h), linear ubiquitin (Merck Millipore, MABS199, clone 1E3, lot n 2980771, validated using recombinant linear polyubiquitin chains in website), RIPK1 (BD, 610459, clone 38, lot n 14414, validated in human endothelial cell lysates in website), RIPK3 (Enzo, ADI-905-242-100, lot n 06041509, validated in mouse 3T3 whole cell lysate in the absence or presence of blocking peptide with RIP3 antibody in website), FADD (Assay Design, AAM-121, lot n 04201742), MLKL (Millipore, MABC604, clone 3H1, validated using MLKL KO mice in Extended Data Fig. 5a), phospho-MLKL (Abcam, ab196436, lot n GR246882-13, validated in L929 cells treated with TNFand ZVAD Extended Data Fig. 7h), Tubulin (Sigma, T9026) and FADD (Santa Cruz, sc-5559, clone H181). All antibodies were used at a 1/1000 dilution.
HRP coupled antibodies for western blotting were the following, Goat anti-IgG1 (catalogue n., 1070-05, lot n G1013-5075E), Goat anti-Rabbit (catalogue n., 4050-05, lot n., I15144-T365L), Goat anti-Rat (catalogue n., 3050-05, lot n., G8212-PK146), Goat anti-Mouse (catalogue n., 1031-05, lot n., FO415-NB76G). All antibodies were purchased and validated by Southern Biotech and used at a 1/10.000 dilution.
The following antibodies were used for Flow Cytometry analysis: CD16/32, clone 93 and 2.4G2 (eBioscience, 45-0161-82, lot n 11-0161-82 and BD553141), CD135, clone A2F10.1 (BD, 553842, lot n 7272953/2342831), Ly-6A/E, clone D7 (Sca-1) (BD, 558162, lot n 38503), CD117 (c-Kit), clone 2B8 (BD, 560185, lot n B249344), CD34, clone RAM34 (BD, 562608, lot n 7187754), mouse Lineage Cocktail, clones 17A2/RB6-8C5/RA3-6B2/Ter-119/M1/70 (Biolegend, 133313, lot n B227259 and BD, 561301), CD16/32, clone 2.4G2 (BioXcell, CUS-HB-197), CD11b, clone M1/70 (Biolegend, 101228, lot n B223927 and eBioscience, 15-0112-81), CD11c, clone HL3 (BD, 561241), F4/80, clone BM8 (Biolegend, 123110), GR-1, clone RB6-8C5 (Biolegend, 108416, lot n B188267 and 108410), CD45, clone 30-F11 (Biolegend, 103128, lot n B241345 and Biolegend, 103112), CD3ε, clone, 145-2C11 (Biolegend, 100310), B220, clone RA3-6B2, (Biolegend, 103210), CD71, clone RI7217 (Biolegend, 113807, lot n B213759) and TER-119, clone TER-119 (Biolegend, 116234, lot n B244218). Antibodies were used at a 1/100 dilution except for the linage cocktail which was used at a 8/100 dilution. Validation for antibodies can be found in manufacturer's website.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No eukaryotic cell lines were used

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	Males and females from 6 weeks up to 24 weeks were used for time matings. Embryos were analysed at every embryonic days from E10.5-E16.5, regardless of gender. MEFs were obtained from E13.5 embryos. Embryos older that E14.5 were humanely culled by cervical dislocation. Adult MLKL/Caspase-8/HOIL-1- or HOIP-deficient of RIPK1/RIPK3/Caspase-8/HOIL-1-deficient mice were analysed around 20-40 days depending on the health status. MLKL/Caspase-8/cpdm mice were anlaysed at around 60 days. The genetic background of mice used in this study is C57BL6 All animal experiments were conducted under an appropriate UK project license in accordance with the regulations of UK home office for animal welfare according to ASPA (animal (scientific procedure) Act 1986). A summary of all strains used in this study is detailed in Extended Data Fig.10a.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	This study did not involve human research participants.

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☐ Initial submission ☐ Revised version ☒ Final submission

Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Single-cell suspensions from mechanically dissociated E13.5 foetal livers or a pool of E11.5 aortas (AGM region) from 3 embryos in PBS supplemented with 10% FCS, were stained for 30 min on ice with antibodies described.
6. Identify the instrument used for data collection.	LSR Fortessa (BD Biosciences) or FACSAria FUSION cell sorter (BD Biosciences).
7. Describe the software used to collect and analyze the flow cytometry data.	FlowJo 7.6.1 software (Treestar)
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Sorted samples were confirmed for purity post-sort via flow cytometry. Sorted populations were of ~98% purity.
9. Describe the gating strategy used.	Fluorescence minus one (FMO) were used as gating controls. Compensation was performed with compensation beads (eBioscience). For quantification of absolute number of cells, a defined number of flow cytometric reference beads (Invitrogen) were mixed to the samples before acquisition. All cells were first gated in FSC/SSC according to cell size and granularity. This population was then gated according to their viability using cell viability dye (eBioscience) (negative population). Subsequently cells were gated according to their lineage positivity or negativity to finally arrive gate according to the specific surface markers as shown in the extended figures for the desired cell population.

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