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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	Nanoparticle tracking: NTA software Gwyddion Mass spectometry: Xcalibur ZEN Zeiss software
Data analysis	Statistical analysis: GraphPrism; Microscopy images: ImageJ; mass spectometry: Xcalibur and MaxQuant; ImageStream: IDEAS software v6.2; Flow cytometry: FlowJo.

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

The full NGS dataset is available at ENA (European Nucleotide Archive) with the identifier 'ena-STUDY-AARHUS UNIVERSITY 12-12-2018-17:12:31:528-124', under accession number 'PRJEB30324'.
Original immuno blots are shown in Supplementary Figure 7.

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	The number of experiments and replicates was based on standard practice. In general, three independent experiments were conducted with three biological replicates. Details for each experiment are included in the individual figure legends.
Data exclusions	No data were excluded from the analysis
Replication	The experimental findings were reproduced. Data from cell lines were generated using at least three independent biological replicates, and independently repeated as specified in the figure legends.
Randomization	For the mouse experiments, animals were randomly placed in the different groups.
Blinding	No blinding was performed

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	Western blotting: CD81 (sc-9158, Santa Cruz), CD63 (sc-5275, Santa Cruz), calnexin (ab-22595, Abcam), nSmase2a (sc-166637, Santa Cruz), cGAS (SAB2100310, Sigma-Aldrich), STING (AF6516, R&D), vinculin (V9131, Sigma), HRP-conjugated actin (ab-49906, Abcam). Corresponding peroxidase-conjugated secondary antibodies were used (Jackson ImmunoResearch). Immunofluorescence: Rab7 (9367, Cell Signaling.), IRF3 (Cell Signaling, 4302), STING (AF6516), phospho S222 MVB12b (GenScript, Antibody production service) and corresponding secondary antibodies.
Validation	Commercially available antibodies were validated by manufactureres usually by Western blotting. Most commercially available antibodies were, additionally validated in the laboratory by comparison of lysates from relevant Wt and KO cells prior to use in the project. Anti S222 Mvb12b was validated by Western blotting and IF on relevant material from wt and Mvb12b KO cells in the presence and absence of dsDNA stimulation.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	THP1 (ATCC)
Authentication	THP1 cells were authenticated by the vendor ATCC, and were not validated further in our laboratory. Cell lines that were obtained and validated by other groups were not further authenticated.

Mycoplasma contamination	All cells imported are tested for mycoplasma. All cells in the laboratory are frequently rested for potential mycoplasma contamination be PCR and/or the MycoAlert Kit (Lonza).
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Animals and other organisms

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

Laboratory animals	12 weeks old C57BL/6N mice. Both genders were included.
Wild animals	N/A
Field-collected samples	N/A
Ethics oversight	Animal experiments have been approved by the Vienna University of Veterinary Medicine institutional ethics committee and performed according to protocols approved by the Austrian law (BMWF 68.205/0032-WF/II/3b/2014).

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	CD3+ cells were isolated from mouse spleens using EasySep kit from STEMCELL Technology. To evaluate the effects of EVs from L.monocytogenes-infected cells on apoptosis in bystander cells, resting, apoptosis-induced (low concentration of FasL, 5 ng/ml, Sigma-Aldrich, catalog no. F0427), or activated (Human T-Activator CD3/CD28 Dynabeads™, ThermoFisher Scientific catalog no 11161D) T lymphocytes were treated with supernatants from macrophages infected for 18h with L.monocytogenes (+/- GW4869). Apoptosis was evaluated by flow cytometry analysis Annexin V staining (ThermoFisher).
Instrument	Fluorescence was measured using LSRFortessa flow cytometer (BD Biosciences)
Software	FlowJo v.10 (Tree Star)
Cell population abundance	CD3+ cells are highly abundant in the mouse spleen, and the yields for the experiments performed in this work were within the normal range. In this experiments the percentage of Annexin V+ CD3+ cells were between 1 and 40% as illustrated in the figures.
Gating strategy	First a FSC/SSC-A plot was made to gate all whole cells. Next, single cells were gated based on plotting of the SSC-H/SSC-A. The apoptosic singlet CD3+ cells were finally identified based on positive annexin V (Alexa 488) staining.

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