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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.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Immunoblot images: Odyssey Infrared Imaging System (version 2.1), LI-COR Biosciences Immunofluorescence staining data: ZEN (version 1.1.2.0), Carl Zeiss ELISA data: SoftMax Pro (version 5.0.1), Molecular Devices Corp. Flow cytometric data: FACSDiva (version 6.1.3), BD Biosciences Luciferase assay data: SoftMax Pro (version 5.4), MDS Analytical Tech.
Data analysis	Protein identification and quantification by mass spectrometry were done with Integrated Proteomics Pipeline (IP2, Integrated Proteomics Applications, Inc. San Diego, CA). Flow cytometry data was analyzed with FlowJo 10.1r7. Microscopy images were processed in ZEN 2012 1.1.2.0 (Carl Zeiss). Colocalization analysis was performed with CellProfiler 2.2.0. Immunoblot quantification was performed in Fiji 1.0 (ImageJ). All other data/graphs were plotted and analyzed using Prism 5.0c or 6.0 (GraphPad). Statistical analysis was performed with Prism 5.0c, 6.0 or 7.0.

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 upon request. 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 datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-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 sizes. Sample sizes for studies with mice were determined by availability of animals of the correct genotypes or were based on the numbers used in previous publications, in which comparable sample sizes resulted in statistically significant results. Statistical tests (as described in the Methods) were used to determine statistical significance.
Data exclusions	No data were excluded from analyses.
Replication	Every experiment was repeated (with similar results) at least twice. In most cases, experiments were repeated greater than 3 times with similar results.
Randomization	Randomization was not appropriate for this study. Littermate mice placed into groups based on their genotypes to enable meaningful comparisons.
Blinding	Investigators were blinded to the genotype of samples for analysis of anti-nuclear antibody staining of serum samples.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	Any unique biological resources will be made readily available to any investigators at non-profit institutions after publication and upon request. Requests from for-profit corporations will be handled and negotiated by our university's technology transfer office. Unique mouse strains will also be made available, given that the investigators provide written assurance that the animals will be used in accordance with their institution's IACUC guidelines.

Antibodies

Antibodies used

The following antibodies were used for immunoblots and immunoprecipitations: anti-HA as purified antibody or matrix (clone 3F10; Ab: 11867423001, 1:1000; matrix: 11815016001, Roche), anti-FLAG as purified antibody or matrix (M2; Ab: F1804, 1:2000; matrix: M8823; Sigma-Aldrich), anti-mLamp-1 (AF4320, 1:1000, R&D Systems), anti-Calnexin (ADI-SPA-860, 1:1000, Enzo Life Sciences), anti-Gapdh (GT239, 1:5000, GeneTex), anti-Myd88 (AF3109, 1ug/ml, R&D Systems), anti-IRAK2 (4367, 1:1000, Cell Signaling), anti-Phospho-p38 (9211, 1:1000, Cell Signaling), anti-p38 (9212, 1:1000, Cell Signaling), anti-Phospho-SAPK/JNK (81E11, 1:1000, Cell Signaling), anti-SAPK/JNK (56G8, 1:000, Cell Siganling), anti-Phospho-p44/42 (ERK1/2) (D13.14.4E, 1:000, Cell Signaling), anti-p44/42 (ERK1/2) (137F5, 1:1000, Cell Siganling), anti-IκBα (9242, 1:1000, Cell Signaling), anti-Syntenin-1 (2C12, 1:125, Novusbio), anti-Unc93b1 (PA5-20510, 1:2000, Thermo Scientific), anti-ubiquitin (P4D1, 1:400, Santa Cruz), anti-K63-linked ubiquitin (human polyclonal, kind gift from Michael Rape, 1:10000), anti-Alix (GTX64925, 1:400, GeneTex), anti-CD63 (9B217345, 1:2000, Abcam), goat anti-mouse IgG-AlexaFluor680 (A21058, 1:10000, Invitrogen), rabbit anti-goat IgG-AlexaFluor680 (A21088, 1:10000, Invitrogen), goat anti-human IRDye 680RD (926-68078, 1:10000, Licor), goat anti-mouse IRDye 800CW (926-32210, 1:10000, Licor), donkey anti-rabbit IRDye 680RD (926-68073, 1:10000, Licor), goat anti-rat IgG-AlexaFluor680 (A21096, 1:10000, Invitrogen).
Antibodies for immunofluorescence were: rat anti-HA (3F10, 11867423001, 1:200, Roche), rabbit anti-Lamp1 (ab24170, 1:500, Abcam), Donkey anti-rat IgG-AlexaFluor488 (712-545-150, 1:500, Jackson Immunoresearch), goat anti-rabbit IgG-AlexaFluor647 (711-606-152, 1:500, Jackson Immunoresearch). Cells were mounted in Vectashield Hard Set Mounting Medium for Fluorescence (H-1400, Vector Laboratories).
For ELISA: anti-mouse TNFα purified (1F3F3D4, 14-7325-85, 1.5ug/ml, eBioscience), anti-mouse TNFα-biotin (XT3/XT22, 13-7326-85, 1ug/ml, eBioscience), Streptavidin-HRP (554066, 1:8000, BD Pharmingen).
Antibodies and reagents used for flow cytometry were: anti-TNFα (MP6-XT22, 17-7321-82, 1:500, eBioscience), purified anti-CD16/32 Fc Block (2.4G2, 553141, 1:500, BD), CD3ε (145-2C11, 100327, 1:100, BioLegend), CD4 (GK1.5, 100453, 1:800, BioLegend), CD8 (53-6.7, 100742, 1:800, BioLegend), CD44 (IM7, 11-0441-85, 1:100, eBioscience), CD62L (MEL-14, 12-0621-83, 1:400, eBioscience), CD69 (H1.2F3, 25-0691-82, 1:400, eBioscience), CD1d (1B1, 17-0011-82, 1:200, eBioscience), B220 (RA3-6B2, 56-0452-82, 1:200, Invitrogen), CD19 (6D5, 115555, 1:800, BioLegend), IgD (11-26c.2a, 405704, 1:200, BioLegend), IgM (eB121-15F9, 25-5890-82, 1:200, eBioscience), CD21 (eBio8D9, 47-0211-82, 1:200, eBioscience), CD23 (B3B4, 101618, 1:200, eBioscience), CD138 (281-2, 142507, 1:200, BioLegend), CD11b (M1/70, 101243, 1:1000, BioLegend), Ly6G (1A8, 60-1276-U100, 1:400, TONBO biosciences), Ly6C (HK1.4, 128012, 1:400, BioLegend), F4/80 (CI:A3-1, MCA497F, 1:100, AbD serotec), MHCII (M5/114.15.2, 47-5321-82, 1:400, eBioscience), CD86 (GL1, 12-0862-82, 1:200, eBioscience), CD11c (N418, 117349, 1:800, BioLegend), CD117 (c-Kit) (2B8, 17-1171-82, 1:400, eBioscience), Sca-1 (D7, 25-5981-82, 1:400, eBioscience). For ANA detection: anti-mouse IgG-AlexaFluor 488 (115546146, 1:300, Jackson Immunoresearch), anti-mouse IgM-FITC (11-5790-81, Invitrogen).
The antibody against phosphorylated Unc93b1 was generated by Invitrogen against synthesized phospho-peptide (YLEEDN(pS)DE(pS)DMEGEQ) using their "Rabbit, 90-Day immunization" protocol. Antibody in sera was enriched with immobilized phospho-peptide, followed by negative absorption with unphosphorylated peptide.

Validation

The custom-designed rabbit polyclonal antibody against phospho-Unc93b1 for IB studies was validated in Extended Data Fig. 7a and b. The custom-designed human polyclonal antibody against K63-ubiquitin for IB studies was validated previously and published in https://doi.org/10.1016/j.cell.2017.09.040. All other commercial antibodies were validated by manufacturers for indicated species and application. Antibodies used for flow cytometry and immunofluorescence microscopy were further validated by staining known populations of cells. When possible, genetic controls were used to confirm specificity of any staining. Antibodies used for biochemistry were further validated by performing pilot experiments using samples with known parameters.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

RAW264 macrophage cell line was obtained from ATCC.
HEK293 cells were obtained from ATCC.

Authentication

Cells lines were authenticated via morphology, flow cytometry (measuring surface markers), and functional assays. Individual lines were not kept in culture for longer than 2 months.

Mycoplasma contamination

Cell lines were screened for mycoplasma. Also, Individual lines were not kept in culture for longer than 2 months. New lines were thawed from cryopreserved stocks previously confirmed as mycoplasma-negative.

Commonly misidentified lines
(See ICLAC register)

None of the cell lines used are listed in the ICLAC database.

Animals and other organisms

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

Laboratory animals

Mouse, C57BL/6, males and females, ages ranging from 3 weeks to greater than 5 months.
Mouse, Unc93b1(PKP) knockin mouse, males and females, ages ranging from 3 weeks to greater than 5 months.
Mouse, Tlr7-/- (on the C57BL/6 background), males and females, ages ranging from 3 weeks to greater than 5 months.

Wild animals

The study did not involve wild animals.

Field-collected samples	The study did not involve field-collected samples.

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	Toll-like receptor signaling in RAW macrophages, BMMs, and BM-DCs: Cells were seeded into non-treated tissue culture 24-well plates or round-bottom 96-well plates. The next day cells were stimulated with the indicated TLR ligands. To measure TNFα production, BrefeldinA (BD GolgiPlug, BD Biosciences) was added to cells 30 min after stimulation, and cells were collected after an additional 5.5 h. Dead cells were excluded using a fixable live/dead stain (Violet fluorescent reactive dye, Invitrogen). Cells were stained for intracellular TNFα with a Fixation & Permeabilization kit according to manufacturer's instructions (eBioscience). (BM-DCs were additionally surface-stained for CD11c, MHCII, and Ly6c). Mouse characterization: Spleens and lymph nodes were digested with collagenase XI and DNase I for 30min at 37°C and single cell suspensions were generated by mechanical disruption. Red blood cells were lysed in ACK Lysing Buffer (Gibco). The remaining cells were stained for flow analysis. Dead cells were excluded using a fixable live/dead stain (Aqua fluorescent reactive dye, Invitrogen) or DAPI and all stains were carried out in PBS containing 1% BSA (w/v) and 0.1% Azide (w/v) including anti-CD16/32 blocking antibody. Cells were stained for 20 min at 4°C with surface antibodies. Data were acquired on a Fortessa or X20 (BD Biosciences). For B cell proliferation assays, spleens were digested with collagenase 8 (Sigma) and DNAse-I for 45 min and red blood cells were lysed using ACK buffer (Gibco). Splenocytes were labeled with 12.5ug/mL CFSE (Invitrogen) for 10min at 37°C and immediately underlayed with 3ml FCS to spin out CSFE. Cells were taken up in media (RPMI/10%FCS/L-glutamine/Pen-Strep/HEPES/Sodium pyruvate/ ME), counted, and seeded at 200,000 cells per well in round-bottom 96-well plates. Cells were incubated in media with various concentrations of CpG-B, R848, or LPS for 72 h. Flow cytometry was used to analyze stimulated cells. Live, singlet cells were pre-gated on CD19+ and cell proliferation was determined by the geometric mean fluorescence intensity (gMFI) of CFSE. For the quantification, the gMFI CSFE of the unstimulated control was divided by the gMFI CSFE of the stimulated sample (CSFEUnstim:CFSESample) and plotted along the ligand titration.
Instrument	BD Fortessa or X20 flow cytometer (BD Biosciences)
Software	FlowJo 10.1r7.
Cell population abundance	Unc93b1-/- Raw macrophages retrovirally transduced with Unc93b1 mutants were sorted on mCherry to 95-99% purity.
Gating strategy	All cells, including RAW macrophages, BMMs, BM-DCs, B cells, bone marrow cells, and mouse splenocytes and lymph node cells were gated in the following order to determine the frequencies of live cells:1) FSC-A/SSC-A, 2) Live/dump, 3) Singlets (FSC-H/FSC-A). Data concerning the frequency of different cell subsets were reported as frequency of live cells (unless otherwise stated). The precise gating strategy for each immune cell type is outlined in Fig. S8. For intracellular TNFa staining, the frequencies of TNFa positive cells of live cells are displayed.

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