nature portfolio

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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	BD FACSymphony
Data analysis	FlowJo version 10.7, GraphPad Prism 8.4.0

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 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 that support the findings of this study are available from the corresponding author upon reasonable request.

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	sex and gender information from PBMC donors was not collected and is unknown
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	PMBC samples were derived from participants between the ages of 25-50 yrs old. Participants were deemed healthy and void of chronic disease or infections
Recruitment	Participants were anonymous and recruited based on informed consent and enrollment in Amgen's blood research donor program.
Ethics oversight	Amgen Research Blood Donor Program

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	Sample size was determined based on the variability from sample to sample for specific asssays, previous results, and effect size. Selected as the most representative example
Data exclusions	not applicable
Replication	All experiments were reproduced at least 2 times and is described in the figure legends
Randomization	Animal randomization was not applicable or applied to studies. Covariates were controlled and compared to vehicle treated groups and/or wild type litter mate controls
Blinding	All histological analysis and image acquisitions were blinded. Due to animal identification system used in studies, blinding was not possible for other studies.

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

The following antibodies were used for western blot analysis. AlexaFluor 800 plus anti-rabbit IgG (Inivtrogen;A32735), AlexaFluor 800 plus anti-mouse IgG (Invitrogen;A32730), AlexaFluor 680 plus anti-mouse IgG (Invitrogen;A32729), AlexaFluor 680 plus antirabbit IgG (Invitrogen;A32734), anti-TLR7 N terminus (Invitrogen;PA112829), anti-GFP(Invitrogen;PA5-22688), anti-p-IRF5 (Invitrogen;PA5-64760), rabbit anti-IRF5 (Abcam;ab181553), anti-IRF7 (CST;4920), anti-Flag tag (CST;14793), anti-HA-tag (CST;3724), anti-beta-actin (CST;ab3700), anti-tubulin (CST;86298), anti-GAPDH (CST;2118), anti-Histone H3 (CST;9715), p-STAT1 (CST;9167), STAT1 (CST;9172), p-IkBa (CST;2859), IkBa (CST;4812), p38 (CST; 8690), p-p38 (CST;4511).The following antibodies were used for FACS- BD Biosciences:
CD8 BUV395 (clone 53-6.7), CD4 BUV496 (GK1.5), and Ly6G BUV395 (1A8), CD21/CD35 PE (7G6), mouse TLR7 PE (A94B10), mouse TLR9 (J15A7). BioLegend: CD3 BV605 (17A2), CD19 BV711 (6D5), Ly6C (HK1.4), B220 BV711 (RA3-6B2), CD45 BV785(30-F11), CD11c FITC or BV421 (N418), CD11b APC-Cy7 (M1/70), CD16/32 antibody (2.4G2), and I-A/I-E PECy7 (M5/114.15.2), CD23 APC (B3B4). eBioscience: CD317/PDCA-1 FITC (eBio927), Siglec-H PE (eBio440c), and F4/80 APC (BM8). The following antibodies were used for immunoprecipitation experiments :Anti-mouse TLR9 TIR domain serum, anti-mouse TLR9 antibody (J15A7), and anti- mouse TLR7 antibody (A94B10) was a gift from K. Miyake (University of Tokyo)

Validation

IRF5,pIRF5 antibodies were validated in supplementary figure 5a. Antibodies used for immunoprecipitation experiments were validated in Onji, M. et al. An essential role for the N-terminal fragment of Toll-like receptor 9 in DNA sensing. Nat Commun 4, 1949, doi:10.1038/ncomms2949 (2013).
Antibodies were validated as described on manufacturer's website and datasheets were referenced for specificity
AlexaFluor 800 plus anti-Rabbit IgG; https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32735
AlexaFluor 800 plus anti-mouse IgG https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32730
AlexaFluor 680 plus anti-mouse IgG; https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32729
AlexaFluor 680 plus anti-rabbit IgG; https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32734
anti-TLR7 N terminus PA112829; https://www.fishersci.com/shop/products/tlr7-polyclonal-antibody-invitrogen/01675820
anti-GFP(PA5-22688); https://www.thermofisher.com/order/genome-database/generatePdf?productName=&assayType=PRANT&productId=PA5-22688
anti-p-IRF5(PA5-64760); https://www.thermofisher.com/antibody/product/Phospho-IRF5-Ser437-Antibody-Polyclonal/PA5-64760
rabbit anti-IRF5 (ab181553); https://www.abcam.com/en-us/products/primary-antibodies/irf5-antibody-epr17067-ab181553
anti-IRF7; https://www.cellsignal.com/products/primary-antibodies/irf-7-antibody/4920
anti-Flag tag; https://www.cellsignal.com/products/primary-antibodies/dykddddk-tag-d6w5b-rabbit-mab-binds-to-same-epitope-as-sigma-aldrich-anti-flag-m2-antibody/14793
anti-HA-tag; https://www.cellsignal.com/products/primary-antibodies/ha-tag-c29f4-rabbit-mab/3724
anti-beta-actin ; https://www.cellsignal.com/products/primary-antibodies/b-actin-8h10d10-mouse-mab/3700
anti-tubulin; https://www.cellsignal.com/products/primary-antibodies/b-tubulin-d3u1w-mouse-mab/86298
anti-GAPDH; https://www.cellsignal.com/products/primary-antibodies/gapdh-14c10-rabbit-mab/2118
anti-Histone H3; https://www.cellsignal.com/products/primary-antibodies/histone-h3-antibody/9715
anti-Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb; https://www.cellsignal.com/products/primary-antibodies/phospho-stat1-tyr701-58d6-rabbit-mab/9167
anti-Stat1 Antibody; https://www.cellsignal.com/products/primary-antibodies/stat1-antibody/9172
anti-Phospho-IκBα (Ser32) (14D4) Rabbit mAb; https://www.cellsignal.com/products/primary-antibodies/phospho-ikba-ser32-14d4-rabbit-mab/2859
anti-IκBα (44D4) Rabbit mAb; https://www.cellsignal.com/products/primary-antibodies/ikba-44d4-rabbit-mab/4812
anti-SAPK/JNK; https://www.cellsignal.com/products/primary-antibodies/sapk-jnk-antibody/9252
anti-phospho-SAPK/JNK Antibody; https://www.cellsignal.com/products/primary-antibodies/phospho-sapk-jnk-thr183tyr185-81e11-rabbit-mab/4668

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK293T cells were purchased from ATCC (CRL-3216)
Genotyped Epstein Barr immortalized B cells (BLCLs) were obtained from Coriell Institute. The following BLCLs catalog numbers were ordered from the Coriell Institute and used in this study:
HG01500
HG01606
HG01625
HG01669
HG01682
HG01756
HG01761
HG02224
HG02231
HG02238
HG01503

HG01608
HG01624
HG01672
HG01680
HG01765
HG01767
HG02221
HG02233
HG02236
HG01786
HG01746
HG01702
HG01628
HG01784
HG01757
HG01704
HG01632
HG00111
HG00102
GM20795
GM20799
GM20832
GM20804
GM20797
GM20822
GM12006
GM12761
GM11994
GM12004
GM12763
GM11993

Authentication	Cell line was authenticated according to manufacturer's website https://www.atcc.org/Products/All/CRL-3216.aspx#documentation, STR profiling
Mycoplasma contamination	Cell line was measured for mycoplasma according to manufacturer's certification - mycoplasma was not detected
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

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	TASL knockout mice and wild type littermate controls were in the Balbc/j background. Females 6-9 weeks of age were used for studies. All animals were housed at Amgen South San Francisco in a 12hr light/12hr dark lighting cycle at 22 degree Celsius
Wild animals	No wild animals were used in this study
Reporting on sex	Findings are not unique to one sex
Field-collected samples	No field collected samples were used in the study
Ethics oversight	The Amgen Institutional Animal Care and Use Committee approved all animal studies under Committee Protocol 2009-00004

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

Plants

Seed stocks	No seed stocks were used in study
Novel plant genotypes	No novel plant geneotypes were used in study
Authentication	Not applicable to study

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	Spleens were harvested from 5-8 week old wildtype or TASLK KO mice. Spleens were homogenized in Miltenyi Biotec C tubes program msSpleen_01 using a Miltenyi gentlemacs dissociator. Homogenates were treated with ACK lysis buffer(Gibco) for removal of erythrocytes and filtered through a 40micron filter prior to FACs staining. Before staining for surface markers, cells were incubated with an anti CD16/32 antibody (2.4G2, BD Biosciences) and dead cells were excluded using a fixable live/dead stain (eBioscience; 65-2860-40) or 7AAD(Biologend; 420403). All stains were carried out in PBS containing 3% FBS (v/v) or Brilliant stain buffer (BD Biosciences). Cells were stained for at least 20 minutes at 4°C with antibodies.
Instrument	Data were collected on LSR II or Symphony (BD Biosciences)
Software	Data was analyzed by FlowJo (FlowJO LLC)
Cell population abundance	Populations were sorted at >90% purity as determined by flow cytometric analysis of post-sort samples.
Gating strategy	Doublets (FSC-H vs FSC-A) and dead cells were excluded, and all cell populations were gated on CD45+. T cells were isolated as CD4+ or CD8+ within CD19- CD3+ subpopulation. B cells were isolated as CD19+. Marginal zone B cells were identified as CD23lo/-CD21hi and follicular B cells were identified as CD23hiCD21int within B220+ population. Myeloid populations were all gated on CD3-. Neutrophils were identified as CD11b+ Ly6G+. Monocytes as CD11b+ Ly6C+ Ly6G-. cDCs as CD11c+ I-A/I-E +Ly6CLy6G-. Macrophages as F4/80+ I-A/I-E+ Ly6C- Ly6G-. pDCs as SiglecH+ B220+ within CD11c+ I-A/I-E+ cDC subpopulation.

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