nature research

Corresponding author(s): Susan K. Pierce

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

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.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.
For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

Describe how sample size was determined.

Sample size was determined based on previous studies of similar nature.

2. Data exclusions

Describe any data exclusions.

No data exclusions have been made

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

All experiments were repeated and experiments contained duplicates, triplicates etc. Key experiments were repeated by different authors at different times using different reagent stocks to minimize batch error and maximize reproducibility. For consistent results, slight optimizations in protocols of experiments involving calcium and ROS stains and CD40L were carried out for each batch. Freeze-Thaw cycles and extended storage were strictly avoided. Once the measures specified above were taken, experiments were reproducible.

4. Randomization

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

For adoptive transfer experiments, age-sex matched animals were used. Animals were randomly assigned to experimental groups.

5. Blinding

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

Injections were carried out by our animal technician who was given single-blinded barcoded samples. She did not know which groups took what treatment.

Note: all in vivo studies must report how sample size was determined and 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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes 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 in all relevant figure captions (with explicit mention of central tendency and variation)

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.	FlowJo (version 10.2) for flow cytometry experiments. Graphpad prism (version 7.03), R Project (version 3.4.3) and JMP (version 13.2.0) for statistical tests, Imaris (version 9.0)and ImageJ (version 1.51m9) for microscopy data.

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 third party.	There are no restrictions for material availability. Commercial source of reagents were all disclosed in the methods section. Primer and probe sequences are listed in supplementary table

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Commercially available antibodies were used based on previous studies. Commercial antibodies were validated by vendors as noted in their websites. No additional validation was carried out except for preliminary experiments and control groups.Antibodies against CD19 (clone: 6D5), CD69 (clone: H1.2f3), CD4 (clone: RM4-5), CD45.1 (clone:A20) , CD45.2 (clone:104), CD25 (clone: PC61), CD16/32 (clone 93) and B220 (clone:RA3-6B2), CD86 (clone: GL-1) (BioLegend); ovalbumin (catalog no: Ab85584),TOM20 (clone F10), COXIV (clone: 20E8C12), VDAC1 (clone: N152B/23) (Abcam); goat IgG light chain (catalog no:205-602-176) and mouse IgG2a (catalog no:115-607-186) (Jackson ImmunoResearch) were used in various flow cytometry experiments. Rabbit anti mouse TOM20 (catalog no:ab56783) (Abcam) followed by ATTO-647N-conjugated anti rabbit IgG secondary (catalog no: 40839) (Sigma-Aldrich) were used for STED microscopy. The following antibodies were used for immunoblots: b-actin (A1978, Sigma-Aldrich), HSP60 (4870S, Cell Signaling Technology), SIRT3 (5490S, Cell Signaling Technology), and TOM20 (sc-11415, Santa Cruz Biotechnology).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	The NIH/3T3 mouse fibroblast cell line (ATCC CRL-1658) and 3A9 mouse T cell hybridoma line (ATCC CRL-3293) were provided by Dr. Oliver Voss (NIAID/NIH) and Dr. Paul Allen (Washington University) respectively. Phoenix Eco retroviral packaging cell line (ATCC CRL-3214) was purchased from American Type Culture Collection (Manassas, VA, USA)
b. Describe the method of cell line authentication used.	No authentication was used.
c. Report whether the cell lines were tested for mycoplasma contamination.	Lines were tested negative for mycoplasma
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 misidentified cell line was 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 all relevant details on animals and/or animal-derived materials used in the study.	C57BL/6 (Stock No: 00664), B6 CD45.1 (Pep Boy) (Stock No: 002014) and B10.BR (Stock No :00465) were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Hen Egg Lyzoyzme (HEL) specific BCR transgenic mice (MD4 a.k.a IgHEL), were purchased from Taconic Farms (Hudson, NY, USA). TLR9-deficient mice were provided by Dr. Silvia Bolland (NIAID, NIH). HEL-specific TCR transgenic mice (3A9 a.k.a TcrHEL3A9) were provided by Dr. Igal Gery. Adult female mice (8-12 weeks old) were used in the experiments

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 does not involve human research participants.

nature research

Corresponding author(s): Susan K. Pierce

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.	Mouse cells were taken from spleens of euthanized animals. Organs were meshed on cell strainers. ACK lysis was performed to remove RBCs. For experiments that required culturing B or T cells were purified using negative selection. Culture condition depended on experimental detail outlined in the methods section. viability marker was included in all experiments to exclude dead cells. For certain experiments that were performed after O/N culture, FACS sorting was used to remove dead cells to prevent errors in downstream assays.
6. Identify the instrument used for data collection.	BD- LSR II, BD X20
7. Describe the software used to collect and analyze the flow cytometry data.	FlowJO version 10.2
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Depending on the experimental condition, viable cells were as low as 40% in presorted population and over 99% in the sorted population.
9. Describe the gating strategy used.	FSC-A vs SSC-A was used to initially gate splenocytes. FSC-H vs FSC-A was used in this gate to determine the singlets. FSC-H vs Live dead marker was used in the singlet gate to exclude dead cells. Further gating depended on experimental design and were clearly described in the manuscript.

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