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	NIS Elements AR 64-bit 5.21.03; Slidebook 6.0.19; LAS X 3.5
Data analysis	PRISM 8.3.0 was used for graphing , FlowJo 10, NSInc was used for statistical analysis (referenced in manuscript)

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

All data generated or analysed during this study are included in this published article (and its supplementary information files).

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 numbers of samples/cells in experiments was determined based on preliminary or internal pilot data, to be adequate for statistical analysis and ensured reproducibility. No statistical methods were used to determine the sample size.
Data exclusions	In assays examining synaptic recruitment, cells were excluded that were not stimulated by the bilayer and thus did not form a synapse.
Replication	All data reported were from at least two independent experiments (each specified in figure legends) with exactly the same conditions established from pilot experiments.
Randomization	In all of the assays the cells were prepped and placed in the experiment by the same person, thus making it difficult to randomize. For proliferation assays, male and female mice were used and selected based on genotype. For analysis, the fields were randomly selected.
Blinding	In all of the assays, the cells were prepped, placed in the experiment and analyzed by the same person, thus making it difficult to blind. For the statistical analysis using NSInc, the biostatisticians were blinded and had no prior knowledge of what the predicted outcome might be.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	CD3e clone 145-2C11 Biolegend cat 100331 lot B302116, CD28 clone 3751 Biolegend cat 102112 lot B31420, biotin-TCRb clone, H57-597 Biolegend cat 109204 lot B201953, pZAP70 clone 65E4 Cell Signal cat 2701 lot 10, pAKT S473 clone D9E Cell Signal cat 4060 lot 24, AKT Cell Signal cat 9272 lot 27, CD4 clone GK1.5 Biolegend cat 100415 lot B144238, CD4 clone RM4-5 Biolegend cat 100520 lot B217327, CD8 clone 53-6.7 Biolegend cat 100716 lot B237103, CD19 clone 6D5 Biolegend Cat115504 lot B288656, B220 clone RA3-6B2 cat 103204 lot B288658, CD25 PC61 Biolegend cat 102004 lot B345686, Ter 119 clone Ter-119 Biolegend cat 116204 lot B336476, TCRg clone GL-3 Invitrogen cat 13-5711-85 lot2220919, CD11c clone N418 Bioloegend Cat 117304 lot B317309, CD11b clone M1/70 Biolegend cat 101204 Lot B307868, TCRb clone H57-597 Biolegend cat 109201 lot B214556, Lck clone 3A5 Santa Cruz cat sc-433 lot E2913, Lck clone 73A5 Cell Signal cat 2787 lot 3, LAG3 clone C9B7W in house, LAG3 clone 410C9 in house, Rat IgG AF488 ThermoFisher cat A21208 lot 2310102, Rat IgG CR488 Biotium cat 20027 lot 16C301, Rat IgG AF594 ThermoFisher cat A21209 lot 1979379, Rabbit IgG AF555 ThermoFisher cat A32794 lot VK307588, Rabbit IgG AF594 ThermoFisher cat A32754 lot T1271728, Mouse IgG AF568 ThermoFisher cat A11031 lot 2026148, Mouse IgG Atto647N Rockland cat 610-158-121 lot 30008, Rabbit IgG Jackson Immuno cat 711-005-152 lot 134479, Rat IgG Jackson Immuno cat 712-005-153 lot 110736, Mouse IgG Jackson Immuno cat 715-005-151 lot 133843, Hamster IgG Jackson Immuno cat 127-005-160 lot 104516. All antibodies were validated according to manufacture
Validation	All the antibodies used are commercially available, validated for applications used in this manscripts and commonly used in the field based on multiple publications. LAG3 antibodies Rat C9B7W and mouse 410C9 were generated by us, validated against LAG3 KO cells and used in mulitple publications from our group as well as other groups. Clone C9B7W is also commercially available.

Animals and other organisms

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

Laboratory animals	All the mice that were used in this manuscript were on a C57BL/6 background and were housed in American Association for the Accreditation of Laboratory Animal Care-accredited, specific-pathogen-free facilities in Animal Resource Center, St. Jude Children's Research Hospital (SJCRH), and Division of Laboratory Animal Resources, University of Pittsburgh Scholl of Medicine (UPSOM). Female and male mice were used. Animal protocols were approved by the Institutional Animal Care and Use Committees (IACUC) of SJCRH and University of Pittsburgh. Mice were housed in SPF conditions with filtered air, watering system, and 12hr light/dark cycle. The temperature and humidity dictated by DLAR/IACUC.
Wild animals	N/A
Field-collected samples	N/A
Ethics oversight	American Association for the Accreditation of Laboratory Animal Care-accredited, specific-pathogen-free facilities in Animal Resource Center, St. Jude Children's Research Hospital (SJCRH), and Division of Laboratory Animal Resources (DLAR), University of Pittsburgh Scholl of Medicine (UPSOM).Animal protocols were approved by the Institutional Animal Care and Use Committees of SJCRH and University of Pittsburgh.

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	Spleens and lymph nodes were removed from C57BL/6 or Lag3–/– mice, processed to single cell suspensions
Instrument	All flow cytometry data was acquired on a BD LSR or Fortessa analyzer or BD Aria sorter for cell purification (BD Biosciences).
Software	Data collected on FACS.DIVA software and analyzed using FLowJO
Cell population abundance	Cells going into the assays were purified by negative bead selection (outlined in M&M) and thus the only cell population that was analyzed.
Gating strategy	Cells were gated on cell size by SSC-A vs. FSC-A lymphocytes and CD4 or CD8.

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