

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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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 <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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	Flow cytometry data was collected on a CyAn ADP (Dakocytomation), a BD LSRFortessa™ X-20 Cell Analyzer (BD Biosciences) or a Cytex Northern Lights (Cytex Biosciences). Western blots were visualized using G:BOX by Syngene or a ChemiDoc XRS Imaging system (BioRad)
Data analysis	Flow cytometry data was analyzed using FlowJo (v9.x and v10.8, v10.10) software (BD). Western blots were quantified using Image Studio Lite ver 5.2 (LI-COR). Statistical analysis and graphs were generated using Prism v10 (GraphPad Software, Inc).

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](#)

No large data sets were generated for this manuscript. All data generated or analyzed during this study are included in this published article and the source file or available upon 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 N/A

Reporting on race, ethnicity, or other socially relevant groupings N/A

Population characteristics N/A

Recruitment N/A

Ethics oversight N/A

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](https://www.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 pilot assays performed in our lab to obtain desired power.
Data exclusions	No data was excluded, except for 1 mouse in each group in the data set shown in Fig 5G-J. There was no immune response in this mouse and we determined that that was due to a failure to immunize. The mouse also lacked an antibody response to SRBC.
Replication	At least two independent experiments and typically three or more - as noted in figure legends - were performed and all experiments were reliably reproduced. The data in Fig 5B-E showed significant variability within the Lyn deficient group. That experiment was repeated 4 times and these data are shown in Supplemental Fig 12. The reproducibility was based on magnitude and consistency of measurable differences between samples. All data points presented represent biological replicates.
Randomization	In most experiments groups were compared that are genetically different (KO vs WT, autoreactive BCR vs non autoreactive BCR). When two conditions were tested on the same cells (inhibitor vs DMSO) both conditions were tested on the same cells from the same spleen.
Blinding	Experiments were not performed in a blinded fashion. Blinding was not relevant to our study, since we are studying and comparing the property of known cell types and genotypes across comparator stimuli using objective data measurements (flow staining e.g.) and there is no subjective human analysis (such as grading disease severity or histologic severity).

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	Stimulatory antibodies polyclonal F(ab')₂ goat anti-mouse IgM (anti-μ) (Jackson ImmunoResearch, cat # 115-006-020) anti-IgDa (clone AMS 9.1, Santa Cruz Biotechnology, cat # sc-53853L) anti- FcγRIIB (clone 2.4G2) was used to block nonspecific binding (produced in house) ELISPOT Biotin-conjugated Mouse Anti-Mouse IgM[a] (Clone:DS-1; BD Biosciences, cat# 553515, lot#) 1:2000 Streptavidin-Alkaline Phosphatase (SouthernBiotech, cat#7100-04) 1:2000 Surface staining flow cytometry B220-Alexa647 (clone RA3-6B2, Biolegend, cat# 103229) 1:500 anti-HEL- Dylight650 conjugated (clone D1.3, produced and conjugated in house). 1:400 B220-BV421 (clone RA3-6B2 Biolegend cat# 103251) 1:500 CD43-APC (clone S11, Biolegend, cat# 143207) 1:200 IgD-PE (clone 11-26c.2a, Biolegend cat# 405705) 1:300 IgM-Dylight 488 (clone B-7-6). B-7-6 was produced in our own laboratory, Fab fragments were made for some applications, and it was conjugated to Dylight 488) Fabs were used at 1:250 and intact antibody at 1:200 CD69-BV786 (clone H1.2F3, BD Biosciences, cat# 564683) 1:200 CD86-BV605 (clone GL1, BD Biosciences, cat# 563055) 1:200 CD95-FITC (clone Jo2, BD Biosciences, cat# 554257), 1:200 CD138-PE-Cy7 (Clone: 281-2; Biolegend) 1:300 Intracellular staining anti-Ars/A1 idotype – Dylight650 (clone E4 was produced and conjugated in our laboratory), 1:300 anti-GFP – Dylight488 (polyclonal Goat anti-GFP Antibody (Cat# 600-101-215, Rockland) conjugated in our laboratory) 1:500 pCD79A-Alexa 647 (pY182) (clone D189, Cell Signaling Technology, cat# 29742S) 1:50 pSyk (pY352) -Alexa 647 (Clone 17A/P-ZAP70, BD Biosciences, cat# 557817) 1:5 pErk (pT202/pY204) -Alexa 647 (clone 20A, BD Biosciences, cat # 612593), 1:5 pPLCβ2 (pY759) -Alexa 647 (clone K86-689.37, BD Biosciences, cat# 558498),1:5 pBLNK (pY84) -Alexa647 (clone J117-1278, BD Biosciences, cat# 558443) 1:5 pAkt (pS473) Alexa647 (clone M89-61, BD Biosciences, cat# 560343), 1:5 IkBα Alexa647 (clone L35A5, Cell Signaling Technology, cat # 8993S), 1:100 pS6 (pS235/pS236)-PE-Cy7 (clone cupk34k, eBioscience/Invitrogen, cat# 25-9007-42), 1:100 Rabbit isotype control -Alexa 647 (Cell Signaling Technology, cat# 3452S), 1:200 mouse IgG1 isotype control Alexa647 (clone MOPC-21, BD Biosciences, cat# 557783)1:5 mouse IgG1-PE-Cy7 isotype control (clone P3.6.2.8.1, eBioscience/Invitrogen, cat# 50-155-68). 1:200 Western blot pAkt (S473) (Cell Signaling, cat# 4058L, lot 33) 1:1000 Akt (Cell Signaling, cat# 9272S, lot 30) 1:1000 p44/42 MAPK (Erk1/2) (pThr202/pTyr204) (Cell Signaling Technology, cat# 9101, lot 32), 1:2000 P44/42 MAPK (Erk1/2) (Cell Signaling Technology, cat# 4695S, lot 40) 1:1000 pSyk (Tyr525/Tyr526) (Cell Signaling Technology, cat# 2711S, lot 15) 1:1000 Syk (Cell Signaling, cat# 2712S, lot 40) 1:1000 pSHIP-1 (Tyr1022, Cell Signaling, cat #3941, lot 3) 1:1000 SHIP-1 (clone D1163, Cell Signaling, cat #2728, lot 2) 1:1000 pSHP-1 (Tyr564, clone D11G5, Cell Signaling, cat #8849, lot 3) 1:1000 SHP-1 (clone C14H6, Cell Signaling, cat #3759, lot 5) 1:1000 Lyn (LYN-01, Abcam, Cat # ab1890, lot 1-17722-5), 1:1000 Lyn (affinity purified polyclonal rabbit IgG raised against Lyn 1-131) 1:1000 actin-HRP conjugated (clone C4, Santa Cruz , cat# sc47778, lot A0924) 1:10,000 Goat anti-Rabbit IgG HRP-conjugated (Cell Signaling Technology, cat# 7074S). 1:20,000
Validation	All commercial antibodies have been validated by the vendors. We have confirmed that the profile of flow antibodies matches the expected pattern (i.e. expressed on the right cell type under the right conditions). For western blot antibodies we confirmed that the detected protein was the right size. Anti-idiotypic antibody E4 was purified from hybridoma supernatant and validated by flow cytometry on B cells that express the Ars/A1 BCR and those that do not. Anti-HEL clone D1.3 was purified from hybridoma supernatant and validated by flow cytometry on SRBC-HEL conjugates. Polyclonal anti-Lyn antibody was previously generated in rabbits and affinity purified. The antibody was validated on lysates from purified Lyn sufficient and deficient B cells.

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	Six- to twenty-week-old mice were used in all experiments. The mice were kept under SPF conditions on a 14 hrs light-10hrs dark cycle. hCD20-TamCre mice were previously described in PMID: 22555432 (Donated by Dr M Shlomchik, University of Pittsburgh). Ars/ A1 mice were previously described in PMID: 11163228 (donated by Dr L Wysocki, University of Colorado). Lynflox/flox mice were previously described in PMID: 23940344. In these mice exons 3 and 4 are flanked by loxp sites (donated by Dr. C. Lowell, UCSF, San Francisco, CA). SHIP-1flox/flox mice were previously described in PMID: 12874264. In these mice exons 10 and 11 are flanked by loxp
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sites (donated by Dr J Ravetch, Rockefeller University). The other mice were originally purchased from Jackson Laboratory: MD4 mice (C57BL/6-Tg(IghelMD4)4Ccg/J; strain #002595), mb1cre mice (B6.C(Cg)-Cd79atm1(cre)Reth/EhobJ; strain #020505), Rosa26-flox-STOPYFP mice (B6.129X1-Gt(ROSA)26Sortm1(EYFP)Cos/J; strain #006148) and C57BL/6J mice (strain #000664)

Wild animals	No wild animals were used in this study
Reporting on sex	In each experiment, mice were age-matched and sex-matched. Both sexes were used in our experiments with similar results.
Field-collected samples	This study contains no field-collected samples
Ethics oversight	All experiments with mice were performed in accordance with the regulations and with approval of the University of Colorado SOM Institutional Animal Care and Use Committee.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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	Single cell suspensions of splenic cells or bone marrow cells, flushed out of the two hindleg femurs, were prepared in 10% FCS complete medium, and red blood cells were lysed using Ammonium Chloride Potassium (ACK) Lysis buffer (15 mM NH ₄ Cl, 10 mM KHCO ₃ , 0.1 mM Na ₂ EDTA, pH 7.4). Cells were resuspended in PBS containing 1% BSA and 0.05% sodium azide and incubated with an optimal amount of directly fluorochrome labeled antibodies. For intracellular staining cells were fixed and permeabilized with BD Fix/Perm and stained in BD permwash, or with fixed with 2% formaldehyde followed by 100% methanol (-80C) after which cells were stained in PBS, 1%BSA. Cells were spun down at 400g before fixation and 700g post fixation.
Instrument	Flow cytometry data was collected on a CyAn ADP (Dakocytomation), a BD LSRFortessa™ X-20 Cell Analyzer (BD Biosciences) or a Cytex Northern Lights (Cytex Biosciences).
Software	Flow cytometry data was analyzed using FlowJo (v9.x and v10.8, v10.10) software (BD).
Cell population abundance	For all B cell flow based ex vivo assays, B cells represented between 10-60% of lymphocyte events., depending if we gated on total B220+ B cells or only B220+ YFP+ B cells. For in vivo adoptive transfer assays the final gate on YFP+ E4+ B cells represents 0.4%-0.002% of the lymphocyte gate.
Gating strategy	In adoptive transfer experiments we gated the lymphocyte population based on FSC/SSC properties but including larger cells to capture plasmablasts, followed by a doublet exclusion based on FSC-A and FSC-H scatter. This was followed by gating on YFP+ E4+ events. For all ex vivo signaling experiments we gated on the lymphocyte population based on FSC/SSC properties, followed by doublet exclusion based on FSC-A and FSC-H scatter. B cells were gated on B220+ events and if YFP+ was used to mark cells that had cre activity we gated on B220+ YFP+ events. Gating strategies are shown in Suppl Figs 1A, 4A, 5A, 6A, and 13A.

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