

Reporting Summary

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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

BD FACS Diva software v8.0.1, SoftMax Pro 5.4.1, ZEN Lite 2012, QuantStudio Real-time PCR Software v1.3, UNICORN 5.01, NIS-Elements F 4.00.00, Immunospot 5.1

Data analysis

GraphPad Prism6 was used for data analysis and plotting.
FlowJo was used for flow cytometry analysis.
ZEN Lite 2012 and Fiji ImageJ were used for immunofluorescence image analysis.

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](https://www.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	We did not predetermine sample size using software. The exact n values used to calculate the statistics are provided and a reasonable sample size was chosen to ensure adequate reproducibility of results.
Data exclusions	No data were excluded from the analysis.
Replication	All experiments were performed at least twice with similar results.
Randomization	Mice were randomized into different groups.
Blinding	Blinding was not performed due to the unambiguous nature of measurements and systematic analyses used in these experiments.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Unique biological materials
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Human research participants

Methods

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

I. For flow cytometry
 I-a. For DC and macrophage analysis and sorting (Fig 4b-d, Fig 5f, Fig S3-S6, Fig S8, Fig S9b)
 CD19 AF700 (Clone: 6D5; Cat. 115528; Lot: 205B249811; Biolegend)
 B220 PE/Cy7 (Clone: RA3-6B2; Cat. 25-0452-82; Lot: E07569-1636; eBioscience)
 CD11c PE (Clone: N418; Cat. 117308; Lot: B256109; Biolegend)
 CD11c FITC (Clone: N418; Cat. 117305; Lot: B244373; Biolegend)
 I-A/I-E APC/CY7 (Clone: M5/114.15.2; Cat. 107628; Lot: B248055; Biolegend)
 CD11b PerCP/Cy5.5 (Clone: M1/70; Cat. 45-0112-82; Lot: 1929457; eBioscience)
 SIGNR1 APC (Clone: 22D1; Cat. 17-2093-80; Lot: 4326927; eBioscience)
 CD169 PE (Clone: 3D6.112; Cat. 142403; Lot: B250778; Biolegend)
 F4/80 PE/Cy7 (Clone: BM8; Cat. 123113; Lot: B237342; Biolegend)
 CD103 FITC (Clone: 2E 7; Cat. 11-1031-82; Lot: 4301319; eBioscience)
 CXCR5 biotin (Clone: SPRCL5; Cat. 13-7185-82; Lot: 4337044; eBioscience)

Streptavidin APC/Cy7 (Cat. 405208; Lot: B215107; Biolegend)
 I-b. For Tfh cells staining (Fig 5a)
 Thy1.1 PerCP/Cy5.5 (Clone: OX-7; Cat. 202516; Lot: B178918; Biolegend)
 TCR Vα2 PE (Clone: B20.1; Cat. 12-5812-82; Lot: E01800-1634; eBioscience)
 CD4 PE/Cy7 (Clone: GK1.5; Cat. 100422; Lot: 1936945; Biolegend)
 PD1 APC (Clone: J43; Cat. 17-9985-82; Lot:4335542; eBioscience)
 CXCR5 biotin (Clone: SPRCL5; Cat. 13-7185-82; Lot: 4337044; eBioscience)
 Streptavidin APC/Cy7 (Cat. 405208; Lot: B215107; Biolegend)
 I-c. For GC B cells staining (Fig 5b)
 B220 PE (Clone: RA3-6B2; Cat. 12-0452; Lot: E01249-1634; eBioscience)
 GL7 Alexa Fluor488 (Clone: GL-7; Cat. 53-5902-82; Lot: E11670-1635; eBioscience)
 FAS PE/Cy7 (Clone: Jo2; Cat. 557653; Lot: 7018663; BD Pharmingen™)
 I-d. For T cell activation (Fig 5d, 5e)
 CD69 PE (Clone: H1.2F3; Cat. 12-0691; Lot: E01333-1634; eBioscience)
 CD25 FITC (Clone: PC61; Cat. 102006; Lot: B126955; Biolegend)
 CD44 APC/Cy7 (Clone: IM7; Cat. 103028; Lot:B262797; Biolegend)
 CD62L AF700 (Clone: MEL-14; Cat. 56-0621-82; Lot: 4306534; eBioscience)
 I-e. For B cell activation (Fig 6a,6g)
 B220 APC (Clone: RA3-6B2; Cat. 103212; Lot: B208579; Biolegend)
 CD86 PE (Clone: GL1; Cat. 12-0862-81; Lot: 4294065; eBioscience)
 CD69 PE (Clone: H1.2F3; Cat. 12-0691-82; Lot: E01333-1634; eBioscience)
 CD45.1 FITC (Clone: A20; Cat. 110705; Lot: B202563; Biolegend)
 CD45.2 PerCP/Cy5.5 (Clone: 104; Cat. 45-0454-82; Lot: 4336370; eBioscience)
 I-f. For human macrophage analysis (Fig S7)
 HLA-DR APC/Cy7 (Clone: L243; Cat. 307617; Lot: B272894; Biolegend)
 CD14 PE (Clone: M5E2; Cat. 301806; Lot: B246225; Biolegend)
 CD209 APC (Clone: 9E9A8; Cat. 330107; Lot: B279429; Biolegend)
 Human TruStain FcXTM (Cat. 422302; Lot: B291618; Biolegend)
 The working concentration of all the antibodies for FACS is 2.5 µg/ml.

II. For immunofluorescence assay (Fig 4a, Fig 4e, Fig 5c, Fig 6e, 6f Fig S3b, Fig S10a)
 Full length GFP polyclonal antibody (Cat. 632592; Lot: 1510024; Clontech; 1:1000.)
 SIGNR1 APC (Clone: 22D1; Cat. 17-2093-80; Lot: 4326927; eBioscience)
 CD169 PE (Clone: 3D6.112; Cat. 142403; Lot: B250778; Biolegend)
 CD11c FITC (Clone: N418; Cat. 117035; Lot: B244373; Biolegend)
 B220 PE (Clone: RA3-6B2; Cat. 12-0452-82; Lot: E01249-1634; eBioscience)
 B220 eFluor 450 (Clone: RA3-6B2; Ref: 48-0452-80; Lot: 4339476; Invitrogen)
 GL7 Alexa Fluor488 (Clone: GL-7; Cat. 53-5902-82; Lot: E11670-1635; eBioscience)
 CD45.1 eFluor 450 (Clone: A20; Cat. 48-0453; Lot: E08504-1632; eBioscience)
 CD45.2 Biotin (Clone: 104; Cat. 13-0454; Lot: E02536-1631; eBioscience)
 Streptavidin FITC (Cat. 11-4317-87; Lot: 4323304; eBioscience)
 The working concentration of all the antibodies for immunofluorescence is 2.5 µg/ml.

III. For immunological histological chemistry (IHC) (Fig 3f, Fig S2c) (1:900 dilution)
 Polyclonal rabbit anti-Hepatitis B virus core antigen (HBcAg) (Cat. B0586; Lot: 10048815; Dako)

IV. For ELISA (1:5000 dilution)
 Horseradish peroxidase (HRP) conjugated goat anti-mouse IgG (H+L) (Cat. ZB2305; Lot: 133499; ZSGB-BIO)
 Peroxidase-conjugated Goat anti-mouse IgG subclass I (Cat. SA00012-1; ProteinTech)
 Peroxidase-conjugated Goat anti-mouse IgG2c (Cat. SA00012-4; ProteinTech)
 Peroxidase-conjugated Goat anti-mouse IgM (Cat. SA00012-6; ProteinTech)

V. For ELISPOT
 Purified anti-mouse IFN-γ (Cat. 51-2525KC; Lot: 5295506; BD Biosciences) (1:200 dilution)
 Biotinylated anti-mouse IFN-γ (Cat. 51-1818KZ; Lot: 6125718; BD Biosciences) (1:250 dilution)
 Streptavidin-HRP (Cat. 51-9000209; Lot: 6225988; BD Biosciences) (1:100 dilution)
 Donkey Anti-Mouse IgG, Biotin Conjugated (Cat: CW0225; Lot: 1915F; CWbio) (1:1000 dilution)

VI. For IFNγ blocking
 InVivoMAb anti-mouse IFNγ (Cat#BE0055; Lot: 699718S1; BioXCell)

Validation

All the antibodies used are from commercial sources and have been validated by the vendors. Validation data are available on the manufacturer's website.

CD19 AF700 (Clone: 6D5; Cat. 115528; Lot: 205B249811; Biolegend)
<https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-cd19-antibody-3391>
 B220 PE/Cy7 (Clone: RA3-6B2; Cat. 25-0452-82; Lot: E07569-1636; eBioscience)
<https://www.thermofisher.com/cn/zh/antibody/product/CD45R-B220-Antibody-clone-RA3-6B2-Monoclonal/25-0452-81>
 CD11c PE (Clone: N418; Cat. 117308; Lot: B256109; Biolegend)
<https://www.biolegend.com/en-us/products/pe-anti-mouse-cd11c-antibody-1816>
 CD11c FITC (Clone: N418; Cat. 117305; Lot: B244373; Biolegend)
<https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd11c-antibody-1815>
 I-A/I-E APC/CY7 (Clone: M5/114.15.2; Cat. 107628; Lot: B248055; Biolegend)
<https://www.biolegend.com/en-us/products/apccyanine7-anti-mouse-i-a-i-e-antibody-5966>

CD11b PerCP/Cy5.5 (Clone: M1/70; Cat. 45-0112-82; Lot: 1929457; eBioscience)
<https://www.thermofisher.com/antibody/primary/query/45-0112>
 SIGNR1 APC (Clone: 22D1; Cat. 17-2093-80; Lot: 4326927; eBioscience)
<https://www.thermofisher.com/cn/zh/antibody/product/CD209b-SIGN-R1-Antibody-clone-eBio22D1-22D1-Monoclonal/17-2093-80>
 CD169 PE (Clone: 3D6.112; Cat. 142403; Lot: B250778; Biolegend)
<https://www.biolegend.com/en-us/products/pe-anti-mouse-cd169-siglec-1-antibody-7522>
 F4/80 PE/Cy7 (Clone: BM8; Cat. 123113; Lot: B237342; Biolegend)
<https://www.biolegend.com/en-us/products/pe-cy7-anti-mouse-f4-80-antibody-4070>
 CD103 FITC (Clone: 2E 7; Cat. 11-1031-82; Lot: 4301319; eBioscience)
<https://www.thermofisher.com/antibody/primary/query/11-1031>
 CXCR5 biotin (Clone: SPRCL5; Cat. 13-7185-82; Lot: 4337044; eBioscience)
<https://www.thermofisher.com/cn/zh/antibody/product/CD185-CXCR5-Antibody-clone-SPRCL5-Monoclonal/13-7185-82>
 Streptavidin APC/Cy7 (Cat. 405208; Lot: B215107; Biolegend)
<https://www.biolegend.com/en-us/products/apc-cy7-streptavidin-1471>
 Thy1.1 PerCP/Cy5.5 (Clone: OX-7; Cat. 202516; Lot: B178918; Biolegend)
<https://www.biolegend.com/en-us/products/percpcyanine55-anti-rat-cd90mouse-cd901-thy-11-antibody-4514>
 TCR Vα2 PE (Clone: B20.1; Cat. 12-5812-82; Lot: E01800-1634; eBioscience)
<https://www.thermofisher.com/antibody/primary/query/12-5812>
 CD4 PE/Cy7 (Clone: GK1.5; Cat. 100422; Lot: 1936945; Biolegend)
<https://www.biolegend.com/en-us/products/pe-cy7-anti-mouse-cd4-antibody-1919>
 PD1 APC (Clone: J43; Cat. 17-9985-82; Lot: 4335542; eBioscience)
<https://www.thermofisher.com/cn/zh/antibody/product/CD279-PD-1-Antibody-clone-J43-Monoclonal/17-9985-82>
 B220 PE (Clone: RA3-6B2; Cat. 12-0452-82; Lot: E01249-1634; eBioscience)
<https://www.thermofisher.com/antibody/primary/query/12-0452>
 GL7 Alexa Fluor488 (Clone: GL-7; Cat. 53-5902-82; Lot: E11670-1635; eBioscience)
<https://www.thermofisher.com/cn/zh/antibody/product/GL7-Antibody-clone-GL-7-GL7-Monoclonal/53-5902-82>
 FAS PE/Cy7 (Clone: Jo2; Cat. 557653; Lot: 7018663; BD Pharmingen™)
<https://www.fishersci.com/shop/products/anti-cd95-pe-cy-7-clone-jo2-bd/bdb557653#?keyword=557653>
 CD69 PE (Clone: H1.2F3; Cat. 12-0691-82; Lot: E01333-1634; eBioscience)
<https://www.thermofisher.com/antibody/primary/query/12-0691>
 CD25 FITC (Clone: PC61; Cat. 102006; Lot: B126955; Biolegend)
<https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd25-antibody-422>
 CD44 APC/Cy7 (Clone: IM7; Cat. 103028; Lot: B262797; Biolegend)
<https://www.biolegend.com/en-us/products/apc-cy7-anti-mouse-human-cd44-antibody-3933>
 CD62L AF700 (Clone: MEL-14; Cat. 56-0621-82; Lot: 4306534; eBioscience)
<https://www.thermofisher.com/antibody/primary/query/CD62L%20AF700>
 B220 APC (Clone: RA3-6B2; Cat. 103212; Lot: B208579; Biolegend)
<https://www.biolegend.com/en-us/products/apc-anti-mouse-human-cd45r-b220-antibody-442>
 CD86 PE (Clone: GL1; Cat. 12-0862-81; Lot: 4294065; eBioscience)
<https://www.thermofisher.com/cn/zh/antibody/product/CD86-B7-2-Antibody-clone-GL1-Monoclonal/12-0862-81>
 CD45.1 FITC (Clone: A20; Cat. 110705; Lot: B202563; Biolegend)
<https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd45-1-antibody-198>
 CD45.2 PerCP/Cy5.5 (Clone: 104; Cat. 45-0454-82; Lot: 4336370; eBioscience)
<https://www.thermofisher.com/antibody/primary/query/45-0454>
 HLA-DR APC/Cy7 (Clone: L243; Cat. 307617; Lot: B272894; Biolegend)
<https://www.biolegend.com/en-us/products/apc-cyanine7-anti-human-hla-dr-antibody-2863>
 CD14 PE (Clone: M5E2; Cat. 301806; Lot: B246225; Biolegend)
<https://www.biolegend.com/en-us/products/pe-anti-human-cd14-antibody-796>
 CD209 APC (Clone: 9E9A8; Cat. 330107; Lot: B279429; Biolegend)
<https://www.biolegend.com/en-us/products/apc-anti-human-cd209-dc-sign-antibody-4886>
 Human TruStain FcXTM (Cat. 422302; Lot: B291618; Biolegend)
<https://www.biolegend.com/en-us/products/human-trustain-fcx-fc-receptor-blocking-solution-6462>
 Full length GFP polyclonal antibody (Cat. 632592; Lot: 1510024; Clontech; 1:1000.)
<https://www.takarabio.com/products/antibodies-and-elisa/fluorescent-protein-antibodies/green-fluorescent-protein-antibodies?catalog=632592>
 B220 eFluor 450 (Clone: RA3-6B2; Ref: 48-0452-80; Lot: 4339476; Invitrogen)
<https://www.thermofisher.com/cn/zh/antibody/product/CD45R-B220-Antibody-clone-RA3-6B2-Monoclonal/48-0452-80>
 CD45.1 eFluor 450 (Clone: A20; Cat. 48-0453-82; Lot: E08504-1632; eBioscience)
<https://www.thermofisher.com/antibody/primary/query/48-0453>
 CD45.2 Biotin (Clone: 104; Cat. 13-0454-82; Lot: E02536-1631; eBioscience)
<https://www.thermofisher.com/antibody/primary/query/13-0454>
 Streptavidin FITC (Cat. 11-4317; Lot: 4323304; eBioscience)
<https://www.thermofisher.com/order/catalog/product/11-4317-87?SID=srch-hj-11-4317-87>
 Horseradish peroxidase (HRP) conjugated goat anti-mouse IgG (H+L) (Cat. ZB2305; Lot: 133499; ZSGB-BIO)
<http://www.zsbio.com/product/ZB-2305>
 Peroxidase-conjugated Goat anti-mouse IgG subclass I (Cat. SA00012-1; ProteinTech)
<http://www.ptgcn.com/Products/HRP-conjugated-Affinipure-Goat-Anti-Mouse-IgG,-Fc%CE%B3Subclass-1-Specific-secondary-antibody.htm>
 Peroxidase-conjugated Goat anti-mouse IgG2c (Cat. SA00012-4; ProteinTech)
<https://www.ptgcn.com/Products/HRP-conjugated-Affinipure-Goat-Anti-Mouse-IgG,-Fc%ce%b3Subclass-2c-Specific-secondary-antibody.htm>
 Peroxidase-conjugated Goat anti-mouse IgM (Cat. SA00012-6; ProteinTech)
<https://www.ptgcn.com/Products/HRP-conjugated-Affinipure-Goat-Anti-Mouse-IgM,-%ce%bc-Chain-Specific-secondary-antibody.htm>

Purified anti-mouse IFN- γ (Cat. 51-2525KC; Lot: 5295506; BD Biosciences)
 Biotinylated anti-mouse IFN- γ (Cat. 51-1818KZ; Lot: 6125718; BD Biosciences)
 Streptavidin-HRP (Cat. 51-9000209; Lot: 6225988; BD Biosciences)
<https://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-1-cells/immunoassays/elispot/mouse/mouse-ifn--elispot-set/p/551083>
 Donkey Anti-Mouse IgG, Biotin Conjugated (Cat: CW0225; Lot: 1915F; CWbio)
<http://www.cwbiotech.com/product/19887.html>
 InVivoMAB anti-mouse IFN γ (Cat#BE0055; Lot: 699718S1; BioXCell)
<https://bxc.com/product/invivomab-anti-m-ifngamma/>

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HepG2-hNTCP cell line was generated by Dr. Wenhui Li (National Institute of Biological Sciences, Beijing, China). Ref: Elife. 2012 Nov 13;3. doi: 10.7554/eLife.00049.
Authentication	No authentication was carried out. Cell morphology and behavior such as infection by HBV was consistent with expectations.
Mycoplasma contamination	The cell line was tested to be negative for Mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Wild type C57BL/6 mice and BALB/c mice were obtained from Vital River Laboratory Animal Technology Co. (Beijing, China). CD11c-DTR mice and OT-II TCR transgenic mice were purchased from The Jackson Laboratory. MD4 transgenic mice were kindly provided by Dr. Baidong Hou (Chinese Academy of Science Key Laboratory for Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China). Cxcr5 ^{-/-} mice were kindly provided by Dr. Hai Qi (Institute for Immunology, School of Medicine, Tsinghua University, Beijing, China). Signr1 ^{-/-} mice were generated by Cyagen Biosciences Inc. (Suzhou, China). For the detection of antibody response in wild type C57BL/6 mice and BALB/c mice, 6-10 weeks-old female mice were used. In the AAV-HBV1.3 associated protective assay, 6-10 weeks-old C57BL/6N male mice were used. In the AAV-HBV1.3 associated therapeutic assay, 4-5 weeks-old C57BL/6N male mice were used. For the using of transgenic mice, age and sex matched female or male mice were used for each experiment.
Wild animals	No wild animals were involved.
Field-collected samples	No field-collected samples were used.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	Human fresh distal mesenteric lymph nodes from neuroblastoma patients of 2.5-14 years old were derived from the patients who underwent surgery at Department of Pediatric Surgical Oncology, Children's Hospital of Chongqing Medical University, Chongqing, P.R China. Patients are HIV, HBV, HCV negative. Patients with acute infection, fever or diarrhoea were also excluded. No endocrine therapy, chemotherapy, or radiotherapy was given to patients before surgery.
Recruitment	We used available mesenteric lymph nodes samples from the patients. The patients were informed, and they signed consent forms acknowledging the use of their resected tissues for research purposes. There was no potential self-selection bias.

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	Lymph nodes were harvested and gently minced using scissors in FACS buffer (PBS containing 2% FBS). The tissues were then digested with 0.5 mg/ml collagenase I (Sigma) and 0.04 mg/ml DNase I (Roche) at 37°C for 1 h. In CXCR5 detection and ferritin-
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FITC binding assay, LNs were digested with 100 U/ml CLSPA (Worthington) and 0.04 mg/ml DNase I (Roche) in 1640 medium at 37°C for 1 h. The suspension was washed with cold FACS buffer, disaggregated by passing through a 70 µm cell strainer (Biologix Group) and centrifuged at 500 g for 5 min. Splenocytes were collected in the same manner, and red blood cells were removed with ammonium-chloride-potassium buffer.

Instrument

LSRFortessa, FACS Aria III

Software

BD FACS Diva 8.0.1 software was used for data collection and FlowJo v.10 was used for data analysis.

Cell population abundance

In mouse lymph nodes, there are ~0.1% of SIGNR1+ rDC, ~0.04% of SIGNR1+ miDC, ~0.7% of SIGNR1+ macrophage, and ~0.2% F4/80+ macrophage in single alive cell gate.
 For GC B cells, ~30% of B220+ cells and 0.4% of FAS+GL7+ cells in ferritin NP vaccine immunized groups and 0.09% in control.
 For Tfh cells, ~2% of OTII+CD4+ cells and 0.1% of CXCR5+PD1+GL7+ cells in ferritin NP vaccine immunized group, ~1% of OTII+CD4+ cells and 0.006% of CXCR5+PD1+GL7+ cells in control.
 In human lymph nodes, there are ~0.4% of CD14+ macrophage and ~0.17% of DC-SIGN+ macrophage in single alive cell gate.
 For flow sorting, post-sort cells were analyzed on BD Aria III and the purity was at least 95%.

Gating strategy

I. For mouse DC and macrophage analysis and sorting
 I-a. For naïve LN, single alive lymphocytes were first gated with CD19 and B220, then non-B cells were gated with CD11c and MHCII, CD11c high cells were resident DC, MHCII high cells were migratory DC, others were non-DCs. DCs were then gated with SIGNR1 and CD11b. CD11b+ non-DCs were then gated with CD169 and F4/80 or SIGNR1 and F4/80.
 I-b. For immunized LN, CD11c+MHCII+ DCs were gated with CD103, CD103- cells were resident DC, CD103+ cells were migratory DC; macrophages were gated as the same as above.

II. For human macrophage analysis
 HLA-DR+ CD14+ macrophages were gated and identified into CD209+ and CD209- populations.

III. For Tfh staining and GC B cells
 Tfh cells: single alive lymphocytes were gated with Thy1.1+TCR Vα2+CD4+, and finally gated with CXCR5+PD-1+ cells or GL7+PD-1+ cells.
 GC B cells: single alive lymphocytes were gated with B220+ cells, and finally gated with FAS+GL7+ cells.

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