

## **Supplementary material**

**Follicular dendritic cell dysfunction contributes to impaired T cell-dependent antigen-specific humoral responses in sepsis-surviving mice.**

**Minakshi Rana<sup>1</sup>, Andrea La Bella<sup>1</sup>, Rivka Lederman<sup>1</sup>, Bruce T. Volpe<sup>2</sup>, Barbara Sherry<sup>3\*</sup>, Betty Diamond<sup>1\*</sup>**

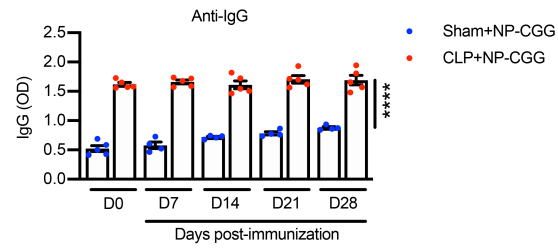
<sup>1</sup>Center for Autoimmune, Musculoskeletal and Hematopoietic Diseases, The Feinstein Institutes for Medical Research, New York, USA; <sup>2</sup>Center for Biomedical Science, The Feinstein Institutes for Medical Research, New York, USA. <sup>3</sup>Center for Immunology and Inflammation, The Feinstein Institutes for Medical Research, New York, USA.

### **\*Correspondence**

Betty Diamond, The Feinstein Institutes for Medical Research, Center for Autoimmune, Musculoskeletal and Hematopoietic Diseases, 350 Community Dr., Manhasset, New York 11030; e-mail: bdiamond@northwell.edu; Phone: 516-562-3830, Fax: 516-562-2953

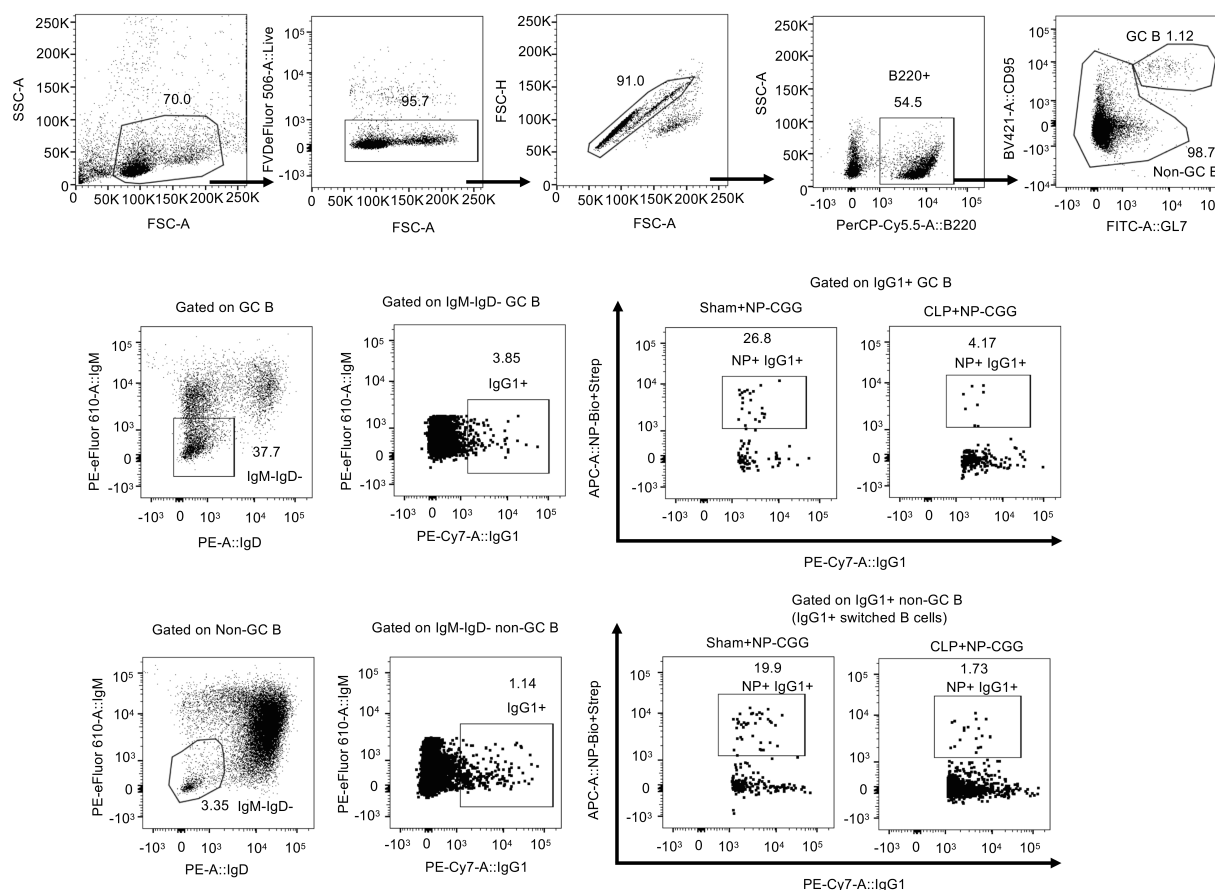
Barbara Sherry, The Feinstein Institutes for Medical Research, Center for Immunology and Inflammation, 350 Community Dr., Manhasset, New York 11030; e-mail: bsherry@northwell.edu; Phone: 516-562-3402, Fax: 516-562-1022

## Supplementary Figure 1



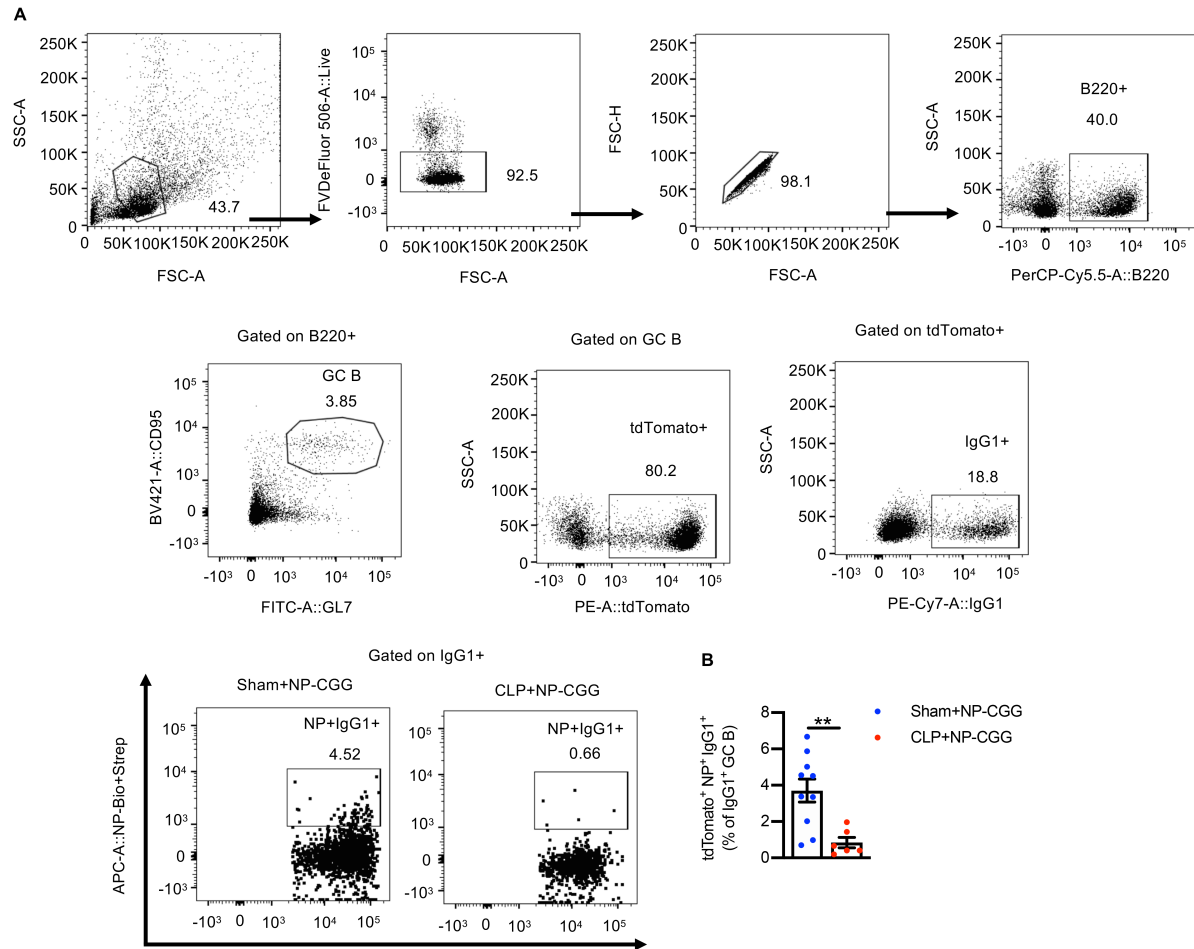
**Supplementary Figure 1.** Sham and CLP mice were immunized with NP-CGG in alum; single i.p. injection four-weeks post-surgery. Serum was analyzed for total anti-IgG (1:20000). Time-dependent response of total anti-IgG (n=4-5, Sham+NP-CGG; n=5, CLP+NP-CGG). Data represent mean  $\pm$  SEM from one of two independent experiments. Sham+NP-CGG vs. CLP+NP-CGG \*\*\*\* $P < 0.0001$  (Mixed-effects model (REML)).

## Supplementary Figure 2



**Supplementary Figure 2. Gating strategy for germinal-center (GC) and Non-GC (memory) B cells.** Sham-operated or CLP mice were immunized with a single i.p. injection of NP-CGG (100  $\mu$ g) in alum (100  $\mu$ L) four-weeks after surgery. At the time points indicated in results section, splenocytes isolated from immunized sham or CLP mice at different time points indicated in result were stained for CD95, GL7, IgM, IgD, IgG1 and NP, and analyzed by flow cytometry. Representative flow cytometry dot plots of splenocytes isolated from an immunized sham-operated mouse depicting gating strategy for NP-specific IgG1<sup>+</sup> GC and Non-GC (class-switched IgG1<sup>+</sup> memory) B cells.

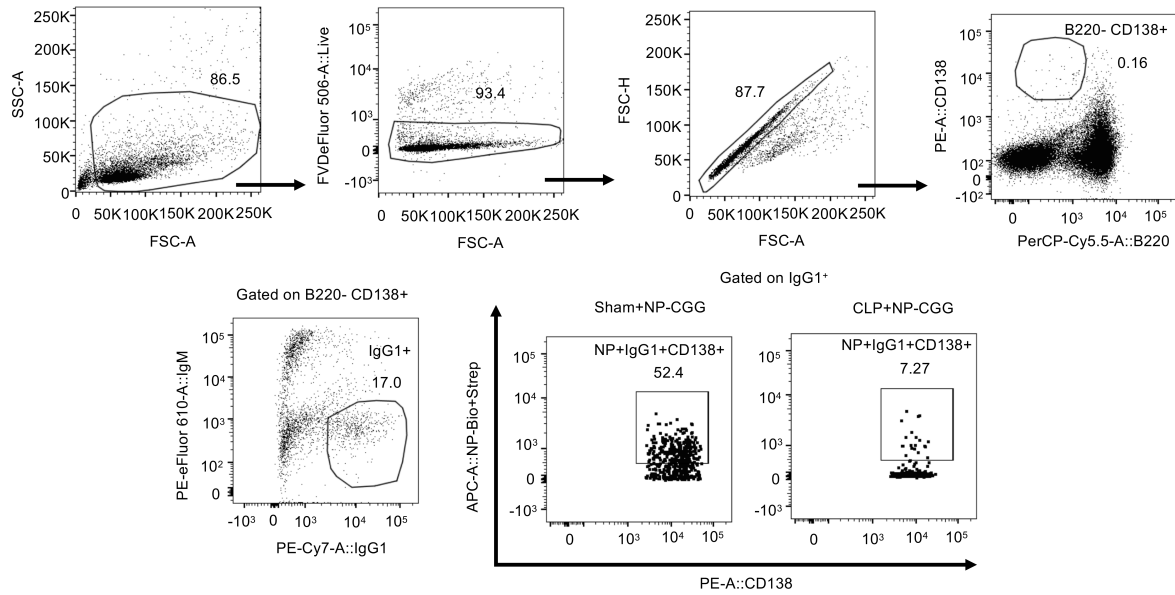
### Supplementary Figure 3



**Supplementary Figure 3. GC-dependent defect in an antigen-specific antibody response.** Sham or CLP surgery was performed in *Slpr2-ERT2cre-tdTomato* reporter mice. Four-weeks post-surgery mice were immunized with a single i.p. injection of NP-CGG (100  $\mu$ g) in alum (100  $\mu$ L) and tdTomato expression in these mice was induced by i.p. administration of 2 mg tamoxifen (Sigma) in 100  $\mu$ L of corn oil (Sigma) for three consecutive days (days 11-13; once per day) starting at day 11 after NP-CGG immunization. Splenocytes isolated from immunized sham or CLP mice on day 14 after immunization were stained for CD95, GL7, IgG1, NP, and analyzed by flow cytometry. **(A)** Representative flow cytometry dot plots of splenocytes isolated from an immunized sham mouse depicting gating strategy for GC-specific  $tdTomato^+$  NP $^+$  IgG1 $^+$  B cells. **(B)** Quantification of percentage of  $tdTomato^+$  NP $^+$  IgG1 $^+$  GC B in IgG1 $^+$  gate. Each dot represents an individual animal ( $n=10$ , Sham+NP-CGG;  $n=6$ , CLP+NP-CGG). Data represent mean  $\pm$  SEM from two independent experiments. Sham+NP-CGG vs. CLP+NP-CGG  $**P < 0.01$ . (Mann-Whitney U-test).

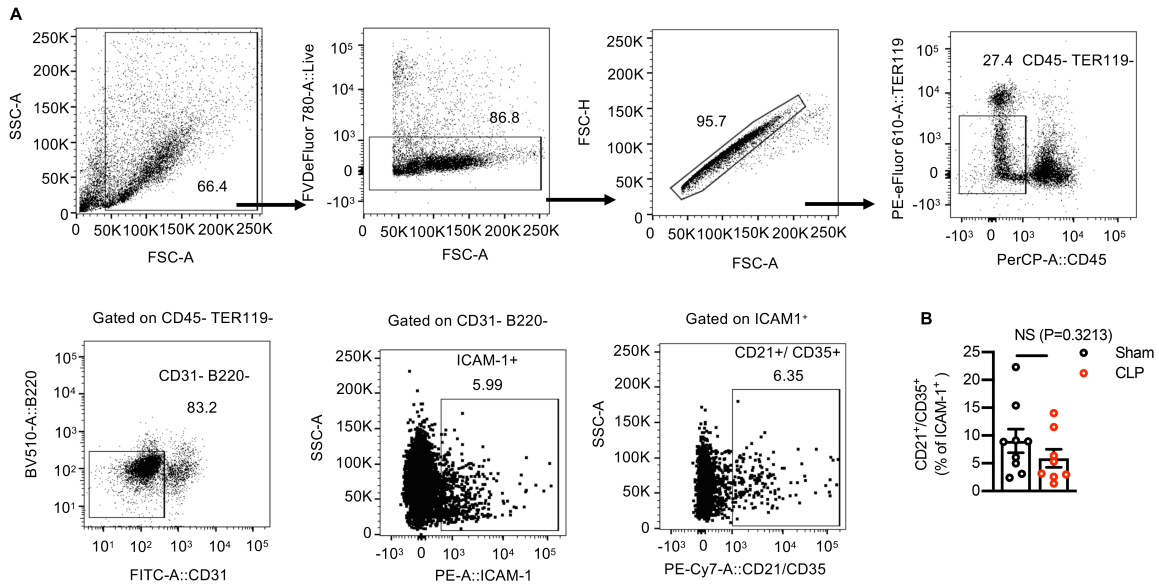


## Supplementary Figure 4



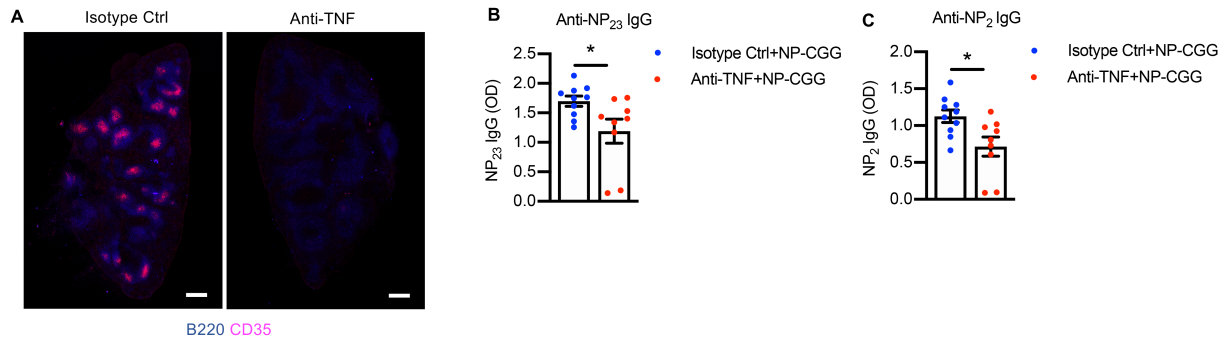
**Supplementary Figure 4. Gating strategy for plasma cells.** Sham or CLP mice were immunized with single i.p. injection of NP-CGG (100  $\mu$ g) in alum (100  $\mu$ L) four-weeks post-surgery. Splenocytes isolated from immunized sham or CLP mice at the time points indicated in result section were surface stained for B220 and CD138 and intracellularly stained for IgM, IgG1 and NP, and analyzed by flow cytometry. Representative flow cytometry dot plots of splenocytes isolated from an immunized sham mouse depicting gating strategy for NP-specific IgG1<sup>+</sup> CD138<sup>+</sup> PCs in IgG1<sup>+</sup> gate.

## Supplementary Figure 5



**Supplementary Figure 5. No significant change was observed in the frequency of FDCs in CLP mice compared to sham mice.** Sham or CLP mice were sacrificed at four-weeks post-surgery. After CD45<sup>+</sup> cells depletion by using anti-CD45 MACS microbeads, stromal cells were surface stained for CD45, TER119, B220, CD31, ICAM-1 and CD21/CD35 antibodies and analyzed by flow cytometry. **(A)** Representative flow cytometry dot plots of CD35<sup>+</sup> stromal cells isolated from a CLP mouse depicting gating strategy for FDC analyses. **(B)** Quantification of percentage of CD21<sup>+</sup>/CD35<sup>+</sup> cells in ICAM-1<sup>+</sup> gate. Each dot represents an individual animal (n=9, Sham; n=8, CLP). Data represent mean  $\pm$  SEM from two independent experiments. Sham vs. CLP NS, not significant. (Mann-Whitney U-test).

## Supplementary Figure 6



**Supplementary Figure 6. Inactivation of TNF results in loss of FDC clusters and an impaired antigen-specific humoral response.** Wild-type C57BL/6J mice were treated with anti-TNF antibody or isotype control (Ctrl) (250  $\mu$ g per mouse in 250  $\mu$ L PBS; i.p.) and immunized with NP-CGG (100  $\mu$ g) in alum (100  $\mu$ L), single, i.p. Mice were sacrificed at day 14 after immunization and serum and spleens were harvested. **(A)** Representative whole spleen sections from an isotype Ctrl and an anti-mouse TNF antibody-treated mouse stained for B220 and CD35. Whole spleen images were acquired by stitching tiled images. Magnification (20X; scale bar = 500  $\mu$ m). **(B)** Low-affinity anti-NP<sub>23</sub> IgG and **(C)** high-affinity anti-NP<sub>2</sub> IgG levels at day 14 post-immunization (n=10, Isotype Ctrl+NP-CGG; n=9, anti-TNF+NP-CGG). Each dot represents an individual animal. Data represent mean  $\pm$  SEM from two independent experiments. Isotype Ctrl+NP-CGG vs. anti-TNF+NP-CGG \* $P$  < 0.05 (Mann-Whitney U-test).

**Table. List of Reagents used in this study.**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Goat anti-mouse IgG, Human ads-UNLB	Southern Biotech	Cat # 1030-01
Goat anti-mouse IgG1, Human ads-AP	Southern Biotech	Cat # 1070-04
Goat anti-mouse IgG, Human ads-AP	Southern Biotech	Cat # 1030-04
Goat anti-mouse IgG2c, Human ads-AP	Southern Biotech	Cat # 1079-04
Goat anti-mouse IgM, Human ads-AP	Southern Biotech	Cat # 1020-04
Goat anti-mouse IgG, Human ads-BIOT	Southern Biotech	Cat # 1030-08
BV421 Hamster Anti-Mouse CD95 (Clone: Jo2)	BD Biosciences	Cat # 562633
FITC Rat Anti-Mouse T- and B-Cell Activation Antigen (Clone: GL7)	BD Biosciences	Cat # 553666
PE Rat Anti-Mouse IgD (Clone: 11-26c.2a)	BD Biosciences	Cat # 558597
PE Rat Anti-Mouse CD138 (Clone: 281-2)	BD Biosciences	Cat # 553714
PE/Cy7 anti-mouse IgG1 (Clone: RMG1-1)	BioLegend	Cat # 406614
PerCP-Cy5.5 anti-Hu/Mo CD45R (B220) (Clone: RA3-6B2)	Invitrogen/eBioscience/ Thermo Scientific	Cat # 45-0452-82
PE-eFluor 610 Anti-Mo IgM (Clone: II/41)	Invitrogen/eBioscience/ Thermo Scientific	Cat # 61-5790-82
PerCP anti-mouse CD45 (Clone: 30-F11)	BioLegend	Cat # 103130
PE/Cy7 anti-mouse CD21/CD35 (CR2/CR1) (Clone: 7E9)	BioLegend	Cat # 123419
BV510 Rat Anti-mouse B220/ CD45R (Clone: RA3-6B2)	BD Biosciences	Cat # 563103
FITC Rat Anti-Mouse CD31 (Clone: MEC 13.3)	BD Biosciences	Cat # 553372
PE-eFluor 610-TER119 (Clone: TER-119)	eBioscience/ Thermo Fisher Scientific	Cat # 61-5921-82
PE anti-mouse CD54 ICAM-1 (Clone: YN1/1.7.4)	Invitrogen/eBioscience	Cat # 12-0541-81
LEAF™ Purified anti-mouse TNF- $\alpha$ (Clone: MP6-XT22)	BioLegend	Cat # 506325
LEAF™ Purified Rat IgG1, $\kappa$ isotype Ctrl (Clone: RTK2071)	BioLegend	Cat # 400427
Biotin-conjugated rat anti-mouse CD35 (Clone: 8C12)	BD Biosciences	Cat # 553816
APC-conjugated hamster anti-mouse CD3e (Clone: 145-2C11)	BD Biosciences	Cat # 553066
eFluor 450-conjugated rat anti-mouse B220 (Clone: RA3-6B2)	Invitrogen/Thermo Fisher Scientific	Cat # 48-0452-82
Purified rat anti mouse CD16/32 Fc block (Clone: 24. G2)	BD Biosciences	Cat # 553142
<b>Chemicals, Peptides, and Recombinant Proteins</b>		
4-hydroxy-3-nitrophenyl) acetyl (NP) <sub>20</sub> -chicken $\gamma$ -globulin (CGG)	Biosearch Technologies	Cat # N-5055C
CGG	Biosearch Technologies	Cat # C-1000
NP-AECM-Ficoll	Biosearch Technologies	Cat # F-1420
Imject alum	Thermo Scientific	Cat # 77161
PBS	Corning	Cat # 21-040-CV
NP <sub>2</sub> -BSA	Biosearch Technologies	Cat # N-5050L

NP <sub>23</sub> -BSA	Biosearch Technologies	Cat # N-5050H
NP <sub>6</sub> -BSA-biotin	Biosearch Technologies	Cat # N-1026
Phosphatase substrate	Sigma	Cat # S0942
Sodium Bicarbonate (NaHCO <sub>3</sub> )	Fisher Scientific	Cat # S233-3
Magnesium chloride (MgCl <sub>2</sub> )	Sigma	Cat # M8266
5-Bromo-4-chloro-3-indolyl phosphate p-toluidine salt (BCIP)	Sigma	Cat # B6777
Triton X	Sigma	Cat # T8787
Tween 20	Fisher Scientific	Cat # BP337
Tamoxifen	Sigma	Cat # T5648
Formalin solution 10%, neutral buffer	Sigma	Cat # HT 5011
UltraPure 0.5 M Ethylene-diaminetetraacetic acid (EDTA), pH 8.0	Invitrogen	Cat # 15575-038
HBSS (1X)	Gibco	Cat # 14175-095
Acetone	Fisher scientific	Cat # A18-4
2-Mercaptoethanol 1000X	Gibco/Life Technologies	Cat # 21985-023
<b>Experimental Models: Organisms/Strains</b>		
C57BL/6J	Jackson Laboratory	Stock no. 000664
<i>Rag1</i> KO (B6.129S7- <i>Rag1</i> <sup>&lt;tm1Mom&gt;/J</sup> )	Jackson Laboratory	Stock no. 002216
<i>Rosa26</i> fl/fl tdtomato	Jackson Laboratory	Stock no. 007914
<i>Slpr2</i> -ERT2cre	Tomohiro Kurosaki	Osaka University, Japan
<b>Software and Algorithms</b>		
FlowJo software v10.6.1	Tree Star	<a href="https://www.flowjo.com/solutions/flowjo">https://www.flowjo.com/solutions/flowjo</a>
Graph Pad Prism v8.3.1	Graph Pad Software, LLC	N/A
<b>Other</b>		
Suture silk 4-0 C13	Fisher Scientific	Cat # NC9404551
Suture, 6/0 27 PDS II VIO MONO TF	Medline	SKU # ETHZ432H
Imipenem/Cilastatin	Fresenius kabi USA, LLC	N/A
0.9% Sodium Chloride	Fisher Scientific	Cat # NC9054335
Bupivacaine HCL 0.5% injection	North America Covetrus	SKU # 054893
Buprenex (buprenorphine HCL) injection, 0.3mg/ml, c3	North America Covetrus	SKU # 055175
Braintree Scientific 9 MM EZ CLIPS 1000/CS	Fisher Scientific	Cat # NC9281117
Isoflurane, USP	North America Covetrus	SKU # 029405
Half-area 96-well flat bottom plates	Corning	Cat # 3690
Immulon 4HBX plates	Thermo Scientific	Cat # 3855
Streptavidin-AP	Southern Biotech	Cat # 7105-04
Fixable Viability Dye eFluor™ 780	Invitrogen/eBioscience /Thermo Fisher Scientific	Cat # 65-0865-18
Streptavidin, Alexa Flour 568 Conjugate	Fisher	Cat # S112266A
Streptavidin, Alexa Fluor™ 488 Conjugate	Thermoscientific Fisher	Cat # S32354

Streptavidin APC crosslinked Conjugate	Thermoscientific Fisher	Cat # S868
Fixable Viability Dye eFluor™ 506	Invitrogen/eBioscience/ Thermo Fisher Scientific	Cat # 65-0866-14
RBC lysis buffer 10X	BioLegend	Cat # 420301
DMEM/High glucose	Sigma	Cat # D5796
EasySep Buffer	Stem Cell Technologies	Cat # 20144
CD45 MicroBeads, mouse	Miltenyi Biotec	Cat # 130-052-301
LS Columns	Miltenyi Biotec	Cat # 130-042-401
Anti-fade Mounting Medium	Vector Labs	Cat # H-1000
Collagenase D	Sigma/Roche	Cat # 11088858001
EasySep Mouse Pan-B Cell Isolation kit	Stem Cell Technologies	Cat # 19844
EasySep Mouse CD4+ T Cell Isolation Kit	Stem Cell Technologies	Cat # 19852A
B-Phycoerythrin Antibody Rabbit Polyclonal	Rockland Immunochemicals	Cat # 200-401-099
B-Phycoerythrin	Thermo Fisher/ Invitrogen	Cat # P800
Directzol RNA micro 200 prep	Zymo Research	Cat # R2062
Dispase II (neutral protease, grade II)	Sigma/Roche	Cat # 4942078001
DNase I	Sigma/Roche	Cat # 11284932001
eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set	eBioscience	Cat # 00-5523-00
Pre-Amp Master Mix (2x)	Thermo Fisher Scientific	Cat # 4391128
MACS BSA stock solution	Miltenyi Biotec	Cat # 130-091-376
Sodium Pyruvate (100 mM)	Gibco/Life Technologies	Cat # 11360-070
HEPES (1 M)	Gibco/Life Technologies	Cat # 15630-080
Penicillin-Streptomycin Solution	HyClone	Cat # SH40003-12
Auto MACS Rinsing Solution	Miltenyi Biotec	Cat # 130-091-222
Fetal Bovine Serum	HyClone	Cat# SH30910.03
Bovine Serum Albumin Fraction V	Roche	Cat # 10735108001
iScript cDNA Synthesis kit	BioRad	Cat # 170-8891
Tissue-tek cryo-OCT compound	Fisher Health Care	Cat # 14-373-65
Corn oil	Sigma	Cat # C8267
Normal Goat Serum (ab7481)	Abcam	Cat # ab7481
TRIzol reagent	Life Technologies	Cat # 15596018
RPMI	Gibco/Life Technologies	Cat # 11875119
LightCycler 480 Probes Master	Roche	Cat # 04887301001
Cell strainer 100 µm	Fisher	Cat # 22-363-549
Difco Agar, Granulated	BD	Cat # 214530
BBL Brain Heart Infusion	BD	Cat # 211059