B–helper neutrophils stimulate immunoglobulin diversification and production in the marginal zone of the spleen

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SUPPLEMENTARY TABLES S1 – S6

SUPPLEMENTARY FIGURES AND LEGENDS S1 – S22

SUPPLEMENTARY MOVIE LEGENDS M1 and M2

SUPPLEMENTARY TABLES

Table S1. Patients with neutrophil disorders.

				(mg/dL)		
Sex	**Age**	**Immunodeficiency**	**Gene mutated**	**IgA**[¶]	**IgG**	**IgM**
M	11 yr	SCN[#]	Unknown	378,3	418,1	117,3
F	11 yr	SCN	Unknown	184,1	1179,5	273,1
F	14 yr	SCN	Unknown	116,2	691,5	166,2
F	15 yr	SCN	Unknown	174,3	961,9	158,2
F	16 yr	SCN	Unknown	204,7	889,2	220,4
F	19 yr	SCN	Unknown	11,3	187,2	42,4
F	32 yr	SCN	Unknown	186,8	479,2	167,3
F	35 yr	SCN	Unknown	388,4	1770,3	242,6
F	36 yr	SCN	Unknown	273,4	479,2	108,1
F	41 yr	SCN	Unknown	N/A*	N/A	N/A
M	49 yr	SCN	Unknown	246,5	604,2	225,0
M	50 yr	SCN	Unknown	326,4	1744,1	153,2
M	17 yr	SCN	*ELANE*	467,9	2063,1	197,1
M	10 yr	SCN	*ELANE*	N/A	N/A	N/A
M	12 yr	SCN	*WAS*	405,6	1712,1	245,3
M	14 yr	SBDS[##]	*SBDSP1*	305,7	1302,1	119,2
F	19 yr	SBDS[##]	*SBDSP1*	65,1	95,3	88,2
F	N/A	WHIM[§] syndrome	*CXCR4*	188,7	1267,2	68,0
F	N/A	WHIM syndrome	*CXCR4*	70,2	176,8	60,4
F	24 yr	CN[¥]	*ELANE*	452,8	1848,8	265,9
F	27 yr	CGD[¤]	*CYBB*	281,6	819,4	262,1
F	12 yr	LAD-1[∞]	*ITGB2*	340,5	1159,6	175,0

[¶]Normal range IgA [142-262]; IgG [920-1480]; IgM [88-184].
[#]SCN, severe congenital neutropenia (< 500 neutrophils/μl).
*N/A, not available.
[##]SBDS, shwachman-Bodian-Diamond syndrome (< 500 neutrophils/μl).
[§]WHIM, warts-hypogammaglobulinemia-infections-myelokathexis syndrome (< 500 neutrophils/μl).
[¥]CN, cyclic neutropenia (< 1000 neutrophils/μl).
[¤]CGD, chronic granulomatous disease (> 1500 neutrophils/μl).
[∞]LAD-1, leukocyte adhesion deficiency-1 (> 1500 neutrophils/μl).

Table S2. Fresh tissue samples.

SAMPLE	SEX	AGE	CONDITION
Spleen	M	4 yr	Organ donor
Spleen	M	22 yr	Organ donor
Spleen	F	51 yr	Organ donor
Spleen	M	56 yr	Organ donor
Spleen	F	58 yr	Organ donor
Spleen	F	62 yr	Organ donor
Spleen	F	68 yr	Organ donor
Spleen	F	68 yr	Organ donor
Spleen	F	68 yr	Organ donor
Spleen	M	74 yr	Organ donor
Spleen	F	78 yr	Organ donor
Spleen	F	86 yr	Organ donor
Spleen	F	71 yr	Organ donor
Spleen	M	12 yr	Trauma
Spleen	M	22 yr	Trauma
Spleen	M	22 yr	Trauma
Spleen	M	34 yr	Trauma
Spleen	F	35 yr	Trauma
Spleen	F	43 yr	Trauma
Spleen	M	44 yr	Trauma
Spleen	M	48 yr	Trauma
Spleen	F	55 yr	Trauma
Spleen	F	57 yr	Trauma
Spleen	F	59 yr	Trauma
Spleen	N/A*	N/A	SCN[#]
Axilar lymph node	F	49 yr	Breast cancer
Pelvic lymph node	F	73 yr	Squamous cell carcinoma
Retroperitoneal lymph node	F	68 yr	Organ donor
Mesenteric lymph node	N/A	N/A	Colon carcinoma
Mesenteric lymph node	N/A	N/A	Colon carcinoma
Tonsils	F	7 yr	Follicular hyperplasia
Tonsils	F	9 yr	Follicular hyperplasia

*Not available.
[#]SCN, severe congenital neutropenia

Table S3. Frozen and paraffin-embedded tissue samples.

SAMPLE	SEX	AGE	CONDITION
Fetal spleen		12 wk	Induced abortion
Fetal spleen		17,5 wk	Spontaneous abortion
Fetal spleen		19 wk	Spontaneous abortion
Fetal spleen		20 wk	Induced abortion
Fetal spleen		21 wk	Induced abortion
Fetal spleen		21 wk	Induced abortion
Fetal spleen		21 wk	Induced abortion
Fetal spleen		22 wk	Induced abortion
Fetal spleen		22 wk	Spontaneous abortion
Fetal spleen		22 wk	Induced abortion
Fetal spleen		32 wk	Spontaneous abortion
Fetal spleen		41 wk	Spontaneous abortion
Neonatal spleen	M	2 d	Congenital cardiopathy
Neonatal spleen	F	10 d	Diaphragmatic hernia
Neonatal spleen	M	30 d	Microangiopathy
Neonatal spleen	M	6 mo	Cardiopathy
Spleen	M	4 yr	Trauma
Spleen	M	6 yr	Brain aneurysm
Spleen	F	14 yr	N/A*
Spleen	F	35 yr	Trauma
Spleen	M	70 yr	Trauma
Spleen	M	58 yr	Colon cancer
Spleen	N/A	N/A	Bleeding disorder
Spleen	N/A	N/A	Systemic lupus erythematosus
Spleen	N/A	N/A	Systemic lupus erythematosus
Spleen	N/A	N/A	HIV infection
Spleen	N/A	N/A	Hereditary spherocytosis
Spleen	N/A	N/A	Severe congenital neutropenia
Spleen	N/A	N/A	Hyper-IgD syndrome
Spleen	F	31 yr	Idiopathic thrombocytopenic purpura
Spleen	M	72 yr	Idiopathic thrombocytopenic purpura
Intestine	M	83 yr	Colon cancer
Intestine	M	67 yr	Colon cancer
Intestine	M	84 yr	Colon cancer
Mesenteric lymph node	M	74 yr	Colon cancer
Mesenteric lymph node	M	49 yr	Colon cancer
Cervical lymph node	F	30 yr	Thyroid cancer
Cervical lymph node	F	30 yr	Thyroid cancer
Cervical lymph node	M	54 yr	Laryngeal cancer
Axillar lymph node	F	49 yr	Breast cancer
Pelvic lymph node	F	68 yr	Prostate cancer
Pelvic lymph node	F	73 yr	Prostate cancer
Tonsils	F	9 yr	Follicular hyperplasia
Tonsils	M	6 yr	Follicular hyperplasia

*Not available.

Table S4. Antibodies used in flow cytometry and immunofluorescence.

a. Antibodies to human antigens.

Antigen	Label	Isotype	Clone (if monoclonal)	Manufacturer	Use
AID		Rat IgG2b	EK2 5G9	Cell Signaling	¶IF
AID		Goat IgG	-	Santa Cruz	
APRIL		Rabbit IgG	ED2	Prosci	#FC /IF
BAFF		Mouse IgG1	1D6	eBioscience	IF
BAFF	PE	Mouse IgG1	1D6	eBioscience	
CD8	FITC	Mouse IgG1	RFT-8	Southern Biotech	FC
CD11b	PE	Mouse-IgG2a	D12	BD Biosciences	FC
CD11c	PE	Mouse IgG1	B-ly6	BD Biosciences	IF
CD15	FITC	Mouse IgM	28	Southern Biotech	FC
CD15	Biotin	Mouse IgG1	W6D3	Biolegend	
CD15		Mouse IgM	28	Southern Biotech	IF
CD16	Biotin	Mouse IgG1	3G8	BD Biosciences	FC
CD16	PE	Mouse IgG1	3G8	BD Biosciences	FC
CD16	Alexa647	Mouse IgG1	3G8	BD Biosciences	
CD19	APC	Mouse IgG1	H1B19	BD Biosciences	
CD19	PE-Cy7	Mouse IgG1	HIB19	Biolegend	FC
CD20	FITC	Mouse IgG1	L27	BD Biosciences	*IHC
CD24	PE	Mouse IgG2a	ML5	BD Biosciences	FC
CD27	PE	Mouse IgG1	M-T271	Ancell	FC
CD31	PE	Mouse IgG1	TDR31.3	Ancell	
CD31	Alexa 647	Mouse IgG2a	M89D3	BD Biosciences	
CD31		Mouse IgG1	M0823	Dako	IF
CD38	PE	Mouse IgG1	HIT2	BD Biosciences	FC
CD54	PE	Mouse IgG1	HCD54	Biolegend	FC
CD62L	PE-Cy7	Mouse IgG1	DREG-56	eBioscience	FC
CD62P	PE	Mouse IgG1	AK4	Biolegend	FC
CD68		Mouse IgG1	KP1	Dako	IF
CD86	PE	Mouse IgG2b	IT2.2 BD	Biosciences	FC
CD95	PE	Mouse IgG1	DX2	BD Biosciences	FC
CD102	PE	Mouse IgG1	CBR-IC2/2	Biolegend	FC
CD154 (CD40L)	PE	Mouse IgG1	24-31	Ancell	FC
CD163		Mouse IgG1	10D6	Thermo Scientific	IF
CD206 (MR)		Mouse IgG1	19.2	BD Biosciences	IF
CD206 (MR)	FITC	Mouse IgG1	19.2	BD Biosciences	
CEACAM-1		Mouse IgG	-	M. López-Botet (IMIM)	IF
IgA	Biotin	Goat IgG	-	Southern Biotech	IF
IgD	FITC	Goat IgG F(ab')2	-	Southern Biotech	FC/IF

IgD	Biotin	Goat IgG F(ab')$_2$	-	Southern Biotech	IF
IL-10	PE	Mouse IgG1	JES3-19F1	Biolegend	IF
LPS		Mouse IgG2b	2D7/1	Abcam	IHC/IF
MHC-I	PE	Mouse IgG2a	3F10	Ancell	FC
MHC-II	PE	Mouse IgG1	TDR31.3	Ancell	FC
Myeloperoxidase	FITC	Mouse IgG1	F0714	Dako	IF
Neutrophil Elastase		Mouse IgG1	NP57	Dako	IF
Neutrophil Elastase		Rabbit IgG	-	Abcam	IF
Pax-5		Rabbit IgG	-	Neomarkers	IF
vWF		Rabbit IgG	-	Dako	IF

b. Antibodies to mouse antigens.

Antigen	**Label**	**Isotype**	**Clone (if monoclonal)**	**Manufacturer**	**Use**
IgD	FITC	Rat IgG2a	11-26c.2a	BD Biosciences	[¶]IF
IgD	Biotin	Mouse-IgG2b	AMS 9.1	BD Biosciences	IF
MOMA-1	Biotin	Rat IgG2a	MOMA-1	Abcam	IF
Ly6G	Cy5	Rat IgG2b	RB6-8C5	Abcam	IF
Ly6G	APC	Rat IgG2b	RB6-8C5	eBioscience	[#]FC
CD11b	PE	Rat IgG2b	M1/70	eBioscience	FC

[¶]IF, immunofluorescence.
[#]FC, flow cytometry.
*IHC, immunohistochemistry.

Table S5. Coating strategy for ELISA.

Purified antigens	**Source**	**Company**	**Antigen concentration or dilution**
LPS	*Escherichia coli*	InvivoGen	10 μg/ml
LPS	*Pseudomonas aeruginosa*	Sigma	1 μg/ml
CPS (serotypes 9N, 14, 19F, 23F)	*Pneumococcus*	ATCC[§]	10 ng/ml
Gal-α-1,3-Gal		Dextra Lab	0,5 μg/ml
LTA	*Bacillus subtilis*	Sigma	1 μg/ml
PGN	*Bacillus subtilis*	Sigma	1 μg/ml
Tetanus toxin	*Clostridium tetani*	Sigma	0.1 μg/ml
Whole Bacteria			
Escherichia coli		Invitrogen	1:1000*
Salmonella typhimurium		G. Dougar	1:1000
Staphylococcus aureus		ATCC	1:1000
Haemophilus influenzae type-b		ATCC	1:1000
Lactobacillus plantarum		ATCC	1:1000
Vaccines			
Anatoxal Tedi (TT[#] + DT[¶])		Berna Biotech	1:1000
Menjugate Kit (Meningococcal CPS conjugated with DT)		Novartis	1:1000

[§]ATCC, American Type Culture Collection.
*Volume:volume.
[#]TT, tetanus toxin.
[¶]DT, diphtheria toxin.

Table S6. Primers used in RT-PCR and qRT-PCR.

Target gene		Primer sequence
ACTB	S	GGATGCAGAAGGAGATCACT
	AS	CGATCCACACGGAGTACTTG
AICDA	S	AGAGGCGTGACAGTGCTACA
	AS	TGTAGCGGAGGAAGAGCAAT
ALDH1A1	S	ATGGCATGATTCAGTGAGTG
	AS	GCAGACGATCTCTTTCGATT
APAF1	S	GTCTGCTGATGGTGCAAG GA
	AS	GATGGCCCGTGTGGATTTC
ARG1	S	ATGGGGACCTGCCCTTTGCT
	AS	CTTCTGCCACCTTGCCAGCC
BAD	S	AGCCAACCAGCAGCAGCCATCAT
	AS	CTCCCCCATCCCTTCGTCGTC
BAK1	S	AGCAGCACCATGGGGCAGGT
	AS	CTCTCAAACAGGCTGGTGGCAATC
BAX	S	AGAGGATGATTGCCGCCGT
	AS	CAACCACCCTGGTCTTGGATC
BCL2	S	CCTCCCTGGCCTGAAGAAGA
	AS	TGTCCTTCGGCGTGGAAATC
BCL2L1	S	GGAAAGCGTAGACAAGGAGATGC
	AS	TCCACAAAAGTATCCCAGCCG
BCL2L11	S	ATGAGAAGATCCTCCCTGCT
	AS	AATGCATTCTCCACACCAGG
BCL10	S	GAACTCCTGGACTCAAGCAA
	AS	GGTGGCTCGTGTCTGTAATC
CARD6	S	GATGACCCAGAGCACGTTGGA
	AS	TCCTCCTCCAATGAAAGGCTGGT
CARD8	S	CTGCCGAGACGGGACAGTGGA
	AS	ATTTGTCACACAGGCCTCAGCCT
CASP1	S	TCTTCCTTTCCAGCTCCTCAGGCA
	AS	TGAGAGCAAGACGTGTGCGGCT
CASP3	S	GCAGCAAACCTCAGGGAAAC
	AS	TGTCGGCATACTGTTTCAGCA
CASP5	S	CCTGCAAGGAATGGGGCTCACTAT
	AS	CTCTGCAGGCCTGGACAATGATGAC
CCL2	S	CCCCAAGCAGAAGTGGGTTC
	AS	TGTCTGGGGAAAGCTAGGGG
CCL3	S	GACTTCAGAAGGACACGGGC
	AS	TGAGCAGGTGACGGAATGTG
CCL4	S	CTGGGTCCAGGAGTACGTGT
	AS	AGGAACTGCGGAGAGGAGTC
CCL5	S	TGAGCTCTGGCTTTGCCTTG
	AS	TCCTCCCCTCCTTGCCTCTA
CCL19	S	CGCCGCAGCAGTTAACCTAT
	AS	CCTTCCTTCTGGTCCTCGGT
CCL20	S	GAACCTTGCTGGGGTTGGAG
	AS	TGGACAAGTCCAGTGAGGCA
CCL21	S	CTGGTTCTGGCCTTTGGCAT

	AS	AAGCTTGGTTCCTGCTTCCG
CD40LG	S	GCTTTGAAATGCAAAAAGGTGAT
	AS	TTGTTTTACTGCTGGCCTCACTT
CXCL1	S	GCGCCCAAACCGAAGTCATA
	AS	TCAGGAACAGCCACCAGTGA
CXCL2	S	AGAAAGCTTGTCTCAACCCCG
	AS	TCCTTCAGGAACAGCCACCA
CXCL3	S	GCGCCCAAACCGAAGTCATA
	AS	TCAGTTGGTGCTCCCCTTGT
CXCL5	S	TGCTGTGTTGAGAGAGCTGC
	AS	TTGGAGCACTGTGGGCCTAT
CXCL10	S	TGAGCCTACAGCAGAGGAA
	AS	TACTCCTTGAATGCCACTTAGA
CXCL12	S	ACCGCGCTCTGCCTCA
	AS	CATGGCTTTCGAAGAATCGG
CXCL13	S	GCAGCCTCTCTCCAGTCCAA
	AS	TGGACACATCTACACCTCAAGCTT
CRLF2	S	GGGGCAAGGAGGAGCAGATTCA
	AS	GTCGTCTCGCTGCTCTGCGT
FAS	S	ATGCTGGGCATCTGGACCCT
	AS	GCCATGTCCTTCATCACACAA
FASLG	S	TGTGCCCAGAAGGCCTGGTC
	AS	AGGCCTGTGCTGTGGTTCCCT
GRN	S	AAGGAGAACGCTACCACGGA
	AS	CGGGACAGCAGTGTATGTGG
ELANE	S	GCGTGGCGAATGTAAACGTC
	AS	CCGTTTTCGAAGATGCGCTG
IFNA2	S	GTGACCTACCACAAACCCACAGCC
	AS	ATTCTCCTCATCTGCGCCAGGA
IFNG	S	CTAATTATTCGGTAACTGACTTGA
	AS	ACAGTTCAGCCATCACTTGGA
IDO1	S	TGTCTGGCTGGAAAGGCAAC
	AS	CAGCAGGACGTCAAAGCACT
IL1B	S	TCCCCAGCCCTTTTGTTGA
	AS	TTAGAACCAAATGTGGCCGTG
IL6	S	GGTACATCCTCGACGGCATCT
	AS	GTGCCTCTTTGCTGCTTTCAC
IL8	S	CCAAACCTTTCCACCC
	AS	ACTTCTCCACAACCCT
IL10	S	ACCTGCCTAACATGCTTCGAG
	AS	TGTCCAGCTGATCCTTCATTTG
IL10RA	S	TCAGACGCTCATGGGACAGA
	AS	ATACCTCAGGAGCGCCACTT
IL10RB	S	ATCAGGGCAGCAAACAAGGG
	AS	TCTGGTGGCTAAGTCCAGGG
IL12A	S	TTCACCACTCCCAAAACCTGC
	AS	GAGGCCAGGCAACTCCCATTAG
IL12B	S	GGGAACTGAAGAAAGATGTTTATG
	AS	CTCTGGTCCAAGGTCC
IL15	S	GTTCACCCCAGTTGCAAAGTAAC

	AS	TATCATGAATACTTGCATCTCCGGA
IL17A	S	CCCCAGTTGATTGGAAGAAA
	AS	GAGGACCTTTTGGGATTGGT
IL18	S	CCAGTGCATTTTGCCCTCCT
	AS	GTTTGTTGCGAGAGGAAGCG
IL21	S	TGTGAATGACTTGGTCCCTGAA
	AS	CAGGAAAAAGCTGACCACTCA
IL22	S	GCAGGCTTGACAAGTCCAACT
	AS	GCCTCCTTAGCCAGCATGAA
MCL1	S	GGTACCTTCGGGAGCAGGCCA
	AS	AAGGCCGTCTCGTGGTTGC
MIR155HG	S	CTCTAATGGTGGCACAAA
	AS	TGATAAAAACAAACATGGGCTTGAC
MPO	S	ATCGGTACCAGCCCATGGAA
	AS	TGACGATTCAGCTTGGCAGG
NLRC4	S	TCCCCGTGCGTCATCGGTGGA
	AS	AGTCAGCAGGGTTCTGGGCAAGG
NLRP3	S	AAAGAGATGAGCCGAAGTGG
	AS	TTCCTGGCATATCACAGTGG
NOS2	S	CCCAGCACAAGGGAGTGCGG
	AS	AGGATGTCCTGAACATAGACCTTGGG
PAX5	S	TTGCTCATCAAGGTGTCAGG
	AS	CTGATCTCCCAGGCAAACAT
PPBP	S	TGTATGCTGAACTCCGCTGC
	AS	TGCAATGGGTTCCTTTCCCG
PRDM1	S	GTGGTATTGTCGGGACTTTGCAG
	AS	TCGGTTGCTTTAGACTGCTCTGTG
PTX3	S	GGGACAAGCTCTTCATCATGCT
	AS	GTCGTCCGTGGCTTGCA
SLPI	S	CCTGGATCCTGTTGACACCC
	AS	CACTTCCCAGGCTTCCTCCT
SOCS1	S	CGATTACCGGCGCATCACGC
	AS	TGTCGCGCACCAGGAAGGTG
SOCS3	S	CACTCTTCAGCATCTCTGTCGGAAG
	AS	CATAGGAGTCCAGGTGGCCGTTGAC
TGFB1	S	AAGGACCTCGGCTGGAAGTGG
	AS	CCGGGTTATGCTGGTTGTA
TNF	S	CCCAGGCAGTCAGATCATCTTC
	AS	AGCTGCCCCTCAGCTTGA
TNFSF13	S	GAAGGCAGGAGACTCTATTCCG
	AS	CCCTTGGTGTAAATGGAAGAC
TNFSF13B	S	ACCGCGGGACTGAAAATCT
	AS	CACGCTTATTTCTGCTGTTCTGA
TSLP	S	CCCAGGCTATTCGGAAACTCAG
	AS	CGCCACAATCCTTGTAATTGTG
TLR1	S	GGCTGGCCTGATTCTTATAAGTG
	AS	CTCTAGGTTTGGCAATAATTCATTC
TLR2	S	CAGCAGGTTCAGGATGTCCG
	AS	GCAGATGTTCCTGCTGGGAG
TLR3	S	TGACTGAACTCCATCTCATGTCC

	AS	CCATTATGAGACAGATCTAATGTG
TLR4	S	CCCTGCGTGGAGGTAT
	AS	GCACCTGCAGTTCTGGGAAA
TLR6	S	AAGCGGAGGTTCTAGGCCAT
	AS	CTGCACTGAACCTGTGTGCT
TLR7	S	GCTGTGACCGAGTGGGTTTT
	AS	TTTTCCAGAACTGGCTGCCC
TLR8	S	TTCCTGTGAGTTATGCGCCG
	AS	TATTTGCCCACCGTTTGGGG
TLR9	S	ACAACAACATCCACAGCCAAGTGTC
	AS	AAGGCCAGGTAATTGTCACGGAG
XBP1	S	AGGAGTTAAGACAGCGCTTGG
	AS	AGAGGTGCACGTAGTCTGAGTGCTG
16SrRNA	S	TGGTGTGTAAATATGTTCTCGTCAA
	AS	TTCGTATTACCGCGGCTGCTGG
V_H3-23	S	GGCTGAGCTGGCTTTTTCTTGTGG
	ASμ	GTTGCCGTTGGGGTGCTGGAC
	ASγ	AAGACCGATGGGCCCTTGGTGG
intV_H3-23	S	GGGGGTCCCTGAGACTCTC
	ASμ	AAGGAAGTCCTGTGCGAGGCAGC
	ASγ	AAGACCGATGGGCCCTTGGTGG

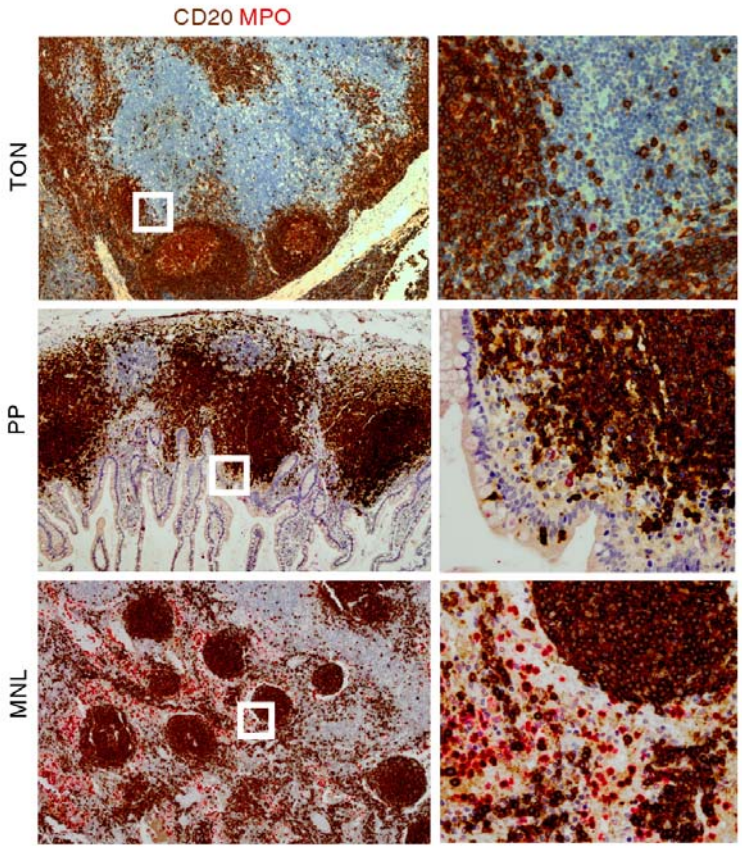

Figure S1. Neutrophils colonize perifollicular areas of mesenteric lymph nodes. Immunohistochemistry of tonsil (TON), intestinal Peyer's patches (PP) and mesentheric lymph node (MLN) stained for CD20 (brown) and myeloperoxidase (MPO, red). Boxes correspond to magnified right images. Original magnification, x4 (left) and x20 (right). Data are from one of five experiments with similar results.

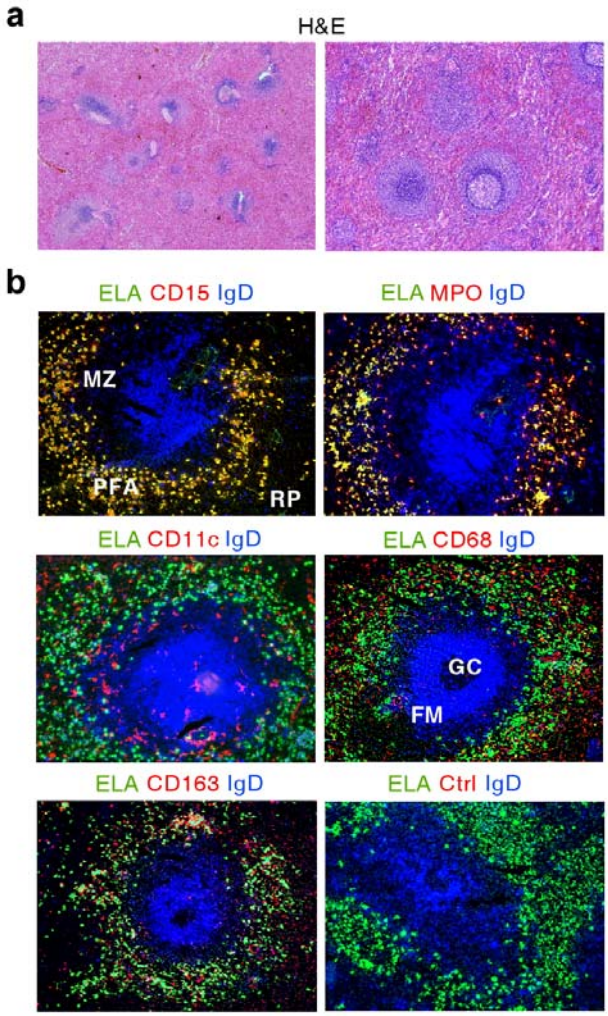

Figure S2. Perifollicular neutrophils colonize spleens that have no histological alterations. (**a**) Light microscopy of normal spleens stained with hematoxylin and eosin (H&E). Original magnification, x4. (**b**) Immunofluorescence of spleens stained for elastase (ELA, green), CD15, myeloperoxydase (MPO), CD11c, CD68, CD163 or mouse IgG1 isotype control (red), and IgD (blue). MZ, marginal zone; PFA, perifollicular area; RP, red pulp; GC, germinal center; FM, follicular mantle. Original magnification, x10. Data are from one of five experiments with similar results.

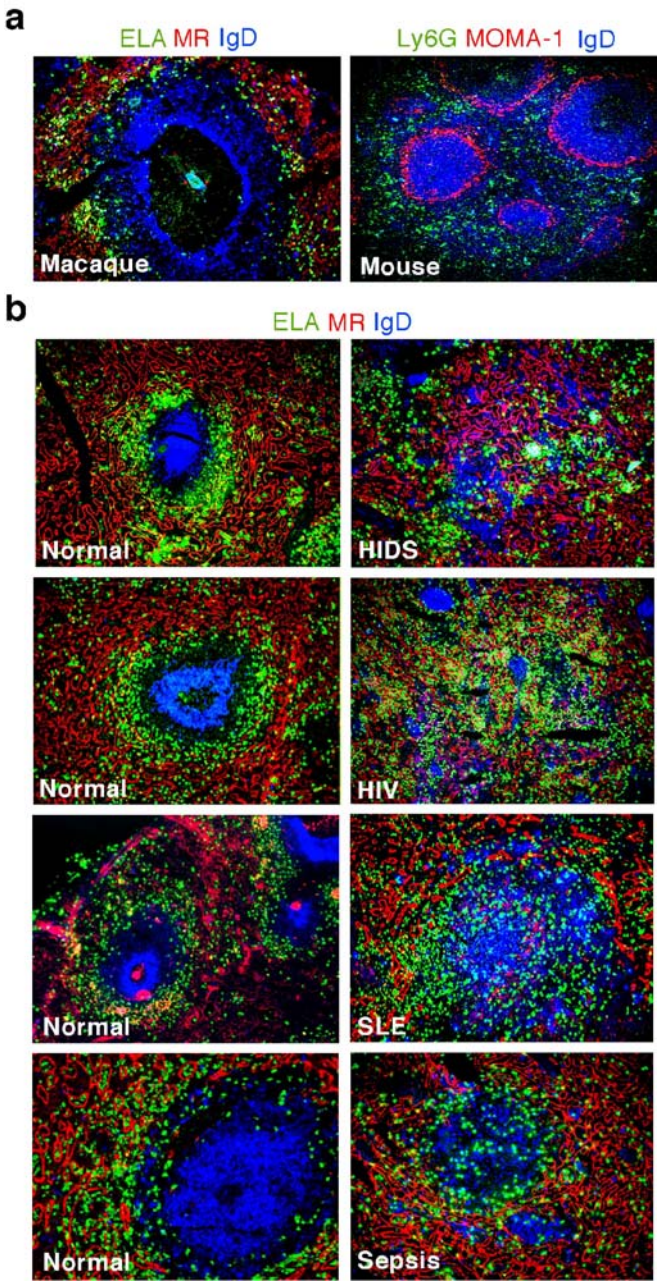

Figure S3. Neutrophils selectively colonize splenic perifollicular areas in healthy humans, rhesus macaques and mice, but invade splenic follicular areas in patients with systemic inflammation or infection. (**a**) Immunofluorescence of normal rhesus macaque spleen (left panel), and normal mouse spleen (right panel) stained for elastase (ELA) or Ly6G (green), MR or MOMA-1 (red), and IgD (blue). Original magnification, x10. (**b**) Immunofluorescence of spleens stained for ELA (green), MR (red), and IgD (blue) from individuals with (right panels) or without (left panels) systemic inflammation or infection, including hyper-IgD syndrome (HIDS), HIV infection, systemic lupus erythematosus (SLE), and sepsis. Original magnification, x10. Data are from one of several experiments with similar results.

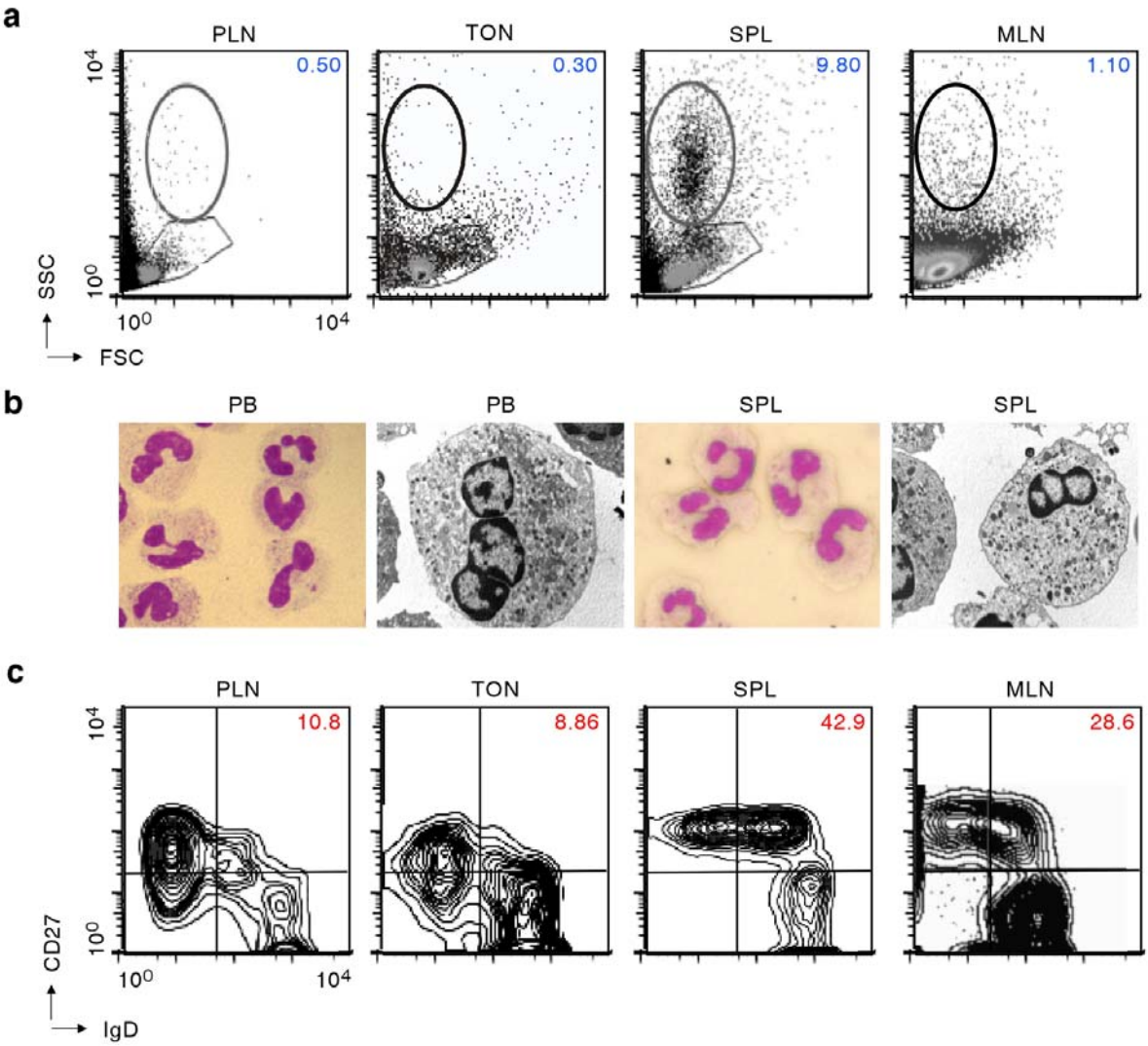

Figure S4. Neutrophil presence in spleens and mesenteric lymph nodes correlates with a higher percentage of MZ B cells. (**a**) Flow cytometry of neutrophils from peripheral lymph node (PLN), tonsil (TON), spleen (SPL) and mesenteric lymph node (MLN) cells. Oval gate includes cells with size (forward scatter, FSC) and intracellular complexity (side scatter, SSC) compatible with those of neutrophils. Numbers indicate percentage of neutrophils. (**b**) Light microscopy (first and third panels from left) and transmission electron microscopy (second and fourth panels from left) of neutrophils from peripheral blood (PB) and spleen (SPL) of healthy donors. Original magnification, x63 (Giemsa) and x790 (electron microscopy). (**c**) Flow cytometry of IgD and CD27 on CD19-gated B cells from PLN, TON, SPL, MLN. Data are from one of five experiments with similar results.

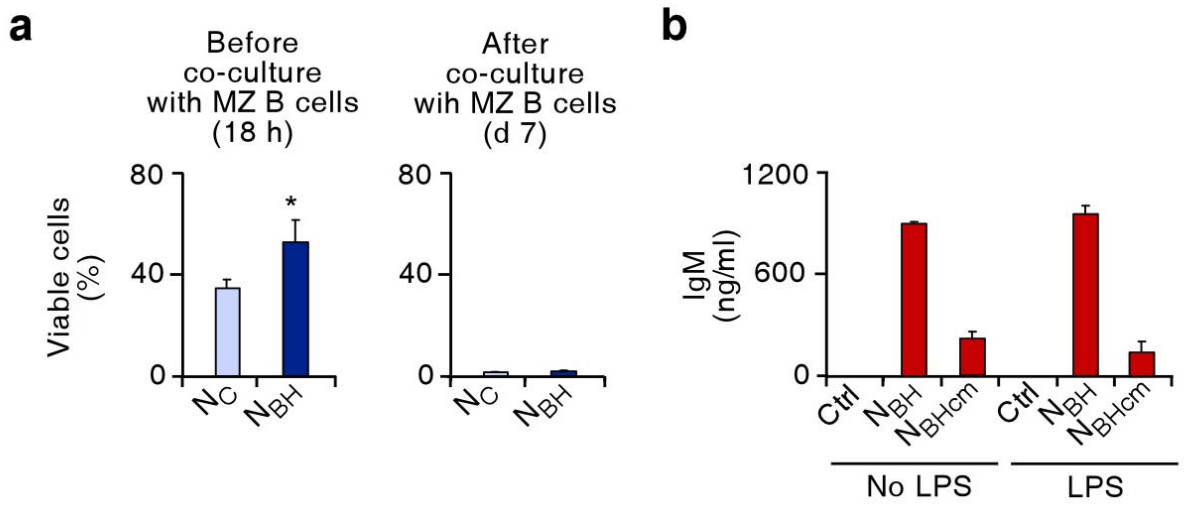

Figure S5. N_{BH} cells show better short-term survival but equivalent long-term survival as compared to N_C cells and activate MZ B cells via both contact-dependent and contact-independent mechanisms. (**a**) Flow cytometry of viable annexin V-negative propidium iodide-negative N_C and N_{BH} cells after 18 h of culture with medium alone (left panel) or after co-culture with MZ B cells for 7d (right panel). (**b**) ELISA of IgM from splenic MZ B cells cultured with N_{BH} or N_{BH} conditioned medium (N_{BHcm}). N_{BH} cells were primed with medium or LPS. Error bars, s.e.m.; * $P < 0.05$ (one-tailed unpaired Student's t-test). Data are from one of three experiments with similar results.

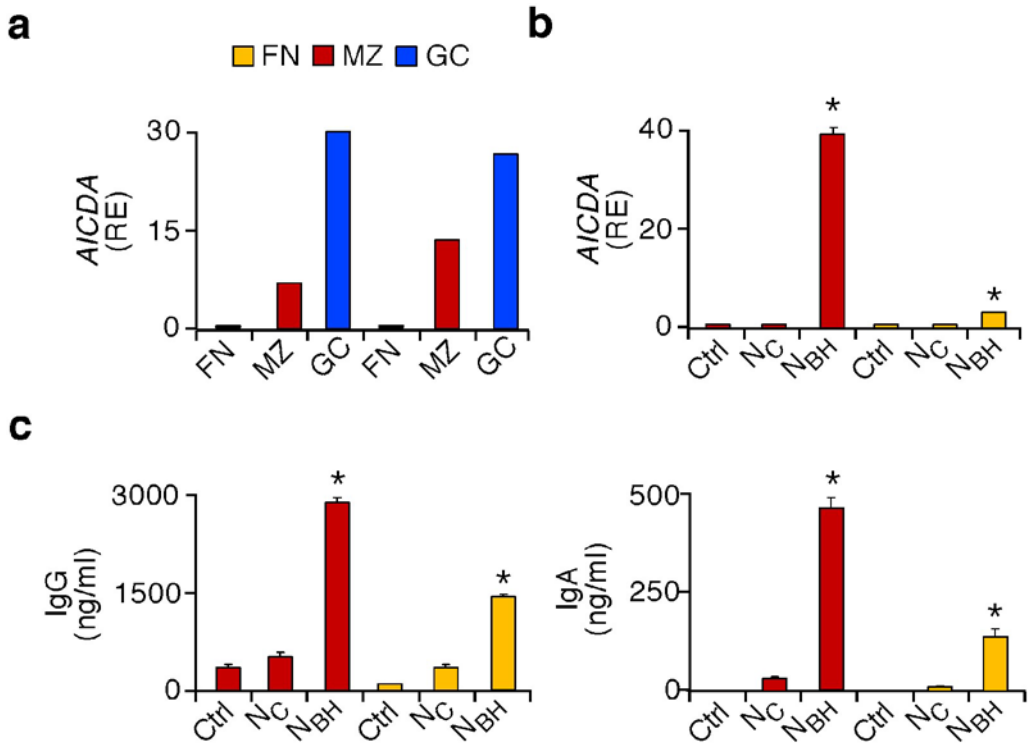

Figure S6. N_{BH} but not N_C cells induce more AID and more IgG and IgA in MZ than FN B cells. (**a**) qRT-PCR of *AICDA* mRNA (encoding AID) from splenic follicular naïve (FN), MZ and germinal center (GC) B cells isolated from two healthy donors. Results are normalized to *ACTB* mRNA (encoding β-actin) and are presented as relative expression (RE) compared with that of FN B cells. (**b**) qRT-PCR of *AICDA* mRNA from splenic MZ and FN B cells cultured for 2 d with medium (Ctrl), N_C cells or N_{BH} cells. Results are normalized to *PAX5* mRNA (encoding the B cell protein Pax5) and are presented as RE compared with that of FN B cells or MZ B cells incubated with medium alone. (**c**) IgG and IgA from splenic MZ and FN B cells cultured as in **a** for 4 d. Error bars, s.e.m.; * $P < 0.005$ (one-tailed unpaired Student's t-test). Data are from one of five experiments with similar results (**a**) or summarize three independent experiments (**b,c**).

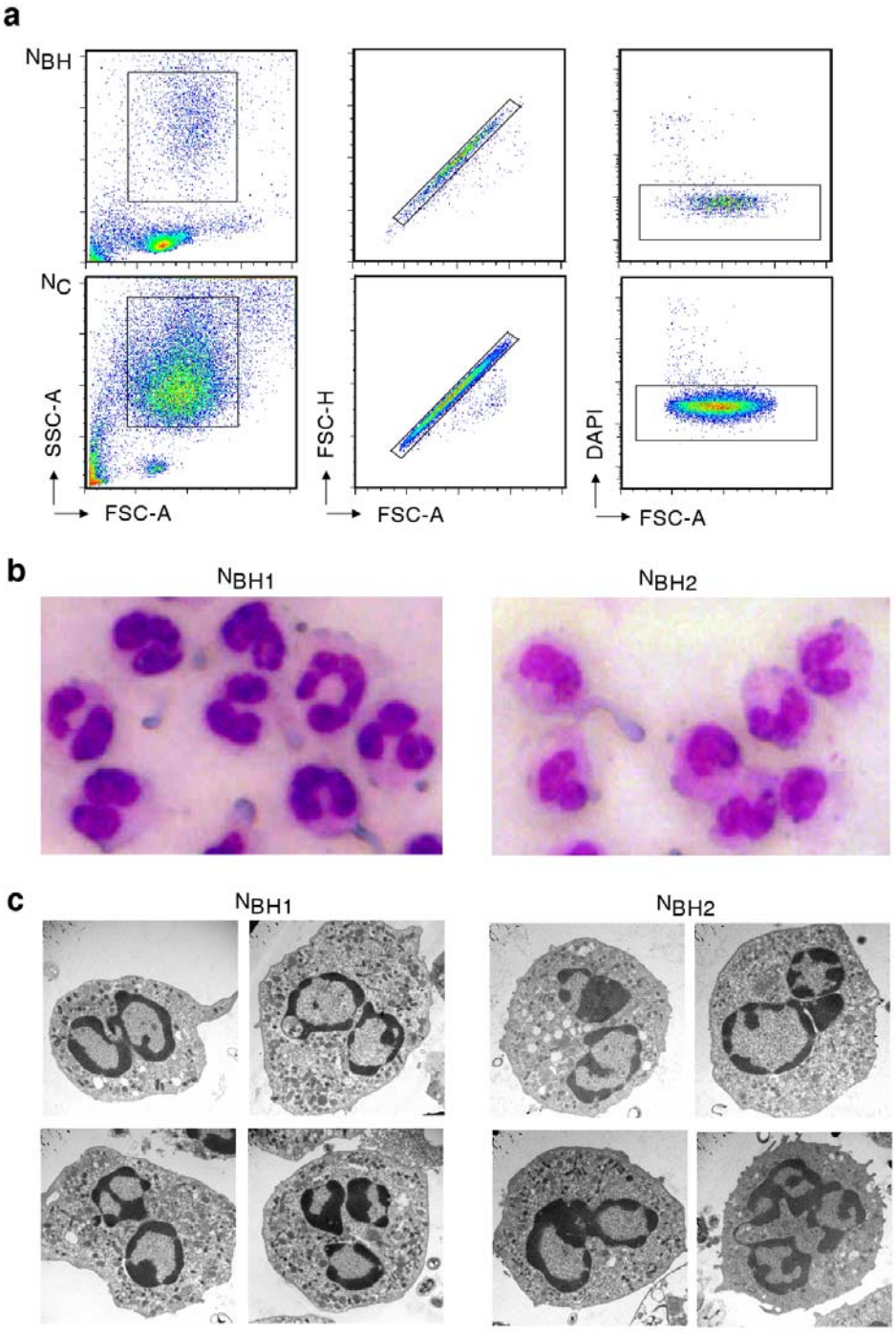

Figure S7. N_{BH1} and N_{BH2} cells have comparable morphological and ultrastructural features. (**a**) Flow cytometry of N_{BH} (top panels) and N_C cells (bottom panels) gated according to canonical forward scatter (FSC) and side scatter (SSC) parameters and negativity for DAPI. (**b** and **c**) Light microscopy (**b**) and transmission electron microscopy (**c**) of N_{BH1} (left panels) and N_{BH2} (right panels) cells from healthy donors. Original magnification, x63 (Giemsa) and x790 (electron microscopy). Data are from one of several experiments with similar results.

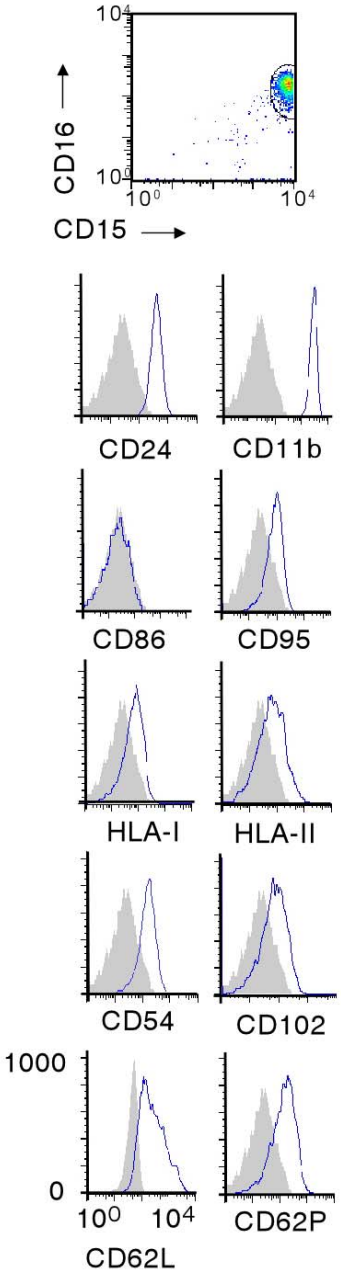

Figure S8. Inflamed splenic tissue contains N_{BH1} but not N_{BH2} cells. Flow cytometry of CD15, CD16, CD24, CD11b, CD86, CD95, HLA-I, HLA-II, CD54, CD102, CD62L and CD62P on N_{BH} cells from the spleen of a patient with hyper-IgD syndrome (HIDS). Gray solid profile, isotype control. Data are from one of four experiments with inflamed spleens from patients affected with HIDS, HIV, lupus or sepsis that yielded similar results.

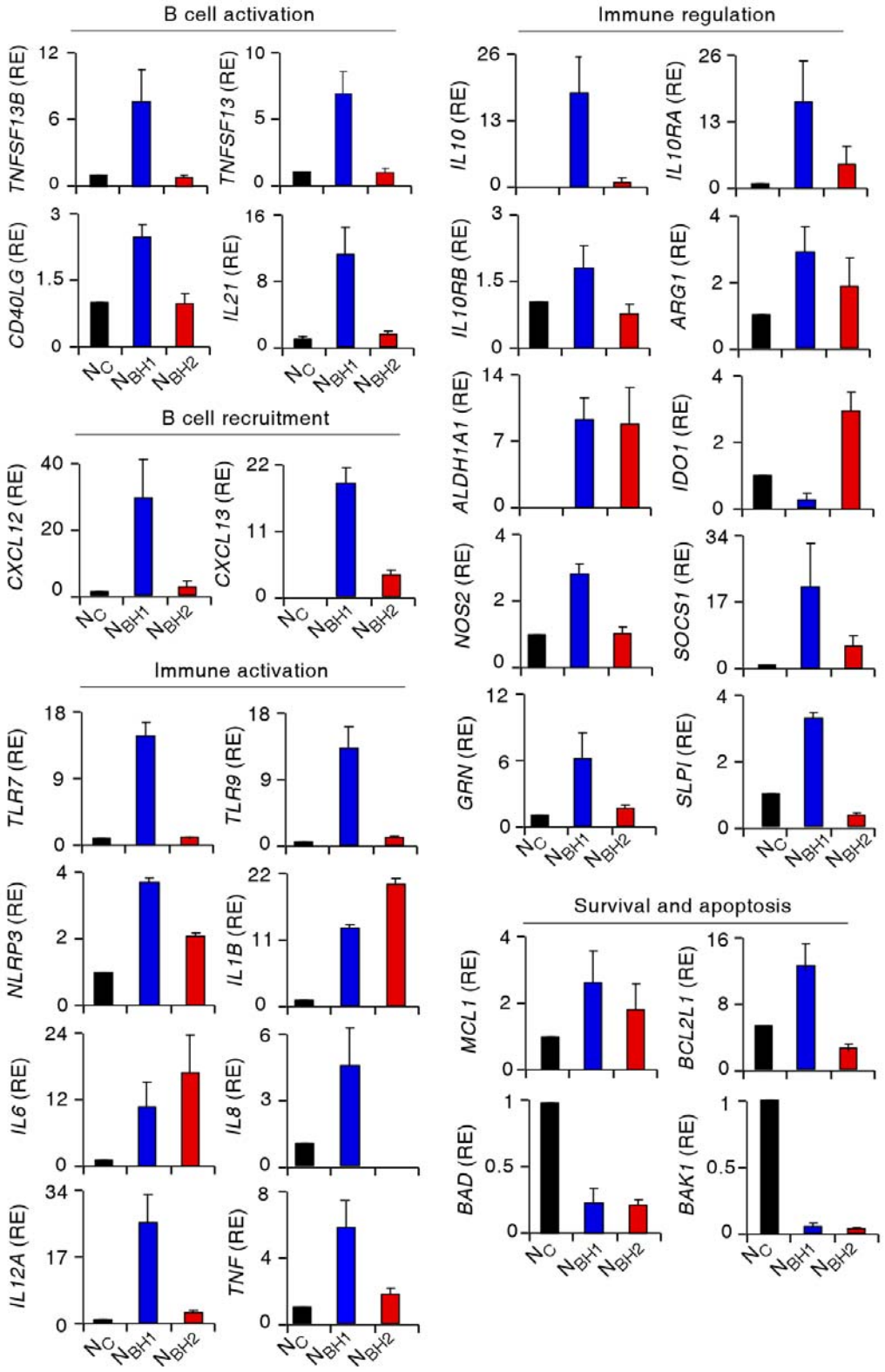

Figure S9. N_{BH1} and N_{BH2} cells express more mRNAs for B cell activation, B cell recruitment, immune activation, immune regulation and cell survival molecules than N_C cells. Gene expression profile of N_C, N_{BH1} and N_{BH2} cells established by customized qRT-PCR arrays. Results are normalized to *ACTB* mRNA (encoding β-actin) and are presented as relative expression (RE)

compared with that of N_C cells. Functional mRNA clusters and highly relevant mRNAs are indicated. *TNFSF13B, TNFSF13, NLRP3*, *IL1B*, *IL12A, IL10RA*, *IL10RB*, *ARG1*, *ALDH1A1, IDO1*, *NOS2*, *GRN SLPI*, and *BCL2L1* mRNAs encode BAFF, APRIL, NALP-3, IL-1β, IL-12p35, IL-10 receptor α, IL-10 receptor β, arginase I, RALDH, IDO, iNOS, progranulin, SLPI and bcl-xL, respectively. Error bars, s.e.m. Data summarize three independent experiments.

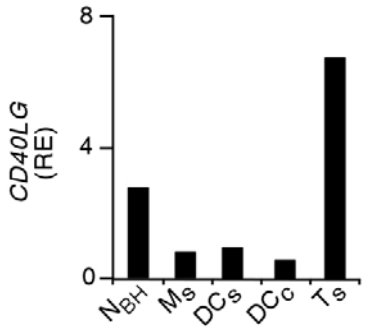

Figure S10. N_{BH} express mRNA for CD40L, although less than splenic $CD4^+$ T cells. qRT-PCR of mRNA for CD40L in N_{BH} cells, splenic macrophages (M_s), splenic dendritic cells (DC_s), circulating dendritic cells (DC_c), and splenic $CD4^+$ T cells (T_s). Results are normalized to *ACTB* mRNA (encoding β-actin) and are presented as relative expression (RE) compared with that of DC_s cells. Data are from one of two experiments with similar results.

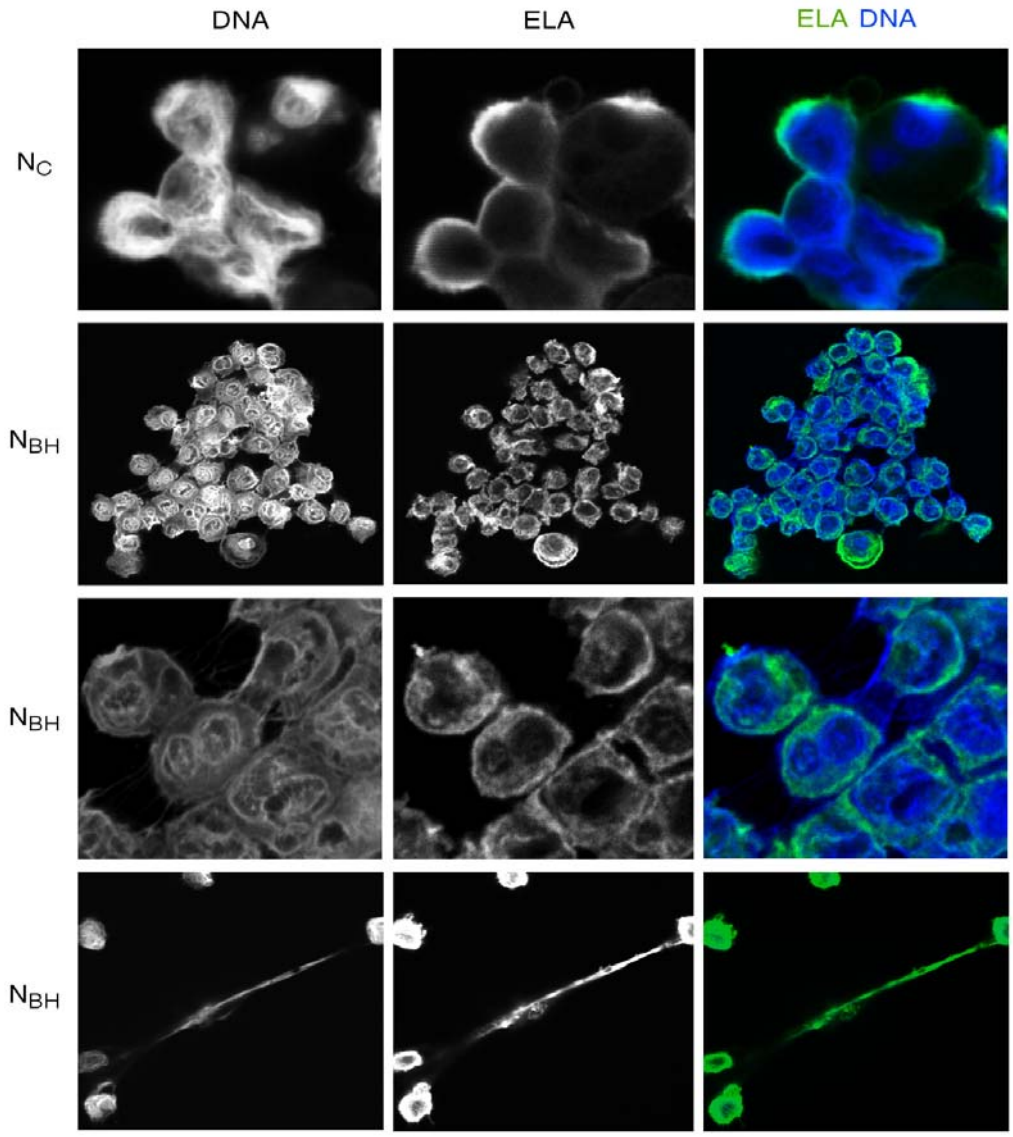

Figure S11. N_{BH} but not N_C cells spontaneously form NET-like structures. Confocal microscopy of N_C and N_{BH} cells stained for elastase (ELA, green) and DNA (blue). Original magnification, x40 (first two rows and fourth row from top) and x63 (third row from top). Data are from one of three experiments with similar results.

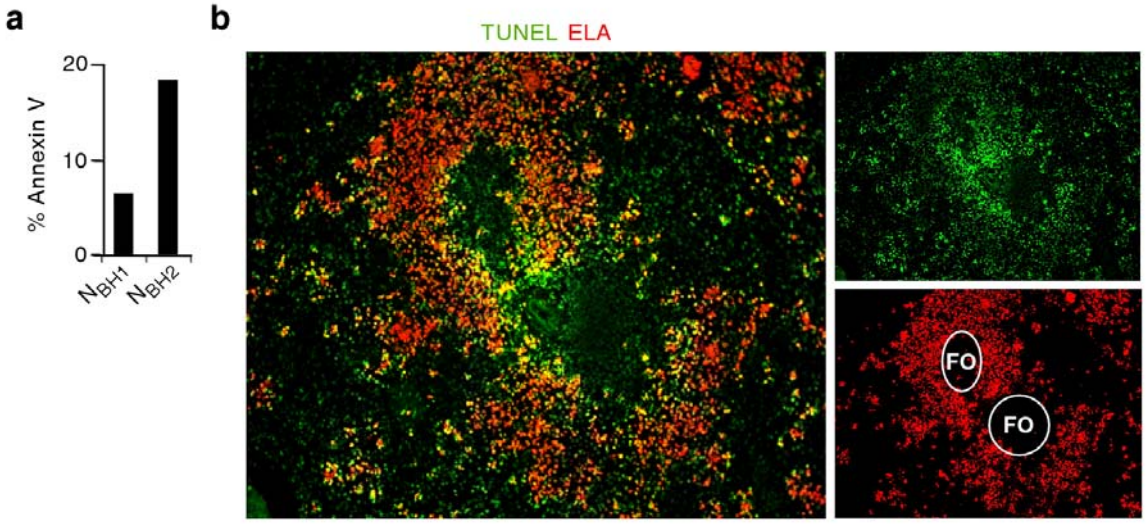

Figure S12. N_{BH2} cells are more apoptotic than N_{BH1} cells and may correspond to apoptotic N_{BH} cells occupying splenic peri-MZ areas. (**a**) Flow cytometric analysis of annexin V on N_{BH1} and N_{BH2} cells. (**b**) Immunofluorescence of spleen processed for TUNEL assay (green) and co-stained for elastase (ELA, red). The same image is shown in Fig. 3d at smaller magnification. FO, follicle. Original magnification, x10. Data are from one of three experiments with similar results.

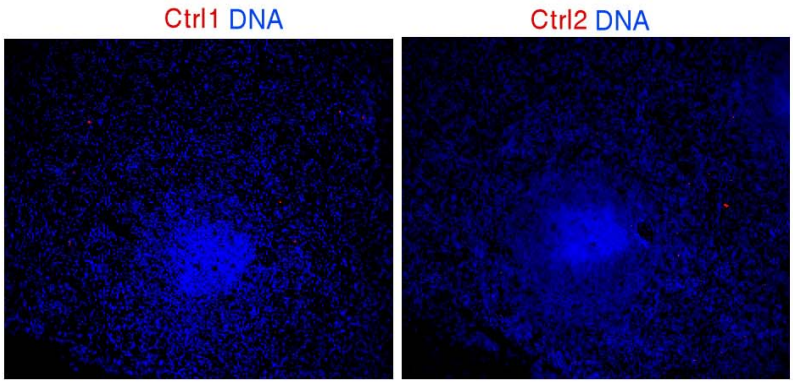

Figure S13. Control antibodies isotype-matched with antibodies reactive for BAFF and APRIL do not stain splenic peri-MZ areas. Fluorescence microcopy of spleens stained for control mouse IgG1 (Ctrl1) or control rabbit IgG (Ctrl2) antibodies (red) and DNA (blue). Original magnification, x10. Data are from one of three experiments with similar results.

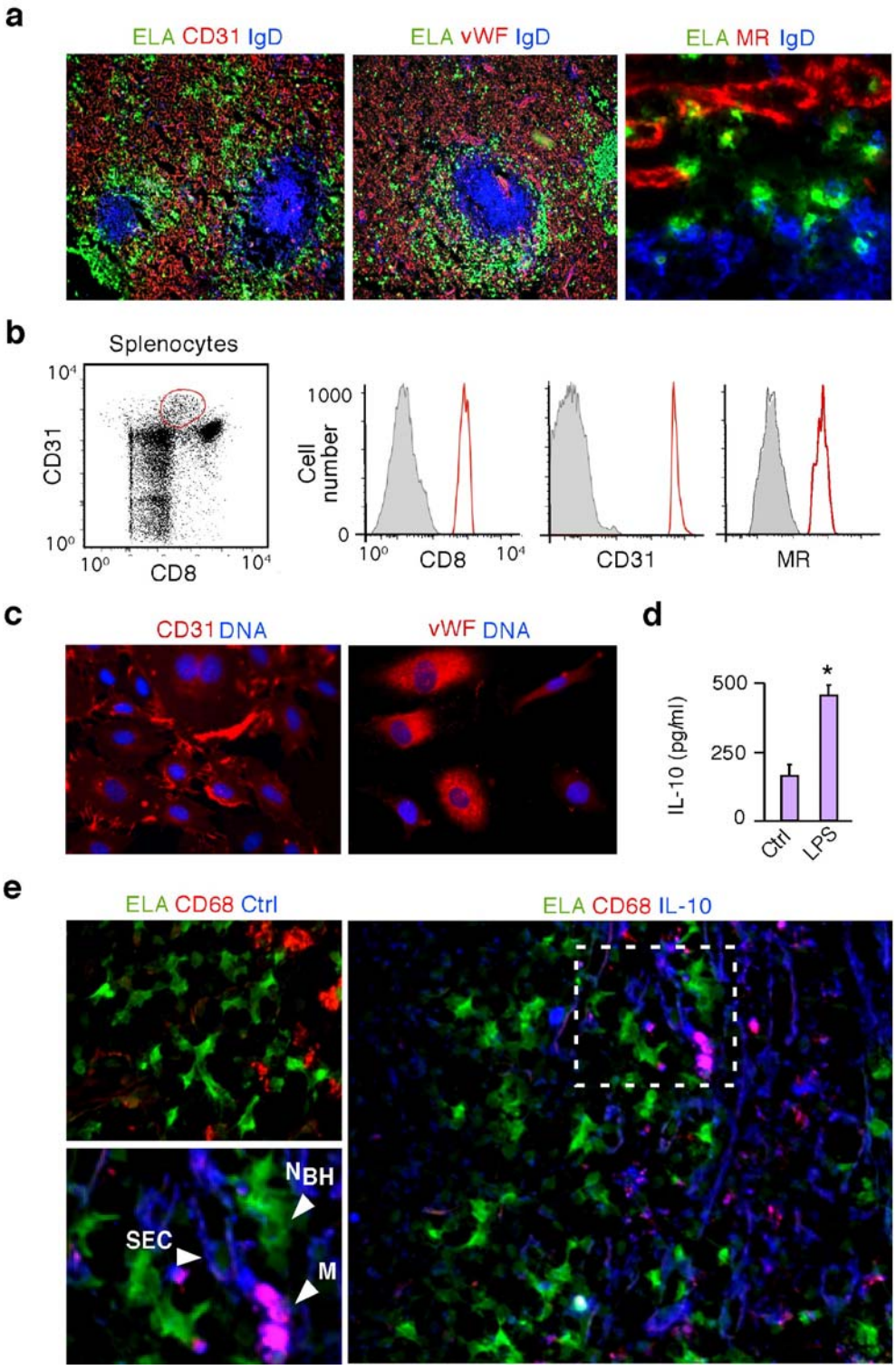

Figure S14. N_{BH} cells are proximal to SECs and macrophages producing IL-10. (**a**) Immunofluorescence of spleen stained for elastase (ELA, green), CD31, von Willebrand factor (vWF) or MR (red), and IgD (blue). Original magnification, x10 (left and mid panels) and x63 (right panel). (**b**) Flow cytometry of CD8, CD31, and MR on SECs. Red circle gates SECs expressing CD8 and CD31. Gray profile, isotype control. (**c**) Immunofluorescence of SECs stained

for CD31 or vWF (red) and DNA (blue). Original magnification, x40. (**d**) ELISA of IL-10 from SECs incubated with medium (Ctrl) or LPS for 24 h. (**e**) Immunofluorescence of SECs stained for ELA (green), CD68 (red), and IL-10 or control mouse IgG1 antibody isotype-matched with the anti-IL-10 antibody (blue). Dashed area corresponds to magnified left-bottom image. Arrowheads point to SEC, N_{BH} and macrophage (M). Original magnification, x40. Error bars, s.e.m.; * $P < 0.05$ (one-tailed Student's t-test). Data are from five (**a-c**) of three (**e**) experiments with similar results or summarize three independent experiments (**d**).

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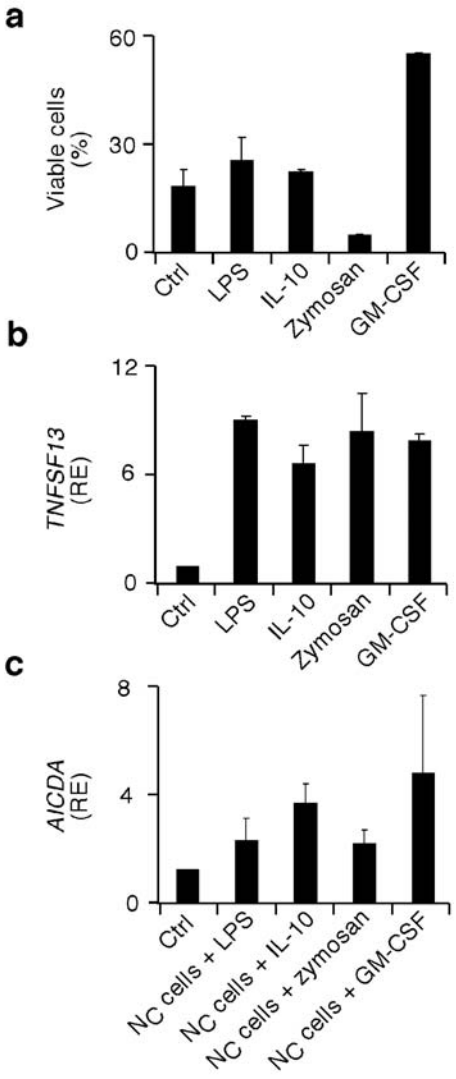

Figure S15. N_C cells acquire N_{BH}-like properties in response to either pro-apoptotic or anti-apoptotic activating signals. (**a**) Flow cytometric analysis of viable annexin V-negative propidium iodide-negative N_C cells cultured for 18 h in the presence of medium (Ctrl), LPS, IL-10, zymosan or GM-CSF. (**b**) qRT-PCR of *TNFRSF13* encoding APRIL in N_C cells cultured as in **a**. Results are normalized to *ACTB* mRNA (encoding β-actin) and presented as relative expression (RE) compared with that of unstimulated cells. (**c**) qRT-PCR of *AICDA* encoding AID in circulating unswitched IgD^+ B cells cultured for 2 d with N_C cells stimulated as in **a**. Results are normalized to *PAX5* mRNA and presented as RE compared with that of B cells cultured with unstimulated N_C cells. Error bars, s.e.m. Data summarize three independent experiments.

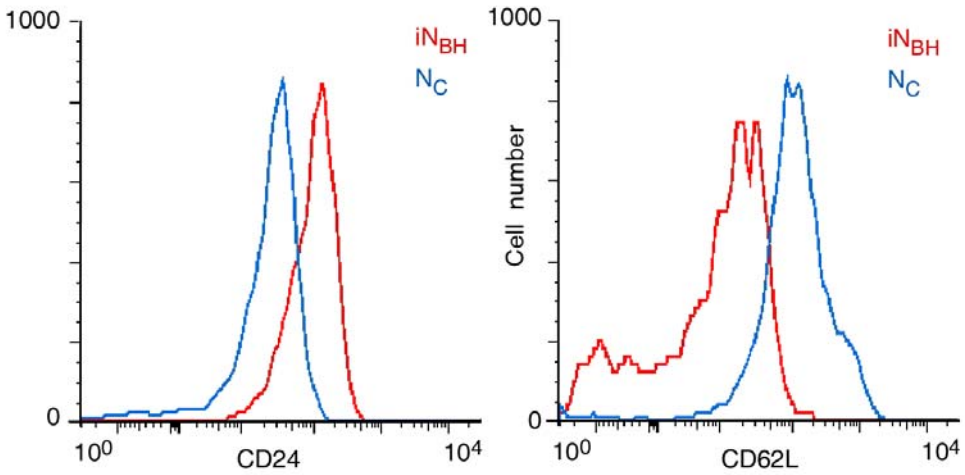

Figure S16. iN$_{BH}$ cells express more CD24 regulatory protein but less CD62L adhesion protein than N$_C$ cells. Flow cytometry of iN$_{BH}$ cells (red profile) obtained 4 h after trans-SEC migration and control non-transmigrated N$_C$ cells (blue profile). Data are from one of three experiments with similar results.

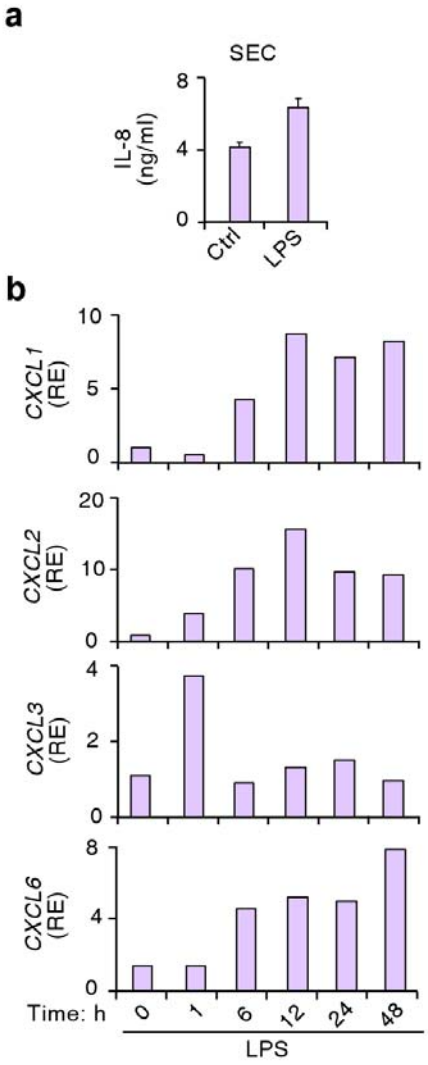

Figure S17. SECs enhance the production of neutrophil-attracting chemokines in response to microbial signals. (**a**) ELISA of IL-8 (CXCL8) from SECs cultured with medium alone (Ctrl) or LPS for 12 h. (**b**) qRT-PCR of mRNA for CXCL1, CXCL2, CXCL3 and CXCL6 from SECs exposed to LPS for 0, 1, 6, 12, 24 or 48 h. Error bars in **a** panel, s.e.m. Data summarize three independent experiments (**a**) or represent one of three experiments with similar results (**b**).

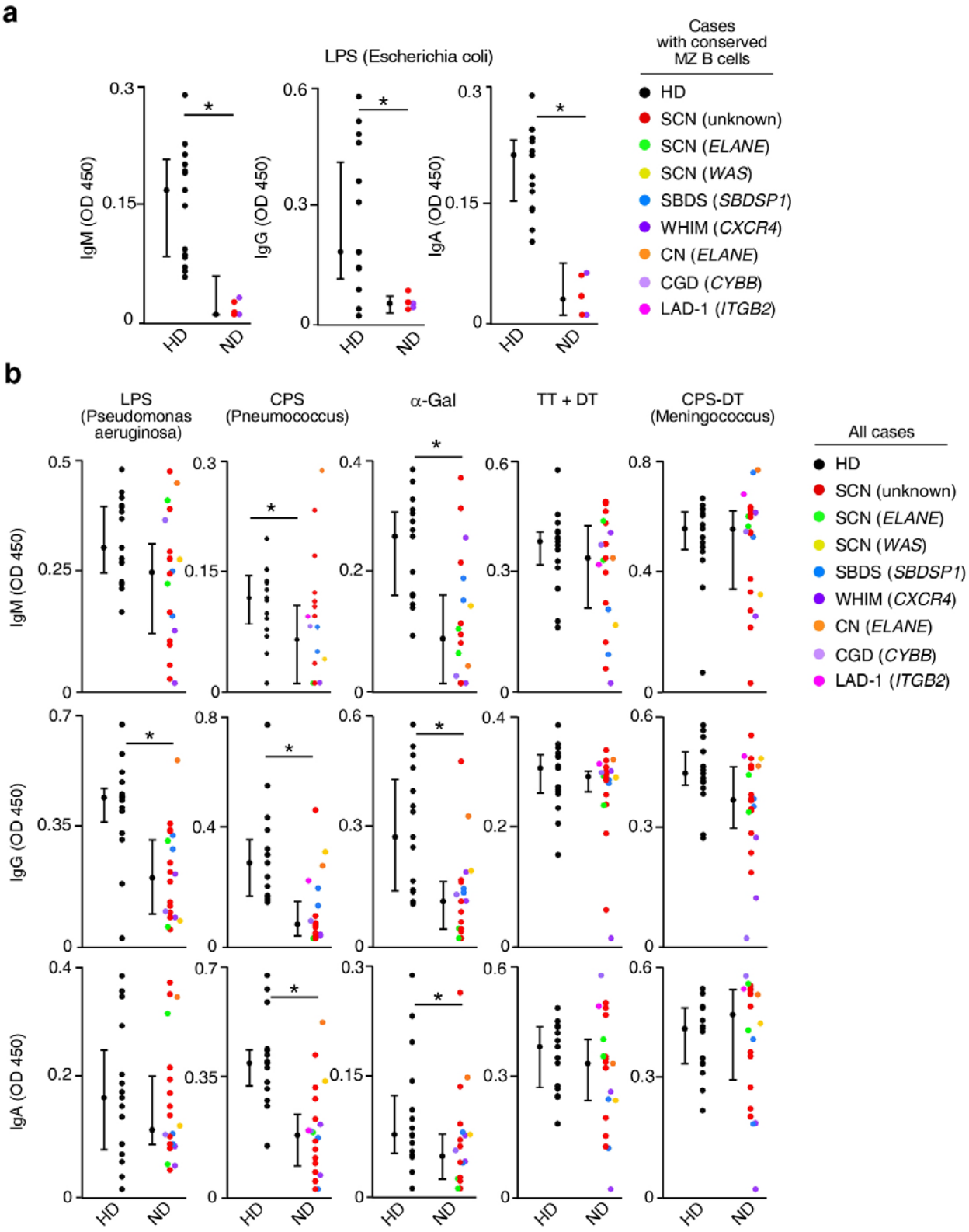

Figure S18. Neutropenia and functional neutrophil disorders decrease preimmune serum concentrations of IgM, IgG and IgA to TI antigens. (**a**) ELISA for IgM, IgG and IgA to LPS from Escherichia coli in serum of age-matched healthy donors (HD) and a subset of patients with

neutrophil disorders (ND) but numerically conserved circulating MZ B cells. OD, optical density. SCN, severe congenital neutropenia; SBDS, Shwachman-Bodian-Diamond syndrome; WHIM, warts-hypogammaglobulinemia-infections-myelokatexis syndrome: CN, cyclic neutropenia; CGD, chronic granulomatous disease; LAD-1, leukocyte adhesion deficiency-1. *ELANE*, *WASP*, *SBDSP1*, *CXCR4*, *CYBB* and *ITGB2* indicate genes encoding elastase, Wiskott-Aldrich syndrome protein, SBDS protein 1, CXCR4, p91-PHOX and CD18, respectively. (**b**) ELISA for IgM, IgG and IgA to LPS from Pseudomonas aeruginosa, CPS from Pneumococcus 9N, 14, 19F and 23F, Gal-α-1,3-Gal (or α-Gal), tetanus toxin (TT) combined with diphtheria toxin (DT), and CPS from Meningococcus conjugated with DT in serum of patients with ND as in **a** and age-matched HD. OD, optical density. Error bars, median and percentile 25 and 75; * $P < 0.05$ (Mann-Whitney U test).

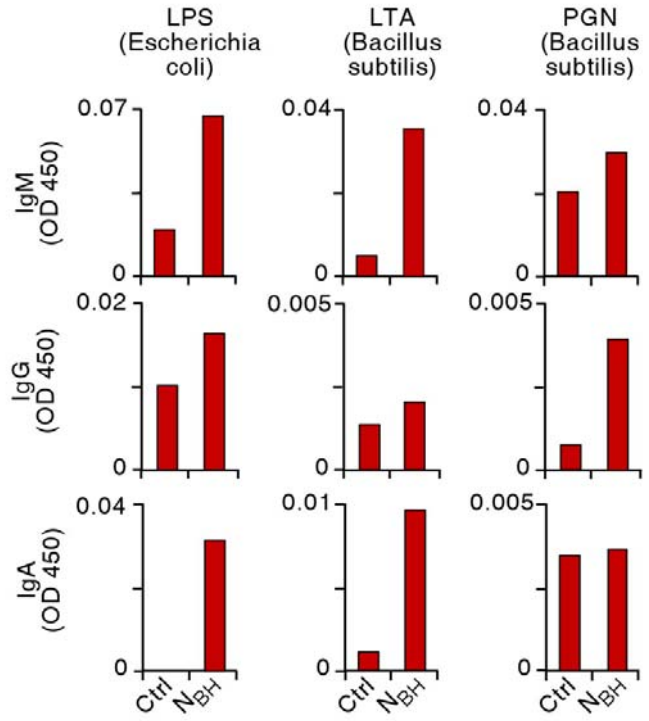

Figure S19. N_{BH} cells induce MZ B cell secretion of IgM, IgG and IgA to TI antigens. ELISA for IgM, IgG and IgA to LPS from Escherichia coli and to LTA and PGN from Bacillus subtilis in splenic MZ B cells cultured with medium alone (Ctrl) or N_{BH} cells for 6 d. OD, optical density. One of three experiments with similar results.

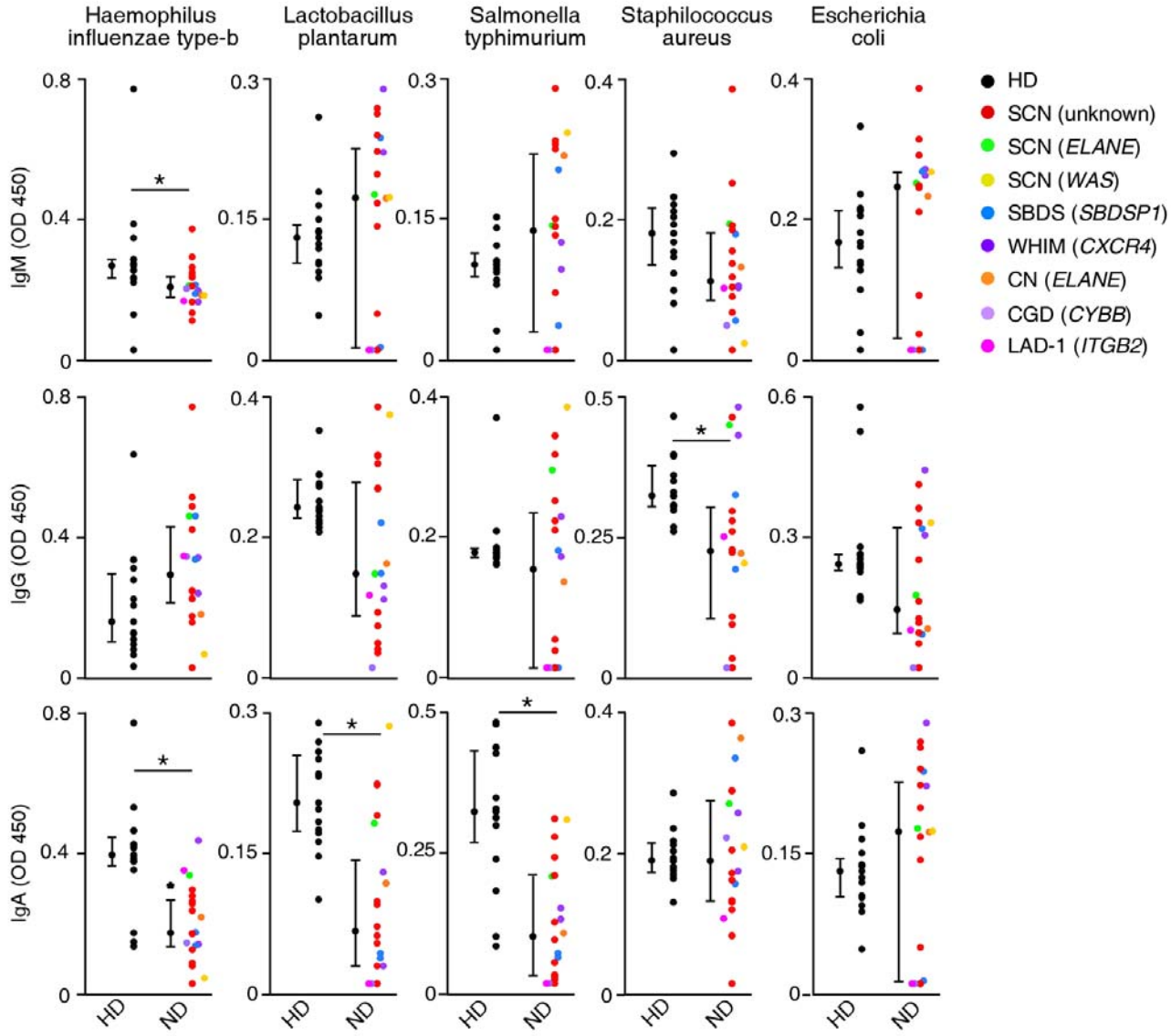

Figure S20. Neutropenia and functional neutrophil disorders decrease preimmune serum concentrations of IgM, IgG and IgA to some mucosal bacteria. ELISA for IgM, IgG and IgA to whole fixed *Haemophilus influenza* type-b, *Lactobacillus plantarum*, *Salmonella typhimurium*, *Staphilococcus aureus* and *Escherichia coli* in the plasma of patients with neutrophil disorders and age-matched healthy donors. SCN, severe congenital neutropenia; SBDS, Shwachman-Bodian-

Diamond syndrome; WHIM, warts-hypogammaglobulinemia-infections-myelokatexis syndrome: CN, cyclic neutropenia; CGD, chronic granulomatous disease; LAD-1, leukocyte adhesion deficiency-1. *ELANE*, *WASP*, *SBDSP1*, *CXCR4*, *CYBB* and *ITGB2* indicate genes encoding elastase, Wiskott-Aldrich syndrome protein, SBDS protein 1, CXCR4, p91-PHOX and CD18, respectively. OD, optical density. Error bars, median and percentile 25 and 75; * $P < 0.05$ (Mann-Whitney U test).

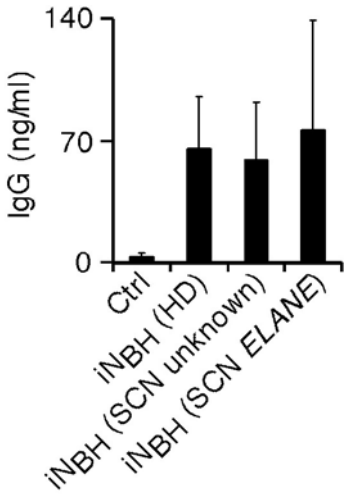

Figure S21. N_C cells from healthy donors and neutropenic patients acquire comparable N_{BH}-like function in response to IL-10. ELISA of IgG secreted by circulating unswitched IgD^+ B cells cultured for 7 d with medium alone or IL-10-induced iN_{BH} cells from healthy donors (HD; n = 3)) or six patients with severe congenital neutropenia (SCN) caused by unknown molecule defects (unknown; n = 3) or deleterious mutations of the gene encoding elastase (*ELANE*; n = 3). Error bars, s.e.m.

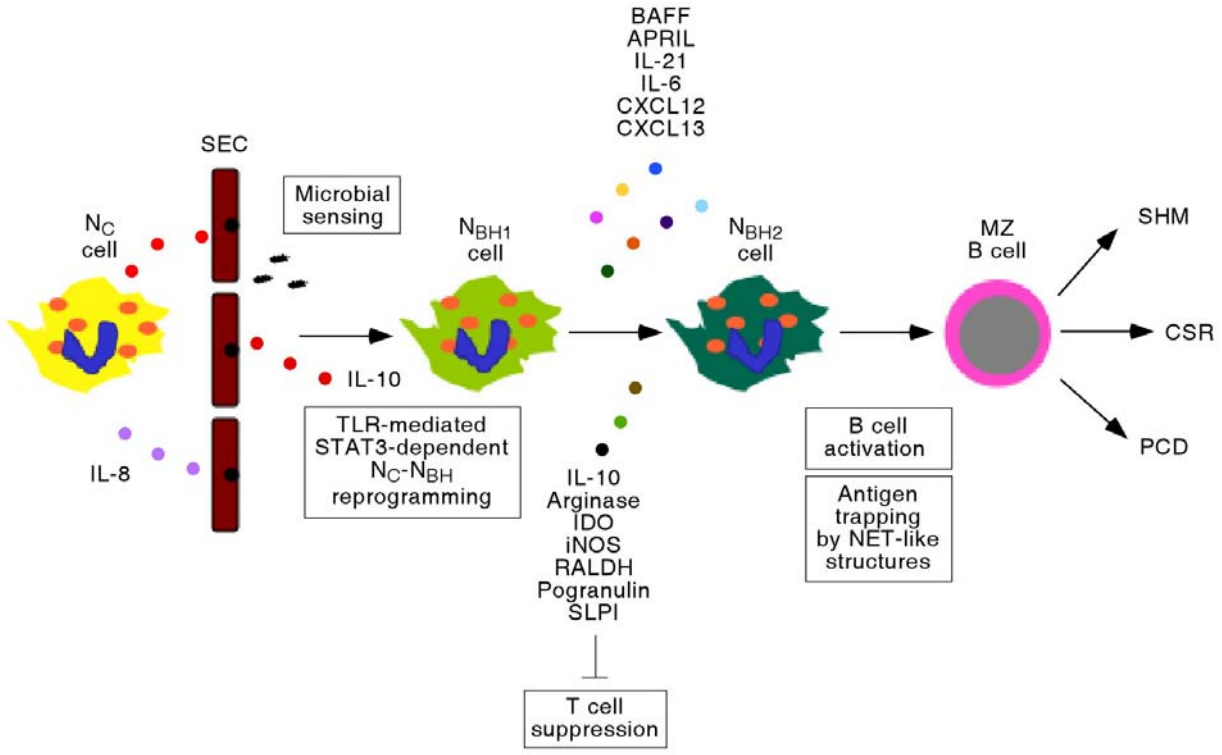

Figure S22. Proposed model for N_{BH} cell reprogramming and function. N_C cells home to the spleen under homeostatic conditions in response to microbial products undergoing splenic filtration after systemic translocation from mucosal surfaces. In the presence of TLR signals, SECs release chemokines such as IL-8 to induce recruitment of N_C cells as well as STAT3-inducing cytokines such as IL-10 to induce reprogramming of N_C cells into N_{BH} cells. These latter include N_{BH1} and N_{BH2} subsets with distinct phenotype, gene expression profile and B-helper activity. N_{BH} cells trigger Ig SHM, CSR, plasma cell differentiation (PCD), including IgM, IgG and IgA production, by activating MZ B cells through a mechanism involving BAFF, APRIL and IL-21 release as well as formation of antigen-trapping NET-like structures. Production of B cell-attracting chemokines such as CXCL12 and CXCL13 as well as PCD-inducing cytokines such as IL-6 may also play a role. Interaction of N_{BH} cells with MZ B cells would facilitate the formation of a circulating pre-immune repertoire of antimicrobial Igs to TI antigens. In addition to activating MZ B cells, N_{BH} cells suppress splenic $CD4^+$ T cells through a contact-independent mechanism that might involve IL-10, arginase, IDO, iNOS, RALDH, progranulin and SLPI. This T cell-suppressor activity could enable N_{BH} cells to sustain tonic TI MZ B cell activation without triggering TD follicular B cell activation and inflammatory tissue damage.

SUPPLEMENTARY MOVIE LEGENDS

Movie S1. N_{BH} cells form NET-like structures to interact with splenic MZ B cells. Confocal microscopy and three-dimensional animation of a spleen section stained for elastase (green), IgD (red) and DNA (blue). The movie was generated by acquiring up to 14 x,y planes with 0.3 μm z spacing. Original magnification, x40. One of three experiments yielding similar results.

Movie S2. N_{BH} cells spontaneously form DNA-containing NET-like projections. Confocal microscopy and three-dimensional animation of N_{BH} cells stained for elastase (green) and DNA (blue). The movie was generated by acquiring up to 25 x,y planes with 0.3 μm z spacing. Original magnification, x63; digital magnification, x2. One of three experiments yielding similar results.