SUPPORTING INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

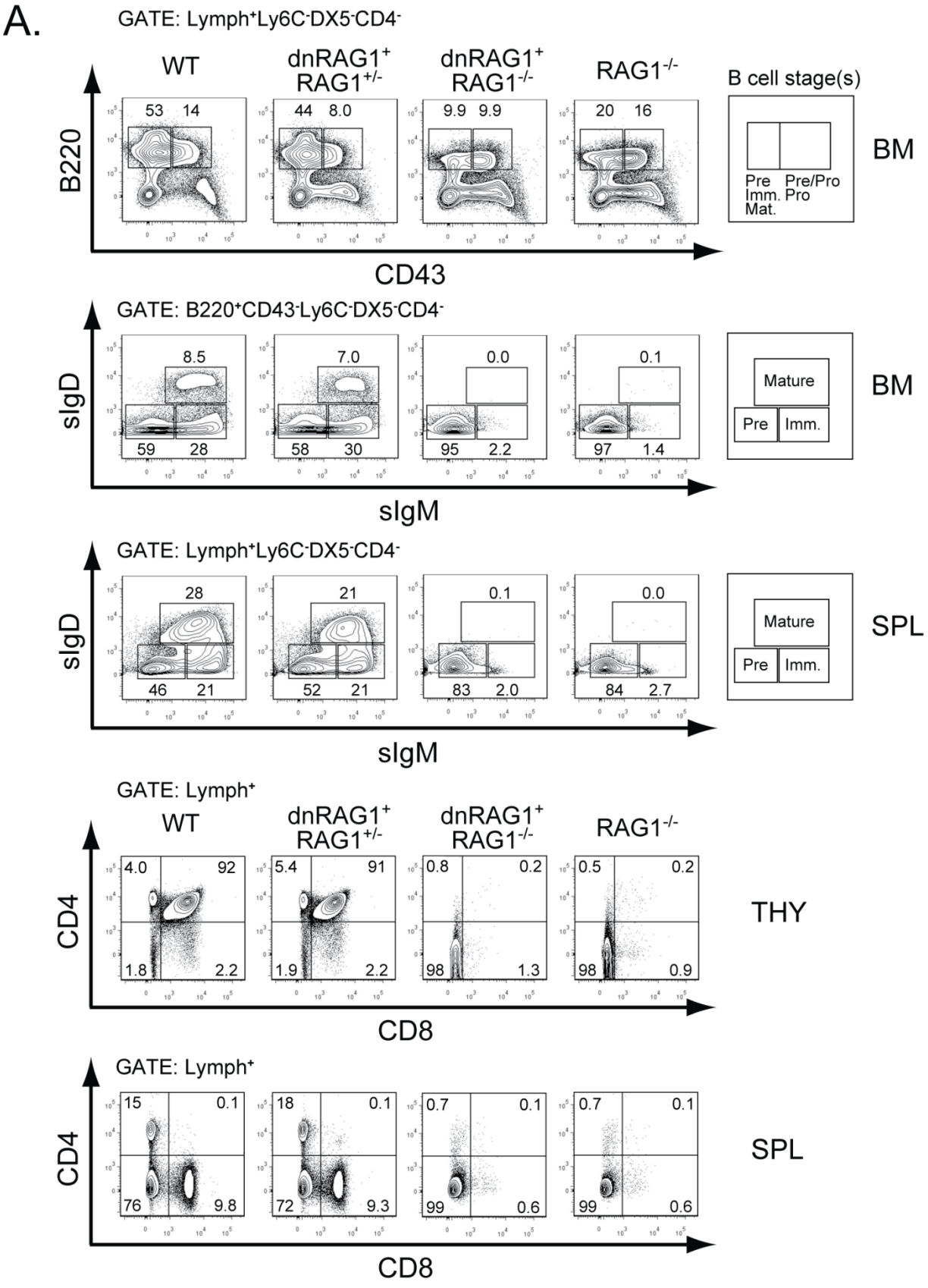
FIG. S1. dnRAG1 mice bred onto a RAG1-deficient background fail to develop mature lymphocytes. Cells prepared from WT, dnRAG1^{+}RAG1$^{+/-}$, dnRAG1^{+}RAG1$^{-/-}$, and RAG1$^{-/-}$ bone marrow (BM), spleen (SPL), or thymus (THY), and identified by the gating parameters shown above each row, were analyzed for the expression of B220 and CD43 (top row), sIgM and sIgD (middle two rows), or CD4 and CD8 (bottom two rows). B cell developmental subsets specified by the staining pattern are indicated below each column with corresponding gates. The percentage of cells within the identified gates is shown for representative animals.

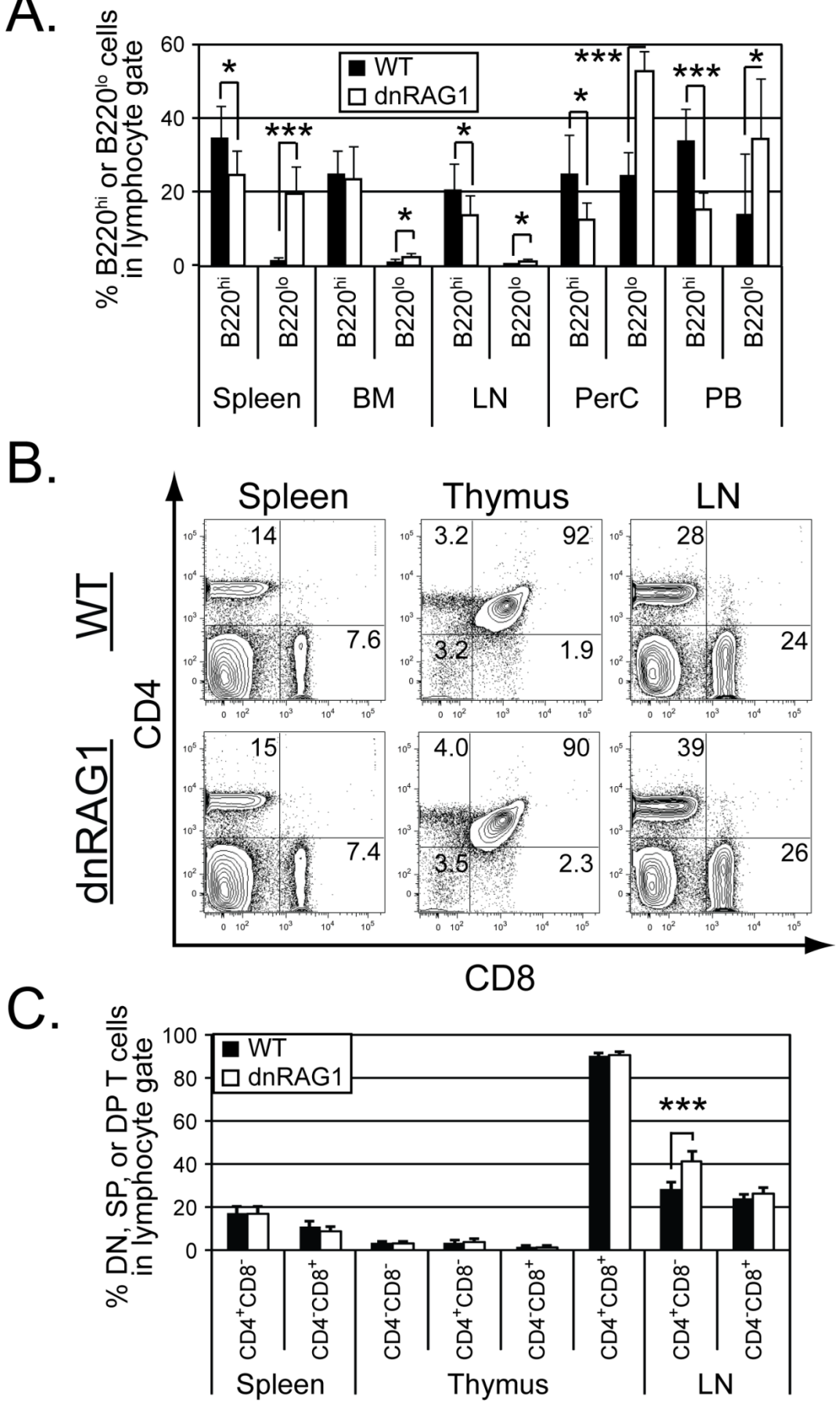
FIG. S2. Summary of data obtained for Fig. 1C and for analysis of T cell populations in the spleen thymus and lymph node. (A) Summary of data obtained for Fig. 1C in bar graph format. (B) Lymphocyte-gated cells prepared from WT or dnRAG1 spleen, thymus, and lymph node (LN) were analyzed for the expression of CD4 and CD8. (C) Summary of data obtained for Fig. S1B in bar graph format. Significance was determined from post-hoc analysis following one-way ANOVA (*, $p<0.05$; **, $p<0.01$; ***, $p<0.005$).

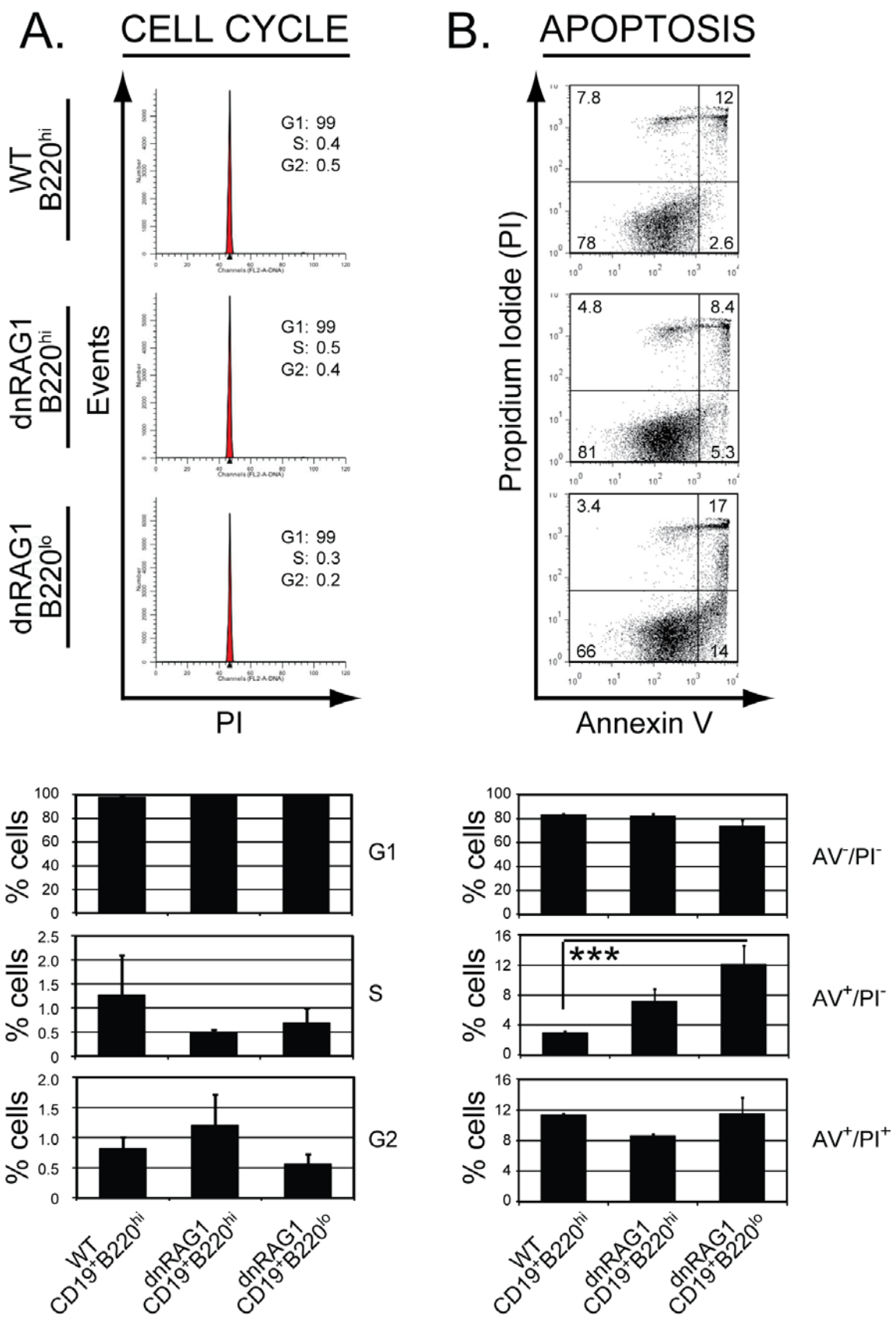
FIG. S3. Comparison of cell cycle status and apoptosis levels between sorted CD19^{+}B220^{hi} and CD19^{+}B220^{lo} B cells purified from WT and dnRAG1 mice. (A) Sorted CD19^{+}B220^{hi} and CD19^{+}B220^{lo} B cells purified from WT and dnRAG1 mice were incubated with Vindelov's reagent and propidium iodide (PI) staining was analyzed by flow cytometry. The percentage of cells in the G1, S, and G2 phase of the cell cycle were determined using the ModFit software

(upper panels). Statistical analysis of data obtained from n≥3 animals displayed in bar graph format (lower panels). (B) Sorted $CD19^{+}B220^{hi}$ and $CD19^{+}B220^{lo}$ B cells purified from WT and dnRAG1 mice were incubated with Annexin V (AV) and PI and analyzed by flow cytometry. The percentage of cells in each quadrant was determined using the FloJo software (upper panels). Statistical analysis of data obtained from n≥3 animals presented as in (A) (lower panels). Significance was determined from post-hoc analysis following one-way ANOVA (*, $p<0.05$; **, $p<0.01$; ***, $p<0.005$).

FIG. S4. Flow cytometric analysis comparing surface expression levels of B220 versus CD43 on BM B cells, and AA4.1 versus B220, IgMa versus IgMb and Igκ vs Igλ on splenic B cells from WT, dnRAG1, 56Rki, and DTG mice. (A) Cells prepared from WT, dnRAG1, 56Rki, and DTG bone marrow or spleen and identified by the gating parameters shown above each row were analyzed for the expression of B220, CD43, and AA4.1. B cell developmental subsets specified by the staining pattern are indicated below each column with corresponding gates. The percentage of cells within the identified gates is shown for representative animals. (B) Cells prepared from WT, dnRAG1, 56Rki, and DTG spleen and identified by the gating parameters shown above each row were analyzed for the expression of IgMa, IgMb, Igκ and Igλ. The percentage of cells within the identified gates is shown for representative animals. The absolute number of cells in each population is shown in the lower panel (***, $p<0.005$).


A.
GATE: Lymph+Ly6C-DX5-CD4-
WT
dnRAG1+ RAG1+/-
dnRAG1+ RAG1-/-
RAG1-/-
B220
CD43
53 14
44 8.0
9.9 9.9
20 16
B cell stage(s)
Pre Imm.
Pre/Pro Pro
Mat.
BM
GATE: B220+CD43-Ly6C-DX5-CD4-
sIgD
sIgM
8.5
59 28
7.0
58 30
0.0
95 2.2
0.1
97 1.4
Mature
Pre
Imm.
BM
GATE: Lymph+Ly6C-DX5-CD4-
sIgD
sIgM
28
46 21
21
52 21
0.1
83 2.0
0.0
84 2.7
Mature
Pre
Imm.
SPL
GATE: Lymph+
WT
dnRAG1+ RAG1+/-
dnRAG1+ RAG1-/-
RAG1-/-
CD4
CD8
4.0 92
1.8 2.2
5.4 91
1.9 2.2
0.8 0.2
98 1.3
0.5 0.2
98 0.9
THY
GATE: Lymph+
CD4
CD8
15 0.1
76 9.8
18 0.1
72 9.3
0.7 0.1
99 0.6
0.7 0.1
99 0.6
SPL


A.
% B220hi or B220lo cells in lymphocyte gate
WT
dnRAG1
B220hi
B220lo
Spleen
BM
LN
PerC
PB
B.
Spleen
Thymus
LN
WT
dnRAG1
CD4
CD8
14
7.6
3.2
92
3.2
1.9
28
24
15
7.4
4.0
90
3.5
2.3
39
26
C.
% DN, SP, or DP T cells in lymphocyte gate
CD4+CD8-
CD4-CD8+
CD4-CD8-
CD4+CD8-
CD4-CD8+
CD4+CD8+
CD4+CD8-
CD4-CD8+
Spleen
Thymus
LN


A.
CELL CYCLE
WT B220hi
dnRAG1 B220hi
dnRAG1 B220lo
Events
G1: 99
S: 0.4
G2: 0.5
G1: 99
S: 0.5
G2: 0.4
G1: 99
S: 0.3
G2: 0.2
PI
B.
APOPTOSIS
Propidium Iodide (PI)
7.8
12
78
2.6
4.8
8.4
81
5.3
3.4
17
66
14
Annexin V
% cells
G1
S
G2
AV-/PI-
AV+/PI-
AV+/PI+
***
WT CD19+B220hi
dnRAG1 CD19+B220hi
dnRAG1 CD19+B220lo

Hassaballa et al. Fig. S4

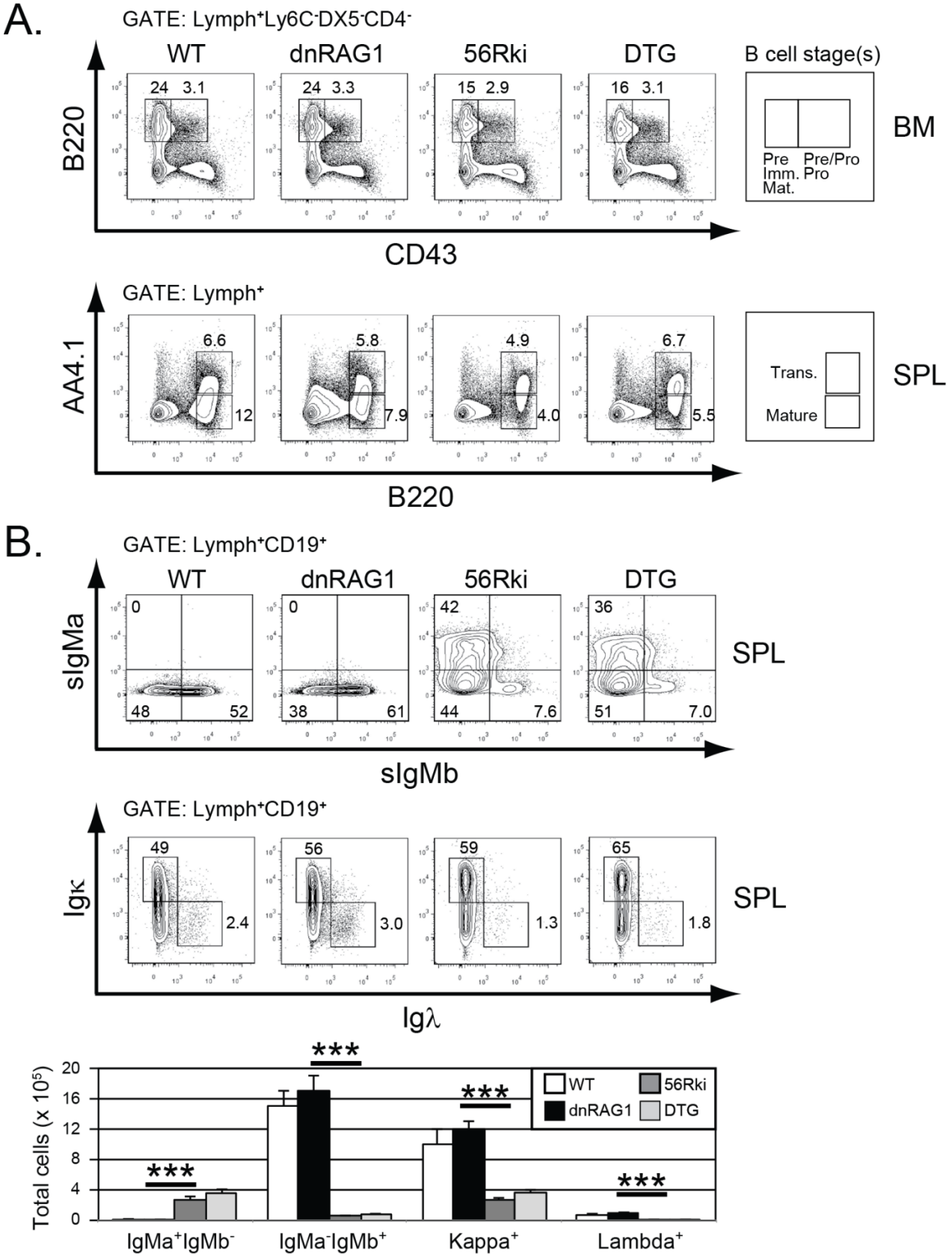


Table S1. Primers used for Igκ gene sequence analysis.

Primer name	**Sequence (5′-3′)**
MuIgκVL5'-A	GGGAATTCATGRAGWCACAKWCYCAGGTCTTT
MuIgκVL5'-B	GGGAATTCATGGAGACAGACACACTCCTGCTAT
MuIgκVL5'-C	ACTAGTCGACATGGAGWCAGACACACTSCTGYTATGGGT
MuIgκVL5'-D	ACTAGTCGACATGAGGRCCCCTGCTCAGWTTYTTGGIWTCTT ACTAGTCGACATGGGCWTCAAGATGRAGTCACAKWYYCWGG
MuIgκVL5'-G	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT ACTAGTCGACATGGATTTWCARGTGCAGATTWTCAGCTT ACTAGTCGACATGGTYCTYATVTCCTTGCTGTTCTGG ACTAGTCGACATGGTYCTYATVTTRCTGCTGCTATGG
MuIgκVL3'-1	CCCAAGCTTACTGGATGGTGGGAAGATGGA

B = C or G or T
D = A or G or T
H = A or C or T
I = inosine
K = G or T
M = A or C
R = A or G
S = C or G
V = A or C or G
W = A or T
Y = C or T

Table S2. Summary of total cell counts of various B cell populations (x 10^5 unless indicated).

	WT	dnRAG1	56Rki	DTG	ONE WAY ANOVA [a]	WT vs. dnRAG1 [b]	dnRAG1 vs. DTG [b]	56Rki vs. DTG [b]
Bone Marrow	**(n=9)**	**(n=8)**	**(n=12)**	**(n=10)**				
Cellularity (x10^7)	1.79±0.25	1.74±0.23	1.87±0.17	1.69±0.21	n.s.	n.s.	n.s.	n.s.
	(n=4)	**(n=3)**	**(n=4)**	**(n=4)**				
Lymphs	57±3	56±1	61±2	56±3	n.s.	n.s.	n.s.	n.s.
B220+ $CD43^+$	2.35±0.34	2.66±0.73	2.29±0.27	2.01±0.21	n.s.	n.s.	n.s.	n.s.
$B220^+$ $CD43^-$	16.02±1.68	14.02±0.25	10.08±0.83	9.56±0.92	†††	n.s.	†	n.s.
Pre- B (IgM^-IgD^-)	9.07±1.10	8.55±0.66	7.47±0.87	8.06±0.80	n.s.	n.s.	n.s.	n.s.
Imm. B (IgM^+ IgD^-)	2.25±0.23	2.34±0.32	0.32±0.07	0.40±0.14	†††	n.s.	†††	n.s.
Mature B (IgM^+IgD^+)	4.43±0.69	2.87±0.14	2.17±0.14	0.98±0.05	†††	†††	†††	†
	(n=9)	**(n=8)**	**(n=12)**	**(n=10)**				
Lymphs	72.8±1.4	71.3±2.5	68.0±2.4	65.0±2.9	n.s.	n.s.	n.s.	n.s.
$IgMa^+$ $IgMb^-$	0.15±0.05	0.098±0.021	1.22±0.17	0.99±0.11	†††	n.s.	†††	n.s.
$IgMa^-$ $IgMb^+$	5.6±0.5	6.34±0.51	0.34±0.03	0.50±0.14	†††	n.s.	†††	n.s.
	(n=7)	**(n=6)**	**(n=10)**	**(n=8)**				
$Kappa^+$	4.33±0.85	4.58± 0.61	1.35±0.17	0.97±0.0	†††	n.s.	†††	n.s.
$Lambda^+$	0.43±0.08	0.45±0.02	0.10±0.02	0.13±0.02	†††	n.s.	†††	n.s.
Spleen	**(n=7)**	**(n=6)**	**(n=10)**	**(n=8)**				
Cellularity (x10^7)	1.5±0.6	0.99±0.64	0.59±0.22	0.64±0.26	n.s.	n.s.	n.s.	n.s.
Lymphs	63±5	47±5	29±2	30±2	†††	††	†††	n.s.
$CD19^+B220^{lo}$	2.6±1.3	8.0±1.2	0.61±0.07	0.80±0.12	†††	†††	†††	n.s.
$CD19^+$ $B220^{hi}$	11±3	6.2±1.5	3.3±0.5	4.2±0.7	†	n.s.	n.s.	n.s.
$B220^+$ $AA4.1^+$	6.3±1.1	3.7±0.7	1.4±0.2	2.0±0.4	†††	†	n.s.	n.s.
T1	1.8±0.2	1.6±0.3	0.34±0.06	0.77±0.17	†††	n.s.	†††	n.s.
T2	1.7±0.4	0.41±0.11	0.24±0.04	0.17±0.0.3	†††	†††	n.s.	n.s.
T3	1.8±0.6	0.38±0.14	0.53±0.11	0.46±0.11	†††	†††	n.s.	n.s.
$B220^+$ $AA4.1^-$	10±1.5	5.4±1.0	2.8±0.3	3.2±0.4	†††	†††	n.s.	n.s.
MZ	0.62±0.09	0.73±0.19	0.54±0.09	0.78±0.09	†††	n.s.	ns	†††
FM	8.1±1.5	2.8±0.6	1.8±0.3	1.2±0.2	†††	†††	n.s.	n.s.
$IgMa^+$ $IgMb^-$	0.081±0.045	0.050±0.038	2.7±0.4	3.5±0.6	†††	n.s.	†††	n.s.
$IgMa^-$ $IgMb^+$	15±2	17±2	0.54±0.05	0.75±0.11	†††	n.s.	†††	n.s.
$Kappa^+$	10±2	12±1	2.7±0.2	3.6±0.4	†††	n.s.	†††	n.s.
$Lambda^+$	0.71±0.14	0.96±0.10	0.033±0.004	0.067±0.011	†††	n.s.	†††	n.s.
Peritoneal Cavity	**(n=7)**	**(n=6)**	**(n=10)**	**(n=8)**				
Cellularity (x10^7)	0.12±0.04	0.16±0.03	0.054±0.017	0.10±0.03	n.s.	n.s.	†	n.s.
Lymphs	2.8±0.5	7.4±1.4	0.90±0.15	3.0±0.4	†††	†††	†††	†
$CD19^+B220^{lo}$	0.95±0.31	4.2±0.6	0.15±0.03	0.65±0.10	†††	†††	†††	n.s.
$CD19^+$ $B220^{hi}$	0.32±0.09	0.47±0.07	0.46±0.09	0.31±0.04	†††	n.s.	n.s.	†††

[a] variance between groups by one-way ANOVA: n.s., not significant; †, $p<0.05$; ††, $p<0.01$; †††, $p<0.005$.
[b] post-hoc analysis by unpaired *t* test: n.s., not significant; †, $p<0.05$; ††, $p<0.01$; †††, $p<0.005$.

Table S3. Igκ gene sequence analysis.

Cell Population	Primer Family	Number	Sequence Name	V region	J segment	Mutations	AA substitutions	Transition	Transversion	Insertions	Total del	V del	J del
WT B220hi	Kappa A	1	Tg-KA1-M13For_B09_2.ab1	IgVk 19-23	Jk5	5	2	4	1		3	2	1
		6	Tg-6KA-M13For_F02_2.ab1	Vk 19-23	Jk2	1	0	1			7	4	3
		7	Tg-7KA-M13For_G02_3.ab1	Vk 19-15, partial	Jk5	1	1	1			3	3	precise
		9	Tg-KA8.1-M13For_C07_3.ab1	Vk 19-17, partial	Jk5	0					3	3	precise
		10	Tg-KA9-M13For_D07_4.ab1	Vk 19-17, partial	Jk2	1	1	1			4	3	1
		11	Tg-KA10-M13For_E07_1.ab1	Vk 19-15, partial	Jk5	1	0	1			3	3	precise
		12	Tg-KA11-M13For_F07_2.ab1	Vk 19-23	Jk5	0					3	3	precise
	Kappa B	1	Tg-1-KB-M13Rev_E03_1.ab1	Vk 21C	Jk1	0					3	1	2
		2	Tg-2-KB-M13For_B02_2.ab1	Vk 21A	Jk2	0					4	3	1
		3	Tg-3-KB-M13For_C02_3.ab1	Vk 21 Subgroup	Jk2	2	0	2			4	3	1
		4	Tg-4-KB-M13For_D02_4.ab1	Vk 21A	Jk2	1	insert = frameshift			1	4	3	1
		6	Tg-11KB-M13For_E11_1ab.1	Vk 21C	Jk1	2	1	2			3	precise	3
	Kappa C	5	Tg-KC6-M13For_A09_1.ab1	Vk 21C	Jk1	0					3	1	2
Cell Population	**Primer Family**	**Number**	**Sequence Name**	**V region**	**J segment**	**Mutations**	**AA substitutions**	**Transition**	**Transversion**	**Insertions**	**Total del**	**V del**	**J del**
dnRAG1 B220hi	Kappa A	3	Tg+HiKA3-M13For_H09_4.ab1	Vk 19-23	Jk5	0					3	2	1
		4	Tg+HiKA4-M13F0r_A10_1.ab1	Vk 19-32	Jk1	0					3	1	2
		5	Tg+Hi5KA-M13For_A03_1.ab1	Vk 19-23	Jk5	1	1	1			3	3	precise
		6	Tg+Hi6KA-M13For_B03_2.ab1	Vk 19-15	Jk4	0					3	3	precise
		7	Tg+Hi7KA-M13For_C03_3.ab1	Vk 19-17, partial	Jk1	1	1		1		3	3	precise
		8	Tg+Hi8KA-M13For_D03_4.ab1	Vk 19-23	Jk5	0					3	2	1
		10	Tg+HiKA10-M13For_A08_1.ab1	Vk 19-15, partial	Jk5	0					3	2	1
		11	Tg+HiKA11-M13For_B08_2.ab1	Vk 19-23	Jk2	0					4	3	1
		12	Tg+HiKA12-M13For_C08_3.ab1	Vk 19-23	Jk5	0					3	2	1
	Kappa B	1	Tg+Hi-1-KB-M13FOr_E02_1.ab1	Vk 21B Subgroup	Jk2	0					4	precise	4
		2	Tg+Hi-2-KB-M13For_F02_2.ab1	Vk 21 Subgroup	Jk2	1	0	1			4	4	precise
		3	Tg+Hi-3-KB-M13For_G02_3.ab1	Vk 21C	Jk2	0					4	3	1
		4	Tg+Hi-4-KB-M13For_H02_4.ab1	Vk 21B Subgroup	Jk2	1	0	1			4	precise	4
		6	Tg+Hi7KB-M13For_G07_3.ab1	Vk 21B Subgroup	Jk1	0					3	1	2
		7	Tg+Hi10KB-M13For_H07_4.ab1	Vk 21B Subgroup	Jk5	1	1	1			3	3	precise
		9	Tg+Hi12KB-M13For_B02_2.ab1	Vk 21C	Jk2	0					4	3	1
		10	Tg+Hi13KB-M13For_C02_3.ab1	Vk 21 Subgroup	Jk1	2	1	2			3	precise	3
	Kappa C	1	Tg+HiKC1-M13For_G08_3.ab1	Vk 8-21	Jk1	1	1	1			perfect	precise	precise
		3	Tg+HiKC3-M13For_A09_1.ab1	Vk 21 Subgroup	Jk2	1	0	1			4	3	1
		4	Tg+HiKC5-M13For_A04_1.ab1	Vk 21C	Jk2	1	1	1			4	3	1
		5	Tg+HiKC6-M13For_B04_2.ab1	Vk 21G	Jk2	2	2	1	1		4	3	1
		6	Tg+HiKC7-M13For_C04_3.ab1	Vk 8-27	Jk2	0					3	2	1
		7	Tg+HiKC8-M13For_D04_4.ab1	Vk 21 Subgroup	Jk1	4	3	4			3	precise	3
		8	Tg+HiKC9-M13For_E04_1.ab1	Vk 8-30	Jk2	0					4	3	1
		9	Tg+HiKC10-M13For_F04_2.ab1	Vk 21 Subgroup	Jk2	1	0	1			4	3	1
		11	Tg+HiKC12-M13For_H04_4.ab1	Vk 21A	Jk1	0					3	precise	3
	Kappa D	1	Tg+HiKD1-M13For_E03_1.ab1	Vk ba9	Jk5	0					3	3	precise
		2	Tg+HiKD2-M13For_F03_2.ab1	Vk ba9	Jk2	2	2	1	1		4	3	1
		*3	Tg+HiKD3-M13F0r_G03_3.ab1	Vk psi-br9 (9-128)	Jk5	0						3	precise
		4	Tg+HiKD4-M13For_H03_4.ab1	Vk ba9	Jk1	1	1	1			3	3	precise
		5	Tg+HiKD5-M13For_E06_1.ab1	Vk 8-28	Jk5	2	insert=frameshift		1	1	3	3	precise
		8	Tg+HiKD9-M13For_G01_3.ab1	Vk bv9	Jk1	2	2	1	1		3	1	2
		9	Tg+HiKD10-M13For_H01_4.ab1	Vk 8-28	Jk5	0					3	3	precise
	Kappa G	1	Tg+HIKG1-M13For_F04_2.ab1	Vk cr1 gene	Jk2	0					4	3	1
		3	Tg+HiKG5-M13For_A03_.1ab1	Vk cr1 gene	Jk2	1	1	1			4	3	1
		5	Tg+HiKG7-M13For_C03_.3ab1	Vk 8-19, partial	Jk5	0					3	3	precise
		6	Tg+HiKG9-M13For_D03_4.ab1	Vk cr1 gene	Jk5	0					3	3	precise
Cell Population	**Primer Family**	**Number**	**Sequence Name**	**V region**	**J segment**	**Mutations**	**AA substitutions**	**Transition**	**Transversion**	**Insertions**	**Total del**	**V del**	**J del**
dnRAG1 B220lo	Kappa A	2	Tg+LoKA2-M13For_C10_3.ab1	Vk 19-32	Jk1	0					3	1	2
		3	Tg+LoKA3-M13For_D10_4.ab1	Vk 19-32	Jk1	0					3	1	2
		4	Tg+Lo5KA-M13For_E03_1.ab1	Vk 19-32	Jk1	1	1	1			3	1	2
		5	Tg+Lo6KA-M13For_F03_2.ab1	Vk 19-32	Jk1	0					3	1	2
		6	Tg+Lo7KA-M13For_G03_3.ab1	Vk 19-32	Jk1	0					3	1	2
		7	Tg+LoKA8-M13For_D08_4.ab1	Vk 19-32	Jk1	1	1	1			6	6	precise
		8	Tg+LoKA9-M13For_E08_1.ab1	Vk 19-32	Jk1	0					3	1	2
		9	Tg+LoKA10-M13For_F08_2.ab1	Vk 19-17	Jk5	0					3	3	precise
		10	Tg+LoKA11-M13For_G08_3.ab1	Vk 19-32	Jk1	0	stop				3	1	2
	Kappa B	5	Tg+Lo5KB-M13For_A08_1.ab1	Vk 21A	Jk2	0					4	3	1
		7	Tg+Lo12KB-M13For_D02_4.ab1	Vk 21C	Jk1	0					3	1	2
	Kappa C	2	Tg+LoKC2-M13For_D08_4.ab1	Vk 21C	Jk1	0					3	1	2
		6	Tg+LoKC6-M13For_B05_2.ab1	Vk 21A	Jk2	0					4	3	1
		7	Tg+LoKC7-M13For_C05_3.ab1	Vk 19-32	Jk1	0					3	1	2
	Kappa D	7	Tg+LoKD8-M13For_D02_.4ab1	Vk ba9	Jk2	0					4	3	1