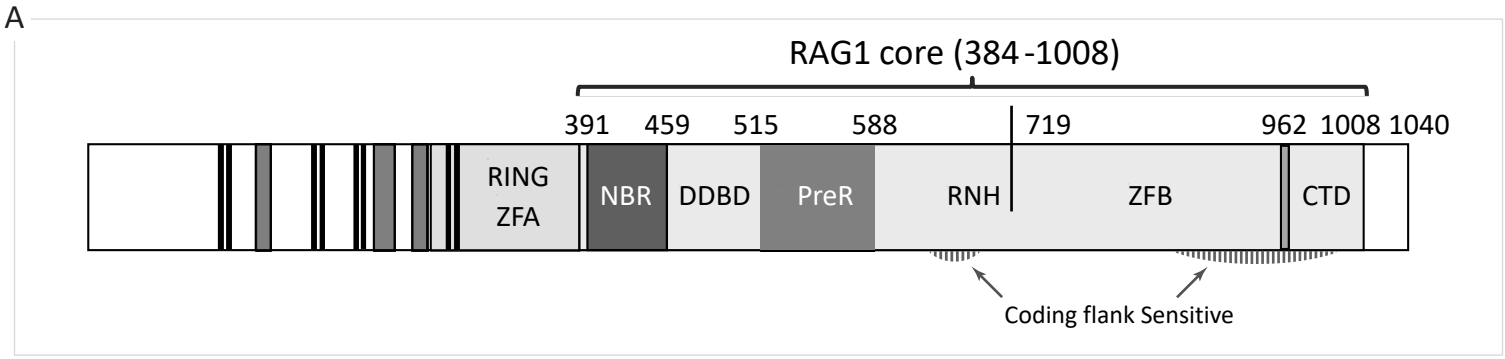
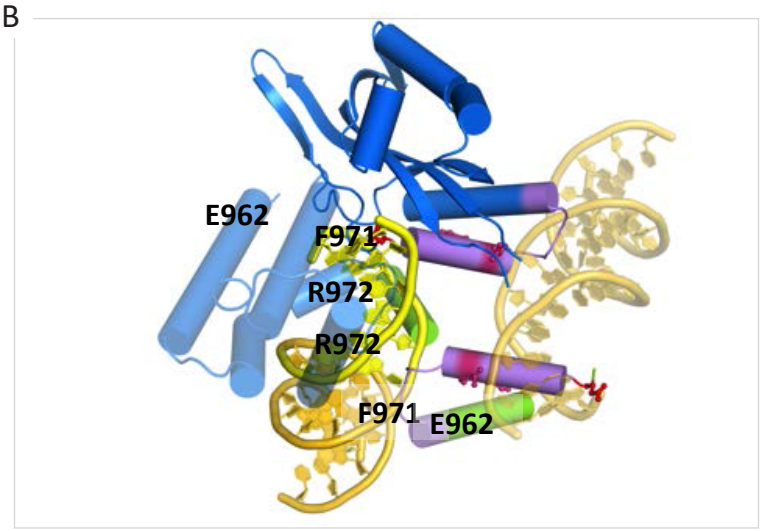
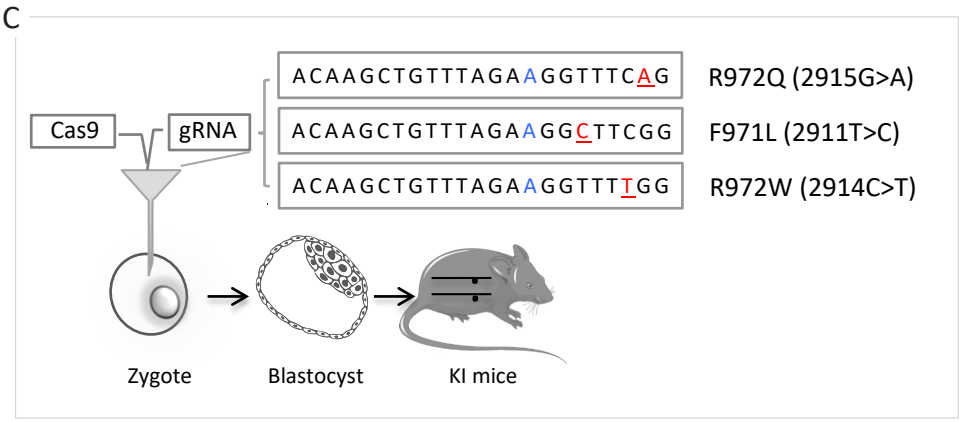






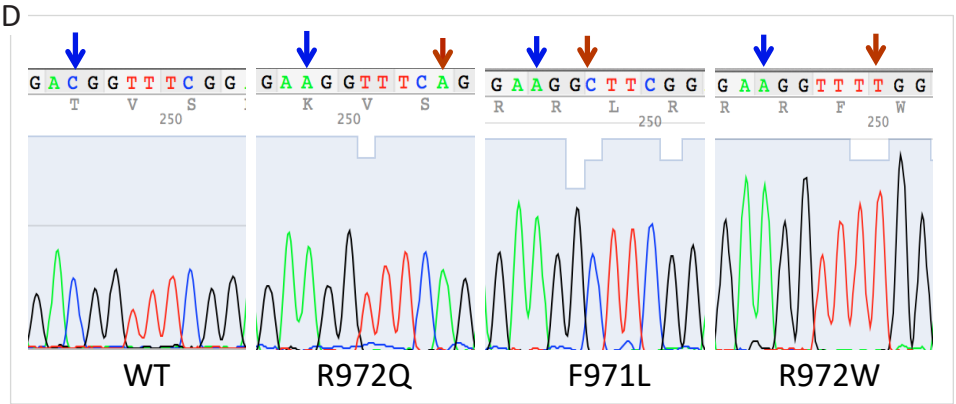


Figure S1

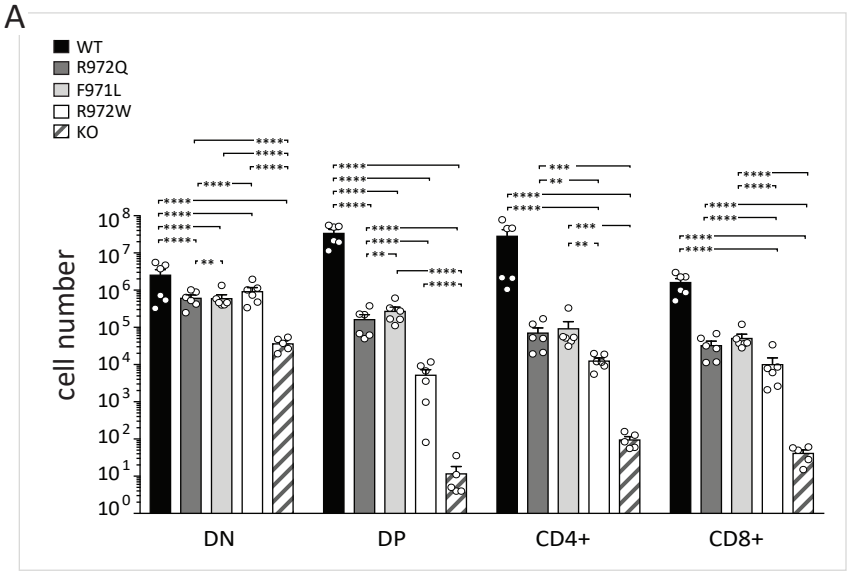
Predicted effect of *Rag1* mutations based on crystallography data, and strategy for generation of *Rag1* mutant mice

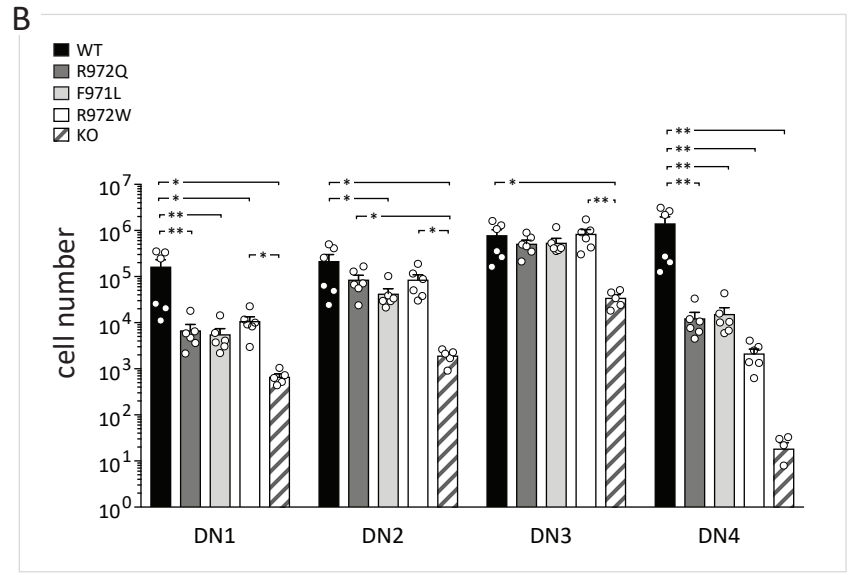
A RAG1 protein structure, showing Zinc Finger A (ZFA) and Zinc Finger B (ZFB), Nonamer binding region (NBR) and DNA dimerization and binding domain (DDBD), pre-RNase (preR), the catalytic RNase H-like (RNH) domain and the C-terminal domain (CTD). Residue numbers are given for the boundaries of the different domains. Two coding flank sensitive regions run from amino acid 606-611 and from 889-974.

B Structural model of RAG1-RSS complex. Both F971 and R972 are located on the bridging α-helix (colored in purple), which is immediately downstream from the catalytic residue E962 and is predicted to link the two RSS DNAs.

C Zygote injection with a mixture of 3 different ssODNs each containing specific point mutations (red) and a common silent variant (blue).

D Sanger sequences of resulting genotypes showing the silent variant (blue arrow) and the specific c.2915G>A, c.2911T>C and α c.2914C>T mutations (red arrows).





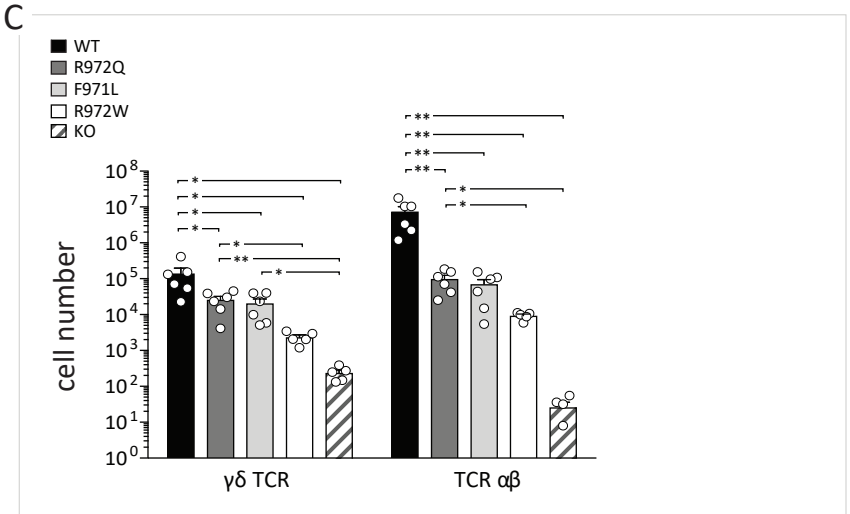


Figure S2

Absolute number of cells at various stages of lymphocyte development in the thymus

A Absolute count of $CD4^-$ $CD8^-$ double negative (DN), $CD4^+$ $CD8^+$ double positive (DP) and single positive ($CD4^+$ and $CD8^+$) cells in the thymus of wild-type (WT), $Rag1^{-/-}$ (KO) and indicated mutant mice.

B Absolute count of DN thymocytes at various stages of development (DN1-DN4) based on CD44 and CD25 expression: $CD44^+$ $CD25^-$ (DN1), $CD44^+$ $CD25^+$ (DN2), $CD44^-$ $CD25^+$ (DN3), and $CD44^-$ $CD25^-$ (DN4).

C Absolute count of γδTCR and αβTCR thymocytes.

In all panels, 6 mice per group were analyzed, and statistical analysis was done with one-way ANOVA (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

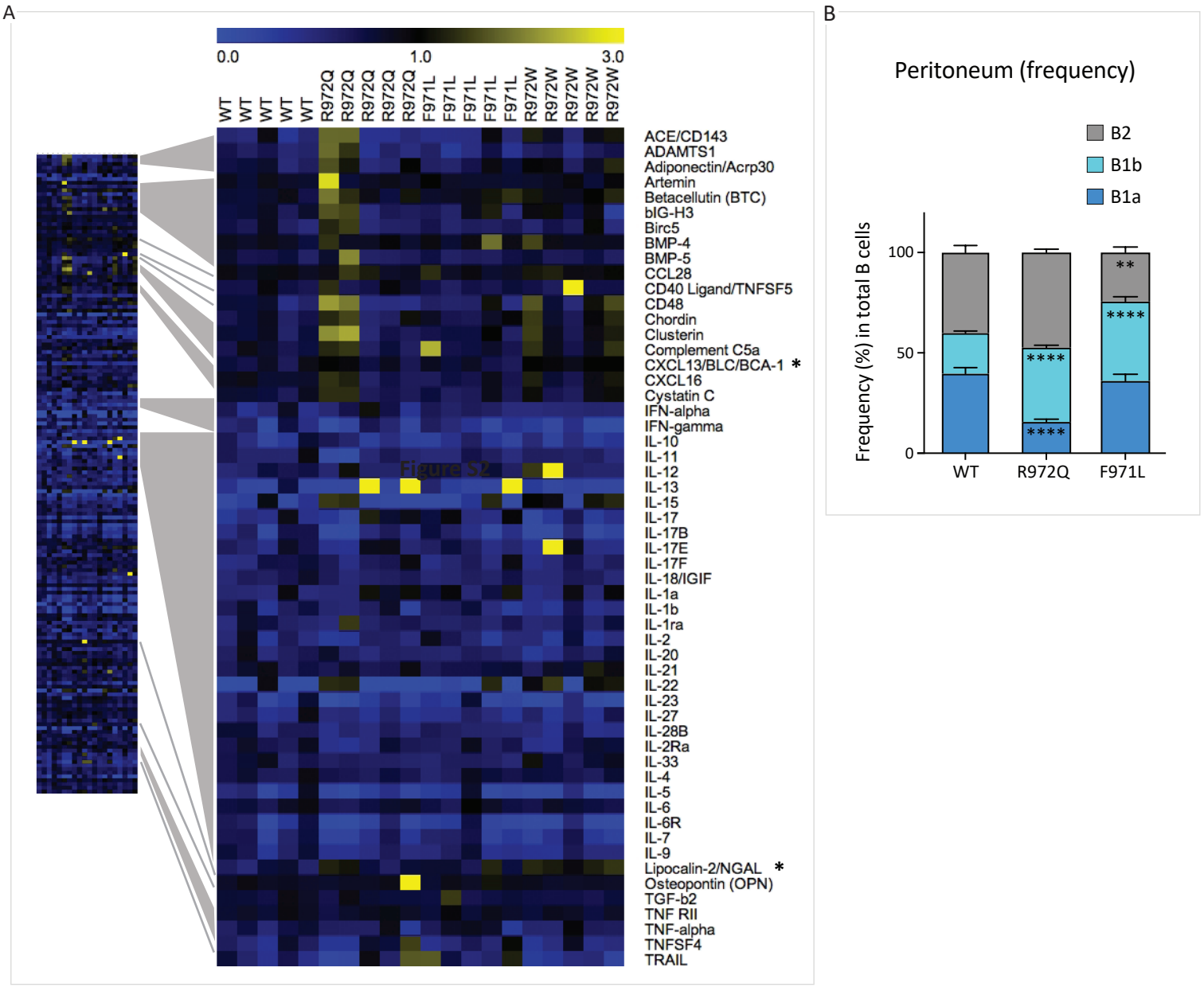


Figure S3

Anti-cytokine antibodies and distribution of peritoneal B1 and B2 subsets

A An array of full-length murine proteins was used to screen for cytokine autoantibodies (IgG) in mutant (n=5 per strain) and WT (n=5) mice. The heatmap displays the ratio of signal intensities to the second SD over the WT mean, and therefore highlights signals that are stronger in the mutant mice. A selection of cytokines, for which autoantibodies had been found in patients previously, or that tested positive in our mice, are shown on the right. SEM test was used to test for statistical significance between groups (* FDR ≤1%). This was only the case for Lipocalin-2/NGAL and CXCL13/BCL/BCA-1 in the R972W model compared to WT.

B Distribution of B1 ($CD19^+$ $CD11b^+$) and B2 ($CD19^+$ $CD11b^-$) cells and B1a ($CD19^+$ $CD11b^+$ $CD5^+$) and B1b ($CD19^+$ $CD11b^+$ $CD5^-$) cells in peritoneal lavage. Statistical analysis was done with one-way ANOVA (** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$). Error bars represent SEM.

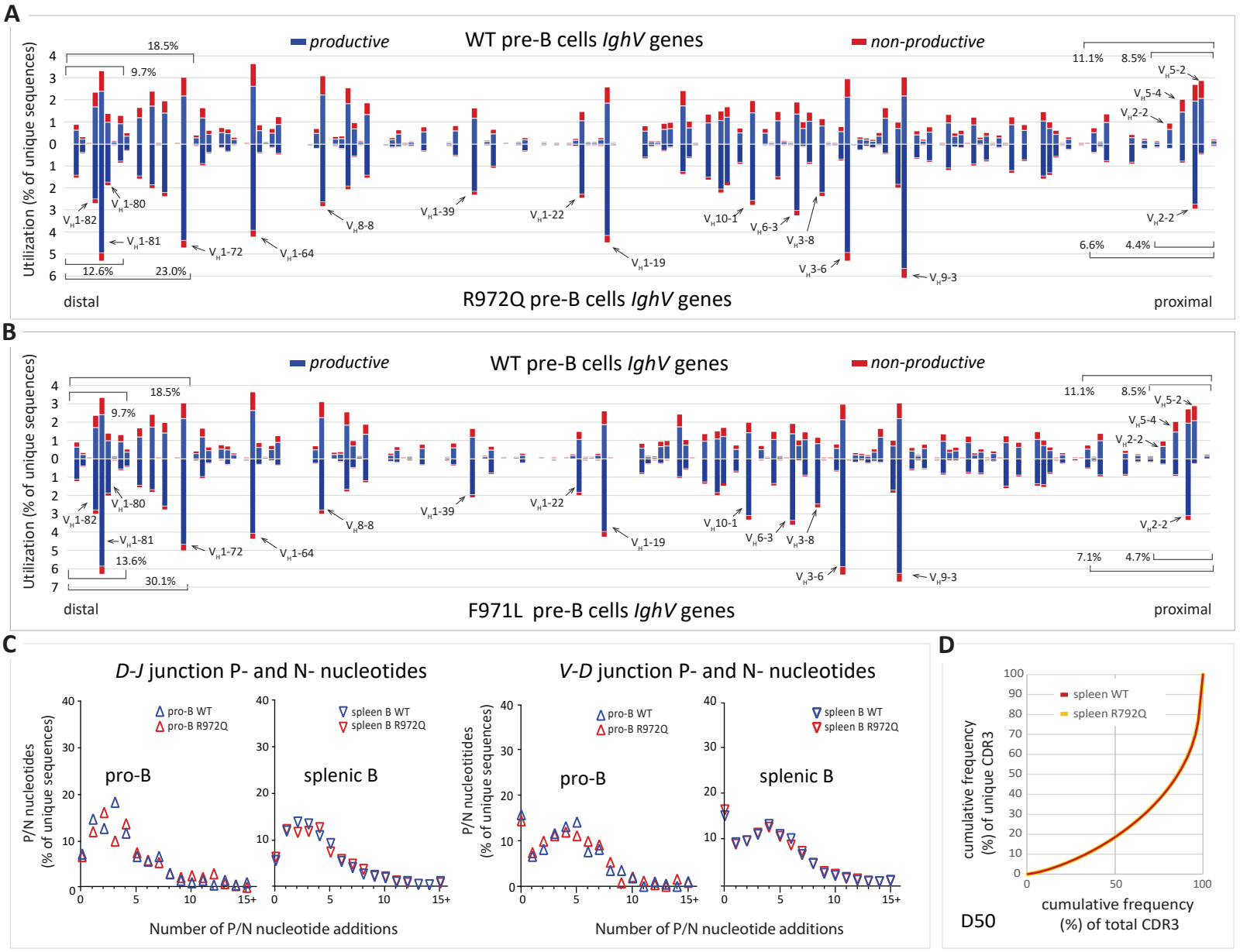


Figure S4

***Igh* repertoire of bone marrow and splenic B cells**

A *V* gene usage in pre-B cells from WT (top, n=3), and R972Q (bottom, n=3) mice.

B *V* gene usage in pre-B cells from WT (top, n=3) and F971L (bottom, n=3) mice.

C Distribution of P and N nucleotide addition in *D-J* and *V-D* junctions in bone marrow pro-B cells and splenic B cells. X-axis: number of P/N nucleotide additions, Y-axis: percentage of unique sequences with given number of P/N nucleotide additions.

D D50, corresponding to the percentage of unique CDR3 sequences that account for 50% of the total number of sequences recovered of mature splenic B cells from R972Q vs WT mice.

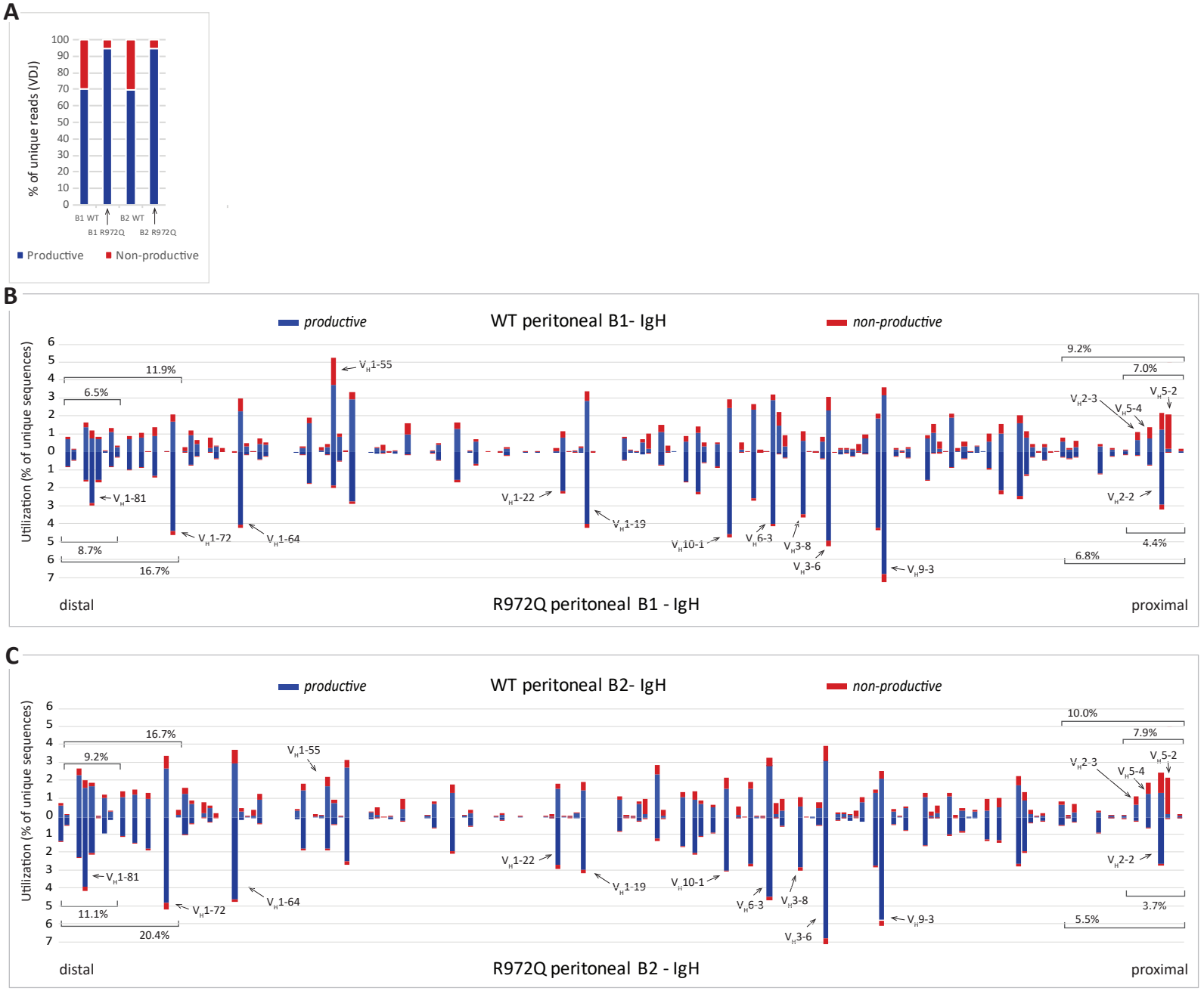


Figure S5

***Igh* repertoire of peritoneal B1 and B2 cells**

A Bar graph showing proportion of productive (blue) and non-productive (red) reads among total unique *Igh VDJ* rearrangements in FACS-sorted peritoneal B1 ($B220^+$ $CD19^+$ IgM^+ $CD11^+$) and B2 ($B220^+$ $CD19^+$ IgM^+ $CD11b^-$) cells.

B Frequency of V gene utilization in unique *Igh VDJ* rearrangements in peritoneal B1 ($B220^+$ $CD19^+$ IgM^+ $CD11b^+$) cells from WT (top) and R972Q (bottom) mice.

C Frequency of V gene utilization in unique *Igh VDJ* rearrangements in peritoneal B2 ($B220^+$ $CD19^+$ IgM^+ $CD11b^-$) cells from WT (top) and R972Q (bottom) mice.

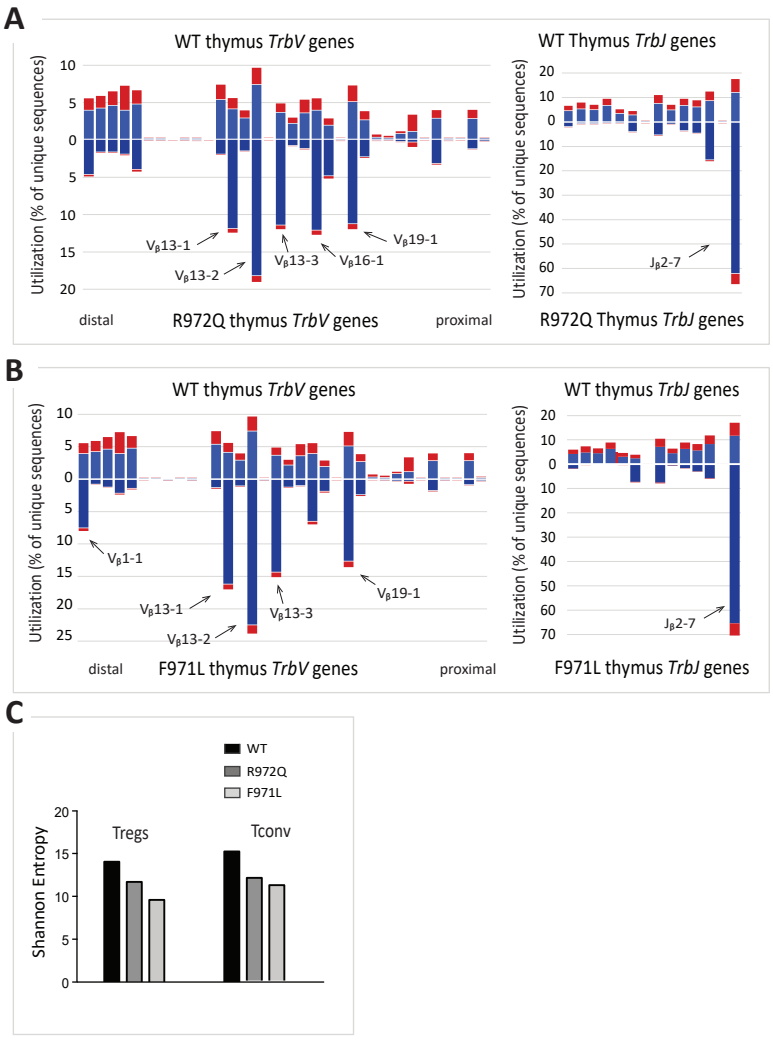


Figure S6

Thymocyte *Trb* repertoire

A Utilization of *V* (left) and *J* (right) genes in *Trb VDJ* rearrangements in unsorted thymocytes from WT (top, n=3) and R972Q (bottom, n=3) mice.

B Utilization of *V* (left) and *J* (right) genes in *Trb VDJ* rearrangements in unsorted thymocytes from WT (top, n=3) and F971L (bottom, n=3) mice.

C Shannon Entropy as a measure of diversity of the *Trb* repertoire of Treg and Tconv cells.

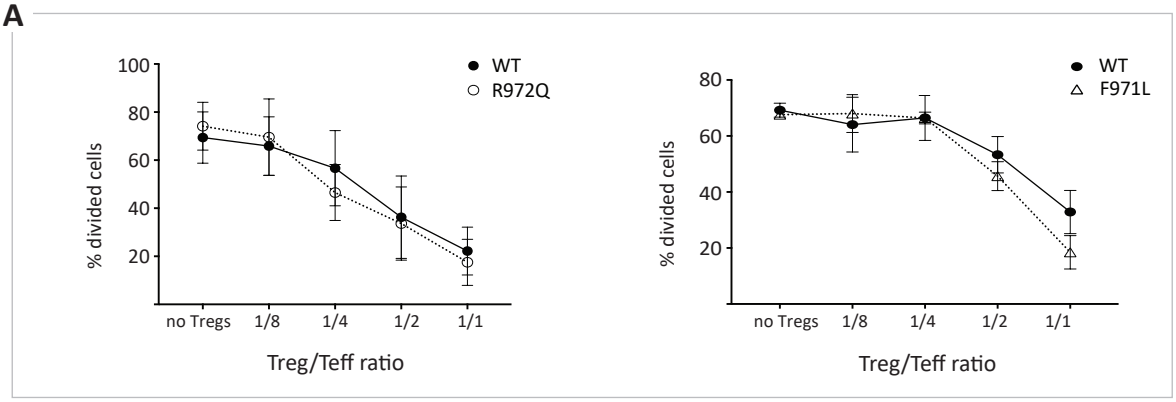


Figure S7

Treg suppressive function

A Treg suppression assay comparing Treg cells of WT mice, R972Q and F971L mice.
Data points represent means and SEM of 2 independent experiments.

Table S1: Genotypes of generated F0 mice (related to figure S1)

	Allele 1	**Allele 2**
1	c. 2911T>C	6bp indel
2 *	c. 2911T>C	c. 2911T>C
3 [1]	c. 2911T>C	c. 2911T>C
4 [2]	c. 2911T>C	c. 2911T>C
5 [3]	c. 2914C>T	c. 2911T>C
6	c. 2911T>C	c. 2911T>C
7	c. 2911T>C	2bp indel
8 *	c. 2915G>A	c. 2915G>A
9 [4]	c. 2915G>A	c. 2914C>T
10	c. 2915G>A	c. 2914C>T
11	c. 2911T>C	6bp indel
12	1bp deletion	c. 2914C>T
13	c. 2914C>T	156bp indel
14	c. 2911T>C	c. 2911T>C
15	c. 2911T>C	c. 2911T>C
16 *	c. 2914C>T	c. 2914C>T
17 *	c. 2915G>A	c. 2908C>A (silent mut)
18	c. 2915G>A	c. 2914C>T
19 *	c. 2911T>C	c. 2914C>T
20 [5]	c. 2911T>C	c. 2915G>A
21	c. 2914C>T	9bp deletion
22	c. 2914C>T	c. 2914C>T
23	c. 2911T>C	c. 2911T>C
24	c. 2911T>C	c. 2911T>C
25	WT	4bp deletion
26	c. 2911T>C	c. 2915G>A

[1] 17bp indel on one allele
[2] 1bp indel on one allele
[3] Additional third sequence with c. 2915G>A due to somatic chimerism
[4] 9 bp indel on one allele
[5] 1bp indel on one allele
* F0 mice that were selected to continue breeding with to generate 2 lines per mutation

Table S2: Repertoire sequencing counts (related to figure 6 and 7 and figure S4 and S5)

Sample	**Total (*VDJ*)**	**Unique (*VDJ*)**	**Unique productive (*VDJ*)**	**HTGTS total VDJ sequences (used for *VDJ/DJ ratio*)**	**HTGTS *DJ* sequences (used for D graph)**
pro-B WT	4439	3149	1729	8178	9448
pro-B R972Q (2)	1543	610	431	1031+1852	1884+4048
Pro-B F971L (2)	482	112	67	286+747	1280+4043
Spleen WT	129721	15617	10674		
Spleen R972Q (2)	87132	10967	10170		
pre-B WT (3)	317993	171193	124139		
pre-B R972Q (3)	187524	56416	52571		
Pre-B F971L (3)	20208	11030	10309		
Thymus WT (3)	1505542	932332	768007		
Thymus R972Q (3)	559557	94890	89866		
Thymus F971L (3)	53583	4870	4152		
DN4 WT	84084	56679	35104		
DN4 R972Q	199	128	95		
DP WT	1459836	1144368	795466		
DP R972Q	515080	74453	70049		
DP F971L (2)	74457	17501	16450		
WT T conv	80705	65569	45410		
WT Treg	44015	33666	22600		
R972Q Tconv	63692	17664	16797		
R972Q Treg	21781	7954	7502		
F971L Tconv	37247	8820	8294		
F971L Treg	10363	2486	2276		

Table S3: Comparison of gene distributions of WT vs. mutant, using Kolmogorov-Smirnov

Gene	Cell	WT vs.	P value
V	pro-B cells	R972Q	1.922E-12
V	pro-B cells	F971L	4.270E-22
V	pre-B cells	R972Q	4.400E-02
V	pre-B cells	F971L	8.360E-07
V	spleen B cells	R972Q	1.980E-03
V	peritoneal B1 cells	R972Q	3.300E-02
V	peritoneal B2 cells	R972Q	2.400E-02
V	DN4 thymocytes	R972Q	3.340E-08
J	DN4 thymocytes	R972Q	1.870E-05
V	DP thymocytes	R972Q	1.584E-05
V	DP thymocytes	F971L	8.800E-07
J	DP thymocytes	R972Q	1.189E-04
J	DP thymocytes	F971L	1.870E-05
V	unsorted thymocytes	R972Q	2.270E-05
V	unsorted thymocytes	F971L	2.270E-05
J	unsorted thymocytes	R972Q	1.189E-04
J	unsorted thymocytes	F971L	1.870E-05
V	CD4+ Tconv	R972Q	9.680E-03
V	CD4+ Tconv	F971L	1.655E-03
J	CD4+ Tconv	R972Q	6.434E-04
J	CD4+ Tconv	F971L	1.189E-04
V	CD4+ Treg	R972Q	9.680E-03
V	CD4+ Treg	F971L	7.290E-05
J	CD4+ Treg	R972Q	6.434E+04
J	CD4+ Treg	F971L	1.189E-04

Table S4: Igh V genes in pro-B and splenic B cells (related to figure 6)

	pro-B unique					spleen unique		
***V* genes**	**WT**	**R972Q**	**P[1]**	**F791L**	**P[1]**	**WT**	**R972Q**	**P[1]**
Ighv1-86	0.03%	0.16%		0.00%		0.01%	0.00%	
Ighv1-85	0.32%	0.66%		0.89%		0.79%	1.38%	****
Ighv1-84	0.25%	0.16%		0.00%		0.34%	0.42%	
Ighv1-83	0.16%	0.00%		0.00%		0.08%	0.01%	*
Ighv1-82	1.87%	1.97%		2.68%		3.28%	2.80%	*
Ighv1-81	2.41%	3.11%		2.68%		2.86%	4.40%	****
Ighv1-80	0.89%	1.64%		4.46%		2.15%	2.22%	
Ighv1-79	0.16%	0.00%		0.00%		0.11%	0.00%	***
Ighv1-78	1.02%	0.66%		0.89%		0.90%	0.78%	
Ighv1-77	0.29%	0.00%		0.00%		0.39%	0.28%	
Ighv8-16	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-76	1.30%	1.15%		0.00%		1.28%	1.31%	
Ighv8-15	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-75	1.62%	1.15%		0.89%		1.25%	1.43%	
Ighv8-14	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-74	0.95%	1.15%		0.89%		1.17%	1.73%	***
Ighv1-73	0.03%	0.00%		0.00%		0.06%	0.00%	
Ighv8-13	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-72	2.06%	4.26%	*	1.79%		2.44%	3.72%	****
Ighv1-71	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-70	0.51%	0.16%		1.79%		0.21%	0.00%	****
Ighv8-12	1.17%	0.49%		0.89%		1.09%	0.80%	*
Ighv1-69	0.83%	0.82%		0.89%		1.96%	0.89%	****
Ighv1-68	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-67	0.98%	0.82%		0.89%		0.63%	0.01%	****
Ighv1-66	0.41%	0.16%		0.00%		0.54%	0.31%	*
Ighv8-11	0.25%	0.33%		0.89%		0.14%	0.01%	***
Ighv1-65	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv8-10	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-64	2.54%	1.80%		2.68%		3.49%	4.17%	*
Ighv1-63	0.57%	0.33%		0.00%		0.47%	0.14%	****
Ighv8-9	0.03%	0.00%		0.00%		0.04%	0.00%	
Ighv1-62-3	0.57%	0.33%		0.00%		0.60%	0.28%	***
Ighv1-62-2	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-62-1	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-62	0.06%	0.00%		0.00%		0.07%	0.01%	*
Ighv1-61	0.19%	0.16%		0.00%		0.65%	0.19%	****
Ighv1-60	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-59	0.60%	0.82%		0.00%		0.79%	0.41%	***
Ighv1-58	0.41%	0.16%		0.00%		0.49%	0.16%	****

[1] P value calculated with Chi square test. * P≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001

Table S4 continued ...

	pro-B unique					spleen unique		
***V* genes**	**WT**	**R972Q**		**F791L**	**p[1]**	**WT**	**R972Q**	**p[1]**
Ighv8-8	1.43%	2.46%		1.79%		1.76%	1.87%	
Ighv1-57	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv8-7	0.51%	0.16%		0.89%		0.22%	0.01%	****
Ighv1-56	0.10%	0.00%		0.00%		0.10%	0.05%	
Ighv1-55	2.92%	1.97%		0.89%		2.88%	2.39%	*
Ighv1-54	0.60%	0.82%		0.89%		0.71%	0.44%	*
Ighv8-6	0.00%	0.00%		0.00%		0.06%	0.00%	*
Ighv1-53	1.68%	1.15%		0.00%		2.43%	2.18%	
Ighv1-52	0.35%	0.49%		0.00%		1.04%	0.56%	****
Ighv1-51	0.00%	0.16%		0.00%		0.00%	0.00%	
Ighv1-50	0.64%	0.66%		0.89%		1.14%	1.09%	
Ighv8-5	0.22%	0.16%		0.00%		0.17%	0.03%	***
Ighv1-49	0.51%	0.33%		0.00%		0.33%	0.04%	****
Ighv1-48	0.00%	0.00%		0.00%		0.01%	0.00%	
Ighv8-4	0.10%	0.00%		0.00%		0.06%	0.00%	*
Ighv8-3	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-47	0.92%	0.33%		0.00%		0.56%	0.16%	****
Ighv1-46	0.03%	0.00%		0.00%		0.00%	0.00%	
Ighv1-45	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-44	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-43	0.00%	0.00%		0.00%		0.04%	0.01%	
Ighv1-42	0.64%	0.33%		0.00%		0.81%	0.62%	
Ighv1-41	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-40	0.06%	0.00%		0.00%		0.02%	0.00%	
Ighv1-39	1.17%	1.64%		1.79%		1.82%	2.09%	
Ighv1-38	0.32%	0.16%		0.00%		0.13%	0.00%	***
Ighv1-37	0.06%	0.00%		0.00%		0.09%	0.00%	*
Ighv1-36	0.44%	0.66%		1.79%		0.47%	0.46%	
Ighv1-35	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-34	0.13%	0.33%		0.00%		0.21%	0.47%	***
Ighv1-33	0.13%	0.16%		0.89%		0.38%	0.16%	*
Ighv1-32	0.06%	0.00%		0.00%		0.05%	0.00%	
Ighv1-31	0.19%	0.16%		0.00%		0.18%	0.20%	
Ighv1-30	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-29	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-28	0.03%	0.00%		0.00%		0.04%	0.00%	
Ighv1-27	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-26	3.49%	4.75%		0.89%		5.48%	6.23%	*
Ighv1-25	0.10%	0.33%		0.00%		0.06%	0.00%	*
Ighv1-24	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-23	0.41%	0.00%		0.00%		0.32%	0.00%	****
Ighv1-22	0.95%	0.82%		2.68%		1.68%	2.59%	****
Ighv1-21	0.00%	0.16%		0.00%		0.02%	0.00%	

Table S4 continued ...

	pro-B unique					spleen unique		
***V* genes**	**WT**	**R972Q**	**p[1]**	**F791L**	**p[1]**	**WT**	**R972Q**	**p[1]**
Ighv1-21-1	0.10%	0.00%		0.00%		0.05%	0.00%	
Ighv1-20	0.19%	0.00%		0.00%		0.15%	0.03%	*
Ighv1-19	2.03%	4.26%	*	3.57%		1.52%	3.15%	****
Ighv1-19-1	0.06%	0.00%		0.00%		0.02%	0.00%	
Ighv1-18	2.41%	2.46%		6.25%		2.71%	3.33%	*
Ighv1-17	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-17-1	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-16	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-15	0.64%	0.66%		0.89%		1.18%	0.81%	*
Ighv1-14	0.03%	0.16%		0.00%		0.08%	0.00%	*
Ighv1-13	0.03%	0.16%		0.00%		0.04%	0.00%	
Ighv1-12	0.51%	0.00%		0.00%		0.81%	0.26%	****
Ighv1-11	1.59%	0.66%		0.89%		0.76%	0.09%	****
Ighv1-10	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-9	1.68%	0.98%		1.79%		2.52%	1.29%	****
Ighv15-2	0.76%	0.33%		0.00%		0.60%	0.16%	****
Ighv1-8	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv10-4	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-7	1.02%	1.48%		0.00%		1.17%	1.69%	***
Ighv1-6	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv10-3	0.95%	1.15%		3.57%		1.12%	1.42%	*
Ighv1-5	1.30%	1.48%		2.68%		1.02%	1.18%	
Ighv10-2	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv1-4	0.48%	0.98%		0.00%		0.58%	0.61%	
Ighv1-3	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv10-1	1.65%	3.28%	*	1.79%		1.08%	1.62%	***
Ighv1-2	1.40%	1.48%		0.89%		0.76%	0.16%	****
Ighv8-2	1.05%	1.31%		1.79%		0.44%	0.01%	****
Ighv6-7	0.03%	0.00%		0.00%		0.03%	0.00%	
Ighv6-6	0.86%	1.31%		1.79%		0.93%	1.81%	****
Ighv6-5	0.06%	0.33%		0.00%		0.07%	0.00%	*
Ighv6-4	0.03%	0.00%		0.00%		0.01%	0.00%	
Ighv6-3	1.27%	1.97%		2.68%		1.20%	3.10%	****
Ighv12-3	0.89%	0.33%		0.89%		0.53%	0.02%	****
Ighv13-2	1.05%	0.49%		1.79%		0.58%	0.25%	****
Ighv8-1	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv3-8	0.83%	2.13%	*	2.68%		0.68%	1.68%	****
Ighv5-21	0.00%	0.00%		0.00%		0.02%	0.00%	
Ighv3-7	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv9-4	0.79%	0.82%		0.00%		0.35%	0.36%	
Ighv3-6	2.38%	4.10%	*	4.46%		2.73%	5.27%	****
Ighv13-1	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv3-5	0.25%	0.33%		0.00%		0.17%	0.17%	

Table S4 continued ...

	pro-B unique					spleen unique		
***V* genes**	**WT**	**R972Q**	**P[1]**	**F791L**	**P[1]**	**WT**	**R972Q**	**P[1]**
Ighv3-4	0.25%	0.16%		0.00%		0.15%	0.05%	*
Ighv7-4	0.10%	0.16%		0.00%		0.10%	0.07%	
Ighv3-3	0.51%	0.00%		0.00%		0.27%	0.00%	****
Ighv14-4	0.83%	0.16%		0.89%		1.22%	0.23%	****
Ighv15-1	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv7-3	0.70%	1.15%		0.00%		0.70%	1.53%	****
Ighv9-3	2.29%	3.93%	*	5.36%		2.64%	5.54%	****
Ighv12-2	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv9-2	0.51%	0.82%		0.00%		0.26%	0.45%	*
Ighv12-1	0.10%	0.00%		0.00%		0.06%	0.01%	*
Ighv9-1	0.38%	0.00%		0.89%		0.53%	0.81%	*
Ighv6-2	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv16-1	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv14-3	0.92%	1.31%		1.79%		0.95%	1.34%	*
Ighv11-2	0.44%	0.16%		0.00%		0.35%	0.03%	****
Ighv3-2	0.54%	1.15%		0.89%		0.57%	0.03%	****
Ighv4-2	0.60%	0.66%		0.00%		0.36%	0.02%	****
Ighv14-2	0.60%	0.33%		0.00%		1.20%	0.67%	****
Ighv11-1	0.38%	0.33%		0.00%		0.13%	0.00%	***
Ighv3-1	0.32%	0.82%		0.00%		0.42%	0.49%	
Ighv4-1	1.33%	0.98%		0.00%		0.62%	0.48%	
Ighv14-1	0.10%	0.00%		0.00%		0.42%	0.13%	****
Ighv7-2	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv7-1	0.98%	1.48%		1.79%		0.60%	0.61%	
Ighv5-19	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv2-9	0.79%	1.31%		0.00%		0.67%	0.33%	***
Ighv2-8	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv5-18	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv5-17	0.76%	0.98%		1.79%		1.56%	1.50%	
Ighv5-16	0.83%	0.98%		3.57%		0.92%	1.40%	***
Ighv5-15	0.32%	0.66%		0.00%		0.24%	0.17%	
Ighv2-7	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv2-6-8	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv5-13	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv2-9-1	0.54%	1.31%	*	0.00%		0.64%	1.11%	****
Ighv5-12-4	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv5-9-1	0.51%	0.16%		0.00%		0.45%	0.28%	*
Ighv2-6	0.76%	0.82%		0.00%		0.44%	0.41%	
Ighv5-12	0.95%	1.48%		0.89%		0.48%	0.22%	***
Ighv5-11	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv2-5	0.86%	0.49%		0.89%		0.56%	0.57%	
Ighv5-10	0.00%	0.00%		0.00%		0.00%	0.00%	
Ighv5-9	0.35%	0.33%		1.79%		0.24%	0.56%	****

Table S5: Trb V gene usage (related to figure 7)

	Spleen Treg					Spleen Tconv					Thymus DN4			Thymus DP				
Trb V	**WT**	**R972Q**		**F971L**		**WT**	**R972Q**		**F971L**		**WT**	**R972Q**		**WT**	**R972Q**		**F791L**	
01-1	5.81%	6.52%	*	14.00%	****	6.92%	6.51%		11.20%	****	4.81%	4.69%		4.90%	4.08%	****	7.91%	****
02-1	6.88%	1.93%	****	0.46%	****	6.90%	2.08%	****	0.66%	****	5.29%	2.34%		5.46%	1.46%	****	0.45%	****
03-1	8.55%	3.66%	****	2.19%	****	7.65%	3.22%	****	1.75%	****	5.66%	0.78%		5.99%	1.22%		0.87%	
04-1	4.83%	1.32%	****	2.11%	****	6.01%	1.42%	****	1.68%	****	6.69%	2.34%		7.19%	1.98%		2.56%	
05-1	7.83%	5.78%	****	2.03%	****	7.29%	5.45%	****	1.48%	****	6.29%	9.38%		6.64%	4.15%		1.40%	
06-1	0.00%	0.00%		0.00%		0.00%	0.00%		0.01%		0.00%	0.78%		0.00%	0.00%		0.01%	
07-1	0.00%	0.00%		0.00%		0.00%	0.00%		0.00%		0.00%	0.00%		0.00%	0.00%	****	0.00%	***
08-1	0.00%	0.00%		0.04%		0.00%	0.00%		0.00%		0.00%	0.00%		0.00%	0.00%		0.00%	
09-1	0.01%	0.00%		0.00%		0.00%	0.00%		0.00%		0.00%	0.00%	*	0.00%	0.00%	****	0.00%	****
10-1	0.06%	0.03%		0.00%		0.08%	0.00%		0.00%		0.09%	0.00%		0.06%	0.00%	****	0.00%	****
11-1	0.01%	0.00%		0.00%		0.00%	0.00%		0.01%		0.00%	0.78%		0.00%	0.00%	****	0.01%	****
12-1	4.69%	3.08%	****	2.40%	****	2.67%	0.82%	****	0.53%	****	8.93%	1.56%		7.92%	2.05%	****	1.29%	****
13-1	5.08%	8.01%	****	9.98%	****	5.85%	9.13%	****	13.71%	****	5.37%	9.38%		5.42%	11.68%		16.60%	
12-2	3.07%	2.23%	****	1.16%	****	1.72%	0.98%	****	0.52%	****	4.88%	1.56%	***	4.22%	1.51%	****	0.89%	****
13-2	11.22%	17.03%	****	23.15%	****	11.98%	18.33%	****	24.51%	****	8.90%	13.28%		8.85%	19.44%	****	26.25%	****
12-3	0.01%	0.00%		0.04%		0.00%	0.00%		0.01%		0.00%	0.00%		0.00%	0.00%	****	0.00%	****
13-3	5.61%	11.34%	****	12.71%	****	6.13%	12.73%	****	15.55%	****	4.64%	10.94%	***	4.38%	12.30%	****	13.85%	****
14-1	1.57%	0.20%	****	0.37%	****	1.91%	0.40%	****	0.42%	****	2.74%	3.13%		3.01%	0.72%	****	1.00%	****
15-1	4.66%	1.39%	****	1.28%	****	3.94%	1.30%	****	0.94%	****	5.38%	7.03%		5.40%	1.24%		1.00%	
16-1	5.43%	13.61%	****	9.23%	****	5.07%	12.46%	****	6.67%	****	5.10%	11.72%		5.15%	12.74%	****	6.03%	****
17-1	1.54%	1.92%	*	0.33%	****	1.61%	1.81%	****	0.53%	****	2.63%	5.47%	*	2.63%	5.25%	****	1.86%	****
18-1	0.00%	0.01%		0.04%		0.00%	0.00%		0.01%		0.00%	0.78%		0.00%	0.00%	****	0.01%	****
19-1	7.70%	14.95%	****	14.04%	****	8.62%	16.42%	****	14.79%	****	6.60%	7.03%		6.85%	12.37%	****	12.62%	****
20-1	4.14%	2.57%	****	2.36%	****	4.26%	2.60%	****	2.70%	****	3.61%	0.00%		3.62%	2.53%	****	2.57%	****
21-1	0.37%	0.01%	****	0.00%	*	0.42%	0.00%	*	0.02%	*	0.60%	0.00%		0.53%	0.00%	****	0.00%	****
22-1	0.34%	0.00%	****	0.00%	*	0.24%	0.00%	*	0.00%	*	0.29%	0.00%		0.26%	0.00%		0.00%	
23-1	0.93%	0.28%	****	0.00%	****	1.01%	0.22%	****	0.11%	****	0.94%	0.00%		0.93%	0.18%	****	0.06%	****
24-1	3.12%	0.08%	****	0.00%	****	2.85%	0.11%	****	0.02%	****	3.71%	1.56%		3.03%	0.20%		0.08%	
25-1	0.00%	0.00%		0.04%		0.00%	0.00%		0.00%		0.00%	0.78%		0.00%	0.00%		0.01%	
26-1	3.95%	3.42%	*	1.74%	****	3.74%	3.38%	****	1.72%	****	3.66%	0.78%		3.77%	3.42%	****	1.73%	****
27-1	0.01%	0.08%		0.21%		0.00%	0.02%		0.04%		0.00%	1.56%		0.00%	0.01%	****	0.10%	***
28-1	0.00%	0.00%		0.00%		0.00%	0.00%		0.00%		0.00%	0.00%		0.00%	0.00%		0.00%	
29-1	2.23%	0.42%	****	0.04%	****	2.98%	0.45%	****	0.26%	****	3.00%	2.34%		3.53%	1.33%		0.74%	
30-1	0.32%	0.13%	*	0.04%	*	0.15%	0.16%	*	0.14%	*	0.19%	0.00%		0.24%	0.13%		0.10%	

Table S6: Trb J gene usage[1](related to figure 7)

	DN4			DP				Spleen Treg				Spleen TConv			
Trb J	**WT**	**R972Q**		**WT**	**R972Q**		**F971L**		**WT**	**R972Q**		**F971L**		**WT**	**R972Q**		**F791L**	
1-01	10.55%	3.91%	*	10.36%	1.56%	****	2.03%	****	8.46%	0.68%	****	1.11%	****	4.27%	0.67%	****	0.89%	****
1-02	6.80%	0.78%	*	7.01%	0.17%	****	0.31%	****	5.59%	0.05%	****	0.05%	****	7.12%	0.09%	****	0.09%	****
1-03	6.08%	1.56%	*	6.22%	0.30%	****	0.23%	****	6.01%	0.16%	****	0.05%	****	6.91%	0.20%	****	0.12%	****
1-04	8.22%	0.00%	*	8.56%	0.10%	****	0.18%	****	7.12%	0.11%	****	0.18%	****	8.49%	0.07%	****	0.14%	****
1-05	3.82%	0.00%	*	4.07%	0.12%	****	0.22%	****	3.14%	0.05%	****	0.09%	****	4.00%	0.07%	****	0.10%	****
1-06	4.17%	4.69%		4.29%	3.28%	****	6.62%	****	2.97%	5.42%	****	11.33%	****	2.87%	4.30%	****	8.56%	
1-07	0.04%	0.00%		0.04%	0.01%	****	0.01%	*	0.02%	0.00%		0.00%		0.02%	0.00%		0.01%	
2-01	9.71%	2.34%	*	9.95%	4.75%	****	7.29%	****	9.96%	4.01%	****	5.73%	****	10.01%	4.34%	****	6.05%	****
2-02	5.86%	4.69%		6.14%	0.37%	****	0.68%	****	6.40%	0.48%	****	0.82%	****	6.77%	0.49%	*	0.93%	****
2-03	8.88%	3.13%	*	9.14%	2.97%	****	1.48%	****	8.07%	1.85%	****	1.63%	****	8.98%	2.33%	****	1.55%	****
2-04	7.97%	4.69%		8.10%	3.86%	****	2.70%	****	9.35%	4.88%	****	3.77%	****	8.15%	4.36%	****	3.12%	****
2-05	10.67%	15.63%		11.35%	14.54%	****	6.52%	****	12.48%	15.80%	****	7.12%	****	12.50%	15.53%	****	7.34%	****
2-06	0.03%	0.00%		0.03%	0.01%	***	0.02%		0.02%	0.00%		0.00%		0.02%	0.00%		0.02%	
2-07	17.07%	58.59%	****	14.66%	67.58%	****	71.70%	****	20.40%	66.49%	****	68.13%	****	19.90%	67.55%	****	71.08%	****

[1] P value calculated with Chi square test. * P≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001

Table S7: Igh V gene Coding end comparison in pro-B cells (related to discussion)

R972Q >WT	**Coding end**		**F971L >WT**	**Coding end**
Ighv1-19	ga		*Ighv1-19*	ga
Ighv1-72	ga		*Ighv1-18*	ga
Ighv3-6	ga		*Ighv3-6*	ga
Ighv9-3	ga		*Ighv9-3*	ga
Ighv10-1	ca		*Ighv5-16*	ga
Ighv3-8	ta		*Ighv3-8*	ta
Ighv1-26	ga		*Ighv10-3*	ga
Ighv8-8	ag		*Ighv1-22*	ga
Ighv2-9-1	aa		*Ighv5-9*	ca
Ighv1-80	ga		*Ighv1-80*	ga
WT > R972Q	**Coding end**		**WT > F971L**	**Coding end**
Ighv14-4	ca		*Ighv1-7*	ga
Ighv8-12	ag		*Ighv2-3*	cc
Ighv1-9	ga		*Ighv1-76*	ga
Ighv1-64	ga		*Ighv4-1*	ca
Ighv1-11	gg		*Ighv1-53*	ga
Ighv1-55	ga		*Ighv5-6*	ca
Ighv2-3	cc		*Ighv1-55*	ga
Ighv5-4	ga		*Ighv5-4*	ga
Ighv2-2	aa		*Ighv1-26*	ga
Ighv5-2	ca		*Ighv5-2*	ca
WT = R972Q	**Coding end**		**WT = F971L**	**Coding end**
Ighv1-18	ga		*Ighv1-69*	ga
Ighv1-82	ga		*Ighv14-4*	ca
Ighv5-6	ca		*Ighv2-5*	aa
Ighv1-2	ga		*Ighv12-3*	ga
Ighv1-76	ga		*Ighv1-74*	ta
Ighv1-22	ga		*Ighv5-12*	ca
Ighv1-69	ga		*Ighv1-67*	ga
Ighv9-4	ga		*Ighv1-78*	ga
Ighv2-6	ga		*Ighv1-72*	ga
Ighv1-50	ga		*Ighv8-12*	ag

Table S8: Trb V gene coding end comparison in DP cells (related to discussion)

R972Q >WT	**Coding end**		**F971L >WT**	**Coding End**
TCRBV13-02	tg		*TCRBV13-02*	tg
TCRBV13-03	tg		*TCRBV13-01*	tg
TCRBV16-01	ga		*TCRBV13-03*	tg
TCRBV13-01	tg		*TCRBV19-01*	ag
TCRBV19-01	ag		*TCRBV01-01*	ga
TCRBV17-01	ga		*TCRBV16-01*	ga
WT> R972Q	**Coding end**		**WT> F971L**	**Coding end**
TCRBV12-02	tc		*TCRBV14-01*	tc
TCRBV24-01	ta		*TCRBV26-01*	tc
TCRBV05-01	ga		*TCRBV29-01*	tc
TCRBV02-01	ga		*TCRBV24-01*	ta
TCRBV15-01	gc		*TCRBV12-02*	tc
TCRBV03-01	gc		*TCRBV15-01*	gc
TCRBV04-01	ga		*TCRBV04-01*	ga
TCRBV12-01	tc		*TCRBV02-01*	ga
WT= R972Q	**Coding end**		**WT= F971L**	**Coding end**
TCRBV30-01	ga		*TCRBV30-01*	ga
TCRBV22-01	tc		*TCRBV22-01*	tc
TCRBV26-01	tc		*TCRBV21-01*	tc
TCRBV21-01	tc		*TCRBV17-01*	ga
TCRBV23-01	tc		*TCRBV23-01*	tc
TCRBV01-01	ga		*TCRBV20-01*	tg

Supplementary Methods

Mice

A guide RNA (gRNA) was designed to target nucleotides 2919 and 2920 of the mouse *Rag1* gene (CCDS16463.1), in a region within the C-terminus domain (CTD).

To introduce the desired mutations into the mouse *Rag1* locus, three single-stranded 160 bp DNA oligonucleotide (ssODN) donor templates (ordered from Integrated DNA Technologies, Coralville, IA) were used, that were highly homologous to the area flanking the cutting site, and containing the following protospacer regions, each containing the desired point mutation (underlined):

ACAAGCTGTTTAGAAGGTTTCAG (R972Q, or c.2915G>A)

ACAAGCTGTTTAGAAGGCTTCGG (F971L, or c. 2911T>C),)

ACAAGCTGTTTAGAAGGTTTTGG (R972W, or c.2914C>T).

In addition to the desired mutation, all ssODNs contained a silent mutation at c. 2908 C>A to prevent additional cutting after the sequence of the ssODN has been incorporated into the targeted mouse genome.

100ng/ul of Cas9 mRNA (purchased from System Biosciences, Palo Alto, CA), 50ng/ul of gRNA (generated from PCR product using Megashortscript T7 kit, Life Technologies, Washington D.C.), and 150 ng/µl of a mixture with equal amounts of the three ssODNs were injected in 200 zygotes of C57BL/6 mice. Embryos were placed back into six recipient C57BL/6 mice, as reported previously[1]. F0 genotypes were analyzed using Sanger sequencing. F0 mice were bred with wt C57BL/6 mice to get heterozygous F1 mice, which were bred to obtain homozygous F2 mice. All mouse experiments were done with F2 or later generations. *Rag*1$^{-/-}$ control mice and the *Foxp3*EGFPCre mice[2] were purchased from the Jackson Laboratory (Bar Harbor, ME). *Foxp3*EGFPCre mice were crossed with homozygous F2 mutants. Wild-type control mice were derived from wild-type F2 littermates. All mice described in this manuscript were on a C57BL/6 background. Offspring of different founders were compared to rule out any effects of offsite targeting. Animal work was conducted adhering to the institution's guidelines for animal use, and followed the guidelines and basic principles in the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the Guide for the Care and Use of Laboratory Animals by certified staff in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited facility.

Cell isolation

Thymic DP ($CD4^+$ $CD8^+$) and DN4 ($CD4^-$ $CD8^-$ $CD44^-$ $CD25^-$) cells, splenic Treg cells ($CD4^+$ $EGFP^+$) and Tconv cells ($CD4^+$ $EGFP^-$), bone marrow pro-B ($B220^{low}$ IgM^- $B220^{lo}IgM^-CD43^+$) and pre-B ($B220^{low}$

IgMB220^{lo}IgM^{-}CD43^{-}) and peritoneal B1 (CD19^{+}CD11b^{+}), B2 (CD19^{+}CD11b^{-}) and B1a (CD19^{+}CD11b^{+}CD5^{+}) and B1b (CD19^{+}CD11b^{+}CD5^{-}) cells were isolated on FACSAria II (BD). Mature splenic IgM^{+} B cells were purified using Miltenyi MACS bead purification.

Flow cytometry antibodies

Lymphocyte subsets were analyzed by flow cytometry using the BD LSRFortessa™ (BD Biosciences, San Jose, CA) with DIVA software, and Flowjo™. The following antibodies were used: CD3-PerCP-Cy 5.5 Clone 145-2c11 (BD Biosciences), CD4-PB clone RM 4-5 (eBioscience, San Diego, CA), CD8-APC-Cy7 Clone 53-6.7 (Biolegend, San Diego, CA), CD44-PE-Cy7 clone IM7 (Biolegend), CD25-Percp-cy5.5 and PE Clone PC61 (BD), CD44-FITC clone IM7 (BD Biosciences), CD62L-PE clone MEL-14 (Biolegend), B220-PB Clone RA3-6B2 (eBioscience), CD117-PE-Cy7 clone 2B8 (Biolegend), CD19-PB clone eBio1D3 (eBioscience), IgM-Percp-Cy5.5 and BV605 (eBioscience), CD43- PE and FITC clone eBioR2/60 (eBioscience), CD93-PE-Cy7 clone AA4.1 (eBioscience), CD21-FITC clone 7G6 (BD), CD23-PE clone B3B5 (eBioscience), lambda-PE clone RML-42 (Biolegend), γδ TCR -FITC clone GL3 (Biolegend), β TCR-PE-Cy7 clone H57-597 (eBioscience). For thymus staining, a lineage negative stain was used to exclude cells that were not thymocytes: B220-APC clone RA3-6B2 (Biolegend), CD11b-APC clone M1/70 (Biolegend), Ter119-APC clone TER119 (Biolegend). Annexin V eBioscience kit was used with Annexin V-APC and PI. For staining of peritoneal B cells, the following antibodies were used: B220 APC-Cy7 clone C363-16A (Biolegend), CD5-APC clone 53-7.3 (Biolegend), CD3e Alexa Fluor 700 clone 500A2 (BD Pharmingen), CD11b PE clone M1/70 (BD Pharmingen), CD19 PerCP-Cy5.5 clone eBio1D3 (eBioscience). For staining of age-associated B cells (ABCs), the following antibodies were used: CD19 FITC (clone eBio1D3, eBioscience), CD11c (clone HL3, BD PharMingen), 7-AAD as a vitality dye, IgG1κ Isotype control eFluor 660 (clone: P3.6.2.8.1, eBioscience) and T-bet eFluor 660 (clone 4B10, eBioscience). Protocol B (One-step protocol: intracellular (nuclear) proteins) of Thermofischer scientific was followed.

Immunoglobulin levels in naïve mice

Total IgG, IgM, and IgE immunoglobulin levels in serum of naïve mice were measured using ELISA as previously described[3]. Briefly, plates were coated overnight using the following antibodies: anti-mouse IgM (Southern Biotech, Birmingham, AL), anti-mouse IgG (Southern Biotech, Birmingham, AL), anti-mouse IgE (BD). Plates were blocked with 1-3% BSA for 2h and serum dilutions were incubated overnight. For IgG detection, an IgG specific antibody conjugated to HRP was used (BD Biosciences). Rag2-deficient serum was used as a negative blank control. For IgM, an alkaline phosphatase-conjugated antibody was used (BD). For IgE determination, a biotinylated anti-IgE (BD Biosciences) primary antibody was used, followed by streptavidin-HRP. OD was measured at 405 nm

and 450 nm for AP- and HRP-mediated reactions, respectively, using an ELISA reader (BioTek Instruments, Winooski, VT). A standard curve was included and exact concentrations could be determined.

Immunizations with TNP-Ficoll, TNP-KLH and pneumococcal WCA

To elicit a T-independent antibody response, 8-12 week old mutant mice and wild type mice were injected with 50 μg TNP(23)Ficoll (Biosearch Technologies, Petaluma, CA). TNP-specific IgM and IgG3 serum levels were determined by ELISA prior to immunization (d0) and 14 days post immunization (d14). IgM and IgG3 levels were measured by ELISA using goat anti-mouse antibody conjugated to AP specific for IgM and IgG3 followed by reaction with *p*-Nitrophenyl Phosphate, Disodium Salt (PNPP) and stopped by 3 N NaOH, and reading of OD at 405 nm. To elicit a T-dependent antibody response, 8-12 week old mice received a primary immunization with 100 μg TNP(18)KLH (Biosearch Technologies) mixed with aluminum adjuvant (volume ratio 2:1) and a booster immunization at d14 with 50 μg TNP(18)KLH with aluminum adjuvant. TNP-specific high affinity IgG was determined prior to immunization, at d14 (prior to booster) and at day 21 (d21) by coating the ELISA plate with TNP(5)BSA (Biosearch Technologies). To measure IgG levels, HRP-conjugated goat anti-mouse IgG (Sigma Aldrich) was used, followed by reaction with TMB that was stopped by sulfuric acid, and OD was read at 450 nm. For both TNP-Ficoll and TNP-KLH ELISAs five-fold serum dilutions were used: 1:100, 1:500, 1:2500, 1: 12500, 1:62500, 1:312000, 1:1562500.

To elicit a Th17 response, 8-12 week old mutant and wild type mice were immunized with 100 μg Pneumococcal whole cell antigen (WCA, a gift of Dr. Richard Malley, Boston Children's Hospital) in the presence of aluminum adjuvant at d0, followed by a booster immunization at d14 with 50 μg WCA without aluminum adjuvant. Antigen specific IgG was determined by coating the 96-well plate with WCA and determining specific IgG at d0, d14 and d21 with HRP-conjugated goat anti-mouse IgG (Sigma Aldrich, Saint Louis, MO), followed by reaction with TMB that was stopped by sulfuric acid, and reading of OD at 450 nm. Serum dilutions at 1:150, 1:450 and 1:1350 were used[4].

Autoantibody microarray

Protein arrays were used to screen for a broad panel of IgM autoantibodies (University of Texas Southwestern Medical Center, Genomic and Microarray Core Facility) as previously described[5]. Serum from naïve 8-12 week old mice was used. Values were normalized using positive IgM controls for each sample, generating a normalization factor. Each signal was multiplied by this factor. Values from negative control samples (wild-type mice) for each antigen were averaged and ratios were calculated between each sample and the average of negative controls plus 2SD. A heat map of these

ratios was generated using Multi experiment viewer software (MeV, DFCI Boston, MA). Significant differences were assessed using the significance analysis of microarray (SAM, Stanford University Labs) with a false discovery rate <1.

Anti-cytokine autoantibody microarray

RayBio® mouse protein array G2 (Cat # PAM-G2, RayBiotech) was used to screen for cytokine autoantibodies (IgG) in mouse sera diluted 1:200. Protein array screening was performed in the recommended buffers according to the manufacturer's protocol. Arrays were scanned using a micro-array scanner (LuxScan HT24, BioCapital). GenePix pro microarray software (v5.1) was used for alignment and data acquisition. Results were quantile normalized to account for interplate variability[6].

Next generation sequencing of *Trb*

T cell receptor β (*Trb*) high throughput sequencing was performed by Adaptive Biotechnologies (Seattle, WA). The DNA was extracted from FACS-sorted lymphocyte populations. Multiplex Polymerase chain reaction (PCR), containing a mixture of all *V* and *J* gene primers, was used to amplify the rearranged CDR3β[7]. PCR products were sequenced using the Illumina HiSeq platform (Illumina, San Diego, CA). The sequences were aligned to a reference genome, and *Trb VDJ* gene definitions were based on the international ImMunoGeneTics (IMGT) system[8]. A complete, synthetic repertoire of TCRs was previously used to establish an amplification baseline and to correct for any amplification bias. Data deposited at: https://doi.org/10.21417/B7MP7Q, with username: OttdeBruin-review@adaptivebiotech.com and password: ottdebruin2017review.

Next generation sequencing of *Igh* rearrangements

The *Igh* locus of pre-B cells ($B220^{lo}IgM^{-}CD43^{-}$) and peritoneal B1 cells ($CD19^{+}CD11b^{+}$), peritoneal B2 ($CD19^{+}CD11b^{-}$) cells, peritoneal B1a ($CD19^{+}CD11b^{+}CD5^{+}$) and peritoneal B1b ($CD19^{+}CD11b^{+}CD5^{-}$) cells was sequenced by Adaptive Biotechnologies using a similar technique as for the *Trb* sequencing. However, with the technology of Adaptive Biotechnologies partial rearrangements (*DJ*) would not be picked up. Therefore, we sought a different in depth technology that would be able to show these partial products. This is particularly important at the pro-B cell stage where *D*-to-*J* and *V*-to-*DJ* rearrangements take place. The *Igh* locus of pro-B cells was therefore sequenced using high-throughput genome-wide translocation sequencing-adapted repertoire sequencing (HTGTS-Rep-seq) as described[9,10]. With this translocation-based sequencing technique, different *J* bait primers are used, so any sequence containing a *J* sequence can be analyzed, including *DJ* rearrangements that do

not contain a *V* gene sequence. Adaptive data deposited at: https://doi.org/10.21417/B7MP7Q, with username: OttdeBruin-review@adaptivebiotech.com and password: ottdebruin2017review. HTGTS-Rep-seq data deposited at: http://www.ncbi.nlm.nih.gov/bioproject/421212

PCR amplification of *Vκ-Jκ* rearrangements

Vκ-to-*Jκ* rearrangement PCRs were performed as previously described[11] in pre-B cells ($B220^{lo}IgM^{-}CD43^{-}$) of wild-type and R972Q mutant mice. Primers flanking exon 6 of the *Dlg5* gene were used as a loading control under the same conditions. PCR products were gel electrophoresed and transferred to determine *VJ* recombination by Southern blotting.

Treg suppression assay

$CD4^{+}$ T cells were isolated with a CD4 negative isolation kit (Miltenyi). The FACSAria II was used to separate Treg cells ($CD4^{+}EGFP^{+}$) and use these as suppressor cells. The remaining $CD4^{+}EGFP^{-}$ cells were labeled with CellTrace Violet Cell Proliferation dye according to the manufacturer's instructions (Life Technologies) and used as responder cells. *Rag1*$^{-/-}$ spleen cells were used as feeder cells as previously described[12] with a 1:4 ratio of responder to feeder cells. Different ratios of suppressor to responder cells were used in serial dilutions (1:1, 1:2, 1:4, 1:8 and no suppressors). 15,000-45,000 responder cells/well were used. Cells were stimulated for 4-5 d with 2 µg/ml of soluble anti-CD3 and cultured in 96-wells, in round-bottomed plates in triplicate. The percentage of divided cells was determined by flow cytometry and the average of the three values was plotted.

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