**Immunity, Volume *28***

# Supplemental Data

# Rearrangement of Mouse Immunoglobulin Kappa Deleting Element Recombining Sequence Promotes Immune Tolerance and Lambda B Cell Production

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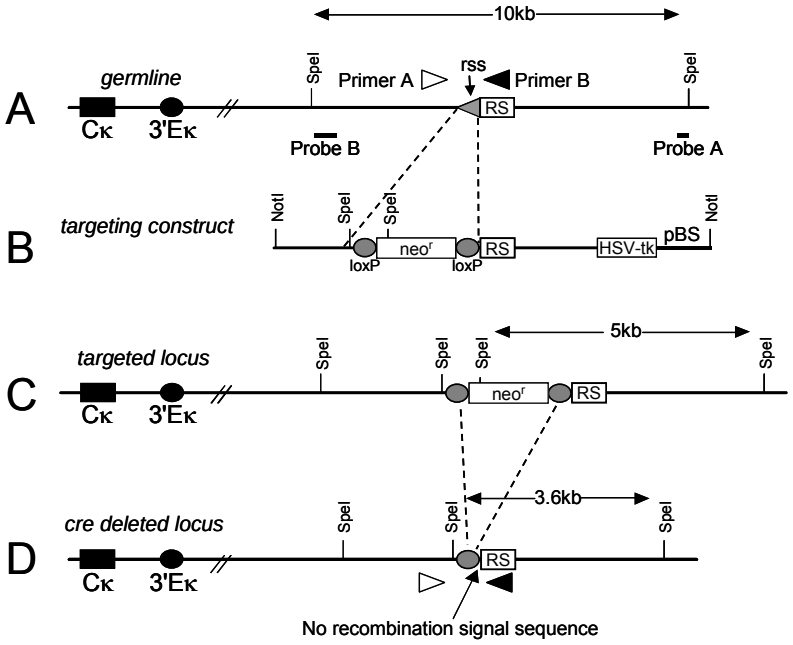


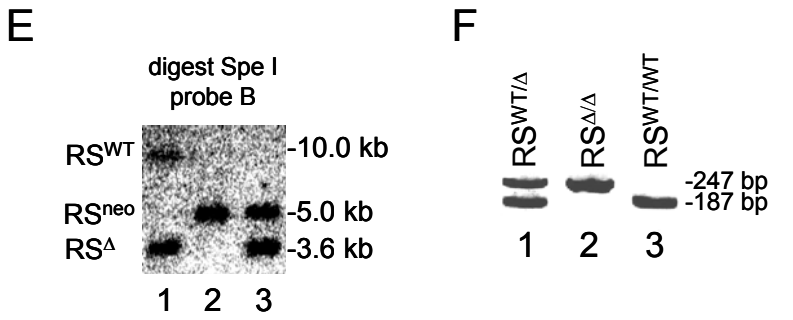


G

GGGACCAACCTGTGAGGGGTACACACTAGTGGATCCCCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATACCGT
CGACCCCCCCGGGTCTAGCCGCGGTCTAGGAATTCTCTAGGATCGATTCTAGGG**ATCCATAACTTCGTATAATGTAT**
**GCTATACGAAGTTAT**CTCGAGCTAGAGGTACCCTAGAAAGCTTCCTAGAAGATCTCCTAGAGTTCGATTCTCAGATT
TGAGCCCTAATGTAGC

**Figure S1. Targeted Mutation of RS Recombination Signal**

(A) Germline *Igk* locus showing relative positions of RS, its recombination signal sequence (rss) and other features of the *Igk* locus, including positions of oligonucleotide primers for PCR detection of modified and germline RS locus (primers A, B), Southern blotting probes A and B, and SpeI restriction sites.

(B) Schematic of targeting construct, including loxP sites, neomycin resistance gene ($neo^r$), herpes simplex virus thymidine kinase gene (*HSV-tk*) and residual bacterial plasmid backbone (pBS).

(C and D) Predicted structure of targeted RS locus before and after cre-mediated deletion of $neo^r$ insertion.

(E) Southern blot analysis of SpeI-digested mouse tail DNA. Lanes 1, 3: (RS targeted x *ZP3-cre Tg*)F2. Lane 1: mouse received wild type allele from its father. Lane 2: homozygous targeted (but not neo deleted) $RS^{neo/neo}$ mouse. Lane 3: mouse received targeted alleles from both parents, but only the maternal allele underwent cre deletion ($RS^{\Delta}$).

(F) PCR analysis of tail DNA using primers A and B, which are indicated in part A.

(G) RS mutated sequence after cre/loxP recombination ($RS^{\Delta}$), as determined by cloning and sequencing PCR product generated as in F (lane 2). Primer sites used are underlined, loxP site is in bold.

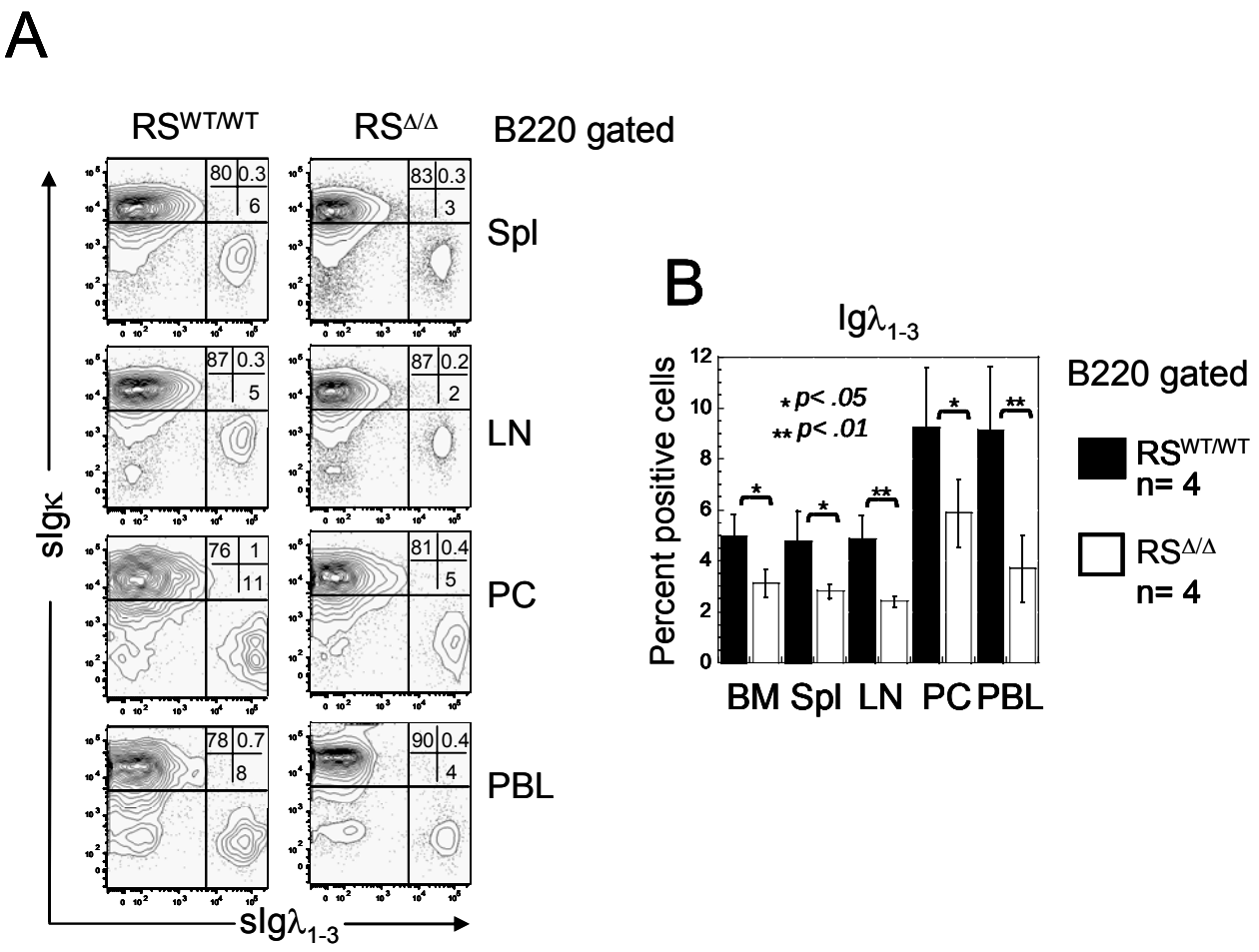


**Figure S2. Reduced Frequency of $\lambda_{1-3}^+$ Cells in Various Lymphoid Tissues of $RS^{\Delta/\Delta}$ Mice Compared to $RS^{WT/WT}$ Controls**

(A) Example of flow cytometry analysis of lymphocyte cell preparations from spleen (Spl), lymph nodes (LN), peritoneal cavity (PC) and blood (PBL).

(B) Statistical analysis of data obtained from 4 mice/group. Shown are means with error bars indicating standard deviations from the mean. Methods were as indicated in Experimental Procedures.

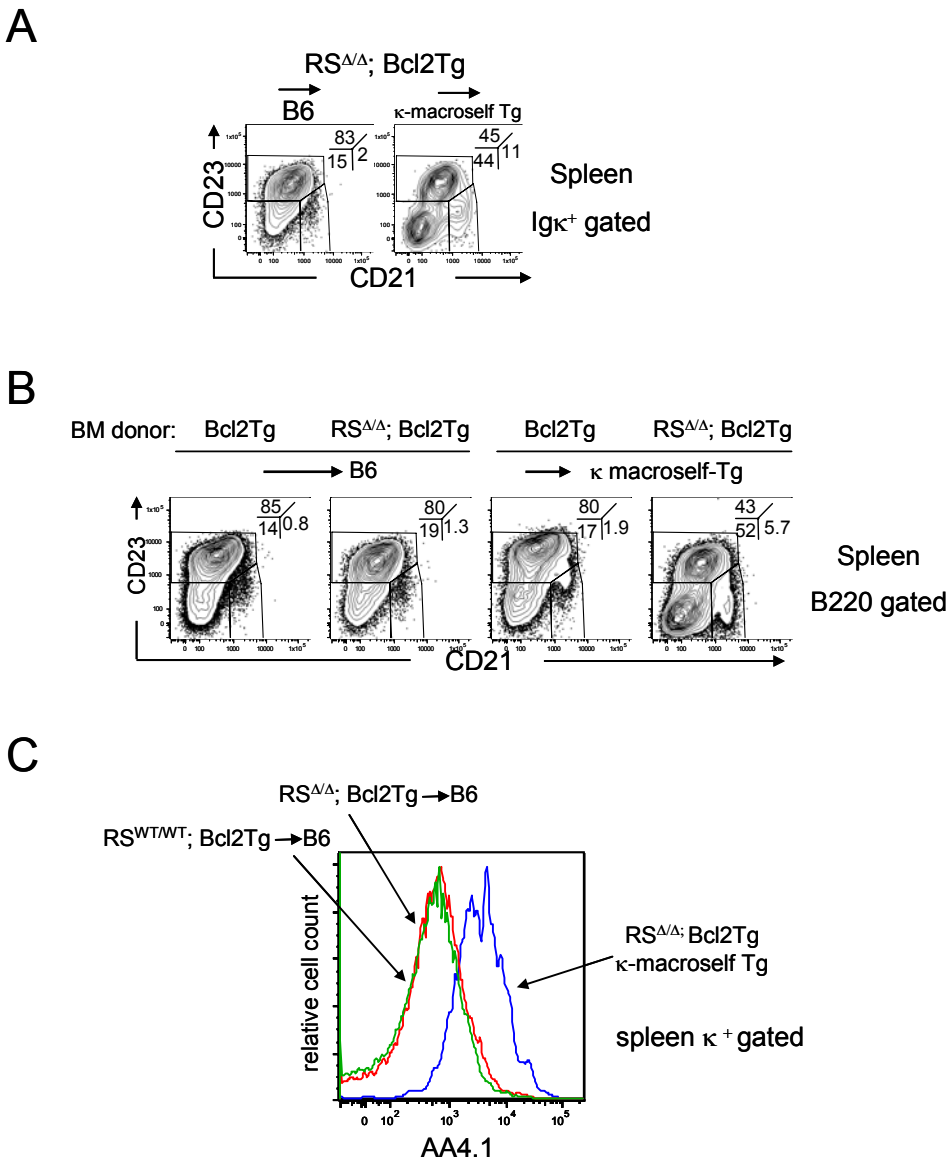


**Figure S3. Analysis of the Effect of RS Mutation on B Cell Tolerance and Cell Surface Phenotype in Apoptosis-Resistant *Bcl2* Tg B Cells**

BM chimeras were generated using donor BM from RS-sufficient or RS-mutant mice that also carried a *Bcl2* transgene enforcing B lineage restricted expression. Irradiated recipient mice were either C57BL6/J.CD45.1 (B6) or littermates that carried a ubiquitously expressed *κ-macroself* transgene (Aït-Azzouzene et al., 2005) to promote negative selection of $\kappa^+$ cells. Radiation chimeras were analyzed at 10 weeks post reconstitution.

(A) CD21,CD23 cell surface phenotype of Igκ+ gated splenic cells in either $RS^{\Delta/\Delta}$; *Bcl2* Tg→B6 or $RS^{\Delta/\Delta}$; *Bcl2* Tg→*κ-macroself* Tg chimeras.

(B) Analysis of CD21,CD23 phenotype of B220 gated splenic cells from the indicated chimeras.

(C) Cell surface expression of AA4.1 marker (CD93) on Igκ+ gated B cells of the indicated chimeric mice. Note: $RS^{WT/WT}$; *Bcl2* Tg→*κ-macroself* Tg group was excluded because of a lack of κ+ cells in the spleens of these mice.

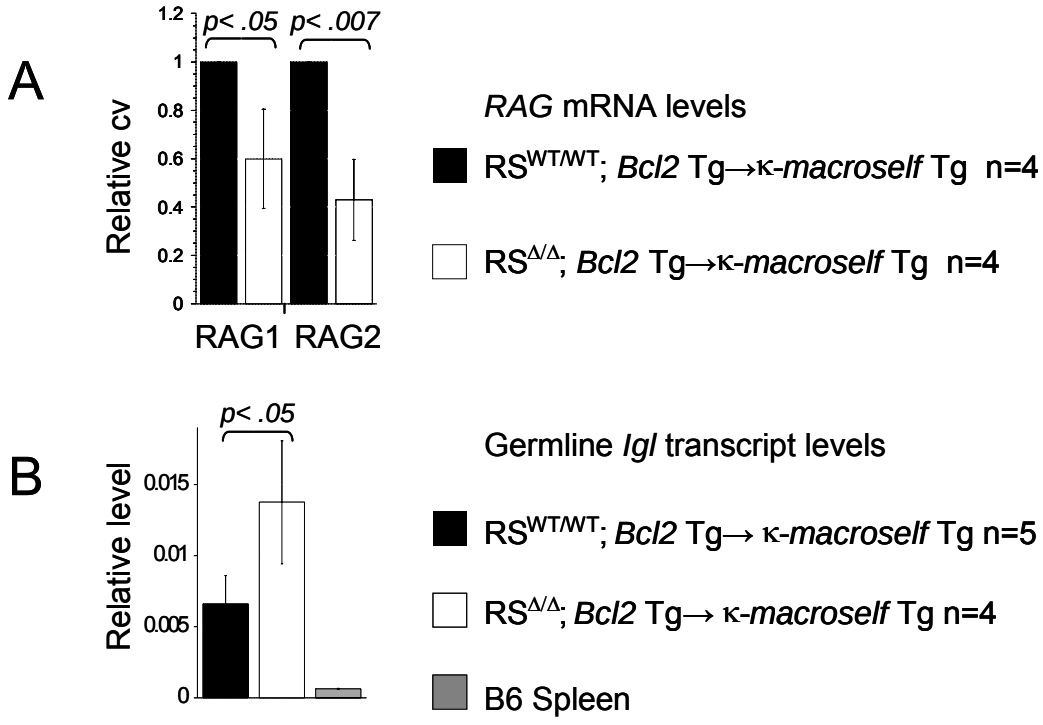


**Figure S4. RNA Analysis of RAG Expression and *Igl* Locus Accessibility in RS$^{\Delta/\Delta}$; *Bcl2* Tg→ *κ-macroself Tg* Chimeras**

RNA was isolated from bone marrow B lineage cells (B220+IgD-) and specific messages quantitated.

(A) RAG mRNA levels measured using real time PCR. Four independent experiments were each normalized to control β–actin levels and combined results depicted in bar graphs. Wild type RAG levels were arbitrarily set to 1 for this comparison and statistical analysis used paired T test.

(B) Expression of the $\lambda_1$ J-C germline transcript. cDNAs were analyzed by semi-quantitative RT-PCR normalized to control β-actin. Graph depicts mean ± SD and p value determined using 2-tailed Student's T-test. Spleen cell cDNA served as negative control.

**Table S1. Absolute Numbers of B Cell Subsets in $RS^{\Delta/\Delta}$ and $RS^{WT/WT}$ Mouse Tissues (Millions)**

| Genotype | tissue | total cells | B220+ | $\lambda_{1-3}$+ | κ+ | κ+$\lambda_{1-3}$+ |
|---|---|---|---|---|---|---|
| $RS^{WT/WT}$ n=18 | bone marrow | 37.8 ± 7.17 | 7.23 ± 1.44 | **0.31 ± 0.08** | 3.07 ± 1.37 | 0.026 ± 0.007 |
| $RS^{\Delta/\Delta}$ n=18 | | 33.1 ± 6.76 | 5.84 ± 1.34 | **0.17 ± 0.06** | 2.33 ± 0.47 | 0.023 ± 0.010 |
| | | | | p= 0.000015 | | |
| $RS^{WT/WT}$ n=18 | spleen | 51.4 ± 12.3 | 23.1 ± 7.02 | **1.38 ± 0.42** | 20.50 ± 6.37 | 0.152 ± 0.095 |
| $RS^{\Delta/\Delta}$ n=18 | | 49.1 ± 16.4 | 20.6 ± 9.40 | **0.83 ± 0.33** | 18.51 ± 8.66 | 0.095 ± 0.069 |
| | | | | p= 0.000097 | | |

Mice were 10-12 weeks of age at time of analysis. Shown are mean values ±SD. p values based on an unpaired 2-tailed Student's T test show significance of difference between $RS^{WT/WT}$ and $RS^{\Delta/\Delta}$ mice in λ B cell frequency in the above-indicated tissues.

**Table S2. Numbers of B Cell Subsets in $RS^{\Delta/\Delta}$; *κ-macroself* Tg and $RS^{WT/WT}$;*κ-macroself* Tg Mice (Millions)**

| Genotype | tissue | total cells | B220+ | $\lambda_{1-3}$+ | κ+ | κ+/$\lambda_{1-3}$+ |
|---|---|---|---|---|---|---|
| $RS^{WT/WT}$;*κ-macroself* Tg n=5 | BM | 46.6 ± 5.18 | 7.39 ± 1.52 | **6.21 ± 3.10** | 0.904 ± 0.27 | 0.029 ± 0.009 |
| $RS^{\Delta/\Delta}$; *κ-macroself* Tg n=5 | | 50.4 ± 7.16 | 8.53 ± 1.28 | **1.92 ± 0.88** | 1.14 ± 0.30 | 0.057 ± 0.015 |
| | | | | p= 0.018 | | p= .007 |
| $RS^{WT/WT}$;*κ-macroself* Tg n=5 | spleen | 44.4 ± 15.1 | 12.4 ± 4.56 | 11.1 ± 3.92 | 0.05 ± 0.02 | 0.159 ± 0.085 |
| $RS^{\Delta/\Delta}$; *κ-macroself* Tg n=5 | | 36.6 ± 11.4 | 9.01 ± 2.28 | 6.94 ± 2.60 | 0.09 ± 0.05 | 0.227 ± 0.053 |
| | | | | p= 0.08 | | |

Mice analyzed were 10 weeks old. Shown are mean values ±SD.

**Table S3. Numbers of B Cell Subsets in $RS^{\Delta/\Delta}$; *Bcl2* Tg→ *κ-macroself* Tg and $RS^{WT/WT}$; *Bcl2* Tg→ *κ-macroself* Tg Chimeras (Millions)**

| Genotype (n) | tissue | total cells | B220+ | λ+ | κ+ | κ+λ+ |
|---|---|---|---|---|---|---|
| $RS^{WT/WT}$; *Bcl2* Tg→*κ-macroself* Tg n=5 | BM | 113.6 ± 35.1 | 15.46 ± 6.6 | 2.74 ± 1.3 | **0.71 ± 0.79** | **0.043 ± .018** |
| $RS^{\Delta/\Delta}$; *Bcl2* Tg→*κ-macroself* Tg n=7 | | 93.71 ± 12.83 | 16.23 ± 1.8 | 1.67 ± 0.3 | **5.48 ± 0.49** | **0.179 ± .032** |
| | | | | p=.06 | $p<10^{-6}$ | $p<10^{-5}$ |
| $RS^{WT/WT}$; *Bcl2* Tg→*κ-macroself* Tg n=5 | Spleen | **86 ± 16.4** | **13.27 ± 2.7** | **8.64 ± 4.8** | **0.05 ± 0.07** | 0.022 ± 0.023 |
| $RS^{\Delta/\Delta}$; *Bcl2* Tg→*κ-macroself* Tg n=7 | | **49.6 ± 5.6** | **8.19 ± 2.7** | **4.31 ± 1.3** | **0.78 ± 0.48** | 0.064 ± 0.068 |
| | | p= .0003 | p= .009 | p= .04 | p= .008 | |
| $RS^{WT/WT}$; *Bcl2* Tg → B6 n=5 | BM | 102.2 ± 14.8 | 18.37 ± 2.2 | 1.90 ± 0.5 | 6.66 ± 1.77 | 0.094 ± .019 |
| $RS^{\Delta/\Delta}$; *Bcl2* Tg → B6 n=6 | | 108.3 ± 25.3 | 16.66 ± 5.4 | 1.49 ± 0.4 | 6.47 ± 3.10 | 0.146 ± .088 |
| | | | | | | |
| $RS^{WT/WT}$; *Bcl2* Tg → B6 n=5 | Spleen | 100.8 ± 14.0 | 40.92 ± 9.3 | 3.94 ± 1.3 | 34.17 ± 7.51 | 0.236 ± .049 |
| $RS^{\Delta/\Delta}$; *Bcl2* Tg → B6 n=6 | | 96.1 ± 20.2 | 37.1 ± 9.7 | 3.61 ± 2.1 | 31.2 ± 7.49 | 0.304 ± .169 |

Chimeras were analyzed 10 weeks after bone marrow transplantation. Shown are mean values ±SD.

## Supplemental References

Aït-Azzouzene,D., Verkoczy,L., Peters,J., Gavin,A., Skog,P., Vela,J.L., and Nemazee,D. (2005). An immunoglobulin C{kappa}-reactive single chain antibody fusion protein induces tolerance through receptor editing in a normal polyclonal immune system. J.Exp.Med. *201*, 817-828.