Supplementary data

Complete *cis*-exclusion upon duplication of Eμ enhancer at the immunoglobulin heavy-chain locus

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Supplementary Results and Discussion

1- B cell development in the fetal liver, bone marrow and peripheral lymphoid organs.

The effect of premature expression of IgG3 on B cell development in various compartments of homozygous mice was analysed by FACS. In the bone marrow, we found a decrease in the $B220^+$ population. In contrast, in the spleen, the Peyer's patches, the peritoneum, and the fetal liver, the percentage of total $B220^+$ was slightly higher in A150 mice than in WT controls (Fig. S2A).

A striking and constant finding was that IgM-expressing B cells were barely detectable (<1%) in every compartment analysed in A150 mice, while IgG3-expressing B cells formed the vast majority of B cell populations (Fig. S2B-S2F). The lack of IgM expression was checked by using a polyclonal anti-IgM antibody or a mAb against the IgM^a allotype, since the mutant allele was derived from 129Sv1 mouse strain.

In the bone marrow, the pro-B cell compartment was slightly affected while pre-B cell population was clearly reduced. Similar ratios of pro-B and pre-B cell subsets were obtained by gating on IgM^- fraction for WT mice, on $IgG3^-$ fraction for A150 mice, or on κ^- for both (Fig. S2G). Immature B cells ($B220^+AA4.1^+\kappa^+$) were slightly decreased in A150 mice, while circulating B cells ($B220^+AA4.1^-\kappa^+$) were increased (Fig. S2H).

The data show that premature expression of γ3 heavy chain can drive B cell development in the bone marrow, with a decrease in pre-B cell population and to some extent immature B cell

population. Despite this impairment, IgG3-expressing B cells can populate the various lymphoid compartments. Importantly, the mutation leads to a complete absence of IgM-surface expression throughout B cell development.

In view of the above findings, together with the data reported in the main text regarding allelic exclusion, it is important to note that despite premature expression, γ3 heavy chain was not as efficient as μ heavy chain in driving early B cell development. The $B220^{+}$ population was decreased in the bone marrow which correlated with a markedly reduced pre-B cell compartment. While proper surface expression of the μ pre-BCR is required to deliver proliferative and survival signals that allow pre-B cells to mature to the next stage (1, 2), it is plausible that surface expression of the γ3 pre-BCR is compromised (3). Additionally, there is evidence that proximal V_H-containing heavy chains do not efficiently pair with surrogate light chains (4), which may impact the pre-BCR function. In this regard, we cannot exclude the possibility that failure of proximal V_H-containing μ heavy chains to pair with surrogate light chains may contribute to *cis*-exclusion in A150 mice. Still, this alone does not explain why V(D)J rearrangements are restricted to proximal V_H segments (see discussion in the main text). Whether γ3 and μ pre-BCRs share the same signalling pathway or whether they are equally efficient in mediating allelic exclusion is presently unknown. From our data that show that *prematurely* expressed γ3 heavy chain can mediate an efficient allelic exclusion, it does not follow that γ3 and μ pre-BCRs would be equally efficient when both the mutant and WT alleles have to undergo V(D)J recombination (3, 5). This would rather imply that μ pre-BCR is actually more powerful in a genuine allelic exclusion.

2- B cell development and homing to peripheral lymphoid organs

In the spleen, IgD was, just as IgM, undetectable in the mutant mice (Fig. S2I). In agreement with previous findings (3), the percentage of marginal zone (MZ) B cells was a ~2-fold higher in

A150, whereas the percentage of follicular (FO) B cells was essentially similar to WT controls (Fig. S2J).

In the peritoneum of mutant mice, the increase in $B220^+$ fraction correlated with an increase in the B-2 population ($B220^+CD43^-$), while the $B220^+CD43^+$ fraction remained essentially unchanged (Fig. S2K, upper panels). Surprisingly, whereas $B220^+CD5^+$, B-1a cells, were abundant in the peritoneum of WT mice, they were very rare in that of A150 mice (Fig. S2K, lower panels). Accordingly, the percentage of $IgG3^+CD5^+$ cells in the peritoneum of A150 mice was less than 2% compared to ~20% of IgM^+CD5^+ (Fig. S2K). This contrasts with the situation in fetal liver where the two populations were comparable (Fig. S2L).

Thus, in the spleen, IgG3-expressing B cells preferentially home to the marginal zone. In stark contrast to WT, the mutant IgG3-expressing B-1a cells are very rare in the peritoneum, indicating that IgG3-expressing B cells do not develop peritoneal $CD5^+$ cells. In contrast, they do so in the fetal liver, excluding the possibility that IgG3 and CD5 are incompatible with regard to surface expression.

3- Light chain expression under allelic inclusion, allelic exclusion and isotypic competition

The variable domains of γ3 heavy chain (in A150) and μ heavy chain (in B1-8) preferentially pair with κ and λ light chains respectively, and are specific for HEL and NP respectively (6-8). In order to check whether γ3 and μ heavy chains would compete for κ or λ light chains, and the potential effect of allelic inclusion on the κ/λ ratio, we looked at the surface expression pattern of κ/λ light chains. Strikingly, while B1-8/B1-8 B cells mainly expressed λ light chains, the vast majority of splenic B cells of other genotypes expressed κ light chains (Fig. S2M).

Remarkably, B cells that prematurely express IgG3 can out-compete IgM-expressing B cells in bone marrow of A150/WT mice (Fig. 1). The ratio is relatively more pronounced in the spleen where 76% of B cells express IgG3 only (Fig. S2N). In A150/B1-8 mice, where both isotypes are

prematurely expressed, the vast majority of B cells (94%) were allelically included and co-produced IgM and IgG3 in the spleen (Fig. S2N).

Thus, IgG3 and IgM can efficiently drive B cell development under allelic inclusion, and competition for κ light chain does not appear to be a limiting factor. Moreover, for allelically included isotypes, there is seemingly a selective advantage for κ-expressing cells.

4- The loss of IgG3 expression in A150 heterozygotes results from a deletional process

We considered a deletional process on the mutant allele that could be associated with either V(D)J recombination (because of early transcription of the ectopic J_{H3} and J_{H4} segments) or CSR (because of a fairly abundant transcription of Sμ and Sγ3). In both V(D)J recombination-, and CSR-associated deletions, different parts of the ectopic transcription unit should be lost. Accordingly, we designed different primers to distinguish between the two processes. Genomic DNA was extracted from sorted bone marrow IgM^{b+} and $IgG3^{+}$ single-expressers, and assayed for V(D)J recombination and CSR.

The integrity of the inserted cassette was first checked and yielded the expected amplicons in the right genotypes (see IμF/3'SpeIR, IμF/3'SphIR, and Iγ3F/3'SphIR. Fig. S3A). Amplicons spanning the insertion site on the WT alleles (5'PmeIF/3'PmeIR) were, as expected, detected in both IgM^{+} and $IgG3^{+}$ heterozygous populations, but not in homozygous A150 control (Fig. S3A). Crucially, by using a reverse primer that binds the 5' part of the ectopic sequence, the expected amplicon (5'PmeIF/3'loxPR) was readily detected in $IgG3^{+}$ population, but was barely detectable in IgM^{+} population (Fig. S3A). A similar pattern was obtained by using primers that amplify the ectopic VDJ exon/intron ($VDJ_{A150}F/5'J_H3R$) (Fig. S3A). Quantification of the signals of 5'PmeIF/3'loxPR and $VDJ_{A150}F/5'J_H3R$ amplicons indicated that the vast majority (>90%) of the signals disappeared from the mutant allele of IgM^{b+} population (Fig. S3A), indicating that the ectopic cassette was deleted in most of IgM^{b+} cells.

If low levels of Sμ/Sγ3 CSR occurred on the mutant allele, then switch products might be amplified by DC-PCR. A very faint signal was detected in one $IgG3^+$ population (Fig. S3B), and only upon a nested PCR. Assuming that it reflects genuine CSR events, a possible explanation is that it represents very rare CSR events that may have occurred after a (or some) productive V-DJ rearrangement(s) took place on the A150 allele. Compared to the control DC-PCR, a rough approximation would be that the percentage of switching alleles might not exceed 1% (Fig. S3B). We conclude that CSR is not the main process that mediates the loss of IgG3 expression in IgM^{b+} population. Nonetheless, even low levels of CSR on A150 allele may be sufficient to generate rare clones, which may be selected for further expansion. In support for the potential involvement of CSR, a low frequency of switch events was detected in the bone marrow (*e.g.* 9, 10) and some transformed pre-B cell lines can undergo CSR *in vitro* (11, 12).

A V(D)J recombination-associated deletion is expected to target either the ectopic J_{H3} or J_{H4} RSSs (hereafter called ectopic V(D)J recombination) (Fig. S3C, upper scheme). Therefore, with a primer that binds 5' of J_{H3} and a degenerate primer that binds the RSSs of most of D segments, one should detect DJ_{H1} and DJ_{H2} recombination on both alleles in the $IgG3^+$ fraction. In contrast, one should expect a decrease of D-J_H recombination signal in IgM^{b+} fraction, since the only DJ_{H1} and DJ_{H2} amplicons will originate from the endogenous D-J_H cluster of the A150 allele (Fig. S3C, S4). That is indeed the case (Fig. S3C). Although this assay does not distinguish between recombined endogenous and ectopic J_{H3} and J_{H4} segments, the very low frequency of DJ_{H1} and DJ_{H2} recombination events in IgM^{b+} population (~6%) strongly suggests that ectopic D-J_H recombination is the major mechanism that led to the loss of A150 unit in the precursors of this population.

An accurate quantification of the contribution of each allelic configuration (Fig. S4) during ectopic V(D)J recombination in heterozygotes is difficult because the sorted populations have been selected at various checkpoints. We can only reconstruct the events that likely occurred in their precursors. A plausible scenario is that the A150 and WT alleles undergo D-J_H recombination in pro-B cells regardless of the J_H segments used on the A150 allele. V_H-DJ_H recombination may head first on one allele, and the outcome would depend on the correct reading-frame for feed-back inhibition. If this requirement is not fulfilled, the second allele then undergoes V_H-DJ_H recombination. In a fraction of the ~11% IgM^{b+} population, 40% and 24% of γ3 heavy chain cDNAs containing proximal V_H and distal V_H segments, respectively, displayed correct reading-frames on the A150 allele (Table S1). In another fraction of IgM^{b+} population, the ectopic transcription unit was intact as indicated by the presence of VDJ_{A150}-Cγ3 transcripts. Our interpretation is that some of the γ3 heavy chains did not pair with surrogate light chains, potentially leading to a situation where allelic exclusion was displayed at the protein level despite allelic inclusion at the genomic level (13). The other γ3 heavy chains that paired with surrogate light chains may have done so in few clones which would give rise to double-expressers, and may be selected and expanded. This scenario does obviously not preclude the productive pairing of γ3 heavy chains with light chains as a further checkpoint. In these scenari, we can not *a priori* exclude the possibility that surface expression of a subset of γ3 pre-BCRs may be compromised and/or that they deliver a weak signal, which would enable a time window during which μ pre-BCRs would be expressed and deliver a stronger signal.

Overall, these interpretations would imply that IgM and IgG3 double-expression confers a selective advantage over single isotype expression. This may explain the relatively high percentage (~7%) of IgM^+IgG3^+ double-expressers in the spleen. In support of this interpretation, we also found that the percentage of double expressers was very high in A150/B1-8 spleens.

5- The primary repertoire is not of fetal origin and out-of-frame V(D)J rearrangements are modestly increased

Proximal V_H-DJ_H recombination frequency was reduced in A150 pro-B cells. Still, this does not exclude the possibility that the residual recombined alleles may actually display a high proportion of unproductive rearrangements. Additionally, IgG3-expressing B cells were readily detected in the fetal liver, which hints to the possibility that the primary repertoire of the adult A150 mice may represent a vestige of the fetal repertoire. The terminal transferase is highly active in pro-B cells, but is barely detectable in fetal liver (14). Therefore, if the repertoire were of fetal origin, one would expect an absence or a drastic decrease in N-region addition. In an attempt to explore this issue, we cloned and sequenced 38 and 43 independent rearranged proximal V_HDJ_{H4} genes from sorted WT and A150 pro-B cells respectively. The junctional diversity flanking the rearranged D segments was used to check the clonality of the sequences.

While there was an overall balanced proportion of productive and non-productive rearrangements in WT controls (55% versus 45% respectively), we found a relative increase of non-productive rearrangements in A150 (61%) (Fig. S6 and Table S2). Inspection of the junction sequences showed no obvious difference between A150 mutants and WT controls. There was no evidence for over-representation of a D gene segment family in A150, nor an anomaly regarding the number of nucleotides inserted or deleted during V(D)J recombination (Fig. S6). We conclude that unproductive rearrangements modestly contribute to *cis*-exclusion of IgM, and that the primary repertoire of A150 mice is unlikely to be of fetal origin.

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Supplementary Figure Legends

Figure S1. Replacement of Iγ3 region by a pre-rearranged V(D)J-Eμ cassette.

(A) Structure of the targeted γ3 locus (not to scale): scheme of a WT allele showing an un-rearranged *IgH* locus, and highlighting the Eμ enhancer, the Iγ3 GL promoter, Sγ3, Cγ3 and the 3'RR lying at ~150 kb downstream of the γ3 region. **(B)** The structure of the targeting construct is shown. The *neo*^{r}-PVH-V(D)J-Eμ cassette was inserted into the ClaI site that replaced PmeI and SphI respectively. The two *lox*P sites are shown as triangles. The homologous recombination event leads to the replacement of the whole Iγ3 region. Ig sequences in the targeting vector and the recombinant ES clones are derived from the 129Sv1 mouse strain. **(C)** The structure of the floxed allele is shown as well as the position of the external 5' probe and the internal 3' probe. B: BamHI, C: ClaI, R: EcoRI, H: HindIII, P: PmeI, S: SphI, X: XhoI. **(D)** A Southern blot analysis of genomic DNA from WT and A150 mice. The digests used are indicated. The position of the probes is shown in (C).

Figure S2. FACS Analysis of B cell populations and Ig expression in B cell compartments.

(A). Total B cell populations. After removal of red blood cells, cells from the BM, spleen, PPs, peritoneum, and FL, were stained with anti-B220. Representative profiles from each compartment in WT and A150 mice are shown. Right panels: the histograms show the standard deviations. BM, n=5, p=0.0005; Spleen, n=6, p=0.0006; PPs, n=2, p=0.1; Peritoneum, n=2, p=0.02; FL, n=2, p=0.2 (pools of 3 embryos, each from a pregnant mouse (day 19 *p.c*), were used).

(B-F). IgH expression in B cell compartments. Single-cell suspensions from the BM (**B**), spleen (**C**), FL (**D**), PPs (**E**), and peritoneum (**F**), derived from WT and 150 mice, were labelled with anti-B220, anti-IgM and/or anti-IgG3, and gated on B220^{+} population. Representative dot plots showing essentially the absence of IgM- and the presence of IgG3-surface expression on A150 B cells are

shown, with the corresponding B cell compartments. BM, n≥5; Spleen, n≥4; FL, n=2 (pools of 3 embryos at day 19 *p.c* from 2 pregnant mice were used), PPs, n=2; Peritoneum, n=2.

(G, H). B cell subsets in the BM. (G) Single-cell suspensions from the BM of WT and A150 mice were stained with anti-B220 and anti-CD43. Representative plots and the corresponding histograms are shown on the right (n≥3). (**H**). Single-cell suspensions from the BM of WT and A150 mice were stained with anti-B220, anti-AA4.1, and anti-κ, and gated on $B220^+$ population. Representative plots are shown on the left, and the corresponding histograms are shown on the right (n=3). (***, p<0.001; **, *p*<0.01; *, *p*<0.05; Data are presented ± SEM).

(I, J). IgH expression in the spleen. (I) After removal of red blood cells, single-cell suspensions were stained with anti-B220, and anti-IgM, or anti-IgD, or anti-IgG3**.** The histograms on the right panels show the standard deviations (n=5, p<0.0001 for IgM^+ cells; n=2, p<0.0001 for IgD^+ cells; n=5, p<0.0001 for $IgG3^+$ cells). (**J**) To check for the ratio of MZ/FO B cells, splenic cells were stained with anti-B220, anti-CD21 and anti-CD23. Representative profiles, gated on $B220^+$ population, are shown in the left panels (n=2, p=0.001 for MZ and p=0.02 for FO B cells).

(K, L). **B cell populations in the peritoneum and fetal liver. (K)** Peritoneal cells were stained with anti-B220 and anti-CD43 (upper plots), with anti-B220 and anti-CD5 (middle plots), or with anti-CD5 and anti-IgM, or anti-IgG3 (lower plots). The B-1a population was defined as $B220^+CD5^+CD43^{high}$, B-1b population as $B220^+CD5^{low}CD43^{low}$, and B-2 population as $B220^+CD5^-CD43^-$. Right panels: the histograms show the standard deviations (n=2, p=0.0005 for B-2 cells; p=0.002 for IgM^+ B-1a cells, and p=0.01 for $IgG3^+$ B-1a cells). **(L)** After removal of red blood cells, cells from the FL were stained with anti-B220 and anti-CD5. Representative profiles from WT and A150 mice are shown. Right panels: the histograms show the standard deviations. n=2, p=0.2 (pools of 3 embryos, each from a pregnant mouse (day 19 *p.c*), were used).

(M). Analysis of surface expression of light chains in the spleen. After removal of red blood cells, splenocytes with the indicated genotypes were stained with anti-B220 and anti-κ and anti-λ, and gated on $B220^+$ population. Representative plots are shown (n=2).

(N). Flow cytometric analyses of allelic exclusion, allelic inclusion and isotypic competition. (Upper panels) Single-cell suspensions from the spleens of mice with the indicated genotypes were stained with anti-B220 and mAbs against IgM^a and IgM^b allotypes, and gated on $B220^+$ population. Representative plots are shown (n=5). **(Lower panels)** Cells from the spleens of mice with the indicated genotypes were stained with anti-B220, anti-IgM and anti-IgG3, and gated on $B220^+$ population. Representative plots are shown (n=4).

Figure S3. PCR analysis of deletion events on the A150 allele in A150/WT mice.

(A). IgM^+-only and $IgG3^+$-only expressing B cells were sorted from the BM of A150/WT mice, and their genomic DNAs were extracted and subjected to PCR using appropriate primers. The relative localisation of the primers is indicated on the right of the corresponding panels. Genomic DNAs from WT and A150/A150 pre-B cells were used as controls. Representative results are shown with, below, the corresponding histograms (n=2, p=0.008 for IμF-3'SpeIR, p=0.76 for Iγ3F-3'SphIR, p=0.06 for IμF-3'SphIR, p=0.7 for 5'PmeIF-3'PmeIR, $p<0.0001$ for 5'PmeI-3'PmeIR, $p<0.0001$ for VDJ_{A150}F-5'J_H3R). **(B).** DC-PCR analysis of CSR events in IgM^+ and $IgG3^+$ populations. Genomic DNAs were extracted from the sorted BM populations, digested with EcoRI, and subjected to DC-PCR. The relative position of the primers is indicated in the top scheme. As a control, genomic DNA from a serial dilution of splenic B cells induced to switch to IgG3 was assayed by using the same primers. The percentage of switched B cells was determined by FACS (n=2). **(C).** Detection of ectopic V(D)J recombination. Genomic DNAs from the sorted populations were assayed for D-J_H recombination by using a forward primer (top scheme) that binds RSSs of

the vast majority of D segments and a backward primer that binds upstream of both the endogenous and the ectopic J_H3 (n=2, $p<0.0001$).

Figure S4. Possible configurations of A150 and WT alleles and expected transcripts in IgM^{b+} and $IgG3^+$ populations.

The A150 and WT alleles in their un-rearranged configuration are shown on the top. For simplicity, a single V_H, D or J_H segment is shown. Possible V_H-DJ_H rearrangements in IgM^{b+} and $IgG3^+$ populations and the corresponding transcripts are indicated. The transcripts that potentially produce μ or γ3 heavy chains are underlined. Assumptions underlying some configurations and the likely order of rearrangements are reported below each scheme (see paragraph 4 of Supplementary results for more details).

Figure S5. **Sequences of the VDJ-Cγ3 cDNAs from sorted IgM^{b+} and $IgG3^+$ A150/WT bone marrow B cells.**

IgM^{b+}-only and $IgG3^+$-only expressing B cells were sorted from the bone marrow of A150/WT mice. Total RNAs were extracted and reverse transcribed. Single-stranded cDNAs were subjected to PCR using a primer that binds either the proximal V_{H7183}, or the distal V_{HJ558} gene segment family, and a reverse primer that binds Cγ3-1 exon. Non-productive sequences are further divided into those with out-of-frame rearrangements, and those with premature stop codons (underlined) within the rearranged D-J_H sequences. The correct reading-frame resulting from J_{H3}-Cγ3 or J_{H4}-Cγ3 splicing is shown below the alignments. The Cγ3-1 exon sequence is indicated in bold.

Figure S6. **Sequences of the V_H-D-J_H4 junctions from WT and A150/A150 pro-B cells.**

The sequences were amplified by using a primer that binds the proximal V_{H7183} gene-family segments, and one primer that binds 3' of J_{H4} segment. The junctional diversity was used to check

for the clonality of the sequences. 38 and 43 sequences were obtained from sorted WT and A150 pro-B cells respectively. Non-productive sequences are further divided in those with out-of-frame rearrangements, and those with premature stop codons (in italics).

Supplementary Materials and Methods

Gene targeting. For A150 targeting construct, a ~2.6 kb PmeI-SphI fragment spanning Iγ3 promoter/exon, and ending at ~250 bp downstream of the Iγ3 exon was replaced by a ~5 kb fragment containing a pre-rearranged V(D)J gene and Eμ enhancer. The PmeI and SphI sites were first modified into ClaI site in a pBluescript II KS$^-$ (Stratagene) vector containing a ~8 kb XhoI-BamHI fragment spanning Iγ3 and Sγ3 sequences. The PVH-V(D)J-Eμ sequence was derived from pYMT8 (1) as a SpeI fragment and cloned into the unique SpeI site of a vector downstream of a *neo*^{r} cassette flanked by two *lox*P sites. The HSV *tk* gene was inserted into XhoI site. The ES cell line CK35 (129Sv1) (kindly provided by C. Kress, Institut Pasteur, Paris) was transfected by electroporation, and selected using G418 (300 μg/ml) and gancyclovir (2 μM). Recombinant clones were identified by PCR and Southern blot analysis after an EcoRI digest, with an external 5' probe (a 1.0 kb EcoRI-XhoI fragment), or after a BamHI digest, with an internal 3' probe (a 2 kb HindIII-BamHI fragment downstream of Sγ3 region). Two ES clones showing homologous recombination were injected into C57Bl/6 blastocysts, the male chimeras were then mated with C57Bl/6 females. Germ-line transmission of the mutation was checked by PCR and Southern blot after EcoRI or BamHI digests and using the external or internal probes. Homozygous N/N mutant mice were mated with EIIa-cre transgenic mice. The progeny was checked by PCR and Southern blot for *Cre*-mediated deletion *neo*^{r} by using the same digests and probes. Additional checks were made by sequencing pertinent regions in the genomic DNA of splenic B cells from *neo*^{r}-deleted mice.

Flow cytometry analyses. Lymphoid organs from 6- to 8-week-old mice were prepared by standard techniques. After disaggregation, washing, and removal of red blood cells, $5x10^5$ cells/assay were stained and gated as indicated in the figure legends. Fetal livers were pooled from embryos at day 19 *post-coitum*, and treated as above. Data were obtained on 2.0×10^4 viable cells

by using a BD FACSCalibur. In all FACS acquisitions, dead cells were excluded by labelling with propidium iodide.

Antibodies. PE-conjugated anti-IgM^{b}, FITC-conjugated anti-IgM^{a}, FITC-conjugated anti-IgG3, PE- and FITC-conjugated anti-CD43, PE-conjugated anti-κ, FITC-conjugated anti-λ, PE-conjugated anti-CD5, PE-conjugated anti-CD4, FITC-conjugated anti-CD8, PE-conjugated anti-AA4.1, FITC-conjugated anti-κ, were purchased from BD-Pharmingen. PE- and APC-conjugated anti-B220, FITC- and PE-conjugated anti-IgM, APC-conjugated anti-CD21, FITC-conjugated anti-CD23 were from BioLegend. PE-conjugated anti-IgD was from Southern Biotech.

Sequencing of V(D)J amplicons. Genomic DNAs from sorted pro-B cells were amplified by using a degenerate primer that binds the proximal V_{H7183} gene segment family and a primer 3' of J_{H4}. Bands corresponding to $V_H(D)J_{H1}$ rearrangements were purified and cloned into pCR2.1-TOPO (InVitrogen) for sequencing.

Sequencing of V(D)J-Cγ3 cDNAs. Single-cell suspensions from the bone marrows of A150/WT mice were stained with anti-B220, anti-IgM^{b}, and anti-IgG3. IgM^{b+} and IgG3^{+} single-expressers were sorted, and total RNAs were extracted and reverse transcribed. PCRs were performed by using a degenerate primer that binds the proximal V_{H7183} or the distal V_{HJ558} gene segment families, and a reverse primer that binds Cγ3-1 exon. Amplicons were purified and cloned into pCR2.1-TOPO (InVitrogen) for sequencing.

DC-PCR. Splenic B cells were purified by using CD43-beads (Miltenyi) and cultured in the presence of 20 μg/ml LPS (Sigma) and 3 ng/ml anti-IgD-dextran (Fina Biosolutions). At day 4, genomic DNA was extracted and assayed by DC-PCR essentially as described (2).

References associated with Supplementary Materials and Methods

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Supplementary tables

Table S1. Productive versus non-productive rearrangements in sorted A150/WT bone marrow B cells.

IgM^+ population	**Productive**	**Non-productive**	**Number of sequences**	**J_{H3} usage**	**J_{H4} usage**
Proximal V_H	17 **(40%)**	25 **(60%)**	42 (100%)	13	29
Distal V_H	9 **(24%)**	29 **(76%)**	38 (100%)	18	20
$IgG3^+$ population	**Productive**	**Non-productive**	**Number of sequences**	**J_{H3} usage**	**J_{H4} usage**
Proximal V_H	40 **(95%)**	2 **(5%)**	42 (100%)	12	30
Distal V_H	42 **(98%)**	1 **(2%)**	43 (100%)	5	38

Ratios of productive/non-productive proximal and distal VDJ-Cγ3 cDNA sequences in sorted IgM^+ and $IgG3^+$ heterozygous B cells from A150/WT mice. IgM^+-only and $IgG3^+$-only cells were sorted from the bone marrow of A150/WT mice. Total RNAs were extracted, reverse transcribed and subjected to PCR using a primer that binds either the proximal V_{H7183}, or the distal V_{HJ558} gene segment family, and a reverse primer that binds 5' of Cγ3.

Table S2. Amplified joints ($V_{H7183}DJ_{H4}$) from sorted pro-B cells.

Genotype	In frame sequences	Out of frame sequences	Total number of sequences
WT	21 **(55%)**	17 **(45%)**	38 **(100%)**
A150/A150	17 **(39%)**	26 **(61%)**	43 **(100%)**

Ratios of productive/non-productive proximal V_H-D-J_H rearrangements in sorted pro-B cells from WT and homozygous A150 mice. Genomic DNAs were prepared from sorted pro-B cells and subjected to PCR using a degenerate primer that binds the proximal V_{H7183} genes family and a primer that binds 3' of J_{H4} segment.

Table S3. *Oligonucleotides used in this study.*

Primer	Sequence (5' to 3')	Tm (°C)	Reference
V(D)J rearrangement assays :			
D_HQ52-Fw	CCACAGGCTCGAGAACTTTAGCG	60	Perlot *et al.* (2005)
D_HL-Fw	TTTTTGTCAAGGGATCTACTACTGTG	60	Perlot *et al.* (2005)
V_H7183-Fw	GCGAAGCTTGTGGAGTCTGGGGGAGGCTTA	63	Perlot *et al.* (2005)
V_HQ52-Fw	GCGAAGCTTCTCACAGAGCCTGTCCATCAC	60	Perlot *et al.* (2005)
$V_HGam3.8$	CAAGGGACGGTTTGCCTTCTCTTTGGAA	60	Fuxa *et al.* (2004)
V_HJ558	GCGAAGCTTARGCCTGGGRCTTCAGTGAAG	60	Perlot *et al.* (2005)
J_H4-Rev	CCTAAAGGCTCTGAGATCCCTAGACAG	60-63	Puget *et al.* (2014)
VκDEG-Fw	GGCTGCAGSTTCAGTGGCAGTGGRTCWGGRAC	60	Dudley *et al.* (2003)
Jκ5.1-Rev	GAACTGACTTTAACTCCTAACATGAAAACC	60	Dudley *et al.* (2003)
HS4-Fw	CCAAAAATGGCCAGGCCTAGG	60	Perlot *et al.* (2005)
HS4-Rev	AGGTCTACACAGGGGCTCTG	60	Perlot *et al.* (2005)
Transcription Analysis / Q-PCR and RT-PCR assays :			
µ0-Fw	GGTGAGTCCTGCATCTGGGG	60	This study
Iµ-Fw	CTCTGGCCCTGCTTATTGTTG	60	Haddad *et al.* (2011)
Cµ-Rev	GAAGACATTTGGGAAGGACTGACT	60-62	Haddad *et al.* (2011)
Iγ3-Fw	TGGGCAAGTGGATCTGAACAC	60	Haddad *et al.* (2011)
Cγ3-Rev	CTCAGGGAAGTAGCCTTTGACA	60-62	Haddad *et al.* (2011)
VDJ_{A150}-Fw	CCAGAGAGAGAGATTATAGGCTTGACTAC	60	This study
AS_{DSP}-Fw	ACTTGGCAGGGATTTTTGTC	60	Guo *et al.* (2011)
AS_{DSP}-Rev	TGAAGAGTCTGCTGGGCATA	60	Guo *et al.* (2011)
pV_H7183 GLT-Fw	ATGGACTTCGGGCTCAGCTTG	60	Guo *et al.* (2011)
pV_H7183 GLT-Rev	GATGCTCTGCAGGAGGTTTT	60	Guo *et al.* (2011)
dV_HJ558 GLT-Fw	ATGGGATGGAGCTGGATCTT	60	Guo *et al.* (2011)
dV_HJ558 GLT-Rev	CTCAGGATGTGGTTACAACACTGTG	60	Perlot *et al.* (2005)
AS J558 5'int-Fw	ATTCCCCTCCCAATAGGAAA	60	Bolland *et al.* (2004)
AS J558 5'int-Rev	TGTCAATCACAATGGGCATC	60	Bolland *et al.* (2004)
AS PAIR4-Fw	ATGGGGCACATAGGTTCTTCC	64	Puget *et al.* (2014)
AS PAIR4-Rev	GGACATCTGAGAGATCATTGAACATC	64	Puget *et al.* (2014)

Primer	Sequence (5' to 3')	Tm (°C)	Reference
L-VDJ_H-Fw	GCAACAGCTACAGGTGTCCACTCCC	60	This study
pV_H7183-Fw	GCGAAGCTTGTGGAGTCTGGGGGAGGCTTA	60	Perlot *et al.* (2005)
dV_HJ558-Fw	GCGAAGCTTARGCCTGGGRCTTCAGTGAAG	60	Perlot *et al.* (2005)
D_H F	TTTTTGTSAAGGGATCTACTACTGTG	62	Puget *et al.* (2014)
Actin 4-Fw	CTGACAGACTACCTCATGAAGATCC	60	Puget *et al.* (2014)
Actin 5-Rev	CATGGATGCCACAGGATTCC	60	Puget *et al.* (2014)
GAPDH-Fw	GGTGAAGGTCGGTGTGAACG	60/64	Puget *et al.* (2014)
GAPDH-Rev	CTCGCTCCTGGAAGATGGTG	60/64	Puget *et al.* (2014)
YWHAZ-Fw	AGATGAAGCAGAAGCAGGAGAAG	60/64	Puget *et al.* (2014)
YWHAZ-Rev	CAGCATGGATGACAAATGGTCAC	60/64	Puget *et al.* (2014)
Analysis of deletion events on the A150 allele :			
Iμ F	CTCTGGCCCTGCTTATTGTTG	60	Haddad *et al.* (2011)
3'SpeI R	CATGAGCTCTATGATTATTGGTTAACAGGCAAC	60	This study
Iγ3 F	TGGGCAAGTGGATCTGAACAC	60	Haddad *et al.* (2011)
3'SphI R	GCTCTCCTGTGGCTGCTCAACTTGG	60	This study
5'PmeI F	AGAAGTGTGTGTGGTCATCAAAGC	60	This study
3'PmeI R	AATTACATACTTTCAGCATTCAATGATACTG	60	This study
3'LoxP R	CTTGGCTGGACGTAAACTCCTC	60	This study
VDJ_{A150} F	CCAGAGAGAGAGATTATAGGCTTGACTAC	60	This study
D_H F	TTTTTGTSAAGGGATCTACTACTGTG	62	Puget *et al.* (2014)
5'J_H3 R	CAGATGGAGGCCAGTGAGGG	60/62	This study
DC-PCR assays :			
5'Sμ R	CATGAGCTCTATGATTATTGGTTAACAGGCAAC	63	This study
3'Sγ3 F	TTGTCAATTCTTGATCTTACAGCACAAAGGG	63	This study
Nested 3'Sγ3 F	ACTCCCTGGGTCGAGAATATACAAGCC	63	This study
nAChRe-Fw	CGGTCGACAGGCGCGCACTGACACCACTAAG	63	Chu *et al.* (1992)
nAChRe-Rev	GCGCCATCGATGGACTGCTGTGGGTTTCACCCAG	63	Chu *et al.* (1992)

References associated with Table S3:

Bolland DJ, Wood AL, Johnston CM, Bunting SF, Morgan G, Chakalova L, Fraser PJ, Corcoran AE. 2004. Antisense intergenic transcription in V(D)J recombination. Nat Immunol **5:**630-637.

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Dudley DD, Sekiguchi J, Zhu C, Sadofsky MJ, Whitlow S, DeVido J, Monroe RJ, Bassing CH, Alt FW. 2003. Impaired V(D)J recombination and lymphocyte development in core RAG1-expressing mice. J Exp Med **198:**1439-1450.

Fuxa M, Skok J, Souabni A, Salvagiotto G, Roldan E, Busslinger M. 2004. Pax5 induces V-to-DJ rearrangements and locus contraction of the immunoglobulin heavy-chain gene. Genes & Dev **18:**411-422.

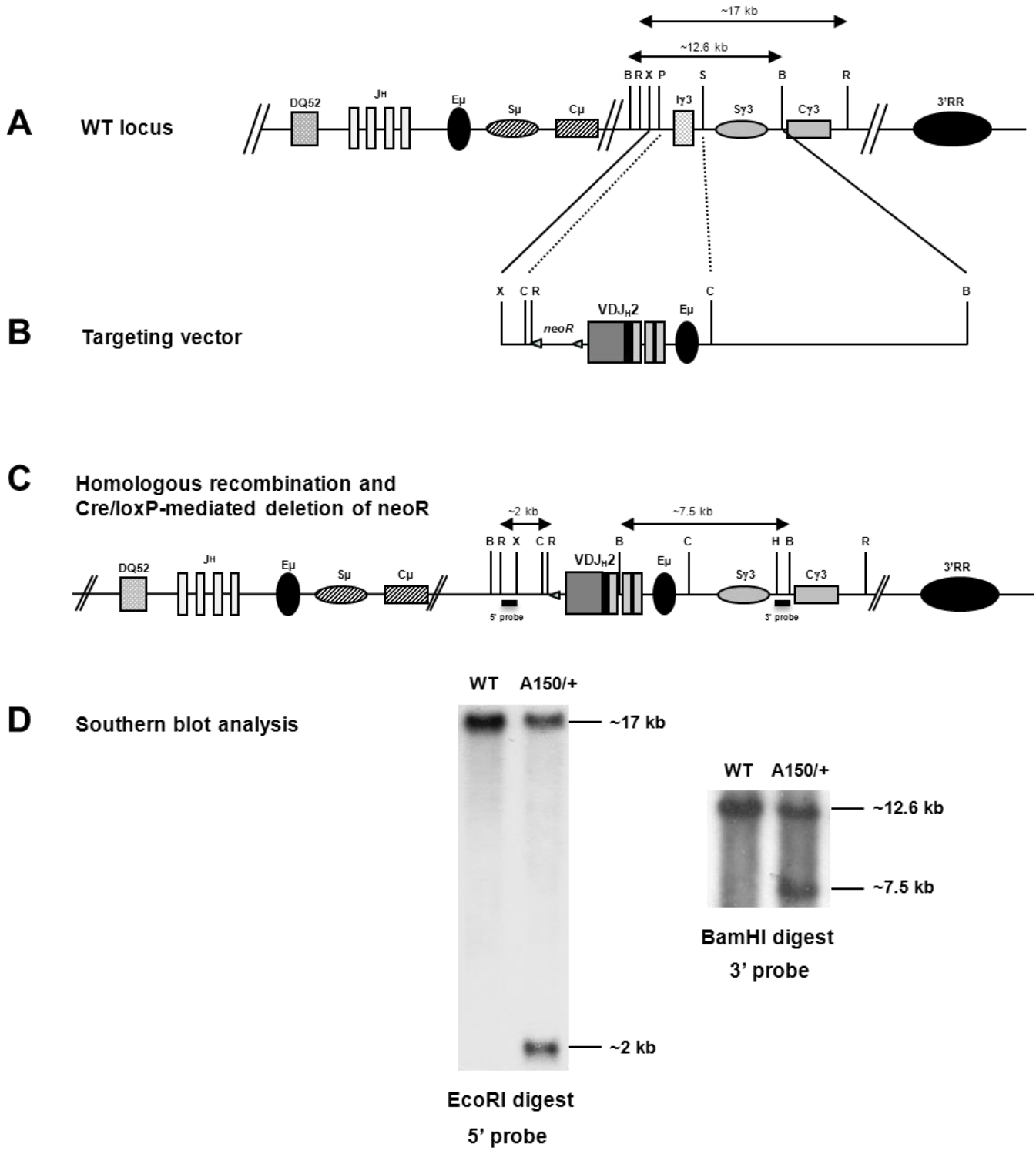
Guo C, Yoon HS, Franklin A, Jain S, Ebert A, Cheng HL, Hansen E, Despo O, Bossen C, Vettermann C, Bates JG, Richards N, Myers D, Patel H, Gallagher M, Schlissel MS, Murre C, Busslinger M, Giallourakis CC, Alt FW. 2011. CTCF-binding elements mediate control of V(D)J recombination. Nature **477:**424-430.

Haddad D, Oruc Z, Puget N, Laviolette-Malirat N, Philippe M, Carrion C, Le Bert M, Khamlichi AA. 2011. Sense transcription through the S region is essential for immunoglobulin class switch recombination. EMBO J **30:**1608-1620.

Perlot T, Alt FW, Bassing CH, Suh H, Pinaud E. 2005. Elucidation of IgH intronic enhancer functions via germ-line deletion. Proc Natl Acad Sci USA **102:**14362-14367.

Puget N, Hirasawa R, Nguyen Hu NS, Laviolette-Malirat N, Feil R, Khamlichi AA. 2015. Insertion of an imprinted insulator into the IgH locus reveals developmentally regulated, transcription-dependent control of V(D)J recombination. Mol Cell Biol **35**:529-543.

Figure S1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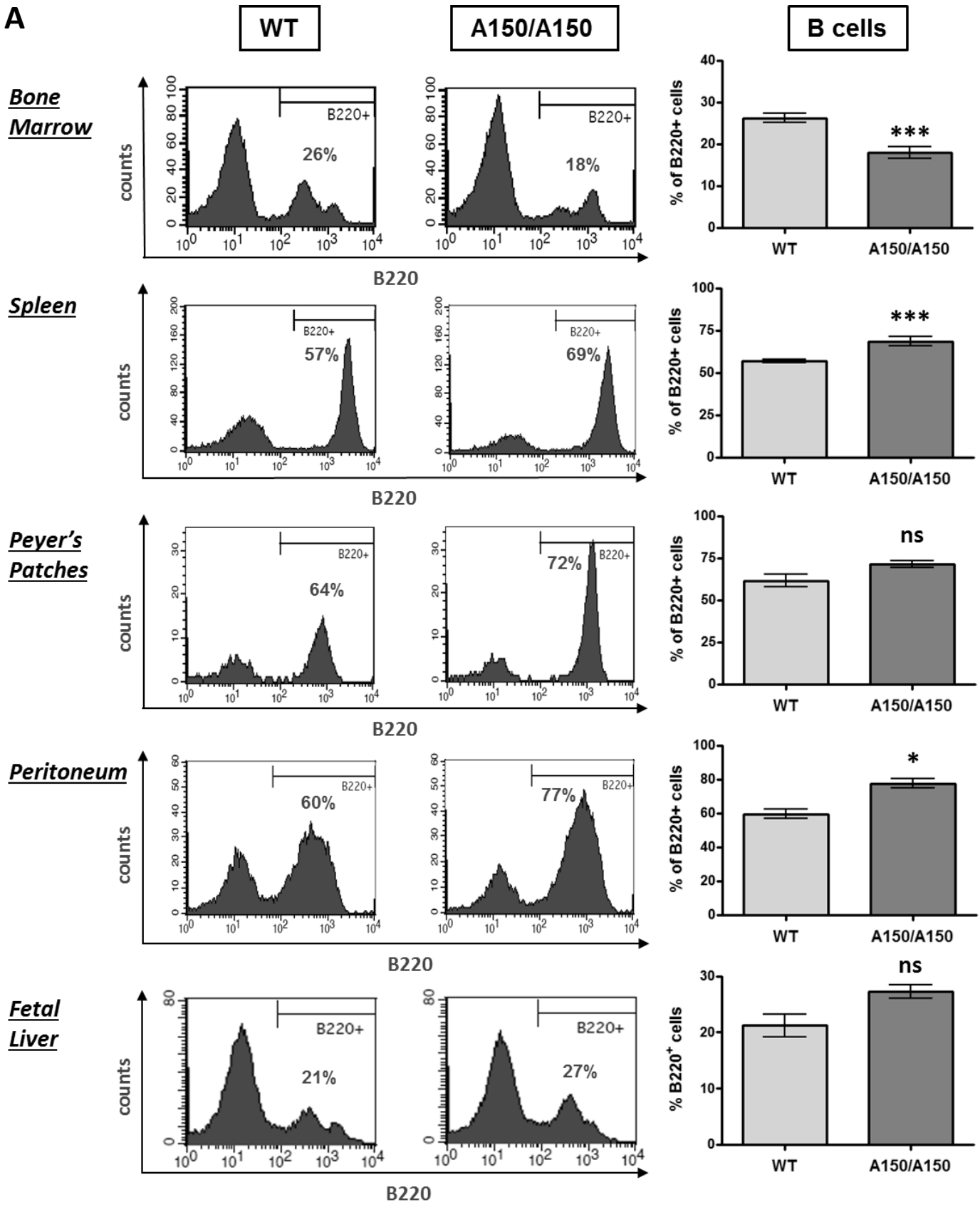
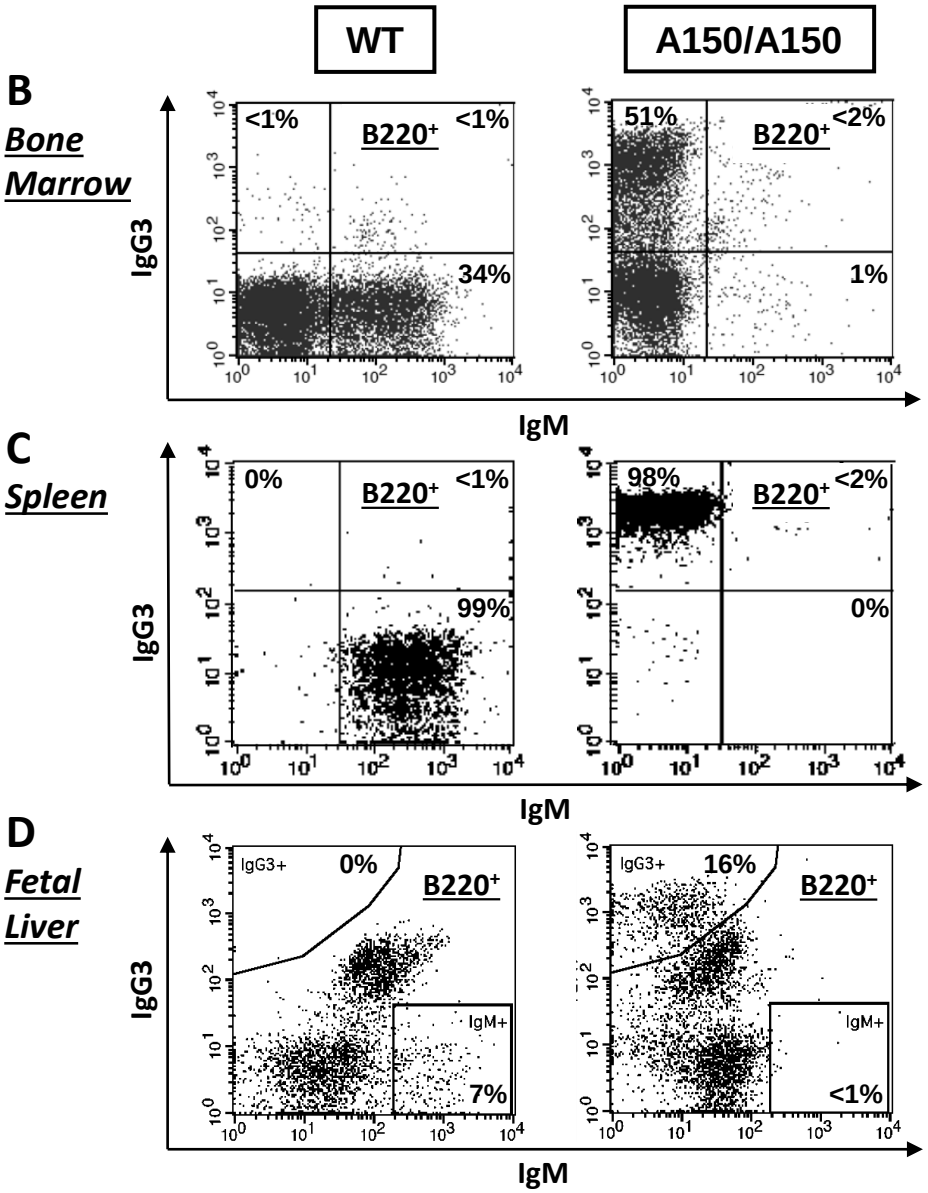
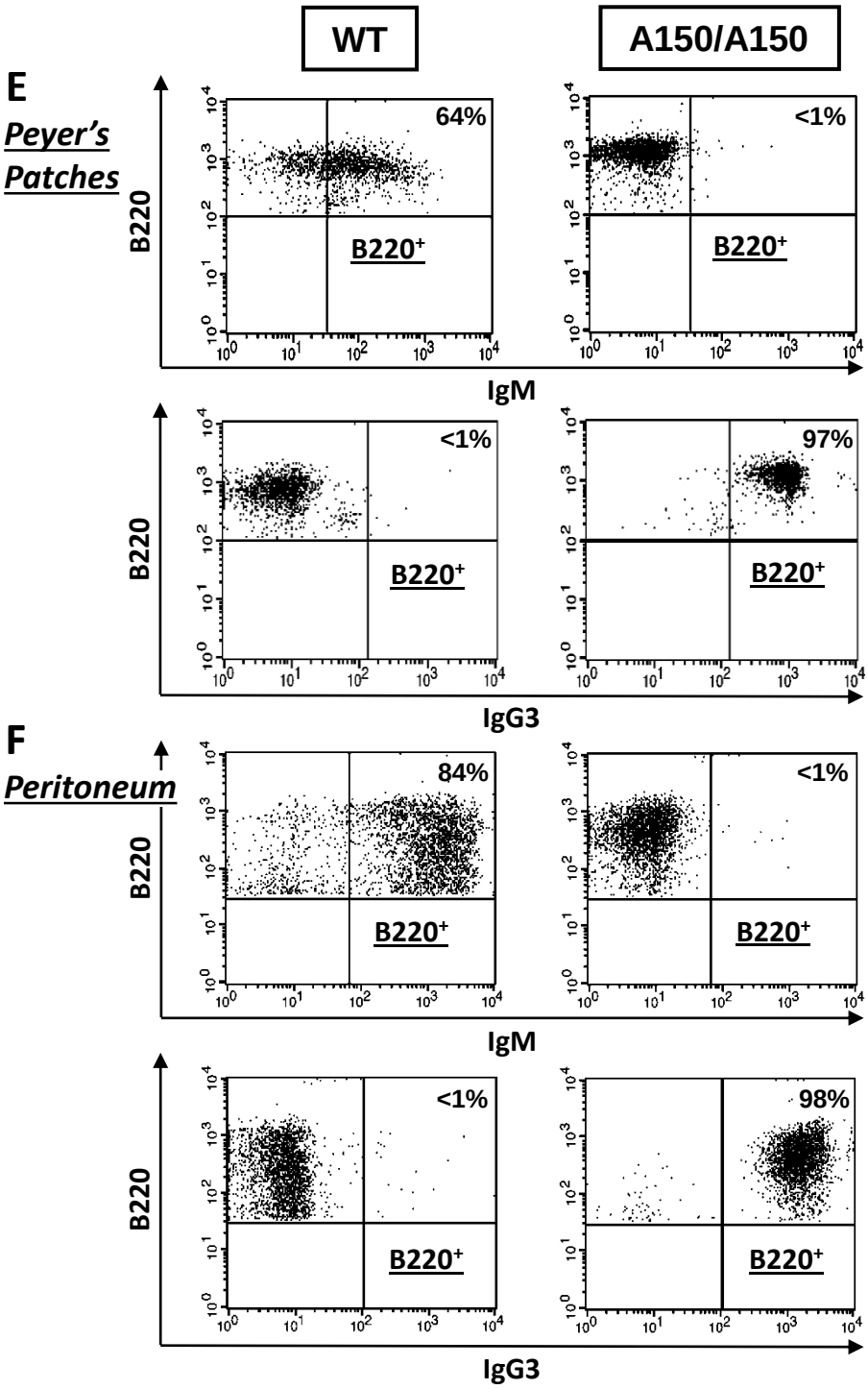
WT locus
~17 kb
~12.6 kb
B R X P
S
B
R
DQ52
JH
Eμ
Sμ
Cμ
Iγ3
Sγ3
Cγ3
3'RR
B
Targeting vector
X C R
C
B
neoR
VDJH2
Eμ
C
Homologous recombination and
Cre/loxP-mediated deletion of neoR
~2 kb
~7.5 kb
B R X C R
B
C
H B
R
5' probe
3' probe
D
Southern blot analysis
WT A150/+
~17 kb
~2 kb
EcoRI digest
5' probe
WT A150/+
~12.6 kb
~7.5 kb
BamHI digest
3' probe

Figure S2

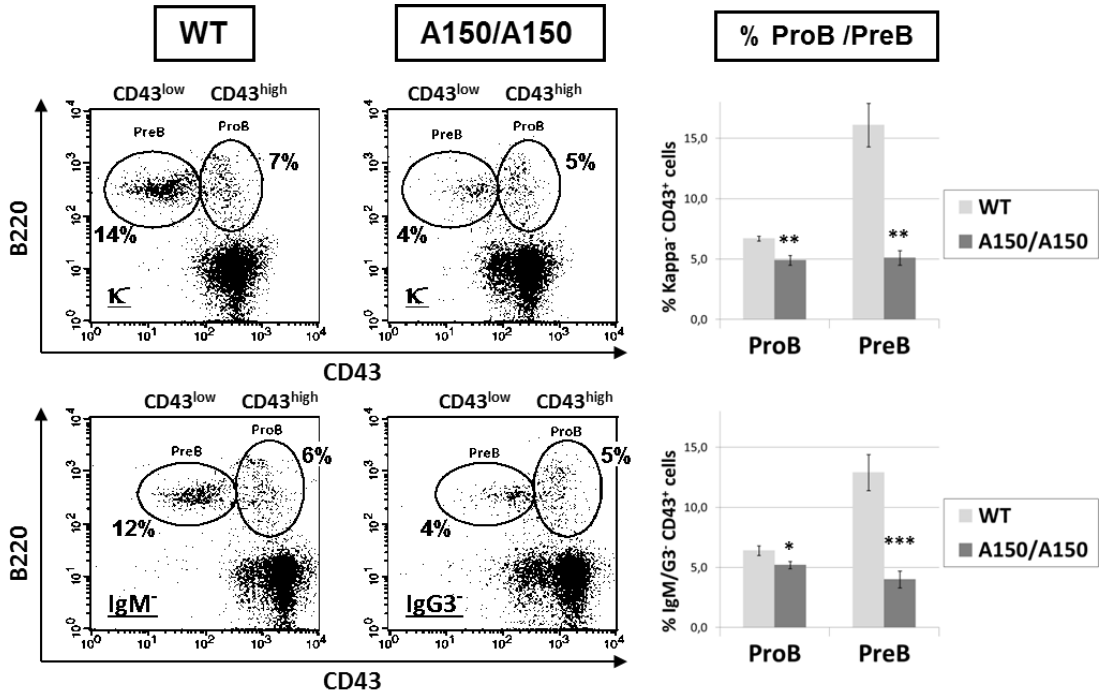

A
WT
A150/A150
B cells
Bone Marrow
Spleen
Peyer's Patches
Peritoneum
Fetal Liver
counts
B220
B220+
26%
18%
57%
69%
64%
72%
60%
77%
21%
27%
% of B220+ cells
% B220+ cells
***
ns
*
WT
A150/A150


WT
A150/A150
B
Bone Marrow
<1%
B220+
<1%
34%
51%
B220+
<2%
1%
IgG3
IgM
C
Spleen
0%
B220+
<1%
99%
98%
B220+
<2%
0%
IgG3
IgM
D
Fetal Liver
IgG3+
0%
B220+
IgM+
7%
IgG3+
16%
B220+
IgM+
<1%
IgG3
IgM


WT
A150/A150
E
Peyer's Patches
64%
<1%
B220+
B220
IgM
<1%
97%
B220+
B220
IgG3
F
Peritoneum
84%
<1%
B220+
B220
IgM
<1%
98%
B220+
B220
IgG3

Bone Marrow

G



H

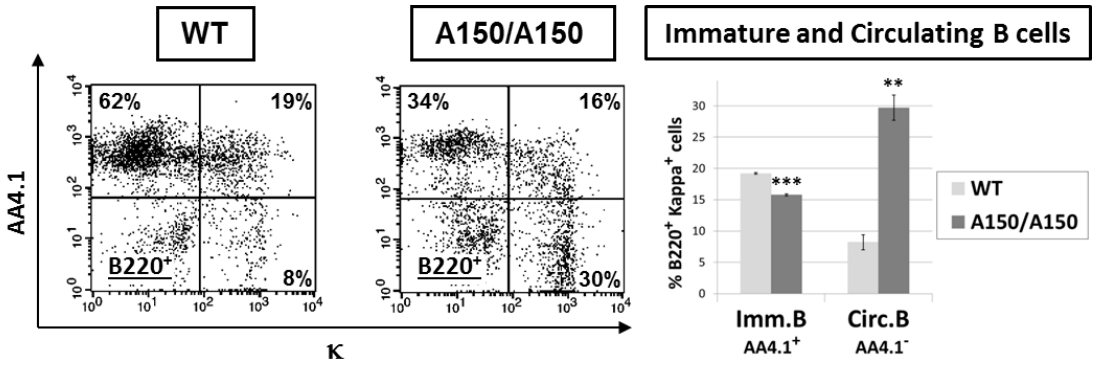


Figure S2

Spleen

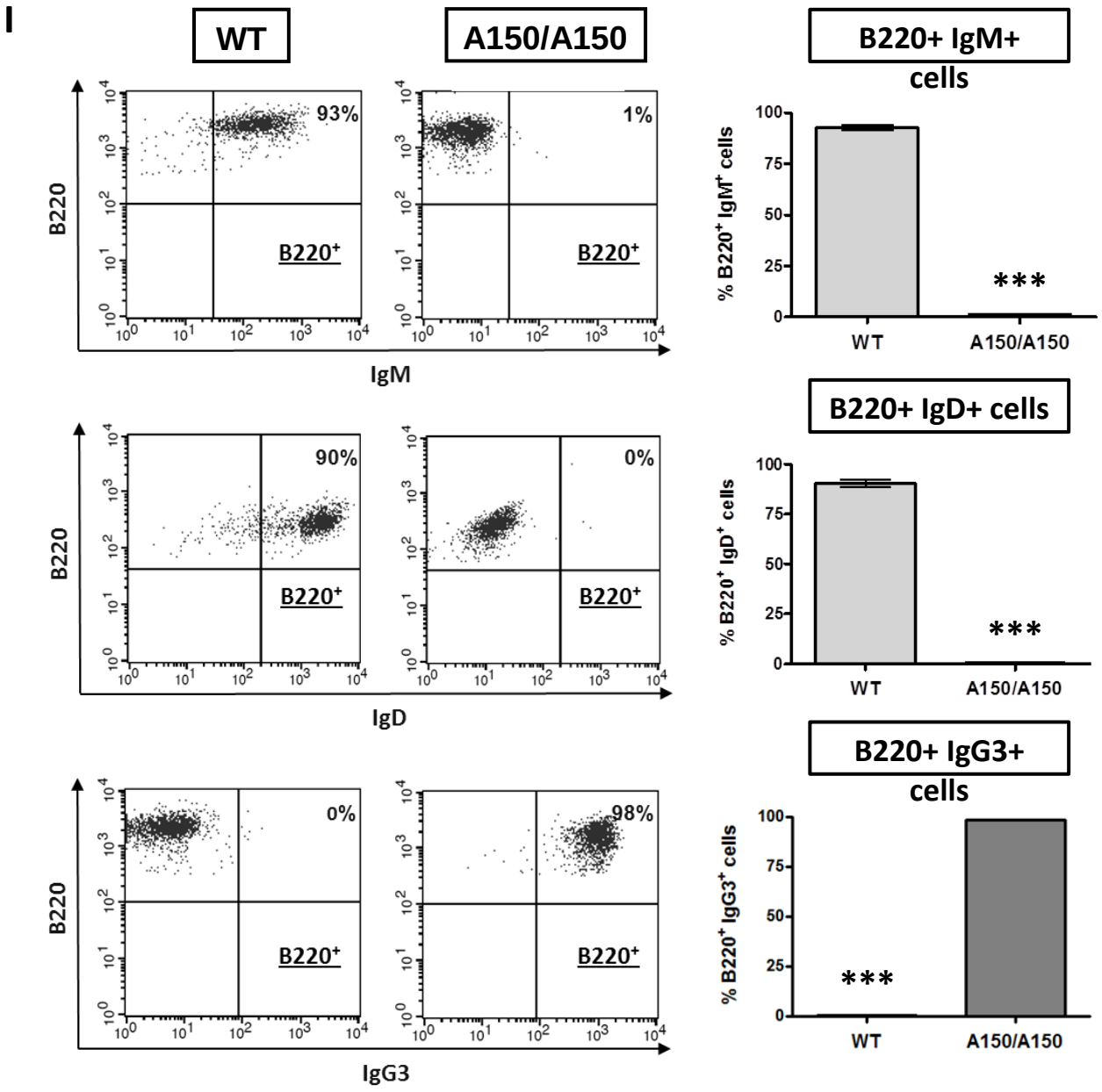
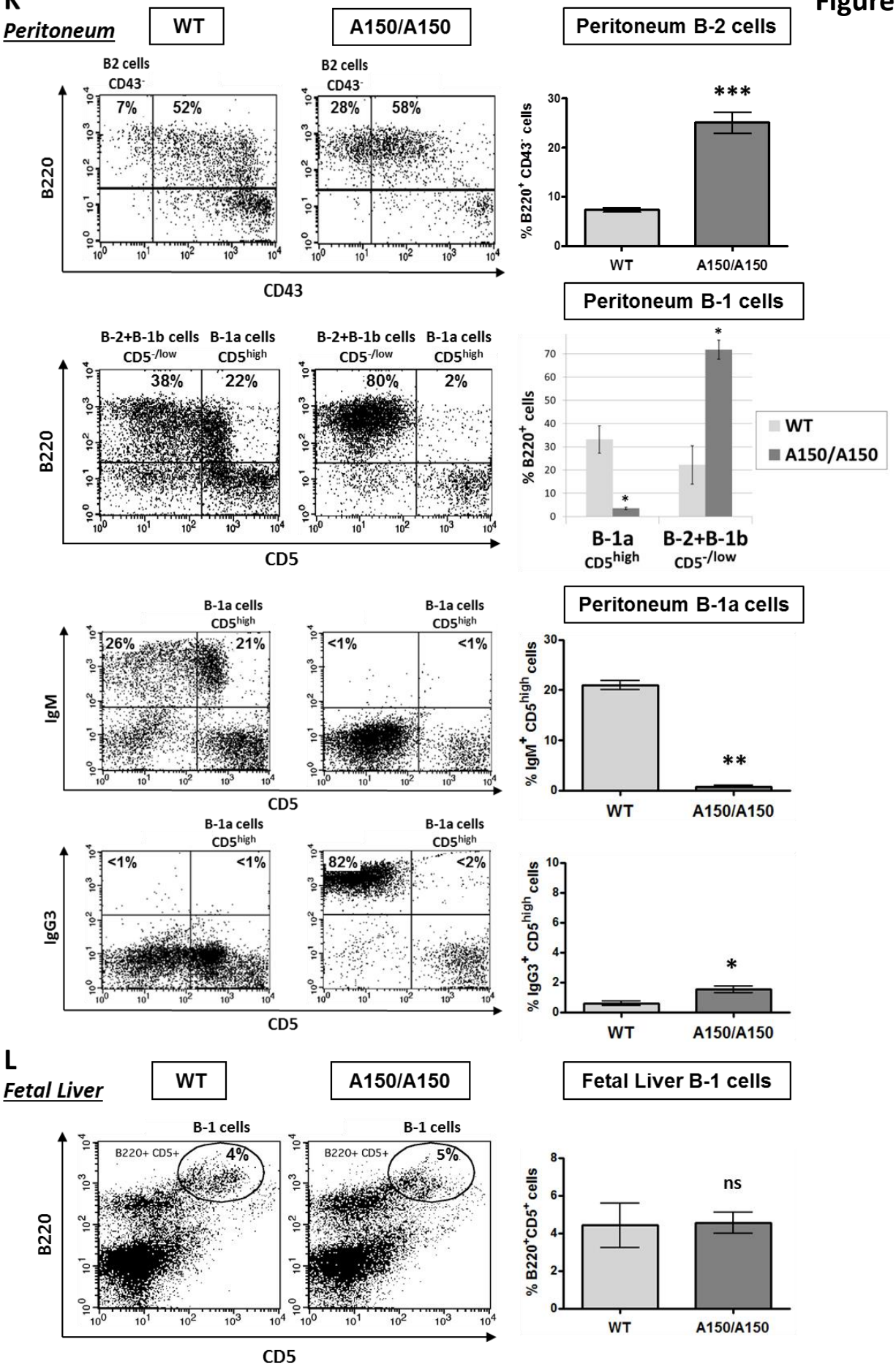
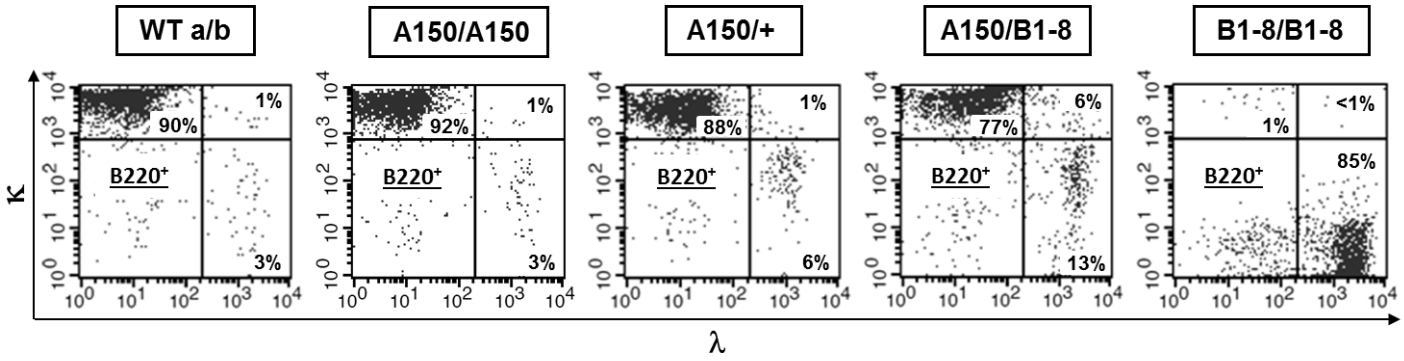


Figure S2


K
Peritoneum
WT
A150/A150
Peritoneum B-2 cells
B2 cells
CD43-
7%
52%
28%
58%
B220
CD43
% B220+ CD43- cells
***
WT
A150/A150
Peritoneum B-1 cells
B-2+B-1b cells
CD5-/low
B-1a cells
CD5high
38%
22%
80%
2%
CD5
% B220+ cells
*
B-1a
CD5high
B-2+B-1b
CD5-/low
Peritoneum B-1a cells
IgM
26%
21%
<1%
<1%
% IgM+ CD5high cells
**
IgG3
<1%
<1%
82%
<2%
% IgG3+ CD5high cells
*
L
Fetal Liver
WT
A150/A150
Fetal Liver B-1 cells
B-1 cells
B220+ CD5+
4%
5%
% B220+CD5+ cells
ns

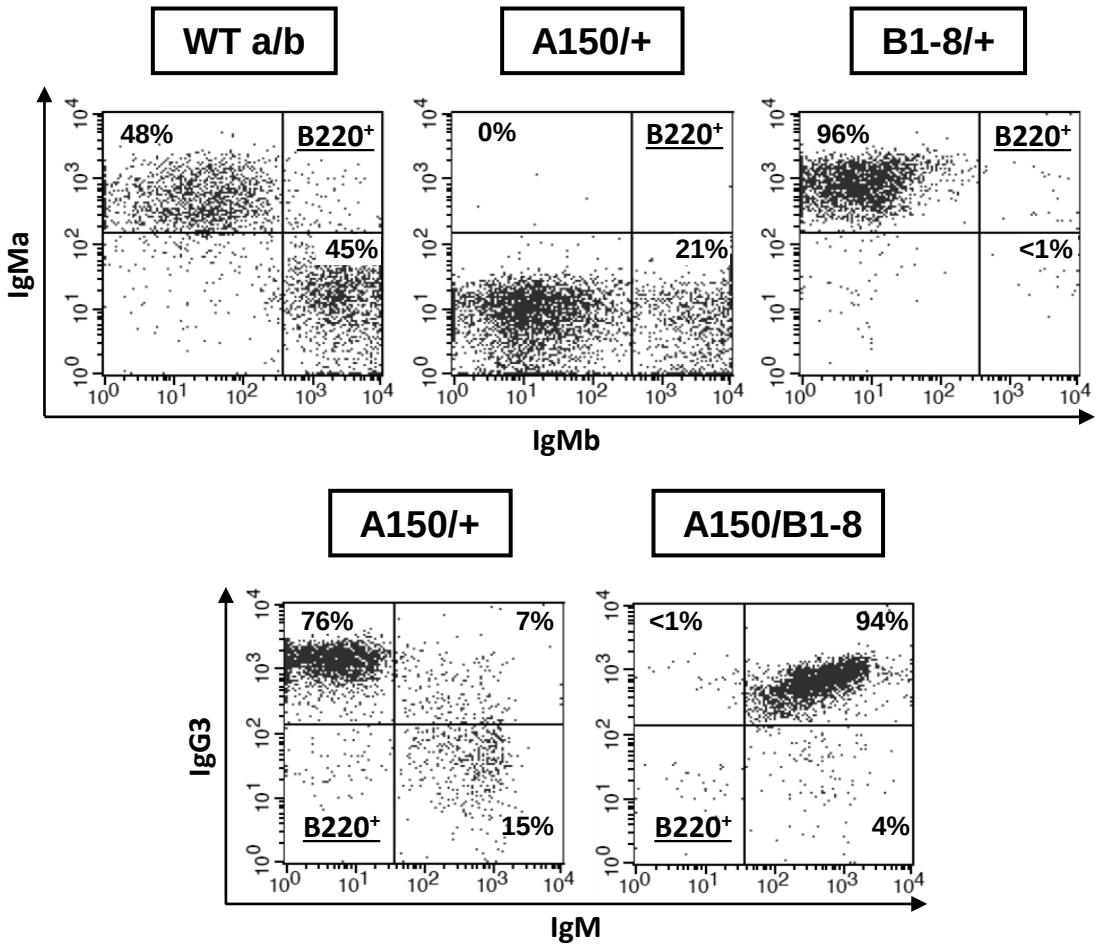
M

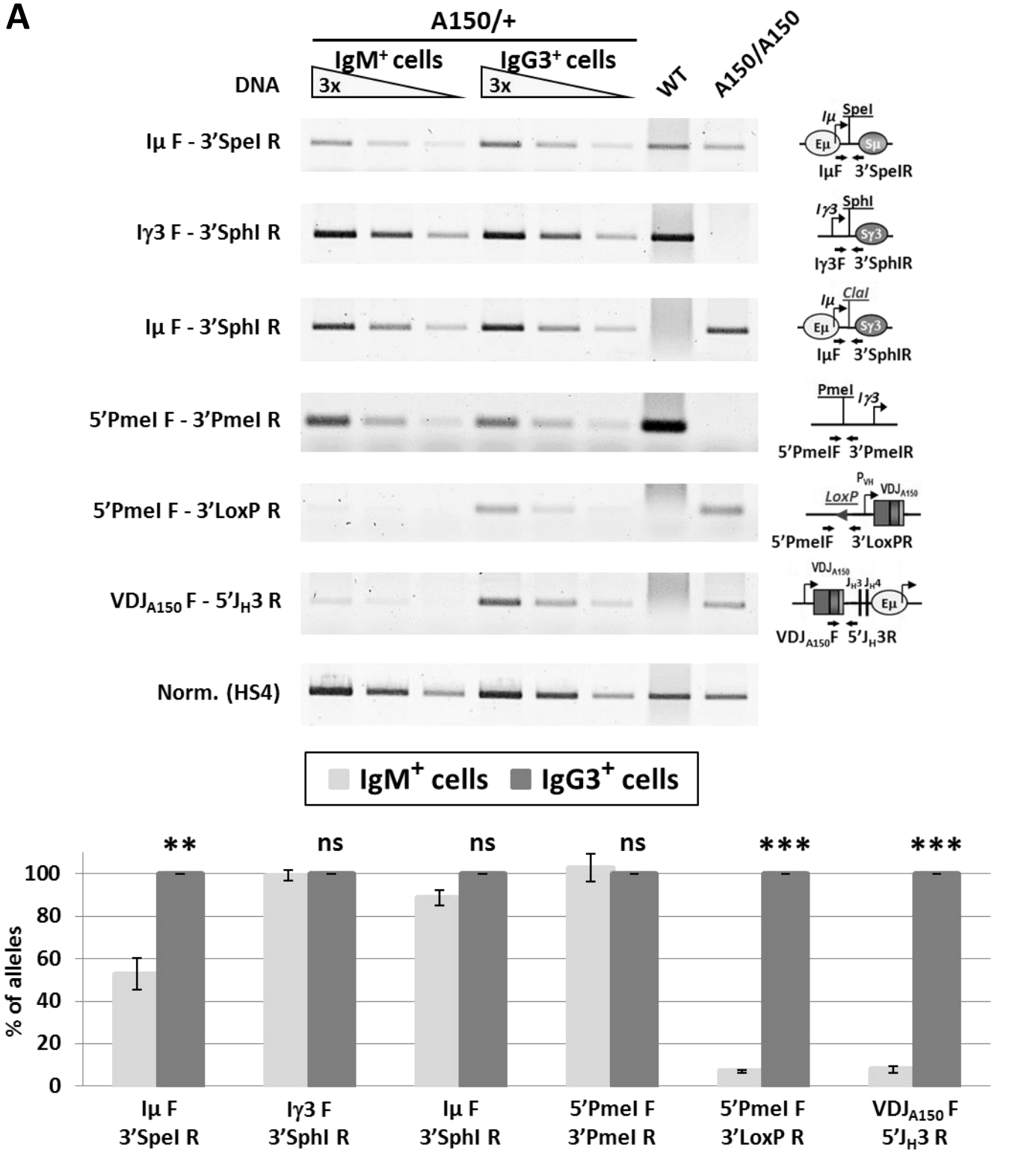
Spleen



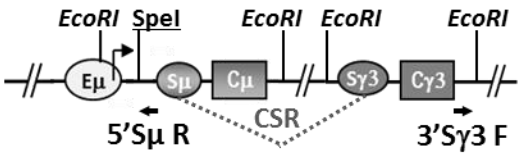
N

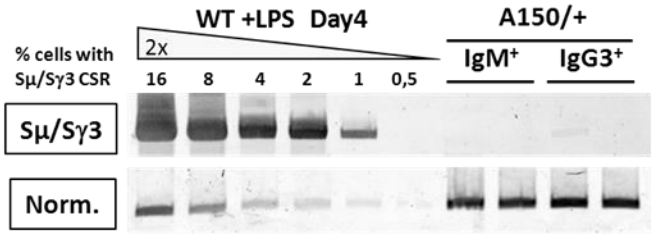
Spleen



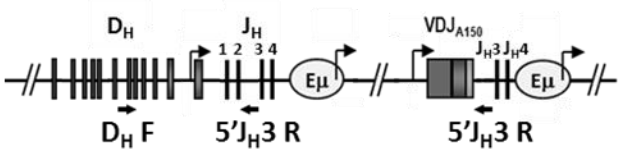

A
A150/+
IgM+ cells
IgG3+ cells
3x
3x
DNA
WT
A150/A150
Iμ F - 3'SpeI R
Iγ3 F - 3'SphI R
Iμ F - 3'SphI R
5'PmeI F - 3'PmeI R
5'PmeI F - 3'LoxP R
VDJA150 F - 5'JH3 R
Norm. (HS4)
Iμ SpeI
Eμ Sμ
IμF 3'SpeIR
Iγ3 SphI
Sγ3
Iγ3F 3'SphIR
Iμ ClaI
Eμ Sγ3
IμF 3'SphIR
PmeI Iγ3
5'PmeIF 3'PmeIR
PVH VDJA150
LoxP
5'PmeIF 3'LoxPR
VDJA150 JH3 JH4
Eμ
VDJA150F 5'JH3R
IgM+ cells
IgG3+ cells
**
ns
ns
ns
***
***
% of alleles
100
80
60
40
20
0
Iμ F 3'SpeI R
Iγ3 F 3'SphI R
Iμ F 3'SphI R
5'PmeI F 3'PmeI R
5'PmeI F 3'LoxP R
VDJA150 F 5'JH3 R

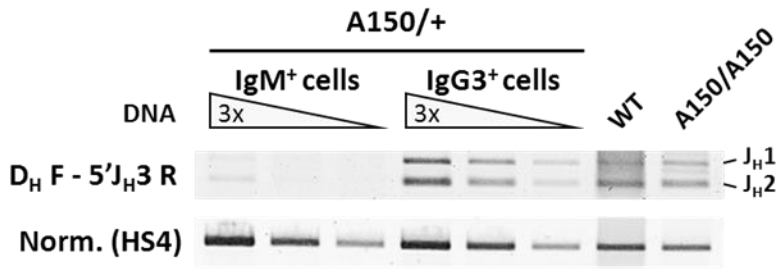
B

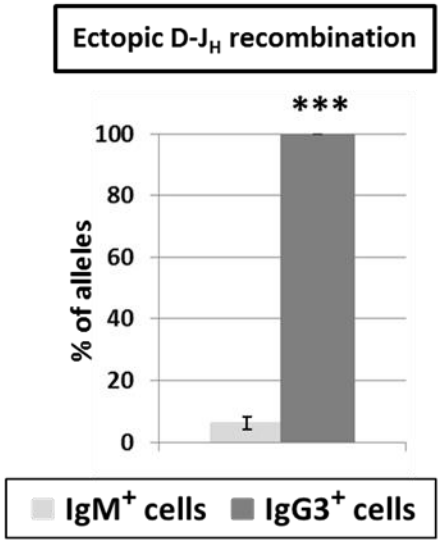

EcoRI
SpeI
EcoRI
EcoRI
EcoRI
Eμ
Sμ
Cμ
Sγ3
Cγ3
CSR
5'Sμ R
3'Sγ3 F

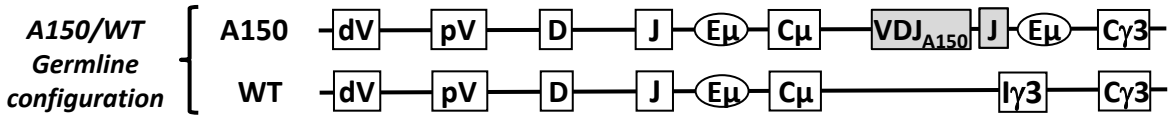

WT +LPS Day4
A150/+
% cells with Sμ/Sγ3 CSR
2x
16
8
4
2
1
0,5
IgM+
IgG3+
Sμ/Sγ3
Norm.

C

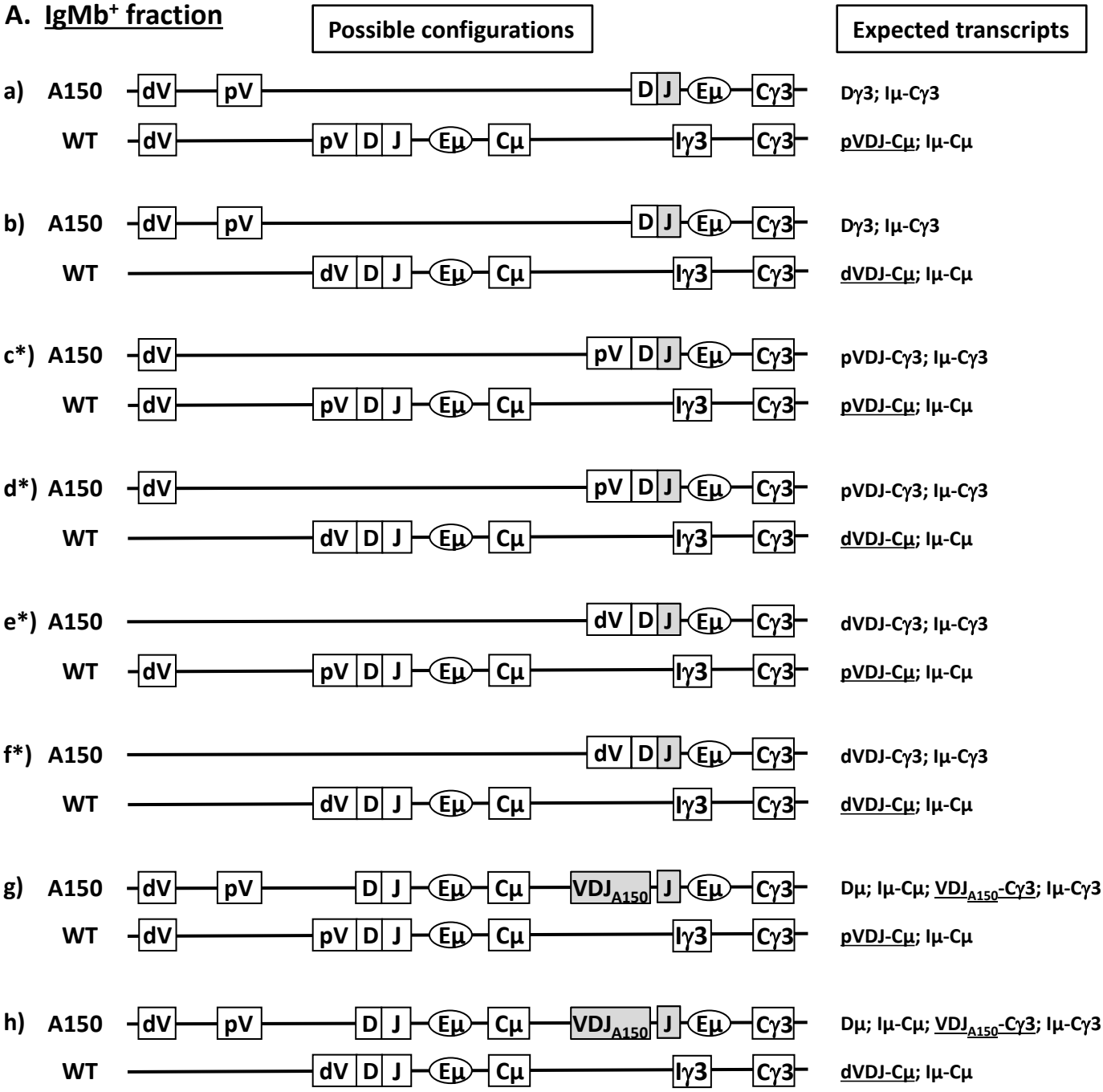

DH
JH
1 2 3 4
VDJA150
JH3 JH4
Eμ
Eμ
DH F
5'JH3 R
5'JH3 R


A150/+
IgM+ cells
IgG3+ cells
DNA
3x
3x
WT
A150/A150
DH F - 5'JH3 R
JH1
JH2
Norm. (HS4)


Ectopic D-JH recombination
***
100
80
60
40
20
0
% of alleles
IgM+ cells
IgG3+ cells



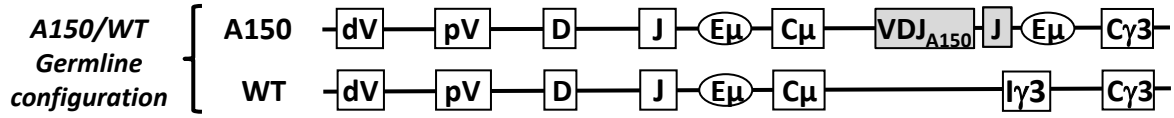
A. IgMb+ fraction



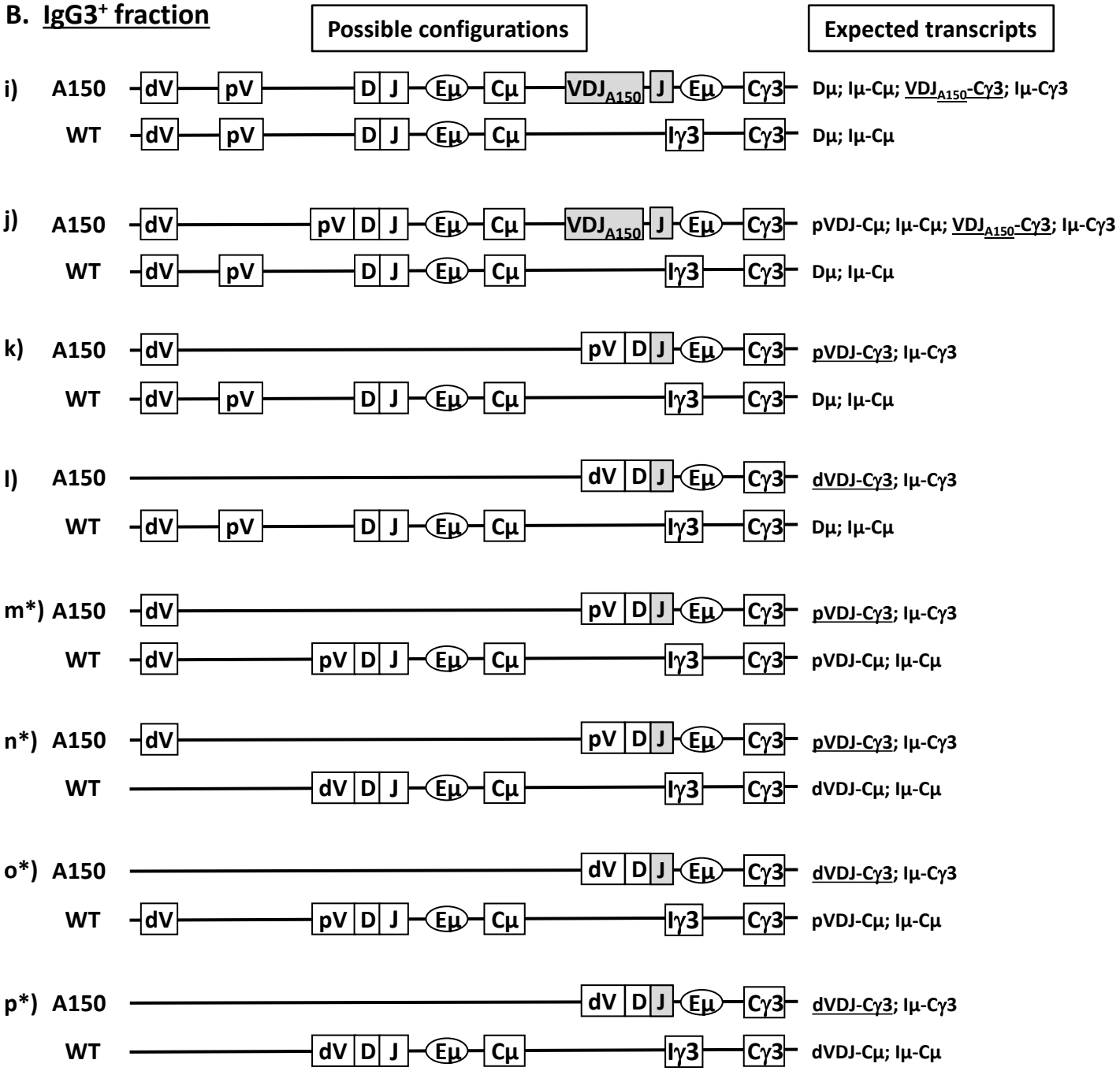
*** V-DJ rearrangements on the A150 allele occur first, but could be:**

1) **non-productive,**
2) **productive but γ3 heavy chains do not pair with surrogate light chains/light chains**
3) **productive and γ3 heavy chains pair with light chains, *en route* to double expression.**

Figure S4



B. IgG3+ fraction



*** V-DJ rearrangements on the WT allele occur first, but may be:**

1) **non-productive,**
2) **productive but μ heavy chains do not pair with surrogate light chains/light chains,**
3) **productive and μ heavy chains pair with light chains, *en route* to double expression.**

Sequences of the VDJ-Cγ3 cDNAs from sorted A150/WT BM B cells.

A. Amplified joints (pV_H7183-D-J_H3) from sorted $IgMb^+$ cells

Seq n°	V_H gene segment	N and P nucleotide additions	J_H3 segment
GL	...tac tgt gca aga		cc tgg ttt gct tac tgg ggc caa …
In frame (4)			
a02	…tac tgt gca aga	*cag ggg tcg g*	cc tgg ttt gct tac tgg ggc caa…
a09	…tac tgt gca aga	*ccc ccc tcc tac tat agg tac gac gta c*	gg ttt gct tac tgg ggc caa...
c09	...tac tgt gca aga	cct ccc tac tat gat tac gac gac gcg cct ct	g ttt gct tac tgg ggc caa...
c11	...tac tgt gca aga	*ccg cct act atg gta act c*	cc tgg ttt gct tac tgg ggc caa...
Out of frame (6)			
a07	…tac tgt gca aga	*gac ata ggt acg ccg*	gtt tgc tta ctg ggg cca agg…
b08	…tac tgt	*acc agg ctc cca act ggg acc c*	gt ttg ctt act ggg gcc aag…
c05	…tac tgt gtg aga	*cat agc tgc tcg atg gtt act gac ggg*	ggt ttg ctt act ggg gcc aag…
c10	...tac tgt gca aga	*ggt ggg cc*	t ttg ctt act ggg gcc aag...
d03	... tac tgt gtg aga	*gat gat tat tac tac ggt agt agc tac tct*	cct ggt ttg ctt act ggg gcc aag...
d09	…tac tgt gca aga	*ccc ccc ttt act ata ggt acg ccc*	cct ggt ttg ctt act ggg gcc aag…
Premature stop codon (3)			
a08	…tac tgt gca aga	*gat gca tga*	
b05	…tac t	*tt gca aga caa cgg tag*	
c07	…tac	*ggg tag*	

J_H3-**Cγ3**

cc tgg ttt gct tac tgg ggc caa ggg act ctg gtc act gtc tct gca g**ct aca aca aca gcc cca tct gtc tat ccc ttg gtc cct ggc tgc agt gac aca tct gga tcc tcg gtg aca ctg gga tgc ctt gtc aaa ggc tac ttc cct gag …**

B. Amplified joints (pV_H7183-D-J_H4) from sorted $IgMb^+$ cells

Seq n°	V_H gene segment	N and P nucleotide additions	J_H4 segment
GL	...tac tgt gca aga		at tac tat gct atg gac tac tgg ggt…
In frame (13)			
a03	…tac tgt gca aga	*ccc ttt gat ggc ggt*	tgg ggt…
a04	…tac tgt	*acc agg ccg aga aga tta cga cgg gg*	t tac tat gct atg gac tac tgg ggt…
a10	… tac	*aag cct act ata ggt acg acc ttc*	tat gct atg gac tac tgg ggt…
b06	…tac tgt gca aga	*cca tac cct*	tat gct atg gac tac tgg ggt…
b07	…tac tgt gca a	*aa gat ccc ccc atc tac tat gat tac gag cc*	c tat gct atg gac tac tgg ggt…
b10	…tac tgt gca a	*ag tat tac tac ggc tac g*	ac tat gct atg gac tac tgg ggt…
c03	…tac tgt gca aga	*agg cct act ata ggc ac*	g gac tac tgg ggt…
c12	...tac tgt gca aga	*cat aga acg atc cga tgg tta ccc gca t*	at tac tat gct atg gac tac tgg ggt...
d01	...tac tgt g	tg aag tat ggt aac tac ctt a	ac tgg ggt...
d04	…tac tgt g	ta acg gta gtc agt tt	c tat gct atg gac tac tgg ggt...
d07	…tac tgt gca aga	*ggg tct acc ggg*	gct atg gac tac tgg ggt…
d10	…tac tgt gca ag	*g cga tct act atg att acc tca t*	at tac tat gct atg gac tac tgg ggt…
d11	…tac tgt gtg aga	*gtg ggg gt*	t atg gac tac tgg ggt…
Out of frame (8)			
a05	…tac tgt gca aga	*gac gcc cga ctg gga cg*	t act atg cta tgg act act agg gtc…
a06	… tac tgt gca aga	*ggt aac tcg cca*	tgc tat gga cta ctg ggg tca…
b02	…tac tgt gca aga	*gat aag gct atg att acg tac ggc ct*	t atg cta tgg act act ggg gtc…
b03	…tac tgt gca aga		…cag **cta**…
c02	…tac tgt gca aga	*cat cct act atg atg gtc cc*	a ctg ggg tca…
c06	…tac tgt gca aga	*gac gcc cga ctg gga cg*	t act atg cta tgg act act ggg gtc…
c08	…tac tgt	*acc agg gtt atg att acg acg aga ggg gac*	cta tgc tat gga cta ctg ggg tca...
d06	... tac tgt gca aga	*cat ggg gat ggt aac cc*	t atg cta tgg act act ggg gtc...
Premature stop codon (7)			
a01	…tac t*ga*	(ata ggt)	
b01	… tac tg	*g gcc cta tga*	
b11	… tac tgt gca aga	*gat ata cgt cta cta tag*	
b12	… tac	*ggg cta tga*	
c01	… tac tgt gca ag	*c ccc cca tag*	
d02	… tac tgt gca aga	*gat ata cgt cta cta tag*	
d08	…tac tgt gca aga	*cga agg ctt cta tag*	

J_H4-**Cγ3**

at cat tat gct atg gac tac tgg ggt caa gga acc tca gtc acc gtc tcc tca g**ct aca aca aca gcc cca tct gtc tat ccc ttg gtc cct ggc tgc agt gac aca tct gga tcc tcg gtg aca ctg gga tgc ctt gtc aaa ggc tac ttc cct gag**

C. Amplified joints ($dV_H J558$-D-$J_H 3$) from sorted IgMb$^+$ cells

Seq n°	V_H gene segment	N and P nucleotide additions	$J_H 3$ segment
GL	...tac tgt gca aga		cc tgg ttt gct tac tgg ggc caa …
In frame (4)			
e08	...tac tgt gca aga	*tcg gta ccg ggc ggg g*	gg ggc caa...
f10	...tac tgt gca aga	*gag aat ggt tac ccc c*	tt gct tac tgg ggc caa...
h01	...ttc tgt gca aga	*gaa gaa gga tta cgg cc*	g ttt gct tac tgg ggc caa...
h05	...tat tgt gca aga	*gag ggg ggt atc t*	cc tgg ttt gct tac tgg ggc caa...
Out of frame (6)			
e02	…tac tgt aca aga	*cgg ggt aac tac gtc*	gtt tgc tta ctg ggg cca agg…
f12	…tac tgt aca aga	*cgg gga tta cgg gg*	c ctg gtt tgc tta ctg ggg cca agg…
g03	…tac tgt gca aga	*ggg ggt act acg gta ccc t*	gg gcc aag…
f03	…tac tgt gca	*cgc tcc tcc gtc tat gat tac gg*	g ctt act ggg gcc aag…
g10	…tac tgt gca aga	*gaa ggg act atg gag gct g*	tg ggg cca agg…
e06	…tac tgt gca aga	*gaa ggg gat ggt aac ag*	c ctg gtt tgc tta ctg ggg cca agg…
Premature stop codon (7)			
e11	…tac tgt gca aga	*tgg gga cta tag*	
g05	…tac tgt gca ag	*t gga cta tag*	
e04	…ttc tgt gca aga	*ggg ggc cta tga*	
f09	…tat tgt gca aga	*tag*	
g04	…ttc tgt gca aga	*tgg taa*	
g07	…tac tgt gca a	*ct tga*	
h11	... tac tgt gca aga	*ggg tac tac ggt agt agc taa*	
Pseudogenes (1)			
f07			

$J_H 3$-**Cγ3**

cc tgg ttt gct tac tgg ggc caa ggg act ctg gtc act gtc tct gca g**ct aca aca aca gcc cca tct gtc tat ccc ttg gtc cct ggc tgc agt gac aca tct gga tcc tcg gtg aca ctg gga tgc ctt gtc aaa ggc tac ttc cct gag …**

D. Amplified joints ($dV_H J_{558}$-D-J_H4) from sorted IgMb$^+$ cells

Seq n°	V_H gene segment	N and P nucleotide additions	J_H4 segment
GL	...tac tgt gca aga		at tac tat gct atg gac tac tgg ggt…
In frame (5)			
e10	…tac tgt gca ag	*g ggg gga tgg tta tt*	t gct atg gac tac tgg ggt…
f04	...tac tgg gca	*tta cta cgg cta cgt gag aga gat tat agg ctt*	gac tac tgg ggc...
g08	...tac tgt gca aga	*tgt ggg ggt aac tac cca caa gtg gga cta*	tac tat gct atg gac tac tgg ggt...
h03	...tac tgt gca	*tgg cgg act ata ggt acg acg gga gac cct*	tat gct atg gac tac tgg ggt...
h08	... gac act gca	*gtc tcc ttc ata ggt at*	c tat gct atg gac tac tgg ggt...
Out of frame (8)			
e03	...tac tgt gca aga	*tgg gcc tac tat ggt aac tac gag ggt tt*	a tta cta tgc tat gga cta ctg ggg...
e05	...tct gcg gcc agg	*cct act ata aac ccg*	act ggg gtc...
f01	...tac tgt gca aga	*gga agg ggc att ccc t*	ct atg cta tgg act act ggg gtc...
f08	...tac tgt gca a	*ct ggg aaa cgg g*	ct atg cta tgg act act ggg gtc…
g06	...tac tgt gca aga	*tct aac tgg gag ggc att t*	ta tgc tat gga cta ctg ggg tca...
g11	…tac tgt gca aga	*ggt ggt tac tac tct atg*	cta tgg act act ggg gtc…
g12	…tac tgt gca aga	*gag gag gca ccg ggg*	cta tgc tat gga cta ctg ggg tca…
h02	...tac	*tac ggt acc cc*	t act atg cta tgg act act ggg gtc…
Premature stop codon (5)			
e01	…tac tgt gca	*ggt ata cta tag*	
e09	…tac tgt gca aga	*agg tag*	
f06	…tac tgt gca	*gaa gat tta cta cgg tag*	
f11	…ttc tgt gca aga	*caa tga*	
h07	… tac tgt	*act cga tgg taa*	
Pseudogenes (2)			
h04			
h05			

J_H4-**Cγ3**

at cat tat gct atg gac tac tgg ggt caa gga acc tca gtc acc gtc tcc tca g**ct aca aca aca gcc cca tct gtc tat ccc ttg gtc cct ggc tgc agt gac aca tct gga tcc tcg gtg aca ctg gga tgc ctt gtc aaa ggc tac ttc cct gag**

E. Amplified joints (pV_H^{7183}-D-J_H3) from sorted $IgG3^+$ cells

Seq n°	V_H gene segment	N and P nucleotide additions	J_H3 segment
GL	...tac tgt gca aga		cc tgg ttt gct tac tgg ggc caa …
In frame (11)			
a04	…tac tgt	*acg gat gta aga ggg aag g*	tt gct tac tgg ggc caa…
b12	… tac tgt gca aga	*gag gat gat tac gcc ca*	g ttt gct tac tgg ggc caa…
c04	… tac tgt gca aga	*ggg ggc cat gat ggt tac tac tcc*	ttt gct tac tgg ggc caa…
c05	… tac tgt gca aga	*cat gtg atg gtt act acg g*	cc tgg ttt gct tac tgg ggc caa…
c06	... tac tgt gca aga	*cat gga cct cc*	g ttt gct tac tgg ggc caa...
c10	...tac tgt gca aga	*cat agc g*	gg ttt gct tac tgg ggc caa...
d04	… tac tgt gtg aga	*caa agc agt t*	cc tgg ttt gct tac tgg ggc caa…
d05	… tac tgt gca aga	*cat gag ggg ggt tac t*	cc tgg ttt gct tac tgg ggc caa…
d07	... tac tgt gca aga	*gga gga caa ctg gga agg tcg g*	cc tgg ttt gct tac tgg ggc caa...
d08	… tac tgt gtg aga	*gag ggt ggt aac tac ggg aaa gga g*	cc tgg ttt gct tac tgg ggc caa…
d09	… tac tgt gca aga	*cat aac tac ggt agt agc tac*	ttt gct tac tgg ggc caa…
Premature stop codon (1)			
c07	... tac tgt gca ag	*g tat tga*	

J_H3-**Cγ3**

cc tgg ttt gct tac tgg ggc caa ggg act ctg gtc act gtc tct gca g**ct aca aca aca gcc cca tct gtc tat ccc ttg gtc cct ggc tgc agt gac aca tct gga tcc tcg gtg aca ctg gga tgc ctt gtc aaa ggc tac ttc cct gag …**

F. Amplified joints (pV_H7183-D-J_H4) from sorted $IgG3^+$ cells

Seq n°	V_H gene segment	N and P nucleotide additions	J_H4 segment
GL	...tac tgt gca aga		at tac tat gct atg gac tac tgg ggt…
In frame (29)			
a01	…tac tgt gca a	*cc ctc ccc tca tta cta cgg ccc tta t*	at tac tat gct atg gac tac tgg ggt…
a02	…tac tgt gca aga	*agc gga cgt g*	at gct atg gac tac tgg ggt…
a03	…tat tgt agt aga	*tcc tgg gcg agg g*	at gct atg gac tac tgg ggt…
a06	…tac tgt gca aga	*tca agg acc ccc c*	ac tat gct atg gac tac tgg ggt…
a07	…tac tgt gca aga	*cat ggg ggg gta cca tgg*	gct atg gac tac tgg ggt…
a09	…tac tgt gca aga	*ggg ggg tac gac g*	at gct atg gac tac tgg ggt…
a10	…tac tgt gca aga	*cat cag gct atg att acc tca t*	at tac tat gct atg gac tac tgg ggt…
a11	…tac tgt gca aga	*cat gac tcc tgg gcc*	tac tat gct atg gac tac tgg ggt…
b01	…tac tgt gca aga	*gat aag gaa a*	at tac tat gct atg gac tac tgg ggt…
b02	…tac tgt gca aga	*gaa ggg gtt acg aca ggg g*	at tac tat gct atg gac tac tgg ggt…
b03	…tac tgt gca aga	*tcc tcc ccc tac cga ggg tac ttc gat gtc*	tgg ggc…
b04	…tac tgt gca aga	*cat gcg gac tac ggt agt agc ccc tgg*	gct atg gac tac tgg ggt…
b05	… tac tgt gca aga	*caa ggg ggg gat ctc tat gat ggt tac tac a*	at gct atg gac tac tgg ggt…
b06	… tac tgt gca aga	*caa gag gga ccc tc*	t tac tat gct atg gac tac tgg ggt…
b07	… tac tgt gca aga	*gaa tcg g*	ac tat gct atg gac tac tgg ggt…
b08	… tac tgt gca aga	*ggc cgg ggg t*	tg gac tac tgg ggt…
b09	…tac tgt gca aga	*gtt tat tac tac ggt agt gag*	gct atg gac tac tgg ggt…
b10	… tac tgt gca ag	*g ggg gtc tac tat ggt tac gac gaa gag a*	at gct atg gac tac tgg ggt…
b11	… tac tgt gca aga	*gaa ggg gtt acg aca ggg g*	at tac tat gct atg gac tac tgg ggt…
c02	… tac tgt gca aga	*ggg gca aac tgg gac tac ttt*	gac tac tgg ggc…
c03	… tac tgt gca ag	*c ctt tgg ccc t*	ct atg gac tac tgg ggt…
c08	… tac tgt gca aga	*cat gaa ggg ggt aag gag ggg aaa atg*	gct atg gac tac tgg ggt…
c09	… tac tgt gca aga	*ggg aaa ctg gga gag ct*	t gct atg gac tac tgg ggt…
c12	… tac tgt gca aga	*acc ctc tac ctc*	tac tat gct atg gac tac tgg ggt…
d01	… tac tgt gca aga	*gaa aac tat agg*	tat gct atg gac tac tgg ggt…
d02	… tac tgt gca ag	*c tct atg att acg ac*	t tac tat gct atg gac tac tgg ggt…
d03	… tac tgt gca aga	*cag agc tg*	c tat gct atg gac tac tgg ggt…
d06	… tac tgt gca aga	*gat cga ggt aat gg*	t gct atg gac tac tgg ggt…
d12	… tac tgt aca aga	*gga ctg gcg*	tac tat gct atg gac tac tgg ggt…
Premature stop codon (1)			
c11	... tac tat gct a	*tg gac tac tag*	

J_H4-**Cγ3**

at cat tat gct atg gac tac tgg ggt caa gga acc tca gtc acc gtc tcc tca g**ct aca aca aca gcc cca tct gtc tat ccc ttg gtc cct ggc tgc agt gac aca tct gga tcc tcg gtg aca ctg gga tgc ctt gtc aaa ggc tac ttc cct gag**

G. Amplified joints (dV_HJ558-D-J_H3) from sorted $IgG3^+$ cells

Seq n°	V_H gene segment	N and P nucleotide additions	J_H3 segment
GL	...tac tgt gca aga		cc tgg ttt gct tac tgg ggc caa …
In frame (5)			
e11	…tac tgt gca aga	*gac ctt*	ttt gct tac tgg ggc caa …
f06	…tac tgt aca aga	*gat acg atg atg gtt act g*	cc tgg ttt gct tac tgg ggc caa…
f10	... ttc tgt gct aga	*gtg agg gac tac tat gat tac gac ggc*	ttt gtt tac tgg ggc caa...
f11	... tac tgt gc	*g cta ggg ggg acc cct ctt*	ttt gct tac tgg ggc caa...
h09	… tac tgt aca aga	*tcg gct atg gta act acg ttg g*	cc tgg ttt gct tac tgg ggc caa…

J_H3-**Cγ3**

cc tgg ttt gct tac tgg ggc caa ggg act ctg gtc act gtc tct gca g**ct aca aca aca gcc cca tct gtc tat ccc ttg gtc cct ggc tgc agt gac aca tct gga tcc tcg gtg aca ctg gga tgc ctt gtc aaa ggc tac ttc cct gag …**

H. Amplified joints (dV_HJ558-D-J_H4) from sorted IgG3$^+$ cells

Seq n°	V_H gene segment	N and P nucleotide additions	J_H4 segment
GL	...tac tgt gca aga		at tac tat gct atg gac tac tgg ggt…
In frame (37)			
e01	…tac tgt gca aga	*gcc tgg gac g*	ac tat gct atg gac tac tgg ggt…
e02	…tat tgt gca aga	*agg aat agt aac tac ttt*	tat gct atg gac tac tgg ggt…
e03	…ttc tgt gca aga	*acg gt*	t tac tat gct atg gac tac tgg ggt…
e05	…tac tgt gca aga	*agc tat ggt a*	ac tat gct atg gac tac tgg ggt…
e06	…tac tgt gga aga	*agg gac tgg gac gt*	t gct atg gac tac tgg ggt…
e07	…ttc tgt gca aga	*tcc ggc gct agg act g*	at gct atg gac tac tgg ggt…
e08	…tac tgt aca aga	*gac tat agg tac gac gaa t*	ac tat gct atg gac tac tgg ggt…
e09	…tac tgt gca ag	*g gag atg gta act gag gtt t*	ac tat gct atg gac tac tgg ggt…
e10	…ttc tgt gca aga	*gga cgg ccg*	tat gct atg gac tac tgg ggt…
e12	…tac tgt aca aga	*tgg gga ctg gga ggg gat t*	ac tat gct atg gac tac tgg ggt…
f01	…tac tgt gca aga	*ggg agg act acg*	gct atg gac tac tgg ggt…
f03	…ttc tgt gc	*c tgt tac tac gac tat c*	ct atg gac tac tgg ggt…
f04	…tac tgt gca ag	*g ccg atc tat gat ggt tac ccc tt*	t gct atg gac tac tgg ggt…
f05	…tac tgt aca aga	*gcg gaa ctg ggc ct*	c tat gct atg gac tac tgg ggt…
f08	…tac tgt gca aga	*cat ggt aac t*	ac tat gct atg gac tac tgg ggt…
f09	…tac tgt gca aga	*gcc cct tac tac ggt cat t*	ac tat gct atg gac tac tgg ggt…
g01	…tac tgt gca ag	*g ggg gtg gga cga cgg gat t*	ac tat gct atg gac tac tgg ggt…
g02	…tac tgt gca aga	*agg aca ggt ct*	c tat gct atg gac tac tgg ggt…
g03	…tat tgt gca aga	*tca ggg tat ggt aac tac gta a*	at gct atg gac tac tgg ggt...
g04	…ttc tgt gca aga	*ggg gga cag gg*	c tat gct atg gac tac tgg ggt…
g05	…tac tgt aca aga	*tcc ctc ggg gcg g*	ac tat gct atg gac tac tgg ggt…
g06	…tac tgt gca aga	*atg gga gta tgg g*	ac tat gct atg gac tac tgg ggt…
g07	…tac tgt aca aga	*gtt aac tgg gac gag*	gct atg gac tac tgg ggt…
g08	…tac tgt gca aga	*acc tcc ct*	c tat gct atg gac tac tgg ggt…
g09	…tac tgt gca a	*ta ggt acg acg agg g*	ac tat gct atg gac tac tgg ggt…
g10	…tac tgt gca aga	*ggg act atg gta act cct*	tat gct atg gac tac tgg ggt…
g11	…tac tgt gca a	*ta ggt acg*	gct atg gac tac tgg ggt…
g12	…tac tgt gca aga	*caa ctg ggt t*	ac tat gct atg gac tac tgg ggt…
h02	…tac tgt gca aga	*ggc ggt tta cta cgg cta c*	ac tat gct atg gac tac tgg ggt…
h03	…tac tgt gca aga	*tcg gag ggt ata ggt acg acg tgg ggt ggg acg*	gct atg gac tac tgg ggt…
h05	…tac tgt gca aga	*gct ggg acc cct*	tat gct atg gac tac tgg ggt…
h06	…tac tgt aca ag	*t tct atg gta act acg tt*	t gct atg gac tac tgg ggt…
h07	…tac tgt gca aga	*gga gct atg atg gtt acc ccc ttg ggg*	gct atg gac tac tgg ggt…
h08	…tac tgt gca aga	*gga gaa ggt t*	ac tat gct atg gac tac tgg ggt…
h10	…tac tgt gca aga	*atc ccg ccg ggt t*	ac tat gct atg gac tac tgg ggt…
h11	…tac tgt gca aga	*ggg cct agt atg gta acc cct t*	ac tat gct atg gac tac tgg ggt…
h12	…ttc tgt gca aga	*tct ccc tat gat t*	ac tat gct atg gac tac tgg ggt…
Pseudogene (1)			
f07			

J_H4-**Cγ3**

at cat tat gct atg gac tac tgg ggt caa gga acc tca gtc acc gtc tcc tca g**ct aca aca aca gcc cca tct gtc tat ccc ttg gtc cct ggc tgc agt gac aca tct gga tcc tcg gtg aca ctg gga tgc ctt gtc aaa ggc tac ttc cct gag**

Sequences of the V_H-D-J_H4 junctions from WT and A150 Pro-B cells.

A. Amplified joints (V_H7183-D-J_H4) from WT Pro-B cells

Seq n°	V_H gene segment	N and P nucleotide additions	J_H4 segment
GL	...tac tgt gca aga		at tac tat gct atg gac tac tgg ggt…
In frame (21)			
01	…tac tgt gca aga	cat gaa gag	atg gac tac tgg ggt…
02	…tac tgt	acc agg agg gcc ccc gcc tac tat agg tac gac gg	c tat gct atg gac tac tgg ggt…
03	…tac tgt gca aga	cat agg tct acg gag	gct atg gac tac tgg ggt…
04	…tac tgt gca aga	cat caa agg ccc ttc att act acg gct acg gga cca ttt a	ac tat gct atg gac tac tgg ggt…
05	…tac tgt gca aga	cat cag gat tac ctc gcc aa	t tac tat gct atg gac tac tgg ggt…
07	…tac tgt gca aga	gat ccc tcc ttc tac tat gat tac gac ggg	tac tat gct atg gac tac tgg ggt…
10	…tac tgt gca aga	cat cga att act acc c	ct atg gac tac tgg ggt…
17	…tac tgt	acc agg cca agg ggg gac tac ggc tgg g	ac tat gct atg gac tac tgg ggt…
18	…tac tgt gca aga	ccg ggg gcg ag	c tac tgg ggt…
19	…tac tgt	acc agg tgt ggt tac ctc tca t	at tac tat gct atg gac tac tgg ggt…
21	…tac tgt gca aga	cat agc ccc cac tac tat ggt aac tac gg	t gct atg gac tac tgg ggt…
22	…tac tgt gca aga	gag gac tat gaa acg t	ct atg gac tac tgg ggt…
23	…tac tgt gca aga	gat gca tgg ggt tac tac g	at gct atg gac tac tgg ggt…
24	…tac tgt gca aga	gat agg gga tta cta cgg cta cgt gag g	tg gac tac tgg ggt…
29	…tac tgt gca aga	ccc ttt att act acg gta gta gcg	tac tat gct atg gac tac tgg ggt…
35	…tac tgt	aca att tat ggt aac tac cgt	tat gct atg gac tac tgg ggt…
37	…tac tgt gca aga	cgc ggt g	ac tat gct atg gac tac tgg ggt…
39	…tac tgt gca aga	cat ggg aac tat agg tac gac aga gg	c tat gct atg gac tac tgg ggt...
40	…cac tgt gca aga	gga att act acg gta gta gag g	at gct atg gac tac tgg ggt…
42	…tac tgt gca aga	gat cgc tat ggt aaa aag ggg gtc cta t	ct atg gac tac tgg ggt…
43	…tac tgt gca aga	cat gaa gag	atg gac tac tgg ggt…
Out of frame (12)			
08	…tac tgt gca aga	aaa ggg ggt aac tac tct	att act atg cta tgg act act ggg gtc…
14	…tac tgt gca aga	cta tgg tta cga ccg g	ta tgc tat gga cta ctg ggg tca…
15	…tac tgt gca aga	gaa cac cct ctc cc*t cta cta tgg tta cga c*ga gg	a cta tgc tat gga cta ctg ggg tca…
27	…tac tgt gca aga	cat agt ggt acg acg ccg ggg	act atg cta tgg act act agg gtc…
30	…tac tgt gca a	aa cct cga tgg	tta cta tgc tat gga cta ctg ggg tca…
31	…tac tgt gca aga	cag gta acc ctc	cta tgc tat gga cta ctg ggg tca…
32	…tac tgt g	ta acc cgg gg	g cta tgg act act ggg gtc…
33	…tac tgt gca aga	cgg atc tat agg tac gac gct	tat gga cta ctg ggg tca…
36	…tac tgt gca a	aa acc tcg atg g	tt act atg cta tgg act act ggg gtc…
41	… tac tgt gca aga	cat gcc tac tat ggt aac ggt	tgg act act ggg gtc…
45	…tac tgt gca aga	gaa ggc aga cag ctc ggg ccc	tta cta tgc tat gga cta ctg ggg tca…
46	…tac tgt gca aga	gcc cgt tat ggt aac tac gat	att act atg cta tgg act act ggg gtc…
Premature stop codon (5)			
09	…tac tgt gca ag	g cta cta *tag*	
12	…tac tgt gca aga	aga cta tgg *taa*	
13	… tac tgt gca aga	cat *tag*	
26	… tac tgt gca aga	cga gga cta *tag*	
44	…tac tgt gca aga	gat gtc cta cta *tag*	

B. Amplified joints (V_H7183-D-J_H4) from A150 Pro-B cells

Seq n°	V_H gene segment	N and P nucleotide additions	J_H4 segment
GL	...tac tgt gca aga		at tac tat gct atg gac tac tgg ggt…
In frame (17)			
03	…tac tgt ac	g ggg ggt aac ctc t	at tac tat gct atg gac tac tgg ggt…
05	…tac tgt aca aga	cac ggg ggt aac tac gtc ggg	tat gct atg gac tac tgg ggt…
06	…tac tgt	acg acc ctc tac ggt agt agc cct aag gcc t	ct atg gac tac tgg ggt…
07	…tac tgt gca aga	cat tcc ccc tat ggc cct g	ac tat gct atg gac tac tgg ggt…
12	…tac tgt gca aga	cat tcc tac tat ggt aac tac g	at tac tat gct atg gac tac tgg ggt…
13	…tac tgt gca ag	g gcc tac tat ggt aaa gg	t gct atg gac tac tgg ggt…
15	…tac tgt	ata gct acg tgg aga	gct atg gac tac tgg ggt…
19	…tac tgt gca a	cc tat gat ggt tac cc	c tat gct atg gac tac tgg ggt…
20	…tac tgt gca ag	c aac tac ggt agt agc tac cta	tat gct atg gac tac tgg ggt…
23	…ttc tgt tca aga	ggc tat ggt aac	tac tat gct atg gac tac tgg ggt…
25	…tac tgt gca aga	cgg atc tat gat ggt tac tac gtg gga act tc	t tac tat gct atg gac tac tgg ggt…
26	…tac tgt gca aga	aaa ctg ggc cgg ggt t	at tac tat gct atg gac tac tgg ggt…
28	…tac tgt gca ag	g gat atg gat tact ac ggt agt agc tac ggc gg	t tac tat gct atg gac tac tgg ggt…
31	…tac tgt gc	c gta gta acc atc a	at gct atg gac tac tgg ggt…
35	…tac tgt gca ag	t gag ggg gat ggt tac cc	c tat gct atg gac tac tgg ggt…
39	…tac tgt gca aga	aaa ctg ggc cgg ggt t	at tac tat gct atg gac tac tgg ggt…
48	…tac tgt gca ag	g att act acg gta gta gag agg ggg	tat gct atg gac tac tgg ggt…
Out of frame (22)			
08	…tac tgt gca aga	cgg gac act acg cct acc gg	c tat gga cta ctg ggg tca…
09	…tac tgt gca aga	cgg gtt ggg atg gg	c tat gga cta ctg ggg tca…
10	…tac tgt gca ag	t ccc tat tac tac ggt aga gg	a cta tgc tat gga cta ctg ggg tca…
11	…tac tgt gca ag	c cgt tat tact ac ggt agt agc tac ga	g cta tgg act act ggg gtc…
17	…tac tgt gca aga	cag ggt acg gta gta gg	t atg cta tgg act act ggg gtc…
21	…tac tgt gca aga	ccg cct tat tac tac ggt agt agc tac gaa gg	t gga cta ctg ggg tca…
24	…tac tgt gca aga	cgg gcg tat ggt aac tac ag	g cta tgg act act ggg gtc…
29	…tac tgt ttg aga	cat gta cgg gta ggg g	ta tgc tat gga cta ctg ggg tca…
32	…tac tgt gca aga	cct cat agg tac gca	act atg cta tgg act act ggg gtc…
33	…tac tgt gca aga	ggt atg gtt acg acc ct	a tta cta tgc tat gga cta ctg ggg tca…
34	…tac tgt gca aga	cat atg att acg ttg	att act atg cta tgg act act ggg gtc…
36	…tac tgt gca aga	gaa gac gga gac tat agg tcc ggt	cta tgg act act ggg gtc…
37	…tac tgt gca aga	cag ggt aca agg gg	g cta tgg act act ggg gtc…
38	…tac tgt gca aga	ttt ctc att tac gac ggg g	ct atg cta tgg act act ggg gtc…
40	…tac tgt gca aga	ctc ctt atg gta aag	atg cta tgg act act ggg gtc…
41	…tac tgt gca aga	caa ggt ccc tac tat agg tac ggc	act atg cta tgg act act ggg gtc…
42	…tac tgt	acc agg ccc cac tat tac tac ggt agt agc c	tt act atg cta tgg act act ggg gtc…
43	…tac tgt gca ag	g ggt tac tat agg tac gac gcg g	ta cta tgc tat gga cta ctg ggg tca…
44	…tac tgt gca aga	gag gta act c	ct atg cta tgg act act ggg gtc…
45	…tac tgt gca aga	cac gac ccc tct a	ga ctg ggg tca…
49	…tac tgt gca ag	g gat cgt gaa ggt acg tca tc	c tat gga cta ctg ggg tca…
50	…tac tgt gca aga	ggg ggt atg att acg acg ccg	tta cta tgc tat gga cta ctg ggg tca…
Premature stop codon (4)			
01	…tac tgt gca ag	g gat tta *tga*	
02	…tac tgt gca ag	g gat cta cta *tga*	
18	… tac tgt gca ag	c ccc gaa gga cta *tag*	
27	… tac tgt gca a	aa gat cta cgg *tag*	