## Supplementary Table Legends

**Supplementary Table 1**: Oligonucleotide primers used in these studies.

**Supplementary Table 2**: Sequence diversity of PCR products obtained from Figure 1. Products shown in Figure 1 were cloned and their sequence determined. The number of unique direct V-to-D rearrangements is indicated above the total number of clones sequenced. WT and cR2 animals are compared.

**Supplementary Table 3**: (**Part 1**) Sequence characterization of a typical Dβ2 coding element (upper case) deletion amplified from thymocyte DNA from a cR2 animal resulting in a perfect signal joint between the RSS12 and RSS23 (lower case). Germline sequence is shown above the line for comparison purposes. (**Part 2**) Sequence characterization of the Dβ1 to Dβ2 deletional rearrangements amplified from WT and cR2 thymocyte DNA. Some the PCR products shown in Figure 6 were cloned and their sequence determined, demonstrating that the RSS12 of Dβ1 was joined to the RSS23 of Dβ2. Several of the joints included portions of the Dβ1 and Dβ2 coding sequence (upper case) and novel nucleotides (N) in between (also upper case). In one sequence (67-5-1) the short stretch of novel nucleotides has been joined to a sequence that is 106bps away from the Dβ2 and appears to be a cryptic RSS sequence. Germline sequences are shown above the line. (**Part 3**) Sequence characterization of various Dβ-to-Dδ chromosomal translocations. Some of the PCR products shown in Figure 7 were cloned and the sequences determined. RSS sequences and their adjacent coding sequence are shown in lower and upper case, respectively. Untemplated nucleotides (N) are shown between the D coding sequences. In one characterization (c5-F) from a cR2 animal, the Dβ1 sequence is 59bps away from the 3' end of the Dβ1 coding sequence and is likely a cryptic RSS. Germline sequences are shown above the line in each case.

## Supplementary Figure Legends

**Supplementary Figure 1.** Representation of the Vβ8.1 coding sequence (boxed) followed by its flanking RSS-23 sequence. Portions of the RSS-23 are labeled above (clear boxes). A cryptic RSS-12 sequence is shown by the shaded boxex and shares the RSS-23 heptamer sequence but in the reverse orientation. Solid vertical arrow represents the normal position of the coding and signal ends breaks when the RSS-23 is utilized. The hollow vertical arrow denotes the cryptic coding and signal end breaks when the cryptic RSS-12 is utilized.

**Supplementary Figure 2.** Frequency of direct Vβ#-to-Dβ# rearrangements in *cR2* and WT mice. DNA purified from the thymus of sets of four animals for each genotype was analyzed by real-time PCR for direct VDβ1 and VDβ2 (C) rearrangements and the mean values (bars) with standard errors (whiskers) are shown.

**Supplementary Figure 3.** Frequency of complete $V_H$-to-$D_H$-to-$J_H$ and direct $V_H$-to-$D_H$ rearrangements in *cR2* and WT mice. Direct VD-del and VD-inv, deletion and inversion utilizing $V_H$J558 and DFL16.1. DNA purified from the indicated tissues was analyzed for complete VDJ (upper) or direct VD joints (middle and lower; deletion or inversion). Hybrid joints (not illustrated) were excluded by placing the D segment primer just within that coding segment. Four animals from each of the three possible genotypes were examined and the mean values (bars) with standard errors (whiskers) are shown.

**Supplementary Figure 4.** Frequency of direct D to J rearrangements in the thymus of *cR2* and WT mice. DNA purified from the thymus were analyzed for direct deletional VD joints (upper) or direct inversional DJ joints (lower). Four animals from each of the three possible genotypes were examined and the mean values (bars) with standard errors (whiskers) are shown.

Supplementary Table 1.

Conventional Primers

| Oligo Name | Sequence |
|---|---|
| Vβ8-F1 | GTGGCAGTAACAGGAGGAAAGGTGAC |
| Vβ8-F2 | CATGTACTGGTATCGGCAGGACACG |
| Dβ1-F1 | CCAGAGGAGCAGCTTATCTGGTGGT |
| Dβ1-F2 | CAAGGGGTAGACCTATGGGAGGGTC |
| Dβ1-R1 | GAGTAATCGCTTTGTGTGCATCACA |
| Dβ1-R2 | CATTCTGGATCTAAACACATCTAGGCTTGC |
| Dβ2-F1 | AAGCTGAACATTGTCCAGTAAGCAACAGG |
| Dβ2-F2 | TCCTGGTAGGGACCTTATCACTTCACTCC |
| Dβ2-R1 | CCCTGGATAGCCTTTCACACTCGTG |
| Dβ2-R2 | CACATAGACTCCTCCTCACATGTTAAATGCA |
| Dδ2-F1 | CCAAAGCACTGGCTGGTTAATGGC |
| Dδ2-F2 | CCAAGTTTGATAACAGGTGAGTCTCTGTGC |
| Dδ2-R1 | GTCACTGTTAGTCCGCTTGATCAATATTGAGG |
| Dδ2-R2 | TTCTTGCAGTGACAATACAGACCAAATATACAGC |
| Dβ2-SJ-R1 | GCACCTCTTCCAGTTGAATCATTGTGCACA |
| Vh101-F1 | ATGGCTGTCTTAGGGCTGCTCTTC |
| Vh101-F2 | GCCAGTCTCCAGGAAAGGGTCTG |
| DhFL16.1-R1 | AGTAGTTCTTATCATTCCTCCCAAAGCA |
| DhFL16.1-R2 | GCTGTTGGGAGGTCAGCCTAGAG |
| Dδ1-R1 | GTCACTGTTAGTCCGCTTGATCAATATTGAGG |
| Dδ1-R2 | TTCTTGCAGTGACAATACAGACCAAATATACAGC |
| Dδ1-F1 | CCAAAGCACTGGCTGGTTAATGGC |
| Dδ1-F2 | CCAAGTTTGATAACAGGTGAGTCTCTGTGC |
| Dδ2-R1 | CCAGGAAACAGATGAGAGACAGAGGC |
| Dδ1-R2 | GCAAGTGGAGGTCATATCTTGTCCAGTC |

qPCR Primers

| Oligo Name | Sequence |
|---|---|
| APRT-F1 | TGACTGAGGAGCTGGCTAGATG |
| APRT-R1 | GAGCCACCAAGCAGTTCCTG |
| APRT-TaqMan | TCACACCCCTGCTCCCAGCAGC |
| Vβ2-F | CCACACGGGTCACTGATACG |
| Vβ2-R | AGGTTCTGCCCTGGCTCAT |
| Vβ2-TaqMan | TGGCCACTTGCAGCCTCAGCT |
| Vβ7-F | GGAAGAAGCGGGAGCATTTC |
| Vβ7-R | GTAGAGCTGTGGATAAACTGCTAGCA |
| Vβ7-TaqMan | CCCTGATTCTGGATTCTGCTAAAACAAACCA |
| Vβ8-F | AGAAAGGAGATATCCCTGATGGATAC |
| Vβ8-R | GGGTAGCCAACTCCAGAATGAG |
| Vβ8-TaqMan | AGGCCTCCAGACCAAGCCAAGAGAACTT |
| Vβ14-F | GCCGAAGGACGACCAATTC |
| Vβ14-R | GGCACAGAGGTAGAAGCCAGAGT |

| | |
|---|---|
| Vβ14-TaqMan | TCCTAAGCACGGAGAAGCTGCTTCTCAG |
| Dβ1-R | GCGACCCAGGAGAAGAGTAGAG |
| Dβ2-R | GCTGAGAGTTGGTGTTTTTTTGG |
| Jβ2.7-R | GGAAGCGAGAGATGTGAATCTTACC |
| Vh81X-F | CCATGGAGAGACGATTCATCATC |
| Vh81X-R | GTCCTCAGACCTCAGACTGCTCAT |
| Vh81X-TaqMan | CCAGAGACAATACCAAGAAGACCCTGTACCTGC |
| Vh101-F | CAAGGCCACAYTSACTGTAGACA |
| Vh101-R | AGGCTGMTGAGCTGCATGT |
| Vh101-MGB | AYCCTCCAGCACAGCC |
| JhS1-R | GCCAGCTTACCTGAGGAAACG |
| JhS4-F | CCAGGGGTGATTCTAGTCAGACTCT |
| JhS4-R | AGACCTGGAGAGGCCATTCTTAC |
| JhS4-TaqMan | TCAAGGAACCTCAGTCACCGTCTCCTCA |
| DhFL16.1-F | TACGGTAGTAGCTACCACAGTGCTATATCCATC |
| DhFL16.1-R | CCGTAGTAATAAACACAGTAGTAGATCCCTTCACAA |
| DhSP2.9-F | TTTTTGTCAAGGGATCTACTACTGTGTCT |
| DhSP2.9-R | TTTTTGTAGCTGGATATATCACTGTGGTA |
| DhQ52-F | GCGGAGCACCACAGTGCTA |
| DhQ52-R | CCACGTGTCACCGTGGTC |

LM-PCR Primers

| Oligo Name | Sequence |
|---|---|
| JhS2-F1 | TCACTGGGTCTATAATTACTCTGATGTCTAGGAC |
| JhS2-F2 | GTCAGGTGAGTCCTGCATCTGGG |
| Vα7.2-F1 | CAACCAAACCGGTAATGTGCCTTTC |
| Vα7.2-F2 | CTGTGTAGCGTGTGCTCTATCTCAGGC |
| Vβ18-R1 | CATTTCTCGTGACTGGTGTCTGTAGTAAGTCCT |
| Vβ18-R2 | TGAACAGGTGTCAGCCGAATCTGTG |
| Vβ14-F1 | ATGTTGTTTTCCAGACTTCAACTTGAC |
| Vβ14-F2 | GACTATCAGCCAGAAATTCAGTGGCAA |
| Vδ4-R1 | CCAACTCAGCTCCAGCCTTTCTTATCAG |
| Vδ4-R2 | AGGAGGTCTCATCTCAGATGTGCTGC |
| Vδ5-F1 | CCCATTGAACCCAGAGTGAGAGGTG |
| Vδ5-F2 | GAAGCCAGTCTTTCCTGTGCCAGG |
| β-Glob-F1 | TTTGGCTAGTCACTTCGGCA |
| B-Glob-R2 | GTGTGTCCGCTATGCCTCCT |

**Supplementary Table 2:** Sequence diversity of products obtained from Figure 1.

| | | WT | cR2 |
|---|---|---|---|
| Vβ8/Dβ1 | Unique | 8 | 12 |
| | Total | 18 | 16 |
| Vβ8/Dβ2 | Unique | 12 | 14 |
| | Total | 12 | 15 |
| Vh101/DhFL16.1 | Unique | 1 | 8 |
| | Total | 8 | 11 |

Supplementary Table 3, Part 1 Dβ2 Coding Deletion

| | Dβ2 RSS12 | Dβ2 Coding | Dβ2 RSS23 |
|---|---|---|---|
| | ctttttttgtatcacgatgtaacattgtg | GGGACTGGGGGGC | cacaatgattcaactggaagaggtgcttttacaaa |
| cR2 4-13 | ctttttttgtatcacgatgtaacattgtg | | cacaatgattcaactggaagaggtgcttttacaaa |

Supplementary Table 3 Part 2 Dβ1 to Dβ2 Deletions

| | | Dβ1 RSS12 | Dβ1 Coding | N | Dβ2 Coding | Dβ2 RSS23 |
|---|---|---|---|---|---|---|
| | | ctttttgtataaagctgtaacattgtg | GGGACAGGGGGC | N | GGGACTGGGGGGGC | cacaatgatt |
| WT | **5-1** | ctttttgtataaagctgtaacattgtg | | | | cacaatgatt |
| | **25-3-10** | ctttttgtataaagctgtaacattgtg | | | | cacaatgatt |
| | **13-2-2** | ctttttgtataaagctgtaacattgtg | | A | C | cacaatgatt |
| | **14-2-3** | ctttttgtataaagctgtaacattgtg | | T | | cacaatgatt |
| | **16-3-1** | ctttttgtataaagctgtaacattgtg | | T | | cacaatgatt |
| | **21-3-6** | ctttttgtataaagctgtaacattgtg | | TC | | cacaatgatt |
| | **22-3-7** | ctttttgtataaagctgtaacattgtg | GG | | | cacaatgatt |
| | **24-3-9** | ctttttgtataaagctgtaacattgtg | GG | | | cacaatgatt |
| | **5-2** | ctttttgtataaagctgtaacattgtg | GGGACAGGGGG | TTG | GGGACTGGGGGGGC | cacaatgatt |
| | **15-2-4** | ctttttgtataaagctgtaacattgtg | GGGACAGGG | | | atgatt |
| | **17-3-2** | ctttttgtataaagctgtaacattgtg | GGGACA | T | GACTGGGGGGGC | cacaatgatt |
| | **18-3-3** | ctttttgtataaagctgtaacattgtg | GGGACAGGGG | ATGA | GGGACTGGGGGGGC | cacaatgatt |
| | **20-3-5** | ctttttgtataaagctgtaacattgtg | GGGACAGGGGG | AG | TGGGGGGGC | cacaatgatt |
| | **23-3-8** | ctttttgtataaagctgtaacat | | | ACTGGGGGGGC | cacaatgatt |
| cR2 | **6-1** | ctttttgtataaagctgtaacattgtg | | | | cacaatgatt |
| | **73-5-7** | ctttttgtataaagctgtaacattgtg | | | | cacaatgatt |
| | **62-4-6** | ctttttgtataaagctgtaacattgtg | | AGA | | cacaatgatt |
| | **64-4-8** | ctttttgtataaagctgtaacattgtg | | | C | cacaatgatt |
| | **66-4-10** | ctttttgtataaagctgtaacattgtg | | | GC | cacaatgatt |
| | **70-5-4** | ctttttgtataaagctgtaacattgtg | | AG | | cacaatgatt |
| | **57-4-1** | ctttttgtataaagctgtaacattgtg | GGGACAGGG | TATTGTGGGGACACA | GGACTGGGGGGGC | cacaatgatt |
| | **58-4-2** | ctttttgtataaagctgtaacattgtg | GGGACAGGGG | | ACTGGGGGGGC | cacaatgatt |
| | **68-5-2** | ctttttgtataaagctgtaacattgtg | GGGACAGGGG | | ACTGGGGGGGC | cacaatgatt |
| | **60-4-4** | ctttttgtataaagctgtaacattgtg | GGGACAGGG | | ACTGGGGGGGC | cacaatgatt |
| | **63-4-7** | ctttttgtataaagctgtaacattgtg | GGGACAGGGG | | CTGGGGGGGC | cacaatgatt |
| | **69-5-3** | ctttttgtataaagctgtaacattgtg | | T | GACTGGGGGGGC | cacaatgatt |
| | **71-5-5** | ctttttgtataaagctgtaacattgtg | GGGACAGGGGGC | G | CTGGGGGGGC | cacaatgatt |
| | **74-5-8** | ctttttgtataaagctgtaacattgtg | GGGACAGGGGGC | | TGGGGGGGC | cacaatgatt |
| | **67-5-1** | ctttttgtataaagctgtaacattgtg | GGGACAGGG | CCT | | agtggactca |

Cryptic RSS +106bps from Dβ2 is shown underlined

Supplementary Table 3, Part 3

Dδ2 > to < Dβ1 Translocations

| | | RSS | Dδ2 Coding | | Dβ1 Coding | RSS |
|---|---|---|---|---|---|---|
| | | ggtttttgcaaagctctgtagcaccgtg | ATCGGAGGGATACGAG | N | GGGACAGGGGGC | cacggtgatt |
| cR2 | c1-F | ggttt | | AGG | | cacggtgatt |
| cR2 | c2-F | ggtttttgcaaagctctgtagcac | | GAG | | acggtgatt |
| cR2 | c6-F | ggtttttgcaaagctctgtagcaccg | | | | cggtgatt |
| cR2 | c5-F | ggttt | | CCTC | | *cacagtcctctac |

*not RSS sequence

Dδ2 > to < Dβ2 Translocations

| | | RSS | Dδ2 Coding | | Dβ2 Coding | RSS |
|---|---|---|---|---|---|---|
| | | ggtttttgcaaagctctgtagcaccgtg | ATCGGAGGGATACGAG | N | GGGACTGGGGGGGC | cacaatgatt |
| WT | d1-F | ggtttttgcaaagctctgtagcaccgtg | | | | cacaatgatt |
| WT | d2-F | ggtttttgcaaagctctgtagcacg | | | GGGACTGGGGGGGC | cacaatgatt |
| WT | d3-F | ggtttttgcaaagctctgtagcaccgtg | | | | cacaatgatt |
| cR2 | d4-F | ggtttttgcaaagctctgtagcaccgtg | | | | cacaatgatt |
| cR2 | d6-F | ggtttttgcaaagctctg | | GA | | gatt |

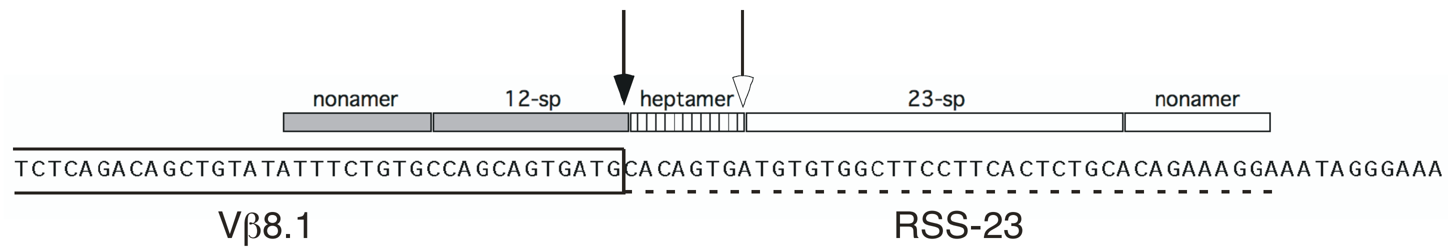
Dβ2 > to < Dδ1 Translocations

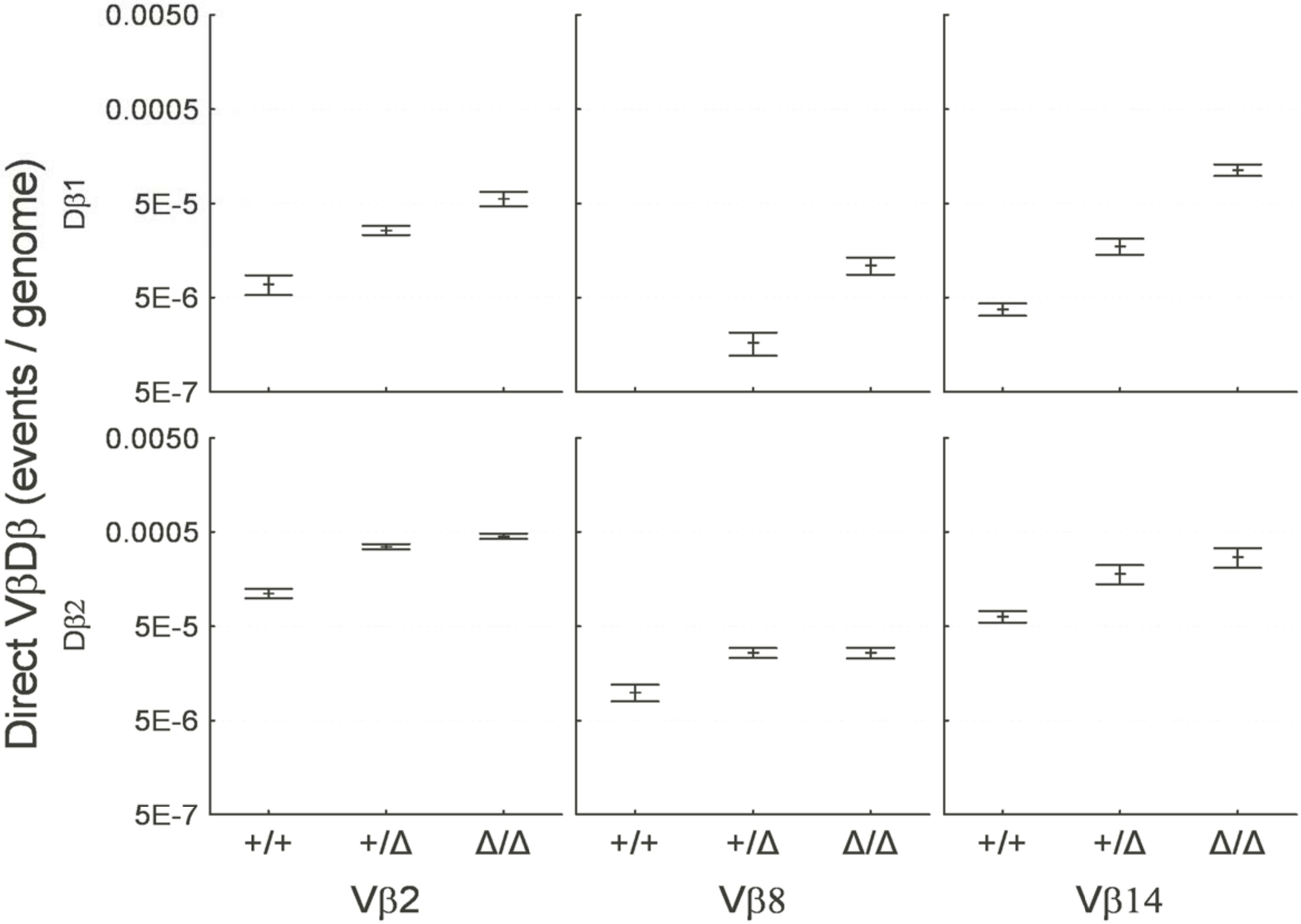
| | | RSS | Dβ2 Coding | | Dδ1 Coding | RSS |
|---|---|---|---|---|---|---|
| | | ctttttgtatcacgatgtaacattgtg | GGGACTGGGGGGGC | N | GTGGCATATCA | cacaggttga |
| WT | a1-F | ctttttgtatcacgatg | | AACCCCCCNN | | caggttga |
| cR2 | a2-F | ctttttgtatcacgatgtaacattgtg | GGGACTGGGGGG | C | | acaggttga |
| cR2 | a4-F | ctttttgtatcacgatgtaacattgtg | GGGACTGGGGGG | | CATATCA | cacaggttga |

Dβ2 > to < Dδ2 Translocations

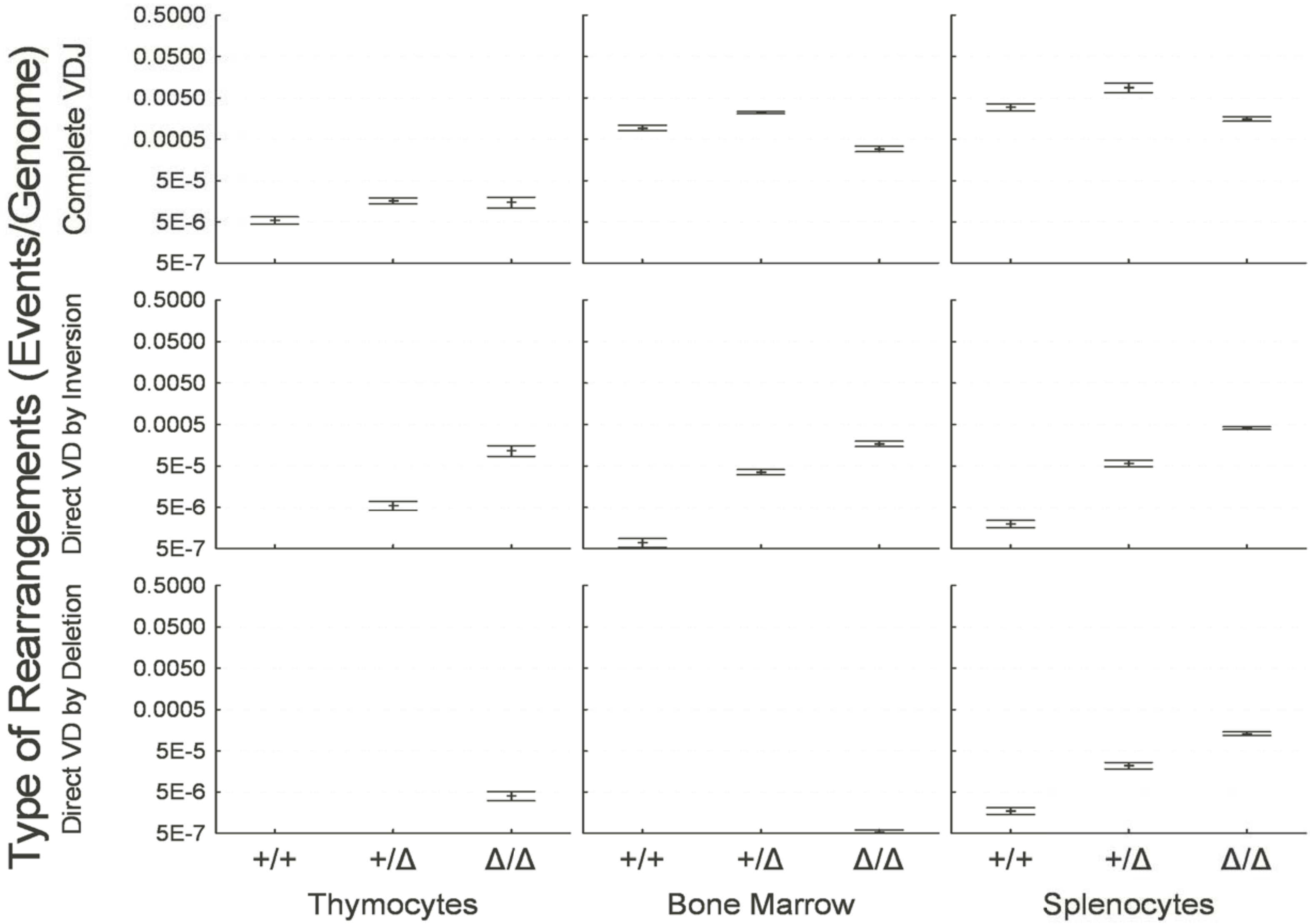
| | | RSS | Dβ2 Coding | | Dδ2 Coding | RSS |
|---|---|---|---|---|---|---|
| | | ctttttgtatcacgatgtaacattgtg | GGGACTGGGGGGGC | N | ATCGGAGGGATACGAG | cacagtgttg |
| WT | b2-F | ctttttgtatcacgatgtaacattgtg | GGGACTGGGGGGGC | GCG | TCGGAGGGATACGAG | cacagtgttg |
| WT | b4-F | ctttttgtatcacgatgtaacattgtg | | TC | | cacagtgttg |
| cR2 | b5-F | ctttttgtatcacgatgtaacattgtg | GGGAC | TGGGTT | | cacagtgttg |
| cR2 | b6-F | ctttttgtatcacgatgtaacattgtg | GGGACTGGGGGGG | ACGTGGCCCGT | | cagtgttg |
| cR2 | b7-F | ctttttgtatcacgatgtaacattgtg | | | | cacagtgttg |

# Supplemental Figure 1

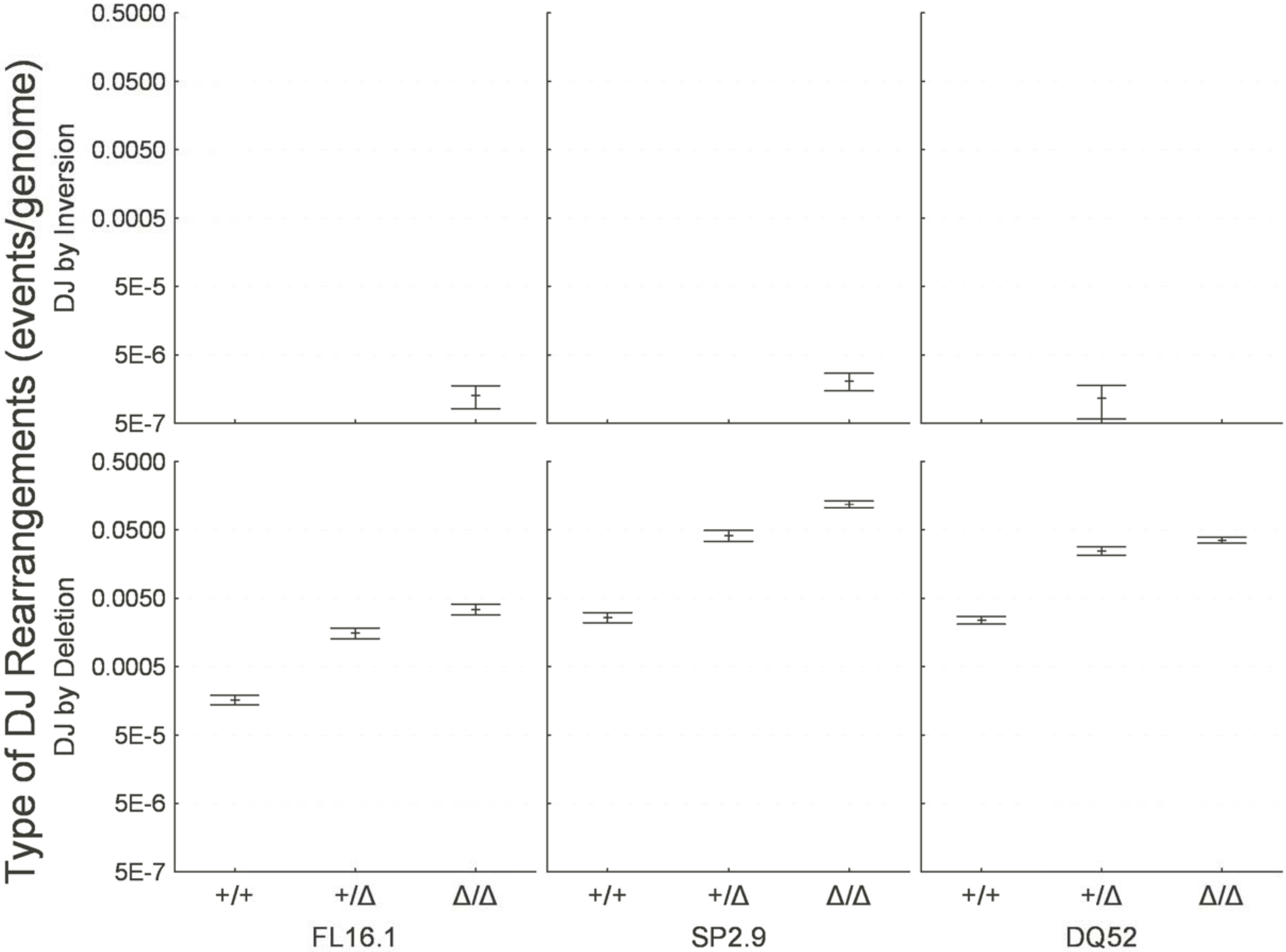





Supplemental Figure 2




Supplemental Figure 3.




Supplemental Figure 4.