SUPPLEMENTAL MATERIALS AND METHODS

Targeting construct cloning and probes for Southern screening

The V<sub>HI</sub> gene was obtained by TA cloning a PCR product containing a V<sub>HI</sub>558 family gene with a 1.5-kb upstream and 500-bp downstream sequences amplified from RAG2<sup>−/−</sup> pro-B cell line DNA using the following primers containing SpeI restriction sites (underlined): SpeI.5F, 5'-GGACTAGTAAAGTTGGTGAGGCTTTTACAGGT-3'; and SpeI300R, 5'-GGACTAGTCATGCGGTGACCCGGGAGATCTGAATTC-3'. Many V<sub>HI</sub> gene segments were amplified using these primers, and the criteria for choosing one particular V<sub>HI</sub> gene were (a) it had an ATG translational start, (b) it had an open reading frame that is continuous to the RSS (taking the intron into consideration), and (c) the RSS had the consensus sequences found in the V<sub>HI</sub>558 family. Southern blot probes used for screening were P1 (74429–73466 of the BAC described in Materials and methods) and P2 (85658–85601 of the BAC; Fig. S2, A and B). Chimeric-targeted mice were bred to EIIa-Cre–expressing mice (B6. FVB-Tg(EIIa-cre)C5379Lmgd/J; The Jackson Laboratory). Neo deletion was monitored by Southern blotting with the inter- nal probe P3 (81564–82746 of the BAC).

Primers used for PCR assays

**ChIP.** The following primers were used: 1, 5'-ATGGCAGACAAAGCAGCATA-3'; 2, 5'-GGTTCCTCAACTGCTGTG-3'; 3, 5'-GACCTGTAAAGCCCACCAA-3'; 4, 5'-CAGGCATTGTCGCTTTGAGAC-3'; and β-globin, (forward) 5'-GATGAGATTTGGTGAGGCCCCCTG-3' and (reverse) 5'-TCTCCAAAGCTATCAAAGTACC-3'.

**Frequency of V<sub>HI</sub>-K<sub>J</sub> gene rearrangement.** The following primers were used: V<sub>HI</sub>558 leader oligo, 5'-CCACGGATC- CATGGRATGGAGYTGKRTCWTYCT-3' and CH oligo, 5'-ATGCAGATCTCTGTTTTTGCCCTCG-3'. BamHI and Bgl II restriction sites underlined in the oligos were included for optional cloning.

**Recombination assays.** V<sub>HI</sub>B8 leader (V<sub>HI</sub>558, 5'-TGATATCATCCTCTCTTTG-3'), V<sub>HI</sub>7183 (5'-CGGTACCAAGAAAMCTGTCTGCAAGATTGAC-3'), and V<sub>HI</sub>Q52 (5'-CGGTACCAAGACATGARCATCACTGAGGCA-3') were paired with a reverse primer (J<sub>HI</sub>3, 5'-GTCCTGATTCTCAACAAGATCGCTCGAGGC-3') and Southern blotted with a probe (J<sub>HI</sub>A, 5'-TGCTCTAGACGTACATCGCTCCTCAGTGTTG-3'). For V<sub>to</sub>-D rearrangement, the same V<sub>HI</sub> family primers were paired with DQ52 R (5'-AGCCTCTCTTACTCCTCA-3') and probed with the DQ52 probe (5'-CCCTGGAGAGCCTCCAACAGAA-3'). As a control, APRT was amplified with APRT forward (5'-TGCTGAGACACCGCAGCCAGGAA-3') and reverse (5'-TGCTGACCGCACCCTCAGAC-3').

**LM-PCR.** RAG-mediated breaks were ligated with the linker (top, 5'-GCGGTGACCCGGAGATCTGAATTCC-3' and bottom, 5'-GAATTAGATCGAC-3') and amplified with the linker-specific primer BW-H (5'-CCGGGAGATCTGCAATCCAC-3'), paired with the following primers and blotted with gene-specific probes: targeted V<sub>HI</sub> gene segment (V<sub>HI</sub>KI first, 5'-GGAAGGTAGTACCGAGGTAT-3') and nested primer (V<sub>HI</sub>kKI nested, 5'-AAGAGCTCTCCTAAATACATCAT-3') with probe (V<sub>HI</sub>kKI probe, 5'-CCACCTTGGCAATGACATCG-3'), 5'-DFL (DFL-Be, 5'-GAAAGCTCTCAGAGAAAGAC-3'), and nested primer (DFL-3, 5'-GACACTGAAACTCACAACCGTGCTCTC-3') with probe (DFL16.1F, 5'-CCCCCAATGCTCTCAACA-3'). Bgl II digestion (BglII100, 5'-ACCTTCTTATCTTCAACTCC-3') and nested primer (BglII1-3205, 5'-CGCAAGCTTAGAGGAGC-3') and nested primer (BglII1-3205, 5'-CGCAAGCTTAGAGGAGC-3') and nested primer (BglII1-3205, 5'-CGCAAGCTTAGAGGAGC-3') and nested primer (BglII1-3205, 5'-CGCAAGCTTAGAGGAGC-3') and nested primer (BglII1-3205, 5'-CGCAAGCTTAGAGGAGC-3').

Bates et al. http://www.jem.org/cgi/content/full/jem.20071787/DC1