SUPPORTING INFORMATION

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1. Supplemental Materials and Methods

Mice. Ter3 transgenic animals were established using the vector pμTer3 described previously (ref. 1). Briefly, we changed codon 3 in the leader peptide of the H chain expression vector pμGPT to an in-frame TGA stop codon and replaced downstream in-frame ATG codons in the V_H region sequence by Ala codons to prevent reinitiation and translation of a truncated H chain protein. To construct transgenic Ter3 mice, a 12.3kb *Sal*I/*Xho*I fragment bearing the IgH promoter, the Ter3 V_H region of the hybridoma 17.2.25 and a genomic Cμ region from a Sv129 mouse (H chain allotype a) was isolated from pμTer3 and injected into the pronucleus of C57/BL6 oocytes. Three independent lines (7204, 7302, and 7307) were established by backcrossing the corresponding founders to C57/BL6 animals. Here we present data of the 7307 line. The insertion sites of the lines 7204 and 7307 were mapped by circular PCR to positions chr5: 109.975.633 and chr5:10.473.198, respectively. Offspring were screened by PCR for the presence of the Ter3 transgene. Wildtype configuration of the allele was detected in with primers flanking the insertion site. For 7204 line, we used the primers 5' of Ter7204 fwd (CAA GAC AAC TCT CGA CTA CAT GTG AG) and 3' of Ter7204 rev (CTG TCC TGA AAC TCA CCT TGT AGA CC) and for 7307 line the primers 5' of Ter7307 fwd2 (AAC ATC AAG TTT CCA AGT AGT GGT GG) and 3' of Ter7307 rev3 (GCT TCT ACT AGA TTC AGT GTA TCT GG). Presence of the transgene was detected with the respective forward primer and the primer IgMTer113 rev (CTG TCA AAG CTA CTT GAT GAG GAT GC), which binds to a sequence in the transgene. QM (ref. 2), V_HB1-8 (ref. 3), Ter5H (pII in ref. 4) and Ter5L (pΔ in ref 4) mice have been described previously. All animal experiments were performed according to institutional and national guide lines.

Antibodies. Fluorochrome-conjugated mAbs against CD19 (clone 1D3, PerCP), c-kit (clone ack45, PE), CD25 (clone PC61, PE), IgM^a (clone DS-1, FITC), and IgM^b (clone AF6-78, PE) were purchased from BD PharMingen and Cy5-conjugated goat abs against mouse μH chain from Southern Biotechnology. The unlabeled monoclonal rat antibody against the V_H17.2.25 idiotype (clone R2.438.8, kind gift of T. Imanishi-Kari, Tufts University, Boston, MA) were detected with a secondary Cy5-conjugated goat-anti-rat IgG serum (Chemicon).

Flow cytometry. Single cell suspensions were prepared from bone marrow, spleen and thymus of 6- to 8-week-old mice. Erythrocytes were removed by incubation with 0.15 M NH_4Cl, 20 mM

HEPES for 5min at RT, and cells were membrane-stained with respective antibodies for 60 min on ice. For intracellular staining, cells were first fixed and permeabilized using the Fix and Perm kit (An der Grub Biotechnologies). Stained cells were examined in a FACSCalibur (BD Biosciences), and data were analyzed with the Cell Quest software (BD Biosciences). Cell sorting was performed on a MoFlo cell sorter (DakoCytomation). Only events falling in the viable lymphocyte gate, as judged by forward/sideward scattering, are shown in histograms and dot plots.

Quantitative TaqMan PCR (qPCR). All qPCRs were performed on Applied Biosystems 7300 or 7500 Real-Time PCR Systems. The amount of Ter5 mRNA was measured in splenocytes from heterozygous V_HB1-8, $Ter5^{High}$ (Ter5H) and $Ter5^{Low}$ (Ter5L) mice using primers V_HB1-8 fwd (GAG CTG TAT CAT CAT CCT CTT CTT G) or Ter5-VHb1-8 fwd (GAG CTG ACT CAT CAT CCT CTT CTT G) with V_HB1-8 rev (CAG GCT GCT GCA GTT GGA) and the fluorescently labeled V_HB1-8 probe (6FAM-AGC AAC AGC TAC AGG TGT CCA CTC CCA-TAM). To detect V_H1-D_H3 recombinations, genomic DNA from FACS-sorted $CD19^+$/c-kit^+ pro-B cells was analyzed using the primers V_H1-FR3 fwd (GAG GAC TCT GCR GTC TAT TWC TGT GC, ref. 5) and J_H3 rev (CCC TGA CCC AGA CCC ATG T) and the fluorescently labeled J_H3 probe (6FAM-TTC AAC CCC TTT GTC CCA AAG TT-TAM). The amount of germline or $DQ_{52}J_H$ rearranged IgH loci was measured on genomic DNA isolated from sorted $CD19^+$/c-kit^+/surface IgM^- pro-B cells using the primers DQ_{52} fwd (CAA GAG ATG ACT GGC AGA TTG G) and DQ_{52} rev (TCA AAA CCT TGC ACC AGT CAG A) and the fluorescently labeled DQ_{52} probe (6FAM-ATA CCC ATA CTC TGT GGC TAG TGT GAG GTT TAA GCC-TAM). These primers amplify a sequence 5' of the D_{Q52} gene segment, including its 5' RSS, which is only present on germline or $D_{Q52}J_H$ rearranged IgH loci, because it is deleted in all other D-to-J_H rearrangements or a V_H-to-D_{Q52} rearrangement**.**

Quantification of Ter3-µH mRNA amounts. To compare the amounts of Ter3 transcripts to that of endogenously encoded sense µH transcripts, Ter3 mice were bred to quasi monoclonal (QM) mice carrying a copy of the productive wildtype $V_H17.2.25$ VDJ exon (i.e., without a nonsense codon) homologously targeted into the IgH locus (2). Thereby, abundances of nonsense $V_H17.2.25$-µH (Ter3) mRNA expressed from the Ter3 transgene and sense $V_H17.2.25$-µH mRNA from the endogenous IgH locus can be compared. Total RNA was isolated from sorted c-kit^+/$CD19^+$ bone marrow and splenic $CD19^+$/ $V_H17.2.25$ $idiotype^+$ B cells from $IgH^{QM/wt}$, $Ter3^{Tg}$ mice. Sense and nonsense transcripts were amplified together by RT-PCR with primers binding to the 5' UTR of both sense $V_H17.2.25$-µH mRNA and Ter3 mRNA (5'UTR of 17.2.25 fwd, CTA CAG ACA CTG AAT CTC AAG GTC C) and the Cµ1 region (mCµ1 rev, GAA GGA AAT GGT GCT GGG CAG G). The 567bp PCR product was gel purified, digested with *Fsp*I, which due to the presence of a mutated Ala codon cleaves only the nonsense Ter3 but not sense $V_H17.2.25$-µH PCR product into a 188bp and a 379bp fragment (Fig. S1), and re-analyzed on an

EtBr agarose gel. The relative amount of Ter3 nonsense mRNA was determined by dividing the band intensity of the 567bp product in digested samples (containing only sense V_H17.2.25-μH mRNAs) by that in undigested samples (containing both sense and nonsense V_H17.2.25 transcripts). The specificity of the digest was controlled with cDNA from Ag8 hybridoma cells transfected with expression plasmids encoding either the sense (pμGPT) or nonsense form (pμTer3) of V_H17.2.25-μH transcripts.

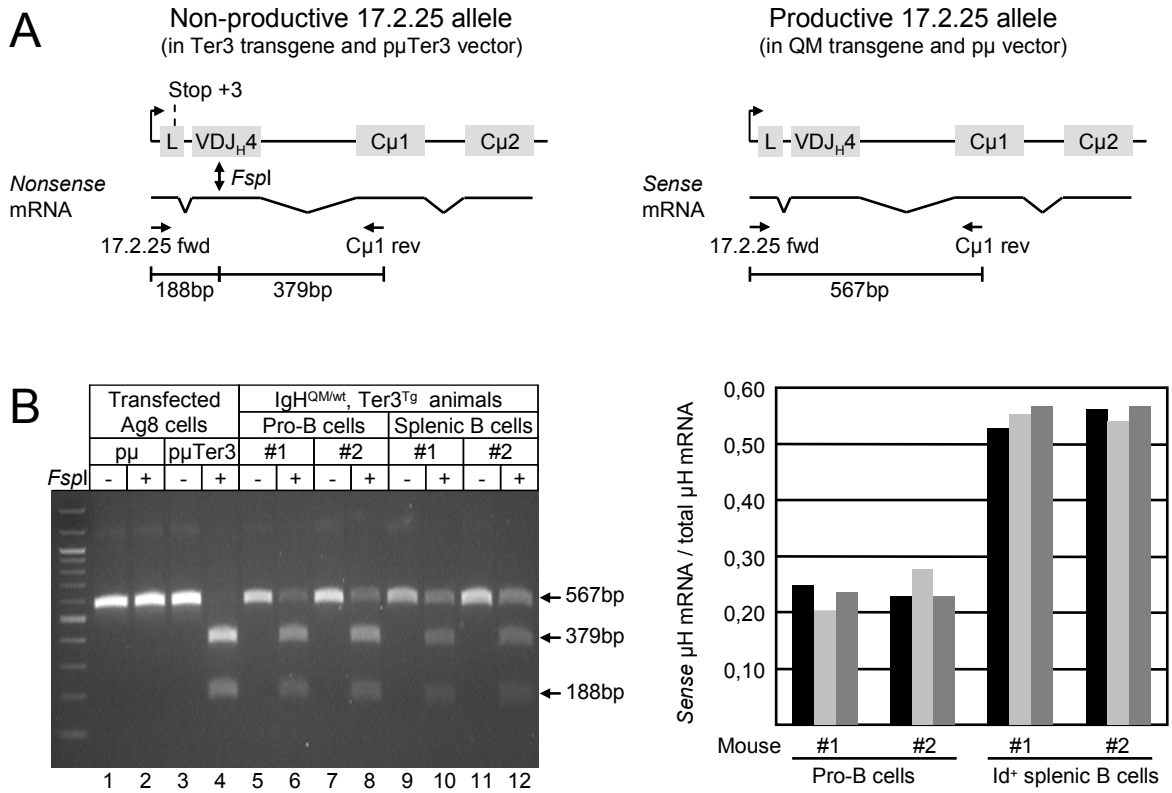
Statistical methods. Statistical analysis was performed using the unpaired t test.

Additional References

1. Lutz J, Müller W, & Jäck HM (2006) VH replacement rescues progenitor B cells with two nonproductive VDJ alleles. *J Immunol* 177(10):7007-7014.
2. Cascalho M, Ma A, Lee S, Masat L, & Wabl M (1996) A quasi-monoclonal mouse. *Science* 272(5268):1649-1652.
3. Sonoda E*, et al.* (1997) B cell development under the condition of allelic inclusion. *Immunity* 6(3):225-233.
4. Fukita Y, Jacobs H, & Rajewsky K (1998) Somatic hypermutation in the heavy chain locus correlates with transcription. *Immunity* 9(1):105-114.
5. Delbos F*, et al.* (2005) Contribution of DNA polymerase eta to immunoglobulin gene hypermutation in the mouse. *J Exp Med* 201(8):1191-1196.

2. Supplemental Figures and Tables

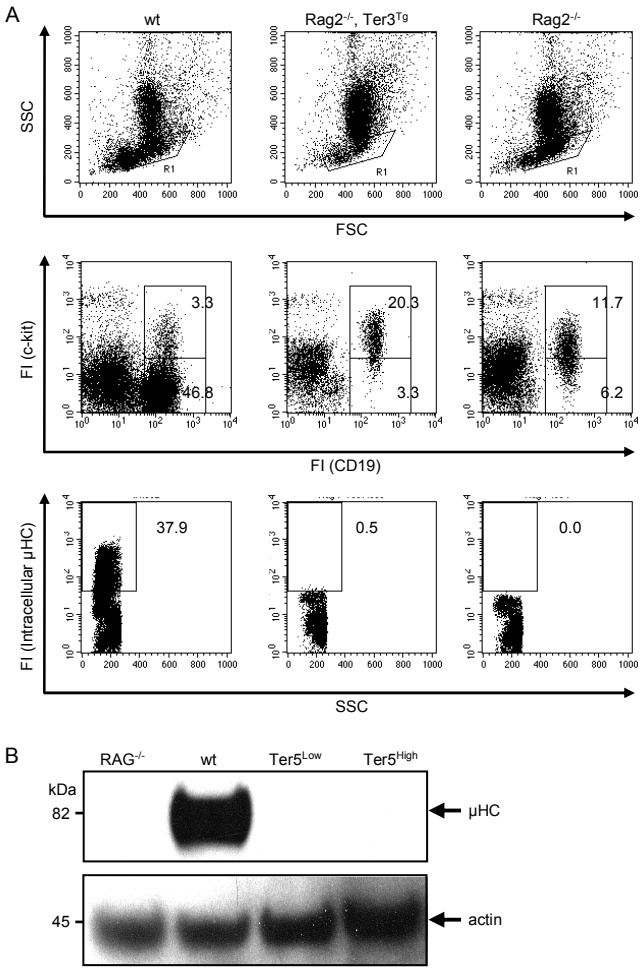
Supplementary Figure 1.



Supplementary Figure 1. Comparison of the abundance of nonsense μH transcripts from the Ter3 transgene (V_H17.2.25 VDJ exon with stop = Ter3) with that of sense μH transcripts from the QM allele (productive V_H17.2.25 VDJ targeted into the IgH locus). (**A**) Genomic organization of the wild-type sense and Ter3 nonsense V_H17.2.25 VDJ exon in the respective transgenes and pμ expression vectors. The conversion of a downstream ATG into an Ala codon created a FspI restriction site in the nonsense allele, which distinguishes the two V_H17.2.25 exons. PCR primer pairs are indicated by arrows. (**B**) RT-PCR analysis of V_H17.2.25 mRNA in Ag8 plasmacytoma cells transfected with the sense pμ and nonsense pμTer3 plasmid and in B lymphoid populations isolated from Ter3 transgenic mice heterozygous for the knock-in wild-type V_H17.2.25 VDJ exon ($IgH^{QM/wt}$, $Ter3^{Tg}$ mice). RNA was isolated from transfected Ag8 cells as well as sorted c-kit^+ pro-B cells and V_H17.2.25 $idiotype^+$ splenic B cells. V_H17.2.25-μH mRNA was amplified by RT-PCR with the primers indicated in panel A and purified and electrophoretically separated before (-) or after (+) FspI digest. The ethidium bromide-stained gel was scanned, and ratios of sense

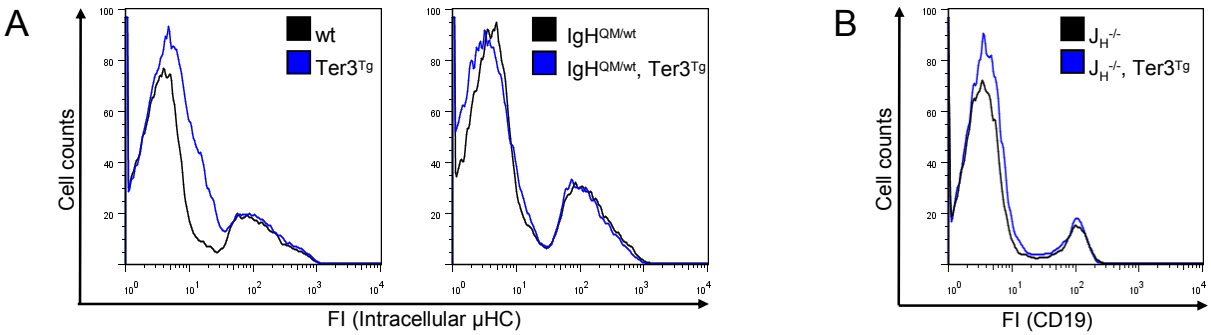
V_H17.2.25-μH mRNA to total V_H17.2.25-μH mRNA were calculated by dividing the intensities of the bands for the full-length 567-bp product in digested samples (containing only indigestible sense V_H17.2.25-μH mRNA) by that from undigested samples (containing both wild-type and Ter3-μH mRNA). Results are from three assays, with RNA from two mice (#1 and #2). The analysis revealed a threefold increase in the abundance of Ter3 mRNA in c-kit$^+$ pro-B cells and about equal amounts of both Ter3 and sense V_H17.2.25-μH transcripts in sorted CD19$^+$/V_H17.2.25 idiotype$^+$ splenic B cells.

Supplementary Figure 2.



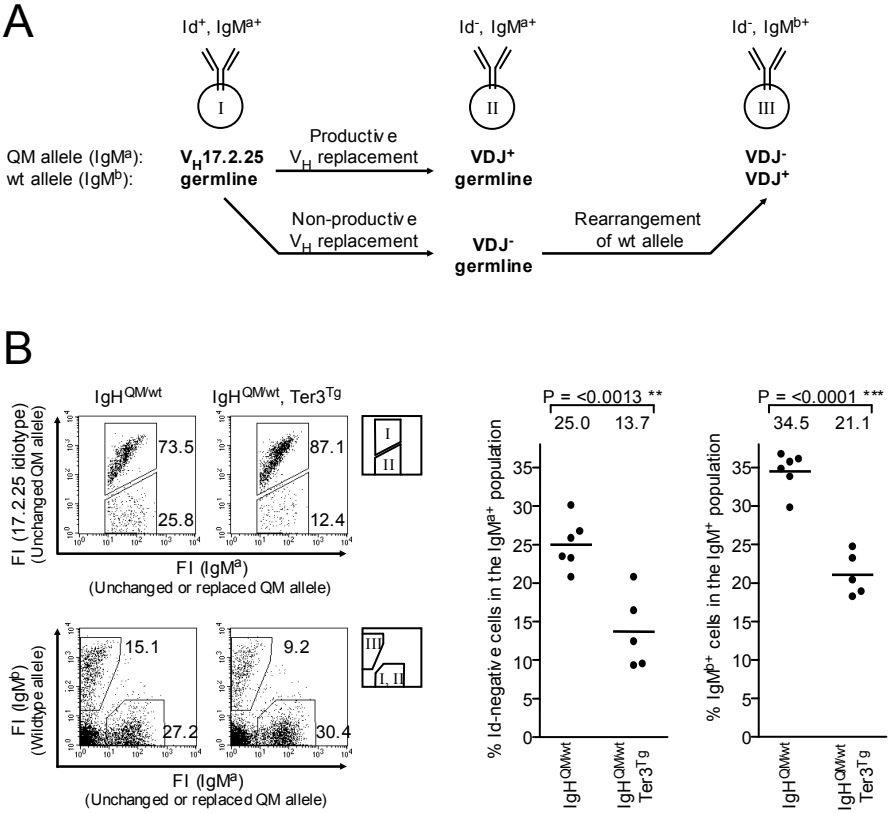
Supplementary Figure 2. Ter3 and Ter5 µH mRNAs are not translated into µH chain protein. (**A**) Bone marrow cells of 6-week-old mice were either membrane stained for c-kit and CD19 or fixed and stained for intracellular µH chain (µHC) using a polyclonal goat anti-IgM (µHC-specific) antibody. Fluorescence intensities (FI) of cells in the lymphocyte gate were determined. The percentages of cells in the individual gates are indicated. FSC, forward scatter; SSC, side scatter. (**B**) Lysates prepared from bone marrow cells of RAG-deficient ($Rag^{-/-}$), wild-type (wt) and homozygous $Ter5^{Low}$ and $Ter5^{High}$ mice were subjected to Western blot analysis using a polyclonal goat anti-µH chain antibody. Actin signals served as control for the integrity and quantity of loaded protein.

Supplementary Figure 3.



Supplementary Figure 3. Ter3 mRNA does not affect expression of IgM and CD19 in Ter3 transgenic mice. (**A**) Bone marrow cells from 6-week-old mice of the indicated genotypes stained for intracellular µH chain (µHC). (**B**) Bone marrow cells from J_H-deficient ($J_H^{-/-}$) mice membrane stained for CD19.

Supplementary Figure 4.



Supplementary Figure 4. Ter3 transcripts interfere with V_H replacement. (**A**) The V_H exon of the productive knock-in V_H17.2.25-H chain gene (QM allele) can be modified by V_H replacement. As a result, B cells in heterozygous $IgH^{QM/wt}$ mice can express any of three types of µH chains: (I) the unchanged V_H17.2.25-µH chain with V_H17.2.25 idiotype (Id^+) and a µH chain of allotype a (IgM^a), (II) an µH chain with a productively replaced V_H domain (Id^-) and a µH chain of allotype a (IgM^a), or (III) a µH chain with allotype b (IgM^b) encoded by a productively rearranged wild-type (wt) allele in cells with a nonproductive replacement (VDJ^-). (**B**) The effect of the Ter3 transgene on the frequency of V_H replacement was determined in the bone marrow of 6-week-old mice by flow cytometry in two ways. In the upper panel, the frequency of V_H17.2.25-Id^+ cells expressing the unchanged QM allele was determined within the IgM^a-positive population. The lower panel shows the analysis of IgM^a-positive cells expressing the QM allele and IgM^b-positive cells expressing a productively rearranged wild-type allele. Expression of the Ter3 transgene reduced the frequencies of both idiotype-negative cells in the IgM^a-positive B cell population and IgM^b-positive cells in the total B cell population. These data suggest a suppressive effect of Ter3 mRNA on VDJ recombination. Results for the entire litter are presented in the diagrams to the right; one dot represents one mouse.

Supplementary Table 1

Cells x10^5	Wildtype	$Ter3^{Tg}$	$Ter3^{Tg/Tg}$
c-kit^+	5.25 (±3.0)	6.84 (-)	12.66 (±2.36)
$CD25^+$	43.85 (±28.27)	36.32 (-)	17.27 (±4.66)
IgM^+	18.73 (±12.64)	18.98 (-)	8.33 (±1.65)

Supplementary Table 1. Average cell numbers of B cell populations in the bone marrow of 6-week-old Ter3 mice and littermate controls. Data are from a representative litter (mean ± SD).