Supporting Information

Kumar et al. 10.1073/pnas.1417988112

SI Materials and Methods

Establishment of IgA Transnuclear Mice. Briefly, cloned embryos at the stage of morula were plated individually in wells of a 96-well plate on a layer of mytomicin C-inactivated mouse embryonic fibroblasts (MEFs) and cultured in ES cell medium for 10 or more days. After this time, embryos were dissociated by trypsin digestion and single-cell suspensions plated on MEFs to ultimately establish ntES cell lines, as previously described (1). The pluripotency of ntES cell lines was confirmed by alkaline phosphatase staining and through the scoring, by immunostaining, of OCT3/4 and Nanog expression. Genomic PCR for IgH and IgL chain rearrangements confirmed the PC origin of ntES lines. One IgA ntES line displaying a normal karyotype was used to inject BALB/c blastocysts to generate chimeric mice. Crossings of IgA chimeras to C57BL/6J animals generated germ line–transmitted animals that were on the C57BL/6 X DBA/2J mixed genetic background.

IgH Southern Blotting Analysis. Genomic DNA (15 μg) was digested overnight with EcoR1, transferred to a positively charged nylon membrane, and hybridized to a ^{32}P-labeled genomic probe mapping downstream of J_H4 (2). To control for DNA loading, membranes were stripped and rehybridized to a probe annealing to a genomic fragment of the *Il4* gene (3). Quantification of bands relative to the V_HQ52^{NT} allele was performed using ImageJ software analysis.

In Vitro Pro–B-Cell Cultures. *Rag2*; *λ5*; *SLP65* triple-mutant pro-B cells were cultured in Iscove's medium (Biochrome AG) containing 10% (vol/vol) heat-activated fetal calf serum (PAN Biotech), 100 U/mL of penicillin, 100 U/mL of streptomycin (Life Technologies), and 2 mM of L-glutamine (Life Technologies) and 50 μM of 2-mercaptoethanol (Life Technologies). We added 1% supernatant of J558L cells stably transfected with a murine IL-7 expression vector to the culture. Primary BM-derived progenitor B-cell cultures were established as previously described (4). Briefly, after red blood cell lysis, BM cell suspensions were plated on OP9 cells in Iscove's modified Dulbecco's medium (GIBCO) supplemented with 20% (vol/vol) FCS, 2 mM of L-glutamine, 100 U/mL of penicillin, 100 U/mL of streptomycin (Life Technologies), and 1% supernatant of mouse IL-7–expressing J558L transfectants (kindly provided by F. Melchers, Max Planck Institute for Infection Biology, Berlin, Germany). Cocultures were fed with fresh medium every 2 d. Analysis of progenitor B cells was performed starting from day 12 of in vitro culture.

Measurement of Anti-DNA Antibodies by ELISA. Salmon sperm (Sigma Aldrich) was used as a source of both single-stranded (after boiling for 10 min) and double-stranded DNA. DNA (10 μg/mL) was immobilized on NUNC Maxisorp plates. Extensive washing with PBS/Tween 0.05% was followed by incubation for 1 h at 37 °C of plates with PBS-3% (vol/vol) BSA blocking solution. Mouse sera were serially diluted and applied to DNA-coated plates. Bound antibodies were revealed using biotin-conjugated rat anti-mouse Igκ (cl. R331.18) followed by incubation with streptavidin-Eu^{3+} (Perkin-Elmer). ELISAs were developed using DELFIA Enhancement Solution (Perkin-Elmer) and plates scanned for time-resolved fluorescence on an EnVision multilabel plate reader platform (Perkin-Elmer). Arbitrary units were determined by multiplying average FRET counts by the dilution factor and dividing by 1×10^6.

1. Wakayama T, et al. (2001) Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer. *Science* 292(5517):740–743.
2. Fukita Y, Jacobs H, Rajewsky K (1998) Somatic hypermutation in the heavy chain locus correlates with transcription. *Immunity* 9(1):105–114.
3. Oberdoerffer P, Novobrantseva TI, Rajewsky K (2003) Expression of a targeted lambda 1 light chain gene is developmentally regulated and independent of Ig kappa rearrangements. *J Exp Med* 197(9):1165–1172.
4. Vieira P, Cumano A (2004) Differentiation of B lymphocytes from hematopoietic stem cells. *Methods Mol Biol* 271:67–76.

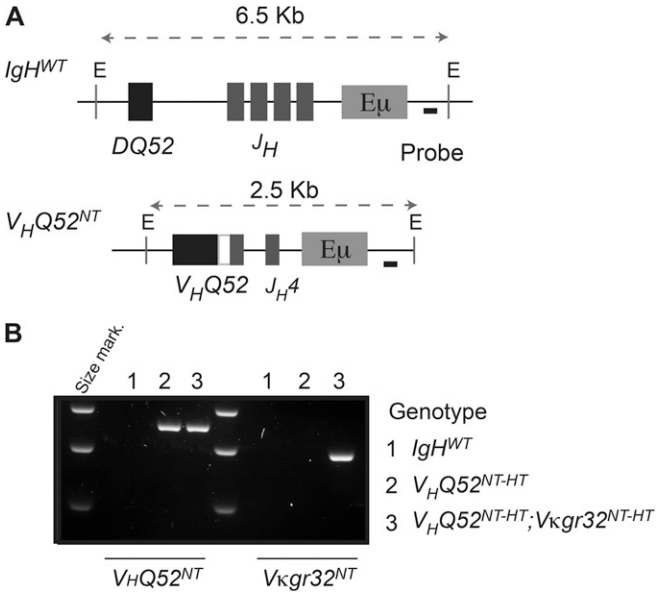

Fig. S1. Germ-line transmission of V_HQ52^{NT}; $V\kappa gr32^{NT}$ Ig rearrangements. (*A*) Southern blotting strategy to screen for germ-line transmission of the V_HQ52^{NT} allele. Genomic DNA was digested with EcoR1 (*E*) and screened with the indicated probe (black bar). Size of genomic fragments (in Kb) corresponding to IgH germ-line and V_HQ52^{NT} alleles, respectively, are shown. (*B*) PCR genotyping of tail DNA of coat-color germ-line transmitted mice using primers specific for V_HQ52^{NT}; $V\kappa gr32^{NT}$ rearrangements. Representative mice inheriting, respectively, the $V_HQ52^{NT\text{-}HT}$ allele alone (no. 2) or in combination with the $V\kappa gr32^{NT\text{-}HT}$ gene (no. 3) are displayed. A wild-type control animal (no. 1) was also included in the analysis.

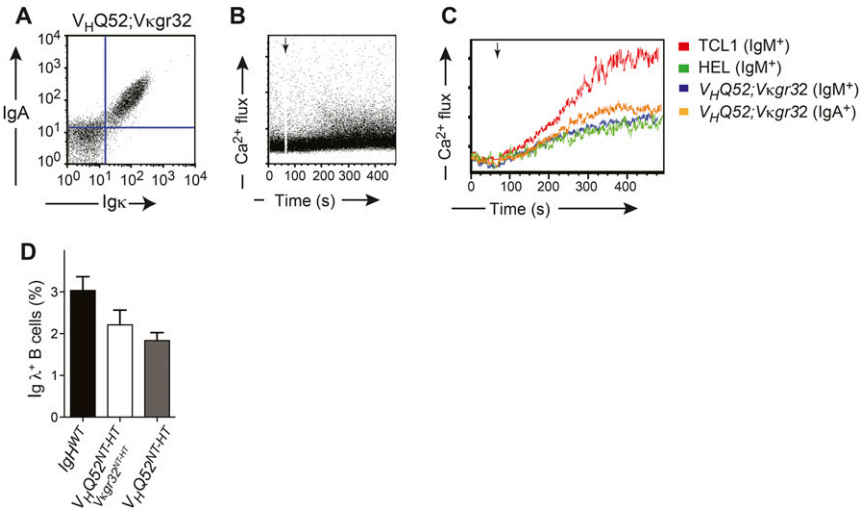

Fig. S2. Transnuclear IgA does not trigger spontaneous Ca^+ mobilization. (*A*) Flow cytometric analysis of TKO pro-B cells reconstituted with transnuclear IgA H/L chains. (*B*) Flow cytometric measurement of spontaneous Ca^{2+} flux upon treatment (arrow) with 4-OHT of BCR-complemented TKO pro-B cells to activate ERT2–SLP65 function. (*C*) Overlay of Ca^{2+} flux measurements in BCR-complemented TKO pro-B cells after addition of 4-OHT. Autonomously active TCL1-derived and nonautonomously active HEL-specific BCRs served as controls. (*D*) Average frequency of sIgλ^+ B220$^+$ B cells in BM of V_HQ52^{WT} ($n = 6$), $V_HQ52^{NT\text{-}HT}$ ($n = 4$), and $V_HQ52^{NT\text{-}HT}$; $V\kappa gr32^{NT\text{-}HT}$ mice ($n = 4$).

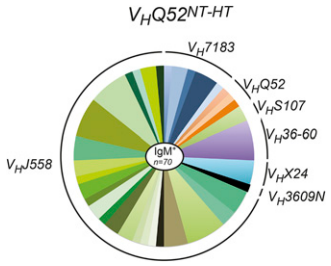

Fig. S3. IgH repertoire of IgM$^+$ B cells in $V_HQ52^{NT\text{-}HT}$ mice. Pie chart representation of V_H rearrangements (n = 70) amplified by RT-PCR from splenic IgM$^+$ B cells of $V_HQ52^{NT\text{-}HT}$ mice using as the reverse primer an oligonucleotide annealing to the Cμ constant region. V_H genes were grouped according to V_H family (n = 3). Each color represents an individual V_H gene. The size of segments is proportional to the frequency of the corresponding V_H rearrangement.

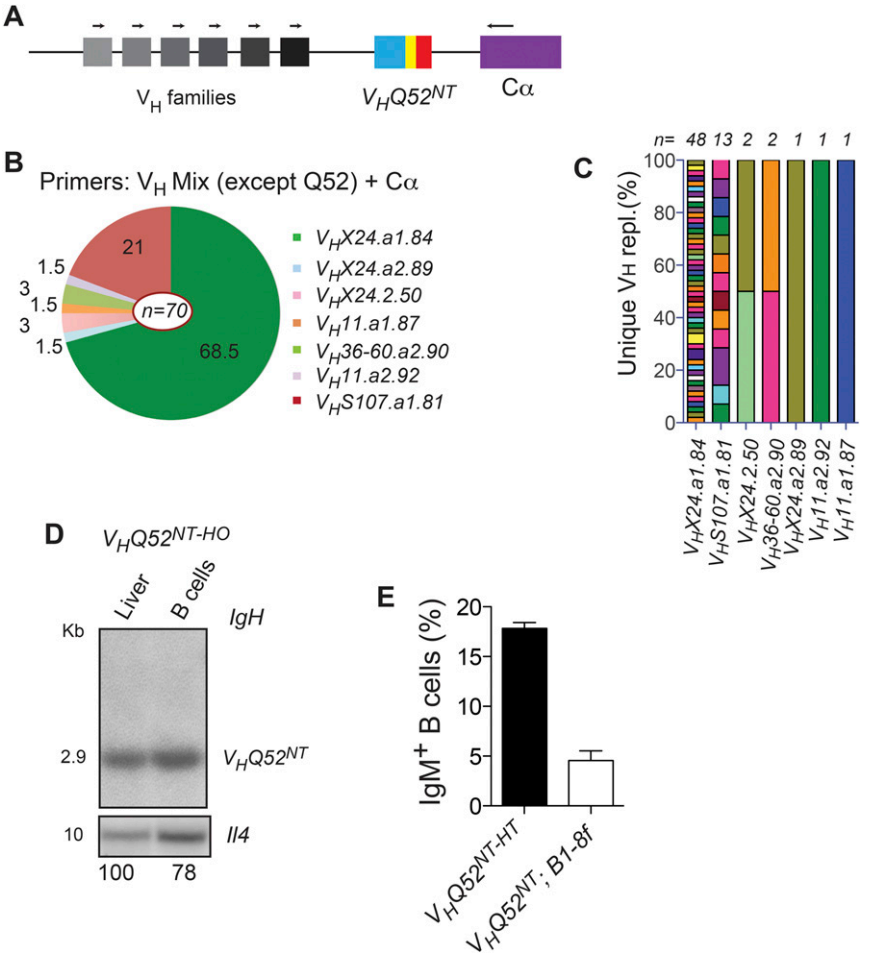

Fig. S4. Analysis of VH replacement in IgM$^+$ B cells of $V_HQ52^{NT\text{-}HT}$ mice. (*A*) Strategy to identify V_H replacements by RT-PCR analysis. Primers are indicated with arrows. The reverse primer anneals to the Cα constant region. (*B*) Frequency of V_H genes recruited in VH replacements (n = 70) amplified by RT-PCR from purified IgM$^+$$V_HQ52^{NT\text{-}HT}$ B cells (n = 6). PCR primer combination is indicated. (*C*) Frequency of unique V_H replacements amplified by RT-PCR from IgM$^+$ B cells of $V_HQ52^{NT\text{-}HT}$ mice, assessed by CDR3 sequence analysis. Replacements using the same indicated V_H germ-line gene were grouped. (*D*) Southern blotting analysis to quantify V_HQ52^{NT} gene copy number. DNA input was normalized using a probe to measure *Il4* gene copy number. Numbers below refer to the fraction (%) of V_HQ52^{NT} alleles in B cells compared with liver cells after normalization for DNA input. (*E*) Average frequency of splenic IgM$^+$ B cells in mice (n = 4) of the indicated genotypes.

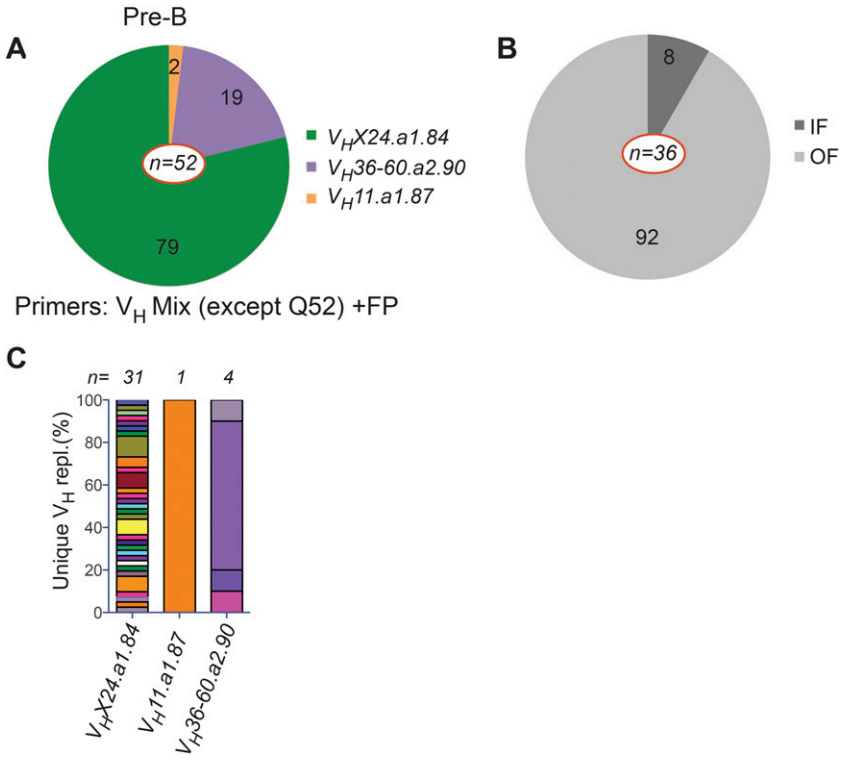

Fig. S5. Analysis of V_H replacement in progenitor B cells of $V_HQ52^{NT\text{-}HT}$ mice. (*A*) Frequency of V_H genes contributing to VH replacements ($n = 52$) amplified by genomic PCR from sorted pre-B cells ($B220^{lo}Ig\kappa^{-}CD43^{-}CD25^{+}$). PCR primers are indicated below. (*B*) Frequency of, respectively, IF and OF unique V_H replacements ($n = 36$) cloned from pre-B cells of $V_HQ52^{NT\text{-}HT}$ mice. (*C*) Frequency of unique V_H replacements ($n = 36$) cloned from pre-B cells of $V_HQ52^{NT\text{-}HT}$ mice, as assessed by CDR3 nucleotide sequence analysis. VH replacements using the same indicated V_H germ-line gene were grouped.

Other Supporting Information Files

Table S1 (DOCX)
Table S2 (DOCX)