Southern blot of DNA from the indicated cell sources (above lanes), hybridized to a probe 3‘ of the IgH locus (3’Igh). This probe maps ~70kb downstream of the 3‘ regulatory region of the IgH locus and detects a 1.5kb EcoRI/BamHI fragment.

Supplemental Table I: somatic hypermutation in germinal center B cells isolated from Peyer's patches

Mouse genotype	Allele	Region analyzed	mutated clones /number clones sequenced (%)	Mutation Frequency (No. of mutations / total nt. sequenced)	point mutations per sequence
$V_HE\mu$/WT	$V_HE\mu$	exon[a]	30/46(65%)	4.20% (696/16560)	0-44
		synonymous[b]	30/46 (65%)	5.51% (201/3649)	0-17
		intron[c]	28/46 (61%)	2.08% (205/9844)	0-24
$V_H\Delta$/WT	$V_H\Delta$	exon[a]	35/53 (66%)	3.46% (661/19080)	0-40
		synonymous[b]	32/53 (60%)	3.45% (145/4204)	0-12
		intron[c]	32/53 (60%)	2.10% (238/11342)	0-23

Germinal center cells(B220+CD95+PNA^{high}) cells from Peyer's patch

[a,b,c] exon = 360 bp covering B1-8 V_H coding sequences. synonymous = 79.33 synonymous sites within the exon (synonymous sites are defined as sites where nucleotide change(s) will not affect amino acid sequence); intron = 214 bp intronic sequences adjacent to the exon and shared by the $V_HE\mu$ and $V_H\Delta$ alleles.

$V_H\Delta$ allele of $V_H\Delta$/WT mice was compared to the $V_HE\mu$ allele of $V_HE\mu$/WT mice (two-tailed Mann-Whitney test, GraphPad Prism, version 5.02 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). No significant difference detected.

Supplemental Table II: somatic hypermutation in splenic germinal center B cells

Mouse genotypes	Allele	Region analyzeda	mutated clones /number clones sequenced (%)	Mutation Frequency (No. of mutations / total nt. sequenced)	Point Mutations per sequence
$V_HE\mu/V_H\Delta$	$V_HE\mu$	exon	31/47 (66%)	0.78% (132/16920)	0-13
SRBC[b]		synonymous	16/47 (34%)	0.70% (26/3729)	0-4
		intron	16/47 (34%)	0.30% (30/10058)	0-5
	$V_H\Delta$	exon	28/49 (57%)	0.49% (86/17640)	0-12
		synonymous	13/49% (27%)	0.59% (23/3887)	0-5
		intron	12/49 (24%)	0.31% (32/10486)	0-13
$V_HE\mu/V_H\Delta$	$V_HE\mu$	exon	31/38 (82%)	1.34% (183/13680)	0-20
SRBC[c]		synonymous	20/38 (52%)	1.43% (43/3015)	0-5
IgG+		intron	24/38 (63%)	0.65% (53/8132)	0-6
	$V_H\Delta$	exon	35/50 (70%)	0.84% (151/18000)	0-15
		synonymous	20/50 (40%)	0.98% (39/3967)	0-5
		intron	19/50 (38%)	**0.40% (43/10700)***	0-5
$V_HE\mu/V_H\Delta$	$V_HE\mu$	exon	19/44 (43%)	0.92% (146/15840)	0-20
NP-CGG[d]		synonymous	12/44 (27%)	1.15% (40/3491)	0-9
IgG+		intron	14/44 (32%)	0.40% (38/9416)	0-12
	$V_H\Delta$	exon	23/46 (50%)	0.68% (112/16560)	0-14
		synonymous	12/46 (26%)	0.69% (25/3649)	0-4
		intron	19/46 (41%)	0.50% (49/9844)	0-6

[a] See Table I legend for description of regions sequenced

[b,c,d:] Germinal center B cells and IgG^+ germinal center B cells, sorted as $B220^+CD95^+PNA^{high}$ (b) and $B220^+CD95^+IgG^+$ (c,d), respectively, were analyzed 7 days after SRBC (b,c) or NP-CGG (d) immunization.

* $p < 0.05$. $V_H\Delta$ allele compared to $V_HE\mu$ allele of $V_HE\mu/V_H\Delta$ mice (two-tailed Mann-Whitney test, GraphPad Prism, version 5.02 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). No other differences between $V_H\Delta$ and $V_HE\mu$ alleles reached statistical significance.

Supplemental Table III. Distribution and nature of mutations

Region	V186.2[a]				exon[b]		intron[c]	
mouse	WT	$V_HE\mu$/WT	$V_H\Delta$/WT		$V_HE\mu/V_H\Delta$, $V_HE\mu$/WT, and $V_H\Delta$/WT			
Allele	WT	$V_HE\mu$	$V_H\Delta$	WT	$V_HE\mu$	$V_H\Delta$	$V_HE\mu$	$V_H\Delta$
No. of point mutations (sequences)	315 (51)	250 (47)	161 (93)	193 (39)	1247 (95)	935 (95)	532 (95)	430 (95)
RGYW/WRCY mutations(%)	50.2	44.8	50.3	38.9	47.1	47.6	18.4	23.2
Mutations at AT (%)	34.5	41.2	54.0	40.3	43.6	41.6	55.8	62.4
Transition mutations (%)	59.0	54.8	60.2	56.4	59.9	57.2	53.4	51.6
Transition at AT (%)	49.6	54.4	54.1	38.5	48.9	47.5	44.5	48.2
Transition at CG (%)	64.1	55.1	67.5	68.6	68.5	63.9	64.8	57.5

[abc] The regions within which mutations were analyzed: V186.2 = the V gene segment shared by WT and knockin B1-8 V_H; exon = all of B1-8 V_H coding sequences (VDJ) ; intron = intronic sequences following B1-8 V_H shared by the $V_HE\mu$ and $V_H\Delta$ alleles. Data derived from Peyer's patch clones and from splenic cDNA clones.

Supplemental Table IV: Base exchange pattern of observed point mutations

Region	Allele(mice)								Region	Allele							
											$V_HE\mu$/WT, $V_H\Delta$/WT, and $V_HE\mu$/$V_H\Delta$ mice						
V186.2[a]		n=315	to	A	C	G	T	Total	Exon[b]		n=1247		A	C	G	T	Total
			A		0.03	0.11	0.11	0.26				A		0.06	0.16	0.11	0.33
		From	C	0.02		0.05	0.14	0.21			From	C	0.04		0.04	0.15	0.22
	WT (WT)		G	0.28	0.06		0.10	0.44		$V_HE\mu$		G	0.24	0.06		0.04	0.34
			T	0.02	0.06	0.00		0.09				T	0.04	0.05	0.02		0.11
		n=250	to	A	C	G	T	Total			n=935		A	C	G	T	Total
			A		0.04	0.18	0.13	0.36				A		0.07	0.12	0.08	0.28
	$V_HE\mu$	From	C	0.08		0.04	0.15	0.26			From	C	0.04		0.06	0.16	0.26
	($V_HE\mu$/WT)		G	0.17	0.08		0.07	0.32		$V_H\Delta$		G	0.21	0.07		0.04	0.32
			T	0.01	0.04	0.00		0.06				T	0.04	0.07	0.02		0.14
		n=161	to	A	C	G	T	Total	Intron[c]		n=532		A	C	G	T	Total
			A		0.06	0.20	0.12	0.39				A		0.07	0.17	0.16	0.40
	$V_H\Delta$	From	C	0.02		0.04	0.15	0.22			From	C	0.02		0.02	0.14	0.17
	($V_H\Delta$/WT)		G	0.16	0.06		0.02	0.24		$V_HE\mu$		G	0.15	0.05		0.06	0.27
			T	0.06	0.09	0.01		0.16				T	0.04	0.07	0.05		0.16
		n=193	to	A	C	G	T	Total			n=430		A	C	G	T	Total
			A		0.04	0.10	0.16	0.30				A		0.07	0.20	0.14	0.42
	WT	From	C	0.01		0.05	0.17	0.22			From	C	0.01		0.06	0.10	0.17
	($V_H\Delta$/WT)		G	0.24	0.09		0.05	0.37		$V_H\Delta$		G	0.11	0.05		0.04	0.20
			T	0.04	0.06	0.01		0.10				T	0.05	0.10	0.06		0.21

N = total number of mutations. The fraction of each type of base exchange (from A to C, etc.) was calculated as the number of such mutations divided by the total number of mutations. The total number of mutations at a given residue divided by all mutations = fraction of "Total"

[abc] The regions within which mutations were analyzed: V186.2 = the V gene segment shared by WT and knockin B1-8 V_H; exon = all of B1-8 V_H coding sequences (VDJ) ; intron = intronic sequences following B1-8 V_H shared by the $V_HE\mu$ and $V_H\Delta$ alleles. Data derived from Peyer's patch clones and from splenic cDNA clones.