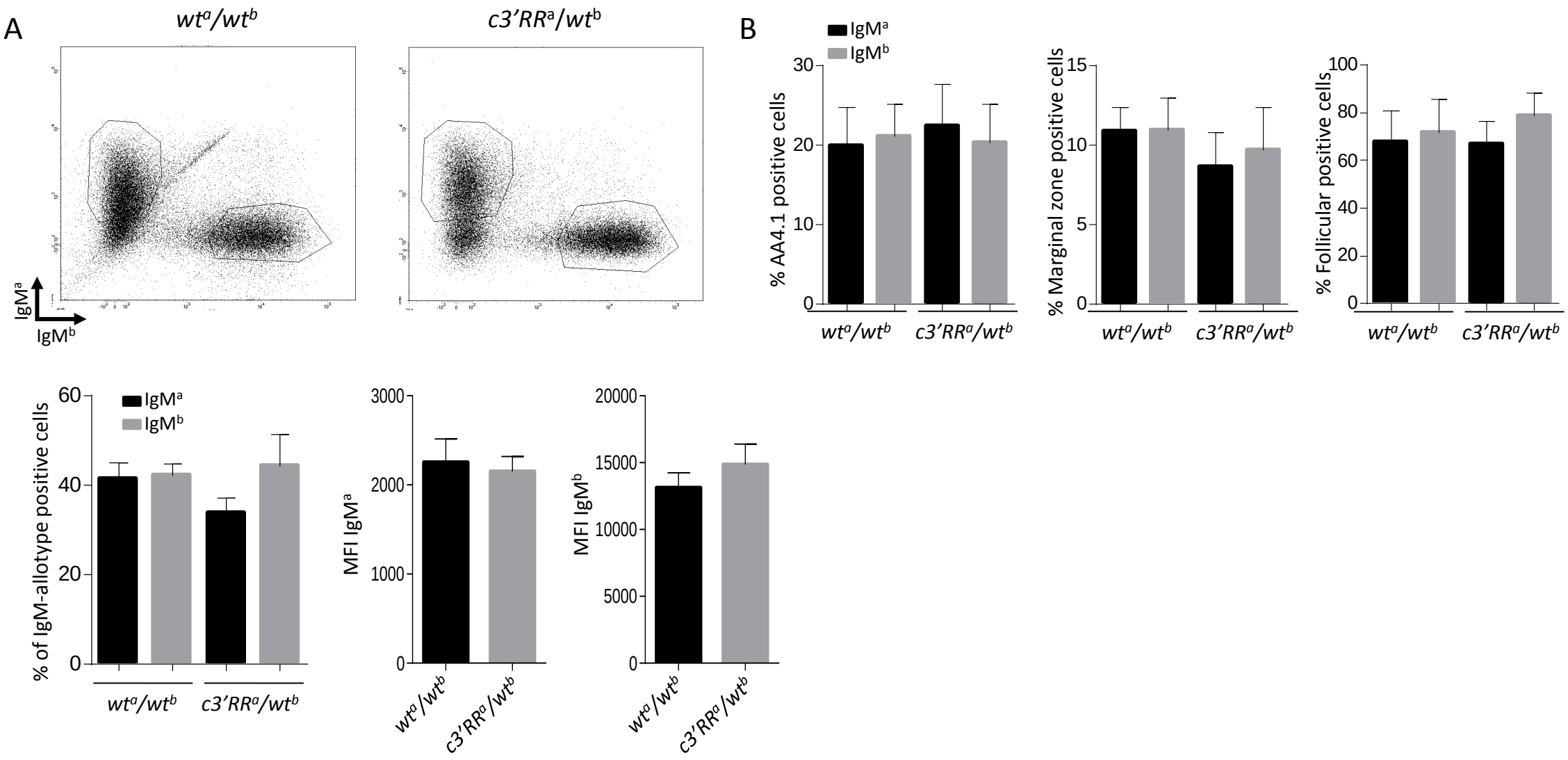
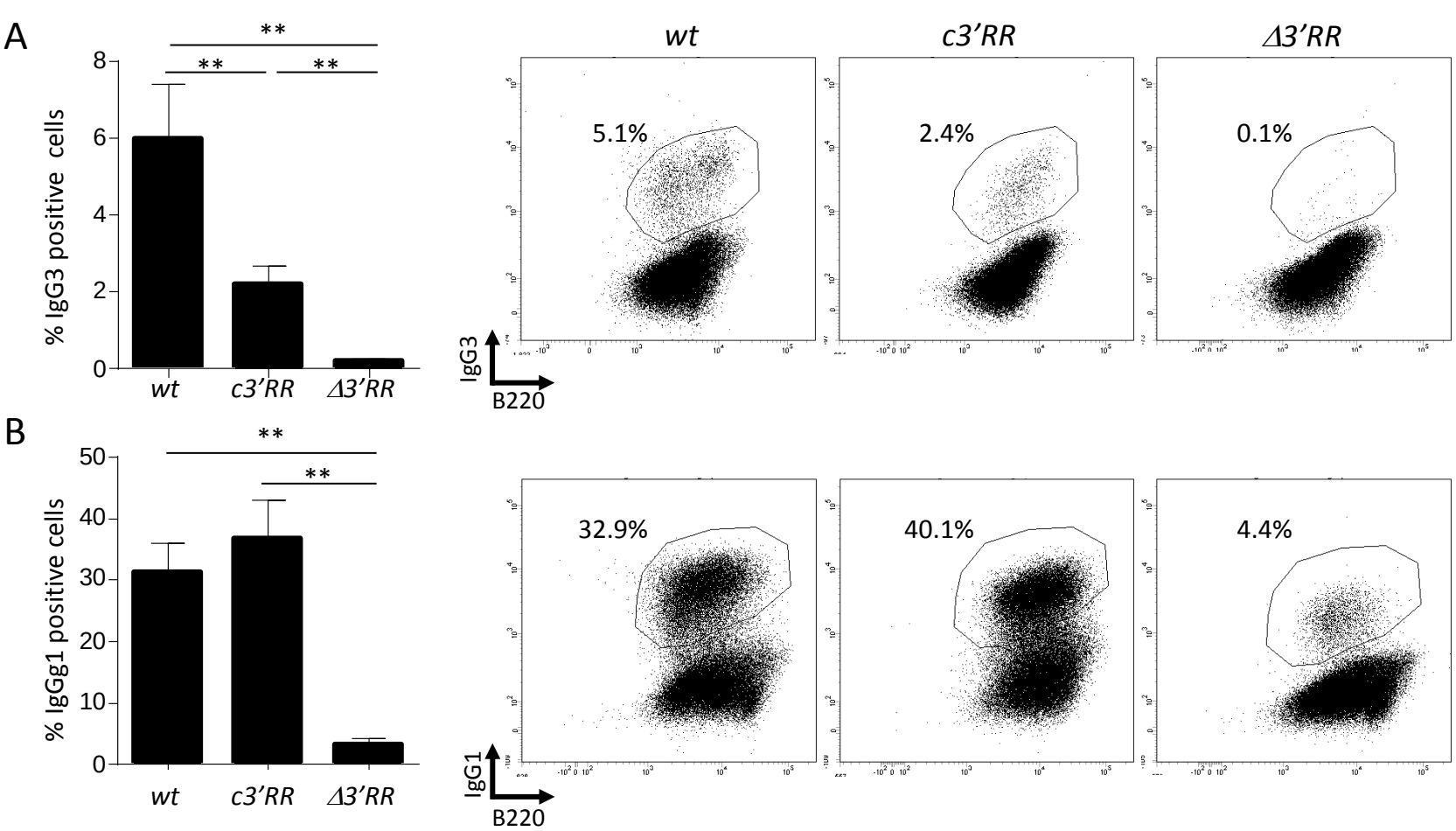
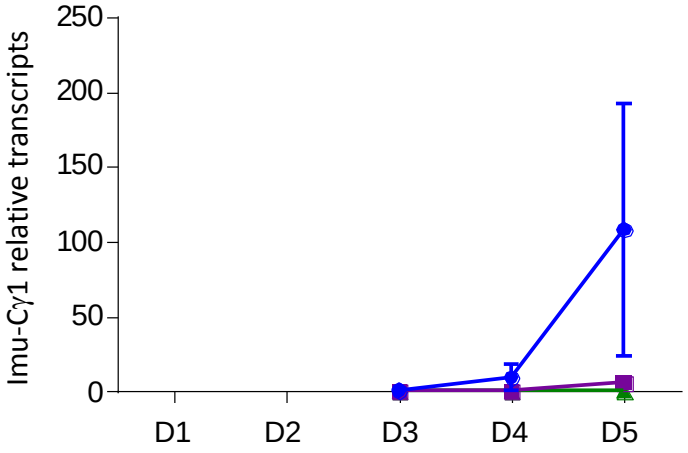
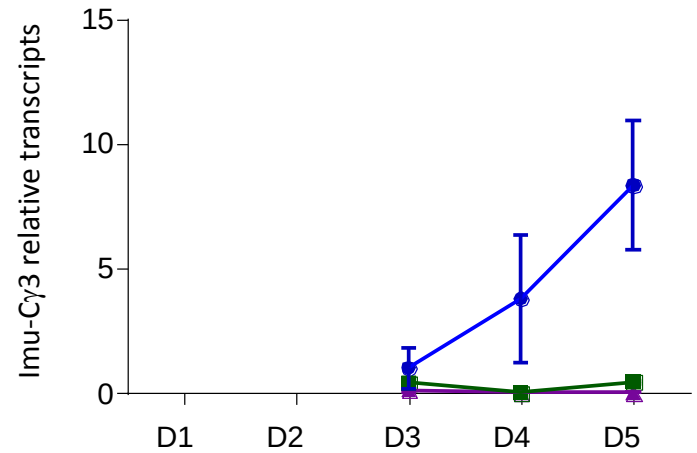


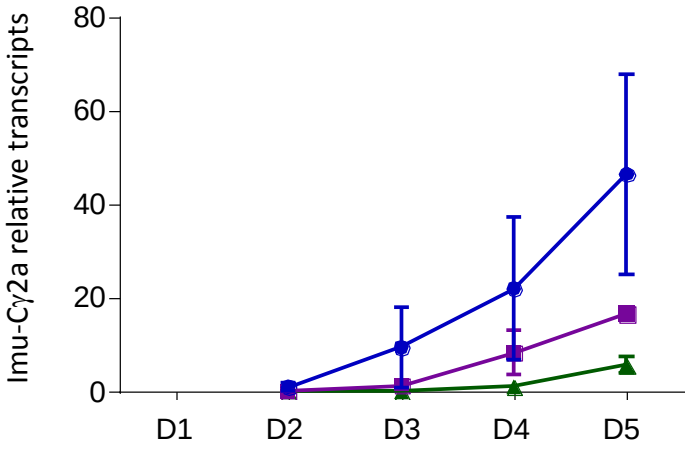
Supplementary Figure S1

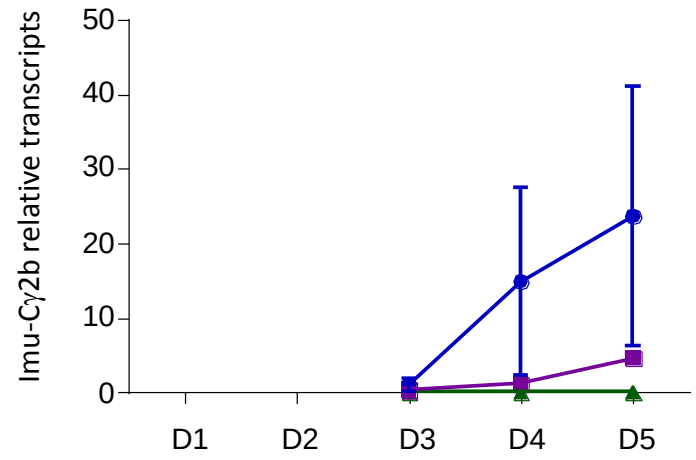


Supplementary Figure S2





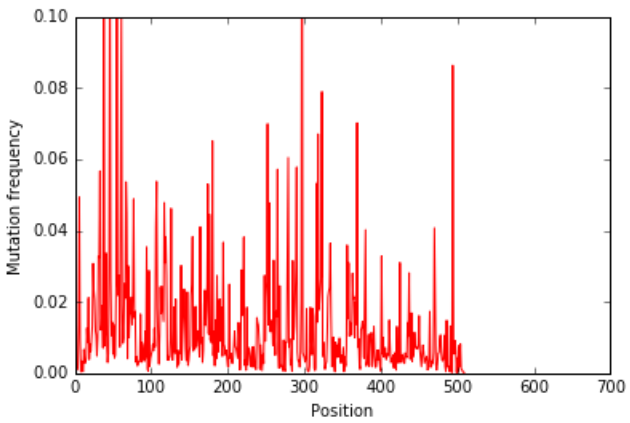




Supplementary Figure S3

3’JH4 mutations

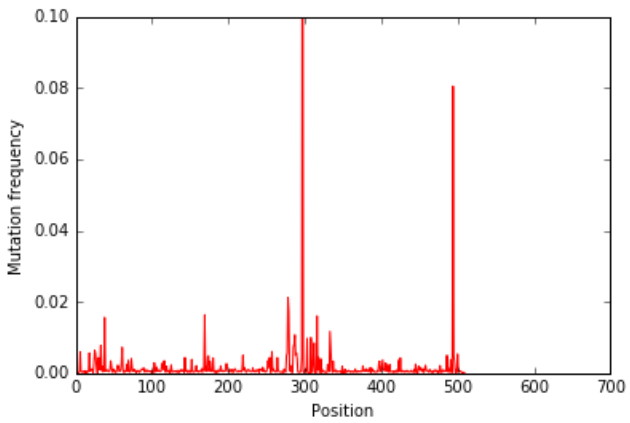
wt



Mutation rate: 12.85 /1000 bases
Mutations: 2 095 296
Aligned bases: 162 999 878

	A	T	C	G	Tot
A	0	11	9	15	35
T	6	0	8	5	19
C	5	13	0	8	26
G	11	5	4	0	20

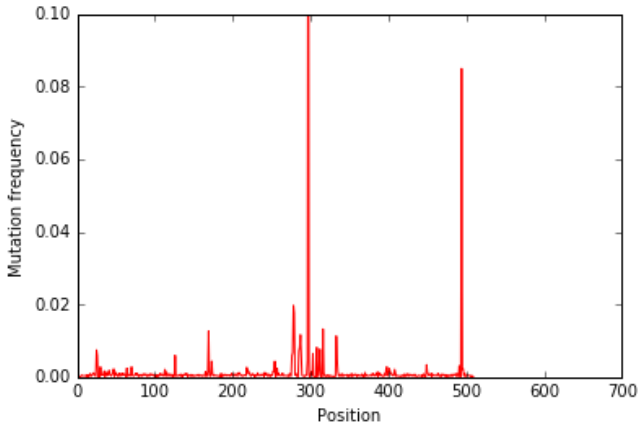
c3’RR



Mutation rate: 1.27 /1000 bases
Mutations: 96 919
Aligned bases: 76 426 949

	A	T	C	G	Tot
A	0	10	4	15	29
T	11	0	8	6	25
C	6	12	0	4	22
G	14	7	3	0	24

Δ3’RR

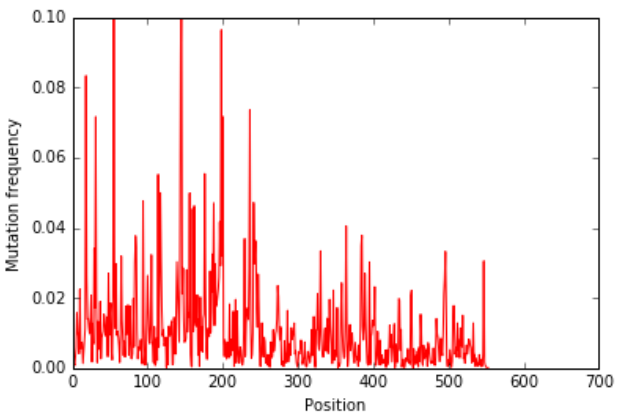


Mutation rate: 0.94 /1000 bases
Mutations: 171 902
Aligned bases: 182 651 508

	A	T	C	G	Tot
A	0	8	6	15	29
T	11	0	9	7	27
C	6	12	0	3	21
G	13	8	3	0	24

3’Jk5 mutations

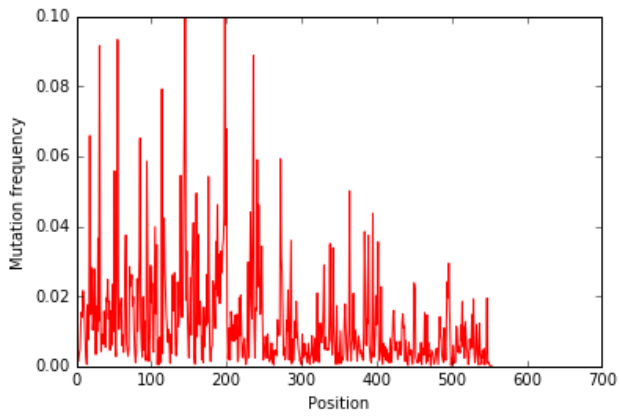
wt



Mutation rate: 11.02 /1000 bases
Mutations: 87 546
Aligned bases: 7 941 973

	A	T	C	G	Tot
A	0	7	8	15	30
T	7	0	9	6	22
C	5	13	0	7	25
G	12	4	6	0	22

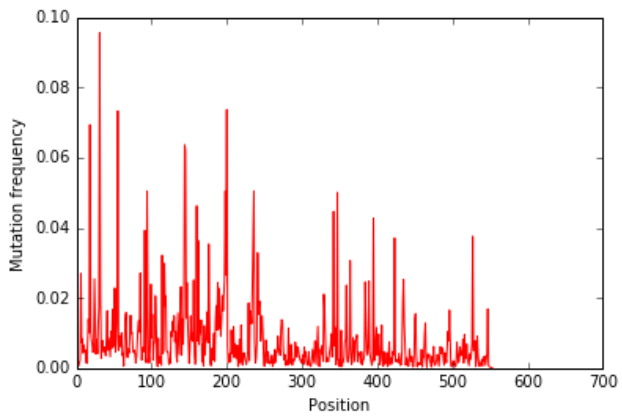
c3’RR



Mutation rate: 13.42 /1000 bases
Mutations: 1 530 524
Aligned bases: 114 047 327

	A	T	C	G	Tot
A	0	8	6	16	30
T	8	0	11	5	24
C	5	16	0	7	28
G	11	3	5	0	19

Δ3’RR



Mutation rate: 8.25 /1000 bases
Mutations: 5 596 862
Aligned bases: 678 247 884

	A	T	C	G	Tot
A	0	9	6	16	31
T	7	0	10	4	21
C	4	13	0	8	25
G	11	4	7	0	22

Supplementary Figure S4

Figure S1: B-cell development

(A) Representative dot plot of IgM^a and IgM^b expression in *wt* and *c3'RR* mice (*top*). Bar graph representing the percentage of IgM^a and IgM^b positive cells [gated on B220 positive cells] in wt^a/wt^b and $c3'RR^a/wt^b$ mice (*bottom left*). Mean ± SEM of membrane IgM^a and IgM^b densities in wt^a/wt^b and $\mu 3'RR^a/wt^b$ mice (*bottom right*). (B) Flow cytometry analysis of transitional ($AA4.1^+$), follicular ($CD21^{low}CD23^{high}$) and marginal zone ($CD21^{high}CD23^{low}$) B-cells in wt^a/wt^b and $c3'RR^a/wt^b$ mice.These experiments were performed on splenic cells from 2 month old mice.

Figure S2: CSR analysis

(A) IgG3 positive B-cells after 4-days LPS *in vitro* stimulation of splenocytes from *wt, c3'RR* and *Δ3'RR* mice, analysed by flow cytometry (*left*). Right panel shows one representative experiment. (B) As in (A) for LPS + IL4 *in vitro* stimulation. Mean ± SEM of 2 independent experiments, with 3 mice each.

Figure S3: Post Switch transcripts

Day1 to 5 kinetics of post-switch transcripts, in LPS +/- cytokines *in vitro* stimulated splenocytes from *Δ3'RR*, *c3'RR* and *wt* mice. Mean ± SEM of an experiment with 2 mice.

Figure S4: Positions and patterns of SHM

Positions and patterns of mutations in the introns flanking rearranged J_H4 and Jκ5 segments, in *wt* compared to *c3'RR* and *Δ3'RR* Peyer's patch GC B-cells. Mutation rate, total number of mutations identified, and total number of aligned bases are indicated. Data are shown for one representative mouse of each genotype.

Primers	Sequence 5' - 3'	Use
5' Arm 1	AAG GAG GCA TAG AAG CAG A	ES cells Screening
5' Arm 2	AGA AGA ACT CGT CAA GA	ES cells Screening
3' Arm 1	AAA GTA TCG TTA GGC CCT AGG	ES cells Screening
3' Arm 2	GTG CAA TCC ATC TTG TTC AAT GGC CGA TCC	ES cells Screening
Screen For wt allele	AGG GAC TGA AGT CCAT CCA TAG	mice screening
Screen rev wt allele	CAG AGC AGA GAA GAC TCC ATC C	mice screening
Screen For mutant allele	ACT CAT TCT AAT AAC TGG TCC C	mice screening
Screen rev mutant allele	CTC TCA CTT CCC TGG GGT GTT	mice screening
CRISP 5' for	CAC CGG GCC ATG ATA CTG GTC ACT	CRISPR/cas 9 primer
CRISP 5' rev	TTT AGT GAC CAG TAT CAT GGC CC	CRISPR/cas 9 primer
CRISP 3' for	CAC CGC CAC AAG CAC CGG CTG CCA	CRISPR/cas 9 primer
CRISP 3' rev	TTT GTG GCA GCC GGT GCT TGT GGC	CRISPR/cas 9 primer
5'Arm For AAtII	TTT GAC GTC AAC GTC AAG AGC CAC TTT CC	µKIKS locus construction
5' Arm Rev ClaI	TTT ATC GAT GAC CAG TAT CAT GGC CCA GT	µKIKS locus construction
Imu-CG3-Fwd Imu2	CTC GGT GGC TTT GAA GGA AC	PST primers
Imu-Cmu-Fwd	ACC TGG GAA TGT ATG GTT GTG GCT T	PST primers
Imu-CG1-Rev	ATG GAG TTA GTT TGG GCA GCA	PST primers
Imu-CG2b-Rev	CGG AGG AAC CAG TTG TAT C	PST primers
Imu-CG2a-Rev	GCT GGG CCA GGT GCT CGA GGT T	PST primers
Imu-CG3-Rev	ACC AAG GGA TAG ACA GAT GGG G	PST primers
PST Imu-CA Fw	ACC TGG GAA TGT ATG GTT GTG GCT T	PST primers
PST Imu-CA Rev	TAA TCG TGA ATC AGG CAG	PST primers
IG3-CG3-Fwd	AAC TAC TGC TAC CAC CAC CAC CAG	GLT primers
IG3-CG3-Rev	ACC AAG GGA TAG ACA GAT GGG G	GLT primers
IG1-CG1-Fwd	GGC CCT TCC AGA TCT TTG AG	GLT primers
IG1-CG1-Rev	ATG GAG TTA GTT TGG GCA GCA	GLT primers
IG2b-Fwd	CCA ACC AGG AAG AGT CCA GAG	GLT primers
CG2b-Rev	ACA GGG ATC CAG AGT TCC AAG T	GLT primers
IG2a-CG2a-Fwd	GCT GAT GTA CCT ACC GAG AGA	GLT primers
IG2a-CG2a-Rev	GCT GGG CCA GGT GCT CGA GGT T	GLT primers
GLT IA-CA Fw	CTA CCA TAG GGA AGA TAG CCT	GLT primers
GLT IA-CA Rev	TAA TCG TGA ATC AGG CAG	GLT primers
sIgM for	TGG AAC TCC GGA GAG ACC TA	secreted IgM transcripts
sIgM rev	CGC TAG CAT GGT CAA TAG CA	secreted IgM transcripts
VHJ558 SHM	CAG CCT GAC ATC TGA GGA CTC TGC	SHM analysis
SHM JH4 3'	CAG CAA CTA CCC TTT TGA GAC CGA	SHM Analysis

Supplementary Table S1 : Name and sequence of the primers used in this study