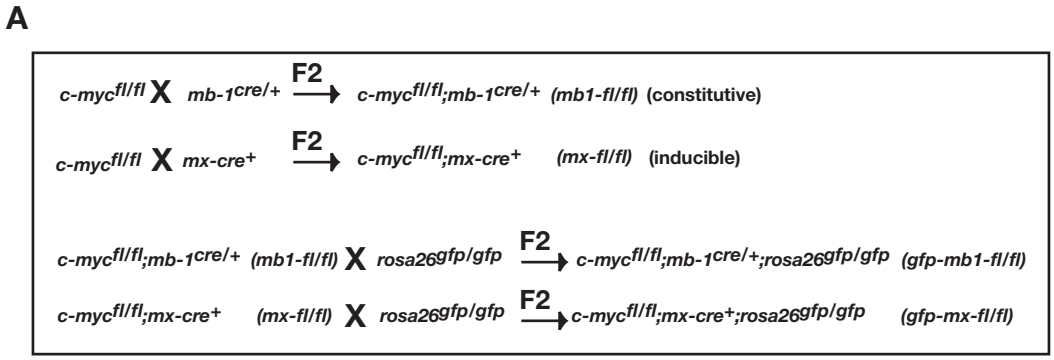

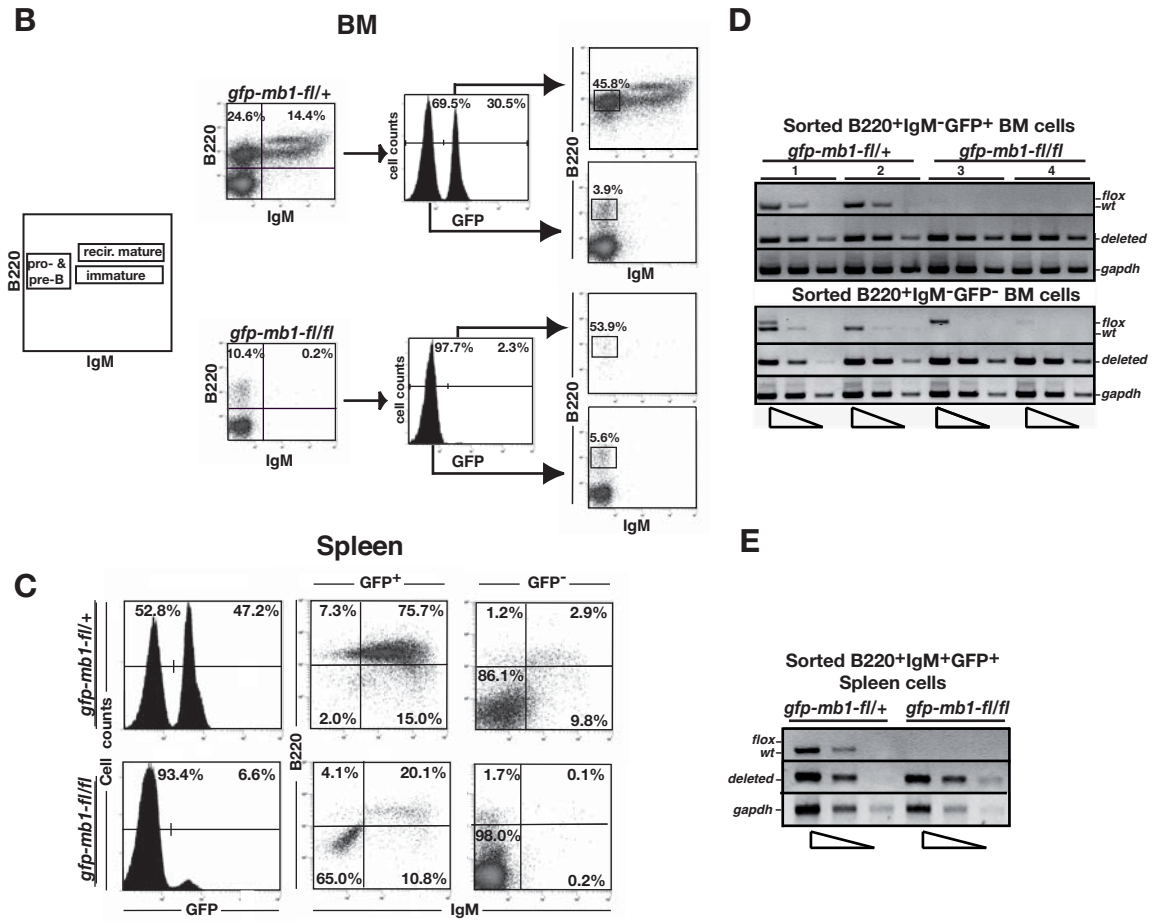

Fig. S1. Deletion of *c-myc* and induction of GFP expression in *gfp-mb1-fl/fl* and heterozygous *gfp-mb1-fl/+* mice. (*A*) Outline of breedings to generate the indicated mouse models. (*B*, *C*) Flow cytometry analysis of BM and spleen cells from mice of the indicated genotype. Experiment representative of at least three independent experiments. (*D*) Genomic PCR analysis of wt, deleted and flox alleles of *c-myc* from sorted B220+IgM- BM cells from the mice shown in (*B*). Numbers indicate individual mice. Experiment representative of two independent experiments with a total of 4 mice for each genotype. (*E*) Genomic PCR analysis of *wt*, *deleted* and *flox* alleles of *c-myc* from sorted B220+IgM+GFP+ spleen cells. *gfp-mb1-fl/fl (c-myc fl/fl;mb1 cre/+;rosa26 gfp/gfp)*. Experiment representative of three independent experiments with total of 3 mice for each genotype.

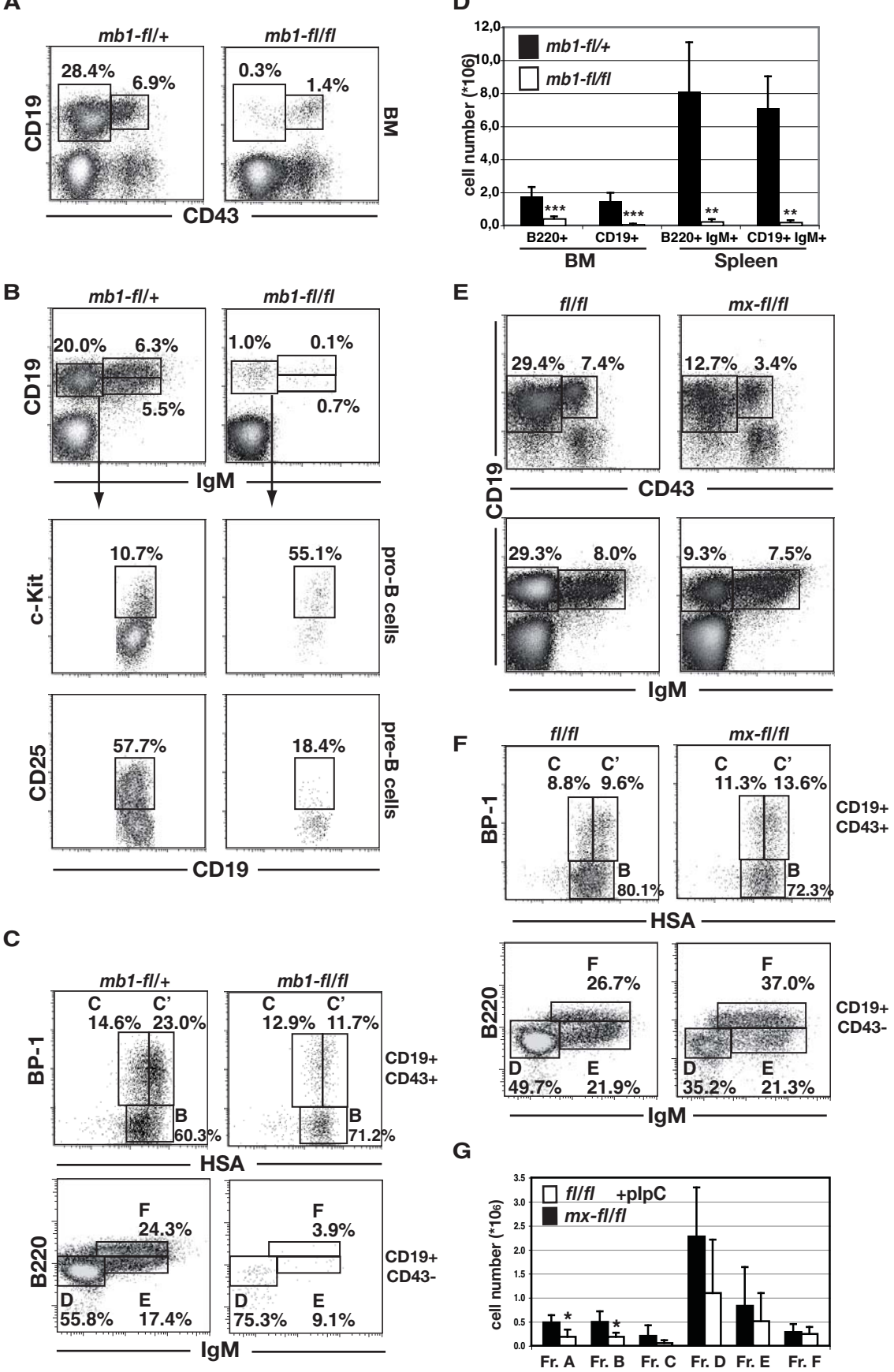

Fig. S2. c-Myc is necessary for B lymphocyte differentiation. *A, B*, and *C*. Flow cytometry analysis of B lymphocytes from BM of *mb1-fl/fl* and *mb1-fl/+* mice. Single-cell suspensions were prepared , stained and analyzed by flow cytometry (see methods). Cells were defined as (Ly6c-NK1.1-DX5-B220+c-Kit+IgM-) pro-B and (Ly6c-NK1.1-DX5-B220+CD25+IgM-) pre-B cells. *D*, Absolute numbers of B lymphocytes in *mb1-fl/fl* and control mouse BM (n=7) and spleen (n=3). *E*, *F* Flow cytometry analysis of B lymphocytes in BM of *mx-fl/fl* and *fl/fl* control mice. B cell populations were defined as in *A*, *B* and *C*. *G*, Absolute numbers of B cells in Hardy Fractions of pIpC-injected *mx-fl/fl* mice. n=4. Data represent one of ≥3 independent experiments. *p* values are ***p<0.001, **p<0.01, *p<0.05.

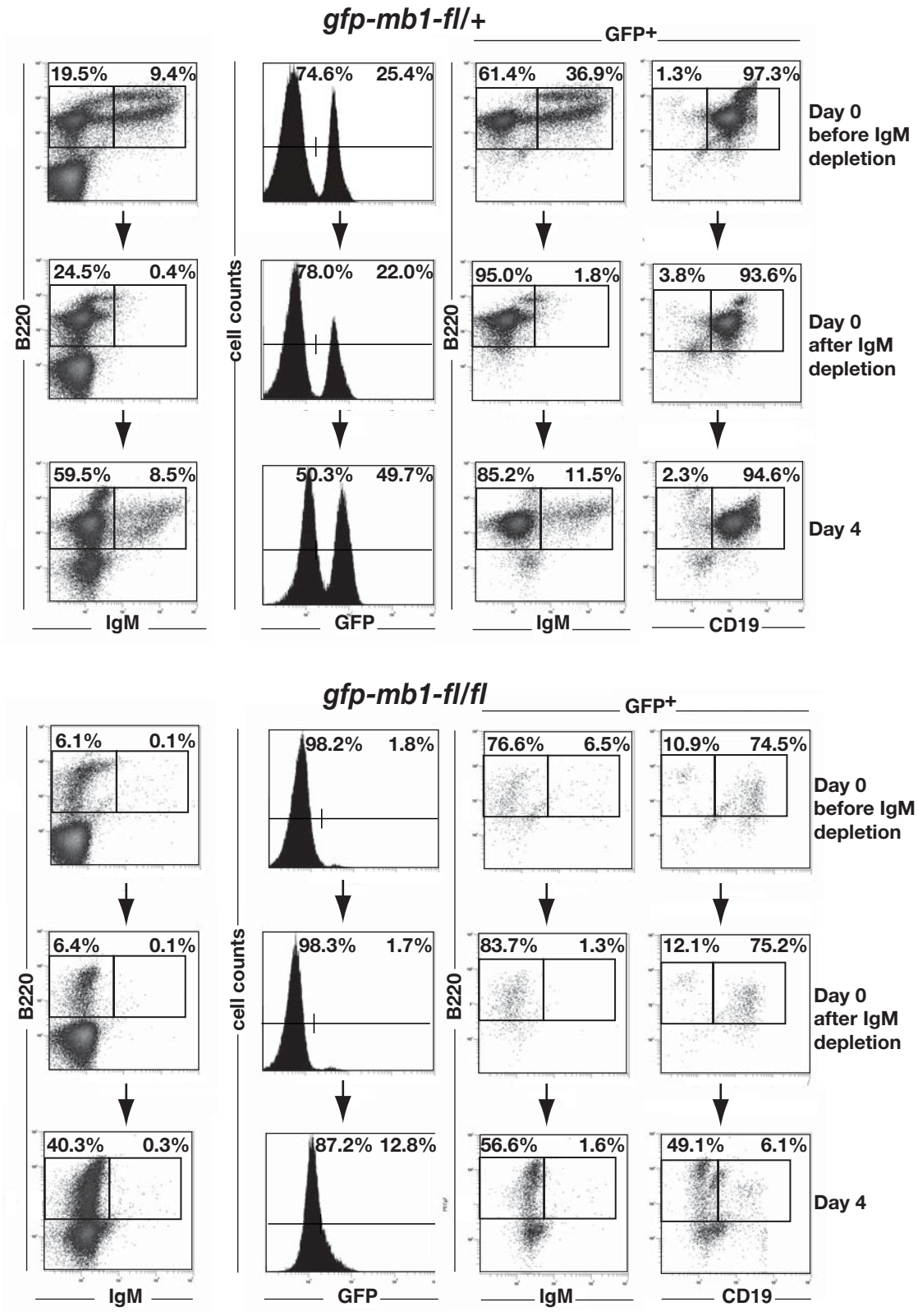

Fig. S3. BM B cell progenitors from *gfp-mb1-fl/fl* do not generate IgM$^+$ cells *in vitro*. BM cells were depleted of IgM$^+$ with beads and cultured with interleukin-7 for 4 days. Experiment representative of three independent experiments. B220$^+$IgM$^-$ cells were isolated from total BM after depletion of IgM$^+$ cells using biotinylated anti-IgM antibody (Southern Biotechnologies) and streptavidin Dynabeads (Invitrogen). Purity of cells was >97% after magnetic separation, as confirmed by flow cytometry. Cells were cultured in 24-well plates (2 x 10^6 cells/ml) and supplemented with recombinant murine stem cell factor (rSCF, 10 ng/ml), rFlt3 ligand (10 ng/ml) and recombinant murine IL-7 (10 ng/ml; all from Pepro-Tech). Cells were analyzed by flow cytometry at day 4.

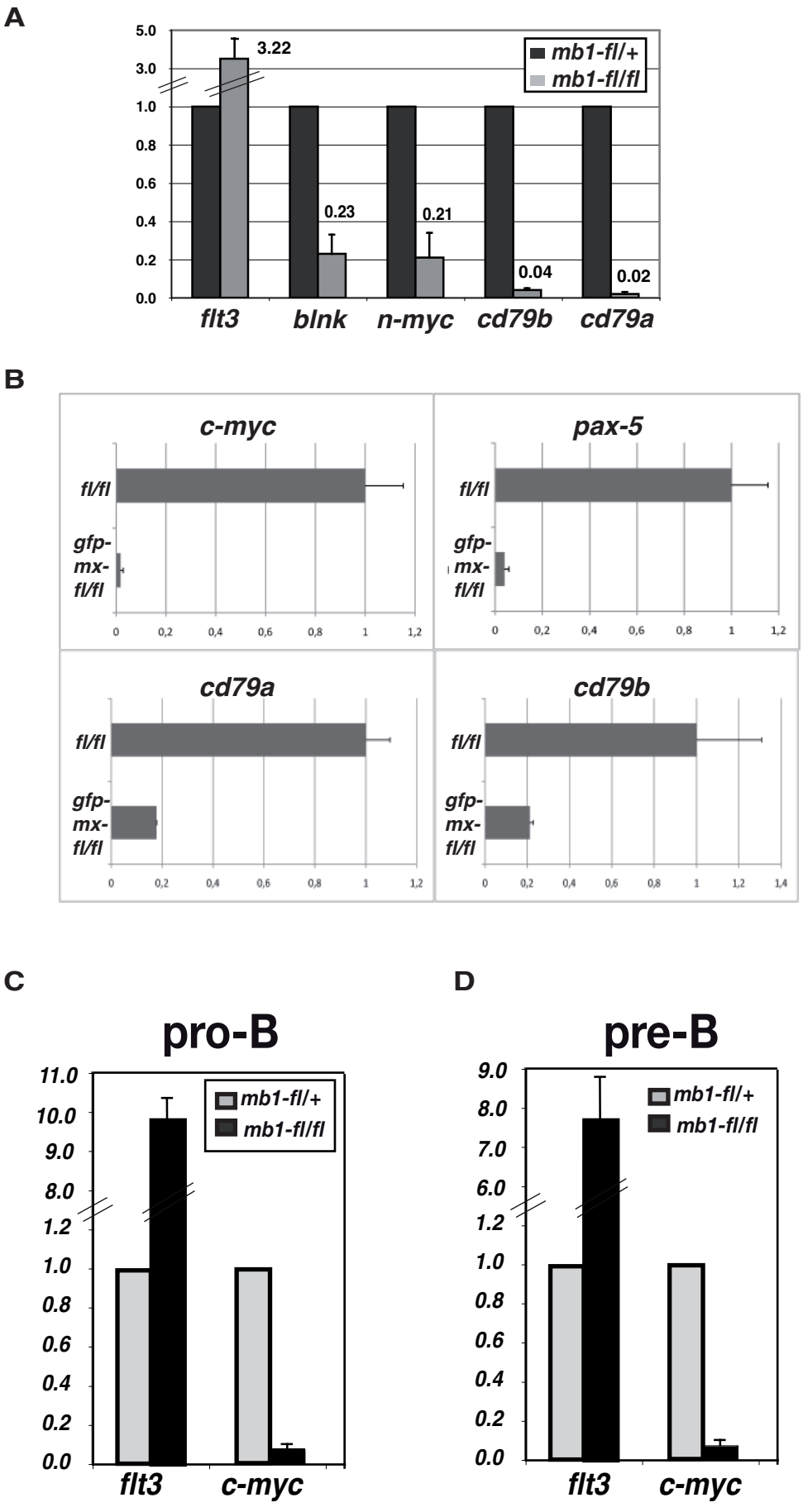

Fig. S4. (*A*) Gene expression by qPCR of sorted pro-B and pre-B cells ($B220^{+}IgM^{-}$) from *mb1 fl/fl* and *mb1-fl/+* control mouse BM (mean ± SD for 3 mutant and 3 control mice); numbers indicate the x-fold change (2-ΔCt). (*B*) Gene expression of $B220^{+}IgM^{-}GFP^{+}$ BM cells from pIpC-injected *gfp-mx-fl/fl* and control *fl/fl* mice. A pool of 3 mice was used for each genotype in two independent experiments. (C and D). Flt3 and c-myc expression by qPCR of purified pro-B (Ly6c-NK1.1-DX5-B220+c-Kit+IgM-) and(Ly6c-NK1.1-DX5-B220+CD25+IgM-) pre-B cells. Experiment represents 3 mice of each genotype.

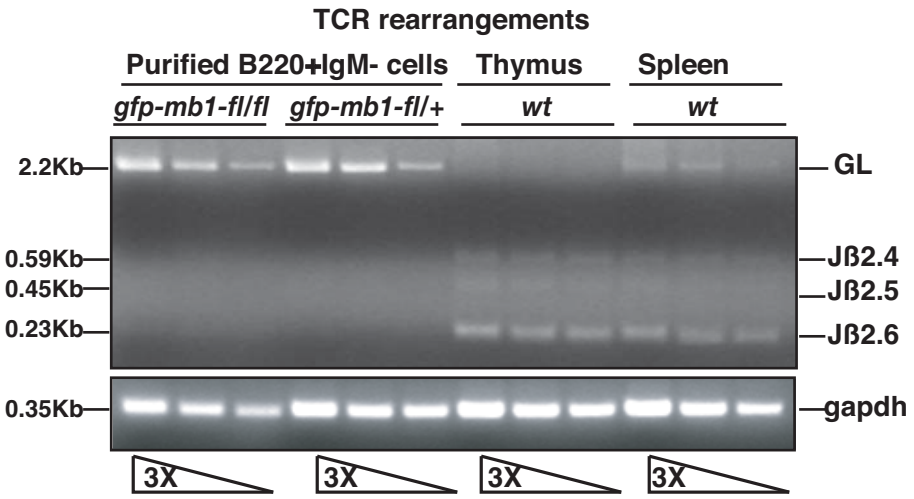

Fig. S5. c-Myc deficient pro- and pre-B lymphocytes do not rearrange the T cell Receptor loci. Sorted B220^{+}IgM^{-}GFP^{+} BM cells from *gfp-mb1-fl/fl*, and *gfp-mb1-fl/+ control* mice were cultured in the presence of the OP9-DL-1 cell line. Subsequently, cells were subjected to genomic PCR analysis to detect TCR rearrangements. Thymus and spleen DNA were used as positive controls. Experiment representative of three independent experiments with a total of three mice of each genotype.

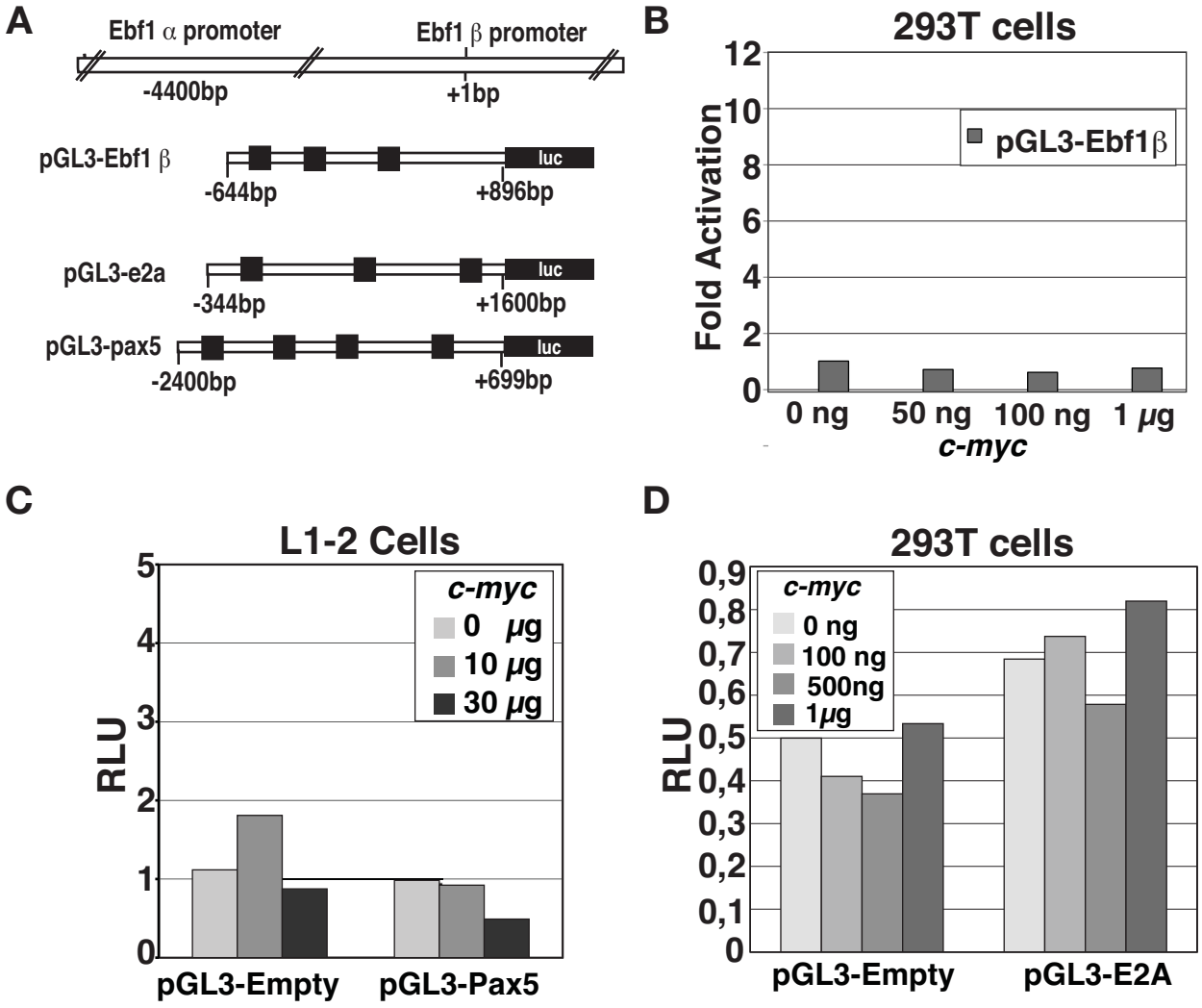

Fig. S6. Luciferase reporter assays. (*A*) Genomic locus of *ebf1* and *pGL3-ebf1* reporter constructs. (*B*, *C*, *D*) *ebf1ß* , *e2a* and *pax-5* genomic loci do not respond to c-Myc. Black squares represent E-boxes. pGL3-E2A contains 2 conserved and one non-conserved E-Boxes. Data representative of three independent experiments.

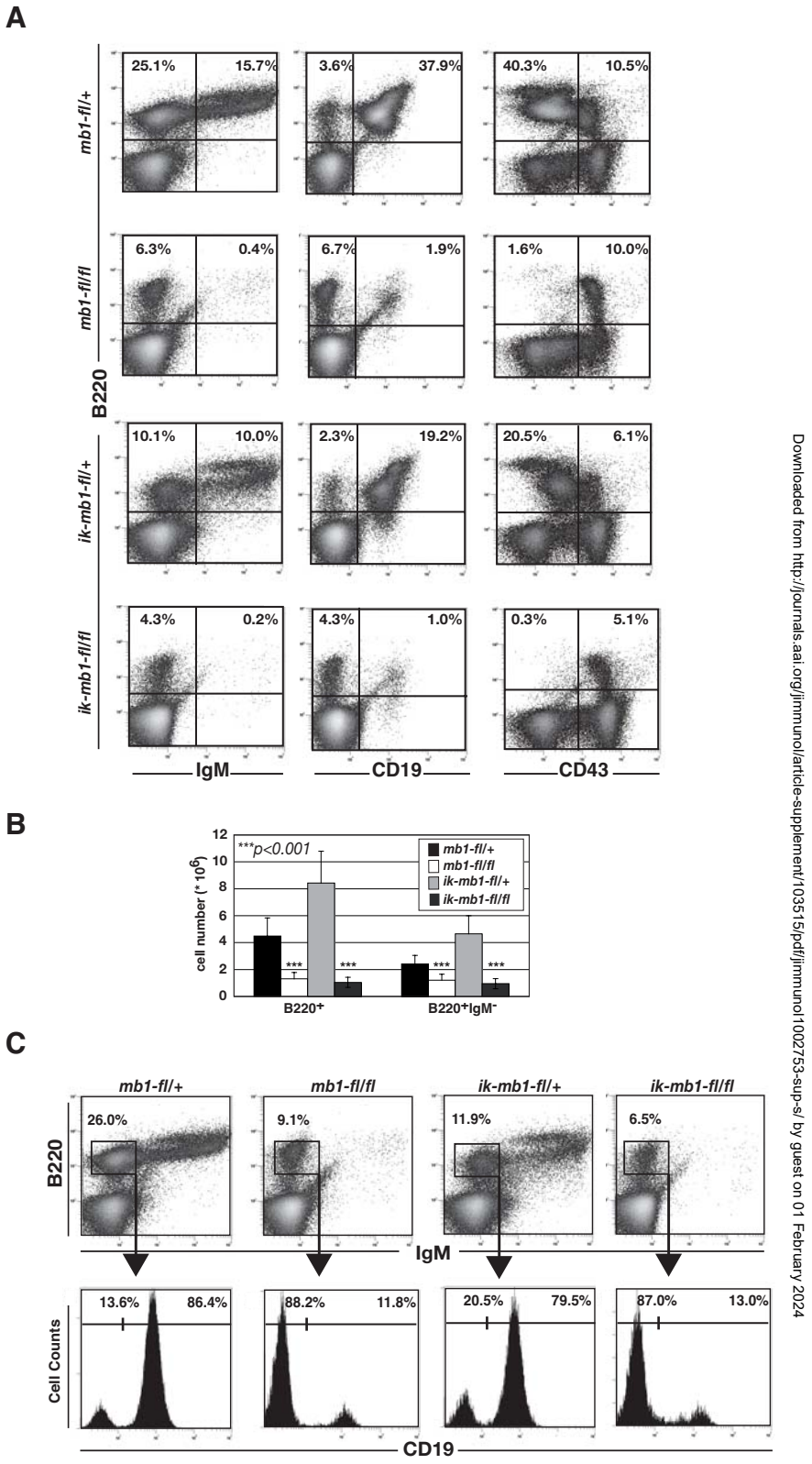

Fig. S7. (*A*) Flow cytometry analysis of the BM of the mice with the indicated genotype. (*B*) BM cellularity. n=5 ***p<0.001. (*C*) CD19 surface expression in $B220^{+}IgM^{+}$ cells in the BM. Flow cytometry experiments are representative of at least three independent experiments.

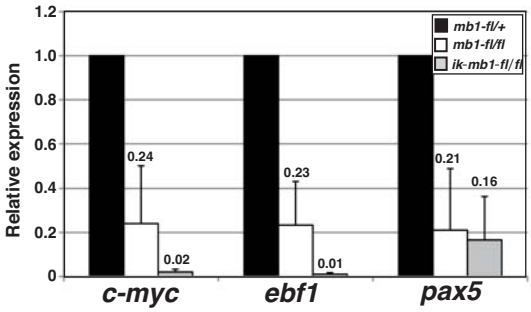

Fig. S8. qPCR of transcriptional levels of *pax5*, *ebf*, and *c-myc* in sorted $B220^{+}IgM^{-}$ from *ik-mb1-fl/fl, mb1-fl/+, and ik-mb1-fl/+* mice. 3 mice of each genotype were used.

mb-1-fl/fl

Seq	V name	3' V region	%homolog	P	N1	P	D	P	N2	P	5' JH3 region (JH3*01)	%homolog	D Name	%homolog	Vmut	Dmut	Jmut
85JH3-1	IGHV1-50*01	tgtgcaaga	59.33		cgtg		....aggtcgg.....				.ctggtttgcttactgg	97.91	IGHD3-2*01	66.66	0	1	0
85JH3-7	IGHV14-3*01	tgtgc....	58.18		ccc		.tttttggtacggtagtagctac		gga		.ctggtttgcttactgg	97.91	IGHD1-1*01	82.60	0	3	0
85JH3-10	IGHV14-1*02	tgtgcaag.	56.57		ggg		........gattacgac		gagg		........gcttactgg	87.5	IGHD2-4*01	71.42	1	0	0
85JH3-11	IGHV1-69*01	tgtgcaaga	59.60		agg		..........ggcctg..		g		cctggtttgcttactgg	100	IGHD3-3*01	66.66	0	1	0
85JH3-12	IGHV1-52*01	tgtgcaag.	60.13		cggg		.gacagctcaggcta.				.ctggtttgcttactgg	97.91	IGHD3-2*02	92.30	0	0	0
85JH3-13	IGHV1S126*01	tgtgcaa..	57.89		nagg		...attact.....		ctt		.....tttgcttactgg	91.66	IGHD1-1*01	71.42	0	0	0
85JH3-16	IGHV1-69*01	tgtgcaag.	60		ta		......atgattacgac		gga	g	cctggtttgcttactgg	100	IGHD2-4*01	84.61	0	0	0
85JH3-18	IGHV1S7*01	tgtgcaa..	56.66		gagcgg		.....actacggtagtagc...		cctg		cctggtttgcttactgg	100	IGHD1-1*01	60.86	0	0	0
85JH3-21	IGHV1S2*01	tgtgcaaga	56.20		ggagg		........ggttactac		agg		...ggtttgcttactgg	93.75	IGHD2-3*01	61.53	0	0	0
85JH3-25	IGHV14-2*02	tgtgcaag.	52.98		tcccct		....ctatgattac...		cctcgg		cctggtttgcttactgg	100	IGHD2-4*01	63.15	1	0	0
85JH3-30	IGHV1S126*01	tgtgcaaga	56.29		gagggaa		......atggttacgac		g		...ggtttgcttactgg	95.83	IGHD2-2*01	57.14	0	0	0
88JH3-1	IGHV1S2*01	tgtgcaag.	56.86		gg		......atgattacgac		gttaa		........gcttactgg	87.5	IGHD2-4*01	77.77	0	0	0
88JH3-3	IGHV1S2*01	tgtgcaag.	54.77		gg		......atgattacgac		gttaa		........gcttactgg	87.5	IGHD2-4*01	77.77	0	0	0
88JH3-12	IGHV1S126*01	tgtaca...	57.61		gtt		....ctatgattacgac		gacccc		....gtttgcttactgg	95.83	IGHD2-4*01	87.5	1	0	0
88JH3-28	IGHV1-69*01	tgtgcaag.	56.52		gg		......atgattacgac		gttaa		........gcttactgg	87.5	IGHD2-4*01	77.77	0	0	0
91JH3-5	IGHV1S126*01	tgtgcaaga	56.37				........gggaccccgg.				cctggtttgcttactgg	100	IGHD2-14*01	100	0	3	0
91JH3-8	IGHV1S126*01	tgtgcaaga	56.66				........gggaccccgg.				cctggtttgcttactgg	100	IGHD2-14*01	100	0	3	0
91JH3-10	IGHV1S126*01	tgtgcaaga	57.04				........gggaccccgg.				cctggtttgcttactgg	100	IGHD2-14*01	100	0	3	0
91-JH313	IGHV1S126*01	tgtgcaaga	56.37				........gggaccccgg.				cctggtttgcttaccgg	97.91	IGHD2-14*01	100	0	3	1
92JH3-2	IGHV1-50*01	tgtgcatga	56.37		g	ga	tctactatgattacgac		gagg		.ctggtttgcttactgg	97.91	IGHD2-4*01	100	1	0	0
92JH3-3	IGHV1S126*01	tgtgcatga	57.04		g	ga	tctactatgattacgac		gagg		.ctggtttgcttactgg	97.91	IGHD2-4*01	100	1	0	0
92JH3-6	IGHV1S126*01	tgtgcaaga	57.04		gg		...attact.....		ctt		.....tttgcttactgg	89.58	IGHD1-1*01	80.0	0	0	0
92JH3-9	IGHV1S126*01	tgtgcaaga	56.66		gg		...attact.....		ctt		.....tttgcttactgg	89.58	IGHD1-1*01	80.0	0	0	0
92JH3-15	IGHV1-50*01	tgtgcaaga	52.34		gggggg		..attactacggtagtagctac			g	cctggtttgcttactgg	86.95	IGHD1-1*01	100	0	0	0

fl/fl

Seq	V name	3' V region	%homolog	P	N1	P	D	P	N2	P	5' JH3 region (JH3*01)	%homolog	D Name	%homolog	Vmut	Dmut	Jmut
20JH3-1	IGHV1S126*01	tgtgcaaga	54.71		tc		.ctactatgattacgac		gtgggccg		.....ctgg	79.16	IGHD2-4*01	90.90	0	0	0
20JH3-5	IGHV1S47*01	tgtgcaag.	55.26		gggt		.....tatggtaactac		gta		..tggtttgcttactgg	95.85	IGHD2-1*01	75.0	0	0	0
20JH3-6	IGHV1-34*01	tgtgcaaga	56.64		g		......atgattacgac		a		...gggttgcttactgg	93.75	IGHD2-4*01	88.88	0	0	1
20JH3-17	IGHV14-2*02	tgt.....	58.04		aatgcatgg		.....tactacggtagtagcta.		ta		cctggtttgcttactgg	100	IGHD1-1*01	78.26	0	0	0
20JH3-30	IGHV1S50*01	tgtgcaaga	56.05		c		........ctacggcta.				cctggtttgcttactgg	100	IGHD1-2*01	88.88	0	0	0
18JH3-4	IGHV1-61*01	tgtgca...	57.14		ttaa		..actataggtacga....		gg		cctggtttgcttactgg	100	IGHD2-14*01	81.25	0	0	0
18JH3-7	IGHV14-1*01	tgt.....	59.60				....gcaag.....		at		cccggtttgcttactgg	97.91	IGHD6-1*01	66.66	0	1	1
18JH3-9	IGHV1S50*01	tgtgcaaga	56.0		tccttcg		........aggtacg.....		cc		...ggtttgcttactgg	95.83	IGHD2-14*01	66.66	0	0	0
18JH3-10	IGHV14-2*02	tgt.....	59.44				.....aacc				cctggtttgcttactgg	100	IGHD6-1*01	100	0	0	0
18JH3-12	IGHV1-23*01	tgtgcaag.	55.33		ggacg		...attactacggt.....		c		.....ttgcttactgg	91.66	IGHD1-1*01	70	1	0	0
18JH3-15	IGHV1S50*01	tgtgcaaga	57.04	t	gggac		ctaactgggac		gtgg		........gcttactgg	87.5	IGHD4-1*01	100	0	0	0
19JH3-2	IGHV1-61*01	tgtgcaaga	55.78		aggtcg		........gattacgac		gtaaaactt		.....tttgcttactgg	89.58	IGHD2-4*01	75	0	0	0
19JH3-3	IGHV1-12*01	tgtgcaaga	55.78		gaggaa	a	tctatgatggttactac		gtaggtacc		........gcttactgg	85.41	IGHD2-3*01	100	0	0	0
19JH33-4	IGHV1S50*01	tgtgcaaga	57.33		cggggaaa		..aactgggac		gg		...ggtttgcttactgg	95.83	IGHD4-1*01	87.5	0	0	0
19JH3-5	IGHV1S56*01	tgtgcaaga	58.50		ggg		.....tactacggtagtacct..				.....cttactgg	83.33	IGHD1-1*01	88.88	0	1	0
19JH3-7	IGHV1-12*01	tgtgcaaga	57.14		tgggggga		....tataggtacgacg..		agggaa		cctggtttgcttactgg	97.91	IGHD2-14*01	68.42	0	0	0
19JH3-8	IGHV1-23*01	tgtgcaaga	56.66		tct		....ctatggttacgac		gag		...ggtttgcttactgg	93.75	IGHD2-2*01	80	1	0	0
19JH3-15	IGHV1S126*01	tgtgcaaga	57.04		gg		.....agggc....		ccg		cctggtttgcttactgg	100	IGHD3-3*01	66.66	0	0	0
30JH3-3	IGHV1-15*01	tgtgcaa..	59.33		tatgg		........gattacgac		gtt		.....tttgcttactgg	91.66	IGHD2-4*01	83.33	1	0	0
30JH3-5	IGHV1S126*01	tgtgcaaga	57.71	tc	ggccg	aaa	ttattactacggtagtag....		atg		........gcttactgg	100	IGHD1-1*01	89.58	0	0	0

Table S1. Sequence analysis of VH558 - JH3 distal rearrangements in sorted BM B220^{+}IgM^{-} cells from *mb1-fl/fl* and *fl/fl* control mice. Analysis of 24 sequences from mutant and 20 from control cells of 4 independent mice from each genotype are shown. The V, D and J family and designation are indicated in the table, as well as the % of homology with the corresponding germline segment. All V sequences belong to the VHJ558 subgroup, except for some segments of the VHSM7 family, which is related to the VHJ558 but maps 3' of it. We also analyzed the extension and frequencies of P and N regions, finding no differences between mutant and control. Sequences are normal in terms of VDJ diversity.