

SUPPLEMENTAL MATERIAL

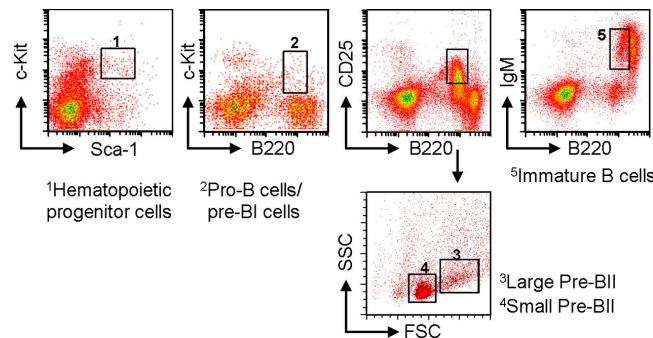
Duy et al., <http://www.jem.org/cgi/content/full/jem.20091299/DC1>

Figure S1. Sorted subsets from mouse bone marrow. HPCs, pro-B cells (fractions B and C), large cycling pre-BII cells (fraction C'), small resting pre-BII cells (fraction D), and immature B cells (fraction E) were sorted from normal mouse bone marrow. FSC, forward scatter; SSC, side scatter.

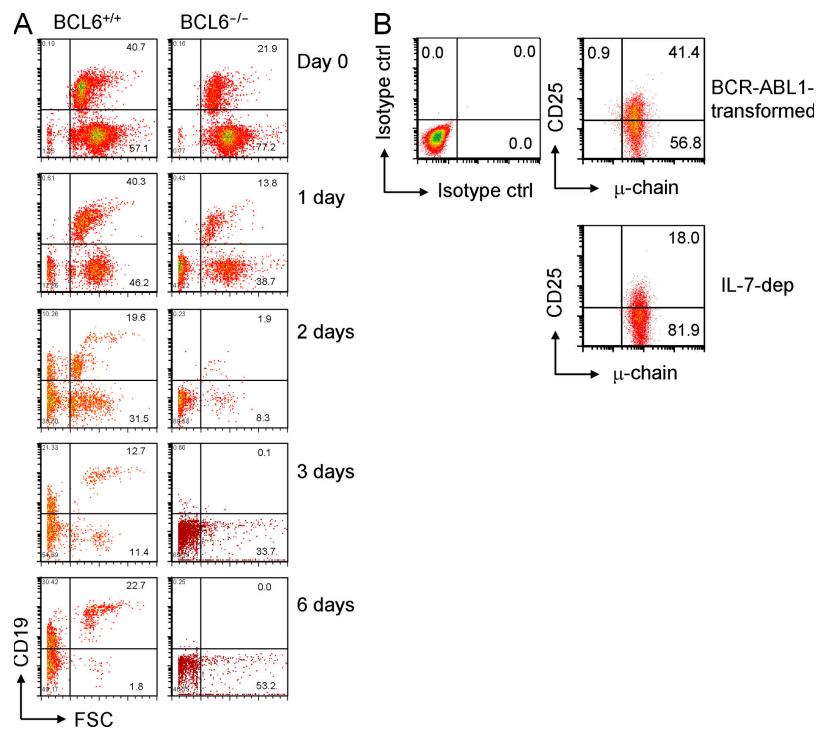


Figure S2. BCL6 is required for pre-B cell self-renewal in vitro. (A) Bone marrow pre-B cells from BCL6^{-/-} mice and wild-type littermates were cultured in the presence of 10 ng/ml IL-7 for the times indicated. After 1, 2, 3, and 6 d, cells were stained with antibodies against CD19 and viability was determined by propidium iodide exclusion. Numbers indicate percentages of viable CD19⁺ and CD19⁻ cells in cell culture. (B) BCR-ABL1-transformed B cell precursors and IL-7-dependent long-term cell cultures (SLP65^{-/-}) were stained for surface expression of CD25 and intracellular expression of μ chain (numbers indicate percentages). It should be noted that BCL6^{-/-} pre-B cells cannot be propagated as IL-7-dependent cell cultures. FSC, forward scatter.

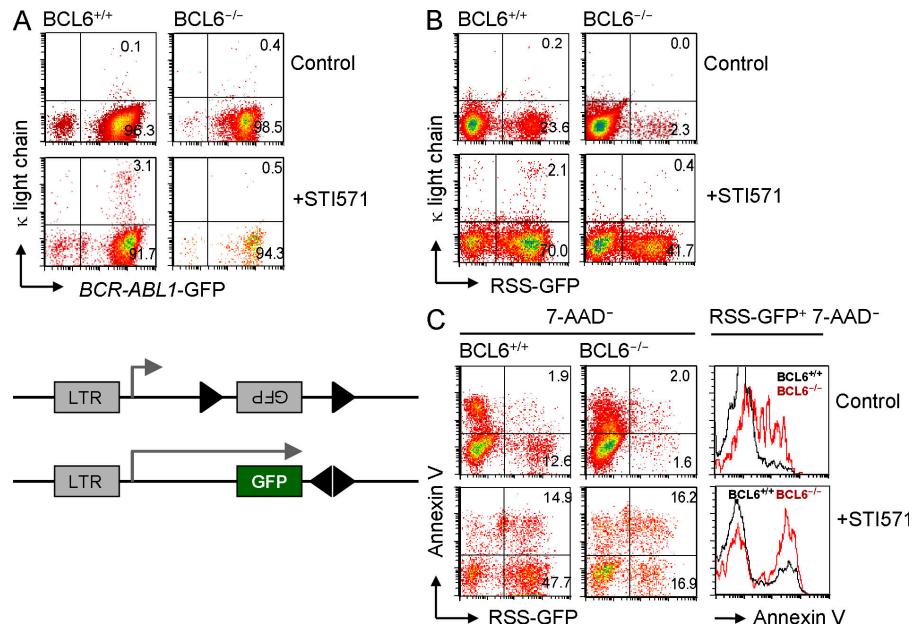


Figure S3. BCL6 protects pre-B cells against apoptosis during V κ -J κ recombination. (A) Bone marrow pre-B cells from BCL6 $^{-/-}$ and wild-type littermates were transformed by BCR-ABL1-GFP and induced to differentiate by inhibition of BCR-ABL1 kinase activity (2 μ mol/liter STI571). The percentages of κ light chain-expressing cells were measured after 3 d of treatment with and without STI571 ($n = 3$). (B and C) In a different set of experiments, BCL6 $^{-/-}$ and BCL6 $^{+/+}$ pre-B cells were transformed with a BCR-ABL1-Neo retrovirus and subsequently transduced with a recombination reporter plasmid carrying an inverted GFP gene flanked by RSS and a puromycin resistance gene ($n = 3$). Transduced cells were subjected to puromycin selection and induced to differentiate as shown in A. κ light chain expression was measured in V(D)J recombining (RSS-GFP $^{+}$) and nonrecombining (RSS-GFP $^{-}$) BCL6 $^{-/-}$ and wild-type pre-B cells after 3 d of STI571 treatment (2 μ mol/liter; B). In addition, apoptosis (annexin V $^{+}$) was measured in V(D)J recombining (RSS-GFP $^{+}$) and nonrecombining (RSS-GFP $^{-}$) BCL6 $^{-/-}$ and BCL6 $^{+/+}$ pre-B cells after 3 d of induced differentiation (STI571) or incubation under control conditions (C). (C, right) A histogram overlay of annexin V staining gated on V κ -J κ rearranging RSS-GFP $^{+}$ pre-B cells from BCL6 $^{-/-}$ and BCL6 $^{+/+}$ mice. Numbers indicate percentages.

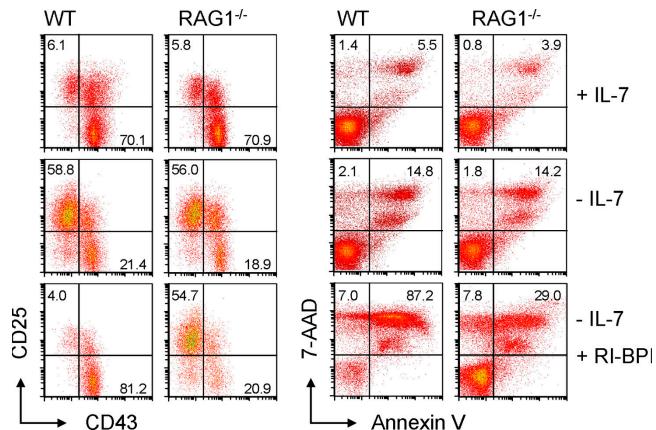


Figure S4. RAG1-dependent V κ -J κ recombination activity causes apoptotic propensity of BCL6-deficient pre-B cells. Bone marrow pre-B cells from RAG1 $^{-/-}$ mice and wild-type littermates were cultured in the presence of 10 ng/ml IL-7 (+IL-7), or IL-7 was withdrawn (-IL-7). In one set of experiments, 5 μ mol/liter of the peptidomimetic BCL6 inhibitor RI-BPI was added upon withdrawal of IL-7 (-IL-7 + RI-BPI). After 3 d, induction of differentiation was measured by flow cytometry (up-regulation of CD25 and down-regulation of CD43) as well as induction of apoptosis (measurement of 7-AAD/annexin V). Unlike other experiments, where de novo expression of κ light chains was measured as an indicator for pre-B cell differentiation, this measurement was impossible because RAG1 $^{-/-}$ pre-B cells cannot rearrange V κ and J κ gene segments and therefore cannot express κ light chains. For this reason, CD25 and CD43 were chosen as positive and negative markers of differentiation, respectively. Numbers indicate percentages.

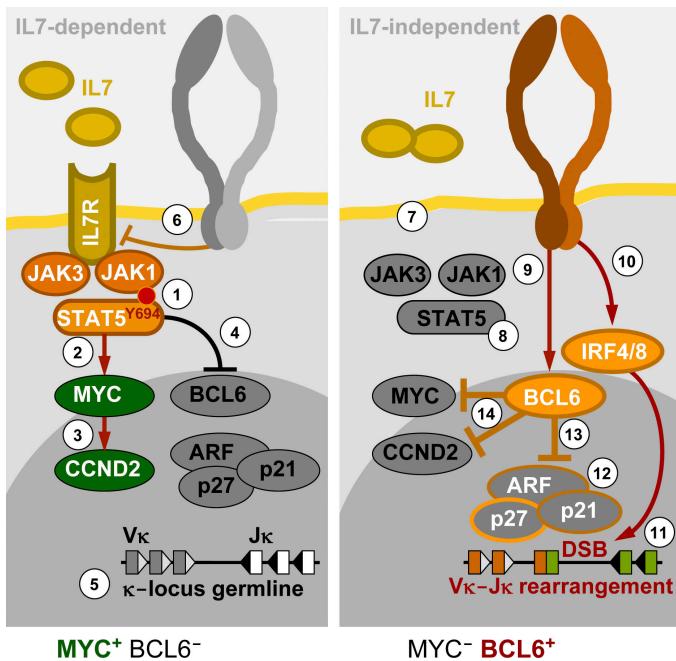


Figure S5. Scenario for the role of BCL6 during the transition from IL-7-dependent to -independent stages of B cell development. (1) Binding of IL-7 to the IL-7 receptor (composed of the IL-7 α chain and common γ chain) activates JAK3 and JAK1, which leads to STAT5 phosphorylation (Fig. 1 C; Palmer et al., 2008). (2) Phosphorylated STAT5 activates MYC (Fig. 1 C) and (3) represses BCL6 expression (Fig. 1 C and Fig. 3 A; Walker et al., 2007). (4) The IL-7-STAT5 signaling mediates the up-regulation of CCND2 (Fig. 1 B; Bouchard et al., 2001) and (5) arrests the κ locus in germline configuration (Mallin et al., 2010). (6) Expression of the pre-B cell receptor induces the down-regulation of IL-7 α expression (Fig. 3 D). (7) Withdrawal of the IL-7 α -STAT5 signaling (Fig. 3, C and D) results in (8) BCL6 up-regulation (Fig. 1, C and D; and Fig. 3, A and B). (9) Pre-B cell receptor mediates the up-regulation of BCL6 (Fig. 3 C) and (10) activates IRF4/8 (Johnson et al., 2008). (11) IRF4 induces $V\kappa$ - $J\kappa$ rearrangement (Johnson et al., 2008), resulting in (12) extensive DNA damage and DNA DSBs (Klein et al., 2005). (13) BCL6 suppresses DNA damage response and checkpoint genes (Fig. 6, A-D; Phan et al., 2005; Ranuncolo et al., 2007), as well as (14) CCND2 (Fernandez de Mattos et al., 2004) and MYC (Fig. 2 B).

A PDF file is also provided that contains Tables S1–S3.

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Table S1: *Phenotypic characterization of bone marrow B cell precursors in BCL6^{-/-} mice*

Phenotype	Gate	Number of cells		% Gated		% All		N (mice per group)	
		BCL6 ^{+/+}	BCL6 ^{-/-}	BCL6 ^{+/+}	BCL6 ^{-/-}	BCL6 ^{+/+}	BCL6 ^{-/-}	BCL6 ^{+/+}	BCL6 ^{-/-}
μ-chain ⁺ CD43 ⁻	PI ⁻ , FSC	1,379 ± 413 p=0.013	419 ± 173	7.4 ± 2.8 p=0.109	2.3 ± 0.6	2.8 ± 0.8 p=0.013	0.8 ± 0.4	4	4
μ-chain ⁺ B220 ⁺	PI ⁻ , FSC	1,687 ± 451 p=0.079	579 ± 168	9.1 ± 3.3 p=0.044	3.3 ± 0.6	3.4 ± 0.9 p=0.076	1.2 ± 0.3	4	4
μ-chain ⁻ B220 ⁺	PI ⁻ , FSC	2,669 ± 641 p=0.014	1,359 ± 471	14.6 ± 4.7 p=0.039	6.8 ± 1.0	5.4 ± 1.3 p=0.024	2.6 ± 0.7	4	4
CD43 ⁻ B220 ⁺	PI ⁻ , FSC	3,218 ± 1,060 p=0.021	942 ± 525	17.4 ± 7.1 p=0.159	5.5 ± 2.9	6.5 ± 2.1 p=0.022	1.9 ± 1.1	4	4
IgD ⁺ B220 ⁺	PI ⁻ , FSC	128 ± 49 p=0.099	65 ± 43	2.1 ± 0.3 p=0.001	0.7 ± 0.3	0.3 ± 0.1 p=0.207	0.2 ± 0.1	4	4
Igκ ⁺ B220 ⁺	PI ⁻ , FSC	933 ± 264 p=0.024	146 ± 51	15.1 ± 3.5 p=0.033	2.1 ± 1.1	1.8 ± 0.6 p=0.016	0.4 ± 0.3	4	4

Notes:

PI, propidium iodide; FSC, forward scatter (lymphocytes); mean values ± S.D. are indicated. P-values were calculated using double-sided T-test.

Table S2: Clonally restricted repertoire of bone marrow and splenic B cells in *BCL6*^{-/-} mice

BCL6^{+/+} Bone marrow		B220 ⁺ κ ⁺ IgD ⁻				
V _H	3'V _H	N1	D _H	N2	5'J _H	J _H
V1-11	tgggaa	a	ttattagcacgttagtc	c	cttgactactgg	J2
V1-11	tgtggaa	c	tttactacgttagct	tttt	actatttgactactgg	J2
V1-11	tgtggaa	cc	ggga	tttt	actatttgactactgg	J2
V1-11	tgtggaga	ct	cagaccggg	gttt	ctactttgactactgg	J2
V1-15	tgtac	ga	tatactaact	c	acttcgatgtctgg	J1
V1-15	tgtacaag	g	aggct	ttt	ctactgg	J2
V1-18	tgtgcaaga	tttaacgg	tgattacgac	caggaattt	tttgactactgg	J2
V1-18	tgtgcaaga	ttaag	ctggg	caaatt	actactttgactactgg	J2
V1-23	tgtacaaga	tcc	tctttgatgggt	cc	actactttgactactgg	J2
V1-23	tgtacaaga	tg	gaccgg	t	ttgactactgg	J2
V1-34	tgtgcaaga	g	tctacta	tttc	actttgactactgg	J2
V1-5	tgtacaaga	c	gggattag	a	ctactttgactactgg	J2
V1-5	tgtacaaga	at	acggtagta	cc	actttgactactgg	J2
V1-82	tgtgcaaga	cgg	actacggttagtc	ca	actttgactactgg	J2
V1-9	tgtgcaa		gggattag	cc	actttgactactgg	J1
V1-9	tgtgcaa		actggg	cc	actttgactactgg	J2
V1-9	tgtgcaa		gttaactacggtt	cc	ggtaacttcgatgtctgg	J1
BCL6^{+/+} Spleen		B220 ⁺ κ ⁺ IgD ⁺				
V1-11	gaag	tca	tctacta	cc	gtacttcgatgtctgg	J1
V1-11	tgtggaa	cccc	tctttgatgggt	caaatttt	actatttgactactgg	J2
V1-11	g	tc	cagaccggg	ccctact	gtacttcgatgtctgg	J1
V1-11	tgtggaga	cg	ctacggtagtagct	gc	actttgactactgg	J2
V1-12	tgtgcaaga		ctatgattac	g	tgaccactgg	J2
V1-15	tgtacaag	gc	tttattactacggtagtagct	tttt	tactttgactactgg	J2
V1-34	tgtgcaaga	ggg	attactacggtagtagctac	gc	ctactggacttcgatgtctgg	J1
V1-5	tgtacaa	aa	agaatggggctac	g	gactactgg	J2
V1-82	tgtgcaaga	gcg	actggg	gc	tgactactgg	J2
V1-82	tgtgcaaga		tacggtagtag	catt	gactactgg	J2
V1-9	tgtgcaaga	tgggc	actacggttagc	agcccctctaagggtt	tgg	J2
V1-9	tgtgcaaga	agggggtga	tctactatggtaa	aggggg	ctactgg	J2
V1-9	tgtgcaag	g	tactacggtagtagct	ccct	tactttgactactgg	J2
V1-9	tgtgcaaga	ggcc	ctggg	g	ttgactactgg	J2
V1-9	tgtgcaaga	ag	actacggttagc	c	ggtaacttcgatgtctgg	J1
BCL6^{-/-} Bone marrow		B220 ⁺ κ ⁺ IgD ⁻				
V _H	3'V _H	N1	D _H	N2	5'J _H	J _H
V1-11	tgtggaa	g	agacagacccgg	gt	actactttgactactgg	J2
V1-11	tgtggaa	g	agacagacccgg	gt	actactttgactactgg	J2
V1-11	tgtggaa	g	agacagacccgg	gt	actactttgactactgg	J2
V1-11	tgtgcaaga	c	gttaactacggtt	ttttt	ttgtacttcgatgtctgg	J1
V1-15	tgtacaaga	gg	aggg	gg	tactgg	J2
V1-15	tgtacaaga	gg	aggg	gg	tactgg	J2
V1-15	tgtacaaga	gg	aggg	gg	tactgg	J2
V1-15	tgtacaaga	gg	aggg	gg	tactgg	J2
V1-82	tgtgcaaga	tcc	gggttattac	acg	tttggactactgg	J2
V1-11	tgtggaa	ggggg	tttattactacggtag	ag	ggaaacttcgatgtctgg	J1
V1-11	tgtggaa	ggggg	tttattactacggtag	ag	ggaaacttcgatgtctgg	J1
V1-15	tgtacaaga		gatgttact	ctc	tttggactactgg	J2
V1-18	tgtgcaaga	ttaag	ctttgatgtt	aatt	tgaccactgg	J2
V1-9	attactgtgc	tgc	acggtagta	aagg	cttcgatgtctgg	J2
V1-18	tattactgtgc	tt	actggg	caat	tactttgactactgg	J2
V1-34	tgtgcaaga	gatccct	ctacggtagt	ccccttt	actactttgactactgg	J2
V1-34	tgtgcaaga	gatccct	ctacggtagt	ccccttt	actactttgactactgg	J2
V1-34	tgtgcaaga	gatccct	ctacggtagt	ccccttt	actactttgactactgg	J2
V1-34	tgtgcaaga	gatccct	ctacggtagt	ccccttt	actactttgactactgg	J2
V1-82	tgtgcaaga	aaaa	tctatgtatggttac	cct	tactttgactactgg	J2
V1-9	tgtgcaaga	tcta	tctatgtatggttac	ctgt	actactttgactactgg	J2
V1-9	tgtgcaaga	tcta	tctatgtatggttac	ctgt	actactttgactactgg	J2
V1-11	tgtggaa	ct	ctacggtagt	ttcccc	tgactactgg	J2
V1-5	tgtacaaga		tactactacggtagtagct	tactttat	actactttgactactgg	J2
V1-5	tgtacaaga		tactactacggtagtagct	tactttat	actactttgactactgg	J2
V1-9	tgtgcaaga		ggga	tt	actactttgactactgg	J2
V1-11	tgtggaa	taga	agtaactac	ttgcg	ctactgg	J2
V1-11	tgtggaa	taga	agtaactac	ttgcg	ctactgg	J2
V1-11	tgtggaa	taga	agtaactac	ttgcg	ctactgg	J2
V1-23	tgtacaaga	tc	ctggg	ag	ctactttgactactgg	J2
V1-82	tgtgcaaga	gcgtcc	tctact	g	tgactactgg	J2
BCL6^{-/-} Spleen		B220 ⁺ κ ⁺ IgD ⁺				
V1-11	tgtggaa	g	agacagacccgg	gt	actactttgactactgg	J2
V1-12	ctgcggcttattt		accggg	gt	actactttgactactgg	J2
V1-12	ctgcggcttattt		accggg	gt	actactttgactactgg	J2
V1-5	actctgcgtct		cagaccggg	gt	actactttgactactgg	J2
V1-15	tcttattact	gccagg	agacagacccgg	gt	actactttgactactgg	J2
V1-11	tcttattac	cgg	agacagacccgg	gt	actactttgactactgg	J2
V1-11	tgtggaaagg		tactacggggg	gg	ctactgg	J2
V1-15	tgtacaaga	gg	aggg	gg	tactgg	J2
V1-12	tgtgcaa	c	ggg	gg	tactgg	J2
V1-15	tgtacaaga	gg	aggtgggg	g	ctttgactactgg	J2
V1-11	tgtgg		gggg	gggg	ctttgactacggg	J2
V1-11	tgtgg		gggg	gggg	ctttgactactgg	J2
V1-11	tgtggaaaagg		ctgggac	gggg	ggtaacttcgatgtctgg	J1
V1-15	tgtacaaga	gatc	atgtatgttac	gggtgg	tttggactactgg	J2
V1-11	tgtggaa	gacgg	gattacgac	gtgtgggg	actactttgactactgg	J2
V1-15	tgt	gggggg	taactggg	t	ctttgactactgg	J2
V1-34	tgtgcaaga	gatccct	ctacggtagt	ccccttt	actactttgactactgg	J2
V1-34	tgtgcaaga	gatccct	ctacggtagt	ccccttt	actactttgactactgg	J2
V1-5	tctgcggcttat	acgtgt	ccccttt	actactttgactactgg	J2
V1-12	gtcttattttctgt	ggtagt	ccccttt	actactttgactactgg	J2
V1-82	tgtgcaaga	gttagggt	ctactatagta	t	ctttgactacccgg	J2
V1-34	tgtgcaaga	ggga	actatagta	tccg	ctttgactactgg	J2
V1-34	tgtgcaaga	ggga	actatagta	tccg	ctttgactactgg	J2
V1-82	tgtgcaaga	gttagggt	ctactatagta	t	ctttgactacccgg	J2
V1-11	tgtggaa		actatgattacgac	gc	gactactgg	J2
V1-9	tgtgcaaga	agagg	gtatggtaactac	gggaccggcc	ttgactactgg	J2
V1-15	tgtacaaga	ggttgg	aactgg	gt	tactttgactactgg	J2
V1-34	tgtgcaaga	tcct	ctacggtagt	tt	tttggactactgg	J2
V1-11	tgtggaaagg	tac	ttactacggtagtagct	tgc	ctactgg	J2

Notes: Light shades denote immunoglobulin rearrangements that were found more than once on one sample (either bone marrow or spleen). Dark shades denote immunoglobulin gene rearrangements that were found both in the bone marrow and the spleen from the same mouse. Complete sequence data are available from EMBL/GenBank under accession numbers FN652762-FN652778.

Table S3: Sequences of oligonucleotide primers used

Clonality and spectratyping analysis

V _H 1_F	5'-AAGGCCACACTGACTGTAGAC-3'
J _H 2_R	5'-GAGGAGACTGTGAGAGTGGTG-3'
C μ _R	5'-TGGCCACCAGATTCTTATCAG-3'
J _H 1-FAM_R	5'-GACGGTGACCGTGGTCCCTGT-3'
J _H 2-FAM_R	5'-GACTGTGAGAGTGGTGCCTG-3'
J _H 3-FAM_R	5'-GACAGTGACCAGAGTCCCTG-3'
J _H 4-FAM_R	5'-GACGGTGACTGAGGTTCTTG-3'
C μ -FAM_R	5'-AGACGAGGGGAAGACATTG-3'

Notes:

V_H1_F binds to rearranged V_H gene segments of the J558 family. FAM denotes dye-labeled oligonucleotide. The FAM-label is attached to the 5' end.

Quantitative RT-PCR

Bcl6_F	5'-CCTGCAACTGGAAGAAGTATAAG-3'
Bcl6_R	5'-AGTATGGAGGCACATCTCTGTAT-3'
Myc_F	5'-ATCATCCAGGACTGTATGTGGAG-3'
Myc_R	5'-TTCTTGCTCTTCAGAGTCG-3'
Cdkn2a_F	5'-GGACCAGGTGATGATGATG-3'
Cdkn2a_R	5'-ATCGCACGATGTCTTGATG-3'
Cdkn1a_F	5'-ACAAGAGGCCAGTACTTC-3'
Cdkn1a_R	5'-CTTGCAGAACCAATCTG-3'
Cdkn1b_F	5'-GTGTCCAGGGATGAGGAAG-3'
Cdkn1b_R	5'-CGGAGCTGTTACGTCTGG-3'
Trp53_F	5'-TCCTTACCATCATCACACTGG-3'
Trp53_R	5'-CGGATCTTGAGGGTGAAATAC-3'
Hprt_F	5'-GGGGGCTATAAGTTCTTC-3'
Hprt_R	5'-TCCAACACTTCGAGAGGTCC-3'

Quantitative chromatin immunoprecipitation (QChIP)

CDKN2A_F	5'-GCGTGCAGCGGTTAGTTA-3'
CDKN2A_R	5'-TCAGGAGGCTGAATGTCAGTT-3'