a

Targeting vector

ClaI   ClaI   1   2   3

QK/TK

Normal *Myc* locus

SalI   1   2   3   EcoRI

1 →   ←

Targeted *Myc* locus

SalI   EcoRI   SalI   1   2   3   EcoRI

1 →   ←
2 →   ←

Indicator fragment of normal locus

Sal/EcoRI: 17.4 kb

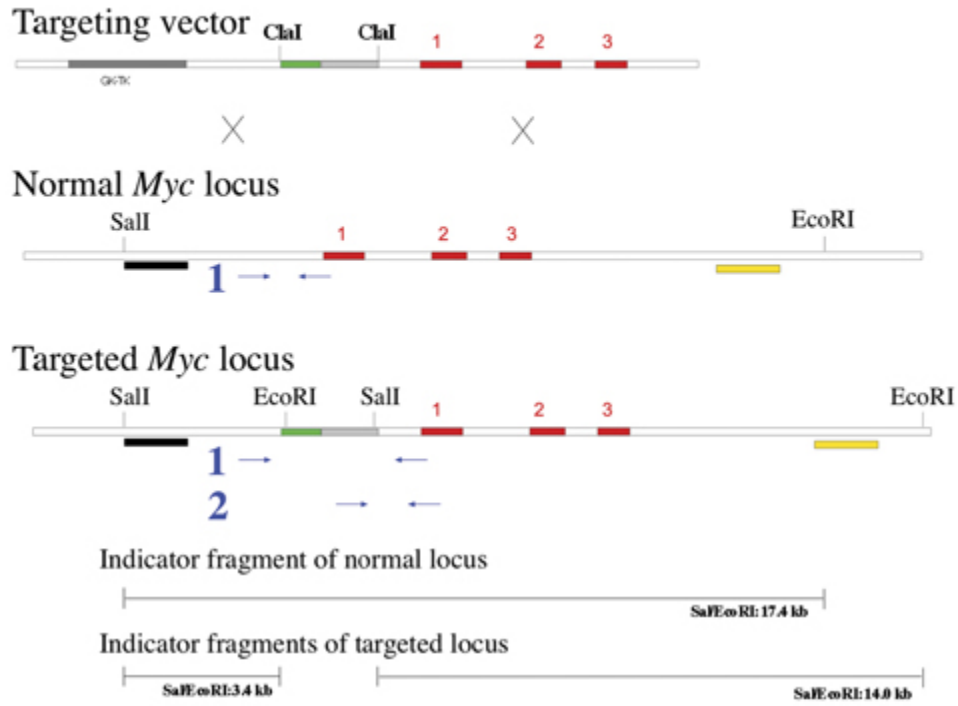Indicator fragments of targeted locus

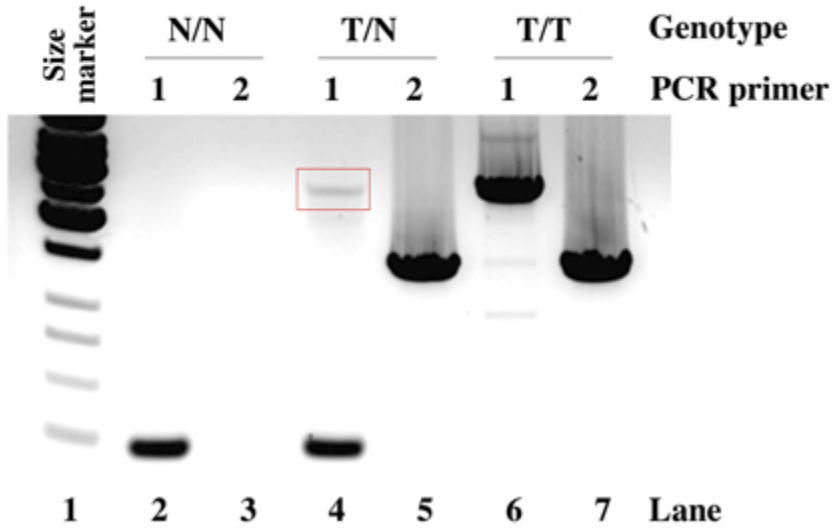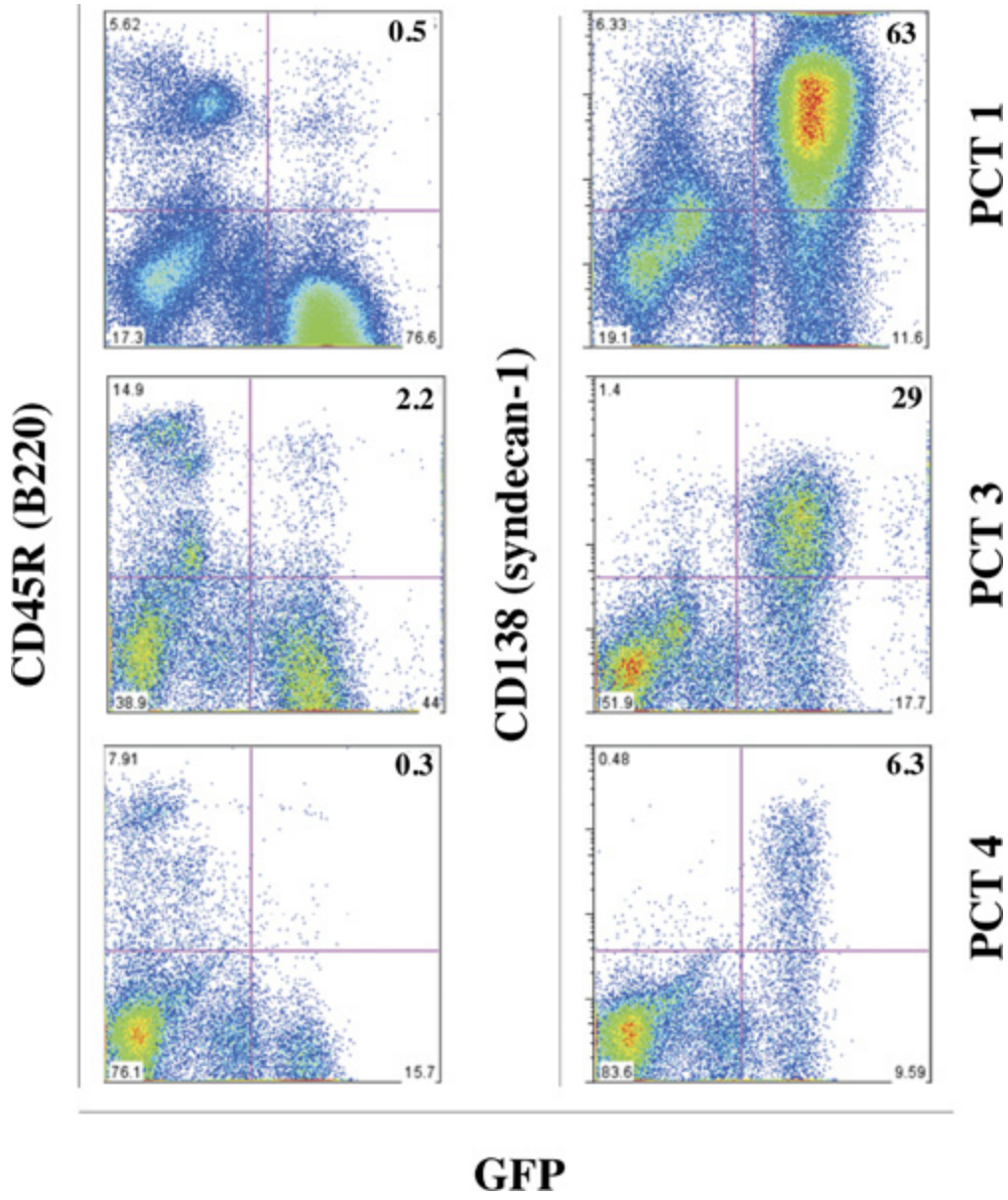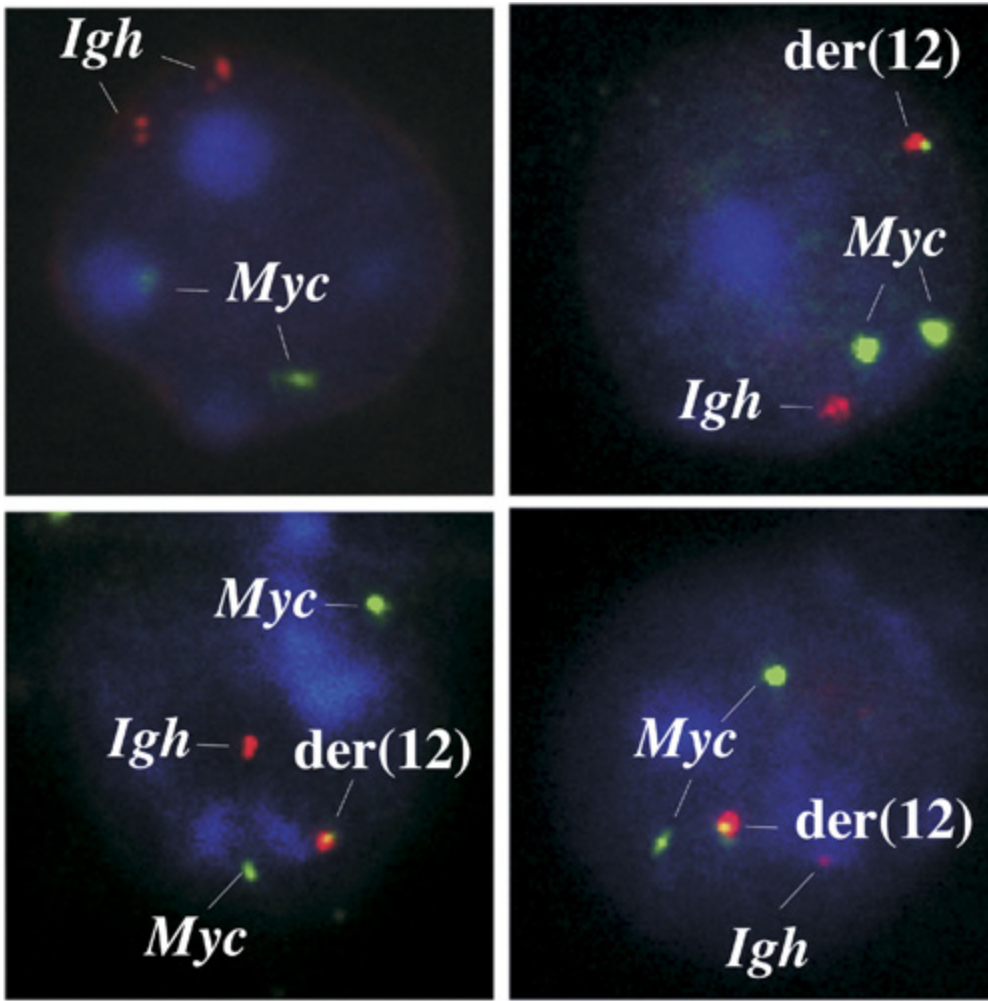Sal/EcoRI: 3.4 kb

Sal/EcoRI: 14.0 kb

b

Supplementary Information – Methods (A), References (B) and Figure Legends (C)

A. Supplementary Methods

Generation of iGFP$^{5'Myc}$ mice

Genomic *Myc* clones were isolated from a phage λ-based mouse 129/SvJ genomic DNA library. A 10-kb

fragment of a *Myc* clone that included the entire coding region was subcloned into a targeting vector that

contains a *loxP*-flanked *Neo* gene and a *GFP* gene whose expression is driven by a V_H promoter

(**Supplemental Figure 1a**). The V_H-GFP construct, which serves as the reporter of chromosomal T(12;15)

translocation in B lymphocytes and plasma cells, was inserted into the BseAI site in *Myc*'s 5' flank, ~1.5 kb

upstream of the first exon of *Myc*. The transcriptional orientation of the V_H-GFP reporter gene was

opposite to that of *Myc*. A glucocorticoid promoter (GK) and thymidine kinase gene (TK) expression

cassette (GK-TK cassette) was added to the 5' arm of homology of the targeting vector, to facilitate the

selection of homologous ES cell recombinants. The targeting vector was introduced into CJ7 ES cells and

selected for by treating the cells with G418 and gancyclovir. ES cell clones were screened by Southern

blotting of SalI/EcoRI digested genomic DNA, using a 5' probe external to the region of vector homology

(**Supplemental Figure 1b**). Mouse chimeras were generated by injecting mutant ES cells into C57BL/6

(B6) blastocysts. Chimeric founder mice were mated with C57BL/6 mice to generate "*Igh-Myc*

translocation reporter" mice, designated iGFP$^{5'Myc}$, on a mixed genetic background of segregating 129 and

B6 alleles. The mice were backcrossed for 6 generations onto strain BALB/c (C) to generate tumor-

susceptible C.iGFP$^{5'Myc}$ N_6 congenic mice, which were used for the studies reported here. Unlike most

inbred strains of mice, including 129 and B6, strain C is genetically susceptible to inflammation (pristane)-

induced peritoneal plasmacytoma (PCT) (Potter *et al.*, 1975). Genotyping of offspring was performed by

PCR, using genomic tail DNA as the PCR template and the following oligonucleotides as PCR primer

pairs:

- For detection of the normal allele—5797U (5'-ctttaagtgccttggggcgaggag-3') and 8518L (5'-

  ctacgctgtgcattctgtacaatc-3'). This is primer set 1 in **Supplemental Figure 1**.

- For detection of the targeted allele—4521U (5'-catcaccacgcctacctaa-3') and 5897L (5'-ggttacaaataaagcaatagcctc-3'). This is primer set 2 in **Supplemental Figure 1**.

Backcrossing of strain C.iGFP$^{5'Myc}$ has been recently completed, and the resulting animals have been added to the mutant mouse repository of The Jackson Laboratory (Bar Harbor, Maine).

Induction, diagnosis and transplantation of PCT

Strains C.iGFP$^{5'Myc}$ and C.BCL2 were intercrossed to generate double-transgenic *Igh-Myc* reporter mice designated C.iGFP$^{5'Myc}$BCL2. Like their single-transgenic C.BCL2 counterparts (Silva *et al.*, 2003), C.iGFP$^{5'Myc}$BCL2 mice were hyper-susceptible to pristane-induced PCT. Mice 4-6 weeks of age were treated with an intraperitoneal injection of 0.5 ml pristane (2,6,10,14-tetramethylpentadecane) (Anderson and Potter, 1969) and monitored for tumor development. Mice remaining tumor-free through day 60 of tumor induction received a second treatment with pristane on that day. Mice in control groups that remained tumor-free through day 120 of tumor induction (C.iGFP$^{5'Myc}$ mice and non-transgenic littermates) received a third treatment with pristane on that day. Incipient tumors were diagnosed based on the finding of aberrant, pleomorphic, hyperchromatic plasma cells in cytofuge specimens of ascites cells stained according to Wright-Giemsa. Small samples (~50 μl) of ascites were obtained at biweekly intervals by abdominal paracentesis of mice with a 20-gauge needle. Mice harboring frank PCTs were humanely euthanized, and these were confirmed to be tumors by histological analysis of tissue sections stained with H&E. PCTs were propagated *in vivo* by transferring PCT cells to pristane-primed C mice. All animal studies were approved under Institutional Animal Care and Use Committee Protocols LG-028 (NCI) and 0006A56361 (University of Iowa).

Flow cytometry

In order to evaluate the expression of GFP and lymphocyte surface markers in heterogeneous cell populations from the abdominal cavity of PCT-bearing mice, we harvested ascites cell specimens and prepared them for FACS analysis. After red blood cells were lysed in hypotonic buffer and the Fc receptor was blocked to prevent non-specific antibody binding, ascites cells were labeled (30 min, 4°C) with antibody to B220 (CD45R, a B-cell marker), CD3 (a T-cell marker) or CD138 (syndecan-1, a plasma-cell

2

marker). GFP expression and B- and T-lymphocyte marker profiles were evaluated using a FACS Calibur Cytometer equipped with CellQuest Software. Flow cytometry hardware and software, and also all antibodies and reagents, were obtained from Becton Dickinson (Franklin Lakes, New Jersey).

Flow cytometric detection of GFP$^+$ splenic B cells from C.iGFP$^{5'Myc}$ mice

In order to evaluate whether GFP$^+$ B cells can be observed in older C.iGFP$^{5'Myc}$ mice, splenocytes from 8-9 month old control BALB/c (C) and C.iGFP$^{5'Myc}$ mice were examined by flow cytometry. Specifically, spleens were harvested from unimmunized C and C.iGFP$^{5'Myc}$ mice, as well as from C.iGFP$^{5'Myc}$ mice challenged three times with sheep red blood cells (SRBC). Mice were immunized on days 0, 21 and 28 with 0.2 ml of a 10% v/v SRBC (Colorado Serum Company, Denver, CO) solution and sacrificed on day 35. Spleen suspensions were washed in balanced salt solution (BSS) and viable mononuclear cells obtained by density centrifugation using Fico/Lite-LM (Atlanta Biologicals, Norcross, GA). The mononuclear cell fraction was further washed in BSS and suspended in staining buffer (BSS supplemented with 5% bovine calf serum). 6×10^6 cells were stained for each sample. Cells were incubated with fluorochrome-conjugated anti-B220 and anti-CD19 monoclonal antibodies (mAbs) for 20 minutes on ice in the presence of 2.4G2 (anti-CD16/32 mAb) and rat serum to saturate Fcγ receptors and non-specific binding sites. After staining, cells were washed, suspended in fixative (1% formaldehyde in 1.25X PBS) and run on a BD FACSCanto flow cytometer (Becton Dickinson Biosciences, San Diego, CA). 3×10^6 events were collected per sample. Data were analyzed using FlowJo software (Tree Star Inc., Ashland, OR).

Immunofluorescence

PCT-containing tissue specimens from mesenteric pristane granulomas or abdominal lymph nodes were fixed for three hours at 4°C in buffered paraformaldehyde (4%), embedded in O.C.T. (Optimal Cutting Temperature) Compound (Sakura Finetek, Inc., Torrance, California), cryosectioned (5 μm) onto silanized slides, and air dried for three hours at ambient temperature. Tissue sections were pretreated for 30 min with 0.1% Triton X and 5% fetal calf serum in PBS, stained with PE (phycoerythrin)-conjugated antibody to CD138 (syndedan-1) or CD45R (B220), and embedded in 4',6-diamidino-2-phenylindole (DAPI)-

containing antifade mounting medium from Vector Laboratories, Inc. Tissue sections were evaluated using

an Olympus BX51 fluorescence microscope equipped with a dedicated filter combination for the detection

of GFP/PE fluorescence, and a CCD camera for the acquisition of photomicrographic images.

Cytogenetic detection of the chromosomal T(12;15) translocation

Protocols for the detection of the T(12;15) translocation have been described previously (Kovalchuk *et al.*,

2001; McNeil *et al.*, 2005). Briefly, chromosome spreads were prepared by lysing tumor cells in hypotonic

75mM KCl, followed by drop-fixation with methanol/acetic acid (3:1). Chromosomal DNA was

hybridized to FISH probes prepared from BAC clones containing mouse *Igh* or *Myc* sequences. The *Igh*

and *Myc* probes were labeled with Spectrum orange and biotin, respectively, using a standard nick-

translation protocol. The *Myc* probe was detected with streptavidin-FITC. The *Igh* probe comprised the

most 3' part of the gene cluster including the Cα locus; thus, the probe only detected derivative 12 (der12)

of the translocation. The *Myc* probe included a sizeable portion of *Myc*'s 5' and 3' flanks; thus, it detected

both der(12) and der(15). For each tumor, at least 10 complete metaphases were evaluated, to determine

the karyotype of that tumor and to detect the T(12;15) translocation reproducibly and unequivocally. In

cases in which this was not possible because fewer than 10 cells were found to be in metaphase (the

fraction of proliferating cells in peritoneal PCT can be very small), 200 interphase nuclei of tumor cells

were evaluated for co-localization of *Myc* and *Igh* probes, the cytogenetic hallmark of the T(12;15)

translocation in non-dividing cells. Since co-localization of that sort also occurs by chance at low

frequency in normal cells, it is important to correct the frequency of *Igh-Myc* co-localization in tumor cells

for that observed in an equal number of normal cells (controls). Splenic B cells prepared from C.BCL2

mice were used for that purpose. Only the cells with numerable signals from both alleles were counted.

Chromosomes were counterstained with DAPI and embedded in anti-fade solution (Vector).

Detection of *Igh-Myc* junction fragments by PCR analysis

Genomic DNA was extracted from approximately one million PCT-containing ascites cells, using the

Gentra Puregene DNA isolation kit according to the manufacturer's instructions (Gentra Systems, Inc.).

PCR primer sets and protocols for the detection of *Igh-Myc* junction fragments, the molecular indicators of

the chromosomal T(12;15) translocation, have been described in detail (Kovalchuk *et al.*, 2000). In most

cases, 400 ng genomic DNA was used as PCR template. PCR fragments were gel-purified using the

QIAquick Gel Extraction Kit (Qiagen) followed by DNA sequencing. The GFP primer used for that

purpose had the following sequence: 5'- GGT GAA CAG CTC CTC GCC CTT GCT CAC CAT-3'.

Importantly, only those *Igh-Myc* junction fragments that were independently confirmed using a different

aliquot of genomic DNA from the same tissue (followed by PCR detection and sequence confirmation)

were interpreted to indicate the presence of T(12;15) in that tissue.

B. Supplementary References

Anderson PN, Potter M (1969). Induction of plasma cell tumours in BALB-c mice with 2,6,10,14-tetramethylpentadecane (pristane). *Nature* **222:** 994-995.

Kovalchuk AL, Esa A, Coleman AE, Park SS, Ried T, Cremer CC *et al* (2001). Translocation remodeling in the primary BALB/c plasmacytoma TEPC 3610. *Genes Chromosomes Cancer* **30:** 283-291.

Kovalchuk AL, Mushinski EB, Janz S (2000). Clonal diversification of primary BALB/c plasmacytomas harboring T(12;15) chromosomal translocations. *Leukemia* **14:** 909-21.

McNeil N, Kim JS, Ried T, Janz S (2005). Extraosseous IL-6 transgenic mouse plasmacytoma sometimes lacks Myc-activating chromosomal translocation. *Genes Chromosomes Cancer* **43:** 137-46.

Potter M, Pumphrey JG, Bailey DW (1975). Genetics of susceptibility to plasmacytoma induction. I. BALB/cAnN (C), C57BL/6N (B6), C57BL/Ka (BK), (C times B6)F1, (C times BK)F1, and C times B recombinant-inbred strains. *J Natl Cancer Inst* **54:** 1413-1417.

Silva S, Kovalchuk AL, Kim JS, Klein G, Janz S (2003). BCL2 accelerates inflammation-induced BALB/c plasmacytomas and promotes novel tumors with coexisting T(12;15) and T(6;15) translocations. *Cancer Res* **63:** 8656-63.

C. Supplementary Figure Legends

Suppl. Figure 1: Generation of iGFP$^{5'Myc}$ translocation reporter mice and genotyping of transgenic offspring.

(a) Molecular scheme of the iGFP$^{5'Myc}$ targeting vector (top), the normal *Myc* locus prior to gene targeting (center) and the modified *Myc* locus after gene targeting (bottom). The three exons of *Myc* (red) are numbered. The V$_H$-GFP reporter gene is indicated by a green box. 5' and 3' probes for Southern blotting of genomic DNA digested with SalI and EcoRI are depicted by black and yellow boxes, respectively, beneath the *Myc* schemes. Restriction of the normal and targeted *Myc* alleles results in a single 17.4-kb indicator fragment and a pair of 3.4 kb and 14.0 kb long fragments, respectively. Annealing sites for PCR primer sets 1 and 2 are indicated by labeled blue, horizonal arrows.

(b) PCR distinguishing non-transgenic offspring carrying two normal *Myc* alleles (N/N) from *Myc*-transgenic (T) offspring that are either heterozygous (T/N) or homozygous (T/T) for the iGFP$^{5'Myc}$ gene-insertion allele. PCR primer pair 1 detects a 221-bp fragment of the normal *Myc* allele in N/N offspring (lane 1) and T/N iGFP$^{5'Myc}$ mice (lane 4). Because the annealing sites of this primer pair are separated by ~2.5 kb in the presence of the V$_H$-GFP gene insertion, this primer pair also detects a 2.7-kb fragment of the targeted *Myc* allele in both T/N (lane 4) and T/T (lane 6) mice. This fragment is of low abundance in T/N mice (indicated by red box), in which the presence of the normal *Myc* allele favors the generation of the small 221-bp fragment. PCR primer pair 2 is specific for the mutated *Myc* allele and detects a 1.5-kb fragment in both T/N (lane 5) and T/T (lane 7) mice, but no band in N/N mice.

Suppl. Figure 2: Variable abundance of GFP-expressing B cells and plasma cells in tumor-bearing C.iGFP$^{5'Myc}$BCL2 mice.

Depicted are scatter plots of ascites cells obtained from transgenic mice injected with PCT1 (top), PCT3 (center) and PCT4 (bottom). Cells from each mouse were labeled with antibodies to B220 (RA3-6B2; left panel) and CD138 (281-2; right panel), in order to distinguish between B cells and plasma cells, respectively. Probing with unrelated, isotype-matched antibodies confirmed the specificity of labeling. Cells were analyzed on a Beckman Coulter FC 500 instrument (Beckman Coulter, Inc., Fullerton,

California). Percentages of marker-positive GFP$^+$ cells are given in the upper right-hand quadrants of the FACS scatterplots. In all three mice, CD138$^+$GFP$^+$ cells (plasma cells; range 6.3% to 63%) were more abundant than B220$^+$GFP$^+$ cells (B cells, range 0.3% to 2.2%). There was no numerical correlation between these two cell populations.

Suppl. Figure 3: Detection of the chromosomal T(12;15) translocation in interphase nuclei of PCT cells by FISH.

Chromosomal DNA was hybridized with FISH probes for *Myc* (FITC, green) and *Igh* (Cy5, red). Merged *Igh-Myc* signals, which are present in all images except the one to the upper left (normal control), indicate that the *Myc*-deregulating product of the T(12;15) translocation, der(12), is present. For the sake of clarity, we have selected images of translocation-bearing tumor cells that were dipoid, although the majority of tumor cells in BCL2-transgenic mice is tetraploid. In diploid tumor cells, the *Myc* probe detects not only der(12), but also the normal *Myc* gene on Chr 15 and the rearranged *Myc* locus on the reciprocal product of translocation, der(15). Because the FISH strategy used here cannot distinguish normal *Myc* from der(15), both signals are labeled *Myc*. Chromosomes were counterstained with DAPI (blue).