Supplementary information

Supplementary materials and methods

Construction of the inserted gene, *Myc*^{His}*Neo*^{loxP}

Myc^{His}*Neo*^{loxP} was constructed from four fragments (Suppl. Fig. 1A). Fragment 1 contained the untranslated exon 1 of mouse *Myc* (striped) together with the P1 and P2 promoters (labeled arrowheads pointing left) and ~1.5 kb of *Myc*'s 5' flank. It was obtained as a XbaI-XhoI fragment by screening the 129/SvJ mouse lambda genomic library from Stratagene. Fragment 2 was the mouse *Myc* cDNA clone, pM c-*myc* 54 (1). Fragment 3 was obtained by two rounds of PCR amplification using a mouse *Myc*-containing 9.1-kb SalI-KpnI fragment obtained from the above-mentioned Stratagene library as the template. The first round of PCR was performed with the primer pair His-1 and M13 (5'-AACAGCTATGACCATG-3') and the second round used the primer pair His-2 and M13 (see panel B for sequences of His-1 and His-2). His-1 contained codons for six histidine residues and one glycine residue adjacent to the stop codon, TAA. His-2 contained a SfuI cloning site and the codons for threonine and arginine residues that completed the 6xHis tag, RTGHHHHHH (Suppl. Fig. 1B). Fragment 3 also contained

the consensus cleavage signal (AAUAAA) for polyadenylation of *Myc* message (black arrowhead labeled "poly A" in panel A). Fragment 4 contained a loxP-flanked *Neo* gene, a kind gift from Dr. Hua Gu, NCI, NIH. The *Myc*^{His}*Neo*^{loxP} gene was assembled by linking fragments 1 and 2 at the XhoI site and fragments 2 and 3 at the SfuI site, which resulted in the in-frame addition of the 6xHis tag at the 3' end of *Myc* (Suppl. Fig. 1C). Fragments 3 and 4 were joined at the indicated SpeI (after extension with a SalI linker) and XhoI sites, which caused the removal of the distal part of fragment 3 (Suppl. Fig. 1C). *Myc*^{His}*Neo*^{loxP} thus consisted of the *Myc* transcription unit surrounded by its genomic 5' and 3' flanks, and *Neo* in the same transcriptional orientation as *Myc*.

Assembly of targeting vector iMyc$^{C\alpha}$

The iMyc$^{C\alpha}$ was constructed from the following four components: *Myc*^{His}*Neo*^{loxP} described above, the short arm of homology, the long arm of homology, and the thymidine kinase gene with the glucocorticoid promoter, *GKTK*, which was used for negative selection of ES clones (Suppl. Fig. 1C). The short arm of homology was a 1.2 kb NheI-AciI fragment containing exon 1 of Cα. The long arm of homology was a 7.2 kb AciI-PmlI fragment containing exons 2 and 3 of Cα. Both arms of homology were obtained by screening the 129/SvJ mouse lambda genomic library from Stratagene. The targeting vector was assembled in plasmid pBluescript (pBS) II SK(-) after transforming the SspI cloning site into a unique AscI site. The AscI site was used to linearize the vector prior to electroporation into ES cells. The 4-step assembly of the targeting vector is illustrated in Suppl. Fig. 1C. First, the short arm of homology was cloned into the SalI site of pBS using SalI linker extension (New

England BioLabs). Second, the long arm of homology was subcloned into the ClaI-NotI sites of pBS using ClaI linker extension at the AciI site and blunt ending of the NotI site. Third, *Myc*^{His}*Neo*^{loxP} was subcloned into the ClaI site of pBS using ClaI linkers on both ends. Since this step was not pre-determined with respect to transcriptional orientation, several clones were screened to identify those with the desired 5' to 5' orientation of *Myc*^{His} and Cα. Fourth, the *GKTK* SalI fragment was inserted into the XhoI site of the multiple cloning site (MCS) of pBS.

Generation of iMyc^{Cα} mice

The generation of strain iMyc^{Cα} is illustrated in Suppl. Fig. 1D. The targeting vector was transfected into CJ7 ES cells by electroporation and selected in 0.4 mg/ml G418 and 1 mM gancyclovir as previously described (2). Homologous recombinants were identified by the detection of a 12.1-kb band on Southern blots of BglII-digested DNA hybridized to a 1.3-kb genomic probe that annealed outside of the 5' recombination site. Homologous recombinants were found at a frequency of 4%. All five recombinant clones chosen for generating gene-targeted chimeras transmitted the mutated *Igh* allele to the progeny. Chimeras and germ-line transmitters were identified by agouti coat color (compared with the black color of the recipient C57BL/6 strain) and confirmed by BglII digests of genomic tail DNA. To delete the *Neo* gene, *Myc*^{His}*Neo*^{loxP} offspring were bred to Cre transgenic mice///. *Neo*-less mice were identified by digestion of tail DNA with ClaI and hybridization to a ^{32}P-labeled *Myc* cDNA probe. Deletion of *Neo* resulted in the reduction of the genomic restriction fragment by 1.2 kb.

Legends of supplementary figures

Suppl. Figure 1

Generation of iMyc$^{C\alpha}$ mice.

(a) Molecular schemes of the four individual components of the *Myc*^{His}*Neo*^{loxP} gene: *Myc* 5' flank and the non-coding part of exon 1 with the P1/P2 promoter (fragment 1); *Myc* coding region consisting of the most 3' part of exon 1, and exons 2 and 3 (fragment 2); the 6xHis tag, and *Myc* 3' untranslated region with the AAUAAA polyadenylation site (black arrowhead; fragment 3); and *Neo*^{loxP} (fragment 4).

(b) Generation of the 6xHis tag by PCR using primers, His-1 and His-2.

(c) Assembly of the targeting vector iMyc$^{C\alpha}$ in plasmid pBS.

(d) Targeting with iMyc$^{C\alpha}$, identification of targeted ES clones by Southern hybridization of the 5' integration site, generation of iMyc$^{C\alpha}$ mice containing *Neo*^{loxP}, and deletion of *Neo*^{loxP} after breeding with Cre transgenic mice.

Suppl. Figure 2

Plasmacytosis in tumor-free Myc/Bcl-X_L mice. Shown are sections of a peripheral lymph node (top), spleen (center), and liver (bottom) stained with antibody to B220 (B cells,

left) and Igκ (plasma cells, right). Plasma cells in spleen and liver are highly abundant compared to the relatively moderate number of plasma cells in the medullary cords of the lymph node.

Suppl. Figure 3

Antibody-forming cells (AFC) are abundant in young Myc/Bcl-X_L mice, but have not yet completed malignant transformation.

(a) Elispot analysis of AFC in the spleen of 8-week-old mice. Myc/Bcl-X_L mice contained two logs more AFC than Bcl-X_L mice (red arrows indicating wells with comparable numbers of AFC) and three logs more than Myc and normal mice (blue arrows). The experiment was repeated three times yielding similar results. The enumeration of AFC in the bone marrow resulted in similar differences among the four mouse strains (not shown).

(b) Summary of transfer experiments of plasma cells obtained from young, tumor-free Myc/Bcl-X_L mice.

Suppl. Figure 4

Increased proliferation and apoptosis in *Myc*-transgenic B cells.

(a) Freshly isolated $B220^+$ splenocytes were stained with PI in vitro (left) or labeled with BrdU in vivo (center) to measure proliferation. Detection of cleaved caspase 3 (right) was used to estimate apoptosis. Results represent the average from three independent experiments.

(b) MACS-purified $B220^+$ splenocytes were cultured for 24 h in serum-supplemented medium and activated by an anti-IgM antibody (10 μg/ml), LPS (50 μg/ml), or both prior to labeling with an antibody for cleaved caspase 3 and evaluation by FACS.

Suppl. Figure 5

Myc/Bcl-X_L transgenic mice harbor actively cycling $CD138^+$ cells.

(a) Shown are immunostained spleen sections that contain dense clusters of CD138-expressing plasma cells and plasmablasts (brown). Many $CD138^+$ cells undergo mitosis, as revealed by positive labeling for phosphohistone 3 (blue, left panel). Some $CD138^+$ cells undergo apoptosis (arrows), as revealed by positive labeling for cleaved caspase 3 (grey, right panel; original magnification 63x).

(b) The balance of proliferation and apoptosis in $CD138^+$ cells from 8-week-old Myc/Bcl-X_L transgenic mice is tipped in favor of proliferation. Shown are serial sections of a peripheral lymph node stained for B cells (B220) and plasma cells/plasmablasts (CD138). Two areas of plasma cell accumulation are indicated by red circles. Enumeration of $CD138^+$ cells immunostained for phosphohistone 3 (~30 cells in this example) or cleaved caspase 3 (5 cells in this example) demonstrated that proliferation exceeds apoptosis. Similar sections from lymph nodes of single transgenic Myc and Bcl-X_L mice were used as controls (results not shown).

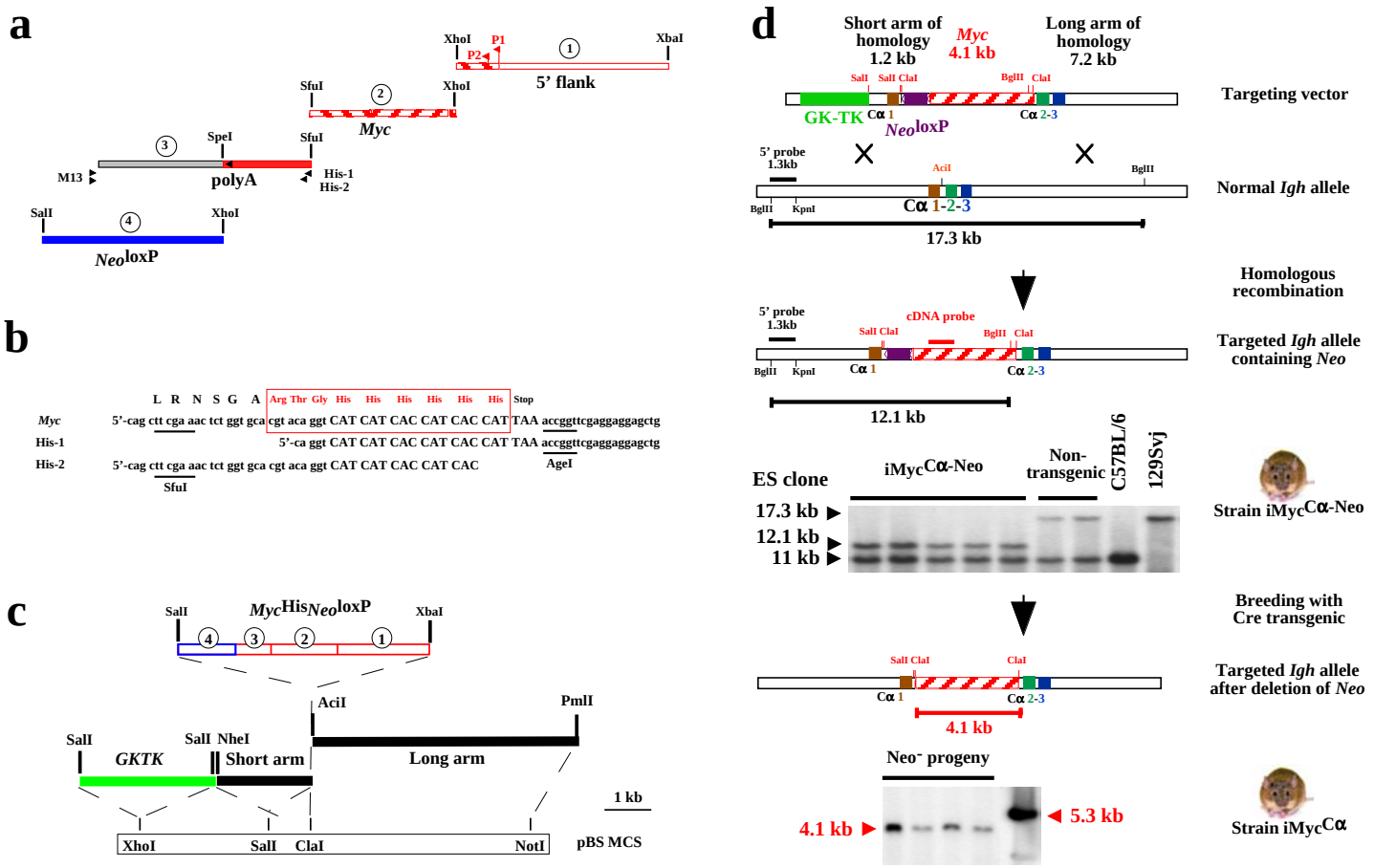
Suppl. Figure 6

Base substitution mutations in the 3' JH4 region of rearranged VH genes. The sequence of the 5' non-transcribed strand from the 129/SvJ germ line is shown. Base substitution

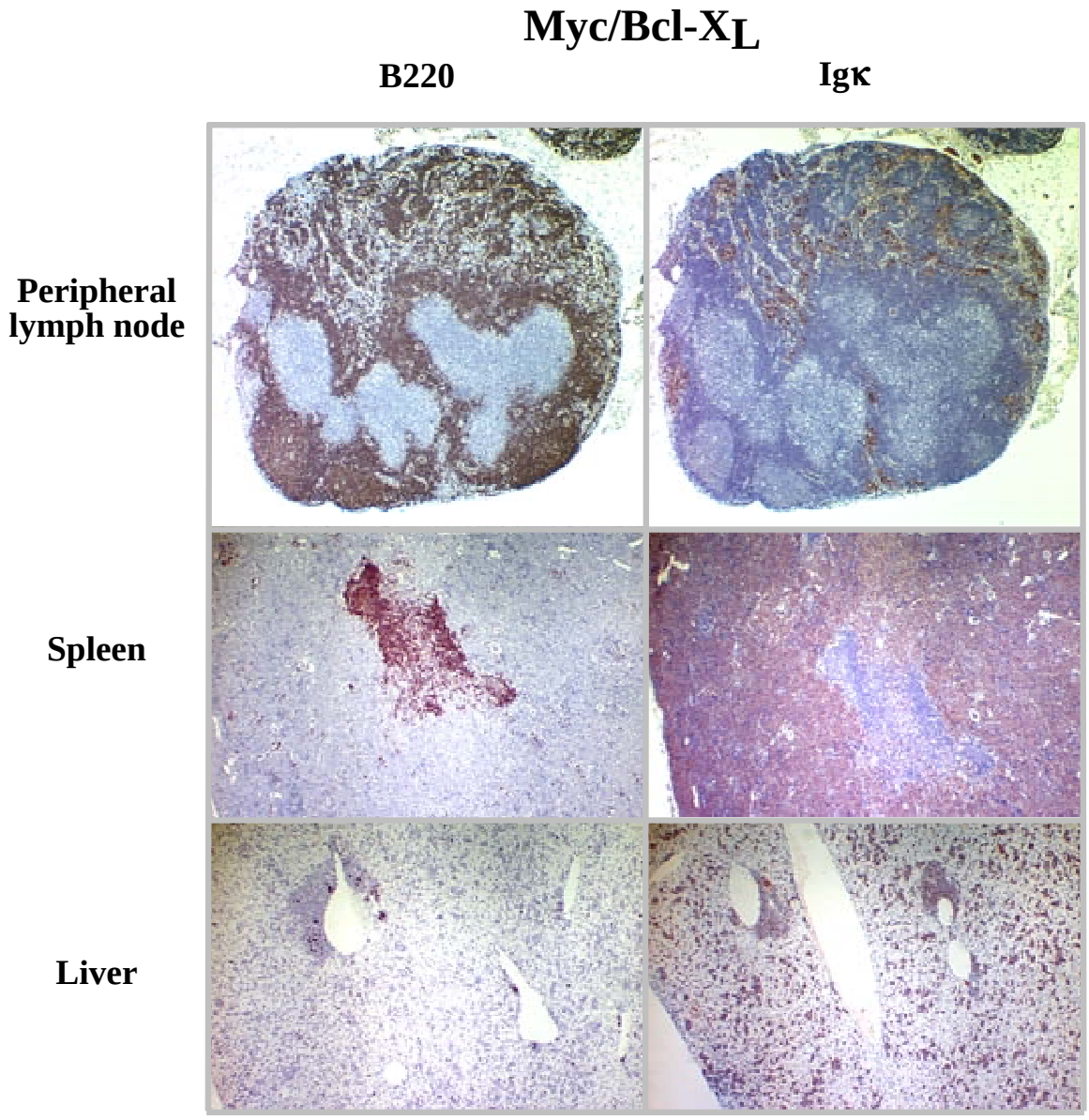
mutations are indicated above the sequence (black, tumor samples; red, control samples). Underlined nucleotides are allelic variations in C57BL/6 and FVB. Blue arrows pointing up denote mutations in tumors that occurred at WA dinucleotides (W = A or T).). One tumor clone contained a single-nucleotide deletion, ΔT (top row).

Suppl. References

1. Stanton, L.W., Watt, R., and Marcu, K.B. 1983. Translocation, breakage and truncated transcripts of c-myc oncogene in murine plasmacytomas. *Nature* 303:401-406.
2. Tessarollo, L., Vogel, K.S., Palko, M.E., Reid, S.W., and Parada, L.F. 1994. Targeted mutation in the neurotrophin-3 gene results in loss of muscle sensory neurons. *Proc Natl Acad Sci U S A* 91:11844-11848.

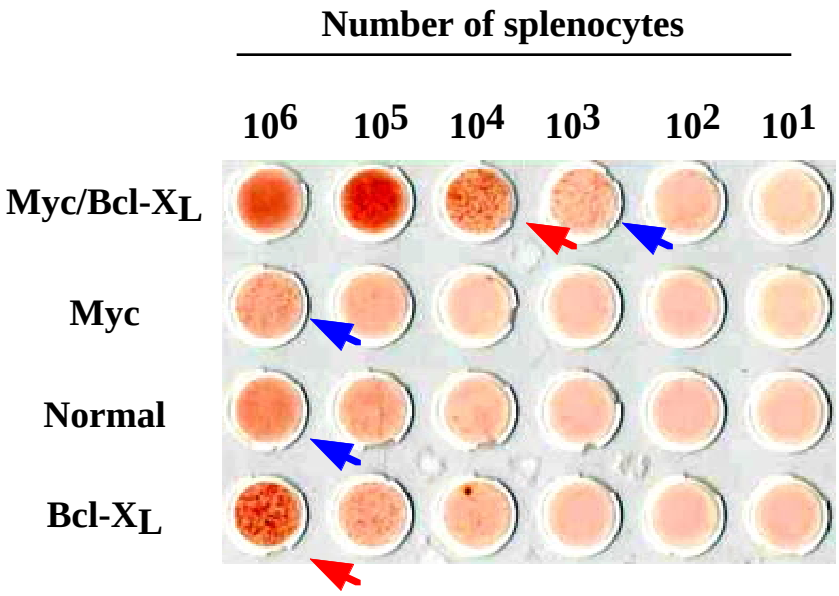


Suppl. Fig. 1: Cheung *et al.*



Suppl. Fig. 2: Cheung *et al.*

a



b

Table 1: Plasma cells from 8-week-old Myc/Bcl-X_L mice are not transplantable into pristane-primed BALB/c nude mice

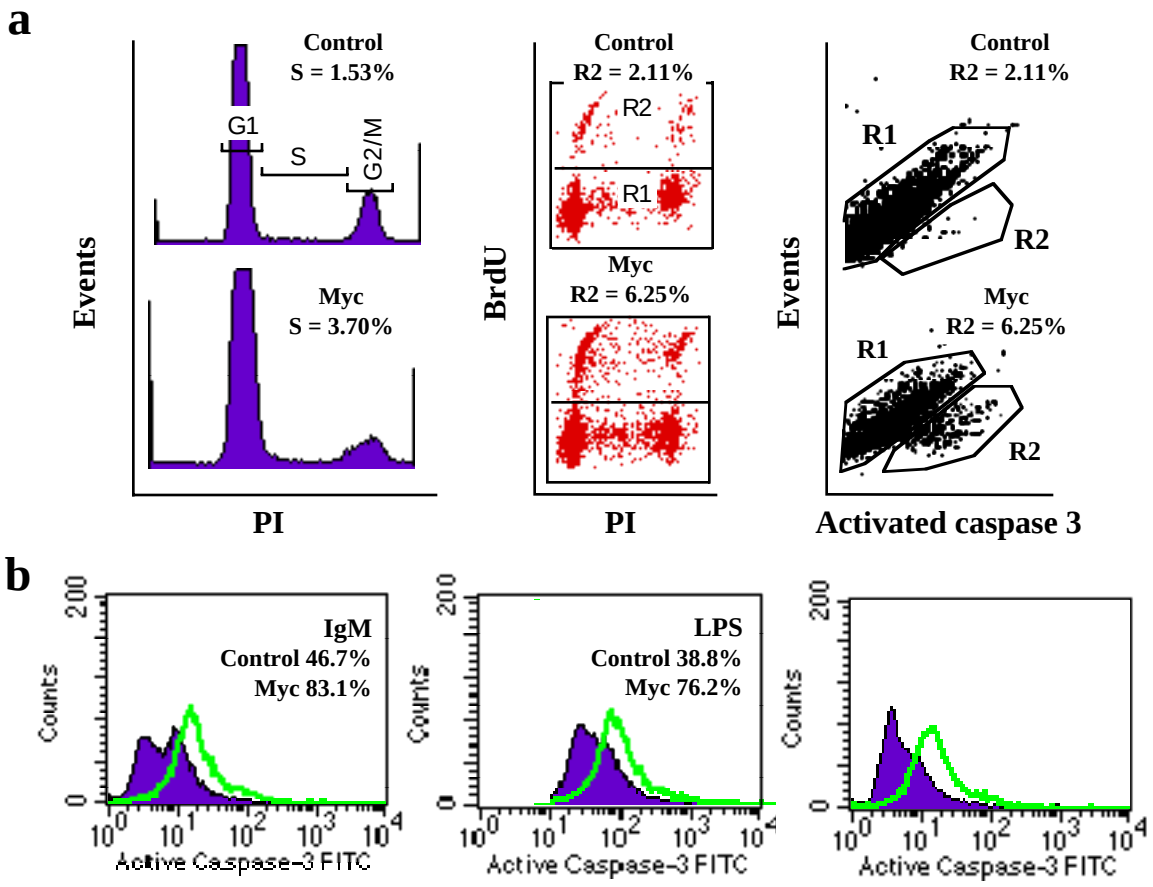
Exp.	Number, age, gender of donor mouse	Tissue source [a] of plasma cells	Number of trans- [b] ferred plasma cells	Number of BALB/c [c] nude recipients
1	#8, 34 days, M	SPL	0.8 x 10^6	2
2	#9, 34 days, F	SPL	1.7 x 10^6	2
3	#23, 34 days, M	MLN	1.4 x 10^6	3
4	#24, 34 days, M	PLF	2.1 x 10^6	1

[a] SPL, spleen; MLN, mesenteric lymph node; PLF, peritoneal lavage fluid

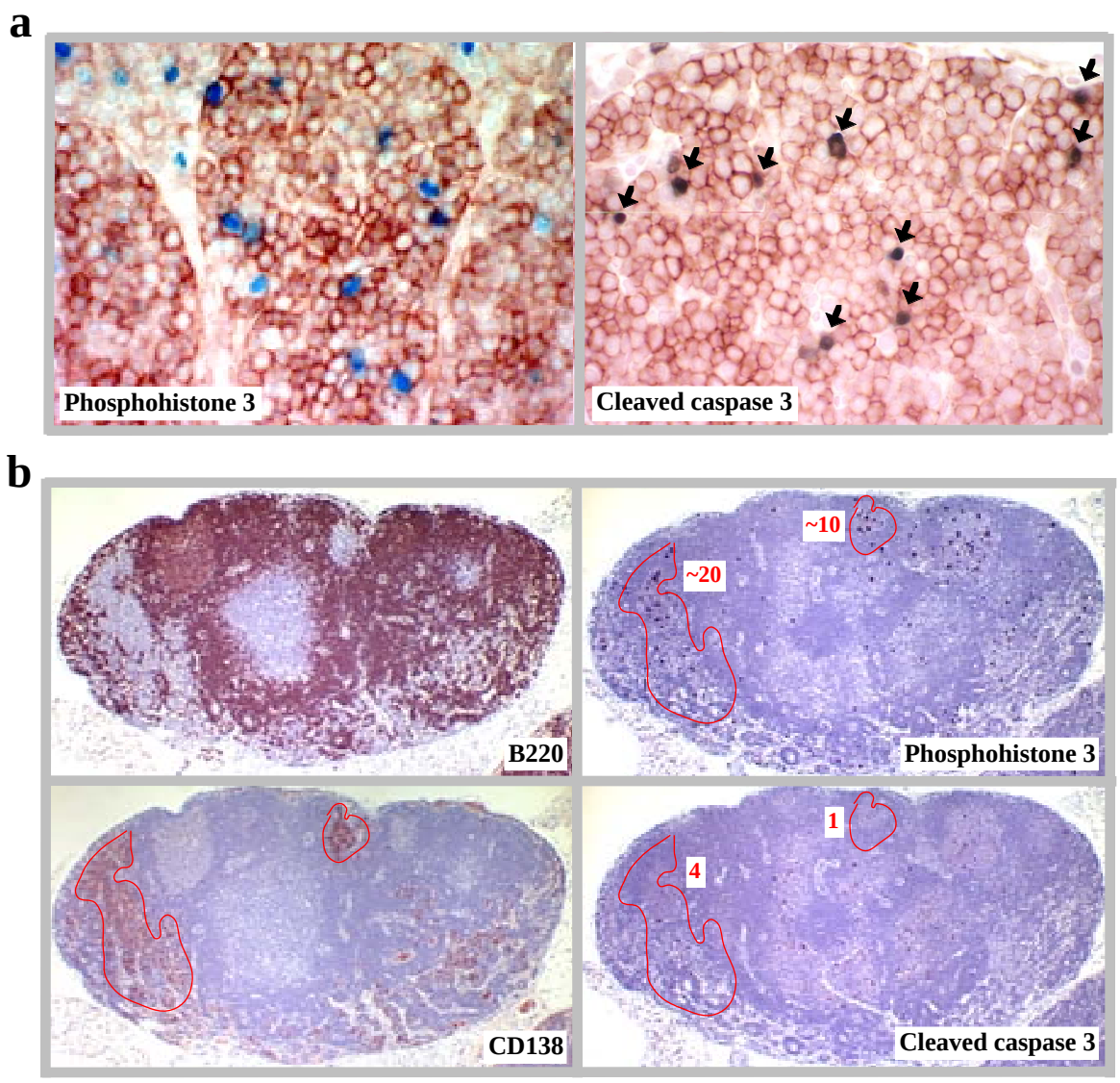
[b] Estimate of live plasma cells (based on FACS analysis of $CD138^+$ cells) contained in single cell suspension of 5 x 10^6 cells total.

[c] T-cell deficient recipient mice were primed i.p. with 0.5 ml pristane two to three weeks prior to cell transfer. Mice were monitored for outgrowth of B cell or plasma cell tumors four and eight weeks after cell transfer by cytological assessment of May-Grünwald-Giemsa stained ascites cell specimens. Mice were sacrificed 12 weeks after cell transfer and evaluated for the presence of incipient B/plasma cell tumors in histological sections of pristane-induced peritoneal granulomas stained according to hematoxylin and eosin.

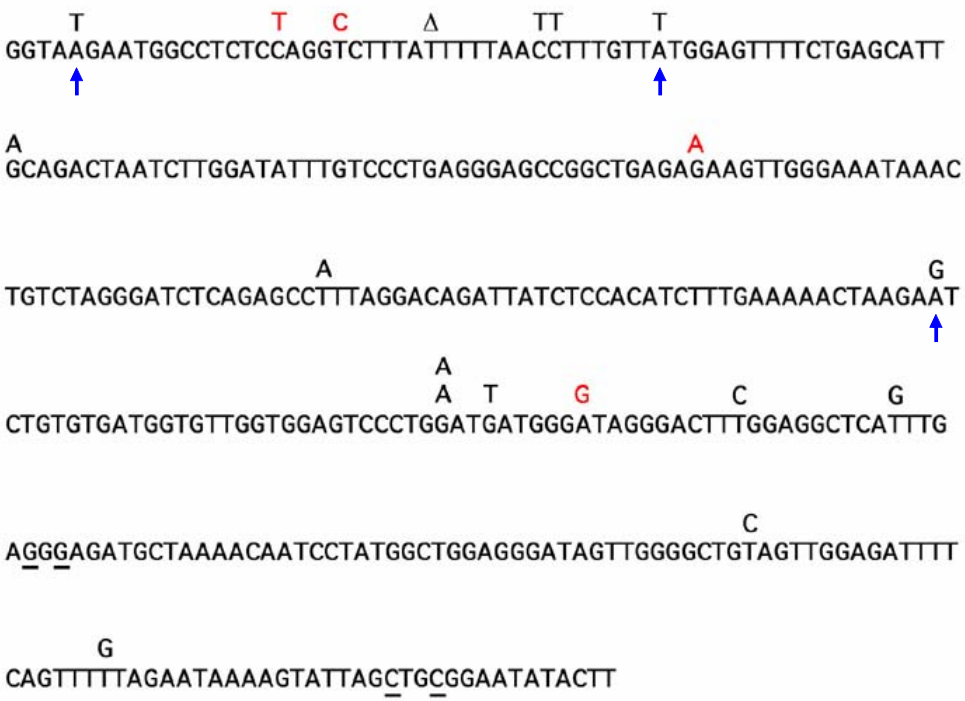
Suppl. Fig. 3: Cheung *et al.*



Suppl. Fig. 4: Cheung *et al.*



Suppl. Fig. 5: Cheung *et al.*



Suppl. Fig. 6: Cheung *et al.*