Figure S1. Purification strategy of either T (Thy 1.2$^+$) or B cells (B220$^+$) by AutoMACS for Western analysis of p16^{INK4a} expression as shown in Fig. 1A, B
Positive selection with Thy 1.2$^+$ or B220$^+$ lead to greater than 95% purity for T or B cells as determined with CD3 and B220 staining respectively.

Figure S2. Absolute and relative numbers of DN thymocyte subsets in mice of indicated ages and genotypes
N=4–5 mice per group; error bars indicate SEM.

Figure S3. *Ex vivo* T-cell activation in response to stimulation through the TCR signaling pathway
(A) Activation of T cells purified from mouse spleens was measured by CD25 FACS staining after stimulation with PBS (−), anti-CD3, anti-CD3 plus anti-CD28, and PMA plus ionomycin (P+I). The percentages of activated (CD25$^+$CD4$^+$7AAD$^-$) T cells are quantified and compared in (B). N=4–5 mice per group; error bars indicate SEM; * indicates $p<0.05$.

Figure S4. Role of p16INK4a in age-related changes in germinal center formation and *ex vivo* B-cell activation
(A) *Ex vivo* activation of splenic B cells from mice of indicated ages and genotypes. Activation was induced by PMA plus ionomycin treatment and measured by CD25$^+$ expression. (B) Representative flow analysis showing germinal center B cells (B220$^+$GL-7$^+$) with and without NP-CGG immunization in mice of indicated ages and genotypes. (C) Quantification of germinal center B cells as determined in (B). N=4 mice per group; error bars indicate SEM.

Figure S5. Tumors in *CD19$^{Cre/+}$ p16$^{L/L}$* mice express B-cell markers
(A) Immunohistochemical staining of tumors involving the CNS from *CD19$^{Cre/+}$ p16L/L* mice with the indicated markers. "Ig" = mouse immunoglobulin. CNS parenchyma (CNS) and tumor (T) are indicated; all original magnifications were 40×. (B) Flow cytometry of the bone marrow and spleen of a 52 week old *CD19$^{Cre/+}$ p16$^{L/L}$* mouse without overt signs of disease. The orange arrow shows a relative decrease in the bone marrow B220Loμ-chainLo population. Red arrows indicate an abnormal B220LoCD93+μ-chainIntermediate population in the bone marrow (upper and middle panel) and spleen (lower panel). Such aberrant B-lineage populations were noted in five of five *CD19$^{Cre/+}$ p16$^{L/L}$* mice analyzed at ages greater than 52 weeks, but not in littermate *CD19$^{Cre/+}$ p16$^{+/+}$* animals (left panels for comparison).

Figure S6. Lymphoma progression in an aged *CD19$^{Cre/+}$ p16$^{L/L}$* mouse
Weekly serial PET/CT images were acquired at the indicated ages in a *CD19$^{Cre/+}$ p16$^{L/L}$* mouse. The upper panel shows fused PET/CT images in coronal views, and the lower panels show matched maximum intensity projection (MIP) PET images with 3-D rendering. The yellow arrows indicate central nervous system (BR), splenic (SPL) or lymph node (LN) involvement. The red arrows indicate lymphomatous involvement of the thorax and abdomen. Background uptake was observed in the heart (HT), bladder (BDR) and retro-orbital gland (RG).

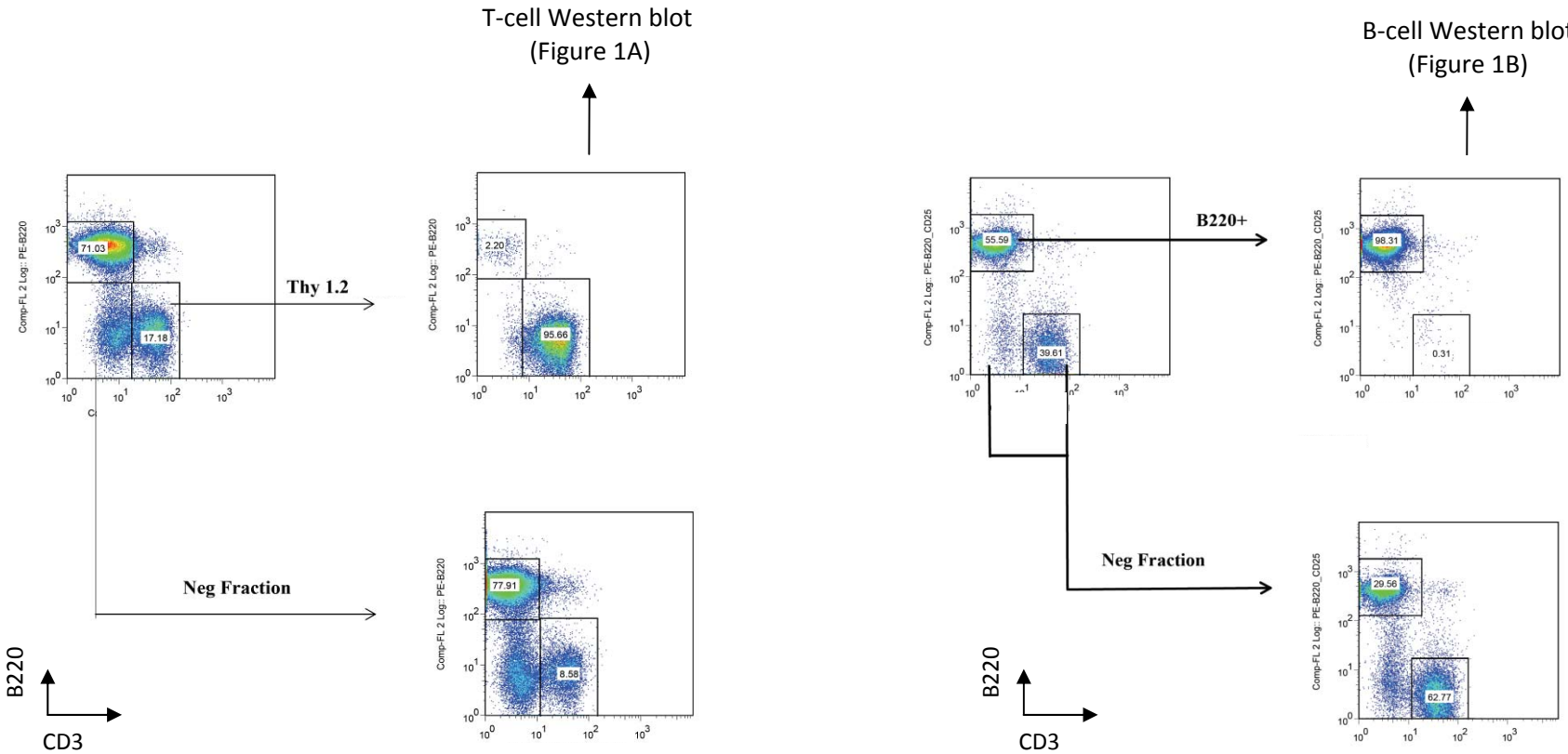
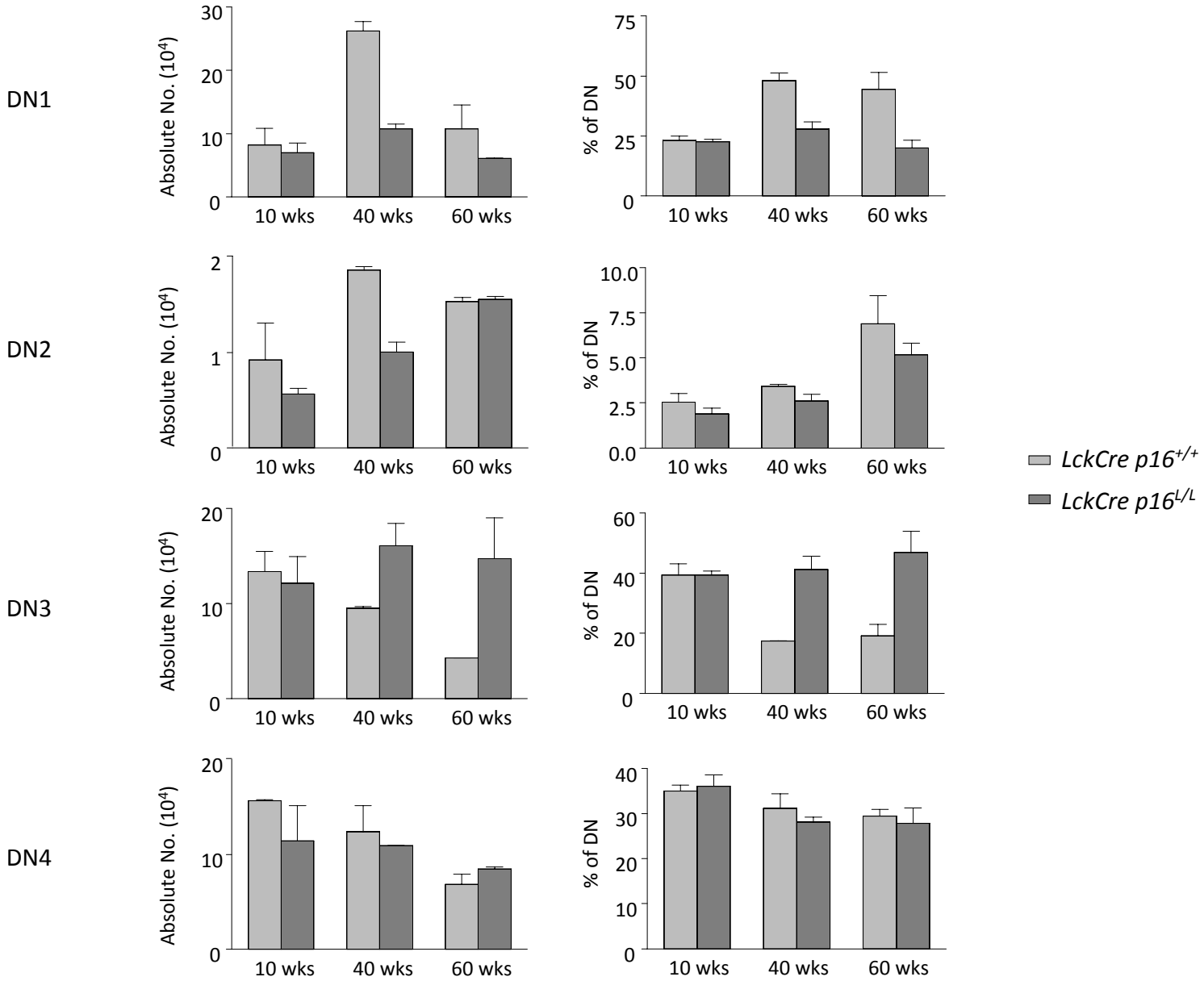
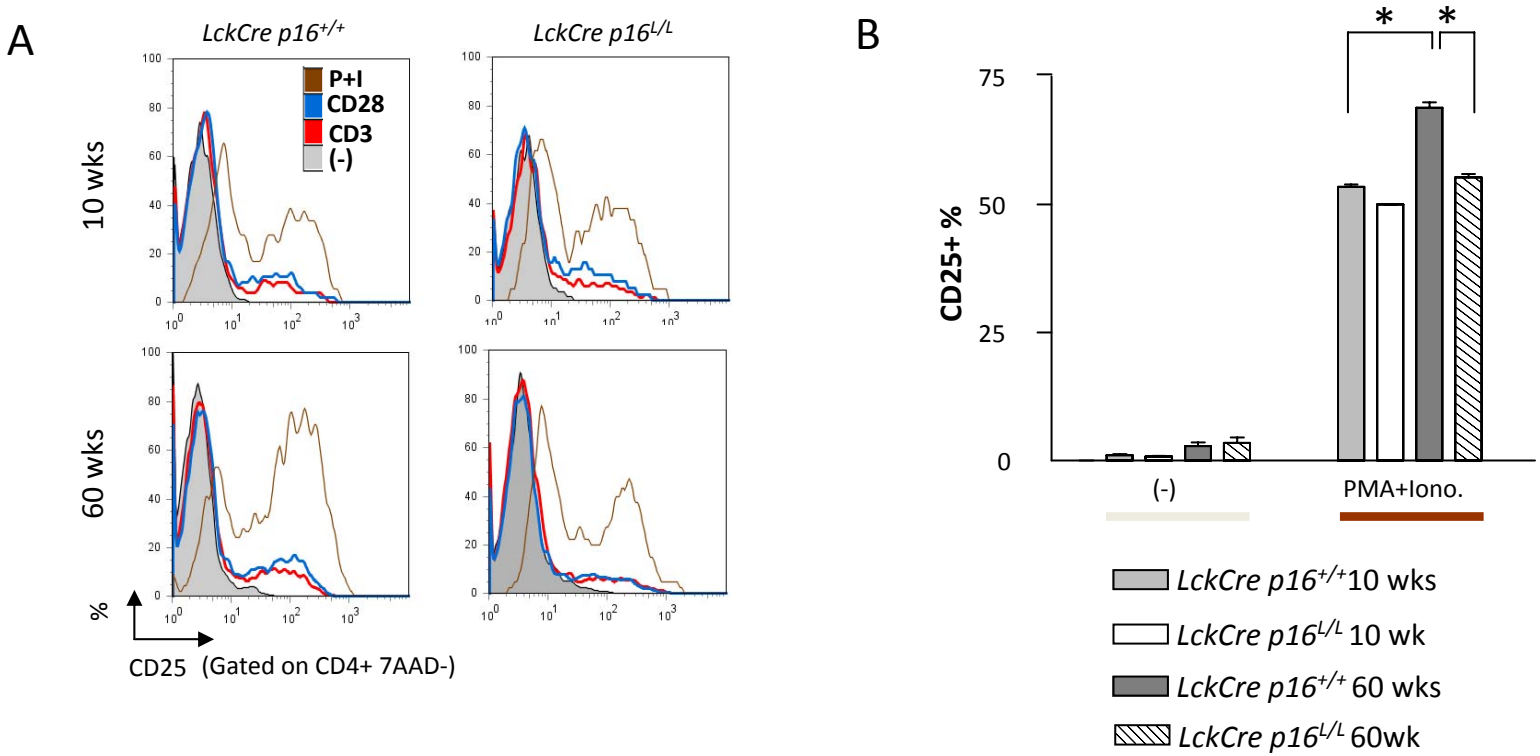

T-cell Western blot
(Figure 1A)
B-cell Western blot
(Figure 1B)
Thy 1.2
Neg Fraction
B220+
Neg Fraction
B220
CD3
B220
CD3
71.03
17.18
2.20
95.66
77.91
8.58
55.59
39.61
98.31
0.31
29.58
62.77

Figure S2


DN1
DN2
DN3
DN4
Absolute No. (10^4)
% of DN
10 wks
40 wks
60 wks
LckCre p16+/+
LckCre p16L/L


A
LckCre p16+/+
LckCre p16L/L
P+I
CD28
CD3
(-)
10 wks
60 wks
%
CD25 (Gated on CD4+ 7AAD-)
B
CD25+ %
75
50
25
0
(-)
PMA+Iono.
LckCre p16+/+10 wks
LckCre p16L/L 10 wk
LckCre p16+/+ 60 wks
LckCre p16L/L 60wk

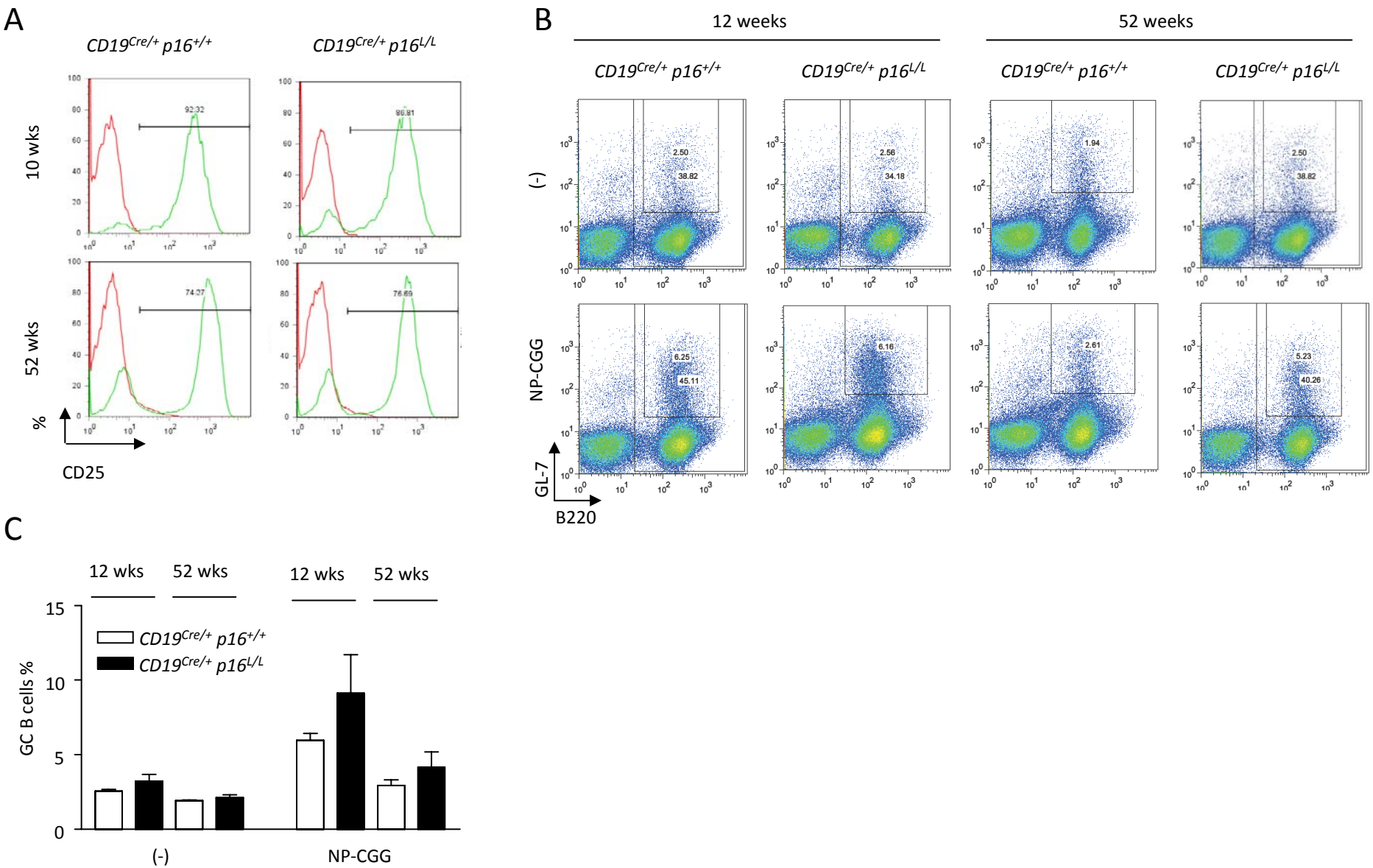
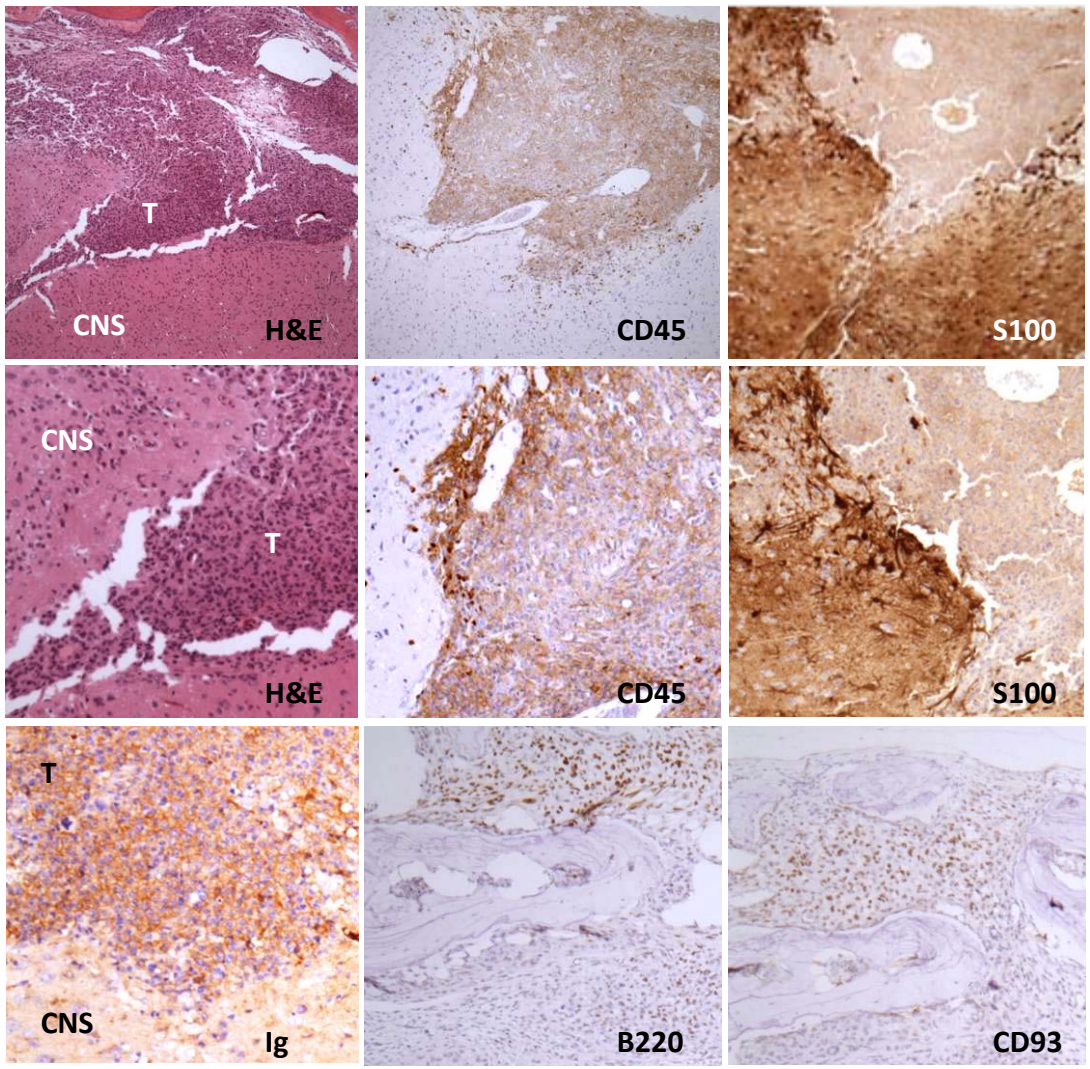

A
CD19Cre/+ p16+/+
CD19Cre/+ p16L/L
10 wks
52 wks
%
CD25
92.32
80.81
74.27
76.69
B
12 weeks
52 weeks
CD19Cre/+ p16+/+
CD19Cre/+ p16L/L
CD19Cre/+ p16+/+
CD19Cre/+ p16L/L
(-)
NP-CGG
GL-7
B220
2.50
38.82
2.56
34.18
1.94
2.50
38.82
6.25
45.11
6.16
2.61
5.23
40.26
C
12 wks
52 wks
12 wks
52 wks
CD19Cre/+ p16+/+
CD19Cre/+ p16L/L
GC B cells %
15
10
5
0
(-)
NP-CGG

Figure S5

A


T
CNS
H&E
CD45
S100
CNS
T
H&E
CD45
S100
T
CNS
Ig
B220
CD93

B

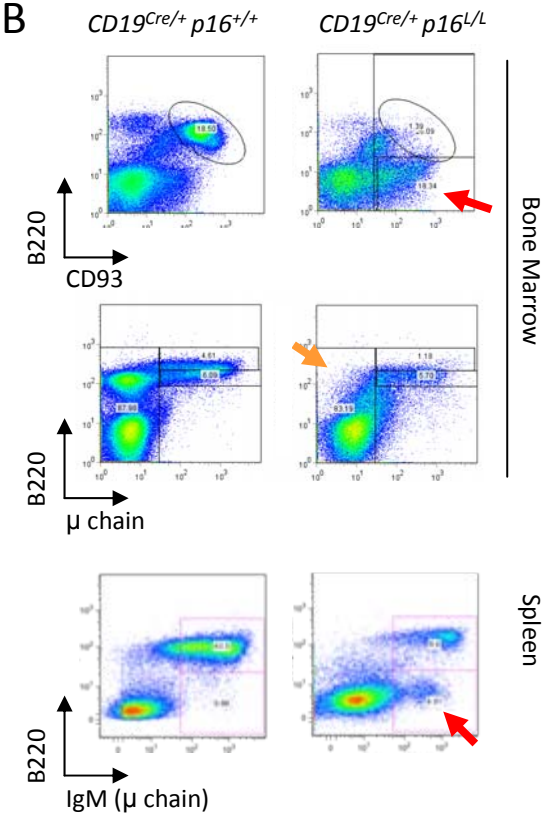
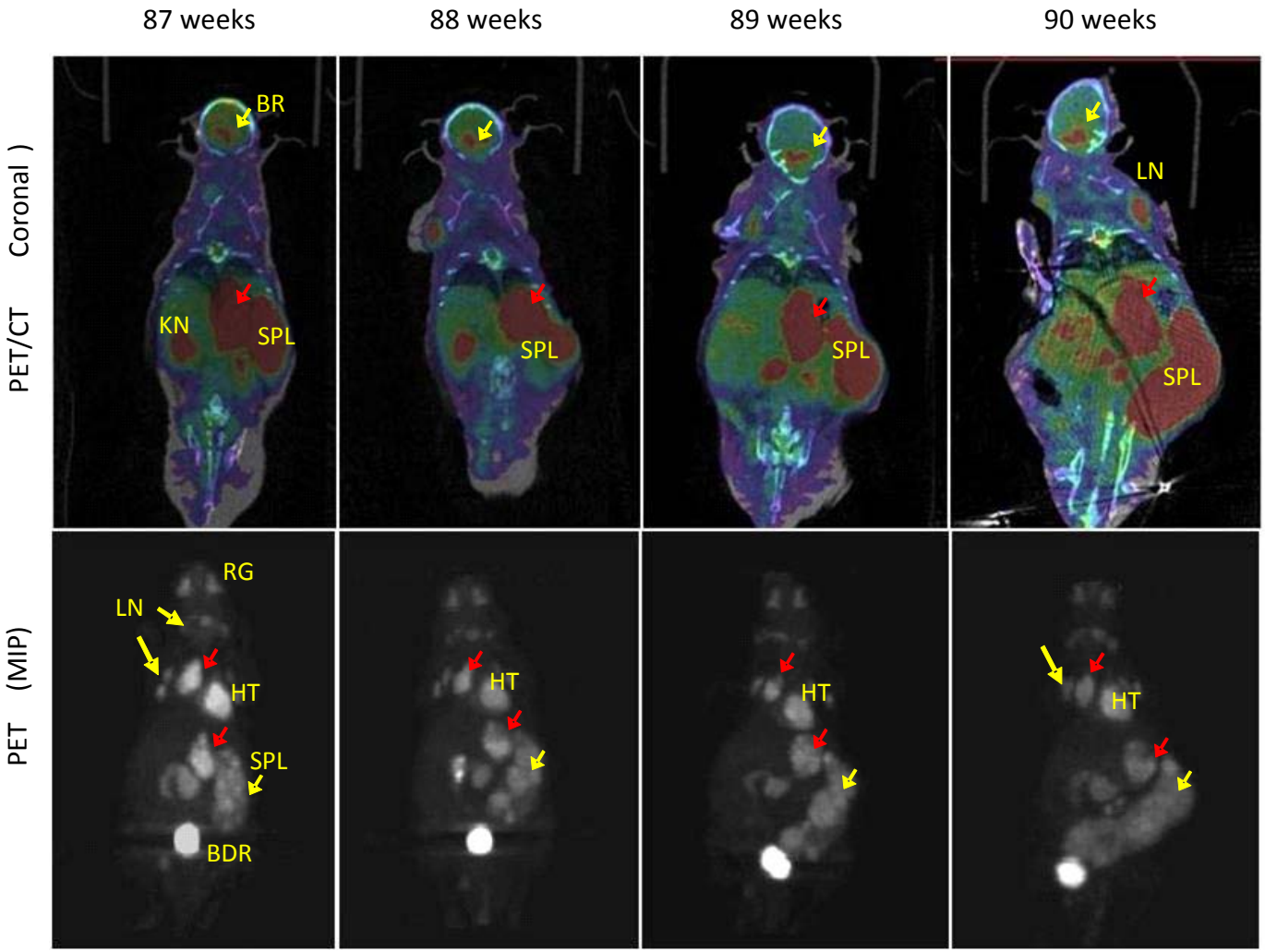

CD19Cre/+ p16+/+
CD19Cre/+ p16L/L
B220
CD93
B220
μ chain
Bone Marrow
B220
IgM (μ chain)
Spleen

Figure S6


87 weeks
88 weeks
89 weeks
90 weeks
PET/CT Coronal)
PET (MIP)
BR
KN
SPL
SPL
SPL
LN
SPL
RG
LN
HT
SPL
BDR
HT
HT
HT