

Legends to Supplemental Figures:

Supplemental Figure 1: Lack of activation/proliferation marker expression in hTNF-tg

PLNs. *A, B*, PLN cells from the indicated sources were analyzed by flow cytometry with antibodies to IgM, B220, CD69, CD80, CD86, GL7 and Ki-67. B cell-gated histograms are shown. PLN B cells from hTNF-tg mice lack expression of common activation markers (*A*) and are not proliferative based on Ki-67 expression (*B*). *C*, total mRNA from PLNs from the indicated sources were reverse transcribed and subject to semi-quantitative RT-PCR reaction to analyze expression of the genes for *hprt*, *mb-1*, the plasma cell marker *Blimp-1* and the germinal center-specific enzyme activation induced cytidine deaminase (*aicda*). Representative results are shown. No significant changes were observed in the expression of these genes, although 2 of 5 hTNF-tg transgenic PLNs displayed very low but detectable levels of *aicda* (significantly lower than PLNs from immunized animals, not shown).

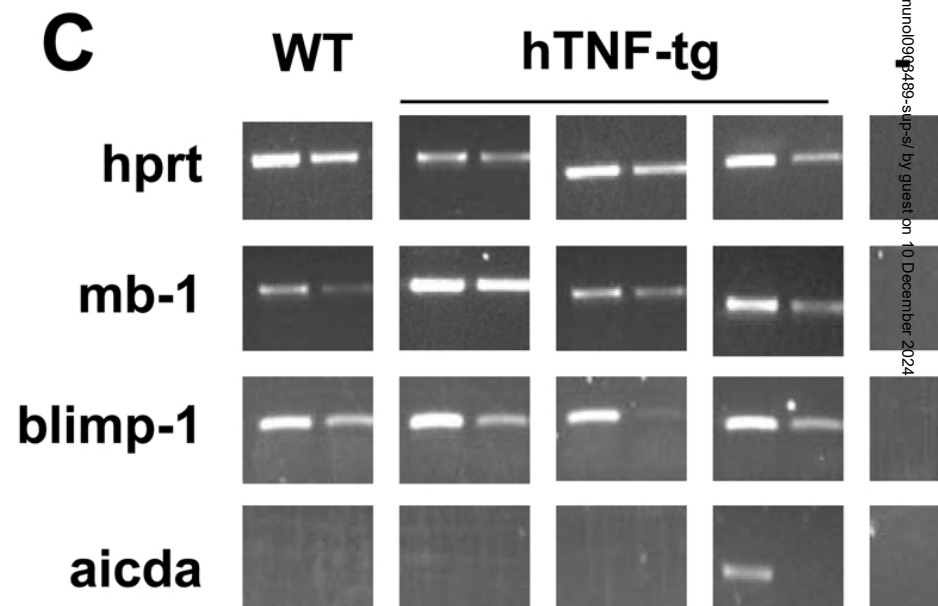
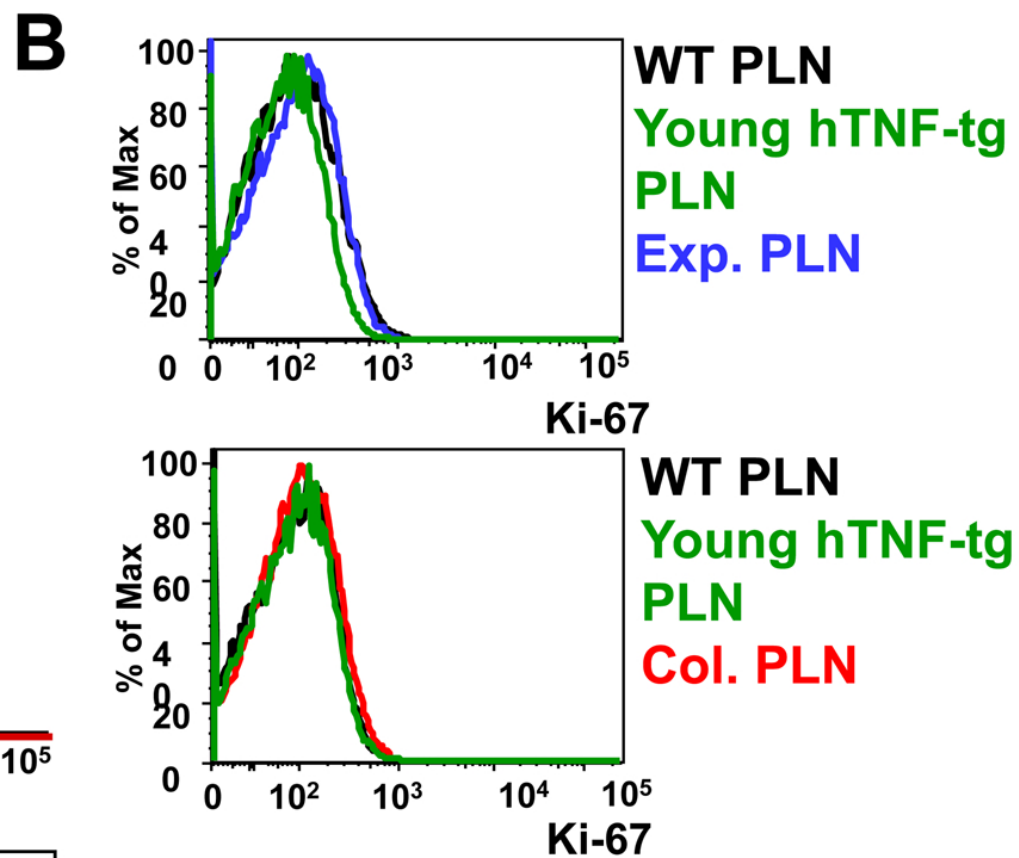
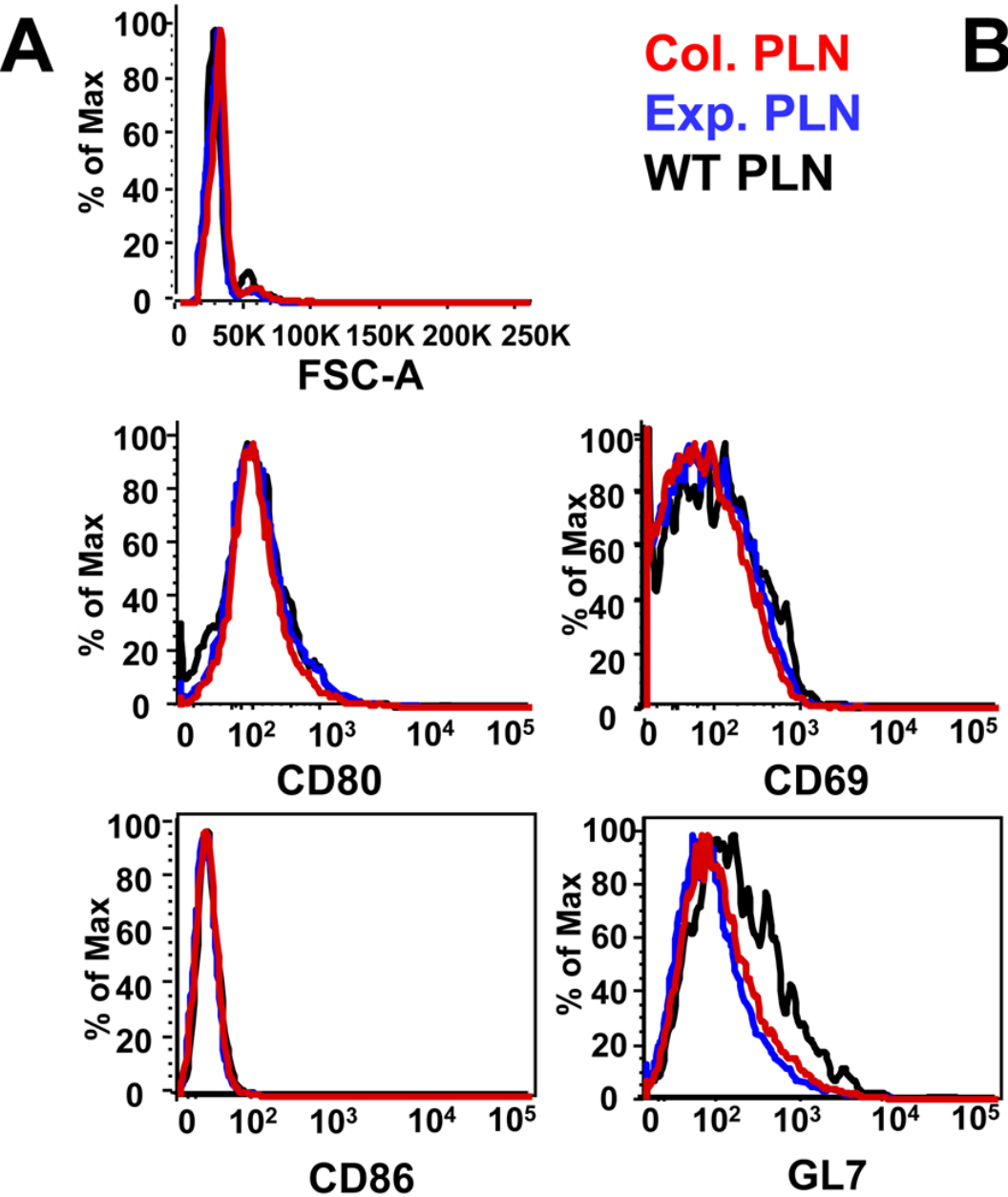
Supplemental Figure 2: Analysis of CD20, TNFR1, TNFR2 and CD27 expression on Bin

cells. PLN cells from C57BL/6 mice (top panels) and hTNF-tg mice (bottom panels) were analyzed by flow cytometry with antibodies to IgM, B220, CD19, CD23, CD21 CD20, CD27, CD120a (TNFR1) and CD120b (TNFR2). CD19-gated B cells were further gated based on CD23 and CD21 expression as described in the main text, and levels of staining with antibodies to CD120a, CD120b, CD27 and CD20 in the three main B cell populations (FoB, Bin and MZB-like) are shown as histograms. CD20 expression is slightly higher in Bin cells from both hTNF-tg and control mice compared to FoB cells. CD27, CD120a and CD120b are similarly low to absent on all cell types from both sources. Panels are representative of two independent experiments.

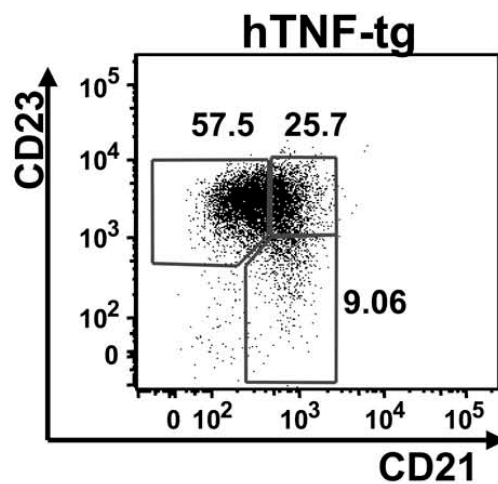
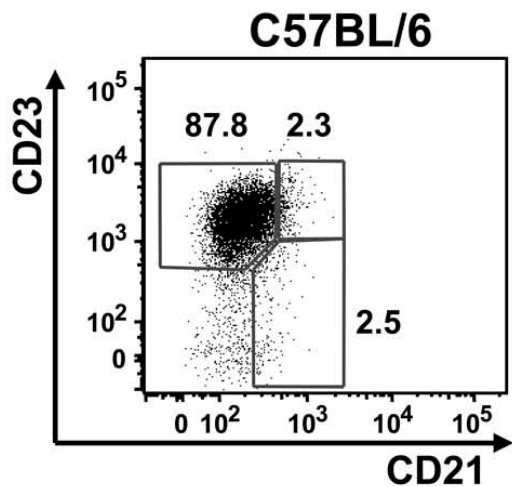
Supplemental Figure 3: Cytokine expression by Bin and follicular B cells.

Sorted Bin (CD21-high, CD23+) and follicular B (FoB) (CD21-low, CD23+) cells from the PLNs of hTNF-tg mice, and FoB cells from C57BL/6 PLNs and CD19+ peritoneal cavity B cells (PCB) cells were used for these experiments (no PLN Bin cells were sorted from control mice due to insufficient numbers). Cells were cultured at 10^6 cells/ml in U-bottom 96-well plates in complete RPMI in the presence of CpG DNA (1 μ g/ml), PMA (50ng/ml) and ionomycin

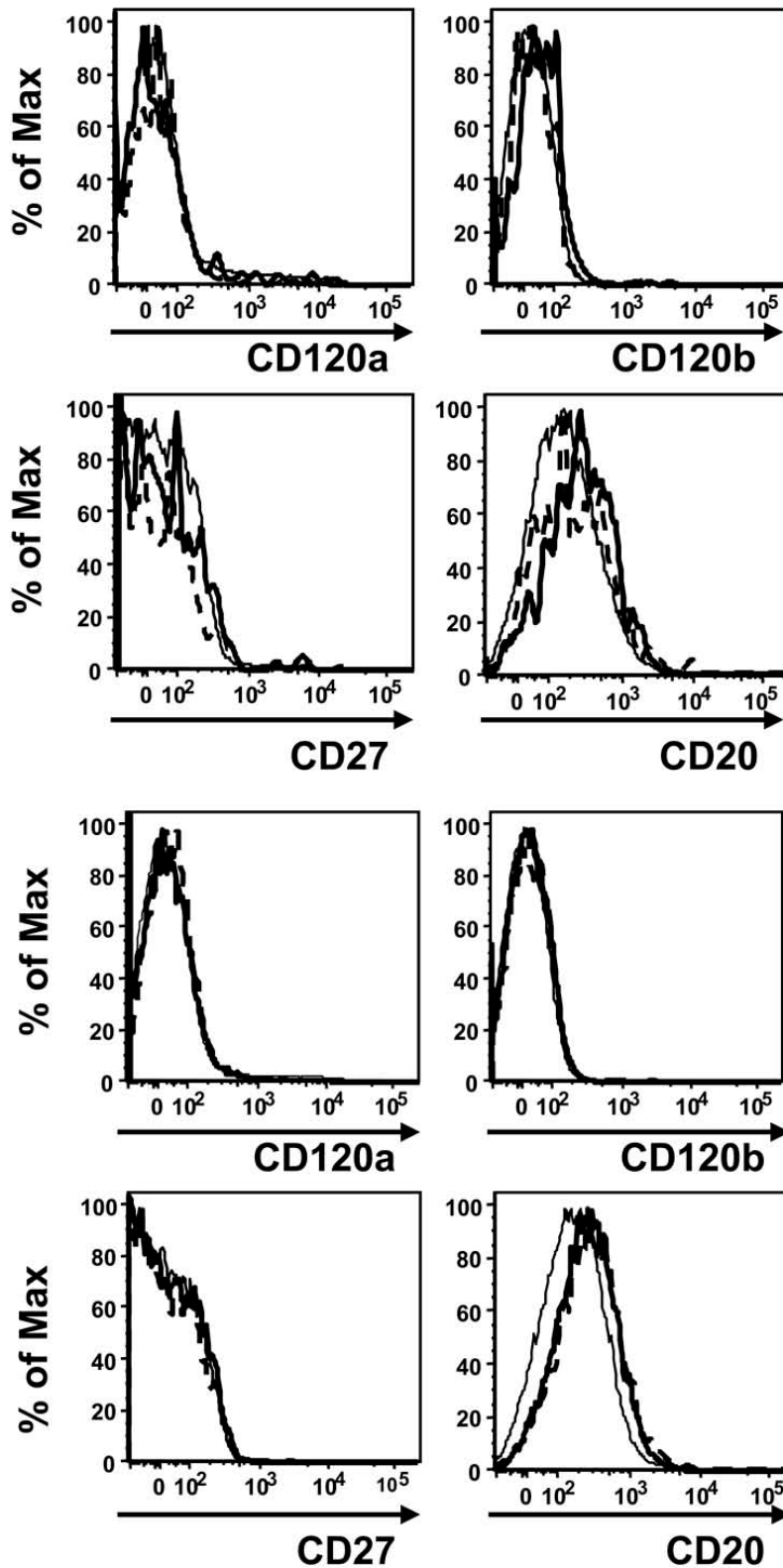
(1.25 μ M) to induce cytokine secretion. **A.** Secretion of IL10, IFN γ and mTNF α in 24-hr culture supernatants was measured by Luminex assay using the Biorad Bio-Plex system with kit reagents. Note the limited and comparable production of cytokines by both Bin and FoB cells in hTNF-tg and controls. Data show mean and standard deviation from 3 parallel cultures of cells from 2 mice/strain. **B.** RT-PCR analysis of mTNF α and hTNF α mRNA expression in in vitro-stimulated cells as described above. PCR products from 2 5-fold serial dilutions of cDNAs are shown for the experimental samples. (C⁺ = DNA template control; dH₂O = no template control).



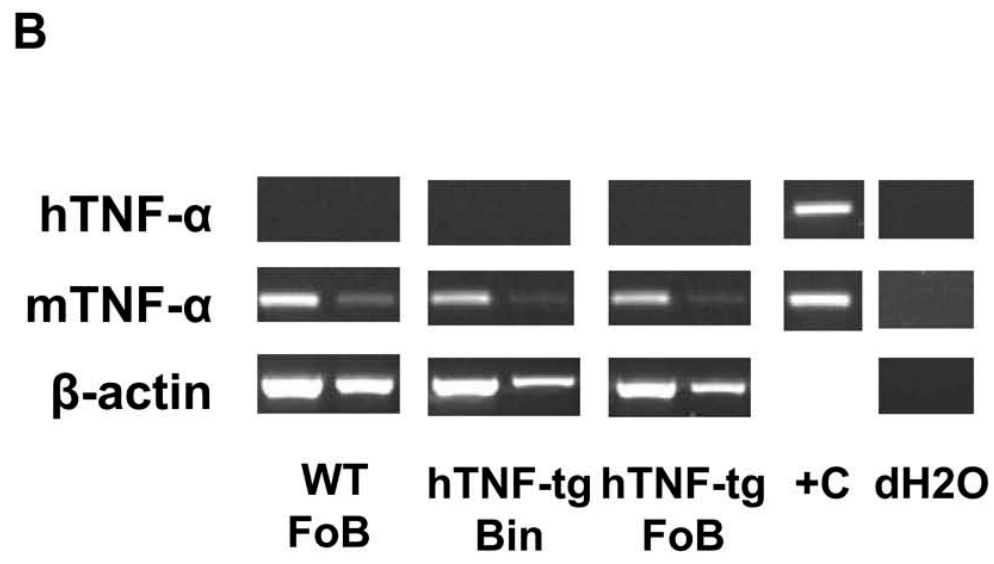
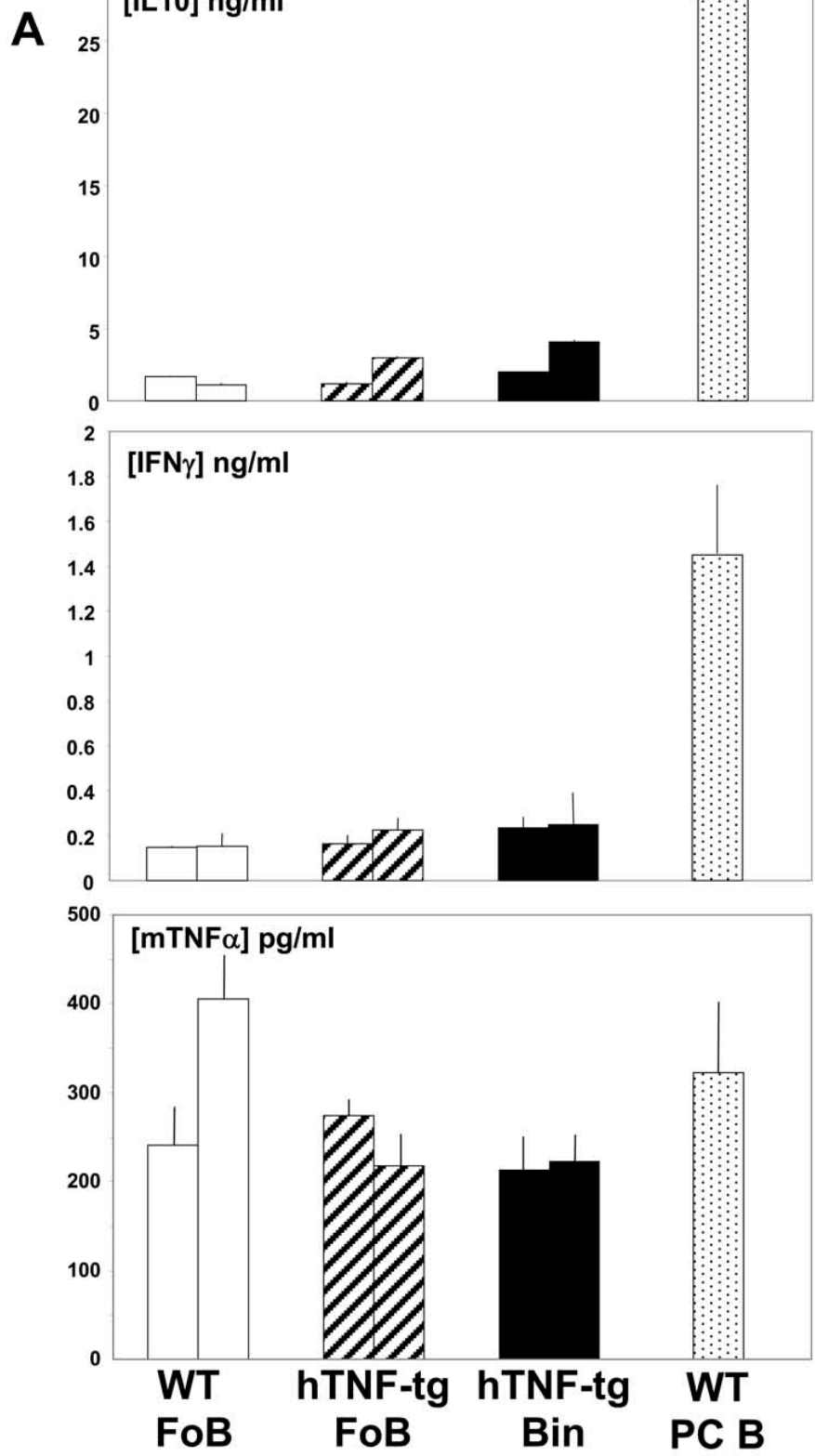
Supplemental Figure 1



FO ———
MZB - - - -
Bin ———



**Supplemental
Figure 2**



Supplemental Figure 3

Supplemental Table I – B cell populations in K/BxN peripheral lymphoid organs

		<u>Healthy littermates</u>	<u>K/BxN</u>
<u>Spleen</u>	Total cell # (x 10 ⁻⁶)	51 ± 13	57 ± 22
	B220+ IgM+ # (x 10 ⁻⁶)	21 ± 4	29 ± 8
	%	42 ± 5	53 ± 11
	CD23+ CD21hi # (x 10 ⁻⁶)	4 ± 1	7 ± 3
	%	18 ± 3	25 ± 6
<u>PLN</u>	Total cell # (x 10 ⁻⁶)	0.9 ± 0.15	2.4 ± 0.2***
	B220+ IgM+ # (x 10 ⁻⁶)	0.16 ± 0.08	1.7 ± 0.7***
	%	18 ± 11	71 ± 8***
	CD23+ CD21hi # (x 10 ⁻⁶)	0.03 ± 0.02	0.6 ± 0.07***
	%	16 ± 6	36 ± 4**
<u>ILN</u>	Total cell # (x 10 ⁻⁶)	0.9 ± 0.2	1.1 ± 0.1*
	B220+ IgM+ # (x 10 ⁻⁶)	0.3 ± 0.06	0.6 ± 0.02***
	%	30 ± 7	52 ± 7**
	CD23+ CD21hi # (x 10 ⁻⁶)	0.05 ± 0.01	0.13 ± 0.03*
	%	20 ± 2.5	22 ± 6
<u>ALN</u>	Total cell # (x 10 ⁻⁶)	2.3 ± 0.3	2.4 ± 0.6
	B220+ IgM+ # (x 10 ⁻⁶)	0.7 ± 0.1	1.1 ± 0.5
	%	28 ± 4	45 ± 16
	CD23+ CD21hi # (x 10 ⁻⁶)	0.1 ± 0.01	0.2 ± 0.15
	%	16 ± 2	20 ± 6
<u>MLN</u>	Total cell # (x 10 ⁻⁶)	5.3 ± 1.5	2.6 ± 2
	B220+ IgM+ # (x 10 ⁻⁶)	2 ± 0.7	5.4 ± 0.2
	%	38 ± 6	48 ± 21
	CD23+ CD21hi # (x 10 ⁻⁶)	0.4 ± 0.17	0.15 ± 0.13*
	%	20 ± 3	10 ± 6*

Abbreviations and cohorts are as described in the text. N = 4 for each group.

* = p < 0.05, ** = p < 0.01, *** = p < 0.001 (2-tailed t-test).