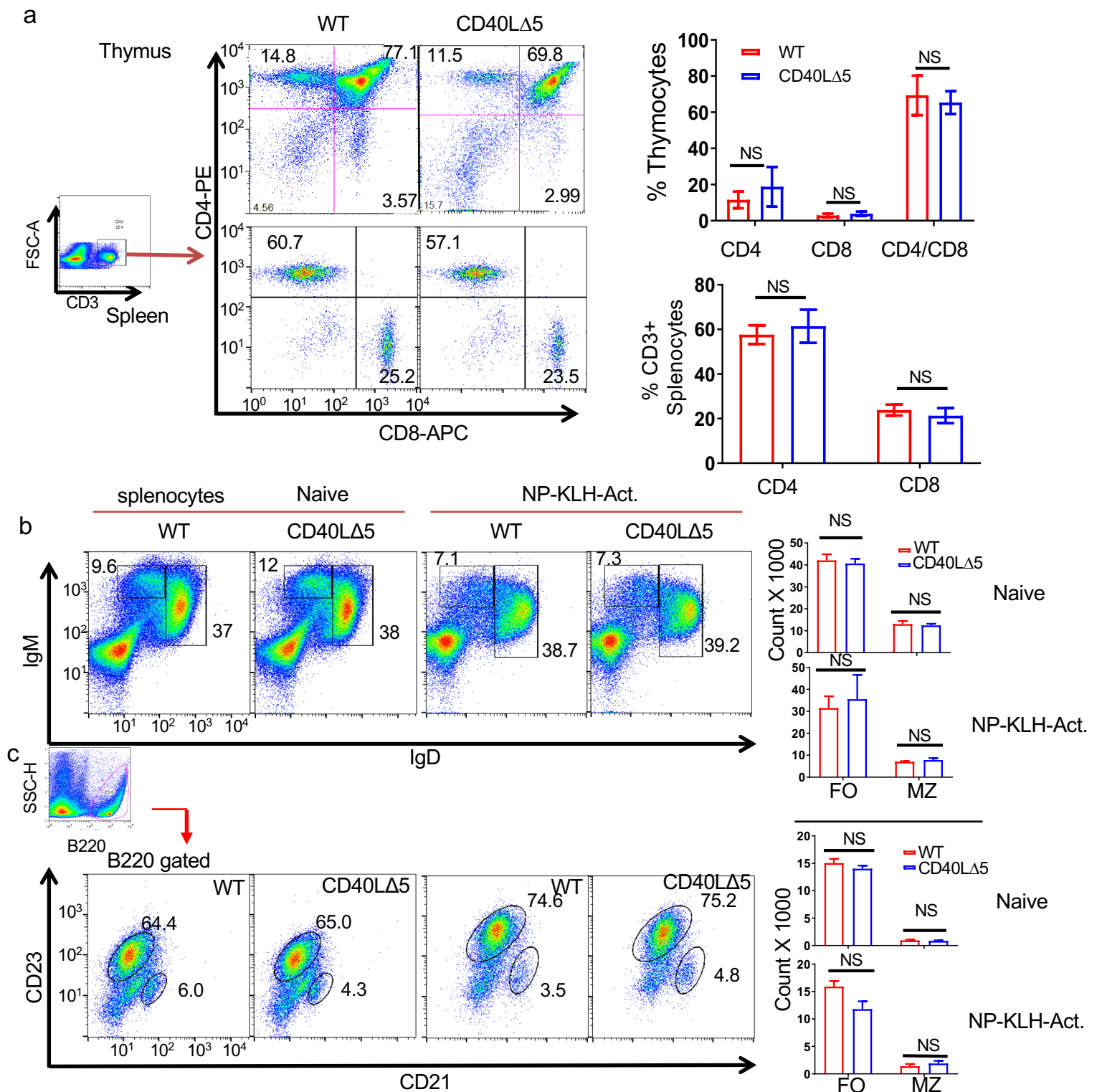


Supplementary Figure 1. (a) Schematic of the mCD40L cDNA showing the coding region, 3'UTR stability element and end of exon 5 (1736). (b) Luciferase assays carried out with regions of the CD40L stability element cloned into the 3' region of the pRL-SV40 vector. Results are normalized to expression of *Renilla luciferase*. (c) Schematic diagrams of the CD40L gene showing targeted regions (exons 4 and 5), CD40L Δ 5 construct, the targeted locus with the Neo^R gene indicated, and the targeted locus after *Cre*-mediated recombination. Indicated in red is the CD40L translational stop codon and polyA site (114 nt from *loxP* site). Location and size of the three CD40L sequences used in the targeting vector, the neo^R and TK cassettes, and *loxP* sites are indicated. PCR primers for screening ES cell clones for homologous recombination are depicted with arrows. Position of probe to analyze Southern blots is indicated as **probe A**. (d) ES cell clones analyzed for proper recombination into the genomic locus; clones 84 and 89 of this analysis were used to generate lines of CD40L Δ 5 mice. (e) Representative PCR from mice generated from clone 84 and carried out with neoF-6567R (right flank) and 7505F-neoR (left flank) primers from outside of the targeting construct. (f) Representative PCR analysis of genomic DNA from mice following deletion of the Neo^R gene showing hemizygous male mutants (y/ Δ 5), heterozygous (+/ Δ 5), homozygous (Δ 5/ Δ 5) and WT females using primers Δ 5F and Δ 5R.



Supplementary Figure 2. Evaluation of T and B cell populations in CD40L Δ 5 mice. (a) Thymus and spleens were collected from 6-10-week-old mice, dissociated and analyzed for distribution of B and T cells. Thymus (top panels) and CD3-gated spleen (bottom panels) were analyzed for CD4 and CD8 expression. Histograms (right) showing the Mean \pm s.e.m. from n=3 experiments. (b) Spleens were collected from naïve (left 2 panels) and day 28 NP-KLH immunized mice (boosted at day 21, right 2 panels). Splenic MZ (IgM^{hi}, IgD^{lo}) and FO (IgM⁺, IgD^{hi}) are boxed and bar graphs of 4 independent experiments are presented on the right. (c) Representative image shows the same groups of cells as in (b) with splenic MZ (B220⁺, CD23^{lo}/CD21^{hi}), and FO (B220⁺, CD23^{hi}/CD21^{lo}) populations from WT and CD40L Δ 5 mice and data from 3 independent experiments compiled in graphs on the right. Significance was determined using student's unpaired t test where a p>0.05 is considered "not significant" (NS).

Supplementary Table 1:

Antibodies used for Flow cytometry and Immunohistochemistry. Antibodies designated in **bold** were used in IHC.

Antigen	Fluor	Catalog #	Source
CD21/35	FITC	123407	Biolegend
CD23	PE	101607	Biolegend
CD19	BV570	115535	BioLegend
IgM	BV510	406531	BioLegend
IgG1	BV650	406629	BioLegend
IgD	APC	405713	BioLegend
CD138	BV711	142519	BioLegend
CD93	PerCp-Cy5	136511	BioLegend
Blimp-1	Alexa 647	150003	BioLegend
CD86	APC-Fire750	105045	BioLegend
CD86	Alexa700	560581	BD
CXCR4	BV421	146511	BioLegend
CXCR4	Alexa647	146504	Biolegend
CD38	Pacific Blue	102719	BioLegend
Fas	BV605	152612	BioLegend
CD73	A700	127229	Biolegend
CD80	PE/Cy7	104733	Biolegend
PD-L2	PE-Dazzle	107215	Biolegend
B220	APC	103211	Biolegend
GL7	Alexa 488	144611	Biolegend
IL-9R	Purified	MAB2134-SP	R&D
2ry α -rat	BV605	405430	Biolegend
IL-10	APC/Cy7	505035	Biolegend
CD4	Alexa 594	100446	Biolegend
CTLA-4	PE/Dazzle 594	106317	Biolegend
IL-9	Alexa 700	IC409N-100ug	R&D
IL-21	PE-Cy7	25-7213-80	ThermoFisher
CD40L	APC	106509	BioLegend
CXCR5	APC-750	145533	BioLegend
PD-1	BV711	135231	BioLegend
IgD	Alexa 647	405707	Biolegend
IL-6	PE	504503	BioLegend
IL-4	BV605	504126	BioLegend
Bcl6	PerCP-Cy5	358507	BioLegend
GATA3	Alexa 488	653807	BioLegend
Tbet	BV785	644835	BioLegend
IFN-g	BV605	505832	BioLegend
Ki-67	Alexa 594	151214	BioLegend
IL-12	V450	561456	BD

Supplementary Table 2.

Primers used to generate and analyze the CD40L Δ 5 construct

Target gene	Purpose	NCBI reference	Forward primer	NCBI reference	Reverse primer
CD40L genomic	Frag. 1	AL672128.8	cgggccgctcagcctcctaacgctgacatcat	same	cggccgcttcaacacagggcagggtcctaact
CD40L genomic	Frag. 2	AL672128.8	tctacagtgggccaagaaagga	same	aaatcacctcagggtgtccagc
CD40L genomic	Frag. 3	AL672128.8	cggccgctcttgggtggaggctgtaccat	same	cgggccgctcaaacatggctgagtaaacctcaagtg
CD40L	deletion-1	AL672128.8	ctcccctcccttcgcacacacacacacac	same	gtgtgtgtgtgtgtgcgaaagggagggggag
CD40L	deletion-2	AL672128.8	ctctcccctcccttctgtctctattatctacc	same	gggtagataataagagaacgaaagggaggggagag
CD40L Δ 5	PCR-5' integration status	AL672128.8	gcagcagcaggcacataactggaaa	Neo	ccatattggctgcaggctcgaaa
CD40L Δ 5	PCR-3' integration status	Neo	gcaaaacaaaattaagggccagctca	AL672128.8	ctcacaatgtagctcaaacatggct
CD40L	Southern blot probe	AL672128.8	atgccgtgctgtgcttaactgac	same	ttcaacacagggcagggtcctaact
CD40L	PCR genotyping	NM_011616	tctcactgaccagacttccatctc	same	ggtatgtcccgtgactgggc

qPCR primers

Target gene	NCBI reference	Forward primer	Reverse primer	Size (bp)
CD40L	NM_011616	acgttgtaagcgaagccaac	tatccttttggcccactg	60
B2M	NM_007393	taacacagttcaccgccctca	gctcggccatactgtcatgctt	188
GAPDH	NM_008084	catggcctccgtgttcta	cctgcttcaccaccttctgat	103
CD69	NM_001033122	ggctctggagggtgcgtgtcc	cgatcatctggagggtgctg	97
CD25	NM_008367	tggtctagggtctgcgcca	agcgtctagagtggcctgct	90
AICDA-1	NM_009645	tctgctacgtggtgaagaggag	ccagtctgagatgtagcgtagg	110
AICDA-2	NM_009645	accgatattggacagcctctg	accacgtagcagaggtagg	103
c-myc	NM_01849.4	gttgaaaccccgagaca	ccagatatcctcactgggcg	178

V186.2 primers

Primer Name	Accession Number	Sequence
Cy1-cDNA	MH397224.1	5'-catggagtagtggggcag-3'
V186.2-leader	J00529	5'-agctgtatcatgctcttctggca-3'
V186.2-nested	J00529	5'-catgctcttctggcagcaacag-3'
Cy1-PCR	MH397224.1	5'-atccaggggcccaggtagtagac-3'