Table S1. Sequences of oligonucleotide primers used for real-time PCR

Gene name	Primer name	Sequence
CCL22	CCL22-fw CCL22-rv	TGGAGTAGCTTCTTCACCCA TCTGGACCTCAAAATCCTGC
CXCL13	CXCL13-fw CXCL13-rv	TTGTGTAATGGGCTTCCAGA AGGTTGAACTCCACCTCCAG
Serpina1a	Serpina1a-fw Serpina1a-rv	AACATCCTCAGCCAGAAAGC AGCTCTGGGACAGCAAGC
St6galnac2	St6galnac2-fw St6galnac2-rv	GTGCTCTTACCCACTGAGCC ACCAGCCAAGTTCAGAAGCA
MadCAM1	MadCAMF MadCAMR	CTGAGCCCTACATCCTGACCT GCTTCACAGAGTAGCTCCCAG
TNF	TNF-fw TNF-rv	CAGCCTCTTCTCATTCCTGC GGTCTGGGCCATAGAACTGA
HPRT	HPRT-fw HPRT-rv	CTCCTCAGACCGCTTTTTG ACCTGGTTCATCATCGCTAA
GAPDH	GAPDH-fw GAPDH-rv	TTGATGGCAACAATCTCCAC CGTCCCGTAGACAAAATGGT

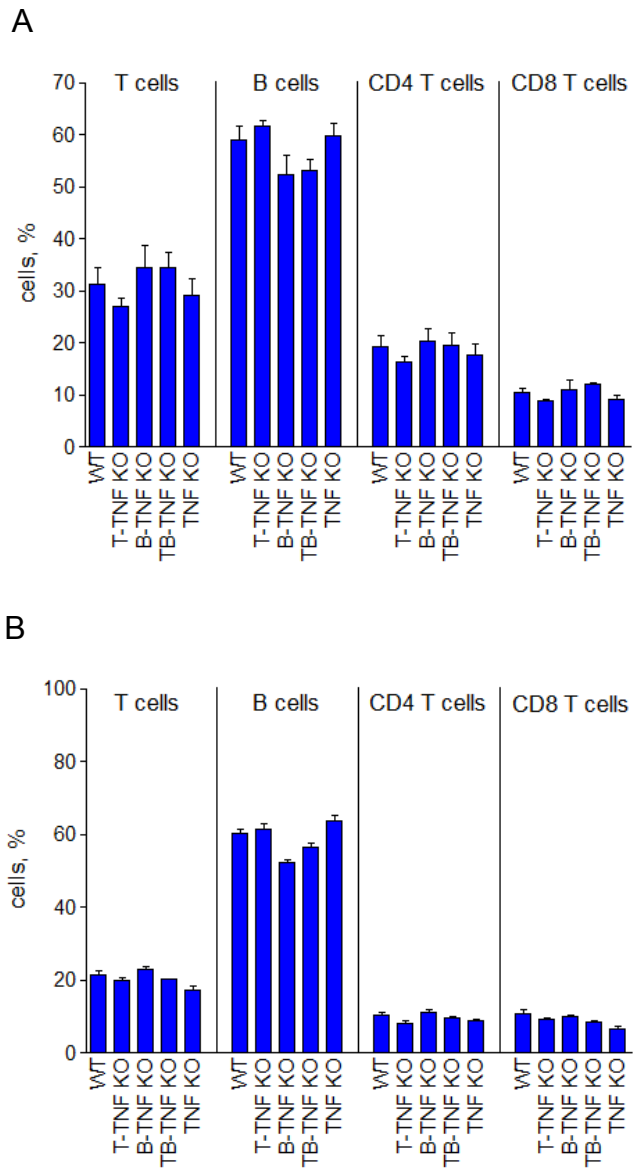


Figure S1. Similar numbers of T and B cells in spleen from T- and B- cell specific TNF deficient mice. Spleens were isolated from either SRBC-immunized (**A**) or naive (**B**) mice, single cell suspensions were prepared using cell strainer, stained with anti-CD19, anti-CD4, anti-CD8 antibodies and analyzed by flow cytometry.

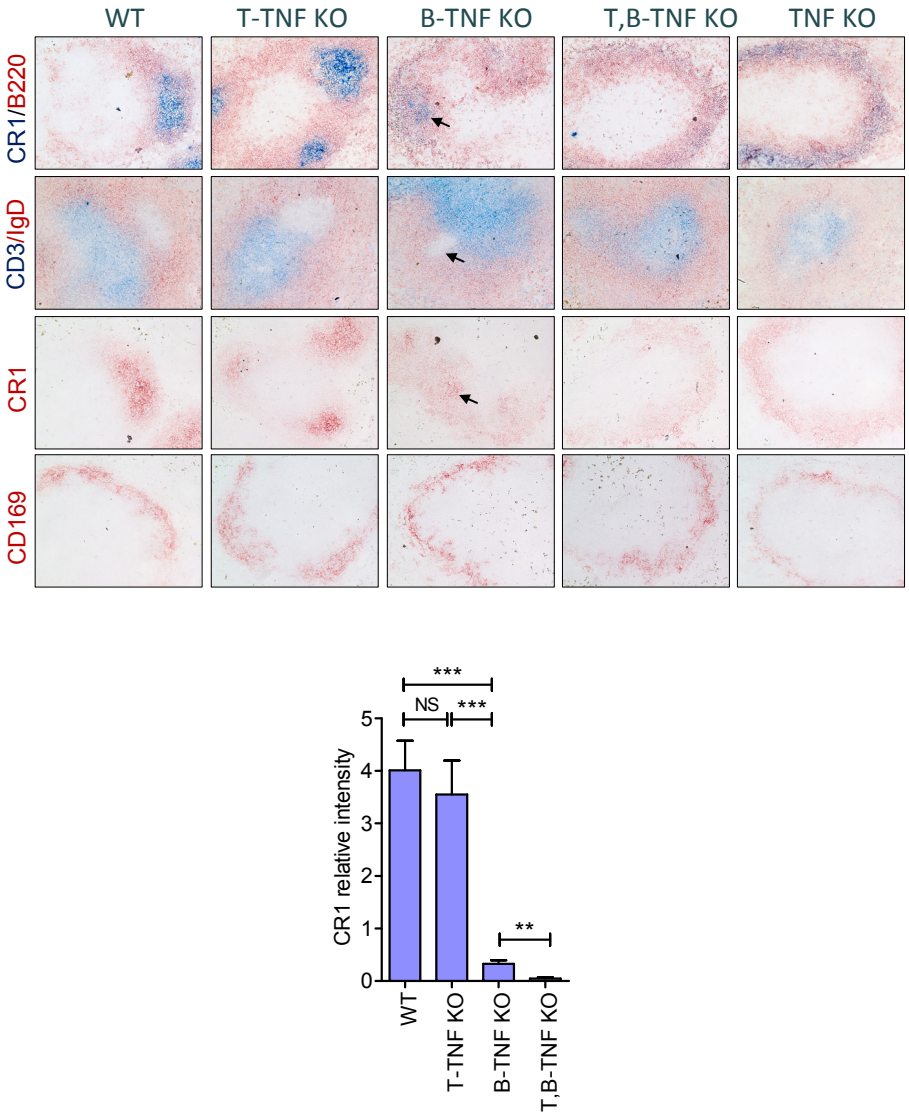


Figure S2. Generation of primary B cell follicles and FDC in *naïve* spleen is primarily controlled by TNF produced by B cells with distinct contribution from TNF produced by T cells. Frozen spleen sections from *naïve* conditional TNF deficient mice were stained with indicated antibodies. B-TNF KO mice lack polarized B cell follicles, and organized FDC networks. Note that some FDC and IgD negative areas are present in the spleens of B-TNF KO mice (shown by arrows), while no FDC and no polarized B cell follicles are observed in spleen of T,B-TNF KO mice. Original magnification is x200. Representative images from one of two independent experiments (n=5 mice per group) are shown. Bottom panel shows quantification of CR1 positive areas. Data represent means ± s.e.m. $^{**}P < 0.01$, $^{***}P < 0.001$.

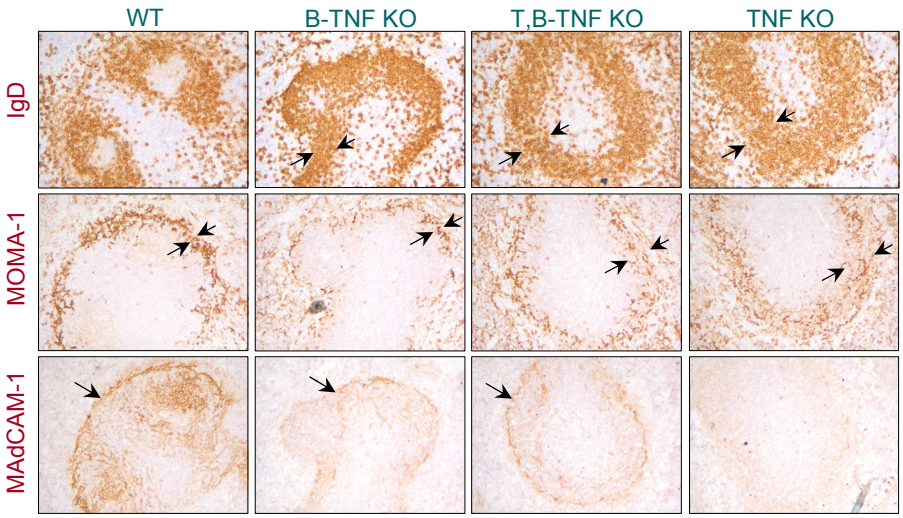


Figure S3. Marginal zone organization in *naïve* spleen is controlled by TNF from B cells with additional contribution of TNF signals from T and non- T,B- cells. Frozen spleen sections from naïve conditional TNF deficient mice were stained with anti-IgD, anti-MOMA-1, and anti-MAdCAM-1 antibodies. Note the extension of marginal zone size, disorganized layer of marginal zone macrophages and marginal sinus in B-TNF, T,B-TNF KO, and TNF KO mice compared to WT mice (shown by arrows). Original magnification is x200. Representative images from one of three independent experiments (n=5 mice per group) are shown.

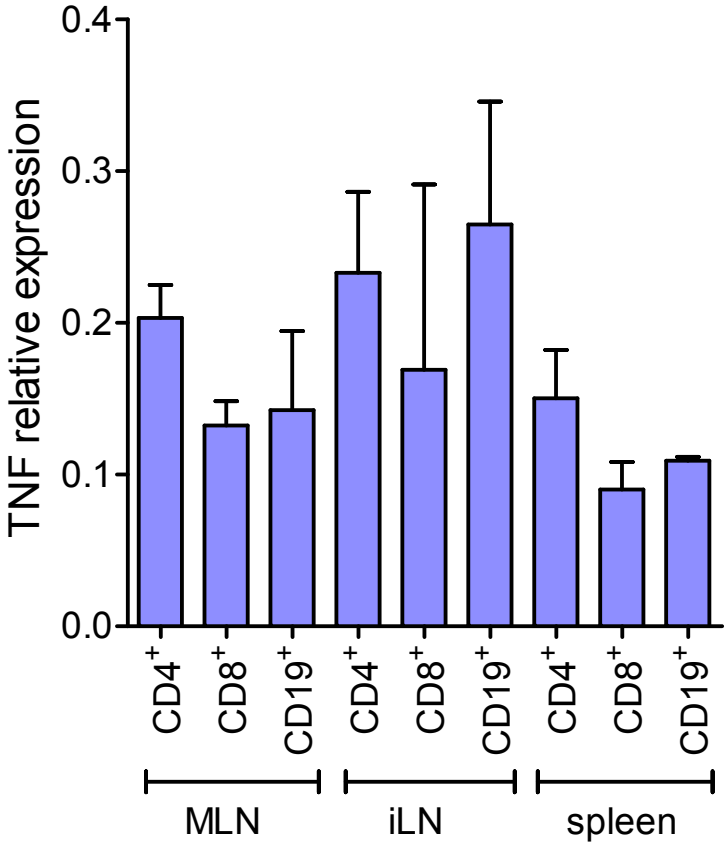


Figure S4. TNF expression in T and B cells from spleen and LN in C57Bl/6 mice. Similar TNF expression levels in purified T and B cells from spleen compared to mesenteric LN (MLN) and inguinal LN (iLN). Spleens and LN were pooled from 3 animals, cells were purified from WT mice using positive selection magnetic bead kit from Stem Cell Technologies, according to the manufacturer's protocol, and TNF expression in CD4+, CD8+, and CD19+ cells measured by real-time PCR with SYBR Green. 2 independetly sorted samples were analyzed. Data represent mean values. The data were normalized to HPRT expression. One of two independent experiments with similar results is shown.

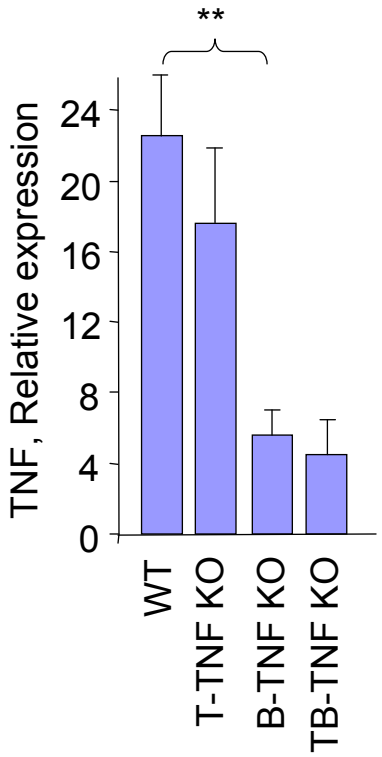


Figure S5. TNF expression in spleen in various cell-specific TNF deficient mice. . RNA was isolated from total spleen, and TNF expression measured by real-time PCR (represents one of two independent experiments, three mice per group). The data were normalized to GAPDH expression. ***P* < 0.01.

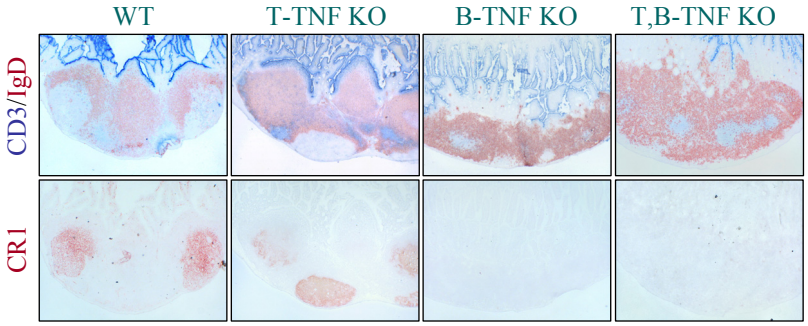


Figure S6. TNF produced by B cells is critical for organization of PP. Mice were immunized i.p. with 108 SRBC and PP sections analyzed on day 8. Frozen sections of PP were stained with antibodies: anti-IgD (red)/anti-CD3 (blue) and anti-CR1 (red). B-TNF KO mice lack FDC and B cell follicles while T-TNF KO mice show normal PP structure. Original magnification is x100. Representative images from one of two independent experiments (n=3 mice per group) are shown.

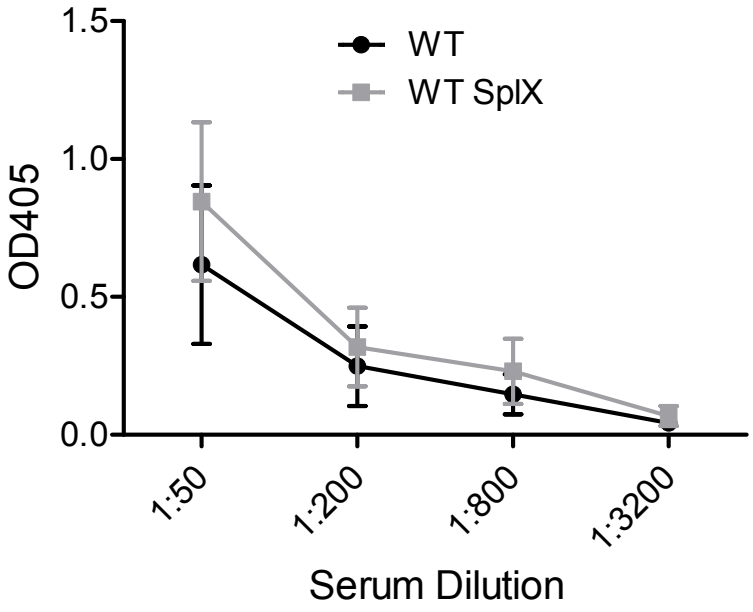


Figure S7. Splenectomized mice are able to generate IgG response after i.p. immunization. Splenectomized mice and control WT mice were immunized i.p. with 108 SRBC and specific IgG response measured at day 21 by ELISA. n=5 mice per group.

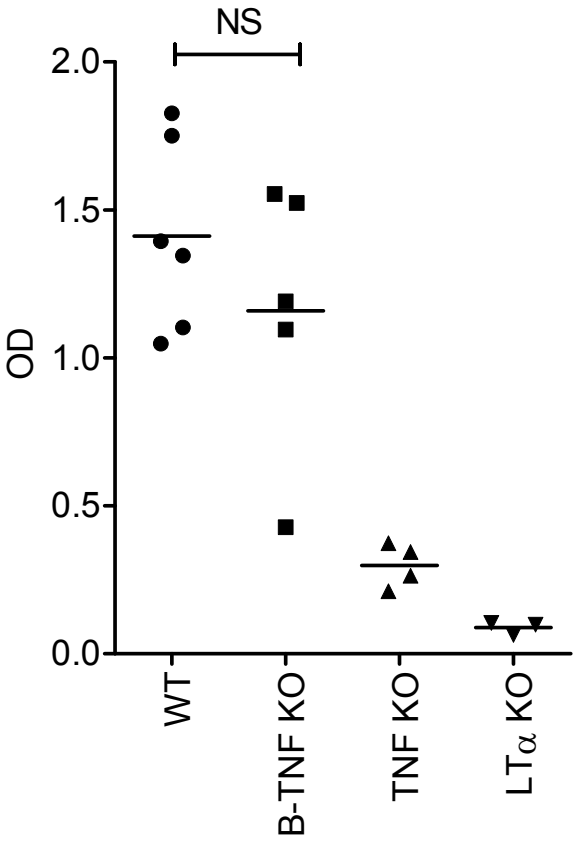


Figure S8. Generation of memory response to SRBC is not impaired in B-TNF KO mice. Mice were re-challenged two months after primary immunization

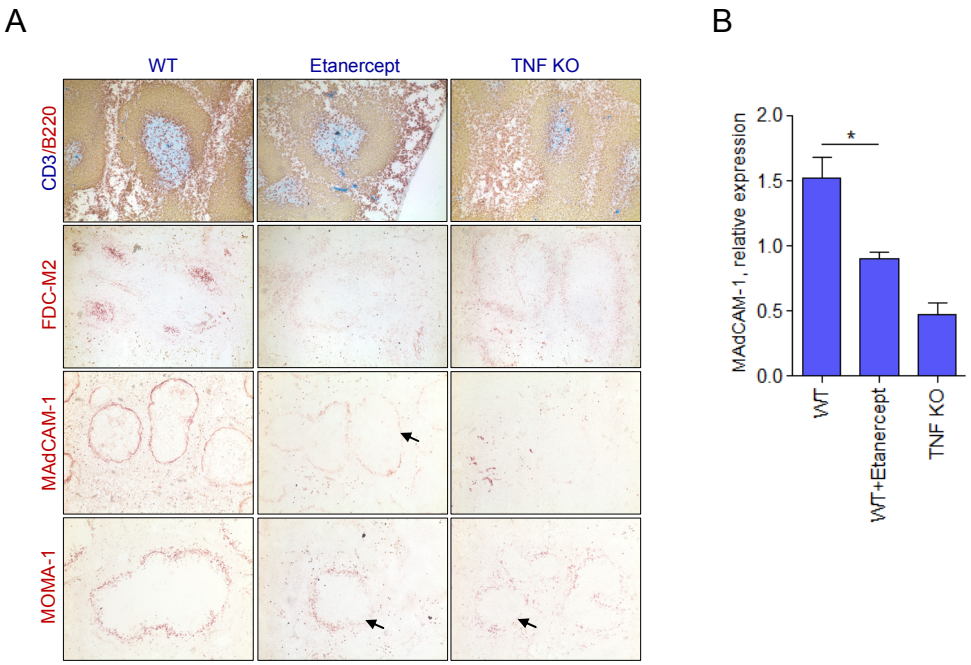


Figure S9. Administration of TNF blocker disrupts lymphoid microarchitecture. WT mice were injected i.p with Etanercept (p75TNFR-Ig, 30mg/kg) at day 0, 3 and 6, and spleen was analyzed by immunohistochemistry at day 7. Representative images from one of two independent experiments (n=5 mice per group) with similar results are shown. Original magnification is x200. Note that B cell follicles were disorganized after treatment and FDC were severely reduced. Interestingly, the staining for marginal zone specific markers MAdCAM-1 and MOMA-1 was also reduced after Etanercept administration, suggesting that the integrity of the marginal zone can be affected after anti-TNF therapy. **B.** Expression of MAdCAM-1 in spleen of indicated mice. n=5 mice per group. Data represent means ± s.d. *$P < 0.05$.

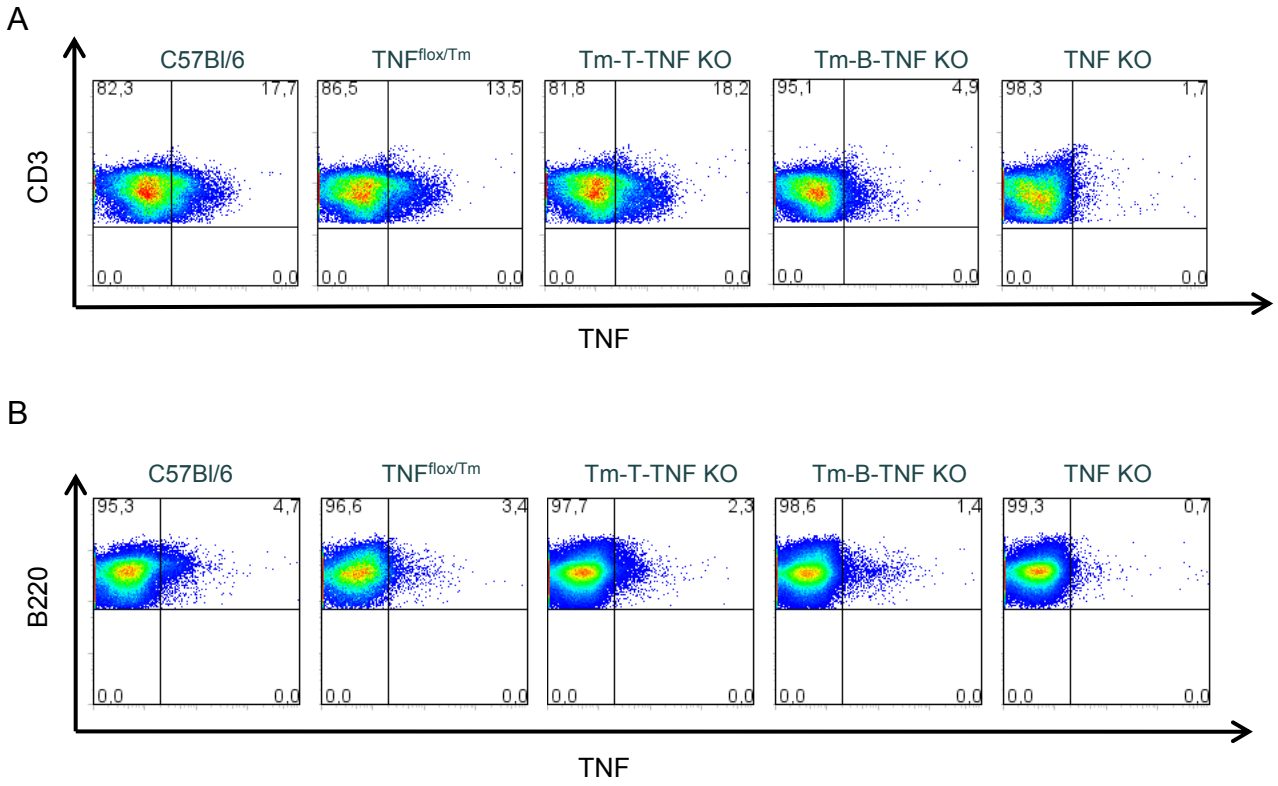
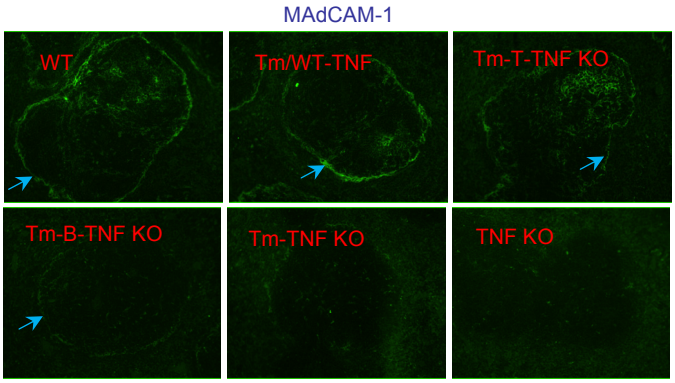


Figure S10. TNF production by T and B cells in conditional TNF mutant mice. T and B cells from Tm-T-TNF KO and Tm-B-TNF KO mice retain the capacity to produce TNF. Splenocytes from indicated mutant mice were stimulated with PMA/ionomycin in the presence of brefeldin A for 4h, and TNF expression by CD3+ and B220+ cells measured by flow cytometry. One of two experiments is shown. N=2 mice per group. Representative plots are shown.

A



B

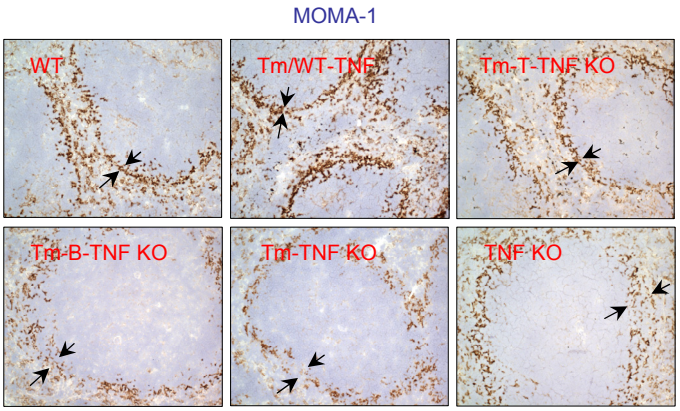


Figure S11. Soluble TNF produced by B cells is essential for organization of the marginal zone. A. Immunofluorescence staining of frozen spleen sections of indicated mutant mice with anti-MAdCAM-1 antibody. **B.** Immunostaining of frozen spleen sections with anti-MOMA-1 antibody. Note gradual reduction of MAdCAM-1 and MOMA expression in Tm-B-TNF KO, Tm-TNF KO and TNF KO mice (shown by arrows). Original magnification is x200. Representative images from one of two independent experiments (n=2 mice per group) are shown.

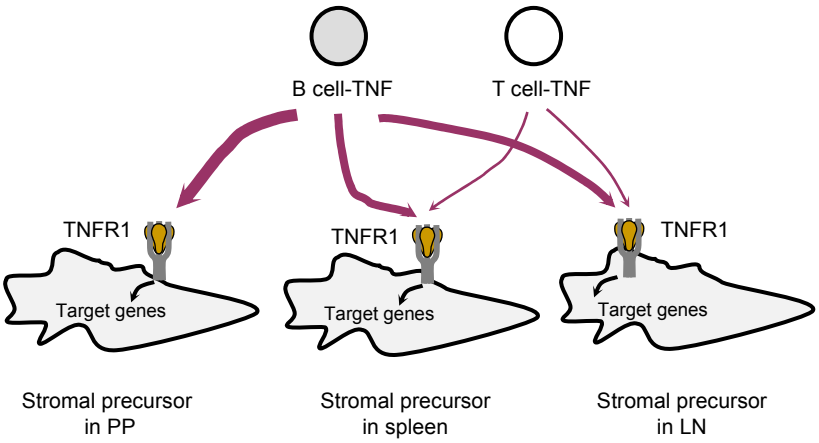


Figure S12. Distinct requirements for cell-type specific TNF production in the organization of different secondary lymphoid tissues. Generation of B cell follicles and FDC in spleen and mesenteric LN is predominantly regulated by soluble TNF from B cells (thick arrow), with a distinct contribution from TNF-expressing T cells (thin arrow). In contrast, PP structure is controlled predominantly by B cell- derived TNF (thick arrow). Stimulation of TNFR1 on stromal cell precursors triggers expression of TNF-dependent target genes required for generation of follicular dendritic cells and B cells follicles.

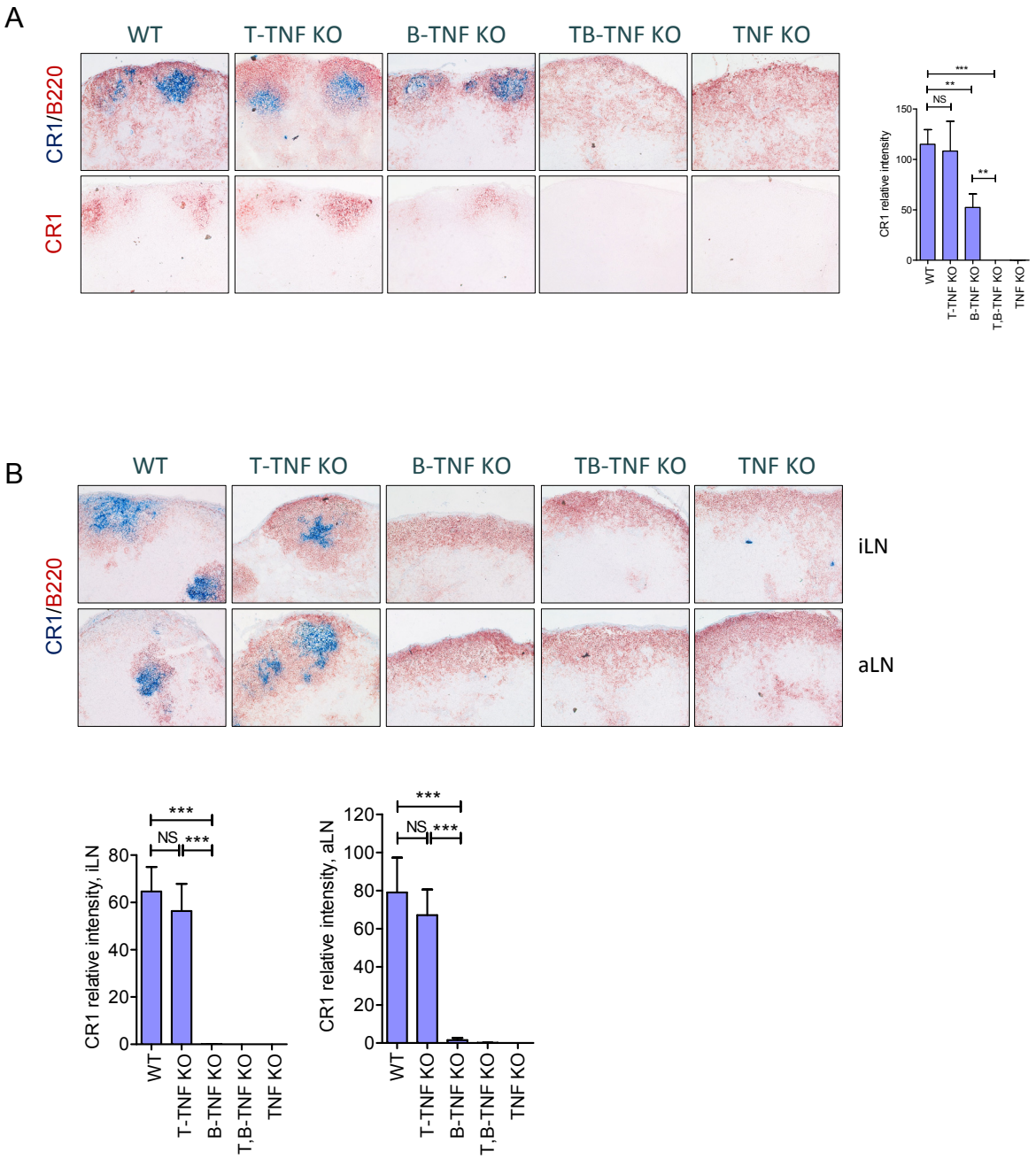


Figure S13. Organization of *naïve* peripheral LNs is primarily dependent on TNF from B cells. Mesenteric LN (**A**) and peripheral LN (**B**) from naïve conditional TNF deficient mice were stained with anti-B220 and anti-CR1 to visualize B cells follicles and FDC, respectively. Representative images from one of two independent experiments (n=3 mice per group) are shown. Original magnification is x200. Quantification of CR1 positive areas is presented as means ± s.e.m. $^{**}P < 0.01$, $^{***}P < 0.001$.