

Table S1. Antibodies and reagents used for flow cytometry

Antigen	Conjugate	Isotype	Clone and source
B220	PerCP	Rat IgG _{2a} κ	RA3-6B2, BD Pharmingen™
CD19	Bv421	Rat IgG _{2a} κ	6D5, BioLegend
CD19	PeCy7	Rat IgG _{2a} κ	1D3, BD Pharmingen™
Flt3	Biotin	Rat IgG _{2a} κ	A2F10, BioLegend
Flt3	PE	Rat IgG _{2a} κ	A2F10.1, BD Pharmingen™
IgM	FITC	Rat IgG _{2a} κ	R6-60.2, BD Pharmingen™
IgD	Percp-e710	Rat IgG _{2a} κ	11-26c, eBioscience
IgG1	Fite	Rat IgG ₁ κ	A85-1, BD Pharmingen™
IL-4R	Biotin	Rat IgG _{2a} κ	mIL4R-M1, BD Pharmingen™
IL-7R	APC	Rat IgG _{2a} κ	A7R34, BioLegend
CD3	V500	Syr. Ham IgG ₂ κ	500A2, BD Horizon™
CD3	PerCp	Ar Ham IgG ₁ κ	145-2C11, BD Pharmingen™
CD4	PB	Rat IgG _{2a} κ	RM4-5, BD Pharmingen™
ICOS	PE	Syr Hams IgG	15F9, BioLegend
CD86	PerCp	Rat IgG _{2a} κ	GL-1, BioLegend
CD80	APC	Ar Hams IgG ₂ κ	16-10A1, BD Pharmingen™
CD40	Percp710	Rat IgG _{2a} κ	1C10, eBioscience
MHCII (I-a/I-e)	APCe780	Rat IgG _{2b} κ	M5/114.15.2, eBioscience
CD138	APC	Rat IgG _{2a} κ	281-2, BD Pharmingen™
CD93	PeCy7	Rat IgG _{2b} κ	AA4.1, eBioscience
GL7	AF647	Rat IgM κ	GL7, BD Pharmingen
Stat6 (pY641)	AF647	Ms IgG ₁ κ	J71-773.58.11, BD Phosflow™
CXCR5	APC	Rat IgG _{2a}	2G8, BD Pharmingen™
Bcl6	AF647	Mouse IgG ₁	K112-91, BD Pharmingen™
CD16/CD32 FC-block		Rat IgG _{2b}	2.4G2, BD Pharmingen™
Streptavidin	PE, APC		BD Pharmingen™
Fixable Viability Dye (FVD)	eFlour™ 506		eBioscience
CellTrace Violet™	eFlour™ 780		
BD Phosflow Perm buffer IV, 10x	405/450		Molecular Probes, invitrogen
BD CytoFix			BD Phosflow™

Table S2. Antibodies and substrates used for Immunohistochemistry

Target	Conjugate	Isotype	Product number and Source
Fc-Block (CD16/32)		Rat IgG _{2a}	553142, BD Pharmingen™
Bcl-6	Alexa Fluor® 647	Mouse IgG ₁	561525, BD Pharmingen™
B220		Rat IgG _{2a}	Ab64100, Abcam
Flt3		Rat IgG ₂	ab73019, Abcam
GL7	Alexa Fluor® 647	Rat LOU	561529, BD Pharmingen™
CXCR4	Biotin	Rat IgG _{2b}	551968, BD Pharmingen™
Streptavidin	Alexa Fluor® 555		S 21381, Molecular Probes™
Anti-Rat	Alexa Fluor® 488	Goat IgG	A11006, Thermo Fisher Scientific
Anti-rabbit	Biotin	Swine F(ab') ₂	E0431, DAKO
ExtraVidin	Alkaline Phosphatase		E2636, Sigma
IgM	Biotin	Goat F(ab') ₂	115-066-075, Jackson Immunoresearch
IgG1	Biotin	Goat F(ab') ₂	1072-08, SouthernBiotech
VECTASTAIN ABC Kit		Goat IgG	PK-6105, Vector Laboratories
ImmPACT™ AEC Peroxidase Kit			SK-4205, Vector Laboratories
NucBlue™ Live Cell Stain	Hoechst 33342		R37605, Molecular Probes™

Table S3. Antibodies used for immunoglobulin ELISA

Target	ELISA	Conjugate	Isotype	Product number and Source
IgM	Total, mBSA	Biotin	Goat F(ab') ₂	115-066-075 Jackson Immunoresearch
IgG		Biotin	Goat F(ab') ₂	115-066-071, Jackson Immunoresearch
IgG1	mBSA	Biotin	Goat (γ1 chain spec)	1070-08, SouthernBiotech
IgG1	Total	Biotin	Goat (γ1 chain spec)	1072-08, SouthernBiotech
IgG2b	mBSA	Biotin	Goat (γ2β chain spec)	1090-08, SouthernBiotech
IgG2b	Total	Biotin	Goat F(ab') ₂ (γ2β chain spec)	1092-08, SouthernBiotech
IgG2c	Total, mBSA	Biotin	Goat (γ2α chain spec)	1179-08, SouthernBiotech
IgG3	mBSA	Biotin	Goat (γ3 chain spec)	1100-08, SouthernBiotech
IgG3	Total	Biotin	Goat F(ab') ₂ (γ3 chain spec)	1102-08, SouthernBiotech
Mouse Igs ExtrAvidin	Total, mBSA	Peroxidase	Goat F(ab') ₂	55491, MP cappel E2886, Sigma

Table S4. Primers used for qPCR

Target	Forward Primer	Reverse Primer	Manufacture
Pax5	TACTCTGCACCGACGCTGAC	GAAGAATACTGAGGGTGGCTGT	Sigma-Aldrich
Bcl6	CTTCCGCTACAAGGGCAAC	CGAGTGTGGGTCTTCAGGTT	Sigma-Aldrich
XBP-1	GAGCAGCAAGTGGTGGATTT	GCGTGTCTTAACTCCTGGTTC	Sigma-Aldrich
IRF4	GGCCCAACAAGCTAGAAAGA	CACCAAAGCACAGAGTCACC	Sigma-Aldrich
Prdma1 (Blimp1)	TCAAGTATGCTGCCAACAACA	GGCATTCTTGGGAAGTGTGT	Sigma-Aldrich
IL-21	ACATTCATCATTGACCTCGTG	GAATCACAGGAAGGGCATT	Sigma-Aldrich
IL-4	CAGAGACTCTTTCGGGCTTT	TGATGCTCTTTAGGCTTTCCA	Sigma-Aldrich
IL-4R	CACTACAGGCTGATGTTCTTCG	CGGCCTATTCATTTCATGT	Sigma-Aldrich
IL-7R	TGGAAGTGATGGAAGTCAAC	TTGCAGCTTGTTAAGAGTTAGGC	Sigma-Aldrich
Common γ -chain	TGTTGGTTGGAACGAATGC	TCAGCCCTTTAGACACACCAC	Sigma-Aldrich
Stat6	AGGAGCCTCACCTGCAAAT	CACAGCATGTTCTGGGACT	Sigma-Aldrich
GAPDH	Available from manufacturer	Available from manufacturer	TATAA Biocenter
γ 1	TCGAGAAGCCTGAGGAATGT	ATAGACAGATGGGGTGTTCG	Sigma-Aldrich
Iyb	GCTCAGAGACAGAGCAGTGACC	AGATCTGGGAACAAGGGCTTC	Sigma-Aldrich
γ 2b	CCAACCAGGAAGAGTCCAGAG	ACAGGGATCCAGAGTTCCAAGT	Sigma-Aldrich
γ 2a	CTGGCAGTACCGATGCAGC	GCCAGTTGTACCTCCACACACAG	Sigma-Aldrich
γ 3	AGAGTCAGCCTCAAGGAGATGAT	CAGGGACCAAGGGATAGACAG	Sigma-Aldrich
Stat3	AGGAGGAGGCATTTGGAAAG	GAACTTGGTCTTCAGGTACGG	Sigma-Aldrich
Stat6	AGGAGCCTCACCTGCAAAT	CACAGCATGTTCTGGGACT	Sigma-Aldrich
IRF8	CAATCAGGAGGTGGATGCTT	AGCACAGCGTAACCTCGTCT	Sigma-Aldrich
AID	GATAGTGCCACCTCCTGCTC	GGTCCCAGTCTGAGATGTAGC	Sigma-Aldrich

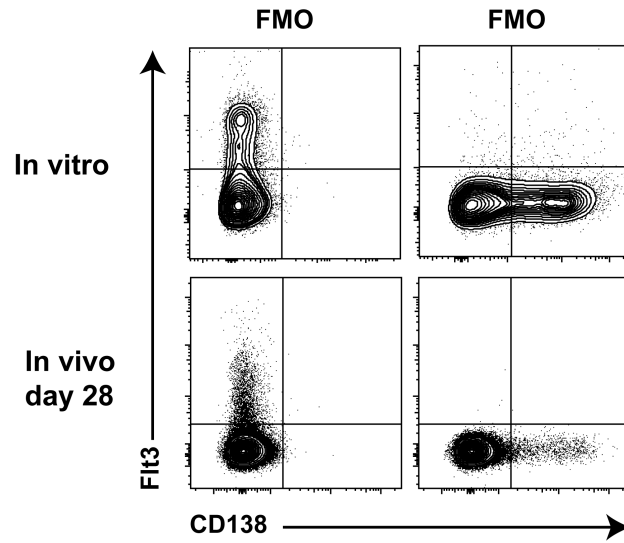


Figure S1. Controls staining used for gating Flt3 and CD138. FMO (fluorochrome minus one control) staining used for gating Flt3 and CD138 on B-cells after *in vitro* activation with LPS and *in vivo* at day 28 after mBSA immunization.

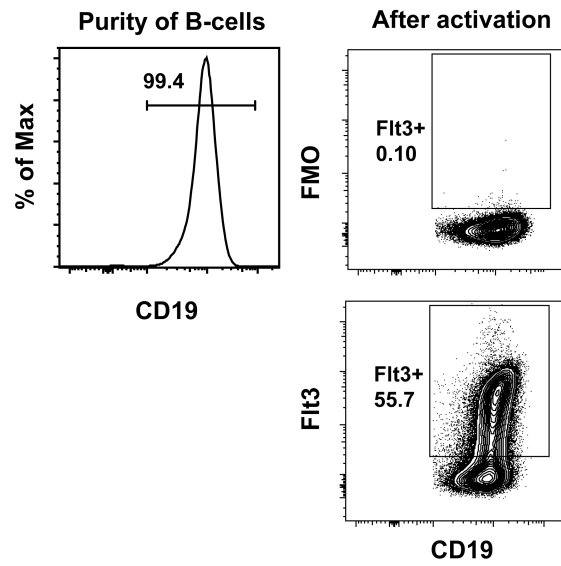


Figure S2. Purity and expression of Flt3 on LPS stimulated B-cell. Purity of spleen CD19+ B cells isolated by EasySep Mouse B cell Isolation Kit (Stemcell) and surface expression of Flt3 on LPS activated B cells prior to FL stimulation *in vitro*. FMO, fluorochrome minus one staining control for Flt3.

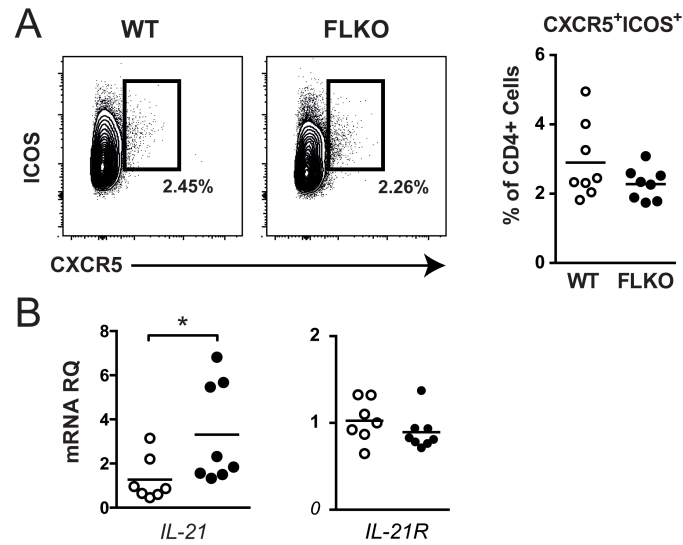


Figure S3. Follicular T-cells in FLKO and WT mice. A) No difference in the frequency of splenic T_{FH} (defined as CD4⁺ICOS⁺CXCR5⁺) was observed between WT and FLKO mice after mBSA immunization. **B)** FLKO mice have increased expression of IL-21 compared to WT mice in the spleen after mBSA immunization. Splenic expression of the IL-21R was similar between FLKO and WT mBSA immunized mice. * P < 0.05. Statistic analysis was performed using unpaired Student's t-test.

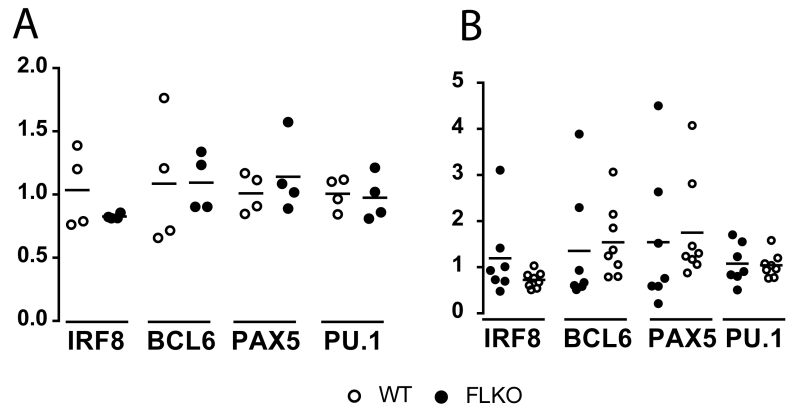


Figure S4. Expression of genes associated with terminal B-cell differentiation in the spleen of FLKO and WT mBSA immunized mice. Splenic gene expression of *IRF8*, *Bcl6*, *Pax5* and *Pu1* at day 14 (A) and day 28 (B) in FLKO and WT mice after mBSA immunization.

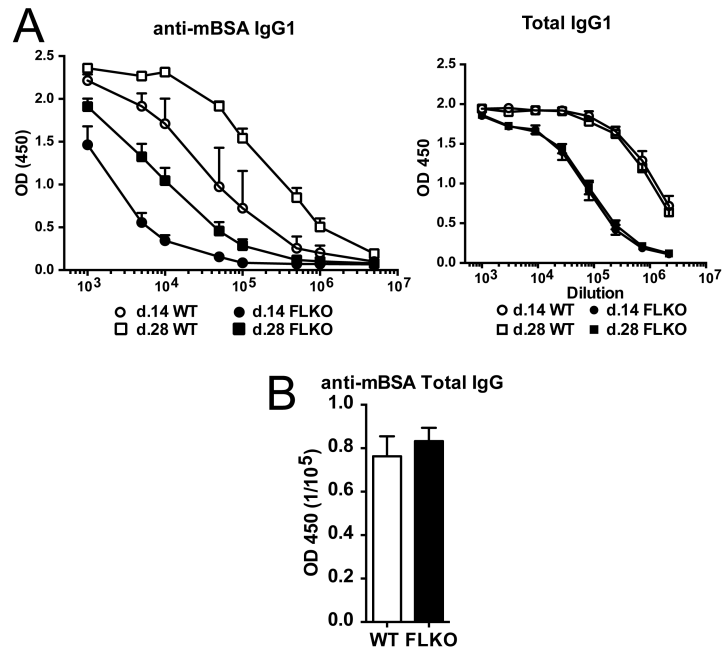


Figure S5. Serum dilution curves for measurement of anti-mBSA and total IgG1 and levels of total anti-mBSA IgG . A) Dilution curves used to determine the serum levels of anti-mBSA and total IgG1 at day 14 and day 28 after mBSA immunization. **B)** No differences in the serum level of total IgG directed against mBSA was found between FLKO and WT mice at day 28 (WT n = 15, FLKO n = 16)

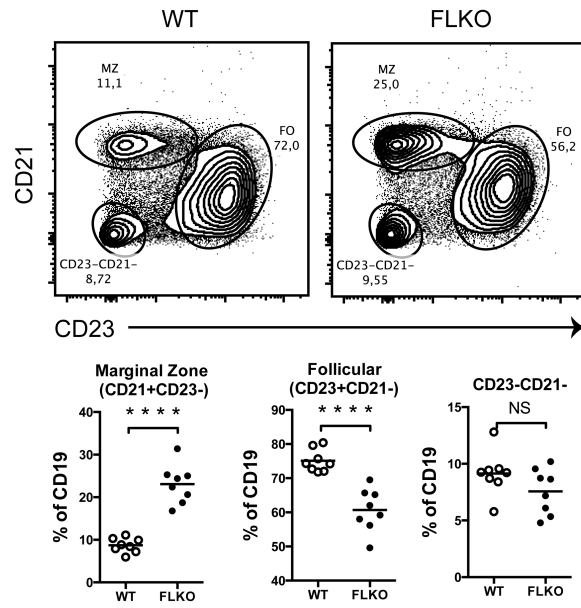


Figure S6. Marginal Zone and follicular B-cells in FLKO and WT mice. FLKO mice have an altered distribution of marginal zone (defined as CD21+CD23-) and follicular B-cells (defined as CD21-CD23+) compared to WT mice. Cells were gated on total CD19+ cells and analyzed in the spleen at day 28 after mBSA immunization.

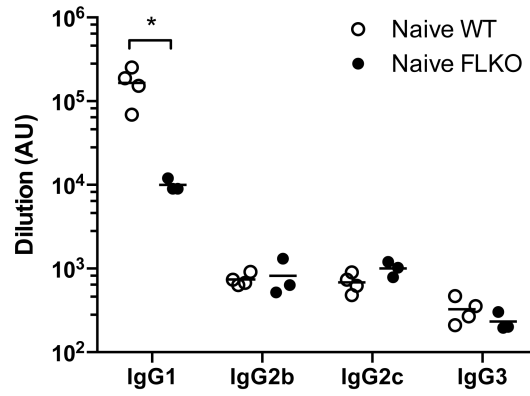


Figure S7. Total levels of IgG subclasses in naïve WT and FLKO mice. Measurement of total levels of IgG1, IgG2b, IgG2c and IgG3 in the serum of naïve and FLKO mice revealed a specific reduction in IgG1 also at baseline, whereas other antibody subclasses were produced at similar levels.

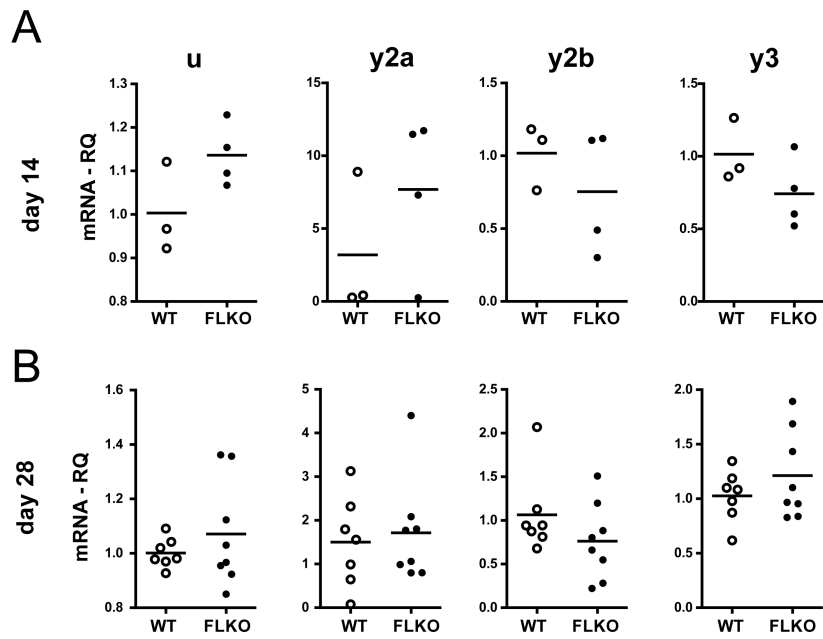


Figure S8. Expression of GLT in the spleen of FLKO and WT mice after mBSA immunization. Expression of GLT for IgM (μ), IgG2b (γ 2b), IgG2c (γ 2a) and IgG3 (γ 3) was evaluated in the spleen of mBSA immunized FLKO and WT mice at day 14 **(A)** and day 28 **(B)**

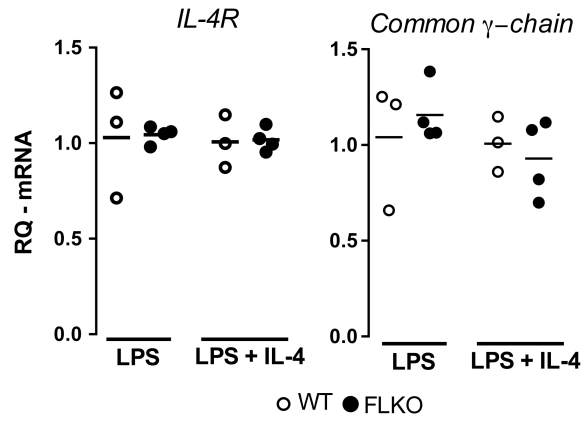


Figure S9. Gene expression of the IL-4R and common γ -chain in FLKO and WT splenocytes after *in vitro* stimulation. Gene-expression of the IL-4R and common γ -chain chain was detected in FLKO and WT splenocytes after *in vitro* stimulation with LPS and LPS+IL-4 for 72h.