

Supplemental Table 1: Antibodies

Target	label	Supplier	clone	Assay
mouse CD19	APC	Biolegend	6D5	FC
mouse CD19	PECy7	Biolegend	6D5	FC
mouse CD19	BV510	Biolegend	6D5	FC
mouse/human B220	Pacific Orange	Thermo fisher	RA-6B2	FC
mouse/human B220	PE	Biolegend	RA-6B2	FC
mouse/human B220	BV421	Biolegend	RA-6B2	FC
mouse CD5	FITC	Biolegend	53-7.3	FC
mouse IgM	eF450	eBioscience	II/41	FC
mouse IgM	FITC	eBioscience	RMM1	FC
mouse IgD	APCCy7	Biolegend	11-26c-2a	FC
mouse IgD	FITC	Biolegend	11-26c-2a	FC
mouse CD21	PE	Biolegend	7E9	FC
mouse CD21	FITC	Biolegend	7E9	FC
mouse CD21	PECy7	Biolegend	7E9	FC
mouse CD23	FITC	Biolegend	B3B4	FC
mouse CD23	PECy7	Biolegend	B3B4	FC
mouse CD24	PerCPCy5.5	Biolegend	M1/69	FC
mouseCD24	APC	Biolegend	M1/69	FC
mouse CD38	PECy7	Biolegend	90	FC
mouse CD43	PECy7	Biolegend	S11	FC
mouse CD69	PE	Biolegend	H1.2F3	FC
mouse CD95	PE	Biolegend	SA367H8	FC
mouse CD138	PECy7	Biolegend	281-2	FC
mouse TACI	PE	Biolegend	8F10	FC
mouse CXCR4	BV421	BD	2B11	FC
mouse CXCR5	PE	Biolegend	L138D7	FC
Mouse/human CD44	BV650	Biolegend	IM7	FC
Mouse/human CD44	APC	Biolegend	IM7	FC
mouse CD49d	PE	BD	9C10	FC
mouse CD29	PE	BD	HMBeta1-1	FC
mouse CD11a	BV421	BD	M17/4	FC
mouse CD18	BV421	BD	M18/2	FC
mouse CD40	APC	Biolegend	3/23	FC
pAkt(pS473)	PE	BD	M89-61	FC
mouse B220/CD45R	unlabeled	BD	RA3-6B2	IF
mouse laminin	unlabeled	Novus	Rabbit polyclonal	IF
Ki67	unlabeled	R&D systems	Sheep polyclonal	IF
mouse CD19	unlabeled	Cell signaling	D4V4B	IHC
mouse/human Kindlin3	unlabeled	Abcam	Rabbit polyclonal	WB
Gamma-tubulin	unlabeled	Cell signaling	Rabbit polyclonal	WB

mouse IgM	unlabeled	Jackson ImmunoResearch	Polyclonal goat F(ab)2	stim
mouse kappa	unlabeled	Southern Biotech	Goat polyclonal	stim
mouse IgD	unlabeled	Bio-Rad	LO-MD-6	stim
Moma-1	Biotin	Abcam	MOMA 1	IF
mouse IgM	Alexa 647	Southern Biotech	Goat polyclonal	IF
mouse/human GL7	unlabeled	Biolegend	GL7	IF
mouse IgG	Biotin	Kindly provided by Palash Maity (home digested and biotinylated)	polyclonal Fab2	IF
PNA	Cy3	Vector Lab	Lectin	IF

FC = Flow cytometry; IF = immunofluorescence; IHC = Immunohistochemistry; WB = western blot; stim = stimulation.

Supplemental methods

Immunohistochemistry and -histology

Spleen pieces were fixed in 4% paraformaldehyde, paraffin embedded, cut into 4 μm sections and stained with Masson-Goldner stain. The antigen retrieval for the CD 19 staining consisted of incubation in citrate buffer (pH 6) at 97°C for 20 minutes. CD 19 IHC was performed using the monoclonal CD19 antibody (Clone D4V4B, XP Rabbit, #90176, Cell Signaling, UK) at a 1:800 dilution, with an incubation time of 30 minutes at room temperature, followed by incubation with the Bright Vision Goat anti Rabbit HRP as secondary antibody (incubation 30 minutes at RT). DAB Quanto (ThermoScientific #TA-125-QHDX) was used as chromogen.

Supplemental figure legends

Supplemental Figure 1: Absolute B cell numbers in the BM of K3 Δ B and CD49d Δ B mice.

CD19 positive and negative fractions were isolated from total splenocytes and B220 positive and negative fractions from the BM of K3fl/fl and K3fl/fl mb1 Cre mice. Kindlin-3 expression was analyzed by western blotting (A). Efficiency of B cell specific CD49d knockout was verified by flow cytometry on cells isolated spleen and BM cells (n= 5 for control and 10 for CD49d KO) (B). An overview of marker expression throughout B cell development is shown in (C). After subsequently gating on B cells (B220 positive) and early B cells (IgM/IgD negative), pre-pro B cells, pro-B cells and pre-B cells are differentiated based on CD43 and CD24 expression as shown. An exemplary FACS plot for differentiating the earliest B cell subsets is shown in (D). BM cells of K3 Δ B and control mice were subjected to a transwell migration assay (5 μ m pore size) for 2h, with the lower well containing medium alone (control) or medium containing CXCL12 (100 ng/ml). Analysis was done by flow cytometry using flow counting beads, B cells subsets were defined by surface staining (B220 and CD19 for B cells, IgM/IgD for the early-immature-mature subsets and CD24/CD43 for differentiating BCR negative cells into Pre-pro-, Pro- and Pre B cells). Dots represent technical replicates, one representative experiment of three is shown (E). Absolute B cell counts were determined using EVE automatic cell counter, and the percentage of B cells among lymphocytes in the BM was determined by flow cytometry (n=14 K3 Δ B and n=9 for CD49d Δ B mice) (F). Groups were compared using unpaired t-test. ns: non-significant; *: P < 0.05; **: P < 0.01 and ***: P < 0.001.

Supplemental Figure 2: Phenotypes in the spleens of K3 Δ B and CD49d Δ B mice. Absolute

B cell counts were determined using EVE automatic cell counter, in combination with lymphocyte percentages determined by flow cytometry. (A). Spleen sections of K3 Δ B mice,

CD49d Δ B mice and control littermates were stained using anti-CD19 IHC for B cell distribution and follicular architecture (**B**), and Masson-Goldner staining for overall architecture (**C**). Dashed lines indicate the borders of B cell follicles, yellow arrows indicate the marginal zone. Groups were compared using unpaired t-test. *: $P < 0.05$.

Supplemental Figure 3: Gating strategy for adoptive transfer using GFP/dTomato Cre reporter mice. B cells were identified by flow cytometry staining as CD19⁺/CD5⁻, T cells as CD19⁻/CD5⁺. Cells without Cre expression expressed dTomato (left row), while cells expressing Cre expressed GFP (middle row). No GFP expression was observed in T cells (right row). An example for the identification of recovered wt and K3 deficient cells after transfer into wt BL6 mice is shown in the lower panel. Absolute numbers were measured using counting beads. The numbers measured were corrected using the percentages of input cells falling into the respective gates that allow discrimination from recipient cells (**A**).

Receptor expression was analyzed by flow cytometry on whole splenocytes of CD49d Δ B mice and control littermates. B cells were defined by CD19 expression (**B**). Groups were compared using unpaired t-test. ***: $P < 0.001$.

Supplemental Figure 4: Overview of differentially expressed genes and gene sets in K3 deficient follicular B cells

The sorting strategy for FO B cells after gating on viable singlets is shown in (**A**). A volcano plot showing the top 25 regulated genes in K3 Δ B vs. control (K3 fl/fl) mice. Color code represents the average intensity across all samples (**B**).

Top 20 up- and down-regulated gene-sets in K3 Δ B vs. control mice. Biocarta (upper panel), KEGG (middle panel) and Hallmark (lower panel) gene-sets were downloaded from MSigDB. Gene-sets are ranked according to their Normalized Enrichment score. Color-code represents the number of leading-edge genes (**C**).

Supplemental Figure 5: Activation markers are increased in K3 KO FO B cells

A relevant selection of activation markers and homing factors from the transcriptome array is displayed including the expression change (LogFC) and p value. A positive logFC indicates increased expression in K3 KO compared to wt FO B cells. (A). Follicular helper T cells (TFH) were defined by co-expression of the markers CD3, CD4 and CXCR5 in K3 Δ B and control mice (n=4) (B). Groups were compared using unpaired t-test.

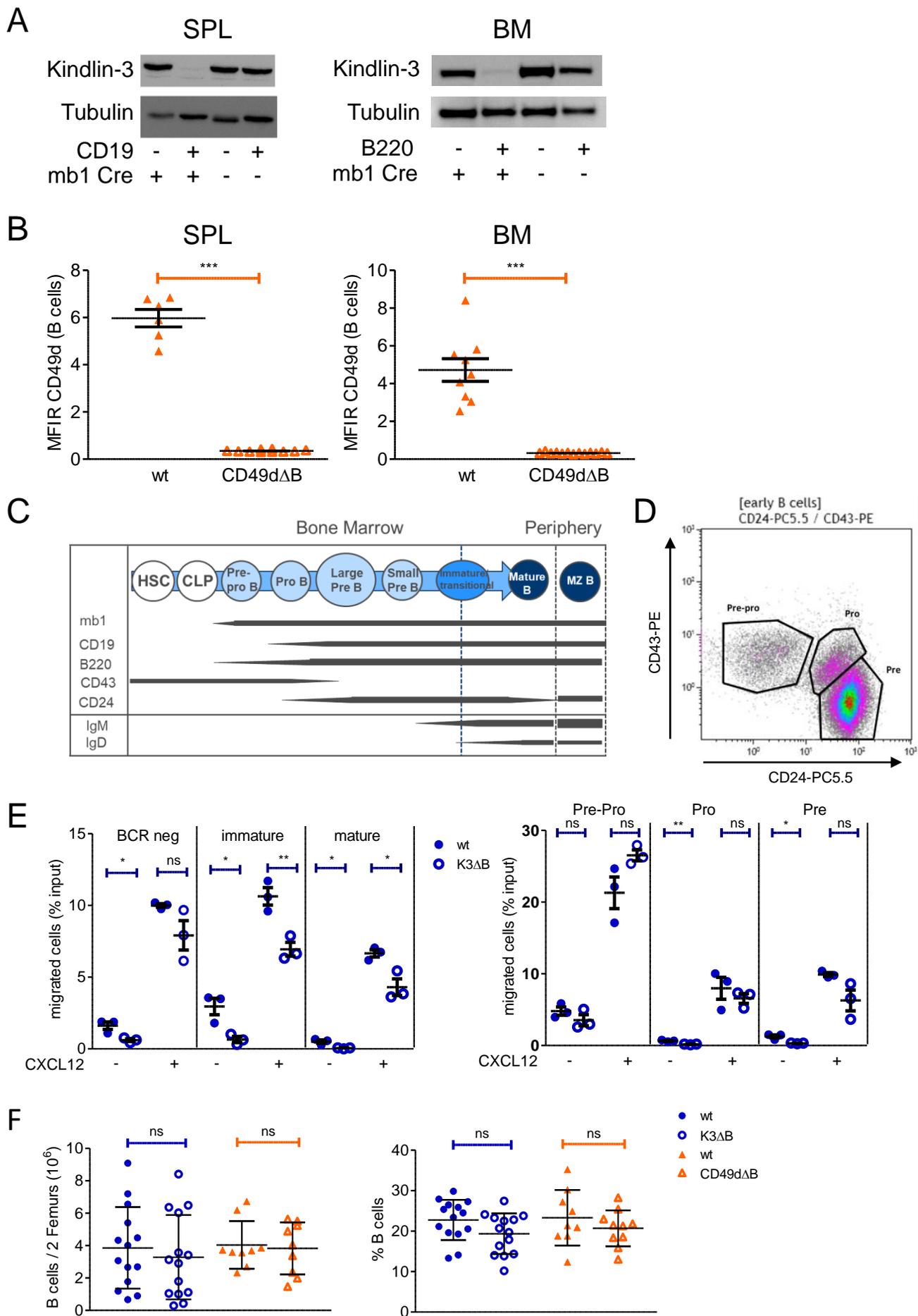
Supplemental Figure 6: Analysis of the plasma cell- and proliferating and germinal center

B cell compartments in K3 Δ B mice. Plasma cells were defined by flow cytometry as shown, gating first on high expression of CD138 and TACI and then further dissecting the three subsets of plasmablasts (PB; B220^{high}/CD19^{low}), early plasma cells (ePCs; B220^{low}/CD19^{med}) and resting plasma cells (rPCs; B220^{low}/CD19^{low}) (A). A quantification of ePCs and rPCs is shown in (B) (n=7). Cryosections of spleens were stained with antibodies against IgM (red) and Ki67 (green) to show proliferating cells. Representative data from 3 pair of K3 Δ B mice and corresponding WT littermate control are shown here. Scale bar, 50 μ m (C). Groups were compared using unpaired t-test.

Supplemental Figure 7: Analysis of chemokine- and BCR signaling in K3 Δ B mice.

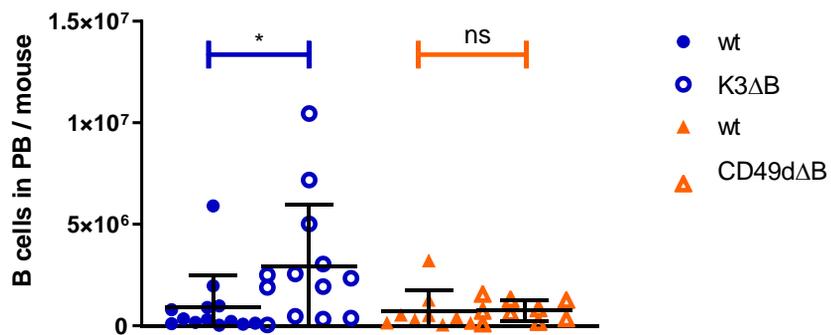
Examples for the quantification of Ca²⁺ mobilization are shown. CXCL13 (500ng/ml) and anti-IgD (10 μ g/ml) were added 60s after start of the measurement as indicated by the arrows (A). Ca²⁺ mobilization response to BCR activation (10 μ g/ml of anti-IgM, anti-kappa or anti-IgD) in wt and K3 deficient FO B cells is shown in (B). Examples of wt and K3KO B cells on collagen substrates with and without CXCL12 and CXCL13 are shown in (C). F-actin was stained using Phalloidin-Alexa488 (green). The lower panel shows an example of a polarized cell (arrow) next to two unpolarized cells. Groups were compared using unpaired t-test.

Supplemental Figure 1

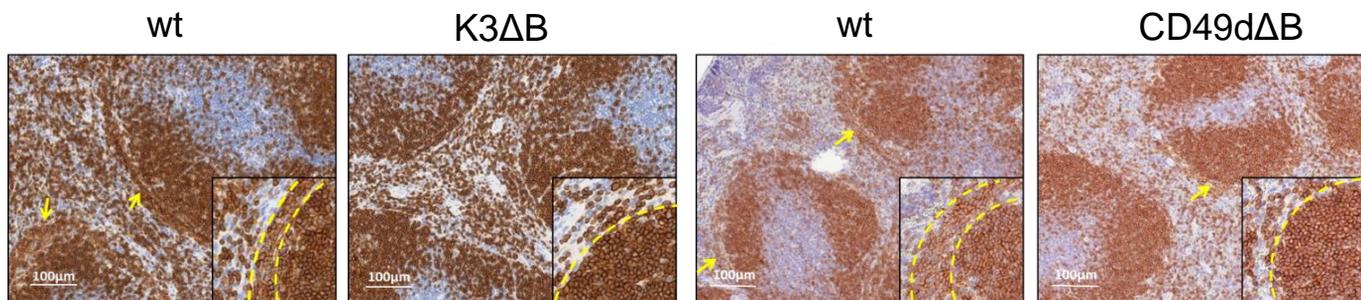


Supplemental Figure 2

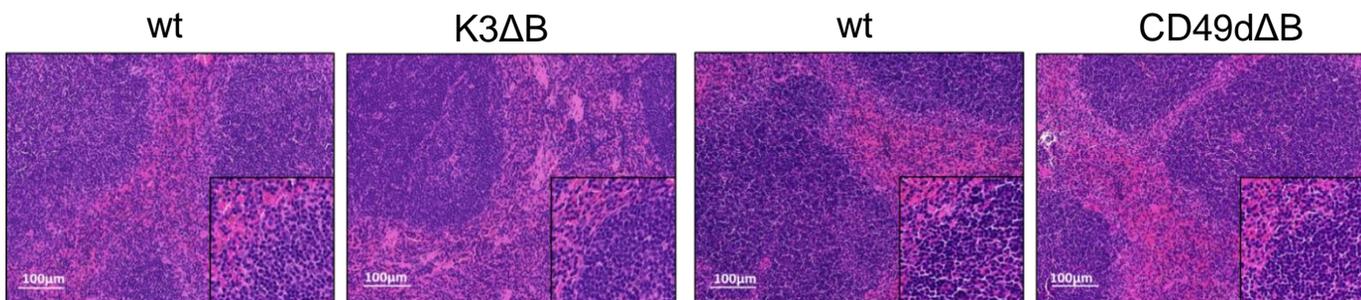
A



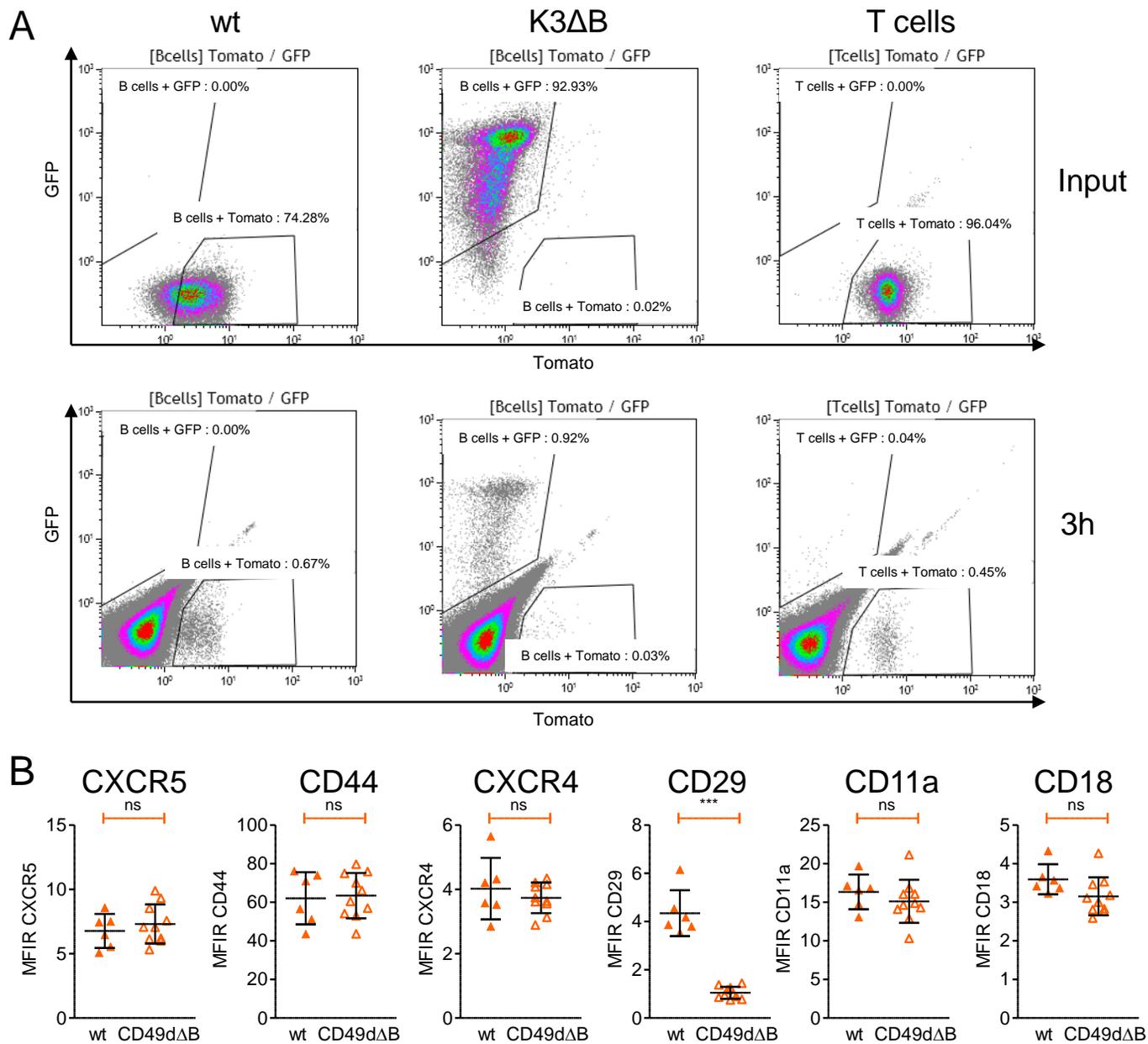
B



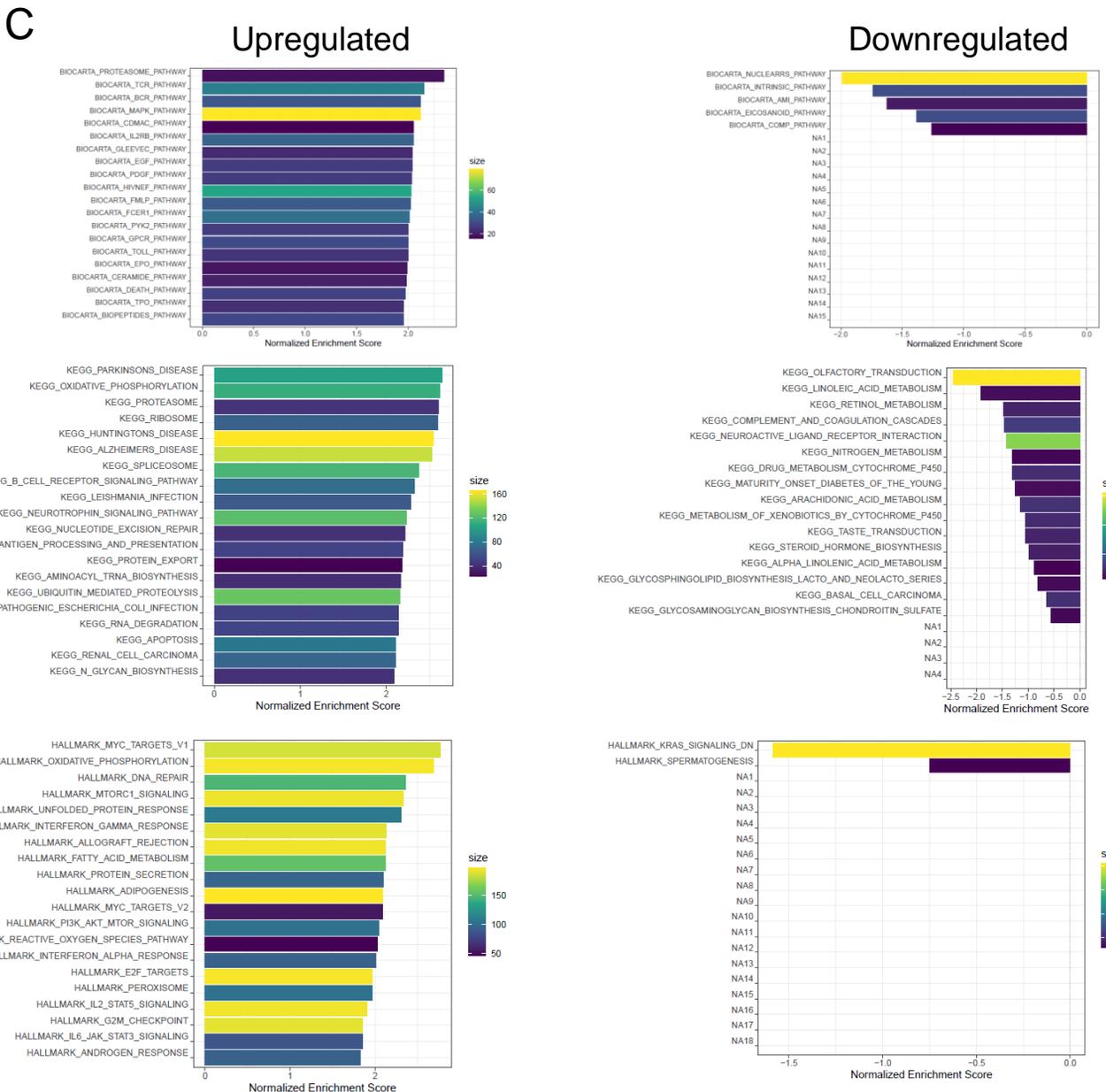
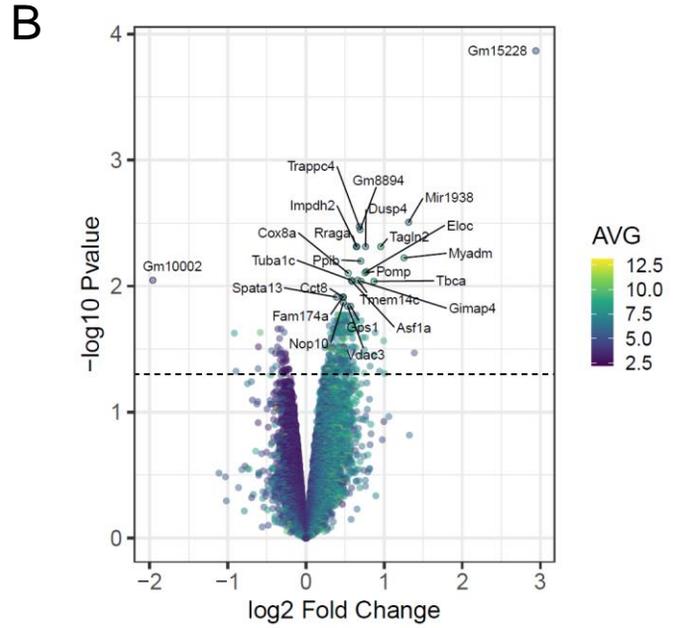
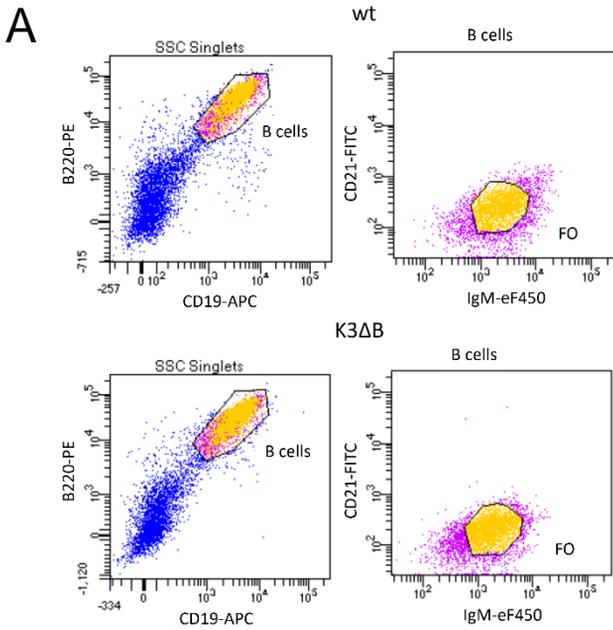
C



Supplemental Figure 3



Supplemental Figure 4

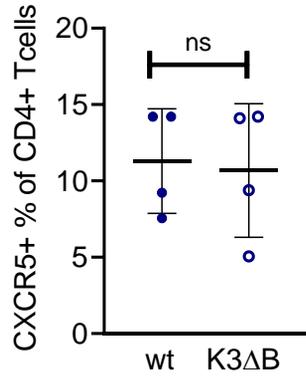


Supplemental Figure 5

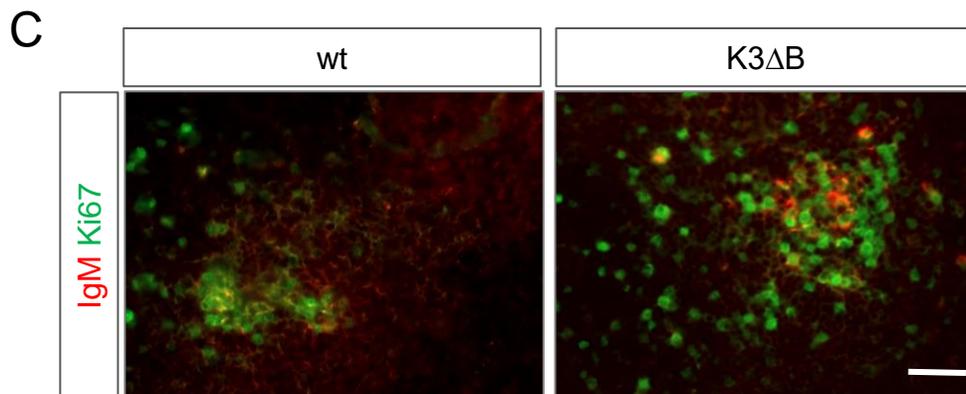
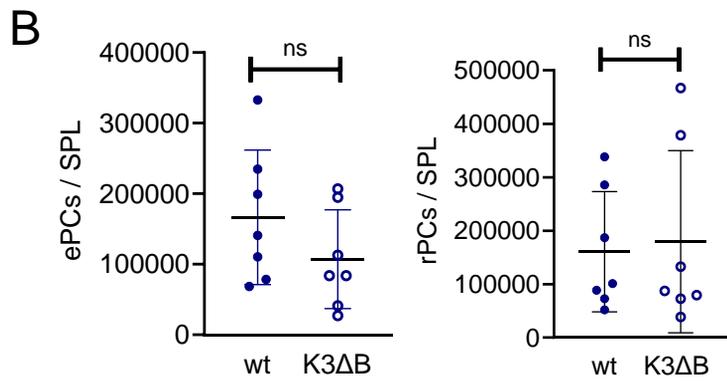
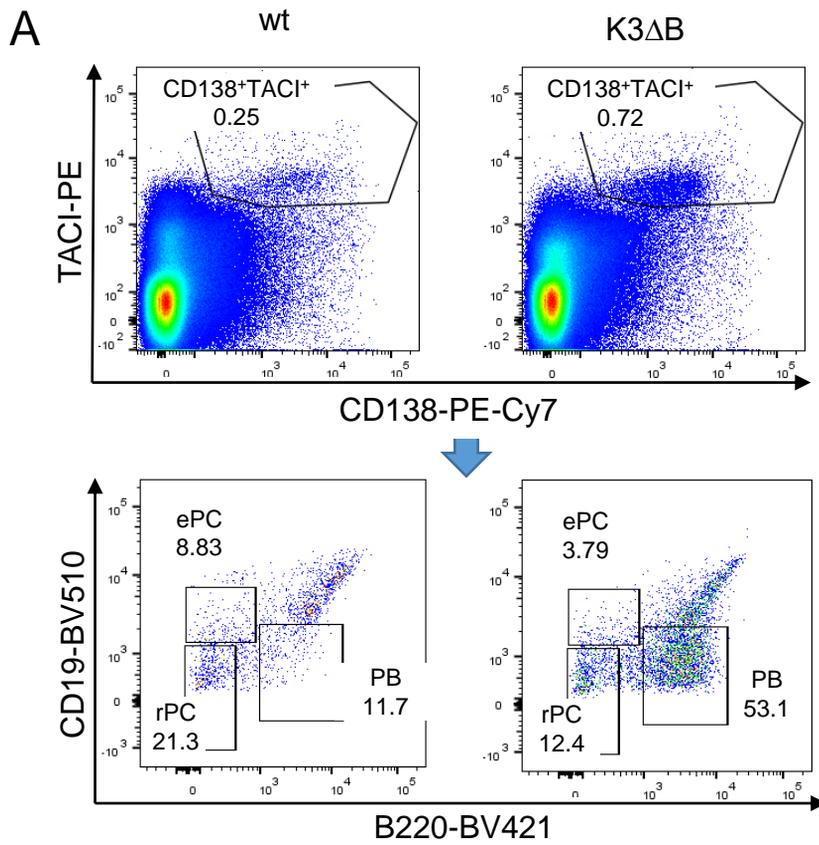
A

Gene name	logFC	P.Value
CD69	0.6259	0.0027
CD40	0.4369	0.0029
CD38	0.5071	0.0024
CD44	0.5383	0.0003
Cxcr5	0.6399	0.00004
GPCR 183 (EBI2)	0.3957	0.0025

B



Supplemental Figure 6



Supplemental Figure 7

