SUPPLEMENTAL FIGURE LEGENDS

Figure S1. *Related to Figure 1.*

A, Schematic depiction of the targeted allele in the *Tbl1xr1*-D370Y mouse model, and the effects of Cre-induced recombination. “E”= Exon; “Neo”= Neomycin resistance cassette. Thin black arrows depict exon orientation.

B, (Top) Scheme depicting the PCR-based strategy used for genotyping the targeted allele in the *Tbl1xr1*-D370Y mouse model. Black arrows represent primers. (Bottom) Representative PCR-based genotyping results on genomic DNA extracted from tail tissue from *Tbl1xr1*$^{WT/WT}$ or *Tbl1xr1*$^{D370Y/WT}$ animals. Numbers designate different animals.

C, Sanger sequencing analysis of *Tbl1xr1* cDNA in sorted splenic GCB from *Tbl1xr1*$^{D370Y/WT}$ or *Tbl1xr1*$^{WT/WT}$ mice, 8 days after SRBC immunization. Data shown for one animal per genotype, representative of a total of three.

D, Quantitative Real Time-PCR analysis of *Tbl1xr1* mRNA expression levels in *Tbl1xr1*$^{D370Y/WT}$ and *Tbl1xr1*$^{WT/WT}$ sorted GCB, 8 days after SRBC immunization. Primers were designed to target *Tbl1xr1* exons 11-13. Results are expressed as fold change, relative to *Tbl1xr1*$^{WT/WT}$, and normalized to GAPDH expression levels.

E, WB analysis of TBL1XR1 expression levels in sorted GCB from three representative *Tbl1xr1*$^{D370Y/WT}$ and *Tbl1xr1*$^{WT/WT}$ animals, obtained as in **C**. ACTIN protein expression levels were used as a loading control.

F, Schematic depiction of the targeted allele in the *Tbl1xr1*-KO mouse model, and the effects of Cre-induced recombination.

G, (Top) Scheme depicting the PCR-based strategy used for verifying Cre-induced *Tbl1xr1* exon 5 excision in the *Tbl1xr1*-KO model. Thin black arrows represent primers. (Bottom) Representative PCR results on genomic DNA from sorted GCB, from *Tbl1xr1*$^{WT/WT}$ or *Tbl1xr1*$^{KO/KO}$ animals, 8 days after SRBC immunization. Numbers designate different animals.

H, Quantitative Real Time-PCR analysis of *Tbl1xr1* mRNA expression levels in GCB obtained as in **G**. Primers were designed to target *Tbl1xr1* exons 11-13. Results are

expressed as fold change, relative to *Tbl1xr1*$^{WT/WT}$ GCB, and normalized to GAPDH expression levels.

I, WB analysis of TBL1XR1 expression levels in sorted GCB from two representative *Tbl1xr1*$^{KO/KO}$ and *Tbl1xr1*$^{WT/WT}$ animals, obtained as in **G**. ACTIN protein expression levels were used as a loading control.

J-K, Representative FC plots (left) and quantification (right) of (**J**) total splenic B-cells or (**K**) GCB in Cγ1Cre-*Tbl1xr1*$^{KO/KO}$ (*Tbl1xr1*$^{KO/KO}$) or *Tbl1xr1*$^{WT/WT}$ mice. See also **Data S1N**.

L, Absolute FC-based quantification of (left) total splenic B-cells or (right) GCB from *Tbl1xr1*$^{KO/KO}$ or *Tbl1xr1*$^{WT/WT}$ mice, 9 days after SRBC immunization.

M, Representative H&E images of splenic sections taken from animals treated as in **J**. Insets (bottom) show zoom of outlined areas. Scale bars = 500μm (top) or 100μm (bottom).

N, Representative images of (top) B220 or (bottom) PNA IHC staining in consecutive splenic sections from specimens in **M**. Scale bars = 100μm.

O-P, Graphs show quantification of (**O**) the number of GC per spleen section, or (**P**) GC size as number of cells (left) or surface area (right), based on PNA staining. Dots in **A** represent different animals. Dots in **P** represent individual GC. Shown are pooled results for 4 animals per genotype.

Q, Representative FC plots (left) and quantification (right) of the relative contribution of *Tbl1xr1*$^{KO/KO}$ and *Tbl1xr1*$^{WT/WT}$ cells to the total B-cell and GCB compartments, based on CD45 allelic frequencies.

R, FC analysis of splenic GCB frequency. Left to right: n = 3, 4, 5, 5 (*Tbl1xr1*$^{WT/WT}$) or 3, 4, 5, 5 mice (*Tbl1xr1*$^{KO/KO}$).

Values represent mean ± SEM. All data were reproducible with at least two repeats. NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001, using unpaired (**D,H,J-L,R**) or paired (**Q**) two-tailed Student's t-test, or Mann-Whitney U-test (**O-P**).

Figure S2. *Related to Figure 2.*

A, Representative FC plots (left) and quantification (right) of AnnexinV/DAPI staining in splenic GCB.

B, Representative FC plots and quantification of (top) total $CD4^+$ or (bottom) GC T follicular helper ($CD4^+CXCR5^{hi}PD\text{-}1^{hi}$) splenic populations.

C, Experimental scheme for experiment in **D-E**.

D, Representative FC plots of (left) GCB formation and (right) the relative contribution of *Tbl1xr1*$^{D370Y/WT}$ and *Tbl1xr1*$^{WT/WT}$ cells to this population, at the indicated time-points.

E, Representative FC plots (left) and quantification (right) of GCB proliferation, based on progressive dilution of a cell proliferation dye. Results are expressed as the frequency of GCB that have undergone a certain number of cell divisions, for three biological replicates, with 3 technical replicates each. Representative plots of stain intensity at Day 0 are included, as a control for initial dye loading levels.

F, Representative FC plots (left) and quantification (right) of EdU incorporation by GCB.

G, FC analysis of cell cycle distribution of splenic GCB for animals in **F**, based on EdU/DAPI staining.

H, RT-qPCR-based quantification of cell cycle checkpoint genes performed on DZ GCB sorted 9 days after SRBC immunization. Results are expressed as fold change of gene-of-interest (G.O.I.), relative to the corresponding *Tbl1xr1*$^{WT/WT}$ controls, and normalized to GAPDH expression levels

Values represent mean ± SEM. All the data were reproducible with at least two repeats. NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001, using unpaired (**A,B,H**) or paired (**F-G**) two-tailed Student's t-test, or two-way ANOVA (**E**).

Figure S3. *Related to Figure 3.*

A, Hierarchical clustering dendrogram, based on Euclidean distance, using log FPKM values of variable genes (top 5th percentile) and Ward's method, for experiment in **Figure 3A**. Squares represent individual animals

B, PCA plot for *Tbl1xr1*$^{D370Y/WT}$ and *Tbl1xr1*$^{WT/WT}$ GCB RNA-Seq samples from **Figure 3A**.

C, Quantitative Real Time-PCR (RT-qPCR) validation of selected differentially expressed genes from **Figure 3A**, performed on an independent set of GCB, sorted 8 days after SRBC immunization. Results are expressed as fold change of gene-of-interest (G.O.I.), relative to *Tbl1xr1*$^{WT/WT}$, and normalized to GAPDH expression levels.

D, Representative FC plots (left) and quantification (right) of S1PR1^{+} splenic GCB.

E, Representative FC plots (left) and quantification (right) of CCR6^{+} splenic GCB.

F, RT-qPCR analysis of *Il9r* mRNA expression levels in *Tbl1xr1*$^{KO/KO}$ and *Tbl1xr1*$^{WT/WT}$ sorted GCB, 8 days after SRBC immunization. Results are expressed as fold change, relative to *Tbl1xr1*$^{WT/WT}$, and normalized to GAPDH expression levels.

G-H, GSEA of gene signatures (left) upregulated or (right) downregulated in *Tbl1xr1*$^{D370Y/WT}$ vs *Tbl1xr1*$^{WT/WT}$ GCB, ranked against: (**G**) the preMB (CCR6^{+}LZ) GCB signature from (Suan et al., 2017); (**H**) an alternative PreMB (mKO2^{hi}) GCB signature from GSE85018.

I, FC analysis of (left) EFNB1^{+} or (right) preMB populations in splenic GCB, at (top) 4 days or (bottom) 15 days post SRBC immunization.

J, FC analysis of (left) EFNB1^{+} or (right) preMB populations in splenic GCB from mice immunized with an hapten conjugate.

Values represent mean ± SEM. All data were reproducible with at least two repeats. NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001, using unpaired two-tailed Student's t-test (**C**-**F**,**I**-**K**).

Figure S4. *Related to Figure 4.*

A-B, Representative histograms (left) and quantification (right) of BLIMP1 expression levels, based on FC data, in (**A**) splenic GCB from *Tbl1xr1*$^{WT/WT}$ or *Tbl1xr1*$^{D370Y/WT}$ mice, 10 days after SRBC immunization, or (**B**) splenic GCB from *Tbl1xr1*$^{WT/WT}$ or *Tbl1xr1*$^{KO/KO}$ mice, 9 days after SRBC immunization. BLIMP1 expression in PC from a representative *Tbl1xr1*$^{WT/WT}$ animal is included on each histogram plot, as a positive control.

C, Representative FC plots for YFP^{+} cells in IgG1(Ctrl Ab)-treated animals in **Figure 4E**.

D-E, Representative FC plots and quantification of (**D**) total YFP^{+} splenocytes and (**E**) the relative cell type composition of this population. aB = Activated B-cells.

Values represent mean ± SEM. All data were reproducible with at least two repeats. NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001, using unpaired two-tailed Student's t-test.

Figure S5. *Related to Figure 5.*

A, RIME analysis of BCL6 interacting partners in OCI-Ly1 cells. A species-matched IgG antibody was used as negative control. Shown is the correlation for two biological replicates for BCL6 immunoprecipitations. Circle sizes are proportional to the fold induction over the IgG control.

B, Co-IP of endogenous TBL1XR1 with BCL6, HDAC3 or SMRT in nuclear extracts obtained from the stated cell lines. A species-matched IgG antibody was included as negative control.

C, WB results showing expression of TBL1XR1 and BirA, in cell lines used for BioID experiments, 48h after Doxycycline induction. ACTIN expression levels were used as a loading control.

D, Co-IP of endogenous BCL6 with TBL1XR1-V5 (WT, D370Y, Y395H, Y446S), in OCI-Ly1 engineered cells from **Figure 5D**. BCL6 immunoprecipitation was conducted on nuclear extracts obtained 48h after Doxycycline induction.

E, WB results showing expression of the stated proteins in nuclear protein extracts from cells transduced to inducibly express WT or mutant (D370Y, Y395H, Y446S) TBL1XR1-V5 fusion proteins. Results shown correspond to extracts obtained 48h after Doxycycline induction. ACTIN expression levels were used as a loading control.

F, Representative histograms (left) and quantification (right) of BCL6 expression levels, based on FC data, in splenic GCB from *Tbl1xr1*$^{WT/WT}$ or *Tbl1xr1*$^{D370Y/WT}$ mice, 8 days after SRBC immunization. BCL6 expression in NB from a representative Tbl1x1$^{WT/WT}$ animal is included on the histogram plot, as a negative control.

G-H, GSEA of gene signatures upregulated and downregulated in *Tbl1x1*$^{KO/KO}$ vs *Tbl1xr1*$^{WT/WT}$ GCB, ranked against: (**G**) the preMB (EFNB1^{+}S1PR2^{lo}) GCB signature from GSE89897 or (**H**) the *IμBcl6-Tbl1xr1*$^{D370Y/WT}$ GCB signature from **Figure 5H**.

I, Representative histograms (left) and quantification (right) of BCL6 expression levels, based on FC data, in splenic GCB from *Tbl1xr1*$^{WT/WT}$ or *Tbl1xr1*$^{KO/KO}$ mice, 8 days after SRBC immunization. BCL6 expression in NB from a representative Tbl1x1$^{WT/WT}$ animal is included on the histogram plot, as a negative control.

J, WB analysis of TBL1XR1 protein expression in representative single-cell-derived CRISPR.KO or Control clones, obtained with 2 different anti-*TBL1XR1* guide RNAs (“1” or “2”).

K, Flow cytometry analysis of cell proliferation by progressive dilution of CellTrace Dye, in CRISPR.KO or Control clones in **J**. Shown are representative histograms (left) and quantification (right). Bar plot presents data as proliferation dye MFI, relative to that on Day 0. Results for two clones per genotype were averaged, considering five technical replicates for each.

L, Co-IP of endogenous SMRT and HDAC3, by BACH2 or BCL6 immunoprecipitation, in nuclear extracts obtained from the stated cell lines. A species-matched IgG antibody was included as negative control.

Values represent mean ± SEM. All data were reproducible with at least two repeats. NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001, using unpaired two-tailed Student's t-test (**F,I**); or two-way (**K**) ANOVA with Tukey's post-test.

Figure S6. *Related to Figure 6.*

A, Representative FC plots (left) and quantification (right) of (top) $IgG1^+$ or (bottom) IgM^+ (bottom) splenic GCB.

B, Representative FC plots and quantification of $IgG1^+$ GCB at (top) 10 days, (center) 15 days, or (bottom) 25 days post SRBC immunization.

C, RT-qPCR-based assessment of *Aicda* mRNA expression in DZ GCB, sorted 9 days after SRBC immunization. Results are expressed as fold change, relative to $Tbl1xr1^{WT/WT}$, and normalized to GAPDH expression levels.

D-E, Representative histograms (left) and quantification (right) of AID expression levels, based on FC data, in (**D**) splenic GCB ($B220^+FAS^+GL7^+$) from $Tbl1xr1^{WT/WT}$ or $Tbl1xr1^{D370Y/WT}$ mice, 8 days after SRBC immunization, or (**E**) GCB from $Tbl1xr1^{WT/WT}$ or $Tbl1xr1^{KO/KO}$ mice, 10 days after SRBC immunization. AID expression in NB ($B220^+FAS^-GL7^-IgD^+$) from a representative $Tbl1xr1^{WT/WT}$ animal (grey), and isotype control staining on GCB (orange) are included on each histogram plot, as negative controls.

F, Representative FC plots (left) and quantification (right) of $IgG1^+$ MB cells at (top) 15 days or (bottom) 25 days post SRBC immunization.

G, ELISA-based assessment of NP-specific serum antibodies in $Tbl1xr1^{D370Y/WT}$ vs $Tbl1xr1^{WT/WT}$ mice, represented as the difference in signal between days 0 and 24 post immunization.

H, ELISA-based assessment of NP-specific serum antibodies in *Tbl1xr1*$^{KO/KO}$ vs *Tbl1xr1*$^{WT/WT}$ mice, represented as the difference in signal between days 0 and 42 post immunization.

I, Relative levels of affinity maturation in *Tbl1xr1*$^{D370Y/WT}$ vs *Tbl1xr1*$^{WT/WT}$ mice, represented as the ratio between high-affinity and low-affinity NP-specific serum immunoglobulin signal, determined as in **G**.

J, Relative levels of affinity maturation in *Tbl1xr1*$^{KO/KO}$ vs *Tbl1xr1*$^{WT/WT}$ mice, represented as the ratio between high-affinity and low-affinity NP-specific serum immunoglobulin signal, determined as in **H**.

K, Bar plot showing SHM burden at the immunoglobulin intron J_H4 loci (as in (Jolly et al., 1997)) in GC B-cells from *Rosa26YFP;Tbl1xr1*$^{D370Y/WT}$ or *Rosa26YFP;Tbl1xr1*$^{WT/WT}$ mice, sorted 9d after SRBC immunization. Genomic DNA extracted from sorted GC B-cells from an *Aicda*$^{KO/KO}$ mouse model (AID KO/KO) was used as negative control for the experiment. Shown are pooled results for 3 [D370Y/WT], 3 [WT/WT] and 1 [AID KO/KO] animals. Numbers in square brackets represent the total numbers of sequenced clones.

L, Representative FC plots (left) and quantification (right) of IgM^+ splenic antigen-specific donor-derived MB cells.

M, Gating strategy and quantification of total antigen-specific donor-derived cells detected in the spleens of recipient mice, 3.5d after immunization, from experiment in **Figure 6F**.

Values represent mean ± SEM. All the data were reproducible with at least two repeats. NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001, using unpaired two-tailed Student's t-test, or Wilcoxon rank sum test (**K**).

Figure S7. *Related to Figure 7.*

A-B, Representative FC plots (left) and quantification (right) of (**A**) total splenic B-cells or (**B**) GCB in *CD19Cre-Tbl1xr1*$^{KO/KO}$ (KO/KO) or *CD19Cre-Tbl1xr1*$^{WT/WT}$ (WT/WT) mice.

C, Representative FC plots and quantification of (top) EFNB1+ or (bottom) preMB populations in splenic GCB for experiment in **A**.

D, Representative FC plots (left) and quantification (right) of splenic PC for experiment in **A**.

E, Representative H&E images of ABC-DLBCL tumors from 4 independent patients, from Scott & Morin cohort (Arthur et al., 2018). Scale bars= 20µm.

F-G, Representative images of B220, CD3, CD138 and KI67 IHC staining in (**F**) spleen or (**G**) kidney sections from animals in **Figure 7A**. Scale bars = 500µm (left) or 15µm (insets).

H-I, *VavP-Bcl2;Cγ1Cre;Tbl1xr1*$^{KO/KO}$ and *VavP-Bcl2;Cγ1Cre;Tbl1xr1*$^{WT/WT}$ mice [n=10 per genotype] received monthly SRBC immunizations, to induce GC formation, from 2m of age until time of sacrifice. Mice were euthanized at 11m of age, and soft organs were collected for pathology studies. (**H**) Representative H&E images of spleen, lung and liver sections. (**I**) Representative images of B220 IHC staining in consecutive sections from specimens in **H**. Insets (right) show zoom of outlined areas. Scale bars= 500µm (left) or 20µm (insets).

J, Representative images (left) and relative quantification (right) of CD38 IHC staining for *TBL1XR1* mutant or WT DLBCL human specimens, from the Scott & Morin cohort (Arthur et al., 2018). Scale bars =20µm.

Values represent mean ± SEM. All the data were reproducible with at least two repeats. NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001, using unpaired two-tailed Student's t-test.