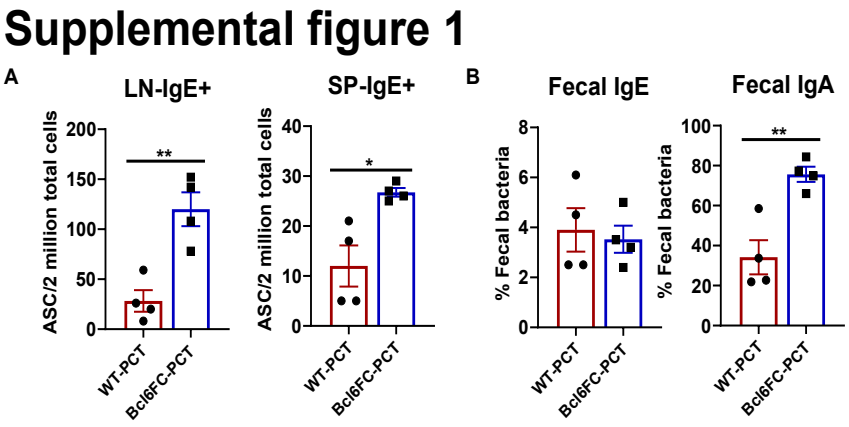
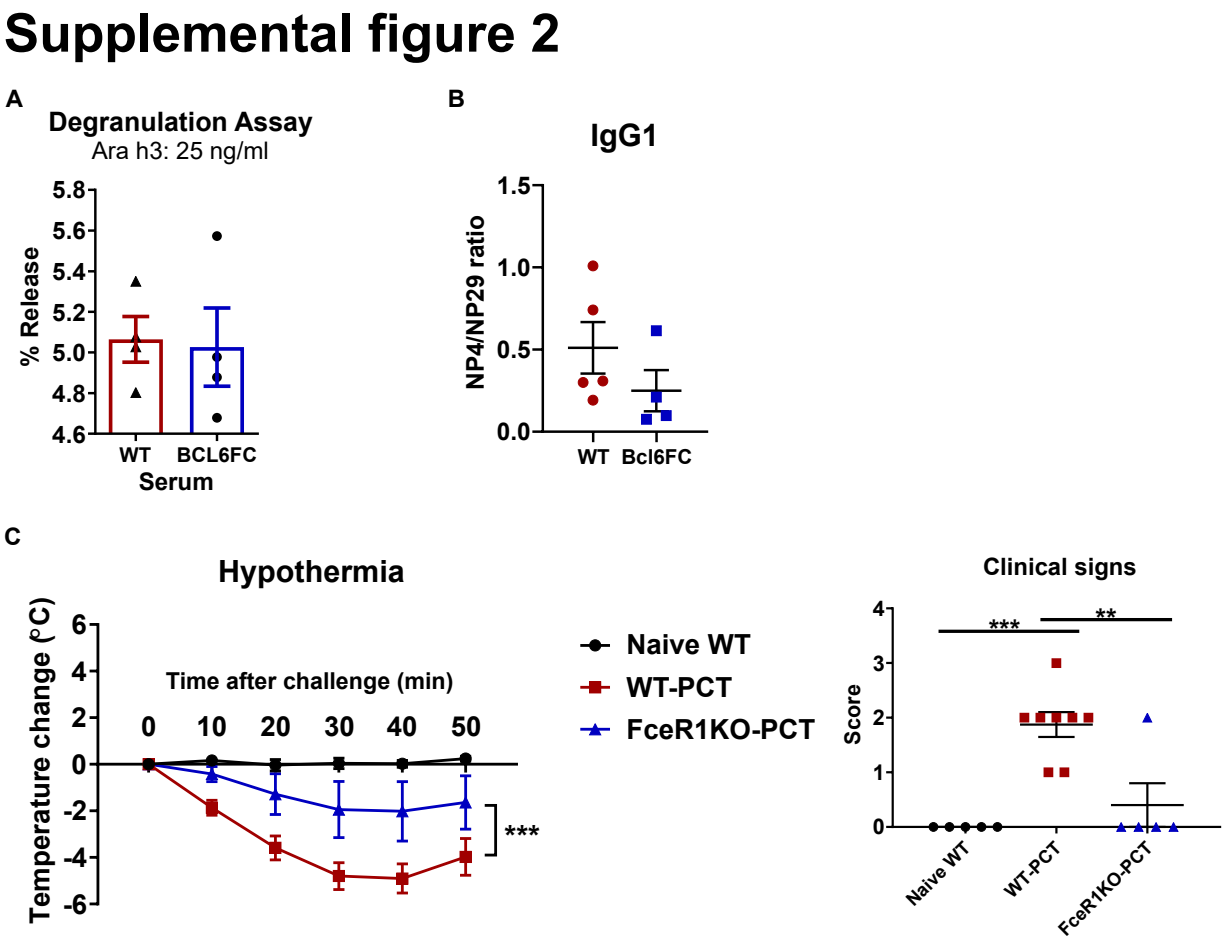
**Supplementary Materials (Supplementary Figures and Supplementary Methods)**

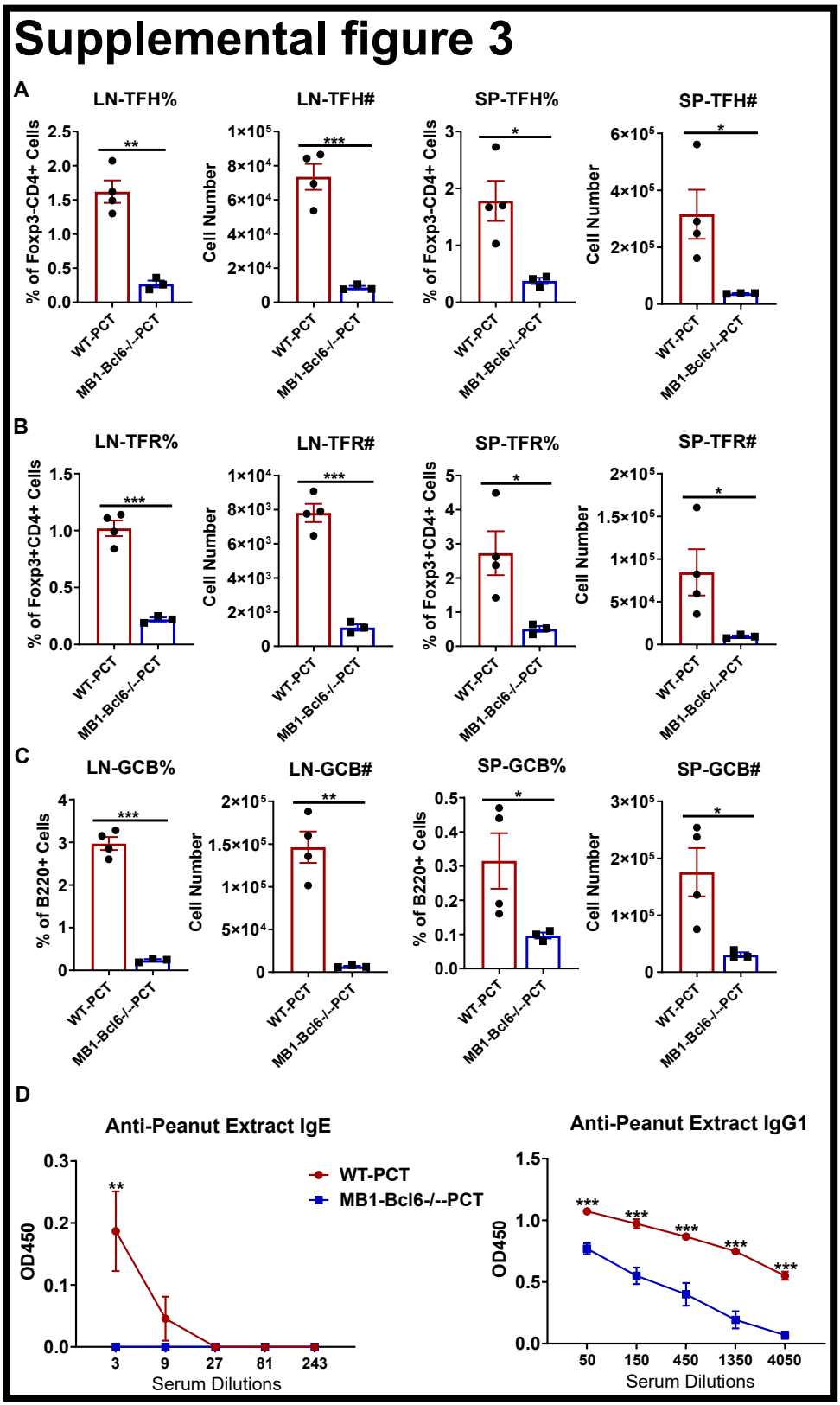
**Supplementary Figure 1. Elevated non-specific IgE and anti-bacterial IgE levels.** (A) Analysis of IgE antibody-secreting cells (ASC) by ELISpot assay using cells from mesenteric lymph node (LN) and spleen (SP) in WT and Bcl6FC mice at day 36 after peanut + cholera toxin (PCT) sensitization. N=4. Data shown is representative of 3 different experiments. ELISpots were performed as in Wu *et al* (1) but used anti-IgE detection reagents. (B) Fecal bacterial from WT and Bcl6FC mice were stained for IgE and IgA binding at day 36 after peanut + cholera toxin (PCT) sensitization. N=4. Data shown is representative of 2 different experiments. Fecal bacteria analysis was performed as in Abdel-Gadir *et al* (2).



**Supplementary Figure 2. Affinity of IgE and IgG1 from Bcl6FC mice and IgE-dependence of anaphylaxis.** (A) Sera from PCT sensitized WT and Bcl6FC mice were tested for affinity of (A) AraH3-specific IgE at day 15 of the response in a RBL mast cell line IgE-dependent degranulation assay (3, 4). (B) Sera from PCT + NP-PE sensitized WT and Bcl6FC mice were tested for affinity of NP-specific IgG1 by using high and low density NP-BSA conjugates in ELISA. The ratio of the NP4 specific response to the NP29 specific response is shown. (C) WT and FcεR1-/- mice were PCT-sensitized and then challenged at day 36 with 2 mg of peanut protein i.p.. Anaphylaxis symptoms were assayed as described in Figure 1 and Methods. Non-sensitized naïve WT mice were used as negative controls. N=5-8.

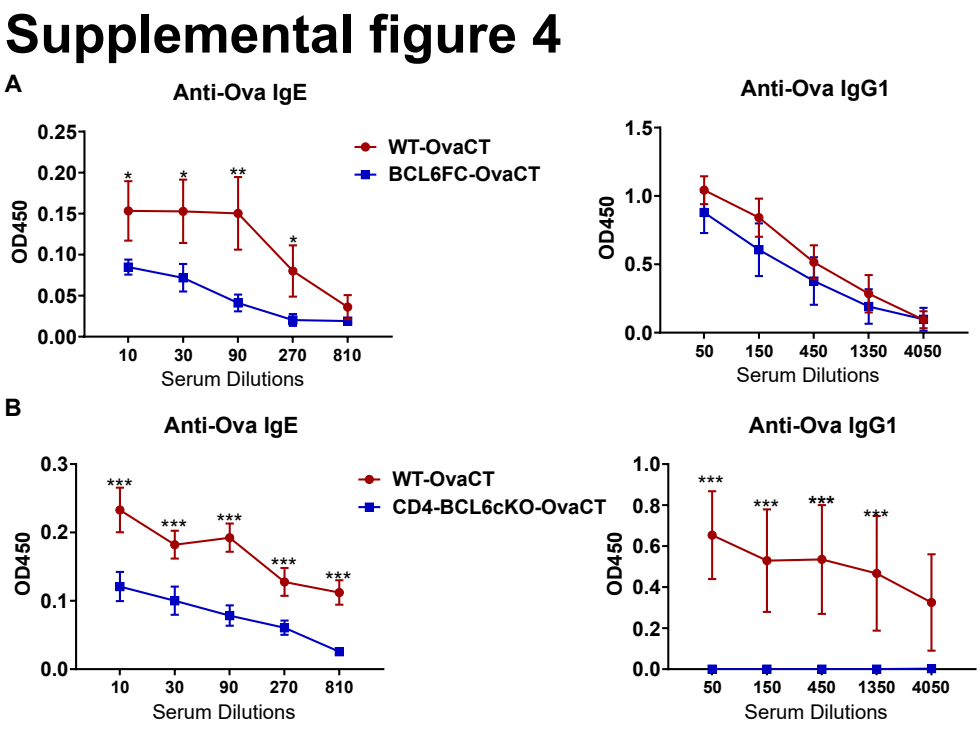


**Supplementary Figure 3. Block of GC B cell development leads to loss of TFH, TFR cells and peanut-specific IgE.** WT and Mb1-cre Bcl6-fl/fl (MB1-Bcl6-/-) mice were sensitized as in Figure 1 with PCT then at D36, mesenteric lymph nodes (LN) and spleens (SP) analyzed for the indicated cell populations by flow cytometry. Graphs show average % of cells as a fraction of parental cell population and total yield of cells.

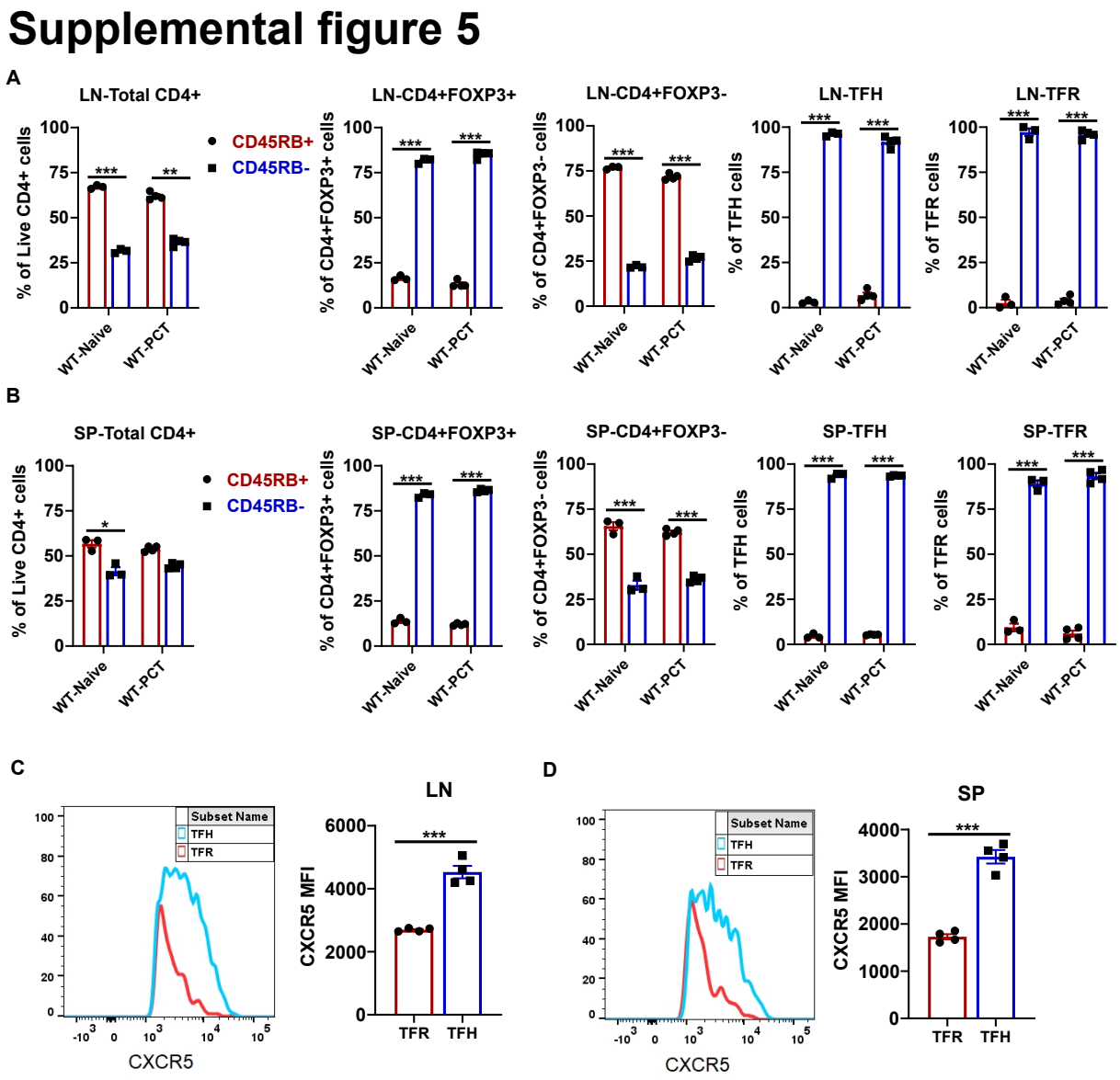



GCB (A), TFH (B) and TFR (C) cells analyzed as in Figure 2. (D) Peanut-specific IgE and IgG1 titers from D36 serum of WT and MB1-Bcl6-/- mice sensitized with PCT. Data are from one experiment representative of two experiments with three to five mice per group. P values were calculated by t test or ANOVA where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

**Supplementary Figure 4. TFR cells control antigen-specific IgE.** (A) Ova-specific IgE and IgG1 titers from D36 serum of WT and Bcl6FC mice sensitized twice with Ovalbumin + cholera toxin. (B) Ova-specific IgE and IgG1 titers from D36 serum of WT and CD4-Bcl6cKO mice sensitized twice with Ovalbumin + cholera toxin. Data are from one experiment representative of two experiments with four to five mice per group. P values were calculated by t test or ANOVA where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

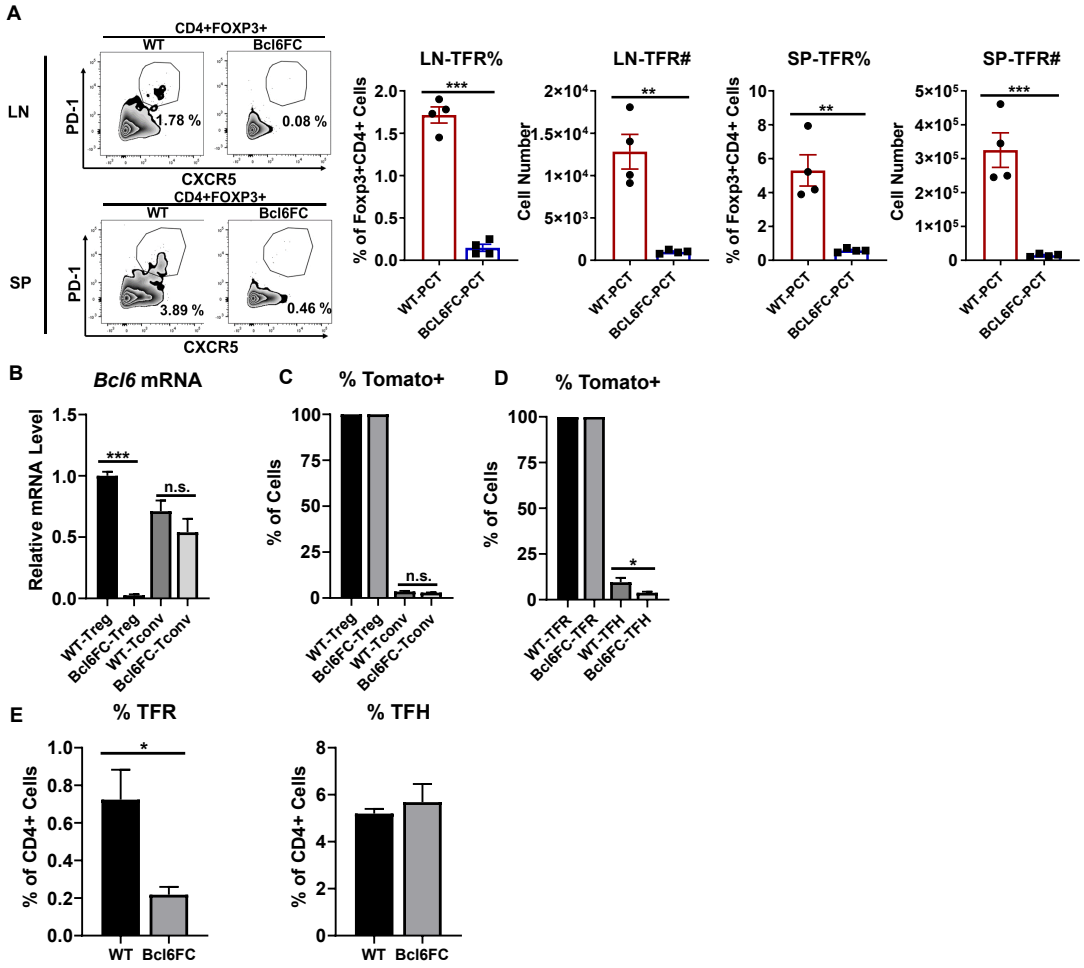


**Supplementary Figure 5. CD45RB and CXCR5 expression on TFH and TFR cells.** Cells taken from mesenteric lymph node (LN: A , C) and spleen (SP: B, D) in WT and Bcl6FC mice at day 36 after peanut + cholera toxin (PCT) sensitization were analyzed by flow cytometry for CD45RB (A, B) and CXCR5 (C, D) expression. For CD45 expression, cells were analyzed as % CD45+ versus CD45RB–. For CXCR5, cells were analyzed for MFI of CXCR5 staining. Data are from one experiment representative of two experiments with 4 mice per group. P values were calculated by t test or ANOVA where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

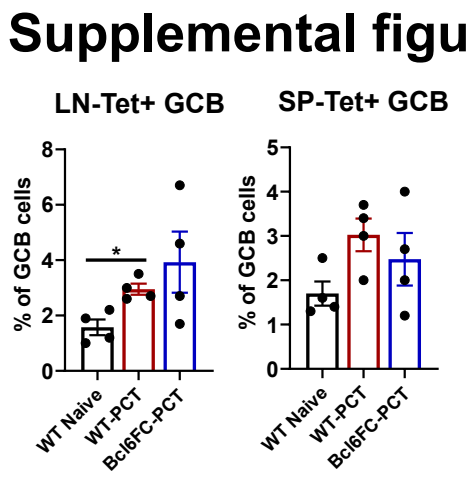


**Supplementary Figure 6. TFR cell deletion and FOXP3-CRE does not delete at a significant level in FOXP3-negative Tconv cells.** (A) WT and Bcl6FC mice were sensitized as in Figure 1 with PCT then at D36, mesenteric lymph nodes (LN) and spleens (SP) analyzed for TFR cells by flow cytometry. (B) Bcl6 mRNA levels were determined by RT-QPCR from YFP+ cells (Tregs) and YFP-negative cells (Tconv) cells FACS isolated from naïve WT and Bcl6FC mice. (C-E) WT and Bcl6FC mice were analyzed 7 days after immunization with Sheep Red Blood Cells (SRBC). CRE-inducible TOMATO expression was used to show FOXP3-CRE activity in (C) Tregs and (D) Tconvs from naïve WT and Bcl6FC mice. (E) Percentages of TFR and TFH cells analyzed by flow cytometry. WT (Foxp3-control) and Bcl6FC ROSA-TOMATO mice were described in Sawant et al (5).

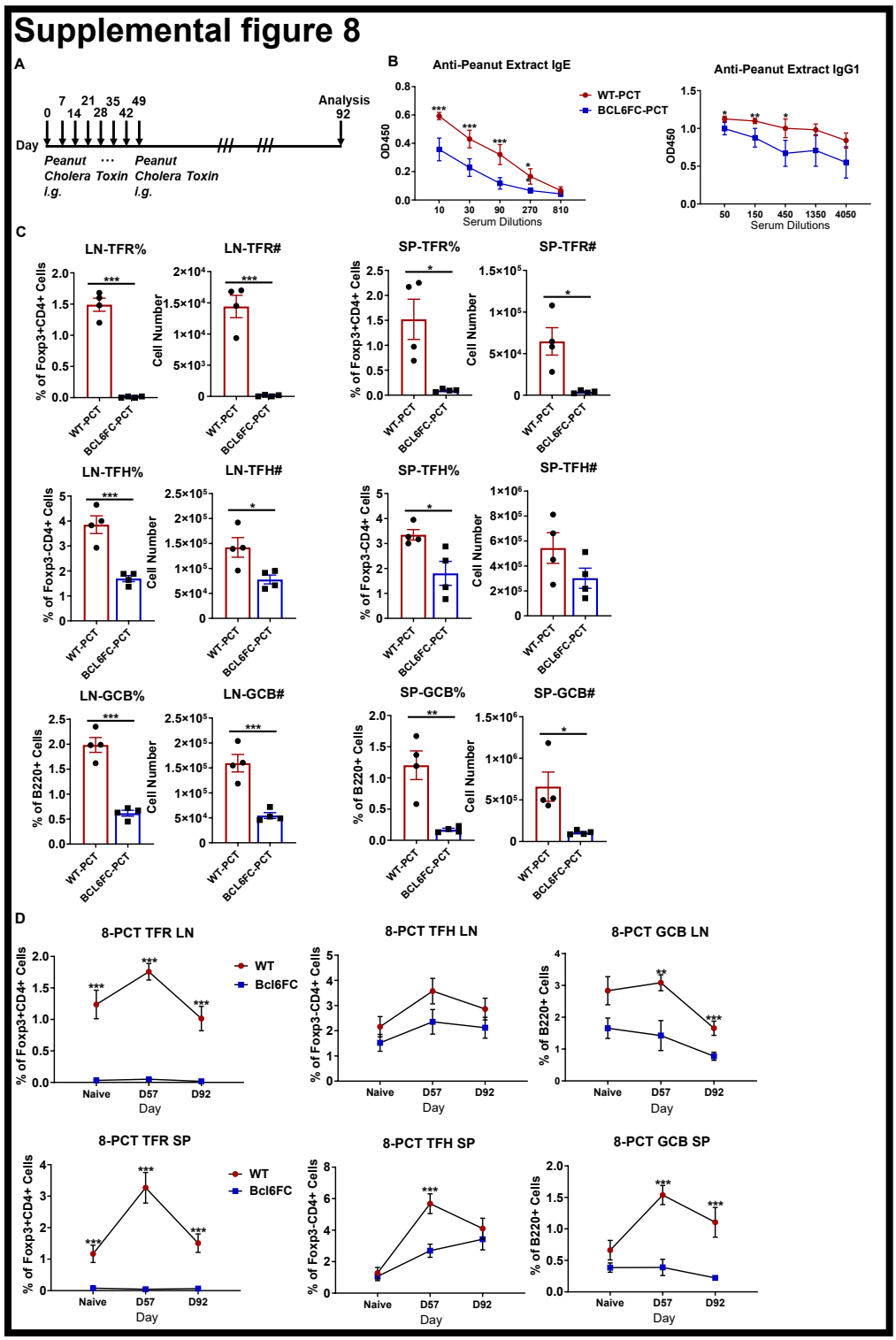
## Supplemental figure 6



**Supplementary Figure 7. Analysis of peanut-specific GC B cells.** Cells were taken from mesenteric LN and SP in WT and Bcl6FC mice at day 36 after peanut + cholera toxin (PCT) sensitization and stained for GCB cell markers and a mixture of 2 different fluorescent tetramers made with the peanut proteins AraH1 and AraH3. Tet+ = tetramer+ GCB cells that stained with the mixture of peanut tetramers. Tetramers were constructed and used as described in Taylor et al (6). Data are from one experiment representative of two experiments with 4 mice per group. P values were calculated by t test or ANOVA where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

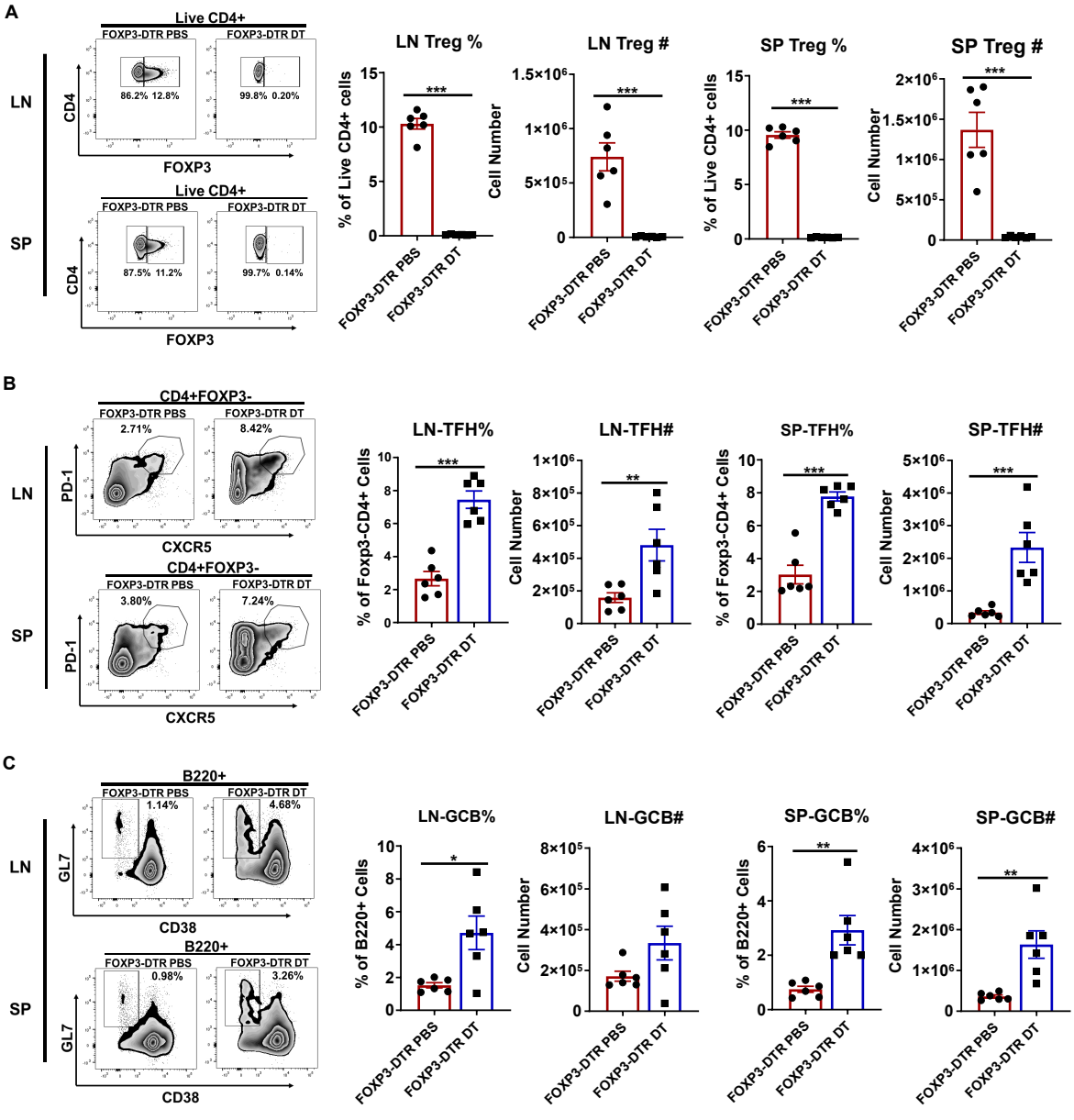


**Supplementary Figure 8. TFR cells are required for normal GC responses and IgE levels using a stronger sensitization system.** (A) This food allergy sensitization system uses 8 i.g. doses of PCT, each 7 days apart, then bleeds at different time-points after sensitization. The mice were analyzed at the day 92 time-point, where immune cells and serum are tested 43 days after the last sensitization. (B) Peanut-specific IgE and IgG1 titers from D92 serum of WT and Bcl6FC mice sensitized 8 times with PCT. (C) WT and Bcl6FC mice were sensitized as in part (A) with PCT then at D92, mesenteric lymph nodes (LN) and spleens (SP) analyzed for the indicated cell populations by flow cytometry. Graphs show average % of cells as a fraction of parental cell population and total yield of cells. GCB, TFH and TFR cells analyzed as in Figure 2. Data for (B) and (C) are from one experiment representative of two experiments with four to five mice per group. (D) Time-course of the TFR, TFH and GCB response after 8 PCT sensitizations. Data for (D) are pooled from naïve (two experiments), D57 (two experiments) and D92 (two experiments) with four to five mice per group. D56 = 7 days after last sensitization, D92 = 43 days after last sensitization.



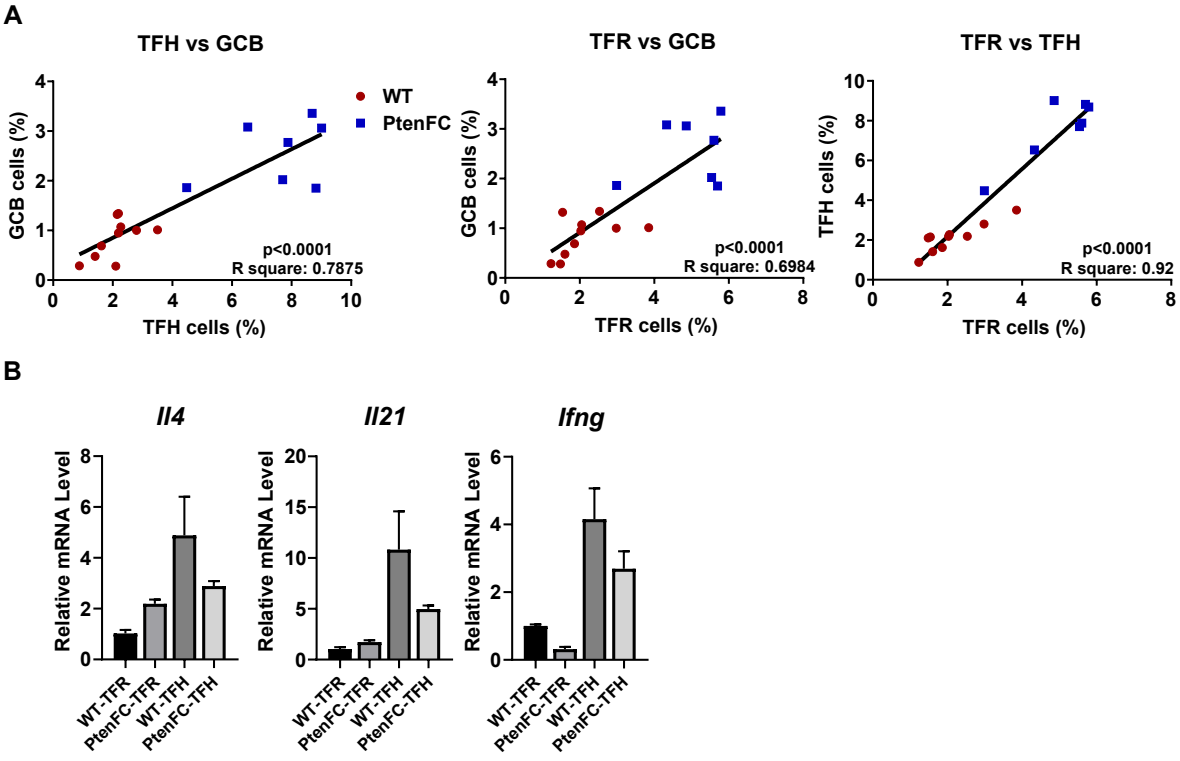
**Supplementary Figure 9. Total Treg deletion leads to up-regulated TFH and GCB cell responses** (A) Foxp3-DTR mice were treated with DT or given PBS as a control, sensitized with PCT at days 0 and 7 as indicated, and then at day 9, draining mesenteric lymph node (LN) and spleen (SP) taken for analysis of Treg (A), TFH (B) and GCB (C) cells by flow cytometry as in Fig. 3C. Representative contour dot plots for each cell staining are shown along with graphs showing average % of cells as a fraction of parental cell population and total yield of cells. (A) Analysis of CD4+FOXP3- PD-1+CXCR5+ TFH cells. TFH cells are quantitated as a percentage of FOXP3- CD4+ T cells, and absolute number per LN or SP. (B) Analysis of B220+CD38- GL7+ GCB cells. GCB cells are quantitated as a percentage of B220+ cells, and absolute number per LN or SP. Data are from one experiment representative of eight experiments with four to six mice per group. P values were calculated by t test where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

## Supplemental figure 9



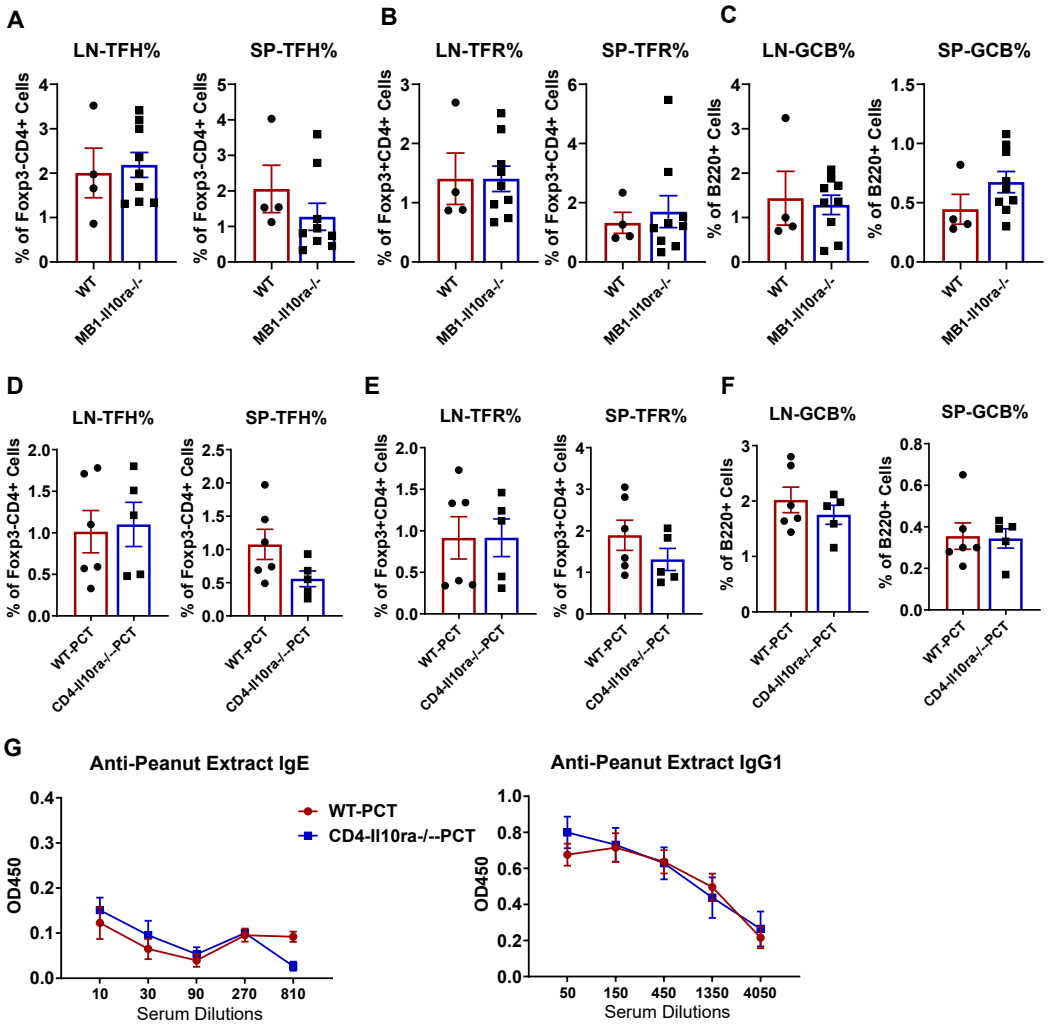
**Supplementary Figure 10. Analysis of cytokine expression by TFR and TFH cells from Pten conditional knockout mice after peanut sensitization.** WT and *Foxp3-cre Pten-fl/fl* (PtenFC) mice were sensitized as in Figure 1 with PCT. (A) Linear correlation analysis between TFH cell % and GCB cell %, between TFR cell % and GCB cell % and between TFR cell % and TFH cell %, using data combined from WT and PtenFC mice. Data was pooled from two experiments with three to five mice per group. P values were calculated by t test or ANOVA where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$. R square values were calculated by Prism Graphpad software. (B) At day 22 of the sensitization system, SP cells were taken to isolate TFR and TFH cells by FACS. *Il4, Il21 and Ifng* mRNA levels in TFR and TFH cells were assayed by RT-QPCR freshly after sorting. Experiment was performed once, N=3.

# Supplemental figure 10

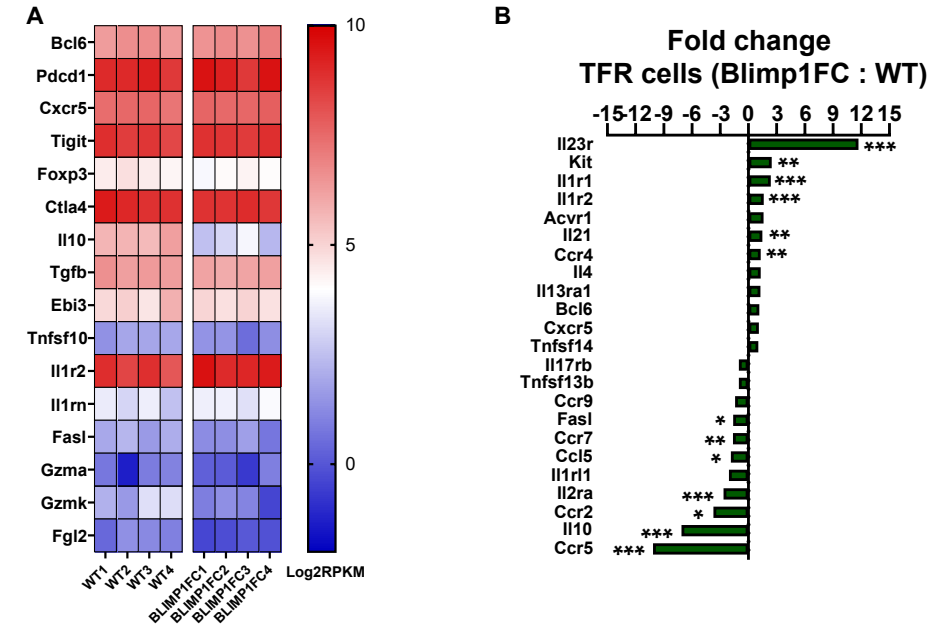


**Supplementary Figure 11. IL-10 signaling on T cells does not affect food allergy GC response and IgE production.** (A-C) LN and SP from naïve unsensitized WT and MB1-Il10ra-/- mice were were stained and analyzed for TFH, TFR and GCB by flow cytometry. Graphs show average % of cells as a fraction of parental cell population and total yield of cells. Data for (A-C) are from one experiment representative of two experiments with four to nine mice per group. (D-F) WT and CD4-cre IL10ra-fl/fl (CD4-Il10ra-/-) mice were sensitized as in Figure 1 with PCT. At D36 of the sensitization system, LN and SP were analyzed for TFR cells, TFH cells and GCB cells by flow cytometry as in Figure 2. Graphs show average % of cells as a fraction of parental cell population and total yield of cells. (G) Peanut-specific IgE and IgG1 titers from D36 serum of WT and CD4-Il10ra-/- mice sensitized with PCT. Data for (D-G) are from one experiment representative of two experiments with five to six mice per group. P values were calculated by t test or ANOVA where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

# Supplemental figure 11

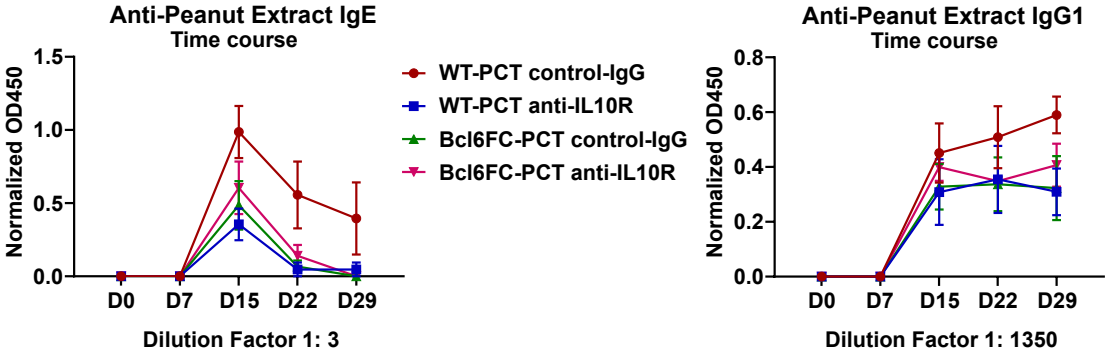


**Supplementary Figure 12. RNAseq analysis of Blimp1-deficient TFR cells are deficient primarily in Il10 expression.** WT and Foxp3-cre Blimp1-fl/fl (Blimp1FC) mice were sensitized as in Figure 1 with PCT. At day 36 of the sensitization system, SP cells were taken to isolate TFR by FACS. (A) Heat map showing RNA transcript levels of key follicular T cell and Treg cell genes in TFR cells from WT and Blimp1FC mice. Transcript levels were determined by RNAseq. TFR cells were isolated by FACS from PCT sensitized mice (N= 4 of each strain). Color scale representing absolute RNA levels is shown on the right as Log2RPKM values. (B) Analysis of selected genes in our RNAseq dataset described as deregulated in Blimp1-deficient TFR cells by Wang *et al* (7).

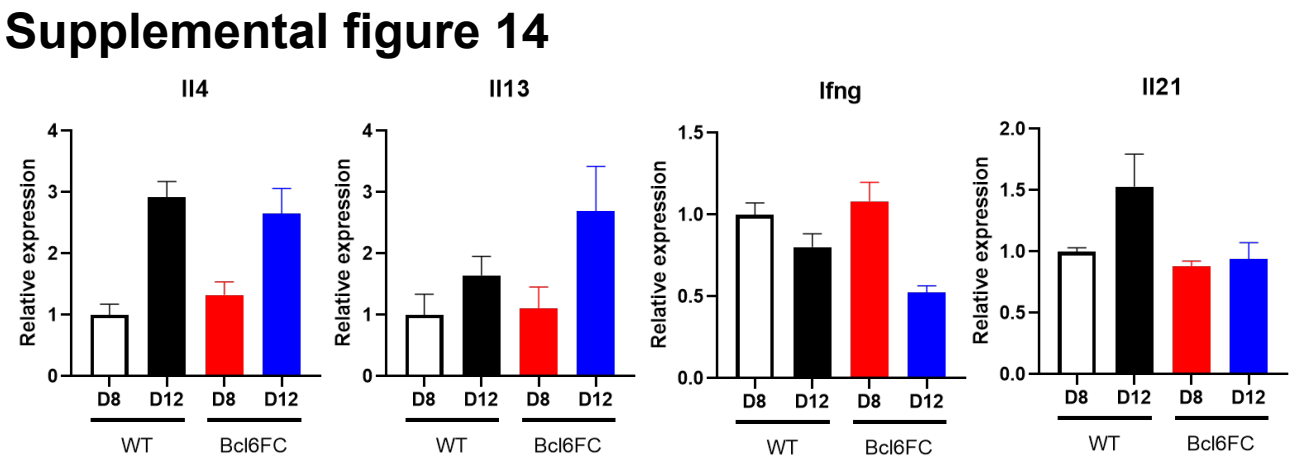


**Supplementary Figure 13. Time-course of peanut-specific IgE and IgG1 titers after IL-10R blockade.** As in Fig. 7F, WT and Bcl6FC mice were subjected to either control or IL-10 receptor blocking Abs and sensitized with PCT. Peanut-specific IgE and IgG1 titers from serum of control and anti-IL10R mice treated as described in Fig. 7F at the indicated timepoints. Data are from one experiment representative of two experiments with three to four mice per group.

# Supplemental figure 13



**Supplementary Figure 14. Analysis of cytokine expression by TFH cells from WT and Bcl6FC mice early after peanut sensitization.** WT and Bcl6FC mice were sensitized with PCT at days 1 and 8. At day 8 and day 12 of the sensitization system, SP cells were taken to isolate TFH cells by FACS. Note, day 8 mice only received one dose of PCT. *Il4, Il13, Il21* and *Ifng* mRNA levels in TFH cells were assayed by RT-QPCR freshly after sorting. Results shown are averaged from 2 different sorting experiments, N= 4-6 mice total.



**Supplementary Methods**

*Depletion of Treg cells and Ab administration*

Foxp3+ cells were deleted by administering 1 μg diphtheria toxin (DT) in PBS per mouse intraperitoneally into Foxp3-DTR mice on indicated dates. For the IL-10R blocking study, 200 μg of anti-IL-10R Ab (1B1.3A; Bioxcell) or control rat IgG1 (HRPN; Bioxcell) were injected intraperitoneally into female C57Bl/6 mice every 3 days starting on day 1 after first i.g. peanut sensitization. Serum was collected from the submandibular vein bleeding. Mice were subjected anaphylaxis at day 36.

*Flow cytometry*

Cell suspensions from mLNs and spleens were prepared and filtered through a 40-μm cell strainer (Fisherbrand). Cells were washed and diluted in PBS with 1% FBS and were stained with Fc block (BioXCell) for 5 min, followed by surface staining for the indicated markers. Following labelled Abs were used: anti-CXCR5 (L138D7), anti-PD-1 (29F.1A12), anti-CD4 (RM4-5), anti-Foxp3 (MF-14), anti-CXCR4 (L276F12), anti-CD86 (GL-1), anti-CD38 (90) and anti-B220 (RA3-6B2) were obtained from BioLegend; GL7 (GL7) was purchased from BD Pharmingen. For intracellular staining, after surface markers were stained, cells were fixed and stained with anti-Foxp3, eBioscience intracellular kit was used. All samples were acquired on an LSR2 flow cytometer (Becton Dickonson) and analyzed with FlowJo software.

*Antibody Titer and Affinity Measurement*

Titers of peanut-specific Ab in serum were measured by ELISA, as previously reported (8). For peanut-specific IgE, 96 well Nunc-Immuno plates (Sigma) were coated with IgE Ab (LO-ME-3, BIO-RAD) overnight at 4° C. Wells were blocked with 1% BSA and diluted serum was added and incubated at room temperature for 2 hours. Peanut extract protein was labelled with biotin (Sigma-Aldrich) and added for one hour followed by adding poly-HRP streptavidin (Pierce Endogen) for half hour. For peanut-specific IgG1, 96 well Nunc-Immuno plates were coated with 5 ug/ml peanut extract protein overnight at 4° C. Wells were blocked with 1% BSA and

diluted serum was added and incubated at room temperature for 2 hours. An anti-mouse IgG1 (BD Pharmingen) was used as secondary Ab with avidin-HRP. Whenever possible, we used serum from wild-type mice sensitized 8 times with peanut plus cholera toxin as a strong peanut-specific IgE positive control, and normalized the IgE ELISA readings with this control. For affinity measurement of peanut-specific IgE, we used purified Ara H3 as a specific antigen (9). IgE+ serum from peanut-sensitized mice in an RBL cell line degranulation assay as described in Handlogten et al (3) and Deak *et al* (4). Briefly, RBL cells were primed with IgE-containing mouse serum, allergen was added to stimulate degranulation, and granule release was measured by a colorimetric assay based on N-acetyl-glucosaminidase activity. Purified AraH3 protein was used to trigger peanut-specific degranulation. Maximal degranulation response was determined by PMA plus ionomycin stimulation of the RBL cells and used to calculate % degranulation. For IgG1 affinity, 0.1 mg NP-PE was added into the normal PCT sensitization. NP-specific IgG1 titers were assayed by ELISA with NP4-BSA and NP29-BSA, and the ratio of the response to the two different NP-conjugated proteins was calculated using the OD values at the mid-range serum dilution.

*Isolation of TFR cells*

CD4+ T cells were isolated from the spleen from D36 PCT sensitized mice via a CD4+ T isolation kit (Miltenyi Biotec). Foxp3+YFP+ CXCR5+ PD1+ cells were further isolated by FACS (FACS Aria). Total RNA was collected from freshly sorted TFR cells using the RNeasy Plus Micro kit (QIAGEN). Total RNA was subjected to RNA-seq or to analyze Il10 RNA levels using RT-QPCR.

*RNA-seq and Bioinformatics Analysis*

RNA-seq was performed by the Indiana University School of Medicine Center for Molecular Genomics. Uniquely mapped sequencing reads were assigned to mm10 refGene genes. Quality control of sequencing and mapping results was summarized using MultiQC. Genes with read count per million (CPM) < 0.5 in more than 4 of the samples were removed. The data was normalized using TMM (trimmed mean of M values) method. Differential expression analysis was performed using edgeR. False discovery rate (FDR) was computed from p-values using the Benjamini-Hochberg procedure. Differentially expressed genes (DEGs) were determined if

their p-values were less than 0.05 after multiple-test correction with FDR-adjustment and the amplitude of fold changes (FCs) were larger than 1.8. Data from WT and Blimp1FC TFR cells is deposited at NCBI as accession GSE143146.

*Bone marrow chimeras*

Bone marrow (BM) cells (2.5×106 each type) from donor mice were injected i.v. into Rag1−/− recipient mice, sublethally irradiated (350 Gy) 24 h prior. One set of mice received Bcl6FC + WT BM and the other set of mice received Bcl6FC + Blimp1FC BM. The lymphoid compartment in the recipients was allowed to constitute for 3-4 months before PCT sensitization.

## Supplemental References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