UNIVERSITY OF CALIFORNIA SAN DIEGO

Cell Intrinsic Role of MicroRNA-23-27-24 Family in Germinal Center B Cells

A thesis submitted in partial satisfaction of the requirements for the degree

Master of Science

in

Biology

by

Ziyue Zhang

Committee in charge:

Professor Li-Fan Lu, Chair
Professor Cornelis Murre
Professor Elina Zuniga

2023

The Thesis of Ziyue Zhang is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

2023

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGEMENTS

Cheng-Jang Wu, Jiayi Dong, and Nimi Marcel contributed to producing data presented in the thesis. The thesis author is the primary author of this thesis.

ABSTRACT OF THE THESIS

Cell Intrinsic Role of MicroRNA-23-27-24 Family in Germinal Center B Cells

by

Ziyue Zhang

Master of Science in Biology

University of California San Diego, 2023

Professor Li-Fan Lu, Chair

Elevated expression of microRNA-23~27~24 (miR-23) family in T cells has been shown to be crucial to restrict follicular helper T (TFH) cell response. Interestingly, like Bcl-6 and many other molecules that play important roles in both TFH cells and germinal center (GC) B cells, two key components in orchestrating proper humoral immunity, we have found that the miR-23 family is also upregulated in germinal center (GC) B cells. Nevertheless, the cell intrinsic role of

this miRNA family in regulating GC B cell response has yet to be characterized. In this study, through employing cell type specific gain-of-function and lost-of function approaches, we show the miR-23 family plays an equally important role in regulating GC B cell responses. However, while the entire miR-23 clusters coordinately limit GC B cell responses, individual members of the miR-23 family contribute to GC B cell regulation in distinct manners. Moreover, despite the fact that GC B cell responses were clearly impacted by the deletion or overexpression of the miR-23 family in B cells, the frequencies of TFH cells in those mice remained unchanged. These results implied that while the miR-23 family serves as negative regulators in limiting GC B cell differentiation and function, they might also play a positive role in promoting GC B/TFH cell interaction. Consistent with this notion, our RNA-seq analysis revealed a network of genes involved in GC B cell biology that are respectively up- and down-regulated in the absence of miR-23 family-mediated regulation. Collectively, our work demonstrates that the miR-23 clusters are pivotal molecular regulators to ensure proper GC B cell responses not only through targeting TFH cells as shown previously but also by directly inhibiting GC B cells in a cell-intrinsic manner.

INTRODUCTION

Chapter 1: Germinal Center Response

Germinal centers (GCs) are specialized structures crucial for mounting humoral response. Activated B cells proliferate and undergo affinity maturation in the GCs, and eventually differentiate into plasma cells (PCs) or memory B cells (Nutt &Tarlinton, 2011; Merlo & Mandik-Nayak, 2013). Humoral immunity is thus capable of providing long term protection against pathogens through generation of sustainable antibody responses and immune memory.

Upon activation through antigen-induced cross-linking of antigen specific B cell receptors (BCRs) and interaction with cognate T cells, a subset of B cells differentiates into GC B cells (Pelet et al., 2008). GC B cell differentiation is initiated by the expression of the B cell lymphoma 6 protein (Bcl-6) and can be characterized by their reactivity with plant lectin peanut agglutinin (PNA) (Coico et al., 1983). GC B cells proliferate and undergo affinity-based selection in the GCs. Activation-induced cytidine deaminase (AID) is upregulated in GC B cells, facilitating somatic hypermutation in the variable regions of BCR. GC B cells bearing high-affinity BCR are rescued from apoptosis by combination of BCR and CD40 signaling provided by antigen-bearing follicular dendritic cells (FDCs) and follicular helper T (TFH) cells. Positively selected B cells proliferate and eventually differentiate into PCs or memory B cells (Luo & Yin, 2021; Shlomchik & Weisel, 2012; Klein & Dalla-Favera, 2008).

TFH cells are a specialized subset of CD4+ helper T cells that can localize to B cell follicles through expression of CXCR5. While Bcl-6 was identified as the master regulator of GCB cell differentiation, it also serves as a key transcriptional factor in TFH cell differentiation, suggesting the existence of a shared regulatory circuits between these two important immune cell subsets. Expression of several surface molecules and production of key cytokines by TFH cells

have been shown to be pivotal for formation and maintenance of GCs through regulating activation and survival of GC B cells (Crotty, 2011). Among them, CD40L expressed by TFH cells is critical to provide activation, proliferation, and survival signal to GC B cells. In the absence of CD40L, GC response is completely inhibited (Foy et al., 1994). PD-1 also plays a role in maintaining GCs. It has been shown that loss of PD-1 in TFH cells or PD-1 ligand on GC B cells results in increased apoptosis in GC B cells and decreased PC population (Good-Jacobson et al., 2010). Moreover, TFH cell-derived cytokines such as IL-4 and IL-21 are also crucial for mounting optimal GC response. While IL-4 has been reported to protect GC B cells from apoptosis and mediate optimal T cell help (Wurster et al., 2002; Yusuf et al., 2010), IL-21 is shown to regulate GC B cell proliferation and differentiation (Avery et al., 2010; Linterman et al., 2010).

It is now well appreciated that the effect of T cell-B cell interaction (T-B interaction) in GCs is bidirectional. While TFH cell defects can lead to impaired GC responses (Chakhtoura et al., 2021), impairment in B cells can also cause defective T cell priming and proliferation (Ron & Sprent, 1987). This can be mediated through bidirectional signaling of surface molecule interaction between TFH and GC B cells. As discussed above, CD40-CD40L interaction provides proliferation and survival signal to GC B cells. At the same time, this interaction is also important for antigen-specific T cell priming and clonal expansion (Grewal et al., 1995). Additionally, GC B cells also possess surface molecules important for TFH cell differentiation. It has been shown that ICOSL expression on GC B cells plays an important role in regulating TFH cell differentiation by interacting with ICOS (Nurieva et al., 2008). As such, positive feedback signals between B cells and TFH cells through the CD40–CD40L and ICOSL–ICOS interactions support the formation of GCs.

Chapter 2: microRNA in Immune Regulation

MicroRNAs (miRNAs) are a class of endogenous small RNA molecules that play key roles in post-transcriptional regulation of gene expression (He & Hannon, 2004). They are first transcribed by RNA polymerase II from genomic DNA into primary miRNA (pri-miRNA) and further processed by the Drosha RNase III endonuclease to form stem loop precursor miRNA (pre-miRNA). The pre-miRNA is actively transported to the cytoplasm, where it is further cleaved by Dicer, another RNase III endonuclease, to produce a double stranded RNA duplex (miRNA:miRNA*) containing the mature miRNA and its complementary strand. The mature miRNA strand is then incorporated into the RNA-induced silencing complex (RISC) by binding protein Argonaute (AGO), while the complementary strand is degraded (Bartel, 2004) (Bushati & Cohen, 2007). AGO plays a critical role in target recognition of RISC. Binding of miRNA to AGO induces conformational change, exposing the 2-8 nucleotide seed sequence of the miRNA (Meister, 2013). The seed sequence guides RISC to their targets mainly through direct binding to the 3' untranslated region (UTR) of the mRNA. This interaction eventually downregulates target gene expression through induction of mRNA degradation or repression of protein translation (Bartel, 2004).

The role of miRNAs in regulating a wide range of biological activities has long been appreciated. Dysregulated miRNAs are associated with the development of various diseases including metabolic disorder, cardiovascular diseases, and cancer (Rupaimoole & Slack, 2017). In the immune system, mounting evidence has also demonstrated that miRNAs serve as important molecular regulators that control the development and function of many immune cell populations. For example, in T cells, absence of Dicer leads to impaired T cell development and aberrant differentiation in T helper cell subsets (Muljo et al., 2005). Likewise, ablation of Dicer

in B cells also results in defective B cell development and antibody responses (Koralov et al., 2008). Specifically, several miRNAs have been recently identified to play a critical role in regulating GC response and the resultant humoral immunity. For instance, miR-155 was previously reported to regulate GC response by negatively regulating AID expression in B cells (Teng et al., 2008). To this end, miR-155 deficiency in B cells leads to defective affinity maturation and failure in producing high affinity antibodies (Thai et al., 2007; Vigorito et al., 2007). In addition, the role of miR-146 in inhibiting GC B and TFH cell responses has also been well documented. Deficiency of miR-146 results in spontaneous accumulation of TFH and GC B cells at GCs (Pratama et al., 2015). Like Bcl-6, miR-146 is highly expressed and can function in a cell intrinsic manner in both TFH and GC B cells. T cell-specific deletion of miR-146 leads to accumulation of TFH cells and a subsequent increase in GC B cell population (Pratama et al., 2015). Similarly, B cell-specific depletion of miR-146 results in an autonomous expansion of GC B cells and a correspondingly increased TFH cell population (Cho et al., 2018). Nevertheless, considering the drastic phenotype observed in B cells devoid of the entire miRNA network, it is thus not surprising that miRNAs other than miR-155 and miR-146 could also play a pivotal role in regulating GC responses. To this end, we have recently demonstrated that the miR-23~27~24 family inhibits TFH cells by targeting multiple molecules essential for TFH cell biology (Wu et al., 2019).

Chapter 3: The miR-23~27~24 family

The miR-23~27~24 family is composed of multiple members and two paralogs: miR-23a~27a~24-2 (miR-23aC) and miR-23b~27b~24-1 (miR-23bC). Individual miRNA members within this family, miR-23a and miR-27a differ by one nucleotide with their corresponding paralogs in mature sequences, while miR-24-1 and miR-24-2 share the same mature sequences (Chhabra et al., 2010).

Previous studies have demonstrated the critical role of miR-23 family in regulating T cell responses. Individual members of the miR-23 family play diverse roles in controlling the differentiation and function of various effector T cell subsets and regulatory T (Treg) cells. While miR-24 and miR-27 collaboratively limit T helper 2 (Th2) cells by inhibiting GATA3 and IL-4, forced expression of miR-24 promotes Th1 and Th17 response through inhibition of TCF1 (Cho et al., 2016; Cho et al., 2017). miR-27 can also inhibit Treg cells by targeting multiple genes important for Treg cell differentiation and function, including c-Rel, IL-10, and GZMB (Cruz et al., 2017). It has also been shown that miR-23 functions as a key regulator that inhibits CD8+ cytotoxic T cell effector function by inhibiting Blimp-1 expression (Lin et al., 2014).

As discussed above, we have previously demonstrated the critical role of miR-23 family in regulating humoral immunity. T cell-specific ablation of miR-23 family resulted in enhanced TFH cell differentiation, which in turn resulted in enhanced GC B cell response and elevated antibody production. Individual members of miR-23 family collaborate with each other to limit TFH cell responses, as enforced expression of any individual member could all result in impaired TFH and the corresponding GC B cell responses. Mechanistically, miR-23~27~24 clusters target a network of genes including a transcription factor, thymocyte selection–associated HMG-box protein (TOX) that are crucial for TFH cell biology (Wu et al., 2019). Considering the

aforementioned notion of a possible presence of shared regulatory circuits between TFH and GC B cells, it is thus plausible that miR-23 family might also play a cell-intrinsic role in regulating GC B cell responses. Indeed, our preliminary studies have demonstrated that like what was observed in TFH cells, a similar up-regulation of miR-23 family could also be found in GC B cells. In this study, by establishing mouse models with B cell-specific deletion or overexpression of miR-23 family, we have identified the miR-23 clusters as cell intrinsic inhibitors of GC B cells. Interestingly, however, while that the entire miR-23 clusters coordinately limit GC B cell responses, unlike what was found in TFH cells, individual members of the miR-23 family contribute to GC B cell regulation in distinct manners. Moreover, despite the fact that GC B cell responses were clearly impacted by the deletion or overexpression of the miR-23 family in B cells, the frequencies of TFH cells in those mice remained unchanged. These results implied that while the miR-23 family serves as negative regulators in limiting GC B cell differentiation and function, they might also play a positive role in promoting GC B/TFH cell interaction. Further RNA-seq analysis has supported this notion as a network of genes that are involved in GC B cell biology and T cell-B cell interactions were respectively up- and down-regulated in the absence of miR-23 family-mediated regulation. Together, our work demonstrates that the miR-23 clusters are pivotal molecular regulators to ensure optimal GC B cell responses not only through targeting TFH cells as shown previously but also by directly inhibiting GC B cells in a cell-intrinsic manner.

RESULTS

Chapter 1: miR-23 Family Inhibits GC B Cell Response

Previously, we have shown that the miR-23 family is crucial for modulating humoral immunity by controlling TFH cell response (Wu et al., 2019). Given that many regulatory circuits were found to be shared between TFH and GC B cells, we hypothesized that the miR-23 family also plays a pivotal role in regulating GC B cell response through cell intrinsic mechanisms. To test our hypothesis, we first examine the expression of miR-23 family in GC B cells versus non-GC B cells. Our results showed that all individual members of the miR-23 family were upregulated in GC B cells (Fig.1), implying a cell intrinsic regulatory role of miR-23 family in GC B cells.

To study the role of miR-23 family in GC B cells, we generated mice with B cell-specific deletion of both miR-23 clusters (DKO) where miR-23a/b cluster floxed (miR-23a/bC$^{fl/fl}$) mice were crossed with CD19-Cre mice. In addition to loss-of-function study, to examine the role of miR-23 family in B cells, we also employed a gain-of-function approach by generating mice with B cell-specific overexpression of miR-23a cluster (CTg) using a similar method (Fig.2).

Upon obtaining the mice, we first examined GC B cell response in the context of acute infection. Mice were infected with lymphocytic choriomeningitis virus (LCMV), and GC B cell response was accessed on day 8 post infection. Consistent with what was observed in the TFH study, the DKO mice exhibited increased GC B cell frequencies, while reduced frequencies of GC B cells was observed in CTg mice when compared to wild-type (WT) mice (Fig.3 A and B). These results suggest that similar to its role in controlling TFH cells, miR-23 family also plays an inhibitory role in regulating GC B cell response in a cell intrinsic manner.

Since elevated GC B cell response was observed in DKO mice, we sought to examine if antibody production, another main functional feature of humoral immunity, is also affected by the disruption of the miR-23 family. To this end, mice were immunized with 4-hydroxy-3-nitrophenylacetyl (NP)-ovalbumin (OVA). This approach not only allowed us to measure GC B cell responses but also permit the assessment of NP-specific antibody production. Analogous to the LCMV infection studies, similar GC B cell phenotype was observed on day 8 post immunization, where mice with miR-23 family-deficient B cells exhibited elevated GC B cell frequencies. On the other hand, enforced miR-23 family expression in B cells resulted in diminished GC B cell responses (Fig.4 A and B). These findings were further supported by histological analysis of GCs, where enlarged GCs were observed in the DKO mice, but decreased in CTg mice (Fig.4 C). Consequently, DKO mice produced higher levels of NP-specific IgG1 antibodies, while CTg mice produced less in comparison to the WT mice (Fig. 4D).

Chapter 2: Distinct Roles of different miR-23 Family Members in Regulating GC B Cells

Thus far, we have identified an inhibitory role of miR-23 family in regulating GC B cell response, but how individual members of miR-23 family contribute to the regulation of GC B cell response is still unclear. Previous study demonstrated that every member of the miR-23 family collaborate with each other to limit TFH cell response (Wu et al., 2019). Given the similar role of miR-23 family in regulating TFH and GC B cell responses, we hypothesized that members of miR-23 family might also modulate GC B cell response in the same manner. To directly test our hypothesis, we generated mice with B cell-specific overexpression of individual miR-23 family members (23Tg, 24Tg, and 27Tg) (Fig.2).

Surprisingly, different from what we expected, under acute infection with LCMV, while overexpression of miR-27 did result in reduced GC B cell frequency, similar to what we observed in CTg mice, enforced expression of miR-23 actually led to elevated GC B cell frequencies. On the other hand, overexpression of miR-24 did not seem to have any detectable effect on GC B cell response (Fig.5 A and B). The same phenotype was also observed in the NP-OVA immunization model (Fig.5 A and C). These results showed that within the miR-23 family, miR-27 plays a dominant role in driving the GC B cell phenotype and that the antagonizing effect from miR-23 might be critical to ensure an optimal GC B cell response required to protect us the host from infection without unwanted autoimmunity.

As discussed above, T-B interaction at GCs is essential for proper GC response. Defects in TFH cells result in compromised GC B cell response, while impaired GC B cells also lead to reduced TFH cell frequencies (Chakhtoura et al., 2021; Ron & Sprent, 1987). Supporting this notion, our previous analysis of mice devoid of the miR-23 family in T cells has also demonstrated an indirect effect of T cell-specific ablation of the miR-23 family on

GC B cell response (Wu et al., 2019). Interestingly, however, inconsistent with our previous studies, modulation of the miR-23 family in GC B cells did not appear to have any impact on TFH cell response. DKO and CTg mice exhibited comparable levels of TFH response in both LCMV infection and NP-OVA immunization models, regardless of changes in GC B cell frequencies (Fig.6 B and C). These results suggest that in addition to serving as negative regulators in limiting GC B cell differentiation and function, the miR-23 family might play an opposite role in promoting GC B/TFH cell interaction.

Chapter 3: miR-23 Family Regulates a Network of Genes in GC B Cells

To investigate the molecular mechanisms underlying miR-23 family-mediated regulation of GC B cell response, we first sought to examine TOX, a transcription factor which was previously identified as the direct target of miR-23 family in TFH cells. As described in our previous study, TOX is induced in TFH cells in a Bcl-6 manner and modulation of miR-23 family in T cells resulted in altered TOX expression (Wu et al., 2019). Consistent with a well-established role of Bcl-6 in GC B cells, TOX was also found to be up-regulated in GC B cells (Fig.7A). Therefore, it is possible that TOX is also targeted by miR-23 family in GC B cells and the miR-23 family-TOX axis is crucial to control GC B cell responses. Interestingly, however, unlike what we have observed in TFH cells, modulation of miR-23 family expression did not affect TOX expression in GC B cells. As shown in Fig. 7B, TOX expression in GC B cells of DKO and CTg mice is comparable to that in WT mice. Moreover, TOX depletion in B cells actually resulted in an elevated GC B cell response, inconsistent with what was observed in CTg mice, where GC B cell response was reduced (Fig.7C and D). These results suggest that while TOX remains a functional important molecule in regulating GC B cell responses, it is not a direct target of miR-23 family in GC B cells, and therefore cannot explain the mechanism of miR-23 family mediated regulation of GC B cell response.

To further investigate the potential molecular mechanisms employed by miR-23 clusters to control GC B cell responses, we took an unbiased approach to examine genes that are differentially regulated in a miR-23 family-dependent manner. To this end, GC B cells sorted from LCMV infected mice of each genotype were subjected to bulk RNA-sequencing. Next, we examined the expression pattern of genes involved in GC B cell response in GC B cells isolated from DKO mice (Fig.8A and B). Differentially expressed genes from Fig.8B were selected as

potential direct and indirect targets of miR-23 family. The expression level of this list of genes was further analyzed in GC B cells isolated from CTg mice (Fig.8C). Considering the opposite GC B cell phenotypes found in DKO and CTg mice, we reasoned that the potential molecules that could account for the observed phenotype should also demonstrate the opposite expression patterns between GC B cells isolated from these two mouse lines. Therefore, genes with opposite expression patterns between GC B cells isolated from DKO and CTg mice in comparison to the WT were selected (Fig.8D). Among them, genes such as IL-4Ra and Myc that have been previously shown to promote formation and maintenance of germinal centers (Gonzalez et al., 2018; Calado et al., 2012), were up-regulated in GC B cells from DKO mice while down-regulated in those from CTg mice. On the other hand, genes, such as TNFAIP3 and Pten, that are known to restrict GC B cell survival and BCR signaling (Tavares et al., 2010; Luo et al., 2019), were down-regulated in DKO GC B cells but found to be up-regulated in CTg GC B cells. As previously discussed, miR-27 likely plays a dominant role in driving the miR-23 family-dependent phenotype in GC B cells (Fig.5). Therefore, genes with similar expression patterns in 27Tg and CTg GC B cells but with the opposite expression patterns in DKO GC B cells were further explored. To this end, BATF and Myc were found to be down-regulated in both CTg and 27Tg GC B cells but up-regulated in DKO GC B cells, while expression of Pten showed the reversed trend (Fig.8D and E). While we could not find any miR-27 binding site in those genes, their upstream regulators could be targets of miR-27.

DISCUSSION

While the importance of humoral immunity in protecting the host from various invading pathogens has been well appreciated, the comprehensive understanding of how humoral immunity is being regulated is still limited. In this study, we identified the miR-23 family as cell intrinsic regulators to ensure optimal GC B cell responses. To this end, our data clearly demonstrated that B cell-specific depletion of the miR-23 clusters resulted in enhanced GC B cell response and antibody production whereas enforced expression of this miRNA family led to impaired GC B cell responses and the resultant humoral immunity.

Prior to this study, our previous work has already demonstrated an important regulatory role of the miR-23 family in controlling humoral immunity. To this end, it was shown that depletion of miR-23 family in T cells resulted in elevated TFH cell frequencies, suggesting that the miR-23 family limits humoral immune responses via targeting TFH cells (Wu et al., 2019). In the current study, while we have observed a similar inhibitory effect of miR-23 family in modulating GC B cell responses, further analysis showed discrepancies between the regulatory mechanisms employed by the miR-23 family in regulating these two immune cell populations. First, individual members of the miR-23 family were found to collaborate with others to regulate TFH cell response, as similarly reduced TFH cell response was observed in mice with T cell-specific overexpression of any miR-23 family member (Wu et al., 2019). On the other hand, despite the overall inhibitory effect of the miR-23 family in controlling GC B cell response, our results demonstrated that only miR-27 overexpression could lead to reduced GC B cell responses, while miR-23 actually promotes them. This result, albeit surprising, is not completely unexpected, as similar antagonizing effects of the each miR-23 family member have also been detected in other T cell subsets. To this end, overexpression of miR-24 resulted in increased Th1

cell differentiation, whereas overexpression of miR-27 resulted in reduced Th1 cell responses (Cho et al., 2016). Secondly, while miR-23 family regulates TFH cell response by directly targeting TOX, our current studies ruled out TOX a target of miR-23 family in GC B cells, as modulation of miR-23 family did not affect TOX expression in GC B cells. Moreover, when TOX was depleted in B cells, enhanced rather than reduced GC B cell response was observed, inconsistent with what we observed in mice with B cell-specific miR-23 family overexpression. Finally, in our previous study, modulating miR-23 family in T cells not only lead to altered TFH cell responses but could also indirectly impact GC B cells due to the bidirectional effects of T-B interaction for mounting proper GC response (Wu et al., 2019; Chakhtoura et al., 2021). However, in our current study, altered GC B cell responses resulted from B cell-specific modulations of the miR-23 family did not lead to any changes in TFH cell frequencies. These results suggested that while the miR-23 family generally plays an inhibitory role in GC B cells, it can also act as a positive regulator to promote T-B interactions.

Our analysis of RNA-seq data further revealed possible molecular mechanisms that could account for miR-23 family-mediated regulation of GC B cells. To this end, BATF, Myc, and Pten were identified as potential indirect targets of miR-27 that can play major regulatory roles in GC B cells. Previously, BATF and Myc are both shown to be crucial in promoting GC formation and maintenance (Inoue et al., 2017; Calado et al., 2012), while Pten is known to function as a negative regulator in BCR signaling pathways (Luo et al., 2019). While we could not find any miR-27 binding site in those genes, their upstream regulators could be targets of miR-27 responsible for the GC B cell phenotype observed in our miR-23 family mutant mice.

In this study, we demonstrated a key role of miR-23 family in regulating GC B cell response and total antigen-specific antibody production. Nevertheless, considering the complex

nature of GC responses and humoral immunity, further studies are needed to investigate the impact of the miR-23 family-mediated gene regulation in controlling PC and memory B cell differentiation. Moreover, additional experiments are also required to validate and identify the direct target of miR-23 family in regulating GC B cell biology. Collectively, it is undoubtedly that the knowledge obtained from these studies will not only help us gain a further appreciation of the pivotal role of miRNAs in regulating humoral immunity but also facilitate the development of novel approaches to target these important molecular regulators to elicit optimal humoral immunity against infection without causing unwanted autoimmunity.

MATERIALS & METHODS

Mice

Mice with B cell specific deletion of miR-23 family were generated by breeding miR-23~27~24a/b$^{fl/fl}$ mice to CD19-Cre mice. Mice carrying miR-23a cluster transgene and transgene of individual miR-23 family members were generated as previously described (Wang et al., 2013). Mice with B cell specific overexpression of miR-23a cluster and individual members were generated by breeding the aforementioned mice to CD19-Cre mice. Mice with B cell specific deletion of TOX were generated by breeding TOX$^{fl/fl}$ mice to CD19-Cre mice. All mice were maintained and handled in accordance with the Institutional Animal Care and Use Guidelines of University of California, San Diego and National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines.

Infection and immunization

For LCMV infection, LCMV Armstrong stocks were prepared and quantified as previously described (Harker et al., 2011). Mice were inoculated intraperitoneally with 2×10^6 plaque-forming unit viruses and taken down on day 8 post infection for GC response study. For NP-OVA, mice were inoculated intraperitoneally with 200μg of NP-OVA (Biosearch technology) aluminum emulsion and taken down on day 8 post immunization for GC response study.

Enzyme-linked immunosorbent assay (ELISA)

For detection of NP-specific antibodies, NP_{27}-BSA antigen was coated onto a 96-well Costar assay plate. Plates were blocked with 1% bovine serum albumin (BSA) in phosphate-

buffered saline (PBS). Serial dilutions of sera in 1% BSA in PBS were applied to the plates and incubated at room temperature for 2 hours. Plates were incubated for 1 hour with horseradish peroxidase-conjugate antibody against total mouse IgG1 (Jackson ImmunoReseach). 3,3′,5,5′-tetramethylbenzidine substrate (BioLegend) was used for color development. Absorbance was measured at 450nm with a microplate reader (Molecular Devices). Plates were washed with 0.05% Tween in PBS between each step before color development.

Immunophenotyping and flow cytometry

Single-cell suspensions were prepared from spleens by slide mechanical grind in PBS. For all FACS analysis, cells were first stained with Ghost Dye Red 780 (Tonbo Biosciences), followed by subsequent staining. Surface staining includes Abs against CD4, CD8α, CD44, B220, and GL7(Thermo Fisher Scientific), and peanut agglutinin (PNA) (Vector Laboratories).For CXCR staining, cells were stained with purified Ab against CXCR5 (BD Biosciences) for 1 hour, followed by staining with biotinylated goat anti-rat IgG (Jackson ImmunoResearch) for 30 min, and then surface staining was performed with indicated Abs as well as fluorescence-labeled streptavidin (Thermo Fisher Scientific). FOXP3/ Transcription Factor Staining kit was used for intracellular staining according to the manufacturer's instructions (Tonbo Biosciences). Intracellular staining of FOXP3, TOX (Thermo Fisher Scientific), and BCL6 (BD Biosciences) was performed after fixation and permeabilization.

Immunostaining

Freshly dissected spleens were rapidly frozen in Tissue-Tek O.C.T. (Sakura). Sections 10 μm in thickness were cut with CryoStar NX50 (Thermo Fisher Scientific), attached on glass

slides, and fixed in cold acetone for 20 minutes, followed by air drying. Sections were blocked with 10% normal rat serum and 1% BSA in PBS for 30 minutes and stained with anti-CD4-phycoerythrin (PE), anti-GL7-AlexaFluor488, and anti-IgD-eFluor450 (Thermo Fisher Scientific) overnight at 4℃ and washed 3 times with 1% Tween in PBS. Images were acquired on an LSM 700 system (Carl Zeiss Inc.).

Quantitative real-time PCR

For detecting TOX expression in GC B cells, total RNA of FACS-sorted GC B cells ($CD4^{-}B220^{+}PNA^{+}GL7^{+}$) was extracted using the RNeasy kit (QIAGEN). Complementary DNAs (cDNAs) were generated by the iScript cDNA synthesis kit (Bio-Rad Laboratories).And real-time PCR was performed using SYBR Green PCR kits (Applied Biosystems). For detecting expression level of miR-23 family members in GC B cells, TaqMan MicroRNA Assay (Thermo FisherScientific) was performed. All real-time reactions were run on a 7900HT Fast Real-Time PCR System (Thermo Fisher Scientific). The primer sequence used are as follows:

TOX: 5’-AACTCGAGTTGCGCACTCAAAGCAGTTC-3’(F),

5’-AAGCGGCCGCTGCAGCTAAGATGCCAGTGA-3’ (R).

Gapdh: 5'-CGTCCCGTAGACAAAATGGT -3’ (F),

5'-TCAATGAAGGGGTCGTTGAT-3’ (R).

Gene expression profiling

GC B cells in the spleen of LCMV infected WT and mutant mice were sorted on a FACSAria II cell sorter (BD Biosciences) followed by total RNA isolation with TRIZOL. Poly-A RNA-sequencing was performed using two biological replicates for each cell population.

Sequenced reads were aligned to mouse reference genome (mm9) with STAR (Dobin et al., 2013)on San Diego Supercomputer Center (SDSC)-Triton Shared Computing Cluter (TSCC) (SDSC, 2022). Sequence read counts were obtained using featureCounts (Liao et al., 2014), and differential expression analysis was performed with DEseq2 (Love et al., 2014). Seaborn was used to generate gene expression heatmaps (Waskom, 2021).

Statistical analysis

Unpaired Student's t test was done on all reported data using Prism software (GraphPad) (*P < 0.05; **P < 0.01; ***P < 0.001)

Cheng-Jang Wu, Jiayi Dong, and Nimi Marcel contributed to producing data presented in the thesis. The thesis author is the primary author of this thesis.

FIGURES & FIGURE LEGENDS

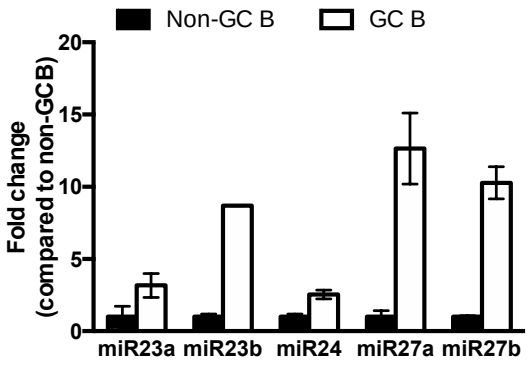


Figure 1. miR-23 family is upregulated in GC B cells. qPCR analysis for the expression of individual miR-23 family members in GC B cells and non-GC B cells. Total splenocytes were sorted for $B220^{+}PNA^{+}GL7^{+}$ GC B cells and $B220^{+}PNA^{-}GL7^{-}$ non-GC B cells.

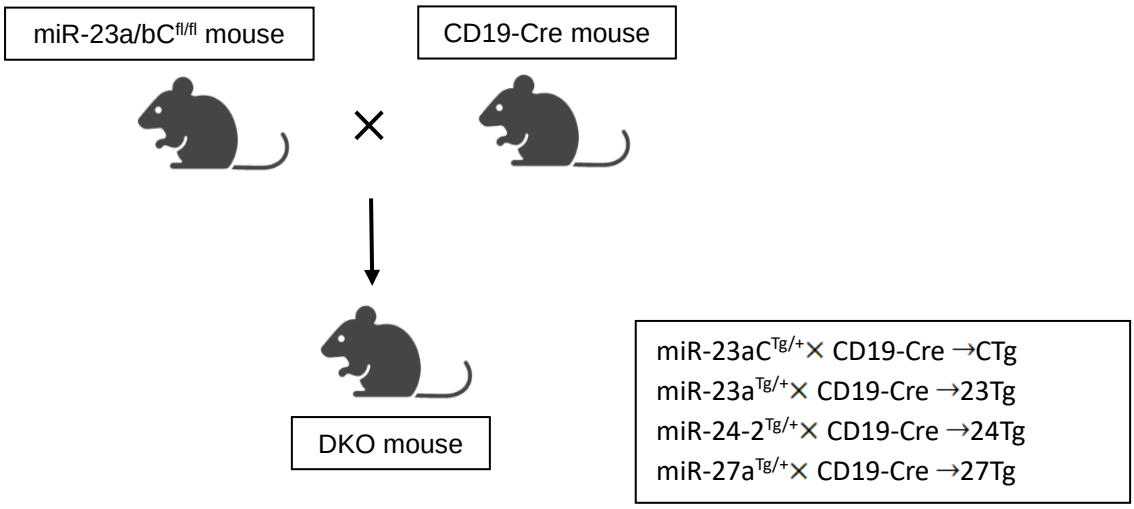


Figure 2. Schematic of the generation of B cell conditional knockout and overexpression mice. Cre mediated recombination under control of CD19 promoter results in B cell specific deletion and overexpression of miR-23 clusters and individual family members.

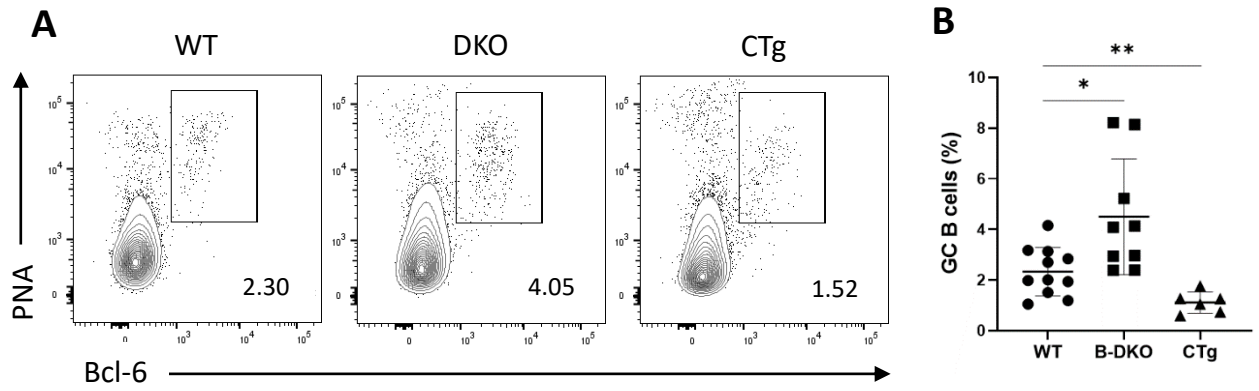


Figure 3. miR-23 family inhibits GC B cell responses during acute infection. (A) Fluorescence activated cell sorting (FACS) analysis, (B) frequencies of $PNA^{+}Bcl\text{-}6^{+}$ GC B cells in the spleen from ~12-week-old DKO and CTg mice compared to WT controls 8 days after LCMV infection.

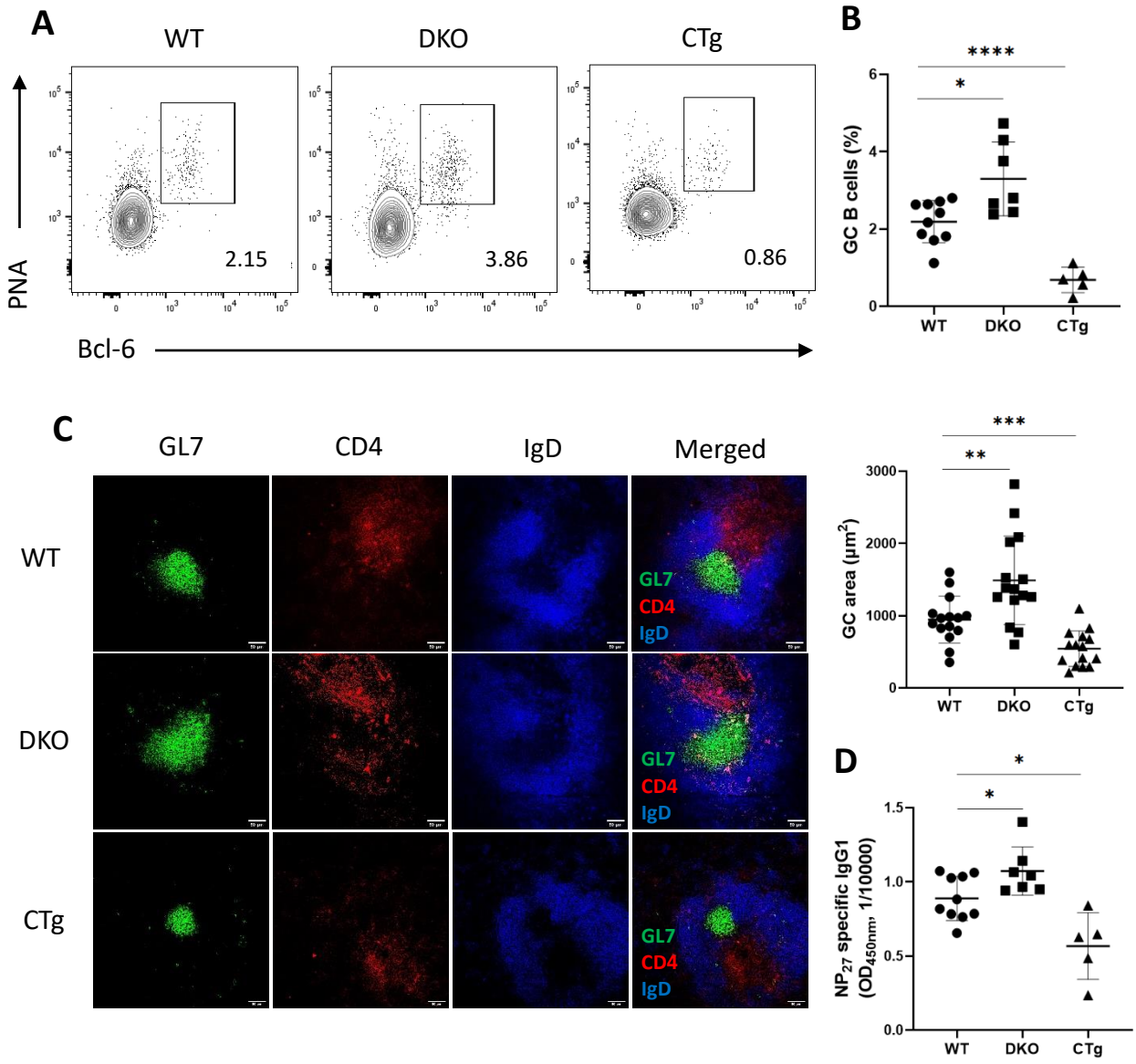


Figure 4. miR-23 family inhibits GC B cell response and antibody production in immunization. (A) FACS analysis, (B) frequencies of $PNA^{+}Bcl\text{-}6^{+}$ GC B cells in the spleen from ~12-week-old DKO and CTg mice compared to WT controls 8 days after NP-OVA immunization. (C) Immunohistological analysis of GC response in NP-OVA immunized spleen sectioned and stained forGL7 (green), CD4 (red), and IgD (blue). (D) Enzyme-linked immunosorbent assay analysis of total NP-specific IgG1 antibodies in sera of DKO or CTg mice compared to WT controls (optical density at 450nm).

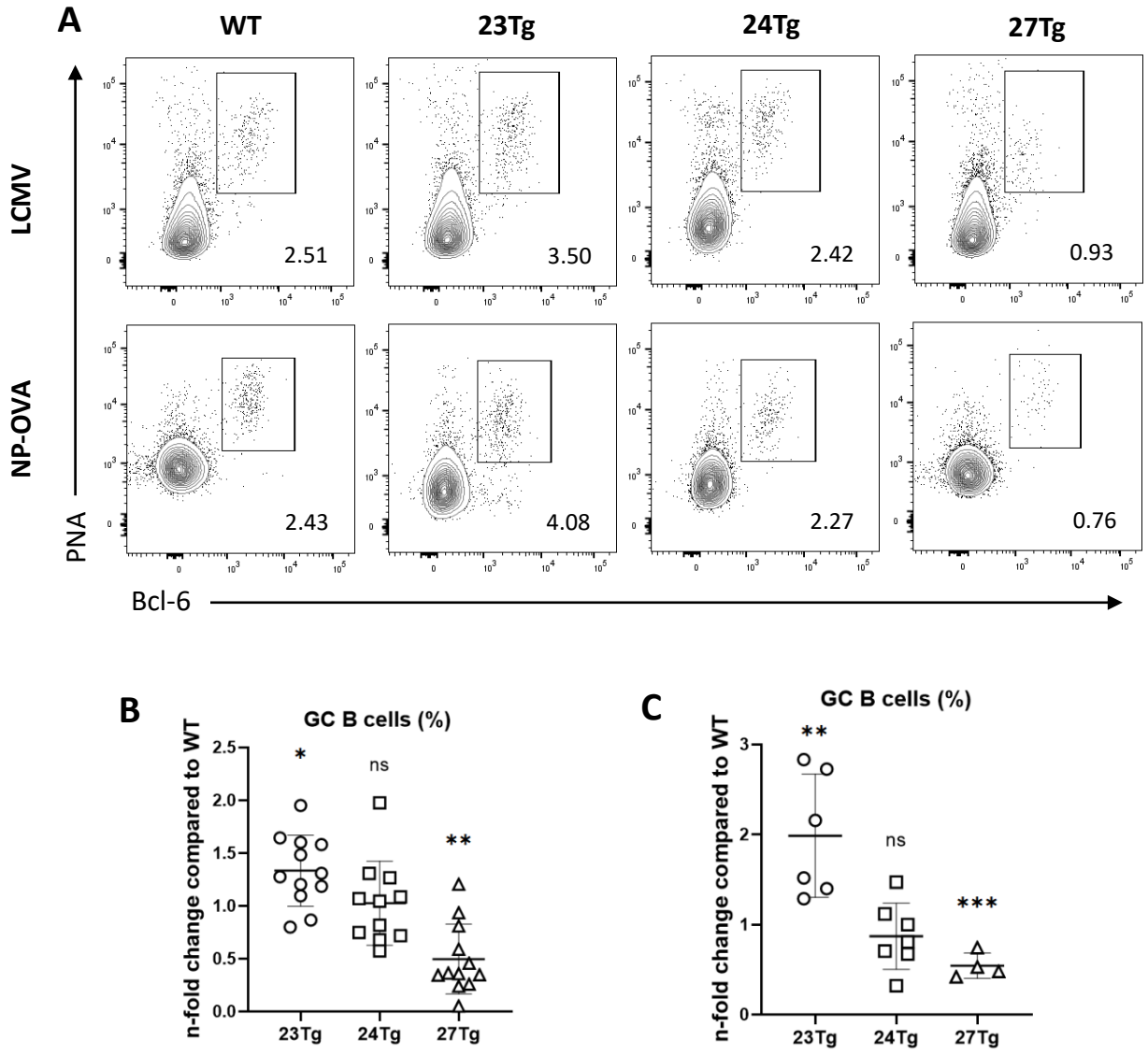


Figure 5. miR-23 family members antagonize each other in regulating GC B cell response. (A) FACS analysis, (B) fold-change of frequencies of $PNA^{+}Bcl\text{-}6^{+}$ GC B cells in the spleen from ~12-week-old 23Tg, 24Tg, and 27Tg mice compared to WT controls 8 days after LCMV infection and (C) NP-OVA immunization.

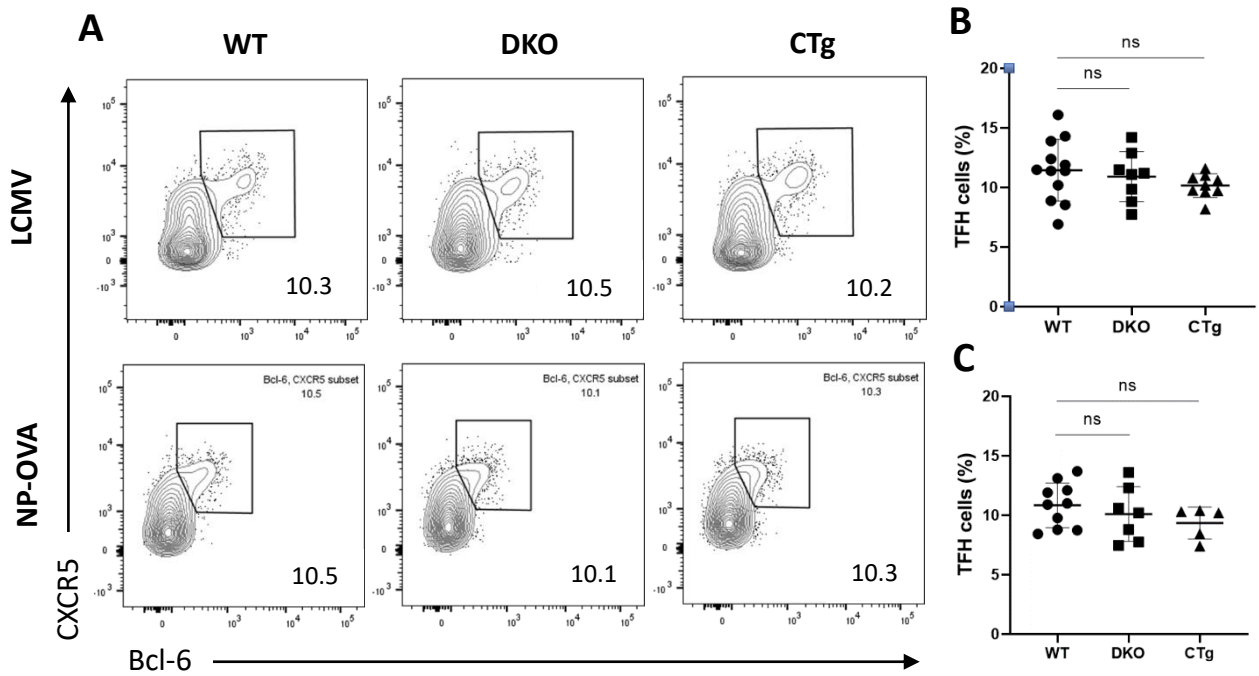


Figure 6. Modulation of miR-23 family in GC B cells does not affect TFH cell responses. (A) FACS analysis, (B) frequencies of $CXCR5^{+}Bcl\text{-}6^{+}$ TFH cells in the spleen from ~12-week-old DKO and CTg mice compared to WT controls 8 days after LCMV infection and (C) NP-OVA immunization.

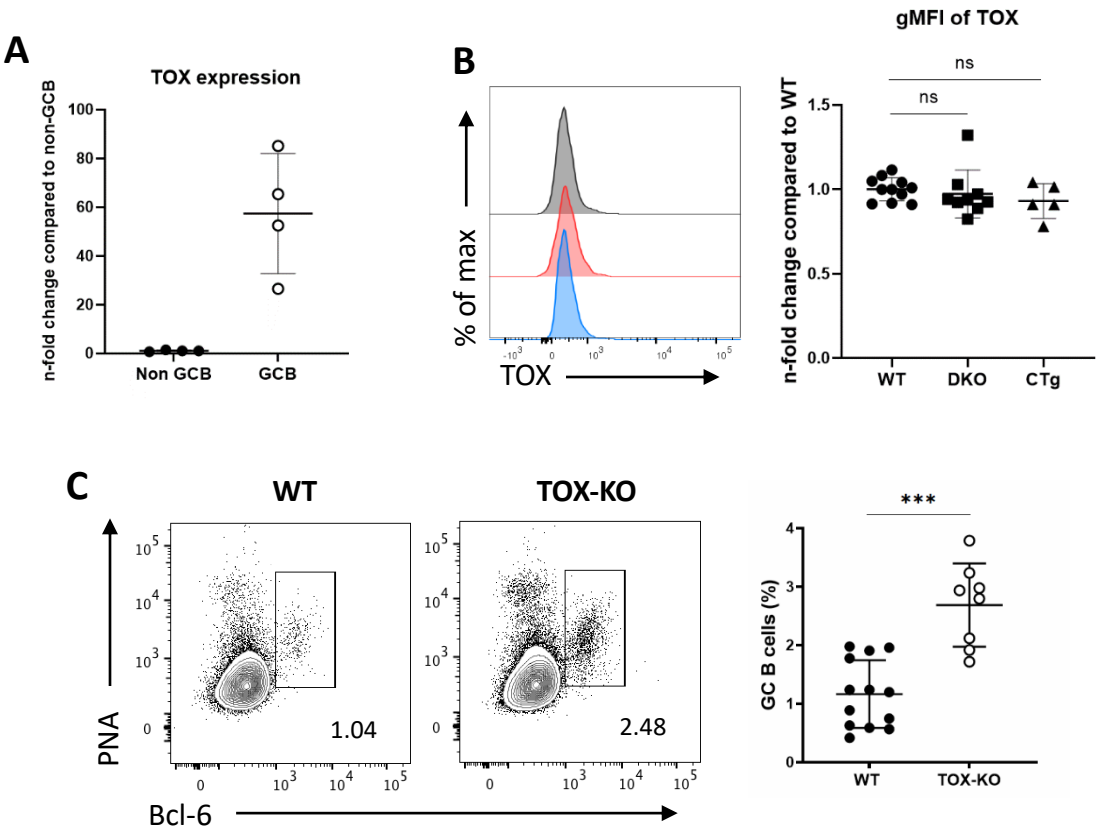
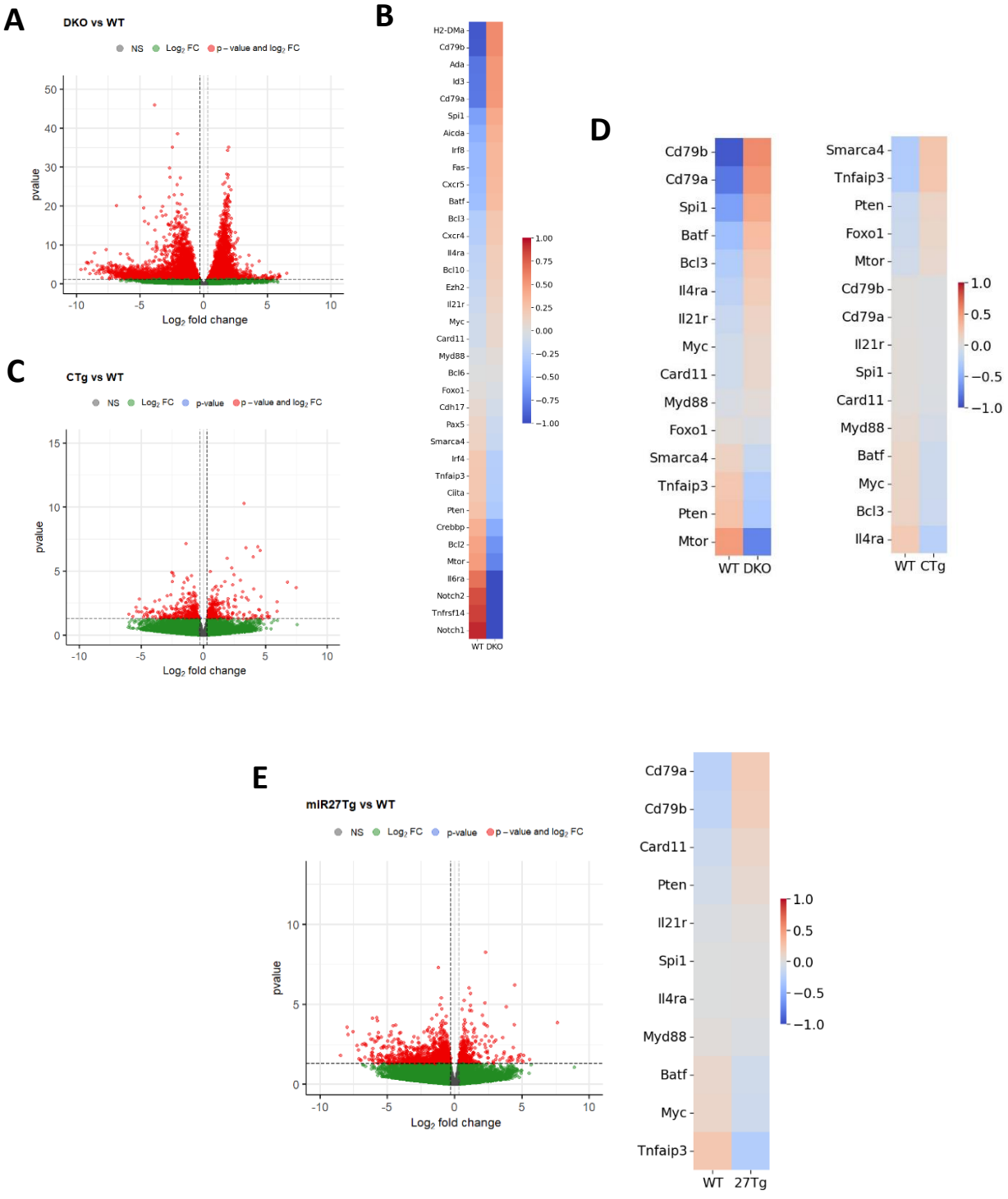


Figure 7. TOX is not a target of miR-23 family in GC B cells. (A) qPCR analysis for the expression of TOX in GC B cells and non-GC B cells. Total splenocytes were sorted for $B220^{+}PNA^{+}GL7^{+}$ GC B cells and $B220^{+}PNA^{-}GL7^{-}$ non-GC B cells. (B) FACS analysis (left)and fold change of TOX gMFIin $PNA^{+}Bcl\text{-}6^{+}$ GC B cells (right) from ~12-week-old DKO and CTg mice compared to WT controls 8 days after NP-OVA immunization. (C) FACS analysis (left) and frequencies of $PNA^{+}Bcl\text{-}6^{+}$ GC B cells(right) from 10-12-week-old B cell specific TOX knockout mice compared to WT controls.

Figure 8. miR-23 family regulates a network of genes in GC B cells. (A and C) Volcanos plot demonstrating gene expression in GC B cells of DKO mice and CTg mice compared to WT controls, respectively. (B) Expression pattern of genes associated with GC B cell development and function in GC B cells of DKO mice compared to WT controls. (D) Gene expression in GC B cellsof DKO and CTg mice that demonstrated opposite trend compared to WT controls. (E) Volcano plot demonstrating gene expression in GC B cells of 27Tg mice compared to WT (left) and expression pattern of the list genes in panel D in GC B cells of 27Tg mice compared to WT controls (right).


A
DKO vs WT
NS
Log2 FC
p – value and log2 FC
pvalue
Log2 fold change
B
H2-DMa
Cd79b
Ada
Id3
Cd79a
Spi1
Aicda
Irf8
Fas
Cxcr5
Batf
Bcl3
Cxcr4
Il4ra
Bcl10
Ezh2
Il21r
Myc
Card11
Myd88
Bcl6
Foxo1
Cdh17
Pax5
Smarca4
Irf4
Tnfaip3
Ciita
Pten
Crebbp
Bcl2
Mtor
Il6ra
Notch2
Tnfrsf14
Notch1
WT DKO
C
CTg vs WT
NS
Log2 FC
p-value
p – value and log2 FC
pvalue
Log2 fold change
D
Cd79b
Cd79a
Spi1
Batf
Bcl3
Il4ra
Il21r
Myc
Card11
Myd88
Foxo1
Smarca4
Tnfaip3
Pten
Mtor
WT DKO
Smarca4
Tnfaip3
Pten
Foxo1
Mtor
Cd79b
Cd79a
Il21r
Spi1
Card11
Myd88
Batf
Myc
Bcl3
Il4ra
WT CTg
E
miR27Tg vs WT
NS
Log2 FC
p-value
p – value and log2 FC
pvalue
Log2 fold change
Cd79a
Cd79b
Card11
Pten
Il21r
Spi1
Il4ra
Myd88
Batf
Myc
Tnfaip3
WT 27Tg

REFERENCES

Avery, D. T., Deenick, E. K., Ma, C. S., Suryani, S., Simpson, N., Chew, G. Y., Chan, T. D., Palendira, U., Bustamante, J., Boisson-Dupuis, S., Choo, S., Bleasel, K. E., Peake, J., King, C., French, M. A., Engelhard, D., Al-Hajjar, S., Al-Muhsen, S., Magdorf, K., Roesler, J., Arkwright, P.D., Hissaria, P., Riminton, D.S., Wong, M., Brink, R., Fulcher, D.A., Casanova, J.-L., Cook, M.C., Tangye, S. G. (2010). B cell–intrinsic signaling through IL-21 receptor and STAT3 is required for establishing long-lived antibody responses in humans. *Journal of Experimental Medicine*, *207*(1), 155–171. https://doi.org/10.1084/jem.20091706

Bartel, D. P. (2004). Micrornas: Genomics, biogenesis, mechanism, and function. *Cell*, *116*(2), 281–297. https://doi.org/10.1016/S0092-8674(04)00045-5

Bushati, N., & Cohen, S. M. (2007). Microrna functions. *Annual Review of Cell and Developmental Biology*, *23*(1), 175–205. https://doi.org/10.1146/annurev.cellbio.23.090506.123406

Calado, D. P., Sasaki, Y., Godinho, S. A., Pellerin, A., Köchert, K., Sleckman, B. P., de Alborán, I. M., Janz, M., Rodig, S., & Rajewsky, K. (2012). The cell-cycle regulator c-Myc is essential for the formation and maintenance of germinal centers. *Nature Immunology*, *13*(11), 1092–1100. https://doi.org/10.1038/ni.2418

Chakhtoura, M., Fang, M., Cubas, R., O'Connor, M. H., Nichols, C. N., Richardson, B., Talla, A., Moir, S., Cameron, M. J., Tardif, V., & Haddad, E. K. (2021). Germinal Center T follicular helper (Gc-tfh) cell impairment in chronic HIV infection involves c-Maf signaling. *PLoS Pathogens*, *17*(7), e1009732. https://doi.org/10.1371/journal.ppat.1009732

Chhabra, R., Dubey, R., & Saini, N. (2010). Cooperative and individualistic functions of the microRNAs in the miR-23a~27a~24-2 cluster and its implication in human diseases. *Molecular Cancer*, *9*(1), 232. https://doi.org/10.1186/1476-4598-9-232

Cho, S., Lee, H.-M., Yu, I.-S., Choi, Y. S., Huang, H.-Y., Hashemifar, S. S., Lin, L.-L., Chen, M.-C., Afanasiev, N. D., Khan, A. A., Lin, S.-W., Rudensky, A. Y., Crotty, S., & Lu, L.-F. (2018). Differential cell-intrinsic regulations of germinal center B and T cells by miR-146a and miR-146b. *Nature Communications*, *9*(1), 2757. https://doi.org/10.1038/s41467-018-05196-3

Cho, S., Wu, C.-J., Nguyen, D. T., Lin, L.-L., Chen, M.-C., Khan, A. A., Yang, B.-H., Fu, W., & Lu, L.-F. (2017). A novel mir-24–tcf1 axis in modulating effector t cell responses. *The Journal of Immunology*, *198*(10), 3919–3926. https://doi.org/10.4049/jimmunol.1601404

Cho, S., Wu, C.-J., Yasuda, T., Cruz, L. O., Khan, A. A., Lin, L.-L., Nguyen, D. T., Miller, M., Lee, H.-M., Kuo, M.-L., Broide, D. H., Rajewsky, K., Rudensky, A. Y., & Lu, L.-F. (2016). Mir-23∼27∼24 clusters control effector t cell differentiation and function. *Journal of Experimental Medicine*, *213*(2), 235–249. https://doi.org/10.1084/jem.20150990

Coico, R. F., Bhogal, B. S., &Thorbecke, G. J. (1983). Relationship of germinal centers in lymphoid tissue to immunologic memory. VI. Transfer of B cell memory with lymph node cells fractionated according to their receptors for peanut agglutinin. *The Journal of Immunology*, *131*(5), 2254–2257. https://doi.org/10.4049/jimmunol.131.5.2254

Crotty, S. (2011). Follicular helper cd4 t cells (T FH). *Annual Review of Immunology*, *29*(1), 621–663. https://doi.org/10.1146/annurev-immunol-031210-101400

Cruz, L. O., Hashemifar, S. S., Wu, C.-J., Cho, S., Nguyen, D. T., Lin, L.-L., Khan, A. A., & Lu, L.-F. (2017). Excessive expression of miR-27 impairs Treg-mediated immunological tolerance. *The Journal of Clinical Investigation*, *127*(2), 530–542. https://doi.org/10.1172/JCI88415

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., &Gingeras, T. R. (2013). STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics (Oxford, England)*, *29*(1), 15–21. https://doi.org/10.1093/bioinformatics/bts635

Foy, T. M., Laman, J. D., Ledbetter, J. A., Aruffo, A., Claassen, E., & Noelle, R. J. (1994). Gp39-cd40 interactions are essential for germinal center formation and the development of b cell memory. *Journal of Experimental Medicine*, *180*(1), 157–163. https://doi.org/10.1084/jem.180.1.157

Gonzalez, D. G., Cote, C. M., Patel, J. R., Smith, C. B., Zhang, Y., Nickerson, K. M., Zhang, T., Kerfoot, S. M., & Haberman, A. M. (2018). Nonredundant roles of il-21 and il-4 in the phased initiation of germinal center b cells and subsequent self-renewal transitions. *The Journal of Immunology*, *201*(12), 3569–3579. https://doi.org/10.4049/jimmunol.1500497

Good-Jacobson, K. L., Szumilas, C. G., Chen, L., Sharpe, A. H., Tomayko, M. M., &Shlomchik, M. J. (2010). PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells. *Nature Immunology*, *11*(6), 535–542. https://doi.org/10.1038/ni.1877

Grewal, I. S., Xu, J., & Flavell, R. A. (1995). Impairment of antigen-specific T-cell priming in mice lacking CD40 ligand. *Nature*, *378*(6557), 617–620. https://doi.org/10.1038/378617a0

Harker, J. A., Lewis, G. M., Mack, L., & Zuniga, E. I. (2011). Late interleukin-6 escalates t follicular helper cell responses and controls a chronic viral infection. *Science*, *334*(6057), 825–829. https://doi.org/10.1126/science.1208421

He, L., & Hannon, G. J. (2004). MicroRNAs: Small RNAs with a big role in gene regulation. *Nature Reviews Genetics*, *5*(7), 522–531. https://doi.org/10.1038/nrg1379

Inoue, T., Shinnakasu, R., Ise, W., Kawai, C., Egawa, T., & Kurosaki, T. (2017). The transcription factor Foxo1 controls germinal center B cell proliferation in response to T cell help. *The Journal of Experimental Medicine*, *214*(4), 1181–1198. https://doi.org/10.1084/jem.20161263

Klein, U., & Dalla-Favera, R. (2008). Germinal centres: Role in B-cell physiology and malignancy. *Nature Reviews Immunology*, *8*(1), 22–33. https://doi.org/10.1038/nri2217

Koralov, S. B., Muljo, S. A., Galler, G. R., Krek, A., Chakraborty, T., Kanellopoulou, C., Jensen, K., Cobb, B. S., Merkenschlager, M., Rajewsky, N., &Rajewsky, K. (2008). Dicer ablation affects antibody diversity and cell survival in the b lymphocyte lineage. *Cell*, *132*(5), 860–874. https://doi.org/10.1016/j.cell.2008.02.020

Liao, Y., Smyth, G. K., & Shi, W. (2014). Featurecounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, *30*(7), 923–930. https://doi.org/10.1093/bioinformatics/btt656

Lin, R., Chen, L., Chen, G., Hu, C., Jiang, S., Sevilla, J., Wan, Y., Sampson, J. H., Zhu, B., & Li, Q.-J. (2014). Targeting miR-23a in $CD8^+$ cytotoxic T lymphocytes prevents tumor-dependent immunosuppression. *The Journal of Clinical Investigation*, *124*(12), 5352–5367. https://doi.org/10.1172/JCI76561

Linterman, M. A., Beaton, L., Yu, D., Ramiscal, R. R., Srivastava, M., Hogan, J. J., Verma, N. K., Smyth, M. J., Rigby, R. J., &Vinuesa, C. G. (2010). IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. *Journal of Experimental Medicine*, *207*(2), 353–363. https://doi.org/10.1084/jem.20091738

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Luo, W., Hawse, W., Conter, L., Trivedi, N., Weisel, F., Wikenheiser, D., Cattley, R. T., &Shlomchik, M. J. (2019). The AKT kinase signaling network is rewired by PTEN to control proximal BCR signaling in germinal center B cells. *Nature Immunology*, *20*(6), 736–746. https://doi.org/10.1038/s41590-019-0376-3

Luo, W., & Yin, Q. (2021). B cell response to vaccination. *Immunological Investigations*, *50*(7), 780–801. https://doi.org/10.1080/08820139.2021.1903033

Meister, G. (2013). Argonaute proteins: Functional insights and emerging roles. *Nature Reviews Genetics*, *14*(7), 447–459. https://doi.org/10.1038/nrg3462

Merlo, L. M. F., & Mandik-Nayak, L. (2013). Chapter 3 - adaptive immunity: B cells and antibodies. In G. C. Prendergast & E. M. Jaffee (Eds.), *Cancer Immunotherapy (Second Edition)* (pp. 25–40). Academic Press. https://doi.org/10.1016/B978-0-12-394296-8.00003-8

Muljo, S. A., Ansel, K. M., Kanellopoulou, C., Livingston, D. M., Rao, A., &Rajewsky, K. (2005). Aberrant T cell differentiation in the absence of Dicer. *Journal of Experimental Medicine*, *202*(2), 261–269. https://doi.org/10.1084/jem.20050678

Nurieva, R. I., Chung, Y., Hwang, D., Yang, X. O., Kang, H. S., Ma, L., Wang, Y., Watowich, S. S., Jetten, A. M., Tian, Q., & Dong, C. (2008). Generation of t follicular helper cells is mediated by interleukin-21 but independent of t helper 1, 2, or 17 cell lineages. *Immunity*, *29*(1), 138–149. https://doi.org/10.1016/j.immuni.2008.05.009

Nutt, S. L., &Tarlinton, D. M. (2011). Germinal center B and follicular helper T cells: Siblings, cousins or just good friends? *Nature Immunology*, *12*(6), 472–477. https://doi.org/10.1038/ni.2019

Peled, J. U., Kuang, F. L., Iglesias-Ussel, M. D., Roa, S., Kalis, S. L., Goodman, M. F., & Scharff, M. D. (2008). The biochemistry of somatic hypermutation. *Annual Review of Immunology*, *26*(1), 481–511. https://doi.org/10.1146/annurev.immunol.26.021607.090236

Pratama, A., Srivastava, M., Williams, N. J., Papa, I., Lee, S. K., Dinh, X. T., Hutloff, A., Jordan, M. A., Zhao, J. L., Casellas, R., Athanasopoulos, V., &Vinuesa, C. G. (2015). MicroRNA-146a regulates ICOS–ICOSL signalling to limit accumulation of T follicular helper cells and germinal centres. *Nature Communications*, *6*(1), 6436. https://doi.org/10.1038/ncomms7436

Ron, Y., &Sprent, J. (1987). T cell priming in vivo: A major role for B cells in presenting antigen to T cells in lymph nodes. *The Journal of Immunology*, *138*(9), 2848–2856. https://doi.org/10.4049/jimmunol.138.9.2848

Rupaimoole, R., & Slack, F. J. (2017). MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases. *Nature Reviews Drug Discovery*, *16*(3), 203–222. https://doi.org/10.1038/nrd.2016.246

San Diego Supercomputer Center (2022): Triton Shared Computing Cluster. University of California, San Diego. Service. https://doi.org/10.57873/T34W2R

Shlomchik, M. J., & Weisel, F. (2012). Germinal center selection and the development of memory B and plasma cells: Germinal center differentiation and selection. *Immunological Reviews*, *247*(1), 52–63. https://doi.org/10.1111/j.1600-065X.2012.01124.x

Tavares, R. M., Turer, E. E., Liu, C. L., Advincula, R., Scapini, P., Rhee, L., Barrera, J., Lowell, C. A., Utz, P. J., Malynn, B. A., & Ma, A. (2010). The ubiquitin modifying enayme a20 restricts b cell survival and prevents autoimmunity. *Immunity*, *33*(2), 181–191. https://doi.org/10.1016/j.immuni.2010.07.017

Teng, G., Hakimpour, P., Landgraf, P., Rice, A., Tuschl, T., Casellas, R., & Papavasiliou, F. N. (2008). Microrna-155 is a negative regulator of activation-induced cytidine deaminase. *Immunity*, *28*(5), 621–629. https://doi.org/10.1016/j.immuni.2008.03.015

Thai, T.-H., Calado, D. P., Casola, S., Ansel, K. M., Xiao, C., Xue, Y., Murphy, A., Frendewey, D., Valenzuela, D., Kutok, J. L., Schmidt-Supprian, M., Rajewsky, N., Yancopoulos, G., Rao, A., &Rajewsky, K. (2007). Regulation of the germinal center response by microrna-155. *Science*, *316*(5824), 604–608. https://doi.org/10.1126/science.1141229

Vigorito, E., Perks, K. L., Abreu-Goodger, C., Bunting, S., Xiang, Z., Kohlhaas, S., Das, P. P., Miska, E. A., Rodriguez, A., Bradley, A., Smith, K. G. C., Rada, C., Enright, A. J., Toellner, K.-M., MacLennan, I. C. M., & Turner, M. (2007). Microrna-155 regulates the generation of immunoglobulin class-switched plasma cells. *Immunity*, *27*(6), 847–859. https://doi.org/10.1016/j.immuni.2007.10.009

Wang, N., Zhu, M., Tsao, S.-W., Man, K., Zhang, Z., & Feng, Y. (2013). MiR-23a-mediated inhibition of topoisomerase 1 expression potentiates cell response to etoposide in human hepatocellular carcinoma. *Molecular Cancer*, *12*(1), 119. https://doi.org/10.1186/1476-4598-12-119

Waskom, M. (2021). Seaborn: Statistical data visualization. *Journal of Open Source Software*, *6*(60), 3021. https://doi.org/10.21105/joss.03021

Wu, C.-J., Cho, S., Huang, H.-Y., Lu, C.-H., Russ, J., Cruz, L. O., Da Cunha, F. F., Chen, M.-C., Lin, L.-L., Warner, L. M., Liao, H.-K., Utzschneider, D. T., Quon, S., Berner, J., Camara, N. O. S., Zehn, D., Belmonte, J. C. I., Chen, L.-C., Huang, S.-F., Kuo, M.-L., Lu, L.-F. (2019). MiR-23~27~24–mediated control of humoral immunity reveals a TOX-driven regulatory circuit in follicular helper T cell differentiation. *Science Advances*, *5*(12), eaaw1715. https://doi.org/10.1126/sciadv.aaw1715

Wurster, A. L., Rodgers, V. L., White, M. F., Rothstein, T. L., &Grusby, M. J. (2002). Interleukin-4-mediated protection of primary b cells from apoptosis through stat6-dependent up-regulation of bcl-xl. *Journal of Biological Chemistry*, *277*(30), 27169–27175. https://doi.org/10.1074/jbc.M201207200

Yusuf, I., Kageyama, R., Monticelli, L., Johnston, R. J., DiToro, D., Hansen, K., Barnett, B., & Crotty, S. (2010). Germinal center t follicular helper cell il-4 production is dependent on signaling lymphocytic activation molecule receptor(Cd150). *The Journal of Immunology*, *185*(1), 190–202. https://doi.org/10.4049/jimmunol.090350