UNIVERSITY OF MARYLAND

The Role of Lipid Metabolism in B Cell Immune Functions

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Neutral lipid accumulation is linked to chronic inflammation

Evidence indicates that neutral lipid accumulation due to obesity triggers low-grade, chronic inflammation, which is correlated with the occurrence of chronic metabolic disorders such as Type 2 diabetes (T2D).[1,2] Recent studies provide evidence for the essential role that B cells play in obesity-induced inflammation and the development of insulin resistance. Previous studies show B cells are recruited to the visceral adipose tissue (VAT), where they produce self-reactive pathogenic antibodies.[3-5] We hypothesize that neutral lipid accumulation in B cells will cause them to infiltrate VAT, trigger autoantibody production, and develop an autoimmune pathology. Preliminary research has led to the generation of a CGI-58 B cell-specific knockout (BKO) mouse model in order to induce neutral lipid accumulation exclusively in B cells. As CGI-58 is an activator of the lipase ATGL, its deletion should cause excess triglyceride buildup in B cells, mimicking the morphology observed in the B cells of obese mice.[6] The goal of this project is to determine the mechanism by which lipid metabolism within B cells regulates their activation and pathogenicity in obesity-associated insulin resistance.

CGI-58 is an intermediate in triglyceride lipolysis

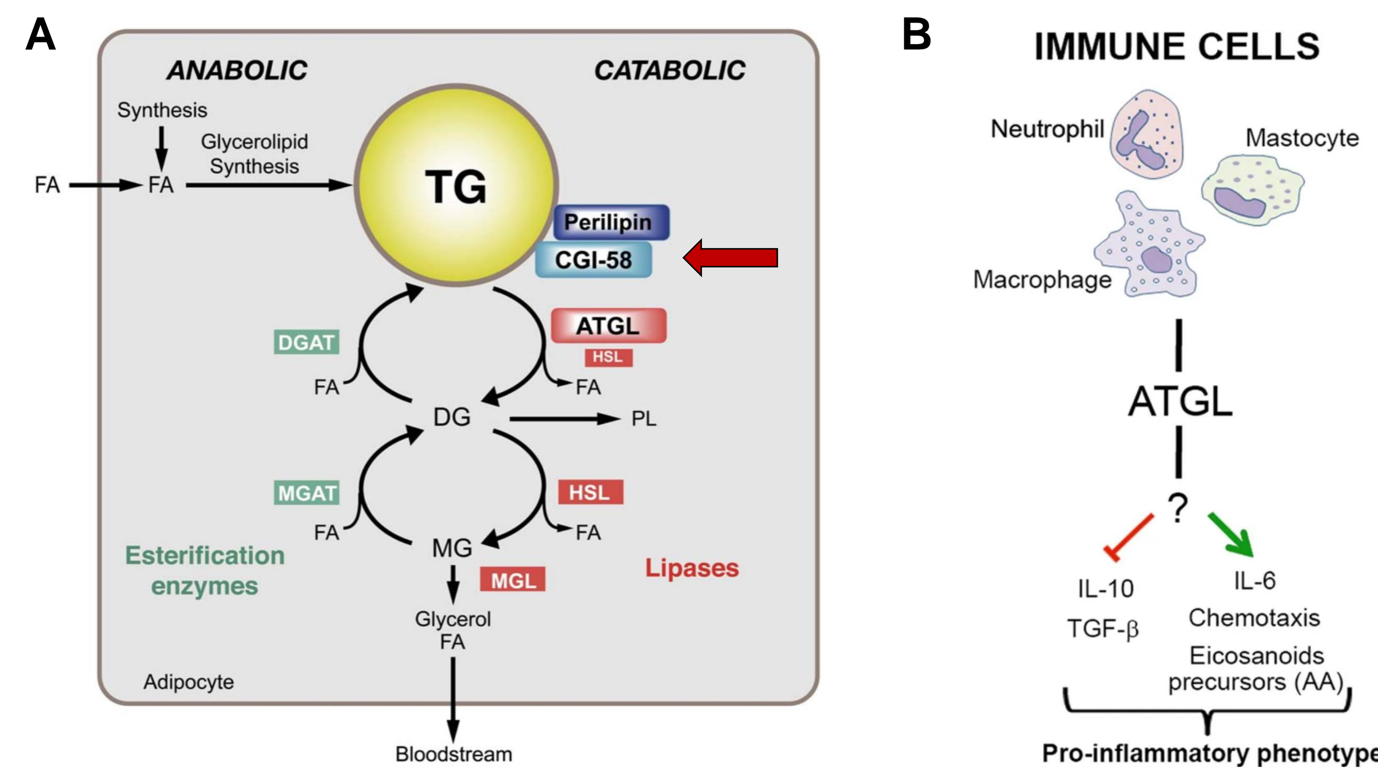


Fig. 1. (A) Anabolic and catabolic pathways that regulate triglyceride levels in adipocytes. CGI-58 is an adiposome-associated protein that activates adipose triglyceride lipase (ATGL) which stimulates lipolysis. Yen and Farese Jr., *Cell Metabolism* (2006) **(B) Involvement of ATGL in immune response.** In most immune cells, ATGL supports pro-inflammatory responses. The downstream pathway of ATGL's effect is still unknown. Adapted from Vegliante et al., *Cell Death and Disease* (2018)

B cells undergo functional changes during obesity-related insulin resistance

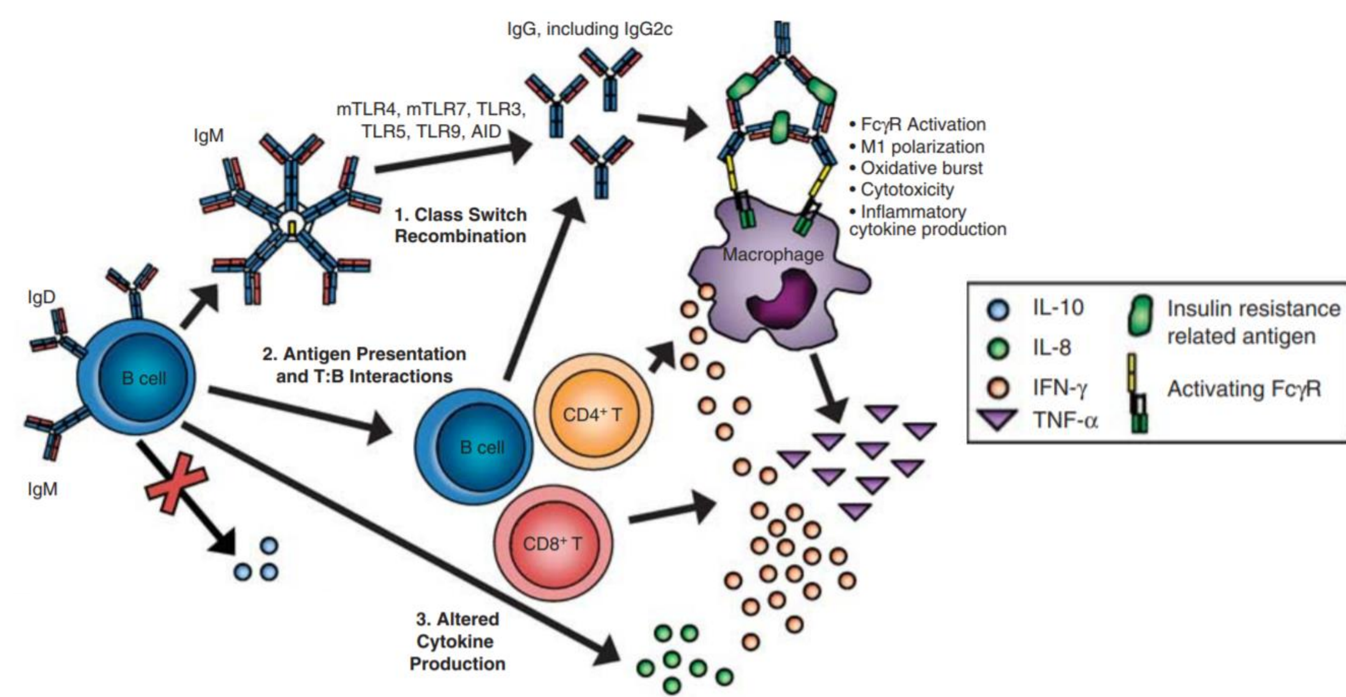


Fig. 2. Immune alterations that exacerbate insulin resistance. B cells are evidenced to class switch to IgG during the development of obesity. IgG antibodies target insulin resistance-related antigens and recruit macrophages. VAT-associated B cells can induce MHC-dependent pro-inflammatory cytokine release. S Winer and DA Winer, *Immunology and Cell Biology* (2012)

Experimental timeline for CGI-58 BKO and Flox control diet trial

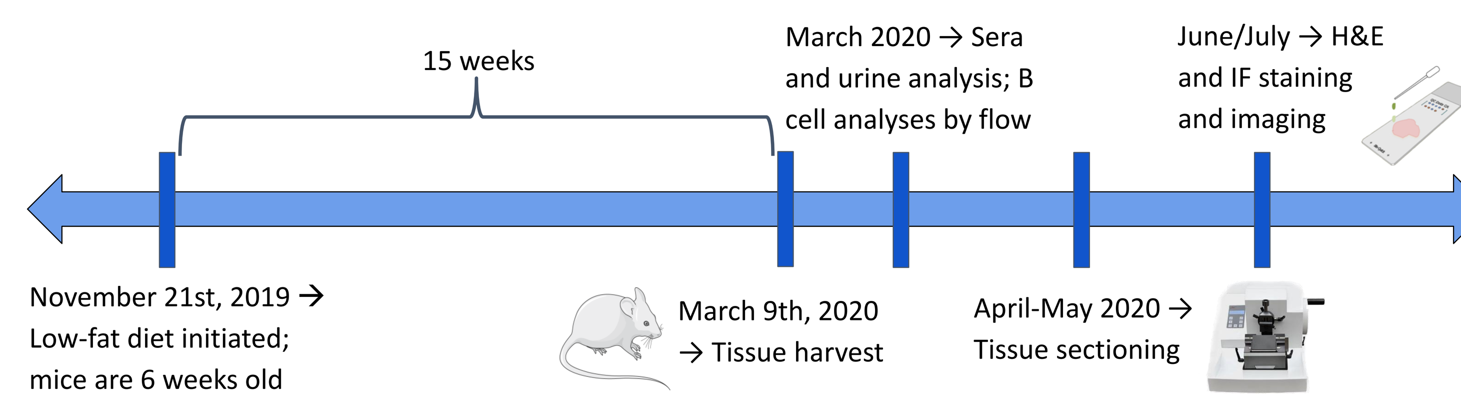


Fig. 3. Timeline for a low-fat diet trial. A low-fat diet (LFD) of 10 kcal% fat was obtained from Research Diets, Inc. 8 Flox control and 8 CGI-58 BKO mice were fed 5g of LFD/day for 15 weeks. On March 9th, they were sacrificed and organs (spleen, pancreas, VAT, kidneys, descending artery) were harvested, weighed, and embedded in Tissue-Tek® O.C.T. compound. Embedded tissue is sectioned and stained to investigate B cell germinal center development (in spleen) and B cell infiltration and autoantibody accumulation (in all other tissues).

Increase in germinal center B cells in the spleens of CGI-58 BKO mice

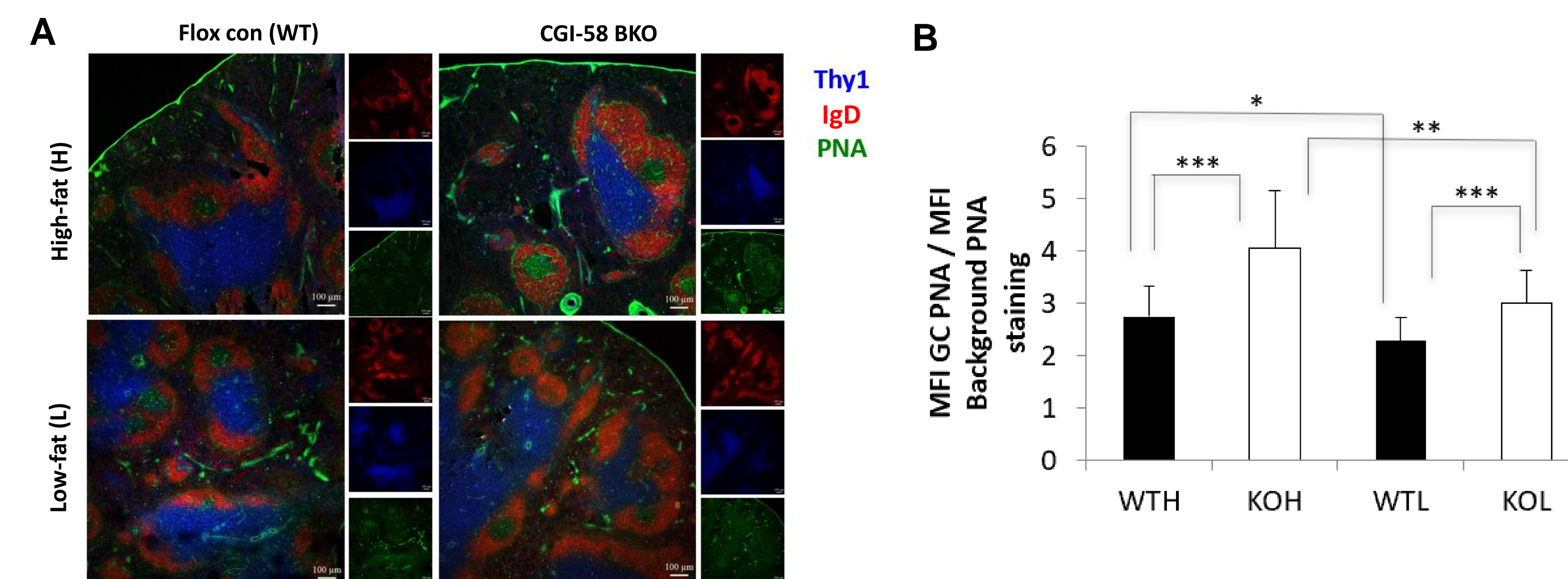


Fig. 4. (A) Representative immunofluorescent images of 12-µm splenic sections of Flox control and CGI-58 KO mice fed either a high-fat (WTH/KOH) or low-fat diet (WTL/KOL) for 15 weeks. Blue is AF488- anti-mouse Thy1.2/CD90.2, red is biotinylated anti-mouse IgD conjugated to AF546-Streptavidin, and green is AF647- anti-mouse PNA. 10x magnification **(B) Quantification of mean fluorescence intensity (MFI) of PNA+ germinal centers (GC).** MFI of PNA+ GC were normalized to MFI of background PNA staining. n = 6 GC per 3 WTH, KOH, WTL, or KOL mice. *p < 0.05, **p < 0.01, ***p < 0.001

Comparing mitochondrial membrane potential, germinal center B cells, and activated B cells in CGI-58 BKO and Flox control mice

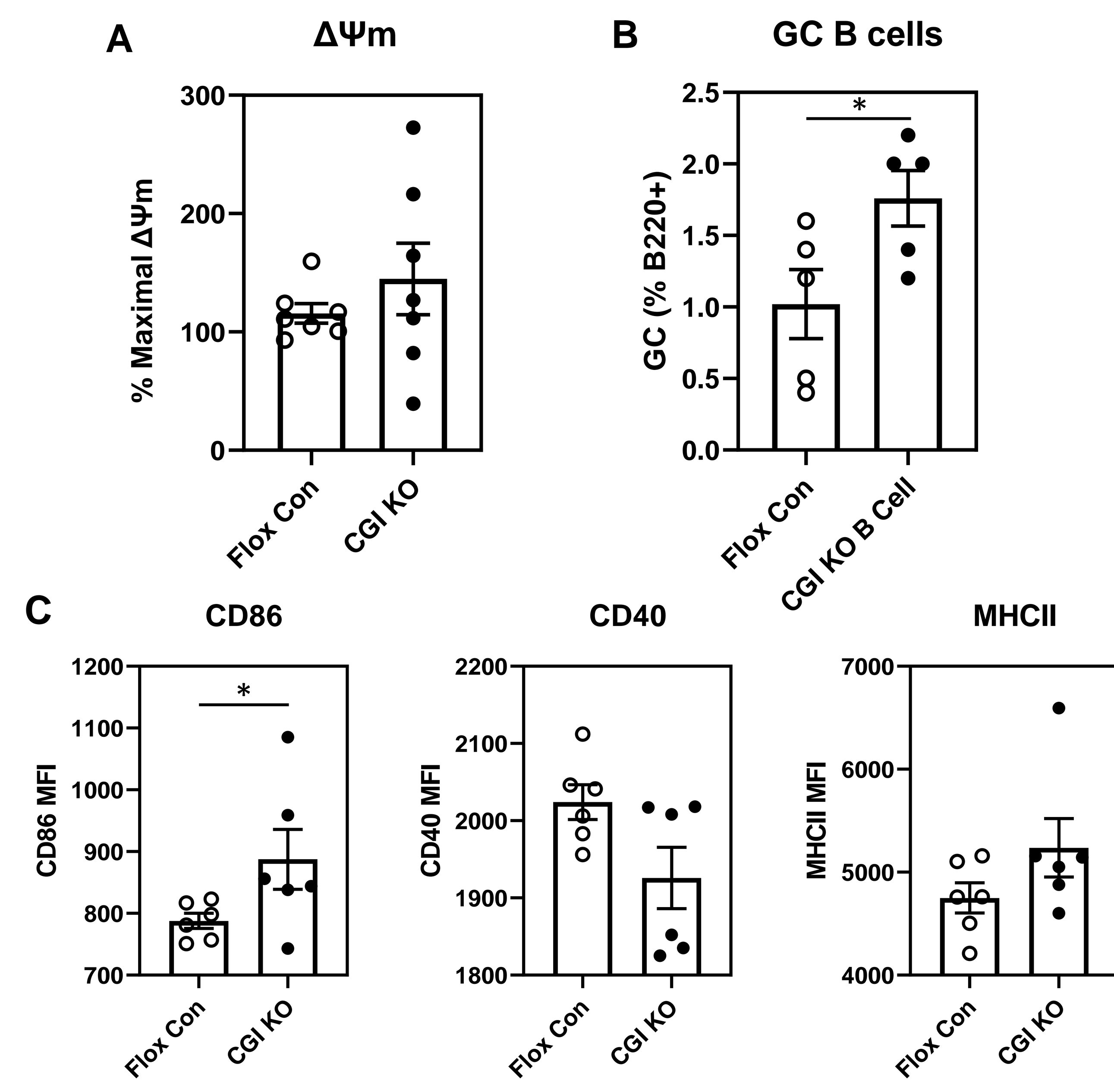
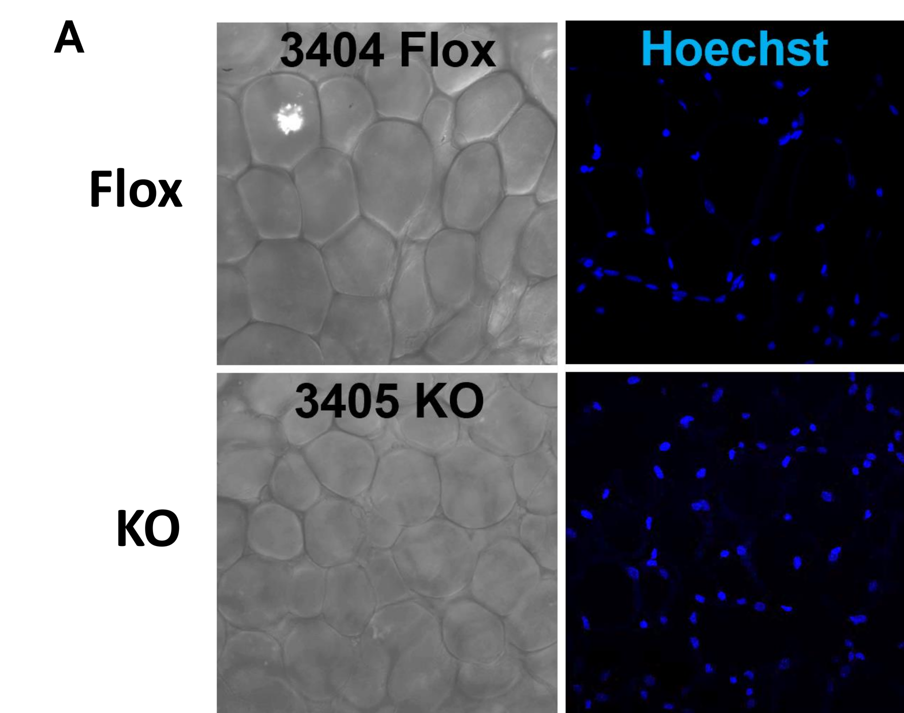


Fig. 5. Splenocytes were isolated using Ficoll-Paque™. They were then labeled with either PerCP Cy5.5- anti-CD45R/B220 (A and B) or AF488 anti-CD19 (C) to stain B cells. Afterwards, various stains/markers were used, and stained samples were analyzed using flow cytometry. **(A) % maximal mitochondrial membrane potential (ΔΨm) of B220+ population.** Cells were stained with Tetramethylrhodamine, methyl ester (TMRM). % ΔΨm determined from the total number of B220+ cells in each sample (n=7). **(B) % of GC7+/CD95+ GC B cells.** Cells were stained with AF488 anti-CD95 and PE anti-GL7. Percentage determined from the total number of B220+ cells in each sample (n=5). *p < 0.05 **(C) Quantification of MFI of activated B cells.** Cells were stained with either biotinylated anti-MHCII conjugated to AF405-Streptavidin, PE- anti-CD86, or APC- anti-CD40 markers. MFI of activated B cells was normalized to MFI of B220+ B cells (n=6). *p < 0.05

Greater number of nuclei present in the VAT of CGI-58 BKO mice



B — Nuclei in Each Frame (● KO, ○ Flox) **

Fig. 6. (A) Immunofluorescent staining of nuclei from VAT of CGI-58 BKO and Flox mice. Approx. 5mm x 5mm VAT tissue sections from 7 Flox control and 7 CGI-58 BKO mice fed a low-fat diet trial were fixed in 4% PFA and stained with PE- anti-mouse B220 (red), AF647- anti-mouse F4/80 (green), and Hoechst DNA marker (blue). 10x magnification **(B) Quantification of nuclei in each frame.** Nuclei in all image frames were quantified for all mice, and quantified nuclei were averaged by genotype (n=7). **p < 0.01

Summary and Future Directions

Summary

Increased accumulation of triglycerides in CGI-58 BKO mice significantly increases the levels of spontaneous activation in B cells, shown by the increases in the number of germinal center B cells, the surface expression levels of B cell activation markers, and the number of infiltrated lymphocytes in VAT, compared to Flox controls.

Future Directions

- IF stain splenic sections with PNA to quantify GC B cells in BKO vs. Flox mice
- Increase staining specificity of infiltrated immune cells in VAT to differentiate B cell nuclei from other immune cell nuclei in tissues harvested in Spring 2020

Acknowledgments

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