UNIVERSITY OF CALIFORNIA SAN DIEGO

The Ablation of *Gnas* in CD11c^{+} Immune Cells, Protects Mice from Obesity-mediated Inflammation and Metabolic Disorders

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by

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The Thesis of Sung Min Lee is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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DEDICATION

I dedicate this thesis paper to my loving family and members of the Raz lab.

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ABSTRACT OF THE THESIS

The Ablation of *Gnas* in CD11c^{+} Immune Cells, Protects Mice from Obesity-mediated Inflammation and Metabolic Disorders

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Sung Min Lee

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Obesity is a common condition characterized by low-grade systemic inflammation that leads to comorbidities such as insulin resistance. Overnutrition causes disturbance in metabolic homeostasis and causes gradual accumulation of CD11c^{+} cells to create an inflammatory state in adipose tissue. Our studies identified the ablation of the *Gnas* gene, which encodes for the

stimulatory G-protein alpha subunit (Gαs) responsible for the generation of cyclic AMP (cAMP), in $CD11c^+$ cells protected mice from obesity-mediated inflammation and insulin resistance. The $Gnas^{\Delta CD11c}$ mice (KO) maintained a lean phenotype when stressed with a high-fat diet (HFD) and results showed improved insulin sensitivity and glucose tolerance. Histological analysis of white adipose tissue (eWAT) of KO mice on HFD showed reduced crown-like structures (CLS) whose formation is often associated with inflammasome activation in macrophages. To further investigate the reduced inflammatory status of KO mice, we cultured and stimulated $CD11c^+$ bone-marrow-derived macrophages (BMDM) to induce inflammasome activation. Notably, the inflammasome gene expression and pro-inflammatory cytokine secretion were significantly blunted. In addition, we found treating KO BMDM with cell-permeable cAMP analog restored the inflammasome-mediated inflammatory response. Collectively, our results demonstrate that Gαs-cAMP signaling in $CD11c^+$ cells play an important role in regulating high fat diet-induced immune responses in mice.

INTRODUCTION

Obesity is a chronic inflammatory disease that has emerged as a leading promotor of various comorbidities[1]. Some of these comorbidities include diseases such as type 2 diabetes (T2D), non-alcoholic fatty liver disease (NAFLD), cancer and insulin resistance which contribute to the increasing mortality rates around the world[1,2,3,4]. Obesity is often associated with the accumulation of immune cells in adipose tissue (AT) in response to overnutrition by which inflammatory cytokines are released, leading to chronic low-grade inflammation and insulin-resistant states[5,6]. For example under metabolic stress, the white adipose tissue (WAT) secretes chemokines such as monocyte chemoattractant protein-1 (MCP-1/CCL2) molecules to recruit circulating monocytes to the site of inflammation[7,8]. The heterogeneous characteristic of monocytes provokes their differentiation into adipose tissue macrophages (ATM) under exposure to growth factors and pro-inflammatory cytokines[9].

The pro-inflammatory M1-like macrophages that reside in the WAT can be characterized by the expression of CD11c (*Itgax*)[10,11,]. The CD11c marker, a type I transmembrane protein, is mainly expressed in immune cells including monocytes, dendritic cells (DCs), macrophages and neutrophils[12]. The exposure to inflammatory cytokines and metabolic stress polarizes these $CD11c^+$ macrophages to a classical M1 state that is associated with a pro-inflammatory profile[13]. The M1 macrophages secrete inflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-6, and interleukin (IL)-1β that play important roles in mediating inflammation in the WAT. Recent studies suggest $CD11c^+$ cells also play an important role in T-cell accumulation and activation in AT, which contribute to insulin resistance[14]. And the ablation of $CD11c^+$ cells is associated with decreased adipose tissue inflammation and reduction in insulin resistance[15].

Therefore, further studies on the mechanisms by which $CD11c^+$ immune cells regulate inflammation seem to be an attractive area of research.

Gαs protein and cAMP

Immune cells, including CD11c+ macrophages, utilize surface molecules to sense and recognize pathogens in their environment to shape their immune responses[16]. G-protein coupled receptors (GPCR) are one of the largest and most diverse cell surface receptors that mediate signal transmission in macrophages[17]. Studies have suggested that the GPCR and agonist interactions regulate macrophage functions and lead to modulation of signaling pathways to activate inflammatory mediators in metabolic tissues (e.g. cytokines and chemokines), clearance of cellular debris, and tissue repair mechanisms[18,19]. The activation of GPCR has also been shown to be involved in the maintenance of metabolic homeostasis. β-adrenergic receptors (β-ARs) are a class of Gαs-linked GPCRs that facilitate lipolysis in both brown and white adipose tissue. The catecholamine norepinephrine (NE) and epinephrine released from the sympathetic nervous system, activate the β-AR and subsequent activation of PKA, promoting the hydrolysis of triglyceride in adipocytes by the phosphorylation of hormone-sensitive lipases (HSL) and adipose triglyceride lipases (ATGL)[20,21,22].

The GPCR coupled G proteins are heterotrimeric proteins meaning they consist of three subunits: alpha (Gα), Beta (Gβ), and Gamma (Gγ). The binding of extracellular ligands, such as neurotransmitters, hormones, growth factors, or lipids[23] to a GPCR leads to the conformational change and activation of intracellular G proteins such as the stimulatory Gα proteins (Gαs)[24]. The activated Gαs exchanges the bound guanosine diphosphate (GDP) for guanosine triphosphate (GTP) through its GTPase activity. The GDP-GTP exchange induces the dissociation of Gα protein

from the Gβ and Gγ subunits and stimulates the effector molecules, such as adenylyl cyclase (AC)[17,25]. The stimulated AC can relay signal transduction by catalyzing the production of important intracellular second messenger such as cyclic adenosine monophosphate (cAMP) from ATP. Intracellular cAMP signaling plays an important role in activating downstream pathways including PKA and EPAC to mediate cell proliferation and metabolic lipid metabolism such as catabolic lipolysis[26]. Although cAMP involvement as a second messenger in numerous signal transduction pathways is extensively studied, little is known about its role in shaping immunity. Previous studies have suggested that disrupting or enhancing the cAMP production in immune cells may have immunoregulatory importance in inflammatory disorders[27]. Decreased cAMP levels showed a tendency to have pro-inflammatory effects on immune cells while increasing cAMP concentrations were correlated with anti-inflammatory effects by inhibiting the NF-kB activity[28,29]. However other studies have also suggested the positive effects of cAMP on NF-kB activation upon stimulation by β-AR agonists such as thrombin and high glucose[30]. Hence the relationship between cAMP and its role in mediating inflammatory response via the NF-kB pathway remains conflicting. Therefore, further studies are necessary to determine the potential involvement of cAMP in the induction of inflammation and insulin resistance in obesity.

NLRP3-inflammasome and Gas-cAMP

Inflammasome activity in adipose tissue has been proposed as a major mediator of low-chronic inflammation and insulin resistance in obesity[31,32]. The NOD, leucine-rich repeat-containing pyrin (NLRP)-3 inflammasome is a multi-component cytosolic complex that induces the cleavage of the proinflammatory cytokine IL-1β[33]. These NOD-like receptors, as well as toll-like receptors (TLRs), allows the macrophage to sense pathogen-associated molecular patterns

(PAMPs) and damage-associated molecular patterns (DAMPs) to initiate cellular signaling for appropriate cellular responses[34]. The activation of NLRP3 requires a two-signal process; the first is a priming signal followed by a second activating signal for the subsequent assembly and activation of the NLRP3 inflammasome components[35,32]. The initial priming step is mediated upon recognition of PAMPS or stress signals by pattern recognition receptors (PRRs) such as TLRs known to activate the NF-kB signaling pathway[36]. The activation of the NF-kB transcription factor causes the binding to the NLRP3 promoter site and upregulates the expression of both NLRP3 and pro-IL-1β[36,37]. A second activating signal is facilitated through the response to various PAMPs and DAMPs including adenosine triphosphate (ATP), uric acid, and fatty acids[38]. Upon activation, the NLRP3 inflammasome recruits the adaptor protein ASC which results in the self-cleavage of pro-caspase-1 to its active effector form caspase-1[39,40]. The activated caspase-1 in turn cleaves cytosolic pro-IL-1β to mature IL-1β[41]. It is well documented that the release of pro-inflammatory cytokines such as IL-1β in macrophages is involved in instigating chronic low-grade inflammation in adipose tissue and disrupting insulin signaling[42,43]. Recent studies have proposed that increased NLRP3 inflammasome activity in adipose tissue and the lack of *Nlrp3* expression in mice lead to the resistance of HFD-induced obesity and protection against insulin resistance[32]. We, therefore, hypothesized the pro-inflammatory states and insulin resistance observed in obesity were instigated by NLRP3 activation and successive release of IL-1β by $CD11c^+$ macrophages. In addition, the crosstalk between cAMP and NLRP3 induction remains conflicting as cAMP is said to have an inhibitory effect on inflammasome assembly by direct binding[44]. Thus, in our study, we will explore the potential mediators that lead to NLRP3 inhibition as a result of cAMP ablation in $CD11c^+$ cells.

Inhibiting Effects of PPARγ on NLRP3 Expression

The Peroxisome proliferator-activated receptor-gamma (PPARγ), a member of the nuclear receptor superfamily, is a lipid-sensing ligand-inducible transcription factor[45]. It is a major regulator of adipogenesis and obesity-induced inflammation in adipose tissue[46]. Activated PPARγ functions to maintain the lipid homeostasis by regulating adiponectin secretion which in turn improves overall whole-body insulin resistance[47,48]. PPARγ is mainly expressed in the WAT and implements anti-inflammatory effects by reversing macrophage infiltration and functions[49,50,]. The PPARγ structure has a three-dimensional structure that consists of a DNA binding domain (DBD) and a ligand-binding domain (LBD). Upon ligand binding, PPARγ translocate to the nucleus and forms a heterodimer with the retinoid X receptors (RXR) to initiate transcriptional activity[51]. The PPAR-RXR heterodimers can interact with DNA sequences known as peroxisome proliferator hormone response elements (PPREs) to repress or activate the transcription of target genes[52].

The NF-kB pathway is one of the known targets of transrepression by PPARγ and which leads to the subsequent inhibition of NLRP3[53]. Previous studies have shown the presence of PPARγ induction antagonizes the NF-kB transcription factor activity thus affecting NLRP3 expression which indicates an inverse relationship[54]. Since papers have also suggested PPARγ expression induces the macrophage to an M2-like polarization state[55], we hypothesized the anti-inflammatory phenotype observed in adipose tissue of *Gnas* ablated mice could be influenced by PPARγ expression in macrophages and its subsequent inhibitory effect on the NF-κB pathway.

Working Hypothesis

In this study, we hypothesized the deletion of the *Gnas* gene in $CD11c^{+}$ cells, protects against diet-induced tissue inflammation and metabolic disorders in mice by inhibiting M1-inflammatory mediators associated with obesity.

RESULTS

$Gnas^{\Delta CD11c}$ KO mice are protected from obesity and insulin resistance

We first used the Cre-loxP system to generate $Gnas^{\Delta CD11c}$ KO mice (C57BL/6 background) with *Gnas* deletion in $CD11c^{+}$ cells (hereafter referred to as KO mice)[56]. These mice have ablation of cAMP signaling in $CD11c^{+}$ cells and exhibit a unique metabolic phenotype **(Fig. 2a)**. The $Gnas^{fl/fl}$ (Flox) control mice and the KO mice were challenged with a normal chow diet (NCD) or with a 60% kcal high-fat diet (HFD) and body weights (BW) were evaluated from the ages of 8 weeks until 16 weeks **(Fig. 1)**. While the male Flox mice showed a gradual increase in BW, the female Flox mice showed a slower rate in change of BW. Sex differences have been reported to affect the susceptibility to diet-induced obesity due to. Levels in estrogen, the major sex hormone in females, have been suggested as the major reason for their protection against obesity and other metabolic syndromes[57]. Both the male and female KO mice showed resistance to change in BW under NCD **(Fig. 2b)**.

Mice groups challenged with HFD also showed a similar trend in which the KO mice showed resistance to BW change despite the high-calorie intake **(Fig. 2c)**. The tissue weights of the liver and brown, epididymal and inguinal white adipose tissue (BAT, eWAT, and iWAT) were measured. In NCD challenged mice, results showed that the tissue weights were similar in both mice groups except for the eWAT, which was lighter in the KO mice **(Fig. 2b)**. In line with this finding, the tissue histologies and the size distribution of eWAT, and iWAT in mice under NCD showed no significant differences between genotypes **(Fig. 3a and b)**. However, mice challenged with HFD showed that the iWAT, eWAT, and BAT were significantly lighter in the KO mice **(Fig. 2c)**. The histologies of the tissues showed whitening of the BAT, and enlargement of eWAT

and iWAT in the HFD-Flox mice, however, the KO mice did not exhibit any of these changes **(Fig. 3c)**.

HFD-induced obese mice often display glucose intolerance and insulin resistance[58,59]. To further characterize the metabolic changes in mice with Gαs deficiency in CD11c$^+$ cells we compared the insulin sensitivity of Flox and KO mice challenged with NCD or HFD using the glucose and insulin tolerance tests (GTT and ITT) **(Fig. 4a-d)**. After i.p injection of D-glucose, the NCD-KO mice exhibited a slight improvement in glucose tolerance and insulin sensitivity compared to the Flox **(Fig 4a and b)**. Under HFD, the KO mice were significantly less hyperglycemic at various time points compared to obese Flox mice **(Fig. 4c)**. Consistent with this data, the KO mice also displayed higher insulin sensitivity than obese Flox mice **(Fig. 4d)**. In further support of this, KO mice on NCD at 5 months of age showed lower fasting insulin and glucose levels, and consequently lower HOMA-IR compared to Flox **(Fig. 4e-g)**. Collectively, these findings suggest that the ablation of *Gnas* and cAMP signaling in CD11c$^+$ cells protected mice from diet-induced obesity and improved glucose and insulin tolerance.

Gnas$^{\Delta CD11c}$ KO mice have reduced adipose tissue inflammation

Obesity is often associated with low-grade inflammation characterized by immune cell accumulation and NLRP3 activation in adipose tissue[60,61]. We thus examined the mRNA expression of various immune cell markers and proinflammatory cytokine genes in the eWAT of Flox and KO mice fed a NCD or HFD by qPCR analysis. In mice challenged with NCD, no significant differences in expression of immune cell markers were detected **(Fig. 5a)**. In contrast, HFD-induced obesity in Flox mice led to increased expression of macrophage markers *Itgax* (CD11c), and *Adgre1* (F4/80) **(Fig. 5a)**. The increase was also seen in the expression of

inflammatory cytokines *Il6* and *Ccl2* **(Fig. 5a and b)**. However, the expressions of these macrophage markers and inflammatory cytokines were decreased in the KO mice fed the same HFD **(Fig. 5a and b)**. Markedly, the expression of anti-inflammatory cytokine *Il10* was greater in the NCD-KO mice in comparison to Flox mice and increased to a greater extent under HFD **(Fig. 5b)**. In line with this result, ELISA analysis showed a 50-fold increase of anti-inflammatory cytokine IL-10 in the KO compared to Flox mice on HFD **(Fig. 5c)**. The circulating IL-6 was also increased in the KO mice as compared to Flox on HFD. However, mRNA expression did not exhibit these changes **(Fig. 5b)**. In addition, plasma levels of TNFα, IL-1β, and CCL2 showed no significant differences between groups. In line with the increased macrophage markers, the histology of eWAT tissues showed more presence of Crown-like structures (CLS) in the Flox mice under HFD, which is a histologic characteristic of macrophage-induced inflammatory response occurring in adipose tissue **(Fig. 6a and b)** [62].

Studies have suggested there is a strong co-localization of NLRP3 with macrophage markers in CLS of adipose tissue[31]. We, therefore, examined the mRNA expression of activated inflammasome complex components *caspase-1* and adaptor protein, apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC)*(Pycard)* in the eWAT of Flox and KO mice under HFD. As expected, the HFD-challenged Flox mice had increased expression of both *Caspase-1* and *Pycard* in the eWATs, but the increases were blunted in the KO **(Fig. 6d)**. No significant differences in mRNA expression of *Nlrp3* and *Il1b* were observed between groups **(Fig. 5b and 6d)**. Next, we measured protein levels of NLRP3 and IL-1β expression in homogenized eWAT by western blotting. Analysis showed low NLRP3 expression in mice on NCD but showed a significant increase in Flox mice challenged with HFD **(Fig. 6c)**. However, the NLRP3 expression was significantly blunted in the KO. The pro-IL-1β expression was detected in

both mice groups on NCD, but the expression of mature IL-1β was low in this condition **(Fig. 6c)**. Under HFD challenge, the pro-IL-1β was reduced in Flox mice however the KO did not exhibit any reduction **(Fig. 6c)**. This suggests that the presence of Caspase-1 and ASC in Flox mice led to successful cleavage of pro-IL-1β into its bioactive form[63,64].

cAMP promotes inflammatory phenotype in the BMDM

The NLRP3 is induced in innate immune cells and mainly expressed in monocytes including macrophages[65]. As we detected decreased level of NLRP3 in the eWAT of KO mice on HFD, we suspected that the NLRP3 detected in eWAT was likely from tissue macrophages. We, therefore, looked at NLRP3 in bone marrow-derived macrophages (BMDM) from the Flox and KO mice. We treated BMDM of Flox and KO mice with LPS to induce an inflammatory response. The mRNA expression by qPCR analysis showed that LPS treatment increased the expression of *Il1β*, *Il6*, and *Nlrp3* in the BMDM of Flox mice **(Fig. 7a)**. However, the increase was dampened in the BMDM of KO mice.

Consistently, the western blot analysis of BMDM with LPS treatment showed significantly increased NLRP3 and Pro-IL-1β level in the BMDM of both groups while the increases were less dramatic in the BMDM of KO compared to that of the Flox **(Fig. 7c)**. LPS treatment alone did not provoke the secretion of IL-1β. Therefore, we treated BMDM with both LPS and ATP for complete inflammasome activation and successful pro-IL-1β cleavage. The addition of both priming and activating signals led to reduced expressions of NLRP3 and Pro-IL-1β compared to LPS-only treatment at the protein level **(Fig. 7c)**. There is degradation of NLRP3 after ATP treatment at the protein level in both Flox and KO BMDM **(Fig. 7c)**, however the IL-1β secretion was not influenced **(Fig. 7b)**. Markedly, the IL-1β secretion in KO was significantly lower than in the Flox

(Fig. 7b). Consistently, we detected a lower level of the inflammatory cytokine IL-6 secretion in the KO regardless of with or without ATP since the IL-6 is independent of inflammasome processing.

Recent studies suggest that PPARγ (labeled as PPARG in figures) induction leads to the degradation of NF-kB/P65 thereby attenuating the NLRP3-induced inflammatory response[53,66]. We, therefore, examined the protein expression of PPARγ in BMDM of Flox and KO groups. Interestingly, the KO-BMDM exhibited higher PPARγ expression than the Flox-BMDM **(Fig. 7c)**. However, when challenged with LPS, the expression of PPARγ was reduced and led to increased expression of NLRP3 which suggests PPARγ and NLRP3 induction may be inversely correlated **(Fig. 7c).**

To investigate whether the reduced NLRP3 inflammasome activity we observed in the KO BMDM is due to defective TLR signaling, we checked the mRNA expression of *Tlrs,* and the TLR adaptor proteins *Trif* and *MyD88.* The expression remained unchanged however a slight increase in *Trif* expression was observed in the KO-BMDM **(Fig. 7d)**. Stimulation with LPS also showed that p38, ERK, and JNK pathway induction was also protected **(Fig. 8a)**. In contrast, the degradation of IκB and phosphorylation of p65 were reduced in the KO-BMDM **(Fig. 8b)**. This data indicates the reduction of NF-κB activation and transrepression in KO mice is mediated by the increased PPARγ expression.

Gαs dependent cAMP signaling regulates the inflammasome-mediated inflammatory response.

Our previous data showed that the NLRP3 is downregulated in the Gαs-deficient BMDM, therefore we reasoned that increasing the intracellular cAMP concentration in $CD11c^{+}$ BMDM

deficient in *Gnas* will re-establish the NF-kB-induced pro-inflammatory response. To test this hypothesis, we incubated $CD11c^+$ BMDM with Long-Acting Beta Agonists (LABA) or with cell-permeable cAMP analog, 8-CPT-cAMP (CPT). We analyzed the protein expression of NLRP3, Pro-IL-1β, and PPARγ in Flox and KO-BMDM under LABA or CPT treatment to activate GPCR signaling. Analysis showed an overall higher baseline-level expression of NLRP3 and Pro-IL-1β in Flox-BMDM compared to KO-BMDM **(Fig. 9c)**. The presence of CPT led to increased induction of NLRP3 and pro-IL-1β in Flox BMDM however the effect was reduced in KO BMDM **(Fig. 9c)**. LABA induced NLRP3 and pro-IL-1β in the Flox BMDM but did not affect KO-BMDM. Consistent with our previous data, the LABA treated KO BMDM had higher *Pparγ* expression while the expression was reduced by CPT in Flox and KO BMDM **(Fig. 9a and b)**. Moreover, incubation with CPT re-established the expression of NLRP3 and reduced the expression of *Pparγ* in KO BMDM **(Fig. 9a)**.

The mRNA expression of *Il1β, Nlrp3,* and *Pparγ was* analyzed to confirm the negative relationship. CPT induced an increase in the mRNA expression of cAMP-dependent gene *Crem* in Flox BMDM stimulated with CPT and LABA **(Fig. 9a and b)**. This confirmed the activation of cAMP signaling. Incubation with CPT increased the *Il1β* and *Nlrp3* expression in KO-BMDM to similar expression levels of Flox-BMDM **(Fig. 9a)**. As expected, the CPT treatment in KO-BMDM led to reduced expression of *Pparγ*. In contrast, LABA treatment induced the expression of *Il1β* and *Nlrp3* in the Flox BMDM but not in KO BMDM **(Fig. 9b)**. Consistent with previous results, the KO BMDMs exhibited higher *Pparγ* expression under LABA treatment. In summary, these data signify that the Gαs-cAMP-dependent signaling is required for an inflammasome-mediated inflammatory response.

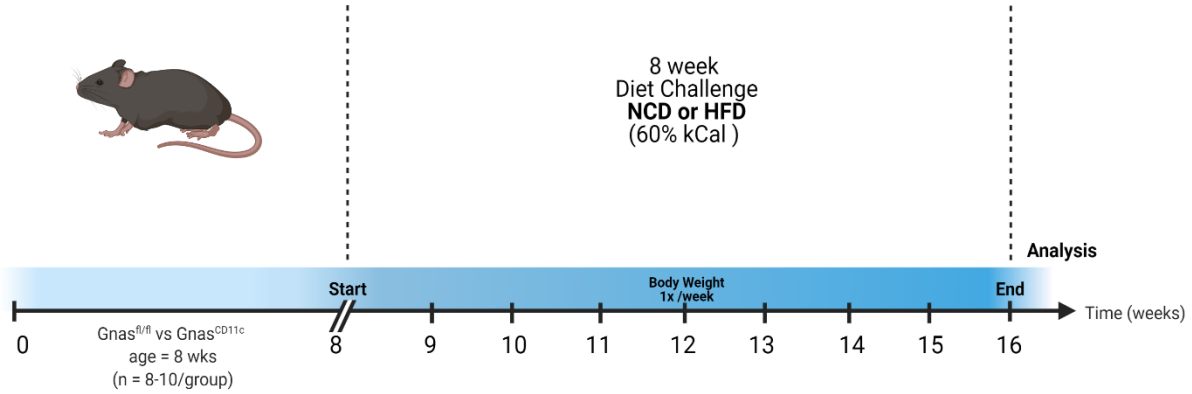

Figure 1. Schematic overview of diet challenge on Gnas$^{fl/fl}$ and Gnas$^{\Delta CD11c}$ mice.
Gnas$^{fl/fl}$ (Flox) and the Gnas$^{\Delta CD11c}$ (KO) mice were challenged with a normal chow diet (NCD) or with a 60% kcal high-fat diet (HFD) and body weights (BW) were evaluated from the ages of 8 weeks until 16 weeks (n= 8-10/group)

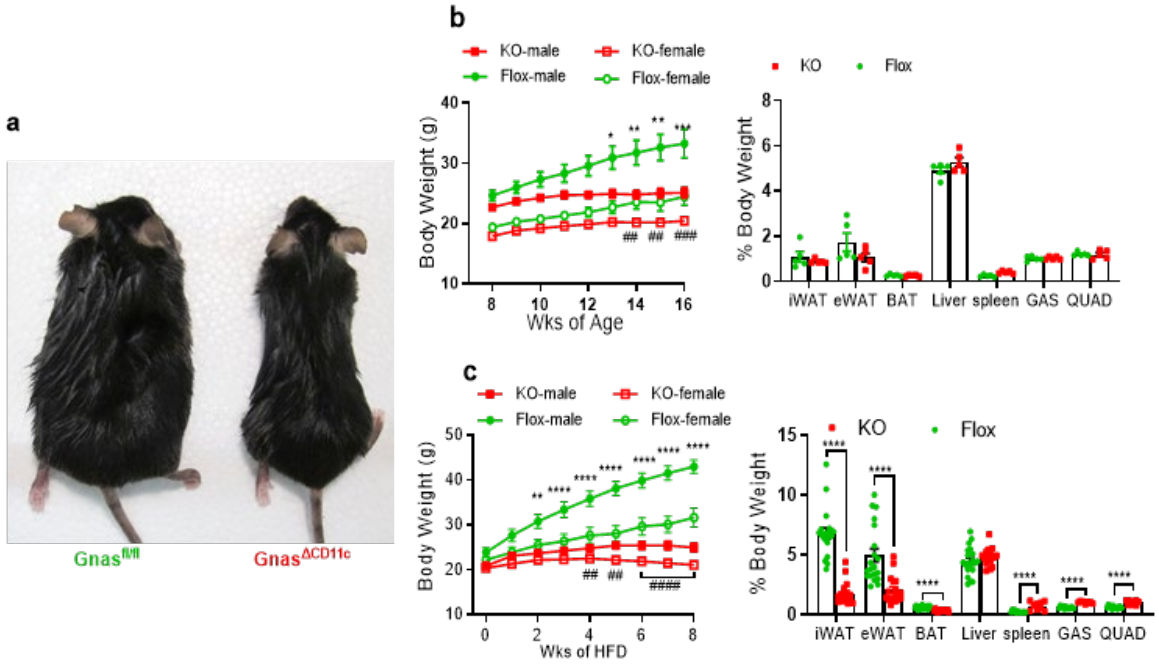

Figure 2. Gnas$^{\Delta CD11c}$ mice are resistant to changes in body weight
For all panels, Gnas$^{fl/fl}$ (Flox) mice are presented in green and Gnas$^{\Delta CD11c}$ (KO) mice in red. **(a)** Body phenotype of Flox and KO mice on HFD. **(b)** Body weights (left), and tissue weights (right) of male and female littermates on normal chow diet (NCD) from age 8 to 16 weeks (n=8-10/group). **(c)** Body weights (left), and tissue weights (right) of male and female littermates on a normal chow diet (HFD) from age 8 to 16 weeks (n=8-10/group). iWAT, eWAT, brown adipose tissue (BAT), liver, spleen, gastrocnemius muscle (GAS), and quadriceps muscle (QUAD). Data are presented as mean ± SEM and asterisks indicate Statistical significance by 2-way repeated-measures ANOVA or unpaired student's t-test as appropriate; *p < 0.05; **p < 0.01; ***p < 0.001; ****p<0.0001. For all panels, Flox mice are presented in green and KO mice in red. Figure 2 has been submitted for publication. Zeng, Liping; Lee, Sung Min "Reciprocal regulation of cAMP signaling in immune cells and adipocytes controls lipolysis, thermogenesis, and obesity" The thesis author is a co-author.

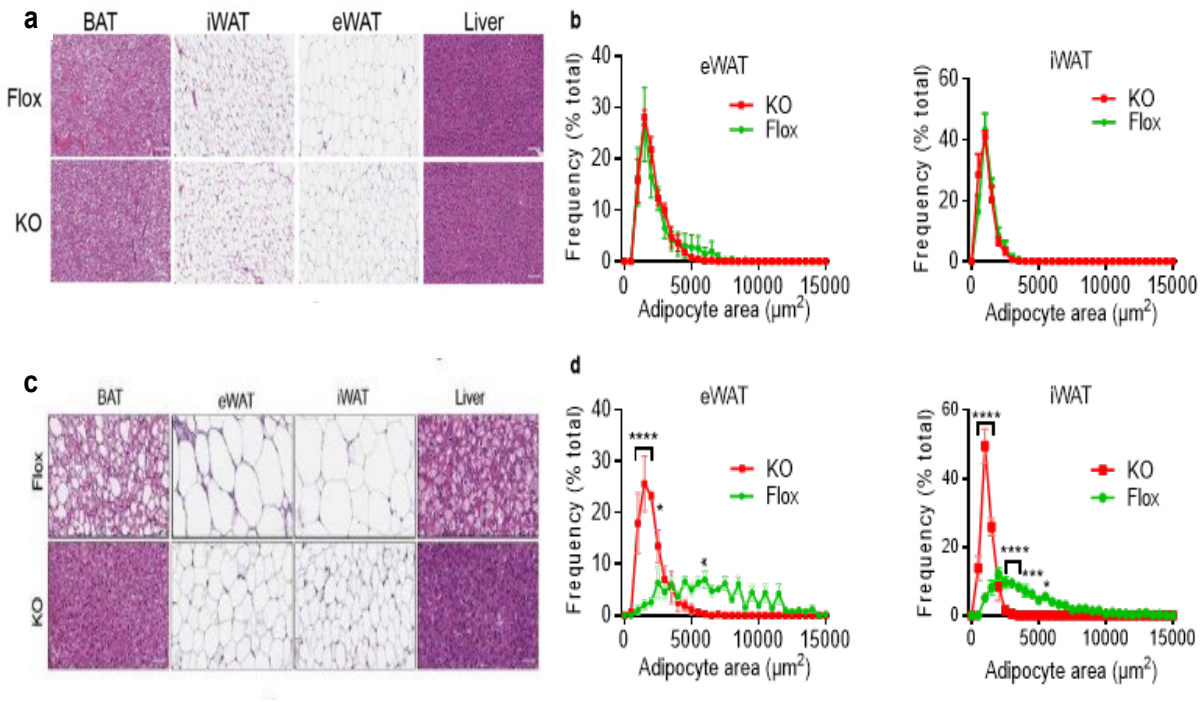

Figure 3. Effects of NCD and HFD challenge on tissues of *Gnas* mice.
(a) Representative H&E staining of BAT, eWAT, iWAT, and liver of NCD-fed Flox and KO mice. Scale bar=100 μm. **(b)** Adipocyte area distribution (μm^2) in eWAT (left) and iWAT (right) from male Flox and KO mice on NCD. **(c)** Representative H&E staining of BAT, eWAT, iWAT, and liver of HFD-fed Flox and KO mice. Scale bar=100 μm. **(d)** Adipocyte area distribution in eWAT (left) and iWAT (right) from male Flox and KO mice on HFD. For all panels, Flox mice are presented in green and KO mice in red. Figure 3 has been submitted for publication. Zeng, Liping; Lee, Sung Min "Reciprocal regulation of cAMP signaling in immune cells and adipocytes controls lipolysis, thermogenesis, and obesity" The thesis author is a co-author.

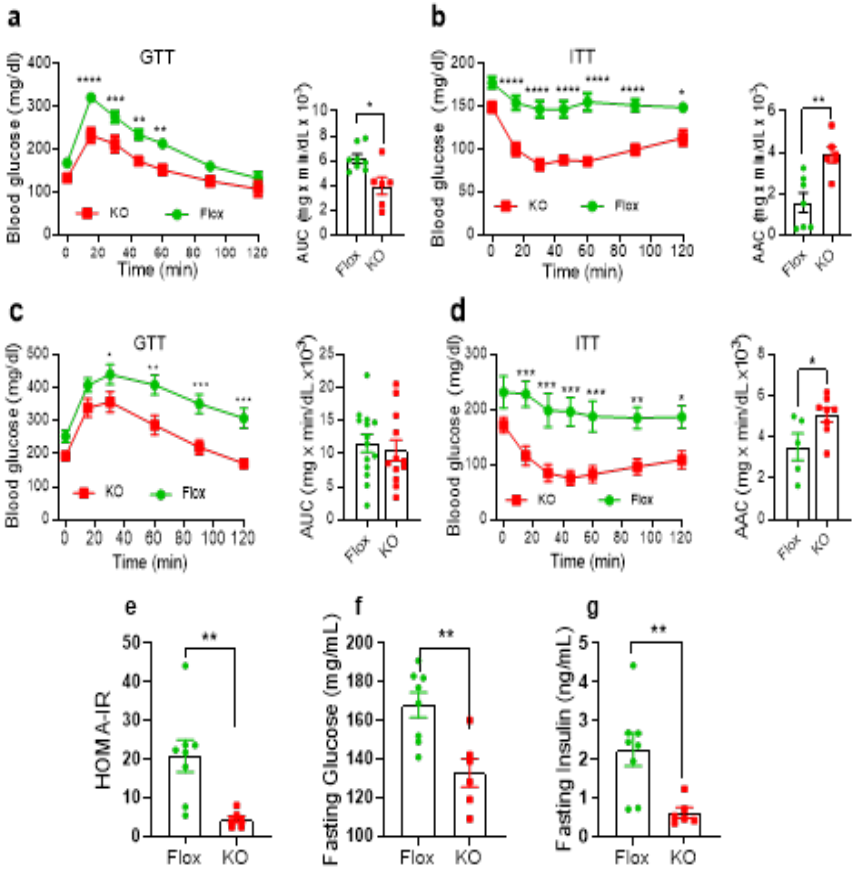

Figure 4. Gnas$^{\Delta CD11c}$ mice are protected from glucose intolerance and insulin resistance
(a) Glucose tolerance tests (GTT) of mice on NCD. Bar graph presents area under the curve (AUC). **(b)** Insulin tolerance tests (ITT) of mice challenged with NCD. Bar graph shows area above the curve (AAC). **(c)** GTT for mice on HFD and area under the curve (AUC). Time ($p<0.0001$), genotype ($p=0.0029$), and interaction ($p=0.055$) by repeated measures 2-way ANOVA. **(d)** ITT for mice challenged with HFD and area above the curve (AAC). **(e,f and g)** Fasting insulin, glucose levels and HOMA-IR of Flox and KO on NCD for 20 weeks. Data are presented as mean ± SEM and asterisks indicate Statistical significance by 2-way repeated measures ANOVA or unpaired student's t-test as appropriate; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. For all panels Flox mice are presented in green, KO mice in red. Figure 4 has been submitted for publication. Zeng, Liping; Lee, Sung Min "Reciprocal regulation of cAMP signaling in immune cells and adipocytes controls lipolysis, thermogenesis and obesity" The thesis author is a co-author.

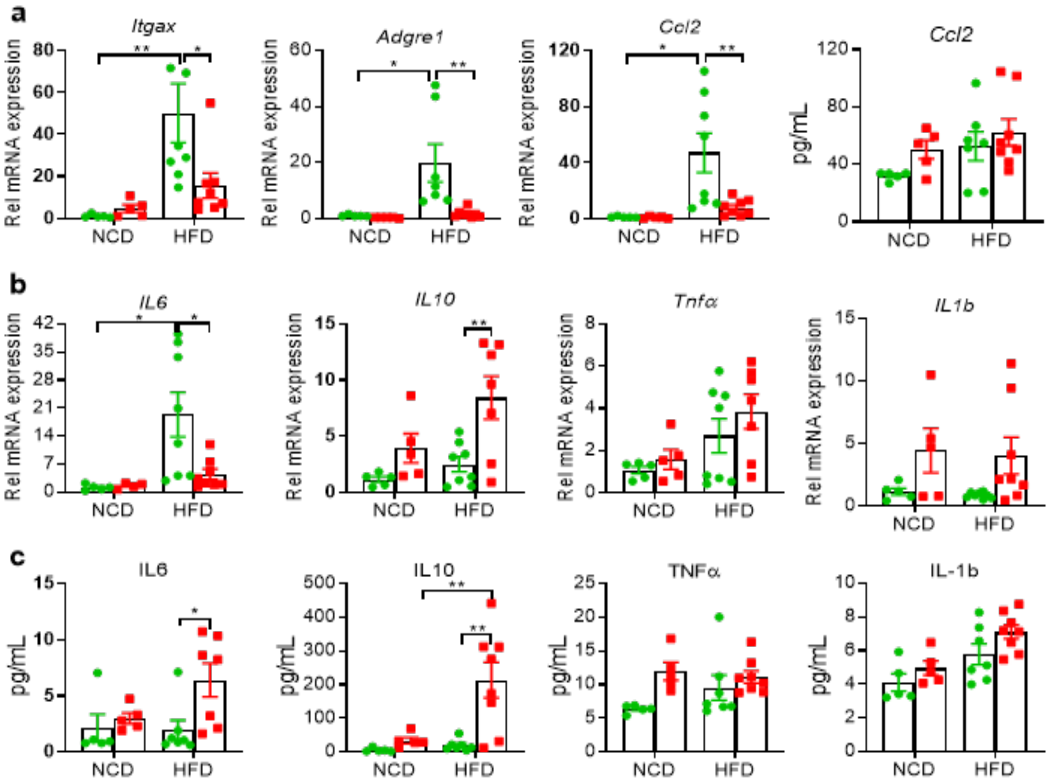

Figure 5. Gnas$^{\Delta CD11c}$ KO mice displayed reduced macrophage infiltration and adipose tissue inflammation. **(a)** qPCR analysis showing mRNA gene expression of *Itgax* (CD11c), *Ccl2* (MCP-1), and *Adgre1* (F4/80) in eWAT from KO and Flox mice challenged with NCD or HFD for 8 weeks. Asterisks indicate significance by 2-way ANOVA (n=5-8/group). **(b and c)** *Il6* (interleukin-6), *Il10* (interleukin-10), *Tnfα* (Tumor necrosis factor-α), and *Il1β* (interleukin-1β) gene expression and plasma protein levels for Flox and KO mice (n=5-8/group). Asterisks indicate statistical significance by unpaired Student's t-test or 2-way ANOVA as appropriate; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. For all panels, Flox mice are presented in green and KO mice in red. Figure 5 has been submitted for publication. Zeng, Liping; Lee, Sung Min "Reciprocal regulation of cAMP signaling in immune cells and adipocytes controls lipolysis, thermogenesis, and obesity" The thesis author is a co-author.

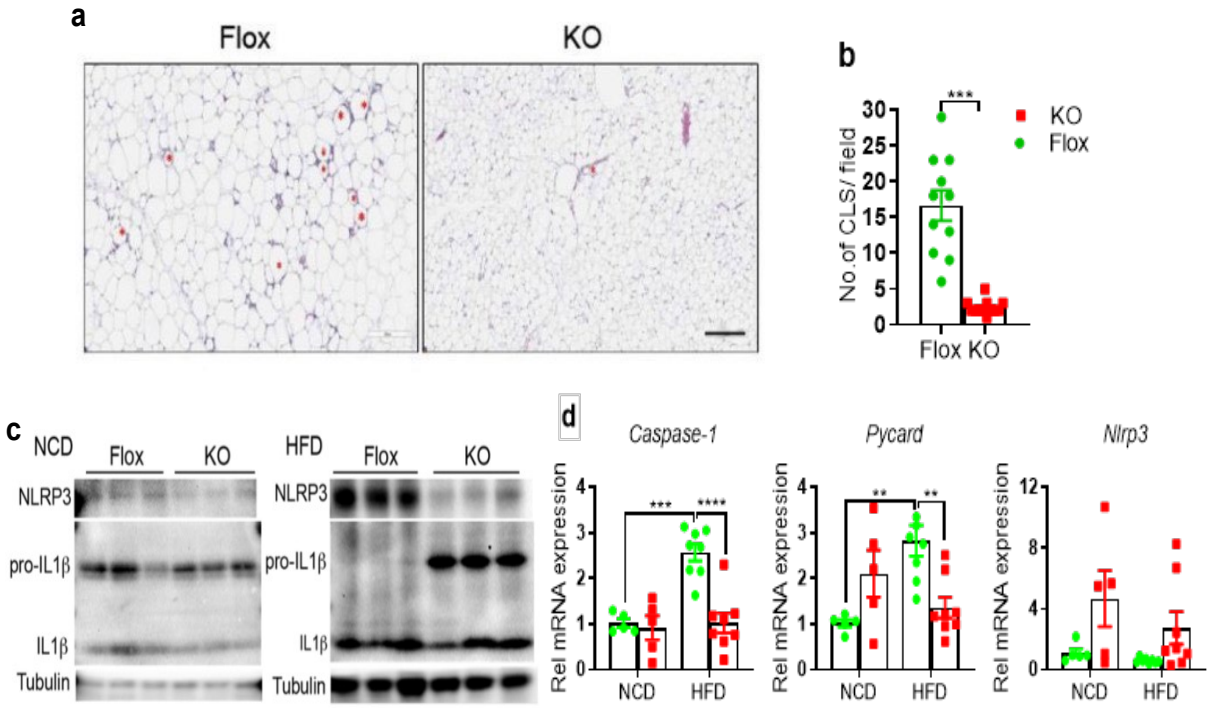

Figure 6. Gnas$^{\Delta CD11c}$ KO exhibit reduced inflammation and NLRP3 expression in adipose tissue and bone marrow-derived macrophages.
(a) Representative H&E staining images of eWAT tissue sections in Flox and KO mice. Crown-like structures (CLS) are indicated with red asterisks. **(b)** Quantification bar graph of CLS density of the H&E-stained sections (n=4/group). **(c)** Western blot analysis showing expression of NLRP3, pro-IL-1β, and β-tubulin as a loading control in eWAT extracts from Flox and KO mice fed with NCD (left) or HFD(right). **(d)** qPCR analysis showing gene expression of *Caspase-1* (Casp1) and *ASC* (Pycard) and *Nlrp3* in eWAT from Flox and KO mice fed with NCD or HFD. Data are presented as mean ± SEM and asterisks indicate Statistical significance by 2-way repeated-measures ANOVA or unpaired student's t-test as appropriate; *p < 0.05; **p < 0.01; ***p < 0.001; ****p<0.0001. For all panels, Flox mice are presented in green and KO mice in red. Figure 6 has been submitted for publication. Zeng, Liping; Lee, Sung Min "Reciprocal regulation of cAMP signaling in immune cells and adipocytes controls lipolysis, thermogenesis, and obesity" The thesis author is a co-author.

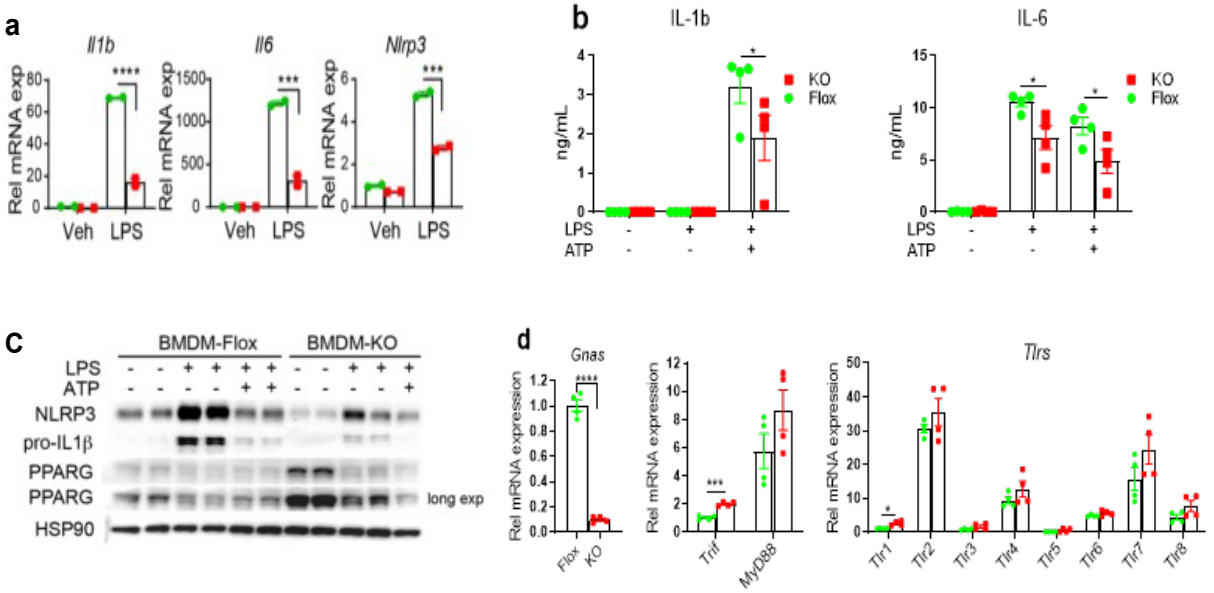

Figure 7. Gnas ablation led to reduced NLRP3 activation and macrophage-mediated inflammatory response. **(a)** qPCR analysis showing gene expression of *Il1β*, *Il6,* and *Nlrp3* in BMDM from Flow and KO mice treated with LPS (100 ng/mL) for 18 h. **(b)** ELISA analysis showed IL-1β and IL-6 levels in the supernatants from BMDM cells from Flox or KO mice treated with LPS +/- ATP (5 mM, 1 h). **(c)** Immunoblot analysis of BMDM cells in **(b)** for NLRP3, pro-IL-1β, PPARG, and β-Actin. Longer exposure of the PPARG blot is provided. **(d)** Relative mRNA expression of *Gnas, Trif, MyD88*, and *Tlr1-8* in BMDM from Flox and KO mice. Data are presented as mean ± SEM and asterisks indicate Statistical significance by 2-way repeated-measures ANOVA or unpaired student's t-test as appropriate; *p < 0.05; **p < 0.01; ***p < 0.001; ****p<0.0001. For all panels, Flox mice are presented in green and KO mice in red. Figure 7 has been submitted for publication. Zeng, Liping; Lee, Sung Min "Reciprocal regulation of cAMP signaling in immune cells and adipocytes controls lipolysis, thermogenesis, and obesity" The thesis author is a co-author.

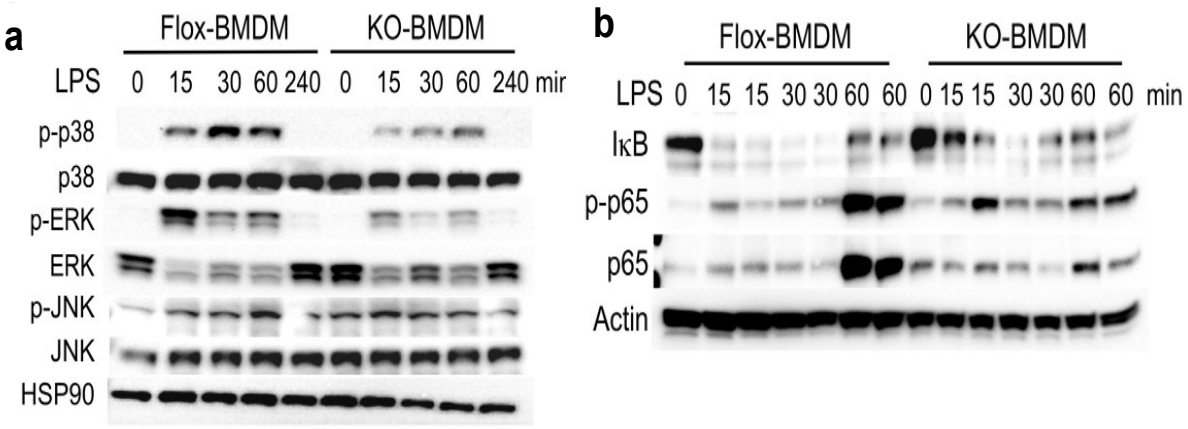

Figure 8. Reduced NF-κB signaling in bone marrow-derived macrophages of Gnas$^{\Delta CD11c}$ KO mice.
(a) Western blot analysis of phospho-p38MAPK, -JNK, and -ERK signaling in BMDM from Flox and KO mice treated with LPS (0, 15, 30, 60, 240 min). **(b)** Western blot analysis of IkB and phospho-p65 RelA (NF-κB) signaling in BMDM from Flox and KO mice treated with LPS (0, 15, 30, 60, 240 min). Figure 8 has been submitted for publication. Zeng, Liping; Lee, Sung Min "Reciprocal regulation of cAMP signaling in immune cells and adipocytes controls lipolysis, thermogenesis, and obesity" The thesis author is a co-author.

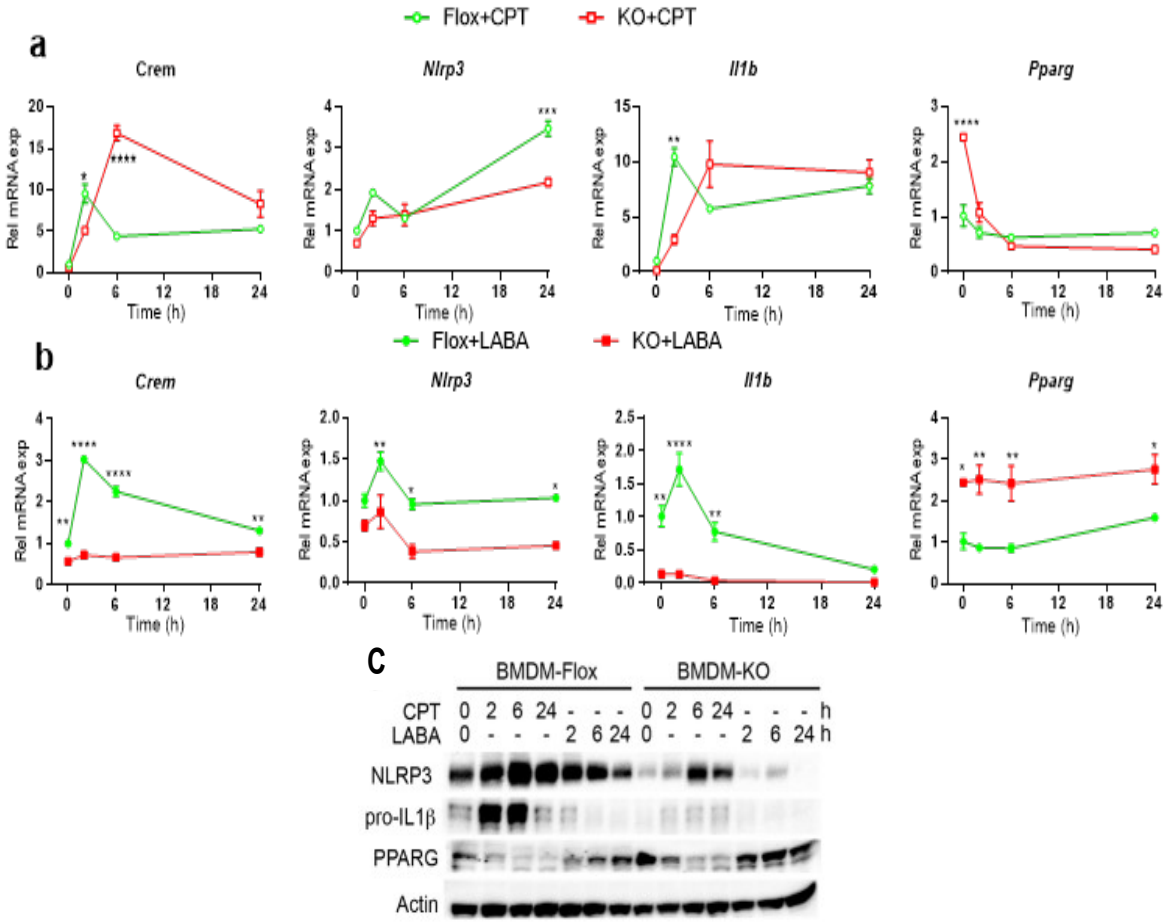

Figure 9 Re-establishment of intracellular cAMP concentration in BMDM of Flox and KO mice, reverses NLRP3 and PPARγ expression.

(a) Time course relative mRNA expression of *Crem, Il1β, Nlrp3,* and *Pparg* gene expression in Flox or KO BMDM treated with cell-permeable cAMP analog (CPT, 50 μM) by qPCR. **(b)** Time course relative mRNA expression of *Crem, Il1β, Nlrp3,* and *Pparg* gene expression in Flox or KO BMDM treated with long-acting b2-agonist formoterol (LABA, 1 μM). **(c)** Western Blot analysis of BMDM from Flox or KO mice treated with CPT or LABA over increasing time immunoblotted against NLRP3, pro-IL1β, PPARG, and β-Actin. Data are presented as mean ± SEM and asterisks indicate Statistical significance by 2-way repeated-measures ANOVA or unpaired student's t-test as appropriate; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p<0.0001$. For all panels, Flox mice are presented in green and KO mice in red. Figure 9 has been submitted for publication. Zeng, Liping; Lee, Sung Min "Reciprocal regulation of cAMP signaling in immune cells and adipocytes controls lipolysis, thermogenesis, and obesity" The thesis author is a co-author.

DISCUSSION

The cAMP signaling pathway is a key player in the underlying pathogenesis of inflammatory metabolic disorders including obesity[27]. Efforts have been made to therapeutically target the cAMP signaling axis to disrupt cAMP production. Studies on the effects of cAMP signaling remain conflicting as increased intracellular cAMP led to different downstream effects depending on cell and tissue type[67,68]. Therefore, the mechanism by which cAMP plays a role in diet-induced tissue inflammation is still unclear. To fill this knowledge gap, we ablated the *Gnas* gene in $CD11c^+$ cells of mice and observed the impact on its metabolism. We challenged Flox and $Gnas^{\Delta CD11c}$ KO mice with HFD to induce obesity and through this, we identified a tendency for a lean phenotype in the KO mice while displaying improved insulin resistance. In both groups challenged with NCD or HFD, the female mice showed a tendency be more resistant in gaining BW **(Fig. 2b and c)**. Sex differences have been reported to affect the susceptibility to diet-induced obesity however the mechanism is yet unclear. Levels in ovarian hormones have been suggested as the major reason for their protection against obesity and other metabolic syndromes[57]. It was shown that an ovariectomized female mice eliminated the protection against body weight gain[69]. In this study, it was revealed macrophage markers and inflammatory cytokines were decreased in the eWAT of KO mice challenged with HFD. In addition, there was lower NLRP3-associated gene expression and cytokine release indicating a reduced pro-inflammatory response. To further explore why this phenomenon is observed, we harvested and isolated $CD11c^+$ BMDM of KO mice and challenged cells with NLRP3 activating signals using LPS and ATP. The KO BMDM displayed reduced expression of NLRP3 while there was an increase in PPARγ expression, suggesting a potential inverse relationship. The data presented here, indicate a potential role of PPARγ in the trans-repression of the NF-κB pathway thereby inhibiting the NLRP3-mediated

inflammatory response in CD11c^{+} BMDM of KO mice. These results define the role of cAMP signaling in CD11c^{+} macrophages in modulating diet-induced tissue inflammation and insulin sensitivity.

First, our data indicate that ablation of Gαs decreased the inflammatory signaling and expression of macrophage markers in the adipose tissue of KO mice. Macrophages respond to the surrounding microenvironment by altering their phenotype to an M1-like (pro-inflammatory) or M2-like (anti-inflammatory) polarization state. In response to stress factors such as fat accumulation, macrophages are classically activated to a M1 state and produce pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α[70]. In contrast, M2 macrophages produce anti-inflammatory cytokines IL-10 to exploit protection against disease progression in obesity[71]. Adipose tissue macrophages (ATMs) of lean mice are known to highly express M2 macrophage markers including *Il10*[72]. The lean phenotype and reduced adipocyte distribution observed in the Gnas$^{\Delta CD11c}$ KO mice may contribute to and support the polarization of CD11c^{+} ATMs to a more M2-directed state **(Fig. 2a)** As our data indicate, the mRNA expression of anti-inflammatory cytokine *Il10* in eWAT and plasma IL-10 level was significantly greater in the KO mice and increased to a greater extent under HFD suggesting a potential increase in presence of M2 macrophages. Another hypothesis for the increase in IL-10 secretion may be a result of suppression of Gαs signaling of CD11c^{+} dendritic cells[29]. Previous studies have shown Gαs signaling of CD11c^{+} BMDC promoted a Th17 biased inflammatory response by the production of pro-inflammatory cytokines IL-1β and IL-6. However the effects were reversed by the suppression of Gαs signaling in Gnas$^{\Delta CD11c}$ BMDC which promoted a Th2 biased response through increased production of IL-4 and IL-10[29,73,]. The increased IL-10 secretion has been reported to modulate macrophages to transcriptionally suppress pro-IL-1β production as well as causing inhibition of

caspase-1 activity[74]. Thus, the increased IL-10 secretion from $Gnas^{\Delta CD11c}$ dendritic cells may potentially contribute to the decreased pro-IL-1β cleavage and *Caspase1* expression in eWAT of HFD-KO mice as shown in our results **(Fig 6c and d)**. However, the exact molecular mechanism of how the ablation of Gαs signaling in $CD11c^+$ cells promote a more anti-inflammatory status needs further research.

In addition to their immunoregulatory role, macrophages that reside in the adipose tissue are known to be recruited to WAT in response to adipocyte stress[75]. Here, ATMs act as scavengers to phagocytose adipocyte debris and consequently produce CLS in the adipose tissue[76]. In a study on macrophage-adipocyte interaction, the cell to cell interaction led to the increase in macrophage-mediated IL-6 secretion in a phagocytosis-dependent manner[77]. In line with this, our results show an upregulation of macrophage markers in the eWAT as well as an increase in CLS structures and higher mRNA expression of *Il6* in the Flox mice **(Fig 5 and 6)**. However, the opposite trend is observed in the KO mice, showing blunted *Il6* expression and significantly less CLS formation. This may be a result of reduced *Ccl2,* and *Adgre1* expression in the eWAT indicating decreased macrophage infiltration and recruitment. Fewer macrophages recruited to the WAT may lead to decreased macrophage-adipocyte interactions, therefore, contributing to the reduction of CLS formation and reduced *Il6* expression as observed in the eWAT of KO mice.

The cause of reduced *Il6* expression may be due to attenuated NF-κB activation in ATM. Studies have shown HFD is an inducer of the IKKβ/NF-κB pathway in adipocytes and macrophages[78]. Upon activation, the transcription factor NF-κB is translocated to the nucleus and regulates the expression of *Il6* and *Il1β*. Our results show NF-κB activation through western blotting of $CD11c^+$ BMDM of Flox mice. However, in KO cells, we observed decreased NF-κB transcriptional activity which may have contributed to the decreased transcription and expression

of *Il6*. Inconsistent with our results, previous studies have demonstrated an increase in intracellular cAMP acting as inhibitors of NF-κB pathway[79]. Therefore, based on our results we propose that the effects of cAMP signaling vary depending on the specificity of cell type and further emphasize the presence of other factors that contribute to the attenuation of NF-κB activation.

In our study, we identified that deletion of *Gnas* in CD11c$^+$ cells resulted in downregulation of NLRP3 expression and secretion of IL-1β. M1-like macrophages and other immune cells infiltrate the adipose tissue and were shown to induce tissue inflammation by increased secretion of pro-inflammatory cytokines such as IL-1β, IL-6 and TNFα[61]. ATMs upon activation by PAMPs or DAMPS such as overnutrition, mediate the activation of the NF-κB pathway through PRRs[80]. Consequently, this initiates the transcription of NLRP3 and pro-IL-1β to prepare for a pro-inflammatory response[31]. The addition of a secondary signal triggers the activation and assembly of the inflammasome, leading to the caspase-1 dependent cleavage of pro-IL-1β to mature IL-1β. In agreement with this, the western blot analysis showed that HFD induced an increase in NLRP3 expression in the eWAT of Flox mice along with increased expression of *Caspase-1* and *Pycard* **(Fig. 6)**. In addition, CD11c$^+$ BMDM from Flox mice challenged with LPS and ATP showed increased secretion of IL-1β **(Fig. 7b)**. However, ablation of Gαs signaling in CD11c$^+$ BMDM led to reduced NLRP3-associated gene expression and IL-1β secretion. The reduction of NLRP3 activation could be caused by the induction of PPARγ as studies suggest PPARγ functions to regulate metabolic pathways by suppression of NLRP3 activation [81,82,83]. In line with this, our results showed an increase in PPARγ induction in CD11c$^+$ BMDM of KO while exhibiting reduced NLRP3 activation **(Fig. 7c)**. Based on our previously mentioned data, NF-κB activation was shown to be reduced in the CD11c$^+$ BMDM of KO mice **(Fig 8)**. This may be an indication of PPARγ possessing inhibitory effects on NF-κB activation as results showed reduced degradation of IκB

and phosphorylation of p65. In support of our results, studies have suggested that PPARγ act as an E3 ubiquitin ligase by utilizing Lys48-linked polyubiquitin bound to the LBD to induce p65 degradation[66]. Since suppression of NF-κB activity could lead to reduced transcription of NLRP3 and related pro-inflammatory cytokines, the E3 ligase activity may play important role in attenuating obesity-mediated tissue inflammation. The potential role of PPARγ to act as an E3 ligase to inhibit NF-κB activity could be critical in suppressing NLRP3-mediated inflammatory response, nevertheless, further research is needed to validate this hypothesis.

The deletion of *Gnas* gene in $CD11c^+$ BMDM ablated the cAMP signaling pathway, but our study revealed that CPT treatment re-established the NLRP3-mediated inflammatory response **(Fig. 9)**. Collectively, our results indicate that Gαs-mediated cAMP signaling is essential for NLRP3 expression and activity. Consistent with the aforementioned result, increasing cAMP signaling via CPT treatment downregulated the expression of PPARγ in Flox and KO BMDM which underlines the potential role of cAMP in establishing a contrary relationship between PPARγ and NLRP3. This opposing relationship could be explained by the cross-talk between cAMP and cyclic guanosine monophosphate (cGMP)[84].

Intracellular cGMP is synthesized by guanyl cyclases (GCs) in response to nitric oxide (NO) and natriuretic peptides and mediates the modulation of metabolic pathways[85]. cGMP production results in downstream activation of cGMP-dependent protein kinase-G (PKG) which exerts opposing effects to PKA activation and vice versa[86]. The cAMP and cGMP signaling pathway leads to activation of phosphodiesterases (PDEs) that hydrolyze these secondary messengers, such that an increase in intracellular cGMP results in decreased cAMP concentration[87]. PDE2 and PDE3 were shown to be the main cGMP-regulated isoforms in macrophages[88]. Therefore, it is possible the ablated cAMP signaling in $CD11c^+$ BMDM of KO mice may result in

increased cGMP signaling. In support of this, a recent study on the role of cGMP in WAT showed that cGMP/PKG signaling caused an increase in PPARγ expression[89]. The study also revealed PKG activation had anti-inflammatory effects by lowering pro-inflammatory adipokine production as well as triggering the browning of WAT which indicates anti-obesity effects. Thus, the deletion of *Gnas* in $CD11c^{+}$ cells and subsequent ablation of cAMP signaling may be a result of increased intracellular cGMP production that led to reduced diet-induced inflammation. In conclusion, future studies exploring cGMP signaling in $Gnas^{\Delta CD11c}$ KO mice may serve as potential therapeutic targets in improving tissue inflammation in metabolic disorders.

Future Directions

Unfortunately, our study did not explore the possible role of other $CD11c^+$ immune cells such as dendritic cells in the $Gnas^{\Delta CD11c}$ KO mice under HFD conditions. Due to overlapping markers and $CD11c^+$ being widely expressed in different cell types, the distinguishment between ATMs and adipose tissue dendritic cells (ATDCs) has been unclear. It is difficult to know whether only one specific cell type or a variety of cell types are contributing to the lean phenotype in KO mice. However a recent study has suggested Flow Cytometry analysis on adipose tissue cells revealed $CD11c^+$ $CD64^+$ markers were specific for ATMs while ATDCs were enriched in $CD11c^+$ $CD64^-$ cells[90]. It is known that ATMs and ATDCs both contribute to obesity-induced inflammation and insulin resistance[91]. Therefore, exploring the possible role of ATDCs could further unravel the underlying mechanisms that are responsible for the phenotype observed in the $Gnas^{\Delta CD11c}$ KO mice. Also, future experiments should be directed towards single-cell sequencing on $CD11c^+$ stromal vascular cells (SVCs) to uncover the different cell types and difference in the transcription profiling caused by the *Gnas* deletion. Through this we can discrete the $CD11c^+$ cell populations that contribute to obesity-induced inflammation.

Further investigation on intracellular cGMP is also necessary to demystify the molecular mechanisms that led to inhibition of M1-like inflammatory mediators in the KO mouse model. Exploring the PKG-cGMP signaling pathway could unravel the exact mechanisms that led to PPARγ induction and the subsequent transrepression of NLRP3 in the KO BMDM. The potential upregulation of cGMP generation caused by ablation of intracellular cAMP could be the major influencer of reduced tissue inflammation in $Gnas^{\Delta CD11c}$ KO mice.

MATERIALS AND METHODS

Mice

Young WT C57BL/6 (B6) mice (ages 2 - 4 months) were purchased from Jackson Laboratories. The $Gnas^{\Delta CD11c}$ ($Gnas^{tm5.1Lsw/tm5.1Lsw}$/Tg(Itgax-cre)1-1Reiz) mice were generated as described in a previous study from the Raz Lab[56]. To generate the $Gnas^{\Delta CD11c}$ mice, the floxed $Gnas^{fl/fl}$ (*$Gnas^{tm5.1Lsw/tm5.1Lsw}$*) mice were crossed with CD11c-Cre mice. Here, the floxed mice were used as littermate controls. In this paper, mice used in experiments were challenged with a high-fat diet (HFD, D12492, 60% kcal from fat; 5.24 kcal/g, Research Diets Inc, New Brunswick, NJ) for 8-10 weeks to induce obesity. Control mice were fed a normal chow diet (NCD, rodent 5001). All mice were bred in the vivarium under standard conditions (24 ± 2 ℃ on a 12 h light/12 h dark cycle, light on at 6 am) and all animals had free access to food and water before being placed in the study groups. All procedures were approved by the Animal Care Committee of the University of California San Diego, School of Medicine.

Glucose and insulin tolerance tests

The glucose tolerance test (GTT) was performed on mice, after fasting for 8h. Mice were injected intraperitoneally (i.p) with D-glucose (2g/kg). The tail bleed was used to measure blood glucose levels at 0 min and monitored at intervals until 12 mins using a glucose meter (Easy Step Blood Glucose Monitoring System, Home Aide Diagnostics, Inc., Deerfield Beach, FL). The insulin tolerance test (ITT) was measured after mice were fasted for 6 h and injected i.p with recombinant human insulin (0.4 U/kg). Terminal fasting glucose was measured after 6 h fasting. Terminal fasting plasma insulin was measured by a mouse insulin kit (Meso Scale Diagnostics, Rockville, MD). The homeostatic model of insulin resistance (HOMA-IR) was calculated as

follows: (fasting serum insulin concentration (mU/ml)) x (fasting blood glucose levels (mg/dl))/(405).

Histopathological Study

Mouse tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. For histopathological evaluation, sections of 5-mm-thick were stained with haematoxylin and eosin according to standard protocols. For adipose tissue, pictures of representative areas from each section in ×100 magnification were taken, and Adiposoft software was used to calculate cell size of 3 images/section/mouse. Minimal 20 mm and maximum 100 mm thresholds were set for automated measurement of adipocyte diameter followed by manual correction. A frequency distribution was calculated for each group. Total adipocyte number within the distribution was subsequently calculated, and the frequency was converted to a percentage of total adipocytes counted.

RNA isolation and real-time PCR

RNA was extracted from cells or tissues using an PureLink™ RNA Mini Kit (ThermoFisher Scientific) in accordance with protocol provided by the manufacturer. 0.5-1 µg RNA was transcribed to cDNA with the reverse transcription system (Invitrogen). Real-time PCR was performed on a Bio-Rad CFX384™ real-time PCR detection system using iTaq™ Universal SYBR® Green Super mix (BioRad).

Enzyme-linked immunosorbent analysis (ELISA)

Cytokine and chemokine levels in serum were obtained using ELISA kits (eBioscience) and UPLEX Adipokine assays (Meso Scale Discovery, Millipore).following the manufacturer's protocol.

Western Blotting and preparation

For western blot sample preparations, the proteins of cells or tissues were lysed using RIPA buffer (Thermo Fisher Scientific) containing protease and phosphatase inhibitors. The lysed samples were incubated on ice (30 min) and centrifuged for 15 min at 10,000 rpm at 4°C. Protein lysate samples were quantified using the micro-BCA protein assay kit (23225, Thermo Fisher Scientific, New York, NY) using BSA as standard. Total protein lysates (10-20 μg) were separated on 10-15% polyacrylamide gels and transferred onto the Polyvinylidene difluoride (PVDF) membrane (IPVH00010, Millipore Sigma). Membranes were blocked in 5% non-fat milk or BSA in PBS with 0.1% Tween 20 (PBST) for 1 h at RT and then incubated at 4˚C overnight with the primary antibody at the indicated concentration. Next, the membranes were washed for 5 min X 4 times with PBST and incubated with HRP-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hour. The membranes were visualized on X-ray films or ChemiDoc Imaging System (BioRad, Hercules, CA) following the reaction with the enhanced chemiluminescence substrate (SuperSignal™ West Pico PLUS Chemiluminescent Substrate, ThermoFisher Scientific).

Cell Culture

Bone marrow cells from mice were isolated by flushing femurs and tibias with PBS, supplemented with 2% FBS. The cells were centrifuged and then filtered through a 70 μm cell strainer before being re-suspended in RPMI-1640 containing 100 U/mL penicillin, and 100 μg/mL streptomycin and supplemented with 10% FBS and differentiated for 7 days in the presence of 10 ng/mL mouse recombinant GMCSF (BioLegend; 576302). On day 3, an additional 50% of the culture medium with 20 ng/ml GM-CSF was added, and on day 6, half of the medium was removed and supplemented with 10 ng/ml GM-CSF culture medium. Adherent cells were collected on day 7 and CD11c+ BMDM were selected by anti-CD11c linked magnetic beads (Stemcell; 18780). CD11c+BMDM Cells were primed by 3 h treatment with ultrapure LPS (100 ng/ml; Invivogen) alone; inflammasome stimulation was provided by treatment with ATP (5 mM; 1h).

Statistics

were analyzed using Prism (Graphpad) and are presented as mean ± SEM or mean ± SD. Statistical significance was determined using the unpaired two-tailed Student's t-test or 1-way or 2-way ANOVA followed by Tukey post-tests for multiple variables. A p-value of < 0.05 was considered significant and is presented as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, or $****p < 0.0001$.

Figures 2-9 and Methods section are currently being prepared for submission for publication of the material. Liping Zeng, D. Scott Herdman, Sung Min Lee, Ailin Tao, Manasi Das, Samuel Bertin, Lars Eckmann, Sushil Mahata, Shwetha Devulapalli, Hemal H. Patel, Anthony J.A. Molina, Olivia Osborn, Maripat Corr, Eyal Raz, and Nicholas J.G. Webster "Reciprocal regulation of cAMP signaling in immune cells and adipocytes controls lipolysis, thermogenesis and obesity" The thesis author is a co-author.

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