

**Treating Obesity and Type 2 Diabetes by Targeting a Phase II Sulfotransferase**

by

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# Treating Obesity and Type 2 Diabetes by Targeting a Phase II Sulfotransferase

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University of Pittsburgh, 2021

Obesity and Type II Diabetes Mellitus and their related comorbidities are one of the leading causes of loss of life and disability globally. Current first-line therapies seem to be inefficient in reducing global trends. The need to find potential therapeutic targets to help curb growing insulin resistance and obesity rates will be vital to stop this health crisis. Given these metabolic diseases involve dysregulated cholesterol levels, examining proteins involved in cholesterol regulation may prove fruitful. SULT2B1b is a sulfotransferase that preferentially conjugates a sulfate group onto cholesterol. Generally, sulfonation by a sulfotransferase is a part of phase II metabolism in which the liver attempts to clear metabolites from the body. However, sulfonation also plays a significant role in modulating the effects of certain endogenous compounds by deactivating their biological effects. In a previous study, cholesterol sulfate, the product of SULT2B1b, was shown to reduce lipogenesis through inhibition of the nuclear receptor Liver X Receptor (LXR). Furthermore, cholesterol sulfate inhibited Hepatic Nuclear Factor 4 $\alpha$  (HNF4 $\alpha$ ), which caused downregulation of gluconeogenesis. Overexpression of SULT2B1b shows protection against diet-induced obesity given its inhibitory roles on excessive glucose and lipid production. However, in this study, we investigated the SULT2B1b genetic knockout to examine whether loss of SULT2B1b in mice challenged with a high-fat diet would worsen outcomes. Surprisingly, SULT2B1b knockout protected mice against fat accumulation and insulin resistance. This may be due to the downregulation of CD36 in SULT2B1b null mice. Given the protection provided through genetic ablation, inhibiting SULT2B1b may be a promising target to combat obesity and T2DM.

**Key Words:** sulfotransferase, type 2 diabetes, obesity, liver, cd36

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## **ABBREVIATIONS**

**SULTs**: sulfotransferases, **SULT2B1b**: sulfotransferase 2B1b, **HFD**: high fat diet, **LXR**: liver X receptor, **CAR**: constitutive androstane receptor, **HNF4 $\alpha$** : hepatic nuclear factor 4 $\alpha$ , **T2DM**: Type II Diabetes Mellitus, **NAFLD**: nonalcoholic fatty liver disease, **HCC**: hepatocellular carcinoma, **ALT**: alanine aminotransferase, **AST**: aspartate aminotransferase, **CD36**: cluster of differentiation 36, **RQ**: respiratory quotient

## **Preface**

I faced many challenges this past year and there are several people whose support and patience were tremendously appreciated. I would like to thank Dr. Wen Xie for being a great mentor during my time at Pittsburgh, challenging me to become a better critical thinker, and giving me ample support and understanding during this challenging year.

I would also like to thank Dr. Maggie Folan, whose support and kindness were also greatly appreciated.

I would like to thank Dr. Robert Gibbs and Dr. Da Yang for being excellent teachers for my Grant Writing course and helping me begin to learn the fundamentals of scientific writing and communication. I would also like to thank them both for agreeing to be on my committee and helping without hesitation at every opportunity they could. I sincerely cannot thank the four of you enough for all the guidance and support you have given me to pursue my scientific career.

From the lab, I would like to thank Meishu Xu, who was always kind and eager to help with any issue I had. I would also like to thank Hung-Chun Tung and Xinran Cai for their assistance in helping me get my experiments off the ground and showing me the ropes.

Lastly, I would like to thank my fiancé Tyler for all of her encouragement, love, and support.

## 1.0 INTRODUCTION

### 1.1 Obesity and Insulin Resistance

#### 1.1.1 Epidemiology

Obesity represents a major health issue affecting an estimated 650 million people worldwide [1, 2] with a steadily increasing prevalence in most regions globally [3]. Obesity is characterized by an excess accumulation of adipose tissue imposing deleterious health effects [1]. It poses a significant economic burden, with an estimated global loss of \$2 trillion due to disability and medical treatments [4]. Furthermore, there is a broad range of comorbidities associated with obesity. These include an extensive list of cancers such as breast [5, 6, 7], renal [7, 8], pancreatic [9, 10], and colorectal [11,12]. Other comorbidities include non-alcoholic fatty liver disease [13], cardiovascular diseases [2, 14], and the development of insulin resistance leading to type 2 diabetes mellitus (T2DM) [15, 16].

T2DM incidence has increased with obesity rates, with T2DM seeing a more than 6-fold increase in the US over the last half-century [16]. While several factors contribute to the development of T2DM, obesity remains a significant risk factor leading to an estimated 170 million people afflicted with obesity-induced T2DM [15]. T2DM is characterized by an increase in blood glucose brought about in part by a diminished sensitivity to insulin [17]. Therefore, reducing body fat accumulation and maintaining appropriate insulin responses proves to be a critical aim in curbing the obesity and T2DM epidemic.

### **1.1.2 Role of the Liver in Obesity and T2DM Development**

The liver has a substantial role in moderating blood glucose levels by serving as the first site of glucose uptake following meal digestion and subsequent absorption [18]. Additionally, the liver controls endogenous glucose production further affecting its role in blood glucose moderation [19]. Given that obesity and T2DM are classified in part by dysfunction of glucose regulation, the liver becomes a natural choice for investigation.

Beyond its role in glucose homeostasis, the liver is also adversely affected by metabolic syndrome. Non-alcoholic fatty liver disease (NAFLD) is characterized by the presence of hepatic steatosis leading to inflammation and fibrosis [12]. This can further progress to necrosis and hepatocellular carcinoma [12, 20]. Much like the other pathologies associated with metabolic syndrome, it is still not fully clear whether NAFLD contributes directly to insulin resistance/obesity, is mediated by the presence of insulin resistance/obesity, or is all part of a common pathology [20, 21]. There is a strong indication, however, that reducing circulating free fatty acids and reducing hepatic inflammation can help alleviate the development and progression of NAFLD in the context of metabolic syndrome [22]. Thus, targeting obesity and insulin resistance may also ameliorate NAFLD.

## **1.2 Sulfotransferases in Metabolic Syndrome**

### **1.2.1 Sulfotransferase Background**

Sulfotransferases (SULTs) refer to a superfamily of low molecular weight, cytosolic proteins which conjugate a sulfate molecule to a substrate [23, 24, 25]. Sulfonation generally decreases the biological activity of the substrate and makes it more soluble in water to allow for greater clearance [26]. SULTs utilize the universal sulfate donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to sulfonate a hydroxyl or amino group on its substrate [27]. SULTs are

indispensable for their role in clearing both endogenous and exogenous compounds. In humans, there are currently 10 known SULTs organized into four sub-families: SULT1, SULT2, SULT4 and SULT6 [24, 25, 28] with an additional 5 theorized through genome database mining [29]. Given the critical role of detoxifying and clearing agents, SULTs are relatively conserved across species; mice, for instance, have 23 SULTs organized into seven sub-families [25]. Not unlike other phase II metabolizers, SULTs have broad and sometimes overlapping specificities to different substrates [30].

SULT1 and SULT2 are the most populated subfamilies primarily sulfonating phenol and hydroxysteroids respectively. The primary substrate of the SULT isoform generally dictates its expression level with most SULTs being expressed in the liver, skin, lung, gastrointestinal tract, breast, and kidney [31].

Recent studies, however, indicate SULTs have a larger impact than clearance alone. SULT activation has been shown to activate procarcinogen molecules and oxidative stressors [23, 28]. This activity has been shown to contribute to the pathogenesis and exacerbation of numerous diseases including hepatocellular carcinoma [32], colorectal cancer [33], bladder cancer [34], NAFLD [35], Acetaminophen injury [36], and acute kidney injury [37,38]. Sulfonation also represents a means of cell signaling [35] and can result in the deactivation of hormones [23, 38, 39]. Therefore, it is becoming increasingly clear that SULTs have a greater physiological impact beyond its role in metabolite clearance.

### **1.2.2 SULT2B1b and its Impact on the Onset of Obesity and Insulin Resistance**

SULT2B1a and SULT2B1b are the two isoforms belonging to the SULT2B group with SULT2B1b being expressed about 7-fold times higher than its counterpart [39]. Both isoforms sulfonate  $3\beta$ -hydroxysteroids like DHEA and pregnenolone [39, 40] as well as sulfonating 25-hydroxycholesterol (25HC) to form 25-hydroxycholesterol-3-sulfate (25HC3S) [39]. Since obesity and obesity-related diseases coincide with high cholesterol, groups began studying the

significance of SULT2B1b converting 25HC to 25HC3S. It was observed that 25HC was a ligand for Liver X Receptor, a nuclear receptor that regulates cholesterol, glucose homeostasis, and lipogenesis [41]. Furthermore, a separate study revealed 25HC3S inhibited LXR [19], thus implicating SULT2B1b as a regulator of LXR and its subsequent targets. Constitutive androstane receptor (CAR) is also activated by 25HC and its inhibition was shown to be protective against obesity and T2DM [42]. Thus, the balance between 25HC and 25HC3S mediates the expression of many genes associated with metabolic syndrome.

Although SULT2B1b is a fundamental mediator of 25HC and 25HC3S levels, it has low basal expression in the liver, where LXR has the highest expression [31]. SULT2B1b is basally expressed in the prostate, lung, small intestine muscle, and skin [31, 39], however, it was shown to be induced in the liver of obese mice when going from a fasted to a fed state [43]. This inducible state further solidified the role SULT2B1b has in mediating the response of cholesterol sensing receptors.

SULT2B1b was shown to influence gluconeogenesis as well. Overexpression of SULT2B1b in the liver or treatment of 25HC3S, led to the downregulation of hepatocyte nuclear factor 4-alpha (HNF4 $\alpha$ ), a pro-gluconeogenic regulator [43]. It was later determined SULT2B1b is a transcriptional target of HNF4 $\alpha$ , which indicates SULT2B1b is part of a negative feedback loop since the enzymatic action of SULT2B1b leads to HNF4 $\alpha$  downregulation [44].

### **1.3 Hypothesis**

SULT2B1b sulfonation leads to decreased expression of gluconeogenic proteins by way of HNF4 $\alpha$  inhibition and decreased expression of lipogenic proteins by way of CAR and LXR inhibition through 25HC3S formation. Thus, genetic ablation of SULT2B1b should cause a relative lack of cholesterol sulfate which would lead to a loss of a regulatory factor of both HNF4 $\alpha$  and CAR/LXR. When challenged with a high-fat diet, mice without SULT2B1b would be

expected to have worse outcomes due to increasing gluconeogenesis and lipogenesis.

Surprisingly, early data showed SULT2B1b knockout caused a protective effect indicating SULT2B1b may modulate an unknown pathway. Additionally, SULT2B1b inhibition may be a target to protect against the onset of metabolic diseases such as obesity, T2DM, and NAFLD.

## 2.0 MATERIALS AND METHODS

### 2.1 *Animals*

SULT2B1b whole-body knockout mice were bred from our lab's extant colonies derived from Strain # 018773 from Jackson Labs. 8-week-old SULT2B1b KO mice were used for the High Fat Diet (HFD) challenge regimen. Male C57BL/6J were purchased at 8 weeks from Jackson Labs and started simultaneously with age-matched SULT2B1b KO mice on HFD. HFD consisted of 20% protein, 20% carbohydrates, and 60% fat (Research Diets D124924). 4 groups of KO (n = 17 total) containing 4 male KO mice were matched against 4 groups of WT C57BL/6J (n = 16 total) in four separate 12-week studies. For the in-house bred WT study, 2 groups of WT mice (n = 8) were matched against 2 groups of KO mice (n = 9). The in-house WT mice were selected from litters from heterozygous SULT2B1b KO parents and matched with SULT2B1b KO littermates. Animal body composition (fat, lean, water mass) was observed using an EchoMRI weekly. Animals were sacrificed using CO<sub>2</sub> euthanasia prior to tissue harvest. All animals were housed in 12 hours light/12 hours dark, pathogen-free conditions. Approval for study and proper training was received prior to beginning any mouse experimentation. Animals were treated in accordance with policies enacted by the University of Pittsburgh and Institutional Animal Care and Use Committee (IACUC).

## **2.2 ITT and GTT**

Insulin Tolerance Tests (ITTs) and Glucose Tolerance Tests (GTTs) were performed every four weeks on all 6 groups. The ITT was performed at the beginning of the week and the GTT at the end of the week to reduce the stress placed on the mice. For the ITT, mice were placed into clean cages without access to food and starting at 9 AM. After 6 hours of fasting, mice were given an injection of 0.50 IU insulin/kg BW by I.P. injection. Blood glucose was monitored right before injection and every 30 minutes following injection for 2 hours using a glycosometer. For the GTT, mice were placed into clean cages without access to food at 5 PM. The following day, mice were given 2g glucose/kg BW by I.P. injection. Blood glucose was monitored in the same manner as the ITT protocol.

## **2.3 Metabolic Cage Study**

Following 12 weeks of study on HFD, two groups (WT n = 8, KO n = 9) were placed in metabolic cages (Columbus Labs Animal Monitoring System) for 72 hours. Mice were individually caged and gas exchange (O<sub>2</sub>, CO<sub>2</sub>) food/water consumption, energy expenditure, and mouse movement were tracked for 48 hours. Following standard procedure [45], data was not recorded for the first 24 hours as this is an acclimation period for the mice to the new environment. 12-hour light/dark cycles were simulated and free access to food and HFD was provided. Indirect calorimetry reported was calculated using the Weir equation [46] which determines energy expenditure from O<sub>2</sub> consumed and CO<sub>2</sub> produced.

## **2.4 Tissue Harvest**

Tissues were harvested quickly following euthanasia. Upon harvesting, tissues were weighed, divided, and one division was flash-frozen in liquid nitrogen. Another section of each tissue was placed in 10% paraformaldehyde solution for histological use. The last part of the division was placed into O.C.T cryostat embedding medium and frozen using isopentane and liquid nitrogen to avoid bubbles.

## **2.5 Histology**

H&E staining: Tissues were embedded in paraffin following storage in 10% paraformaldehyde for 1 week and moved to 70% ethanol. Section were 4 microns. Sections were stained with hematoxylin and counter-stained with eosin.

Oil Red O/IF: Tissues frozen in O.C.T. were sectioned using a cryostat. Sections were 8 microns. For ORO, sections were stained in ORO for 15 minutes and counterstained with hematoxylin. For IF, sections were blocked, washed, probed against CD36 using CD36 primary antibody (ab252923). CD36 antibody was visualized with Alexafluor 488, and the nuclei were visualized through DAPI staining. Images were taken using confocal microscopy. Exposure time was 170ms.

## **2.6 Liver and Serum Biochemical Analysis**

Blood was collected via cardiac puncture and spun down in a centrifuge at 2,000 g for 10 minutes to separate the serum. Serum was tested for cholesterol and triglyceride content via Stanbio Cholesterol LiquiColor (SB1010225) and Stanbio Triglycerides LiquiColor (SB2200225) respectively. The liver was homogenized and lipids were extracted via the Folch Method as

described previously [47]. The resulting solution was tested with the same kits listed above. AST and ALT of homogenized liver tissue were tested using Stanbio AST/SGOT Liqui-UV (SB2920430) and Stanbio ALT/SGOT Liqui-UV (SB2930430) respectively.

### **2.7 Real-Time PCR**

Tissue was homogenized and RNA was extracted using TRIzol reagent through phenol-chloroform extraction. RNA was isolated using isopropanol, then ethanol, and then stored in water at -80C. Real-time PCR was performed using SuperScript™ III Reverse Transcriptase kit from Invitrogen. mRNA levels were measured using quantitative PCR, measured with SYBR green fluorescence using a cyclophilin as a normalizing control.

### **2.8 Western Blot**

Tissues were lysed with a protease inhibitor cocktail. Pierce™ BCA Protein Assay Kit was used to assess protein concentration and to dilute samples accordingly. Protein samples were run on a 10% SDS-PAGE. Proteins were transferred to PVDF and CD36 (ab252923) and Beta-Actin were probed against. Bands were visualized using ECL western blotting substrate.

### **2.9 Statistical Testing**

Data was presented as mean  $\pm$  standard error of the mean (SEM). Statistical analysis between groups was conducted by Student's t-test with a p-value of <0.05 considered significant. Data conducted over intervals (i.e. MRI/BW/ITT/GTT) was analyzed by Student's t-test at each interval. Data was compiled and presented using GraphPad Prism 8.

## 3.0 RESULTS

### 3.1 SUL2B1b Genetic Knockout Protects Mice from Body Fat Accumulation when Challenged with High Fat Diet

To assess the role that SUL2B1b has in mediating the development insulin resistance and obesity, male SUL2B1b null mice and C57Bl/6J purchased from Jackson Laboratories mice were challenged with 12 weeks of High Fat Diet (HFD). Given the protective role SUL2B1b has in mediating lipogenesis and gluconeogenesis, it was predicted that the loss of SUL2B1b would lead to uncontrolled glucose regulation, increased lipogenesis and thus worse outcomes when challenged with HFD. Each week food consumption, body mass and body composition were recorded. To measure changes to insulin sensitivity, both groups had insulin tolerance tests (ITTs) and glucose tolerance tests (GTTs) every four weeks after starting on HFD (**Figure 1A**).

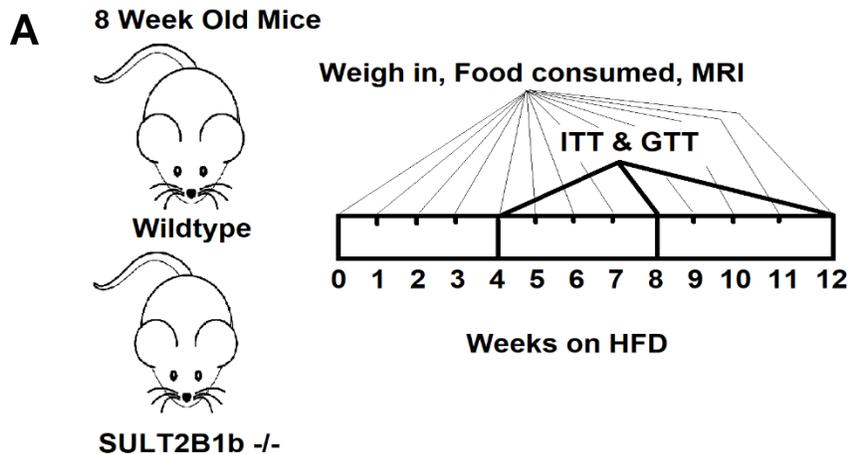
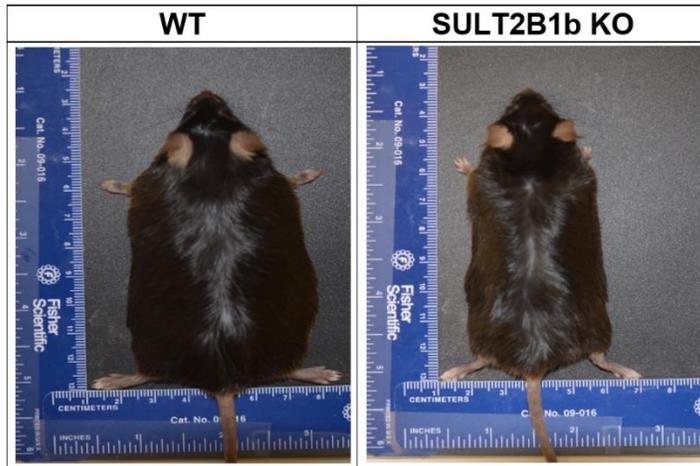


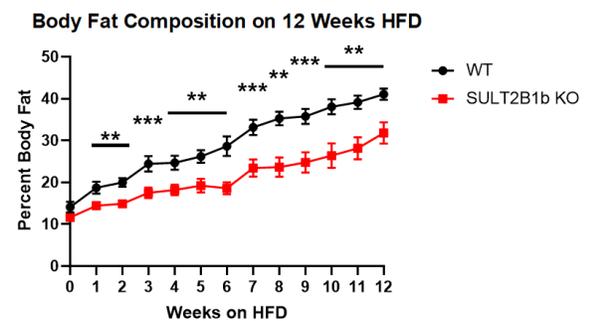
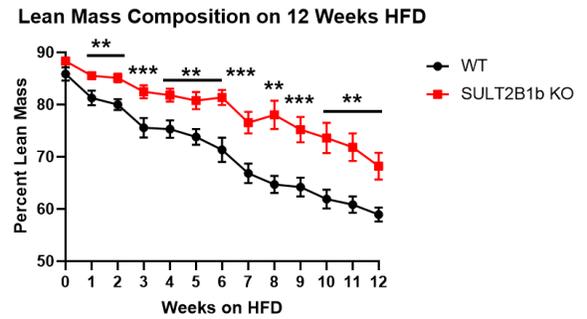
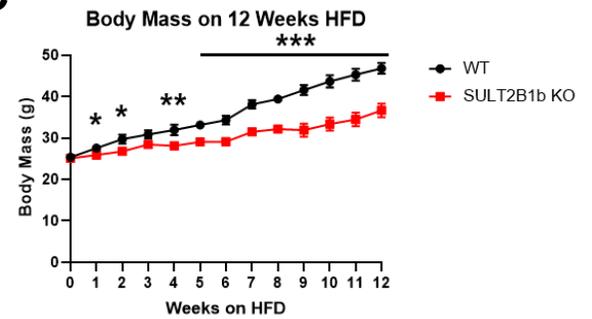
Figure 1A: Schematic of HFD Challenge

Surprisingly, the SULT2B1b null mice were visibly smaller compared to the wildtype mice following 12 weeks of HFD (**Figure 1B**). MRI revealed both groups started with similar body mass and body fat compositions, however the SULT2B1b knockout mice were protected from body mass and fat accumulation and lean mass loss associated with HFD consumption (**Figure 1C**).

**B**

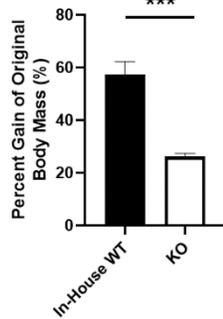


**C**

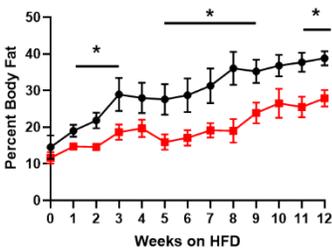


**D**

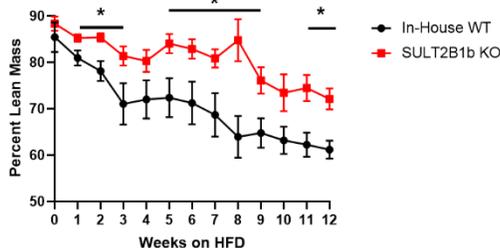
Percent Body Mass Gain Following 12 Weeks of HFD



Body Fat Composition on 12 Weeks HFD (In-House WT)



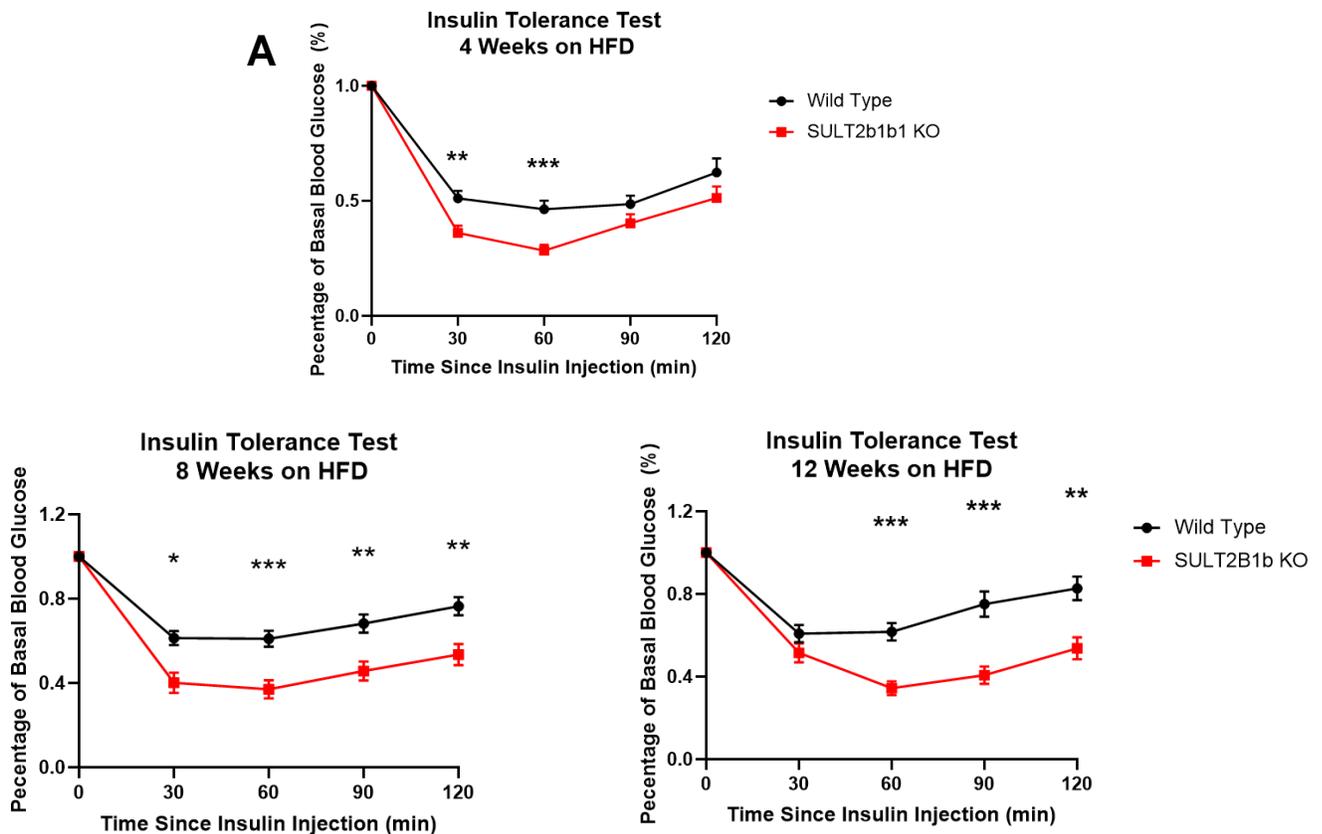
Lean Mass Composition on 12 Weeks HFD (In-House WT)



To confirm the differences between the wildtype and the SULT2B1b knock out groups represented meaningful differences, we bred SULT2B1b heterozygous mice to perform the same challenge again using the resulting WT and SULT2B1b knockout littermates. A similar trend was found showing that the WT mice bred in-house from the SULT2B1b heterozygous mice accumulated significantly more body mass and body fat, and lost more lean mass compared to the SULT2B1b null mice. (Figure 1D).

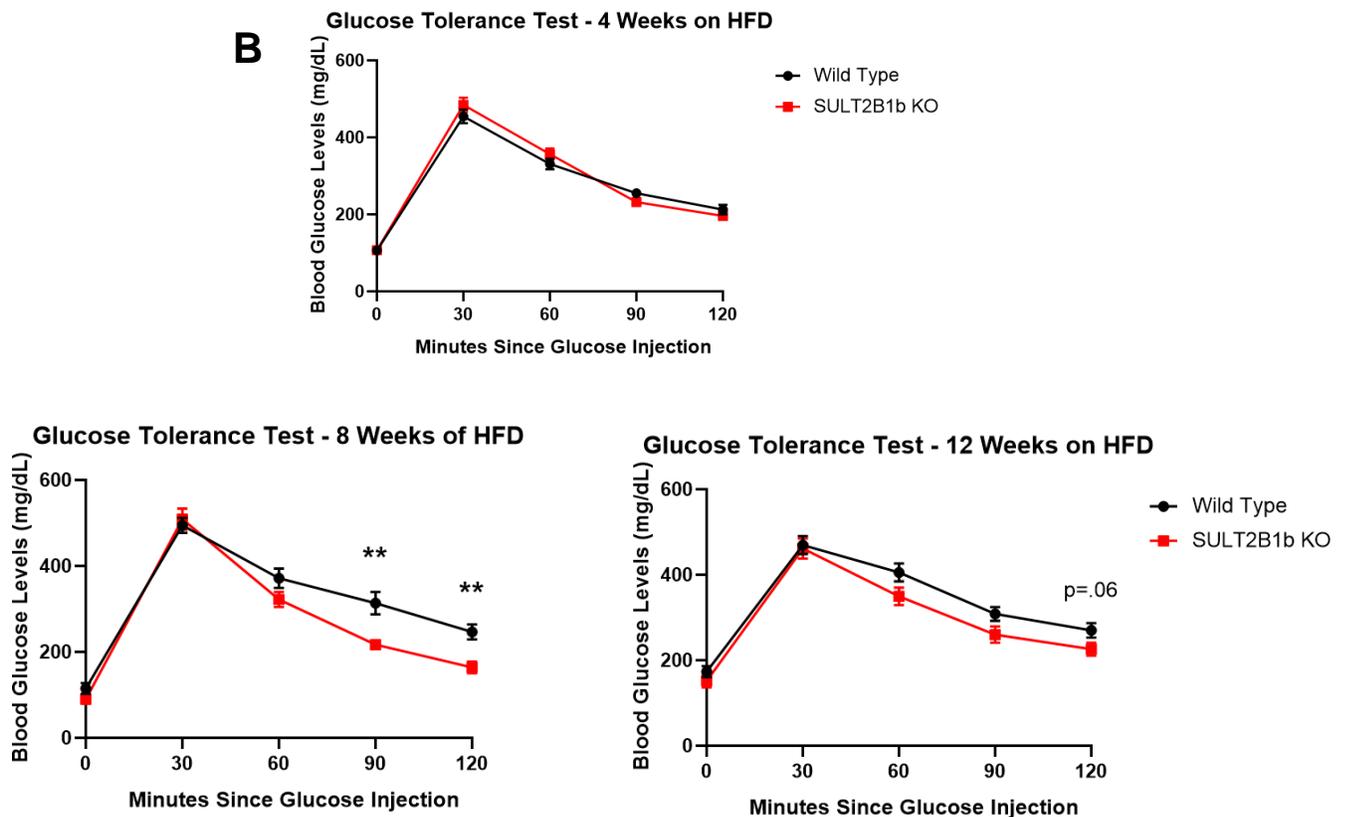
### 3.2 Loss of SULT2B1b Protects Mice from Developing Insulin Resistance

As shown in Figure 1A, insulin tolerance test and glucose tolerance tests were conducted every four weeks to determine whether SULT2B1b loss affected insulin sensitivity. Consistent with the protection SULT2B1b loss provided against body fat accumulation, SULT2B1b null mice were more sensitive to insulin 30- and 60-minutes following insulin injection on the 4<sup>th</sup> week compared to wildtype mice (Figure 2A).



Moreover, on the 8<sup>th</sup> and 12<sup>th</sup> week, SULT2B1b null mice were significantly more sensitive to insulin than the wildtype mice at 4 and 3 time points respectively. Perhaps more importantly, the reaction to insulin by the SULT2B1b group stays relatively consistent throughout the study indicating a protection against insulin resistance.

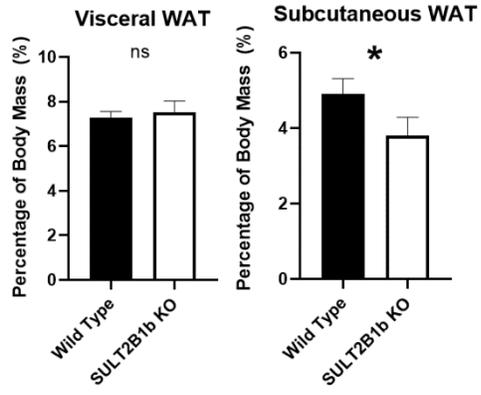
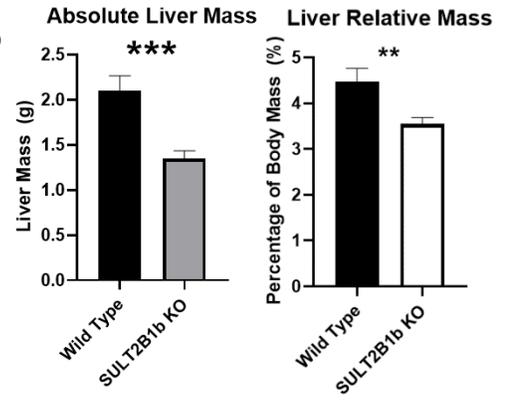
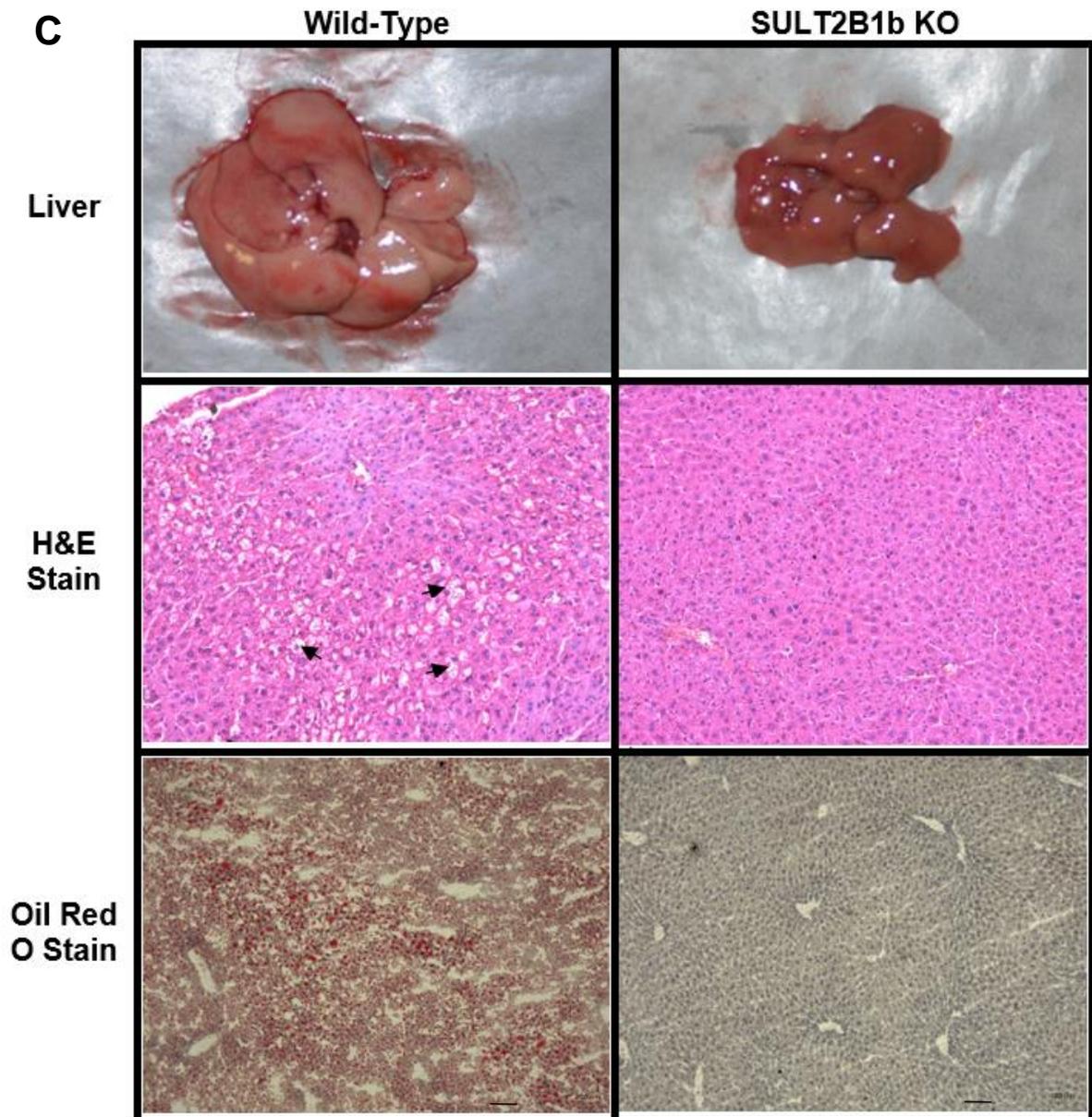
The stark contrast between the two groups is lost on the GTTs (**Figure 2B**). The 8-week test showed some improvement in removing glucose from the blood in the knockout compared to the wildtype towards the end of the time period. This moderate protection, however, was diminished by the 12<sup>th</sup> week.

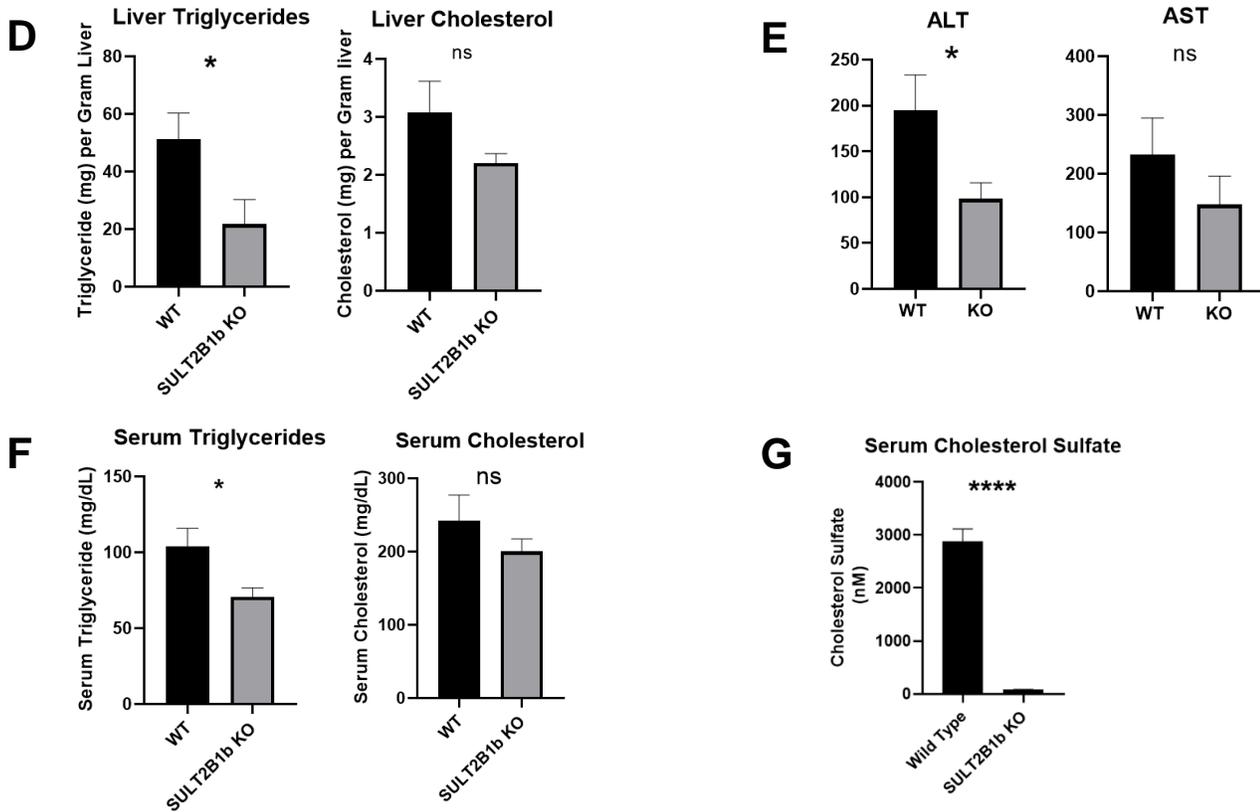


### **3.3 SULT2B1b Null Mice were Protected from Liver Steatosis, Accumulation of Subcutaneous White Adipose Tissue, and Hyperlipidemia**

Following 12 weeks of HFD, SULT2B1b knockout mice and wildtype mice were sacrificed for tissue harvesting. Visceral and subcutaneous white adipose tissue depots, skeletal muscle, blood, and liver were harvested for histological and biochemical investigation. **Figure 3A** shows the mass of both visceral and subcutaneous WAT relative to the body mass of the animal it was harvested from. The relative mass of the subcutaneous WAT was significantly higher in WT mice compared to SULT2B1b null.

A dramatic difference between the liver phenotypes of the two groups was also revealed. The livers of the WT mice were significantly larger even when controlling for the animal's body mass (**Figure 3B**). Furthermore, histological examination shows hepatocyte ballooning in the H&E staining (examples denoted with arrows) and a meaningful difference in lipid accumulation through Oil Red O staining (**Figure 3C**). Biochemical analysis of liver triglycerides confirms the histological finding and also reveals that the WT mice had greater than 50mg triglyceride per gram liver which is a clinical parameter for hepatic steatosis (**Figure 3D**). The WT mice had a trend for increased liver cholesterol; however, the difference was not significant. Increased aspartate aminotransferase (AST) and significantly increased alanine aminotransferase (ALT) within the serum indicated that liver damage was present (**Figure 3E**). Finally, serum analysis uncovered increased triglycerides in the WT versus the knockout, further defining SULT2B1b loss to have a protective role (**Figure 3F**). Analysis of the serum showed the loss of SULT2B1b dramatically reduced the circulating cholesterol sulfate (**Figure 3G**).

**A****B****C**

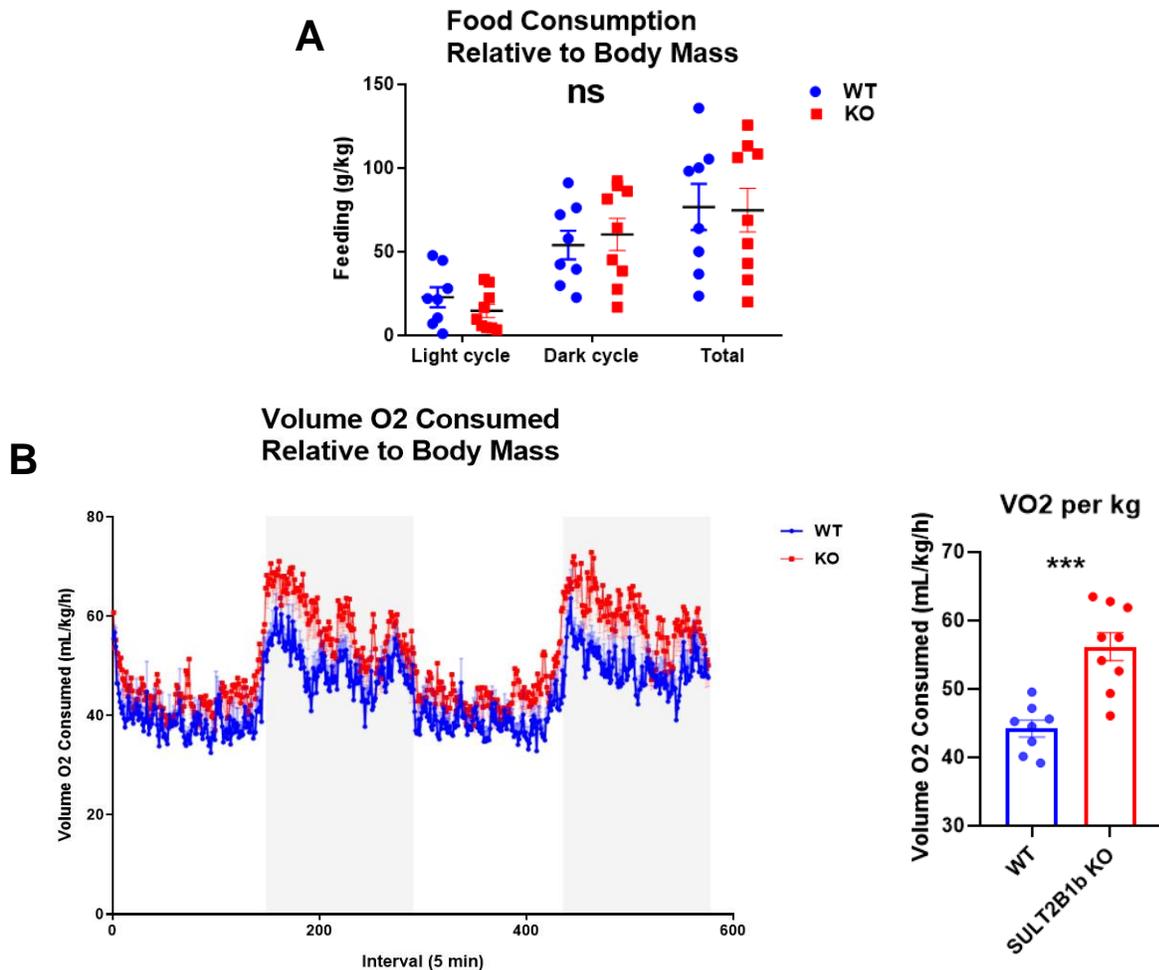


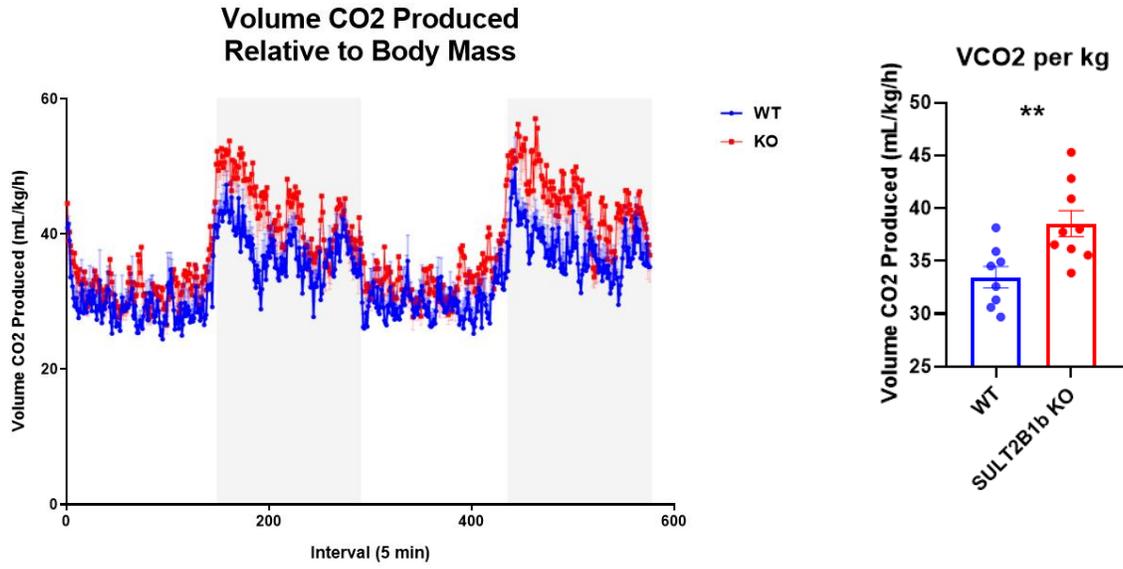
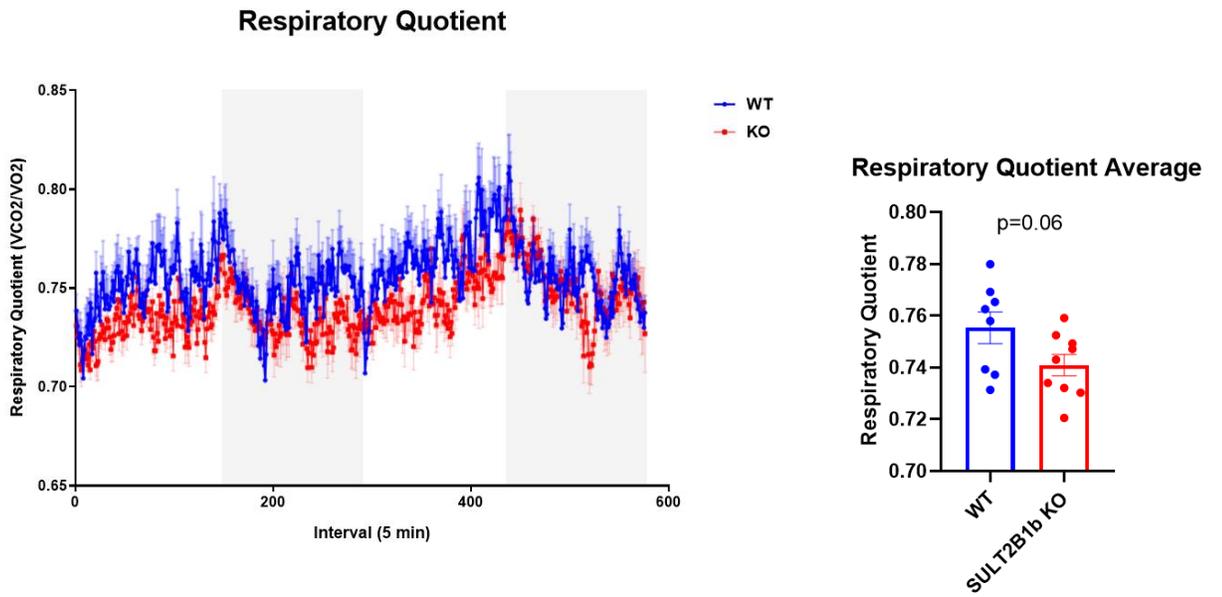
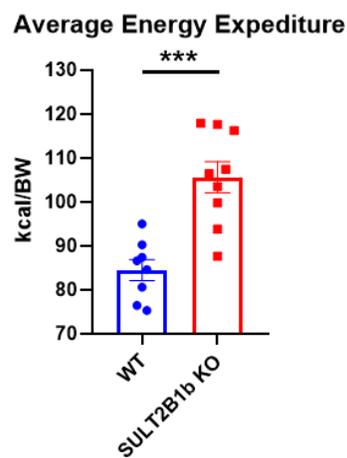
### 3.4 Cage Metabolic Study Revealed SULT2B1b Knockout Mice Expended More Energy Compared to WT Mice

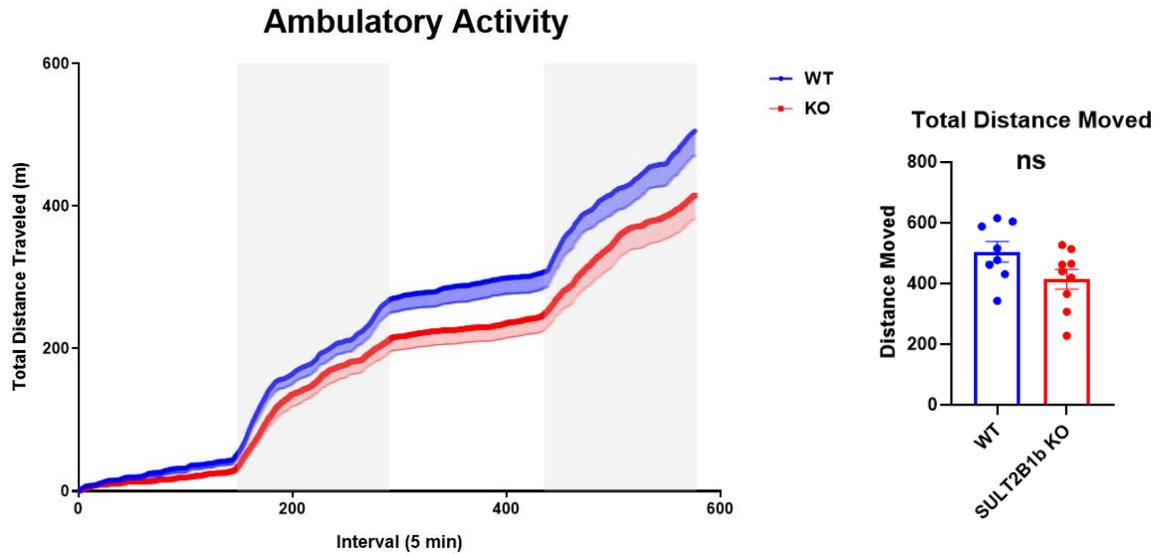
Following 12 weeks of HFD, two groups of SULT2B1b KO and WT mice were placed into singly-house metabolic cages which monitored food consumption, mouse movement and gas exchanges for 48 hours. When controlling for body mass, the WT and SULT2B1b KO group consumed similar amounts of food (**Figure 4A**). The KO mice, however, consumed significantly more oxygen and produced significantly more carbon dioxide (**Figure 4B, C**). The SULT2B1b null mice also had a significantly different respiratory quotient (RQ); the RQ hints at the fuel source used by the animal [48, 49]. An RQ of exactly 1 indicates the fuel source are carbohydrates [48]. When proteins or fats are being used as a fuel source, the RQ is reduced and will fall between 1.0 and 0.7 with proteins favoring an RQ of 0.8 and fats favoring an RQ of

0.7 [46, 48, 49]. **Figure 4D** shows the SULT2B1b KO mice had a lower RQ, which indicated that those mice may be using a fattier fuel source.

Furthermore, indirect calorimetry was used to examine the energy expenditure differences between the groups (**Figure 4E**) and found that the SULT2B1b group expended significantly more energy than the WT mice when controlling for body mass. Fascinatingly, this cannot be accounted for due to difference in movement; in fact, the SULT2B1b mice trended to move less than their WT counterparts (**Figure 4F**).



**C****D****E**

**F**

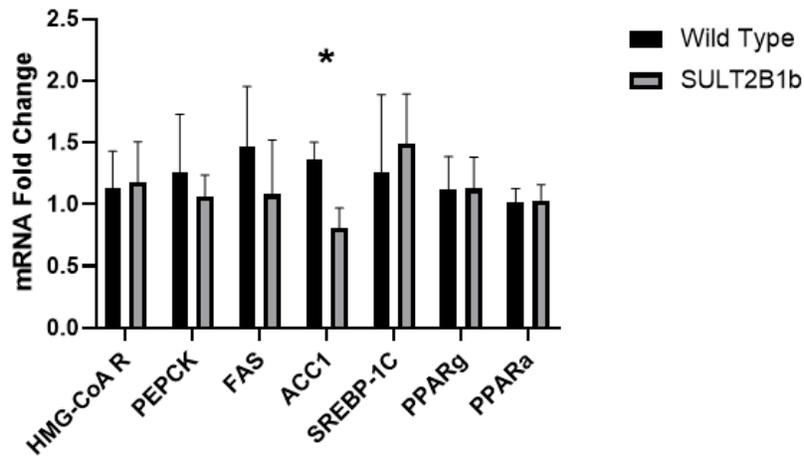
### 3.5 Loss of SULT2B1b May Cause Down Regulation of CD36 Expression in the Liver, Alleviating Fat Accumulation in the Liver

Realtime PCR was performed in liver samples in order to determine a potential mechanism to identify how the loss of SULT2B1b was exerting its protective effect. Analysis of some gluconeogenic (PEPCK), lipogenic (FASN, ACC1, SREBP-1C), lipolytic (HMG-CoA Reductase) and nuclear receptors (PPAR $\alpha$ , PPAR $\gamma$ ) which have a role in metabolism revealed small differences between the SULT2B1b null mice and the WT groups (**Figure 5A**). ACC1 involved in the irreversible step of converting acetyl-CoA to malonyl-CoA, a key step in fatty acid synthesis, was found to be significantly higher in the WT group, although the difference was relatively low. CD36, a receptor protein involved in fatty acid trafficking, had a 6-fold difference in mRNA transcription in the WT compared to the KO (**Figure 5B**).

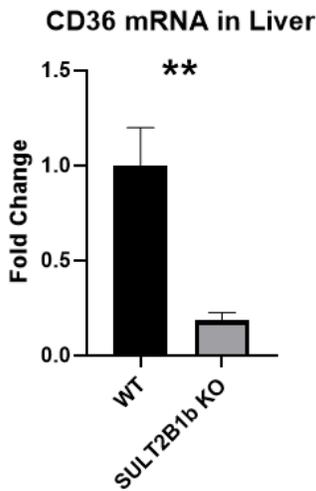
To confirm whether the difference in transcription level was actualized on the protein level, a western blot was performed with 5 representative samples from the SULT2B1b null and WT groups (**Figure 5C**). Previously our lab established a transgenic mouse model overexpressing

CD36 in the liver; this was used as our positive control. The positive western prompted a histological analysis using immunofluorescence to examine if the increased level CD36 expression was trafficked to the cell surface where it is functionally active (**Figure 5D**). Once again, the transgenic CD36 mouse liver was used as a positive control.

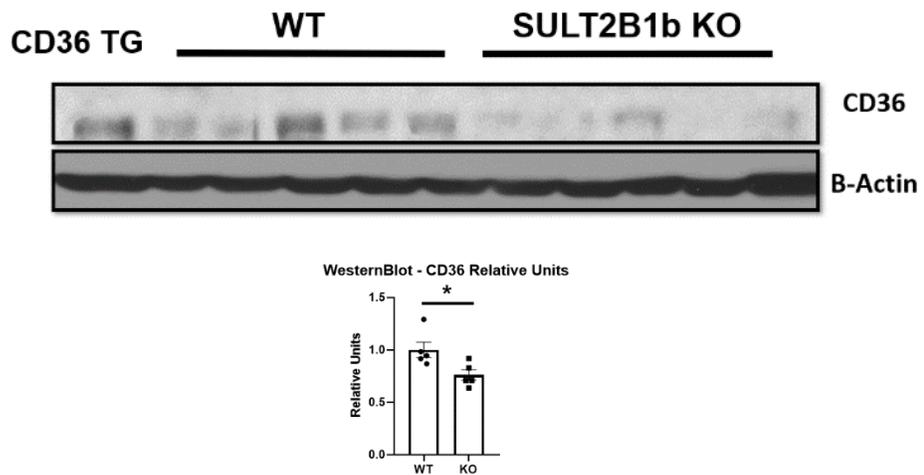
**A mRNA in the Liver following 12 Weeks HFD**



**B**



**C**

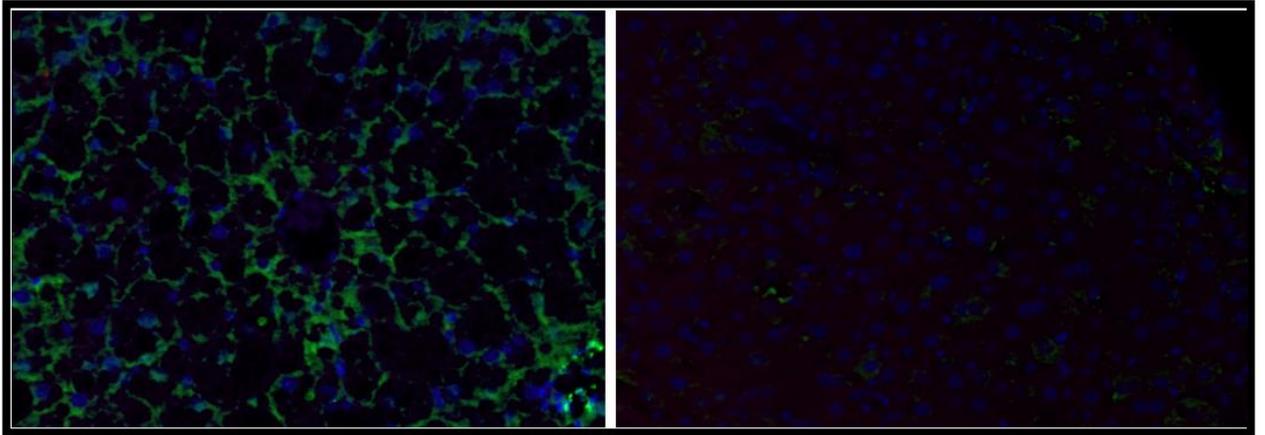


**D**

**Wild Type**

**SULT2B1b KO**

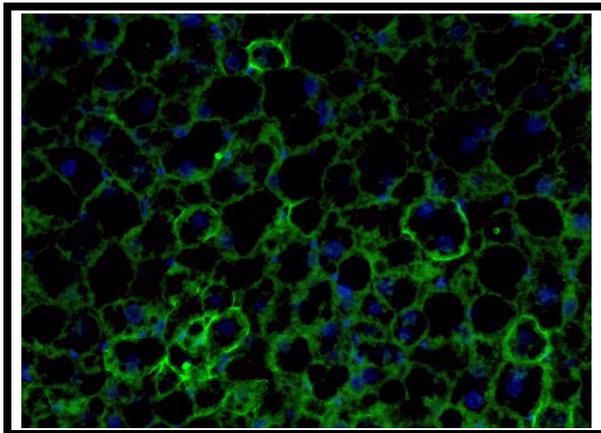
**CD36 IF**



**CD36 TG**

**Positive Control**

**CD36  
IF**



## 4.0 DISCUSSION

We initially hypothesized the loss of SULT2B1b would lead to worse outcomes in mice challenged with HFD due to the regulatory role it has in gluconeogenesis and lipogenesis. However, our data showed the loss of SULT2B1b to be protective against fat accumulation, especially in the liver and subcutaneously. Hepatic fat accumulation was particularly high, with visible ballooning, steatosis, and a significantly increased serum ALT, which is a sign of liver disease. Visceral fat, regarded as the more dangerous fat associated with insulin resistance [55], was notably similar between the two groups when controlling for body mass and not significantly different when measuring absolute values (data not shown). The SULT2B1b null mice were protected from developing insulin resistance as shown by the improvement in the ITTs. Given the tangled nature of metabolic syndrome, it is difficult to assert whether this protection is due to a decreased fat accumulation in white adipose depots, a reduction in lipid accumulation in the liver, or other means.

The metabolic study showed SULT2B1b null mice had a greater energy expenditure than their WT littermates relative to their body weight; though this is a trend that has been observed clinically when comparing obese individuals to non-obese individuals [50]. When not correcting for body mass, there is no significant difference between either group. The fact that the SULT2B1b null mice are expending more energy as measured through indirect calorimetry, however, still remains to be an interesting discovery. The metabolic cage study also found a difference in the respiratory quotient, indicating a potentially different use of fuel sources. Taken together, it is possible that the loss of SULT2B1b leads to a higher metabolic activity that uses a fattier fuel source. However, continued experimentation is required to support that speculation.

Finally, CD36 was shown to be dramatically upregulated in the liver of the wildtype mice when challenged with HFD. This upregulation is lost in the mice which lacked SULT2B1b. Understanding the mechanism by which the loss of SULT2B1b leads to protection against these negative outcomes still warrants further investigation. In previous studies, the product of SULT2B1b, cholesterol sulfate, was shown to have a protective effect by inhibiting both HNF4 $\alpha$  and CAR/LXR [42-44, 51]. The data confirms a dramatic reduction in the presence of cholesterol sulfate in the serum potentially indicating the potentially harmful effects of cholesterol sulfate loss is being outweighed by the action of a different pathway. One possible explanation is the loss of SULT2B1b leads to the downregulation of CD36.

CD36 is a scavenger receptor protein that carries out fatty acid uptake [52]. Upregulation of CD36 has been implicated in the development of NAFLD [52, 53]. Furthermore, genetic disruption of CD36 has been shown to have a protective effect against NAFLD in mice challenged with HFD [53]. CD36 is also a transcriptional target of LXR [54]; given that 25HC3S is an LXR antagonist, the loss of 25HC3S could lead to increased CD36 transcription. It is still, however, impossible to conclude from this data that CD36 downregulation is a result of SULT2B1b loss rather than a due to a reduction in hepatic lipid accumulation. Further study is required to examine whether CD36 has lower expression levels in SULT2B1b KO directly, due to SULT2B1b mediation or indirectly, through a reduction in hepatic steatosis. An additional study could examine whether basal levels of CD36 are lower in SULT2B1b without HFD challenge to further investigate the role SULT2B1b has on mediating CD36 expression.

Regardless, SULT2B1b genetic deletion promotes a healthier hepatic metabolic state compared to its wild-type counterpart.

Limitations of this study include the use of all-male cohorts. It is known that estrogen has a protective role in some comorbidities associated with obesity [5, 6, 9]. Therefore, a future study would require the use of both sexes to ascertain the loss of SULT2B1b protective effect on female mice. Additionally, this study focused primarily on the liver, due to SULT2B1b

upregulation in obese mice in the liver. A continuation of this study would examine other tissues to determine the extent of crosstalk between the liver and other metabolically active tissues.

Conditions relating to metabolic syndrome such as obesity, insulin resistance, and nonalcoholic fatty liver disease pose a growing problem that has yet to be properly addressed. While proper diet and exercise remain the first line of defense against such issues, therapeutical approaches offer an enticing option to help treat the affected population. Given the option of diet and exercise has yet to slow the growth of obesity-related diseases, finding new drug targets remains quintessential to improving public health.

In this study, we showed that whole-body loss of SULT2B1b has a surprisingly protective effect against obesity and insulin resistance. This may be due to a novel pathway that has yet to be elucidated. Further investigation is required to determine the mechanisms which govern this protection. SULT2B1b could be a promising target in the fight against obesity and T2DM.

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