Adipocyte-specific deletion of IL-6 does not attenuate obesity-induced weight gain or glucose intolerance in mice

Whitham, Martin; Pal, Martin; Petzold, Tim; Hjorth, Marit; Egan, Casey L; Brunner, Julia S; Estevez, Emma; Iliades, Peter; Zivanovic, Borivoj; Reibe, Saskia; Hughes, William E; Findeisen, Maria; Hidalgo, Juan; Febbraio, Mark A

DOI:
10.1152/ajpendo.00206.2019

Document Version
Peer reviewed version

Citation for published version (Harvard):
Whitham, M, Pal, M, Petzold, T, Hjorth, M, Egan, CL, Brunner, JS, Estevez, E, Iliades, P, Zivanovic, B, Reibe, S, Hughes, WE, Findeisen, M, Hidalgo, J & Febbraio, MA 2019, 'Adipocyte-specific deletion of IL-6 does not attenuate obesity-induced weight gain or glucose intolerance in mice', *American Journal of Physiology - Endocrinology and Metabolism*, vol. 317, no. 4, pp. E597-E604. https://doi.org/10.1152/ajpendo.00206.2019

Adipocyte specific deletion of IL-6 does not attenuate obesity-induced weight gain or glucose intolerance in mice

Martin Whitham[1,2*], Martin Pal[1], Tim Petzold[1], Marit Hjorth[1], Casey L Egan[1], Julia S Brunner[1,3], Emma Estevez[1], Peter Iliades[4], Borivoj Zivanovic[4], Saskia Reibe[1], William E Hughes[1,5], Maria Findeisen[1], Juan Hidalgo[6], and Mark A Febbraio[1,7*]

[1]Cellular & Molecular Metabolism Laboratory, Division of Diabetes & Metabolism, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia, [2]School of Sport, Exercise & Rehabilitation Sciences, University of Birmingham, Edgbaston, United Kingdom, [3]Institute for Physiology, Center for Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria, [4]Baker Heart & Diabetes Institute, Melbourne, VIC, Australia, [5]St Vincent's Clinical School, University of New South Wales, Sydney, New South Wales, Australia, [6]Institute of Neurosciences, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [7]Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria, Australia.

*****Addresses for Correspondence:

Mark A. Febbraio: mark.febbraio@monash.edu

Martin Whitham: m.whitham@bham.ac.uk

Abstract

Objectives: It has been suggested that interleukin-6 (IL-6) produced by adipocytes in obesity leads to liver insulin resistance, although this hypothesis has never been definitively tested. Accordingly, we did so by generating adipocyte specific IL-6 deficient (AdipoIL-6$^{-/-}$) mice and studying them in the context of diet-induced and genetic obesity.

Methods: Mice carrying 2 floxed alleles of IL-6 (C57Bl/6J) were crossed with Cre recombinase overexpressing mice driven by the adiponectin promoter to generate AdipoIL-6$^{-/-}$ mice. AdipoIL-6$^{-/-}$ and floxed littermate controls (FL) were fed a standard chow (CHOW) or high fat diet (HFD) for 16 weeks and comprehensively metabolically phenotyped. In addition to a diet-induced obesity (DIO) model, we also examined the role of adipocyte derived IL-6 in a genetic model of obesity and insulin resistance by crossing the AdipoIL-6$^{-/-}$ mice with leptin deficient (*ob/ob*) mice.

Results: As expected, mice on a HFD and *ob/ob* mice displayed marked weight gain and increased fat mass compared with chow fed and *ob/+* (littermate control) animals, respectively. However, deletion of IL-6 from adipocytes in either model had no effect on glucose tolerance or fasting hyperinsulinemia.

Conclusion: Adipocyte specific IL-6 does not contribute to whole body glucose intolerance in obese mice.

1. Introduction

Interleukin-6 (IL-6) is an inflammatory cytokine that has been implicated in the etiology of obesity and type 2 diabetes (T2D), since patients with metabolic syndrome display elevated levels of this cytokine(2, 21). Furthermore, pre-clinical studies have demonstrated that IL-6 causes insulin resistance, particularly in the liver, by activating Suppressor of Cytokine Signaling -3 (SOCS-3) leading to defective tyrosine phosphorylation of Insulin Receptor Substrate-1 (IRS1) (17, 18).

However, the notion that IL-6 can cause insulin resistance is unclear since there are many lines of evidence that this cytokine can, in fact, be protective against insulin resistance. Firstly, in humans, infusion of recombinant human IL-6 during a hyperinsulinemic euglycemic clamp results in a small increase, rather than decrease, in glucose infusion rate (3). Vigorous physical exercise can increase IL-6 over 100-fold in the circulation from the contracting muscles (19) and following exercise insulin sensitivity is markedly increased (23). Furthermore, mice lacking IL-6 are prone to obesity (22), liver inflammation and insulin resistance when fed a high fat diet (12), while mice that overexpress IL-6 present with the opposite phenotype, in part via enhanced leptin sensitivity (16). More recently, IL-6 was shown to enhance the incretin response in mice (5) which is a drug target for T2D. Notwithstanding this evidence, two important findings suggest that IL-6 release from adipose tissue in obesity can result in liver insulin resistance and inflammation. In a study by Davis and co-workers, the authors observed that a c-Jun NH2-terminal kinase 1 (JNK1) -dependent secretion of IL-6 by adipose tissue caused increased expression of liver SOCS3, and hepatic insulin resistance (15). Shoelson and colleagues blocked IL-6 using an antibody in transgenic mice where IkappaB kinase β (IKKβ) was constitutively active in liver, and observed an improvement in insulin resistance (1). Taking all of the data together, it appears that if IL-6 is released from skeletal muscle during exercise it may have beneficial effects, but that IL-6 released from adipose tissue in obesity can lead to liver insulin resistance. This latter hypothesis has, to date, not been comprehensively tested. Accordingly, in the present study, we generated adipocyte specific IL-6 deficient mice in the context of diet induced

and genetic obesity. We demonstrate, contrary to prevailing dogma, that deletion of IL-6 specifically from adipocytes, has no effect on obesity-induced metabolic disturbances.

2. Methods

2.1 Mouse models of obesity

2.1.1 *AdipoIL-6$^{-/-}$ mice*

Adipocyte specific, IL-6 knock out (AdipoIL-6$^{-/-}$) mice were generated via breeding of C57BL/6 mice carrying loxP inserts surrounding exon 2 of the IL-6 gene as previously described (14). Prior to crossing these mice with C57BL/6 mice carrying the Cre recombinase gene under the control of the adiponectin promotor (Jackson laboratories B6.FVB-Tg(Adipoq-cre)1Evdr/J), we backcrossed these mice to C57BL/6 mice a further 10 generations. Mice were housed at the Alfred Medical Research and Education Precinct Animal Centre, Melbourne, Australia, in a pathogen free facility under a 12:12 h light:dark cycle. From 8 weeks of age, mice were fed either a normal chow diet (NC; 14.3 MJ/kg, 76% of kJ from carbohydrate, 5% fat, and 19% protein), or a high fat diet (HFD) [19 MJ/kg, 36% of kJ from carbohydrate, 43% fat (42.9% saturated, 35.24% monounsaturated, and 21.86% polyunsaturated fatty acids), and 21% protein; both Specialty Feeds, Glen Forrest, Australia) for a total of 16 weeks. Food and water was provided *ad libitum*. All experiments were performed in male mice and on cessation of the study, mice were euthanized after a 5h fast. Animal experiments were approved by the Alfred Medical Research and Education Precinct Animal Ethics Committee and conducted in accordance with the National Health and Medical Research Council of Australia Guidelines for Animal Experimentation.

2.1.2 *AdipoIL-6$^{-/-}$ ob/ob mice*

To examine the effects of adipocyte specific deletion of IL-6 in a genetic model of obesity, AdipoIL-6$^{-/}$ mice were crossed with *ob/ob* mice (B6.Cg-Lep<ob>/JAusb=). This resulted in mice either KO or wild type for IL-6, specifically in adipocytes, that regardless, exhibited hyperphagia and rapid weight gain typical of leptin deficient *ob/ob* mice. Mice were housed at the Behavioural Testing Facility,

Garvan Institute of Medical Research, Australia in a 12h:12h light:dark cycle with food and water provided *ad libitum*. All experiments were approved by the Garvan Institute Animal Ethics Committee in accordance with the Australian code for the care and use of animals for scientific purposes (2013).

2.2 Metabolic phenotyping

2.2.1 *Body composition*

Fat mass and lean body mass were measured with a 4-in-1 EchoMRI body composition analyzer (Columbus Instruments, USA) and standard laboratory scales.

2.2.2 ***Oral Glucose Tolerance Test (OGTT)***

Glucose tolerance was assessed via gavage of 2 g/kg lean body mass 25% glucose solution and assessment of blood glucose sampled from the tail vein, via handheld glucometer (Accu-check, Roche, Castle Hill, Australia). In all instances, mice were fasted for 5 h. At time points 0 and 15mins, a ~20ul blood sample was taken for assessment of plasma insulin.

2.3 Plasma analysis

Plasma insulin and IL-6 were determined using mouse ELISA (80-INSMSU-E01ALPCO, Salem, NH, USA) and (P08505, RayBiotech, Norcross, GA, USA) respectively, as per manufacturer's instructions.

2. 4 Liver histology

Haematoxylin and Eosin staining was carried out on 3 liver sections from a minimum of 7 mice per group. 3 representative images of each section were taken using a Leica DM4000 microscope with a 20x NA 0.5 HCX PL Fluotar objective. Particle analysis in ImageJ (Bethesda, MD, USA) was

performed to identify and measure likely lipid droplets, with data from 9 images for each animal combined.

2.5 *PCR and microarray*

For confirmation of loxP-cre recombination, in both AdipoIL-6-/- and AdipoIL-6-/- ob/ob mice, genomic DNA was isolated using the Isolate II Genomic DNA kit (Bioline, Alexandria, Australia) as per manufacturer's instructions. PCR was run using the Phire Hot Start II DNA Polymerase (ThermoFisher Scientific) and primer sequences FOR CCCACCAAGAACGATAGTCA and REV ATGCCCAGCCTAATCTAGGT. For quantitative real time PCR and microarray, RNA was isolated using the Nucleospin RNA extraction kit (Macherey-Nagel, Duren, Germany) according to manufacturer's instructions. For Microarray analysis, RNA was assessed on a Bioanalyzer using an RNA 6000 Nano chip (Agilent, Santa Clara, CA, USA), setting the threshold for RNA quality at RIN values >7. Samples (N=3) were subsequently hybridized to a GeneChip Mouse Gene 2.0 ST Array and run according to manufacturer's instructions (Affymetrix). For qRT-PCR, cDNA synthesis was carried out via Tetro reverse transcriptase (Bioline, Alexandria, Australia) after DNAse treatment. Inventoried Taqman primer sequences (Assay on demand, Applied Biosystems, ThermoFisher Scientific) were used for IL6 (Mm00446190_m1), TNFα (Mm00443258_m1), MCP-1 (Mm00441242_m1) and B2M (Mm00437762_m1) with the latter used as a housekeeping gene.

2. 6 Statistics

For OGTT analysis, incremental area under the curve was calculated in Prism (La Jolia, CA, USA). All data were analyzed by ordinary or repeated measures two-way ANOVA, or paired T test, where appropriate. For microarray analysis, pre-processing entailed use of the Robust Multi-Array average (RMA) algorithm in R to detect true signal from background followed by background subtraction, normalisation and summarization using median-polish (7, 8). For detection of differentially expressed

genes, limma was used (11) which implements linear models to assess differential expression between groups. Genes were considered significantly different when the adj.p.value (q-value) was equal or smaller than 0.05 (Benjamini-Hochberg correction for multiple testing). All other data are presented as Mean ± standard deviation and significance was set at 0.05.

3. Results

3.1 Adipocyte specific deletion of IL-6 does not affect high fat diet-induced obesity, or glucose intolerance

After generating AdipoIL-6-/- mice, we first phenotyped floxed control, cre control and wild type control mice after 16 weeks of high fat feeding. There were no differences in phenotype when comparing all control animals (data not shown). Floxed animals were, therefore, used in all subsequent experiments as controls. We initially confirmed loxP-cre recombination exclusively in white and brown adipose tissue in AdipoIL-6-/- mice (KO) but not littermate floxed controls (FL) (Figure 1A). In order to study the role of IL-6 in adipose tissue on high fat diet-induced obesity and glucose intolerance, we first placed AdipoIL-6-/- mice and floxed control animals on a chow or HFD for 16 weeks. As expected, the dietary intervention increased body weight (Figure 1B) and fat mass (Figure 1C) irrespective of genotype. No differences were observed in these measures when comparing genotypes (Figure 1B,C). After 16 weeks on HFD, animals were characteristically hyperinsulinemic, a hallmark of insulin resistance, but again we observed no differences when comparing genotypes (Figure 1D). To determine whether glucose tolerance was influenced by adipocyte derived IL-6, we next performed OGTT. As shown previously in our laboratory (20), after only one week of HFD, glucose tolerance was impaired. This effect was observed irrespective of genotype (Supplementary Figure 1A, available at https://doi.org/10.25500/edata.bham.00000338). The HFD-induced glucose intolerance was maintained after 8 weeks (Supplementary Figure 1B, DOI: 10.25500/edata.bham.00000338) and 16 weeks (Figure 1E-G). Importantly, however, adipocyte specific deletion of IL-6 did not affect glucose tolerance at any time point irrespective of

diet (Figure 1, Supplementary Figure 1). These data suggest that adipocyte derived IL-6 plays no role in the HFD-induced insulin resistance and glucose intolerance seen in mice.

3.2 Adipocyte specific deletion of IL-6 does not affect high fat diet-induced liver steatosis or inflammatory gene expression

Two previous studies suggested that IL-6 release from adipose tissue in obesity can result in liver insulin resistance and inflammation (1, 15). Accordingly, we measured liver steatosis and the mRNA expression of inflammatory genes (IL-6), tumour necrosis factor alpha (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) in liver. As expected, 16 weeks on a high fat diet increased lipid droplet formation (Figure 2A) and the mRNA expression of IL-6, TNF-α and MCP-1 (Figure 2B). While, as expected the high fat diet did not increase liver IL-6 mRNA in AdipoIL-6$^{-/-}$there were no other differences in these measures, when comparing the AdipoIL-6$^{-/-}$ with Floxed controls (Figure 2A,B). These data suggest that adipocyte derived IL-6 plays no role in the HFD-induced hepatic steatosis and inflammation.

3.3 Adipocyte specific deletion of IL-6 does plays a minimal role in modulating global gene expression in adipose tissue

To this point, we were surprised that the adipocyte specific deletion of IL-6 had almost no effect on any measured parameters. To ascertain the impact of such deletion on global gene expression we performed microarray experiments on adipose tissue from AdipoIL-6$^{-/-}$ and floxed control mice on both a HFD and chow diet and compared the gene expression profile between diets and genotypes. As demonstrated in Figure 3A,B, compared with animals on a chow diet, the HFD had a dramatic effect on the adipose tissue gene expression profile in both the AdipoIL-6$^{-/-}$ and floxed control mice, with many genes being significantly affected, including a significant enrichment of genes involved

in inflammation (P<0.0001). In contrast, however, when the gene expression profile of AdipoIL-6$^{-/-}$ was compared with that of the floxed control mice, only two genes, apolipoprotein A-IV (APOA4) and cytochrome P450 respectively, were significantly increased in the AdipoIL-6$^{-/-}$ adipose tissue compared with the floxed control on a HFD, with no genes being significantly decreased (Figure 3C). When fed a chow diet, there were no differences whatsoever when comparing AdipoIL-6$^{-/-}$ adipose tissue with tissue obtained from the floxed control (Figure 3C).

3.4 Adipocyte specific deletion of IL-6 does not affect obesity, or glucose intolerance in ob/ob mice

The proposed mechanism by which IL-6 is thought to mediate liver insulin resistance and inflammation is by acting as an adipokine. Despite a pronounced increase in adiposity after prolonged high fat feeding (Figure 1C), this did not result in a detectable increase in circulating IL-6 in floxed control animals (Figure 4A). One could argue, therefore, that the experimental model, that being diet-induced obesity was an insufficient metabolic perturbation to test the hypothesis. Accordingly, we next crossed the AdipoIL-6$^{-/-}$ mice with a genetic model of obesity, the leptin deficient (*ob/ob*) mouse. This model results in a more severe obese phenotype. We first verified that IL-6 was indeed deleted from the adipocytes but not other tissues in the AdipoIL-6$^{-/-}$ *ob/ob* mice (Figure 4B). Critically, in this experimental model circulating IL-6 was ~3-fold higher in floxed *ob/ob* control compared with AdipoIL-6$^{-/-}$ *ob/ob* (Figure 4C), making it an appropriate model to test the adipokine hypothesis. As expected *ob/ob* mice had higher body weight (Figure 4D) and fat mass (Figure 4E) compared with *ob/+* controls (deletion of only one leptin allele). No differences were observed in these measures when comparing AdipoIL-6$^{-/-}$ *ob/ob* with floxed *ob/ob* control mice or AdipoIL-6$^{-/-}$ *ob/+* with floxed *ob/+* control mice (Figure 4D,E). Despite the fact that circulating IL-6 was ~3 fold lower in the AdipoIL-6$^{-/-}$ *ob/ob* mice, this had no effect on insulin resistance, as measured by circulating basal insulin (Supplementary Figure 2 DOI: 10.25500/edata.bham.00000338) or glucose tolerance (Figure 4F,G).

4. Discussion

It is generally accepted that elevated circulating IL-6 due to obesity results in systemic insulin resistance, in part by reducing hepatic insulin action secondary to increased expression of SOCS3, liver inflammation and hepatic steatosis. This hypothesis has, to date, not been experimentally tested. Here, using two models of obesity, we report that adipocyte specific deletion of IL-6 plays no role in diet induced or genetic obesity driven insulin resistance or glucose intolerance.

The current thinking that elevated circulating IL-6 mediates liver insulin resistance is largely based on two previous studies. In the study by Davis and colleagues (15), the authors deleted JNK1 from adipose tissues and observed a complete amelioration of circulating IL-6 and improvements in liver inflammation, steatosis, SOCS3 protein expression and liver insulin action. Intriguingly, however, in this previous paper, the high fat diet induced IL-6 levels in wild type animals was ~30 pg/ml. These levels were 30 fold higher than we report here. The reason for these discrepancies are not readily apparent, however, high fat diet induced increases in IL-6 of 30 pg/ml are at odds with most values reported in the literature. In the study by Shoelsen and colleagues (1), the circulating IL-6 levels in the control mice were consistent with those reported by us, but increased ~2fold in the liver specific IKK transgenic mice. Neutralising IL-6 in this model ameliorated insulin resistance. It must be pointed out, however, that in the aforementioned study, IKK was overexpressed in the liver and, hence, the IL-6 levels in the liver of the mice was directly increased. Taking our current data together with this previous study, we can only conclude that liver derived IL-6 as a result of increased IKK results in systemic insulin resistance, but the IL-6 in adipocytes plays no role in obesity-induced systemic insulin action.

In the DIO model of obesity, while high fat feeding led to pronounced adiposity, this did not translate to any observable increase in circulating IL-6. Therefore, it was possible that the DIO model was not appropriate to test our hypothesis. This necessitated further experiments using a genetic model of

obesity. The *ob/ob* mice irrespective of IL-6 presence/absence in adipocytes weighed ~55g at the cessation of the study and in this model, the circulating IL-6 in the floxed *ob/ob* mice were approximately 30 pg/ml which was reduced to ~10 pg/ml in the AdipoIL-6$^{-/-}$ *ob/ob* mice (Figure 4C). These levels were roughly similar to the previous paper discussed above (15). Despite the ~3 fold decrease in circulating IL-6, glucose tolerance (Figure 4 F,G) and hyperinsulinemia (Supplementary Figure 2) were unaffected. Of note when we deleted IL-6 from adipocytes specifically in this model, circulating IL-6 was nonetheless ~10 fold higher than we reported in our DIO model. It must be noted that the Adipoq Cre line exhibits no recombination in other tissues, whereas another common adipose specific cre mouse, the adipocyte protein 2 (aP2) -Cre mouse results in recombination in many other cells and tissues (10). Indeed, when IL-6 is deleted driven by aP2-Cre promoter differences were observed in metabolism of female mice (13). The *ob/ob* mouse is known to display systemic inflammation (6) and it is likely that other cells, such as those of the immune system, contributed to this elevation. This may have been sufficient to blunt any effect of reduced adipocyte IL-6 release. Notwithstanding this, it is clear from our data that adipocyte specific deletion of IL-6 plays no role in obesity-induced metabolic disturbances.

To our surprise, adipocyte specific deletion of IL-6 had almost no effect on global gene transcription in the adipose tissue. While the HFD had major effects on the transcriptome (Figure 3), no genes were significantly changed when comparing the AdipoIL-6$^{-/-}$ with floxed control mice on a chow diet, while only two genes were increased by adipocyte IL-6 deletion on a HFD. ApoA4 is known to play a role in chylomicrons and very low density lipoprotein secretion and catabolism (9). Cytochrome p450 belongs to a group of hemoproteins which perform varied roles such as the oxidation of structurally unrelated compounds, including steroids and fatty acids (4). Whether the upregulation of these genes in the adipose tissue of high fat fed AdipoIL-6$^{-/-}$ mice has functional significance requires further investigation. What is clear, however, is that deleting IL-6 from the adipocytes of mice has almost no effect on global gene transcription.

In summary, we demonstrate that deleting IL-6 specifically from adipocytes in obese mice has no role in mediating glucose metabolism. Hence our data do not support the prevailing model that in obese states IL-6 is released from adipose tissue to induce liver steatosis, inflammation and insulin resistance.

Acknowledgements

We would like to acknowledge the Ramaciotti Centre for Genomics (UNSW Sydney, Australia) as the service provider for microarray analysis and staff of the AMREP (Melbourne, Australia) and Australian Bioresources (Moss Vale, Australia) animal research facilities for assistance in breeding and back-crossing animals. This study was supported by a grant from the National Health and Medical Research Council of Australia (NHMRC project grant APP1062436 awarded to MAF and MW). MAF is a Senior Principal Research Fellow of the NHMRC (APP1116936). JH is supported by SAF2014-56546-R.

Figure legends

Figure 1. Conditional knock out of IL-6 from adipocytes does not lead to glucose intolerance in diet-induced obesity. (A) PCR indicating specific recombination of loxP sites and Cre recombinase, exclusively in adipose tissue of AdipoIL-6$^{-/-}$ (KO), but not floxed (FL) control animals. Arrow indicates deletion band (B) Body mass and (C) fat mass of chow and high fat diet (HFD) fed KO and floxed animals. (D) Basal insulin at 16 weeks of HFD or chow. Mice presented with no genotype effect on oral glucose tolerance assessed by (E), glucose curve or (F), area under the curve or (G) insulin response to oral glucose tolerance test. Data reported as Mean ± SD, N=7-10. # main effect of diet, no interaction, no main effect of genotype.

Figure 2. Conditional knock out of IL-6 from adipocytes does not lead to (A) hepatic steatosis (lipid droplet area) or (B) hepatic inflammation following 16 weeks of HFD. Data reported as Mean ± SD, N=7-10. # main effect of diet, no interaction, no main effect of genotype.

Figure 3. Microarray analysis of adipose tissue reveals minimal effects of conditional KO of IL-6 from adipocytes in chow and HFD fed mice. (A) Hierarchical clustering of genes in AdipoIL-6$^{-/-}$(KO) or floxed (FL) control animals after 16 weeks normal chow (nch) or HFD (B) Volcano plots of effects of diet or (C) genotype (Log fold change vs –log10 P value). Solid line indicates adjusted P=0.05, N=3 per group

Figure 4. (A) High fat feeding did not induce increased plasma IL6 in KO or floxed animals. Subsequent conditional knock out of IL-6 from adipocytes in *ob/ob* mice (B) PCR indicating specific recombination, exclusively in adipose of AdipoIL-6$^{-/-}$ *ob/ob* but not floxed *ob/ob* mice. Arrow indicates deletion band (C) AdipoIL-6$^{-/-}$ *ob/ob* mice had significantly lower circulating IL-6 but showed no difference in (D) body mass or (E) fat mass at 24 weeks of age. Conditional knock out of

IL6 from the adipocyte had no effect on glucose tolerance (F) or insulin (G, change from 0-15min during OGTT expressed as a mean. Data reported as Mean ± SD, N=6-8, **P<0.01

References

1. **Cai D, Yuan M, Frantz D, Melendez P, Hansen L, Lee J, and Shoelson S**. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* 11: 183-190, 2005.
2. **Carey AL, Bruce CR, Sacchetti M, Anderson MJ, Olsen DB, Saltin B, Hawley JA, and Febbraio MA**. Interleukin-6 and tumor necrosis factor-alpha are not increased in patients with Type 2 diabetes: evidence that plasma interleukin-6 is related to fat mass and not insulin responsiveness. *Diabetologia* 47: 1029-1037, 2004.
3. **Carey AL, Steinberg GR, Macaulay SL, Thomas WG, Holmes AG, Ramm G, Prelovsek O, Hohnen-Behrens C, Watt MJ, James DE, Kemp BE, Pedersen BK, and Febbraio MA**. Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes* 55: 2688-2697, 2006.
4. **Danielson BPB**. The Cytochrome P450 Superfamily: Biochemistry, Evolution and Drug Metabolism in Humans. *Curr Drug Metab* 3: 561-597, 2002.
5. **Ellingsgaard H, Hauselmann I, Schuler B, Habib A, Baggio L, Meier D, Eppler E, Bouzakri K, Wueest S, Muller Y, Hansen A, Reinecke M, Konrad D, Gassmann M, Reimann F, Halban P, Gromada J, Drucker D, Gribble F, Ehses J, and Donath M**. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat Med* 17: 1481-1489, 2011.
6. **Frühbeck G, Catalán V, Rodríguez A, Ramírez B, Becerril S, Portincasa P, and Gómez-Ambrosi J**. Normalization of adiponectin concentrations by leptin replacement in ob/ob mice is accompanied by reductions in systemic oxidative stress and inflammation. *Sci Rep* 7: 2752, 2017.
7. **Huber W, Carey VJ, Gentleman R, Anders S, Carlson M, Carvalho BS, Bravo HC, Davis S, Gatto L, Girke T, Gottardo R, Hahne F, Hansen KD, Irizarry RA, Lawrence M, Love MI, MacDonald J, Obenchain V, Oleś AK, Pagès H, Reyes A, Shannon P, Smyth GK, Tenenbaum D, Waldron L, and Morgan M**. Orchestrating high-throughput genomic analysis with Bioconductor. *Nat Methods* 12: 115-121, 2015.
8. **Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, and Speed TP**. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 4: 249-264, 2003.
9. **Karathanasis SK, Oettgen P, Haddad IA, and Antonarakis SE**. Structure, evolution, and polymorphisms of the human apolipoprotein A4 gene (APOA4). *Proc Natl Acad Sci U S A* 83: 8457-8461, 1986.
10. **Lee KY, Russell SJ, Ussar S, Boucher J, Vernochet C, Mori MA, Smyth G, Rourk M, Cederquist C, Rosen ED, Kahn BB, and Kahn RC**. Lessons on Conditional Gene Targeting in Mouse Adipose Tissue. *Diabetes* 62: 864-874, 2013.
11. **Liu R, Holik AZ, Su S, Jansz N, Chen K, Leong H, Blewitt ME, Asselin-Labat M-L, Smyth GK, and Ritchie ME**. Why weight? Modelling sample and observational level variability improves power in RNA-seq analyses. *Nucleic Acids Res* 43: 2015.
12. **Matthews V, Allen T, Risis S, Chan M, Henstridge D, Watson N, Zaffino L, Babb J, Boon J, Meikle P, Jowett J, Watt M, Jansson JO, Bruce C, and Febbraio M**. Interleukin-6-deficient mice develop hepatic inflammation and systemic insulin resistance. *Diabetologia* 53: 2431-2441, 2010.
13. **Navia B, Ferrer B, Giralt M, Comes G, Carrasco J, Molinero A, Quintana A, Leclerc J, Viollet B, Señarís RM, and Hidalgo J**. Interleukin‐6 deletion in mice driven by aP2‐Cre‐ERT2 prevents against high‐fat diet‐induced gain weight and adiposity in female mice. *Acta Physiologica* 211: 585-596, 2014.
14. **Quintana A, Erta M, Ferrer B, Comes G, Giralt M, and Hidalgo J**. Astrocyte-specific deficiency of interleukin-6 and its receptor reveal specific roles in survival, body weight and behavior. *Brain Behav Immun* 27: 162-173, 2013.

15. **Sabio G, Das M, Mora A, Zhang Z, Jun JY, Ko HJ, Barrett T, Kim JK, and Davis RJ**. A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. *Science* 322: 1539-1543, 2008.
16. **Sadagurski M, Norquay L, Farhang J, D'Aquino K, Copps K, and White MF**. Human IL6 enhances leptin action in mice. *Diabetologia* 53: 525-535, 2010.
17. **Senn J, Klover P, Nowak I, and Mooney R**. Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes* 51: 3391-3399, 2002.
18. **Senn J, Klover P, Nowak I, Zimmers T, Koniaris L, Furlanetto R, and Mooney R**. Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J Biol Chem* 278: 13740-13746, 2003.
19. **Starkie RL, Rolland J, Angus DJ, Anderson MJ, and Febbraio MA**. Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-alpha levels after prolonged running. *Am J Physiol Cell Physiol* 280: C769-774, 2001.
20. **Turner N, Kowalski GM, Leslie SJ, Risis S, Yang C, Lee-Young RS, Babb JR, Meikle PJ, Lancaster GI, Henstridge DC, White PJ, Kraegen EW, Marette A, Cooney GJ, Febbraio MA, and Bruce CR**. Distinct patterns of tissue-specific lipid accumulation during the induction of insulin resistance in mice by high-fat feeding. *Diabetologia* 56: 1638-1648, 2013.
21. **Vozarova B, Weyer C, Hanson K, Tataranni P, Bogardus C, and Pratley R**. Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes Res* 9: 414-417, 2001.
22. **Wallenius V, Wallenius K, AhrÃ©n B, Rudling M, Carlsten H, Dickson S, Ohlsson C, and Jansson J-O**. Interleukin-6-deficient mice develop mature-onset obesity. *Nat Med* 8: 75-79, 2002.
23. **Wojtaszewski JF, Hansen BF, Gade, Kiens B, Markuns JF, Goodyear LJ, and Richter EA**. Insulin signaling and insulin sensitivity after exercise in human skeletal muscle. *Diabetes* 49: 325-331, 2000.

Author contribution statement

MAF and MW conceived and designed the research, JH provided the animal model, MW, MP, TP, JSB, EE, PI, BZ, SR, WEH, CLE, MH and MF carried out data collection. MW and MAF interpreted results, MW, MAF, SR, MP, TP and JSB prepared the figures, MW and MAF wrote the first draft of the manuscript and all authors approved the final submission

Competing interests

The authors declare no competing interests.

Figure 1

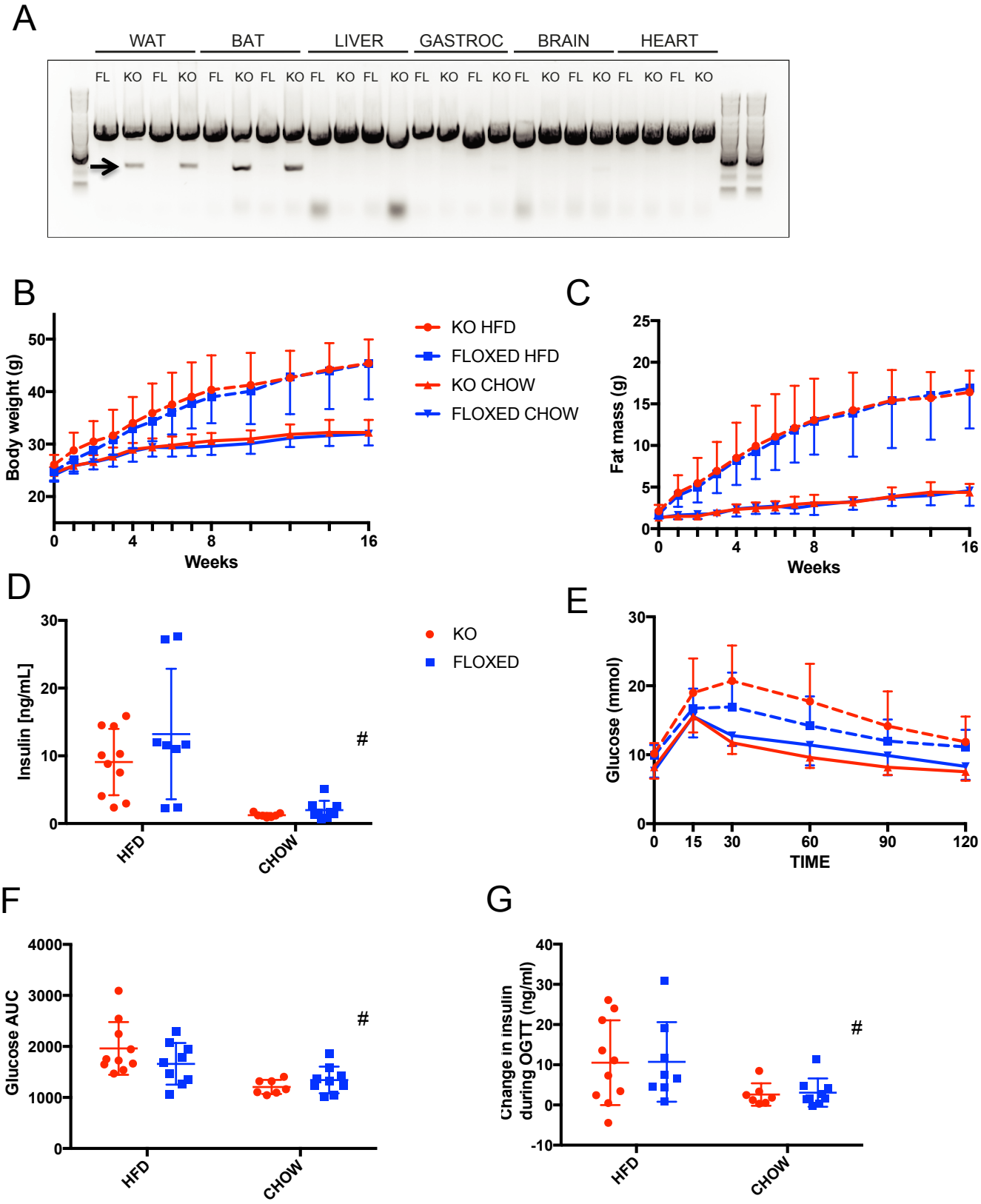

A
WAT
BAT
LIVER
GASTROC
BRAIN
HEART
FL KO FL KO FL KO FL KO FL KO FL KO FL KO FL KO FL KO FL KO FL KO FL KO
B
Body weight (g)
Weeks
KO HFD
FLOXED HFD
KO CHOW
FLOXED CHOW
C
Fat mass (g)
Weeks
D
Insulin [ng/mL]
KO
FLOXED
#
HFD
CHOW
E
Glucose (mmol)
TIME
F
Glucose AUC
#
HFD
CHOW
G
Change in insulin during OGTT (ng/ml)
#
HFD
CHOW

Figure 2

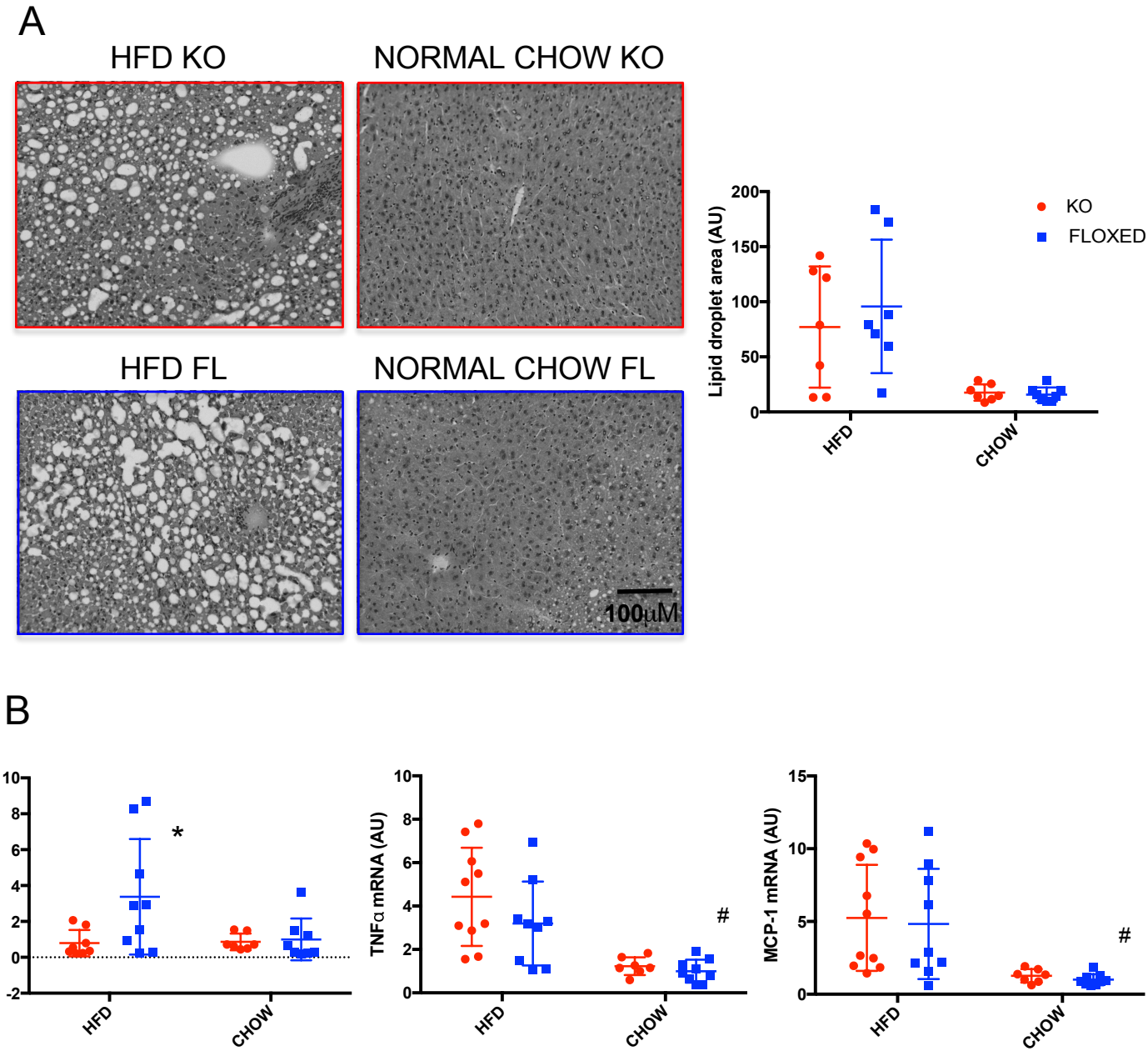
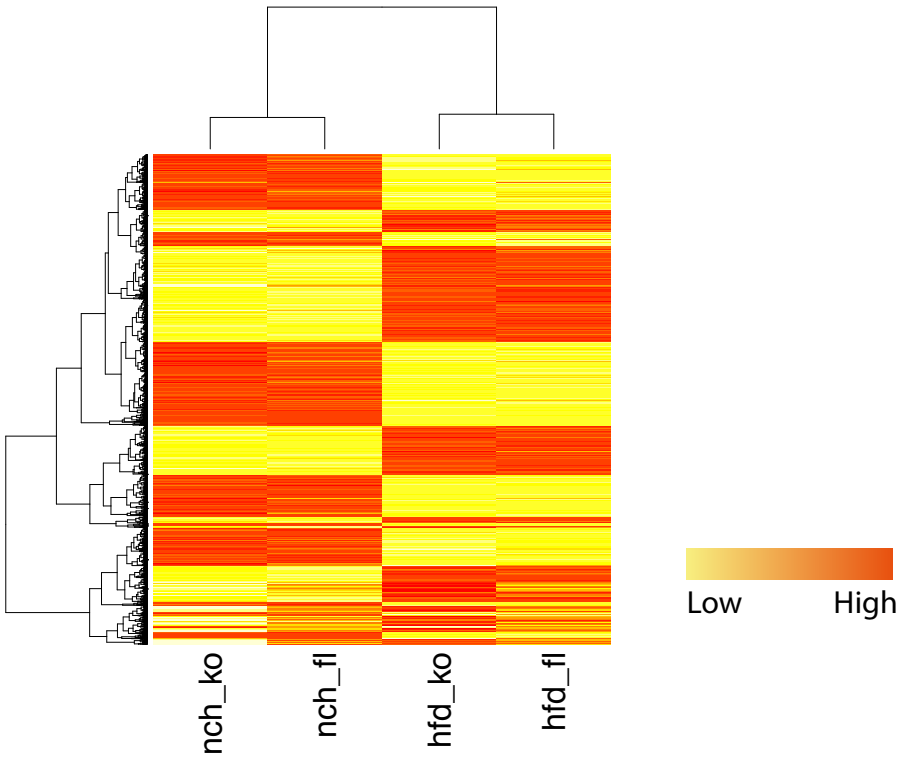
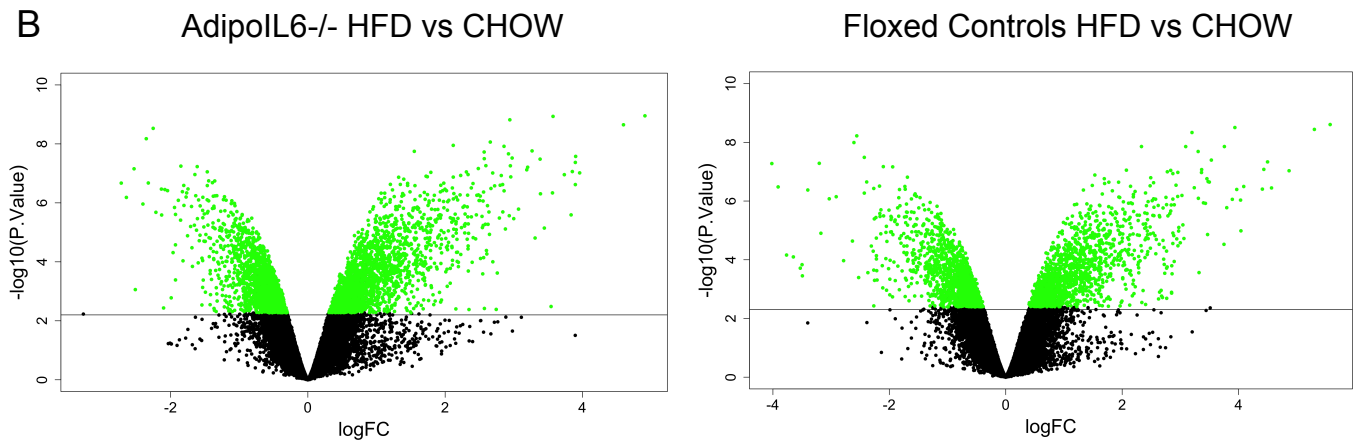

A
HFD KO
NORMAL CHOW KO
HFD FL
NORMAL CHOW FL
100μM
Lipid droplet area (AU)
200
150
100
50
0
KO
FLOXED
HFD
CHOW
B
IL6 mRNA (AU)
10
8
6
4
2
0
-2
*
HFD
CHOW
TNFα mRNA (AU)
10
8
6
4
2
0
#
HFD
CHOW
MCP-1 mRNA (AU)
15
10
5
0
#
HFD
CHOW

Figure 3

A


nch_ko
nch_fl
hfd_ko
hfd_fl
Low
High

B


AdipoIL6-/- HFD vs CHOW
Floxed Controls HFD vs CHOW
-log10(P.Value)
logFC

C

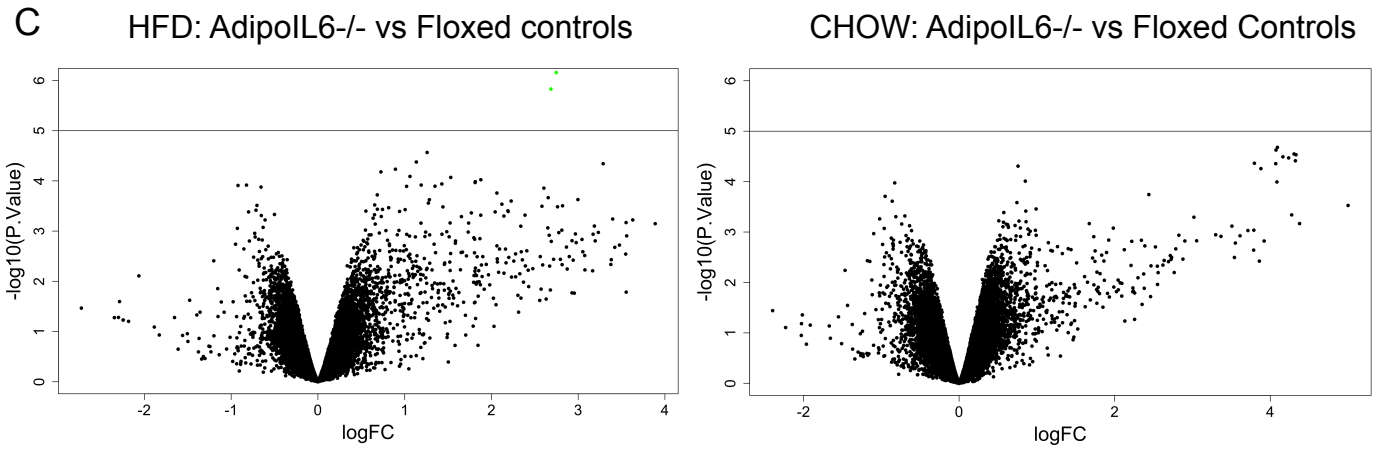
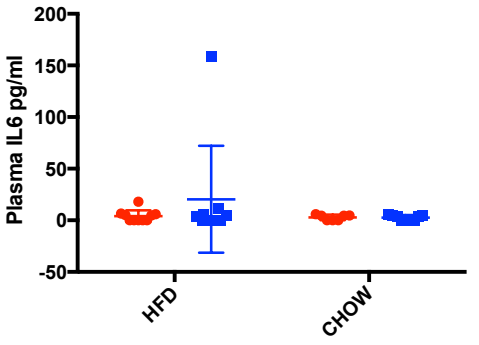
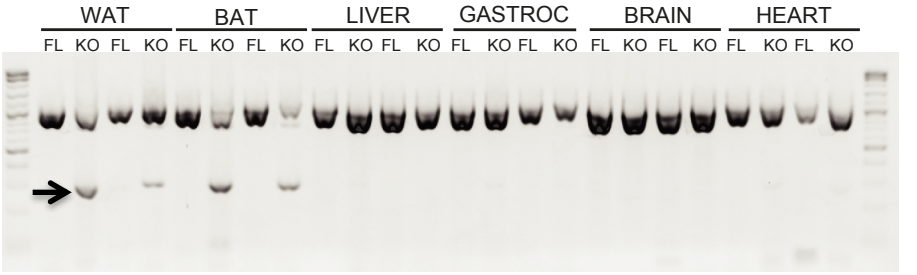

HFD: AdipoIL6-/- vs Floxed controls
CHOW: AdipoIL6-/- vs Floxed Controls
-log10(P.Value)
logFC

Figure 4

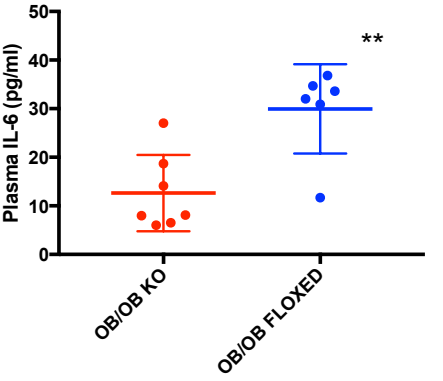
A



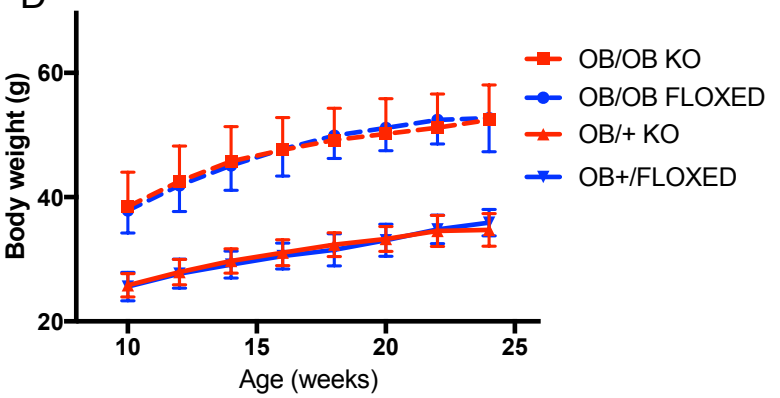
B



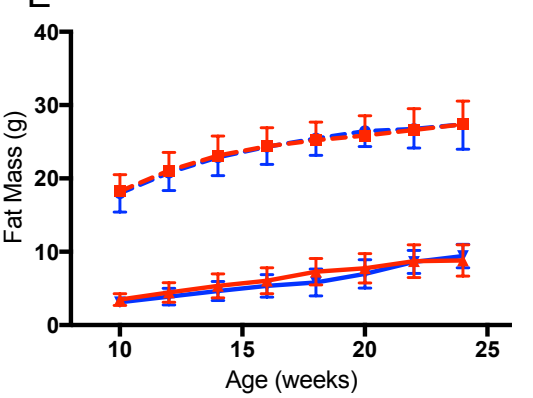
C



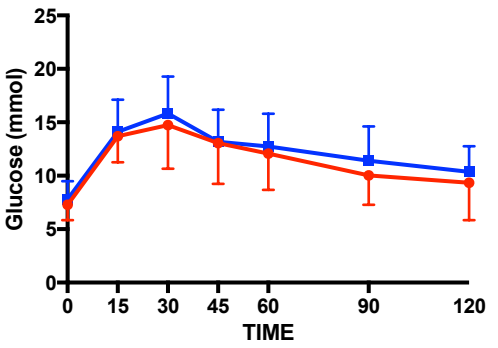
D



E



F



G