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Targeted deletion of *Tcf7l2* in adipocytes promotes adipocyte hypertrophy and impaired glucose metabolism

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ABSTRACT

Objective: Activation of the Wnt-signaling pathway is known to inhibit differentiation in adipocytes. However, there is a gap in our understanding of the transcriptional network regulated by components of the Wnt-signaling pathway during adipogenesis and in adipocytes during postnatal life. The key intracellular effectors of the Wnt-signaling pathway occur through TCF transcription factors such as TCF7L2 (transcription factor-7-like 2). Several genetic variants in proximity to TCF7L2 have been linked to type 2 diabetes through genome-wide association studies in various human populations. Our work aims to functionally characterize the adipocyte specific gene program regulated by TCF7L2 and understand how this program regulates metabolism.

Methods: We generated *Tcf7l2*^{F/F} mice and assessed TCF7L2 function in isolated adipocytes and adipose specific knockout mice. ChIP-sequencing and RNA-sequencing was performed on the isolated adipocytes with control and TCF7L2 knockout cells. Adipose specific TCF7L2 knockout mice were challenged with high fat diet and assessed for body weight, glucose tolerance, and lipolysis.

Results: Here we report that TCF7L2 regulates adipocyte size, endocrine function, and glucose metabolism. *Tcf7l2* is highly expressed in white adipose tissue, and its expression is suppressed in genetic and diet-induced models of obesity. Genome-wide distribution of TCF7L2 binding and gene expression analysis in adipocytes suggests that TCF7L2 directly regulates genes implicated in cellular metabolism and cell cycle control. When challenged with a high-fat diet, conditional deletion of TCF7L2 in adipocytes led to impaired glucose tolerance, impaired insulin sensitivity, promoted weight gain, and increased adipose tissue mass. This was accompanied by reduced expression of triglyceride hydrolase, reduced fasting-induced free fatty acid release, and adipocyte hypertrophy in subcutaneous adipose tissue.

Conclusions: Together our studies support that TCF7L2 is a central transcriptional regulator of the adipocyte metabolic program by directly regulating the expression of genes involved in lipid and glucose metabolism.

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Keywords Diabetes; Adipose tissue; Obesity; Wnt signaling; Lipolysis

1. INTRODUCTION

The Wnt signaling pathway is an integral regulator of cellular proliferation and development and has more recently been shown to regulate cellular metabolism [1,2]. Canonical Wnt signaling is initiated when extracellular wnt ligands activate cell surface receptors on target cells. This leads to stabilization and subsequent translocation of β -catenin to the nucleus where it activates TCF/LEF transcription factors [3,4]. In the absence of Wnt ligand, TCF transcription factors are bound to TLEs, a family of transcriptional repressors that silence Wnt-mediated gene expression [5]. Several components of the Wnt signaling pathway are known to regulate metabolic disease in humans including the TCF/LEF family member, TCF7L2 [6]. Genome-wide association studies in humans aimed at uncovering novel genetic

variants associated with type 2 diabetes have identified polymorphisms (rs12255372 and rs7903146) in *Tcf7l2* that correlate with type 2 diabetes across multiple populations [7–40], [41–60], [61–100], [101–131]. Remarkably, polymorphisms in *Tcf7l2* have a greater impact on risk for developing type 2 diabetes than any other risk allele that has been identified in humans, including those found in PPAR γ , a transcription factor that regulates adipocyte function [10,19,132].

A causal relationship between *Tcf7l2* and type 2 diabetes has been explored in mice expressing multiple copies of *Tcf7l2* using a BAC transgenic system. This led to overexpression of the human *Tcf7l2* gene in multiple tissues, which resulted in impaired glucose handling [133]. Efforts to understand how TCF7L2 functions in various tissues to regulate systemic glycemia have yielded mixed results. Mice

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lacking TCF7L2 in pancreatic β -cells show normal β -cell function and insulin secretion, which might be expected since patients who harbor *Tcf7l2* risk alleles have increased expression of TCF7L2 in the pancreas [134]. In contrast, mice with deletion of TCF7L2 in the liver have improved glucose tolerance and reduced gluconeogenesis, while mice overexpressing TCF7L2 in the liver are glucose intolerant [134]. Additional studies are needed to characterize the role of TCF7L2 in other metabolically active tissues to gain a full understanding of how TCF7L2 contributes to the complex etiology of type 2 diabetes.

One tissue that regulates both carbohydrate and lipid metabolism is the adipose tissue. White adipocytes synthesize and store triglycerides from newly synthesized fatty acids or from dietary sources via chylomicrons. Adipocytes also operate as endocrine cells, secreting bioactive peptides that regulate glucose and lipid metabolism including leptin, adiponectin, resistin, and adipisin [135–138]. Previous studies show that TCF7L2 regulates adipocyte differentiation. Expression of a dominant negative form of TCF7L2 in fibroblasts blocks Wnt-signaling and stimulates cells to differentiate into adipocytes [139]. During adipocyte differentiation Wnt-responsive genes are actively repressed by TLE3 through a direct interaction with TCF7L2 [140]. Given the central role of adipose tissue in type 2 diabetes pathology and the role of TCF7L2 in adipogenesis, defining the direct transcriptional targets of TCF7L2 in adipocytes will provide opportunities to understanding how this pathway drives metabolic control.

Here we show that *Tcf7l2* is highly expressed in adipocytes, and its expression is reduced in adipose tissue of mice fed high-fat diet and in genetic models of obesity. We demonstrate that conditional deletion of *Tcf7l2* in adipocytes leads to adipocyte hypertrophy, and impaired glucose handling on a high-fat diet. Genome-wide analysis of TCF7L2 binding and gene expression analysis supports that TCF7L2 is a key metabolic regulator in adipocytes. TCF7L2 directly regulates genes involved in triglyceride hydrolysis and lipogenesis, and adipocyte depletion of TCF7L2 ultimately promotes lipid storage and adipocyte hypertrophy. These studies highlight that TCF7L2 has a role beyond development that extends to metabolic control in adipocytes. Together our findings suggest that targeted therapies that enhance *Tcf7l2* expression in adipocytes are likely to improve glucose metabolism in individuals carrying this susceptibility gene.

2. MATERIALS AND METHODS

2.1. Animals

Mice were maintained in 12 h light/12 h dark with free access to water and standard chow (Tekland Global 2920X-030818). For high fat diet (HFD) studies, mice were fed rodent diet with 60 kcal% fat diet (D12492, Research Diets, Inc). *Tcf7l2^{F/F}* mice were generated from JM8.F6 ES cells originally derived from C57BL/6N mice through the EUCOMM collection, *Tcf7l2^{tm1a(EUCOMM)Wtsi}* (EM:06158). The L1L2_Bact_P cassette was inserted at position 55907842 of Chromosome 19 upstream of exon5 (Build GRCm38) (Exon5: TCGAACAAAG TACCGGTGGT GCAACACCCC CACCATGTCC ACCCACTCAC GCCTCTCATC ACGTACAGCA ATGAACACTT CACCCGGGA AATC-CACCTC CGCACTTACC AGTGACGTA GACCCCAAAA CAGG). The cassette is composed of an FRT flanked lacZ/neomycin cassette followed by a loxP site. An additional loxP site is inserted downstream of the targeted exon5 at position 55908926 (Supplemental Figure 2A). Exon 5 is thus flanked by loxP sites (Supplemental Figure 2B). A “conditional ready” (floxed) allele was created by crossing mice with Actin-*flip* mice to induce recombinase expression in the germline.

Removal of LacZ and Neomycin cassettes were confirmed by PCR using primers flanking FRT sites, forward (CTGCCCCAATACAACATACACA), and reverse (CTCATAAATTAACCGGACAATGATG). *Flip* induced recombination leads to a 306 bp PCR product, which was observed. Subsequent breeding of *Tcf7l2^{F/+}* mice was completed using adiponectin-Cre mice [141] to induce deletion of exon5 in adipocytes. The *Tcf7l2^F* allele was genotyped for the presence of the terminal loxP site primers forward (TCCTAAGCAAAGTAGGTAGCAATGA) and reverse (TTCAGTTGTACATCTGTAATAATGGG) as well as for the presence of the FRT site primers forward (CTGCCCCAATACAACATACACA) and reverse (CTCATAAATTAACCGGACAATGATG) (Supplemental Figure 2C).

For glucose tolerance tests (GTTs) mice were fasted for 6 h, then given an intraperitoneal injection of glucose (1 mg glucose/g body weight). Insulin tolerance tests (ITT) was performed by intraperitoneal injection of 0.75U insulin/kg body weight (Lilly). In both of these experiments, blood glucose was monitored via tail vein bleed using a glucometer (Contour).

2.2. Body composition

Non-anesthetized mice were weighed and then placed in Bruker Minispec LF50 NMR to measure lean body mass, fat mass, and water mass. Measurements were made from mice fed chow or high-fat diets as indicated in figure legends.

2.3. In vivo lipolysis

Tcf7l2^{F/F};Adp-Cre and littermate *Tcf7l2^{F/F}* control mice received an intraperitoneal injection of CL-316,243 administered at a dose of 1 mg/kg body weight or a volume matched vehicle control injection of PBS. Food was removed at T0 and the mice were allowed free access to water. Blood was taken by tail vein immediately before the injection for T0 and then taken at 30 min, 1 h, 3 h, and 6 h post-injection. Blood was spun at 2,000 × *g* for 20 min in a refrigerated centrifuge and serum was collected and transferred to a new tube. Glycerol measurements were performed by colorimetric assay following manufacturer instructions (Cayman). Free fatty acids were measured using a Free Fatty Acid Quantitation Kit (Sigma) according to manufacturer's instructions. Insulin measurements were performed on the serum of mice fasted for 6 h by ELISA (Crystal Chemical).

2.4. Cell culture

Preadipocytes were isolated from minced inguinal white adipose tissue of *Tcf7l2^{F/F}* mice by digestion with Collagenase type II (Sigma). Cells from the stromal vascular fraction were then resuspended in HBSS and stained with Biotin CD31 (Biolegend), Biotin CD45 (Biolegend), Biotin Ter-119 (Biolegend), FITC Streptavidin (Biolegend), PE CD34 (BD Biosciences), V500 Sca1 (BD Biosciences), PE/Cy7 CD29 (Biolegend), and Sytox Blue (Thermo Fisher). Dead cells were excluded by uptake of Sytox blue and lineage positive cells were excluded by FITC Streptavidin. Preadipocytes were positively selected for by CD34, Sca1, and CD29 staining. Cells were sorted on a BD FACS Aria cell sorter and analyzed with BD FACSDiva software. Isolated preadipocytes were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10%FBS(RMBIO) and maintained in a 5% CO₂ atmosphere and were then immortalized by retroviral expression of the SV40 large T antigen using a hygromycin selection marker. To generate an inducible knockout cell line immortalized cells were then infected with retroviral vector pMSCV CreERT2 using a puromycin selection marker. For differentiation cells were plated, grown to confluence, and then stimulated with differentiation media (1 μ M dexamethasone, 0.5 mM isobutylmethylxanthine, and 5 μ g/mL insulin)

for two days. The cells were maintained in 10% FBS DMEM with 5 μ g/mL insulin.

2.5. Histology

Tissues were excised and fixed in a 4% buffered formaldehyde for 24 h then transferred to 70% ethanol. White adipose tissue was embedded in paraffin and sliced into 10 μ m sections and stained for hematoxylin and eosin.

2.6. RNA isolation

Adipose tissue was excised and then placed in 1 mL Trizol reagent (Thermo Fisher Scientific). Tissue was lysed using a tissuelyzerll (Qiagen). Homogenates were then spun at 12,000 g for 10 min at 4 °C and top lipid bilayer removed. RNA extraction was then carried out by manufacturer's instructions for Trizol. cDNA was synthesized with SuperScript IV VIL0 Master Mix (Thermo Fisher Scientific) and qPCR was performed on ABI Quant Studio 6 flex with KAPA SYBR FAST (Kapa Biosystems).

2.7. Gene expression

Human gene expression data was acquired from GTEx database. The most abundant transcript of *Tcf7l2* in white adipose tissue corresponds to TCF7L2v-003 which encodes for 3802 bp transcript corresponding to NM_030756 and gene code ID ENST00000369397. This transcript includes the following exons, 1,2,3,4,8,9,10,11,12,13,14,15,16,19, that encode for a 596 amino acid protein. The data used for the analyses described in this manuscript were obtained from the GTEx Portal on 05/17/17.

2.8. Western blot analysis

2.8.1. Tissue

Epididymal white adipose tissue was excised from *Tcf7l2^{F/F}* and *Tcf7l2^{F/F}; +Adp-Cre* mice and processed using RIPA and glass dounce. Adipose tissue lysate was spun at 12,000 rpm, and top lipid layer was removed. This was repeated twice to ensure that lipids were removed from lysate. Protein was quantified using a BCA Protein Assay Kit (Pierce) and 50 μ g of protein was diluted into Laemmli sample buffer, heated at 95 °C for 5 min, loaded on 4–12% TPX gel (Biorad), and transferred to Protran Premium 0.45 nitrocellulose (GE-Amersham). The membrane was blocked with 5% BSA in PBS-T. TCF7L2 was detected using antibody TCF4 (C48H11) Rabbit mAb #2569 (Cell Signaling) in 5% BSA in PBS-T overnight at 4 °C. HMGB1 was detected using antibody HMGB1 antibody #ab18256 (Abcam) in 5% milk in PBS-T overnight at 4 °C. Blots were probed with secondary antibody Goat Anti-Rabbit IgG (H + L) secondary antibody, HRP #656120 (Thermo Fisher Scientific).

2.8.2. Cells

Cells were homogenized using RIPA buffer plus proteinase inhibitor and incubated on ice for 20 min. Cell homogenate was spun at 12,000 \times g for 10 min at 4 °C. Protein was quantified using a BCA Protein Assay Kit (Pierce). Samples were diluted in Laemmli sample buffer, heated at 95 °C for 5 min and loaded on 4–12% TPX gel (Biorad) and transferred to Protran Premium 0.45 nitrocellulose (GE-Amersham). The membrane was blocked with 5% milk in PBS-T. TCF7L2 was detected using antibody TCF4 (C48H11) Rabbit mAb #2569 (Cell Signaling) in 5% BSA in PBS-T overnight at 4 °C. HMGB1 was detected using antibody HMGB1 antibody #ab18256 (Abcam) in 5% milk in PBS-T overnight at 4 °C. Blots were probed with secondary antibody Goat Anti-Rabbit IgG (H + L) secondary antibody, HRP #656120 (Thermo Fisher Scientific) and run on SDS page.

2.9. Chromatin immunoprecipitation and next generation sequencing

Immortalized *Tcf7l2^{F/F}* preadipocytes expressing pMSCV CreERT2 were treated with either 500 nM of (z) 4-hydroxytamoxifen (Sigma) or vehicle and then induced to differentiate with DMEM containing 10% FBS, 1 mM dexamethasone, 0.5 mM isobutylmethylxanthine, 20 nM GW1929, and 5 mg/ml insulin for 2 days, followed by 5 mg/ml insulin and 20 nM GW1929 alone. After 8 days of differentiation, cells were fixed with 1% formaldehyde for 10 min at RT. Fixation was quenched with the addition of 125 mM glycine for 5 min at RT. Cells were washed 2 times in cold PBS and harvested in Farnham Lysis Buffer and nuclei isolation was performed using a dounce. Homogenates were then spun at 4000 rpm for 5 min at 4 °C. Nuclei were resuspended in ChIP RIPA buffer (1xPBS, 1%NP-40, 0.5% NaDoc, 0.1%SDS, protease inhibitor cocktail (Roche)) and sonicated in Diagenode Pico according to manufacturer's instructions. Samples were then spun at 10,000 g for 5 min at 4 °C and supernatant was incubated with antibody TCF4 (C48H11) Rabbit mAb #2569 (Cell Signaling) or Rabbit IgG #2729 (Cell Signaling) overnight at 4 °C. Protein A/G magnetic beads #88802 (Thermo Fisher Scientific) were then added to Ab-Ag complexes for 4 h at 4 °C. ChIP reactions were washed in ChIP wash buffer 1 (0.1% SDS, 0.1% NaDoc, 1% Triton, 0.15M NaCl, 1 mM EDTA, 20 mM Tris pH 8.0, protease inhibitor cocktail (Roche)) for 5 min at 4 °C, ChIP wash buffer 2 (0.1% SDS, 0.1% NaDoc, 1% Triton, 0.5M NaCl, 1 mM EDTA, 10 mM Tris pH 8.0, protease inhibitor cocktail (Roche)) for 5 min at 4 °C, LiCl wash buffer (100 mM Tris pH 7.5, 500 mM LiCl, 1% NP-40, 1% NaDoc, protease inhibitor cocktail (Roche)) for 3 min at 4 °C 5 times, and TE 1 min at 4 °C. Chromatin was eluted with Elution buffer (1% SDA, 0.1M NaHCO₃) at 65 °C for 1 h. Crosslinks were reversed by incubated samples in 0.2M NaCl overnight at 65 °C. Samples were then incubated with 10 μ g of RNase A for 1 h at 37 °C followed by 20 μ g of Proteinase K for 2 h at 45 °C. Samples were then purified using Zymo Chip DNA purification and concentrator kit (Zymo). ChIP-seq Library was prepared with DNA SMART ChIP-seq Kit (Takara) according to manufacturer's instructions and purified using AMPure XP beads #A63880 (Beckman Coulter). Sequencing libraries (25 pM) were chemically denatured and applied to an Illumina HiSeq v4 single read flow cell using an Illumina cBot. Hybridized molecules were clonally amplified and annealed to sequencing primers with reagents from an Illumina HiSeq SR Cluster Kit v4-cBot (GD-401-4001). Following transfer of the flowcell to an Illumina HiSeq 2500 instrument (HCSv2.2.38 and RTA v1.18.61), a 50-cycle single-read sequence run was performed using HiSeq SBS Kit v4 sequencing reagents (FC-401-4002). RNA-seq and ChIP-seq data is deposited in GEO DataSets.

2.10. Integration of ChIP-seq and RNA-seq analysis

FASTQ sequences were aligned to the mouse genome (UCSC build mm10) with Novocraft Novoalign (version 3.7, <http://www.novocraft.com>), allowing for one random alignment for reads with multiple alignments. Alignments had moderately high duplication levels; therefore, alignments at duplicate positions were subsampled to an overall duplication rate of 15% and maximum duplicate depth of 30 using `bam_partial_dedup` (https://github.com/tjarnell/HCI-Scripts/blob/master/BamFile/bam_partial_dedup.pl) to avoid false peaks while preserving natural signal strength. Fragment coverage bedGraph files were generated with BioToolBox (version 1.52, <https://github.com/tjarnell/biotoolbox>) `bam2wig` using 200 bp as an extension, excluding mitochondrial chromosome and Encode black list regions, depth-normalizing to 1 million reads, and taking the mean of replicates. Peaks were called manually with Macs2 (version 2.1.1.20160309, <https://github.com/taoliu/MACS>) using a lambda

control bedGraph generated from the input with bam2wig (small and local lambda set to 1 kb and 10 kb, respectively) and peak calling thresholds of q-value of 2 (1% FDR), minimum peak length 250 bp, and peak gap of 100 bp. ChIP peaks were annotated with the R package ChIPSeeker (version 1.12.1) using UCSC knownGene transcript and Ensembl annotation tables. Promoters were defined as ± 3 kb from the TSS. Unique transcript start sites were collected using BioToolBox `get_gene_regions`. Heat maps were generated with deepTools (version 2.5.4, <http://deeptools.readthedocs.io/en/develop/>) `computeMatrix` and `plotHeatMap`. Transcription factor site motif was performed using Homer (version 4.9, <http://homer.ucsd.edu/homer/>) `findMotifsGenome`, and Jaspar http://159.149.160.88/pscan_chip_dev/.

RNA was purified using Qiagen RNeasy mini Kit and library preps were made with Illumina TruSeq Stranded Total RNA Sample Prep Kit with Ribo-Zero Gold. Samples were sequenced on Illumina HiSeq 2000 with HiSeq 125 Cycle paired-end Sequencing v4. Reads were aligned to mm10.M_musculus_Dec_2011, GRCm38 using Bioconductor RNA-Seq workflow to find differentially expressed genes using DESeq2 and the `hciR` package on Github to simplify the R code.

2.11. HMDP analysis

The Hybrid Mouse Diversity Panel arrays and clinical traits were generated as previously described [142,143]. Gene expression and PC vector correlations were assessed using the `BicorAndPvalue` function in the R package WGCNA [144]. Principle Component coordinates for each mouse was extracted from the base R function `prcomp` and plotted using the package `factextra` as previously described [145]. Pathway enrichment was performed on the top 500 genes, either positively or negatively correlated with *Tcf7l2* within the adipose tissue expression arrays using The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 [146,147].

2.12. In vitro lipolysis assay

Immortalized *Tcf7l2*^{F/F} preadipocytes expressing pMSCV CreERT2 were treated with either 500 nM of (z) 4-hydroxytamoxifen (Sigma) or vehicle and then induced to differentiate with DMEM containing 10% FBS, 1 mM dexamethasone, 0.5 mM isobutylmethylxanthine, 20 nM GW1929, and 5 mg/ml insulin for 2 days, followed by 5 mg/ml insulin and 20 nM GW1929 alone. After 10 days of differentiation cells were washed with PBS and incubated in Krebs Ringer buffer for two hours. Cells were then treated with 100 nM CL 316,243 for four hours. Fatty acids from media were then quantified using a Free Fatty Acid Quantitation Kit (Sigma) according to manufacturer's instructions.

2.13. Statistical analysis

Student T-test or ANOVA followed by Tukey posthoc analysis. Analysis was completed using excel or Prism 7.

3. RESULTS

3.1. *Tcf7l2* expression is reduced in adipose tissue of high fat fed mice and genetic models of obesity

The strong association of *Tcf7l2* with type 2 diabetes prompted us to explore the regulation of TCF7L2 in adipose tissue. To test whether *Tcf7l2* expression changes with high-fat diet, mice were placed on 10% or 60% high-fat diet for 3-days. When compared to 10% high-fat diet, mice fed 60% high-fat diet had increased body weight (Figure 1A) and reduced mRNA of *Tcf7l2* and *adiponectin* in epididymal white adipose tissue (eWAT) (Figure 1B), while mRNA of *Tcf7l2* and *adiponectin* was similar in inguinal white adipose tissue (iWAT) (Figure 1C).

To determine whether *Tcf7l2* expression remains low with prolonged high-fat diet, we measured expression of *Tcf7l2* in eWAT and iWAT after 4-months of high-fat diet, and found reduced gene expression of *Tcf7l2*, *Adiponectin* and *Cfd* (adipsin) (Figure 1E,F). The decreased TCF7L2 expression in HFD was also observed by western blot analysis (Figure 1G). To test whether genetic models of obesity had altered expression of *Tcf7l2*, we measured gene expression in eWAT of *ob/ob* and *db/db* mice and found reduced expression of *Tcf7l2* (Figure 1H). To determine the normal distribution of TCF7L2, we completed western blot analysis on multiple tissues, and found that TCF7L2 had the highest expression in epididymal and inguinal white adipose tissue in mice (Figure 1I). To develop a broader view of *Tcf7l2* expression in humans, we queried the Genotype-Tissue Expression (GTEx) portal, where gene expression data from RNA sequencing is available from multiple human tissues post mortem. Expression data is shown as RPKMs (number Reads Per Kilobase gene model and Million mapped reads) using 0.5 RPKM as threshold for detection. In humans, we found that *Tcf7l2* expression was high in subcutaneous and visceral adipose tissue, while expression in pancreas, heart and skeletal muscle was relatively low (Figure 1J). To understand where *Tcf7l2* is expressed, we fractionated adipose tissue, and found that *Tcf7l2* expression is highest in white adipocytes, with lower expression in the stromal vascular fraction (Figure 1K). The decrease in expression in both diet-induced and genetic models of obesity, and the high expression in the adipocyte fraction, raised the possibility that reduced *Tcf7l2* expression in adipocytes might lead to impaired glucose or lipid metabolism.

3.2. Inducible deletion of *Tcf7l2* promotes adipocyte differentiation and lipid accumulation

To test the *in vitro* role of TCF7L2 on adipocyte differentiation and lipid storage, we generated mice from embryonic stem cells engineered by EMMA for C57BL/6NTac-Tcf7l2<tm1a (EUCOMM)Wtsi>/Wtsi (EM:06158). Cells were injected into fertilized embryos and chimeras were bred with C57BL6J mice. Pups from the F1 cross were genotyped and those carrying the targeted allele were bred with Actin-F1pe mice to remove the neomycin cassette. *Tcf7l2*^{F/F} mice have *loxP* sites flanking exon 5, which is expressed in all *Tcf7l2* transcripts. *Tcf7l2*^{F/F} preadipocytes were immortalized by retroviral expression of LargeT antigen and subsequently infected with viral vectors for CreERT2 [148,149]. Western blot analysis of cells treated with (z)-4-hydroxy tamoxifen resulted in greatly diminished TCF7L2 protein (Figure 2A) and mRNA (Figure 2C). Subsequent differentiation of TCF7L2 null preadipocytes led to enhanced differentiation as supported by increased expression of differentiation markers, including *Adiponectin*, *aP2*, and *Dgat1* expression (Figure 2C) as well as increased lipid accumulation as measured by bodipy staining (Figure 2B). To develop a global assessment of the role of TCF7L2 in adipocytes, we measured differential gene expression by RNA-sequencing analysis (Figure 2D). Gene set enrichment analysis (GSEA) on differentially expressed genes from control (Vehicle) or TCF7L2 null cells (4-OH-Tam) identified several metabolic pathways, including those involved in the respiratory electron transport chain, pyruvate metabolism, fatty acid and triglyceride metabolism, glucose metabolism, amino acid metabolism, and adipocyte differentiation (Figure 2E).

3.3. Genome-wide analysis of TCF7L2 binding sites suggests direct transcriptional regulation of metabolic and proliferative pathways

To define the transcriptional pathways that are directly regulated by TCF7L2, we performed ChIP-seq analysis using preadipocytes from *Tcf7l2*^{F/F} mice. Cells were engineered to express CreERT2 enabling

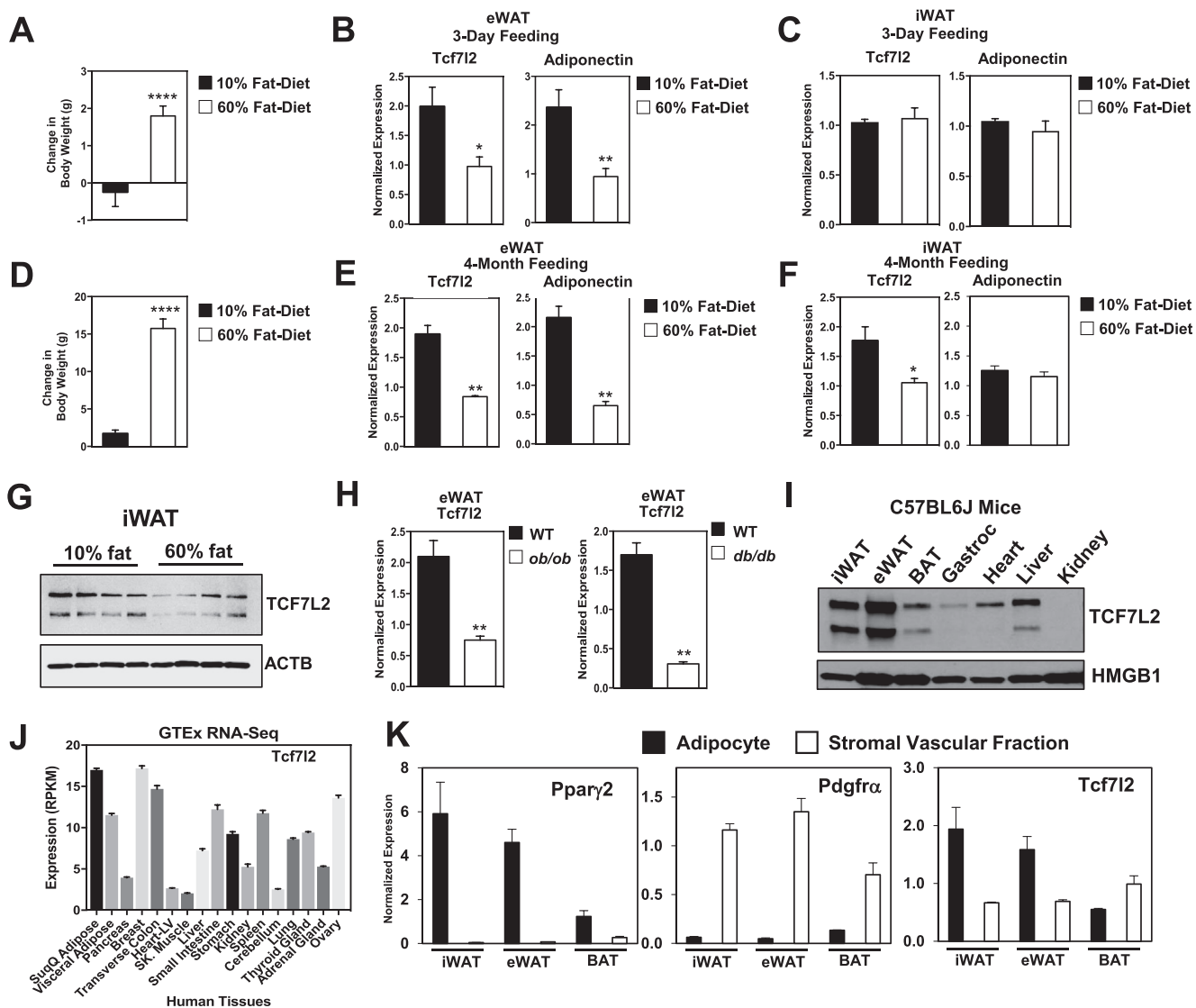


Figure 1: TCF7L2 expression is reduced in white adipose tissue with high-fat diet and genetic models of obesity. (A) Change in body weight of C57Bl/6J mice placed on 10% or 60% HFD for 3 days (males, $n = 5$, 3-months of age). (B) *Tcf7l2* and *adipoq* (adiponectin) gene expression in eWAT and (C) iWAT. (D) Change in body weight of C57Bl/6J mice placed on 60% HFD for 4 months. (males, $n = 5$ for each group, 60% HFD started at 14 weeks old) (E) *Tcf7l2* and *adipoq* gene expression in eWAT and (F) in iWAT, gene expression normalized to *Rps3* expression. (G) Western blot analysis in iWAT of TCF7L2 and ACTB (β -actin) after 4-month HFD. (H) *Tcf7l2* expression in eWAT of control, ob/ob mice and db/db mice, gene expression was normalized to *Rps3* expression. (I) Western blot analysis of TCF7L2 and HMGB1 expression in mouse tissues. (J) *Tcf7l2* expression in human tissues from publicly available GTEx RNA-Seq data set. (K) Gene expression in fractionated adipose tissue depots from C57Bl/6J 3-month old male mice. P-values were determined using Student's t-test, * $p < 0.05$.

conditional deletion of TCF7L2 with a single tamoxifen treatment. Genome-wide occupancy of TCF7L2 showed 12,073 total binding sites which mapped to 7,207 genes. As expected, occupancy was greatly diminished in *Tcf7l2*^{Cre} cells receiving tamoxifen (Figure 2A,C). Notably, TCF7L2 binding sites were largely localized near the transcriptional start site (Figure 3A). Although the majority of TCF7L2 binding sites were located within promoter regions, we observed binding in intronic, 5' UTR, 3'UTR, and distal intergenic regions (Figure 3B). To identify direct targets of TCF7L2, we completed integrated analysis of RNA-seq and ChIP-seq data and found that 1,427 genes that were differentially regulated had a TCF7L2 binding site nearby (Figure 3D). We then performed GO analysis on transcripts that were differentially regulated and found

that upregulated genes were enriched in metabolic pathways such as the TCA cycle, respiratory electron transport chain, lipid and triacylglycerol metabolism, while downregulated genes were highly enriched in cell cycle, DNA replication, translation and transcriptional pathways, pathways involved in proliferation (Figure 3F). To determine whether other transcription factors associate with TCF7L2 binding sites, we performed motif analysis on the ChIP fragments using Jaspar. Through this analysis we identified transcription factor binding motifs of Jun and ATF4, which are known to regulate proliferation. We also identified motifs for transcription factors that regulate various aspects of metabolism such as CEBP, EBF, and KLF13 (Figure 3C). These findings provide evidence for a direct role for TCF7L2 in regulating gene expression of metabolic pathways.

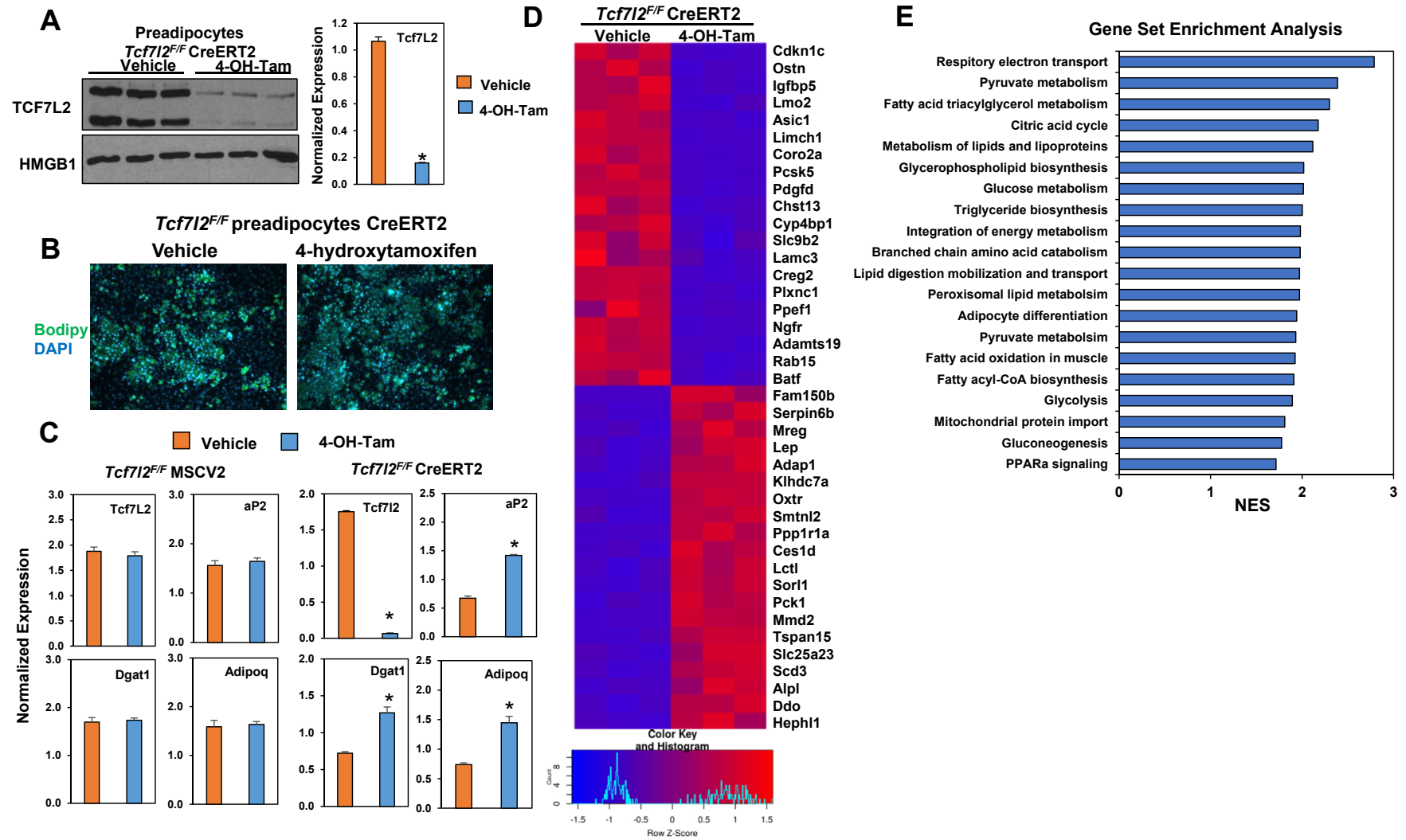


Figure 2: Inducible deletion of TCF7L2 results in enhanced adipocyte differentiation and lipid accumulation. (A) Western blot of TCF7L2 and HMGB1 expression in preadipocytes harvested from *Tcf712^{F/F}* mice. CreERT2 expressing preadipocytes were treated with vehicle (ethanol) or 50 nM 4-hydroxytamoxifen, n = 3. Corresponding changes in Tcf712 transcript (bar graph). (B) Bodipy and DAPI staining of differentiated *Tcf712^{F/F}* preadipocytes expressing CreERT2. (C) Gene expression changes were measured by real-time PCR and normalized to *Rps3* expression. (D) Heat map of differential gene expression changes in control and TCF7L2-null cells. (E) Gene Set Enrichment Analysis of differentially expressed genes in control and TCF7L2-null cells. n = 3. P-values were determined using Student's t-test, *p < 0.05.

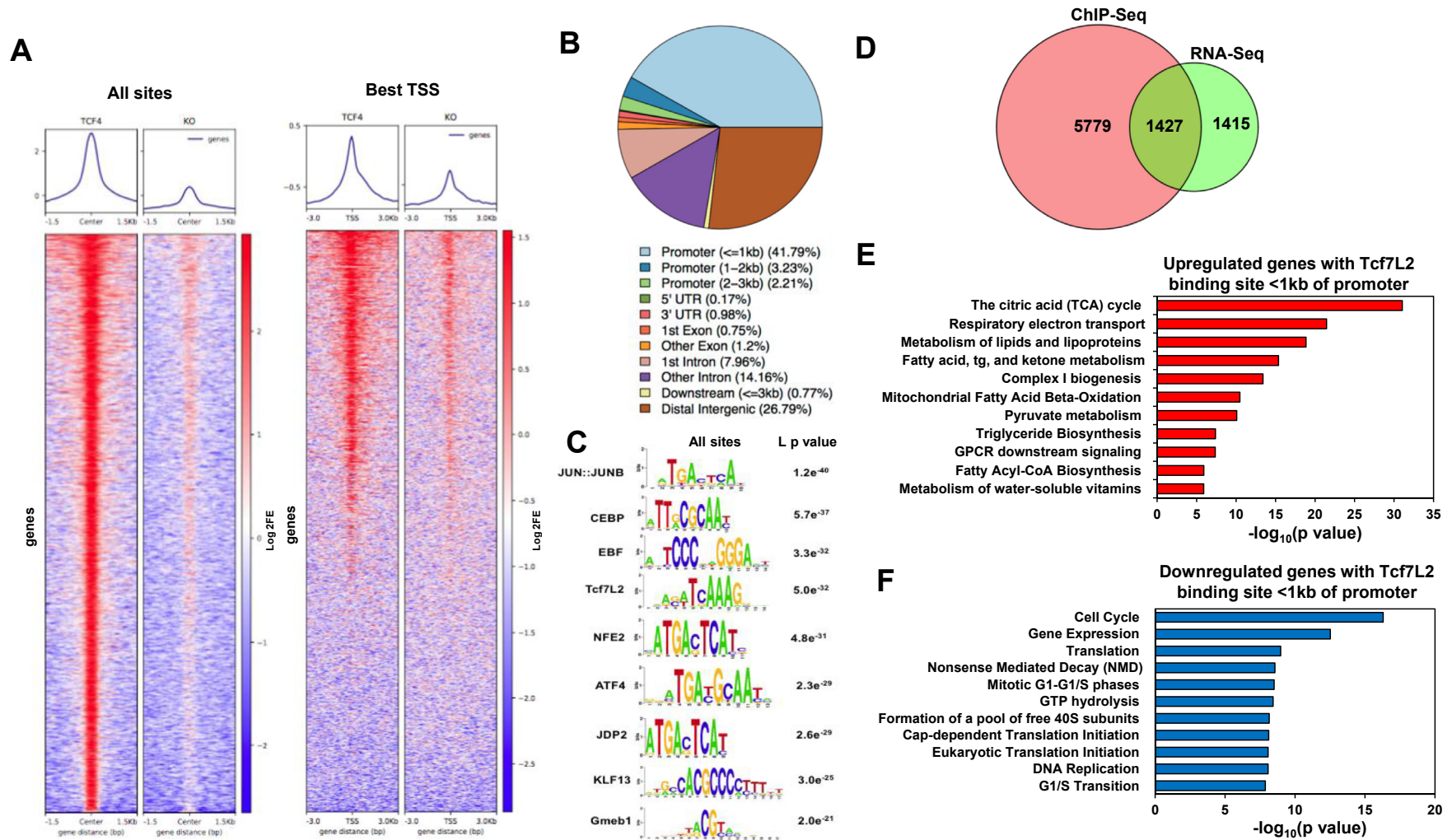


Figure 3: Genome wide analysis of TCF7L2 binding sites shows direct regulation of metabolic and proliferative pathways. (A) Heat map of ChIP-seq data of TCF7L2 binding in proximity to the transcriptional start site. (B) Genomic distribution of TCF7L2 occupancy. (C) Motif analysis of TCF7L2 ChIP fragments using JASPAR. (D) Venn diagram showing overlap between differential gene expression and genes occupied by TCF7L2. (E) GO analysis of upregulated genes with TCF7L2 binding within 1 kb of promoter region. (F) GO analysis of downregulated genes with TCF7L2 binding within 1 kb of promoter region. $n = 3$.

3.4. *Tcf712* expression correlates with HOMA-IR, fat mass, and metabolic genes across Hybrid Mouse Diversity Panel

Given that we observed a negative correlation with *Tcf712* expression and adiposity in C57BL6/J male mice, and this mouse strain is susceptible to obesity, we next asked if similar patterns of variation persisted across genetically diverse populations. We interrogated the Hybrid Mouse Diversity Panel (HMDP) in order to assess how natural variation in *Tcf712* expression relates to metabolic phenotypes. This panel consists of over a hundred classical and recombinant inbred mouse strains whereby environmental influence can be tightly controlled, thus maximizing our ability to see how genetic architecture plays a role in complex phenotypes [150,151]. We specifically analyzed a population which was fed a high-fat/high-sucrose diet for 8 weeks as this seemed particularly relevant for studies of metabolism and diet-induced metabolic dysfunction [142,143]. Here, we observed striking negative correlations between *Tcf712* expression in adipose tissue and both fat mass and HOMA-IR (Figure 4A, B). Furthermore, we analyzed the strongest negatively or positively correlated genes with *Tcf712* in adipose tissue where we observed strong enrichments in cell cycle and mitochondrial processes, respectively (Figure 4C, D). These data show that the patterns of natural variation of adipose tissue *Tcf712* recapitulate a significant role in regulating pertinent gene expression pathways in fat and consequent changes in metabolic homeostasis, consistent with our observations generated through targeted deletion of *Tcf712*.

We next asked if integration of the data generated from ChIP-sequencing experiments could further inform the mechanism by which *Tcf712* was exerting its functions across a population. We therefore implemented a principal component approach used to capture patterns of variation of larger gene sets across genetically diverse individuals [145]. The basic intuition underlying this approach is as follows: ChIP-seq experiments informed direct binding regions and downstream genes being regulated directly by TCF7L2. Given that TCF7L2 is likely exerting its effects on metabolism through its regulations on gene expression, those transcripts identified in the ChIP-seq should also vary in a manner consistent with *Tcf712*. Therefore, capturing a metric of the variance in the genes across a population should further identify key regulatory nodes allowing *Tcf712* to exert robust effects and thus show stronger correlations across relevant traits. Along these lines, principle component analysis prioritized two vectors which explained the maximal HMDP variance in gene expression from the ChIP-seq profiles (Figure 4C, D). We note that several genes, such as *Ccl2*, *Ncf4* and *Slc44a3* showed substantial individual contribution to the first PC vector. Importantly, this vector which explained the most total variance in gene expression (PC1) showed patterns similar, but substantially more significant than *Tcf712* itself to clinical traits (Figure 4E). This integrative analysis not only validated the physiologic implications of the *Tcf712* ChIP-seq data but additionally prioritized several key genes in which TCF7L2 could regulate to exert its effects.

3.5. Selective deletion of TCF7L2 in adipocytes leads to glucose intolerance and insulin resistance on a high-fat diet

Because of the striking negative correlations between *Tcf712* expression in adipose tissue with both fat mass and HOMA-IR (Figure 4A, B), we hypothesized that functional role of TCF7L2 in adipocytes is important in the etiology of type 2 diabetes associated with obesity. To test this hypothesis, we generated adipose specific knockout mice by breeding *Tcf712^{F/F}* mice with adiponectin-cre (Adp-Cre), an adipocyte selective Cre driver. Glucose tolerance tests showed similar glucose excursion curves between *Tcf712^{F/F}* and *Tcf712^{F/F};+Adp-Cre* mice on a

chow diet. In contrast, when mice were challenged with a high-fat diet, *Tcf712^{F/F};+Adp-Cre* mice showed impaired glucose tolerance relative to *Tcf712^{F/F}* controls (Figure 5A). To assess the insulin responsiveness of these mice, we performed an insulin tolerance test on mice fed a chow and high-fat diet. Mice had a similar drop in glucose in response to 0.75U/kg of insulin with chow, while on a high-fat diet *Tcf712^{F/F};+Adp-Cre* mice had a greater resistance to insulin's glucose lowering effects when compared to *Tcf712^{F/F}* controls (Figure 5A). The observed glucose intolerance found in *Tcf712^{F/F};+Adp-Cre* mice was accompanied by increased serum insulin levels (Figure 5C). The impaired glucose tolerance and elevated insulin levels suggest that the TCF7L2 knockout mice developed insulin resistance, which is supported by calculation of the HOMA-IR (Figure 5C). The impaired glucose tolerance was seen within 3-days of high-fat feeding, and mice became insulin resistant during this period as well, suggesting that the insulin resistance is driving the impairment in glucose handling. After 30 min from the initial administration of insulin, *Tcf712^{F/F}* control mice showed a decrease in blood glucose levels by 132 mg/dL, while *Tcf712^{F/F};+Adp-Cre* mice had a 73 mg/dL drop. In response to this short 3-day high-fat diet feeding, insulin lost its glucose lowering ability by almost 2-fold *Tcf712^{F/F};+Adp-Cre* mice (Figure 5B). Glucose intolerance can result from increased gluconeogenesis in the liver; however, we did not see changes in PEPCK or G6Pase gene expression (Supplemental Figure 5C).

Adipose tissue is able to regulate systemic metabolism through secretion of adipokines, to assess the impact of TCF7L2 loss on adipokine transcripts we measured gene expression by real-time PCR in adipose tissue depots. In eWAT, we found similar expression of several adipokines including *Leptin*, *Resistin*, and *adiponectin* in *Tcf712^{F/F}* and *Tcf712^{F/F};+Adp-Cre* mice, while components of the Wnt pathway such as *Wnt11* were increased in TCF7L2 knockout mice. In contrast, in the subcutaneous iWAT depot, *adipsin* and *resistin* were decreased, while *Wnt11* was elevated, and leptin expression remained the same between *Tcf712^{F/F}* and *Tcf712^{F/F};+Adp-Cre* mice. Similarly, *Glut4* expression was reduced in iWAT of *Tcf712^{F/F};+Adp-Cre* mice and not in eWAT (Figure 5D). These results supported that the loss of *Tcf712* in adipocytes leads to impaired glucose metabolism and altered expression of adipokines in mice.

3.6. TCF7L2 regulates adipose tissue mass and adipocyte size

Due to the negative correlations between *Tcf712* expression in adipose tissue with body fat mass (Figure 4B), we wanted to assess the importance of TCF7L2 in weight gain. We measured body weights of *Tcf712^{F/F}* mice and *Tcf712^{F/F};+Adp-Cre* mice on chow and high-fat diet. On chow diet, both groups had similar gains in body weight and only started to diverge as the mice aged, at 1 year *Tcf712^{F/F};+Adp-Cre* mice started to gain more than *Tcf712^{F/F}* control mice (Figure 6A). To observe subtle changes in body weight, we increased the n number of mice monitored for body weight on chow diet and observed significant increases in body weight but little changes in body composition as measured by Nuclear Magnetic Resonance (NMR) (Figure 6B). For high-fat diet experiments, mice were placed on a 60% high-fat diet and body weights were measured over a 12-week period. Body weight curves diverged after 8-weeks of 60% high-fat diet and showed that selective deletion of TCF7L2 increased weight gain in response to a high-fat diet (Figure 6A). To determine whether loss of TCF7L2 increased adiposity on a high-fat diet, we measured whole body composition using NMR, and found increased fat mass and body mass in *Tcf712^{F/F};+Adp-Cre* mice, while lean body mass was unchanged (Figure 6C). To determine

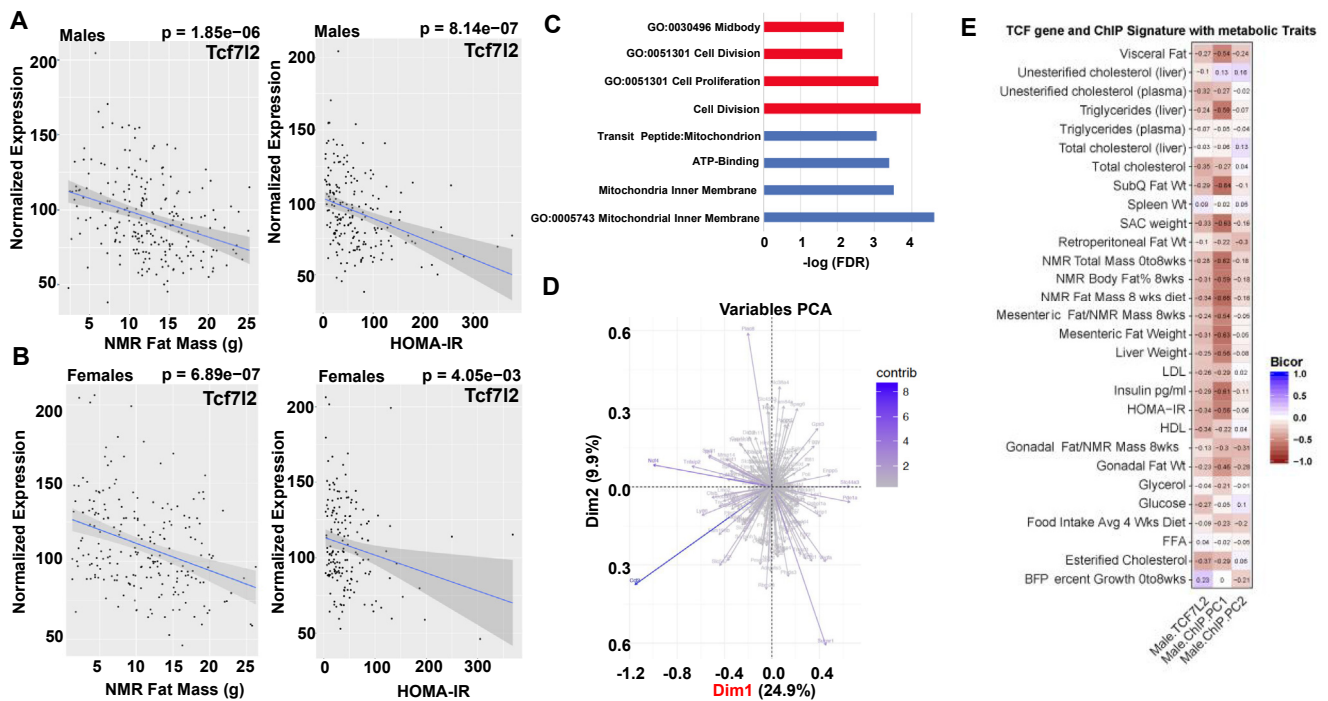


Figure 4: *Tcf7l2* expression correlates with HOMA-IR, fat mass, and metabolic genes across mouse populations (A) Analysis of Hybrid Mouse Diversity Panel (HMDP) fed a high-fat/high-sucrose diet for 8 weeks for correlation of *Tcf7l2* expression in male mice with HOMA-IR and fat mass in female mice (B). (C) Gene ontology of principle component analysis prioritized two vectors which explained the total HMDP variance in gene expression of the top 500-enriched genes from the ChIP-Seq profiles and Gene Ontology (D). *Tcf7l2* gene and ChIP signature integrated with metabolic traits across HMDP.

which adipose tissue depots were contributing to the differences in fat mass on a high-fat diet, the fat pads were excised and weighed. We observed increased iWAT weight, however eWAT and BAT were unchanged (Figure 6C). To test if adipocyte size was altered in iWAT, we completed histological analysis using hematoxylin and eosin (H&E) staining of iWAT and found that TCF7L2 null adipose tissue had increased adipocyte size relative to controls (Figure 6D). Adipocyte size was quantified and found to be twice as large in TCF7L2 null adipocytes (Figure 6E). These findings suggest that during conditions of high fat diet TCF7L2 regulates adipocyte size and adipose tissue mass in a depot specific manner.

3.7. TCF7L2 regulates lipogenic and lipolytic gene expression programs

To identify transcriptional targets of TCF7L2 that alter adipocyte size we performed RNA-seq analysis on subcutaneous adipose tissue from both *Tcf7l2^{F/F}* and *Tcf7l2^{F/F};+Adp-Cre* mice. Because adiponectin is expressed after the initiation of differentiation in adipocytes, the RNA-seq from the *Tcf7l2^{F/F};+Adp-Cre* iWAT will inform on a subset of TCF7L2 targets separate from those in the isolated *Tcf7l2^{F/F}* adipocytes where TCF7L2 was knocked-out before differentiation (Figure 2). Similar to the RNA-seq in the isolated *Tcf7l2^{F/F}* adipocytes, we found altered expression of metabolic pathways. Notably lipolytic genes, triglyceride hydrolases (*Tgh*) and *Tgh2* expression were downregulated in the hypertrophic tissue of *Tcf7l2^{F/F};+Adp-Cre* mice (Figure 7A). Gene set enrichment analysis showed altered gene expression in translational control, DNA synthesis, cell cycle, cholesterol metabolism, and triglyceride biosynthesis and fatty acyl-CoA biosynthesis (Figure 7B). Review of our ChIP-seq data in

isolated adipocytes assessing genes in the lipogenic pathways showed TCF7L2 binding sites within 1 kb of the promoter region in genes that encode for enzymes involved in triglyceride synthesis and *de novo* lipogenesis (Figure 7C). These results were confirmed by real-time PCR analysis where we found that *Tgh* and *Tgh2* expression were reduced in iWAT of *Tcf7l2^{F/F};+Adp-Cre*, while several transcripts implicated in lipogenesis were increased, an outcome that may drive the larger adipocyte size (Figure 7D). The decreased *Tgh* and *Tgh2* expression prompted further exploration of lipolysis and plasma free fatty acid levels. When compared to *Tcf7l2^{F/F}* controls, *Tcf7l2^{F/F};+Adp-Cre* mice showed reduced free fatty acid levels, which suggested that lipolysis was decreased (Figure 7E).

3.8. Tcf7l2 regulates fasting-induced weight loss and adipose tissue lipolysis

Tcf7l2^{F/F};+Adp-Cre mice on a HFD had reduced levels of *Tgh* and *Tgh2* expression in iWAT and serum free fatty acid levels were decreased, which suggested that TCF7L2 regulates adipose tissue lipolysis. These results let us to challenged *Tcf7l2^{F/F}* and *Tcf7l2^{F/F};+Adp-Cre* mice with 24 h of fasting to stimulate adipose tissue lipolysis. We observed that *Tcf7l2^{F/F};+Adp-Cre* mice lost less body fat as measured by NMR, than *Tcf7l2^{F/F}* mice (Figure 8A). In this paradigm, we also measured free fatty acid (FFA) levels in the serum throughout the 24 h fast and saw a rise in FFAs in both *Tcf7l2^{F/F}* and *Tcf7l2^{F/F};+Adp-Cre* mice, however the induction in *Tcf7l2^{F/F};+Adp-Cre* mice was lower compared to control *Tcf7l2^{F/F}* mice (Figure 8B). We measured expression of lipolytic genes such as *Lipe* (encodes hormone sensitive lipase) and *Atgl* (adipose triglyceride lipase) and saw minimal differences only in the ad lib state. We measured *Tgh* and *Tgh2* expression and again saw reduced expression in *Tcf7l2^{F/F};+Adp-Cre* mice, however only in the *ad libitum*

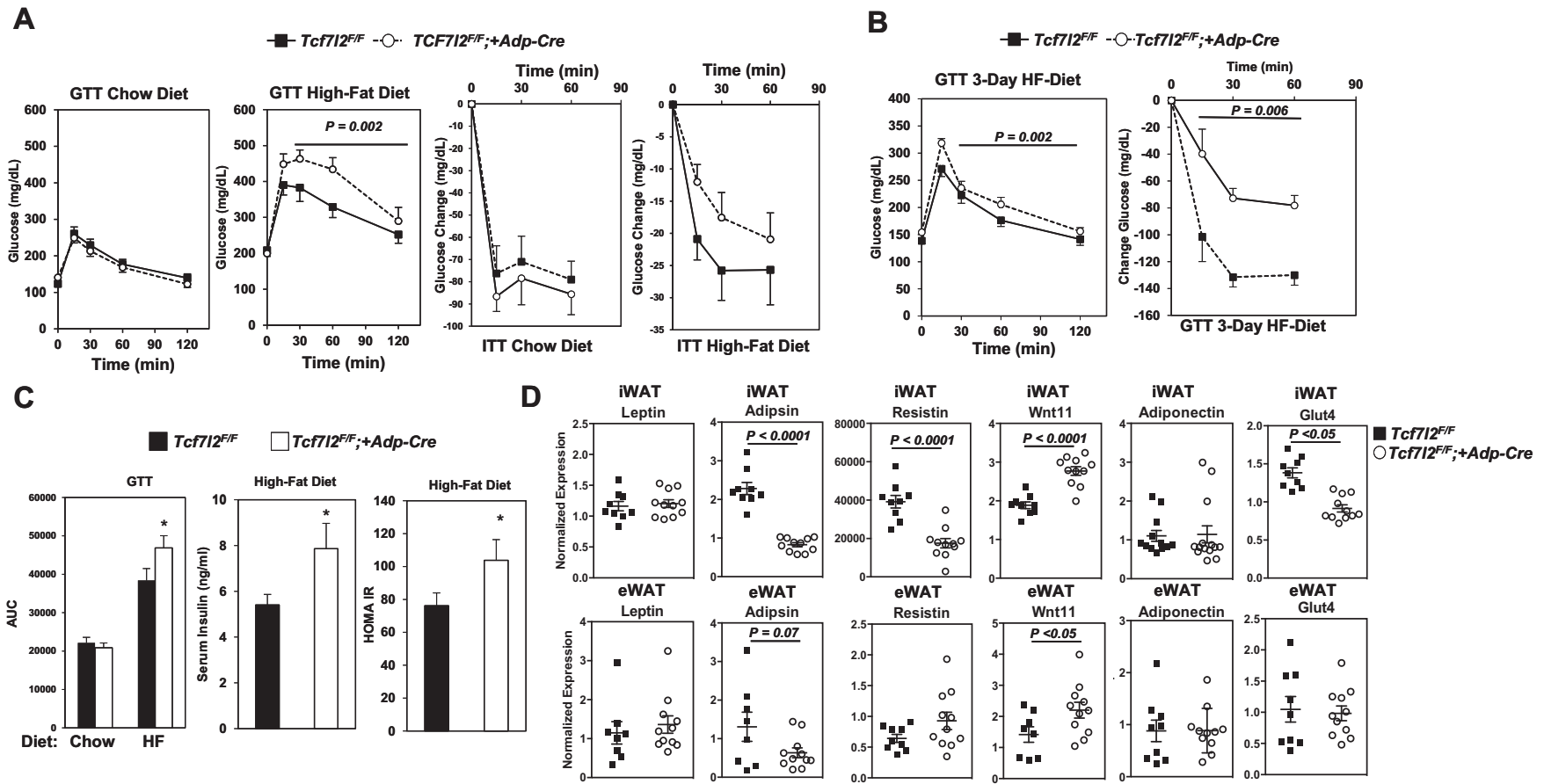


Figure 5: Adipocyte specific deletion of TCF7L2 leads to glucose intolerance, insulin resistance and altered adipokine expression on HFD. (A) Glucose tolerance test (GTT) and insulin tolerance test (ITT) of *Tcf7l2^{F/F}* and *Tcf7l2^{F/F};+Adp-Cre* mice on control and HFD for 12 weeks (males $n = 9$ for controls and $n = 11$ for *Tcf7l2^{F/F};+Adp-Cre* at age 6 months) (B) GTT and ITT for *Tcf7l2^{F/F}* and *Tcf7l2^{F/F};+Adp-Cre* mice on control and HFD for 3 days. (males $n = 9$ for controls and $n = 11$ for *Tcf7l2^{F/F};+Adp-Cre* at age 3 months) (C) AUC for GTT, fasting insulin, and HOMA IR. (D) gene expression in iWAT and eWAT of adipokines and *Glut4*, gene expression normalized to *Rps3* expression. (males $n = 9$ for controls and $n = 11$ for *Tcf7l2^{F/F};+Adp-Cre* at age 6 months) P-values were determined using ANOVA or student T-test, * $p < 0.05$.

state (Figure 8C). Utilizing the ChIP-seq data from the isolated *Tcf7l2^{F/F}* adipocytes, we were able to observe TCF7L2 binding in close proximity to the genes that encode *Tgh* and *Tgh2* (Supplemental Figure 8A). To test whether response to a lipolytic stimulus was impaired with the loss of *Tcf7l2*, mice were administered a single dose of CL-316,243, and glycerol was measured in the blood. We found a 1.5 fold reduction in glycerol levels in *Tcf7l2^{F/F}*; +Adp-Cre mice when compared to *Tcf7l2^{F/F}* controls (Figure 8D). Together, these findings suggest that TCF7L2 regulates lipolysis and directly regulates *Tgh* and *Tgh2* expression.

4. DISCUSSION

One of the strongest genetic predispositions for type 2 diabetes in humans is a variant of the *Tcf7l2* gene [7]. Polymorphisms in *Tcf7l2* have also been linked to body weight, triglyceride metabolism, and gestational diabetes [152–154]. In this study, we report that the Wnt-effector TCF7L2 is enriched in both human and mouse white adipose tissue and its expression is reduced in white adipose tissue of both genetic and diet-induced models of obesity. The conditional deletion of TCF7L2 in adipocytes leads to impaired glucose metabolism, insulin resistance, altered adipokine and GLUT4 expression, and adipocyte hypertrophy on a high-fat diet. Additionally, loss of TCF7L2 in pre-adipocytes leads to enhanced differentiation and lipid accumulation. Genome-wide analysis of TCF7L2 binding suggests a direct role in regulation of genes involved in both lipogenesis and lipolysis, an outcome that likely results in adipocyte hypertrophy. Knockout of TCF7L2 in adipocytes led to reduced expression of triglyceride hydrolases (*Tgh*)1 and 2 as well as increased expression in lipogenic genes. The *in vivo* impact of this impaired lipid processing was observed in adipose specific TCF7L2 knockout mice during fasting, where the knockout mice had reduced weight loss and reduced serum free fatty acid levels compared to the control. These studies underscore a direct role for TCF7L2 in the regulation of glucose and lipid metabolism in the mature adipocyte.

Mechanisms that lead to activation of Wnt-signaling have been shown to block differentiation of adipocytes [139]. While loss of function studies targeting β -catenin in preadipocytes show enhanced adipocyte differentiation [155]. Similarly, expression of a dominant negative form of TCF7L2 in fibroblasts results in enhanced adipocyte differentiation [139]. Our findings are consistent with these previous studies where we observed that the loss of *Tcf7l2* in preadipocytes led to enhanced differentiation and lipid accumulation. Combined ChIP-seq and RNA-seq analysis shows that loss of TCF7L2 led to an induction of genes that are enriched in metabolic pathways such as the TCA cycle, respiratory electron transport chain, lipid and triacylglycerol metabolism. Downregulated genes were highly enriched in pathways that are involved in proliferation such as cell cycle, DNA replication, and translation. These data suggest that the block in adipocyte differentiation likely occurs through direct regulation of transcriptional targets involved in proliferation and a shift in metabolism. Perhaps a shift in the metabolic program that favors a switch from glycolysis towards oxidative respiration [156]. However, our findings are in contrast with recently published results from Chen et al. (2018) in which knockdown of *Tcf7l2* led to impaired adipocyte differentiation [157]. This may be due to the use of different genetic models, they employed shRNA technology in 3T3-L1 cells while we employed *Tcf7l2^{F/F}* pre-adipocytes engineered with retroviral expression of tamoxifen inducible Cre recombinase, CreERT2. These two genetic models may differ in the timing of TCF7L2 knockout or knockdown and TCF7L2 may be required for generation of preadipocytes and inhibition of subsequent

differentiation. In other tissues, TCF7L2 is required for the maintenance of Lgr5+ stem cells in the adult small intestine [158]. The essential role of TCF7L2 in maintaining stemness, may be apparent in adipocyte progenitors, an outcome that needs to be further explored. Unlike other anti-adipogenic factors like Wnt10b and Wnt6, TCF7L2 expression is maintained in the mature adipocyte, leading to the possibility that it may have separate roles in adipogenesis and in differentiated adipocytes [159]. Other components of the Wnt signaling pathway have been shown to have roles in both differentiation and in the mature adipocyte, an example of this is found in the TCF/LEF repressor TLE3 which also coactivates PPAR γ [140]. Previous studies on TCF7L2 in the mature adipocyte have focused on assessing the impact of metabolic disease conditions on TCF7L2 expression in adipocytes. Kuzmicki et al.(2011) observed significant reduction in TCF7L2 expression in visceral white adipose tissue in gestational diabetes [160]. However, when corrected for BMI, the changes in expression are no longer apparent, suggesting that adiposity is driving the reduced expression of TCF7L2 in human adipose tissue and not diabetes. In our studies we have observed reduced *Tcf7l2* expression during high fat diet and genetic models of obesity in mice on the C57BL6J background. Typically, metabolic studies are often carried out in the inbred strain C57BL6J mice which are somewhat susceptible to obesity. However, one inbred mouse strain does not adequately reflect the genetic diversity seen in human populations, which has thought to be a major reason for poor translation of a substantial number of metabolic studies. The Hybrid Mouse Diversity Panel (HMDP) which consists of a similar variation of SNPs to most human populations were therefore used to assess how natural variation in *Tcf7l2* expression relates to traits such as HOMA-IR, fat mass, liver triglycerides and liver weight. We also observed trends consistent with observations from direct modification of *Tcf7l2* with respect to metabolic phenotypes and hypothesize that we would see similar correlations in diverse human populations.

Our work characterized the role of TCF7L2 in mature adipocytes during obesogenic growth. We pursued these questions with use of *Tcf7l2^{F/F}* mice where cre expression was driven by the adiponectin promoter which is activated post-adipocyte commitment. These adipose specific TCF7L2 knockout mice were phenotyped using both chow and high fat diet. In our studies, loss of TCF7L2 in adipocytes enhanced weight gain associated with high-fat diet and aging. On a high-fat diet loss of TCF7L2 leads to adipocyte hypertrophy, an outcome that may be driven by an increase in lipogenesis and impaired lipolysis. In our ChIP-seq data TCF7L2 was found in close proximity to genes that encode for enzymes involved in lipogenesis. Furthermore, we observed increased lipid accumulation in preadipocytes where *Tcf7l2* was knocked-out before differentiation, suggesting that TCF7L2 normally has an inhibitory role in adipocyte differentiation through direct regulation of lipogenesis. Adipocyte hypertrophy may also be driven by impaired lipolysis. In addition to reduced expression of *Tgh1* and *Tgh2* with the loss of *Tcf7l2*, ChIP-Seq analysis showed TCF7L2 occupancy in the genomic loci that includes the *Ces1* family that contain *Ces1f,d* (*Tgh1* and *Tgh2*). Similar to *TCF7L2^{F/F}*; +Adp-Cre mice, mice lacking TGH develop adipocyte hypertrophy and have lower serum free fatty acid levels, suggesting that decreased TGH expression promotes adipocyte hypertrophy [161]. Although TGH1 and 2 are triglyceride hydrolases, they do not associate with the lipid droplet in adipocytes but are located in the endoplasmic reticulum and they are not activated by adrenergic stimulation such as isoproterenol [162]. In *Drosophila*, there is evidence that activation of wnt-signaling using an *axin* gain of function allele leads to reduced lipid droplet size, which is associated with elevated free fatty acid levels [168]. Based on these observations,

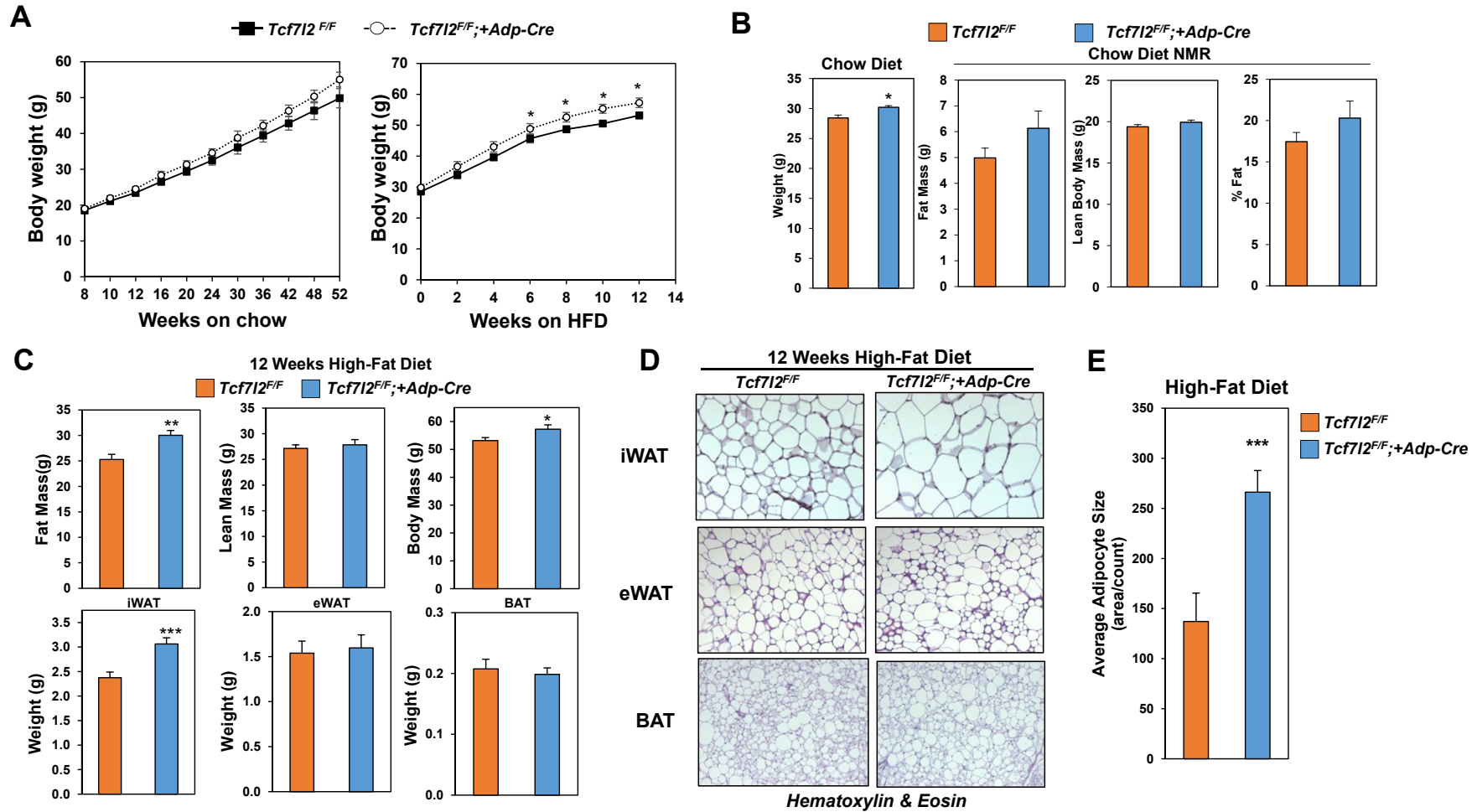


Figure 6: Selective deletion of TCF7L2 in adipocytes promotes weight gain and subcutaneous adipocyte hypertrophy. (A) Body weights of *Tcf712^{F/F}* and *Tcf712^{F/F};+Adp-Cre* mice on Chow (males, n = 6 per group) and HFD (males n = 9 for controls and n = 11 for *Tcf712^{F/F};Adp-Cre* mice at age 6 months). (B) NMR on chow diet (males n = 9 for controls and n = 11 for *Tcf712^{F/F};Adp-Cre* mice at age 3 months). (C) NMR and tissue weight on 12 week HFD. (D) H + E staining of eWAT, iWAT and BAT after 12 weeks HFD. (E) Average adipocyte size in iWAT of mice fed HFD. P-values were determined using Student's t-test, *p < 0.05.

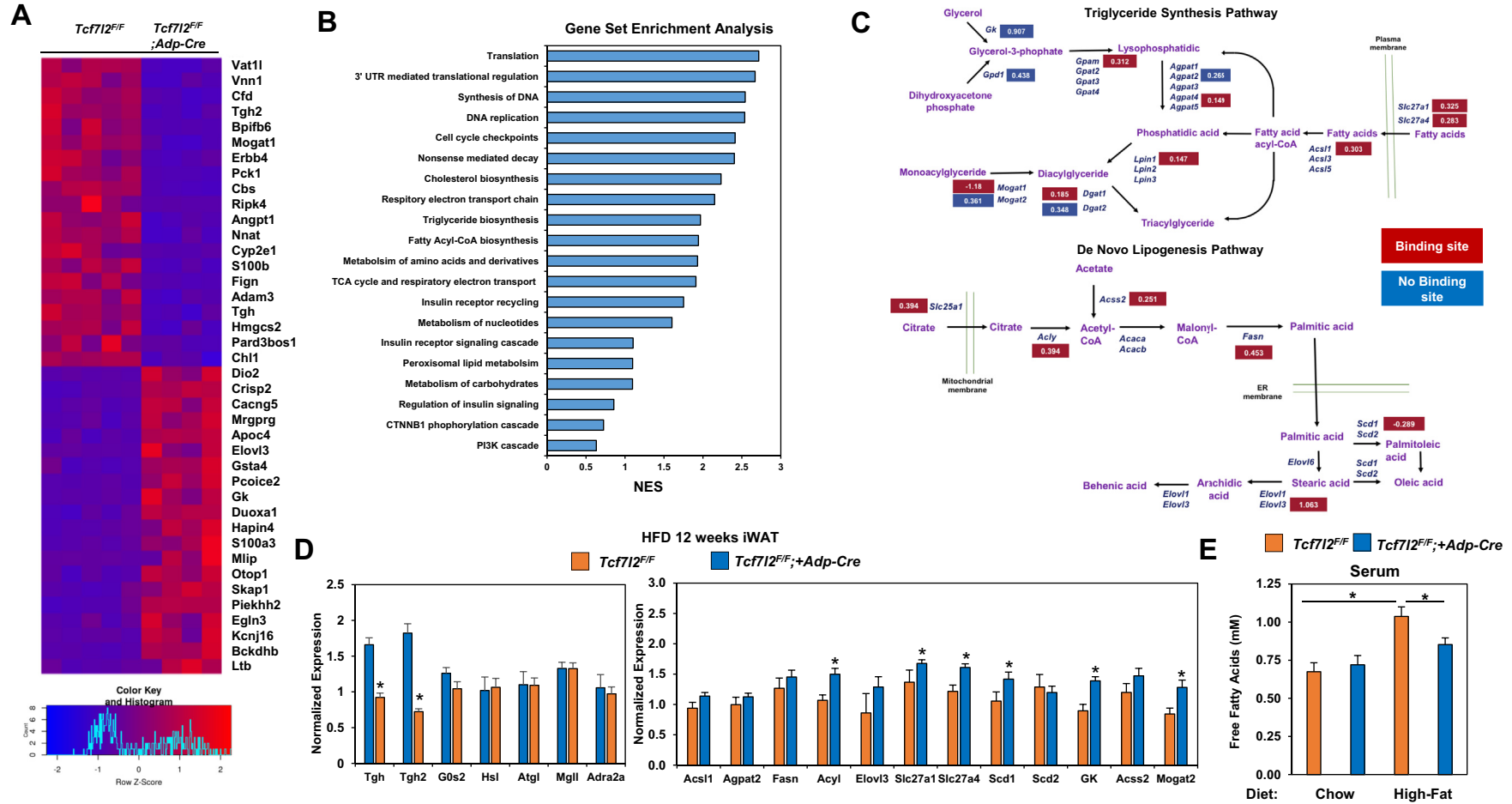


Figure 7: TCF7L2 regulates lipogenic and lipolytic gene expression programs. (A) Heat map of differential gene expression changes in iWAT of *Tcf7l2^{F/F}* and *Tcf7l2^{F/F};+Adp-Cre* mice on 12 week HFD (males, $n = 5$ for control and $n = 4$ for *Tcf7l2^{F/F};+Adp-Cre* mice at age 6 months). (B) Gene Set Enrichment Analysis of gene expression changes in iWAT. (C) Schematic of triglyceride synthesis and *de novo* lipogenesis pathway with gene expression changes from RNA-Seq analysis. Red boxes indicate TCF7L2 occupancy within 1 kb of the promoter, while blue boxes indicate that there is a lack of nearby TCF7L2 binding site. (D) Gene expression of lipolytic and lipogenic genes in iWAT, gene expression normalized to *Rps3* expression. (E) Free fatty acid levels in serum. P-values were determined using student's t-test, * $p < 0.05$.

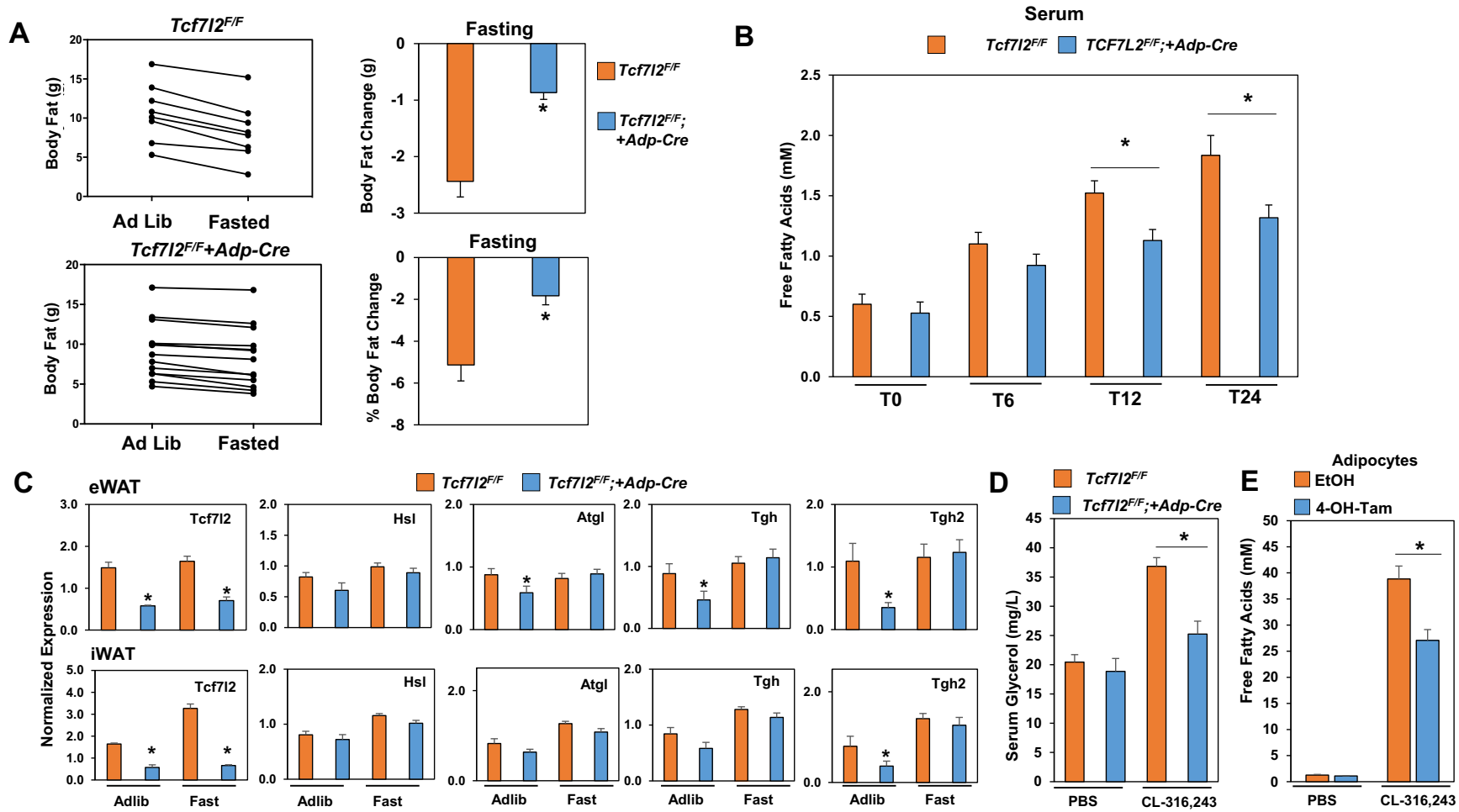


Figure 8: Loss of TCF7L2 leads to impaired adipocyte lipolysis. (A) Body weight of *Tcf7l2^{F/F}* and *Tcf7l2^{F/F};+Adp-Cre* mice fasted for 24 h compares to Ad Lib controls. (B) Fasting time course of serum free fatty acid levels. (C) Gene expression of lipolytic genes in eWAT and iWAT, gene expression normalized to *Rps3* expression. (males $n = 8$ for controls and $n = 12$ for *Tcf7l2^{F/F};+Adp-Cre* mice at age 5 months). (D) *Tcf7l2^{F/F}* and *Tcf7l2^{F/F};+Adp-Cre* were administered 1 mg/kg of CL-316,243 or vehicle control. Serum glycerol was measured 30 min after treatment. (E) Immortalized *Tcf7l2^{F/F}* preadipocytes retrovirally expressing CreERT2 were treated with vehicle or 500 nM 4-hydroxytamoxifen for 24 h 10 days post differentiation. Cells were then treated with either PBS or 100 nM CL316,243 for 4 h and media was measured for concentration of free fatty acids. P-values were determined using Student's t-test or in the case of multiple group comparison an ANOVA with a Tukey Posthoc Analysis, $*p < 0.05$.

Wnt-signaling may have a conserved role in regulating lipolysis. Recent studies on a human prospective cohort of spontaneous weight change, showed reduced adipose tissue lipolysis in subcutaneous white adipose tissue. This showed that disturbed lipolysis increased the risk for impaired glucose metabolism [169]. This leads to the possibility that the changes in adipose tissue lipolysis seen with loss of *Tcf7l2* in adipocytes, may drive the changes in glucose metabolism that we observe. Our data suggest that the Wnt effector, TCF7L2 directly regulates lipid handling through direct inhibition of lipogenic programs and activation of lipolytic gene expression program.

The glucose intolerance and reduced insulin sensitivity that we observed with the loss of TCF7L2 occurred at 3-days and 3-months of high-fat feeding. These findings would suggest that the loss of TCF7L2 manifests early and predisposes mice to diabetes when challenged with a high-fat diet. These findings are consistent with prior observations that adipocytes are key regulators of systemic glucose metabolism. Other components of the Wnt-signaling pathway have been reported to affect glucose metabolism as well. Gain of function studies in transgenic mice overexpressing Wnt10b in adipocytes show protection against high-fat diet induced obesity, and obesity in *ob/ob* and *Agouti* mice [163]. These mice also show improvements in glucose handling [163]. These findings suggest that the reduced levels of TCF7L2 that are observed in white adipose tissue in genetic and diet-induced models of obesity contributes to the development of diabetes.

One mechanism through which TCF7L2 in the adipocyte could be contributing to the development of type 2 diabetes is through regulation of adipokine expression. Adipokines released from adipocytes have pleiotropic effects on distant tissues to ultimately regulate glucose, lipid, and energy balance. Adipokines like adiponin are reduced in genetic and diet-induced models of obesity, and, when levels are restored to normal, there are whole body improvements in glucose control. Recently adiponin was shown to improve β -cell function in diabetes by enhancing glucose stimulated insulin secretion [137]. In our studies, we found that loss of TCF7L2 in adipocytes leads to reduced expression of adiponin, which in the long-term may lead to impaired β -cell function. These changes were most apparent in inguinal white adipose tissue in high-fat fed mice. It has been well established that TCF7L2 regulates β -cell function and insulin secretion in mice and humans [32,164]. Humans who carry the T risk allele rs7903146 have increased expression of Tcf7L2 in the pancreas and have a higher proinsulin to insulin ratio in their plasma resulting from higher expression of proinsulin processing genes [165]. Previous studies observed no changes in Tcf7L2 expression in adipose tissue in humans carrying risk T risk allele rs7903146 but only a subset of Tcf7L2 transcripts were assayed [166]. More recent studies have found reduced expression of some isoforms in subcutaneous adipose tissue that also inversely correlated with BMI in non-carriers [167]. Although Tcf7L2 seems to have a direct role in β -cell function and insulin secretion, our studies suggest that TCF7L2 activity in adipose tissue also may affect pancreatic β -cells indirectly through adiponin.

Our work describes the functional role of TCF7L2 in adipogenesis, mature adipocytes, and in obesogenic growth. We observed that the loss of TCF7L2 in adipocytes leads to glucose intolerance and insulin resistance. Adipose specific knockout of *Tcf7l2*, led to adipocyte hypertrophy that was accompanied by impaired lipolysis in response to fasting. This inability to mobilize the triglyceride pool is likely a driving factor leading to adipocyte hypertrophy. Developing therapies that modulate TCF7L2 expression may provide opportunities to treat type 2 diabetes in individuals that carry a risk allele in the TCF7L2 locus.

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CONFLICT OF INTEREST

No conflict of interests to report.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.molmet.2019.03.003>.

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