# A genome-wide ATLAS of liver chromatin architecture reveals that sex dictates diet-induced nucleosome dynamics

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**Abstract**

The three-dimensional organization of the genome plays an important role in cellular function. Alterations between open and closed chromatin states contributes to DNA binding, collaborative transcriptional activities and informs post-transcriptional processing. The liver orchestrates systemic metabolic control and has the ability to mount a rapid adaptive response to environmental challenges. We interrogated the chromatin architecture in liver under different dietary cues. Using ATAC-seq, we mapped over 120,000 nucleosome peaks, revealing a remarkably preserved hepatic chromatin landscape across feeding conditions. Stringent analysis of nucleosome rearrangements in response to diet revealed sex as the dominant factor segregating changes in chromatin accessibility. A lipid-rich diet led to a more accessible chromatin confirmation at promoter regions in female mice along with enrichment of promoter binding CCAAT-binding domain proteins. Male liver exhibited stronger binding for nutrient sensing nuclear receptors. Integrative analysis with gene expression corroborated a role for chromatin states in informing functional differences in metabolic traits. We distinguished the impact of gonadal sex and chromosomal sex as determinants of chromatin modulation by diet using the Four Core Genotypes mouse model. Our data provide mechanistic evidence underlying the regulation for the critical sex-dimorphic GWAS gene, *Pnpla3*. In summary, we provide a comprehensive epigenetic resource in murine liver that uncovers the complexity of chromatin dynamics in response to diet and sex.

**Keywords:** chromatin accessibility, sex differences, transcription factor

## Introduction

An important way that cells control transcript biogenesis and crosstalk with other steps in gene regulation is through epigenetic checkpoints (*1*). DNA is typically stored in highly condensed structures known as nucleosomes which then congregate to form chromatin (*2*). Chromatin can convert from an active to inactive state through a variety of mechanisms (*3*). Chemical modifications on DNA or histone proteins, for example, can alter the three-dimensional chromatin structure and nucleosome positioning (*4, 5*). Since rearrangements in nucleosomes often precede transcription factor binding or activation (*6, 7*), information about nucleosome position can be used to infer active regulatory regions. Genome-wide interrogation of accessible chromatin regions grew in popularity with the development of ATAC-seq (*8, 9*). This robust technique leverages a Tn5 enzyme that permits the determination of a genome-wide epigenetic map. Despite an increase in ATAC-seq datasets that describe spatial organization of chromatin states in tissues under basal conditions or during development (*10-12*), our understanding of how chromatin rearrangements inform metabolic health and disease states remains limited. In particular, few studies have carefully examined sex-differences in nucleosome positioning in response to dietary cues.

The liver is an important metabolic center that transduces signals from the diet to influence gene expression and metabolism (*13, 14*). For example, high fat diet feeding imparts unique perturbations in liver metabolism to accommodate the intake of excess calories and high fat content. Similarly, a diet rich in cholesterol leads to maladaptive changes in liver, but not precisely the same as high fat diet feeding (*15-18*). Both high fat and cholesterol-rich diets can increase the risk of Metabolic Associated Fatty Liver Disease (MAFLD) (*19-21*).

Besides diet, it is well established that many metabolic traits, including MAFLD, are influenced by biological sex (*22-24*). For example, there are known differences in hepatic lipid composition between males and females at baseline and after feeding lipid-rich diets (*16, 24*). Mediators that are proposed to confer sex differences in hepatic gene expression and metabolism include the estrogen receptor, growth hormone, X-chromosome factors, and the BCL6-STAT5 axis (*16, 25*). Relatively little is known about the interaction between sex and diet at the level of chromatin organization and transcriptional dynamics. Here, we present an unbiased analysis of transcriptional regulation by diet and sex determinants, which has uncovered previously unappreciated relationships.

One level of control by which the liver responds to different challenges is by regulating the chromatin landscape. Changes in chromatin can lay the foundation for transcription or interface with pathways involved in transcript splicing, modification, and export (*26-30*). To what extent does the chromatin environment in the liver change with different cues, and how does it contribute to gene expression and function? In this study, we map the chromatin profile in mouse liver and show that sex primes the chromatin landscape in liver and biases responses to lipid-rich diet. Using an example a of disease-relevant gene, *Pnpla3*, we reveal the molecular underpinning of sex-differences in MAFLD that consolidates well with known clinical observations.

## Results

### Chromatin accessibility profiling in liver is dictated by sex at promoter regions.

To better understand how chromatin rearrangements are informed by diet composition we performed ATAC-seq in liver of male and female mice fed with chow, western diet (WD, rich in

cholesterol) or high fat diet (HFD, low cholesterol but rich in fat content) for 2 weeks (Fig. 1A). Across conditions, we mapped a total of 122,587 unique peaks (Fig. 1B) (Table S1), the majority of which are located in Intergenic (49 %) or Intronic DNA regions (38 %), with 7 % of peaks located in “promoter-TSS” regions (Fig. 1B). Principal component analysis–a dimensionality technique that explores contributors to variance–of liver ATAC-seq showed that chow-fed female and male had largely similar profiles for principal component PC1 and PC2 (Fig.1C). PC1 captured >98% of variance observed across conditions. Interestingly, WD or HFD feeding induced completely distinct responses on chromatin landscape with respect to sex. Feeding of HFD or WD further segregated changes in chromatin landscape with WD leading to more dramatic differences across PC1. This result revealed that hepatic chromatin accessibility differences in response to dietary lipid excess are orchestrated in a highly sex-biased manner.

Detailed analyses indicated that females are more responsive than males to diet-induced changes in chromatin access. While most peaks did not change access in response to diet (constitutive peaks), females exhibited both more loss and gain of accessible chromatin sites compared to males (Fig. 1D). The percentage of differential chromatin changes (DCC) resulting from WD and HFD did not reveal any sex-specific differences in genomic localization (Fig. 1E). Peak distribution analysis revealed that sex-induced chromatin differences cluster around the TSS region, with comparable distribution for both sexes in other regions (Fig. 1F, G, fig. S1A, S1B). Compared to chow baseline, WD-fed or HFD-fed female mice showed more differential chromatin changes (DCC), for both gain and loss of access sites, at promoters compared to WD-fed or HFD-fed male mice (Fig. 1H, fig. S1C). Collectively, our findings suggest male and female mice exhibit distinct changes in chromatin rearrangements, particularly at promotor regions, in response to a hyperlipidemic diet.

**Motif analysis reveals a sex-biased TF preference in response to diet challenges.**

We next analyzed motif preferences using the DCCs from male and female mice to decipher the transcription factors involved in the sex-specific chromatin regulation. Since sex bias was noted in chromatin accessibly at promoter regions, we ranked all promoter-specific differential peaks based on changes in accessibility (HFD vs chow diet, or WD vs chow diet) in both sexes and binned them into equal-sized bins (six bins each), followed by motif enrichment analysis (see Methods for details). This strategy allows us to compare the enrichment of each motif across all bins using a significance enrichment score (Fig. 2A). While most motifs exhibited similar enrichment between males and females, some motifs showed a sex-biased enrichment pattern for certain bins or groups of genes, including ETS family transcription factors (TFs) (Elk4, ETS1, EVT4, GABPA) as well as the promoter-binding transcription factor NFY (Fig. 2A). While enriched in both male and female mice, NFY showed differential enrichment between males and females in the HFD and WD group (Fig. 2B). Among the top 10 enriched motifs from each group, 9 were found to be commonly enriched (Fig. 2C). While stronger enrichment was observed for certain transcription factors in males (KLF3, KLF5, Elk1, Elk4, and Sp5), NFY emerged as a strongly enriched transcription factor in females (Fig. 2D). Collectively, these results suggest that binding patterns of most TFs at promotor regions respond similarly to diet in both sexes, but there is sex bias in some groups of TFs, including NFY, that are known to be directly involved in regulating chromatin access and collaborative TF binding.

To further disentangle the factors that led to sex-specific chromatin responses to hyperlipidemia at a genome-wide scale, we separated signatures of male-biased and female-biased DCCs (Fig. 3A): C1 and C8 quadrants (C1&C8) represent male-specific enhanced chromatin opening in response to WD (group I); C5&C6 represent the female-specific enhanced chromatin opening in response to WD (group II); C4&C5 represent the male-specific reduced chromatin opening in

response to WD (group III), and C1&C2 represent the female-specific reduced chromatin opening in response to WD (group IV). Motif analysis of the top or bottom 100 peaks for each sex (Fig. 3B) revealed distinct positional bias for each group (Fig. 3C). For example, Cux2 was specifically enriched in “female loss” of accessibility cluster (group IV), consistent with previous reports showing that Cux2 tends to be more enriched at the genomic region exhibiting male-higher accessible chromatin than in female mouse liver (*31*). Female-biased clusters also showed enrichment of estrogen receptor motifs, as expected. The “male gain” (group I) and “female loss” (group IV) peaks preferentially exhibited enrichment of motifs of well-known nutrient-sensing nuclear receptor family members such as LXRE, RXRE and PPARE, and the “female gain” (group II) and “male loss” (group III) peaks specifically enriched the motif of Zinc Finger family (Zf), ETS family and NFY (CCAAT) (Fig. 3C). Notably, the top enriched motif for “male gain” of chromatin access is LXRE while the top enriched motif for “female gain” of chromatin access is NFY (Fig. 3D).

To focus on the motifs that show the greatest sex bias, we reanalyzed the motifs using quadrants C1, C3, C5 and C7 in Fig. 3A, representing concordant sex responses (“male & female gain” and “male & female loss”) or divergent sex responses (“male gain & female loss” and “female gain & male loss”). Once again, the results demonstrate that LXRE was more strongly enriched in “male gain & female loss” group (C1), while NFY was preferentially enriched in the “female gain & male loss” group (C5) (Fig. 3E). Taken together, these results hint that in response to diet feeding, males may be more sensitive to chromatin rearrangements involving nutrient-sensing transcription factors whereas females are more sensitive to promotor-binding factors such as NFY.

**Nucleosome dynamics of sex-specific transcription factors are associated with distinct transcriptomic responses.**

To gain insight into the consequences of the hepatic chromatin accessibility/TF bias between males and females in driving differential gene expression, we determined the hepatic transcriptome differences between male and female mice responding to 2 or 4 weeks of WD or HFD feeding compared to chow (Fig. 4A). Consistent with ATAC-seq results, RNA-seq revealed that WD and HFD induced or suppressed gene expression in similar patterns (Supplemental Fig. 4A). While the majority of genes were unchanged with a lipid-rich challenge, there were approximately 2,000 differentially expressed genes (DEGs) in response to WD and HFD (fig. S4B and S4C) (Table S2). The vast majority of these DEGs (~80%) exhibited sex-biased expression based on fold changes and P-values (described in Materials and Methods) (Fig. 4B, C). This observation is in line with other evidence showing that a significant proportion of hepatic genes show sex-biased regulation (*13*). To decipher sex-specific DEGs, we compared the female and male DEGs from 2 and 4 weeks lipid-rich dietary challenge (fig. S4D) and functionally annotated these genes by Metascape analysis (*32*). Shared DEGs between males and females showed “Metabolism of lipid” as the top enriched pathway (fig. S4E). Female- and male-specific DEGs in response to diet exhibited differences in pathway enrichment. Male DEGs showed enrichment of metabolic pathway changes whereas female DEGs showed stronger changes in cell migration and MCM (minichromosome maintenance) remodeling (fig. S4F-I).

To explore the relationship of genes with sex-specific differential expression to chromatin accessibility changes, we overlapped DEGs from male or female RNA-seq with female or male ATAC-seq changes (Fig. 4D and 4E). Clustering of gene expression by integrating ATAC-seq showed that WD and HFD feeding have similar overall effects on gene expression (Fig. 4F). However, both WD and HF feeding showed divergent gene expression patterns with respect to

feeding time (Fig. 4F). Furthermore, based on gene expression patterns, we predicted the top enriched motifs of male-biased and female-biased genes (fig. S2J). Our analysis identified enrichment of androgen receptor (AR) motif among male-biased genes with low enrichment in females. Reciprocally, estrogen receptor (ESR1) was strongly enriched among female-biased genes but not males. These results align well with known hormonal regulation of hepatic gene expression in the two sexes. Unlike our ATAC-seq analysis, which showed higher LXR enrichment in males (Fig. 3E), LXR was enriched in both male and female clusters based on transcriptomics (fig. S2J). Thus, undertaking an RNA-centric analysis strategy reinforced most but not all of the sex-biased results of chromatin dynamics. These results hint that additional layers in gene regulation beyond transcription and epigenetic control (e.g., transcript stability, export, and processing) may contribute to steady-state transcriptome levels.

**Sex-biased diet responsive genes are influenced by gonadal and chromosomal sex**

There are two primary determinants of biological sex—sex chromosomes (XX or XY) and gonads (ovaries or testes and hormones secreted by these tissues) (*33-35*). To further investigate the role of chromosomal and gonadal sex determinants in sex-biased gene expression in response to a HF diet, we employed the Four Core Genotypes (FCG) mouse model, which includes four sex genotypes (XX with ovaries, XX with testes, XY with ovaries, XY with testes) (Fig. 4G) (*25, 36-39*). We assessed the determinants of gene expression for the 148 sex-biased genes shown in Fig. 4F in FCG mice having intact gonads or after surgical removal of gonads (GDX) from adult mice followed by HF diet feeding. About 80% genes that responded to lipid-enriched diets in a sex-biased manner in standard male and female mice were also regulated by a sex determinant (gonadal sex, chromosomal sex, or both) in the FCG model (Fig. S2K and S2L) (Table S3).

Gonadal status emerged as the most prevalent determinant of the sex bias, although many genes were influenced by both gonadal and chromosomal sex (Fig. 4H, fig S2L, Table S3). Cluster 1 included genes whose expression was diminished when acute gonadal hormones were depleted by removal of gonads, while Cluster 2 consisted of genes inhibited by gonadal secretions, as their expression was enhanced following GDX (Fig. 4H). Within these groups, many genes were influenced by chromosomal sex, with clear patterns of greater expression in XX compared to XY liver (for example, cluster 2a, XX>XY in GDX mice), or greater expression in XY compared to XX liver (for example, cluster 1b in intact mice and cluster 2c in GDX mice). These findings reveal the complex regulation of sex-biased diet response genes by both genetic and hormonal sex determinants. In many cases, the regulation by sex chromosome genotype is more apparent after gonadal hormones are removed, suggesting that sex chromosome complement may be an important determinant of diet response under conditions of reduced gonadal hormone levels, such as following menopause and andropause in humans. In summary, integrating hepatic ATAC-seq and RNA-seq under different conditions shows overlap in sex-biased signatures and suggests, at least in part, changes in chromatin dynamics under different conditions are functionally relevant and inform gene expression changes.

**Chromatin architecture informs mechanisms of sex differences at disease relevant genes.**

To more carefully interrogate how diet composition related to sex-differences in gene expression we stringently defined female-biased and male-biased genes as outlined in our methods. We ultimately identified 25 female-biased DEGs and 32 male-biased DEGs (Fig 5A). Many of these genes showed sex-biased expression in human liver (fig. S3A-S3C) (Table S4). Notably, Patatin-like phospholipase domain-containing protein 3 (*Pnpla3*) emerged as one of the top

female-biased in both mouse and human liver (Fig. 5A, fig. S3B and S3D). Hepatic *PNPLA3* is of substantial interest because variants at *PNPLA3* have been associated with risk of MAFLD (*40-43*). We obtained consistent results by analyzing public RNA-sequencing datasets (*44*), which suggested that *Pnpla3* is one of the top-ranked female-biased genes (Fig. 5B). We confirmed the female-biased expression of *Pnpla3* by qRT-PCR on liver samples from mice fed different diets (Fig. 5C). Notably, 16 of the female-biased genes we identified overlapped with those from previously published RNA-sequencing datasets (*44*), with *Pnpla3* showing the greatest difference between males and females (fig. S3E and S3F).

*Pnpla3* is known to be impacted by LXR, and our analysis showed that LXR chromatin dynamics differs between the sexes, although these LXR changes minimally inform global transcriptome signatures. To carefully examine a role for LXR in underlying sex-differences in hepatic *Pnpla3*, we employed a variety of approached. We assessed changes in canonical LXR target genes during physiologic perturbations. RNA-seq under different dietary conditions showed no major sex differences in the expression of LXR target genes except for *Scd1* under certain conditions (fig. S3G). In addition, we orally administered the LXR agonist GW3965 to male and female mice and examined gene expression by qRCR. LXR targets were induced with GW3965, but we did not observe substantial differences in LXR gene regulation comparing male and female liver (fig. S3H). Collectively, these results suggest that there may be subtle differences at select LXR targets, but response to LXR agonist showed no major 'global' sex effect (fig. S3G). However, even with maximal LXR activation, *Pnpla3* was confirmed to be a female-biased gene by qPCR (Fig. 5D). Collectively, our data suggest that LXR regulates *Pnpla3* expression, but is not a critical factor in sex-differences in its expression.

Prior work reported that PNPLA3 underlies sex-differences in MAFLD (*45*). A recent study reported higher hepatic expression of *PNPLA3* in female liver may be driven by estrogen receptor-α (ER-α) (*46*). We examined the chromatin architecture at the *Pnpla3* locus in male and female liver. We identified a cluster of accessible regions at the *Pnpla3* promoter region extending into the gene body (red, Fig. 5E) and another upstream region (blue). The upstream region (blue) was near estrogen receptor binding sites and showed minimal regulation between the sexes. This led us to reexamine a role for estrogen receptor in dictating the sex bias in *Pnpla3* expression. We treated wild-type male and female mice with an adeno-associated virus (AAV) under control of a TBG promoter, expressing either ER-α (AAV-TBG-ESRa) or GFP (AAV-TBG-GFP). We confirmed robust overexpression by immunoblotting as well as the enhanced expression of *Polg1*, an ER-α regulated gene we previously demonstrated (Fig. 5F, fig. S4A). *Pnpla3* expression was measured by qPCR, and no significant difference was observed between AAV-GFP and AAV-ESRa in either males or females (Fig. 5G), hinting that estrogen may not be the dominant factor contributing to sex-dependent differences in the regulation of *Pnpla3*. We evaluated *Pnpla3* expression using the FCG mouse model, comparing the effects of sex chromosomes, gonadal status, and ovary/testes-specific influences (Fig. 5H). Expression levels were higher in the genotypes with ovaries compared to those with testes (Fig. 5I), with no significant difference between the XX chromosome and XY chromosome groups (Fig. 5J). We further investigated which gonadal factor regulated *Pnpla3* expression. Removal of testes led to increased *Pnpla3* expression (Fig. 5K), whereas removal of ovaries had no effect. (Fig. 5L). Overall, these results suggested that *Pnpla3* was suppressed by testosterone rather than regulated by ovarian hormones.

The accessible region at promotors at *Pnpla3* showed strong regulation between male and females fed different diets (Fig 5E, red). Notably, accessibility at the *Pnpla3* promoter was dramatically reduced in male mice (Fig. 5E). PROMO (*47, 48*), a method that constructs positional weight matrices from known transcription factor binding sites for a given gene,

supports the idea there are potential hotspots around the promoter region of *Pnpla3* that may underlie differences in expression across conditions (fig. S4B). Interestingly, the promoter accessible region overlapped with key histone marks that may gate chromatin access (Fig. 5E). Females also had higher expression of activating chromatin signature H3K27ac (fig. S4C). H3K27ac ChIP analysis in female liver revealed stronger binding of H3K27ac at the *Pnpla3* locus compared to males (fig. S4D). The balance or activating and repressive histone marks can be orchestrated by chromatin modifying factors Ezh1 and Ezh2. Ezh1 and Ezh2 have been linked with sex-biased regulation and gonadal hormone balance (*40, 44, 49*), which led to us hypothesize that Ezh proteins could contribute to differences in *Pnpla3* expression between the sexes. Double knockout of Ezh1/2 (DKO) in liver resulted reduced differences in *Pnpla3* expression between the sexes (Fig. 5M). Collectively, these data suggests that that histone modifying factors that gate promoter regions contribute to sex-differences in regulation of *Pnpla3*.

## Discussion

Our data provides a comprehensive catalog of changes in chromatin dynamics between the sexes under different dietary conditions. Selective gene regulation is strongly influenced by the epigenetic landscape (*50-52*). Therefore, mapping nucleosomes dynamics across conditions can be used to infer critical transcriptional regulators and determine their cooperative and hierarchical interactions. Our analysis pointed to a number of unexpected findings. First, chromatin accessibility differed by diet composition by was dominantly informed by sex. Second, feeding a western or high fat resulted in greater differences in the chromatin landscape between the sexes. The differences were particularly more pronounced at promotor regions. While we observed that sex was the pivotal driver of chromatin responses, we do point out that our dietary perturbations were only for 2 weeks. It possible that longer diet feeding would have shown different results. However, we doubt that this would be the case since we found minimal differences in gene expression in liver comparing mice at 2 and 4 weeks. Other studies found that genetic control of metabolic response to dietary challenge is most pronounced within the first 2 weeks (*53*), hence the focus on early timepoints.

Multiple motifs showed common enrichment between male and female liver, but our analysis also identified critical differences in nucleosome binding patterns. For example, females had stronger enrichment of promotor binding factors, like the trimeric complex NFY and ETS transcription factors. These results were corroborated by gene expression integration and align with our observation that female livers had more accessible promoter regions. Male liver showed more extensive nucleosome rearrangements related to nuclear receptor pathways. As expected, we observed differences in established hormonal pathways known to differ between the sexes such as estrogen receptor and androgen receptor.

Integrating chromatin dynamics and gene expression revealed substantial overlap in sex-biased signatures and provides evidence that nucleosome rearrangements inform gene expression changes. However, this was not the case for every transcription factor. For example, LXR-dependent chromatin changes between the sexes were pronounced, yet we observed minimal differences in LXR-dependent gene expression. It is possible there are differences in LXR binding patterns or activity between the sexes but mechanisms beyond RNA biogenesis could dictate steady state transcript levels. For example, change in mRNA stability, export or translation efficiency between the sexes is likely important.

Our unsupervised analysis of sex biased genes highlighted a number of critical factors involved in lipid metabolism and lipid droplet regulation. *PNPLA3* is one of the few genes consistently implicated in MAFLD GWAS (*40-43*). Using epidemiologic and molecular studies, recent evidence suggested that regulation of *PNPLA3* by estrogen contributes to the rise in MAFLD in older females (*46*). These studies do not consolidate well with known changes in estrogen levels that occur during menopause. Examination of chromatin dynamics at *Pnpla3*, corroborate our overarching findings that changes at promoters appear to underlie differences in hepatic expression of *PNPLA3* between males and females. Integrating our findings with public datasets, we use knockout and overexpression models to show that sex differences in PNPLA3 transcript levels are regulated by male gonadal factors and histone modifiers acting at promoters, rather than estrogen. Our work does not entirely rule out a role for estrogen signaling in *PNPLA3* regulation, but leveraging a 'chromatin-centric' platform suggests that other factors may be contributing. Future experiments can more thoroughly interrogate the molecular pathways that regulate sex-differences in *PNPLA3* expression. In summary, we provide a resource that can used to uncover mechanisms of gene regulation in hepatic response to lipid excess between the sexes.

## Materials and methods

### Animals and diet

All animal experiments were approved by the UCLA Animal Research Committee and strictly adhered to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health. The mice used in this study were of the C57BL/6 background and housed in a temperature-controlled room at 22°C with 50–65% humidity, under a 12-hour light/dark cycle in pathogen-free conditions. Wild-type mice (strain 000664) were obtained from The Jackson Laboratory and were fed ad libitum on either a standard chow diet (PicoLab Rodent Diet 20, 5053), Western diet (WD: Research Diets, D12079B), or high-fat diet (HFD: Research Diets, D12492). Four Core Genotypes mice on a C57BL/6 congenic background from a UCLA maintained colony were used as we described previously (*52, 53*). These mice have a deletion of the testis determining gene Sry on the Y chromosome and insertion of Sry transgene onto an autosomal chromosome, which allows for independent segregation of gonad development and sex chromosome complement (*54*). For the ATAC-seq experiments, mice were subjected to diet treatments for 2 weeks, while RNA-seq treatments lasted either 2 or 4 weeks. For the FCG mice experiment, gonadectomy was performed at 10-11 weeks of age under isoflurane anesthesia by aseptic procedures as previously described(*55*). FCG mice were fed ad libitum a high-fat diet for 10 weeks (intact gonad cohort) or 16 weeks (gonadectomized cohort). Prior to sacrifice, mice were fasted for 6 hours, unless specified otherwise, such as in the LXR agonist treatment experiment. Liver tissues were harvested, flash-frozen in liquid nitrogen, and stored at -80°C for subsequent analysis.

### Adeno-associated virus (AAV) transduction

For AAV-mediated ESR overexpression, eight-week-old male or female C57BL/6J wild-type mice received intravenous injections of AAV8.TBG.GFP or AAV8.TBG.ESRa (purchased from VectorBiolabs) at a dose of $1 \times 10^{11}$ GC per mouse, administered via the lateral tail vein. One week after AAV transduction, mice were placed on a HFD for 5 weeks. Mice were sacrificed following a 6-hour fasting period.

### LXR agonist treatment

The LXR agonist treatment experiments in mice were conducted following the protocol described in previous studies (*54, 55*). In brief, 9-week-old mice were administered GW3965 (Sigma) at a dose of 40 mg/kg, given via oral gavage 17 hours and again 8 hours before

sacrifice. Mice were fasted for 4 hours prior to sacrifice. GW3965 was dissolved in dimethyl sulfoxide (DMSO, Sigma) and delivered in canola oil (Sigma). DMSO was used as the vehicle control.

**RNA extraction and qRT-PCR**

Total RNA was extracted using TRIzol reagent (Invitrogen). RNA was reverse transcribed using a homemade reverse transcriptase as described in our previous publication (*56*). Real-time PCR (qRT-PCR) was used to quantify cDNA, utilizing SYBR Green Master Mix (Diagenode) on a BioRad Real-Time PCR instrument. The primers for amplifying *Pnpla3* were sourced from a previous publication (*57*). Primers amplifying *Polg1* was from our previous publication (*58*). Gene expression level was normalized to the housekeeping gene 36B4 or as indicated in Figure Legends.

**Immunoblot analysis**

Proteins were extracted using RIPA lysis buffer (Boston Bioproducts) supplemented with a complete protease inhibitor cocktail (Roche). The protein samples were resolved on a 4–12% NuPAGE Bis-Tris Gel (Invitrogen), transferred to a Hybond ECL membrane (GE Healthcare), and then probed with primary antibodies. The following antibodies were used: anti-PDI (Cell Signaling Technology, 3501, 1:1000 dilution) and anti-Estrogen Receptor α (Sigma-Aldrich, 06-935, 1:1000 dilution).

**ATAC-Sequencing and ATAC-Sequencing analysis**

ATAC-seq using frozen liver samples were performed using our previous optimized protocol (*59*). The number of nuclei in each sample was counted using Trypan Blue (Thermo Fisher) and a hemocytometer, and 75,000 nuclei were extracted and centrifuged at 500 g for 5 min at 4°C and used for the transposition reaction. ATAC-Seq libraries were constructed using the Nextera Tn5 Transposase and DNA library preparation kit (Illumina) as described (*9*). We performed size selection usign AMPure XP magnetic beads. Libraries were sequenced by NovaSeq 6000 (Read Length: 2× 100) at the Broad Stem Cell Research Center Sequencing Core (BSCRC) at UCLA.

Sequenced reads were aligned to the mouse genome mm9 (NCBIv37/mm9) using Bowtie2 (*60*). Duplicated reads or those that mapped to the mitochondrial genome or aligned to unmapped contiguous regions were removed. MACS2 (*61*) was applied for peak calling using the option: –nomodel –keep-dup all -q 0.01 –llocal 10000. We merged the reproducible peak sites in each sample and obtained RPKM by SeqMonk (Babraham Bioinformatics). Bedgraph files were generated by HOMER (*62*) and transformed to TDF by IGV tools and visualized (*63*). Gene annotation was performed by Homer annotation function using the genome location of peaks. Motif enrichment analysis was performed by HOMER Motif Analysis (*62, 64*). Promoter specific motif analysis was performed for Figure 2 and global motif analysis was performed for Figure 3. PCA was performed with R package PCAtools (*65*). Peaks including annotation were provided in supplemental Table S1.

**RNA-Sequencing and RNA-Sequencing analysis**

RNA sequencing was performed as we previously described (*16*). Briefly, total RNA for RNA-sequencing was extracted using TRIzol reagent (Invitrogen) and purified by Qiagen RNeasy Mini Kit (Qiagen). RNA concentration was measured by Nanodrop and sequenced at UCLA Technology Center for Genomics and Bioinformatics (TCGB) using Hiseq3000 (Read Length: 1× 50 bp). RNA-seq analysis was performed as described in our previous publication (*16*). Differential expressed genes (DEGs) in diets challenging experiments were defined as: fold change [(WD or HFD)/Chow] > 1.5 & P-value < 0.05 (for up-regulated genes), and fold change [Chow/ (WD or HFD)] > 1.5 & P-value < 0.05 (for down-regulated genes). FPKM and DEGs

were provided in supplemental Table S2. Ontologies and Transcription analysis were performed by EnrichR (*66-68*), or Metascape (*32*) as described in the results or Figure legends. Venn diagrams were generated by Venny (*69*) or Venn diagram online tool from Bioinformatics & Evolutionary Genomics (https://bioinformatics.psb.ugent.be/webtools/Venn/). Heatmaps were generated by Morpheus (https://software.broadinstitute.org/morpheus) or ClustVis (*70*). For the liver RNA-sequencing data obtained from public datasets, we utilized the processed data generated by Lau-Corna et al. (*44*). Related to Figure 5, sex-specific gene expression was compared by calculating the ratio of FPKM values: "Control female" FPKM divided by "Control male" FPKM. To identify female- or male-biased DEGs, we applied the following criteria: 1). Female-biased DEGs: Fold change (FC) of WD/Chow > 1.2 at both 2 and 4 weeks in females, while FC (WD/Chow) ≤ 1 at least at one time point in males; or FC (Chow/WD) > 1.2 at both 2 and 4 weeks in females, with FC (Chow/WD) ≤ 1 at least at one time point in males. 2). Male-biased DEGs: FC (WD/Chow) > 1.2 at both 2 and 4 weeks in males, while FC (WD/Chow) ≤ 1 at least at one time point in females; or FC (Chow/WD) > 1.2 at both 2 and 4 weeks in males, with FC (Chow/WD) ≤ 1 at least at one time point in females. For the RNA-Sequencing analysis of FCG model, reads were aligned to GRCm39 reference mouse genome and read count per gene quantified with STAR (version 2.7.10a). DEG analysis was performed with DESeq2 (version 1.36) with contrast tests with reduced models to evaluate effect of each variable: gonadal effect (mice with intact ovaries vs. mice with intact testes), chromosomal effect (mice with XX chromosomes vs. mice with XY chromosomes), ovariectomy effect (ovariectomized mice vs. mice with intact ovaries), and castration effect (castrated mice vs. mice with intact testes).

**ChIP-Sequencing analysis**

ChIP-seq from public datasets are as listed in figure legends. We reanalyzed the ChIP-seq using downloaded fastQ files. Reads were aligned to the mouse genome mm9 (NCBIv37/mm9) using Bowtie2 (*60*). MACS2 (*61*) was applied for peak calling. To visualize the peaks, HOMER software was applied to generate bedgraph files, which were then transformed to TDF and visualized by IGV tools (*63, 64*). Gene annotation was performed by SeqMonk annotated to "mRNA" (Babraham Bioinformatics).

**Data availability**

The ATAC-sequencing and RNA-sequencing datasets have been deposited to the National Center for Biotechnology Information and are available at accession number: ATAC-seq: (Pending). RNA-sequencing for diets treatment: (Pending). RNA-Sequencing for FCG mouse model: (Pending).

**Competing interest statement**

The authors declare no competing interests.

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**Footnotes**

This article is accompanied by supplemental material.

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**Figure Legands:**

**Figure 1. Sex segregates liver chromatin accessibility in response to diet.** (**A**) Design of liver ATAC-seq experiments. (**B**) Distribution of genomic features for all peaks. (**C**) PCA plots of chromatin accessibility of liver samples using mean peak score from each group (n=122,587). (**D**) Comparison of the proportion of genes. FWD: female WD peaks compared to female chow peaks; FHFD: female HFD peaks compared to female chow peaks; MWD: male WD peaks compared to male chow peaks; MHFD: male HFD peaks compared to male chow peaks. Peaks with fold change (FC)> 2 were considered 'gain of accessibility' and FC< 0.5 were considered 'loss of accessibility'. (**E**) Distribution of genomic features amount differential peaks (FC> 2 or FC< 0.5) in female or male mice liver. (**F**) and (**G**) Normalized ATAC-seq signal intensity for differential peaks of WD compared to chow ± 3000 nt (F) or ± 500 nt (G) from TSS. (**H**) Number of differential female and male peaks in the Promoter-TSS region according to Homer annotation. Chow: chow diet; WD: western diet; HFD: high fat diet.

**Figure 2. Promoter-binding factors are enriched in female liver.** (**A**) Heatmap of motif accessibility across promoter specific peaks ranked based on accessibility difference between HFD and chow diet samples or WD and chow diet samples, separated to 6 bins for each group. Female and male are calculated separately. Motif analysis performed by Homer. The numbers indicated $-Log_{10}$ (P-value) of each motif. Motifs with maximum $-Log_{10}$ (P-value) > 5 included. (**B**) Heatmap showing maximum $-Log_{10}$ (P-value) of male or female enriched motifs from Fig. 2A. Cluster A represents female-preferred transcription factors. Cluster B represents male-preferred transcription factors. Values indicated $-Log_{10}$ (P-value). (**C**) Venn diagram showing the overlap of the top 10 enriched TFs from each group. (**D**) Bar plot showing the maximum $-Log_{10}$ (P-value) for females ($F^{max}$) minus that for males ($M^{max}$).

**Figure 3. Diet induces sex-divergent motif enrichment in the liver.** (**A**) Correlation analysis of male and female ATAC-seq peaks under WD. Blue lines indicate 1.2-fold change. (**B**) Motif analysis group strategy. (**C**) Enriched motifs and motif families in different group in B as analyzed by Homer. Color in heatmap represents the P-value. (**D**) Top 1 motif from each group from C. (**E**) Top 5 enriched motifs from specific quadrants and their enriched P-value from other quadrants.

**Figure 4. Integrative analysis identifies sex-dimorphic chromatin-gene expression connections in liver tissue.** (**A**) Schematic showing experiment design of RNA-seq in

mice liver with diet feeding. (**B**) and (**C**) Differentially expressed genes (DEGs) in male or female with 2 weeks or 4 weeks of WD (B) or HFD feeding (C). Cutoff: |FC| > 1.5 & P< 0.05. (**D**) DEGs of female in 2 weeks and 4 weeks overlapped with “female hypo” ATAC-seq peaks (Bottom 200 peaks based on female in C1&C2 clusters of Fig. 3A) or “female hyper” ATAC-seq peaks (Top 200 peaks based on female in C5&C6 clusters of Figure 3A). (**E**) DEGs of male in 2 weeks and 4 weeks overlapped with “male hypo” ATAC-seq peaks (Bottom 200 peaks based on male in C4&C5 clusters of Fig. 3A) or “male hyper” ATAC-seq peaks (Top 200 peaks based on male in C1&C8 clusters of Figure 3A). (**F**) Heatmap was generated based on fold changes calculated by RNA-seq RPKM of overlapped genes from Fig. 4D and Fig. 4E. F-2W: female fold changes calculated by WD compared to Chow or HFD compared to Chow at 2-week time point; F-4W: female fold changes calculated by WD compared to Chow or HFD compared to Chow at 4-week time point; M-2W: male fold changes calculated by WD compared to Chow or HFD compared to Chow at 2-week time point; M-4W: male fold changes calculated by WD compared to Chow or HFD compared to Chow at 4-week time point; (**G**) Schematic of the Four Core Genotypes (FCG) mouse model. (**H**) The expression of diet-induced, sex-biased genes, as shown in Fig. 4F, was detected using RNA-seq in the FCG mouse model in (G). Clusters were generated based on CPM. Ov: ovaries, Te: ovaries.

**Figure 5. *Pnpla3* is regulated by male gonads and epigenetic factors**. (**A**) Fold changes of potential sex-biased genes are displayed in the heatmap. The left panel shows female-biased DEGs as described in methods, while the right panel presents male-biased DEGs calculated using the same method as the female-biased DEGs. (**B**) Hepatic sex-specific DEGs were identified by RNA sequencing from Lau-Corona et al., 2020 (detailed in Methods). (**C**) Gene expression of *Pnpla3* detected by qPCR from livers of mice fed with chow, WD or HFD for 2 weeks. n = 6 (Male Chow and male WD); n = 8 (male HFD); n=5 (Female Chow, WD and HFD). Data are represented as mean ± SEM. P-values were calculated by two-way ANOVA. **: P< 0.01; ***: P< 0.001; ns: not significant. (**D**) LXR agonist treatment and gene expression of *Pnpla3* detected by qPCR. n = 8 (Female Vehicle, male Vehicle and male GW3965); n = 7 (Female GW3965). *: P< 0.05 calculated by unpaired t test. (**E**) Peaks of ATAC-seq from this study and ChIP-seq of histone mark from ENCODE project with GEO number: GSM1000153 (H3K9ac); GSM1000140 (H3K27ac). ATAC-seq peaks were overlayed from all 3 samples of each group. (**F**) Schematic of ESRa overexpression by AAV and immunoblotting of ESRa in murine livers. (**G**) Gene expression of *Pnpla3* detected by qPCR from livers of mice from Fig. 5F. N= 5; not significant by either t-test or ANOVA. (**H**) Schematic comparing *Pnpla3* expression using the FCG model. (**I**) Hepatic *Pnpla3* expression in mice with intact ovaries (Ov) or with intact testes (Te). N=12 (Ov), N=11 (Te). *: P< 0.05 by unpaired t-test. (**J**) Hepatic *Pnpla3* level in mice with chromosome XX and XY. N= 23 (XX). N= 24 (XY). ns: P> 0.05 by unpaired t-test. (**K**) Hepatic *Pnpla3* level in mice with intact testes (Te Intact) or with testes GDX (Te GDX). N= 11 (Te Intact). N= 12 (Te GDX). *: P< 0.05 by unpaired t-test. (**L**)Hepatic *Pnpla3* level in mice with intact ovary (Ov Intact) or ovary GDX (Ov GDX). N= 12. ns: P> 0.05 by unpaired t-test. (**M**) Fold change calculated using FPKM of *Pnpla3* from Lau-Corona et al., 2020. Data shown as FPKM relative to Female control. DKO: Ezh1 and Ezh2 double-KO.

**Figure S1. ATAC-seq in mice liver (Related to Figure 1).** (**A**) and (**B**) Normalized ATAC-seq signal intensity for differential peaks of HFD compared to chow ± 3000 nt (A) or ± 500 nt (B) from TSS. (**C**). Number of differential female and male peaks in Promoter-TSS region according to Homer annotation.

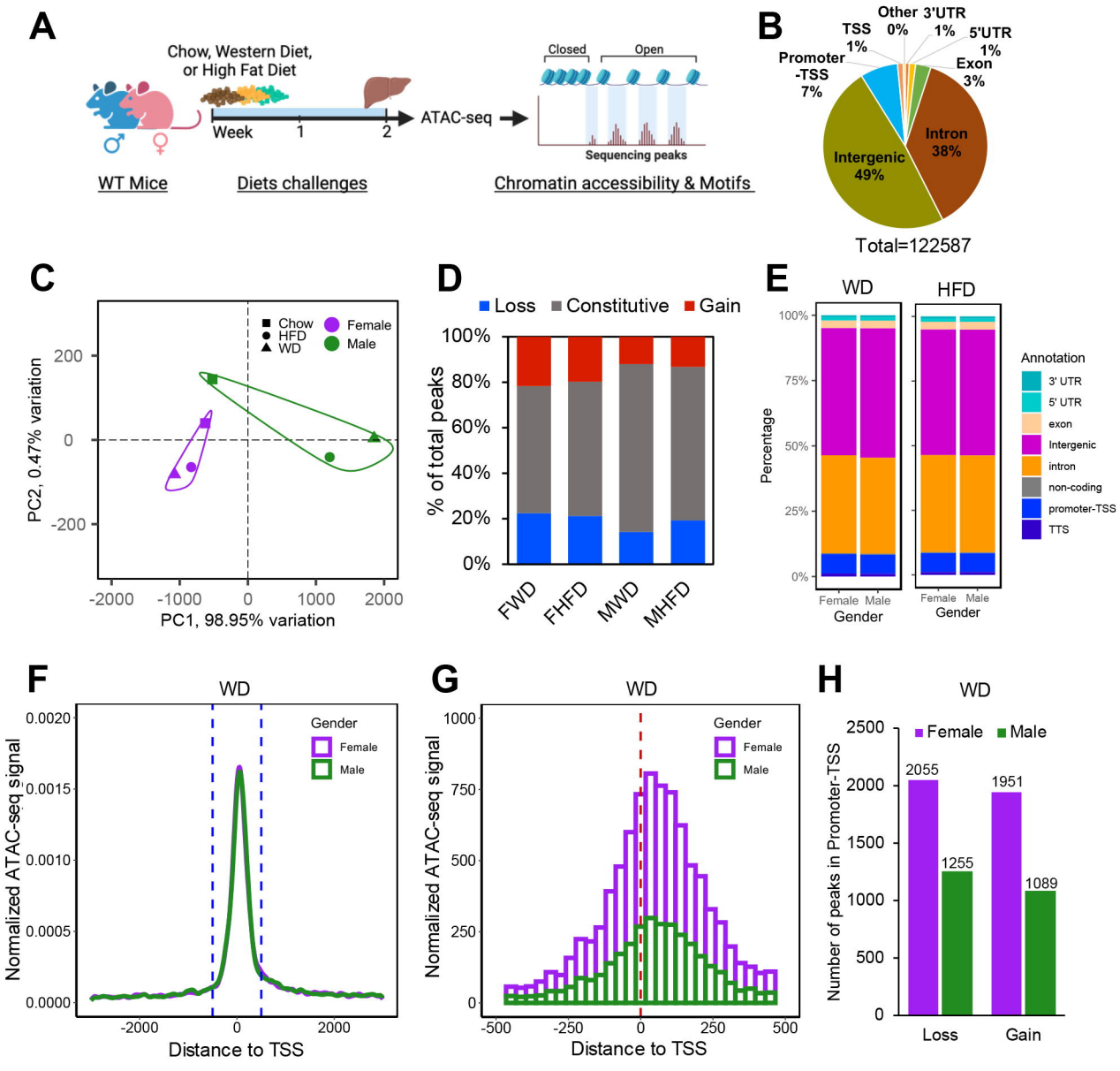
**Figure S2. Sex-specific hepatic gene expression profiles exhibit different motif preferences (Related to Figure 4).** (**A**) Heatmap showing data of all the genes detected by RNA-seq in mice liver samples. (**B)** Differentially expressed genes (DEGs) in male or female with 2 weeks or 4 weeks WD feeding. (**C**) DEGs in male or female with 2 weeks or 4 weeks HFD feeding. (**D)** Venn diagram showing female and male common DEGs and specific DEGs. (**E**) Metascape analysis of male and female common DEGs. Top 5 terms shown. (**F-I).** Top 10 Metascape terms shown for female or male-specific up or down regulated genes. (**J**) Motifs enrichment prediction of male-biased or female-biased genes in Fig. 4F. (**K**) Genes from Fig. 4F matching to DEGs identified by RNA-seq using FCG mouse model. P-value< 0.05 was considered as significant effect. (**L**) Venn diagram exhibiting overlap of diets induced sex difference genes from Fig. 4F and DEGs identified by RNA-seq using FCG mouse model.

**Figure S3. Gene expression from human liver corroborates analysis from murine studies (Related to Figure 5).** (**A**) Heatmap was generated based on TPM of genes from Fig. 5A in human livers. TPMs were obtained from GETx Portal. Genes from Fig. 5A with no related human genes, or with expression level of “0” were taken out. (**B** and **C**) Venn diagram showing the overlap of female (B) or male (C) diets induced DEGs from Fig. 5A and DETx genes. (**D**) Expression of human PNPLA3 in top 10 tissues. Data from GETx Portal. (**E**) Venn diagram showing the overlap of DEGs from Fig. 5F and liver expressed sex-biased genes from Lau-Corona et al., 2020. (**F**) Fold-change of sex-biased DEGs from Lau-Corona et al., 2020. Fold changes shown as Males/Females (control mice from Lau-Corona et al., 2020). (**G**) LXR target gene expression based on RNA-seq from Fig. 4A. CF2: chow diet fed female mice for 2 weeks. WF2: WD fed female mice for 2 weeks. CF4: chow diet fed female mice for 4 weeks. WF4: WD fed female mice for 4 weeks. CM2: chow diet fed male mice for 2 weeks. WM2: WD fed male mice for 2 weeks. CM4: chow diet fed male mice for 4 weeks. WM4: WD fed male mice for 4 weeks. (**H**) Expression of LXR target genes detected by qPCR from liver. N=8 per group. P-value was calculated by unpaired t-test. *: P-value< 0.05; **: P< 0.01; ***: P< 0.001; ns: not significant.

**Figure S4. Chromatin architecture and regulation of *Pnpla3* (Related to Figure. 5).** (**A**) Gene expression of *Polg1* detected by qPCR from livers of mice from Fig. 5F, shown as relative to male or female AAV-TBG-GFP values respectively. N= 5; * P< 0.05 by t-test. (**B**) PROMO predicted TFs binding sites around TSS of *Pnpla3*, as indicated in the red box in Fig. 5E. “Level” revealed=s the density of binding sites of TFs, higher “level” indicated the higher density of TFs binding. (**C**) H3K27ac ChIP-seq peaks from public datasets under GEO#: SRR6756579 (female Rep 1), SRR6756580 (female Rep 2), SRR6756581 (female Rep 3), SRR6756582 (female Rep 4), SRR6756571 (male Rep 1), SRR6756572 (male Rep2), SRR6756573 (male Rep3) and SRR6756574 (male Rep

4). (**D**) Fold change calculated based on ChIP-seq enrichment of H3K27ac performed in male or female liver (from Lau-Corona et al., 2020). Gene annotation was performed by Seqmonk annotated to mRNA.

# Figure 1


A
Chow, Western Diet, or High Fat Diet
Week
1
2
ATAC-seq
Closed
Open
Sequencing peaks
WT Mice
Diets challenges
Chromatin accessibility & Motifs
B
Other 0%
3'UTR 1%
5'UTR 1%
TSS 1%
Promoter -TSS 7%
Exon 3%
Intron 38%
Intergenic 49%
Total=122587
C
Chow
HFD
WD
Female
Male
PC2, 0.47% variation
PC1, 98.95% variation
D
Loss
Constitutive
Gain
% of total peaks
FWD
FHFD
MWD
MHFD
E
WD
HFD
Percentage
Annotation
3' UTR
5' UTR
exon
Intergenic
intron
non-coding
promoter-TSS
TTS
Female
Male
Gender
F
WD
Gender
Female
Male
Normalized ATAC-seq signal
Distance to TSS
G
WD
Gender
Female
Male
Normalized ATAC-seq signal
Distance to TSS
H
WD
Female
Male
Number of peaks in Promoter-TSS
2055
1255
1951
1089
Loss
Gain

# Figure 2

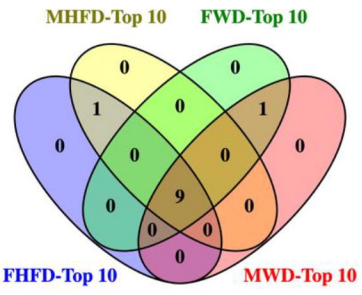
**A**

| Motif | Family | Female 1 | Female 2 | Female 3 | Female 4 | Female 5 | Female 6 | Male 1 | Male 2 | Male 3 | Male 4 | Male 5 | Male 6 | Female 1 | Female 2 | Female 3 | Female 4 | Female 5 | Female 6 | Male 1 | Male 2 | Male 3 | Male 4 | Male 5 | Male 6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HFD | | | | | | | | | | | | WD | | | | | | | | | | | |
| GFY | ? | 10 | 7 | 10 | 9 | 7 | 8 | 6 | 13 | 5 | 12 | 9 | 12 | 6 | 5 | 15 | 9 | 7 | 8 | 9 | 6 | 8 | 17 | 7 | 11 |
| GFY-Staf | ?,Zf | 12 | 6 | 10 | 10 | 7 | 12 | 6 | 11 | 7 | 11 | 10 | 15 | 11 | 4 | 14 | 10 | 11 | 8 | 9 | 8 | 8 | 15 | 9 | 15 |
| Usf2 | bHLH | 1 | 6 | 3 | 3 | 2 | 6 | 3 | 2 | 1 | 5 | 2 | 5 | 1 | 5 | 9 | 4 | 2 | 1 | 3 | 2 | 3 | 1 | 6 | 5 |
| E-box | | 3 | 5 | 2 | 1 | 1 | 6 | 3 | 1 | 2 | 5 | 3 | 4 | 2 | 1 | 7 | 4 | 2 | 2 | 4 | 1 | 3 | 2 | 4 | 6 |
| TFE3 | | 2 | 5 | 4 | 1 | 2 | 7 | 3 | 3 | 3 | 5 | 1 | 5 | 1 | 3 | 7 | 4 | 2 | 2 | 5 | 3 | 2 | 3 | 4 | 2 |
| CLOCK | | 2 | 4 | 3 | 1 | 1 | 3 | 2 | 0 | 1 | 5 | 1 | 3 | 1 | 2 | 6 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 3 | 3 |
| MITF | | 1 | 4 | 1 | 0 | 2 | 6 | 2 | 2 | 2 | 3 | 2 | 1 | 1 | 3 | 4 | 2 | 3 | 0 | 2 | 3 | 2 | 1 | 3 | 2 |
| CRE | bZIP | 7 | 3 | 5 | 7 | 4 | 8 | 7 | 3 | 11 | 7 | 3 | 4 | 5 | 5 | 4 | 4 | 10 | 5 | 6 | 5 | 6 | 6 | 9 | 2 |
| Atf1 | | 10 | 5 | 8 | 4 | 2 | 5 | 10 | 4 | 9 | 6 | 4 | 2 | 6 | 9 | 4 | 2 | 5 | 7 | 3 | 8 | 9 | 6 | 6 | 2 |
| NFIL3 | | 9 | 3 | 5 | 2 | 3 | 3 | 7 | 7 | 5 | 3 | 0 | 4 | 4 | 4 | 3 | 3 | 5 | 3 | 1 | 5 | 5 | 5 | 4 | 3 |
| Atf7 | | 6 | 4 | 4 | 3 | 3 | 4 | 6 | 3 | 9 | 3 | 2 | 1 | 4 | 6 | 3 | 2 | 5 | 4 | 2 | 3 | 5 | 9 | 5 | 1 |
| Atf2 | | 5 | 2 | 5 | 2 | 2 | 3 | 5 | 3 | 5 | 4 | 1 | 2 | 3 | 4 | 2 | 2 | 5 | 2 | 1 | 3 | 3 | 7 | 4 | 1 |
| JunD | | 6 | 3 | 3 | 2 | 2 | 2 | 6 | 3 | 4 | 5 | 1 | 0 | 4 | 3 | 1 | 3 | 4 | 2 | 2 | 5 | 2 | 4 | 4 | 1 |
| c-Jun-CRE | | 5 | 2 | 5 | 1 | 1 | 2 | 5 | 2 | 5 | 4 | 1 | 1 | 2 | 4 | 3 | 2 | 4 | 1 | 1 | 3 | 2 | 6 | 4 | 0 |
| **NFY** | **CCAAT** | 28 | 25 | 40 | 27 | 55 | 29 | 31 | 36 | 37 | 33 | 42 | 22 | 30 | 36 | 34 | 27 | 40 | 34 | 36 | 26 | 30 | 34 | 37 | 36 |
| E2F4 | E2F | 3 | 2 | 3 | 2 | 5 | 3 | 1 | 4 | 7 | 6 | 1 | 2 | 4 | 3 | 2 | 2 | 4 | 3 | 3 | 6 | 3 | 4 | 3 | 2 |
| Elk4 | ETS | 27 | 21 | 20 | 26 | 30 | 20 | 27 | 15 | 22 | 23 | 30 | 30 | 17 | 24 | 22 | 23 | 24 | 20 | 21 | 16 | 22 | 26 | 33 | 31 |
| Elk1 | | 29 | 19 | 17 | 24 | 26 | 17 | 27 | 13 | 21 | 18 | 30 | 24 | 14 | 24 | 19 | 22 | 24 | 17 | 20 | 15 | 19 | 21 | 29 | 27 |
| ELF1 | | 26 | 17 | 18 | 23 | 21 | 18 | 26 | 15 | 18 | 16 | 26 | 26 | 14 | 27 | 16 | 21 | 21 | 18 | 20 | 15 | 16 | 22 | 28 | 26 |
| ETS | | 27 | 17 | 22 | 23 | 19 | 16 | 22 | 16 | 21 | 18 | 25 | 24 | 15 | 22 | 18 | 23 | 18 | 16 | 18 | 16 | 22 | 21 | 27 | 23 |
| Fli1 | | 19 | 14 | 14 | 21 | 25 | 11 | 19 | 14 | 15 | 15 | 24 | 17 | 14 | 19 | 15 | 14 | 14 | 19 | 19 | 11 | 13 | 16 | 22 | 25 |
| ETS1 | | 17 | 7 | 13 | 16 | 14 | 7 | 11 | 11 | 15 | 10 | 11 | 17 | 9 | 14 | 11 | 14 | 9 | 14 | 13 | 6 | 13 | 7 | 14 | 25 |
| ETV4 | | 21 | 11 | 11 | 22 | 18 | 12 | 17 | 12 | 16 | 12 | 17 | 21 | 11 | 19 | 12 | 15 | 17 | 16 | 13 | 8 | 16 | 17 | 17 | 24 |
| GABPA | | 20 | 11 | 15 | 19 | 15 | 12 | 15 | 13 | 15 | 12 | 19 | 21 | 11 | 22 | 10 | 17 | 15 | 13 | 14 | 9 | 11 | 19 | 21 | 22 |
| ETV1 | | 18 | 8 | 9 | 16 | 13 | 7 | 10 | 9 | 10 | 9 | 14 | 18 | 8 | 16 | 8 | 13 | 11 | 9 | 10 | 5 | 8 | 13 | 16 | 19 |
| Etv2 | | 14 | 5 | 12 | 14 | 11 | 4 | 8 | 9 | 13 | 8 | 5 | 15 | 10 | 14 | 7 | 9 | 8 | 10 | 9 | 5 | 7 | 10 | 10 | 19 |
| Elf4 | | 14 | 6 | 11 | 16 | 13 | 11 | 11 | 9 | 14 | 10 | 13 | 14 | 8 | 15 | 11 | 11 | 11 | 12 | 14 | 6 | 8 | 13 | 15 | 17 |
| ERG | | 14 | 7 | 10 | 13 | 10 | 6 | 7 | 10 | 13 | 8 | 8 | 13 | 11 | 11 | 9 | 10 | 7 | 10 | 11 | 5 | 8 | 8 | 13 | 17 |
| EHF | | 10 | 3 | 9 | 10 | 7 | 7 | 6 | 9 | 7 | 5 | 10 | 7 | 4 | 10 | 8 | 7 | 5 | 9 | 7 | 2 | 5 | 8 | 10 | 15 |
| EWS:FLI1-fusio | | 11 | 6 | 9 | 10 | 13 | 5 | 7 | 7 | 13 | 10 | 7 | 11 | 9 | 8 | 7 | 9 | 11 | 8 | 10 | 5 | 4 | 10 | 14 | 14 |
| ELF5 | | 5 | 4 | 8 | 11 | 7 | 10 | 7 | 6 | 9 | 5 | 8 | 8 | 5 | 6 | 9 | 7 | 5 | 7 | 8 | 4 | 4 | 5 | 13 | 9 |
| SPDEF | | 5 | 2 | 10 | 4 | 2 | 5 | 5 | 5 | 5 | 3 | 4 | 4 | 1 | 7 | 3 | 5 | 4 | 4 | 4 | 2 | 3 | 4 | 5 | 7 |
| ELF3 | | 4 | 3 | 4 | 6 | 4 | 5 | 5 | 5 | 5 | 2 | 5 | 2 | 3 | 4 | 5 | 4 | 3 | 4 | 4 | 0 | 4 | 4 | 5 | 6 |
| SpiB | | 0 | 1 | 1 | 6 | 3 | 2 | 1 | 2 | 3 | 1 | 1 | 2 | 0 | 5 | 1 | 2 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 3 |
| ETS:RUNX | ETS,Runt | 2 | 1 | 5 | 2 | 2 | 1 | 0 | 2 | 3 | 2 | 2 | 1 | 2 | 2 | 6 | 1 | 1 | 2 | 3 | 1 | 2 | 1 | 1 | 3 |
| Hoxa9 | Homeobox | 3 | 1 | 3 | 2 | 7 | 3 | 3 | 1 | 2 | 4 | 1 | 6 | 1 | 4 | 2 | 3 | 6 | 2 | 5 | 1 | 3 | 1 | 1 | 6 |
| Nanog | | 1 | 2 | 4 | 1 | 3 | 2 | 3 | 3 | 1 | 4 | 1 | 3 | 1 | 2 | 6 | 3 | 1 | 3 | 3 | 1 | 3 | 2 | 1 | 4 |
| IRF8 | IRF | 2 | 2 | 4 | 2 | 1 | 2 | 3 | 2 | 1 | 2 | 1 | 3 | 2 | 5 | 1 | 0 | 4 | 1 | 2 | 0 | 3 | 2 | 6 | 1 |
| NRF1 | NRF | 11 | 3 | 4 | 6 | 5 | 9 | 4 | 5 | 6 | 9 | 7 | 4 | 5 | 5 | 6 | 7 | 7 | 5 | 4 | 4 | 10 | 8 | 7 | 4 |
| NRF | | 11 | 5 | 6 | 7 | 5 | 8 | 6 | 8 | 5 | 8 | 10 | 3 | 6 | 7 | 8 | 7 | 8 | 5 | 5 | 8 | 7 | 7 | 8 | 4 |
| Stat3 | Stat | 9 | 2 | 1 | 2 | 1 | 3 | 3 | 2 | 1 | 1 | 5 | 2 | 3 | 2 | 0 | 2 | 4 | 3 | 3 | 2 | 2 | 4 | 4 | 2 |
| Stat3+il21 | | 7 | 1 | 0 | 0 | 0 | 3 | 1 | 1 | 0 | 2 | 3 | 1 | 0 | 2 | 0 | 2 | 3 | 1 | 2 | 3 | 1 | 1 | 2 | 1 |
| Ronin | THAP | 14 | 8 | 11 | 10 | 9 | 12 | 8 | 11 | 7 | 13 | 10 | 17 | 12 | 4 | 14 | 10 | 12 | 7 | 8 | 10 | 9 | 15 | 11 | 16 |
| Sp2 | Zf | 37 | 32 | 38 | 25 | 35 | 34 | 37 | 36 | 31 | 34 | 29 | 29 | 31 | 28 | 36 | 32 | 40 | 33 | 30 | 29 | 45 | 34 | 38 | 31 |
| Sp1 | | 30 | 40 | 35 | 37 | 39 | 39 | 40 | 38 | 34 | 34 | 32 | 33 | 38 | 34 | 32 | 36 | 42 | 31 | 31 | 40 | 38 | 40 | 39 | 33 |
| Sp5 | | 33 | 32 | 31 | 32 | 34 | 34 | 35 | 30 | 31 | 35 | 29 | 33 | 35 | 29 | 24 | 33 | 34 | 35 | 25 | 34 | 38 | 40 | 35 | 30 |
| KLF3 | | 22 | 27 | 30 | 28 | 26 | 30 | 36 | 22 | 21 | 31 | 23 | 24 | 26 | 25 | 21 | 34 | 34 | 18 | 22 | 25 | 28 | 24 | 36 | 30 |
| KLF5 | | 23 | 22 | 25 | 26 | 24 | 24 | 30 | 24 | 19 | 25 | 18 | 25 | 25 | 22 | 19 | 25 | 26 | 22 | 19 | 24 | 22 | 27 | 28 | 26 |
| KLF14 | | 17 | 21 | 24 | 19 | 22 | 23 | 22 | 19 | 20 | 23 | 21 | 19 | 21 | 16 | 16 | 27 | 24 | 22 | 17 | 16 | 26 | 23 | 27 | 20 |
| KLF6 | | 14 | 16 | 16 | 18 | 17 | 21 | 25 | 14 | 14 | 16 | 15 | 16 | 17 | 13 | 16 | 15 | 17 | 20 | 11 | 22 | 15 | 20 | 19 | 18 |
| Klf9 | | 15 | 14 | 19 | 17 | 14 | 15 | 20 | 9 | 15 | 15 | 14 | 18 | 16 | 15 | 11 | 15 | 18 | 13 | 9 | 15 | 16 | 18 | 18 | 19 |
| Klf4 | | 11 | 15 | 13 | 11 | 12 | 11 | 15 | 13 | 11 | 11 | 13 | 10 | 9 | 15 | 12 | 16 | 10 | 11 | 9 | 14 | 11 | 9 | 14 | 17 |
| KLF10 | | 8 | 12 | 10 | 5 | 6 | 4 | 8 | 9 | 6 | 8 | 7 | 4 | 8 | 7 | 7 | 13 | 6 | 4 | 7 | 11 | 4 | 8 | 5 | 10 |
| Maz | | 5 | 2 | 4 | 6 | 5 | 8 | 5 | 5 | 3 | 6 | 4 | 5 | 6 | 4 | 3 | 6 | 4 | 3 | 5 | 2 | 6 | 3 | 6 | 7 |
| ZNF143\|STAF | | 5 | 4 | 1 | 3 | 4 | 6 | 2 | 3 | 2 | 5 | 4 | 6 | 2 | 1 | 3 | 2 | 6 | 4 | 3 | 2 | 2 | 4 | 4 | 5 |
| YY1 | | 4 | 6 | 4 | 4 | 3 | 5 | 5 | 3 | 6 | 3 | 4 | 4 | 4 | 5 | 5 | 3 | 5 | 4 | 5 | 5 | 4 | 6 | 4 | 3 |
| EKLF | | 2 | 5 | 1 | 1 | 0 | 0 | 1 | 1 | 2 | 2 | 2 | 0 | 0 | 3 | 0 | 6 | 0 | 0 | 2 | 1 | 2 | 0 | 1 | 2 |

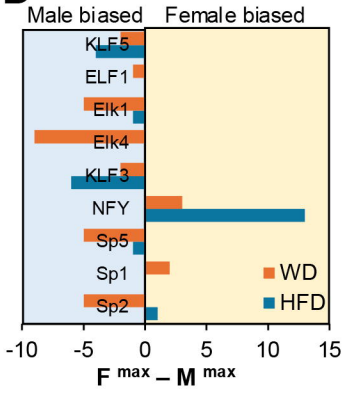
**B**

| | $F^{max}$ (HFD) | $M^{max}$ (HFD) | $F^{max}$ (WD) | $M^{max}$ (WD) | |
|---|---|---|---|---|---|
| Cluster A | 55 | 42 | 40 | 37 | NFY |
| | 6 | 3 | 4 | 3 | MITF |
| | 6 | 3 | 5 | 3 | SpiB |
| | 12 | 9 | 13 | 11 | KLF10 |
| | 5 | 2 | 6 | 2 | EKLF |
| | 7 | 5 | 7 | 5 | TFE3 |
| | 5 | 3 | 6 | 3 | ETS:RUNX |
| | 6 | 5 | 9 | 6 | Usf2 |
| | 6 | 5 | 7 | 6 | E-box |
| | 27 | 25 | 23 | 27 | ETS |
| | 16 | 14 | 15 | 17 | Elf4 |
| | 11 | 9 | 9 | 13 | ELF5 |
| | 11 | 9 | 7 | 10 | NRF1 |
| | 8 | 6 | 6 | 7 | Maz |
| | 25 | 24 | 19 | 25 | Fli1 |
| | 22 | 21 | 19 | 24 | ETV4 |
| | 14 | 13 | 11 | 17 | ERG |
| | 6 | 5 | 5 | 6 | ELF3 |
| | 4 | 3 | 5 | 6 | IRF8 |
| | 38 | 37 | 40 | 45 | Sp2 |
| | 4 | 5 | 6 | 3 | CLOCK |
| | 8 | 11 | 10 | 9 | CRE |
| | 10 | 10 | 9 | 9 | Atf1 |
| | 5 | 5 | 5 | 7 | Atf2 |
| | 6 | 6 | 4 | 5 | JunD |
| | 5 | 5 | 4 | 6 | c-Jun-CRE |
| | 30 | 30 | 24 | 33 | Elk4 |
| | 26 | 26 | 27 | 28 | ELF1 |
| | 17 | 17 | 14 | 25 | ETS1 |
| | 18 | 18 | 16 | 19 | ETV1 |
| | 10 | 10 | 10 | 15 | EHF |
| | 13 | 13 | 11 | 14 | EWS:FLI1-fusion |
| | 15 | 15 | 16 | 17 | Klf4 |
| | 6 | 6 | 5 | 6 | YY1 |
| Cluster B | 29 | 30 | 24 | 29 | Elk1 |
| | 20 | 21 | 22 | 22 | GABPA |
| | 14 | 15 | 14 | 19 | Etv2 |
| | 34 | 35 | 35 | 40 | Sp5 |
| | 19 | 20 | 18 | 19 | Klf9 |
| | 5 | 7 | 4 | 6 | E2F4 |
| | 10 | 13 | 15 | 17 | GFY |
| | 12 | 15 | 14 | 15 | GFY-Staf |
| | 6 | 9 | 6 | 9 | Atf7 |
| | 14 | 17 | 14 | 16 | Ronin |
| | 26 | 30 | 26 | 28 | KLF5 |
| | 21 | 25 | 20 | 22 | KLF6 |
| | 30 | 36 | 34 | 36 | KLF3 |
| | 4 | 4 | 6 | 4 | Nanog |
| | 40 | 40 | 42 | 40 | Sp1 |
| | 6 | 6 | 6 | 5 | ZNF143\|STAF |
| | 10 | 5 | 7 | 7 | SPDEF |
| | 9 | 5 | 4 | 4 | Stat3 |
| | 7 | 3 | 3 | 3 | Stat3+il21 |
| | 9 | 7 | 5 | 5 | NFIL3 |
| | 7 | 6 | 6 | 6 | Hoxa9 |
| | 11 | 10 | 8 | 8 | NRF |
| | 24 | 23 | 27 | 27 | KLF14 |

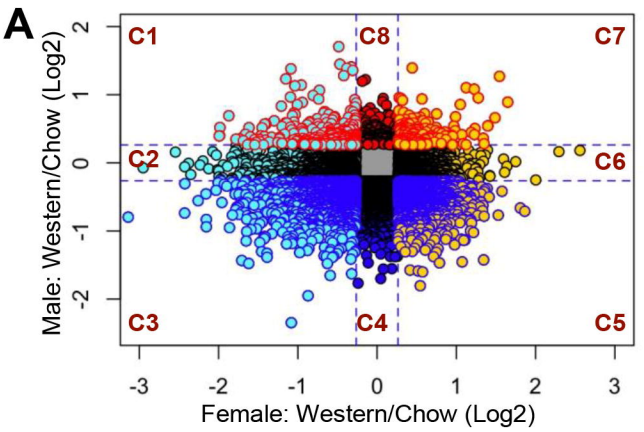
**C**

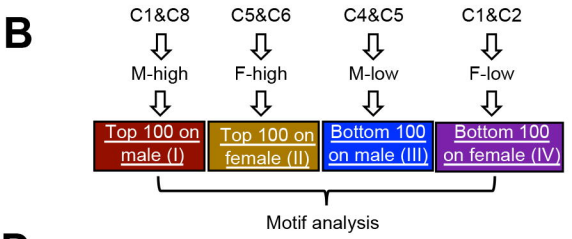


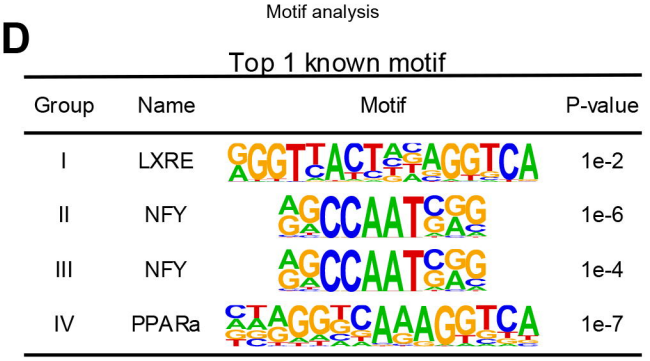

**D**

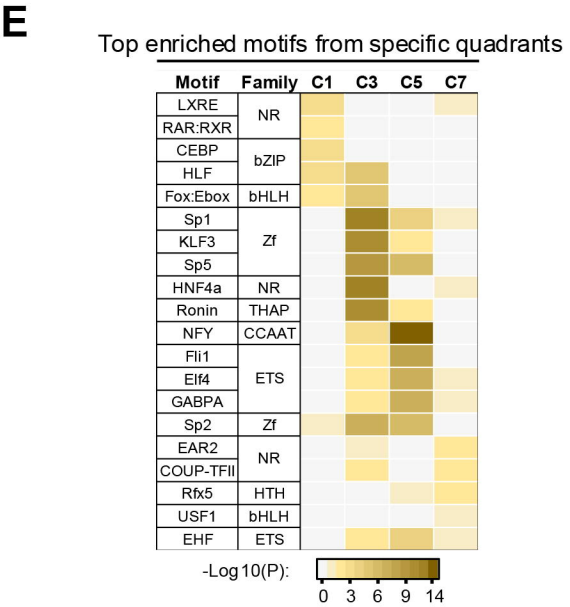


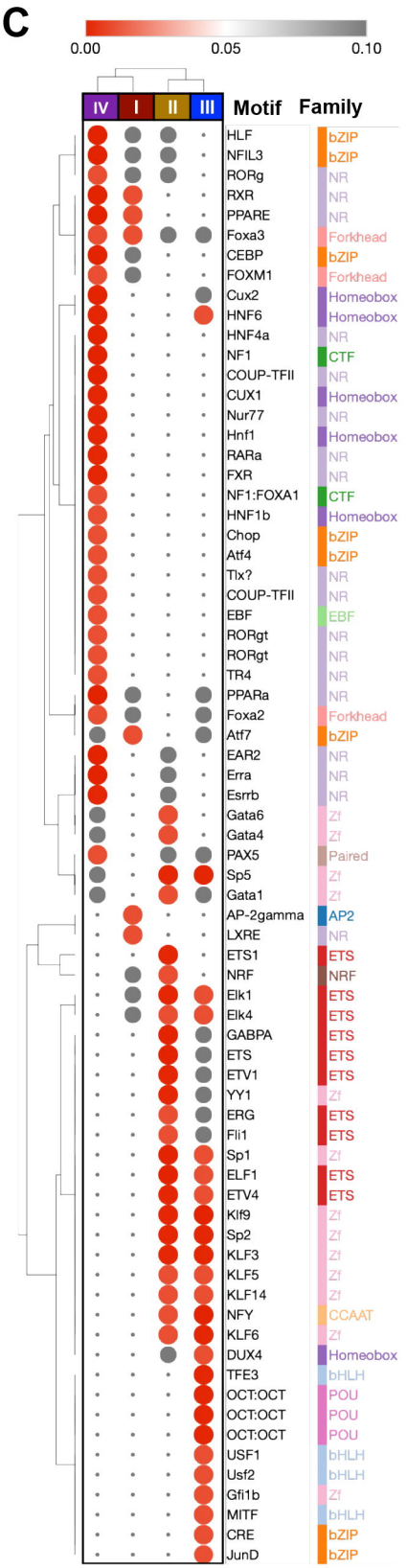
# Figure 3


A
C1
C8
C7
C2
C6
C3
C4
C5
Male: Western/Chow (Log2)
Female: Western/Chow (Log2)
-3
-2
-1
0
1
2
3

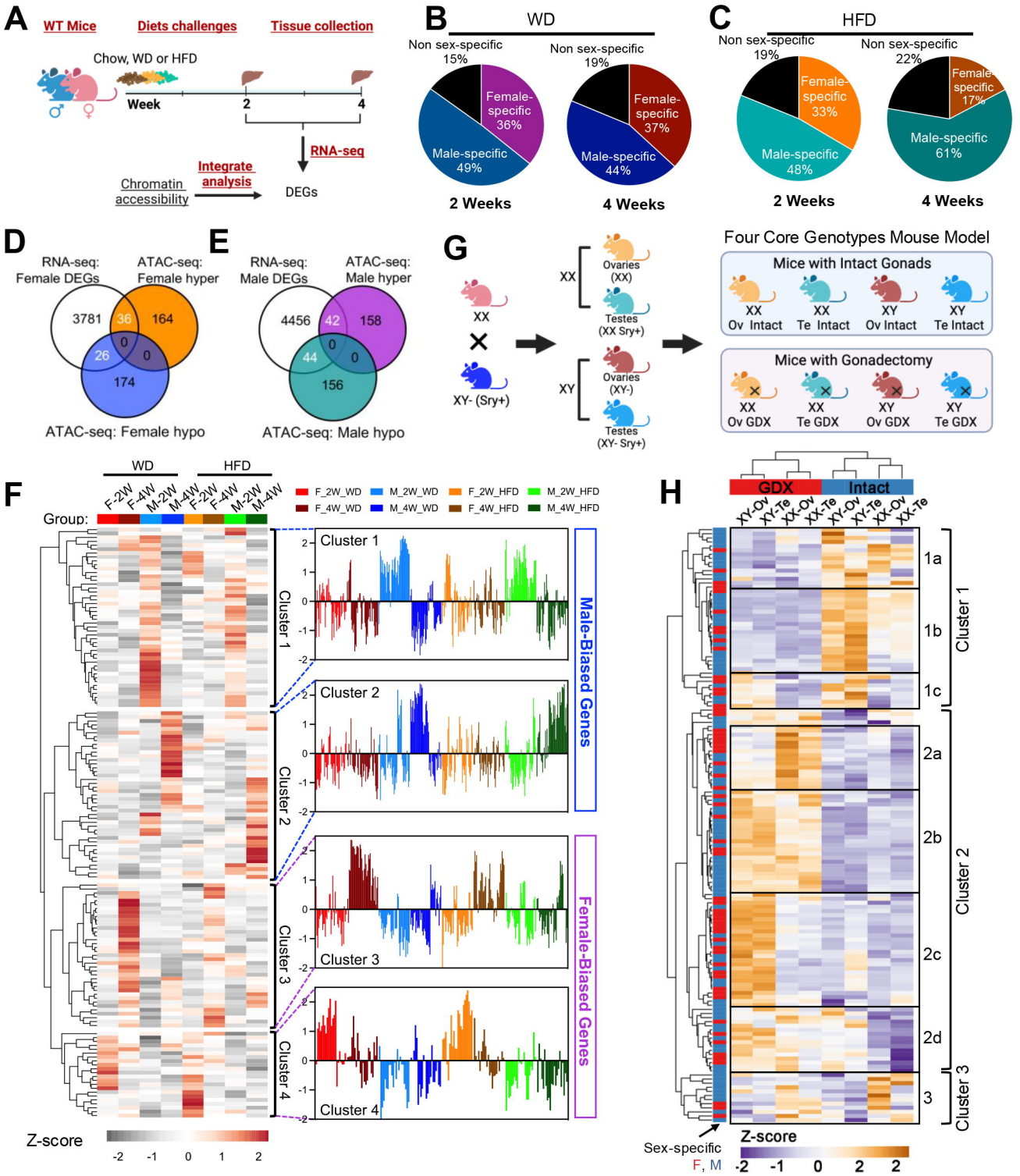

B
C1&C8
C5&C6
C4&C5
C1&C2
M-high
F-high
M-low
F-low
Top 100 on male (I)
Top 100 on female (II)
Bottom 100 on male (III)
Bottom 100 on female (IV)
Motif analysis


D
Top 1 known motif
Group
Name
Motif
P-value
I
LXRE
1e-2
II
NFY
1e-6
III
NFY
1e-4
IV
PPARa
1e-7

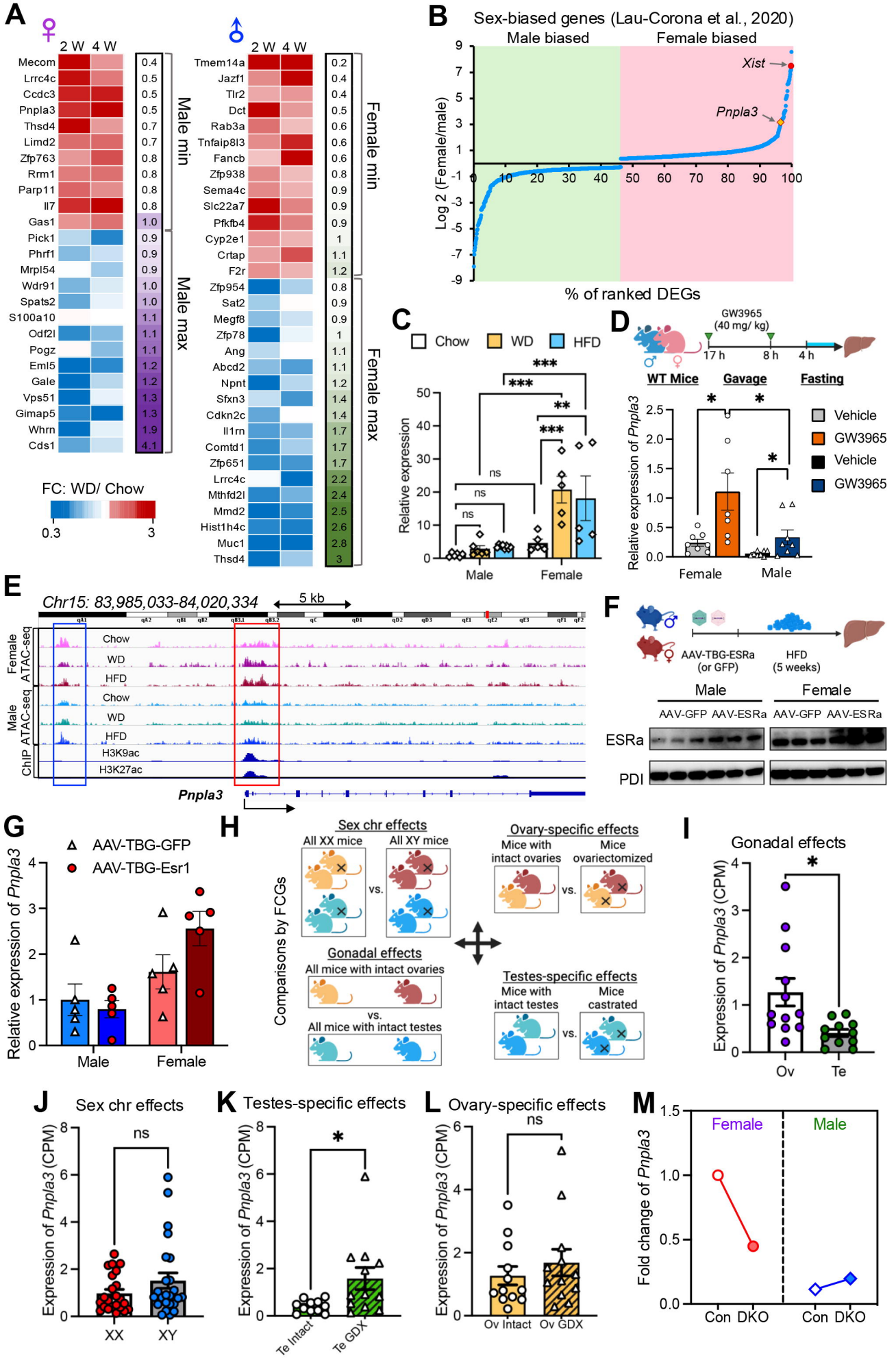

E
Top enriched motifs from specific quadrants
Motif
Family
C1
C3
C5
C7
LXRE
RAR:RXR
NR
CEBP
HLF
bZIP
Fox:Ebox
bHLH
Sp1
KLF3
Sp5
Zf
HNF4a
NR
Ronin
THAP
NFY
CCAAT
Fli1
Elf4
GABPA
ETS
Sp2
Zf
EAR2
COUP-TFII
NR
Rfx5
HTH
USF1
bHLH
EHF
ETS
-Log10(P):
0
3
6
9
14


C
0.00
0.05
0.10
IV
I
II
III
Motif
Family
HLF bZIP
NFIL3 bZIP
RORg NR
RXR NR
PPARE NR
Foxa3 Forkhead
CEBP bZIP
FOXM1 Forkhead
Cux2 Homeobox
HNF6 Homeobox
HNF4a NR
NF1 CTF
COUP-TFII NR
CUX1 Homeobox
Nur77 NR
Hnf1 Homeobox
RARa NR
FXR NR
NF1:FOXA1 CTF
HNF1b Homeobox
Chop bZIP
Atf4 bZIP
Tlx? NR
COUP-TFII NR
EBF EBF
RORgt NR
RORgt NR
TR4 NR
PPARa NR
Foxa2 Forkhead
Atf7 bZIP
EAR2 NR
Erra NR
Esrrb NR
Gata6 Zf
Gata4 Zf
PAX5 Paired
Sp5 Zf
Gata1 Zf
AP-2gamma AP2
LXRE NR
ETS1 ETS
NRF NRF
Elk1 ETS
Elk4 ETS
GABPA ETS
ETS ETS
ETV1 ETS
YY1 Zf
ERG ETS
Fli1 ETS
Sp1 Zf
ELF1 ETS
ETV4 ETS
Klf9 Zf
Sp2 Zf
KLF3 Zf
KLF5 Zf
KLF14 Zf
NFY CCAAT
KLF6 Zf
DUX4 Homeobox
TFE3 bHLH
OCT:OCT POU
OCT:OCT POU
OCT:OCT POU
USF1 bHLH
Usf2 bHLH
Gfi1b Zf
MITF bHLH
CRE bZIP
JunD bZIP

# Figure 4


A
WT Mice
Diets challenges
Tissue collection
Chow, WD or HFD
Week
2
4
RNA-seq
Integrate analysis
Chromatin accessibility
DEGs
B
WD
Non sex-specific 15%
Female-specific 36%
Male-specific 49%
2 Weeks
Non sex-specific 19%
Female-specific 37%
Male-specific 44%
4 Weeks
C
HFD
Non sex-specific 19%
Female-specific 33%
Male-specific 48%
2 Weeks
Non sex-specific 22%
Female-specific 17%
Male-specific 61%
4 Weeks
D
RNA-seq: Female DEGs
ATAC-seq: Female hyper
3781
36
164
0
26
0
174
ATAC-seq: Female hypo
E
RNA-seq: Male DEGs
ATAC-seq: Male hyper
4456
42
158
0
44
0
156
ATAC-seq: Male hypo
G
XX
XY- (Sry+)
XX
Ovaries (XX)
Testes (XX Sry+)
XY
Ovaries (XY-)
Testes (XY- Sry+)
Four Core Genotypes Mouse Model
Mice with Intact Gonads
XX Ov Intact
XX Te Intact
XY Ov Intact
XY Te Intact
Mice with Gonadectomy
XX Ov GDX
XX Te GDX
XY Ov GDX
XY Te GDX
F
WD
HFD
F-2W
F-4W
M-2W
M-4W
F-2W
F-4W
M-2W
M-4W
Group:
F_2W_WD
F_4W_WD
M_2W_WD
M_4W_WD
F_2W_HFD
F_4W_HFD
M_2W_HFD
M_4W_HFD
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Male-Biased Genes
Female-Biased Genes
Z-score
-2
-1
0
1
2
H
GDX
Intact
XY-Ov
XY-Te
XX-Ov
XX-Te
XY-Ov
XY-Te
XX-Ov
XX-Te
1a
1b
1c
2a
2b
2c
2d
3
Cluster 1
Cluster 2
Cluster 3
Sex-specific
F, M
Z-score
-2
-1
0
2
2

# Figure 5


A
♀
2 W 4 W
Mecom 0.4
Lrrc4c 0.5
Ccdc3 0.5
Pnpla3 0.5
Thsd4 0.7
Limd2 0.7
Zfp763 0.8
Rrm1 0.8
Parp11 0.8
Il7 0.8
Gas1 1.0
Pick1 0.9
Phrf1 0.9
Mrpl54 0.9
Wdr91 1.0
Spats2 1.0
S100a10 1.1
Odf2l 1.1
Pogz 1.1
Eml5 1.2
Gale 1.2
Vps51 1.3
Gimap5 1.3
Whrn 1.9
Cds1 4.1
Male min
Male max
♂
2 W 4 W
Tmem14a 0.2
Jazf1 0.4
Tlr2 0.4
Dct 0.5
Rab3a 0.6
Tnfaip8l3 0.6
Fancb 0.6
Zfp938 0.8
Sema4c 0.9
Slc22a7 0.9
Pfkfb4 0.9
Cyp2e1 1
Crtap 1.1
F2r 1.2
Zfp954 0.8
Sat2 0.9
Megf8 0.9
Zfp78 1
Ang 1.1
Abcd2 1.1
Npnt 1.2
Sfxn3 1.4
Cdkn2c 1.4
Il1rn 1.7
Comtd1 1.7
Zfp651 1.7
Lrrc4c 2.2
Mthfd2l 2.4
Mmd2 2.5
Hist1h4c 2.6
Muc1 2.8
Thsd4 3
Female min
Female max
FC: WD/ Chow
0.3
3
B
Sex-biased genes (Lau-Corona et al., 2020)
Male biased
Female biased
Xist
Pnpla3
Log 2 (Female/male)
% of ranked DEGs
C
Chow
WD
HFD
Relative expression
Male
Female
ns
***
**
D
GW3965
(40 mg/ kg)
17 h
8 h
4 h
WT Mice
Gavage
Fasting
Vehicle
GW3965
Vehicle
GW3965
Relative expression of Pnpla3
Female
Male
E
Chr15: 83,985,033-84,020,334
5 kb
Female ATAC-seq
Male ATAC-seq
ChIP
Chow
WD
HFD
H3K9ac
H3K27ac
Pnpla3
F
AAV-TBG-ESRa
(or GFP)
HFD
(5 weeks)
Male
Female
AAV-GFP AAV-ESRa AAV-GFP AAV-ESRa
ESRa
PDI
G
AAV-TBG-GFP
AAV-TBG-Esr1
Relative expression of Pnpla3
Male
Female
H
Sex chr effects
All XX mice
All XY mice
vs.
Ovary-specific effects
Mice with intact ovaries
Mice ovariectomized
Comparisons by FCGs
Gonadal effects
All mice with intact ovaries
All mice with intact testes
Testes-specific effects
Mice with intact testes
Mice castrated
I
Gonadal effects
Expression of Pnpla3 (CPM)
Ov
Te
J
Sex chr effects
ns
Expression of Pnpla3 (CPM)
XX
XY
K
Testes-specific effects
Te Intact
Te GDX
L
Ovary-specific effects
ns
Ov Intact
Ov GDX
M
Female
Male
Fold change of Pnpla3
Con DKO
Con DKO