

Supplementary information

High fat diet-induced changes of mouse hepatic transcription and enhancer activity can be reversed by subsequent weight loss

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Supplementary table S1. Summary of Illumina sequencing. Sequencing data was aligned to mm9 using TopHat (RNA-seq [Ribozero]), Bowtie (DNase-seq) or STAR (RNA-seq [PolyA], DNase-seq and ChIP-seq). RNA-seq, PolyA represents RNA-seq data from polyA-enriched RNA. RNA-seq, Ribozero represents RNA-seq data produced from RNA-depleted ribosomal RNA.

Sample ID	Method	Uniquely aligned reads
Chow, rep1	RNA-seq, PolyA	11720171
Chow, rep2	RNA-seq, PolyA	11783129
Chow, rep3	RNA-seq, PolyA	10588812
HFD, rep1	RNA-seq, PolyA	10369184
HFD, rep2	RNA-seq, PolyA	10279207
HFD, rep3	RNA-seq, PolyA	7455851
HFD-chow, rep1	RNA-seq, PolyA	11438832
HFD-chow, rep2	RNA-seq, PolyA	11224943
HFD-chow, rep3	RNA-seq, PolyA	14074351
Chow-chow, rep1	RNA-seq, PolyA	11927319
Chow-chow, rep2	RNA-seq, PolyA	11897382
Chow-chow, rep3	RNA-seq, PolyA	9777484
Chow, rep1	RNA-seq, Ribozero	49261677
Chow, rep2	RNA-seq, Ribozero	50060785
Chow, rep3	RNA-seq, Ribozero	51125127
HFD, rep1	RNA-seq, Ribozero	55636861
HFD, rep2	RNA-seq, Ribozero	43146179
HFD, rep3	RNA-seq, Ribozero	39882859
Chow, rep1	DNase-seq 40U	11751937
Chow, rep2	DNase-seq 40U	13081505
HFD, rep1	DNase-seq 40U	11496699
HFD, rep2	DNase-seq 40U	13026425
Chow, rep1	DNase-seq 60U	30797401
Chow, rep2	DNase-seq 60U	28945099
HFD, rep1	DNase-seq 60U	35524039
HFD, rep2	DNase-seq 60U	33956408
Chow, rep1	H3K27Ac ChIP-seq	15077420
Chow, rep2	H3K27Ac ChIP-seq	14388057
Chow, rep3	H3K27Ac ChIP-seq	12629852
HFD, rep1	H3K27Ac ChIP-seq	10621099
HFD, rep2	H3K27Ac ChIP-seq	13060025
HFD, rep3	H3K27Ac ChIP-seq	7298490
HFD-chow, rep1	H3K27Ac ChIP-seq	11659004
HFD-chow, rep2	H3K27Ac ChIP-seq	12156868
HFD-chow, rep3	H3K27Ac ChIP-seq	12057716
Chow-chow, rep1	H3K27Ac ChIP-seq	11053602
Chow-chow, rep2	H3K27Ac ChIP-seq	11411917
Chow-chow, rep3	H3K27Ac ChIP-seq	12211452

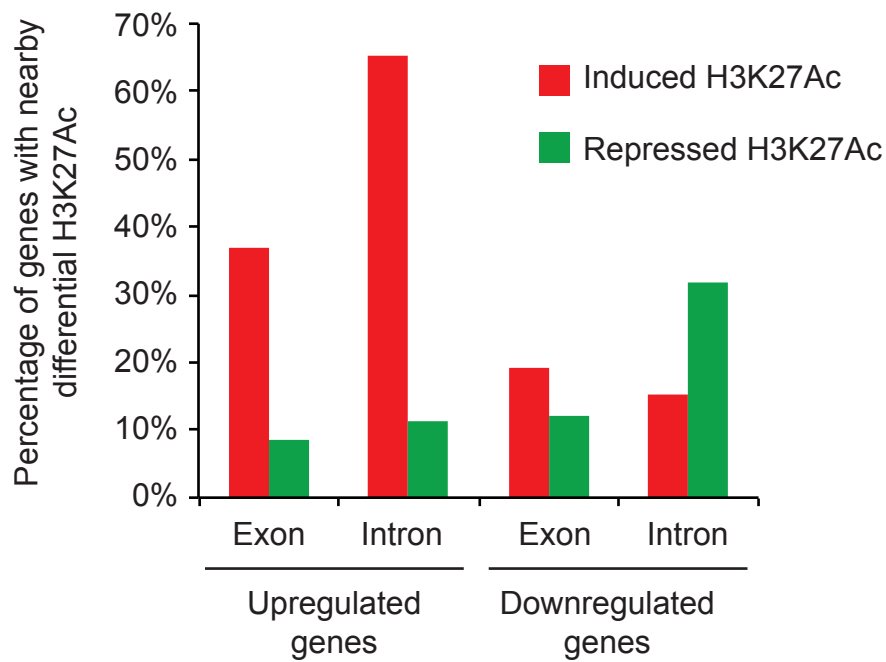
Supplementary table S2. Gene ontology analysis using PANTHER.
Overrepresentation test using Bonferroni correction for multiple testing.

PANTHER GO-Slim Biological Process	Fold Enrichment	P-value
Lipid metabolic process (GO:0006629)	5.01	1.08E-14
Steroid metabolic process (GO:0008202)	7.79	2.35E-10
Fatty acid metabolic process (GO:0006631)	6.91	2.15E-09
Sensory perception of chemical stimulus (GO:0007606)	< 0.2	9.21E-05
Cholesterol metabolic process (GO:0008203)	7.61	9.55E-04
Metabolic process (GO:0008152)	1.39	1.18E-03
Sensory perception of smell (GO:0007608)	< 0.2	4.06E-03
Primary metabolic process (GO:0044238)	1.39	1.21E-02
Neurological system process (GO:0050877)	0.33	1.78E-02
Sensory perception (GO:0007600)	0.23	1.94E-02
Cellular amino acid metabolic process (GO:0006520)	3.45	5.92E-02
Gluconeogenesis (GO:0006094)	13.22	6.51E-02
Anion transport (GO:0006820)	2.97	5.74E-01

Supplementary table S3. Reactome curated pathway analysis.

Reactome pathway analysis	FDR
Activation of gene expression by SREBF (SREBP)	6.18E-08
Regulation of cholesterol biosynthesis by SREBP (SREBF)	9.27E-07
Circadian Clock	0.001020081
Fatty acid, triacylglycerol, and ketone body metabolism	0.001020081
Unwinding of DNA	0.017989684
Fatty Acyl-CoA Biosynthesis	0.017989684
Mitotic G1-G1/S phases	0.018982142
ChREBP activates metabolic gene expression	0.034287085
Triglyceride Biosynthesis	0.037763381
PPARA activates gene expression	0.040182535

Supplementary figure S4



Supplementary figure S4. Frequency of HFD-induced up- and downregulated genes with nearby accessible regions that are differentially acetylated at H3K27 by HFD. Induced H3K27Ac is marked green and reduced H3K27Ac is marked red. Accessible regions within 100kb of the transcriptional start sites were selected for analysis. Differential gene expression was calculated using in intron or exon reads.

Supplementary figure S5

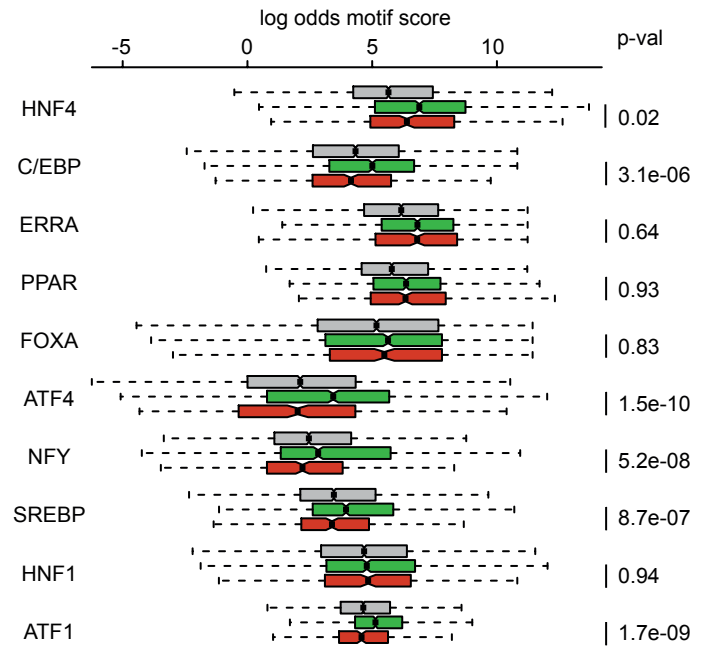
a

Motifs in regions with HFD-regulated DHS and increased H3K27Ac

Factor	Top 10 most enriched known motifs	p-val	%target/backgr
HNF4		1e-104	32/10
C/EBP		1e-61	28/11
ERRA		1e-42	61/41
PPAR		1e-35	35/19
FOXA		1e-30	37/22
ATF4		1e-29	13/4
NFY		1e-27	27/15
SREBP		1e-23	11/4
HNF1		1e-22	7/2
ATF1		1e-21	21/12

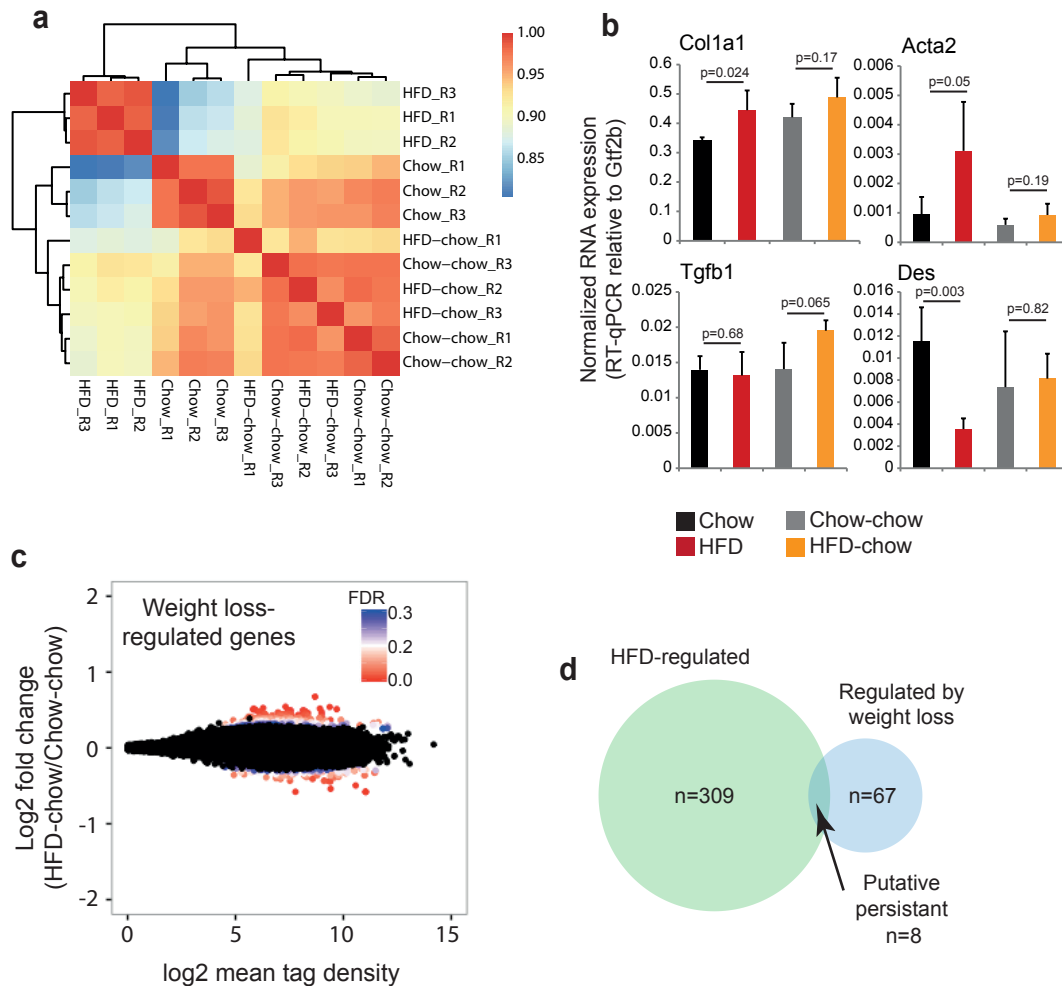
b

■ Random DHS
 ■ Increased H3K27Ac
 ■ Reduced H3K27Ac



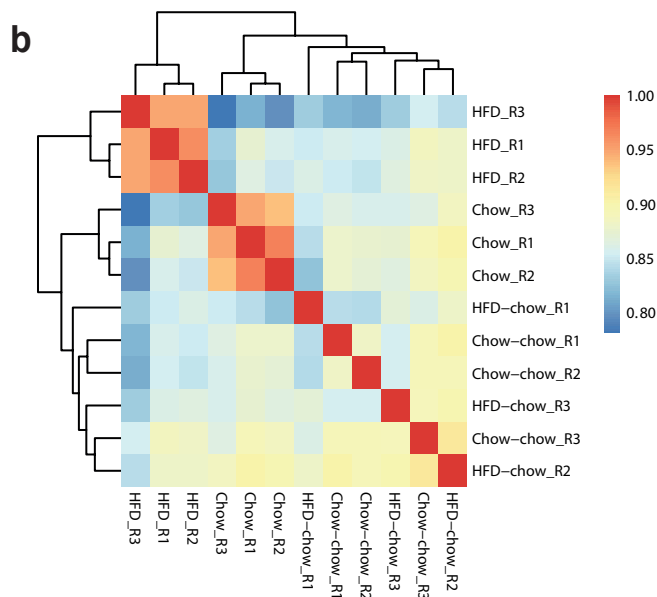
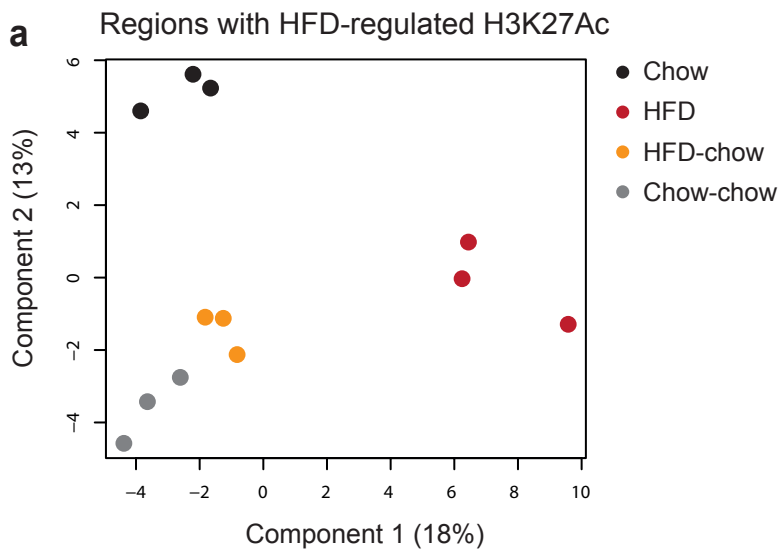
Supplementary figure S5. DNA motif analysis of HFD-regulated enhancers. **a)** DNA-sequence at DNase accessible regions with differentially regulated H3K27Ac by HFD was extracted and subjected to enriched motif analysis by HOMER. Top 10 of the most enriched known motifs. **b)** Motif strength of the most enriched motifs curated by HOMER at regions with either increased (green) or decreased (red) H3K27Ac or randomly selected DNase accessible regions (grey). Statistical test was performed by Wilcoxon signed-rank test.

Supplementary figure S6



Supplementary figure S6. a) Pairwise correlation analysis (Pearson) of RNA-seq replicates from Chow, HFD, HFD-chow and Chow-chow feeding regimens. RNA-seq data was generated from polyA-enriched RNA. Pairwise correlation analysis was clustered using euclidian distance computation. Heatmap shows Pearson correlation coefficient. **b)** Expression of genes associated with hepatic fibrosis measured by RT-qPCR (n=3-4). Statistical test was performed by two-tailed t-test. Error bars represent SEM. **c)** Differential RNA expression (RNA-seq) in livers isolated from Chow-chow compared to HFD-chow treated mice. DESeq2 was used to compute FDR. FDR>0.3 is colored black, while FDR<0.3 is colored according to the heatmap. Red represents most significantly regulated genes. **d)** Unique and common regulated genes by HFD and genes regulated as a result of weight loss. Differentially regulated genes are scored at FDR<0.05 computed by DESeq2. Common regulated genes (8 in total) represents putative persistent regulated genes as a result of HFD.

Supplementary figure S7



Supplementary figure S7. a) Principle component analysis and **b)** Pearson pairwise correlation analysis of H3K27Ac ChIP-seq replicates. Analysis was performed on regions demonstrating differential H3K27Ac in response to HFD ($p < 0.05$). Pairwise correlation analysis was clustered using euclidean distance computation. Heatmap shows Pearson correlation coefficient.