

Supplementary Materials. DNA methylation alters transcriptional rates of differentially expressed genes and contributes to pathophysiology in mice fed a high fat diet by Zhang et al.

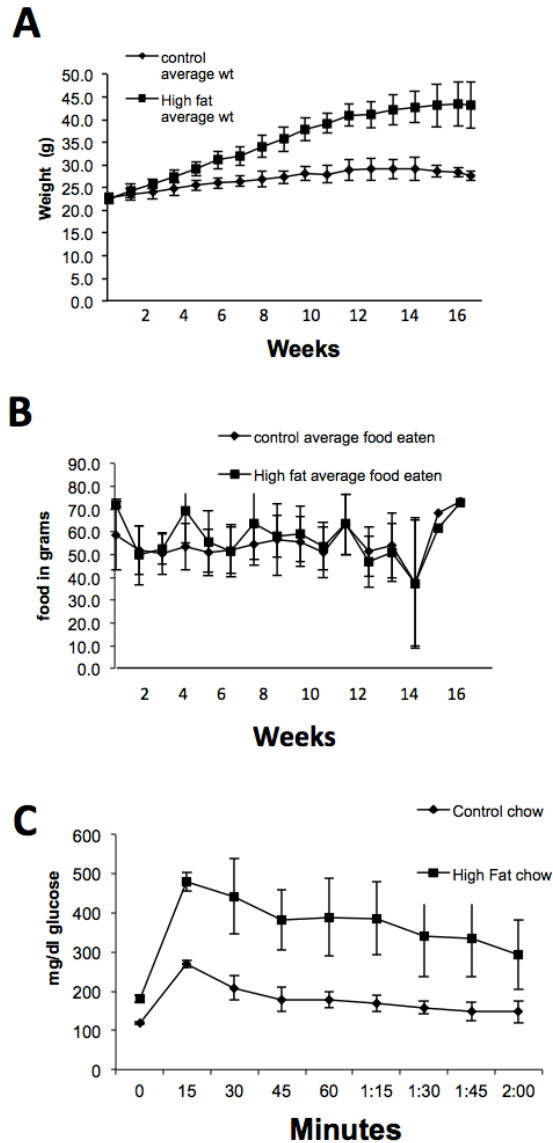
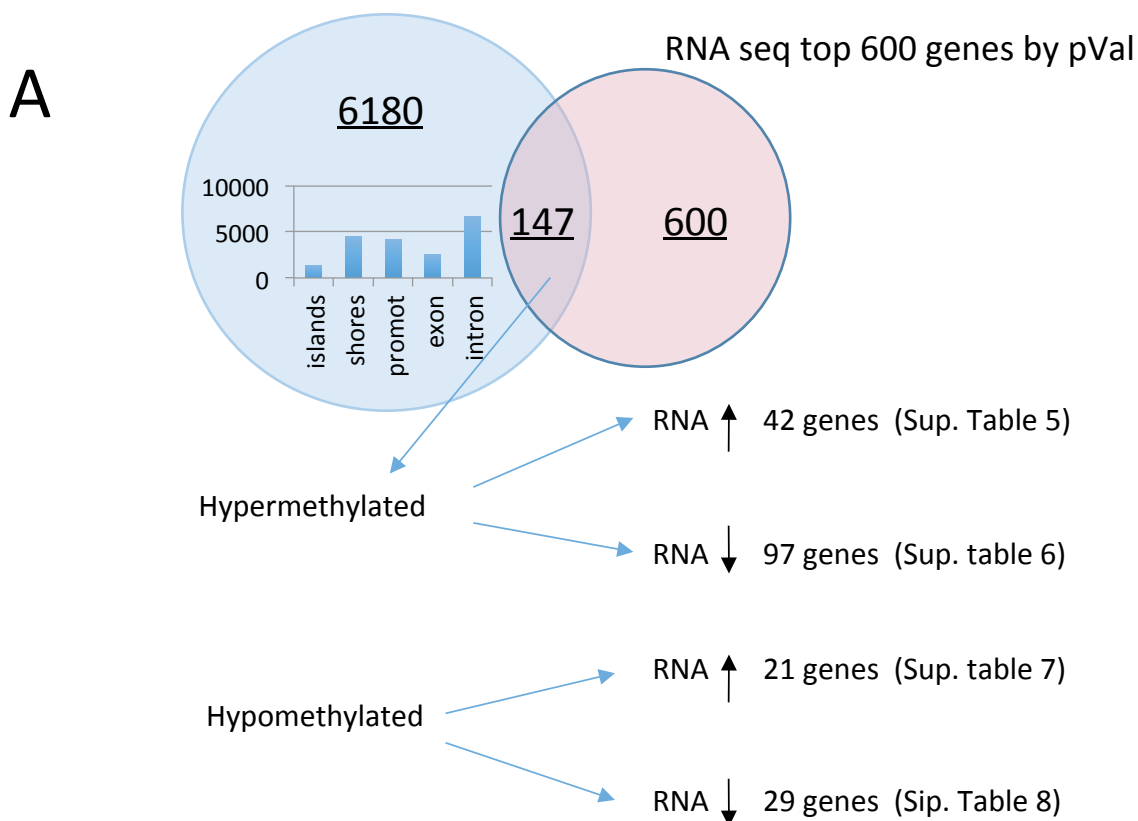


Figure S1. Characterization of mice on a high fat diet. C57BL/6 mice were placed on a high fat or normal chow diet for 14 weeks. A. Average weight of the animals over the course of the experiment. B. Food intake was measured in grams. C. An intraperitoneal glucose tolerance test was performed and blood glucose was measured for the times indicated. (data are the mean +/- SEM, *, P < 0.05)

Figure S2.

DifMeth +/- 10,000 bp from TSS



B

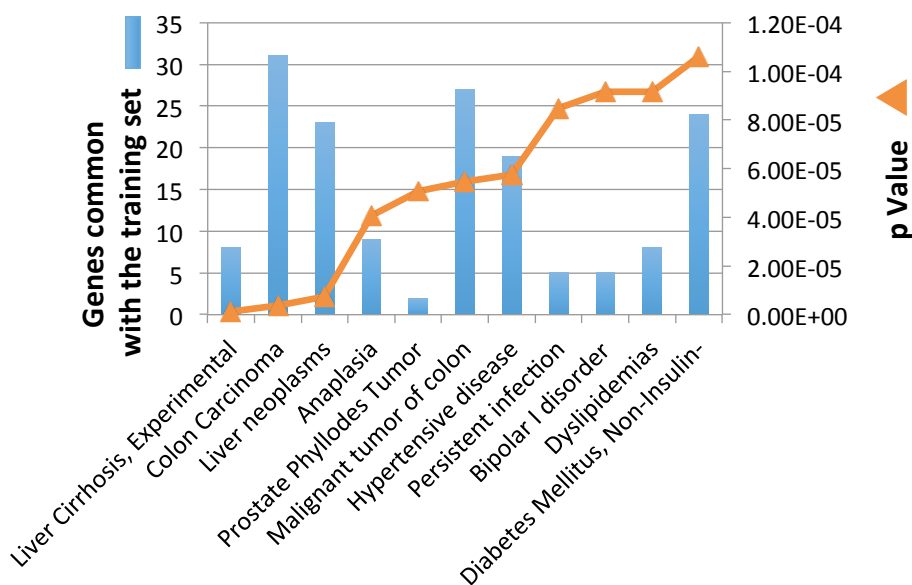


Figure S2. Analysis of differentially expressed genes with differentially methylated CpGs after 14 weeks on a high fat diet. A. 8870 significantly differentially methylated CpGs, representing 6180 genes, were found within 10,000 bp of transcription start sites (TSS). When intersected with the top 600 differentially expressed RNAs, 147 genes were found in common. Numbers of genes found to be increased or decreased with corresponding hypermethylated or hypomethylated CpGs are shown (lists of those genes in are found in supplementary tables 5-8). Note that many genes have multiple differentially methylated CpGs, and genes may have CpGs that are both hypo- and hypermethylated. B. Toppgene [1] analysis of disease prioritization showing number of genes in common with the training set and the p Value. Diseases correlating with altered genes from the high fat diet include cirrhosis, cancer, hypertensive disease, dyslipidemia and diabetes – all consistent with the development of the metabolic syndrome [2].

Supplemental Table 1. RT-PCR and ChIP oligonucleotides

Name	Left primer	Right primer
pre-mRNA		
<i>Slc16a5</i>	GCATGGCGGATTAACCTCATT	GGTAGCGTTCTCCATTTTGG
<i>Gnmt</i>	GAGGCCTAAGCAGATGGTGT	GGGCAGCCCAACCTTATAGT
<i>Phlda1</i>	TCCGTGTCCTTTCTTTCCAG	GCTTCCTGCAACTGTGATGA
<i>Foxa3</i>	TTCGCCTTTCTCTTTCTCCA	CCTCGGCTCTTGGTGTACTT
<i>Onecut 1</i>	GGCTGTGAGGAATGGTTGAC	TTGGACGGACGCTTATTTTC
ChIP		
<i>Phlda1</i> promoter	CCTGCCTGGATCTGCTACC	GCAGGTTCTGGAAACTCCTG
<i>Phlda1</i> EXON1	GAAGGATGCTGGAGAACAGC	ACTAGGTTGGGACGGTTCCG
<i>Phlda1</i> EXON2	TCCGTGTCCTTTCTTTCCAG	GCTTCCTGCAACTGTGATGA
<i>Onecut1</i> promoter	CCTCGGTTCAATTTGTGCTG	GGCTCGGTTACTGTTGCAGA
<i>Onecut1</i> Exon1	ACAAGATGCTCACCCCAAAT	ATCTCTTCCATCTGCCCTGA
<i>Onecut1</i> Exon2	GAAAATAAGCGTCCGTCCAA	AAGTGCTCGATGAGGACGAT
<i>Onecut1</i> Exon2 end	GGAAAATAAGCGTCCGTCCA	TTCCTCCCTCATGCTTTGGT
<i>Foxa3</i> Exon1	GGGGCTAGTGCCTGTAGAGA	ATCCCCGAGGACACACC
<i>Foxa3</i> Exon2	TTGAACCCACTCAGC TCTCC	CCTTTGCCATCTCTTTTCCA
<i>Foxa3</i> Exon2 end	TTCCTTTCTTTCCACGGTTG	GTGCCTTTTTAATGGCCAAA
<i>Foxa3</i> promoter	TTCCTTTCTTTCCACGGTTG	GGATAACGGGAAAGTCATCG
mRNA		
<i>β-actin</i>	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA
<i>Gnmt</i>	AAGAGGGCTTCAGCGTGATG	CTGGCAAGTGAGCAAACCTGT
<i>Slc16a5</i>	ATCACGGTAGTGGGCTTGTAT	TGGAGTAAGATGCCACCGAAG
<i>Slc25a3</i>	CATCACCAAGAAGCACCTGA	GTACCCTTATAGCCCGCACA
<i>Irx3</i>	GACGAGGAAGAGAGCAAACG	TCGTCCGAGTCGCTAGTTTT
<i>Cish</i>	TCGGGAAGTGGATGCTTATC	AAACTCCAGACACCCCACTG
<i>Socs2</i>	TGGCTGCTCAAGATCAAATG	TGCCTCCTGGAAATGGAAG

<i>Foxa1</i>	CAGCACAAGCTGGACTTCAA	AGCACGGGTCTGGAATACAC
<i>Foxa2</i>	GACATACCGACGCAGCAGCTACA	GGCACCTTGAGAAAGCAGTC
<i>Onecut1</i>	GGCAACGTGAGCGGTAGTTT	TTGCTGGGAGTTGTGAATGCT
<i>Phlda1</i>	GGGCTACTGCTCATACCGC	AAAAGTGCAATTCCTTCAGCTT
<i>Foxa3</i>	CTACATGACCTTGAACCCACTC	GGGCTACATACCCGGAAGC

Supplemental Table 2. Summary statistic for RNA-seq

Library	total reads	aligned reads	% aligned
normal			
1_1BC	42,315,569	36,343,322	0.858864074
1_1LC	21,658,077	17,575,957	0.811519739
1_1NC	59,685,957	50,084,723	0.839137471
1_1RC	34,954,648	28,458,348	0.81415061
2_1LC	31,907,946	26,294,512	0.824074104
2_1NC	31,732,872	26,096,217	0.822371735
2_1RC	53,368,278	42,173,702	0.790239138
high fat			
1_4BC	53,863,635	43,665,966	0.810676183
1_4LC	37,439,032	28,227,112	0.753948767
1_4NC	38,131,178	29,035,979	0.761476055
1_4RC	34,835,729	26,676,785	0.765788051
2_4LC	20,302,121	17,692,470	0.871459194
2_4NC	41,644,336	36,539,067	0.877407842
2_4RC	23,634,778	19,678,396	0.832603378

Supplemental Table 3. Summary Statistics for DNA Methyl-Seq

Sample ID	#reads	# pairs	# pairs removed after trimming	#pairs remaining	# Paired end alignment with unique best hit		# duplicated pairs	pairs analyzed	% pairs analyzed	approximate fold coverage (52Mb capture)
Normal										
G1_1RC	108,787,410	54,393,705	1,213,575	53,180,130	36,727,668	69.1%	11,143,605	25,584,063	47.03%	
G1_1LC	128,228,736	64,114,368	1,450,274	62,664,094	43,654,179	69.7%	9,550,798	34,103,381	53.19%	131.16685
G2_1LC	124,264,108	62,132,054	1,400,257	60,731,797	42,685,346	70.3%	1,270,902	41,414,444	66.66%	159.2863231
G1_1NC	120,760,734	60,380,367	1,392,279	58,988,088	40,589,058	68.8%	4,894,023	35,695,035	59.12%	137.2885962
G2_1RC	117,629,094	58,814,547	1,343,298	57,471,249	38,964,159	67.8%	11,124,280	27,839,879	47.34%	107.0764577
G2_1NC	113,885,548	56,942,774	1,297,220	55,645,554	38,621,945	69.4%	9,178,454	29,443,491	51.71%	113.2441962
G1_1BC	116,203,544	58,101,772	1,318,334	56,783,438	39,496,146	69.6%	9,994,402	29,501,744	50.78%	113.4682462
High Fat										
G1_4NC	169,802,970	84,901,485	2,034,858	82,866,627	57,067,889	68.9%	14,046,240	43,021,649	50.67%	165.4678808
G1_4BC	157,695,914	78,847,957	1,957,171	76,890,786	52,182,910	67.9%	9,264,249	42,918,661	54.43%	165.0717731
G1_4RC	186,815,332	93,407,666	2,058,068	91,349,598	62,090,548	68.0%	29,997,052	32,093,496	34.36%	123.4365231
G2_4RC	149,406,108	74,703,054	1,618,152	73,084,902	51,158,472	70.0%	24,190,973	26,967,499	36.10%	103.72115
G2_4LC	131,049,978	65,524,989	1,534,427	63,990,562	42,881,093	67.0%	12,239,682	30,641,411	46.76%	117.8515808
G2_4NC	98,751,052	49,375,526	1,176,190	48,199,336	33,022,488	68.5%	10,364,887	22,657,601	45.89%	87.14461923

Supplementary Table 5. Genes with Hypermethylated CpGs increased expression.

Acot4	Cidea	Gngt1	Ntrk2	Rtn4	Tpm2
Ar	Corin	Kbtbd11	Osbp13	Sh2d4a	Trdmt1
Car3	Cpeb1	Lgals1	Pax8	Slc13a4	Unc13a
Cdca2	Cxcl11	Mab21l3	Pdlim2	Slc22a26	Wfdc2
Cenpf	Cyp26a1	Mfap3l	Plscr2	Smc2	Zfp442
Cenpw	Fsip1	Mogat1	Prrx1	Snai2	
Cep55	Gadd45b	Neb1	Raet1d	Sult1c2	

Supplementary Table 6. Genes with Hypermethylated CpGs decreased expression.

3930402G23Rik	Cadm4	Dact1	Foxa3	Hp	Lepre1	Phlda1	Serpina1a	Srd5a1	Xirp1
Al118078	Cd4	Dnajc12	Fzd5	Hspa5	Llgl2	Pitx3	Serpina1e	Srgap3	Zbtb7c
Abtb2	Chrna2	Dpy19l3	Fzd7	Hyal1	Lrfn3	Plcx2	Shb	Susd4	Zfhx3
Adcy1	Cldn1	Efna3	Got1	Id2	Myo1e	Prhoxnb	Sid2	Syvn1	Zfp217
Agap2	Cobl	Egfr	Grm8	Id3	Nudt7	Qsox1	Sik3	Tbc1d30	Zfp568
Ankrd33b	Creld2	Erbp3	H13	Igfbp2	Oaf	Rcl1	Slc13a2	Tgm1	Zfp598
Apoa1	Crip2	Fabp5	Hhex	Il22ra1	Onecut1	Rnase4	Slc25a34	Tnfrsf81l	Zfpm1
Arid5b	Crybb3	Fga	Hip1r	Irf6	Orm1	Scara5	Socs3	Tspan9	
Atp2a2	Cyp1a2	Fgb	Hist2h3c2	Irs2	Pck1	Sel1l3	Sorbs3	Usp6nl	
C8b	Cyp21a1	Fkbp11	Hopx	Lad1	Pde9a	Serpina10	Sox12	Vtn	

Supplementary Table 6. Genes with Hypomethylated CpGs increased expression.

A530016L24Rik	Aatk	B430212C06Rik	Caprin2	Car3	Fam126a	Far1
Mad2l1	Mpped2	Neb1	Pdlim2	Pdcp	Rtn4	Smc2
Stap1	Stil	Tpm2	Trdmt1	Tsc22d1	Unc13a	

Supplementary Table 6. Genes with Hypomethylated CpGs decreased expression.

Ada	Ccdc11	Cd276	Cish	Crip2	Dact1	Fzd7	Gfra1	Gm11194	Ifitm2
Igfbp2	Myl1	Myo1e	Onecut1	Pck1	Pitx3	Sel1l3	Serpine2	Sik3	Slc41a3
Slco2a1	Tmem120b	Tnk2	Tspan9	Tuba8	Tuft1	Wnk4	Zfp217	Zfpm1	

Supplemental Table 9. Characteristics of genes differentially methylated and expressed in liver after a high fat diet.

Gene	DNA Methylation HF vs SC	RNAseq HF vs SC	RT-PCR HF vs SC	Function/ Role in Obesity/Diabetes
<i>Foxa3</i>	6.12*	0.64*	0.26*	Development, glucose homeostasis in adult liver
<i>Hip1R</i>	13.35*	0.54*	0.75	Huntington's interacting protein 1 related, endocytosis
<i>Notch3</i>	20.37*	0.53*	0.72	Hypertension
<i>Phlda1</i>	13.03*	0.27*	0.33*	Associated with steatosis: KO animals have liver steatosis
<i>Scara5</i>	-22.75*	1.61*	0.62	Scavenger receptor
<i>Sik3</i>	15.85*	0.63*	0.78	Salt inducible kinase 3, member of AMPK family
<i>Trdmt1</i>	23.83*	0.20*	0.61	tRNA aspartic acid methyl-transferase 1
<i>Onecut1</i>	21.7*	0.22*	0.41	Liver development

Mean of fold change comparing high fat diet (HF) to standard chow diet (SC) using data from RNAseq or RT-PCR. For DNA Methyl-seq, data are relative differences in percentage methylation in CpGs within 1,000 bp of the TSS of the indicated gene. The *Phlda1* assignment was a CpG ~10,000 bp from the TSS. n=7, * p < 0.05

References for Supplemental Material.

- [1] Chen, J., Bardes, E. E., Aronow, B. J., and Jegga, A. G., 2009. ToppGene Suite for gene list enrichment analysis and candidate gene prioritization. *Nucleic acids research* 37: W305-311
- [2] Kaur, J., 2014. A comprehensive review on metabolic syndrome. *Cardiology research and practice* 2014: 943162