Fig. S1

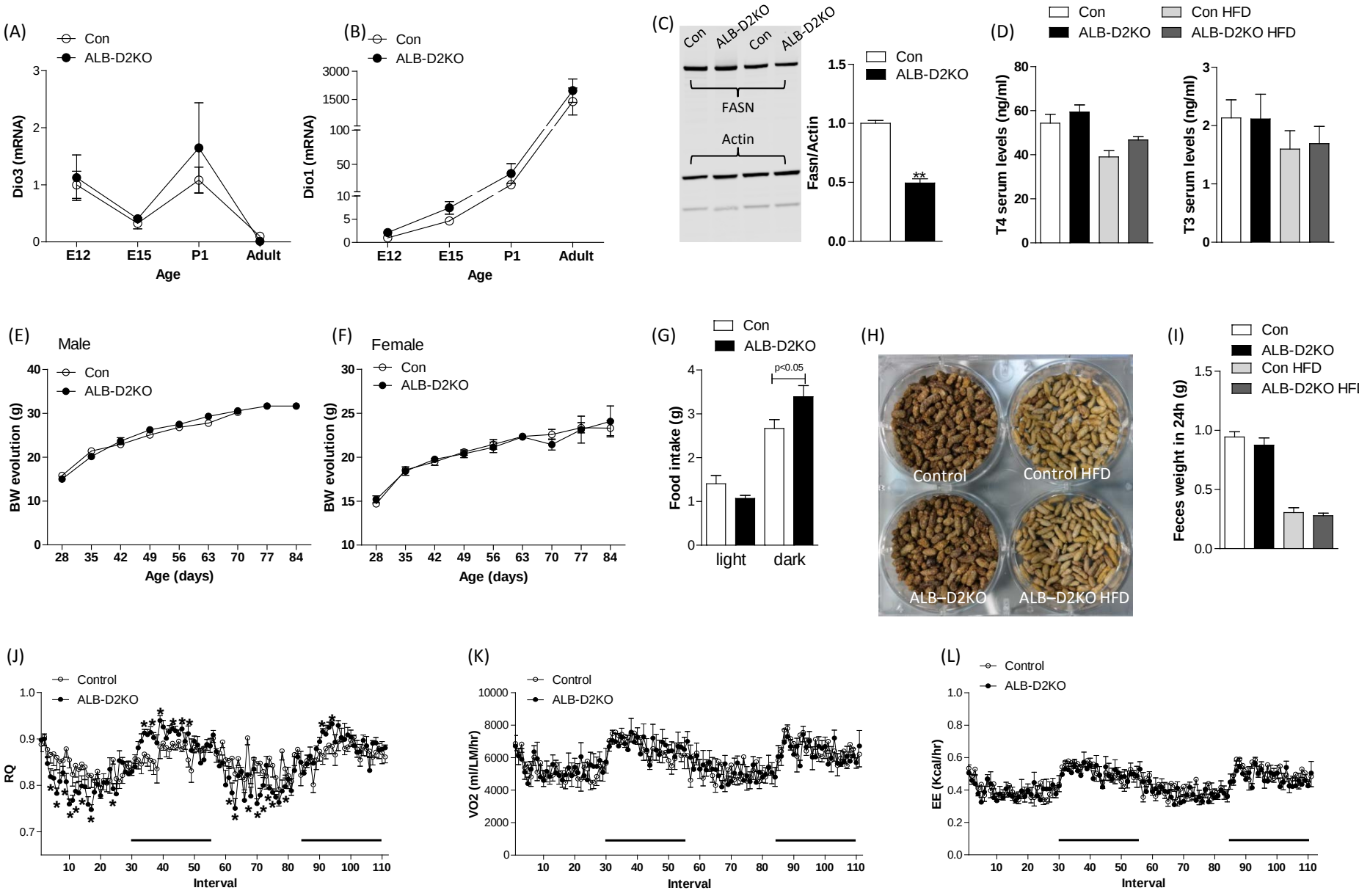
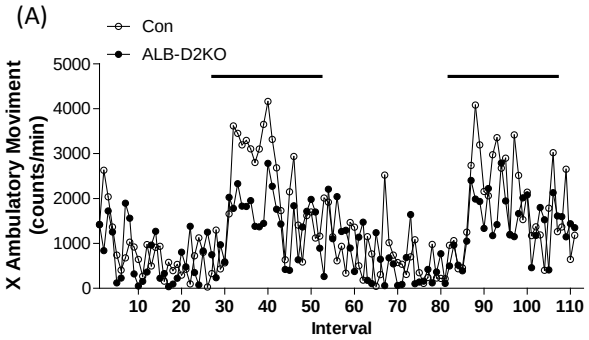
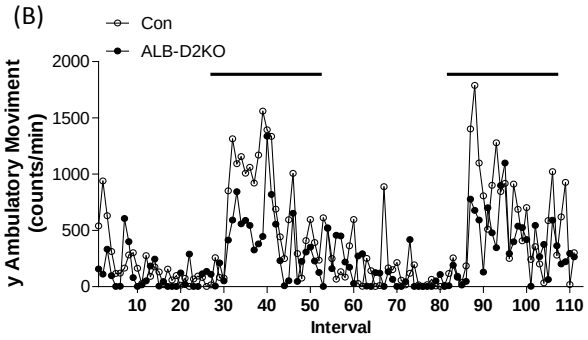
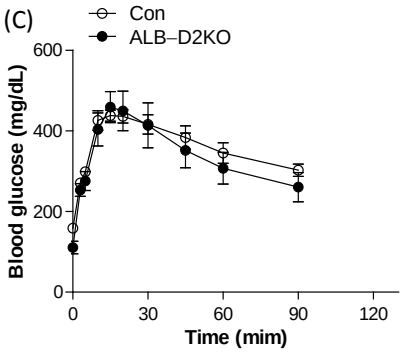
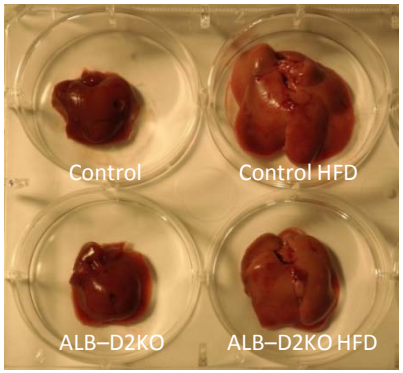

(A)
Con
ALB-D2KO
Dio3 (mRNA)
E12
E15
P1
Adult
Age
(B)
Con
ALB-D2KO
Dio1 (mRNA)
E12
E15
P1
Adult
Age
(C)
Con
ALB-D2KO
Con
ALB-D2KO
FASN
Actin
Con
ALB-D2KO
Fasn/Actin
**
(D)
Con
ALB-D2KO
Con HFD
ALB-D2KO HFD
T4 serum levels (ng/ml)
T3 serum levels (ng/ml)
(E)
Male
Con
ALB-D2KO
BW evolution (g)
Age (days)
(F)
Female
Con
ALB-D2KO
BW evolution (g)
Age (days)
(G)
Con
ALB-D2KO
p<0.05
Food intake (g)
light
dark
(H)
Control
Control HFD
ALB–D2KO
ALB–D2KO HFD
(I)
Con
ALB-D2KO
Con HFD
ALB-D2KO HFD
Feces weight in 24h (g)
(J)
Control
ALB-D2KO
RQ
Interval
(K)
Control
ALB-D2KO
VO2 (ml/LM/hr)
Interval
(L)
Control
ALB-D2KO
EE (Kcal/hr)
Interval

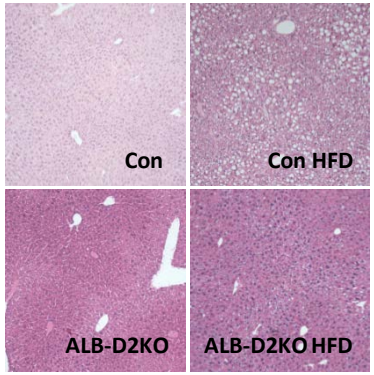
Fig. S2

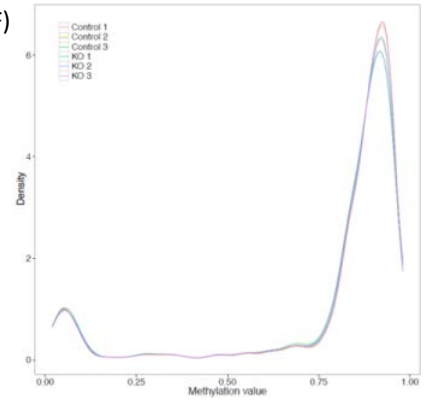

(A)
Con
ALB-D2KO
X Ambulatory Moviment (counts/min)
Interval

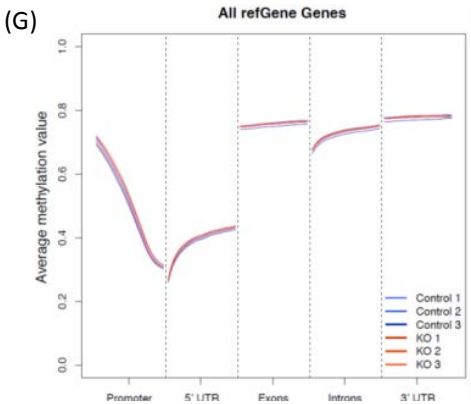

(B)
Con
ALB-D2KO
y Ambulatory Moviment (counts/min)
Interval

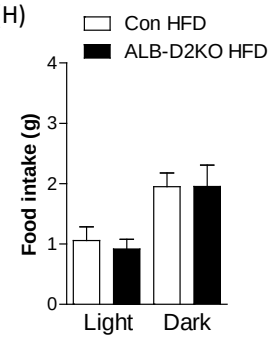

(C)
Con
ALB–D2KO
Blood glucose (mg/dL)
Time (mim)

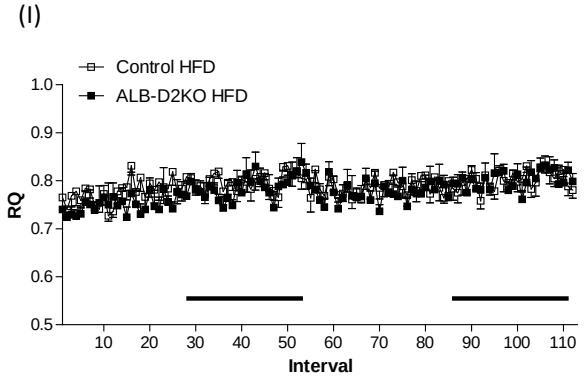

(D)
Control
Control HFD
ALB–D2KO
ALB–D2KO HFD


(E)
Con
Con HFD
ALB-D2KO
ALB-D2KO HFD


(F)
Control 1
Control 2
Control 3
KO 1
KO 2
KO 3
Density
Methylation value


(G)
All refGene Genes
Average methylation value
Control 1
Control 2
Control 3
KO 1
KO 2
KO 3
Promoter
5' UTR
Exons
Introns
3' UTR


(H)
Con HFD
ALB-D2KO HFD
Food intake (g)
Light
Dark


(I)
Control HFD
ALB-D2KO HFD
RQ
Interval

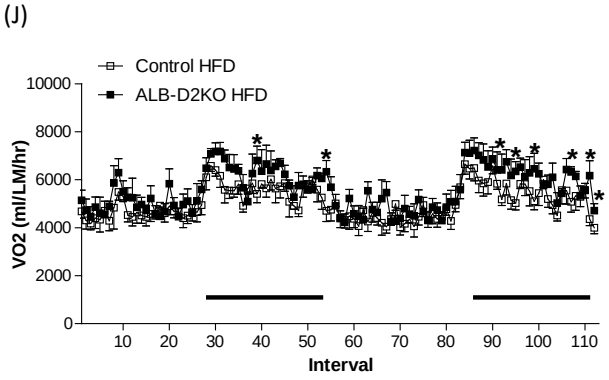

(J)
Control HFD
ALB-D2KO HFD
VO2 (ml/LM/hr)
Interval

Fig. S3

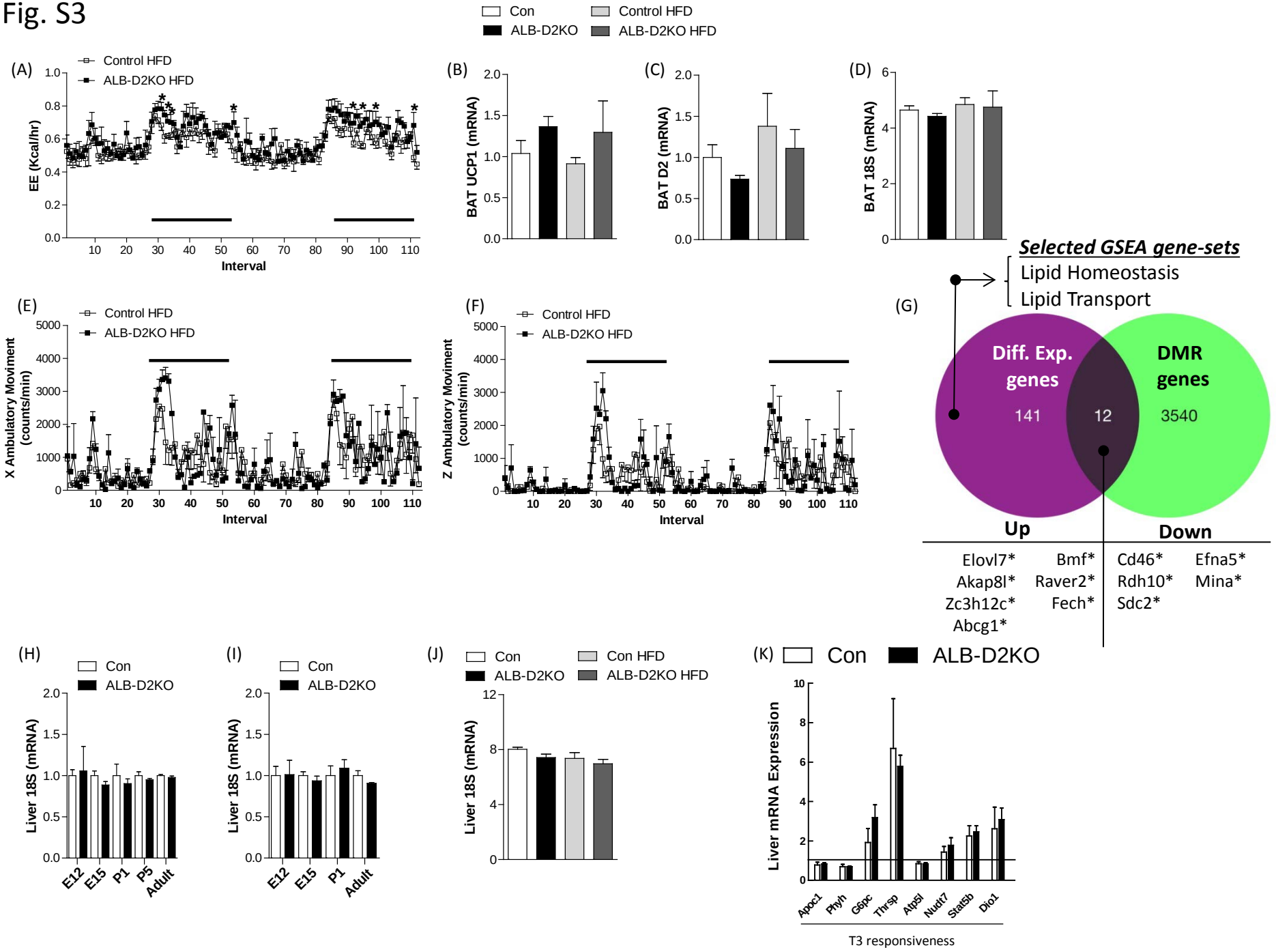

Con
ALB-D2KO
Control HFD
ALB-D2KO HFD
(A)
EE (Kcal/hr)
Interval
(B)
BAT UCP1 (mRNA)
(C)
BAT D2 (mRNA)
(D)
BAT 18S (mRNA)
(E)
X Ambulatory Moviment (counts/min)
(F)
Z Ambulatory Moviment (counts/min)
(G)
Selected GSEA gene-sets
Lipid Homeostasis
Lipid Transport
Diff. Exp. genes
141
12
DMR genes
3540
Up
Down
Elovl7*
Akap8l*
Zc3h12c*
Abcg1*
Bmf*
Raver2*
Fech*
Cd46*
Rdh10*
Sdc2*
Efna5*
Mina*
(H)
Liver 18S (mRNA)
E12
E15
P1
P5
Adult
(I)
(J)
Con HFD
ALB-D2KO HFD
(K)
Liver mRNA Expression
Apoc1
Ptyh
G6pc
Thrsp
Atp5l
Nudt7
Stat5b
Dio1
T3 responsiveness

Fig. S4

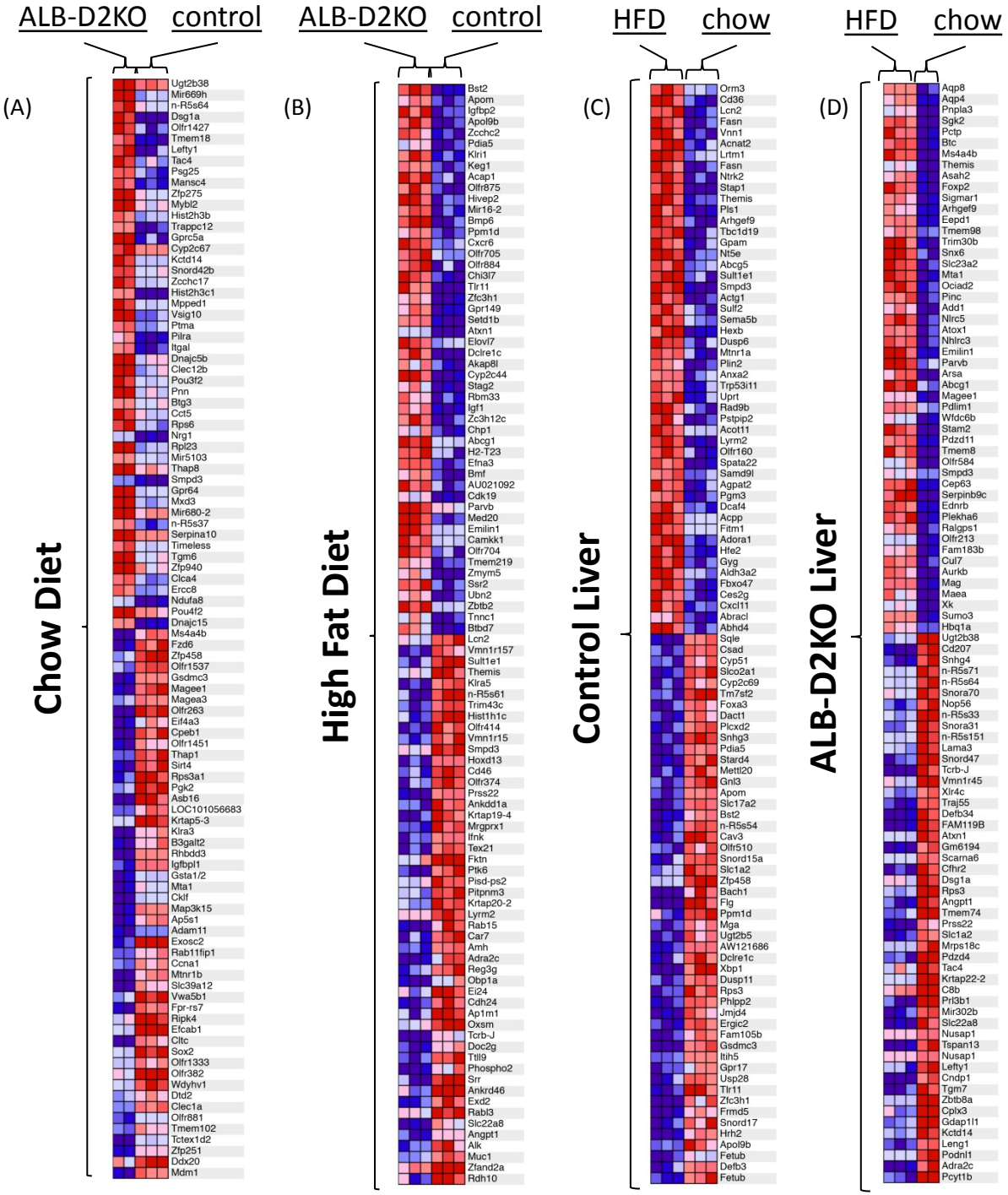
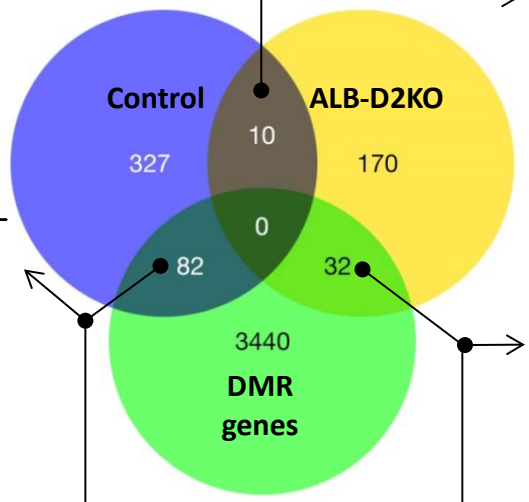

ALB-D2KO
control
(A)
Chow Diet
Ugt2b38
Mir669h
n-R5s64
Dsg1a
Olfr1427
Tmem18
Lefty1
Tac4
Psg25
Mansc4
Zfp275
Mybl2
Hist2h3b
Trappc12
Gprc5a
Cyp2c67
Kctd14
Snord42b
Zcchc17
Hist2h3c1
Mpped1
Vsig10
Ptma
Pilra
Itgal
Dnajc5b
Clec12b
Pou3f2
Pnn
Btg3
Cct5
Rps6
Nrg1
Rpl23
Mir5103
Thap8
Smpd3
Gpr64
Mxd3
Mir680-2
n-R5s37
Serpina10
Timeless
Tgm6
Zfp940
Clca4
Ercc8
Ndufa8
Pou4f2
Dnajc15
Ms4a4b
Fzd6
Zfp458
Olfr1537
Gsdmc3
Magee1
Magea3
Olfr263
Eif4a3
Cpeb1
Olfr1451
Thap1
Sirt4
Rps3a1
Pgk2
Asb16
LOC101056683
Krtap5-3
Klra3
B3galt2
Rhbdd3
Igfbpl1
Gsta1/2
Mta1
Cklf
Map3k15
Ap5s1
Adam11
Exosc2
Rab11fip1
Ccna1
Mtnr1b
Slc39a12
Vwa5b1
Fpr-rs7
Ripk4
Efcab1
Cltc
Sox2
Olfr1333
Olfr382
Wdyhv1
Dtd2
Clec1a
Olfr881
Tmem102
Tctex1d2
Zfp251
Ddx20
Mdm1
ALB-D2KO
control
(B)
High Fat Diet
Bst2
Apom
Igfbp2
Apol9b
Zcchc2
Pdia5
Klri1
Keg1
Acap1
Olfr875
Hivep2
Mir16-2
Bmp6
Ppm1d
Cxcr6
Olfr705
Olfr884
Chi3l7
Tlr11
Zfc3h1
Gpr149
Setd1b
Atxn1
Elovl7
Dclre1c
Akap8l
Cyp2c44
Stag2
Rbm33
Igf1
Zc3h12c
Chp1
Abcg1
H2-T23
Efna3
Bmf
AU021092
Cdk19
Parvb
Med20
Emilin1
Camkk1
Olfr704
Tmem219
Zmym5
Ssr2
Ubn2
Zbtb2
Tnnc1
Btbd7
Lcn2
Vmn1r157
Sult1e1
Themis
Klra5
n-R5s61
Trim43c
Hist1h1c
Olfr414
Vmn1r15
Smpd3
Hoxd13
Cd46
Olfr374
Prss22
Ankdd1a
Krtap19-4
Mrgprx1
Ifnk
Tex21
Fktn
Ptk6
Pisd-ps2
Pitpnm3
Krtap20-2
Lyrm2
Rab15
Car7
Amh
Adra2c
Reg3g
Obp1a
Ei24
Cdh24
Ap1m1
Oxsm
Tcrb-J
Doc2g
Tll9
Phospho2
Srr
Ankrd46
Exd2
Rabl3
Slc22a8
Angpt1
Alk
Muc1
Zfand2a
Rdh10
HFD
chow
(C)
Control Liver
Orm3
Cd36
Lcn2
Fasn
Vnn1
Acnat2
Lrtm1
Fasn
Ntrk2
Stap1
Themis
Pls1
Arhgef9
Tbc1d19
Gpam
Nt5e
Abcg5
Sult1e1
Smpd3
Actg1
Sulf2
Sema5b
Hexb
Dusp6
Mtnr1a
Plin2
Anxa2
Trp53i11
Uprt
Rad9b
Pstpip2
Acot11
Lyrm2
Olfr160
Spata22
Smd9l
Agpat2
Pgm3
Dcaf4
Acpp
Fitm1
Adora1
Hfe2
Gyg
Aldh3a2
Fbxo47
Ces2g
Cxcl11
Abracl
Abhd4
Sqle
Csad
Cyp51
Slco2a1
Cyp2c69
Tm7sf2
Foxa3
Dact1
Plcxd2
Snhg3
Pdia5
Stard4
Mettl20
Gnl3
Apom
Slc17a2
Bst2
n-R5s54
Cav3
Olfr510
Snord15a
Slc1a2
Zfp458
Bach1
Fig
Ppm1d
Mga
Ugt2b5
AW121686
Dclre1c
Xbp1
Dusp11
Rps3
Phlpp2
Jmjd4
Ergic2
Fam105b
Gsdmc3
Itih5
Gpr17
Usp28
Tlr11
Zfc3h1
Frmd5
Snord17
Hrh2
Apol9b
Fetub
Defb3
Fetub
HFD
chow
(D)
ALB-D2KO Liver
Aqp8
Aqp4
Pnpla3
Sgk2
Pctp
Btc
Ms4a4b
Themis
Asah2
Foxp2
Sigmar1
Arhgef9
Eepd1
Tmem98
Trim30b
Snx6
Slc23a2
Mta1
Ociad2
Pinc
Add1
Nlrc5
Atox1
Nhlrc3
Emilin1
Parvb
Arsa
Abcg1
Magee1
Pdlim1
Wfdc6b
Stam2
Pdzd11
Tmem8
Olfr584
Smpd3
Cep63
Serpinb9c
Ednrb
Plekha6
Ralgps1
Olfr213
Fam183b
Cul7
Aurkb
Mag
Maea
Xk
Sumo3
Hbq1a
Ugt2b38
Cd207
Snhg4
n-R5s71
n-R5s64
Snora70
Nop56
n-R5s33
Snora31
n-R5s151
Lama3
Snord47
Tcrb-J
Vmn1r45
Xlr4c
Traj55
Defb34
FAM119B
Atxn1
Gm6194
Scarna6
Cfhr2
Dsg1a
Rps3
Angpt1
Tmem74
Prss22
Slc1a2
Mrps18c
Pdzd4
Tac4
Krtap22-2
C8b
Prl3b1
Mir302b
Slc22a8
Nusap1
Tspan13
Nusap1
Lefty1
Cndp1
Tgm7
Zbtb8a
Cplx3
Gdap1l1
Kctd14
Leng1
Podnl1
Adra2c
Pcyt1b

Fig. S5




***Up regulated***

Acpp, Aga, Agxt2l2, Anxa2, Athl1, C4b, Cbr4, Ccnh, Celsr1, Col15a1, Cxcl11, Dennd2d, Dusp6, Fam92a, Fut8, **Gpam**, Hfe2, Hsbp1, Khdrbs3, Klhl5, **Lclat1**, Lgals8, Lrtm1, Mina, Mms22l, Mut, Nrg1, Ntrk2, Pex12, Pgm3, Rad9b, Rhbdf2, Sema5b, Sesn3, Shkbp1, Slc25a21, Slc2a5, Slc38a6, Sulf2, Supt7l, Tbc1d19, Tmem126b, Tnfrsf19, Top1mt, Tpm2, Trp53i11, Wfdc2, Zbtb37

***Down regulated***

Akap8l, Amfr, Atoh7, B3gnt2, Bach1, Cdc42bpb, Csrp2, Eif1a, Ephb3, Fam109a, Fech, Folr4, Frmd5, Golga4, Hectd1, Kcmf1, Nt5c3, Onecut2, Prdm2, Ptpn3, Ptpru, Rab20, Slc38a2, Slc6a6, Slc7a14, Stard4, Tbx15, Terf2ip, Tmem183a, Ubp1, Vwa5b1, Wdfy3, Wipi1, Zmym2

Arhgef9, Asah2, Nudt21, Ralgps1, Rps3, Slc1a2, Smpd3, Tcrb, Themis, Zfand2a

***Up regulated***

**abcg1**, aqp4, arsa, cdkl4, cul7, ednrb, eepd1, foxp2, mef2c, nlrc5, plekha6, slc23a2, slc39a12, stam2, tmem8, vegfc

***Down regulated***

Brd3, Cd207, Dmrt1, Enthd1, Fam78a, Kctd14, Mgst2, Mpped1, Podnl1, Skor2, Srcin1, Syt6, Tac4, Tmem74, Tspan13, Zfp236

## Supplemental Figures Legends

**Suppl. 1** – Relative liver Dio3 (A) and Dio1 (B) mRNA levels at the indicated ages; (C) Representative Western blot analysis of FASN and quantitation by densitometry; Actin was used as internal control; (D) Serum T4 and T3 Levels of control and ALB-D2KO mice (n=5-6); (E) Body weight evolution in males of ALB-D2KO and controls; (F) Body weight evolution in female ALB-D2KO and control animals; (G) Food intake measured by the C.L.A.M.S. during light and dark cycles in ALB-D2KO and control animals fed with chow diet; (H) Gross fecal morphology in ALB-D2KO mouse and controls after 7 weeks of chow diet or HFD and (I) Quantification of the fecal volume collected during 72h; the result is shown as total content in 24h period; Metabolic profile of ALB-D2KO and controls animals: Respiratory coefficient (J), oxygen consumption (K) corrected by lean body mass in grams and energy expenditure (L) in animals fed with chow diet; all the measures were made on the third day, after 48h of acclimatization to individual metabolic cages in the C.L.A.M.S. Entries are mean ± SEM of 4-6 animals *$p<0.05$ and ** $p<0.01$ vs. respective control.

**Suppl. 2** - (A) X ambulatory movement and (B) Y ambulatory movement measured by the C.L.A.M.S. during light and dark cycles in ALB-D2KO and control animals fed with chow diet; (C) Blood glucose concentrations at the indicated time points after IP glucose injection (2g/kg) in 2-month-old ALB-D2KO and controls animals; (D) Gross liver morphological findings in ALB-D2KO mouse and controls after 7 weeks of chow diet or HFD; (E) representative liver slide stained with H&E (original magnification, ×10); (F) Global methylation profile in the ALB-D2KO and control animals; (G) Methylation profile of different regions - promoter, 5UTR, exons, introns and 3UTR in the ALB-D2KO and control animals; (H) Food intake measured by the C.L.A.M.S. during light and dark cycles in ALB-D2KO and control animals fed with HFD for 7 weeks; Metabolic profile of ALB-D2KO and controls animals fed with high fat diet: Respiratory coefficient (I) and oxygen consumption (J) corrected by lean body mass in grams. Entries are mean ± SEM of 4-6 animals. *$p<0.05$ vs. respective control.

**Suppl. 3** - (A) Energy expenditure in animals fed with high fat diet; Relative Ucp-1mRNA (B) and Dio2 mRNA (C) levels in BAT of ALB-D2KO and control mice fed with chow diet and HFD. Results are expressed using 18S mRNA levels as internal control and normalized to the levels observed in the control animals fed with chow diet; (D) Relative 18S mRNA levels in adult BAT of ALB-D2KO and control mice fed with chow diet and HFD used to normalized the gene expression levels observed in B and C; (E) X ambulatory movement and (F) Y ambulatory movement measured by the C.L.A.M.S. during light and dark cycles in ALB-D2KO and control animals feeding with HFD; (G) Venn diagram indicating common genes between (i) differential expression analysis of HFD-ALB-D2KO vs. HFD-control mouse liver microarray (Dataset S6) and (ii) differentially methylated regions (DMRs) (Dataset S3); some of the gene sets by GSEA and the 12 overlapping genes are indicated; (H) Relative 18S mRNA levels in embryonic (E12 and E15), neonatal (P1), newborns with 5 days of age (P5) and adult

livers of ALB-D2KO and controls; this data was used to normalized the gene expression levels observed in the figure 1A; (I) Relative 18S mRNA levels in embryonic (E12 and E15), neonatal (P1) and adult livers of ALB-D2KO and control; this data was used to normalized the gene expression levels observed in the figure 2; (J) Relative 18S mRNA levels in adult liver of ALB-D2KO and control mice fed with chow diet and HFD used to normalize the gene expression levels observed in figure 5; (K) Relative mRNA levels of T3-responsive genes in hypothyroid mice treated with T3. Results are expressed using 18S mRNA levels as internal control and normalized to the levels observed in the respective control hypothyroid (black line); Entries are mean ± SEM of 3-7 animals. *$p<0.05$ vs. respective control.

**Suppl. 4** - Effect of D2 inactivation on the Liver transcriptome in ALB-D2KO mouse. (A) heat map of the 50 most enriched and 50 most down-regulated genes in adult ALB-D2KO liver and controls by differential expression analysis where expression values are represented as colors (red: higher level; blue: lower level) with the degree of color saturation indicating degree of expression; (B) Same as in A, but except that comparison is made between both groups in HFD and (C-D) Same as in A, but except that comparison is made between chow and HFD for both groups.

**Suppl. 5** – Liver transcriptome modifications in response to HFD in ALB-D2KO vs control mice. Venn diagram indicating common HFD-enriched genes between control –Dataset S8- (blue circle) and ALB-D2KO –Dataset S9- (yellow circle) animals vs DMRs of the same samples (Dataset S3); all overlapping genes area are indicated.

# Supplemental Tables

**Table S1**- Mendelian distribution between genotypes

| | | **Flox D2 allele** | | **Cre allele** | |
|---|---|---|---|---|---|
| Percentage | WT | FloxD2 Homozygous | FloxD2 Heterozygous | WT | CRE |
| *Expected* | *25%* | *25%* | *50%* | *25%* | *75%* |
| acquire | 26% | 24% | 51% | 27% | 73% |

Note: percentage was calculated using 180 animals born from 8 different breeders. The average number of pups from each litter was 9.

**Table S2-** Morphometric data from ALB-D2KO vs. control mice kept on chow diet.

| Tissue | Control | ALB-D2KO |
|---|---|---|
| BAT | 0.05±0.002 | 0.05±0.005 |
| Brain | 0.18±0.002 | 0.19±0.006 |
| Epidid. fat | 0.24±0.01 | 0.21±0.03 |
| Liver | 0.53±0.02 | 0.56±0.03 |
| Muscle | 0.05±0.001 | 0.06±0.004 |
| Pancreas | 0.09±0.007 | 0.09±0.005 |
| BMD | 55.6±0.0008 | 56.4±0.0008 |

All tissues are express in *g of tissue/tibia length cm*. BMD: bone mineral density ($mg/cm^2$). Tissues weigh and BMD was performed with animals in 14 weeks of age. All values in the table are ±SEM (n=3-10).

**Table S3**- Enriched GO terms in methylated regions (DMRs) as observed in GREAT analysis obtained from adult ALB-D2KO and control mice kept on a chow diet.

| # GREAT version 2.0.2<br># Term Name | Binom Rank | Binom Raw P-Value | Binom FDR Q-Val | Binom Fold Enrichment | Binom Observed Region Hits | Binom Region Set Coverage | Hyper Rank | Hyper FDR Q-Val | Hyper Fold Enrichment | Hyper Observed Gene Hits | Hyper Total Genes | Hyper Gene Set Coverage |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Enriched in ALB-D2KO on chow diet* | | | | | | | | | | | | |
| Neuromuscular process controlling balance | 98 | 0.000003 | 0.0002 | 2.18 | 44 | 0.014 | 313 | 0.0009 | 2.41 | 21 | 46 | 0.005 |
| Myoblast cell fate commitment | 106 | 0.000004 | 0.0004 | 4.91 | 13 | 0.004 | 436 | 0.0048 | 5.28 | 5 | 5 | 0.001 |
| Skin development | 109 | 0.000005 | 0.0004 | 2.15 | 43 | 0.014 | 432 | 0.0046 | 2.11 | 22 | 55 | 0.006 |
| Exploration behavior | 118 | 0.000008 | 0.0006 | 4.61 | 13 | 0.004 | 516 | 0.0150 | 3.96 | 6 | 8 | 0.002 |
| Negative regulation of lipid biosynthetic process | 125 | 0.000011 | 0.0008 | 2.73 | 25 | 0.008 | 414 | 0.0040 | 2.56 | 15 | 31 | 0.004 |
| Regulation of dendritic spine morphogenesis | 128 | 0.000012 | 0.0008 | 3.37 | 18 | 0.006 | 630 | 0.0381 | 3.08 | 7 | 12 | 0.002 |
| Negative regulation of lipid metabolic process | 153 | 0.000045 | 0.0025 | 2.32 | 29 | 0.009 | 492 | 0.0107 | 2.16 | 18 | 44 | 0.005 |
| Ureter development | 187 | 0.000139 | 0.0064 | 2.62 | 20 | 0.006 | 516 | 0.0150 | 3.96 | 6 | 8 | 0.002 |
| Peyer's patch development | 193 | 0.000155 | 0.0069 | 3.67 | 12 | 0.004 | 614 | 0.0318 | 3.52 | 6 | 9 | 0.002 |
| Mesonephros development | 224 | 0.000330 | 0.0126 | 2.33 | 22 | 0.007 | 482 | 0.0102 | 2.93 | 10 | 18 | 0.003 |
| Nephron tubule formation | 230 | 0.000352 | 0.0131 | 2.27 | 23 | 0.007 | 304 | 0.0008 | 3.77 | 10 | 14 | 0.003 |
| Metanephric collecting duct development | 252 | 0.000484 | 0.0165 | 3.72 | 10 | 0.003 | 333 | 0.0012 | 5.28 | 6 | 6 | 0.002 |
| Negative regulation of reproductive process | 259 | 0.000561 | 0.0186 | 2.15 | 24 | 0.008 | 414 | 0.0040 | 2.56 | 15 | 31 | 0.004 |
| Positive regulation of cell morphogenesis involved in differentiation | 264 | 0.000600 | 0.0195 | 2.06 | 26 | 0.008 | 351 | 0.0016 | 2.99 | 13 | 23 | 0.003 |
| Mammary gland epithelial cell differentiation | 265 | 0.000602 | 0.0195 | 2.46 | 18 | 0.006 | 509 | 0.0143 | 3.25 | 8 | 13 | 0.002 |
| Regulation of neurotransmitter transport | 276 | 0.000682 | 0.0212 | 2.05 | 26 | 0.008 | 463 | 0.0074 | 2.35 | 16 | 36 | 0.004 |
| Metanephric nephron morphogenesis | 285 | 0.000817 | 0.0246 | 2.09 | 24 | 0.008 | 267 | 0.0004 | 3.52 | 12 | 18 | 0.003 |
| Lens induction in camera-type eye | 290 | 0.000909 | 0.0269 | 2.85 | 13 | 0.004 | 443 | 0.0052 | 4.53 | 6 | 7 | 0.002 |
| Embryonic camera-type eye morphogenesis | 295 | 0.000965 | 0.0281 | 2.03 | 25 | 0.008 | 456 | 0.0066 | 2.76 | 12 | 23 | 0.003 |
| Actin cytoskeleton reorganization | 307 | 0.001060 | 0.0296 | 2.05 | 24 | 0.008 | 421 | 0.0041 | 2.64 | 14 | 28 | 0.004 |

Binom Rank: Binomial rank; Binom Raw P-Value: Binomial raw P-Value; Binom FDR q-val: Binomial false discovery rate q-value; Biom Fold Enrichment: Binomial Fold Enrichment; Binom Observed Region Hits: Binomial observed region hits and Binom Region Set Coverage: Binomial region set coverage.
Hyper Rank: Hyperbrowser rank; Hyper FDR q-val: Hyperbrowser false discovery rate q-value; Hyper Fold Enrichment: Hyperbrowser fold enrichment; Hyper observed gene Hits: Hyperbrowser observed gene hits; Hyper total genes: Hyperbrowser total genes and Hyper gene Set Coverage: Hyperbrowser gene set coverage.
The test set of 3,077 genomic regions picked 3,828 (19%) of all 20,221 genes.
GO Biological Process has 8,583 terms covering 15,210 (75%) of all 20,221 genes, and 605,197 term - gene associations.
8,583 ontology terms (100%) were tested using an annotation count range of [1, Inf].

**Table S4-** Genomic Regions Enrichment of Annotations Tool platform of positive DMRs in the Liver of ALB-D2KO and control mice. (ALB-D2KO: n = 3, control: n = 3). Gene ontology biological process - GO:0051055 Negative regulation of lipid biosynthetic process.

| Gene Symbol | Description | DMR region |
|---|---|---|
| Atp1a1 | ATPase, Na+/K+ transporting, alpha 1 polypeptide | chr3:101621500-101622000<br>chr3:101213500-101214000 |
| Fgf15 | fibroblast growth factor 15 | chr7:152095000-152095500 |
| Hrh1 | histamine receptor H1 | chr6:114358500-114359000 |
| Insig2 | insulin induced gene 2 | chr1:123222500-123223000<br>chr1:123217000-123217500<br>chr1:122860500-122861000 |
| Mup5 | major urinary protein 5 | chr4:61377000-61377500<br>chr4:61363000-61363500<br>chr4:61360000-61360500<br>chr4:61238500-61239000<br>chr4:61225500-61226000 |
| Mup7 | major urinary protein 7 | chr4:60133500-60134000 |
| Mup8 | major urinary protein 9 | chr4:60133500-60134000 |
| Pdgfa | platelet derived growth factor, alpha | chr5:139432500-139433000<br>chr5:139351500-139352000 |
| Pdgfb | platelet derived growth factor, B polypeptide | chr15:79908500-79909000<br>chr15:79780500-79781000 |
| Prox1 | prospero-related homeobox 1 | chr1:192140000-192140500 |
| Sik1 | Salt inducible kinase 1 | chr17:31871500-31872000 |
| Snai1 | snail homolog 1 (Drosophila) | chr2:167399000-167399500<br>chr2:167400500-167401000 |
| Sod1 | superoxide dismutase 1, soluble | chr16:90098000-90098500<br>chr16:90126000-90126500 |
| Trib3 | tribbles homolog 3 (Drosophila) | chr2:152195000-152195500 |
| Wnt4 | wingless-related MMTV integration site 4 | chr4:136628000-136628500 |

DMRs: Differentially methylated regions; chr: chromosome

**Table S5-** Genomic Regions Enrichment of Annotations Tool platform of positive DMRs in the Liver of ALB-D2KO and control mice. (ALB-D2KO: n = 3, control: n = 3). Gene ontology biological process - GO:0045833 Negative regulation of lipid metabolic process.

| Gene Symbol | Description | DMR region |
|---|---|---|
| Adra2a | adrenergic receptor, alpha 2a | chr19:54123500-54124000<br>chr19:54963500-54964000 |
| Atp1a1 | ATPase, Na+/K+ transporting, alpha 1 polypeptide | chr3:101621500-101622000<br>chr3:101213500-101214000 |
| Cidea | cell death-inducing DNA fragmentation factor, alpha | chr18:67459000-67459500 |
| Cnr1 | cannabinoid receptor 1 (brain) | chr4:33613500-33614000 |
| Fgf15 | fibroblast growth factor 15 | chr7:152095000-152095500 |
| Hrh1 | histamine receptor H1 | chr6:114358500-114359000 |
| Insig2 | insulin induced gene 2 | chr1:123222500-123223000<br>chr1:123217000-123217500<br>chr1:122860500-122861000 |
| Mup5 | major urinary protein 5 | chr4:61377000-61377500<br>chr4:61363000-61363500<br>chr4:61360000-61360500<br>chr4:61238500-61239000<br>chr4:61225500-61226000 |
| Mup7 | major urinary protein 7 | chr4:60133500-60134000 |
| Mup8 | major urinary protein 9 | chr4:60133500-60134000 |
| Pdgfa | platelet derived growth factor, alpha | chr5:139432500-139433000<br>chr5:139351500-139352000 |
| Pdgfb | platelet derived growth factor, B polypeptide | chr15:79908500-79909000<br>chr15:79780500-79781000 |
| Prox1 | prospero-related homeobox 1 | chr1:192140000-192140500 |
| Sik1 | salt inducible kinase 1 | chr17:31871500-31872000 |
| Snai1 | snail homolog 1 (Drosophila) | chr2:167399000-167399500<br>chr2:167400500-167401000 |
| Sod1 | superoxide dismutase 1, soluble | chr16:90098000-90098500<br>chr16:90126000-90126500 |
| Trib3 | tribbles homolog 3 (Drosophila) | chr2:152195000-152195500 |
| Wnt4 | wingless-related MMTV integration site 4 | chr4:136628000-136628500 |

DMRs: Differentially methylated regions; chr: chromosome.

**Table S6**- Molecular signature pathway in methylated regions (DMRs) as observed in GREAT analysis obtained from adult ALB-D2KO and control mice kept on a chow diet.

| # GREAT version 2.0.2<br># Term Name | Binom Rank | Binom Raw P-Value | Binom FDR Q-Val | Binom Fold Enrichment | Binom Observed Region Hits | Binom Region Set Coverage | Hyper Rank | Hyper FDR Q-Val | Hyper Fold Enrichment | Hyper Observed Gene Hits | Hyper Total Genes | Hyper Gene Set Coverage |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Enriched in ALB-D2KO on chow diet* | | | | | | | | | | | | |
| Glioma | 1 | 0.0000001 | 0.00009 | 2.45 | 45 | 0.015 | 8 | 0.006 | 2.16 | 25 | 61 | 0.007 |
| Ca++/ Calmodulin-dependent Protein Kinase Activation | 4 | 0.0000004 | 0.00008 | 3.96 | 20 | 0.006 | 11 | 0.008 | 3.66 | 9 | 13 | 0.002 |
| Genes involved in CREB phosphorylation through the activation of CaMKII | 7 | 0.0000026 | 0.00032 | 3.36 | 21 | 0.007 | 28 | 0.030 | 2.97 | 9 | 16 | 0.002 |
| Transcription factor CREB and its extracellular signals | 8 | 0.0000151 | 0.00166 | 2.68 | 25 | 0.008 | 43 | 0.043 | 2.35 | 12 | 27 | 0.003 |
| Genes involved in CREB phophorylation through the activation of Ras | 15 | 0.0002025 | 0.01188 | 2.31 | 24 | 0.008 | 35 | 0.036 | 2.44 | 12 | 26 | 0.003 |
| Cyclins and Cell Cycle Regulation | 16 | 0.0002253 | 0.01239 | 2.87 | 16 | 0.005 | 27 | 0.030 | 2.54 | 12 | 25 | 0.003 |
| Genes involved in HDL-mediated lipid transport | 18 | 0.0002525 | 0.01234 | 4.04 | 10 | 0.003 | 7 | 0.006 | 4.23 | 8 | 10 | 0.002 |
| Genes involved in Collagen-mediated activation cascade | 19 | 0.0002813 | 0.01303 | 2.36 | 22 | 0.007 | 13 | 0.008 | 2.86 | 13 | 24 | 0.003 |
| Genes involved in G1 Phase | 20 | 0.0004051 | 0.01783 | 3.11 | 13 | 0.004 | 28 | 0.030 | 2.97 | 9 | 16 | 0.002 |
| Genes involved in Integrin alphaIIbbeta3 signaling | 31 | 0.0010389 | 0.02949 | 2.58 | 15 | 0.005 | 22 | 0.014 | 2.76 | 12 | 23 | 0.003 |
| Genes involved in Platelet Aggregation (Plug Formation) | 41 | 0.0021308 | 0.04573 | 2.24 | 17 | 0.006 | 21 | 0.014 | 2.64 | 13 | 26 | 0.003 |
| Actin cytoskeleton reorganization | 307 | 0.001060 | 0.0296 | 2.05 | 24 | 0.008 | 421 | 0.0041 | 2.64 | 14 | 28 | 0.004 |

Binom Rank: Binomial rank; Binom Raw P-Value: Binomial raw P-Value; Binom FDR q-val: Binomial false discovery rate q-value; Biom Fold Enrichment: Binomial Fold Enrichment; Binom Observed Region Hits: Binomial observed region hits and Binom Region Set Coverage: Binomial region set coverage.
Hyper Rank: Hyperbrowser rank; Hyper FDR q-val: Hyperbrowser false discovery rate q-value; Hyper Fold Enrichment: Hyperbrowser fold enrichment; Hyper observed gene Hits: Hyperbrowser observed gene hits; Hyper total genes: Hyperbrowser total genes and Hyper gene Set Coverage: Hyperbrowser gene set coverage.
The test set of 3,077 genomic regions picked 3,828 (19%) of all 20,221 genes.
MSigDB Pathway has 880 terms covering 5,986 (30%) of all 20,221 genes, and 37,583 term - gene associations.
880 ontology terms (100%) were tested using an annotation count range of [1, Inf].

**Table S7-** Genomic Regions Enrichment of Annotations Tool platform of positive DMRs in the Liver of ALB-D2KO and control mice. (ALB-D2KO: n = 3, control: n = 3). Molecular signature pathway – Systematic name M5056: Genes involved in HDL- mediated lipid transport.

| Gene Symbol | Description | DMR region |
|---|---|---|
| Abca1 | ATP-binding cassette, sub-family A (ABC1), member 1 | chr4:53122000-53122500 |
| Abcg1 | ATP-binding cassette, sub-family G (WHITE), member 1 | chr17:31262000-31262500 |
| Alb | albumin | chr5:90860500-90861000 |
| Amn | amnionless | chr12:112595500-112596000 |
| Apoa1 | apolipoprotein A-I | chr9:45907000-45907500<br>chr9:46007000-46007500 |
| Cubn | cubilin (intrinsic factor-cobalamin receptor) | chr2:13413000-13413500 |
| Lcat | lecithin cholesterol acyltransferase | chr8:108485000-108485500 |
| Scarb1 | scavenger receptor class B, member 1 | chr5:125822000-125822500<br>chr5:125679500-125680000 |

DMRs: Differentially methylated regions.

**Table S8**- Mouse Genome Informatics – phenotype in 437 DMRs overlapped with k4me1/3 and K36me3 (active chromatin state) as observed in GREAT analysis obtained from adult ALB-D2KO mice.

| # GREAT version 2.0.2<br># Term Name | Binom Rank | Binom Raw P-Value | Binom FDR Q-Val | Binom Fold Enrichment | Binom Observed Region Hits | Binom Region Set Coverage | Hyper Rank | Hyper FDR Q-Val | Hyper Fold Enrichment | Hyper Observed Gene Hits | Hyper Total Genes | Hyper Gene Set Coverage |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| abnormal hepatobiliary system physiology | 3 | 7.91E-07 | 0.0019 | 2.49 | 36 | 0.0824 | 44 | 0.0046 | 2.27 | 30 | 363 | 0.04 |
| abnormal thymus size | 4 | 9.22E-07 | 0.0017 | 2.47 | 36 | 0.0824 | 18 | 0.0005 | 2.57 | 32 | 342 | 0.04 |
| small thymus | 5 | 1.21775E-06 | 0.0018 | 2.61 | 32 | 0.0732 | 22 | 0.0010 | 2.64 | 28 | 291 | 0.04 |
| abnormal liver physiology | 6 | 1.36707E-06 | 0.0017 | 2.55 | 33 | 0.0755 | 48 | 0.0060 | 2.30 | 28 | 334 | 0.04 |
| abnormal thymus morphology | 7 | 1.70104E-06 | 0.0018 | 2.17 | 44 | 0.1007 | 15 | 0.0003 | 2.35 | 40 | 467 | 0.05 |
| increased organ/body region tumor incidence | 11 | 9.45961E-06 | 0.0063 | 2.05 | 43 | 0.0984 | 57 | 0.0102 | 2.01 | 35 | 479 | 0.05 |
| abnormal double-negative T cell morphology | 13 | 1.69402E-05 | 0.0095 | 2.51 | 27 | 0.0618 | 36 | 0.0030 | 2.65 | 24 | 249 | 0.03 |
| increased gland tumor incidence | 14 | 1.79919E-05 | 0.0094 | 2.68 | 24 | 0.0549 | 96 | 0.0233 | 2.52 | 18 | 196 | 0.02 |
| increased susceptibility to autoimmune hemolytic anemia | 18 | 2.7241E-05 | 0.0111 | 53.99 | 3 | 0.0069 | 51 | 0.0069 | 27.47 | 3 | 3 | 0.00 |
| abnormal trophoblast layer morphology | 19 | 3.27147E-05 | 0.0126 | 3.37 | 16 | 0.0366 | 90 | 0.0212 | 3.13 | 13 | 114 | 0.02 |
| branchial arch hypoplasia | 22 | 5.17537E-05 | 0.0172 | 7.52 | 7 | 0.0160 | 82 | 0.0176 | 8.59 | 5 | 16 | 0.01 |
| decreased circulating HDL cholesterol level | 25 | 5.5644E-05 | 0.0163 | 4.07 | 12 | 0.0275 | 50 | 0.0070 | 4.66 | 10 | 59 | 0.01 |
| increased mammary gland tumor incidence | 26 | 6.22775E-05 | 0.0175 | 4.02 | 12 | 0.0275 | 104 | 0.0294 | 3.62 | 10 | 76 | 0.01 |
| increased CD8-positive T cell number | 27 | 6.30996E-05 | 0.0171 | 3.53 | 14 | 0.0320 | 61 | 0.0111 | 3.47 | 13 | 103 | 0.02 |
| increased single-positive T cell number | 29 | 8.83508E-05 | 0.0223 | 2.86 | 18 | 0.0412 | 84 | 0.0175 | 2.70 | 17 | 173 | 0.02 |
| thymus hypoplasia | 32 | 9.10019E-05 | 0.0208 | 2.67 | 20 | 0.0458 | 92 | 0.0224 | 2.62 | 17 | 178 | 0.02 |
| abnormal trophoblast giant cells | 34 | 9.43171E-05 | 0.0203 | 3.85 | 12 | 0.0275 | 130 | 0.0477 | 3.31 | 10 | 83 | 0.01 |
| decreased double-positive T cell number | 37 | 0.00013366 | 0.0264 | 2.67 | 19 | 0.0435 | 92 | 0.0224 | 2.62 | 17 | 178 | 0.02 |

Binom Rank: Binomial rank; Binom Raw P-Value: Binomial raw P-Value; Binom FDR q-val: Binomial false discovery rate q-value; Biom Fold Enrichment: Binomial Fold Enrichment; Binom Observed Region Hits: Binomial observed region hits and Binom Region Set Coverage: Binomial region set coverage.

Hyper Rank: Hyperbrowser rank; Hyper FDR q-val: Hyperbrowser false discovery rate q-value; Hyper Fold Enrichment: Hyperbrowser fold enrichment; Hyper observed gene Hits: Hyperbrowser observed gene hits; Hyper total genes: Hyperbrowser total genes and Hyper gene Set Coverage: Hyperbrowser gene set coverage.

The test set of 437 genomic regions picked 736 (4%) of all 20,221 genes.

Mouse Phenotype has 7,310 terms covering 6,642 (33%) of all 20,221 genes, and 456,354 term - gene associations.

7,310 ontology terms (100%) were tested using an annotation count range of [1, Inf].

**Table S9**- Osborne Annotated Disease Ontology in 437 DMRs overlapped with k4me1/3 and K36me3 (active chromatin state) as observed in GREAT analysis obtained from adult ALB-D2KO mice.

| # GREAT version 2.0.2<br># Term Name | Binom Rank | Binom Raw P-Value | Binom FDR Q-Val | Binom Fold Enrichment | Binom Observed Region Hits | Binom Region Set Coverage | Hyper Rank | Hyper FDR Q-Val | Hyper Fold Enrichment | Hyper Observed Gene Hits | Hyper Total Genes | Hyper Gene Set Coverage |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| hepatitis C | 10 | 1.47E-04 | 0.033 | 2.58 | 20 | 0.046 | 78 | 4.17E-02 | 2.21 | 18 | 224 | 0.024 |
| hepatitis | 14 | 2.37E-04 | 0.038 | 2.04 | 30 | 0.069 | 75 | 2.41E-02 | 1.92 | 28 | 401 | 0.038 |

Binom Rank: Binomial rank; Binom Raw P-Value: Binomial raw P-Value; Binom FDR q-val: Binomial false discovery rate q-value; Biom Fold Enrichment: Binomial Fold Enrichment; Binom Observed Region Hits: Binomial observed region hits and Binom Region Set Coverage: Binomial region set coverage.

Hyper Rank: Hyperbrowser rank; Hyper FDR q-val: Hyperbrowser false discovery rate q-value; Hyper Fold Enrichment: Hyperbrowser fold enrichment; Hyper observed gene Hits: Hyperbrowser observed gene hits; Hyper total genes: Hyperbrowser total genes and Hyper gene Set Coverage: Hyperbrowser gene set coverage.

The test set of 437 genomic regions picked 736 (4%) of all 20,221 genes.

Disease Ontology has 2,220 terms covering 7,558 (37%) of all 20,221 genes, and 224,634 term - gene associations.

2,220 ontology terms (100%) were tested using an annotation count range of [1, Inf].

**Table S10**- Enriched GO biological process in 276 DMRs overlapped with k27me3 (repressed chromatin state) as observed in GREAT analysis obtained from adult ALB-D2KO mice.

| # GREAT version 2.0.2<br># Term Name | Binom Rank | Binom Raw P-Value | Binom FDR Q-Val | Binom Fold Enrichment | Binom Observed Region Hits | Binom Region Set Coverage | Hyper Rank | Hyper FDR Q-Val | Hyper Fold Enrichment | Hyper Observed Gene Hits | Hyper Total Genes | Hyper Gene Set Coverage |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| embryonic morphogenesis | 3 | 4.15E-06 | 0.012 | 2.19 | 39 | 0.141 | 6 | 2.59E-09 | 3.65 | 40 | 461 | 0.083 |
| pattern specification process | 4 | 9.05E-06 | 0.019 | 2.30 | 33 | 0.120 | 9 | 7.46E-08 | 3.66 | 34 | 390 | 0.071 |
| embryonic organ development | 9 | 1.75E-05 | 0.017 | 2.38 | 29 | 0.105 | 17 | 1.77E-06 | 3.68 | 28 | 320 | 0.058 |
| embryonic organ morphogenesis | 11 | 3.17E-05 | 0.025 | 2.62 | 23 | 0.083 | 22 | 3.67E-06 | 4.32 | 22 | 214 | 0.046 |
| Regionalization | 13 | 5.31E-05 | 0.035 | 2.47 | 24 | 0.087 | 29 | 1.91E-05 | 3.62 | 24 | 279 | 0.050 |
| positive regulation of tooth mineralization | 14 | 5.46E-05 | 0.033 | 42.61 | 3 | 0.011 | 151 | 3.21E-02 | 42.04 | 2 | 2 | 0.004 |
| negative regulation of Notch signaling pathway | 17 | 8.32E-05 | 0.042 | 18.02 | 4 | 0.014 | 111 | 7.06E-03 | 15.29 | 4 | 11 | 0.008 |
| sensory organ development | 19 | 9.14E-05 | 0.041 | 2.04 | 33 | 0.120 | 23 | 4.11E-06 | 3.23 | 31 | 403 | 0.064 |

Binom Rank: Binomial rank; Binom Raw P-Value: Binomial raw P-Value; Binom FDR q-val: Binomial false discovery rate q-value; Biom Fold Enrichment: Binomial Fold Enrichment; Binom Observed Region Hits: Binomial observed region hits and Binom Region Set Coverage: Binomial region set coverage.

Hyper Rank: Hyperbrowser rank; Hyper FDR q-val: Hyperbrowser false discovery rate q-value; Hyper Fold Enrichment: Hyperbrowser fold enrichment; Hyper observed gene Hits: Hyperbrowser observed gene hits; Hyper total genes: Hyperbrowser total genes and Hyper gene Set Coverage: Hyperbrowser gene set coverage.

The test set of 276 genomic regions picked 481 (2%) of all 20,221 genes.

GO Biological Process has 8,583 terms covering 15,210 (75%) of all 20,221 genes, and 605,197 term - gene associations.

8,583 ontology terms (100%) were tested using an annotation count range of [1, Inf].

**Table S11**– Oligonucleotide Primers

| Gene | | Sequence |
|---|---|---|
| deiodinase, iodothyronine, type II | DIO2 Forward (Primer 1) | TCCTAGATGCCTACAAACAGGTTA |
| | DIO2 Reverse (Primer 1) | GTCAGGTGGCTGAACCAAAG |
| deiodinase, iodothyronine, type II | DIO2 Forward (Primer 2) | GTCCGCAAATGACCCCTTT |
| | DIO2 Reverse (Primer 2) | CCCACCCACTCTCTGACTTTC |
| glycerol-3-phosphate acyltransferase, mitochondrial | GPAM Forward | ACAGTTGGCACAATAGACGTTT |
| | GPAM Reverse | CCTTCCATTTCAGTGTTGCAGA |
| 1-acylglycerol-3-phosphate O-acyltransferase 2 | AGPAT2 Forward | CAGCCAGGTTCTACGCCAAG |
| | AGPAT2 Reverse | TGATGCTCATGTTATCCACGGT |
| stearoyl-Coenzyme A desaturase 1 | SCD1 Forward | TTCTTGCGATACACTCTGGTGC |
| | SCD1 Reverse | CGGGATTGAATGTTCTTGTCGT |
| fatty acid synthase | Fasn Forward | GGAGGTGGTGATAGCCGGTAT |
| | Fasn Reverse | TGGGTAATCCATAGAGCCCAG |
| sterol regulatory element binding transcription factor 1 | SREBF-1c Forward | TGACCCGGCTATTCCGTGA |
| | SREBF-1c Reverse | CTGGGCTGAGCAATACAGTTC |
| ELOVL family member 6, elongation of long chain fatty acids | ELOVL6 Forward | GAAAAGCAGTTCAACGAGAACG |
| | ELOVL6 Reverse | AGATGCCGACCACCAAAGATA |
| ATP citrate lyase | ACLY Forward | ACCCTTTCACTGGGGATCACA |
| | ACLY Reverse | GACAGGGATCAGGATTTCCTTG |
| cluster of differentiation 36 | CD36 Forward | AGATGACGTGGCAAAGAACAG |
| | CD36 Reverse | CCTTGGCTAGATAACGAACTCTG |
| liver x receptor (NR1H3) | LXR Forward | CTCAATGCCTGATGTTTCTCCT |
| | LXR Reverse | TCCAACCCTATCCCTAAAGCAA |
| low density lipoprotein receptor | LDLR Forward | TGACTCAGACGAACAAGGCTG |
| | LDLR Reverse | ATCTAGGCAATCTCGGTCTCC |
| proprotein convertase subtilisin/kexin type 9 | PCSK9 Forward | GAGACCCAGAGGCTACAGATT |
| | PCSK9 Reverse | AATGTACTCCACATGGGGCAA |

**Table S12** – Oligonucleotide Primers

| Gene | | Sequence |
|---|---|---|
| peroxisome proliferator activated receptor alpha | PPARa Forward | AGAGCCCCATCTGTCCTCTC |
| | PPARa Reverse | ACTGGTAGTCTGCAAAACCAAA |
| 3-hydroxy-3-methylglutaryl-CoA reductase | HMGCoAR Forward | AGCTTGCCCGAATTGTATGTG |
| | HMGCoAR Reverse | TCTGTTGTGAACCATGTGACTTC |
| acetyl-CoA carboxylase alpha | ACC Forward | GCCTCTTCCTGACAAACGAG |
| | ACC Reverse | TGACTGCCGAAACATCTCTG |
| diacylglycerol O-acyltransferase 2 | DGAT2 Forward | GCGCTACTTCCGAGACTACTT |
| | DGAT2 Reverse | GGGCCTTATGCCAGGAAACT |
| sterol regulatory element binding transcription factor 2 | SREBF2 Forward | GCAGCAACGGGACCATTCT |
| | SREBF2 Reverse | CCCCATGACTAAGTCCTTCAACT |
| 18S rRNA | 18S Forward | GTAACCCGTTGAACCCCATT |
| | 18S Reverse | CCATCCAATCGGTAGTAGCG |
| cytochrome P450, family 7, subfamily A, polypeptide 1 | CYP7A1 Forward | ACCCAGACAGCGCTCTTTGA |
| | CYP7A1 Reverse | CCATGATGCAAAACCTCCAAT |
| peroxisome proliferator-activated receptor gamma | PPARg Forward | CCCACCAACTTCGGAATCA |
| | PPARg Reverse | TGCGAGTGGTCTTCCATCAC |
| thyroid hormone responsive (Thrsp) | SPOT14 Forward | CTCGGAGGAGCTGGACCTA |
| | SPOT14 Reverse | GTGATGGAGGCTGCAGAAGT |
| carnitine palmitoyltransferase 1a | CPT1 Forward | TGAGTGGCGTCCTCTTTGG |
| | CPT1 Reverse | CAGCGAGTAGCGCATAGTCA |
| carbohydrate-responsive element-binding protein (MLXIPL) | ChREBP Forward | AGATGGAGAACCGACGTATCA |
| | ChREBP Reverse | ACTGAGCGTGCTGACAAGTC |
| patatin-like phospholipase domain containing 3 | PNPLA3 Forward | TCACCTTCGTGTGCAGTCTC |
| | PNPLA3 Reverse | CCTGGAGCCCGTCTCTGAT |
| apolipoprotein A-I | APOA1 Forward | GGCACGTATGGCAGCAAGAT |
| | APOA1 Reverse | CCAAGGAGGAGGATTCAAACTG |
| ATP-binding cassette, sub-family A (ABC1), member 1 | ABCA1 Forward | GCTTGTTGGCCTCAGTTAAGG |
| | ABCA1 Reverse | GTAGCTCAGGCGTACAGAGAT |
| phospholipase A1 member A | PLA1A Forward | GGTTGTGGGGACCACTTTTATG |
| | PLA1A Reverse | CACCTTGAGGTTGGTGCCT |
| solute carrier family 27 (fatty acid transporter), member 5 | SLC27A5 Forward | TCTATGGCCTAAAGTTCAGGCG |
| | SLC27A5 Reverse | CTTGCCGCTCTAAAGCATCC |
| phosphate cytidylyltransferase 2, ethanolamine | PCYT2 Forward | CGATGGCTGCTATGACATGGT |
| | PCYT2 Reverse | GCCCCTTATGCTTGGCAATCT |
| uncoupling protein 1 | UCP1 Forward | AAGCTGTGCGATGTCCATGT |
| | UCP1 Reverse | AAGCCACAAACCCTTTGAAAA |

**Table S13-** Metabolic panel of ALB-D2KO and control animals kept on chow and high fat diet.

| ng/ul | *Chow diet* | | *HFD* | |
|---|---|---|---|---|
| | **Control** | **Liver-D2KO** | **Control** | **Liver-D2KO** |
| Insulin | 5.0±1.3 | 3.3±1.2 | 6.6±0.9 | 4.9±0.6 |
| Leptin | 3.5±0.3 | 2.8±0.3 | 8.9±3.0[a] | 5.8±1.3 |
| C-peptide | 14±4.9 | 6.9±2.5 | 8.5±2.7 | 10±3.2 |
| PYY | 0.3±0.08 | 0.2±0.02* | 0.2±0.01 | 0.1±0.02* |
| PP | 0.2±0.07 | 0.1±0.03* | 0.06±0.04 | 0.06±0.01 |
| Resistin | 9.7±0.9 | 7.1±1.3 | 13±3.1 | 17±3.7[b] |

*p<0.05 vs respective control; a p<0.01 vs Control Chow Diet and ALB-D2KO chow Diet (ANOVA); b p<0.05 vs ALB-D2KO (ANOVA); values are the mean ± SEM of 5 independent samples.

## Supplemental Experimental Procedures

*Histology and in situ hybridization:* Serial 12-µm thick coronal sections (Leica Microsystems GmbH, Wetzlar, Germany) were mounted on gelatine coated slides, air-dried at 42ºC overnight and stored at -80°C. A 801 bp of mouse D2 coding region (bases 155–956 of GeneBank) was cloned into pGemT vector (Promega) and used as probe. Mouse D2 cRNA antisense probe was generated with SP6 (Promega Corp., Madison, WI) polymerase after NcoI digestion in the presence of [S35] labelled UTP. Sense probe was generated after NotI digestion with T7 polymerase. Both probes were purified with Quick spin columns (Roche Applied Science, Mannheim, Germany). On the day of hybridization, 12 µm thick cryostat sections were thawed and fixed with 4% PFA for 10 min (1). After post-hybridization the sections were dehydrated with ascending concentrations of alcohol, air dried and dipped into Kodak NTB emulsion. Autoradiograms were developed kept at at 4oC and developed 6 weeks later. Counterstaining was with 0.005% Cresyl violet.

*Microarray Analysis:* Expression values (signal) of individual genes were log2 transformed. One-way ANOVA was used to calculate p-values for each fold change (linear); multi-testing correction was then performed using the Benjamini-Hochberg Step-Up FDR-controlling procedure for all the expressed genes; differentially expressed genes were tabled (Table S1; Table S16 and Table S18-19). Then gene ontology analysis was used to determine differences in enrichment of gene sets between phenotypes (Gene Set Enrichment Analysis (GSEA), Broad Institute). Expression values for all genes from all liver samples of both genotypes were used in the analysis. GSEA included calculation of enrichment scores (ES), estimation of significance level of ES (nominal p-value), and adjustment for multiple hypothesis testing including the normalized enrichment score (NES) and false discovery rate (FDR). Core enrichment of individual genes within these gene sets was indicated by GSEA and defined as those genes contributing to the leading edge subset (Table S2 and Table S17 ).

*Methylome Analysis:* MeDIP (Methylated DNA immunoprecipitation) and MRE (Methylation-sensitive Restriction Enzyme) sequencing libraries were constructed as previously described (2). Simply, genomic DNA from three controls and three ALB-D2KO animals kept on chow diet was extracted by phase lock gel (5 PRIM, USA). For MeDIP-seq, 500 ng genomic DNA was sonicated to ~100-500 bp with a Bioruptor sonicator (Diagenode). Sonicated DNA was end-repaired, A-tailed, and ligated to pair-end adapters. After AMPure XP beads purification (Beckman Coulter) to remove unligated adapters, adapter-ligated DNA was used for each immunoprecipitation using a mouse monoclonal anti-methylcytidine antibody (1 mg/ml, Eurogentec), 2 µl of rabbit anti-mouse IgG secondary antibody (2.5 mg/ml, Jackson Immunoresearch) and 100 µl Protein A/G beads (Pierce Biotechnology). After immunoprecipitation a total of 7-9 IP

washes were performed with ice cold IP buffer. The immunoprecipitated DNA was released from beads and purified by Qiagen MinElute columns. Twelve cycles of PCR were performed on the immunoprecipitated DNA using the pair end Illumina PCR primers. The resulting reactions are purified over Qiagen MinElute columns, after which a final size selection (220-520 bp) was performed by electrophoresis in 2% agarose. Libraries were QC'd by Qubit (Life Technologies) and Agilent DNA Tape Station and Bioanalyzer (Agilent) analysis. An aliquot of each library was diluted in Qiagen EB buffer to 10nM and 1 µl used as template in 4 independent PCR reactions to confirm enrichment for methylated and de-enrichment for unmethylated sequences, compared to input (sonicated DNA). Two positive controls (*SNRPN* and *MAGEA1* promoters) and 2 negative controls (a CpG-less sequence on Chr15 and *GAPDH* promoter) were amplified by a real-time PCR. For MRE-seq, five methylation sensitive restriction endonucleases were used to digest intact genomic DNA, each digestion was performed with 500 ng of DNA. Five parallel digested DNA was precipitated with ethanol and combined into one tube. Combined DNA was size-selected by electrophoresis on a 1% agarose TAE gel. A 100-500 bp gel slice was excised using a sterile scalpel and gel-purified using Qiagen Qiaquick columns. Library construction was performed by end repair, A tailing and adapter ligation. The modified DNA was used as template for PCR with 15 cycles. After the second size selection (220-520 bp), DNA was gel purified with Qiagen MinElute columns, each library was examined by Qubit (Life Technologies) and Agilent DNA Tape Station and Bioanalyzer (Agilent).

Raw reads from sequencing machine were first applied adapter trimming and quality filtering using cutadapt tool (https://code.google.com/p/cutadapt/). The filtered reads were then aligned to mouse reference genome mm9 using BWA aligner (version 0.7.10, the mem alignment algorithm, http://bio-bwa.sourceforge.net/bwa.shtml). The alignment results were processed by methylQA (http://methylqa.sourceforge.net/) to generate reads density files visualized in WashU EpiGenome Browser (http://epigenomegateway.wustl.edu/browser/). M&M algorithm (3) (http://epigenome.wustl.edu/MnM/) was used to find differentially methylated region among control and KO samples with a p-value cutoff 1e-4, and methylCRF algorithm (4) (https://methylcrf.wustl.edu/) was used to estimate single CpG resolution methylation values. All processed data can be visualized from the WashU EpiGenome Browser hub at:
http://epigenomegateway.wustl.edu/browser/?genome=mm9&datahub=http://cgs.wustl.edu/~dli/bianco/hub. Coordinates of DMRs were used for function enrichment analysis using GREAT tool (http://great.stanford.edu, version 2.0.2) using default settings (http://www.nature.com/nbt/journal/v28/n5/abs/nbt.1630.html). We used seven-state chromHMM model data for liver from mouse ENCODE project (http://www.nature.com/nature/journal/v515/n7527/full/nature13992.html), and verified overlap of all DMRs with the active/suppressed chromatin states. The center position of

one DMR was used for overlap checking. For calculating enrichment of any state, we divided while genome to 500-bp window as background, and same overlap was applied. Then the enrichment was calculated based on ratio over background. To compare overlap between gene expression from the liver microarray GSEAs and differentially methylated local regions (DMRs), individual genes demonstrating core enrichment within the enriched gene sets were entered into VENNY (http://bioinfogp.cnb.csic.es/tools/venny/index.html) where Venn diagrams were used to identify common genes. The statistical significance of overlap was calculated using hypergeometric test in R environment (http://www.r-project.org/).

*Tissue T3 content:* P1 Mice were anesthetized with isoflurane and perfused with liver perfusion medium (Gibco) through a needle placed in the left ventricle (LV). Tissues were snap frozen in liquid nitrogen and stored at – 80°C. T3 was extracted from whole liver using a method described previously (5) and T3 content was measured by RIA as previously detailed (6). Recovery was monitored by the addition of (125)-I-T3 before tissue extraction.

*Western blot analysis:* Tissues were lysed in 0.25M sucrose PE containing 10mM DTT. The lysates were diluted with 4X sample loading buffer (Invitrogen) and 20µg of total protein were run on 4-12% NuPAGE Bis-Tris Gels (Life Technologies, Carlsbad, CA). Samples were transferred to Immobilon-FL PVDF transfer membrane (Millipore, Billerica, MA) and probed with antibodies as indicated at a 1:1000 dilution overnight. Fluorescent labeled secondary antibodies (LiCOR Biosciences, Lincoln, NE) were used at 1:2500 for 1 h. All blots were imaged using LiCOR Odyssey instrument per manufacturer's instructions (LiCOR Biosciences, Lincoln, NE).

*T3 production in intact cells:* Fresh P1 livers were macerated and posterior washed in 4ml of Leibowitz medium containing 0.1% BSA. 500,000K $^{125}$I-T4 was added to the medium of intact cells and incubated for 4h with 0.1nM of T4 and 1mM of PTU. At the end of the experiment, the cells were centrifuged and 400uL of medium was transferred to a new tube where the reaction was stopped with 2:1 horse serum/TCA. Samples were centrifuged and$^{125}$I was counted on the gamma counter.

*T3 responsiveness:* Hypothyroidism was induced by iodine deficient diet with 0.15% PTU (TD 95 125; Harlan Teklad, Indianapolis, IN) and 0.05% of MMI in drinking water for 2 weeks. 2h before the sacrifice the animals were injected with 5ug T3 in a single injection ip.

## Supplemental References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