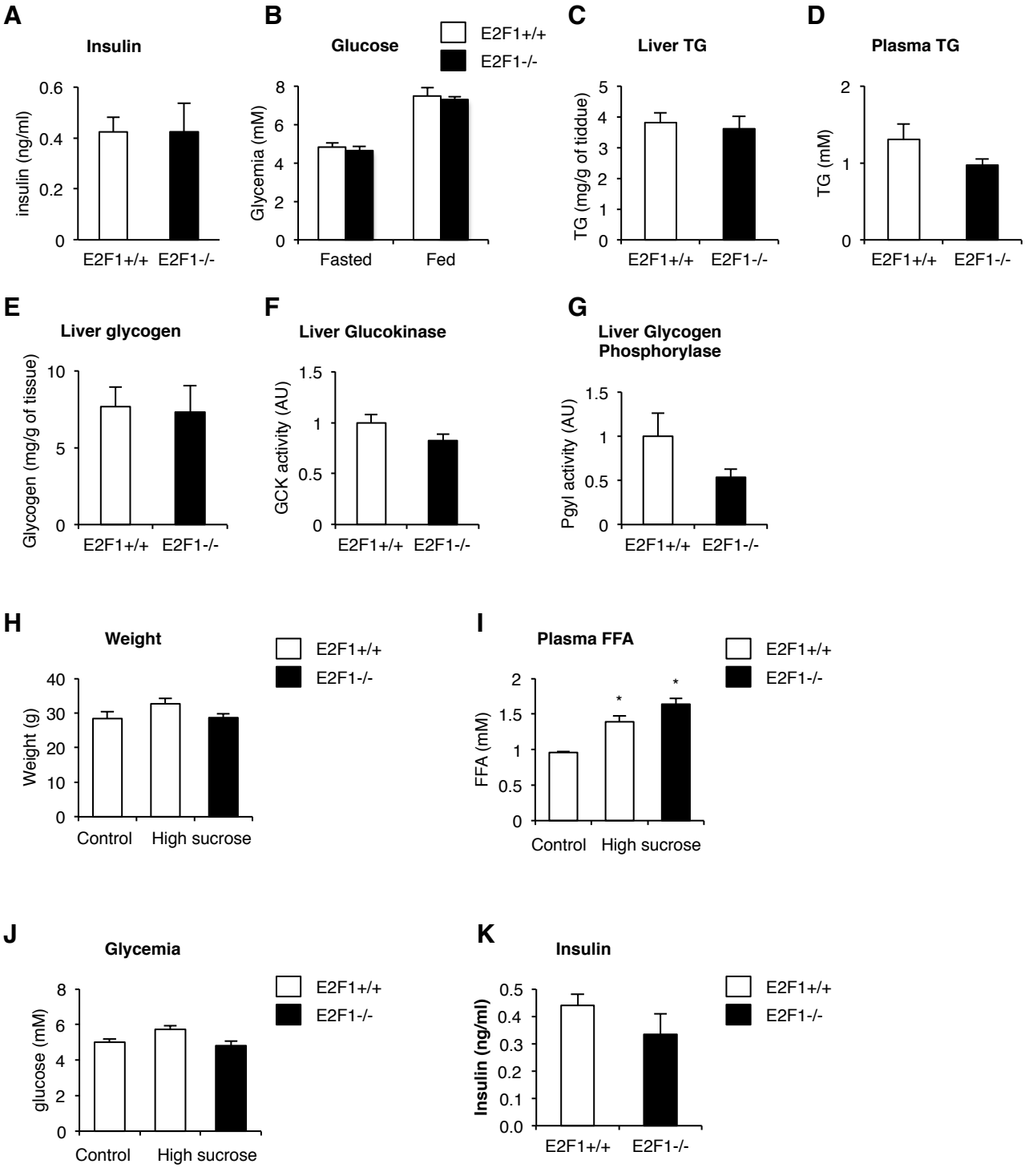
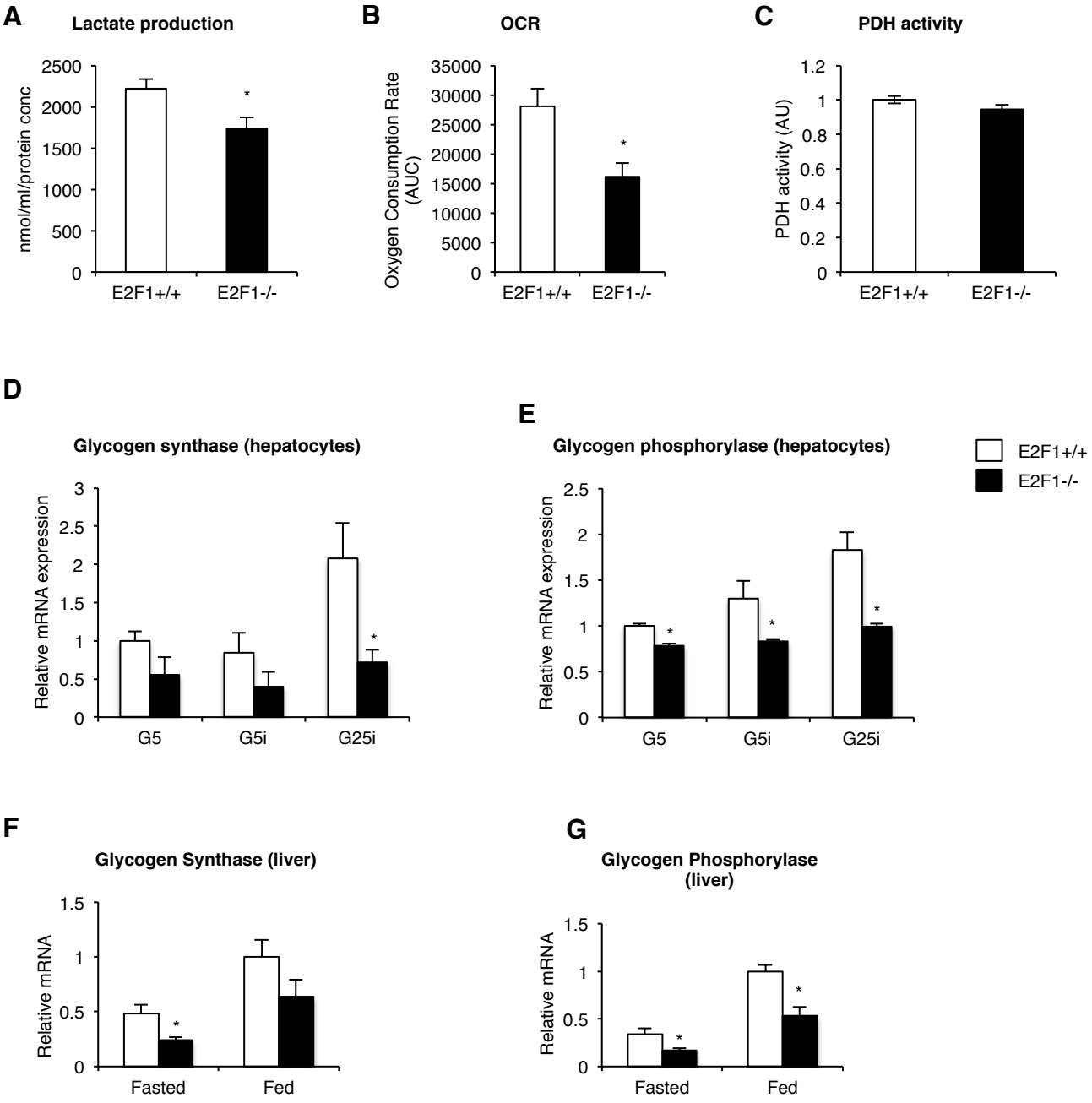
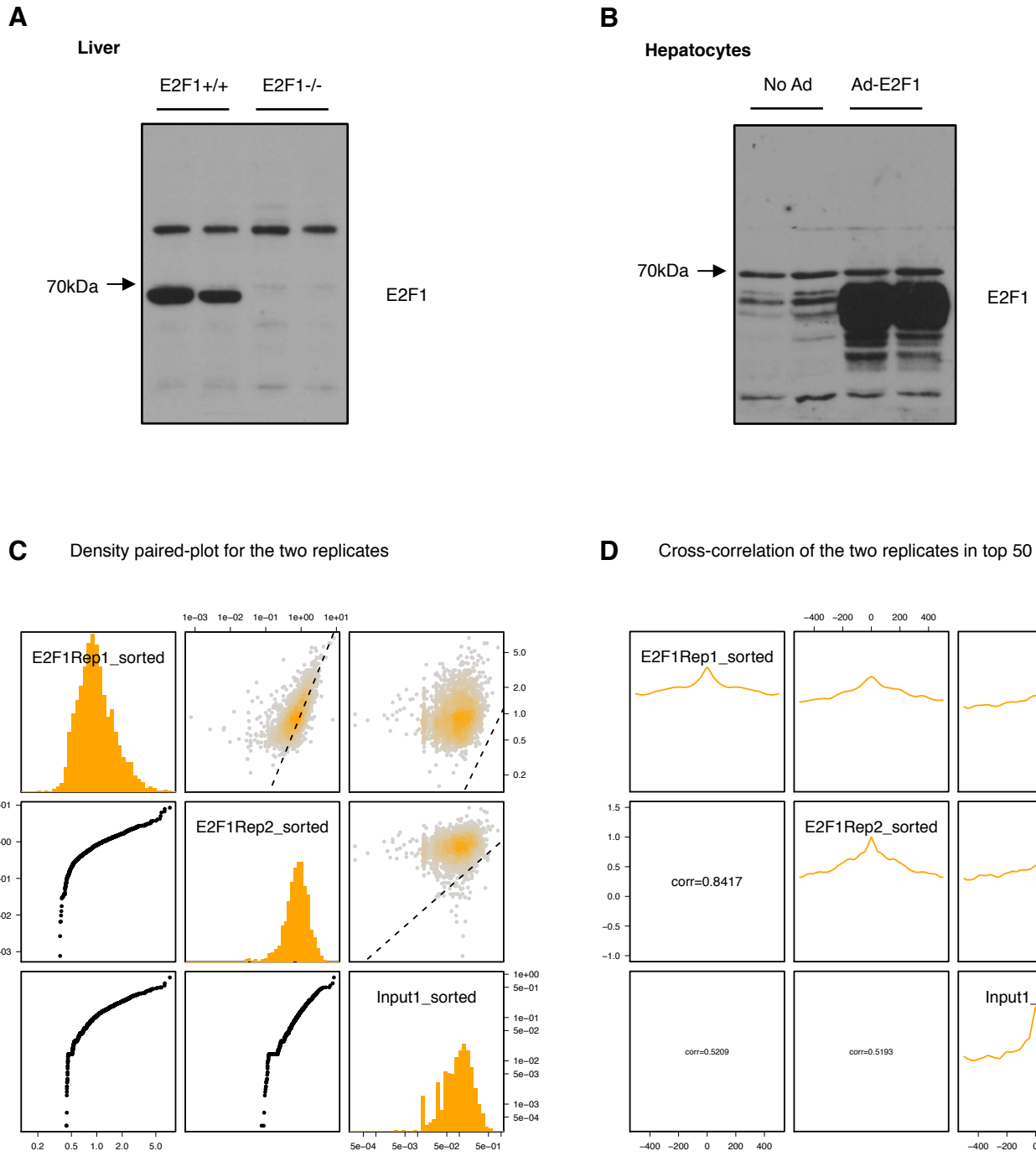


Supplemental Figure 1. **Metabolic parameters of E2F1 knockout mice.**
(A) Plasma insulin of fed E2F1+/+ and -/- mice. (B) Glycemia of the mice after overnight fasting and during fed state (n=5). (C), liver TG content, (D) plasma TG level and (E) Liver glycogen content of fed E2F1+/+ and E2F1-/- mice. (F) Glucokinase activity and (G) Glycogen phosphorylase activity in livers from fed E2F1+/+ and E2F1-/- mice. (H) Body weight, (I) fasting plasma FFA and (J) fasting glycemia of E2F1+/+ and E2F1-/- mice submitted to 9 weeks of high sucrose diet. (K) Plasma insulin of fed E2F1+/+ and E2F1-/- mice after 7 weeks of high sucrose diet (n=3-6).
An asterisk indicates statistically significant differences ($p<0.05$) from control.



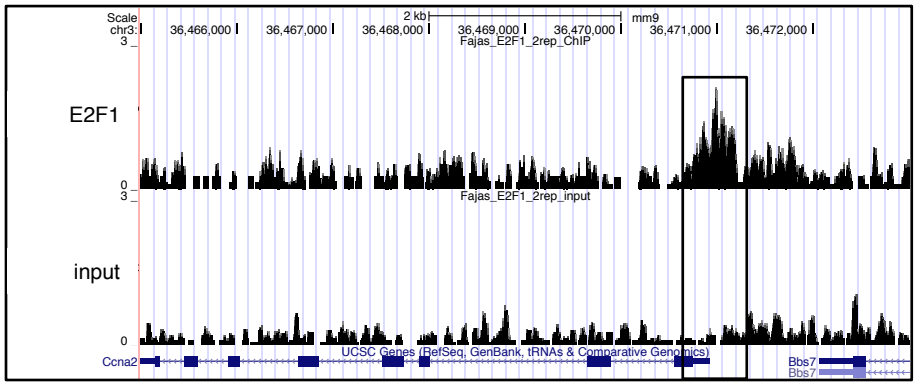
Supplemental Figure 2. Lactate production, glucose oxidation, PDH activity and glycogen metabolism gene expression.

(A) Lactate release in the medium by E2F1+/+ and E2F1-/- hepatocytes after a Seahorse glycolysis experiment,related to figure 2C. (B) Oxygen consumption rate of E2F1+/+ and E2F1-/- hepatocytes after glucose injection in the medium, related to figure 2C. (C) PDH activity in E2F1+/+ and E2F1-/- hepatocytes. (D) Glycogen Synthase (Gys2) and (E) Glycogen phosphorylase (Pygl) gene expression in E2F1+/+ and E2F1-/- hepatocytes treated for 24h in low glucose 5 mM (G5), low glucose 5 mM plus insulin 100nM (G5i) or high glucose 25 mM plus insulin 100 nM (G25i). (F) Liver Glycogen Synthase (Gys2) and (E) liver Glycogen phosphorylase (Pygl) gene expression in fasted 6h or Fed E2F1+/+ and E2F1-/- mice.
An asterisk indicates statistically significant differences ($p<0.05$) from control.

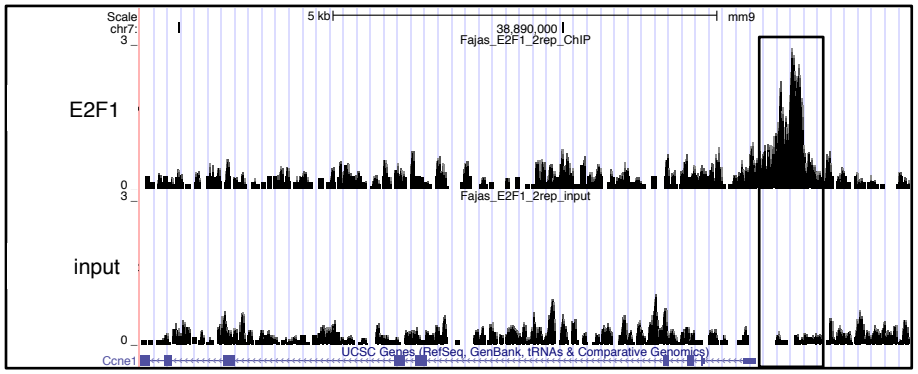


Supplemental Figure 3. E2F1 ChIP-seq validation.
E2F1 c20 antibody validation: (A) Full western blot of E2F1 on liver protein extract from E2F1+/+ and E2F1-/- mice. (b) Full western blot of E2F1 on control hepatocytes (no Ad) and hepatocytes adenofected by Ad-E2F1.
ChIP-Seq replicates validation. (C) Density paired-plot for the two replicates: paired qq-plots (lower matrix), global density (diagonal) and scatter plots upper matrix) of the ChIP-seq signals for rep1 and rep2, in peaks obtained from rep1. A good correlation between the two replicates is observed in both the qq-plot and scatter-plot. The input signal for replicate 1 has been added for negative control purpose. (D) Cross-correlation of the two replicates: paired cross-correlation and correlation of the ChIP-seq signals for rep1 and rep2, in the best 50 peaks obtained from rep1 in term of deconvolution score. Peak regions have been refined to the summit+/-150bp. The input signal for replicate 1 has been added for negative control purpose.

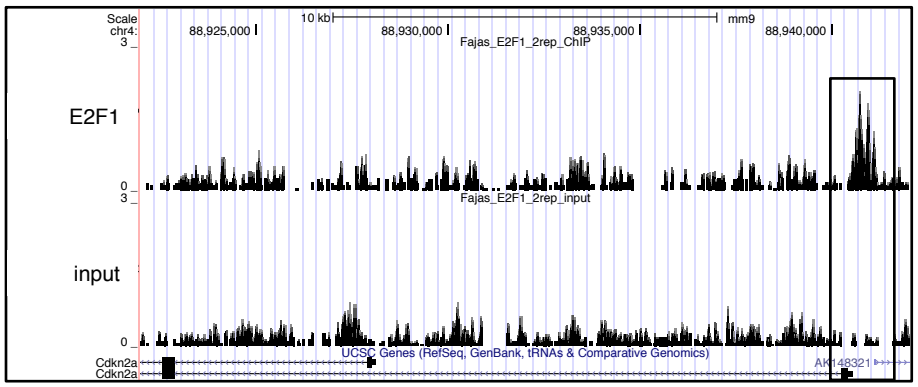
Ccna2



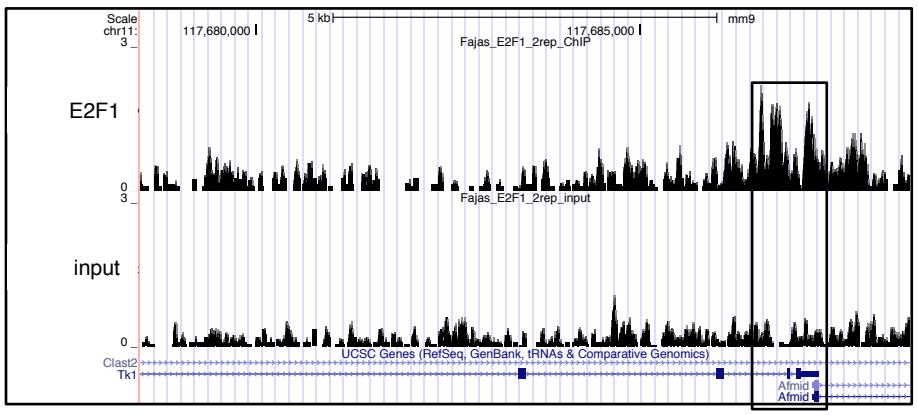
Ccne1



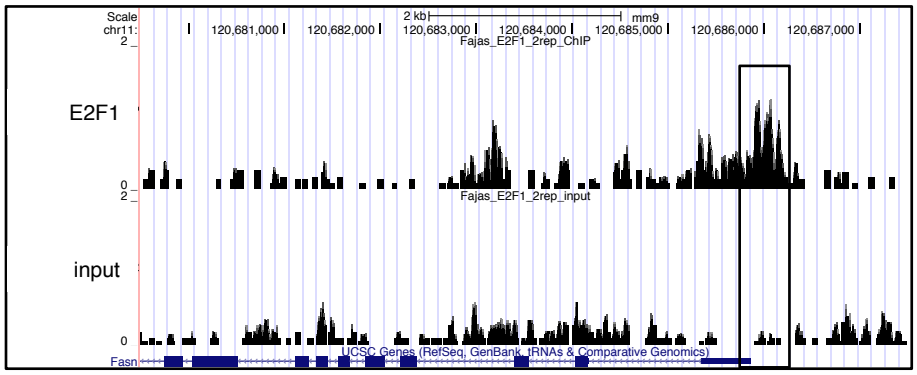
Cdkn2a



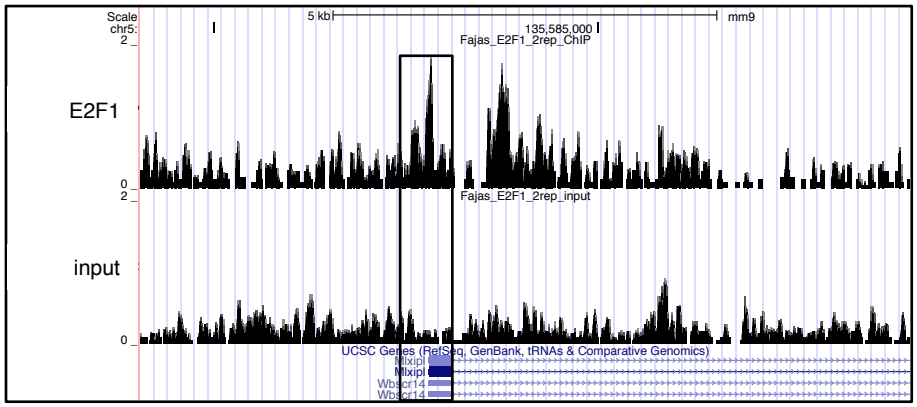
Tk1



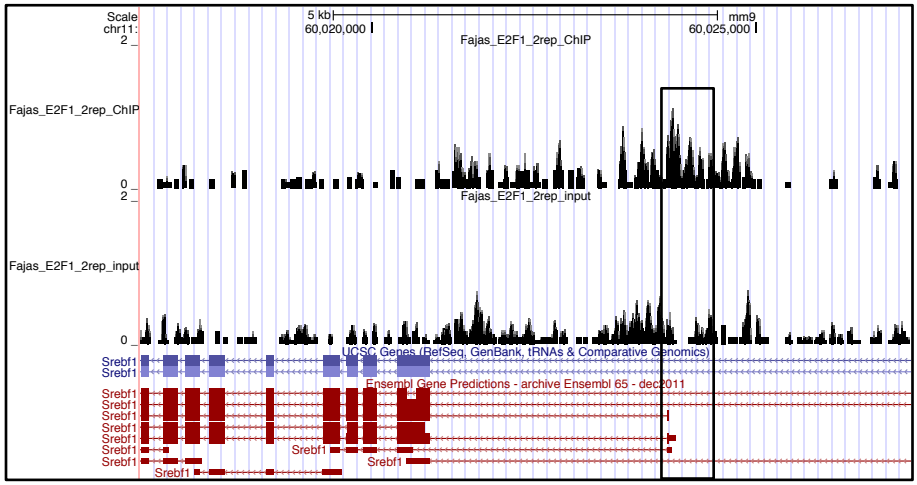
Fasn



ChREBP

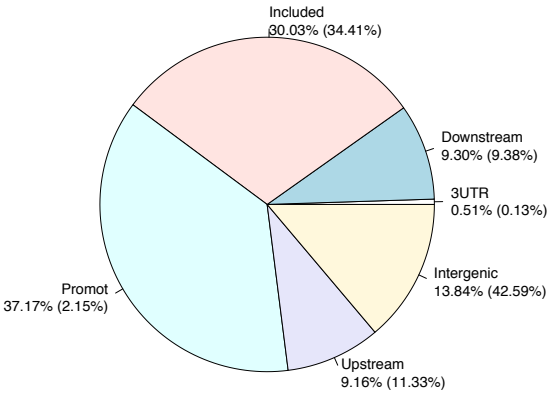


SREBP-1c

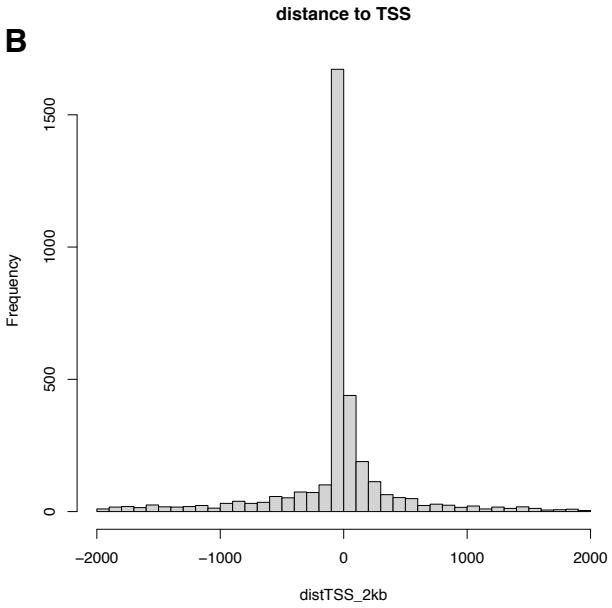


Supplemental Figure 4. **E2F1 ChIP-seq browser shot.**
Browser shots from the UCSC genome browser website: http://genome.ucsc.edu/. Input and E2F1 ChIP alignements are shown on Mouse genome (NCBL37/mm9) with USCC genes predictions. Peaks from Ccna2, Ccne1, cdkn2a, Tk1, Fasn, Chrebp (mlxipl), Srebp-1c (Srebf1) promoters are highlighted.

A



B

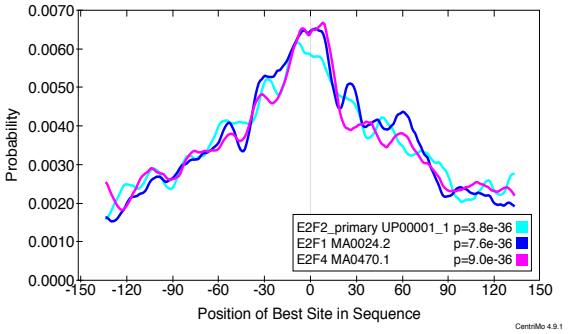


C

Enriched motifs fund by centiMo

	ID	Name	E-value	Region Width	Region Matches
1	UP00001_1	E2F2_primary	2.20E-33	123	1063
2	MA0024.2	E2F1	4.50E-33	155	892
3	MA0470.1	E2F4	5.30E-33	85	797

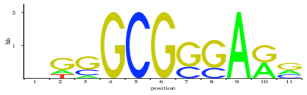
Motif position probability graph



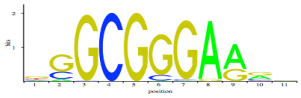
Motifs logo

Motif 1: E2F2_primary

Motif 2: E2F1



Motif 3: E2F4



Supplemental Figure 5. **Ad-E2F1 ChIP-seq analysis.**
(A) Distribution of the location of the peaks relative to genes. The promoter regions were defined as 2kb upstream and 500bp downstream of the TSS. As a reference, between brackets are the values observed in a set of randomly located peaks (10 randomized locations for each original peak). It shows a clear enrichment at promoter regions and a depletion in intergenic regions (Chi-squared p-values < 2.2e-16 in both cases).
(B) Distribution of distance to closest gene: Distribution of the distances to the nearest transcription start site (TSS).
(C) CentriMo Central Motif Enrichment Analysis: The region of maximum central enrichment in peaks found in promoters was studied with CentriMo. The sequences submitted correspond to the sequence surrounding the summit of the peaks +/-150bp. The region of maximum central enrichment in peaks found in promoters was studied with CentriMo. The databases JASPAR_CORE_2014_vertebrates.meme and uniprobe_mouse.meme were used.

Liver Genes enrichment bound by E2F1

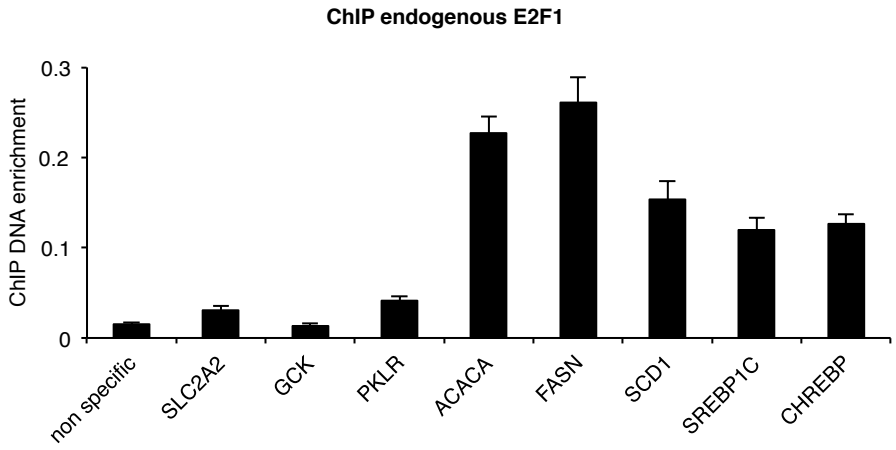
Biological Processes	p-value
cellular response to stress	9,7E-9
response to DNA damage stimulus	2,1E-6
oxidation reduction	4,7E-6
fatty acid metabolic process	7,0E-6
protein localization	2,2E-5
homeostatic process	2,2E-5
DNA metabolic process	2,2E-5
regulation of cell cycle	2,7E-5
generation of precursor metabolites and energy	3,0E-5
mitotic cell cycle	3,5E-5
DNA repair	5,8E-5

Liver Genes enrichment bound by E2F1

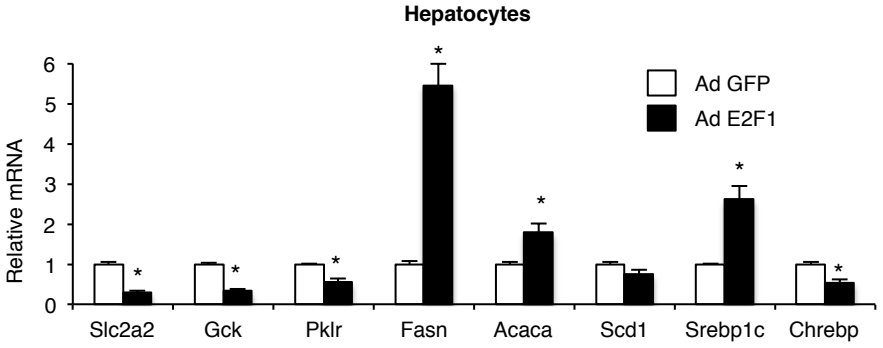
KEGG Pathway	p-value
Fatty acid metabolism	1,8E-5
Prostate cancer	2,3E-4
Adipocytokine signaling pathway	1,7E-3
Insulin signaling pathway	1,9E-3
PPAR signaling pathway	3,2E-3
Propanoate metabolism	3,5E-3
Endometrial cancer	4,7E-3
Adherens junction	5,7E-3
Non-small cell lung cancer	6,3E-3
Huntington's disease	9,1E-3
DNA replication	9,4E-3

Supplemental Figure 6. **Ad-E2F1 ChIP-seq analysis.**
Genes associated to E2F1 were selected based on their liver specific expression with the UP_TISSUE database. Liver specific genes bound by E2F1 were clustered using the Gene Ontology Biological Pathway database (GOTERM_BP) and the KEGG (Kyoto Encyclopedia of Genes and Genomes) Pathway database. Lipid metabolism pathways are highlight in bold. Fisher Exact P-Value = 0 represents perfect enrichment. P-Value is equal or smaller than 0.05 to be considered strongly enriched in the annotation categories.

A



B



Supplemental Figure 7. E2F1 ChIP analysis

(A) Endogenous E2F1 ChIP in HepG2 cells (one representative experiment out of three is shown).

(B) Relative mRNA expression of relevant glycolytic and lipogenic genes in primary hepatocytes infected with Ad GFP or Ad E2F1 (n=3), related to ChIP experiments in Figure 3B.

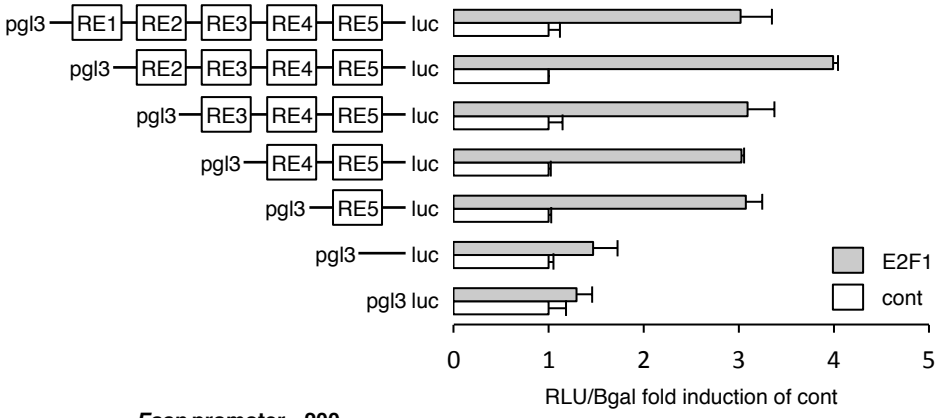
A ***Fasn*** **mouse promoter**

```
-600
CGAAGTGCTTTGCCGCTGTTGCCGGCCCATCACCCTATTGCCTAGCAACGCCCACCCGCGCGCCGCCATTGGGCCACCGAGAACGGCCTCGGTGTCCAAT
       putatif E2FRE1
TGGTTTCGATGTGGAGCAGGCCACGCCCCTCGGTTCCGCGCGCGCTACGATCGCGGCGTGGAATCGCAGCGACACGGACCTGTCCCCCCGCGTGGCCCTG
                              putatif E2FRE2
GTGTCCTCCTCAGTGCAGAGTTTCCAGTGTGACCAAGCACGCCCGACCCACACTGCGCGCGCACAGTGCACACCTGGCACCGGCCGCGAGGGGGTGGGGG
                                                        putatif E2FRE3  Ebox -332
TGGGAGGACAGAGATGAGGGCGTCGGGATGAGCCCCGCGTGGCCCGCGCGAGGCCGGGGGCGGGGACGGAAGCAGGCGGGGGCTGCGCGTTCCTTGTGCT

CCAGCGCGCGCCCGTGCAGGGTCCCGGCTGGGGGCGGCGCGCGCGGGCATCACCCCACCGGCGGCGGCGCGCCGGGTCCCGGGGCGCAGCCCCGACGCTC
putatif E2FRE4                          E2FRE5        SRE -150
ATTGGCCTGGGCGGCGCAGCCAAGCTGTCAGCCCATGTGGCGTGGCCGCGCGGGGATGGCCGCGGTTTAAATAGCGCCGGCGCGGCCTAGAGGGAGCCAG
-100                mouse +1      Ebox -65                                                          human +1
```

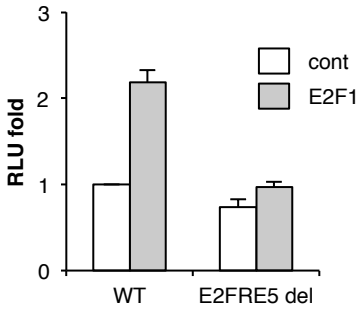
B

Truncated ***Fasn*** **promoter**



Fasn **promoter - 200**

C

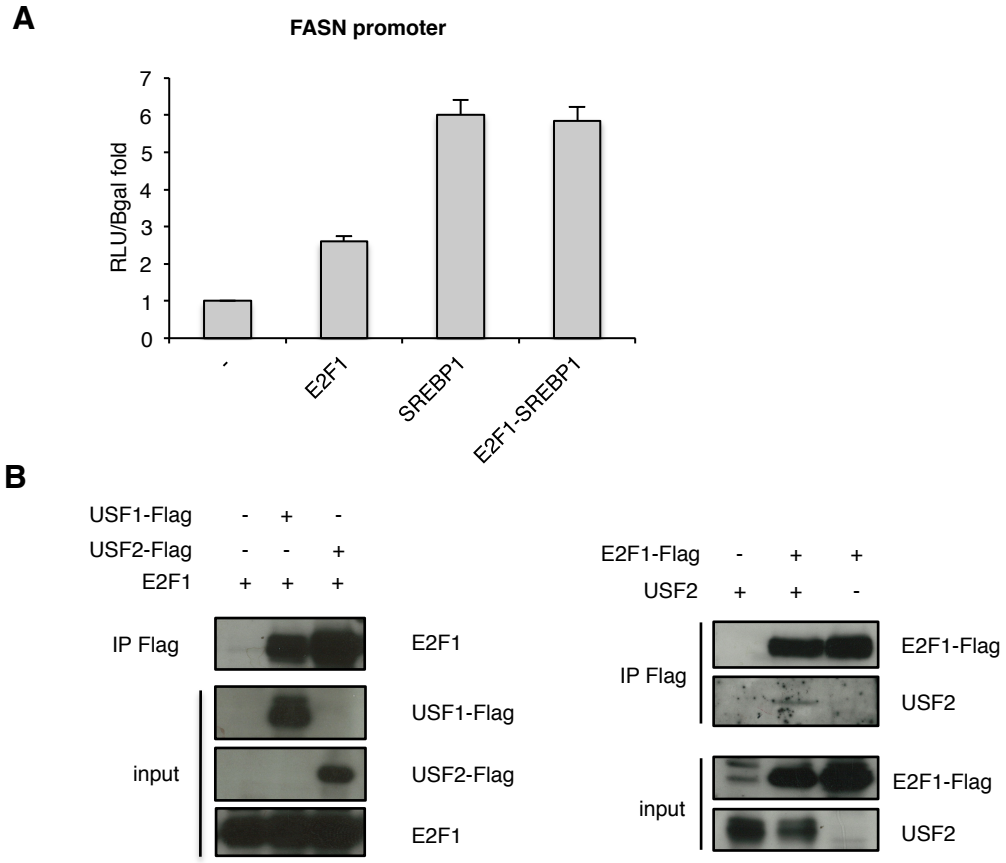


Supplemental Figure 8. Mouse *Fasn* promoter analysis

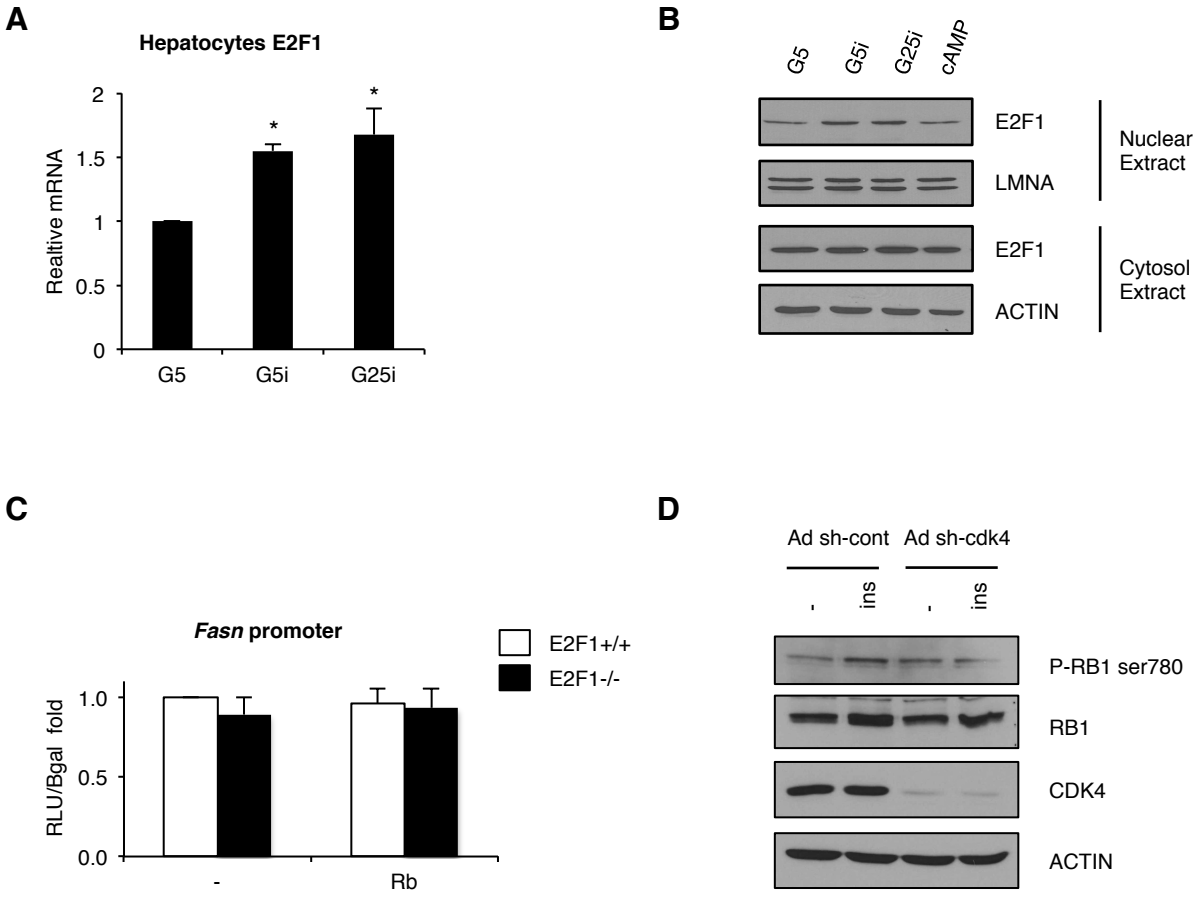
(A) 5 putative E2F binding sites were found on mouse *Fasn* promoter.
Ebox -332, SRE -150 and Ebox-65 are shown.

(B) Mouse *Fasn* promoter truncation experiment to define E2F1 transactivation site. Sequential deletion of mouse *Fasn* promoter were performed to define the E2F1 responsive element. RE1, RE2, RE3, RE4, RE5 represent the putative E2FRE shown in (A)

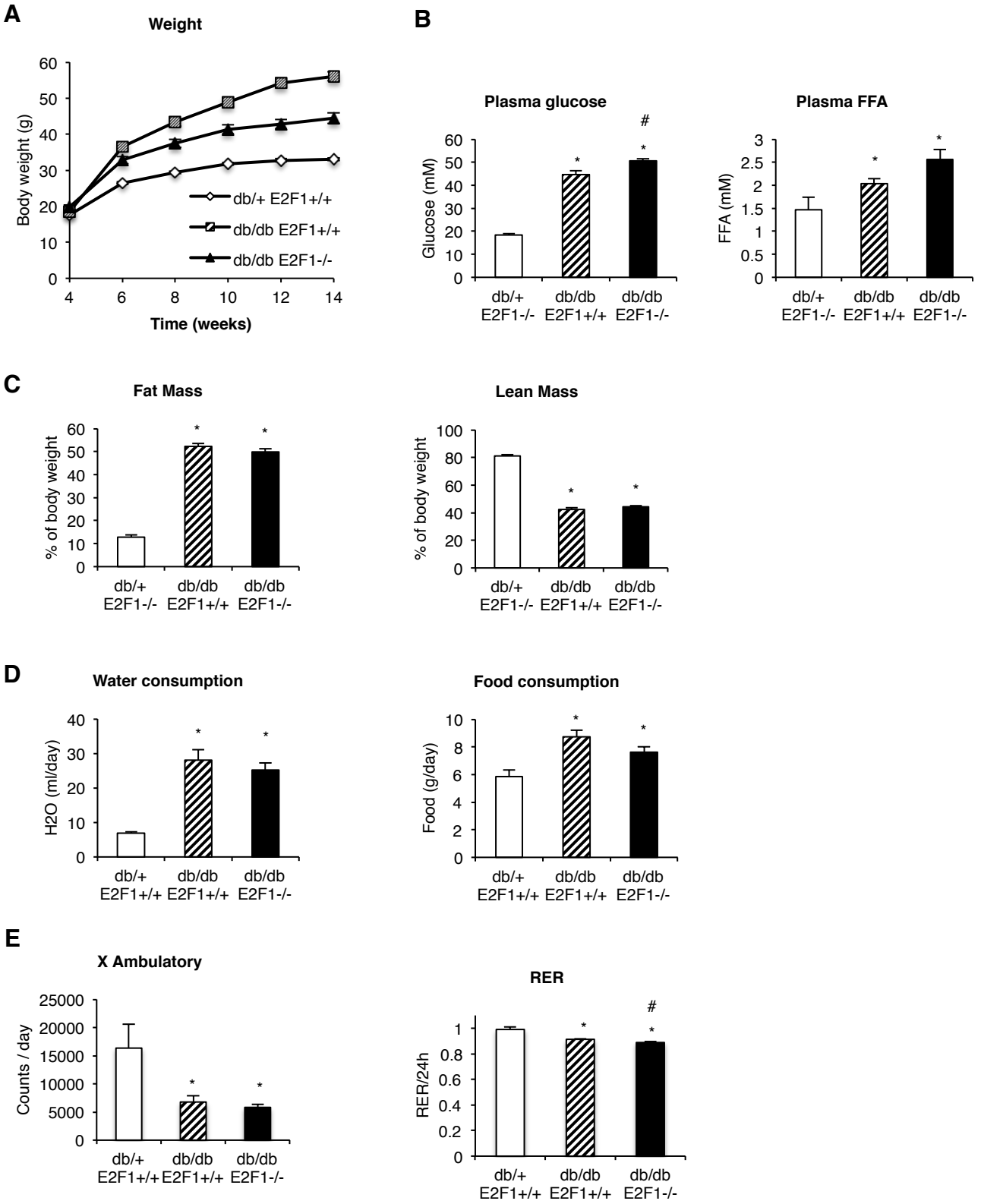
(C) Mouse *Fasn* promoter deleted for the E2FRE site.

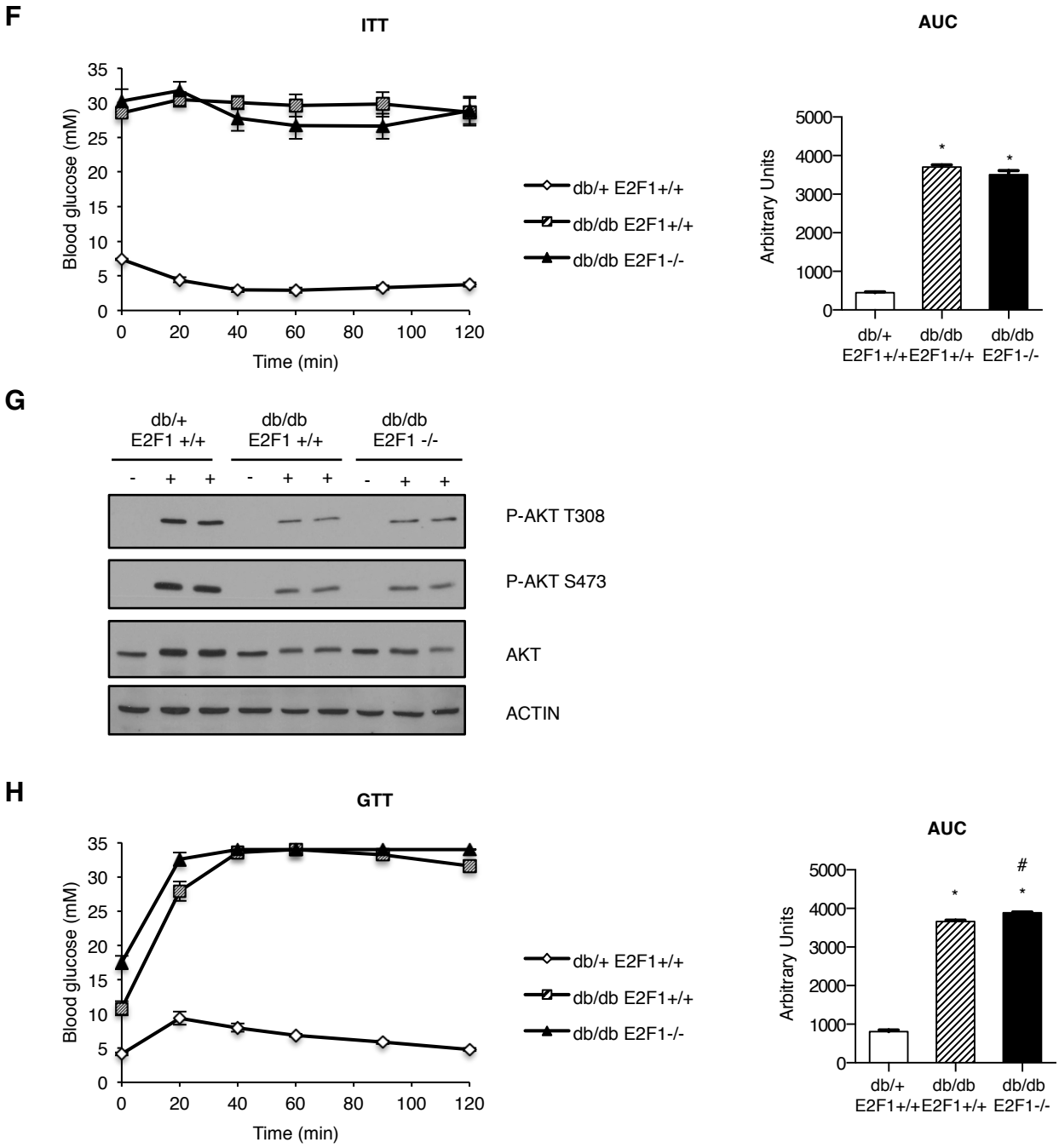


Supplemental Figure 9. **E2F1 protein interacts with USF1/2.**
(A) Mouse *Fasn* promoter activity in hepatocytes transfected with empty vector, E2F1 or SREBP1, as indicated.
(B) E2F1 interacts with USF1 and USF2. Co-immunoprecipitation experiments of E2F1, USF1 and USF2 in HepG2 hepatocytes. Cells were transfected with USF1-flag, USF2-flag and E2F1 or E2F1-flag and USF2 as indicated, and proteins were immunoprecipitated with an anti flag antibody.



Supplemental Figure 10. **Rb-E2F1 regulation in hepatocytes.**
(A) Relative E2F1 mRNA expression in hepatocytes cultured 24h in low glucose 5mM (G5), low glucose 5mM plus insulin 100nM (G5i) or high glucose 25mM plus insulin 100nM (G25i).
(B) Protein expression analysis in the indicated cellular fractions in hepatocytes treated 24h in G5, G5i, G25i and low glucose 5mM + dibutyril cAMP (100μM).
(C) Mouse *Fasn* promoter activity in hepatocytes from E2F1+/+ and E2F1-/- mice transfected with empty vector or pRb, as indicated.
(D) Ser780 RB1 phosphorylation in hepatocytes expressing Ad sh-cont or Ad sh-cdk4, starved or treated with insulin (100nM) for 1h.


A
Weight
Body weight (g)
Time (weeks)
db/+ E2F1+/+
db/db E2F1+/+
db/db E2F1-/-
B
Plasma glucose
Glucose (mM)
Plasma FFA
FFA (mM)
C
Fat Mass
% of body weight
Lean Mass
% of body weight
D
Water consumption
H2O (ml/day)
Food consumption
Food (g/day)
E
X Ambulatory
Counts / day
RER
RER/24h



Supplemental Figure 11. **Metabolic parameters of E2F1-/- db/db mice.**

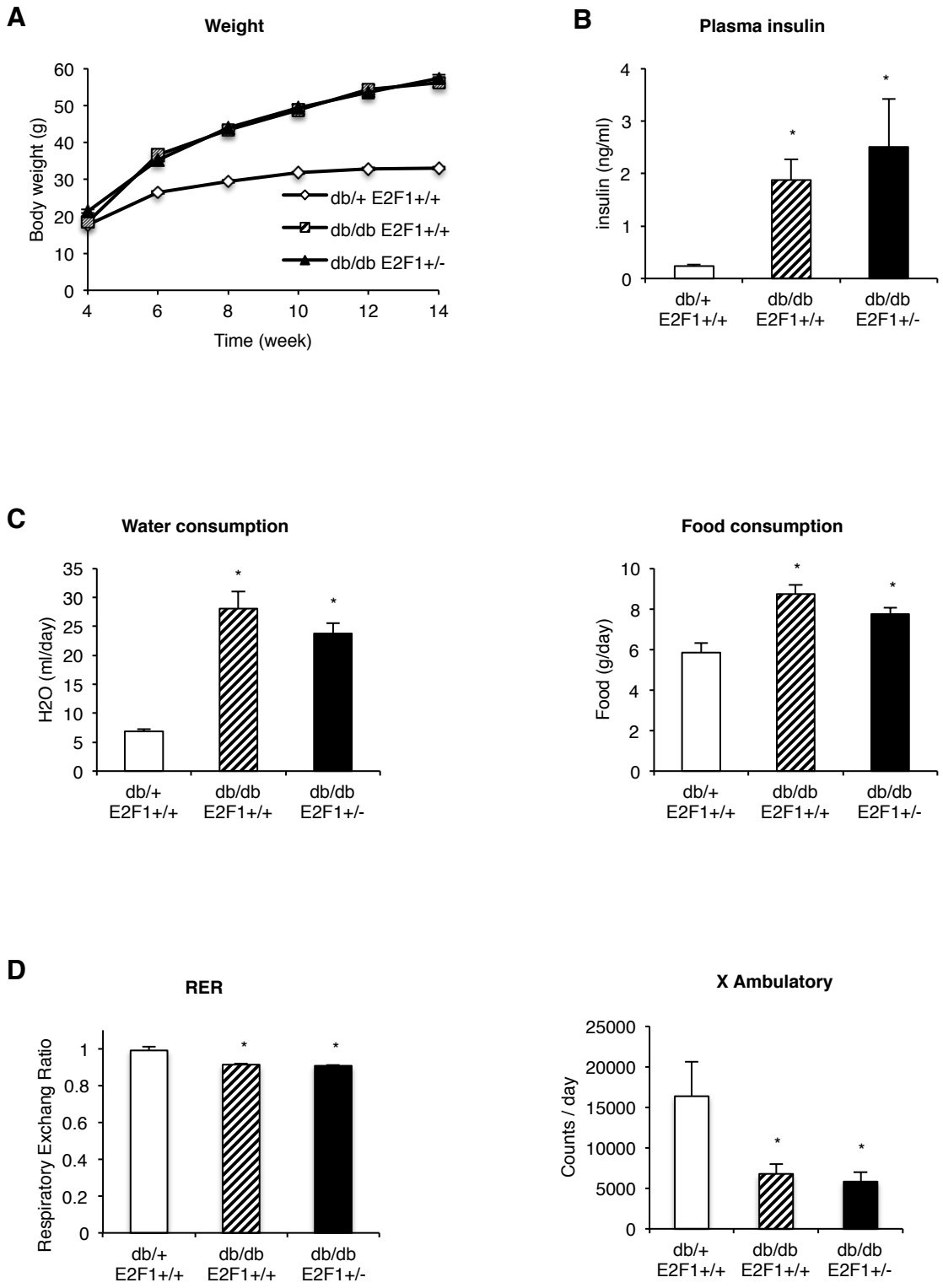
(A) Body weight of E2F1+/+ db/+ , E2F1+/+ db/db and E2F1-/- db/db mice (n>10). (B) Quantification of plasma glucose and FFA of the indicated mice genotype (n=5-9). (C) Body composition, (D) water-food consumption and (E) Ambulatory activity and Respiratory Exchange Ratio of the indicated mice genotype (n=6-10).

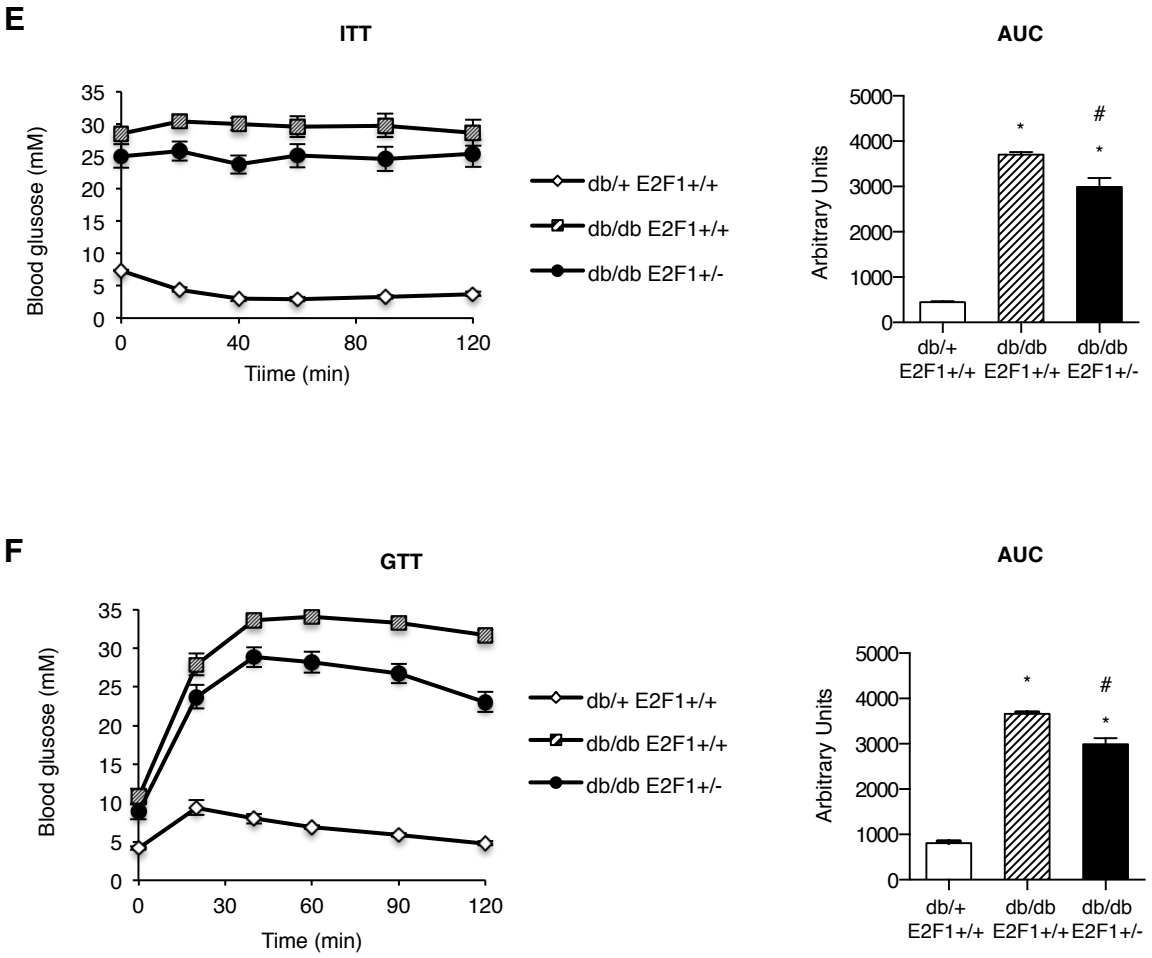
(F) Insulin tolerance test was performed in E2F1+/+ db/+ , E2F1+/+ db/db and E2F1-/- db/db mice. After 6h fasting, mice were injected intraperitoneally with 1 U/kg of insulin. Blood glucose was determined using the Aviva Accu-Chek glucometer. The area under the curve is shown (n=5-9). An asterisk indicates statistically significant differences ($p<0.05$) from db/+ mice.

(G) Insulin signaling experiments, after overnight fasting, mice were injected with 1 U/kg of insulin in the portal vein . Three minutes after injection, livers were removed and snap frozen in liquid nitrogen. Total AKT, Ser473 and Thr308 phosphorylation of AKT and ACTIN in livers of E2F1+/+ db/+ , E2F1+/+ db/db and E2F1-/- db/db mice are shown. A representative western blot is shown.

(H) Glucose tolerance test was performed in E2F1+/+ db/+ , E2F1+/+ db/db and E2F1-/- db/db mice. After overnight fasting, mice were injected intraperitoneally with 0.75 g/kg of glucose. Blood glucose was determined using the Aviva Accu-Chek glucometer. The area under the curve is shown (n=5-7).

An asterisk indicates statistically significant differences ($p<0.05$) compared db/+ mice. An # indicates statistically significant differences ($p<0.05$) in E2F1-/- db/db compared to E2F1+/+ db/db .


A
Weight
Body weight (g)
Time (week)
db/+ E2F1+/+
db/db E2F1+/+
db/db E2F1+/-
B
Plasma insulin
insulin (ng/ml)
C
Water consumption
H2O (ml/day)
Food consumption
Food (g/day)
D
RER
Respiratory Exchang Ratio
X Ambulatory
Counts / day



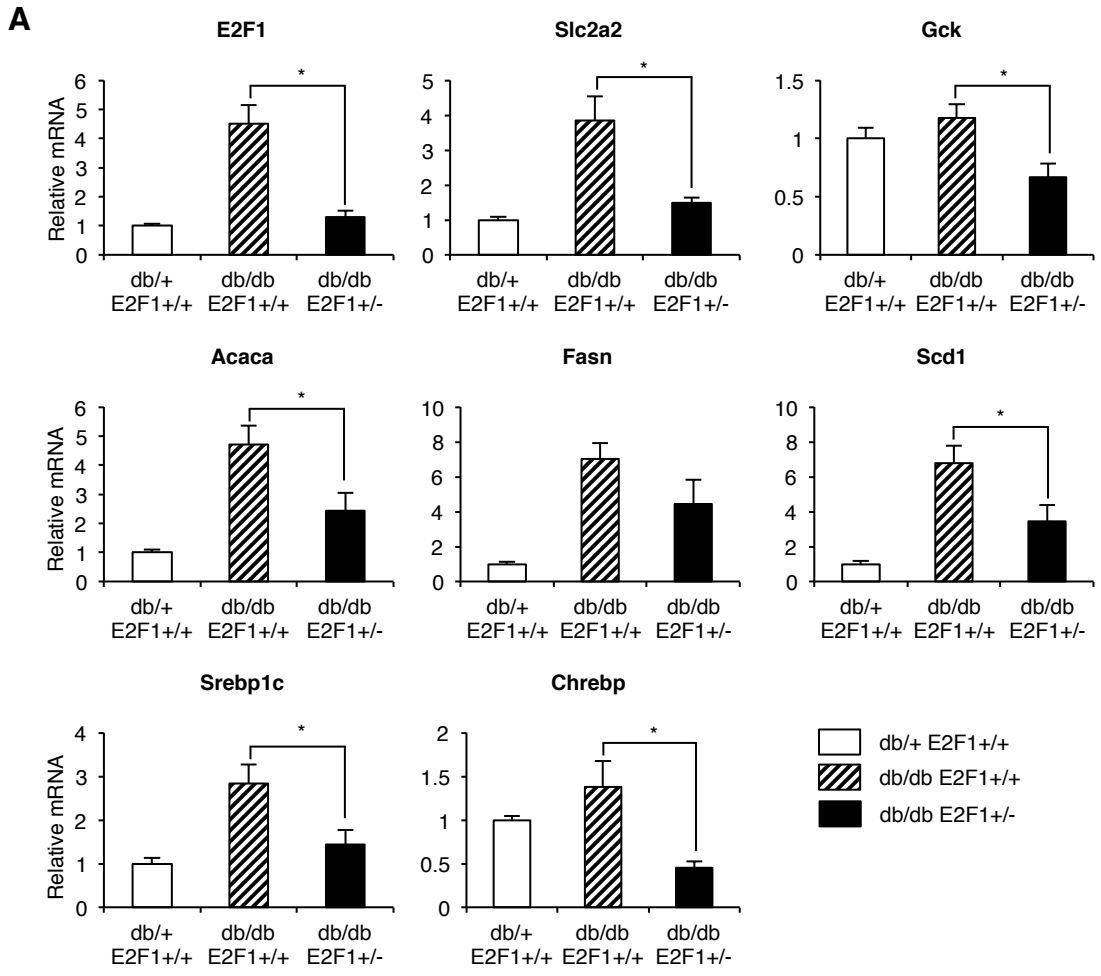
Supplemental Figure 12. **Metabolic parameters of E2F1+/- db/db mice.**

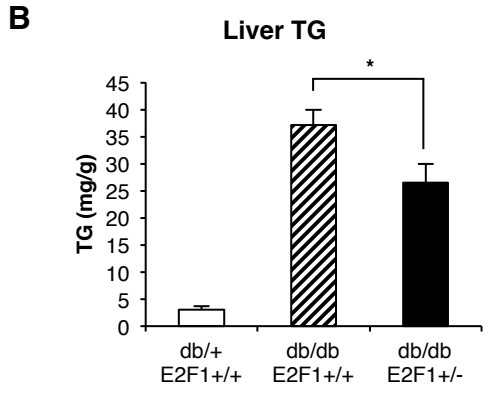
(A) Body weight of E2F1+/+ db/+ , E2F1+/+ db/db and E2F1+/- db/db mice (n>10). (B) Quantification of plasma insulin of the indicated mice genotype (n=6-11). (C) Water and food consumption and (D) Ambulatory activity and Respiratory Exchange Ratio of the indicated mice genotype (n=6-10).

(E) Insulin tolerance test was performed in E2F1+/+ db/+ , E2F1+/+ db/db and E2F1+/- db/db mice. After 6h fasting, mice were injected intraperitoneally with 1 U/kg of insulin. Blood glucose was determined using the Aviva Accu-Chek glucometer. The Area under the curve is shown (n=5-9).

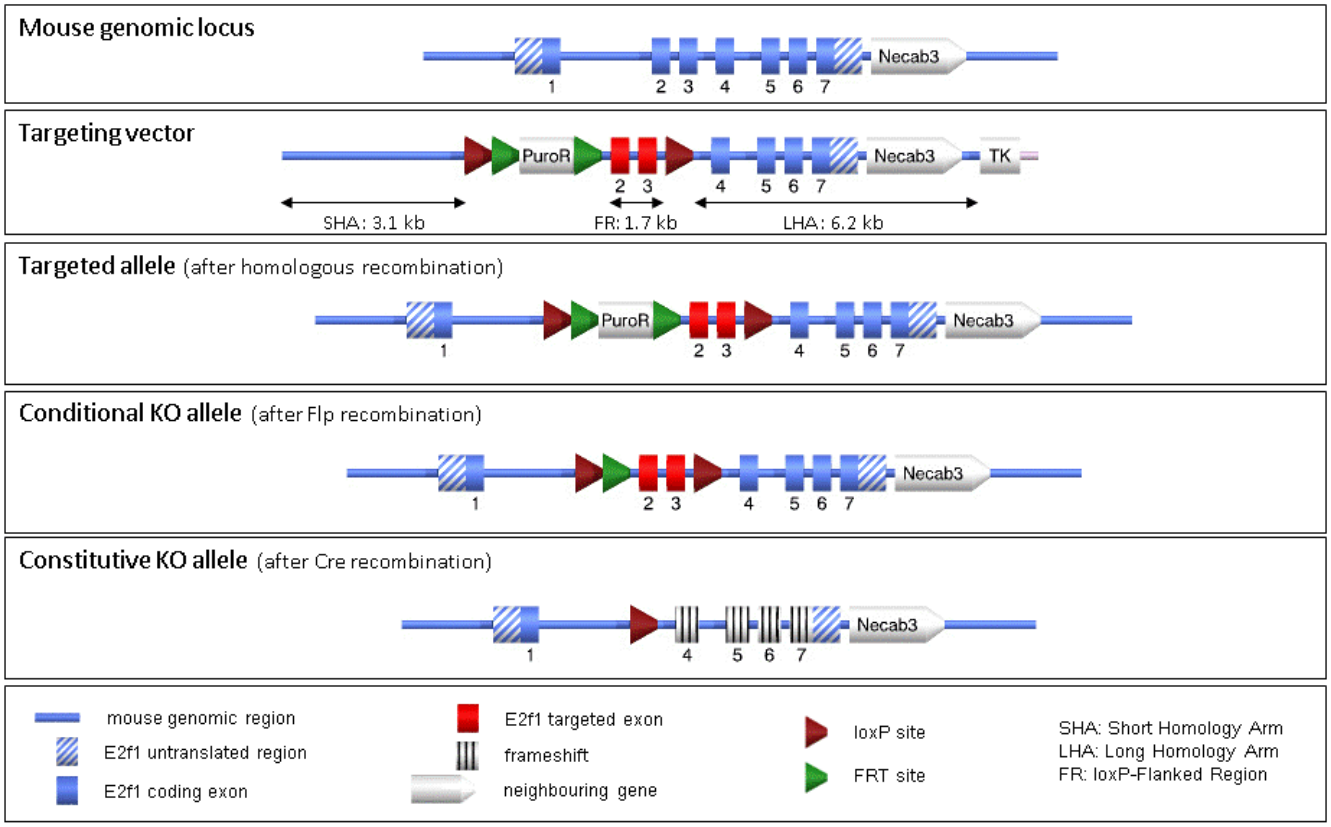
(F) Glucose tolerance test was performed in E2F1+/+ db/+ , E2F1+/+ db/db and E2F1+/- db/db mice. After overnight fasting, mice were injected intraperitoneally with 0.75 g/kg of glucose. Blood glucose was determined using the Aviva Accu-Chek glucometer. The area under the curve is shown (n=5-7).

An asterisk indicates statistically significant differences ($p<0.05$) from db/+ mice. An # indicates statistically significant differences ($p<0.05$) in E2F1-/- db/db compared to E2F1+/+ db/db .





Supplemental Figure 13. **Liver glycolitic and lipogenic programs and hepatic steatosis are reduced in E2F1+/- db/db mice.** (A) Relative liver mRNA expression of the indicated genes in the annotated mouse genotypes (n=5-9). (B) Quantification of liver TG in the indicated mouse genotypes (n=5-9). An asterisk indicates statistically significant differences ($p<0.05$).



Supplemental Figure 14. Generation of conditional E2F1 KO mice. Targeting strategy.

Supplemental Materials and Methods:

Hepatocyte glucose oxidation: Glucose oxidation was measured using a Seahorse analyzer. Briefly, hepatocytes were seeded in Seahorse XF24 plates (Seahorse Bioscience) and then glucose-starved overnight. They were then washed and placed in an unbuffered DMEM based medium containing 2mM glutamine for 1.5 hours. After basal oxygen consumption rate (OCR) was measured, glucose (25mM) was injected into the cells directly with the seahorse apparatus. Three consecutive OCR measurements, normalized by protein content, one hour after glucose injection were used to determine glucose oxidation. The graphs shown are the average of 20 wells per genotype, from two independent experiments.

Hepatocyte lactate production: Hepatocytes were seeded in Seahorse XF24 plates and then glucose-starved overnight. They were then washed and placed in an unbuffered DMEM based medium containing 2mM glutamine for 1.5 hours, glucose (25mM) were injected into the cells directly with the seahorse apparatus. After one hour of glucose exposure, the culture supernatant was recovered and used to determine lactate production. The culture medium samples were centrifuged at 12.000 rpm for 5 min at 4°C. 50μlof sample were used to determine lactate concentration using the L-Lactate Assay Kit (Colorimetric/Fluorometric) (abcam ab65330) according to the manufacturers protocol. The lactate concentration in each well (nmol/ml) was normalized by the total protein amount in each well. The graphs shown are the average of 20 wells per genotype, from two independent experiments.

Hepatocyte PDH activity: Hepatocytes were seeded in 6 well plates at a density of 1 million cells per well. 48 hours after isolation, the cells were washed and then and placed in an unbuffered DMEM based medium containing 2mM glutamine for 1.5 hours, glucose (25mM) was added into the cells directly. The samples were harvested 30 min after and used to determine PDH activity with using the Pyruvate dehydrogenase enzyme activity dipstick assay kit (abcam ab109882) according to the manufacturers protocol. Briefly, the cells were washed with ice-cold PBS and resuspended in 130μl of sample buffer, 15μl of detergent were added and the sample was lysed on ice for 10 min. The samples were then centrifuged for 10 min at 3000 rpm, and the protein levels in the supernatant were determined with the BCA method (ThermoFisher 23225). 75μg per sample were then used for PDH activity determination. The graphs shown are the average of 12 wells per genotype, from two independent experiments.

Metabolic mesurements: Energy expenditure was measured with an Oxymax apparatus (Columbus Instruments). Before starting calorimetric mesurements, mice were adapted to the individual chambers for one day. Animals had free access to food and water during the experiment. Mice activity, water and food consumption were determined.

Insulin and glucose tolerance test: Insulin tolerance test were performed after a 6h fasting. Mice were injected intraperitoneally with 1 Unit/kg of insulin (Actrapid, NovoNordisk). Glucose tolerance test was performed after an overnight fasting. Mice were injected intraperitoneally with 0.75 g/kg of glucose. Blood glucose was determined using the Aviva Accu-Chek glucometer.

***In vivo* insulin stimulation:** Mice were anesthetized after an overnight fasting and then injected or not with 1 Unit/kg of insulin via the portal vein. Three minutes after injection of insulin bolus, livers were removed and snap frozen in liquid nitrogen.

Mesurement of liver glucokinase and glycogen phosphorylase enzymatic activity: Liver samples were prepared as previously described (Dentin et al, JBC 2004). Briefly, 250mg of liver samples were homogenized in 1 ml of homogenization buffer consisting of 50 mM triethanolamine hydrochloride (pH 7.3), 100 mM KCl, 1 mM dithiothreitol, 5% glycerol, 1 mM EDTA, 1 mM EGTA, Na azide 0,02% and protease inhibitor (Sigma Aldrich). Homogenates were centrifuged at 10,000 g for 15 minutes at 4°C after an incubation period of 15 minutes with 25% polyethylene glycol. Glucokinase activity was measured as previously described (Dentin et al, JBC 2004). Glycogen phosphorylase activity was mesured according to sigma protocol. 20μl of liver lysate were added in the reation buffer (50mM KH2PO4, 1,5mM MgCl2, 0,0003% glucose 1,6-biphosphate, 0,1mM EDTA,0,15% glycogen, 1mM NADP, Glucose 6-Phosphate deshydrogenase (G6PDH) 2U/ml, phosphoglucomutase 2U/ml pH6.8). Phosphorylase-a activity was determined by spectrometric detection of NADP to NADPH conversion by phosphorylase coupled to phosphoglucomutase and G6PDH reactions. Activity was normalized witht protein content.

	age	BMI	fasting glycemia	HBA1c	fasting insulinemia	HOMAIR
lean	39±4	24.13±1.06	4.42±0.20	5.43±0.09	5.72±0.59	0.82±0.06
Glucose intolerant obese	47±3	47.75±1.54***	6.40±0.11***	5.90±0.18*	9.97±0.64***	1.6±0.1***

Supplemental Table 1. **Metabolic parameters of patients used in this study (10 female patients per group)**
* And *** indicates statistically significant differences (respectively $p<0.05$ and $p<0,0001$),

Mouse mRNA primers :	
Rs9	CGGCCCGGGAGCTGTTGACG
	CTGCTTGCGGACCCTAATGTGACG
Cyclophilin B	TGGAGAGCACCAAGACAGACA
	TGCCGGAGTCGACAATGAT
E2F1 (exon 4-5)	ACAGCTGCAACTGCTTTCGGAG
	AGCTTGTAGTTGGGTCTCAGGAGG
E2F1 (exon 3-4)	CAAACGCTTCTTGGAGCTGCTGAG
	GGCTGCCTAGCCACTGGATATGAT
Slc2a2	ACCCTGTTCCTAACCGGG
	TGAACCAAGGGATTGGACC
Gck	GCTCAGTGAACCCCGGTCAGC
	TGTGCGCAGCTGCTCTGAGG
Pklr	ATCTGGTGATTGTGGTGACAGG
	GGGGTGTGGGTTGAAAGAAA
Acaca	ACATCCCGCACCTTCTTCTACTGG
	CCTTCACTGCGCCTTCAACTTCTA
Fasn	TGCTCCCAGCTGCAGGC
	GCCCGGTAGCTCTGGGTGTA
Scd1	AAAGAGAAGGGCGGAAAACT
	GCGTTGAGCACCAGAGTGTA
Srebp1c	GGA GCC ATG GAT TGC ACA TT
	GCT TCC AGA GAG GAG GCC AG
ChREBP	GGGTAATTACTGGAAGCGGCGCAT
	TGGACTTACGGAGCCGCTTTTTG
Human mRNA primers :	
Rps9	AAGGCCGCCCGGGAACTGCTGAC
	ACCACCTGCTTGCGGACCCTGATA
Actb	GGCACTCTTCCAGCCTTCCT
	GCAATGCCAGGGTACATGGT
E2f1	ACAAGGCCCGATCGATGTTT
	AGAGACTGGCTGGGATCTGT
Mouse ChIP primers :	
Slc2a2 promoter	CCCATTTCTGCCACACTTAT
	TCCAATCAATACACCCTTTACC
Gck promoter	CCAAGGACTTCTGCACTAAT
	ATCCCACGTGGTTCTTTG
Pklr promoter	CCCACTGACAAAGGCAGAGT
	CCTCCAAGTTCCCTCCATCT
Acaca promoter	CTGACCTGCTGTCACTTTC
	GGCAGCCTCAGTTTCTTT
Fasn promoter	AATTGGTTTCGATGTGGAGCAGGC
	TGGTCACACTGGAAACTCTGCACT
Scd1 promoter	GGCAGGACAAGGTGGCACCAA
	GAGGCGCCGGGATGCTGAAG
Srebp1c promoter	TTACTGGCGGTCACTGT
	AGAGCTTCCGGGATCAAA
ChREBP promoter	AAAGTGCTCTCAACAGAAAGA
	CCTTAGTTGGCAGGTGATG
Human ChIP primers :	
Slc2a2 promoter	TACAAACCAGAGGCAATCAC
	GCTCAGCATATCTCATCTTCTAC
Gck promoter	AACTTTGGTGTGACCCTTAC
	CCAAAGCATCTACCTCTTAGC
Pklr promoter	CCTATGTTCCATGGCTTCTG
	TTCGGTCATGGGTCTCTAA
Acaca promoter	GCGTAGCTCTACCAAGAATG
	CTCAGCTCTAACAGGGTACT
Fasn promoter	GTTACTGCCGGTCATCG
	CGGGAAGCTGCTAAGGA
Scd1 promoter	GAGAAGCTGAGAAGGAGAAAC
	TTGGCCGAAGGGAATTTG
Srebp1c promoter	GGTAACTGTCACACCTTCTC
	TTTATACAATGCCTCCGTCTC
ChREBP promoter	CGACGACACCATGAAGTG
	ACTCGGACACAGACTCG
non specific region	GCCACAGGATATGAGCATTAG
	GGTAAAGAAGTGGAGGAGTAGA

Supplemental Table 2. Primers used for qPCR