Supplemental information

Supplemental Figure legends

Figure S1

(A) Summary of TFs identified through mass spectrometry of nuclear proteins isolated with a Biotin-Pck1p pulldown from Vehicle (V), Dexamethasone/cAMP (6hrs, D/C) and D/C (6hrs) plus insulin (last 30min, D/C/I) treated primary hepatocytes.

(B) Quantification of TOX4 protein from mass spectrometry of Pck1p pulldown samples under V, D/C and D/C/I treatment.

(C) Tox4 mRNA expression in WT primary hepatocytes treated with different combination of hormones. mRNA levels in each sample were determined by qPCR analysis (n=3).

(D) Tox4 mRNA levels in liver samples from WT 8-week(wk)-old male killed in ad lib (n=5), 16hr-fasting (n=7) and 16hr-fasting followed 4hr-refeeding (n=4) conditions. (ns= not significant).

(E) TOX4 protein in different tissues from 10-wk-old male mice.

* $p<0.05$, ** $p<0.01$, **** $p<0.0001$ by One-way ANOVA analysis. ns: not significant. Data are presented as means ± SEM.

Figure S2

(A) WB of *Ctrl sh*- or *Tox4 sh*-treated WT primary hepatocytes lysates.

(B) SiRNA-mediated silencing of *Tox4* in WT primary hepatocytes as determined by qPCR. Primary hepatocytes were transfected with control (*Ctrl*) siRNA or *Tox4* siRNA for 72 hrs (n=12). **** $p<0.0001$, 2-tailed student's t-test.

(C-D) *Pck1* (C) and *G6pc* (D) mRNA levels determined by qPCR in *Ctrl* or *Tox4* siRNA-treated primary hepatocytes after incubation with V, D/C for 6hrs, D/C for 6 hrs then 100nM insulin for the last 2hrs (D/C/I 2hrs), or D/C/I for 6hrs (n=3).

(E) WB of TOX4, FoxO1, and ACTIN in Ad-GFP and Ad-Flag-TOX4-treated primary hepatocytes.

(F) Glucose production in primary hepatocytes under the same conditions indicated in D.

(G-H) PTT and GTT in 10-wk-old mice after Ad-GFP or Ad-TOX4 injection on Day 6 and Day 12 (n=7,8).

(I) WB of TOX4, FoxO1 in livers of Ad-GFP- and Ad-TOX4-treated mice.

*/a p<0.05, **/aa p<0.01, ***/aaa p<0.001, **** p<0.0001. 2-tailed student's t-test in B, G, H and 2-way ANOVA in C,D,F. Asterisks indicate comparison within group. a indicates comparison with *Ctrl* siRNA or Ad-GFP group. Data are presented as means ± SEM.

Figure S3

10-wk-old C57BL/6J mice were injected with adenovirus *Ctrl sh* (n=7) or *Tox4 sh* (n=8) and sacrificed after 2 wks post injection.

(A) Expression of *Tox* isoforms in Liver (n=6,7).

(B) Body weight (BW).

(C) Liver weight, normalized to BW.

(D) Plasma insulin levels at indicated (n=6-8 for each group);

(E-H) Plasma triglyceride (TG) (E); non-esterified fatty acids (NEFAs) (F), total cholesterol (G) and Alanine Amino-transferase (ALT, H) determined by colorimetric assays (n=7 for each group).

(I) Expression of lipogenic and lipolysis genes in EWAT of 4-hr fasted mice.

(J) *Gckp*-luciferase assays of TOX4, HNF4α and their combinations.

(K) PAS staining of liver sections from 16-hr fasted mice. Scale bar, 200μm.

(L) Glycogen content in livers from 16-hr fasted and 4-hr refed mice.

* p<0.05, ** p<0.01, *** p<0.001, compared with *Ctrl sh* group by 2-tailed student's t-test. Data are presented as means ± SEM.

Figure S4

(A) Heatmap of expression clustering of differentially expressed genes in *Tox4 sh*-treated liver vs *Ctrl sh*-treated liver.

(B-C) Functional enrichment of differentially expressed genes in A. Top 20 enriched biological processes (BP) of up-regulated (B) and down-regulated (C) genes.

(D) List of up-regulated genes enriched in BPs (GO Term) associated with glucose metabolism.

(E) Liver *Gcgr* mRNA level in *Ctrl sh*- or *Tox4 sh*-treated mice.

* $p<0.5$ by 2-tailed student's t-test. Data are presented as means ± SEM.

Figure S5

(A) Diagram of breeding strategy to generate TOX4 LKO mice.

(B) Genotyping results of Tox4 loxP (f) and Albumin-Cre alleles.

(C) *Tox2, Tox3, Tox4* expression in TOX4 f/f (n=5) and TOX4 LKO (n=6) mouse liver.

(D) WB of PCK1 and G6PC in TOX4 f/f and LKO primary hepatocytes.

(E) Body weight of 12-wk-old male TOX4 f/f and LKO mice fed with chow diet (CD, n=11,9) or high fat diet for 4 weeks (HFD, n=8,8). Mice were weighed after a 16-hr fast.

(F) Fasting glucose (16-hr fast) in mice from (E).

(G) Plasma insulin level in TOX4 f/f and LKO mice on CD (4-hr fast, n=6,8) or HFD (6-wk HFD, 4-hr fast, n=6,8).

(H) Insulin tolerance test (ITT) of TOX4 f/f and LKO mice under HFD (n=5,6).

(I-J) mRNA levels of glucose metabolic genes in TOX4 f/f and LKO mice fed with CD (16-hr fasting, n=8,6) (I) or HFD mice (4-hr fasting, n=7,8)(J).

** $p<0.01$, *** $p<0.001$, 2-tailed student's t-test. Data are presented as means ± SEM.

Figure S6

17-wk-old male FoxO1 f/f (n=8), TOX4 LKO (TLKO, n=6), FoxO1 LKO (O1LKO, n=7) and TOX4/FoxO1 double LKO (DKO, n=6)

(A) Body weight (BW).

(B) Fasting glucose (F16h).

(C) Plasma insulin after 16-hr fasting.

(D) Liver weight (normalized to BW).

(E-F) Liver glycogen content (E) and PAS staining of liver sections (F) after 4-hr fasting. Scale bar, 200μm.

(G) WB of RFP- or Flag-TOX4-transfected AML12 cells after 30min treatment with either 0.1mM cAMP or 100nM Insulin.

** p<0.01, *** p<0.001 by One-way ANOVA analysis. No significant differences were observed among the three genotypes in A-C and F. Data are presented as means ± SEM.

Figure S7

(A-B) QPCR (A) and WB (B) of livers from 14- to 15-week-old f/f (n=9), LIRKO (n=9) and LIRTDKO (n=5) mice. *p<0.05, **** p<0.0001, 2-way ANOVA.

(C-D) Body weight (C, 16-hr fast) and PTT (D) of 12-wk-old IR f/f (n=4) an LIRKO (n=6) male mice fed with chow diet.

(E) Liver histology, HE and PAS staining. Scale bar, 200 μm.

* p<0.05, ** p<0.01, by 2-tailed student's t-test, as compared with f/f mice. Data are presented as means ± SEM.

Figure S8

TOX4 locus shows strong genetic association with glycemic traits. Colored bars summarize bottom-line meta-analyzed associations for phenotypes in the glycemic trait group. P-value for each phenotype indicated in the bracket. Phenotypes directly associated with TOX4 gene are marked by red check marks (Type 2 Diabetes Knowledge Portal).

Figure S9

(A) Body weight of mice after 1 month of HFD at day 6 and day 10 post adenovirus injection.

(B) Liver weight of *Ctrl sh*- and *Tox4 sh*-treated HFD mice at Day12 post adenovirus administration.

(C-H) Plasma insulin (C), TG (D), cholesterol (E), NEFA (F), ALT (G) and liver glycogen (H, n=7,7) from *Ctrl sh*-and *Tox4 sh*-treated HFD mice determined by ELISA or colorimetric assays.

(I) Liver mRNA expression of glucose metabolic genes as indicated in *Ctrl sh*- or *Tox4 sh*-treated mice (n=9,10).

* $p<0.05$ by 2-tailed Student's t-test. Data are presented as means ± SEM.

Figure S10

(A) Tox4 mRNA in muscle from Ctrl sh- and Tox4 sh-treated *db/db* mice (n=8,8).

(B) Body weight (BW) of 12-wk-old male *db/db* mice before (Day 0) and after adenovirus treatment (Day 10).

(C) Liver weight of Ctrl sh- and Tox4 sh-treated *db/db* mice. Liver weight was normalized to BW.

(D) Plasma insulin in Ctrl sh- and Tox4 sh-treated *db/db* mice after a 4-hr fast.

(E) Plasma TG in Ctrl sh- and Tox4 sh-treated *db/db* mice after a 4-hr fast.

(F) Plasma NEFA in Ctrl sh- and Tox4 sh-treated *db/db* mice after a 4-hr fast.

(G) Plasma ALT levels in Ctrl sh- and Tox4 sh-treated *db/db* mice after a 4-hr fast.

(H) Liver glycogen content.

* $p<0.05$ by 2-tailed student's t-test. Data are presented as means ± SEM.

A

TFs	Σ# PSMs	Negative control	V	D/C	D/C/I
TOX4	4	0	4527041.7	4282688.77	0
HMGA1	5	0	14835013	24341980	0
NFIC	7	0	31151610	40653993.7	6478798.1
NFIX	8	0	51391508	45622390.1	13666289
NFIA	10	0	31151610	39953339.7	15396101
NFIB	8	0	28291065	38657421.5	17766508
NFIL3	22	0	28553090	41551205.5	31609071
YBX1	8	51470227	76836287	50852203	41831809
TFAM	12	0	118442654	50746815.3	43474472
UBP1	5	0	7015711.1	8391422.63	7537677.6
CDC5L	20	2707361	25752706	18686856.2	25588380
MTA2	5	2421430	4514363	4413410.91	6235892.6
CEBPA	4	0	4389757.8	3748911.77	6182215.8
AHCTF1	67	1719952	39543349	42571608.3	76869056
CEBPB	36	8030686	31684415	106483642	192592272
SMARCC2	5	0	4044330.9	4160207.24	10261075
ZBTB20	9	8983750	13644700	8982654.31	23704815
SMARCA5	8	2523090	11412245	2919820.43	10514397
ONECUT1	4	0	8380033.6	0	15673577
PDS5B	4	0	13474771	0	9937279.2

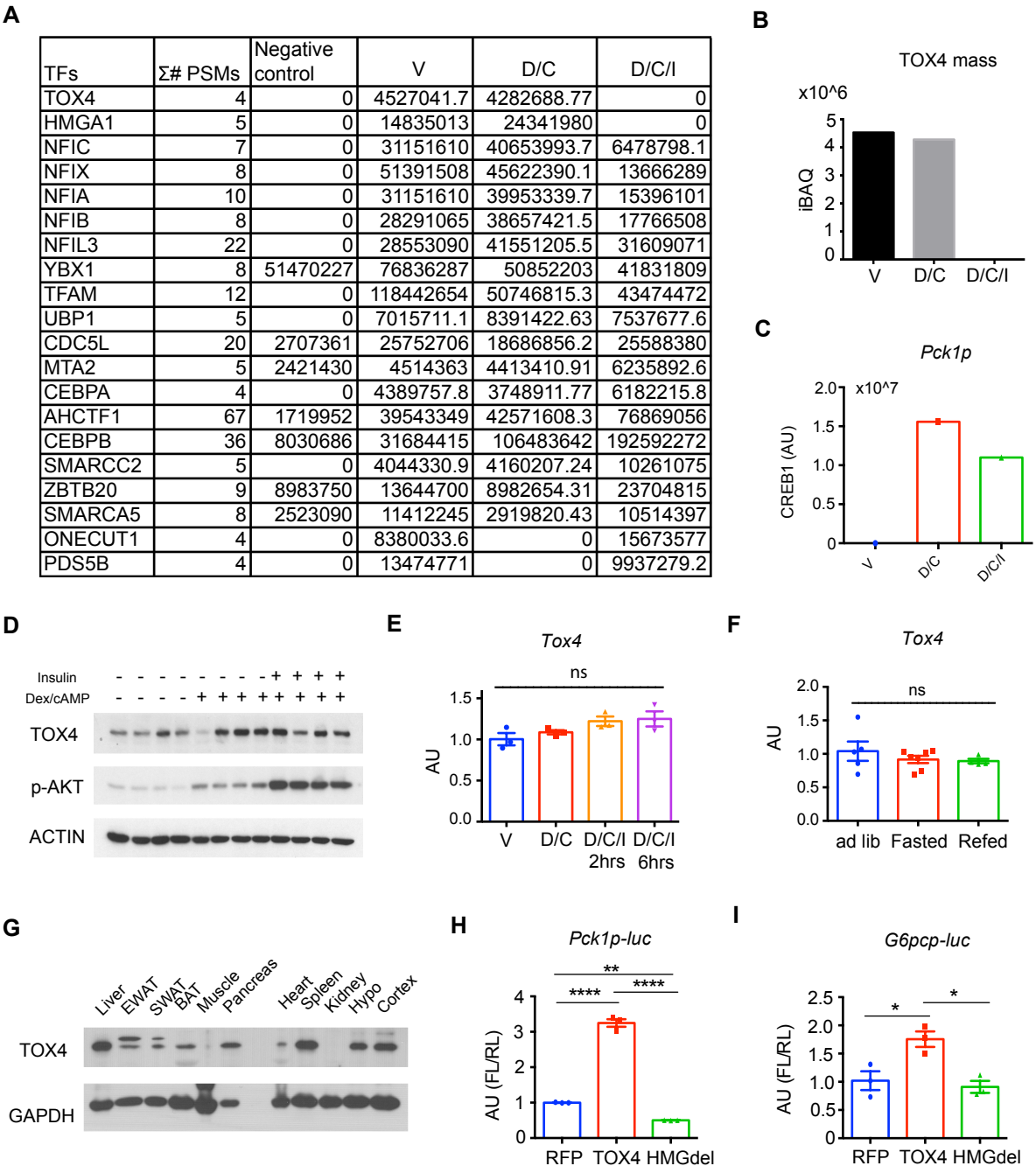

Figure S1. Identification of TOX4 as an insulin-regulated TF from *Pck1p*-binding proteins. Related to Figure 1.

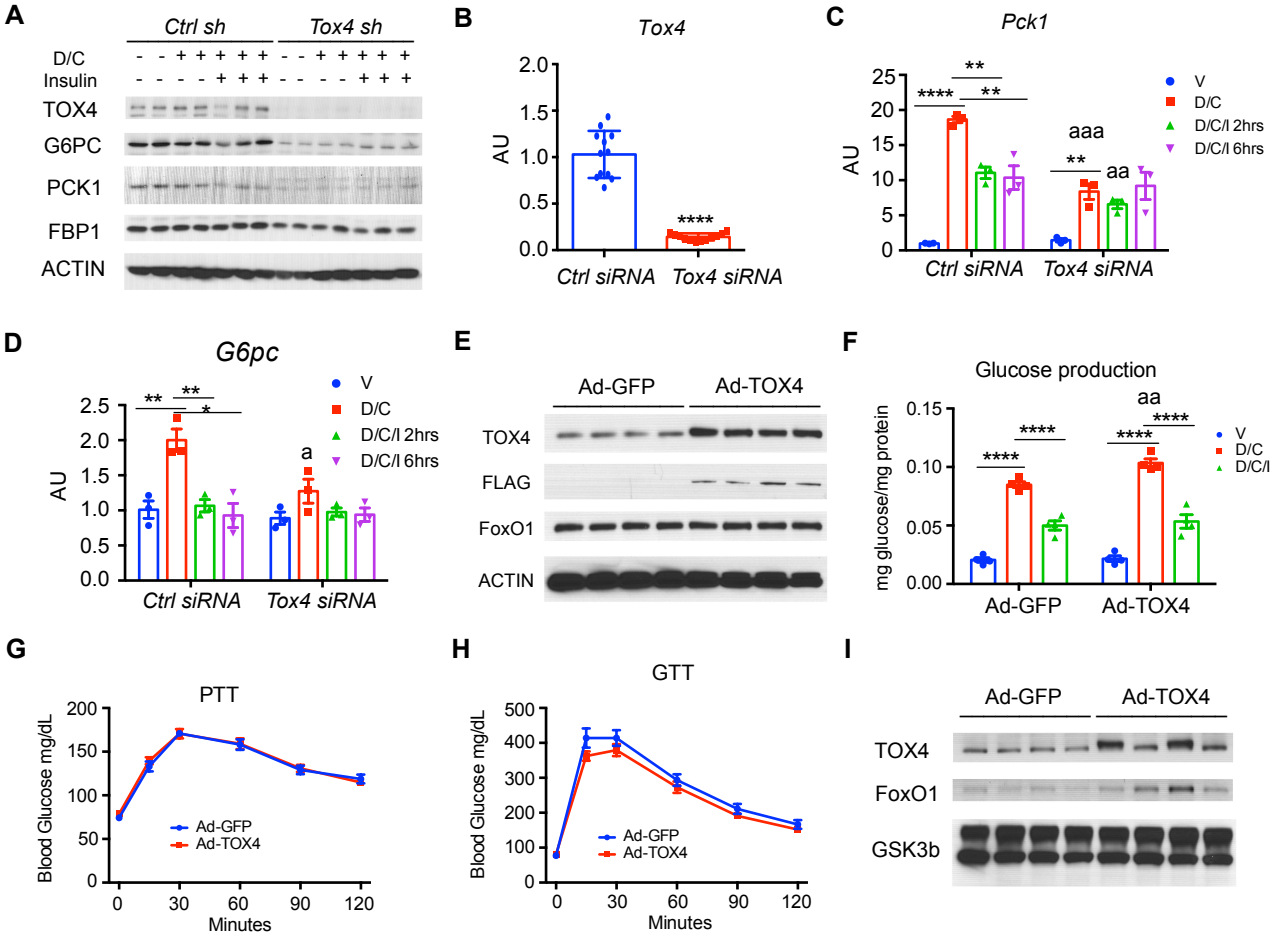

Figure S2. Silencing *Tox4* in primary hepatocytes decreased gluconeogenesis. Related to Figure 2.

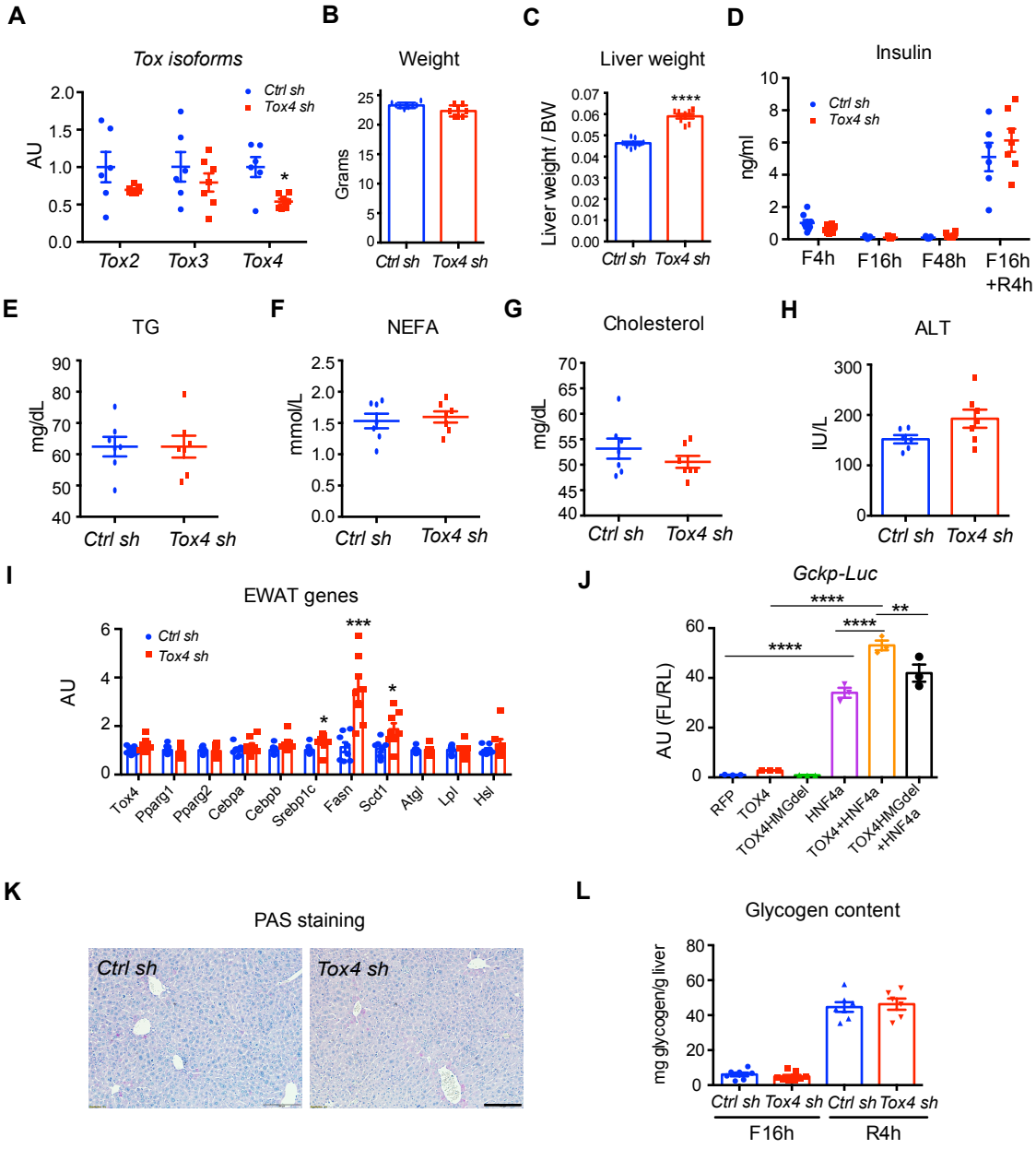

Figure S3. Metabolic characterization of TOX4 KD mice. Related to Figure 2.

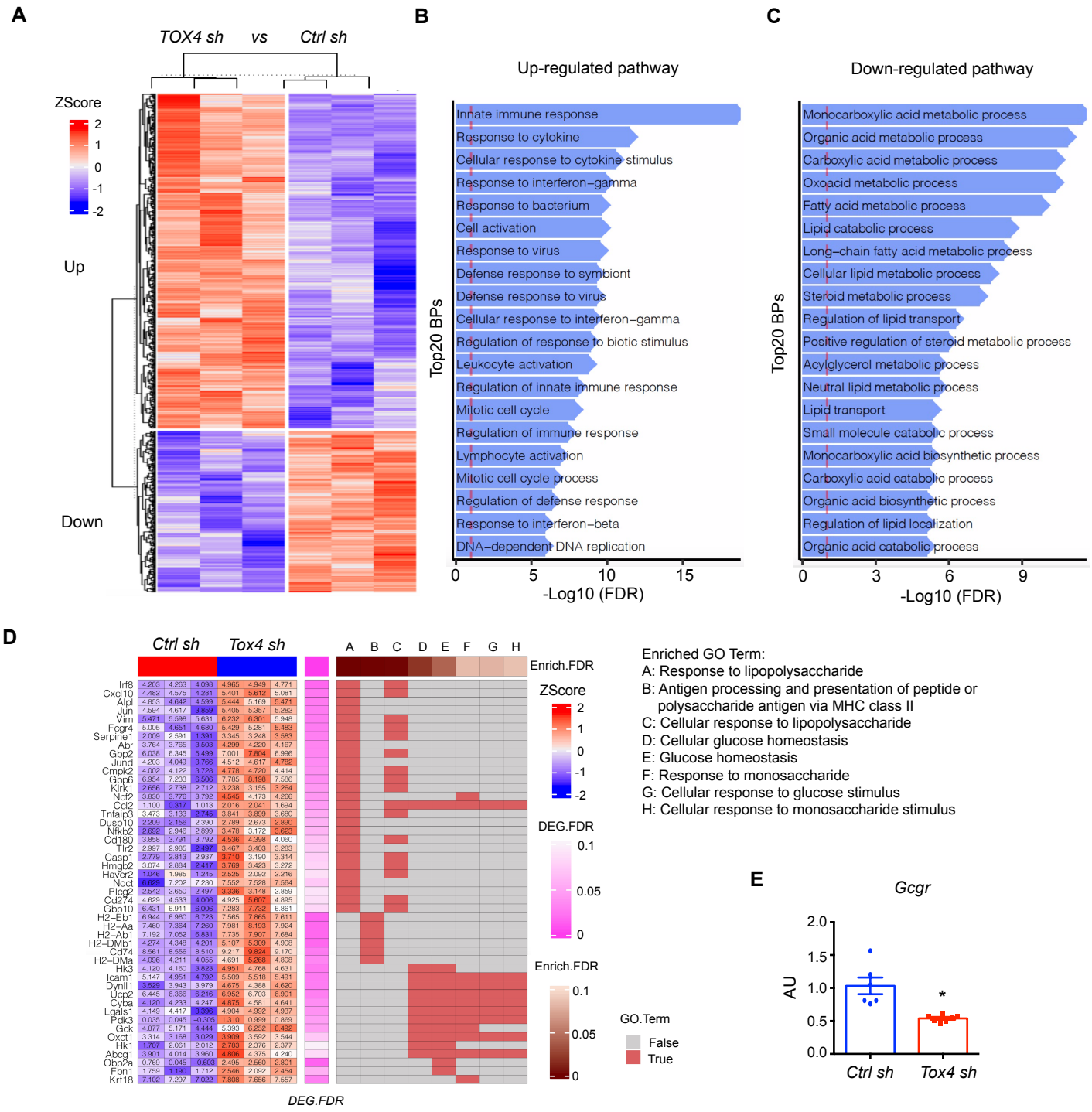

Figure S4. RNA-seq of livers from TOX4 KD mice. Related to Figure 2.

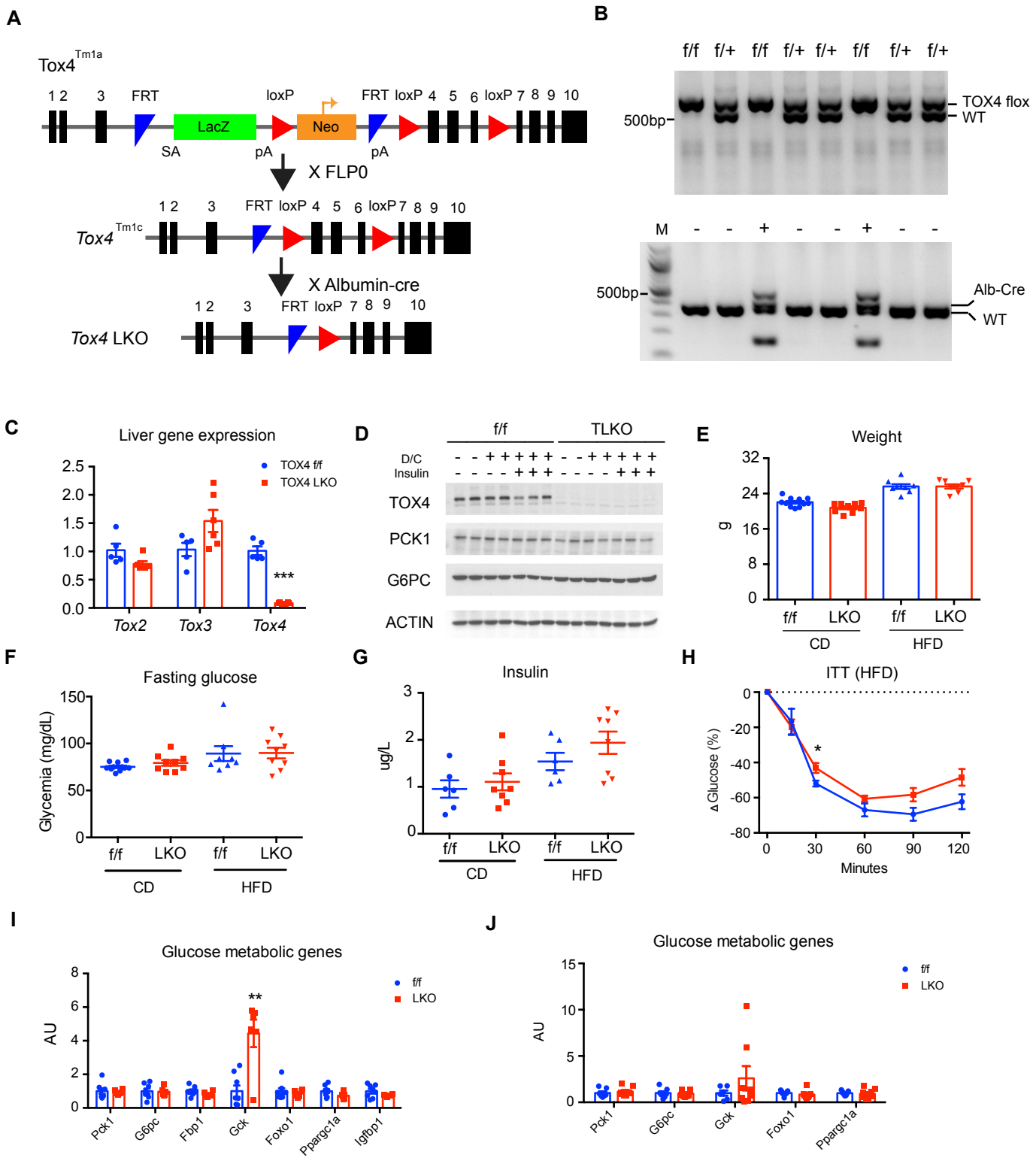

Figure S5. Generation and metabolic characterization of TOX4 LKO mice. Related to Figure 3.

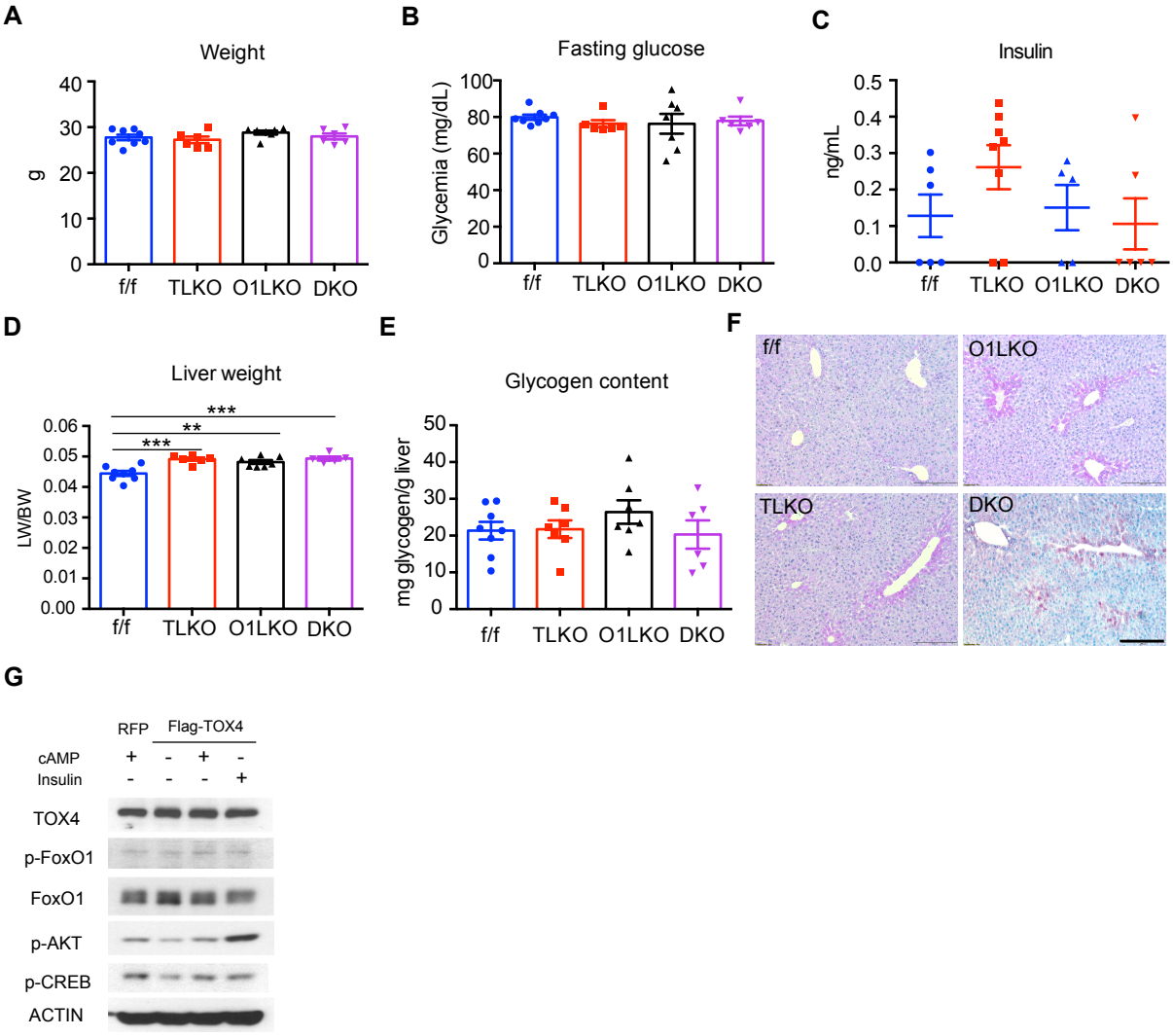

Figure S6. Metabolic characterization of TOX4/O1 DKO mice. Related to Figure 4.

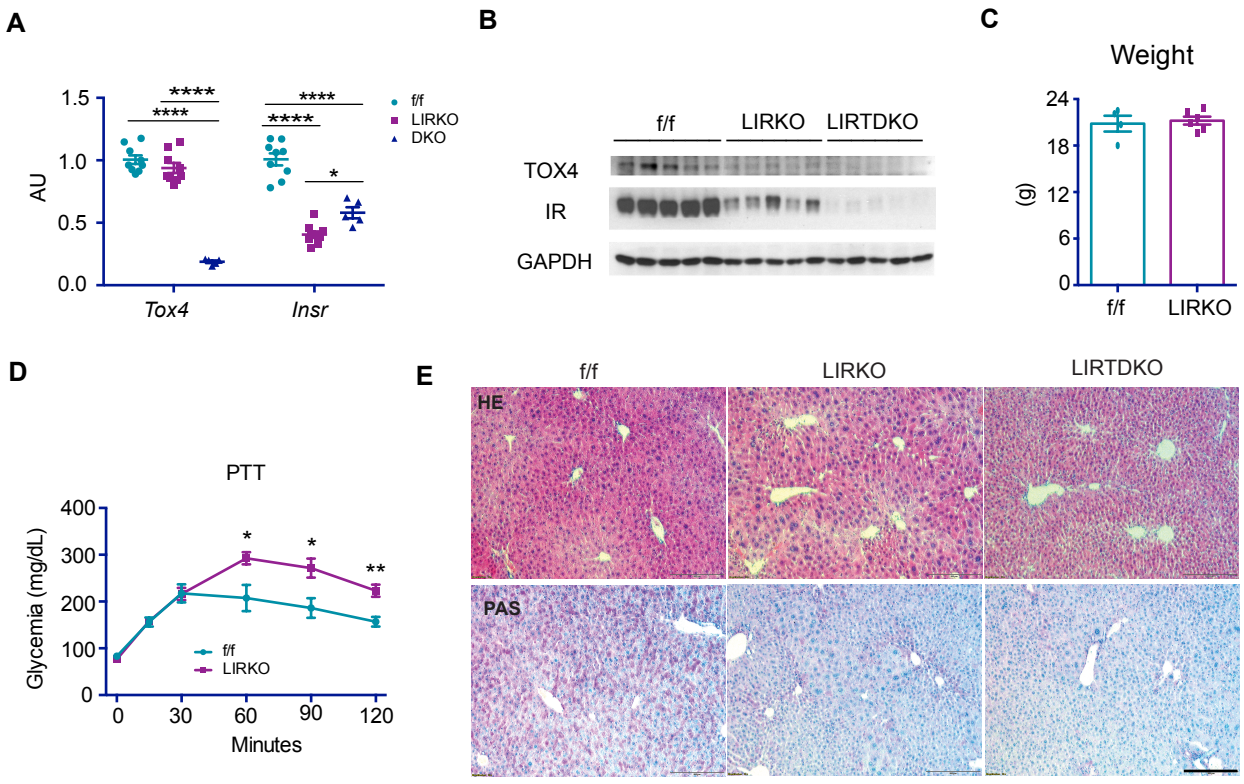

Figure S7. Metabolic characterization of LIRKO and LIRTDKO mice. Related to Figure 5.

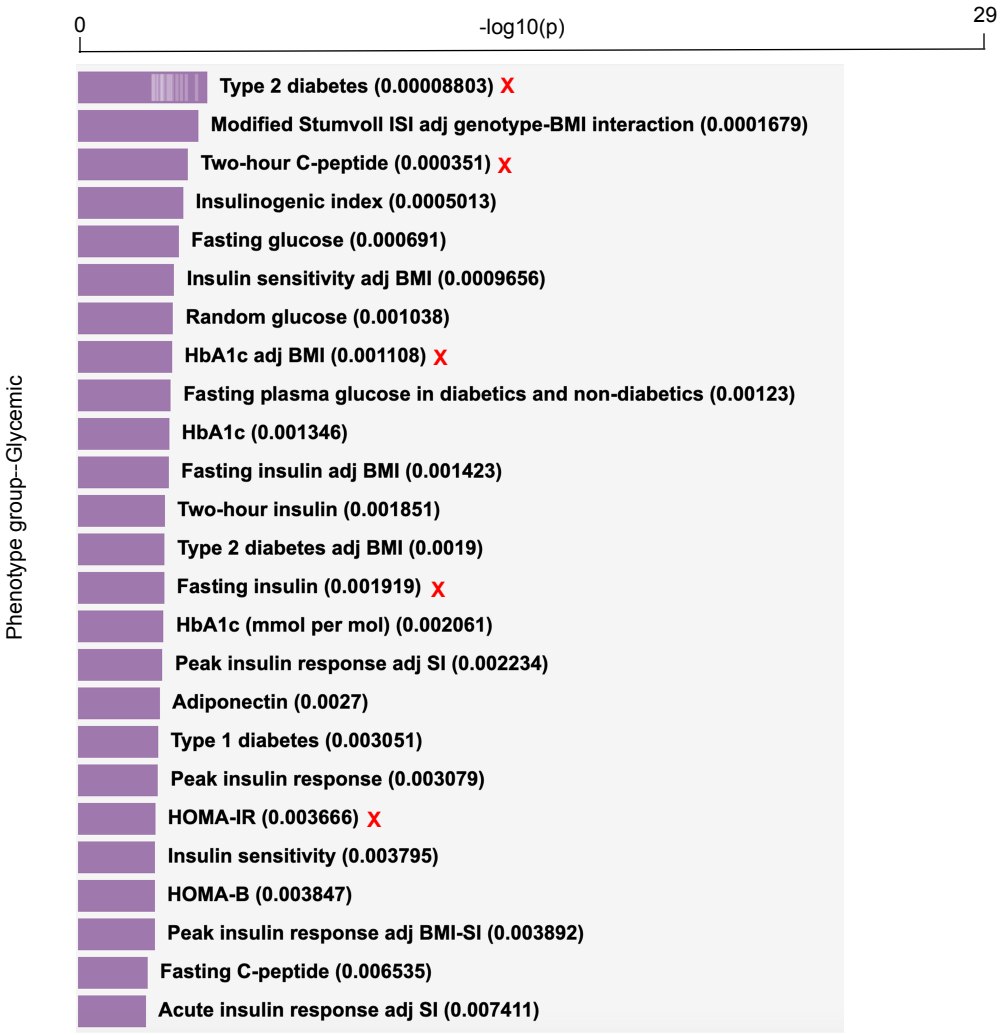

Figure S8. TOX4 locus is associated with glycemic traits in human. Related to Figure 6.

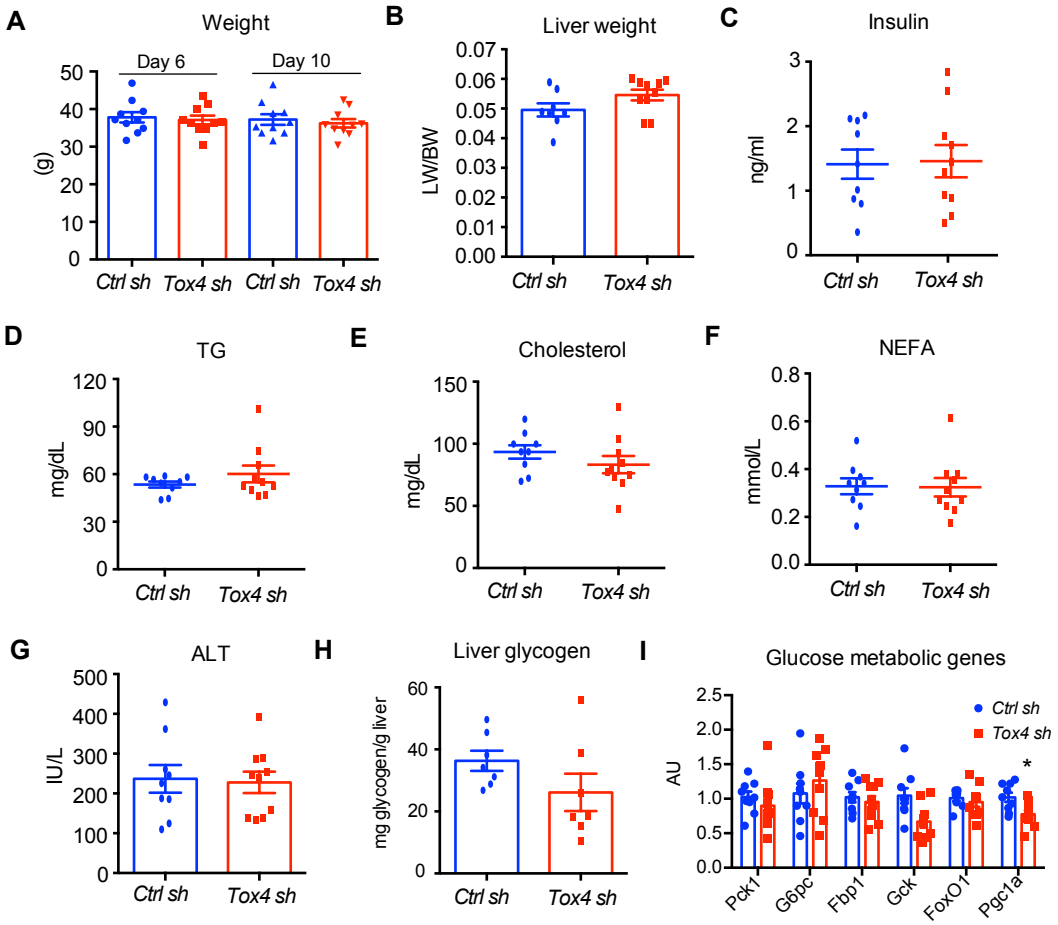

Figure S9. Metabolic characterization of TOX4 KD DIO mice. Related to Figure 6.

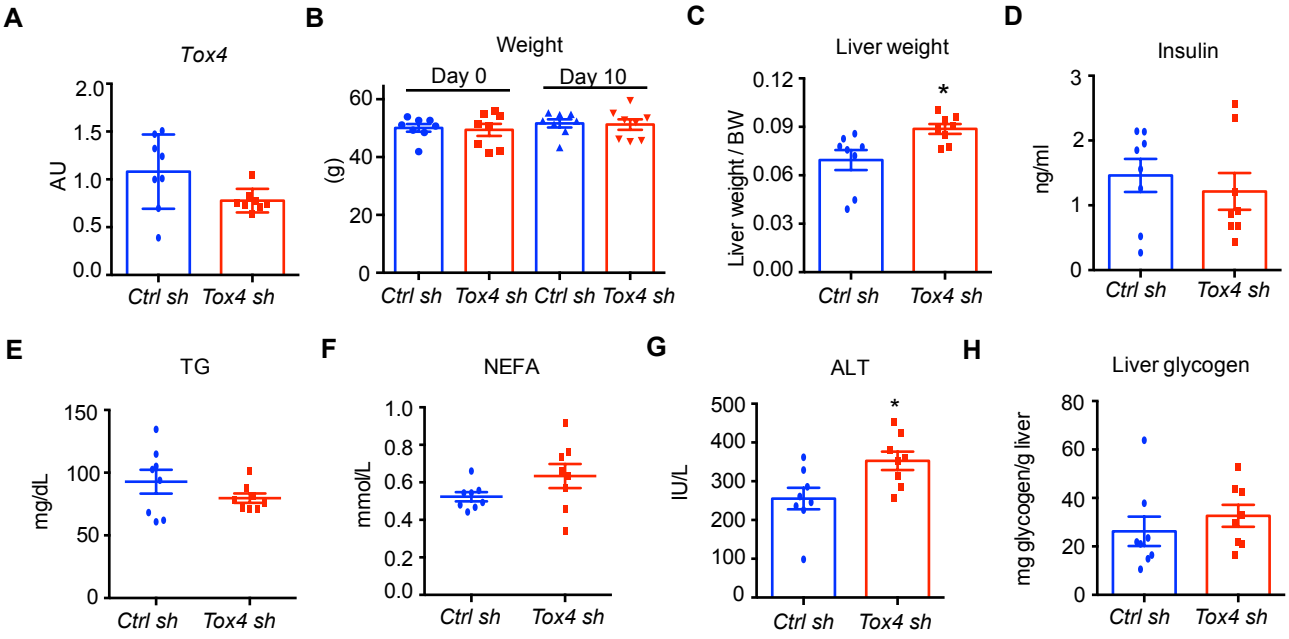

Figure S10. Metabolic characterization of TOX4 KD db/db mice. Related to Figure 7.

Table S1. Information of human subjects included in this study. Related to Figure 6.

ID	Age	Gender	T2D/Obese	Liver diagnoses
1	13	male	NA	Normal
2	10	male	NA	Normal
3	59	male	NA	Normal
4	36	female	NA	Normal
5	50	male	NA	Normal
6	67	female	NA	Normal
7	53	male	T2D	fatty liver
8	67	female	T2D	cirrhosis
9	64	female	T2D	fatty liver +HCC*
10	62	female	Obese	fatty liver
11	43	female	Obese	fatty liver
12	57	female	T2D	fatty liver

*HCC: Hepatocellular carcinoma