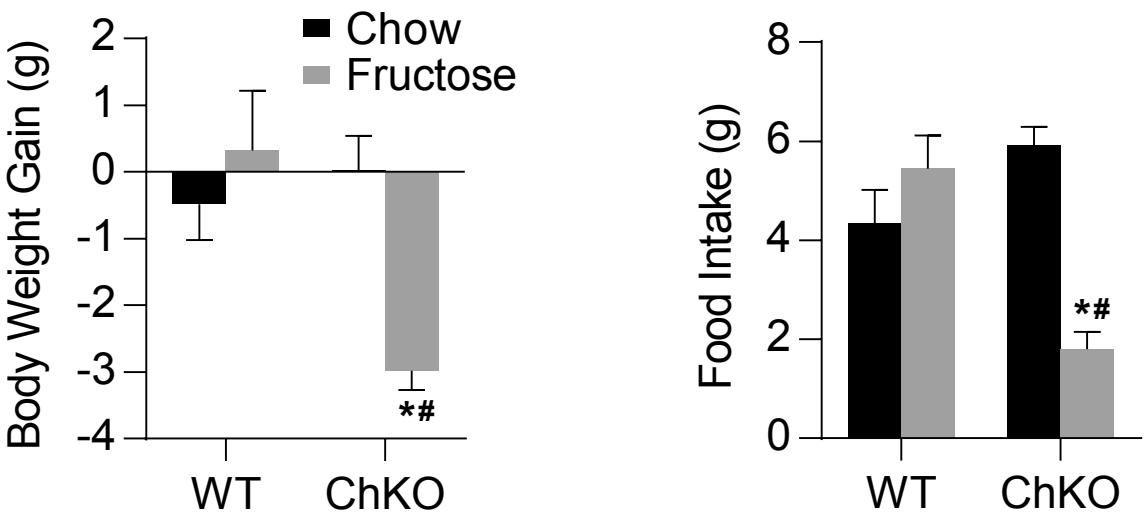
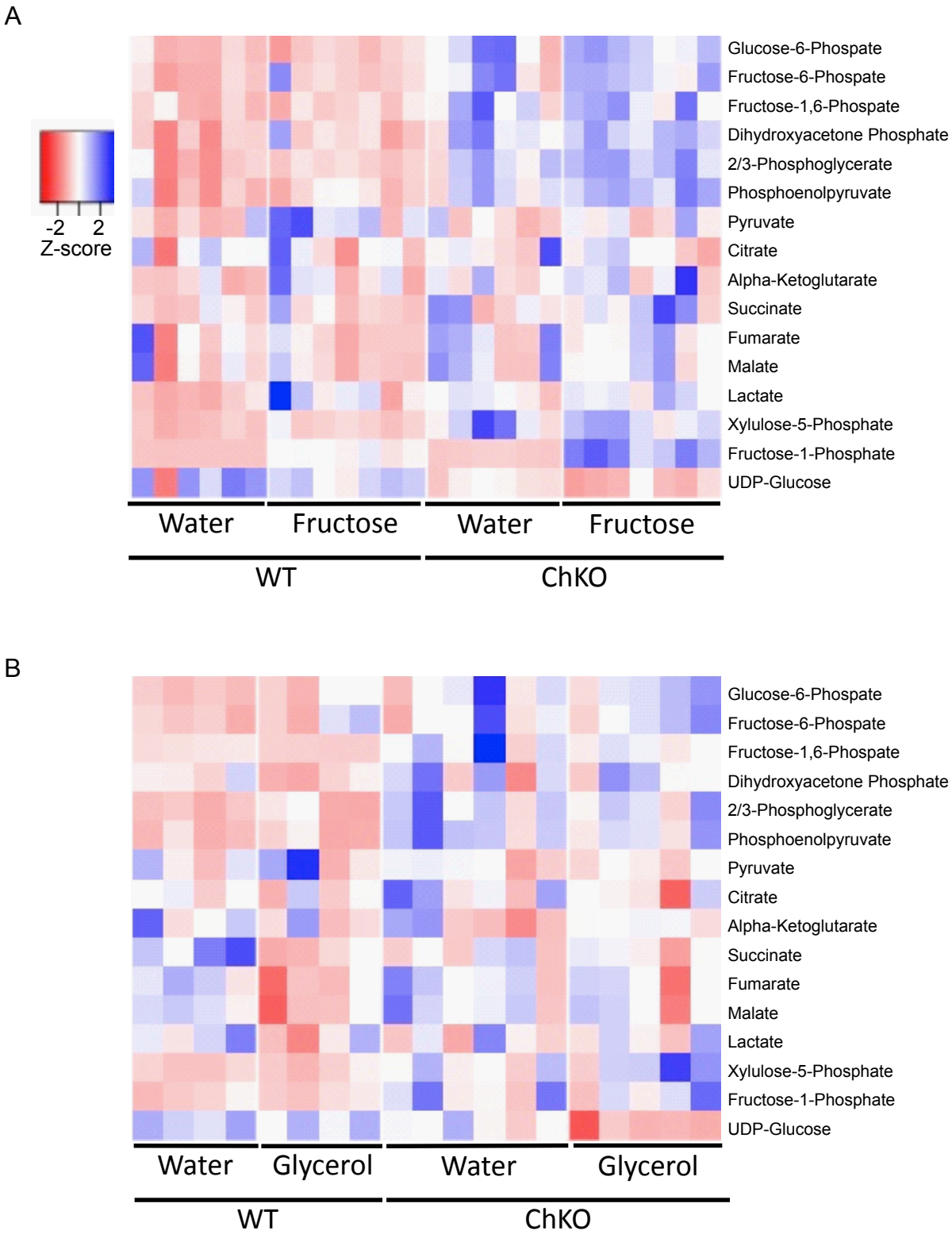
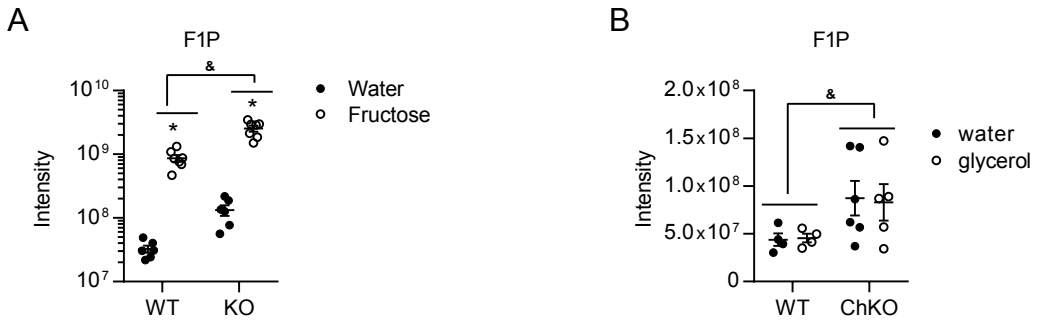


Supplemental Figure 1. **ChREBP KO mice cease eating and rapidly lose weight on a high-fructose diet**. (A) Changes in body weight and (B) food intake in 15-week-old, female WT and ChKO mice fed chow or high-fructose diet for 36 hours. *P<0.05 compared to chow within genotype. # P<0.05 compared to WT within diet (n=5 per group).



Supplemental Figure 2. **Semi-quantitative targeted metabolite measurement** by LC-MS in freeze-clamped liver from WT and ChKO mice gavaged with (A) water or fructose and (B) water or glycerol. Each column represents an individual animal. Metabolite levels are reported as z-scores.



Supplemental Figure 3. **Semi-quantitative measurement of F1P** by LC-MS in freeze-clamped liver from WT and ChKO mice gavaged with (A) water or fructose and (B) water or glycerol. & $P<0.05$ main effect of genotype by ANOVA. *$P < 0.05$ compared to gavage treatment within genotype.

A **model: G6P Concentration ~ log(G6PC activity)**

All Mice

R squared	Adjusted R Squared	P-Value
0.468	0.455	3.06E-07

Coefficients:

	Estimate	Std. Error	T-Value	P-Value
Intercept	0.131	0.011	11.95	2.24E-15
log(G6PC activity)	-0.124	0.02	-6.079	3.06E-07

B **model: G6P Concentration ~ log(G6PC activity)**

Wild-type or Control Mice Only

R squared	Adjusted R Squared	P-Value
0.429	0.413	6.17E-06

Coefficients:

	Estimate	Std. Error	T-Value	P-Value
Intercept	0.129	0.007	17.52	2.00E-16
log(G6PC activity)	-0.094	0.018	-5.267	6.17E-06

Supplemental Figure 4. **Regression analysis of G6P Concentration versus G6PC activity across three mouse cohorts** including (A) all mice (n=44), and (B) excluding ChREBP KO mice (n=39).

model: log(HGP) ~ G6PC activity

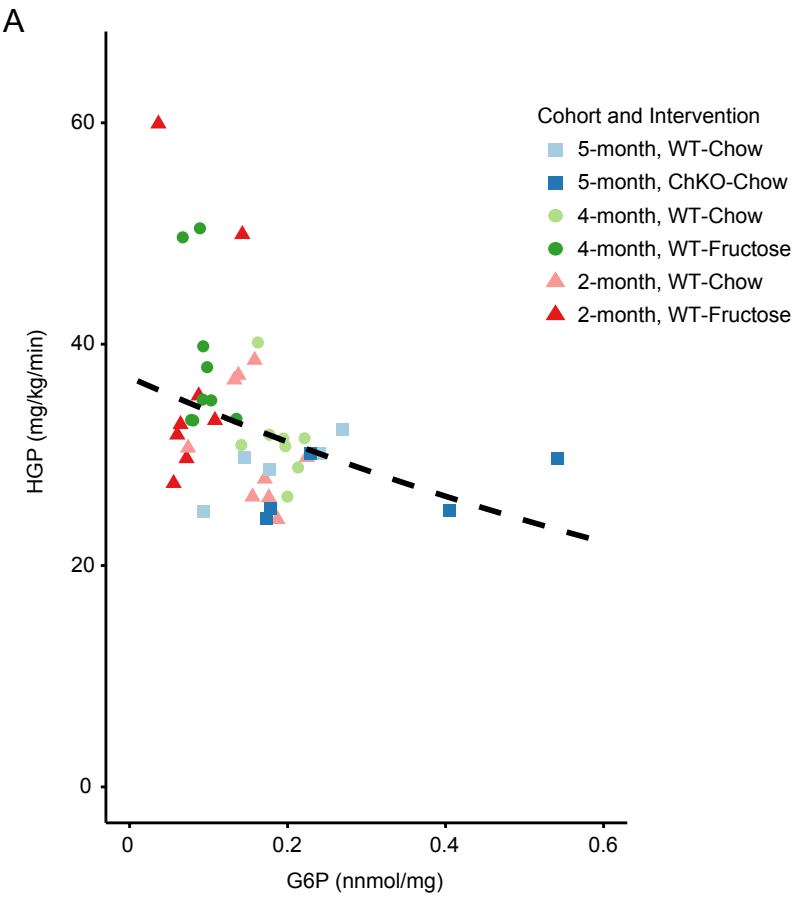
R squared	Adjusted R Squared	P-Value
0.336	0.321	3.68E-05

Coefficients:

	Estimate	Std. Error	T-Value	P-Value
Intercept	3.24	0.06	55.55	2.00E-16
G6PC Activity	0.261	0.057	4.615	3.68E-05

Supplemental Figure 5. **Regression analysis of HGP versus G6PC Activity** across three mouse cohorts 4 hours after food removal. Each point represents an individual mouse (n=44).

A



B

model: log(HGP) ~ G6P Concentration

R squared	Adjusted R Squared	P-Value
0.15	0.13	0.009

Coefficients:

	Estimate	Std. Error	T-Value	P-Value
Intercept	3.611	0.057	63.67	2.00E-16
G6P concentration	-0.858	0.315	-2.724	0.009

C

model: log(HGP) ~ G6P Concentration + G6PC activity

R squared	Adjusted R Squared	P-Value
0.338	0.306	2.13E-04

Coefficients:

	Estimate	Std. Error	T-Value	P-Value
Intercept	3.262	0.114	28.58	2.00E-16
G6P concentration	-0.097	0.359	-0.272	0.787
G6PC activity	0.249	0.073	3.41	0.001

Supplemental Figure 6. **Regression analysis of G6P levels versus HGP**. (A) Graph showing hepatic G6P levels versus HGP across three mouse cohorts 4 hours after food removal. Each point represents an individual mouse (n=44). Regression analysis assessing the effects of (B) hepatic G6P levels on HGP and (C) the effects of G6P levels and G6PC activity on HGP.

A

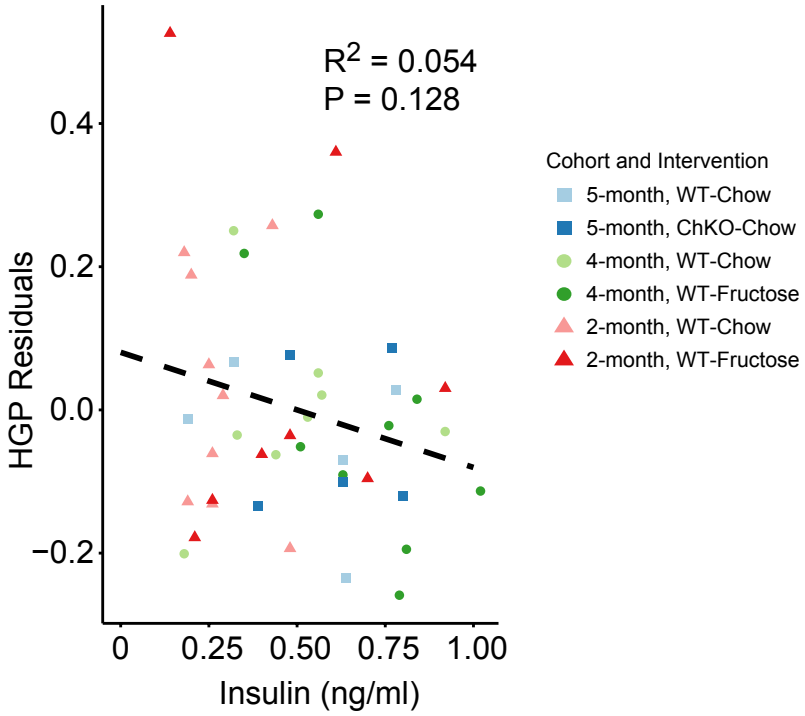
model: log(HGP) ~ G6PC activity + Insulin

R squared	Adjusted R Squared	P-Value
0.377	0.347	6.13E-05

Coefficients:

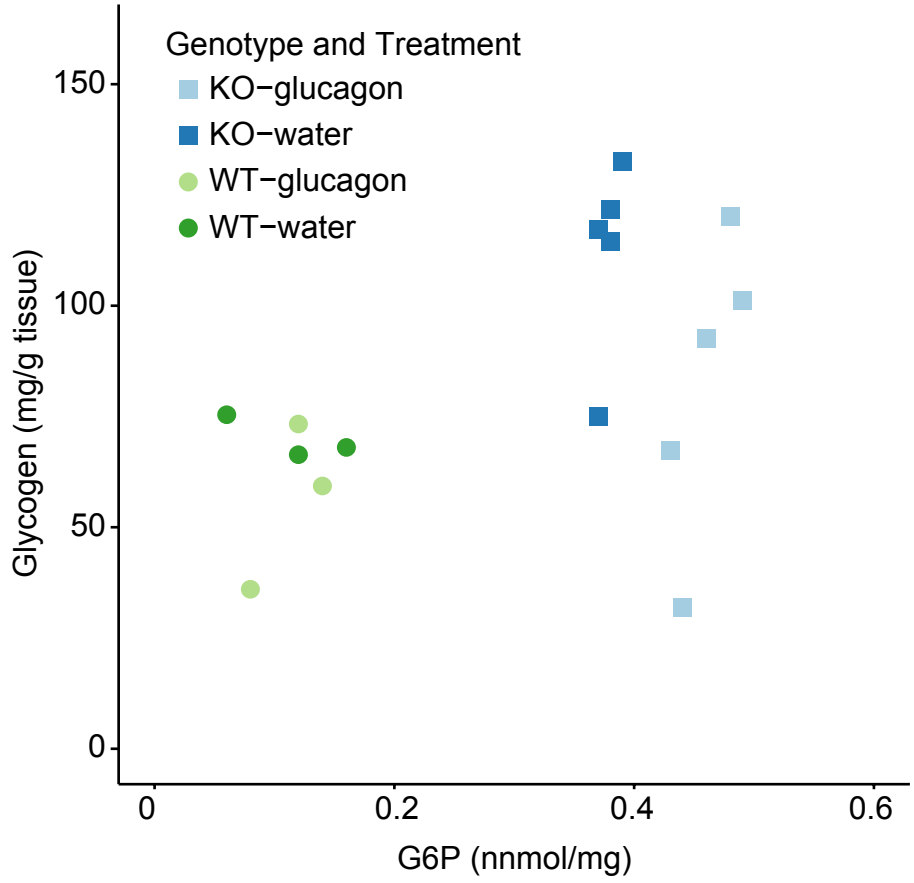
	Estimate	Std. Error	T-Value	P-Value
Intercept	3.28	0.068	48.35	2.00E-16
G6PC Activity	0.293	0.059	4.981	1.19E-05
Insulin	-0.181	0.111	-1.634	0.11

B



Supplemental Figure 7. **Regression analysis of HGP versus G6PC Activity and Insulin Levels**. (A) Regression analysis assessing the effects of serum insulin on the relationship between HGP and G6PC activity across three mouse cohorts 4 hours after food removal. Each point represents an individual mouse (n=44). (B) Graph showing serum insulin levels versus HGP residuals after regressing out the effects of G6PC activity.

A



B

Control Mice (n = 6)

model: Glycogen ~ G6P Concentration + Treatment (+/- glucagon)

R squared	Adjusted R Squared	P-Value
0.302	-0.163	0.583

Coefficients:

	Estimate	Std. Error	T-Value	P-Value
Intercept	48.6	22.98	2.12	0.125
G6P	67.02	186.7	0.359	0.743
Treatment	13.73	12.69	1.082	0.358

C

ChREBP KO Mice (n = 10)

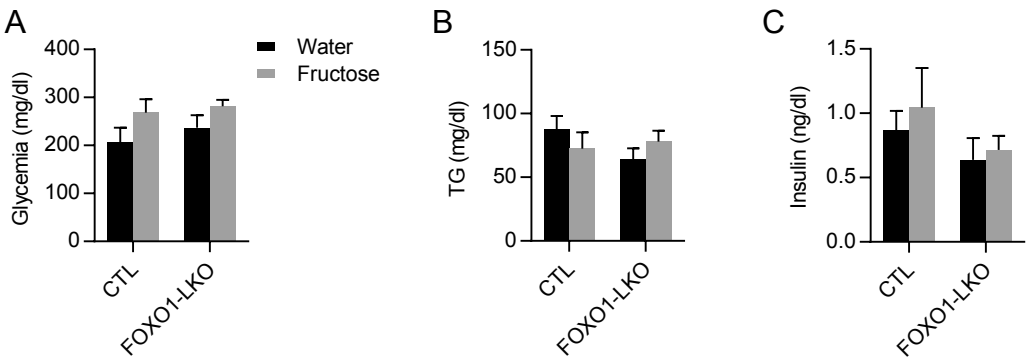
model: Glycogen ~ G6P Concentration + Treatment (+/- glucagon)

R squared	Adjusted R Squared	P-Value
0.683	0.592	0.018

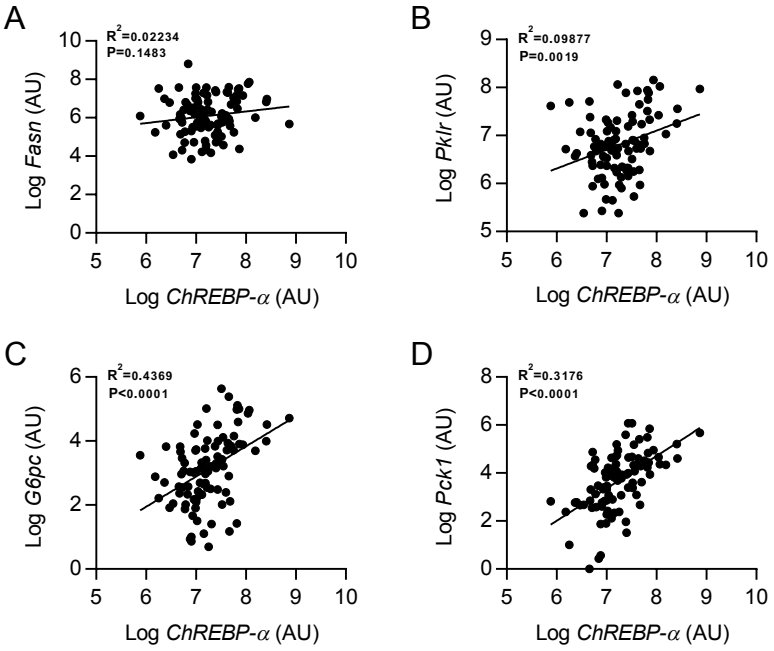
Coefficients:

	Estimate	Std. Error	T-Value	P-Value
Intercept	-456	171	-2.6	0.035
G6P	1148	372	3.09	0.018
Treatment	124	33	3.75	0.007

Supplemental Figure 8. **Analysis of hepatic G6P and glycogen levels in ChKO mice versus control**. Mice were fed HDD for 2 weeks. Hepatic glycogen levels and hepatic G6P levels were measured 20 minutes after injection with either glucagon (20 ug/kg body weight) or water (n=3-5 per group). (A) Graph showing G6P versus glycogen levels. Each point represents an individual mouse. Regression analysis among (B) Control (n=6) and (C) ChKO (n=10) mice for the effects of G6P and glucagon treatment on glycogen levels.



Supplemental Figure 9. **Serum metabolic measurements in water and fructose-gavaged liver-specific Foxo1 knockout mice.** (A-C) 5 hour fasted, 8-10 week-old wild type and Foxo1 liver knockout male mice were gavaged with water or fructose (4 g/kg body weight) and sacrificed 100 minutes later. (A) Glycemia, (B) hepatic triglyceride levels and (C) serum insulin levels were measured.



Supplemental Figure 10. Correlations between ChREBP-α and ChREBP transcriptional targets including: (A) Fasn (R^2=0.02234, P=0.1483), (B) Pklr (R^2=0.09877, P=0.0019), (C) G6pc (R^2=0.1909, P<0.0001), and (D) Pck1 (R^2=0.3176, P<0.0001) in liver biopsy samples from 95 overnight fasted human subjects with NAFLD. Each point represents an individual person.

Dependent Variable: G6pc

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
	0.582	.339	.325	.88366

ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
Regressio n		36.470	2	18.235	23.353	.000[a]
	Residual	71.057	91	.781		
	Total	107.527	93			

Coefficients

Model	Beta Coeff.	t	Sig.
(Constant)		-3.966	.000
ChREBPa	.275	2.921	.004
ChREBPb	.409	4.333	.000

Dependent Variable: Pklr

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
	.683[a]	.467	.455	.47015

ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
Regressio n		17.816	2	8.908	40.301	.000[a]
	Residual	20.336	92	.221		
	Total	38.152	94			

Coefficients

Model	Beta Coeff.	t	Sig.
(Constant)		3.993	.000
ChREBPa	.028	.336	.738
ChREBPb	.671	7.970	.000

Dependent Variable: Fasn

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
	0.652	.425	.413	.78803

ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
Regressio n		42.282	2	21.141	34.044	.000[a]
	Residual	57.132	92	.621		
	Total	99.414	94			

Coefficients

Model	Beta Coeff.	t	Sig.
(Constant)		1.646	.103
ChREBPa	-.149	-1.702	.092
ChREBPb	.702	8.028	.000

Dependent Variable: Pck1

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
	.393[a]	.154	.136	1.19229

ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
Regressio n		23.893	2	11.947	8.404	.000[a]
	Residual	130.783	92	1.422		
	Total	154.676	94			

Coefficients

Model	Beta Coeff.	t	Sig.
(Constant)		-2.095	.039
ChREBPa	.411	3.879	.000
ChREBPb	-.048	-.456	.649

Supplemental Figure 11. **Multiple Regression Analysis for ChREBP-α, ChREBP-β and indicated transcriptional targets** in liver biopsy samples from 95 overnight fasted human subjects with NAFLD.

Supplemental Methods

G6PC activity assay. To prepare hepatic microsomes, approximately 200 mg of frozen liver was homogenized in 1.5 ml homogenization buffer (250 mM sucrose, 5 mM HEPES, pH. 7.4) with dounce homogenizer, and centrifuged at 8000Xg for 10 min. The supernatant was centrifuged at 105,000Xg for 45 min in a Beckman TLA 100.2 rotor. The resulting pellet was resuspended in homogenization buffer (~100 ul per initial 100 mg liver).

Chromatin immunoprecipitation. Briefly, 30 mg of liver was minced and cross-linked using 2 mM disuccinimidyl glutarate in PBS at room temperature for 45 min and washed with PBS. The minced liver was cross-linked using 1% formaldehyde for 5 min, followed by adding 0.125 M glycine for 5 min to stop cross-linking. Cross-linked liver was dounce homogenized in buffer A (10 mM HEPES pH7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 0.3 % NP-40, and protease inhibitors) and crude nuclei were collected by centrifugation at 1000 x g and washed in PBS once. Nuclear pellets were resuspended in sonication buffer (50 mM Tris-Cl pH8.0, 10 mM EDTA, 0.25 % SDS and protease inhibitors) and sonicated using a Covaris S220 to achieve a DNA fragment size of 200-500 bp. The sonicate was centrifuged to remove debris, and the chromatin was diluted in 0.5X RIPA buffer followed by preclearing with protein G-Sepharose. The pre-cleared chromatin was incubated overnight at 4℃ with either 2 ug of anti-ChREBP or anti-rabbit IgG antibodies. Bound chromatin was eluted with 350 ul of digesting buffer (50 mM Tris-Cl pH 8.0, 1 mM EDTA, 100 mM NaCl, 0.5 % SDS) for overnight at 65℃. DNA was extracted with using phenol-chloroform and ethanol-precipitated with 50 ug of glycogen. Purified DNA was used for qPCR validation using ABI 7900 with SYBR green master mix. See Table S2 in the supplemental material for primer sequences.

Liver metabolite extraction and LC-MS. Liver tissue samples were bead homogenized (Biospec Products Minibeadbeater-96+ (Bartlesville, OK, USA) for 2 min) in a solution of 4:1 methanol/water extraction solvent cooled on ice 30 min prior to use at a tissue/solvent ratio of 20mg tissue/1mL solvent. Sample material was then

sonicated with a Cole-Parmer 4710 series ultrasonic homogenizer (Vernon Hills, IL, USA) for 10 sec. Homogenized material was placed in a -20 ºC freezer for 30 min, and centrifuged at 14,000 rcf for 5 min. Aliquots of supernatant were dried in a vacuum concentrator. Extracted metabolites were resuspended in 40 µL of Mobile Phase A and transferred to a 96-well 2 mL-polypropylene plate (Analytical Sales & Services) prior to injection. Samples were injected onto a Waters Acquity 2.1x100 mm HSS T3 column (Waters Corporation, Milford, MA, USA) using a Shimadzu Prominence chromatography system (Shimadzu Corporation, Kyoto, Japan) coupled to a Thermo Scientific Q-Exactive mass spectrometer (Thermo Fisher Scientific). Mobile phase A consisted of 95/5 water/methanol, 10 mM tributylamine, and 15 mM acetic acid. Mobile phase B was isopropyl alcohol. The gradient was 0-5 min - 0% B, 5-10 min - increase to 2% B, 10-11 min - increase to 9% B, 11-16 min - 9% B, 16-18 min - increase to 25% B, 18-19 min - increase to 50% B, 19-25 min - increase to 50% B, 25-26 min - increase to 0% B, 26-36 min - 0% B. The flow rates were 0-10 min - 0.40 mL/min, 10-11 min - increase to 0.35 mL/min, 11-16 min - increase to 0.25 mL/min, 16-18 min - 0.25 mL/min, 18-19 min - Linear to 0.15 mL/min, 19-26 min - 0.15 mL/min, 26-32 min - Linear to 0.40 mL/min, and 32-36 min - 0.40 mL/min. Column temperature was kept constant at 35 ˚C. MS data was collected in negative ion mode with a 66.7-1000 m/z scan range for 35 min at 70 k resolution. The automatic gain control, sheath gas, auxiliary gas, spray voltage, and capillary temperature were set to 1e6, 50, 10, 3.5 kV, and 320 ˚C respectively. The injection volume was 5 µL. A blank, an external standard, and a sample pool were injected every 10 samples. The external standard consisted of 2-phosphoglycerate, 3-phosphoglycerate, 3-methyl-2-oxovaleric acid, α-ketoisocaproic acid, citric acid, dihydroxyacetone phosphate, dimethylallyl-pyrophosphate, fructose, fructose-1-phosphate, fructose-6-phosphate, glucose, glucose-1-phosphate, glucose-6-phosphate, glyceraldehyde-3-phosphate, glycerol phosphate, isocitric acid, isopentenyl-5-pyrophosphate, methylmalonic acid, phosphoenolpyruvate, ribose-5-phosphate, ribulose-5-phosphate, and succinic acid at 45.5 µg/mL. An F2,6BP standard was not available when this analysis was performed. Therefore, we are not certain that separation of F1,6BP and F2,6BP was achieved. Reported metabolites were identified based on accurate mass and retention time match to authentic standards. A sample

pool spiked with the external standard was also included to confirm appropriate hexose phosphate identification in matrix.

LC-MS for fructose clearance. Measurement of fructose was carried out on an Agilent 1290 Infinity UHPLC system (Agilent Technologies, Palo Alto, CA) and maXis impact UHR time-of-flight mass spectrometer system (Bruker Daltonics Inc, Billerica, MA) equipped with an electrospray ionization (ESI) source. Data were acquired with Bruker Daltonics HyStar software version 3.2 for UHPLC & Compass OtofControl software version 3.4 for mass spectrometry, and processed with Bruker Compass DataAnalysis software version 4.1. Plasma samples were extracted using acetone/chloroform (9:4 v/v) and dissolved in acetonitrile/water (4:1 v/v). For the UHPLC system, 8 µLof samples were injected onto the UHPLC including a G4220A binary pump with a built-in vacuum degasser and a thermostatted G4226A high performance autosampler. A Luna NH_2 analytical column (2 x 150 mm, 3 µm) (47) and a guard column (NH_2 2.0 x 4 mm) from Phenomenex (Torrance, CA) were used at the flow rate of 0.2 mL/min (47) using 80% acetonitrile in water as the mobile phase for the isocratic mode. The column temperature was maintained at room temperature. For the MS detection, the ESI mass spectra data were recorded on a negative ionization mode for a mass range of m/z 50 to 1200; calibration mode, HPC; spectra rate, 1.00 Hz; capillary voltage, 3400 V; nebulizer pressure, 10.0 psi; drying gas (N_2) flow, 6.0 L/min; drying gas (N_2) temperature, 220ºC. A mass window of ± 0.005 Da was used to extract the ions of m/z 179.0561 and 185.0762 representing $[M-H]^-$ of unlabeled and labeled fructose. Fructose was considered detected when the mass accuracy was less than 5 ppm and there were a match of isotopic pattern between the observed and the theoretical ones and a match of retention time between those in real samples and standards.

Primer Name	Sequence
Rplp0-for	AGATTCGGGATATGCTGTTGGC
Rplp0-rev	TCGGGTCCTAGACCAGTGTTC
ChREBP-α-for	AGCATCGATCCGACACTCAC
ChREBP-α-rev	TTGTTCAGCCGGATCTTGTC
ChREBP-β-for	TCTGCAGATCGCGTGGAG
ChREBP-β-rev	CTTGTCCCGGCATAGCAAC
SREBP-1c-for	GGAGCCATGGATTGCACATT
SREBP-1c-rev	GGCCCGGGAAGTCACTGT
Pklr-for	CATCCCTGCCTTGATCATCT
Pklr-rev	TATCGACTCAGAGCCTGTGG
Gpi1-for	AAAGTCCAATGGCTGACCAC
Gpi1-rev	CACGGCCAAAGTGAAAGAGT
Eno1-for	ATCTTTGACTCCCGTGGGAATC
Eno1-rev	AGCGGGTCTTATCATTGTCTCG
Fasn-for	GCTGCGGAAACTTCAGGAAAT
Fasn-rev	AGAGACGTGTCACTCCTGGACTT
Acly-for	GCCAGCGGGAGCACATC
Acly-rev	CTTTGCAGGTGCCACTTCATC
Acaca-for	TGTACAAGCAGTGTGGGCTGGCT
Acaca-rev	CCACATGGCCTGGCTTGGAGGG
Aldob-for	GCTGGGCAATTTCAGAGAGC
Aldob-rev	GAGGACTCTTCCCCTTTGCT
Khk-for	GGACAGTGCAGGAGTTGGAT
Khk-rev	GGACATCATCAATGTGGTGG
Dak-for	AGCACACCCTTCCACAGAAT
Dak-rev	CGGCATCTCAGAGCAGAAG
G6pc-for	GTGTCCAGGACCCACCAATA
G6pc-rev	ACTGTGGGCATCAATCTCCT
Pck1-for	TCTGGATGGTTTTAATGGCA
Pck1-rev	TGCCTGGATGAAGTTTGATG
Slc37a4-for	GACTGGCTGCTTGTGATGAG
Slc37a4-rev	ACCGCAAAACCTTCTCCTTT
human ChREBP-α-for	AGTGCTTGAGCCTGGCCTAC
human ChREBP-α-rev	TTGTTCAGGCGGATCTTGTC
human ChREBP-β-for	AGCGGATTCCAGGTGAGG
human ChREBP-β-rev	TTGTTCAGGCGGATCTTGTC
human Fasn-for	TCTCCGACTCTGGCAGCTT
human Fasn-rev	GCTCCAGCCTCGCTCTC
human Pklr-for	GAGAAGTTGAGTCGCGCAAT
human Pklr-rev	CAGTACCAGCATCATTGCCA
human G6pc-for	GTATACACCTGCTGTGCCCAT
human G6pc-rev	CTACAGCAACACTTCCGTGC
human Pck1-for	ATGCCGATCTTTGACAGAGG
human Pck1-rev	GAGAAAGCGTTCAATGCCAG
human Rplp0-for	TCTGCATTCTCGCTTCCTG
human Rplp0-rev	GGACTCGTTTGTACCCGTTG

Supplemental Table 1. qPCR Primer sequences. Primers are for mice unless specified as human.

Primer Name	Sequence
ChIP-ChREBP-β-for	TGATTGGCAGGCTCCTGAG
ChIP-ChREBP-β-rev	TGTCCTTTGCCCCTTGGTC
ChIP-G6pc-for	TGAAGATAGCGCTCTAGGTTCC
ChIP-G6pc-rev	AAAGCATCCAAAGGCCACTG
ChIP-negative control-for	TGGACATTTGACTCCAGAGC
ChIP-negative control-rev	AACATGGAGAAGAAGGCAGTG

Supplemental Table 2. Primer sequences for ChIP PCR.