SUPPLEMENTARY DATA

Sleeve gastrectomy improves glycemia independent of weight loss by restoring hepatic insulin sensitivity.

Supplementary Information

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Supplementatry Figure S1. accompanying Figure 2. Quantification of pancreatic markers in SG, sham operated mice and lean controls.
A-C: Insulin (green), Pdx1 (blue) and Ki67 (red) in sham operated *db/db* mice 1 week after surgery (A), SG operated *db/db* mice 1 week after surgery (B) and lean mice (C). Red nuclear staining in insulin and Pdx1 positive cells indicates β-cell proliferation.
D: Quantification of A-C.
E-H: Insulin (green), DAPI (blue) and 53BP1 (red) in sham operated *db/db* mice 1 week after surgery (E), SG operated *db/db* mice 1 week after surgery (F), SG operated *db/db* mice 1 month after surgery (G), and lean mice (H). Red foci in insulin positive cells indicate DNA damage in β-cells.
I. Quantification of E-H.
J-M: Insulin (green), Pdx1I (blue) and Nkx6.1 (red) in sham operated *db/db* mice 1 week after surgery (J), SG operated *db/db* mice 1 week after surgery (K), SG operated *db/db* mice 1 month after surgery (L), and lean mice (M). Nuclear magenta staining in insulin positive cells indicates co-localization of Pdx1 and Nkx6.1 in β-cells.
N. Quantification of E-H.
O-Q. Insulin (green), glucagon (blue) and gastrin (red) in sham operated *db/db* mice 1 week after surgery (J), SG operated *db/db* mice 1 week after surgery (K), SG operated mice 1 month after surgery (L), and lean mice (M).
S-U: Pdx1 (blue) and Ucn3 (red) in sham operated *db/db* mice 1 week after surgery (S), SG operated *db/db* mice 1 week after surgery (T), SG operated mice 1 month after surgery (U). S is an example of a low Ucn3, U-T example of high Ucn3.
V: Quantification of S-U. All images taken under same settings. Quantification shows pooled islets from all mice, as there was a high intra-mouse variability among islets.
** $p<0.01$, * $p<0.05$, unpaired students t-test. n=5-7 mice in all groups.

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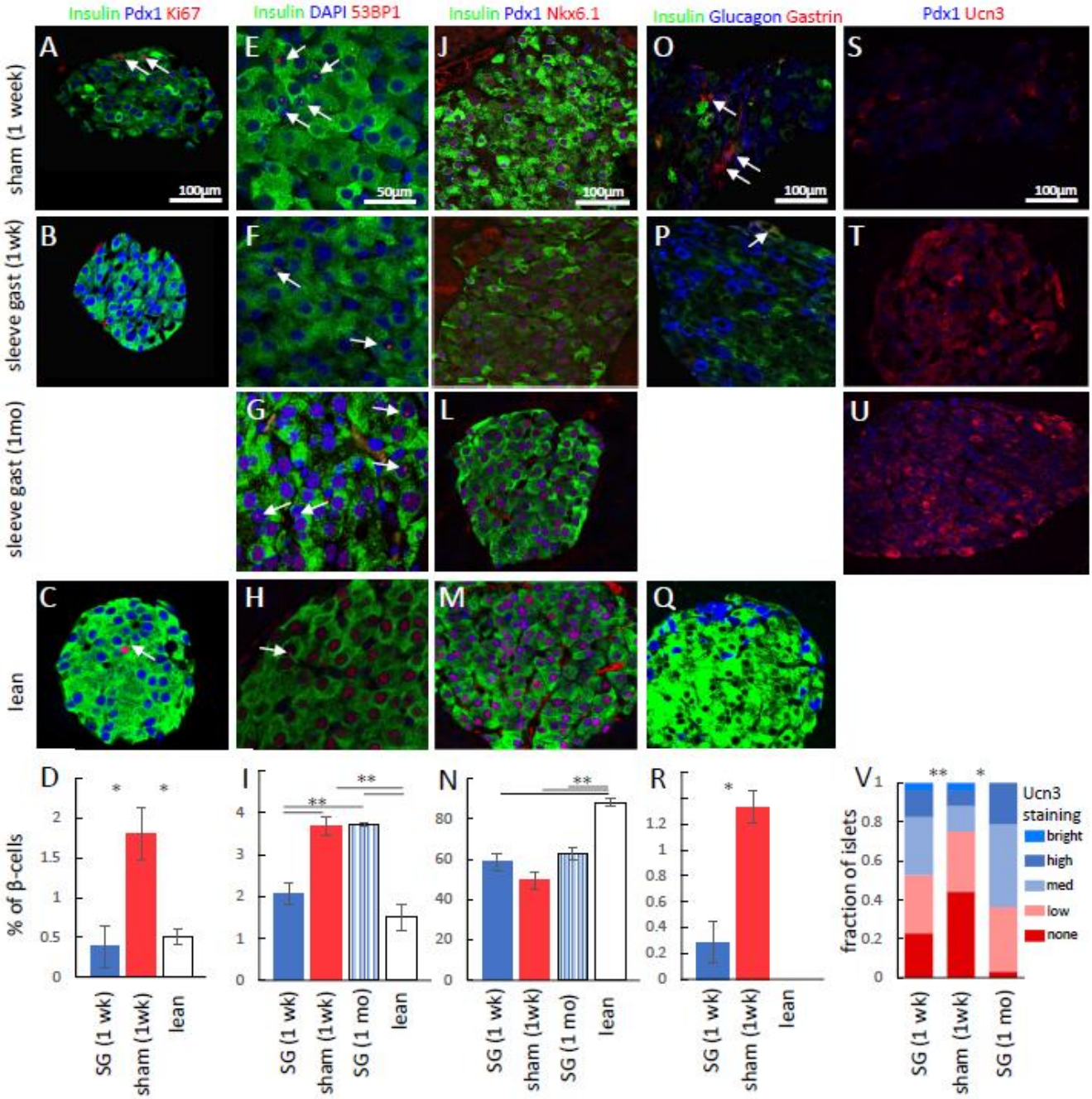

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Supplementary Figure S2. accompanying Figure 3. Weight and glucose in diet-induced obese (DIO) mice prior to and 7 days after sleeve gastrectomy or weight-matched sham operated controls.

A: Weight of DIO sleeve gastrectomy operated mice at day of surgery (purple) and seven days after surgery (purple stripes), and of DIO mice that were sham operated and weight-matched at day of surgery (green) and 7 days after surgery (green stripes).

B: Change in weight of DIO sleeve gastrectomy operated mice (purple) and weight-matched sham operated mice (green).

C: Non-fasting glucose levels of DIO sleeve gastrectomy operated mice one day before surgery (purple) and seven days after surgery (purple stripes), and of DIO mice that were sham operated and weight-matched one day before surgery (green) and 7 days after surgery (green stripes).

D: Change in non-fasting glucose of DIO sleeve gastrectomy operated mice (purple) and weight matched sham operated mice (green).

** $p<0.01$, * $p<0.05$. A,C: Two-way ANOVA with Bonferroni corrected post-hoc tests. B,D: unpaired students t-test. n=13-16 mice in all groups.

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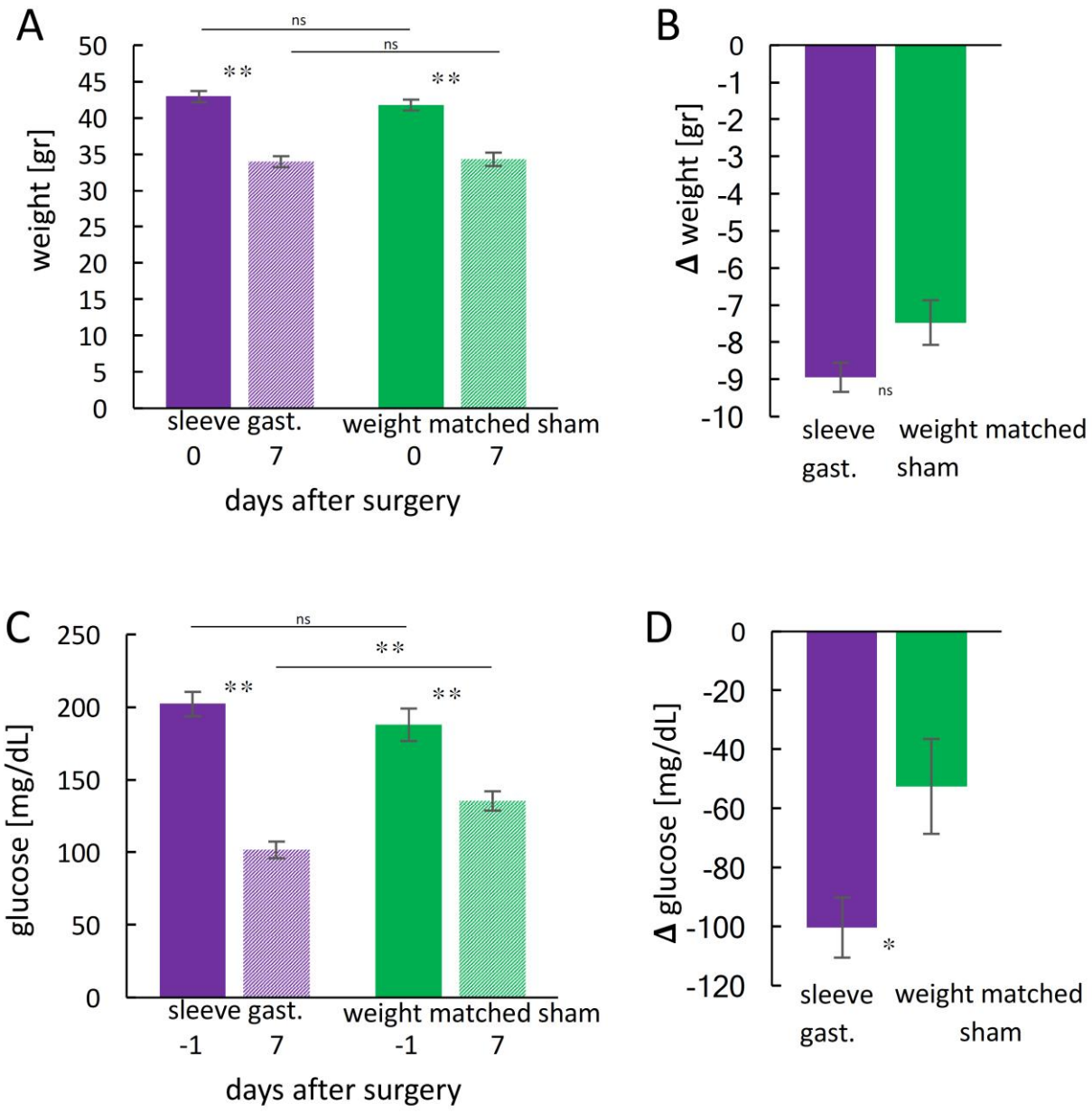

Supplementary Figure S3. accompanying Figure 4. Western blot and gene expression analysis of specific gene sets.

A. Western blot of S6, pS6 and tubulin from weight-matched sham operated and sleeve gastrectomy operated *db/db* mice.

B-F. Heatmap of log2 fold change in expression of amino acid catabolic enzymes (B), FXR target genes (C), fatty acid oxidation enzymes (D), HNF4α targets (E) and PPARα targets (F) from livers of mice after sleeve gastrectomy or pair-fed sham operated mice harvested after a hyperinsulinemic-euglycemic clamp. PPARα expression shown in F. Expression was normalized to mean of pair-fed sham operated mice. Dashed line denotes q=0.05. Selected genes shown in the right of the heatmaps. See tables S2-S4,S6 for full gene list.

G. Representative Western blots of PPARα, PKA-C and GAPDH. PKA-C levels were not altered.

Color map for all panels shown in the bottom.

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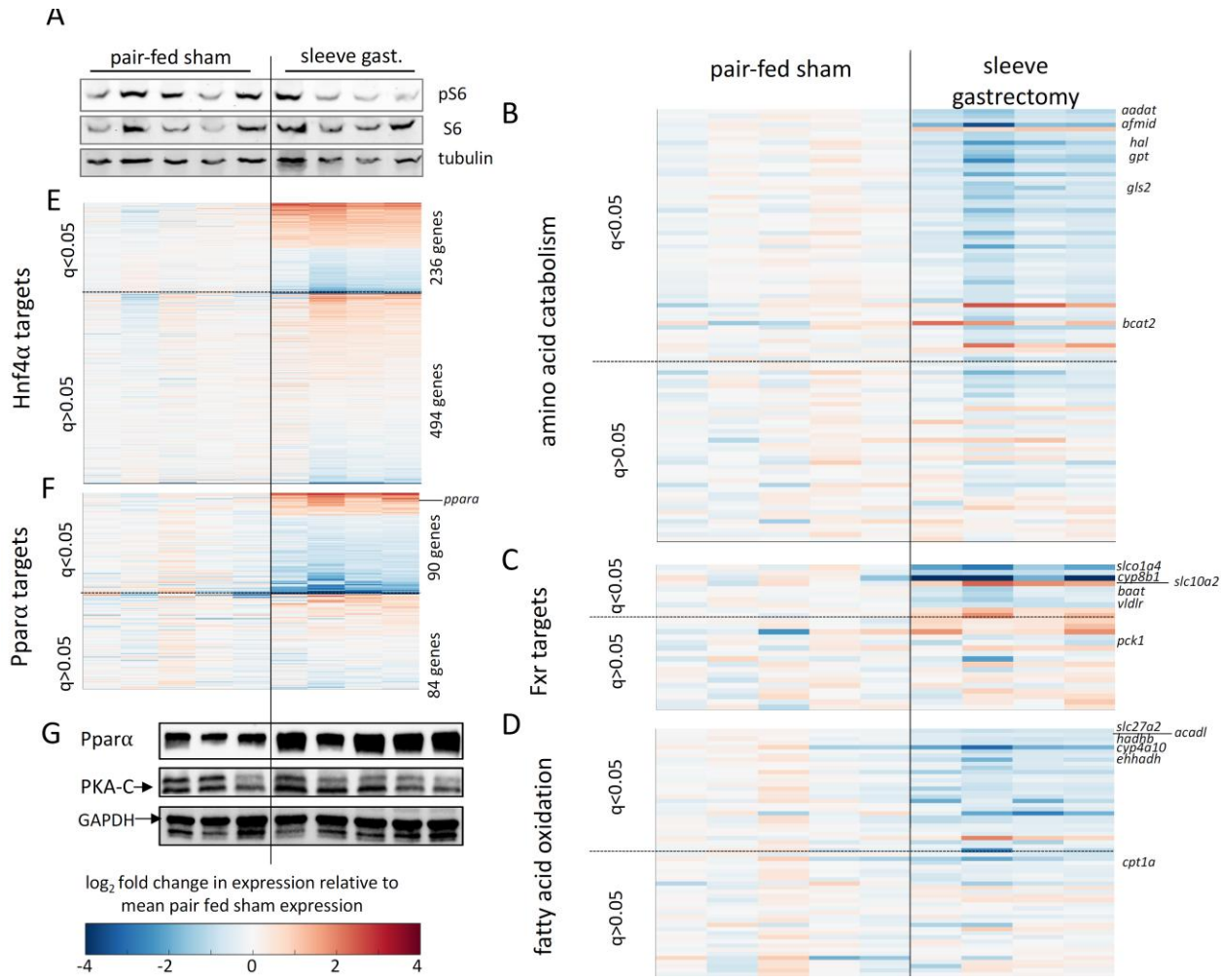

Supplementary tables.

S1: gene names, log2 fold change and adjusted p-value for differentially regulated metabolic genes shown in figure 4F.

S2: gene names, log2 fold change and adjusted p-value for genes coding for amino acid catabolic proteins shown in figure S3.

S3: gene names, log2 fold change and adjusted p-value for targets of the transcription factor FXR shown in figure S3.

S4: gene names, log2 fold change and adjusted p-value for genes coding for protein involved in fatty acid oxidation shown in figure S3.

S5: Enriched DNA binding factors binding significantly (q<0.01) regulated genes in our dataset.

S6: gene names, log2 fold change and adjusted p-value for targets of the transcription factors HNF4α and PPARα in figure S3.

S7: gene names, log2 fold change and adjusted p-value for all genes with an adjusted p-value smaller than 0.05.

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Supplementary Experimental Procedures

Mice:

db/db Mice: 8-13-week-old male *db*/*db* mice (The Jackson Laboratories, USA) underwent sleeve gastrectomy or sham surgery. Sleeve Gastrectomy was performed as described previously1. Mice were fed normal chow prior to surgery. Briefly, a 1 cm midline incision was made, and the stomach was exposed. Using a Ligaclip Applier (Ethicon Inc., Somerville, NJ), a 12-mm clip was placed horizontally across the greater curvature of the stomach, thereby excluding the entire inferior aspect of the stomach and creating a gastric sleeve. The excluded part of the stomach was then excised and discarded and the cut edges of the stomach were irrigated with saline before closure of body wall and skin. The surgery lasted approximately 15 minutes. Sham surgeries included the abdominal incision and closure of body wall and skin. Mice were fasted the day prior to surgery and the day of surgery but could drink *ad libitum*, and were then allowed to feed on normal chow. Mice received 0.5mL dextrose injection and meloxicam immediately after surgery and at the day after surgery. Meloxicam treatment was continued three days after surgery. Lean controls were *db*/+ littermates of *db*/*db* mice.

Diet induced obese (DIO) mice: Eight weeks old C57Bl6 male mice were fed a high fat high sucrose diet (TD.08811, Envigo) for 16 weeks. Sleeve gastrectomy was performed as described above. At the day after surgery, mice returned to high fat high sucrose diet. Sleeve gastrectomy operate mice were allowed to eat *ad libitum*. Weight matched shams were fed approximately two grams of high fat diet each day to match sleeve gastrectomy operated mice weight.

The joint ethics committee (IACUC) of the Hebrew University and Hadassah Medical Center and the Institutional Animal Care and Use Committee of the University of Pennsylvania approved the animal experiments carried out in Jerusalem and Philadelphia, respectively.

Euglucemic-Hyperinsulinemic clamp:

Clamp studies, triglyceride, cholesterol and NEFA measurements were performed by the mouse phenotyping core of the Diabetes Research Center at the University of Pennsylvania, 5 days after SG or sham surgery.

An indwelling catheter was inserted in the right internal jugular vein under sodium pentobarbital anesthesia and extended to the right atrium. Five days after recovery the mice were fasted for 6 hours (0800–1300), tail blood glucose measured and a bolus injection of 5 μCi of [3–^{3}H] glucose administered intravenously, followed by continuous intravenous infusion at 0.05 μCi min^{-1}. Baseline glucose kinetics were determined for 120 min followed by hyperinsulinemic clamp for 120 min. A priming dose of regular insulin (150 mU kg−1, Humulin; Eli Lilly, Indianapolis, IN) was given intravenously, followed by continuous infusion at 20 mU kg^{-1} min^{-1}. A variable intravenous infusion of 20% glucose was infused to attain blood glucose levels of 140–160 mg/dL. At the end of the clamp, 10 μCi 2-deoxy-D-[1-^{14}C] glucose was injected to measure glucose uptake. The mice were euthanized, and liver, perigonadal adipose tissue, and gastrocnemius muscle samples excised, frozen in liquid nitrogen and stored at −80 °C for analysis of glucose uptake. Rates of whole body glucose uptake and basal glucose turnover were measured as the ratio of the [^{3}H] glucose infusion rate (d.p.m.) to the specific activity of plasma glucose. Hepatic glucose production (HGP) during clamp was measured by subtracting the glucose infusion rate (GIR) from the whole-body glucose uptake (Rd). Under basal (fasting) conditions, the rate of Rd = HGP.

Non-esterified fatty acids, total cholesterol and triglyceride levels were measured by the mouse phenotyping core service of the Diabetes Research Center at the University of Pennsylvania.

Insulin and glucose quantification

Insulin: Insulin was measured using an ultra-sensitive mouse insulin ELISA kit (Crystal Chem Inc.) according to the manufacturer's instructions.

Glucose: blood glucose concentrations were measured at 11am by tail bleeding using a glucometer (Accu-check, Roche).

Histology and immunohistochemical staining:

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Tissues were fixed overnight in 4% formaldehyde and transferred to paraffin blocks. Tissues were cut and deparaffinaized using Xylene and washed in PBS. Antigen retrieval was performed in citrate buffer (pH 6.0) in a pressure cooker. Tissues were blocked in CAS block (Thermo Fisher Scientific). Primary antibodies were stained overnight in CAS block, and washed 3 times in PBS. Secondary antibodies were diluted in PBS with 1% BSA overnight. Imaging was done using an ECL system Nikon C1 confocal microscope and quantified using ImageJ or MATLAB.
Western blots were performed as described[2].

Antibody	**Manufacturer**	**Concentration**
G.PαInsulin	Dako	1:400
MsαGlucagon	Abcam	1:800
RbαGastrin	Cell Marquee	1:200
RbαKi67	Thermo 9106	1:200
Rbα53bp1	Leica CM5	1:400
Goat αPdx1	a gift from Chris Wright	1:2500
MsαNkx6.1	BCBC	1:400
RbαUcn3	Generous gift from Prof. A. Chen, Weizmann Institute of science (Phoenix Pharmaceuticals Inc. H-019-29)	1:300
RαPka-c-a	Cell signaling	1:500
mαGapdh	Abcam	1:1000
mαPpara	Santa Cruz Biotechnology	1:1000
Secondary antibodies were purchased from Jackson ImmunoResearch		
Alexa488 donkey anti guiena pig	Jackson ImmunoResearch	1:200

Alexa647 donkey anti goat	Jackson ImmunoResearch	1:500
Alexa647 donkey anti mouse	Jackson ImmunoResearch	1:500
Cy3 donkey anti rabbit	Jackson ImmunoResearch	1:500
Cy3 donkey anti mouse	Jackson ImmunoResearch	1:500

Hematoxylin and Eosin staining:

Liver samples were deparaffinized and incubated for 10 minutes with freshly filtered Mayer's Hematoxylin solution (Sigma MHS32). Slides were washed with running water and dipped once in acid alcohol (100:1 80% EtOH:37% HCl), washed for 10 minutes under running water, dipped in 95% Ethanol and incubated with alcoholic Eosin Y with phloxin (Sigma HT110332). Dehydration from 95% Ethanol to Xylene was carried out and dried slides were sealed with Entellan mounting medium (Mercury 1079600600).

Image analysis:

Image analysis was performed blindly. Pancreatic sections were quantified using ImageJ, hepatic sections were quantified using a custom-built MATLAB script.

Statistics:

The unpaired two-tailed student's t-test was used to compare two measurements; Shapiro-Wilk method was used to test for normal distribution of the data. Values shown are means ± standard error unless written otherwise.

Weight and glucose time series: two-way ANOVA with Tukey HSD post-hoc test was used to determine differences between groups.

RNA-Seq: differential expression analysis was performed using *voom*[3] as implemented in the EdgeR and limma packages. Gene list were curated from the gene ontology database or Kegg database. Enriched gene sets were determined using gene set enrichment analysis [4] using the gene ontology and Kegg databases, and using the gene ontology enrichment and analysis and visualization tool[5]. Differential expression was defined as statistically significant with a corrected Benjamini-Hochberg corrected p-value smaller than 0.05.

ENCODE enrichment: Genes with FDR<0.01 were compared with the ENCODE database[6] for binding with Hep2G cells.

Gene enrichment: Enrichment of Pparα and Hnf4α targets was determined using the hypergeometric distribution with all differentially regulated genes in our dataset as background.

References

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3. Law, C. W., Chen, Y., Shi, W. & Smyth, G. K. voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol.* **15,** R29 (2014).

4. Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U. S. A.* **102,** 15545–50 (2005).

5. Eden, E., Navon, R., Steinfeld, I., Lipson, D. & Yakhini, Z. GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. *BMC Bioinformatics* **10,** 48 (2009).
6. Auerbach, R. K., Chen, B. & Butte, A. J. Relating genes to function: identifying enriched transcription factors using the ENCODE ChIP-Seq significance tool. *Bioinformatics* **29,** 1922–1924 (2013).