SUPPLEMENTARY INFORMATION

Supplementary Methods

Animals. Indirect calorimetry was performed in PhenoMaster cages (TSE Systems, Bad Homburg, Germany) with individually housed mice at 22°C. Calculations of resting metabolic rate (RMR) and body-weight-adjusted oxygen consumption were performed as described (Tschop et al, 2011). All mice were maintained on a 12 hr light/dark cycle at 24 C with regular unrestricted diet unless stated otherwise.

Human VLDL clearance. Human VLDL was isolated from fasting serum samples by ultracentrifugation as described (Redgrave et al, 1975). Briefly, 3.5 ml serum was put in a SW40Ti polyallomer tube and mixed with 1.39 g KBr, overlayered with 332.8 ml of a NaCl/KBr solution (D = 1.063, 1.019, and 1.006 g/ml) and run for 18 hr at 40,000 rpm. Human VLDL (20 mg) was injected into each animal, and serum samples were taken at 2, 10, 30, 60, and 120 min. Serum human ApoB-100 levels were measured using a human-specific ApoB ELISA. For the ELISA, we used a primary coating antibody generated against human apoB-100 (mAb47, kindly supplied by J. Witztum, University of San Diego, USA), in a concentration of 2 mg/well IgG protein in TBS/EDTA/BHT and a secondary biotinylated polyclonal antibody raised in goat against human ApoB in a concentration of 4 ug/well in 1.5% BSA/TBS/0.1% Tween. To prevent nonspecific binding, plates were blocked with 1.5% BSA/TBS/0.1% Tween. Samples were diluted 1:25. Absorbance was

read 30 min after addition of TMB and termination of the reaction with 2 M H_2SO_4 at 450 nm (Groot et al, 1991).

Trichloric acid / Acetone Protein Precipitation. For precipitation of protein from FPLC fraction, 200 µl of FPLC fractions 35-37 was mixed with 1.6 ml ice cold acetone and 200 µl trichloric acid (100% w/v). The mix was kept at -20°C for 1 h for precipitation. Then, the mix was centrifuged at 11500 rpm for 15 min at 4°C. The pellet was washed 4 times with 1 ml ice-cold acetone and centrifuged for 15 min at 4°C. The acetone was removed and the pellet air dried. The pellet was resupenden in loading buffer and subjected to Western Blot analysis.

Supplementary References

Groot PH, van Stiphout WA, Krauss XH, Jansen H, van Tol A, van Ramshorst E, Chin-On S, Hofman A, Cresswell SR, Havekes L (1991) Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. *Arterioscler Thromb* **11**(3)**:** 653-662

Redgrave TG, Roberts DC, West CE (1975) Separation of plasma lipoproteins by density-gradient ultracentrifugation. *Anal Biochem* **65**(1-2)**:** 42-49

Tschop MH, Speakman JR, Arch JR, Auwerx J, Bruning JC, Chan L, Eckel RH, Farese RV, Jr., Galgani JE, Hambly C, Herman MA, Horvath TL, Kahn BB, Kozma SC, Maratos-Flier E, Muller TD, Munzberg H, Pfluger PT, Plum L, Reitman ML, Rahmouni K, Shulman GI, Thomas G, Kahn CR, Ravussin E (2011) A guide to analysis of mouse energy metabolism. *Nat Methods* **9**(1)**:** 57-63

Supplementary Figure Legends

Supplementary Figure 1. (a) Quantitative PCR analysis of TSC22D1 expression from liver extracts from control or TSC22D1 shRNA adenovirus injected wild-type C57BL/6J mice. (b) Representative Western Blot of liver extracts from same mice as in a, using TSC22D1 (D1) or actin specific antibodies, (c) body weight, (d) liver weight, (e) abdominal fat weight, (f) liver triglycerides. (Representative cryosections stained with Oil Red O), (g) serum non-esterified fatty acids (NEFA), (h) serum ketone bodies, (i) blood glucose, (j) serum TG, for refed mice, levels for fasted mice were not detectable, (k) mean VO2, as determined by indirect calorimetry. (l) respiratory exchange ratio as determined by indirect calorimetry, (m) Quantification of Western Blot of liver extracts from control or TSC22D1 cDNA adenovirus injected wild-type C57BL6/J mice using TSC22D1 (D1) or actin specific antibodies. Western Blot was quantified with ImageJ. (n) Quantitative qPCR analysis of TSC22D1 expression in liver, abdominal fat (abd), inguinal fat (ing), kidney or gastrocnemius muscle (GC) in the fasted state in same mice as in m. (o) Liver cholesterol levels of same mice as in m. (p) Body weight of 24 h fasted or 6 h refed control or TSC22D1 cDNA adenovirus injected wild-type C57BL/6J mice, (q) liver triglycerides, (r) liver weight, (s) blood glucose, (t) serum non-esterified fatty acids (NEFA), (u) serum ketone bodies, (v) serum triglycerides (TG),. Statistical tests student's t-test. *, $p<0.05$, **, $p<0.01$

Supplementary Figure 2: (a) Quantitative qPCR analysis of TSC22D1 expression in liver, abdominal fat (abd), inguinal fat (ing), kidney or gastrocnemius muscle (GC) in the fasted state in of refed control or miRNA TSC22D1 adeno-associated virus injected wild-type C57BL/6N (b)Liver cholesterol ofsame mice as in a, (c) serum triglycerides (TG), (d) body weight, (e) liver weight, (f) blood glucose, (g) serum non-esterified fatty acids (NEFA) in same mice as in a. (means±SEM, n=5), Statistical tests student's t-test

Supplementary Figure 3. (a) Quantitative PCR analysis of hepatic TSC22D1 of control or TSC22D1 shRNA adenovirus injected wild-type C57BL/6J mice.(b) Clearance of human ApoB from serum of control or TSC22D1 shRNA adenovirus injected wild-type C57BL/6J mice. 20 mg of human VLDL were injected into each animal and serum samples were taken at the indicated time points. Human ApoB levels were determined by human-specific ELISA (means±SEM, n=6). (c) Western Blot of liver extracts from control or TSC22D1 shRNA adenovirus injected wild-type C57BL/6J mice using Apolipoprotein A1 (ApoA1) or valosin containing protein (VCP) specific antibodies. (d) Western Blot of serum from same mice as in c, using ApoA1 specific antibody. (e) Western Blot of precipitated FPLC fractions 35-37 from same mice as in c, using ApoA1 antibody.(f) Quantification of Western Blot of liver extracts from mice fed a high-fat diet (HFD) compared to control mice on a low-fat diet (LFD), using TSC22D1 (D1) or actin specific antibodies. Western Blot was quantified with ImageJ. (g) Serum TGFbeta1 levels in wt and ob/ob mice. (h) Quantification of Western Blot of liver extracts from Balb/C mice treated with PBS or $1.5x10^6$ colon 26 (C26) cells over 3 weeks, using TSC22D1 (D1) or actin specific antibodies. Western Blot was quantified with ImageJ. (i) Relative lesion area in LDLRKO mice with or without streptozotocin treatment (STZ). The surface lesion area was quantified with ImageJ software. means±SEM, n=5). Statistical test: student's t-test. *, $p<0.05$

Supplementary Figure 4. (a) Liver cholesterol levels of 24 h fasted or 6 h refed control or miRNA TSC22D1 adeno-associated virus injected ob/ob mice, (b) body weight, (c) blood glucose, (d) liver weight, (e) serum non-esterified fatty acids (NEFA), (f) serum ketone bodies, (g) serum triglycerides (TG) in same mice as in a. (means±SEM, n=5), Statistical tests student's t-test: *, $p<0.05$

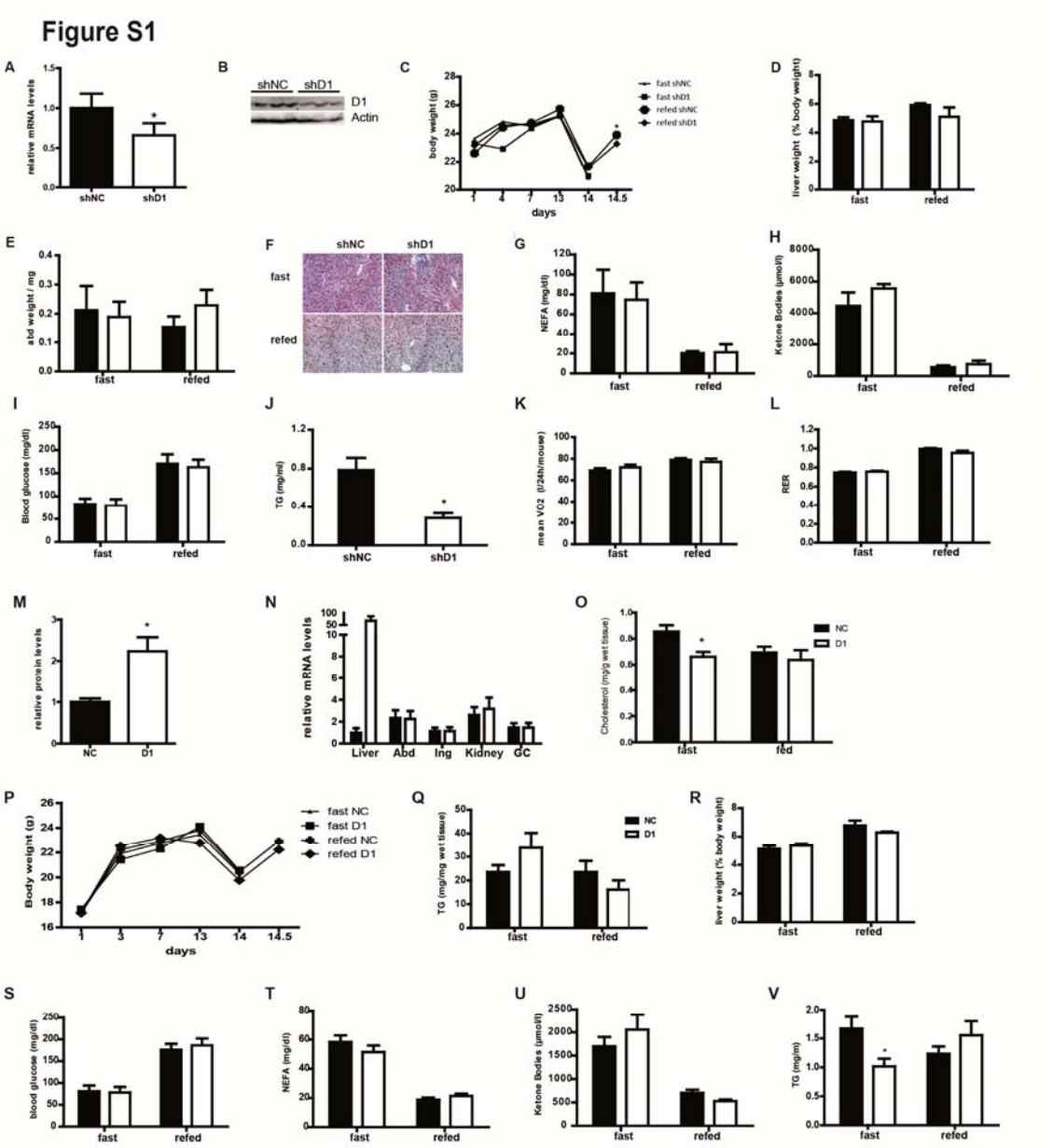


Figure S1
A
relative mRNA levels
shNC
shD1
B
shNC
shD1
D1
Actin
C
body weight (g)
days
fast shNC
fast shD1
refed shNC
refed shD1
D
liver weight (% body weight)
fast
refed
E
abd weight / mg
F
shNC
shD1
fast
refed
G
NEFA (mg/dl)
H
Ketone Bodies (µmol/l)
I
Blood glucose (mg/dl)
J
TG (mg/ml)
K
mean VO2 (l/24h/mouse)
L
RER
M
relative protein levels
NC
D1
N
relative mRNA levels
Liver
Abd
Ing
Kidney
GC
O
Cholesterol (mg/g wet tissue)
NC
D1
fast
fed
P
Body weight (g)
days
fast NC
fast D1
refed NC
refed D1
Q
TG (mg/mg wet tissue)
NC
D1
R
liver weight (% body weight)
S
blood glucose (mg/dl)
T
NEFA (mg/dl)
U
Ketone Bodies (µmol/l)
V
TG (mg/ml)

Figure S2

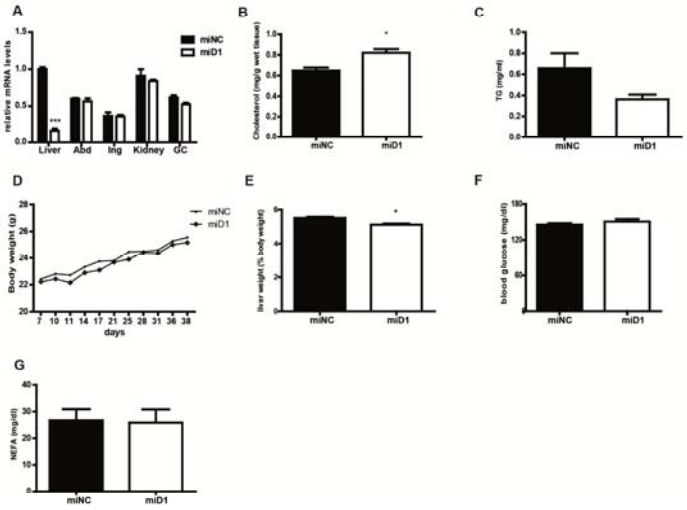

A
relative mRNA levels
miNC
miD1
***
Liver
Abd
Ing
Kidney
GC
B
Cholesterol (mg/g wet tissue)
*
miNC
miD1
C
TG (mg/ml)
miNC
miD1
D
Body weight (g)
miNC
miD1
7 10 11 14 17 21 25 28 31 36 38
days
E
liver weight (% body weight)
*
miNC
miD1
F
blood glucose (mg/dl)
miNC
miD1
G
NEFA (mg/dl)
miNC
miD1

Figure S3

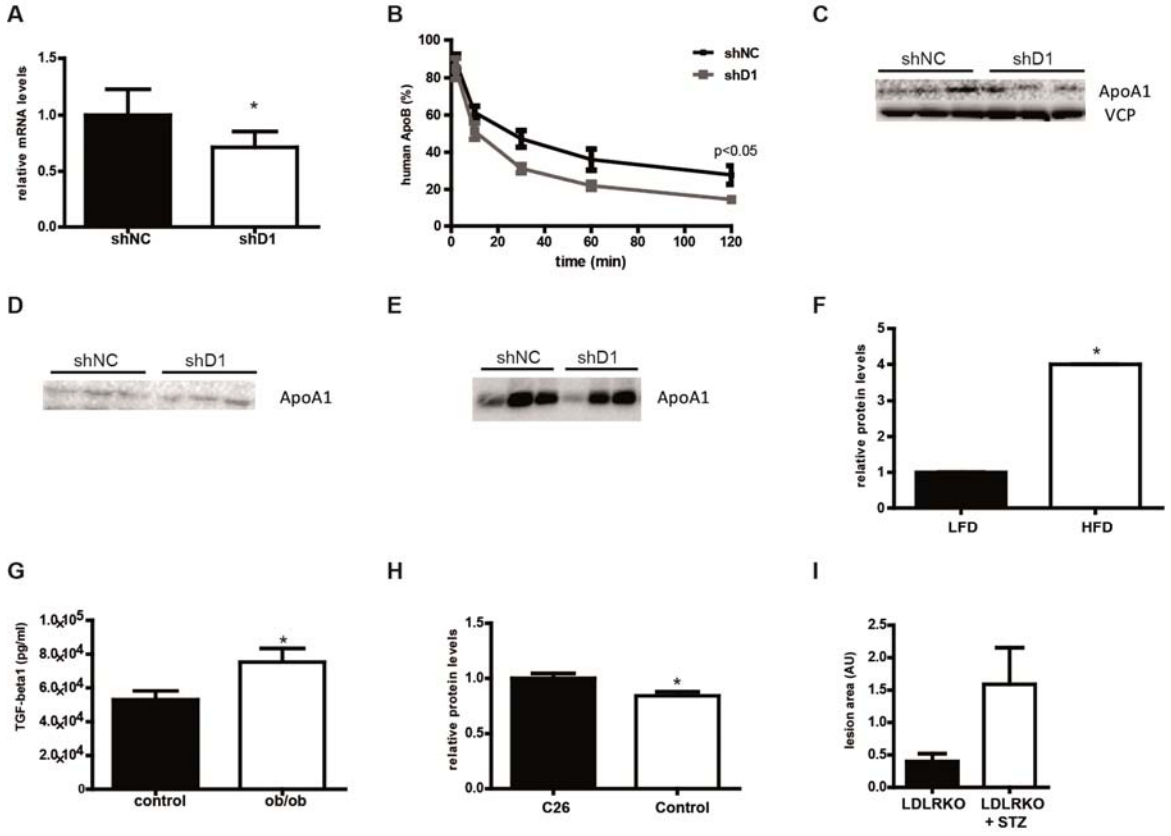
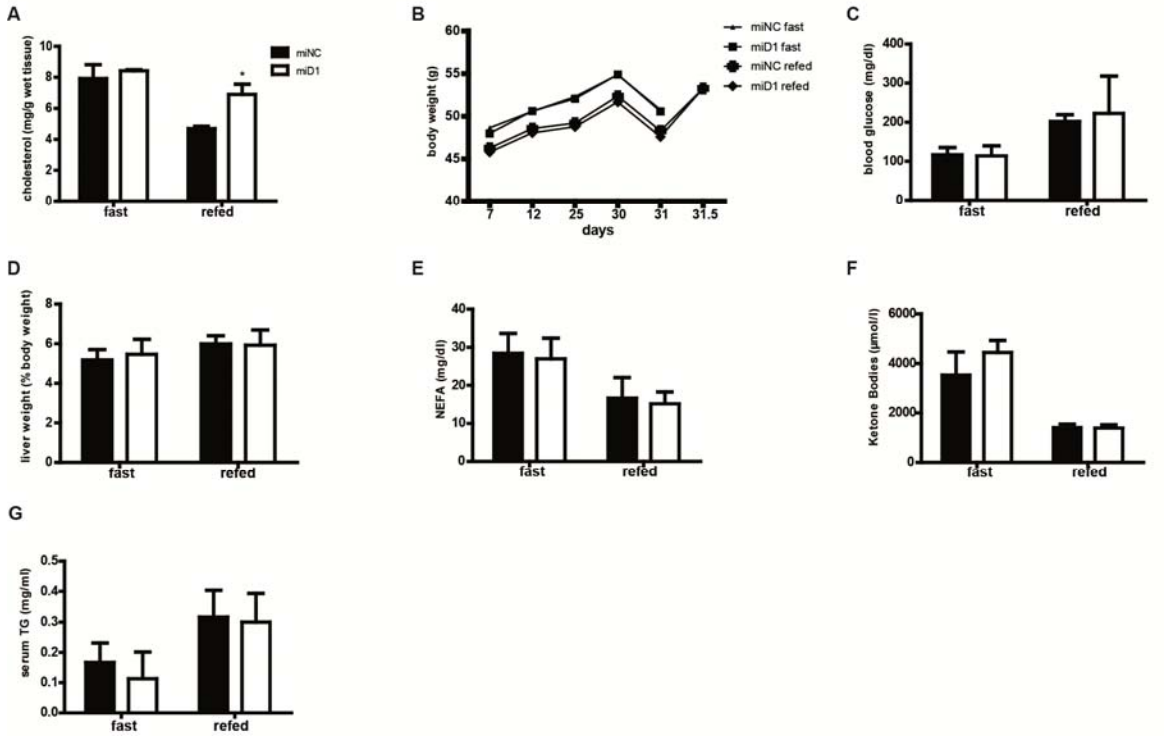

A
relative mRNA levels
1.5
1.0
0.5
0.0
*
shNC
shD1
B
human ApoB (%)
100
80
60
40
20
0
shNC
shD1
p<0.05
0
20
40
60
80
100
120
time (min)
C
shNC
shD1
ApoA1
VCP
D
shNC
shD1
ApoA1
E
shNC
shD1
ApoA1
F
relative protein levels
5
4
3
2
1
0
*
LFD
HFD
G
TGF-beta1 (pg/ml)
1.0x10^5
8.0x10^4
6.0x10^4
4.0x10^4
2.0x10^4
0
*
control
ob/ob
H
relative protein levels
1.5
1.0
0.5
0.0
*
C26
Control
I
lesion area (AU)
2.5
2.0
1.5
1.0
0.5
0.0
LDLRKO
LDLRKO + STZ

Figure S4


A
cholesterol (mg/g wet tissue)
miNC
miD1
fast
refed
B
body weight (g)
miNC fast
miD1 fast
miNC refed
miD1 refed
7
12
25
30
31
31.5
days
C
blood glucose (mg/dl)
fast
refed
D
liver weight (% body weight)
fast
refed
E
NEFA (mg/dl)
fast
refed
F
Ketone Bodies (µmol/l)
fast
refed
G
serum TG (mg/ml)
fast
refed