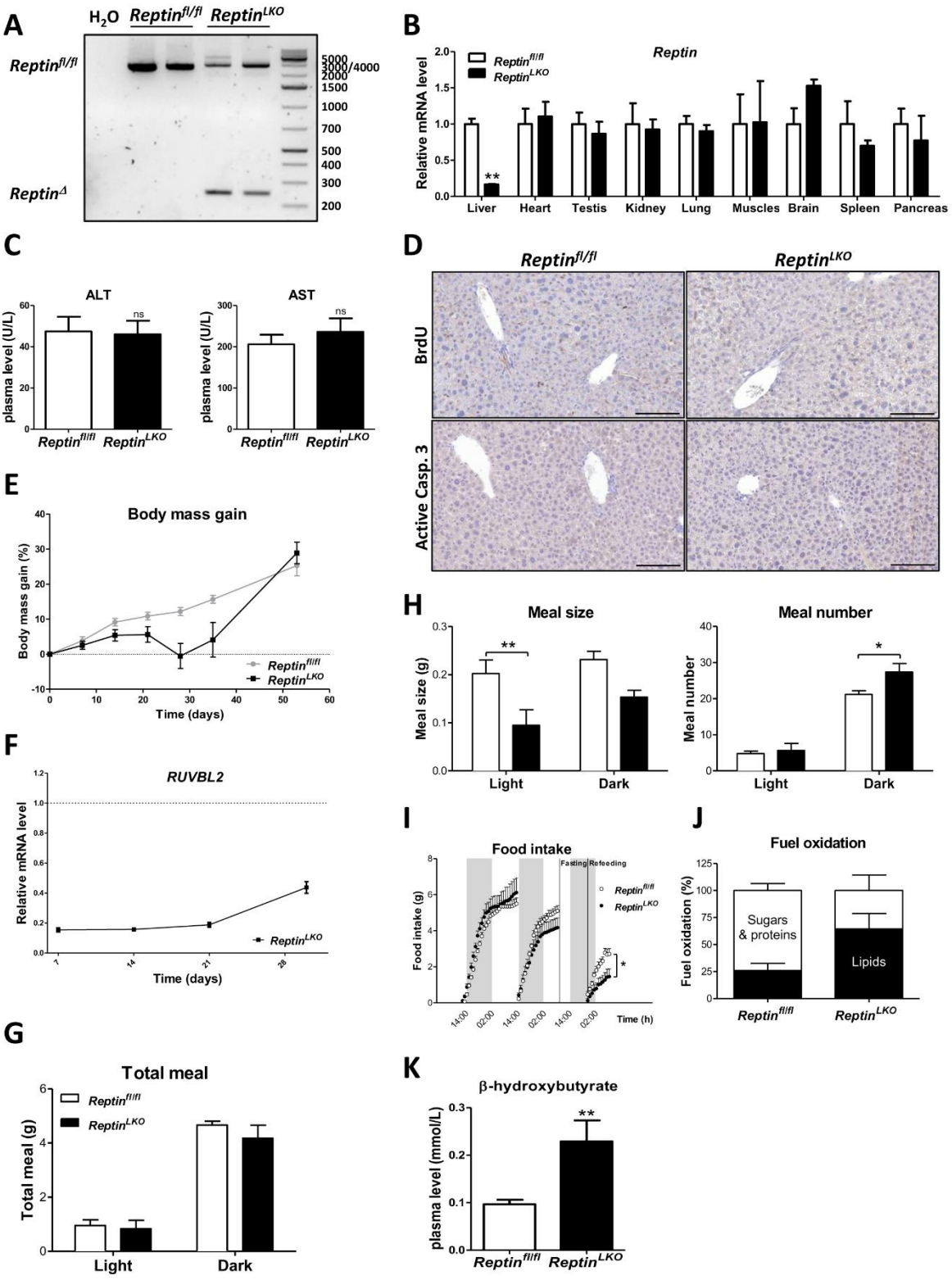
SUPPLEMENTARY FIGURES

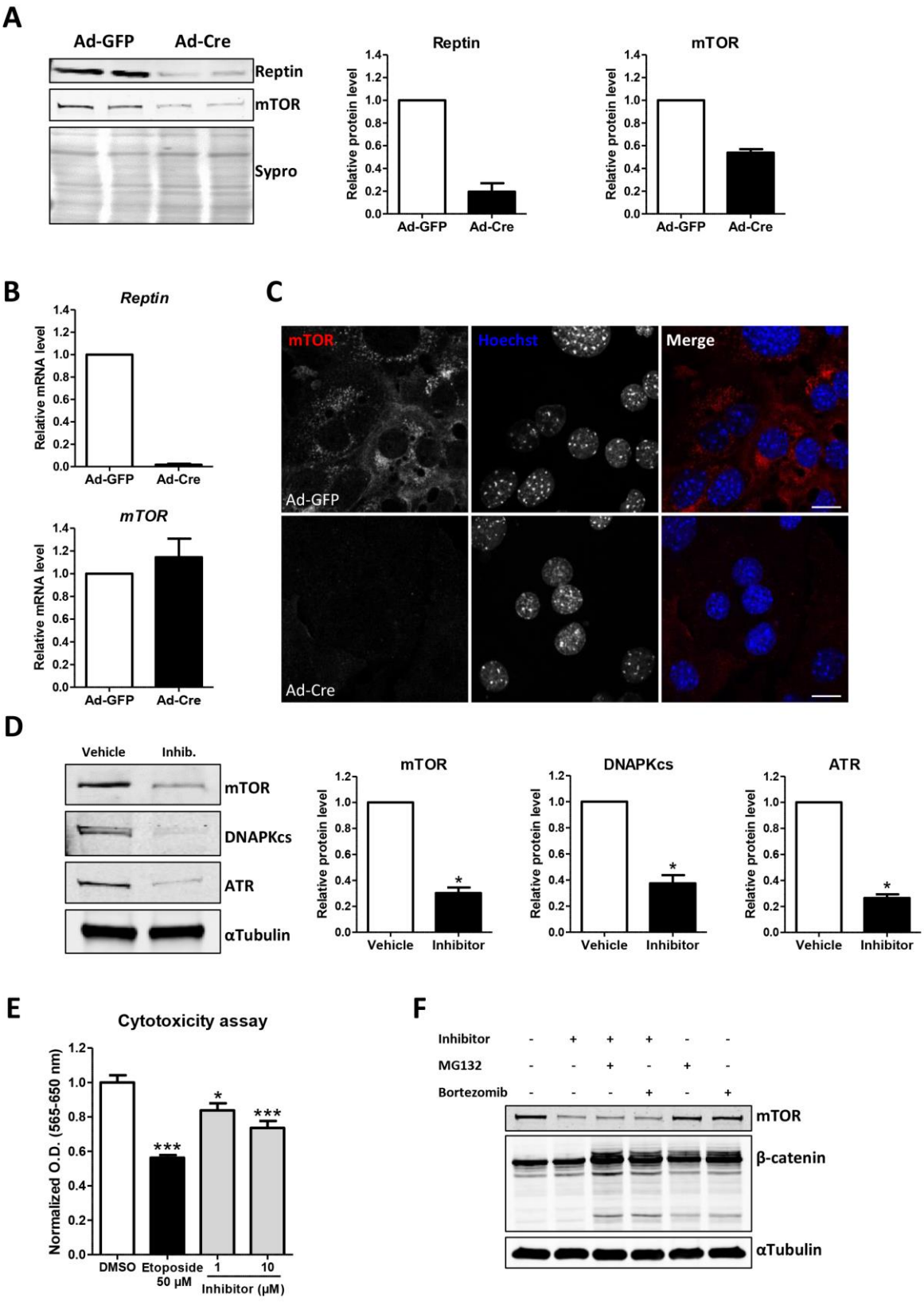
Sup Figure 1: Characterization of liver-specific Reptin knock-out mice (*Reptin^{LKO}*)

(A) PCR analysis of *Reptin* gene on genomic DNA extracted from whole livers of *Reptin$^{fl/fl}$* and *Reptin^{LKO}* mice at D7 post-tamoxifen injection PCR analysis of *Reptin* (primers F1, R) **(B)** Relative Reptin mRNA expression level from liver (n=5), heart, testis, kidney, lung, muscles, brain, spleen (n=3) and pancreas (n=2) extracts from *Reptin$^{fl/fl}$* and *Reptin^{LKO}* mice D15 post-TAM. **(C)** Plasma ALT and AST measurements (n=3). **(D)** Representative pictures of BrdU and active-caspase 3 immunostaining of liver sections from *Reptin$^{fl/fl}$* and *Reptin^{LKO}* mice at D15 post-TAM. Scale bar, 100 µm. **(E)** Body mass gain calculation and **(F)** Relative mRNA expression of Reptin in liver extracts from Reptin$^{fl/fl}$ and Reptin^{LKO} mice from D0 to 6 weeks post-tamoxifen injection (n=3 per group). **(G)** Total meal per day in mice fed ad libitum; **(H)** Size and number of meals per day and; **(I)** Food intake ad libitum and during fasting and refeeding as indicated in *Reptin$^{fl/fl}$* and *Reptin^{LKO}* mice (n=6). **(J)** Fuel oxidation ratio from *Reptin$^{fl/fl}$* and *Reptin^{LKO}* mice during refeeding (n=6). **(K)** β-hydroxybutyrate plasma levels from *Reptin$^{fl/fl}$* and *Reptin^{LKO}* mice at D15 post-TAM fed AL (n=5). All graphs represent mean ± SEM and significance is indicated as follows: * $p \leq 0.05$, ** $p \leq 0.01$.


A
H2O
Reptin fl/fl
Reptin LKO
Reptin fl/fl
Reptin Δ
5000
3000/4000
2000
1500
1000
700
500
400
300
200
B
Reptin
Reptin fl/fl
Reptin LKO
Relative mRNA level
Liver
Heart
Testis
Kidney
Lung
Muscles
Brain
Spleen
Pancreas
C
ALT
AST
plasma level (U/L)
ns
Reptin fl/fl
Reptin LKO
D
Reptin fl/fl
Reptin LKO
BrdU
Active Casp. 3
E
Body mass gain
Body mass gain (%)
Time (days)
F
RUVBL2
Relative mRNA level
Time (days)
G
Total meal
Total meal (g)
Light
Dark
H
Meal size
Meal size (g)
Meal number
Light
Dark
I
Food intake
Fasting
Refeeding
Food intake (g)
Time (h)
J
Fuel oxidation
Fuel oxidation (%)
Sugars & proteins
Lipids
K
β-hydroxybutyrate
plasma level (mmol/L)

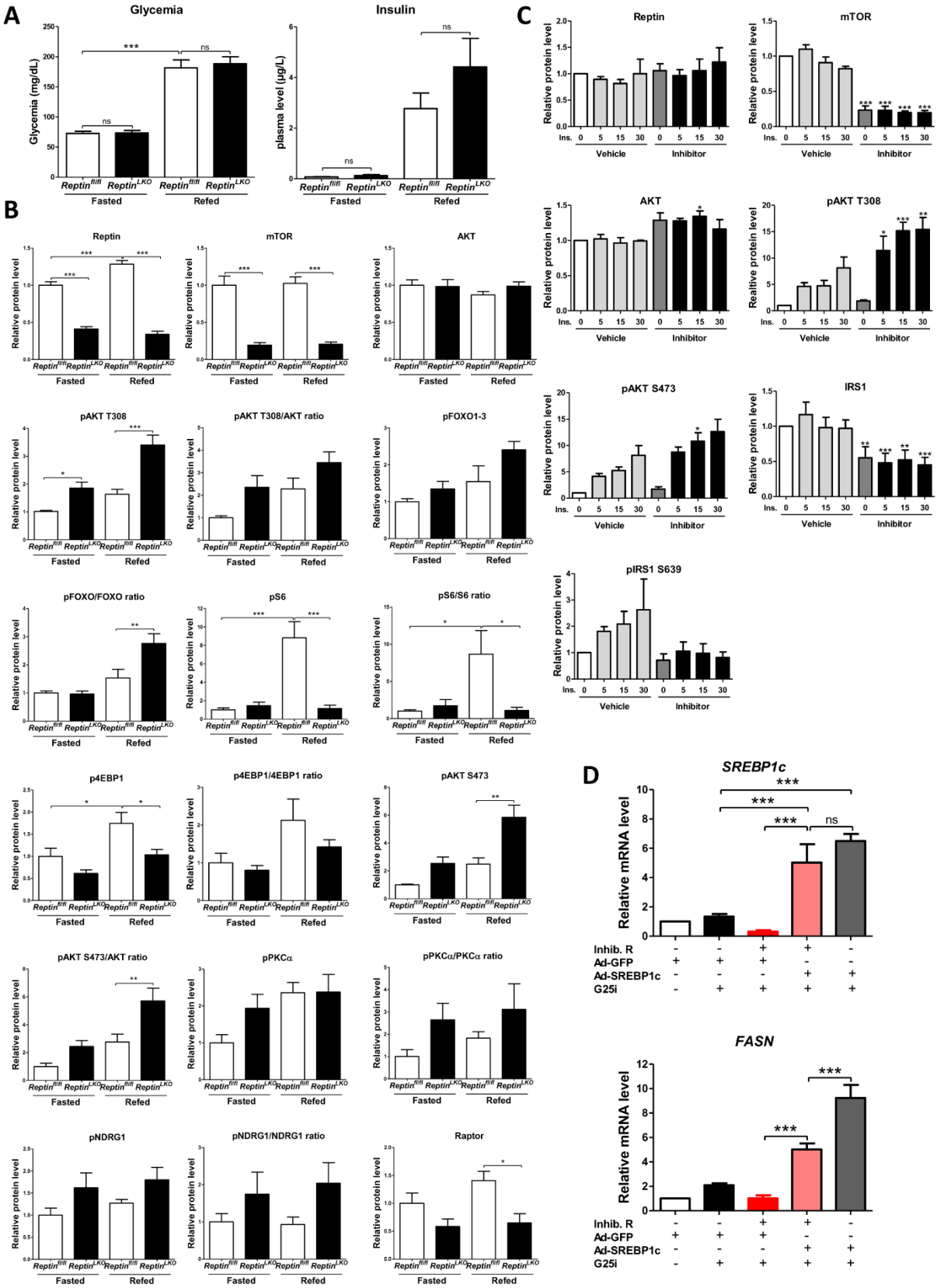
Sup Figure 2: Loss of hepatic Reptin leads to decreased mTOR protein level in a cell-autonomous-manner

(A) Representative immunoblotting and relative protein expression of Reptin and mTOR and **(B)** relative mRNA expression of mTOR in *Reptin*$^{lox/lox}$ mouse primary hepatocytes extracts after infection with Cre- or GFP-expressing adenovirus at MOI 10 (n=2). **(C)** mTOR immunofluorescence in *Reptin*$^{lox/lox}$ mouse primary hepatocytes infected with Cre- or GFP-expressing adenovirus at MOI 10. Scale bar, 20 µm. All graphs represent mean ± SEM. **(D)** Representative immunoblotting of mTOR, DNA-PKcs and ATR in primary hepatocytes treated with Reptin/Pontin ATPase inhibitor (1µM) or vehicle (DMSO) (n=3). DNA-PKcs and ATR are other members of the PIKK family also known to be chaperoned by the R2TP complex (6) **(E)** Sulforhodamine-B cytotoxicity assay in primary hepatocytes treated with Reptin/Pontin ATPase inhibitor at indicated concentrations or vehicle (DMSO). Etoposide has been used as a positive control. **(F)** Representative immunoblotting of mTOR in primary hepatocytes treated with Reptin/Pontin ATPase inhibitor (1uM). Cells were then treated with proteasome inhibitors MG132 (10 µM) or Bortezomib (10 µM) 9 hours before harvest. The accumulation of phosphorylated β-catenin demonstrates the efficiency of proteasome inhibition. Tubulin was used as loading controls.


A
Ad-GFP
Ad-Cre
Reptin
mTOR
Sypro
Reptin
Relative protein level
Ad-GFP
Ad-Cre
mTOR
Relative protein level
Ad-GFP
Ad-Cre
B
Reptin
Relative mRNA level
Ad-GFP
Ad-Cre
mTOR
Relative mRNA level
Ad-GFP
Ad-Cre
C
mTOR
Hoechst
Merge
Ad-GFP
Ad-Cre
D
Vehicle
Inhib.
mTOR
DNAPKcs
ATR
αTubulin
mTOR
Relative protein level
Vehicle
Inhibitor
DNAPKcs
Relative protein level
Vehicle
Inhibitor
ATR
Relative protein level
Vehicle
Inhibitor
E
Cytotoxicity assay
Normalized O.D. (565-650 nm)
DMSO
Etoposide 50 μM
1
10
Inhibitor (μM)
F
Inhibitor
MG132
Bortezomib
mTOR
β-catenin
αTubulin

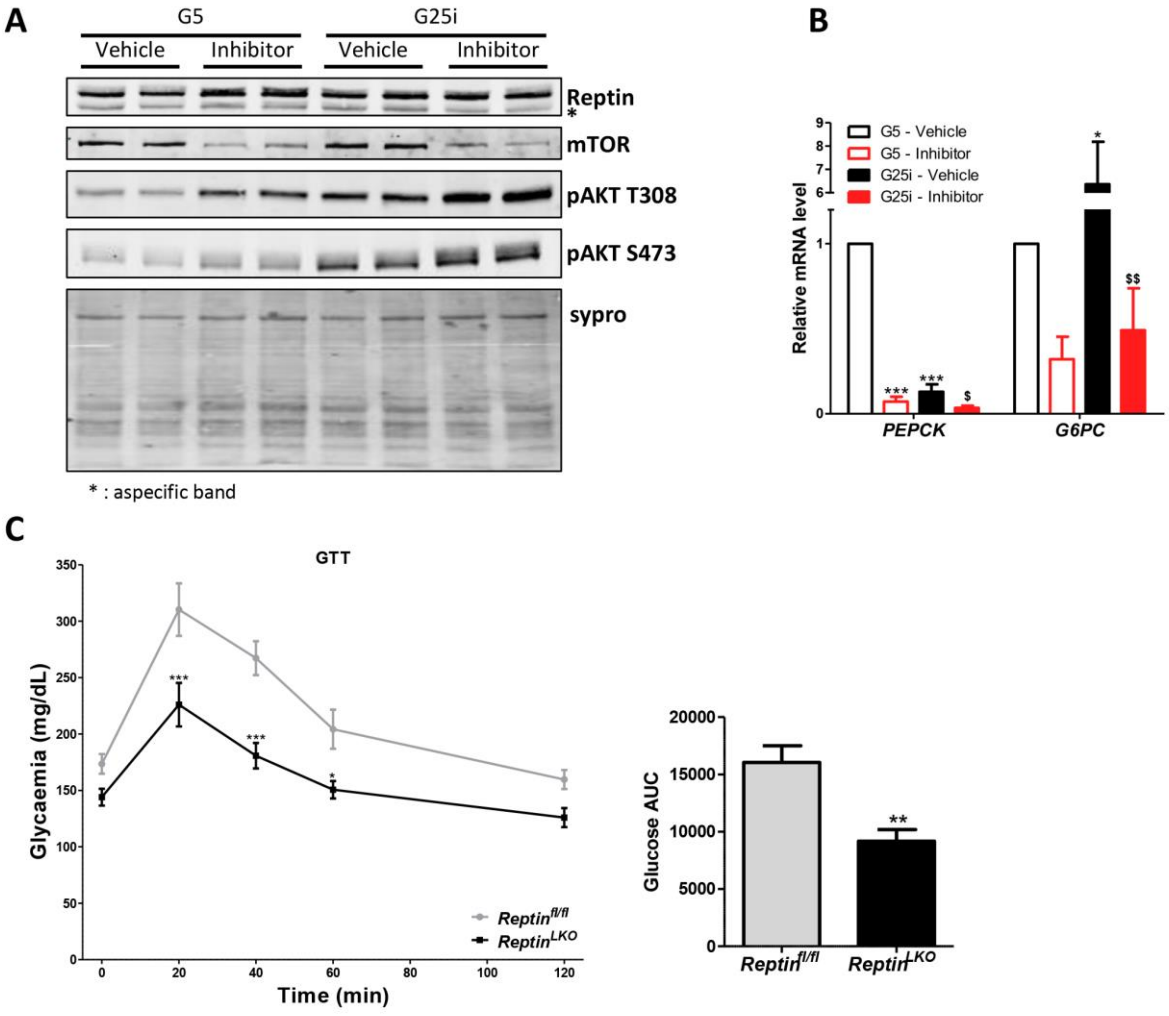
Sup Figure 3: Opposite effects on mTORC1 and mTORC2 signaling upon loss of hepatic Reptin.

(A) Glycemia and plasma insulin levels measured upon 13 hours fasting and 6 hours refeeding in *Reptin*$^{fl/fl}$ and *Reptin*^{LKO} mice. **(B)** Relative protein expression of Reptin, mTOR, AKT, mTORC1 and mTORC2 signaling in liver extracts from *Reptin*$^{fl/fl}$ and *Reptin*^{LKO} mice in fasted and refed state (n=6, from 2 independent experiments). **(C)** Relative protein expression of insulin signaling in mouse primary hepatocytes extracts after treatment with 1 µM Reptin/Pontin ATPase inhibitor and stimulation by 10 nM insulin as shown on the graph (n=3). In these experiments, significance is shown between the conditions vehicle and Reptin ATPase inhibitor at every time point. **(D)** Relative mRNA levels of *DNL genes SREBP1c and FASN* in primary hepatocytes treated with Reptin/Pontin ATPase inhibitor (1uM) and transduced with Ad-SREBP1c active or Ad-GFP as control (MOI 1). Cells were stimulated with glucose 25mM and insulin 100 nM for 24h (n=4). All graphs represent mean ± SEM and significance is indicated as follows: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns (not significant).


A
Glycemia
Glycemia (mg/dL)
Insulin
plasma level (µg/L)
Reptin fl/fl
Reptin LKO
Fasted
Refed
B
Reptin
mTOR
AKT
pAKT T308
pAKT T308/AKT ratio
pFOXO1-3
pFOXO/FOXO ratio
pS6
pS6/S6 ratio
p4EBP1
p4EBP1/4EBP1 ratio
pAKT S473
pAKT S473/AKT ratio
pPKCα
pPKCα/PKCα ratio
pNDRG1
pNDRG1/NDRG1 ratio
Raptor
Relative protein level
C
Reptin
mTOR
AKT
pAKT T308
pAKT S473
IRS1
pIRS1 S639
Relative protein level
Ins.
Vehicle
Inhibitor
D
SREBP1c
FASN
Relative mRNA level
Inhib. R
Ad-GFP
Ad-SREBP1c
G25i

Sup Figure 4: Loss of Reptin in the liver improves glucose tolerance.

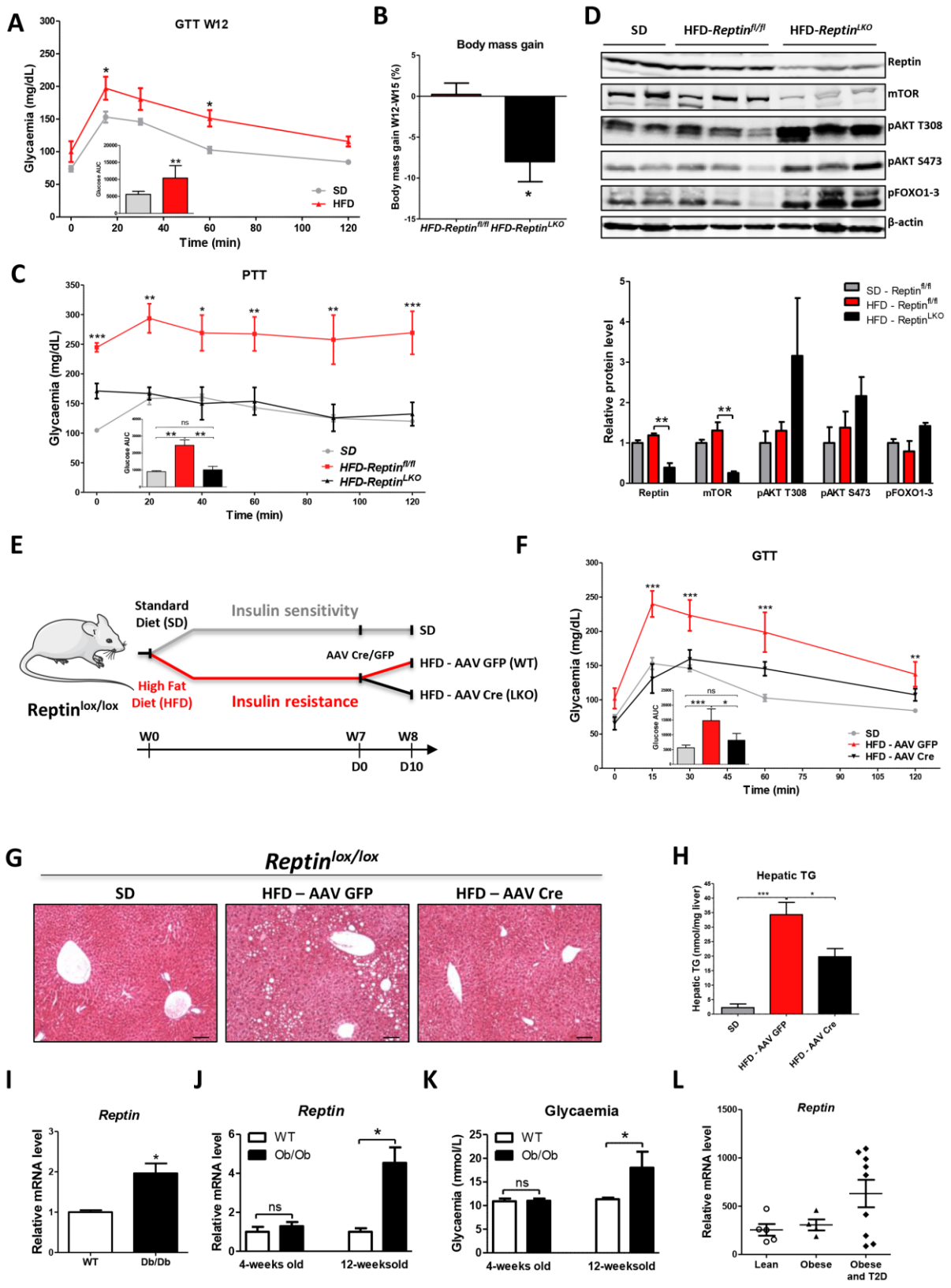
(A) Representative immunoblotting of mTOR and pAKT Ser473 and Thr308 and **(B)** relative mRNA expression of gluconeogenic genes (*PEPCK and G6PC*) genes expression in mouse primary hepatocytes extracts stimulated for 24h with 5 mM (G5) or with 25 mM glucose and 100 nM insulin (G25i) after treatment with 1 µM Reptin ATPase inhibitor or control-Vehicle (n=3). All graphs represent mean ± SEM; significance versus 5 mM low glucose control (G5-Vehicle) is indicated as follows: * p≤0.05, ** p≤0.01, *** p≤0.001 and significance versus 25 mM high glucose + 100 nM insulin control (G25i-Vehicle) is indicated as follows: $ p≤0.05, $$ p≤0.01, $$$ p≤0.001. **(C**) Oral Glucose Tolerance Test (1g/kg) with quantification of the area under the curve from *Reptin*$^{fl/fl}$ and *Reptin*^{LKO} mice after 8 hours of fasting (n=7). All graphs represent mean ± SEM and significance is indicated as follows: * p≤0.05, ** p≤0.01, *** p≤0.001.



Sup Figure 5: Loss of Reptin rescues HFD-induced insulin resistance and hepatic steatosis

(A) Oral Glucose Tolerance Test (1g/kg) after 12 hours of fasting with quantification of the area under the curve from SD (n=3) and HFD (n=8) mice 12 weeks after HFD introduction and prior induction of Reptin deletion. Significance is shown between SD and HFD at every time point. **(B)** Body weight gain calculation from HFD-*Reptin*$^{fl/fl}$ (n=3) and HFD-*Reptin*^{LKO} (n=5) mice 18 days after tamoxifen injection. **(C)** Hepatic glucose production analysis by Pyruvate Tolerance Test (PTT) 2g/kg after 10 hours of fasting with quantification of the area under the curve calculated from baseline y=100 from SD (n=3), HFD-*Reptin*$^{fl/fl}$ (n=3) and HFD-*Reptin*^{LKO} (n=5) mice 16 days after TAM injection. Significance is shown between SD and HFD-*Reptin* $^{fl/fl}$ mice at every time point. **(D)** Representative immunoblotting of insulin signaling in liver extracts from SD, HFD-*Reptin*$^{fl/fl}$ and HFD-*Reptin*^{LKO} 18 days after Reptin deletion. **(E)** Experimental strategy for Reptin deletion by retro-orbital injection of Cre-expressing AAV in Reptin$^{lox/lox}$ model with 8 weeks HFD-induced metabolic syndrome. Three groups: standard diet (SD n=3), HFD-AAV GFP (n=3) and HFD-AAV Cre (n=3). **(F)** Glucose tolerance was restored back to normal in mice with AAV-Cre Reptin-deficient livers. **(G)** Hepatic steatosis was abolished as revealed by H&E staining of liver sections (scale bar, 100 µm) and **(H)** hepatic TG content was decreased in AAV-Cre Reptin-deficient livers. Significance is shown between SD and HFD-AAV GFP mice at every time point. **(I-J)** Relative mRNA of Reptin in liver extracts of **(I)** diabetic Db/Db mice (n=5), and **(J)** of 4- and 12- weeks- old obese Ob/Ob mice (n=3) with **(K)** respective glycemia levels (n=3). All graphs represent mean ± SEM and significance is indicated as follows: * p≤0.05, ** p≤0.01, *** p≤0.001. **(L)** Reptin mRNA level in liver of obese or obese and T2D subjects compared to lean subjects (7).

Data from ME Patti's lab, Boston (GEO analysis): https://www-ncbi-nlm-nih-gov.gate2.inist.fr/geo/query/acc.cgi?acc=GSM391710; https://www-ncbi-nlm-nih-gov.gate2.inist.fr/geo/tools/profileGraph.cgi?ID=GDS3876:201459_at.


A
GTT W12
Glycaemia (mg/dL)
Time (min)
SD
HFD
B
Body mass gain
Body mass gain W12-W15 (%)
HFD-Reptin fl/fl
HFD-Reptin LKO
D
SD
HFD-Reptin fl/fl
HFD-Reptin LKO
Reptin
mTOR
pAKT T308
pAKT S473
pFOXO1-3
β-actin
C
PTT
Glycaemia (mg/dL)
Time (min)
SD
HFD-Reptin fl/fl
HFD-Reptin LKO
Relative protein level
SD - Reptin fl/fl
HFD - Reptin fl/fl
HFD - Reptin LKO
E
Standard Diet (SD)
Insulin sensitivity
SD
AAV Cre/GFP
HFD - AAV GFP (WT)
High Fat Diet (HFD)
Insulin resistance
HFD - AAV Cre (LKO)
Reptin lox/lox
W0
W7
W8
D0
D10
F
GTT
Glycaemia (mg/dL)
Time (min)
SD
HFD - AAV GFP
HFD - AAV Cre
G
Reptin lox/lox
SD
HFD – AAV GFP
HFD – AAV Cre
H
Hepatic TG
Hepatic TG (nmol/mg liver)
SD
HFD - AAV GFP
HFD - AAV Cre
I
Reptin
Relative mRNA level
WT
Db/Db
J
Reptin
Relative mRNA level
WT
Ob/Ob
4-weeks old
12-weeksold
K
Glycaemia
Glycaemia (mmol/L)
WT
Ob/Ob
4-weeks old
12-weeksold
L
Reptin
Relative mRNA level
Lean
Obese
Obese and T2D

Sup Figure 6: Loss of Reptin increases mTORC2 signalling.

(A) Relative mRNA expression of *INSIG2A* in liver extracts from *Reptin*$^{fl/fl}$ and *Reptin*^{LKO} mice as indicated (n=6, from 2 independent experiments). **(B)** Venn diagrams of UP and DOWN-regulated genes that are affected both in fasted and refed conditions in liver extracts from *Reptin*^{LKO} mice. **(C)** Relative mRNA expression of *Rictor* in liver extracts from *Reptin*$^{fl/fl}$ and *Reptin*^{LKO} mice as indicated (n=6, from 2 independent experiments). All graphs represent mean ± SEM and significance is indicated as follows: * p≤0.05, ** p≤0.01, *** p≤0.001, ns (not significant).