Supporting Online Material

Defective Hepatic Autophagy in Obesity Promotes ER Stress and Causes Insulin Resistance

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Figure S1

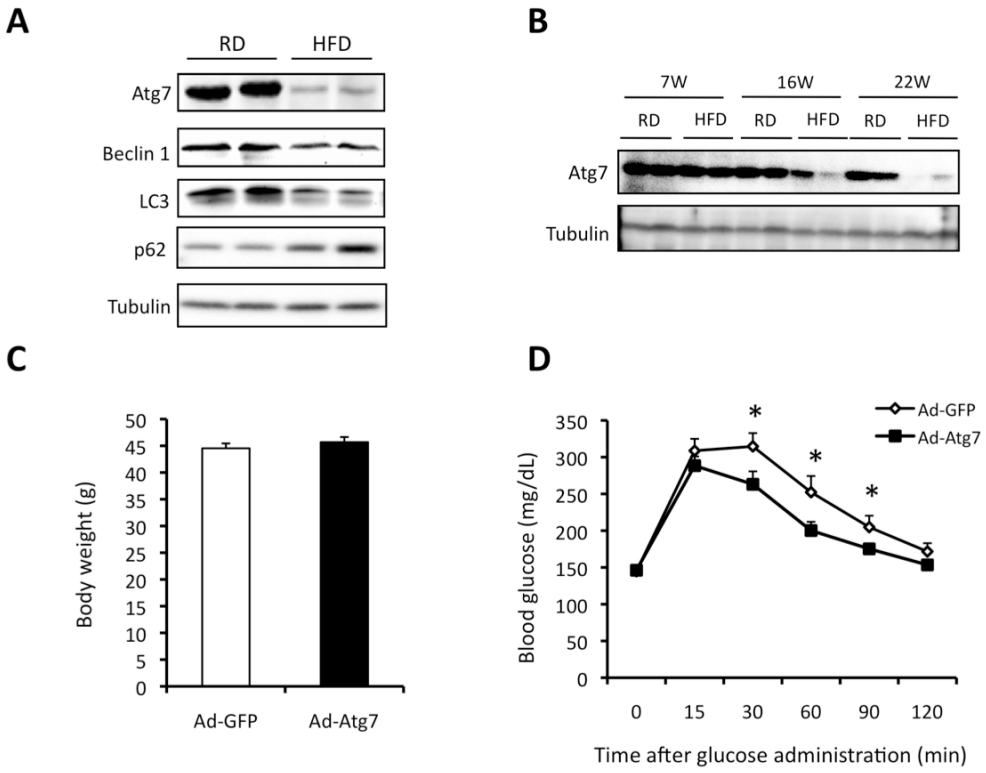

Figure S1. ***Regulation of systemic insulin action by Atg7 in a high fat diet-induced obesity model.*** **A.** Autophagy was examined in liver of mice fed with high fat diet (5 month on HFD) and regular diet (RD) by western blot analysis, using the conversion of LC3-I to LC3-II, expression of Beclin 1 and Atg7 as autophagy markers. **B**. Atg7 expression level during the course of HFD feeding was examined by western blot assay. The C57BL/6J mice were fed with high-fat diet after weaning for 20 weeks, followed by injection of adenovirus carrying GFP (Ad-GFP, n=11) or Atg7 (Ad-Atg7, n=11). After 7 days, body weights were measured **(C)** and glucose tolerance tests **(D)** were performed. Data are shown as mean±SEM. Asterisk indicates statistical significance determined by repeated measures two-way ANOVA followed by post-test (* indicates $p<0.05$).

Figure S2

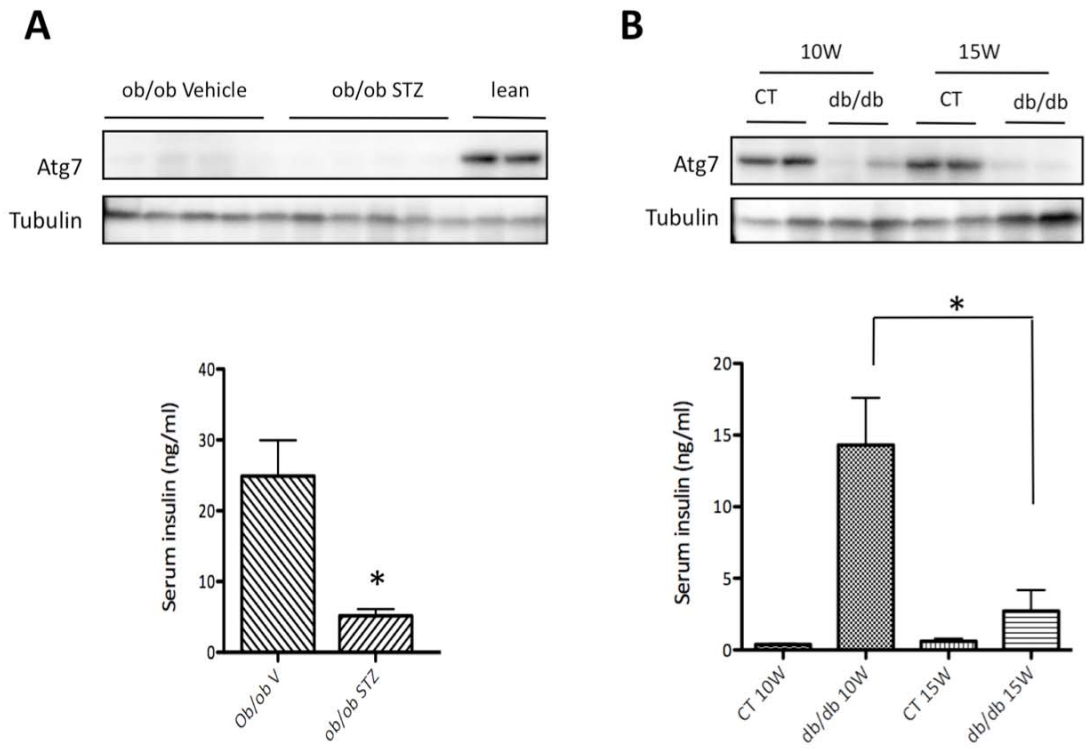

Figure S2. ***The effect of hyperinsulinemia on Atg7 expression in obese mice.*** Atg7 expression level was examined in liver tissue of *ob/ob* mice (**A**) treated with STZ or *db/db* mice at different ages (**B**). Serum insulin levels were measured in mice after 6 hours daytime food withdrawal, and shown at the bottom of each blot. Data are shown as mean±SEM. Asterisk indicates statistical significance determined by student's-*t* test (*$p<0.05$).

Figure S3

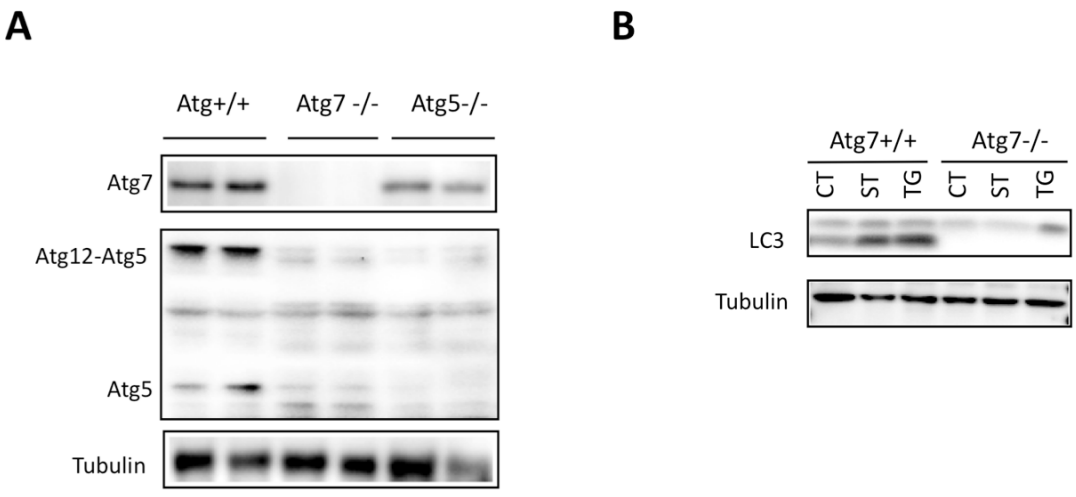

Figure S3. ***Regulation of autophagy in Atg7- or Atg5-deficient MEFs.*** Atg7, Atg12-Atg5, and Atg5 expression levels in MEF cells lacking Atg7 or Atg5 (**A**) and the conversion of LC3 (**B**) in Atg7-/- mouse embryonic fibroblasts (MEF) in the presence or absence of autophagy inducers: starvation (ST) and thapsigargin (TG, 200nM) were examined.

Figure S4

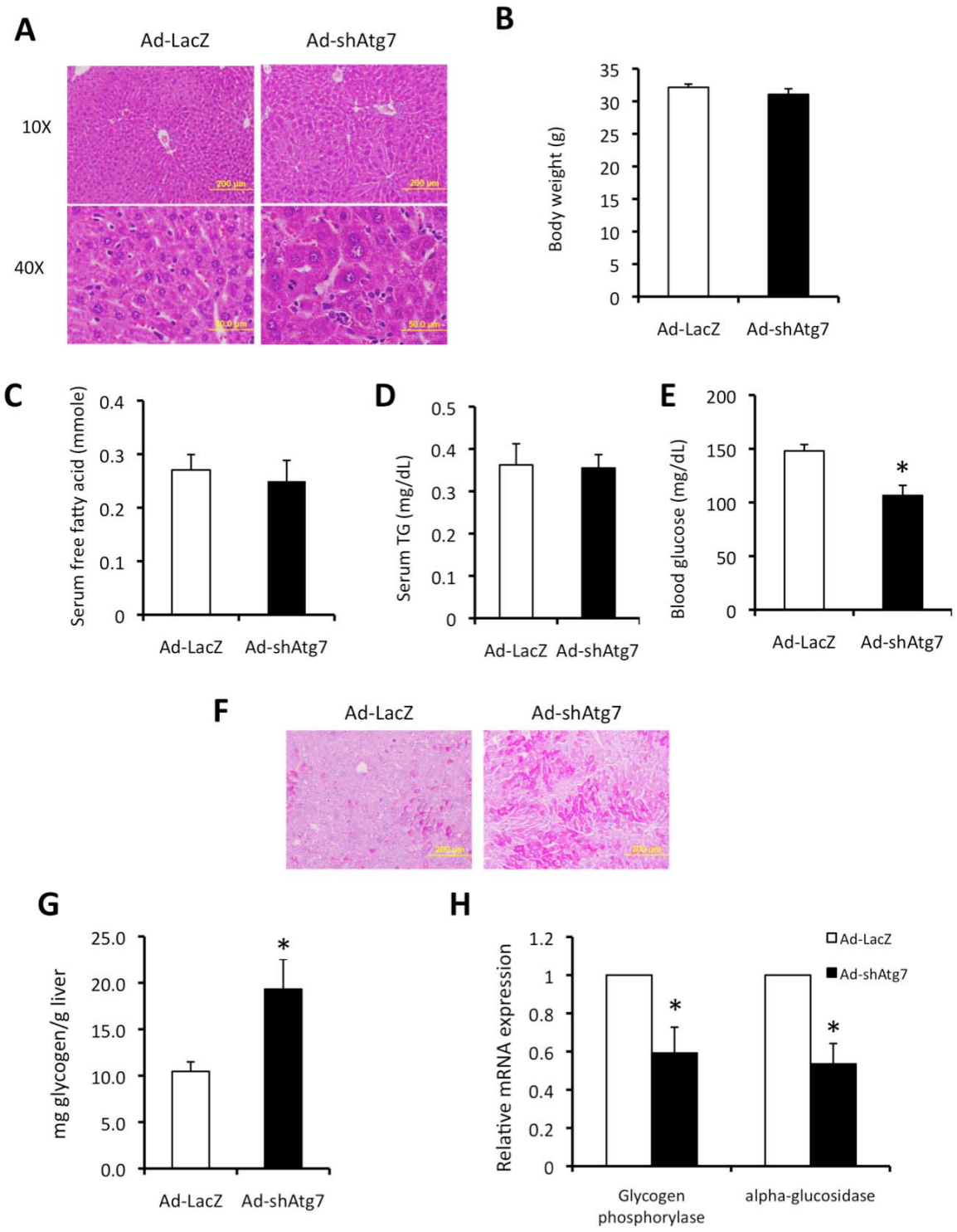
A
Ad-LacZ
Ad-shAtg7
10X
40X
B
Body weight (g)
35
30
25
20
15
10
5
0
Ad-LacZ
Ad-shAtg7
C
Serum free fatty acid (mmole)
0.4
0.3
0.2
0.1
0
Ad-LacZ
Ad-shAtg7
D
Serum TG (mg/dL)
0.5
0.4
0.3
0.2
0.1
0
Ad-LacZ
Ad-shAtg7
E
Blood glucose (mg/dL)
200
150
100
50
0
*
Ad-LacZ
Ad-shAtg7
F
Ad-LacZ
Ad-shAtg7
G
mg glycogen/g liver
25.0
20.0
15.0
10.0
5.0
0.0
*
Ad-LacZ
Ad-shAtg7
H
Relative mRNA expression
1.2
1
0.8
0.6
0.4
0.2
0
Ad-LacZ
Ad-shAtg7
*
*
Glycogen phosphorylase
alpha-glucosidase

Figure S4. ***Metabolic effects of Atg7 suppression in liver tissue of lean mice.*** **A.** Representative images of H&E staining of liver tissue sections from lean mice (male, 14-weeks-old, C57BL/6J) injected with the control adenovirus expressing LacZ shRNAi to LacZ (Ad-LacZ) or Atg7 (Ad-shAtg7) shRNAi. Body weight (**B)**, Serum free fatty acid (**C**)**,** triglyceride (TG) (**D**) and glucose levels (**E**) were measured in lean mice after 6 hours food withdrawal following Atg7 knockdown. **F.** Representative images of PAS staining in liver tissue sections from lean mice (male, 14-weeks-old, C57BL/6J) injected with the control (Ad-LacZ) or Atg7 (Ad-shAtg7) shRNAi containing adenoviruses after 6 hours fasting. **G.** Glycogen levels were measured in liver tissue collected from mice injected with Ad-LacZ or Ad-shAtg7. **H.** mRNAs coding for glycogen phosphorylase and α-glucosidase were examined by quantitative RT-PCR and the results presented as gene expression levels in Atg7 group normalized to controls (Ad-LacZ). All data are shown as mean±SEM. Asterisk indicates statistical significance determined by student's *t* test (*$p<0.05$).

Figure S5

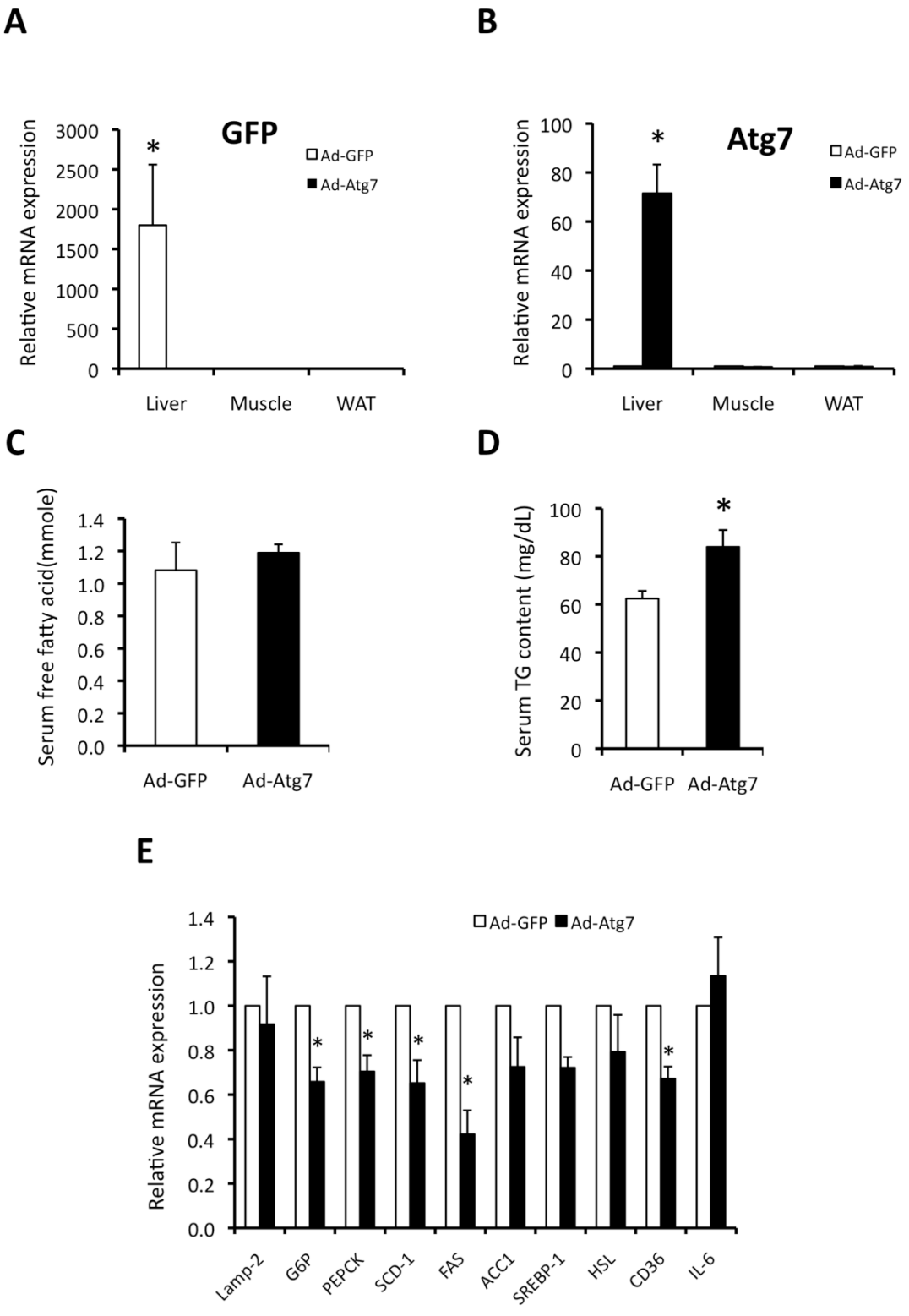
A
GFP
Ad-GFP
Ad-Atg7
Relative mRNA expression
3000
2500
2000
1500
1000
500
0
*
Liver
Muscle
WAT
B
Atg7
Ad-GFP
Ad-Atg7
Relative mRNA expression
100
80
60
40
20
0
*
Liver
Muscle
WAT
C
Serum free fatty acid(mmole)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Ad-GFP
Ad-Atg7
D
Serum TG content (mg/dL)
100
80
60
40
20
0
*
Ad-GFP
Ad-Atg7
E
Ad-GFP
Ad-Atg7
Relative mRNA expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Lamp-2
G6P
PEPCK
SCD-1
FAS
ACC1
SREBP-1
HSL
CD36
IL-6

Figure S5. ***Metabolic effects of liver Atg7 restoration in obese mice.*** mRNAs coding for GFP (**A**) or Atg7 (**B**) were examined in liver, muscle and white adipose tissue (WAT) of *ob/ob* mice injected with Ad-GFP or Ad-Atg7 by quantitative RT-PCR. Results are presented as gene expression levels in Ad-GFP group normalized to Ad-Atg7 group (**A**), or in Ad-Atg7 group normalized to Ad-GFP controls (**B**). Serum free fatty acid **(C)** and serum triglyceride (TG) **(D)** were measured in *ob/ob* mice expressing Atg7 (n=8) or control vector (n=8). **E.** mRNA coding for genes involved in gluconeogenesis and lipogenesis in liver were examined by quantitative RT-PCR. Results are presented as gene expression levels in Ad-Atg7 group normalized to Ad-GFP controls. All data are shown as mean±SEM. Asterisk indicates statistical significance determined by student's-*t* test (*$p<0.05$).

Figure S6

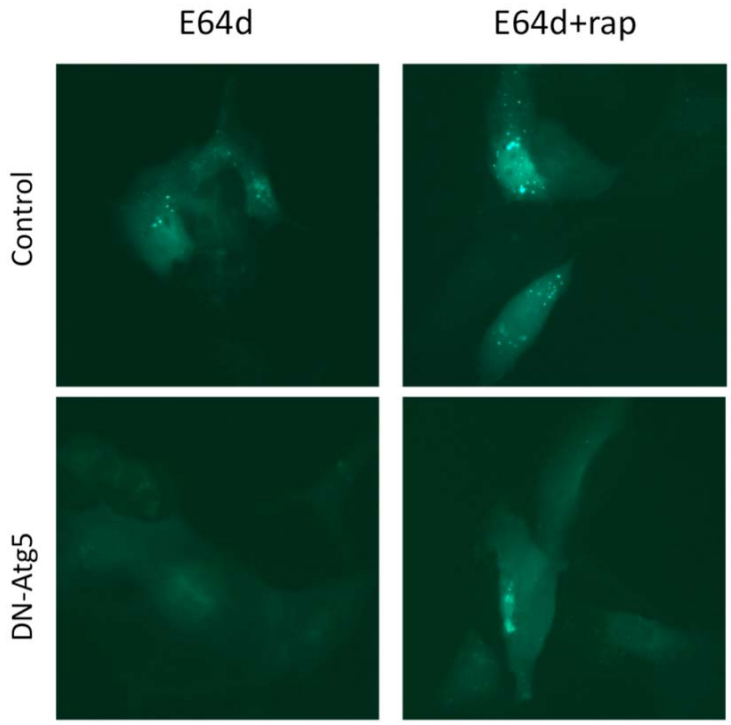

Figure S6. ***Suppression of autophagy by dominant-negative Atg5.*** Representative fluorescence microscopy images (40x) of GFP-LC3 punctate structures in human hepatoma cell line, Huh-7 cells expressing GFP-LC3 alone (CT), or GFP-LC3 plus DN-Atg5 (DN-Atg5), after treatment with rapamycin (100nM) for 4 hours in the presence of the lysosomal proteases inhibitor, E64d. These experiments were performed to verify the efficiency of the dominant-negative (DN)-Atg5 to block autophagy.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Mouse models: Male *db/db* mice at 10 weeks of age and its controls (C57Bl.KS/J) were purchased from Jackson Labs, kept on a 12-hrs light cycle, and fed with regular rodent diet. For streptozotocin (STZ) experiments, male *ob/ob* mice (8 weeks) were injected with vehicle (0.05M sodium citrate) or STZ at 200mg/kg dose, every seven days, for two times via intraperitoneal route. The 6-hour fasting blood glucose levels were monitored in *db/db* mice and STZ-treated *ob/ob* mice every three days.

Glycogen assays: Liver glycogen content in mice transduced with Ad-shAtg7 or control virus (Ad-LacZ) was measured, as described in (Le Lay et al., 2009). Briefly, 100mg of liver tissue samples were homogenized in 6% PCA. After centrifugation, the supernatant was collected, combined with one volume of H_2O, adjusted to pH:6.7 with KOH, and incubated with amyloglucosidase at 40^{o}C for 2hours. Glucose concentrations were then determined using an Amplex Red Glucose/Glucose Oxidase assay system (Invitrogen).

Quantitative real time RT-PCR: The primers used to quantitate the lipogenesis and gluconeogenesis genes were described as before (Cao et al., 2008; Furuhashi et al., 2007); the primers used for mouse liver glycogen phosphorylase are: forward: 5'-GCGACTACTACTTCGCCCTTG-3'; reverse:5'-GAGGTAATACACCCTCTTGGGA-3'; the primers used for mouse acid alpha-glucosidase are: forward:5'-CACCAGGAAGGTT ACAAGCCA-3'; reverse: 5'-GTCGGGGGCACAGTCAAAG-3'. The primers for mouse Grp78 are: forward: 5'-TCATCGGACGCACTTGGAA-3', reverse: 5'-CAACCACCTTG

AATGGCAAGA-3’; the primers for mouse PDI are: forward: 5-CAAGATCAAGCCCCACCTGAT-3’, reverse: 5’-AGTTGCCCCAACCAGTACTT-3’; the primers for mouse Erdj4 are: forward: 5’-CCCCAGTGTCAAACTGTACCAG-3’, reverse: 5’-AGCGTTTCCAATTTTCCATAAATT-3’; the primers for mouse Gadd34 are: forward: 5’-GAGGGACGCCCACAACTTC-3’, reverse: 5’-TTACCAGAGACAGGGGT AGGT-3’; the primers for mouse Chop are: forward: 5’-CCACCACACCTGAAAGCAG AA-3’, reverse: 5’-AGGTGAAAGGCAGGGACTCA-3’; the primers for mouse Lamp2 were used as described (Yanagawa et al., 2007); the primers for Atg5 were purchased from SABioscience; the primers used for mouse Atg7 are: forward: 5’-TGCCTATGATG ATCTGTGTC-3’, reverse:5’-CACCAACTGTTATCTTTGTCC-3’.

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