

Macroautophagy is inhibited in siATG7 and 3-methyladenine-treated cells. (A) Immunoblots of total protein from VEC and siATG7 cells untreated and treated with ammonium chloride and leupeptin (A/L) and probed for LC3 or β -actin. (B) Immunoblots of wild-type cells untreated (Con) or treated with 3-methlyadenine (3MA) in the absence or presence of ammonium chloride/leupeptin. The LC3-I and LC3–II forms are indicated by arrows.



An inhibition of autophagy does not induce 3T3-L1 cell death. The percentages of apoptotic (Apop) and necrotic (Nec) cells were determined after acridine orange/ethidium bromide costaining by fluorescence microscopy 24 h after the induction of adipocyte differentiation in VEC and siATG7 cells (**A**), and wild-type cells untreated (Con) or treated with 3-methyladenine (3MA) (**B**). Acridine orange stained cells were evaluated for morphological characteristics of apoptosis and cells staining positive for ethidium bromide were considered necrotic. Results are mean + SEM (n=3-4).



Knockdown of *atg5* in 3T3-L1 cells blocks TG accumulation and adipocyte differentiation. (A) Total protein was isolated from VEC cells and cells stably infected with a lentivirus expressing one of two different shRNAs for *atg5* (siATG5 #1 and siATG5 #2 cells) and immunoblotted with antibodies for Atg5 and β -actin. (B) TG levels in VEC, siATG5 #1 and siATG5 #2 cells at Day 6 after initiation of adipocyte differentiation. Results are mean + SEM (*n*=6-7). **P*<0.006 versus VEC cells. (C) Immunoblots of proteins isolated from VEC, siATG5 #1 and siATG5 #2 cells on the days of differentiation listed and probed with the indicated antibodies.



Effects of the adipose-specific loss of *atg7* on body weight and food intake. (A) Total body weights of control (con) and knockout (ko) mice fed a RD (n=5). *P<0.04 versus control. (B) Body weights of HFD-fed animals (n=4). *P<0.001 versus control. (C) Average daily oral food intake measured over a two-week period in RD and HFD-fed mice (n=19-24). Results are mean ± SEM.



Knockout mice have decreased serum leptin levels and no change in adiponectin levels. Serum levels of leptin (A) and adiponectin (B) in control (con) and knockout (ko) mice. Results are mean + SEM (n=5-11). *P<0.04 versus control.



Weights in non-adipose tissues. (A) Weights of liver (Lv), heart (Ht), lung (Ln), kidney (Kd), spleen (Sp) and skeletal muscle (Sm) from control (con) and knockout (ko) mice fed RD. (B) Organ weights from HFD-fed mice. Results are mean + SEM (n=3-9). *P<0.03 versus control.



Serum lipid levels in RD- and HFD-fed mice. Serum levels of fatty acids (A), triglycerides (B) and total cholesterol (C) in control (con) and knockout (ko) mice. Results are mean + SEM (n=3-11). *P<0.04 versus control.



TG content does not increase in non-adipose tissues. (A) Hepatic TG content. (B) Heart TG content. Results are mean + SEM (n=3-10). *P<0.05 versus control fed the same diet.



Adipocytes in WAT from ATG7^{F/F}-aP2-Cre mice contain more mitochondria. (A) Representative electron micrographs of WAT from control and knockout mice. Inserts show areas at higher magnification to illustrate the presence of "vacuolated structures" (white arrows) and mitochondria (black arrows). L: lipid. Scale bars: 1 μ m; inserts: 0.5 μ m. The percentage of cellular area occupied by mitochondria (**B**) and the number of mitochondria per microscopic field (**C**) were calculated from 9 different micrographs of control (con) and knockout (ko) mice. **P*<0.001 versus control. Results are mean + SEM.



Total body and adipose tissue weights do not change in young knockout animals. (A) Body weights in RD-fed 3-week old control (con) and knockout (ko) mice. (B) Gonadal WAT weights. (C) Interscapular BAT weights. Results are mean + SEM (n=4-7).



The WAT progenitor cell population is unchanged by a knockout of autophagy. The numbers of white adipocyte progenitor cells identified as Lin⁻, CD29⁺, CD34⁺, Sca-1⁺ and CD24⁺ cells as a percentage of the population of adipose stromal vascular cells in control (con) and knockout (ko) mice. Results are mean + SEM (n=4-9).