Supporting Information

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SI Text

Tissue Processing and Cell Size Quantification. At the age of 18–20 weeks, a set of control and mutant mice were killed. Fat tissues were weighed and fixed with phosphate buffered formalin (Surgipath Medical Industries, Inc.), and embedded in paraffin. Slides were stained with hematoxylin and eosin (H&E) for morphological analysis. Pictures were taken with a Universal Microscope Axioplan 2 imaging system (Carl Zeiss) with phase contrast objectives (magnification, ×100).

For cell size quantification, pictures of the H&E stained slides were taken. The area of the picture (S) and the total number of cells (nuclei, N) in the picture were then determined. The average radius (r) of the cells was calculated as the square root of S/(pi*N) and the average volume was determined. For each data point, six random pictures were used. For quantification of the size of lipid droplets, the diameters of lipid droplets in the pictures were measured with Adobe Photoshop software (Adobe Systems, Inc.) and the total volume of lipid droplets was calculated. For each data point, 50 random lipid droplets in a representative picture were measured and calculated.

EM Analyses. The white fat tissue (gonadal fat pad) and brown fat tissue (interscapular fat pad) were fixed with 2.5% gluteraldehyde/4% paraformaldehyde in 0.1M cacodylate buffer for 2 h. The samples were processed and thin sections (90-nm) were cut on a Reichert Ultracut E microtome. Sections were viewed at 80 kV with a JEOL 1200EX transmission electron microscope. Micrographs were taken in the Philips CM12 (15–20 per sample) by random sampling with magnification form X3000–X20000. Mitochondria numbers were counted for 25 cells, averaged, and expressed as the number of mitochondria per nucleus.

Adipocyte Differentiation of Primary MEFs and Lipid Staining. The MEFs were prepared from 13.5-day *atg7*+/+ and *atg7*−/− embryos according to standard protocol. The primary MEF cells of passages three through five were treated under standard protocol to induce adipocyte differentiation. Briefly, cells were seeded in six-well plates with cover slips and propagated to confluence. Forty-eight hours later, which was designated as Day 0, differentiation was initiated using DMEM containing 10% FBS, 5 μg/mL insulin, 1 μM dexamethasone, 0.5 mM 3-Isobutyl-1-methylxanthine (IBMX), and 10 μM troglitazone (Sigma). Two days after initiation, the medium was replaced with a maintenance medium (DMEM containing 10% FBS, 5 μg/mL insulin, and 10 μM troglitazone). Fresh maintenance medium was replaced every 2 days thereafter. Live cells were observed under an Olympus IX70 microscope with relief contrast objectives (magnification, ×10) and phase contrast objectives (magnification, ×10).

The differentiating cells were fixed with phosphate buffered formalin and stained with 0.01 mg/mL BODIPY 493/503 for 15 min (Invitrogen). Stained cells were observed with a Universal Microscope Axioplan 2 imaging system (Carl Zeiss) with 20X phase contrast objectives. Images were processed using Image J software (National Institutes of Health, Bethesda, MD, US). Oil Red-O (Sigma) staining was performed with method described in ref. 17. To quantify staining, Oil Red-O was extracted from cells on the slides with isopropanol containing 4% Nonidet P-40, and optical density (OD) was then measured at a wavelength of 520 nm.

Lipolysis Assay. For lipolysis assay in vitro, primary white adipocytes were isolated and cultured. Briefly, epididymal fat pads from the control and conditional knockout mice were removed, minced, and digested in KRBH buffer (Krebs-Ringer-bicarbonate HEPES buffer, pH 7.4) containing fatty acid free BSA (10 mg/mL, Sigma), glucose (2.5 mM), and collagenase II (1 mg/mL, Sigma) at 37 °C for 1 h. The digested mixtures were filtered through 300-mm nylon and the suspended primary white adipocytes were collected after centrifuge at 280 × *g* for 1 min. Adipocytes were washed three times with KRBH buffer and counted. With/without 10 μM isoproterenol treatment, 5×10^5 cells were cultured in KRBH buffer containing fatty acid free BSA (20 mg/mL) and glucose (2.5 mM) for 2 h before the media were collected and subjected to FFA and glycerol measurement.

FFA Oxidation Measurement. FFA oxidation was measured in primary white adipocytes as described previously with some modification. Equal number of freshly isolated and cultured primary white adipocytes (5×10^5) were incubated in 50-mL polypropylene tubes in 1 mL KRBH buffer containing fatty acid free BSA (20 mg/mL), glucose (2.5 mM), oleic acid (0.4 mM, Sigma), and 1.0 μCi of 1-^{14}C oleic acid (GE Healthcare). A 1.5-mL centrifuge tube was placed in the center of the 50-mL tube to hold a loosely folded filter paper soaked with hyamine hydroxide (PerkinElmer). After incubation for 3 h, 0.5 mL of 4 M H_2SO_4 was added to the tubes and the tubes were maintained sealed for additional 30 min before the filter papers were carefully removed and the $^{14}CO_2$ it trapped was measure by scintillation counter.

Quantitative RT-PCR. RNA was extracted from tissue samples using TRIzol reagent (Invitrogen). Subsequent RNA quality assessment, cDNA amplification, and quantitative RT-PCR reactions were carried out by the Bionomics Research and Technology center (BRTC) of the Environmental and Occupational Health Science Institute (EOSHI) at Rutgers University, Piscataway, NJ according to standard institutional protocols (http://www.eohsi-brtc.com/frontend/protocols/protocols.php). Seven genes relevant to adipocyte differentiation, glycerol-3-phosphate acyltransferase (*gpam*), CCAAT/enhancer binding protein, alpha (*cebpa*), peroxisome proliferator activated receptor gamma (*pparg*), fatty acid binding protein 4 (*fabp4*), uncoupling protein 1 (*ucp1*), 1-acylglycerol-3-phosphate O-acyltransferase 2 (*agpat2*), and perilipin (*plin*), and two control genes, glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and *β-actin*, were selected for quantitative real-time PCR analysis. Gene expression was examined using Taqman chemistry with probes and primers designed using the Roche Universal Probe Library design algorithm (www.universalprobelibrary.com). Probe sequences were as follows: *gapm* forward, GAGGCAAGGACATTTATGTGG and reverse, GGTGCTTTCACAATCACTCG; *cebpa* forward, CTGGCTCTGGGTCTGGAA and reverse, AGCCACAGGGGTGTGTGTA; *pparg* forward, CTCTCAGCTGTTCGCCAAG and reverse, CACGTGCTCTGTGACGATCT; *fabp4* forward, GCACGGTCTCTCTGCAATC and reverse, ACAATCAATCAGCGCAGGA; *agpat2* forward, TTCCCACCTCAAGCCTGT and reverse, TGCCTTGTGGTCTTGTGG; and *plin* forward, CTCCGGCCTTTCCTCTCTA and reverse, GGGGGAGTGATGACATGG. Standard normalizer gene probes were obtained from Applied Biosystems for *gapdh* and *β-actin* genes. The following dye combinations for probe generation were used for detection and

data normalization: FAM (for the genes of interest), HEX (for normalizer genes, see below) and BHQ1 (non-fluorescent quencher) and ROX (reference).

An additional three brown fat cell marker genes, cell death-inducing DFFA-like effector a (*cidea*), elongation of very long chain fatty acids like 3 (*elovl3*), PR domain-containing protein 16 (*prdm16*), and cell death-inducing DFFA-like effector protein C (*fsp27*, a.k.a. *cidec*) were chosen for secondary quantitative real-time PCR analysis. Two genes relevant to lypolysis, hormone-sensitive lipase (*lipe*) and adipose triglyceride lipase (*atgl*, a.k.a. *pnpla2*) were selected for secondary quantitative real-time PCR analysis. All primers and probe sets were obtained from Applied Biosystems TaqMan Copy Number Assay catalog. The catalog numbers are as follows: *cidea* (Mm00432554_m1), *cidec* (FSP27, Mm00617672_m1), *elovl3* (Mm00468164_m1), *prdm16* (Mm00712556_m1), *lipe* (*hsl*, Mm00495359_m1), *pnpla2* (*atl*, Mm00503040_m1). Relative quantification using the comparative threshold cycle (C_T) method was performed as noted above.

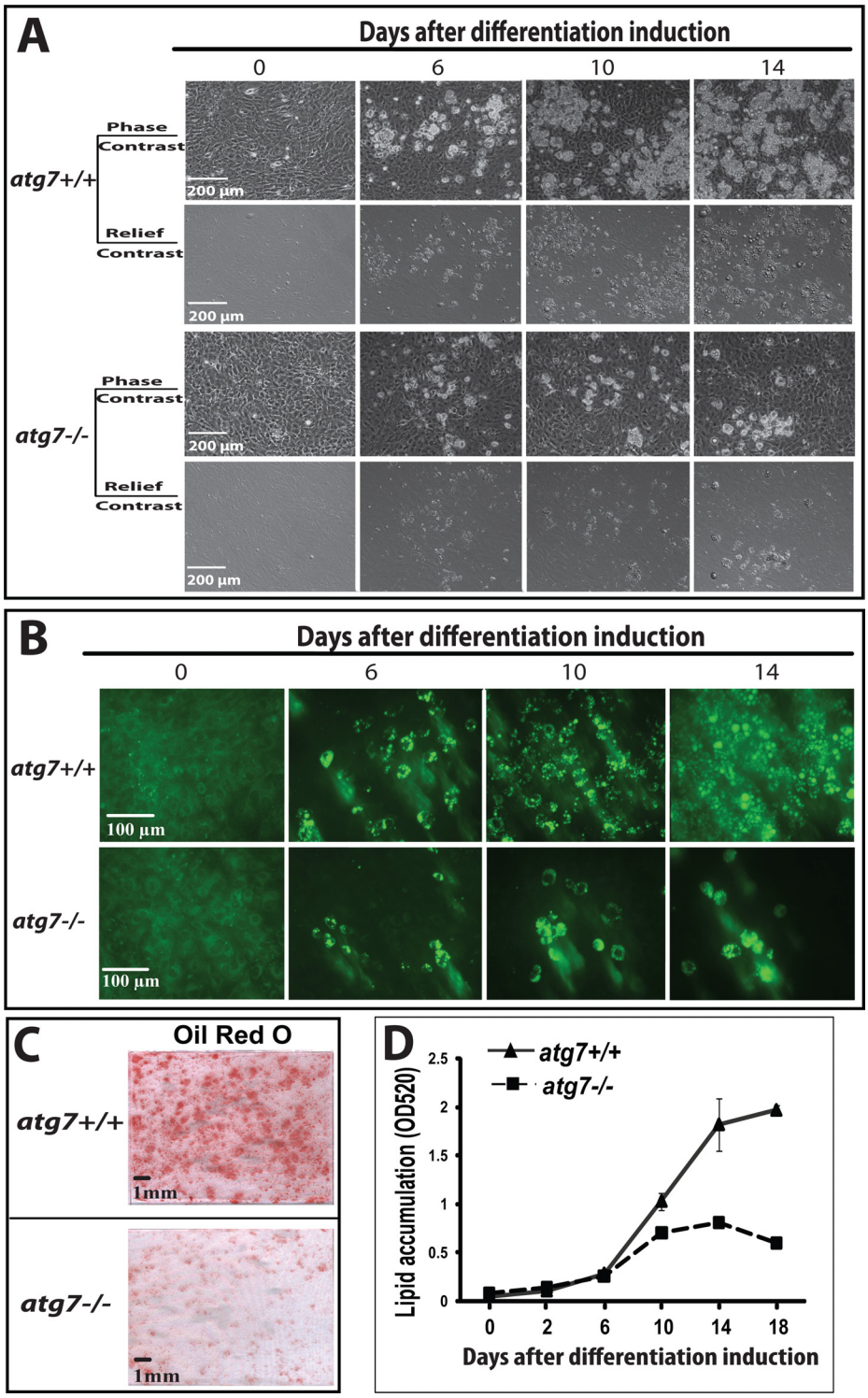

Fig. S1. Autophagy deficient primary *atg7*−/− MEFs exhibited reduced efficiency in adipogenesis. Primary *atg7*+/+ or *atg7*−/− MEFs were induced for adipogenesis. At indicated time points, the progress of differentiation was observed and analyzed. (*A*) Cells were observed under a microscope equipped with relief contrast (magnification, ×10) and phase contrast (magnification, ×10) objectives. The differentiation of the *atg7*−/− MEFs exhibited a drastically reduced efficiency as compared to the wild-type cells. (*B*) Cells were stained with the lipid dye Bodipy 493/503, a fluorescent dye that specifically stains intracellular lipid droplets, and observed with microscopy under phase contrast objectives (magnification, ×20). (*C*) Fourteen days after differentiation inductions, cells grown on cover slips were stained with the lipid dye Oil Red-O, another dye that specifically stains the lipid droplets, and scanned. (*D*) Cells grown on cover slips were stained with Oil Red-O at indicated time points and Oil Red-O was extracted and measured by spectrometry. These data represent results from experiments with cells derived from two pairs of embryos.

Table S1. Quantitative RT-PCR analysis of the gene expression in WAT of control and adipocyte-specific *atg7* knockout mice

Gene	Expression relative to *gapdh* mRNA		
	Control	Knockout	*P* value*
agpat2	0.5251 ± 0.2712	0.5288 ± 0.2052	0.490678852
cebpa	5.3809 ± 1.8286	4.6604 ± 1.0457	0.233191289
fabp4	0.1016 ± 0.0497	0.1005 ± 0.0767	0.489521209
gpam	0.0077 ± 0.0034	0.0082 ± 0.0026	0.4050495
plin	0.0079 ± 0.0054	0.0049 ± 0.0022	0.472965439
cidea	0.0659 ± 0.0893	0.0260 ± 0.0448	0.199001276
elovl3	0.0006 ± 0.0008	0.0005 ± 0.0006	0.393783219
prdm16	0.0003 ± 0.0001	0.0002 ± 0.0001	0.200577026
ucp1	Negligible	Negligible	—
lipe	0.5487 ± 0.2689	0.4181 ± 0.1244	0.176623917
pnpla2	0.0562 ± 0.0181	0.0564 ± 0.0272	0.137781604
cidec	0.4887 ± 0.2242	0.6151 ± 0.5582	0.191915253

All results were mean ± SEM from independent experiments on gonadal fat pads from control mice (*atg7flox/flox*, $n = 5$) and *atg7* knockout mice (*atg7flox/flox*; aP2-*Cre*, $n = 5$). All quantitative RT-PCR reactions were run in triplicate with either custom-designed or ″off-the-shelf″ primers and probes.
*Student's *t* test.