Supplementary Materials and Methods

**Bacteria culture and administration.** *B. acidifaciens* (BA) and *B. sartorii* were grown in peptone-yeast-glucose (PYG) broth at 37°C for 48 hr anaerobically with BBL™ GasPak 100™ EZ gas generating container (Becton Dickinson, Sparks, MD). After the bacteria were concentrated by centrifuging for 15 min at 5,000 g, the supernatants were taken and the pellets were suspended with sterile PBS. For therapeutic studies, mice were orally administered BA (5 × 10⁹ CFU /100 μl) or *B. sartorii* (5 × 10⁹ CFU / 100 μl) daily for 10 weeks by oral zonde needle (Fuchigami, Kyoto, Japan). The actual bacterial dose given was confirmed by plating serial dilutions onto Eggerth-Gangon (EG) blood agar plates.

**Fecal microbiota transplantation.** Transfer of feces was done as previously described.¹ In brief, fresh feces were harvested from three mice, pooled together, and then thoroughly mashed with sterile PBS containing 0.05% cysteine HCl (Sigma-Aldrich, St. Louis, MO) on 100-μm cell strainers. The resulting suspension after passing through strainers was briefly centrifuged at 100 g to remove large aggregates and then administered daily for 18 weeks to mice by needleless intubation tool.

**Co-housing (CH) experiments.** CH was done as previously described.² In brief, sibling *Atg7^ΔCD11c* or *Atg7^ff* mice were weaned together in the same cage and genotyped at age 4 weeks in order to match the number of conditional knockout mice and littermate control mice.

**Food consumption.** Food intake was calculated as previously described ². In brief, food weight was measured for *Atg7^ΔCD11c* and *Atg7^ff* mice and for PBS-, BA-, and *B. sartorii*-fed mice. Mice were given normal chow diet (10% fat, Cat #D12450B; Research Diets, New York, NY).
Brunswick, NJ) or high-fat diet (60% fat, Cat #D12492; Research Diets). Successive food weights were calculated with the formula: \([(\text{Food weight})_{\text{Day}(N)} - (\text{Food weight})_{\text{Day}(N+1)}]\) and these values were adjusted for the number of mice for 2 weeks. Five mice were housed per cage.

**Magnetic resonance imaging (MRI) analysis.** All MRI experiments were performed at 9.4 T / 160 mm by MRI scanner (Agilent Technologies, Santa Clara, CA) using a millipede-shaped volume radiofrequency coil. All animals were anesthetized through a mask by spontaneous inhalation of 1.5 ~ 2% isoflurane. Shimming was performed to minimize B0 inhomogeneity prior to MR scanning both automatically and manually. The axial T1-weighted (T1-WI) fast spin echo images was used to cover both kidneys completely. The parameters of T1-WI image were TR = 1100 msec, kzero = 1, echo spacing (ESP) = 9.82 msec (effective TE = 48 msec), 48 segments, echo train length (ETL) = 4, 4 average, matrix = 192 × 192, field of view (FOV) = 25 × 30 mm, slice thickness = 1.0 mm; and total scan time = 3 min 33 sec. During MR scanning, external triggering was used to eliminate respiratory motion artifacts.

**MRI data analysis.** Image J software (US National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/ij/) was used for segmentation and measurement of MR image compartments. We chose two representative MRI sections at the center of kidney level and at the kidney low pole. Then, the regions of interest were manually drawn to encompass the entire abdomen in order to calculate the total abdominal area and to encompass the peritoneal cavity for calculation of a visceral fat area. Contours of the visceral fat area were then generated semi-automatically based on the threshold of signal intensity to select fatty tissue. If there was a non-fat component within the contours, we manually removed those
components.

**Glucose tolerance test (GTT) and insulin tolerance test (ITT).** GTT and ITT were done as previously described.³ In brief, for GTT, mice that had fasted 16 hr were injected intraperitoneally (i.p.) with 2 mg of glucose per gram of body weight. For ITT, after 6 hr of fasting, mice were injected i.p. with 0.75 U of insulin. The concentration of glucose in the plasma was monitored at 0, 15, 30, 60, 90, and 120 min after injection of glucose or insulin.

**FISH analysis.** Localization of BA in gut mucosa was determined by *FISH* (*fluorescence in situ* hybridization) as previously described.⁴ In brief, the distal region of large intestines were isolated and fixed with 4% formaldehyde and dehydrated with 15% and 30% sucrose in PBS consecutively. Then dehydrated tissues were embedded in OCT compound (Sakura Finetek, Tokyo, Japan), frozen, sliced into 5-µm sections, and dried thoroughly. **We cut approximately 10 sections from the distal colon of each mouse and total mice numbers are five mice per each group.** For the quantitation of BA in the feces, we obtained feces from individual mice after BA feeding and suspended the feces in sterile PBS. Then, we dropped 100 µl of suspension onto a slide glass and fixed the specimen. Hybridization buffer containing 5 ng of oligonucleotide probe µl⁻¹ (Bacid2 [5’-AACATGTTTCCACATTATTCAGG-3’]) was applied to the slide and incubated at 50°C for 2 hr. Oligonucleotide probes labeled with fluorescein were synthesized by Bioneer Corporation (Daejeon, Korea). The slides were rinsed with washing buffer at 50°C for 10 min. **After staining with probes, we photographed more than 20 areas of each slide and counted the positively stained cells. No cells were stained in the positive control group, which had no probes in the reagents.** After mounting with PermaFluor (Thermo Scientific, Fremont, CA), slides were viewed under a confocal laser microscope (Zeiss, Göttingen, Germany).
**Cytokine levels in serum.** Cytokine levels in serum were measured by cytometric bead array–mouse inflammation kit (BD Biosciences, San Jose, CA) according to the manufacturer’s instructions.

**Histology.** Visceral adipose tissues and intestines were washed with PBS and fixed in 4% formaldehyde for 1 hr at 4°C. Tissues were dehydrated by gradually soaking in alcohol and xylene and then embedded in paraffin. The paraffin-embedded specimens were cut into 5-µm sections, stained with hematoxylin-eosin, and viewed by digital light microscope (Olympus, Tokyo, Japan). Adipocyte size was determined by *i*-Solution™ program (IMT *i*-Solution, Burnaby, Canada).

**Real-time PCR for tissues.** Tissue RNA was extracted using TRIZol® (Invitrogen, Carlsbad, CA), and total RNA (0.5 µg) was reverse-transcribed into cDNA according to the manufacturer’s instructions. All signal mRNA was normalized to GAPDH mRNA. Specific primer sets are listed in supplementary table 2. All reactions were performed in the same manner: 95°C for 10 sec, followed by 45 cycles of 95°C for 15 sec and 60°C for 1 min. The results were analyzed with real-time system AB 7900HT software (Life Technologies, Waltham, MA), and all values were normalized to the levels of GAPDH.

**Analysis of metabolic parameters.** Serum glucose, total cholesterol, and triglyceride concentrations were determined using commercially available enzymatic assay kits (Asan Pharmacology, Seoul, Korea). Serum insulin levels were measured with an ultra-sensitive mouse insulin ELISA kit (Shibayagi, Gunma, Japan).
**Hyperinsulinemic-euglycemic CLAMP.** Hepatic and peripheral insulin sensitivity was determined by a hyperinsulinemic-euglycemic clamp as previously described. In brief, at $t = 0$ min, a primed-continuous intravenous infusion of $[3^{-3}H]$-glucose ($1 \mu$Ci bolus, $0.1 \mu$Ci / min; PerkinElmer, Waltham, MA) was initiated and maintained throughout. A pancreatic euglycemic clamp began at $t = 60$ min and lasted until 150 min, during which somatostatin (SRIF; $8.3 \mu$g / kg / min) and insulin ($3 mU / kg / min$) were infused concurrently to suppress endogenous insulin and glucagon secretions and maintain basal insulin concentrations, respectively. An infusion of 10% glucose solution was also started and periodically adjusted to maintain plasma glucose concentration. Samples for determination of $[3^{-3}H]$-glucose-specific activity were obtained at 10 min intervals.

**Comprehensive laboratory animal monitoring system (CLAMS).** Individually, 11- to 12-week-old BA-fed mice and controls were placed in CLAMS (Columbus Instruments, Columbus, OH) cages and monitored over a 5-day period. The hourly file displays all measurements for each parameter: volume of oxygen consumed ($V_{O_2}$; ml/kg/h), volume of carbon dioxide produced ($V_{CO_2}$; ml/kg/h), respiratory exchange ratio, heat (kcal/h), activity (XY total-, XY ambulatory-, and Z activity). The data were recorded during 30-sec sampling periods.

**Bacterial survival assays.** Bone marrow-derived dendritic cells were differentiated for 6–8 days and CD11c+ cells were sorted out using MACS® separation columns (Miltenyi Biotec, Bergisch Gladbach, Germany). After co-incubation of CD11c+ cells with BA for 6 or 24 hr, cells were lysed with 0.5 ml of 0.2% Triton X-100. After vigorous pipetting, the plates were incubated at room temperature for 10 min until the cells were lysed. Bacteria released into the supernatant after cell lysis was serially diluted in PYG broth, and 100 µl of several
dilutions was plated onto EG agar plates. After anaerobic incubation at 37°C for 48 hr, the number of colonies was counted and the total number of bacteria recovered from wells was calculated.
References


Supplementary Table 1. Mean alpha diversity indices for *Atg7*<sup>f/f</sup> and *Atg7*<sup>ΔCD11c</sup> mice from fecal samples. Chao1, Shannon, and Simpson scores were analyzed from fecal samples using taxonomic classifications in terms of alpha-diversity (n=3 mice/group). Statistically significant values are indicated by an asterisk (*P*<0.05) compared to *Atg7*<sup>f/f</sup> group. Data are presented as mean ± s.e.m.