Supporting Information

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SI Materials and Methods

Food Intake, Body Composition, and Oxygen Consumption. For monitoring food intake, we fed mice a high-fat diet (HFD) in individual cages and then weighed their food and pooled the data for 18 wk. Mice were analyzed for body composition by dual-energy X-ray absorptiometry with a LUNAR PixiMus2 scanner (GE Healthcare) after being fed a HFD for 18 wk. Oxygen consumption was measured every 3 min for 24 h in the fasting mice using an O_2/CO_2 metabolism measurement device (model MK-5000; Muromachikikai). After being fed a HFD for 18 wk, each mouse was placed in a sealed chamber (560 mL volume) with an airflow rate of 500 mL/min at room temperature, and cumulative recordings were collected over 36 h. The amount of oxygen consumed was converted to milliliters per minute by multiplying it with the flow rate.

Adipocyte Size. Epididymal white adipose tissue and s.c. fat were routinely processed for paraffin embedding, and 4-μm sections were cut and mounted on silanized slides. The adipose tissue sections were stained with H&E, and the total adipocyte area was manually traced and analyzed using Win ROOF software (Mitani). The white adipocyte area was measured in 200 or more cells per mouse in each group.

Plasma Lipids, Adiponectin, and Leptin. Plasma levels of triglyceride (TG) (Roche Diagnostics) and nonesterified fatty acids (NEFA) (Wako) were analyzed using the corresponding enzyme-based measurement kits. Plasma levels of adiponectin (Otsuka Pharmaceutical) and leptin (R&D Systems) were measured by ELISA.

Limited Feeding to Match the Body Weight of both Genotypes. To match the body weight gain between *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice fed a HFD, we fed them with a limited HFD. Before metabolic studies, mice were fed ad libitum for 1 wk (Fig. S3).

Human Subjects. We acquired s.c. adipose tissue from healthy female donors undergoing liposuction of the abdomen or thighs after obtaining their consent. We examined expression of CD68a in the tissue. We purified total RNA using TRIzol (Invitrogen) and determined relative mRNA levels using real-time PCR. This study was approved by the ethics committee of the University of Tokyo Hospital.

Bone Marrow Transplantation. For bone marrow transplant (BMT) studies, whole bone marrow was prepared from the femur and tibia of female *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice and injected i.v. (5×10^6 cells/recipient) into lethally irradiated 5-wk-old male C57BL/6J mice (9.5 Gy) as recipients. Then C57BL/6J recipients were fed a HFD to promote obesity.

Macrophage Culture. Peritoneal macrophages were harvested from *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice intraperitoneally injected with 1.0 mL of thioglycolate broth 3 d before. Macrophages were cultured in RPMI1640 supplemented with 10% FCS and allowed to adhere to the culture dish for 5–6 h and then rinsed with PBS to remove unattached cells and further cultured overnight. To evaluate the induction of mRNA encoding proinflammatory cytokines, we stimulated cells with 100 ng/mL of LPS for 8 h. To evaluate the effect of phosphoinositide-3 kinase (PI3K) inhibitor on Akt (also known as protein kinase B, PKB) phosphorylation, cells were cultured with inhibitors or DMSO for 30 min before stimulation with monocyte chemotactic protein-1 (MCP-1) (50 ng/mL; R&D Systems) or colony-stimulating factor 1 (CSF-1) (100 ng/mL; Peprotech) for 30 min. Immunoblotting was performed using antibody for Akt, Ser473-phosphorylated Akt, and p110γ subunit (Cell Signaling Technology). For in vitro differentiation to bone marrow-derived macrophages (BMDM), wild-type and *Pik3cg*$^{-/-}$ bone marrow cells were isolated from femurs and tibias and cultured in DMEM supplemented with 10% FCS and 20% L929 conditioned media. To evaluate alternative activation, we stimulated DMEM with recombinant IL-4 (10 ng/mL) for 36 h and quantified the mRNA induction levels of M2 macrophage markers by using the real-time PCR method.

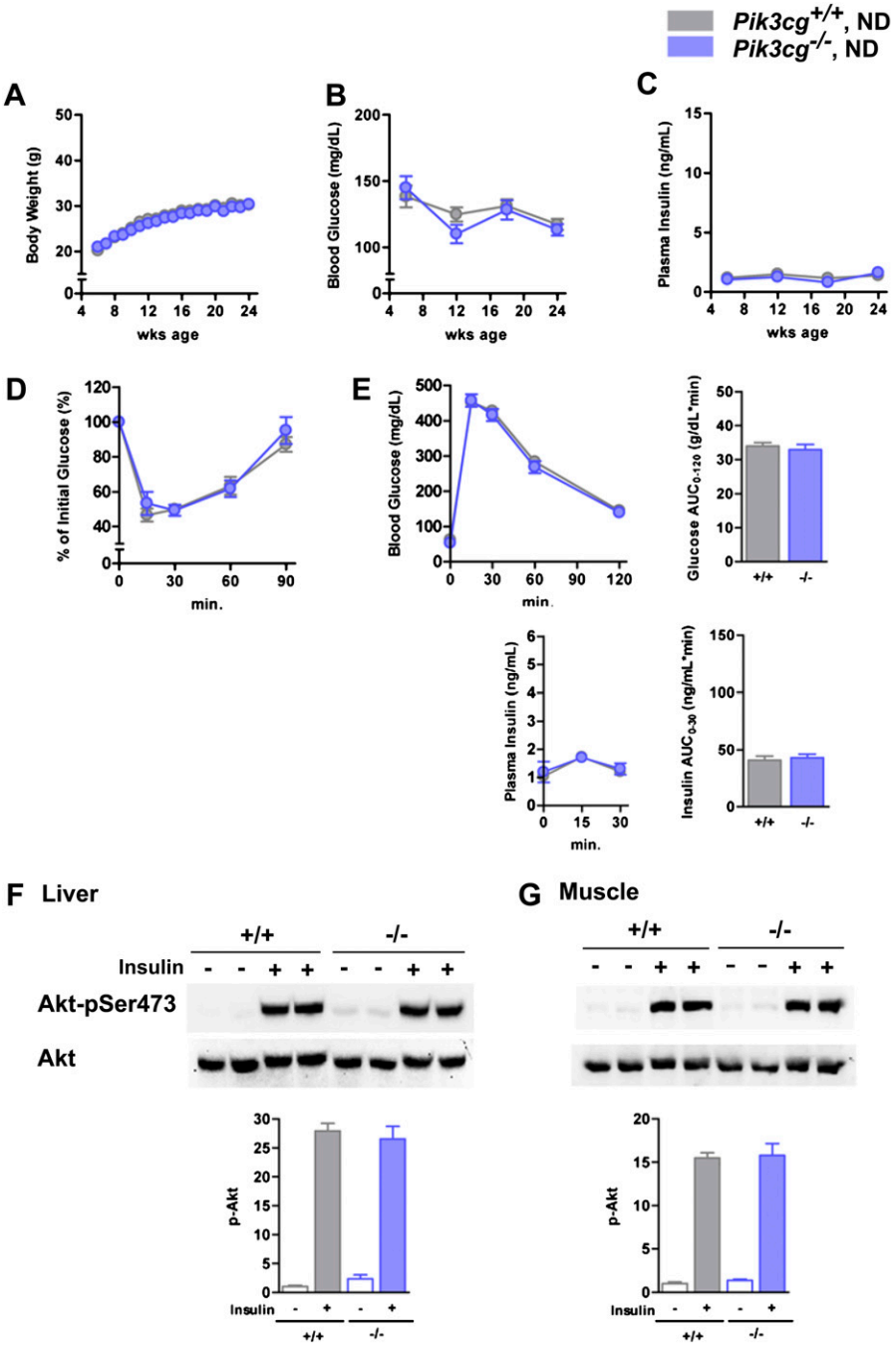

Fig. S1. Deletion of PI3Kγ had no effect on glucose metabolism on normal diet (ND)-fed mice. (*A*–*C*) Time course change of body weight (*A*), blood glucose (*B*), and plasma insulin levels (*C*) in *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice fed ad libitum with a ND up to 24 wk of age (n = 7–8). (*D*) Glucose levels during insulin tolerance test (ITT) in *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice fed a ND were determined at the indicated times after i.p. injection with a bolus of insulin (1.0 U·kg^{-1} BW) (n = 7–8). (*E*) Glucose and insulin levels during glucose tolerance test (GTT) in *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice on a ND were determined at the indicated times after i.p. injection with a bolus of glucose (2.0 g·kg^{-1} BW) (n = 7–8). (*F* and *G*) Phosphorylation of Akt induced by a bolus injection of insulin was assessed in livers (*F*) and skeletal muscles (*G*) of *Pik3cg*$^{+/+}$ ($^{+/+}$) and *Pik3cg*$^{-/-}$ ($^{-/-}$) mice fed a ND (n = 3–4). Light gray, ND-fed *Pik3cg*$^{+/+}$ mice; light blue, ND-fed *Pik3cg*$^{-/-}$ mice. Results are represented as the mean ± SEM.

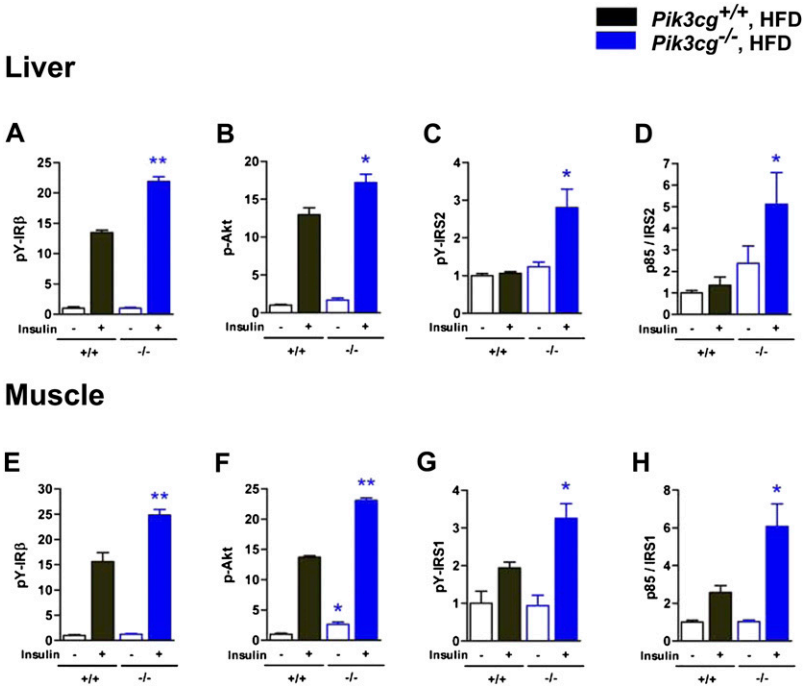

Fig. S2. Enhanced insulin signaling in liver and muscle of *Pik3cg*$^{-/-}$ mice on a HFD. (*A* and *E*) Phosphorylation of insulin receptor β-subunit. (*B* and *F*) Phosphorylation of Akt. (*C* and *G*) Phosphorylation of insulin receptor substrate-1 (IRS1) (*C*) or IRS2 (*G*). (*D* and *H*) p85 associated with IRS1 (*D*) or IRS2 (*H*). Band intensities of phosphorylated proteins from Fig. 1*E* (*A–D*, liver) and from Fig. 1*F* (*E–H*, muscle) were measured. Data were normalized by those from *Pi3cg*$^{+/+}$ mice without insulin (n = 3–4). *$P < 0.05$, **$P < 0.01$.

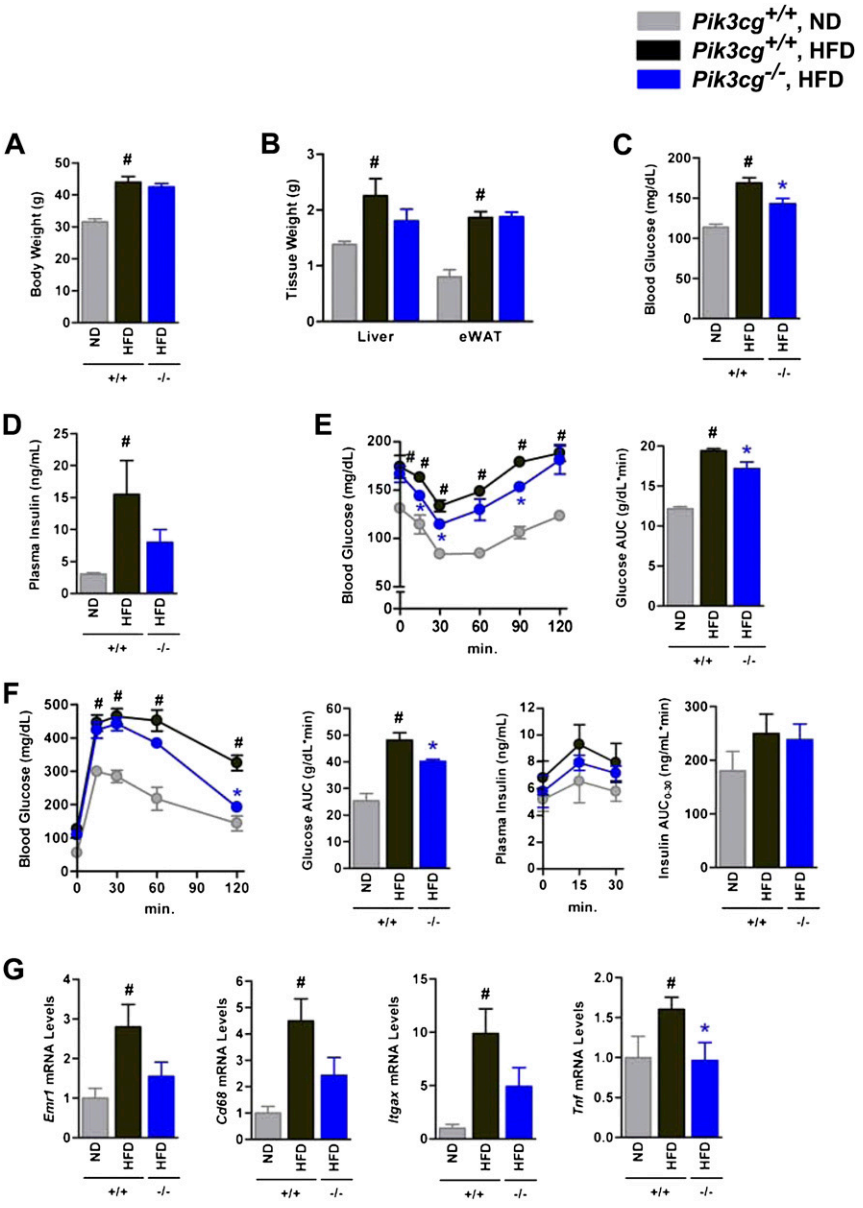

Fig. S3. The improvements of insulin sensitivity and glucose intolerance in *Pik3cg*$^{-/-}$ mice were independent of body weight. *Pik3cg*$^{+/+}$ mice were limitedly fed a HFD to match their weight gain to that of *Pik3cg*$^{-/-}$ mice. (*A*–*D*) Body weight (*A*), liver and epididymal adipose tissue (eWAT) weight (*B*), blood glucose levels (*C*), and plasma insulin levels (*D*) in male *Pik3cg*$^{+/+}$ ($^{+/+}$) and *Pik3cg*$^{-/-}$ ($^{-/-}$) mice fed a ND or a HFD. (*E*) Glucose levels during an ITT in male *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice fed a ND or a HFD were determined at the indicated time points after i.p. injection with a bolus of insulin (1.0 U·kg^{-1} BW). (*F*) Glucose and insulin levels during a GTT in male *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice fed a ND or a HFD were determined at the indicated times after i.p. injection with a bolus of glucose (1.5 g·kg^{-1} BW) (*G*) Abundance of genes encoded macrophage-related protein in eWAT of male *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice fed a ND or a HFD. (*F*) TG content in liver of male *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice on a ND or a HFD. Light gray, ND-fed *Pik3cg*$^{+/+}$ mice; black, HFD-fed weight-matched *Pik3cg*$^{+/+}$ mice; dark blue; HFD-fed *Pik3cg*$^{-/-}$ mice. Results are represented as the mean ± SEM (n = 4–5). #$P < 0.05$ for HFD compared with a ND. *$P < 0.05$ for HFD-fed *Pik3cg*$^{-/-}$ mice compared with HFD-fed weight-matched *Pik3cg*$^{+/+}$ controls.

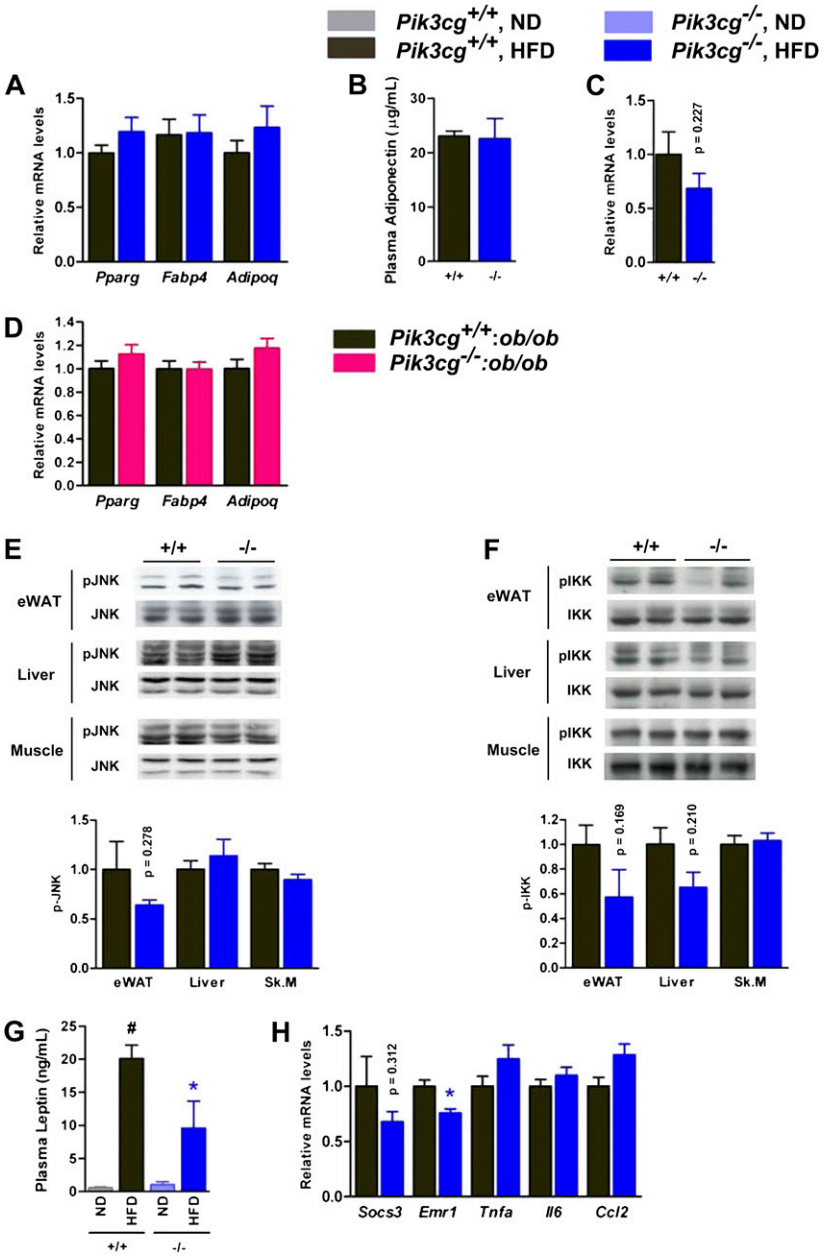

Fig. S4. The genes involved in adipocyte function were not altered by *Pik3cg* deficiency in obese mice. (*A*) mRNA expression levels of genes encoded peroxisome proliferator-activated receptor γ (*Pparg*), aP2 (*Fabp4*), and adiponectin (*Adipoq*) in eWAT of *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice on a HFD. (*B*) Plasma adiponectin levels of *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice fed a HFD. (*C*) *Cd8* expression levels in eWAT of *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice fed on a HFD (n = 7–8). Black, *Pik3cg*$^{+/+}$ mice on a HFD; Dark blue, *Pik3cg*$^{-/-}$ mice on a HFD. (*D*) mRNA expression levels of *Pparg*, *Fabp4*, and *Adipoq* in eWAT of *Pik3cg*$^{+/+}$*:ob/ob* and *Pik3cg*$^{-/-}$*:ob/ob* mice (n = 7–8). (*E* and *G*) Phosphorylation levels of c-jun N-terminal kinase (*E*) or IκB kinase (*G*) were assessed in eWAT, liver, and skeletal muscle of *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice fed on a HFD. (*H*) Plasma leptin levels of *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice fed on a ND or a HFD (n = 7–8). (*I*) mRNA expression levels of genes encoding F4/80 (*Emr1*), SOCS3 (*Socs3*), TNFα (*Tnf*), IL-6 (*Il6*), and MCP-1 (*Ccl2*) in hypothalamus of *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice fed on a HFD (n = 7–8). Light gray, ND-fed *Pik3cg*$^{+/+}$ mice; light blue, HFD-fed *Pik3cg*$^{-/-}$ mice; black, HFD-fed *Pik3cg*$^{+/+}$ mice; dark blue, HFD-fed *Pik3cg*$^{-/-}$ mice (*A*, *B*, *C*, *E*, *F*, *G*, and *H*); black, *Pik3cg*$^{+/+}$*:ob/ob* mice; pink, *Pik3cg*$^{-/-}$*:ob/ob* mice (*D*). Results are represented as the mean ± SEM. #$P < 0.05$ for HFD compared with ND. *$P < 0.05$ for *Pik3cg*$^{-/-}$ mice compared with *Pik3cg*$^{+/+}$.

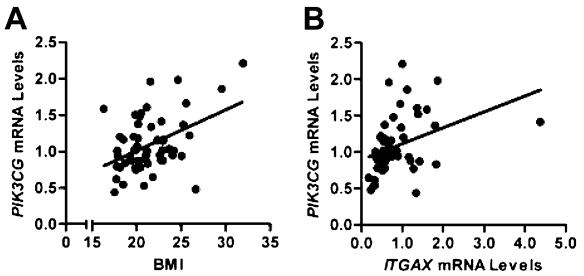

Fig. S5. *PIK3CG* expression in human s.c. adipose tissue. mRNA expression levels in s.c. adipose tissue surgically obtained from 54 human subjects were analyzed. (*A*) Correlation between the *PIK3CG* expression level and body mass index (BMI) (r = 0.44 by Pearson's correlation coefficient test; P = 0.0009). (*B*) Correlation between the *PIK3CG* and *ITGAX* expression level (r = 0.35, P = 0.0087). Each dot represents an individual donor (n = 54).

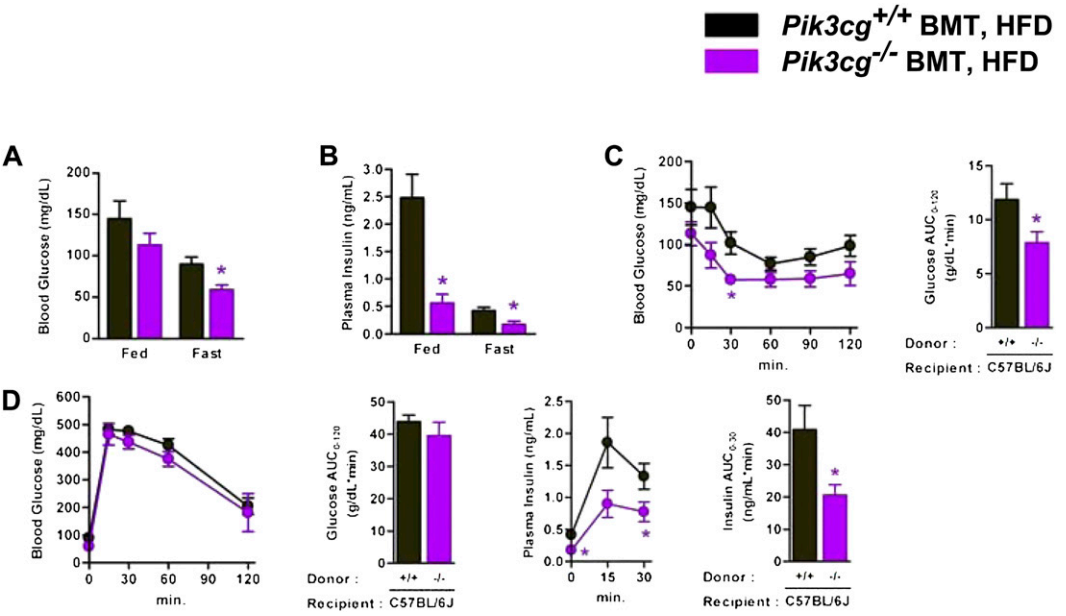

Fig. S6. Bone marrow-specific deletion of PI3Kγ ameliorated HFD-induced insulin resistance. Bone marrow-specific PI3Kγ knockout mice (*Pik3cg*$^{-/-}$ BMT) were generated by BMT and then fed a HFD for 24 wk. (*A* and *B*) Blood glucose (*A*) and plasma insulin levels (*B*) in HFD-fed *Pik3cg*$^{+/+}$ BMT or *Pik3cg*$^{-/-}$ BMT mice. (*C*) Glucose levels during an ITT in HFD-fed *Pik3cg*$^{+/+}$ BMT or *Pik3cg*$^{-/-}$ BMT mice were determined at the indicated time points after i.p. injection with a bolus of insulin (1.0 U·kg^{-1} BW). (*D*) Glucose and insulin levels during GTT in HFD-fed *Pik3cg*$^{+/+}$ BMT or *Pik3cg*$^{-/-}$ BMT mice were determined at the indicated time points after i.p. injection with a bolus of glucose (1.5 g·kg^{-1} BW). Black, *Pik3cg*$^{+/+}$ BMT; purple, *Pik3cg*$^{-/-}$ BMT. Results are represented as mean ± SEM (n = 4–7). *P < 0.05.

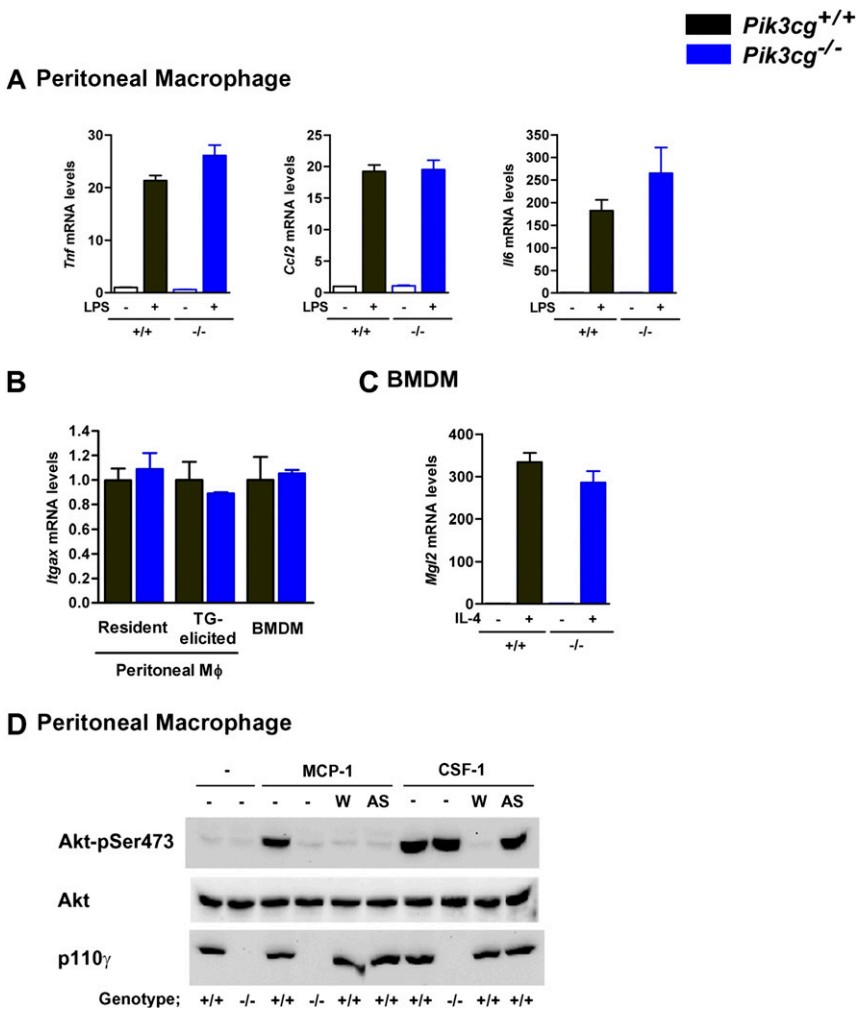

Fig. S7. (*A*) The expression levels of proinflammatory genes induced by LPS in peritoneal macrophage from *Pik3cg*$^{+/+}$ or *Pik3cg*$^{-/-}$ mice. (*B*) mRNA expression levels of *Itgax* (CD11c) in resident or TG-elicited peritoneal macrophage (Mϕ) and in vitro-differentiated BMDM of *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice. (*C*) mRNA expression levels of *Mgl2*, a M2 macrophage marker, induced by IL-4 in BMDM of *Pik3cg*$^{+/+}$ and *Pik3cg*$^{-/-}$ mice. Results are represented as mean ± SEM (n = 3). (*D*) AS-605240 selectively inhibited PI3Kγ signaling. Effect of AS-605240 (AS, 1 μM) or Wortmannin (W, 10 nM) on MCP-1– or CSF-1–mediated Akt phosphorylation in cultured peritoneal macrophages from *Pik3cg*$^{+/+}$ ($^{+/+}$) and *Pik3cg*$^{-/-}$ mice ($^{-/-}$).

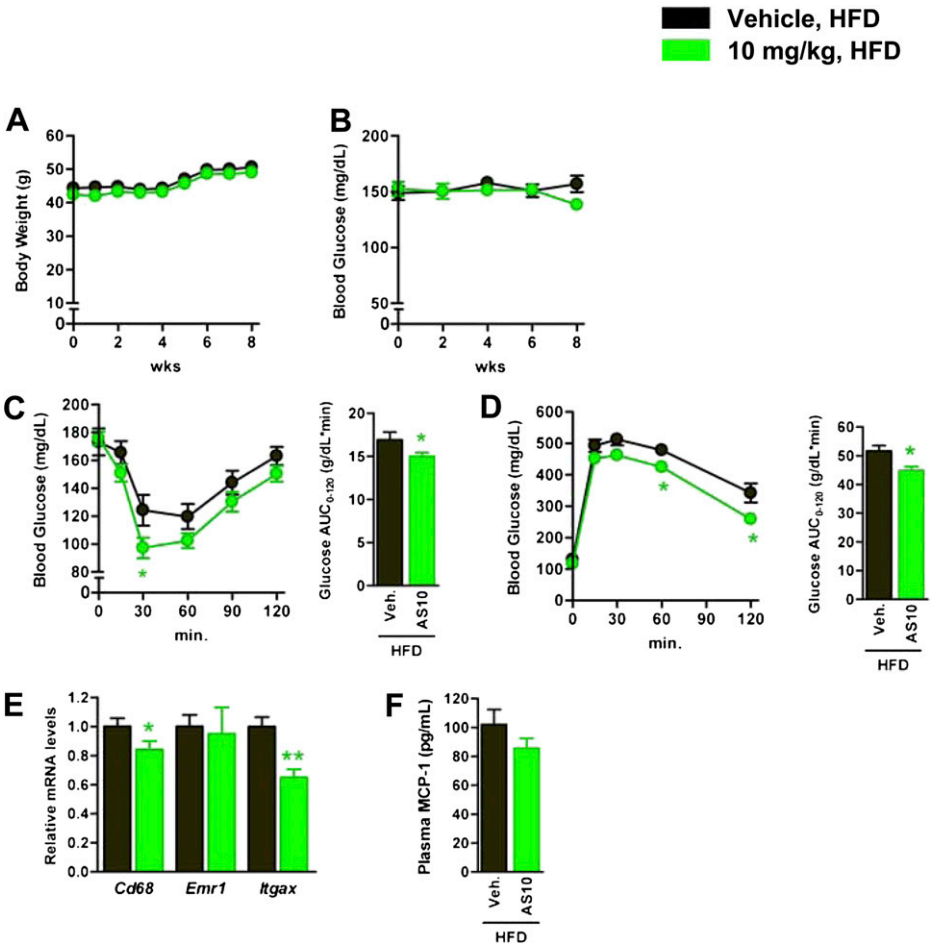

Fig. S8. C57BL/6J mice were fed a HFD for 10 wk and then randomly assigned to two groups and administered either vehicle or 10 mg/kg/d of AS-605240 for another 8 wk and maintained on a HFD. (*A* and *B*) Time-course change of body weight (*A*) and blood glucose levels (*B*) in vehicle- or AS-605240–treated HFD-fed mice. (*C* and *D*) Glucose levels during an ITT (*C*) or a GTT (*D*) in vehicle- or AS-605240–treated HFD-fed mice were determined at the indicated times after i.p. injection with a bolus of insulin (1.0 U·kg^{-1} BW) for an ITT or glucose (1.5 g·kg^{-1} BW) for a GTT. (*E*) An abundance of genes encoded the macrophage-related protein in the eWAT of vehicle- or AS-605240–treated HFD-fed mice. (*F*) Serum MCP-1 levels in vehicle- or AS-605240–treated HFD-fed mice. Black, vehicle treated-HFD-fed mice; green, 10 mg/kg of AS-605240–treated HFD-fed mice. Results are represented as mean ± SEM (n = 7–8). *P < 0.05, **P < 0.01.

Table S1. Gross findings of *Pik3cg*$^{-/-}$ mice on a HFD

	HFD		
	Pik3cg$^{+/+}$	*Pik3cg*$^{-/-}$	*P* value
Body weight (g)	46.0 ± 0.7	37.3 ± 1.9	0.001
Food intake (g/day)	6.1 ± 0.3	5.9 ± 0.3	0.714
Liver weight (g)	2.35 ± 0.30	1.77 ± 0.46	0.318
eWAT weight (g)	1.46 ± 0.07	1.22 ± 0.24	0.339
Adipocyte size (μm^2)	4371.5 ± 379.9	3553.7 ± 350.5	0.148
Fat (%)	40.4 ± 1.2	42.0 ± 0.6	0.405
VO_2 (mL/min/kg)	40.7 ± 0.6	40.4 ± 1.0	0.844
RQ (–)	0.67 ± 0.001	0.67 ± 0.001	0.130
TG (mg/dL)	63.7 ± 7.2	57.9 ± 9.1	0.624
NEFA (mEq/L)	1.11 ± 0.09	0.90 ± 0.11	0.152

Values are represented as means ± SEM (n = 5–10). eWAT, epididymal white adipose tissue; RQ, respiratory quotient.