DETAIL EXPERIMENTAL PROCEDURES

Generation of knockout animals and genotyping

Heterozygous *PIKE*$^{-/-}$ C57BL/6 mice with a targeted deletion of exon 3 to 6 of *CENTG1* were generated under contract by Ozgene (Australia). Genotyping of offspring was performed by PCR using genomic DNA isolated from the tail tip. Tail samples were digested overnight in lysis solution (0.4X SSC, 10mM Tris pH7.5, 1mM EDTA, 1% SDS and 0.5mg/ml protease K). Genomic DNA was than purified by ethanol precipitation. PCR was performed using a combination of primer D (5'ACAGGATCAGTGCATCATCTC3'), H (5'CTGCCCAGCTACAGGAGTAG3'), primer A (5'TCAGTTGACTGGAAGCTCTG3') and C (5'CCAGAGCCTATCTATGCCTAG3').

Immunopreciptiation and Western blotting

Tissue extracts were prepared by homogenizing the tissues in buffer as reported (1). Cell debris was removed by centrifugation and the supernatants were collected. Immunoprecipitation using antibodies as indicated was performed as reported (1). Antibodies used in the Western blot analysis were obtained from SantaCruz Biotechnology, USA (IR, Akt) and Cell Signaling Technology, USA (anti-phosphor-Thr^{308} of Akt and, anti-phosphor-Thr^{172} of AMPK, anti-phosphor-Ser^{79} of ACC, anti-AMPKα and anti-ACC).

Southern blot analysis

Genomic DNA was extracted from mouse tail as described above, digested with *Nhe* I and size separated on an agarose gel followed by capillary transfer to a Hybond N+ nylon membrane (Amersham Biosciences, USA). Hybridization using 50 ng α-^{32}P-dCTP (10mCi/ml, Amersham Biosciences, USA) labeled 5' probe was performed as previously described (2).

Analytical procedures

All animal experiments were performed according to the care of experimental animal guidelines from Emory University. Twelve-week-old female mice were fed with chow or HFD (Research Diets Inc, USA) for 20 weeks. Blood glucose level was measured using whole blood collected from tail vain by ACCU-CHEK Advantage Blood Glucose Meter (F. Hoffmann-La Roche Ltd., Switzerland). Serum insulin was measured by ELISA (Crystal Chem Inc., USA). Serum triglyceride levels were measured by Serum Triglyceride Determination Kit (Sigma-Aldrich, USA). Serum TNFα was measured by ELISA (BD Biosciences, USA). Glucose tolerance tested was performed on female mice fasted for overnight. Blood sample was collected from tail vein immediately before peritoneal injection of 200 µl D-glucose (2g/kg of body weight) or PBS. Blood was sampled at indicated time interval and glucose concentration was measured using ACCU-CHEK Advantage Blood Glucose Meter.

Adipocyte differentiation assay

Adipocyte differentiation assay from MEF was performed under isobutylmethylxanthine/dexamethasone/insulin (MDI) induction as reported (3). MEF were grown to 2 days post-confluence in DMEM with 10% bovine serum. Cells were then induced to differentiate by changing the medium to DMEM containing a standard induction cocktail of 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine, 1 µM dexamethasone, and 1.7 µM insulin. After 48 h, this medium was replaced with DMEM supplemented with 10% FBS and 1.7 µM insulin, and cells were maintained in this medium for additional 48 h. Lipid accumulation was examined by

oil red O staining, followed by extraction of the absorbed dye with 100% isopropanol and measurement of the OD490 as reported (4).

In vivo insulin stimulation

Experiments were performed in female mice fasted for 16 hr. Animals were anesthetized by intraperitoneal administration of sodium pentobarbital (50mg/kg of body weight). Saline or 5U human insulin (Elli Lily, USA) was injected through inferior vena cava. After 5 min, liver, hindlimb muscles and inguinal fat were removed and frozen in liquid nitrogen.

PI3K assay

In vitro PI3K assay were performed using anti-p110α (SantaCruz Biotechnology, USA) as described previously (5).

RT-PCR

Total RNA from was prepared by using Trizol Isolation Reagent (Invitrogen, USA). First-strand cDNA from 5μg total RNA was synthesized using Superscript III reverse transcriptase (Invitrogen, USA) and Oligo-dT17 as primer with recommended procedures. Amplification of Pref-1, aP2, PPARγ, and C\EBPα were performed using the following primers: mPref1-F (5'-GACCCACCCTGTGACCCC-3'), mPref1-R (5'-CAGGCAGCTCGTGCACCCC-3');,maP2-F (5'-CAAAATGTGTGATGCCTTTGTG-3'), maP2-R (5'-CTCTTCCTTTGGCTCATGCC-3'), mPPARg2-F (5'-ATGCTGTTATGGGTGAAACT-3'), mPPARg2-R (5'-CTTGGAGCTTCAGGTCATATTTGTA-3'), mC\EBPa-F (5'-ATCCCAGAGGGACTGGAGTT-3'), mC\EBPa-R (5'-AAGTCTTAGCCGGAGGAAGC-3'). Expression of PIKE-A was determined using primers D and H as described in the genotyping assay. GAPDH fragment was also amplified as internal standard using primers 5'-CGCATCTTCTTGTGCAGTGCC-3' (forward) and 5'-GGCCTTGACTGTGCCGTTGAATTT-3' (reverse). PCR product was quantitated by computer software ImageJ (NIH, USA). A kinetic profile of the amount of PCR product generated at different PCR cycles was constructed and the cycle number used for individual gene expression study was chosen within the exponential region of the amplification curve. This is to ensure that the amount of the PCR product reflects the amount of template in the original sample.

In vitro 2-deoxyglucose uptake

In vitro 2-deoxglucose uptake in soleus muscle and inguinal fat pad were performed in the presence or absence of human insulin (Elli Lily, USA) as reported previously (6). Briefly, soleus muscles and inguinal fat pad were pre-incubated in Krebs-Ringer bicarbonate buffer (KRBB) containing 8 mM glucose for 20 min at 37 °C. The tissues were then transferred to KRBB containing 8 mM glucose, 2 mCi/ml 2-^{3}H-deoxyglucose and 0.3 mCi of ^{14}C-mammitol with or without 10mU/ml insulin for 30 min at 37 °C. After incubation, the tissues were blotted, frozen and dissolved in 1 M KOH. After neutralization by 1 M HCl, the samples were measured by scintillation counting.

Fatty acid oxidation assay

Fatty acid oxidation was measured by determining the production of $^{3}H_2O$ from [9,10-^{3}H]palmitate as reported (7). Briefly, hepatocytes and muscle cells isolated from 3-month-old mice were plated (0.5 X 10^5 cell per well) in a 12-well plate and allowed to growth for 6 days.

On the day of experiment, albumin-bound tritiated palmitate was added and incubated for 2 h at 37 °C. After incubation, the medium was removed and added to a tube containing cold 10% trichoroacetic acid. The supernatant was then neutralized with NaOH, applied to ion-exchange resin to separate the ^3H_2O and counted by scintillation counting.

Hyperinsulinemic-euglycemic clamp and metabolic cage studies.

In vivo glucose metabolisms including glucose infusion rate, glucose turnover rate and glycogen synthesis were determined by hyperinsulinemic-euglycemic clamp as reported previously (8). Metabolic cages studies were performed using the TSE Systems (Bad Hombery, Germany) (9).

Statistical analysis

Results were expressed as mean ± S.E.M. and were considered significant when $P<0.05$. Statistic analysis of the data were performed using either Student's *t*-test, one-way ANOVA or two-way ANOVA followed by Tukey's multiple comparison test or Bonferroni post-tests by the computer program GraphPad Prism (GraphPad Software, USA).

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SUPPLEMENTAL FIGURES

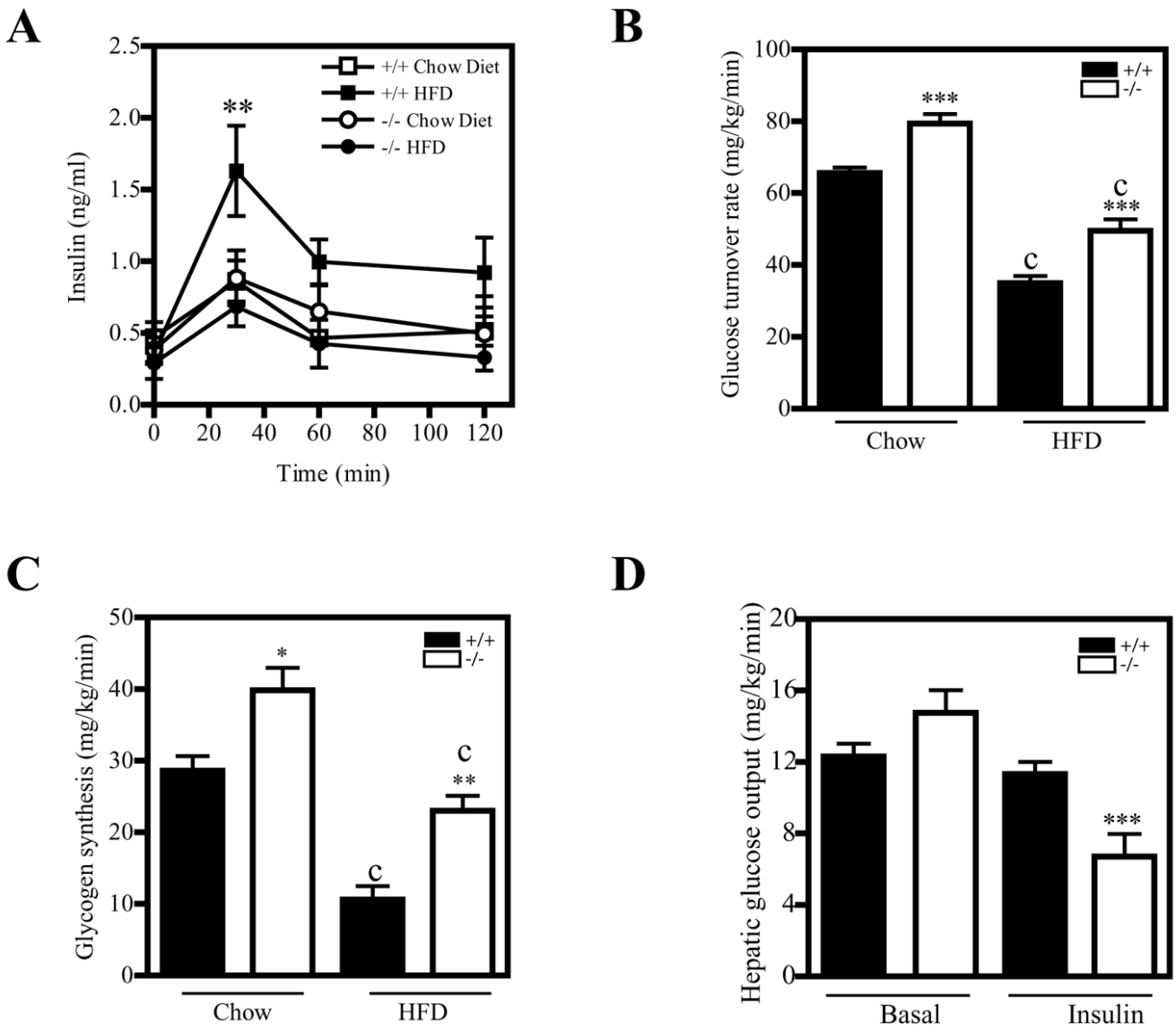
Supplemental Figure 1. Enhanced systemic insulin sensitivity in *PIKE*$^{-/-}$ mice.

(A) Circulating insulin concentration in mice (8 to 9-month-old) that have been fed with chow or HFD for 20 weeks during the glucose tolerance test. Results were expressed as mean ± S.E.M. (n=7; **: P<0.01; vs the same genotype; two-way ANOVA).

(B) Glucose turnover rate in wild-type (+/+) and knockout (-/-) mice (8 to 9-month-old) that have been fed with chow or HFD for 20 weeks during the hyperinsulinemic-euglycemic clamp experiment. Results were expressed as mean ± S.E.M. (n=9; **: P<0.01 vs the same diet; c: P<0.001 vs the same genotype; one-way ANOVA).

(C) Whole body glycogen synthesis in wild-type (+/+) and knockout (-/-) mice (8 to 9-month-old) that have been fed with chow or HFD for 20 weeks during the hyperinsulinemic-euglycemic clamp experiment. Results were expressed as mean ± S.E.M. (n=9; *: P<0.05, **: P<0.01 vs the same diet; c: P<0.001 vs the same genotype; one-way ANOVA).

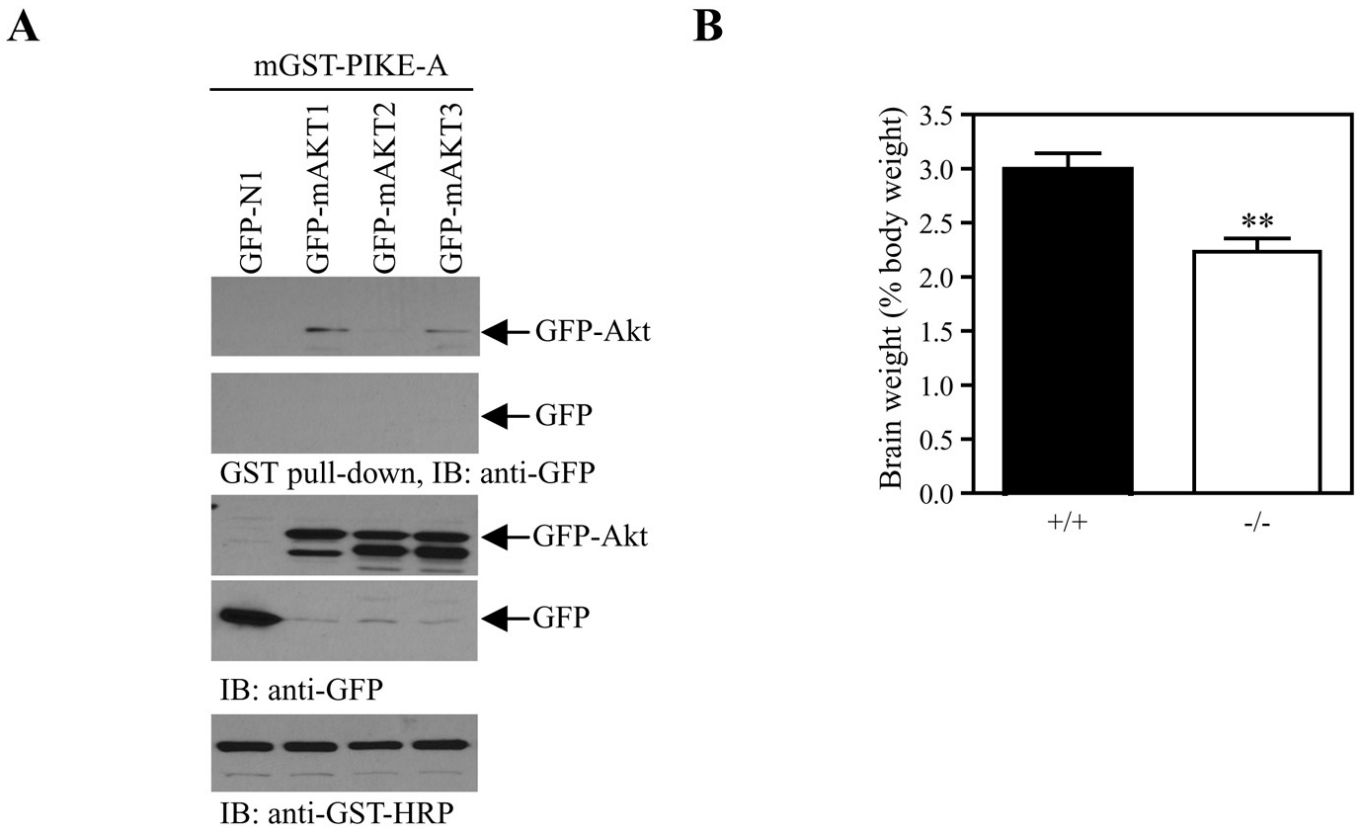
(D) Hepatic glucose output in wild-type (+/+) and knockout (-/-) mice (8 to 9-month-old) that have been fed with HFD for 20 weeks during hyperinsulinemic-euglycemic clamp experiment. Values are the mean ± S.E.M (***: P<0.01 vs the same genotype, One-way ANOVA, n=6).



Supplemental Figure 2 PIKE-A preferentially interacts with Akt1 and 3.

(A) PIKE-A interacts differentially with Akt1 and Akt3. HEK293 cells were co-transfected with mGST-PIKE-A and different GFP-Akt isoforms. The GST proteins were then pull-downed by glutathione beads and the associated Akt proteins were detected by using anti-GFP antibody (1st and 2nd panels). The expression of various GFP-tagged proteins (3rd and 4th panels) and GST-tagged PIKE-A (5th panel) were also examined.

(B) Ratio of brain weight to total body weights. Brain of 3-month-old wild-type (+/+) and *PIKE*$^{-/-}$ (-/-) mice (n=5) were collected, measured and expressed against the corresponding body weight. Results are expressed as mean ± S.E.M (**: P<0.01, Student's t-test).



Supplemental Figure 3 PIKE-A ablation does not alter the AMPK and ACC phosphorylation in hypothalamus and MEF.

(A) Analysis of ACC and AMPK phosphorylations in wild-type (+/+) and knockout (-/-) mice (8 to 9-month-old) that have been fed with chow or HFD for 20 weeks. Hypothalamic extracts were prepared and immunobloted with phosphor-Thr172-AMPK, phosphor-Ser79-ACC, total AMPK and ACC antibodies.

(B) Analysis of ACC and AMPK phosphorylations in wild-type (+/+) and knockout (-/-) MEF. Cell extracts were prepared and immunobloted with phosphor-Thr172-AMPK, phosphor-Ser79-ACC, total AMPK and ACC antibodies.