SUPPLEMENTARY DATA

Perilipin 3 deficiency stimulates thermogenic beige adipocytes through PPARα activation

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Supplementary Figure 1. Generation of the *Plin3* targeting vector and *Plin3* KO mice. The *Plin3* floxed-Neo construct had three major locus modifications. A *loxP* site was introduced into intron 3; a KpnI¨*FRT*-Neo-*FRT*-*LoxP*-SpeI cassette was introduced into intron 6, and a flanking thymydate kinase (*TK*) gene was added downstream of exon 8. The linearized *Plin3*-Fl-Neo vector was electroporated into 129/SvEv ES cells, and ES cell clones with the desired homologous recombination event were identified by PCR and Southern blot hybridization, using the indicated 5' (upstream-KpnI) and 3' (downstream-EcoRV) probes. Positive ES cells were micro-injected into blastocysts derived from C57BL/6J females and transferred into pseudo-pregnant C57BL/6J recipients. Chimeric mice were bred with C57BL/6J mice for germline transmission. The presence of the targeted allele in the agouti-colored offsprings was confirmed by PCR and Southern blot hybridization. These mice were mated with mice expressing Ella-Cre recombinase, and additionally crossed with C57BL/6J to obtain *Plin3* KO mice, which were confirmed for deletion of exons 3, 4, and 5 by Southern blot, PCR and RT-qPCR. The *Plin3* KO mice were backcrossed into C57BL/6J for more than 10 generations prior to experiments. **(A)** The levels of Plin3 proteins in various tissue by western blot analyses. GAPDH was used as a loading control. **(B)** *Plin3*-targeting vector and *Plin3* KO allele. Yellow boxes are non-coding exons and black boxes are coding exons. The Plin3 antibody recognition region is indicated. **(C)** RT-qPCR analysis for *Plin3* gene expression in BAT from WT and *Plin3* KO mice using primers located within the deletion target region (left). Each mRNA level was normalized to cyclophilin mRNA, and mRNA expression levels are relative to WT. Immunoblots for *Plin3* protein in BAT from WT and *Plin3* KO mice (right). GAPDH was used as a loading control. **(D)** Morphology of littermate WT and *Plin3* KO mice at 8 weeks of age. Data represent the mean ± SEM.

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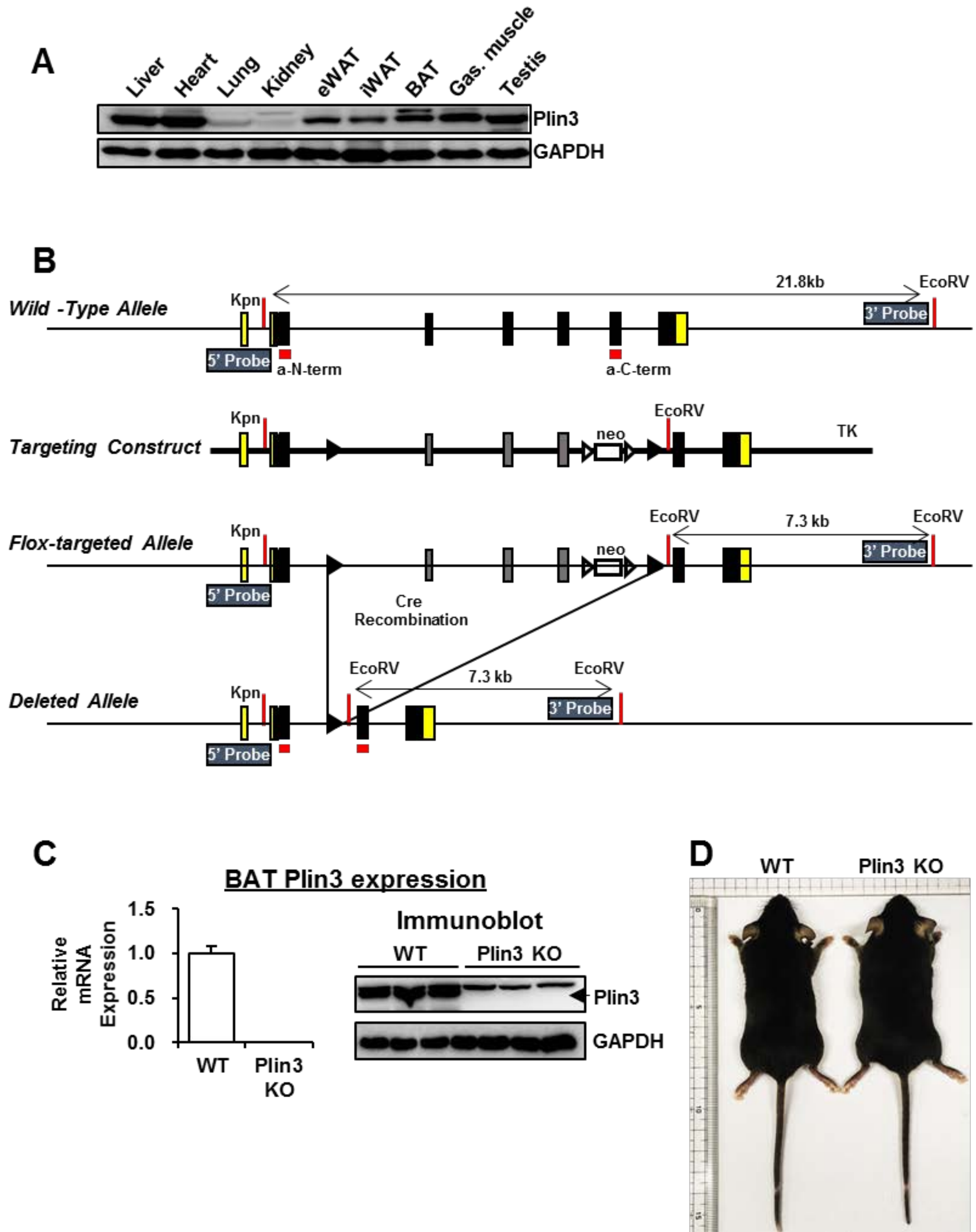

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Supplementary Figure 2. Metabolic phenotypes of *Plin3* KO mice. (A) Body and tissue weights of WT and *Plin3* KO mice exposed to 30°C or 4°C for 6 days (n = 6 mice/group). **(B)** RT-qPCR analysis of beige adipocytes specific gene expression in iWAT from WT and *Plin3* KO mice exposed to 30°C or 4°C (n = 3 mice/group). Each mRNA level was normalized to cyclophilin mRNA, and mRNA expression levels are relative to WT at 30°C. For analysis of rectal temperature by administration of β3-adrenergic receptor agonist CL-316,243, 10-week-old male mice were injected intraperitoneally with CL-316,243 at a dose of 0.5 mg/kg then measured body temperature using rectal probe. Whole-body oxygen consumption was measured using a TSE phenomaster. Briefly, mice were housed singly in TSE phenomaster cages and adapted for 48 h and injected intraperitoneally with a β3-adrenergic receptor-specific agonist CL-316,243 at a dose of 0.5 mg/kg. Data on energy consumption rate (VO_2, VCO_2, RER) were recorded every 7 min for a 1 h with temperature at 22°C. **(C)** Whole body rectal temperature for 30 min after CL-316,243 (0.5 mg/kg) under normal chow diet fed mice (n = 6 mice/group, 3 independent experiment). **(D)** Whole body energy consumption measured by TSE after CL-316,243 (0.5 mg/kg) injection under normal chow diet fed mice (n = 6 mice/group, 2 independent experiment). **(E)** RT-qPCR analysis of thermogenesis, fatty acid oxidation and mitochondrial related gene expression in iWAT from WT and *Plin3* KO mice (n = 3 mice/group). Each mRNA level was normalized to cyclophilin mRNA, and mRNA expression levels are relative to WT controls. **(F)** Whole body energy metabolic phenotypes were measured by TSE under normal chow diet fed mice (n = 6 mice/group, 2 independent experiment). Data represent the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ indicate significant differences between groups as determined by either two-tailed unpaired Student's *t* tests or two-way ANOVA versus control.

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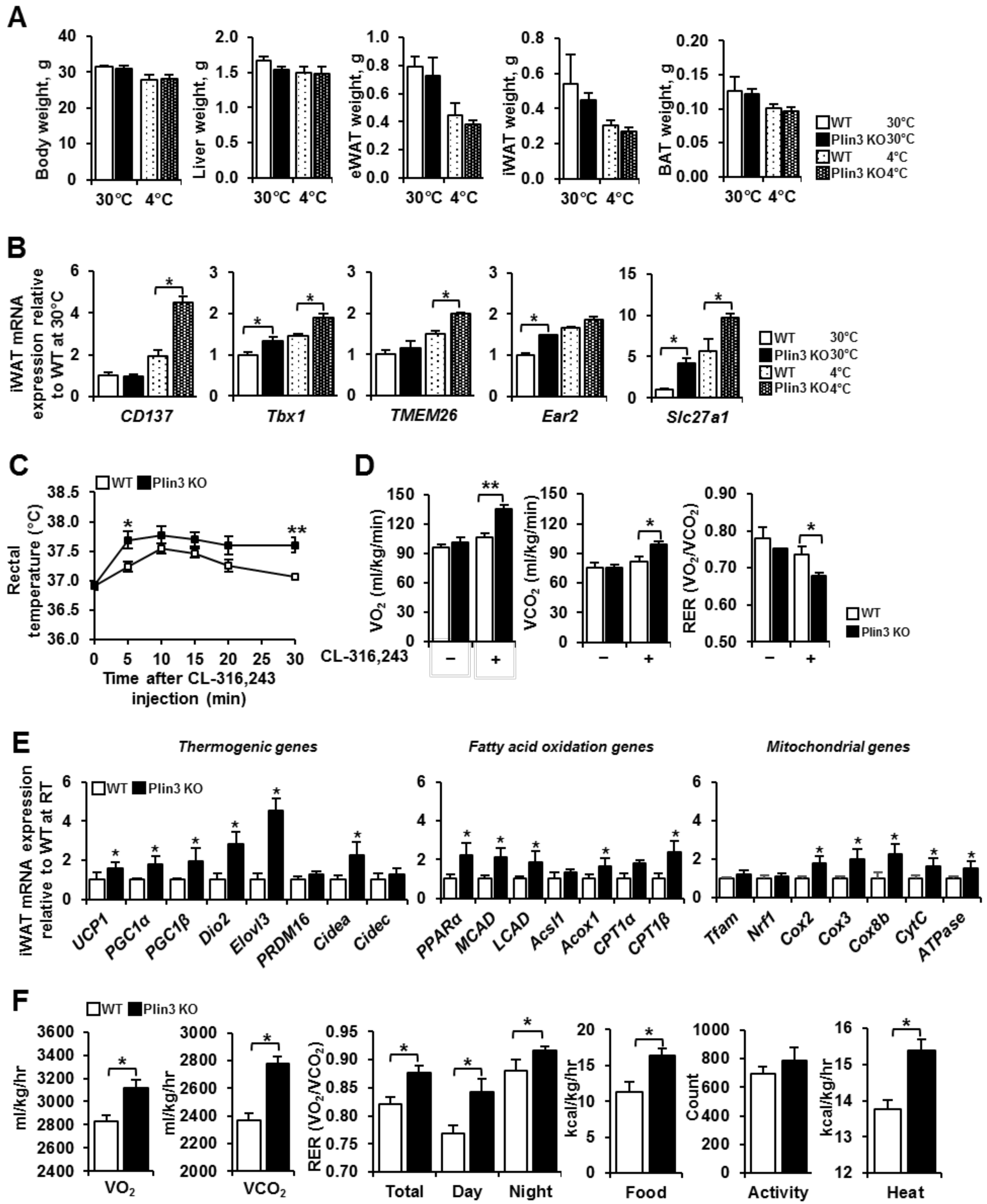

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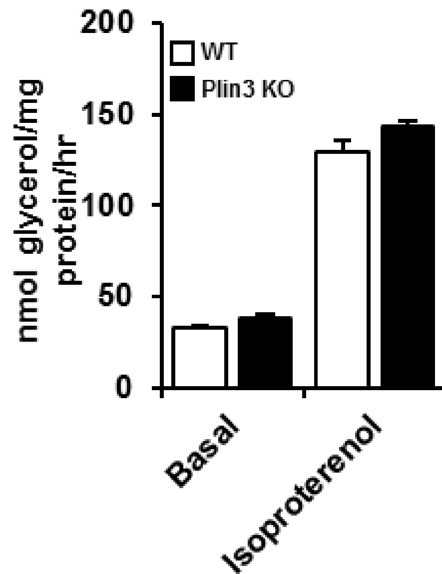
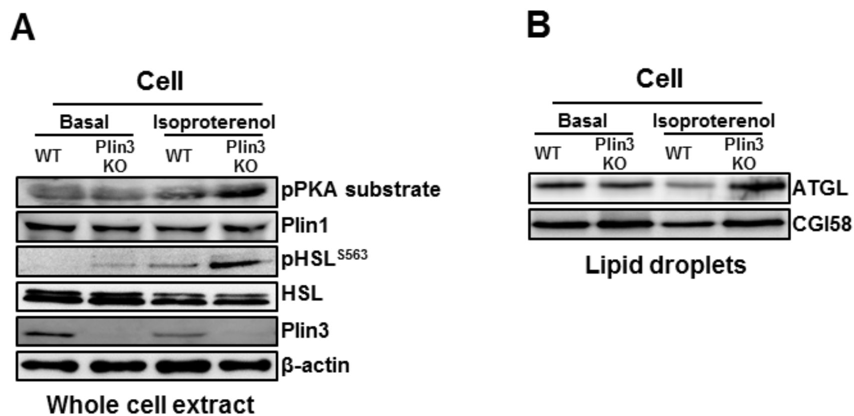
Supplementary Figure 3. Lipolysis in *Plin3* KO brown adipocytes. Stromal vascular cells were isolated from BAT of WT and *Plin3* KO mice and were fully differentiated into adipocytes. For basal and stimulated lipolysis, differentiated adipocytes were treated with or without isoproterenol (1 μM) for 3 h. The levels of glycerol were measured from conditional media. Data represent the mean ± SEM.



Supplementary Figure 4. Lipolytic capacity in *Plin3*-deficient adipocytes. (A) Western blot analysis of lipolytic activity in differentiated adipocytes from WT and *Plin3* KO mice. Differentiated adipocytes were treated with or without isoproterenol (3 μM) for 6 h. β-actin was used as a loading control. **(B)** Immunoblots for lipolytic activity in isolated lipid droplet fraction from differentiated adipocytes of WT and *Plin3* KO mice. Differentiated adipocytes were treated with or without isoproterenol (3 μM) for 6 h.



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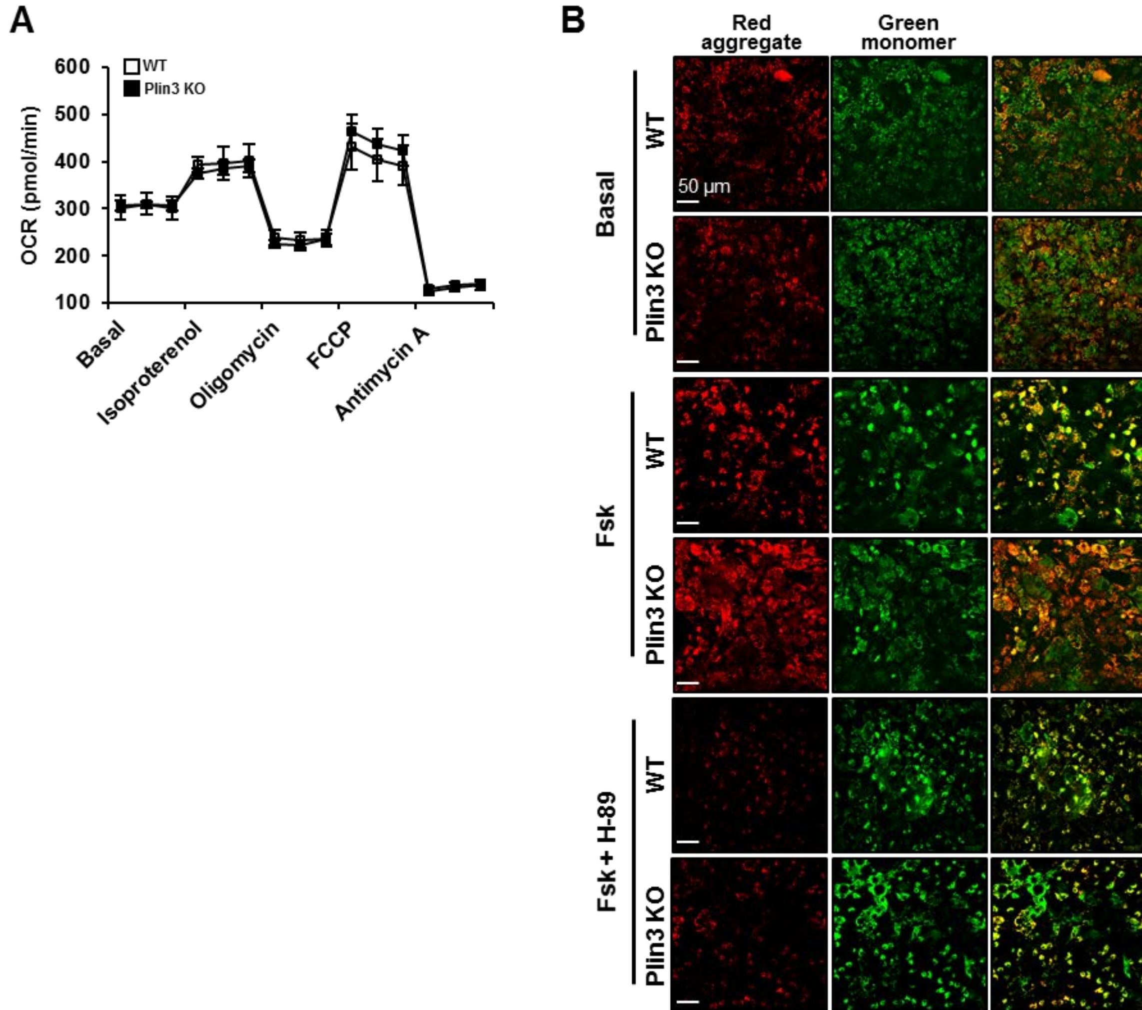
Supplementary Figure 5. Oxygen consumption rate in *Plin3* KO brown adipocytes and mitochondrial activity of *Plin3* KO adipocytes on cAMP/PKA. **(A)** Oxygen consumption rates of differentiated adipocytes from BAT of WT or *Plin3* KO mice. Data represent the mean ± SEM. **(B)** JC-1 using a mitochondrial membrane potential staining in differentiated adipocytes pretreated with or without H-89 (50 μM) followed by forskolin (10 μM) for 3 h. Bars represent 50 μm.



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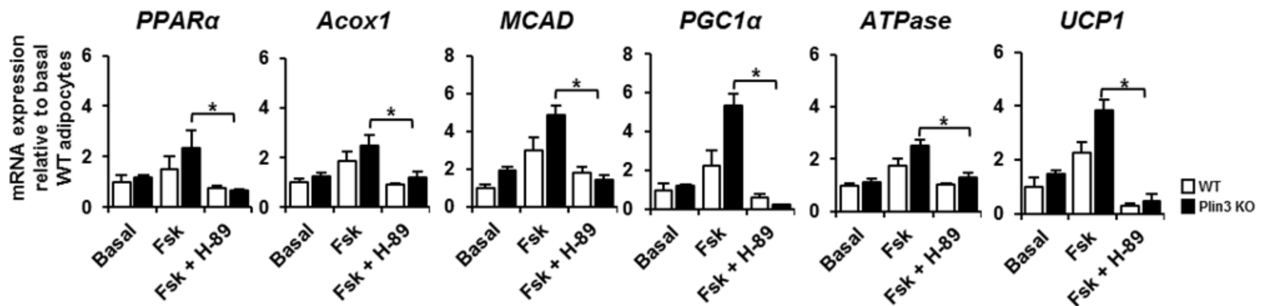
Supplementary Figure 6. PPARα expression of *Plin3* KO adipocytes on cAMP/PKA. RT-qPCR analysis of *PPARα* and target genes in differentiated adipocytes pretreated with or without H-89 (50 µM) followed by forskolin (10 µM) for 3 h. Each mRNA level was normalized to cyclophilin mRNA, and mRNA expression levels are relative to untreated WT controls. Data represent the mean ± SEM. **p* < 0.05 indicates significant differences between *Plin3* KO + Fsk *vs.* WT with Fsk + H-89 and/or *Plin3* KO with Fsk + H-89 as determined by two-tailed unpaired Student's *t* tests.



Supplementary Figure 7. Effect of *Plin3* siRNA in differentiated WT adipocytes. **(A)** RT-qPCR analysis of *Plin3* and *PPARα* in differentiated adipocytes with or without *Plin3* siRNA. Differentiated adipocytes were treated with or without isoproterenol (3 µM) for 8 h. Each mRNA level was normalized to cyclophilin mRNA, and mRNA expression levels are relative to untreated WT controls. **(B)** RT-qPCR analysis of thermogenic marker genes in differentiated adipocytes with or without *Plin3* siRNA in the presence or absence of isoproterenol (3 µM) for 8 h. Each mRNA level was normalized to cyclophilin mRNA, and mRNA expression levels are relative to untreated WT controls. **(C)** Stromal vascular cells were isolated from iWAT of WT and *Plin3* KO mice and were fully differentiated into adipocytes. For basal and stimulated lipolysis, differentiated adipocytes were treated with or without isoproterenol (1 µM) for 3 h. The levels of glycerol were measured from conditional media. **(D)** Oxygen consumption rates of differentiated adipocytes from iWAT of WT or *Plin3* KO mice. Differentiated adipocytes were treated with or without *Plin3* siRNA. Data represent the mean ± SEM. **p* < 0.05, ***p* < 0.01 indicate significant differences between groups as determined by either two-tailed unpaired Student's *t* tests or two-way ANOVA versus control.

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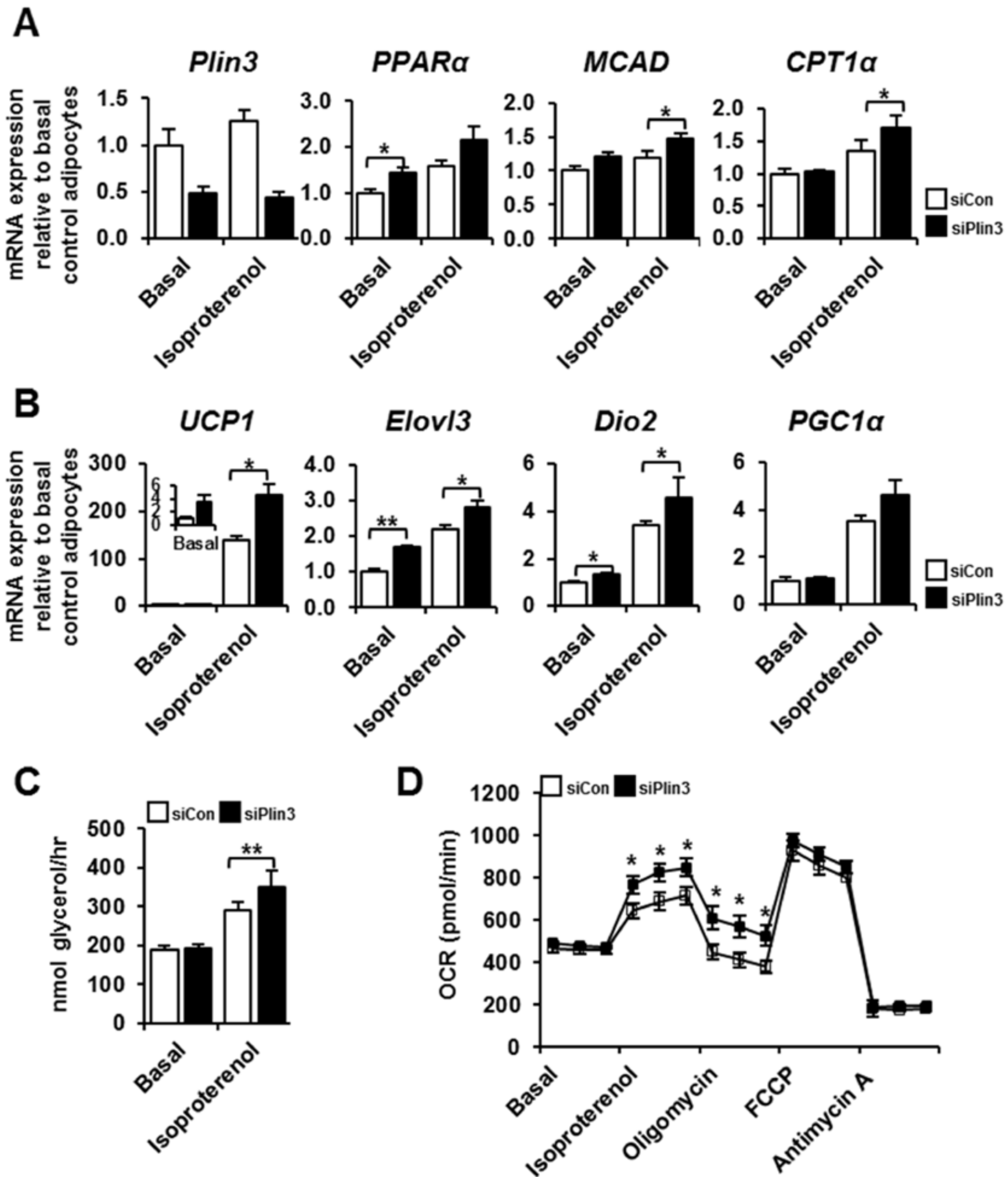

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Supplementary Figure 8. Overexpression of *Plin3* in differentiated adipocytes. (A) eGFP (negative control) or GFP-*Plin3* were transfected in differentiated adipocytes from WT and *Plin3* KO mice for 8 h. Differentiated adipocytes were treated with or without isoproterenol (3 μM) for 6 h. Each mRNA level was normalized to cyclophilin mRNA, and mRNA expression levels are relative to WT eGFP controls. **(B)** Western blot analysis of Plin3 protein expression in differentiated adipocytes from WT and *Plin3* KO mice in the presence or absence of *Plin3*-GFP. Differentiated adipocytes were treated with or without isoproterenol (3 μM) for 6 h. β-actin was used as a loading control. **(C)** RT-qPCR analysis of *PPARα* and target genes in differentiated adipocytes treated with or without isoproterenol (3 μM) for 6 h. Each mRNA level was normalized to cyclophilin mRNA, and mRNA expression levels are relative to WT eGFP controls. **(D)** RT-qPCR analysis of thermogenic genes in differentiated adipocytes treated with or without isoproterenol (3 μM) for 6 h. Each mRNA level was normalized to cyclophilin mRNA, and mRNA expression levels are relative to WT eGFP controls. **(E)** Lipolysis assay in differentiated adipocytes from WT and *Plin3* KO mice in the presence or absence of *Plin3*-GFP. Differentiated adipocytes were treated with or without isoproterenol (3 μM) for 6 h. The levels of glycerol were measured from conditional media. **(F)** Oxygen consumption rates of differentiated adipocytes from iWAT of WT or *Plin3* KO mice. Differentiated adipocytes were treated with or without *Plin3*-GFP. Data represent the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ indicate significant differences between groups as determined by either two-tailed unpaired Student's *t* tests or two-way ANOVA versus control. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ indicate significant differences between *Plin3* KO + Iso. (eGFP) *vs. Plin3* KO + *Plin3*-GFP as determined by two-tailed unpaired Student's *t* tests.

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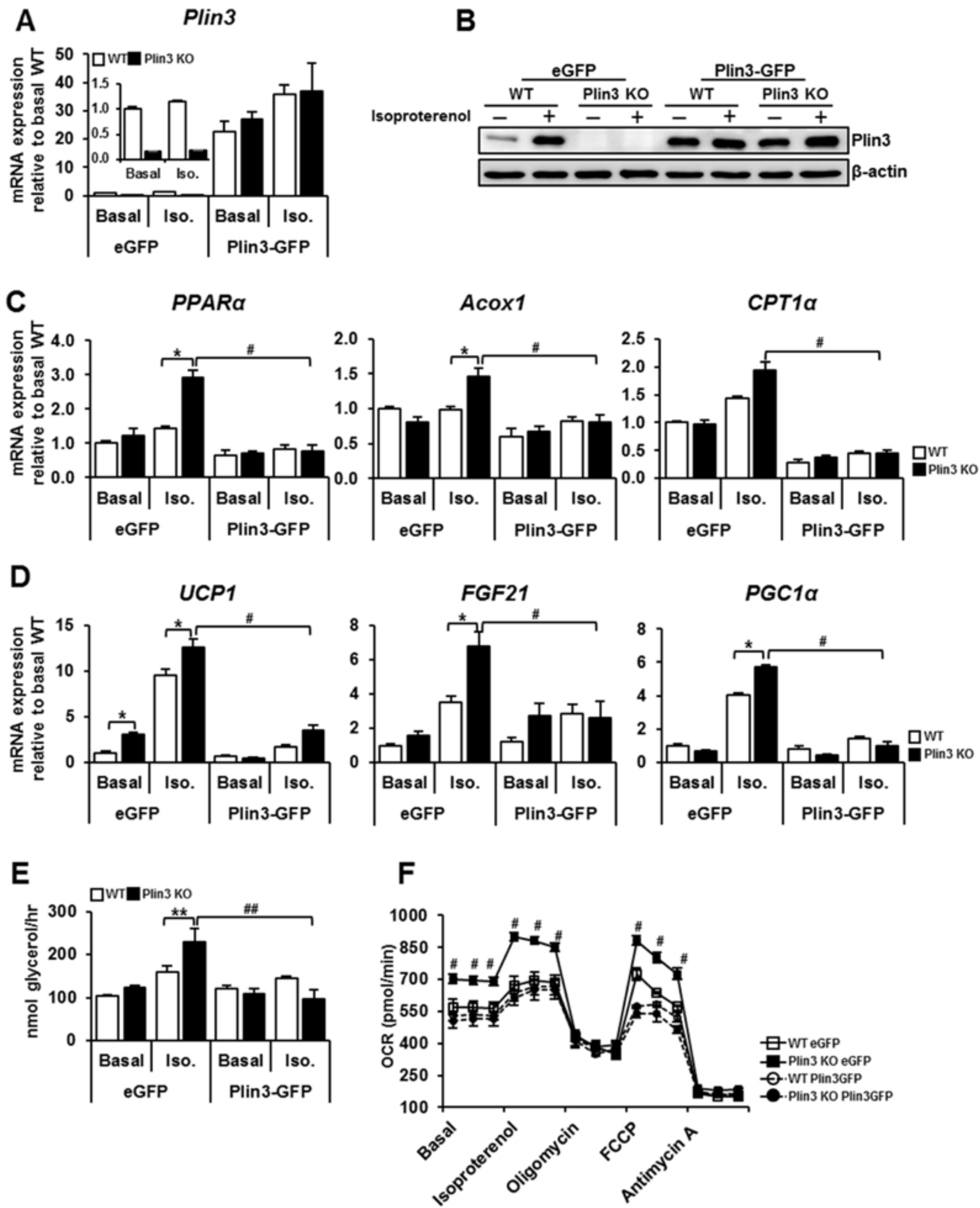
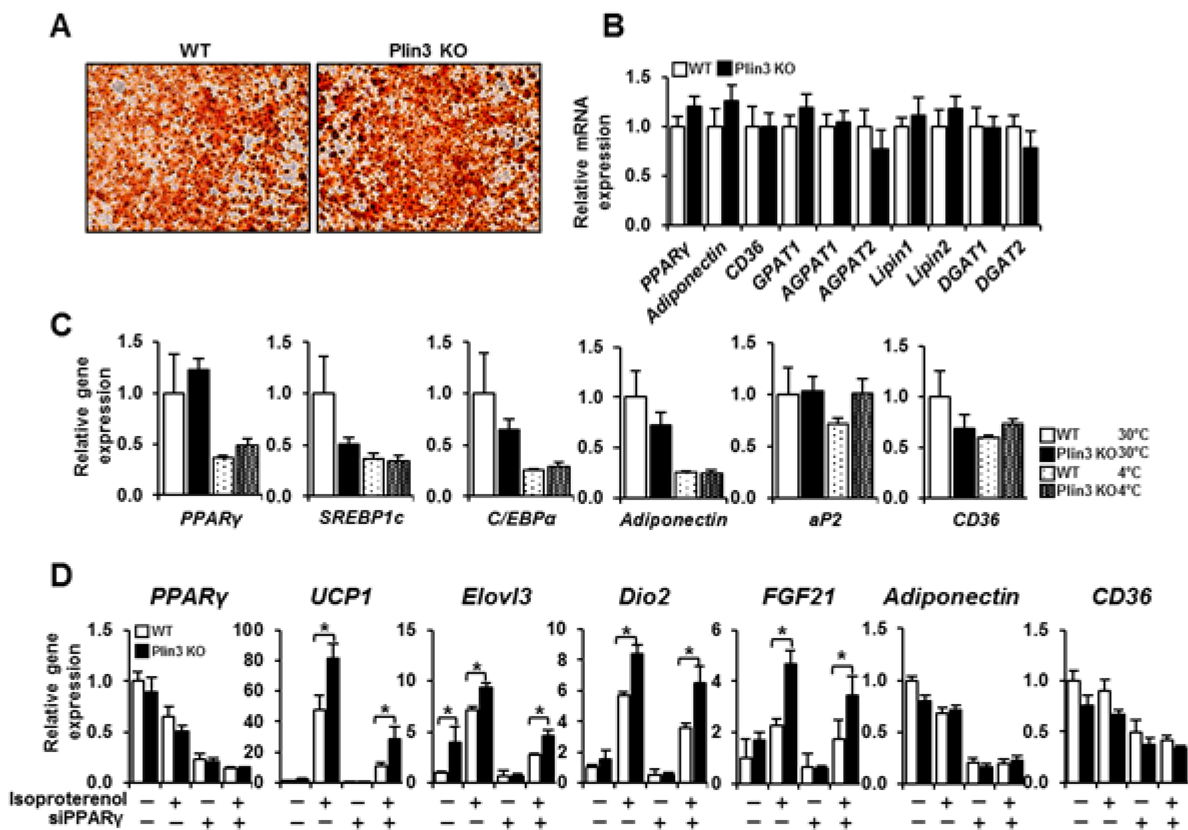


A
Plin3
mRNA expression relative to basal WT
WT
Plin3 KO
Basal
Iso.
eGFP
Plin3-GFP
B
eGFP
Plin3-GFP
WT
Plin3 KO
Isoproterenol
Plin3
β-actin
C
PPARα
Acox1
CPT1α
D
UCP1
FGF21
PGC1α
E
nmol glycerol/hr
F
OCR (pmol/min)
WT eGFP
Plin3 KO eGFP
WT Plin3GFP
Plin3 KO Plin3GFP
Basal
Isoproterenol
Oligomycin
FCCP
Antimycin A

SUPPLEMENTARY DATA

Supplementary Figure 9. Neutral lipid accumulation and PPARγ activation in *Plin3* KO iWAT. **(A)** Oil Red O staining of differentiated adipocytes from WT and *Plin3* KO mice (n = 6 mice/group, 3 independent experiments). Magnification was 100x with microscopy. **(B)** RT-qPCR analysis of TAG synthesis gene expression in differentiated adipocytes from WT and *Plin3* KO mice. Each mRNA level was normalized to cyclophilin mRNA, and mRNA expression levels are relative to WT. **(C)** RT-qPCR analysis of *PPARγ* and its target genes in iWAT from WT and *Plin3* KO mice exposed to 30°C or 4°C for 6 days (n = 3 mice/group). Each mRNA level was normalized to cyclophilin mRNA, and mRNA expression levels are relative to WT at 30°C. **(D)** RT-qPCR analysis of *PPARγ* and thermogenic marker genes in differentiated adipocytes treated with or without *PPARγ* siRNA. Differentiated adipocytes were treated with or without isoproterenol (3 μM) for 8 h. Each mRNA level was normalized to cyclophilin mRNA, and mRNA expression levels are relative to untreated WT controls. Data represent the mean ± SEM. $^{*}p < 0.05$ indicates significant differences between groups as determined by two-tailed unpaired Student's *t* tests in comparison to control.



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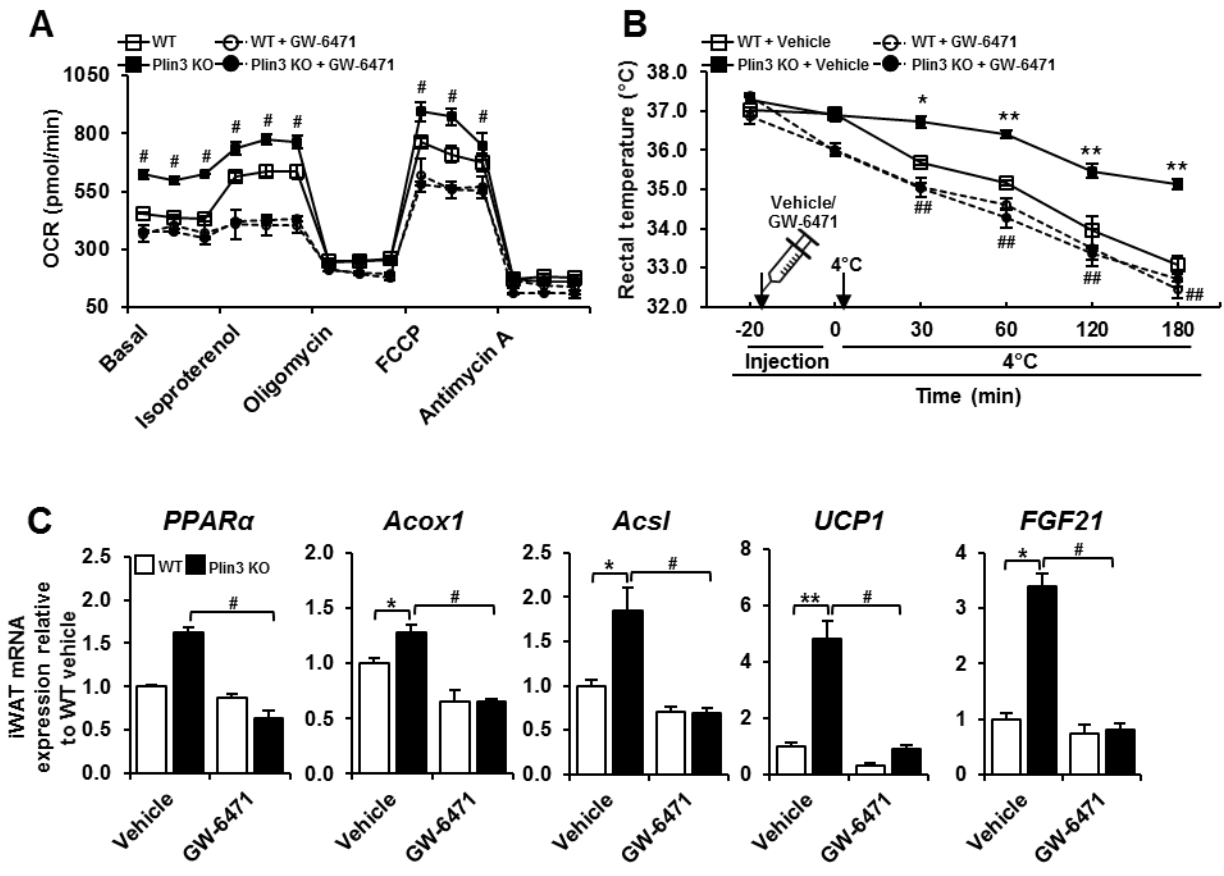
Supplementary Figure 10. Inactivation of PPARα in *Plin3* KO mice on thermogenic capacity. (A) Oxygen consumption rates of differentiated adipocytes from iWAT of WT or *Plin3* KO mice. Differentiated adipocytes were treated with PPARα antagonist (GW-6471, 10 μM) for 48 h. **(B)** Changes in rectal temperature by GW-6471 (10 mg/kg) injection for 20 min followed by 3 h cold exposure (n = 3-5 mice/group). PBS injected mice were used as control vehicle group. **(C)** RT-qPCR analysis of *PPARα, UCP1,* and *FGF21* gene expression in iWAT from WT and *Plin3* KO mice administered GW-6471 followed by 4°C exposure (n = 3-5 mice/group). Each mRNA level was normalized to cyclophilin mRNA, and mRNA expression levels are relative to WT vehicle. Data represent the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ indicate significant differences between groups as determined by either two-tailed unpaired Student's *t* tests or two-way ANOVA versus control. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ indicate significant differences between KO vehicle *vs.* WT + GW-6471 and/or *Plin3* KO + GW-6471 as determined by two-tailed unpaired Student's *t* tests.



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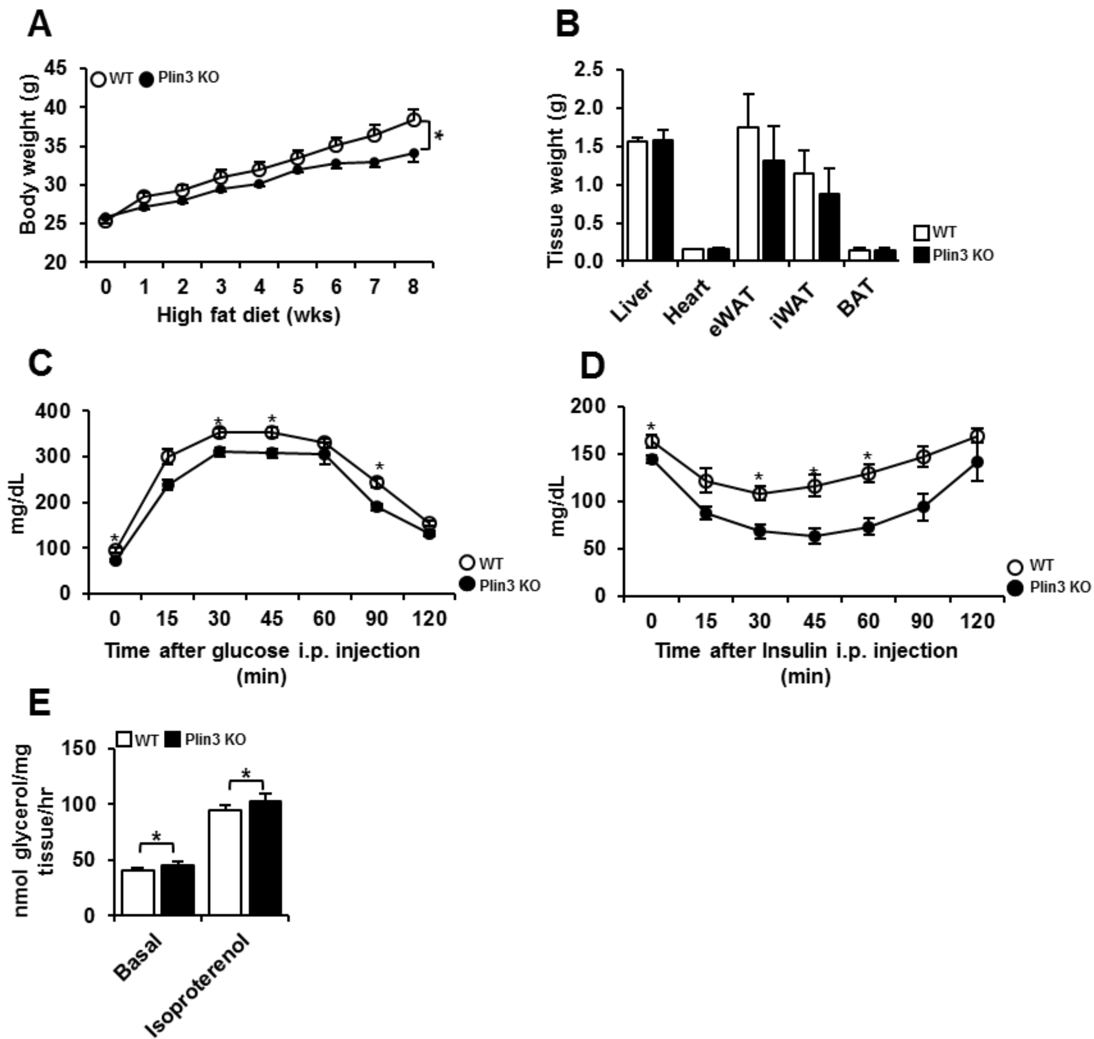
Supplementary Figure 11. Metabolic phenotypes of *Plin3* KO mice upon high fat diet (HFD). WT and *Plin3* KO mice were fed HFD (n = 10-11) for 8-10 weeks from 8 weeks of age. **(A)** Body weights of WT and *Plin3* KO mice on high fat diet (n = 10–11 mice/group). **(B)** Tissue weights in WT and *Plin3* KO mice after 8 weeks of HFD (n = 10-11 mice/group). **(C)** Intraperitoneal glucose tolerance test (GTT). **(D)** Intraperitoneal insulin tolerance test (ITT). **(E)** *Ex vivo* iWAT lipolysis assay from HFD-fed mice for 8-10weeks (n = 6 mice/group). Data represent the mean ± SEM. *$p < 0.05$ indicates significant differences between groups as determined by two-tailed unpaired Student's t tests in comparison to control.



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Supplementary Table 1. Sequence information of the RT-qPCR primers used in this study.

Gene	*5′sequence*	*3′sequence*
Mouse UCP1	GGCCCTTGTAAACAACAAAA	GGCAACAAGAGCTGACAGTA
Mouse Plin3	CTGAGAAAGGCGTCAAGACC	TTTCTTGAGCCCCAGACACT
Mouse PGC1α	AAGGTCCCCAGGCAGTAGAT	GCTCTTTGCGGTATTCATCC
Mouse PGC1β	CTCCAGGCAGGTTCAACCC	GGGCCAGAAGTTCATCCC
Mouse Elovl3	TGTTGGCCAGACCTACATGA	ATCTGACTACGGCGTCATCC
Mouse Dio2	TTCTCCAACTGCCTCTTCCTG	CCCATCAGCGGTCTTCTCC
Mouse Cidea	TCCTATGCTGCACAGATGACG	TGCTCTTCTGTATCGCCCAGT
Mouse Cidec	CTGGAGGAAGATGGCACAAT	GGGCCACATCGATCTTCTTA
Mouse CytC	GGCTGCTGGATTCTCTTACACA	CCAAATACTCCATCAGGGTATCCT
Mouse ATPase	CACCACCAAGAAGGGATCGA	GCAGGGTCAGTCAGGTCATCA
Mouse Cox2	TCTCCCCTCTCTACGCATTCT	TCATTGGTGCCCTATGGTTT
Mouse Cox3	CGGAAGTATTTTTCTTTGCAGGAT	CAGCAGCCTCCTAGATCATGTG
Mouse Cox8b	TGCGAAGTTCACAGTGGTTC	ATGCTGCGGAGCTCTTTTTA
Mouse Tfam	CCACAGAACAGCTACCCAAATTT	TCCACAGGGCTGCAATTTTC
Mouse Nrf1	AAGAGACAGCAGACACGTTTGC	TCCCACTCGCGTCGTGTAC
Mouse PPARα	ATGCCAGTACTGCCGTTTTC	GGCCTTGACCTTGTTCATGT
Mouse CPT1α	CATCACCCCAACCCATATTC	CGTGTTGGATGGTGTCTGTC
Mouse CPT1β	TGTCATGGCAACAGTTGGTT	GACTCCGGTGGAGAAGATGA
Mouse CPT2	CTATCTGCCCAGCTTCCATC	ATAGAGCTCAGGCAGGGTGA
Mouse Acsl1	GTCCTGGGCACAGAAGAGAG	GTCAGAAGGCCGTTGTCAAT
Mouse Acox1	CAGGAAGAGCAAGGAAGTGG	CCTTTCTGGCTGATCCCATA
Mouse MCAD	AGGTTTCAAGATCGCAATGG	CTCCTTGGTGCTCCACTAGC
Mouse LCAD	TGCGAAGTTCACAGTGGTTC	TCAGGGATGTGCAACTTCA
Mouse FGF21	AGCATACCCCATCCCTGACT	TCCTCCCTGATCTCCAGGTG
Mouse PPARγ	TTGCTGSSCGTGAAGCCCATCGAGG	GTCTTGTAGATCTCCTGGAGCAG
Mouse adiponectin	GGCAGGAAAGGAGAACCTGG	AGCCTTGTCCTTCTTGAAGAG

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Mouse CD36	GAGCAACTGGTGGATGGTTT	GCAGAATCAAGGGAGAGCAC
Mouse GPAT1	ACAGTTGGCACAATAGACGTTT	CCTTCCATTTCAGTGTTGCAGA
Mouse AGPAT1	TAAGATGGCCTTCTACAACGGC	CCATACAGGTATTTGACGTGGAG
Mouse AGPAT2	CTTCAAGTACGTGTATGGCCTT	CTGTGAACATTAGCTCACGCT
Mouse Lipin1	CTCCGCTCCCGAGAGAAAG	TCATGTGCAAATCCACGGACT
Mouse Lipin2	GAAGTGGCGGCTCTCTATTTC	AGAGGGTTACATCAGGCAAGT
Mouse DGAT1	CTGATCCTGAGTAATGCAAGGTT	TGGATGCAATAATCACGCATGG
Mouse DGAT2	GCGCTACTTCCGAGACTACTT	GGGCCTTATGCCAGGAAACT
Mouse SREBP1c	GGAGCCATGGATTGCACATT	CAGGAAGGCTTCCAGAGAGG
Mouse CD137	CGTGCAGAACTCCTGTGATAAC	GTCCACCTATGCTGGAGAAGG
Mouse Tbx1	GGCAGGCAGACGAATGTTC	TTGTCATCTACGGGCACAAAG
Mouse Ear2	CCTGTAACCCCAGAACTCCA	CAGATGAGCAAAGGTGCAAA
Mouse Tmem26	ACCCTGTCATCCCACAGAG	TGTTTGGTGGAGTCCTAAGGTC
Mouse Slc27a1	CTGGGACTTCCGTGGACCT	TCTTGCAGACGATACGCAGAA
Mouse C/EBPα	ATCGACTTCAGCCCCTACCT	CTCTGGGATGGATCGATTGT
Mouse aP2	AAGAAGTGGGAGTGGGCTTT	GCTCTTCACCTTCCTGTCGT
Mouse 18s rRNA	TCATAAGCTTGCGTTGATTA	TAGTCAAGTTCGACCGTCTT
Mouse 16s rRNA	ACATCCCAATGGTGTAGAAG	AAGTTGAGAGCGCTTATTTG
Mouse cyclophilin	CAGACGCCACTGTCGCTTT	TGTCTTTGGAACTTTGTCTG

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