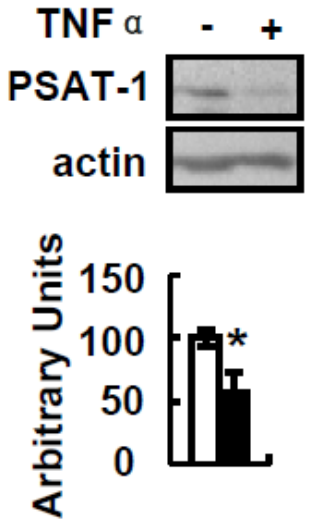
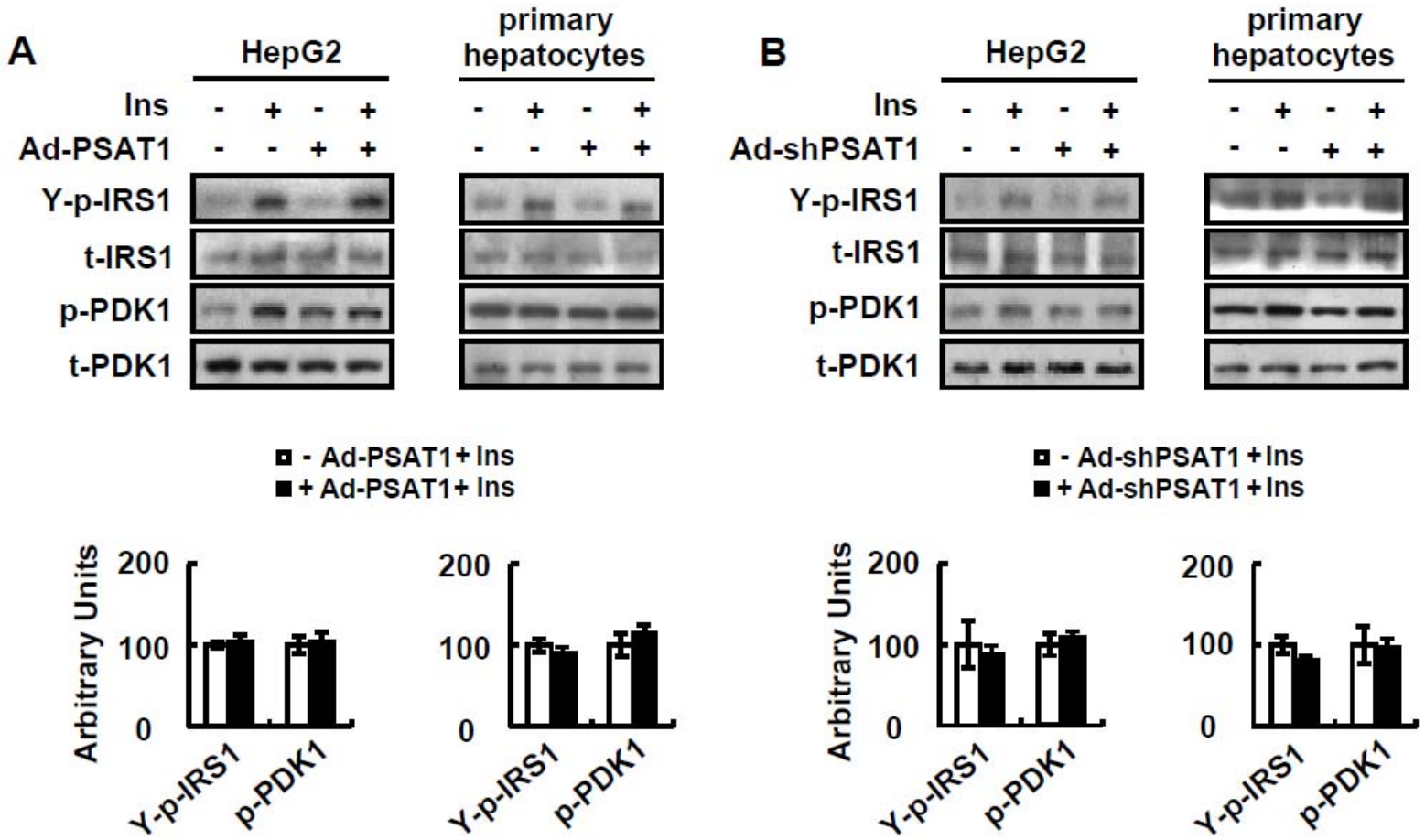
SUPPLEMENTARY DATA

Supplementary Figure 1. PSAT1 expression is decreased in TNFαtreated-HepG2 cells
HepG2 cells were treated with 40 ng/ml TNFα (+ TNFα, Sigma) or without (- TNFα) for 24 h, followed by examination of PSAT expression. Means ± SEMs shown are representative of at least three independent in vitro experiments. Statistical significance was calculated using the two-tailed student *t-test* for the effects of with versus without TNFα (*: $p < 0.05$). PSAT1 protein (*top*, western blot; *bottom*, quantitative measurement of PSAT1 protein relative to actin).



SUPPLEMENTARY DATA

Supplementary Figure 2. PSAT1 has no effect on insulin-stimulated phosphorylation of IRS1 and PDK1 in vitro
(A and B) Cells were infected with Ad-PSAT1 (+ Ad-PSAT1) or Ad-GFP (- Ad-PSAT1) for 48 h in A, or Ad-shPSAT1 (+ Ad-shPSAT1) or Ad-scrambled (- Ad-shPSAT1) for 72 h in B; both cases were followed with (+ Ins) or without (- Ins) 100 nM insulin stimulation for 20 min. Data were obtained with at least three independent in vitro experiments and are presented as means ± SEMs. Statistical significance was calculated using the two-tailed student *t-test* for the effects of Ad-PSAT1 or Ad-shPSAT1 versus corresponding control following insulin stimulation (*: $p < 0.05$). (A and B) p-IRS1 (tyrosine 612/608-human/mouse) and p-PDK1 (ser241) (*top*, western blot; *bottom*, quantitative measurements of p-IRS1, p-PDK1 relative to their total protein).

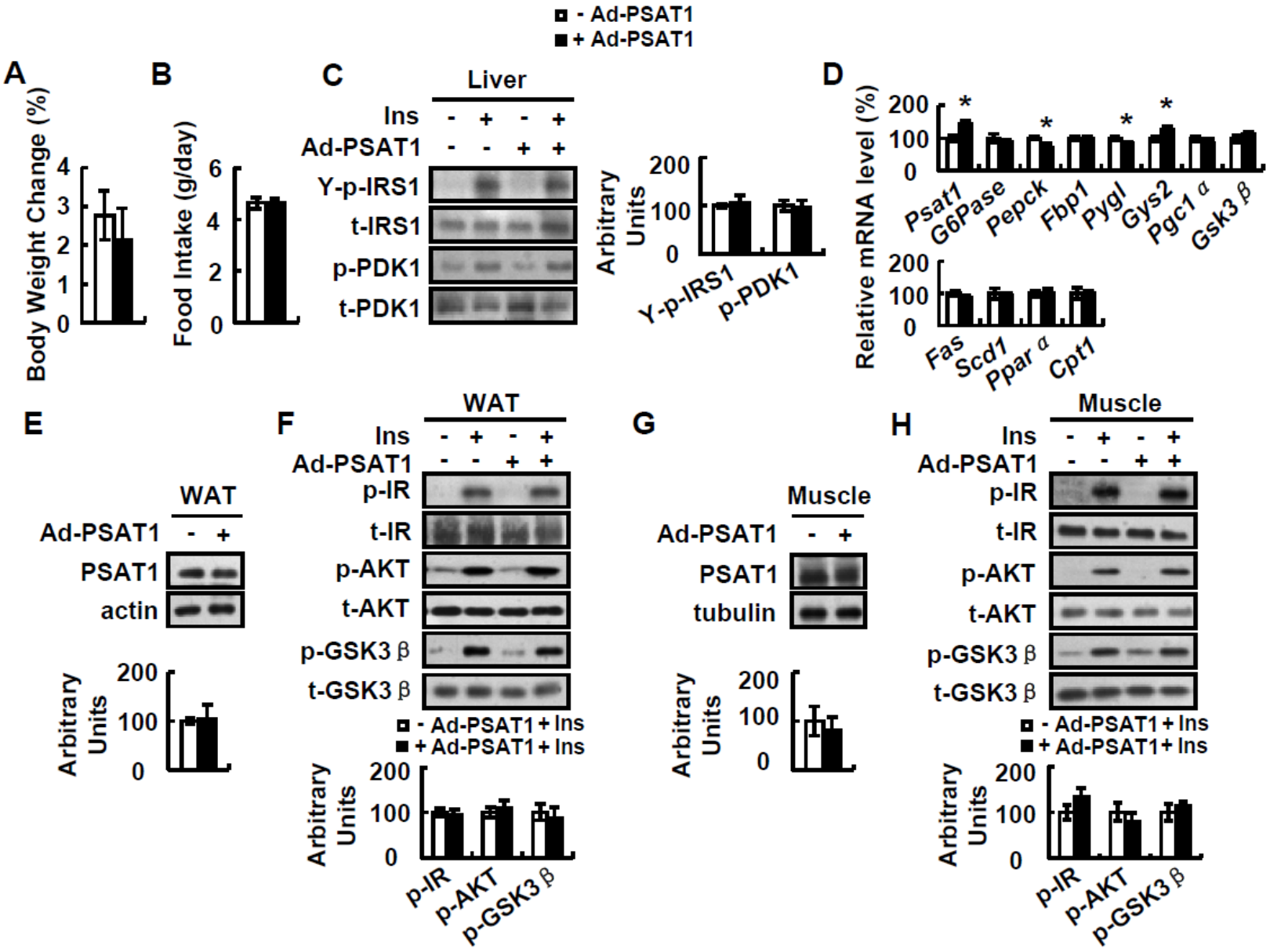


SUPPLEMENTARY DATA

Supplementary Figure 3. Various metabolic effects of PSAT1 over-expression in wild-type (WT) mice

Male C57 BL/6J WT mice were injected with Ad-PSAT1 (+ Ad-PSAT1) or Ad-GFP (- Ad-PSAT1) for 7 days, followed by measurement of body weight and food intake in A and B; examination of insulin signaling before (- Ins) and after (+ Ins) 2 U/kg insulin stimulation for 3 min in liver, 4 min in white adipose tissue (WAT) and 5 min in muscle at day 7 in C, F and H; detection of glucose and lipid metabolism genes in D; examination of PSAT1 expression in WAT and muscle in E and G. Data were obtained with mice described above (n = 10-14 mice per group) and are presented as means ± SEMs. Statistical significance was calculated using the two-tailed student *t-test* for the effects of Ad-PSAT1 versus corresponding control without insulin treatment in A, B, D, E and G, or with insulin treatment in C, F and H (*: $p < 0.05$). (A) Body weight change; (B) Food intake; (C) p-IRS1 (tyrosine 612/608-human/mouse) and p-PDK1 (ser241) (*top*, western blot; *bottom*, quantitative measurements of p-IRS1, p-PDK1 relative to their total protein); (D) mRNA levels of genes related to glucose and lipid metabolism. The sequences of primers were listed in Table S2; (E and G) PSAT1 protein (*top*, western blot; *bottom*, quantitative measurement of PSAT1 protein relative to actin or tubulin); (F and H) p-IR (tyr1150/1151), p-AKT (ser473) and p-GSK3β(ser9) protein (*top*, western blot; *bottom*, quantitative measurements of p-IR, p-AKT, p-GSK3β relative to their total protein).

SUPPLEMENTARY DATA

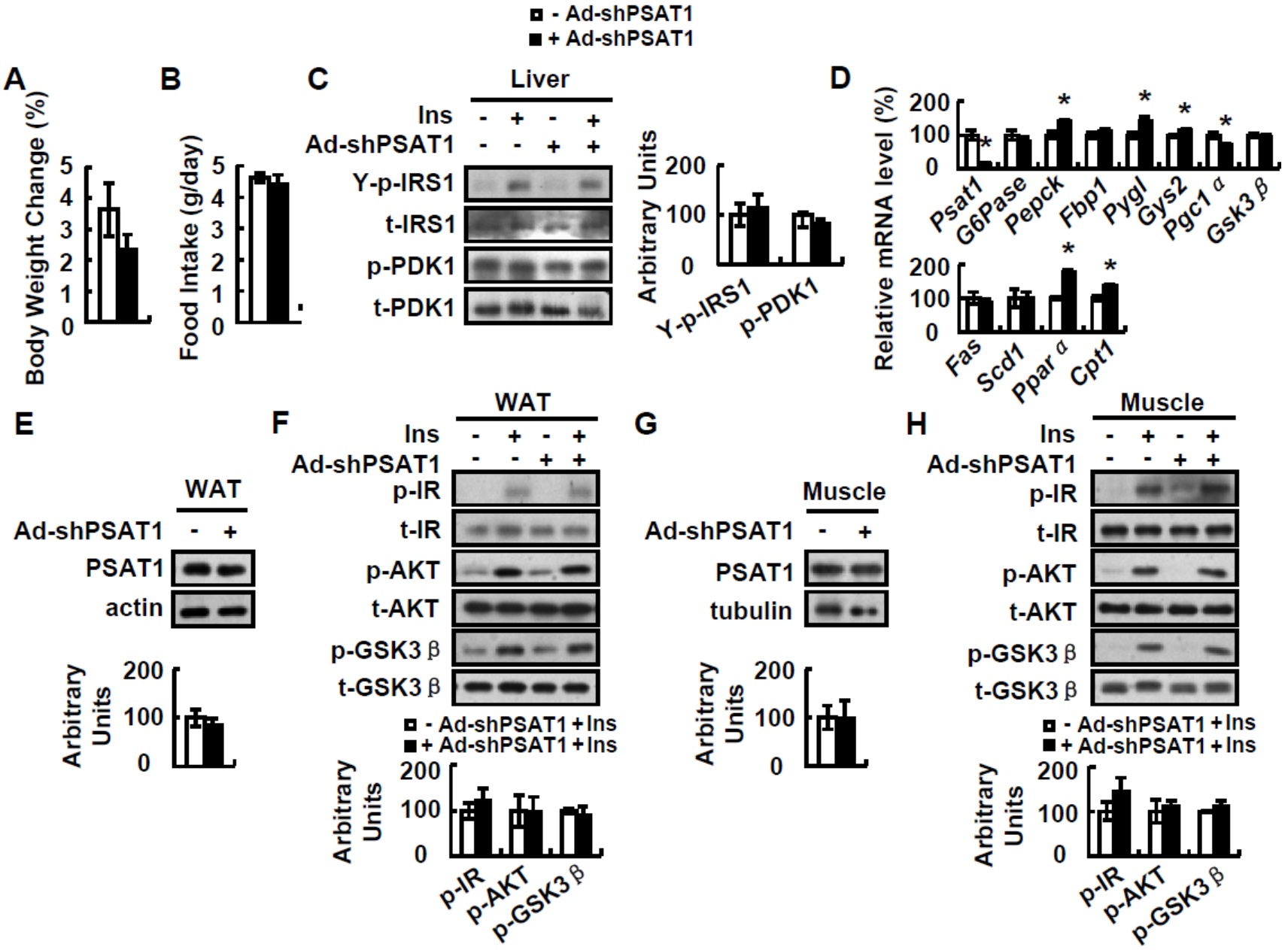


SUPPLEMENTARY DATA

Supplementary Figure 4. Various metabolic effects of PSAT1 knock down in wild-type (WT) mice

Male C57 BL/6J WT mice were injected with Ad-shPSAT1 (+ Ad-shPSAT1) or Ad-scrambled (- Ad-shPSAT1) for 13 days, followed by measurement of body weight and food intake in A and B; examination of insulin signaling before (- Ins) and after (+ Ins) 2 U/kg insulin stimulation for 3 min in liver, 4 min in WAT and 5 min in muscle at day 13 in C, F and H; detection of glucose and lipid metabolism gene in D; examination of PSAT1 expression in white adipose tissue (WAT) and muscle in E and G. Data were obtained with mice described above (n = 10-14 mice per group) and are presented as means ± SEMs. Statistical significance was calculated using the two-tailed student *t-test* for the effects of Ad-shPSAT1 versus corresponding control without insulin treatment in A, B, D, E and G, or with insulin stimulation in C, F and H (*: $p < 0.05$). (A) Body weight change; (B) Food intake; (C) p-IRS1 (tyrosine 612/608-human/mouse) and p-PDK1 (ser241) (*top*, western blot; *bottom*, quantitative measurements of p-IRS1, p-PDK1 relative to their total protein); (D) mRNA levels of genes related to glucose and lipid metabolism. The sequences of primers were listed in Table S2; (E and G) PSAT1 protein (*top*, western blot; *bottom*, quantitative measurement of PSAT1 protein relative to actin or tubulin); (F and H) p-IR (tyr1150/1151), p-AKT (ser473) and p-GSK3β(ser9) protein (*top*, western blot; *bottom*, quantitative measurements of p-IR, p-AKT, p-GSK3β relative to their total protein).

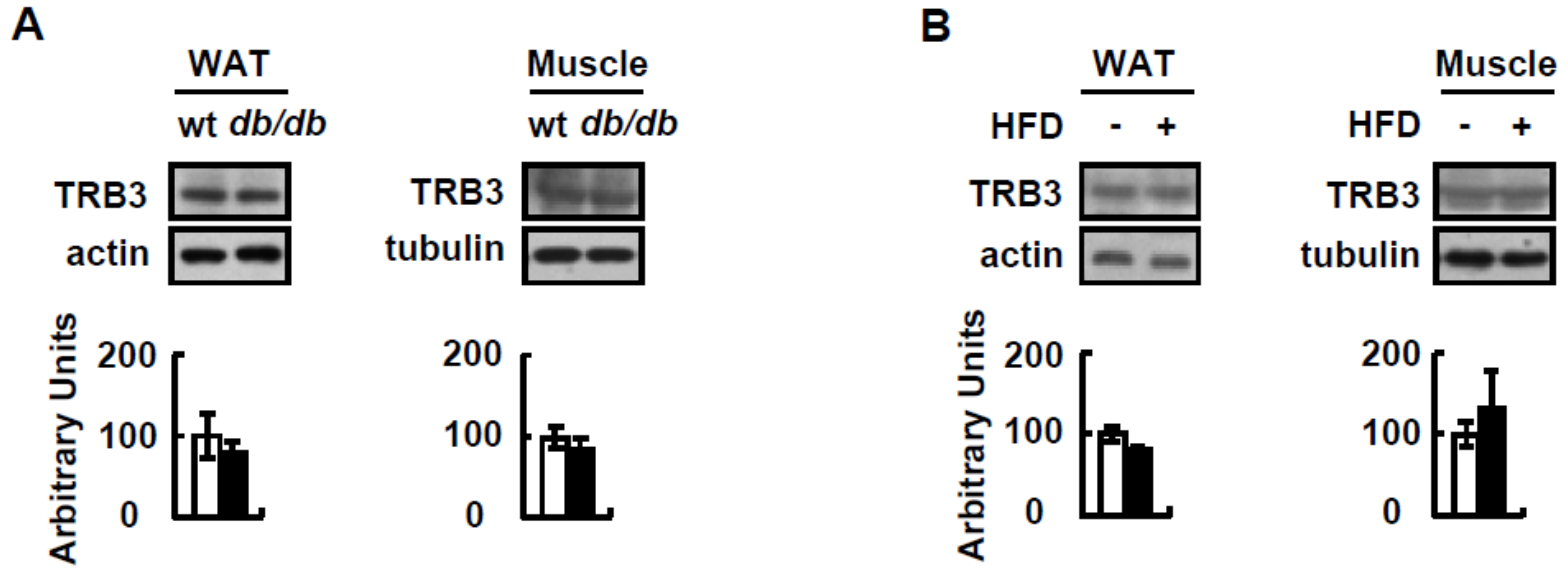
SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure 5. No differences in TRB3 expression are observed in white adipose tissue (WAT) and muscle of insulin-resistant mice

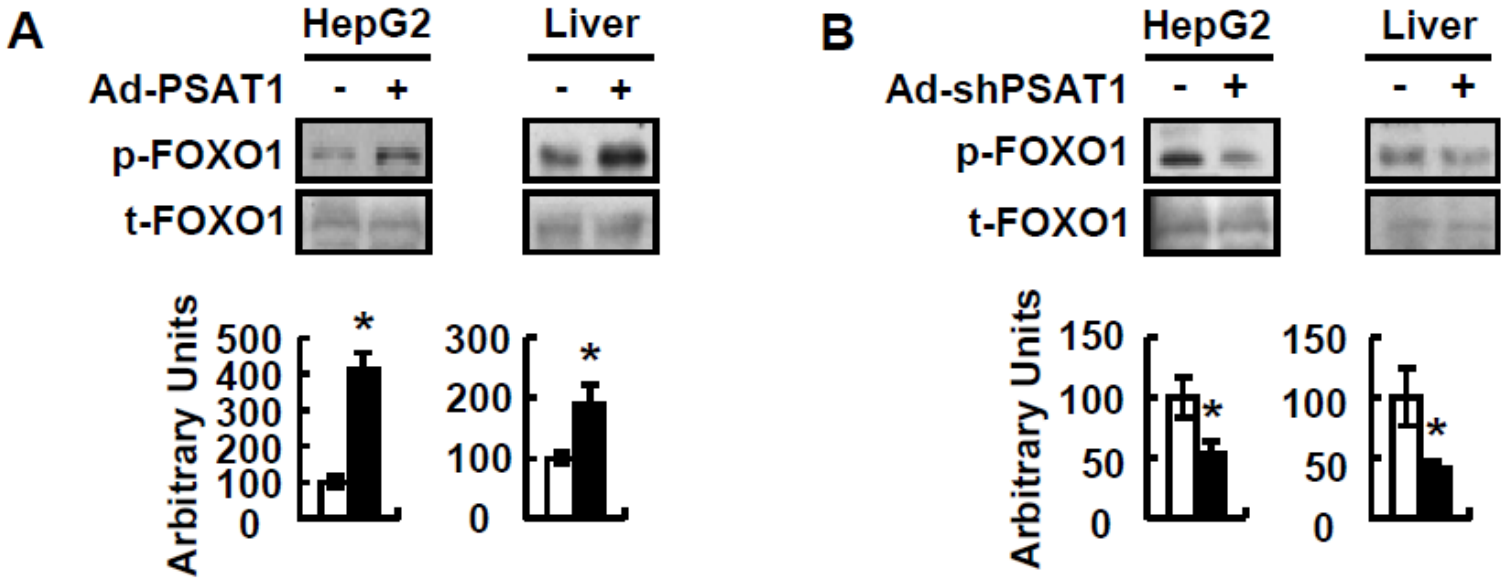
TRB3 expression were analyzed in WAT and muscle of wild-type (wt) and *db/db* mice in A, or WT mice fed a control (- HFD) or HFD (+ HFD) for 16 weeks in B. Data were obtained with mice described above (n = 10-14 mice per group) and are presented as means ± SEMs. Statistical significance was calculated using the two-tailed student *t-test* for the effects of *db/db* or HFD mice versus their relevant control mice (*: $p < 0.05$). (A and B) TRB3 protein (*top*, western blot; *bottom*, quantitative measurement of TRB3 protein relative to tubulin or actin).



SUPPLEMENTARY DATA

Supplementary Figure 6. Hepatic FOXO1 phosphorylation is increased by Ad-PSAT1 and decreased by Ad-shPSAT1 in vitro and in vivo

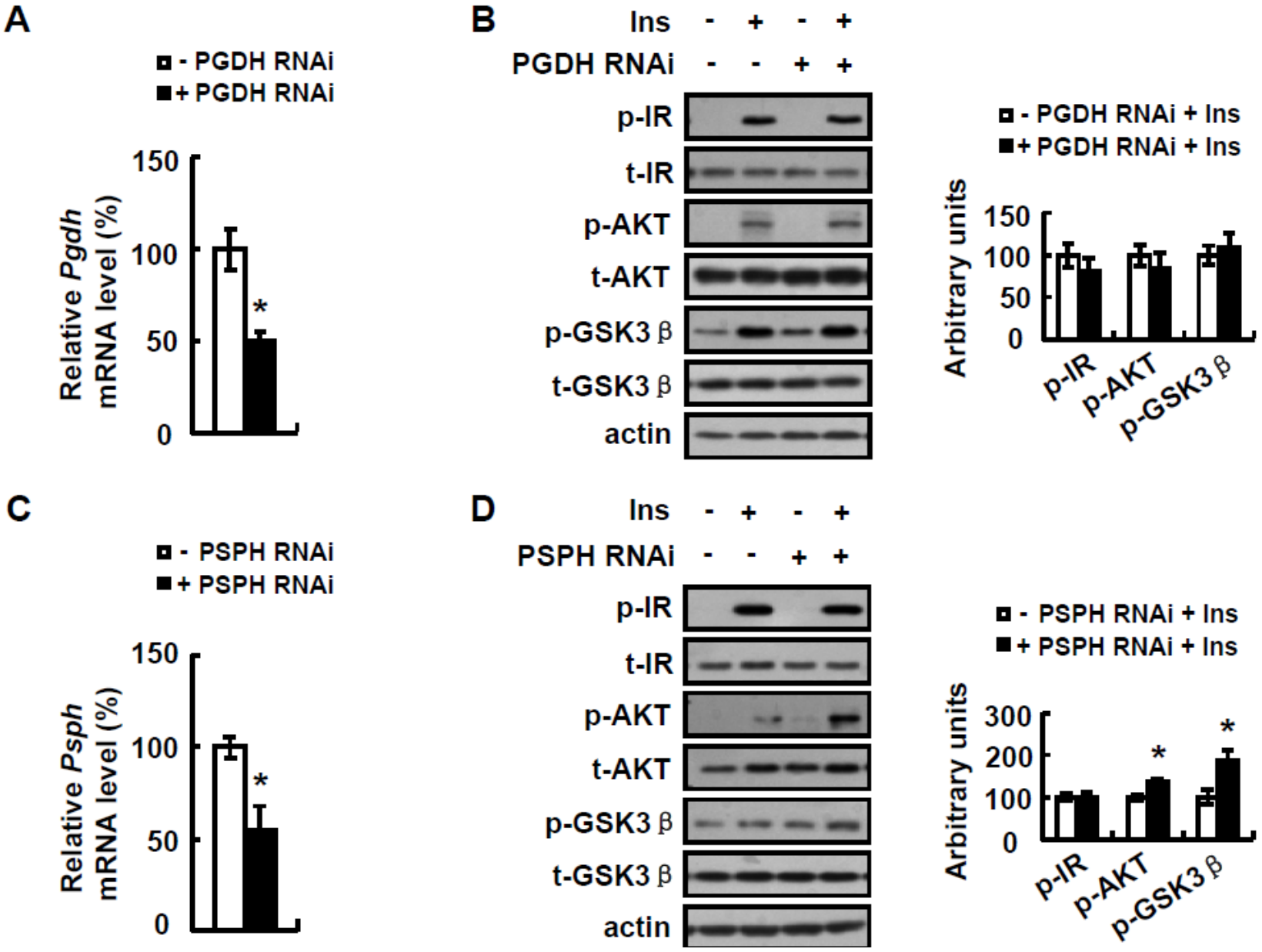
(A) HepG2 cells were infected with Ad-PSAT1 (+ Ad-PSAT1) or Ad-GFP (- Ad-PSAT1) for 48 h or male C57 BL/6J wild-type (WT) mice were injected with Ad-PSAT1 (+ Ad-PSAT1) or Ad-GFP (- Ad-PSAT1) via tail-vein injection, followed by examination of FOXO1 in liver at day 7; (B) HepG2 cells were exposed to Ad-shPSAT1 (+ Ad-shPSAT1) or Ad-scrambled (- Ad-shPSAT1) for 72 h or male C57 BL/6J WT mice were injected with Ad-shPSAT1 (+ Ad-shPSAT1) or Ad-scrambled (- Ad-shPSAT1) via tail vein injection, followed by examination of FOXO1 in liver at day 13. Data were obtained with mice described above (n = 10-14 mice per group) or at least three independent in vitro experiments and are presented as means ± SEMs. Statistical significance was calculated using the two-tailed student *t-test* for the effects of the Ad-PSAT1 or Ad-shPSAT1 versus the control group (*: $p < 0.05$). (A and B) p-FOXO1 and FOXO1 protein (*top*, western blot; *bottom*, quantitative measurements of p-FOXO1 protein relative to total FOXO1)



SUPPLEMENTARY DATA

Supplementary Figure 7. Insulin signaling is not inhibited in PGDH RNAi or PSPH RNAi-treated HepG2 cells
HepG2 cells were treated with PGDH siRNA (+ PGDH RNAi) or control reagent (- PGDH RNAi) for 48 h in A, PSPH siRNA (+ PSPH RNAi) or control reagent (- PSPH RNAi) for 48 h in B, both cases were followed with (+ Ins) or without (- Ins) 100 nM insulin stimulation for 20 min. Means ± SEMs shown are representative of at least three independent in vitro experiments. Statistical significance was calculated using the two-tailed student *t-test* for the effects of PGDH RNAi or PSPH RNAi versus corresponding control following insulin stimulation (*: $p < 0.05$). (A) *Pgdh* mRNA; (B and D) p-IR (tyr1150/1151), p-AKT (ser473) and p-GSK3β(ser9) protein (*left*, western blot; *right*, quantitative measurements of p-IR, p-AKT, p-GSK3β relative to their total protein); (C) *Psph* mRNA.

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Table 1. Metabolic parameters in different mice

mice	wt (8w)	*db/db* (8w)	Cont (4w wt mice under chow diet for 16 w)	HFD (4w wt mice under HFD for 16w)
Blood glucose (mg/dl)	162.3±4.51	316.2±18.57*	156.3±6.82	200.7±5.15*
Serum insulin (ng/ml)	0.33±0.01	14.42±4.14*	0.49±0.08	1.17±0.13*
Serum glucagon (pg/ml)	194.17±1.46	103.56±20.08*	212.47±26.92	135.87±17.96*
Serum TG (mg/dl)	19.56±2.10	301.33±53.57*	27.46±8.41	9.37±1.46*
Serum TC (mM/l)	1.93±0.06	4.62±0.12*	2.03±0.25	4.61±0.47*
Serum FFA (mEq/l)	0.57±0.03	1.15±0.10*	0.40±0.03	0.50±0.02*
Liver TG (mg/g)	21.14±2.64	61.31±5.76*	21.71±2.85	66.97±6.61*
Liver TC (mg/g)	15.56±0.88	19.98±1.85*	17.99±0.62	21.07±1.12*
Liver FFA (mM/g)	0.05±0.001	0.09±0.01*	0.05±0.002	0.10±0.001*

SUPPLEMENTARY DATA

Supplementary Table 2. List of oligonucleotide primer pairs used in RT-PCR analysis

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Mmu-GAPDH	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGAT
Mmu-G6PASE	ATGACTTTGGGATCCAGTCG	TGGAACCAGATGGGAAAGAG
Mmu-PEPCK	CGGAAGAGGACTTTGAGAAAGC	TGGTGCGGCCTTTCATG
Mmu-FBP1	GCTGCGGCTGCTGTATGA	ACCGGCCTTCTCCATGA
Mmu-PYGL	GGTAGCCATCCAGCTGAATGAC	TCAATGTCCACAAAAATCCTCATC
Mmu-GYS2	GCACGGAGAGGCTCTCAGAT	AGGTGTCTGGCATGCTGGTAA
Mmu-PGC1α	GATGGCACGCAGCCCTAT	CTCGACACGGAGAGTTAAAGGAA
Mmu-GSK3β	TTGGACAAAGGTCTTCCGGC	AAGAGTGCAGGTGTGTCTCG
Mmu-FAS	AGGTGGTGATAGCCGGTATGT	TGGGTAATCCATAGAGCCCAG
Mmu-SCD1	GCGATACACTCTGGTGCTCA	CCCAGGGAAACCAGGATATT
Mmu-PPARα	CTGCAGAGCAACCATCCAGAT	GCCGAAGGTCCACCATTTT
Mmu-CPT1	GCGAAGTGTCGGCAGACCTA	TGTTCCGATTCGTCCAACGT