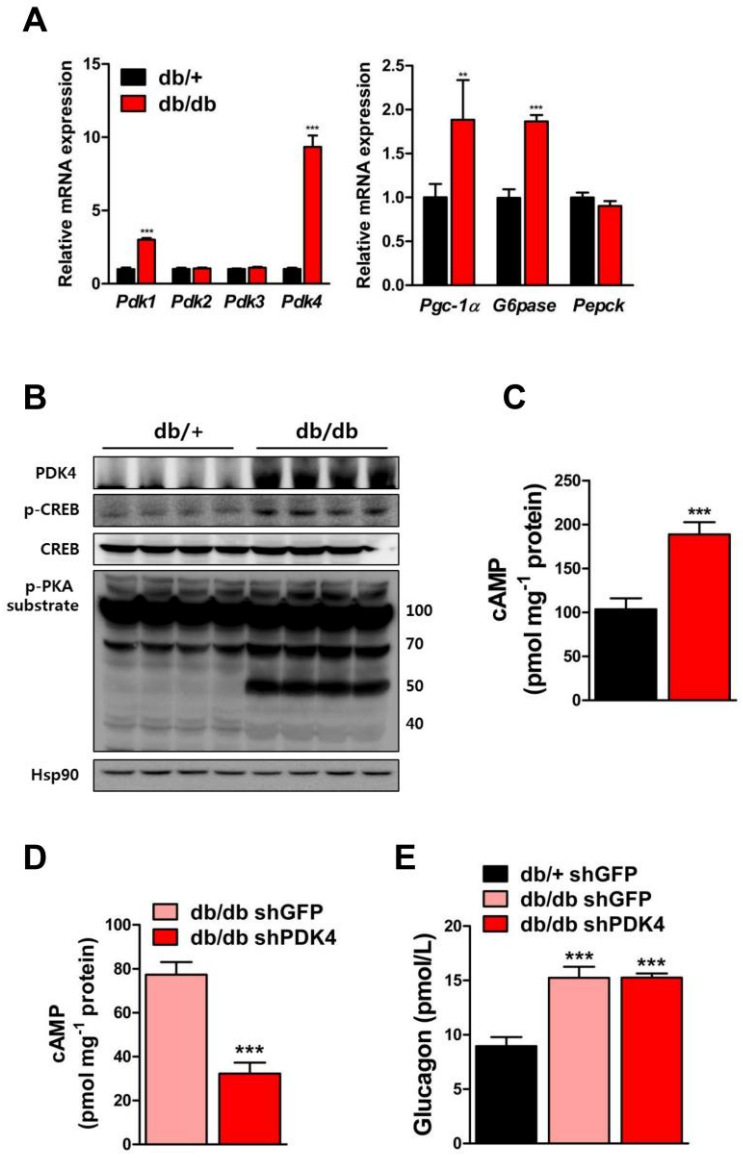
SUPPLEMENTARY DATA

Supplementary Figure 1. Hepatic PDK4 expression is positively correlated with gluconeogenic signaling

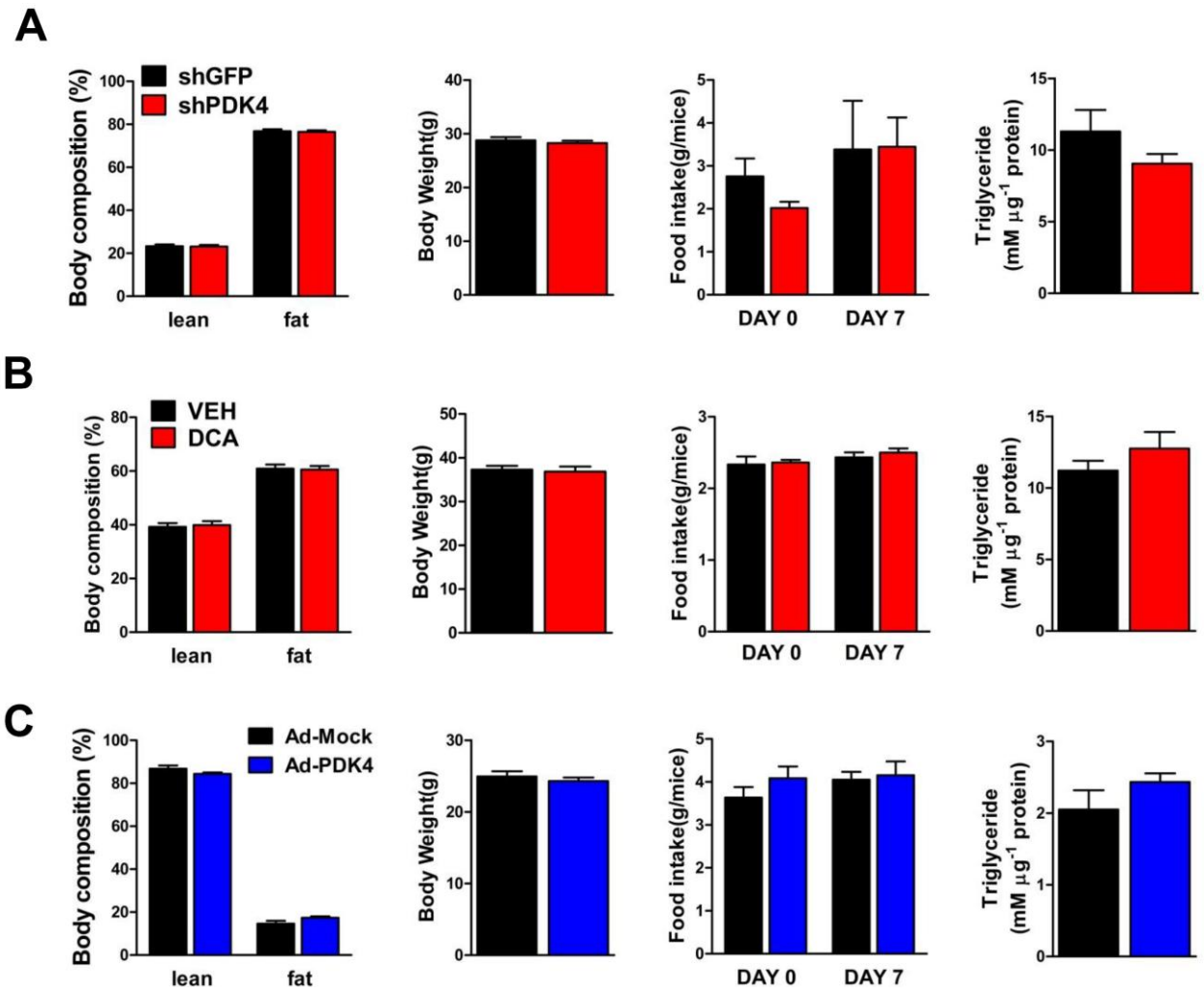
(A) Relative mRNA expression for PDK isoforms and gluconeogenic genes in liver of *ad libitum* fed control db/+ and diabetic db/db mice (n=6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with db/+ control mice. (B) Western blot data showing PDK4, p-CREB and p-PKA substrates in db/+ and db/db mice. Hsp90 served as endogenous control. (C) Comparison of cAMP levels in livers of db/+ control mice and db/db diabetic mice after 16 h fasting (n=6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with db/+ control mice. (D) Comparison of cAMP levels in livers of db/db diabetic mice injected with either shGFP or shPDK4 (n=6-8). $^{***}P < 0.001$ compared with shGFP injected db/db. (E) 16-hr fasted plasma glucagon levels of db/+ injected shGFP and db/db mice injected with either shGFP or shPDK4 (n=6-8).



SUPPLEMENTARY DATA

Supplementary Figure 2. Genetic modulation of PDK4 abundance in the liver or short-term pharmacological PDK inhibition does not affect body weight, composition, food intake or hepatic triglyceride levels.

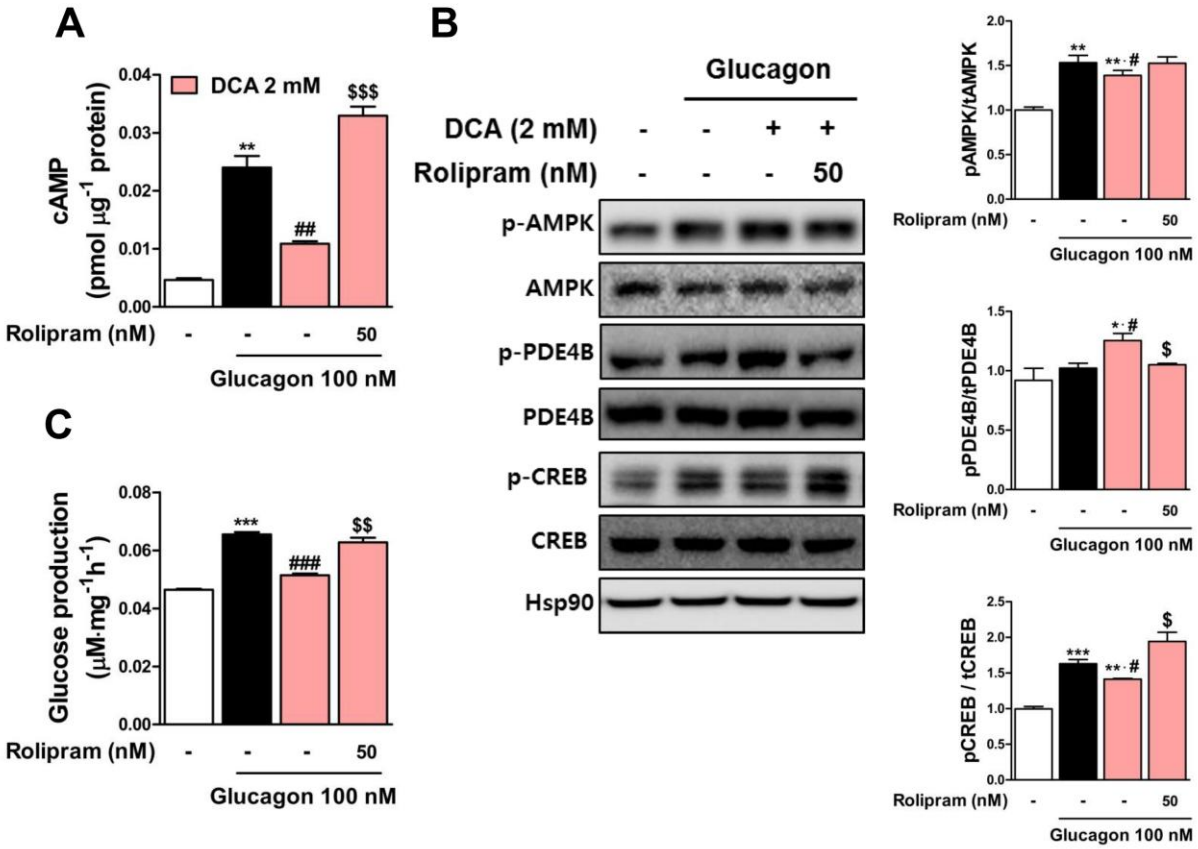
(A) Body composition, body weight, food intake (day 0 and day 7) and hepatic triglyceride levels of shGFP and shPDK4-injected diet-induced obesity (DIO) mice (n=6-8). (B) Body composition, body weight, food intake (day 0 and day 7) and hepatic triglyceride levels of intraperitoneally vehicle (VEH) and DCA (300mg/kg)-treated DIO mice (n=7-9). (C) Body composition, body weight, food intake (day 0 and day 7) and hepatic triglyceride levels of Ad-Mock and Ad-PDK4-injected mice (n=10-11).



SUPPLEMENTARY DATA

Supplementary Figure 3. Restoration of cAMP signaling cancels the effect of PDK4 inhibition on gluconeogenesis

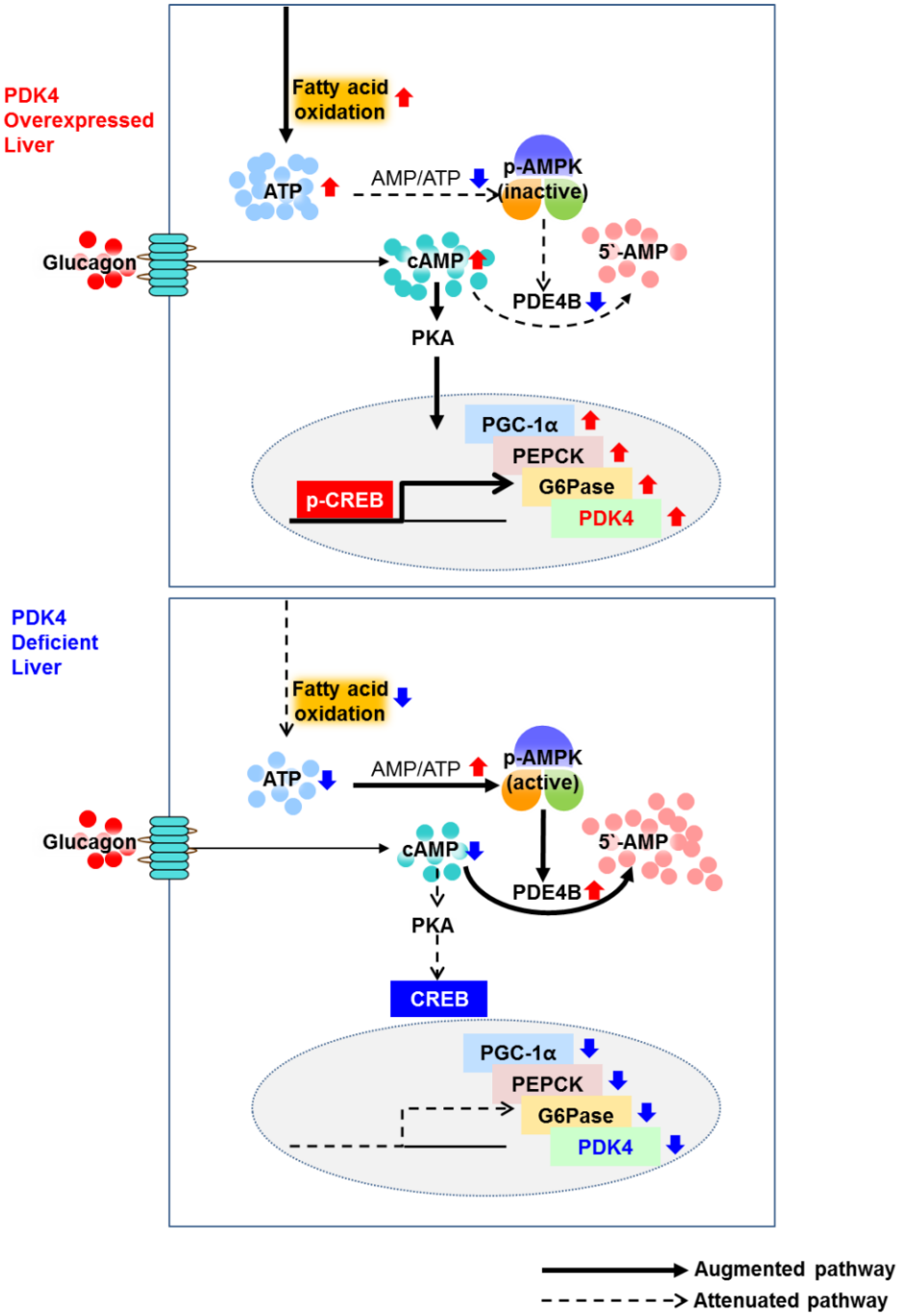
(A-C) Effect of rolipram (50 nM) on intracellular cAMP concentration (A), p/t-CREB, p/t-AMPK, and p/t-PDE4B levels (B), and hepatic glucose production in primary mouse hepatocyte treated with 2 mM of DCA (pink bars) (C). Hepatocytes were exposed with or without 100 nM of glucagon. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with untreated hepatocytes (control; white bars). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with glucagon treated-hepatocyte (black bars). $^{\$}P < 0.05$, $^{\$\$}P < 0.01$, $^{\$\$\$}P < 0.001$ compared with both glucagon- and DCA-treated hepatocyte.



SUPPLEMENTARY DATA

Supplementary Figure 4. Schematic model showing the mechanism by which PDK4 deficiency inhibits hepatic gluconeogenesis

In diabetic condition, PDK4 expression in the liver is increased by augmented glucagon signaling pathway. PDK4 deficiency in the liver decreases FAO flux and corresponding ATP concentration, which is sufficient to modulate AMP/ATP ratio and AMPK phosphorylation. This, in turn, increases PDE4B phosphorylation which is responsible for cAMP degradation. Reduction of cAMP level as a result of increased cAMP degradation in the hepatocytes attenuates PKA-CREB signaling pathway and decreases gluconeogenic gene expression. Of note, attenuation of the signaling further decreases PDK4 transcription.



SUPPLEMENTARY DATA

Supplementary Table 1. Mouse primer sequence for real-time PCR

Gene	Forward (5`-3`)	Reverse (5`-3`)
Gapdh	GAGGGTGGAGCCAAAAG	GCTGACAATCTTGAGTGAGTTG
Pdk1	CACCACGCGGACAAAGG	GCCCAGCGTGACGTGAA
Pdk2	CCCCGTCCCCGTTGTC	TCGCAGGCATTGCTGGAT
Pdk3	GGAGCAATCCCAGCAGTGAA	TGATCTTGTCCTGTTTAGCC
Pdk4	CCATGAGAAGAGCCCAGAAGA	GAACTTTGACCAGCGTGTCTACAA
Pgc-1α	TGCGGGATGATGGAGACA	GCGAAAGCGTCACAGGTGTA
G6pase	CAACCGCCATGCAAAGG	CTGGCCTCACAATGGGTTTC
Pepck	CGCCCAGCAGCCAAGTT	TCTTTGTCCTTCCGGAACCA
Cpt-1	CGATCATCATGACTATGCGCTACT	GCCGTGCTCTGCAAACATC