SUPPLEMENTARY DATA

Prostaglandin $F_{2\alpha}$ facilitates hepatic glucose production through CaMKIIγ/p38/FoxO1-signaling pathway in fasting and obesity

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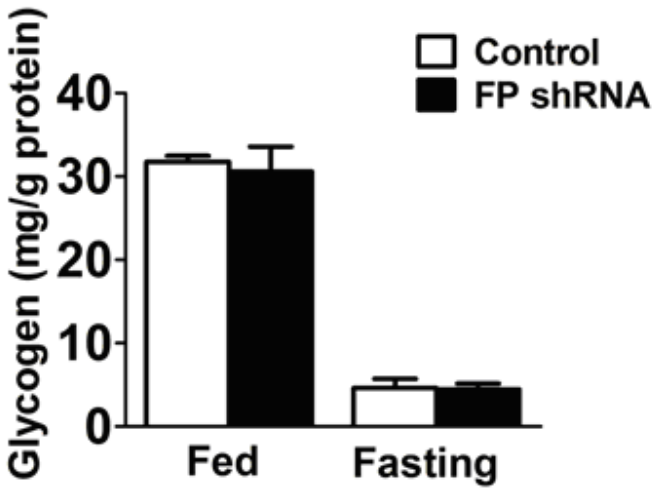
Supplementary Figure 1. Hepatic glycogen content in fed or fasted mice after FP shRNA adenovirus infection (n = 4).



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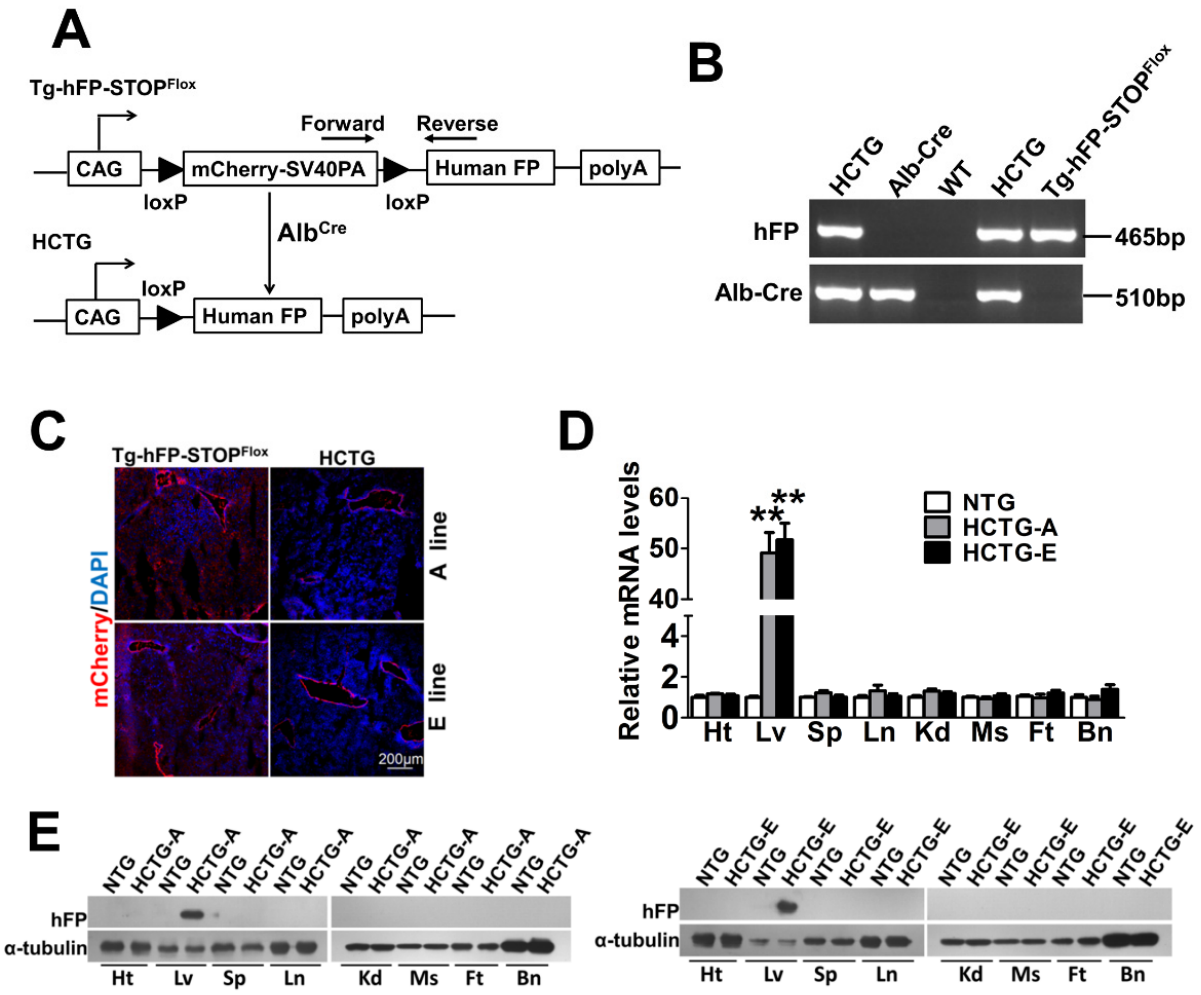
Supplementary Figure 2. Characterization of hFP transgenic mice. (A) The Cre-loxP strategy for generation of HCTG mice. **(B)** PCR genotyping of tail biopsies of offspring from the mating of hFP Tg-Stop with Alb^{Cre} mice. HCTG mice expressing both hFP (465 bp) and Alb^{Cre} (510 bp). **(C)** Representative liver immunofluorescence-staining images of mCherry in Tg-hFP-$STOP^{Flox}$ mice and HCTG mice (mCherry-negative). **(D)** mRNA levels of hFP in different tissues from NTG and HCTG mice. $^{**}P < 0.01$ vs. NTG ($n = 6$). Ht, heart; Lv, liver; Sp, spleen; Ln, lung; Kd, kidney; Ms, muscle; Ft, fat; Bn, brain. **(E)**Immunoblotting assays of hFP protein in different tissues from NTG and HCTG mice.



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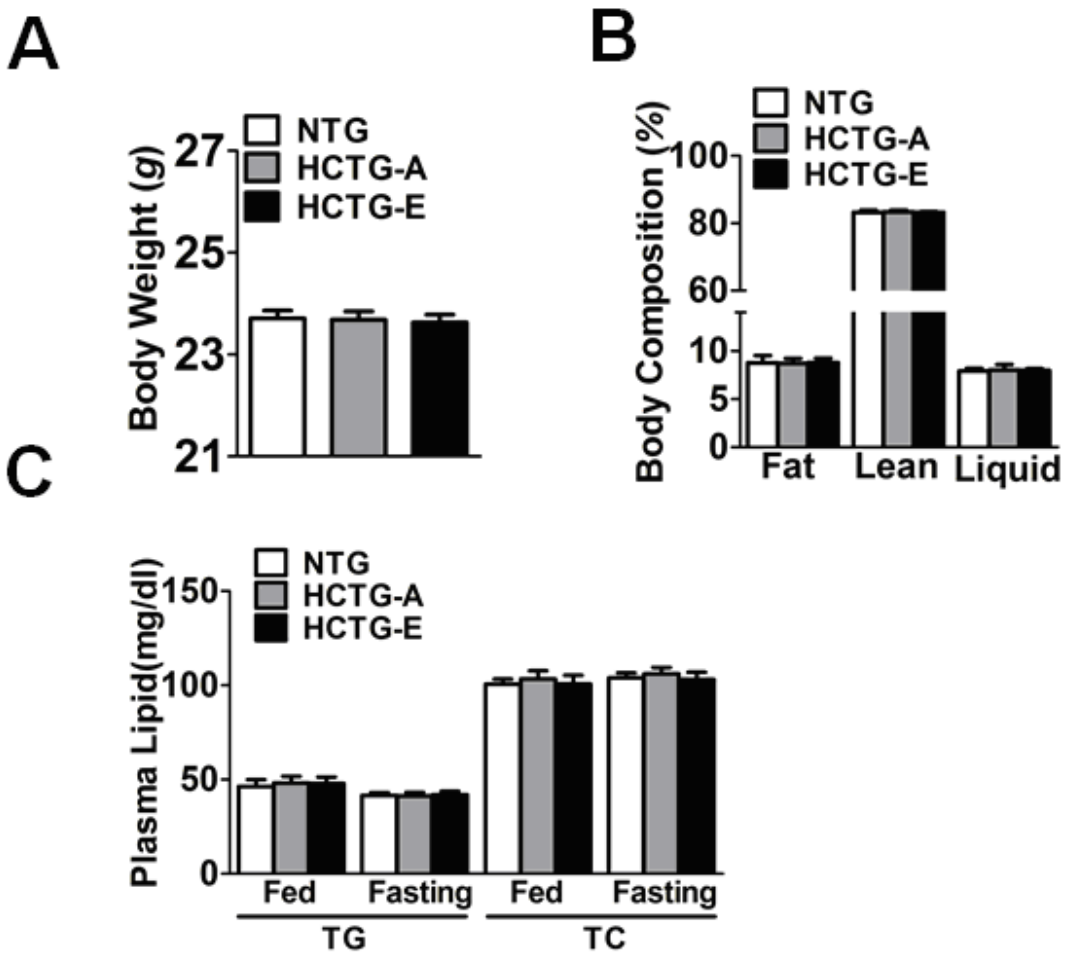
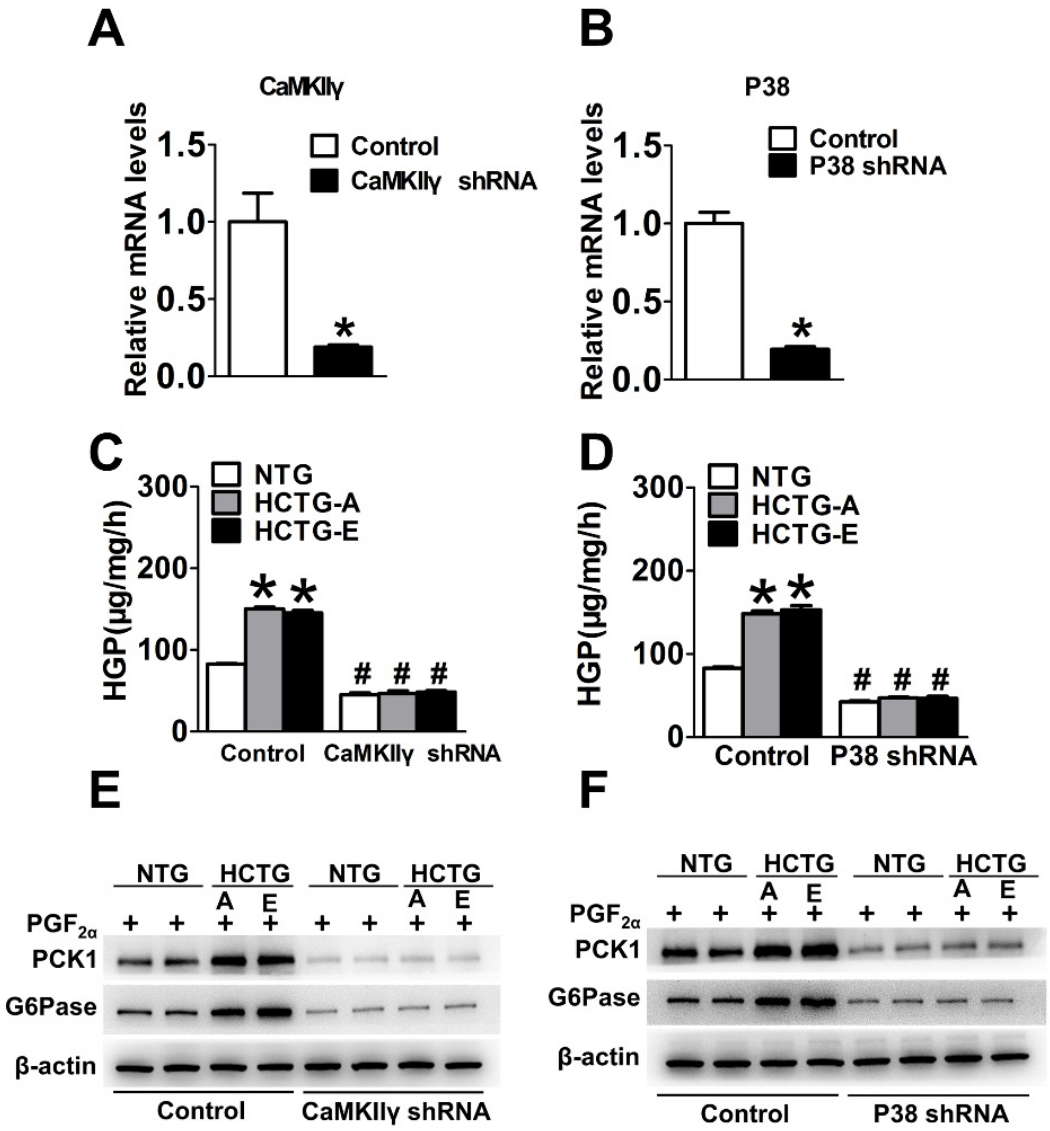
Supplementary Figure 3. Body weight, body composition, and lipid profiles in hFP HCTG mice. (A) Body weights and **(B)** body compositions of HCTG and NTG littermates (8-weeks old) (*n* = 7). **(C)** Plasma total triglyceride and cholesterol levels in HCTG and NTG littermates (8-weeks old) after feeding or 8 h of fasting.



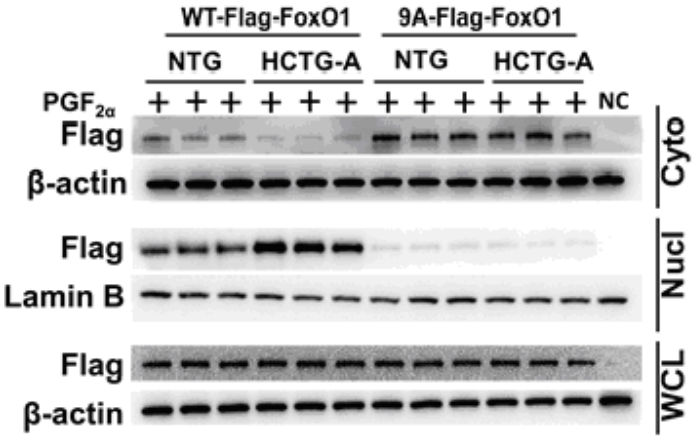
SUPPLEMENTARY DATA

Supplementary Figure 4. Silencing CaMKIIγ or p38 significantly attenuates $PGF_{2\alpha}$-induced hepatic glucose production (HGP). (A) CaMKIIγ and p38α mRNA expression levels in primary hepatocytes treated with shRNA adenovirus. **P* < 0.01 vs. Control (*n* = 3). **(B,C)** Hepatic glucose production in $PGF_{2\alpha}$-stimulated NTG and HCTG hepatocytes after infection with CaMKIIγ shRNA or P38 shRNA adenovirus, respectively. **P* < 0.01 vs. NTG, # *P* < 0.05 vs. Control(*n* = 3). **(D,E)** Expression of PCK1 and G6Pase protein in NTG and HCTG hepatocytes after infection with CaMKIIγ shRNA or P38 shRNA adenovirus, respectively.

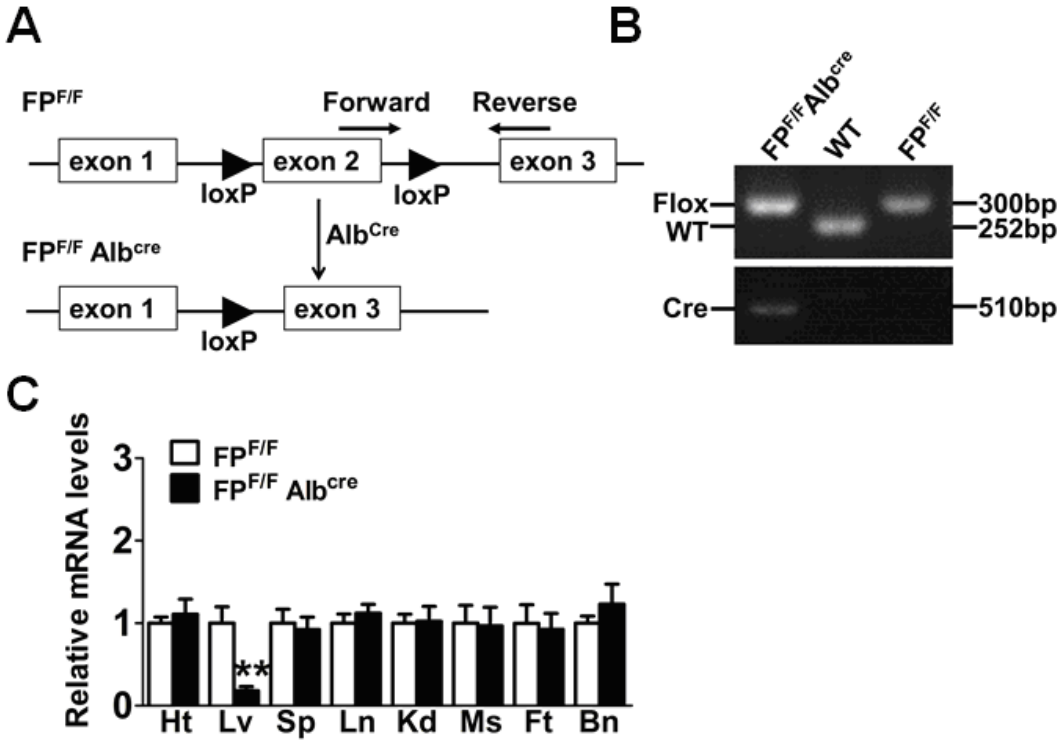


SUPPLEMENTARY DATA

Supplementary Figure 5. Mutation of p38 phosphorylation sites in FoxO1 protein leads to nuclear translocation defect in response to $PGF_{2\alpha}$ in HCTG hepatocytes. NTG and HCTG hepatocytes were transfected with adenovirus encoding WT-FLAG-FoxO1 or 9A-FLAG-FoxO1 mutant, then stimulated with $PGF_{2\alpha}$. Nuclear and cytosolic distribution of FoxO1 were assayed by immunoblot.



Supplementary Figure 6. **Construction and characterization of hepatocyte-specific FP-deficient mice. (A)** The Cre-loxP strategy for generation of hepatocyte-specific FP-knockout mice ($FP^{F/F}Alb^{cre}$). **(B)** PCR genotyping of tail biopsies of offspring from wild-type, $FP^{F/F}$, and $FP^{F/F}Alb^{cre}$ mice. $FP^{F/F}Alb^{cre}$ mice showed both bands of $FP^{F/F}$ (300 bp) and Alb^{cre} (510 bp). **(C)** mRNA levels of FP in different tissues from $FP^{F/F}$ and $FP^{F/F}Alb^{cre}$ mice. $^{**}P < 0.01$ vs. $FP^{F/F}$ ($n = 6$). Ht, heart; Lv, liver; Sp, spleen; Ln, lung; Kd, kidney; Ms, muscle; Ft, fat; Bn, brain.



SUPPLEMENTARY DATA

Supplementary Table S1. Primers for PCR.

	Forward primer (5′–3′)	Reverse primer (5′–3′)
hFP	ACCTCCCCCTGAACCTGAAA	CGATGGCAAGGCTGTTTGAC
Alb-Cre	CCAGGCTAAGTGCCTTCTCTACA	AATGCTTCTGTCCGTTTGCCG
CaMKIIα	TGCCTGGTGTTGCTAACCC	CCATTAACTGAACGCTGGAACT
CaMKIIβ	GCACGTCATTGGCGAGGAT	ACGGGTCTCTTCGGACTGG
CaMKIIγ	ACCGACGACTACCAGCTTTTC	GCAGCATATTCCTGCGTAGATG
CaMKIIδ	TCAAGGCCGGAGCTTACGA	GAGGCTGTGATACGTTTGGCA
β-actin	AACGCAGCTCAACAGTCC	TGGAATCCTGTGGCATCCATG

Supplementary Table S2. Primers for real-time PCR.

	Forward primer (5′–3′)	Reverse primer (5′–3′)
FP	GAAGTTCAGAAGCCAGCAGCATA	AGCAACGACTGGCAAGTTTATAC
hFP	CCATTCGGAGAGCAAAAAGT	AGCTCCTGGCGATAATGTGT
G6Pase	CCATGGGCGCAGCAGGTG	AGGTAGATCCGGGACAGACAGACG
PCK1	CGCTGGATGTCGGAAGAG	AGTCTGTCAGTTCAATACCAATC
Pygl	GCCATCGCCGTGTTGAC	GCCCTGACGGCAGCATT
P38	CTGACCGACGACCACGTTC	CTTCGTTCACAGCTAGGTTGC
CaMKIIα	TGCCTGGTGTTGCTAACCC	CCATTAACTGAACGCTGGAACT
CaMKIIβ	GCACGTCATTGGCGAGGAT	ACGGGTCTCTTCGGACTGG
CaMKIIγ	ACCGACGACTACCAGCTTTTC	GCAGCATATTCCTGCGTAGATG
CaMKIIδ	TCAAGGCCGGAGCTTACGA	GAGGCTGTGATACGTTTGGCA
h18S	CAGCCACCCGAGATTGAGCA	TAGTAGCGACGGGCGGTGTG
18S	AGGGGAGAGCGGGTAAGAGA	GGACAGGACTAGGCGGAACA
β-actin	CGTGCGTGACATCAAAGAGAAG	CGTTGCCAATAGTGATGACCTG

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Supplementary Table S3. List of antibodies

Antibody	Manufacturer	Catalog Number
PCK1	Proteintech, China	16754-1-AP
p38	Proteintech, China	14064-1-AP
lamin B	Proteintech, China	12987-1-AP
G6Pase	Santa Cruz Biotechnology, USA	sc-25840
CaMKIIγ	Santa Cruz Biotechnology ,USA	sc-1541
α-tubulin	Cell Signaling Technology, USA	2125
phospho-Thr287 CaMKIIγ	Cell Signaling Technology, USA	12716
phospho-p38	Cell Signaling Technology, USA	4511
FoxO1	Cell Signaling Technology, USA	2880
HA-Tag	Cell Signaling Technology, USA	5017
hFP	Epitomics, USA	5523-1
β-actin	Sigma-Aldrich, USA	A5441

Supplementary Table S4. shRNA Sequences

shRNA	Forward (5′–3′)	Reverse (5′–3′)
FP	GATCCCG***CC*****TTAAAGGTTGCTGCT** **ATTT**GAAGCTTG**AAATAGCAGCAA** **CCTTTAAGG**TTTTTT	CTAGAAAAAA**CCTTAAAGGTTGCTGCT** **ATTT**CAAGCTTC**AAATAGCAGCAACCT** **TTAAGG**CGG
CaMKIIγ	GATCCCG**TTAATGATTTCTTGTTTT** **CTC**GAAGCTTG**GAAAACAAGAAAT** **CATTAAGA**TTTTTT	CTAGAAAAAA**TTAATGATTTCTTGTTT** **TCTC**CAAGCTTC**GAAAACAAGAAATCA** **TTAAGA**CGG
P38	GATCCCG**TGACCGGAAGAACGTT** **GTTTC**GAAGCTTG**GAAACAACGTT** **CTTCCGGTCA**TTTTTT	CTAGAAAAAA**TGACCGGAAGAACGTT** **GTTTC**CAAGCTTC**GAAACAACGTTCTT** **CCGGTCA**CGG