Supplementary information

Statin-activated nuclear receptor PXR promotes SGK2 dephosphorylation by scaffolding PP2C to induce hepatic gluconeogenesis

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Supplementary Figure 1. Statin induction of the *G6Pase* gene mediated by SGK2 and PXR. (A) Relative expression of G6Pase mRNA levels measured by qRT-PCR in SGK2 siRNA (left) or PXR siRNA (right)-transfected human primary hepatocytes treated with simvastatin (Simva, 10 μM) for 24 h. Results are shown as fold change relative to DMSO treated control siRNA transfected cells. (n = 3, mean ± s.d.) *P < 0.05, ***P < 0.001. NS, not significant, determined by One-way ANOVA. (B) Relative expression of G6Pase mRNA levels measured by qRT-PCR in SGK2 siRNA (left) or PXR siRNA (right)-transfected ShP51 cells treated with simvastatin (Simva, 10 μM) for 2.5 and 5 h. Results are shown as fold change relative to DMSO treated control siRNA transfected cells. (n = 3, mean ± s.d.) **P < 0.01, ***P < 0.001. NS, not significant, determined by One-way ANOVA.

Supplementary Figure 2. Effect of phosphatase inhibitors on simvastatin induction of gluconeogenic genes. Relative expression of PEPCK1 (left) and G6Pase (right) mRNA levels measured by qRT-PCR in ShP51 cells which are pre-treated with (A) sanguinarine chloride (3 μM), (B) okadaic acid (10 nM) or (C) tautomycin (20 nM) and fostriecin (100 nM) for 30 min, followed by co-treatment with simvastatin (Simva, 10 μM) for additional 3

h. Results are shown as fold change relative to DMSO treated cells. (n = 3, mean ± s.d.) **P < 0.01, ***P < 0.001. NS, not significant, determined by One-way ANOVA. (D) Immunoprecipitation of FLAG-SGK2 and Western blot analysis of PP2Cα, PP1α, PP2A and FLAG-SGK2 in pcDNA/FLAG/SGK2 T193A or pcDNA/FLAG/SGK2 T193D-transfected ShP51 cells treated with simvastatin (Simva, 10 μM) for 60 min.

Supplementary Figure 3. SGK2 binding sites at 107/141 and 334/348 of PXR. Top, immunoprecipitation of FLAG-PXR WT and FLAG- PXR Δ107/141, Δ334/348 and Western blot analysis of SGK2, PP2Cα and FLAG-PXR in pcDNA/SGK2 and pCR3/FLAG/PXR or pCR3/FLAG/PXR Δ107/141, Δ334/348 transfected HepG2 cells treated with simvastatin (Simva, 10 μM) for 60 min. Bottom, schematic representation of domain structure of human PXR WT and PXR Δ107/141, Δ334/348. DBD: DNA binding domain, LBD: ligand binding domain.

Supplementary Figure 4. Scheme of identification of PSRE within the upstream region of gluconeogenic genes. 10 K upstream region of human *G6Pase* gene was searched for PXR binding sequences analyzed by GCG Seqlab. 27 putative binding sites

were aligned with 10 K upstream region of human *PEPCK1*gene, resulting in 5 putative sites found. ChIP assays were subjected to examine PXR levels at regions A (-8,460/-8,033), B (-7,146/-6,824), C (-5,083/-4,633) and D (-1,759/-1,590, PSRE) of the human *G6Pase* promoter in ShP51 cells treated with rifampicin (10 μM) for 1 h.

Supplementary Figure 5. Statin-treatment increase the human *PEPCK1* IRS promoter in ShP51 cells. Luciferase activity of human *PEPCK1* promoter in ShP51 cells exposed to simvastatin (Simva, 10 μM) for 24 h. Results are shown as fold change relative to DMSO treated cells (n = 3, mean ± s.d). **P < 0.01, determined by Student's t test.

Supplementary Figure 6. Statin-treatment did not affect phosphorylated status of SGK2 in mouse livers. Left, relative expression of PEPCK1 mRNA levels measured by qRT-PCR in livers of simvastatin (50 mg/kg)-administrated mice. Results are shown as fold change relative to control mouse livers (n = 3, mean ± s.e.) NS, not significant, determined by Student's t test. Right, Western blot analysis of p-SGK2, SGK2 and β-actin from whole cell lysates from livers of simvastatin (50 mg/kg)-administrated mice.

Supplementary Table 1 Primer sequences used for plasmid constructions

	Primer Sequences (5'-3')
hSGK2 full length	ACCATGAACTCTAGCCCAGCTGGGACCC;
	CTAGCAATCCAAGATGTCATCATCCTCTGG
Mutation of SGK2 T93A	TGAAGACACCACATCCGCATTCTGTGGTACCCC;
	GGTACCACAGAATGCGGATGTGGTGTCTTCA
Mutation of SGK2 T193D	TGAAGACACCACATCCGACTTCTGTGGTACCCC;
	GGGGTACCACAGAAGTCGGATGTGGTGTCTTCA
FLAG-SGK2	AACCCAGAATTCAATGAACTCTAGCCCAGCTGGGA;
	CGTGCTGGATCCCTAGCAATCCAAGATGTCATCA
PXR Δ107/141	CGCAAGTGCCTGGAGAGCGGCATGGGGCTGAC;
	CATCCGCTGCTCCTCTGTCAGCCCCATGCCGC
PXR Δ334/348	TTCCACTACATGCTGAAGAAGCTGGAGGAGGA;
	CTGCAGCTGGTCCACCACGCGGTGATGCAGCT

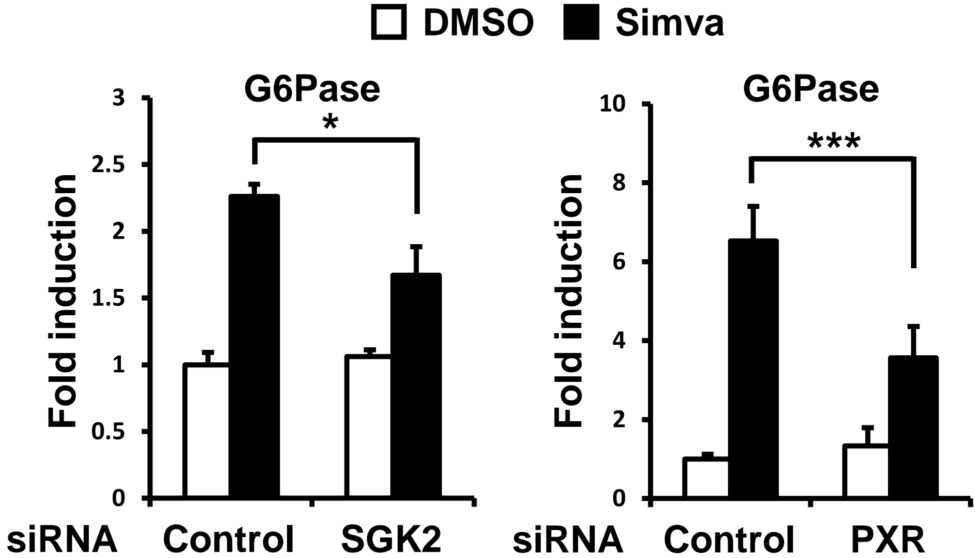
Supplementary Table 2 Primer sequences used for ChIP assays

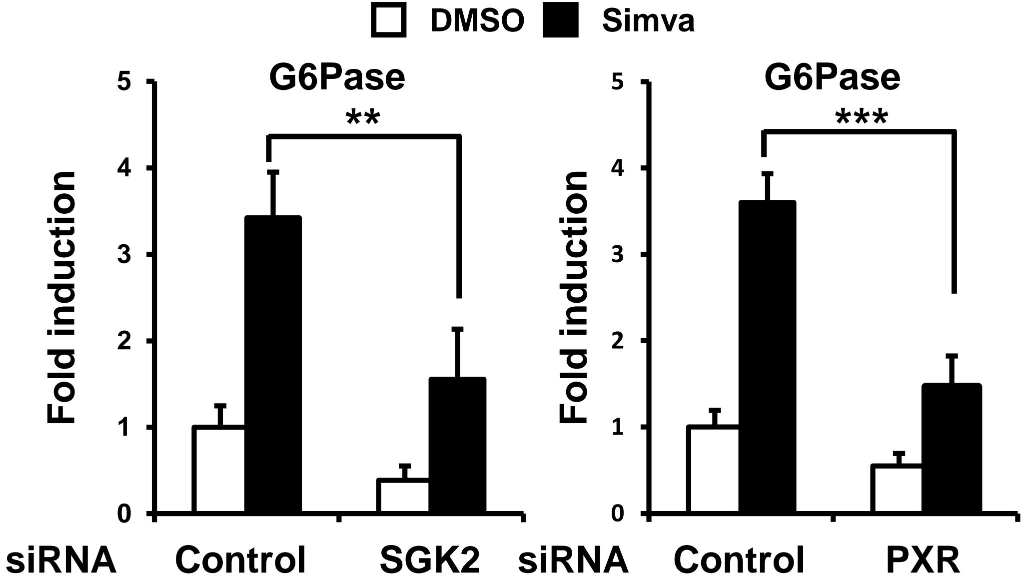
	Primer Sequences (5'-3')
PEPCK1 PSRE	TGACACCTGAGAGGTGGCCCT; TGCAGGAGGGGGCCAGACAG
PEPCK1 IRS	CCCAAGTTAGGGTGCATCCTTCCCA; ACAGGCAGGTGGGTCAAGGACA
G6Pase A	AGGCCGAGGCGGGTAGATCG; TTACAGGCACGCGCCACCAC
G6Pase B	CCTCTGCCTCTGTAGTGCGCC; CCTGCCTCAGCCTCCCGAGT
G6Pase C	CCCAGCAGGAGTGCAGTGGC; GTGCAGTGGCTCACGCCTGT
G6Pase D (PSRE)	AGACCAGCCTGGGCCGCATA; ACACTTGGGTCACGGAGGACTGT
G6Pase IRS	TGTCCTGTGTCTCTGGCCTGGT; TCAGGTCAACCCAGCCCTGATCT

Supplementary Table 3 Consensus PXR binding site and PSRE sequences

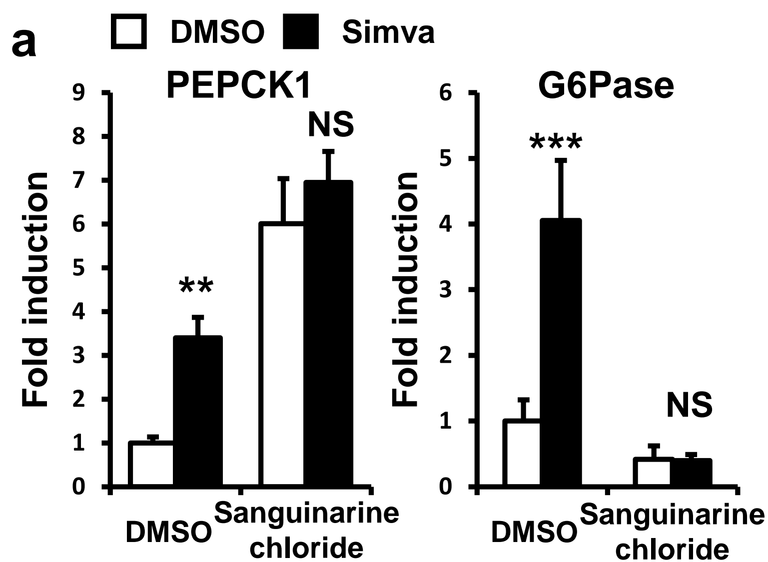
	PSRE Sequences (5'-3')
Consensus PXR binding site	TGTACTCCGTGACCC
G6Pase PSRE	AGTCCTCCGTGACCC
PEPCK1 PSRE	GGGTCTGCCATGACT (Complementary sequence is similar.)

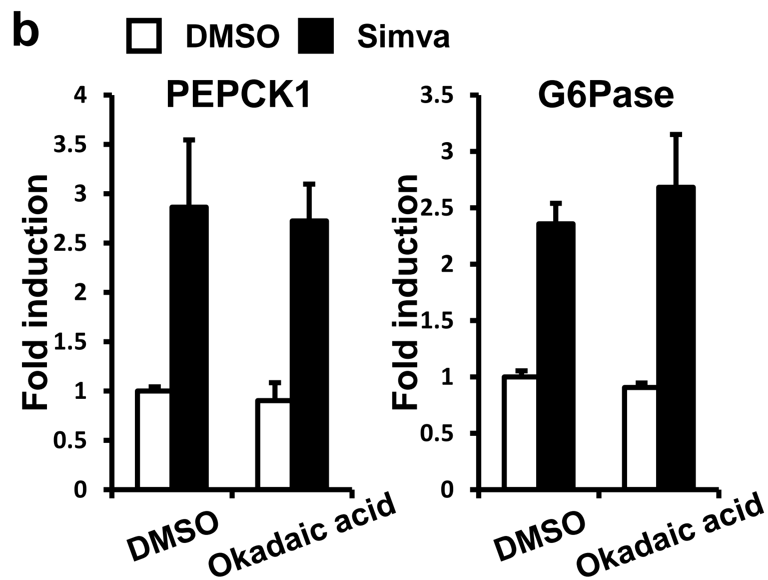
Supplemental Figure 1

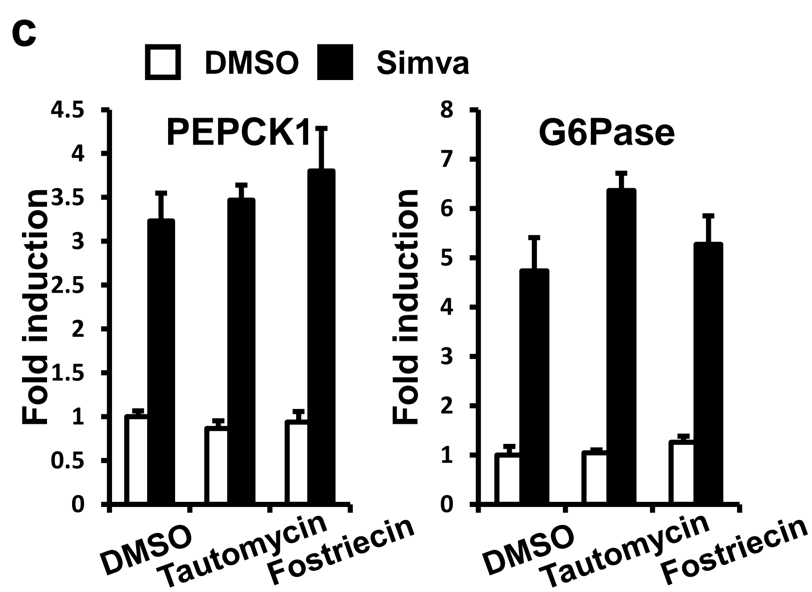


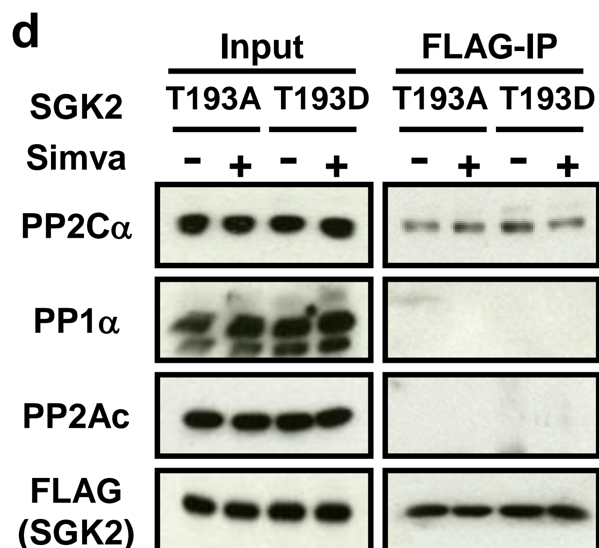


Supplemental Figure 2

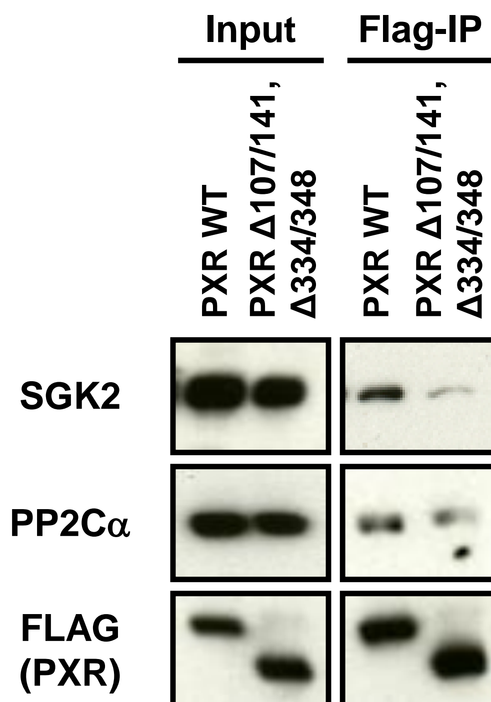




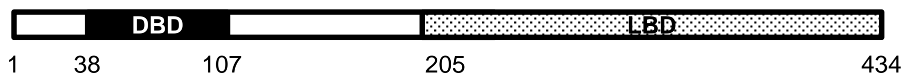




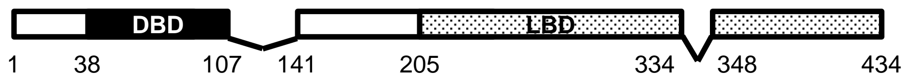
Supplemental Figure 3



PXR WT



PXR Δ107/141, Δ334/348



Supplemental Figure 4

Within -10K region of the human *G6Pase* gene

Seqlab

27 sites selected

Aligning these 27 sites within the *PEPCK1* gene

GENETYX

5 conserved

-10kb

-5kb

G6Pase

Putative PXR binding site

A B C D

ChIP assays with α-PXR antibody

Input

α-PXR

Rifampicin – + – +

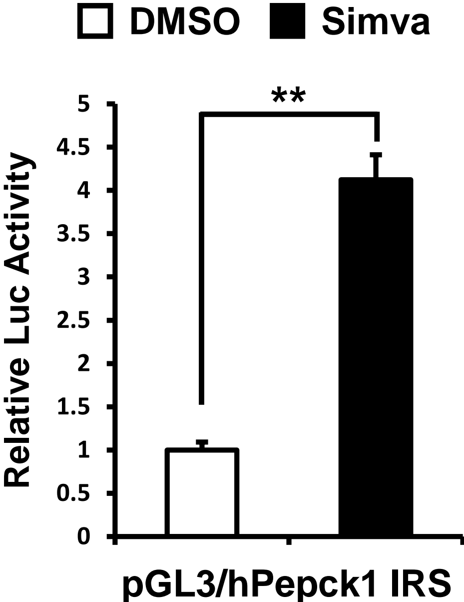
B

C

D (PSRE)

1 found (D)

Supplemental Figure 5



Supplemental Figure 6