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Drug repurposing in alternative medicine: herbal digestive Sochehwan exerts multifaceted effects against metabolic syndrome

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Supplementary methods

Chemicals and reagents

For in vitro study, Oil-Red O, oleic acid (OA), palmitic acid (PA), 3-isobutyl-1-methylxanthine (IBMX), and dexamethasone were all purchased from Sigma Chemicals (St. Louis, MO, USA). 2-NBDG (2-(2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose)) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Antibodies to phosphorylated acetyl-CoA carboxylase (p-ACC), acetyl-CoA carboxylase (ACC), phosphorylated 5' AMP-activated protein kinase (p-AMPK), 5' AMP-activated protein kinase (AMPK), CCAAT/Enhancer Binding Protein (C/EBP), and carnitine palmitoyltransferase 1 (CPT1) were purchased from Cell Signaling Technology (Beverly, MA, USA). Peroxisome proliferator-activated receptor gamma (PPAR-γ) antibodies were purchased from Abcam (Cambridge, UK). Leptin, GLUT2, β-actin antibodies were purchased from Santa Cruz (Santa Cruz, California, USA). HRP-conjugated secondary antibodies were purchased from Santa Cruz (USA). Alexa fluor 488 conjugated goat anti-rabbit antibody was purchased from Thermo Fisher Scientific (USA). Oligo nucleotide primers used in real-time PCR were purchased from Macrogen (Seoul, South Korea).

Determination of cell viability

Effect of SCH on cell viability was determined by EZ-Cytox cell viability assay kit (Daeil Lab Service, Seoul, Korea) following the kit protocol. HepG2 cells and 3T3-L1 cells were seeded at 96 well plates with density of $1x10^4$ cells per well and incubated for 24 h. Cells were incubated with various concentrations (0-100 μg/ml) of SCH for 24 h. EZ-Cytox kit reagent was added to each well and then incubated at 37℃ for 2 h. Optical density of supernatant was measured at a wavelength of 450 nm using microplate spectrophotometer (VersaMax, Molecular Devices, Sunnyvale, CA, USA)

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Western blotting for insulin signaling proteins

Steatosis of HepG2 cells were induced by conditions described in manuscript (1 mM, OA:PA = 2:1, for 24 h). Steatotic HepG2 cells were washed twice with DPBS before changing culture medium. Cells were further incubated in fresh DMEM with SCH for 24 h. Human recombinant insulin was added to culture medium (50 nmol) for 30 min. Cells were scraped and lysed with RIPA (Thermo Fisher Scientific, USA) containing protease inhibitor and phosphate inhibitor (Gendepot, USA). Whole protein was subjected to Western blotting with same protocol as described in Methods section.

Gene expression profiling by RT^2 PCR array

Steatosis of HepG2 cells were induced by conditions described in 'Cell culture and differentiation' section of Methods (1 mM, OA:PA = 2:1, for 24 h). Intact RNA was extracted from steatotic HepG2 cells after 3 h of SCH treatment and reverse transcription was performed for preparation of quantitative real-time PCR array. Genes involved in Human liver steatosis were investigated by Human Fatty Liver RT^2 Profiler PCR Array kit (PAHS-157Z, Qiagen, Hilden, Germany) according to manufacturer's protocol. All data acquired from PCR array were analyzed by following calculation and analysis process on web Data Analysis Center

(http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php)

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Supplementary Figure and Table

Parameters	Animal Groups			
	ND	HFD	HFD+SCH LD (100 mg/kg)	HFD+SCH HD (200 mg/kg)
0 week B.W (g)	18.75 ± 1.40	18.70 ± 0.92	18.43 ± 1.49	18.82 ± 1.01
15 week B.W (g)	28.02 ± 2.99	42.70 ± 2.92#	38.85 ± 10.54	39.93 ± 8.26
FER per group (0~15 week)	0.040	0.110	0.096	0.094
Liver weight (g)	1.00 ± 0.11	2.13 ± 0.33#	1.85 ±1.04	1.82 ± 0.52
Intestine fat weight (g)	0.34 ± 0.11	1.15 ± 0.46#	0.97 ± 0.68	1.12 ± 0.53
Gonadal fat weight (g)	1.02 ± 0.31	2.62 ± 0.29#	2.15 ± 0.89	2.25 ± 0.51
Renal fat weight (g)	0.26 ± 0.09	0.93 ± 0.14#	0.78 ± 0.34	0.90 ± 0.26

Table S1. Effect of 15 week SCH administration on body weight, food efficiency ratio (FER) and organ weight in high fat diet-fed mice. All data are expressed as mean±SD. # shows statistically significant difference at $p<0.05$ as compared with the ND group. * shows statistically significant difference at $p<0.05$ as compared to the HFD group

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Primer	Sequence	
	Sequence of Forward Primer (5’-3’)	Sequence of Reverse Primer (5’-3’)
C/EBPα	GCGCAAGAGCCGAGATAAAG (20 mer)	CACGGCTCAGCTGTTCCA (18 mer)
PPARγ	ATGCCAAAAATATCCCTGGTTTC (23 mer)	GGAGGCCAGCATGGTGTAGA (20 mer)
SCD1	ATATCCTGGTTTCCCTGGGT (20 mer)	CAGCGGTACTCACTGGC (17 mer)
SREBF	GGAACAGACACTGGCCGA (18 mer)	AAGTCACTGTCTTGGTTGTTGAT (23 mer)
FABP4	CAGAAGTGGGATGGAAAGTCG (21 mer)	CGACTGACTATTGTAGTGTTTGA (23 mer)
ACC1	TGGCGTCCGCTCTGTGATA (19 mer)	CATGGCGACTTCTGGGTTG (19 mer)
β-Actin	GACGGCCAGGTCATCACTATTG (22 mer)	CCACAGGATTCCATACCCAAGA (22 mer)

Table S2 Primers used in quantitative real-time PCR

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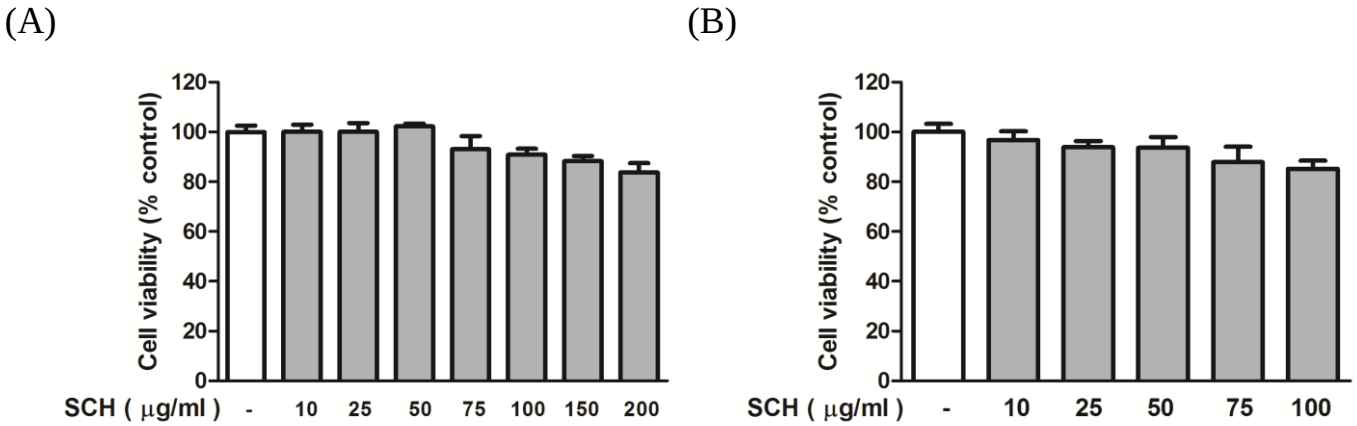

Fig. S1 Impact of SCH treatment on (A) 3T3-L1 cell viability and (B) HepG2 cell viability. Results are expressed as percentage of non-treated control. All data are expressed as mean±SD.

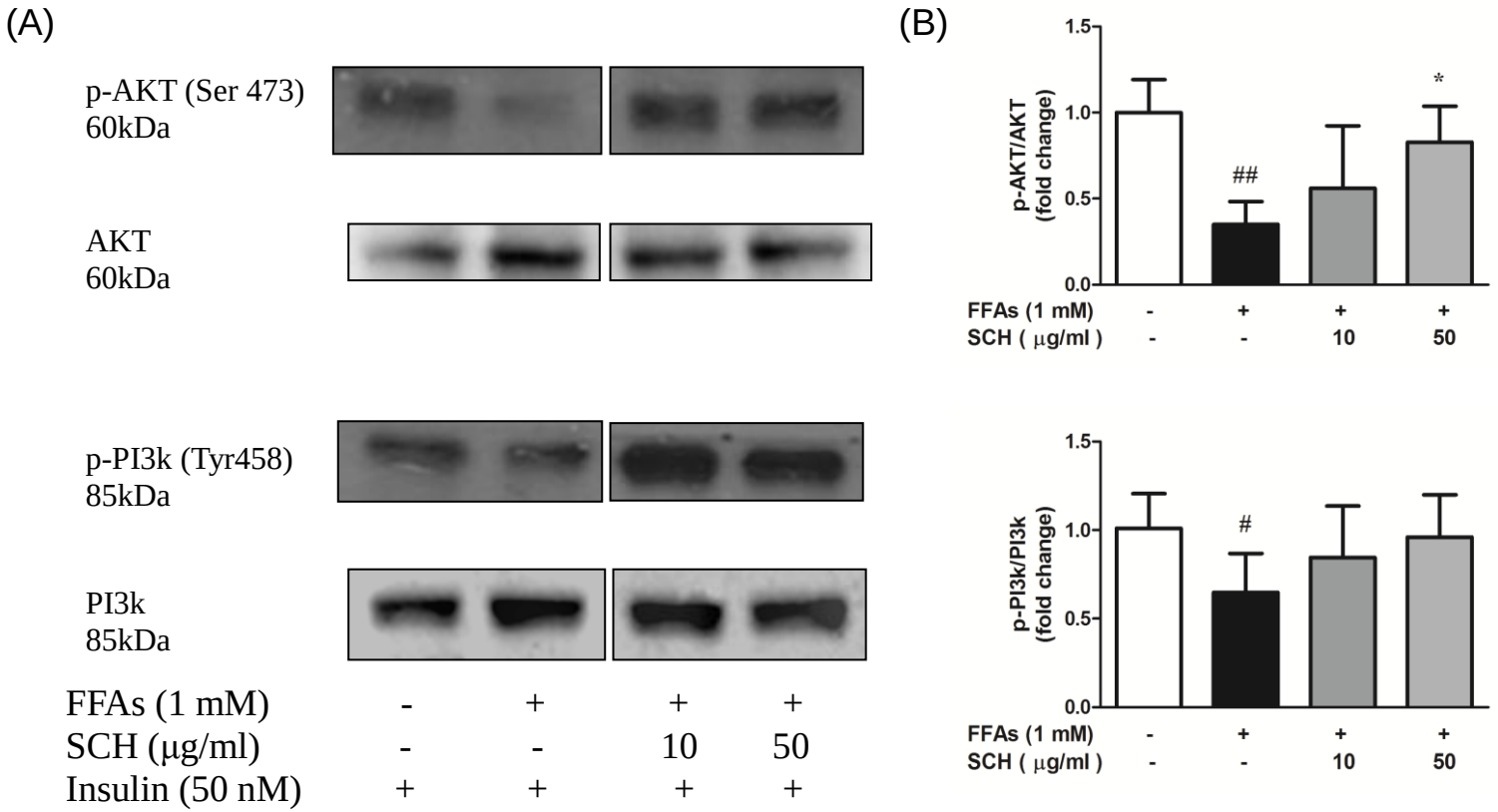

Fig. S2 Western blot analysis showing the effect of SCH on phosphorylation of proteins involved with insulin signaling pathway after insulin stimulation in FFA-induced steatotic HepG2 cells. (A) Representative blots of proteins are depicted. (B) Band intensity was measured with densitometric analysis and normalized to the intensity of non-phosphorylated AKT or PI3k. Data are expressed as mean±SD.

Full-length blots are represented in Supplementary Figure 4.

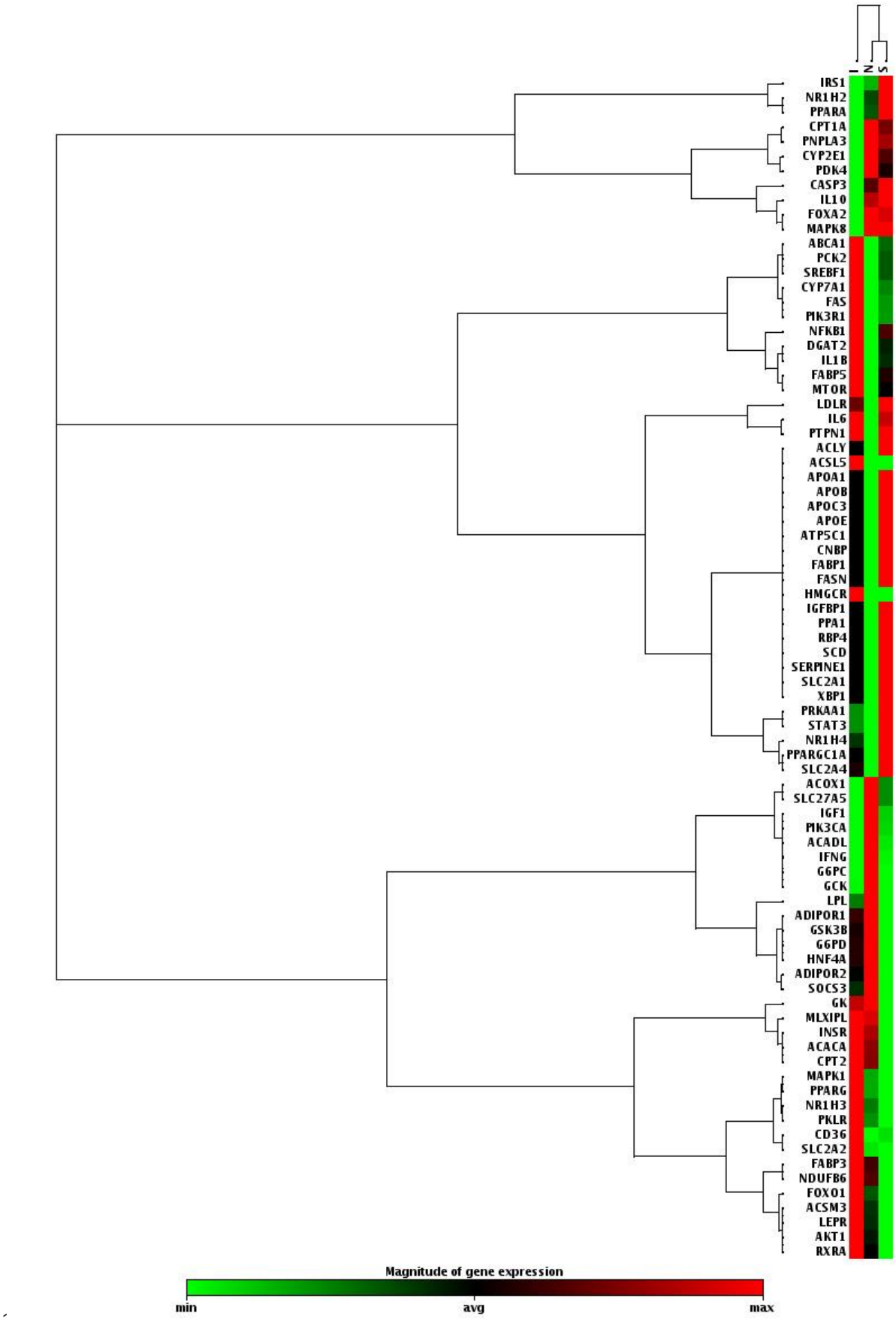
I
N
S
IRS1
NR1H2
PPARA
CPT1A
PNPLA3
CYP2E1
PDK4
CASP3
IL10
FOXA2
MAPK8
ABCA1
PCK2
SREBF1
CYP7A1
FAS
PIK3R1
NFKB1
DGAT2
IL1B
FABP5
MTOR
LDLR
IL6
PTPN1
ACLY
ACSL5
APOA1
APOB
APOC3
APOE
ATP5C1
CNBP
FABP1
FASN
HMGCR
IGFBP1
PPA1
RBP4
SCD
SERPINE1
SLC2A1
XBP1
PRKAA1
STAT3
NR1H4
PPARGC1A
SLC2A4
ACOX1
SLC27A5
IGF1
PIK3CA
ACADL
IFNG
G6PC
GCK
LPL
ADIPOR1
GSK3B
G6PD
HNF4A
ADIPOR2
SOCS3
GK
MLXIPL
INSR
ACACA
CPT2
MAPK1
PPARG
NR1H3
PKLR
CD36
SLC2A2
FABP3
NDUFB6
FOXO1
ACSM3
LEPR
AKT1
RXRA
Magnitude of gene expression
min
avg
max

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Fig. S3 Hierarchical clustering result of PCR array of genes associated with human hepatic steatosis in HepG2 cells. The heatmap diagram denotes the level of gene expression of each group normalized to that of the FFA-induced group. Dendrogram height represents relative measure of dissimilarity between clusters. N : control group, I : FFAs-induced steatosis group, S : exposed to FFAs with SCH group.

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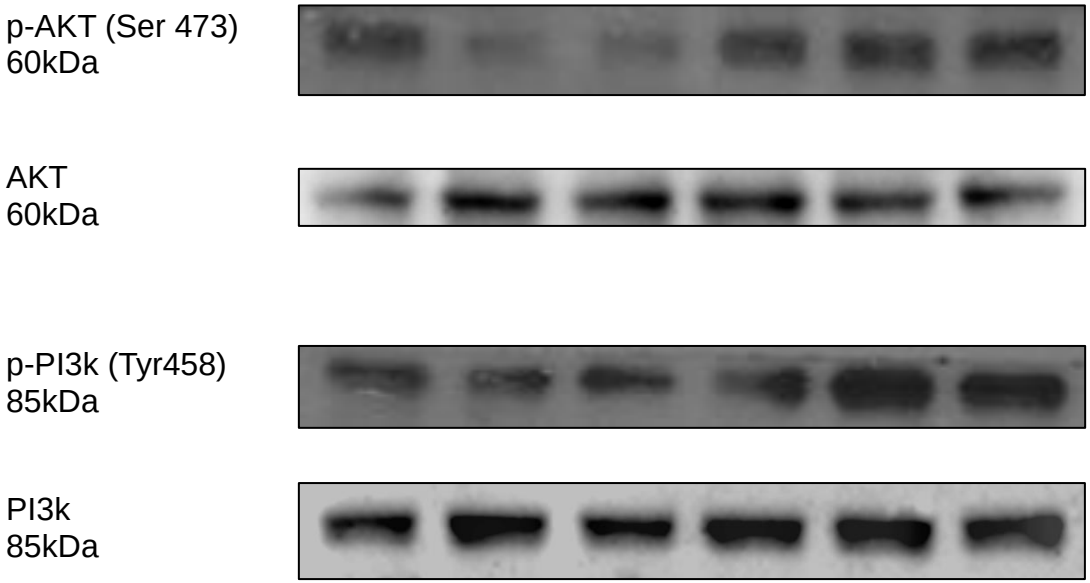

Fig. S4 Full-length blots of Supplementary Figure 2. Proteins from HepG2 Cells treated with other test samples were loaded at Lane 3 and Lane 4.