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Supplemental Information

**Soluble Klotho Improves Hepatic Glucose
and Lipid Homeostasis in Type 2 Diabetes**

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Supplementary Materials

Materials and Methods

Measurement of fasting glucose, insulin and glucose tolerance tests

For QUICKI calculation ($1 / [\log (\text{fasting insulin mU/mL}) + \log (\text{fasting glucose mg/dL})]$), fasting plasma glucose and insulin were determined respectively with an Accu-Chek (Performa) and by ELISA (ThermoScientific, EMINS). For GTT, briefly, 1 mg/g body weight of D-(+)-glucose 47829) was administered by intraperitoneal route to mice fasted for 12h. Glycemia was checked blood from tail vein 15 min before glucose injection and then at various times after glucose administration.

Measurement of Liver Lipid and Function

Levels of TG, TC, NEFA, LDL and HDL were measured using corresponding commercial determination kits (F001-1-1 for TG, F002-1-1 for TC, A042-2-1 for NEFA A112-2-1 for HDL A113-2-1 for LDL; Jiancheng, Nanjing, China) with liver tissue or serum samples. Liver function valued by serum levels of ALT and AST was measured using an ADVIA 2400 Chemistry System analyzer (Siemens, Tarrytown, NY) according to the manufacturer's instructions.

Co-immunoprecipitation assay

Co-immunoprecipitation was performed in L02 cells using Pierce™ Co-Immunoprecipitation Kit (Thermo-Fisher Scientific, 26149) according to the manufacturer's protocol. Briefly, immunoprecipitations of IGF1R were performed using an anti-IGF1R antibody overnight at 4 °C. After washing, anti-Klotho antibody was used for western blot analysis.

Supplementary Tables

Table S1. Primers for qPCR.

Gene	Sequence 5'→3'
human <i>GCK</i>	F:GGAGGAAACTGTGACTGAACCTCAA R: AGGCTTCTGAAGTGGAAATATCCAA
human <i>PEPCK</i>	F: GTTCAATGCCAGGTTCCCAG R: TTGCAGGGCCAGTTGTTGAC
human <i>PPARγ</i>	F: GGGATCAGCTCCGTGGATCT R: TGCAC TTTGGTACTCTTGAAGTT
human <i>SREBP1c</i>	F: ACAGTGACTTCCTGGCCTAT R: GCATGGACGGGTACATCTTCAA
human <i>SCD1</i>	F: GCCCCTCTACTTGAAGACGA R:AAGTGATCCCATACAGGGCTC
human <i>FASN</i>	F: ACAGCGGGGAATGGGTACT R: GACTGGTACAACGAGCGGAT
human <i>Cd36</i>	F: AATGCCAGTTGGAGACCTGC R: TCAGCGTCCTGGGTACATT
human <i>FATP1</i>	F: AAGGGCATGGACGATCGTCTTTTCT R: CGAAGGCTGCCATGCGGTAGTACCT
human <i>CIDEA</i>	F: ATTGATGTGGCCCGTGTAACG R: CAGCAGTGCAGATCATAGGAAA
human <i>PPARα</i>	F: ATGGTGGACACGGAAAGCC R: CGATGGATTGCGAAATCTCTTGG
human <i>ACOX1</i>	F: TGGCCGCTATGATGGGAATG R: CAGTGACTTCAGGTGCTTGT

human <i>CPT1α</i>	F: TCCAGTTGGCTTATCGTGGTG R: CTAACGAGGGGTTCGATCTTGG
human <i>β-actin</i>	F: CATGTACGTTGCTATCCAGGC R: CTCCTTAATGTCACGCACGAT
mouse <i>GCK</i>	F: AGGAAGACCTGAAGAAGGTGATGA R: GTAGGTGGGCAACATCTTTACACT
mouse <i>PEPCK</i>	F: TGAGGAAGTTCGTGGAAGGCAATG R: TCATATTTCTTCAGCTTGCGGATG
mouse <i>PPARγ</i>	F: AAAAAAATCTGACACCTAAGAAAT R: TTGTAAATGTATCTTTATAAACA
mouse <i>SREBP1c</i>	F: CACTTCTGGAGACATCGCAAAC R: ATGGTAGACAACAGCCGCATC
mouse <i>SCD1</i>	F: TCTTCCTTATCATTGCCAACACCA R: GCGTTGAGCACCAGAGTGTATCG
mouse <i>FASN</i>	F: GGAGGTGGTGATAGCCGGTAT R: TGGGTAATCCATAGAGCCCAG
mouse <i>Cd36</i>	F: GACTGGGACCATTGGTGATGA R: AAGGCCATCTCTACCATGCC
mouse <i>FATP1</i>	F: TCTGTTCTGATTCGTGTTCCG R: CAGCATATAACCACTACTGGCG
mouse <i>CIDEA</i>	F: TGTCGTGTTAGCACCCGAG R: TTGCGCTGTTCTGATGGGG
mouse <i>PPARα</i>	F: TATTCGGCTGAAGCTGGTGTAC R: CTGGCATTGTTCCGGTTCT
mouse <i>ACO1</i>	F: GTCTCCGTCATGAATCCCGA R: TGCGATGCCAAATTCCCTCA

mouse *CPT1 α*

F: AGGACCCTGAGGCATCTATT

R: ATGACCTCCTGGCATTCTCC

mouse *β -actin*

F: GTGACGTTGACATCCGTAAAGA

R: GCCGGACTCATCGTACTCC

Table S2. Yeast two-hybrid system primers

Gene	Sequence 5'→3'
pGBKT7- mouse <i>IGF1R</i>	F: ATGGAGGCCGAATTCCCGGGGATCC ATGAAGTCTGGCTCCGGAGGAGGGT R: GGCCGCTGCAGGTCGACGGATCC TCAGCAGGTCGAGGACTGGGGCAGAG
pGADT7- mouse <i>IGF1R</i>	F: CGGGTGGGCATCGATACGGGATCC ATGAAGTCTGGCTCCGGAGGAGGGT R: CTGCAGCTCGAGCTCGATGGATCC TCAGCAGGTCGAGGACTGGGGCAGAG
pGADT7- mouse <i>sKL</i>	F: CGGGTGGGCATCGATACGGGATCC ATGCTAGCCCGCGCCCCTCCTCGCC R: CTGCAGCTCGAGCTCGATGGATCCC AGATAGACATTCGGGTCAGTAAACT
pGBKT7- mouse <i>sKL</i>	F: ATGGAGGCCGAATTCCCGGGGATCC ATGCTAGCCCGCGCCCCTCCTCGCC R: GGCCGCTGCAGGTCGACGGATCC AGATAGACATTCGGGTCAGTAAACT

Table S3. Analysis of the interactions of sKL with IGF1R in yeast

No	Strain	Selective agar plates			
		DDO	DDO/A	QDO	QDO/X/A
Q	Y187(pGADT7- sKL)× Y2HGold(pGBKT7-IGF1R)	White	White	White	Blue
Q1	Y187(pGADT7)× Y2HGold(pGBKT7-IGF1R)	White	No	No	No
Q2	Y187(pGADT7- sKL)× Y2HGold(pGBKT7)	White	No	No	No
P	Y187(pGADT7-IGF1R)× Y2HGold(pGBKT7- sKL)	White	White	White	Blue
P1	Y187(pGADT7)× Y2HGold(pGBKT7- sKL)	White	No	No	No
P2	Y187(pGADT7-IGF1R)× Y2HGold(pGBKT7)	White	No	No	No
M	Y187(pGADT-T)× Y2HGold (pGBKT7-53)	White	White	White	Blue
N	Y187(pGADT-T)× Y2HGold (pGBKT7-Lam)	White	No	No	No

DDO (SD/-Leu/-Trp); DDO/A (SD/-Leu/-Trp/AbA); QDO (SD/-Ade/-His/-Leu/-Trp); QDO/x/A (SD/-Ade/-His/-Leu/-Trp/X- α -gal/AbA).