Supporting Information

METHODS

Stability assay of PsTag fusion proteins in the presence of mouse plasma and renal homogenate

80 μg of PsTag600-FGF21 in phosphate buffer saline (PBS) was incubated in a BALB/c mouse plasma from untreated animals (83% v/v) at 37 ℃ for up to 48 h. Samples were taken at different time points (0 h, 1 h, 3 h, 6 h, 18 h, 24 h and 48 h), immediately diluted 1:4 with SDS-PAGE running buffer and adjusted with SDS gel loading buffer (5 × concentrated) to 2.5 % v/v 2-mercaptoethanol, followed by boiled for 5 min. Then, the samples were subjected to 10% SDS-PAGE (0.8 μg PsTag600-FGF21 per lane), and a Western blot was carried out with a rabbit anti-human FGF21 antibody (1:1000 dilutions, ab64857, Abcam, Cambridge, MA) as primary antibody and horseradish peroxidase-conjugated goat anti-rabbit Ig antibody (1:5000 dilutions, Sangon Biotech, China) as secondary antibody for detection of FGF21. The protein concentration was determined using the Bradford Protein Assay Kit (Beyotime Institute of Biotechnology, China).

A kidney from a BALB/c mouse was mechanically at 4 ℃ in PBS and the homogenate was cleared by centrifugation. 10 μg of PsTag600-FGF21 in 20 μl PBS was mixed with 60 μl of the kidney homogenate at varying dilution (1:5, 1: 10, 1:50, 1:100, 1:1000) in PBS and incubated at 37 ℃ for 1 h. Then, the samples were subjected to 10% SDS-PAGE (1 μg PsTag600-FGF21 per lane), and a Western blot was performed as described above.

Immunogenicity

Female BALB/c mice were injected subcutaneously at 1 mg ₭g^{-1} of FGF21 and 3.7 mg ₭g^{-1} of PsTag600-FGF21 (as FGF21 equivalent of 51 nmol ₭g^{-1}) every 2 days for three weeks, for a total of 8 injections per animal. Native FGF21 was without polyhistidine tag. Each antigen was injected into two groups, either alone or

pre-mixed with adjuvant. Groups including adjuvant received complete Freund's adjuvant with the first injection and incomplete Freund's adjuvant for all subsequent injections. IgG titers specific for corresponding antigen were continuously detected for 5 weeks after the final immunization.

A plasma sample was isolated from each animal one week following the final immunization. Antibody titer was measured by direct ELISA carried out by 12 h immobilization of 1250 ng/well antigen in PBS, followed by blocking with 10% fetal cow serum (Gibco, New Zealand). After 3 washes with PBS, plasma samples were serially diluted (1:50-1:10000) across the plate in PBS containing 2% BSA. After incubation and washing, plates were developed by incubation with horseradish peroxidase-conjugated anti mouse IgG (Sangon Biotech, China) followed by o-Phenylenediamine (OPD) substrate and read after 30 minutes at 492 nm.

Reverse- Phase HPLC

The purified protein solution in PBS was adjusted to 5% v/v acetonitrile, 0.065% v/v trifluoroacetic acid (TFA) and 50 µl was applied to a SepaxGP-C18 column (Sepax Technologies, Inc.). The conditions of RP-HPLC were as following: High Performance Liquid Chromtography System (Agilent Technologies) equipped with an autosampler. The mobile phase comprised two buffers: Buffer A (distilled H_2O, 0.065% TFA) and Buffer B (acetonitrile, 0.065%). The RP column was first subjected to an isocratic 5% v/v acetonitrile-water gradient for 10 min, followed by a 5% to 60% (v/v) acetonitrile-water gradient over 20 min, at a total solvent flow-rate of 1 ml min^{-1}. Absorbance was measured at 219 nm using a UV detector.

Figures and figure legends

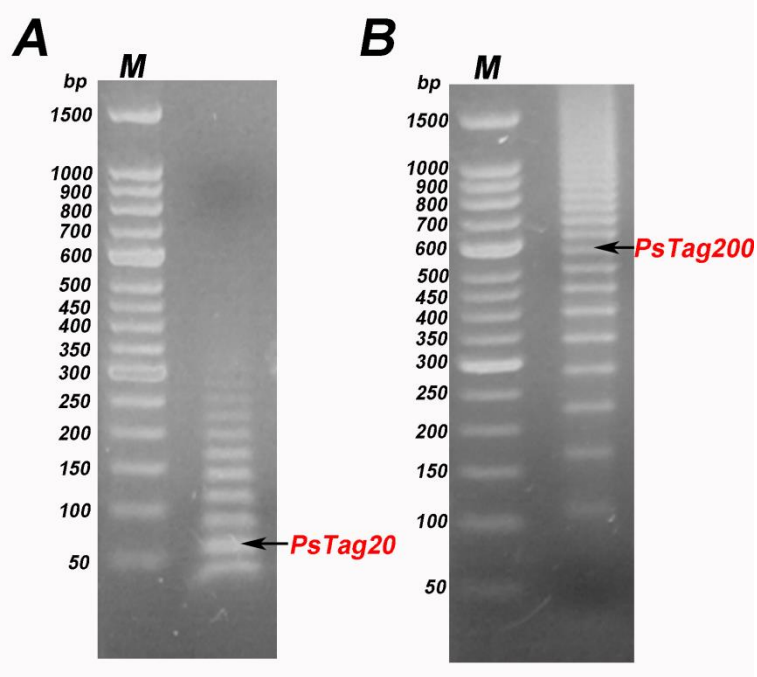

Figure S1. Analysis of the building block by 2% agarose gel electrophoresis after phosphorylation and ligation. DNAs bands were stained with Goldview^{TM}. (A) 60 nucleotide fragments encoding 20 amino acids were isolated. (B) 600 nucleotide fragments encoding 200 amino acids were isolated.

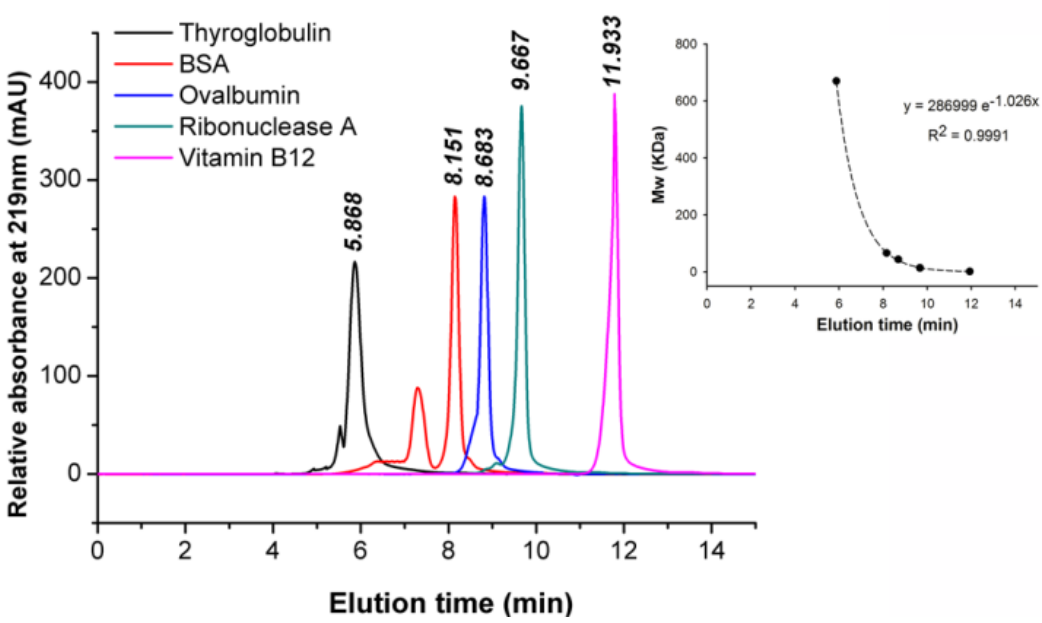

Figure S2. SEC-HPLC of standard proteins

Analytical SEC was performed on a Zenix-C 300 column (Sepax Technologies, Inc.) at a flow rate of 1 ml min^{-1} using High Performance Liquid Chromtography System (Agilent Technologies) equipped with an autosampler. The standard proteins were applied with 150 mmol L^{-1} sodium phosphate buffer (pH 7.0) and respective elution volumes were measured. Protein standards were each run independently in successive experiments. The molecular weights (MWs) of marker proteins (Thyroglobulin, 670 kDa; BSA, 66 kDa; Ovalbumin, 44 kDa; Ribonuclease A, 13.7 kDa; Vitamin B12, 1.35 kDa) was plotted versus their elution time (black circles) and fitted by a nonlinear regression model (R^2 = 0.9991).

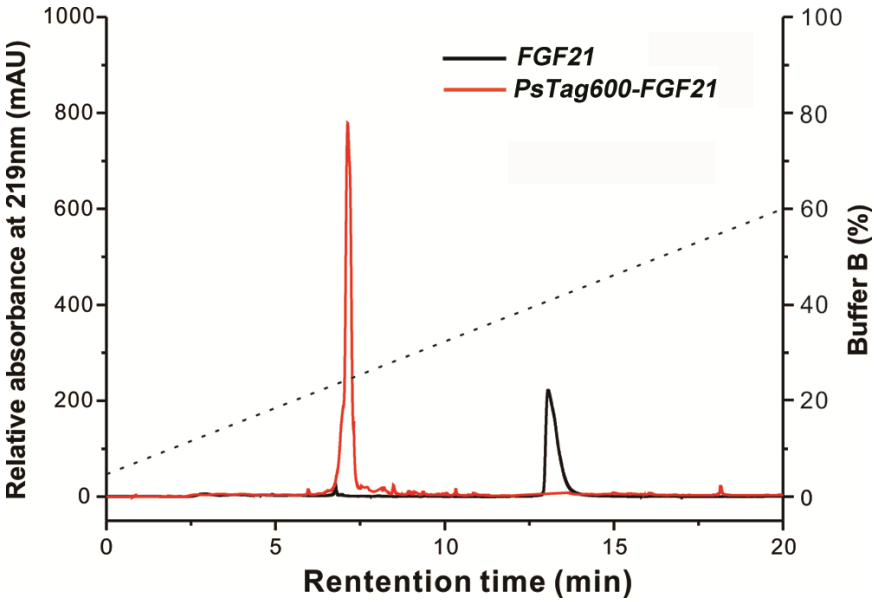

Figure S3. Purity analysis of native FGF21 and PsTag600-FGF21 by RP-HPLC.

The purities of FGF21 and PsTag600-FGF21 were performed on a SepaxGP-C18 column using an elution gradient from 5% v/v acetonitrile, 0.065% v/v TFA to 60% v/v acetonitrile, 0.065% TFA. Both profiles showed a single homogeneous peak with obviously earlier elution of PsTag600-FGF21(24.6% acetonitrile) in comparison with the corresponding unfused FGF21 (40.9% acetonitrile).

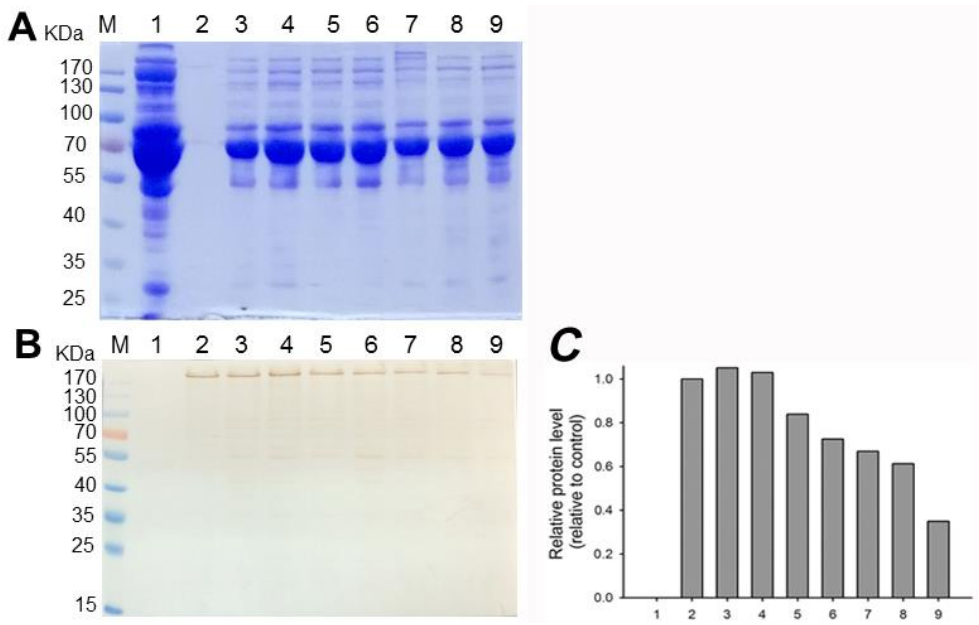

Figure S4. Analysis of plasma stability for PsTag600-FGF21

Purified PsTag600-FGF21 was incubated in the presence of BALB/c mouse plasma at 37 ℃ for up to 48 h. Samples taken at different time points were subject to (A) 10% SDS-PAGE and (B) Western blotting with a rabbit anti-human FGF21 antibody. Lane 1, serum only (as control); lane 2, PsTag600-FGF21; lane 3-9: samples taken at 0, 1, 3, 6, 18, 24, and 48 h, respectively. Full length PsTag600-FGF21 protein was detectable for up to 48 h, demonstrating high stability against serum proteases. (C) Western blotting quantitative analysis of Figure S4B by Quantity One ® (Bio-Rad).

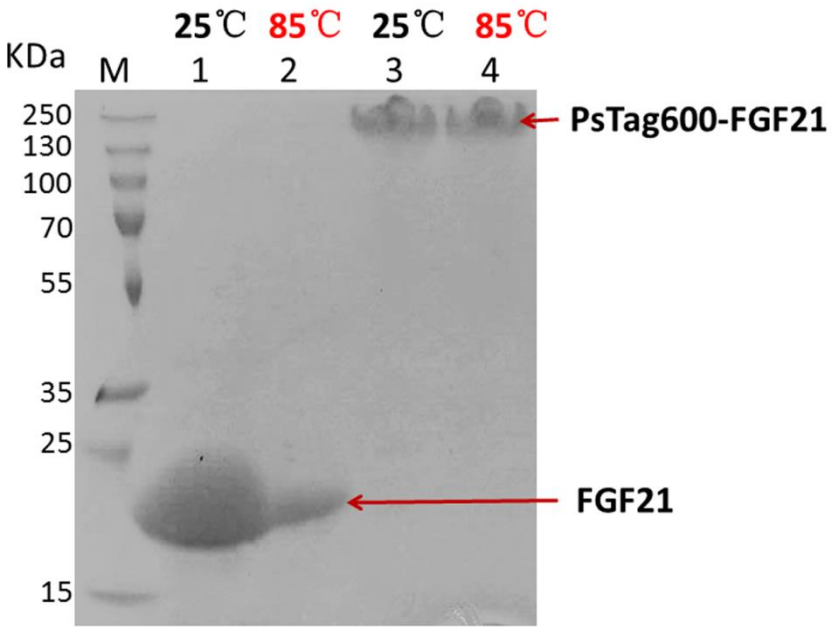

Figure S5. Human FGF21 was stabilized by PsTag polypeptides

To demonstrate the stabilization of FGF21 conferred by PsTag polypeptides, FGF21 and PsTag600-FGF21 with the concentration of 5 mg·mL^{-1} in PBS were incubated at 25℃ (lanes 1 & 3) and 85℃ (lanes 2 & 4) for 15 min at which time any insoluble protein was rapidly removed by centrifugation at 12000 rpm for 20 min. The soluble fraction was then analyzed by 12% SDS-PAGE. PsTag600-FGF21 remained soluble after heating while visible precipitate was observed after heat treatment of FGF21 alone.

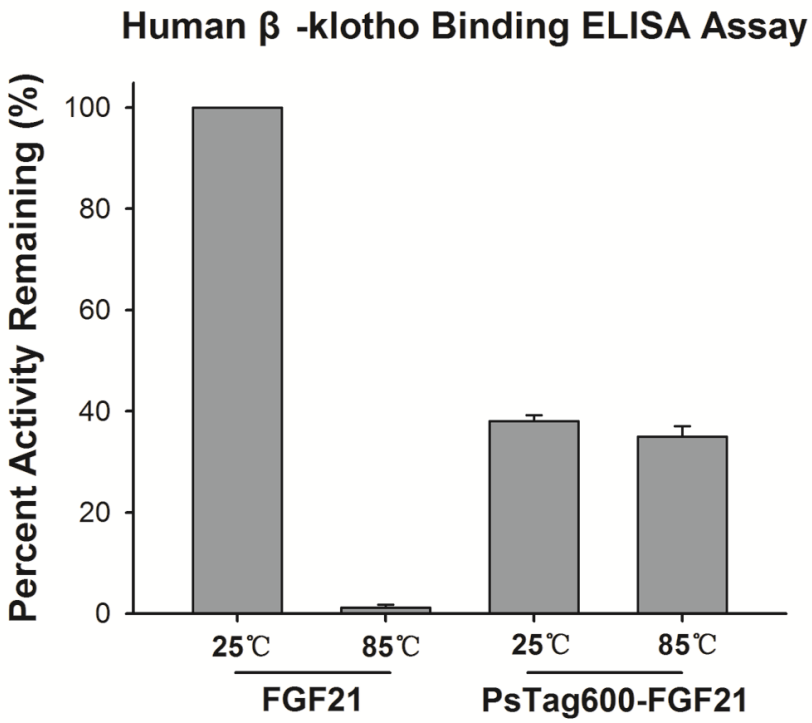

Figure S6. PsTag600-FGF21 retained activity following heat treatment

Human β-klotho binding was measured for FGF21 and PsTag600-FGF21 after incubation for 15 min at 85℃ versus untreated controls (N = 3). Here binding activities of FGF21 and PsTag600-FGF21 were determined by measuring the EC_{50} for binding to human β-klotho (58890KB, R&D) in a direct binding ELISA. 25 ng/well human β-klotho was coated in a 96-wells ELISA plate (Costar, USA), and various concentrations of FGF21 and PsTag fusion proteins were added into the pre-coated plate. The bound protein complexes was detected by HRP labeled FGF21 antibody for each concentration, FGF21 antibody was pursued from Abcam and labeled by HRP labeling kit (Thermo). The OD_{450nm} absorance was plotted against protein concentrations and EC_{50} values were fitted with a single-site binding model using GraphPad Prism 5. All values were reported as relative to FGF21 incubated at 25℃ (set to 100%). FGF21 and PsTag600-FGF21 samples were prepared as described in Figure S5. Thermal denaturation of FGF21 resulted in a significant loss of activity consistent with the observed precipitation (Figure S5), while PsTag600-FGF21 retained the majority of its starting activity.

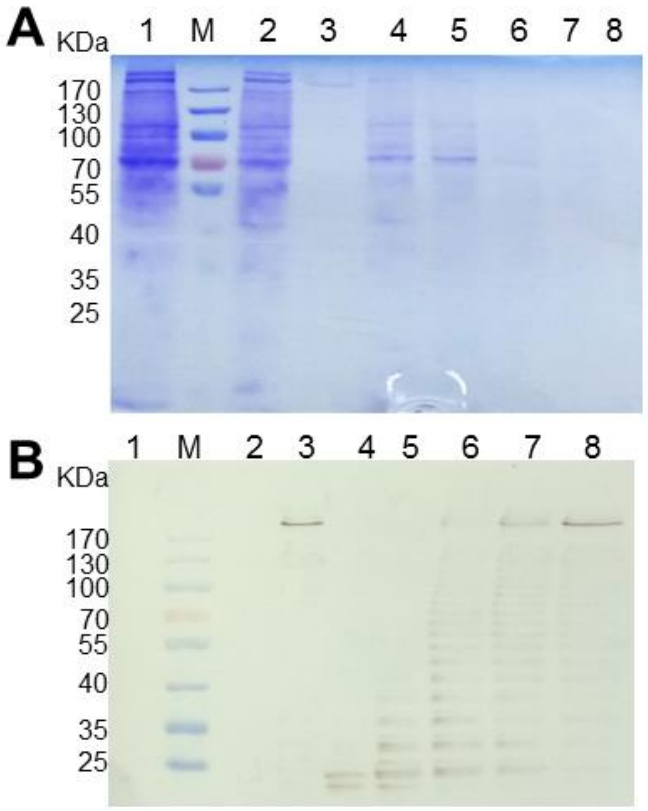

Figure S7. Analysis of biodegradability for PsTag600-FGF21

Purified PsTag600-FGF21 was incubated in the presence of a mouse renal homogenate with varying dilution at 37 ℃ for 1 h and subjected to (A) 10% SDS-PAGE and (B) Western blotting with a rabbit anti-human FGF21 antibody. Lane 1, PsTag600-FGF21 with homogenate; lane 2, 1:5 dilution of homogenate (as control); lane 3, purified PsTag600-FGF21; lane 4-8, PsTag600-FGF21 incubated with the renal homogenate at 1:5, 1:10, 1:50, 1:100 and 1:1000 dilution, respectively. The band for PsTag600-FGF21 disappears with decreasing dilution of the kidney extract, showing that PsTag600-FGF21 was rapidly degraded. Thus, in contrast with the poorly degradable chemical polymer PEG, PsTag polypeptides should be easily metabolized.

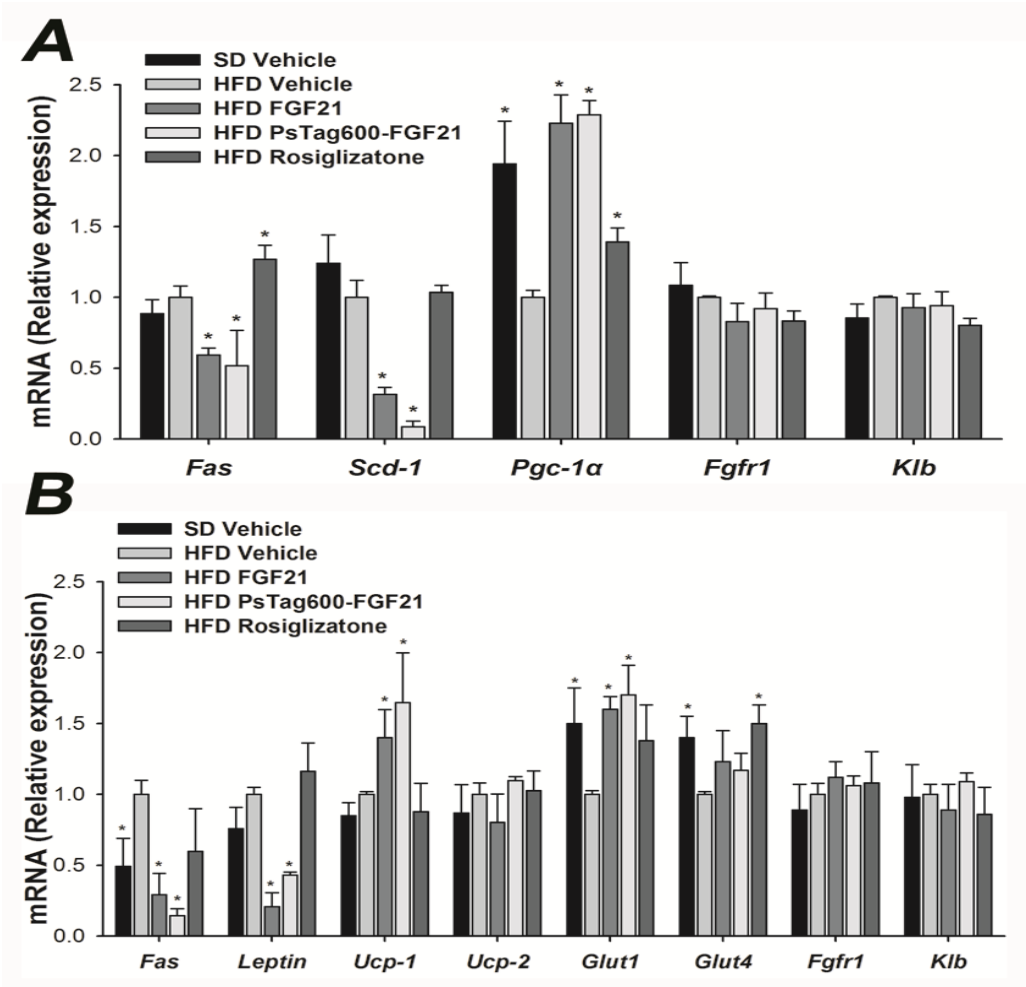

Figure S8. Gene analysis of liver and iWAT.

(A) Relative mRNA levels of key genes in liver. *Fas*, Fatty acid synthase; *Scd-1*, Stearoyl-CoA desaturase 1; *Pgc-1α*, PPARγ-coactivator-1α; *Fgfr1*, Fibroblast growth factor receptor 1; *Klb*, β-Klotho. (B) Relative mRNA levels of key genes in WAT. *Ucp*, Uncoupling protein; *Glut*, Glucose transporter. (N = 6/group. Data are means ± SD. * $p<0.05$ vs vehicle-treated HFD mice.).

Table S1

A library of 10-amino-acid segments

Name of sequence	Amino acid sequence
PT01-1	GPSASTGPST
PT01-2	GPASTSGPST
PT01-3	GPASTGSPST
PT01-4	GPASTGPSTS
PT01-5	GPSTSGPTAS
PT01-6	GPTSGSPTAS
PT01-7	GPTSGPSTAS
PT01-8	GPTSGPTSAS
PT01-9	GPSTASGTPS
PT01-10	GPTSASGTPS
PT01-11	GPTASGSTPS
PT01-12	GPTASGTSPS
PT01-13	GPSTASGPTS
PT01-14	GPTSASGPTS
PT01-15	GPTASGSPTS
PT01-16	GPTASGPSTS
PT01-17	GSPAGSPTST
PT01-18	GTSSATPSGP
PT01-19	GTSTPSGSAP
PT01-20	GSPATSGSTP

Table S2

PCR Primer Sequences

Gene name	Forward primer sequence (F)	Reverse Primer sequence (R)
Fas	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
Scd1	TTCTTGCGATACACTCTGGTGC	CGGGATTGAATGTTCTTGTCGT
Pgc1α	TATGGAGTGACATAGAGTGTGC	CCACTTCAATCCACCCAGAAAG
Glut1	GGATCTCTCTGGAGCACAGG	TCCTCCTGGACTTCACTGCT
Glut4	CCGGATTCCATCCCACAAG	TCATGCCACCCACAGAGAAG
Fgfr1	AGAGTCCAAGAGTAAAAGCAGC	CTTCCGAGGTTCAGCTCTCC
Klb	TGTTCTGCTGCGAGCTGTTAC	TACCGGACTCACGTACTGTTT
Leptin	GAGACCCCTGTGTCGGTTC	CTGCGTGTGTGAAATGTCATTG
Ucp1	AGGCTTCCAGTACCATTAGGT	CTGAGTGAGGCAAAGCTGATTT
Ucp2	ATGGTTGGTTTCAAGGCCACA	CGGTATCCAGAGGGAAAGTGAT
β-actin	TAAGGCCAACCGTGAAAAAG	ACCAGAGGCATACAGGGACA

PsTag200 sequence

GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPTSGPSTAS GPSTASGPTS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPTSGPSTAS GPSTASGPTS

PsTag400 sequence

GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPTSGPSTAS GPSTASGPTS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPTSGPSTAS GPSTASGPTS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPTSGPSTAS GPSTASGPTS

PsTag600 sequence

GPTSGPSTAS GPSTASGPTS GPASTGSPST GPTSGPSTAS GPTSGPSTAS GPSTASGPTS GPTSGPSTAS GPSTASGPTS GPTSGPSTAS GPSTASGPTS GPTSGPSTAS GPSTASGPTS GPASTGSPST GPTSGPSTAS GPTSGPSTAS GPSTASGPTS GPTSGPSTAS GPSTASGPTS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPTSGPSTAS GPSTASGPTS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPTSGPSTAS GPSTASGPTS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPASTGSPST GPTSGPSTAS GPTSGPSTAS GPSTASGPTS