# Mitochondria-derived peptide SHLP2 regulates energy homeostasis through the activation of hypothalamic neurons

Seul Ki Kim[1,2], Le Trung Tran[1,2], Cherl NamKoong[3], Hyung Jin Choi[3,4], Hye Jin Chun[5], Yong-ho Lee[5], MyungHyun Cheon[6], ChiHye Chung[6], Junmo Hwang[7], Hyun-Ho Lim[7], Dong Min Shin[1,2], Yun-Hee Choi[1], and Ki Woo Kim[1,2 *]

[1]Division of Physiology, Department of Oral Biology, Yonsei University College of Dentistry, Seoul, 03722, Korea

[2]Department of Applied Life Science, BK21 FOUR, Yonsei University College of Dentistry, Seoul, 03722, Korea

[3]Neuroscience Research Institute, Seoul National University College of Medicine, Seoul, 03080, Korea

[4]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, 03080, Korea

[5]Department of Internal Medicine, Yonsei University College of Medicine, Seoul, 03722, Korea

[6]Department of Biological Sciences, Konkuk University, Seoul, 05029, Korea

[7]Neurovascular Unit Research Group, Korea Brain Research Institute (KBRI), Daegu, 41068, Korea

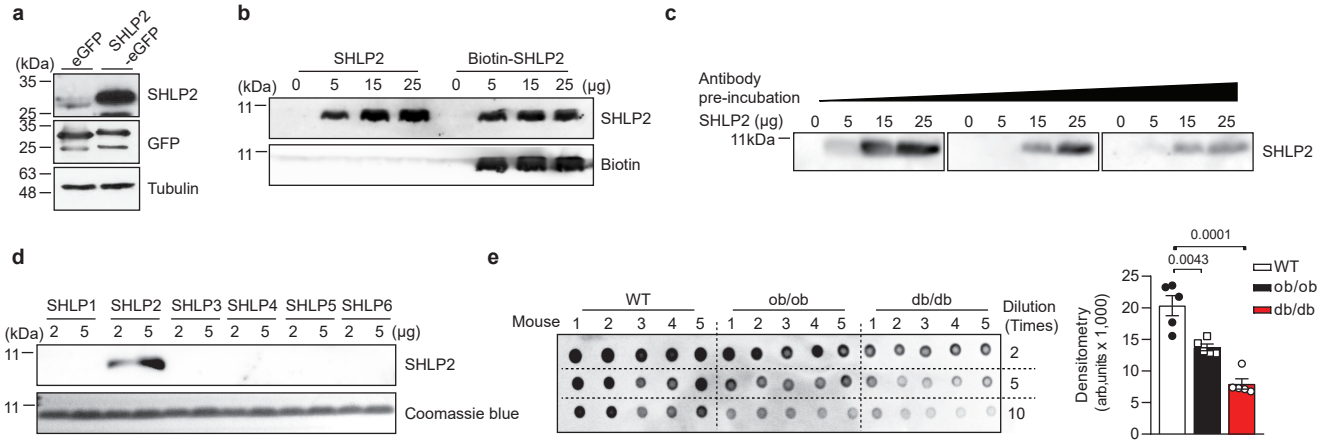
[*]Correspondence and reprints, materials should be addressed to:

Ki Woo Kim (kiwoo-kim@yuhs.ac)

**SUPPLEMENTARY INFORMATION**

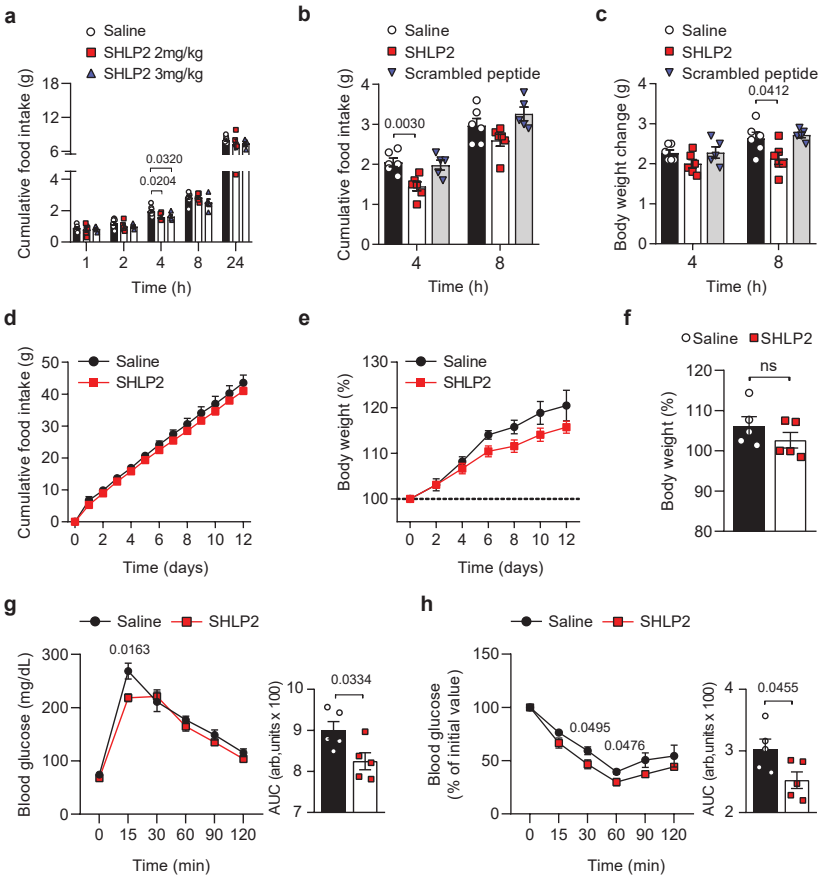
**Supplementary Figure 1-6**

**Supplementary Tables 1-3**



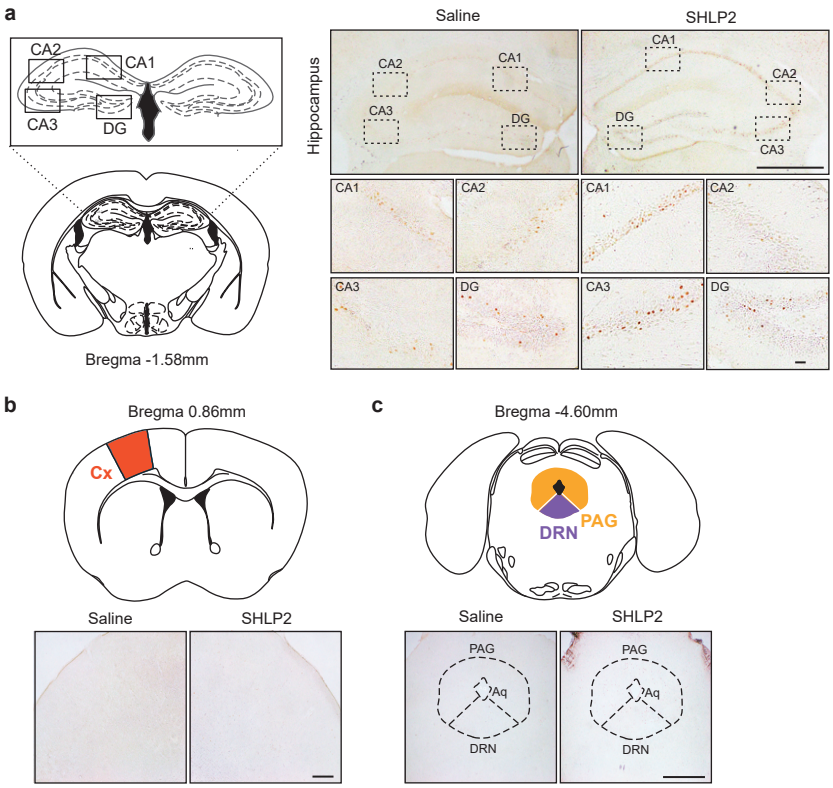

**Supplementary Fig. 1: Validation of SHLP2 antibody.**

**a**, Detection of SHLP2 after overexpression of SHLP2-eGFP construction in HEK 293 cells. Western blots were performed using anti-SHLP2, anti-GFP or anti-tubulin antibodies. **b**, Detection of SHLP2 and biotinylated SHLP2 peptides using anti-SHLP2 and anti-biotin antibodies. **c**, Peptide competition assays using SHLP2 peptides. Anti-SHLP2 antibody was incubated in the presence of increasing amounts (1, 5, 15, 25 μg) of each peptide indicated. **d**, Detection of SHLP1 to 6 using anti-SHLP2 antibody. **e**, Dot-blot image (left) and relative blot densitometry (right) detecting plasma SHLP2 levels in WT, *ob/ob* and *db/db* male mice ($n = 5$ each group). Data shown are representative of three independent experiments with similar results (**a-d**). *n* indicates the number of biologically independent mice examined. Data were presented as mean ± SEM. Two-tailed Student's t-tests were used. Source data are provided as a Source Data file.



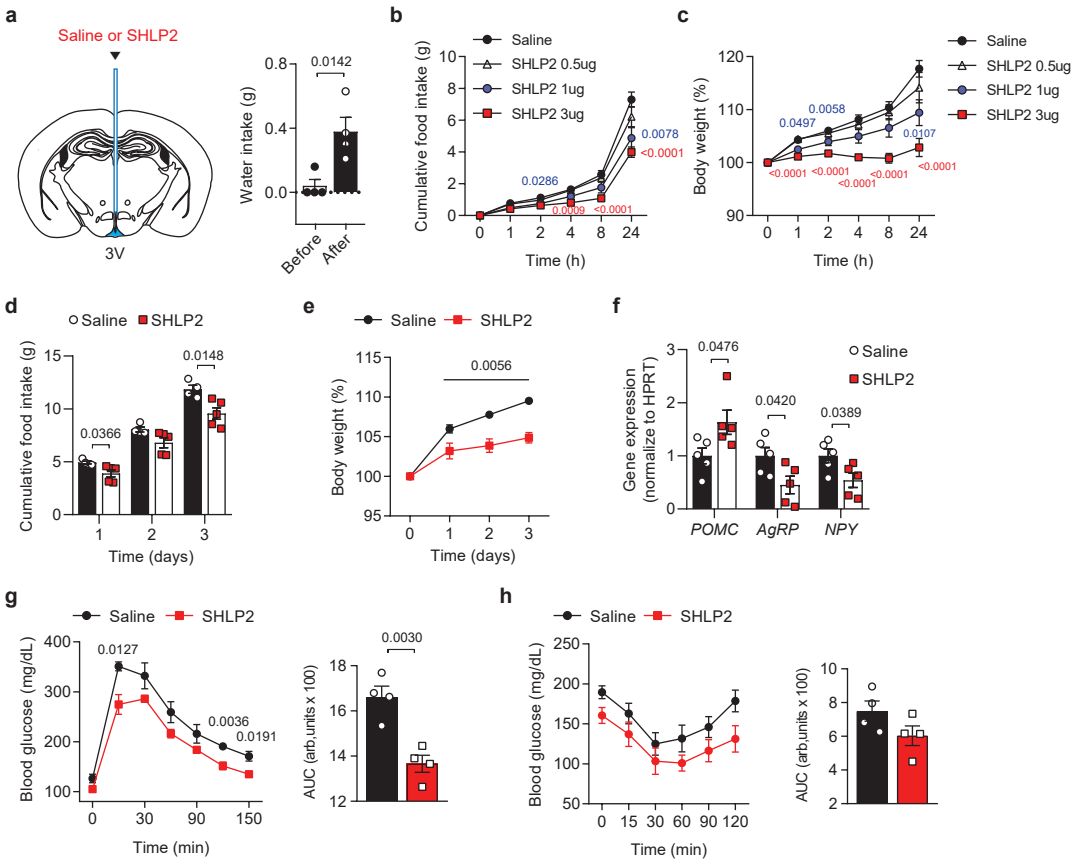

**Supplementary Fig. 2: Temporal changes in food intake and body weight, as well as the results of the ITT and GTT, in normal chow-fed mice after an IP injection of SHLP2**

**a,** Cumulative food intake (left) of male mice fed with NC after IP injection of saline or 2, 3mg/kg SHLP2 ($n$ = 9 saline, $n$ = 5 each SHLP2 group). **b-c,** Cumulative food intake (**b**) and body weight change (**c**) of male mice fed with NC after IP injection of saline or SHLP2 or scrambled peptide following an overnight fasting ($n$ = 6 saline, $n$ = 6 SHLP2, $n$ = 5 scrambled peptide). **d-e**, Cumulative food intake (**d**) and body weight change (% of initial) (**e**) were monitored after daily IP injection of either saline or SHLP2 in male mice ($n$ = 3 saline, $n$ = 5 SHLP2). **f**, Body weight (% of initial) of male mice fed with NC after daily IP injection of saline or SHLP2 for 3 weeks ($n$ = 5 each group). **g-h**, GTT (**g**) and ITT (**h**) were performed in NC-fed male mice after 3 weeks of IP administration of saline or SHLP2 ($n$ = 5 each group). $n$ indicates the number of biologically independent mice examined. Data were presented as mean ± SEM. Two-tailed Student's t-tests were used. Source data are provided as a Source Data file.



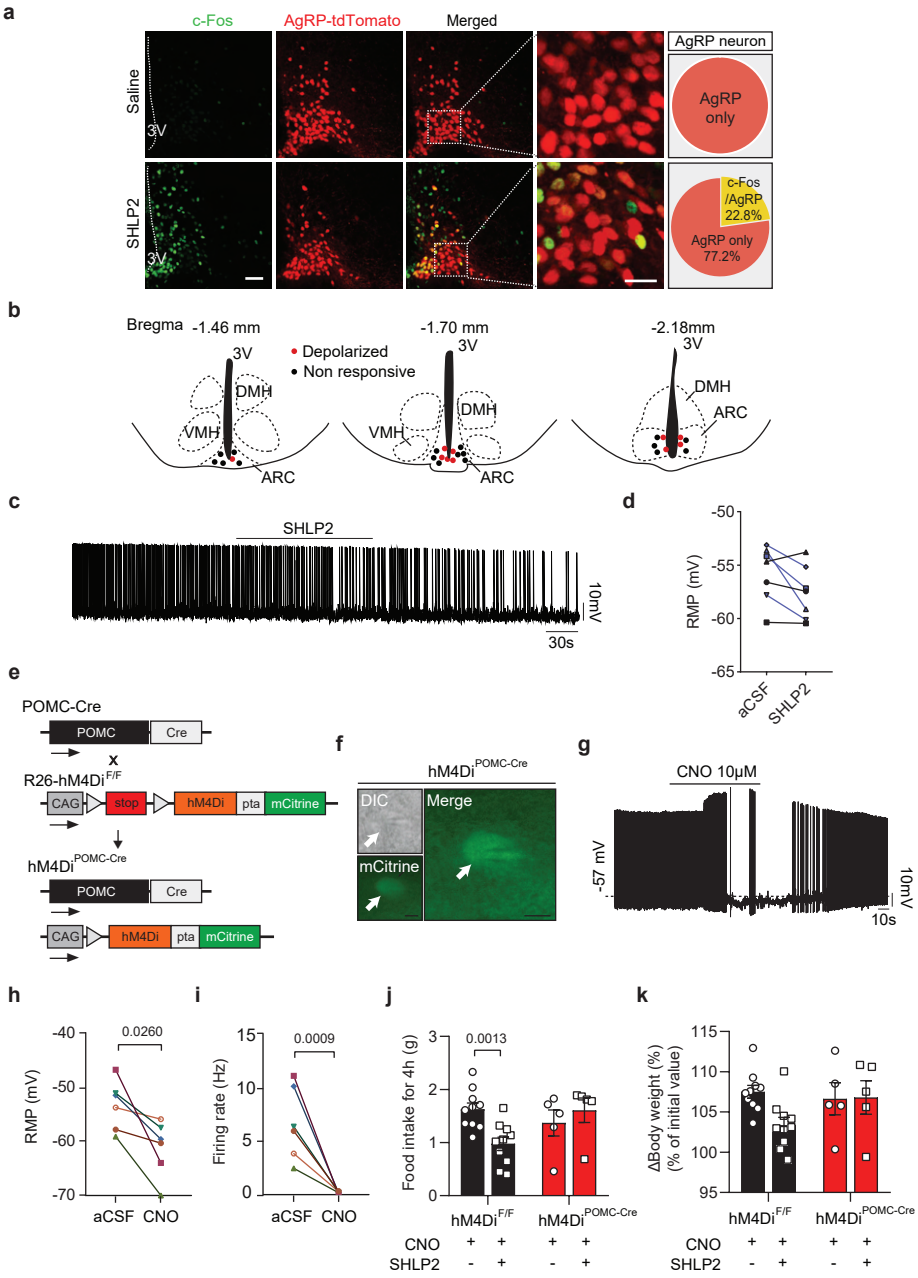

**Supplementary Fig. 3: c-Fos activation in different brain regions in response to SHLP2.**

**a-c**, c-Fos immunoactivity after SHLP2 administration (IP) in the hippocampus (**a**)**,** cortex (Cx. **b**), periaqueductal gray (PAG, **c**) and dorsal raphe nucleus (DRN, **c**) (*n* = 4 each group). CA, cornu amonis. DG, dentate gyrus. Aq, aqueduct. *n* indicates the number of biologically independent mice examined. Scale bars, 100μm. Source data are provided as a Source Data file.



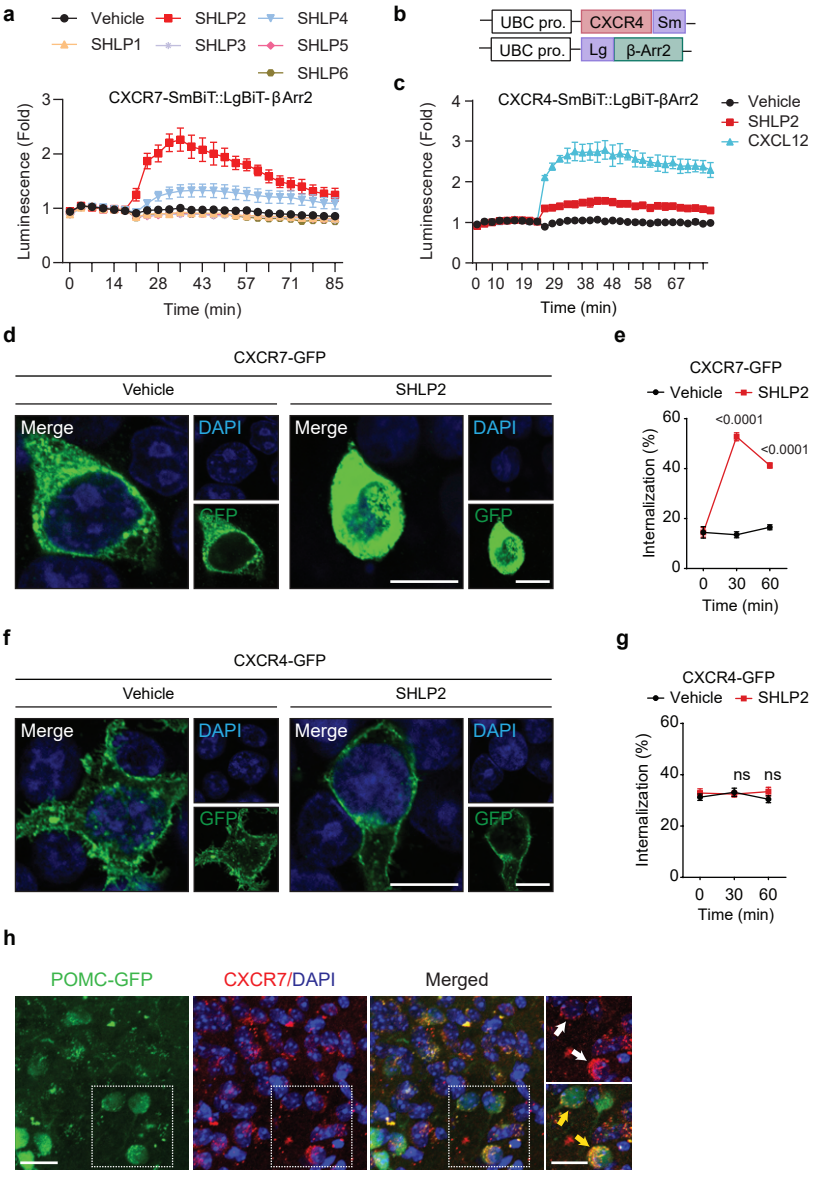

**Supplementary Fig. 4: Long-term SHLP2 treatment ameliorates HFD-induced obesity.**

**a**, Schematic for experimental configuration. A guide cannula was implanted to the 3V to deliver saline or SHLP2 (left). Water intake (right) following an acute ICV administration of angiotensin II ($n = 4$ each group). **b-c**, Cumulative food intake (**b**) and body weight (% of initial) (**c**) of male mice fed with NC after ICV injection of saline or 0.5, 1, 3μg SHLP2 ($n = 10$ each group). **d-e**, Cumulative food intake (**d**) and body weight (% of initial) (**e**) of HFD-fed male mice following a single ICV injection of saline or SHLP2 ($n = 4$ saline, $n = 5$ SHLP2). **f**, The *Pomc*, *Agrp* and *Npy* expression in the hypothalamus of male mice fed HFD after one hour of ICV injection of Saline or SHLP2 ($n = 4$ saline, n = 5 SHLP2). **g-h**, GTT (**g**) and ITT (**h**) were performed in HFD-fed male mice after 4 hours of ICV administration of saline or SHLP2 ($n = 4$ each group). *n* indicates the number of biologically independent mice examined. Data were presented as mean ± SEM. Two-tailed Student's t-tests were used. and two-way ANOVA with Bonferroni post-hoc tests were used in figure (**e**). Source data are provided as a Source Data file.


a
c-Fos
AgRP-tdTomato
Merged
AgRP neuron
Saline
SHLP2
3V
AgRP only
c-Fos /AgRP 22.8%
AgRP only 77.2%
b
Bregma
-1.46 mm
-1.70 mm
-2.18mm
Depolarized
Non responsive
DMH
VMH
ARC
c
SHLP2
10mV
30s
d
RMP (mV)
aCSF
SHLP2
e
POMC-Cre
POMC
Cre
R26-hM4Di F/F
CAG
stop
hM4Di
pta
mCitrine
hM4Di POMC-Cre
f
hM4Di POMC-Cre
DIC
Merge
mCitrine
g
CNO 10μM
-57 mV
10mV
10s
h
RMP (mV)
0.0260
aCSF
CNO
i
Firing rate (Hz)
0.0009
aCSF
CNO
j
Food intake for 4h (g)
0.0013
hM4Di F/F
hM4Di POMC-Cre
CNO
SHLP2
k
ΔBody weight (%) (% of initial value)
hM4Di F/F
hM4Di POMC-Cre
CNO
SHLP2

**Supplementary Fig. 5: Effect of SHLP2 on POMC and AgRP neurons.**

**a**, Percentage of c-Fos activation in AgRP neurons after an ICV administration of SHLP2 (*n* = 3). Scale bars, 20μm. **b**, Diagram showing the location and responses of recorded POMC neurons across the mediobasal hypothalamus. **c-d,** Representative trace (**c**) of AgRP neurons and effects on resting membrane potential (RMP) (**d**) of a subset of AgRP neurons. **e**, Schematic strategy for recombinant transgenes to generate hM4Di$^{POMC\text{-}Cre}$ male mice. **f**, Fluorescent and DIC figures of targeted POMC neurons expressing the Cre-dependent modified form of the hM4Di receptor in fusion with the fluorescence protein mCitrine. The arrows indicate the targeted cell (*n* = 6 from 2 animals). **g-i**, Representative inhibition of POMC neuron (**g**) by CNO application. CNO induced hyperpolarization of POMC neurons expressing hM4Di, showing through decreases in the resting membrane potentials (**h**) and firing rates (**i**) (*n* = 6 from 2 animals). **j-k,** Cumulative food intake (**j**) and Δbody weight (%) (**k**) in hM4Di$^{POMC\text{-}Cre}$ male mice after ICV SHLP2 administration with CNO injection (*n* = 10 each hM4Di$^{F/F}$ group, *n* = 5 each hM4Di$^{POMC\text{-}Cre}$ group). Scale bars, 5μm. *n* indicates the number of biologically independent mice examined. Data were presented as mean ± SEM. Two-tailed Student's t-tests were used. Wilcoxon matched-pairs signed rank test were used in electrophysiological result. CAG, CMV immediate enhancer/β-actin promoter; hM4Di, Gi-coupled hM4D DREADD; pta, porcine Teschovirus cleavage site; mCitrine; citrine fluorescence. Source data are provided as a Source Data file.


a
Vehicle
SHLP2
SHLP4
SHLP1
SHLP3
SHLP5
SHLP6
CXCR7-SmBiT::LgBiT-βArr2
Luminescence (Fold)
Time (min)
b
UBC pro.
CXCR4
Sm
UBC pro.
Lg
β-Arr2
c
CXCR4-SmBiT::LgBiT-βArr2
Vehicle
SHLP2
CXCL12
Luminescence (Fold)
Time (min)
d
CXCR7-GFP
Vehicle
SHLP2
Merge
DAPI
GFP
e
CXCR7-GFP
Vehicle
SHLP2
<0.0001
<0.0001
Internalization (%)
Time (min)
f
CXCR4-GFP
Vehicle
SHLP2
Merge
DAPI
GFP
g
CXCR4-GFP
Vehicle
SHLP2
ns
ns
Internalization (%)
Time (min)
h
POMC-GFP
CXCR7/DAPI
Merged

**Supplementary Fig. 6: SHLP2 interacts specifically with CXCR7, but not CXCR4.**

**a,** The β-Arrestin2 recruitment to CXCR7 by SHLP 1 to 6 ($n = 4$ each group). Note that the only SHLP2 exhibited specific activity. **b**, The DNA constructs used to for the NanoLuc complementation-based β-Arrestin2 recruitment to the chemokine receptor CXCR4. **c**, Representative result of β-Arrestin2 recruitment assay to CXCR4 ($n = 3$ saline, $n = 3$ CXCL12, $n = 4$ SHLP2). Note that there is no notable activity by SHLP2 **d,** Representative immunofluorescent staining of CXCR7 upon the application of SHLP2. **e**, The percentage of internalized receptors CXCR7 at different time points after the treatment of SHLP2 ($n = 3$ each group). **f**, Representative immunofluorescent staining of CXCR4 upon the application of SHLP2. **g**, The percentage of internalized receptors CXCR4 at different time points after the treatment of SHLP2 ($n = 3$ each group). Scale bars, 10μm. **h**, Representative images of CXCR7 expression in POMC neurons ($n = 3$ each group). Scale bars, 50μm. *n* indicates the number of biologically independent experimental groups examined. Data were presented as mean ± SD. ns: no significance by two-way ANOVA followed by Sidak multiple comparison tests (**e and g**). Source data are provided as a Source Data file.

## Supplementary Table 1. The clinical characteristics of the sutdy subjects

| | Healthy (n= 7) | Obese (n= 6) | Diabetic (n= 7) |
|---|---|---|---|
| Age (year) | 55.1± 14.9 | 41.3± 23.7 | 42.3± 18.7 |
| BMI (kg/m$^2$) | 22.6± 5.1 | 31.9± 2.9 | 31.4± 2.3 |
| TG (mg/dL) | 85.4± 101.8 | 192.2± 164.8 | 174.4± 190.6 |
| Cholesterol (mg/dL) | 195.3± 43.7 | 217.2± 32.2 | 202.3± 28.7 |
| AST (U/L) | 24.9± 9.1 | 36.8± 21.2 | 34.4± 18.6 |
| ALT (IU/L) | 22.7± 19.3 | 60.6± 42.4 | 62.0± 39 |
| Gamma-GT (U/L) | 14.7± 11.3 | 46.8± 45.2 | 57.9± 61.1 |

BMI, Body mass index; TG, Triglyceride; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; Gamma-GT, Gamma-glutamyl transferase

## Supplementary Table 2. Primer sequences used for RT-PCR analyses

| **Gene** | Forward primer (5'-3') | Reverse primer (5'-3') |
|---|---|---|
| *Ucp1* | GGCAACAAGAGCTGACAGTA | GGCCCTTGTAAACAACAAAA |
| *Cox2* | ATAACCGAGTCGTTCTGCCAAT | TTTCAGAGCATTGGCCATAGAA |
| *Rps18* | TGTGTTAGGGGACTGGTGGACA | CATCACCCACTTACCCCCAAAA |
| *Pomc* | CAGGTCCTGGAGTCCGAC | CATGAAGCCACCGTAACG |
| *Npy* | CTACTCCGCTCTGCGACACT | AGTGTCTCAGGGCTGGATCTC |
| *Agrp* | CGGCCACGAACCTCTGTAG | CTCATCCCCTGCCTTTGC |
| *Cart* | AGAAGAAGTACGGCCAAGTC | GGACAGTCACACAGCTTCC |
| *Pgc1α* | AACCACACCCACAGGATCAGA | TCTTCGCTTTATTGCTCCATGA |
| *Dio2* | TTCTCCAACTGCCTCTTCCTG | CCCATCAGCGGTCTTCTCC |
| *Pparγ* | CAAGAATACCAAAGTGCGATCAA | GAGCTGGGTCTTTTCAGAATAATAAG |
| *Srebp-1c* | GGAGCCATGGATTGCACATT | GGCCCGGGAAGTCACTGT |
| *Fasn* | GGTGTGGTGGGTTTGGTGAATTGT | TCACGAGGTCATGCTTTAGCACCT |
| *Hprt* | CTCATGGACTGATTATGGACAGGAC | GCAGGTCAGCAAAGAACTTATAGCC |
| *Scd1* | AGTGCAGCAGGACCATGAGAATGA | TCCCTCCGGAAATGAACGAGAGAA |
| *Acaca* | AGGAAGATGGTGTCCCGCTCTG | GGGGAGATGTGCTGGGTCAT |
| *Fabp4* | AAGAGAAAACGAGATGGTGACAA | CTTGTGGAAGTCACGCCTTT |
| *Prdm16* | CGTCCACACGGAAGAGCGTGA | TGGAGGTTGCTGGGGTCCGT |
| *Nrf1* | CGATGGGATTCCAGTCTCTGT | TGAGCATCTCTGGGATAAATGC |

## Supplementary Table 3. Sequences of peptides

| Peptide name | Sequence |
|---|---|
| Small humanin-like peptide 1 (SHLP1) | MCHWAGGASNTGDARGDVFGKQAG |
| Small humanin-like peptide 2 (SHLP2) | MGVKFFTLSTRFFPSVQRAVPLWTNS |
| Small humanin-like peptide 3 (SHLP3) | MLGYNFSSFPCGTISIAPGFNFYRLYFIWVNGLAKVVW |
| Small humanin-like peptide 4 (SHLP4) | MLEVMFLVNRRGKICRVPFTFFNLSL |
| Small humanin-like peptide 5 (SHLP5) | MYCSEVGFCSEVAPTEIFNAGLVV |
| Small humanin-like peptide 6 (SHLP6) | MLDQDIPMVQPLLKVRLFND |
| Scrambled peptide | MKSFRVTVRPASLTSGVFTNLQFPWF |